PLEXON
Neurotechnology Research Systems

User Guide

Release 20, May 2021

# OmniPlex®

Neural Data Acquisition System

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**Caution**

**Electrostatic Discharge**

Some devices can be damaged by improper handling. Use appropriate electrostatic discharge (ESD) procedures when handling these devices. See http://www.esda.org/ for additional information on ESD procedures.

# OmniPlex®

Neural Data Acquisition System

User Guide

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# Publication History

### Change Log and Release Notes

The OmniPlex® User Guide is periodically updated and reissued, typically following a new software release. You can see a summary of changes that have been implemented in the software by accessing the **Change Log** for this product on the Plexon® website, www.plexon.com.

For additional details about a specific release, see the OmniPlex **Release Notes** document, which the software installer places on the hard drive during installation.

**Note:** The March 2018 version of this user guide reflected the new software naming convention. Release 16 was the same as Version 1.16.

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# Overview and System Components

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## 1.1 Overview

The Plexon® OmniPlex® Neural Data Acquisition System (OmniPlex System) is a modular, high-performance system for the acquisition, timestamping, recording, and visualization of neural signals, associated non-neural signals, and hardware digital events.

With the appropriate front-end data acquisition subsystem, the OmniPlex System can be configured to operate with either analog or digital headstages. Each of the front-end subsystems delivers continuously-digitized wideband data to the OmniPlex chassis. The subsystem descriptions are as follows:

- DigiAmp™ subsystem—This data acquisition subsystem is for use with Plexon analog headstages. It supports low-noise, wideband signal acquisition at a sampling rate of 40 kHz per channel. The electrically isolated DigiAmp Digitizing Amplifier is available in two sizes: the MiniDigi™ Amplifier for 16, 32, 48 and 64 channels, and the DigiAmp Amplifier for 64, 128, 192 and 256 channels.
- Digital Headstage Processor (DHP) subsystem—This data acquisition subsystem is for use with Plexon digital headstages. It enables up to 512 channels of neural recording, decreased sensitivity to ambient electrical noise, and lighter headstage cables with fewer wires for greater freedom of animal movement. It provides real-time upsampling to 40kHz and adjustment of multiplexer timing offsets (equivalent to simultaneous sampling) for improved sorting quality, trodal acquisition and software referencing.

The OmniPlex System processes the incoming data with a set of software modules which support flexible, user-configurable separation of spikes and field potentials, spike detection and alignment, and state-of-the-art spike sorting algorithms. Regardless of which data acquisition subsystem is being used, many of the OmniPlex features are the same.

Visualization of signals, spikes, and events, along with control of all processing and recording parameters, is provided by PlexControl, a customizable software application which is the main user interface to the OmniPlex System. Experimental data acquired by the OmniPlex System can also be sent in real time to MATLAB®, NeuroExplorer®, C/C++ and Python™ client programs and across a TCP/IP or UDP network to remote clients.

**Note:** This user guide is not intended to cover the features of the earlier analog version of the OmniPlex System, which includes an analog amplifier. However, some features of the analog and digital systems are similar. See Section 1.2.4, “Digital OmniPlex System versus Analog OmniPlex System” on page 7 for additional details.

## 1.2 Components

### 1.2.1 OmniPlex System Components

The OmniPlex System consists of the following hardware and software components:

- A rack-mountable OmniPlex chassis containing digital input, analog input, link, and timing / synchronization cards, plus one or more BNC breakout panels. There are two versions of the chassis, as shown below.

Basic OmniPlex chassis—operates with all software releases

OmniPlex eChassis—requires software Release 18 or later

- A Plexon supplied custom-configured and performance-optimized Windows® 10 PC, which runs the OmniPlex System acquisition and control software and has a high-speed link to the OmniPlex chassis.

**Note:** OmniPlex Systems are supported only with PCs that have been provided and configured by Plexon.

If you are installing additional programs on your PC, please note the following information: The PCs currently being provided by Plexon for OmniPlex Systems run the Windows 10 64-bit operating system.

The OmniPlex software runs only on Windows 7 and Windows 10 systems.

If you have an older OmniPlex System which is still installed on a Windows XP computer, please migrate the system to a Windows 10 PC. It is also recommended that you consider migrating any OmniPlex System PC from Windows 7 to Windows 10. Plexon Support (+1 214-369-4957 or support@plexon.com) can answer questions about how to move your system from Windows 7 or Windows XP to Windows 10.

- The OmniPlex System acquisition and control software, consisting of OmniPlex Server, PlexControl, PlexNet, and a software development kit (SDK) which allows online interfacing to MATLAB, C/C++ and Python™ programs. The latest software and SDKs can be downloaded from the Plexon website.

**Note:** The SDKs support the OmniPlex Native Client API, a set of functions for reading online spike, continuous, and event data, with full support for OmniPlex sources. This API is present in Release 18 and later. Each of the SDKs contains a set of sample client programs that illustrate how to use various capabilities of the Native Client API, plus documentation of Native Client API function parameters and usage.

For the C/C++ online SDK, the Native Client API is in the file CandC++ClientDevelopmentKit.zip.

For the MATLAB online SDK, the Native Client API is in MatlabClientDevelopKit-mexw.zip.

For the Python online SDK, the Native Client API is in PythonClientDevelopmentKit.zip.

Earlier (legacy) versions of the API were originally designed for use with Plexon MAP ("Harvey Box") systems. Clients written using the legacy API may still be used with all versions of the OmniPlex System. However, the Native Client API is recommended for all new OmniPlex client development, because it has full support for OmniPlex sources, which makes development more straightforward than using the legacy APIs.

- A front-end data acquisition subsystem—Either a Plexon DigiAmp subsystem *or* Plexon DHP subsystem. See Section 1.2.2, "DigiAmp Subsystem Components" on page 5 or Section 1.2.3, "DHP Subsystem Components" on page 6 as applicable.

This user guide assumes that your OmniPlex System has been installed, configured and tested by a Plexon Sales Engineer; if not, please contact Plexon (+1 214-369-4957 or support@plexon.com) for installation assistance before attempting to operate the system. Appendix J: Hardware Pinouts, Connections and LEDs contains information on pinouts and cabling which may be useful when connecting the OmniPlex System to external devices and systems, such as a behavioral control system.

It is also assumed that you are familiar with basic concepts of neural electrophysiology, such as spikes (action potentials) and field potentials.

### 1.2.2 DigiAmp Subsystem Components

The DigiAmp subsystem consists of a Plexon DigiAmp™ or MiniDigi™ Digitizing Amplifier, containing 16 to 256 channels of analog preamplification and signal conditioning, analog-to-digital conversion, and a high-speed proprietary digital interface to the OmniPlex System chassis. The MiniDigi subsystem supports 8 and 16 channel analog headstages (HST8 and HST16). The DigiAmp subsystem supports 8, 16 and 32 channel analog headstages (HST8, HST16 and HST32).

**Note:** The DigiAmp subsystem operates with the basic OmniPlex chassis, but not with the (newer) OmniPlex eChassis. The eChassis operates with the DHP subsystem, as described in Section 1.2.3, “DHP Subsystem Components” on page 6.

DigiAmp Digitizing Amplifier (DigiAmp Amplifier)

MiniDigi Digitizing Amplifier (MiniDigi Amplifier)

### 1.2.3 DHP Subsystem Components

The Digital Headstage Processor (DHP) subsystem consists of a DHP unit which performs upsampling and time alignment for 16 to 512 channels of digital headstages. It connects to the OmniPlex chassis through a high-speed proprietary digital interface. The DHP subsystem supports 8, 16, 32, 64 and 128 channel digital headstages (HST8D Gen2, HST16D, HST16D Gen2, HST32D, HST64D, HST64DS, HST64DSH and HST128DS).

The DHP subsystem operates with both the basic OmniPlex chassis and the (newer) OmniPlex eChassis.

#### Performance considerations

If you are upgrading an existing DHP subsystem to greater than 256 channels, certain Windows system settings must be updated for correct operation.

For performance reasons, systems with greater than 256 channels cannot record to the older PLX file format; recording to Plexon's newer, high performance PL2 format is required.

For optimal OmniPlex system reliability, especially at high channel counts, it is important to maintain the performance of the Windows PC on which the OmniPlex software runs. Typically this involves not running other CPU-intensive or real-time applications at the same time as the OmniPlex software. However, if you plan to use 256 or more channels, additional steps may be required to ensure optimal performance. Please refer to the discussion and procedures in "Appendix E: Lowest Latency Operation and PC Performance Considerations" on page A-31.

Please contact Plexon Support (+1 214-369-4957 or support@plexon.com) if you have any questions about Windows settings or migration from PLX to PL2.

### 1.2.4 Digital OmniPlex System versus Analog OmniPlex System

This user guide is for users of the digital OmniPlex System, as opposed to the earlier, pre-DigiAmp Amplifier version, the analog OmniPlex System. The terms “digital system” and “analog system” are somewhat misleading, since the main difference is whether the analog-to-digital (A/D) conversion is done before the data reaches the OmniPlex chassis (as in the “digital system”) or after the data reaches the OmniPlex chassis (as in the “analog system”).

Much of the user guide is applicable to both systems; in particular, digital filtering, thresholding, and spike sorting are identical in both functionality and user interface. The main difference from an operational point of view is that the analog system has per-channel analog gain control, and corresponding software functionality for automatically setting gains, whereas the digital system takes a more global approach to the gain settings.

OmniPlex Systems are supported only with PCs that have been provided and configured by Plexon. If you are installing additional programs on your PC, please note the following information: The PCs currently being provided by Plexon for all OmniPlex Systems (analog or digital) run the Windows 10 64-bit operating system.

## 1.3 Getting the Most Out of this User Guide

#### Step by Step Instructions

The sections containing step by step instructions are designed to get you started as easily as possible, guiding you through typical OmniPlex System tasks. Therefore, you will change very few of the default OmniPlex System settings and options, such as filter cutoffs, sorting methods, etc. Once you are comfortable with the basics of using the OmniPlex System, you can refer to the Appendices sections to learn about additional options and features that will allow you to use the full capabilities of the system and configure it for your particular experiments. You should work through all the numbered steps in the applicable procedural sections to acquire a basic proficiency in using the OmniPlex System.

#### Concepts

Many sections contain background material that you will find useful in understanding the “why” behind the “how-to” of each section of step by step instructions. Items marked with **TIP (** **)**, and the Appendices, contain shortcuts, additional techniques and more detailed information, but you don't have to read them in order to complete the step by step tasks successfully.

#### Examples—DigiAmp Subsystems

The OmniPlex System that will be used for most of the examples is a 64 channel MiniDigi system with a 32 channel AuxAI card; your system may differ in the number of DigiAmp channels (16 to 256) and the headstage connectors on the DigiAmp Amplifier (16 channels per connector on the MiniDigi Amplifier versus 32 channels per connector on the DigiAmp Amplifier), but for the most part, the instructions will apply to any DigiAmp subsystem. When the instructions read

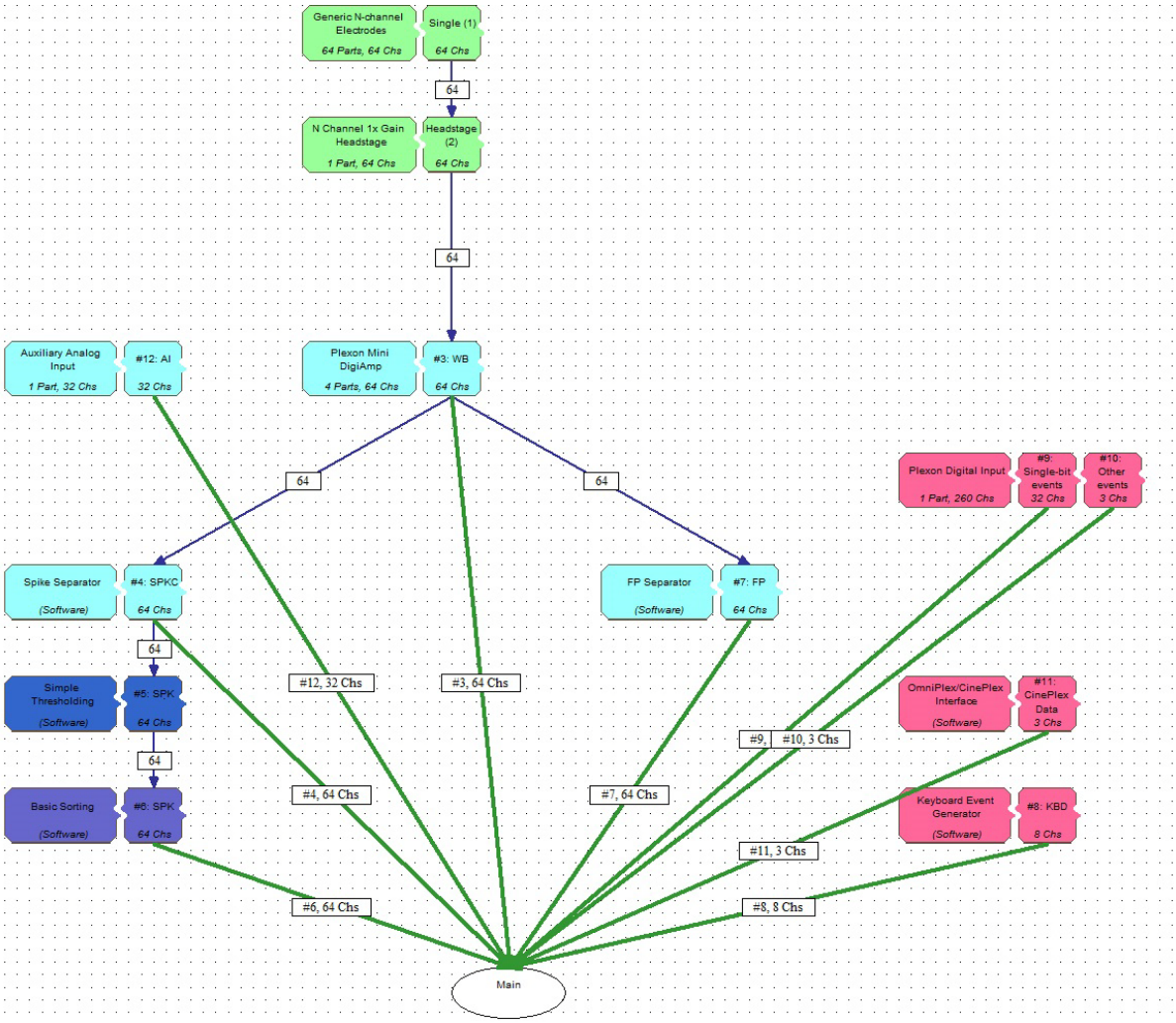
"DigiAmp Amplifier," this should be read as either a DigiAmp Amplifier or MiniDigi Amplifier, as appropriate for your system.

### Examples—DHP Subsystems

In many cases, the examples that are based on the DigiAmp subsystem (see above) are applicable to the DHP subsystem also. When individualized instructions are provided for the DHP subsystem, the OmniPlex System that will be used for most of the examples is a 128 channel DHP subsystem with a 32 channel AuxAI card; your system may differ in the number of channels (16 to 512), but for the most part, the instructions will apply to any DHP subsystem.

## 1.4 Understanding Devices and Sources

This section describes the types of signals and data that can be acquired and derived by the OmniPlex System. These are shown in the topology diagram in the OmniPlex Server application, which shows the flow of data from hardware devices into software processing modules (both of which are referred to as devices in OmniPlex System terminology) and eventually flowing into the Main Datapool at the bottom of the diagram. (You don't need to start Server to follow this explanation - this is only background information to give you an overview of Server's functionality before starting the step-by-step instructions.)




In the topology diagram, the colors of the rectangles are based on the types of data flowing into and out of the OmniPlex System:

- The green rectangles correspond to devices that output analog signals (for example, electrodes and headstages)
- Light blue rectangles correspond to devices that output continuously digitized sample data (for example, a DHP unit or DigiAmp Amplifier, digital filters and analog input card)

- Red rectangles correspond to devices that output digital event data (for example, digital input card, Plexon® CinePlex® System interface and keyboard event detector)

**Note:** For information about the interface between the OmniPlex and CinePlex Systems, see the CinePlex User Guide, which is available on the Plexon website.

- The remaining rectangles correspond to devices that have unique functions (for example, thresholding device for spike detection and sorting device for spike sorting)

Each device in the topology (the larger rectangular boxes) has associated with it one or more *sources* (the smaller square boxes to the right of each device), where **a source is defined as a contiguous range of channels output by a hardware or software device**. The topology diagram provides an excellent high-level view of what processing is applied in what order, and what source types are associated with which devices.

The Main Datapool can be thought of as a continuously updated buffer area which is the destination of all the input and processing chains, and from which the acquired and processed data, of all different types, is made available to PlexControl, MATLAB, C/C++ client programs, PlexNet, and NeuroExplorer.

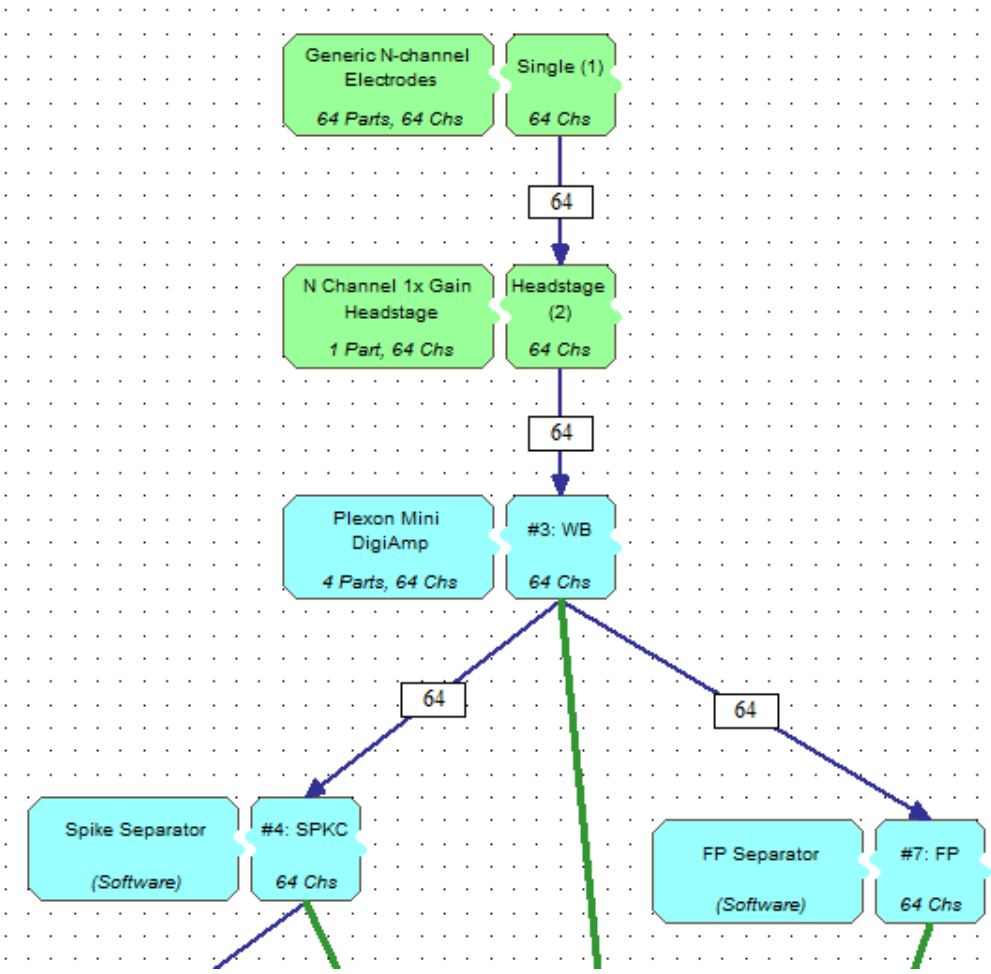
The list of predefined source types that will be found in a standard OmniPlex System includes:

- WB: Continuously digitized wideband neural data from a DHP unit or an A/D device, such as a DigiAmp Amplifier.
- SPKC: The result of highpass filtering and optional lowpass filtering a WB source, i.e. the “spike-filtered” continuous signal.
- SPK: Extracted spike waveforms, the result of performing spike detection on a SPKC source.
- FP: The result of lowpass filtering and optional highpass filtering a WB source, i.e. the field potentials.
- EVT: Individual digital events, e.g. discrete single-bit events or strobed multi-bit data words.
- AI: Continuously digitized non-neural data, typically at a low sampling rate (e.g. 1 kHz), e.g. eye position, etc.
- KBD: Similar to single-bit digital events, but generated by pressing Alt-1 through Alt-8 on the keyboard, for manually marking events during an experiment.
- CPX: Data that is generated by a CinePlex System and sent to the OmniPlex System to be included in recordings.
- Optionally, there may be an AIF source: The result of filtering an AI source.

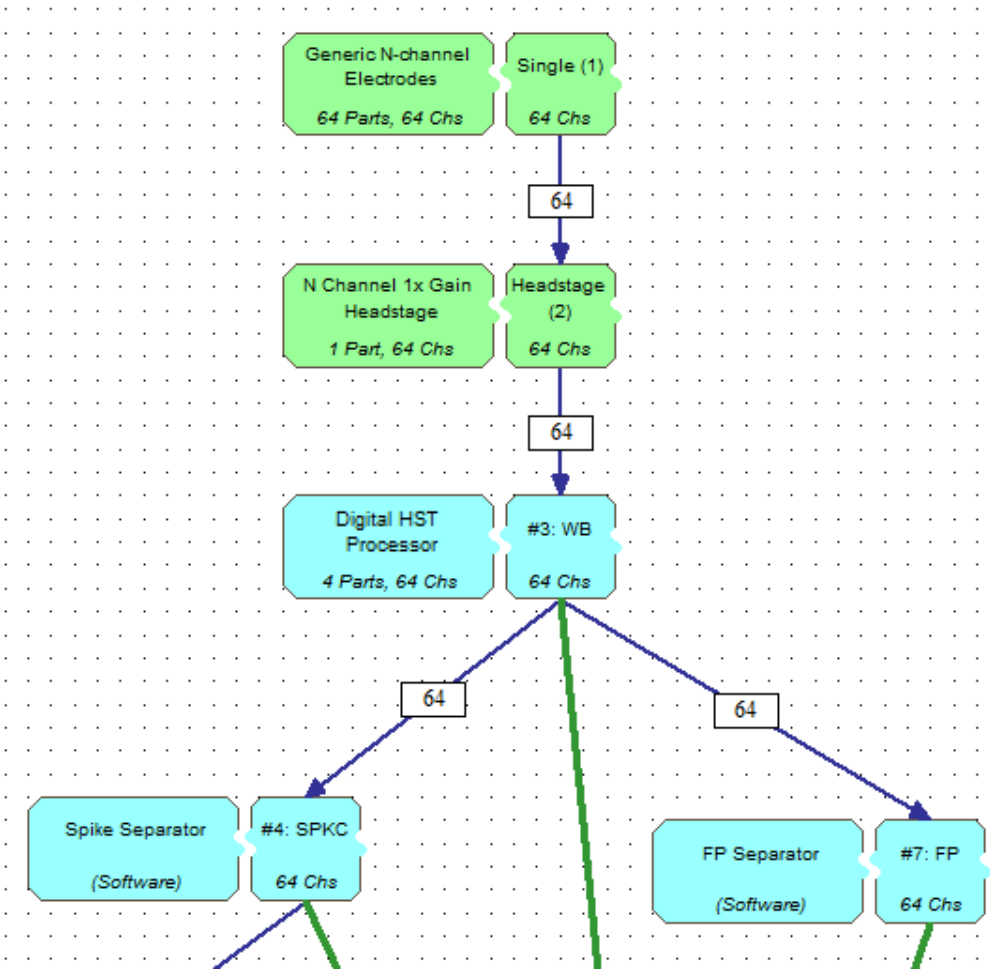
There can actually be more than one source of a given type in a topology (for example, WB channels 1-48 could be one source, and WB channels 49-64 a second source), but in most systems this will *not* be the case, and you can assume for the purposes of this user guide that when we say, for example, "the SPKC source," this means a single source, the one that consists of all the channels of data that are output by the Spike Separator device in the topology. In this context, **a source is simply all the channels of data produced by a hardware or software device.**

The list below provides a more detailed explanation of the flow of data through the devices in the topology. It traces one of the data channels (channel 1) in a typical system, as shown on the overall topology diagram on page 9:

- Example—OmniPlex System with a DigiAmp subsystem:
  Input channel 1 of the DigiAmp Amplifier receives an analog signal from a headstage connected to an electrode. The DigiAmp Amplifier outputs continuously digitized data for this channel at a 40 kHz sample rate on channel WB001. The WB001 data is sent in parallel to three destinations: the Spike Separator and FP Separator devices and the Main Datapool:

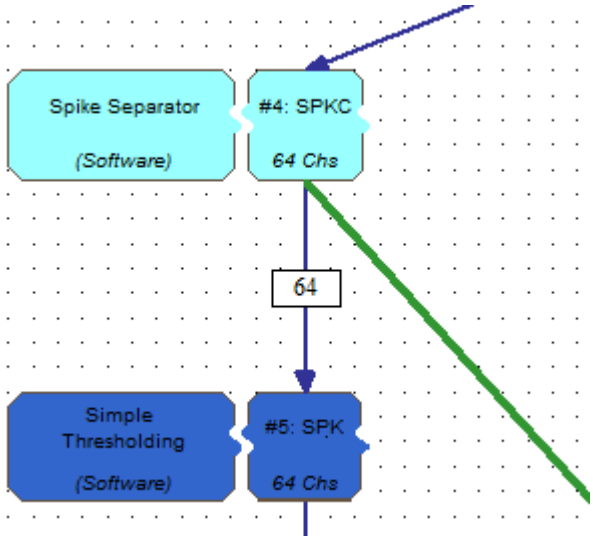


- Example—OmniPlex System with a DHP subsystem:
  Input channel 1 of the DHP unit (labeled **Digital HST Processor** in the topology view, below) receives data from a Plexon digital headstage connected to an electrode. The DHP unit outputs continuously digitized data for this channel at an effective 40 kHz sampling rate on channel WB001. The WB001 data is sent in parallel to three destinations: the Spike Separator and FP Separator devices and the Main Datapool:

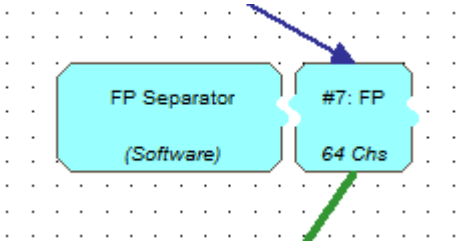



**Note:** The DHP unit performs real-time digital signal processing which optimizes the time-alignment of the data across multiple channels.

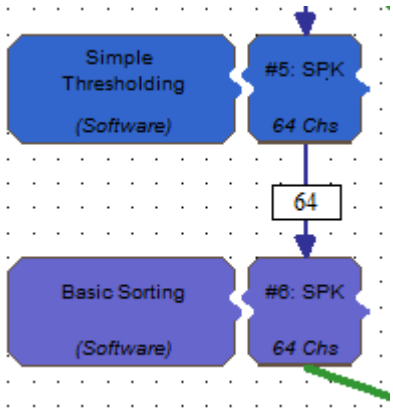
- The Spike Separator performs highpass filtering and optional lowpass filtering on its input data, outputting the result on channel SPKC001; which is sent, in parallel, to the Main Datapool and to the thresholding device:




- The FP Separator performs lowpass filtering, optional highpass filtering and downsampling to a 1 kHz sample rate, outputting the result on channel FP001, which is sent directly to the Main Datapool:



- The thresholding device which extracts spikes from the continuous highpass-filtered data on SPKC001 (using the current thresholding parameters for that channel), outputting the result on channel SPK001, which is sent to the sorting device:

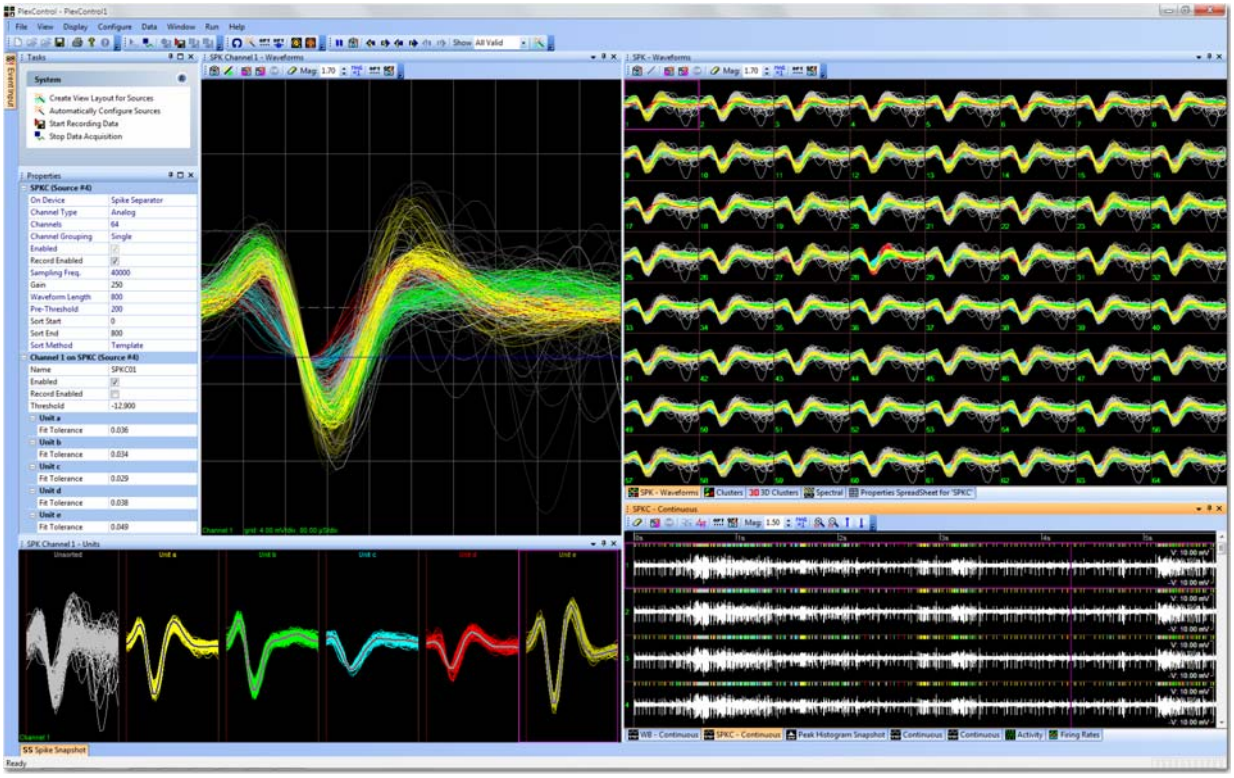



- The sorting device fills in the unit numbers for spikes on SPK001 (using the sorting parameters in effect for that channel) and outputs the result, still on channel SPK001, which is sent to the Main Datapool.

For the example in the above list, the same description applies to the remaining 63 neural channels, e.g. WB002 - WB064. That is, the WB source consists of 64 channels, WB001 - WB064, and similarly for the other sources.

Non-neural sources, such as digital input and keyboard sources, send their data directly to the Main Datapool rather than through a chain of processing devices.

Later, you will see that the multi-window, tabbed user interface in PlexControl has windows or tabs within windows that display each of the sources. Here's a preview:



**TIP**

**Become familiar with OmniPlex System sources**

If all this discussion about topologies, devices, and sources seems intimidating, all that you really need to remember is the above list of sources, especially the first five or six. These will quickly become familiar as you learn to use the OmniPlex System.

# Chapter 2
# Startup (with DigiAmp Subsystem)

2.1 Connections and Power-up ..... 18
2.2 Starting and Configuring the OmniPlex Server ..... 28
2.3 Starting PlexControl ..... 38
2.4 Starting Data Acquisition ..... 40
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2.6 Setting the Wideband Gain with a Live Neural Signal ..... 57
2.7 Separating Wideband Signal into Field Potentials and Spikes ..... 58
2.8 Stopping Data Acquisition ..... 60

## 2.1 Connections and Power-up

### How to use Chapter 2 and Chapter 3

This chapter—Chapter 2, Startup (with DigiAmp Subsystem)—applies to OmniPlex® Systems that use the DigiAmp subsystem (including the DigiAmp™ Digitizing Amplifier or MiniDigi™ Digitizing Amplifier) for data acquisition. In this document, these are referred to as the "DigiAmp Amplifier" and "MiniDigi Amplifier."

**Note:** **If you have a DHP subsystem, use** Chapter 3, Startup (with DHP Subsystem) **instead.**

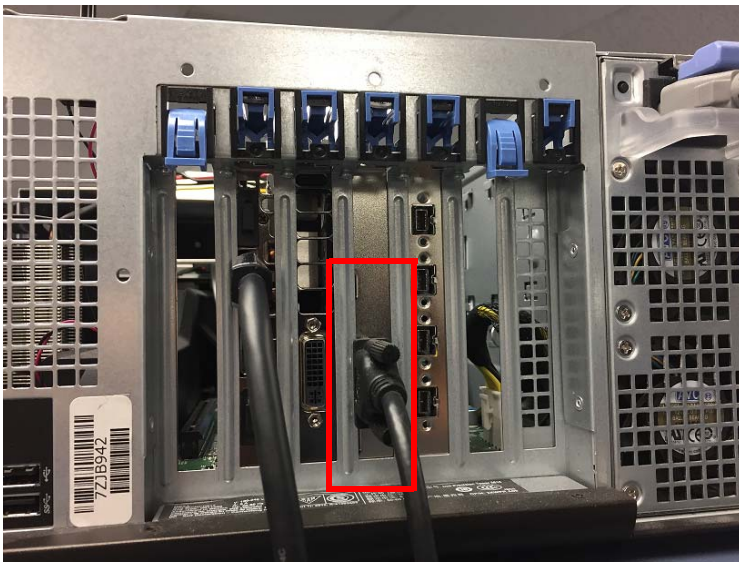
### DigiAmp subsystem connections and power-up procedures

If you already have a running system with all the cables connected and a headstage tester unit (HTU) connected to the audio output of the PC, you can skip this section and go directly to the section Section 2.2, "Starting and Configuring the OmniPlex Server" on page 28.

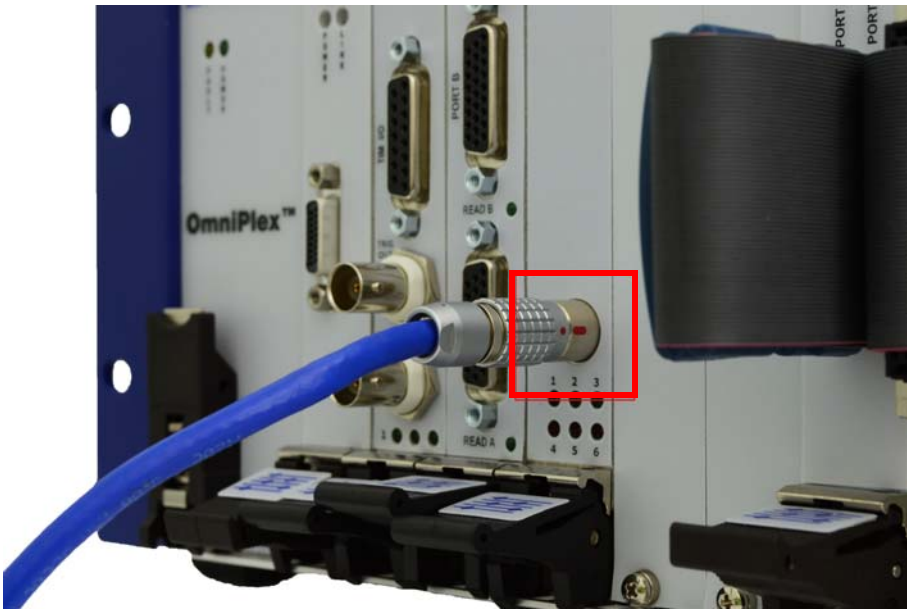
### Initial settings

1. If the PC is on, close all programs and shut it down (close Windows® and fully power down - not just Sleep or Hibernate).
2. If the power switch on the OmniPlex chassis is in the "on" position (—), switch it to the "off" position ( **O** ) now by pushing the rocker switch on the rear panel of the chassis (see the image, below).
3. Plug in the power cable to the chassis. Leave the rocker switch in the "off" position.

**4** Connect the black signal cable from the **COMPUTER LINK** card in the chassis to the connector on the back of the PC.



5 Connect the blue link cable is connected from the **AMP LINK** card in the chassis to the connector on the DigiAmp Amplifier. The red markings on the cable connectors should line up before inserting the cable. (See the images below.)

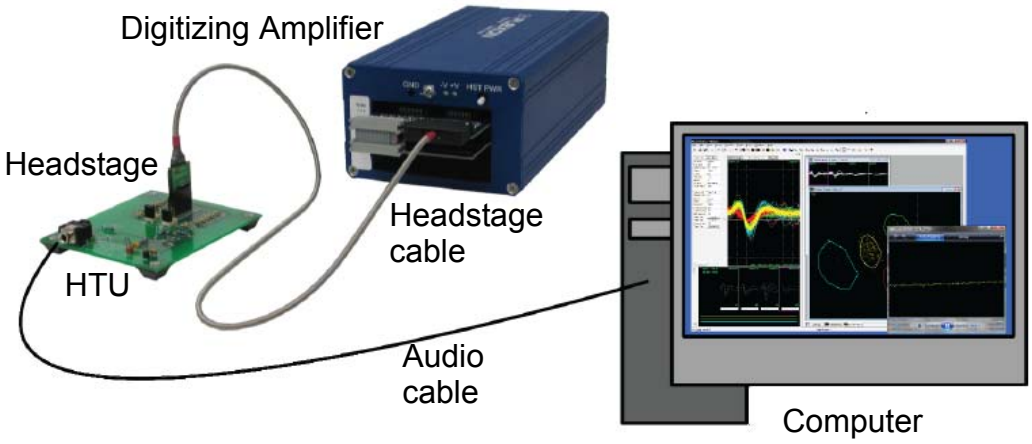


If you ever need to disconnect the blue link cable from the MiniDigi Amplifier, DigiAmp Amplifier or the AMP LINK card in the chassis, unplug the connector by grasping the connector (shown inside the red rectangle in the images above) and pulling straight out. **Warning: Do not pull on the blue cable itself, and do not twist the connector. Never bend or kink the blue cable.**

| | |
|---|---|
| | **CAUTION**<br>**Once Server is running, do not plug/unplug the blue cable**<br>Never plug/unplug the blue cable if the Server application is running. Doing so could damage the circuitry. |

### Connecting to the Headstage Tester Unit

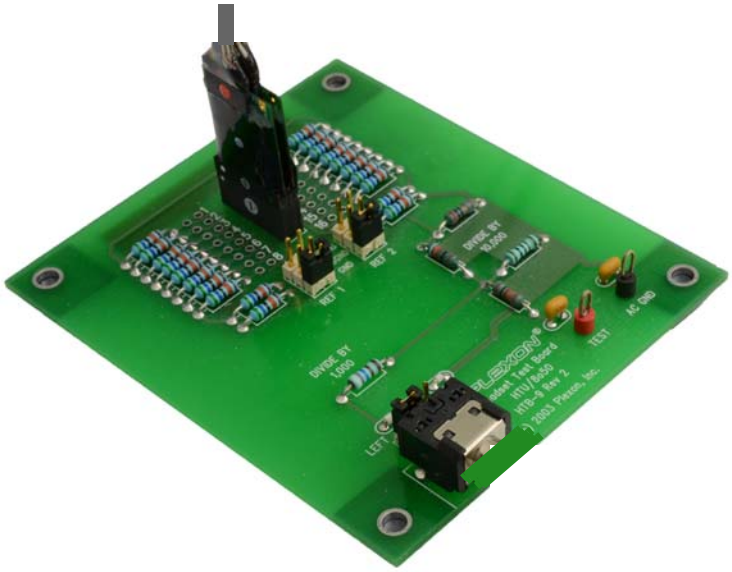
The next few steps will connect the headstages and audio cable to the Headstage Tester Unit (HTU). The general connection scheme is shown in the following diagram.




6 Obtain the headstages and headstage cables that you are going to test. If not already done, connect each headstage to a headstage cable.

7 Connect the headstage input connector to the connector on the HTU. (Repeat if you have multiple headstages and HTUs.)

**Headstage input connected to HTU**
**(There are several models of the HTU. This is an example.):**

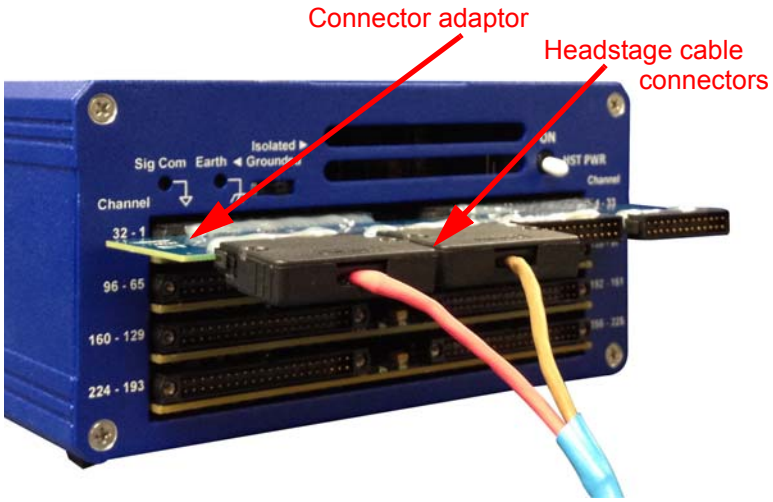


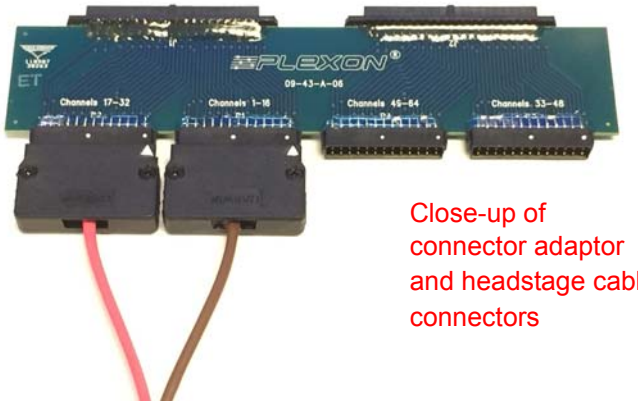
**8** Connect the other end of the headstage cable to the connector on the DigiAmp or MiniDigi Amplifier. See the step applicable to the type of amplifier unit you have (below). (Repeat this step as needed if you have multiple headstages and headstage cables.)

If you have a DigiAmp Amplifier:

**8a** Plug in the **connector adaptor** to the DigiAmp input connectors. Then connect one or more headstage cables to the connector adaptor. Begin on the left side with Channels 1-16 and 17-32, as shown below.

**Headstage connectors connected to a DigiAmp Amplifier through the connector adaptor—Note the brown wire is for Ch 1-16 and 33-48, and the red wire is for Ch 17-32 and 49-64**




Close-up of connector adaptor and headstage cable connectors

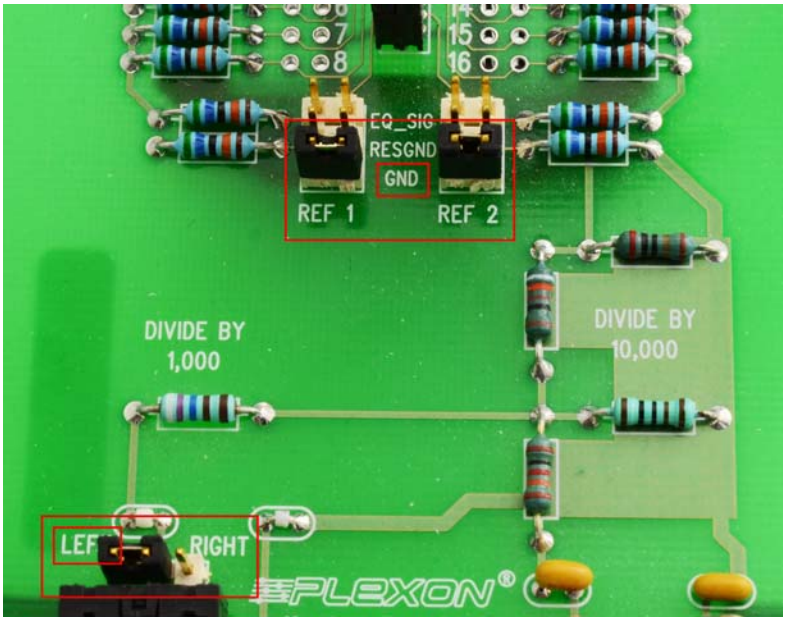
If you have a MiniDigi Amplifier:

**8b** Connect one or more headstage cables to the connector adaptor. Begin at the top with Channels 1-16 and 17-32, as shown below.

**Headstage connectors connected to a MiniDigi Amplifier**
**Note that the brown wire is for Ch 1-16 and 33-48,**
**and the red wire is for Ch 17-32 and 49-64**

**9** Verify that the **REF** jumper(s) on the HTU are positioned on **GND**. Verify that the left/right jumper is positioned on **LEFT**.

**(There are several models of the HTU. This is an example.**
**Your HTU might have one REF header or multiple headers)**

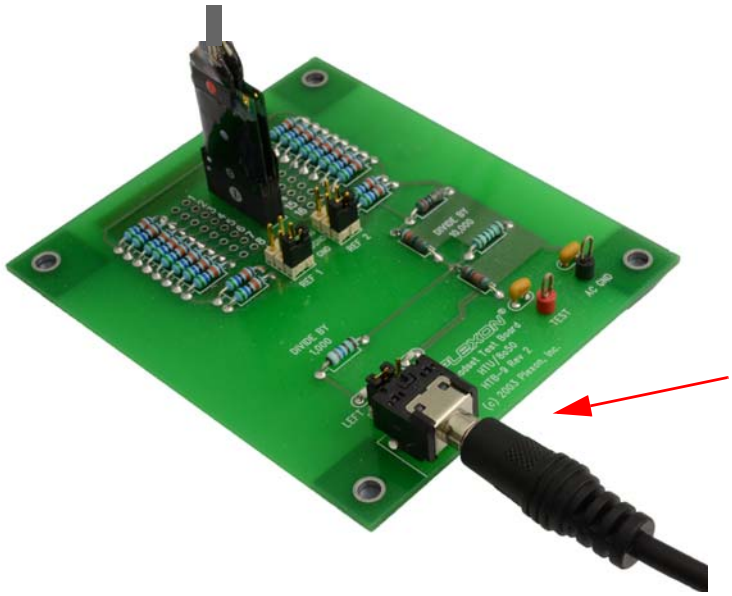


**10** (This step is applicable to some OmniPlex Systems, and depends upon the audio capabilities of the specific PC that was included in your system.) All OmniPlex components, including the high speed PC, are fully integrated and tested prior to shipment. PC models change from time to time as computer technology improves. Most PC models have good quality audio ports, but some do not. If the audio port on a PC model is not sufficiently high quality, or if it is noisy, Plexon® includes a USB audio interface that will provide higher quality audio for OmniPlex testing purposes.

If you have a USB audio interface, plug the interface into any USB port on the PC.

**Note:** A test audio file will be played in the procedure in Section 2.4, “Starting Data Acquisition” on page 40.

**11** Connect a 1/8” stereo audio cable from the USB audio interface output jack (or from the 1/8” line output jack on the front or back of the PC) to the 1/8” input jack on the HTU.



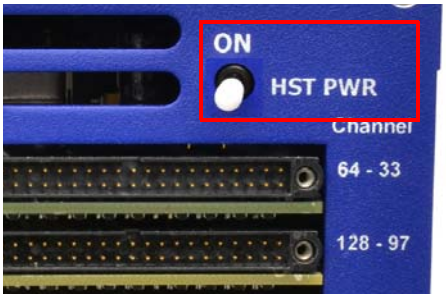
**12** Turn the chassis power on. You should see the two green **POWER** LEDs light up at the left end of the front panel.

**13** Start the PC and allow Windows to boot up normally. You should see the green **LINK** LED light up on the COMPUTER LINK card.

**Note:** If the LINK LED does not come on, it is possible that the black link cable to the PC is not connected properly. In this case, verify that both ends of the cable are properly connected, then repeat the procedure starting from Step 1.

**14** Turn the **HST PWR** switch on (up).

**DigiAmp Amplifier:**

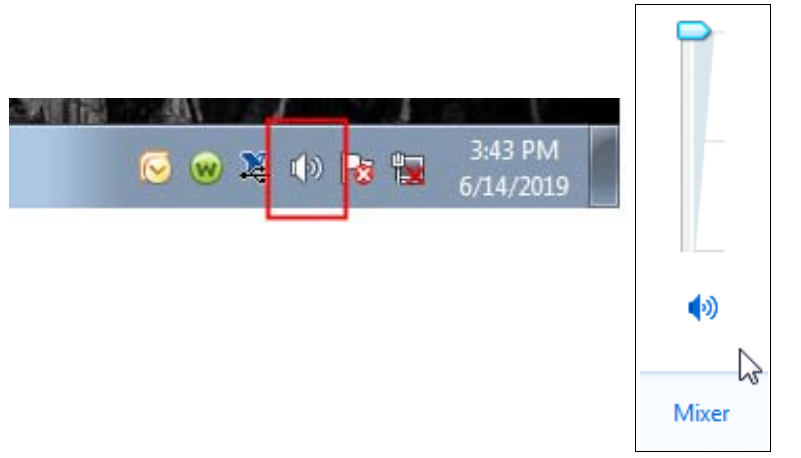



DigiAmp Amplifier
HST PWR ON

**MiniDigi Amplifier:**

MiniDigi Amplifier
HST PWR ON

15 Do not play any audio on the PC yet, but open the Windows volume control or audio mixer and make sure that Line Out and Headphone out (some PCs will only have one or the other output option) are enabled (not muted), and set the volume to maximum.




**Note:** A test audio file will be played in the procedure in Section 2.4, “Starting Data Acquisition” on page 40.

### If you are connecting a Plexon CinePlex® System to your OmniPlex System

For information about the interface between these two systems, see the CinePlex User Guide, which is available on the Plexon website.

## 2.2 Starting and Configuring the OmniPlex Server

The OmniPlex Server is the first of the two primary software components of the OmniPlex System. Server is the "engine" which receives data from hardware devices, sends commands to the hardware devices, and contains the topology (network) of software modules which perform filtering, thresholding, sorting, and other signal processing functions. Once Server has been configured for your hardware (which may have already been done by a Plexon Sales Engineer), you will probably find that you spend relatively little time interacting with it, since the main user interface to the OmniPlex System is provided by PlexControl, which is described in Section 2.3, "Starting PlexControl" on page 38.

### Launching Server and setting the topology

**TIP**

**Press Ctrl key to prevent auto-loading of pxs file**

If you ever need to prevent the auto-loading of the last-used pxs file, for example, for troubleshooting, you can hold down the Ctrl key before double-clicking on the Server desktop shortcut; in this case, you will be "starting from scratch" and will need to either load some other pxs file, or use the Topology Wizard to create a new one, as described in the steps below.

1 From the Windows desktop, double-click the **OmniPlex Server** shortcut:

**CAUTION**

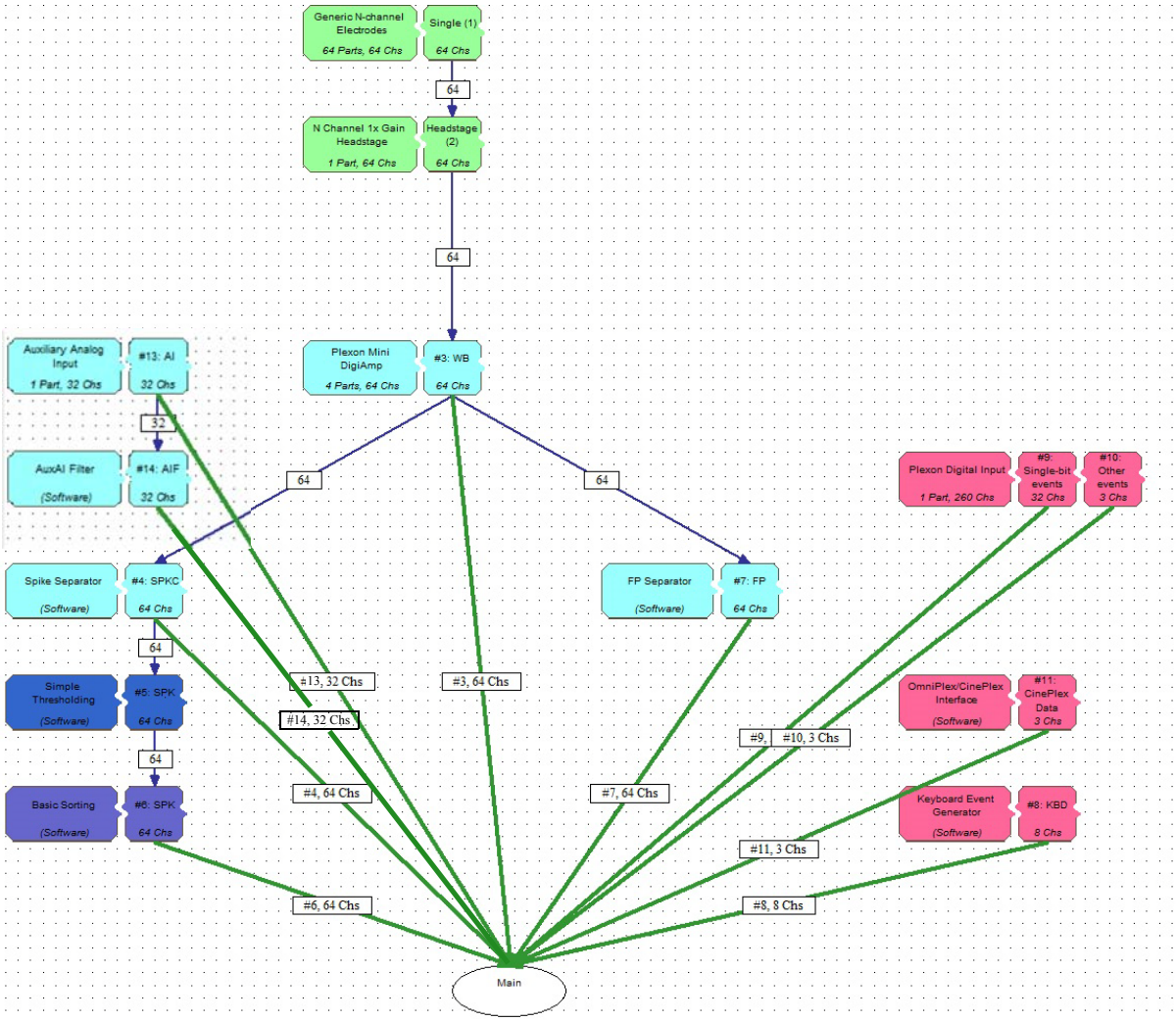
**Once Server is running, do not plug/unplug the blue cable**

Never plug/unplug the blue cable if the Server application is running. Doing so could damage the circuitry.

2 If a system topology (configuration) diagram similar to the one below is displayed, wait for the green progress bar at the bottom of the Server window to finish. This diagram represents the topology that is currently saved in the system from a previous run. If the topology is already set up the way you want, you may proceed directly to Section 2.3, "Starting PlexControl" on page 38.

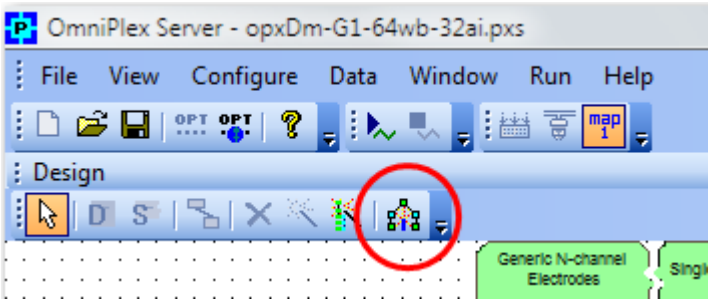
If you do not see a topology diagram, or you want to modify it, perform Step 3 through Step 17 to create a new topology for the system.

**Note:** When you upgrade to a new OmniPlex System software version, or if you add or replace any of the hardware in your system, you should create a new topology (a new .pxs file) to ensure compatibility. Perform Step 3 through Step 17.

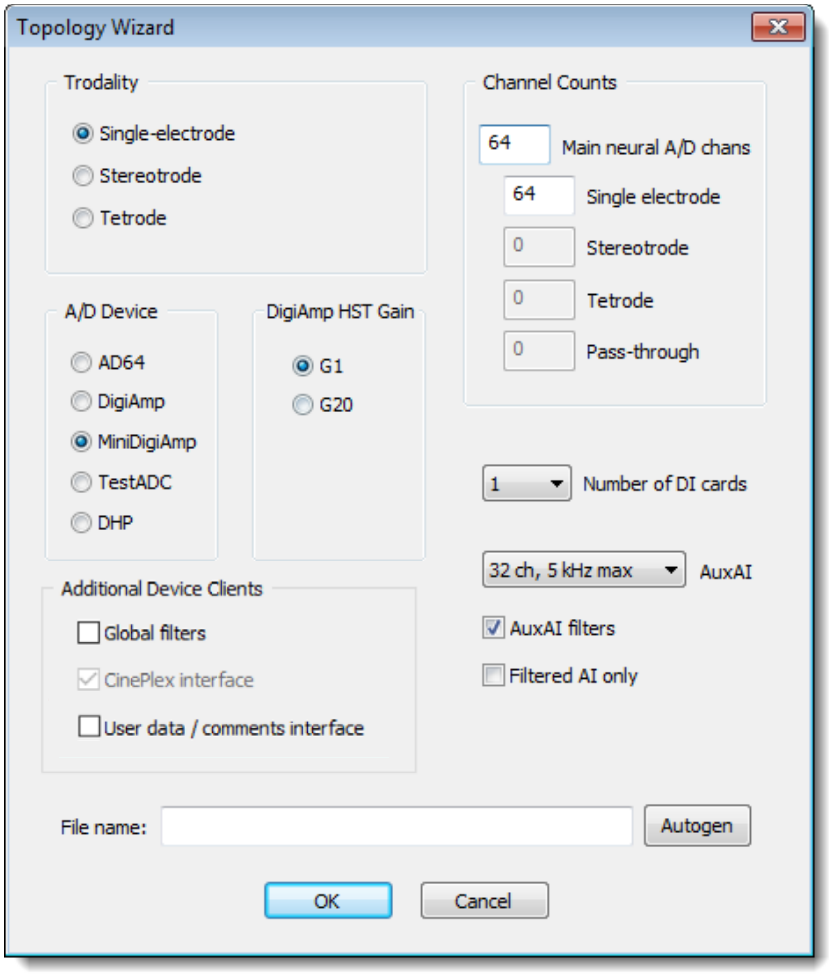



**Note:** Data acquisition must be stopped to load or create a topology. See Section 2.8, “Stopping Data Acquisition” on page 60.

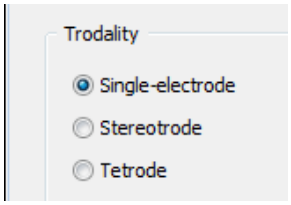
**3** You can use Server's Topology Wizard to specify the topology of your OmniPlex System. To do so, click on the **Topology Wizard** button in the toolbar:



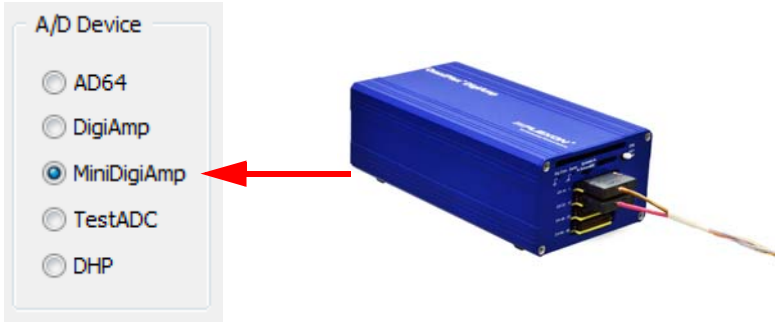
The **Topology Wizard** dialog is displayed:




**4** Select the type of electrode in your experiment. You can choose only one type for each topology. (The examples below assume **Single-electrode** is selected.)



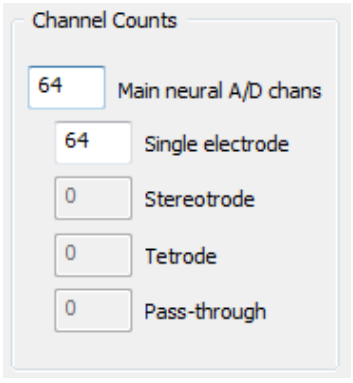
5 In the **A/D Device** section, click on either the **DigiAmp** button (if you have a "big" DigiAmp Amplifier) or the **MiniDigiAmp** button (if you have a MiniDigi Amplifier).




6 In the **Channel Counts** section, enter the total number of actual channels in your DigiAmp Amplifier in **Main neural A/D chans**. A MiniDigi Amplifier has from 16 to 64 channels, with 16 channels per board; a "big" DigiAmp Amplifier has 64 to 256 channels, with 64 channels per board. If you are unsure of the number of channels, contact Plexon for assistance. When you enter a channel count in **Main neural A/D chans**, the corresponding number is automatically entered in the **Single electrode** field.

If you are using a MiniDigi Amplifier, enter 16, 32, 48 or 64 for **Main neural A/D chans**.

If you are using a DigiAmp Amplifier, enter 64, 128, 192 or 256 for **Main neural A/D chans**.




**Note:** You can define fewer channels than are physically present in your system. This can be useful if you will be running experiments using a limited number of channels. For example, if you have a system

physically capable of 192 channels, but only plan to use 128 of those channels, you can enter 128 for **Main neural A/D chans** and the system will ignore (and not attempt to display) the unused 64 channels.

You can also disable individual channels in the Properties Spreadsheet.

It is important to understand how the system handles the number of channels that you enable/disable. For a complete explanation of these options, see Appendix F: Disabling Unused Boards to Reduce Channel Counts.

### AuxAI configuration

Perform Step 7 through Step 9 if you have an Auxiliary Analog Input (AuxAI) card in your chassis and want to use the channels associated with the AuxAI subsystem. Otherwise, skip to Step 10.

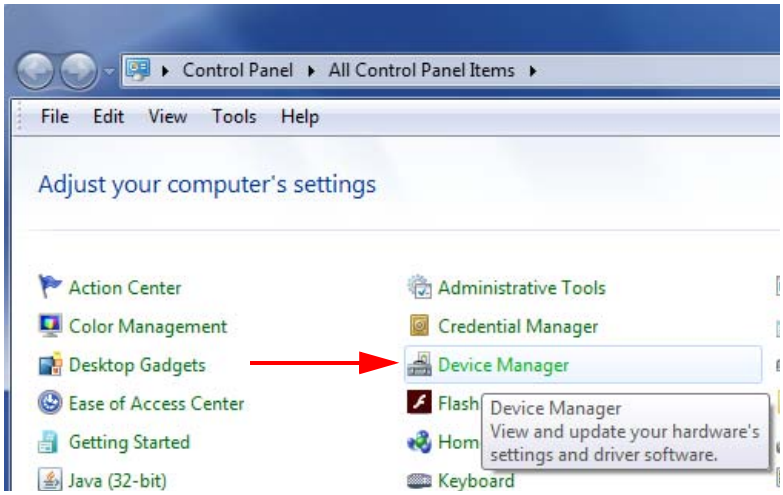
**Note:** The AuxAI hardware is shown in the photo in Section 12.2, “5 kHz, 10 kHz and 20 kHz Maximum Sampling Rates” on page 366.

**7** There are several types of AuxAI cards.

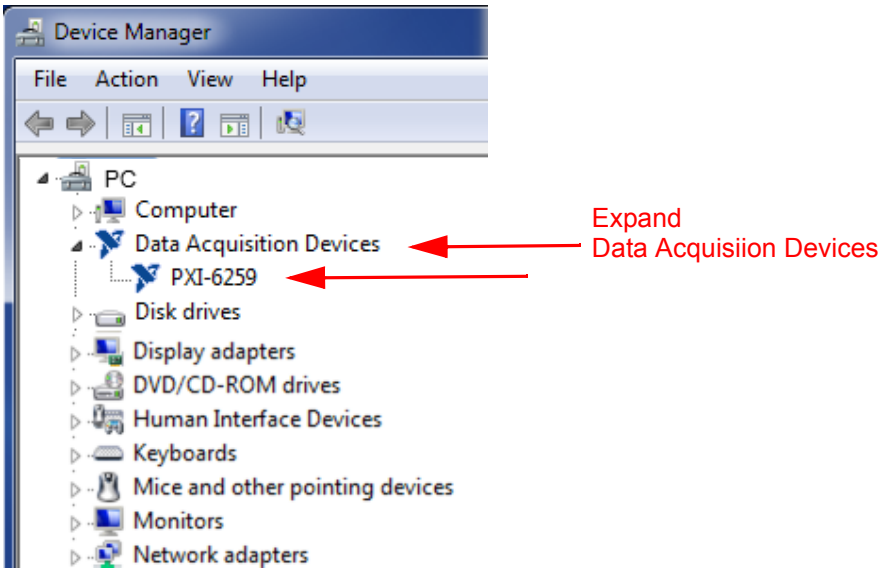
- The standard AuxAI module, suitable for sampling rates up to 10 kHz maximum, is the 16 channel PXI-6341. (Some systems use the 5 kHz 32 channel PXI-6224.)
- The “fast” AuxAI module, suitable for sampling rates up to 250 kHz maximum, is PXI-6259.

If you already know which card is installed, proceed to Step 8. If you are unsure whether you have a standard or “fast” card, you can determine this by the following method:

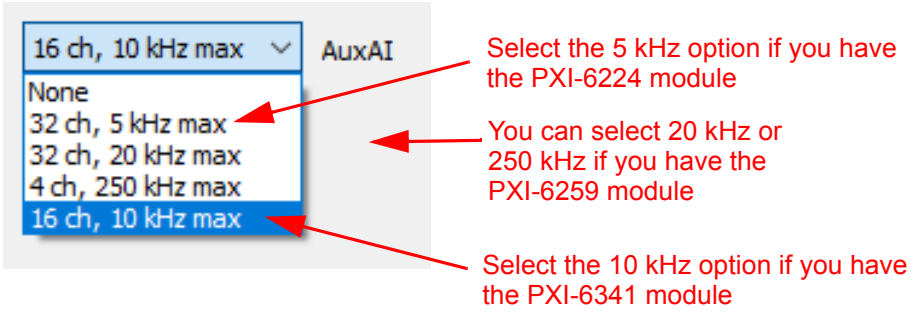
**7a** Left click the **Start** icon and navigate to the **Device Manager**.



**7b** In **Device Manager**, view the display to determine which module is installed in your PC:



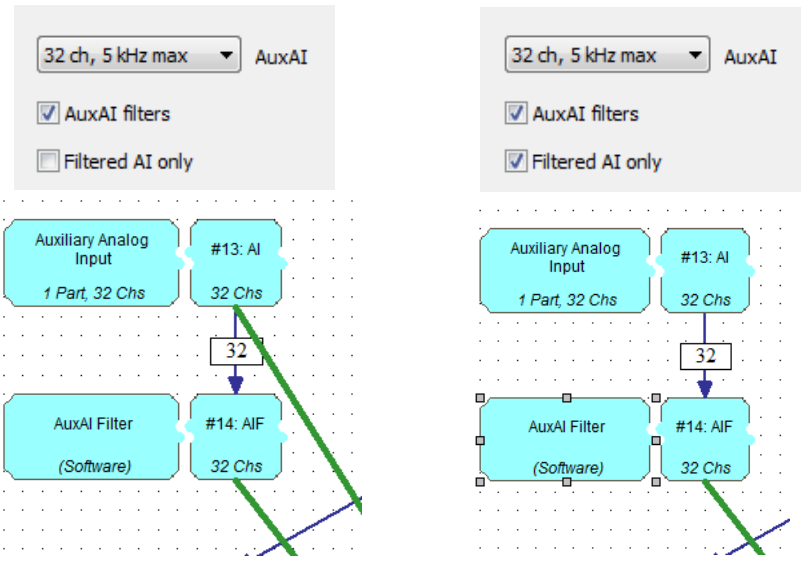

**8** In the Topology Wizard, select the desired combination of channels and max sampling rate from the **AuxAI** dropdown list.




**9** If you want to enable AuxAI filters, click in the **AuxAI filters** checkbox. This will cause the system to acquire both the filtered and unfiltered AuxAI signals.

If you want the system to acquire *filtered AuxAI data only*, click in the **Filtered AI only** checkbox.

**Note:** The **Filtered AI only** option cannot be selected by itself.

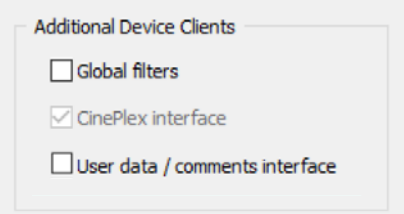



In the example on the left, we did not check **Filtered AI only**, so the green lines (which are pointing towards the main datapool, not shown here) indicate that both AuxAI and AIF sources will be available in the OmniPlex GUI. In the example on the right, we checked both **AuxAI filters** and **Filtered AI only**, so only the green line from AIF is present.

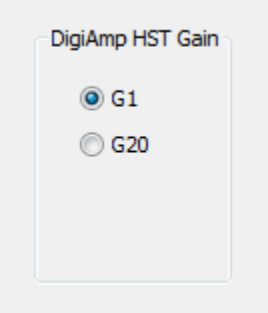
For further discussion of AuxAI functions, see Chapter 12, Auxiliary Analog Input.

10 The **Global filters** option in the **Additional Device Clients** section relates to a legacy feature. It is generally not needed and can be left unchecked. Information about this option is available in Appendix B: Separation of Spikes and Field Potentials Using Digital Filters on page A-4.

The **User data / comments interface** option can be selected if you would like to have the ability to add comments during recording (see Section 7.3, “Adding Comments Before and During Recording” on page 217).




11 Leave all other Topology Wizard settings at their default values. However, for future reference, note that there is an option which indicates whether you are using a unity-gain (G1) or gain-of-20 (G20) headstage.

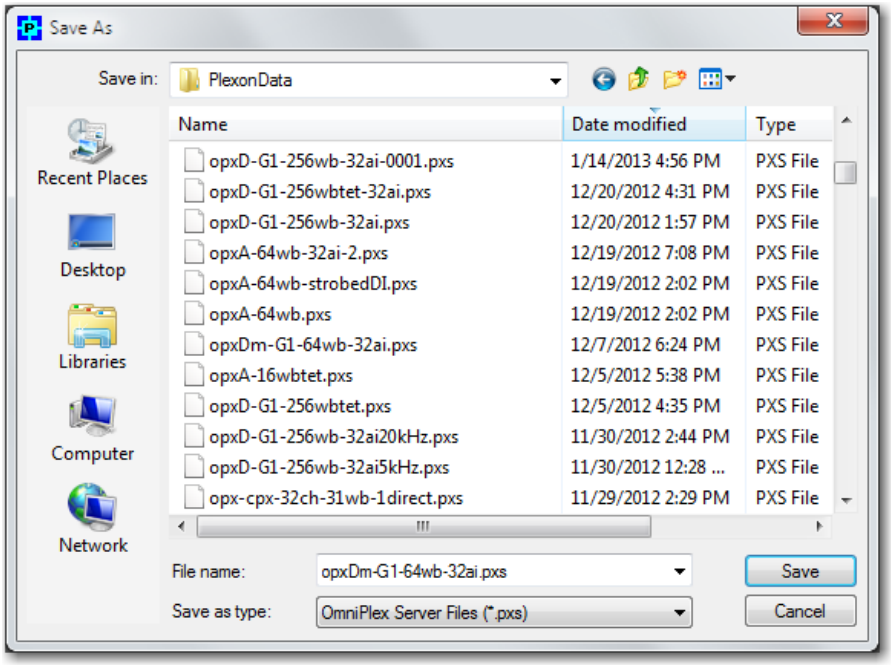



For the purposes of the user guide, we will use the G1 option, but in actual use, make sure that your topology includes the correct headstage gain setting.

12 Click **OK**. Wait for Server to generate a new topology diagram and go through the initialization sequence, as indicated by the green progress bar at the bottom of the window.

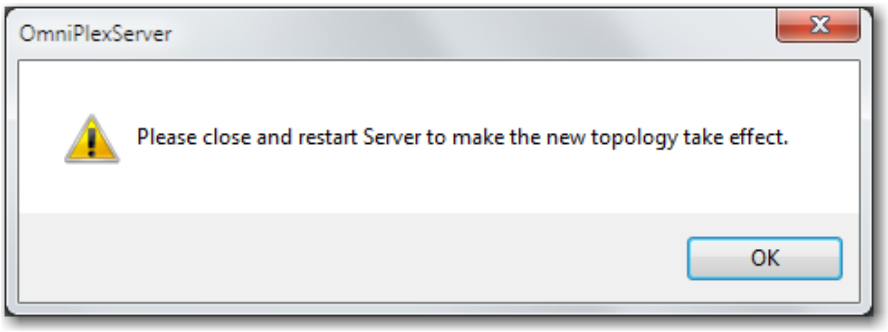
13 Server will display a **Save As** dialog asking you for the name for your new topology (a topology is saved in a file with the extension “.pxs”). It will display an automatically generated and appropriate name, for example,

opxDm-G1-64wb-32ai.pxs



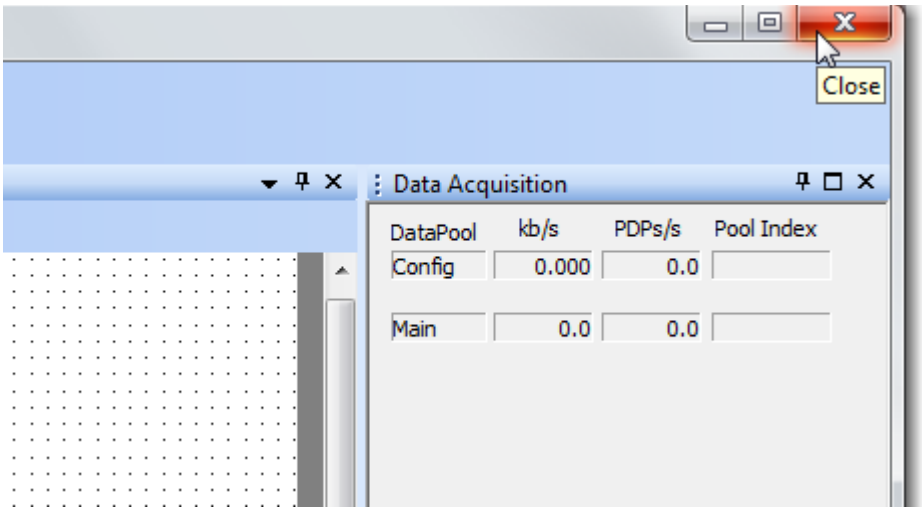

Click **Save** to accept the default filename, or edit the name if you wish, and then save the pxs file.

**14** Server will display a message box:



15 Click **OK**.

16 Close Server.




17 Restart Server as described in Step 1, wait for the .pxs file to load and for the green progress bar to finish.

Server now has auto-loaded a .pxs topology file, either one that was used in a previous OmniPlex session, or one that you just created in Step 3 through Step 16 above using the Topology Wizard. In either case, from now on, when you start Server from the desktop, by default it will automatically load the last-used pxs file.

**TIP**

**When to use Topology Wizard**

You should use the Topology Wizard to create a new pxs file when the hardware configuration of your system changes (for example, adding more boards to a DigiAmp or MiniDigi Amplifier or adding a new card to the chassis), or when you are updating software.

**TIP**

**Additional device settings—filtering, referencing, latency**

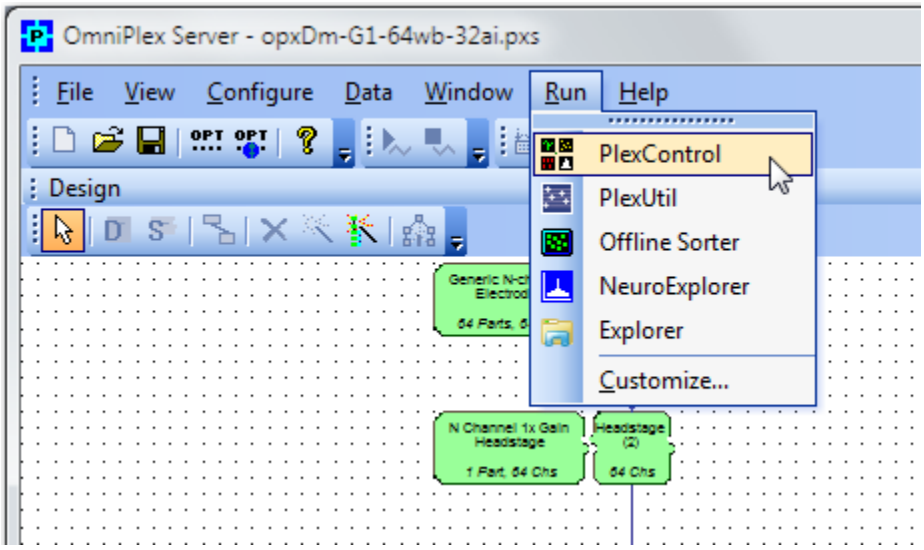
If you need to make adjustments to the default headstage settings in the **DigiAmp Device Settings** dialog, such as filter cutoffs, referencing and latency, see Appendix C: DigiAmp Device Settings—Filtering, Referencing and Latency on page A-15.

## 2.3 Starting PlexControl

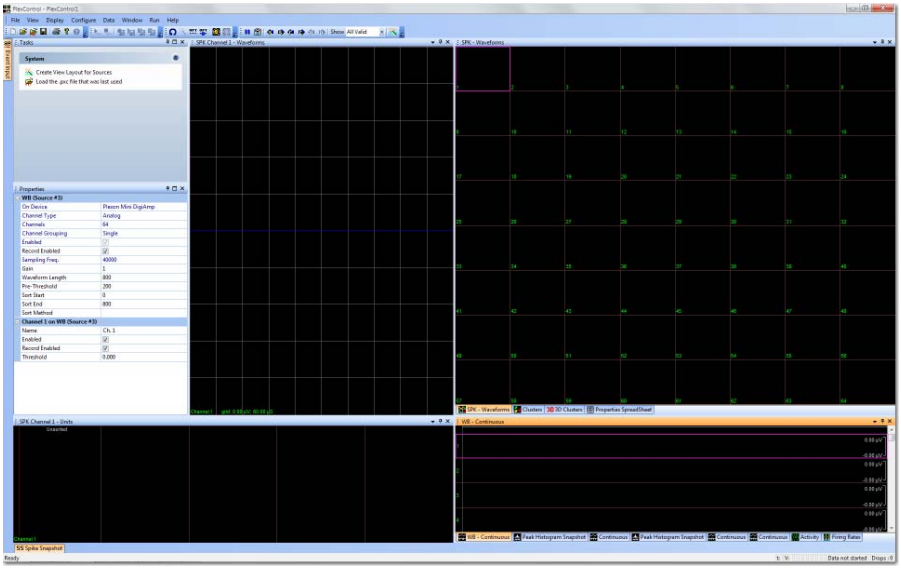
This section assumes that you have already started Server, as described in the previous section.

1 Start PlexControl either by double-clicking its desktop shortcut:

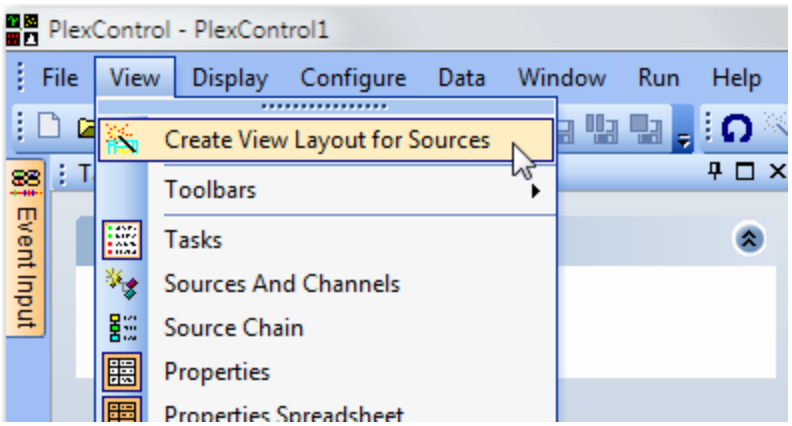
or select **PlexControl** from the **Run** menu in Server:



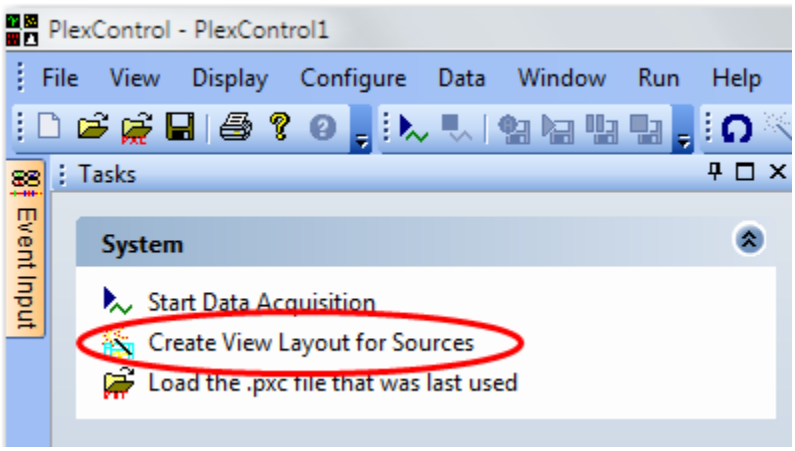

2 The PlexControl application window is displayed. It should look something like this:



If you would prefer to restore the display to the default view, select **Create View Layout for Sources** from the **View** menu.




or from the Tasks view at upper-left:




You can do this at any time to restore the user interface layout to a default state, without affecting any of the actual parameters for acquisition, sorting, recording, etc. In other words, it is a purely cosmetic operation.

**TIP**

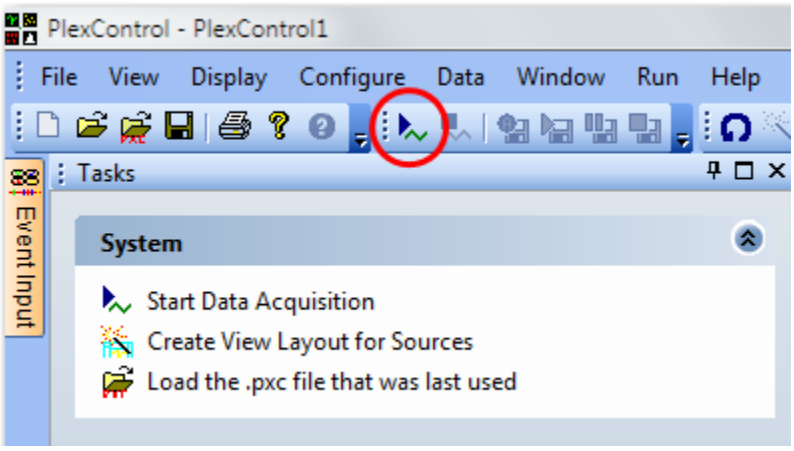
**Do not perform Create View Layout for Sources while recording**

Due to the amount of system activity involved in recreating all the views from scratch, especially on systems with high channel counts, it is recommended that you not perform **Create View Layout for Sources** while recording data.

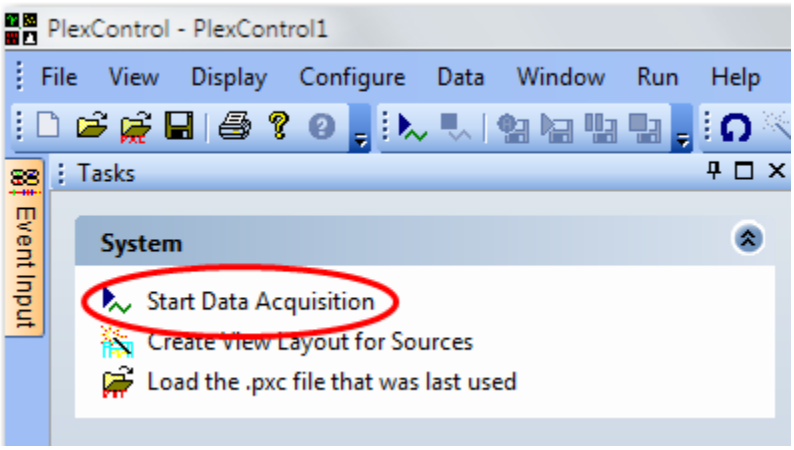
## 2.4 Starting Data Acquisition

This section explains how to start data acquisition.

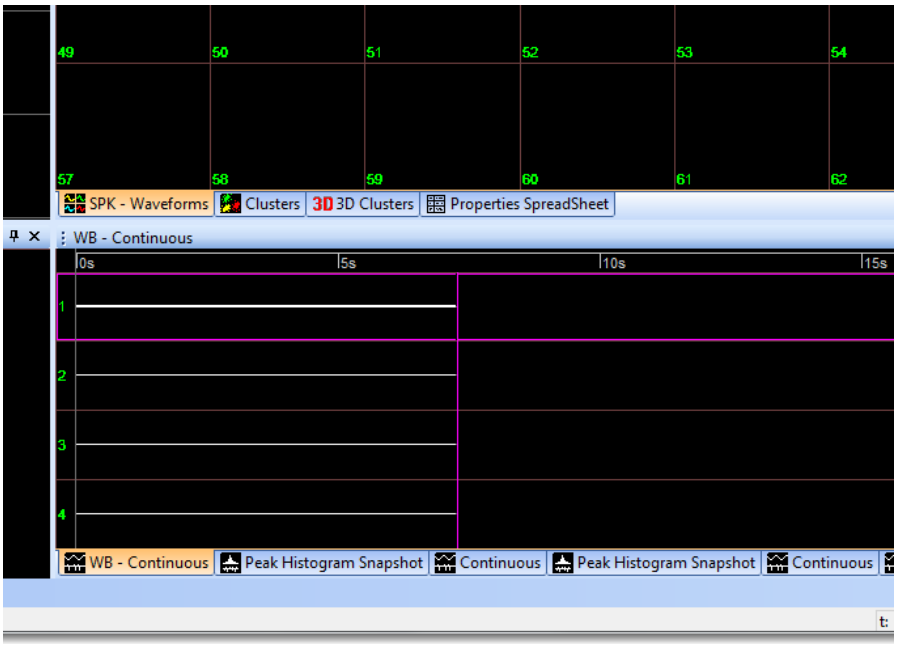
1 Click **Start Data Acquisition**, either in the main toolbar:




or in the Tasks view:



2 After a few seconds you should see signal traces being drawn in the view labeled “WB - Continuous” in the lower-right part of the window:




”WB - Continuous” refers to continuously-digitized signals from the WB source. The vertical colored sweep line shows the current position; once it reaches the far right end of the window, it “wraps around” to the leftmost position and overwrites the oldest data in the view. The time labels on the horizontal axis indicate relative time for this specific view only; later you will learn how to adjust the horizontal sweep speed and other viewing parameters.

**TIP**

**Some settings can be changed only when data acquisition is stopped**

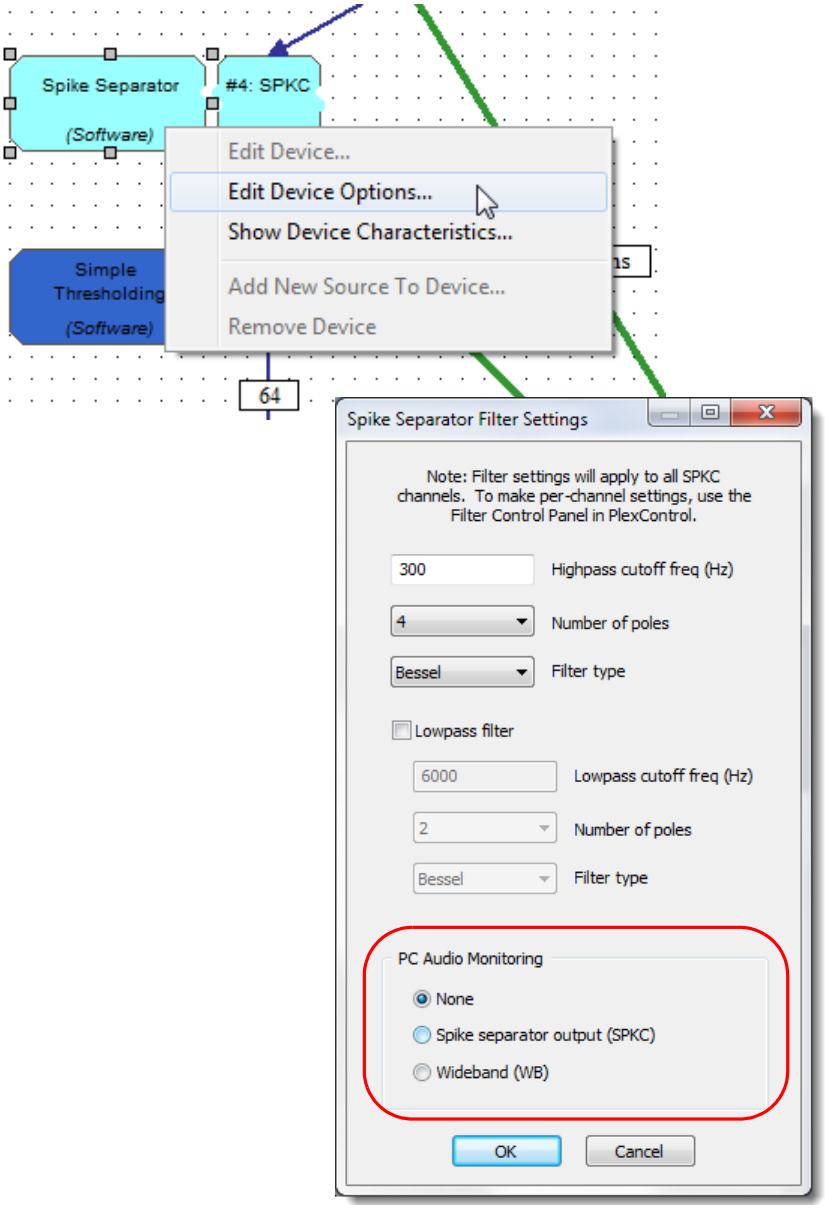
Later, you will see that there are some settings which can only be changed when data acquisition is stopped. For example, you *must* stop data acquisition before changing the headstage options.
In addition, the OmniPlex System will not allow you to modify the spike waveform length or pre-threshold length, or change the sorting method, while data acquisition is running. In such cases, simply stop data acquisition, perform the desired operation, then start data acquisition again. See the instructions on stopping data acquisition, below. See Section 2.8, “Stopping Data Acquisition” on page 60

### Playing a test audio file

3 Before you play a test audio file, you need to ensure that the OmniPlex audio monitoring feature is disabled. Otherwise the OmniPlex System could generate an audio feedback signal that would mix with the intended test signal. Audio monitoring is for live data only.

If you are operating a new OmniPlex System for the first time, the audio monitoring feature is already disabled; this is the factory default setting.

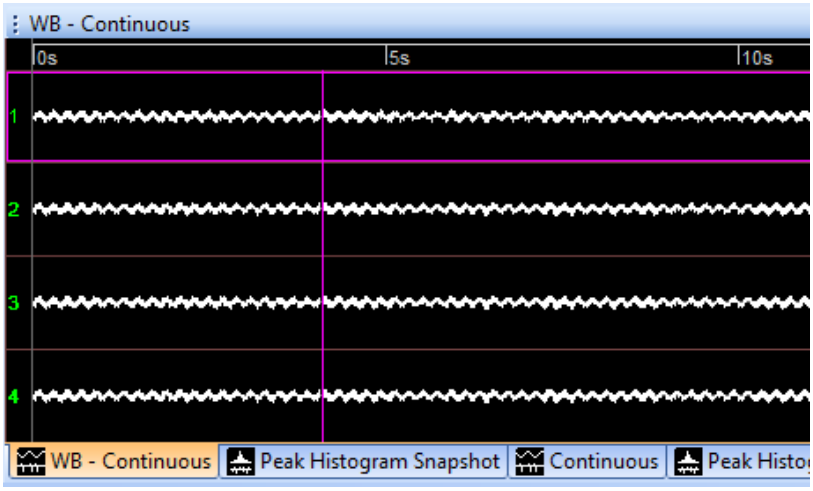
To ensure that the audio monitoring is disabled, right-click on the **Spike Separator** in Server, and select **Edit Device Options**. Then, in the **Device Options** dialog, ensure **PC Audio Monitoring** is set to **None**. If necessary, set it to **None**. Then click **OK**.




**Note:** You can enable this feature later when you are working with live data.

4 Start playback of a test audio file by double-clicking on its desktop shortcut. (Test audio files are available on the Plexon website under **Support | Software Downloads | Data Samples**.)

5 Depending on the signal level from the PC's audio output, some activity may now be visible on the traces in the WB - Continuous view. The colored rectangle identifies the currently-selected channel within the wideband source, by default channel 1; selection of sources and channels within sources will be described later.




If you still see only completely flat traces in the WB Continuous view (as in Step 2), you may wish to recheck that audio is being played into the HTU. If you need to troubleshoot, here are a few things you can try.

**WARNING**

**Be aware of safe audio level**

Be aware that the sound level at this point in the procedure is at maximum (from Step 15 on page 27). If you are using a headphone set or unusually powerful speakers, consider turning down the sound level temporarily during this audio test.

5a If you are using a USB audio interface—Go to Windows **Control Panel > Sound**, and ensure the **USB Audio Device** is set as the default device. If the USB Audio Device is not displayed at all, open Device Manager and ensure the USB Audio Device is enabled. Also make sure you don't have the audio muted on the PC.

If there is still no signal in the WB - Continuous display, you can

verify the audio file is being transmitted by the interface and through the 1/8” audio cable. To do this, attach the end of the audio cable to a speaker, or plug a headphone set into the audio outlet of the interface. Listen for the sound of the audio file.

**5b** If you are using the PC system audio—Briefly unplugging the audio cable from the PC's headphone or line out jack should cause the audio file to be heard from the PC speaker. If not, make sure you don't have audio muted on the PC.

Some PCs have both front panel and rear panel audio input and output jacks, and you can use whichever is more convenient. However, in rare cases, you may find that one output jack provides a noticeably cleaner audio signal than the other, and using that output will make working with the test audio file easier.

**TIP**

**Play the audio file with continuous repeat selected**

For convenience, set the audio to repeat continuously while you are performing the above step.

**5c** If you have verified that the PC is playing back audio correctly, try briefly unplugging the audio cable from the HTU, which will usually cause a brief noise transient to appear in the wideband trace display. If not, check to make sure all the cables and adaptors are firmly and evenly seated.

For the DigiAmp Amplifier:

— Audio cable and headstage connected to the HTU
— Headstage cable(s) seated fully on the connector adaptor at one end, and connected to the headstage(s) at the other end
— Connector adaptor seated into the DigiAmp connectors

For the MiniDigi Amplifier:

— Audio cable and headstage connected to the HTU
— Headstage cable(s) seated fully into the MiniDigi connectors at one end, and connected to the headstage(s) at the other end

Also verify the HTU jumpers are in the proper positions, as described in Section 2.1, “Connections and Power-up” on page 18 above.

**5d** If none of the above steps locate the problem, try using a different audio cable (1/8” stereo). If you have a USB audio interface, move it to a different USB port on the PC.

**Note:** If the problem persists, contact Plexon support at +1 214-369-4957 or support@plexon.com.

**TIP**

**Using “zoom” and “magnification” for a better view**

Zoom and magnification are two functions that you will find useful for viewing signals.

For historical reasons, the OmniPlex System term “zoom” is used to describe the process of double clicking on a particular channel so this single channel expands to fill an entire view. See Section 4.4.1, “Using the Zoom Feature” on page 120.

You can also use the “magnification” function, which is the OmniPlex System term for viewing a display at varying degrees of enlargement or reduction, for example, 1.5 times or 0.8 times the default height. See Section 4.4.2, “Changing the Magnification” on page 121.

## 2.5 Setting the Wideband Gain

The DigiAmp Amplifier provides a choice of three global gain values (50, 250, and 1000), which are primarily intended for complementing the gain of the headstage, so that the total analog gain is 1000. For example, if you are using a unity-gain headstage (G1), then you should start with a DigiAmp gain of 1000; if you are using a G20 headstage, then you should start with a DigiAmp gain of 50. The intermediate DigiAmp gain value of 250 is provided for situations where the gain of 50 is too low when using a G20 headstage, or the gain of 1000 is too high when using a G1 headstage. However, once you have selected an appropriate gain value, you should not need to adjust the gain again during an experiment, unless the signal amplitude increases so much that clipping of the wideband signal occurs, in which case you should reduce the DigiAmp gain to prevent distortion of the signal. Note that the gain value applies to all the channels in the DigiAmp Amplifier. See Appendix A: Signal Amplitudes and Gain for additional information on gain, clipping, and related issues.

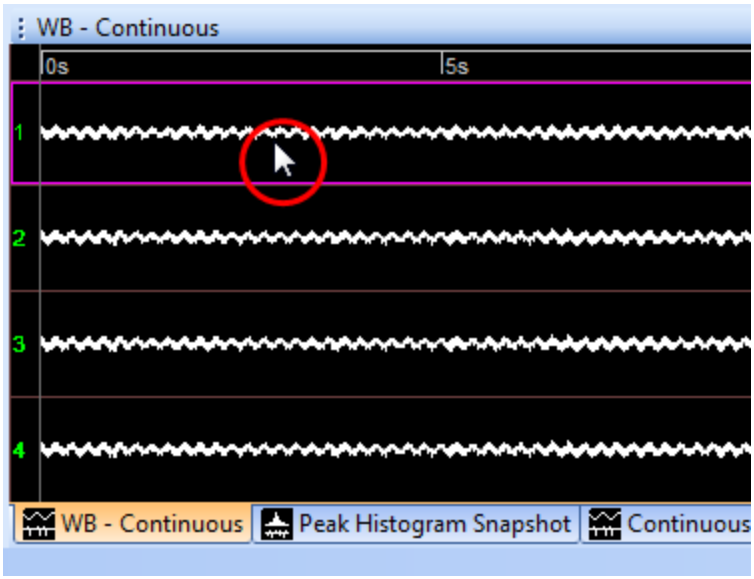
**TIP**

**Use magnification for an enlarged view of signals**

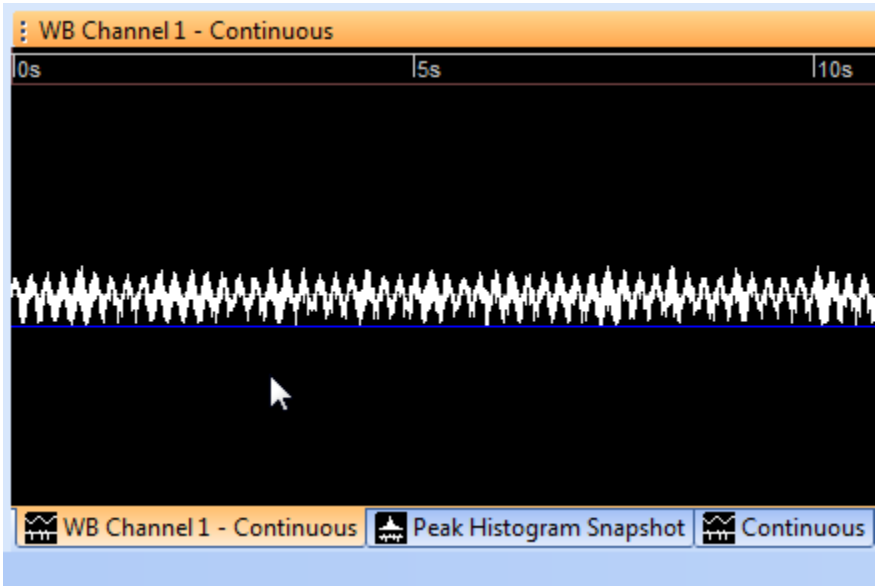
To avoid clipping of the signal, it is advisable not to set the gain too high. If a lower gain setting causes the signals on some channels to appear small in the displays, you can use the magnification feature to enlarge the displays as much as necessary. See Section 4.4.2, “Changing the Magnification” on page 121.

For the purposes of this user guide, the situation is slightly different than it would be in an actual experiment, since we are using an audio file being played through the HTU voltage divider as our test signal. What you will do next depends on the amplitude of the wideband test signal coming from the PC's audio output, as shown in the WB - Continuous view.

1 First, to get a better look at the signal, double-click in the WB - Continuous view, inside of the first row, labeled “1” at the left.




This will expand that channel's display so that it occupies the entire WB - Continuous view; note that the view's title bar now shows “WB Channel 1 - Continuous” instead of the previous “WB - Continuous”:

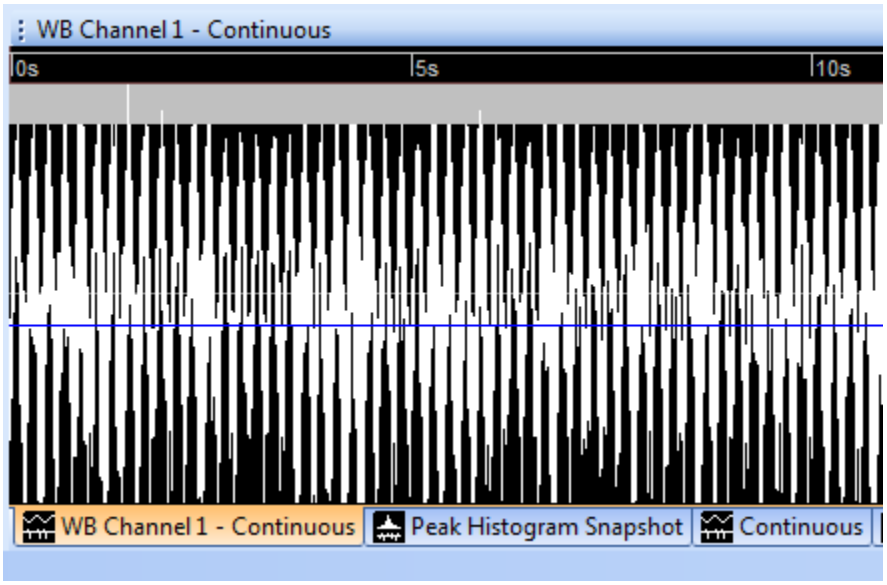



If you now double-click on the zoomed-in single channel, it will revert back to the multi-channel view. Before proceeding, make sure the view is zoomed in on channel 1 as shown above.

**TIP**
**Understanding “zoom” and “magnification”**

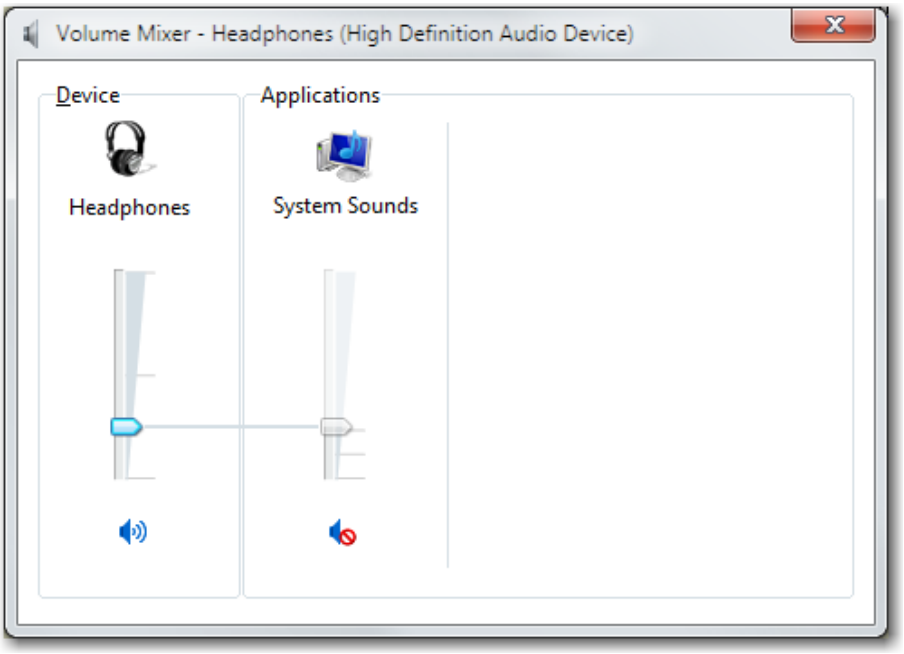
For historical reasons, the OmniPlex System term for this display mode, where only a single channel expands to fill an entire view, is “zoom.” Later, we will describe “magnification,” which is the OmniPlex System term for viewing a display at varying degrees of enlargement, for example, 1.5 times larger.

**2** View the wideband signal in the WB Channel 1 - Continuous display. If the wideband signal looks like the image below (that is, if it exceeds the allowable maximum amplitude in either the positive and/or negative direction), this is referred to as *clipping*.

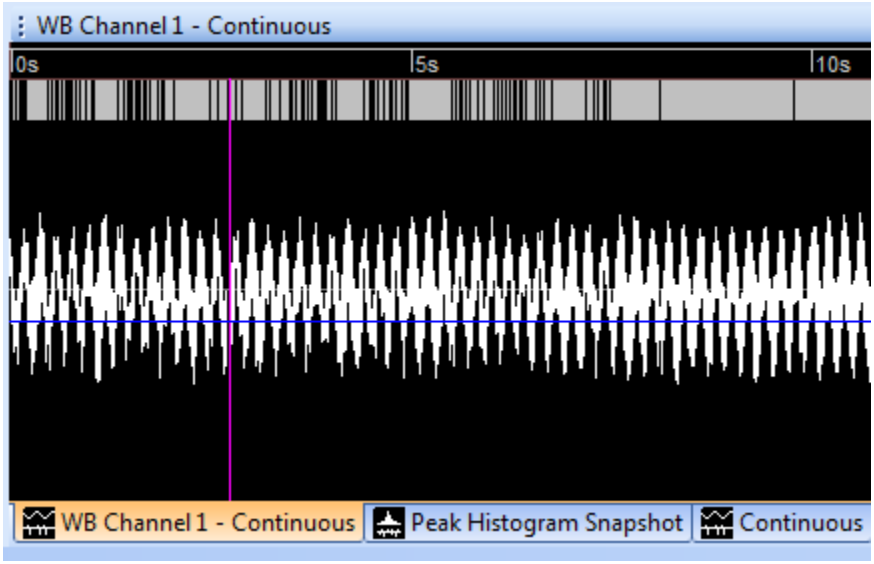



This situation occurs either when the analog gain is set too high, or in an artificial situation when using PC audio as a test signal, where the audio output can produce such a high amplitude signal that the voltage divider built into the HTU is not enough to reduce it to a reasonable level. You should only rarely encounter a situation in an actual experiment where using a unity-gain headstage (G1) with the lowest DigiAmp gain setting of 50 still results in a clipped wideband signal.

If you see clipping with the test signal as shown above, check to make sure your gain is set to 50, which is the lowest setting (and the default setting) on the DigiAmp Amplifier. If the gain is set to 50, go to your PC audio mixer or Windows volume control and turn down the volume:




When reducing the test signal level using the PC's volume control, try to set it to a level such that the largest peaks of the wideband signal occupy about 1/2 to 2/3 the vertical range, as shown in the WB - Continuous view:




The goal is to prevent the wideband signal from clipping the A/D converters, that is, exceeding their allowed maximum input range. See Appendix A: Signal Amplitudes and Gain for more detailed information on this topic.

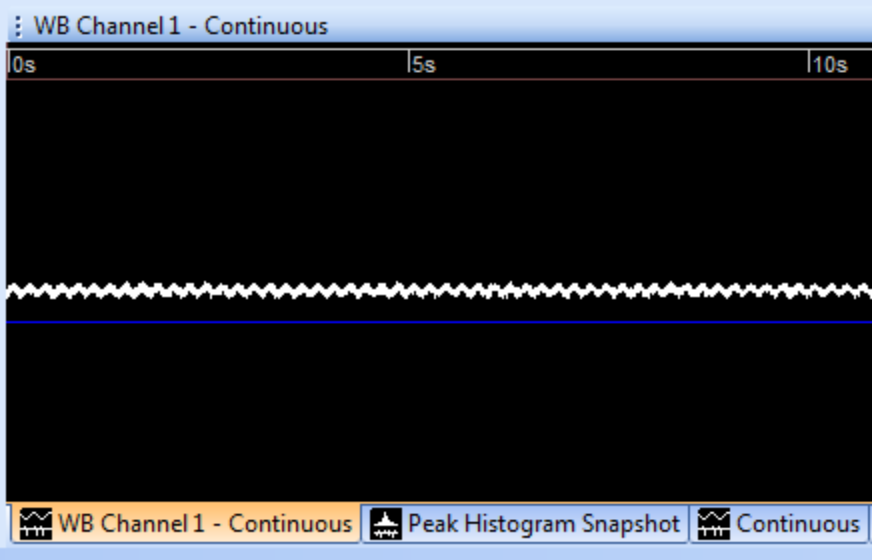
Look at the WB - Continuous view (see the image above) to determine whether clipping is occurring:

- The maximum positive voltage limit before clipping is the bottom of the gray row of tick marks just below the time labels. (These tick marks merge together into a horizontal bar when they are very close to each other.)
- The greatest negative voltage limit is the bottom edge of the view.

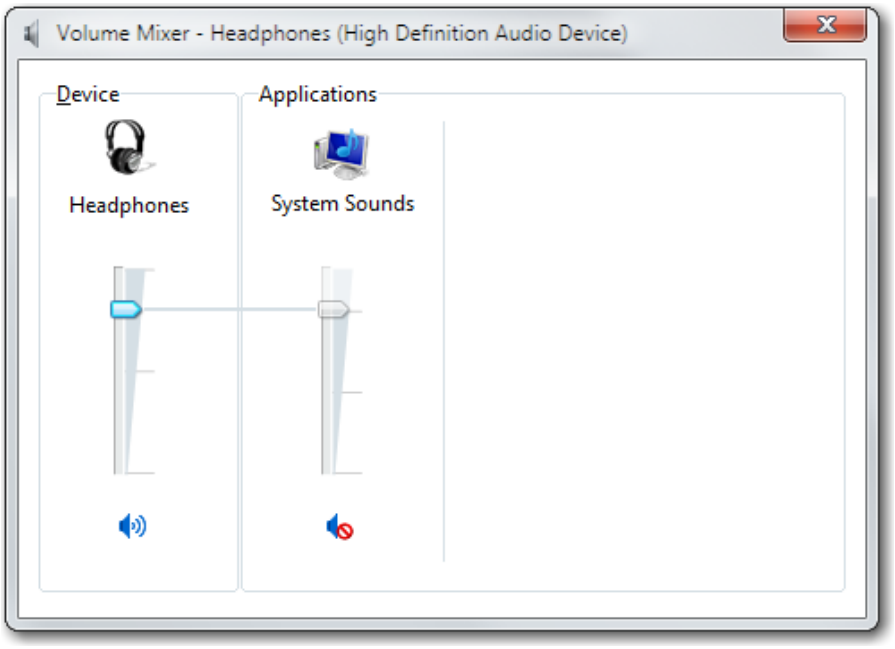
(For now, you can ignore the gray row of tick marks, as well as the thin blue horizontal line in the WB display; these will be explained later.)

If your wideband signal occupies about 1/2 to 2/3 the vertical range, you can skip Step 3 and Step 4 since they cover the opposite case, where the signal amplitude is too small.

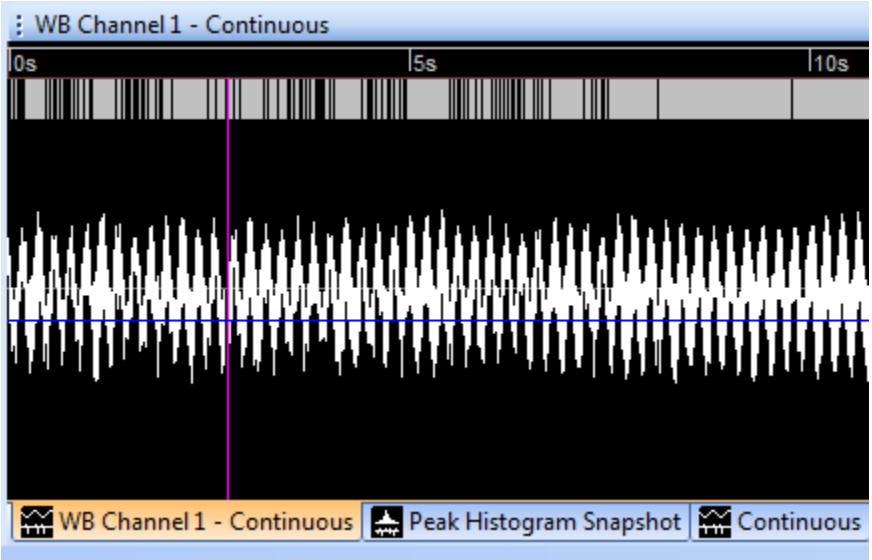
**3** If the wideband signal looks like the image below (that is, it occupies only a small fraction of the available amplitude range), it means that the volume level on your PC needs to be raised:



To raise the volume level on your PC, increase the volume level of the PC's Line Out or Headphones Out, using the Windows volume control or audio mixer:




When increasing the test signal level using the PC's volume control, try to set it to a level such that the largest peaks of the wideband signal occupy about 1/2 to 2/3 the vertical range, as shown in the WB - Continuous view:




The goal is to increase the wideband signal level high enough that it can be digitized accurately, while at the same preventing it from clipping the A/D converters, that is, exceeding their allowed maximum input range. See Appendix A: Signal Amplitudes and Gain for more detailed information on this topic.

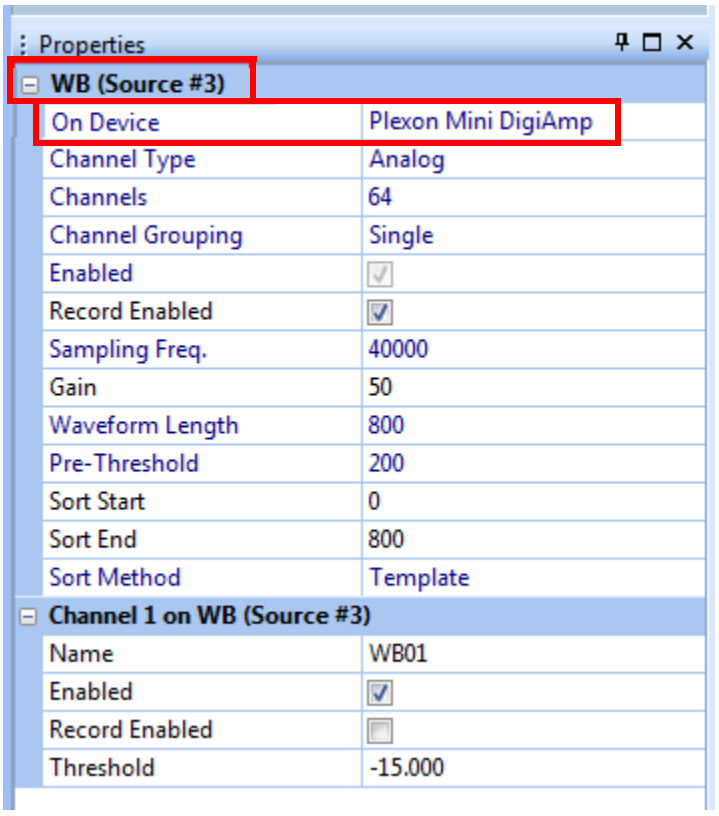
Note that maximum positive voltage limit before clipping corresponds to the top edge of the black background area, just below the time labels, while the greatest negative voltage limit is the bottom edge. The gray row of spike tick marks, which can merge together into a horizontal bar when the firing rate is high, can potentially obscure the very tops of signal peaks. If the signal peaks are obscured, it is possible that the wideband signal is being clipped, and you should reduce the gain as described below. This row of spike ticks serves as a “danger zone” into which continuous signals should not cross.

If the wideband signal is now displayed at a suitable amplitude in PlexControl, you can proceed to Step 5. If you increase the output level from the PC's Line Out to its maximum, but the wideband signal displayed in PlexControl is still too low in amplitude, then you will need to increase the DigiAmp gain, as described in Step 4.

**4** To change the DigiAmp (or miniDigi) gain, which is the analog gain applied to all channels of the wideband signal before A/D conversion (digitization), first verify that the Properties view at the left side of the PlexControl window shows the properties for source WB.

In the image below, the **On Device** row displays the device in the current topology, which in this example is a MiniDigi Amplifier (“Plexon Mini DigiAmp”).

**Note:** Depending on your topology, the source number might be different than the source number shown in the image below. Source numbers can generally be ignored.



The upper section of the Properties view displays the properties that are common to all the channels in that source, while the lower section displays per-channel properties. The Properties view is context-dependent—it displays the properties of the currently selected source, and the currently selected channel within that source. For example, if you clicked on channel 27 in the multichannel spike window, the Properties view would display the properties for source SPK, channel 27.

If the properties for the WB source are displayed, skip to Step 6. If some other source's properties are displayed (i.e. not WB), continue to Step 5.

**TIP**

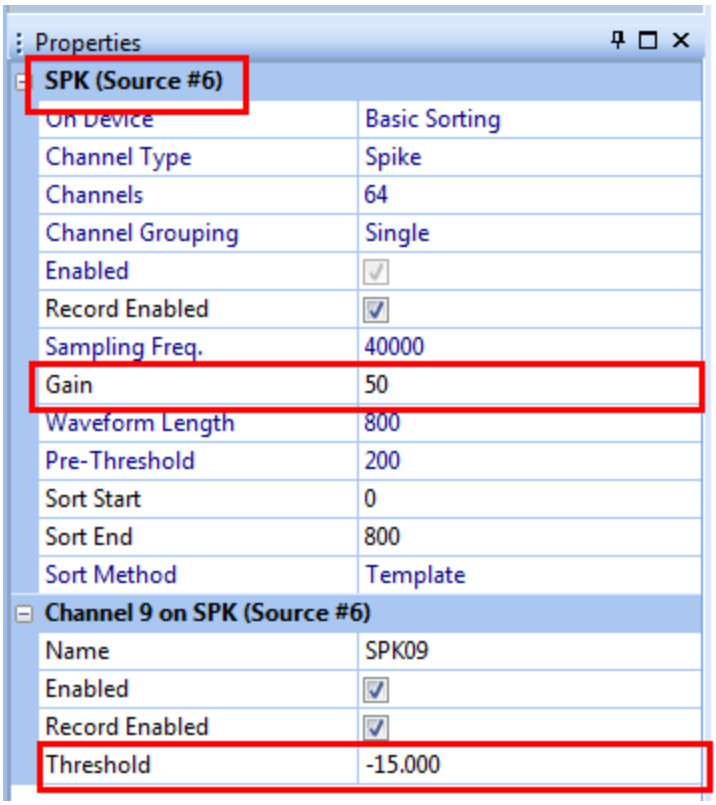
**Double-click to return to multichannel display**

Remember that if a multichannel continuous display (or any multichannel display) is zoomed, that is, only displaying a single channel, you can double-click it to return to a multichannel display, where you can select individual channels by single-clicking within that channel's rectangle.

Note that there are some properties listed for the WB source that are actually properties of other sources. For example, the Waveform Length is a property of the thresholder device, and Sort Method is a property of the sorting device. This is done as a convenience so you don't have to constantly select sources to change common properties.

The OmniPlex System automatically determines which upstream or downstream source is referred to relative to the one shown in the Properties view sub-title. For example, WB is upstream from SPKC and SPK, while SPK is downstream from SPKC and WB.

For example, if you selected a channel in the multichannel spike window, the Properties view might show something like the following image:




**Gain** is displayed as a property, even though the sorting device has no gain control. When you change the gain in this case, it actually changes the gain in the DigiAmp Amplifier, which is the first device upstream from the sorter. Likewise, channel 9's Threshold value is displayed and can be adjusted, and editing this parameter affects the thresholding device which is immediately upstream from the sorter.

However, if you select a source for which this consolidation of properties is not possible, e.g. one that is not downstream from the DigiAmp Amplifier when you want to change the gain, you will need to explicitly select a source for which the desired properties can be set. You can select the desired source by clicking on a view that contains that source.

**TIP**

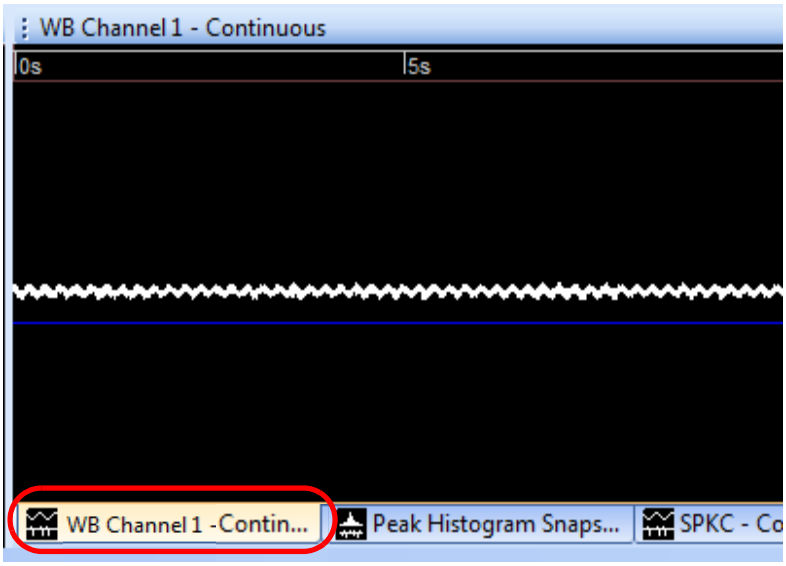
**Properties for upstream and downstream sources**

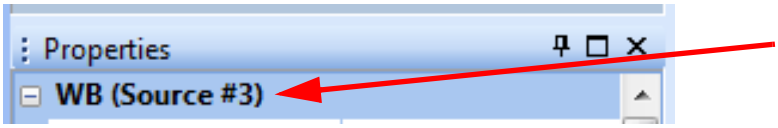
Later in this section you will see a description of the larger, multichannel Properties Spreadsheet, which displays certain properties for upstream and downstream sources, and provides consolidation of properties as described above.

You can also select sources, channels, and units by using the **Previous/Next Source/Channel/Unit** arrows in the toolbar:

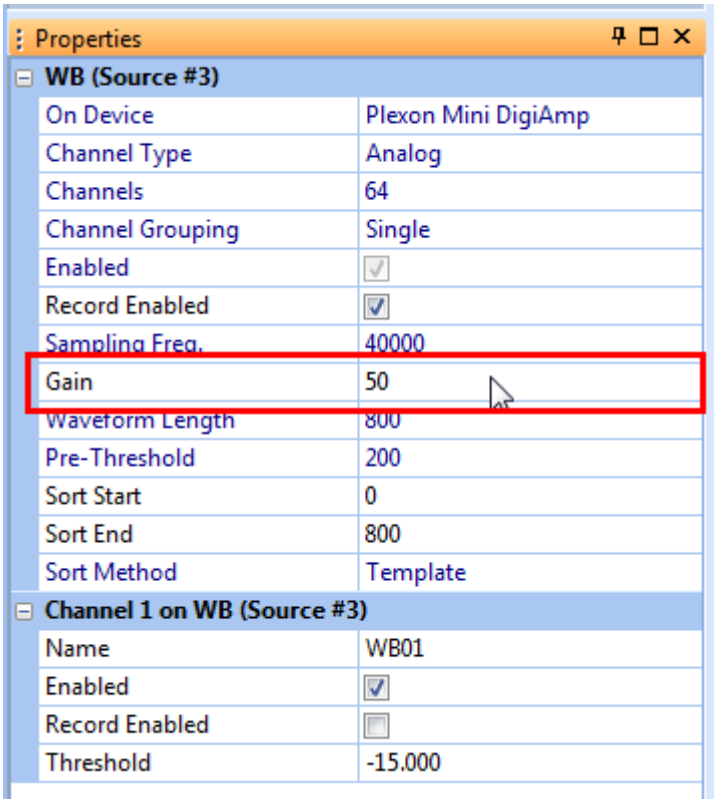
As you repeatedly click the **Next Source** button (right-arrow S), you will see the Properties view step through all of the sources in the topology. Similarly, the **Previous/Next Channel** buttons will step through the channels within the current source. Selecting a source or channel never changes any properties of that source or channel; it only highlights that source/channel in the displays and causes its properties to be displayed.

**5** If you do not see “WB” in the bar just below the “Properties” title in the Properties view, this is probably because you previously clicked on a view that is displaying a different source. To cause the Properties view to show the WB properties, simply click on the WB tab in the lower-right window so that it is the currently selected source:

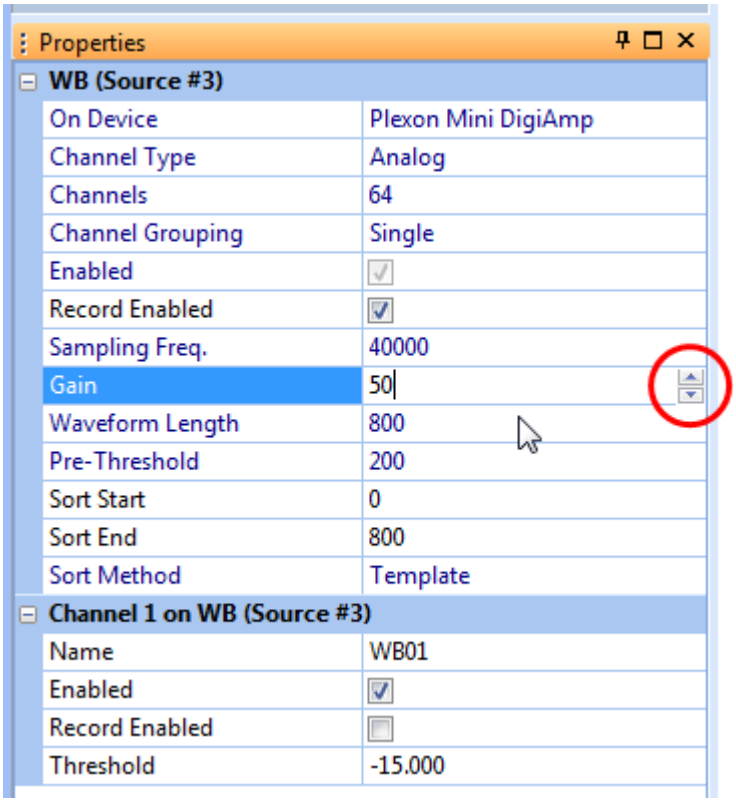


6 Now that the properties for the WB source are displayed in the Properties view, single-click anywhere in the **Gain** row:



You will see up/down arrows appear at the right end of the **Gain** row:




**7** Click the up arrow once to increase the gain from 50 to 250. If you see clipping in the WB - Continuous view (as shown at the start of Step 2), click the down arrow to return the gain to the original value of 50. On the other hand, if the signal level is still too low (this is very unlikely when using a strong signal such as the audio from a PC), you can try increasing the gain one more step to its maximum value of 1000. Remember, if you see clipping in the wideband signal, reduce the gain one step at a time until the largest peaks of the signal fit comfortably within the display window for the channel in the WB - Continuous view.

## 2.6 Setting the Wideband Gain with a Live Neural Signal

When you are working with a live neural signal, rather than a test signal from a PC, the gain-setting process is usually simpler than the procedure described above. Now that you know how to adjust the gain and monitor the wideband signal for clipping, these two guidelines should cover most situations:

- If you are using a unity-gain headstage (G1), set the DigiAmp gain to 1000; if clipping of the wideband signal occurs, reduce the gain as necessary until it occupies no more than 1/2 to 2/3 of the maximum amplitude range
- If you are using a gain-of-20 headstage (G20), set the DigiAmp gain to 50; if the wideband signal occupies a very small portion of the maximum amplitude range, carefully increase the DigiAmp gain until the signal occupies no more than 1/2 to 2/3 of the maximum amplitude range

Remember that, unlike the test signal, with a live neural signal you will often have different signal amplitudes on different channels. In such cases, make sure to keep the gain low enough to prevent clipping on the channel with the highest amplitude signals. Chapter 4, PlexControl User Interface, describes how to change the number of channels that are displayed at one time and other viewing parameters.

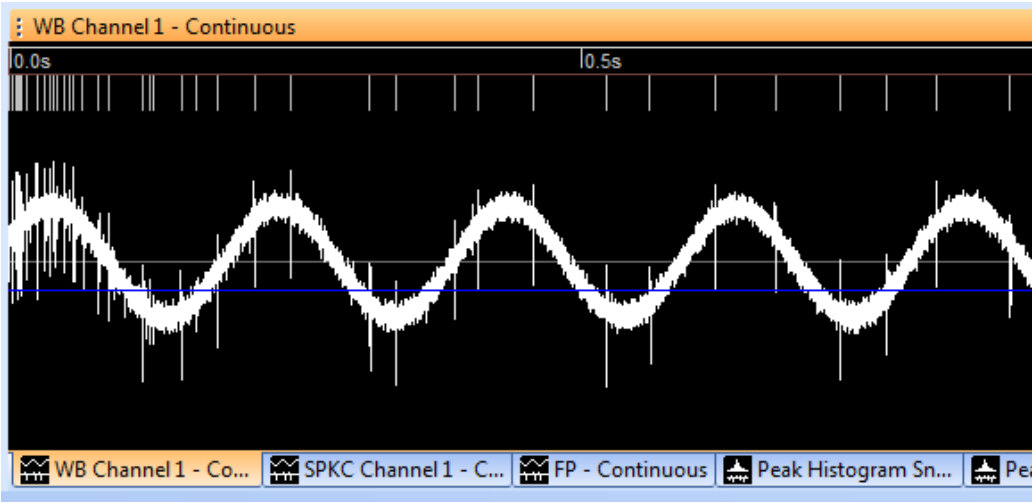
**TIP**

**Use magnification for an enlarged view of signals**

To avoid clipping of the signal, it is advisable not to set the gain too high. If a lower gain setting causes the signals on some channels to appear small in the displays, you can use the magnification feature to enlarge the displays as much as necessary. See Section 4.4.2, “Changing the Magnification” on page 121.

## 2.7 Separating Wideband Signal into Field Potentials and Spikes

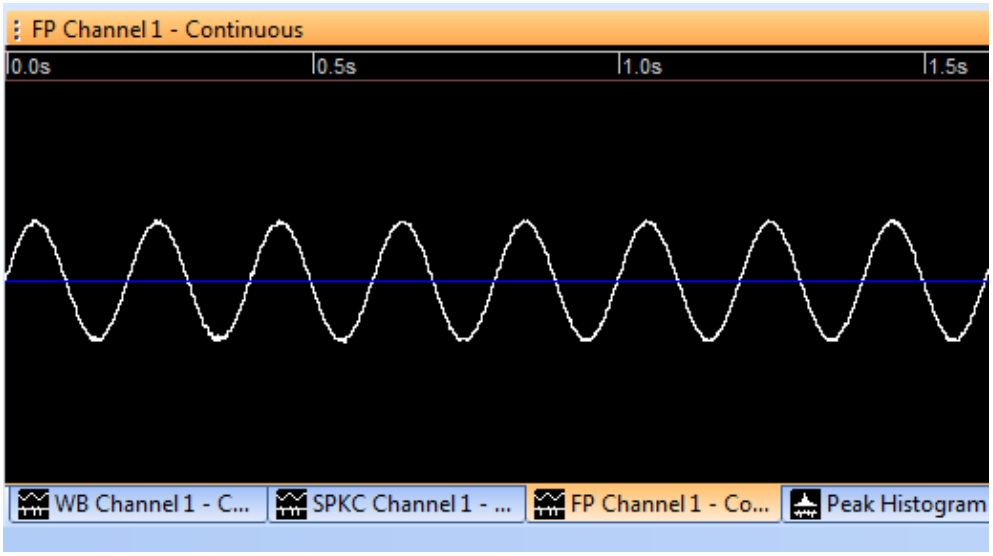
So far, you have been working with the wideband signal (WB source), which after preamplification (analog gain), is digitized at a sampling rate of 40 kHz. The digitized wideband signal for each channel contains field potentials at lower frequencies plus a spike-band signal at higher frequencies. Since the field potentials are typically of a much larger amplitude than the spikes, the net effect is of spikes “riding on the waves” of field potentials:




In live neural signals, the spike amplitudes are often small compared to the field potential amplitudes (even smaller than what is shown in the sample image above).

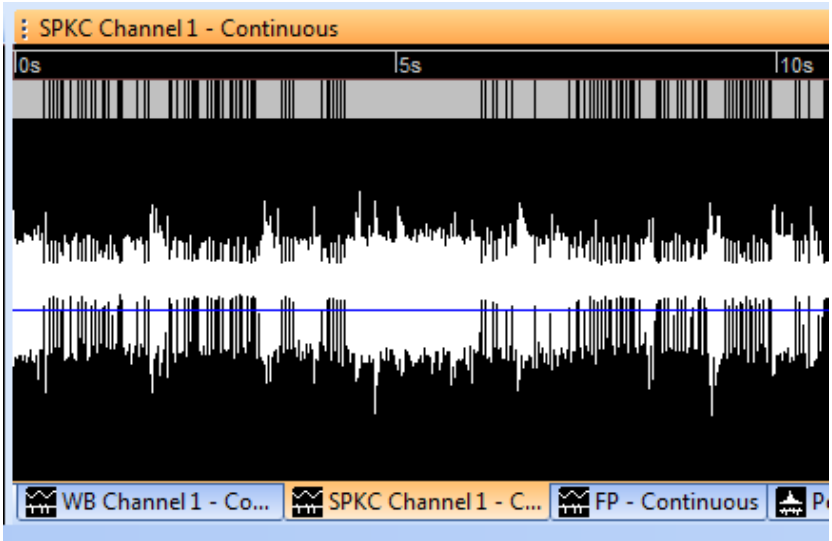
The OmniPlex System uses software DSP filters in Server to separate the WB signal into its two primary components:

- Lowpass filtering with a cutoff of approximately 200-300 Hz yields the field potentials, which are then downsampled to a default sampling rate of 1 kHz (FP source). The downsampled signal can be processed more efficiently and helps reduce the size of recording files. As a rule, the sampling rate must be at least twice the highest frequency component in the signal, and a factor of four or more is preferable.




**Note:** This is an artificial test signal where the “FP component” is a simple low-frequency (5 Hz) sine wave. Real FPs would be more complex low-frequency signals.

- Highpass filtering with a cutoff of approximately 200-300 Hz yields the continuous spike signal (SPKC source), sampled at the same 40 kHz rate as the original wideband signal.

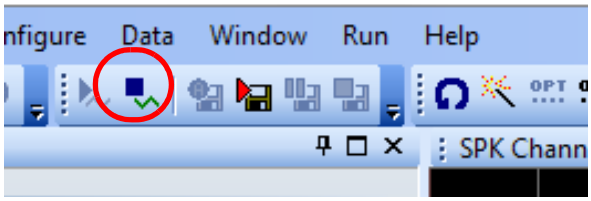


Informally, you can think of removing the field potentials as “flattening the baseline” of the wideband signal; without this flattening, it would be impossible to detect spikes by comparing the continuous signal amplitude against a fixed voltage threshold.

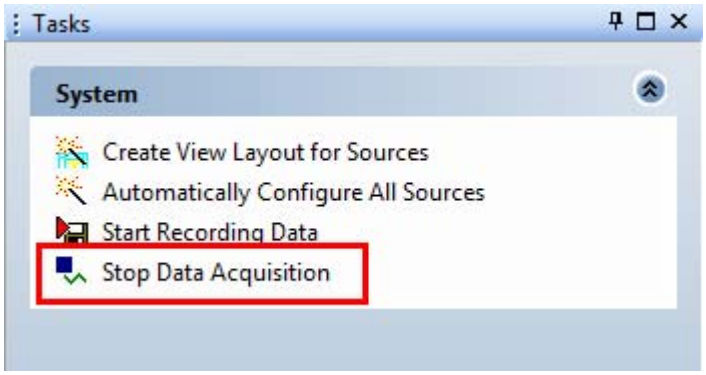
The OmniPlex System allows you to configure the characteristics of the spike and FP separator filters and the downsampling, but for these examples, you will use the default settings. See Appendix B: Separation of Spikes and Field Potentials Using Digital Filters for details on how to change the default settings and some of the tradeoffs involved.

## 2.8 Stopping Data Acquisition

The **Stop Data Acquisition** button is to the right of the **Start Data Acquisition** button:




Or, from the Tasks view, select **Stop Data Acquisition** (which is only displayed while data acquisition is running):




Parameters which you had previously set while data acquisition was running, such as thresholds, etc. will be preserved when you restart data acquisition.

# Chapter 3
# Startup (with DHP Subsystem)

3.1 Connections and Power-up ..... 62
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3.3 Digital Headstage Ports ..... 82
3.4 Specifying Digital Headstage Types ..... 85
3.5 Port and Channel Assignment Guidelines ..... 89
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3.7 Starting PlexControl ..... 93
3.8 Starting Data Acquisition ..... 95
3.9 Separating Wideband Signal into Field Potentials and Spikes ..... 101
3.10 Stopping Data Acquisition ..... 103

## 3.1 Connections and Power-up

### How to use Chapter 2 and Chapter 3

This chapter—Chapter 3, Startup (with DHP Subsystem)—applies to OmniPlex® Systems that use the DHP (Digital Headstage Processor) subsystem for data acquisition.

**Note:** **If you have a DigiAmp™ subsystem, use** Chapter 2, Startup (with DigiAmp Subsystem) **instead.**

### DHP subsystem connections and power-up procedures

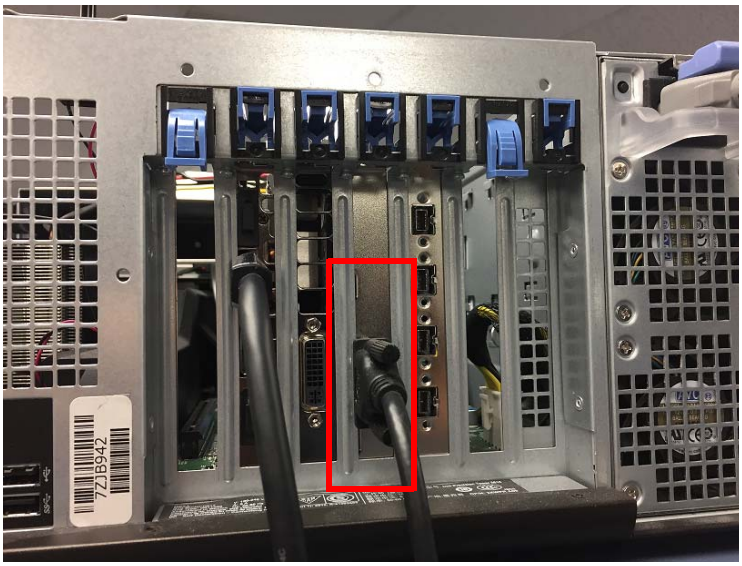
If you already have a running system with all the cables connected and a headstage tester unit (HTU) connected to the audio output of the PC, you can skip this section and go directly to the section Section 3.2, “Starting and Configuring the OmniPlex Server” on page 73.

### Initial settings

1. If the PC is on, close all programs and shut it down (close Windows® and fully power down - not just Sleep or Hibernate).
2. If the power switch on the OmniPlex chassis is in the “on” position (—), switch it to the “off” position ( **O** ) now by pushing the rocker switch on the rear panel of the chassis (see the image, below).
3. Plug in the power cable to the chassis. Leave the rocker switch in the “off” position.

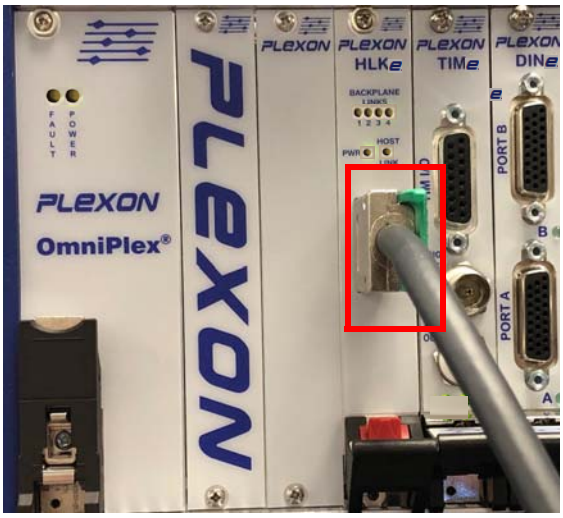
**4** (This step applies to the basic OmniPlex chassis and the COMPUTER LINK card. If you have the OmniPlex eChassis and the HLKe card, skip this step and use Step 5 instead.)

Connect the black signal cable from the **COMPUTER LINK** card in the chassis to the connector on the back of the PC.

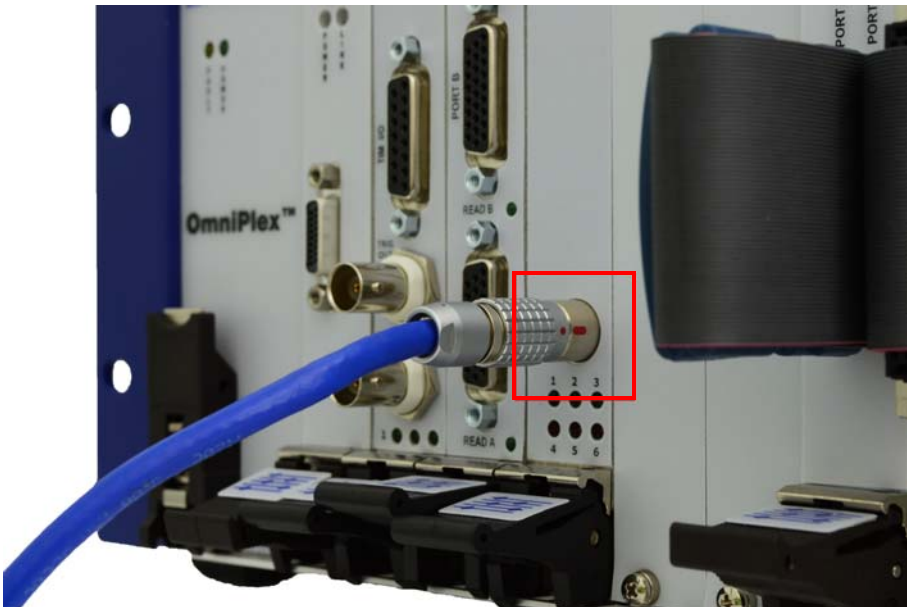


**5** (This step applies to the OmniPlex eChassis and the HLKe card. If you have the basic OmniPlex chassis and the DATA LINK card, skip this step.)

Connect the PC signaling cable from t**he HLKe ca**rd in the eChassis to the connector on the back of the PC.



6 Connect the blue link cable from the **DATA LINK** card in the basic chassis (or the **PDLe** card in the eChassis) to the connector on the Digital Headstage Processor (DHP) unit. The red markings on the cable connectors should line up before inserting the cable.

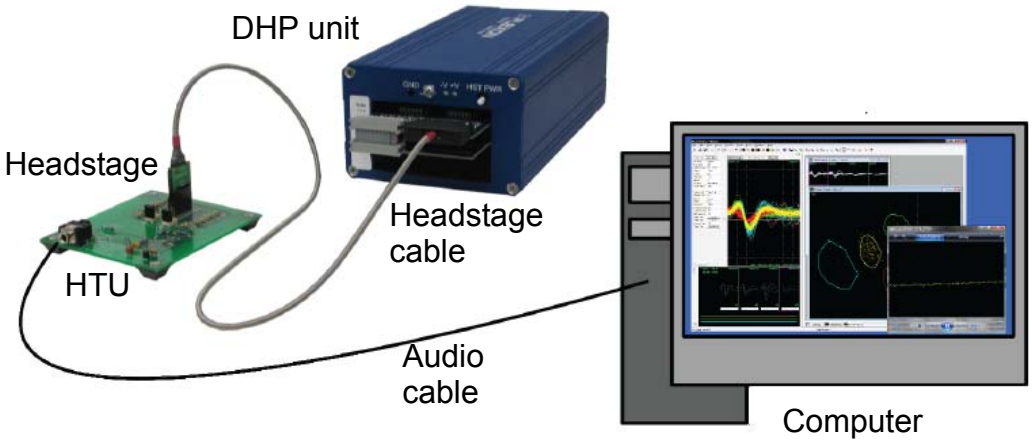


If you ever need to disconnect the blue link cable from the DHP unit, DATA LINK card or PDLe card, unplug the connector by grasping the connector (shown inside the red rectangle in the images above) and pulling straight out. **Warning: Do not pull on the blue cable itself, and do not twist the connector. Never bend or kink the blue cable.**

| | |
|---|---|
| | **CAUTION**<br>**Once Server is running, do not plug/unplug the blue cable**<br>Never plug/unplug the blue cable if the Server application is running. Doing so could damage the circuitry. |

### Connecting to the Headstage Tester Unit

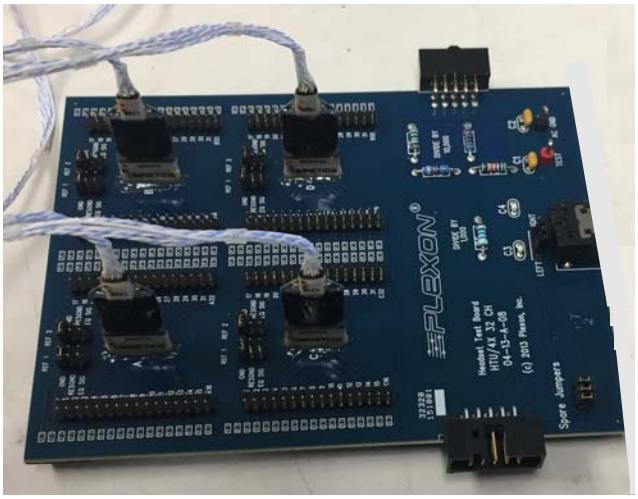
The next few steps will connect the headstages and audio cable to the Headstage Tester Unit (HTU). The general connection scheme is shown in the following diagram.




**7** Obtain the headstages and headstage cables that you are going to test. If not already done, connect each headstage to a headstage cable.

**8** Connect the headstage input connector to the connector on the HTU. (Repeat if you have multiple headstages and HTUs.)

**HTU shown with four headstages connected**
**(There are several models of the HTU. This is an example.):**



**9** Connect the other end of the headstage cable to the connector on the DHP unit. (Repeat if you have multiple headstages and headstage cables.)

**DHP unit shown with one headstage cable connected:**

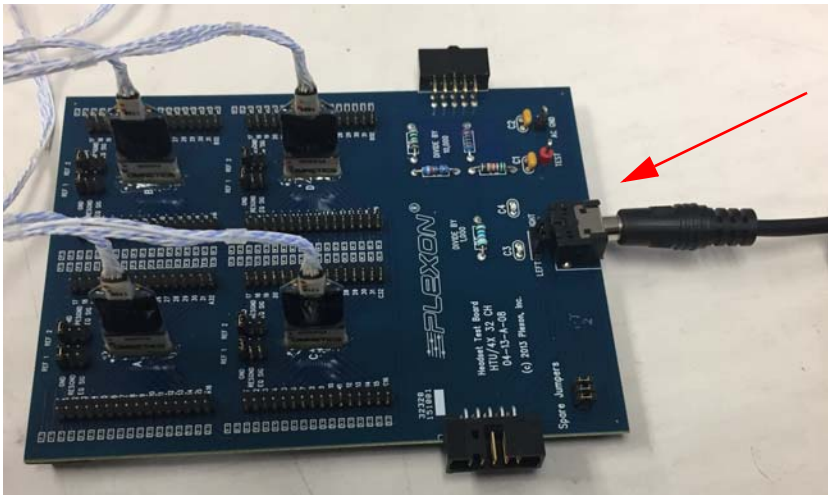
**10** (This step is applicable to some OmniPlex Systems, and depends upon the audio capabilities of the specific PC that was included in your system.) All OmniPlex components, including the high speed PC, are fully integrated and tested prior to shipment. PC models change from time to time as computer technology improves. Most PC models have good quality audio ports, but some do not. If the audio port on a PC model is not sufficiently high quality, or if it is noisy, Plexon® includes a USB audio interface that will provide higher quality audio for OmniPlex testing purposes.

If you have a USB audio interface, plug the interface into any USB port on the PC.

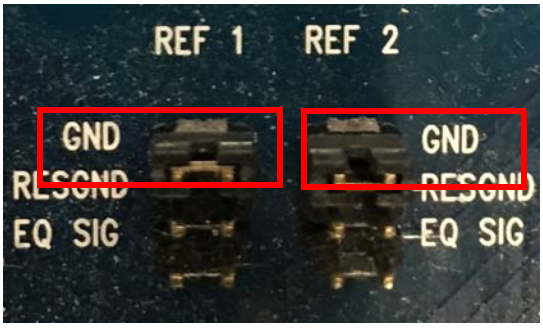
**Note:** A test audio file will be played in the procedure in Section 3.8, “Starting Data Acquisition” on page 95.

**11** Connect a 1/8” stereo audio cable from the USB audio interface output jack (or from the 1/8” line output jack on the front or back of the PC) to the 1/8” input jack on the HTU.

**HTU shown with four headstages and audio cable connected:**



**12** Verify that the **REF** jumper(s) on the HTU are positioned on **GND**. Verify that the left/right jumper is positioned on **LEFT**.



### Power up

**13** Turn the chassis power on. You should see the two green **POWER** LEDs light up at the left end of the front panel.

Basic OmniPlex chassis:

OmniPlex eChassis:

**Note:** The four LEDs labeled “BACKPLANE LINKS” indicate communication activity with the PC and do not require any user action.

**14** Start the PC and allow Windows to boot up normally. You should see the green LED light up—the **LINK** LED on the COMPUTER LINK card or the **HOST LINK** LED on the HLKe card.

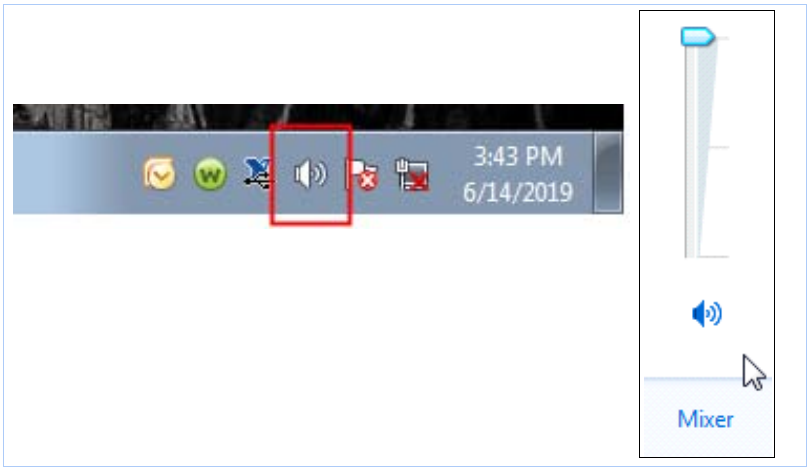
**Note:** If the link LED does not come on, it is possible that the black link cable to the PC is not connected properly. In this case, verify that both ends of the cable are properly connected, then repeat the procedure starting from Step 1.

Basic OmniPlex chassis:

OmniPlex eChassis:

**Note:** The **HOST LINK** LED blinks when the PC is connected and turned on.

**15** Do not play any audio on the PC yet, but open the Windows volume control or audio mixer and make sure that Line Out and Headphone out (some PCs will only have one or the other output option) are enabled (not muted), and set the volume to maximum.




**Note:** A test audio file will be played in the procedure in Section 3.8, “Starting Data Acquisition” on page 95.

### If you are connecting a Plexon CinePlex® System to your OmniPlex System

For information about the interface between these two systems, see the CinePlex User Guide, which is available on the Plexon website.

## 3.2 Starting and Configuring the OmniPlex Server

The OmniPlex Server is the first of the two primary software components of the OmniPlex System. Server is the "engine" which receives data from hardware devices, sends commands to the hardware devices, and contains the topology (network) of software modules which perform filtering, thresholding, sorting, and other signal processing functions. Once Server has been configured for your hardware (which may have already been done by a Plexon Sales Engineer), you will probably find that you spend relatively little time interacting with it, since the main user interface to the OmniPlex System is provided by PlexControl, which is described in Section 3.7, "Starting PlexControl" on page 93.

### Launching Server and setting the topology

**TIP**

**Press Ctrl key to prevent auto-loading of pxs file**

If you ever need to prevent the auto-loading of the last-used pxs file, for example, for troubleshooting, you can hold down the Ctrl key before double-clicking on the Server desktop shortcut; in this case, you will be "starting from scratch" and will need to either load some other pxs file, or use the Topology Wizard to create a new one, as described in the steps below.

1 From the Windows desktop, double-click the **OmniPlex Server** shortcut:

**CAUTION**

**Once Server is running, do not plug/unplug the blue cable**

Never plug/unplug the blue cable if the Server application is running. Doing so could damage the circuitry.

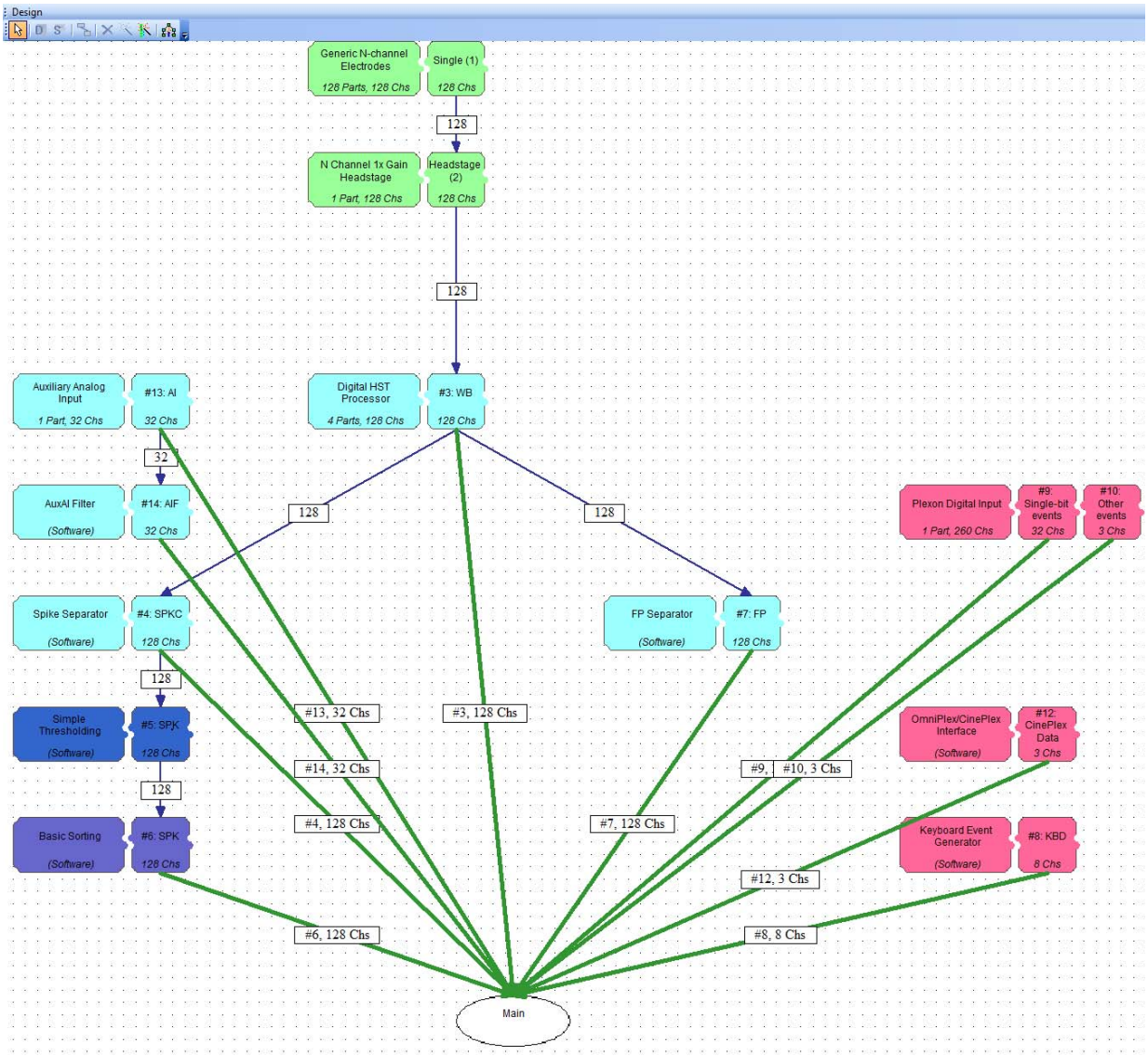
2 If you have recently upgraded the OmniPlex Server software, the system might display a dialog "DHP Firmware Update Warning." Upgrading your firmware is recommended, because it will provide updates such as enhanced protection from severe transient noise in the environment, operation with additional chassis cards and higher channel counts. To perform the firmware upgrade, see Appendix K: Firmware Upgrade for DHP Unit on page A-84.

3 If a system topology (configuration) diagram similar to the one below is displayed, wait for the green progress bar at the bottom of the Server window to finish. This diagram represents the topology that is currently saved in the system from a previous run. If the topology is already set up the way you

want, you may proceed directly to Section 3.3, “Digital Headstage Ports” on page 82.

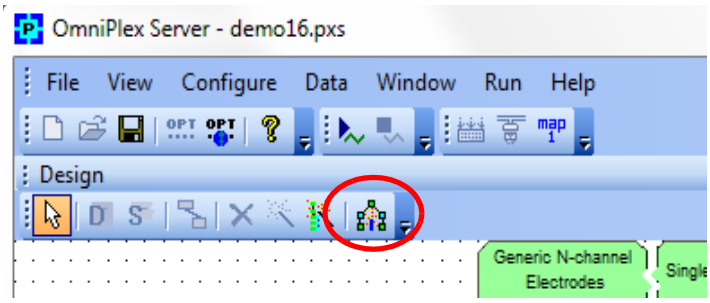
If you do not see a topology diagram, or you want to modify it, perform Step 4 through Step 19 to create a new topology for the system.

**Note:** When you upgrade to a new OmniPlex System software version, or if you add or replace any of the hardware in your system, you should create a new topology (a new .pxs file) to ensure compatibility. Perform Step 4 through Step 19.

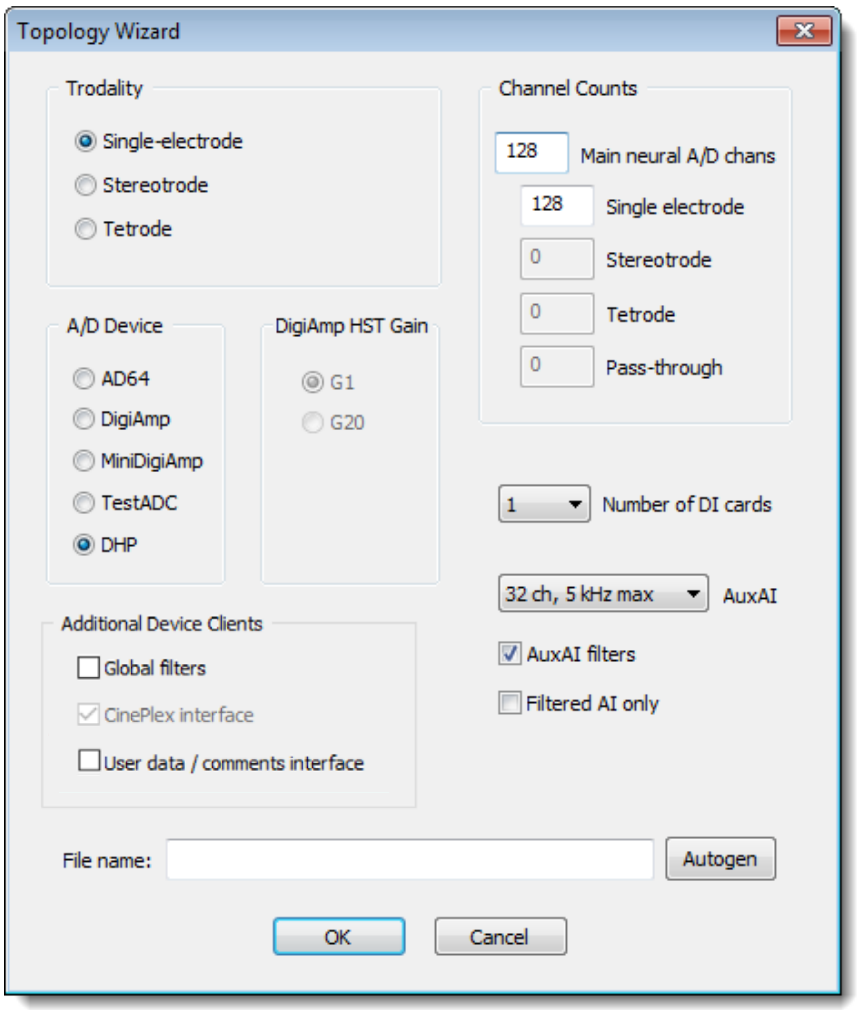



**Note:** Data acquisition must be stopped to load or create a topology. See Section 3.10, “Stopping Data Acquisition” on page 103.

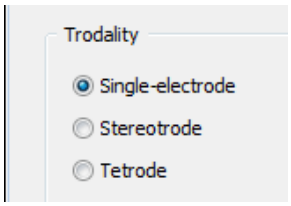
4 You can use Server's Topology Wizard to specify the topology of your OmniPlex System. To do so, click on the **Topology Wizard** button in the toolbar:



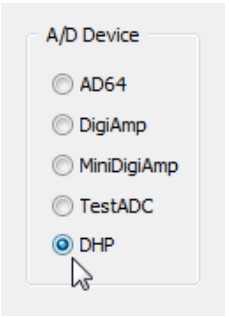

5 The **Topology Wizard** dialog is displayed:



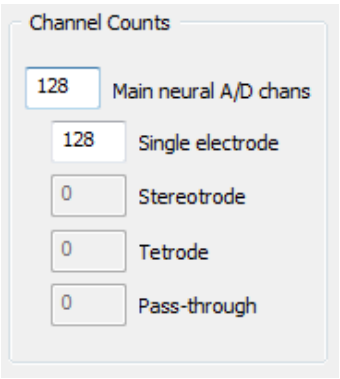
6 Select the type of electrode in your experiment. You can choose only one type for each topology. (The examples below assume **Single-electrode** is selected.)




7 In the **A/D Device** section, click on the **DHP** button.




8 In the **Channel Counts** section, for **Main neural A/D chans**, enter the total number of channels you will use in your DHP unit. If you are unsure of the number of channels, contact Plexon for assistance. When you enter a channel count in **Main neural A/D chans**, the corresponding number is automatically entered in the **Single electrode** field.
The number you enter should correspond to the maximum number of headstage channels that you will use. For example, if you will be using four 32 channel headstages, enter 128 for **Main neural A/D chans**. The same would apply if you were using eight 16 channel headstages, or two 32 channel headstage and four 16 channel headstages.




**Note:** You must enter a number that is a multiple of 16, that is, 16, 32, 48, ... 512, but not higher than the maximum channel count of your system license.

If you are using a single HST/8D Gen2 headstage (only a single 8 channel digital headstage) with the OmniPlex System, you need to configure your topology for 16 channels (16 channels is the minimum topology). As displayed in PlexControl, the first 8 channels will

contain your data, and the next 8 channels will display all zeros.

You can define fewer channels than are physically present in your system. This can be useful if you will be running experiments using a limited number of channels. For example, if you have a system physically capable of 192 channels, but only plan to use 128 of those channels, you can enter 128 for **Main neural A/D chans** and the system will ignore (and not attempt to display) the unused 64 channels.

You can also disable individual channels in the Properties Spreadsheet.

It is important to understand how the system handles the number of channels that you enable/disable. For a complete explanation of these options, see Appendix F: Disabling Unused Boards to Reduce Channel Counts on page A-40.

### AuxAI configuration

Perform Step 9 through Step 11 if you have an Auxiliary Analog Input (AuxAI) card in your chassis, and want to use the channels associated with the AuxAI subsystem. Otherwise, skip to Step 12.

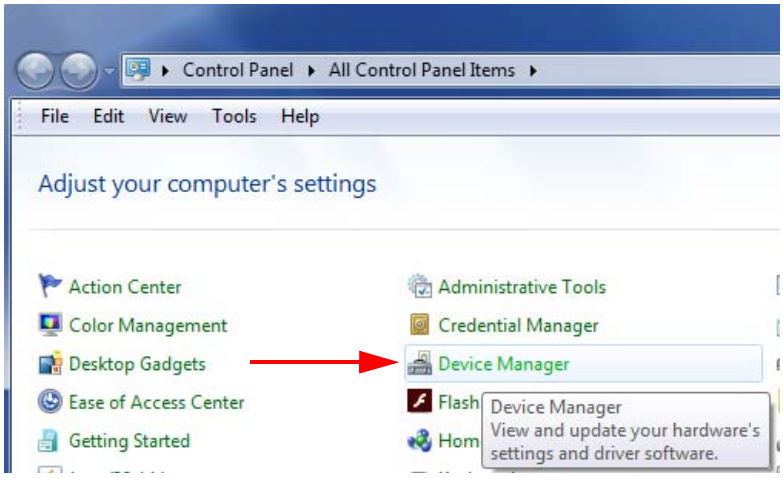
**Note:** The AuxAI hardware is shown in the photo in Section 12.2, “5 kHz, 10 kHz and 20 kHz Maximum Sampling Rates” on page 366.

**9** There are several types of AuxAI cards:

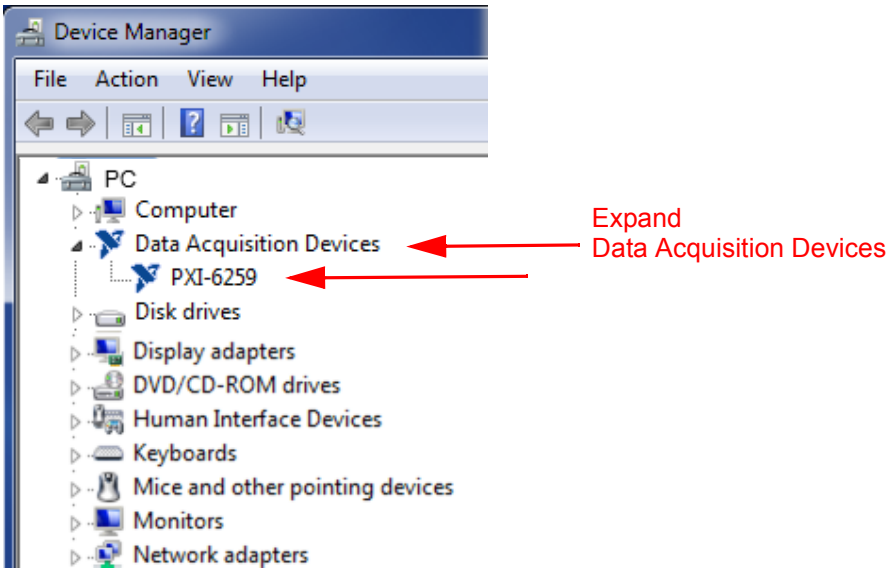
- The standard AuxAI module, suitable for sampling rates up to 10 kHz maximum, is the 16 channel PXI-6341. (Some systems use the 5 kHz 32 channel PXI-6224.)
- The “fast” AuxAI module, suitable for sampling rates up to 250 kHz maximum, is PXI-6259.

If you already know which card is installed, proceed to Step 10. If you are unsure whether you have a standard or “fast” card, you can determine this by the following method:

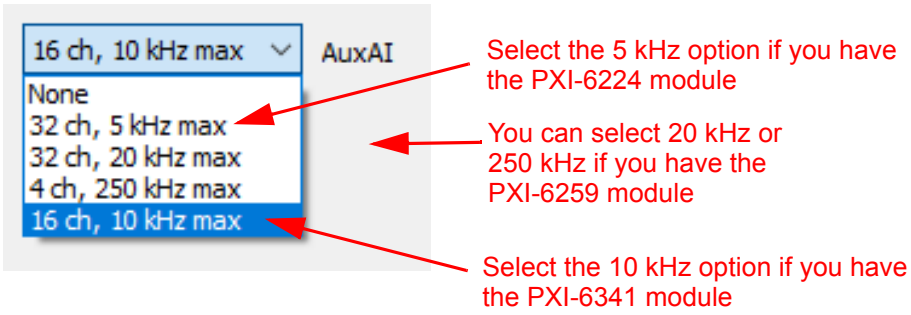
**9a** Left click the **Start** icon and navigate to the **Device Manager**.



**9b** In **Device Manager**, view the display to determine which module is installed in your PC:



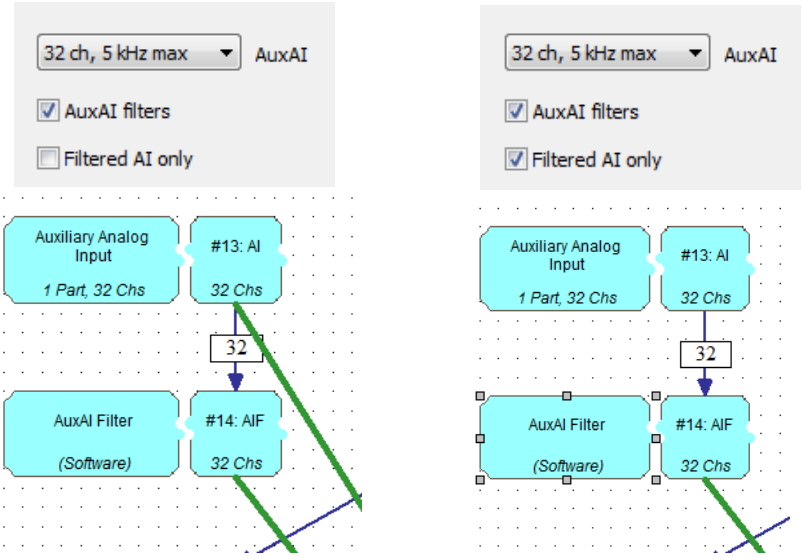

**10** In the Topology Wizard, select the desired combination of channels and max sampling rate from the **AuxAI** dropdown list.



11 If you want to enable AuxAI filters, click in the **AuxAI filters** checkbox. This will cause the system to acquire both the filtered and unfiltered AuxAI signals.

If you want the system to acquire *filtered AI data only*, click in the **Filtered AI only** checkbox.

**Note:** The **Filtered AI only** option cannot be selected by itself.



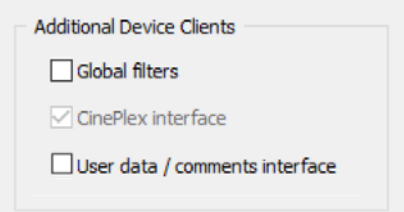

In the example on the left, we did not check **Filtered AI only**, so the green lines (which are pointing towards the main datapool, not shown here) indicate that both AuxAI and AIF sources will be available in the OmniPlex GUI. In the example on the right, we checked both **AuxAI filters** and **Filtered AI only**, so only the green line from AIF is present.

For further discussion of AuxAI functions, see Chapter 12, Auxiliary Analog Input.

### Final topology configuration steps

12 The **Global filters** option in the **Additional Device Clients** section relates to a legacy feature. It is generally not needed and can be left unchecked. Information about this option is available in Appendix B: Separation of Spikes and Field Potentials Using Digital Filters on page A-4.

The **User data / comments interface** option can be selected if you would like to have the ability to add comments during recording (see Section 7.3, “Adding Comments Before and During Recording” on page 217).

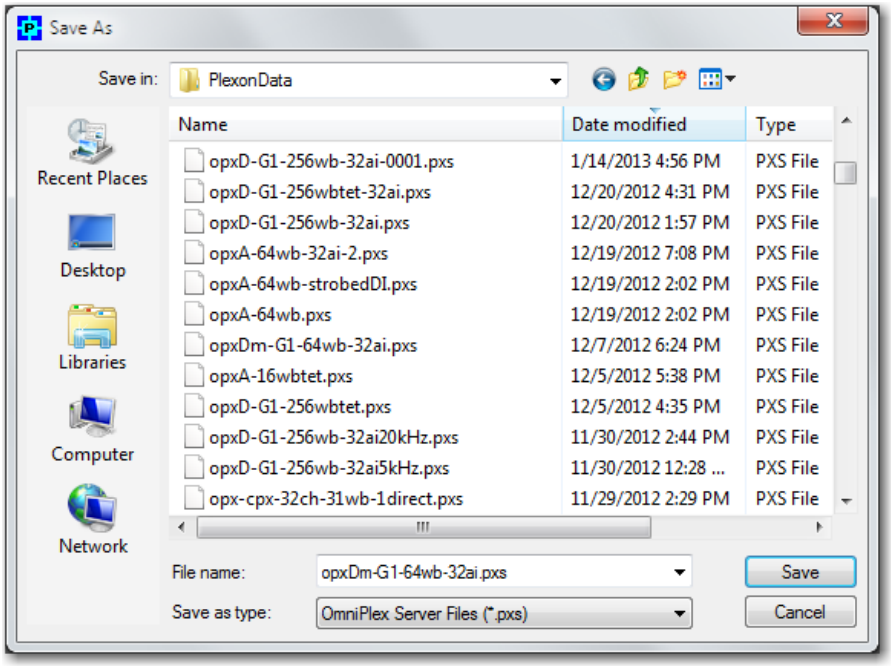


13 Leave all other Topology Wizard settings at their default values. Note also that the **DigiAmp HST Gain** parameter is not applicable to the DHP unit and there is no need to be concerned with it.

14 Click **OK**. Wait for Server to generate a new topology diagram and go through the initialization sequence, as indicated by the green progress bar at the bottom of the window.

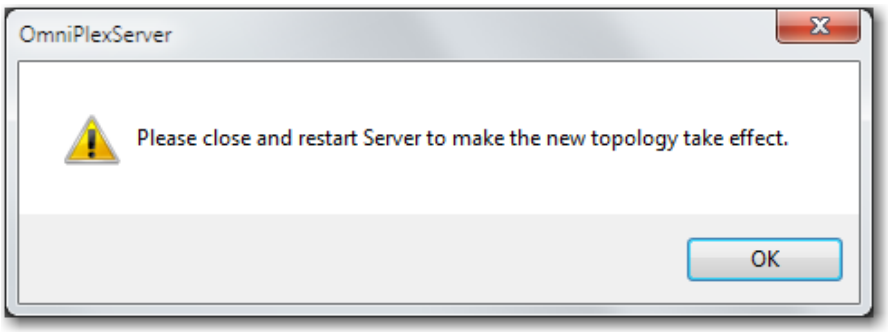
15 Server will display a **Save As** dialog asking you for the name for your new topology (a topology is saved in a file with the extension ".pxs"). It will display an automatically generated and appropriate name, for example,

DHP-64wb-32ai.pxs



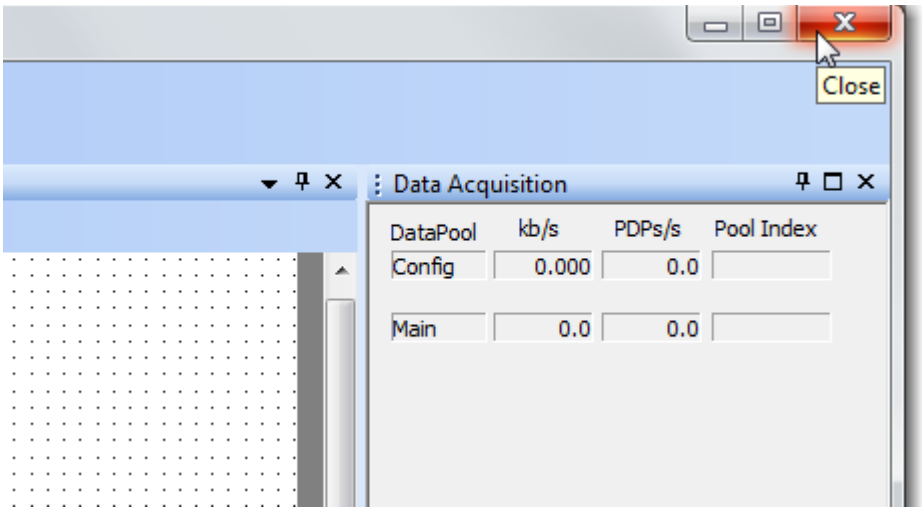

Click **Save** to accept the default filename, or edit the name if you wish, and then save the pxs file.

16 Server will display a message box:



17 Click **OK**.

18 Close Server.




19 Restart Server as described in Step 1, wait for the .pxs file to load and for the green progress bar to finish.

Server now has auto-loaded a .pxs topology file, either one that was used in a previous OmniPlex session, or one that you just created in Step 4 through Step 19 above using the Topology Wizard. In either case, from now on, when you start Server from the desktop, by default it will automatically load the last-used pxs file.

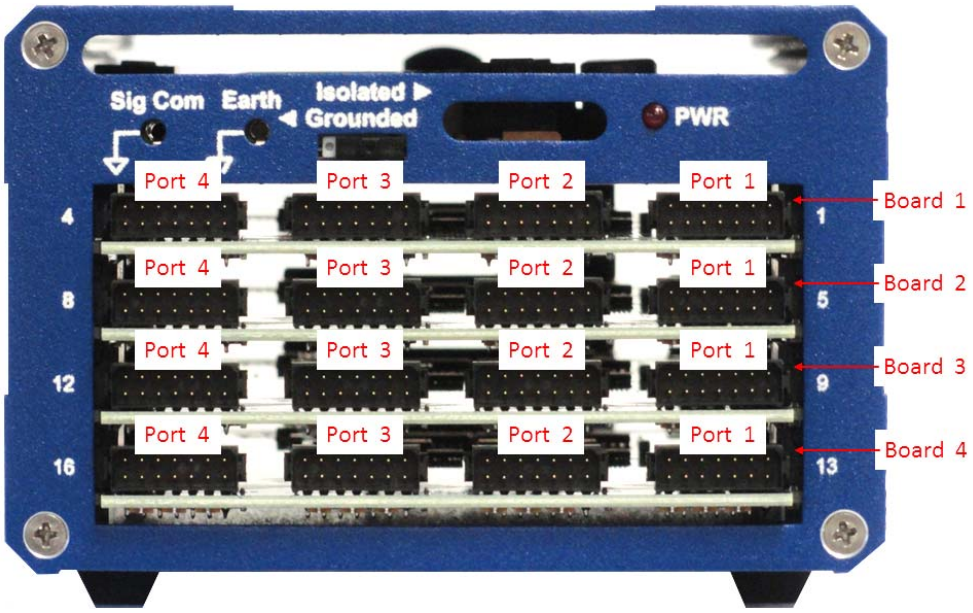
**TIP**

**When to use Topology Wizard**

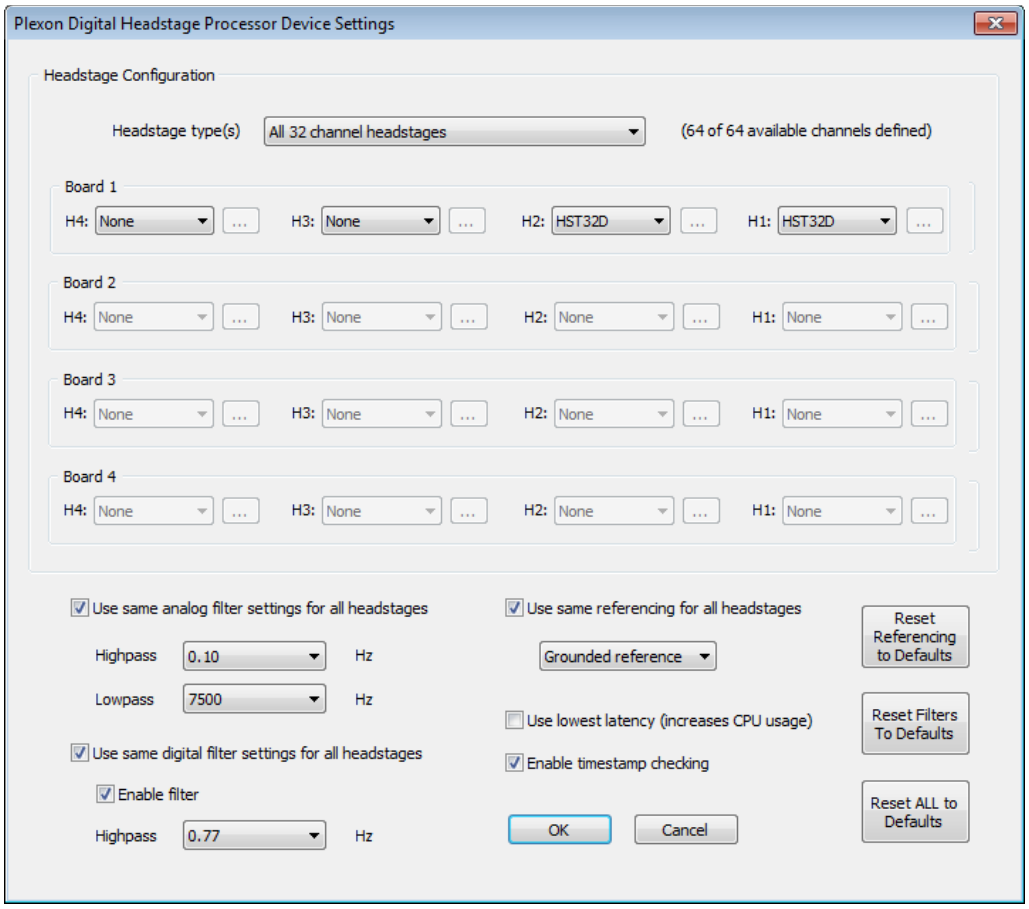
You should use the Topology Wizard to create a new pxs file when the hardware configuration of your system changes (for example, adding more boards to a DHP unit or adding a new card to the chassis), or when you are updating software.

## 3.3 Digital Headstage Ports

The DHP unit can contain from one to four circuit boards. Each board includes four digital headstage connectors, called ports. Each port can interface to an 8, 16, 32, 64 or 128 channel digital headstage. The ports on each board are numbered in right to left order, and the topmost board is Board 1. The image below shows the board and port numbers.




**Note:** As shown in the image below, the **Device Settings** dialog of the OmniPlex user interface displays Ports 1, 2, 3 and 4 as H1, H2, H3 and H4 for each board. We will explain how to use the **Device Settings** dialog in the steps below.




Every DHP system has at least one board, with four ports, although not all ports may need to be used on smaller systems. When used with four 32 channel headstages, two 64 channel headstages or one 128 channel headstage, each board supports a maximum of 128 channels. With 16 channel headstages, the maximum is 64 channels per board.

### If you are using only 32 channel headstages:

The system default in the **Device Settings** dialog assumes that all headstages are of the 32 channel type. If you are using only 32 channel headstages, there is no need to change any of the headstage connection types in this dialog.

### If you have a single 8 channel headstage:

If you are using a single HST/8D Gen2 headstage (only a single 8 channel digital headstage) with the OmniPlex System, you need to configure your topology for 16 channels (16 channels is the minimum topology). As displayed in PlexControl, the first 8 channels will contain your data, and the next 8 channels will display all zeros.

### If you have a 16 channel OmniPlex System:

If you have a 16 channel OmniPlex System, the **Device Settings** dialog assumes you have a single 16 channel headstage. In that case it is not necessary to manually set these options for each port.

### Working with headstages

You *must* stop data acquisition on the OmniPlex System before changing the headstage options, as well as before connecting or disconnecting digital headstages from the DHP unit.

**CAUTION**

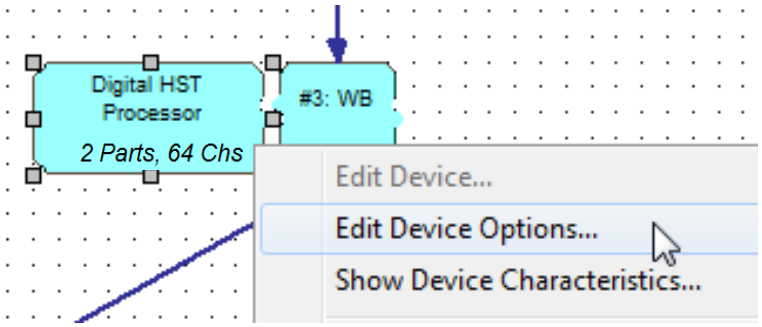
**Stop data acquisition before connecting or disconnecting headstage cables**

Do not connect or disconnect headstage cables from the DHP unit, or from the digital headstage while data acquisition is occurring. Doing so will create invalid signals if you then reconnect the cables or headstages. See Section 3.10, “Stopping Data Acquisition” on page 103.

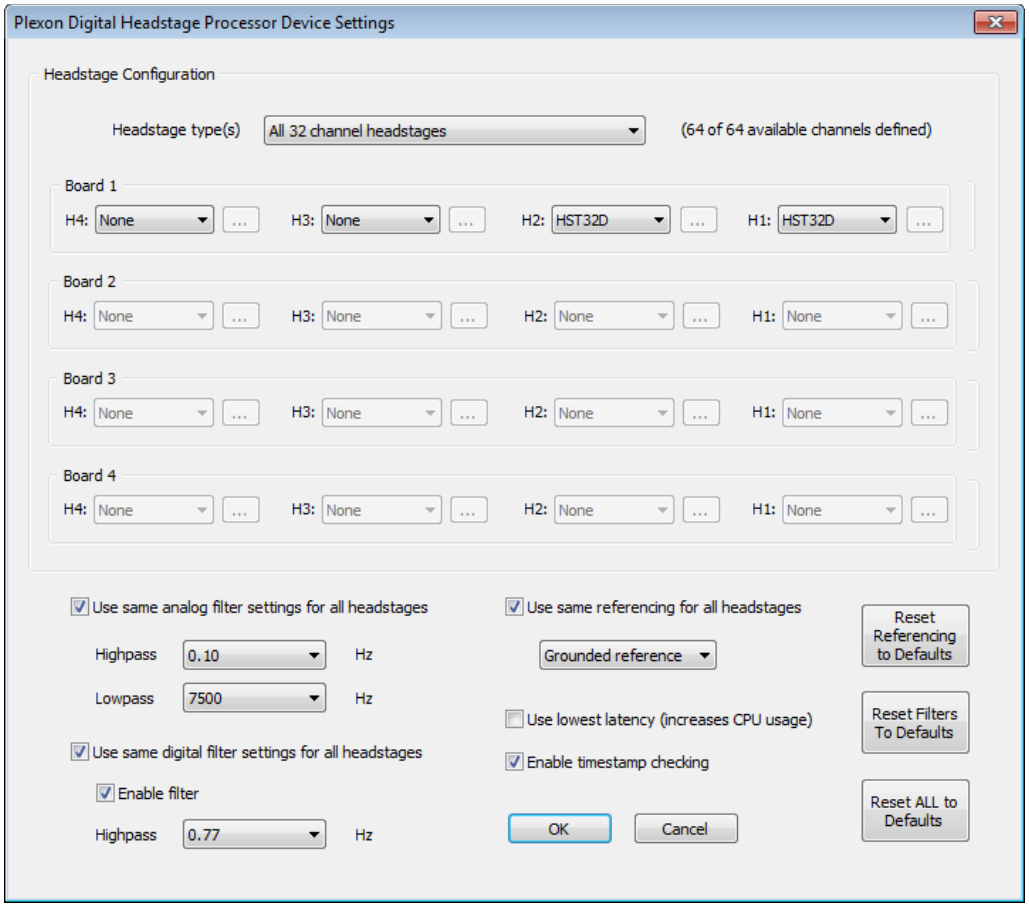
See Section 3.6, “Working with Headstages and the DHP Unit” on page 91 for more details about digital headstages.

## 3.4 Specifying Digital Headstage Types

1 To display the current DHP device options, make sure that data acquisition is first stopped (see Section 3.10, “Stopping Data Acquisition” on page 103), then right click on the **Digital HST Processor** device in the topology and select **Edit Device Options**.

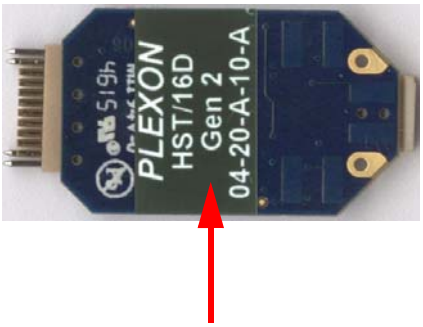


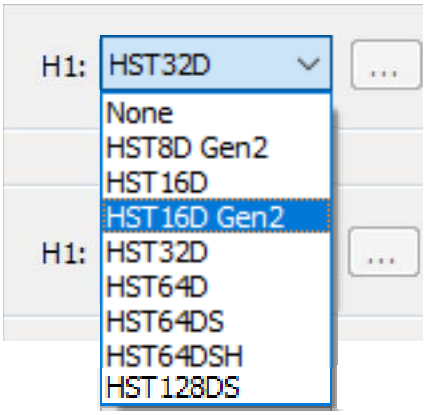

The system displays the **Plexon Digital Headstage Processor Device Settings** dialog:

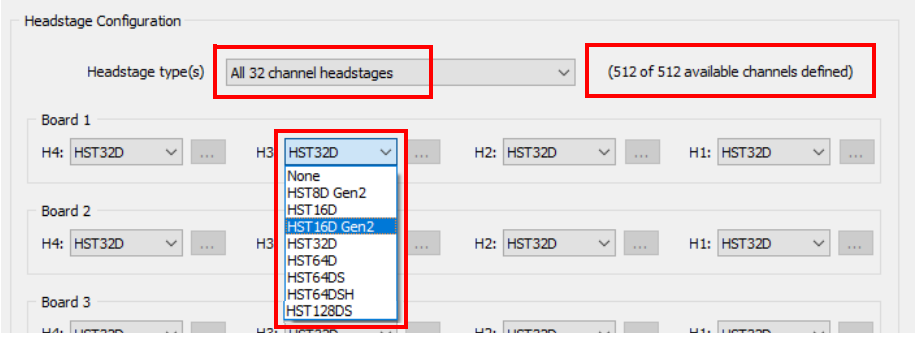


2 The system default assumes that all headstages for an installed board are of the 32 channel type. Set the configuration on each of the ports by using the individual dropdown lists.

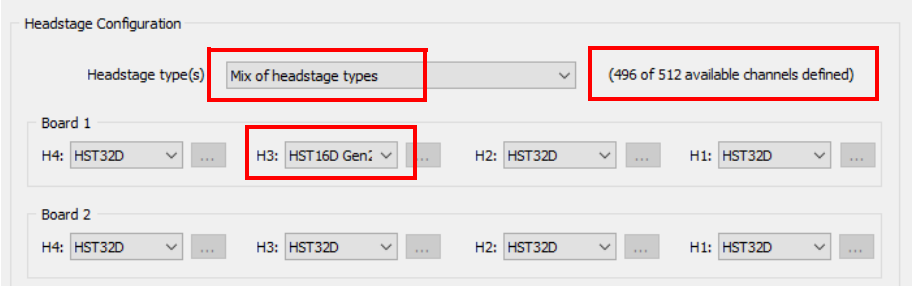
**Note:** Selecting **HST16D** vs. **HST16D Gen2**—If you have a 16 channel headstage, look at the label marked on the headstage. If the label includes the letters “Gen 2” you should select **HST16D Gen2** from the dropdown list. Otherwise, select **HST16D**. See the example below.




In the above example, when you change headstage H3 from HST32D to HST16D Gen2, the summary display at the top changes accordingly:




You can use any combination of 8, 16, 32, 64 and 128 channel digital headstages whose channel counts add up to the number of channels in your topology (pxs file). For example, a 128 channel topology could use one HST128DS, or two HST64D, or one HST64D plus one HST32D plus two HST16D Gen2 headstages. The only additional constraints are as follows:

- When a 128 channel headstage is used, it must be plugged into Port 1, the rightmost port on a DHP signal board when viewed end-on, and no other headstages can be plugged into the same board.

- When a 64 channel headstage is used (HST64D, HST64DS or HST64DSH), it can only be plugged into every other port, starting from the rightmost port (Port 1) on each board. Even though the 64 channel headstage physically plugs into a single port, you should think of it as "occupying" two adjacent ports on the DHP. In other words, **if an HST64D is plugged into Port 1, do not plug any headstage into Port 2; if an HST64D is plugged into Port 3, do not plug any headstage into Port 4.**

**TIP**

**Dropdown lists identify ports that have the 64 and 128 channel options**

Consistent with the use of 64 channel headstage as described above, the dropdown lists for Ports 1 and 3 (labeled H1 and H3) display the 64 channel option (HST64D, HST64DS or HST64DSH), but the dropdown lists for Ports 2 and 4 (labeled H2 and H4) do not.

Consistent with the use of the 128 channel headstage as described above, the dropdown list for Port 1 (labeled H1) displays the 128 channel option (HST128DS), but the dropdown lists for Ports 2, 3 and 4 (labeled H2, H3 and H4) do not.

When you start data acquisition in the OmniPlex System, an error message will be displayed if an incorrect headstage configuration is detected. Refer to the Server message window for the specific board and port on which the error was detected.

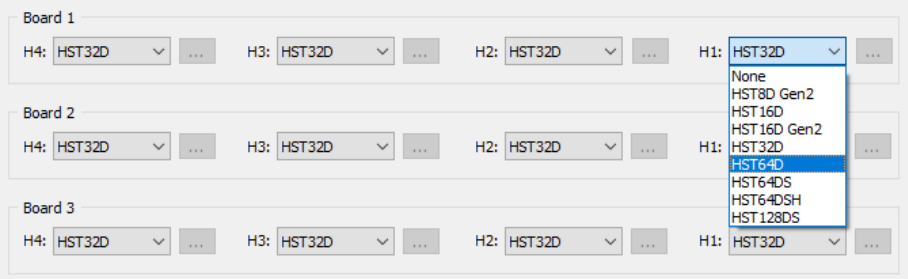
**CAUTION**

**Ensure firmware is up to date for HST128DS**

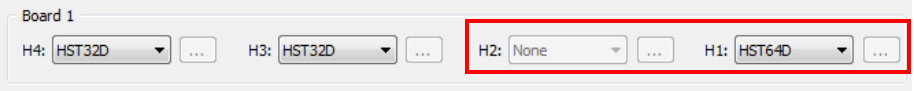
Server will display a warning if you need to update your DHP firmware (to DADH firmware version 2.9) to support HST128DS. See Appendix K: Firmware Upgrade for DHP Unit on page A-84 for more information on updating the DHP firmware.

### Example—Modifying the headstage assignments (HST32D and HST64D)

To change the headstage on Board 1, Port 1 from 32 to 64 channels, begin by selecting **HST64D** in the dropdown list for Port 1 on Board 1.

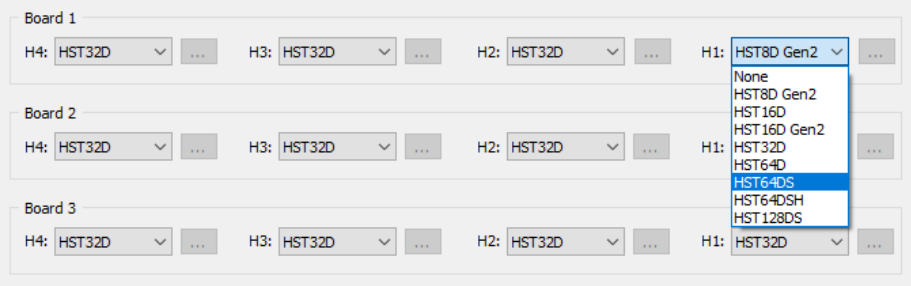


Once you have selected a 64 channel headstage, note that the controls for the adjacent port are set to **None** and are disabled to indicate that the adjacent port is not available:




### Headstage assignments for HST64DS and HST64DSH

The 64 channel digital headstages, HST64DS and HST64DSH, are "thumbtack" designs which use Samtec™ connectors (hence the "S"); the "H" version differs in that its connector is oriented horizontally.




### Headstage assignments for HST128DS

In the device options dialog, select HST128DS from the dropdown list for port H1. Note that when you do this, the headstage selection dropdowns for the remaining three ports on the board are grayed out to indicate that the HST128 is "using" the full 128 channel capacity of the board.

You can configure up to a total of four 128 channel headstages, one per DHP signal board, with each occupying the rightmost port on a board. You can use any combination of 8, 16, 32, 64 and 128 channel headstages on a single DHP, as long as you have the required number of DHP signal boards.

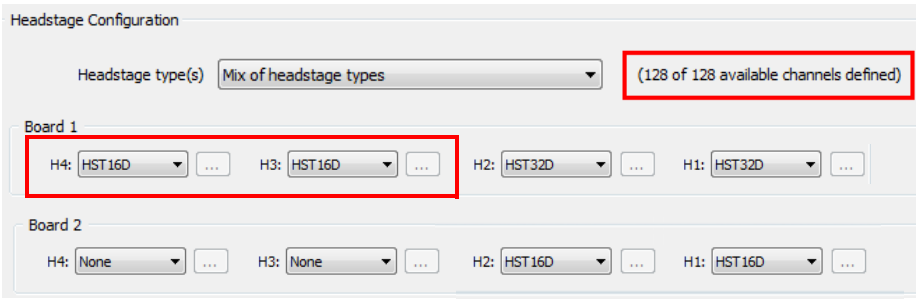
**TIP**

**Additional device settings—filtering, referencing, latency**

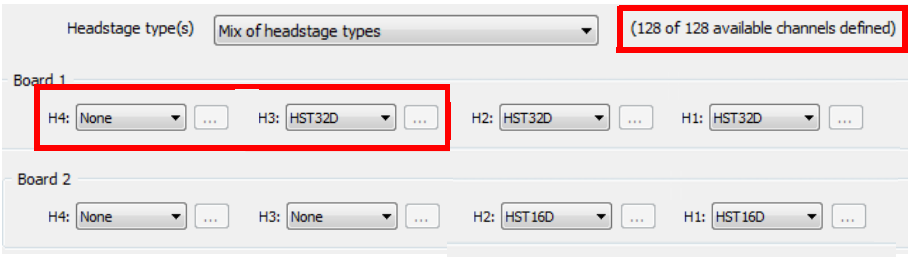
If you need to make adjustments to the default headstage settings in the **Plexon Digital Headstage Processor Device Settings** dialog, such as filter cutoffs, referencing and latency, see Appendix D: DHP Device Settings—Filtering, Referencing and Latency on page A-22.

## 3.5 Port and Channel Assignment Guidelines

As a general rule, it is recommended that you assign your headstages as shown below, with all the headstages of a given channel count grouped together in consecutive port positions.




However, the system allows considerable flexibility, and configurations such as the following are also acceptable:




When the above headstage channels are viewed in the OmniPlex user interface, the correspondence between headstage channels and OmniPlex channels, e.g. WB001 – WB128, is determined by the following rules:

- The rightmost (lowest-numbered) headstage on the topmost (lowest-numbered) board corresponds to the lowest-numbered channels.
- Channel numbers then increase with increasing port number (right to left) and increasing board number (top to bottom).
- Any ports that are set to **None** have no effect on the channel numbering. In the above example, the channel numbering would be as follows:

  Board 1, H1: channels 001 - 032

  Board 1, H2: channels 033 - 064

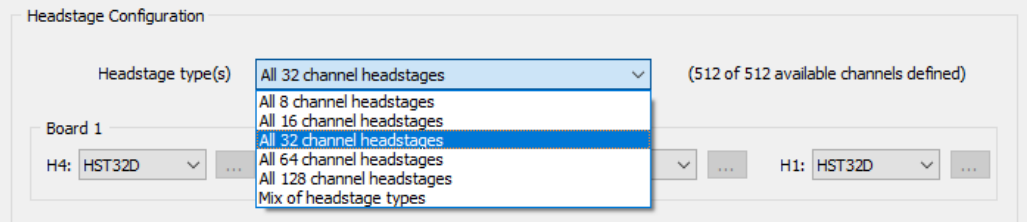
  Board 1, H3: channels 065 - 096

  Board 2, H1: channels 097 - 112

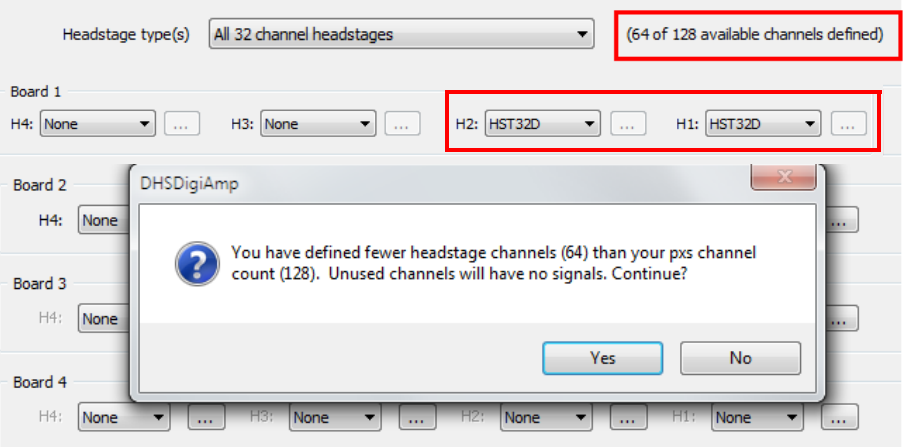
  Board 2, H2: channels 113 - 128

You may have noticed that **Headstage type(s)** in the summary at the top of the dialog is a dropdown control. It changes automatically depending on the type of

headstages you assign, but you can also use it as a shortcut command. If you set it to **All 8 channel headstages**, **All 16 channel headstages**, **All 32 channel headstages, All 64 channel headstages**, or **All 128 channel headstages**, the headstage configuration will be reset to use only that type of headstage, with the appropriate number of headstages defined for the current pxs channel count.




Note that you can configure fewer headstage channels than the available number of channels defined in the pxs; unused channels will display no signals when viewed in the OmniPlex user interface. However, if you do so, the system will warn you when you click **OK** to exit the **Device Settings** dialog:




If you click **Yes**, in this example you will still have a 128 channel configuration, but signals will only appear on the first 64 channels. If you click **No**, you will return to the **Device Settings** dialog where you can assign additional headstages to fill out the available channel count.

When you created a new topology (pxs file) or loaded an existing pxs file, the DHP device options were automatically set to default settings that are suitable for most uses. However, if you want to change the headstage highpass and lowpass filter cutoff frequencies, or make adjustments to the referencing or latency default settings, see Appendix D: DHP Device Settings—Filtering, Referencing and Latency on page A-22.

**TIP**

**Additional device settings options**

If you need to make adjustments to the default headstage settings in the **Plexon Digital Headstage Processor Device Settings** dialog, such as filter cutoffs, referencing and latency, see Appendix D: DHP Device Settings—Filtering, Referencing and Latency on page A-22.

## 3.6 Working with Headstages and the DHP Unit

### Headstage selection and specifications

Please refer to the Plexon *Digital Headstage Technical Guide* and the *Headstages Data Sheet* for specifications and additional information about the digital headstages.

### Important note on connecting and disconnecting digital headstages

An important difference between the DHP subsystem and the DigiAmp subsystem is that with the DHP subsystem, **you should only connect and disconnect cables from the DHP unit, or from the digital headstage, when data acquisition is stopped in the OmniPlex application.** Typically, unplugging a headstage or headstage cable during data acquisition will only cause loss of signal on the corresponding channels, but if you attempt to reconnect a disconnected headstage during acquisition, the corresponding channels will display invalid signals until data acquisition is stopped and restarted, which re-establishes the digital communication link to the headstages. In any case, headstages other than the one being disconnected or reconnected are not affected and their data acquisition continues normally.

**CAUTION**

**Stop data acquisition before connecting or disconnecting headstage cables**

Do not connect or disconnect headstage cables from the DHP unit, or from the digital headstage while data acquisition is occurring. Doing so will create invalid signals if you then reconnect the cables or headstages. See Section 3.10, “Stopping Data Acquisition” on page 103.

However, you can disconnect the headstage itself from the source of analog signals (e.g. the electrodes) without affecting the digital communications with the DHP unit. In other words, the input to the headstage is analog; but everything else (headstage-to-cable connection, cable-to-DHP connection) is digital. Another way of thinking of this is that only the very first connection in the chain, typically “at the animal,” can be changed without first stopping data acquisition.

### Other digital headstage considerations

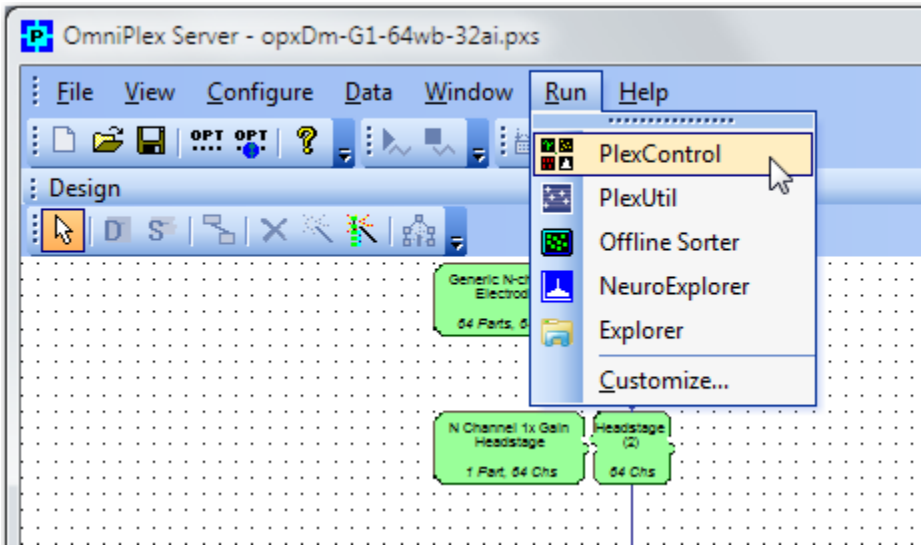
Note that the digital headstages use a fixed gain, and so there is no need to adjust gain or match gain depending on headstage type as with previous OmniPlex Systems. The maximum allowed input voltage at the headstage is 10mv peak-to-peak (mV pp); voltages slightly exceeding this, up to approximately 12mV, can be applied to the headstage without causing A/D clipping but are not recommended for best results. Note that if you find that unwanted large-amplitude low frequency signals or DC drift at the electrode are causing the input voltage to exceed this range, you may be able to reduce this by raising the cutoff frequency of the headstage analog highpass (low-cut) filter, as described in Appendix D: DHP Device Settings—Filtering, Referencing and Latency on page A-22.

## 3.7 Starting PlexControl

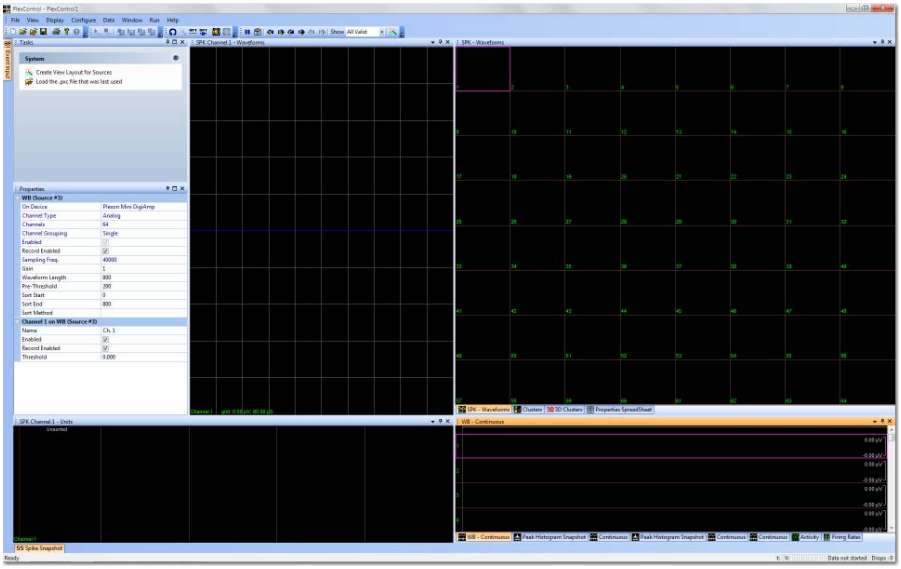
This section assumes that you have already started Server and set up your digital headstage ports, as described in the previous sections.

1 Start PlexControl either by double-clicking its desktop shortcut:

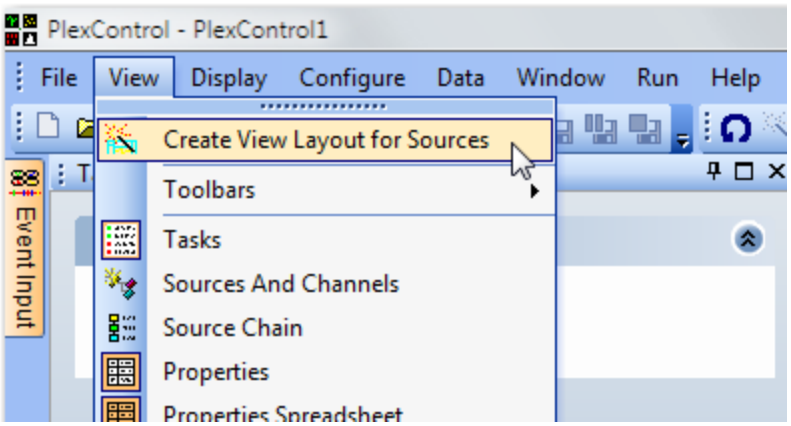
or select **PlexControl** from the **Run** menu in Server:



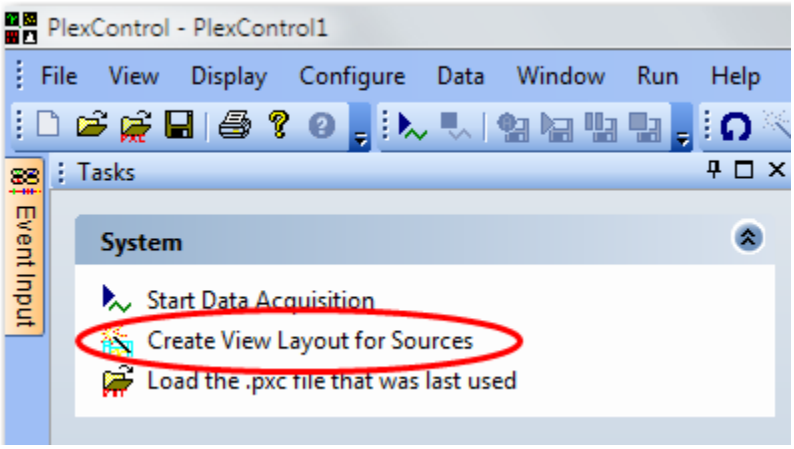

2 The PlexControl application window is displayed. It should look something like this:



If you would prefer to restore the display to the default view, select **Create View Layout for Sources** from the **View** menu.




or from the Tasks view at upper-left:




You can do this at any time to restore the user interface layout to a default state, without affecting any of the actual parameters for acquisition, sorting, recording, etc. In other words, it is a purely cosmetic operation.

**TIP**

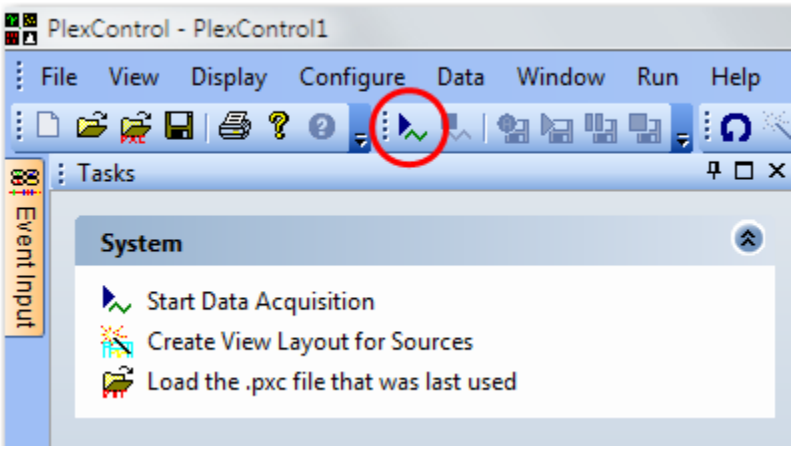
**Do not perform Create View Layout for Sources while recording**

Due to the amount of system activity involved in recreating all the views from scratch, especially on systems with high channel counts, it is recommended that you not perform **Create View Layout for Sources** while recording data.

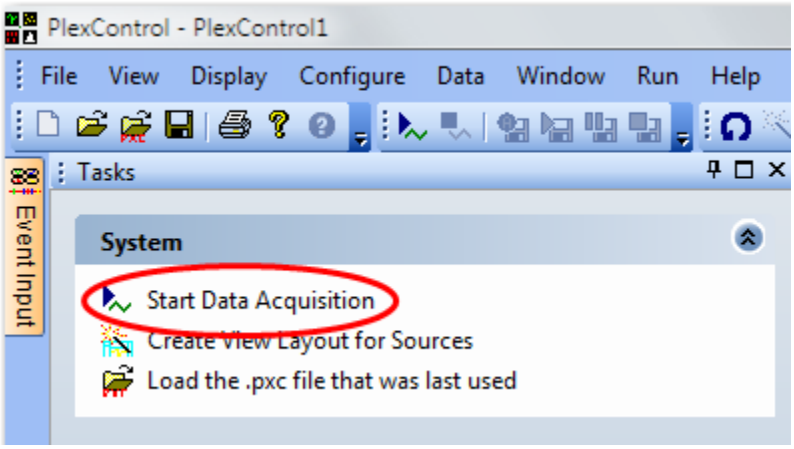
## 3.8 Starting Data Acquisition

This section explains how to start data acquisition.

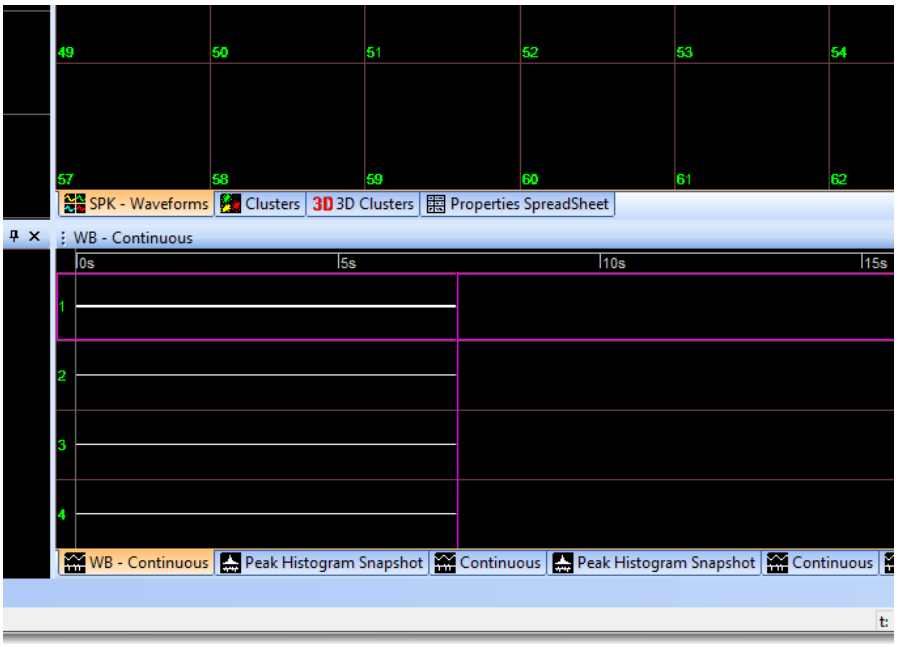
1 Click **Start Data Acquisition**, either in the main toolbar:




or in the Tasks view:



2 After a few seconds you should see signal traces being drawn in the view labeled “WB - Continuous” in the lower-right part of the window:




”WB - Continuous” refers to continuously-digitized signals from the WB source. The vertical colored sweep line shows the current position; once it reaches the far right end of the window, it “wraps around” to the leftmost position and overwrites the oldest data in the view. The time labels on the horizontal axis indicate relative time for this specific view only; later you will learn how to adjust the horizontal sweep speed and other viewing parameters.

**TIP**

**Some settings can be changed only when data acquisition is stopped**

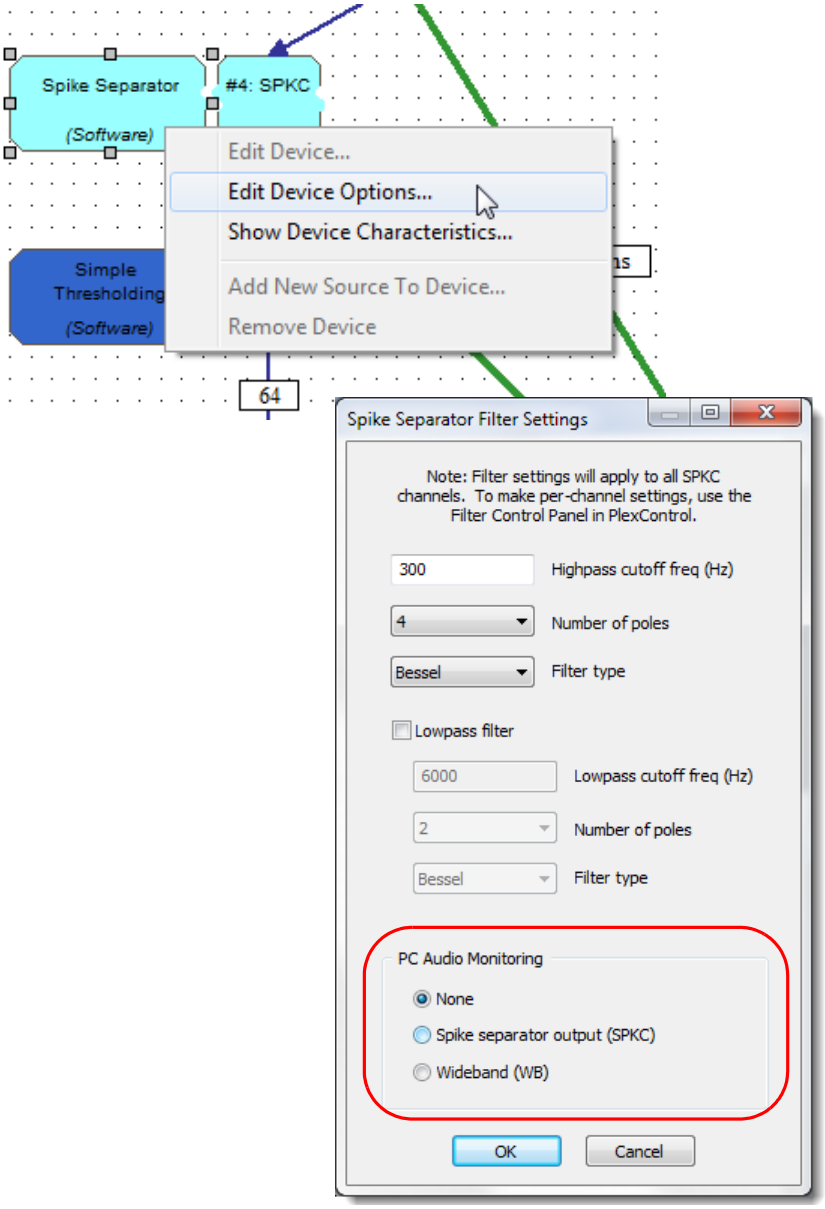
Later, you will see that there are some settings which can only be changed when data acquisition is stopped. For example, the OmniPlex System will not allow you to modify the spike waveform length or pre-threshold length, or change the sorting method, while data acquisition is running. In such cases, simply stop data acquisition, perform the desired operation, then start data acquisition again. See the instructions on stopping data acquisition, Section 3.10, “Stopping Data Acquisition” on page 103.

### Playing a test audio file

3 Before you play a test audio file, you need to ensure that the OmniPlex audio monitoring feature is disabled. Otherwise the OmniPlex System could generate an audio feedback signal that would mix with the intended test signal. Audio monitoring is for live data only.

If you are operating a new OmniPlex System for the first time, the audio monitoring feature is already disabled; this is the factory default setting.

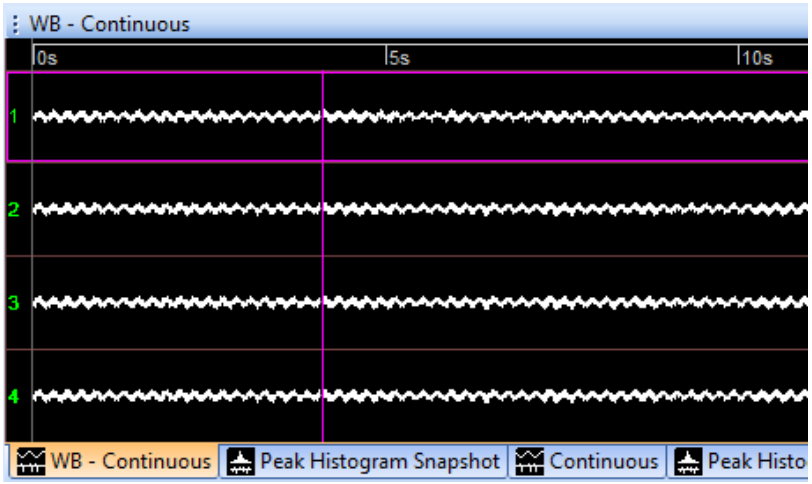
To ensure that the audio monitoring is disabled, right-click on the **Spike Separator** in Server, and select **Edit Device Options**. Then, in the **Device Options** dialog, ensure **PC Audio Monitoring** is set to **None**. If necessary, set it to **None**. Then click **OK**.




**Note:** You can enable this feature later when you are working with live data.

4 Start playback of the test audio file by double-clicking on its desktop shortcut. (Test audio files are available on the Plexon website under **Support | Software Downloads | Data Samples**.)

5 Depending on the signal level from the PC's audio output, some activity may now be visible on the traces in the WB - Continuous view. The colored rectangle identifies the currently-selected channel within the wideband source, by default channel 1; selection of sources and channels within sources will be described later.




If you still see only completely flat traces in the WB Continuous view (as in Step 2), you may wish to recheck that audio is being played into the HTU. If you need to troubleshoot, here are a few things you can try.

**WARNING**

**Be aware of safe audio level**

Be aware that the sound level at this point in the procedure is at maximum (from Step 15 on page 72). If you are using a headphone set or unusually powerful speakers, consider turning down the sound level temporarily during this audio test.

5a If you are using a USB audio interface—Go to Windows **Control Panel > Sound**, and ensure the **USB Audio Device** is set as the default device. If the USB Audio Device is not displayed at all, open Device Manager and ensure the USB Audio Device is enabled. Also make sure you don't have the audio muted on the PC.

If there is still no signal in the WB - Continuous display, you can verify the audio file is being transmitted by the interface and through the 1/8" audio cable. To do this, attach the end of the audio

cable to a speaker, or plug a headphone set into the audio outlet of the interface. Listen for the sound of the audio file.

**5b** If you are using the PC system audio—Briefly unplugging the audio cable from the PC's headphone or line out jack should cause the audio file to be heard from the PC speaker. If not, make sure you don't have audio muted on the PC

Some PCs have both front panel and rear panel audio input and output jacks, and you can use whichever is more convenient. However, in rare cases, you may find that one output jack provides a noticeably cleaner audio signal than the other, and using that output will make working with the test audio file easier..

**TIP**

**Play the audio file with continuous repeat selected**

For convenience, set the audio to repeat continuously while you are performing the above step.

**5c** If you have verified that the PC is playing back audio correctly, try briefly unplugging the audio cable from the HTU, which will usually cause a brief noise transient to appear in the wideband trace display. If not, check to make sure all the cables are firmly and evenly seated:

— Audio cable and headstage connected to the HTU
— Headstage cable(s) seated fully into the DHP connectors at one end, and connected to the headstage at the other end

Also verify the HTU jumpers are in the proper positions, as described in Section 3.1, “Connections and Power-up” on page 62.

**5d** If none of the above steps locate the problem, try using a different audio cable (1/8” stereo). If you have a USB audio interface, move it to a different USB port on the PC.

**Note:** If the problem persists, contact Plexon support at +1 214-369-4957 or support@plexon.com.

**TIP**

**Using “zoom” and “magnification” for a better view**

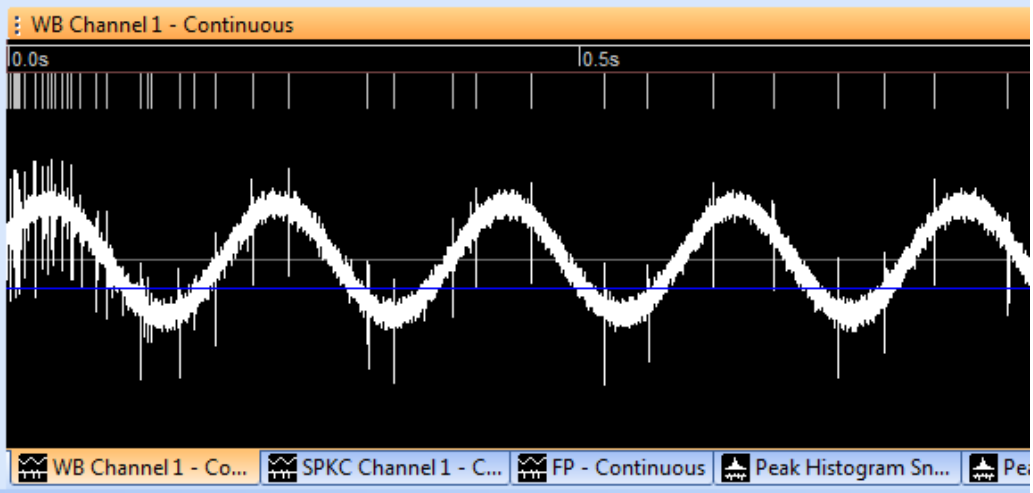
Zoom and magnification are two functions that you will find useful for viewing signals.

For historical reasons, the OmniPlex System term “zoom” is used to describe the process of double clicking on a particular channel so this single channel expands to fill an entire view. See Section 4.4.1, “Using the Zoom Feature” on page 120.

You can also use the “magnification” function, which is the OmniPlex System term for viewing a display at varying degrees of enlargement or reduction, for example, 1.5 times or 0.8 times the default height. See Section 4.4.2, “Changing the Magnification” on page 121.

## 3.9 Separating Wideband Signal into Field Potentials and Spikes

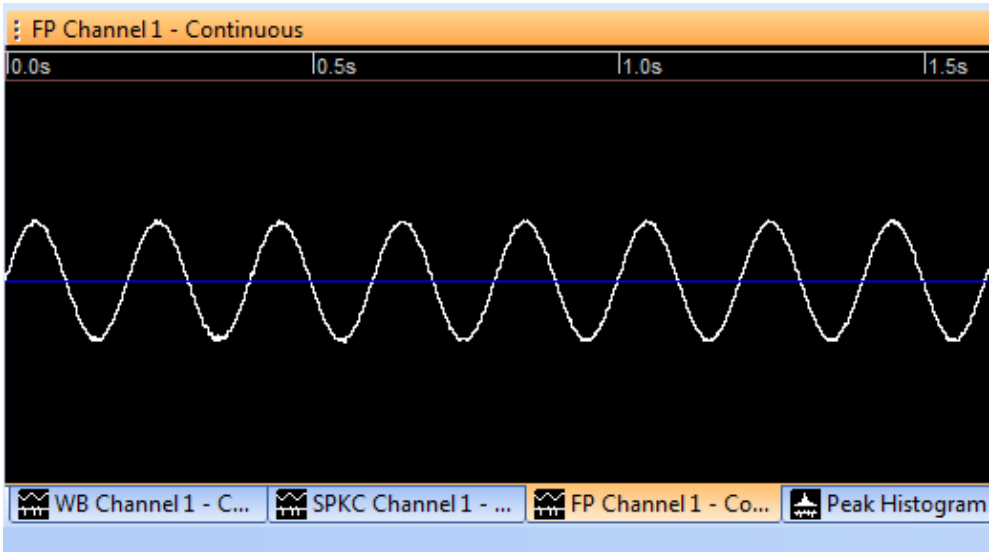
So far, you have been working with the wideband signal (WB source), which after preamplification (analog gain), is digitized at a sampling rate of 40 kHz. The digitized wideband signal for each channel contains field potentials at lower frequencies plus a spike-band signal at higher frequencies. Since the field potentials are typically of a much larger amplitude than the spikes, the net effect is of spikes “riding on the waves” of field potentials:




In live neural signals, the spike amplitudes are often small compared to the field potential amplitudes (even smaller than what is shown in the sample image above).

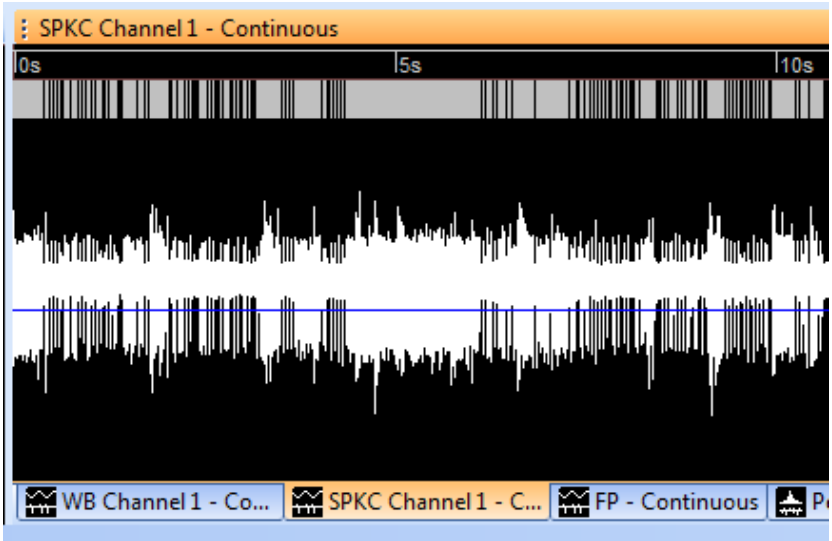
The OmniPlex System uses software DSP filters in Server to separate the WB signal into its two primary components:

- Lowpass filtering with a cutoff of approximately 200-300 Hz yields the field potentials, which are then downsampled to a default sampling rate of 1 kHz (FP source). The downsampled signal can be processed more efficiently and helps reduce the size of recording files. As a rule, the sampling rate must be at least twice the highest frequency component in the signal, and a factor of four or more is preferable.




**Note:** This is an artificial test signal where the “FP component” is a simple low-frequency (5 Hz) sine wave. Real FPs would be more complex low-frequency signals.

- Highpass filtering with a cutoff of approximately 200-300 Hz yields the continuous spike signal (SPKC source), sampled at the same 40 kHz rate as the original wideband signal.



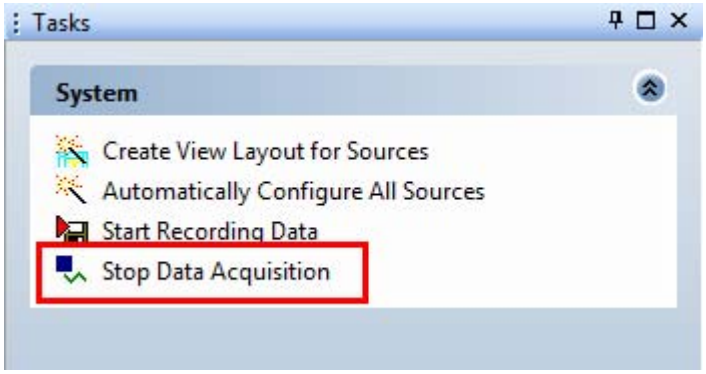
Informally, you can think of removing the field potentials as “flattening the baseline” of the wideband signal; without this flattening, it would be impossible to detect spikes by comparing the continuous signal amplitude against a fixed voltage threshold.

The OmniPlex System allows you to configure the characteristics of the spike and FP separator filters and the downsampling, but for these examples, you will use the default settings. See Appendix B: Separation of Spikes and Field Potentials Using Digital Filters on page A-4 for details on how to change the default settings and some of the tradeoffs involved.

## 3.10 Stopping Data Acquisition

The **Stop Data Acquisition** button is to the right of the **Start Data Acquisition** button:

Or, from the Tasks view, select **Stop Data Acquisition** (which is only displayed while data acquisition is running):




Parameters which you had previously set while data acquisition was running, such as thresholds, etc. will be preserved when you restart data acquisition.

# Chapter 4
# PlexControl User Interface

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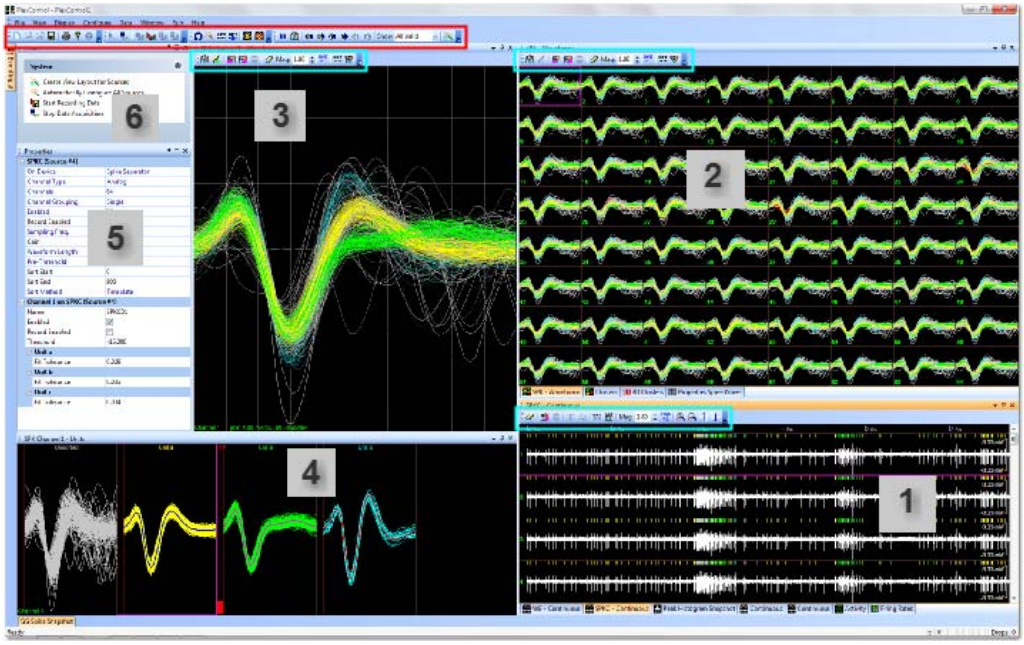
## 4.1 Overview

This chapter provides guidance on using the PlexControl user interface.

To assist you in viewing and interpreting continuous signals (WB, SPKC and FP), it will be helpful to learn a few more things about the PlexControl user interface. Although the examples will focus on continuous signals, most of this information also applies to the other views in PlexControl, such as the ones displaying detected spike waveforms.

After you start data acquisition, your PlexControl user interface should look something like the image below. The spikes may look somewhat larger or smaller - this is not a problem, as long as they aren't clipping.

**Note:** As discussed in the previous chapters, for DigiAmp subsystems you might need to adjust the wideband gain setting to a reasonable value to see spikes clearly in the user interface. If the spikes appear to be clipped, you should reduce the wideband gain to avoid clipping. (This note applies to DigiAmp subsystems only, not to DHP subsystems. In DHP subsystems, the gain is preset and not user configurable.)

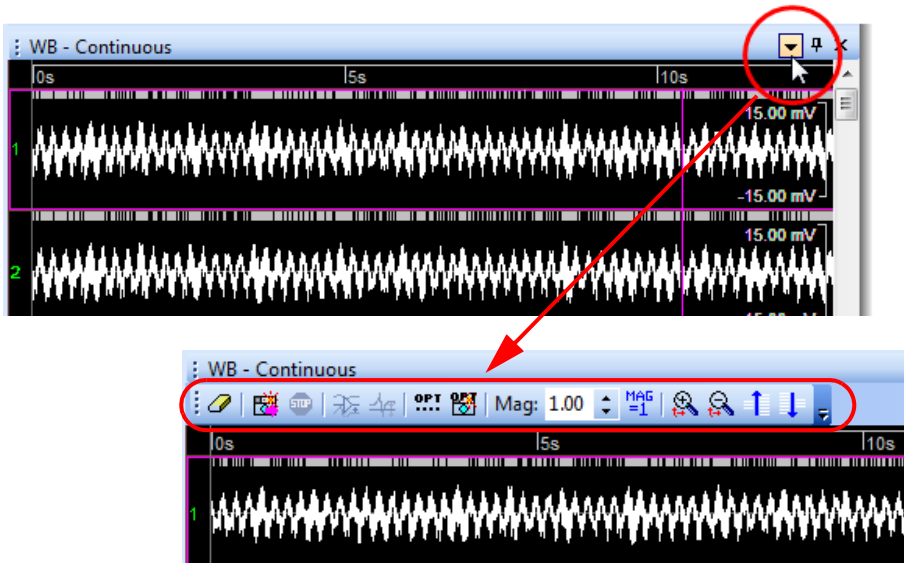



If your layout doesn't look like the image above, select **Create View Layout for Sources** from the **View** menu, or from the Tasks view at the upper-left of the main window, as described in Section 2.3, "Starting PlexControl" on page 38 (if you are using the DigiAmp subsystem) or Section 3.7, "Starting PlexControl" on page 93 (if you are using the DHP subsystem).

**Note:** **Create View Layout for Sources** is in location 6 in the above diagram.

In the default layout (as shown in the image above), PlexControl shows the following items in its views - you will already be familiar with some of them from having worked through the previous tasks:

1. Continuous signals, in multiple tabbed views at lower right.
2. Detected spikes in the multichannel spike view at upper right.
3. An enlarged view of detected spikes for the currently-selected channel in the main spike view at center-left.
4. Sorted units for the currently-selected channel in the units windows at lower left.
5. A Properties view at the far left showing settings and properties of the currently-selected source and the channel within that source. Note that what is displayed in this view, and therefore which parameters you can access from it, depends on which source you have selected, either by clicking on a window displaying that source or by using the source selection button in the toolbar (as described later).
6. A tasks view showing some common high-level commands, such as starting and stopping data acquisition and recording, above the Properties view.
7. A set of global mini toolbars in a row just below the menu bar and title bar at the top of the screen (highlighted in red above).
8. For most of the views, a per-view toolbar which can be toggled on and off (highlighted by blue rectangles in locations 1, 2 and 3 in the image above). These toolbars are hidden by default, but can be easily toggled on and off. For example, in the upper right corner of a view displaying continuous signals, click the down-arrow to display the toolbar:



We previously described how double-clicking on any of the multichannel views causes it to toggle into a single-channel display, and back again. When in multichannel mode (as in the example shown above), each of the displays has a current channel, which is indicated by the colored selection rectangle (frame). Clicking on a channel in a view which is displaying a particular source, for example, an SPK channel within the multichannel spike view, causes that source and channel to be selected and its properties to be displayed in the Properties view at the left side of the window (location 5 in the previous image).

Also, when you select a channel in any of the main sources (WB, SPKC, SPK, or FP), the corresponding channel in all the other multichannel source views is identified with a colored rectangle. For example, selecting SPK014 in the multichannel spike view will cause channels WB014, SPKC014, and FP014 to be highlighted as the current channel within each of their respective multichannel views. However, selecting a channel within a source which does not originate from the DigiAmp, such as digital event (DI) channels and Auxiliary Analog Input (AuxAI) channels, does not affect the current channel within other sources.

Besides single-clicking on a specific channel in any multichannel view to select it, you can select a channel by double-clicking on that channel's row in the **Properties Spreadsheet** or **Extended Properties Spreadsheet**. The currently selected channel's row number is prefixed with “>>” in the spreadsheet.

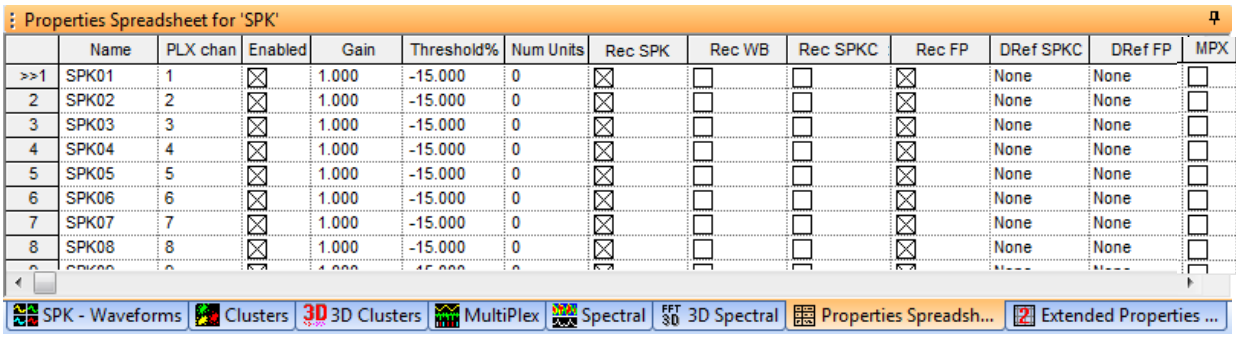
**Note:** To view the **Properties Spreadsheet** or the **Extended Properties Spreadsheet**, select the appropriate tab under the multichannel spike window (location 2 in the previous image). For additional details on the spreadsheets, see Section 4.2, “Properties Spreadsheet and Extended Properties Spreadsheet” on page 108.

You can rearrange the views and nest them within each other (which causes them to appear as a row of tabs) to customize the interface slightly or totally reconfigure it. See Section 13.8, “Advanced User Interface Features” on page 442 for more details. When first learning to use the OmniPlex® System, it is recommended that you not make major changes to the layout, so that it will be easier to follow the examples shown here. However, you may want to make some of the views larger or smaller, depending on what you are working on and the aspect ratio of your monitor. The easiest way to do this is to drag the splitter bar that divides adjacent views, making one larger and the other smaller. As an example, you can enlarge the lower-right view, which is displaying the continuous sources.

## 4.2 Properties Spreadsheet and Extended Properties Spreadsheet

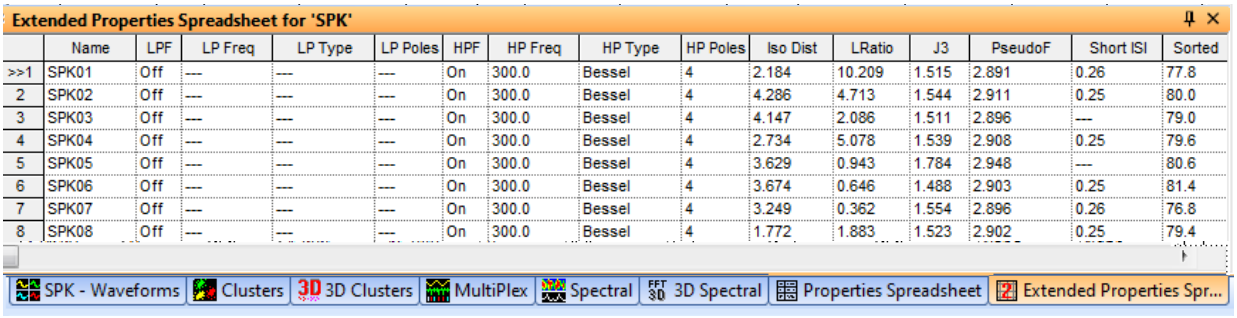
The main Properties Spreadsheet and Extended Properties Spreadsheet display the properties (settings or parameters) for each channel in the currently selected source. Depending on the selected source, the set of properties displayed will vary. Most of properties in the main Properties Spreadsheet can be edited, as described throughout this user guide, while the Extended Properties Spreadsheet is not editable.

**Properties Spreadsheet:**




| | Name | PLX chan | Enabled | Gain | Threshold% | Num Units | Rec SPK | Rec WB | Rec SPKC | Rec FP | DRef SPKC | DRef FP | MPX |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| >>1 | SPK01 | 1 | ☒ | 1.000 | -15.000 | 0 | ☒ | ☐ | ☐ | ☒ | None | None | ☐ |
| 2 | SPK02 | 2 | ☒ | 1.000 | -15.000 | 0 | ☒ | ☐ | ☐ | ☒ | None | None | ☐ |
| 3 | SPK03 | 3 | ☒ | 1.000 | -15.000 | 0 | ☒ | ☐ | ☐ | ☒ | None | None | ☐ |
| 4 | SPK04 | 4 | ☒ | 1.000 | -15.000 | 0 | ☒ | ☐ | ☐ | ☒ | None | None | ☐ |
| 5 | SPK05 | 5 | ☒ | 1.000 | -15.000 | 0 | ☒ | ☐ | ☐ | ☒ | None | None | ☐ |
| 6 | SPK06 | 6 | ☒ | 1.000 | -15.000 | 0 | ☒ | ☐ | ☐ | ☒ | None | None | ☐ |
| 7 | SPK07 | 7 | ☒ | 1.000 | -15.000 | 0 | ☒ | ☐ | ☐ | ☒ | None | None | ☐ |
| 8 | SPK08 | 8 | ☒ | 1.000 | -15.000 | 0 | ☒ | ☐ | ☐ | ☒ | None | None | ☐ |

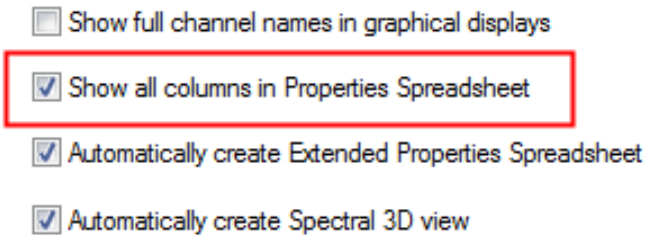
**Extended Properties Spreadsheet**



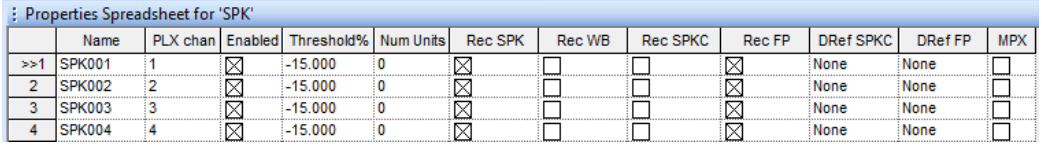

| | Name | LPF | LP Freq | LP Type | LP Poles | HPF | HP Freq | HP Type | HP Poles | Iso Dist | LRatio | J3 | PseudoF | Short ISI | Sorted |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| >>1 | SPK01 | Off | --- | --- | --- | On | 300.0 | Bessel | 4 | 2.184 | 10.209 | 1.515 | 2.891 | 0.26 | 77.8 |
| 2 | SPK02 | Off | --- | --- | --- | On | 300.0 | Bessel | 4 | 4.286 | 4.713 | 1.544 | 2.911 | 0.25 | 80.0 |
| 3 | SPK03 | Off | --- | --- | --- | On | 300.0 | Bessel | 4 | 4.147 | 2.086 | 1.511 | 2.896 | --- | 79.0 |
| 4 | SPK04 | Off | --- | --- | --- | On | 300.0 | Bessel | 4 | 2.734 | 5.078 | 1.539 | 2.908 | 0.25 | 79.6 |
| 5 | SPK05 | Off | --- | --- | --- | On | 300.0 | Bessel | 4 | 3.629 | 0.943 | 1.784 | 2.948 | --- | 80.6 |
| 6 | SPK06 | Off | --- | --- | --- | On | 300.0 | Bessel | 4 | 3.674 | 0.646 | 1.488 | 2.903 | 0.25 | 81.4 |
| 7 | SPK07 | Off | --- | --- | --- | On | 300.0 | Bessel | 4 | 3.249 | 0.362 | 1.554 | 2.896 | 0.26 | 76.8 |
| 8 | SPK08 | Off | --- | --- | --- | On | 300.0 | Bessel | 4 | 1.772 | 1.883 | 1.523 | 2.902 | 0.25 | 79.4 |

## 4.2.1 Option to Show All Columns in Properties Spreadsheet

An option in the PlexControl **Global Options** dialog allows the main Properties Spreadsheet to be displayed in a reduced format, where controls for sources related to the currently selected source are not shown. The default is to show all columns.

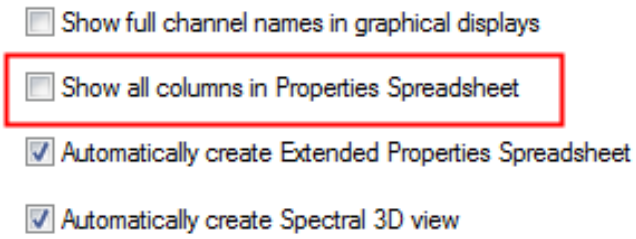



For example, if the SPK (spikes) source is currently selected, the Properties Spreadsheet will look like this when “all columns” are displayed. Note that columns are shown for sources related to SPK: WB (wideband), SPKC (spike continuous) and FP (field potential).

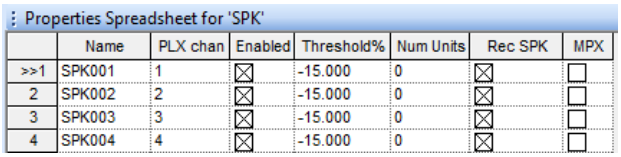



| | Name | PLX chan | Enabled | Threshold% | Num Units | Rec SPK | Rec WB | Rec SPKC | Rec FP | DRef SPKC | DRef FP | MPX |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| >>1 | SPK001 | 1 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ | ☒ | None | None | ☐ |
| 2 | SPK002 | 2 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ | ☒ | None | None | ☐ |
| 3 | SPK003 | 3 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ | ☒ | None | None | ☐ |
| 4 | SPK004 | 4 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ | ☒ | None | None | ☐ |

However, if you uncheck **Show all columns**:



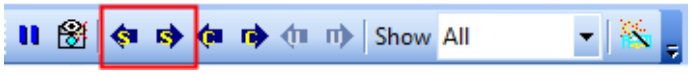

then only columns that control the selected source are displayed, for example:

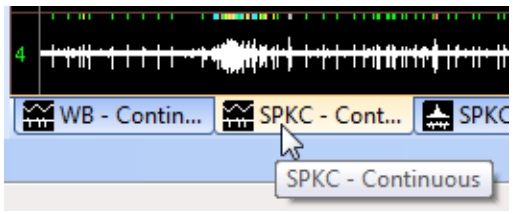



| | Name | PLX chan | Enabled | Threshold% | Num Units | Rec SPK | MPX |
|---|---|---|---|---|---|---|---|
| >>1 | SPK001 | 1 | ☒ | -15.000 | 0 | ☒ | ☐ |
| 2 | SPK002 | 2 | ☒ | -15.000 | 0 | ☒ | ☐ |
| 3 | SPK003 | 3 | ☒ | -15.000 | 0 | ☒ | ☐ |
| 4 | SPK004 | 4 | ☒ | -15.000 | 0 | ☒ | ☐ |

The tradeoff is that the reduced view is less cluttered and more logically consistent, while the original “show all” display gives immediate access to more controls without having to switch to different sources to see them.

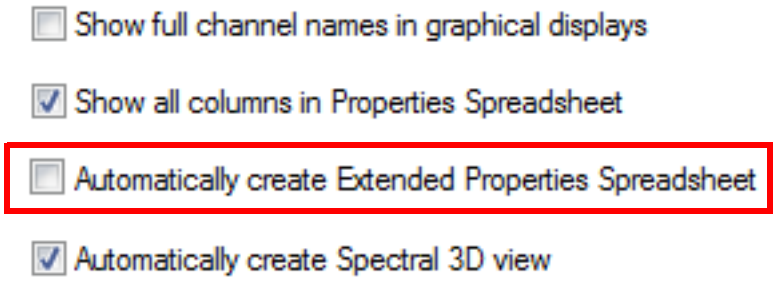
For example, in the above reduced view, you would have to select the SPKC source (either using the Previous Source / Next Source buttons in the main toolbar, or by clicking on the tabbed window displaying the SPKC channels) in order to view and change the Rec SPKC and DRef SPKC options.



### 4.2.2 Creating the Extended Properties Spreadsheet

By default, the system creates the **Extended Properties Spreadsheet** on startup and when a **Create View Layout for Sources** is performed. If you do not want it to be created automatically, deselect the appropriate checkbox on the **General** page of the **Global Options** dialog.




### 4.2.3 Contents of the Extended Properties Spreadsheet

The **Extended Properties Spreadsheet** is a tabular view that is similar in appearance to the standard main **Properties Spreadsheet**. It is used for informational and monitoring purposes, as opposed to adjusting settings such as thresholds or referencing. The digital filter properties of the currently selected source are displayed, and if a spike-related source is selected, spike sorting quality metrics, if any, are displayed in the Iso Dist, LRatio, J3, PseudoF, Short ISI and Sorted columns, as shown here (the red lines were added to emphasize this):

Extended Properties Spreadsheet for 'SPK'

| | Name | LPF | LP Freq | LP Type | LP Poles | HPF | HP Freq | HP Type | HP Poles | Iso Dist | LRatio | J3 | PseudoF | Short ISI | Sorted |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| >>1 | SPK01 | Off | --- | --- | --- | On | 300.0 | Bessel | 4 | 2.184 | 10.209 | 1.515 | 2.891 | 0.26 | 77.8 |
| 2 | SPK02 | Off | --- | --- | --- | On | 300.0 | Bessel | 4 | 4.286 | 4.713 | 1.544 | 2.911 | 0.25 | 80.0 |
| 3 | SPK03 | Off | --- | --- | --- | On | 300.0 | Bessel | 4 | 4.147 | 2.086 | 1.511 | 2.896 | --- | 79.0 |
| 4 | SPK04 | Off | --- | --- | --- | On | 300.0 | Bessel | 4 | 2.734 | 5.078 | 1.539 | 2.908 | 0.25 | 79.6 |
| 5 | SPK05 | Off | --- | --- | --- | On | 300.0 | Bessel | 4 | 3.629 | 0.943 | 1.784 | 2.948 | --- | 80.6 |
| 6 | SPK06 | Off | --- | --- | --- | On | 300.0 | Bessel | 4 | 3.674 | 0.646 | 1.488 | 2.903 | 0.25 | 81.4 |
| 7 | SPK07 | Off | --- | --- | --- | On | 300.0 | Bessel | 4 | 3.249 | 0.362 | 1.554 | 2.896 | 0.26 | 76.8 |
| 8 | SPK08 | Off | --- | --- | --- | On | 300.0 | Bessel | 4 | 1.772 | 1.883 | 1.523 | 2.902 | 0.25 | 79.4 |

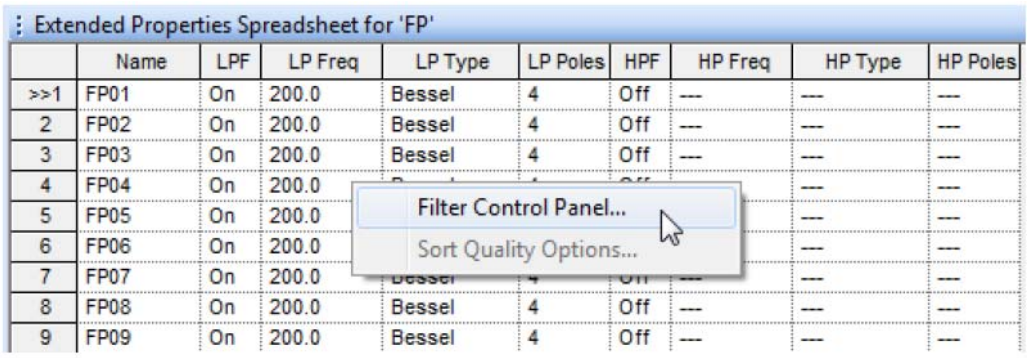
Also note that even though SPK and not SPKC is the selected source in this example, the relevant SPKC filter settings are displayed for convenience. Likewise, when SPKC is selected, the SPK sort quality metrics are displayed. (The sort quality metrics and options are described further in Section 8.10, “Spike Sorting Quality Metrics” on page 290.)

**Note:** Some sources, such as the wideband (WB) and digital input (DI) sources, do not currently display any information besides their channel names in the Extended **Properties Spreadsheet** when they are the currently selected source.

### 4.2.4 Extended Properties Spreadsheet and Filter Control Panel (FCP)

The filter settings shown in the **Extended Properties Spreadsheet** update immediately when you change them in the **Filter Control Panel (FCP)**. This is particularly useful when you are using the FCP to apply different filter settings to different ranges of channels. You cannot edit the filter settings "in place" in the spreadsheet—instead, you edit these settings in the FCP.

You can access the FCP from the right-button popup menu in the spreadsheet, as shown in the image below, and from the **View** menu dropdown list item or **Filter Control Panel** toolbar button ( ).

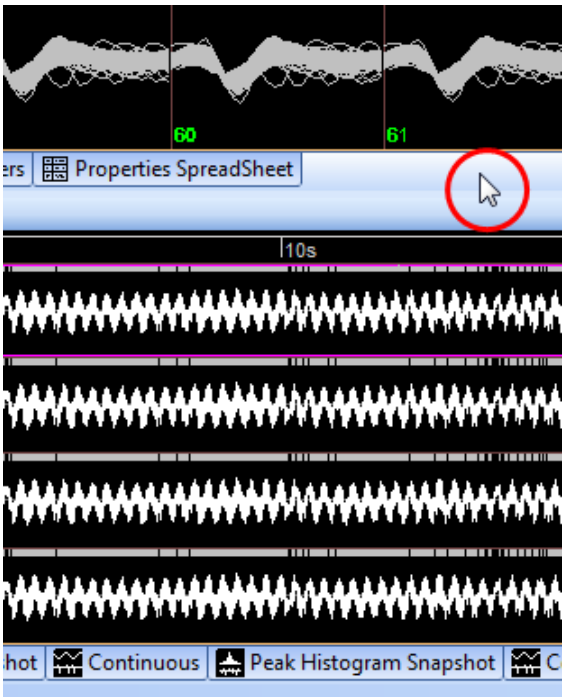


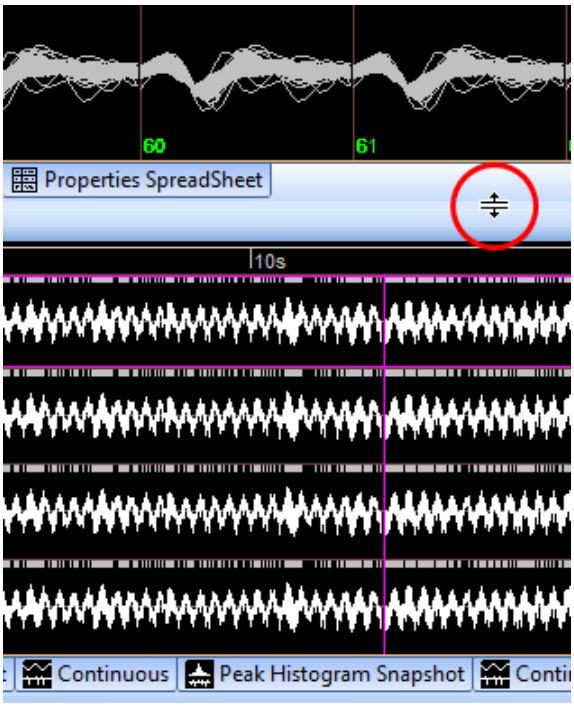

| | Name | LPF | LP Freq | LP Type | LP Poles | HPF | HP Freq | HP Type | HP Poles |
|---|---|---|---|---|---|---|---|---|---|
| >>1 | FP01 | On | 200.0 | Bessel | 4 | Off | --- | --- | --- |
| 2 | FP02 | On | 200.0 | Bessel | 4 | Off | --- | --- | --- |
| 3 | FP03 | On | 200.0 | Bessel | 4 | Off | --- | --- | --- |
| 4 | FP04 | On | 200.0 | | | | | --- | --- |
| 5 | FP05 | On | 200.0 | | | | | --- | --- |
| 6 | FP06 | On | 200.0 | | | | | --- | --- |
| 7 | FP07 | On | 200.0 | | | | | --- | --- |
| 8 | FP08 | On | 200.0 | Bessel | 4 | Off | --- | --- | --- |
| 9 | FP09 | On | 200.0 | Bessel | 4 | Off | --- | --- | --- |

**Note:** The features of the FCP are described in Section 13.5, "Filter Control Panel" on page 420.

## 4.3 Resizing Windows Using the Splitter Bars

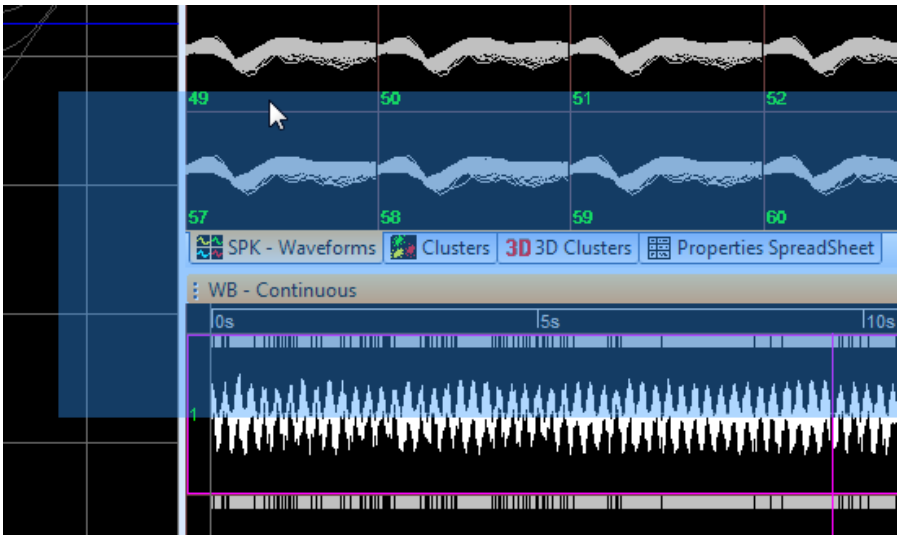
1 Move the mouse cursor to the horizontal bar between views and, without holding down the mouse button, move the mouse up and down across the bar until you see the cursor change from the standard arrow cursor to the splitter-dragging cursor:



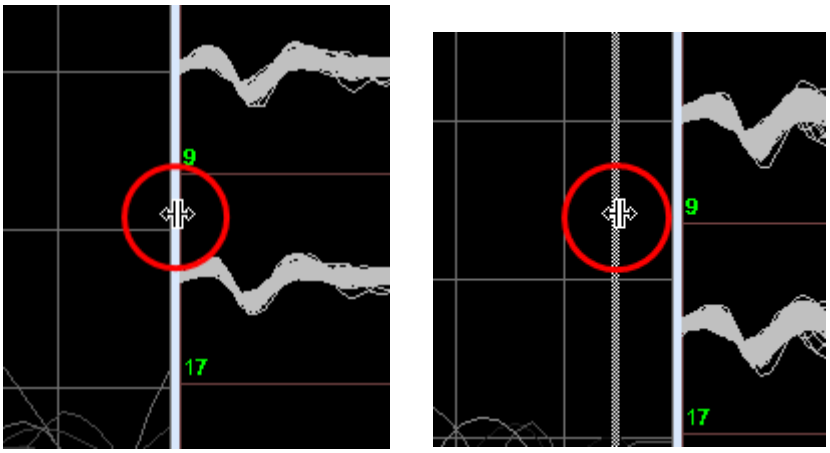



2 When you see the cursor change, hold down the left mouse button and drag to move the divider between the two windows, as shown below. The divider displays as a gray bar while you are dragging it. Release the left button when you are done resizing the windows.

If you accidentally drag the entire window (as shown by a large shaded rectangle being dragged by a normal arrow cursor) instead of dragging the divider bar, you can cancel this by hitting the ESC key and then releasing the mouse button.



3 Similar instructions apply for adjusting the width of a view; in this case a vertical splitter-dragging cursor will appear when you hover over the splitter bar between adjacent views, and you can drag the splitter left or right:




Remember, if you ever have a problem with the window layout, you can always use the **Create View Layout for Sources** menu item to restore everything to the default layout.

**TIP**

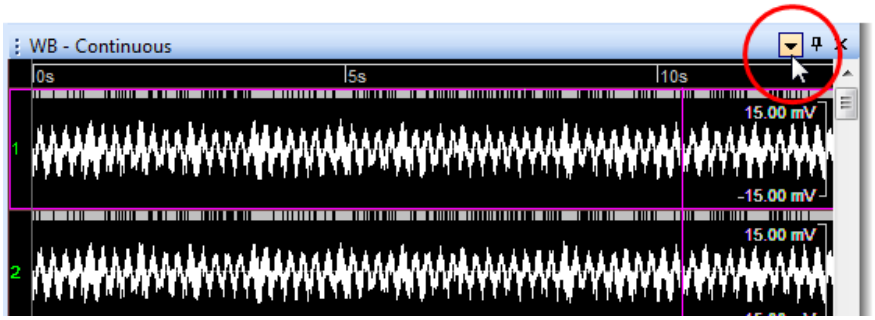
**Adjust text size and channel labels**

The system allows you to show full channel names in multi-channel displays (as opposed to the channel number. only, which is the default). In addition, you can make the font size larger or smaller than the default size (8 point); these options can be used to optimize readability across a range of monitor sizes and system channel counts. To access these options, go to the **General** tab of the **Global Options** page.

## 4.4 Using the View Toolbars and Options

In addition to the main set of toolbars located near the top of the PlexControl main window, several of the views have their own toolbars, for accessing functionality specific to each view. These toolbars are hidden by default, but can be easily toggled on and off.

1 For example, in the upper right corner of a view displaying continuous signals, click the down-arrow to display the toolbar:

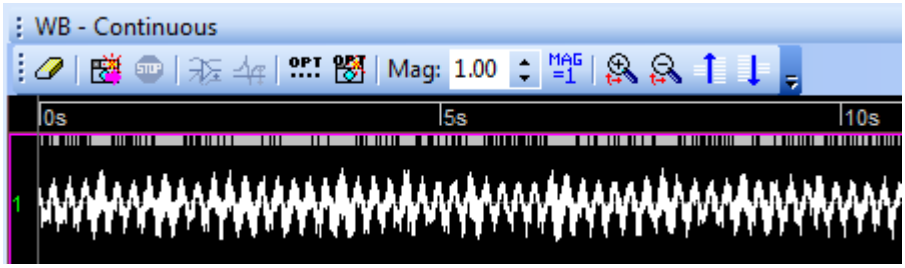


**TIP**

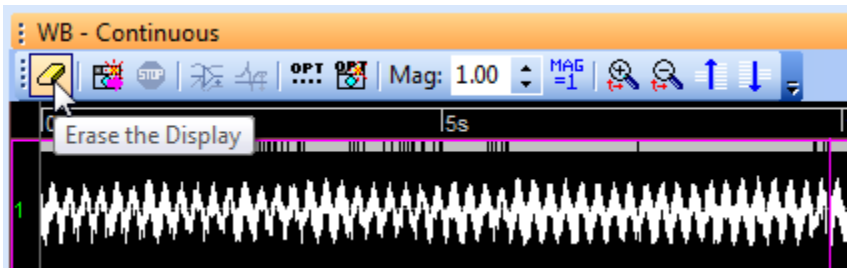
**Double-click to return to multichannel display**

When a view has the focus (indicated by its title bar displaying in orange), you can press the "T" key on the keyboard to toggle its toolbar on and off.

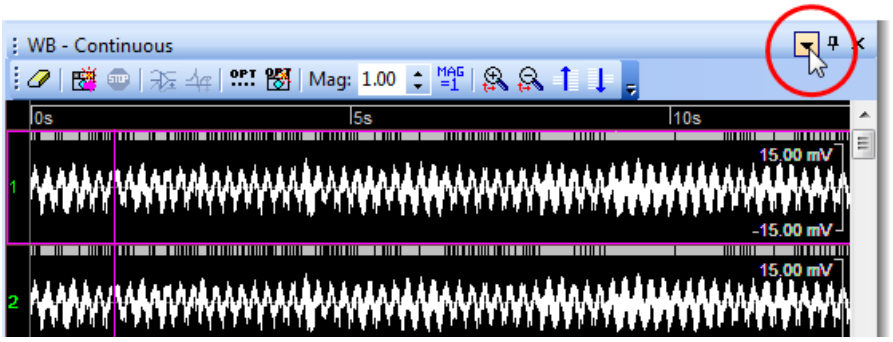
The view's toolbar is displayed:



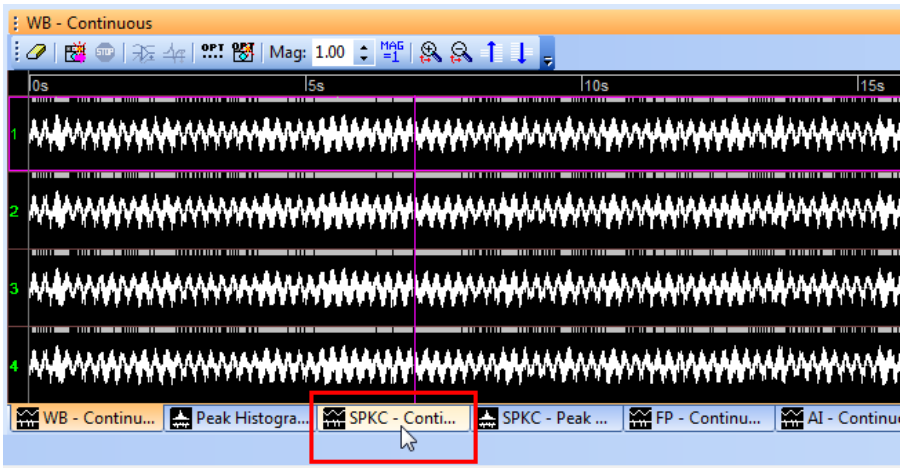

2 You can hover the cursor over each of the buttons in the toolbar to see a tooltip description of the button's function:



3 If you want to hide the toolbar, for example to save vertical display space, click the down-arrow in the upper-right corner again:




4 Certain of the functions available in the continuous-view toolbars are particularly useful in working with the spike-continuous data. For example, since the amplitude of spikes is usually much smaller than the amplitude of the wideband signal, you will typically use the magnification feature more often when working with spikes. To view the spike-continuous (SPKC) view, click on the tab labeled “SPKC - Continuous”:

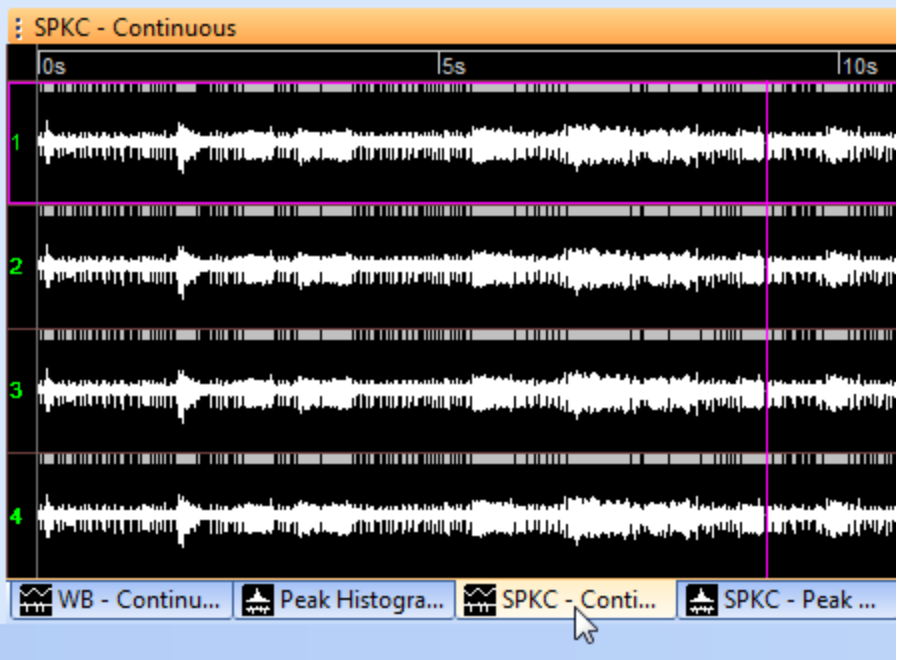


**TIP**

**Click on tabs to display their labels**

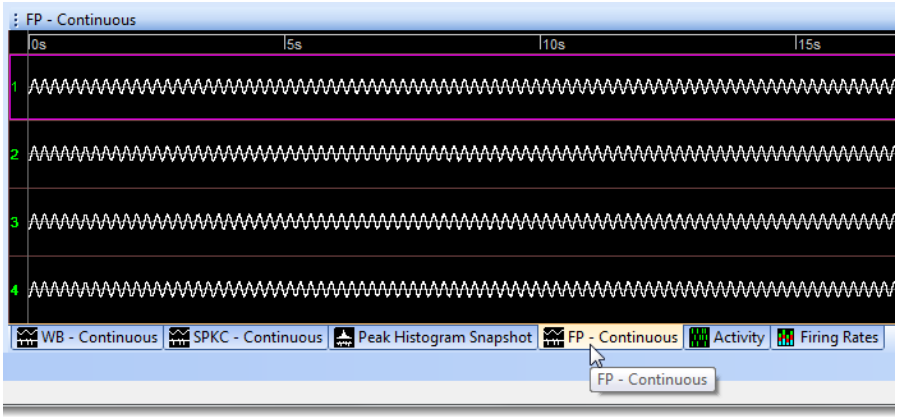
If the tabs are not displaying their source abbreviations (WB, SPKC, etc), you can click on the tabs to cause the source abbreviations to appear. After the first time you click on a tab, it will maintain its labeling.

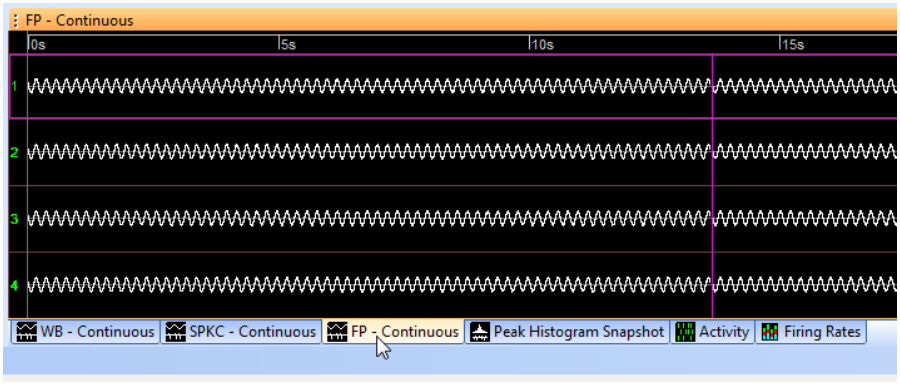
The SPKC - Continuous view is displayed:



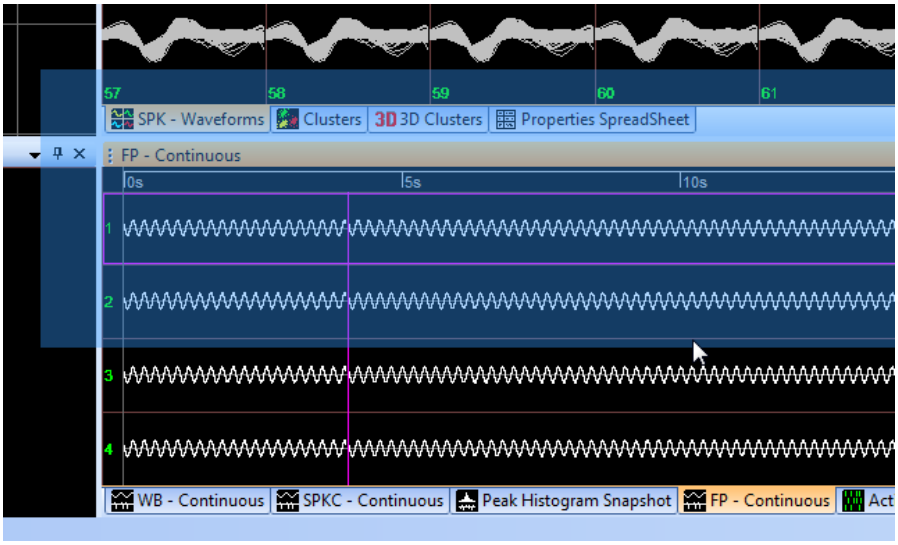

**5** Note that a toolbar is not displayed - this is because each view can have its own toolbar, which can be hidden or displayed. To display the toolbar for the SPKC view, left-click the down arrow at the upper right, as you did with the WB view.

6 If you wish, you can drag the tabs into a different left-to-right order. You can drag tabs to the right or left. For example, to move the FP - Continuous tab next to the SPKC - Continuous tab, place the cursor on the tab that you want to move, hold down the left mouse button, and drag to the left. You will see the tab labels change as the tab you are dragging “steps” left, one tab position at a time. Release the left button when the tab you are dragging reaches the desired position:



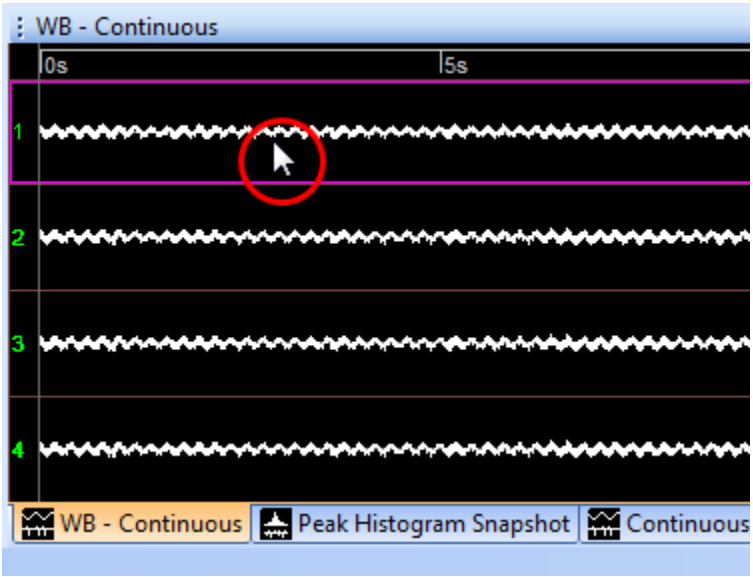
If you accidentally drag the entire view (as shown by a large shaded rectangle being dragged by a normal arrow cursor) instead of dragging the tab, you can cancel this by hitting the ESC key and then releasing the mouse button.



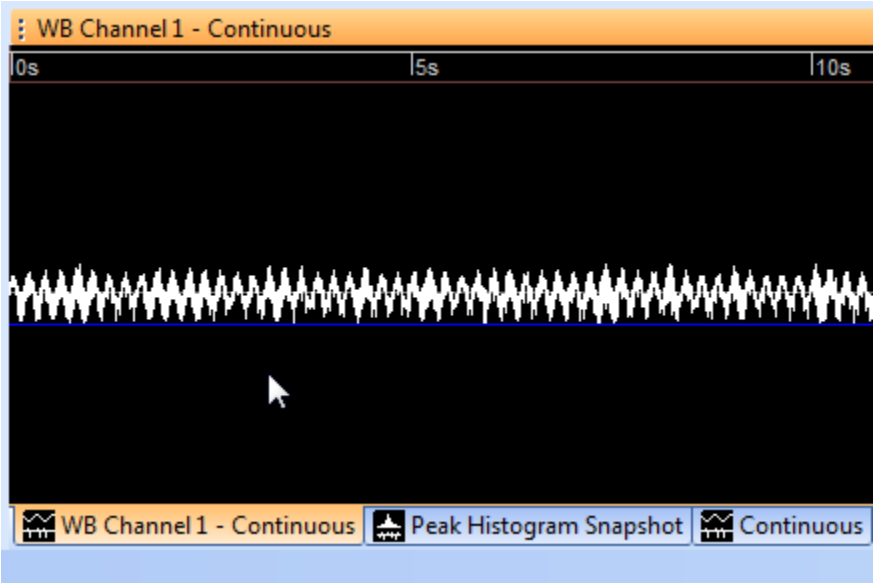

Once you have adjusted the size of the views to your liking, the three most common viewing adjustments that you will make for continuous data are changing the magnification (vertical scaling), the sweep rate, and the number of channels displayed in a view.

### 4.4.1 Using the Zoom Feature

First, to get a better look at the signal from an individual channel, for example Channel 1 in the image below, double-click inside of the first row, labeled “1” at the left.



This will expand that channel's display so that it occupies the entire WB - Continuous view; note that the view's title bar now shows “WB Channel 1 - Continuous” instead of the previous “WB - Continuous”:




If you now double-click on the zoomed-in single channel, it will revert back to the multi-channel view. Before proceeding, make sure the view is zoomed in on channel 1 as shown above.

### 4.4.2 Changing the Magnification

In the OmniPlex System, “magnification” refers to the vertical scaling of the contents of a view, usually either continuous signals or the waveforms of detected spikes. It does not affect the data itself, only your view of it. You can change the magnification by any of the following methods:

- Rolling the mouse wheel
- Typing a new magnification factor into the “Mag:” value in the toolbar
- Clicking on the up/down arrows to the right of the magnification value
- Clicking on the **MAG=1** button to restore the magnification to 1.0

These methods are described below.

Rolling the mouse wheel:

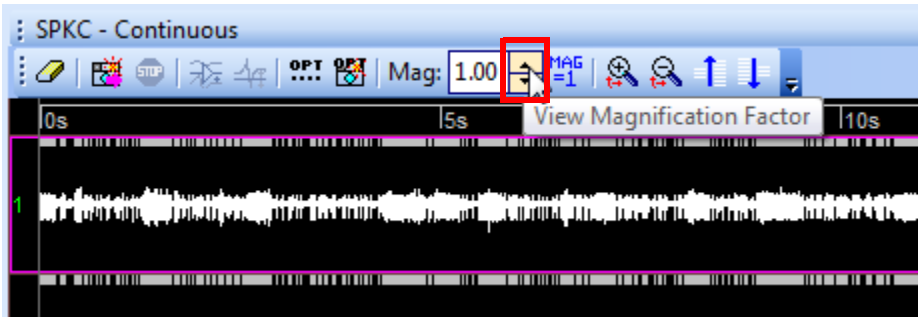
You can use the mouse wheel to adjust the magnification in zoomed (single-channel) spike and continuous views, in the same way as in the 2D and 3D cluster views. Adjusting the magnification in the main spike window (current channel display) will by default also change the magnification in the multichannel spike window. The effect is exactly the same as using the magnification up/down arrows in the corresponding toolbar (see below). In a multichannel continuous view, you must double-click an individual channel to zoom it into single-channel

mode before the mouse wheel can be used to adjust magnification; this is because in multi-channel mode, the mouse wheel is used to scroll the display up and down through the list of channels, and so is not available for magnification control.

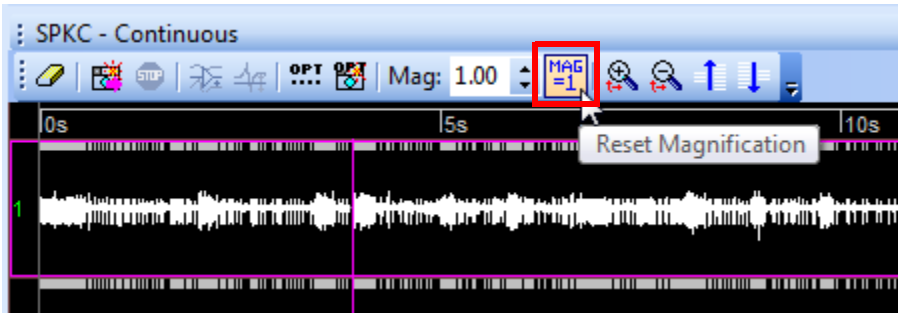
**Note:** You can also use the mouse to adjust magnification by holding down the right mouse button and dragging vertically in a window while holding down the Shift key. This method is somewhat awkward, but it allows slightly more control over the degree of magnification than the mouse wheel method.

Typing in a new magnification factor:

Clicking on the up/down arrows to change magnification factor:

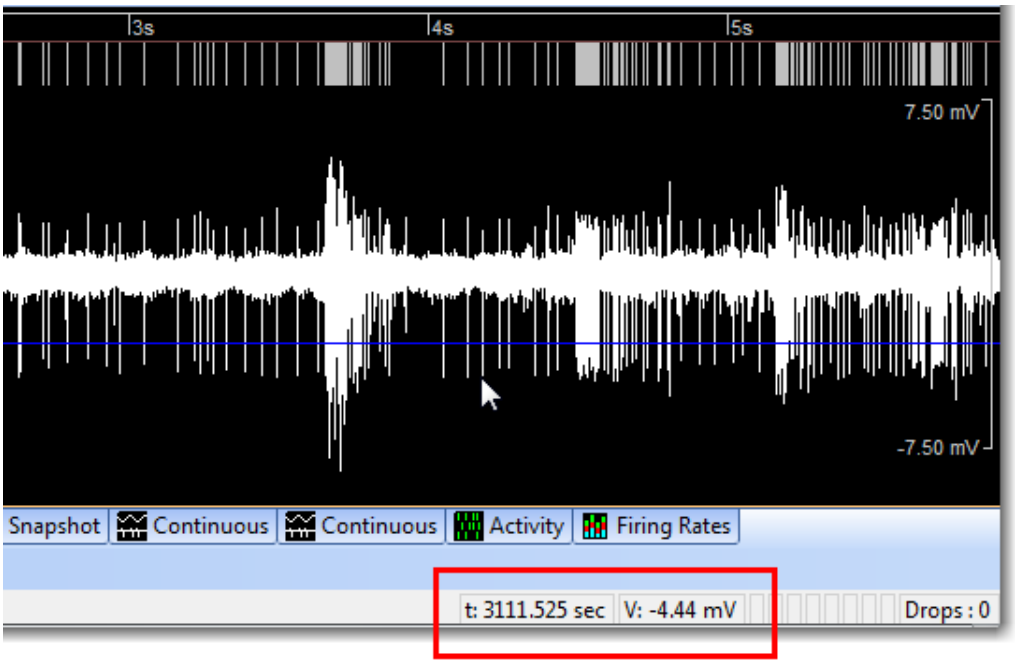



Clicking on the “MAG=1” button to restore the magnification to 1.0:




If you double-click within one channel's display area within the view, which expands it to fill the entire view, additional information becomes available. At the bottom of the PlexControl main window, the status bar continuously displays the time and voltage corresponding to the cursor position, as you move the cursor within the view. For example, you can point to a spike peak to get an approximate

measurement of its amplitude. Note that the time displayed here is absolute time since data acquisition started.

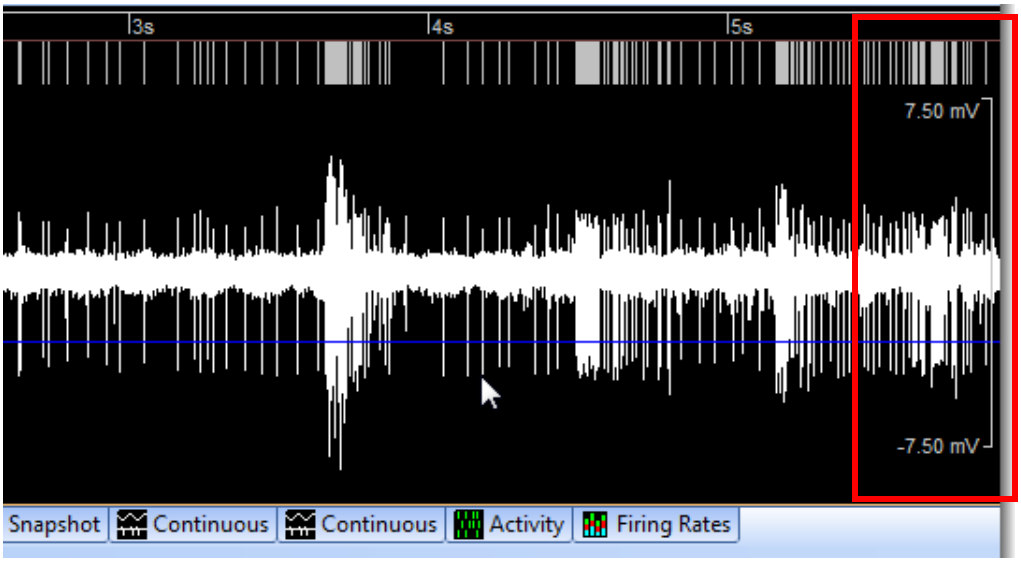


**TIP**

**Pause the display for convenient viewing**

Pausing the display, as described later, makes it easier to point at specific parts of the displayed signal. The time/voltage readout in the status bar will still track the moving cursor position, even if the display is paused.

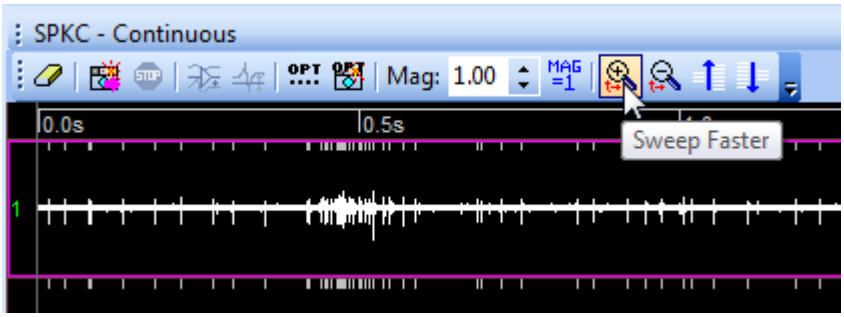
Note that the amplitude bar at the right end of the continuous displays is marked with minimum and maximum values, but the top and bottom of the bar are at 75% of the actual minimum and maximum values for the display. In the example shown below, the actual amplitude range of the display extends beyond the top and bottom of the scale bar, all the way to the very top and bottom of the black background area. In this case, the actual minimum and maximum displayable amplitudes are -10 mV and +10 mV.

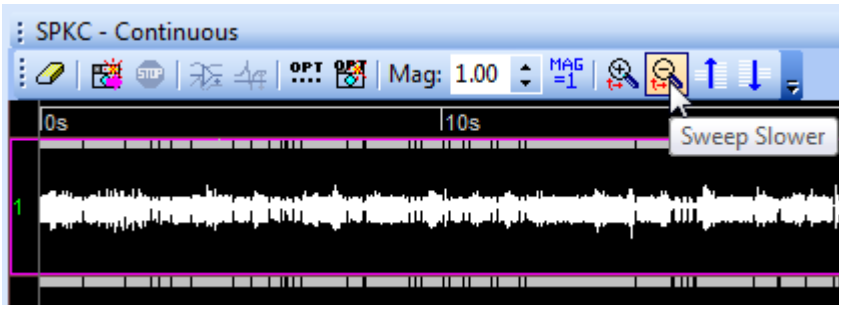



**Note:** To set all magnifications to the same value, see Section 4.4.7, “Using the Same Magnification for All Channels” on page 130.

### 4.4.3 Changing the Sweep Rate

You can change the horizontal sweep (scroll) rate of the continuous displays using the **Sweep Faster** ("+" icon) and **Sweep Slower** ("–" icon) buttons in the toolbar:

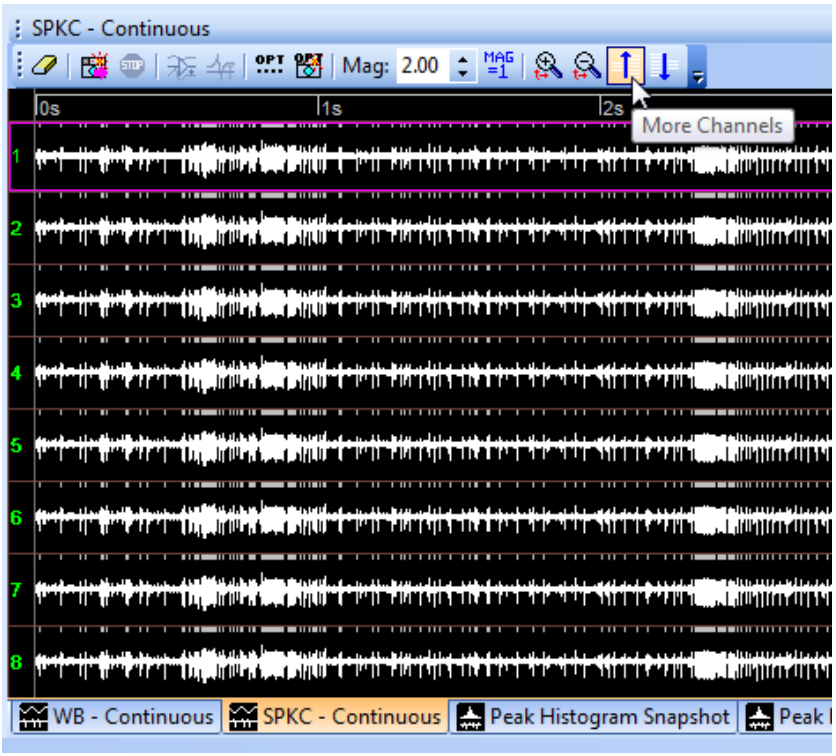


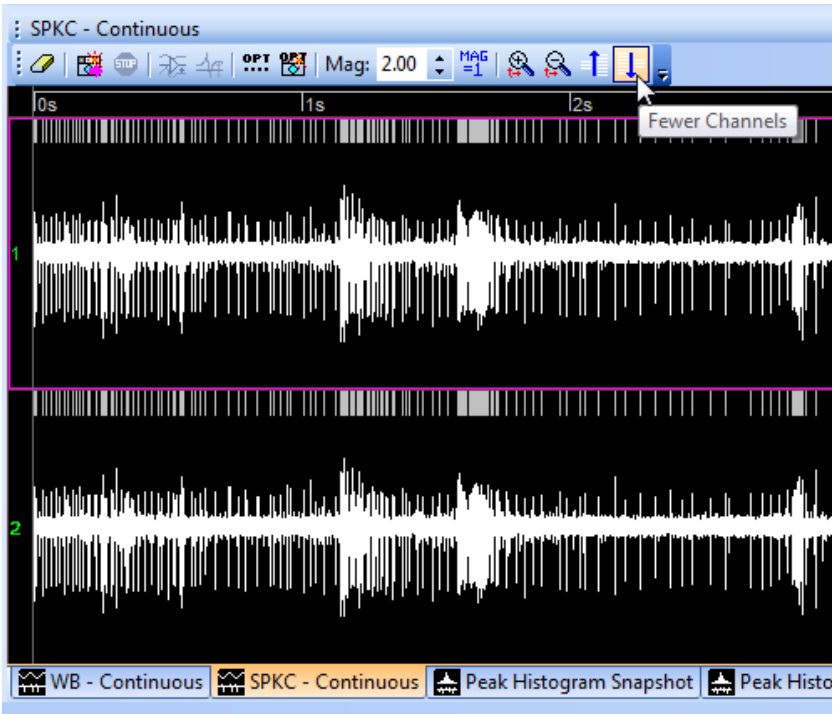




The time scale along the top edge of the continuous view is redrawn to show the new sweep speed.

### 4.4.4 Changing Number of Channels Displayed in a View (Continuous Channels)

You can change the number of channels displayed together in a view with the **More Channels** and **Fewer Channels** buttons in the toolbar.

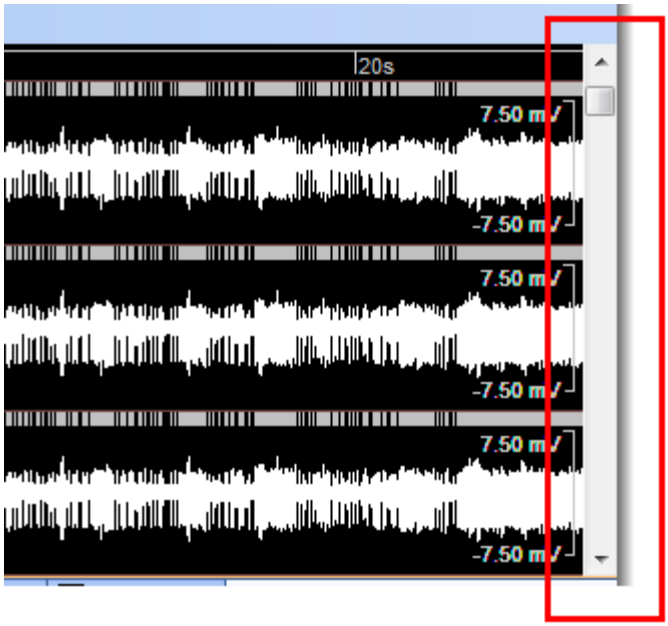


You can use the **Shift** and **Ctrl** keys on your keyboard for additional options when displaying more channels or fewer channels.

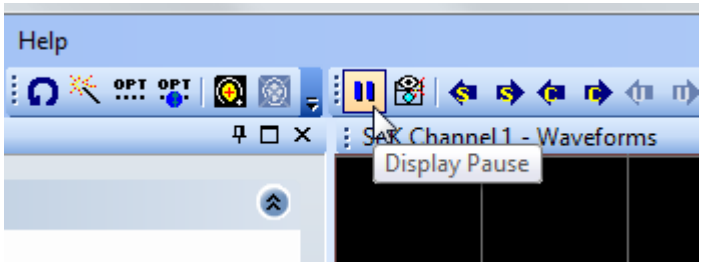
- Pressing the **Ctrl** key while clicking the **More Channels** or **Fewer Channels** button toggles the number of channels displayed to twice the original number or half of the original number.
- Pressing **Shift+Ctrl** while clicking the **More Channels** or **Fewer Channels** button toggles between showing all channels and showing one channel.

If you are viewing fewer than the total number of channels, you can use the scrollbar and scroll arrows at the right side of the view to scroll other channels in and out of the view:




### 4.4.5 Pausing the Displays

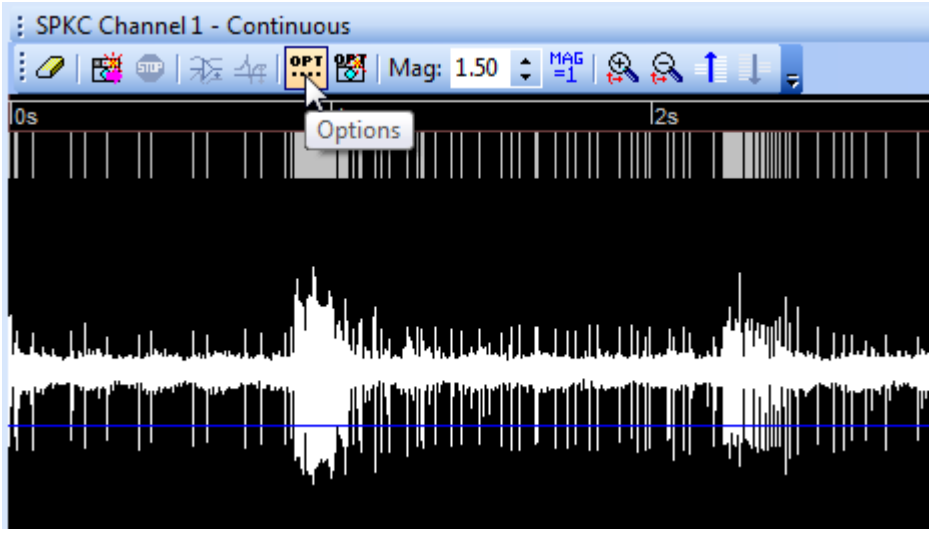
A feature related to changing the sweep speed, but which applies to all views, not just continuous views, is **Display Pause**, which freezes all graphical displays. To pause the display, click on the **Display Pause** button in the main OmniPlex toolbar at the top of the main window:



All animated views will be paused until you click the button again. Note that this has no effect on the acquisition, processing, or recording of data, which will continue as before. It is simply a handy way to “freeze” data views so that you can inspect them in a static state. Do not confuse this with snapshots, which are described later. Pausing the display does not take a snapshot, and displaying a snapshot only freezes the view.

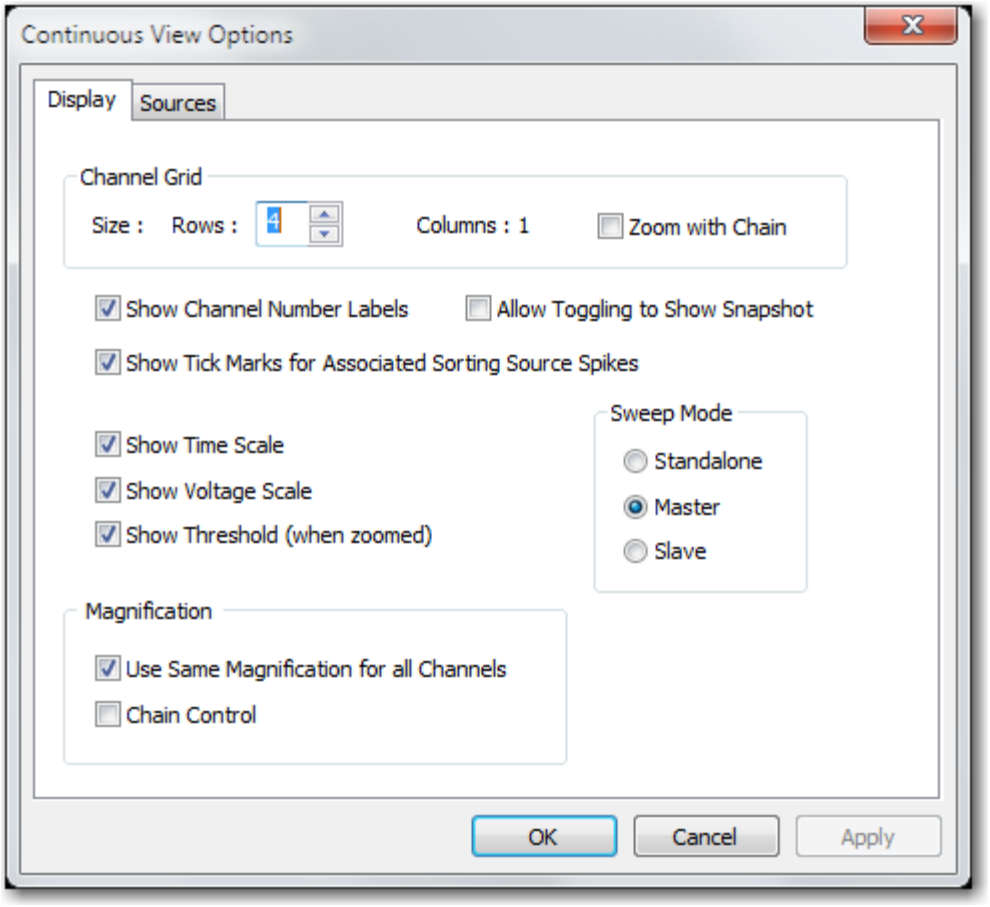
### 4.4.6 Using the Continuous View Options

There are a number of display options associated with each view. Each view's options, which can be set independently, are accessed by clicking the **Options** button in its toolbar:




**Note:** The other **Options** button—**Global Options** ( )—accesses snapshot options, which will be described later.

When you click the **Options** button, the Continuous View Options dialog is displayed:

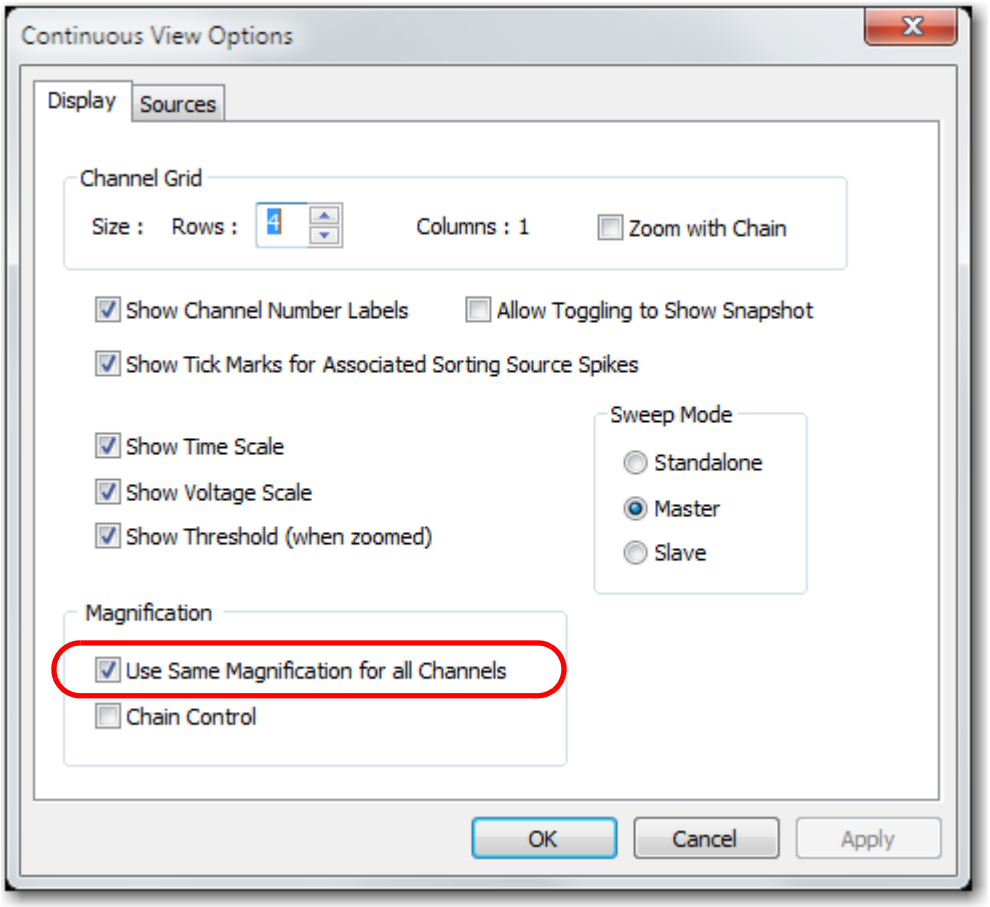



Some of the options should be self-explanatory, or are advanced options, and will not be described here. Two that will be described are **Use Same Magnification for all Channels**, and **Chain Control**.

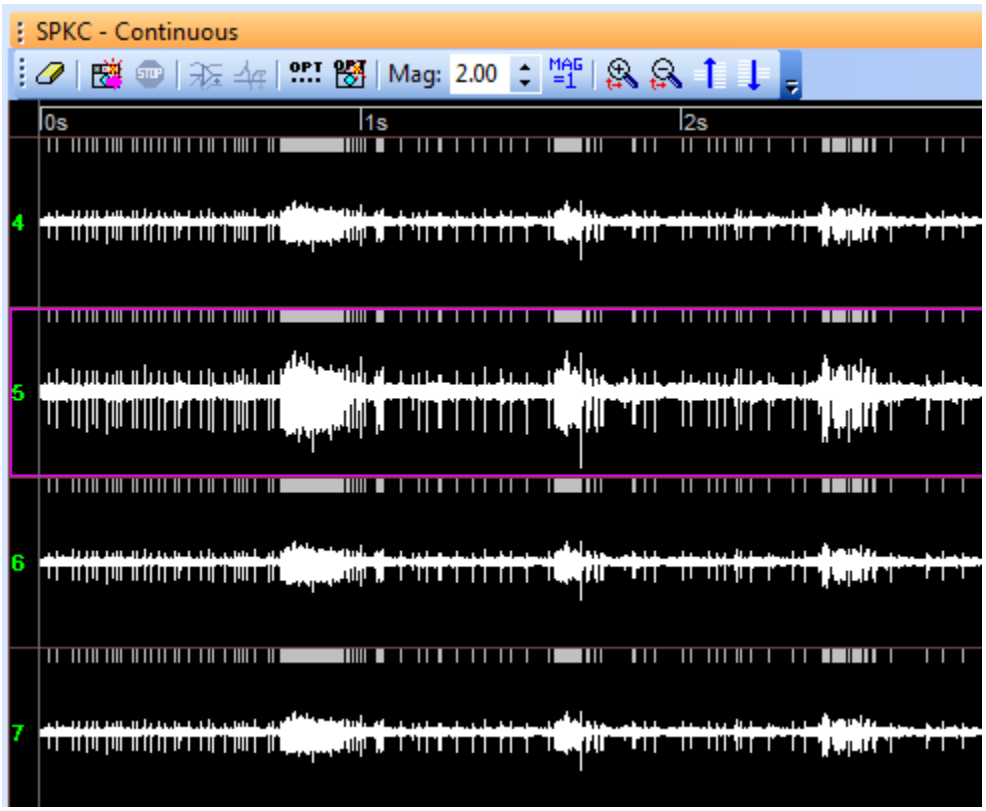
### 4.4.7 Using the Same Magnification for All Channels

**Note:** For basic zoom and magnification options, see Section 4.4.1, “Using the Zoom Feature” on page 120 and Section 4.4.2, “Changing the Magnification” on page 121.

By default, changing the magnification value in a view's toolbar affects all the channels in a view. However, by unchecking **Use Same Magnification for all Channels**, each channel can have its own magnification value, which can be useful when the signal amplitude varies widely from channel to channel.



When the **Use Same Magnification for all Channels** option is unchecked, you must select a channel by clicking on it before adjusting its magnification. In this example, only channel 5, which is selected, has had its magnification increased to 2.0, while the other channels remain at their default magnification of 1.0:



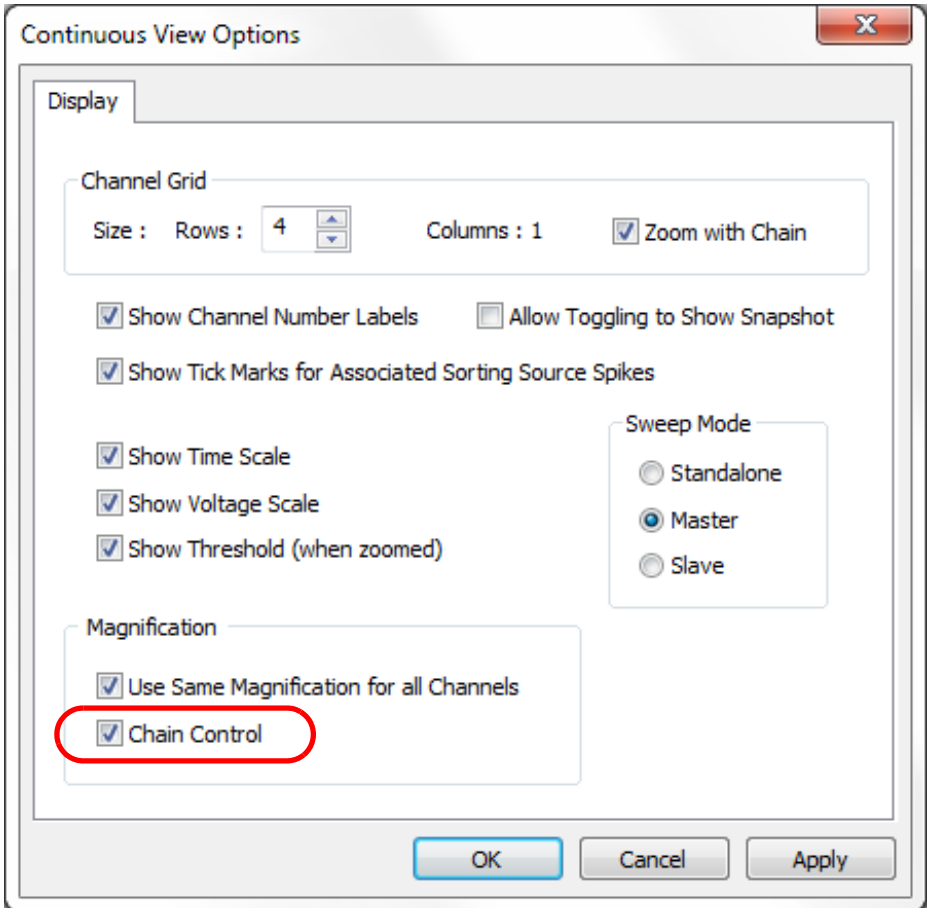
**TIP**

**Changing magnification on the selected channel**

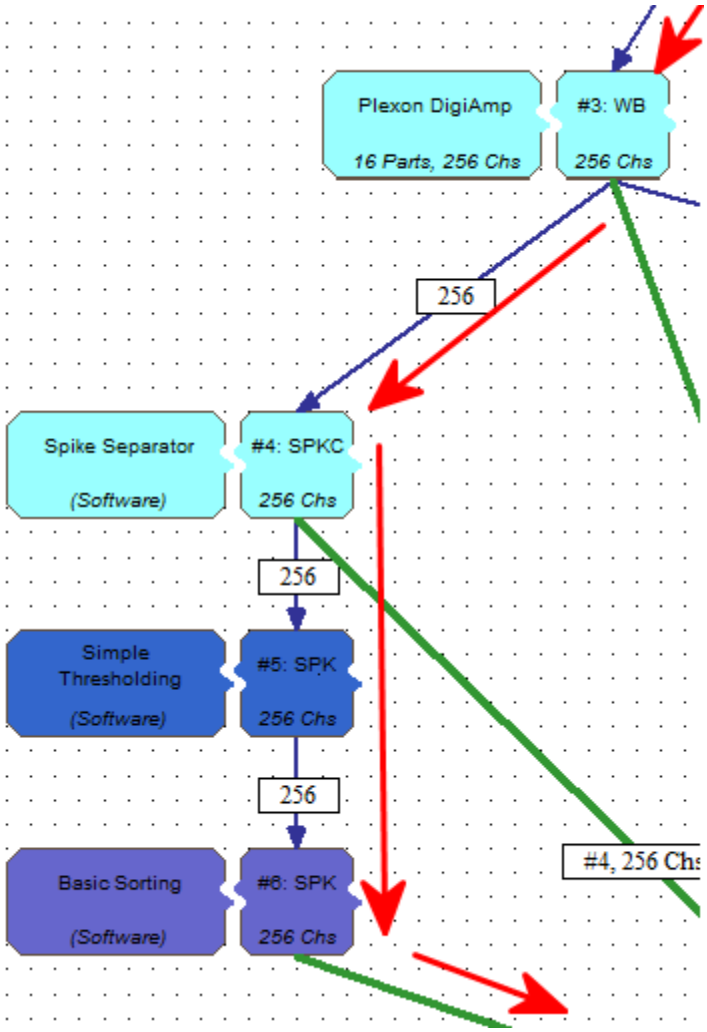
If you change the magnification value in the toolbar and don't see any change in the magnification, check to make sure that the selected channel is visible; you may need to use the scrollbar to bring it into view.

### 4.4.8 Using Chain Control

Chain Control magnification is selectable in the Continuous View Options window.




To understand how **Chain Control** works, remember the topology diagram that is displayed in Server, which shows chains of hardware and software devices, feeding data downward along chains, ending in the Main Datapool where it is read by PlexControl. Here, we are interested in the chain of sources from the DigiAmp, through the spike separator, thresholder, and spike sorter - you can think of this as the “spike chain”:




Three of the sources in the spike chain have their data displayed in views in PlexControl: continuous wideband (WB) data from the DigiAmp Amplifier is displayed in one continuous view, continuous spike data (SPKC) in another continuous view, and sorted spikes (SPK) in two other views (the multichannel spike view and the current channel spike view. The idea behind chain control is that changing the magnification in one view will automatically change the magnification in other views which also have **Chain Control** enabled. The usual case where you would use **Chain Control** is to cause a magnification change in any spike view (SPKC view or the two SPK views) to affect the other spike views. To do so, simply enable **Chain Control** in the **Continuous View Options** dialog, in the views which you want to respond to magnification changes from other views for the same source chain.

Note that you will usually *not* want to enable **Chain Control** for a view displaying wideband (WB) data. This is because the wideband and FP signals are usually of a larger amplitude than the spikes, and so the spike views will typically use a higher magnification value than wideband or FP views.

You can use **Chain Control** and **Use Same Magnification for all Channels** in combination; for example, if **Chain Control** is enabled and **Use Same Magnification for all Channels** is disabled, changing the magnification of channel 23 in the SPKC view will affect only the magnification of channel 23 in the two SPK views, and vice versa.

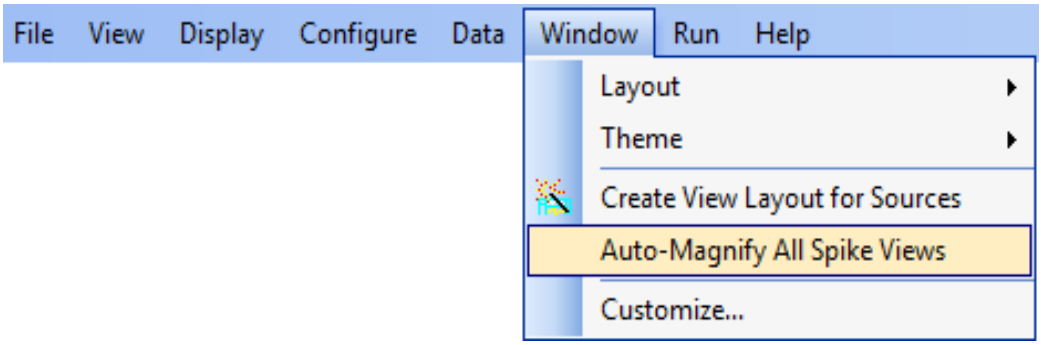
**TIP**

**Understanding the source associated with the thresholding device**

The observant reader will have noticed that the source associated with the thresholding device is not sent to the Main Datapool. This is because it consists of unsorted spikes, and the only use for those spikes is to serve as the input for the sorting device. You can think of the source associated with the thresholding device as an “internal source” which can be safely ignored.

### 4.4.9 Auto-Magnify All Spike Views

The Auto-Magnify All Spike Views command is accessed from the Window menu.




The default behavior for this command is for each channel to be auto-magnified independently, so that all channels' signals are clearly visible.

If you hold down the CTRL key when you select this command, the system determines the magnification for the SPKC channel with the largest amplitude, and applies that same magnification factor to all other SPKC channels, so the relative magnitudes are preserved visually.

Also, when you perform an Auto-Magnify All Spike Views command, the setting of the Use Same Magnification for all Channels option in the affected views will be automatically adjusted if appropriate. For example, if you do an Auto-Magnify with independent per-channel magnifications (the default behavior), the Use Same option will be disabled; conversely, if you Auto-Magnify with the same magnification applied to all channels, the option will be enabled. This usually prevents unexpected results when you manually adjust magnification after having done an Auto-Magnify, but you can change the setting of the Use Same option at any time if desired.

Note that when you first start PlexControl, the SPKC (spike continuous) view is hidden under the WB (wideband continuous) view, and in fact the SPKC view is not initialized until the first time you click its tab to view it. If you perform an Auto-Magnify before the SPKC view has been viewed for the first time, SPKC will not be updated by the Auto-Magnify. If you see that SPKC has not been auto magnified, simply select the Auto-Magnify command again to update all the SPK and SPKC views.

## 4.5 Using the Toolbar Auto Hide Button

The illustration below shows a typical window title bar. It contains (from left to right) a **Title**, an **Auto Hide** button, a **Maximize** button, and a **Close** button.

- **Auto Hide Button** - The **Auto Hide** button "pins" a window to the screen to keep it visible or "rolls up" a visible window into a tab. When the window is pinned, the push pin in the **Auto Hide** button points in a vertical direction. If the window is rolled up, the push pin points in a horizontal direction.

- **Maximize Button** - The **Maximize** button may not appear on all windows. It is the standard Windows® maximize button. Clicking the **Maximize** button on a window will maximize the original window and hide other windows occupying the same horizontal or vertical space. Clicking the **Maximize** button again will restore the previous layout. When clicked the image on the button toggles between one window and overlapping windows.

- **Close Button** - The **Close** button closes the window.

## 4.6 Advanced User Interface Options

If you would like to further customize the layout of views and toolbars in PlexControl, see Section 13.8, "Advanced User Interface Features" on page 442.

# Chapter 5
# Spike Detection

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## 5.1 Spike Detection by Thresholding

In spike detection, the amplitude of the SPKC signal is compared, point-by-point, against a per-channel threshold amplitude value. When the SPKC signal crosses the threshold value, a segment of the SPKC signal (by default in the OmniPlex® System, eight points preceding the threshold crossing and 24 points following the threshold crossing, for a total length of 800 microseconds) is used to define a spike waveform, which is then output on the corresponding channel of the SPK source. Spike detection operates continuously on the incoming SPKC signal, outputting spike waveforms in sequential order as they are detected on each channel.

Spikes on a channel typically have a larger negative peak amplitude, and so the default thresholding in the OmniPlex System is "on the negative side," but a positive threshold can be defined, if the spikes on a given channel have larger positive peaks. The threshold value must be large enough (far enough from the zero volts baseline, in whichever direction) to avoid false triggering on low-amplitude noise, but not so high that low-amplitude spikes are missed.

A common mistake is to set thresholds too low, motivated by a desire to "not miss any spikes"; however, in addition to incorrectly detecting noise as spikes, this can cause parts of valid spikes to be missed, if a noise-triggered detection occurs immediately before a valid spike. While thresholds can be set manually for each channel, as described later, the problems just described can largely be avoided by taking a continuous snapshot of the SPKC signal and using PlexControl's auto-thresholding feature to set the thresholds in a consistent, well-defined way. Auto-thresholding is also much faster than setting thresholds manually, especially for systems with many channels.

## 5.2 Working with Snapshots

The OmniPlex System has the ability to take snapshots of continuous data or detected spike waveforms. A snapshot can be thought of as a temporary copy of the incoming data, containing either a given number of seconds of continuous data, or either a given number of seconds worth of spikes, or a given fixed number of spikes. Here are some examples of typical snapshots:

- A snapshot consisting of 10 seconds of continuous spike (SPKC) data for each of 64 channels, with the snapshots collected (taken) in parallel; a 10-second snapshot will contain 400,000 samples per channel at a 40 kHz sampling rate
- A snapshot consisting of 500 spikes on each of 64 channels, with the snapshot collection starting at the same time on each channel, but ending on each channel at the time when 500 spikes have been collected on that channel; depending on the firing rates, some channels will complete their snapshot collection before others
- A snapshot consisting of 10 seconds of spikes on each of 64 channels; depending on the firing rates, some channels will have more spikes in their snapshot than others

Snapshots are useful purposes such as determining the statistics of a continuous signal for use in auto-thresholding, or for capturing a set of spikes for use in manual or automatic spike sorting. When you are manually defining units for spike sorting, as described later, you can define the units either directly “on” the live, animated data, or on a static snapshot; which you use is a matter of preference, and of the dynamics of the incoming data. You can capture a fresh snapshot at any time, and the snapshots for different sources (e.g. SPKC versus SPK) are independent for the most part.

**TIP**

**Toggling between viewing live spikes and snapshots**

The OmniPlex System allows you to define, edit and delete sorted units for a spike channel while viewing either the live incoming spikes or a snapshot of previously captured spikes. You can toggle between these two viewing modes without causing any interruption to defining, editing or deleting sorted units. See Section 8.5, “Snapshot Mode versus Live Display” on page 255.

**TIP**
**Forward and backward snapshots**

For performance reasons, the OmniPlex System only allows a forward snapshot to be taken of continuous sources. You can think of a forward snapshot as a command to "start collecting continuous data for the snapshot, starting now and going forward" for the duration of the snapshot, e.g. for the next 10 seconds. On the other hand, a spike source (SPK) can have either a forward snapshot or a backward snapshot. A backward snapshot is in effect a command to use the last N seconds or N spikes of "old" data as the snapshot. A backward snapshot is useful in that you see the data that will be captured for the snapshot, then capture it; in comparison, when you start a forward snapshot, you have yet to see the data that will be collected for the snapshot. Also, backward snapshot can be taken instantly, whereas you must wait for a forward snapshot to be collected. Forward and backward snapshots only differ in how the data is collected; once the snapshot has been taken by either method, there is no difference in the way it is used.

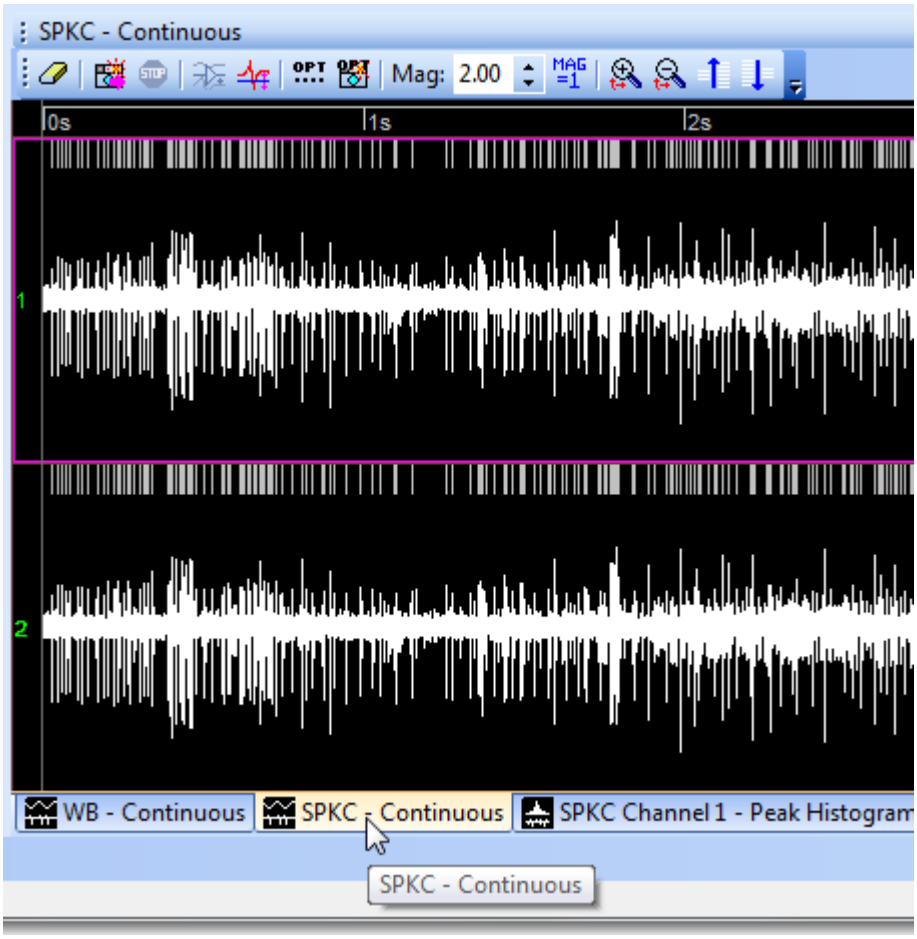
**TIP**
**Collecting a snapshot for an individual channel**

You can also collect snapshots for individual channels one at a time, by collecting a snapshot in a view where only a single channel is displayed, as opposed to in a multichannel view. Details of this will not be discussed here. Usually, you will want to take a snapshot of all the channels in a source at the same time.

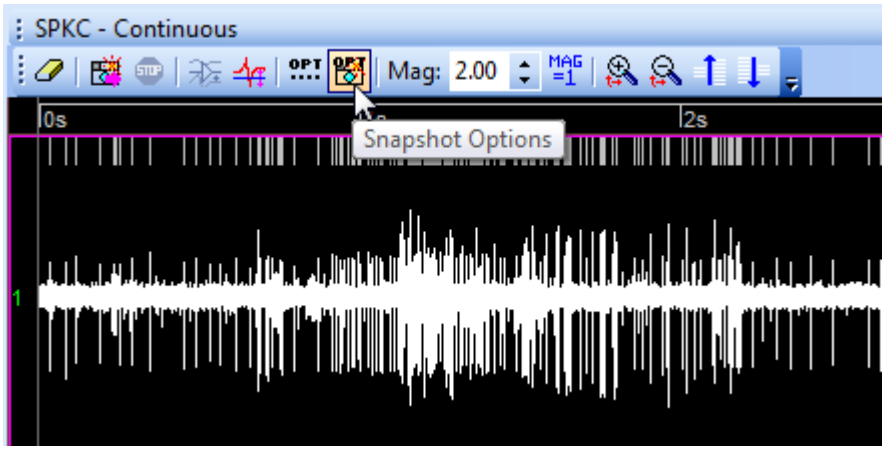
The most common use of the continuous snapshot is to automatically set the thresholds that are used for spike detection, as described in the following sections. Using snapshots for spike sorting will be described in a later section.

## 5.3 Using a Continuous Snapshot to Set Thresholds Automatically

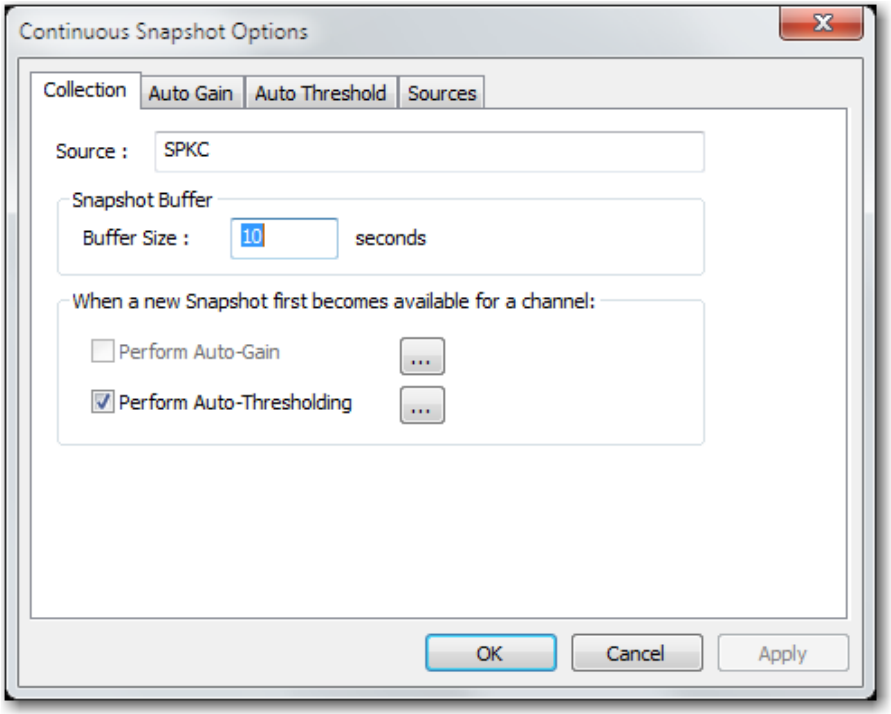
1 If it is not already visible, click the SPKC - Continuous tab to display the continuous spike signal. If only one channel is displayed, double-click anywhere within the display area to return to multichannel display mode. You do not need to display every channel; as long as two or more channels are displayed, you are in multichannel mode, and snapshot collection and analysis will be applied to all channels, even the ones that are not visible onscreen.



2 Click on the **Snapshot Options** button in the toolbar (not the Options button to its left):




3 The **Snapshot Options** for the SPKC view are displayed. You can see that the default length of a snapshot of continuous data is 10 seconds, and that the first time that you collect a SPKC snapshot, the snapshot will be used to automatically set the thresholds for the SPKC source:



**TIP**

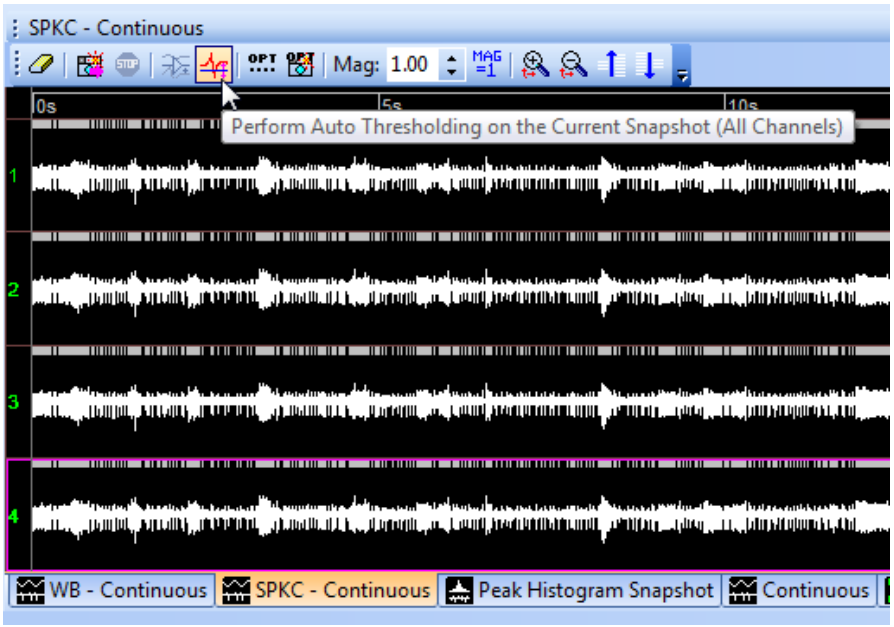
**Increase snapshot length with caution**

Increasing the length of the snapshot above the default length of 10 seconds should be done with caution, since this will also increase the amount of time require to process the snapshot.

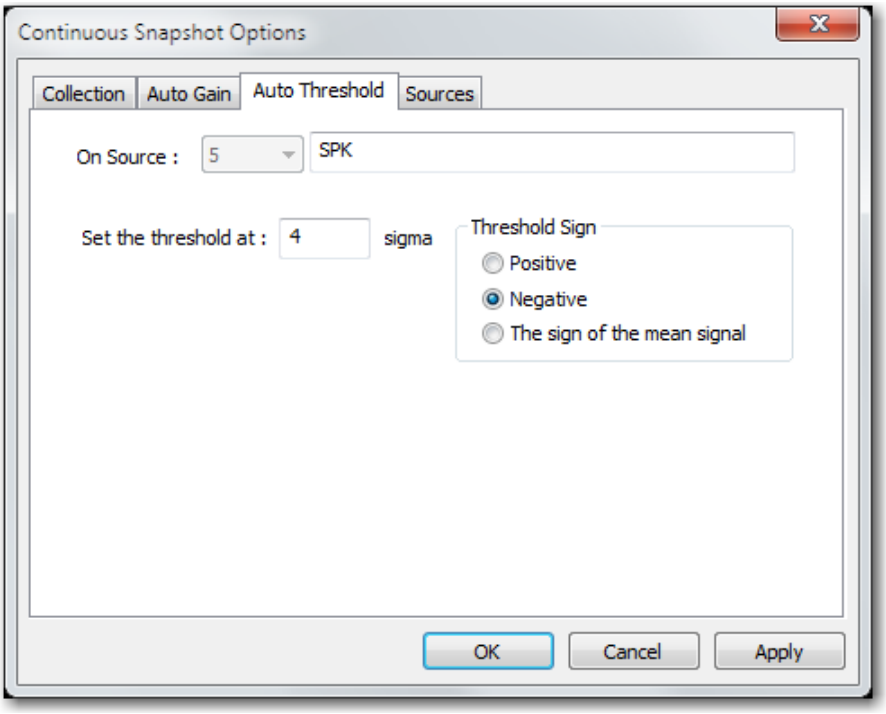
**TIP**

**Auto-Threshold option and button**

If you disable the “Perform Auto-Thresholding” option, snapshots can still be collected, but you will have to use the Auto-Threshold button in the toolbar to perform an auto-threshold, using the most recently collected snapshot.

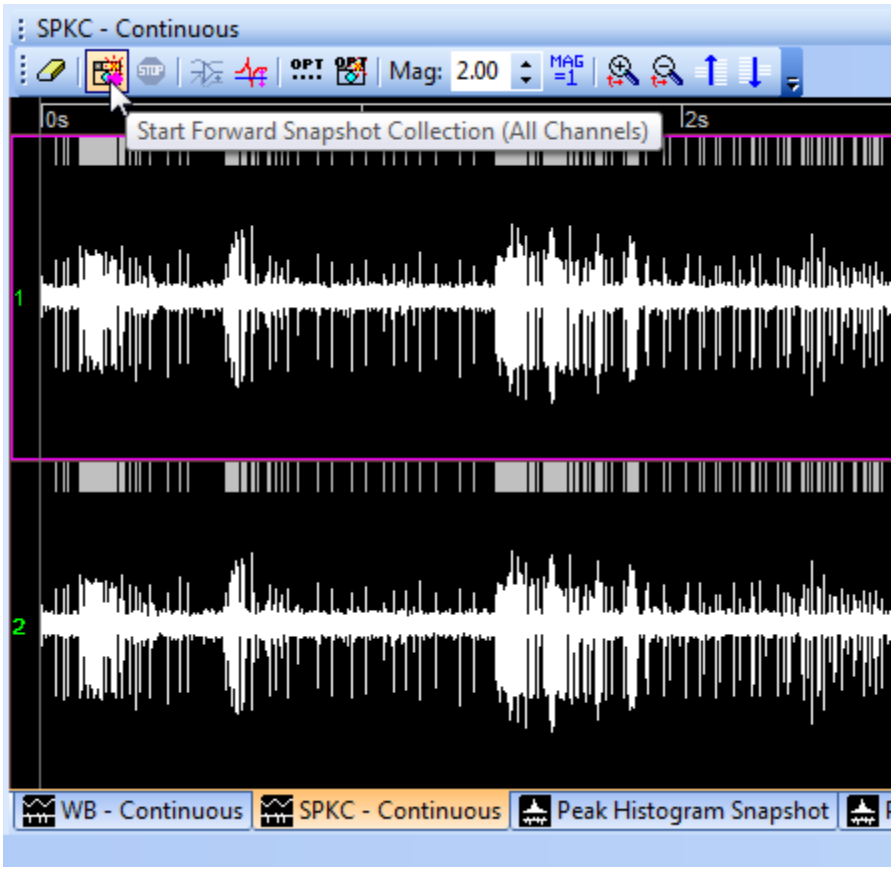


4 If you select the **Auto Threshold** tab, you will see the default settings that will be used for auto-thresholding:

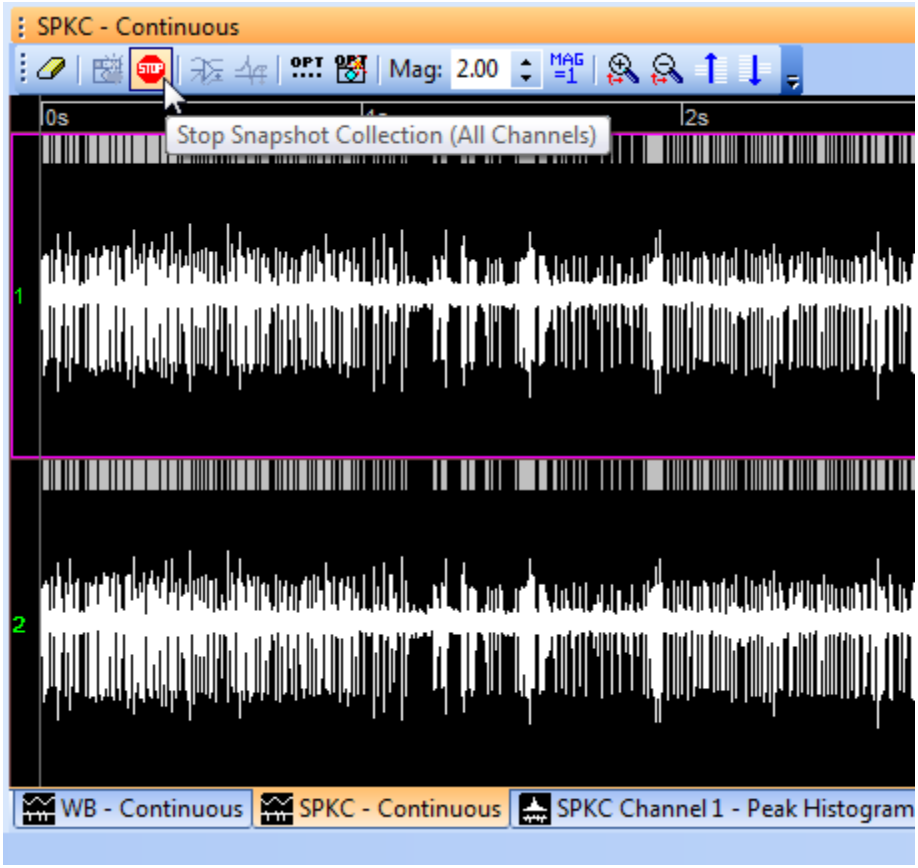



As you will see below, when a continuous snapshot is collected, the OmniPlex® System uses it to derive per-channel histograms of the peaks in the continuous signal. The auto-threshold procedure sets the threshold at a certain number of standard deviations (sigmas) from the mean of this distribution. Click **OK** or **Cancel** to dismiss the **Snapshot Options** dialog.

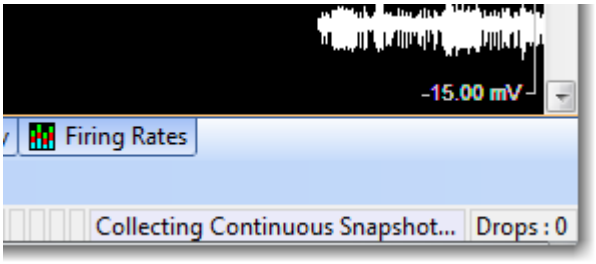
5 Click the **Start Forward Snapshot** button in the toolbar to begin the snapshot collection.



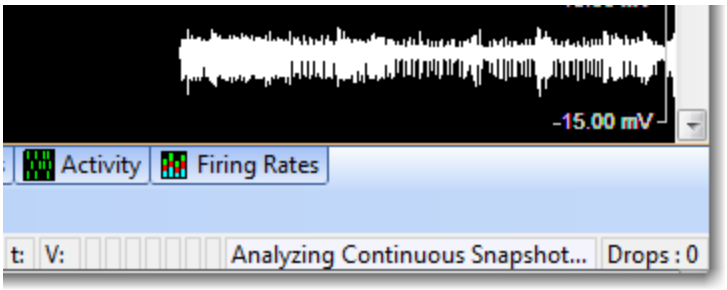
6 While the snapshot is being collected (by default, for 10 seconds), the **Start Forward Snapshot** button changes to the **Stop Snapshot Collection** button, a stop sign. Do not click the button, or the snapshot will be aborted.



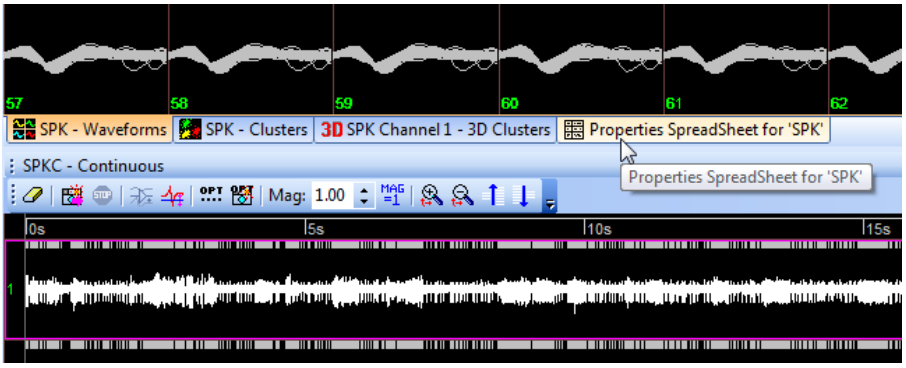

Also note that while the snapshot is being collected, the status bar at the bottom of the main PlexControl window indicates that continuous snapshot collection is in progress:



7 Once the collection has completed, the status bar shows that analysis of the continuous snapshot is in progress; in this case, the analysis consists of the auto-thresholding.




8 When the status bar no longer shows “Analyzing Continuous Snapshot,” the auto-threshold operation is complete. To view the newly-set threshold values, select the Properties Spreadsheet tab under the multichannel spike window:




The Properties Spreadsheet shows properties for the channels of the currently selected source, in this case the SPKC (continuous spike) source. The Threshold% column shows the threshold values that were just set by the auto-thresholding of the SPKC snapshot:

Properties SpreadSheet for 'SPKC'

| One | Name | PLX chan | Enabled | Threshold% |
|---|---|---|---|---|
| >>1 | SPKC001 | 257 | ☑ | -10.747 |
| 2 | SPKC002 | 258 | ☑ | -10.697 |
| 3 | SPKC003 | 259 | ☑ | -10.695 |
| 4 | SPKC004 | 260 | ☑ | -10.604 |
| 5 | SPKC005 | 261 | ☑ | -10.764 |
| 6 | SPKC006 | 262 | ☑ | -10.704 |
| 7 | SPKC007 | 263 | ☑ | -10.624 |
| 8 | SPKC008 | 264 | ☑ | -10.756 |
| 9 | SPKC009 | 265 | ☑ | -10.686 |
| 10 | SPKC010 | 266 | ☑ | -10.677 |
| 11 | SPKC011 | 267 | ☑ | -10.659 |
| 12 | SPKC012 | 268 | ☑ | -10.718 |
| 13 | SPKC013 | 269 | ☑ | -10.705 |
| 14 | SPKC014 | 270 | ☑ | -10.774 |

The threshold value in percent is relative to the maximum amplitude that can be digitized without clipping. For example, if the SPKC view has a range of -5 mV to +5 mV, and the threshold is -10%, then

5 mV * -0.10 = -0.5 mV or -500 microvolts

Remember that, as mentioned previously, the amplitude scale bar at the right end of the continuous displays indicates the values for -75% and +75% of the actual minimum and maximum displayable amplitude values. In other words, if the scale bar extends from -3.75 mV to +3.75 mV, the actual voltage limits before clipping are -5 mV to +5 mV.

### Additional resources and methods

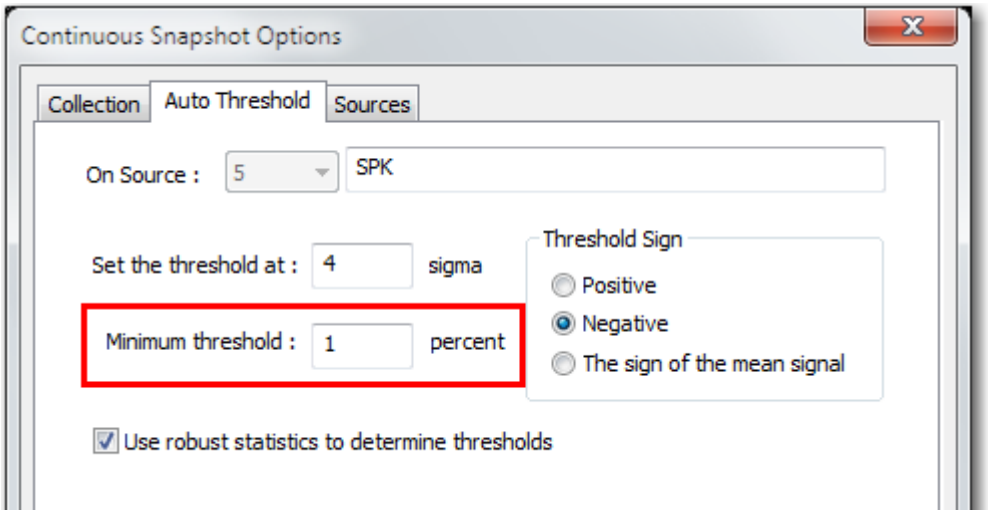
For additional background information on thresholding, refer to Section 5.1, “Spike Detection by Thresholding” on page 138.

In addition to, or as an alternative to using auto-thresholding to set the thresholds on all channels at a given number of sigmas from zero, you can adjust thresholds manually, as described in the next section.

## 5.4 Minimum Threshold for Auto-thresholding

In cases where a channel's signal contains only noise, PlexControl's auto-thresholding procedure may attempt to set a threshold so low that the spike detector triggers on noise rather than valid spikes. To prevent such behavior, you can set a minimum threshold value in percent. If the auto-thresholding procedure attempts to set a threshold whose absolute value is less than the specified percentage, it will instead set that channel's threshold to -99%, in effect disabling the channel's spike detector. However, since the channel is not completely disabled, you can still view the wideband (WB) and spike continuous (SPKC) signals for that channel, allowing you to continue to monitor it for spiking activity, adjust the threshold manually, and to take a new snapshot and re-auto-threshold it if desired. The minimum threshold value only affects auto-thresholding; you can manually set the threshold to any desired value at any time, either by dragging the threshold line or by editing the threshold value numerically.

An appropriate value for the minimum threshold will be data-dependent, but should be small relative to the thresholds of channels which have obvious spiking activity, in order to avoid disabling spike detection on channels with valid low amplitude spikes. For example, if you find that auto-thresholding is producing thresholds of approximately -10% on spiking channels, you might set a minimum threshold of 10% / 10 = 1%.



## 5.5 Adjusting Thresholds Manually

There are several different ways that you can manually set or adjust the threshold on a channel:

- Changing the numeric value of the threshold in the Properties Spreadsheet
- Changing the numeric value of the threshold in the per-channel Properties view
- Dragging the blue threshold line in the SPKC view
- Dragging the blue threshold line in the single-channel spike view
- Dragging the blue threshold line in the SPKC snapshot peak histogram view

Each of these methods will be described below.

**TIP**
**Consider using the automatic threshold settings**
Keep in mind that automatic threshold setting (Section 5.3, “Using a Continuous Snapshot to Set Thresholds Automatically” on page 141) works well for many scenarios, so manual setting might not be required for your particular experiment.

### 5.5.1 Changing the Value of the Threshold in the Properties Spreadsheet

1 1. Click on the threshold cell for the channel you wish to adjust. An edit cursor appears in the cell and up/down arrow controls are displayed:

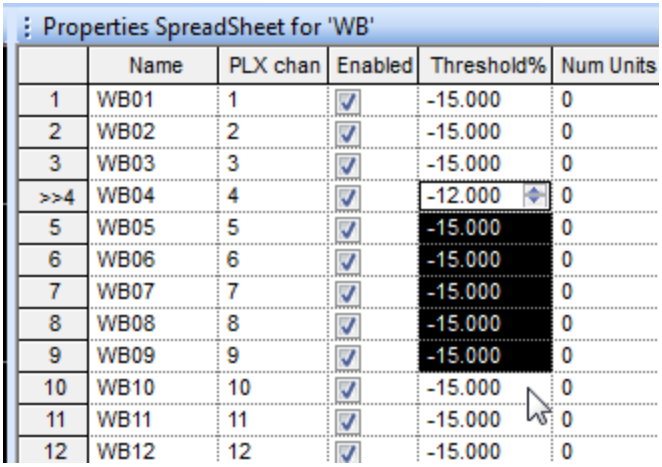
Properties SpreadSheet for 'WB'

| | Name | PLX chan | Enabled | Threshold% | Num Units |
|---|---|---|---|---|---|
| 1 | WB01 | 1 | ☑ | -15.000 | 0 |
| 2 | WB02 | 2 | ☑ | -15.000 | 0 |
| 3 | WB03 | 3 | ☑ | -15.000 | 0 |
| 4 | WB04 | 4 | ☑ | -15.000 | 0 |
| 5 | WB05 | 5 | ☑ | -15.000 | 0 |
| 6 | WB06 | 6 | ☑ | -15.000 | 0 |
| 7 | WB07 | 7 | ☑ | -15.000 | 0 |

2 Click the up/down arrows to adjust the threshold in increments of 1%, or directly edit the numeric value.

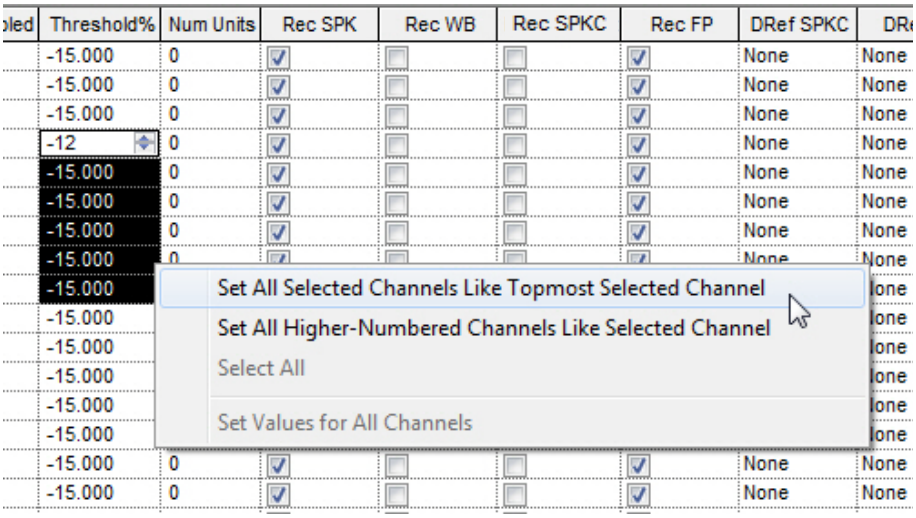
**TIP**
**Double-clicking to edit numerical value**
If you double-click on the numeric value, everything but the minus sign (if present) will be selected, and you can type in a new value, which will replace the old value.

3 To set several successive channels to the same threshold value, set the first (lowest numbered) channel to the desired value, as described in steps 1 and 2. Then hold down the left mouse button on the first channel and drag downward until range of channels up through the last (highest numbered) channel is selected, and release the left mouse button to complete the selection:

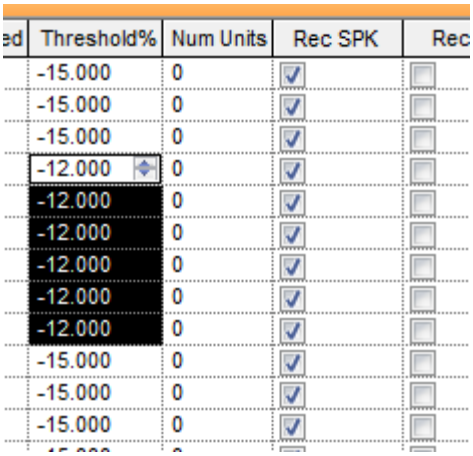



| | Name | PLX chan | Enabled | Threshold% | Num Units |
|---|---|---|---|---|---|
| 1 | WB01 | 1 | ☑ | -15.000 | 0 |
| 2 | WB02 | 2 | ☑ | -15.000 | 0 |
| 3 | WB03 | 3 | ☑ | -15.000 | 0 |
| >>4 | WB04 | 4 | ☑ | -12.000 | 0 |
| 5 | WB05 | 5 | ☑ | -15.000 | 0 |
| 6 | WB06 | 6 | ☑ | -15.000 | 0 |
| 7 | WB07 | 7 | ☑ | -15.000 | 0 |
| 8 | WB08 | 8 | ☑ | -15.000 | 0 |
| 9 | WB09 | 9 | ☑ | -15.000 | 0 |
| 10 | WB10 | 10 | ☑ | -15.000 | 0 |
| 11 | WB11 | 11 | ☑ | -15.000 | 0 |
| 12 | WB12 | 12 | ☑ | -15.000 | 0 |

4 Right-click to display a menu:

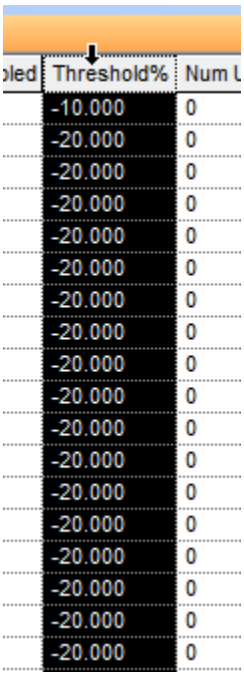


5 Click on **Set All Selected Channels Like Topmost Selected Channel**. The threshold values of all the channels are changed accordingly.



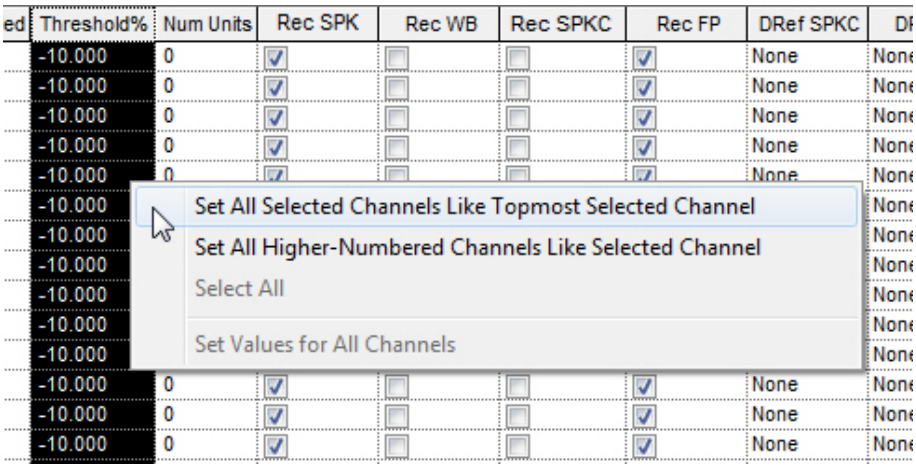
| ed | Threshold% | Num Units | Rec SPK | Rec |
|---|---|---|---|---|
| | -15.000 | 0 | ☑ | ☐ |
| | -15.000 | 0 | ☑ | ☐ |
| | -15.000 | 0 | ☑ | ☐ |
| | -12.000 | 0 | ☑ | ☐ |
| | -12.000 | 0 | ☑ | ☐ |
| | -12.000 | 0 | ☑ | ☐ |
| | -12.000 | 0 | ☑ | ☐ |
| | -12.000 | 0 | ☑ | ☐ |
| | -12.000 | 0 | ☑ | ☐ |
| | -15.000 | 0 | ☑ | ☐ |
| | -15.000 | 0 | ☑ | ☐ |
| | -15.000 | 0 | ☑ | ☐ |

6 You can set all channels to the same threshold value using the following technique. First, set the threshold for channel 1 as desired, as described in steps 1 and 2. Next, left-click on the Threshold column header; clicking on a column header in the Properties Spreadsheet always selects that entire column:



| bled | Threshold% | Num U |
|---|---|---|
| | -10.000 | 0 |
| | -20.000 | 0 |
| | -20.000 | 0 |
| | -20.000 | 0 |
| | -20.000 | 0 |
| | -20.000 | 0 |
| | -20.000 | 0 |
| | -20.000 | 0 |
| | -20.000 | 0 |
| | -20.000 | 0 |
| | -20.000 | 0 |
| | -20.000 | 0 |
| | -20.000 | 0 |
| | -20.000 | 0 |
| | -20.000 | 0 |
| | -20.000 | 0 |
| | -20.000 | 0 |
| | -20.000 | 0 |

7 Now right-click and select either of the **Set All** commands; all channels will now have the same threshold as the value you set for channel 1:



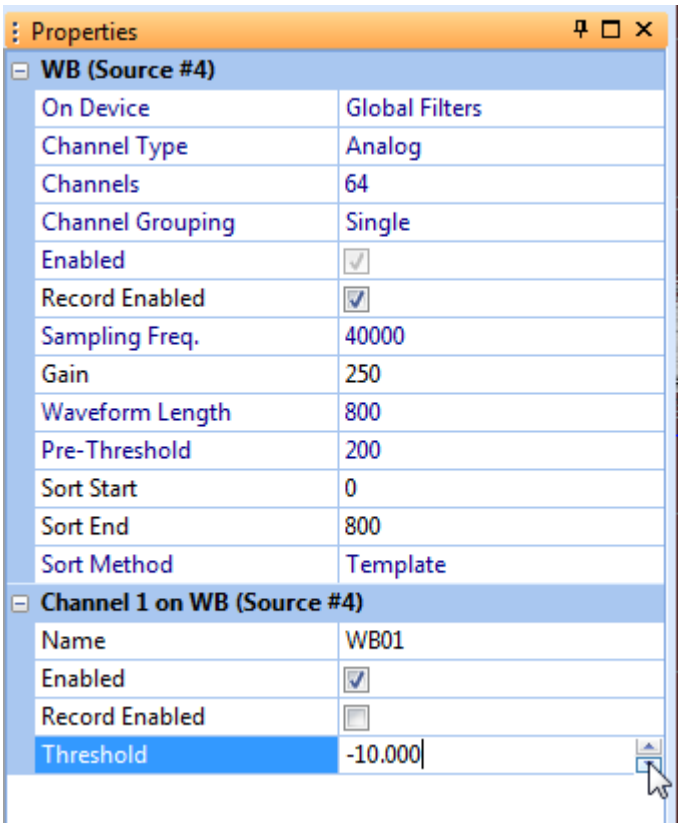
**TIP**

**Set All command can also be used for other columns**

The same technique of selecting the column header and then using the **Set All** commands can be used for the other columns; for example, enabling or disabling checkboxes within the "Rec" columns of recording options.

### 5.5.2 Changing the Value of the Threshold in the Per-channel Properties View

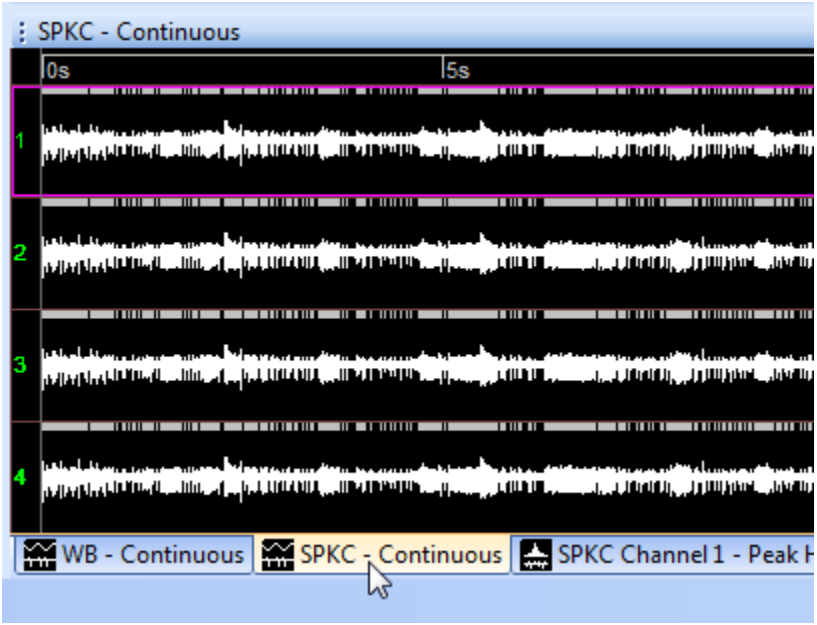
The Properties view at the left side of the screen, which displays the properties for the currently selected source and channel, can also be used to inspect and adjust the threshold value. Click within the cell to display a set of up/down arrows, just as in the multichannel Properties Spreadsheet.



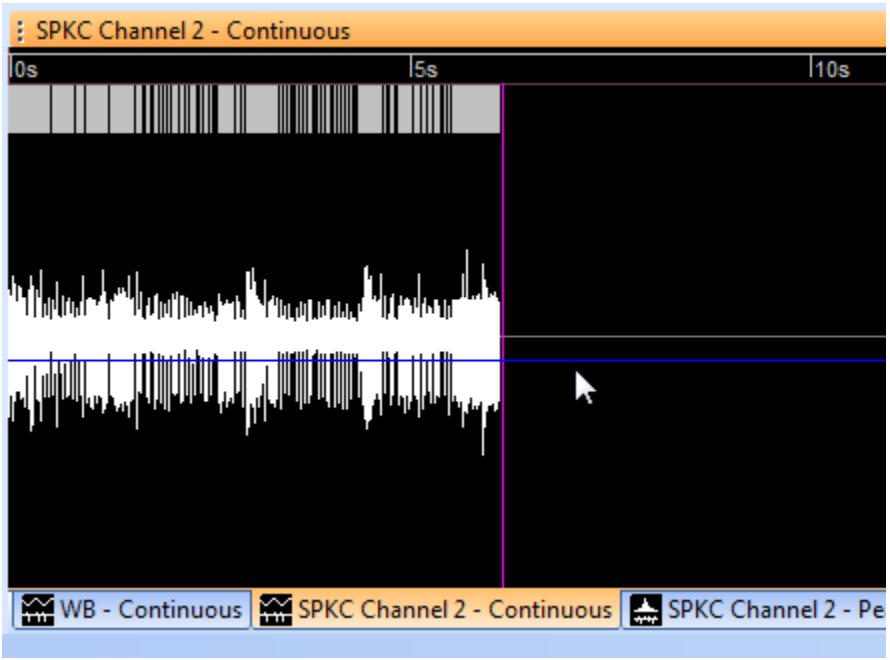

Use of the arrows and direct editing of the numeric threshold value is as described previously.

### 5.5.3 Dragging the Threshold Line in the SPKC View

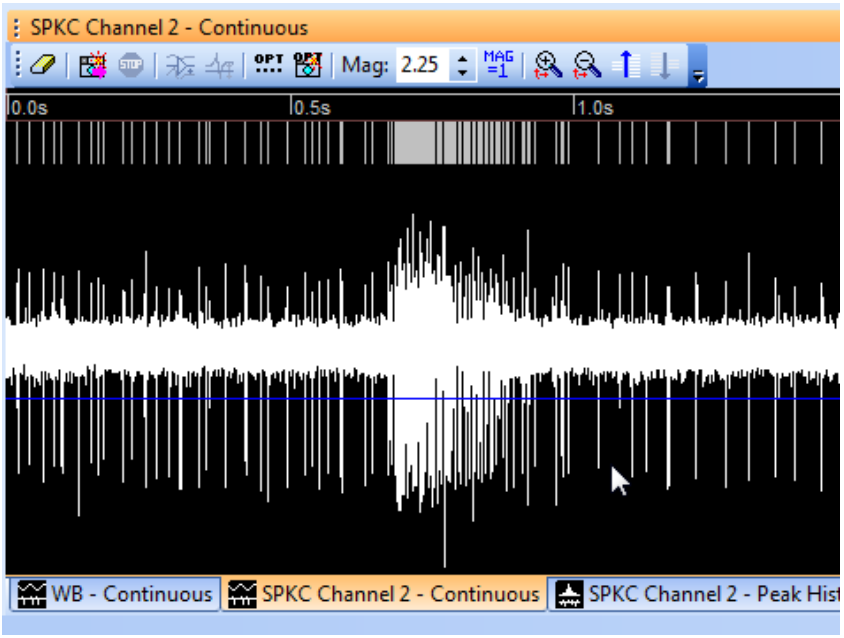
You can also adjust the threshold value graphically, on a display of the continuous spike signal (SPKC). To do so, click on the SPKC - Continuous tab to display the SPKC view, if it is not already visible:




The threshold is only displayed when the SPKC view is in single-channel mode, so double-click on the desired channel to toggle the view into single-channel mode:



You can use the toolbar buttons to adjust the magnification and sweep speed so that the SPKC signal is easier to see and work with. Here, we have increased both the magnification and the sweep speed:




Move your mouse cursor over the blue threshold line (see the blue line in the image above). Notice that the cursor changes to an up/down cursor to indicate that you can adjust the threshold. Click and drag to move the threshold to the desired position.

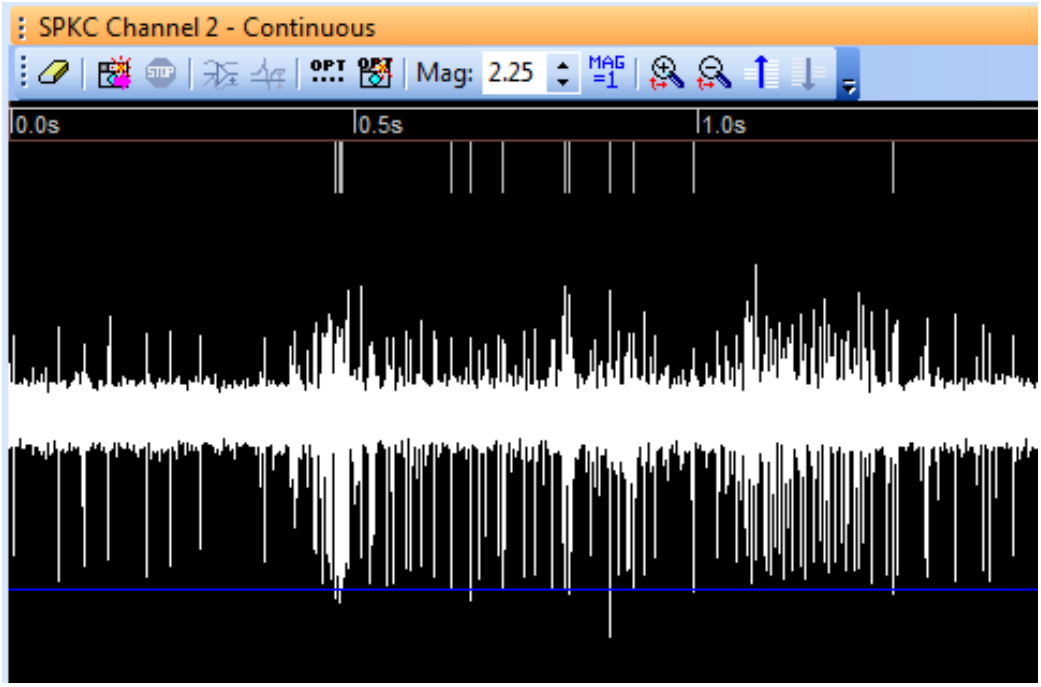
**TIP**

**Thresholds at high magnification**

At very high magnifications and large threshold values (e.g. 15x magnification with a 40% threshold), the blue threshold line can disappear, due to it being beyond the top or bottom of the displayed amplitude range, i.e. it's "off the screen." In such cases, you may need to set the threshold value numerically, using one of the previously described methods, or temporarily reduce the magnification, in order to bring the threshold close enough to zero that it remains visible when you restore the high magnification.

If you set a threshold value that is unnecessarily large (i.e. too conservative), it might cause low-amplitude spikes to be missed, as shown in the following example.

**Threshold set too high (low amplitude spikes might be missed):**



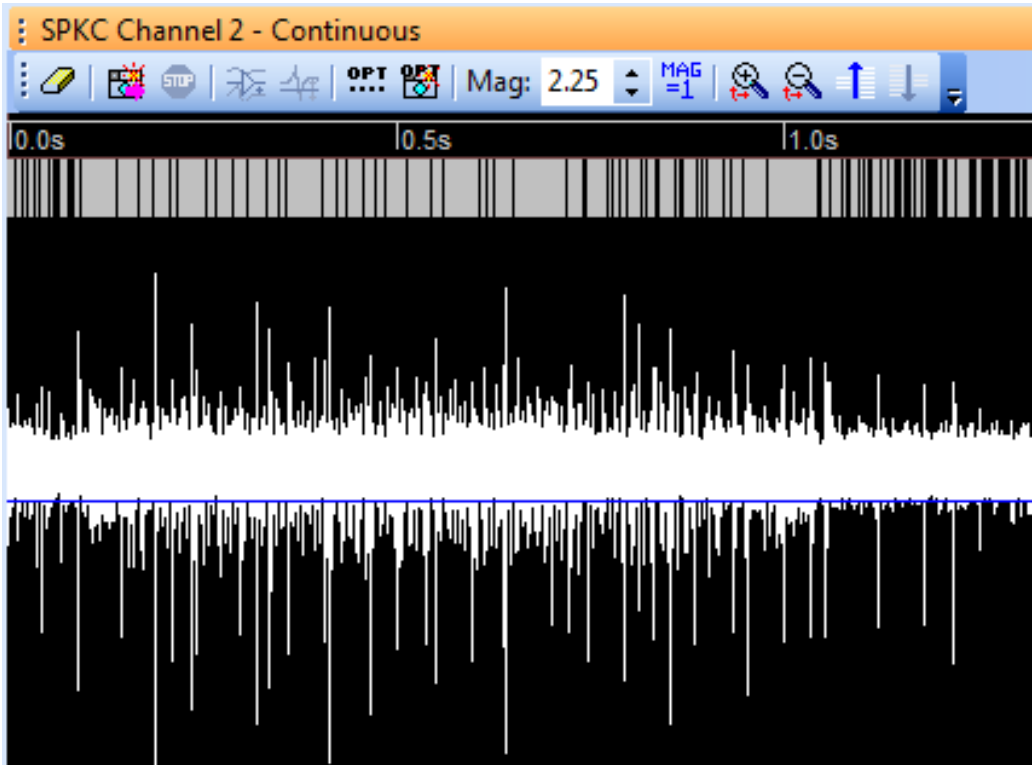
**TIP**

**View the raster-tick display to see the detected spikes**

The raster-tick display along the top edge of the view (in each of the images above) gives immediate visual feedback of the effect of your threshold changes on the detection of spikes. The tick marks indicate the times of detected spikes. In the “threshold too large” example (above), it is clear that many “obvious” spikes are not being detected.

If you set a threshold value that is “down in the noise” (very close to zero), as shown in the following example, you can potentially flood the system with the high number of noise spikes that are generated.

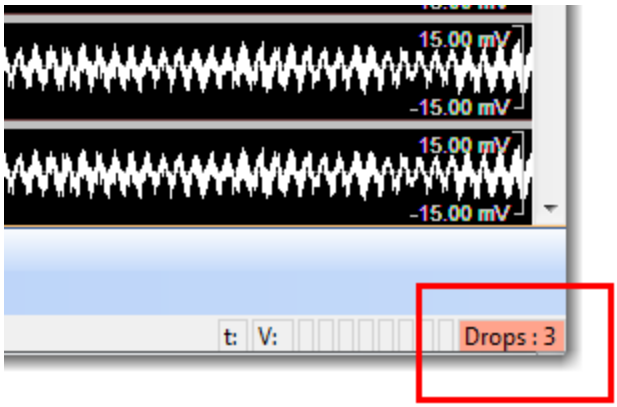
**Threshold set too low (in the noise):**

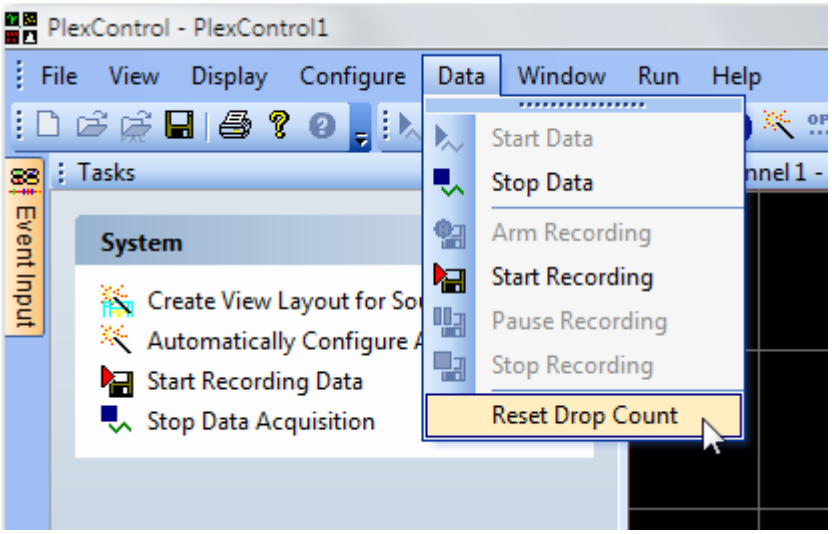



You can use the threshold rate limiting feature to help defend against some of these thresholding problems. See Section 15.4.2, “Threshold Crossing Rate Limiting” on page 492.

It is highly recommended that you perform several “dry run” tests in the intended usage scenario(s) for your experiment. Based on the results of these tests, you might decide to set the threshold value higher or lower.

During the dry run tests, check the lower right corner of the status bar and make sure that the Drop indicator does not appear. If the Drop indicator does appear, it is possible that your threshold setting is too low and is causing the system to be flooded with noise spikes. You can reset the Drop indicator to zero using the Reset Drop Count command in the Data menu. Drops are brief gaps in the data, but are not errors in timestamping, and data outside of the gaps is not affected.




**Note:** The presence of the Drop indicator could also indicate interference from a CPU-intensive application running on the same machine as the OmniPlex System, so it is advisable to close any unnecessary applications on your PC. For further guidance on managing CPU usage, see "Performance considerations in lowest-latency operation" on page A-35.

**Note:** Unexpectedly high spike rates can also be produced by non-neural interference picked up from external noise sources such as electronic devices, power lines, motors, etc. In such cases, you should try to reduce the interference by removing or shielding the sources of noise. The OmniPlex System also provides software filters which can be useful in reducing high frequency noise. See "Spike lowpass filter" on page A-8.

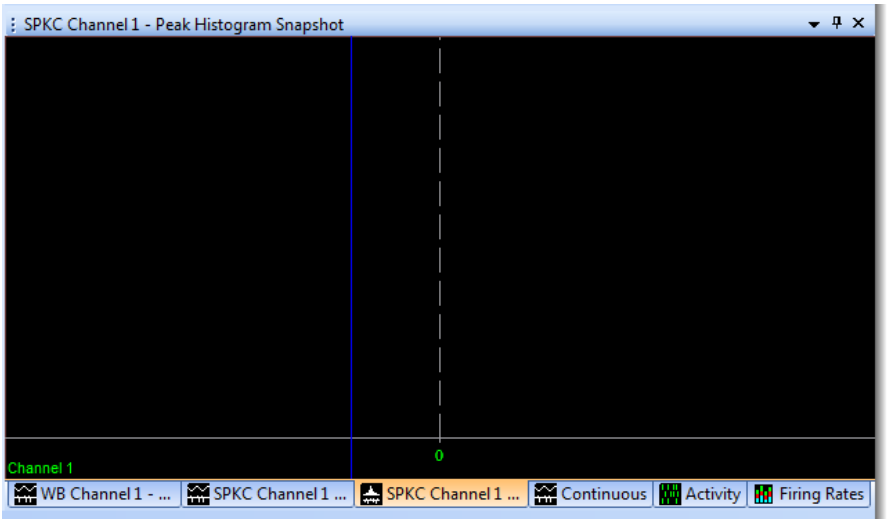
For additional background information on thresholding, refer to Section 5.1, “Spike Detection by Thresholding” on page 138.

If you would like additional guidance on optimizing your threshold settings, please contact Plexon® at +1 214-369-4957 or support@plexon.com.

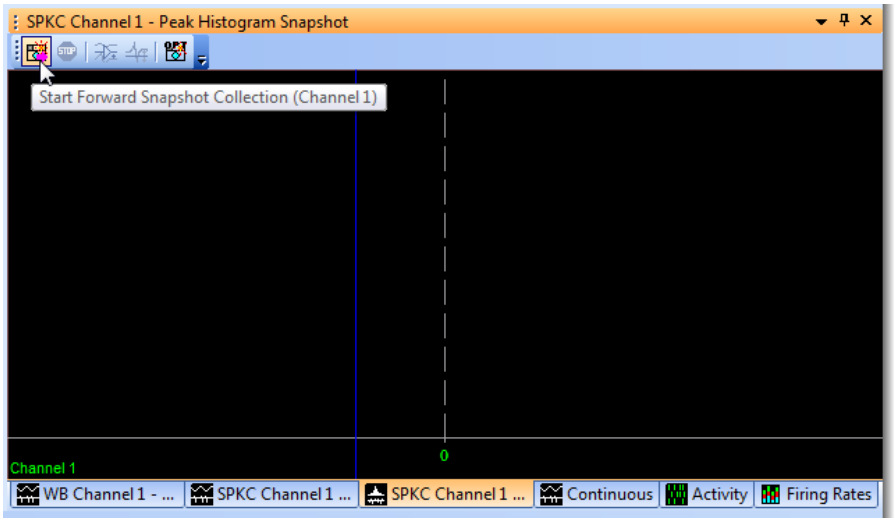
### 5.5.4 Dragging the Threshold Line in the SPKC Snapshot Peak Histogram View

Although, as described previously, a snapshot of a channel of continuous data consists of a certain duration of that signal, the OmniPlex System does not display a SPKC snapshot as a “frozen” segment of the SPKC data; rather, it displays the peak histogram for the snapshot data, which is considerably more useful.

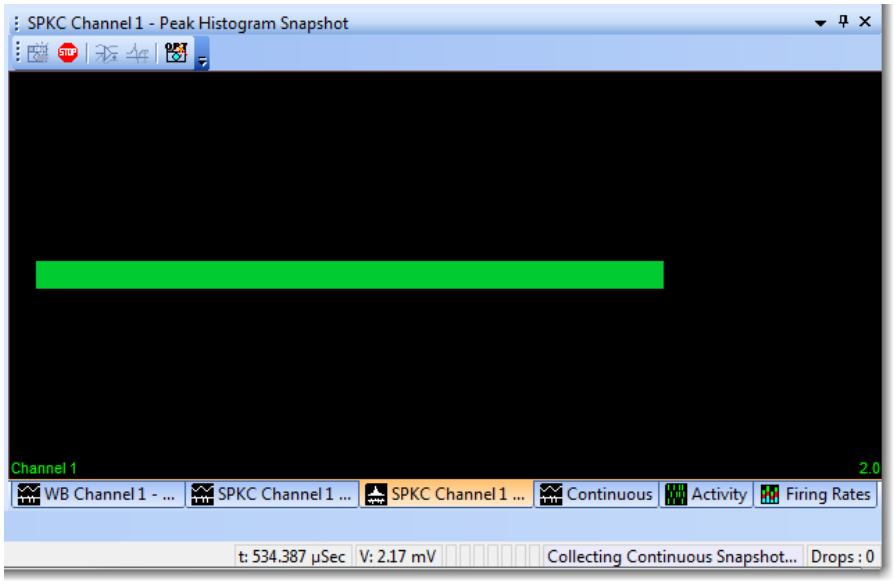
1 Click the SPKC - Channel tab which shows the Peak Histogram icon SPKC Channel...; if you have not yet collected a snapshot for that channel, you will see something like this:



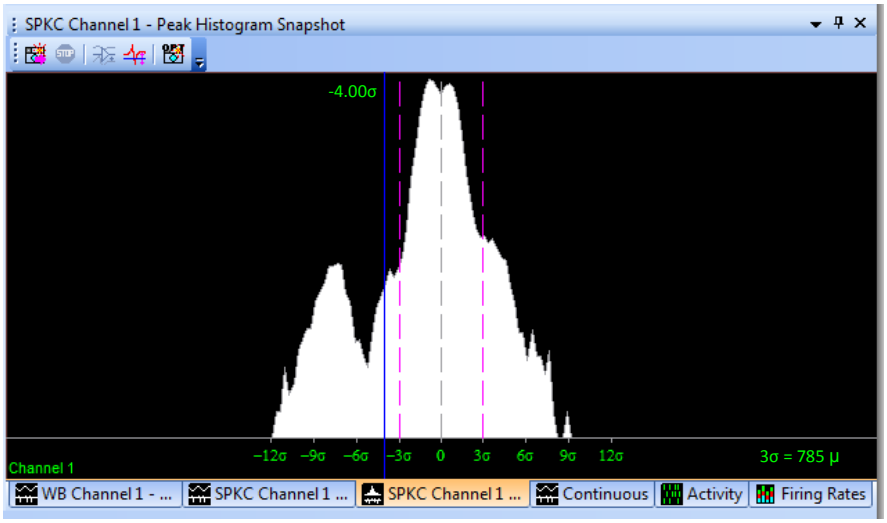

2 Display the toolbar (if it is not already visible) and click the **Start Forward Snapshot Collection** button; note that since we are only viewing one channel, only that channel's snapshot will be collected:



3 A progress bar is displayed as the continuous snapshot is being collected:




4 When collection is complete, the peak histogram for the channel is displayed:



**TIP**

**Use the mousewheel to change magnification**

You can click anywhere in the histogram window and then use the mouse wheel to increase or decrease the horizontal scale as desired. Changes to this horizontal scale do not affect the threshold value. For more information about this feature, see the discussion later in this section,

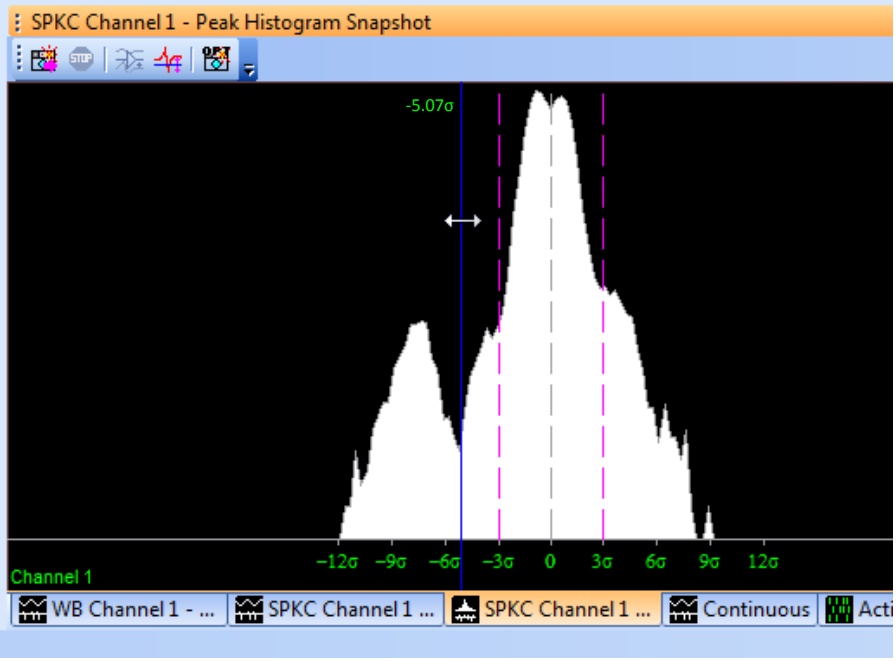
The peak histogram represents the distribution of peak (local maxima or minima) values in the SPKC snapshot. The horizontal axis represents amplitude, but in terms of standard deviations (sigmas) on either side of the mean. Note that since

the default options for SPKC snapshots are to perform an auto-threshold after the snapshot is collected, and the default auto-threshold is placed at -4 sigmas, there is a blue line representing the automatically-set threshold, expressed in terms of sigmas rather than percent.

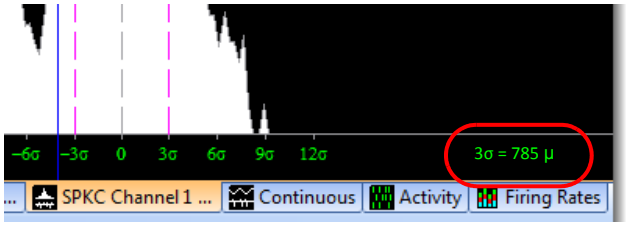
In short, the basic idea behind using the peak histogram is that for typical signals, there will be a large peak in the histogram, centered on zero sigmas, which corresponds to the noise in the signal - this is because spikes represent a relatively small percentage of the total duration of the SPKC signal, so noise samples will tend to be predominant in the distribution. By setting the threshold at or beyond the point where the “shoulder” of this distribution has its first clear discontinuity, we reduce the chance that noise will be incorrectly detected as spikes.

Outside of the central noise peak, significant peaks in the histogram, such as the very prominent one centered at approximately -8 sigmas in the example, usually represent spikes. You can see how the section of the histogram between approximately -3 sigmas and -5 sigmas is probably the sum of the tails of the central noise distribution and the distribution that is centered at -8 sigmas. In this case, the default auto-threshold value of -4 sigmas is a good compromise between rejecting low-amplitude noise and missing low-amplitude spikes.

Now that you understand what the blue threshold line means in the histogram display, you can use the mouse to directly adjust it if desired. Click and drag the threshold line; when you release the mouse button, the numeric threshold values, and the blue threshold lines in the other displays, will be updated to show the new threshold.

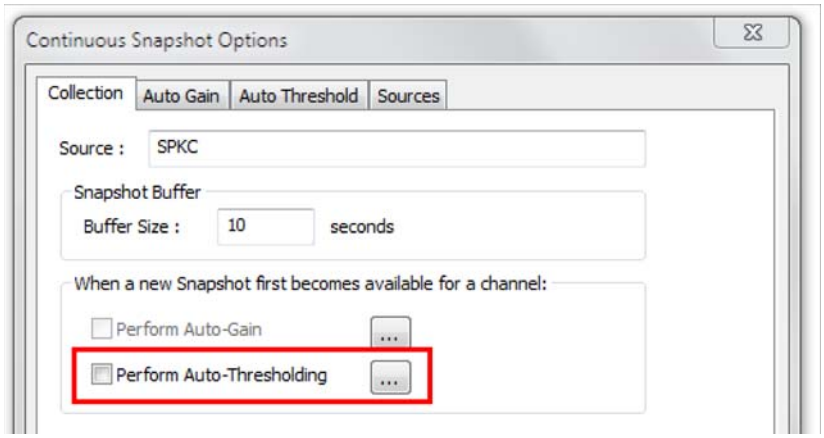


To provide an indication of the noise level on each spike continuous (SPKC) channel, the voltage value corresponding to three standard deviations in the SPKC peak histogram is displayed (in microvolts) in the lower right corner of the histogram.




Since the value of sigma is based on the peak histogram, i.e. based on local peaks in the SPKC signal (which is more informative than the histogram of the raw SPKC signal, for the purpose of setting thresholds), this value can differ from the sigma of the raw SPKC signal. Nonetheless, it can be useful as an indication of relative noise levels and changes in noise levels.

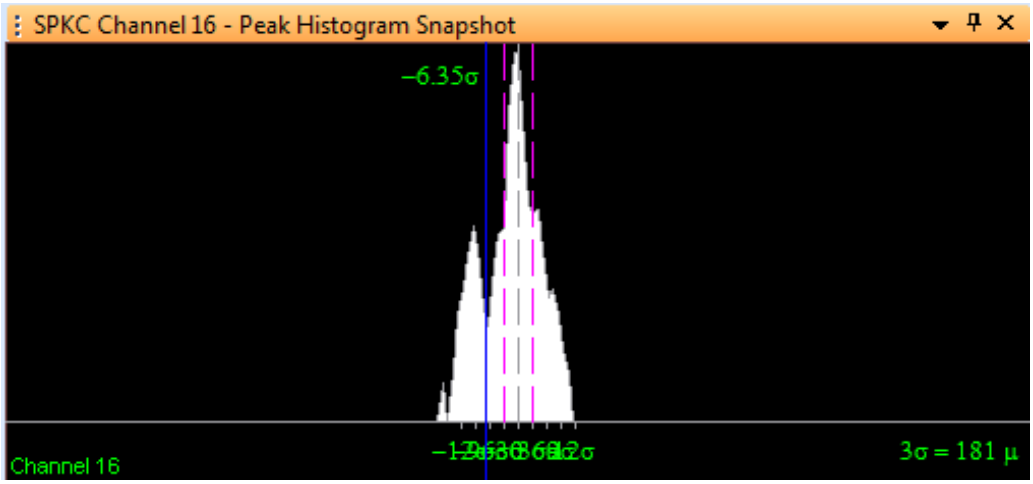
Remember that if you periodically take a new SPKC snapshot to update the sigma display, you should disable the **Perform Auto-Threshold** option in the SPKC snapshot options dialog before taking the additional snapshots, unless you do want the thresholds to be updated each time.



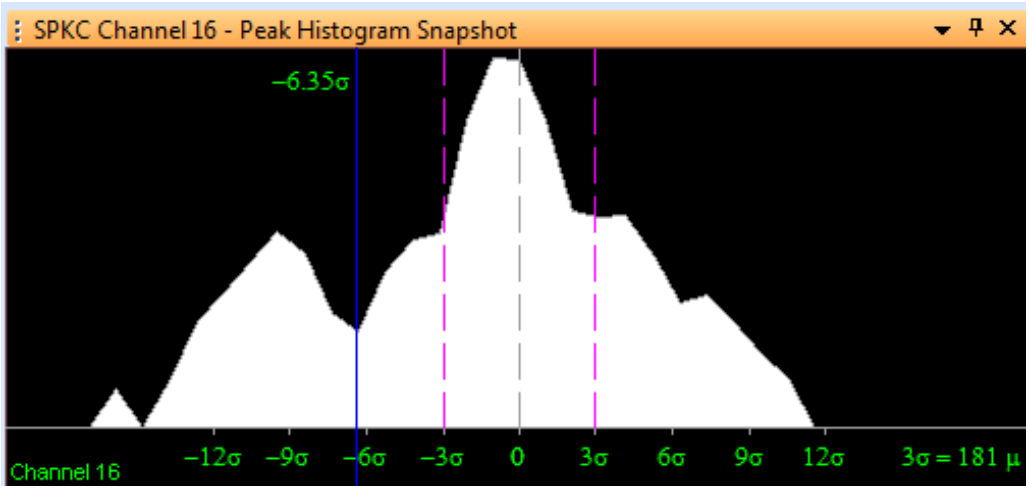
### Mouse wheel control of magnification in SPKC histogram view

Similar to the way in which you can use the mouse wheel to change the magnification in single-channel spike and continuous views, you can adjust the magnification of the SPKC histogram. Simply click in the histogram window and use the mouse wheel to increase or decrease the magnification as desired.

Before increasing magnification:




After increasing magnification:

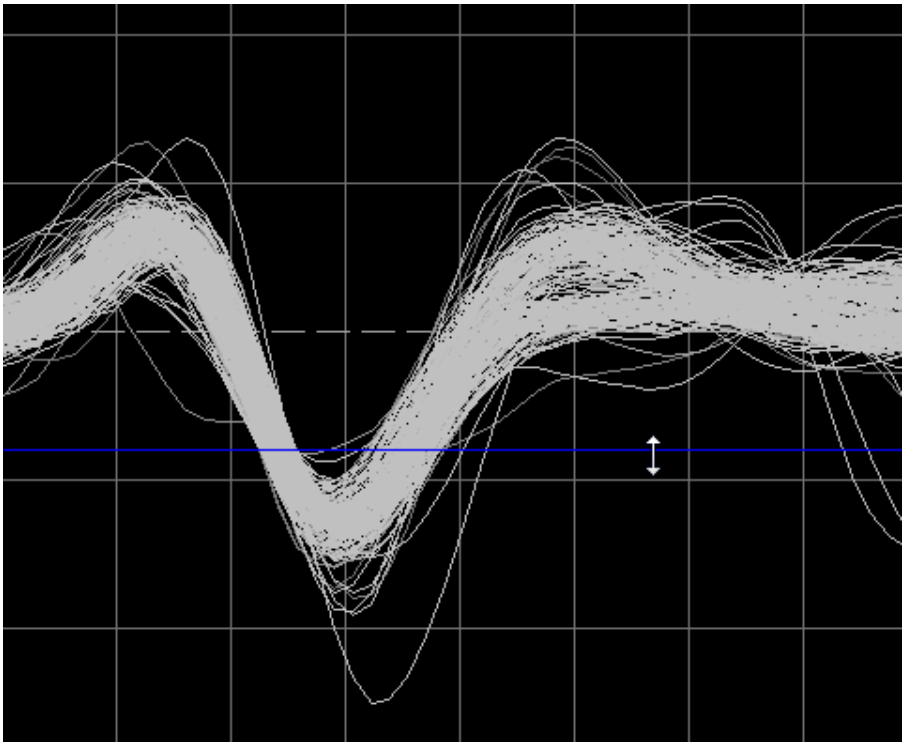



Note that the threshold is unaffected; only the graphical display of the histogram is scaled.

### 5.5.5 Dragging the Threshold Line in the Single-channel Spike View

You can also use the mouse to drag the blue threshold line that is shown in the main spike view. This is the SPK window which displays an enlarged view of the channel that is currently selected in the multichannel spike view, with a background time/voltage grid.

**1** Move the cursor over the threshold line until it changes to an up/down arrow cursor, then click and drag to move the threshold as desired:



**2** When you release the mouse button, the Properties view and Properties Spreadsheet will display the new threshold value as a percentage, and the blue threshold lines in the other displays will update as well.

Note in particular that the new amplitude threshold is shown on the peak histogram as the equivalent threshold in sigmas, and vice versa.

**TIP**

**Be careful when adjusting thresholds in the main spike window**

Be careful when adjusting thresholds in the main spike window, since you are only viewing the detected spikes, not the continuous spike (SPKC) signal, which is what spike detection is actually performed on. For example, if you set the threshold very far away from zero, you will not see any spikes at all in the main spike window, although of course the SPKC signal is still present and can be viewed in the SPKC - Continuous view. Similarly, even if you have an appropriate threshold value set, if the firing rate on the channel is low, you may only occasionally see spikes, making it difficult to judge the effect of adjusting the threshold. In such cases, you should use the SPKC - Continuous view and/or the SPKC peak histogram to set the threshold, as previously described.

### 5.5.6 Additional Resources and Methods

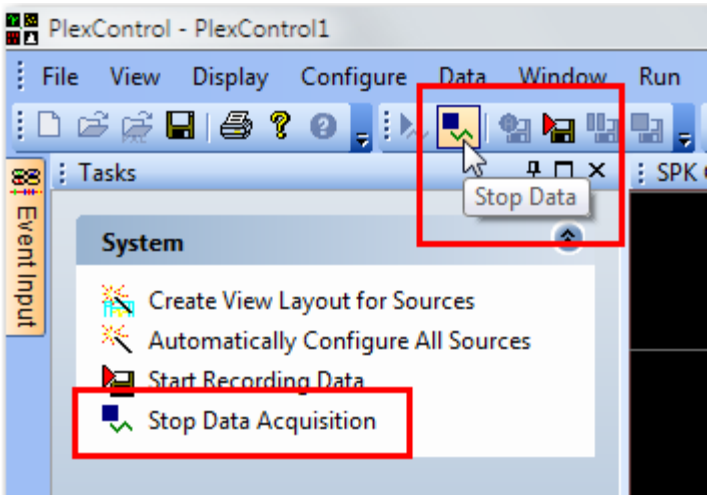
For additional background information on thresholding, refer to Section 5.1, “Spike Detection by Thresholding” on page 138.

In addition to, or as an alternative to manually adjusting the threshold as discussed in this section, you can use auto-thresholding to set the thresholds on all channels at a given number of sigmas from zero. See Section 5.3, “Using a Continuous Snapshot to Set Thresholds Automatically” on page 141.

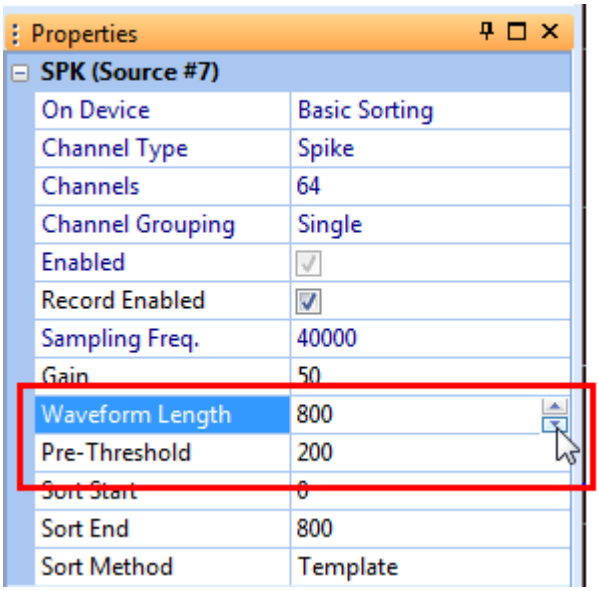
## 5.6 Changing the Spike Extraction Parameters

By default, when the OmniPlex System detects a threshold crossing in the SPKC data stream, it extracts an 800 microsecond segment and outputs it to the corresponding channel on the SPK source. The 800 microsecond waveform segment consists, by default, of a 200 microsecond pre-threshold interval and a 600 microsecond post-threshold interval, with the timestamp for the spike defined as the time of threshold crossing. You can change these values, for example to increase the waveform length to capture long, complex action potentials, or to decrease the length, to avoid capturing an unwanted "noise tail" when the spikes are shorter.

1 To change the thresholding (spike detection) parameters, you must first stop data acquisition, either from the main toolbar or the Tasks view:

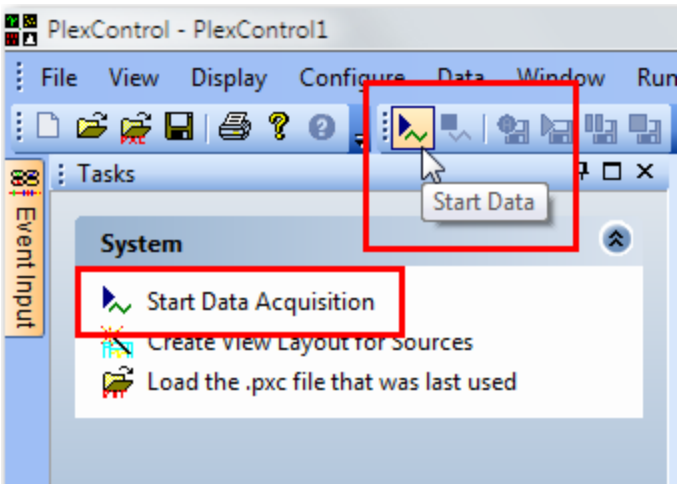



2 Once data acquisition has stopped, click in the Properties view at the left to change the **Waveform Length** and **Pre-Threshold**:



The post-threshold length is not editable, as it is always the difference between the waveform length and the pre-threshold time (800-200 = 600 microseconds, in this example).

3 To make the new settings take effect, start data acquisition again:




Note that changing the waveform length will invalidate a number of the spike sorting parameters (for example, sorting templates and principal component analysis (PCA) projections depend on the waveform length, as described later) so you will usually make any changes to the thresholding parameters before proceeding to set up spike sorting. If in doubt as to a good waveform length, you should make the waveform length no longer than necessary to capture the main features of the spike shape, as seen in the main spike window. Long waveforms (> 1000 microseconds) should be avoided in most cases.

**TIP**

**Waveform length and pre-threshold cannot be changed during recording**

Since you must first stop data acquisition, note that this also means that the waveform length and pre/post threshold intervals cannot be changed during a recording, or while online client programs are reading live data from the OmniPlex System.

For guidance on increasing the maximum spike waveform length, see Section 5.7, “Maximum Spike Waveform Length” on page 170.

### Dead time

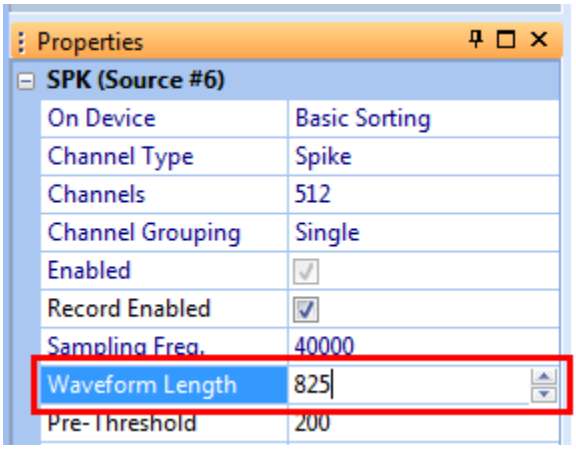
Some thresholding algorithms, such as the one in Plexon's Offline Sorter™, have the concept of “dead time,” which is an interval of time after each spike detection during which a thresholding algorithm ignores subsequent threshold crossings. Currently, the OmniPlex System does not support a fixed dead time. That is, on the next sample point after the end of an extracted spike, the thresholder starts scanning the continuous spike signal for the next threshold crossing. If the next threshold crossing occurs within the next few samples after the end of the previous spike, it is possible that the pre-threshold interval of the second spike will overlap the last few points of post-threshold interval of the first spike. If this causes problems, either in spike sorting or in your data analysis, you should consider reducing the waveform length and/or the post-threshold interval.

### Aligned Extraction

See Section 15.4.4, “Thresholding By Aligned Extraction” on page 495 for details on aligned extraction, which is an alternative extraction method which can give results superior to standard thresholding in many cases.

## 5.7 Maximum Spike Waveform Length

In most cases, the default waveform length (800 microseconds) should be adequate. However, the system supports a maximum spike length of 224 points or (224 * 25 microseconds) = 5600 microseconds = 5.6 milliseconds. To change the waveform length from its default of 800 microseconds, stop data acquisition and modify the length in PlexControl:

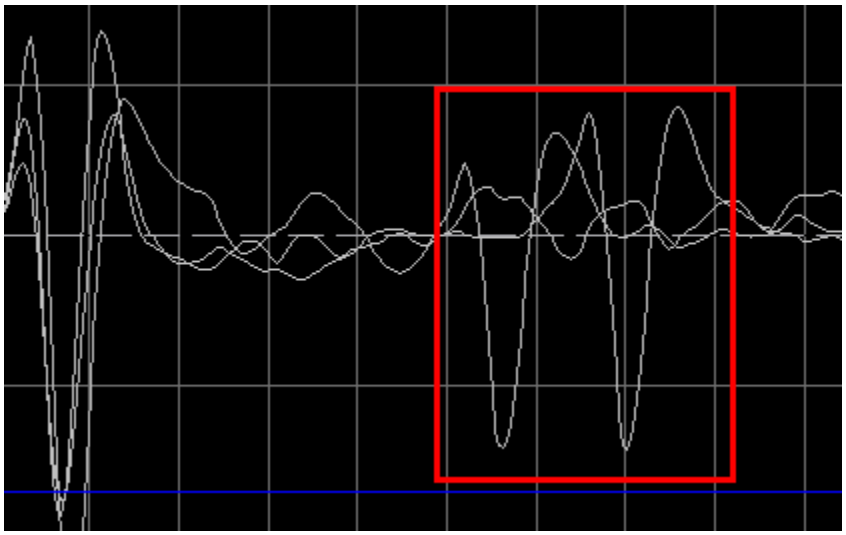



Depending on the shape of the spike waveforms, you may also wish to modify the pre-threshold interval.

The following considerations should be kept in mind when using long waveforms.

### Overlaps / superpositions in the tail of spike waveforms

A common mistake is to set a very long waveform length "to make sure we get the entire spike." While it is desirable to capture the full action potential, remember that the tail of a spike decays into some combination of noise and action potentials from nearby units. If the waveform length is longer than necessary, it is more likely that the tail of the spike will unintentionally include the superposition of a portion or all of other action potentials:



This is problematic in that these “tail overlaps,” which could be subsequent firings of the same unit, or action potentials from other cells, will not be detected and timestamped as separate spikes. In addition, their superposition onto the initial spike’s shape can be thought of as large-amplitude noise spikes which make it difficult or impossible to sort the distorted spike shape. When viewed in PCA feature space, these overlap-corrupted waveforms tend to appear as outliers.

The Plexon Offline Sorter software, Version 4.0 and later, has functionality which can in some cases “untangle” such overlapped spikes, but this can be a labor-intensive process, may not succeed in extreme cases, and requires that you record the continuous wideband or spike-continuous signal. Therefore, you should only increase the spike length in cases where the action potentials from the cells that you are recording are in fact significantly longer than the default 800 microseconds, and you should visually check the resulting detected spikes for excessive overlaps. However, a small percentage of overlaps are unavoidable in many cases, especially when recording highly active cells, and this can usually be tolerated without adversely affecting subsequent analyses.

Note that the line sorting and band sorting methods are more robust to the presence of overlaps in the tail of spike waveforms. In line sorting, you can avoid drawing sort lines in the tail region, so that sorting is immune to shape distortions in the tail. With band sorting, you can set narrow band widths in the “important” middle part of the spike’s shape, and larger widths in the tail. In comparison, with template sorting you can only adjust a single sum-of-squared-errors tolerance value for the entire spike, with no way to indicate to the sorting algorithm that some segments of the spike waveform are more likely to be “clean” than others (e.g. the tail). Note that although line and band sorting are better able to tolerate shape distortions that only occur in a subset of the spike, overlapped waveforms that are unintentionally captured in the tail are still “missed waveforms” which are not captured and timestamped separately.

### Increased processor load

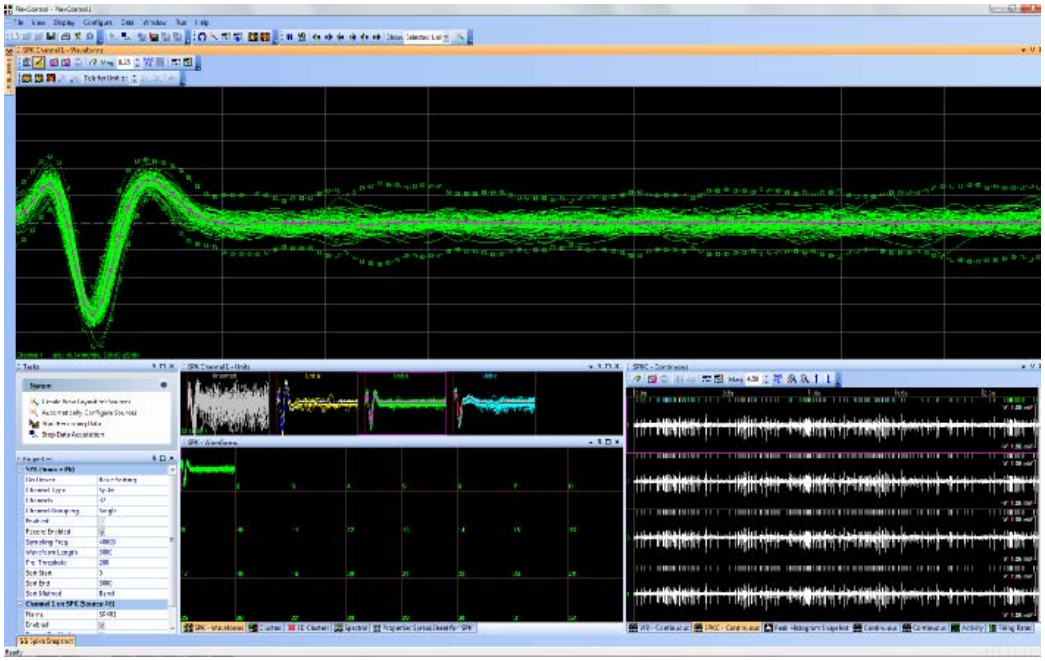
Acquiring very long spikes can increase the amount of processing power required to sort, display, and record spikes. As with normal-length spikes, processor load is also dependent on the number of channels, the spike firing rates, and can be exacerbated by unnecessarily low thresholds. If setting a large waveform length appears to cause performance problems or data drops, see "Appendix E: Lowest Latency Operation and PC Performance Considerations" on page A-31 for more information on how to monitor processor usage and optimize performance.

### Recording: PL2 versus PLX

The PLX legacy file format cannot record spikes longer than 56 points (1400 microseconds). If you attempt to record longer waveforms to a plx file, they will be truncated in the file so that only the first 56 points are recorded. The PL2 format can record the full 224 point maximum length. Note that with either file format, acquisition and recording of continuous data (WB, SPKC, FP) are not affected by the spike waveform length.

### User interface

When working with very long spikes, you may find that user interface elements in the main spike window become too crowded to work with comfortably. In particular, the editing handles on lines, bands, and templates can overlap, making it difficult to select and move them. In such cases, making the main spike window as wide as possible, and/or dragging it to a separate monitor, will make defining and editing units easier. See Chapter 4, PlexControl User Interface, and Section 13.8, “Advanced User Interface Features” on page 442 for information on how to resize and rearrange windows within PlexControl.



## 5.8 Advanced Threshold Configuration Options

The **Thresholding Configuration** dialog offers several tools for adjusting the thresholding behavior, such as Aligned Extraction. See Section 15.4, “Thresholding Configuration Options” on page 490.

# 5 Spike Detection

# Chapter 6
# Basic Spike Sorting

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## 6.1 Overview

Now that you have learned how to configure the OmniPlex® System to correctly detect spike waveforms, the next step is spike sorting. Spike sorting is the process of determining, for each detected spike waveform on each channel, which neuron near the corresponding electrode tip fired. For example, the electrode for channel 5 might be picking up action potentials from three nearby neurons, call them SPK05a, SPK05b, and SPK05c. Informally, we would say “there are three units on channel 5.” Spike sorting is in effect a classification problem, where incoming unsorted spike waveforms are sorted into classes a, b, c, etc, each class corresponding to one neuron. Most spike sorting algorithms are based on the assumption that each neuron produces action potentials whose shape is sufficiently distinct from the shape of other neurons' spikes to allow reliable classification.

### 6.1.1 The Two Elements of Spike Sorting

It should be emphasized that there are two distinct procedures involved in spike sorting. The actual sorting of incoming spikes is done in Server, and once configured, is a process that generally requires little or no user intervention. But first, PlexControl must determine the spike sorting parameters that Server will use; for example, how many distinct units are there on each channel, and what are the sorting parameters for each of those units? The OmniPlex System supports several methods for both defining the sorting parameters, and for the actual spike sorting.

### 6.1.2 OmniPlex System Spike Sorting Methods

The following spike sorting methods, which will be described later, are currently supported by the OmniPlex System:

- Template sorting
- Line sorting
- Band sorting
- Box sorting
- 2D polygon sorting

The first four sorting methods operate directly on the set of waveforms samples, i.e. the raw detected spike, while polygon sorting operates on a projection of the waveform into a two-dimensional feature space. These are the algorithms that run in the sorting device in Server, sorting incoming spikes in real-time and writing them to the Main Datapool.

### 6.1.3 OmniPlex System Unit Definition Methods

The OmniPlex System supports three methods for manually defining units, given a set of spikes. These are the techniques that are available in PlexControl for your use; the unit definitions that are created are sent to the sorting device in Server to perform the actual sorting.

- Waveform crossing
- 2D PCA cluster circling
- 3D PCA cluster circling

Any of the unit definition methods can be used to define units for any of the sorting methods, with the exception of the line and box sorting methods. Line sorting and box sorting are different in that the unit definition method and the sorting method are identical for each method; in these two sorting methods, you graphically specify the sorting parameters, directly "on" the waveforms, as opposed to the OmniPlex System calculating the sorting parameters from a set of waveforms.

In this edition of the user guide, we will only cover a subset of the sorting methods and unit definition methods; others will be described in separate sections at a later date. You can also contact Plexon® technical support (+1 214-369-4957 or support@plexon.com) for more information.

### 6.1.4 Automatic Spike Sorting

In addition to methods for manually defining units, the OmniPlex System provides several auto-sorting algorithms. Automatic sorting, also known as unsupervised unit classification, can be a challenging problem, especially in cases where the spike waveforms do not have clearly distinct, unit-specific shapes, or are noisy. Even in cases where automatic sorting does not provide a perfect first-pass solution, it can be valuable, especially at high channel counts, in that it gives you a set of initial unit definitions which you can then adjust and improve manually. See Section 9.1, "Introduction to Automatic Sorting (Automatic Unit Finding)" on page 298.

### 6.1.5 OmniPlex System Spike Sorting as a Toolbox

It's easy to be a bit overwhelmed by the variety of spike sorting and unit definition techniques that are available in the OmniPlex System, but none of them, taken individually, are overly complex. Some methods are especially easy to use when defining units manually (e.g. line sorting), while others are more suited for use with automatic sorting (e.g. band sorting). As with any toolbox of techniques, each user will find that they have preferences as to which methods work best for them, their working methods, and their data.

First we will describe the simplest, and probably most widely used sorting method, template sorting, and the simplest unit definition method, waveform crossing. Once you are familiar with this, you will understand a number of techniques which are applicable to the other methods, which you can then learn at your convenience.

## 6.2 Selecting Sort Method and Entering Unit Editing Mode

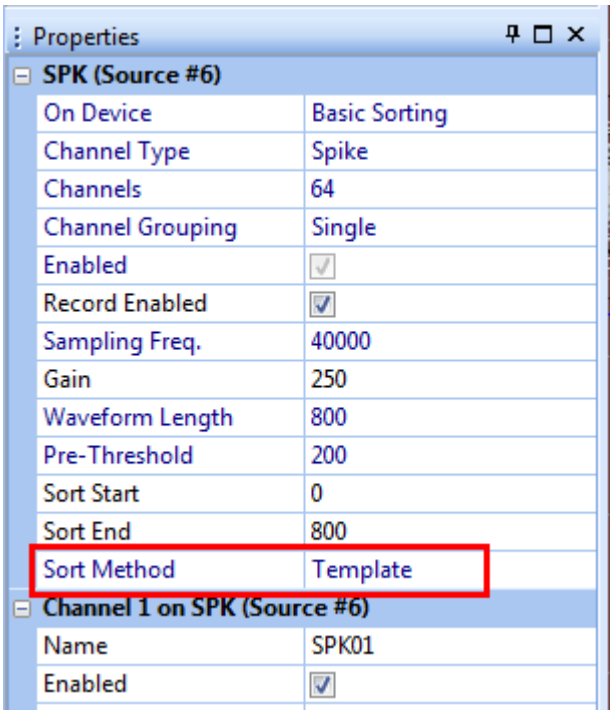
1 As described in previous sections, start data acquisition, set an appropriate gain and collect an SPKC snapshot, so that suitable thresholds are set on all channels and spikes are appearing in the multichannel spike window.

**Note:** Setting the gain is applicable to DigiAmp subsystems, not DHP subsystems.

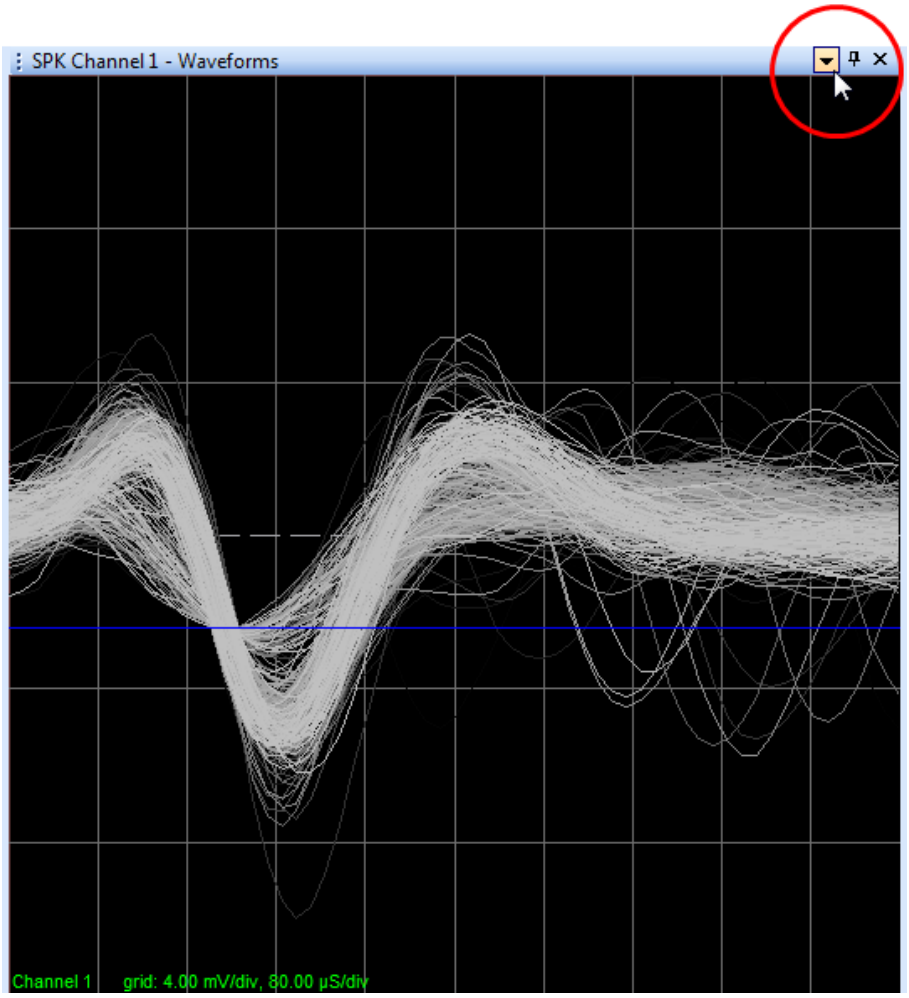
**TIP**

**Set the sorting mode before starting data acquisition**

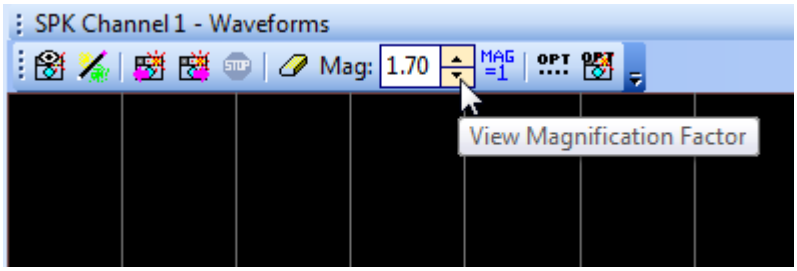
Before starting data acquisition, make sure that the OmniPlex System is set to the correct sorting mode, as shown in the image below.



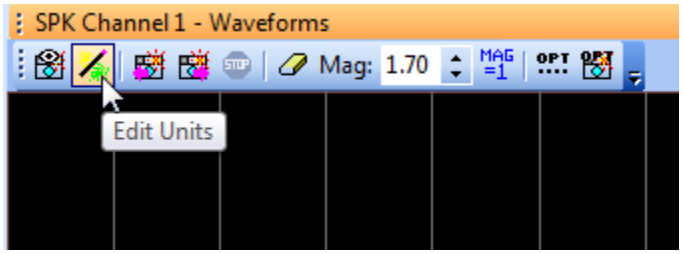
2 In the main spike window, display its toolbar by clicking on the **down-arrow** at the upper-right corner of the window:



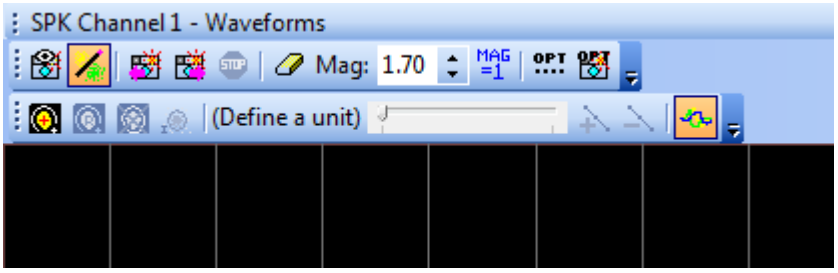

3 Make sure that the incoming spikes are displayed at a large enough size that you can see them clearly; use the Magnification control in the toolbar to increase the magnification if necessary:



4 After adjusting the magnification, click on the **Edit Units** button:




This enters unit editing mode, in which you can add, delete, and modify units on the currently selected channel. The unit editing toolbar is displayed below the main spike toolbar:




Note how the **Edit Units** button remains highlighted in the main spike toolbar, to indicate that you are in unit editing mode. If you click on the **Edit Units** button again, you will exit from unit editing mode, and the unit editing toolbar will disappear. But for now, remain in unit editing mode.

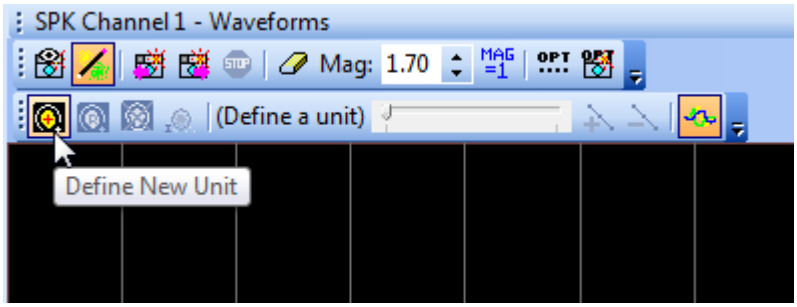
**TIP**

**Click to select a current channel**

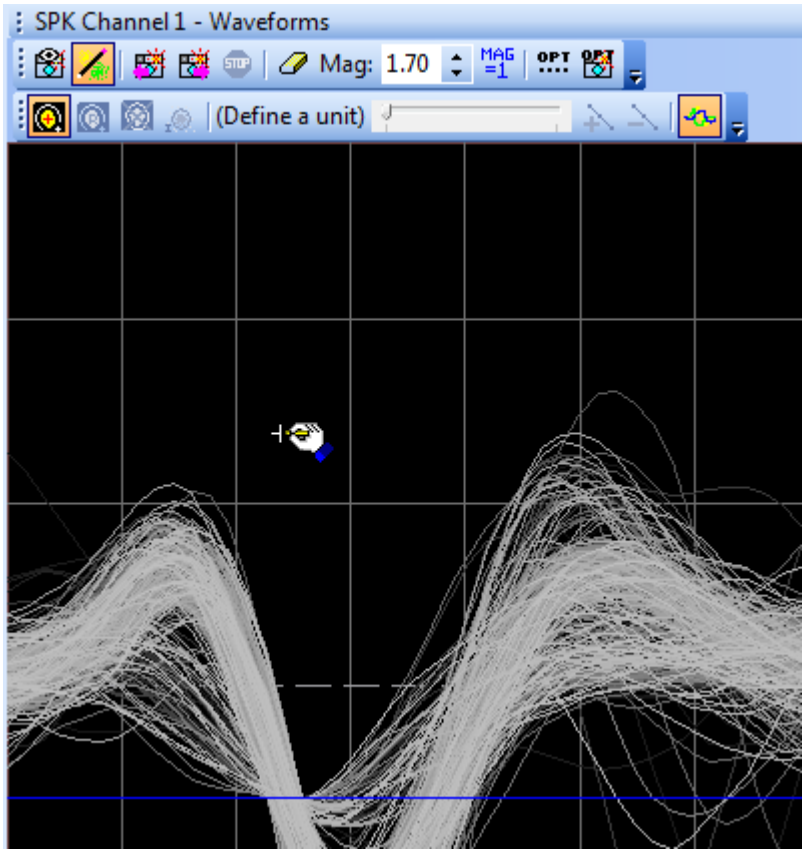
While in unit editing mode, or at any other time, you can click on any channel in the multichannel spike window to select it as the current channel. In other words, you don't have to jump in and out of unit editing mode to add and delete units on different channels.

## 6.3 Adding a New Unit

1 Click the **Define New Unit** button in the unit editing toolbar:



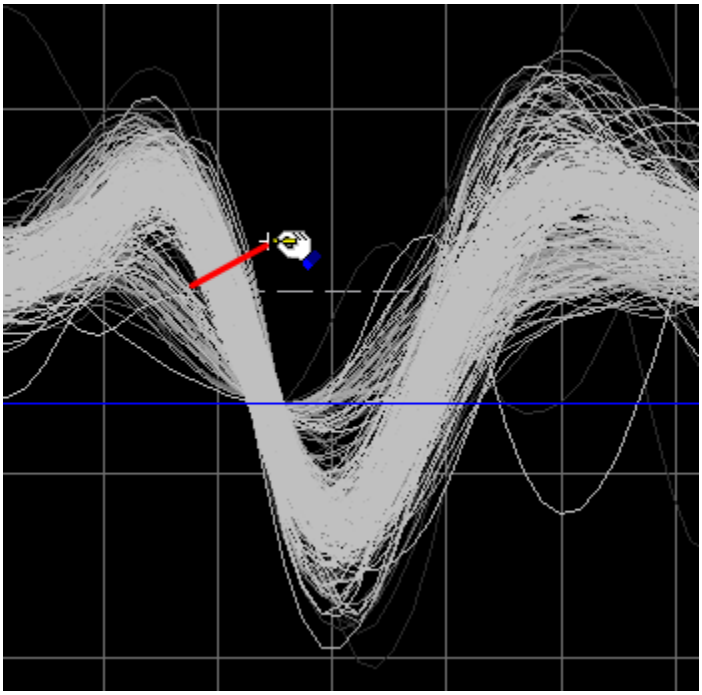

If you move the cursor over the main area where spikes are being displayed (do not click yet), you will see that it is now shown as the **Add Unit** cursor:




What you will do next is based on the following scheme. As incoming spikes are displayed, spikes of similar shape, which are assumed to be spikes originating from the same neuron, will naturally tend to be grouped into visually identifiable subsets or "bundles" of waveforms. You will draw a line across each of these bundles to indicate that those waveforms should be used to calculate a mean waveform that will be used for template sorting. In other words, the spike waveforms that cross (intersect) the line you draw will be averaged together and sent to the spike sorting device in Server, which will use that template waveform to sort incoming spikes. When defining a unit, the best place to draw a crossing

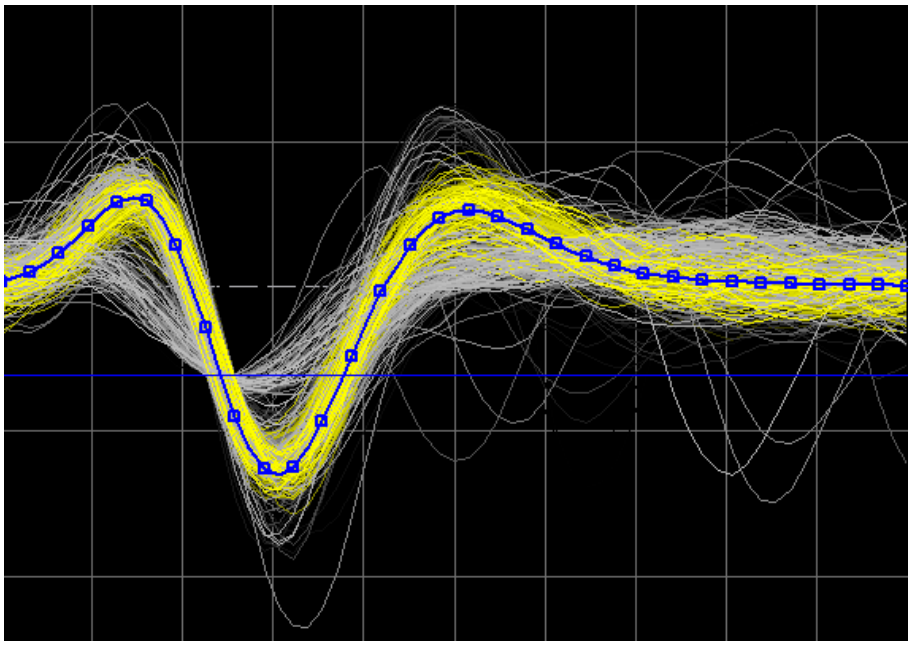
line is where the bundles of waveforms are most distinctly separated from each other. Don't worry if you don't get good results right away - the OmniPlex System lets you delete a template and start over, and with a little practice, you will get an intuitive feel for the most effective placement of waveform crossing lines.

**2** To add a unit by drawing a waveform crossing line, place the cursor where the line should start, press the left mouse button, drag to draw the line, and release the mouse button at the point at which the line should end:



**3** When you release the mouse button, the crossing line is removed, and the template that was created is displayed, with small squares indicating the points of the template waveform. This is the first defined unit on this channel,

so it is unit “a.” Incoming spikes that match the new template are now displayed in a unit-specific color:



So how does the OmniPlex System determine which spikes match a template? The algorithm for template sorting is based on computing an error measure for each incoming spike, relative to each template. The error measure is the sum of squared amplitude differences between the spike and the template waveform, where the differences are taken between corresponding points (i.e. sample values) on the spike waveform and the template waveform. Also, when a new template is computed from a set of waveforms that you selected via waveform crossing, a multiple of the standard deviation of the set of waveforms is used as the fit tolerance for that unit, by default one sigma.

In other words, for each incoming spike, the sum-of-squares error between it and each of the templates for that channel is calculated. To match a template, the error value for the spike, relative to that template, must be less than the fit tolerance. If a spike is within the fit tolerance of more than one template, then the unit whose template yielded the lowest error (closest template fit) wins. If the incoming spike is not within the fit tolerance of any of the templates that are defined on its channel, it remains unsorted.

Therefore, the definition of each unit consists of the template waveform and the associated fit tolerance. Once created, the unit definition is sent from PlexControl to the sorting device in Server, which immediately begins using it to sort incoming spikes, which are then displayed in PlexControl in their unit colors.

**Note:** You can use the mouse to move individual template points, as marked by the small squares. However, this can be a time consuming process and is generally unnecessary.

**TIP**

**“Unit” and “Unit definition”**

Informally, you will often see the terms “unit” and “unit definition” used interchangeably.

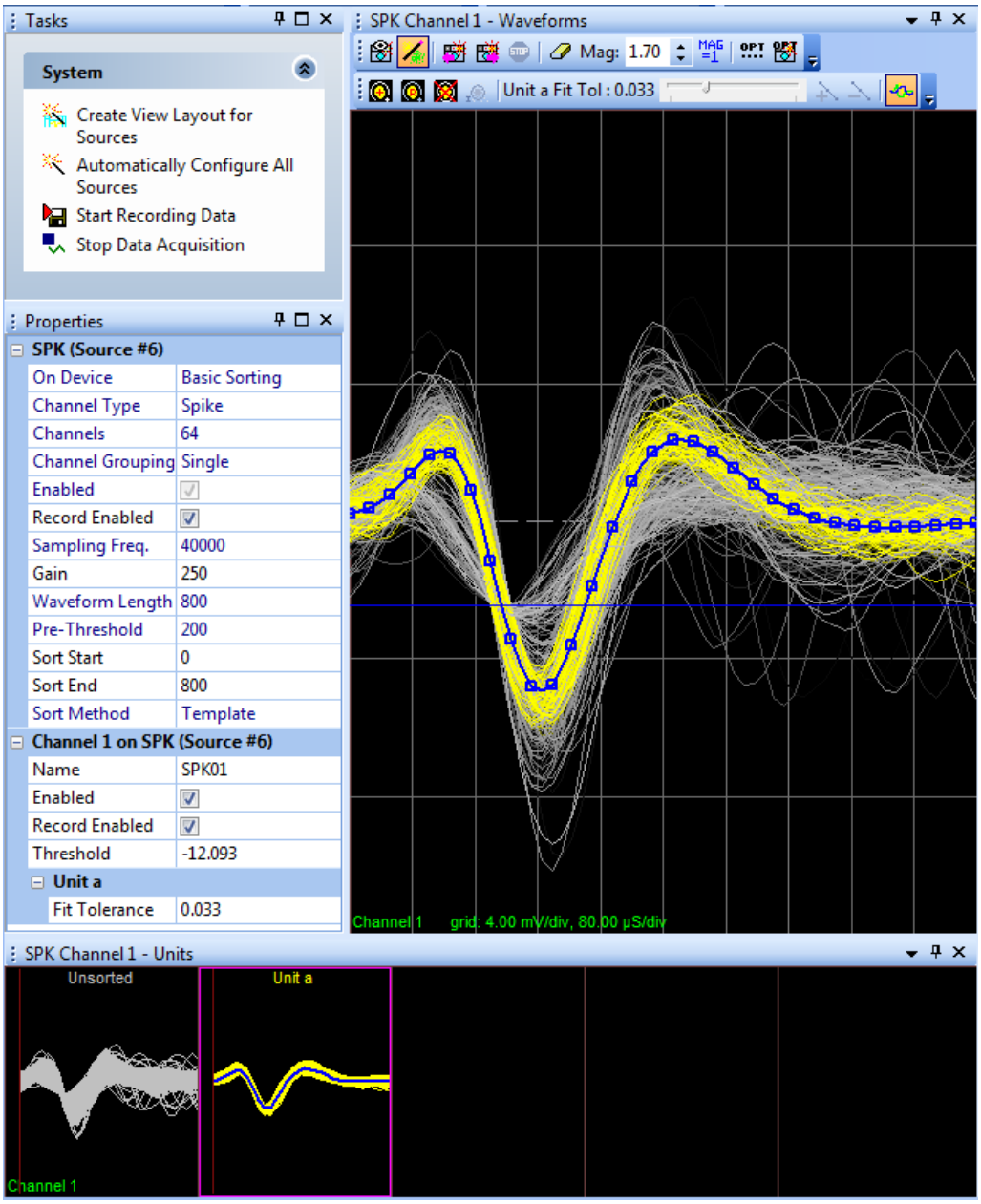
**TIP**

**Add several units using this shortcut**

Rather than clicking the Add Unit button in the toolbar each time you wish to add a new unit, you can simply hold down the CTRL key and draw a crossing line in the main spike window, which is interpreted as an Add Unit command. An experienced user can quickly add several units using this shortcut.

## 6.4 Changing the Fit Tolerance for a Unit

Sorted and unsorted units for the current channel are also displayed in the Units window below the main spike window:

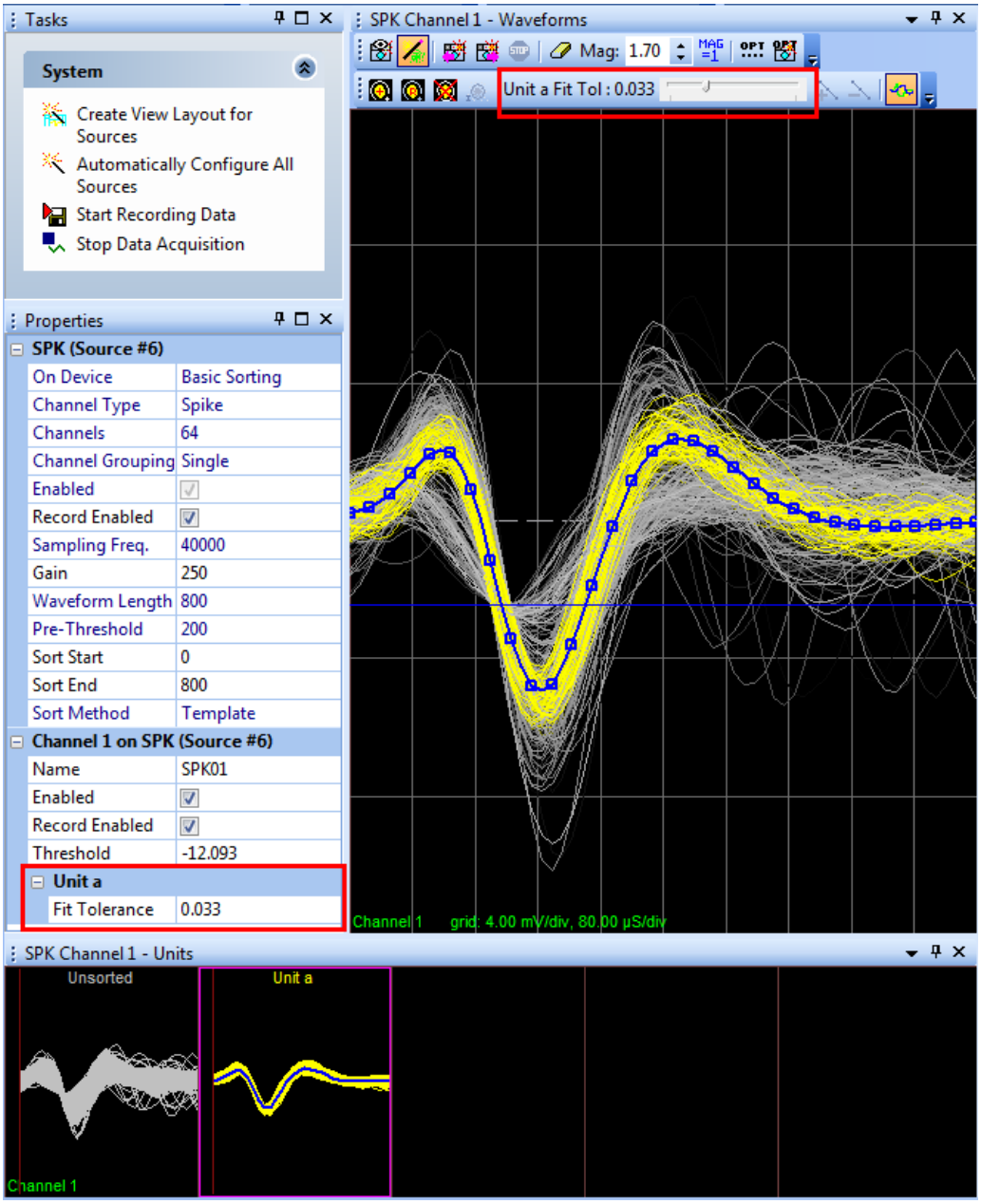



This is useful, because it reduces the visual clutter and allows you to see what is being “sorted into” each unit in isolation.

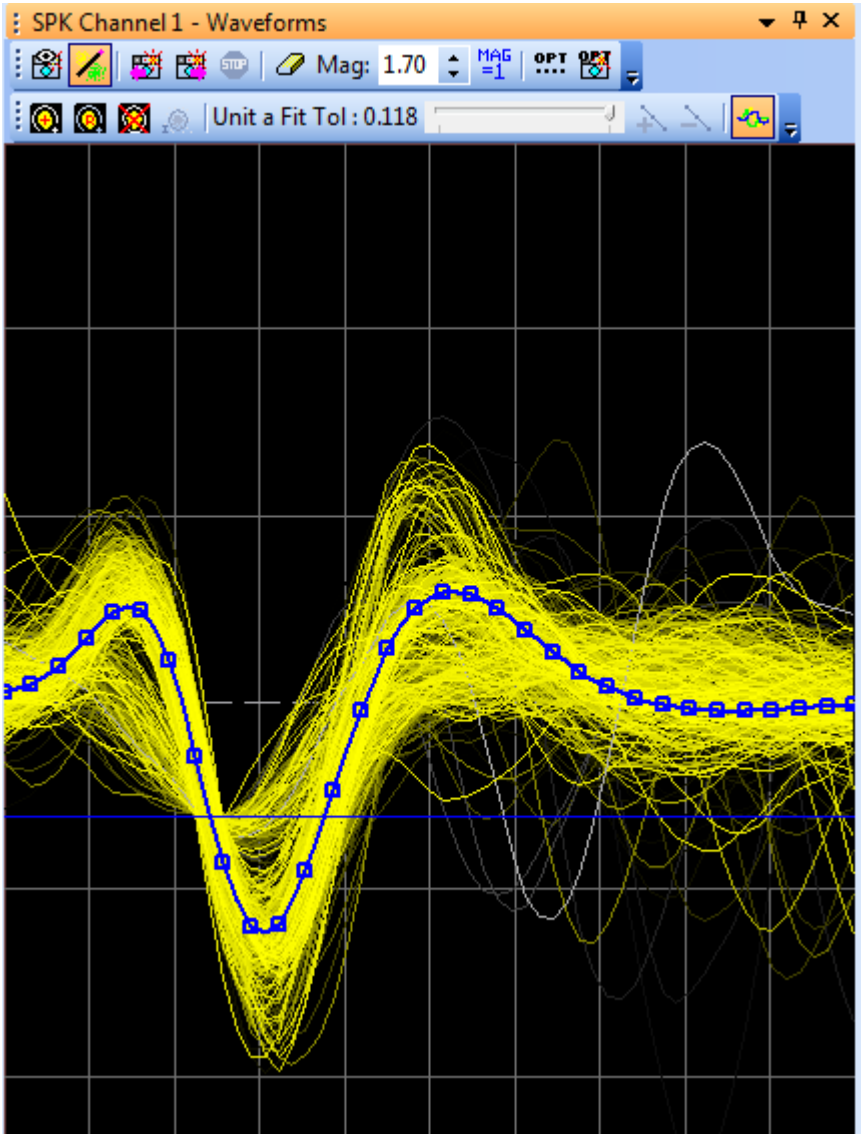
Within the Units Window, the colored highlight rectangle indicates the currently selected unit, which is analogous to the currently selected source and channel.

You can click on a unit in the Units Window to select that unit, which allows you to perform tasks on it such as changing its fit tolerance or deleting it.

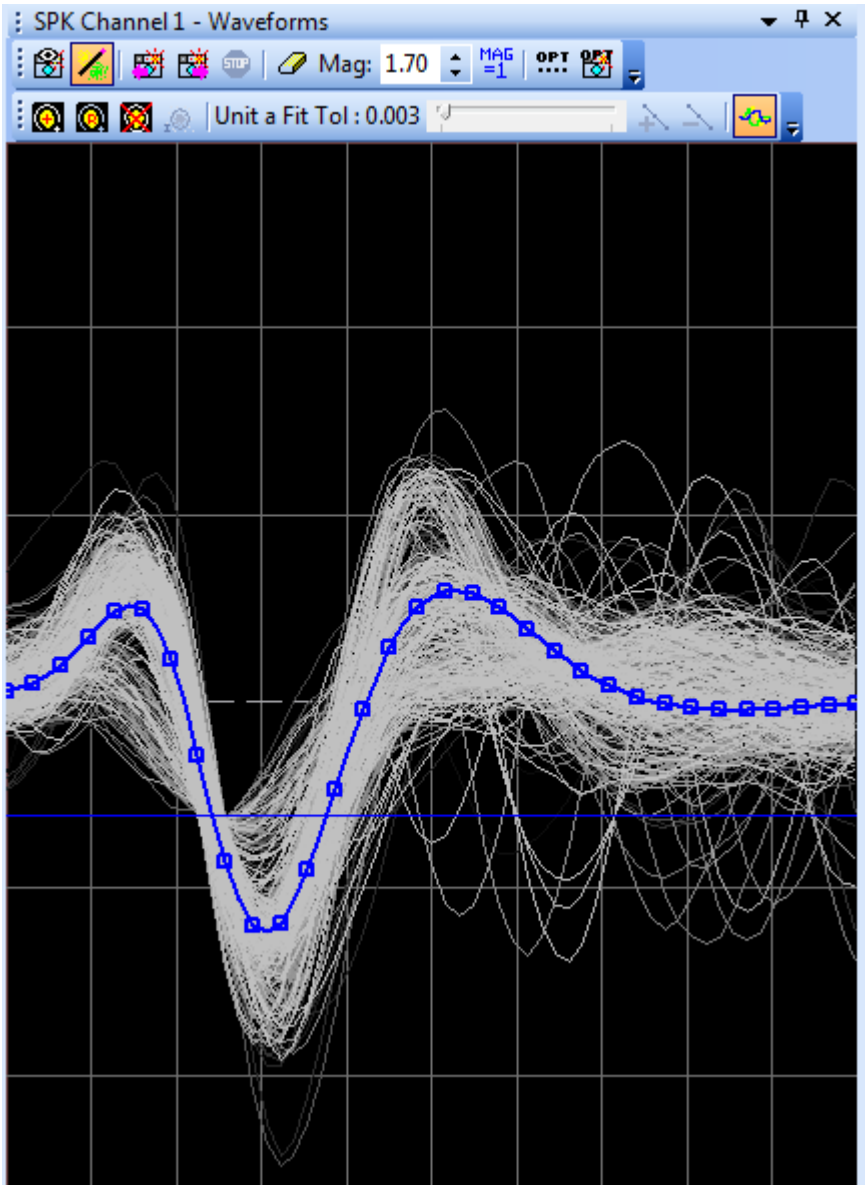
When a unit is selected in the Units Window, its fit tolerance is displayed as both an editable value in the Properties view at the left, and as a value with an adjustable slider in the unit editing toolbar:



If you decrease the fit tolerance, the net effect is to make the template matching “stricter” - incoming spikes must more closely match the template. If you increase the tolerance, the template matching becomes “looser” - the error measure between the spike and the tolerance can be larger, yet still qualify as a match to the template. As an extreme example, if you only have a single unit defined on a channel, but the fit tolerance is set to the maximum, then most or all detected spikes will be sorted as unit “a”:



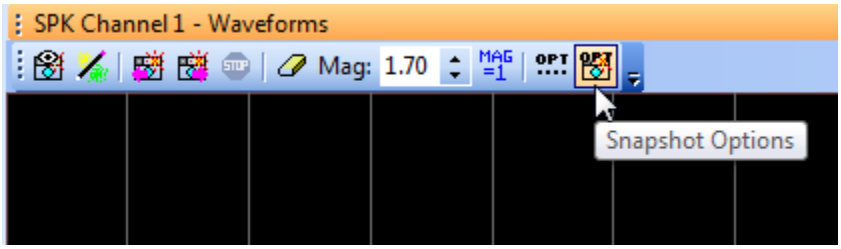
Conversely, if the fit tolerance is too low, then no units will be sorted as unit “a”:



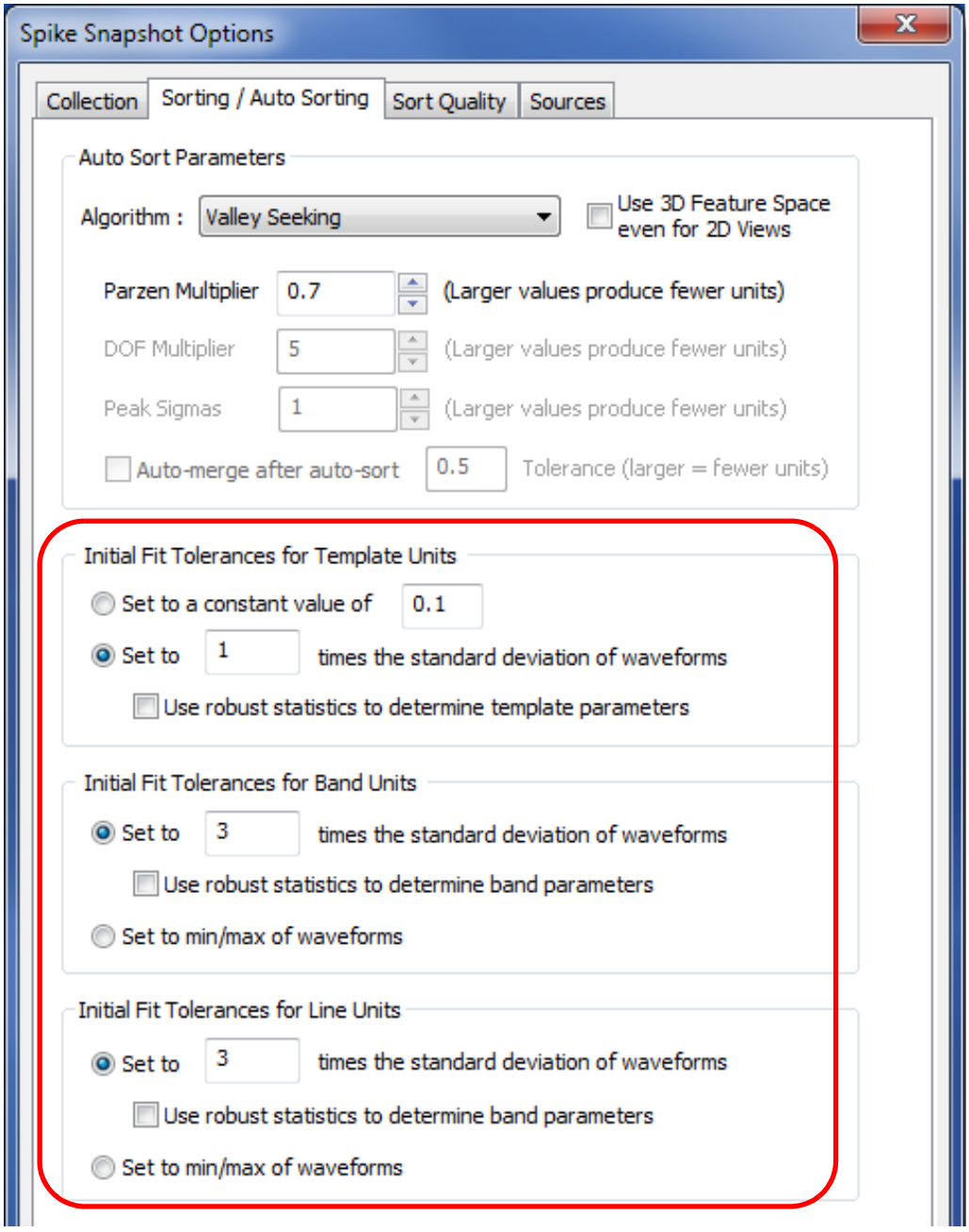

Decreasing the tolerance can “tighten up” the sorting of a unit, but risks excluding valid matches; increasing the tolerance avoids being “too strict,” but risks creating false matches. PlexControl provides some useful visualization options that help you to evaluate the effect of your changes to sorting parameters, and these options can be used with any of the sorting methods.

## 6.5 Changing the Default for the Initial Fit Tolerance

As mentioned, the default fit tolerance for a newly created template is one standard deviation, relative to the set of waveforms used to create the template. You can change this default value in the **Spike Snapshot Options** dialog. To access it, click the toolbar button:

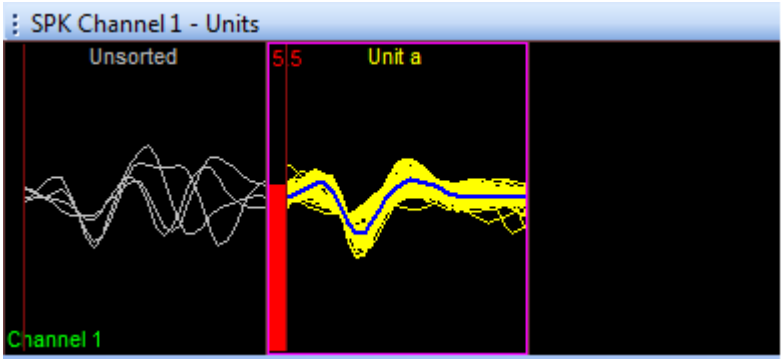



The **Spike Snapshot Options** dialog is displayed. Adjust the initial fit tolerance as desired and click **OK** to accept your changes.


Spike Snapshot Options
Collection
Sorting / Auto Sorting
Sort Quality
Sources
Auto Sort Parameters
Algorithm :
Valley Seeking
Use 3D Feature Space even for 2D Views
Parzen Multiplier
0.7
(Larger values produce fewer units)
DOF Multiplier
5
(Larger values produce fewer units)
Peak Sigmas
1
(Larger values produce fewer units)
Auto-merge after auto-sort
0.5
Tolerance (larger = fewer units)
Initial Fit Tolerances for Template Units
Set to a constant value of
0.1
Set to
1
times the standard deviation of waveforms
Use robust statistics to determine template parameters
Initial Fit Tolerances for Band Units
Set to
3
times the standard deviation of waveforms
Use robust statistics to determine band parameters
Set to min/max of waveforms
Initial Fit Tolerances for Line Units
Set to
3
times the standard deviation of waveforms
Use robust statistics to determine band parameters
Set to min/max of waveforms

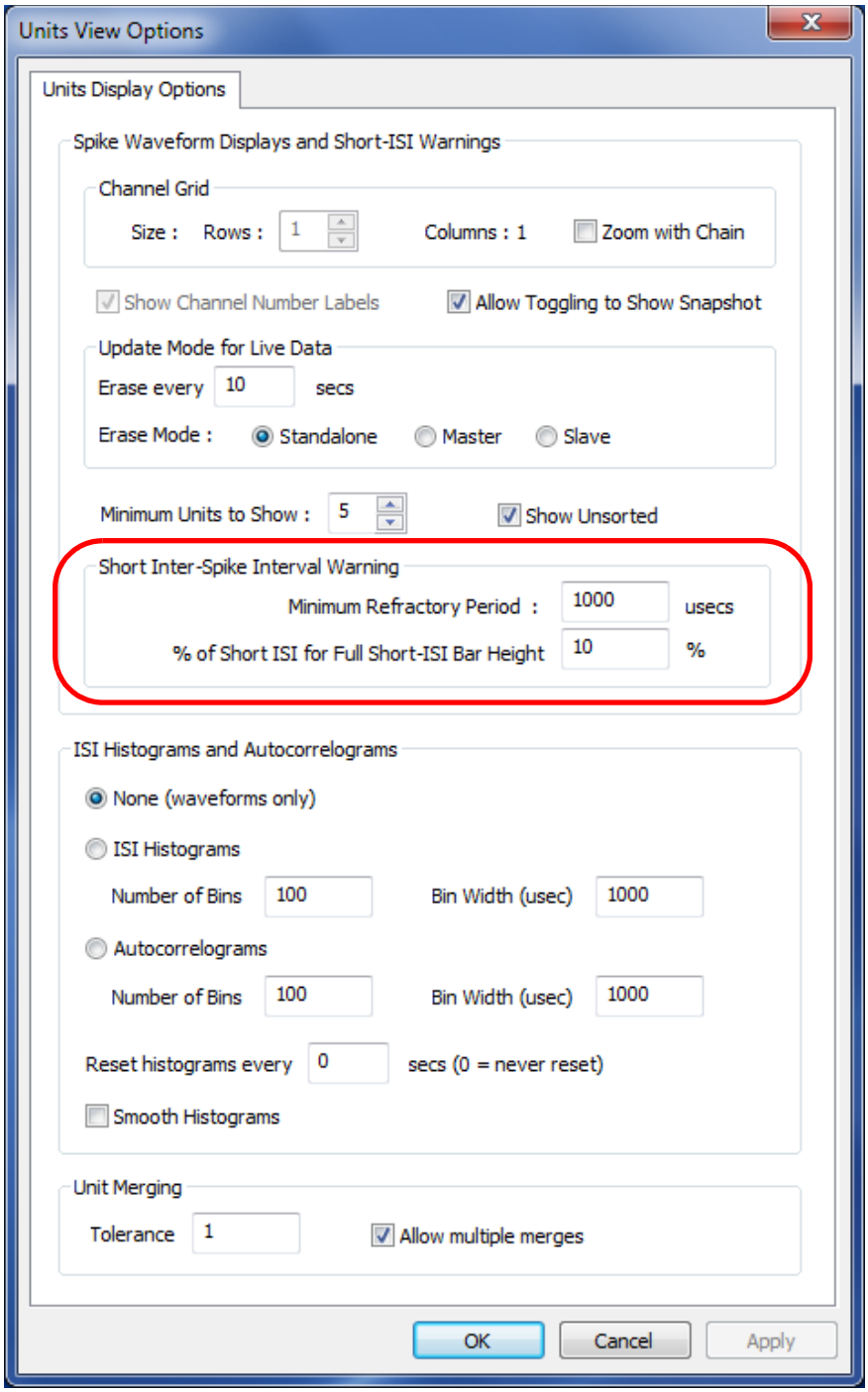
## 6.6 The Short-ISI Indicator

In cases where “too-loose” sorting parameters are causing too many spikes to be sorted into a given unit, one possible indication of the problem is that the firing rate of the spikes for that unit is too high; in other words, it's unlikely that one neuron could have produced that many action potentials per second. In the Units window, the Short-ISI Indicator is a red bar and associated value for the percentage of spikes that have an inter-spike interval (ISI) less than the refractory period (i.e. recovery interval) of a single neuron:

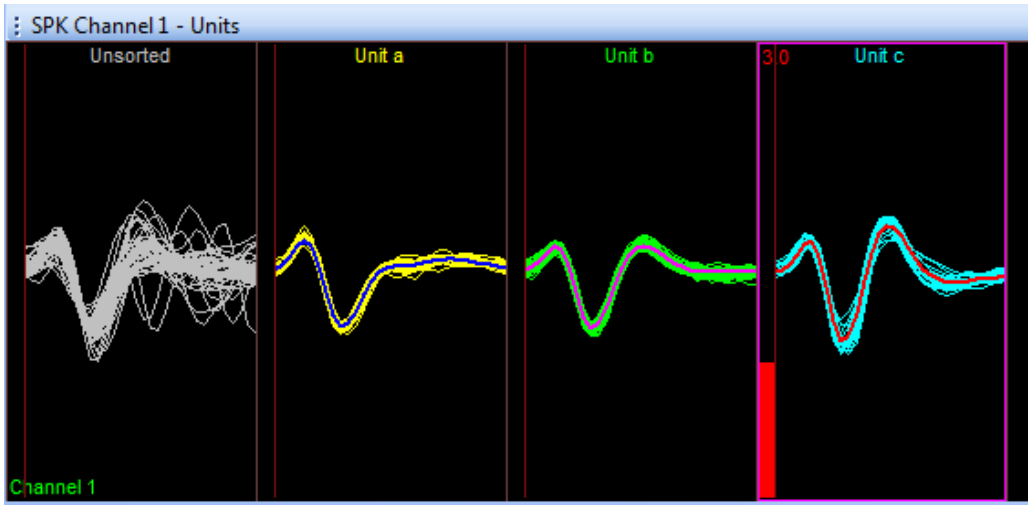



In effect, the Short-ISI Indicator is a warning that the current sorting parameters are resulting in sorted spikes that do not correspond to a physiologically plausible scenario. By default, the OmniPlex System assumes that the minimum refractory period for a unit is one millisecond, i.e. a 1 kHz maximum firing rate. You can change this default (typically to reduce it) by displaying the toolbar for the Units window and clicking the **Options** button:

The **Units View Options** dialog is displayed. You can set the value of the minimum ISI, as well as the scaling of the red warning bar:



When multiple units are defined on a channel, each is shown in the Units window with its name and Short-ISI bar:




Assuming that you have set a reasonable value for the **Minimum Refractory Period** in the Options dialog, the two most common reasons for a high percentage of Short-ISI spikes are:

- The threshold is set too low and is resulting in a significant number of "noise spikes" which are being detected at a firing rate higher than would be possible for an actual neuron
- Two or more units are being incorrectly sorted into a single unit; as an extreme example of this, if you drew a waveform crossing line across all the waveforms on a channel, resulting in all spikes being sorted into unit "a," you would very likely see a high percentage of Short-ISI spikes, unless the overall activity on the channel was very low

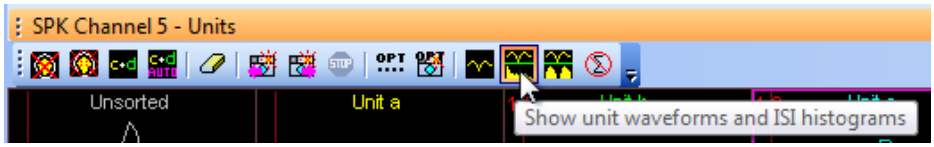
Note that the Short-ISI bar does not affect the sorting in any way; it is simply a monitoring tool to alert you to potential sorting problems.

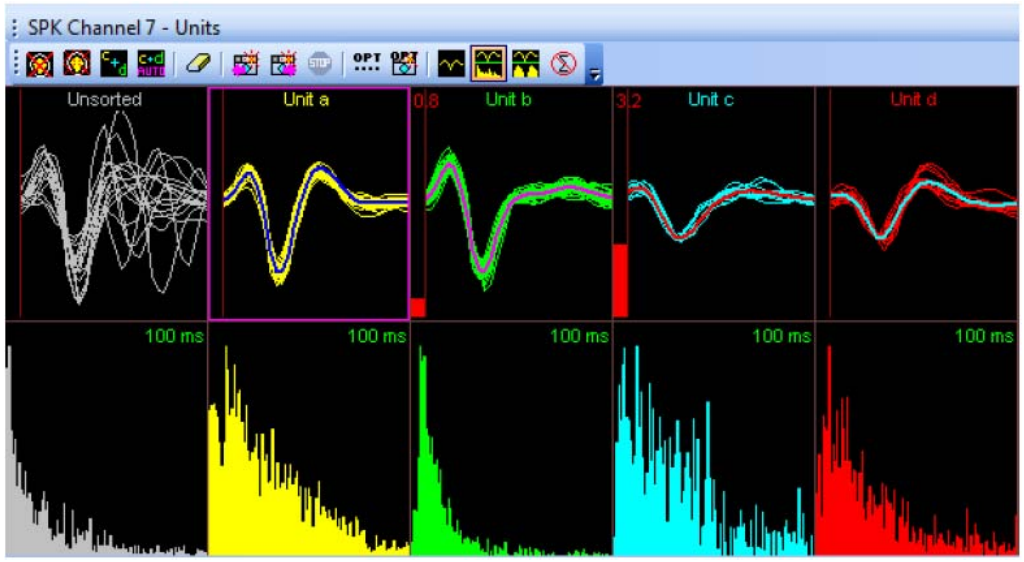
The Short ISI values are displayed in the Extended Properties Spreadsheet, as described in Section 8.10, "Spike Sorting Quality Metrics" on page 290.

## 6.7 Live ISI and Autocorrelation Histograms in the Units View

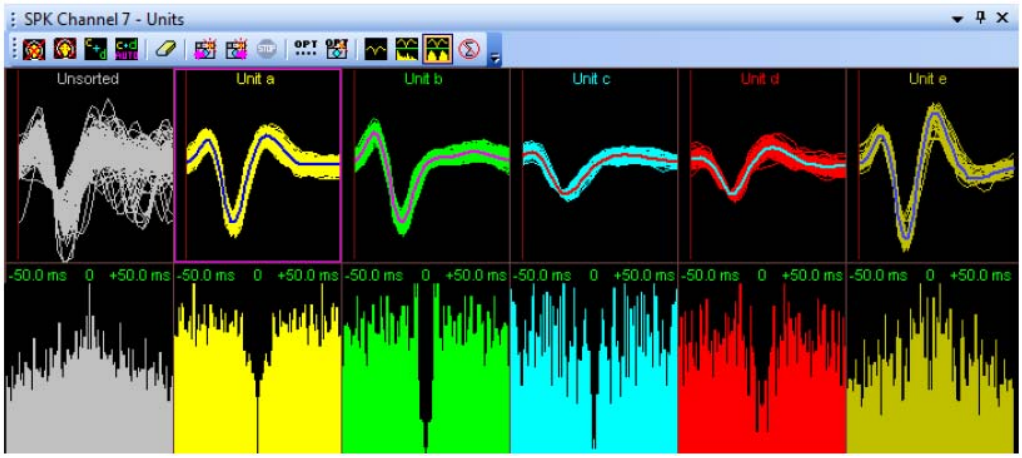
The **Units** view can optionally display either per-unit interspike interval (ISI) histograms or autocorrelograms, in addition to the usual unit waveforms. These provide an additional means for monitoring spiking activity and verifying correct sorting. Three toolbar buttons set the display mode to waveforms-only, waveforms and ISI histograms, or waveforms and autocorrelograms.

To display ISI histograms:

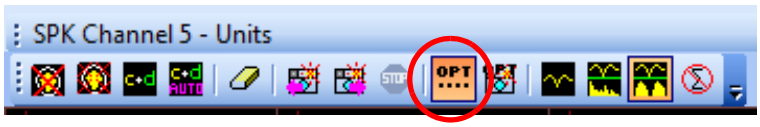


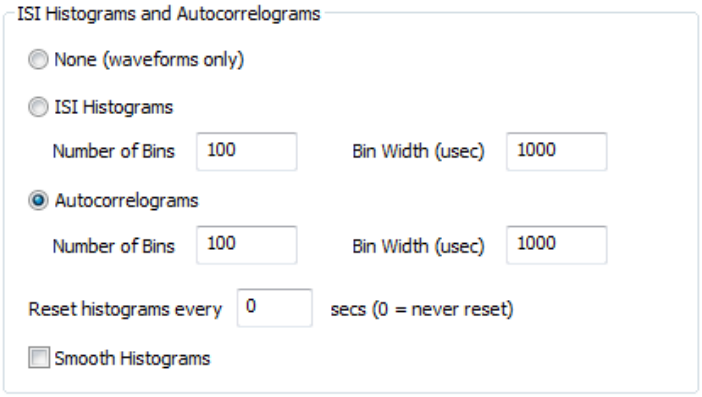




To display autocorrelograms:




Options for the ISI histograms and autocorrelograms can be configured in the **Units** view **Options** dialog:




The total histogram time width is given by the **Number of Bins** multiplied by the **Bin Width**. If the total time is T, then for ISI histograms, the time axis runs from 0 to T. For autocorrelograms, the time axis runs from –T/2 to +T/2.

Histograms start with bin counts of 0 and by default, only increment and never reset; for stable units, this allows the bin counts to become large enough that the shape of the histogram becomes evident, but you may wish to try finite refresh times, e.g. 30 seconds, to check for changes in the histogram over time. But be aware that after a reset, the histograms will appear somewhat random until the bin counts increase.

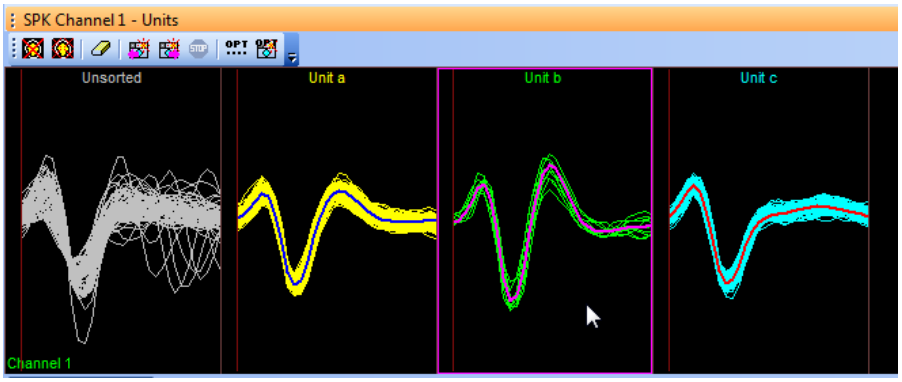
Each histogram is displayed with its height normalized, i.e. the largest bin count equals full height, no matter what that largest bin count is. This is done so that the histogram of a slowly firing unit doesn't "disappear" relative to a fast-firing unit.

In other words, it is assumed that what is important, for the purposes of monitoring sorting quality, is the shape of this histogram, not the absolute height.

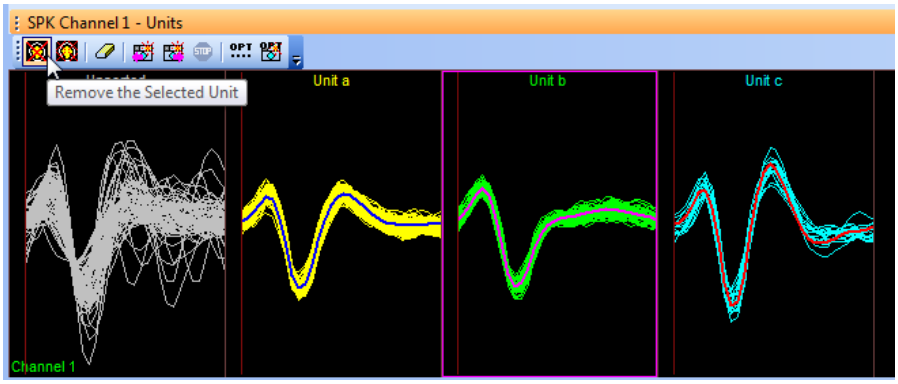
**Smooth Histograms** applies a simple smoothing filter to the bin counts before display. This reduces the noisiness of the histogram, but be aware that it will also smooth out the critical area near 0, and make any “notch” at 0 (a sign of a good unit) less sharp.

## 6.8 Deleting a Unit

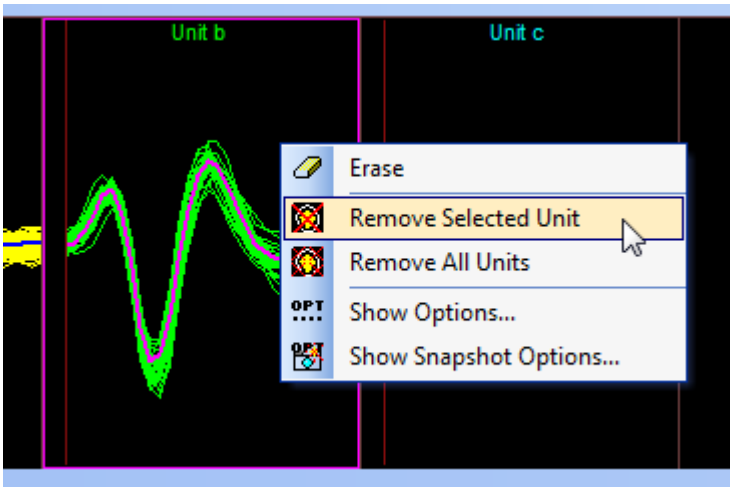
1 To delete a unit, select it by clicking on it in the Units window:




2 To delete the selected unit, click the Delete Unit button in the toolbar:




Alternatively, you can right-click in the Units window to display a menu and select **Remove Selected Unit**.



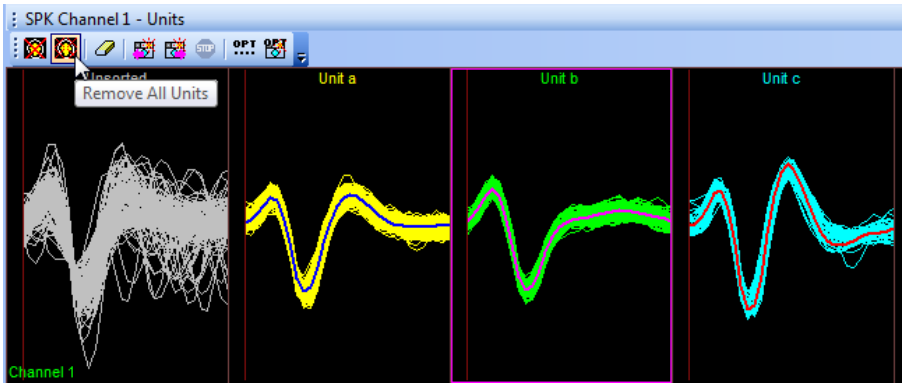

The selected unit is deleted, and any units which followed it in alphabetical order are renamed accordingly. For example, if you have units a, b, c, and d, and delete unit c, unit d will be renamed to unit c.

**TIP**

**Disabling units without removing and renaming**

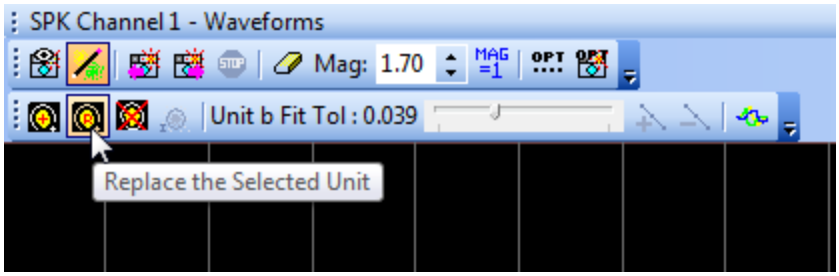
If you need to disable a unit, but for some reason want to avoid the automatic reassignment of unit names that results from deleting a unit, set the undesired unit's fit tolerance to the minimum value instead of deleting it. In most cases this will effectively remove that unit from the sorting process.

**3** If you wish to delete all the units on a channel, select **Remove All Units** from the right-button menu, or use the **Remove All Units** button in the toolbar:



## 6.9 Replacing an Existing Unit

Replacing a unit is similar to adding a new unit, except that the new unit will replace the unit that was selected at the time you clicked the **Replace Unit** button:



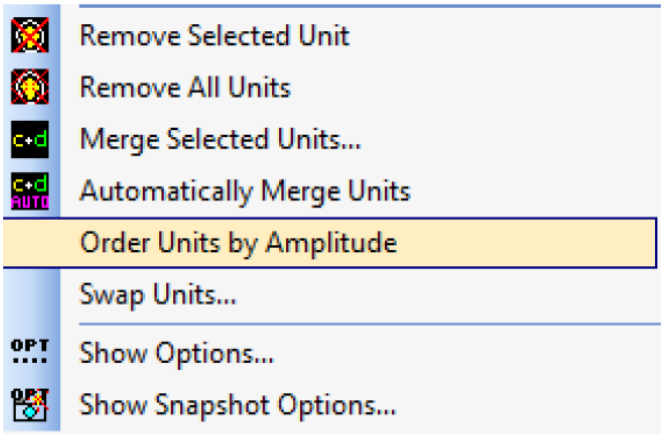

For example, if you have units a, b, c, and d, and unit b is selected, clicking **Replace Selected Unit** will then overwrite the unit definition for unit b. The procedure is otherwise identical to that previously described for adding a new unit. Note that this is not the same as selecting an existing unit and changing its sorting parameters, such as template tolerance or editing its points; the Replace Units command completely overwrites the old unit with the new one.

## 6.10 Changing the Order of Sorted Units

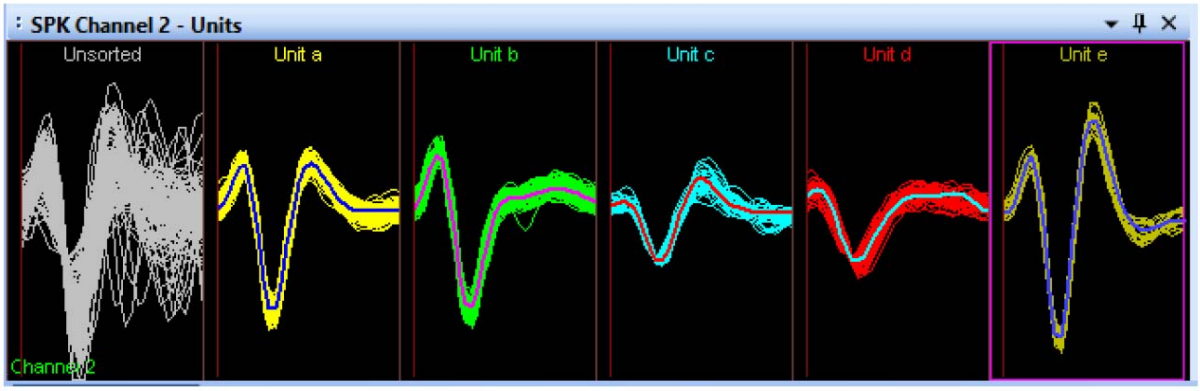
When you manually define units on a channel, they are labeled in alphabetic order (a, b, c, d…) as you add successive units. When you use automatic sorting, the unit order is somewhat arbitrary, since in most cases the unit label order has no meaning – units a and b could equally well be labeled as b and a. However, some analysis software may expect a particular unit order convention, such as amplitude order (unit a = largest template amplitude), quality order (unit a = "best" unit), etc. To support such workflows, the OmniPlex® software allows you to change the order of units, i.e. the unit labeling, on a channel. You can either automatically sort the unit order using peak-to-peak template amplitude, or manually specify the unit order by swapping units.

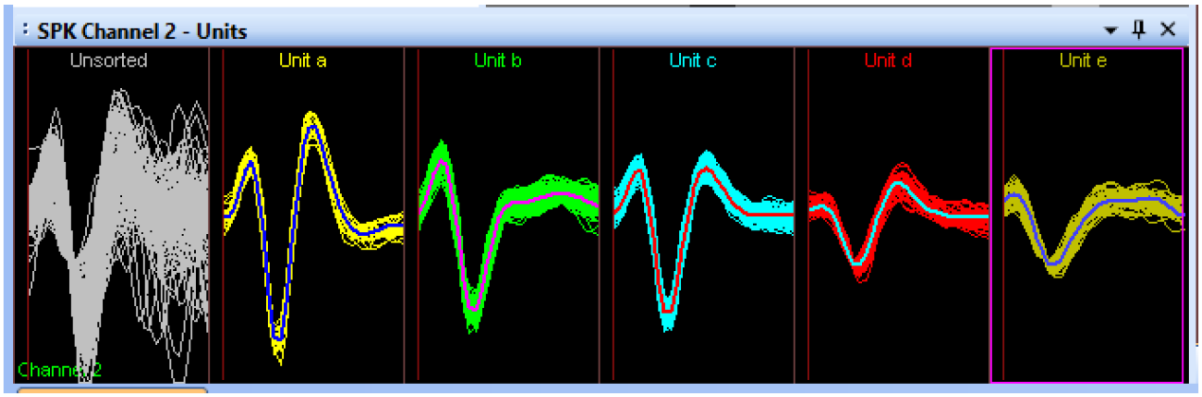
### Ordering units by amplitude

To sort units in order of decreasing peak-to-peak amplitude, use the **Order Units by Amplitude** command in the right-button menu of the Units view.




Here is a before-and-after example:



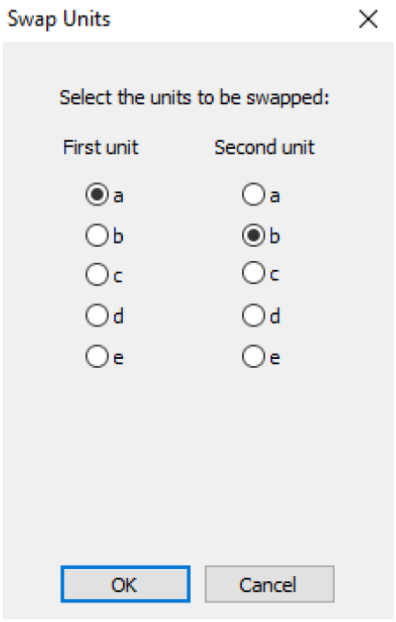



The units are reordered such that the peak-to-peak template amplitudes are in decreasing order. Note that this command is only supported for sorting methods which have explicitly defined templates, i.e, template and band sorting.

Note that the changing the unit order by amplitude is a command, not a mode. In other words, if one or more templates are later modified, deleted or added, the unit order is not automatically updated; you may need to perform another **Order Units by Amplitude** if you wish to maintain the decreasing-amplitude unit ordering.

### Changing unit order by swapping units

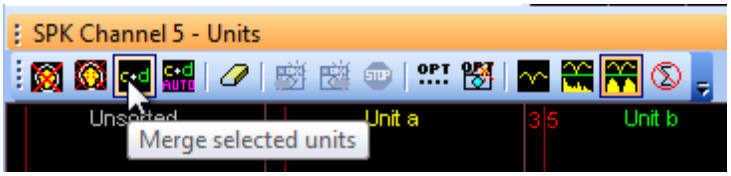
You can define any desired unit order by manually swapping pairs of units. From the same right-button menu shown above, select the **Swap Units** command. The **Swap Units** dialog is displayed:



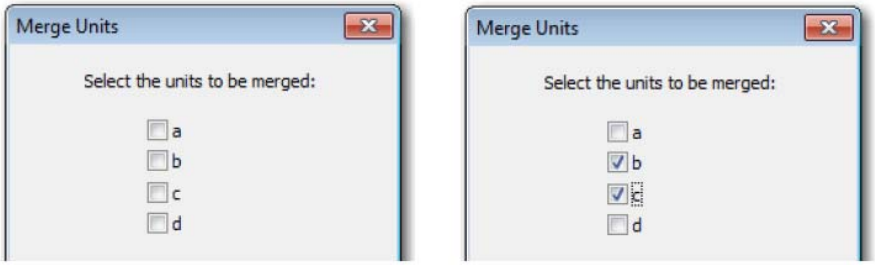

Select the pair of units to be swapped and click **OK**. You can repeat this procedure as needed to define a desired unit order.

## 6.11 Manual and Automatic Unit Merging

The Units view now has functions for manually or automatically merging similar units. If you know which units you want to merge, you can do a manual merge and specify exactly which units should be combined into one. To do this, click the toolbar button for **Merge selected units**:



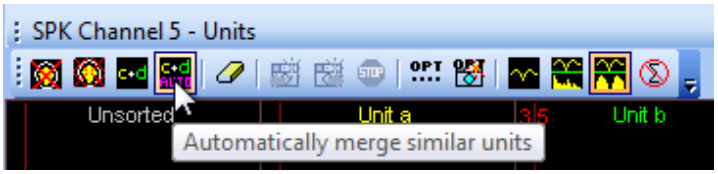

A dialog box is displayed which allows you to select two or more units to be merged:




When you select the desired units and click **OK**, the units are merged. The results of a merge are to form the union of all the spikes in the snapshot for the specified units, and then to define a new unit from that set of spikes in the usual way for the current sorting method in effect. For example, if you merged units b and c with template sorting, the template for the merged unit will be the mean of all the waveforms in the former units b and c. The merged unit is added as the last unit for the channel, the units that were merged are deleted, and units are relabeled to remove gaps. For example, if you had units a, b, c, d and merged units b and c, the result would be:

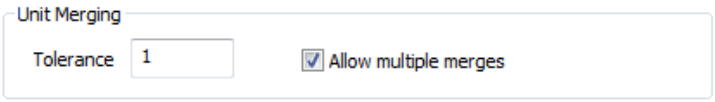
```
new unit a = old unit a
new unit b = old unit d
new unit c = merge(old unit b, old unit c)
```

There is also an automatic merge function which compares all unit templates (for one channel) against each other, finds groups of units that are similar to each other, and merges the units within each group:

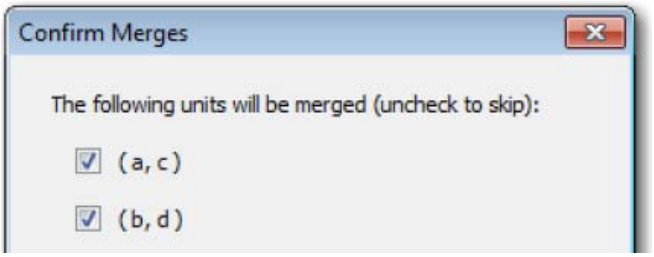



In this case, a merge tolerance (a normalized measurement of the similarity between two unit templates) is used as a threshold for similarity decisions.

Typical values are in the range 0.5 to 1.5. You can control the merge tolerance using the **Tolerance** setting in the **Unit Merging** section of the **Units** view options:

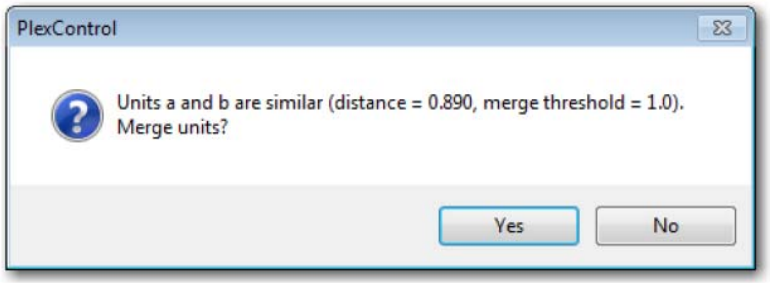



When the **Allow multiple merges** option is enabled, clicking the **Automatically merge similar units** button displays a dialog showing the groups of similar units which it has found:

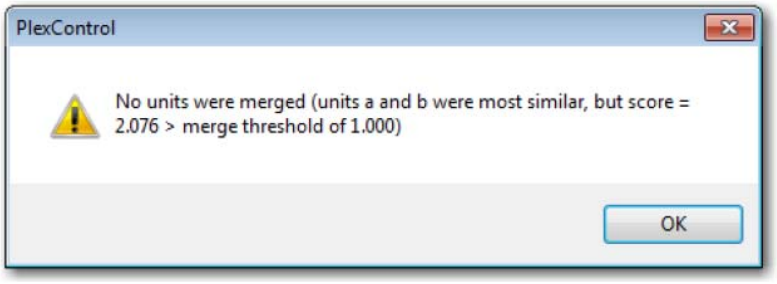



Each potential merge group (list of units which will create a new, merged unit) is shown on a separate line. Click **OK** to accept all the merges, or uncheck any which you do not wish to accept and then click OK.

When the **Allow multiple merges** option is disabled, only the most similar pair of units (which is within the merge tolerance) is a candidate for merging, and a dialog box asks you to confirm the merge:




If no pairs of units were found which were within the merge tolerance, a dialog will be displayed:



## 6.12 Spike Display Modes

PlexControl provides several spike display modes and options which can be very useful when adding units, adjusting sorting parameters, and monitoring the results of spike sorting. See Section 13.2, “PlexControl Spike Display Modes” on page 384 for more information.

# Chapter 7
# Recording

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## 7.1 Overview

Once you have configured the OmniPlex® System for suitable gain, thresholds, and spike sorting parameters, you will eventually want to record your data. The OmniPlex System can record data from any or all of its sources to a recording file in either or both of two Plexon® file formats.

**Note:** Setting the gain is applicable to DigiAmp subsystems, not DHP subsystems.

### 7.1.1 PLX Format

PLX format is an industry-standard recording format originated by Plexon in the 1990s. It is supported by many applications, including Plexon's Offline Sorter™ application, NeuroExplorer®, and used by hundreds if not thousands of custom MATLAB® scripts and application programs written by users. An enormous amount of neurophysiological data has been recorded by hundreds of labs around the world in PLX format. PLX recording is supported for legacy use, for those cases where an application does not yet support reading PL2 files.

### 7.1.2 PL2 Format

PL2 is Plexon's next-generation file format. It can contain all the same information as a PLX file, including the exact same sample values, but it is an extensible format that supports the OmniPlex System concept of sources and allows for new types of data to easily be added. In addition, PL2 handles large amounts of continuous data more efficiently than PLX. For example, when the system is recording wideband (WB) or continuous spike (SPKC) channels, the time required to load continuous channels from the resulting PLX file into an application or MATLAB script can be prohibitive. PL2 typically reduces this load time by a factor of hundreds or even thousands, due to its more efficient storage and indexing scheme, compared to PLX.

Besides Offline Sorter and NeuroExplorer, PL2 files can be read by existing MATLAB and C/C++ programs with little or no modification, using a backwards-compatible file-reading API. Refer to the documentation for the PL2 SDK for more details.

Before recording files in the PL2 format, first make sure that the applications that you intend to use with those files support the PL2 format. If you are using an older version of an application, you may need to update it to a newer version which can read PL2 files.

### 7.1.3 What to Record

The OmniPlex System allows you to record every channel of every source that is available in PlexControl (see the list in the discussion of sources and Server topologies). However, even though the PL2 format greatly reduces load times for large files, it is usually not a good idea to record everything, unless your recordings are short or you have a specific reason. The main contributors to file size are wideband data (WB) and continuous spike data (SPKC), both of which consist of 40,000 samples of 16 bits each, per second per channel, or 80 kilobytes per second per channel, plus a small amount of overhead. Recording the WB data alone results in recording rates of approximately:

64 channels * 80 kb/s = 5 MB/sec = 300 MB/min

256 channels * 80 kb/s = 20 MB/sec = 1.2 GB/min

Recording both WB and SPKC will double the above figures, and should in general be avoided, although the OmniPlex System is capable of doing so, as long as you are careful to not run other performance-intensive applications on the same machine at the same time.

The default recording behavior for the OmniPlex System is to record all channels on all sources, except *no* WB or SPKC channels. This gives the smallest files, but has a number of potential drawbacks; for example, it becomes difficult to impossible to re-threshold the data in Plexon Offline Sorter™ (the threshold cannot be lowered towards zero). To avoid these problems, but at the same time trying to avoid unnecessarily huge file sizes, the question then becomes whether to record WB *or* record SPKC. The tradeoffs are as follows.

Recording the wideband signal ensures that the original digitized signal, unchanged by any digital filtering or other processing, is available for later use. This allows for widest range of offline processing to be done. For example, you wish to try a lower cutoff frequency for the spike highpass filter, you must have the WB signal. However, if it is desired to be able to use the WB data to regenerate the exact same SPKC data as was generated by the OmniPlex System during the experiment, then the offline application must support all of the same digital signal processing functionality as the OmniPlex System. If the SPKC source is recorded, then this issue is avoided.

Digital events, keyboard events and Plexon CinePlex® System events are always recorded; there are no enable/disable checkboxes for these sources.

### 7.1.4 Additional Recording Features

The basic recording procedure is presented in Section 7.2, “Recording” on page 209. For additional recording features, see the information in the following sections:

- Section 7.3, “Adding Comments Before and During Recording” on page 217
- Section 7.4, “PL2 Recording Protection” on page 222

- Section 7.5, “PLX Channel Numbering for Continuous Channels” on page 224
- Section 7.6, “Low Disk Space During Recording” on page 224
- Section 7.7, “Event-Triggered Recording” on page 224

## 7.2 Recording

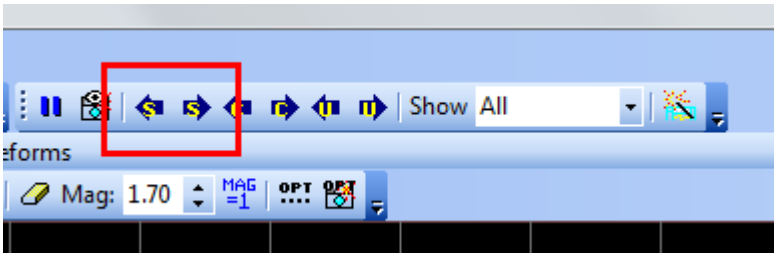
1 Before recording a file, select the sources and channels which you wish to record. Click on the Properties Spreadsheet tab to view the record-enable options:

Properties SpreadSheet for 'SPK'

| | Name | PLX chan | Enabled | Threshold% | Num Units | Rec SPK | Rec WB | Rec SPKC | Rec FP |
|---|---|---|---|---|---|---|---|---|---|
| >>1 | SPK01 | 1 | ☒ | -15.000 | 3 | ☒ | ☐ | ☐ | ☒ |
| 2 | SPK02 | 2 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ | ☒ |
| 3 | SPK03 | 3 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ | ☒ |
| 4 | SPK04 | 4 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ | ☒ |
| 5 | SPK05 | 5 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ | ☒ |
| 6 | SPK06 | 6 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ | ☒ |
| 7 | SPK07 | 7 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ | ☒ |
| 8 | SPK08 | 8 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ | ☒ |
| 9 | SPK09 | 9 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ | ☒ |
| 10 | SPK10 | 10 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ | ☒ |
| 11 | SPK11 | 11 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ | ☒ |
| 12 | SPK12 | 12 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ | ☒ |
| 13 | SPK13 | 13 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ | ☒ |
| 14 | SPK14 | 14 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ | ☒ |
| 15 | SPK15 | 15 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ | ☒ |
| 16 | SPK16 | 16 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ | ☒ |
| 17 | SPK17 | 17 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ | ☒ |
| 18 | SPK18 | 18 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ | ☒ |
| 19 | SPK19 | 19 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ | ☒ |
| 20 | SPK20 | 20 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ | ☒ |
| 21 | SPK21 | 21 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ | ☒ |
| 22 | SPK22 | 22 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ | ☒ |
| 23 | SPK23 | 23 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ | ☒ |
| 24 | SPK24 | 24 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ | ☒ |
| 25 | SPK25 | 25 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ | ☒ |

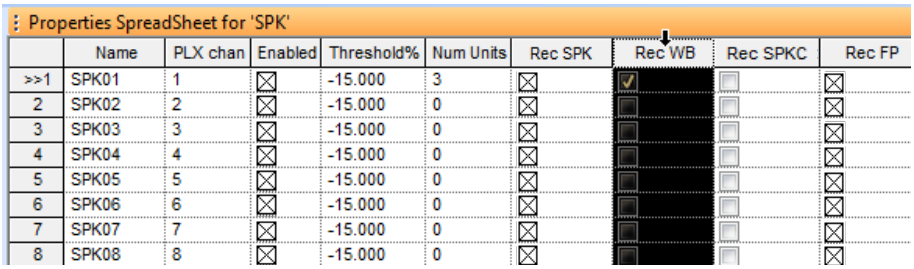
SPK - Waveforms | SPK Channel 1 - Clusters | 3D SPK Channel 1 - 3D Clusters | Properties SpreadSh

2 If any of the sources WB, SPKC, SPK, or FP are currently selected, you will see the record-enable columns for all the others, as shown above. If some other source is selected and you do not see the above Rec columns, use the previous/next source buttons in the main toolbar to step through the available sources until the desired Rec columns are displayed.

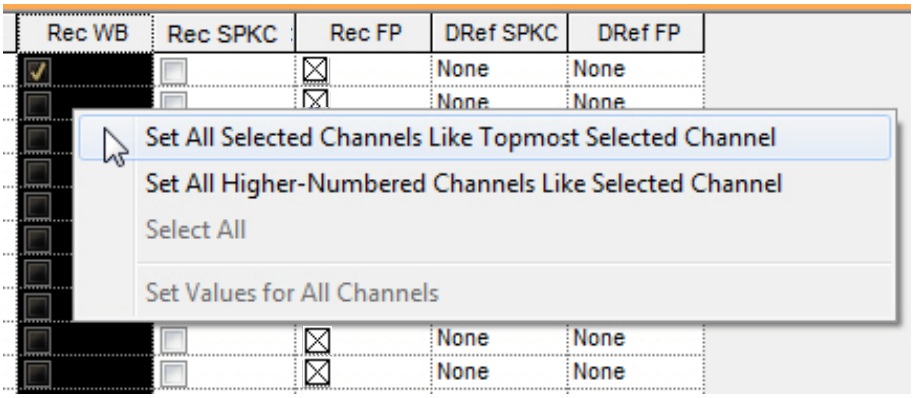



3 Check the appropriate boxes to indicate which channels of which sources you wish to record. Remember that you can select multiple channels within a column and set them all to the same setting as the topmost row, as described

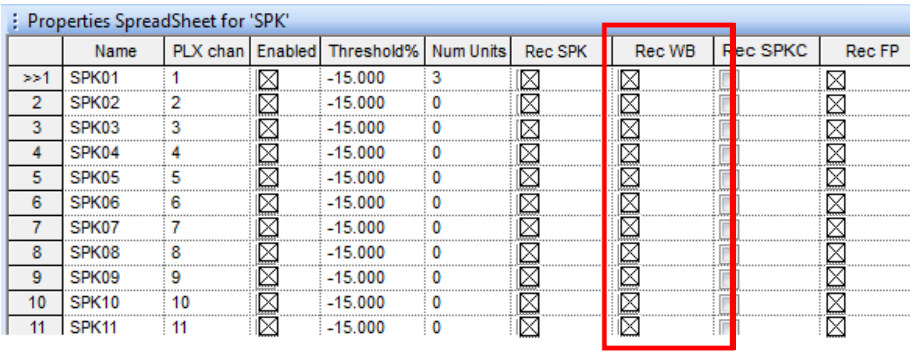
previously. For example, to enable recording for all WB channels, enable WB recording for the top channel, then click on the “Rec WB” column header:




**4** Select **Set All Channels Like Topmost Selected Channel** from the right-button menu:

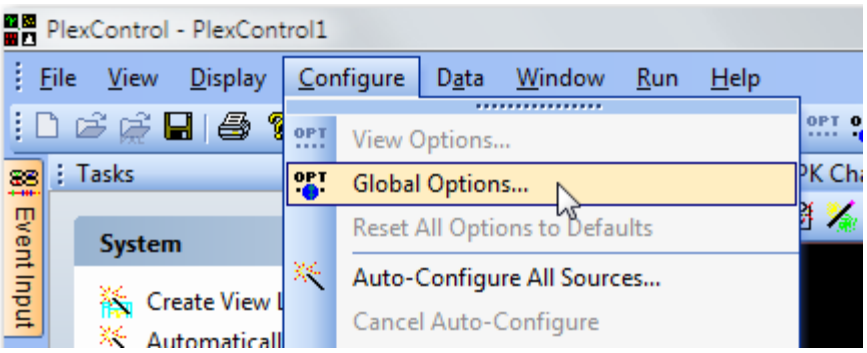



**5** All WB channels will be enabled for recording:

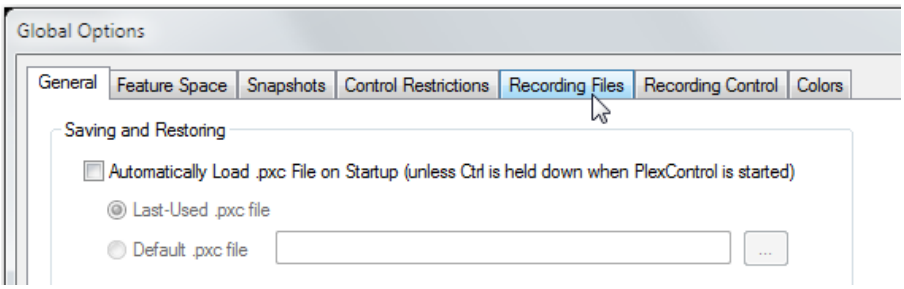



**6** At this point, you could begin recording, and by default the data would be recorded in PLX format. However, it is useful to be aware of some of the

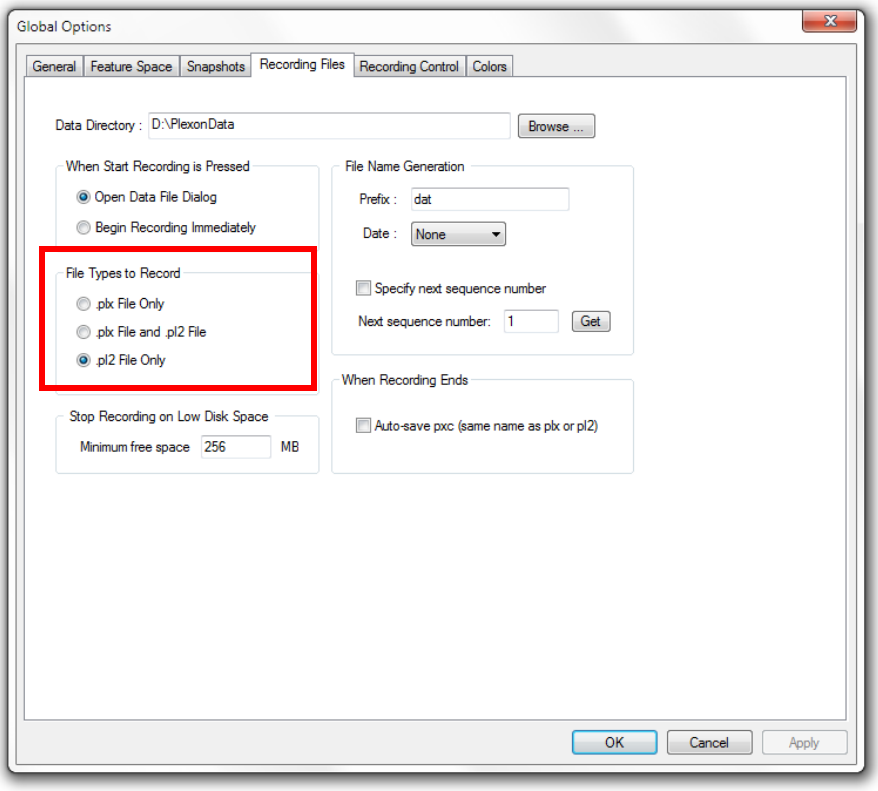
recording options that are available. To view the main recording options, select **Global Options** from the **Configure** menu:



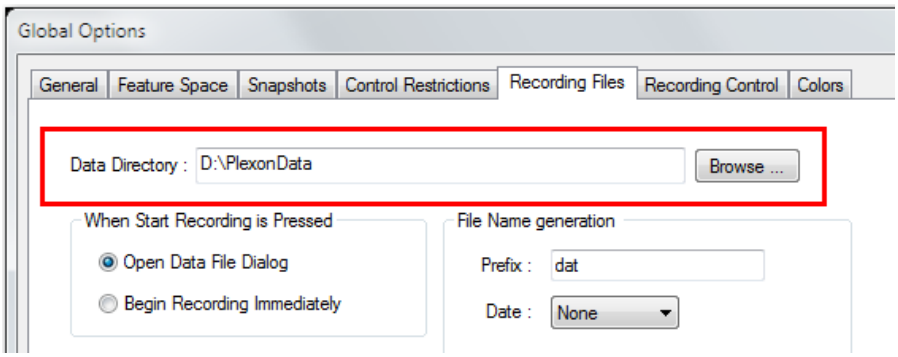

**7** When the **Global Options** dialog is displayed, click the **Recording Files** tab:



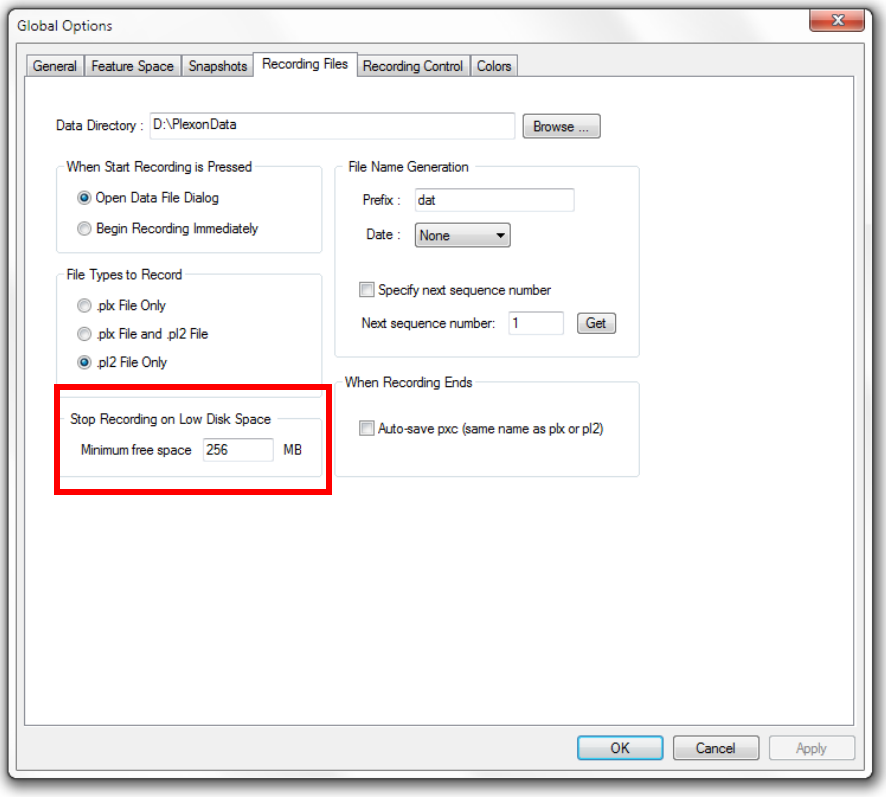
8 In the **Recording Files** options page, the **File Types to Record** section controls whether recordings are written in PLX format, PL2 format, or both formats in parallel. You will usually want to choose one or the other format, but the parallel recording option may be useful if you want to compare the two files, e.g. for sizes and load times.




9 You can also use the **Data Directory** field to set the folder to which files will be recorded:



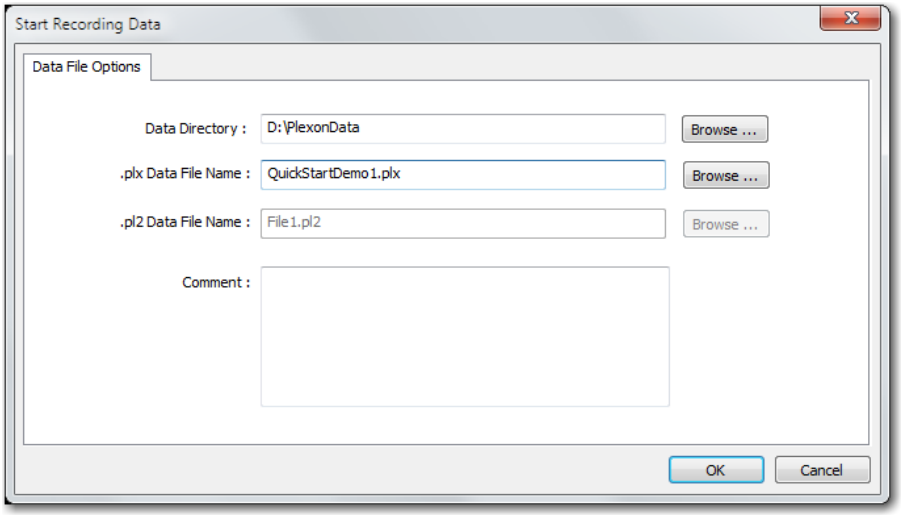
10 Set the desired value for **Stop Recording on Low Disk Space**. The system will automatically stop recording if the free disk space on the PC drops below this value. (The default is 256MB.)



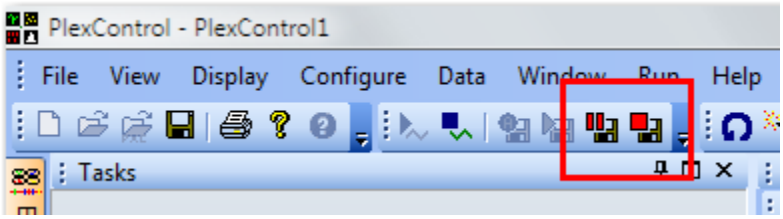

11 Click **OK** to save your changes. Note that you do not have to set these options each time that you record a file, and PlexControl will retain these settings each time you run it.

12 When you are ready to begin the recording, click on **Start Recording** in the main toolbar:

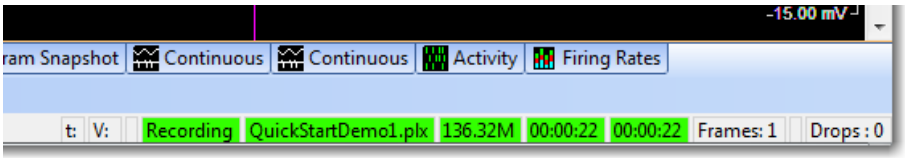
13 The **Start Recording Data** dialog appears:




14 Specify the name of the PLX and/or PL2 files to record. You can also enter a block of comment text which will be recorded in the file header; you can use this for brief descriptive notes or archival information. Click **OK** to start recording. As recording starts, the **Start Recording** button is disabled and the **Pause** and **Stop Recording** buttons are enabled:

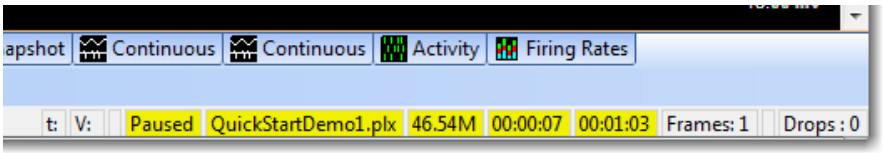



15 In addition, the file size and time indicators in the status bar appear in green:



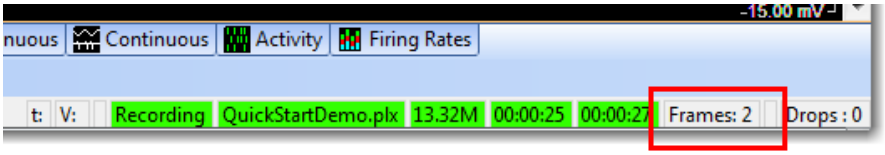

This indicates the recording status (recording versus paused), filename, current file size, and elapsed time.

16 If you click the **Pause** button while recording, the status bar changes its display:



Note that the first time figure now shows the total elapsed recording time, while the second figure shows the total elapsed “clock time” since the start of the recording, whether paused or recording.

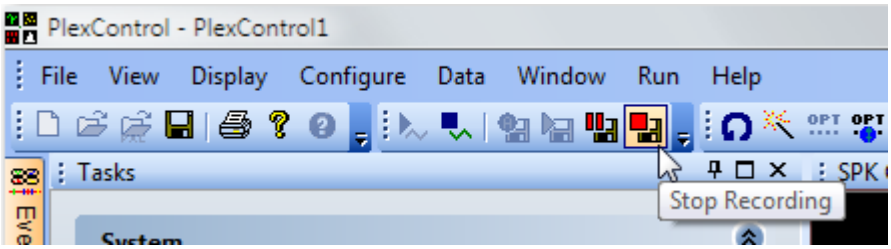
**17** If you now click the **Pause** button again, recording resumes. The Frames count in the status bar indicates how many separate frames (segments) of data have been recorded in the file so far:




**18** If desired, enter additional comments while PL2 recording is in progress.

**Note:** For a description of the comments feature, see Section 7.3, “Adding Comments Before and During Recording” on page 217.

**19** When you want to complete the recording and close the recording file, click the **Stop Recording** button in the toolbar:




**IMPORTANT: It is strongly recommended that you make a short test recording** and examine it in NeuroExplorer, MATLAB, Offline Sorter, or whatever the intended destination for the subsequent use or analysis of your data might be, before proceeding to make important recordings in an actual experiment. This will help reveal any problems in terms of which sources were recorded, signal quality, proper triggering on digital event inputs, etc. Large amounts of time and effort can be wasted if you make a long recording, or a series

of recordings, before discovering that some setting was not correct, that an input cable was bad, etc.

**TIP**

**Avoid changing OmniPlex System settings while recording**

If at all possible, avoid making changes to the OmniPlex System settings while a recording is in progress, unless you are aware of the potential consequences in the recorded data. For example, if you have defined units a, b, and c on a channel, and in the middle of a recording you delete unit b, unit c is by default renamed to unit b, and any analysis of the channel will have to account for this. Likewise, changing thresholds, fit tolerances, and other parameters during a recording may create issues for subsequent analysis of the data. On the other hand, there are cases where the input signals drift during a long recording, and adjusting parameters in PlexControl during a recording is necessary to “track a unit” or compensate for other changes.

**TIP**

**Disable channels before starting the recording**

For performance reasons, PlexControl does not allow you to change the record-enable checkboxes during a recording, so make sure the enables are set as desired before starting to record.

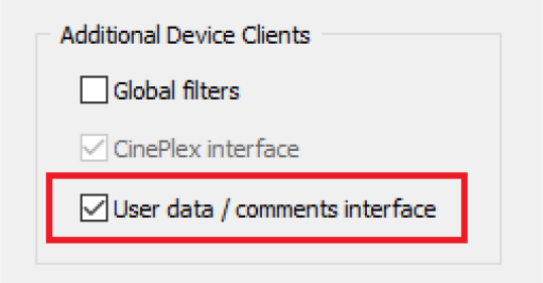
## 7.3 Adding Comments Before and During Recording

The previous procedure (Section 7.2, “Recording” on page 209), showed you how to add a comment to PLX and PL2 files before recording starts. The procedure also included a step that showed how to add more comments to PL2 files during recording, provided certain prerequisites are met. Each comment you enter during recording is timestamped and added to the PL2 file and can later be extracted using the offline file reading SDKs.

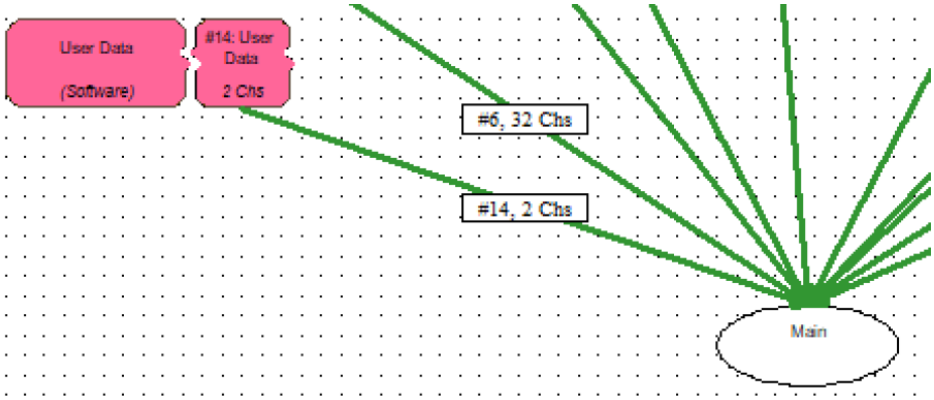
### Topology prerequisite

To enable adding comments during a recording, your topology must include the **User Data** device client, shown in the image below. You can view your topology diagram to verify the **User Data** device client is present.

**Note:** If the client is not present, open the Topology Wizard dialog, select the **User data / comments interface** checkbox, click **OK**, then restart the OmniPlex® software. The User Data device client should now be present.




The generated topology will show the User Data device client and its associated source:



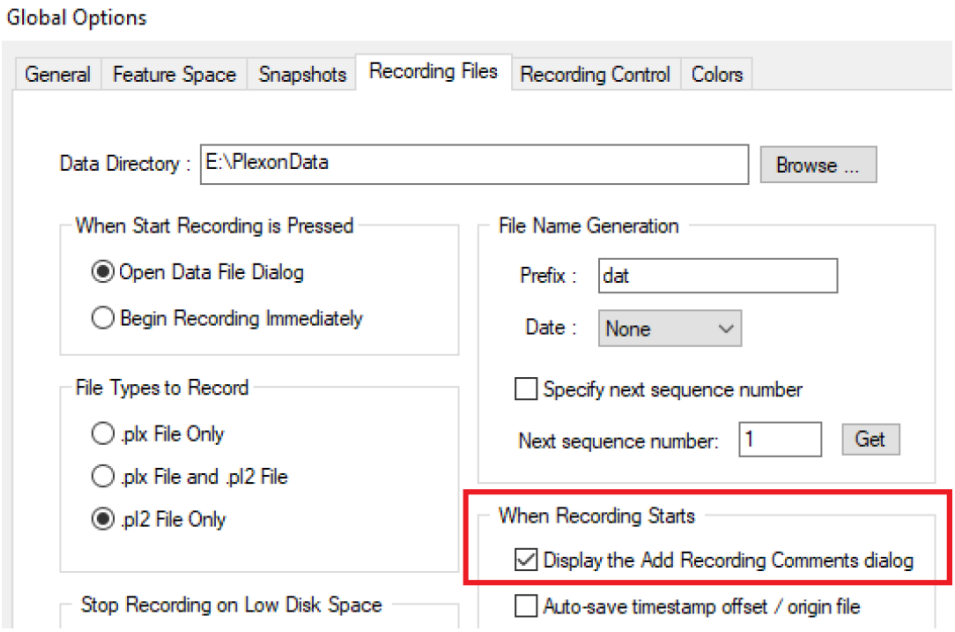

Add comments to a recording with the **Add Recording Comments...** command, as described below.

Note that the **Add Recording Comments...** command is disabled if you are recording only a PLX format file, since recording comments during recording is supported for PL2 files only. If you are recording both PL2 and PLX “in

parallel," you can add comments, but they will only be available in the PL2 file. The single comment block in the **Start Recording Data** dialog is still supported for both file formats.

### Global Options—Display the Add Recording Comments dialog

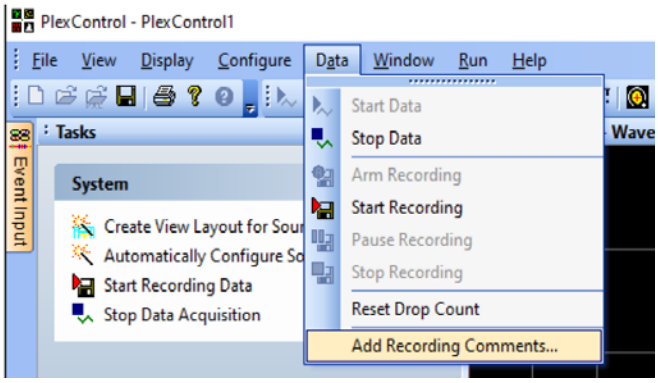
If you would like PlexControl to automatically display the **Add Recording Comments** dialog at the start of each recording, enable the following option in the **Recording Files** page of the **Global Options** dialog:



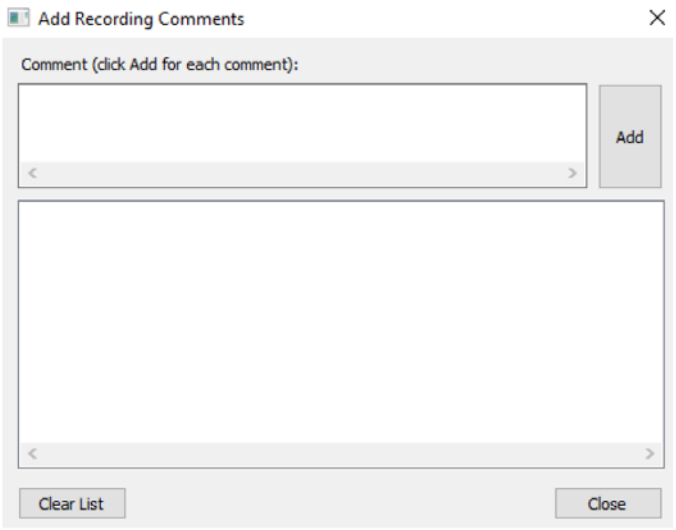
### Adding comments during recording of PL2 files

Follow these steps to add comments during recording.

1 Select the **Add Recording Comments...** command. (This command is supported for PL2 recordings only, and is disabled if you are recording only PLX files.)

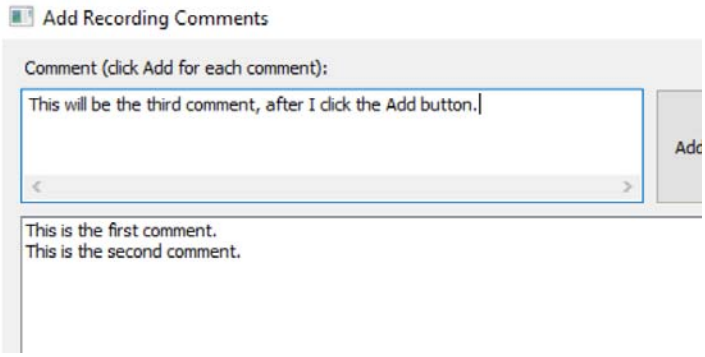



After you click **Add Recording Comments...**, the **Add Recording Comments** dialog is displayed:

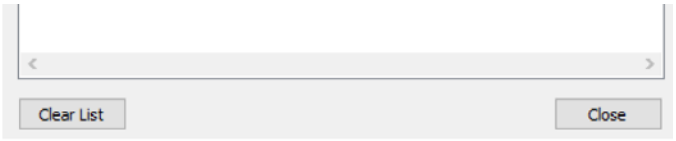



2 Enter each comment in the Comment section at the top, then click the **Add** button to add the comment to the recording. Note that the **Add** button is only enabled after you have started a recording.

Each comment can contain one or more lines, and be up to 1000 characters in length, although multiple, shorter comments are usually easier to work with. After you click **Add**, the comment is also added to the list of comments in the lower section:




3 (Optional) Click the Clear List button if you want to clear the existing comments field.




The **Clear List** button clears the contents of the lower window. It does not remove comments from the recording; it only “cleans up” the display.

4 When you are finished adding comments, click the **Close** button.

The **Close** button dismisses the dialog, but you can open it again during the same recording if you later wish to add more comments. Note that any previously entered and recorded comments are not shown when you reopen the dialog.

### Reading recorded comments from PL2 files

Recording comments can be read from PL2 files using the latest version of the C/C++ and MATLAB offline file reading SDKs, or using third-party applications which can read PL2 files, such as NeuroExplorer (NEX).

**IMPORTANT:** Before recording comments in a PL2 file, you should first verify that your third-party application is capable of reading such files; otherwise, you may encounter an error. For example, in the case of NeuroExplorer (NEX), version 5.306 or newer is required. Check the neuroexplorer.com website for the latest version of the software.

It is recommended that you make a short test recording containing comments and verify that it can be read by the relevant application(s) without error. Note that some applications may choose to read these files without error but ignore the comments, for application-specific reasons.

In the C/C++ offline SDK, refer to the functions **PL2_GetCommentsInfo** and **PL2_GetComments** in the header file PL2FileReader.h.

```
PL2FILEREADER_API int PL2_GetCommentsInfo(
  int fileHandle,
  unsigned long long *numComments,
  unsigned long long *totalNumberOfCommentBytes);

PL2FILEREADER_API int PL2_GetComments(
  int fileHandle,
  long long* timestamps,
  unsigned long long* commentLengths,
  char* comments);
```

An example of how to use these functions to read comments from a PL2 file can be found in the function **PrintSampleOfComments** in PL2FileReaderTest.cpp in the SampleCode folder of the C/C++ offline SDK.

When reading a PL2 file in MATLAB using the offline SDK, recording comments are available in the **NumberOfRecordingComments** field and **RecordingComments** array in the pl2 structure returned by the **PL2GetFileIndex** function; no separate function is needed to read the comments. An example of how to read the comments is the following:

```
PL2GetFileIndex(''); % clear the index
[pl2] = PL2GetFileIndex('myfile.pl2');
fprintf('There are %u recording comments\n', pl2.NumberOfRecordingComments);
for i=1:pl2.NumberOfRecordingComments
  fprintf('comment %u: t = %f, text = "%s"\n', ...
    i, pl2.RecordingComments(i).Timestamp, pl2.RecordingComments(i).Text);
end
```

Since MATLAB programs which read a PL2 file already call the **PL2GetFileIndex** function, any recording comments in the file are automatically available after this function has been called.

When reading a PL2 file from a Python application using the PyPL2 API, you can use the function **pl2_comments** to read comments from the file, as in the following example:

```
from pypl2 import pl2_comments
timestamps, comments = pl2_comments('myfile.pl2')
for n in range(len(timestamps)):
  print("{} {}".format(timestamps[n], comments[n]))
```

## 7.4 PL2 Recording Protection

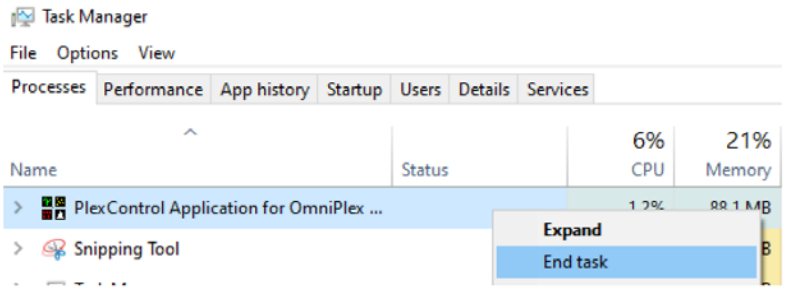
Plexon's PL2 file format allows large recordings, including files containing large amounts of high-bandwidth wideband (WB) and spike-continuous (SPKC) data, to be read efficiently in applications such as Offline Sorter (OFS) and NeuroExplorer (NEX), as well as in custom analysis software written in C/C++, MATLAB and Python using the offline file reading SDKs. In order to support this, when OmniPlex software records a PL2 file, it uses various caching and indexing schemes supported by PL2 files to allow applications and scripts to subsequently read channels of data at high speed.

If the OmniPlex application unexpectedly terminates or if there is a power failure during a recording, the resulting incomplete file could be unreadable. PL2 recording protection addresses this issue by periodically updating the PL2 file's indexes and caches so that in the event of an unexpected termination, the resulting file will in most cases remain readable. You can enable recording protection in the **Recording Files** page of the **Global Options** dialog:

The **Update Interval** determines how frequently the recording protection feature is run. Note that the minimum **Update Interval** is 30 seconds. In the event of the unexpected termination of a recording, at most the data acquired since the most recent protection update may be lost.

You might wonder, why not set an **Update Interval** of one second, to reduce the maximum amount of potential data loss to one second's worth? There are two main reasons. First, the periodic update flushes all cached data to the file, which, if done too frequently, would reduce some of the efficiencies of the PL2 recording scheme – the resulting files would not load as quickly. Second, doing the updates too frequently would noticeably increase the load on the system, especially at high channel counts. At an **Update Interval** of 30 seconds or longer, both of these factors are negligible.

Keep in mind that recording protection cannot guarantee that a recording will be readable in cases such as hard drive hardware failure, unprotected power line surges that cause hardware damage, operating system failure or malware that corrupts the file system, etc. However, you can verify that recording protection works as intended by using the Windows® Task Manager to perform an "End Task" on PlexControl.exe in the middle of a recording, and then reading the resulting file.

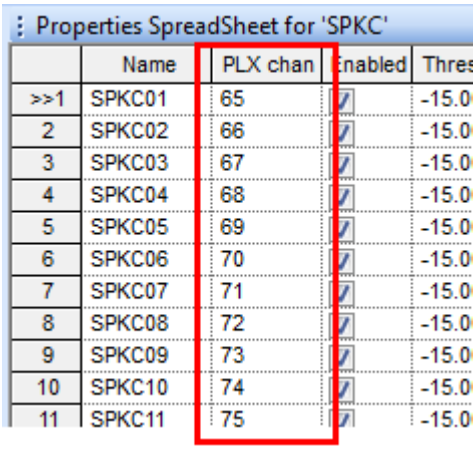



Of course, Plexon does not recommend that you intentionally terminate a recording containing valuable data. Use the test wav file and an HTU, or unconnected headstages, for any such test.

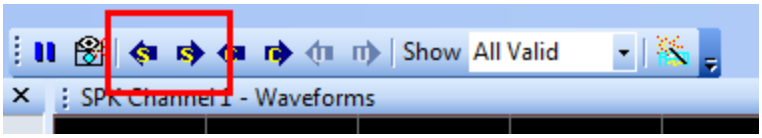
Note that recording protection only applies to PL2 files, since the legacy PLX format is recorded using a much simpler scheme which is usually relatively unaffected by the unexpected termination of a recording, although at the expense of the much higher reading speed and other features of the PL2 format.

## 7.5 PLX Channel Numbering for Continuous Channels

Within the OmniPlex System, channels are source-relative; for example, in a 64 channel systems, the WB channels are numbered 64, the SPKC channels are numbered 64, etc. However, for historical reasons, all the continuous channels in a PLX recording file are numbered in a single continuous range. Therefore, when a PLX file is recorded, the continuous channels in each source in the OmniPlex System are automatically mapped into this single linear range of channels. In the Properties Spreadsheet, the "PLX chan" column shows the PLX channel which the channels of each source will be recorded as, for example:




You can use the previous/next source buttons in the main toolbar to quickly step through the sources to see what PLX channel range will be assigned to each source, using the Properties Spreadsheet.




## 7.6 Low Disk Space During Recording

The system provides an option to stop recording automatically if the free disk space on the PC drops below a set value. The default value is 256MB; the value can be modified in the **Global Options** dialog.

## 7.7 Event-Triggered Recording

In addition to manually starting, stopping, pausing, and resuming recording, you can configure PlexControl to perform any or all of these actions when it sees a user-specified digital event. See Chapter 11, Triggered Recording.

# Chapter 8
# Additional Sorting Methods and Quality Metrics

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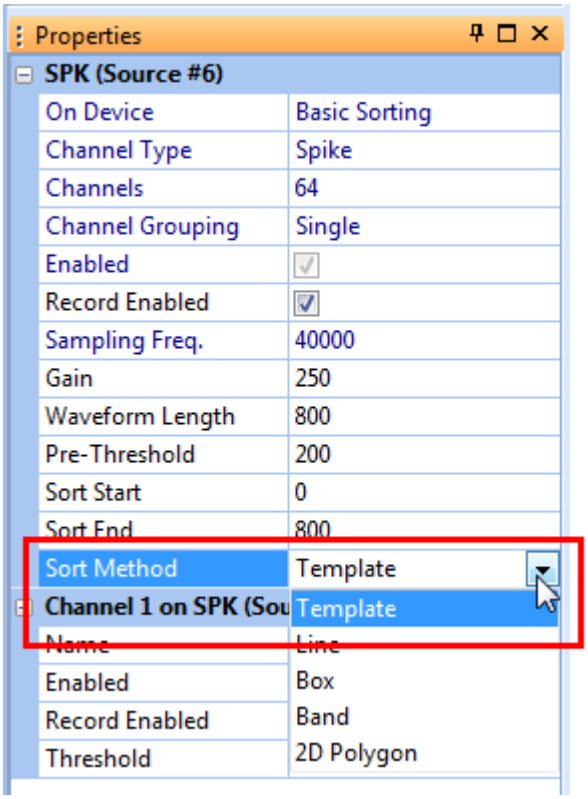
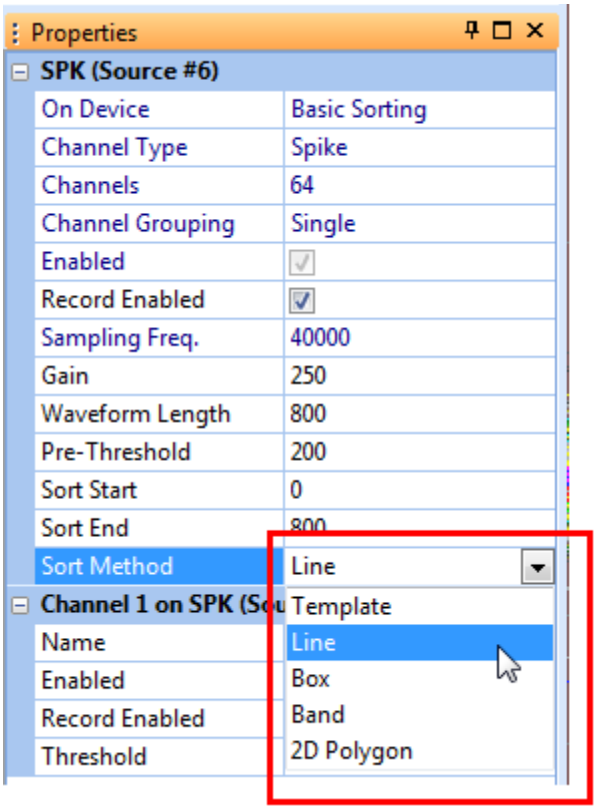
## 8.1 Line Sorting

As opposed to template sorting, which is based on an error criteria (sum of squared errors between a spike and a template), line sorting is what could be called a geometric sorting method. Since you used waveform crossing to select bundles of waveforms which were averaged to form sorting templates, you are in fact already familiar with the basic idea behind line sorting. In line sorting, you draw one or more crossing lines on spikes in the main spike window, as in template sorting, but the crossing lines themselves are what is sent to Server to perform the actual sorting of incoming data. When defining units for line sorting, you can draw more than one crossing line per unit definition, and incoming spikes must pass through all the crossing lines to be sorted into that unit. This is a very intuitive way to interactively specify and refine a unit definition.

1 The OmniPlex® System requires that data acquisition be stopped before the sorting method is changed. Stop data acquisition as previously described, then click on the **Sort Method** entry in the Properties window at the left:

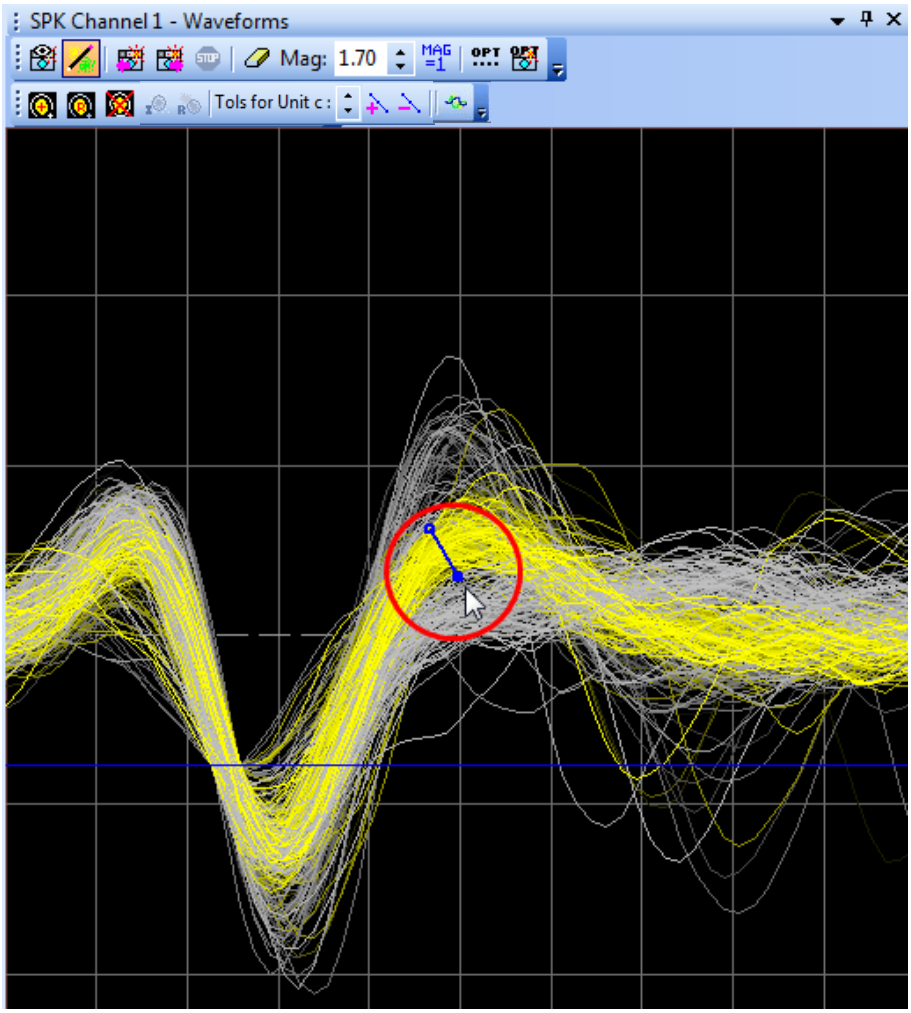


2 Note that **Template** sorting is currently selected. Click on **Line** to switch to line sorting:

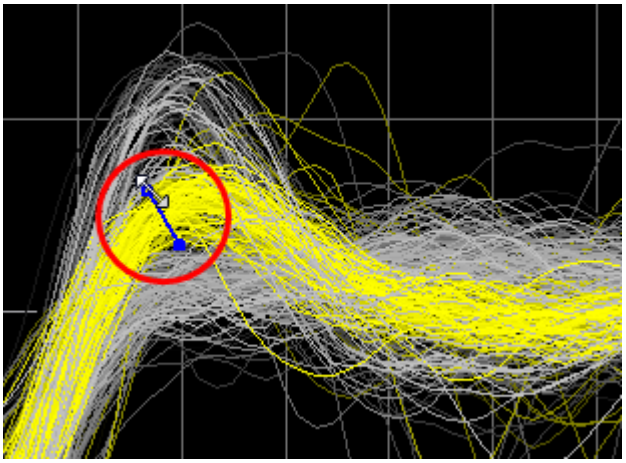



3 Restart data acquisition. Note that since the sorting method changed, all existing unit definitions are deleted and all spikes on all channels are unsorted again.

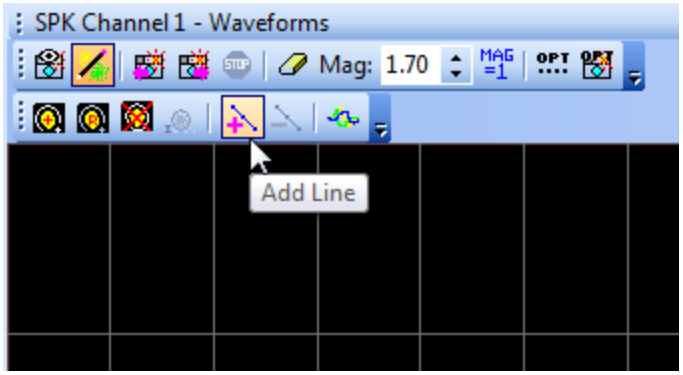
4 Enter unit editing mode and then add a new unit using the waveform crossing method, as previously described for template sorting. Note that the crossing line does not disappear when you finish drawing the line:



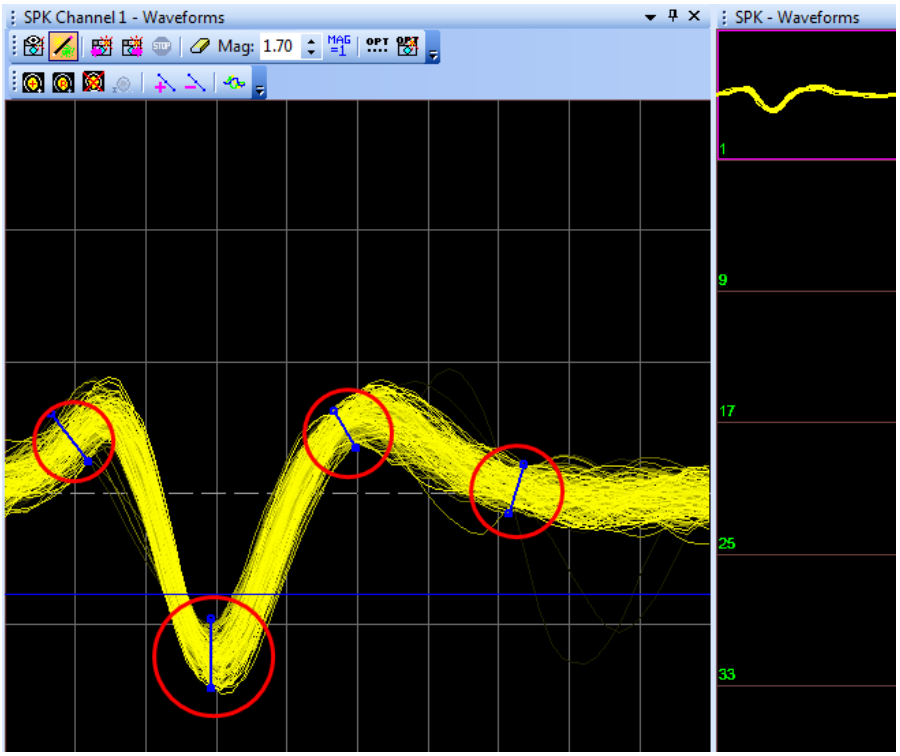

5 Also note that if you move the cursor tip over either end of the line, it changes to a line-edit cursor, to indicate that you can move either end of the line:



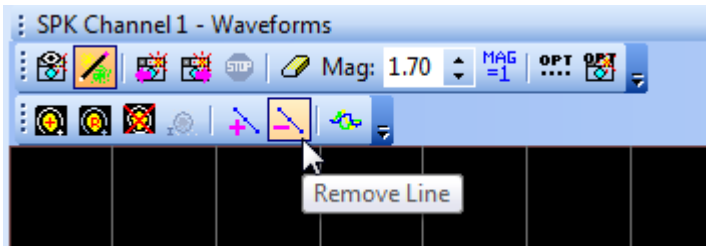
6 You can see that there are some spikes that are incorrectly being sorted into unit a, because one crossing line is sometimes not an adequate sorting criteria. To add another crossing line for the same unit, click the **Add Line** button in the toolbar:




This is a case where the Selected Unit mode for the **Show** filter (see Section 13.2, “PlexControl Spike Display Modes” on page 384) is particularly useful, as it makes the addition of more lines to tighten up the sorting criteria easier. Here is an example after a total of four lines have been added, with the **Show** filter set to **Selected Unit**:

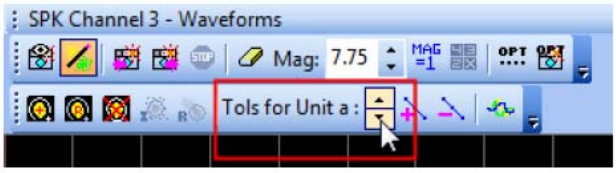


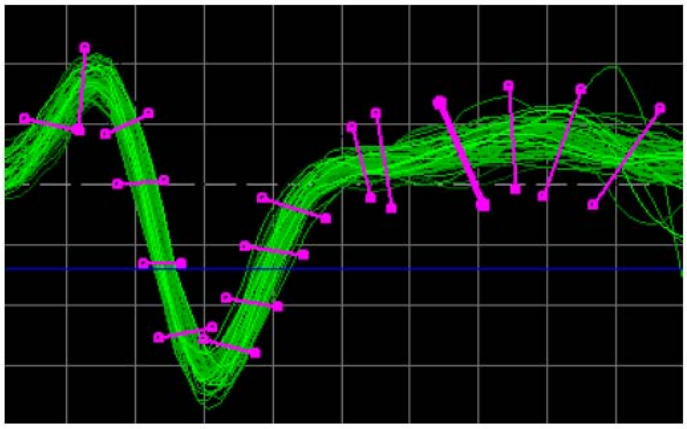
7 You can also use the **Remove Line** command to delete the most recently added line:

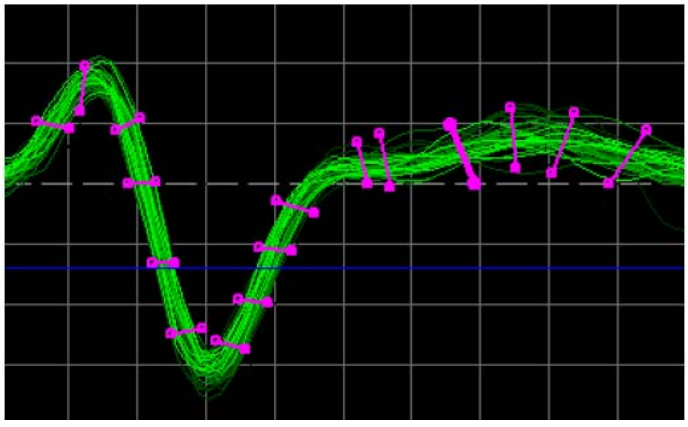



You can add as many lines per unit definition as you like, although in practice you should find that no more than three or four lines are needed in most cases.

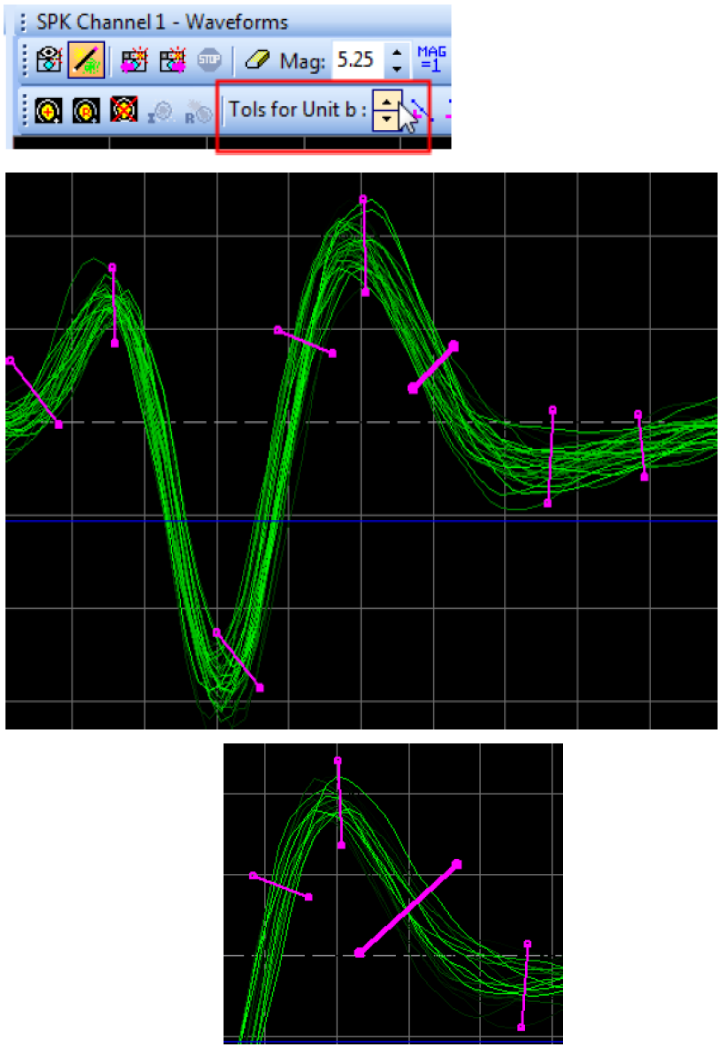
8 You can use the spin arrows in the unit editing toolbar to make all the sorting lines for a unit longer or shorter, similar to adjusting band tolerances in band sorting. This example shows the tolerances being reduced, i.e. shortening all sorting lines:





Alternatively, you can use the spin arrows to change the length of an individual sorting line, simply by holding down the CTRL key while clicking the spin arrows. When this is done, only the currently selected sorting line (drawn as a bold line) will be lengthened or shortened.

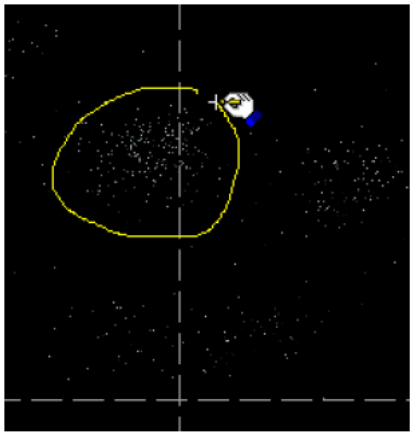


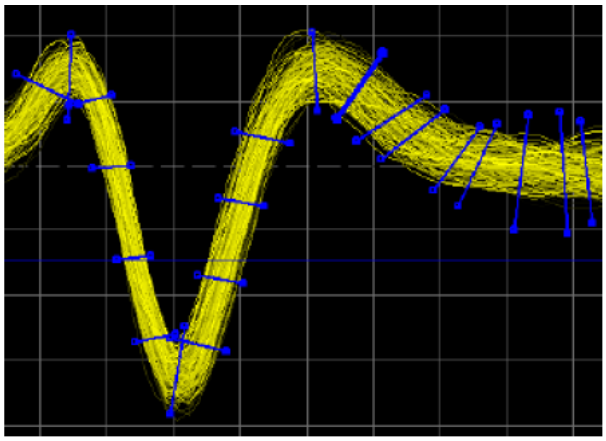

If you wish to adjust the length of a different sorting line, first select it by clicking on either endpoint.

9 You can continue to define more units on the same channel, using the **Add Unit** button again.

### Defining line sorting units by drawing contours in cluster views

You can also define line sorting units by drawing contours around clusters in feature space, in the same way as for the other sorting methods (except box sorting, which does not support this, nor can it be used with automatic spike sorting). The system will generate sorting lines for the spikes enclosed in the drawn contour, using the same algorithm that is applied to the output of the automatic sorting algorithms.





**TIP**

**Using the sorting lines**

Line sorting is quite effective in terms of being able to very closely specify the desired shape of the bundle of waveforms that constitute a unit. You will sometimes hear this referred to as “making the spike jump through hoops.” However, keep in mind that some parts of spikes, in particular the tail, tend to have more amplitude variance than the region around the peaks. As a general rule, the sorting lines should be “tighter to the bundle” in the more well-defined part of the spike, and looser, if used at all, in the tail and other higher-variance regions.

**TIP**

**Lines per unit versus units per channel**

Novice users sometime confuse adding lines with adding units: remember that you can have multiple units per channel, and multiple lines per unit. On a given channel, unit a could have three lines, unit b only one line, unit c two lines, etc. While editing a particular unit, you can only modify the lines used to define that unit.

**Note:** The OmniPlex System requires that all the channels on a spike source use the same sorting method. In practice, this means that all the spike channels in the system must use the same sorting method.

## 8.2 Principal Component Analysis (PCA)

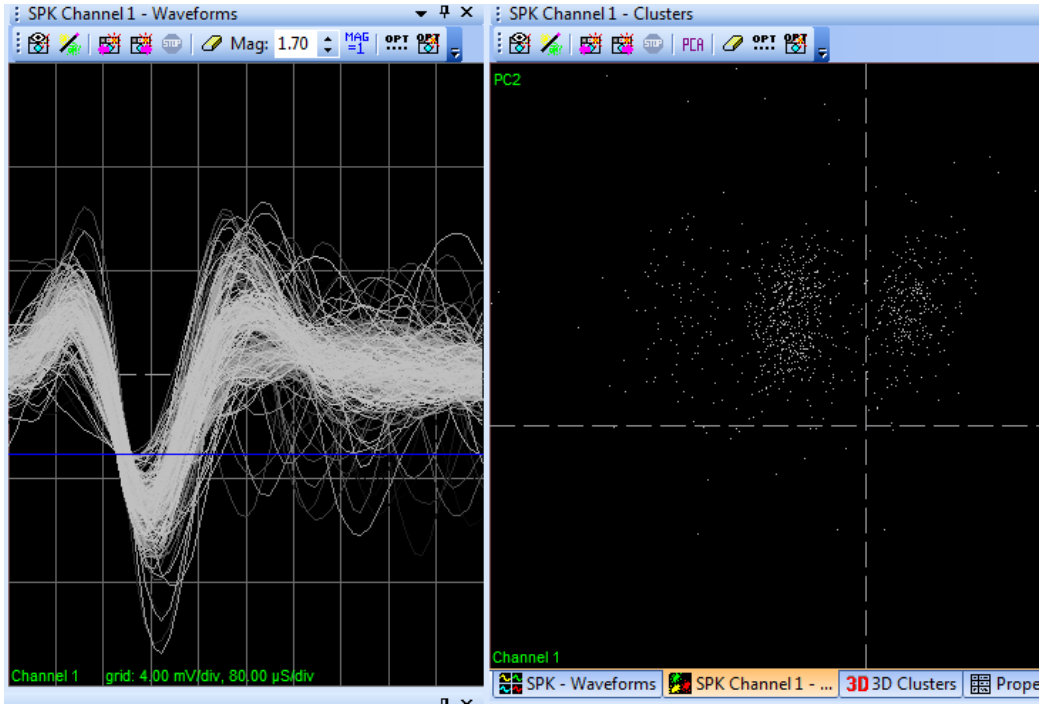
While you can use the OmniPlex System as has been described so far, using template or line sorting to sort spikes using their time/voltage values, i.e. the raw sample values, the OmniPlex System also has support for working with spikes in feature space.

By feature space, we mean a low-dimensional coordinate system into which raw spike data is projected in a way that emphasizes some useful property. The feature space approach treats each spike waveform as a single point in a high-dimensional space (e.g. a 32D space for a 32 point, 800 microsecond spike), where the coordinate in the first dimension is the amplitude at the first sample point, the coordinate in the second dimension is the amplitude at the second point, and so on. Working directly in such a high dimensional space is generally difficult and computationally intractable, and so what is called dimensionality reduction is applied. The idea here is that we want to project each point in the high-D space into the low-D feature space where we can view it and work with it more efficiently. For the purposes of spike sorting, we would like the projection to cause spikes of similar shape, which originated from the same neuron, that is, a unit, to form clusters of points in the low-D feature space.

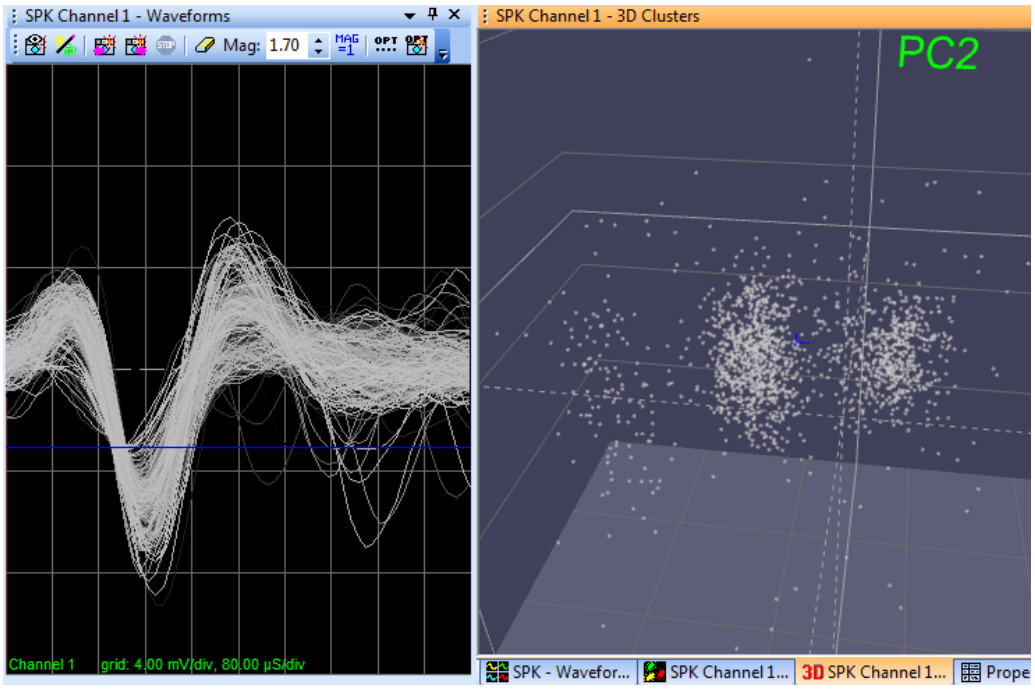
A standard feature space (i.e. projection method) that is used for spike data is principal component analysis (PCA). A full treatment of PCA is beyond the scope of this user guide, but it is a linear projection whose key property in terms of spike sorting is that those sample positions which have high variance across a given set of spikes are given greater weight in calculating the projection. The motivation behind this is that samples where there is more “amplitude spread” within a group of spikes are more likely to have separation between the amplitude values corresponding to different units.

The PCA projection of a 32 point spike results in a 32D point in PCA space; however, PCA orders the coordinates in order of decreasing variance, so that the first few coordinates “contain” most of the variance, and the higher order coordinates represent mainly noise and can be discarded. By taking the first two or three PCA coordinates and dropping the others, we obtain a projection into 2D or 3D PCA feature space and achieve the desired dimensionality reduction.

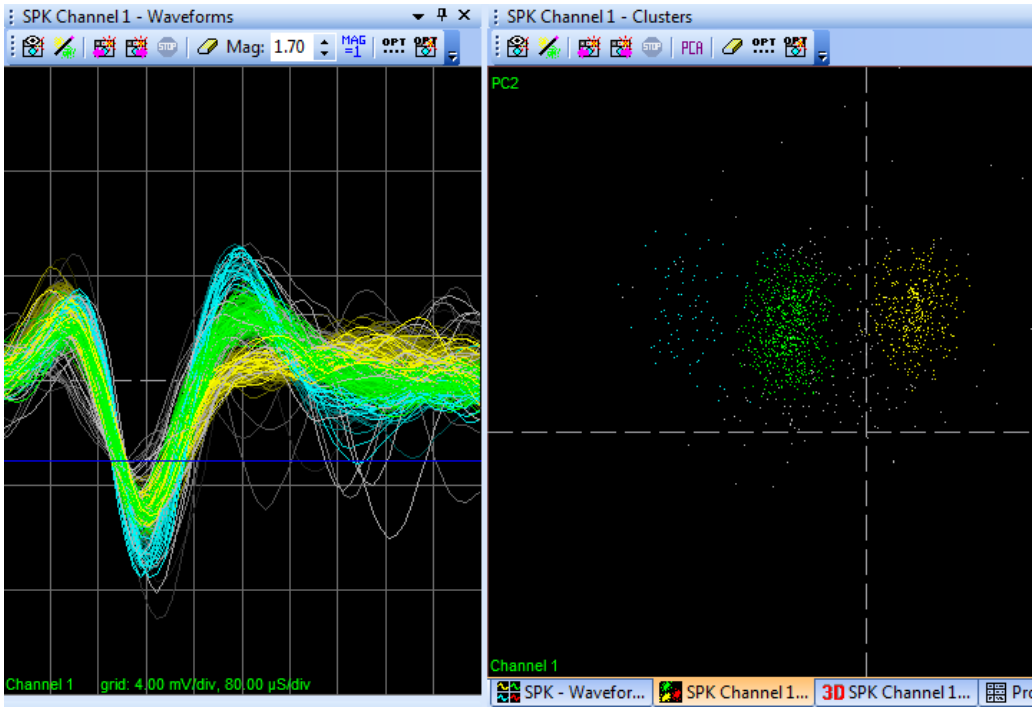
Here is an example of spikes and their corresponding 2D PCA projections, where each point in the PCA display corresponds to one spike in the main spike window:




And similarly, the 3D projections:



There are several uses for PCA in spike sorting. First, we can manually draw boundaries around visible clusters as an alternative to waveform crossing as a method of selecting waveforms. For example, we can indicate that we wish for all the spikes corresponding to the points in a PCA cluster to be averaged to form a template. Second, we can use them as a visualization method for assessing the results of spike sorting. For example:



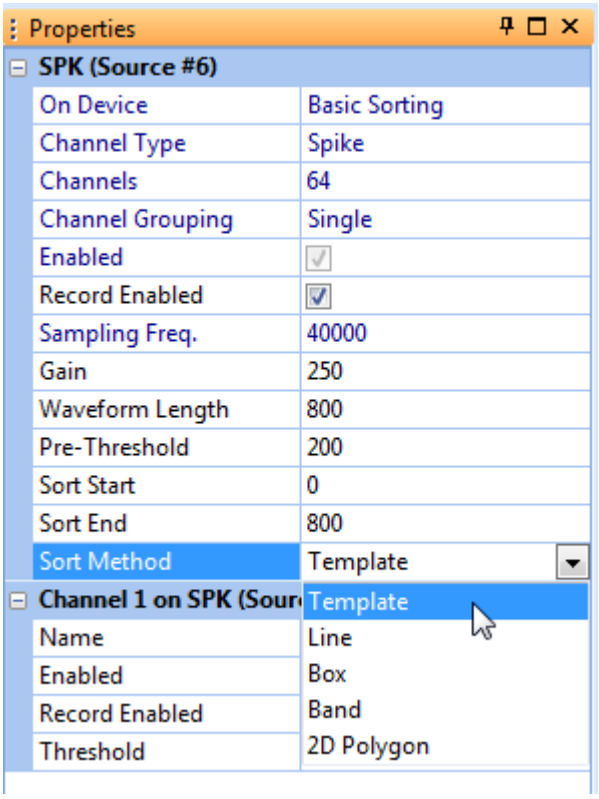

Third, we can use PCA as a basis for a sorting method, where we manually draw contours around visible clusters in the PCA feature space in order to define units, then perform the real-time sorting by projecting each incoming spike into PCA space and testing it against each of the contours. Fourth, PCA is suitable for use by a fully automatic spike sorting algorithm, which identifies clusters in PCA space and generates either templates or PCA contours from them. Each of these uses will be described in the following sections.

The OmniPlex System also supports an improved version of PCA called Enhanced PCA, which yields improved cluster separation and compactness in many cases. For more information, see "Appendix H: Selectable 2D/3D Feature Space and PCA Options" on page A-46.

## 8.3 Taking a Spike Snapshot and Viewing PCA Clusters

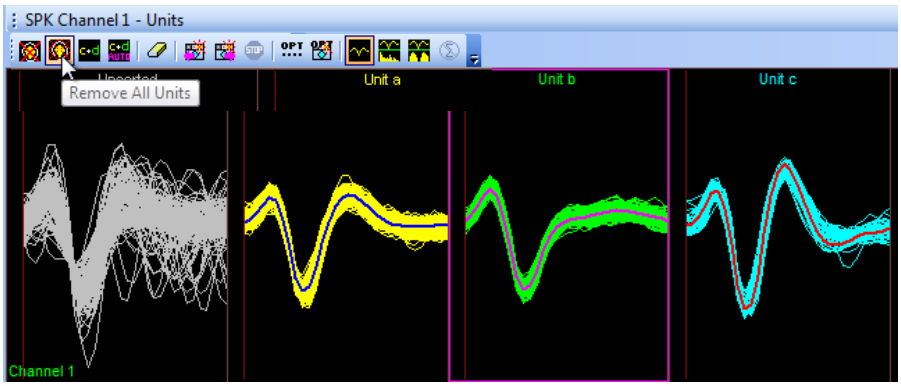
This section describes how to use 2D PCA as an alternative to waveform crossing for defining units for template sorting. All the other techniques that were discussed in the section on template sorting still apply; we will merely be using a different method to select the spikes which will be averaged and used as the template.

1 If you are not currently using template sorting, stop data acquisition and set the sorting method to **Template**, then restart data acquisition.



2 If necessary, set the gain and thresholds as described in previous sections, so that you have incoming spikes on all channels. If you have any defined units on the currently selected channel, delete them using **Remove All Units**, as described previously.

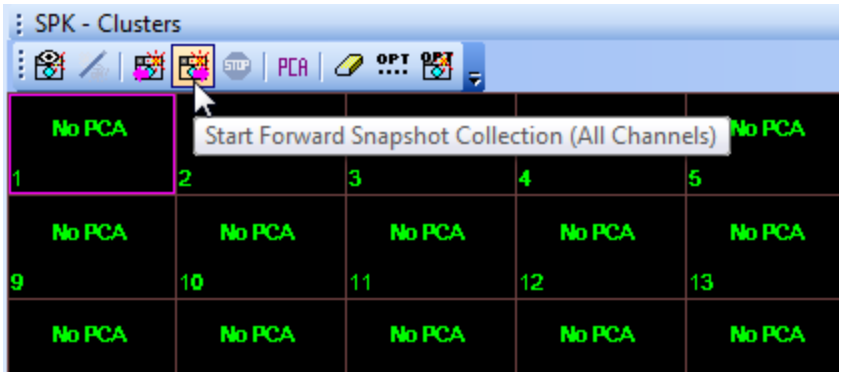
**Note:** Setting the gain is applicable to DigiAmp subsystems, not DHP subsystems.



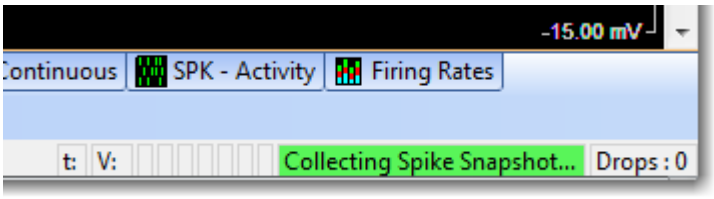

3 Click on the SPK - Clusters tab to display the multichannel 2D PCA window:

4 Note how all the channels show “No PCA.” This is because the initial calculation of the PCA projection requires a spike snapshot, similar to the way

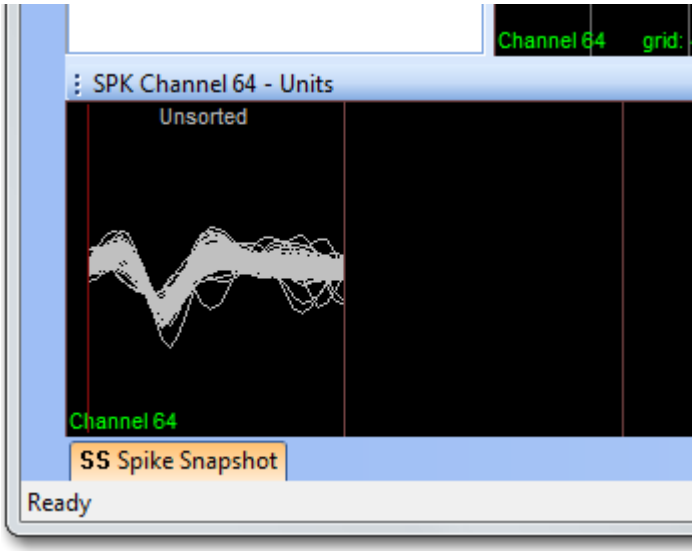
in which auto-thresholding requires a SPKC snapshot. To take a spike snapshot, click the **Forward Snapshot** button in the toolbar:



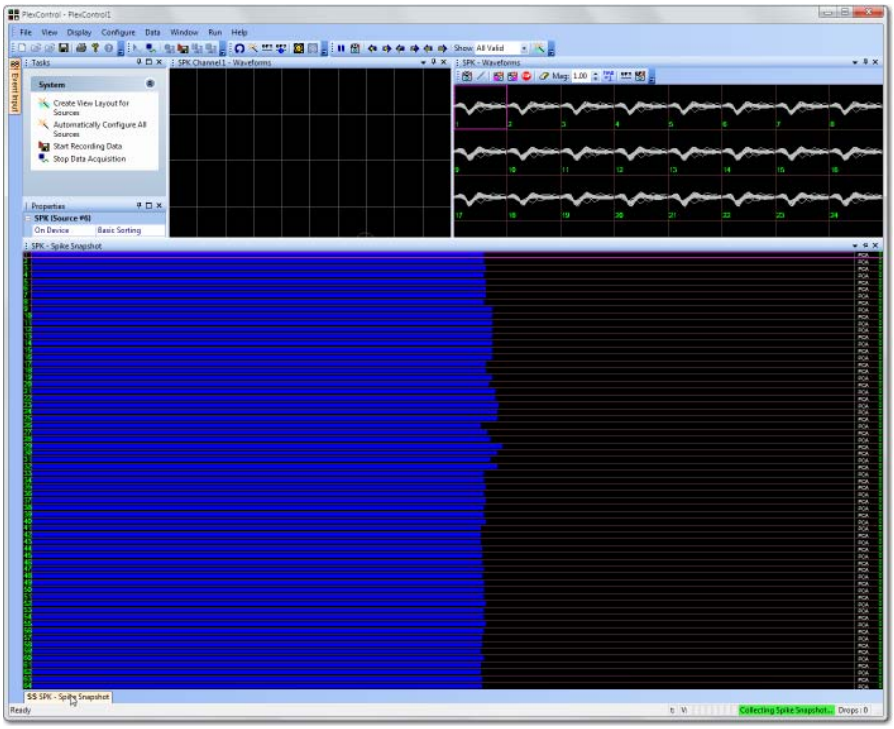

5 While spikes are being collected into the spike snapshot, the status bar displays “Collecting Spike Snapshot...”



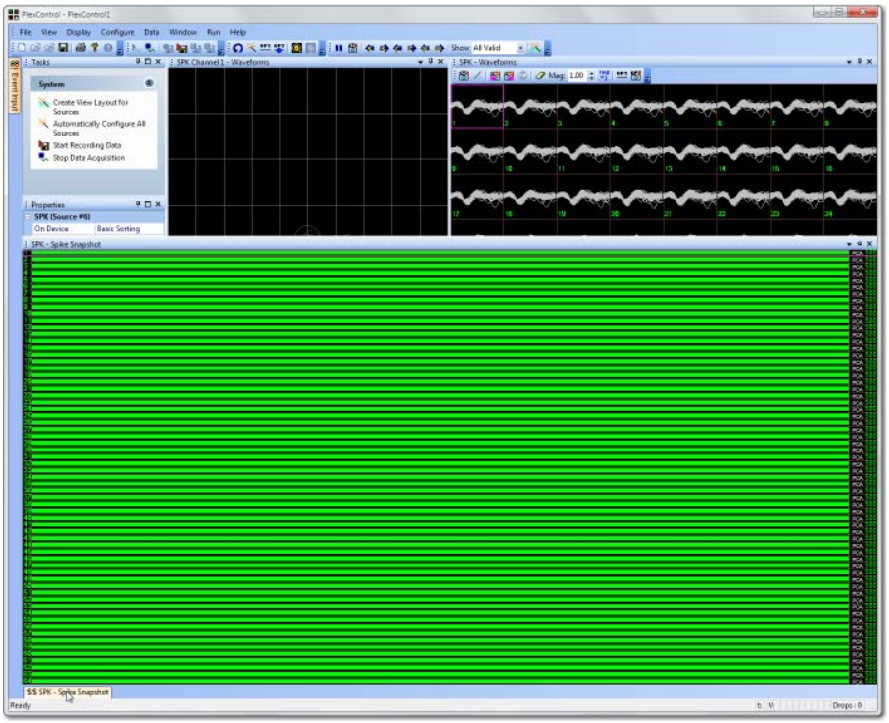

6 You can monitor the progress of spike snapshot collection in more detail by hovering the cursor over the Spike Snapshot tab at the lower-left of the PlexControl window:



7 After hovering the cursor over the tab for a moment, the Spike Snapshot view will pop up:

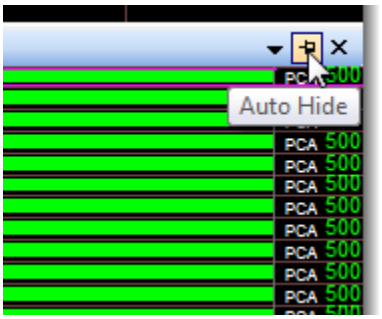


8 The blue bars indicate the progress of snapshot collection; when each channel's bar reaches the right side, the bar turns green to indicate that the snapshot has been collected:

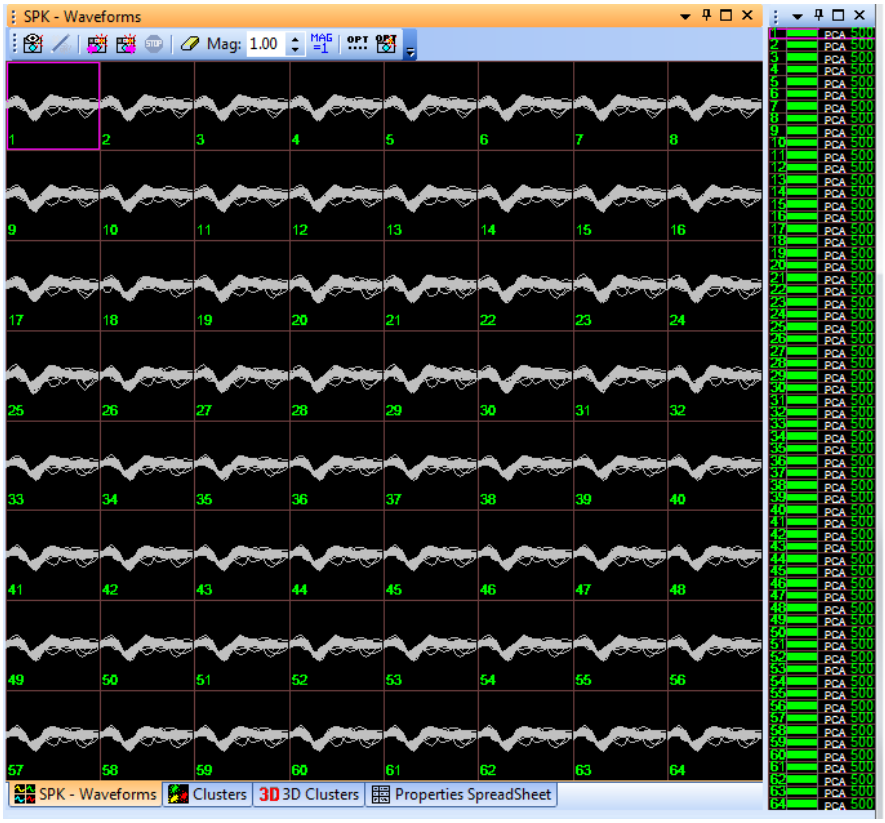


9 Moving the cursor outside of the Spike Snapshot Progress view will cause it to collapse back into the tab at the bottom left of the window.

10 Note that with real data, some channels will complete their snapshots quickly, but others may take much longer, depending on the per-channel firing rates. In such cases, you may wish to “pin” the view so that it remains visible without having to pop it up from its tab each time. To do so, click on the pushpin in its upper-right corner:




11 The Spike Snapshot Progress View then attaches itself to the right side of the multichannel window:



12 When the required number of spikes has been collected (500 spikes by default) for each SPK channel, the “No PCA” for that channel is replaced by a display of PCA clusters:

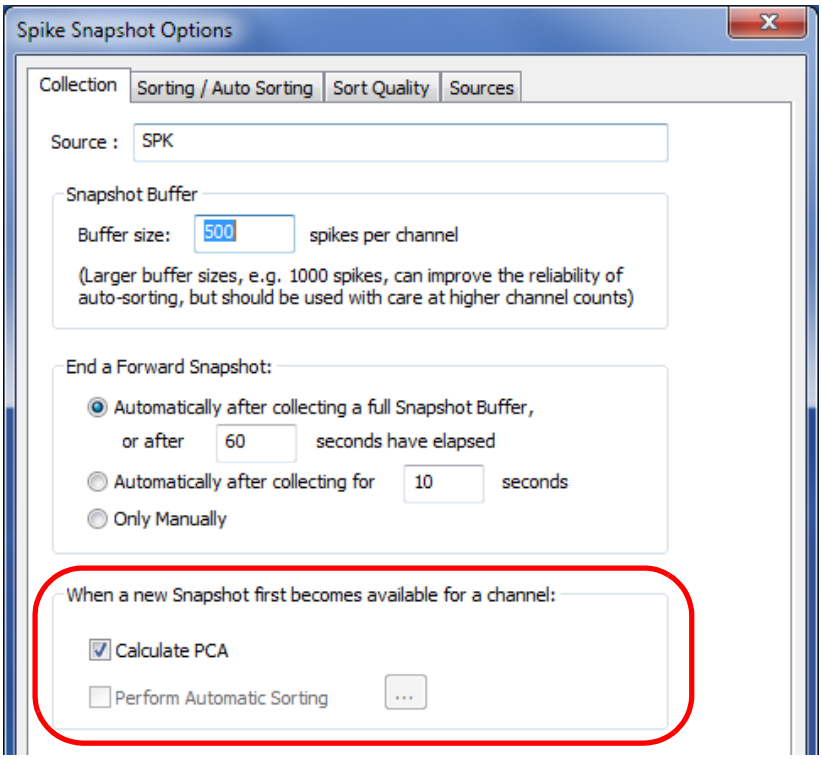
**TIP**

**Taking snapshots**

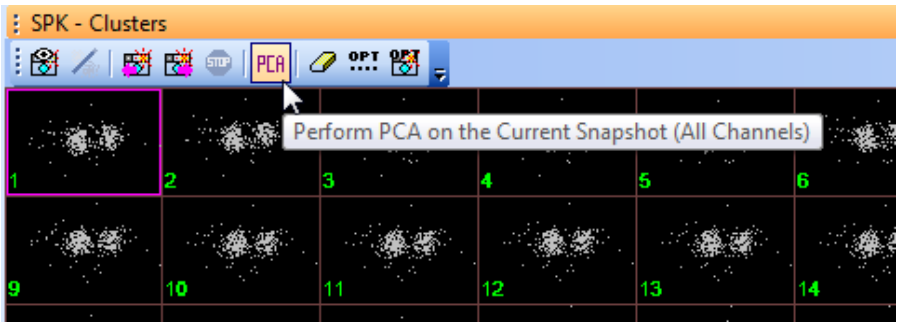
You do not have to wait for every channel to finish its snapshot to begin working with the channels that are ready.

**Note:** You will sometimes see, especially with test signals where every channel is nearly identical, that the PCA clusters for some channels will appear to be flipped or mirrored vertically and/or horizontally, compared to other channels. This is because the mathematical definition of PCA is invariant to sign changes, since PCA is based on variance. In other words, two PCA displays, one of which is the mirror image of the other, are mathematically equivalent.

**13** Note that the default for spike snapshot collection is to calculate PCA when the first snapshot collection is complete, as shown in the spike snapshot options:



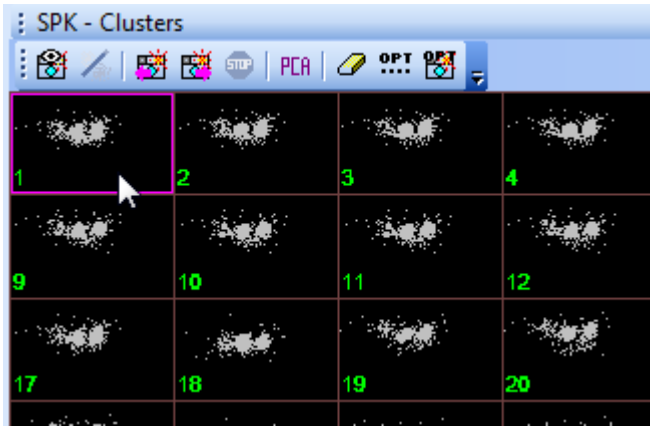

This is generally the most convenient method; however, you can also manually initiate the PCA calculation (for example, if you wish to recalculate PCA based on a later snapshot) using the **PCA** button in the toolbar:

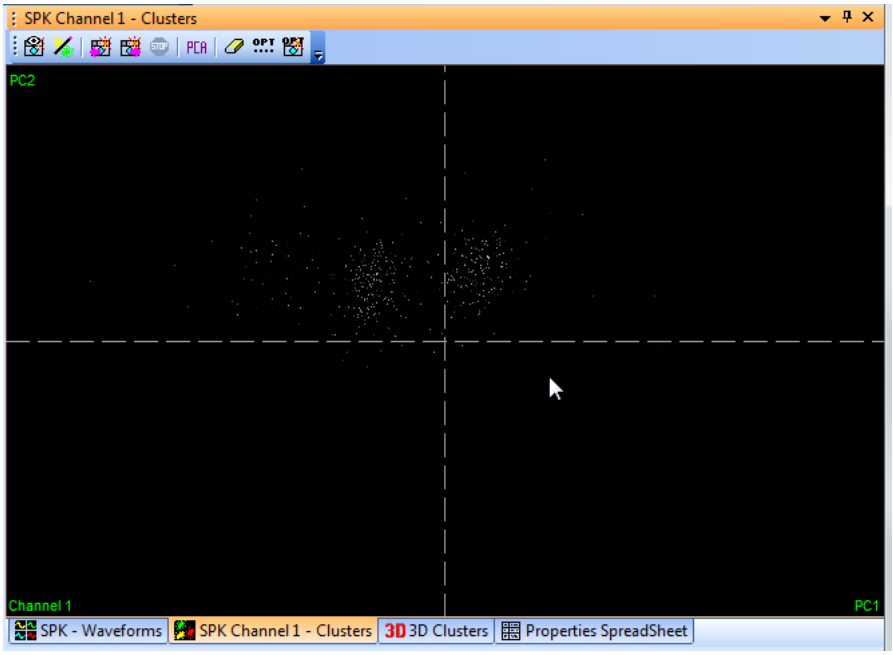



However, you still must collect a spike snapshot before the PCA can be calculated.

| | |
|---|---|
| | **CAUTION**<br>**Caution when recalculating PCA based on a new snapshot**<br>If you are using the 2D Polygon sorting method (as described in Section 8.8, "2D Polygon Sorting " on page 271), recalculating PCA based on a new snapshot will usually require that you create new sorting contours in order to sort correctly with the new PCA features. |

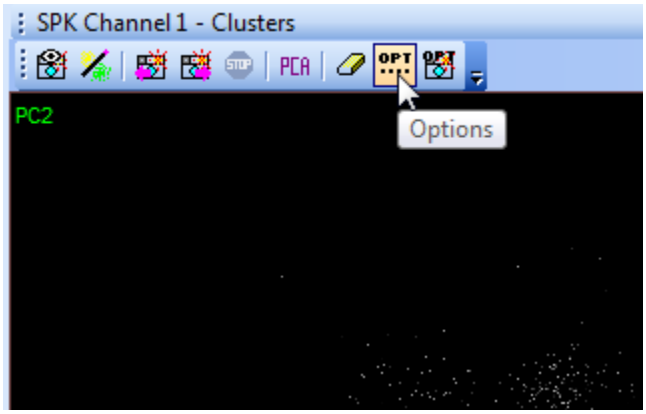
**14** Double-click on channel 1 in the multichannel PCA display to zoom it to single-channel mode:



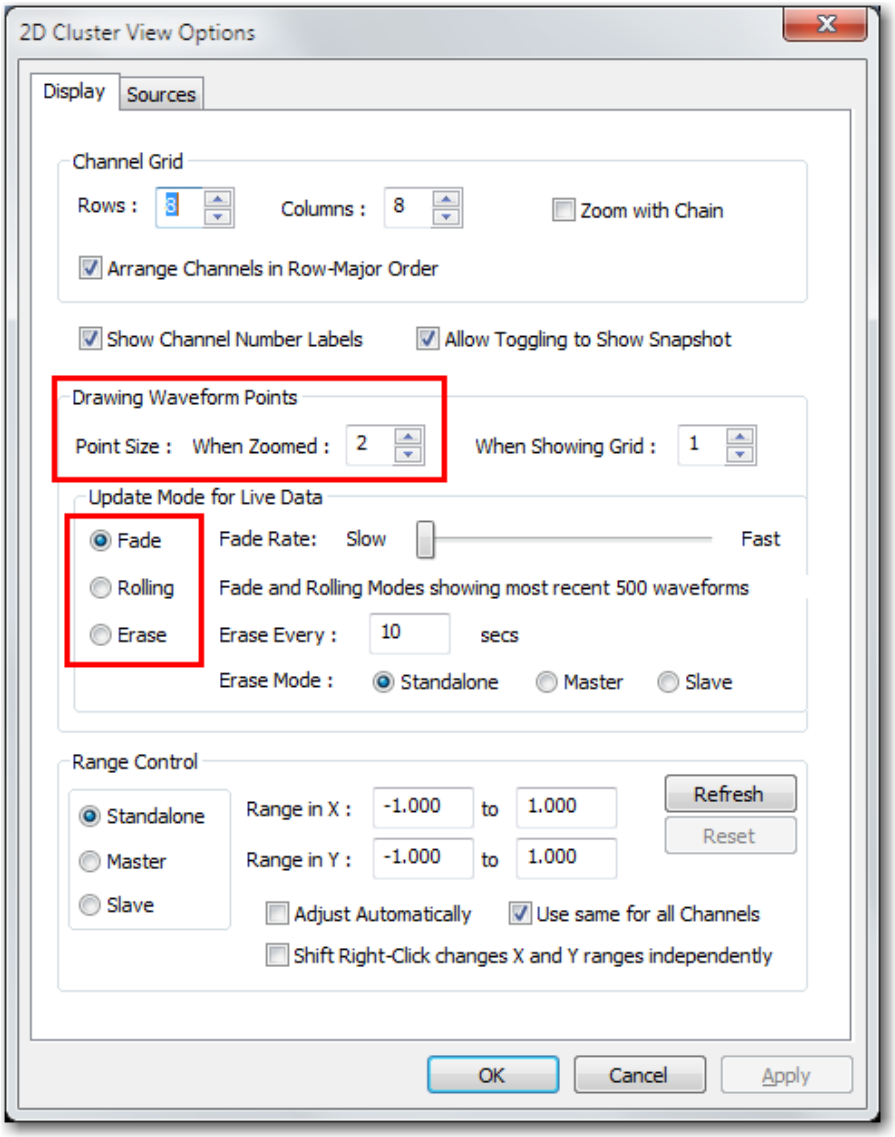




**15** Depending on your monitor and your preferences, you may wish to use a larger point size for the PCA dots. You can also switch to Rolling or Erase

modes, just as with the main spike windows. If you wish to change the display settings, click on the **Options** button in the toolbar:

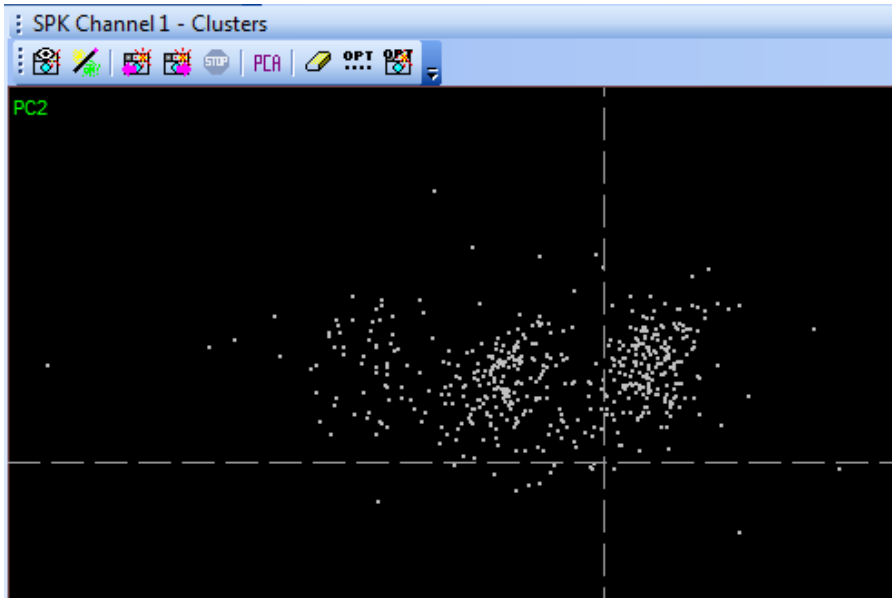


16 In the **2D Cluster View Options** dialog, you can change the **Point Size** and **Update Mode for Live Data** as desired:

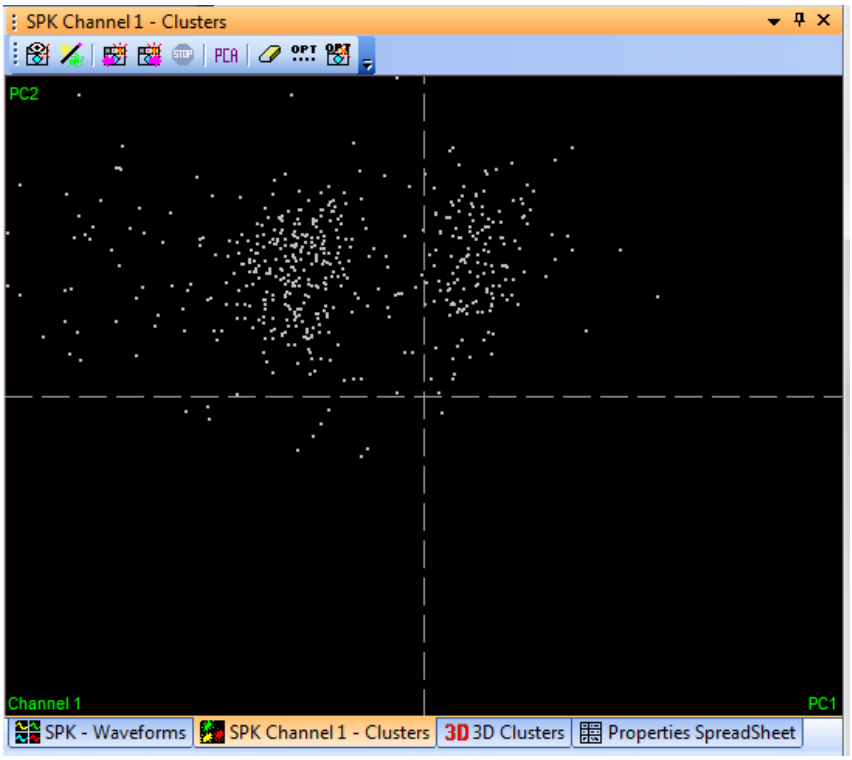


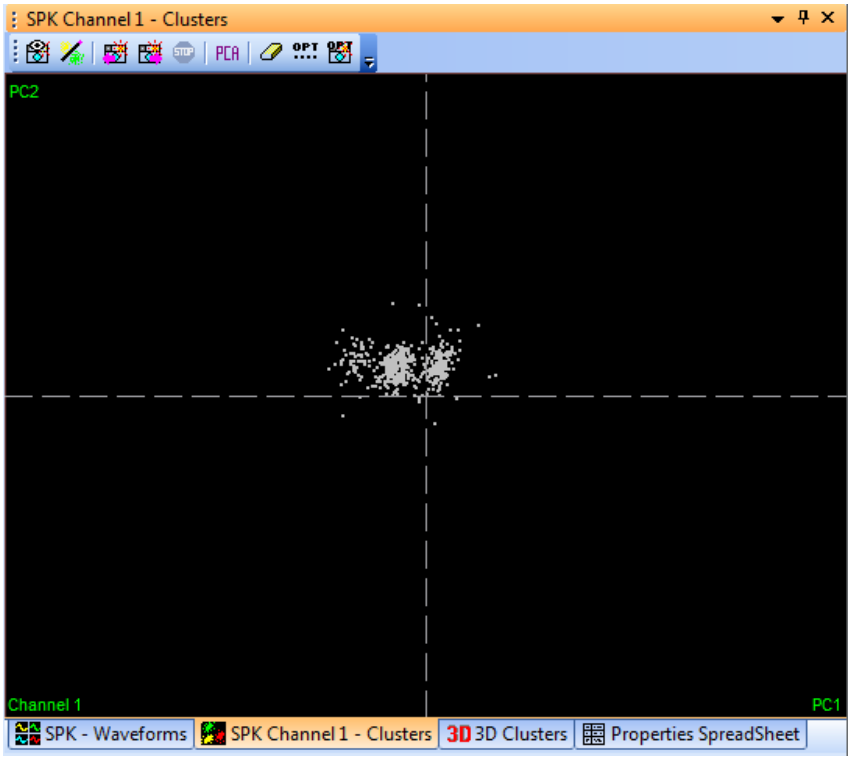
**17** When you change the settings, you can click **Apply** to see the effect of the changes on the display immediately, then click **OK** when you are done making changes.

Here is an example of the effect of setting the point size to 2 pixels and the update mode to Rolling:

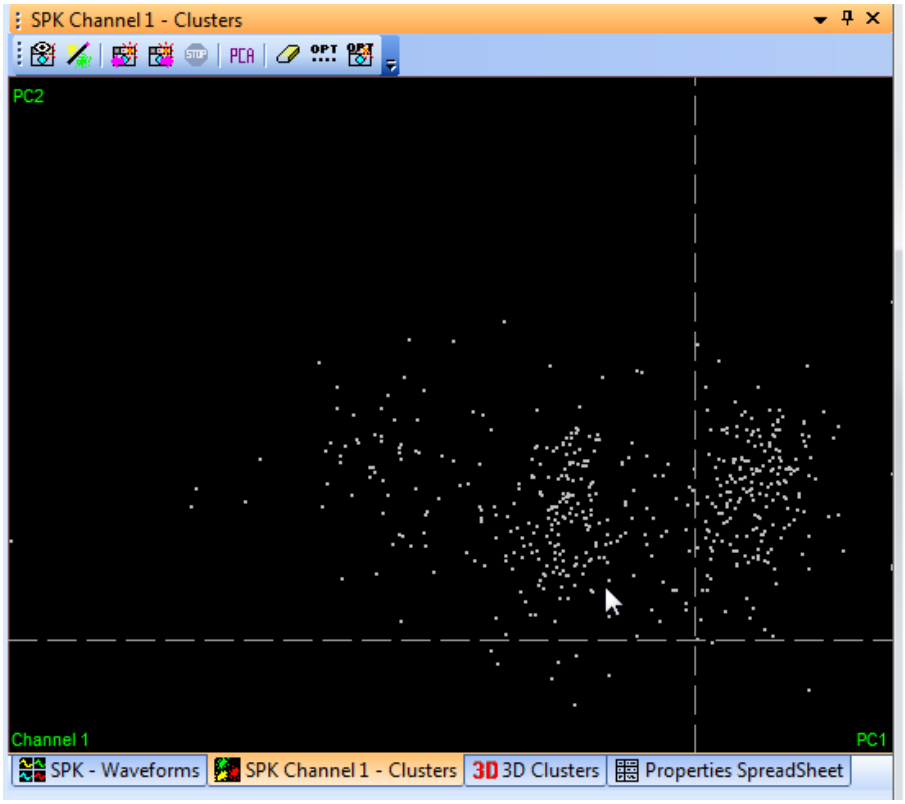


18 The size and position of PCA clusters can vary considerably, so the zoomed PCA display allows you to interactively adjust the magnification and panning (position) of the display. To change the magnification, simply single-click the cursor anywhere in the zoomed PCA display and roll the mouse wheel:



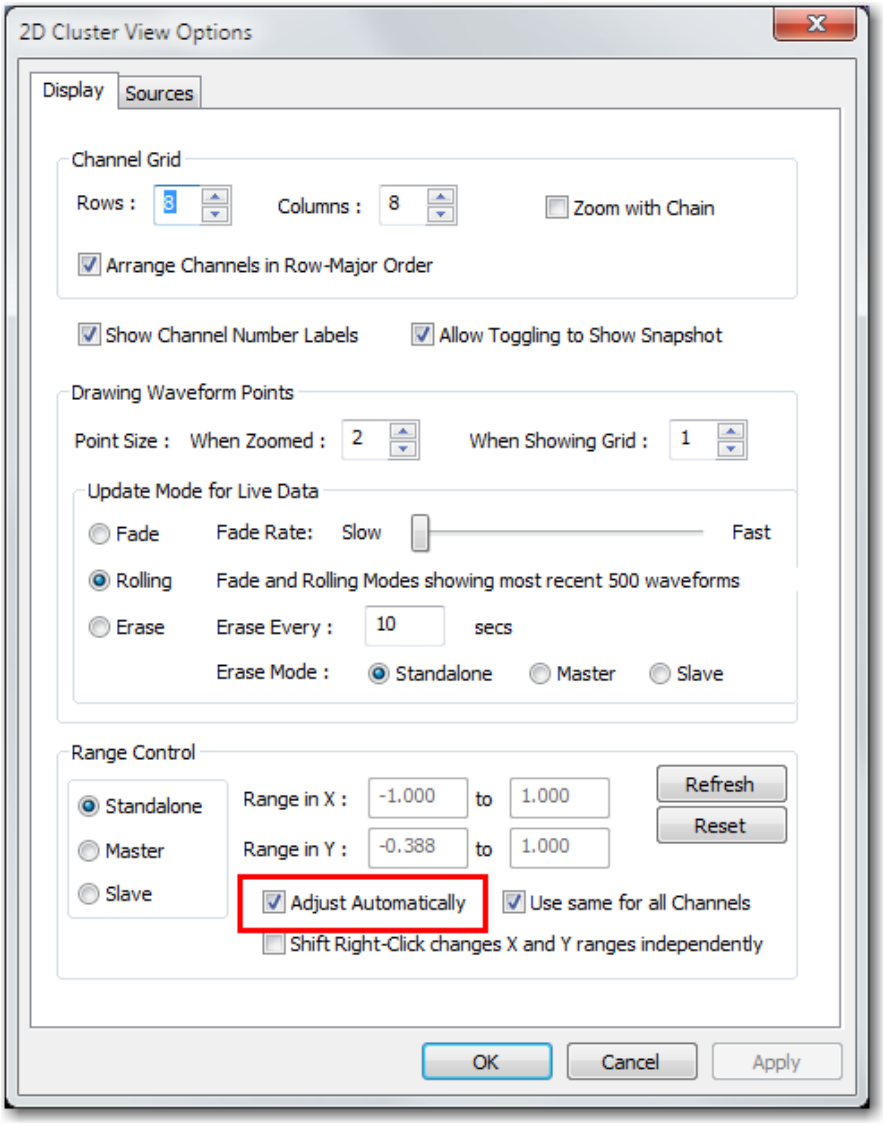



**19** To pan (reposition or drag) the display, place the cursor anywhere in the PCA display, hold down the Shift key, then hold down the left mouse button and move the mouse to pan the display. Release the Shift key and left mouse button when you are done. The X/Y axes show the new position of the origin in PCA space.




Note that the X axis represents PC1, the first PCA coordinate, which represents the direction of largest variance in the original spike data, while the Y axis represents PC2, the second PCA coordinate, which represents the direction of second-largest variance.

**20** Optionally, the PCA display can automatically adjust the magnification and viewing position, so that the clusters can never “go offscreen”:



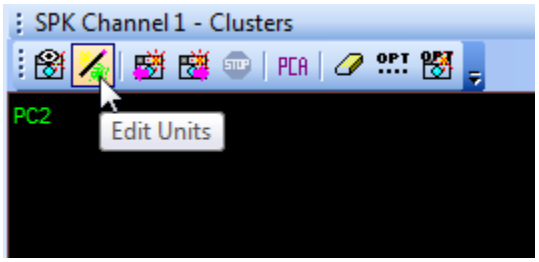

Every few seconds, the PCA display will adjust the magnification and pan as needed to keep the PCA clusters fully onscreen.

You can only adjust the magnification and panning of the PCA display while you are zoomed in, but if you have the **Use same for all Channels** option enabled, your changes will be applied to all the PCA displays. This usually works well, but if the PCA clusters on different channels are substantially different in size and position, you may need to uncheck **Use same for all Channels** and adjust each channel's PCA display individually.

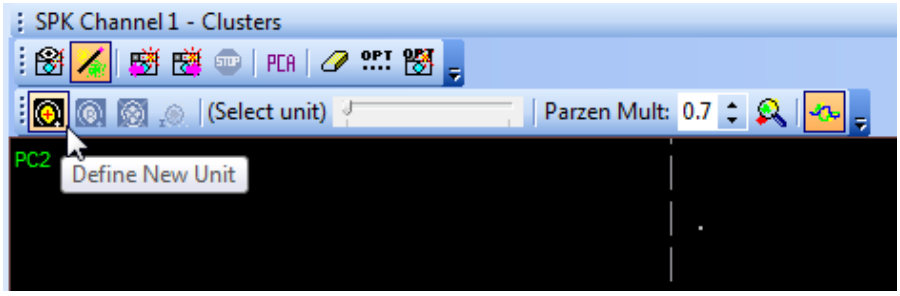
## 8.4 Defining Units for Template Sorting using PCA Contour Drawing

You are now ready to define units for template sorting by drawing contours (outlines) around 2D PCA clusters, instead of by waveform crossing as you did previously. Keep in mind that you can define any unit on any channel by either method; you could define unit “a” by waveform crossing, then unit “b” by contour drawing. Once you have defined the unit, the method used to create the unit definition is no longer relevant.

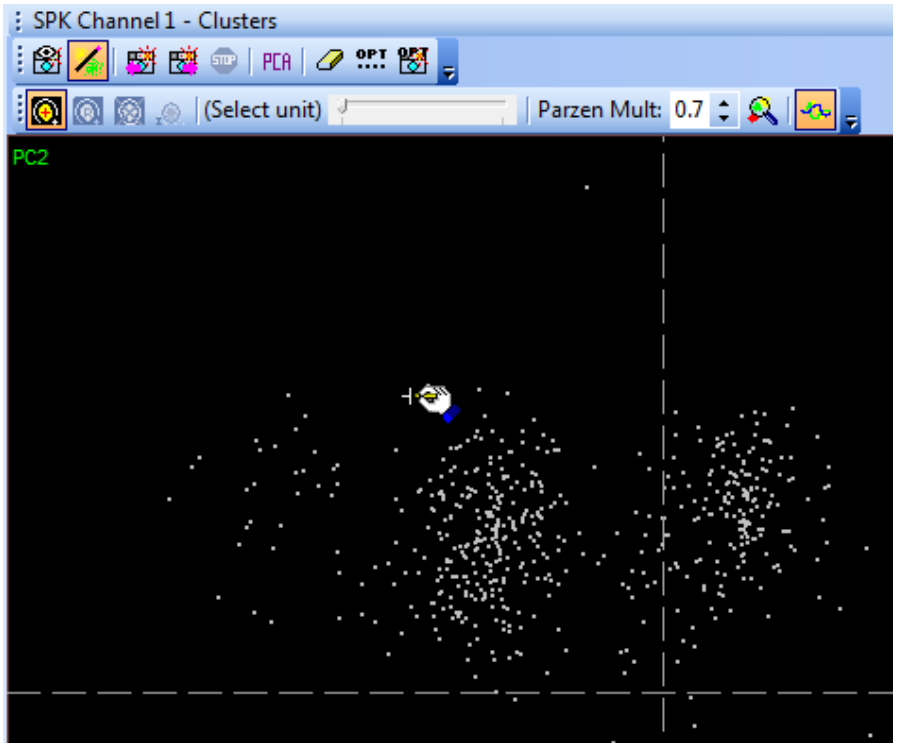
1 Make sure the PCA display is zoomed in on a single channel and that clusters are clearly displayed, as described in the previous section. Display the toolbar for the PCA display and click the **Edit Units** button:



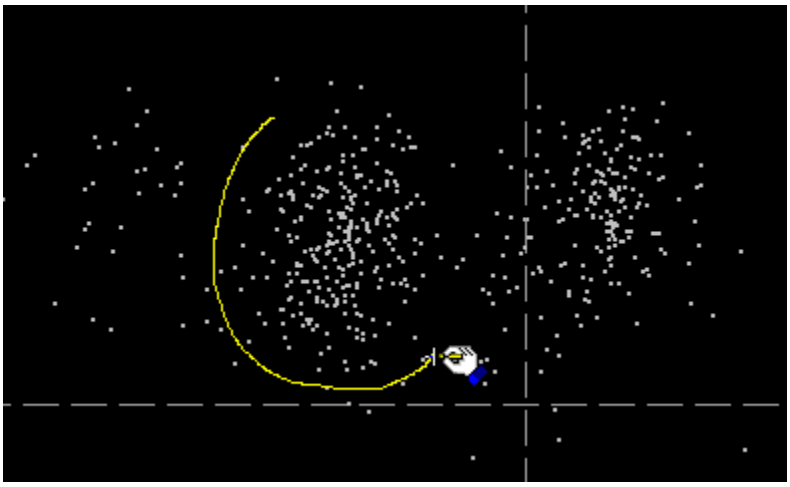

2 The unit editing toolbar is displayed. Click the **Define New Unit** button to add a unit:



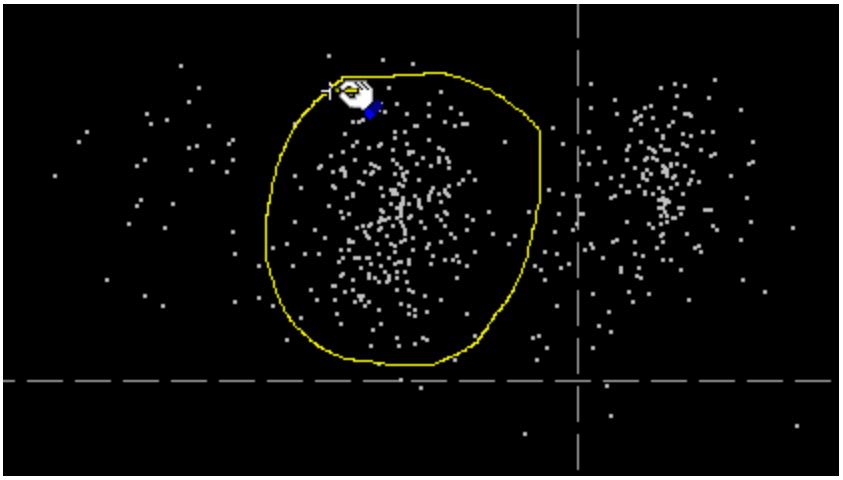
3 The cursor changes to the Add Unit cursor, indicating that you can draw a contour around a PCA cluster:




4 Hold down the left mouse button and draw a contour that encloses the most prominent cluster. Don't worry if the contour isn't smooth - the main thing is to encircle the points that belong to one cluster, while avoiding including points belonging to nearby clusters as much as possible.



5 When you have drawn all the way around the cluster and returned to the approximate starting point of your contour, release the left mouse button:

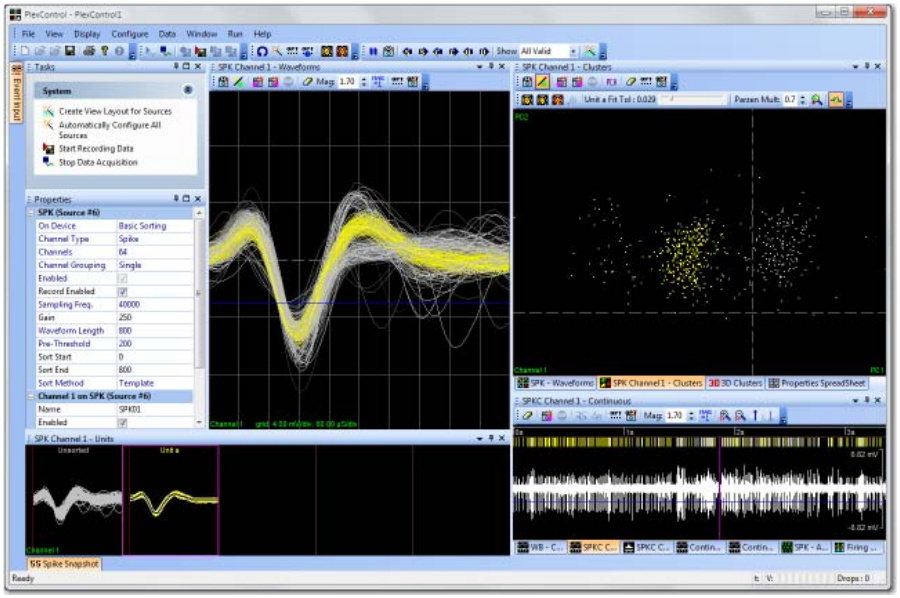


**TIP**

**Use the CTRL key as a shortcut for adding a new unit**

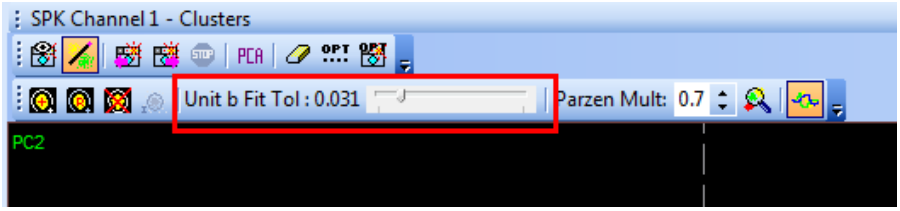
Just as with waveform crossing, you can use the **CTRL** key as a shortcut for adding a new unit, so that you don't have to click the **Add Unit** button before each unit. Hold down **CTRL** before pressing the left mouse button and drawing the contour.

6 The contour which you drew is erased and the spike waveforms corresponding to the PCA points which you encircled are averaged and used to define a new unit template for the current channel. You can see incoming spikes being sorted using this template in the main spike window, the Units window, the zoomed PCA window, and the SPKC window:



7 Just as with defining new units by waveform crossing, you can continue to add more units by circling additional clusters, delete or replace units, etc. Note that while you are seeing the sorted and unsorted spikes being projected into the 2D PCA display, the actual sorting is being done by template sorting, and is controlled by the template and the fit tolerance.

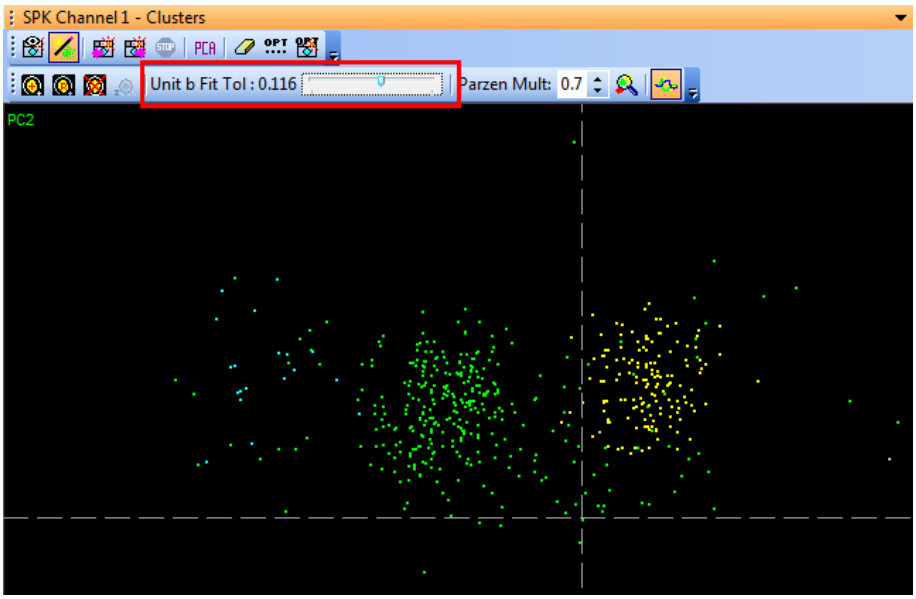
8 When the sorting method is template sorting, a fit tolerance slider is displayed in the PCA unit editing toolbar, just in the main spike window. This can be used to adjust the tolerance for the currently selected unit while viewing clusters, without having to “hop over” to the main spike window.




Increasing the template fit tolerance has the effect in the PCA display of expanding the area of the clusters which consists of sorted spikes, while decreasing the tolerance has the effect of shrinking the sorted area.

Since there is not a one-to-one correspondence between the results of a template sort and the results of testing PCA points for containment in a 2D contour, you may notice that a few points around the edges of a PCA cluster remain unsorted. While this can be considered a form of beneficial outlier rejection, if you wish you can increase the template fit tolerance slightly to in effect expand the area of the cluster that will be sorted, so that the template

sorting more accurately corresponds to the contour your drew. However, increasing the tolerance too much can result in spikes in nearby clusters being incorrectly sorted, as in this example, where the green unit's tolerance is too large, and green spikes are appearing well outside the desired cluster:

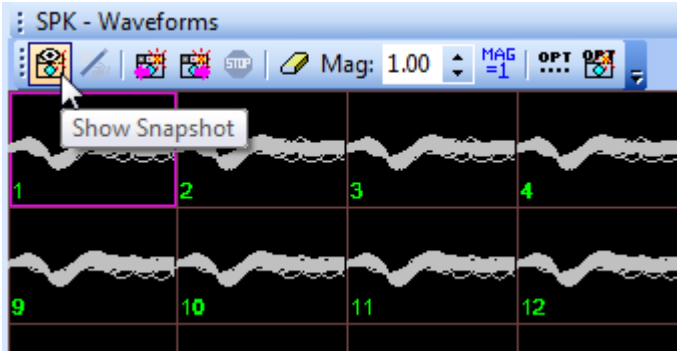


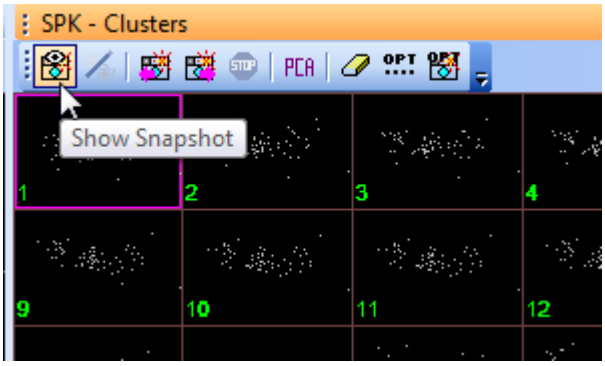

**9** Note that if you wish, you can define another new unit, on the same channel, by waveform crossing instead of PCA; if you do so, you can then use the PCA window to monitor the results of the sorting in PCA space, adjust the fit tolerance, etc.

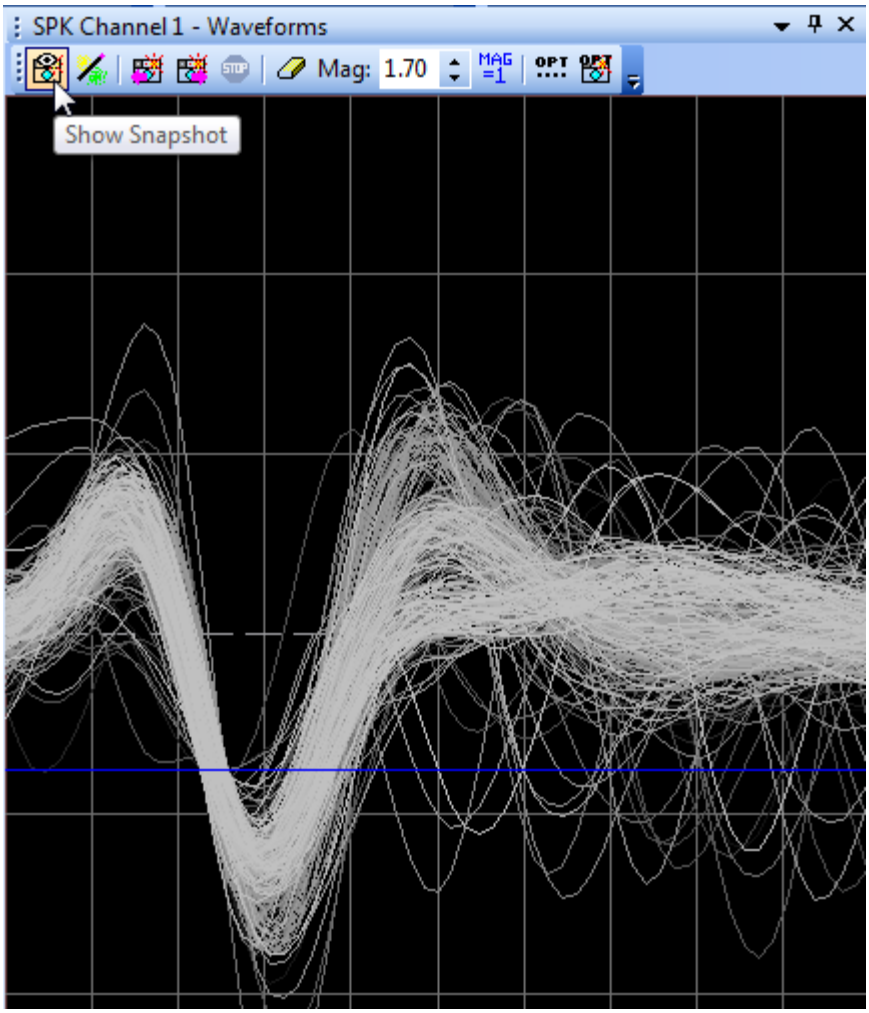
In general, many users find that circling clusters in PCA space is easier than trying to find a good location to cross waveforms in the main spike window. In the main spike window, spikes sometimes overlap over much of their length and the display can be quite "busy," especially at high firing rates, while in many cases the corresponding PCA clusters are more distinct and easier to work with, since the first two PCA components tend to emphasize those parts of the spike where waveforms are more clearly separated, and the drawing of each spike in the PCA display consists of only a single point. However, the choice of one unit definition method over the other can be very data-dependent, so it is best to view both the spikes and the PCA clusters before deciding how to proceed.

## 8.5 Snapshot Mode versus Live Display

So far, we have collected a spike snapshot for the purposes of working with PCA clusters. However, we haven't actually looked at the spike snapshot itself. The OmniPlex System in fact allows you to toggle all the spike and PCA displays between either displaying the live, incoming spikes (the default mode, which we have used for all examples so far) and displaying the spike snapshot. Toggling between live and snapshot modes can be done using the same button from any of the spike or PCA views' toolbars:





**TIP**

**Toggling between live and snapshot modes**

You can also press the "**S**" key on the keyboard to toggle between snapshot and live modes.

Clicking **Show Snapshot** results in all the other displays being toggled as well — you cannot view live spikes in one view while viewing the snapshot in another. Basically, you are either in a mode of "working on live spikes" or "working on the spike snapshot." Note that the continuous displays are totally separate and unaffected by whether live or snapshot spikes are being displayed in the spike and PCA views.

It is important to remember that switching between live and snapshot modes is purely a PlexControl user interface behavior, to allow you to interact with the snapshot data at your leisure. All the processing in Server continues uninterrupted

in the background: filtering, spike detection and sorting, and so on, all continue as before, using the current thresholds, templates, etc.

When you switch to displaying the spike snapshot, all the spike and PCA displays stop animating, because they are now displaying a fixed set of 500 spikes (by default), the same spikes that were collected when you previously clicked on **Collect Forward Snapshot**. These were also the spikes that were used to calculate the PCA projection.

**TIP**

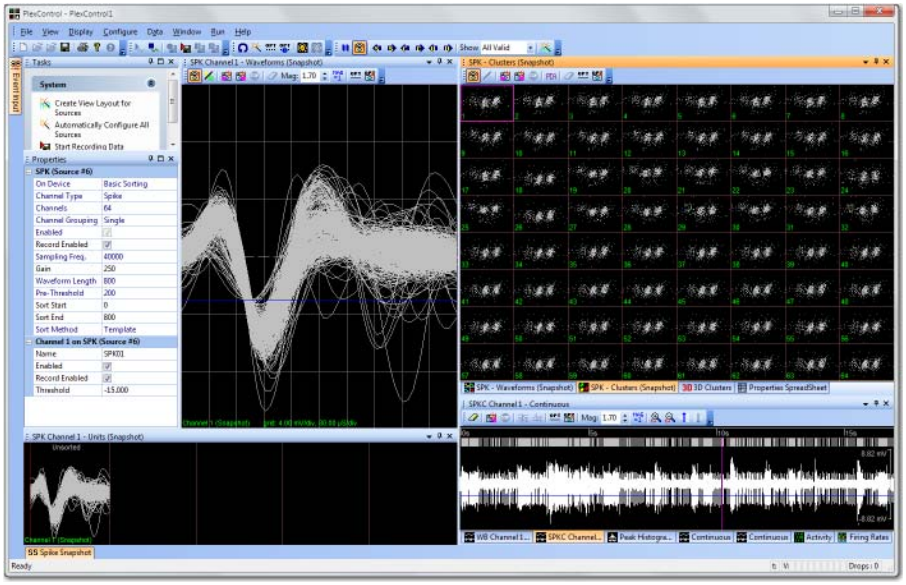
**Understanding snapshot mode vs. Pause**

Don't confuse snapshot mode with pausing the live display using the Pause button in the main toolbar. You must collect a spike snapshot, which is a sample of the live data, whereas pause mode simply freezes the live displays but does not collect a snapshot.

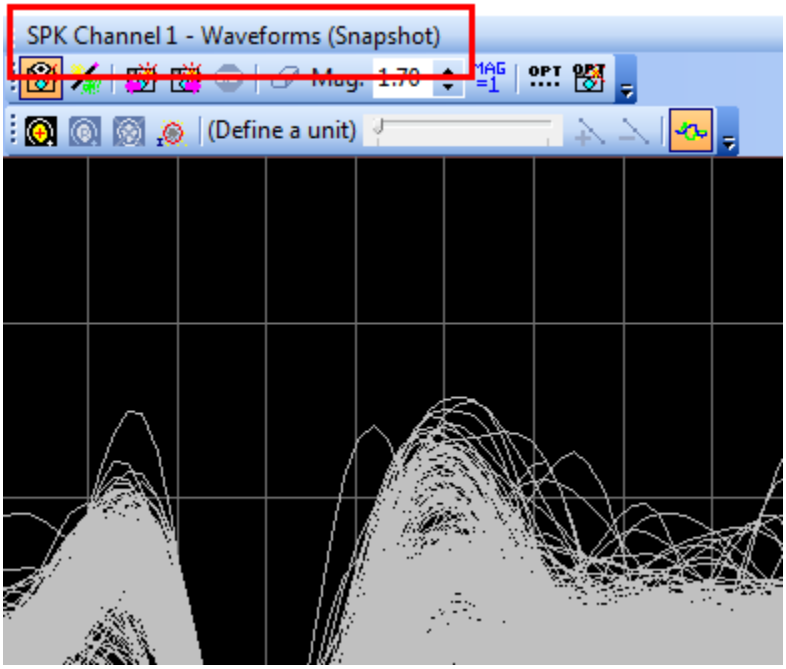
You might wonder why you would want to work with the snapshot, since it consists of "old" data. This is true, but the tradeoff is that it can be easier to define units with respect to a fixed set of data that is not constantly changing and redrawing from second to second. In many cases, spike shapes are quite stable over fairly long periods of time, so the "old data" issue is not a significant problem. Also, you can take a new snapshot, which will overwrite the previous snapshot, at a later time if you wish

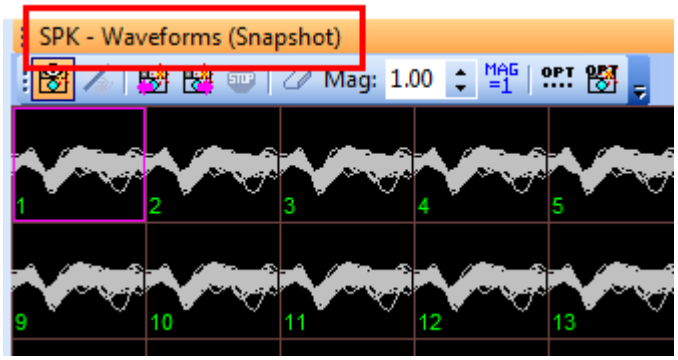
## 8.6 Defining Units Using Spike Snapshots

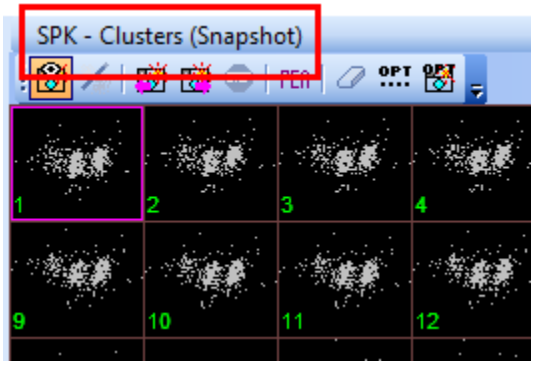
1 Click **Show Snapshot** in any of the spike or PCA views to toggle all the spike and PCA views into snapshot mode. The spike views display the spikes in the most recently collected snapshot; the PCA views show the PCA projection of the spikes in the snapshot.



The title bars of the affected views will indicate “(Snapshot).”



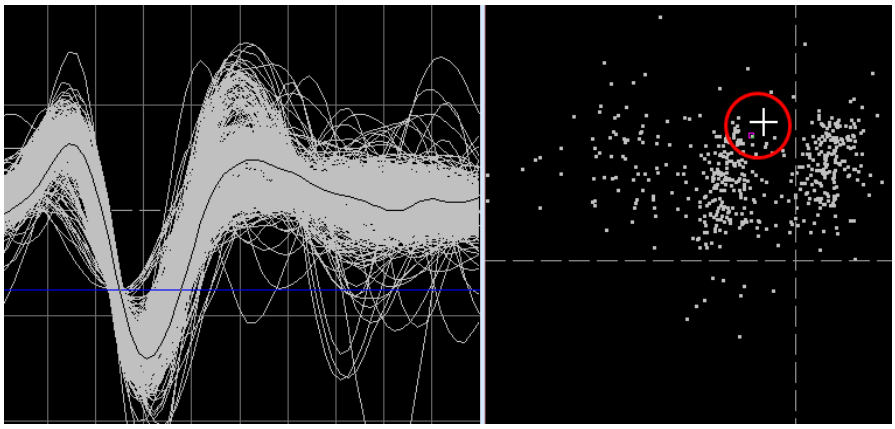


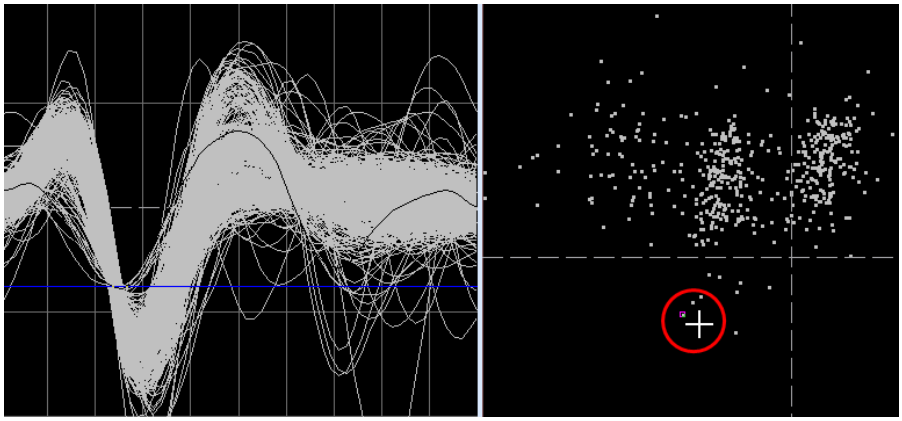

Note that since the spike and PCA views do not animate while in snapshot mode, display options such as Fade, Rolling, Erase and Scope do not apply. However, when the PCA display is zoomed into single-channel mode, you can still use the interactive magnification and panning functions, and the PCA point size setting is still applied.

**2** You can now perform all the same unit definition commands that you used while in live mode, including waveform crossing and PCA contour drawing. When you select waveforms to be used to form a unit template, all units are being defined relative to the same set of waveforms. In comparison, when you define units by selecting waveforms from live data, the “pool” of waveforms available for selection is constantly changing, so in this sense, defining all units based on a snapshot is more consistent.

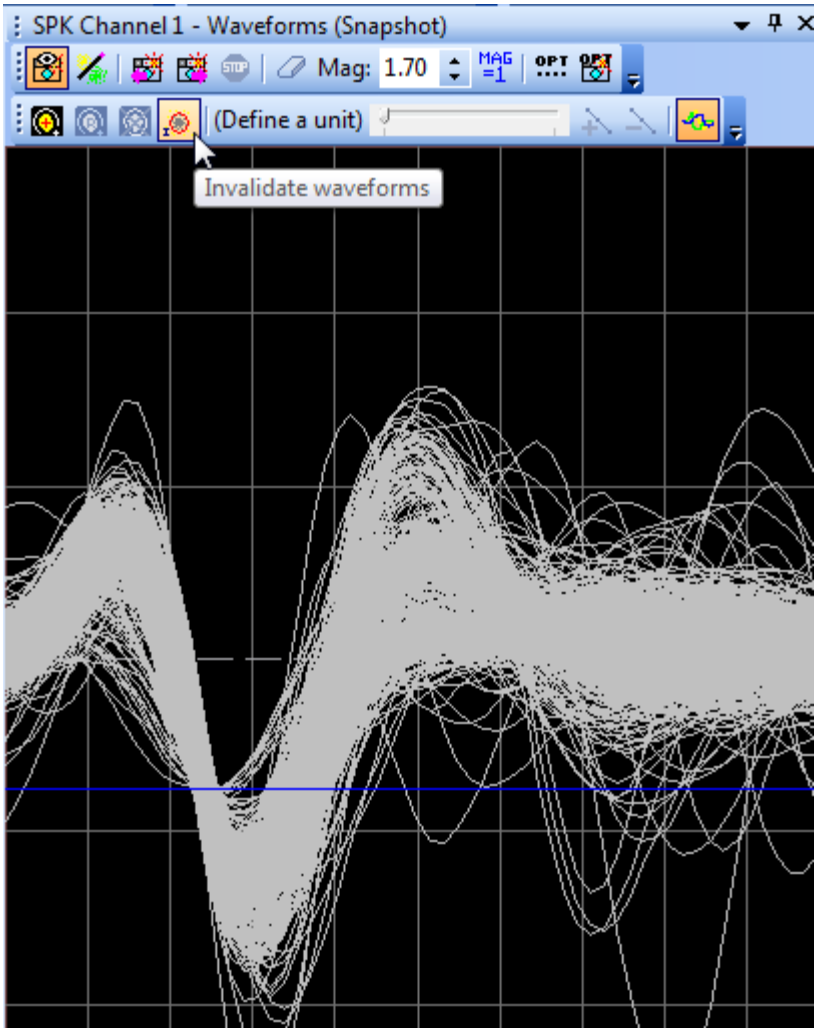
3 While in snapshot mode, you can use the mouse to explore PCA clusters and interactively view the spike waveform corresponding to any PCA dot. To do so, place the cursor in the PCA view, hold down the left mouse button, and move the mouse. The PCA dot nearest to the cursor is highlighted in color, and the corresponding spike waveform is drawn inverted (black for white and white for black) in the main spike window.



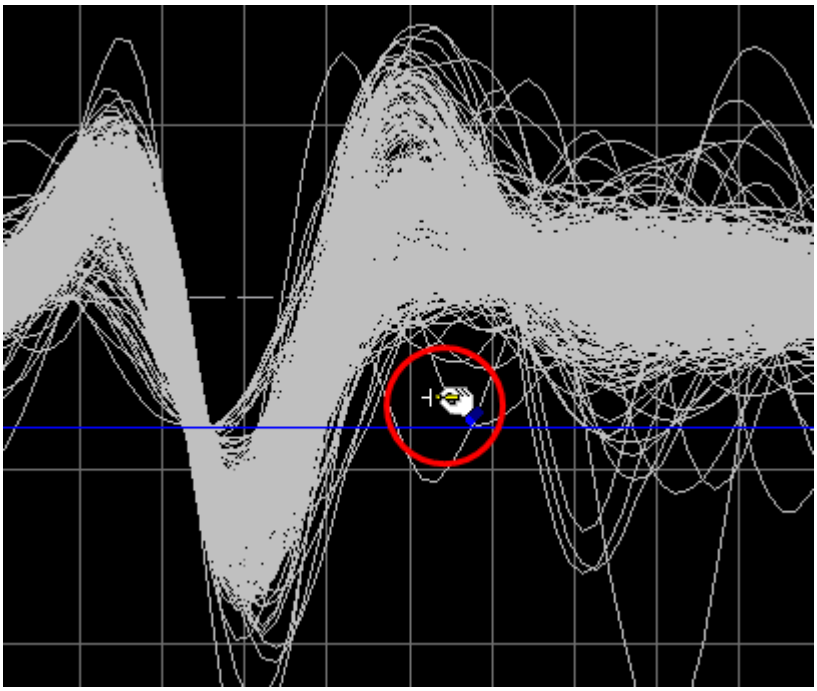
This can be a useful and rather entertaining way to learn about the correspondence between spike waveforms and PCA - for example, outliers that are far from the center of a cluster usually correspond to rare “difficult” spikes which could be the superposition of multiple spikes or other anomalies.



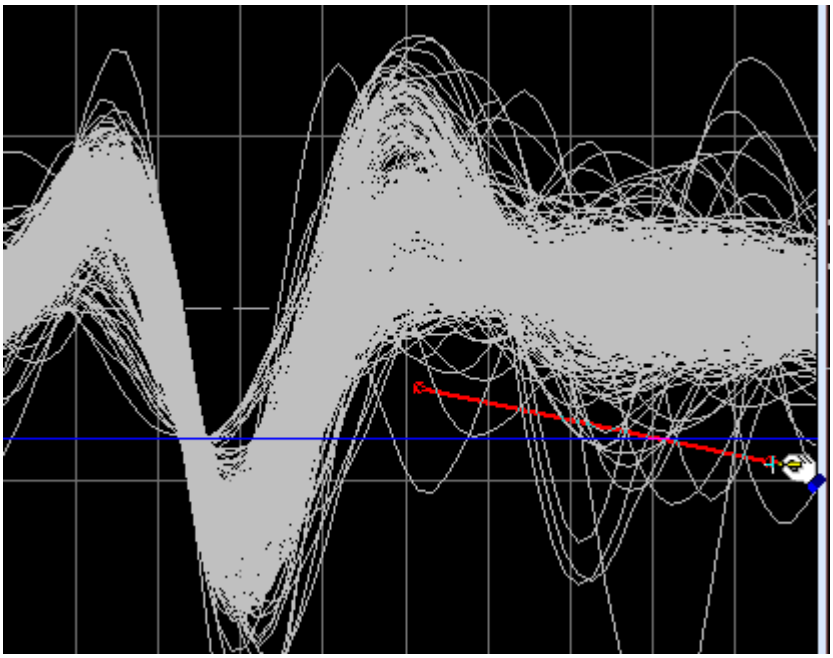
4 Another useful function which is available only in snapshot mode is spike invalidation. When the main spike window is in snapshot mode and unit edit mode, you can use the invalidate tool to remove waveforms from the snapshot. The main use of invalidation is to remove spikes that are clearly outliers, stimulation artifacts, superpositions, etc. After doing this, you can define units using the remaining spikes in the snapshot without the corrupted or outlier spikes “polluting” the results. To invalidate waveforms in the snapshot, click on the Invalidate button in the unit editing toolbar:



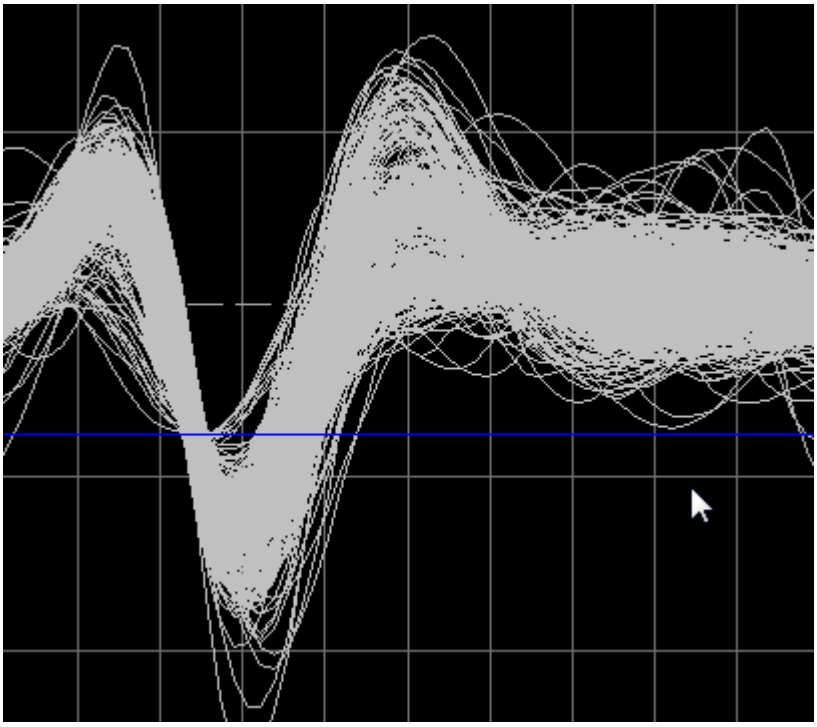
**5** The cursor changes to indicate that you are ready to mark waveforms for invalidation. You can think of this as the inverse of defining units by waveform crossing, in that the crossed waveforms will be removed rather than used to define a unit.



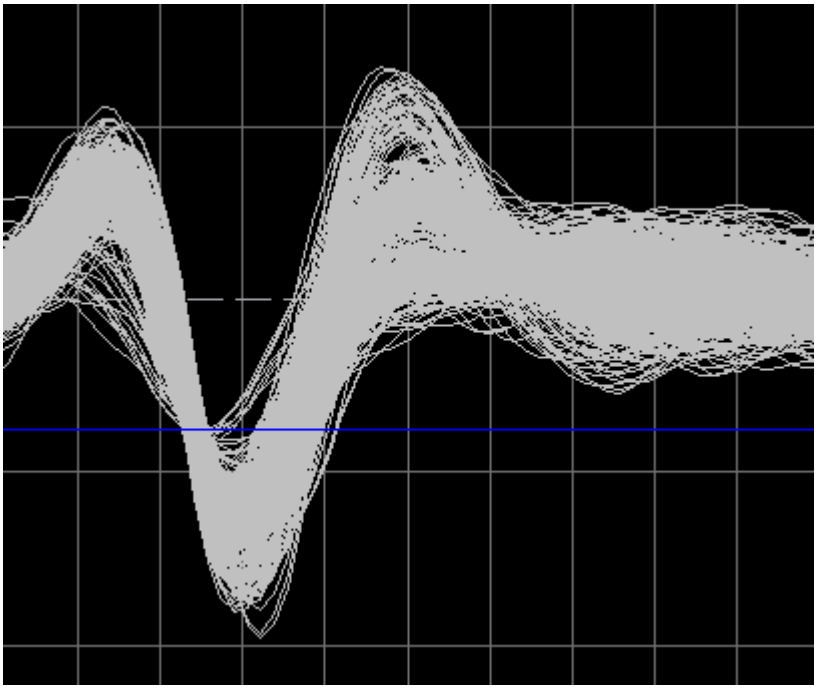
**6** Click and drag to draw a line across any spikes that you wish to invalidate.



7 All spikes in the snapshot which intersect the line are removed from the snapshot.



8 You can continue invalidating waveforms if you wish, until you have finished “cleaning up” the snapshot as desired.



Once the snapshot is satisfactory, you can use waveform crossing on the snapshot to create unit templates for template sorting, or draw sorting lines for line sorting, as previously described.

## 8.7 Band Sorting

### 8.7.1 Overview

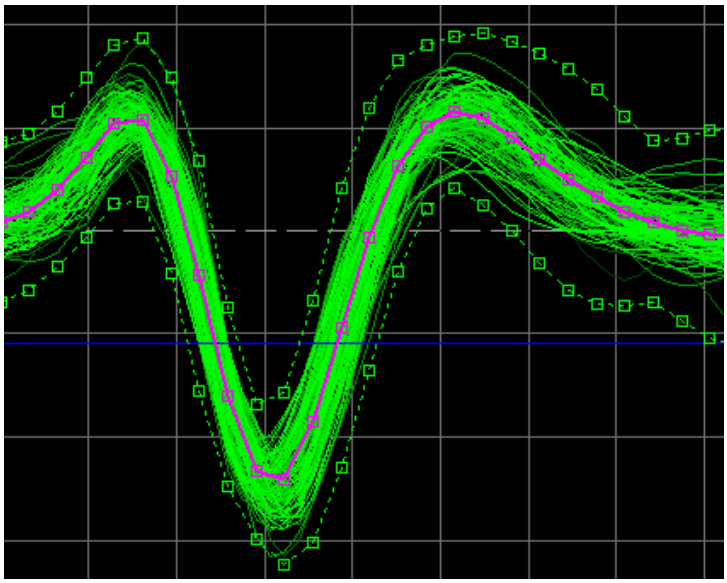
Band sorting is a generalization of template sorting. In both methods, each unit is defined relative to its template, that is, the mean or median of a group of waveforms. The group of waveforms can either be selected manually, or selected automatically by an automatic sorting algorithm, as described in these sections:

- Section 6.2, “Selecting Sort Method and Entering Unit Editing Mode” on page 178
- Section 8.4, “Defining Units for Template Sorting using PCA Contour Drawing ” on page 250
- Section 9.1, “Introduction to Automatic Sorting (Automatic Unit Finding)” on page 298.

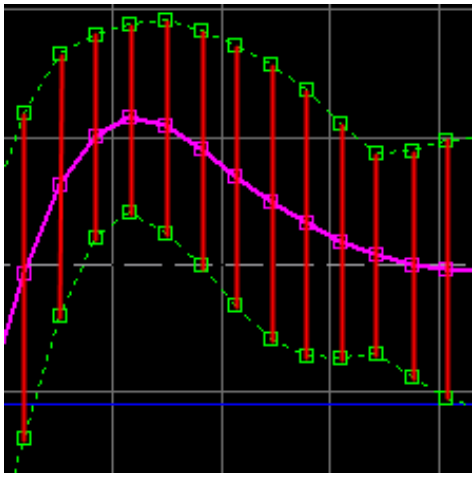
However, band sorting and template sorting use different criteria for determining whether each incoming, unsorted spike matches a given unit template.

With template sorting, the sum of squared errors between each point of the unit template and the corresponding point on the incoming spike is calculated, and this single summed error value is compared against the error tolerance for the template. Regardless of the error at any one point on the waveform, as long as the total sum of squared errors is less than the tolerance, the waveform is assigned to the unit.

However, with band sorting, the OmniPlex System maintains a set of tolerance values, one tolerance per template point, which in effect forms a band enclosing the template (shown below in blue) and defining the per-point amplitude limits within which an incoming spike must fall in order to be classified into that unit. (Note that we have the **PlexControl Show Filter** set to **Sorted Unit** so that only sorted spikes are shown, for clarity.)



You can visualize band sorting in terms of per-point amplitude “error bars” which define the maximum allowable amplitude difference between the template and spikes. To illustrate this, we have drawn additional red lines onto the original PlexControl display to show how at each sample time (x position), the points of the sorting band are equidistant (in y, or amplitude) above and below the corresponding template point:



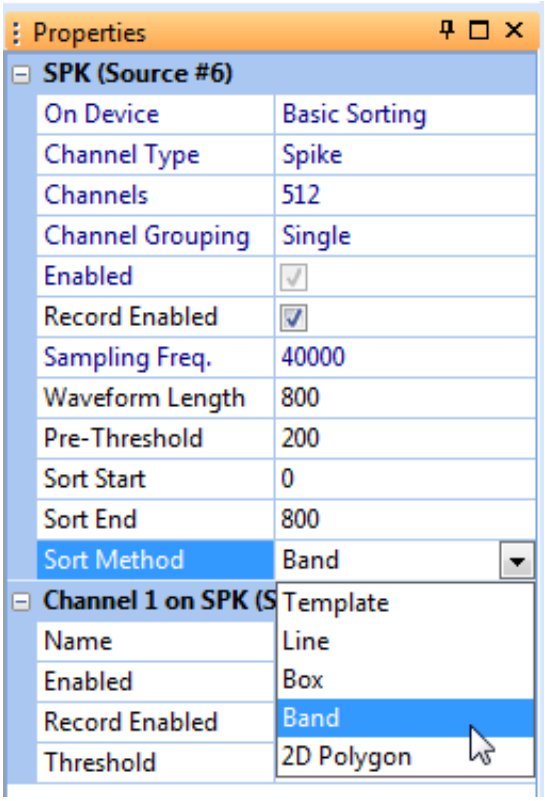
Only waveforms which fall entirely within the sorting band for a unit will be sorted into that unit. This corresponds to the intuitive notion that spikes from the same unit form a “bundle” of waveforms which fall within an envelope or band. By allowing different tolerances to be defined at each point, sections of the waveform which have less amplitude variance can be defined by a narrower band, while points which have more variance, such as those in the tail, can have a larger tolerance.

Now that you understand how band sorting sorts spikes, you can learn how to define and edit units with band sorting.

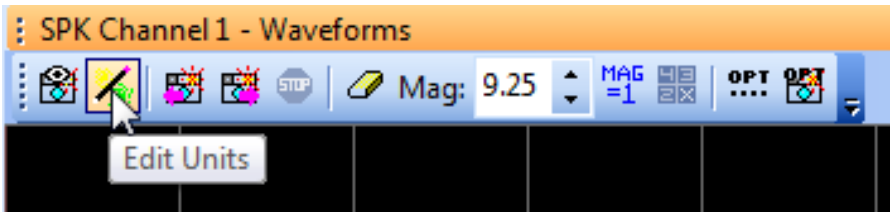
### 8.7.2 Defining and Editing Units with Band Sorting

The following assumes that you are already familiar with defining and editing units using template sorting as described in Section 6.2, “Selecting Sort Method and Entering Unit Editing Mode” on page 178.

To select the band sorting method, stop data acquisition and set the **Sort Method** to **Band**:



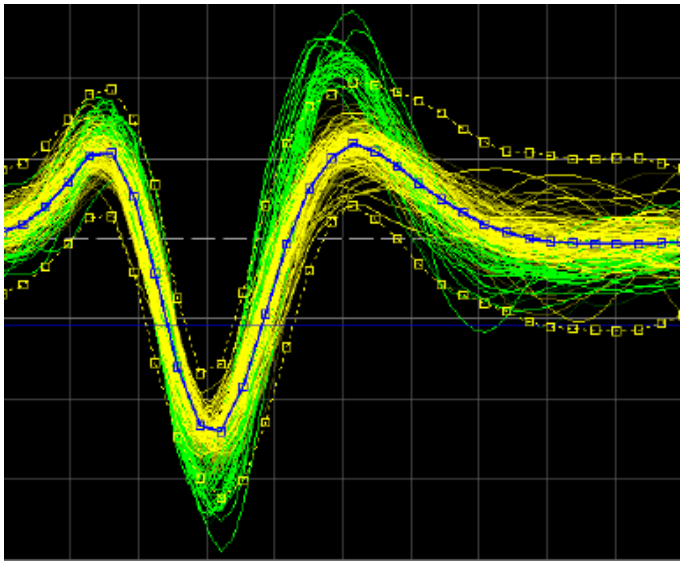

Then restart data acquisition and if necessary, set appropriate thresholds and collect a spike snapshot, as previously described. Once you have done this and are viewing either live spikes or a snapshot of the spikes in the main spike window, click **Edit Units**:



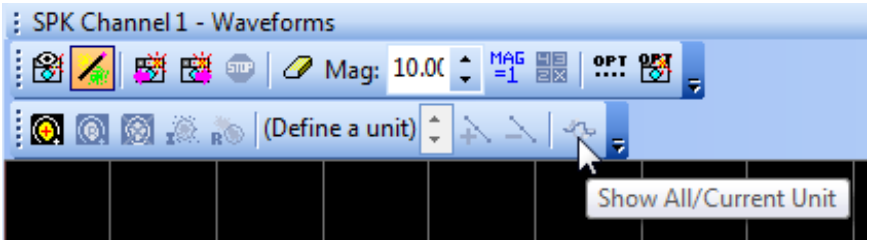

You can now define band sorting units using exactly the same methods as for template sorting, that is, drawing waveform crossing lines in the main spike window and/or drawing contours around clusters in the cluster display, or by using the automatic sorting methods. As with template sorting, all of these

techniques are ways of manually or automatically selecting a set of spike waveforms which the OmniPlex System then uses to create a unit definition. The difference is that with band sorting, the “tolerance” part of the unit definition consists of the set of per-point tolerance values which specify the amplitude width (height in Y) of the band at each point of the template, rather than the single sum-of-squared-errors tolerance that is used by template sorting.

For each unit that you define using any of the manual or automatic methods, one template and its associated band (set of tolerance values) are created. Note that when you are defining units using the band sorting method, only the currently selected unit’s band can be displayed:



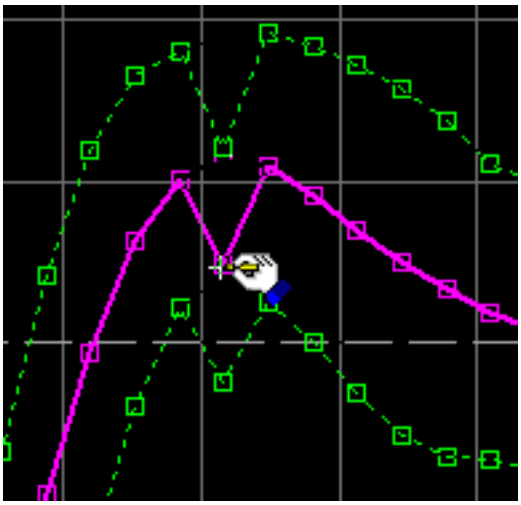
The **Show Current / Show All** option is not available because displaying the bands for more than one band sorting unit at the same time almost always results in a display that is too cluttered to be usable.



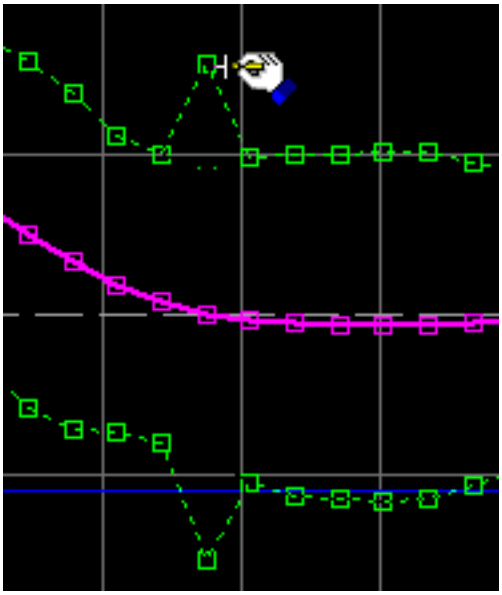

Once you have defined one or more units using band sorting, you may wish to adjust their templates and/or bands. There are three ways in which you can do this.

1. Use the mouse to drag a template point up or down, as with template sorting. The associated pair of band handles, above and below the template point, will also be automatically moved up and/or down by the same amount, so the tolerance remains unchanged relative to the template point. We show an

exaggerated template change here for illustration.

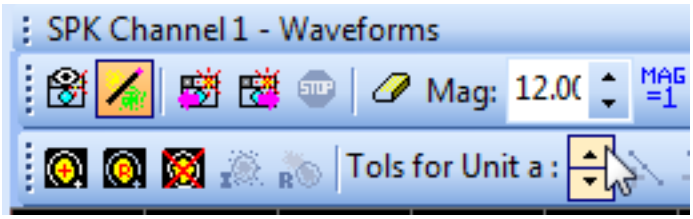


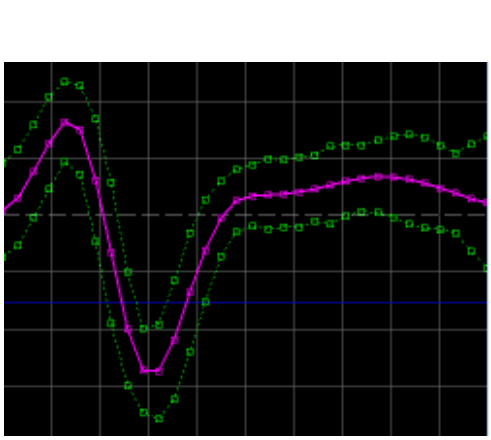
2 Use the mouse to drag a band handle up or down, to change the width of the band, that is. to increase or decrease the tolerance at that point). Note that when you drag either band handle, the band handle on the opposite side of the template moves by the same amount and in the opposite direction. In other words, if you reduce the tolerance (make the band narrower), this is always done symmetrically on either side of the template; you cannot define different tolerances above and below a template point. Editing points on the band does not change the template itself, only the band.

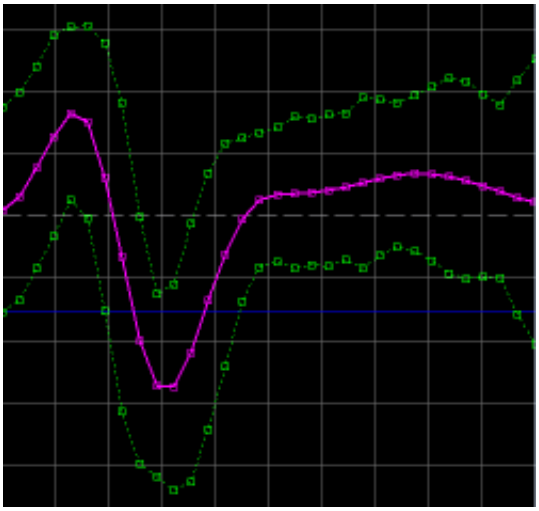


3 Use the **Tols for Unit** (fit tolerance) arrows in the toolbar to increase or decrease the width of the band at all points at the same time. All the band

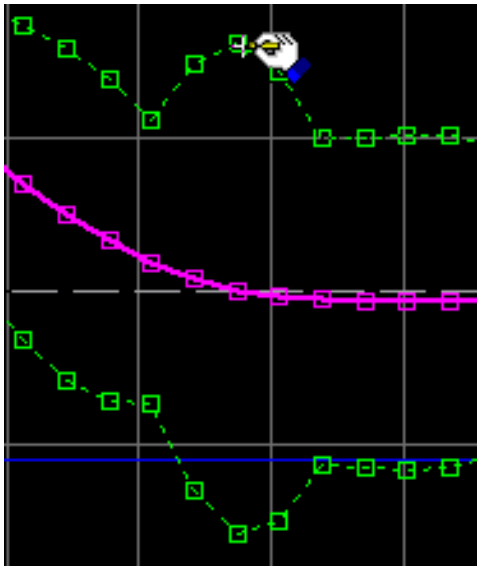
handles are moved closer or farther away from the template as the fit tolerance is adjusted.





With Methods 1 and 2, an additional option is enabled when you hold down the **Shift** key while dragging a template or band point—the two points adjacent to the dragged point are also moved, but not as much as the middle point. This can reduce the amount of point-by-point editing required to produce a desired change in the band shape. Here is an example like the one above for Method 2, but with **Shift** held down:



You cannot add or delete template points or band points. However, if you increase the tolerance at a given band point or points to the maximum, those points will have no influence on the sorting results, since all spikes will fall inside the band there. You can use this technique to cause the sorter to “ignore” any section(s) of a waveform which are noisy or corrupted by artifacts, while closely fitting the “bundle” of spikes in other sections.

### 8.7.3 Band Sorting Compared to Template, Line and PCA Sorting

Band sorting uses all of the raw amplitude information in both the unit template and the spikes being sorted. You can think of a spike waveform as being a single point in N-dimensional (N-d) amplitude space, for example, 32-dimensional space for a 32 point waveform. The template is also a point in this N-d space, and the per-point tolerances are in effect the dimensions of an N-d box centered on the N-d template point. The band sorting algorithm is then a point-in-box containment test in N-d space.

By comparison, template sorting uses a looser sorting metric, in that the N independent tolerances of band sorting are replaced with a single sum-of-squared-error error tolerance over the entire template. PCA sorting reduces each spike to a single point in 2D PCA space, discarding the principal components which contribute little variance, and then doing a containment test using a sorting polygon.

One of the most productive ways to use band sorting is by using it in combination with the automatic sorting methods. Band sorting preserves more of the sorting information produced by an automatic sort than the other sorting methods in the OmniPlex System and often gives very good results.

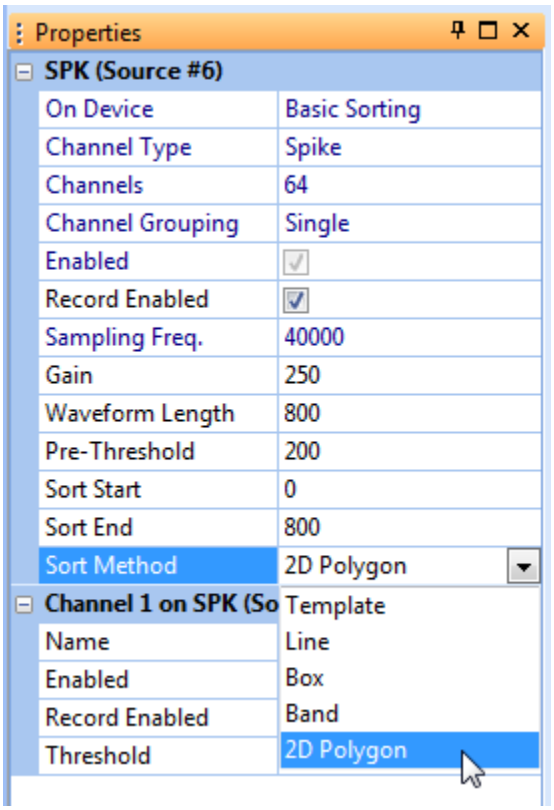
Band sorting can be thought of as a restricted form of line sorting where there are 32 sorting lines but they are constrained to be vertical lines at the sample times. Band sorting is, like template sorting, based on the amplitude differences between spikes and templates, measured at sample times, whereas line sorting is more of a geometric method.

## 8.8 2D Polygon Sorting

In the same way that line sorting uses the technique of waveform crossing to perform live sorting of spike waveforms, 2D polygon sorting uses 2D PCA contours to perform sorting of the projections of spikes in PCA feature space. Since you already know how to draw PCA contours in live and snapshot modes, you know almost all the steps involved in 2D polygon sorting. The main difference is that the contours you draw will be used directly as the actual unit definitions.

**Note:** You can define units for polygon sorting in either live or snapshot mode; for the examples, we will use live mode.

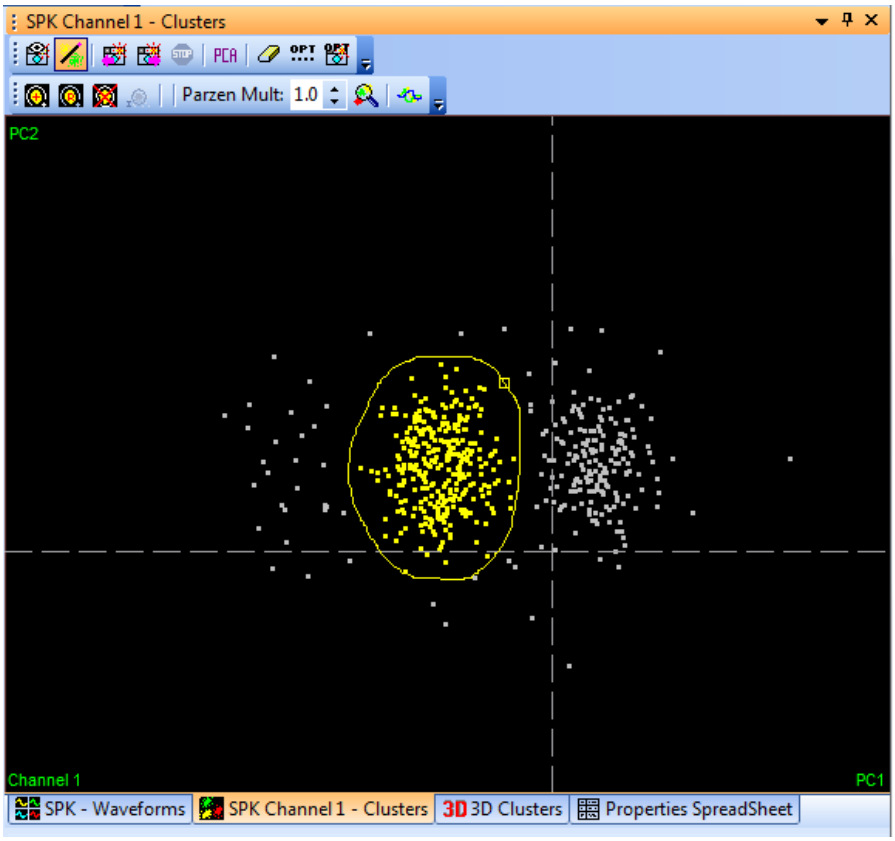
1 Stop data acquisition, change the sorting mode to **2D Polygon**, and restart data acquisition.



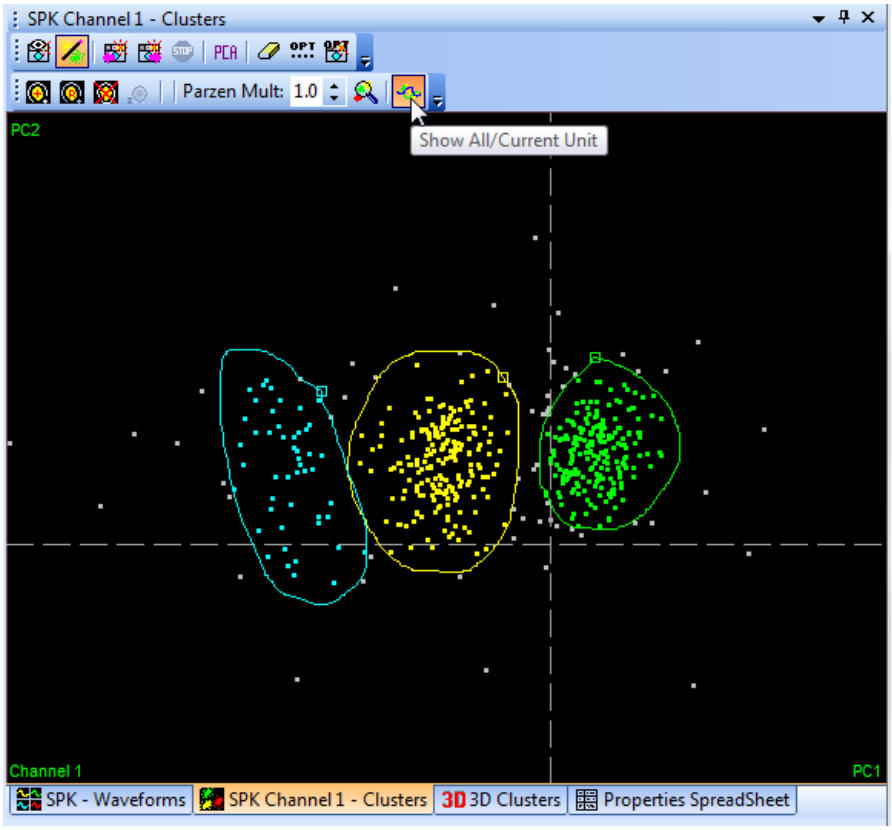

2 Set gain and thresholds if not already set appropriately, and collect a spike snapshot.

**Note:** Setting the gain is applicable to DigiAmp subsystems, not DHP subsystems.

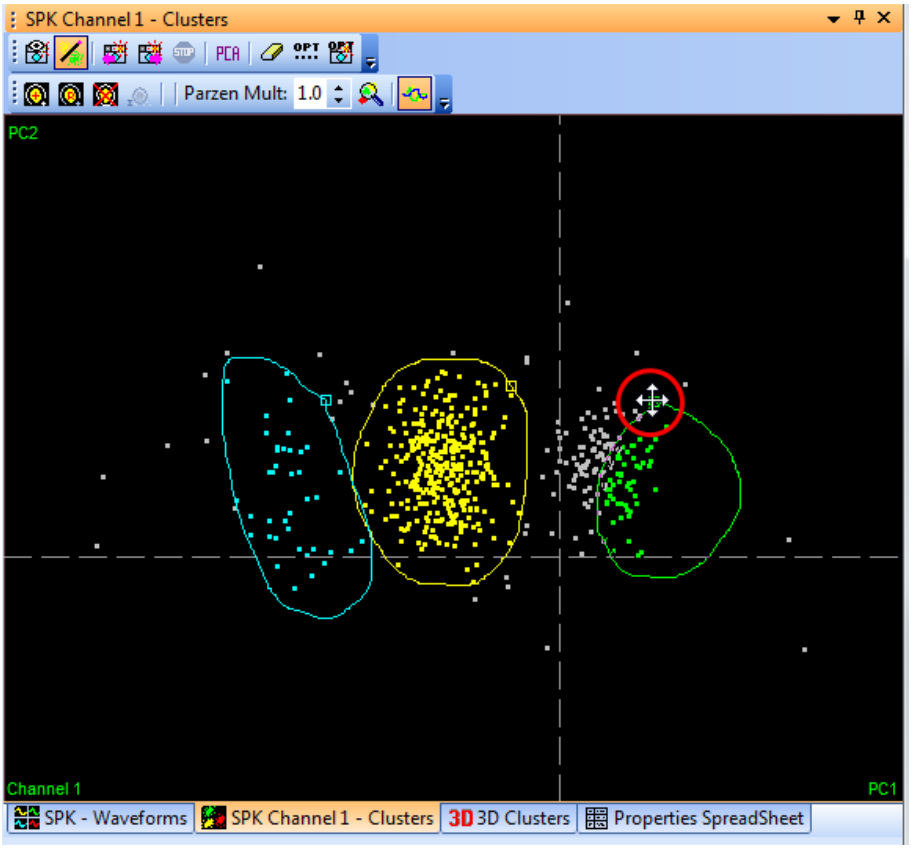
3 Perform steps 1 through 5 from the section Section 8.4, “Defining Units for Template Sorting using PCA Contour Drawing ” on page 250. In other words, enter unit editing mode and add a unit by drawing a contour around a cluster in the zoomed 2D PCA view. But now, rather than the contour disappearing once you have finished drawing it, as was the case in template sorting, the contour remains onscreen:



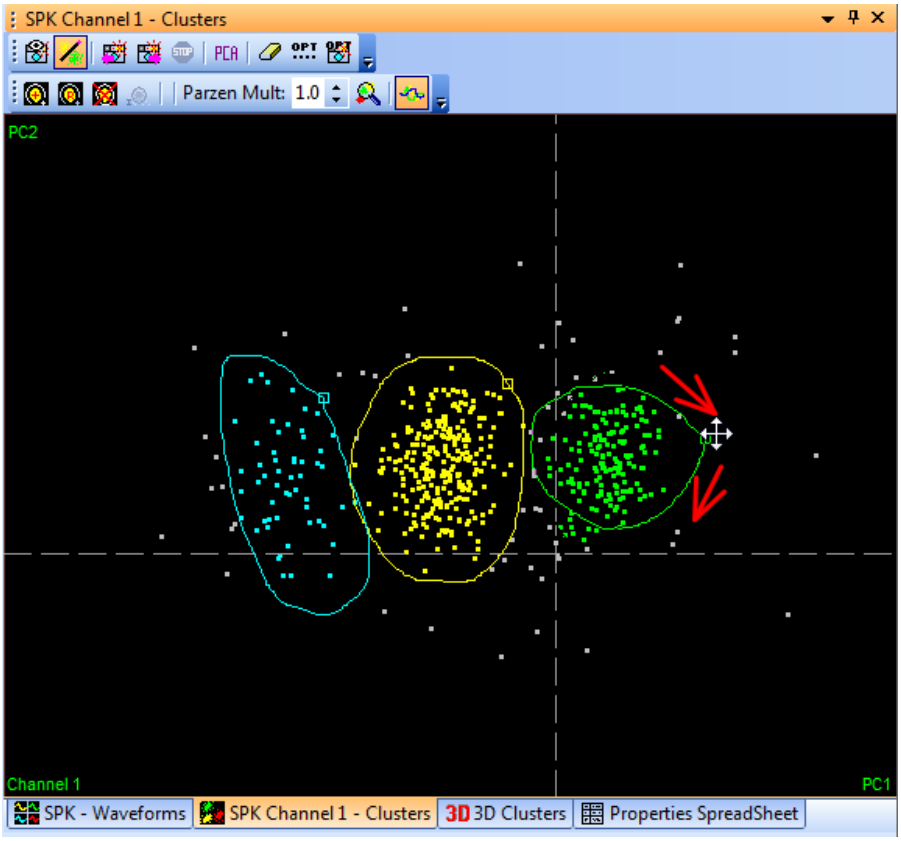
4 The feature space contour is sent to the sorting device in Server, and each incoming spike is projected into PCA feature space and tested against the contour. Points which fall inside a contour are sorted into the corresponding unit. You can define additional units by drawing their contours. You can use the **Show All / Current Unit** button to control whether all the PCA contours are shown, or only the contour for the currently selected unit.



5 Once you have created a contour, you can adjust it if desired. You can move it by dragging it by the small square handle; the cursor changes to a four-way arrow while you are moving the contour:

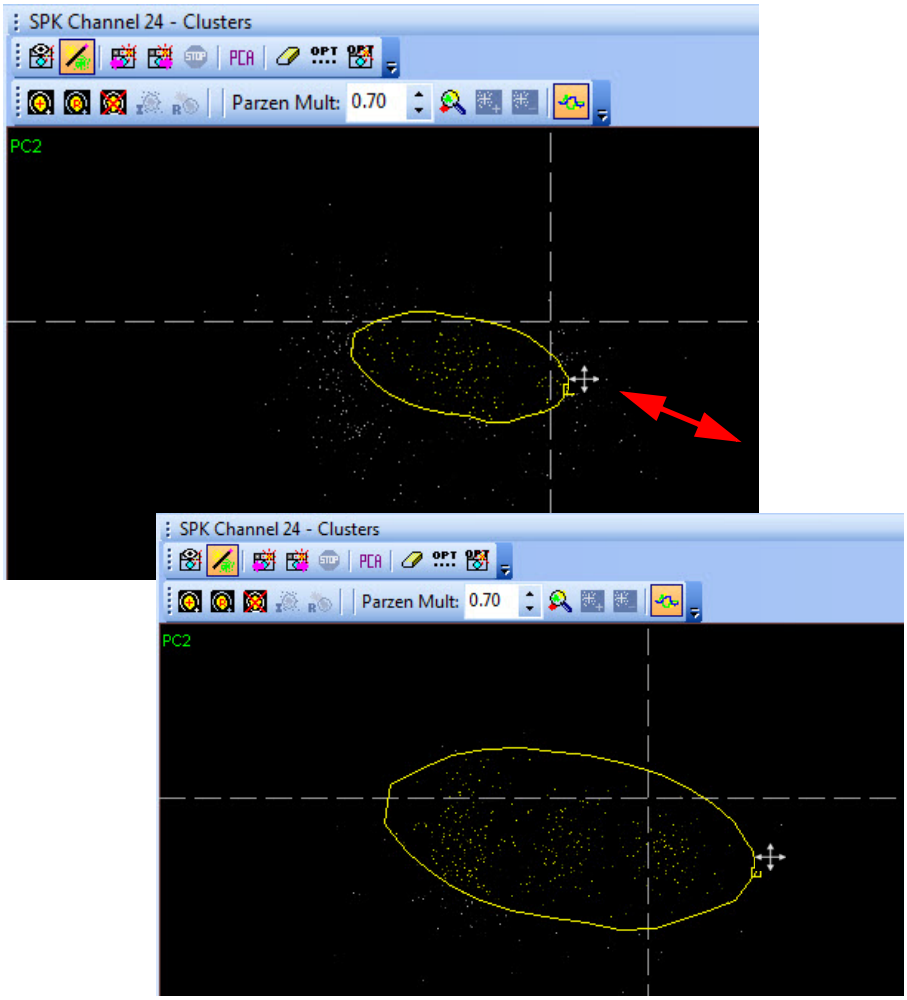


6 You can rotate the contour by holding down the Shift key while dragging the handle:



7 You can resize the contour by holding down both the Shift and Ctrl keys while dragging the handle (the small square) towards or away from the center of the contour:

**Note:** The combination Shift-Ctrl is used because Ctrl-drag is already in use as a shortcut for drawing a new unit's contour.

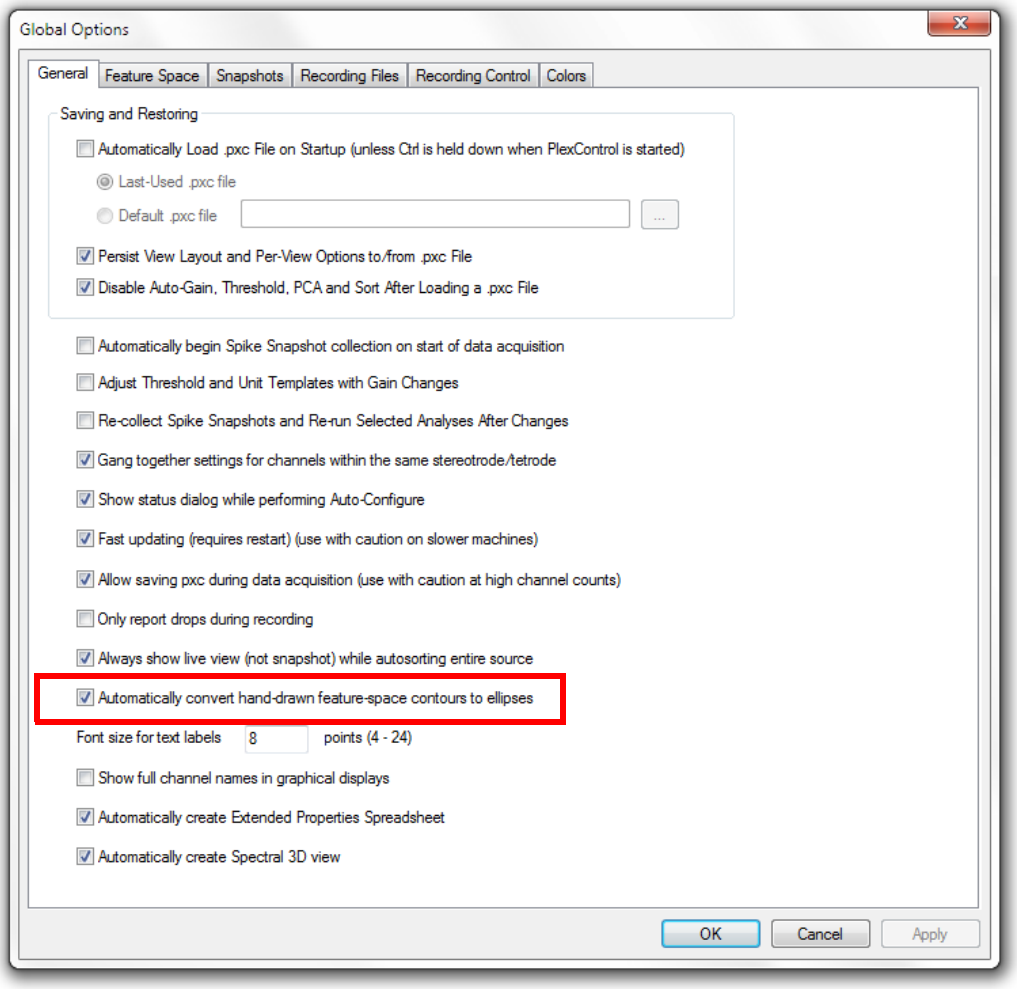



The sorting parameters are updated (i.e. sent to Server) as soon as you release the mouse button.

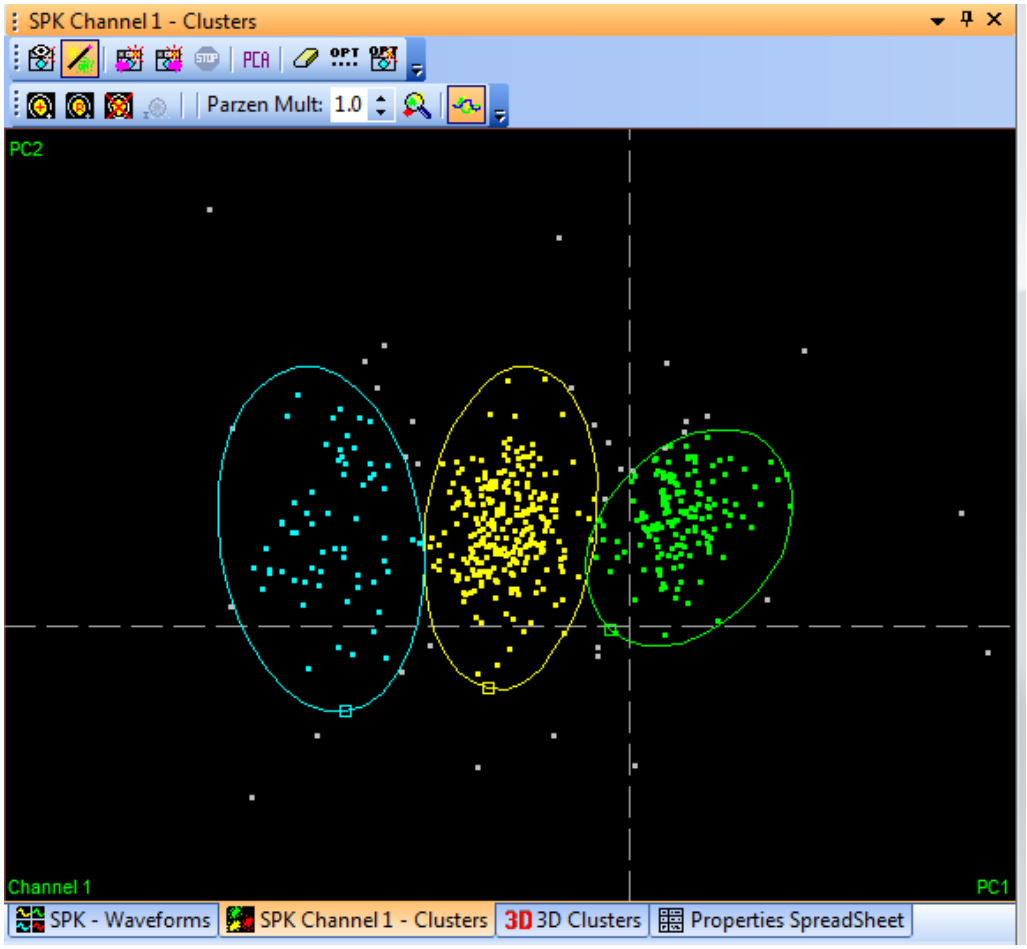
### 8.8.1 Cleanup of Hand-drawn PCA Contours

There is an option which automatically "cleans up" your hand-drawn contours by converting them to ellipses; since clusters are often elliptical in shape, this is a reasonable assumption. To enable this feature, display the **Global Options** dialog from the **Configure** menu and select the option **Automatically convert hand-drawn feature-space contours to ellipses**.

**Note:** This will not affect any previously-drawn contours, only ones that you draw after enabling the option.

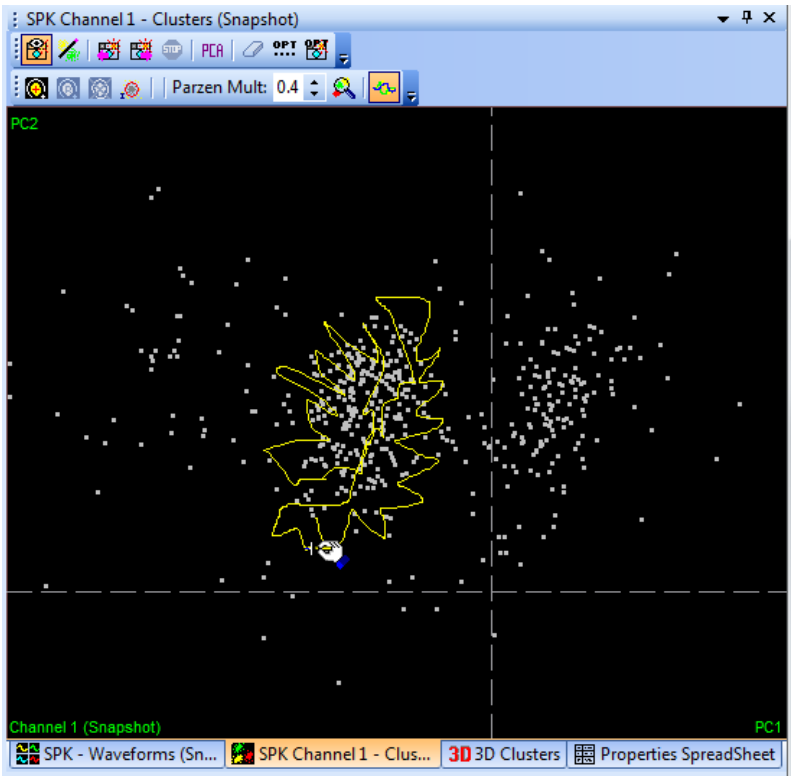


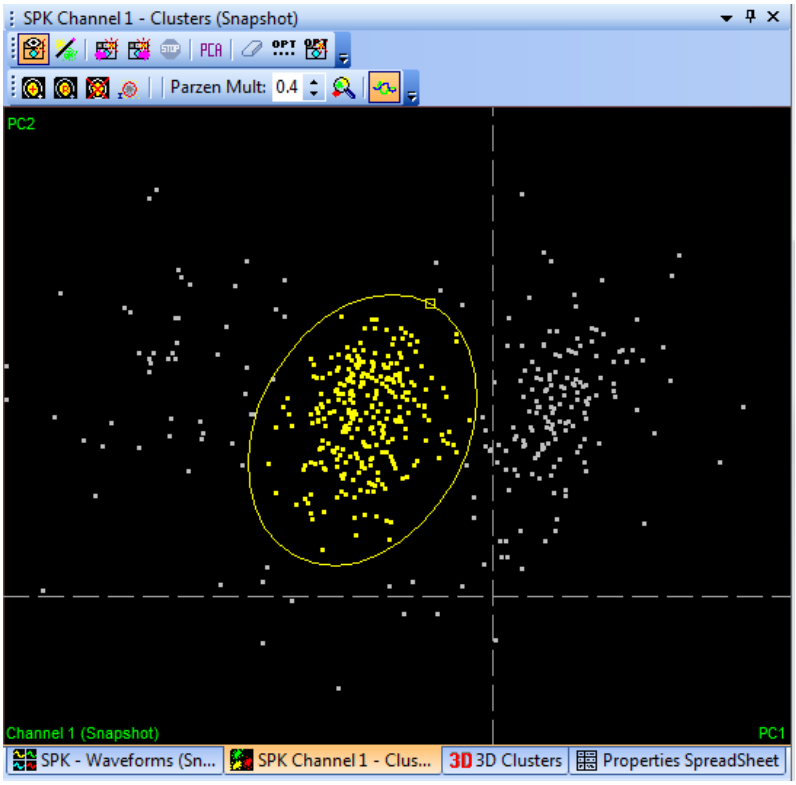
Click **OK** and note how subsequently-drawn contours are replaced with ellipses as you finish drawing each one:




You can move, rotate and resize these contours in the same way as previously described for the non-elliptical contours.

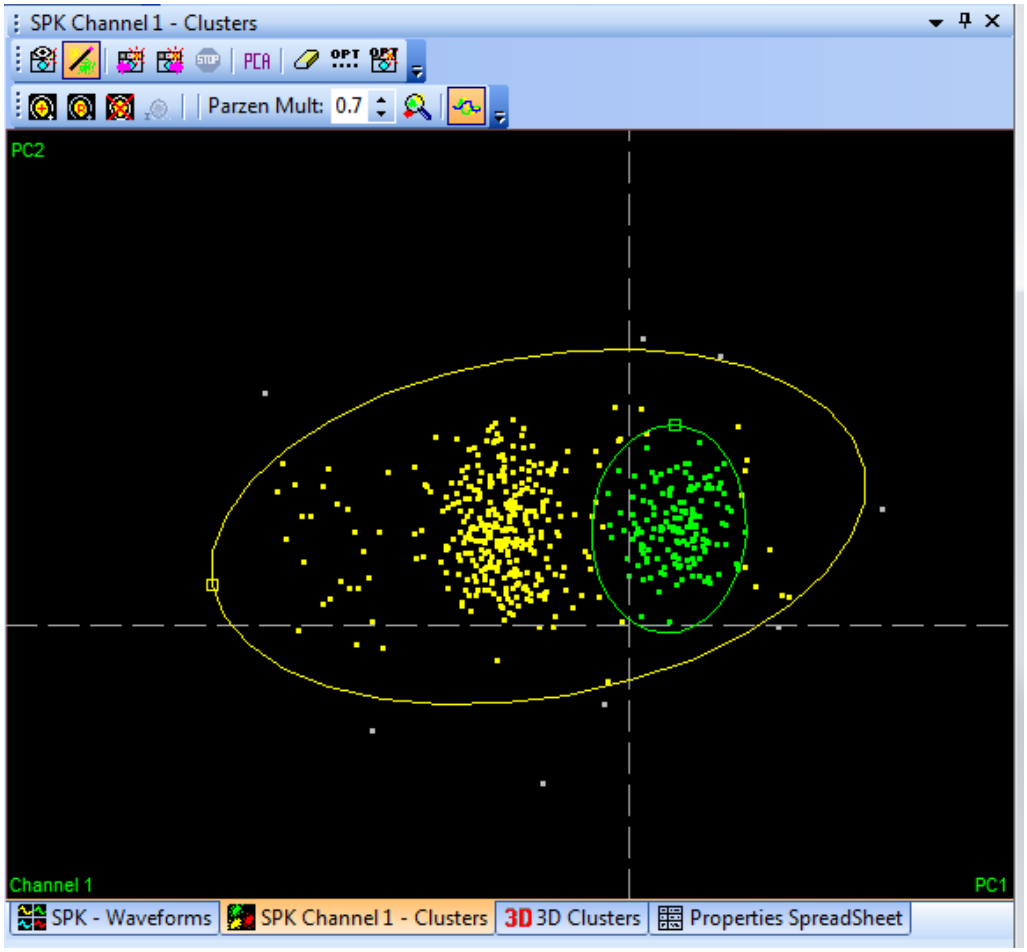
Note that a heuristic procedure, not a standard fitting procedure, is used for the cleanup of hand-drawn contours, which gives an excellent fit in typical cases, and a reasonable result even in pathological cases, such as this one:



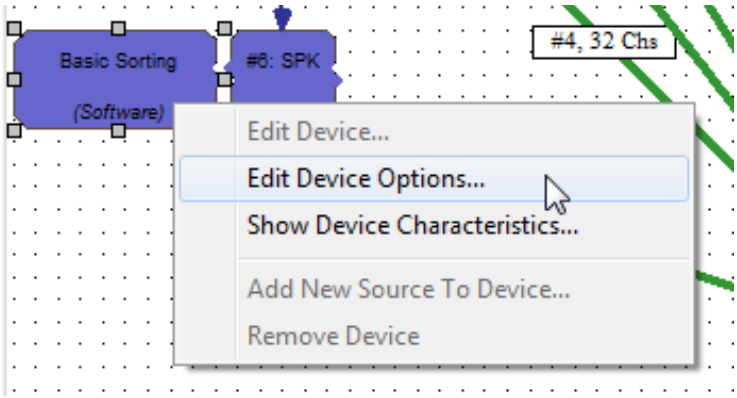
### 8.8.2 Contour Overlap Handling

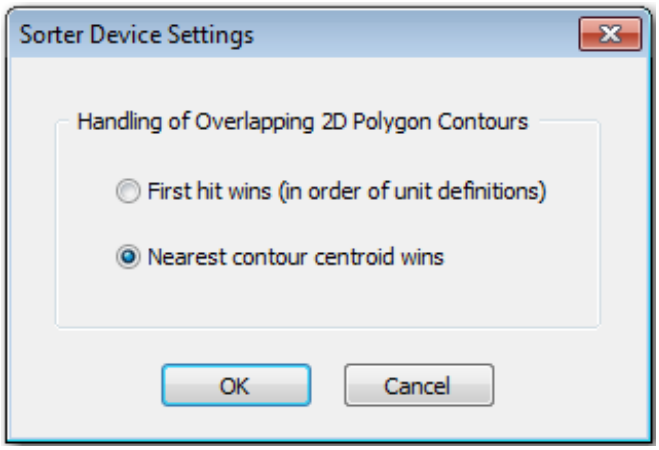
In cases where a spike's PCA projection falls within the overlap of two or more contours, by default, the 2D distance from the spike's projection to the center of each overlapping contour is used as a tie-breaker. This has the beneficial effect of allowing a small cluster that falls within the “outskirts” of a larger cluster to be sorted more correctly. The following is not a realistic example, but gives some idea of how this works:




Spikes within the green ellipse also fall within the large yellow ellipse, but most are sorted into the green unit since they are closer to its center.

An option allows you to choose either the default behavior described above, or an alternate method where overlaps are resolved on a “first unit wins” basis. This option is accessed from the Device Options for the Basic Sorting device in the topology in Server.

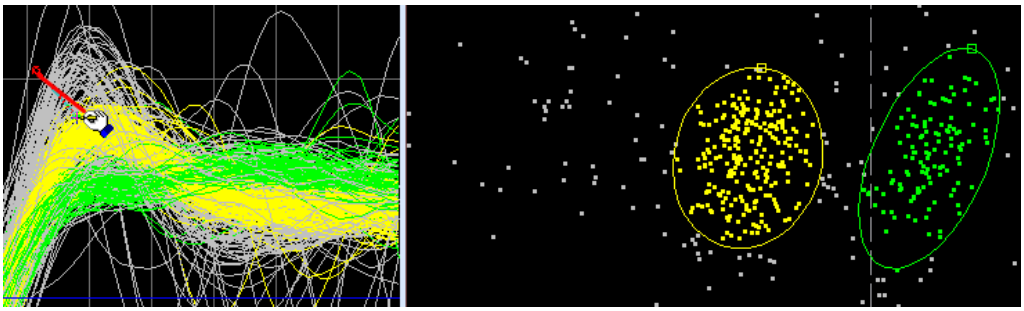


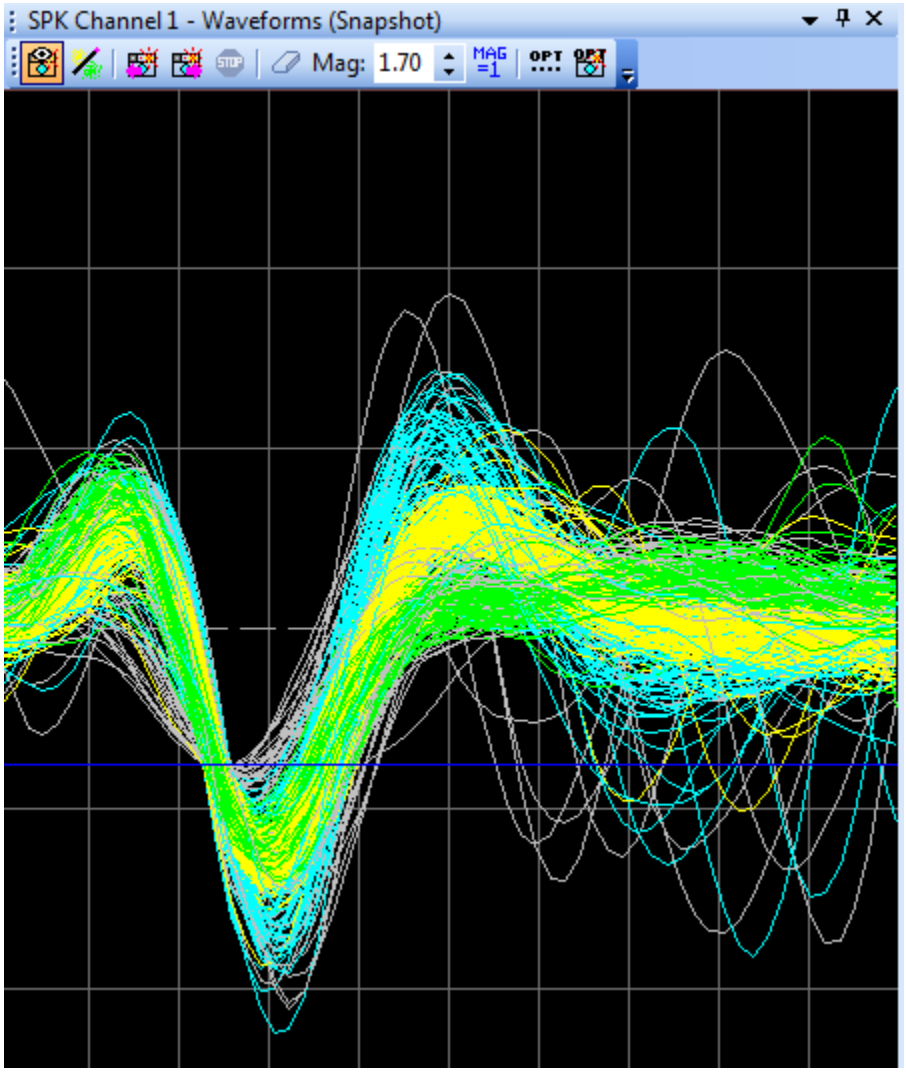



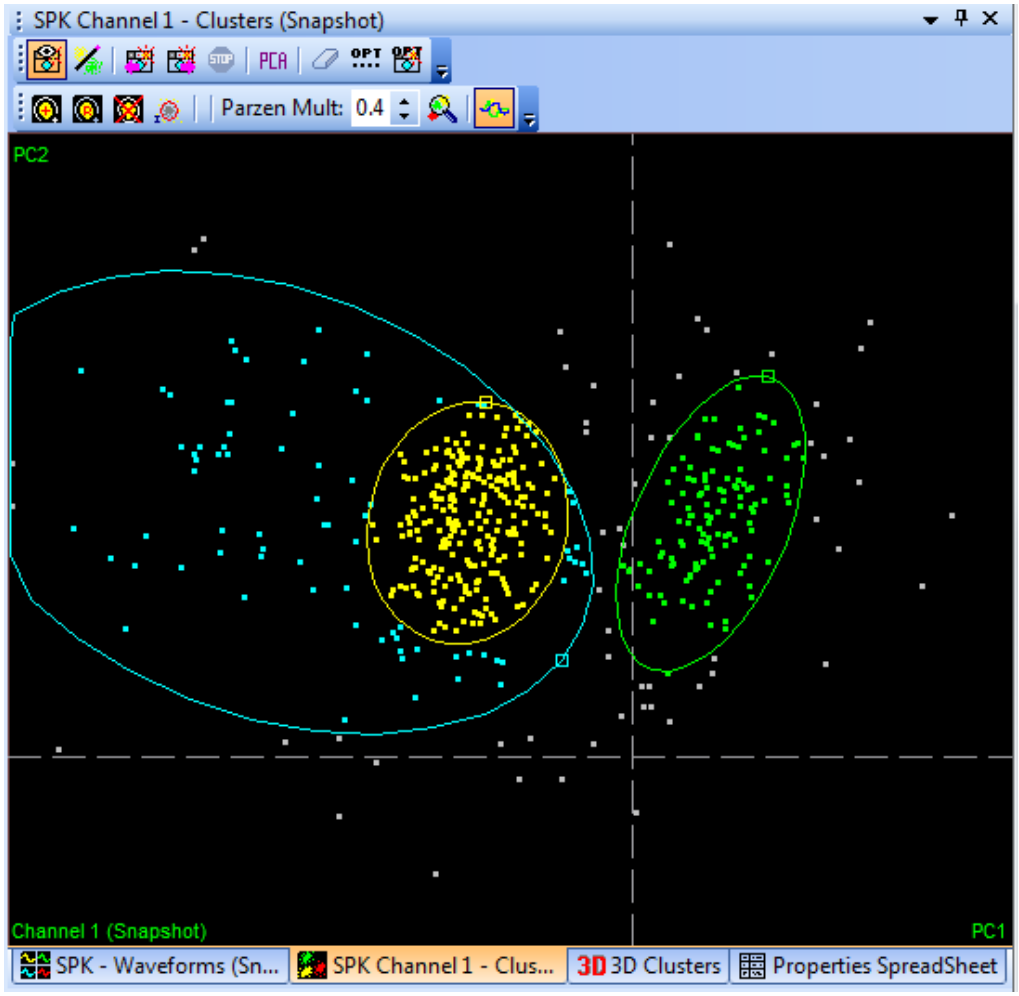

You can use this to create a user-defined overlap resolution. The order in which you define units will be used as the priority order for overlap resolution. For example, if the contours for units b and c overlap, spikes within the overlapped area will always be sorted into unit b, regardless of the relative overall sizes and positions of the contours.

### 8.8.3 Defining Units by Line Crossing with 2D Polygon Sorting

You can use waveform crossing in the main spike window to select bundles of similarly shaped waveforms, as described before, to create unit definitions for polygon sorting. For example, after using automatic unit finding to create the above two unit definitions, here a group of waveforms are crossed to add a third unit:







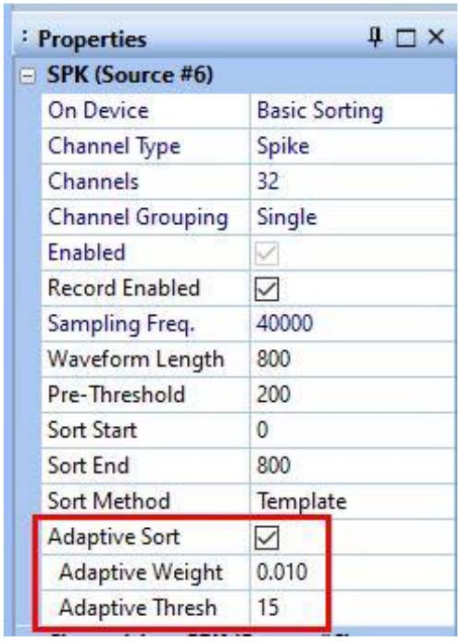

As with automatic unit finding in polygon sorting, an ellipse is fitted to the cloud of points corresponding to the group of waveforms, with the tightness of fit controlled by the ellipse fit tolerance previously described.

## 8.9 Adaptive Template, Band and 2D Polygon Sorting

In adaptive template sorting, the template, band and 2D polygon sorting methods can automatically update their sorting parameters over time, in response to gradual changes in incoming spike waveforms. This also allows the system to refine and improve sorting in some cases where the initial sorting parameters were not optimal. Adaptive sorting is performed independently for each unit.

### 8.9.1 Enabling Adaptive Sorting

To enable adaptive sorting, start data acquisition and define one or more sorted units. Make sure that the SPK source is selected, so the current spike sorting parameters are displayed in the per-source properties spreadsheet which is by default displayed at the left side of the main PlexControl window. You can then enable Adaptive Sort:




### 8.9.2 Template and Band Sorting

For the template and band sorting methods, each unit's template is updated using the following procedure. Note that template and band tolerances are not modified and remain at their original "pre-adaptive" values, although you can modify them manually at any time if desired.

For each defined unit, a temporary template is initialized to be the same as the unit's sorting template (the template that is being used to sort incoming spikes). The unit's temporary template is updated using each incoming spike that is sorted into that unit by the current sorting template (details are given in the next section). These updates to the temporary template do not affect the sorting template until the difference between the two templates exceeds a threshold, as described below. Once the template difference threshold is exceeded, the temporary template then replaces the sorting template, and the process continues,

with incoming sorted spikes being used to update the temporary template until the next time that the difference threshold is exceeded and the temporary template again replaces the sorting template.

Note that unsorted spikes have no effect on the adaptive sorting procedure.

### 8.9.3 Updating of the Temporary Template

For each incoming sorted spike, the temporary template for that unit is updated using a weighted average of the incoming spike and the current value of the temporary template, using the corresponding points on the two templates. The Adaptive Weight parameter determines how much each incoming sorted spike affects the temporary template. If we use the following notation:

```
T = temporary template (an array of points)
S = incoming spike waveform (an array of points)
N = number of points in S and T arrays (the waveform length)
i = index into the S and T arrays
W = adaptive weight (a value between 0.005 and 0.1)
```

then a pseudocode description of the update process for each incoming sorted spike is:

```
for i = 1 to N
  T[i] = ( (1 - W) * T[i] ) + ( W * S[i] );
```

For example, if W = 0.01 (the default value), then:

```
for i = 1 to N
  T[i] = ( 0.99 * T[i] ) + ( 0.01 * S[i] );
```

The default W value of 0.01 can also be thought of as, “each incoming spike contributes 1% to updating the value of the temporary template.”

You can see that for a larger value of W, the “influence” of each incoming spike will be greater, and the temporary template will potentially change more rapidly, if the incoming waveforms are changing. However, if W is too large, then the temporary template will be unduly affected by short-term waveform changes in individual spikes caused by background noise and minor waveform shape changes. The goal is for the temporary template to track longer-term changes in the mean unit waveform, and so the default value for the Adaptive Weight (W) is small and should only be increased with caution. Adaptive sorting is not intended to track brief waveform changes, such as decaying spike amplitudes during a burst.

### 8.9.4 Updating of the Sorting Template

As described in preceding section, the temporary template will change slightly as it is updated using each incoming spike, and it will only replace the sorting template if and when the difference between the two templates exceeds a

threshold, defined by the **Adaptive Thresh** parameter. The difference threshold is based on the sum of squared differences of the two templates, where each squared difference is taken between the corresponding points on the two templates. Smaller threshold values can result in more frequent updates of the sorting template, while larger values cause less frequent updates.

In addition to the **Adaptive Weight** and **Adaptive Thresh** parameters, the rate at which the sorting template can be updated is also controlled by the **Min Update Interval** parameters in the Sorter device options in Server, as described later. However, you can leave **Min Update Interval** set to its default values until you are familiar with the use of the basic parameters, **Adaptive Weight** and **Adaptive Thresh**.

### 8.9.5 2D Polygon Sorting

For 2D polygon sorting, the general concept of using each incoming spike to modify a set of temporary sorting parameters, until they are different enough from the current sorting parameters to replace them, is the same. However, here the sorting parameters are the points of a 2D polygon, which may have originally been defined either manually by drawing, or automatically by an auto-sort procedure. For purposes of adaptive sorting, the system calculates the centroid (center of mass) of the polygon, and uses the feature space projection of each incoming sorted spike to slowly update the position of the temporary centroid. If we use the following notation:

```
cx = temporary centroid (first feature space coordinate)
cy = temporary centroid (second feature space coordinate)
sx = incoming spike waveform (first feature space coordinate)
sy = incoming spike waveform (second feature space coordinate)
W = adaptive weight (a value between 0.0 and 1.0)
```

then a pseudocode description of the update process for each incoming sorted spike is:

```
cx = ( (1 - W) * cx ) + ( W * sx );
cy = ( (1 - W) * cy ) + ( W * sy );
```

For example, if W = 0.01 (the default value), then:

```
cx = ( 0.99 * cx ) + ( 0.01 * sx );
cy = ( 0.99 * cy ) + ( 0.01 * sy );
```

The effect of varying W is analogous to that described for template and line sorting, but using the centroid rather than the template.

### 8.9.6 Updating of the Sorting Polygon

For 2D polygon sorting, the **Adaptive Thresh** parameter determines the minimum 2D distance between the temporary centroid and the centroid of the current sorting polygon required in order for the temporary centroid to be used to

update the sorting polygon. Note that the shape of the sorting polygon is not affected by adaptive sorting, only its position. In other words, the current sorting polygon is translated (offset) by the difference between its centroid and the temporary centroid.

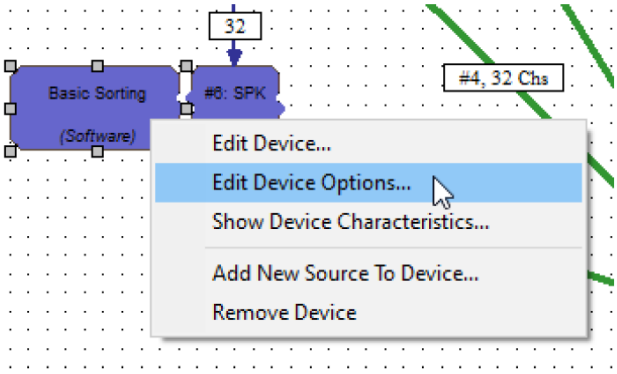
### 8.9.7 Minimum update interval

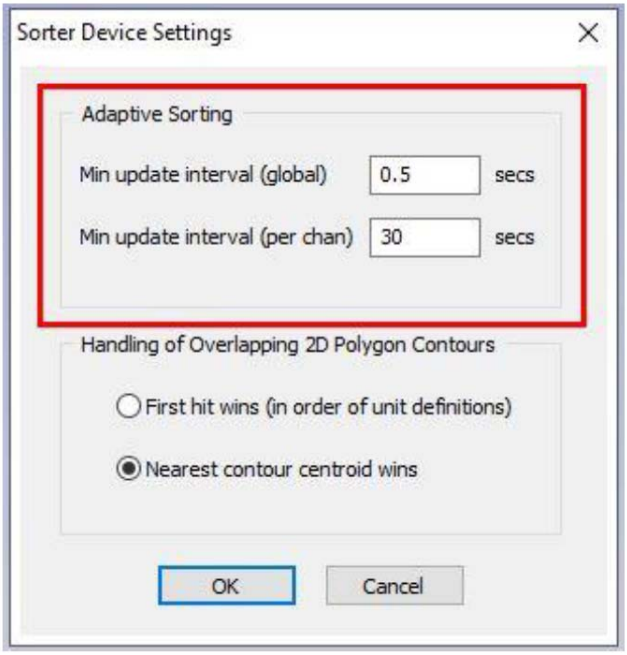
If the **Adaptive Weight** and **Adaptive Thresh** parameters are set to values that cause very frequent updating of the sorting template or polygon, there can be two undesired consequences.

First, if many channels are updating their sorting parameters frequently, significant system resources can be required, especially on high channel count systems. For example, if four units are defined on each channel of a 512 channel system, and the parameters are set such that each template or polygon is updated once per second, up to 4 * 512 = 2048 template or polygon updates per second are possible.

Second, independent of any system performance considerations, any one channel should not normally be updating its sorting parameters too rapidly, since this will result in tracking undesired short-term waveform variations caused by noise, intra-burst decay, and so on.

In order to protect against these scenarios, there are two additional adaptive sorting parameters which “throttle” the maximum rate of template or polygon updates. These are the **Min Update Interval** parameters, and are found in the Device Options dialog for the Sorter device in OmniPlex Server. To modify these options, stop data acquisition and right-click on the Basic Sorting device in the topology diagram in Server:



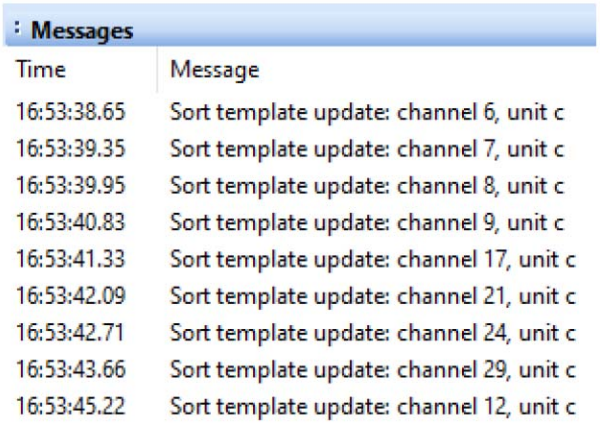



The **Min update interval (global)** parameter determines the minimum time (or equivalently, fastest rate) between template or polygon updates on any channel. For example, the default value of 0.5 seconds means that no more than two templates or polygons can be updated per second, whether on the same channel or on different channels. In effect, this value limits the maximum load on the system caused by adaptive sorting. You should only reduce this value with caution, and when doing so, be aware of any signs of excessive system load, such as stuttering displays, pauses, data drops, etc.

The **Min update interval (per chan)** parameter determines the minimum time (fastest rate) between template or polygon updates on any one channel. Every channel in the system can update at this rate, as long as doing so does not result in a global update rate that exceeds the limit set by the Min update interval (global) parameter. Since adaptive sorting is intended to track slow changes to waveform shapes, you should resist the temptation to set it to very short intervals (e.g. less than 10 seconds), although it can be instructive to do so when you are first learning the effects of varying the **Adaptive Weight** and **Adaptive Thresh** parameters.

### 8.9.8 Adaptive Sorting Notifications in Server Message Log Window

Each time that a template or sorting polygon is adaptively updated, a notification message is displayed in Server's message log window:




These messages can provide useful feedback to aid in tuning of the adaptive sorting parameters. Although the frequency of adaptive sorting updates is in general data-dependent, a reasonable default approach is to leave the **Adaptive Weight** parameter at its default value of 0.01, and then increase or decrease the **Adaptive Thresh** parameter until parameter update messages are only seen occasionally. You can also increase the **Minimum update interval (per chan)**, so that a larger number of spikes are used for each update.

Of course, you can use the main current-channel spike display (or for polygon sorting, the 2D cluster display) in unit editing mode to view the unit templates (or polygons) for a channel in order to observe the changes that are made at each adaptive update; these changes should be incremental and smooth, not large and abrupt. If this is not the case, you can adjust the parameters described above so as to prevent the adaptive algorithm from reacting to short-term fluctuations in waveform shape that are due to noise, within-burst amplitude variations, etc.

## 8.10 Spike Sorting Quality Metrics

The system can calculate one or more of a set of metrics which are intended to measure the quality of unit clusters in feature space. There are multiple metrics because there is no universal agreement on the best criteria for cluster quality, although as of this writing, **Isolation Distance** and **LRatio** are widely used.

### 8.10.1 Sort Quality Metrics in the Extended Properties Spreadsheet

The system displays the sort quality metrics in the right hand part of the **Extended Properties Spreadsheet** (the red lines were added to emphasize this).

Extended Properties Spreadsheet for 'SPK'

| | Name | LPF | LP Freq | LP Type | LP Poles | HPF | HP Freq | HP Type | HP Poles | Iso Dist | LRatio | J3 | PseudoF | Short ISI | Sorted |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| >>1 | SPK01 | Off | --- | --- | --- | On | 300.0 | Bessel | 4 | 2.184 | 10.209 | 1.515 | 2.891 | 0.26 | 77.8 |
| 2 | SPK02 | Off | --- | --- | --- | On | 300.0 | Bessel | 4 | 4.286 | 4.713 | 1.544 | 2.911 | 0.25 | 80.0 |
| 3 | SPK03 | Off | --- | --- | --- | On | 300.0 | Bessel | 4 | 4.147 | 2.086 | 1.511 | 2.896 | --- | 79.0 |
| 4 | SPK04 | Off | --- | --- | --- | On | 300.0 | Bessel | 4 | 2.734 | 5.078 | 1.539 | 2.908 | 0.25 | 79.6 |
| 5 | SPK05 | Off | --- | --- | --- | On | 300.0 | Bessel | 4 | 3.629 | 0.943 | 1.784 | 2.948 | --- | 80.6 |
| 6 | SPK06 | Off | --- | --- | --- | On | 300.0 | Bessel | 4 | 3.674 | 0.646 | 1.488 | 2.903 | 0.25 | 81.4 |
| 7 | SPK07 | Off | --- | --- | --- | On | 300.0 | Bessel | 4 | 3.249 | 0.362 | 1.554 | 2.896 | 0.26 | 76.8 |
| 8 | SPK08 | Off | --- | --- | --- | On | 300.0 | Bessel | 4 | 1.772 | 1.883 | 1.523 | 2.902 | 0.25 | 79.4 |

**Iso Dist**, **LRatio**, **J3**, and **Pseudo-F** are all cluster quality metrics calculated in feature space, while **Short ISI** and **Sorted** are metrics which do not relate to feature space.

#### Iso Dist

**Iso Dist** (Isolation Distance) is a measure of how distant the non-unit spikes are from the spikes in the unit under consideration. It can be visualized as the radius of an ellipsoid in feature space that contains an equal number of within-unit spikes and non-unit (noise) spikes. Isolation Distance is therefore not defined for situations where a unit contains more spikes within it than exist outside of the unit. Large values of Isolation Distance indicate a well-separated unit; the ellipsoid radius had to be made very large to include as many non-unit spikes as there were within-unit spikes.

**Note:** For the mathematical definitions of the L-Ratio and Isolation Distance, see: N. Schmitzer-Torbert, J. Jackson, D. Henze, K. Harris, A.D. Redish. *Quantitative Mesaures of Cluster Quality for use in Extracellular Recordings*, Neuroscience, 131 (2005) 1-11

#### LRatio

**LRatio** (also referred to as L-Ratio) is a measure of the amount of noise near the unit, where noise in this case means waveforms that are not assigned to the unit under consideration. A low value of L-Ratio indicates that the unit is well-separated and not contaminated by nearby spikes that are not assigned to the unit. The value of L-Ratio is essentially a weighted sum over waveforms not assigned to the unit under consideration, with the weight determined by how close the 'foreign' waveform is to the unit center. Foreign spikes that are close to the unit center hurt more (and contribute more to the value of L-Ratio) than those further away.

### J3

**J3** is a non-parametric measure of the quality of sorting (i.e. no normality assumptions are involved).

J3 = J2/J1, where J1 = ΣΣE(f(u,i)-m(u)) is a measure of the average distance in feature space between points in a cluster (f) from their center (m). E(x) represents the Euclidean distance squared ($\tilde{x}$ * x). The summations are over units u, and over feature vectors (points) in each unit i.

J2 = ΣN(u)E(m(u)-m) is a measure of the average distance between unit clusters. The summation is over units u, N(u) is the number of points in unit u, E(x) represents the Euclidean distance squared. m(u) is the cluster center for unit u, and m is the grand center of all points in all units.

So J3 takes on a maximum value for compact, well-separated clusters.

**Note:** For further information on the J3 value, see Wheeler, Bruce C., *Automatic Discrimination of Single Units* in *Methods for Neural Ensemble Recordings*, ed. by Nicolelis, M., CRC Press, Boca Raton, 1999

### Pseudo-F

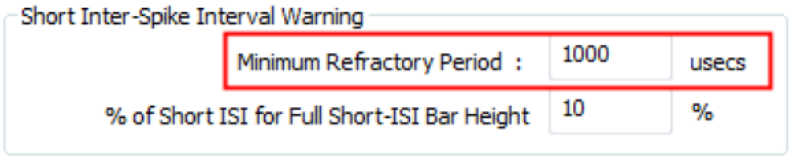
The P**seudo-F** statistic is closely related to the **J3** statistic. It is essentially J3 that has been adjusted for the number of waveforms and the number of units.

Pseudo-F = (N-g)/(g-1) J3

where N is the total number of waveforms, and g is the total number of units.

### Short ISI

**Short ISI**, is the percentage of spikes whose interspike interval (ISI) is less than the **Minimum Refractory Period** defined in the Units window options:




See Section 6.6, “The Short-ISI Indicator” on page 191 for more information about the short ISI warning.

### Sorted

The **Sorted** column reports the per-channel percentage of spikes which are sorted, that is, assigned to any unit.

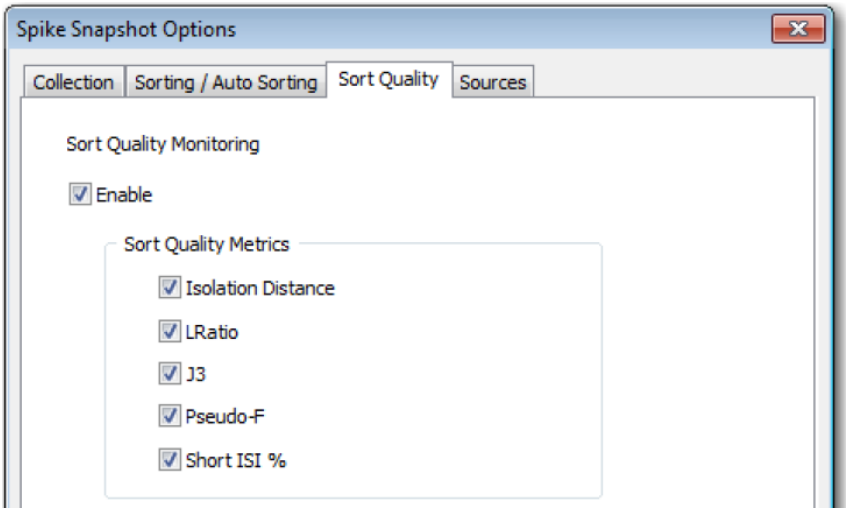
As with the other sort quality metrics, the percent-sorted is updated periodically on a rolling basis, using the most recent 500 spikes on each channel.

Note that percent-sorted is not a sort quality metric in the same sense as the other metrics, where a larger or smaller value is directly proportional to some aspect of the sort quality. For example, you could define a single unit on a channel which actually contains multiple units, but with a very large sorting tolerance which results in 100% of the spikes being sorted into that unit. However, the actual sorting quality, as indicated by the other metrics, would be very poor. For example, in this hypothetical “one unit for all the spikes” case, the Short ISI percentage would probably be very high, the Iso Dist would be very small, and so on, indicating that more than one unit is being “sorted into” a single unit. Ideally, you would have a high percentage of sorted spikes, in conjunction with good values for the other sort quality metrics, but it is generally not recommended to pursue a high percent-sorted as your primary goal in spike sorting.

Also keep in mind that if you have set an unusually low threshold on a channel (this would be more likely with manually-set thresholds where a user is “trying to find a spike in the noise,” and less often when auto-thresholding is used), it is likely that a significant number of “spikes” are actually thresholded noise. In such a case, you would want these noise spikes to remain unsorted, with a corresponding reduction of the percent-sorted metric.

### 8.10.2 Displaying Sort Quality Metrics

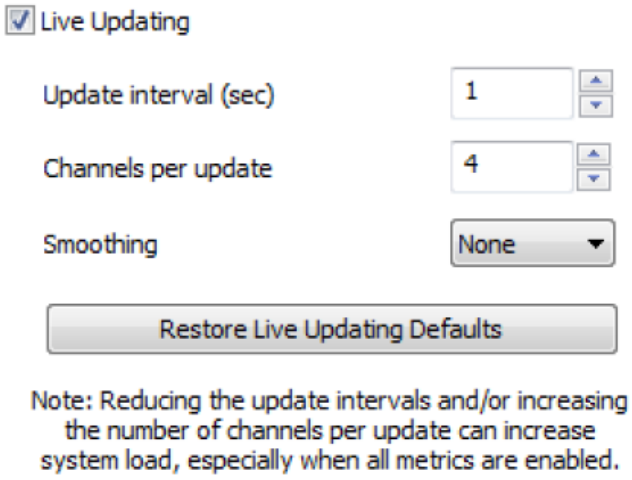
You can choose which metrics are displayed in the **Extended Properties Spreadsheet** using the **Sort Quality** page of the **Spike Snapshot Options** dialog:




Disabling metrics that are not of interest simplifies the spreadsheet (the corresponding columns are not displayed) and reduces the amount of processing needed to periodically update the metrics, which can be significant at high channel counts or fast update rates.

### 8.10.3 Metrics Calculation

The system calculates the metrics whenever units are added or removed on a channel, based upon the most recent sorting of the channel's spike snapshot. It can also periodically recalculate the metrics based on the sorting of the live incoming spikes. This is done by recalculating the metrics on the most recent spikes on each channel, 500 spikes per channel by default. Since some metrics are somewhat expensive to calculate, particularly **Isolation Distance** and **LRatio**, this is done on a rolling scheme whereby a given number of channels are periodically updated. For example, by default, each second the next four channels are updated:

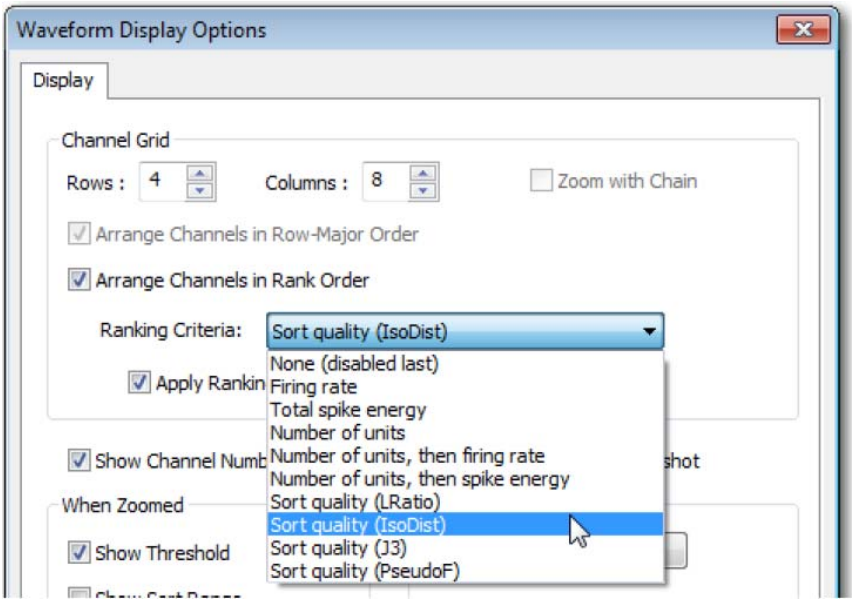



Therefore, on a 32 channel system, it will take eight seconds to update all 32 channels. The default is fairly conservative (a moderate update rate) but you should increase the update rate with caution.

By default, each time a channel's metrics are recalculated, the results based on the most recent 500 spikes are displayed. In some cases, you may wish to apply a moving average filter to smooth the results. If a **Smoothing** option other than **None** is selected, a moving average of the previous metric and the most recent metric is performed.

### 8.10.4 Channel Ranking Based On Sort Quality Metrics

You can use any of the sort quality metrics as the criteria for channel ranking. To do so, simply select the desired metric from the **Ranking Criteria** dropdown list:



Recall that channel ranking is calculated from the spike snapshot and is not updated from the live data. For additional information on channel ranking, see Section 13.7, “Channel Ranking” on page 437.

# 8 Additional Sorting Methods and Metrics

# Chapter 9
# Automatic Spike Sorting

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9.3 Automatic Sorting—TDEM Method ..... 308
9.4 Automatic Sorting—Peak Seeking Method ..... 310
9.5 Semi-automatic Sorting—Guided Spike Sorting ..... 311
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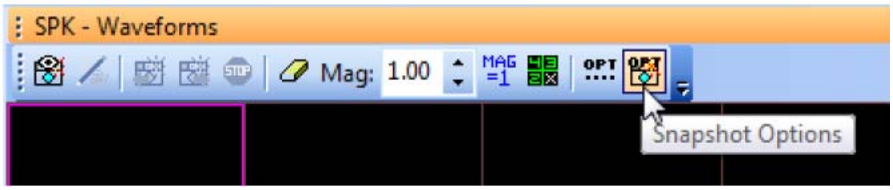
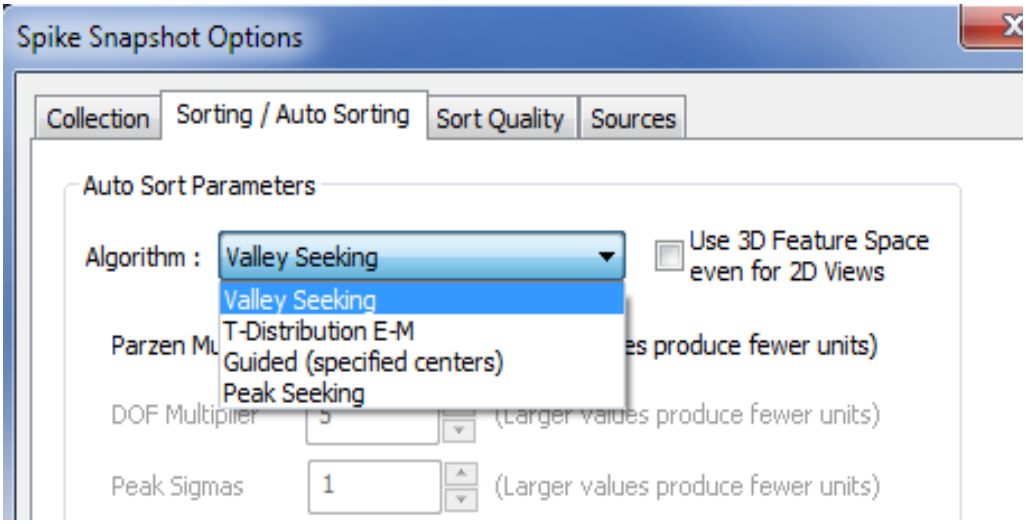
## 9.1 Introduction to Automatic Sorting (Automatic Unit Finding)

### 9.1.1 Automatic Sorting Methods

There are several types of automatic sorting algorithms. These are selectable in the **Spike Snapshot Options** dialog, which is accessed by clicking the **Snapshot Options** button in the **SPK-Waveforms** window.




Automatic sorting might be more accurately described as automatic unit finding or unit definition. Automatic unit finding can be performed while PlexControl's displays are in either live or snapshot modes, but the actual unit finding algorithm always creates unit definitions based on the spikes in the snapshot. These unit definitions are then sent to Server and used to sort the live incoming data, just as with manually defined units.

- **Valley Seeking**: A non-parametric method based on local density measures. See Section 9.2, “Automatic Sorting—Valley Seeking Method” on page 301.
- **T-Distribution EM**: T-Distribution Expectation Maximum (TDEM)—A method which assumes that the clusters in feature space (each cluster representing the spikes from one neuron) can be modeled as t-distributions. See Section 9.3, “Automatic Sorting—TDEM Method” on page 308.
- **Peak Seeking**: A non-parametric, density-based automatic spike sorting method based on the concept that good cluster centers are points of high local density which are maximally distant from points of higher density. See Section 9.4, “Automatic Sorting—Peak Seeking Method” on page 310.

- The **Guided (specific centers)** option is a semi-automatic algorithm. See Section 9.5, “Semi-automatic Sorting—Guided Spike Sorting” on page 311.

### Choosing the best auto-sorting algorithm and parameter values

The choice of auto-sorting algorithm and the appropriate value for the Parzen radius (for the valley seeking method), the degree of freedom (DOF) multiplier (for the TDEM method), or Sigmas (for the peak-seeking method) is somewhat data-dependent. A description of these issues is beyond the scope of this document, but you should work with both methods and observe the effects of adjusting their respective tuning parameter in order to get a feel for the differences and tradeoffs between them. The sort quality metrics provide one way of comparing the results of the different auto-sorting methods (see Section 8.10, “Spike Sorting Quality Metrics” on page 290). Note that you can use Plexon® Offline Sorter™ software as a convenient platform for gaining experience with the auto-sorting methods using your previously-recorded data files, as opposed to live data. Refer to the Offline Sorter user guide for additional information and references on valley seeking and TDEM.

In addition to template sorting, automatic unit finding can produce unit definitions for the line, band and 2D polygon sorting methods. For information on those methods, see:

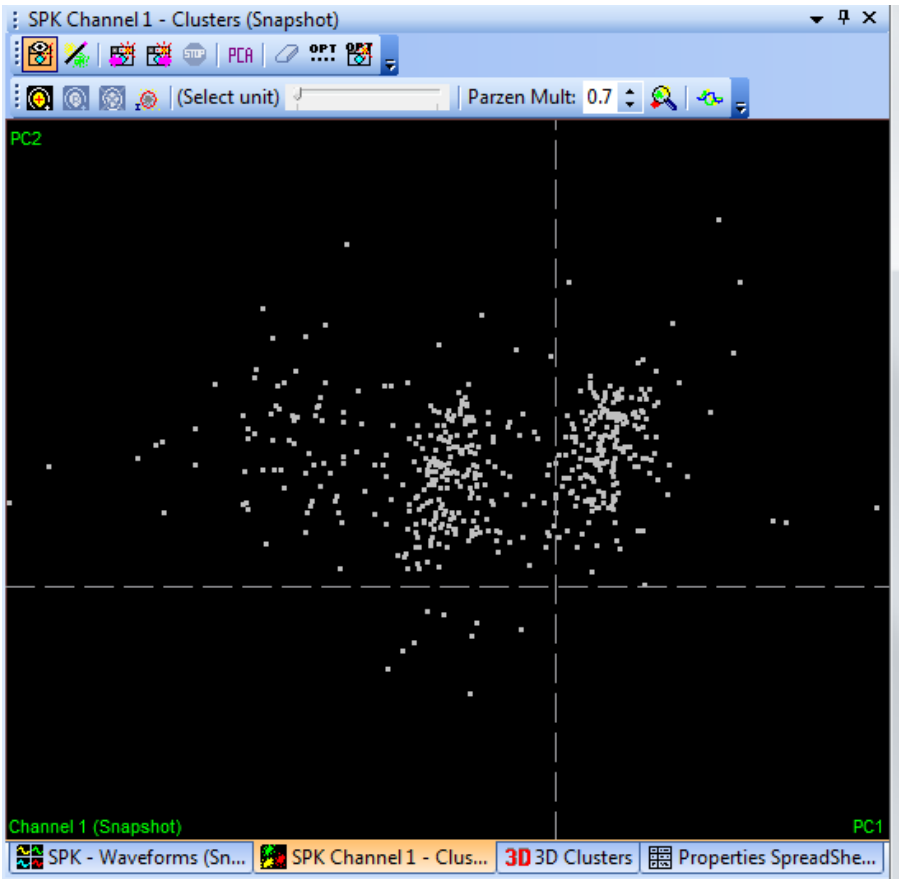
- Section 8.1, “Line Sorting” on page 226
- Section 8.7, “Band Sorting ” on page 264
- Section 8.8, “2D Polygon Sorting ” on page 271

**Note:** Automatic unit finding cannot be used with box sorting.

### 9.1.2 Initial Procedure

1 To see how automatic unit finding works, first make sure that you have collected a spike snapshot, and have toggled into snapshot mode, as described in the previous section.

2 If the PCA view is not already in single-channel (zoomed) mode, double-click on a channel in the multichannel PCA view to zoom it. You should now see something like this:



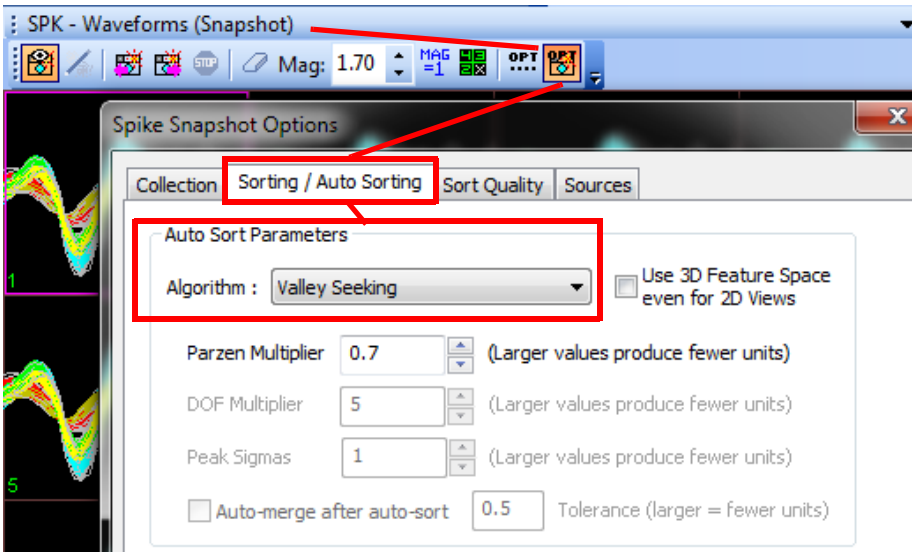

3 Continue configuring the system according to the method you want to use:

- **Valley Seeking**: Section 9.2, “Automatic Sorting—Valley Seeking Method” on page 301.
- **T-Distribution EM**: Section 9.3, “Automatic Sorting—TDEM Method” on page 308.
- **Peak Seeking**: Section 9.4, “Automatic Sorting—Peak Seeking Method” on page 310.
- **Guided (specific centers)**: Section 9.5, “Semi-automatic Sorting—Guided Spike Sorting” on page 311.

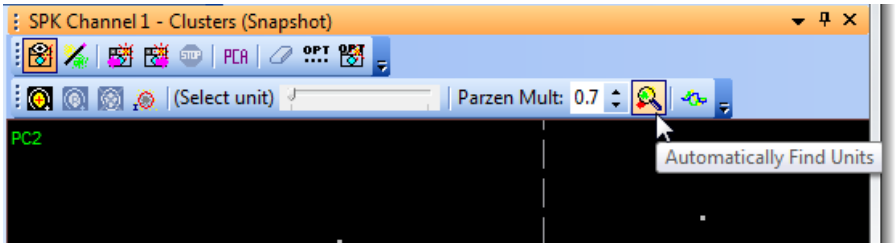
## 9.2 Automatic Sorting—Valley Seeking Method

Follow these steps to use the valley seeking autosorting method.

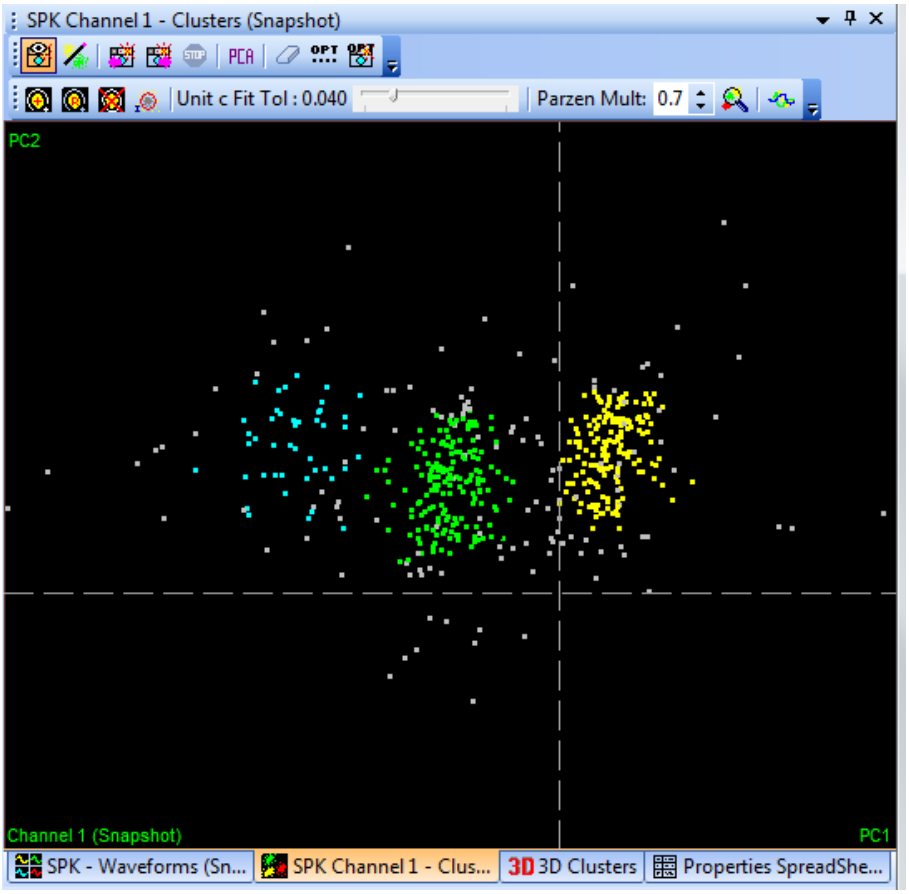
1. Perform the steps in Section 9.1.2, “Initial Procedure” on page 300.
2. To choose Valley Seeking, use the **Algorithm** drop-down list in the **Sorting/ Auto-Sorting** page of the spike snapshot options:



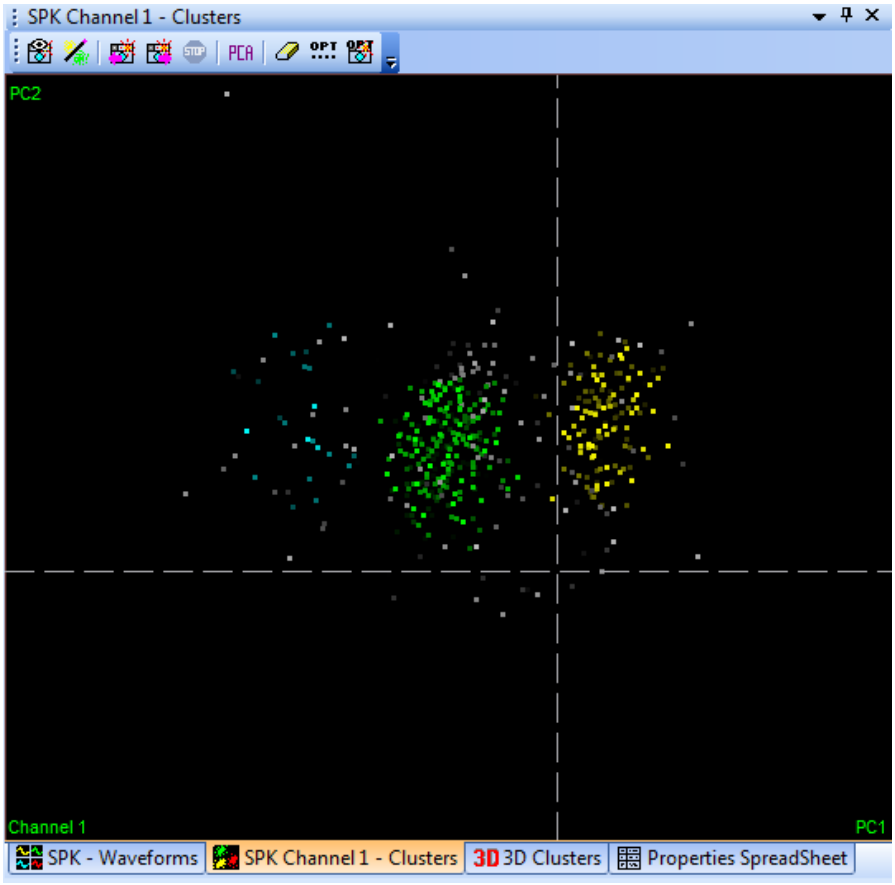

3. Click on the **Automatically Find Units** button in the toolbar:



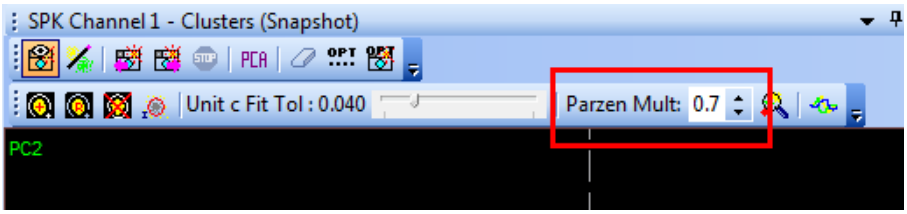
4 The automatic unit finding algorithm sorts the spikes in the snapshot by looking for clusters of their projected PCA points, then averaging the corresponding waveforms for each sorted unit to create a new unit definition (template, bands, lines, or contours). In other words, the unit finding is performed in PCA space, then the results of the sorting are used to produce unit definitions for the actual sorting of incoming data.



5 You can use the **Show Snapshot** button to toggle back to viewing the live data, to see the results of the unit finding (which was performed based on the snapshot) being applied to incoming spikes:



6 If you are not satisfied with the results, there are two different ways in which you can modify the results of the automatic unit finding process. For template, line, or band sorting, you can adjust fit tolerances for any of the found units, just as you would for units that were created manually. However, this assumes that the automatic unit finding located an appropriate number of units based on its default settings, which was the case in the above example, where it found the same three units that we would have defined manually. However, depending on your data, you may decide that the automatic procedure was "too sensitive," e.g. unnecessarily split one cluster into two or more units, or was "too coarse," i.e. inappropriately merged two or more separate clusters into a single sorted unit. To address these issues, the automatic unit finding algorithm provides what is in effect a unit-finding sensitivity control, called the Parzen Multiplier, which is a parameter of the underlying valley-seeking algorithm.




Unlike the fit tolerance, which is a unit definition parameter that can be adjusted retroactively, i.e. after a unit template has been defined, the value of the Parzen Multiplier is used the next time that automatic unit finding is performed. In other words, if you wish to change the value of the Parzen Multiplier, you must then perform another round of automatic unit finding, whose results (i.e. unit definitions) will then overwrite the previous ones.

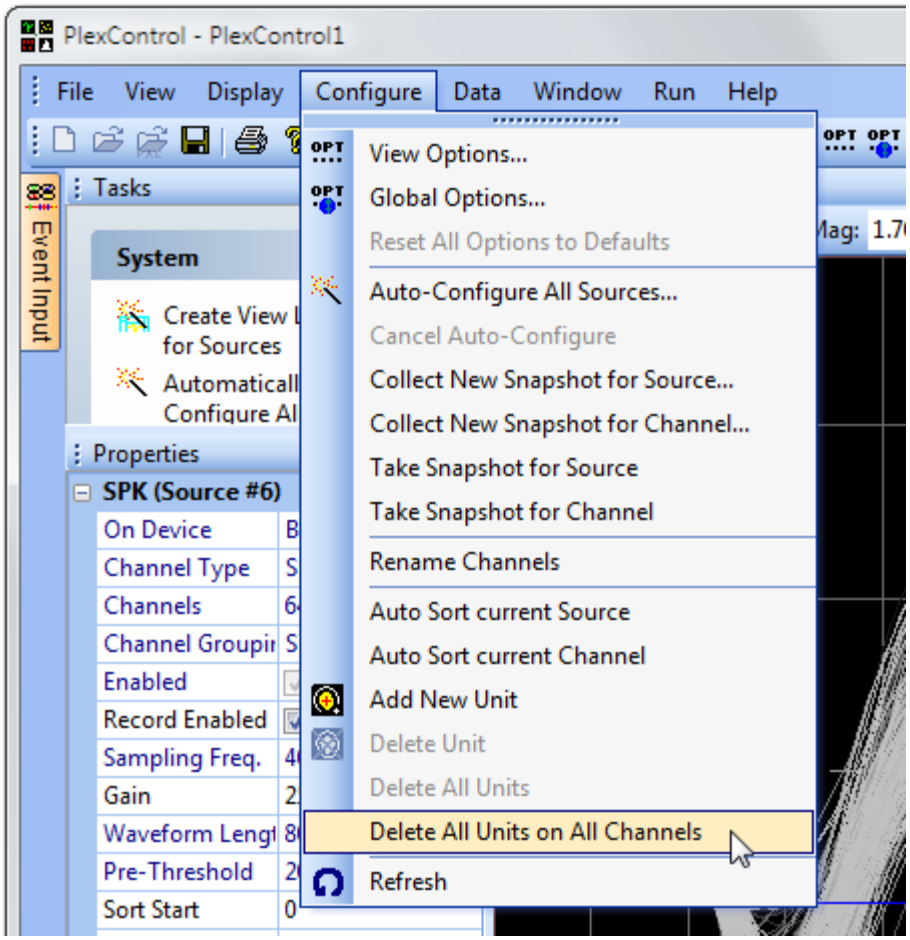
As a general guideline, to find *more* clusters, *reduce* the Parzen Multiplier value; to find *fewer* clusters, *increase* the Parzen Multiplier. However, automatic unit finding, which is in essence an unsupervised classification problem, is a complex task, especially when dealing with noisy data, and you will sometimes find counter-intuitive results when you change the Parzen Multiplier. Fortunately, the default value of 0.7 works well with many types of spike data.

### Automatic unit finding on all channels

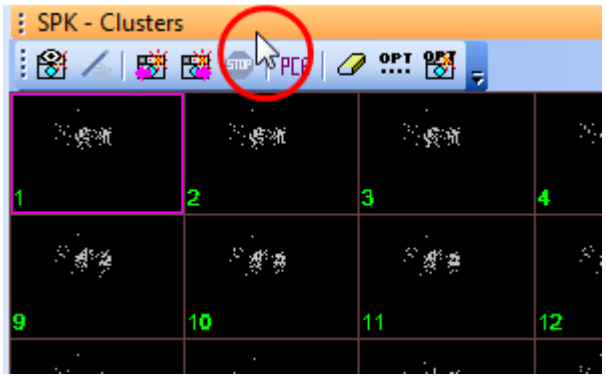
1 The OmniPlex System can also perform automatic unit finding on all spike channels, instead of one at a time. To do this, it is recommended that you first unzoom the PCA display, i.e. return to a multichannel PCA view, by double-clicking in the view if it is zoomed. This is so that you can see the progress of the automatic unit finding as it works through all the spike channels. You can view either the multichannel spike or multichannel PCA views, according to your preference. Also, make sure that you have collected a spike snapshot.

2 If you already have units defined on some or all channels, you may wish to remove them before starting the automatic unit finding. This is optional, since the unit finder will delete any existing units on each channel it processes, but it makes it easier to view the channel-by-channel progress of the unit finding if

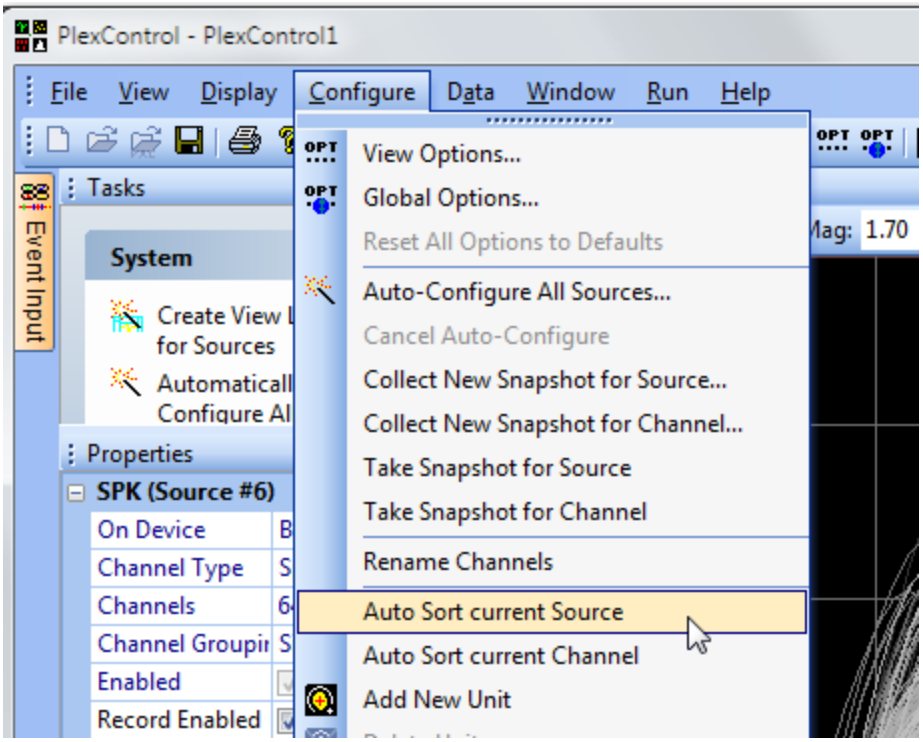
you start from scratch. To do this, use the **Delete All Units on All Channels** command in the **Configure** menu:




3 After all units have been deleted (this may take a few seconds on high channel count systems), make sure that the SPK source is selected; you can do this by clicking on either the main spike window, or the multichannel spike or cluster views:



4 Select **Auto Sort current Source** from the **Configure** menu to begin the automatic unit finding:



**TIP**

**Auto Sort grayed out**

If the **Auto Sort** menu item is grayed out, it is probably because the SPK source is not currently selected.

5 As the automatic unit finding proceeds, you can see which channels have been processed by watching the multichannel spike or PCA views:

6 For performance reasons, it is recommended that you view the live data, *not* the spike snapshot, while a multichannel auto-sort (unit finding) is in progress. In fact, if you have the snapshot displayed and begin a multichannel auto-sort, PlexControl will toggle the views back into live mode, to avoid this problem. However, once the auto-sort has completed, you can switch back and forth between live and snapshot modes as usual.

**TIP**
**Monitor auto-sorting**

If you select the highest-numbered SPK channel in the multichannel display (e.g. channel 64 in the example above), you can watch the main spike window to see when sorted spikes start to appear, as an easily visible indicator of when auto-sorting has finished on all channels.

**TIP**
**Allow auto-sort to complete**

Do not start another auto-sort while an auto-sort is already in progress; in general, allow an auto-sort to complete before adding, deleting, or replacing units.

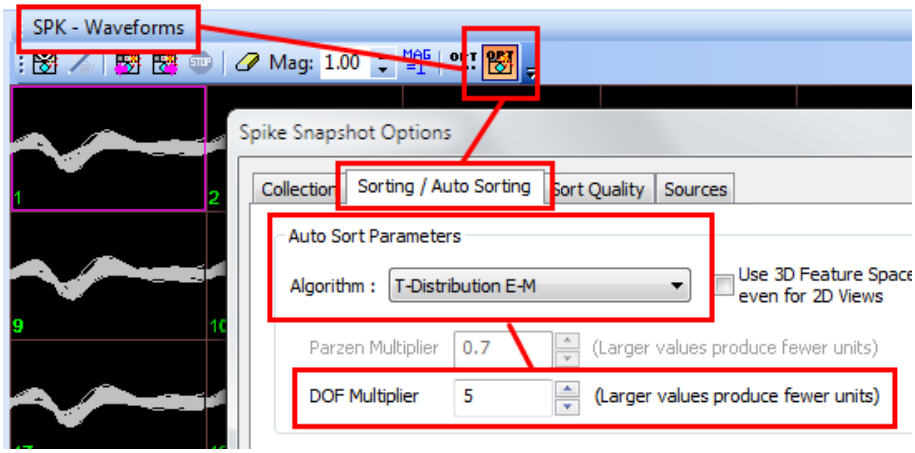
Remember that automatic unit finding is just another tool for creating unit definitions, and the unit definitions that it produces can be used as-is or augmented manually. For example, you may wish to set the Parzen Multiplier to only find large, obvious clusters, then manually inspect the results and manually add units corresponding to smaller or less distinct clusters that are not as easily found automatically. You might decide to delete or manually replace some of the automatically-found units, but leave the majority of them as is. Such approaches can give the best of both worlds, automatic and manual, while saving considerable time compared to defining every unit on every channel manually.

## 9.3 Automatic Sorting—TDEM Method

Section 9.1, “Introduction to Automatic Sorting (Automatic Unit Finding)” on page 298 includes a description of an auto-sorting algorithm, known as valley seeking. Valley seeking is a non-parametric method based on local density measures, and gives very good results in many cases, but it is not always ideal. The system also supports an auto-sorting algorithm called t-distribution expectation maximization, or TDEM for short. TDEM works on the assumption that the clusters in feature space (each cluster representing the spikes from one neuron) can be modeled as t-distributions, so that the entire set of clusters on each channel is a mixture of t-distributions. The TDEM algorithm solves a global optimization problem to find the set of t-distributions which best fit the observed clusters on each spike channel.

Follow these steps to use the TDEM autosorting method

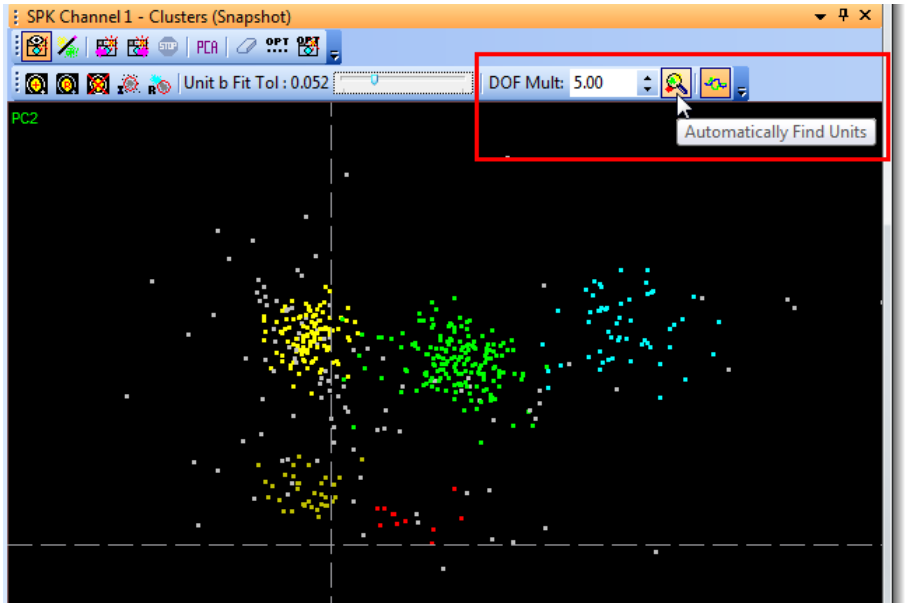
1. Perform the steps in Section 9.1.2, “Initial Procedure” on page 300.
2. To choose TDEM, use the **Algorithm** drop-down list in the **Sorting/Auto-Sorting** page of the spike snapshot options:




3. Adjust the value of the parameter and then click the **Automatically Find Units** button to redo the auto-sort using the current value of the parameter.

   The degrees-of-freedom multiplier, or DOF multiplier for short, is analogous to the Parzen multiplier used in valley seeking. It is a tuning parameter that

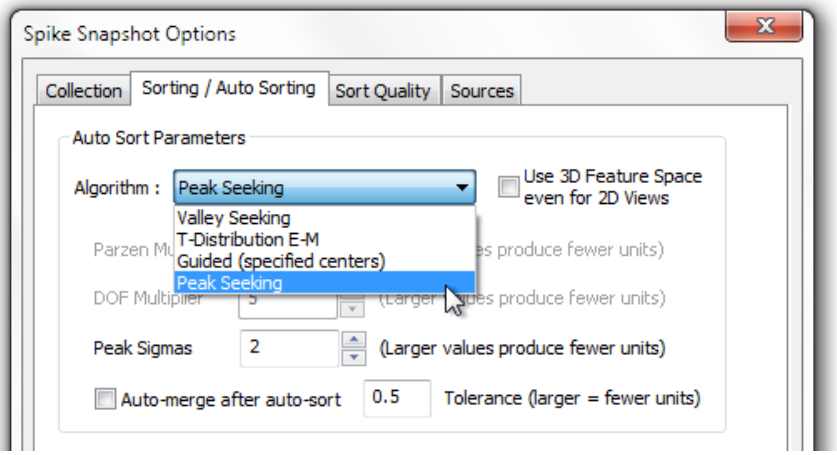
allows you to influence the number of clusters that are found by auto-sorting. With either algorithm, you can see the effect of adjusting the parameter while viewing the PCA snapshot, since clusters are found in the PCA snapshot and then used to derive the sorting parameters for the currently selected sorting method (template, line, band, or 2D polygon) which are then applied to the live incoming spike data.



## 9.4 Automatic Sorting—Peak Seeking Method

Peak seeking is a non-parametric, density-based automatic spike sorting method. It is based on the concept that good cluster centers are points of high local density which are maximally distant from points of higher density.

1. To begin using the peak seeking method, perform the steps in Section 9.1.2, “Initial Procedure” on page 300.
2. Then select **Peak Seeking** from the list of auto sort algorithms in the **Sorting/ Auto Sorting** page of the **Spike Snapshot Options** dialog:




Similarly to the other automatic sorting algorithms, peak seeking has a tuning parameter, in this case named **Peak Sigmas**, which controls the sensitivity of the algorithm. The default value of 2 will usually detect reasonably well-defined clusters and will avoid overfitting. Larger values will detect only the largest and most well-defined clusters, and so are even “safer,” but low density clusters may be missed. A value of 1 will result in more clusters being found; values less than 1 are generally not recommended.

There is also an optional auto-merge step that post-processes the clusters found by peak seeking, merging clusters with similar templates (note that this works even if you are not using template sorting). Peak seeking using low values of **Peak Sigmas** can detect spurious clusters that are the result of sampling jitter splitting a single cluster in two. Note that you can use aligned extraction (see Section 15.4.4, “Thresholding By Aligned Extraction” on page 495) to minimize this jitter. These can be faintly distinct clusters in feature space that are in fact the waveforms from a single unit.

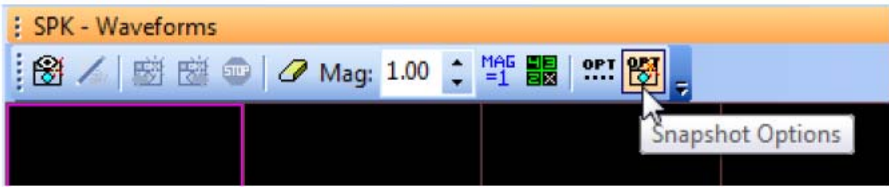
Because such clusters may have mean waveforms that are similar in shape but slightly offset in time, standard Euclidean distance is a poor similarity metric

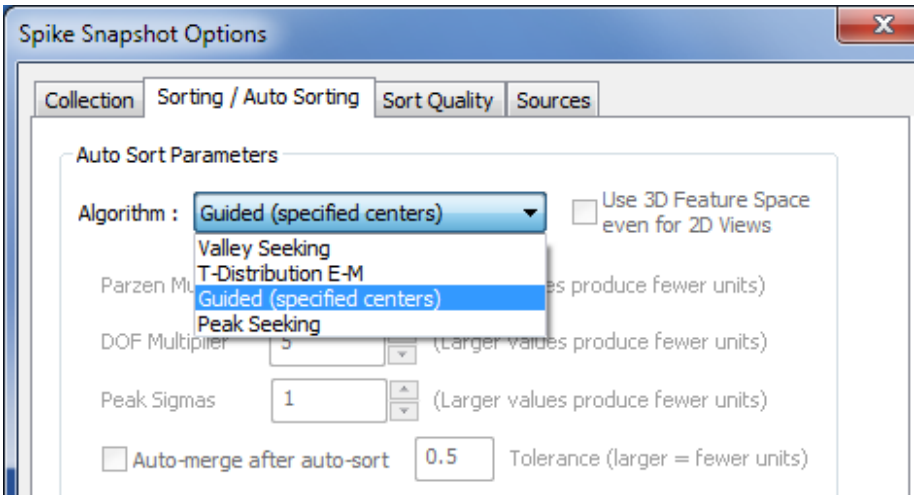
for auto-merging them, and the system instead uses a distance measure which accounts for minor time offsets between templates. When you enable **Auto-merge after auto-sort**, you can specify the merge tolerance, where larger values result in more merges being performed and therefore fewer units. Typical values for the merge tolerance are in the range 0.5 – 1.0. Please note that if you use the default **Peak Sigmas** value of 2, auto-merging is usually not needed and should be left disabled.

## 9.5 Semi-automatic Sorting—Guided Spike Sorting

Guided sorting is a semi-automatic method for defining units. Like the fully automatic methods, it classifies (clusters) spikes in the spike snapshot. However, Guided sorting requires "hints" from you, in that you click the mouse to indicate the approximate center of each unit's cluster. In other words, you provide the initialization for the automatic sorting algorithm. This can be useful in situations where some units fire very infrequently, making them difficult for a fully automatic method to find, and/or clusters are very poorly separated, leading to sub-optimal results from the fully automatic methods.

1. To begin using the peak seeking method, perform the steps in Section 9.1.2, "Initial Procedure" on page 300.
2. Click on the **Snapshot Options** button, then select **Guided (specific centers)** from the **Algorithm** dropdown.

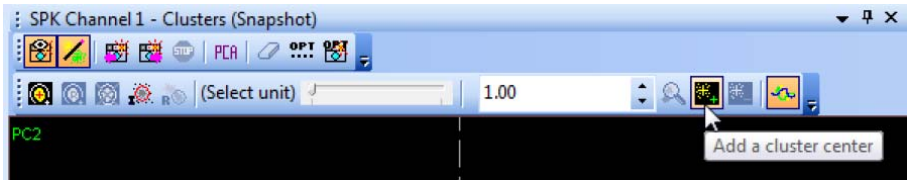


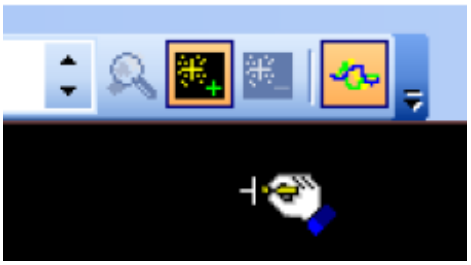
3 Click **OK** (at the bottom of the **Spike Snapshot Options** page).

4 Go to the **Clusters 2D** view, enter unit editing mode and view the snapshot.

5 Notice that the **Find Units** toolbar button and menu item are initially grayed out, because you haven't defined any cluster centers yet. However, the **Add a cluster center** button to its right is enabled. Click this button to begin defining clusters:

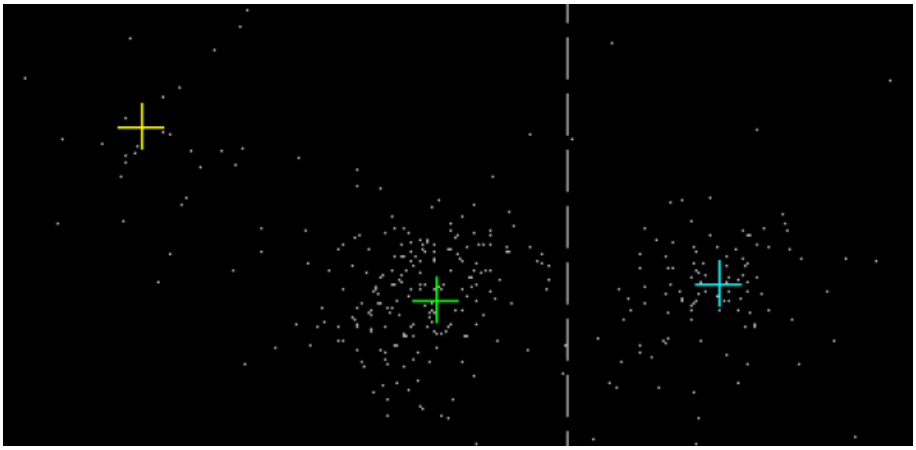



The cursor changes to a crosshair cursor:

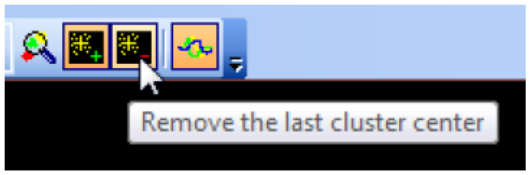


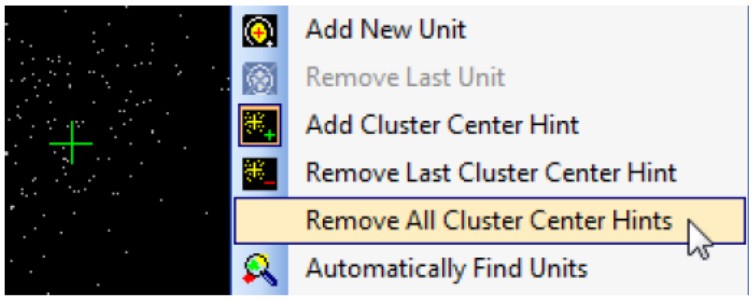
6 Click on one or more cluster centers.

**Note:** When you click on a cluster center, the center position is shown as a colored crosshair marker. Each click defines the approximate center of a unit. Note that no spikes have been sorted yet.

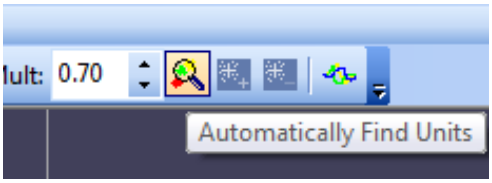


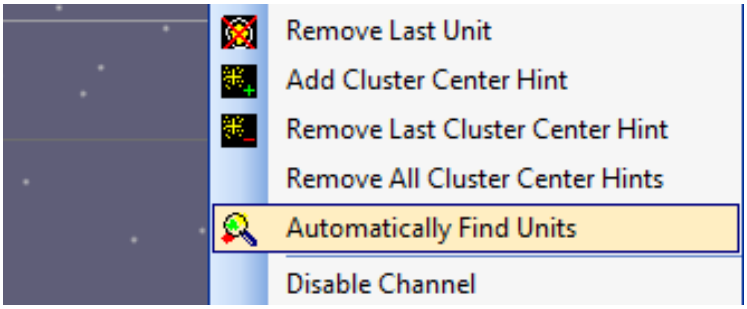
7 (Optional) If you want to remove the last cluster center (i.e. undo it), or start over, there is a toolbar button for **Remove Last Cluster Center**, and there are right-button menu items for removing the last or all centers:




8 When you are done defining all the cluster centers, click **Automatically Find Units**, either from the menu or the toolbar, to define units based on the hints you provided.




Guided sorting is guaranteed to find the exact number of clusters you specified and unless you provide a very poor set of hints (e.g. clicking between clusters instead of near their centers), will result in cluster means that are close to the center hints you provided.

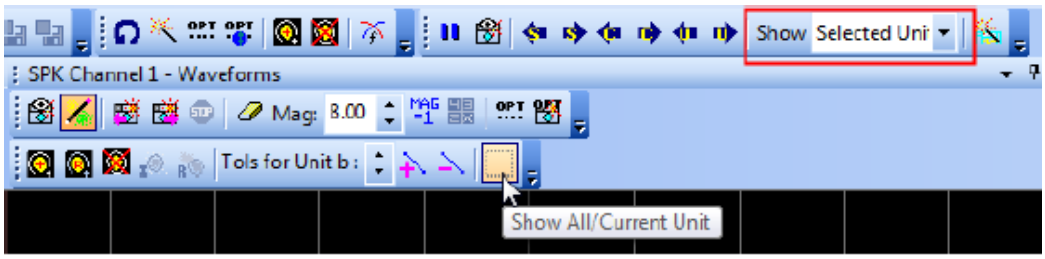
Note that the cluster center hints are temporary; they only exist until you click **Automatically Find Units**. They cannot be edited (except for removing the most recent hint), and if you switch to a different channel while in the middle of adding hints they will be removed.

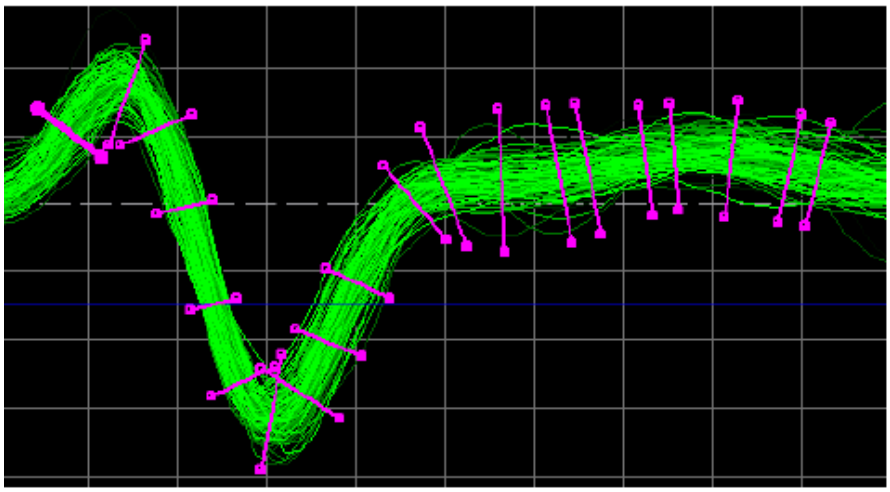
Once you have familiarized yourself with Guided sort, you will find that defining units this way can be very fast, since you don't have to draw contours around clusters, only click once for each unit.

## 9.6 Automatic Spike Sorting in the Line Sorting Method

**Note:** For a manual (non-automatic) method of line sorting, see Section 8.1, “Line Sorting” on page 226.

Automatic and guided spike sorting can be used with the line sorting method. The result is a set of sorting lines that follow the “path” of the spike waveforms for each unit. In the following picture we have set the **Show** filter to **Selected Unit** and set **Show All /Current Unit** to the “Show Current Unit” mode, to remove the waveforms and sorting lines for the other units on this channel. (The **Show All / Current Unit** button will turn blue and show the template for the selected unit only.)

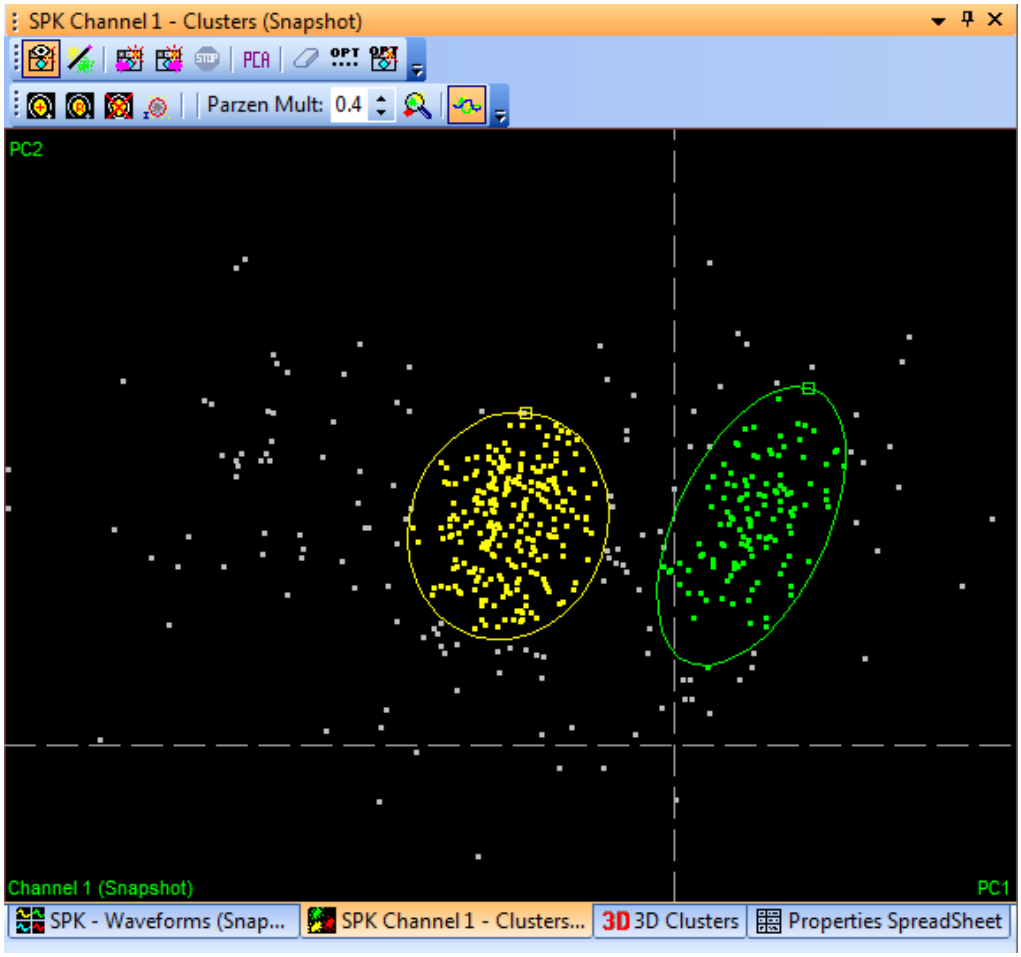


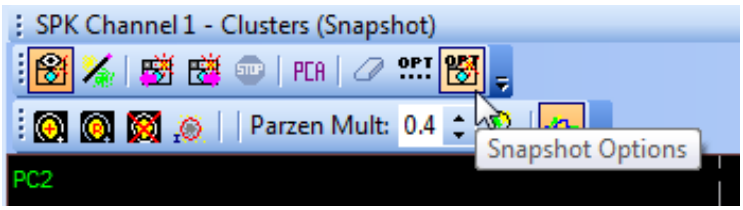
**Note:** After a set of sorting lines has been created by automatic sorting, or as the result of drawing a contour in a cluster view, you can manually modify or delete any of the generated lines, or add more lines, as previously described.

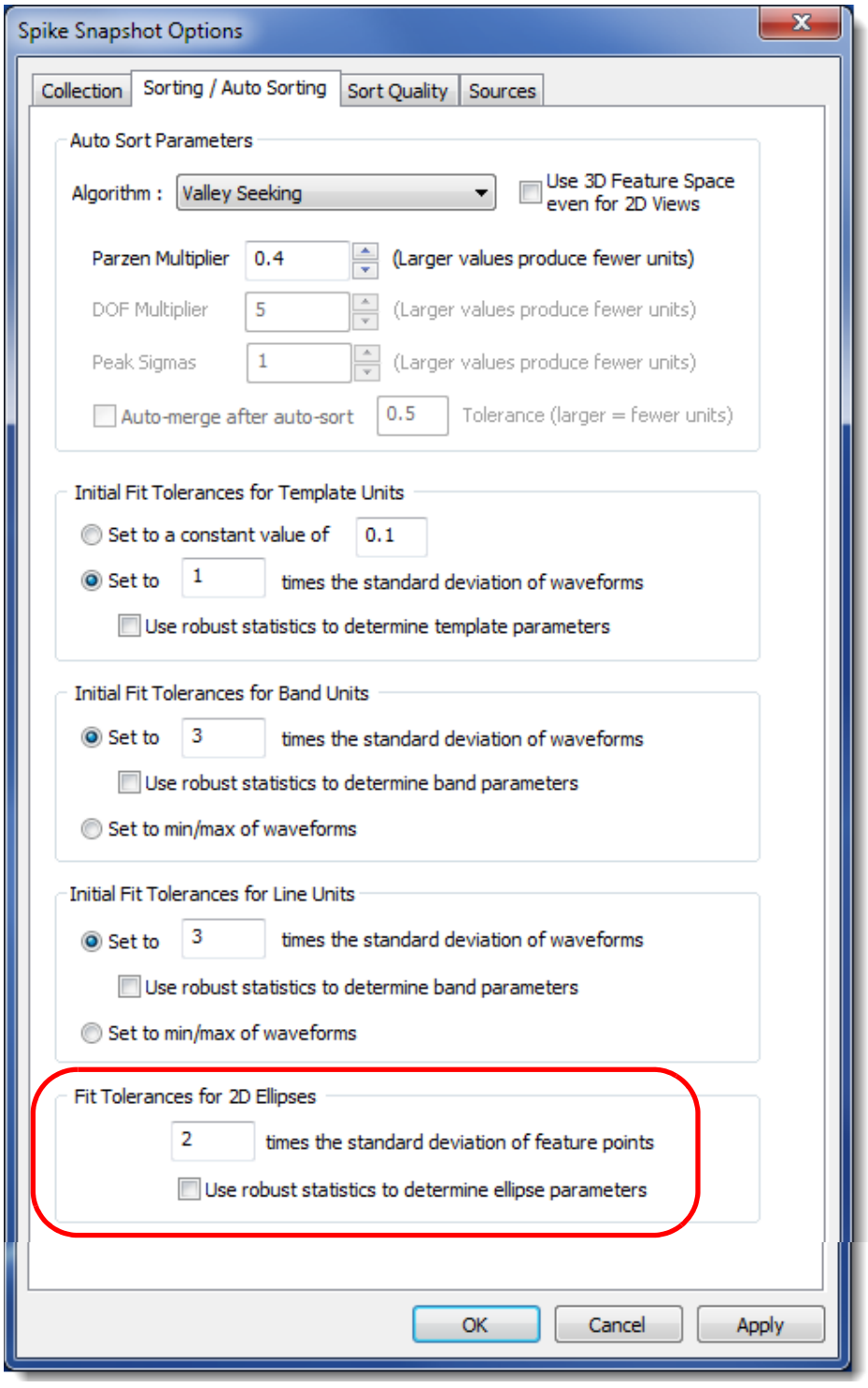
## 9.7 Automatic Unit Finding with 2D Polygon Sorting

You can also perform automatic or guided unit finding while in 2D polygon sorting mode. The main difference relative to template sorting is that the unit finding creates unit definitions consisting of ellipses that are fit to the clouds of sorted PCA points.




You can adjust how tightly the ellipses are fit to the clouds of sorted points from the unit finding algorithm. To do so, display the **Spike Snapshot Options** dialog, using the toolbar button:


SPK Channel 1 - Clusters (Snapshot)
PCA
OPT
Parzen Mult: 0.4
Snapshot Options
PC2


Spike Snapshot Options
Collection
Sorting / Auto Sorting
Sort Quality
Sources
Auto Sort Parameters
Algorithm :
Valley Seeking
Use 3D Feature Space even for 2D Views
Parzen Multiplier
0.4
(Larger values produce fewer units)
DOF Multiplier
5
(Larger values produce fewer units)
Peak Sigmas
1
(Larger values produce fewer units)
Auto-merge after auto-sort
0.5
Tolerance (larger = fewer units)
Initial Fit Tolerances for Template Units
Set to a constant value of
0.1
Set to
1
times the standard deviation of waveforms
Use robust statistics to determine template parameters
Initial Fit Tolerances for Band Units
Set to
3
times the standard deviation of waveforms
Use robust statistics to determine band parameters
Set to min/max of waveforms
Initial Fit Tolerances for Line Units
Set to
3
times the standard deviation of waveforms
Use robust statistics to determine band parameters
Set to min/max of waveforms
Fit Tolerances for 2D Ellipses
2
times the standard deviation of feature points
Use robust statistics to determine ellipse parameters
OK
Cancel
Apply

This value (which should not be confused with the fit tolerance for template sorting) controls the tightness of fitting of all generated ellipses, except for those that result from cleaning up hand-drawn contours.

You can move and rotate the automatically-generated contours, just like manually-created ones. Of course. you can also modify the Parzen Multiplier and run another round of automatic unit finding, as previously described.

# Chapter 10
# Digital Inputs

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## 10.1 Digital Input Card Configuration Types

If you have the basic OmniPlex® chassis, the Digital Input (DI) card may be installed. If you have the newer eChassis, the DIN card (also known as the DINe card) may be installed.

You can use the basic chassis and DI card with all OmniPlex software releases. You must have Release 18 or later to use the eChassis and DINe card.

**Note:** If you have recently upgraded the OmniPlex Server software, the system might display a dialog “DHP Firmware Update Warning.” Upgrading your firmware is recommended, because it will provide updates such as enhanced protection from severe transient noise in the environment, operation with additional chassis cards and higher channel counts. The DIN update (DIN 2.2) fixes a problem with the low-true logic option in Individual Events mode. Note that the DIN update is only needed by and applicable to the new eChassis systems; look for “DIN” or “DINe” on the front panel of the digital input card. To perform the firmware upgrade, see "Appendix K: Firmware Upgrade for DHP Unit" on page A-84.

## 10.2 General Description, DI Card and DINe Card

The DI (or DINe) card allows you to input signals from external digital sources, such as behavioral systems, switches, levers, etc. These digital input signals are detected by the DI/DINe card, timestamped with a resolution of 25 microseconds, and written to the Main Datapool as channels within one of two digital input sources, either-**Individual events** or **Event word**, as described below.

The DI/DINe card has two front panel 26-pin connectors, referred to as Port A (lower) and Port B (upper). The two ports are identical except that the RSTART (level-triggered recording) input is only present on Port A (pin 24). On both ports, pin 22 is the data-ready (strobe) flag, used only when the port is configured for **Event word**. Each port can be configured to detect individual events or an event word, and operate with high-true or low-true logic.

For details on the DI and DINe card pinout assignments, see Appendix J: Hardware Pinouts, Connections and LEDs.

**CAUTION**

**Do not apply voltage <0V or >+5.5V to the pins**

Input voltages to the pins on the digital input card (DI and DINe versions) must always be between 0V and +5.5V. Voltages outside this range can damage the card. Never apply negative voltages to the pins.

**Note:** The DI/DINe cards have internal protection circuitry, and are designed to accept voltages within the specified range (0V to +5.5V) whether the OmniPlex chassis is powered on or off. However, it is good practice to avoid driving voltages, such as TTL pulses, into unpowered devices.

**Note:** These voltage limits (0V and +5.5V) correspond to currents of approximately 0.33mA out and 0.22mA in, respectively.

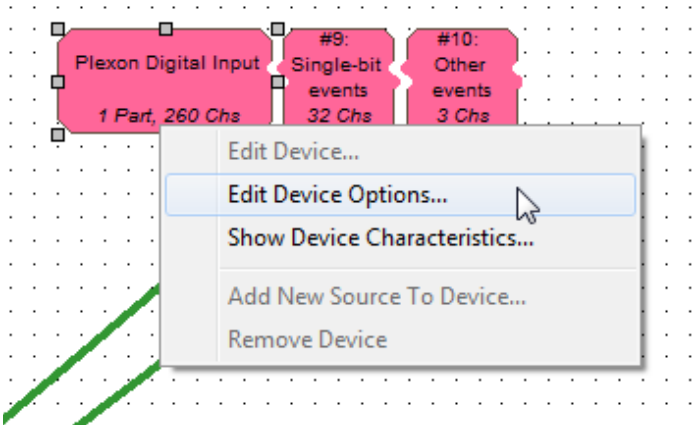
## 10.3 Viewing the Digital Input Card Settings

### 10.3.1 Viewing the DI Card Settings (Basic Chassis)

**Note:** This section is applicable only to the DI card, which is installed in the basic OmniPlex chassis. If you have the DINe card (applicable to the OmniPlex eChassis), go to Section 10.3.2, “Viewing the DINe Card Settings (eChassis)” on page 323.

To view the DI card settings, perform the following steps.

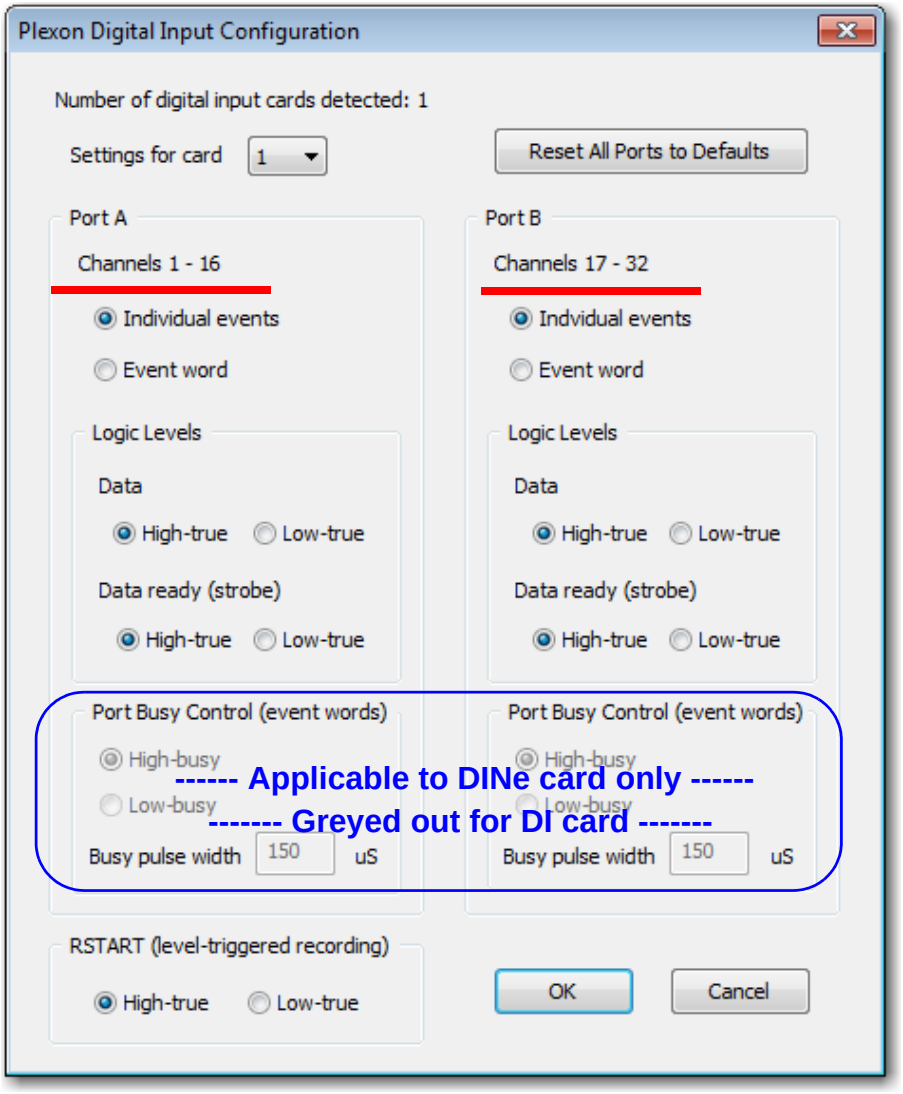
1. Stop data acquisition (see Section 2.8, “Stopping Data Acquisition” on page 60 if you have a DigiAmp subsystem, or Section 3.10, “Stopping Data Acquisition” on page 103 if you have a DHP subsystem).
2. Go to the Server window, and right click the **Plexon Digital Input** device in the topology. Select **Edit Device Options** to display the options dialog:




**Note:** If the option **Edit Device Options** is greyed out (not selectable), make sure that data acquisition is stopped.

The **Plexon Digital Input Configuration** dialog box opens. Notice that with Individual events selected (the default setting), the dialog displays the channel numbering for each port.

The Port Busy Control options are applicable to the DINe card only. These options are greyed out and not used if you have a DI card.




3 If you want to modify any of the DI card settings, see the applicable section(s):

- Section 10.4, “Configuring the Port Event Mode ” on page 325
- Section 10.5, “Configuring the Logic Level Settings” on page 328
- Section 10.7, “Configuring RSTART and RSTOP Events” on page 334

**TIP**

**Optional—Convenient method to set all ports to defaults**

You can click the **Reset All Ports to Defaults** button to reset all ports to **Individual events** and **High-true** logic.

**Note:** For details on the DI card pinout assignments, see Appendix J: Hardware Pinouts, Connections and LEDs.

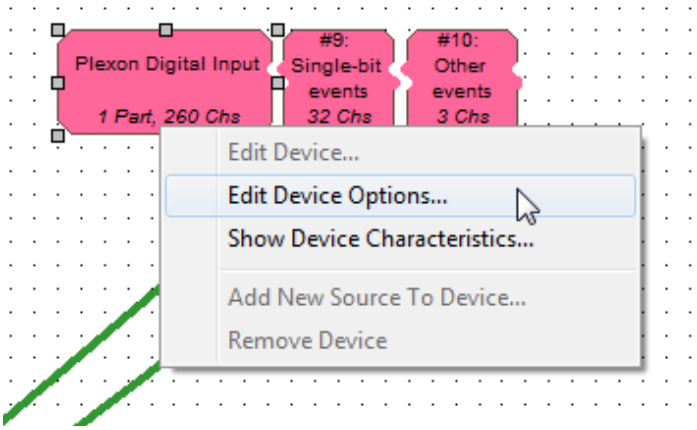
**Note:** Most systems have a single DI card. If your system has two DI cards, the Settings for card dropdown list is enabled, and there are some additional parameters to configure. See "Appendix I: Option for Two Digital Input (DI) Cards" on page A-52.

### 10.3.2 Viewing the DINe Card Settings (eChassis)

**Note:** This section is applicable only to the DINe card, which is installed in the OmniPlex eChassis. If you have the DI card (applicable to the basic OmniPlex chassis), go to Section 10.3.1, “Viewing the DI Card Settings (Basic Chassis)” on page 321.

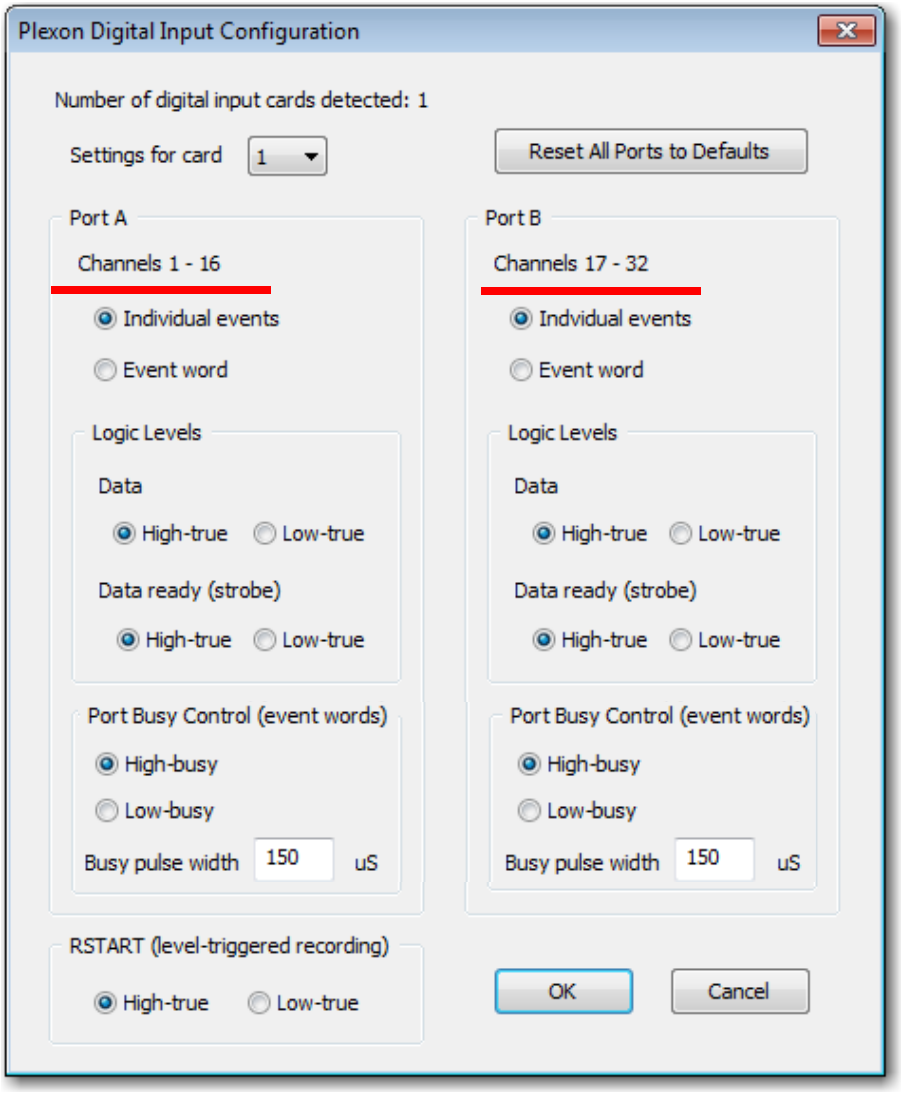
To view the DINe card settings, perform the following steps.

1. Stop data acquisition (see Section 3.10, “Stopping Data Acquisition” on page 103).
2. Go to the Server window, and right click the **Plexon Digital Input** device in the topology. Select **Edit Device Options** to display the options dialog:




**Note:** If the option **Edit Device Options** is greyed out (not selectable), make sure that data acquisition is stopped.

The **Plexon Digital Input Configuration** dialog box opens. Notice that with Individual events selected (the default setting), the dialog displays the channel numbering for each port.




**3** If you want to modify any of the DINe card settings, see the applicable section(s):

- Section 10.4, “Configuring the Port Event Mode ” on page 325
- Section 10.5, “Configuring the Logic Level Settings” on page 328
- Section 10.6, “Configuring the Port Busy Controls (DINe Card Only)” on page 331
- Section 10.7, “Configuring RSTART and RSTOP Events” on page 334

**TIP**

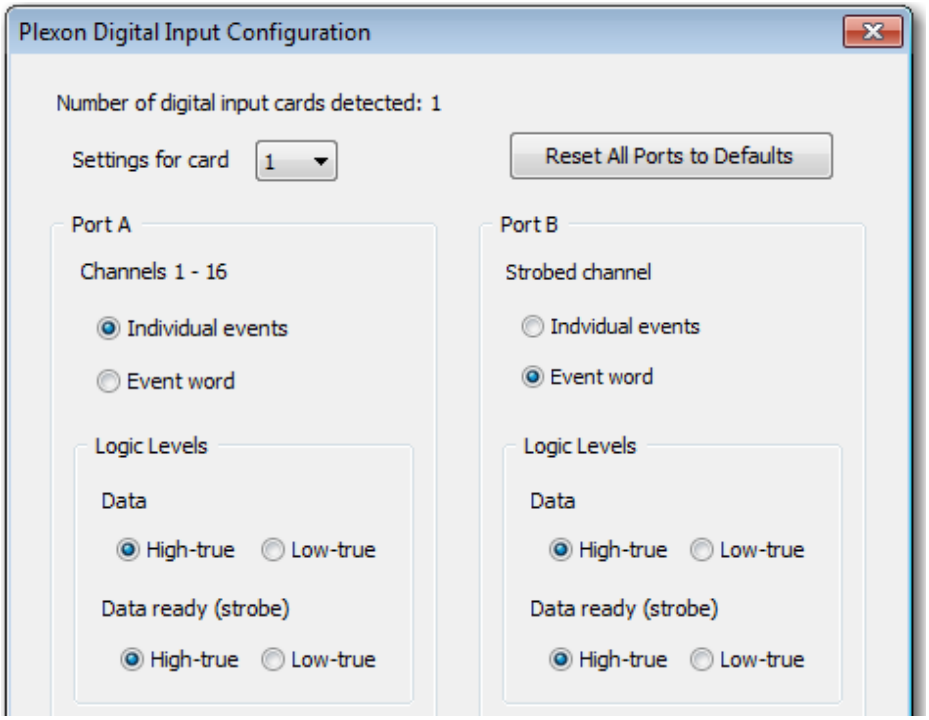
**Optional—Convenient method to set all ports to defaults**

You can click the **Reset All Ports to Defaults** button to reset all ports to **Individual events** and **High-true** logic.

**Note:** For details on the DINe card pinout assignments, see Appendix J: Hardware Pinouts, Connections and LEDs.

## 10.4 Configuring the Port Event Mode

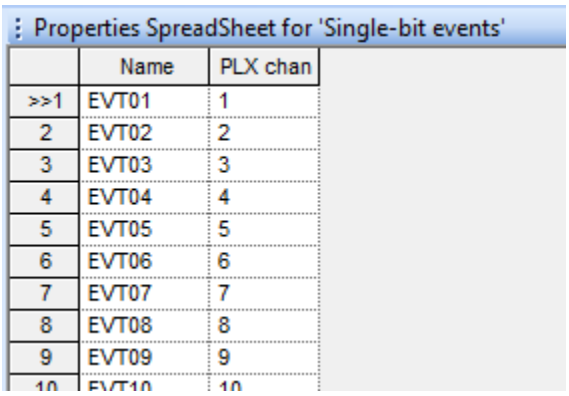
This section describes the port event modes on the digital input cards. These settings are common to both the DI and DINe cards. In the example shown below, Port A is set to **Individual events** mode and Port B is set to **Event word** mode. Ports A and B can be independently configured for **Individual events** or **Event word** in any combination. Each of these modes is explained in the sections that follow.




### 10.4.1 Individual Events Mode

The **Individual events** option configures the port to recognize each event input pin as an independent digital input channel. This option is typically used when there are few actions that need to be recorded. For example, lever presses or IR beam breaks can be sent into the DI (or DINe) port provided that they produce a clean detectable voltage edge. Noisy edges can result in unwanted multiple event detections.

Up to 16 separate, individually timestamped event channels can be sent into a port that is configured for **Individual events**. When the voltage on the event channel's input pin goes high (or goes low, depending on the **High-true**/**Low-true** setting), a timestamped digital event on that channel will be generated. For Port A, these channels will be named EVT1-EVT16; for Port B, they will be named EVT17-EVT32. In PlexControl, single-bit events are shown as a single contiguous range from channels 1-32 in the **Properties Spreadsheet**, as shown in the image below.


Properties SpreadSheet for 'Single-bit events'

| | Name | PLX chan |
|---|---|---|
| >>1 | EVT01 | 1 |
| 2 | EVT02 | 2 |
| 3 | EVT03 | 3 |
| 4 | EVT04 | 4 |
| 5 | EVT05 | 5 |
| 6 | EVT06 | 6 |
| 7 | EVT07 | 7 |
| 8 | EVT08 | 8 |
| 9 | EVT09 | 9 |
| 10 | EVT10 | 10 |

These single-bit event channels contain no additional information beyond their timestamp and channel number.

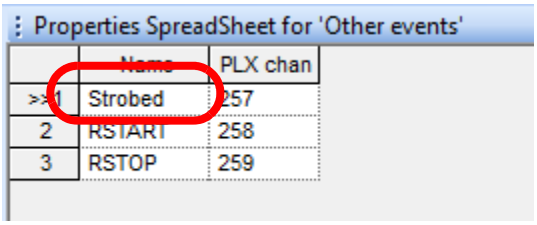
### 10.4.2 Event Word Mode

The **Event word** option configures the port to monitor changes in the level of a data-ready bit on a specially designated pin (pin 22). When the system detects a high-true (or low-true) event on this pin, it recognizes that the remote device is sending a multi-bit Event word. (Historically, this data-ready bit was called the strobe bit or strobe input.) The **Event word** option is typically used when there are conditions or data that need to be timestamped and recorded in real-time during an experiment. The Event word value can be used to encode a trial number, stimulus type or other experimental data.

**Note:** When a port is configured for **Event word**, if the level of the data-ready bit (strobe bit) does not become active, the system ignores the changes on the 16 pins of that port.

Multi-bit digital events are represented as Channel 1 (shown as “Strobed” in PlexControl) in the “Other events” source on the DI device.

In .plx files, for historical reasons these multi-bit events are represented as digital event channel 257 in the recorded file. In .pl2 files, for multi-bit events, the PL2 channel number is given by the number in the leftmost column, as with any other source's PL2 channel number. See the image of the **Properties Spreadsheet** below.


Properties SpreadSheet for 'Other events'

| | Name | PLX chan |
|---|---|---|
| >>1 | Strobed | 257 |
| 2 | RSTART | 258 |
| 3 | RSTOP | 259 |

### If one port is configured in Event word mode

When the data-ready bit (strobe bit) goes high (or goes low, depending on the **High-true**/**Low-true** setting), a 16 bit word value is read from the same 16 inputs that are read as separate channels when in **Individual events** mode. Think of the input pins as representing positions in a binary number in **Event word** mode. All 16 bits on each port (Port A and Port B) are available for user input, and values from 0 to 65535 can be read. Data channel 1 (pin 1) is the least significant bit, and data channel 16 (pin 16) is the most significant bit.

### If both ports are configured in Event word mode

When both ports are configured in **Event word** mode, there is still only one 16 bit event channel to represent the multi-bit events from both ports. The system must provide a means of identifying from which port the multi-bit event originated. It handles this by overwriting the most significant bit (bit 16 / pin 16) as follows:

- If the multi-bit event is from Port A, the high bit (bit 16) will be set to 0.
- If the multi-bit event is from Port B, the high bit will be set to 1.

When the OmniPlex System overwrites bit 16 to indicate the port, any signals sent into bit 16 will be ignored. In effect, only the lower 15 bits (pin 1-15) will be available for user input of values of 0 to 32767.

### Special considerations for third-party applications

It is important to realize that, when both ports are configured for **Event word** mode, third-party applications, MATLAB® scripts, etc, must use the value of the high bit to determine which port each multi-bit word is from, and only interpret the lower 15 bits as user data. The values recorded in the file for Port B will all have 32768 added to them, due the high bit having been overwritten with 1.

But if only one port is configured for **Event word** mode, and therefore bit 16 is not overwritten by the OmniPlex software to indicate the port, if the third-party software incorrectly interprets bit 16 to indicate the port (A or B), but in fact bit 16 contains valid user data, the third-party software will incorrectly assign 16 bit Event words from a single port to either Port A or Port B, depending on whether bit 16 of the user data was 0 or 1 respectively. Thus, when only one port is in **Event word** mode, the third-party device must be configured to read all 16 bits of Event word data.

## 10.5 Configuring the Logic Level Settings

This section explains how to set the **Logic Level** parameters on the digital input card, and how the system applies the configured **Logic Level** values.
These settings are common to both the DI and DINe cards.

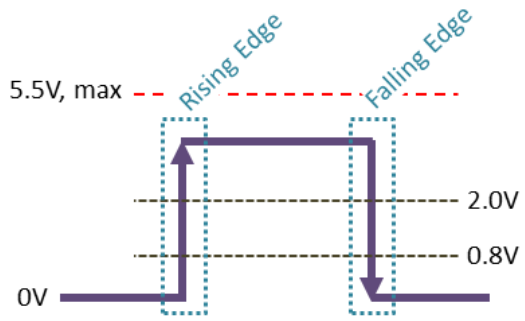
### 10.5.1 Definitions

**Timestamp**—The recorded system time at which a digital event is detected.

**Digital Event**—A rising or falling edge on the voltage level detected on the pin(s) of the DI (or DINe) card.
The system places a timestamp on each event. The response of the system to the rising or falling edge depends on the value of the **Logic Levels** parameter configured in the **Plexon Digital Input Configuration** dialog box: **High-true** or **Low-true**.

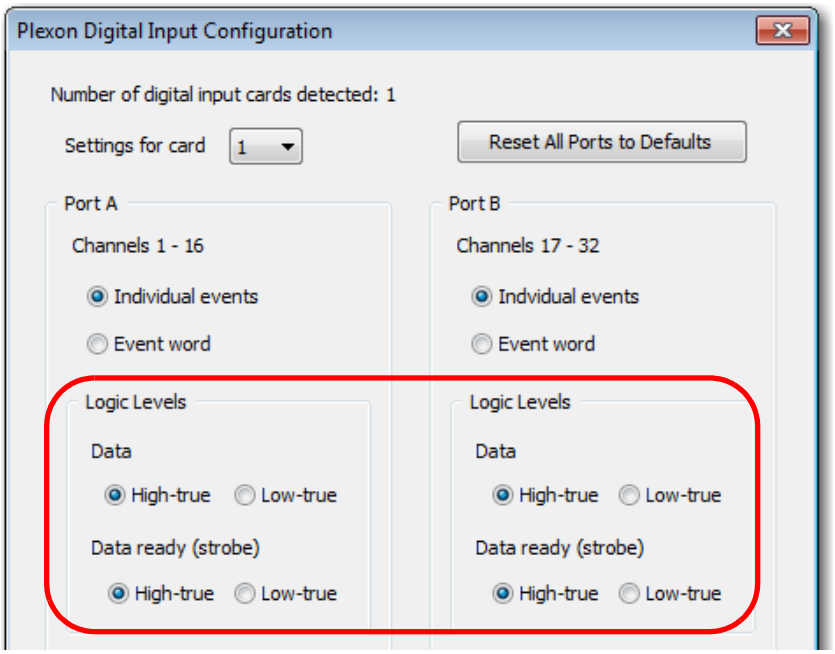
**Rising edge**—A transition of the input signal from less than +0.8V to greater than +2.0V.

**Falling edge**—A transition of the input signal from greater than +2.0V to less than +0.8V.



### 10.5.2 Setting the Logic Levels

The **High-true** and **Low-true** options for Port A and Port B can be set in the **Plexon Digital Input Configuration** dialog box. The default settings for these parameters are all **High-true**.




#### Logic Level Voltages

If the DI card (or DINe card) is configured as **High-true** (the default setting), the system interprets voltage levels as follows:

- A voltage level < +0.8V is interpreted as logic 0
- A voltage level > +2.0V is interpreted as logic 1

If the card is configured as **Low-true**:

- A voltage level < +0.8V is interpreted as logic 1
- A voltage level > +2.0V is interpreted as logic 0

### 10.5.3 High-true and Low-true Examples

For **Logic Levels** set to **High-true**:

- **Individual events** input signals—A rising edge (an input voltage that transitions from below +0.8V to above +2.0V) is recorded as a digital event.
- **Event word** (multi-bit) input signals—If the signal on an individual data pin is greater than +2.0V at the time of the multi-bit event, it contributes to the

value of the multi-bit word. For example, if the signal on data pin 15 is greater than +2.0V and the signals on data pins 1 through 14 are less than 0.8V at the time of the multi-bit event, the value of the **Event word** will be 2^(15-1) = 16384. (Note that the data pins are numbered 1 - 16, not 0 - 15.)

For **Logic Levels** set to **Low-true**:

- **Individual events** input signals—A falling edge (an input voltage that transitions from above +2.0V to below +0.8V) is recorded as a digital event.
- **Event word** (multi-bit) input signals—If the signal on an individual data pin is less than +0.8V at the time of the multi-bit event, it contributes to the value of the multi-bit word. For example, if the signal on data pin 15 is greater than +2.0V and the signals on data pins 1 through 14 are less than 0.8V at the time of the multi-bit event, the value of the **Event word** will be 2^(14-1) + 2^(13-1) + ... + 2^(1-1) = 16383.

## 10.6 Configuring the Port Busy Controls (DINe Card Only)

The **Port Busy Control** is an optional feature that can be useful if you are connecting certain types of external hardware, specifically, external hardware that will be generating digital events and transmitting them to the DINe card in the OmniPlex chassis. Each DINe port generates a port-busy pulse on pin 17, which you can connect to a pin on your external hardware.

The purpose of this feature is simply to mimic the “Input busy” feature of the Plexon® MAP box.

### Compatible with existing external hardware

If you previously connected external hardware to a DI card in a basic OmniPlex chassis, you can connect that same hardware to a DINe card without regard to the **Port Busy Control** settings. Your external device does not need to detect or process the port-busy signal coming from the DINe card.

### How the feature works

When the external hardware asserts the data-ready (strobe) signal to indicate that an event word is available, the DINe card reacts by asserting port-busy for a specified period of time (default 150 microseconds) on pin 17. If desired, you can program your external hardware to wait until port-busy is de-asserted on the DINe card before providing the next event word.

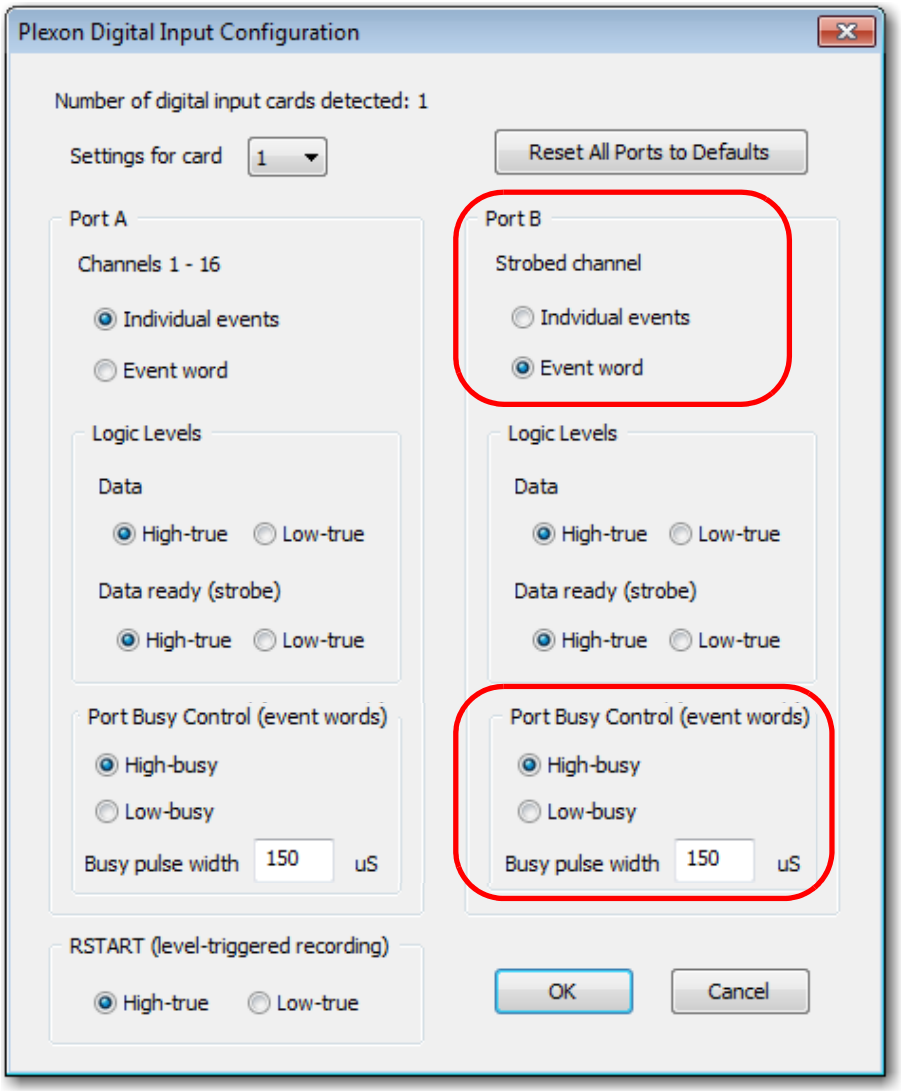
**Note:** The pinout details for this card are described in "Digital Input (DINe) Card in the OmniPlex eChassis" on page A-63.

If you are considering using this function and would like additional information, please contact Plexon Support at +1 214-369-4957 or support@plexon.com.

### Configuring the port-busy parameters

This section explains how to set the values for the **Port Busy Control** and **Busy pulse width** parameters in the Digital Input Configuration dialog.

1. Configure the applicable port for **Strobed channel** (**Event word**).
2. Configure the **Port Busy Control (event words)** parameter for either **High-busy** (the default value) or **Low-busy** operation. The voltage on pin 17 of the DINe card will go high or low (depending on the configured setting) when the internal port-busy pulse is being generated by the OmniPlex System.
3. The **Busy pulse width** value determines the minimum wait time between successive event words, and should usually be left at its default value of 150 microseconds, corresponding to a maximum event word rate of approximately 6000 event words per second per port. Values of less than 150µs should be used with caution, and you may wish to run tests to confirm that your system can reliably acquire event words at these rates.

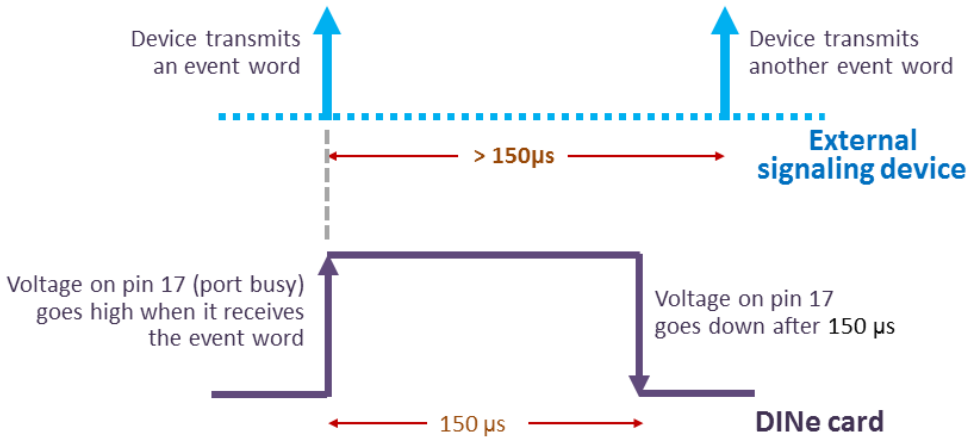


### Port Busy Examples

Assuming the values are set to their defaults (High-busy and 150µs), the voltage on pin 17 on the port connector will go high for 150µs to acknowledge the system has received an event word (strobed data). The examples below illustrate how this feature operates, assuming these default settings. As mentioned above, the use of this feature is optional, and you may or may not want your external hardware to take advantage of it.

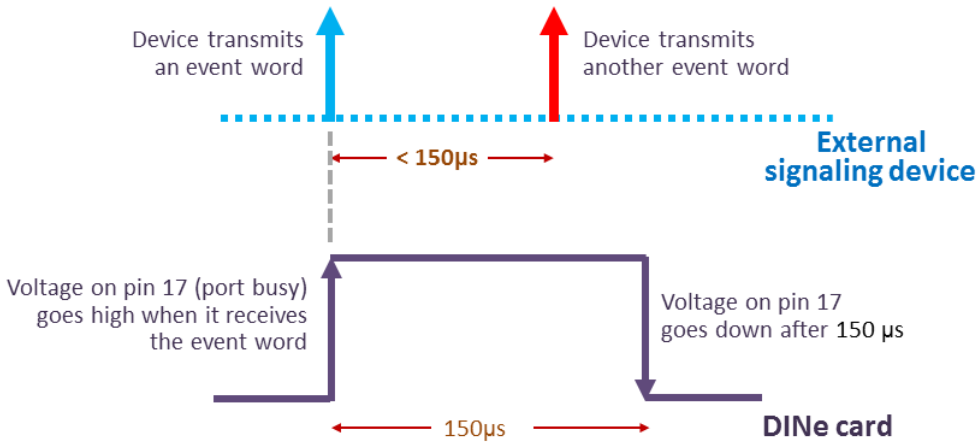
#### Example 1—Event received:
#### Second event word transmitted after 150µs has elapsed

The second event word (second blue arrow) is received by the DINe, because it was sent *after* the port-busy line deasserted (went low).




#### Example 2—Event not received:
#### Second event word transmitted before 150µs has elapsed

The second event word (red arrow) may not be received by the DINe, because it was sent *before* the port-busy line deasserted (went low). Therefore, you should adjust the settings on your external device so it will wait at least 150µs before sending the next event word.

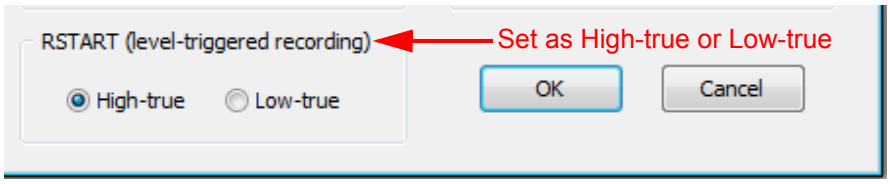


## 10.7 Configuring RSTART and RSTOP Events

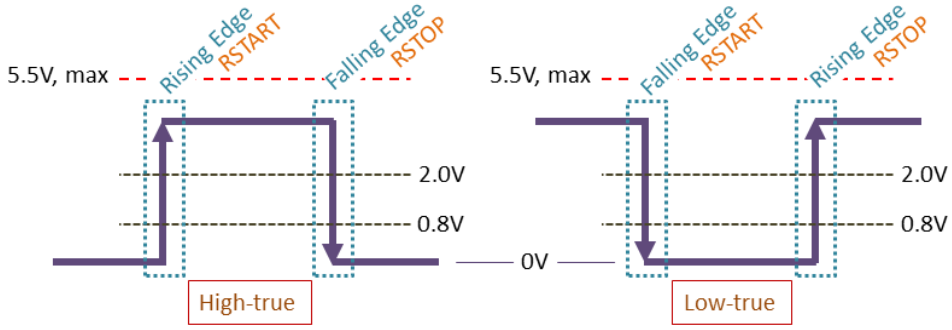
This information in this section applies to both the DI and DINe cards.

Port A has one additional input that is not present on Port B, which is the RSTART line. RSTART is different from the other digital inputs, in that it generates events on both the leading and trailing edges of input pulses. These signals are used for so-called level-triggered recording in PlexControl, where they start and stop recording, so "pulses" on RSTART are in fact typically seconds, minutes, or even hours in duration. The system can generate RSTART events regardless of the individual Port A and Port B data settings (**Individual events** or **Event word**).

The configuration options in the **Digital Input Configuration** dialog are shown below.




- When the **RSTART (level-triggered recording)** option is set to **High-true** in the **Plexon Digital Input Configuration** dialog, a rising edge on the RSTART line will create an RSTART event. The system can be configured to start (or resume) recording on an RSTART event.
  A falling edge on the RSTART line will create an RSTOP event. The system can be configured to stop (or pause) recording on an RSTOP event.
- When the **RSTART (level-triggered recording)** option is set to **Low-true** in the **Plexon Digital Input Configuration** dialog, a falling edge on the RSTART line will create an RSTART event. The system can be configured to start (or resume) recording on an RSTART event.
  A rising edge on the RSTART line will create an RSTOP event. The system can be configured to stop (or pause) recording on an RSTOP event.




To configure PlexControl to start/resume and stop/pause recording using the signal on the RSTART pin, use the **Recording Control** tab in the PlexControl **Global Options** dialog (**Configure > Global Options**). See Chapter 11, Triggered Recording, for examples of using the RSTART event.

## 10.8 Floating Inputs—Pull Up and Pull Down Settings

A floating input occurs when no wire is attached to a particular data pin on the digital input card connector, or when no voltage is being applied through that wire. The system handles this condition as described below. Note that the behavior for the DI card (in the basic chassis) is different from the behavior of the DINe card (in the eChassis).

### 10.8.1 Floating Inputs on the DI Card (in Basic Chassis)

**Note:** This section is applicable only to the DI card, which is installed in the basic OmniPlex chassis. If you have the DINe card (applicable to the OmniPlex eChassis), go to Section 10.8.2, "Floating Inputs on the DINe Card (in eChassis)" on page 336.

For the DI card, floating data pins (inputs) are always pulled up to a fixed voltage of +3.3V. This should not be a problem for ports configured for **Individual events**, since only rising or falling edge transitions are detected.

For ports configured as **Event word**, the system does the following:

- For ports configured as **Event word** and **High-true** logic, floating inputs will be pulled up and appear as 1 (true) bits in the multi-bit word value, causing them to be counted as part of the multi-bit word. It is recommended that you ground all unused inputs to avoid such problems. If you do not do this, you will have to postprocess your digital event data to mask out (set to zero) the bits in the event word corresponding to the floating inputs.
- For ports configured as **Event word** and **Low-true** logic, floating inputs will be pulled up and appear as 0 (false) bits in the multi-bit word value. Thus, they will not be counted as part of the multi-bit word. This will usually be acceptable behavior. Nonetheless, you should consider grounding all unused inputs if there is any chance that you will be changing this setting in the future.

### 10.8.2 Floating Inputs on the DINe Card (in eChassis)

**Note:** This section is applicable only to the DINe card, which is installed in the OmniPlex eChassis. If you have the DI card (applicable to the basic chassis), go to Section 10.8.1, “Floating Inputs on the DI Card (in Basic Chassis)” on page 335.

If a data input pin is floating, the DINe card automatically applies either a pull up or pull down such that the resulting logic bit will be 0 (false). In terms of voltage:

- If the port is set to **High-true** logic, floating inputs are pulled down to a fixed voltage of 0V (ground), resulting in a logic 0 for the corresponding bits.
- If the port is set to **Low-true** logic, floating inputs are pulled up to a fixed voltage of +5.0V or +3.3V, again resulting in a logic 0 for the corresponding bits.

  **Note:** This voltage level (+5.0V or +3.3V) is set with a jumper on the DINe card. The factory default position is +5.0V. For information on setting this jumper, see "Pin 20—Power output option, +5V / +3.3V" on page A-65.

## 10.9 Timing Considerations for Rising and Falling Edges

Transitions (rising and falling edges) on the digital input lines are detected by the DI hardware at a rate of 2 MHz, but since the default timestamp resolution of digital events in the OmniPlex System is 40 kHz (25 µsec), events that are sent into the DI (or DINe) card at a rate higher than 40 kHz will result in multiple digital events with the same 40 kHz timestamp. Therefore, consider throttling your digital inputs accordingly. The maximum recommended sustained event rate (total of all event inputs, both individual and strobed) is 4 kHz, with short bursts at a rate of 8 kHz.

**IMPORTANT:** If you anticipate sending events into the DI at rates approaching these maximum values, it is strongly recommended that you make a short test recording. Verify the results are as expected before proceeding to record important experiment data.

Even if you do not intentionally input high-frequency digital events, if your digital signals are very noisy or have ringing on the edges, this can produce redundant digital events. See the discussion in Section 10.10, “Avoiding Noise On the Digital Input Signal” on page 337.

## 10.10 Avoiding Noise On the Digital Input Signal

The DI card (and DINe card) inputs have a Schmitt-trigger functionality, which means that a small amount of noise in the signal will generally not cause redundant or spurious digital events. However, if you suspect you are seeing redundant digital events, check your input signals for excessive noise. Examine your signals on a scope to verify that they are not noisy and that they have clean edge transitions, and make sure that proper grounding is used.

**TIP**
**Make a test recording**
It is always a good idea to make a test recording to verify correct connectivity and reliable acquisition of digital events before recording actual experimental data.

## 10.11 Viewing Digital Events in PlexControl Activity View

Digital event rasters (ticks) can be viewed in the **Activity** view in PlexControl.

Multi-bit event activity is displayed as a single tick mark in PlexControl but the numeric multi-bit data values are not shown.

See Section 13.1, “PlexControl Activity Display” on page 380 for more information on viewing digital events.

## 10.12 Recording of Digital Events

Digital inputs are always recorded and there are no enable/disable settings.

When the system is recording multi-bit words, the voltage level on the data input pins must be stable for at least 100ns before a pulse is sent into the multi-bit pin (pin 22). If you design your inputs in a manner that causes the signal to be stable for less than the required 100ns, the system will not record the multi-bit events properly. Therefore, plan your inputs to avoid this condition.

## 10.13 Saving the Digital Input Card Settings

When you save the pxs (topology) file in Server, the current settings of the DI card (or DINe card) are also saved in the file.

# 10 Digital Inputs

# Chapter 11
# Triggered Recording

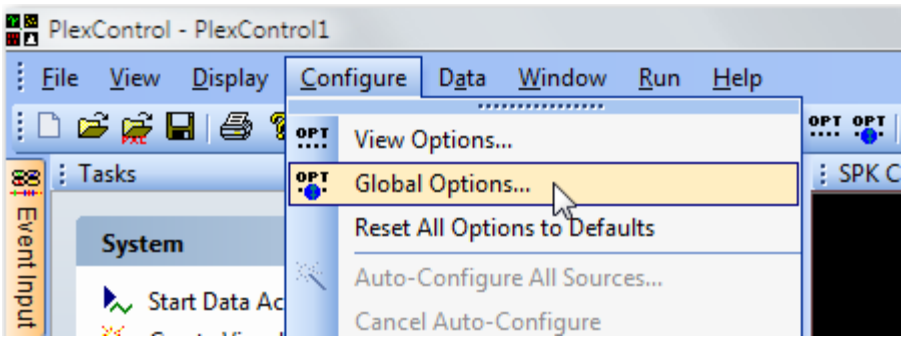
11.1 Timed and Event-triggered File Recording ..... 340
11.2 Examples—Recording a Single File with Pause/Resume Triggers ..... 345
11.3 Understanding Multiple File Recording ..... 349
11.4 Examples—Multiple File Recording ..... 353
11.5 Ensuring Keyboard Events Are Active ..... 358
11.6 Timing Considerations—Timed and Event-triggered Recording ..... 361

## 11.1 Timed and Event-triggered File Recording

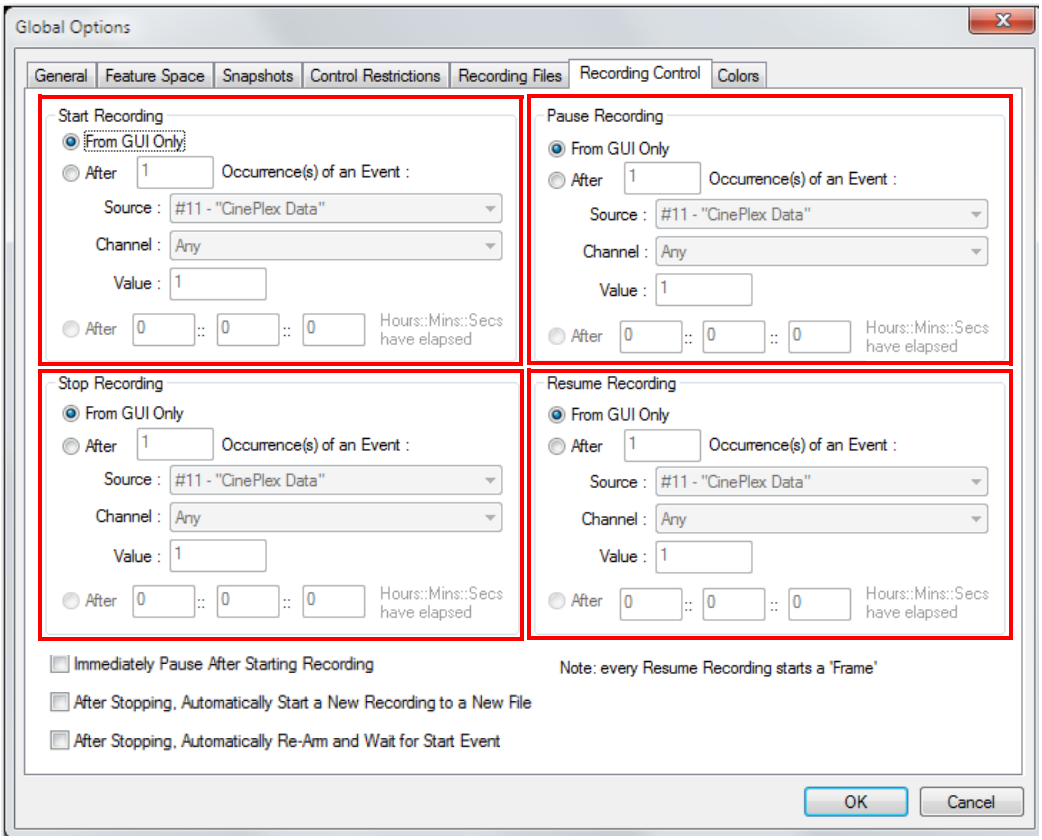
In addition to starting, stopping, pausing, and resuming recording manually from the GUI, you can configure PlexControl to perform any or all of these same actions automatically when a user-specified time has elapsed or when a user-specified event occurs.

### 11.1.1 Accessing the Recording Control Options

The **Global Options** dialog is accessed from the **Configure** menu:




Clicking the **Recording Control** tab opens this page (highlighting added):

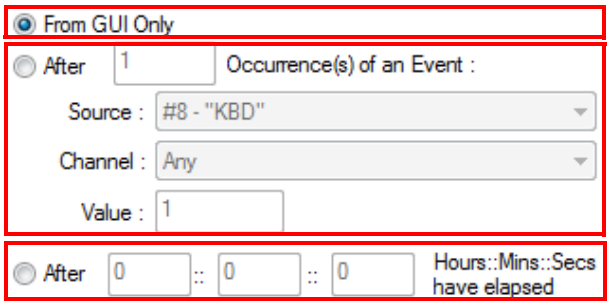


As shown in the image above, there are four control areas that you can configure:

- Start Recording
- Stop Recording
- Pause Recording
- Resume Recording

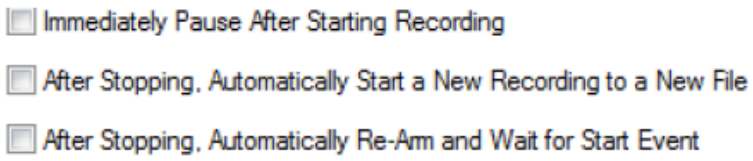
Each of the control areas has three options (see the detail in the image below):

- From the GUI only—Toolbar button or menu command (manual control)
- After the occurrence (or multiple occurrences) of an Event
- After a specified time has elapsed




Each of the four actions—start, stop, pause, and resume—can be independently controlled either from the standard buttons in the main toolbar (**From GUI Only**), triggered by a specified event (**After ... Occurrence(s) of an Event**) or triggered after a specified elapsed time (**After ... Hours::Mins::Secs have elapsed**).

The remaining three options on the **Recording Control** page allow you to specify actions to be taken at the start and/or end of each recording.



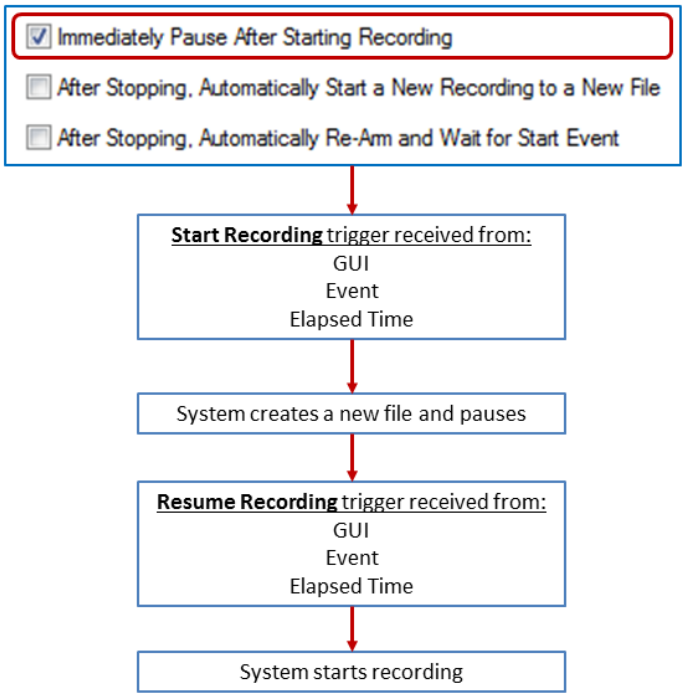
### 11.1.2 Timed and Event-triggered File Recording—Process Diagrams

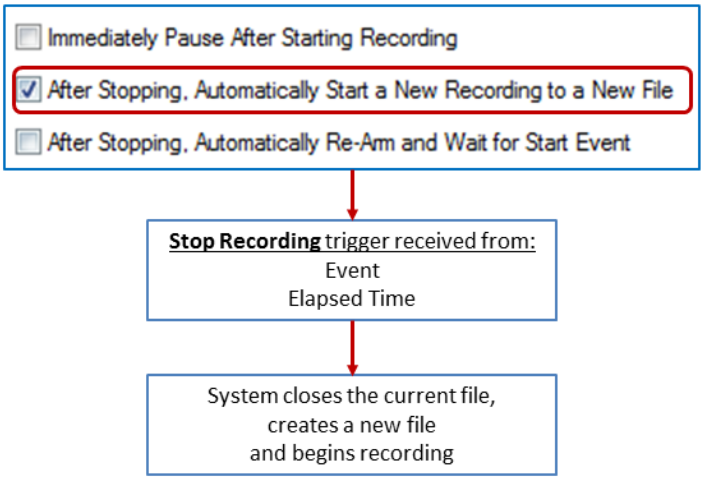
The diagrams that follow illustrate the effects of the **Immediately Pause...** and **After Stopping...** options on the **Recording Control** page of the **Global Options** dialog box.

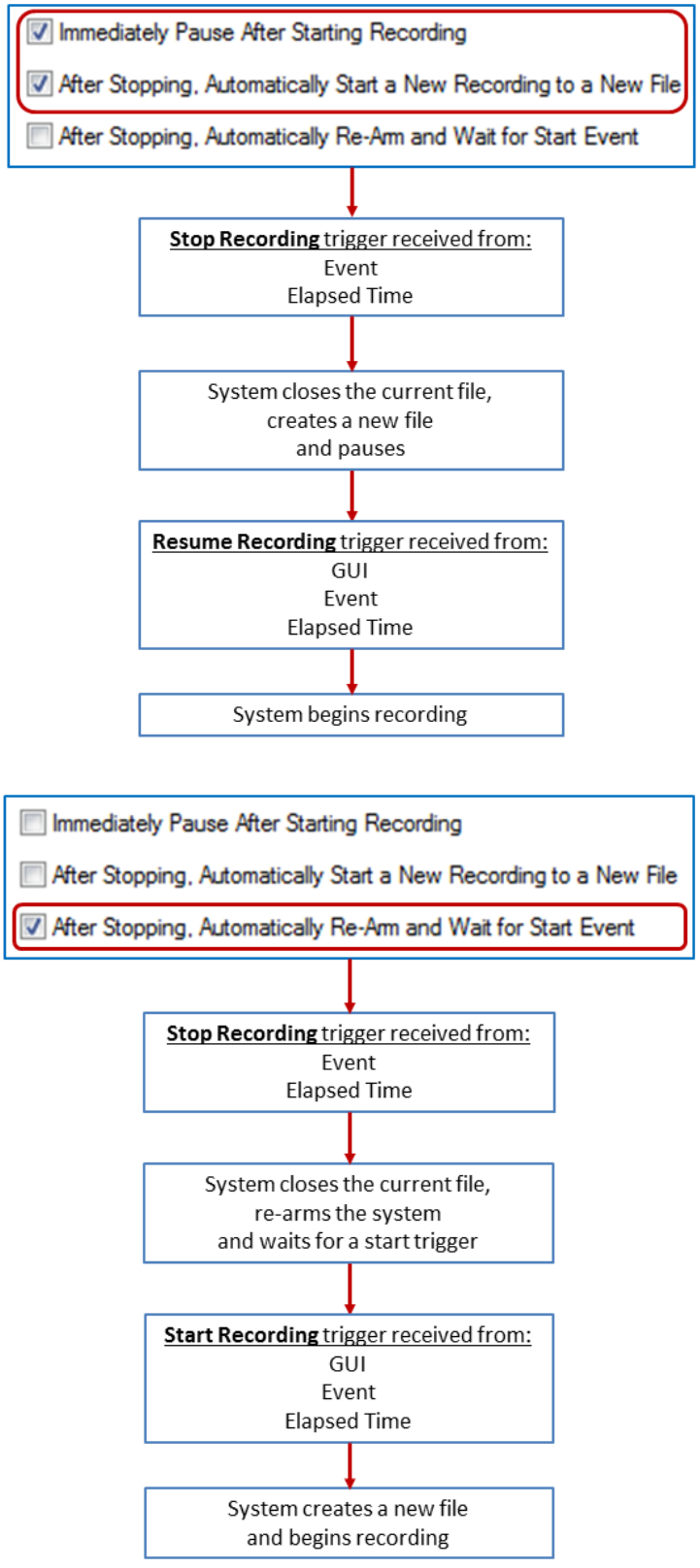
**TIP**

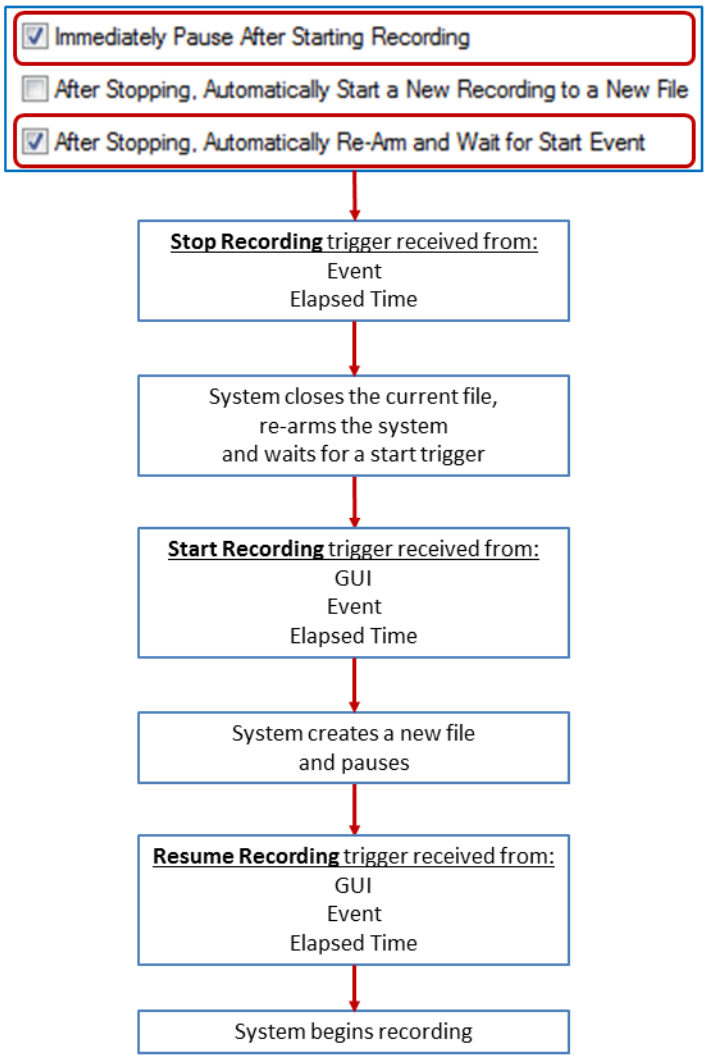
**Stop Recording button always means stop and no new file**

Manual stop (clicking the **Stop Recording** toolbar button or selecting the **Stop Recording** command from the **Data** menu) always causes the system to stop all recording activities completely and take no further action until you click the **Start Recording** button (or select the **Start Recording** command from the **Data** menu).

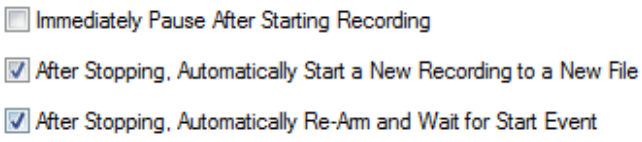



Immediately Pause After Starting Recording
After Stopping, Automatically Start a New Recording to a New File
After Stopping, Automatically Re-Arm and Wait for Start Event
Stop Recording trigger received from:
Event
Elapsed Time
System closes the current file,
creates a new file
and pauses
Resume Recording trigger received from:
GUI
Event
Elapsed Time
System begins recording
Immediately Pause After Starting Recording
After Stopping, Automatically Start a New Recording to a New File
After Stopping, Automatically Re-Arm and Wait for Start Event
Stop Recording trigger received from:
Event
Elapsed Time
System closes the current file,
re-arms the system
and waits for a start trigger
Start Recording trigger received from:
GUI
Event
Elapsed Time
System creates a new file
and begins recording




**Note:** The two "**After Stopping...**" options (shown below) are mutually exclusive. You should *not* select both of them. However, if you *do* select both, the option that the system performs is **After Stopping, Automatically Start a New Recording to a New File**.



**CAUTION**

**Make a test recording before gathering experimental data**

As with all recording, it is strongly recommended that you make a short test recording first, to be sure your settings are giving the desired overall behavior, before you begin actual experimental runs.

## 11.2 Examples—Recording a Single File with Pause/Resume Triggers

The previous section (Section 11.1, “Timed and Event-triggered File Recording” on page 340) explained how to access and configure the **Recording Control** page in the **Global Options** dialog box. The following examples show how the **Recording Control** features are typically used to record single files:

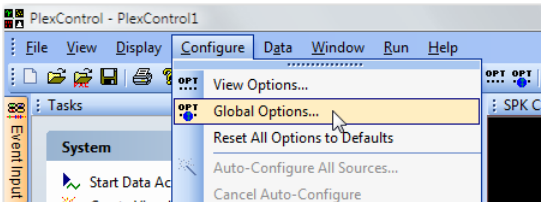
- Section 11.2.1, “Example 1: Single File, Manual Start with RSTART Level-triggered Recording” on page 346
- Section 11.2.2, “Example 2: Single File, Manual Start with Single-bit Events” on page 347
- Section 11.2.3, “Example 3: Single File with Multiple Frames, Keyboard Event Trigger” on page 348

Additional information on single file recording is provided in these sections:

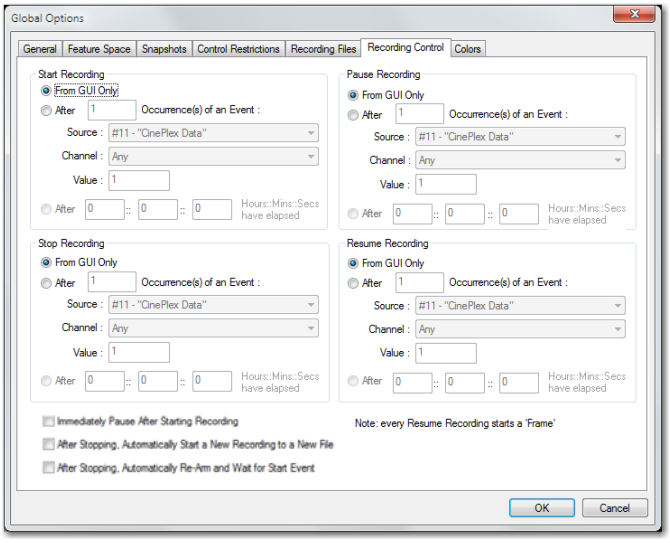
- Section 11.2.4, “Notes on Single File Pause/Resume Examples” on page 348
- Section 11.2.5, “Timing Considerations” on page 348

### Getting Started with Recording Control Options

The **Global Options** dialog is accessed from the **Configure** menu.




Clicking the **Recording Control** tab opens this page.



### 11.2.1 Example 1: Single File, Manual Start with RSTART Level-triggered Recording

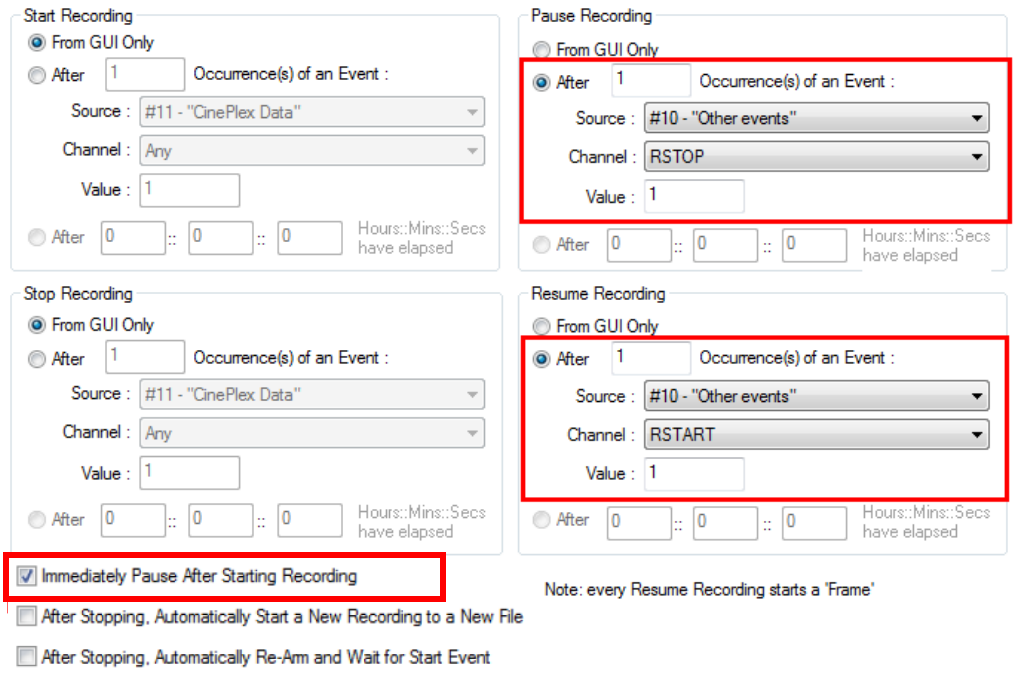
Here is an example of a common usage scenario, where we want the following behavior:

- Clicking the **Start Recording** toolbar button (or menu command) will cause the system to create a new recording file and then pause—it does not record any data yet.
- When the RSTART line on the Digital Input (DI) card sees a transition to a "true" level (by default, a rising edge, but this is configurable in the DI device options), actual recording of data will resume. Recording continues as long as RSTART remains in the true state.
- When the RSTART line sees a transition to a "false" level (by default, a falling edge, but this is also configurable), recording of data will pause, but the file will remain open for additional recording when RSTART goes "true" again.

  **Note:** For more information about RSTART, see Section 10.7, "Configuring RSTART and RSTOP Events" on page 334.

- Clicking the **Stop Recording** toolbar button (or menu command) will finalize the recording and close the file.

Here are the corresponding settings that should be used:

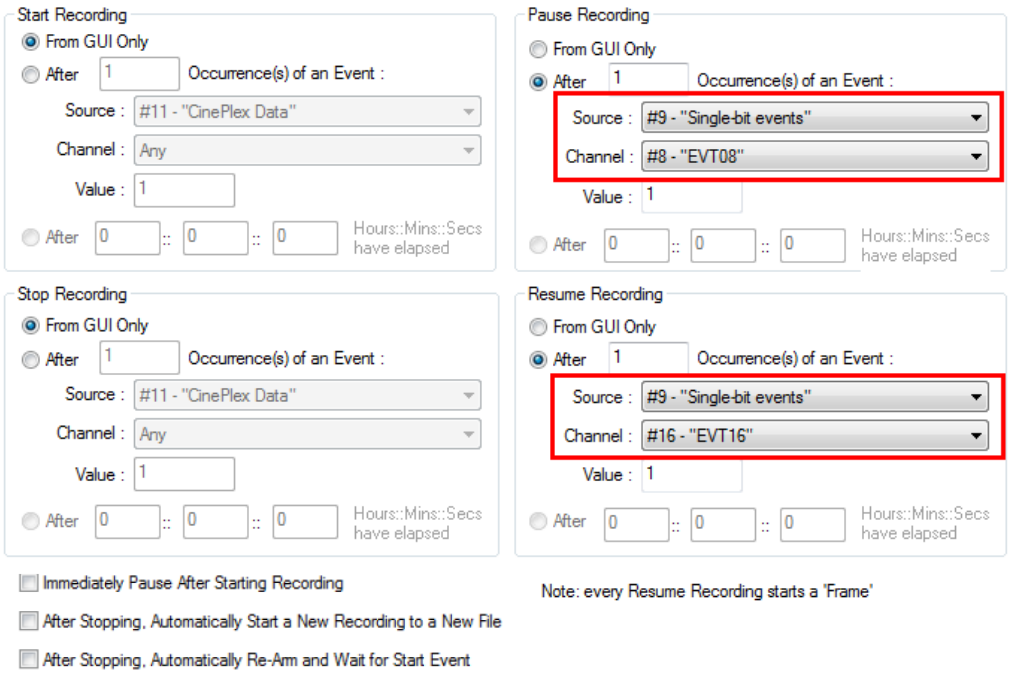



In the file, each frame of recorded data will be bracketed by Start and Stop events (event channels 258 and 259 respectively).

### 11.2.2 Example 2: Single File, Manual Start with Single-bit Events

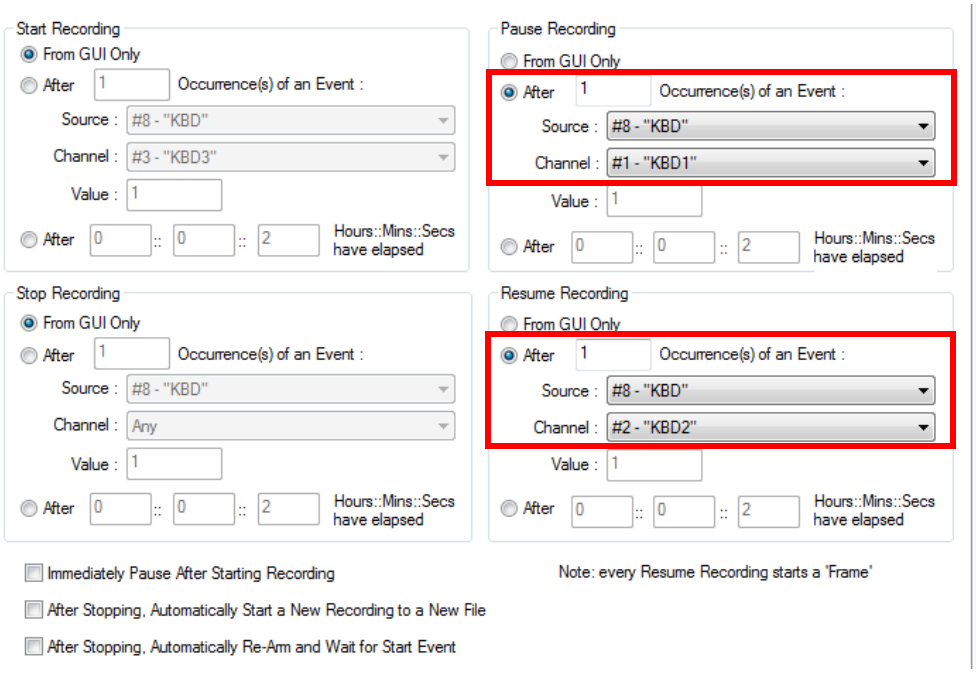
Here is a variation where instead of using level transitions on RSTART to pause and resume recording, we use single-bit events on two separate channels to pause and resume.

**Note:** In this example, we have *not* selected any of the options **Immediately Pause...** or **After Stopping...**



### 11.2.3 Example 3: Single File with Multiple Frames, Keyboard Event Trigger

In this example, we use keyboard events (Alt-1, Alt-2) to pause and resume recording:




### 11.2.4 Notes on Single File Pause/Resume Examples

When you use events to pause and resume, the effect is exactly as if you had clicked on the **Pause Recording** button in the toolbar to pause and resume recording.

When you click the **Stop Recording** button in the toolbar, the recording is ended and the file closed, regardless of whether the state of the recording is currently paused or not.

### 11.2.5 Timing Considerations

See Section 11.6, “Timing Considerations—Timed and Event-triggered Recording” on page 361 for important information about time durations required by the system for starting new files and resuming in-progress files that are currently paused.

## 11.3 Understanding Multiple File Recording

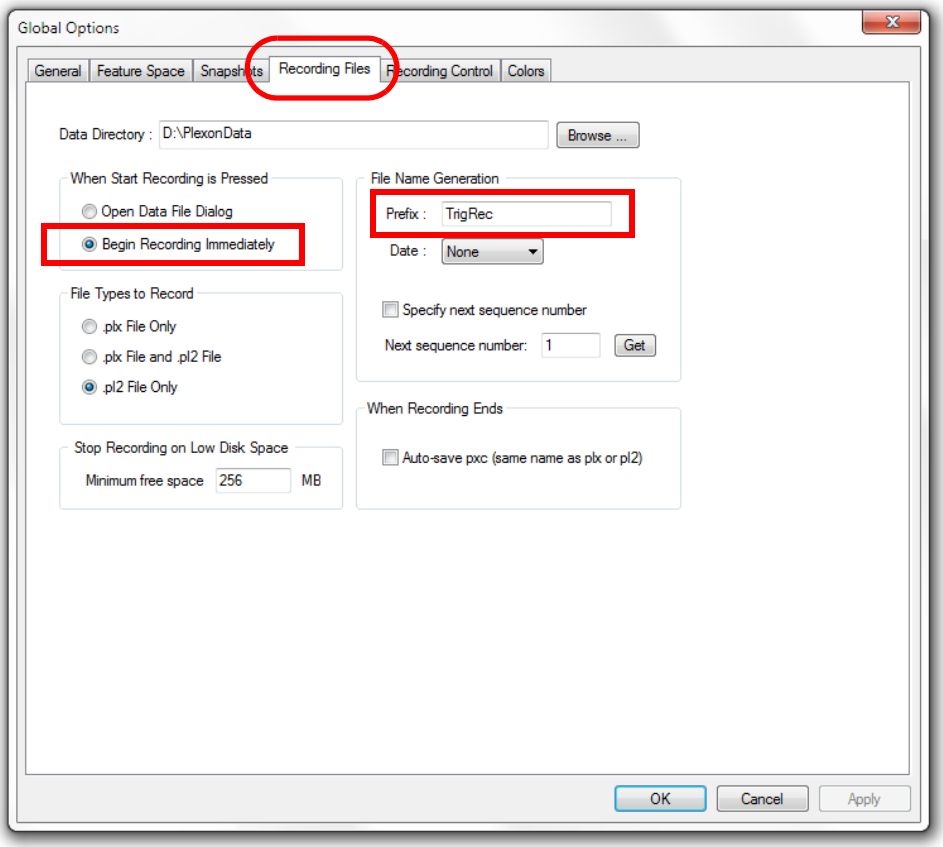
You can use options on the **Recording Control** page of the **Global Options** dialog to create multiple file recordings automatically. With these options, the system can record multiple plx or pl2 files in sequence, based on an elapsed time or triggered by a digital or keyboard event. You can direct the system to perform either of the following functions after the previous file is closed (stopped):

- Automatically start file creation and data recording process for the next file
- Automatically re-arm the system then wait for a Start event before creating the next file

You can also direct the system to immediately pause after it creates a new recording file.

### 11.3.1 Generating File Names

When the system is creating multiple-file recordings, PlexControl can automatically generate a filename for each recording file using the options that you have set on the **Recording Files** page in the **Global Options** dialog:




In the above example, the recording files will be named TrigRec001, TrigRec002, TrigRec003, etc. Filenames will always continue at one greater than the highest numbered file that is present in the recording folder; for example, if one series of

recordings ended with TrigRec078, the next time you click Start Recording, the next file to be recorded will be TrigRec079. You can use the **Specify next sequence number** option to override this default behavior.

**Note:** Make sure that the above options are set as desired before clicking **Start Recording**. Otherwise, you will have to specify the name of each file manually.

**Note:** Note that the **Auto-save pxc** option (and the corresponding **Auto-save pxs** option in Server) cannot be used in timed or triggered multiple-file mode. In such cases, you should manually save the pxc/pxs before starting the timed/ triggered sequence of recordings.

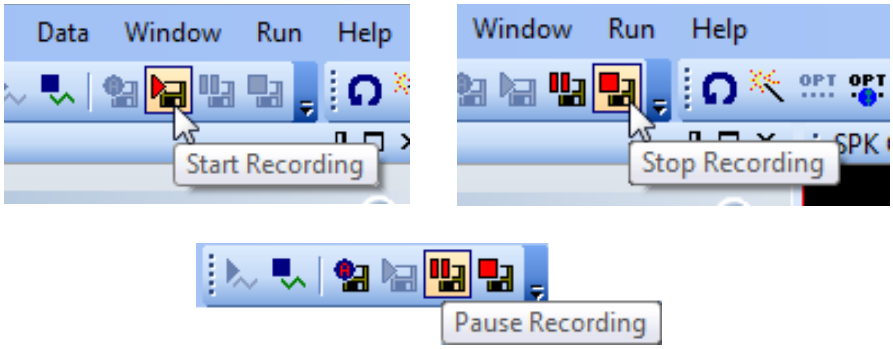
### 11.3.2 Understanding Manual and Event-triggered Recording Options

#### Using the Manual Controls (Toolbar Buttons)

When you click the **Start Recording** button in the toolbar (or click the **Start Recording** command in the **Data** menu), the system creates a file and is ready to record data. Depending on other parameters that you can set on the **Recording Control** page of the **Global Configuration** dialog box, data recording begins immediately or begins after a specified trigger.

When recording is occurring, if you click the **Pause Recording** button in the toolbar (or click the **Pause Recording** command in the **Data** menu), the recording is paused. Clicking on **Pause Recording** again causes the recording to resume.

When you click the **Stop Recording** button in the toolbar (or click the **Stop Recording** command in the **Data** menu), the recording is ended and the file closed, regardless of whether the state of the recording is currently paused or not.




#### Using the Recording Control Options (Events and Elapsed Time)

When you use the options on the **Recording Control** page to start recording, the effect is exactly as if you had clicked on the **Start Recording** button in the toolbar (or clicked the **Start Recording** command in the **Data** menu) to begin recording.

When you use the **Recording Control** options to pause and resume recording, the effect is exactly as if you had clicked on the **Pause Recording** button in the toolbar (or clicked the **Pause Recording** command in the **Data** menu) to pause and resume recording.

However, when you use the **Recording Control** options to stop the recording, the effect is not necessarily the same as if you had clicked on the **Stop Recording** button in the toolbar (or clicked the **Stop Recording** command in the **Data** menu) to stop recording:

- Stopping the recording with the **Recording Control** options allows the system to start new recordings automatically (with events or elapsed time) in the future, if you have configured the system to start recordings automatically.

- Stopping the recording with the manual controls (**Stop Recording** command in the **Data** menu), causes the system to stop all recording activities (until you click the **Start Recording** button or select the **Start Recording** command again).

### Using the Recording Control Options to Create Multiple Recordings

You can use the Events and Elapsed Time options on the **Recording Control** page to make multiple recordings automatically. After you see that the system has completed the desired recordings, click the **Stop Recording** button in the GUI, and the system will end the series of recordings and stop all recording activities.

**TIP**

**Stop Recording button always means stop and no new file**

Manual stop (clicking the **Stop Recording** toolbar button or selecting the **Stop Recording** command from the **Data** menu) always causes the system to stop all recording activities completely and take no further action until you click the **Start Recording** button (or select the **Start Recording** command from the **Data** menu).

## 11.4 Examples—Multiple File Recording

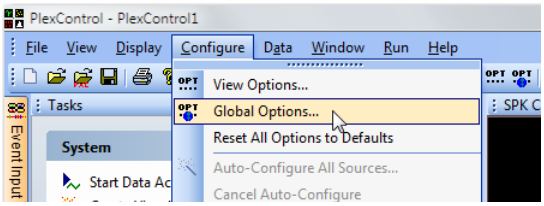
The following examples show how the **Recording Control** features are typically used to record multiple files:

- Section 11.4.1, “Example 1: Multiple Files, Manual Start and Single-bit Event Trigger” on page 354
- Section 11.4.2, “Example 2: Multiple Files, Manual Start and Keyboard Event Trigger” on page 355
- Section 11.4.3, “Example 3: Multiple Files, Manual Start, Specified File Duration” on page 356
- Section 11.4.4, “Example 4: Multiple Files with Multiple Frames Controlled by Single-bit Events” on page 357

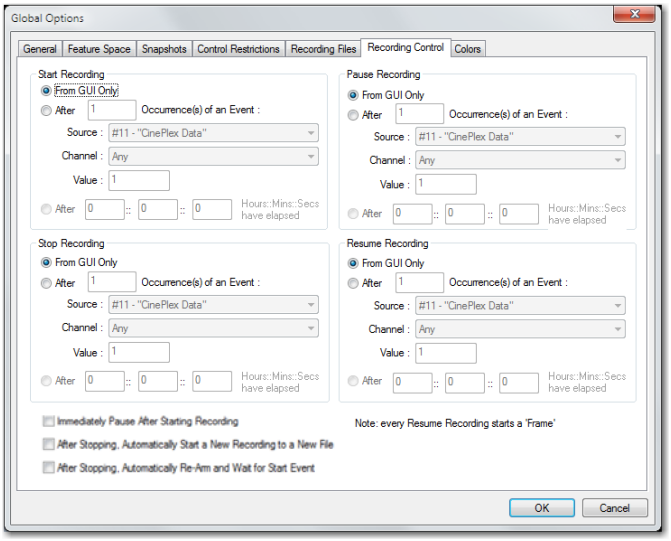
**Note:** See “Timing Considerations—Timed and Event-triggered Recording” on page 11-361 for important information about time durations required by the system for starting new files and resuming in-progress files that are currently paused.

### Getting Started with Recording Control Options

The **Global Options** dialog is accessed from the **Configure** menu.



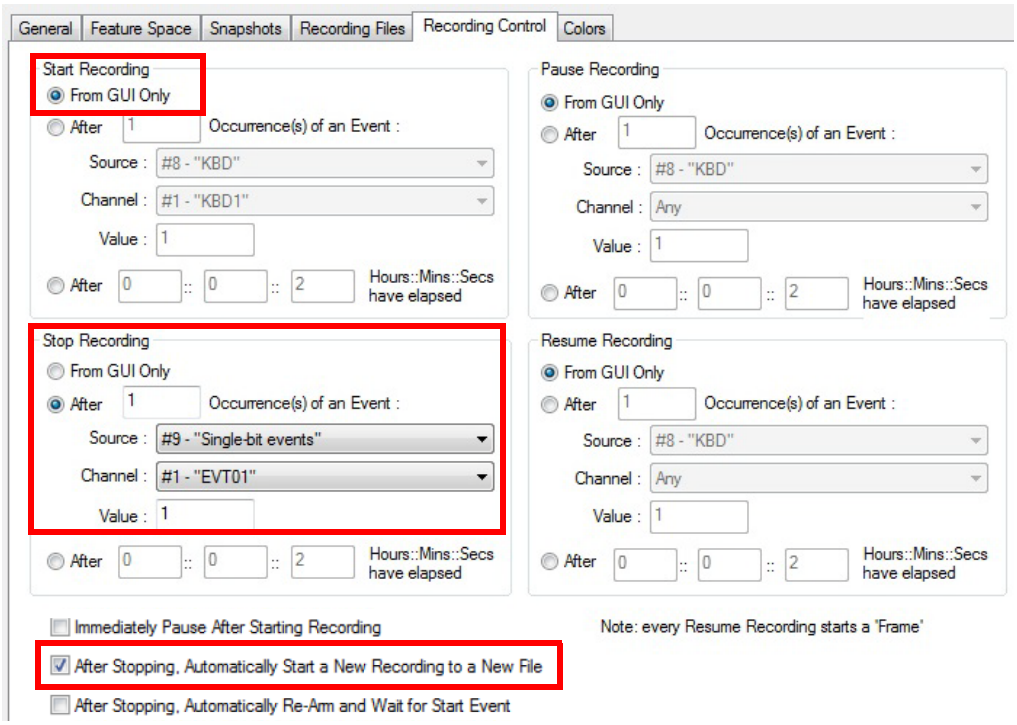

Clicking the **Recording Control** tab opens this page.



### 11.4.1 Example 1: Multiple Files, Manual Start and Single-bit Event Trigger

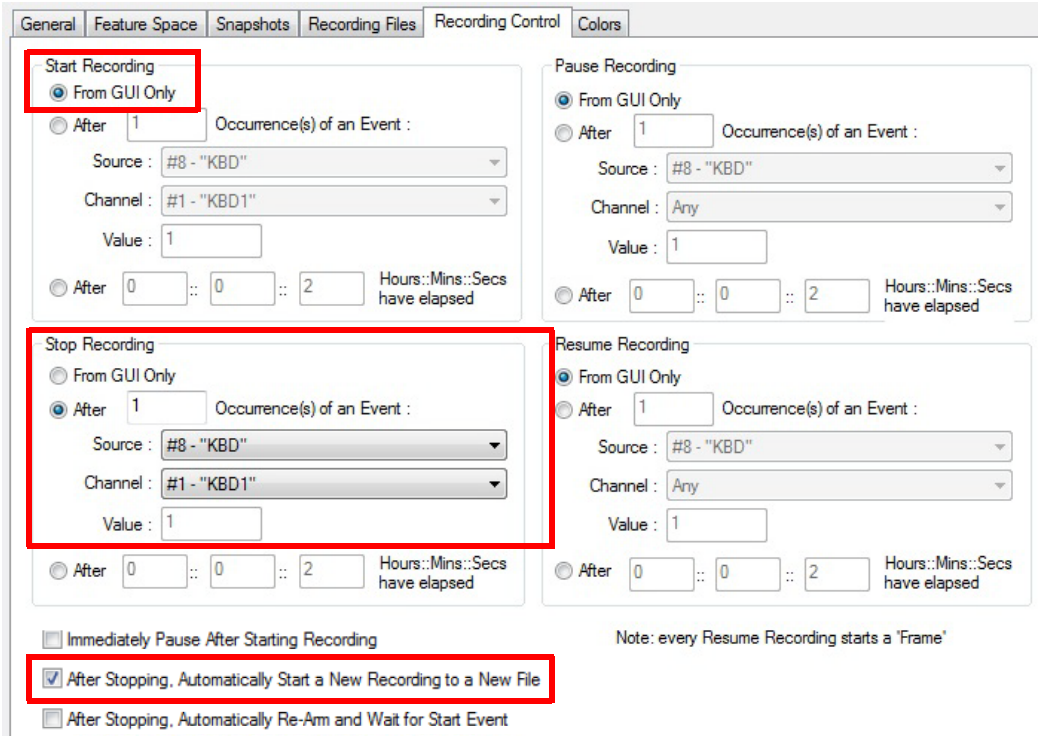
Here is an example for a common usage scenario, where we want the following behavior:

- Clicking the **Start Recording** toolbar button will create a new file and start recording data
- Each time the single-bit digital event EVT01 is received, the current recording file is closed and a new file starts recording.
- Clicking the **Stop Recording** toolbar button will end all recording activity and close the last file.



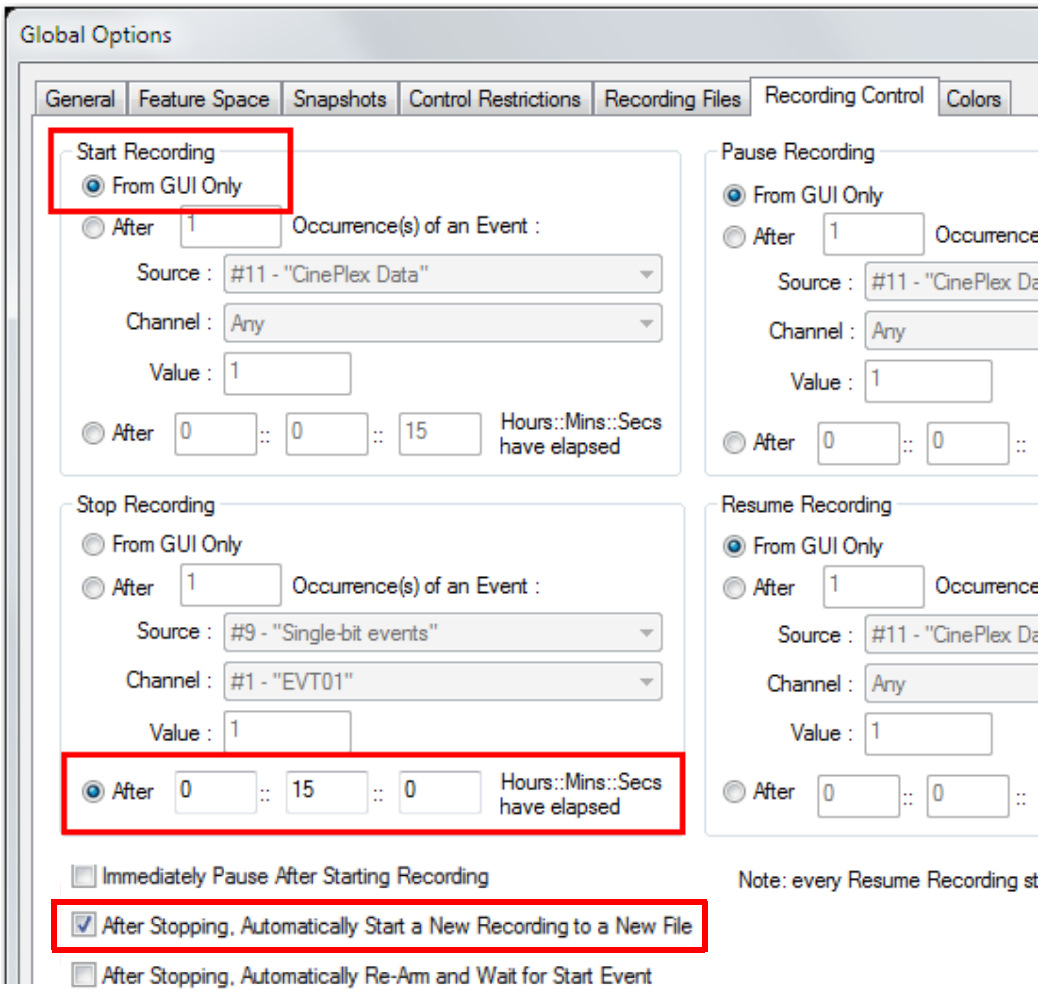
### 11.4.2 Example 2: Multiple Files, Manual Start and Keyboard Event Trigger

In this example, we use a keyboard event (Alt-1) to stop recording. When the keyboard event is received, the system closes the current file and automatically starts a new recording to a new file:



### 11.4.3 Example 3: Multiple Files, Manual Start, Specified File Duration

Here is an example of a how to enable recording of multiple files of a specified duration. In this example, you start the first recording manually by clicking the **Start Recording** button in the toolbar or selecting the **Start Recording** command in the **Data** menu. After 15 minutes of recording, the system stops recording and closes the current file. Then it automatically starts a new recording to a new file.

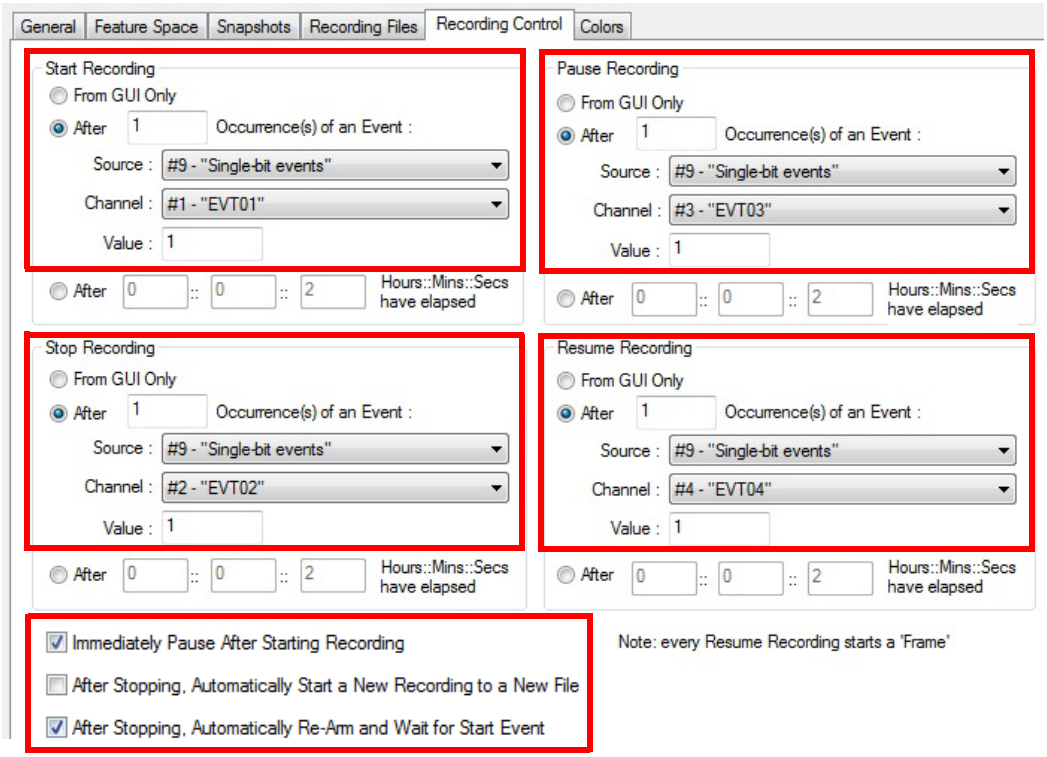


### 11.4.4 Example 4: Multiple Files with Multiple Frames Controlled by Single-bit Events

Here is an example of a how to enable recording of multiple files using four single-bit events to control the start/stop/pause/resume functions.

You must first click the **Arm Recording** button in the main toolbar , after which all recording is controlled by single-bit events. After you have armed the system, recording proceeds as follows:

1. The system waits for an EVT01 event, then creates a new recording file but remains paused.
2. The system waits for an EVT04 event, which begins the actual recording of data.
3. When the system receives an EVT03 event, it pauses the recording.
4. Each EVT04/EVT03 sequence of events causes one frame of data to be recorded in the file, just as with RSTART/RSTOP.
5. When the system receives an EVT02 event, it closes the file. The system then re-arms and waits for another EVT01 event.
6. When the next EVT01 event is received, the system creates a new file but remains paused.



## 11.5 Ensuring Keyboard Events Are Active

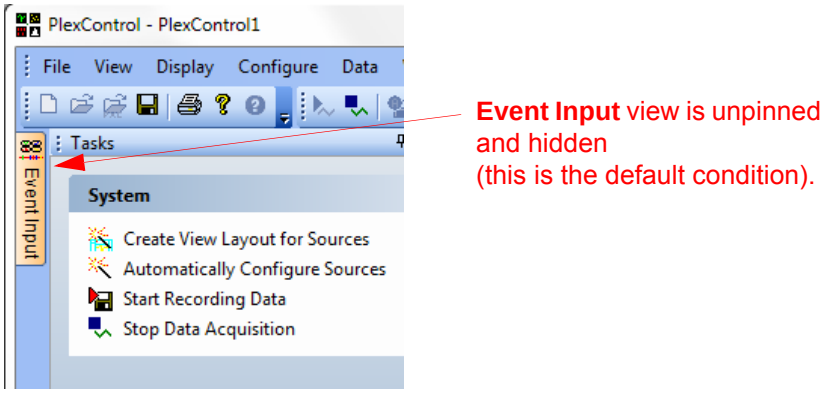
When you are ready to trigger an event by means of a keyboard event, ensure the **Event Input** view is visible. If it is *not* visible, it is *not* active, and your keyboard events will not be detected.

| | |
|---|---|
| | **CAUTION**<br>**Closing the Event Input view disables keyboard events**<br>If you close the **Event Input** view, keyboard events will *not* be detected. If you want to use keyboard events, see the information in this section and ensure that the keyboard events are enabled. |

There are several ways to ensure that the **Event Input** view is visible in some form—either pinned, unpinned or tabbed—and therefore, that keyboard events are enabled:

- If the **Event Input** view is unpinned and minimized (hidden), the **Event Input** function is active and the keyboard event function is enabled.

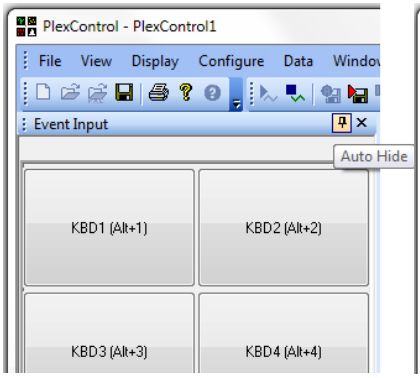
Event Input view is unpinned and hidden—
Keyboard events are enabled:



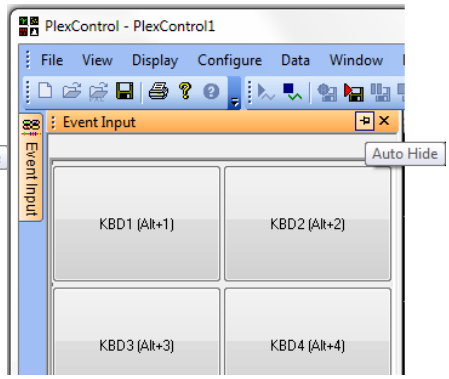
- If the Event Input view is open (pinned or unpinned), keyboard events are enabled. You can click on the toolbar pushpin once to pin it, then again to unpin and minimize it.

Event Input view is open—Keyboard events are enabled:

Open (pinned)

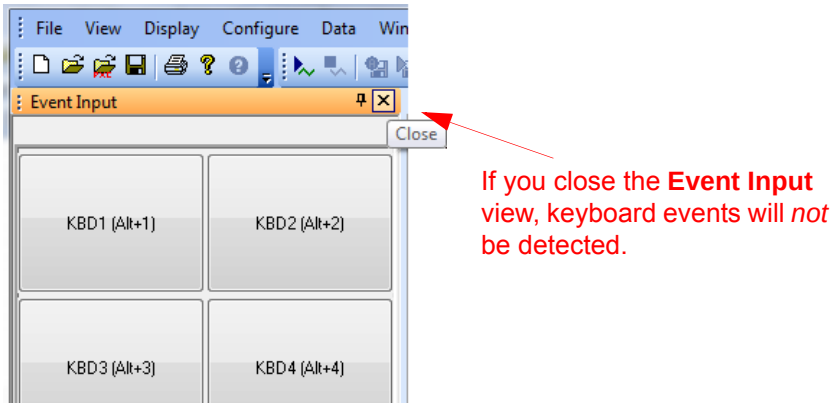



Open (unpinned)



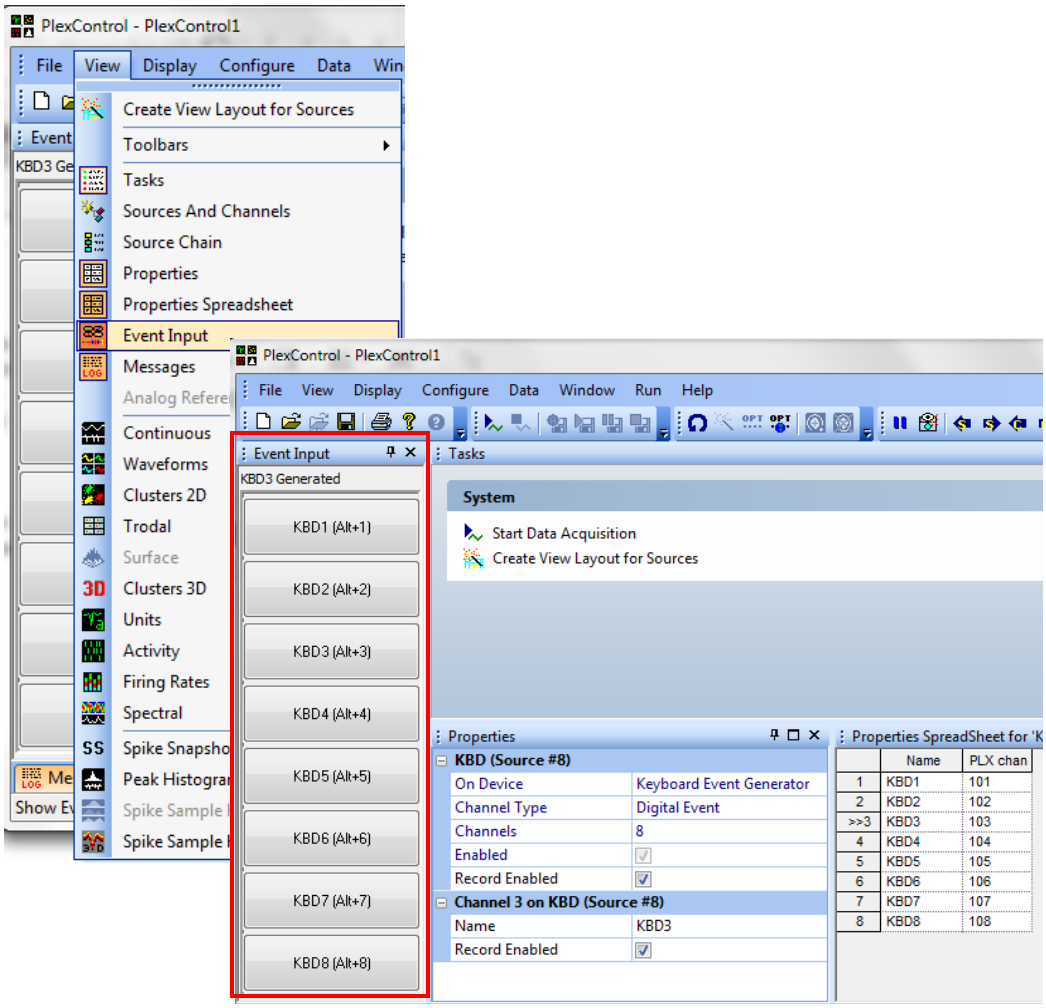

- If you close the **Event Input** view by clicking the "x" in the upper right corner of the view, keyboard events will *not* be detected.

Clicking the "x" will close the Event Input view and disable keyboard events:



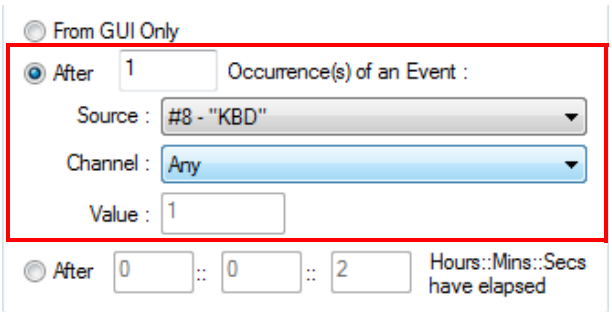

**Note:** You can re-enable the keyboard event function as described below.

To open the **Event Input** view (if it is currently closed), select it from the **View** menu, as shown below. This will ensure that the system can detect keyboard events.




Trigger keyboard events, when desired, by pressing the appropriate keyboard key or by clicking on the appropriate button in the **Event Input** view (above).

Be sure that you have specified the action the system should take for each keyboard event on the **Recording Control** page of the **Global Options** dialog:



## 11.6 Timing Considerations—Timed and Event-triggered Recording

The timed/triggered recording feature is not intended to be used to record very short files. The shortest allowed file duration is approximately two seconds, and attempts to trigger recording more frequently will be ignored by PlexControl, until at least two seconds have elapsed.

You should expect a gap of approximately 1-2 seconds between each pair of recorded files, which should not be a problem for recordings of typical durations. Timestamping will still be correct within each file, but the brief interval of data that occurred between the closing of one file and the opening of the next will not be recorded. In situations where you require short, precisely timed recordings, for example recording exactly two seconds of data each time an experimental stimulus is applied, you should instead record a single longer file and use digital events recorded in the file as markers for the intervals of interest.

Similar considerations are true for pausing and resuming recording in any file. When you use event-triggered recording, there is a small amount of delay between when the RSTART/RSTOP occurs and when recording actually starts and stops. This delay is typically around 100 ms, but can be longer in some cases; to be safe, you should allow at least one second between an RSTOP and the next RSTART. If you need to record very short trials (say, less than 10 seconds each), then instead of using RSTART/RSTOP to start and stop recording, use the following technique: Record the entire series of trials, including the "dead time" between trials, continuously without stopping, but use digital events (e.g. EVT001 and EVT002) to mark the exact start and end of each trial within the recording. You can then use offline tools (such as NeuroExplorer® interval features) to extract the data corresponding to each precisely event-bracketed trial.

As with all recording, it is strongly recommended that you make a short test recording to confirm that event-triggered recording is working as expected, before proceeding to recording important experiment data.

# 11 Triggered Recording

# Chapter 12
# Auxiliary Analog Input

12.1 Auxiliary Analog Input (AuxAI) ..... 364
12.2 5 kHz, 10 kHz and 20 kHz Maximum Sampling Rates ..... 366
12.3 250kHz Maximum Sampling Rate ..... 371
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## 12.1 Auxiliary Analog Input (AuxAI)

The Auxiliary Analog Input (AuxAI) device is a multichannel A/D card in the OmniPlex System chassis which is provided for direct acquisition of non-neural, low-frequency signals (250kHz or less) such as those output by position or orientation sensors. By "direct," we mean that the OmniPlex System provides no preamplification or other analog signal conditioning for these inputs.

The AuxAI chassis cards are shown in the photo in Section 12.2, "5 kHz, 10 kHz and 20 kHz Maximum Sampling Rates" on page 366.

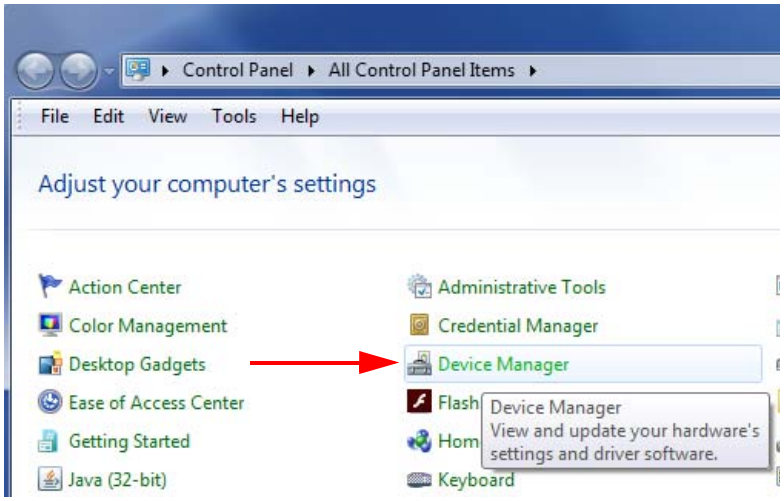
There are several types of AuxAI cards:

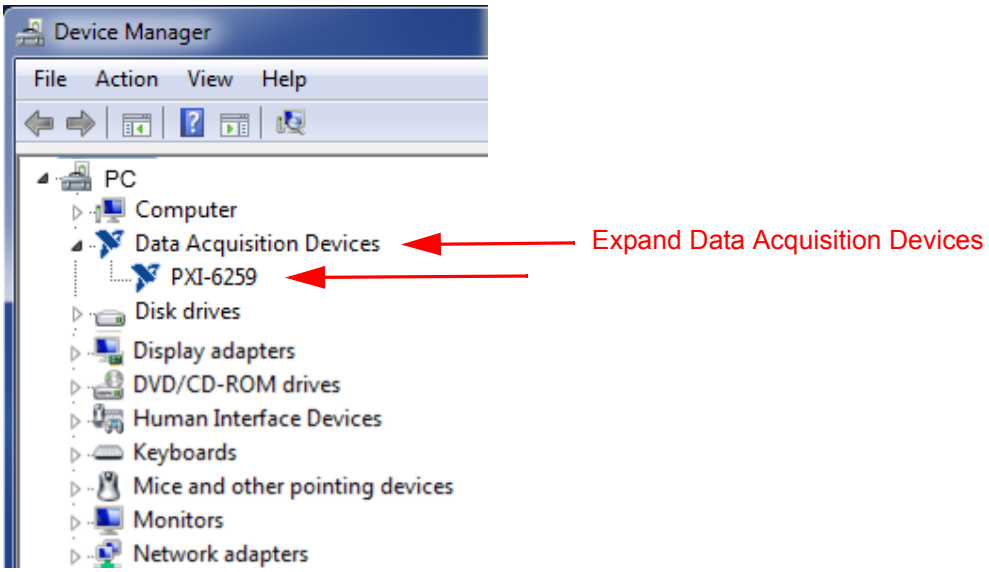
- The standard AuxAI module, suitable for sampling rates up to 10 kHz maximum, is the 16 channel PXI-6341.

  **Note:** (Some systems use the 5 kHz 32 channel PXI-6224.)

- The "fast" AuxAI module, suitable for sampling rates up to 250 kHz maximum, is PXI-6259.

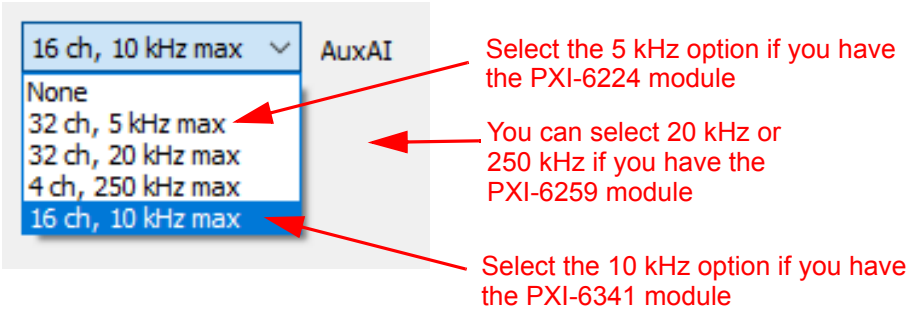
If you are unsure whether you have a standard or "fast" card, you can determine this in the Device Manager of your PC:





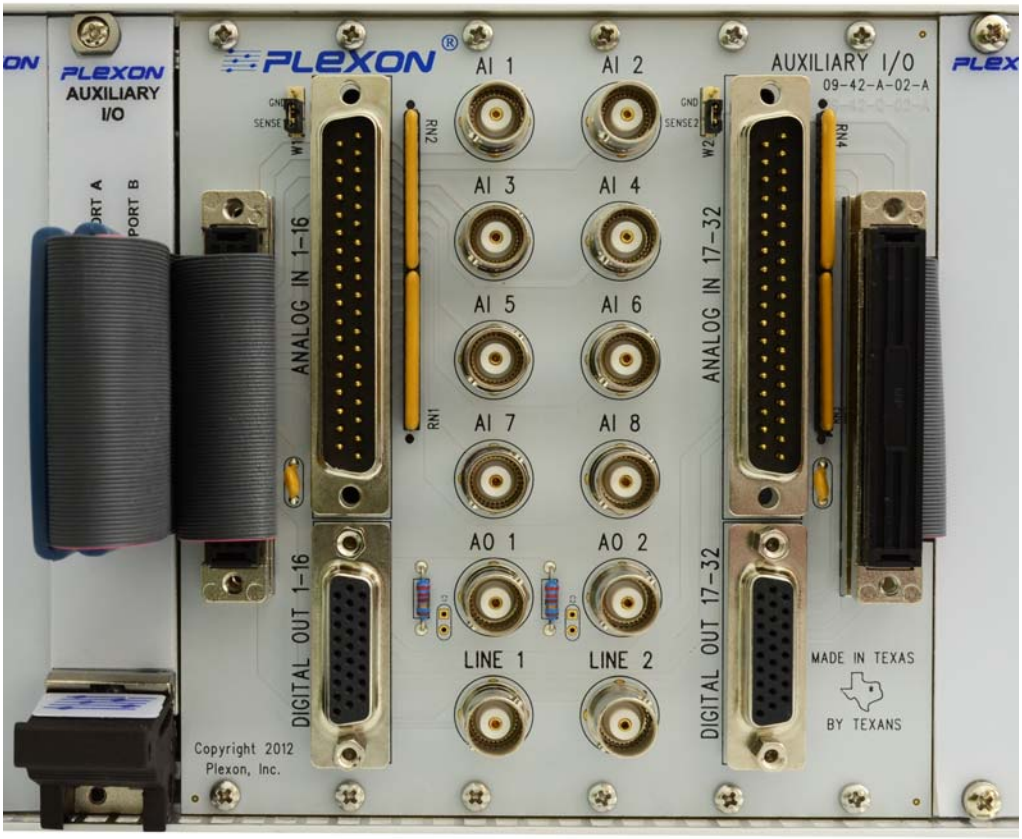

If you need additional assistance, contact Plexon® support.

The supported maximum sampling rates are shown in the Topology Wizard dropdown list:



## 12.2 5 kHz, 10 kHz and 20 kHz Maximum Sampling Rates

The image below shows the AuxAI card.




### 16 channel card

Maximum sampling rates of up to 10 kHz per channel are supported for the PXI-6341 AuxAI card, but the default rate is 1 kHz, the same rate that is used for digitizing field potentials. You can use all 16 channels at these sampling rates. Note that the 16 inputs are sampled in multiplexed fashion within each sampling period, unlike the simultaneous sampling implemented in the DigiAmp Amplifier, MiniDigi Amplifier and DHP unit. However, the same master clock (from the TIM or TIMe card) is used to drive both devices, as well as the digital input (DI, DIN or DINe) card, ensuring synchronized sampling across all devices.

Easy access to the 16 input channels is provided via a BNC / D-sub breakout panel located immediately to the right of the AuxAI card in the chassis.

### 32 channel cards

Maximum sampling rates of up to 5 kHz per channel for the PXI-6224 card, or 20 kHz per channel for the "fast" version of the AuxAI card (PXI-6259), are supported, but the default rate is 1 kHz, the same rate that is used for digitizing field potentials. You can use all 32 channels at these sampling rates. Note that the

32 inputs are sampled in multiplexed fashion within each sampling period, unlike the simultaneous sampling implemented in the DigiAmp Amplifier, MiniDigi Amplifier and DHP unit. However, the same master clock (from the TIM or TIMe card) is used to drive both devices, as well as the digital input (DI, DIN or DINe) card, ensuring synchronized sampling across all devices.

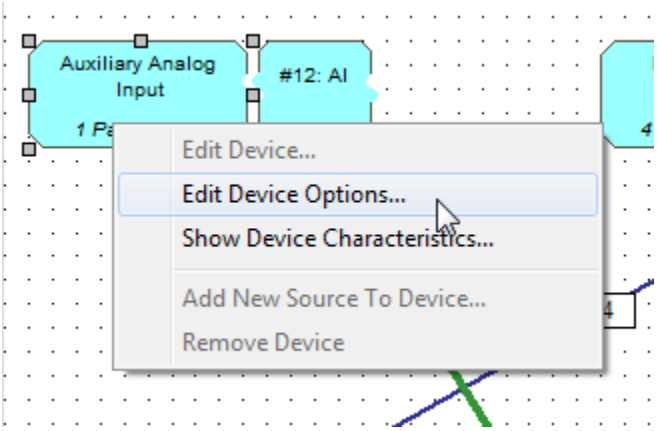
Easy access to the 32 input channels is provided via a BNC / D-sub breakout panel located immediately to the right of the AuxAI card in the chassis.

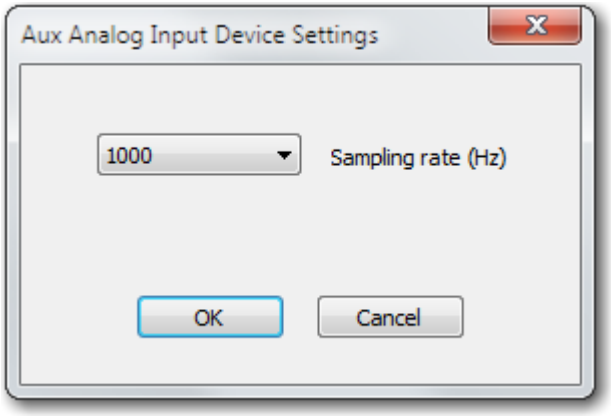
### Input range

The input range of the AuxAI channels is a fixed +/- 5V, with 16 bit resolution. Even for an input signal with an amplitude of only +/- 0.5V, the signal will still be digitized to better than 12 bits of resolution. For applications requiring a greater input range than 10V peak-to-peak, a voltage divider can be inserted between the external device and the AI inputs.

See Appendix J: Hardware Pinouts, Connections and LEDs for details on connecting to the AuxAI breakout panel.

Continuous data from the AuxAI card is an independent source in the Server topology, and data for the AI source is written directly to the Main Datapool without any signal processing. The only device option for the AuxAI card is the per-channel sampling rate. You can access the **Aux Analog Input Device Settings** dialog by right clicking in the appropriate box in Server and selecting **Edit Device Options...**.

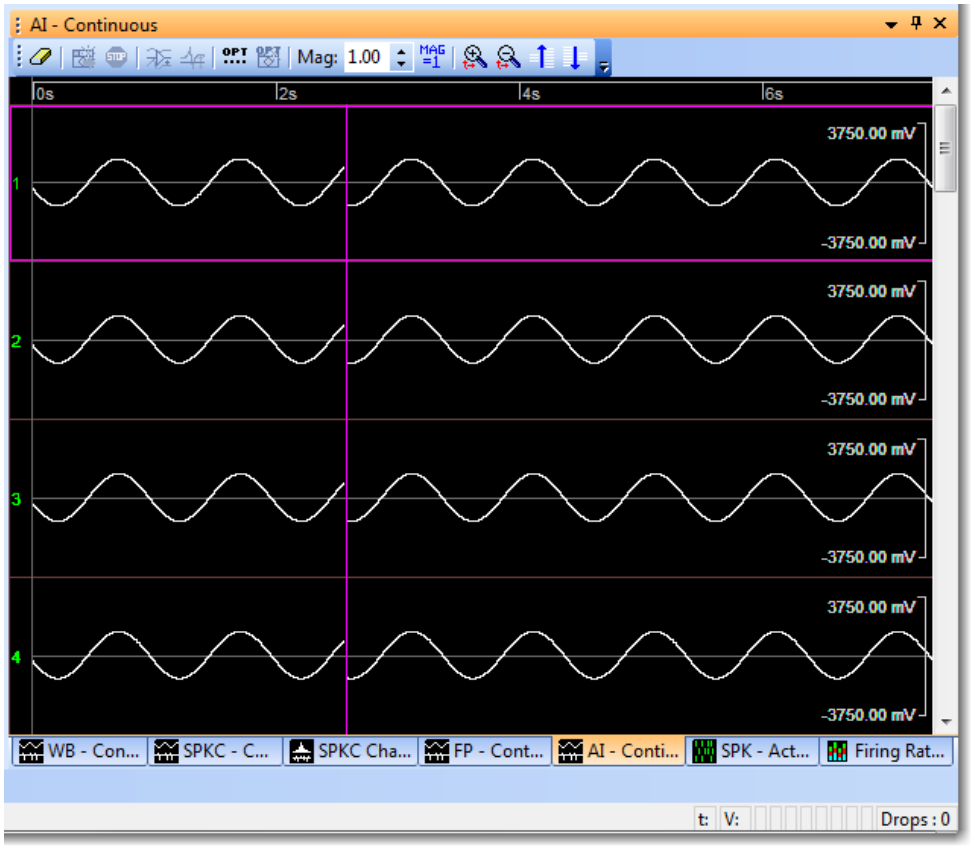





Note that you must stop data acquisition before changing the sampling rate.

After you restart data acquisition, the system will apply the adjusted sampling rate value to all AuxAI channels. When you save the .pxs (topology) file in Server, or the .pxc file in PlexControl, the current settings of the SPKC and FP separators are also saved in the file.

AI channels are shown in their own tab in PlexControl, labeled **AI - Continuous**.



The sweep rate, magnification, and number of channels can be adjusted, as with the other continuous views, but none of the gain control or thresholding functionality is applicable. However, you can use the Properties Spreadsheet (with the AI source selected) to enable and disable recording of individual channels:

Properties SpreadSheet for 'AI'

| | Name | PLX chan | Enabled | Record |
|---|---|---|---|---|
| >>1 | AI01 | 193 | ☒ | ☒ |
| 2 | AI02 | 194 | ☒ | ☒ |
| 3 | AI03 | 195 | ☒ | ☒ |
| 4 | AI04 | 196 | ☒ | ☒ |
| 5 | AI05 | 197 | ☒ | ☒ |
| 6 | AI06 | 198 | ☒ | ☒ |
| 7 | AI07 | 199 | ☒ | ☒ |
| 8 | AI08 | 200 | ☒ | ☒ |
| 9 | AI09 | 201 | ☒ | ☒ |
| 10 | AI10 | 202 | ☒ | ☒ |
| 11 | AI11 | 203 | ☒ | ☒ |
| 12 | AI12 | 204 | ☒ | ☒ |
| 13 | AI13 | 205 | ☒ | ☒ |
| 14 | AI14 | 206 | ☒ | ☒ |
| 15 | AI15 | 207 | ☒ | ☒ |
| 16 | AI16 | 208 | ☒ | ☒ |
| 17 | AI17 | 209 | ☒ | ☒ |
| 18 | AI18 | 210 | ☒ | ☒ |

**TIP**

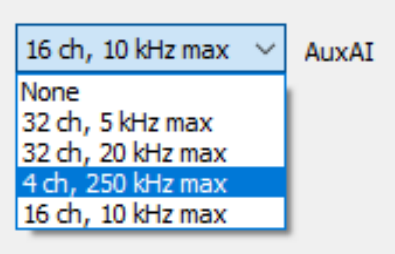
**Loading a .pxc file with a different sampling rate**

If you attempt to load a .pxc file which was saved with a different AuxAI sampling rate than the rate currently in effect in Server, the sampling rate loaded from the .pxc will override the Server rate.

## 12.3 250kHz Maximum Sampling Rate

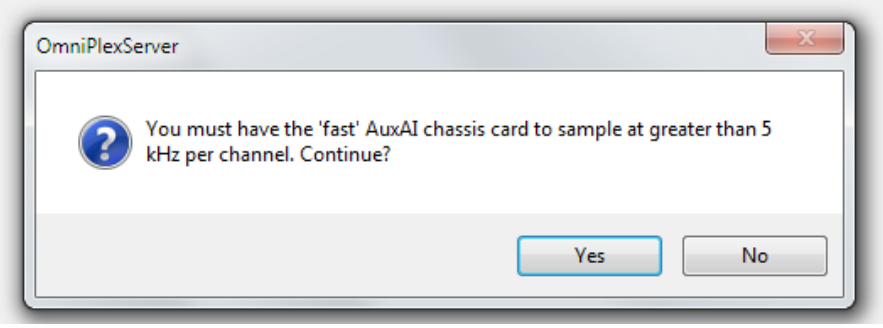
Sampling rates of up to 250 kHz per channel are supported with the “fast” AuxAI card, with a maximum of four channels. This is useful for researchers who wish to record high-frequency audio or other high-frequency experimental data. Note that since the AuxAI device is intended for digitization of non-neural auxiliary signals, the digitized signals cannot be thresholded or spike sorted, and no digital filtering or other processing is currently supported on AuxAI channels.

To enable sampling rates greater than 20 kHz per channel, you must create a topology (pxs) with the appropriate AuxAI option using the Topology Wizard in Server. In the Topology Wizard **AuxAI** dropdown control, select **4 ch, 250 kHz max**:




This allows the AuxAI card to be used at digitizing rates of up to 250 kHz per channel, but at these rates, a maximum of four channels (AI1 – AI4) can be used.

After selecting the 250 kHz max option, a message reminds you that you must have the “fast” version of the AuxAI card for rates greater than 5 kHz per channel:

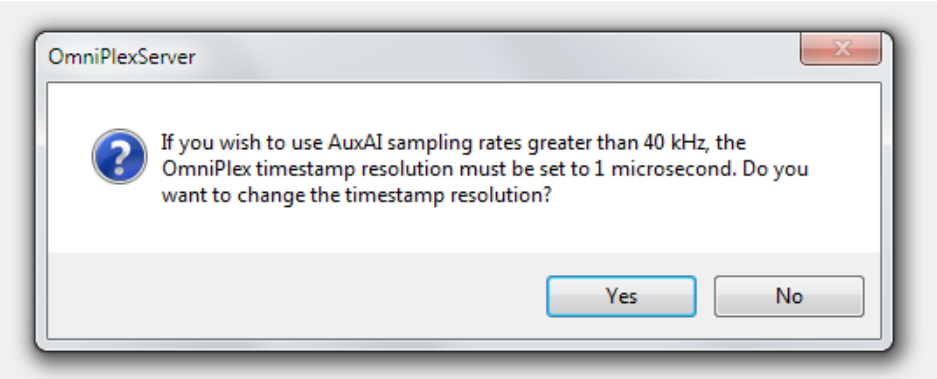



If you are not sure which version of the AuxAI card you have, use the method provided in Section 12.1, “Auxiliary Analog Input (AuxAI)” on page 364 to identify your card.

If you wish to upgrade from the standard AuxAI card to a faster card, please contact support@plexon.com for assistance.

Digitizing rates greater than 40 kHz require that the OmniPlex® timestamp resolution be set to one microsecond, rather than the default 25 microseconds. If

the timestamp resolution is not currently set to one microsecond, the following message will ask whether it should be adjusted:




Select **Yes** to set the timestamp resolution to one microsecond.

Timestamp resolution is explained in more detail in the next section.

## 12.4 Timestamp Resolution

Timestamp resolution refers to the smallest time increments, or "ticks" in which timestamps are stored in recording files and in data that is sent to online client programs. Both in the OmniPlex System and in applications such as Offline Sorter™ (OFS) software and NeuroExplorer® software, the timestamps are displayed in terms of seconds, fractions of a second, microseconds, etc, and the underlying "tick" resolution is of little or no concern.

**Note:** If you do not work with raw integer timestamp values, for example in client programs or custom code that directly reads blocks of data from a plx file, you can safely skip over this section.

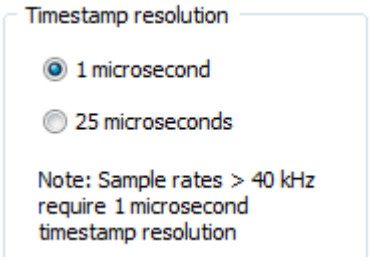
The default timestamp resolution in Plexon systems is 25 microseconds, i.e. 40 kHz. This is not the same as the digitizing rate, but the maximum digitizing rate cannot exceed the timestamp resolution.

The default timestamp resolution of 40 kHz means that in the raw integer timestamps stored in a file or sent to clients, two timestamps that differ by 40,000 represent a time difference of one second. Successive samples on a wideband (WB) or spike-continuous (SPKC) channel will typically have timestamps that increment by one tick for each sample, since in this case the 40 kHz wideband digitizing rate coincides with the 40 kHz timestamp resolution.

By comparison, successive samples from an FP or AuxAI channel, at their default 1 kHz digitizing rate, will differ by 40 ticks. This is because 40 ticks * 25 microseconds = 1000 microseconds = 1 millisecond, and 1 second / 1 millisecond = 1 kHz. Another way of looking at this is to consider that the FP or AuxAI channel is being digitized at 1/40 the rate of a wideband channel, so successive timestamps are 40 ticks apart rather than 1 tick apart. By the same logic, a channel that is digitized at 10 kHz would have timestamps that increment by four ticks from one sample to the next.

It is important to remember the distinction between the digitizing rate and the timestamp resolution. The timestamp resolution is simply the scaling factor that converts raw integer timestamps into floating point timestamps in seconds, or vice versa. Different sources, e.g. WB versus FP, may have different digitizing rates, but they will represent their timestamps in terms of the same timestamp resolution. The timestamp resolution is a single system-wide property, whereas each source can have its own digitizing rate.

In the context of the above discussion, it should be clear why the default timestamp resolution of 40 kHz is inadequate for digitizing rates greater than 40 kHz: timestamps less than one 25 microsecond tick apart cannot be represented as integers. Therefore, in order to support higher digitizing rates, the system supports a one microsecond timestamp resolution mode. Any time that you set a digitizing rate greater than 40 kHz, the system will request permission to set the timestamp resolution to one microsecond, as described in the previous section. You can also manually set the timestamp resolution in Server's **Global Options** dialog:




You must restart the system after changing the timestamp resolution.

For sources whose digitizing rates are 40 kHz or less, note that the accuracy of timestamping is not affected; the only effect is to multiply the raw timestamps by 25, and this effect is invisible when you view the timestamps in Offline Sorter software, NeuroExplorer, and most other applications. In general, unless you are using an AuxAI digitizing rate greater than 40 kHz, you should leave the system timestamp resolution set to the default 25 microseconds.

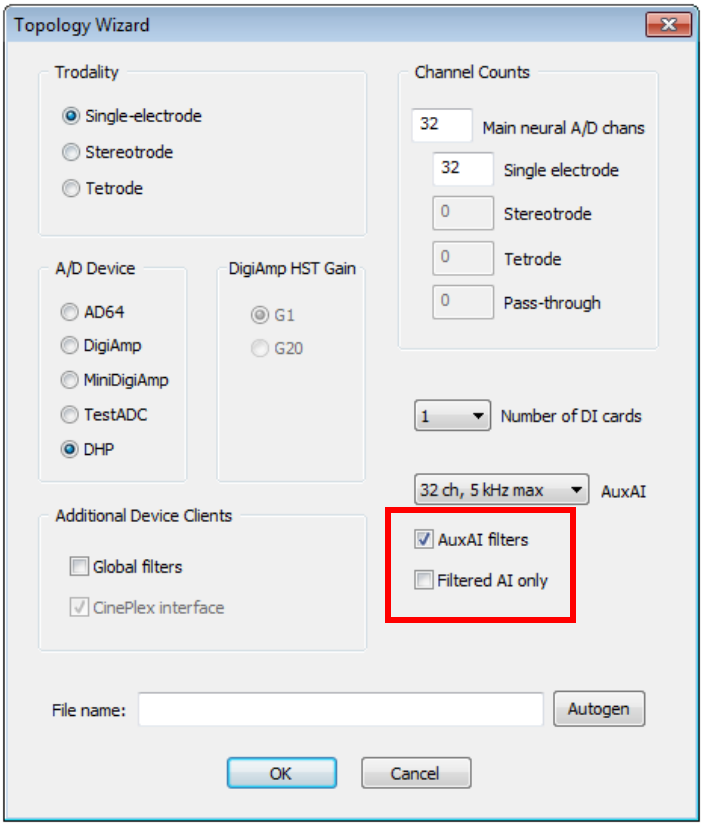
Note that setting the timestamp resolution to one microsecond will cause the low-order 32 bits of the raw integer timestamps to roll over 25 times faster (71 minutes versus 29.8 hours). This is only an issue for legacy code which does not use the full 64 bit integer timestamp value (40 bits in plx files and in online client data).

## 12.5 AuxAI Filter (AIF)

The auxiliary analog input (AuxAI) device supports optional digital highpass and lowpass filters, similar to those available for the SPKC and FP sources. These can be useful for noise and artifact removal, smoothing signals from sensors, and similar purposes. However, unlike the SPKC and FP filters, the AuxAI filters are not included in the OmniPlex topology by default. To include the AuxAI filter device, you must enable the **AuxAI filters** checkbox in the Topology Wizard in Server when creating a new topology or modifying an existing one. The procedure for enabling the these filters is provided in Section 2.2, "Starting and Configuring the OmniPlex Server" on page 28 if you have a DigiAmp Amplifier or MiniDigi Amplifier, or in Section 3.2, "Starting and Configuring the OmniPlex Server" on page 73 if you have a DHP unit.

To access the AuxAI filter features, an AuxAI card must be present in the topology.

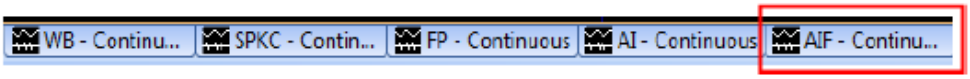
Note that you must stop data acquisition and close PlexControl before changing the topology.



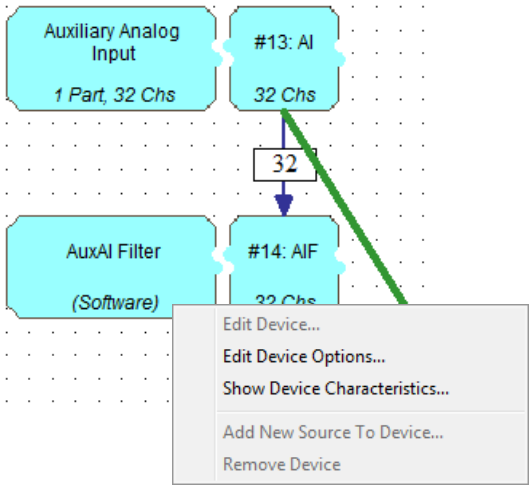

If you only check **AuxAI filters**, then both the standard AI source (unfiltered) and the AIF source (filtered) will be available. If you *also* check **Filtered AI only**, then only the filtered AIF source is available, although you will still be able to disable its filters. In this context, you can think of AI as analogous to the WB (wideband) source for neural signals. Remember that since the AI source is

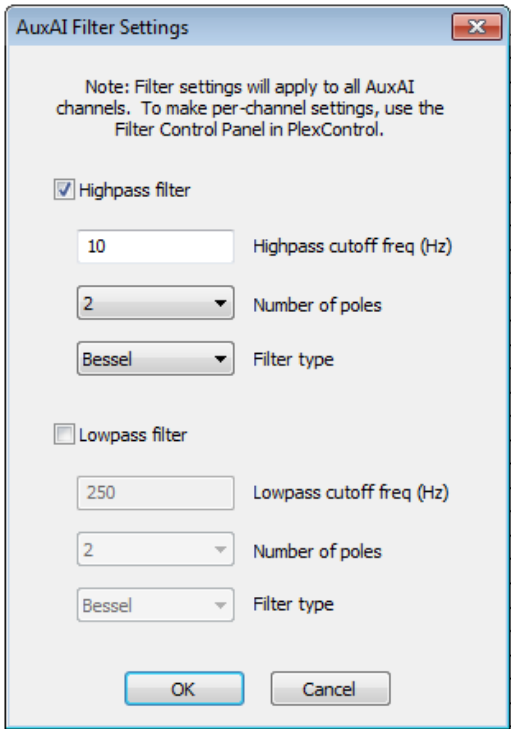
usually digitized at a lower rate than the main neural source (e.g. a few kHz), recording both the raw and filtered versions often will not result in an objectionable increase in file size or processing resources.

In PlexControl, the AIF source is displayed in its own tabbed view, just like the other continuous sources. The image below displays tabs for both **AI-Continuous** and **AIF-Continuous**, which corresponds to checking **AuxAI filters** but not checking **AI filters only** in the Topology Wizard dialog:



You can modify the **AuxAI Filter** settings by right clicking the **AuxAI Filter** icon in the topology, then selecting **Edit Device Options...**




After you restart Server, the system will apply your adjusted AI filter values to all AuxAI channels. When you save the pxs (topology) file in Server, the current settings of the SPKC and FP separators are also saved in the file.

The note at the top of the **AuxAI Filter Settings** dialog refers to per-channel settings in the **Filter Control Panel** in PlexControl. For these settings, see Section 13.5, “Filter Control Panel” on page 420.

# 12 Auxiliary Analog Input

# Chapter 13
# Additional User Interface Views and Features

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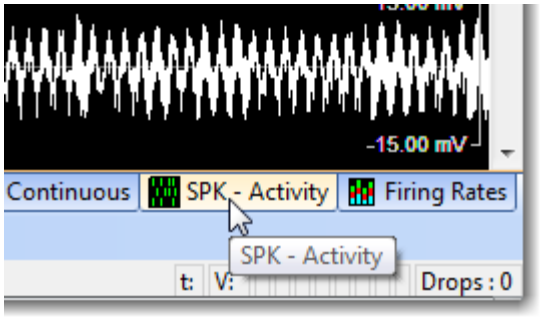
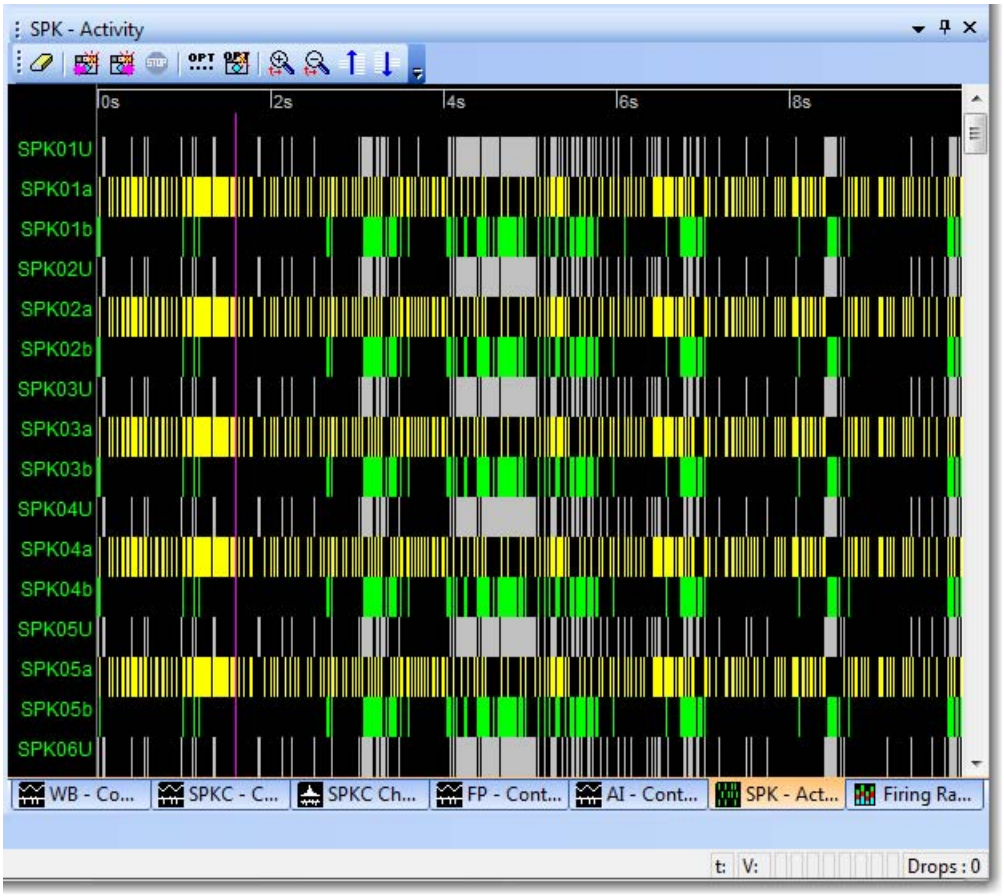
## 13.1 PlexControl Activity Display

The Activity view displays a raster view of activity on spike and event channels. To display it, click the Activity tab in the lower right part of the PlexControl window:




The Activity view is displayed:



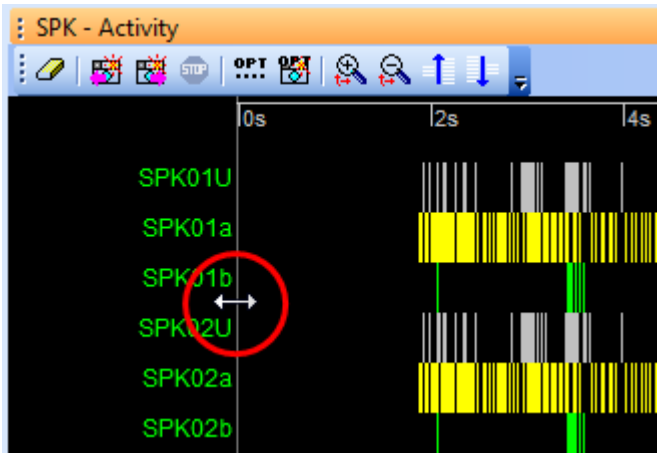

The Activity display is a sweeping, time-based display like the continuous views, but instead of continuous signals, vertical tick marks are display in rasters, each tick indicating the timestamp of a spike or other event. Just as with the continuous

views, you can change the sweep rate and the number of channels that are displayed, as described previously.

The Activity view displays ticks for all OmniPlex System sources which consist of timestamped events, in the following top to bottom source order:

- Spike channels 1 - N
- Keyboard events 1 - 8
- DI event channels 1 - 32 (or 1 - 64 for two DI cards)
- Strobed events
- RSTART (start or resume recording event)
- RSTOP (stop or pause recording event)
- CPX1 (CinePlex® System events)

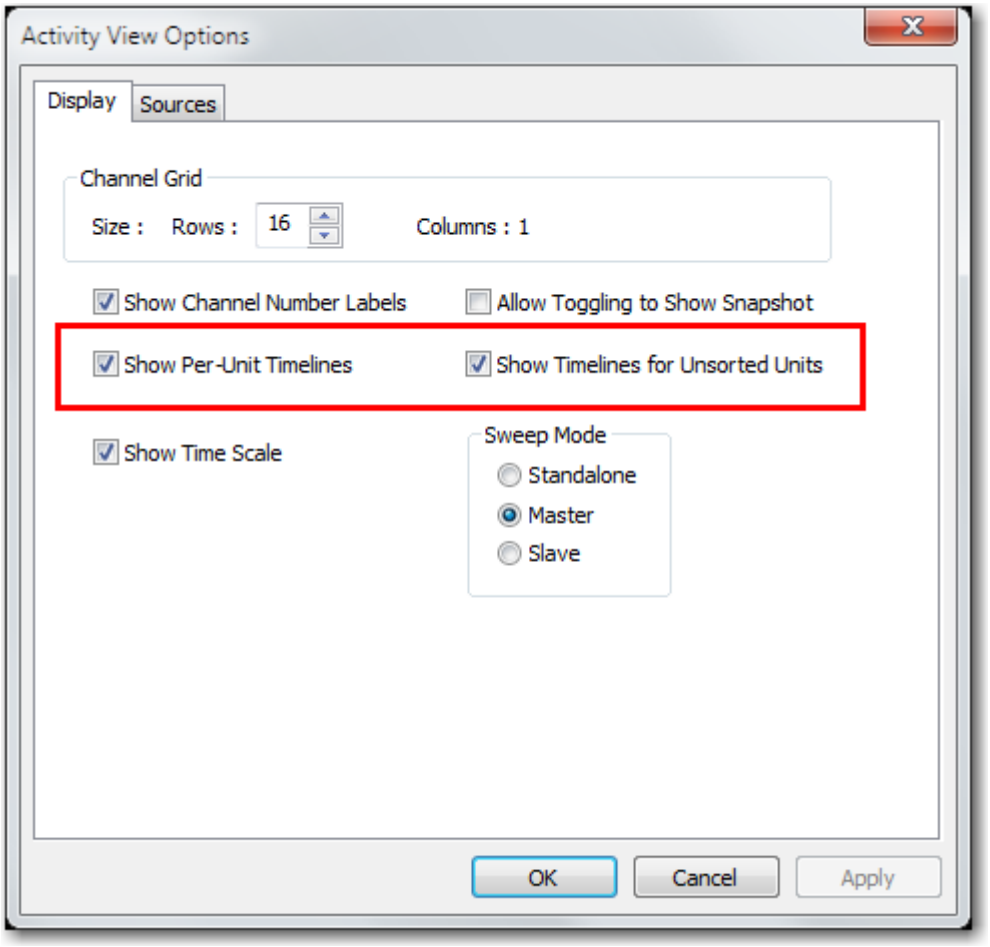
As with the continuous views, the vertical scroll bar allows you to move up and down through the set of channels. In addition, you can adjust the amount of space that is used for the channel labels at the left, by dragging the vertical separator line:



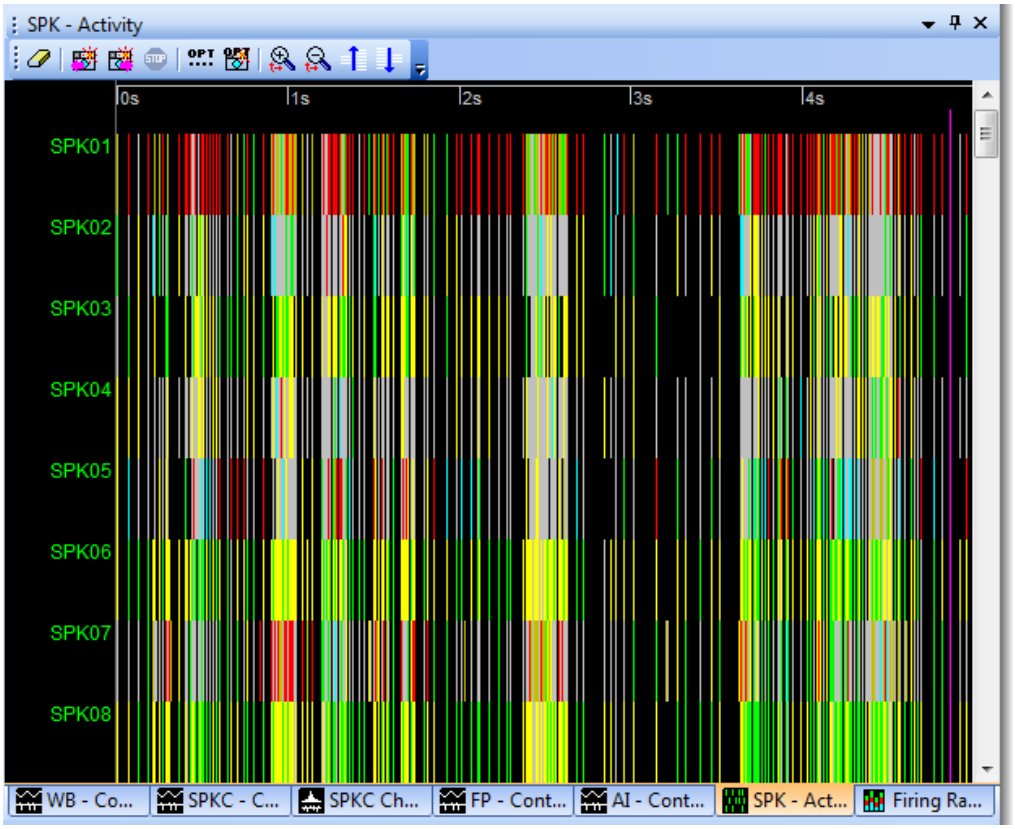

You can use the **Shift** and **Ctrl** keys on your keyboard for additional options when displaying more channels or fewer channels.

- Pressing the **Ctrl** key while clicking the **More Channels** or **Fewer Channels** button toggles the number of channels displayed to twice the original number or half of the original number.
- Pressing **Shift+Ctrl** while clicking the **More Channels** or **Fewer Channels** button toggles between showing all channels and showing one channel.

There are also options for customizing the display in the **Activity View Option** dialog:



The two options that are highlighted determine how spike timestamps are displayed. If you turn off **Show Per-Unit Timelines**, all the ticks for a given spike channel are displayed interleaved on the same row, similar to the display in the small strip at the top of each SPKC channel in its continuous view:



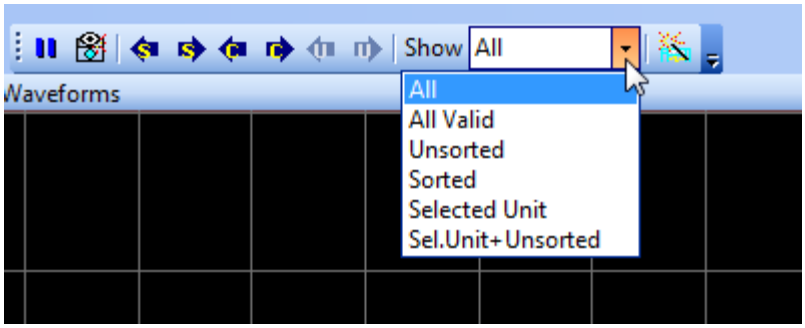

**Note:** Activity ticks can also be displayed in the MultiPlex view. See Chapter 16, MultiPlex Multi-source View.

## 13.2 PlexControl Spike Display Modes

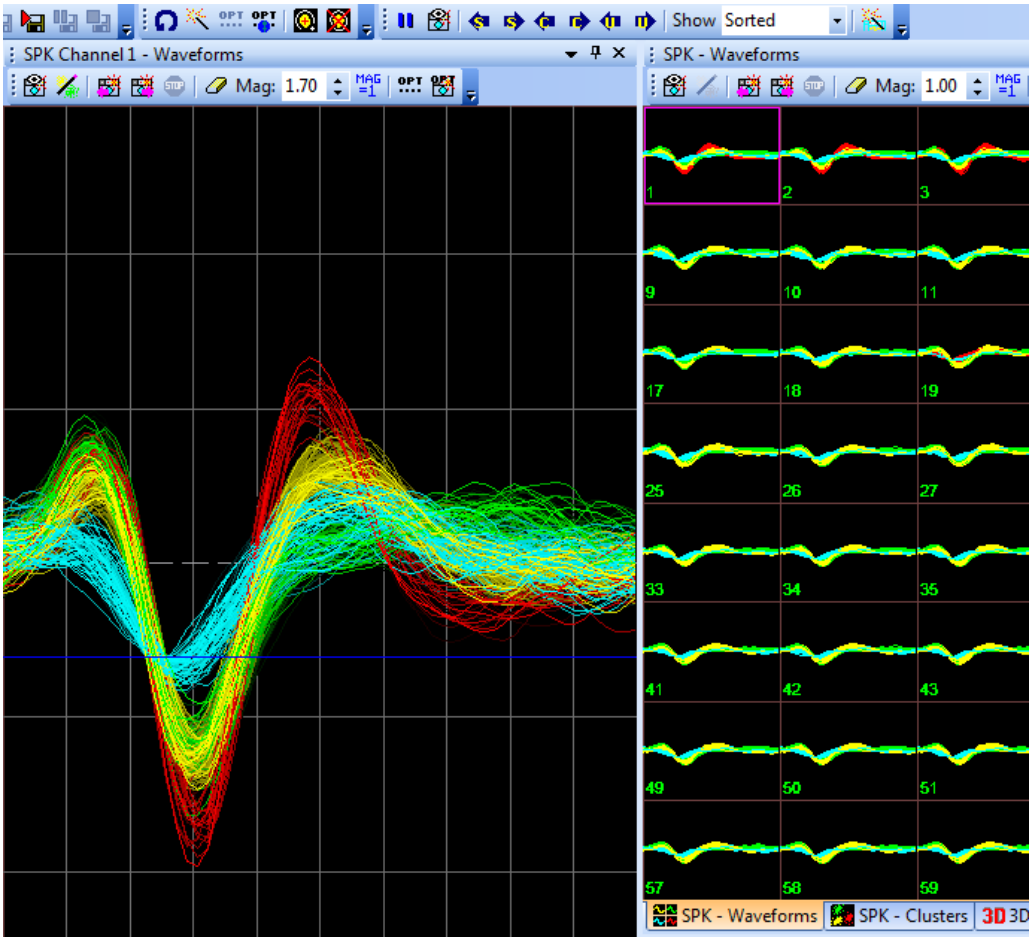
PlexControl's user interface provides a number of display modes and options which are designed to make it easier to view the incoming spikes, define units, and adjust sorting parameters. These include the **Show** filter, **Show All / Current Unit**, and **Fade / Rolling / Erase / Scope** modes.

### Spike Show Filter

The spike **Show** filter is not a filter in the sense of signal processing; it is an option which "filters" which types of spikes are displayed in PlexControl. It does not affect the actual sorting or recording of data. To see the available Show modes, click the down-arrow in the **Show** control in the main toolbar:

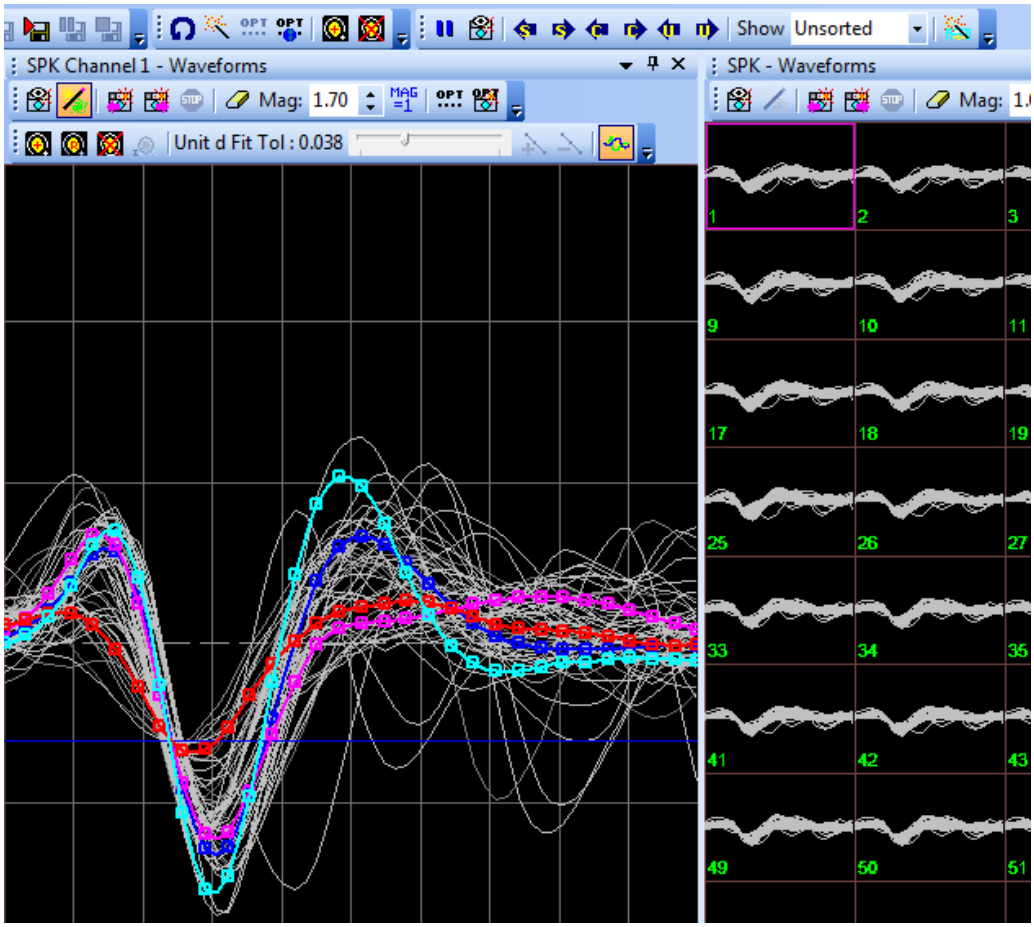


The **Show** options affect the main spike window and the multichannel spike window, but not the Units window. The default is to display All spikes. We will leave the All Valid setting for later. Choose the **Sorted** setting to display only spikes that are sorted, i.e. that match one of the templates:



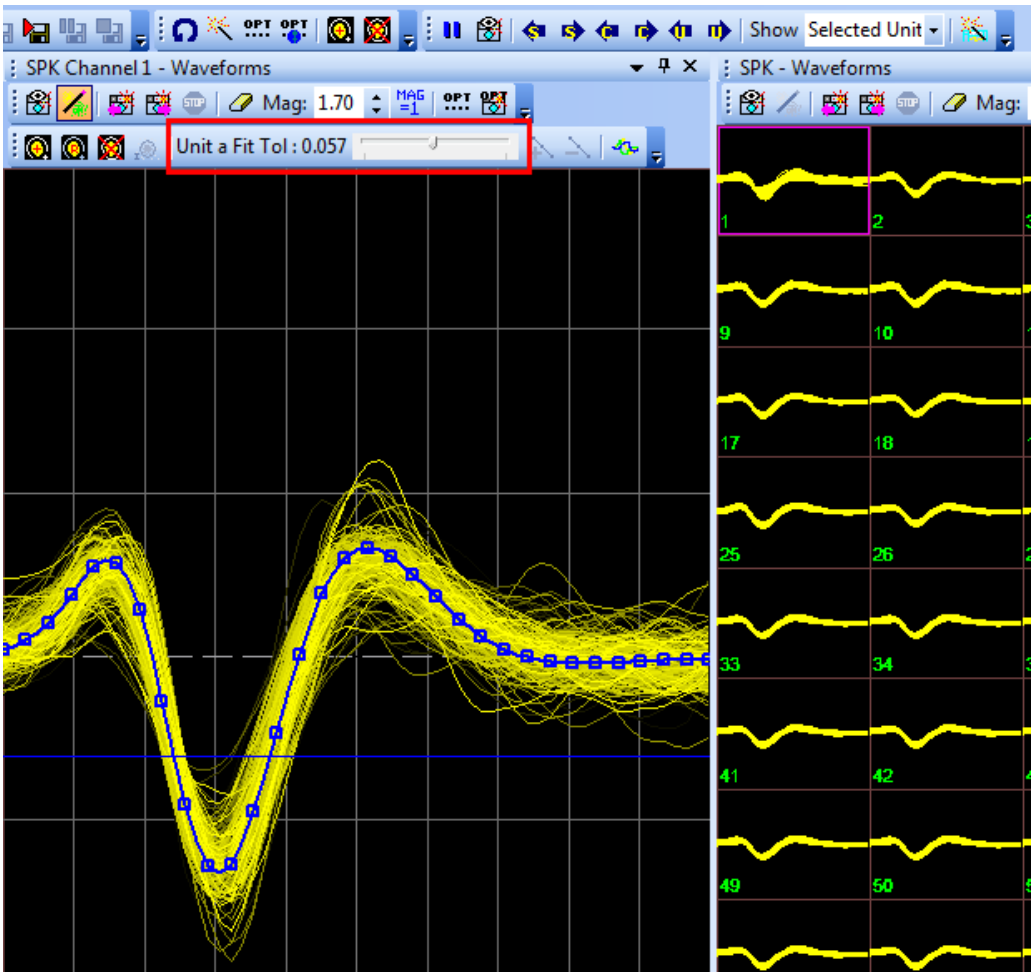
This mode is useful for removing the clutter of unsorted spikes, but be careful, since it also omits all the spikes that almost, but not quite, match a template, making your sorting look unrealistically “clean.” However, it is a good way to see whether each of the units is distinct from the others.

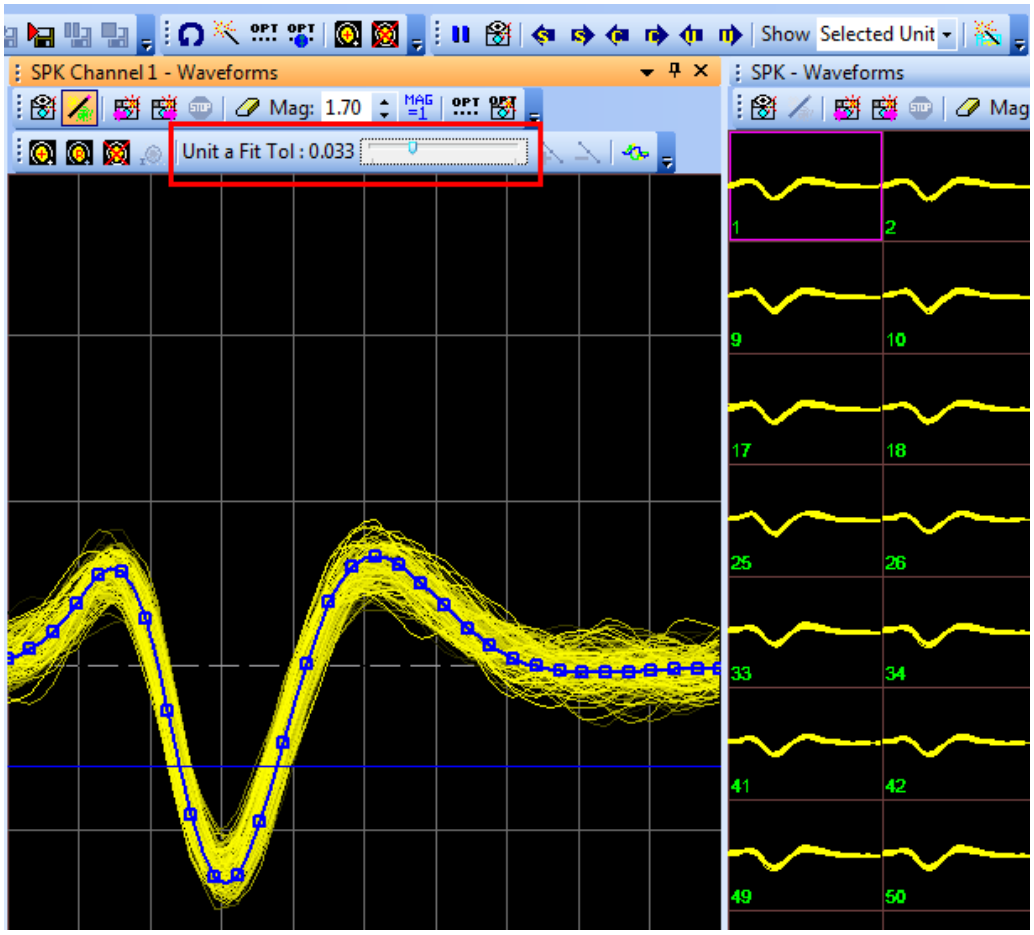
The **Unsorted** mode shows, as the name implies, only the spikes that do not match any template. This mode gives a more pessimistic view of your sorting results.



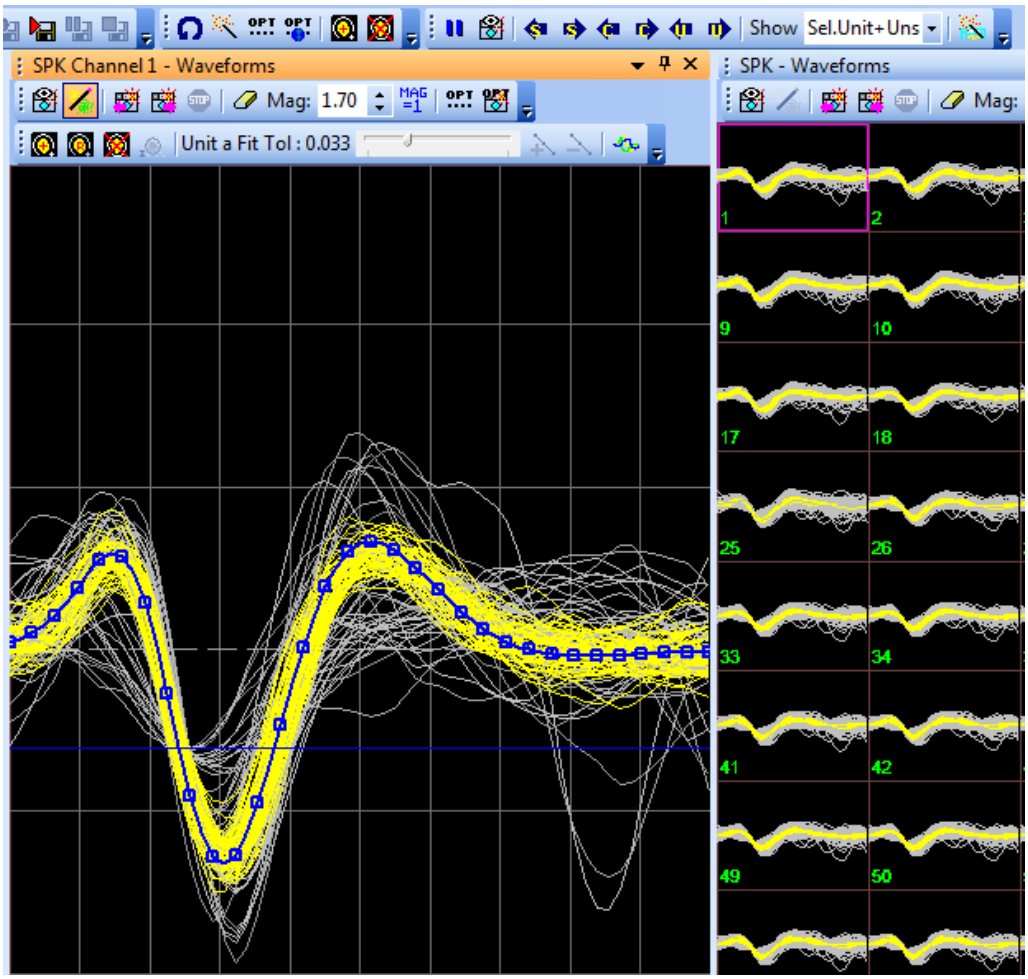
Ideally, the only spikes that you see in this view should be ones that are not valid matches for any defined unit. In practice, you will likely see some that should have been matched to one of your templates. In this case, you may wish to try increasing the fit tolerance for the relevant unit; however, be aware that due to noise and issues such as superposition, there will always be some small percentage of spikes that you can visually identify with a specific unit, but which cannot be sorted without increasing the tolerance so much that there are also invalid matches to that template.

The **Selected Unit** mode displays only the sorted spikes for the currently selected unit; this is the same as what is displayed in each subwindow of the Units window, but larger and with the template waveform displayed. This mode is very handy for fine-tuning the fit tolerance; typically you would start with a a larger tolerance value, and then reduce the tolerance until the bundle of waveforms "comes into focus," without extraneous, invalid waveforms. For example:




Show
Selected Unit
SPK Channel 1 - Waveforms
Mag: 1.70
Unit a Fit Tol : 0.033
SPK - Waveforms
Mag
1
2
9
10
17
18
25
26
33
34
41
42
49
50

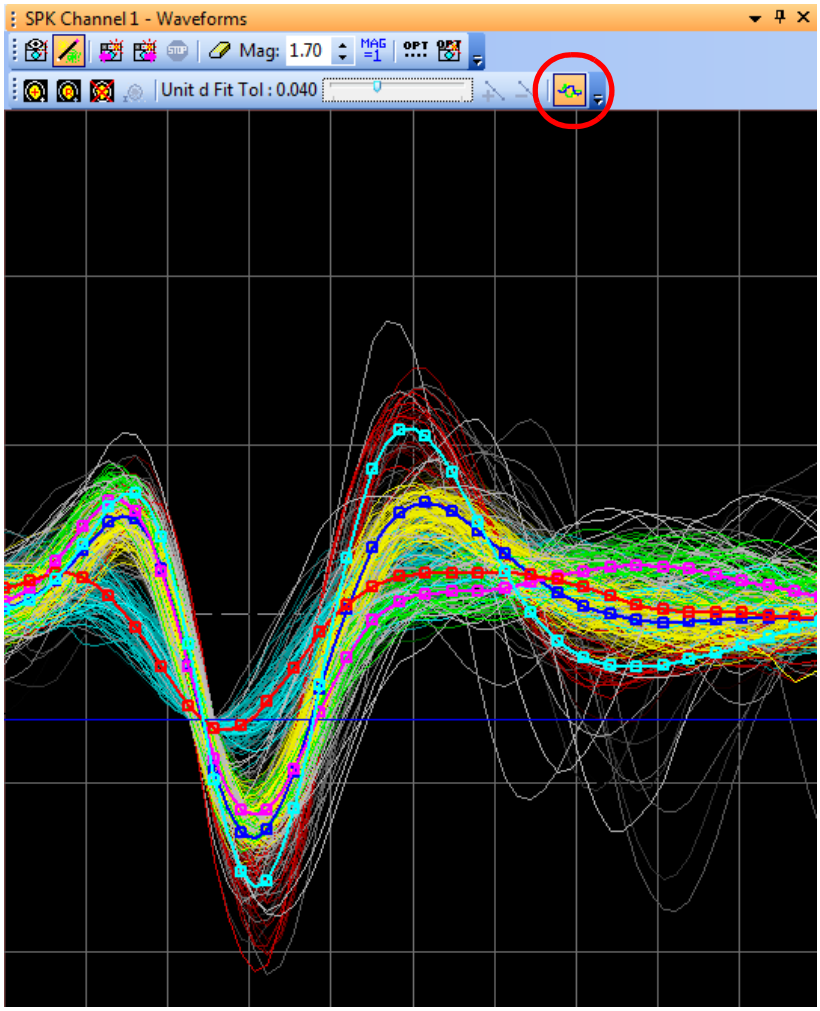
Finally, **Selected Unit + Unsorted** shows both the selected unit and all unsorted spikes, and provides more visual information than Selected Unit mode.



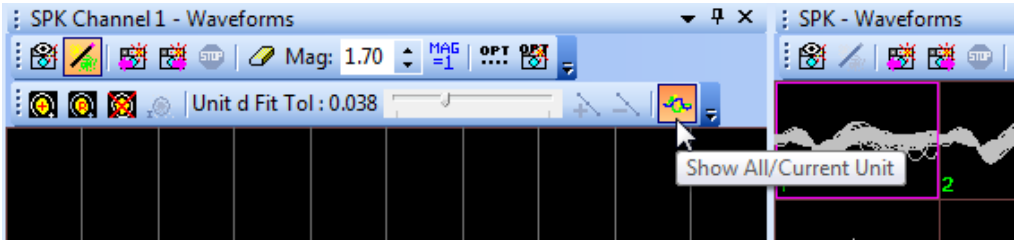

While you will probably spend most of your time with this option set to All, you will find it very useful to toggle back and forth between the display modes as you are defining and refining unit definitions. Note that the **Show** mode applies to both the main spike window and the multichannel spike window.

### Show All / Show Current Unit

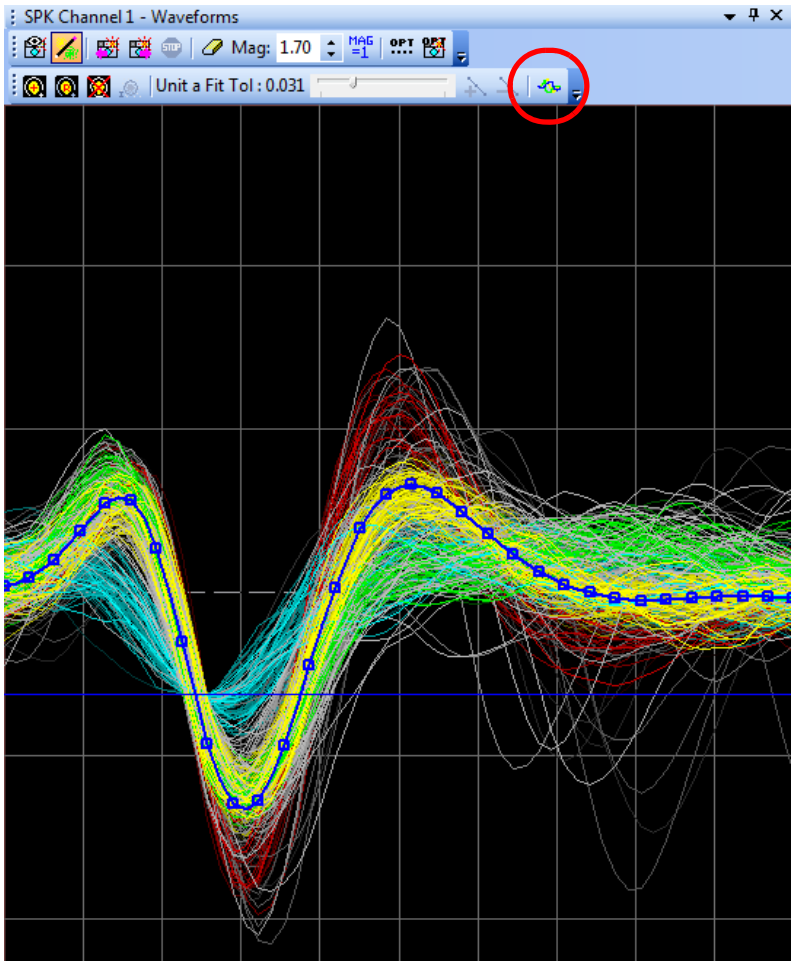
When there are several units defined on the same channel, by default their waveforms are all displayed on top of each other in unit editing mode. The **Show All / Current Unit** button is orange when it is in the "Show All" mode, as in this image:




If you click on this button, it will be deselected, and the system will display the template for the selected unit only.

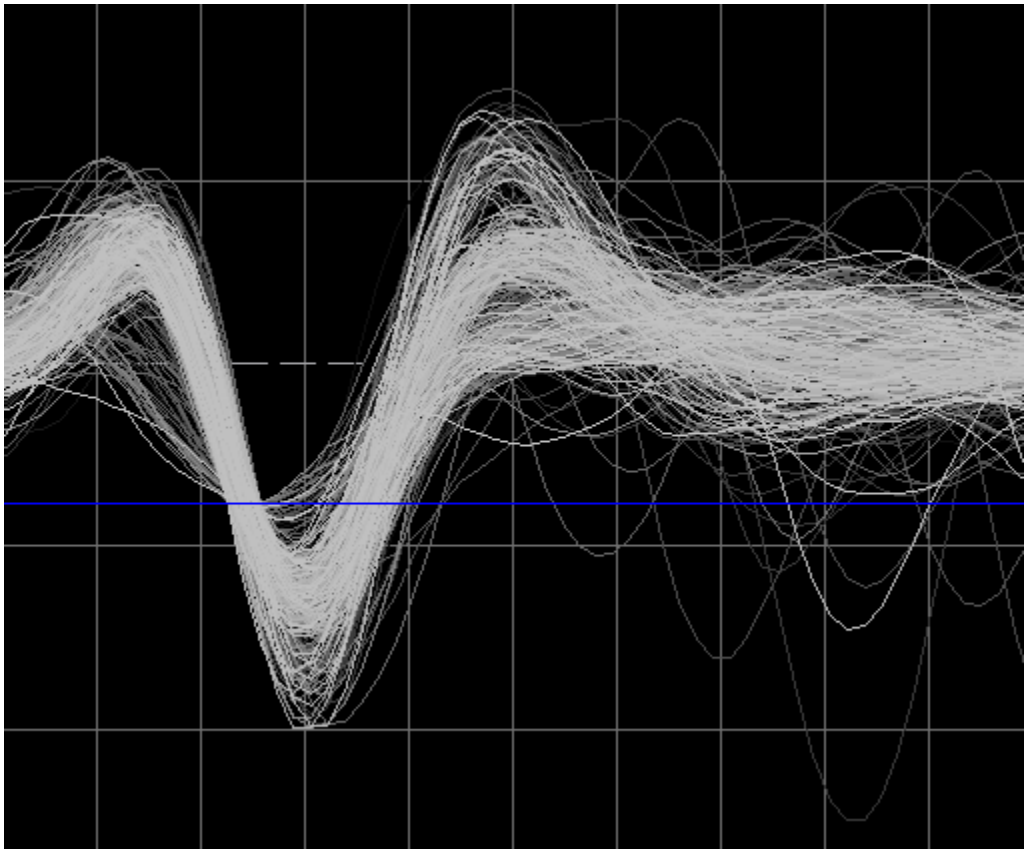


This is the appearance of the “Show Current Unit” mode, in which only the template for the selected unit is displayed:

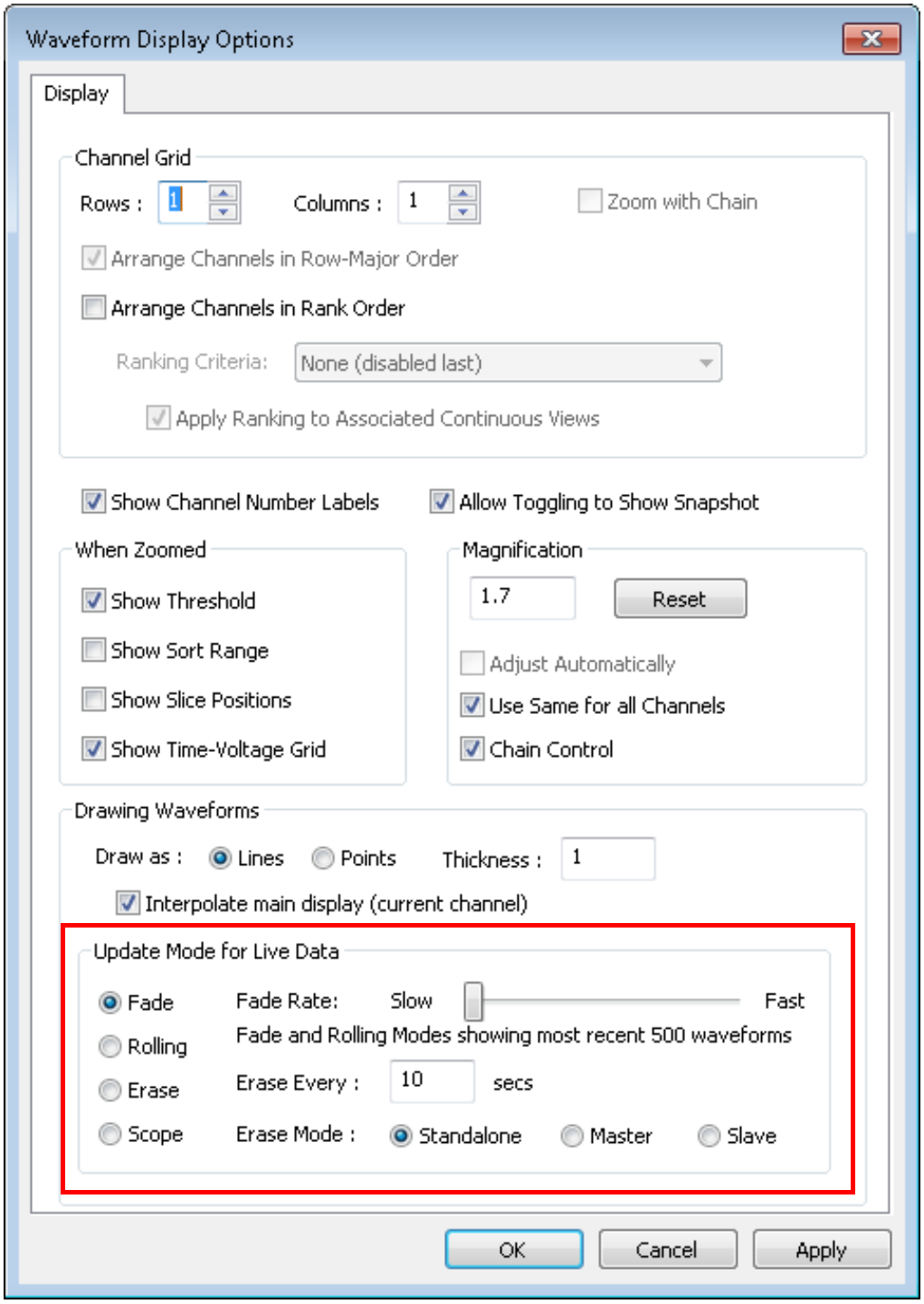


### Fade / Rolling / Erase / Scope

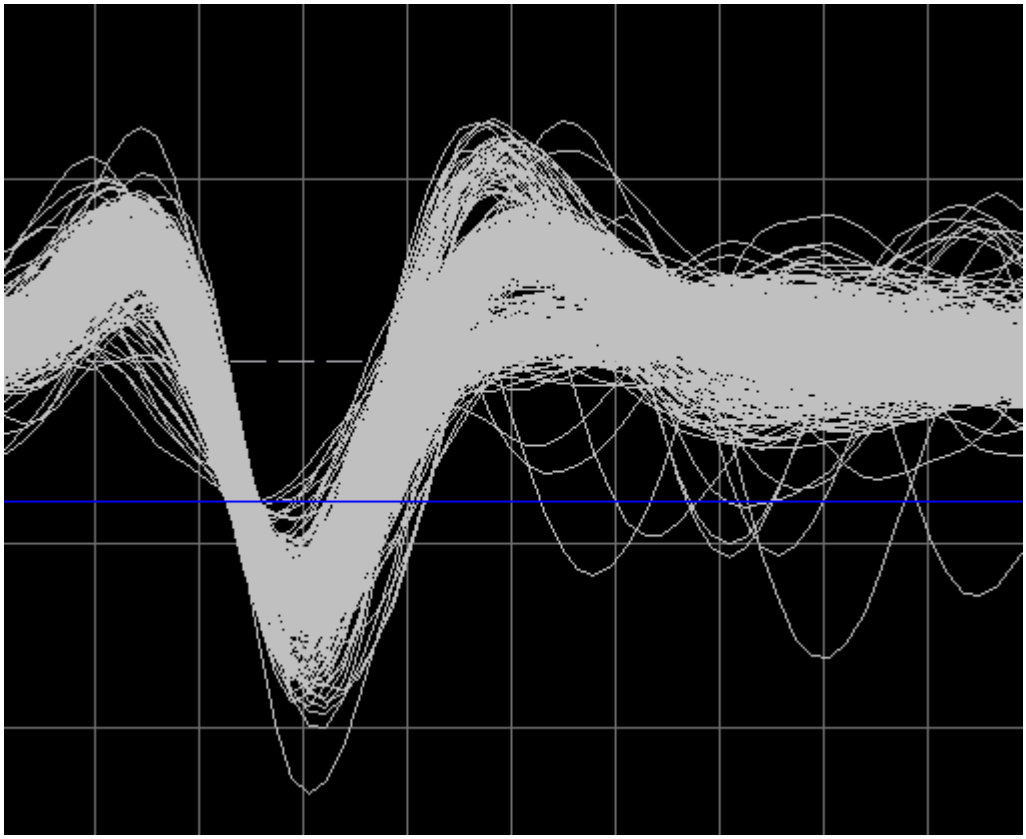
You may have noticed that in the main spike window, after a few seconds, spikes don't suddenly disappear; rather, they slowly fade away. This is referred to as “Fade” mode and is the default display mode for the main spike window.



It is intended to reduce the visual clutter of “old” spikes, and to make it more obvious which action potentials are the most recent ones. The **Waveform Display Options** dialog allows you to select between **Fade** mode and three other modes, **Rolling**, **Erase** and **Scope**:

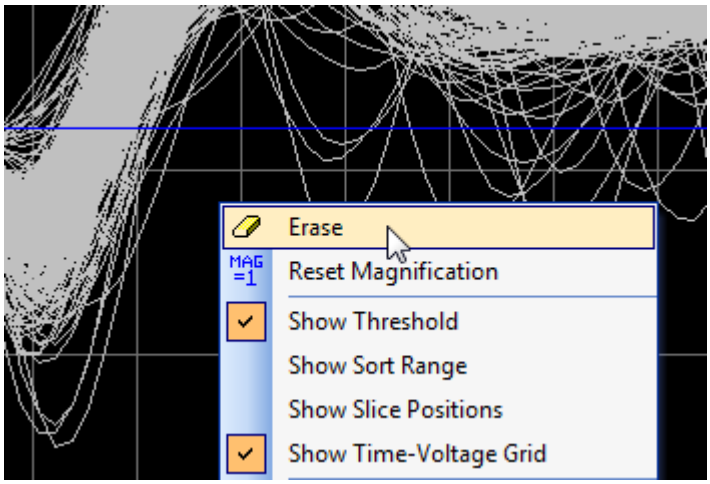


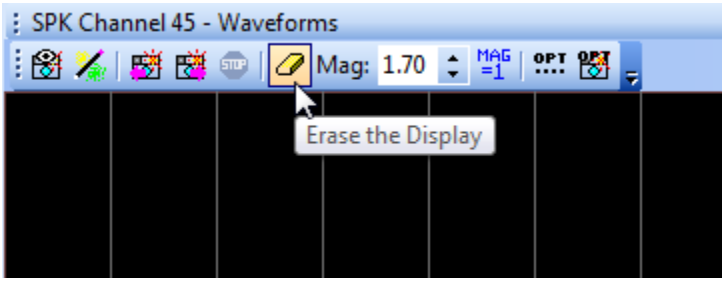
In **Rolling** mode, the most recent 500 waveforms (the same number of waveforms as in snapshot) are displayed; older waveforms suddenly disappear, as opposed to fading out. This mode gives every one of the recent waveforms equal visual “weight”:



In **Erase** mode, incoming waveforms are drawn on top of each other with no fading or rolling, until the specified erase time elapses, at which time the window is erased and the process starts over from scratch. When using Erase mode, be aware that while very old waveforms may not be erased from the screen, only the most recent 500 spikes are available for interactive operations such as waveform crossing, just as with the other two modes. In other words, imagine that you set the erase time to 3600 seconds, or one hour, and allow the display to accumulate thousands or millions of waveforms in that time; then, after an hour, you do a waveform crossing on a spike that was drawn an hour ago - you may see an error message indicating that no waveforms were crossed, and this is because those waveforms are not among the 500 most recent incoming spikes.

When using **Erase** mode with a long redraw interval, note that you can manually erase the display at any time, using either **Erase** from the right-button menu for the main spike window or the **Erase** button in the toolbar:




Some users prefer to use this method, so that they have total control over when the display is erased. Note that the other spike and continuous displays have their own individual Erase commands.

**Scope** mode is very similar to the Scope mode in the MultiPlex display, where incoming spikes are drawn and erased as rapidly as possible in an oscilloscope-like manner. This allows you to follow the dynamics of the spiking activity on a channel without the "waveform pile-up" effect that can obscure spikes, especially small spikes, in some cases.

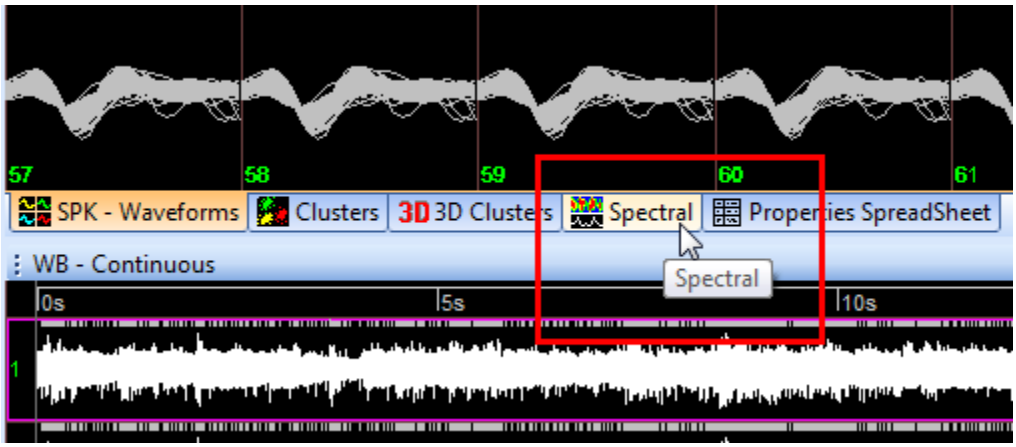
Note that Scope mode is automatically disabled if you enter unit-editing mode, since Scope mode is not helpful when editing units. Scope mode is generally intended for use after you have set thresholds and sorting for a channel.

The 2D and 3D PCA displays also support **Fade**, **Rolling**, **Erase** and **Scope** modes (2D PCA only when in zoomed single-channel mode).

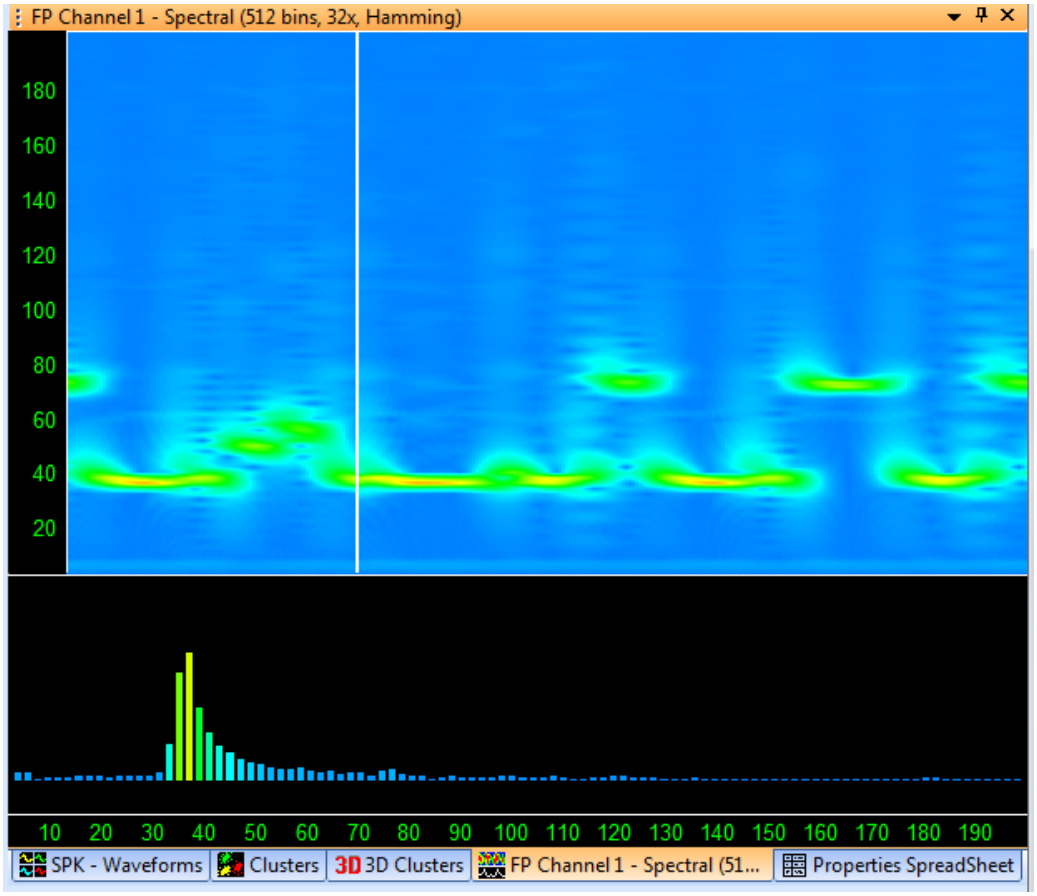
## 13.3 Spectral 2D View

### Overview

The **Spectral 2D** view (also referred to simply as **Spectral** view) displays a rolling color-coded spectrogram of the currently selected channel on the FP (field potential) source, plus an animated spectral graph below it. This allows you to monitor changes in spectral content, such as increase in energy in a range of frequencies, as a function of time. To view the Spectral display, click on the Spectral tab:



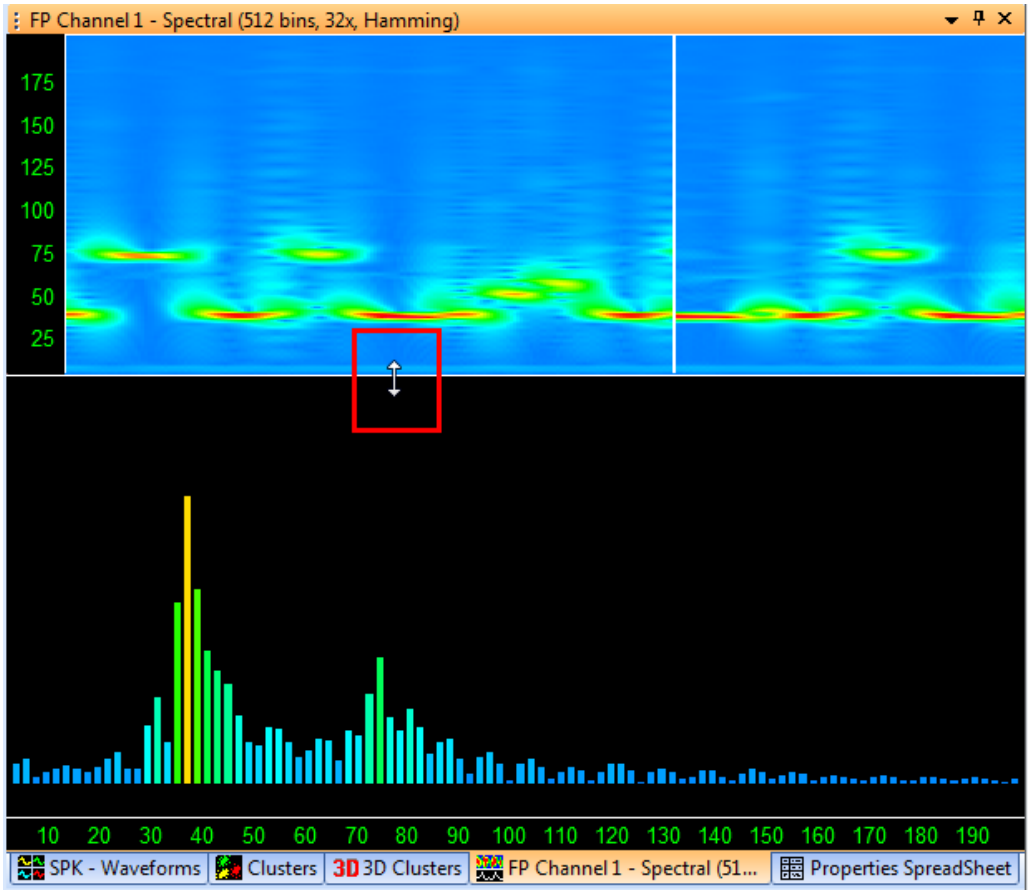
The **Spectral** view is displayed:



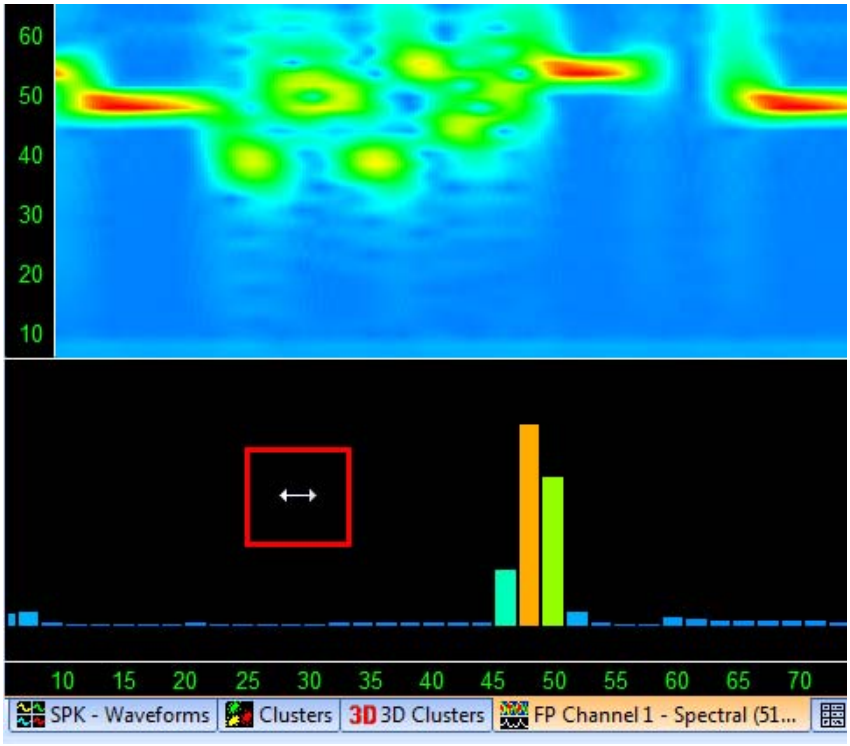

The upper area is the spectrogram, where time increases along the x axis, the y axis represents frequency, and the color at any particular time and frequency represents the relative amplitude of the frequency component, with colors progressing from blue to green to yellow to red as amplitude increases.

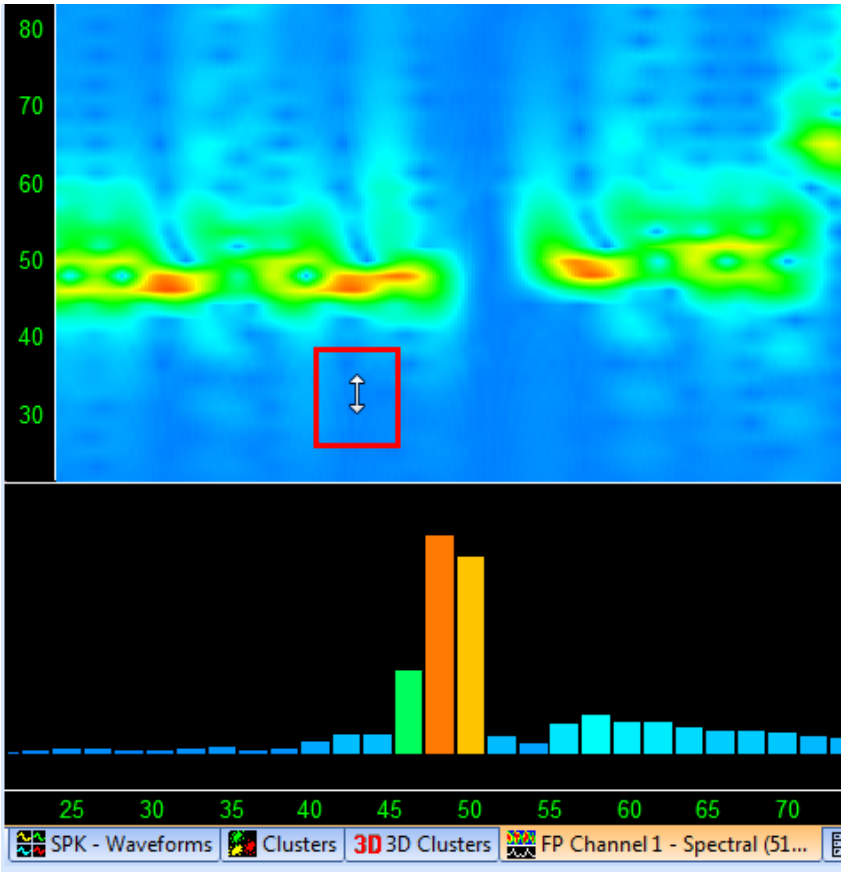
The lower area is the spectral graph, which can be thought of as a vertical "slice" of the spectrogram at the current position of the white sweep line. Here, the x axis represents frequency and the y axis represents amplitude. By default, the spectral graph is shown as a bar graph, where both the height of each bar and its color represent the amplitude at a frequency (or more precisely, the amplitude within one bin of the FFT that is used to perform the spectral analysis).

You can change the relative sizes of the spectrogram and the spectral graph by dragging the divider between them with the mouse:



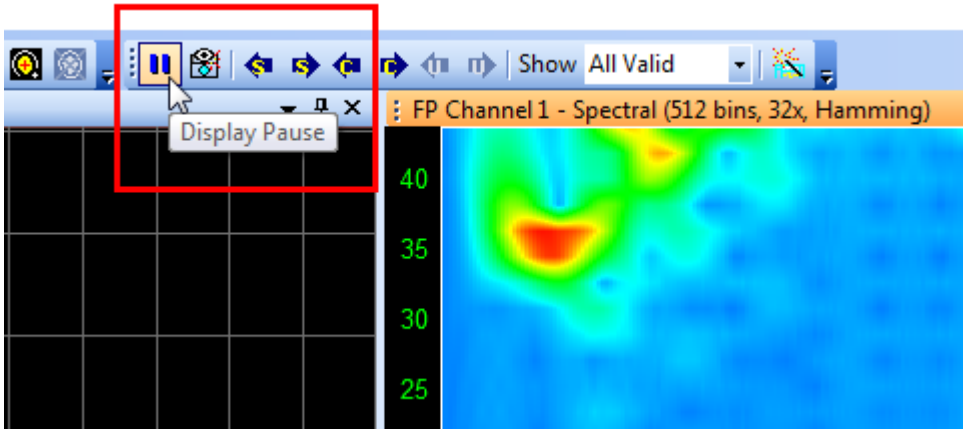

The mouse can also be used to interactively adjust the frequency range that is displayed. In either display, rolling the mouse wheel zooms in and out in frequency, i.e. a larger or smaller range of frequencies are displayed. Click in the spectral display and drag left or right, or click and drag vertically in the spectrogram, to drag the display frequency range accordingly. Note that adjusting the frequency range in one display automatically makes the corresponding change in the other display.


60
50
40
30
20
10
10 15 20 25 30 35 40 45 50 55 60 65 70
SPK - Waveforms
Clusters
3D 3D Clusters
FP Channel 1 - Spectral (51...

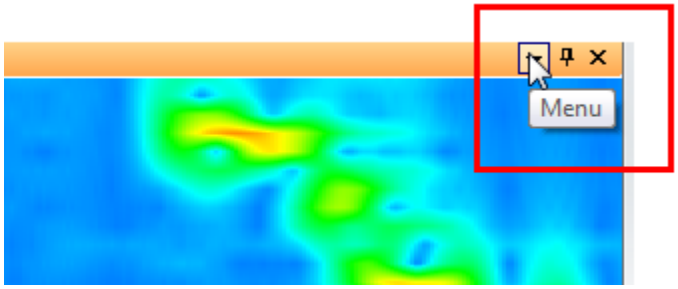

80
70
60
50
40
30
25 30 35 40 45 50 55 60 65 70
SPK - Waveforms
Clusters
3D 3D Clusters
FP Channel 1 - Spectral (51...

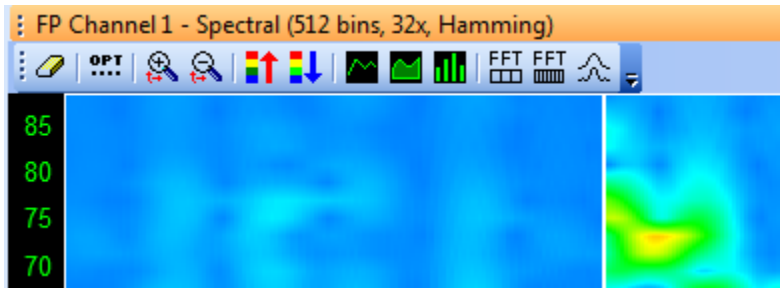
Zooming in frequency only affects the display, not the underlying FFT; in other words, the bin width remains the same, and bars (each of which represents an FFT bin) will become correspondingly larger or smaller in the spectral graph. However, the spectrogram is interpolated so as to remain smooth. When the spectral graph is in line or area mode (described below), these displays are also interpolated, although this can be disabled.

The rolling spectrogram and the spectral graph can be paused by using the global **Display Pause** button in the main toolbar:



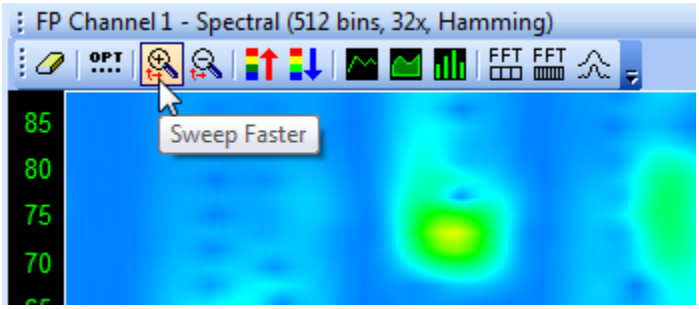

Click the down-arrow in the title bar to display the toolbar for the Spectral view:

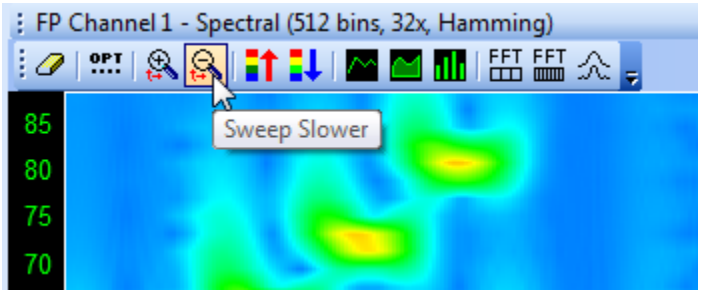



The leftmost button is **Erase**, which clears the display. To its right is **Show Options**; the **Options** dialog will be described later.

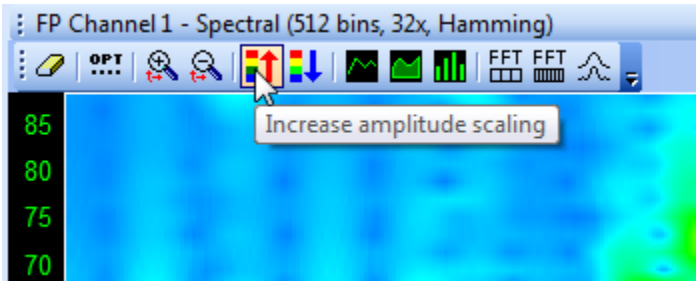
**Sweep Faster** and **Sweep Slower** adjust the speed of horizontal scrolling in the spectrogram; they also affect the rate at which the spectral graph is updated.

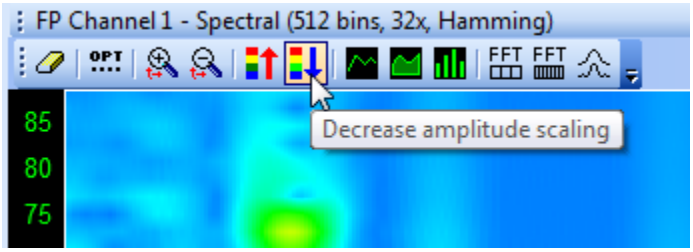


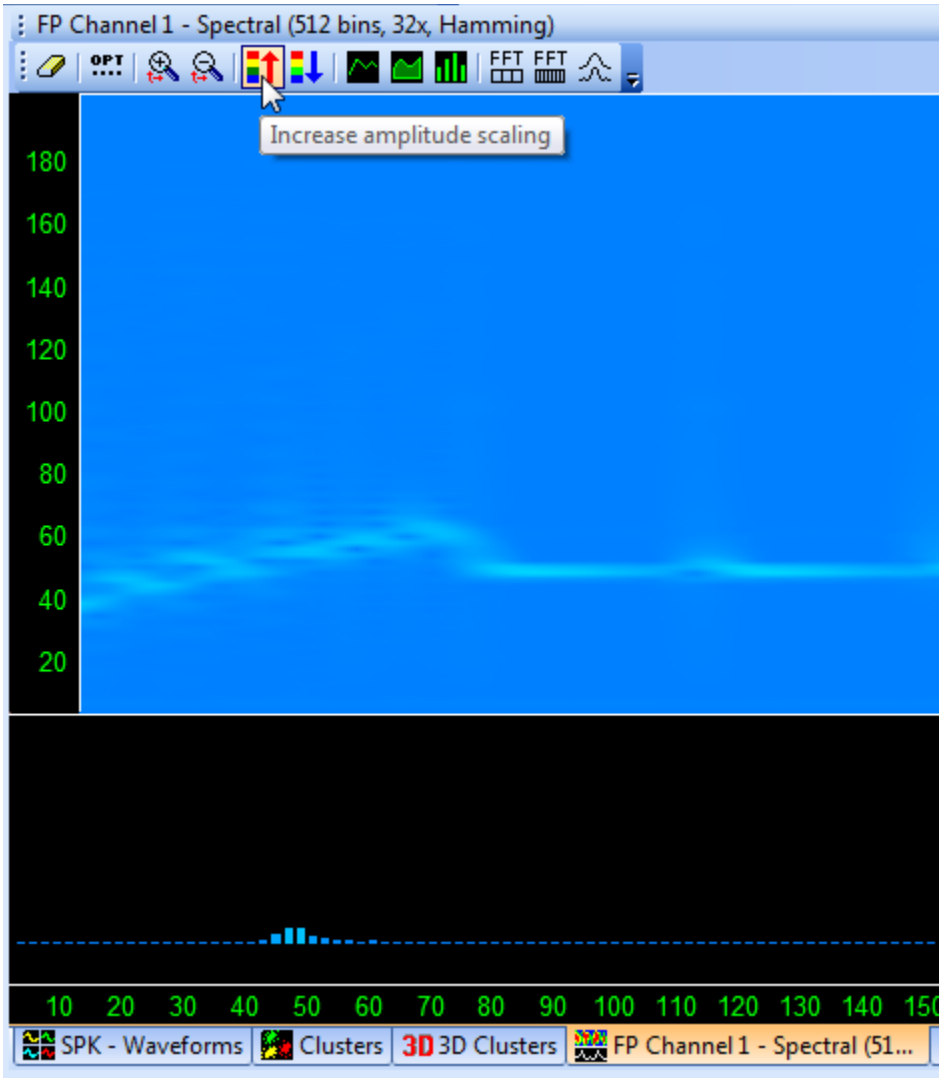

Note that the minimum and maximum scroll/update rates are also a function of the FFT size.

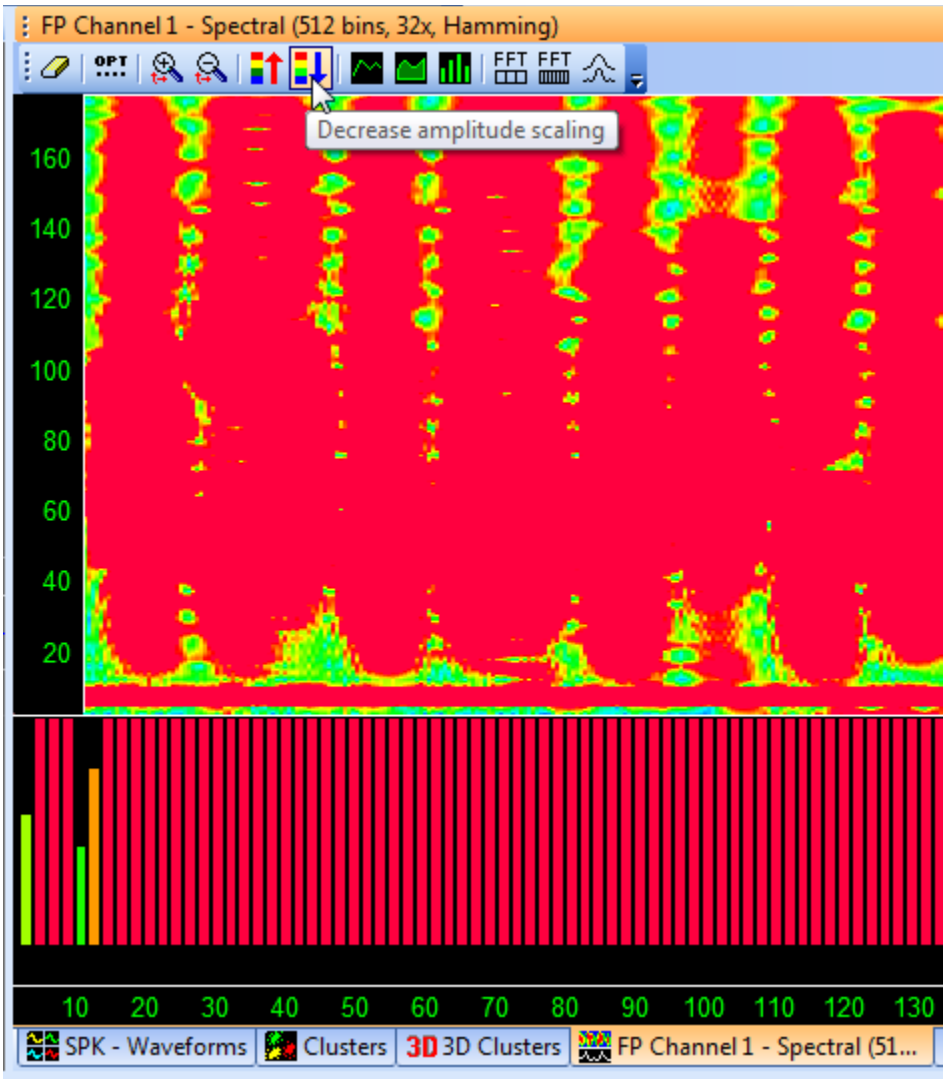
The next two buttons adjust the color scaling of amplitude; you can think of them as "more red" and "more blue" respectively, as indicated by the red up-arrow and blue down-arrow:



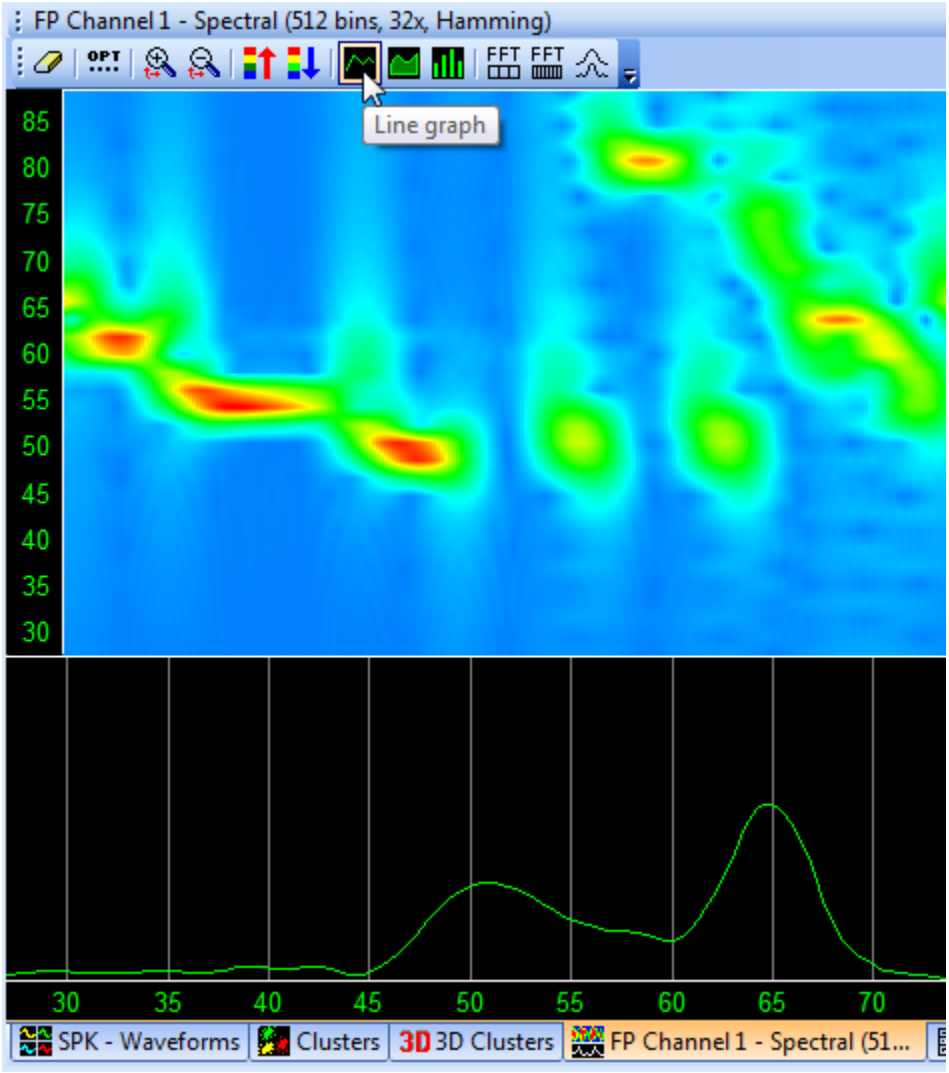
Here are examples of spectrograms where the amplitude should be increased and decreased to give a more informative spectral display:



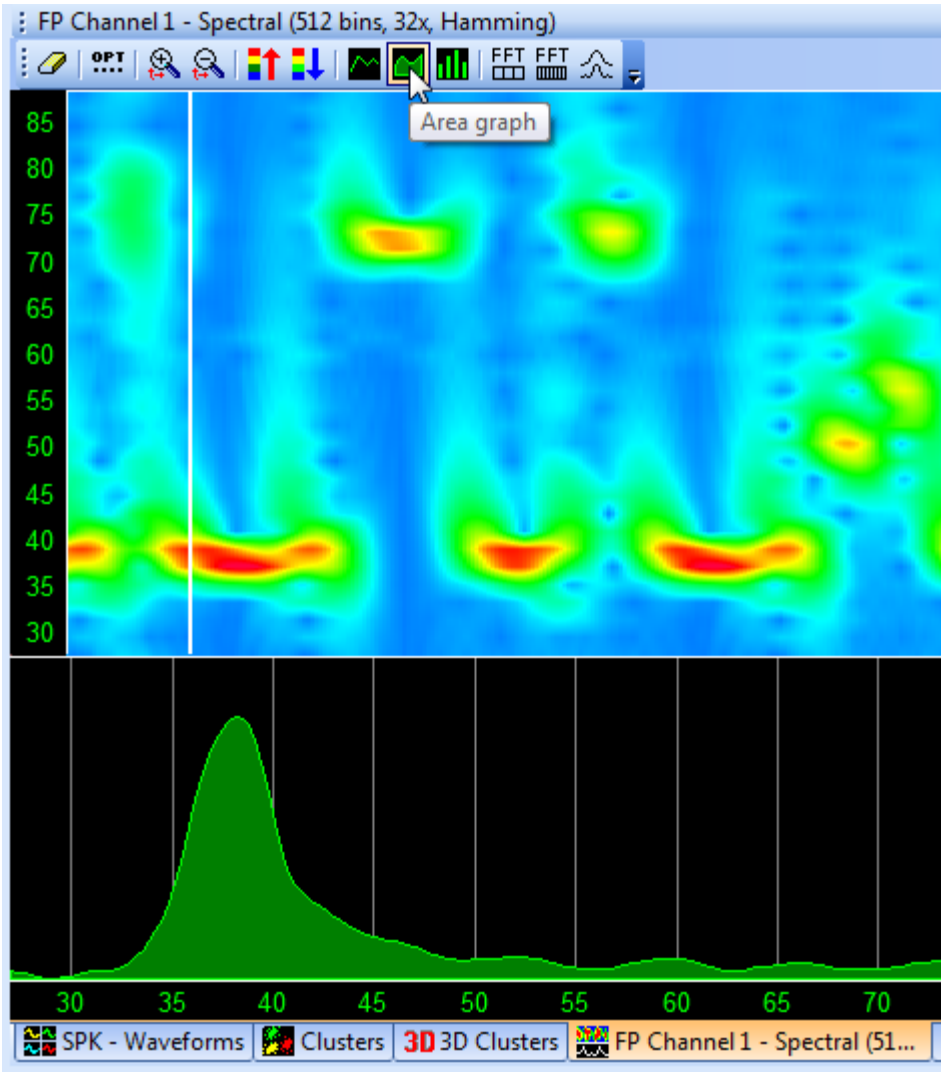

FP Channel 1 - Spectral (512 bins, 32x, Hamming)
Decrease amplitude scaling
160
140
120
100
80
60
40
20
10 20 30 40 50 60 70 80 90 100 110 120 130
SPK - Waveforms
Clusters
3D Clusters
FP Channel 1 - Spectral (51...

The three graph-mode buttons set the display mode for the spectral graph, as shown below.

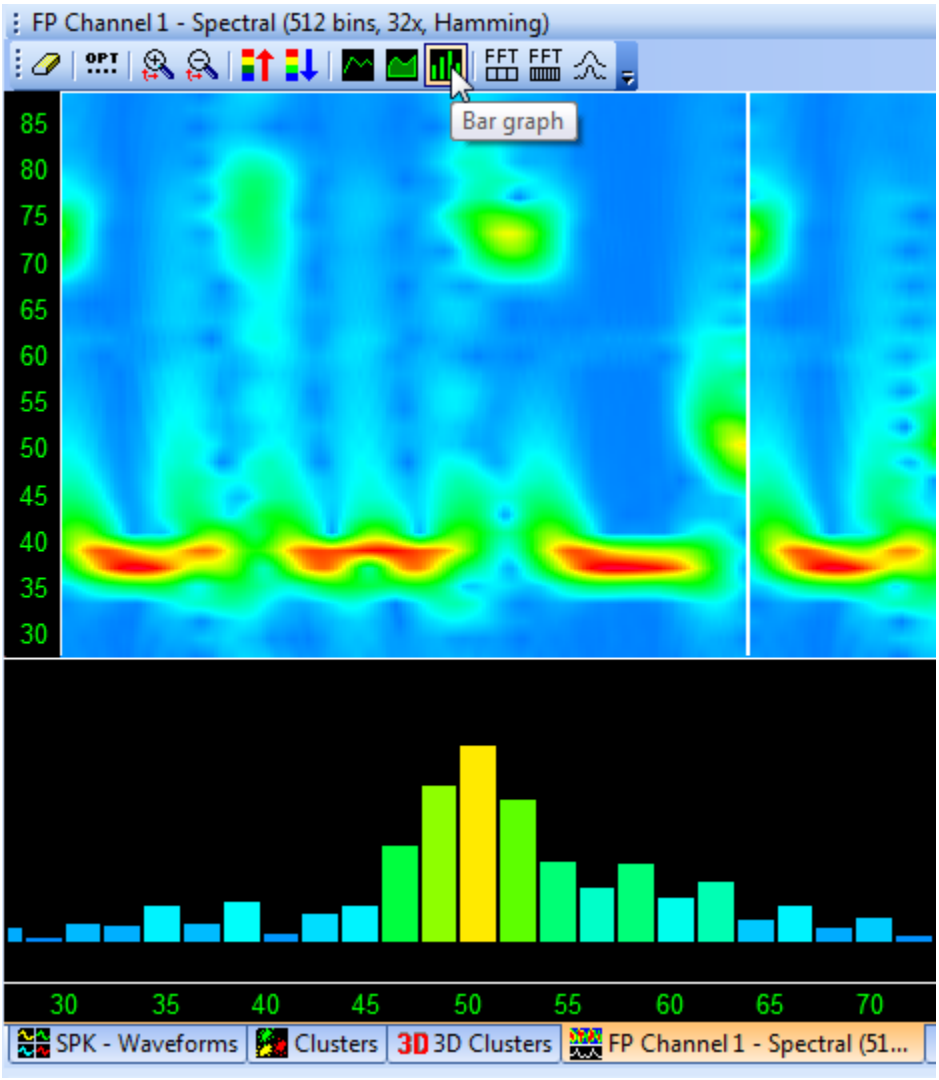
Line graph:



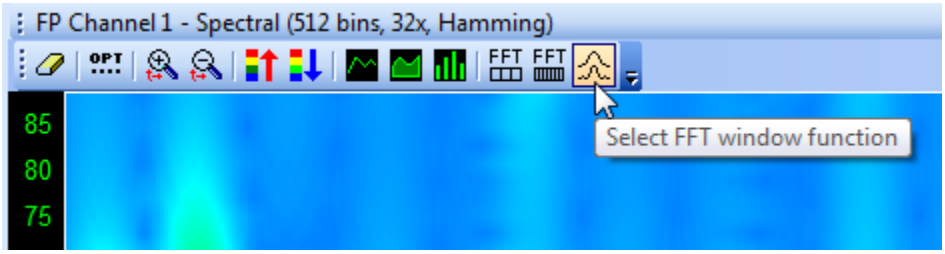
Area graph:


FP Channel 1 - Spectral (512 bins, 32x, Hamming)
Area graph
85
80
75
70
65
60
55
50
45
40
35
30
30
35
40
45
50
55
60
65
70
SPK - Waveforms
Clusters
3D 3D Clusters
FP Channel 1 - Spectral (51...

Bar graph (default):



The next two buttons decrease and increase the size of the FFT, in powers of two, and the rightmost button selects the FFT window function, selecting the next available window function each time it is clicked:




Note that the Spectral view's title bar always displays the current FFT size, oversampling (which is proportional to the sweep speed of the spectrogram), and window function:

The FFT size determines the frequency resolution of the spectral display, and the width of an FFT frequency bin is:

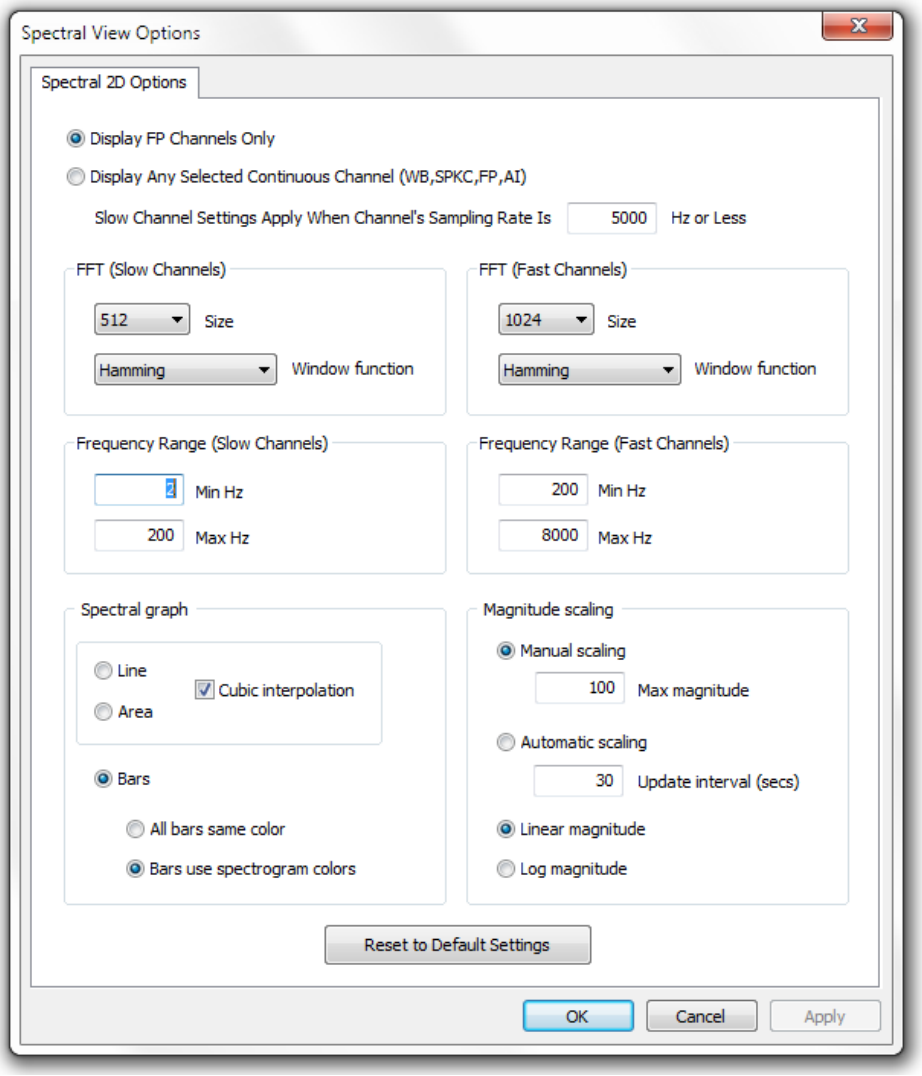
bin width = (sampling rate) / (FFT size)

The choice of window function affects spectral selectivity, side-lobe leakage pattern, and other factors. For most uses it can be left at the default (Hamming). Note that when you change the window function, you may need to adjust the amplitude scaling, unless you have enabled automatic scaling, in which case the amplitude scaling will be corrected at the next update interval.

Consult a reference on digital signal processing or spectral analysis for additional information on the tradeoffs involved in using different FFT sizes and window functions.

### Options dialog

Click on the **OPT...** button to display the **Spectral View Options** dialog for the Spectral view:




Several of these options will already be familiar from their equivalent toolbar buttons, or interactive mouse operations. These include the FFT parameters, the spectral graph display modes, and the frequency range. Others can only be set via the options dialog.

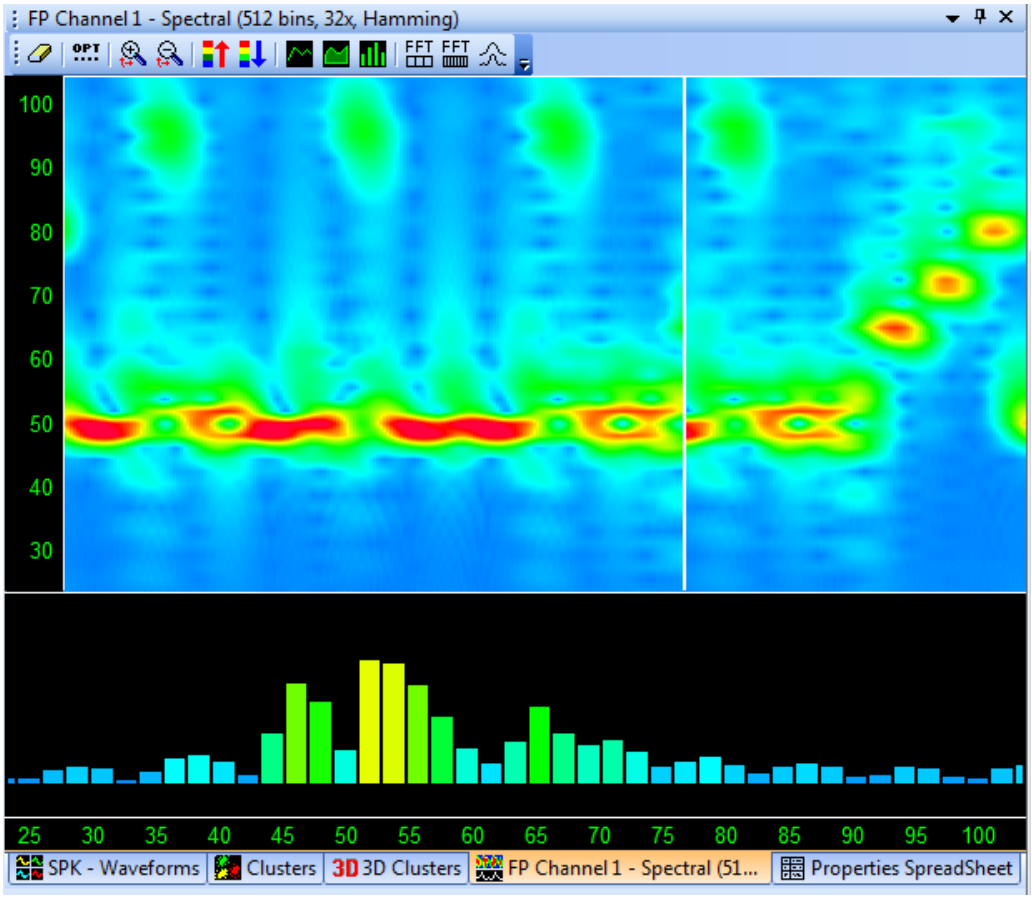
As you can see in the image above, spectrograms can either be “locked” to view only the FP source, or can view the spectra of any continuous source, including wideband (WB), spike-continuous (SPKC) or AuxAI (AI / AIF). The dialog has separate sets of options for “fast” and “slow” channels, and you can define the sampling rate “breakpoint” between fast and slow channels.

**Note:** When you update from previous versions of OmniPlex® software, your current Spectral 2D options will appear as the slow channel options.

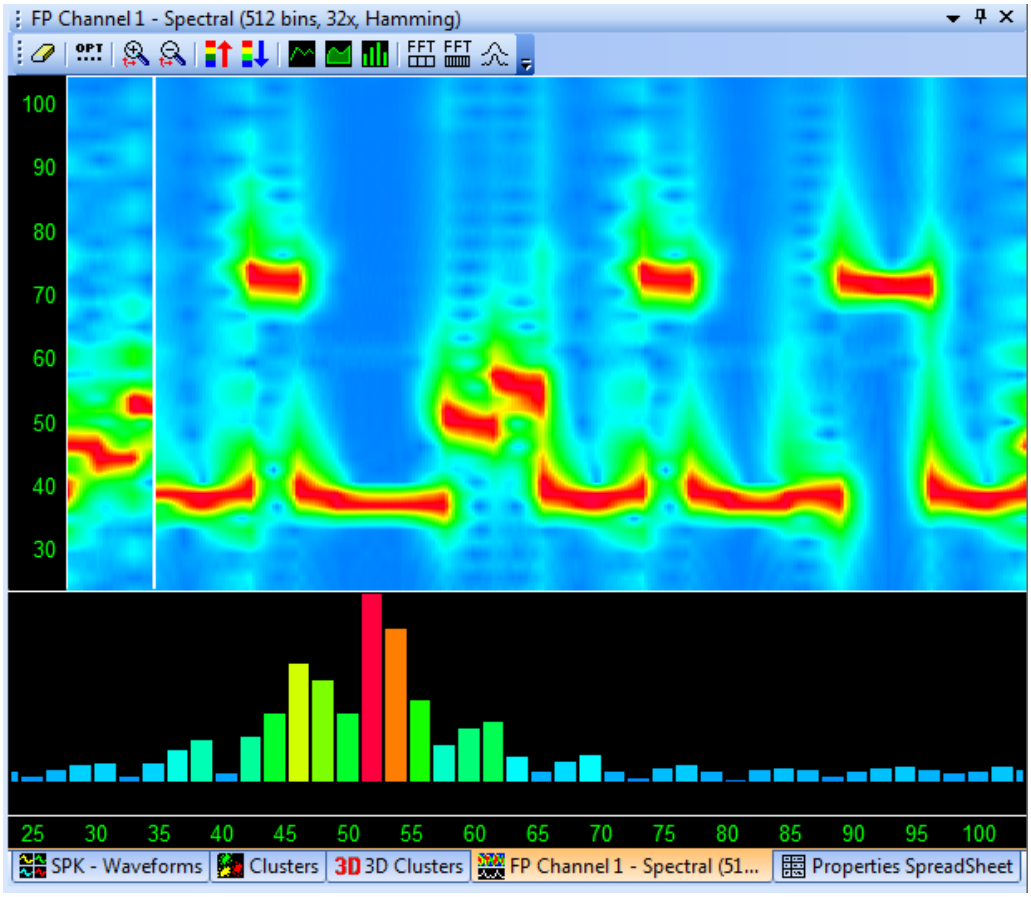
### Manual / automatic scaling

The default, manual scaling, allows you to control amplitude scaling using either the toolbar buttons or by specifying a numeric value. If you select automatic scaling, the OmniPlex System monitors the selected FP channel to determine its maximum amplitude in any frequency bin within a specified time interval, and periodically updates the amplitude scaling using that maximum value. For example, the default update interval of 30 seconds causes the amplitude scaling to be updated every 30 seconds, using the maximum amplitude detected within the preceding 30 seconds. This guarantees that the spectral displays will be "in the ballpark" without manual intervention, but it also means that the amplitude scaling could abruptly change when an update occurs. Note that the special case of an update interval of 0 seconds means that the amplitude scaling will be continuously updated; in effect, this makes the spectral displays show only changes in the *relative* spectral content over time. Here is an example of normal scaling and relative scaling.

Normal scaling:



Relative scaling:

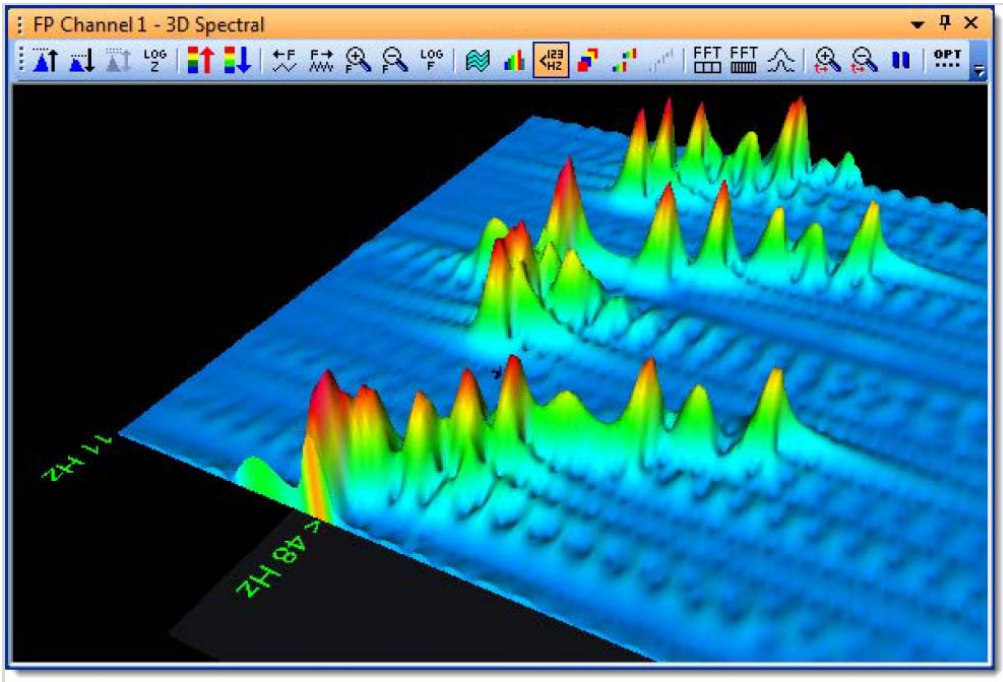



Note how with relative scaling of the spectrogram, at any given instant (x position), there is always at least one frequency (y position) that is displayed in red, i.e. scaled to maximum amplitude. The equivalent behavior in the spectral graph is that the graph will be continuously scaled such that at least one bin in the graph will always be at full height.

## 13.4 Spectral 3D View

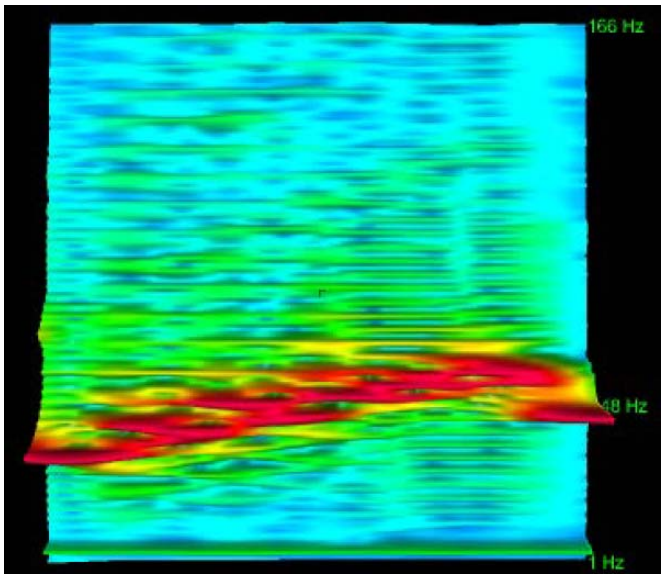
### Overview

The Spectral 3D view is a 3D generalization of the Spectral 2D (scrolling spectrogram) view. In the Spectral 2D view, spectral power at a given time (X position) and frequency (Y position) is displayed as a color, where blue represents low power and red represents high power. In the Spectral 3D view, power is displayed as the relative height of points on a 3D surface (Z position above the X/Y plane), and/or as a color. In other words, the same spectral information is presented in the 2D and 3D spectrograms, and both are based on a short time FFT (STFFT), but the 3D version can be more visually intuitive and expressive.



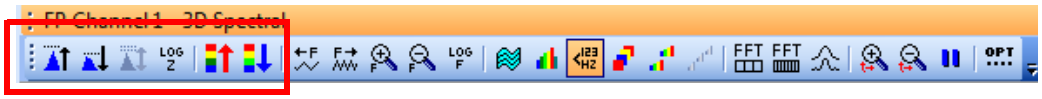

If you are not familiar with the Spectral 2D view, you may wish to consult Section 13.3, “Spectral 2D View” on page 396, since many of the concepts and features in the Spectral 3D view, such as FFT size and window functions, are the same, and will not be covered in detail here. Also, the basic 3D view manipulation techniques, such as rotating the view and zooming in and out using the mouse, are the same as in the other OmniPlex 3D views, such as the Clusters 3D and Spike Sample Histogram 3D views, and will only be mentioned briefly.

Note that the initial 3D viewing angle for the Spectral 3D view is “straight down,” as if you were looking at the flat Spectral 2D view.

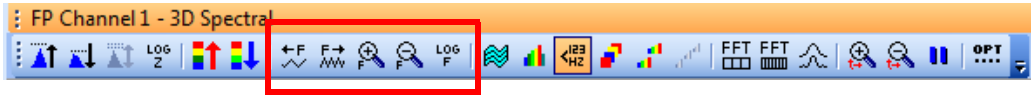



Press the left mouse button and move the mouse to rotate the view. Press the left mouse button, hold down the SHIFT key, and move the mouse to shift the view left/right/up/down. Use the mousewheel to zoom in and out. Clicking both mouse buttons together will reset the view to the default.

The features and options of the Spectral 3D view are accessed through the toolbar and the options dialog box. The following is a summary of the toolbar features:

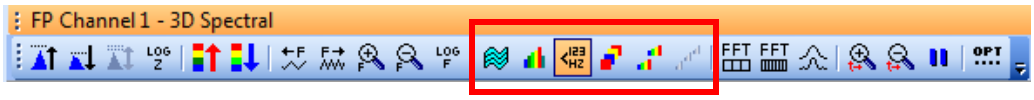


- Scale surface height up
- Scale surface height down
- (reserved for future use)
- Toggle logarithmic height mode
- Scale surface colors towards red
- Scale surface colors towards blue

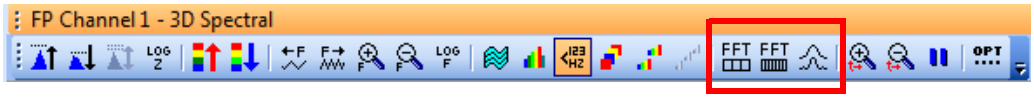



- Shift frequency range lower
- Shift frequency range higher
- Zoom in (narrower frequency range)
- Zoom out (wider frequency range)
- Toggle logarithmic frequency mode

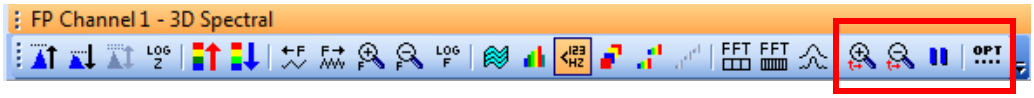
Note that unlike the Spectral 2D view, frequency zooming and shifting (offsetting) are done with toolbar buttons, not mouse moves in the display. This is because the mouse is already being used for 3D view manipulation.




- Toggle surface grid (see below)
- Toggle bar graph on front edge of surface (see below)
- Toggle peak-frequency indicator (tracks the FFT bin with maximum amplitude)
- Toggle surface/slice mode
- Decrease the number of FFTs displayed (default: 256, front to back)
- Increase the number of FFTs displayed




- Decrease the number of bins per FFT (coarser frequency resolution)
- Increase the number of bins per FFT (finer frequency resolution)
- Select FFT window function (each click cycles to the next window function)

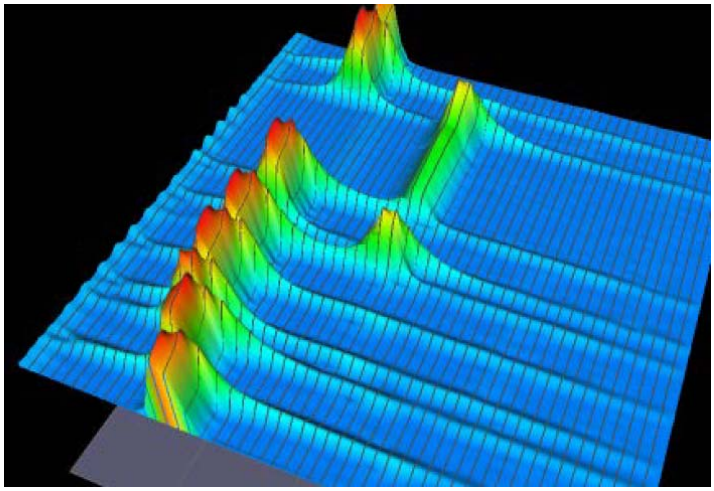



- Sweep faster
- Sweep slower
- Pause (only this display)
- Display the Options dialog (see below)

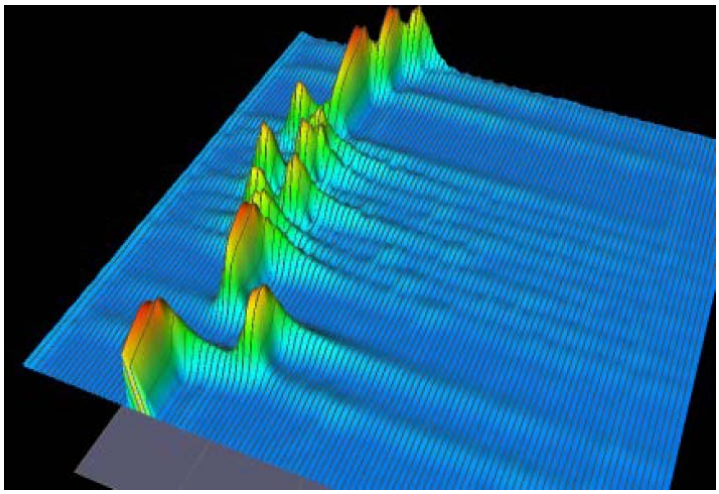
### Display features—surface grid

When the surface grid is enabled, the spacing between grid lines represents FFT bins. Note that for very large numbers of FFT bins, the grid is not drawn.

Fewer FFT bins:



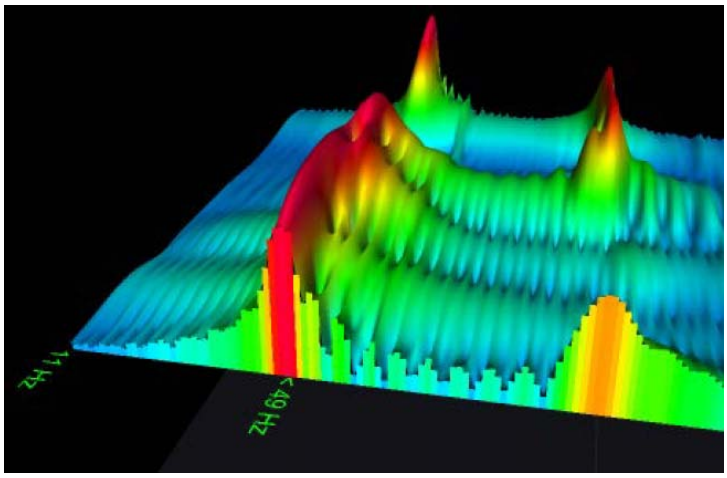
More FFT bins:



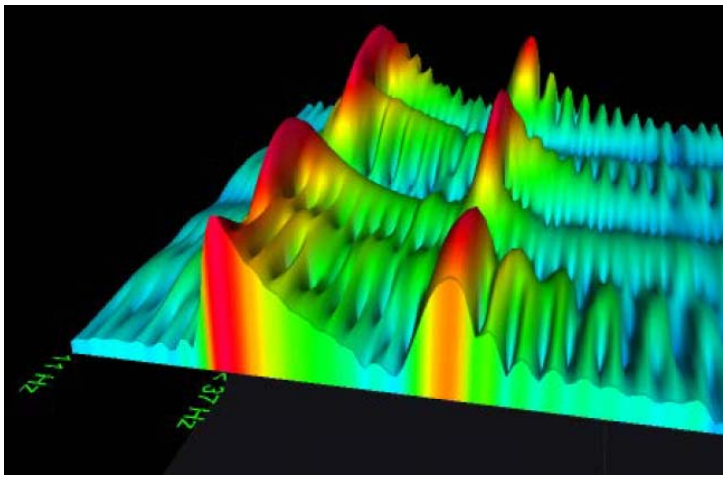
### Display features – bar graph

By default, the “front edge” of the 3D surface, representing the most recently acquired FFT, is displayed as a smoothly interpolated surface, with interpolated colors across its face. However, in bar graph mode, the FFT is shown as a bar graph, with each bar as a single solid color.

Bar graph mode:

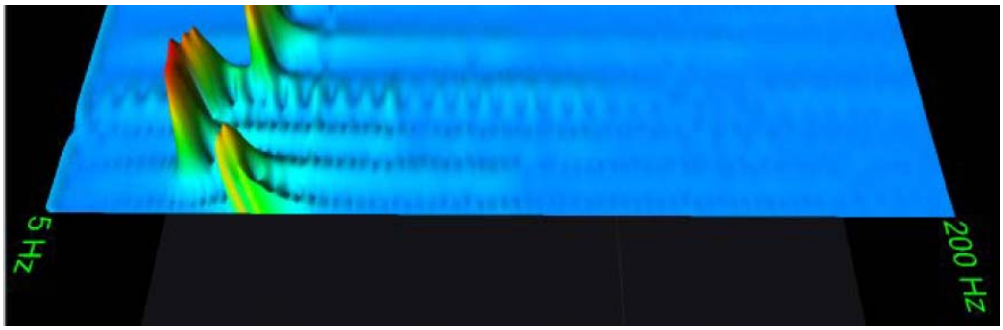



Default mode:

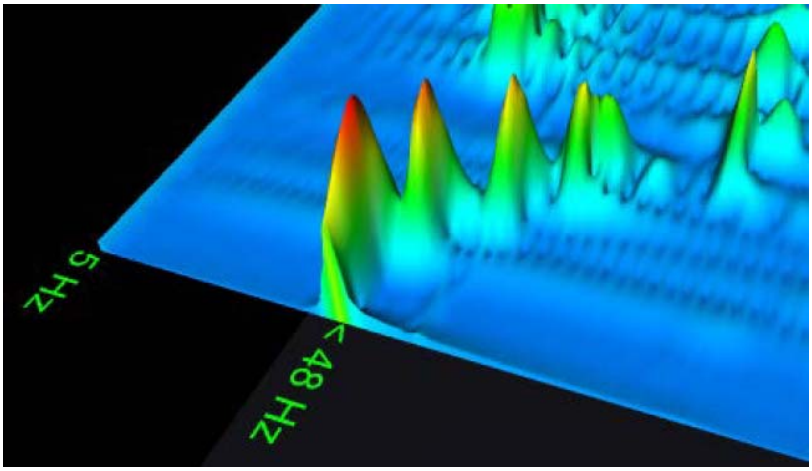


### Display features – frequency labels

Labels are shown at the two corners of the spectrogram which represent the highest and lowest frequencies displayed, at the most recent time, i.e. the newest FFT.

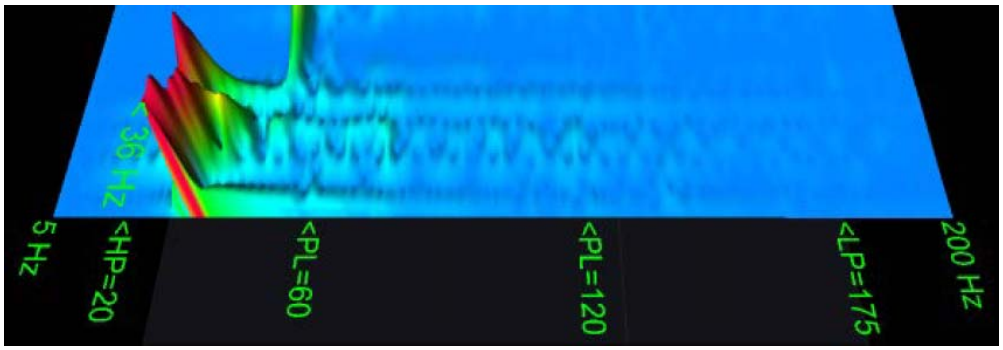



In addition, a toolbar button can be used to toggle peak frequency tracking, which displays a live indicator showing the frequency bin with the highest amplitude in the most recent FFT:



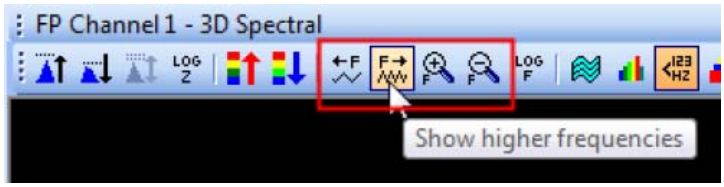

If highpass, lowpass filters, and/or the power line noise filter are enabled, you will see frequency labels for these items as well, as long as their frequencies are within the currently visible range. For example, here frequencies from 5 – 200 Hz are being displayed, with the following filters enabled:

- highpass filter at 20 Hz (“<HP=20”)
- lowpass filter at 175 Hz (“<LP=175”)
- power line noise filter at 60 Hz with second harmonic at 120 Hz (“<PL=60” and “<PL=120”)



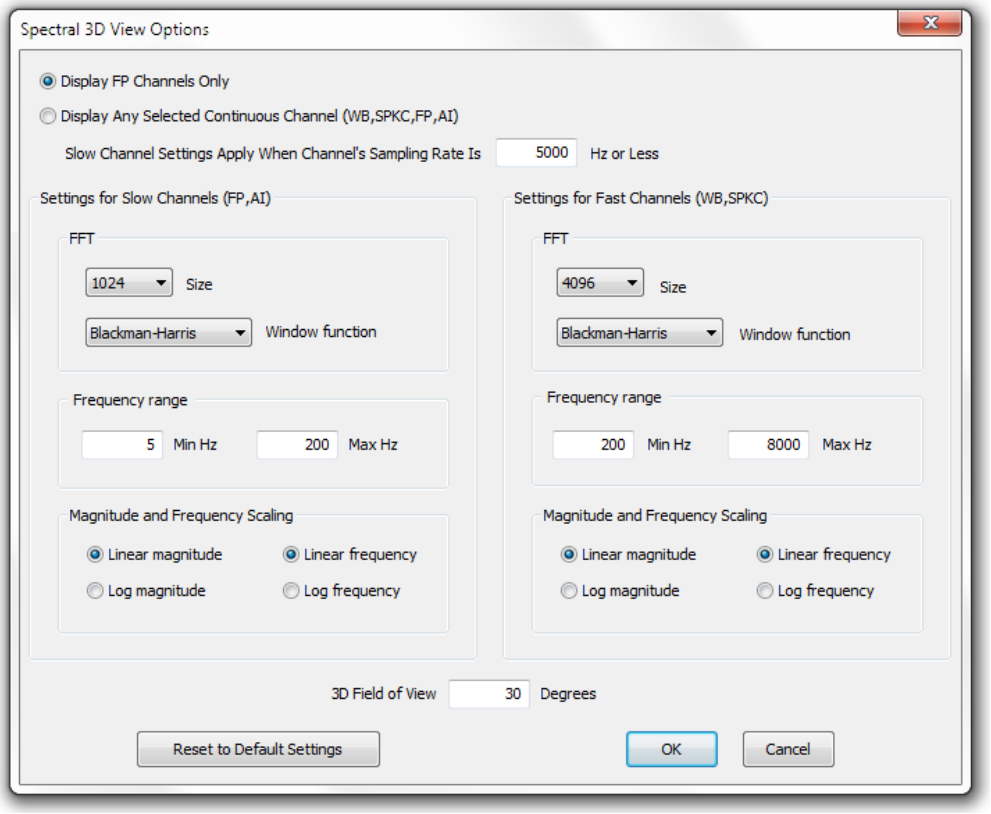

When the highpass, lowpass, and/or power line noise filters are labeled, note that the peak frequency indicator is drawn at the peak bin's height, rather than "on the ground," where it would be more likely to overlap the other labels. It is normal for the peak label to float up and down as the amplitude at the peak bin varies.

Remember that you can use the frequency zoom and shift controls in the toolbar to adjust the range of frequencies displayed, within the range of 0 Hz to half the sampling rate. For example, to shift the display towards higher frequencies:



### Spectral 3D options

The **Options** dialog contains settings for FFT size, window function, frequency range, and scaling of magnitude and frequency. Note that there is a logarithmic frequency scaling option which is not present in the Spectral 2D view; this is mainly useful when viewing the spectrogram of wideband or spike-continuous signals. Field potentials are commonly viewed on a linear frequency scale.



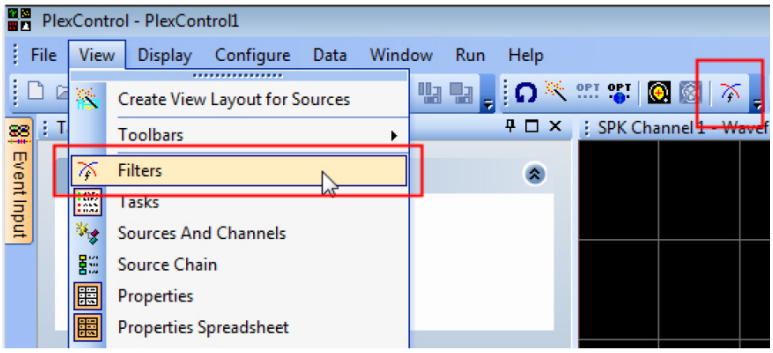

You can "lock" the spectrogram to the FP source with the **Display FP Channels Only** option, or allow it to follow the currently selected continuous source (WB, SPKC, FP, AI, or AIF) with the **Display Any Selected** option. In the latter mode, you can define separate settings for "fast" and 'slow" channels, based upon whether the selected continuous source's sampling rate is above or below a specified frequency. This removes the need to manually set appropriate frequency ranges, FFT sizes, etc, each time you switch between fast and slow sources.

The **3D Field of View** option allows you to in effect control the "camera angle," where larger values give a wide-angle lens effect, and smaller angles give a telephoto effect. You can think of larger field of view angles as "more perspective."

The **Reset to Default Settings** button restores all of the options in the dialog to their default values.

## 13.5 Filter Control Panel

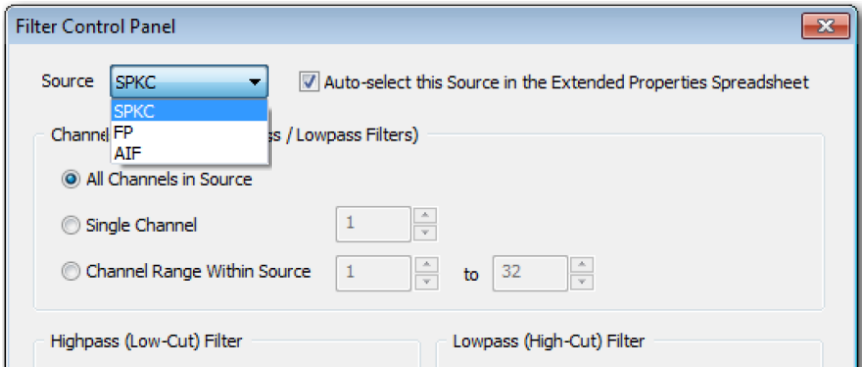
The Filter Control Panel (FCP) allows you to adjust the primary digital filters in the OmniPlex System without having to stop and restart data acquisition. It can be accessed either from the main toolbar in PlexControl, or from the **Views** menu.




### 13.5.1 FCP Controls

The FCP is a modeless dialog box, i.e., you can continue to interact with the rest of the OmniPlex user interface while the FCP is displayed, without having to repeatedly open and close the dialog. Note that data acquisition must be running in order to use the FCP.

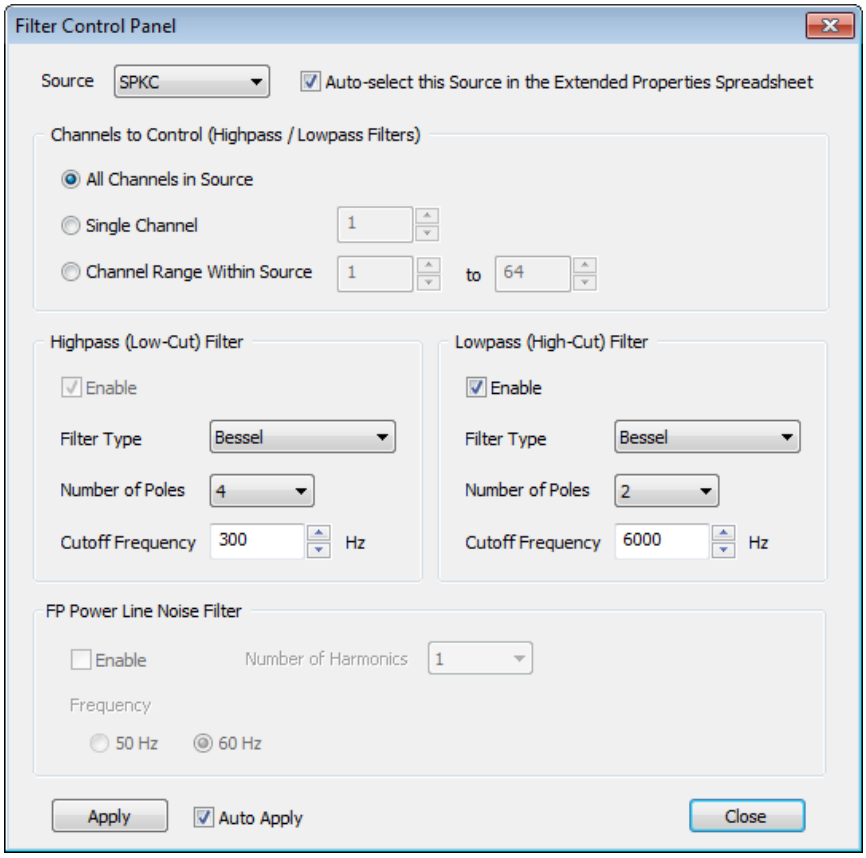
The **Source** is selectable from the dropdown list at the top:




The parameters for each of the sources (SPKC, FP and AIF) are the same, but each source (and optionally, each channel within each source) has its own independent set of parameter values. See the images and parameter descriptions on the following pages. Each source can have highpass and/or lowpass filters, and for the FP source, a power line noise filter is also available.

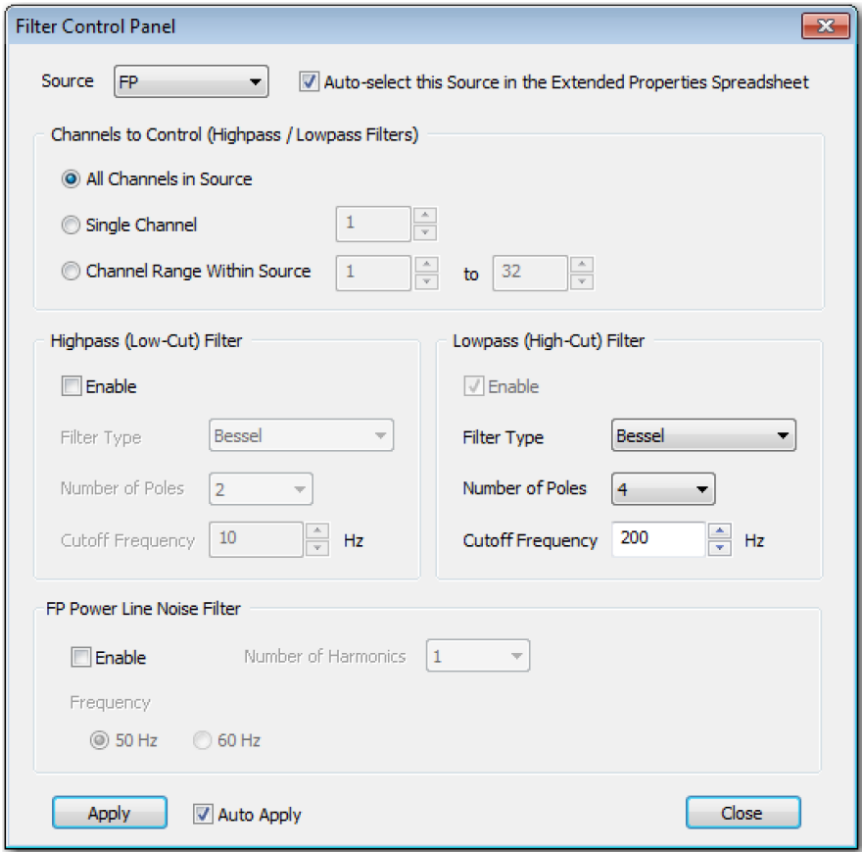
### FCP for spike (SPKC) source

For SPKC, the highpass filter is always enabled, but the lowpass filter can be enabled or disabled as desired.



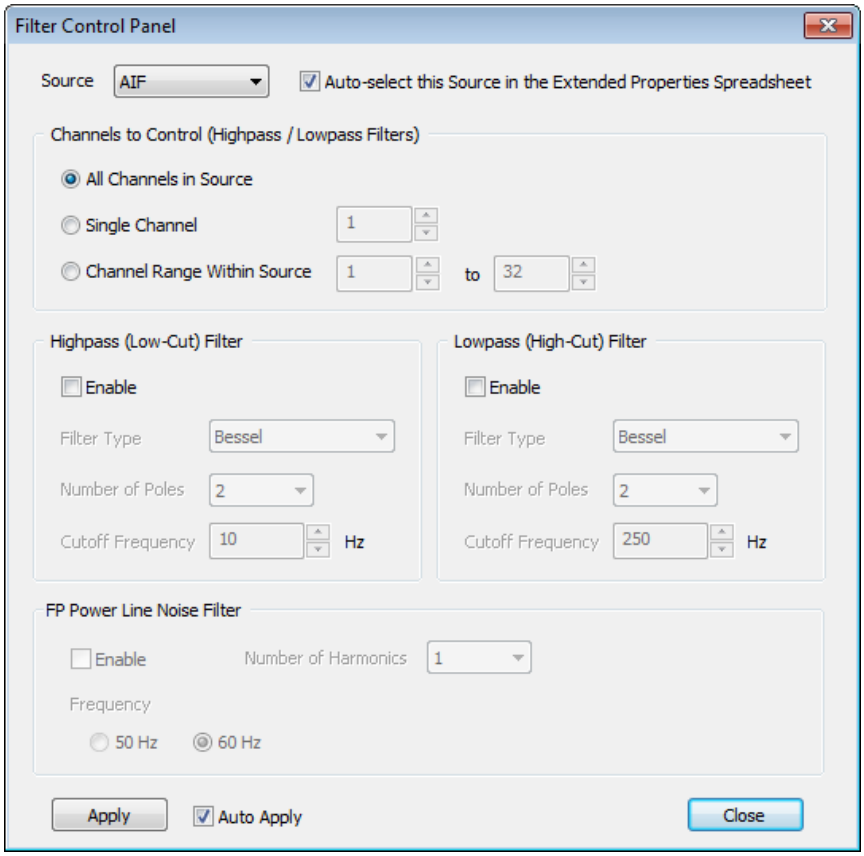
### FCP for field potential (FP) source

For FP, the lowpass filter is always enabled, but the highpass and power line noise filters can be enabled or disabled as desired.



### FCP for AuxAI Filter (AIF) source

For AIF, the highpass and lowpass filters can both be enabled or disabled as desired.




### Auto-select this Source in the Extended Properties Spreadsheet

If you select this checkbox, the **Extended Properties Spreadsheet** will automatically select the same source as you select in the FCP. This allows you to see the per-channel filter settings for all the channels in a source at the same time. (For information on the spreadsheets, see Section 4.2, “Properties Spreadsheet and Extended Properties Spreadsheet” on page 108.)

### Channels to Control

Below the **Source** dropdown in the FCP dialog is the **Channels to Control** section, which determines which channels on the selected source are affected when you change the highpass and/or lowpass filter settings.

By default the first option, **All Channels in Source**, is selected, so any changes you make will be applied to all the channels on the selected source. This is equivalent to changing the filter settings in the **Device Options** in the topology in Server.

The second option, **Single Channel**, will apply any filter settings changes to only the selected channel. For example, you might have two or more channels with

similar signals, and by changing the filtering on only one of them, you can observe the differences between the channels as you adjust the filter settings.

The third option, **Channel Range Within Source**, causes changes to the filter settings to be applied only to the specified range of channels. This can be useful when, for example, different headstages acquire different types of neural signals, with different spectral characteristics or different noise profiles, or when different types of electrodes are being used on different headstages.

If you apply different settings for different channels within a source, you can see a list of the settings for all channels in the **Extended Properties Spreadsheet**.

#### Highpass and lowpass filters

For the **SPKC** source, the highpass filter cannot be disabled, since it is used to remove field potentials from the wideband signal. The lowpass filter is optional. A typical use would be to remove high-frequency noise.

For the **FP** source, the lowpass filter cannot be disabled, since it is used to remove spikes from the wideband signal. The highpass filter is optional. A typical use would be to remove low frequency motion artifacts or near-DC drift.

For the **AIF** source, both the highpass and lowpass filters may be enabled or disabled as desired. Note that the AIF source itself is not present by default, and must be enabled when a topology is created, as described in a later section.

#### FP Power Line Noise Filter

The **FP Power Line Noise Filter**, if enabled, always applies to all channels on the FP source, regardless of the setting of the **Channels to Control** options. For a description of this filter, see FP Adaptive power line noise removal on page A-9.

#### Auto Apply

When the **Auto Apply** checkbox (at the bottom of the dialog box) is enabled, any change that you make using the FCP is immediately applied to the selected source and channels.

Note that the one exception for **Auto Apply** is that when you manually type in a numeric value for a filter cutoff frequency, these changes are not applied until you either click on a different control, or click the **Apply** button. To understand why this is the case, consider an example where you type in the value 300 for the SPKC highpass frequency. As you type the digits “3,” “0,” “0,” the filter cutoff would first be 3 Hz, then 30 Hz, then 300 Hz. It would be distracting (and in some cases nonsensical) to change the cutoff frequency to these “partially entered” values.

### 13.5.2 Uses of the Live Filter Adjustment

Here are some examples of how you can use the ability to adjust the filters in real-time while viewing the signals (continuous, spike, and spectrograms) to easily observe the effects and tradeoffs of different filter settings on spikes and field potentials.

Gradually lowering the cutoff frequency of the SPKC highpass filter will at some point allow unwanted field potentials into the SPKC signal, causing visible baseline “wobble” and making it difficult to reliably detect spikes. Changing the filter type and number of poles will also affect the degree of removal of FPs.

In addition, the SPKC filter settings can affect the shape of spikes; for example, the default Bessel filter causes the least spike shape change, but has a more gradual rolloff (for a given cutoff and number of poles) than the Elliptic filter, which has a sharp rolloff but causes more shape distortion, such as overshoot on steep edges. This can also affect the shape and separation of clusters in feature space.

Enabling the SPKC lowpass filter (e.g. to remove high frequency noise) and then reducing its cutoff frequency will result in smoother, less noisy spikes, but when the cutoff is reduced too far, you will see spike shapes become less distinct from each other, and the corresponding feature space clusters will become poorly separated.

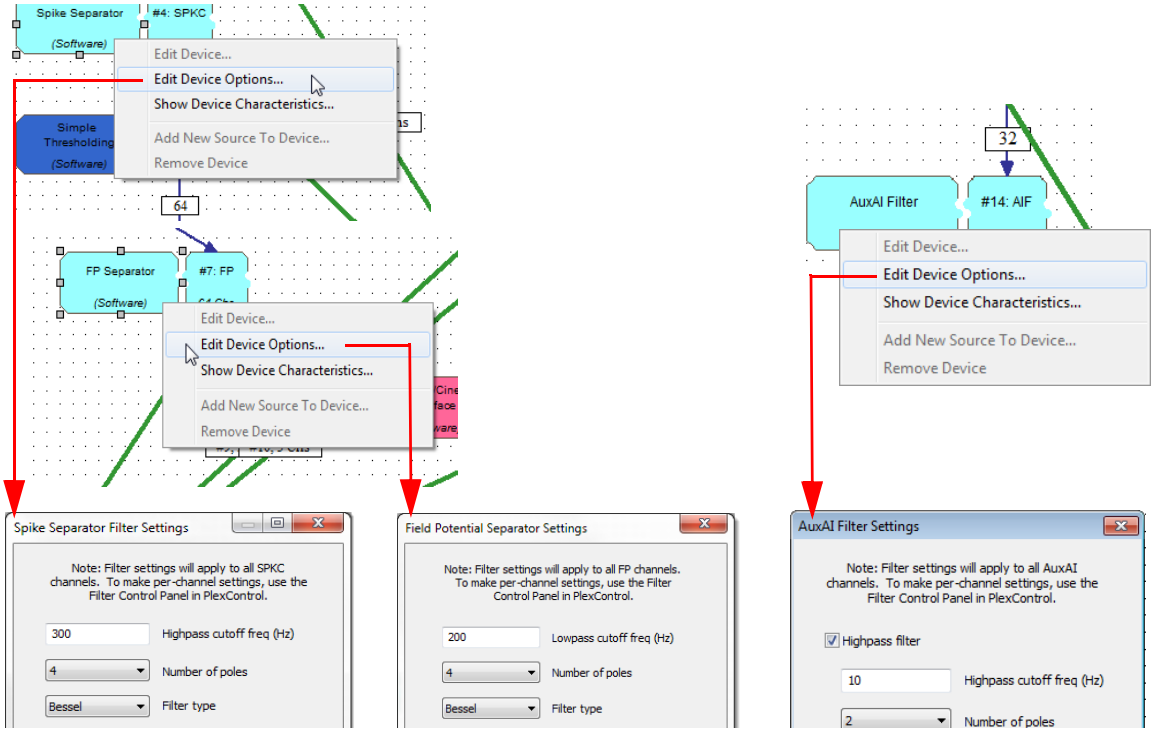
For field potentials, the lowpass filter must remove spikes, which you can verify by comparing the FP and SPKC signals while adjusting the cutoffs. Also, spectral energy above the Nyquist rate (half the downsampled rate, e.g. 500 Hz for a 1 kHz sampling rate) must be removed before the FP signal is downsampled, or aliasing (“foldover” of high frequencies into the lowest frequencies) will result. You can observe the FP spectrogram views while adjusting the FP lowpass cutoff to verify that there is no significant spectral energy at frequencies at or above the Nyquist rate.

### 13.5.3 Setting highpass/lowpass filters in Server vs. FCP

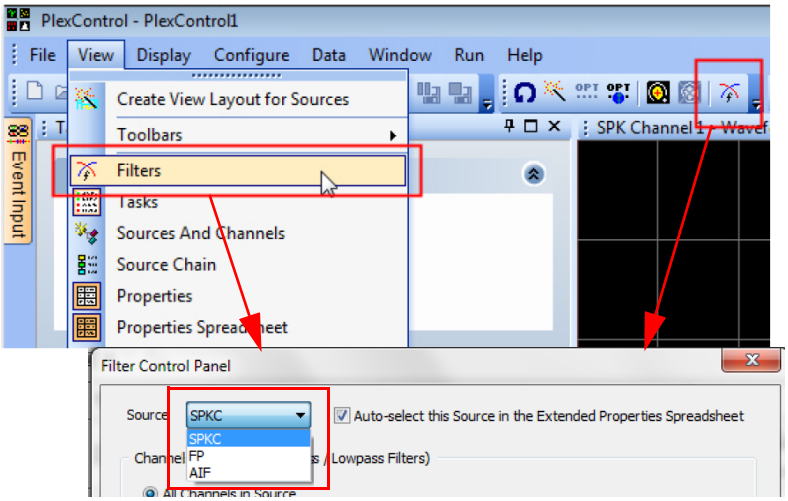
The highpass and lowpass filter parameters in the FCP (type, number of poles, and cutoff frequency) are exactly the same as the ones set with the **Edit Device Options** commands for the **FP Separator**, **Spike Separator**, or **AI Filter** devices in Server. Note that the filter settings in Server act as default values which are in effect unless you override them using the FCP in PlexControl.

The diagrams below show how to access device options in Server and PlexControl (with the FCP dialog).

Accessing Device Options in Server




Accessing Device Options in PlexControl using the Filter Control Panel



It is important to note that if you change the filter settings in the **Device Options** for the **FP Separator**, **Spike Separator**, or **AI Filter** devices with the **Edit Device Options** function in Server, these settings will apply to all channels on the corresponding device, and will override any per-channel filter settings that were made previously with the **Filter Control Panel**.

Also, note that the SPKC, FP, and AIF filter settings are saved in the .pxc file, and loading a .pxc with customized filter settings will also override any filter settings which were in effect before the .pxc was loaded.

### SPKC and FP separation—additional information

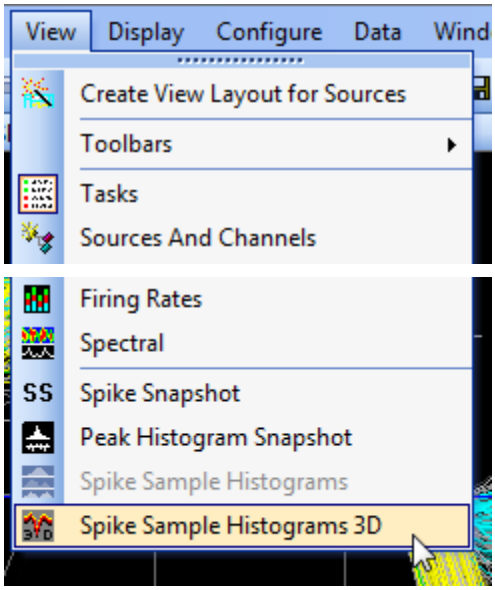
See "Appendix B: Separation of Spikes and Field Potentials Using Digital Filters" on page A-4.

### AuxAI Filter—additional information

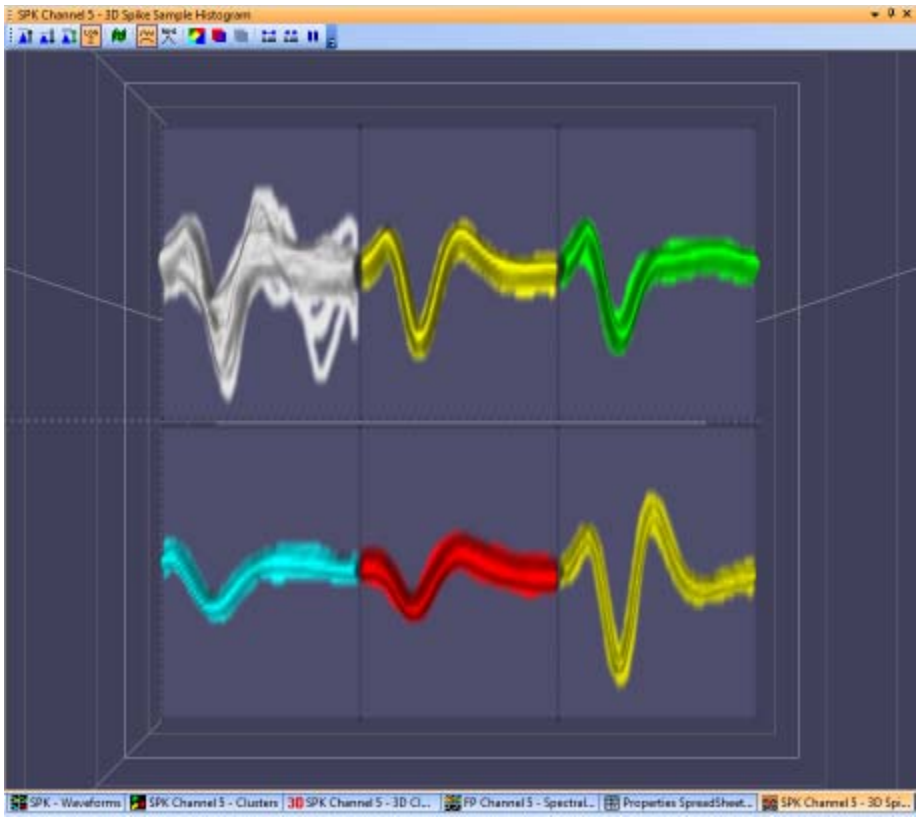
See Section 12.5, “AuxAI Filter (AIF)” on page 375.

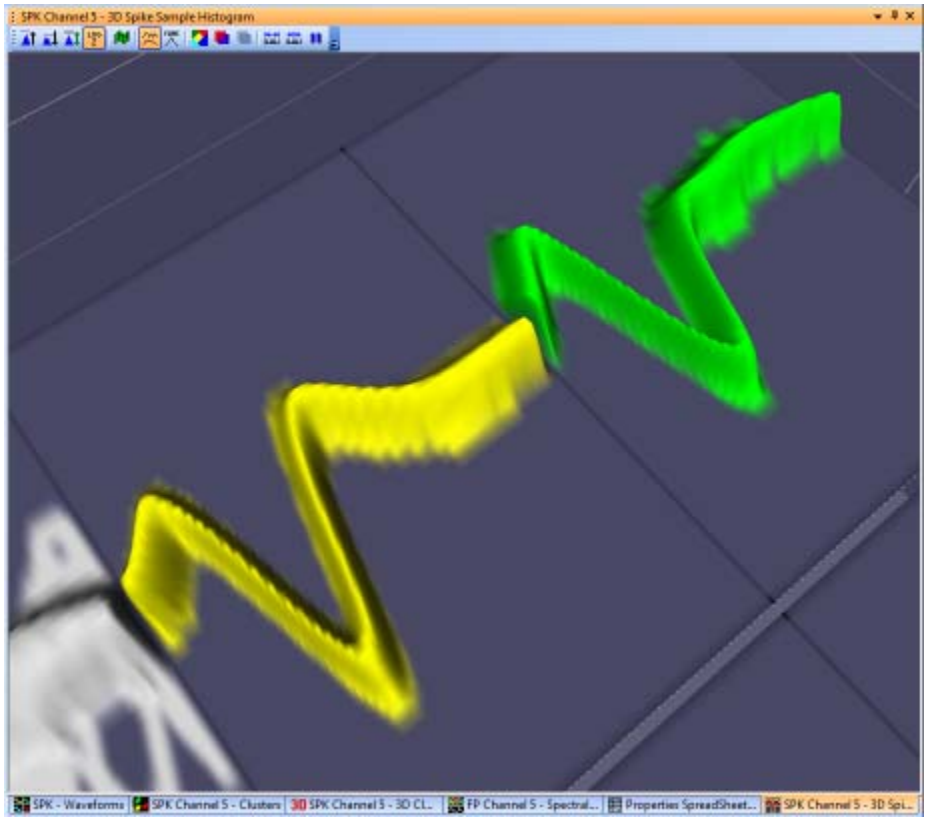
## 13.6 Spike Sample Histogram 3D View

The Spike Sample Histogram 3D view (SSH3D) provides an alternative form of visualization for the currently selected spike channel. It displays the same spikes as the standard main spike window, but in a way that models the distribution of spike sample (amplitude) values and the firing rate of each sorted or unsorted unit as one or more solid or semi-transparent 3D surfaces. To display the SSH3D view, select it from the PlexControl View menu:



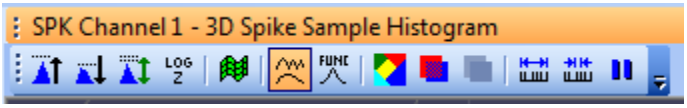

The default view angle looks straight down on the sample histograms, but you can rotate and zoom the display to view the histograms as desired, using the same mouse actions as the 3D PCA view.


SPK Channel 5 - 3D Spike Sample Histogram
SPK - Waveforms
SPK Channel 5 - Clusters
SPK Channel 5 - 3D Cl...
FP Channel 5 - Spectral...
Properties SpreadSheet...
SPK Channel 5 - 3D Spi...

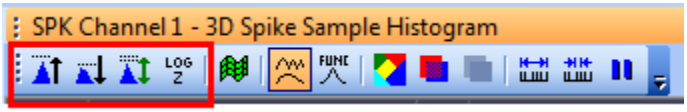

SPK Channel 5 - 3D Spike Sample Histogram
SPK - Waveforms
SPK Channel 5 - Clusters
SPK Channel 5 - 3D CL...
FP Channel 5 - Spectral...
Properties SpreadSheet...
SPK Channel 5 - 3D Spi...

Or, for an automatic “tour” of the histograms, click anywhere in the view and press the “F” key to enter an automatic “flyaround” demo mode. Press “F” again to stop the flyaround.

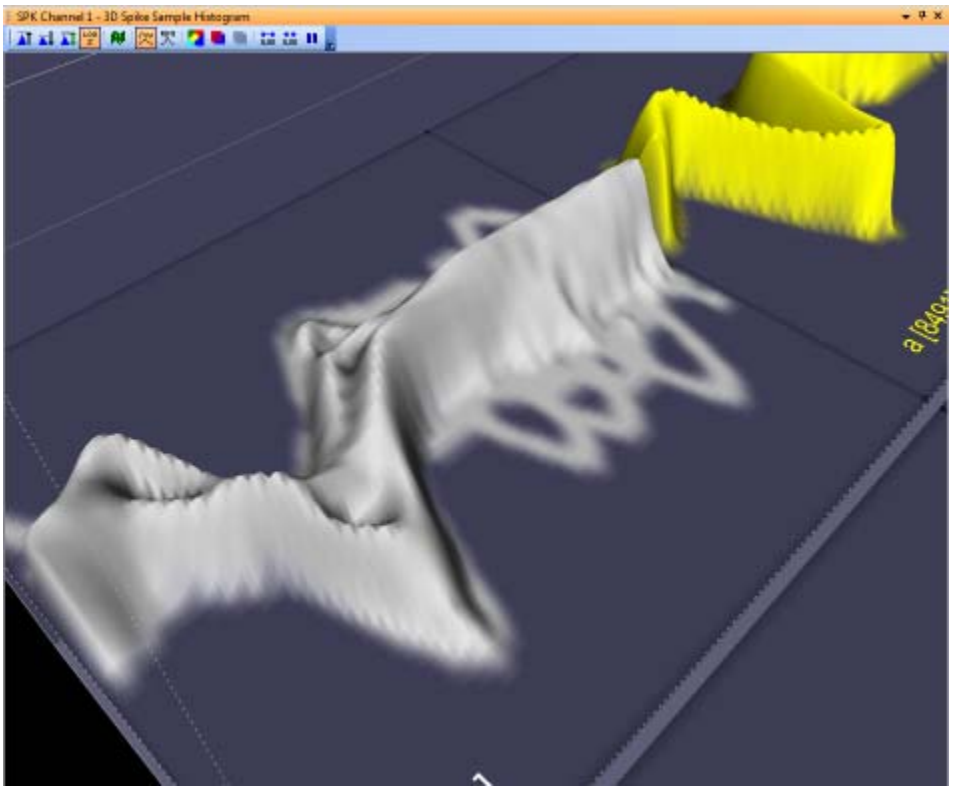
The dropdown toolbar for the SSH3D contains a number of options that are useful for adjusting the parameters used to calculate and display the sample histograms.



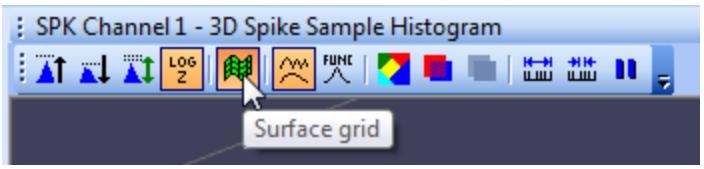

The leftmost four buttons adjust the vertical scaling of the histogram surfaces; in left to right order, they are *Scale up, Scale down, Auto-scale*, and *Log scale*:



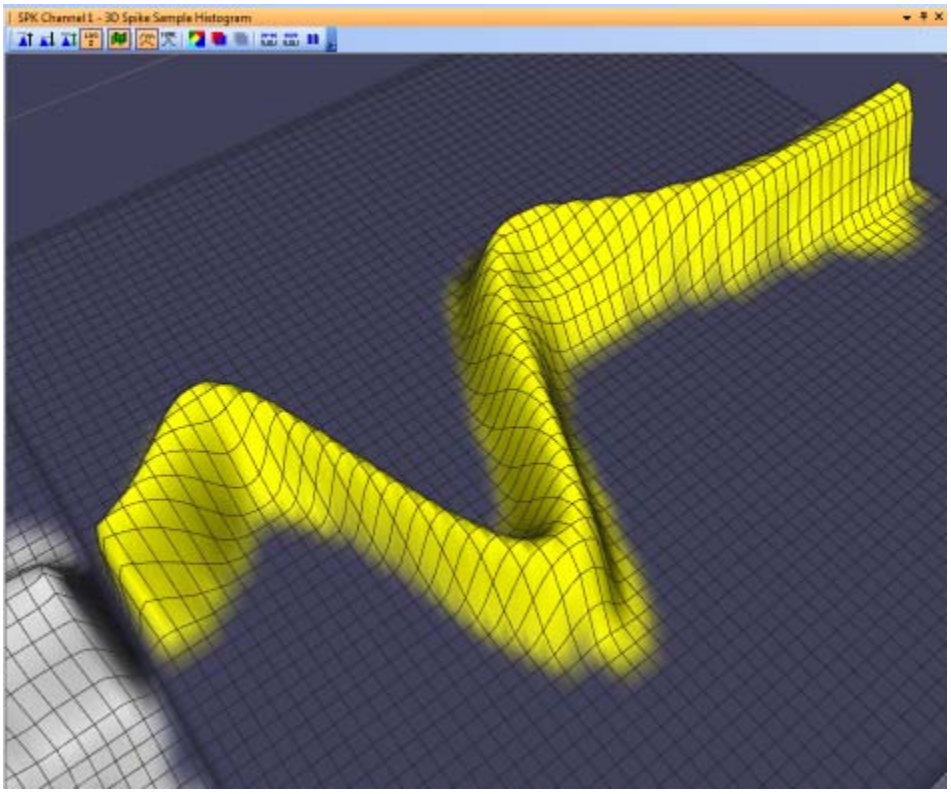

For example, after increasing the vertical scaling and zooming in:



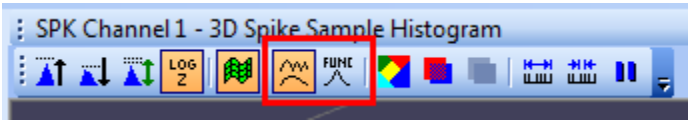
The *Surface grid* button toggles the drawing of a grid on the histogram surface:



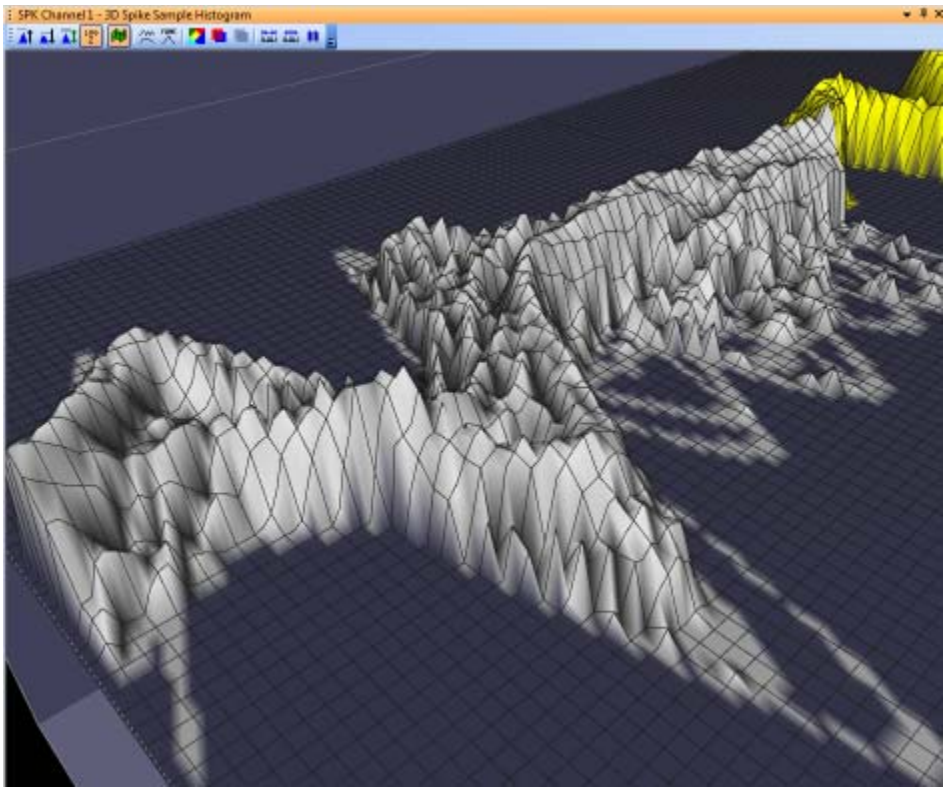

This can help emphasize the shape of the surface:

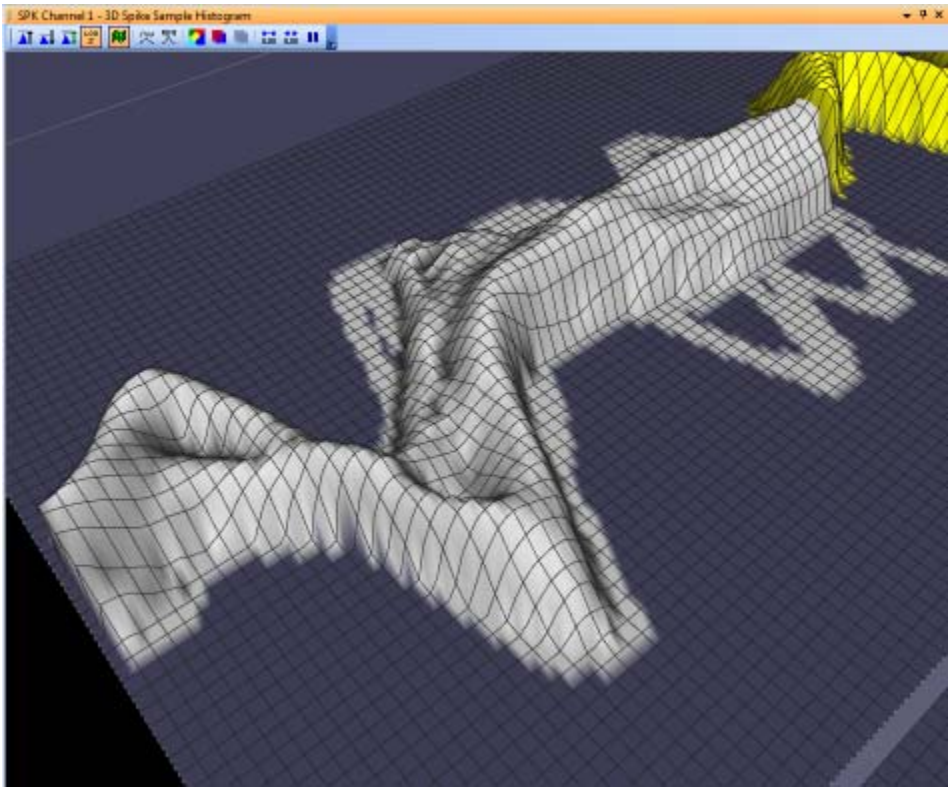


The next two buttons toggle surface smoothing on and off, and select the kernel function that is used to create the histogram from the spike waveform sample values.

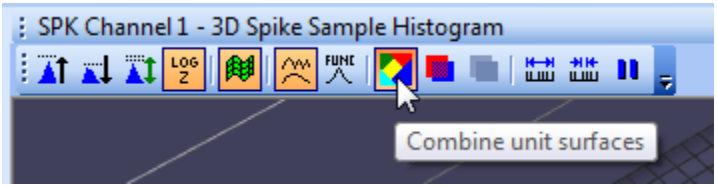


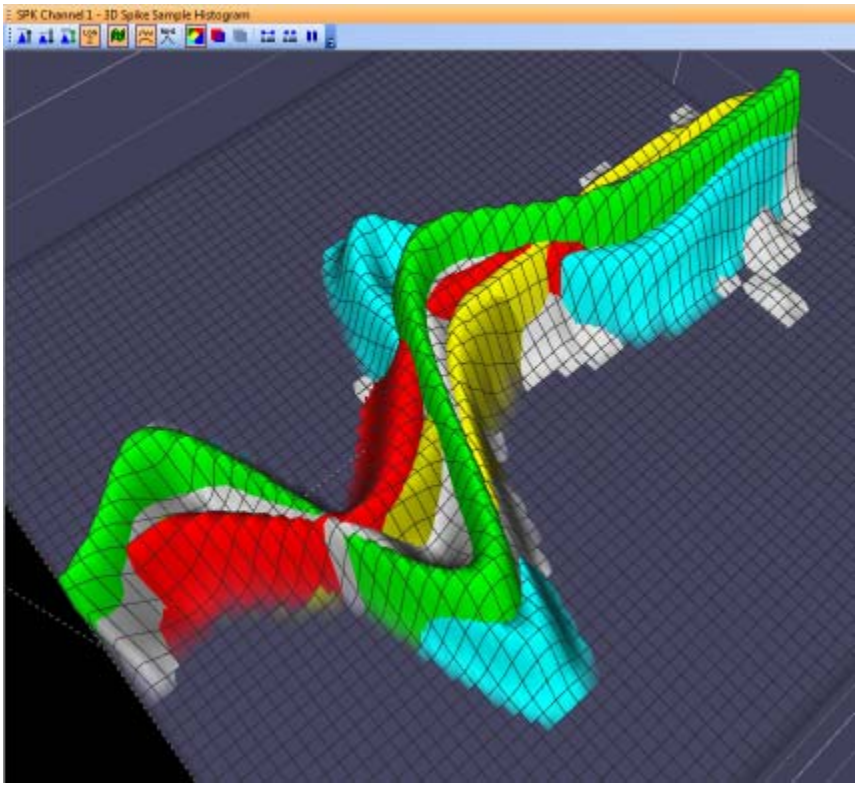

Each time you click the *Kernel function* button, the next function, from a group of five, is selected; the differences are more apparent when you disable surface smoothing.


SPK Channel 1 - 3D Spike Sample Histogram

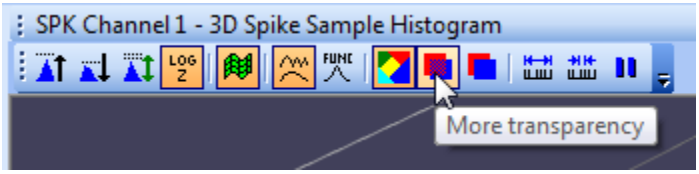

SPK Channel 1 - 3D Spike Sample Histogram

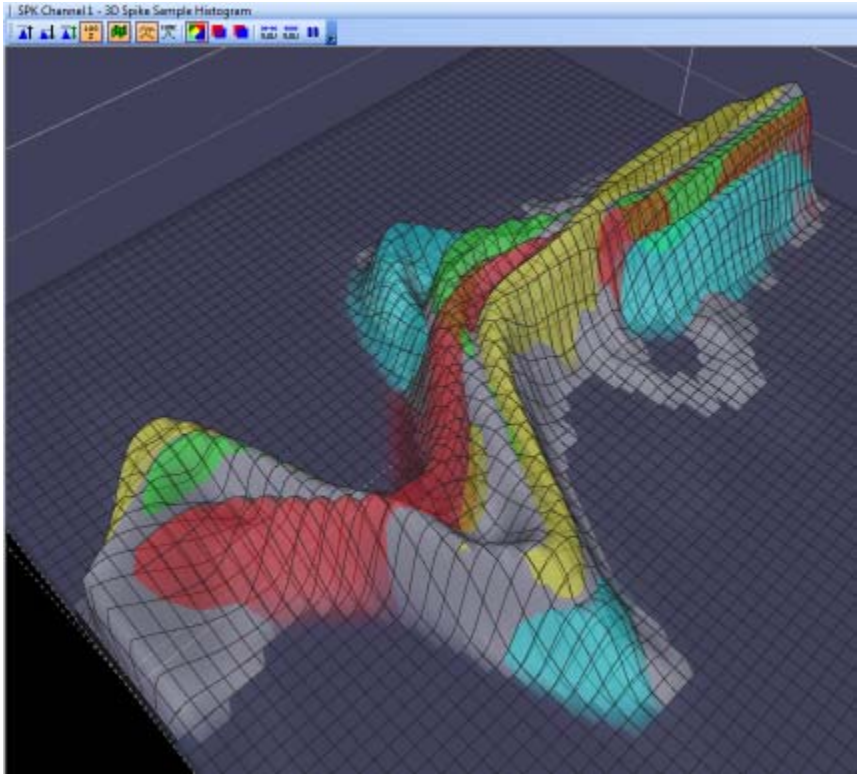
So far, the SSH3D has shown each unit's histogram separately. If you click *Combine unit surfaces*, all the units (and the unsorted unit) are shown superimposed on each other:



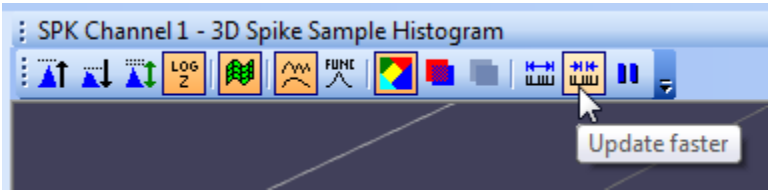
When *Combine surfaces* is enabled, the next two buttons allow you to control the degree of transparency of the surfaces, allowing you to "see through" the outer histograms:



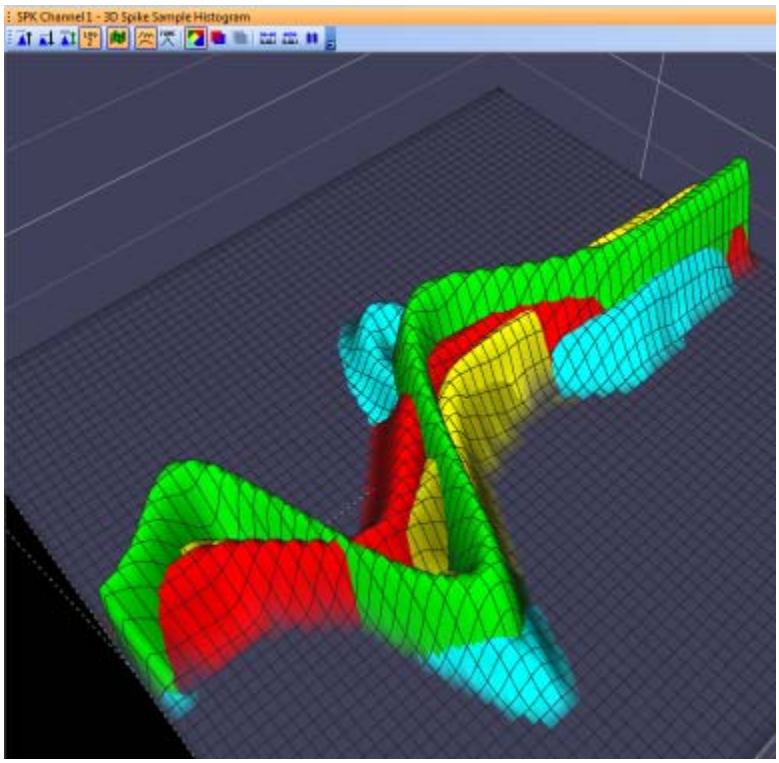


The rightmost three buttons can be used to make the animated histograms update more slowly, faster, or to pause the animation. The fastest update rate will most closely follow changes in the firing rates of the displayed units, while a slower update rate tends to smooth out short-term fluctuations in activity.

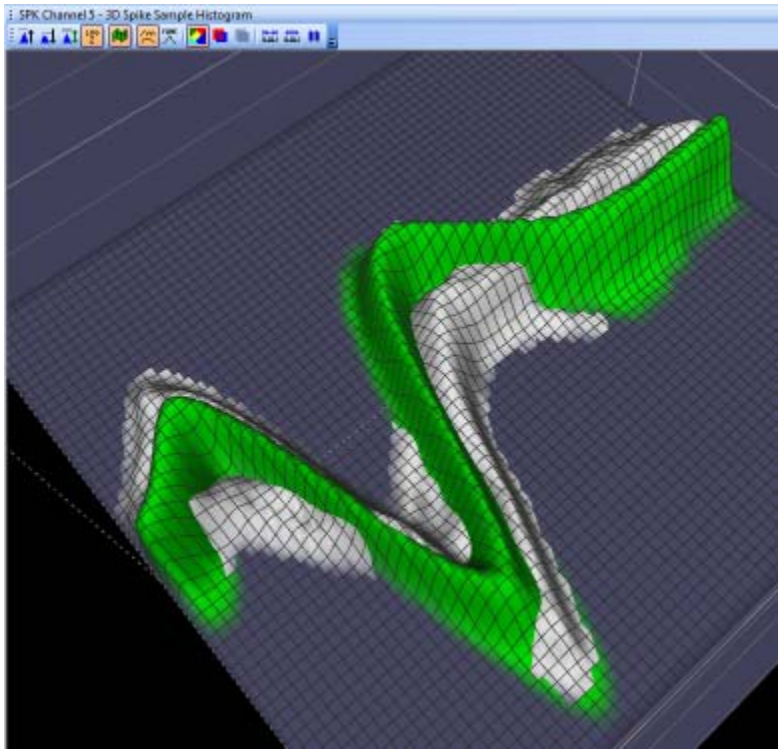
Note that the *Pause* button here is independent of the *Pause* button in the main PlexControl toolbar, so that the SSH3D view can be paused without affecting any other displays.




Note that the spike Show Filter in the main PlexControl toolbar also affects the SSH3D view. For example, setting the Show Filter to *Sorted* causes only sorted spikes to be displayed in the SSH3D:




The other Show Filter modes have analogous effects, e.g. Selected Unit + Unsorted:



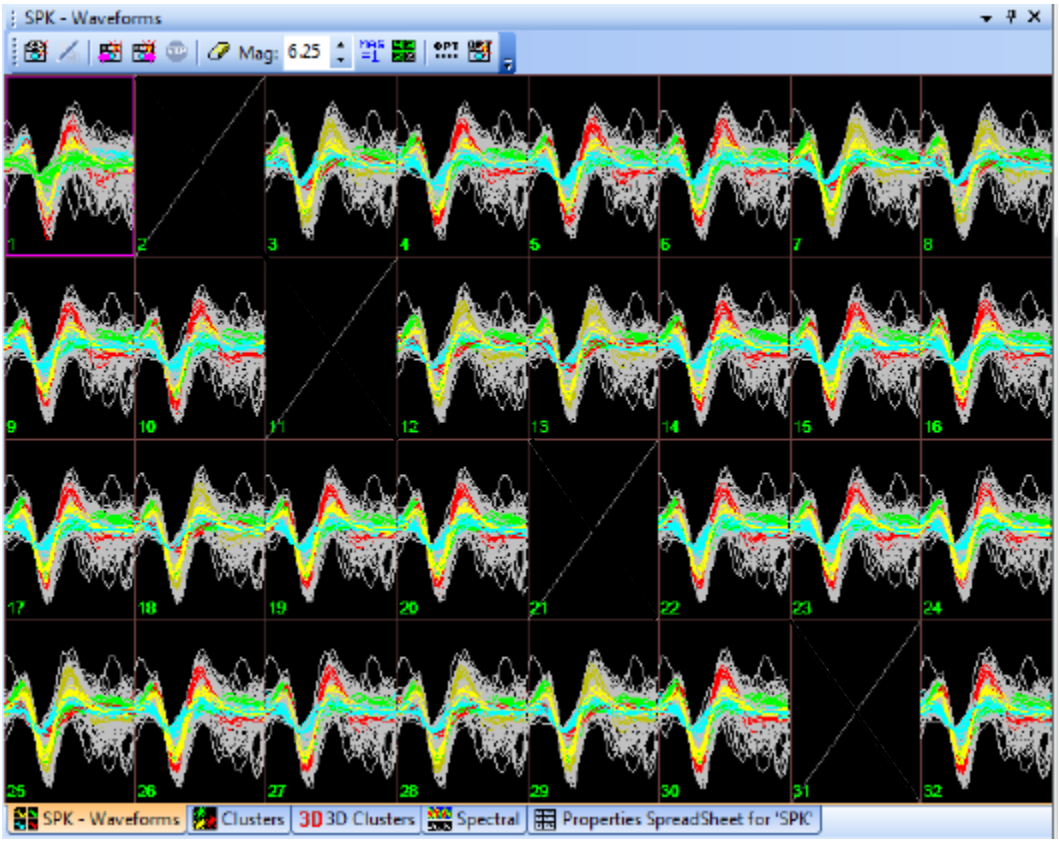
The SSH3D provides intuitive visual feedback about the quality of unit definitions and the relative activity of the different units on a channel. The unit displayed in green above is a good example of what a well-defined unit might look like, with a Gaussian distribution centered on a well-defined “spine” corresponding to the mean waveform.

Note that in stereotrode and tetrode modes, the SSH3D currently only displays the first channel of each stereotrode or tetrode.

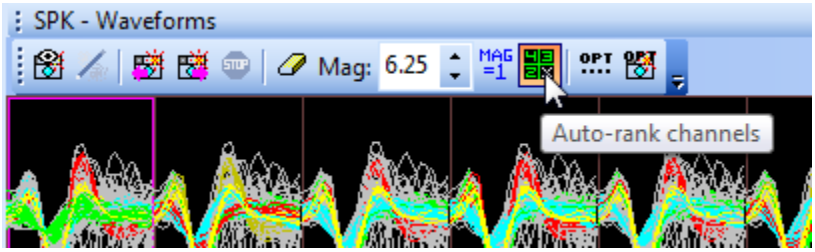
## 13.7 Channel Ranking

The channel ranking feature allows the order of display in the multichannel spike, spike-continuous (SPKC), wideband (WB), and field potential (FP) views to be determined by one or more selected criteria. For example, channels can be ranked such that the channel(s) with the largest number of sorted units is/are displayed first. This can help draw your attention to “interesting” channels and reduces display clutter, especially at higher channel counts and/or if you have a significant number of inactive channels.

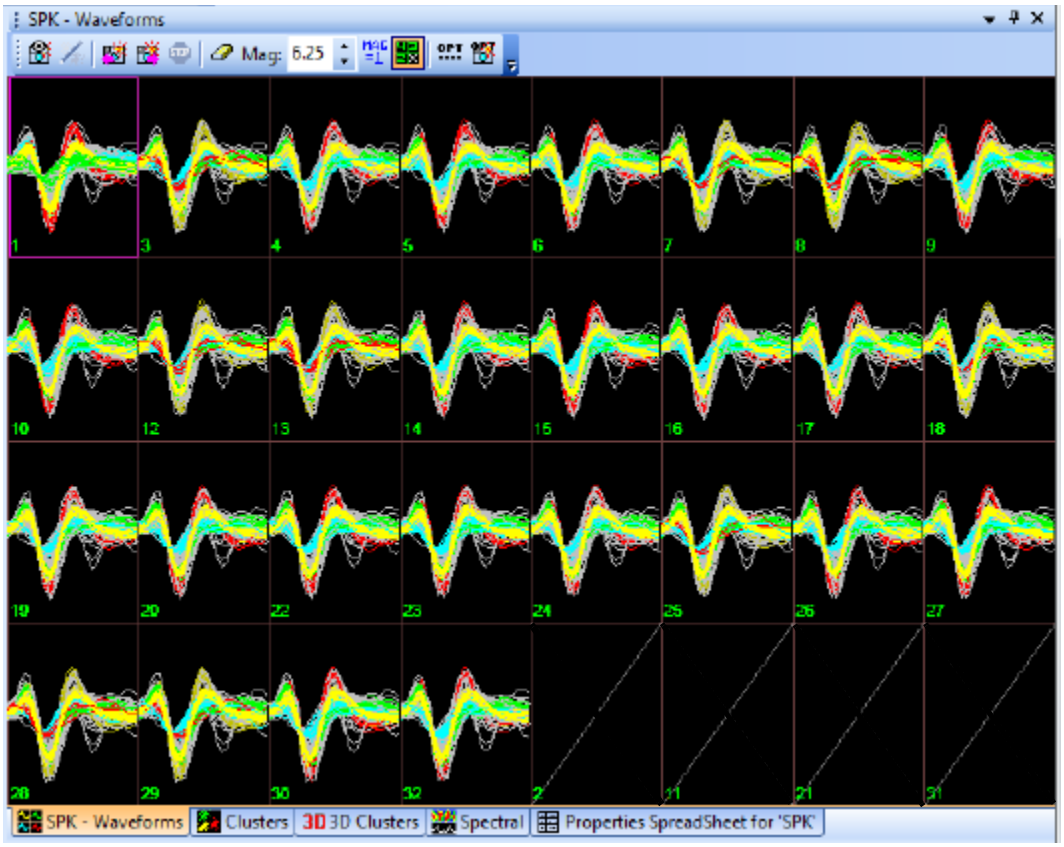
In its simplest form, channel ranking can be used to place all disabled channels last in display order. By default, disabled channels are displayed like this:



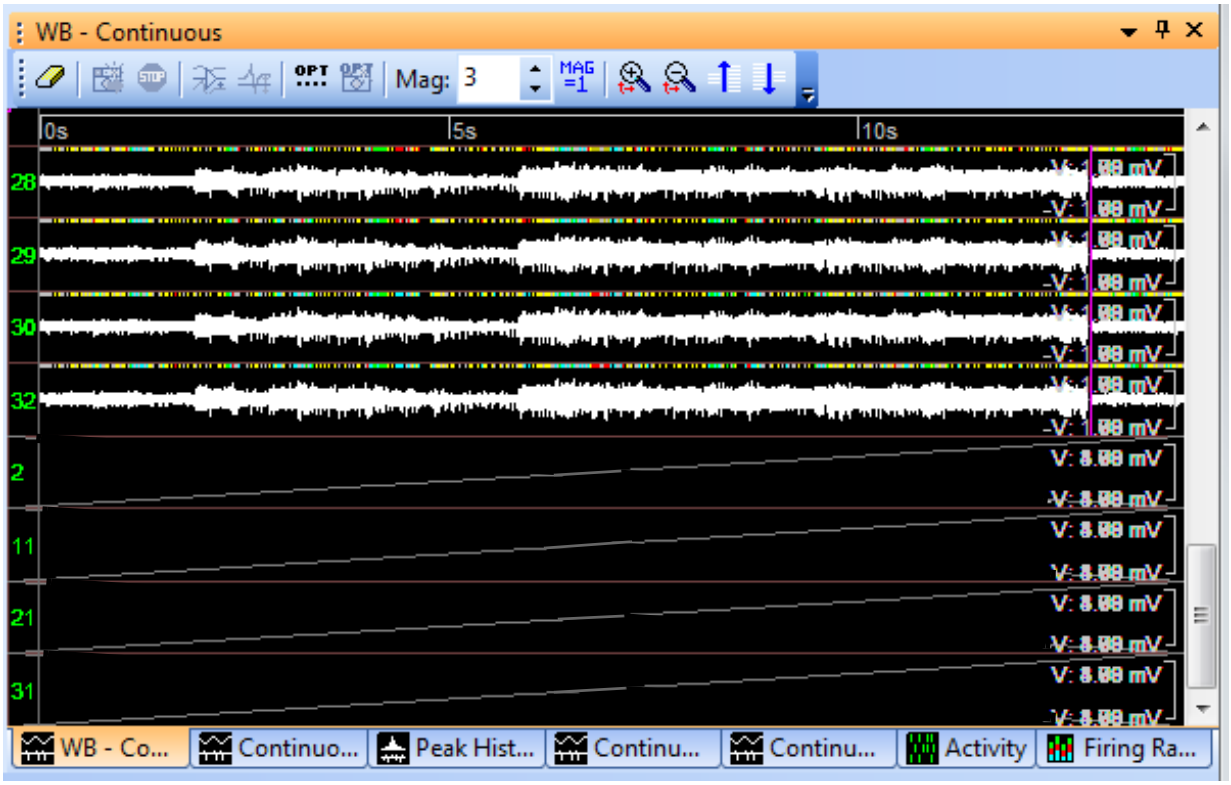

Click on the **Auto-rank channels** button in the multichannel spike window’s toolbar to enable channel ranking:



With channel ranking enabled, disabled channels are pushed to the end of the display:

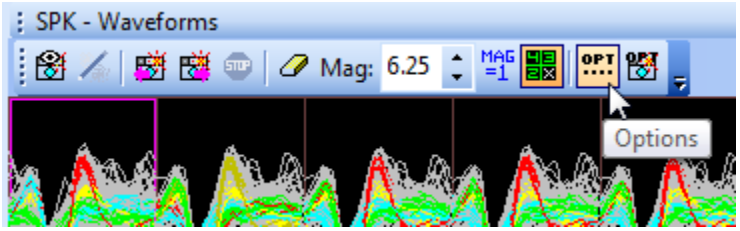


The WB, SPKC, and FP continuous views will display their channels in the corresponding order (channels 1-27 are not visible here but are in normal order above channels 28-32):

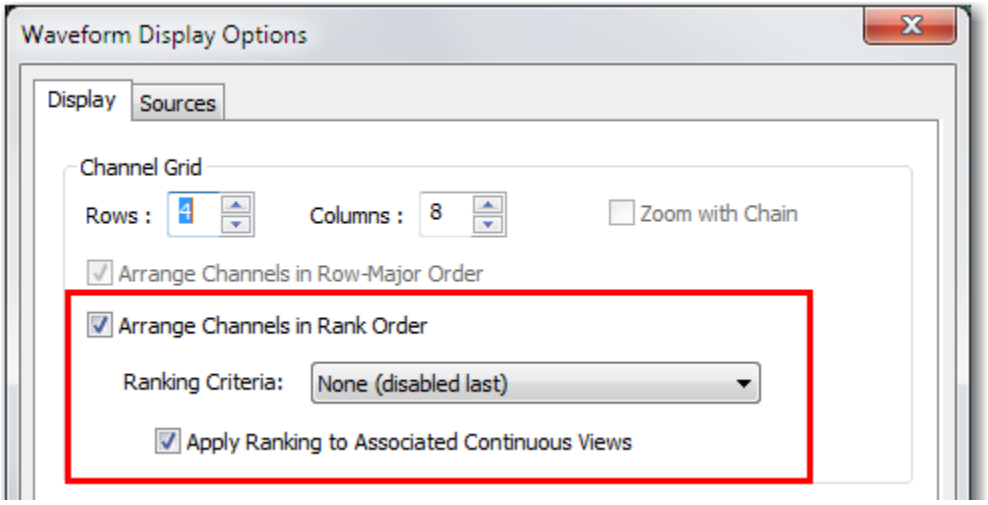



Click the **Auto-rank channels** button again to return to the original unranked channel order. Note that channel ranking does not affect the data itself, the numbering or order of channels in recording files, clients, etc. It is purely an option for organizing the multichannel displays. Also note that the channel ranking is always a function of the channels in the SPK source; for example, if you disable different channels in the SPK and FP sources, the disabled SPK channels will determine the channel ranking.

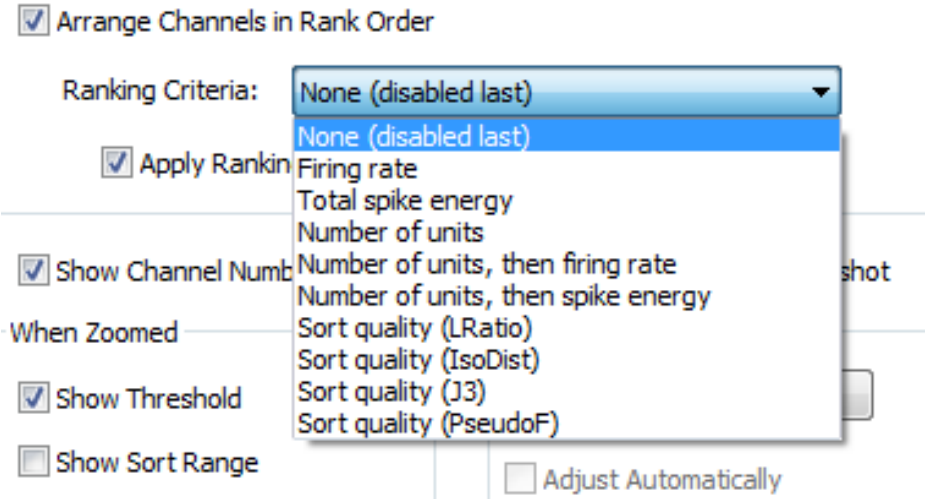
Channel ranking can be based on criteria other than (or in addition to) channels' disable status. To see the available ranking options, click on the **Options** button in the spike window toolbar:




The **Waveform Display Options** dialog is displayed, with the channel ranking options in the upper area as highlighted below:




**Arrange Channels in Rank Order** has the same effect as the toolbar button, i.e. you can use either to toggle channel ranking on and off. **Apply Ranking to Associated Continuous Views** determines whether the channel ranking in the multichannel spike (SPK) window is applied to the WB, SPKC, and FP views. The **Ranking Criteria** dropdown list allows you to use additional criteria in determining the channel ranking.




**None** means that no additional criteria are used, and only disabled channels determine the ranking. The remaining criteria are based on the most recent spike snapshot, so you must take a snapshot before using them.

**Firing rate** is the per-channel mean firing rate, within the snapshot. For example, for the default 500 spikes per channel snapshot, the mean firing rate of each channel would be:

MeanRate[channel] = 500 / (TimestampOfLastSpike[channel] – TimestampOfFirstSpike[channel])

**Total spike energy** for a channel is the sum of the individual spike energies for all spikes in the snapshot, where a spike's energy is defined as the sum of its squared waveform sample values. If different channels have the same number of spikes in their snapshots, the respective total spike energies will depend entirely on the relative spike energies. If different channels have different numbers of spikes in their snapshots, either because the snapshot was taken "by time" instead of "by count," or if one or more channels' snapshots timed out before their count was reached, then the total spike energies will depend on both the spike energies and the number of spikes in the snapshot.

**Number of units** is the number of sorted units on each channel. The three remaining criteria are based on number of units first, and then within channels that have the same number of units, firing rate or total spike energy are used as a secondary ranking criteria. For example, *Number of units, then spike energy* would assign the highest ranking to the channel with the most sorted units *and* the largest spikes.

The system can rank the channels according to a specific sort quality parameter—**LRatio**, **IsoDist**, **J3** or **PseudoF**. For definitions of these parameters, see Section 8.10, "Spike Sorting Quality Metrics" on page 290.

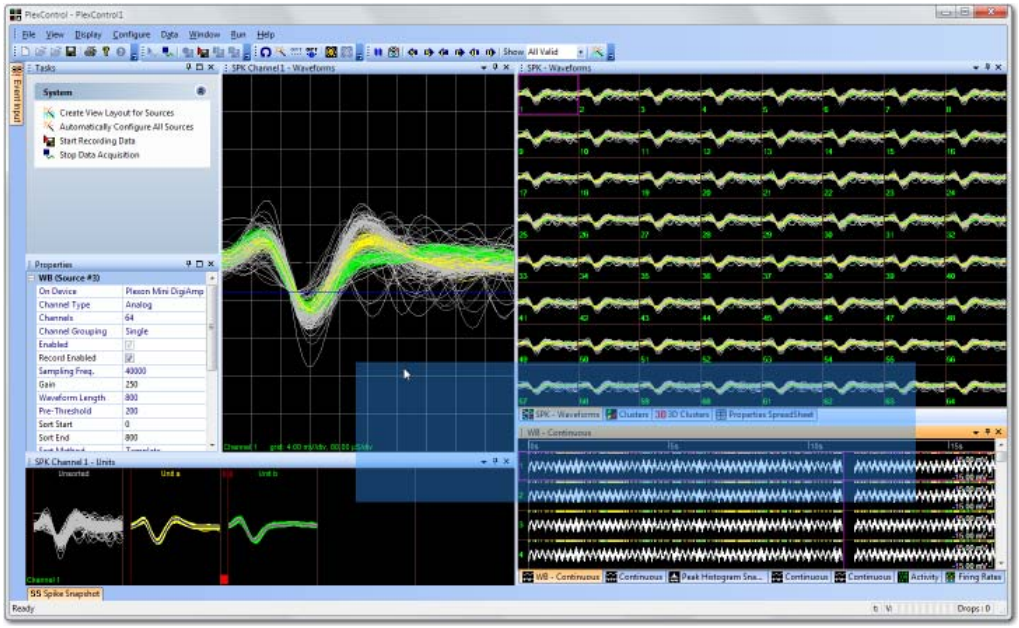
Note that channel ranking is not dynamically updated, i.e. channels do not "move around" in the multichannel display in real-time as their firing rates or number of sorted units change. In the case where disabled channels are displayed last but no other ranking criteria are being used, you can refresh the display order by clicking the Channel Ranking button twice, to disable and then re-enable ranking. For cases where criteria based on the spike snapshot are being used, you must take a new spike snapshot before toggling the ranking off and then back on. When the ranking is re-enabled, it will use the most recently taken spike snapshot.

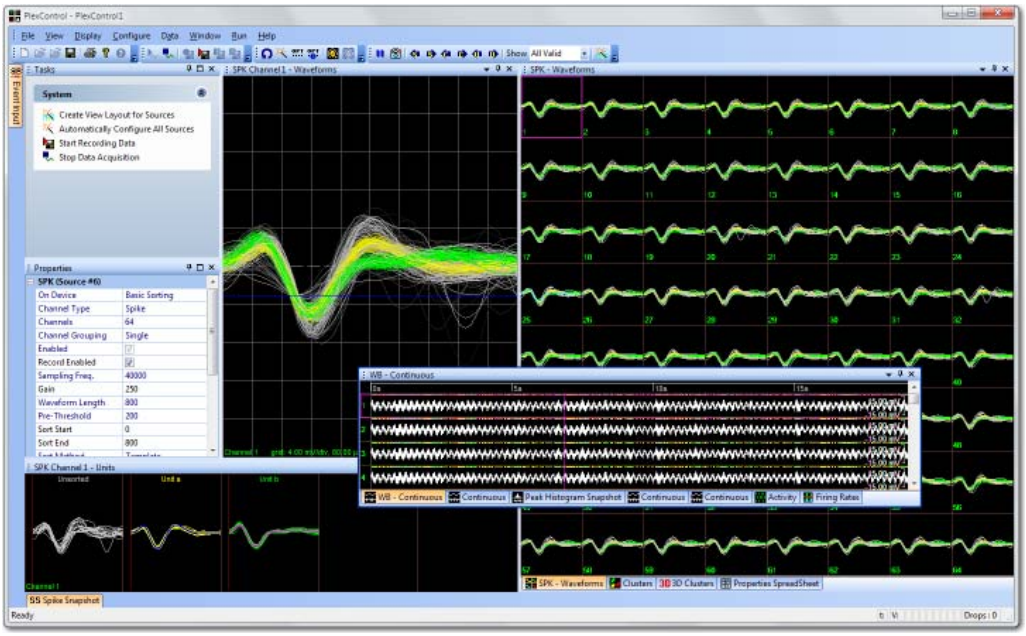
## 13.8 Advanced User Interface Features

The basic features of the OmniPlex System user interface are described in the main body of this user guide. The following more advanced features are handy for users who wish to more extensively customize the layout of views and toolbars in PlexControl.

### Floating a window

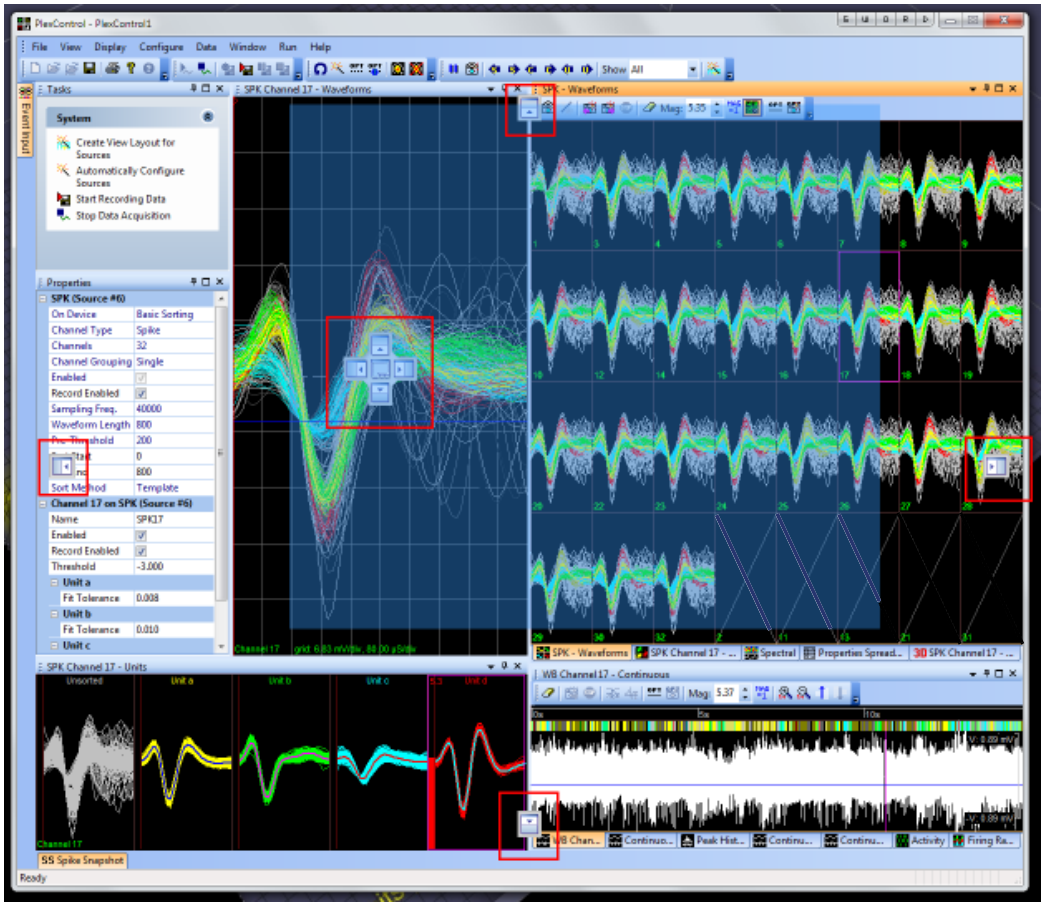
If you place the cursor on the title bar of a window, hold down the left button and drag, you will undock that window, and any tabbed views that are contained within it. If you then release the window while it is not attached to the edge of any other window, it will become a floating window that covers the windows beneath it. Here is an example:



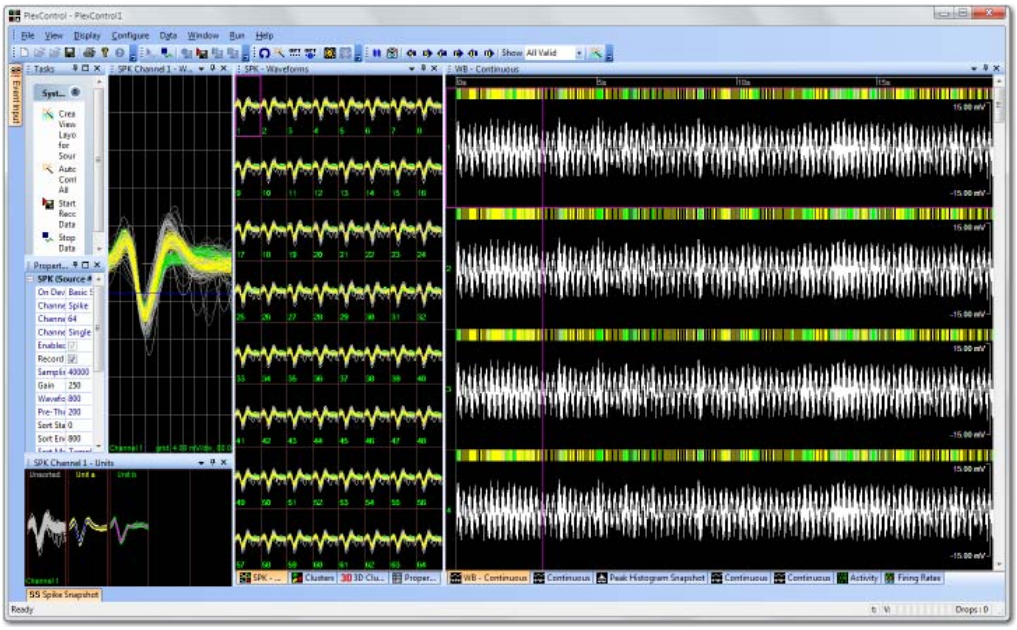

PlexControl - PlexControl1
File View Display Configure Data Window Run Help
Tasks
System
Create View Layout for Sources
Automatically Configure All Sources
Start Recording Data
Stop Data Acquisition
SPK Channel 1 - Waveforms
SPK - Waveforms
Properties
SPK (Source #6)
On Device Basic Sorting
Channel Type Spike
Channels 64
Channel Grouping Single
Enabled
Record Enabled
Sampling Freq. 40000
Gain 250
Waveform Length 800
Pre-Threshold 200
Sort Start 0
Sort End 800
SPK Channel 1 - Units
Unsorted
Unit a
Unit b
WB - Continuous
WB - Continuous
Continuous
Peak Histogram Snapshot
Continuous
Continuous
Activity
Firing Rates
SPK - Waveforms
Clusters
3D Clusters
Properties SpreadSheet
SS Spike Snapshot
Ready
Drops : 0

### Docking a window

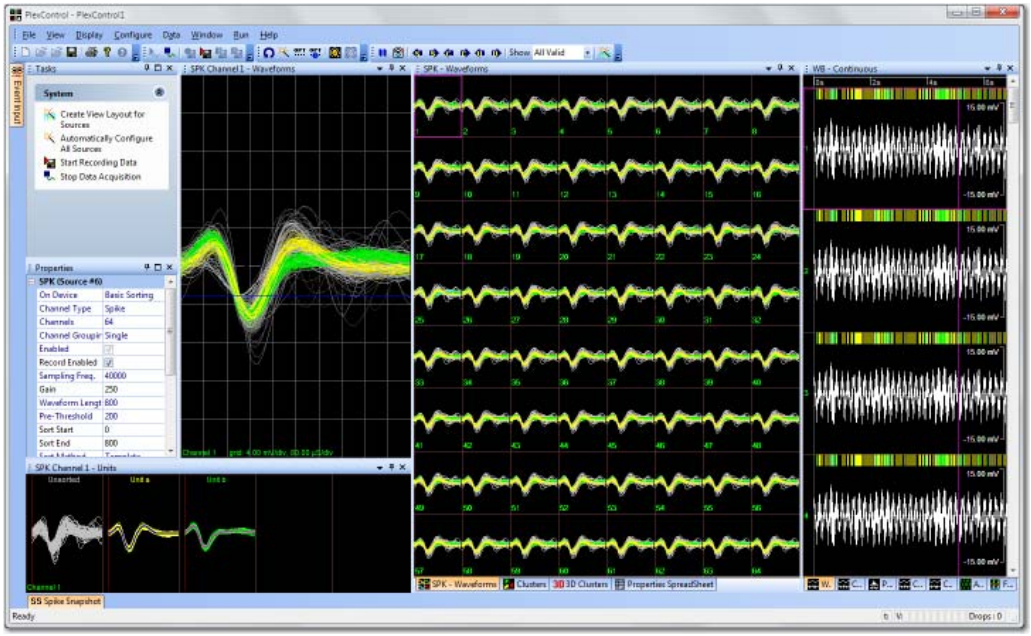
As you are dragging a window, you will see sets of blue arrows appear at the four sides of the main PlexControl window (indicated by red circles below), and a four-way arrow in the center of the nearest window (indicated by a red square below).



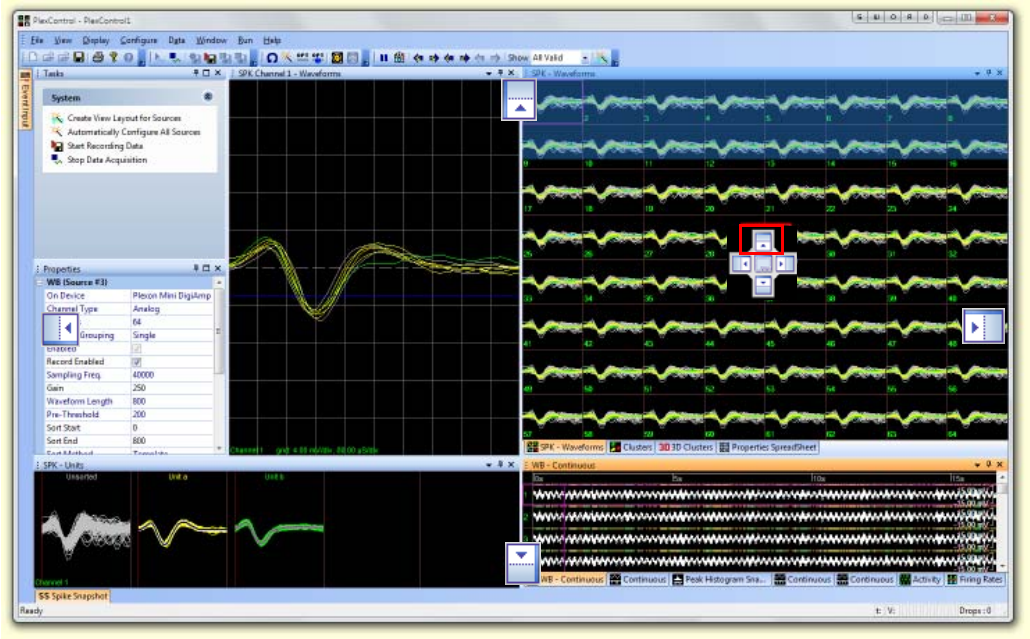
If you move the cursor to any of the four “outer” arrows, the window you are dragging will be docked to that edge. For example, if we dock the window to the right edge:

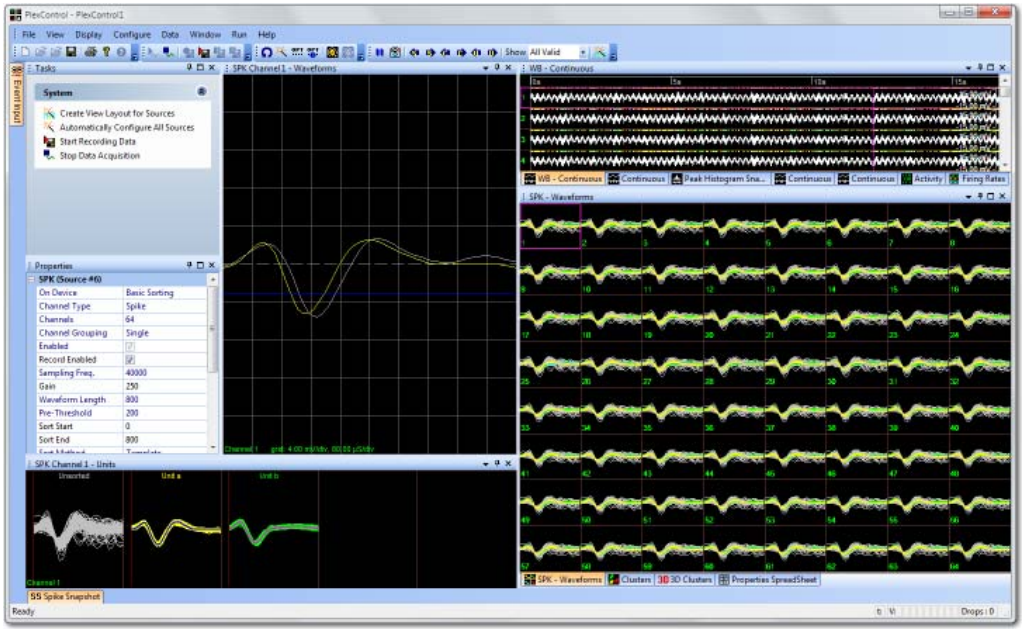


We could then use the splitter bars to resize the windows, within this layout:

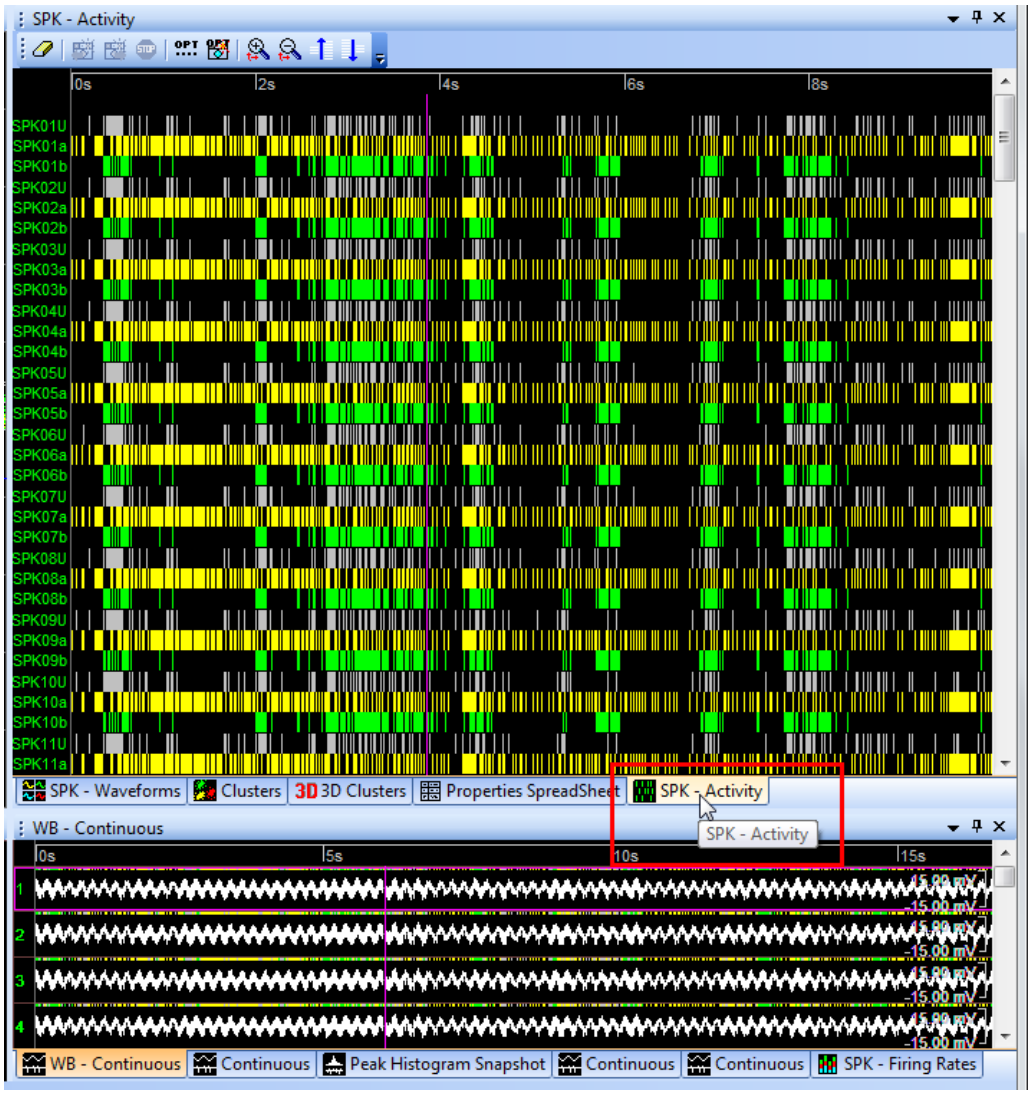


On the other hand, if we release the cursor within one of the four arrows in the four-way arrow, the window will dock to that edge, but nested within that specific window, as opposed to the outer, main PlexControl window. For example:

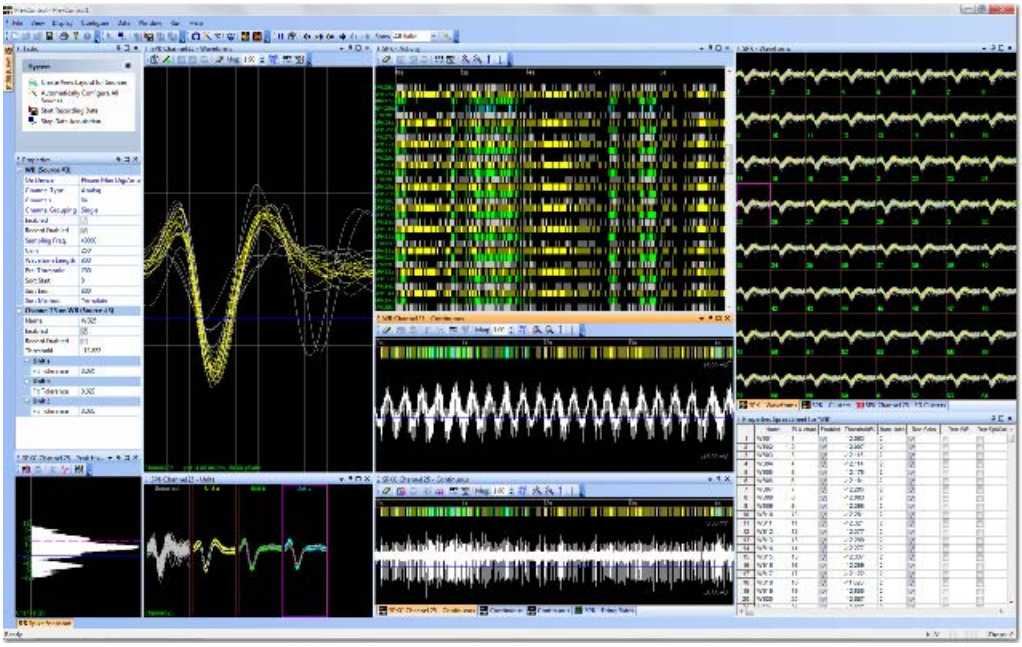


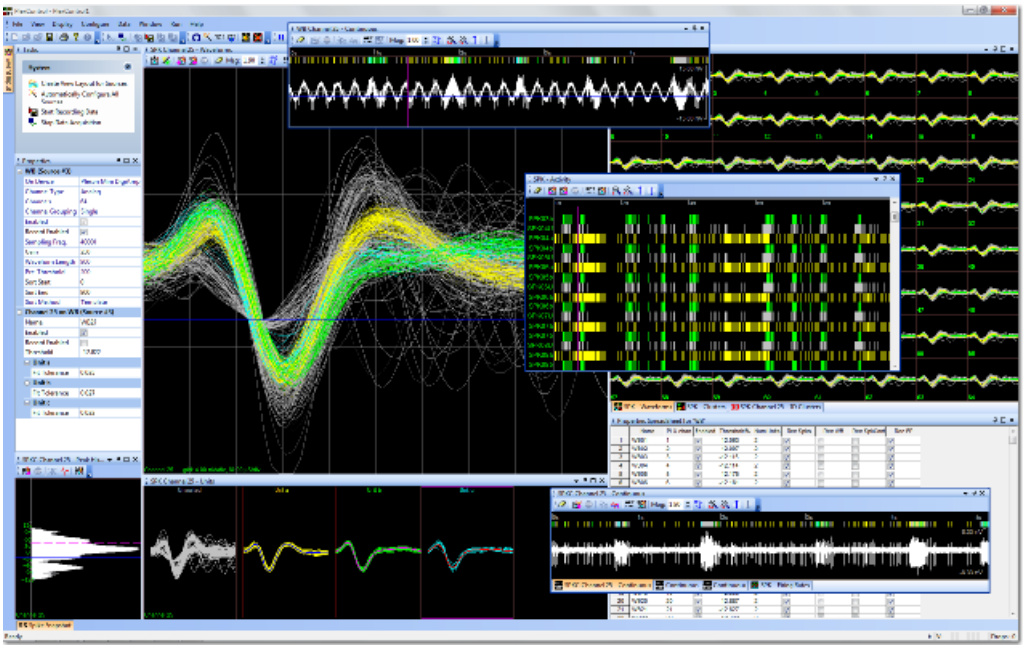


If you drag a window and release while the cursor is in the center of the four-way arrow set, then the dragged window will be added as a tab within the window. This is particularly useful in that you can drag an individual tab from one window into another. For example, we could drag the Activity window tab from the bottom-right window containing the continuous views, into the window which contains the multichannel spike and PCA views:



Sometimes it requires a bit of thought and experimentation, but virtually any window layout can be achieved by a sequence of these operations.





## 13.9 PlexControl Keyboard Shortcuts

The following keyboard shortcuts can be typed as either upper or lower case characters.

T: Toggles display of the currently selected view's toolbar

N: Go to the next channel

P: Go to the previous channel

O: Display the currently selected view's Options dialog

S: Toggles display of snapshot versus live data

space: Redraw view

# Chapter 14
# Saving Settings, Startup/Shutdown, and Troubleshooting

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14.2 Automatically Maintaining Compatible Sets of PXS and PXC Files ..... 453
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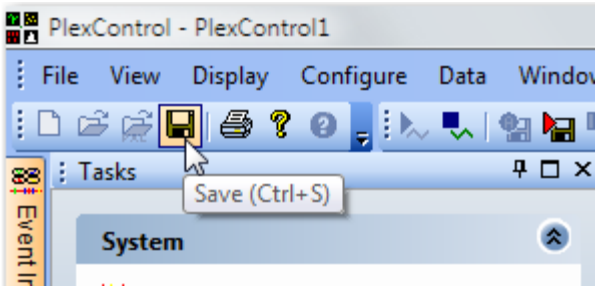
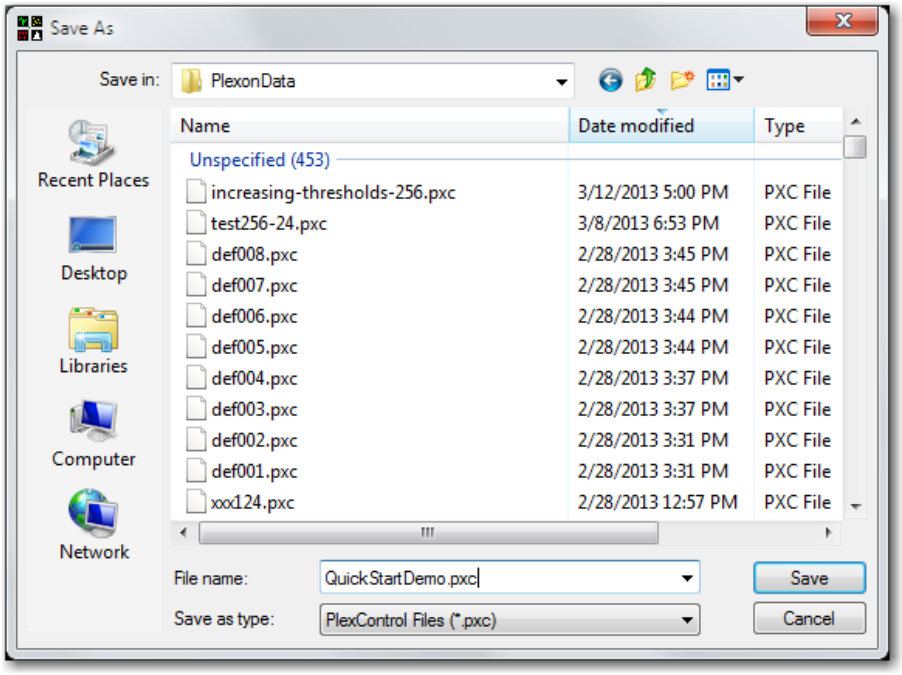
## 14.1 Saving and Loading PlexControl Settings

1 At any time, you can save the settings of all parameters in PlexControl (gain, thresholds, sorting parameters, etc) to a settings file, so that you can restore them later. To do this, simply click the **Save** button in the toolbar:.

**Note:** Setting the gain is applicable to DigiAmp subsystems, not DHP subsystems.

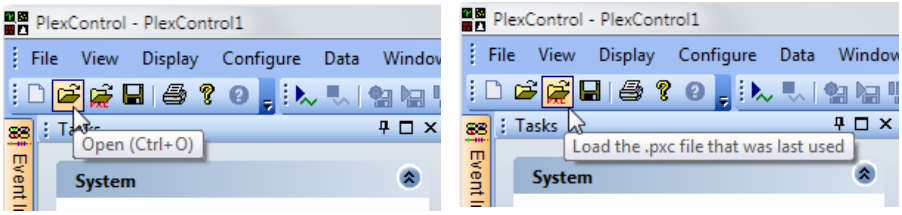



2 Specify a filename using the standard Windows® Save dialog.




**Note:** PlexControl settings files have the extension .pxc.

3 To load a previously saved pxc file, you can use either the **Open** or **Open Most Recently Saved** buttons in the toolbar:

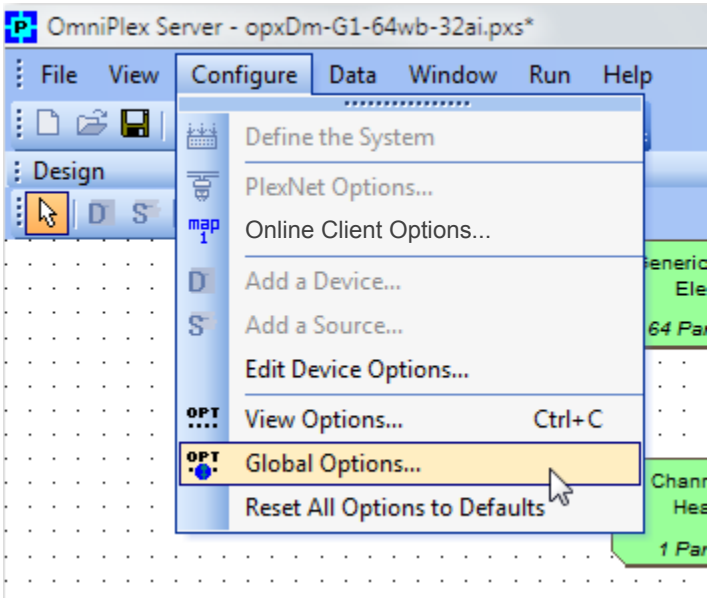



Note that you must stop data acquisition before loading a pxc file, and start data acquisition again after the file has been loaded. However, you do not have to stop data acquisition to save the current settings to a pxc file.

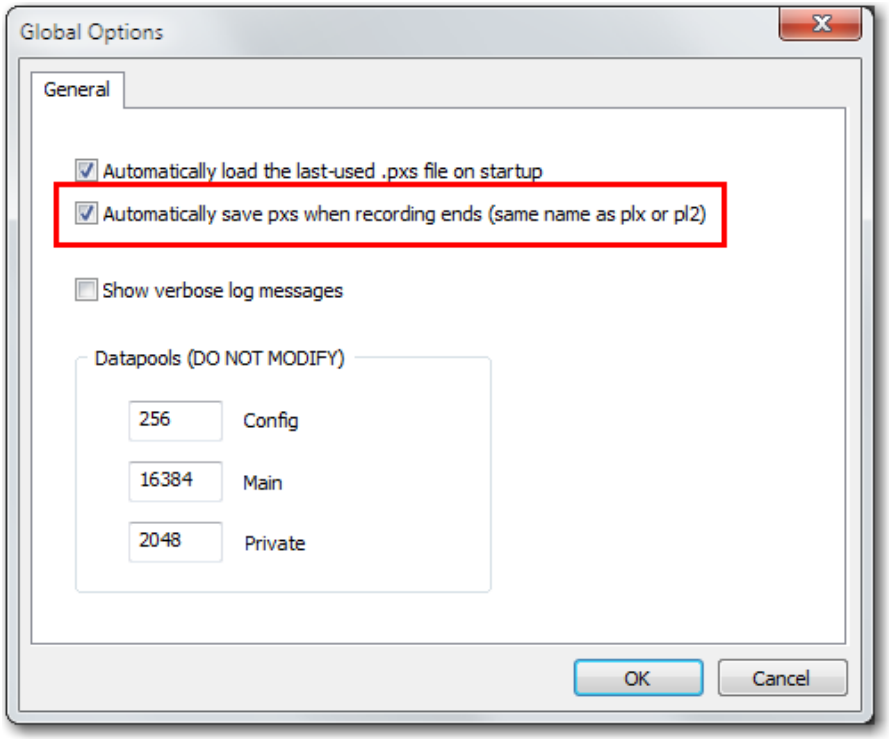
## 14.2 Automatically Maintaining Compatible Sets of PXS and PXC Files

Settings in the OmniPlex® System are saved in two files: Server saves its topology and settings in a PXS file, and PlexControl saves its settings in a PXC file. For many users, a single PXS is used, and only the PXC file changes from experiment to experiment. However, other users use a number of different PXS files, and the issue of PXS/PXC compatibility arises. Specifically, if a PXC file was created while one PXS file was loaded in Server, but at later date, the same PXC file is loaded while a different PXS file is loaded in Server, incompatibilities, some difficult to detect, can occur. If your usage includes such "mix and match" scenarios, you may wish to enable a set of options in the OmniPlex System which will automatically save your PXC and PXS files so that you know that you have a compatible pair of settings files.

1 To enable PXS auto-save in Server, select **Global Options** from the **Configure** menu:

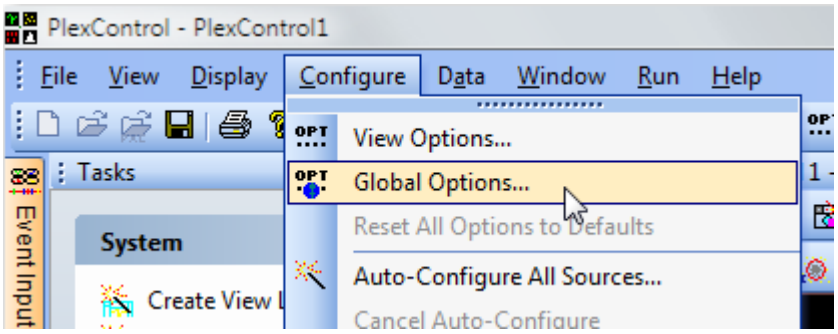


2 Enable **Automatically save pxs when recording ends**.

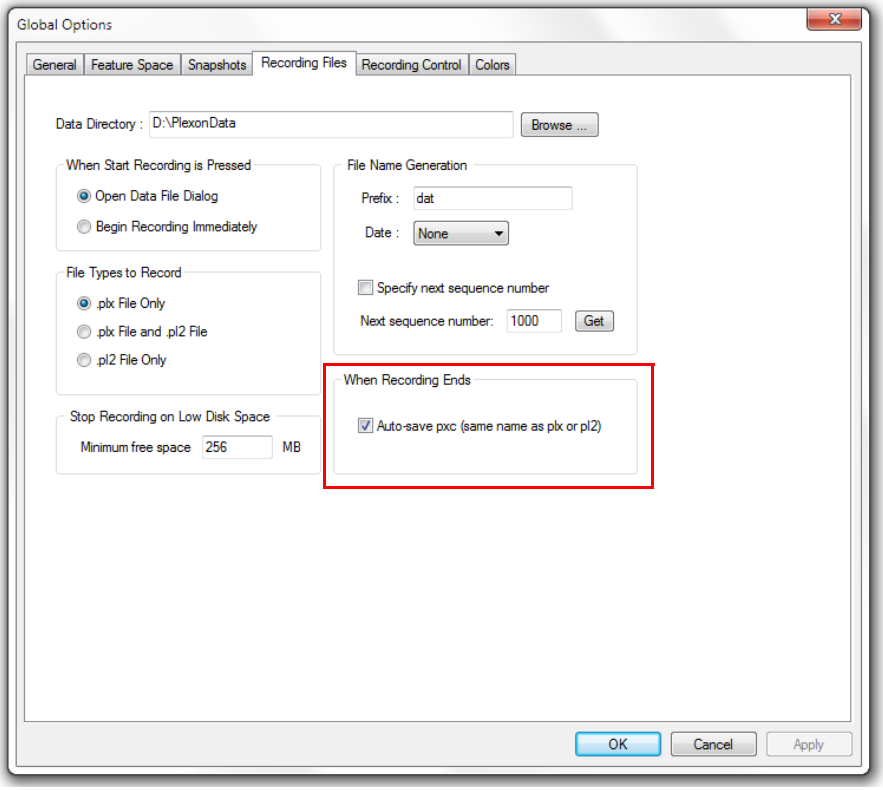



3 Click **OK**.

4 In PlexControl, select **Global Options** from the **Configure** menu:



5 Select the Recording Files tab and enable **Auto-save pxc**.




Now, each time that you record a plx or pl2 file, the current settings in Server and PlexControl will automatically be saved as XXX.pxs and XXX.pxc, where "XXX" is the name of the plx/pl2 file. In this scheme, for each plx/pl2 file you record, PlexControl will create three files with the same name, but with the extensions .plx/pl2, .pxc, and .pxs. If you later wish to run another experiment using the same settings as a previous session, you can load the corresponding pxs and pxc files in Server and PlexControl respectively.

## 14.3 Starting and Shutting Down Server and PlexControl

There are a few "best practices" to keep in mind regarding the sequence in which Server and PlexControl are started and shut down.

The recommended startup sequence is to run Server, wait for the initialization sequence to complete (indicated by the green progress bar at the bottom of the Server window), then start PlexControl, then start data acquisition. It is possible to first run PlexControl, which will then automatically start Server before allowing you to continue; however, if this method ever fails, you should fall back to the recommended sequence.

When you are completely finished with the system and want to shut down the OmniPlex System, the recommended sequence is to stop data acquisition from PlexControl, then close PlexControl, then close Server. In other words, the shutdown sequence is the reverse of the startup sequence.

### Closing and Opening Server (Applicable with DigiAmp Subsystem)

When the Server application is closed, the power to the DigiAmp Amplifier is turned off. The blue link cable between the AMP LINK card in the chassis and the DigiAmp Amplifier can then be safely unplugged. You should never plug or unplug the blue link cable while Server is running, even when data acquisition is stopped. You can verify the DigiAmp power status via the row of three green LEDs on the AMP LINK card in the chassis; if all three LEDs are on, power to the DigiAmp Amplifier is on; if only the leftmost LED is on, power to the DigiAmp Amplifier is off, and the cable can be plugged or unplugged.

**Note:** If you are connecting the blue link cable to the DigiAmp or MiniDigi unit or the AMP LINK card in the chassis, make sure the red markings on the cable connectors line up before inserting the cable. Grasp the connector and push straight in.
To unplug the cable, grasp the connector and pull straight out. See the caution statement below.

The headstages can only be powered when the AMP LINK power to the DigiAmp Amplifier is on, as indicated by the three green LEDs. Of course, the HST PWR toggle switch on the DigiAmp Amplifier must also be on to enable the headstage power..

**CAUTION**
**Handle the blue cable correctly to avoid damage**

=Do not pull on the blue cable itself, and do not twist the connector.
=Never bend or kink the blue cable.
=Never plug/unplug the blue cable if the Server application is running.

### Closing and Opening Server (Applicable with DHP Subsystem)

When the Server application is closed, the power to the DHP unit is turned off. The blue link cable between the DATA LINK card in the basic chassis (or PDLe card in the eChassis) and the DHP unit can then be safely unplugged. You should never plug or unplug the blue link cable while Server is running, even when data acquisition is stopped. If the red “Power” LED on the DHP unit is lit, power to the DHP unit is on and you should *not* uplug the blue cable.

**Note:** If you are connecting the blue link cable to the DHP unit or the DATA LINK or PDLe card in the chassis, make sure the red markings on the cable connectors line up before inserting the cable. Grasp the connector and push straight in.
To unplug the cable, grasp the connector and pull straight out. See the caution statement above.

The headstages are powered when the red PWR LED on the DHP unit is lit.

### Shutting Down and Reopening PlexControl

You will find that if you shut down PlexControl without first stopping data acquisition, data acquisition continues to run in Server, which is expected. If you now re-open PlexControl, it will automatically reconnect to Server while data acquisition is running, and continue displaying incoming data as it did before you shut it down. However, this has the potential to cause occasional problems, particularly at high channel counts. Therefore, it is not recommended that you make a habit of leaving Server running without PlexControl attached to it. As a general rule, when you are done using PlexControl, you should also shut down Server.

## 14.4 Interpreting LEDs on AMP LINK and DATA LINK Cards

This section explains how to interpret the LEDs on the front panel of the AMP LINK and DATA LINK cards in the basic OmniPlex chassis. Note that there is no need to pay attention to these LEDs in normal operation, and this information is only included for informational and troubleshooting purposes.

**Note:** If you have an OmniPlex eChassis. and a PDLe card, see Section 14.5, “Interpreting LEDs on the PDLe Card (eChassis Only)” on page 459.

AMP LINK cards are present in systems with the DigiAmp or MiniDigi subsystem. DATA LINK cards are present in systems with the DHP subsystem. The LEDs are the same on these two cards.

The term “blue box” below refers to the DigiAmp, MiniDigi or DHP unit, as applicable.

LED1 (green)—Power
LED2 (green)—Power to blue box
LED3 (green)—Communication lock (with blue box) OK
LED4 (orange)—Sampling clock
LED5 (orange)—Sampling status
LED6 (orange)—(internal use)

The LEDs can be interpreted as described below for typical system states, where

+ means ON (fully lit)
– means OFF (not lit)
* means partly lit (lit but dim)

Server not running:

+ - -
- - +

Server started, but acquisition stopped:

+ + +
- - +

Data acquisition running:

+ + +
* + -

## 14.5 Interpreting LEDs on the PDLe Card (eChassis Only)

This section explains how to interpret the green LEDs on the front panel of the PDLe card in the OmniPlex eChassis. Note that there is no need to pay attention to these LEDs in normal operation, and this information is only included for informational and troubleshooting purposes.

**Note:** If you have a basic chassis and an AMP LINK or DATA LINK card, see Section 14.4, “Interpreting LEDs on AMP LINK and DATA LINK Cards” on page 458.

LED1—DHP Power
LED2—Communication lock (with DHP unit)
LED3—Acquisition in progress
LED4—Power
LED5—PCIe OK
LED6—System sync

The LEDs can be interpreted as described below for typical system states, where

+ means ON (fully lit)
– means OFF (not lit)
A means partly lit (blinking rapidly to indicate data acquisition)
S means blinking slowly to indicate synchronization

PC on, but Server not running:

| 1 | 2 | 3 |
|---|---|---|
| – | – | – |
| + | + | – |
| 4 | 5 | 6 |

Server started, but acquisition is stopped:

| 1 | 2 | 3 |
|---|---|---|
| + | + | – |
| + | + | – |
| 4 | 5 | 6 |

Data acquisition running:

| 1 | 2 | 3 |
|---|---|---|
| + | + | A |
| + | + | S |
| 4 | 5 | 6 |

**Note:** LED 6 blinks to indicate synchronization between the PDLe card and the DHP unit, which occurs when the Server application is on and data acquisition is running. This LED continues to blink for up to 20 seconds when acquisition is stopped.

## 14.6 Troubleshooting Startup Problems

If you ever encounter a situation where data acquisition fails to start, and/or you receive any error message from Server, the first thing to try before contacting Plexon® support (+1 214-369-4957 or support@plexon.com) is to exit from PlexControl and Server, check all cables and power, and restart the OmniPlex System. If this does not help, use the rocker switch on the back panel of the chassis to turn the power off and then back on (never do this while the OmniPlex software is running!), then reboot Windows and try again. However, in most cases where the problem is an unplugged cable, wrong type of DigiAmp Amplifier, etc, Server will display an error message informing you of the problem.

Problems involving incorrect or inconsistent application settings can often be solved with the software reset procedure described in the next section.

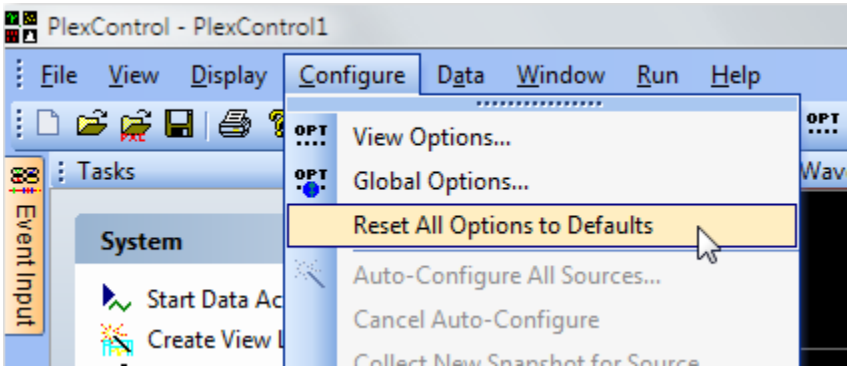
## 14.7 Resetting All OmniPlex System Options to Defaults

If you ever need to reset all PlexControl and Server options to their defaults, use the following procedure. The one setting that will not be reset is the pxs file which is auto-loaded by Server; however, if you want to prevent the last-loaded pxs file from auto-loading in Server, hold down the **Ctrl** key while starting Server.

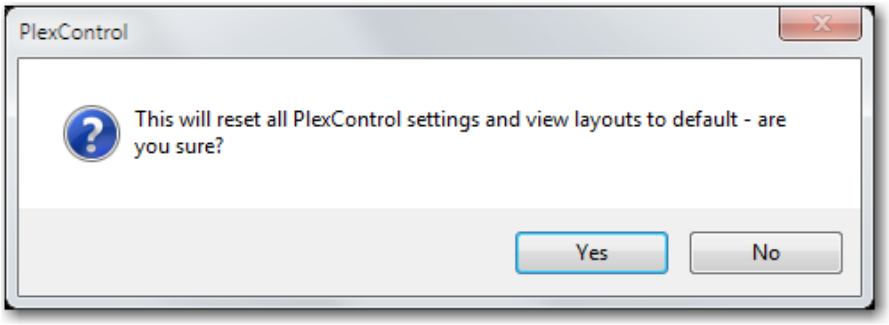
The reset procedure can be useful for troubleshooting, or if you simply want to ensure that you are starting from scratch, without carrying over any settings changes from a previous OmniPlex System session.

To reset all software options and settings in both PlexControl and Server:

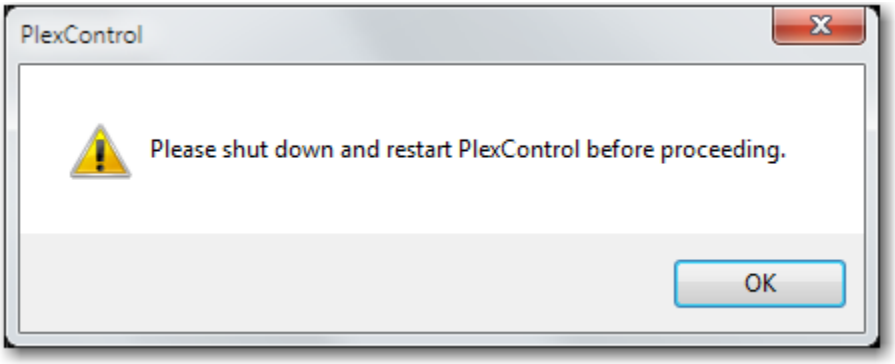
1. Stop data acquisition as described previously.
2. In PlexControl, select the **Reset All Options to Defaults** command:



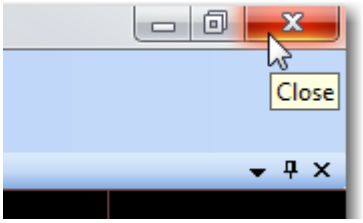

3. You will be asked to confirm the reset:



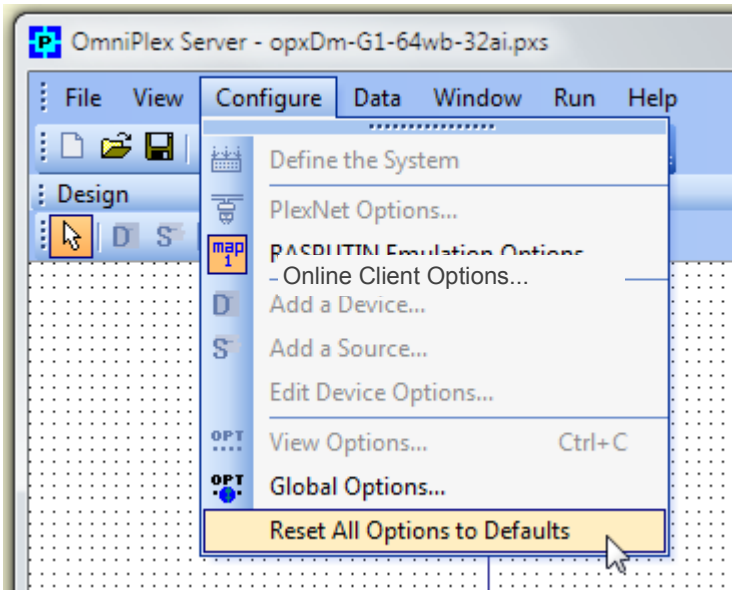
4 Click **Yes** to confirm the reset. You will be prompted to shut down PlexControl:



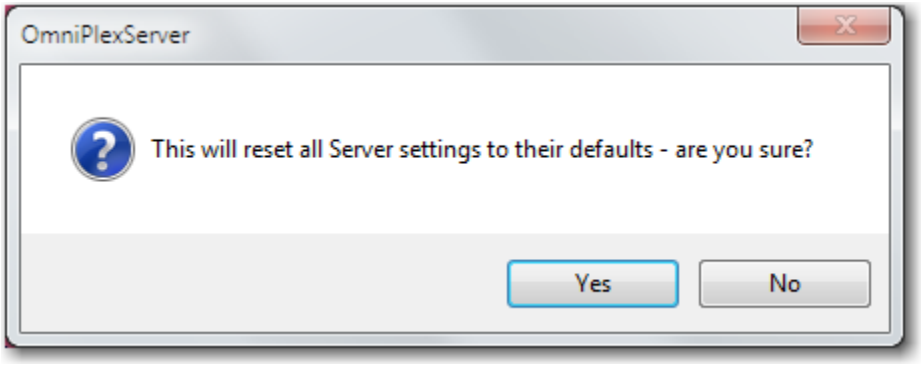

5 Click **OK**, then close PlexControl (but do not restart it yet).



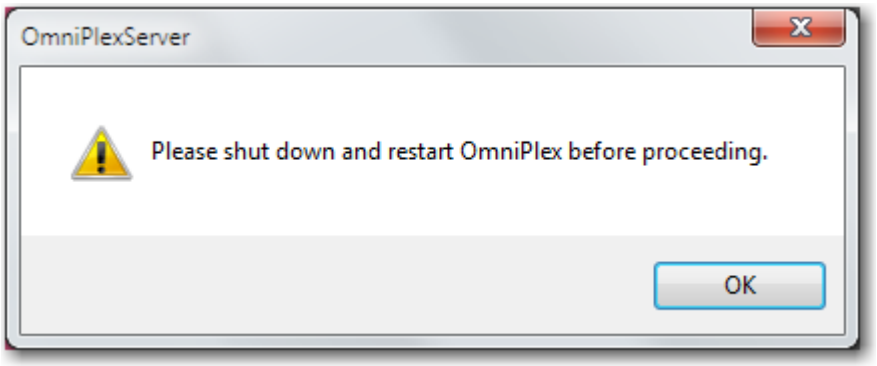

6 In Server, select the **Reset All Options to Defaults** command:



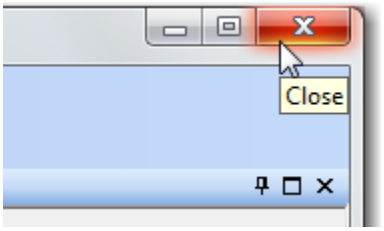
7 You will be prompted to confirm the reset:




8 Click **Yes** to confirm the reset. You will be prompted to shut down Server:




9 Click **OK**, then close Server.




10 Run Server and then PlexControl as usual. All software options and user interface layouts are now restored to their default settings.

# Chapter 15
# Additional Features

15.1 Audio Monitoring of Wideband or Spike Continuous Signals ..... 466
15.2 Digital Referencing ..... 469
15.3 Channel Mapping ..... 477
15.4 Thresholding Configuration Options ..... 490
15.5 Spike and Continuous Data Width—Export to External Clients ..... 501

## 15.1 Audio Monitoring of Wideband or Spike Continuous Signals

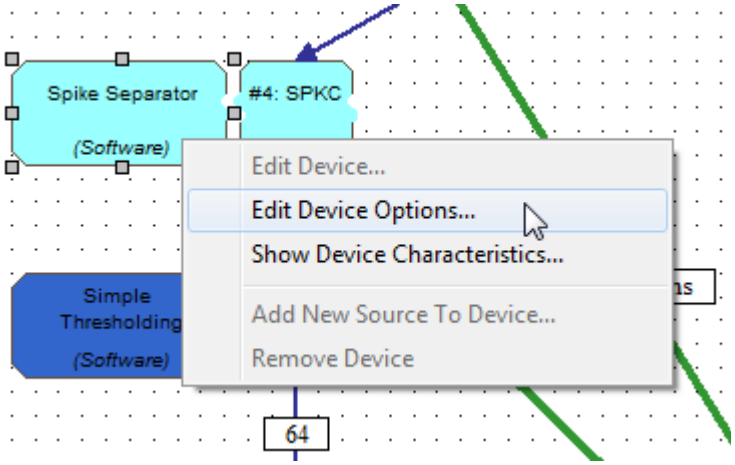
The OmniPlex® System supports online monitoring of the currently selected wideband (WB) or spike-continuous (SPKC) channel using the standard audio outputs of the computer on which the OmniPlex software is running. You may find it useful to listen to the live signal as an aid to electrode placement and for ongoing monitoring of spiking activity once electrodes have been positioned.

To use this feature, you can listen to the audio using the speaker built into the computer, but for better quality you can plug headphones or external speakers into the audio output jack on the front or rear of the computer. The output jack will typically be marked with an icon of a pair of headphones or a speaker, or an arrow pointing outward from the jack. You can use either mono or stereo headphones or speakers, but the audio will be mono in either case, since you are listening to a single channel.

**Note:** Be sure to check that the audio is not muted in the Windows® audio control panel or mixer.

### Enable audio monitoring

To enable audio monitoring and to choose wideband or spike-continuous monitoring, stop data acquisition, right-click on the **Spike Separator** in Server, and select **Edit Device Options**.




In the **Device Options** dialog, select the desired audio monitoring option and click **OK**.

When you start data acquisition, the selected monitoring option is enabled. When you select a channel in PlexControl, the corresponding WB or SPKC channel is automatically selected for audio monitoring.

### Helpful tips for using audio monitoring

In most cases, you will probably want to monitor the spike-continuous (SPKC) signal, since field potential signals (i.e. frequencies below the spike band) tend to have poor audibility and are not very informative. The option to monitor the unfiltered wideband (WB) signal is provided primarily for users who wish to use an external equalizer to do custom filtering of the audio signal.

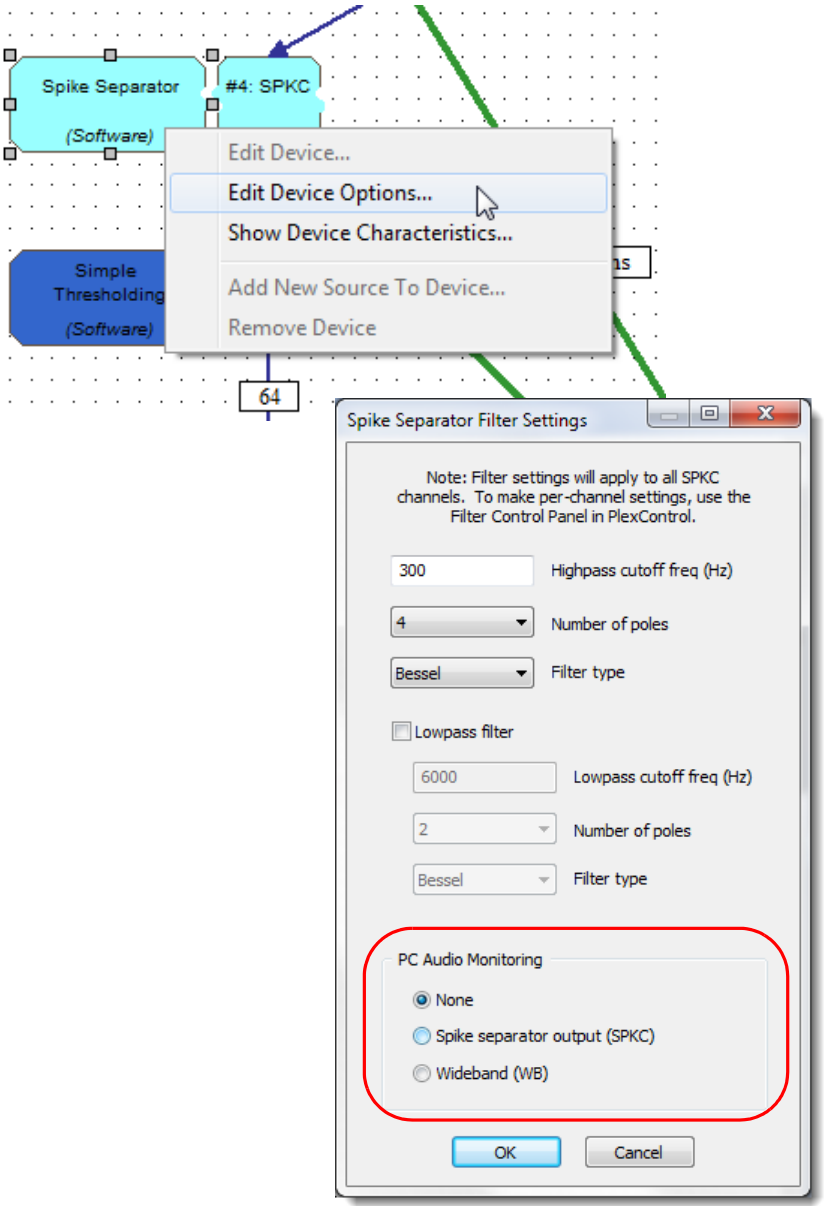
Note that due to the constraints of the computer's audio output subsystem, it is possible that you may occasionally hear a very brief dropout or glitch in the audio. Also, the built-in PC audio may add a small amount of coloration or distortion, relative to the signal which is processed and recorded by the OmniPlex System. If you find the audio quality inadequate, try using the audio output jack on the rear panel of the computer, as in some cases this provides a cleaner signal.

The above caveats should not be problematic in normal use, but it is important to keep in mind that the audio output is only intended for live monitoring, and is not necessarily suitable for recording or analysis.

### IMPORTANT—
### Disable audio monitoring before playing an audio file from audio output

You *must* disable the audio monitoring feature before you start playing a test audio file. Otherwise the system could generate an audio feedback signal that would mix with the intended test signal. Audio monitoring is for live data only.

To disable audio monitoring, right-click on the **Spike Separator** in Server, and select **Edit Device Options**. Then, in the **Device Options** dialog, select **None** for **PC Audio Monitoring** and click **OK**.




**Note:** You can enable this feature later when you are working with live data.

## 15.2 Digital Referencing

### 15.2.1 Overview

Digital referencing can be configured on a per-channel basis for continuous spike (SPKC) channels and/or field potential (FP) channels. For either source, the referencing (digital subtraction of sample values) is performed immediately before the filtering operation, and consequently for the FP source, before the post-lowpass downsampling. In effect, two separate copies of the original wideband (WB) signal are subjected to independent referencing before being passed to the spike separator and FP separator. This ensures that there is no unwanted interaction between the referencing operation and the chosen filters, and that it is performed at the full 40 kHz wideband sampling rate for maximum precision.

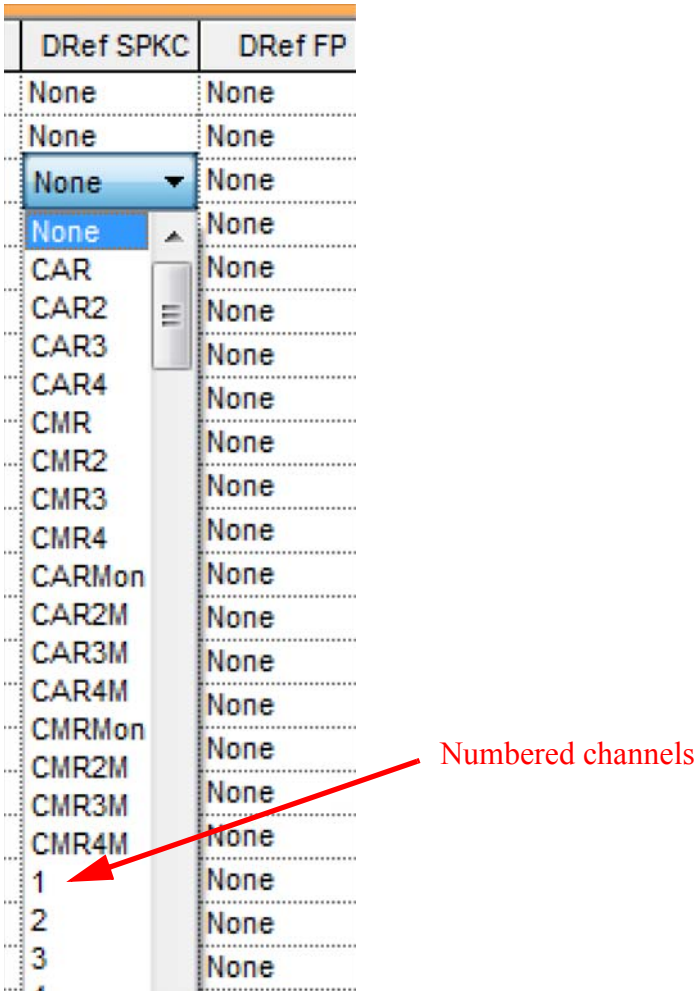
### 15.2.2 Configuring Referencing in PlexControl

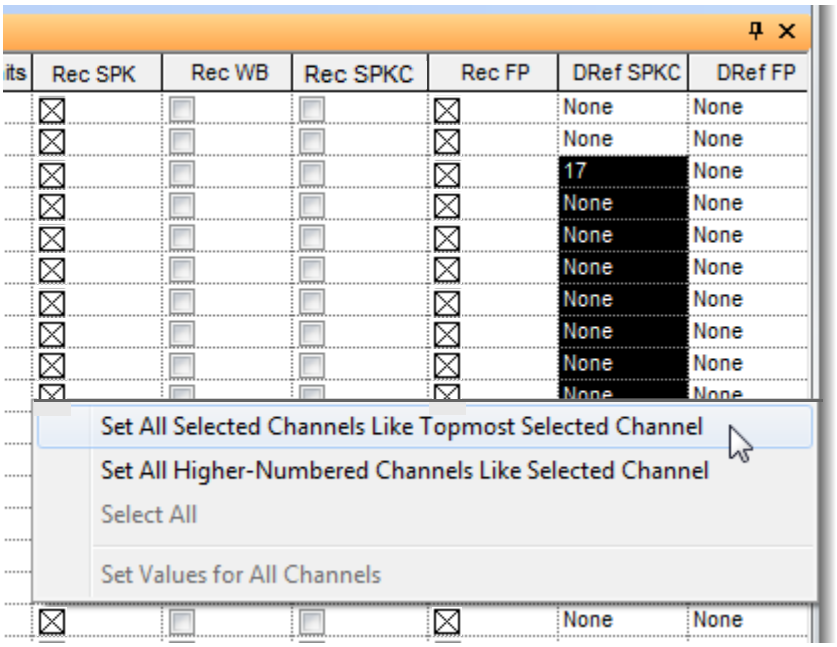
Digital referencing is controlled via the multichannel Properties Spreadsheet in PlexControl. The two columns "DRef SPKC" and "DRef FP" display the digital reference settings for each channel of the SPKC and FP sources:

Properties SpreadSheet for 'SPK'

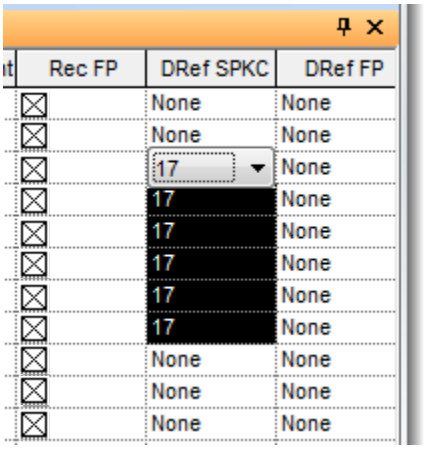
| | Name | PLX chan | Enabled | Threshold% | Num Units | Rec SPK | Rec WB | Rec SPKC | Rec FP | DRef SPKC | DRef FP |
|---|---|---|---|---|---|---|---|---|---|---|---|
| >>1 | SPK01 | 1 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ | ☒ | None | None |
| 2 | SPK02 | 2 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ | ☒ | None | None |
| 3 | SPK03 | 3 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ | ☒ | None | None |
| 4 | SPK04 | 4 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ | ☒ | None | None |
| 5 | SPK05 | 5 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ | ☒ | None | None |
| 6 | SPK06 | 6 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ | ☒ | None | None |
| 7 | SPK07 | 7 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ | ☒ | None | None |
| 8 | SPK08 | 8 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ | ☒ | None | None |
| 9 | SPK09 | 9 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ | ☒ | None | None |
| 10 | SPK10 | 10 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ | ☒ | None | None |
| 11 | SPK11 | 11 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ | ☒ | None | None |
| 12 | SPK12 | 12 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ | ☒ | None | None |
| 13 | SPK13 | 13 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ | ☒ | None | None |
| 14 | SPK14 | 14 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ | ☒ | None | None |
| 15 | SPK15 | 15 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ | ☒ | None | None |
| 16 | SPK16 | 16 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ | ☒ | None | None |

Determine which channel you wish to use as a reference, typically by selecting a channel where unwanted artifacts are clearly present but very few spikes are seen. Select this channel number in the DRef column(s) in the rows corresponding to the channels which are to use this reference. Clicking on a cell displays a drop-down list of the available reference options (the CAR and CMR options will be described later):


DRef SPKC
DRef FP
None
None
None




The selected channels are all set the same reference channel as the topmost channel:




The default reference setting of **None** indicates that no referencing is to be performed on that channel.

There a few simple rules to keep in mind. You cannot reference a channel against itself; there is no valid reason to do so (the result is guaranteed to be exactly zero, since the subtraction is in the digital domain), and you will usually want to be able to monitor the reference signal. If you use one of the right-button functions to set a group of channels to the same reference, and the reference channel is within the range of channels, it will be automatically skipped and its reference set to None, to prevent self-referencing. Also, a channel which is being used as a reference *for* other channels cannot itself be referenced *to* another channel - its reference must be set to **None**.

References within the FP source are assigned in exactly the same way as for SPKC channels. However, you should be cautious when referencing FP channels, since in many cases the field potentials can be attenuated by referencing, due to the same signal being picked up by more than one electrode.

You should use the SPKC and FP displays to monitor the effects of referencing, to verify that artifacts are being reduced or removed, while not harming the signals of interest.

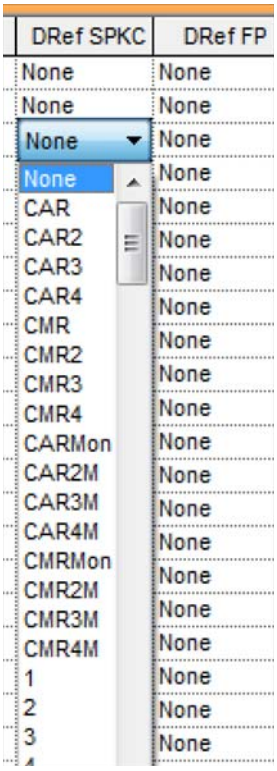
Note that no matter what referencing you apply to SPKC and/or FP channels, the wideband (WB) channels are unaffected. By recording the wideband channels, you can ensure that you have a record of the original signal, including artifacts, which you can later compare against the results of referencing, possibly apply offline referencing to, etc.

### 15.2.3 Common Average Referencing (CAR) and Common Median Referencing (CMR)

In addition to standard referencing, where signals are subtracted pairwise (i.e. channel *j* is subtracted from channel *k*) in either the analog domain or digitally, the OmniPlex System supports two additional methods of digital referencing which combine multiple channels to form a composite reference signal which is then subtracted to remove or reduce unwanted components.

The basic idea of both CAR and CMR is that if many signal channels are averaged together, the averaged signal being the result of averaging corresponding sample values across channels, then artifact signals that are the same across all channels (such as AC power line noise) will "survive the averaging" in the reference signal, whereas components unique to each channel, such as spikes, will tend to "cancel out" and have a mean near zero. The difference between CAR and CMR is that with CAR, the mean of sample values at each sample time is used as the reference signal, whereas with CMR, the median is used. In many cases, CMR will give results that are more robust in the presence of amplitude outliers, i.e. it will produce a better reference signal. However, the choice of standard digital referencings versus CAR versus CMR is data-dependent and should be evaluated on a case by case basis.

To select a channel for CAR (or CMR), select CAR (or CMR) from the channel's drop-down list in the Properties Spreadsheet. Notice that you have the option of selecting up to four independent CAR groups and up to four independent CMR groups simultaneously. If you are considering using multiple CAR or CMR groups, first read the discussion below—"Important considerations for using multiple CAR and CMR groups."


DRef SPKC
DRef FP
None
None
None

### Selecting and Monitoring CAR/CMR (for single CAR and CMR groups)

You can select any number of channels to be included in CAR and/or CMR, although it is recommended that for best results, you use only one or the other method, and include as many channels as is practical, in order to achieve a good average or median reference signal. Note than unlike conventional referencing, the channels that are selected for CAR or CMR are both used to form the reference signal, and this signal is then subtracted from all the selected channels.

Since the common average or common median will be subtracted from all CAR/CMR channels, the OmniPlex System provides a facility for monitoring and recording the CAR/CMR composite reference signal. If you designate a channel's reference as CARMon or CMRMon, that channel's original signal will be replaced by the actual CAR/CMR signal which is being subtracted from the channels whose reference is CAR or CMR respectively. For example, you can locate a channel which is otherwise unusable (e.g. due to a bad electrode) and designate it as the CARMon or CMRMon channel; the common average and common median reference signals can then be viewed in the SPKC or FP display, and recorded if desired. Alternately, you could use a standard procedure such as always allocating the last channel in SPKC/FP as the CAR/CMR monitor channel. Here is a "kitchen sink" example which should give some idea of the flexibility of this scheme; in practice, you would be unlikely to need such a complex set of references in a system with only 32 channels.

Properties SpreadSheet for 'SPK'

| | PLX chan | Enabled | Threshold% | Num Units | Rec SPK | Rec WB | Rec SPKC | Rec FP | DRef SPKC | DRef FP |
|---|---|---|---|---|---|---|---|---|---|---|
| >>1 | 1 | ☒ | -15.000 | 0 | ☒ | ☐ | ☒ | ☒ | 4 | CAR |
| 2 | 2 | ☒ | -15.000 | 0 | ☒ | ☐ | ☒ | ☒ | 4 | 7 |
| 3 | 3 | ☒ | -15.000 | 0 | ☒ | ☐ | ☒ | ☒ | CMR | None |
| 4 | 4 | ☒ | -15.000 | 0 | ☒ | ☐ | ☒ | ☒ | None | 3 |
| 5 | 5 | ☒ | -15.000 | 0 | ☒ | ☐ | ☒ | ☒ | CMR | 7 |
| 6 | 6 | ☒ | -15.000 | 0 | ☒ | ☐ | ☒ | ☒ | 4 | 7 |
| 7 | 7 | ☒ | -15.000 | 0 | ☒ | ☐ | ☒ | ☒ | 4 | None |
| 8 | 8 | ☒ | -15.000 | 0 | ☒ | ☐ | ☒ | ☒ | 4 | 7 |
| 9 | 9 | ☒ | -15.000 | 0 | ☒ | ☐ | ☒ | ☒ | 4 | 7 |
| 10 | 10 | ☒ | -15.000 | 0 | ☒ | ☐ | ☒ | ☒ | CMR | CAR |
| 11 | 11 | ☒ | -15.000 | 0 | ☒ | ☐ | ☒ | ☒ | CMR | CAR |
| 12 | 12 | ☒ | -15.000 | 0 | ☒ | ☐ | ☒ | ☒ | CMR | CAR |
| 13 | 13 | ☒ | -15.000 | 0 | ☒ | ☐ | ☒ | ☒ | CMRMon | CAR |
| 14 | 14 | ☒ | -15.000 | 0 | ☒ | ☐ | ☒ | ☒ | CMR | 7 |
| 15 | 15 | ☒ | -15.000 | 0 | ☒ | ☐ | ☒ | ☒ | CMR | 7 |
| 16 | 16 | ☒ | -15.000 | 0 | ☒ | ☐ | ☒ | ☒ | CMR | CAR |
| 17 | 17 | ☒ | -15.000 | 0 | ☒ | ☐ | ☒ | ☒ | CMR | 3 |
| 18 | 18 | ☒ | -15.000 | 0 | ☒ | ☐ | ☒ | ☒ | 4 | CAR |
| 19 | 19 | ☒ | -15.000 | 0 | ☒ | ☐ | ☒ | ☒ | 4 | CAR |
| 20 | 20 | ☒ | -15.000 | 0 | ☒ | ☐ | ☒ | ☒ | CMR | CAR |
| 21 | 21 | ☒ | -15.000 | 0 | ☒ | ☐ | ☒ | ☒ | CMR | 7 |
| 22 | 22 | ☒ | -15.000 | 0 | ☒ | ☐ | ☒ | ☒ | CMR | CARMon |
| 23 | 23 | ☒ | -15.000 | 0 | ☒ | ☐ | ☒ | ☒ | CMR | CAR |
| 24 | 24 | ☒ | -15.000 | 0 | ☒ | ☐ | ☒ | ☒ | 4 | CAR |
| 25 | 25 | ☒ | -15.000 | 0 | ☒ | ☐ | ☒ | ☒ | CMR | CAR |
| 26 | 26 | ☒ | -15.000 | 0 | ☒ | ☐ | ☒ | ☒ | CMR | CAR |
| 27 | 27 | ☒ | -15.000 | 0 | ☒ | ☐ | ☒ | ☒ | CMR | 7 |
| 28 | 28 | ☒ | -15.000 | 0 | ☒ | ☐ | ☒ | ☒ | CMR | 3 |
| 29 | 29 | ☒ | -15.000 | 0 | ☒ | ☐ | ☒ | ☒ | CMR | 3 |
| 30 | 30 | ☒ | -15.000 | 0 | ☒ | ☐ | ☒ | ☒ | CMR | CAR |
| 31 | 31 | ☒ | -15.000 | 0 | ☒ | ☐ | ☒ | ☒ | CMR | CAR |
| 32 | 32 | ☒ | -15.000 | 0 | ☒ | ☐ | ☒ | ☒ | 4 | CAR |

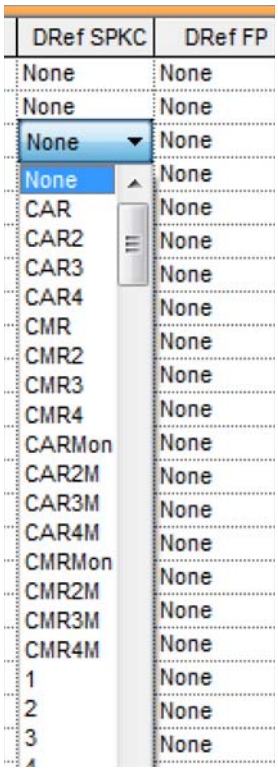
Note that each non-CAR/CMR channel which is being used as a reference (SPKC channel 4 and FP channels 3 and 7) has its own reference set to None, as required.

### Important considerations for using multiple CAR and CMR groups

When you have multiple CAR groups, the channels within each CAR group (e.g. CAR3) are averaged together and that average is subtracted from only the channels of that CAR group. Likewise, the sample-by-sample median of the channels within each CMR group (e.g. CMR4) is calculated and that median is subtracted from only the channels of that CMR group. Each CAR and CMR group can have an associated monitor channel. Any SPKC or FP channel can be assigned to any of the CAR or CMR groups, and the CAR and CMR groups are completely independent between SPKC and FP. For example, you could define one CAR group and three CMR groups on the SPKC channels, but two CAR groups and one CMR group on the FP channels.

Defining more than one CAR and/or CMR group can be useful in cases where various subsets of channels each exhibit unique common noise or artifacts. For example, electrodes from one headstage may be implanted in a different part of the brain than another headstage, or multiple types of electrodes may be in use simultaneously, each with varying susceptibility to noise and artifacts.

The user interface for defining multiple CAR/CMR groups and the associated monitor channels is a straightforward extension of the previous single-CAR / single-CMR scheme. When you click on a channel's entry in the DRef SPKC or DRef FP columns of the main Properties Spreadsheet, you will see the choices shown in the image below.


DRef SPKC
DRef FP
None
None
None

For each channel, you can select a CAR group or a CMR group or a specific channel number as the reference.

- Select one of CAR, CAR2, CAR3, or CAR4 to assign a channel to CAR group 1, 2, 3, or 4 respectively. CARMon, CARM2, CARM3, and CARM4 are the corresponding CAR monitor channels.
- Select one of CMR, CMR2, CMR3, or CMR4 to assign a channel to CMR group 1, 2, 3, or 4 respectively. CMRMon, CMRM2, CMRM3, and CMRM4 are the corresponding CMR monitor channels.
- Selecting a specific channel number as the reference (1, 2, 3… n) simply subtracts that specific channel, rather than using common average referencing or common median referencing.

In addition to the usual caveats about the use of CAR/CMR, using multiple CAR/CMR groups can require additional caution. For the same overall channel count, defining more than one group implies that each group will contain fewer channels than if a single group were used, but it is important that each group contain enough channels that the average or median reference signal contains only artifacts and not identifiable spikes.

The most important tool for ensuring that digital referencing is being applied appropriately is the monitor channel for each group. Each monitor channel should display artifacts, not spikes, and the artifacts on each monitor channel should be different than the other monitor channels; if the artifacts are the same on different monitor channels, this indicates that too many referencing groups have been defined, and you will in fact achieve better results by defining fewer groups, since more channels will then be included in the average or median of each group, helping to “average out” the spikes and to emphasize the artifacts.

Remember that by recording the monitor channel(s), you are recording “what is being subtracted out” from the channel in each referencing group and you could potentially apply your own offline post-processing to add the reference signal(s) back to the channels in the corresponding groups and thereby reconstitute the original, unreferenced signals.

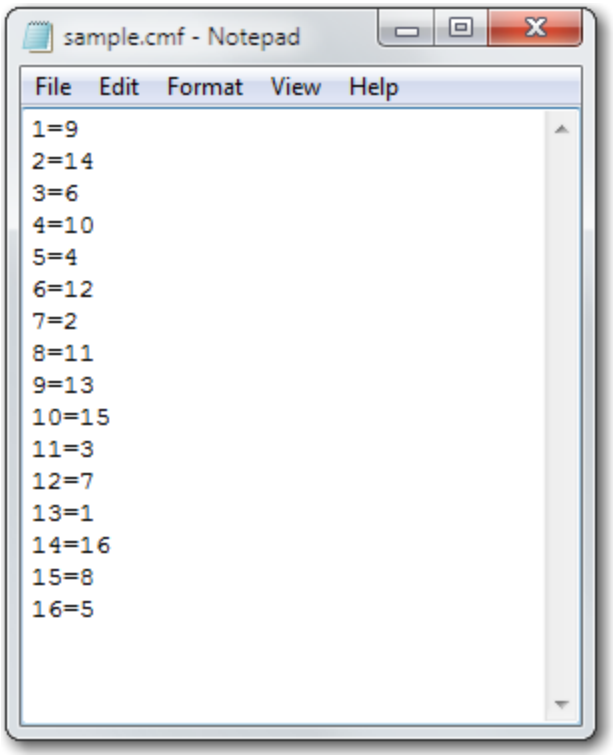
## 15.3 Channel Mapping

Channel mapping allows users of electrodes with nonstandard or inconvenient physical channel numbering, e.g. silicon probes, to map (renumber) the channels, such that the original physical channel numbering is hidden from the OmniPlex System, without the use of custom adaptors or cables which were previously required. Note that currently only neural channels can be mapped; channels such as digital input and auxiliary analog (AuxAI) channels are not affected by channel mapping in any way.

The OmniPlex channel mapping process supports an arbitrary one-to-one mapping (renumbering) of electrode channels (also referred to as input channels) and the OmniPlex System channels (also referred to as output channels). Each input channel must map to exactly one output channel, and vice versa. A channel mapping is specified by loading a cmf file (channel mapping file) in the OmniPlex Server. Once a cmf is loaded, it remains in effect until it is either manually disabled, a different pxs (topology) file is loaded or created in Server, or Server is started without a pxs file. You must close PlexControl before loading a new channel mapping file or disabling channel mapping in Server.

### 15.3.1 Creating a cmf File

A cmf file is a simple text file which you can create using Windows Notepad or any other text editor which can create plain text files. After creating the file in an editor, renaming the file extension from .txt to .cmf will make the OmniPlex System recognize it as a channel mapping file. Here is an example of a typical cmf file:



This cmf is for a 16 channel system, so all channel numbers in the file are within the range 1-16. Each line in this example specifies the mapping of one channel. The channel number on the left side of the = sign is the OmniPlex System channel number as seen in PlexControl, in recording files, clients, etc. The channel number on the right side of the = sign is the original physical electrode channel number. In other words, the mapping commands are of the form:

outputchannelnumber = inputchannelnumber

or to put it another way:

visiblechannelnumber = hiddenchannelnumber

For example, say that the original electrode device was a 1D linear array probe, and the actual physical electrode channel numbers, starting from the tip of the probe, were the following:

[probe tip] 9, 14, 6, 10, 4, 12, 2, 11, 13, 15, 3, 7, 1, 16, 8, 5 [probe top]

but what you would like to see in the OmniPlex System is a convenient list of uniform, increasing channel numbers like this:

[probe tip 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 [probe top]

The above cmf file will accomplish this channel renumbering. In actual use, you will need to consult the documentation for your electrodes, or contact the manufacturer, to determine the electrode channel numbering, i.e. the device pinout. In a case such as the 1D probe example above, it may be obvious what the most useful channel mapping is; in other cases, where the electrodes are arranged in a complex geometry, an appropriate mapping may be a matter of preference, or there may be considerations unique to your experiment or data analysis. For example, if you are using a probe with multiple tetrode sites along a shank, you should assign consecutive channel numbers to the four channels within each tetrode, such that the tetrodes appear in the OmniPlex System as channels 1-4, 5-8, etc.

In many cases, a cmf file similar to the one above will be all that is necessary to define the desired mapping. However, the cmf file supports additional functionality that can make the definition of certain types of mappings easier. If you like, you can skip to Section 15.3.6, “Loading a cmf file in Server” on page 483 on a first reading, and read about the additional features later.

### 15.3.2 Default mapping

In the above example, we explicitly specified the mapping for every single channel. This was because, as in many real-world cases, every channel on the device has to be renumbered. However, imagine that we have a probe or array where only the first eight channels have "inconvenient" physical channel numbers, and the last eight channels are numbered 9 through 16 as with a "normal" set of electrodes. The corresponding cmf might look something like this:

```
1=7
2=5
3=6
4=2
5=7
6=3
7=1
8=4
9=9
10=10
11=11
12=12
13=13
14=14
15=15
16=16
```

In other words, the last eight channels are to retain their original channel numbers. In a case like this, you need only list the channels that require actual mapping; all other channels are assumed to be unchanged:

```
1=7
2=5
3=6
4=2
5=7
6=3
7=1
8=4
```

### 15.3.3 Range mapping

In some cases, you may wish to renumber contiguous blocks of channel numbers, which can be done with a *range mapping:*

```
1..4=9..12
5..8=13..16
9..12=1..4
13..16=5..8
```

The notation 1..4 means channels 1 through 4, and likewise for the other channel ranges shown. For example, the range mapping 1..4=9..12 has the same effect as the following series of mapping commands:

```
1=9
2=10
3=11
4=12
```

You can see that the above cmf commands are in effect permuting four-channel blocks within a total of 16 channels. Swapping the first 32 channels and the last 32 channels in a 64 channel system could be done with these mappings:

```
1..32=33..64
33..64=1..32
```

Note that you can use a combination of single-channel and range mappings within the same cmf file; for example:

```
1..4=9..12
5=7
6=5
7=8
8=6
9..12=1..4
13=16
14=13
15=14
16=15
```

### 15.3.4 Formatting and comments

When the OmniPlex System reads a cmf file, it ignores all spaces within each line of the file, so that the following commands are all equivalent:

```
1..32=33..64
1..32 = 33..64
1 .. 32 = 33 .. 64
1 . . 3 2 = 3 3 . . 6 4
```

You can insert a comment line by using either ";" as the first character of a line, or "//" as the first two characters. Blank lines are also allowed. For example:

```
; Mapping file for MySiliconProbe
; July 19, 2013
 // the next two lines are range mappings
 1..4 = 9..12
 9..12 = 1..4
 // these are single-channel mappings
 5=7
 6=5
 7=8
 8=6
 13=16
 14=13
 15=14
 16=15
```

However, note that you cannot currently put a comment on the same line as a mapping command:

```
1..4 = 9..12 // this comment is illegal
```

### 15.3.5 Overwriting mappings and strict mode

By default, the OmniPlex System allows you to specify mapping commands in any order, as long as the final result maps every input channel to one and only one output channel. This can include sequences of mapping commands that overwrite, or partially overwrite, a previous mapping, for example:

```
1..32=33..64
33..64=1..32
10=10
42=42
```

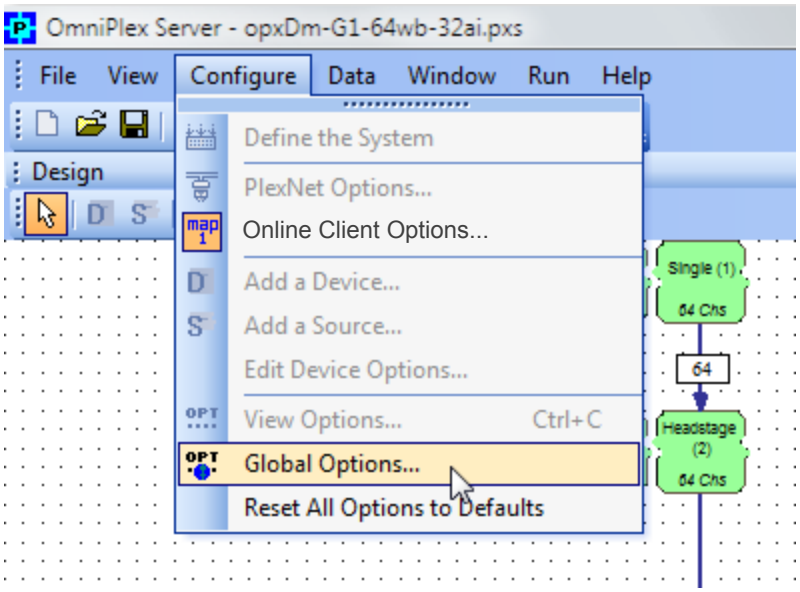
The net effect will be to swap the first 32 and last 32 channels, *except* for channels 10 and 42, which will not be mapped. This is easier than writing out a full sequence of 64 single-channel mapping commands. However, in cases where you do not need to use overwriting, you may wish to disable it, to enable the OmniPlex System to detect cases where an error in your cmf file results in a mapping command which unintentionally overwrites another mapping. If you add a line with the special keyword "strict" before any other mapping command in the cmf, the OmniPlex System will disable support for overwriting and will report an error when a mapping command attempts to overwrite a previous mapping.

```
strict
1..32=33..64
33..64=1..32
// the next two lines will give errors when the cmf is loaded
// since they overwrite the mapping of channels 10 and 42
10=10
42=42
```

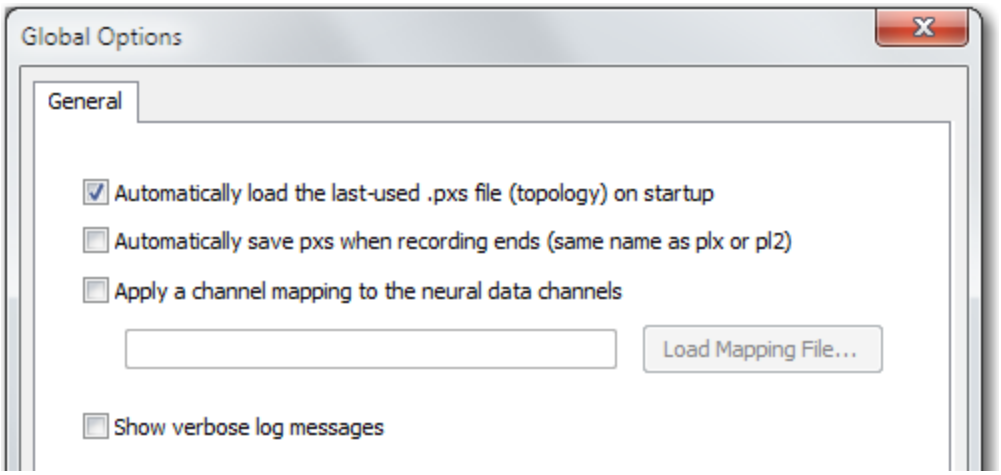
### 15.3.6 Loading a cmf file in Server

**Note:** This section describes how to load a cmf file in Server. As described in Section 15.3.7, “Loading a cmf file in PlexControl” on page 488, the name of the cmf file, if any, can be specified on a per-pxc basis in PlexControl.

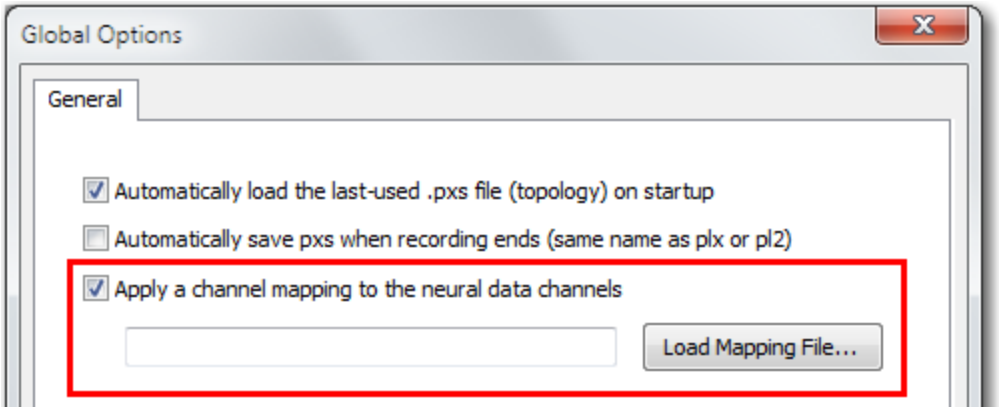
Once you have defined a channel mapping in a cmf file, loading the file in the OmniPlex Server puts the mapping into effect. To load a channel mapping file, first close PlexControl, if it is open. Next, go to **Configure** >> **Global Options**:

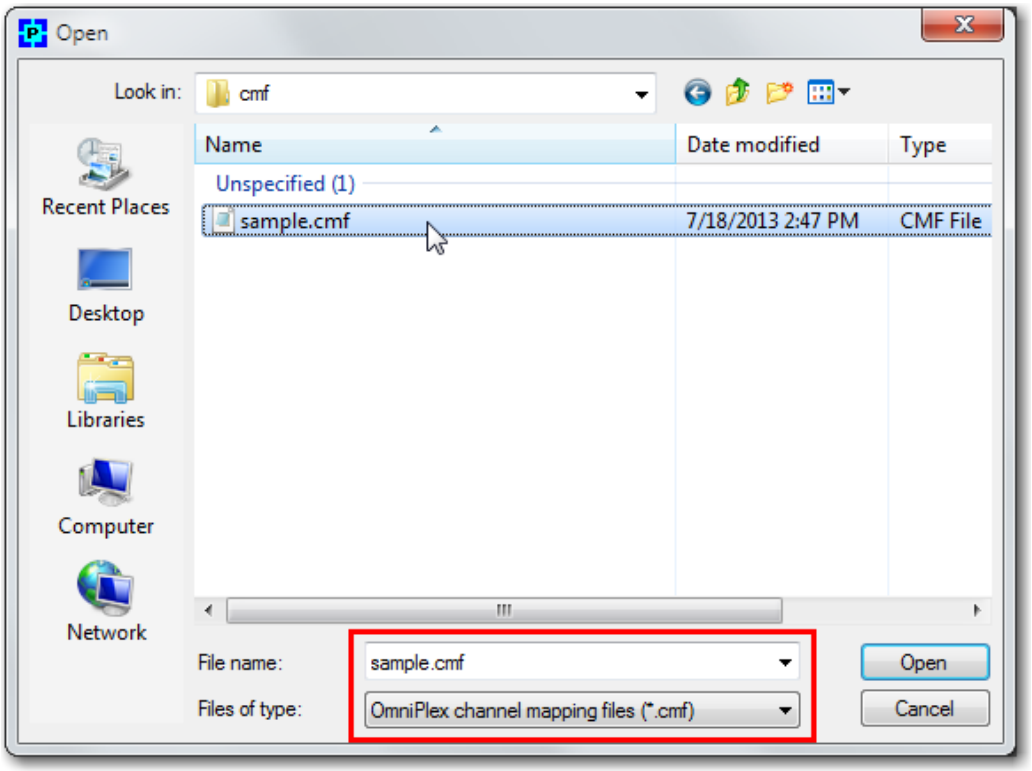



The **Global Options** dialog is displayed:

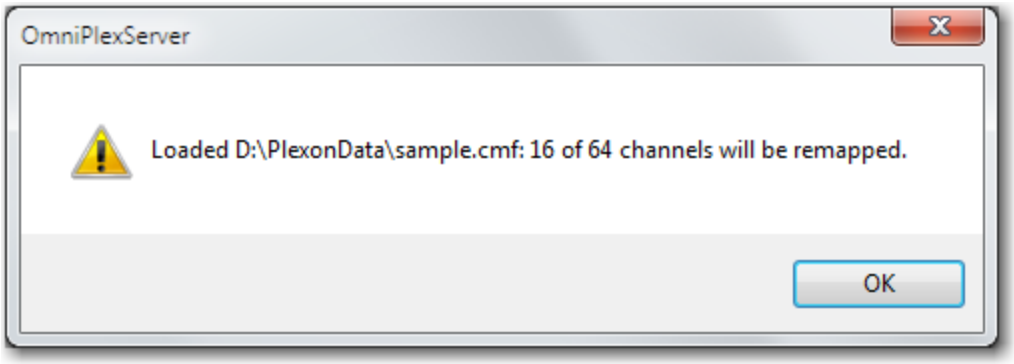


To enable channel mapping, click **Apply a channel mapping to the neural data channels** and then click the **Load Mapping File...** button to specify the desired cmf file:

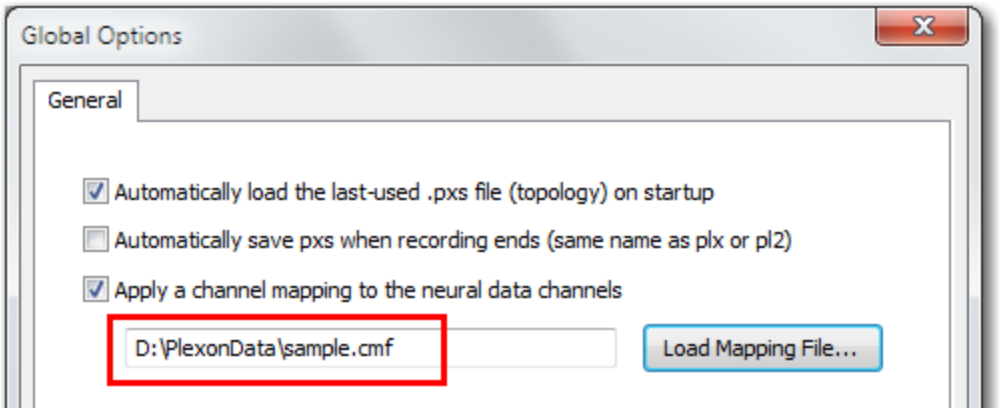


You should see a confirmation that the cmf was loaded successfully:

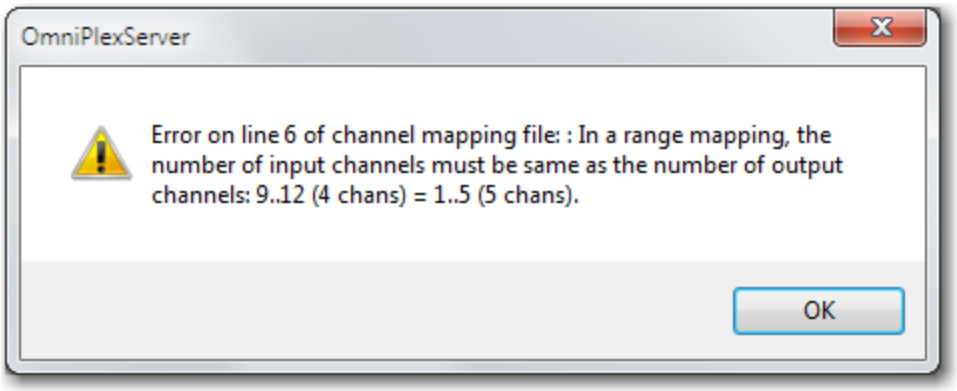



In the above example, 16 of 64 channels will be mapped (renumbered), while the other 48 will not be affected.




The channel mapping is now in effect and the original physical electrode channel numbers are no longer used in the OmniPlex System user interface, in recorded data files, or in online client data. As far as an OmniPlex System user is concerned, the original channel numbering is completely hidden. If for any reason you later need to identify which physical input channel corresponds to a given OmniPlex System channel, for example when analyzing your data, you can refer to the cmf file as a record of the mapping that was in effect.

If any errors are detected when the cmf file is read by the OmniPlex System, an error message is displayed, such as:

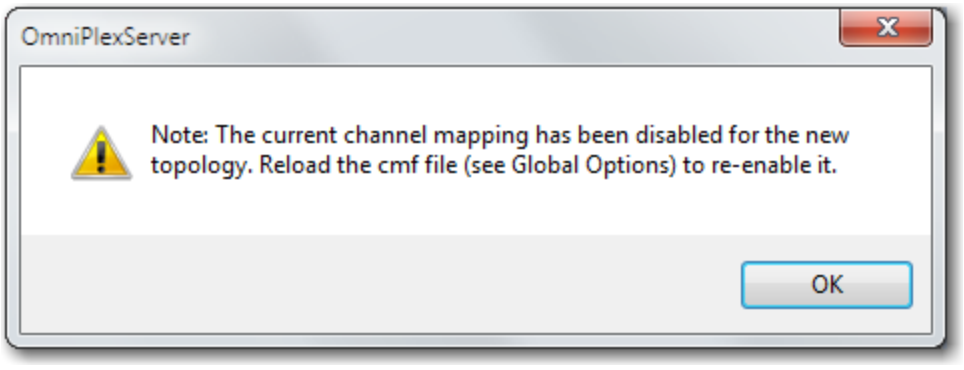



When an error is detected, the OmniPlex System automatically temporarily disables channel mapping, to prevent an invalid mapping from being applied. After correcting the error in the cmf (by editing the file in Notepad, etc), you will need to re-enable channel mapping and load the file again.

Once a cmf has been loaded without error, the OmniPlex Server will automatically load the mapping file every time it starts, until you do one of the following:

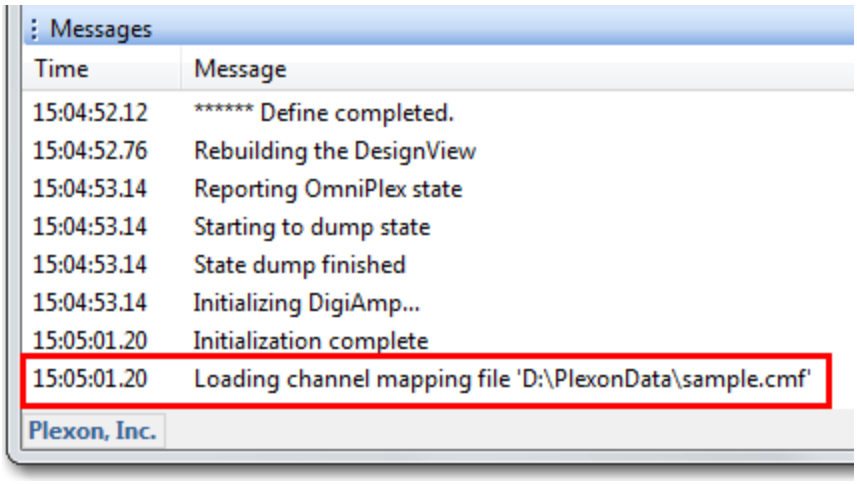
- Manually disable channel mapping in the Global Options dialog
- Load a different pxs file (topology) in Server
- Use the Topology Wizard to generate a new topology
- Start Server without auto-loading a pxs file, for example by holding down the CTRL key while launching Server

If the OmniPlex System automatically disables a channel mapping that was previously in effect, a warning message is displayed, for example:




Channel mapping can also be automatically disabled if Server attempts to auto-load a cmf on startup but the file has been deleted or corrupted since it was last loaded.

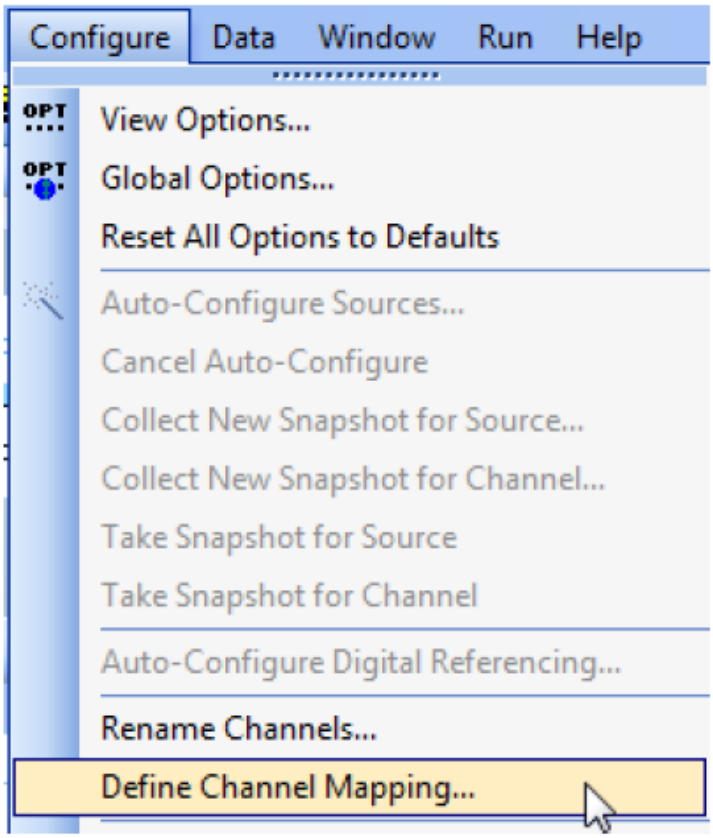
You can easily confirm the channel mapping file that was auto-loaded by viewing Server's Messages window as it starts up:



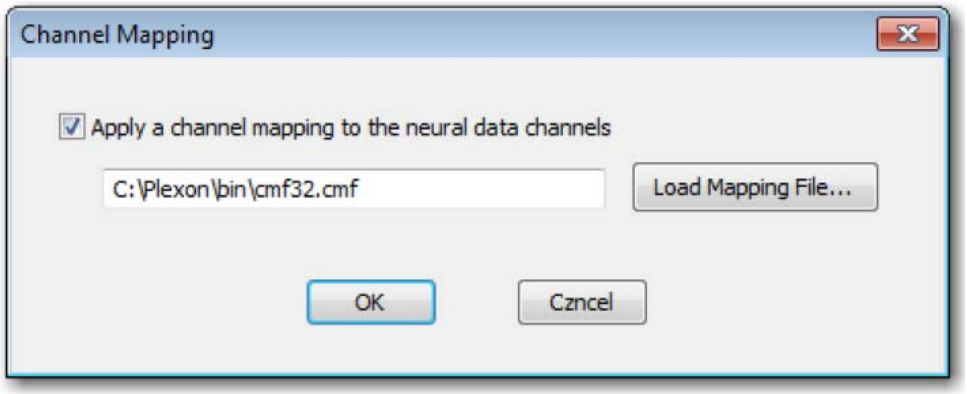

To change the mapping, simply display the **Global Options** dialog and follow the procedure previously described. Note that you must stop data acquisition and close PlexControl before loading a new channel mapping file or manually disabling channel mapping.

### 15.3.7 Loading a cmf file in PlexControl

OmniPlex channel mapping files (cmf files) can be specified on a per-pxc basis. As described in Section 15.3.6, “Loading a cmf file in Server” on page 483, the name of the cmf file, if any, can be specified in Server’s **Global Options**. But it is also possible to specify the cmf file to be used via PlexControl’s **Define Channel Mapping** menu item in the **Configure** menu:




This displays a dialog which allows you to specify the name of the desired cmf file.



This is similar to how the cmf is specified in Server, but has some advantages over that method. First, you can change the cmf file while data acquisition is running, whereas doing so in Server requires stopping and restarting data acquisition. Second, when you specify the cmf file in PlexControl, the name of the cmf is saved in the PlexControl settings file (pxc file), so that the appropriate cmf is loaded for a given pxc file, as opposed to having to load both pxc and cmf files manually.

Note that the cmf file specified in Server, if any, behaves as the default cmf that is loaded when Server starts up, and if you do not manually specify a cmf in PlexControl, and do not load a pxc file that contains a cmf filename, Server's cmf determines the channel mapping.

However, if Server and PlexControl specify different cmf files, PlexControl's cmf takes priority. This is so that no matter what your cmf setup, loading a pxc which specifies a cmf always has the intended effect.

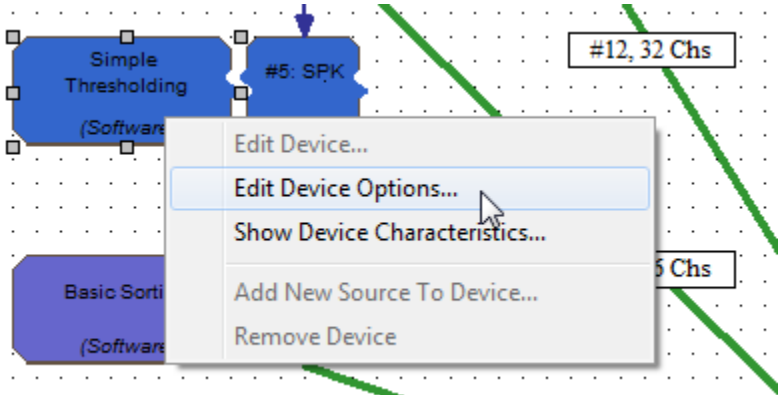
If you manually change the cmf filename in either Server or PlexControl, the cmf in both places is changed to the new filename, and the next time you save a pxc, the new cmf filename will be saved.

Remember that the two components of a channel mapping are the cmf filename, and the contents of the cmf file itself. When you save a pxc file that points to a cmf file, you are only saving the path and name to the cmf file; the contents of the cmf file itself are not saved in the pxc, only a pointer to the cmf file. If you modify the contents of the cmf file and resave it to the same filename, any pxc file that refers to that cmf file will now acquire the modified cmf behavior.

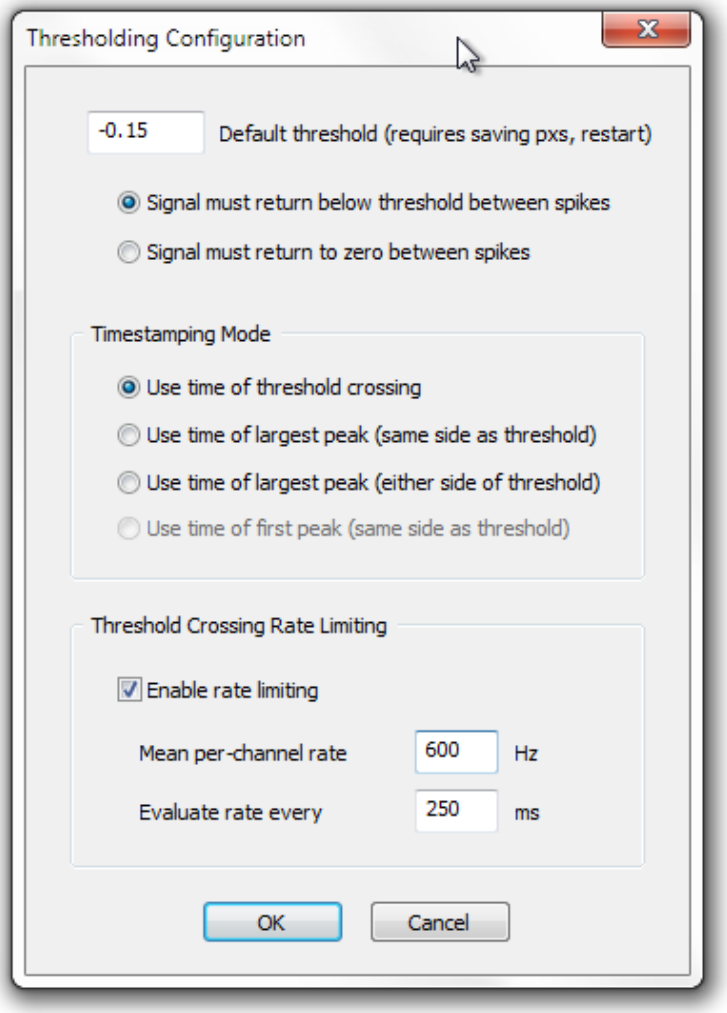
For example, if you made a channel renumbering error when you created a cmf file, fixing the error and resaving the cmf file without changing the filename will cause all pxc files that refer to this cmf file to automatically acquire the corrected mapping, without having to modify the pxc files in any way.

## 15.4 Thresholding Configuration Options

To access the threshold options, first make sure that data acquisition is stopped, then right-click on the Thresholding device in the topology in Server and select **Edit Device Options**:

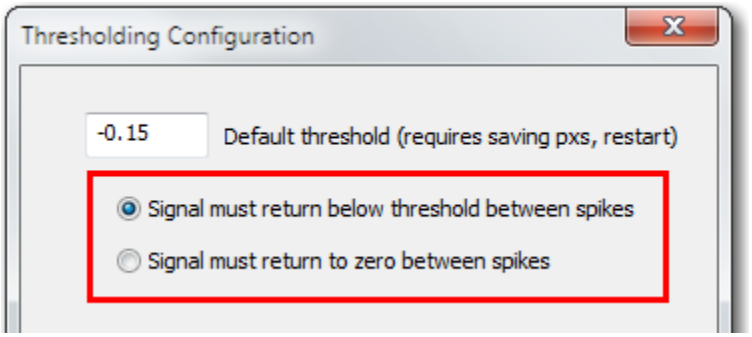



The **Thresholding Configuration** dialog is displayed:



### 15.4.1 Return to Zero Thresholding Option

The thresholding option, **Sigma must return to zero between spikes**, can yield improved spike detection in situations where the signal to noise ratio is poor and/or the threshold is set close to the noise level.




By default, the spike detector re-arms ("starts looking for another spike") after the end of each detected spike only after the signal level has dropped below the threshold level (i.e. towards zero); until this occurs, another threshold crossing (i.e. away from zero) by definition cannot occur. However, if the *Signal must return to zero between spikes* option is selected, a stricter rule for re-arming is used: the signal must not only drop below the threshold, it must actually return to zero before a subsequent spike can be detected.

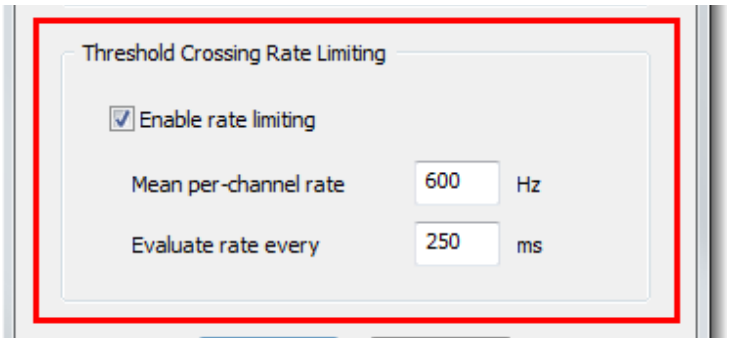
If the threshold is set conservatively, for example by using the system default autothresholding method (threshold at three or four sigmas from the mean or median), the difference between the two re-arming options will often be small. However, in cases where the threshold is set closer to the edge of the "noise band" on a channel, using the return to zero option can reduce the amount of false detection and premature triggering.

Note that the return to zero option is currently only implemented for single-electrode thresholding, not for stereotrodes or tetrodes.

### 15.4.2 Threshold Crossing Rate Limiting

The OmniPlex thresholding algorithm extracts waveform segments from continuous signal channels in the SPKC spike-continuous source, triggered by threshold crossings. In normal circumstances, when threshold values are set appropriately, and in the absence of excessive noise, the resulting waveform segments are valid action potentials, i.e. spikes. However, in atypical situations, such as signals containing high amplitude, high frequency noise, and/or thresholds that the user sets too close to zero, the system will still proceed to extract potentially large numbers of spikes at firing rates that are physiologically improbable. For example, with the default spike length of 800 microseconds, setting a zero threshold on background noise can result in a sustained "firing rate" of 1.25 kHz per channel. In such scenarios, it would be more correct to refer to the *threshold crossing rate* rather than the firing rate, since neurons cannot fire at such rates for more than a fraction of a second, if at all.

Whether due to incorrect threshold settings or intervals of unavoidable high amplitude noise, it is preferable to suppress these spurious "noise spikes," so that they do not unnecessarily increase recording file size, clutter displays, increase the processing and memory requirements for online and offline analysis programs, or degrade system performance. The **Threshold Crossing Rate Limiting** option provides a method for automatically detecting the atypical situations described above and temporarily suppressing such physiologically unlikely threshold crossing rates.




Note that rate limiting is enabled by default. You can completely disable it if you wish, although this is not recommended, especially with high channel count systems (96 channels or more). The default rate limit is proportional to the system channel count, ranging from 1500 Hz per channel at 48, 32, or 16 channels, down to 300 Hz per channel for a 256 or 512 channel system.

Rate limiting is controlled by two parameters, the **Mean per-channel rate** and the rate evaluation interval (**Evaluate rate every** parameter). The default values for these parameters have been chosen to ensure that rate limiting is applied to the thresholding process only when pathologically high threshold crossing rates are detected, and you should understand the following description of how they affect rate limiting before modifying them.

### 15.4.3 Rate Limiting Example

At an interval controlled by *the rate evaluation interval* parameter, by default every 250 ms, the rate limiter examines the total number of threshold crossings, summed over all spike channels, to determine the mean-per channel rate over the preceding evaluation interval. For example, if there are 128 spike channels and the total number of threshold crossings was 1078 over a 250 ms interval, the mean per-channel rate would be

(1078 total spikes / 128 channels) / 0.25 sec = 33.7 Hz

This is far below the rate limit of 600 Hz per channel, so no rate limiting will occur, and the spike data will be passed on to the sorter unchanged.

Now consider the other extreme, the "zero threshold in noise" scenario previously described. If the total number of threshold crossings in a 250 ms interval was 39137, the per-channel rate would be

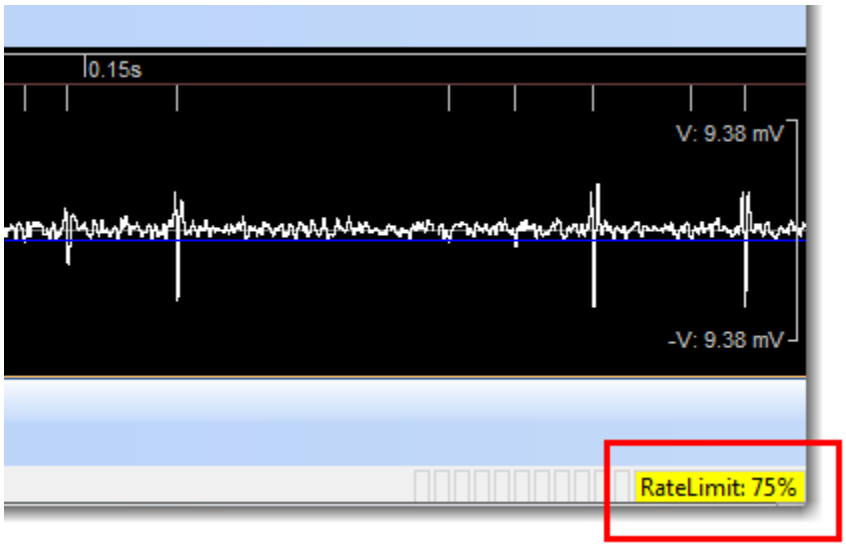
(39137 total spikes / 128 channels) / 0.25 sec = 1223 Hz

This is more than double the 600 Hz rate limit, so starting with the next 250 ms interval, spikes will be automatically culled from the data stream as necessary to limit the firing rate to 600 Hz; in this example, approximately every other spike will be removed. Every 250 ms, the aggregate spike rate across all channels will be re-evaluated, and the number of spikes culled will be updated as necessary to stay within the rate limit. As soon as the aggregate spike rate drops below the limit, rate limiting immediately stops until an excessive rate is detected again.

Note that even when rate limiting is being performed, *any individual channel on which fewer than the maximum number of threshold crossings occur within an evaluation interval will not be affected*. For example, if a brief burst of high frequency noise occurs on all channels, and therefore initiates rate limiting, but in the next 250 ms interval, only one channel's firing rate exceeds 600 Hz, no spikes on any other channel will be affected.

Since rate limiting does not occur until the interval after the interval within which the excessive rate was detected, brief bursts (by default, up to 250 ms) of even physiologically implausible activity will be passed through without change, while protecting against sustained non-neural threshold crossing rates.

When rate limiting is taking place, and spikes are being removed on one or more channels, a rate limiting indicator in the PlexControl status bar displays the rate limiting in effect:




In this example, the spike rate is being limited to 75% of what it would be if rate limiting were not applied. In other words, 25% of the threshold crossings are being removed, implying that the mean crossing rate is approximately 600 Hz / 0.75 = 800 Hz. When the yellow **Rate Limit** indicator is not displayed, 100% of spikes are available. Again, remember that channels whose firing rate is less than the rate limit will not be affected.

Remember that rate limiting has no effect in a situation when one, or a small percentage of channels have an excessive threshold crossing rate. It is intended only to suppress pathological firing rates when they occur across many channels simultaneously, for a period longer than the evaluation interval.

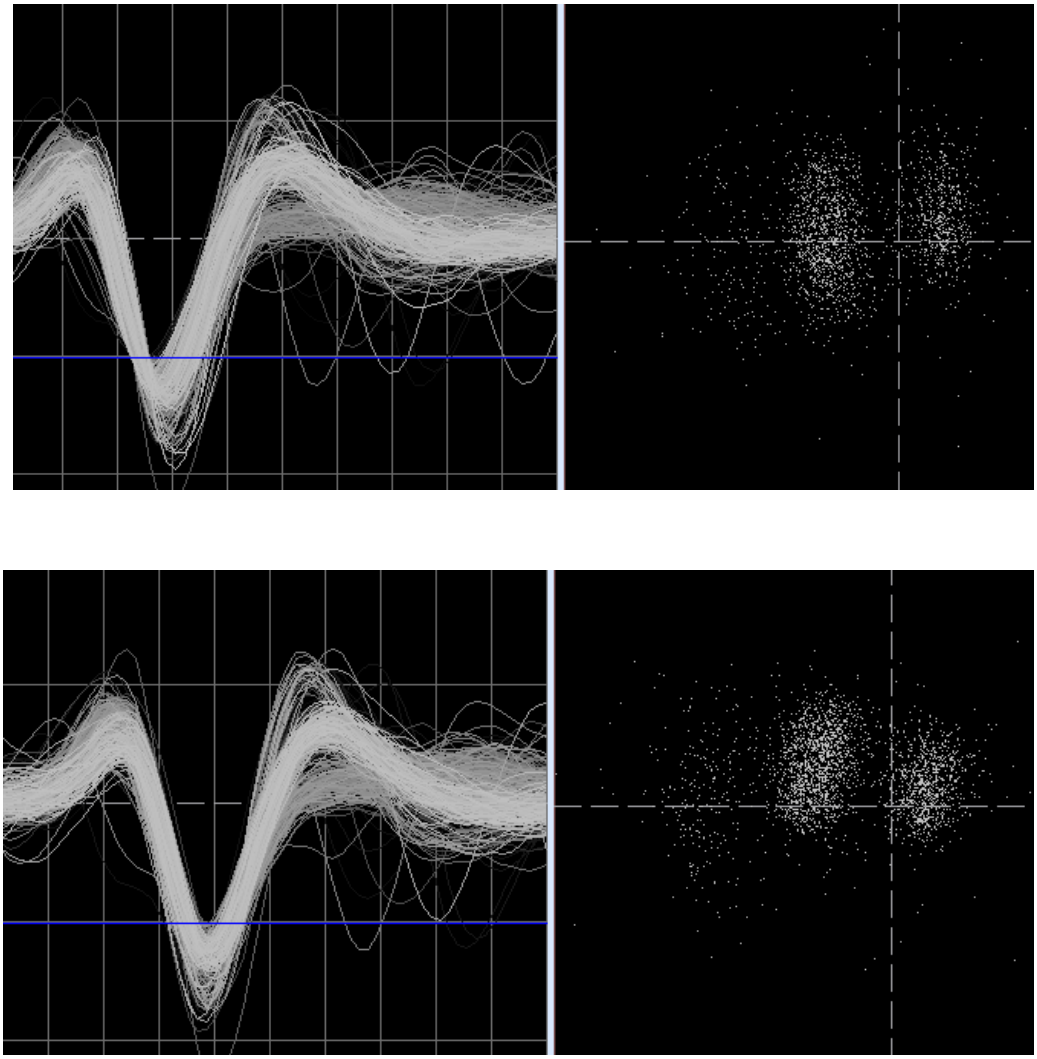
Rate limiting has no effect on the latency of spikes, whether rate limiting is in effect or not. Rate limiting only affects spikes (waveform segments) and has no effect on continuous data. If you record the continuous wideband (WB) and/or spike-continuous (SPKC) sources, you will be able to apply appropriate thresholds and extract valid spikes from the recorded data offline, assuming that any rate limiting during the recording was the result of a too-low threshold, and not caused by excessive noise in the original analog signals.

### 15.4.4 Thresholding By Aligned Extraction

By default, the OmniPlex System uses a standard thresholding (spike extraction) algorithm which defines the time at which the spike waveform crosses (exceeds) the threshold as its timestamp. While this is a classic method of thresholding, it has some drawbacks. First, it means that as the threshold is raised or lowered, the threshold "hits" the spike at different points on a rising or falling edge, potentially resulting in the timestamp changing by one, two, or more sample positions. Another manifestation of the same effect is that spikes of different amplitudes will have slight timestamp offsets relative to each other. While the size of these timestamp errors or "jitter" is too small to cause problems in most spike train analyses, it does have a significant effect on spike sorting: it causes smearing of clusters in feature space.

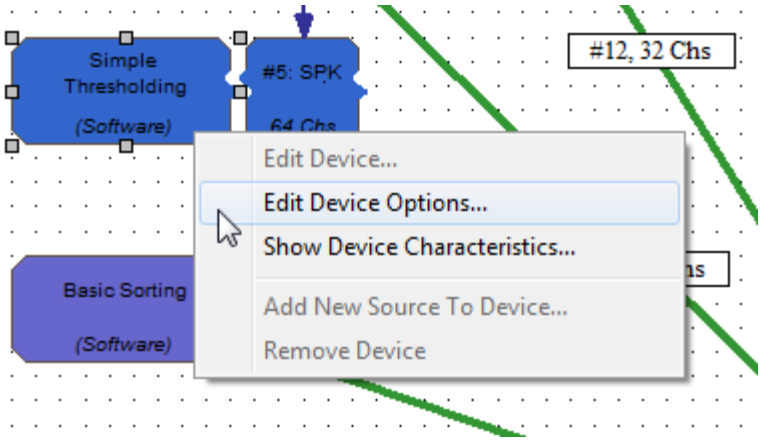
The basic problem with threshold timestamping is that it does not use an unambiguous feature (in time/voltage space) of the spike as the reference to which a timestamp is attached. The obvious choice for such a reference is the largest peak within the spike. The OmniPlex System aligned extraction thresholding algorithm uses this approach.

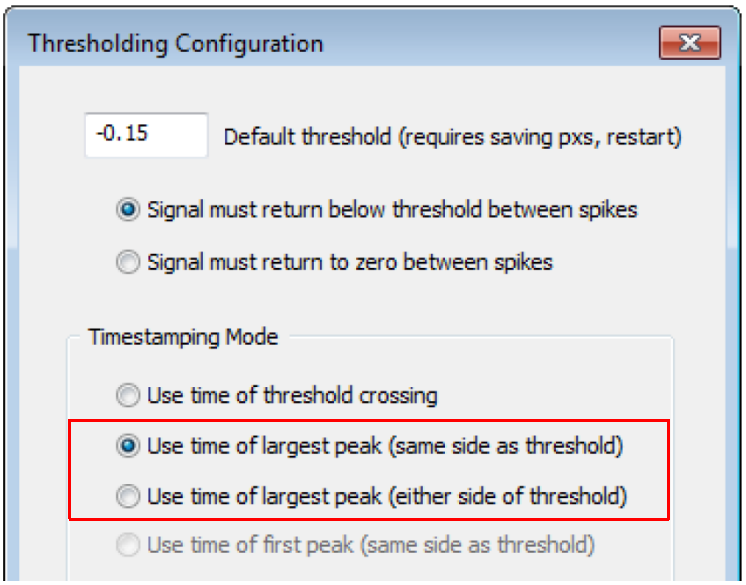
Here is an example of the difference between standard threshold crossing (top) and aligned extraction (bottom). Note how the waveform “bundles” and the corresponding PCA clusters are more well-defined when aligned extraction is used:



Besides the improved timestamping accuracy and jitter reduction, the more well-defined clusters improve the performance of the automatic unit finding algorithm.

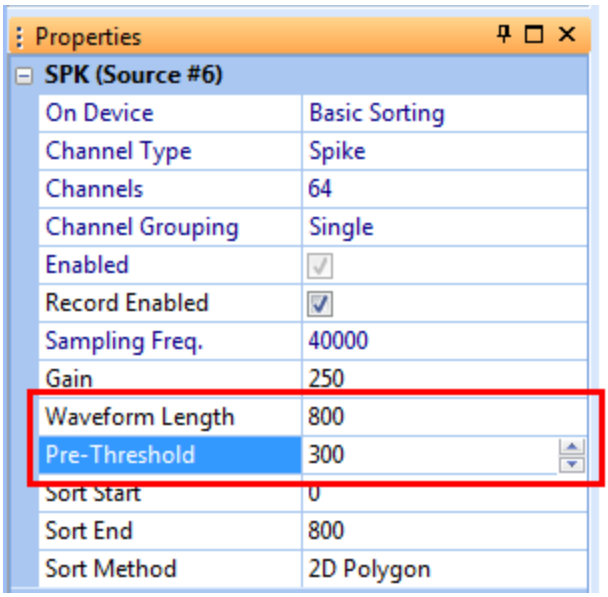
Aligned extraction can be enabled in the device options for the thresholding device in Server:




The first setting is standard thresholding, while the next two are two varieties of aligned extraction. The “time of largest peak (same side as threshold)” setting is recommended (as shown above), because typically you will set the threshold for a channel on the side where the larger-amplitude peaks occur.

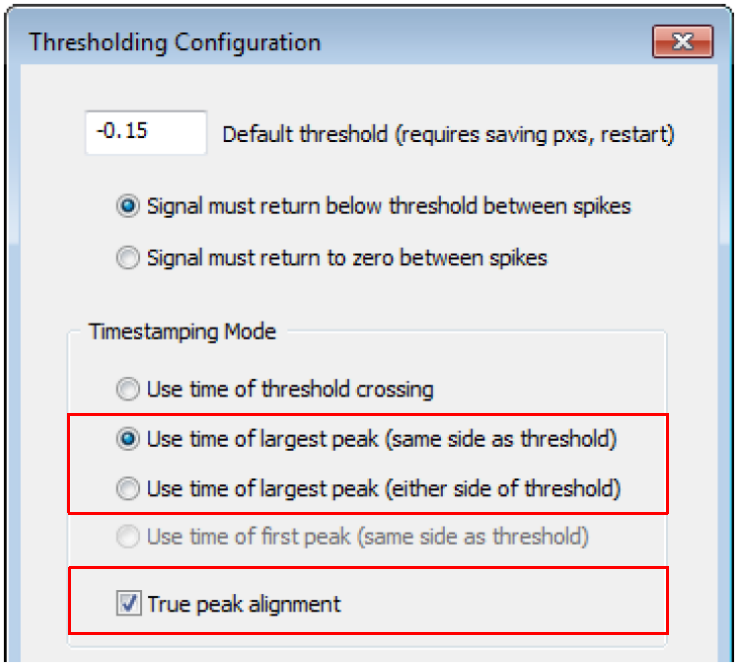
You must stop data acquisition before changing the thresholding mode. Note that there is one other change which you will probably wish to make when using aligned extraction. Due to the peak, rather than the threshold crossing, being used as the timestamp of spikes, the ratio of pre-threshold to post-threshold time tends to be different when aligned extraction is used; in other words, a longer pre-threshold time is appropriate. As a typical example, the default the OmniPlex System settings are a waveform length of 800 microseconds and a pre-threshold time of 200 microseconds; when aligned extraction is enabled, it is suggested that the pre-threshold time be increased to 300 microseconds.




However, the correct values for waveform length and pre-threshold will be somewhat dependent upon the type and shape of action potentials that you are recording. You should ensure that the waveform length and pre-threshold are long enough to capture the features of the spike, without including unwanted noise and artifacts at the head or tail of the spike.

### True peak alignment for aligned extraction thresholding

A **True peak alignment** option can be enabled for the aligned extraction thresholding methods (**Use time of largest peak…**):

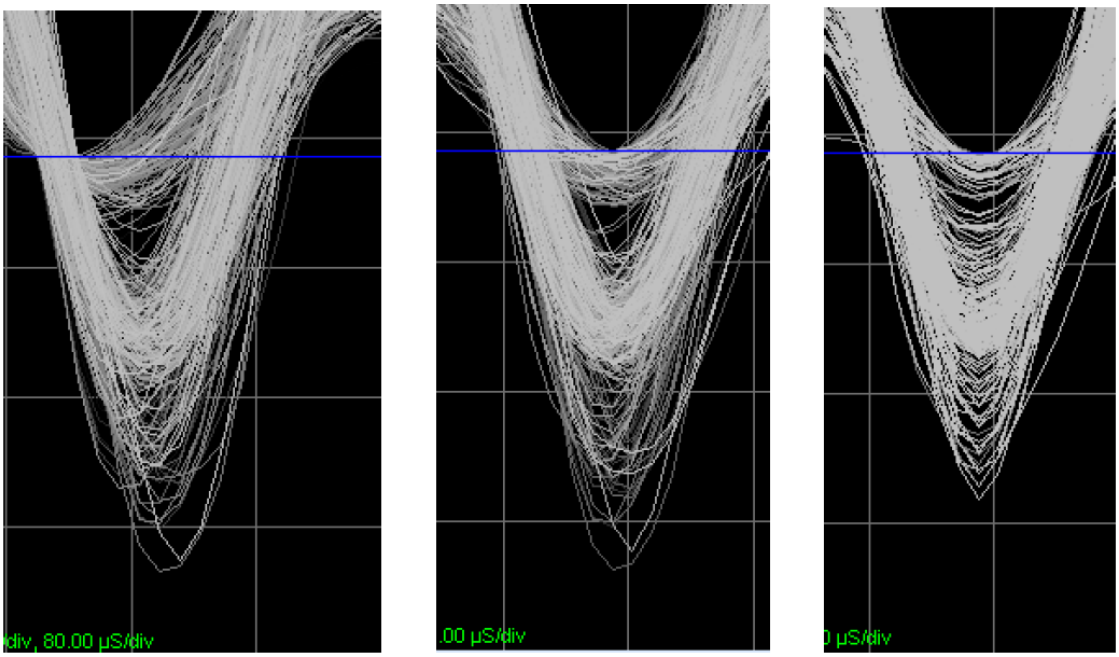


When **True peak alignment** is disabled, the normal aligned extraction method uses the timestamp of the waveform sample with the largest peak amplitude as the timestamp of the spike. However, the true peak of a waveform almost never falls directly on a sample point (time), but somewhere in between two successive samples. The result is that even if the waveform shape is constant (i.e. every spike has the same shape), the timestamp assigned to the spike can vary or "jitter" by +/- 1 sample tick. When many spikes from the same unit are extracted this way, the effect is that the "bundle" of waveforms is "smeared" in time/voltage space, or even split into what appears to be two or three time-offset groups of waveforms, which can appear as a single unit being split into two or three units. Analogous adverse effects occur in derived feature spaces, such as PCA.

However, when **True peak alignment** is enabled, the OmniPlex algorithm improves the result of an aligned extraction as follows. Each incoming detected spike is interpolated using a spline function, and the true sub-sample (between-sample) peak of the spline is determined. The spike is then resampled using the spline, such that the true peak is now defined as falling exactly on a sample tick. In order to avoid large, unexpected changes to the spike waveform in unusual cases, this resampling is only performed if the time shift of the peak would be less than or equal to one sample tick on either side of the original peak timestamp. In practice, almost all waveforms can be aligned using +/- 1 tick of offset or less.

The net result is that waveform peaks are precisely aligned with each other, as can be seen in the following example. From left to right:

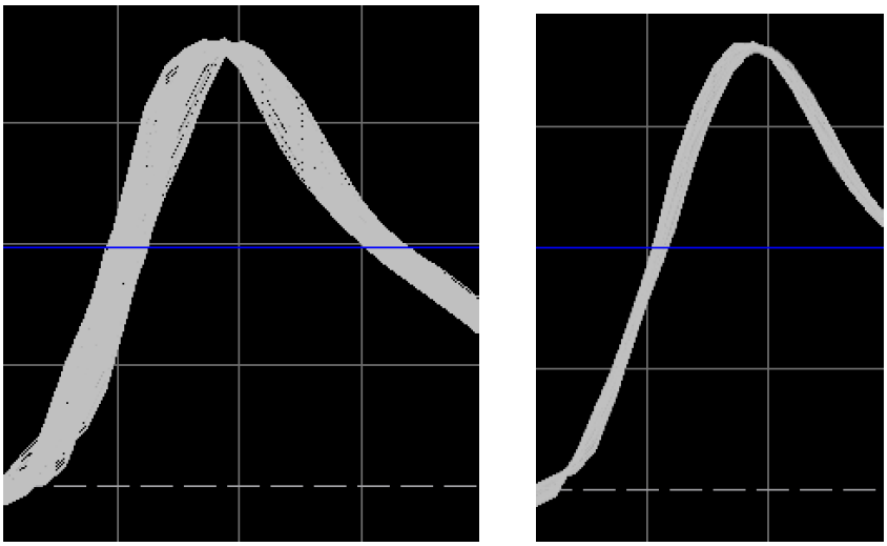
1. Standard threshold crossing, without aligned extraction
2. Aligned extraction, with **True peak alignment** disabled
3. Aligned extraction, with **True peak alignment** enabled




( 1 ) ( 2 ) ( 3 )

Note that the set of spikes in each of the three screen shots is not identical (for example, (3) has fewer high-amplitude spikes), but taken as a whole they are representative of the differences between the three thresholding methods.

Here is another example, using a function generator waveform, with **True peak alignment** disabled (left), then enabled (right). The function generator insures that, for all practical purposes, each detected waveform has exactly the same shape before it is digitized. The tighter "waveform bundle" is clearly visible.



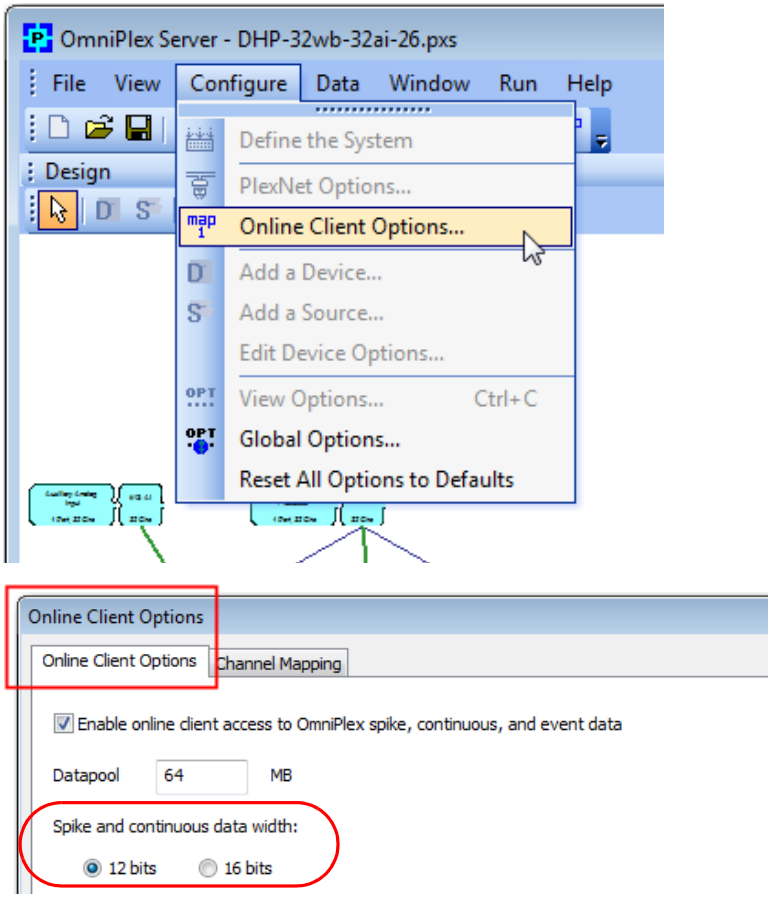
Since the OmniPlex System has access to the live continuous data preceding and following the extracted waveform, there are no issues with "zero padding" at the ends of the waveform, and the waveform is correctly extended with valid sample data as required.

The optimized implementation of this method allows the OmniPlex System to perform online aligned extraction with true peak alignment on a full 512 channels of spike data, with no performance or latency issues compared to standard aligned extraction.

## 15.5 Spike and Continuous Data Width—Export to External Clients

In the **Online Clients Options** dialog (see the diagrams below) you can select the resolution of the continuous and spike waveform data sent to online clients:

- **12 bits**—The system will encode all continuous and spike waveform data sent to online clients as signed 12 bit values within a 16 bit word. In other words, the raw unscaled sample values occupy a range from –2048 to +2047. This option should be selected if you need compatibility with legacy online clients which expect 12 bit values, as were used in the original Plexon® MAP ("Harvey Box") system.
- **16 bits**—The system will encode all continuous and spike waveform data sent to online clients as full 16 bit words, that is, full 16 bit resolution with sample values in the range –32768 to +32767. This provides an additional four bits (a factor of 16) more resolution than 12 bit values.



**Note:** When 16 bit mode is enabled for data sent to external clients that are designed for processing 12 bit data, you *must* set the client programs to divide sample values by an additional factor of 16 (preferably in floating point, so as to take advantage of the four bits of additional precision) to maintain correct voltage scaling.

**CAUTION**

**Client programs must scale 16 bit data correctly**

If you enable 16 bit mode for data sent to external clients, you must set the client programs to scale the sample values correctly. Otherwise, scaled voltages on the external client will be larger than the true values.

Follow these steps to change the data width:

1. In the Online Client Options dialog, select 12 bits or 16 bits for the data width.
2. Save the pxs file.
3. Shut down and restart the OmniPlex System, start data acquisition, then run any client programs as usual.

# Chapter 16
# MultiPlex Multi-source View

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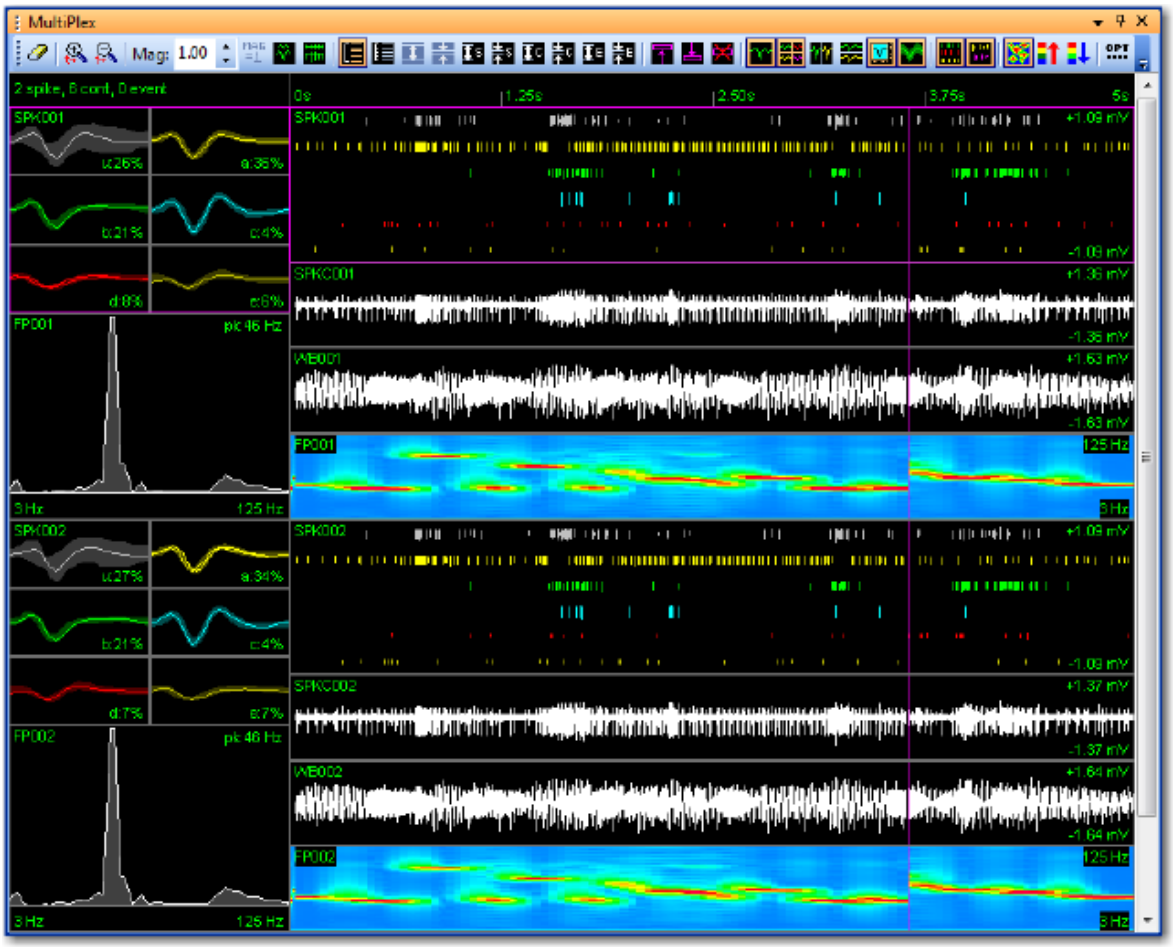
## 16.1 Introduction

This chapter describes the MultiPlex multi-source view and how to use it.

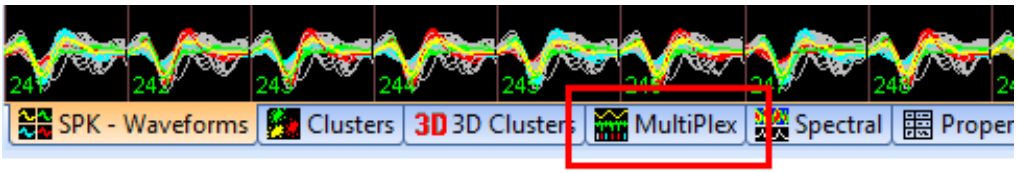
The MultiPlex view allows you to view a customized set of spike, continuous, and event channels from any combination of sources, in any order. Channels are displayed in rows, as in the Activity view, but each row can contain any one of the three types of channels, and each type of row can be sized independently. Besides the rows of sweeping signal traces and/or ticks, an optional column of "scope" windows can be displayed to their left. For example, for a spike channel, where the main display shows spike ticks, the scope displays the waveforms for that channel, together or in individual unit windows. "Slow" continuous channels (sampling rate <= 5 kHz) can be displayed as either signal traces or as spectrograms; when in spectrogram mode, the scopes for these channels display the associated spectral graph (FFT). Here is an example of a MultiPlex view.




Many options are available for adjusting the way in which channels are displayed, for adding and removing channels, etc. Besides the flexible display configuration, there are other new visualization features such as rolling waveform variance envelopes, an oscilloscope-like spike display mode, and tracking of spectral peak frequencies. You can scroll through hundreds of channels of online spike, continuous, and spectrogram displays, with modest CPU usage.

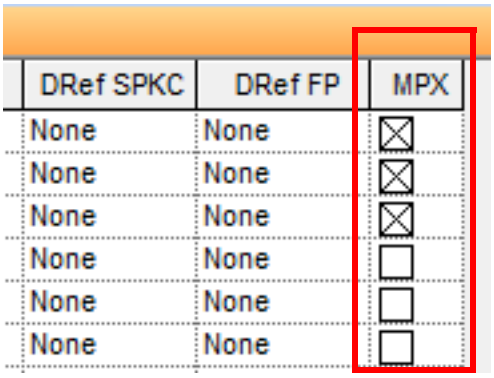
## 16.2 Getting Started with the MultiPlex View

The MultiPlex view is automatically created when PlexControl starts up, like most other views:

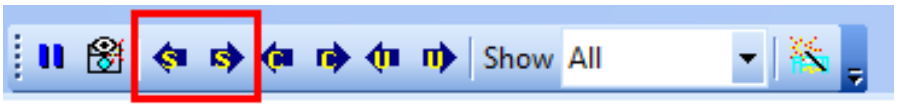



However, it is different in that by default it initially displays no channels. When you select the **MultiPlex** tab, a message is displayed in the middle of the empty window, prompting you to add channels. This can be done in two ways:

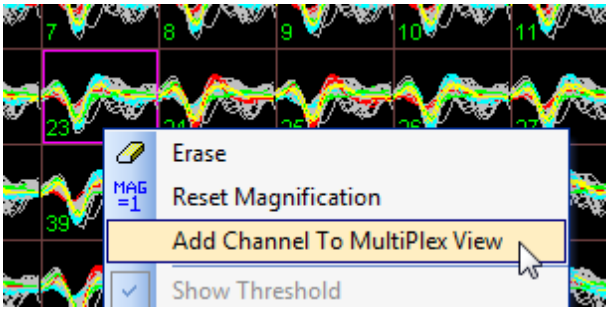
1. Checking a box in the **MPX** column of the main **Properties Spreadsheet** adds that channel to the MultiPlex view. Unchecking the box removes the channel from the MultiPlex view. You can add and remove channels at any time.




Remember that you can make a source visible in the **Properties Spreadsheet** either by clicking on a view that is displaying that source, or by using the **Previous Source** / **Next Source** buttons in the main PlexControl toolbar to cycle through all the available sources, including sources like CinePlex® events and keyboard events.

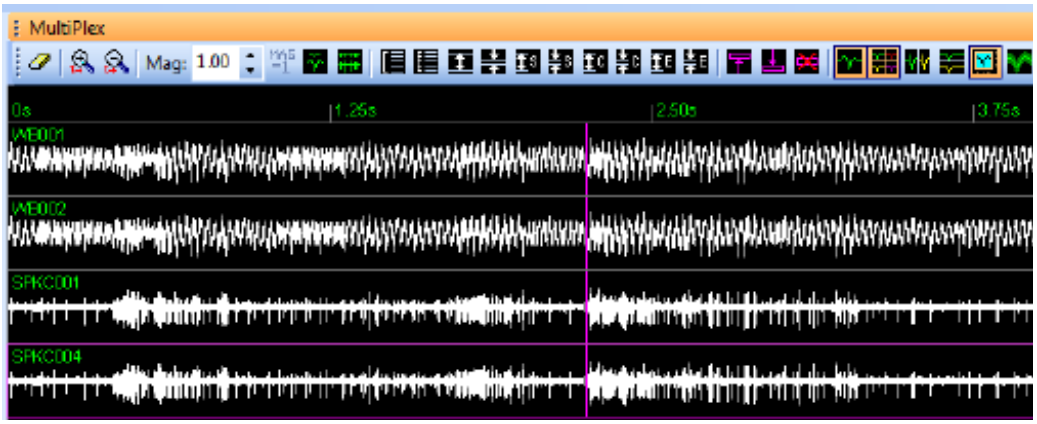


2 Right-clicking on a channel in any spike, continuous, or activity view, and selecting **Add Channel to MultiPlex View** from the right-click menu.




One exception to note is that from the Activity view, you can select and add *spike channels* using the right-click menu, but *not event channels*. However, you can use the **Previous Source** / **Next Source** toolbar buttons in the main toolbar to select the desired event source into the **Properties Spreadsheet** and then use the **MPX** column to add event channels.

Using either method, each added channel results in a row being added to the MultiPlex view.



**TIP**

**Use the keyboard and mouse shortcuts in MultiPlex view**

There are a number of keyboard and mouse shortcuts you can use to manipulate the rows in the MultiPlex display. See Section 16.20, “Keyboard and Mouse Shortcuts for the MultiPlex View” on page 532.

## 16.3 Accessing Features in MultiPlex View

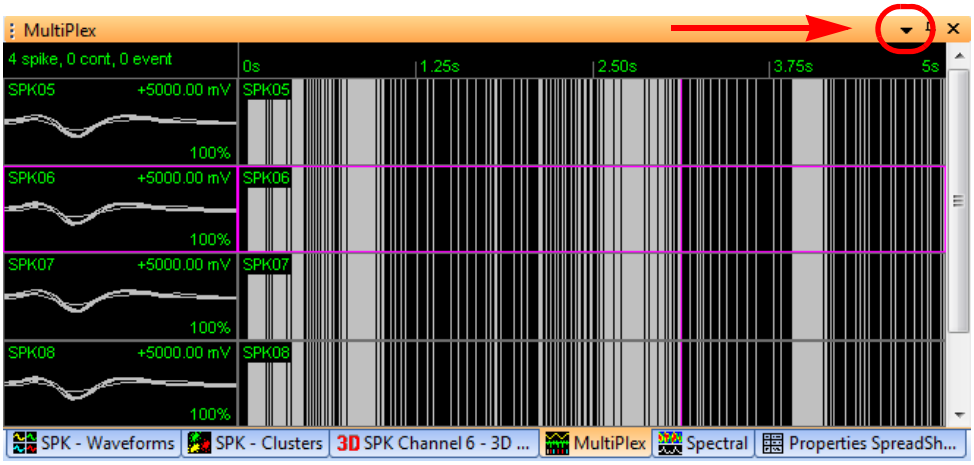
The MultiPlex view has many commands that give you a great deal of control over the displayed channels and data. There are several methods you can use to access all of the MultiPlex view features. Some of the features are available in only one method, and some features are available by means of multiple methods. These methods are summarized in the following sections.

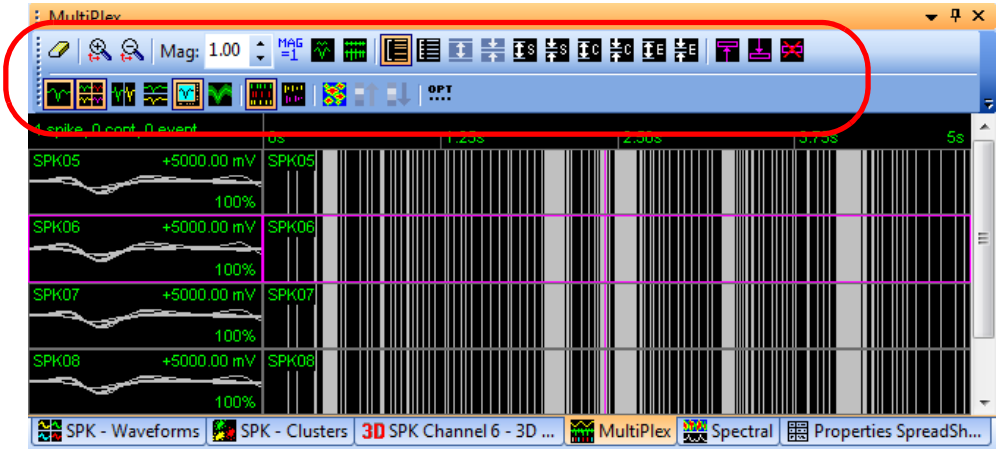
- Section 16.3.1, “MultiPlex View Toolbar Commands” on page 507
- Section 16.3.2, “MultiPlex Right-click Menu” on page 508
- Section 16.3.3, “Options Dialog” on page 509
- Section 16.20, “Keyboard and Mouse Shortcuts for the MultiPlex View” on page 532

You should also familiarize yourself with the information in Section 16.19, “Limitations in MultiPlex View” on page 531.

### 16.3.1 MultiPlex View Toolbar Commands

To view the toolbar commands, click the down arrow in the MultiPlex view header.



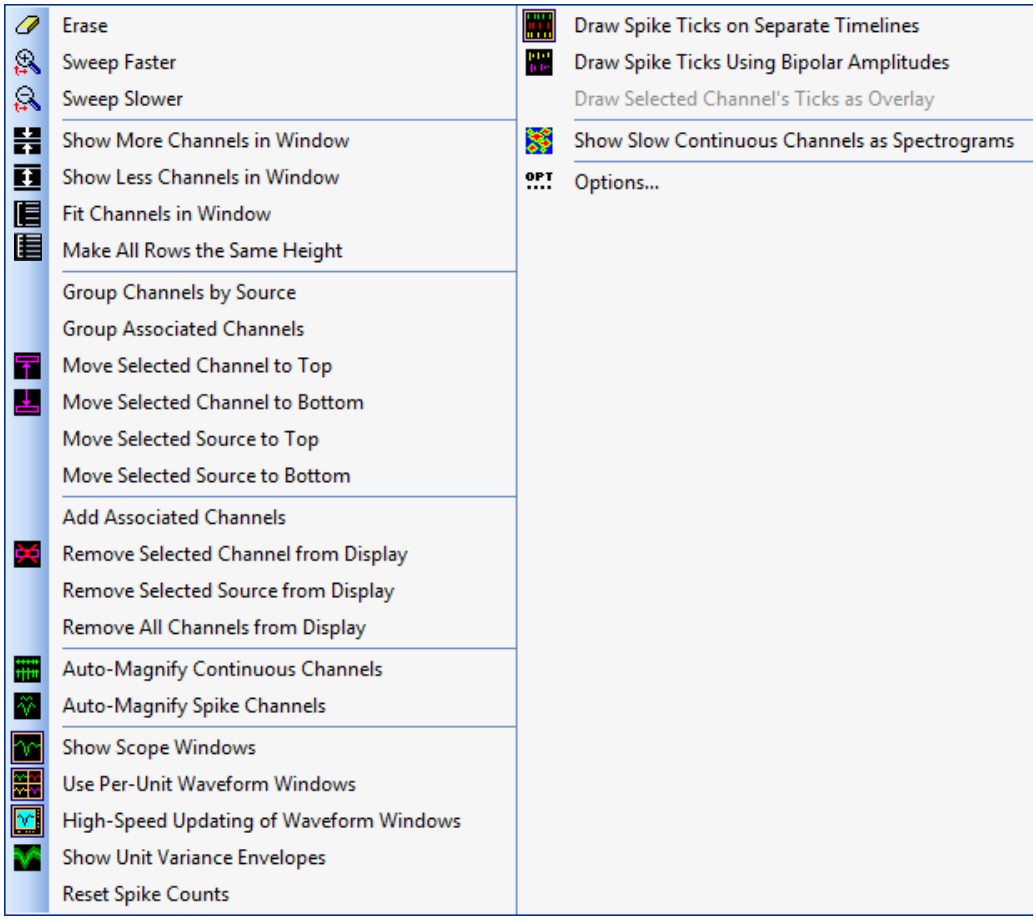



The uses of these toolbar commands are discussed in the following sections:


- Section 16.4, “Toolbar Commands—Moving and Removing Channels” on page 510
- Section 16.5, “Toolbar Commands—Sweep and Magnification Controls” on page 511
- Section 16.6, “Toolbar Commands—Row Layout and Sizing Tools” on page 513
- Section 16.7, “Toolbar Commands—Show Scope Windows” on page 515
- Section 16.8, “Toolbar Commands—Customize Spike Displays” on page 516
- Section 16.9, “Toolbar Commands—Adjust Display of Ticks” on page 520
- Section 16.10, “Toolbar Commands—Spectrograms and Spectral Graphs” on page 521


### 16.3.2 MultiPlex Right-click Menu

You can access a wide range of MultiPlex commands by right-clicking anywhere in the MultiPlex view:



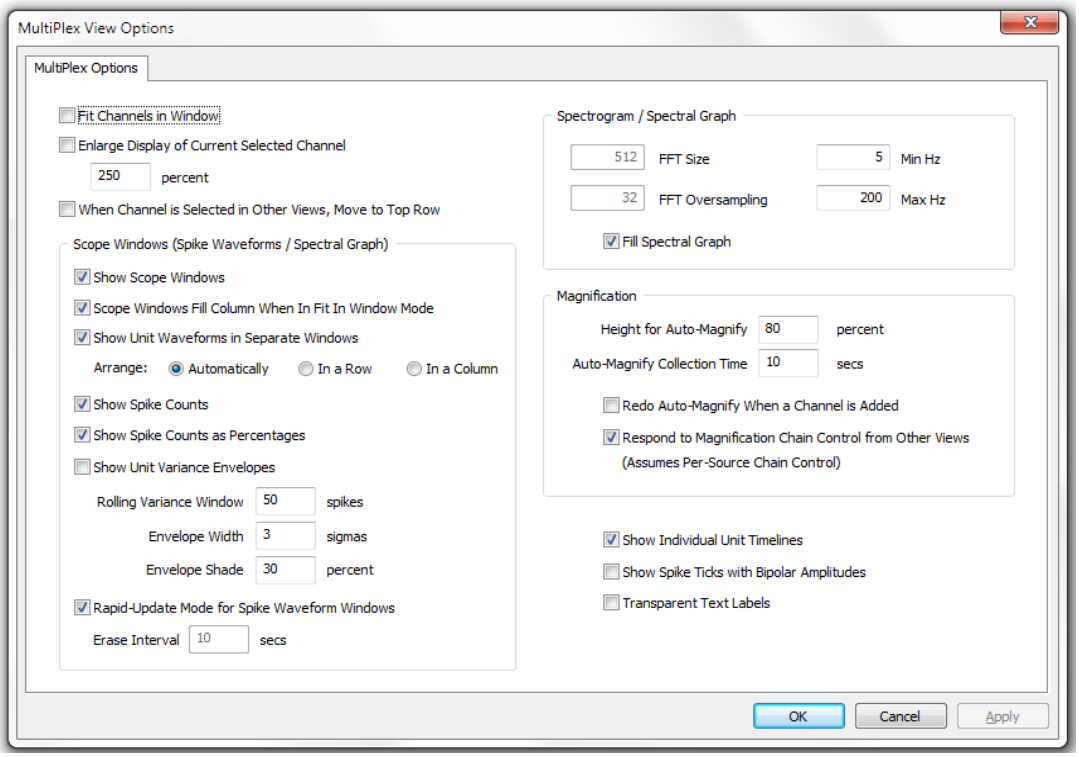
As shown in the image above, some of the commands in the right-click menu display icons that are associated with toolbar commands, which signifies that those commands are available through *both* the toolbar *and* through the right-click menu. Those commands function identically whether you access them using the toolbar or from the right-click menu. For a description of those toolbar commands, see the applicable sections in this chapter.

Some of the commands in the right-click menu are available *only* through this menu, and they are discussed in the following sections:

- Section 16.11, “Right-Click Menu—Group Channels” on page 524
- Section 16.12, “Right-Click Menu—Move Selected Source” on page 525
- Section 16.13, “Right-Click Menu—Add/Remove Source or Channels” on page 525
- Section 16.14, “Right-Click Menu—Reset Spike Counts” on page 526
- Section 16.15, “Right-Click Menu—Draw Selected Channel’s Ticks as Overlay” on page 527

### 16.3.3 Options Dialog

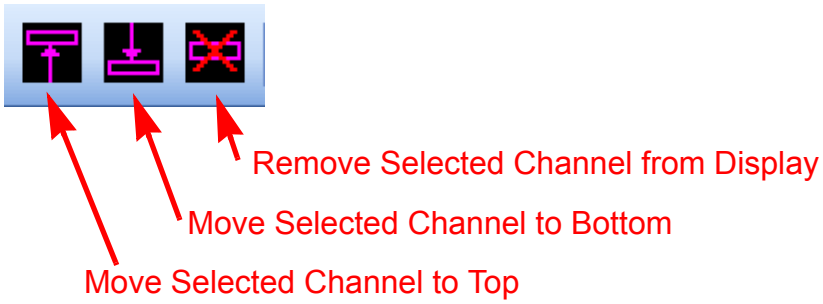
One of the important features accessible from the MultiPlex toolbar and right-click menu is the **Options** dialog. (It is also accessible by pressing the “O” key on the keyboard while the MultiPlex window is selected.) Several of the topics in this chapter will refer to the controls in this dialog. See Section 16.16, “MultiPlex View Options Dialog” on page 528.



## 16.4 Toolbar Commands—Moving and Removing Channels

Channels are displayed in the order in which they are added, but changing this order is easy. Select a channel and, holding down the left mouse button, drag up or down to move the channel up or down in the list of channels. Alternately, you can do this by selecting a channel and then using the up-arrow/down-arrow keys while holding down Shift.

In addition, there are toolbar and menu commands that move channels and sources (i.e. all the MultiPlex channels from the same source as the selected channel) to the top or bottom of the view. For example, these toolbar commands move the selected channel to the top or bottom of the display, or remove it from the view:




There are other ways to remove channels from the display:

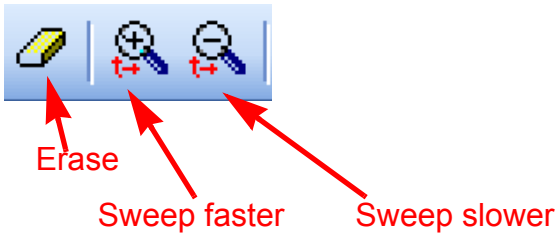
- You can delete a channel by selecting it and pressing the **Delete** key.
- The **Remove All Channels from Display** command in the MultiPlex right-click menu resets the view to the empty state (a message box will ask you to confirm that you really want to remove all channels).

Disabled channels are not displayed in the MultiPlex view. If a channel in the MultiPlex view is disabled from anywhere in PlexControl, it is automatically removed from the view.

## 16.5 Toolbar Commands—Sweep and Magnification Controls

### 16.5.1 Sweep Controls

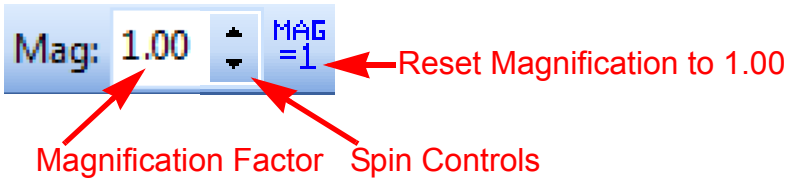
The leftmost toolbar command is **Erase**, which clears the display. **Sweep Faster** and **Sweep Slower** adjust the speed of horizontal scrolling of the displayed data; they also affect the rate at which the data are updated.




### 16.5.2 Magnification Controls

These tools give you direct control of the magnification factor. You can perform any of the following actions:

- Type in a new magnification factor and press the **Enter** key
- Use the spin controls (increase or decrease magnification by clicking the up or down arrow)
- Click the Reset Magnification button to reset the magnification to 1.00




#### Important considerations when managing MultiPlex magnification

Magnification is slightly more complicated than in other views, since the MultiPlex view can contain channels from different sources. By default, changing the magnification using the spin controls changes the magnification on all spike and continuous channels in the view. If you hold down **Ctrl** or **Shift** while clicking the spin arrows, the behavior is as follows:

- Ctrl = change magnification for only the currently selected channel
- Shift = change magnification for all channels of the selected source (i.e. the source for the currently selected channel)

A handy way to remember this is "C and S": Ctrl = Channel, Shift = Source.

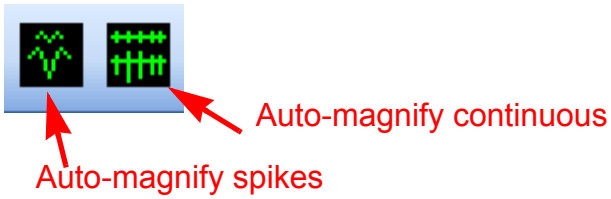
#### Using the mousewheel for magnification

You can also adjust magnification using the mousewheel. See Section 16.20, “Keyboard and Mouse Shortcuts for the MultiPlex View” on page 532.

### 16.5.3 Auto-magnify Spikes and Continuous

#### Auto-magnify commands

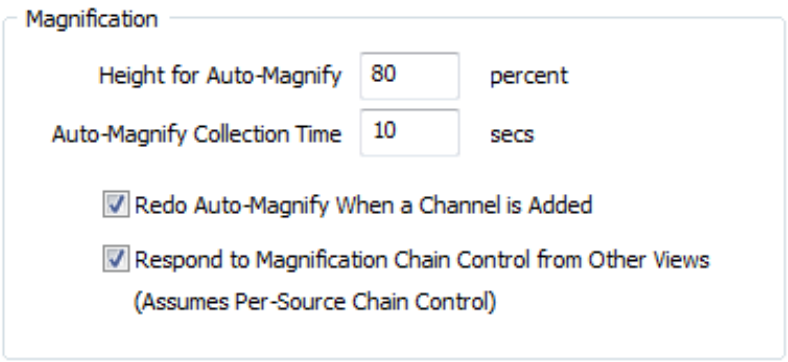
Using these commands, you can let the system automatically adjust magnification.




In practice, you may find that you don't need to manually adjust the magnification very often, but can use the auto-magnify tools instead. When you click on either of the above toolbar commands, all spike or continuous channels are monitored for a given interval (10 seconds by default, but this can be changed in the Options dialog) and then the magnification is set automatically on a per-channel basis. A handy shortcut is simply to press the 'A' key on your keyboard (note that the MultiPlex view must be selected first), which starts the auto-magnify procedure for all channels, both spike and continuous.

#### Perform auto-magnify when adding a channel

There is an option in the Options dialog to automatically perform an auto-magnify whenever you add a channel to the view:




The last option, **Respond to Magnification Chain Control from Other Views**, causes magnification changes from other spike and continuous views to also adjust the magnification of the corresponding source in the MultiPlex view.

## 16.6 Toolbar Commands—Row Layout and Sizing Tools

### 16.6.1 Fit Channels in Window

Clicking the **Fit channels in window** command fits all the channels in the MultiPlex view into the window, so that you never need to scroll to see them all. However, to keep the view from becoming unusably crowded, the system limits the number of channels (rows) to 64; if there are more than 64 channels in the MultiPlex view, this command is disabled.

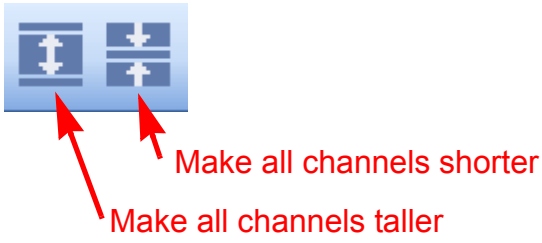
Note that once you enable **Fit channels in window**, it is a mode that is in effect. If you add another 20 channels, it will still make everything fit in the window. If you only have one channel, it will expand that one channel to fill the full height of the window, which may not be the ideal appearance. If the layout appears awkward, consider whether you have **Fit channels in window** turned on, and whether it's appropriate for what you are trying to display.

### 16.6.2 Make All Rows the Same Height

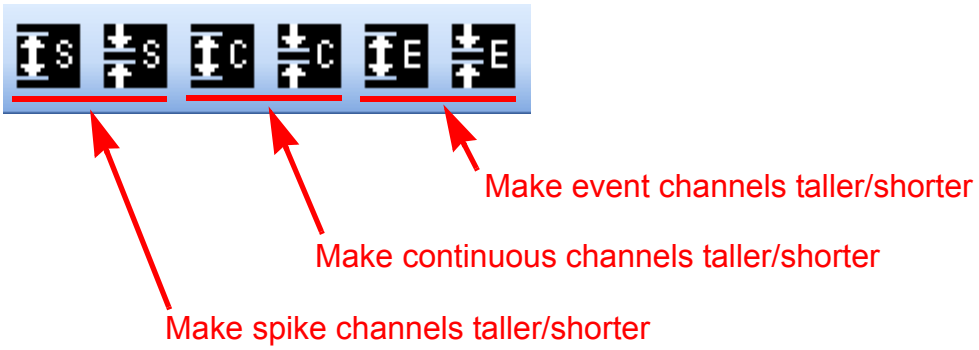
This command makes all rows in the MultiPlex view the same height.

### 16.6.3 Make Channels Taller/Shorter

These commands make all the channels in the MultiPlex view (spike, continuous, and event) appear taller or shorter.

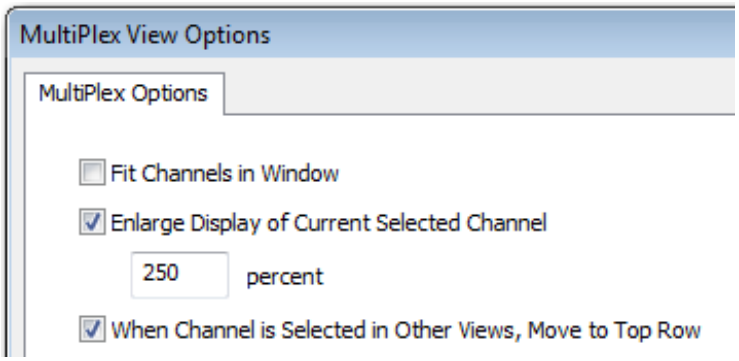


Each of the commands in the image below applies only to a specific category of channels in the MultiPlex view. The “S” commands make all spike channels taller/shorter; The “C” commands make all continuous channels taller/shorter; the “E” commands make all event channels taller/shorter:




Note that the default row heights are not the same—Spike channels are the tallest and event channels are the shortest, since that works well in typical use. You can always use the above commands to size them any way you like.

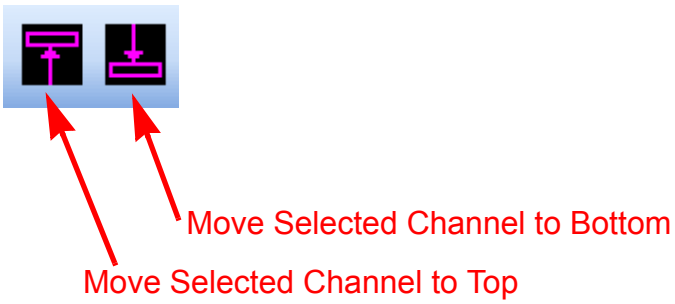
There is also a feature than currently can only be toggled from the Options dialog which automatically enlarges the currently selected channel to a specified percentage of its normal height; for example, the default value of 250% means that the selected channel is 2.5 times as tall as when it is not selected. Another option allows the currently selected channel to be automatically moved to the top row.




The combination of these two options in effect "features" a channel in the MultiPlex view, without it "taking over" the entire window like the usual OmniPlex® "double-click to zoom channel" functionality.

Note that the latter option (**When Channel is Selected in Other Views, Move to Top Row**) only applies when a channel is selected from "outside" the MultiPlex view. (You actually wouldn't want selecting a channel in the MultiPlex view to automatically [and suddenly] pop it to the top row of the display.)

**Note:** If you do want to move a channel to the top or bottom of the display, use the following commands:



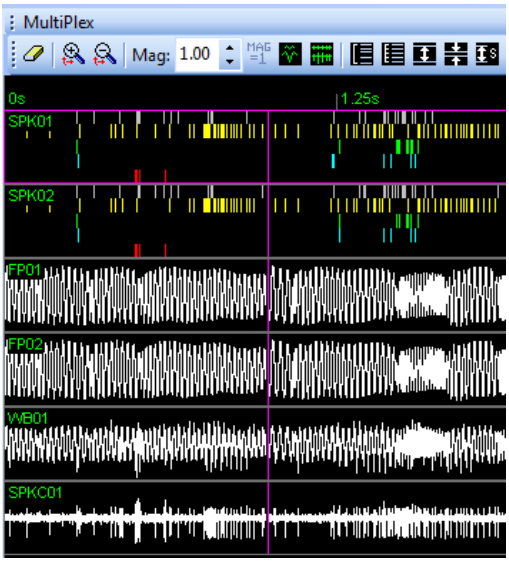

Note that there are also MultiPlex right-click menu commands for **Move Selected Source to Top/Bottom**, which act similarly to **Move Selected Channel** except that all the channels in the same source as the selected channel are moved to the top or bottom of the window.

### 16.6.4 Grouping Channels

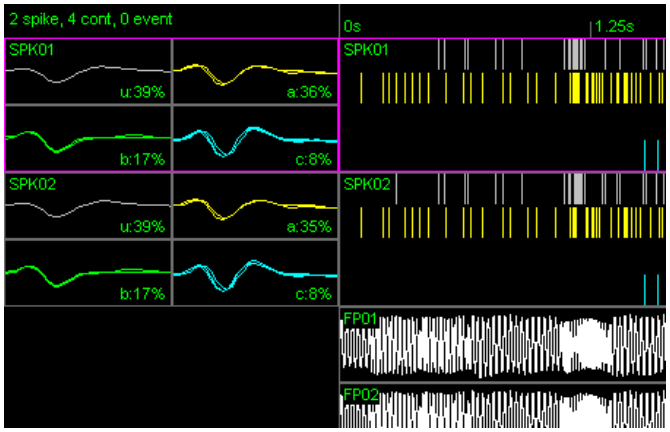
Two of the right-click menu commands affect the top to bottom order of all the channels in the MultiPlex view: **Group Channels by Source** and **Group Associated Channels**. For this information, see Section 16.11, “Right-Click Menu—Group Channels” on page 524.

## 16.7 Toolbar Commands—Show Scope Windows

This command toggles the display of the column of "scope" windows to the left of the main channel rows. For example, with scopes off:



Scopes on, and with the spike channels made larger:



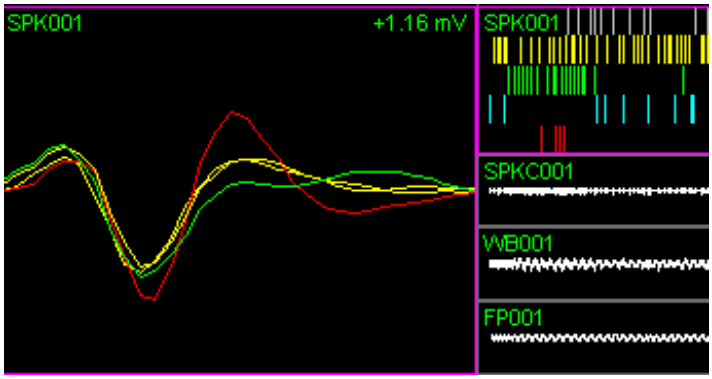

The unit windows display the current relative number of spikes detected. There is a menu command for resetting the spike counts (see Section 16.14, “Right-Click Menu—Reset Spike Counts” on page 526), and an option in the **Options** dialog for displaying raw counts instead of percentages (see Section 16.16, “MultiPlex View Options Dialog” on page 528).

## 16.8 Toolbar Commands—Customize Spike Displays

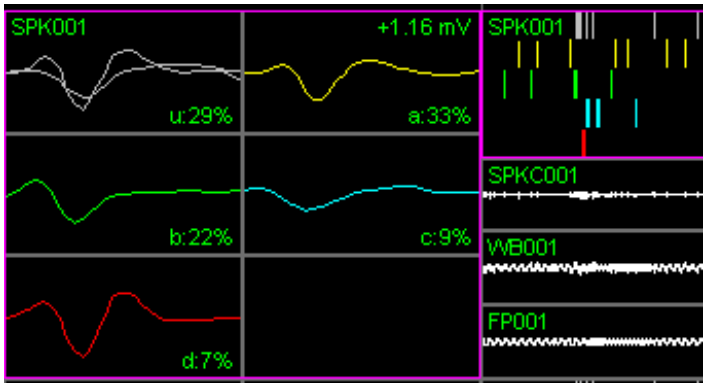
These commands allow you to customize the spike and tick displays.

### 16.8.1 Show Units in Separate Windows

This command toggles the display for a particular spike signal to show all units for the spike or to show only one individual unit belonging to the spike signal. For example, with separate unit windows off:

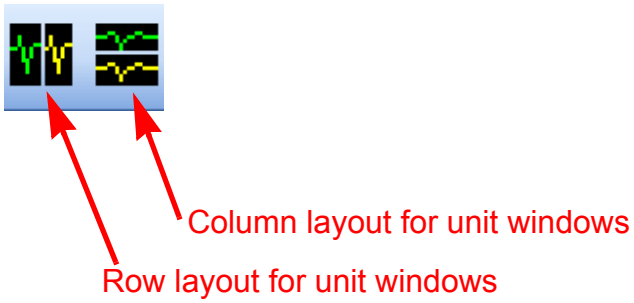


With separate unit windows on:

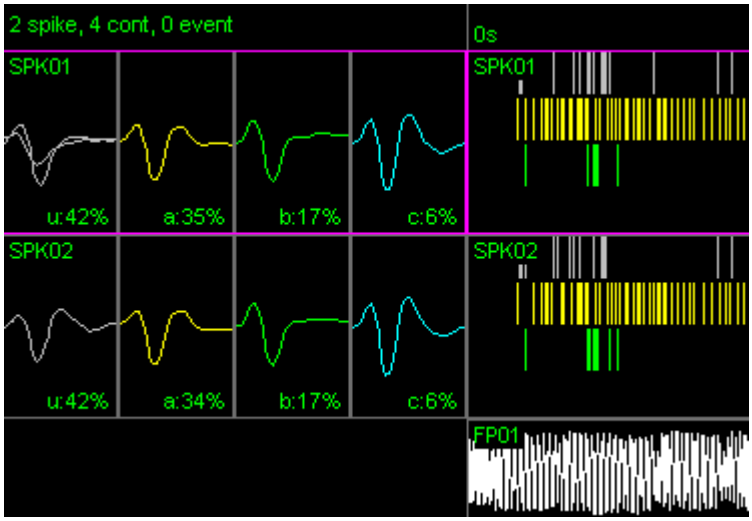



### 16.8.2 Row and Column Layouts for Unit Windows

By default, the MultiPlex view automatically arranges the unit windows in rows and columns. However, these two commands allow you to force it to display the unit windows in rows or columns:




For example, with separate unit windows and a row layout:

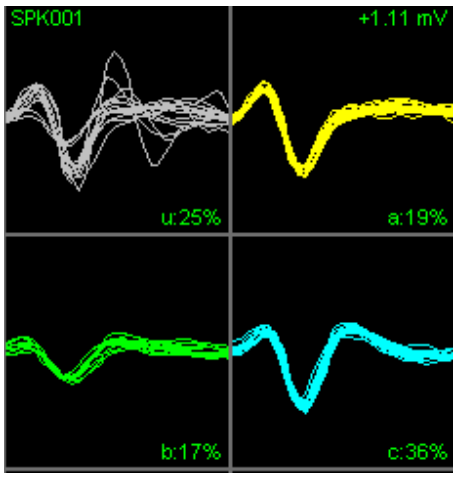


### 16.8.3 High-speed Update Mode

This command toggles high speed updating mode (“scope mode”).

When this mode is enabled, the spike waveform displays operate in a quasi-oscilloscope mode, where each new spike erases the one before it, but the most recent spike remains until another one is seen. This gives a result similar to using an external oscilloscope, one for each channel in the MultiPlex view.

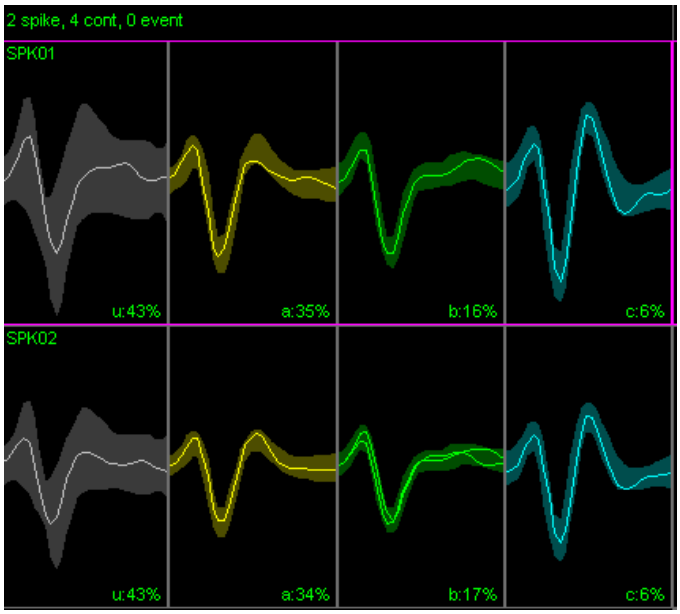
If you disable high speed updating, the display returns to the classic "accumulate and periodic erase" mode, with an erase time that you can set in the Options dialog.



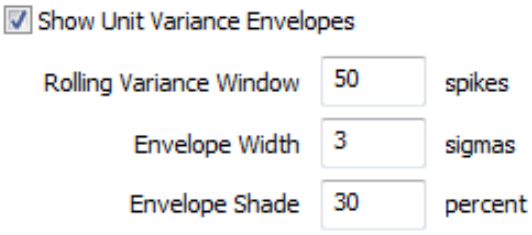

### 16.8.4 Show Unit Variance

This command toggles display of rolling variance envelopes.

For example, with display of variance enabled:



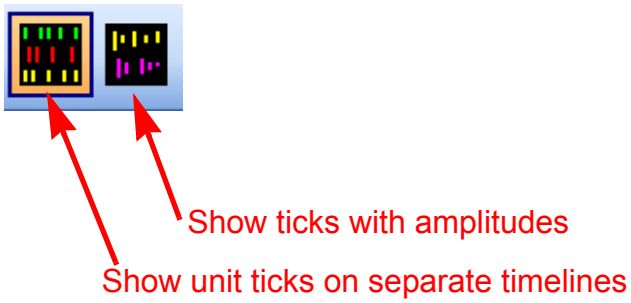

The shaded envelope indicates plus or minus N sigmas from the mean. The mean waveform itself is not displayed (it would clutter the display) but is by definition the centerline down the middle of the variance envelope. Both the mean and the variance are calculated from a moving window M spikes long, and incrementally updated on every spike. Both the length of the moving window (in spikes) and the distance of the variance envelope from the mean (in sigmas) can be set in the Options dialog.




The basic idea is that a well-defined unit should have a tight variance envelope. For example, you can see that the unsorted units above have a large variance, as you would expect, since they are collections of dissimilar waveform shapes that may represent superpositions, artifacts, etc. You can think of the variance envelope as one kind of online sort quality metric - a tight variance envelope is the time domain equivalent of a tight, well-defined PCA cluster.

Note that the variance envelope does not appear until [window length] spikes have fired on a unit after you enable variance envelopes, so depending upon firing rates, it may take a few seconds before the variance envelope is displayed.

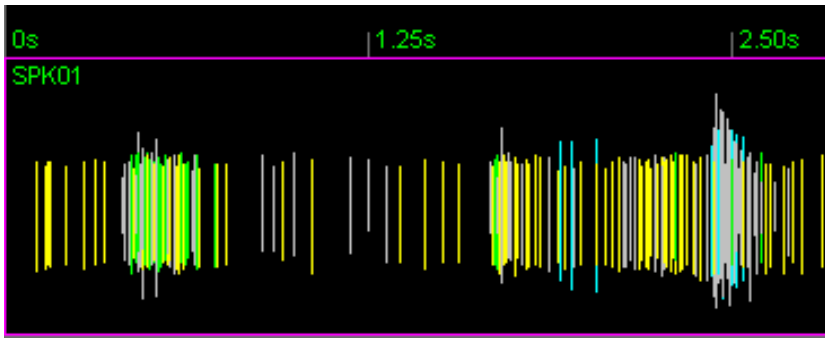
## 16.9 Toolbar Commands—Adjust Display of Ticks



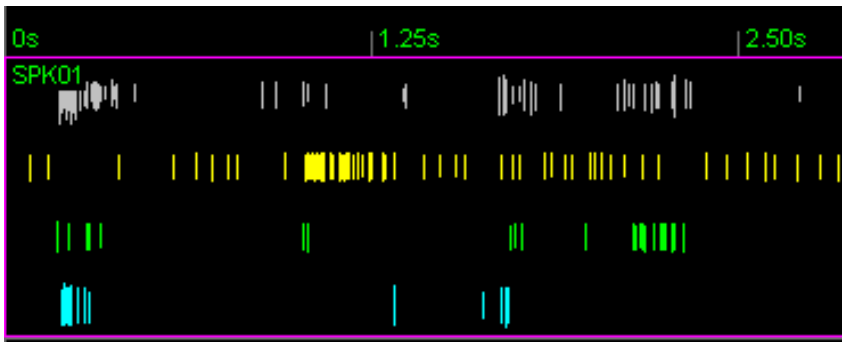

The function of the **Show unit ticks on separate timelines** command is self explanatory.

The **Show ticks with amplitudes** command causes ticks to be drawn not with uniform heights, but according to the peak positive and negative amplitude (bipolar amplitude) of each spike.

Here is an example of all units being displayed on the same timeline, and with bipolar amplitude of the ticks enabled (**Show ticks with amplitudes** command selected):




This image shows the units being displayed on separate timelines and with amplitudes enabled (**Show unit ticks on separate timelines** and **Show ticks with amplitudes** commands selected):

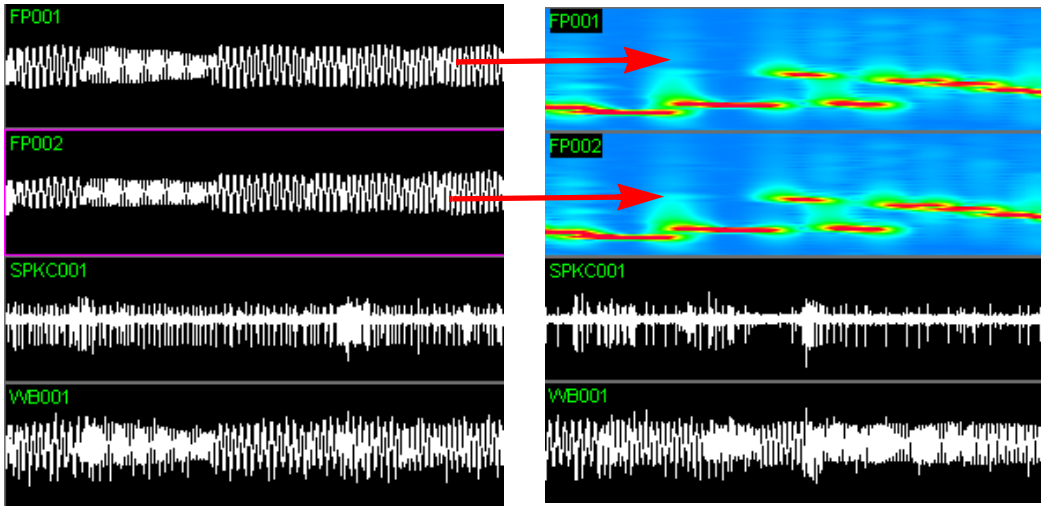


## 16.10 Toolbar Commands—Spectrograms and Spectral Graphs

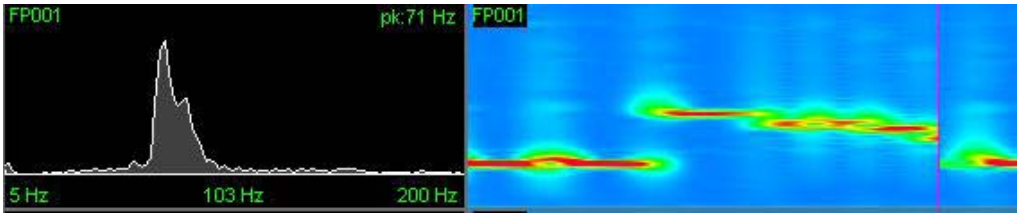
For "slow" continuous channels (sampling rate ≤ 5 kHz), there is an alternate display mode which shows a rolling spectrogram instead of the continuous signal, similar to the standard OmniPlex Spectral view, but with the sweep synchronized to the other channels in the view. This is enabled and controlled via the next three toolbar commands:

Show All Slow Continuous Channels as Spectrograms

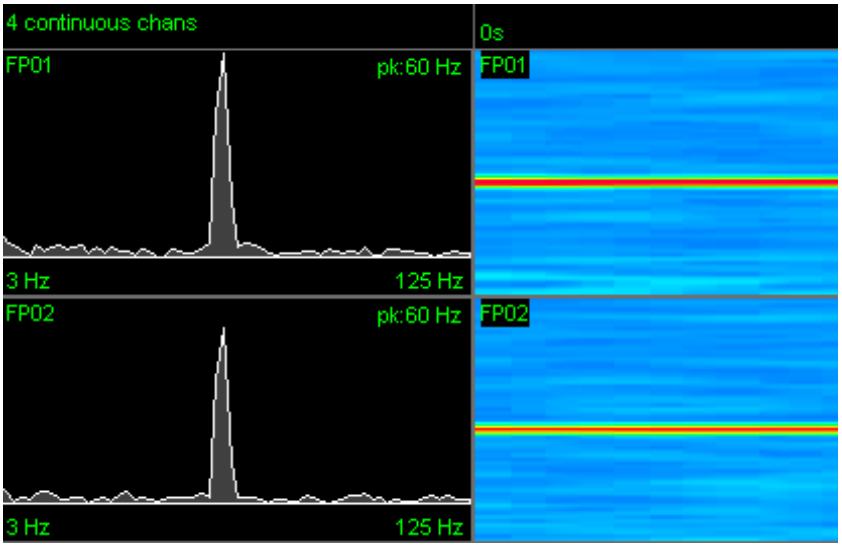
The **Show All Slow Continuous Channels as Spectrograms** command toggles spectrogram mode on and off. For example, the following images show the MultiPlex display before and after clicking the **Show All Slow Continuous Channels as Spectrograms** command for the channels FP001 and FP002:



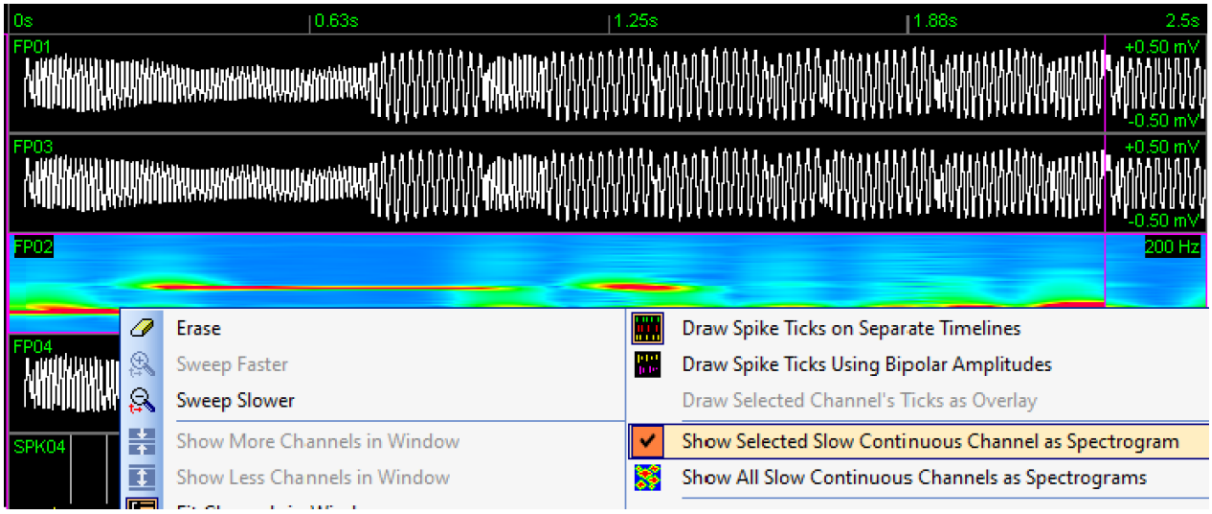
If scopes are also enabled, then in spectrogram mode a spectral graph scope appears to the left of each spectrogram. As with the standard OmniPlex Spectral view, the spectral graph can be thought of as a vertical “slice” of the spectrogram at the current sweep time:




The readout in the upper-right corner of each spectral graph indicates the frequency of the FFT bin with peak magnitude, continuously updated. For example, if you leave the inputs to the neural channels on your signals floating (disconnected), you may see a spectral peak at the 50 or 60 Hz power line frequency being picked up by the floating inputs, i.e. hum:

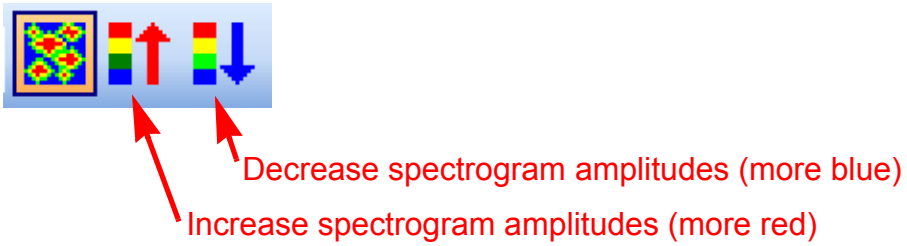


You can toggle between normal and spectrogram modes on a per-channel basis, using the **Show Selected Slow Continuous Channel as Spectrogram** right-button menu command:

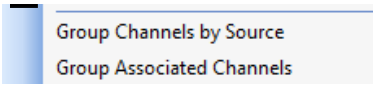



In the above example, FP01, FP03 and FP04 are displayed as their waveforms, while FP02 is displayed as its spectrogram.

The **Increase/Decrease spectrogram amplitude** commands control the amplitude scaling, and work the same way as in the main OmniPlex Spectral view: the red arrow scales up (more red, less blue), the blue arrow scales down (more blue, less red).



## 16.11 Right-Click Menu—Group Channels




Two of the right-click menu commands affect the top to bottom order of all the channels in the MultiPlex view: **Group Channels by Source** and **Group Associated Channels**.

For example, if you had the first two channels of WB, SPKC, SPK, and FP in the MultiPlex view, grouping them by source would yield this top to bottom order:

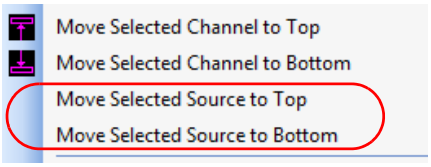
SPK01
SPK02
WB01
WB02
SPKC01
SPKC02
FP01
FP02

On the other hand, grouping associated channels would produce this order:

SPK01
SPKC01
WB01
FP01
SPK02
WPKC02
WB02
FP02

Note that unlike **Fit Channels in Window**, these are commands, not modes; they change the display layout when invoked, but you can then continue to change the row order with other commands and/or by manually dragging channels up or down in the list. There is currently no way to specify a different ordering of sources, or of associated channels when the above commands are used (e.g. perhaps you'd prefer the source order WB, SPKC, SPK, FP). However, grouping by source, followed by use of the **Move Source to Top/Bottom** command, can achieve the same effect.

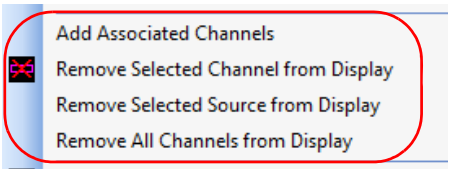
## 16.12 Right-Click Menu—Move Selected Source




**Move Selected Source to Top/Bottom** moves all of the channels belonging to the source (that is, the same source as the selected channel) to the top/bottom of the MultiPlex View display.

**Note:** For **Move Selected Channel to Top/Bottom**, see "Toolbar Commands—Moving and Removing Channels" on page 16-510.

## 16.13 Right-Click Menu—Add/Remove Source or Channels



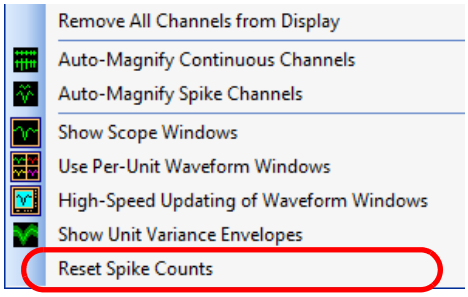

**Add Associate Channels** adds the "parallel channels" derived from the same wideband channel. For example, if you select SPK02 and click **Add Associated Channels**, channels WB02, FP02, and SPKC02 will be added if they are not already in the MultiPlex view.

**Remove Selected Channel from Display**—See Section 16.4, "Toolbar Commands—Moving and Removing Channels" on page 510.

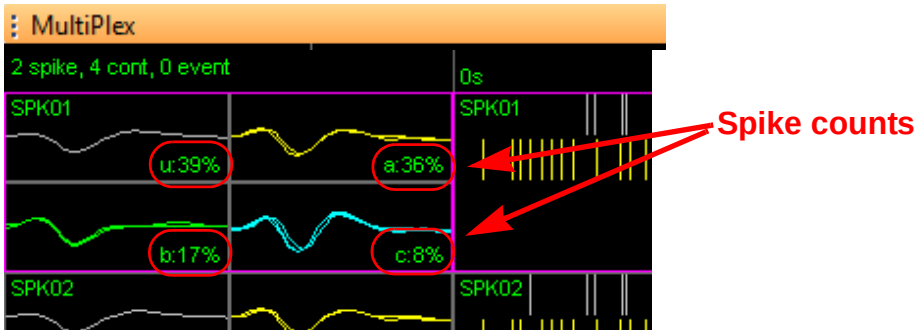
**Remove Selected Source from Display** removes all channels belonging to the source (that is, the same source as the selected channel) from the MultiPlex View display.

If you select **Remove All Channels from Display**, the system prompts you to make sure that is what you want to do. If you click **OK**, the system removes all channels from the MultiPlex view. You can display channels again by opening the **Properties Spreadsheet** and clicking the **MPX** checkboxes for the desired channels.

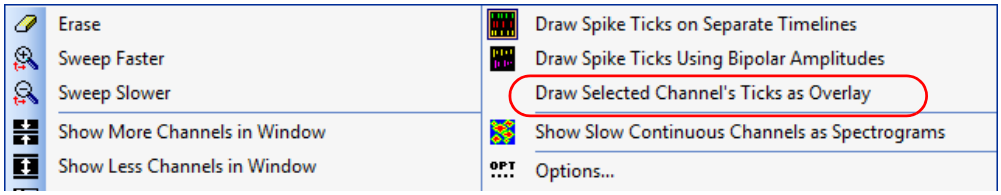
## 16.14 Right-Click Menu—Reset Spike Counts



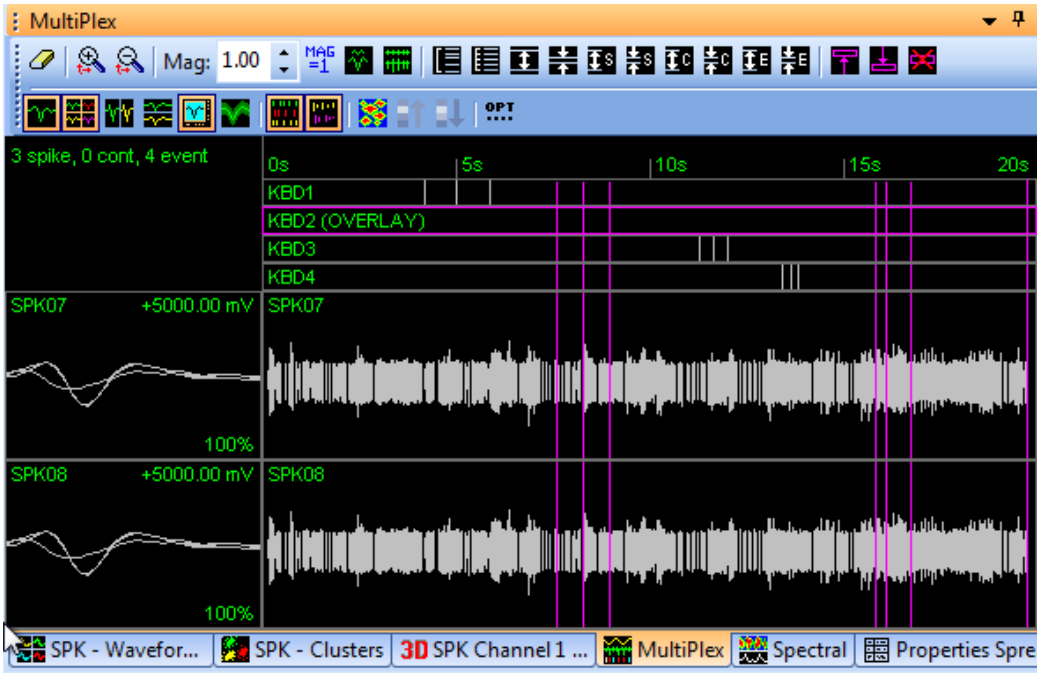

**Reset Spike Counts** sets the spike count(s) to 0 for all channels.



## 16.15 Right-Click Menu—Draw Selected Channel's Ticks as Overlay

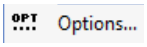



**Draw Selected Channel's Ticks as Overlay** only applies to event channels that are displayed in the MultiPlex view. The image below shows several event channels and two spike channels. For event channel KBD2, the user has selected **Draw Selected Channel's Ticks as Overlay** in the right-click menu. Therefore, the system extends the tick lines for KBD2, causing them to be overlaid on all the other channels in the MultiPlex view. Notice that the event channels KBD1, KBD3 and KBD4 have not been selected for overlay, so their tick marks are not extended.

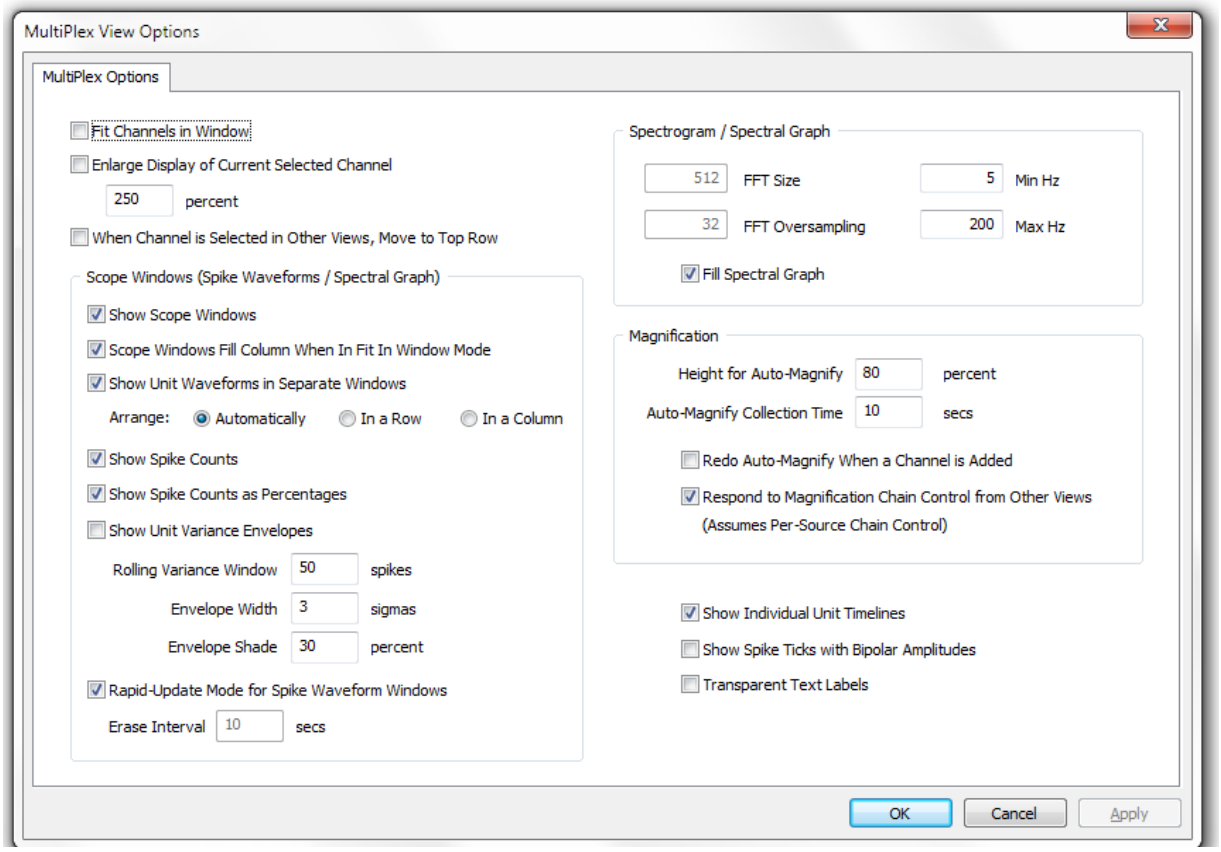


## 16.16 MultiPlex View Options Dialog

The **MultiPlex View Options** dialog contains a number of options and settings that let you customize the default appearance of the data displayed in the MultiPlex view. You can access this dialog in several ways:

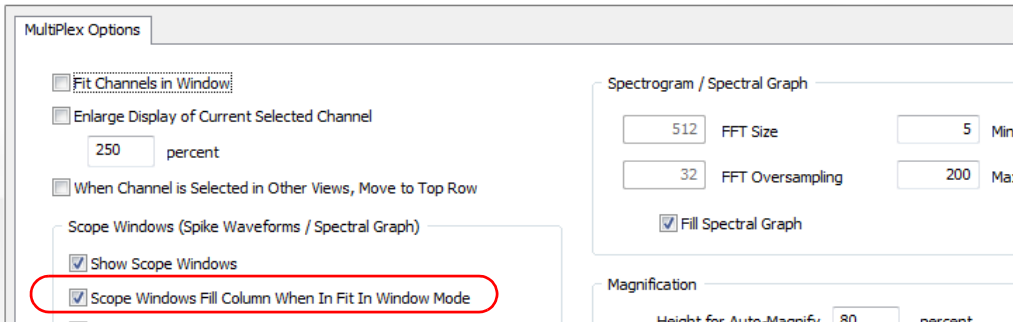
- From the MultiPlex toolbar
- From the MultiPlex right-click menu 
- By pressing the “O” key on the keyboard while the MultiPlex window is selected

In the image below, the default values are displayed.

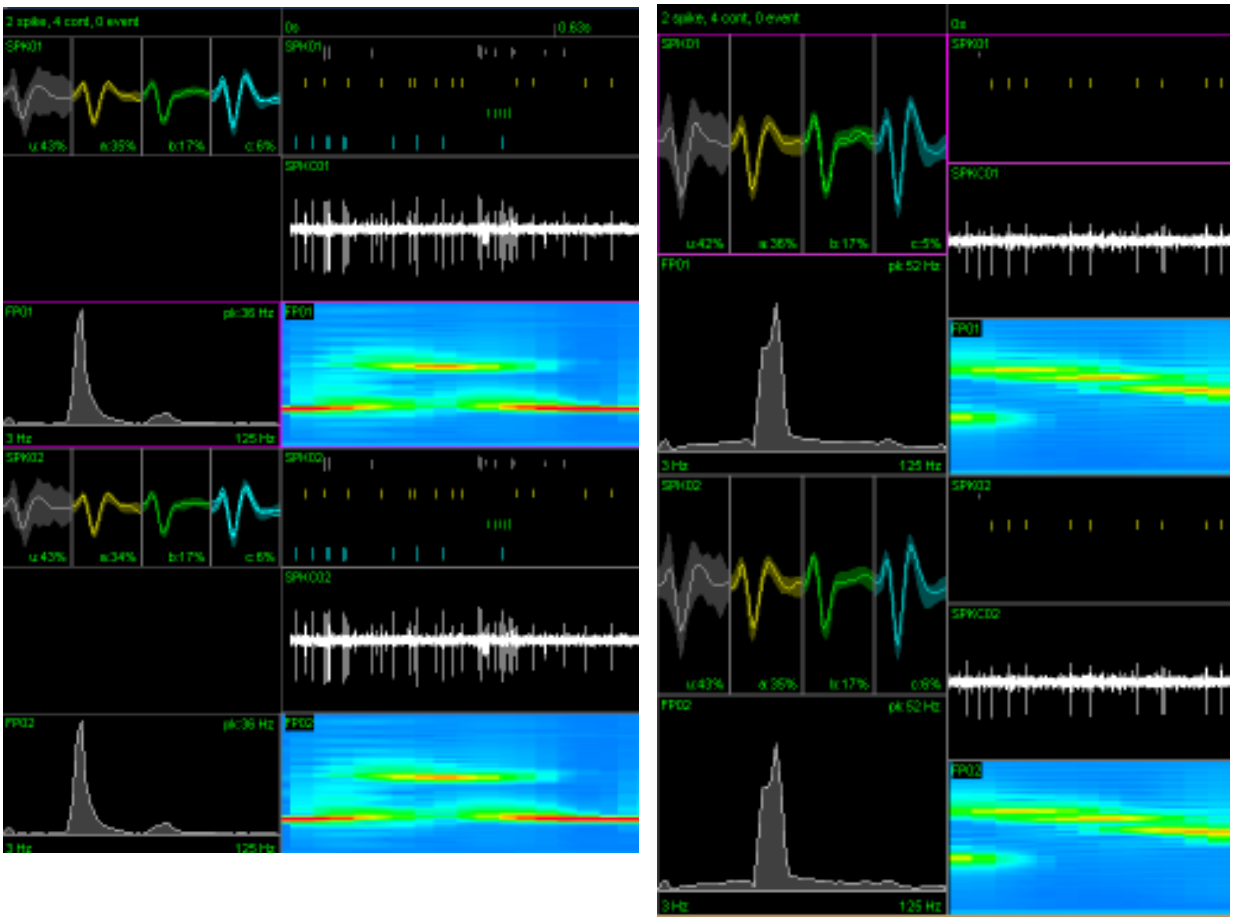


## 16.17 Scopes Fill Column in Fit Channels in Window Mode

The usual rule with the scope windows is that each scope sits directly to the left of the main "channel strip" for that channel, and both are the same height. If you move a channel's row up or down, both the channel strip and its scope move together as a single row. However, when **Fit channels in window** mode ( ) is enabled, there is an option in the **Options** dialog for **Scope Windows Fill Column**.




Here is an example with the option off and then on:

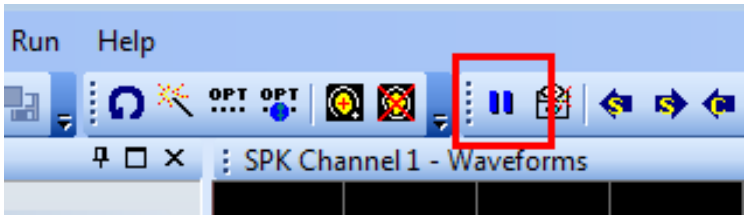


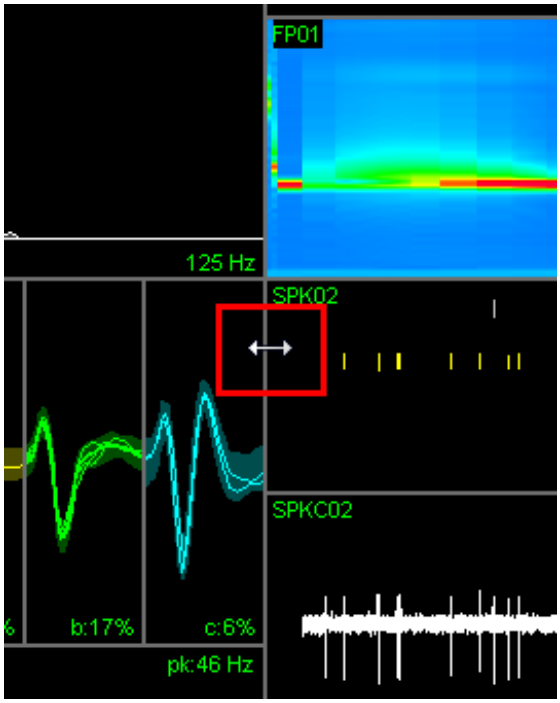
Each scope window's height is simply the total display height divided by the total number of scopes.

Note that there is a tradeoff, in that it is now not quite so obvious which scope "goes with" which channel strip. However, when you select a channel, both the channel strip and the corresponding scope are both highlighted, to help identify that they belong together.

## 16.18 Vertical Splitter

The vertical splitter between the scopes on the left and the channel strips on the right can be dragged to the left or right, changing the proportion of the horizontal display space allocated to each. However, you cannot do this while the display is running; you must use the **Display Pause** button in the main PlexControl toolbar to pause, then drag the splitter left/right, then click the **Display Pause** button again to unpause.



## 16.19 Limitations in MultiPlex View

Continuous channels with sampling rates greater than 5 kHz cannot be displayed as spectrograms. In spectrogram mode, these channels will be displayed as normal continuous signal traces.

The MultiPlex view does not currently support stereotrode or tetrode channels.

Amplitude scaling changes in the spectrogram/spectral graph apply to all channels and there is no auto-scaling of spectrograms.

## 16.20 Keyboard and Mouse Shortcuts for the MultiPlex View

T = toggle toolbar

O = show options dialog

F = toggle fit in window mode

H = make all row heights the same

I = toggle display spike ticks on individual timelines

A = auto-magnify (both spike & continuous)

W = toggle waveform / spectral windows

G = toggle display of slow continuous as spectrograms

B = toggle display of bipolar amplitude ticks

V = toggle spike variance envelopes

S = group by source (note that ‘S’ toggles snapshots in other views)

P = group by associated channels

D = add the first two channels of WB, SPKC, SPK, and FP, turn on fit in window mode, start an auto-magnify (demo mode)

up/down arrow = select prev/next chan (row)

SHIFT up/down arrow = move selected chan up/down in list

Delete = remove the selected channel from this display

CTRL +/- = make the selected row type (spike, continuous, event) taller/shorter

CTRL-SHIFT +/- = make all row types taller/shorter (less/more channels in window)

mouse drag of selected channel = move channel up/down in list

mousewheel = scroll up/down

CTRL-mousewheel = change magnification of selected channel

SHIFT-mousewheel = change magnification of all channels on the selected source

CTRL-SHIFT-mousewheel = change magnification of all channels

# Appendices

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## Appendix A: Signal Amplitudes and Gain

**Note:** Setting the gain is applicable to DigiAmp subsystems, not DHP subsystems.

Using the proper gain value is important, since a too-low gain will result in the signal being digitized with too few bits (out of a maximum of 16 bits) being used to represent the signal. On the other hand, a too-high gain will cause some or all of the signal to exceed the input range of the A/D converter and to be "clipped," resulting in the tops and bottoms of field potential waveforms to be flattened, and spikes to be distorted or even lost entirely. While very occasional clipping may be acceptable, for example clipping of undesired stimulation or motion artifacts which greatly exceed the amplitude of the neural signal, in general *you should be careful not to set the gain any higher than necessary, since clipping causes more damage to the digitized signal than digitizing it at a slightly lower-than-ideal resolution*.

A common mistake with new users is to set the gain higher than necessary, because "the spikes look too small" when the gain is kept at a safe value which avoids clipping the wideband signal. This is partly because the amplitude of spikes is typically much smaller than the amplitude of the field potentials upon which they are superimposed. In other words:

(wideband amplitude) = (field potential amplitude) + (spike amplitude)

where a typical case might be something like:

(wideband amplitude 2.2 mv pp) = (field potential amplitude 2.0 mv pp) + (spike amplitude 200 uv pp)

**Note:** pp = peak-to-peak

But there is no need to use analog gain as a substitute for visual magnification, since the OmniPlex® System provides highly accurate, low-noise A/D conversion of even low-level signal components such as small spikes, and a set of software magnification tools to allow you to view spikes at a comfortable size. In fact, if you "crank up the gain" while looking only at spikes, you may not even be aware that you are causing clipping of the wideband signal.

The maximum allowable signal level at the recording electrode, before amplification, is:

10 V pp / (total gain)

For example, if you are using a unity-gain (G1) headstage and a DigiAmp™ gain setting of 1000:

10 V pp / 1000 = 10 mV pp, i.e. +/- 5 mV

Voltages exceeding this value will cause clipping at the A/D converter, unless you reduce the DigiAmp gain.

In summary: *Always view the wideband signal, when setting gain, and avoid setting the DigiAmp gain so high that it causes clipping of the wideband signal on any channel; then use magnification to visually enlarge the spikes in the displays as necessary.*

# Appendix B: Separation of Spikes and Field Potentials Using Digital Filters

## High sample rate digitization of the wideband signal

The OmniPlex System digitizes neural signals at a sampling rate of 40 kHz (25 microsecond resolution) in order to acquire the wideband (WB) signal with maximum accuracy. While classic signal processing theory emphasizes the Nyquist limit, which states the sampling rate need only be twice the frequency of the highest spectral component of interest, this assumes an ideal analog lowpass filter preceding A/D conversion, and ideal reconstruction of the digitized signal. However, in practice, the highest quality sampling is obtained by using a high sample rate in the A/D converter, and this is the approach that Plexon® neural data acquisition systems have always taken in using a 40 kHz sample rate. The fixed analog anti-aliasing filter in the DigiAmp is a four-pole Bessel with an 8 kHz cutoff, so in practice this means that a 40 kHz sample rate yields an oversampling factor of at least four, and greater for lower spectral components of the neural signal. This results in accurate capture of the time/voltage waveform samples, which is important for time domain spike sorting algorithms (template, line, band, and box sorting) which rely on relatively small differences in spike shape to perform unit discrimination. In addition, the high sample rate preserves the shape of clusters in PCA feature space, which is important for automatic unit finding operations which work in the feature space.

## Spike/FP separation

The digitized wideband signal is typically separated into field potentials (FPs) and a continuous spike signal (SPKC) using the OmniPlex System digital filters, with the separation parameters determined by the user according to his or her needs; there is no universally applicable definition of the upper frequency limit of a field potential or the lower frequency limit of spikes. Depending on the experiment and type of neural signals being acquired, it is often preferable to set the filter cutoff frequencies for spikes and FPs independently, as opposed to attempting to “split” the spectral content of the wideband signal at a single frequency. Also, the class of filter, for example Butterworth versus Bessel, and the steepness of the filter (number of poles) may be different for the lowpass FP filter and the highpass spike filter. The OmniPlex System provides default filter settings that are based on typical usage scenarios, but allows these settings to be easily customized.

One consideration is that the cutoff of the FP lowpass filter must be appropriate for the downsampling which is performed on the lowpass-filtered signal. For example, for the default FP downsampling rate of 1 kHz, there should be no significant spectral content above 500 Hz after lowpass filtering, so the lowpass filter cutoff frequency should be well below this frequency; otherwise, aliasing will result.

The highpass filter used to extract the continuous spike signal (SPKC) must remove low frequency non-spike signals, whether they be legitimate field

potentials, or low frequency artifacts such as motion artifacts, stimulation artifacts, power line hum, etc. Low frequency content leaking into the SPKC can result in a “wandering baseline” which will adversely affect spike detection, since the thresholding operation assumes that there is no significant low frequency content.

### Setting filter parameters in Server

You can access the parameters for each filter, which applies to all channels in the respective sources (SPKC or FP), in the Server topology diagram. To do so, first stop data acquisition, go to the Server window, and right click on either the **Spike Separator** or **FP Separator** device in the topology. Select **Edit Device Options** to display the options dialog.

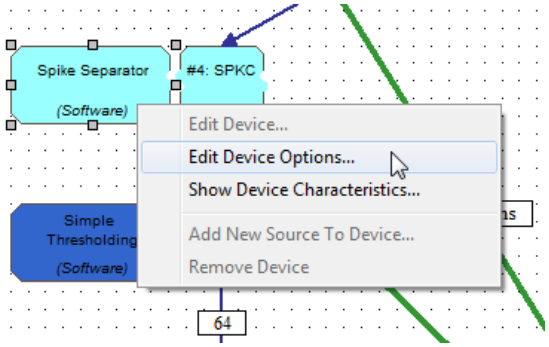
When you save the pxs (topology) file in Server, the current settings of the spike and FP separators are also saved in the file. It is important to note that if you change the filter settings in the **Device Options** for the FP Separator or Spike Separator devices with the **Edit Device Options** function in Server, these settings will apply to all channels on the corresponding device, and will override any per-channel filter settings that were made previously with the **Filter Control Panel** (FCP). (For information about the FCP, see Section 13.5, “Filter Control Panel” on page 420.)

Also, note that the SPKC and FP filter settings are saved in the .pxc file, and loading a .pxc with customized filter settings will also override any filter settings which were in effect before the .pxc was loaded. In general, saving the .pxs file in Server saves the filter settings as the defaults, and saving the .pxc file in PlexControl saves the filter settings for a particular experiment.

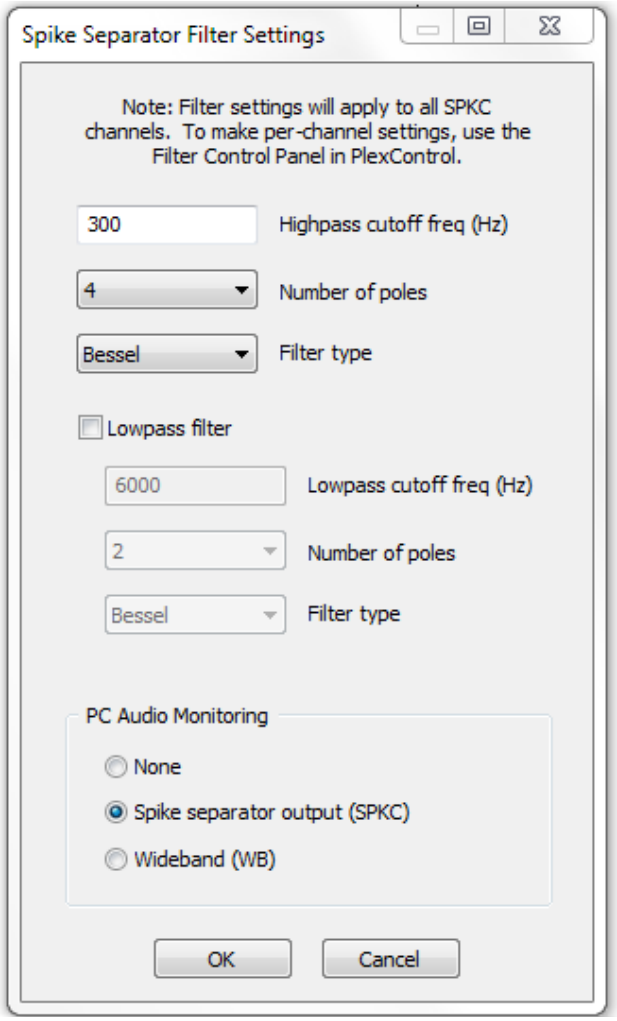
#### Example

Starting Server with a saved .pxs file, then starting PlexControl and loading a saved .pxc file results in the .pxc filter settings overriding the .pxs filter settings. When you open PlexControl without loading a .pxc, it uses the current state that has been set by Server. The intent is that the .pxs has the default settings; when you change the settings in PlexControl (for a particular experiment), you can save these customized settings in a .pxc, or if you save the pxs, you are in effect saving them as new defaults.

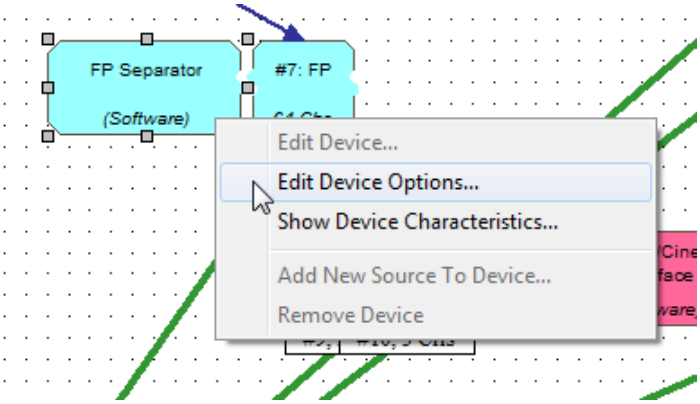
For the Spike Separator device:

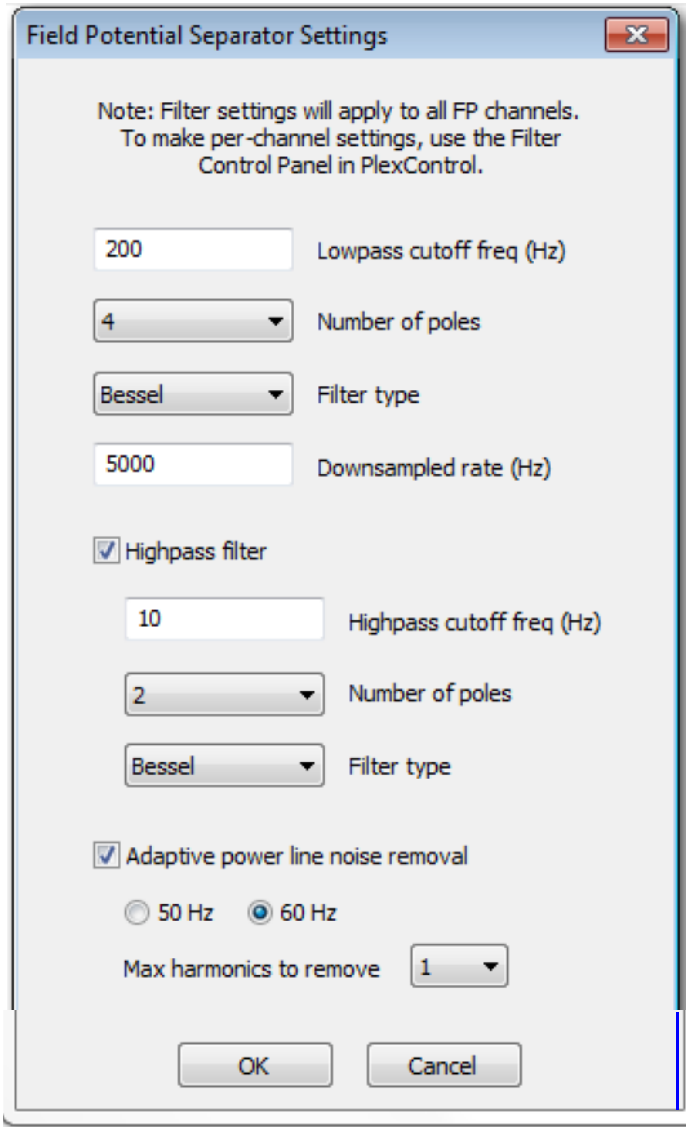



The **Spike Separator Filter Settings** dialog opens, as shown below.



For the FP Separator device:



### Filter parameters in detail

The three parameters which are available for both the spike and FP separator filters are:

- Cutoff frequency (defaults to 200 Hz for the FP lowpass and 300 Hz for the SPKC highpass). See the discussion about these cutoff frequencies in the "Spike/FP separation" section on page page A-4.
- Number of poles, also known as filter order (defaults to four poles for both filters)
- Filter type (Bessel, Butterworth, or Elliptic)

The **Number of poles** affects the steepness of the filter cutoff. You might think that "the steeper the better," but using very high order filters can cause problems due to frequency-dependent group delay of field potentials, and spike waveform shape changes. However, Plexon's FPAlign offline utility is available for removing phase shift and group delay induced by the FP filters; see the FPAlign documentation for more information. In general, it is recommended that you not go beyond the four pole default settings, unless you find that they are inadequate for removing spikes from your FP signal, or low frequency content is leaking into the SPKC signal.

The **Filter type** setting defaults to Bessel for both filters, for the following reasons. Bessel filters induce the least amount of frequency-dependent time shift (group delay), so that the time domain relationship between spikes and field potentials is preserved. Also, for spikes, Bessels cause the least change to the shape of spike waveforms; by comparison, Butterworth filters cause more overshoot, and Elliptic (also known as Cauer) filters cause even more shape distortion. Bessels are therefore the most benign filters to use for spike/FP separation.

However, the other two filter types have advantages in some situations. Butterworths have a maximally-flat amplitude characteristic in the passband, so if the relative amplitude or power of different FP spectral components is of primary interest, you may wish to use a Butterworth filter for the FP lowpass filter. Also note that, for the same number of poles and cutoff frequency, Butterworth filters have a somewhat sharper frequency cutoff characteristic than Bessels.

Elliptic filters have the steepest cutoff behavior, at the expense of more severe group delay variation and spike shape alteration. You should only use an Elliptic filter when it is difficult to achieve adequate spike/FP separation using the other filter types.

### Spike lowpass filter

The **Spike Separator Filter Settings** dialog has an optional lowpass filter. This is not the same as the lowpass filter which is used for FP separation, which is used with a cutoff frequency below the spike band. The spike lowpass filter is intended for use in reducing high frequency noise. The analog anti-aliasing filter

in the DigiAmp Amplifier, preceding the A/D conversion stage, is a four-pole Bessel with a cutoff of 8 kHz; if the spike lowpass filter is used, its cutoff should be less than 8 kHz. For example, a cutoff of 6 kHz will to some extent emulate versions of the Plexon MAP system which employed a 6 kHz analog filter before A/D conversion. The combination of the standard spike highpass filter, plus the spike lowpass filter, results in what is in effect a bandpass characteristic, although you are not constrained to use the same filter type or number of poles for the highpass and lowpass filters.

The spike lowpass filter should only be used when necessary, because excessive lowpass filtering of the wideband signal can result in spikes that are unnecessarily "smoothed off," blurring the differences between the waveform shapes from different units and making spike sorting more difficult. To see this effect, you can try enabling the lowpass filter and setting a very low cutoff, e.g. 2 kHz, to get an idea of the potential issues. If you believe your wideband signal has excessive high frequency noise, it is recommended that you use the least amount of lowpass filtering that is necessary to remove the noise, starting with the default settings and only lowering the cutoff frequency and/or increasing the filter order with caution.

#### Spike PC audio monitoring function

See Section 15.1, "Audio Monitoring of Wideband or Spike Continuous Signals" on page 466

#### FP downsampled rate

The downsampled rate (Hz) refers to the sampling rate which field potentials are reduced to, from the original 40 kHz sampling rate. This is done because field potentials are low frequency signals which do not require the 40 kHz sampling rate, and they can be processed and recorded more efficiently at the lower rate. The downsampled rate must divide evenly into 40 kHz, e.g. 1, 2, 4, 5 kHz.

It is important to note that only frequencies below half the sampling rate can be represented accurately in the downsampled FP signal. For example, at the default 1 kHz rate, frequencies less than 500 Hz can be represented. If frequencies greater than 500 Hz are downsampled to a 1 khz rate, they will alias, or "fold over" into the lowest frequencies. Therefore it is important to specify a cutoff frequency for the FP lowpass filter which eliminates spectral content above this frequency (i.e. spikes and high frequency noise). The default lowpass setting is a four pole filter with a cutoff of 200 Hz. Note that the filter's response does not cut off abruptly at the cutoff frequency, so it should be well below the frequency limit (e.g. a 200 Hz cutoff to remove content above 500 Hz). You can view the effect of the filter settings on the spectral content of the signal using the Spectral 2D and Spectral 3D views.

#### FP Adaptive power line noise removal

For the FP source, an optional power line noise filter (LNF)—displayed as **Adaptive power line noise removal** in the dialog—is available. This is an adaptive "hum filter" which specifically "looks for" components within the FP

signal at a fundamental frequency of either 50 or 60 Hz (the power line frequency, e.g. 60 Hz in the US) and the harmonics (multiples) of the fundamental. This is necessary because while the AC power waveform at the source may be sinusoidal or very nearly so, in practice the waveform shape of AC line noise picked up by electrodes is significantly distorted, which equates to the presence of harmonic spectral components with amplitudes that decrease with increasing frequency.

The OmniPlex System also has a notch filter which is available in the optional Global Filter device in Server, but the LNF has several advantages. First, the notch filter only removes line noise at the fundamental frequency, while the harmonics are unaffected. Second, the notch filter affects frequencies near the fundamental, and increasing the narrowness of the notch (the Q value) to avoid this can cause artifacts in the signal and also make the filter less effective if the power line frequency drifts from the ideal 50/60 Hz value.

By comparison, the LNF affects only the specified fundamental frequency and harmonics, and adaptively determines the phase and amplitude of each component so that it can in effect “cancel out” the power line noise. As opposed to the tradeoffs involved with the notch filter, the LNF only requires the fundamental frequency (50 or 60 Hz) and the number of harmonics to be specified, up to a maximum of five. For example, with a 60 Hz power line frequency, selecting three harmonics would result in adaptive noise removal at 60, 120, and 180 Hz. The amplitude of the higher harmonics in power line noise decreases with increasing frequency, so that unless the line noise is very heavily distorted, removing two or three harmonics will be sufficient. Specifying too many harmonics will not harm the signal, since the noise removal is applied adaptively, but at high channel counts it will use unnecessary processing resources and should be avoided.

In general, it is recommended that you use the 2D and/or 3D spectrogram views and the FP continuous view to check for the presence of line noise and its harmonics. An instructive “offline exercise” is to run OmniPlex with the HTU (headstage tester unit) and a function generator as the input signal, set to 50 or 60 Hz. Observe this signal in the spectrogram and FP continuous views and experiment with the results of enabling and disabling the LNF filter and varying the number of harmonics removed. You may wish to start with a pure sine wave from the function generator (no harmonics), then switch to a triangle wave, which has odd harmonics. i.e. setting the LNF to remove five harmonics will remove the fundamental, third harmonic, and fifth harmonic.

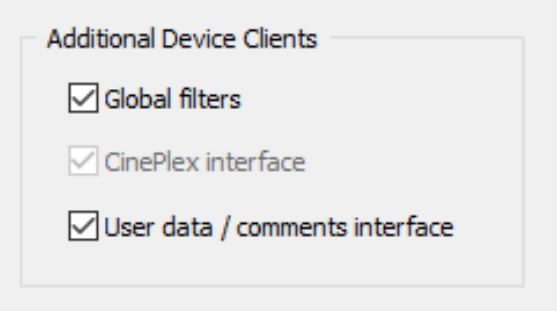
Note that since the LNF is an adaptive filter, it does not instantly respond to changes in line noise amplitude. Typically it causes a significant reduction in line noise within a few seconds, then exhibits a slowly oscillating convergence to maximum noise removal. It can take up to roughly one minute for maximum noise removal to be achieved, but this will be somewhat signal-dependent. As long as you account for this initial settling time it should not be a problem, except in circumstances where the line noise varies rapidly, e.g. a freely-moving animal that is picking up much more line noise in one part of a cage than another. In such

cases, the notch filter may be preferable, keeping in mind the disadvantages that were previously described.

A final caveat is that the line noise filter (and the notch filter) are not substitutes for proper electrical shielding. Remember that these filters are applied to the digitized signal and so cannot remove noise that is of such high amplitude that it exceeds the input range of the A/D converters and clips. You should always try to remove sources of power line noise from the area of an experiment, apply best practices for grounding and shielding, and only use the digital filters in OmniPlex to remove any remaining noise. For more information on filters and noise removal, see Section 13.5, “Filter Control Panel” on page 420.

### Global Filters (legacy, deprecated)

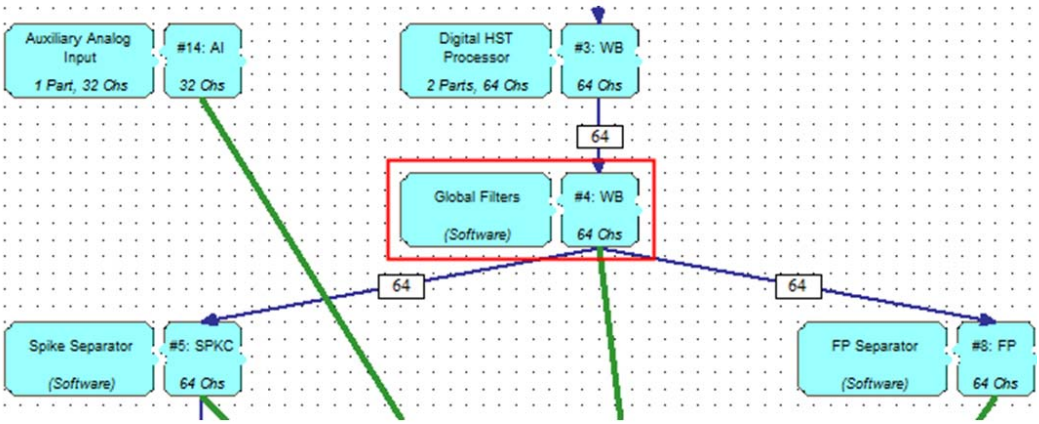
When a new topology is created using the Topology Wizard, an option is available to include a **Global filters** device in the topology:




**Note:** For general information on using the Topology Wizard, see the startup procedure in the chapter that is applicable to your system: Chapter 2, Startup (with DigiAmp Subsystem) or Chapter 3, Startup (with DHP Subsystem).

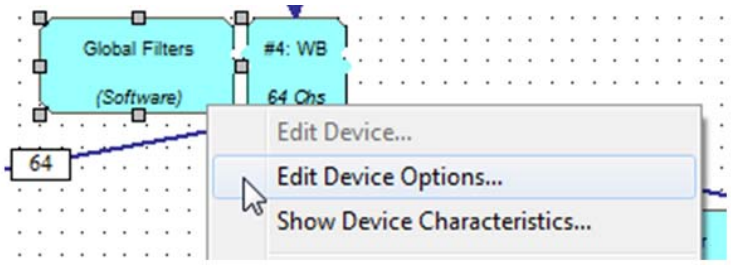
Although the **Global filters** option is available, in most cases there is more recent OmniPlex functionality which provides better results with less processing resources, as described below. Therefore, **Global filters** should be considered a legacy feature which is maintained primarily for compatibility with existing topologies.

If you enable the **Global filters** checkbox in the Topology Wizard, the generated topology (pxs) will include an additional device which performs digital filtering on the wideband (WB) signal, i.e. the output from the main neural A/D device (e.g. DigiAmp, MiniDigi or DHP unit).

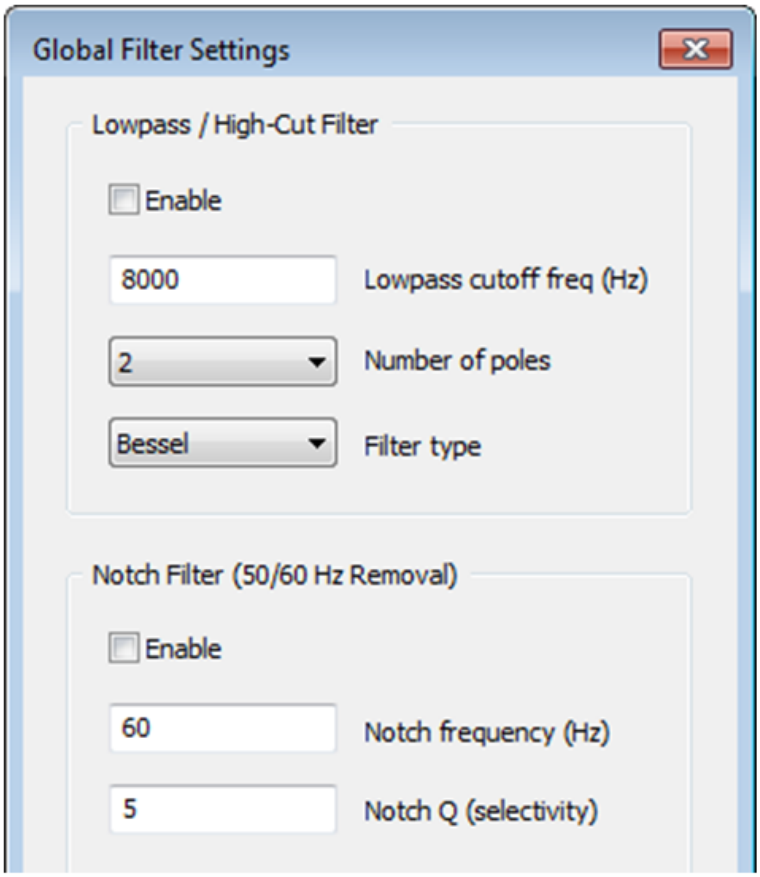



Note that the name of the **Global Filters** source is also WB, so you can think of it as a post-processing stage within the wideband source, rather than a device like the Spike Separator, Sorter, or other device which creates a new source with a unique name (SPKC, SPK, etc). *It is important to note that the unprocessed wideband output of the main neural A/D device is not available as an independent source when the **Global Filters** function is post-processing its output.* This is one of the reasons why **Global Filters** should only be used if absolutely necessary; *any filtering performed using **Global Filters** cannot be "undone" and the original raw wideband data cannot be recorded.*

To see the filtering functions provided by **Global Filters**, stop data acquisition and right click on it in the topology:



The Global Filter Settings dialog is displayed:




Two types of digital filters, lowpass and notch, can be independently enabled, and both are disabled by default.

The **Lowpass / High-Cut Filter** allows you to perform lowpass filtering on the wideband signal. Typically you would use this to remove high frequency noise. However, a lowpass filter is also available in the **Spike Separator**, via either its device options, or the **Filter Control Panel** in PlexControl (Section 13.5, "Filter Control Panel" on page 420). *It is strongly recommended that you use the Spike Separator lowpass filter rather than the one in the **Global Filters**, since the former affects only the SPKC source, leaving the wideband (WB) source unaffected.*

The **Notch Filter** is a narrowband rejection filter intended for removing power line noise (hum) at either 50 or 60 Hz. In addition to the center frequency of the notch, you can specify the Q, or selectivity of the notch. Low Q values (e.g. 1) result in a broader notch, while high values (e.g. 10) result in a very narrow, deep notch. Although it might seem that a very high Q value would be preferable, in order to remove as much spectral content at the exact power line frequency as possible while not affecting desired spectral components at nearby frequencies, this is not entirely true. First, the power line frequency is rarely exactly 50 or 60 Hz, and tends to drift slightly depending on power grid load, time of day, and

other factors. Second, very high Q values can cause resonance artifacts in signals, such as ringing. Therefore, using a notch filter is often a tradeoff between effective noise removal and these side effects.

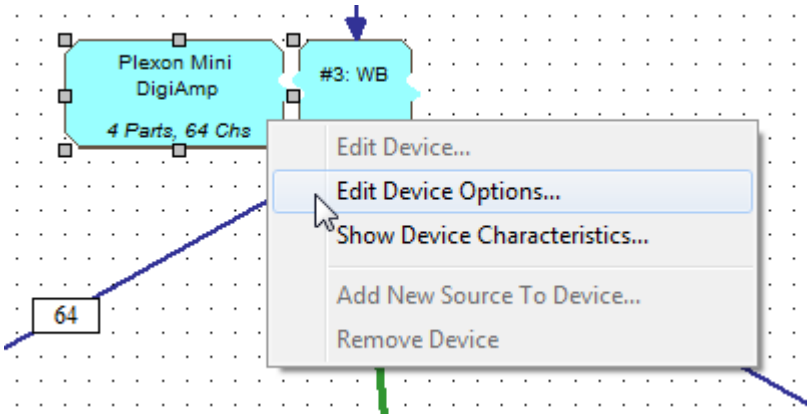
Another problem is that power line noise is often not a pure sine wave with a single component at 50 or 60 Hz. Rather, it tends to consist of a fundamental at 50 or 60 Hz plus one or more harmonics at multiples of the fundamental frequency. In some cases the harmonics may be stronger than the fundamental, or the fundamental may be missing. A notch filter can only remove a single component, the fundamental.

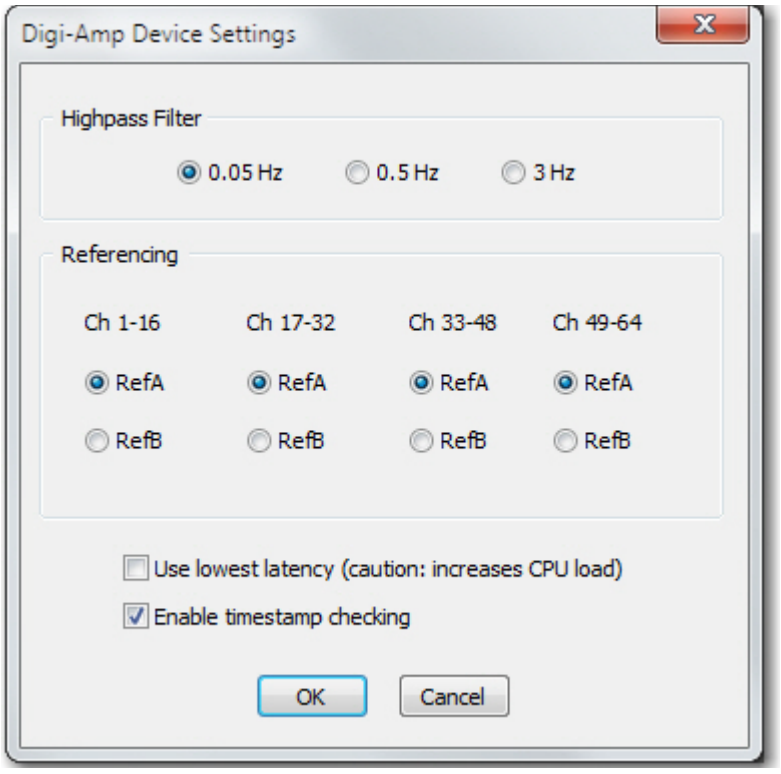
In order to overcome these shortcomings of the **Notch Filter,** the OmniPlex System has an adaptive power line noise filter which can be enabled in the **Filter Control Panel** in PlexControl or the device options for the **FP Separator** in Server. This filter is capable of removing both the fundamental and its harmonics. In addition, it is extremely selective, like a very high-Q notch filter, but without the artifacts caused by a high-Q notch, and it dynamically tracks the power line frequency, so it is effective even when the frequency drifts. *For all these reasons, use of the **Notch Filter** is not recommended except in special cases.* At a minimum, you should try using the adaptive power line noise filter first, and use the **Notch Filter** only if you are unable to achieve satisfactory results.

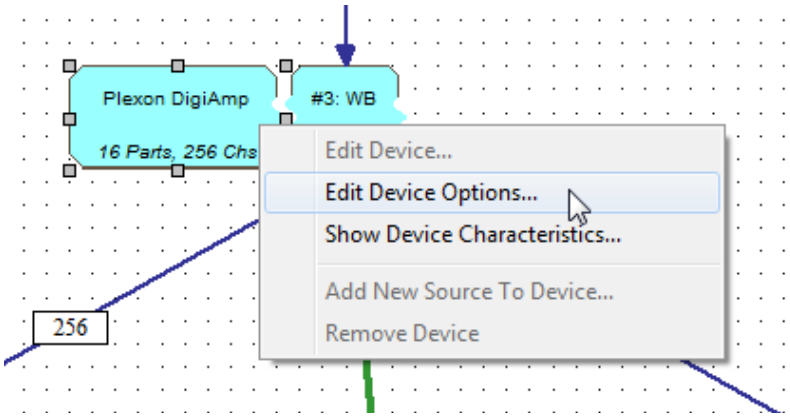
An additional reason to avoid use of the **Global Filters** is that they add processing overhead, since they operate on all channels of the wideband signal at full sampling rate (40 kHz). At high channel counts, this can cause performance issues, especially on older PCs. The Topology Wizard will display a warning message if you attempt to include the **Global Filters** in a topology with more than 64 channels. You can do so, but you should carefully monitor the system for high CPU usage, "stuttering" of scrolling displays, data drops, or other signs of performance problems.

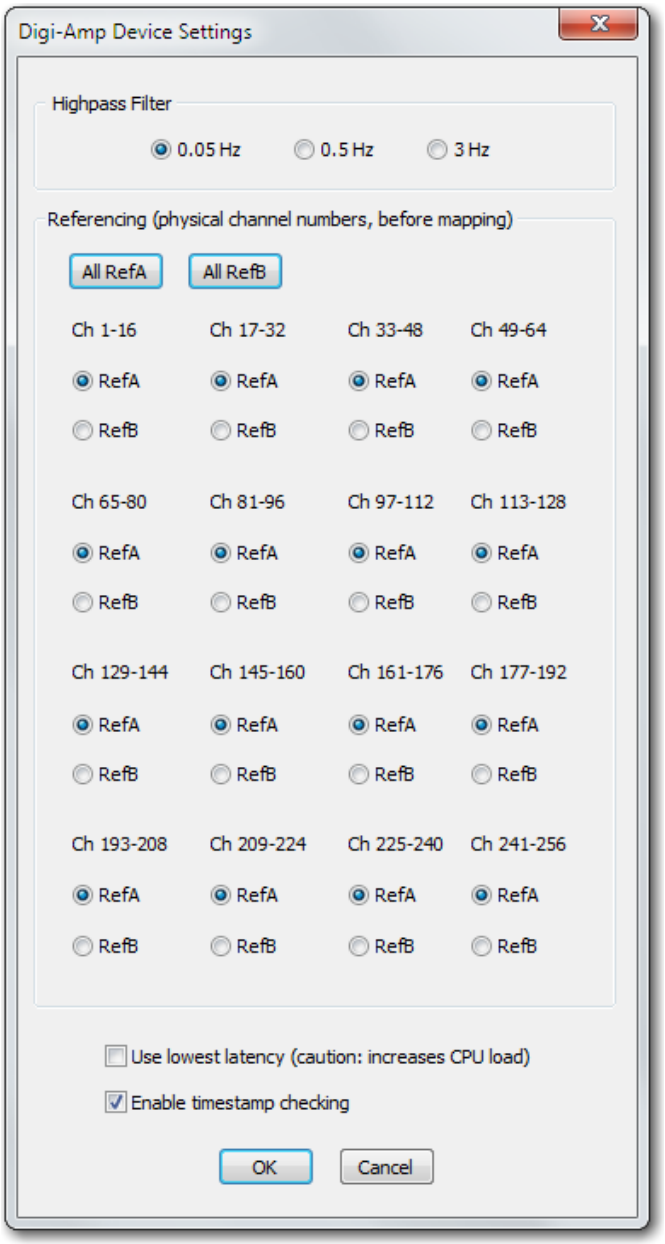
# Appendix C: DigiAmp Device Settings—Filtering, Referencing and Latency

Besides the DigiAmp™ gain, which is controlled from PlexControl, there are other user-accessible analog parameters, which are set in the DigiAmp device options in Server. The first two images below apply to MiniDigi™ options. The next two images apply to DigiAmp options.




Plexon DigiAmp
16 Parts, 256 Chs
#3: WB
Edit Device...
Edit Device Options...
Show Device Characteristics...
Add New Source To Device...
Remove Device
256


Digi-Amp Device Settings
Highpass Filter
0.05 Hz
0.5 Hz
3 Hz
Referencing (physical channel numbers, before mapping)
All RefA
All RefB
Ch 1-16
Ch 17-32
Ch 33-48
Ch 49-64
Ch 65-80
Ch 81-96
Ch 97-112
Ch 113-128
Ch 129-144
Ch 145-160
Ch 161-176
Ch 177-192
Ch 193-208
Ch 209-224
Ch 225-240
Ch 241-256
RefA
RefB
Use lowest latency (caution: increases CPU load)
Enable timestamp checking
OK
Cancel

### Highpass Filter

The Highpass Filter options determine the frequency of the analog highpass (low-cut) filter which blocks DC offsets and very low frequency artifacts. The highpass filter precedes the analog gain and A/D conversion stages, so the cutoff frequency selected here affects the wideband signal and all signals derived from it (SPKC, FP). By default, this is set to the lowest frequency, 0.05 Hz, which enables the recording of the lowest frequency field potentials without significant phase shift (group delay) issues. However, such a low cutoff can be inconvenient when recording of very low frequencies is not needed; in particular, if there is DC offset on the input signal, you may have to wait several seconds after data acquisition is started or a headstage is powered on before the baseline settles to zero, due to the large time constant of the filter. In such cases, you may wish to use a cutoff of 0.5 Hz. If you are experiencing problems due to high amplitude low frequency artifacts (e.g. motion artifacts), you may wish to try the highest cutoff frequency of 3 Hz, although this may cause phase shift issues with recording of field potentials below 10 Hz. However, remember that the FPAlign offline utility may be used to remove most of the phase shifts induced by the filters.

### Referencing

The Referencing section controls analog reference selection, with individual control of each group of 16 channels. Consult the documentation for the specific headstages which you are using for additional details.

Use the procedure on the following pages to verify proper functioning of the referencing feature.

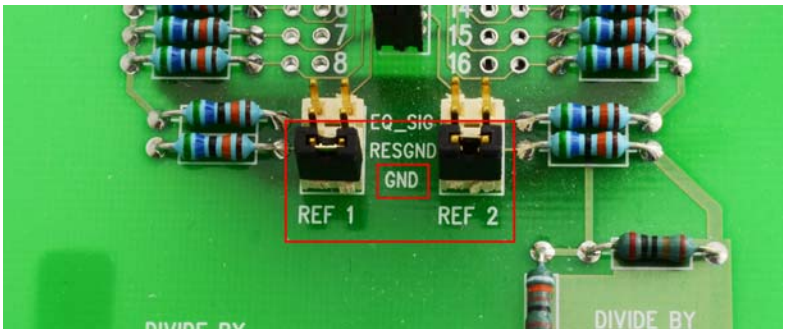
## Testing the Referencing Function

If you want to verify that the referencing function is working properly in both the hardware and software, follow this procedure.

**Note:** In “true reference” mode the system subtracts the signal on the reference input from the other signals and in “grounded reference” mode, it does not.

1. Connect the audio cable and a headstage to the Headstage Tester Unit (HTU). Verify that an audio signal is present in the audio cable.
2. Verify the REF jumper on the HTU is in its original (**GND**) position.

   **(There are several models of the HTU. This is an example. Your HTU might have one REF header or multiple headers)**




Follow these steps to verify correct “true reference” operation

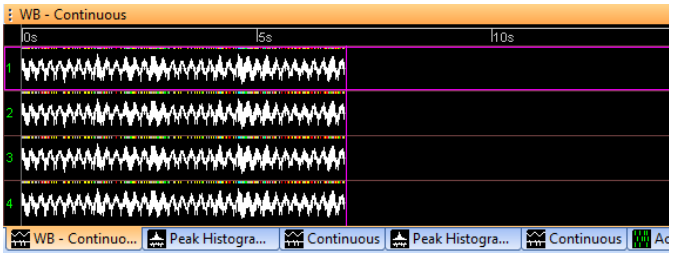
3. In Server, right click on the **Plexon DigiAmp** or **Plexon Mini DigiAmp** icon and select the **Edit Device Options** command.
4. In the **Digi-Amp Device Settings** dialog, verify that the **Referencing** option is set to **RefA** or **RefB** in a manner that ensures the software is subtracting the signal from the headstage reference input. Consult the *Analog Headstage Technical Guide* (available on the Plexon website) to see the proper setting for the specific headstage you are testing.
5. Click OK.

**TIP**

**Naming conventions—Ref1/Ref2 and RefA/RefB**

For some analog headstages, the *Analog Headstage Guide* procedure refers to **Ref1** or **Ref2**.
Be aware that **Ref1** in the *Guide* is equivalent to **RefA** in the **Digi-Amp Device Settings** dialog, and **Ref2** is equivalent to **RefB**.

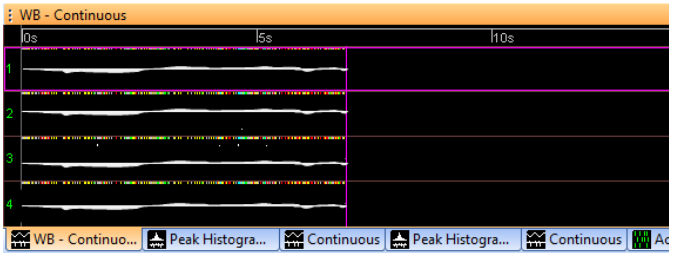
6 Verify that you are seeing the expected (non-zero) signals on the appropriate channels.




7 Move the HTU jumper from the **GND** position to the **EQ SIG** position. Note that when the jumper is on the **EQ SIG** position, the HTU sends the same signal to the headstage referencing pin(s) as it is sending to all the other channels in the headstage.

**Note:** It is acceptable to move the REF jumper on the HTU while data is flowing through the headstage.

8 As you place the jumper on the **EQ SIG** position, you should see the signals on the channels go to zero, because the audio signal is being subtracted from itself. Verify that you see the signal go to zero, confirming that the referencing software and hardware are working correctly.




9 If the signals do not go to zero (or very close to zero) in the previous step, try repeating this testing procedure. If you still do not see the signals go to zero, gather information about your software, hardware and headstage and contact Plexon Support for assistance.

Follow these steps to verify correct “grounded reference” operation

**10** In Server, right click on the **Plexon DigiAmp** or **Plexon Mini DigiAmp** icon and select the **Edit Device Options** command.

**11** In the **Digi-Amp Device Settings** dialog, verify that the **Referencing** option is set to **RefA** or **RefB** in a manner that ensures the software is NOT using the signal on the headstage referencing pin. Consult the *Analog Headstage Technical Guide* (available on the Plexon website) to see the proper setting for the specific headstage you are testing.

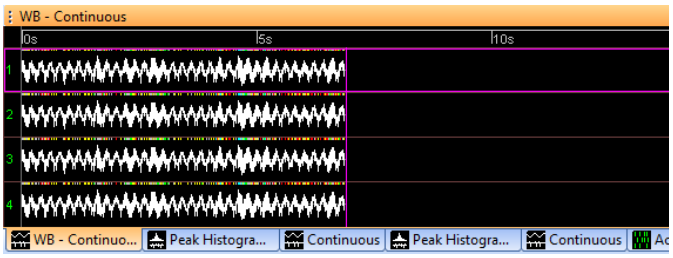
**12** Click OK.

**TIP**

**Naming conventions—Ref1/Ref2 and RefA/RefB**

For some analog headstages, the *Analog Headstage Guide* procedure refers to **Ref1** or **Ref2**.
Be aware that **Ref1** in the *Guide* is equivalent to **RefA** in the **Digi-Amp Device Settings** dialog, and **Ref2** is equivalent to **RefB**.

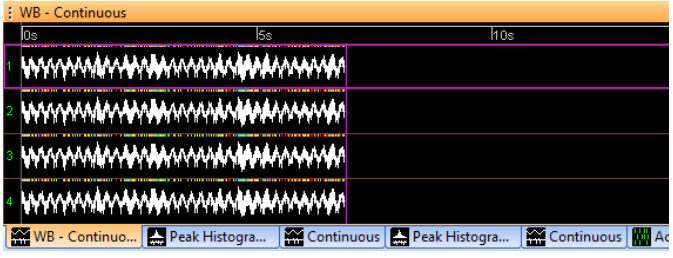
**13** Verify that you are seeing the expected (non-zero) signals on the appropriate channels.




**14** Move the HTU jumper from the **GND** position to the **EQ SIG** position. Note that when the jumper is on the **EQ SIG** position, the HTU sends the same signal to the headstage referencing pin(s) as it is sending to all the other channels in the headstage. However, in this test, you have set the software to ignore the signal on the headstage referencing pin.

**Note:** It is acceptable to move the REF jumper on the HTU while data is flowing through the headstage.

**15** As you place the jumper on the **EQ SIG** position, you should see no change in the signals on the channels (except for transient signals while you are moving the jumper), because the system is not using the signal on the referencing pin. Verify that you see the signal remain the same, confirming that the referencing software and hardware are working correctly.



16 If the signals do not stay the same (or very nearly the same) in the previous step, try repeating this testing procedure. If you still see the signals change in the previous step, gather information about your software, hardware and headstage and contact Plexon Support for assistance.

Final steps before performing additional experiments

17 Restore the REF jumper on the HTU to its original (**GND**) position.

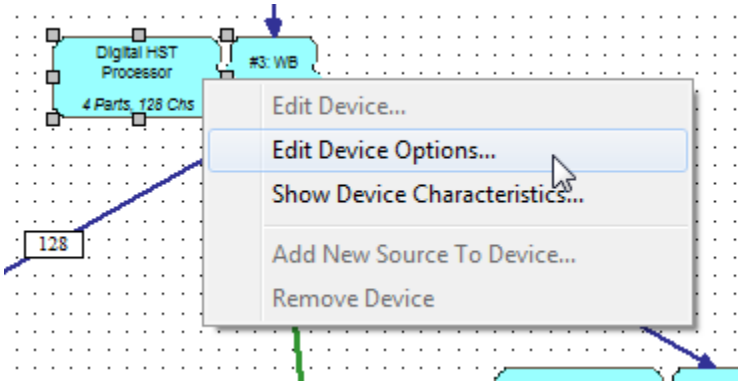
18 In the **Digi-Amp Device Settings** dialog, verify that the **Referencing** option is restored to **RefA** or **RefB** in a manner consistent with your experiments.

## Lowest Latency

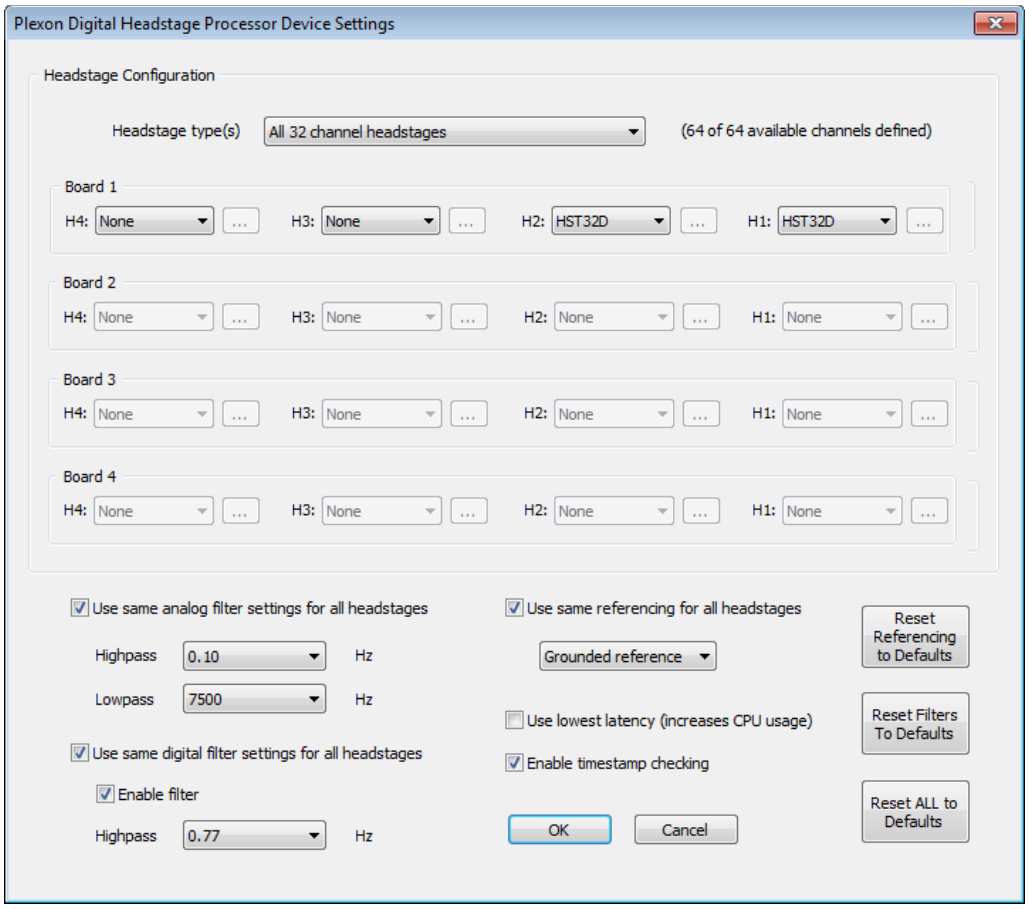
See Appendix E: Lowest Latency Operation and PC Performance Considerations on page A-31.

## Appendix D: DHP Device Settings—Filtering, Referencing and Latency

When you created a new topology (pxs file) or loaded an existing pxs, the DHP device options were automatically set to default settings that are suitable for most uses. However, you may wish to change the headstage highpass and lowpass filter cutoff frequencies, or make other adjustments to the default settings. To display the current DHP device options, make sure that data acquisition is first stopped, then right click on the Digital HST Processor device in the topology and select **Edit Device Options**.



The system displays the **Plexon Digital Headstage Processor Device Settings** dialog:




In addition to the headstage port assignments and channel counts, you can modify the settings for

- Headstage filtering and referencing, either globally (one setting for all headstages), or on a per-headstage basis.
- Lowest Latency setting—See Appendix E: Lowest Latency Operation and PC Performance Considerations on page A-31.

  **Note:** DHP systems of greater than 256 channels do not require a separate lowest-latency mode, so the **Use lowest latency** option in the DHP device options dialog will be grayed out on such systems.

## Purpose of the filtering parameters

Each digital headstage has an analog highpass (low-cut) filter and an analog lowpass (high-cut) filter preceding the analog-to-digital converter in the headstage. The highpass filter allows you to filter out very low frequency signals such as slow baseline drift and animal movement artifacts. The lowpass filter allows you to remove high-frequency noise and helps prevent aliasing caused by frequencies greater than the Nyquist rate (half the sampling rate). The

considerations and tradeoffs involved in choosing the cutoff frequencies are similar for all OmniPlex Systems.

In addition, each headstage allows control of an optional digital highpass filter which is applied to the output of the A/D converter, i.e. to the digitized samples. This digital filter performs two useful functions. First, it eliminates any DC offset appearing at the output of the A/D converter; this offset can otherwise be present as a byproduct of the headstage, even when the analog highpass filter is enabled. Second, by enabling both the analog and digital highpass filters, with the digital highpass set to a higher frequency than the analog filter, very low frequencies in the original analog signal can be removed in the most effective manner. This is partly due to the additional pole of highpass filtering, but for other reasons as well, as described below. If you are not concerned with low frequency phase shift correction, you can skip the following paragraph.

Consider that the analog highpass filters on each digital headstage have a small amount of inherent variation of cutoff frequency from channel to channel, due to analog component tolerances. This means that, for applications where it is important to remove any filter-induced low frequency phase shifts (group delay), for example using Plexon's FPAlign application, there will unavoidably be a small residual phase shift after correction, since FPAlign has no way of "knowing" of these slight imperfections. A digital headstage filter has no such imperfections, but by definition cannot be applied before A/D conversion, and cannot remove low frequency artifacts which can cause clipping in the A/D conversion; only an analog filter can do this.

However, if we use both the analog and digital highpass headstage filters, but set the latter's cutoff significantly higher, the effect of the variation in the analog highpass will be greatly reduced. You might think of the digital highpass as the "precision trim" that follows the "approximate first cut" from the analog highpass. Note that since both the analog and digital filters are relatively gentle one-pole filters (6 dB./octave rolloff), the digital highpass cutoff should be well above that of the analog highpass, e.g. by a factor of 4-10. The default cutoff frequencies are 0.1 Hz for the analog highpass and 0.77 Hz for the digital highpass.

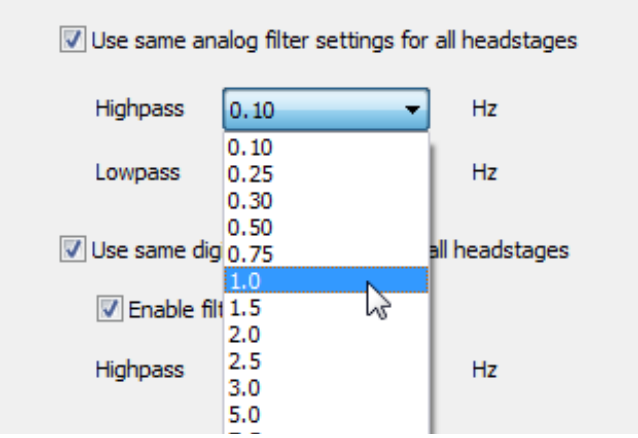
### Purpose of the referencing parameter

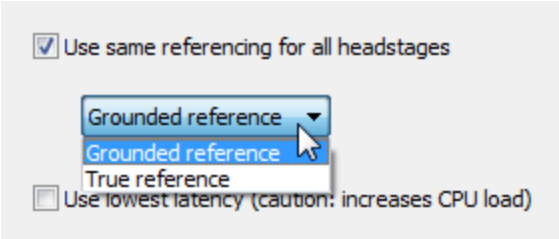
Each digital headstage supports two analog referencing (analog signal subtraction) options, either grounded referencing or true referencing. True referencing subtracts the signal on the headstage's reference electrode from every other channel before A/D conversion. Grounded referencing is equivalent to connecting the reference electrode to headstage ground.

### Setting the filtering and referencing parameters

Global setting of the filter cutoffs and referencing can be done directly in the options dialog. Set the desired option, making sure that the corresponding **Use same analog filter settings for all headstages** and **Use same referencing for all headstages** checkboxes are checked.

Note that list of frequencies is restricted to the frequencies which are implemented in the digital headstage hardware.




You can use the procedure in Testing the Referencing Function on page A-28 to verify proper functioning of the referencing feature.

**TIP**

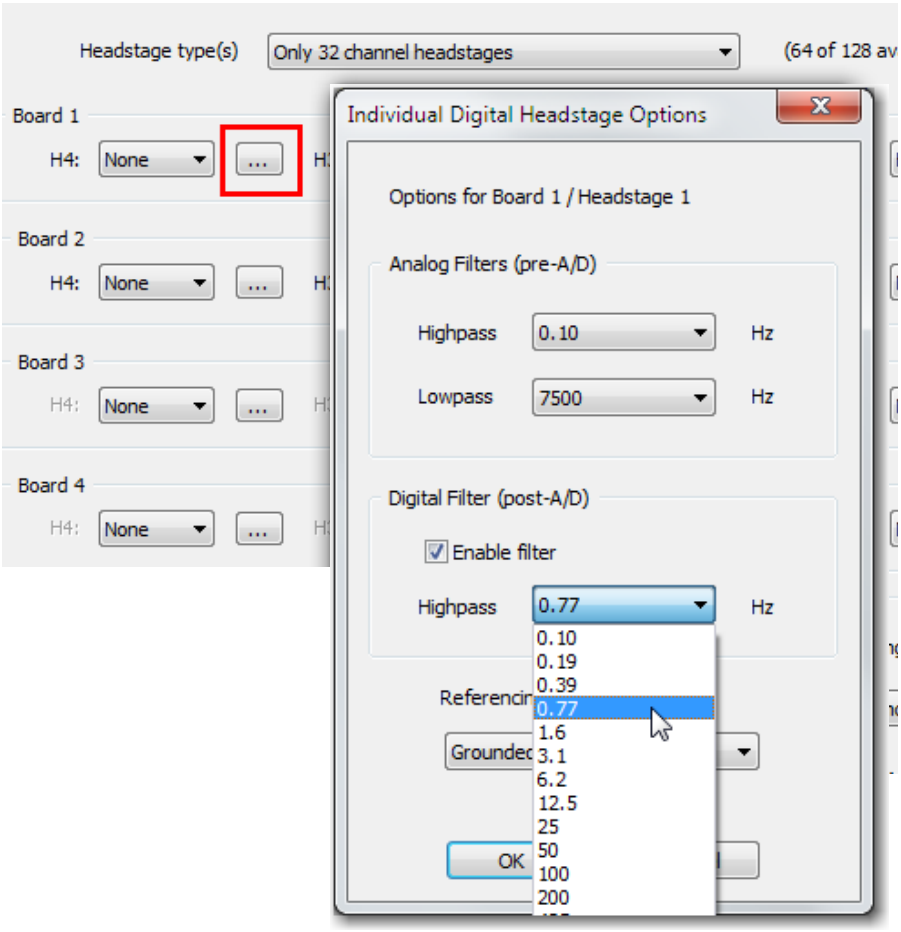
**Referencing considerations for 64 channel digital headstage**

If you are configuring the system for a 64 channel headstage labeled “GR” or “TR,” the above referencing option is not applicable and will be grayed out for the 64 channel headstages.

Instead, the referencing is accomplished in hardware (not configurable in software). Look at the labeling on the headstage (examples shown below). If the label includes the designation “GR” the headstage reference pins are connected to ground. If the label includes “TR” the headstage reference pins are isolated—not connected to ground.

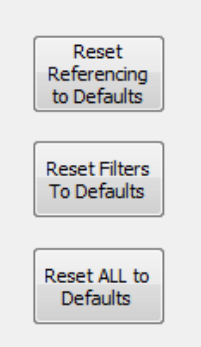
The **Use same referencing for all headstages** checkbox and dropdown will be grayed out if you are using one or more 64 channel headstages. Likewise, the per-headstage **Referencing** dropdown will be grayed out for any port which is configured for a 64 channel headstage. Although configuring any port to use a 64 channel headstage will cause the **Use same referencing for all headstages** option to be disabled for all ports, the referencing configuration on any other headstages which you have previous set will not be affected. For example, if your configuration used four 32 channel headstages and you used **Use same referencing for all headstages** to set them all to true reference, and you then replaced two of the headstages with a single 64 channel headstage, the remaining two 32 channel headstages would still be set to true referencing.

If you wish to set the filter cutoffs and/or referencing individually for each headstage, uncheck the corresponding **Use same** checkbox(es) and then click the "**…**" options button next to the headstage whose settings you wish to modify:

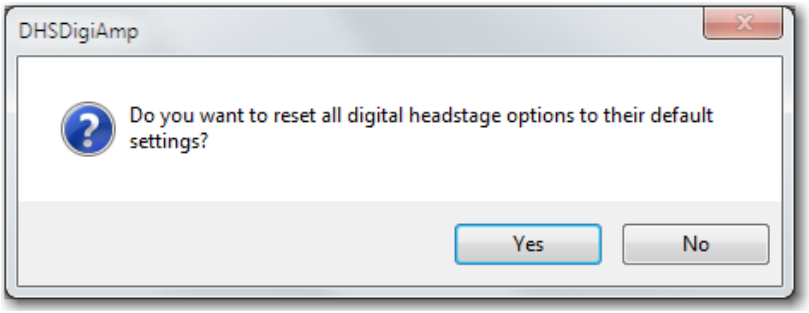



Note that if you later re-enable the **Use same** checkbox(es) in the main options dialog (the **Plexon Digital Headstage Processor Device Settings** dialog box), the global settings will then override any per-headstage changes that you previously made.

If you need to restore all the headstage options, including both global and per-headstage settings, to their default values, click the appropriate **Reset** button. Note that the **ALL** reset includes the configuration of 16 versus 32 channel headstages, which will revert by default to the appropriate number of 32 channel headstages required by the total channel count.




You will be prompted to confirm that you want to reset all options:




**Reset All** can be useful in cases where you have modified the individual headstage options and want to start over.

## Testing the Referencing Function

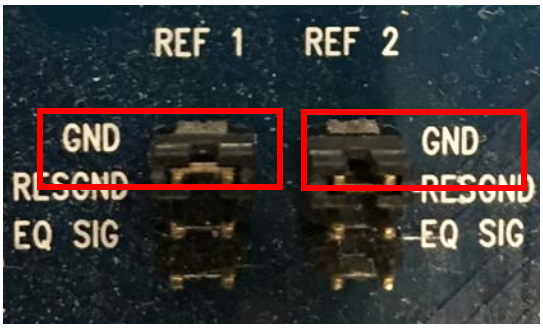
If you want to verify that the referencing function is working properly in both the hardware and software, follow this procedure.

**Note:** In “true reference” mode the system subtracts the signal on the reference input from the other signals and in “grounded reference” mode, it does not.

**Note:** This procedure is not applicable for certain 64 channel digital headstages. See page A-25.

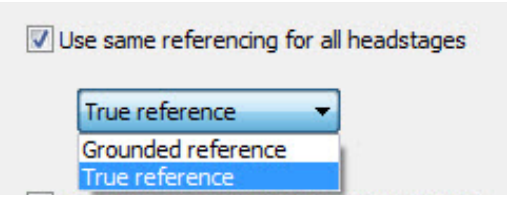
1. Connect the audio cable and a headstage to the Headstage Tester Unit (HTU). Verify that an audio signal is present in the audio cable.
2. Verify the REF jumper on the HTU is in its original (**GND**) position.

   **(There are several models of the HTU. This is an example. Your HTU might have one REF header or multiple headers)**

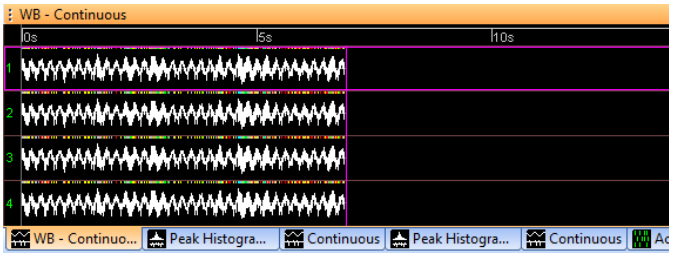



Follow these steps to verify correct “true reference” operation

3. In Server, right click on the **Digital HST Processor** icon and select the **Edit Device Options** command (see the image on page A-22).
4. In the **Plexon Digital Headstage Processor Device Settings** dialog, verify that the referencing option is set to **True reference** and click **OK**. This setting ensures the system uses the actual voltage incident on the reference pin (and is *not* shorting the reference pin to ground).



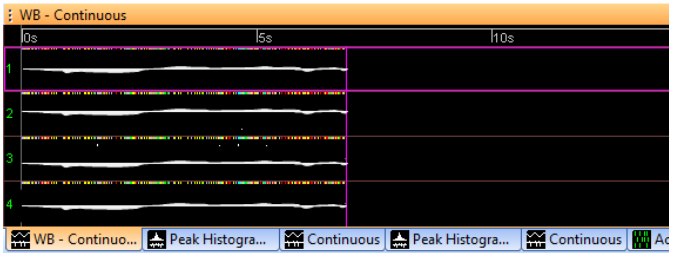
5 Verify that you are seeing the expected (non-zero) signals on the appropriate channels.




6 Move the HTU jumper from the **GND** position to the **EQ SIG** position. Note that when the jumper is on the **EQ SIG** position, the HTU sends the same signal to the headstage referencing pin(s) as it is sending to all the other channels in the headstage.

**Note:** It is acceptable to move the REF jumper on the HTU while data is flowing through the headstage.

7 As you place the jumper on the **EQ SIG** position, you should see the signals on the channels go to zero, because the audio signal is being subtracted from itself. Verify that you see the signal go to zero, confirming that the referencing software and hardware are working correctly.

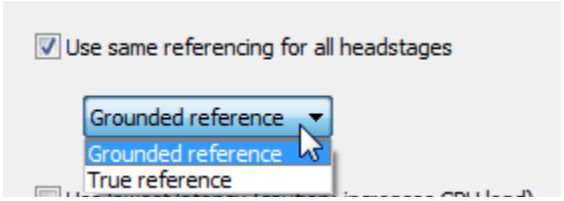



8 If the signals do not go to zero (or very close to zero) in the previous step, try repeating this testing procedure. If you still do not see the signals go to zero, gather information about your software, hardware and headstage and contact Plexon Support for assistance.

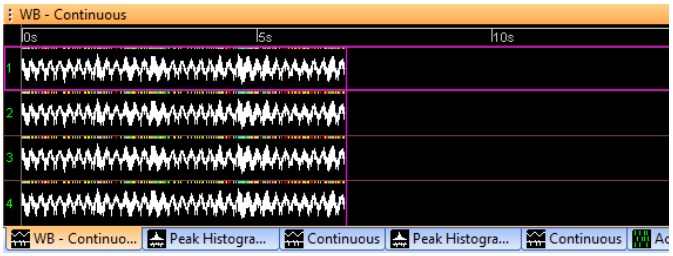
Follow these steps to verify correct “grounded reference” operation

9 In Server, right click on the **Digital HST Processor** icon and select the **Edit Device Options** command (see the image on page A-22).

10 In the **Plexon Digital Headstage Processor Device Settings** dialog, verify that the referencing option is set to **Grounded reference** and click **OK**. This setting ensures the system is shorting the reference pin to ground (and *not* using the voltage incident on the reference pin).



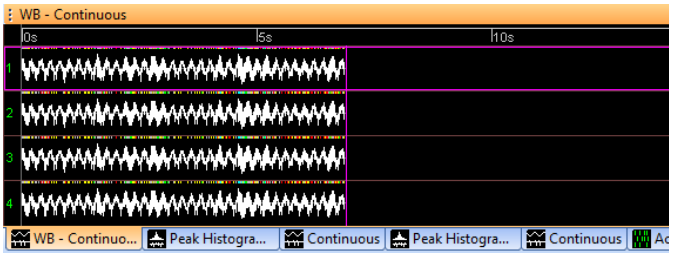
**11** Verify that you are seeing the expected (non-zero) signals on the appropriate channels.




**12** Move the HTU jumper from the **GND** position to the **EQ SIG** position. Note that when the jumper is on the **EQ SIG** position, the HTU sends the same signal to the headstage reference pin(s) as it is sending to all the other channels in the headstage. However, in this test, you have set the software to use ground as a reference signal.

**Note:** It is acceptable to move the REF jumper on the HTU while data is flowing through the headstage.

**13** As you place the jumper on the **EQ SIG** position, you should see no change in the signals on the channels (except for transient signals while you are moving the jumper), because the system is not using **True reference**, that is, the system is not using the signal incident on the referencing pin. Verify that you see the signal remain the same, confirming that the referencing software and hardware are working correctly.




**14** If the signals do not stay the same (or very nearly the same) in the previous step, try repeating this testing procedure. If you still see the signals change in the previous step, gather information about your software, hardware and headstage and contact Plexon Support for assistance.

### Final steps before performing additional experiments

**15** Restore the REF jumper on the HTU to its original (**GND**) position.

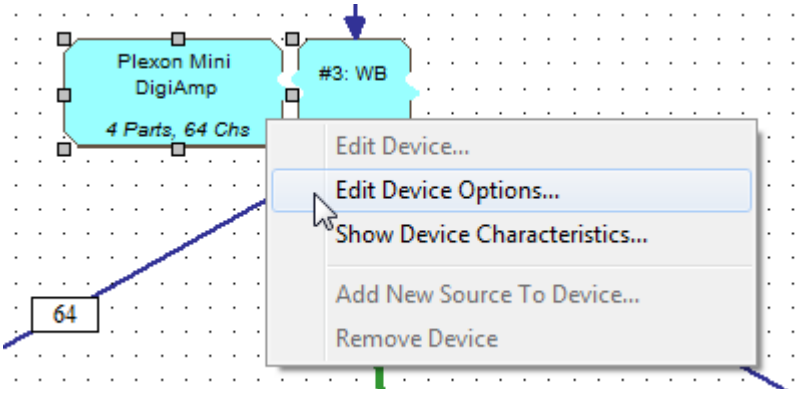
**16** In the **Plexon Digital Headstage Processor Device Settings** dialog, verify that the referencing option is set in a manner consistent with your experiments.

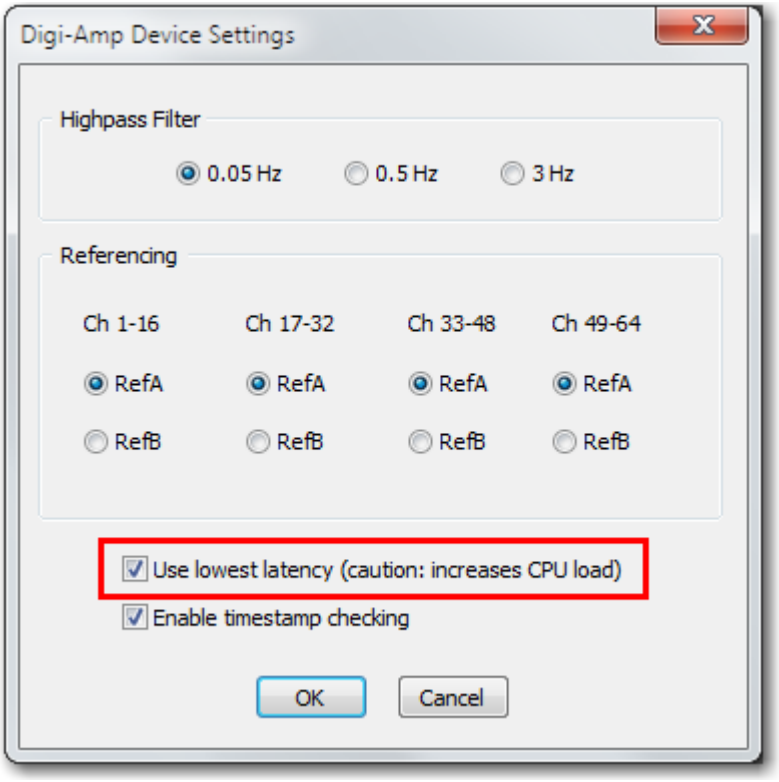
# Appendix E: Lowest Latency Operation and PC Performance Considerations

For applications such as brain-machine interface (BMI) experiments, closed-loop stimulation, etc, it is important to operate the OmniPlex System with the lowest possible latency. By latency we mean the total time for data acquisition, signal processing, spike sorting, and delivery of sorted spikes to external programs such as clients and MATLAB® scripts.

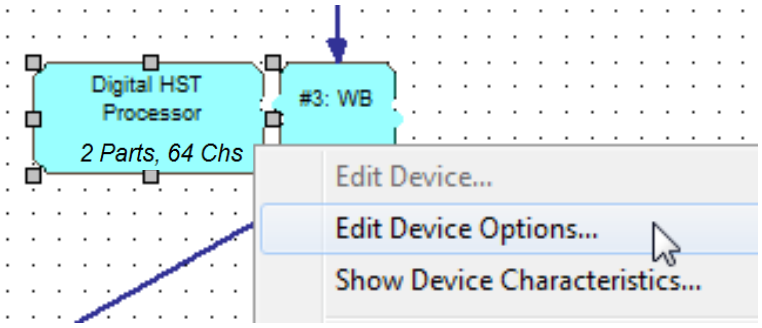
**Note:** In this procedure, you will see that there is some difference between dialog boxes that appear when you have a DigiAmp subsystem vs. a DHP subsystem. However, the basic procedure is the same.

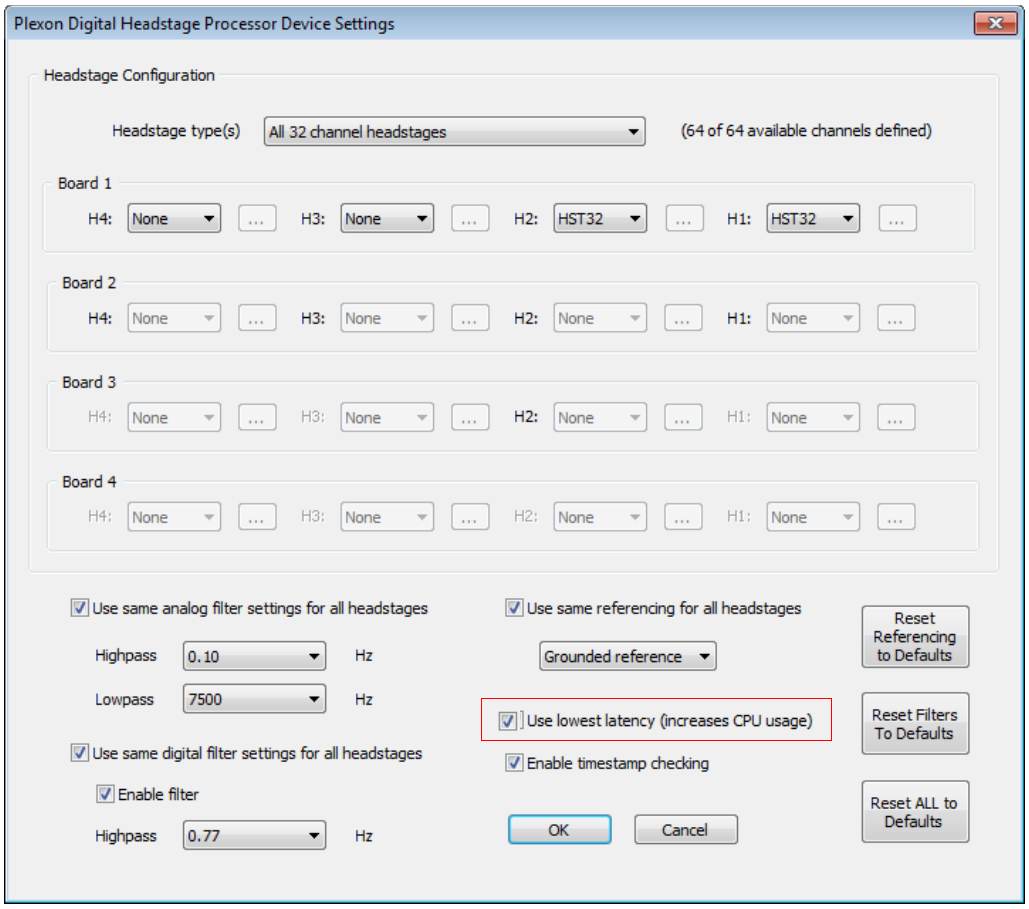
**1** (Applicable with DigiAmp subsystem) In the DigiAmp Device Settings, make sure that “Use lowest latency” is enabled.



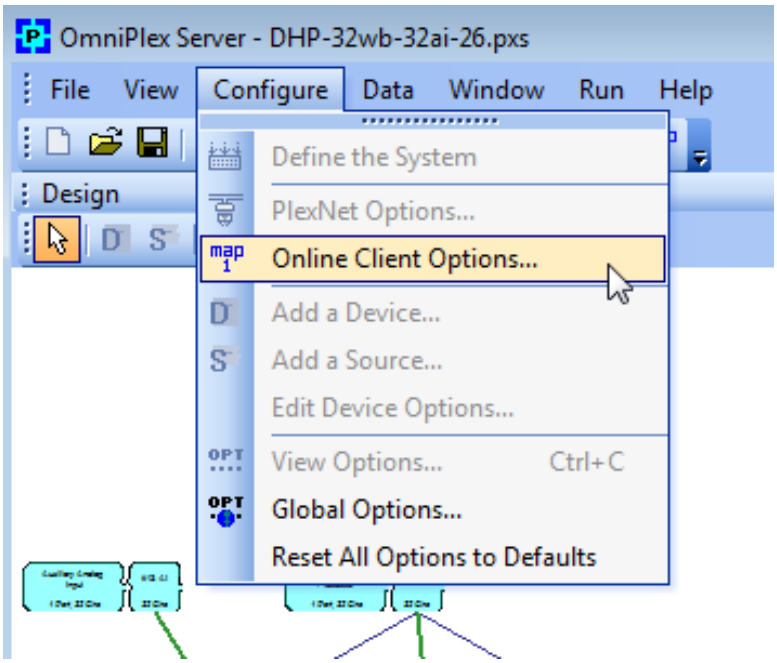



**2** (Applicable with DHP subsystem) In the Plexon Digital Headstage Processor Device Settings, make sure that “Use lowest latency” is enabled.

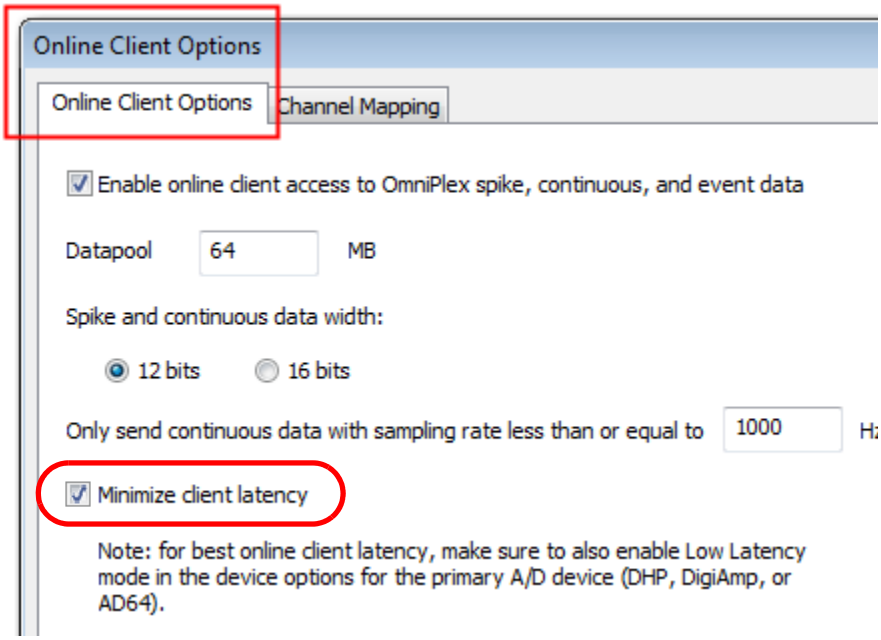





**3** In Server, select **Online Client Options** from the Configure menu:



4 In the **Online Client Options** dialog, enable **Minimize Client Latency**:



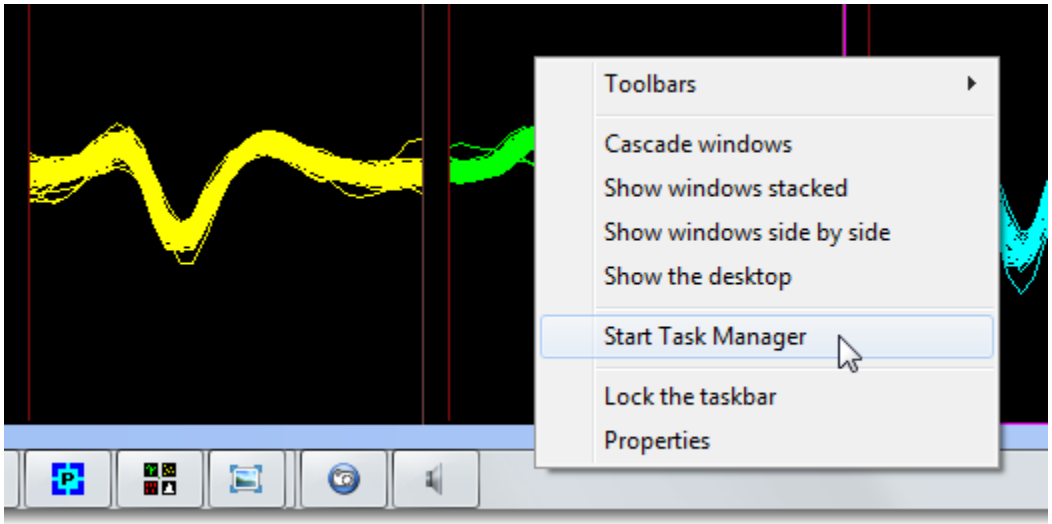
### Client considerations for lowest-latency operation

Note that “Minimize client latency” can result in over 2000 Server synchronization events per second being sent to all client programs, in order to update clients rapidly. Client programs must be designed to handle this high an update rate. For example, a client which redraws its entire user interface, or complex graphical displays, on every update, will probably use excessive CPU and quickly fall behind the incoming data stream. Typically, clients will need to perform only the necessary low-latency processing, such as controlling a neural prosthesis, stimulator, etc, on each update, but perform UI and display updates at a lower rate, e.g. 10-60 Hz, possibly on a separate thread. Consult the Plexon Client SDK documentation for more information on writing real-time clients.

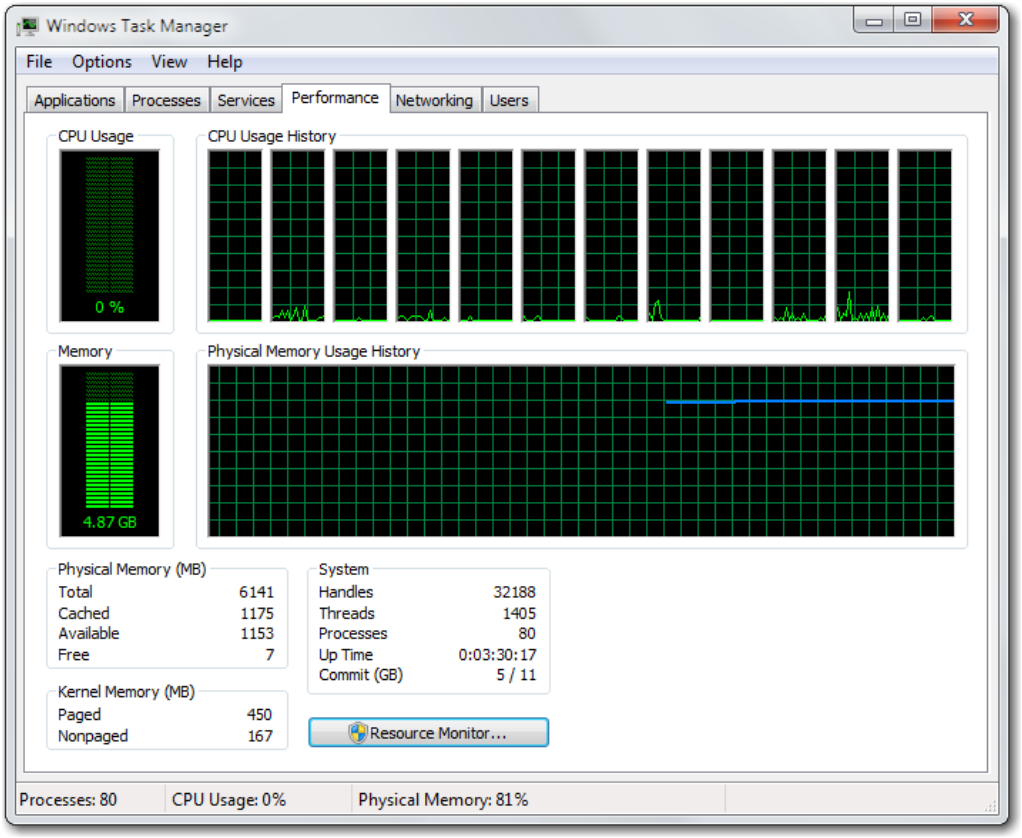
### Performance considerations in lowest-latency operation

Enabling “Use lowest latency” in the DigiAmp or DHP options will increase the CPU usage of the OmniPlex System, although CPU usage will still remain within the available resources on a properly configured PC provided by Plexon. However, the “margin of horsepower” available to other applications running on the same machine will be reduced, and the chances for conflict with other applications which have latency demands or run at elevated priority may be greater. When you configure the OmniPlex System for lowest latency operation, it is highly recommended that you perform several “dry run” tests in the intended usage scenario. During these dry run tests, check the lower right corner of the status bar and make sure that the Drop indicator does not appear. If the Drop indicator does appear, you may need to consider disabling the “Use lowest-latency” option. (Note that high CPU usage might also affect other applications running on your PC.)

In addition to the dry run tests, you should check the CPU usage directly in Windows® Task Manager. To run Task Manager, right-click on the Windows taskbar and select Start Task Manager:



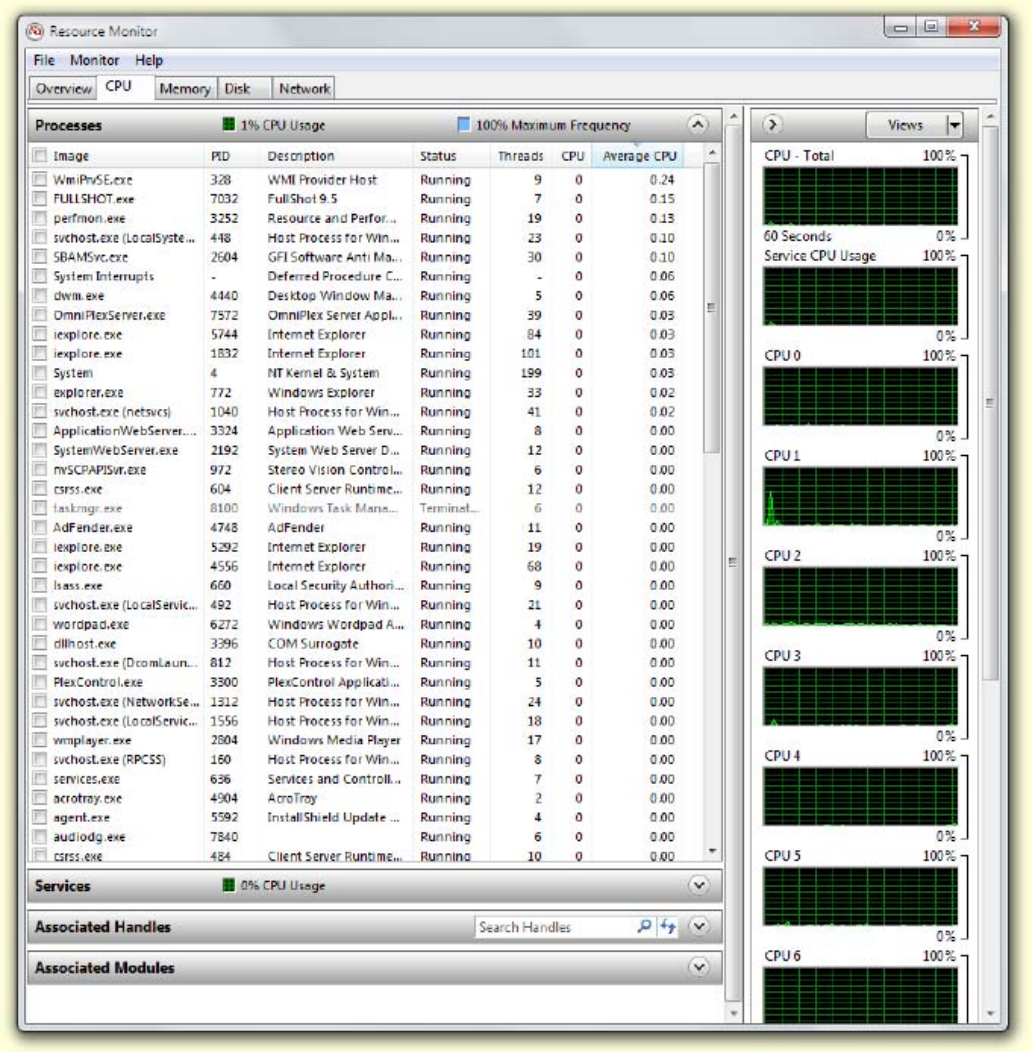
In the Windows Task Manager, select the Performance tab:



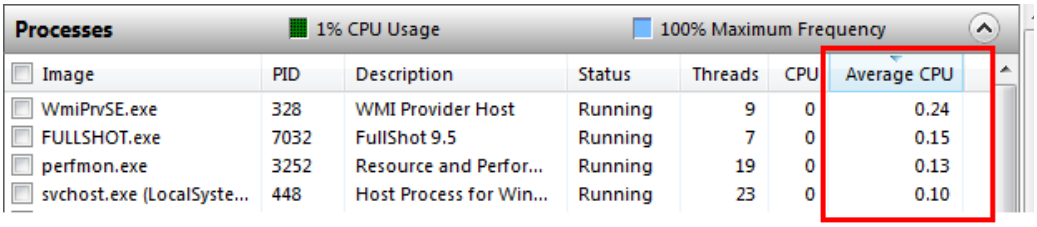

As seen in the above image, the row of small windows under “CPU Usage History” displays the history of CPU usage for each core in the system. As a rough rule of thumb, if any one core exceeds 75% for an extended period, or the total usage (as displayed in the “CPU Usage” window) exceeds 50%, you should verify that all PC applications are operating normally, as explained earlier in this discussion.

Another important indicator of normal operation is the rapid response of the display on your monitor. If the response appears to slow down, for example, if the windows scroll slowly or the interface does not update smoothly, you should check for high CPU usage and the possibility of interference from other applications with high CPU usage.

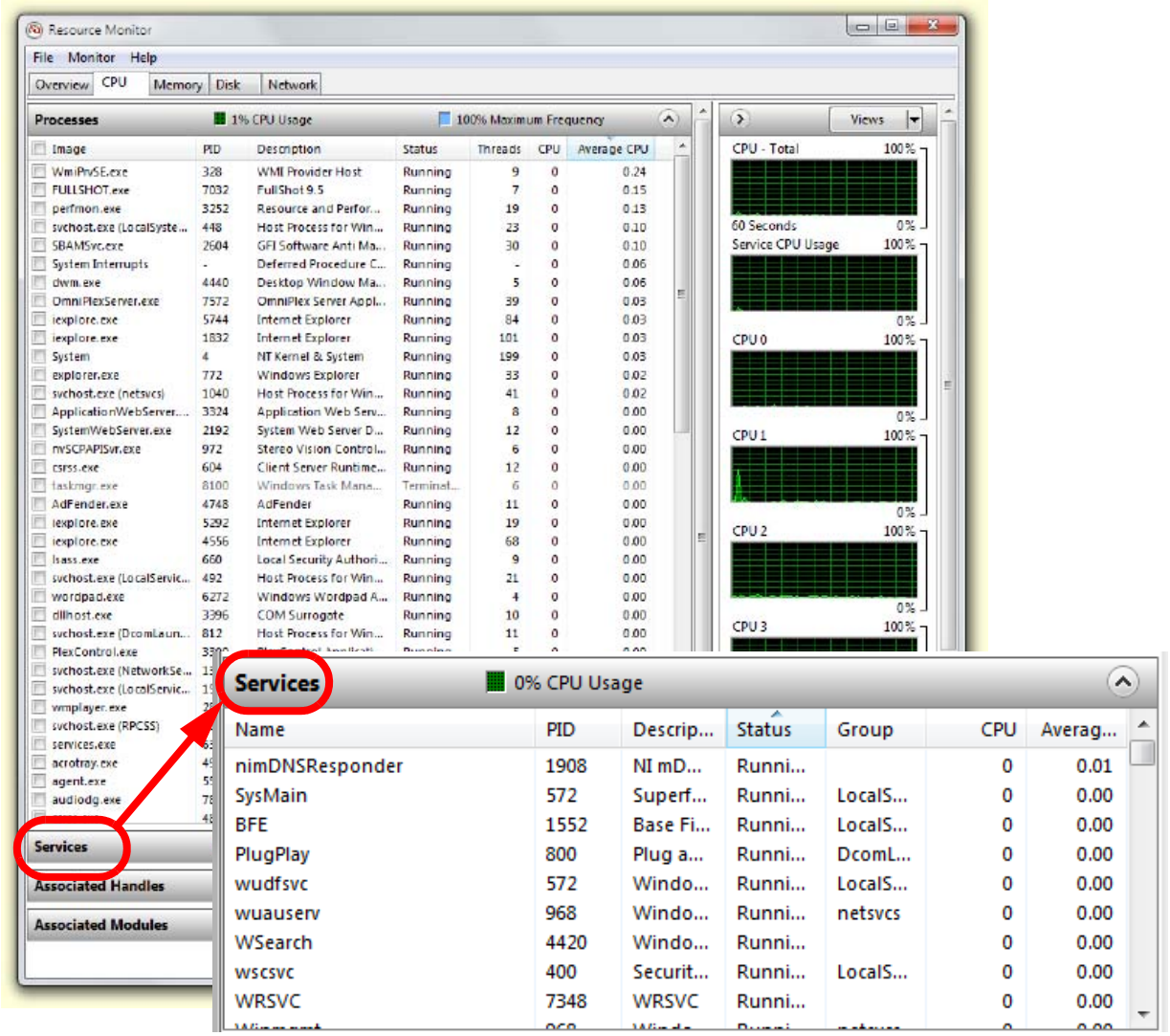
You can resize the Task Manager to make the per-core graphs easier to view. You can also click the **Resource Monitor** button in the Task Manager (shown in the image above) to view more detailed performance information.




Clicking on the Average CPU column header will sort applications in order of decreasing CPU usage, which allows you to quickly focus on any applications that might be using excessive CPU.



It is also useful to check the PC services status and verify that none of the services is using excessive CPU.




### Spectre / Meltdown mitigations and OmniPlex performance

For optimal OmniPlex system reliability, especially at high channel counts, it is important to maintain the performance of the Windows PC on which the OmniPlex software runs. Typically this involves not running other CPU-intensive or real-time applications at the same time as OmniPlex. However, Microsoft has released Windows Update patches which include mitigations (fixes) for the Spectre and Meltdown security vulnerabilities (see https://bit.ly/2Cvedgo for more information). These mitigations can cause performance issues for high channel count OmniPlex systems, especially if operating in lowest-latency mode. Therefore, we have provided a method for disabling these specific patches, which restores normal OmniPlex performance, as well as a method for re-enabling them.

It must be understood that disabling the patches could expose your OmniPlex PC to the risk of Spectre or Meltdown based malware; however, as of this writing, the Spectre / Meltdown vulnerabilities are very difficult for malware creators to exploit, and no malware based on them has been observed on Windows systems. Still, you may wish to consult with your local IT personnel or network administrator to advise them that you intend to disable these protections. Plexon cannot be responsible for any adverse effects or consequential damage as a result of disabling Spectre / Meltdown mitigations.

If you wish to proceed, two batch files are provided in the following folder. Right-click on the batch file name and select Run as administrator, then follow the directions given in the command window that is displayed. Reboot your system after running either batch file to make the changes take effect.

C:\Program Files (x86)\Plexon Inc\Common Files\disable_mitigations.bat

C:\Program Files (x86)\Plexon Inc\Common Files\enable_mitigations.bat

The first file disables the Spectre / Meltdown mitigations, improving OmniPlex performance. The second file re-enables the mitigations, with the accompanying decrease in OmniPlex performance.

It is possible that even after running disable_mitigations.bat, a future Windows Update will silently re-enable the mitigations, in which case any performance issues will reappear and it will be necessary to run disable_mitigations.bat again. This has not happened so far, but you should be aware of the possibility.

Keep in mind that if you are not experiencing performance issues in the OmniPlex System (for example, drop counts in PlexControl, hesitation or “stuttering” of displays), the above procedures are optional. It has been shown that it is definitely necessary at 512 channels and is recommended at 256 channels, especially if operating in lowest-latency mode. If in doubt, you can use the batch files to toggle the mitigations on and off to see their effect, if any, on performance. Remember to reboot after running either batch file.

## Appendix F: Disabling Unused Boards to Reduce Channel Counts

There are two ways to use fewer channels than are physically present in the DigiAmp subsystem or DHP subsystem hardware:

- Specify the number of channels that you want to be active (monitored and displayed) by entering the number in the Topology Wizard in the Server.
- Disable individual channels in the PlexControl Properties Spreadsheet.

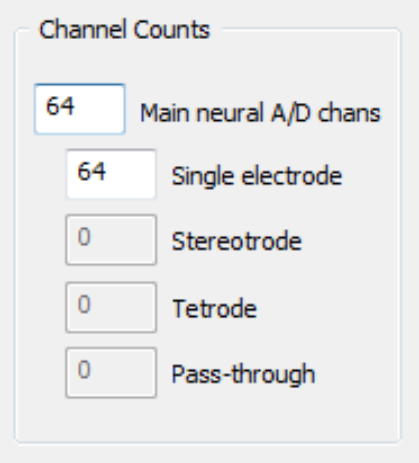
You can also use a combination of these two methods.

### Specify the number of channels in the Topology Wizard

In the **Main neural A/D chans** line of the **Channel Counts** section of the Topology Wizard, enter the total number of actual physical channels that you want the system to use. (The system will ignore any additional channels that might be physically present. It will disable those additional channels and remove them entirely from the user interface.)

Note that a MiniDigi Amplifier has from 16 to 64 channels, with 16 channels per board; a "big" DigiAmp Amplifier has 64 to 256 channels, with 64 channels per board, and the DHP unit has up to 512 channels with up to 128 channels per board. If you are unsure of the number of channels, contact Plexon for assistance. When you enter a channel count in **Main neural A/D chans**, the corresponding number is automatically entered in the **Single electrode** field.

- If you are using a MiniDigi Amplifier, enter 16, 32, 48 or 64 for **Main neural A/D chans**.
- If you are using a DigiAmp Amplifier, enter 64, 128, 192 or 256 for **Main neural A/D chans**.
- If you are using a DHP unit, enter in **Main neural A/D chans** a number that is a multiple of 16, that is, 16, 32, 48, ... 512, but not higher than the maximum channel count of your system license.



Using this method, you will not see "X'ed out" disabled channels in PlexControl, as is the case when you disable channels individually (as shown below); instead, the system will behave as if the DigiAmp, MiniDigi or DHP box contains fewer boards.

Keep in mind that although you can in effect run the system as different-sized configurations by creating or loading an appropriate pxs file, any pxc file that you save in PlexControl must be used with a pxs file that supports the same number of channels, just as if that were the actual hardware configuration. For example, if you have a 256 channel DigiAmp but create a 128 channel pxs topology for it, that 128 channel configuration will only be compatible with 128 channel pxc files.

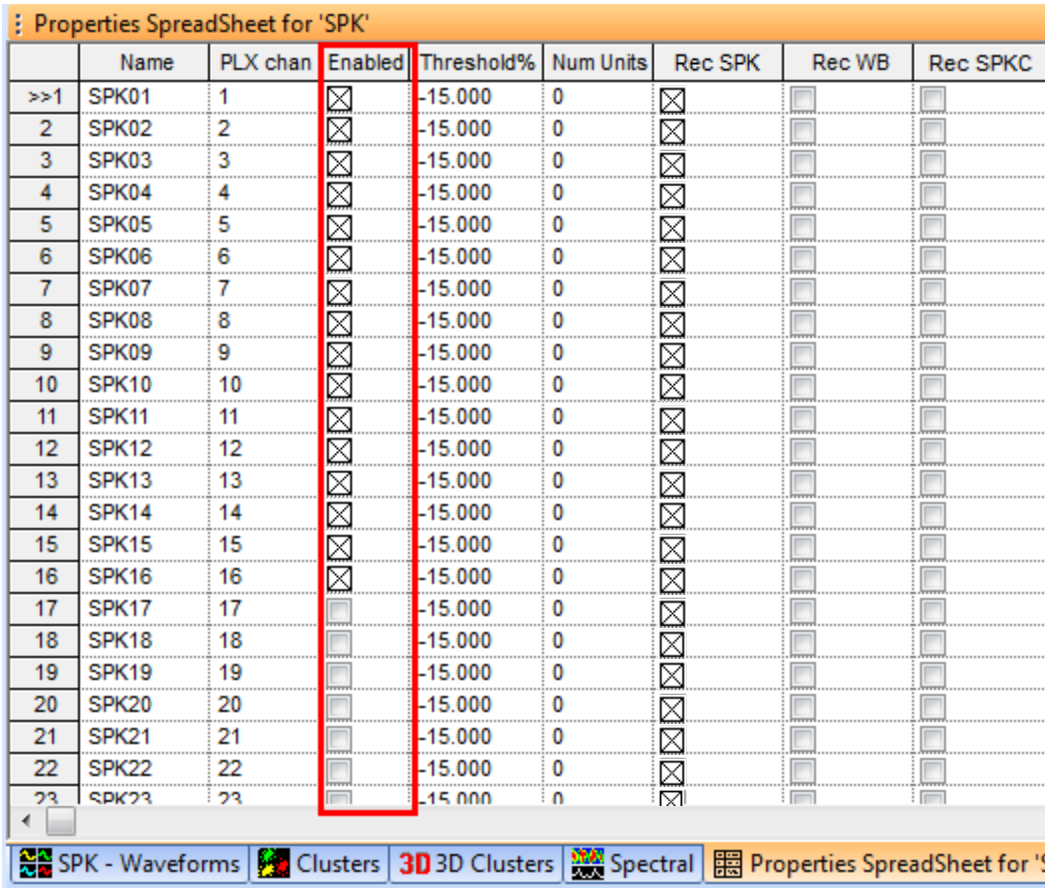
**CAUTION**

**Use caution when entering reduced channel counts in the Topology Wizard**

If you reduce the channel count (entering fewer channels than the number that are physically present in your system), the resulting pxs topology will only be compatible with pxc files with that same number of channels.

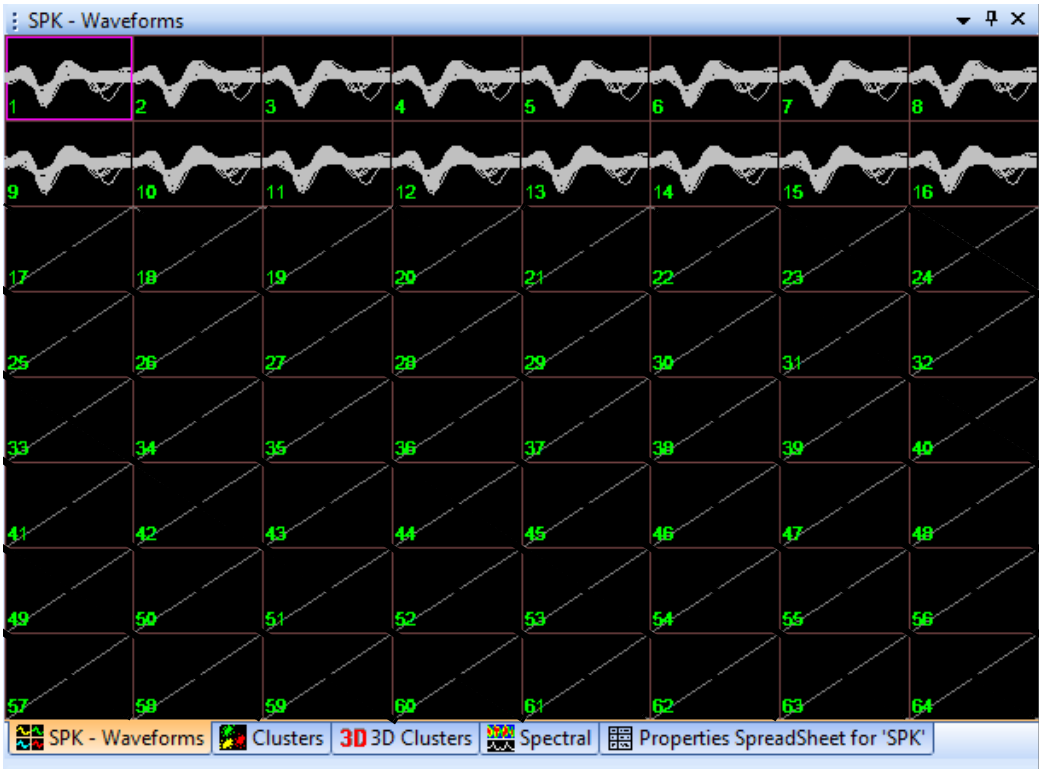
### Disable individual channels in the PlexControl Properties Spreadsheet

In the Properties Spreadsheet, you can select or deselect individual channels, as shown in the image below.




| | Name | PLX chan | Enabled | Threshold% | Num Units | Rec SPK | Rec WB | Rec SPKC |
|---|---|---|---|---|---|---|---|---|
| >>1 | SPK01 | 1 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ |
| 2 | SPK02 | 2 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ |
| 3 | SPK03 | 3 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ |
| 4 | SPK04 | 4 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ |
| 5 | SPK05 | 5 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ |
| 6 | SPK06 | 6 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ |
| 7 | SPK07 | 7 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ |
| 8 | SPK08 | 8 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ |
| 9 | SPK09 | 9 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ |
| 10 | SPK10 | 10 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ |
| 11 | SPK11 | 11 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ |
| 12 | SPK12 | 12 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ |
| 13 | SPK13 | 13 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ |
| 14 | SPK14 | 14 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ |
| 15 | SPK15 | 15 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ |
| 16 | SPK16 | 16 | ☒ | -15.000 | 0 | ☒ | ☐ | ☐ |
| 17 | SPK17 | 17 | ☐ | -15.000 | 0 | ☒ | ☐ | ☐ |
| 18 | SPK18 | 18 | ☐ | -15.000 | 0 | ☒ | ☐ | ☐ |
| 19 | SPK19 | 19 | ☐ | -15.000 | 0 | ☒ | ☐ | ☐ |
| 20 | SPK20 | 20 | ☐ | -15.000 | 0 | ☒ | ☐ | ☐ |
| 21 | SPK21 | 21 | ☐ | -15.000 | 0 | ☒ | ☐ | ☐ |
| 22 | SPK22 | 22 | ☐ | -15.000 | 0 | ☒ | ☐ | ☐ |
| 23 | SPK23 | 23 | ☐ | -15.000 | 0 | ☒ | ☐ | ☐ |

The system continues to monitor and display these channels, so you will see a display similar to the one shown in the following image. The disabled channels are displayed but “X’ed out.”




### Using a combination of methods

You can combine the two methods. For example, with a 128 channel "big" DigiAmp, you could use the Topology Wizard to create a 64 channel (one board) topology, and then in PlexControl manually disable any number of individual channels within those 64 channels.

### Applicable procedures

Refer to the applicable procedures in this user guide:

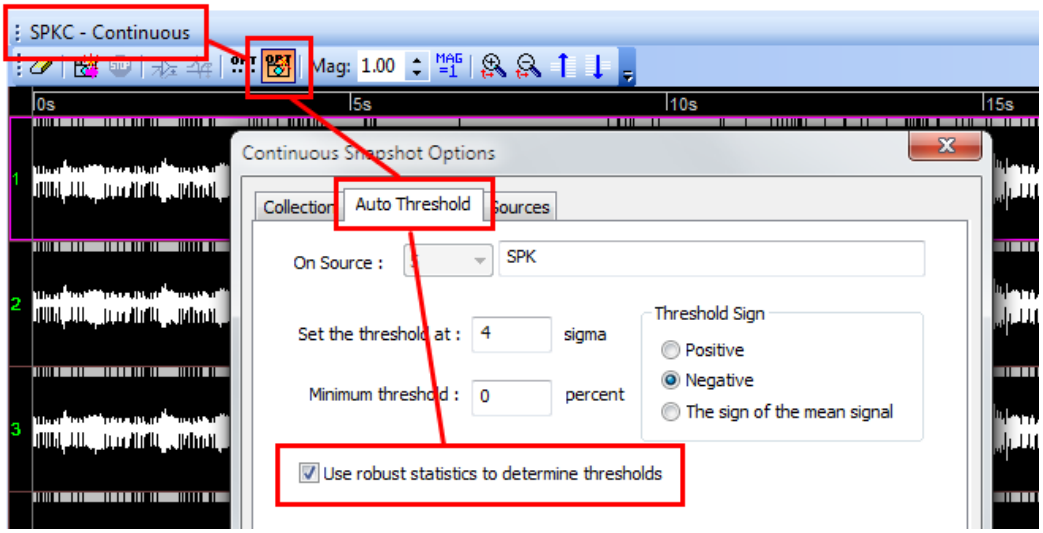
- Section 2.2, “Starting and Configuring the OmniPlex Server” on page 28—Applicable to DigiAmp subsystems
- Section 3.2, “Starting and Configuring the OmniPlex Server” on page 73—Applicable to DHP subsystems

## Appendix G: Robust Statistics

PlexControl provides options for using robust statistics in the determination of auto-thresholding and sorting parameters. The primary advantage of robust statistics is that they are more resistant to the effect of outliers. Some examples are the use of the median as a robust statistic instead of the mean, or the use of the median absolute deviation (MAD) instead of the standard deviation.

### Robust auto-thresholding

To enable robust statistics for auto-thresholding, set the option in the Auto Threshold page of the SPKC snapshot options dialog:



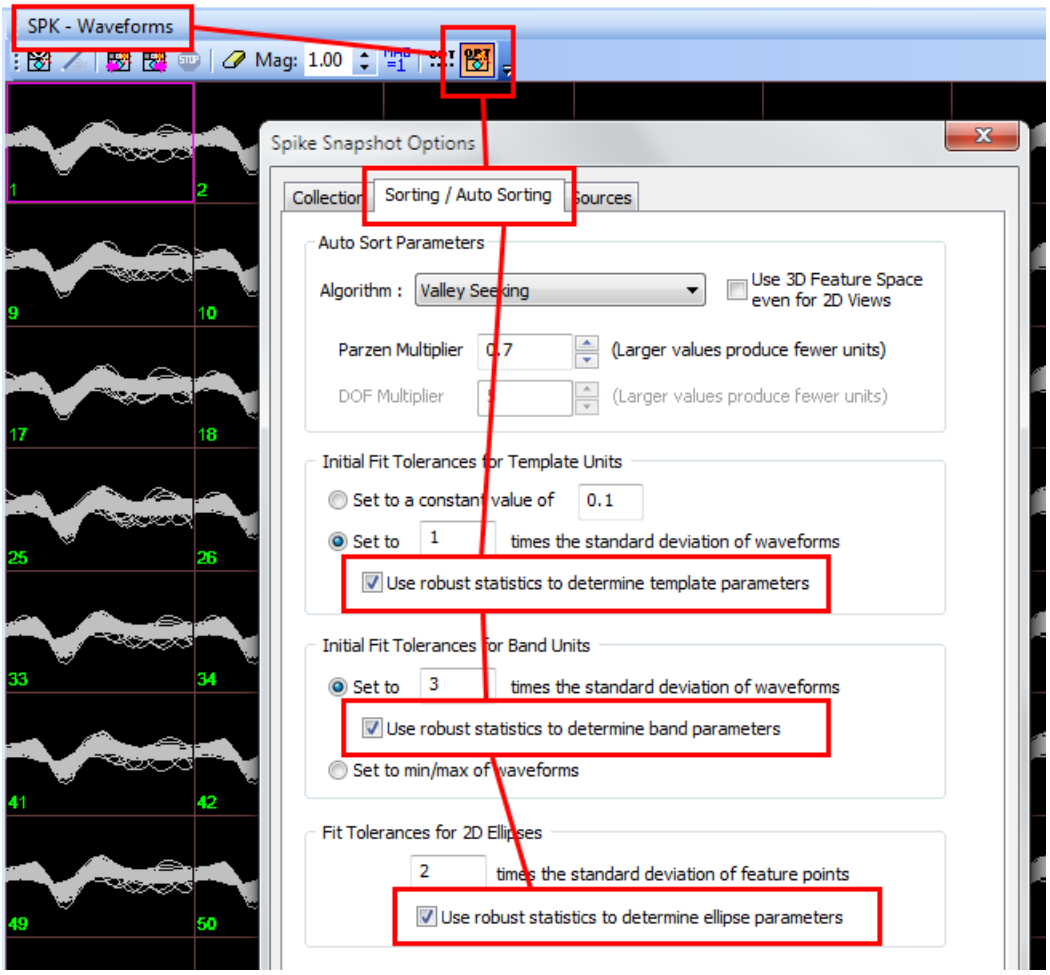

When this option is enabled, the median and MAD of the peak histogram are calculated instead of the mean and standard deviation. The threshold is still expressed in terms of sigmas, but where sigma is derived from MAD by the formula:

$$\text{sigma} = 1.4826 * \text{MAD}$$

You will typically find that using robust statistics produces a more accurate estimate of the noise distribution, with the presence of spikes having less influence on the auto-thresholding process; with conventional auto-thresholding, the more spikes that are present, the higher the threshold produced by auto-thresholding.

### Robust template, band, and ellipse sorting parameters

To enable robust statistics for template, band, and ellipse sorting parameters, set the corresponding option in the Sorting / Auto Sorting page of the SPK snapshot options:



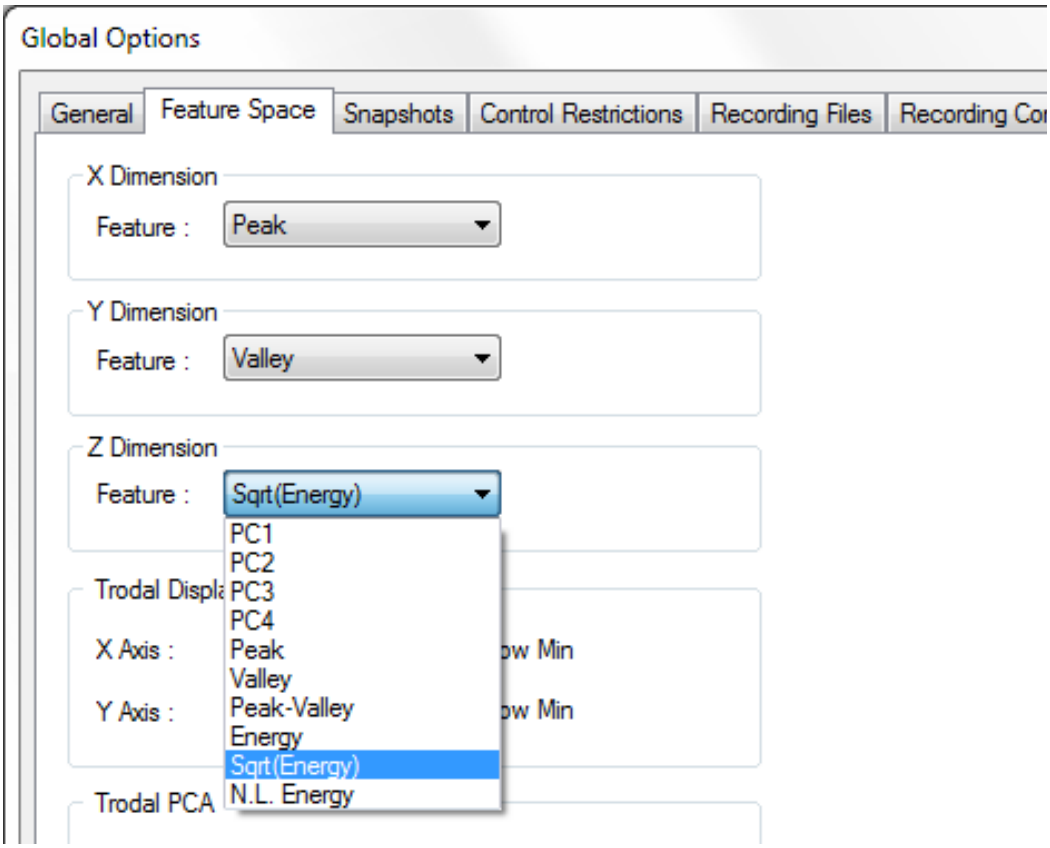

For template sorting, MAD is calculated and used to derive sigma, using the conversion formula described in the section on auto-thresholding, above (sigma = 1.4826 * MAD). For band sorting, MAD is used to derive a sigma value at each sample time within the waveform. For ellipse generation for 2D polygon sorting, the median is used instead of the mean in the standard PCA calculation. After the first two eigenvectors of the covariance matrix are obtained in the usual way, the MAD is calculated with respect to those two vectors and used to derive the sigma values for the major and minor ellipse axes. In all three sorting methods, the actual sorting of incoming spikes is performed using the standard algorithms; robust statistics are used in the calculation of the sorting parameters.

Note that for the case of hand-drawn contours (**Automatically convert hand-drawn contours into ellipses** option, Section 8.8.1, “Cleanup of Hand-drawn PCA Contours” on page 277), 2D ellipse generation does not use robust statistics, nor the standard deviation for that matter. In this case, a special geometric algorithm is used which produces better results; a description of this algorithm is beyond the scope of this document.

# Appendix H: Selectable 2D/3D Feature Space and PCA Options

## Selectable 2D/3D Feature Space

By default, the system uses principal component analysis (PCA) as the feature space into which spike waveforms are projected for cluster displays and, in the 2D Polygon mode, for spike sorting. The first two or three principal components (PCs) are displayed in the 2D Cluster and 3D Cluster views respectively. You can select from a list of several features for any or all of the feature axes. To assign features, use the **Feature Space** page of PlexControl's **Global Options** dialog:




Which features are most useful in identifying clusters is very data-dependent, but for experimentation, you might try starting by leaving the first two features as PC1 and PC2, then varying the third feature. Note that you can click the **Apply** button to update the cluster displays with the new feature space. Depending on the features selected, you may need to adjust the scaling and position of the cluster displays to judge the results.

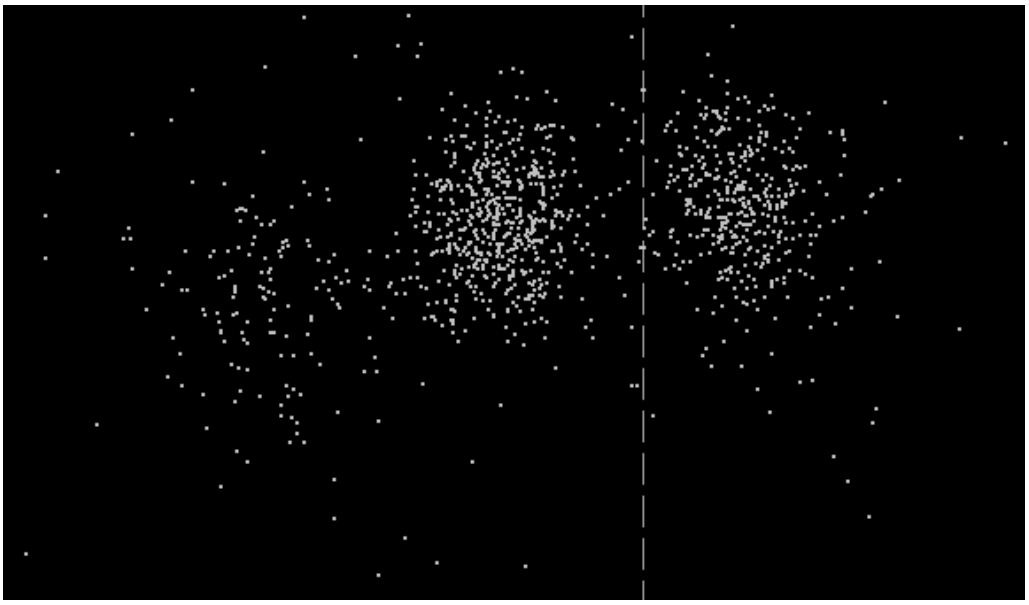
Stereotrode and tetrode modes use the "Trodal Display" and "Trodal PCA" options and are not affected by the choice of the X, Y, Z features.

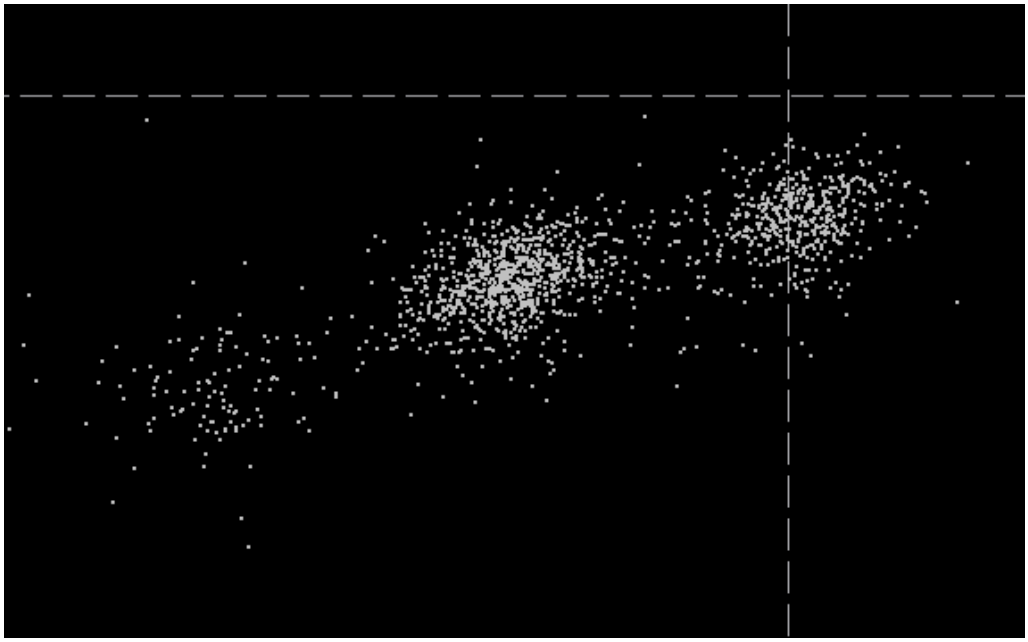
**Note:** PCA features and procedures are presented in more detail in Chapter 8, Additional Sorting Methods and Quality Metrics.

## Enhanced PCA

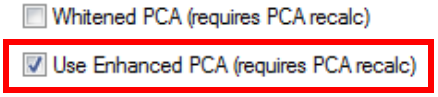
In addition to the standard PCA (principal component analysis) feature space, the OmniPlex System supports an improved version (Enhanced PCA) which was developed by Plexon specifically for spike sorting applications. Standard PCA determines a projection in which the first component is in the direction of maximum variance in the original data, the second component is in the direction of second-greatest variance, and so on. Enhanced PCA uses not only variance, but separability of the data, in calculating the projection. For example if there is a sample position where a group of spikes has a large amount of amplitude variance, but it is basically noise, without any structure within the distribution of amplitudes at that sample time, Enhanced PCA will de-emphasize that component of the data in the resulting projection. In contrast, samples where there is a high degree of separability, even though the amplitude variance may not be large, are emphasized in the projection.

In many cases, the net result of Enhanced PCA is to make the feature space clusters more compact and distinct. However, the degree of improvement is data-dependent, and so you should compare standard PCA and Enhanced PCA to determine which is optimal for your application. Here is an example of standard PCA (top) versus Enhanced PCA (bottom):

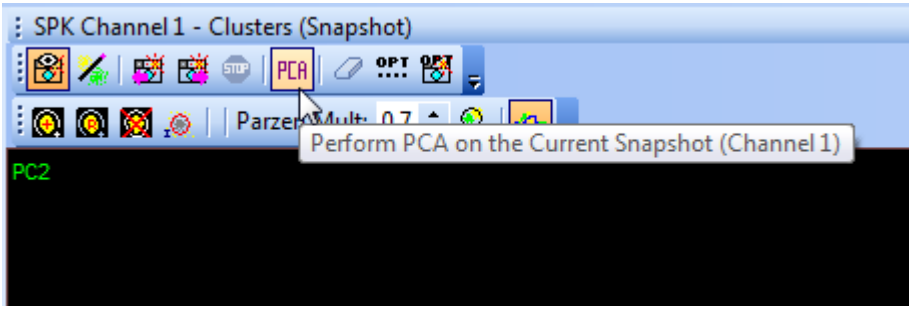




To enable Enhanced PCA, select the **Use Enhanced PCA** checkbox on the **Feature Space** page.




Note that enabling Enhanced PCA will not affect any existing PCA or sorting parameters. The next time you take a spike snapshot, or click the PCA button in the toolbar, the PCA calculation will use the new setting.



**TIP**

**2D Polygon sorting method**

If you are using the 2D Polygon sorting method, you will need to delete the existing units and create new ones.

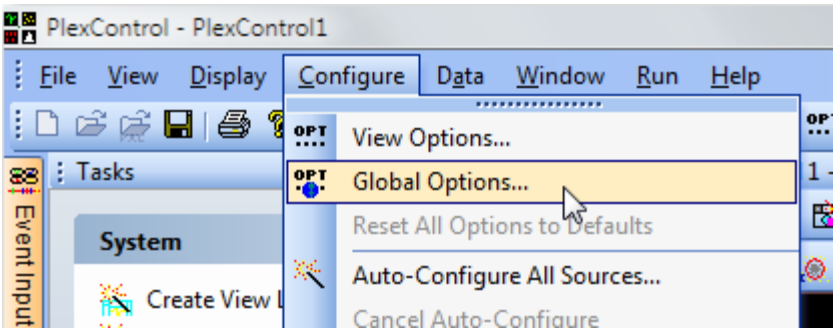
**TIP**
**Increase magnification in PCA view**
Enhanced PCA sometimes results in clusters with a different overall scaling, so that you may need to increase the magnification in the PCA view.

### Whitened PCA

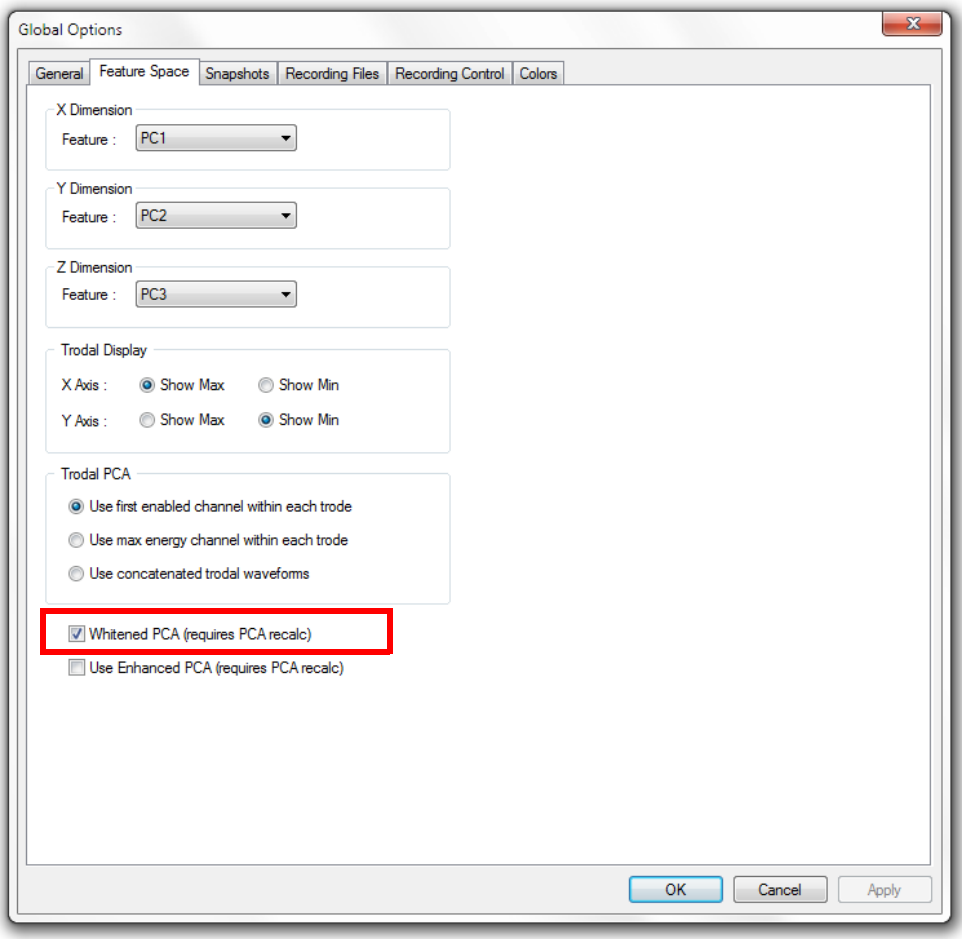
PCA whitening modifies the projection vectors used to project spike waveforms into 2D or 3D PCA space. Each projection vector is divided by the square root of its corresponding eigenvalue. This has the effect of making the PCA projection of the spike snapshot have unit variance in each direction. For this reason, PCA whitening is sometimes referred to as "sphering" the data.

The effect of whitening is data-dependent, but it can improve the performance of auto-sorting. Whitening can be applied to either standard or enhanced PCA, i.e. the Whitened and Enhanced options are independent and can be combined.

To enable Whitened PCA, select **Global Options** in the **Configure** menu:

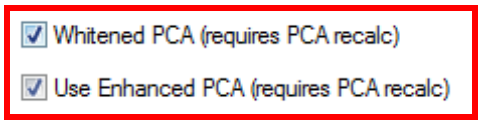


Select the **Feature Space** tab and enable **Whitened PCA**.

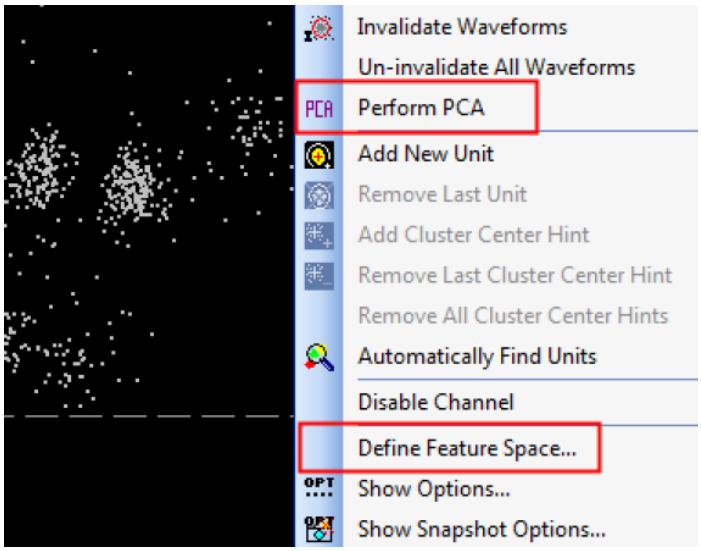



### Combining Whitened PCA and Enhanced PCA

The Whitened and Enhanced options are independent and can be combined.




To experiment with the PCA options, capture a spike snapshot and view the PCA clusters in either the 2D or 3D cluster views. **Define Feature Space** and **Perform PCA** are available on the right button popup menu, and **Perform PCA** is also available as a toolbar button. Viewing the snapshot rather than the live PCA makes it easier to see the effect of the options.


Invalidate Waveforms
Un-invalidate All Waveforms
PCA
Perform PCA
Add New Unit
Remove Last Unit
Add Cluster Center Hint
Remove Last Cluster Center Hint
Remove All Cluster Center Hints
Automatically Find Units
Disable Channel
Define Feature Space...
OPT
Show Options...
OPT
Show Snapshot Options...

## Appendix I: Option for Two Digital Input (DI) Cards

**Note:** For basic instructions on configuring DI card parameters, see Section 10.1, “Digital Input Card Configuration Types” on page 320.

For information on DI card connectors, see Digital Input (DI) Card in Basic OmniPlex Chassis on page A-59.

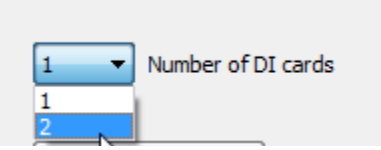
The system includes support for an optional second Digital Input (DI) card. The Topology Wizard in OmniPlex Server allows you to specify the number of DI cards in your system (the default is 1), and the **Plexon Digital Input Configuration** dialog box has been extended to allow you to configure the port settings for each card.

When two DI cards are installed, the maximum number of individual (single-bit) events is 64. This is because each card is capable of generating 32 channels of single-bit events (16 channels each on Port A and Port B):

(maximum number of single-bit events) =
(number of DI cards) * (2 ports per card) * (16 channels per port)

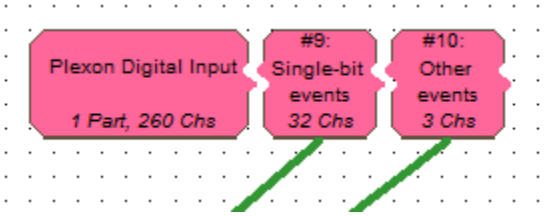
In addition, each card can be configured to have one or more **Event word** sources, containing one multi-bit (“strobed”) channel and the RSTART and RSTOP recording-control events. In addition, the system with multiple DI cards has an additional source named **Multi-DI strobed**, which contains only the strobed event channels for the second DI card.

The Topology Wizard includes a dropdown control that you can use to specify the number of DI cards in your OmniPlex chassis:

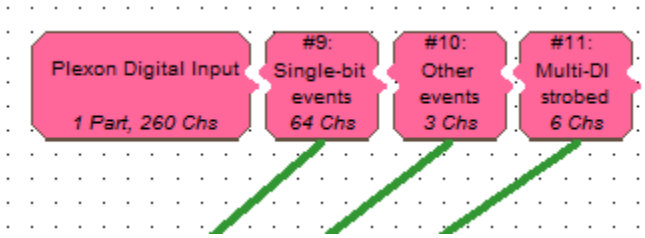



After you have created a topology which includes two DI cards and restarted the OmniPlex application with this topology, you can use all four ports (two per card) for digital input. The per-port options can then be configured, as described below.

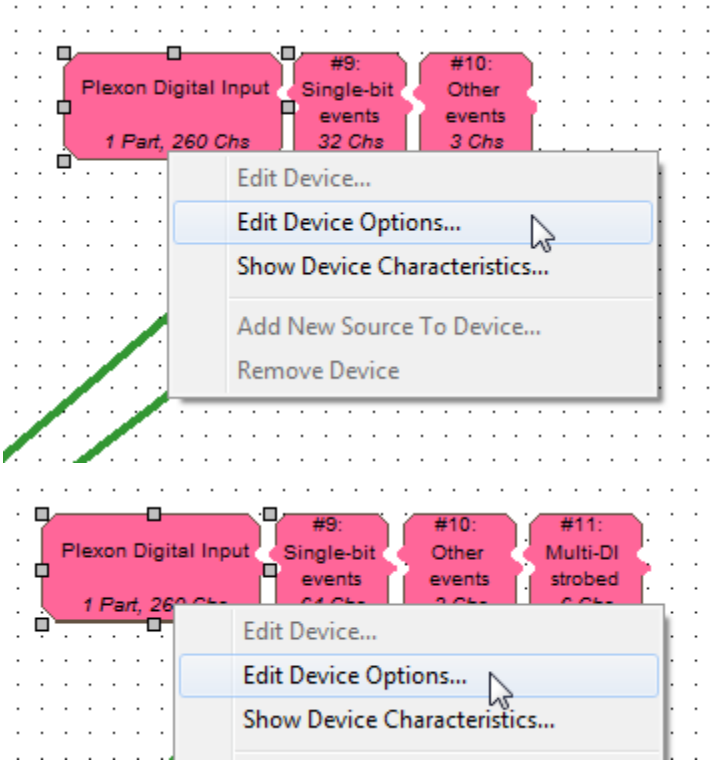
If you have one DI card configured, the topology will appear like this:



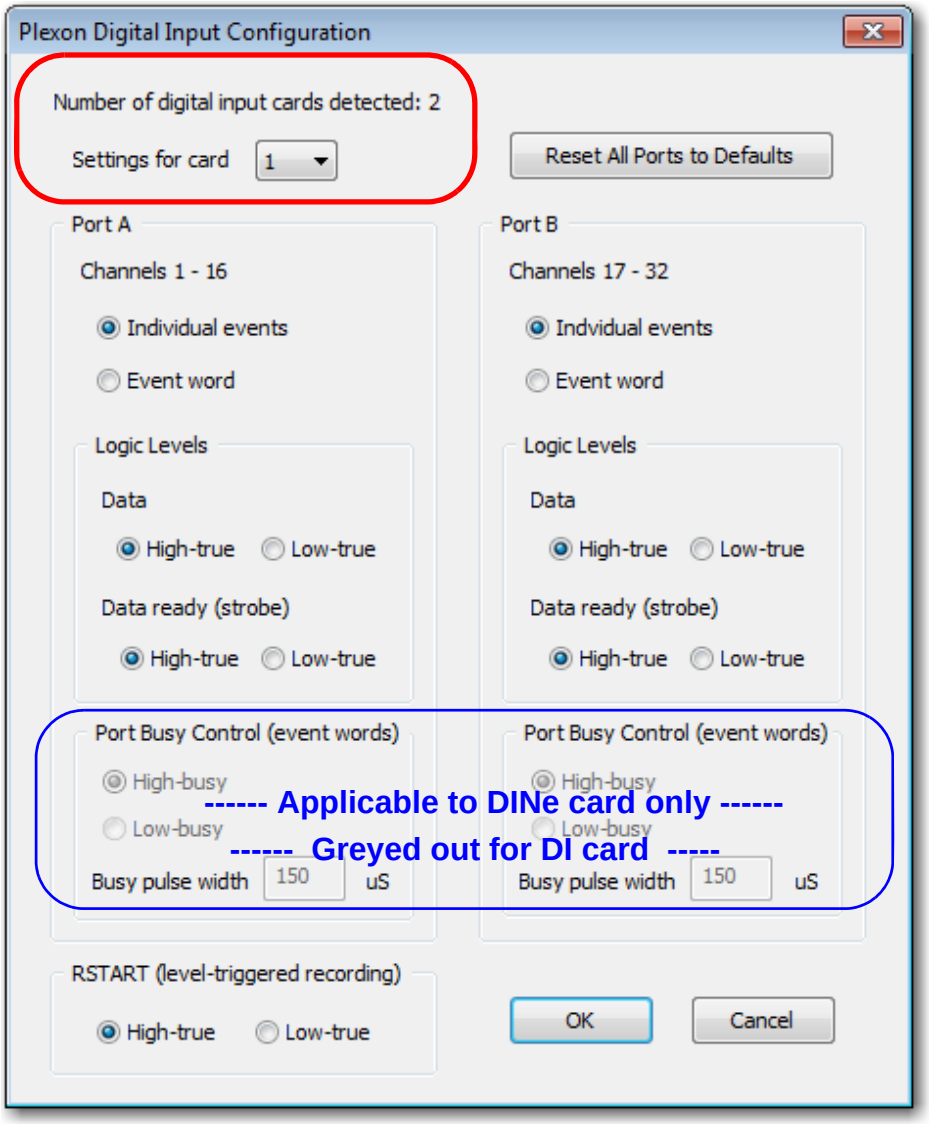

With two DI cards, the topology will appear like this:



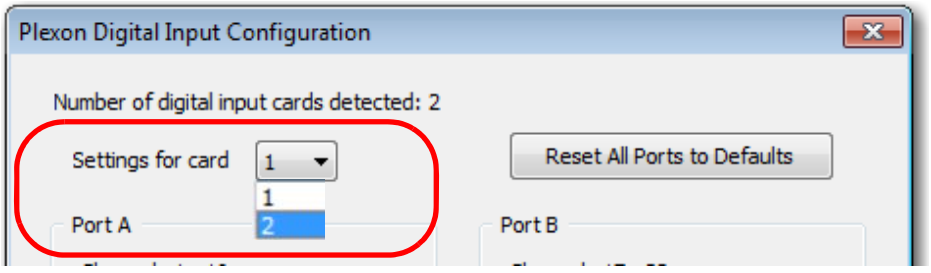

To view or modify the DI card settings, first stop data acquisition, go to the Server window, and right click on the **Plexon Digital Input** device in the topology. Select **Edit Device Options** to display the options dialog.



The **Plexon Digital Input Configuration** dialog box opens. In this example (see the image below), notice that the system has detected two digital input cards, and the settings being configured are for card 1. The numbering of DI cards corresponds to their left-to-right order in the OmniPlex chassis, i.e. the leftmost DI card is card 1.




In the next image, the user is preparing to configure the settings for card 2:

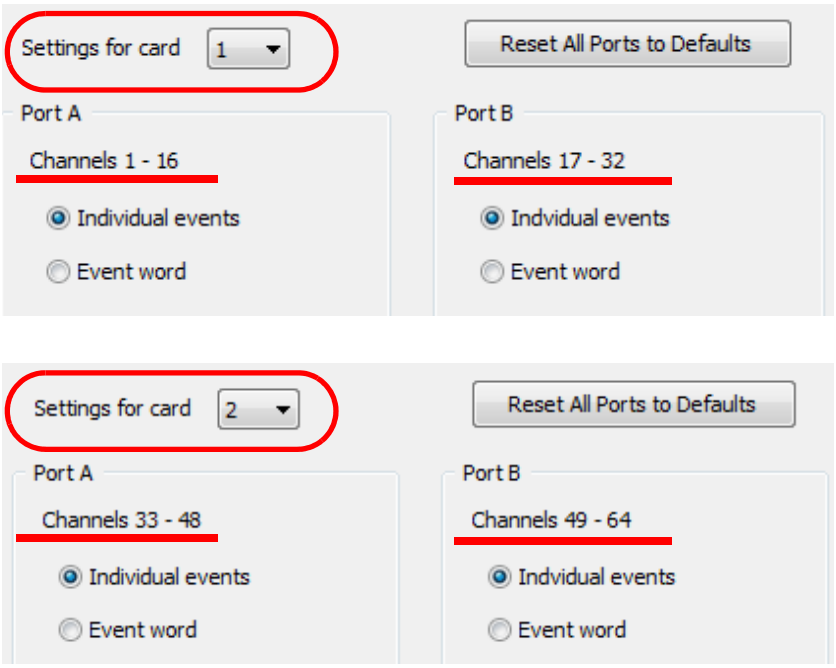


The **Settings for card** control allows you to view and edit the settings for each available DI card in turn. When you change the card number, the settings for Ports A and B for the selected card are displayed. You can switch between each card's settings without losing any changes that you make, but the settings are not actually changed until you click **OK**. You can also use the **Reset All Ports to Defaults** button to reset all ports to **Individual events** and **High-true** logic.

Note that the section for configuring **RSTART** is not port-specific.

### Event channel numbering

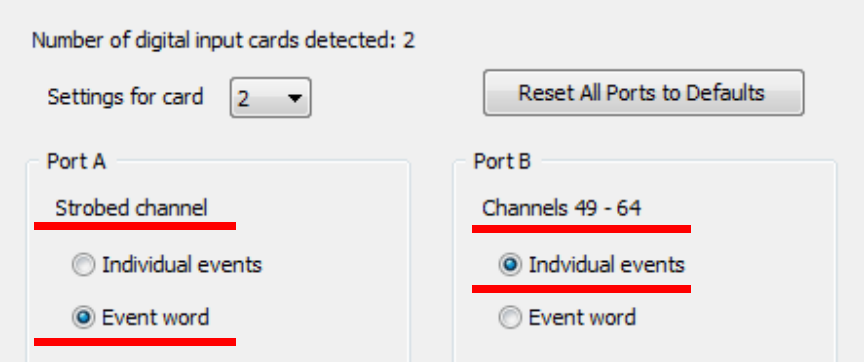
When a port is set to **Individual events** (single-bit events), the range of channel numbers used by that port is displayed, for example:




For single-bit events, the channel numbering is the same for plx and pl2 recordings, as well as for event data sent to online clients:

| DI card+port | Event chan# |
|---|---|
| 1A | 1-16 |
| 1B | 17-32 |
| 2A | 33-48 |
| 2B | 49-65 |

When a port is set to **Event word** (multi-bit events), this labeling is replaced with **Strobed channel.** For example, Port A is set to **Event word** in the image below:




Note that a specific channel number is not shown for multi-bit (strobed) channels; this is because the numbering of multi-bit channels is different for plx and pl2 recording files (online client data uses the same numbering as plx files).

The following table gives the multi-bit channel numbering; remember that for pl2 files, event channels are identified by the combination of the source name and the channel number within that source:

| DI card+port | PLX, client event chan# | PL2 source/chan# |
|---|---|---|
| 1A | 257 | Other events / ch1 |
| 1B | 257* | Other events / ch1 |
| 2A | 241 | Multi-DI strobed / ch1 |
| 2B | 242 | Multi-DI strobed / ch2 |

The asterisk * indicates the special case of Port B on the first DI card. For compatibility with the Plexon plx file format conventions, **Event word** (multi-bit) data from Port B "shares" plx channel 257 with Port A when both ports are set to **Event word** mode. In this case, event words from Port A will always have their high bit set to 0 by the OmniPlex application, while words from Port B will have their high bit set to 1; thus the high bit can be used by readers of the data to distinguish the two ports. This means that when both Ports A and B are in **Event word** mode, the high bit (bit 16) is not available for user input, since it is overwritten by the OmniPlex application. The use of channel 257 is for plx files and online clients. In pl2 files, multi-bit events from either port of the first DI card are recorded to channel 1 of the *Other events* source, but the "special high bit" convention still applies.

For the second DI card, the multi-bit channel numbering is more straightforward. Multi-bit events from Ports A and B always appear on two distinct channels, whether one or both ports are set to **Event word** mode. For plx files and online client data, channels 241 and 242 are used, while in pl2 files, channels 1 and 2 of the *Multi-DI strobed* source are used.

In a two-DI configuration, you can configure the four ports as **Individual events** or **Event word** in any combination; for example, to avoid the "special high bit" issues in a system where two ports are to be used for event word input, you might use ports A and B on the second card for event word data, and ports A and B on the first card for individual events data.

## Appendix J: Hardware Pinouts, Connections and LEDs

The following pinouts and connections are covered in this appendix:


- Digital Input (DI) Card in Basic OmniPlex Chassis, page A-59
- Digital Input (DINe) Card in the OmniPlex eChassis, page A-63
- Auxiliary Analog Input Card, page A-67
- AuxAI digital outputs, page A-69
- Other AuxAI outputs, page A-70
- Timing Control (TIM and TIMe) Board Front Panel Connections and LEDs, page A-71
- DigiAmp Connections and Pinouts, page A-76
- MiniDigi Connections and Pinouts, page A-79
- DHP Connections and Pinouts, page A-82


### Additional LED information

If you have the basic OmniPlex chassis:

- Information about LEDs in the DI and TIM cards is provided in the applicable sections listed at the top of this page.
- For interpretation of LEDs in the AMP LINK and DATA LINK cards, see Section 14.4, “Interpreting LEDs on AMP LINK and DATA LINK Cards” on page 458.
- For interpretation of LEDs in the COMPUTER LINK card, see Chapter 2, Startup (with DigiAmp Subsystem).

If you have the OmniPlex eChassis:

- Information about LEDs in the DINe and TIMe cards is provided in the applicable sections listed at the top of this page.
- For interpretation of LEDs in the PDLe card, see Section 14.5, “Interpreting LEDs on the PDLe Card (eChassis Only)” on page 459.
- For interpretation of LEDs in the HLKe card, see Chapter 3, Startup (with DHP Subsystem).

### Digital Input (DI) Card in Basic OmniPlex Chassis

This section is applicable to the DI card, which is installed in the basic OmniPlex chassis, and operates with all OmniPlex software releases. If you have the DINe card (applicable to the OmniPlex eChassis), go to Digital Input (DINe) Card in the OmniPlex eChassis on page A-63.

The DI card has two input ports, A and B, where A is the lower port. The two ports are identical except that the RSTART (level-triggered recording) input is only present on Port A (pin 24). On both ports, pin 22 is the data-ready pin ("strobe input"), used only when the port is configured as **Event word**. See Section 10.1, "Digital Input Card Configuration Types" on page 320 for information on how to configure each port to operate in either **Individual events** mode (16 individual data bits) or **Event word** mode (15 or 16 bit word input).

**CAUTION**

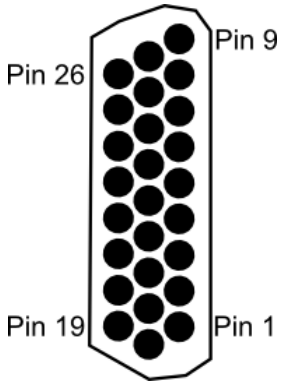
**Do not apply voltage <0V or >+5.5V to the pins**

Input voltages to the pins on the Digital Input card must always be between 0V and +5.5V. Voltages outside this range can damage the card. Never apply negative voltages to the pins.

**Note:** The DI card has internal protection circuitry, and is designed to accept voltages within the specified range (0V to +5.5V) whether the OmniPlex chassis is powered on or off. However, it is good practice to avoid driving voltages, such as TTL pulses, into unpowered devices.

**Note:** These voltage limits (0V and +5.5V) correspond to currents of approximately 0.33mA out and 0.22mA in, respectively.

### Pinout Information—DI Card




| Port A | | Port B | |
|---|---|---|---|
| **Pin #** | **Function** | **Pin #** | **Function** |
| 1 | Data 1 | 1 | Data 17 |
| 2 | Data 2 | 2 | Data 18 |
| 3 | Data 3 | 3 | Data 19 |
| 4 | Data 4 | 4 | Data 20 |
| 5 | Data 5 | 5 | Data 21 |
| 6 | Data 6 | 6 | Data 22 |
| 7 | Data 7 | 7 | Data 23 |
| 8 | Data 8 | 8 | Data 24 |
| 9 | Data 9 | 9 | Data 25 |
| 10 | Data 10 | 10 | Data 26 |
| 11 | Data 11 | 11 | Data 27 |
| 12 | Data 12 | 12 | Data 28 |
| 13 | Data 13 | 13 | Data 29 |
| 14 | Data 14 | 14 | Data 30 |
| 15 | Data 15 | 15 | Data 31 |
| 16 | Data 16 | 16 | Data 32 |
| 17 | Unused | 17 | Unused |
| 18 | Unused | 18 | Unused |
| 19 | Ground | 19 | Ground |
| 20 | +5V | 20 | +5V |
| 21 | Ground | 21 | Ground |
| 22 | Data ready (strobe) | 22 | Data ready (strobe) |
| 23 | Ground | 23 | Ground |
| 24 | RSTART | 24 | Unused |
| 25 | Ground | 25 | Ground |
| 26 | Unused | 26 | Unused |

#### Pin 20, +5V power output

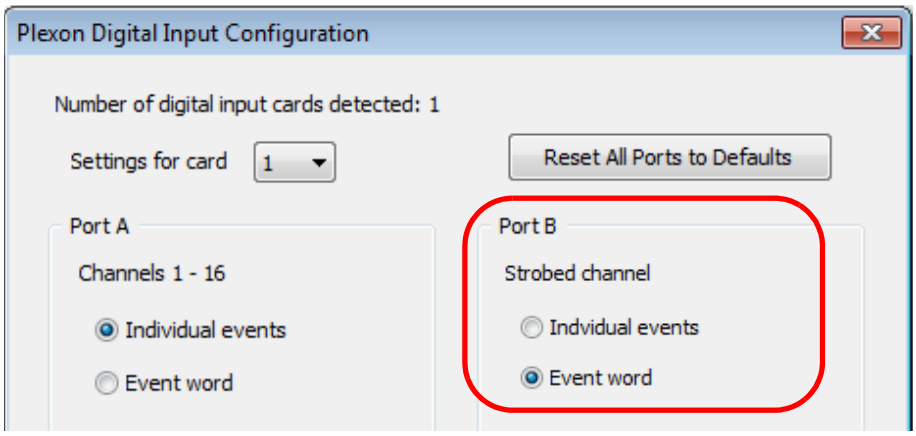
Pin 20 provides electrical power at +5V that you can use to run other equipment in your experiment. The upper limit on the current supported on this pin is 0.5mA at 0 to +5V.

**IMPORTANT:** When using pin 20 for power, be sure to connect the ground of your external device to one of the grounding pins on the same DI port, that is, Pin 19, 21, 23 or 25.

#### Pin 22—Data ready (strobe)

If a port is configured as **Strobed channel** (**Event word**) as highlighted in the example below, the system is ready to accept an event word from an external device. When the strobe input on this pin goes active, the system captures the

data word on the data pins (pins 1-16). To see how to interpret the data on these pins, see Section 10.4, “Configuring the Port Event Mode ” on page 325.



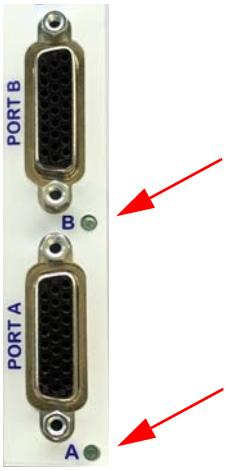
### LED Information—DI Card

The green LED next to Port A (or Port B), turns on to indicate that activity is being detected on the applicable port.

- When the DI card port is configured for **Individual events,** single events detected on pins 1 through 16 cause the LED to turn on for three seconds. Note that if events are coming in more frequently than one every three seconds, the LED effectively stays on because it starts a new three-second blink for every incoming event.

  In **Individual events** mode, strobe inputs are not recognized. Instead, the system interprets logic transitions on the input pins as individual events, which causes the LED to turn on. Verify that you have configured the port for **Event word** if you want the port to receive strobe inputs (event words).

- When the DI card port is configured for **Event word**, a strobe input causes the LED to turn on for three seconds. Single events (individual bit inputs on pins 1 through 16) have no effect on the LED.



### Digital Input (DINe) Card in the OmniPlex eChassis

This section is applicable to the DINe card, which is installed in the OmniPlex eChassis, and operates only with Release 18 and later of the OmniPlex software. If you have the DI card and the basic OmniPlex chassis, go to Digital Input (DI) Card in Basic OmniPlex Chassis on page A-59.

**Note:** You must have Release 18 or later to use the eChassis and DINe card. You can use the basic OmniPlex chassis and DI card with all OmniPlex releases.

The DINe card has two input ports, A and B, where A is the lower port. The two ports are identical except that the RSTART (level-triggered recording) input is only present on Port A (pin 24). On both ports, pin 22 is the data-ready pin ("strobe input"), used only when the port is configured as **Event word**. See Section 10.1, "Digital Input Card Configuration Types" on page 320 for information on how to configure each port to operate in either **Individual events** mode (16 individual data bits) or **Event word** mode (15 or 16 bit word input).

**CAUTION**

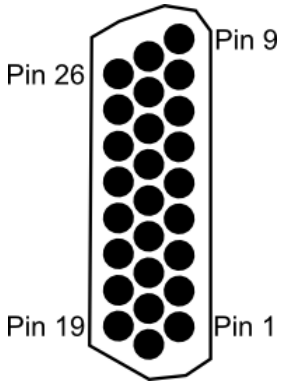
**Do not apply voltage <0V or >+5.5V to the pins**

Input voltages to the pins on the Digital Input card must always be between 0V and +5.5V. Voltages outside this range can damage the card. Never apply negative voltages to the pins.

**Note:** The DINe card has internal protection circuitry, and is designed to accept voltages within the specified range (0V to +5.5V) whether the OmniPlex chassis is powered on or off. However, it is good practice to avoid driving voltages, such as TTL pulses, into unpowered devices.

**Note:** These voltage limits (0V and +5.5V) correspond to currents of approximately 0.33mA out and 0.22mA in, respectively.

### Pinout Information—DINe Card




| | Port A | | Port B |
|---|---|---|---|
| **Pin #** | **Function** | **Pin #** | **Function** |
| 1 | Data 1 | 1 | Data 17 |
| 2 | Data 2 | 2 | Data 18 |
| 3 | Data 3 | 3 | Data 19 |
| 4 | Data 4 | 4 | Data 20 |
| 5 | Data 5 | 5 | Data 21 |
| 6 | Data 6 | 6 | Data 22 |
| 7 | Data 7 | 7 | Data 23 |
| 8 | Data 8 | 8 | Data 24 |
| 9 | Data 9 | 9 | Data 25 |
| 10 | Data 10 | 10 | Data 26 |
| 11 | Data 11 | 11 | Data 27 |
| 12 | Data 12 | 12 | Data 28 |
| 13 | Data 13 | 13 | Data 29 |
| 14 | Data 14 | 14 | Data 30 |
| 15 | Data 15 | 15 | Data 31 |
| 16 | Data 16 | 16 | Data 32 |
| 17 | Port A busy | 17 | Port B busy |
| 18 | Unused | 18 | Unused |
| 19 | Ground | 19 | Ground |
| 20 | +5V / +3.3V | 20 | +5V / +3.3V |
| 21 | Ground | 21 | Ground |
| 22 | Data ready (strobe) | 22 | Data ready (strobe) |
| 23 | Ground | 23 | Ground |
| 24 | RSTART | 24 | Unused |
| 25 | Ground | 25 | Ground |
| 26 | Unused | 26 | Unused |

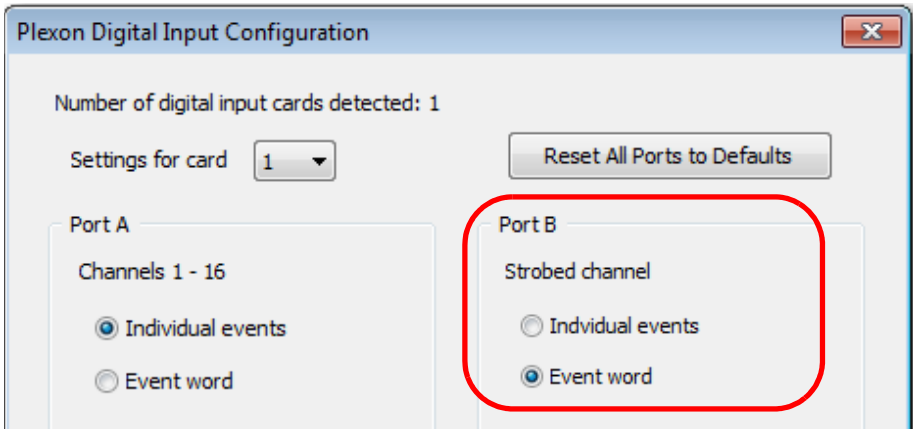
#### Pin 17—Port A (B) busy

The purpose of this pin is similar to that of the “Input busy” pin in the Plexon MAP box. It is used when a port is configured for **Strobed channel** (**Event word**). The voltage on pin 17 goes high or goes low (depending on the configured setting) when the internal **Port busy** pulse is being generated by the OmniPlex System. The **Port busy** signal allows an external device to be aware when the OmniPlex System is ready to accept the next event word (strobe). The settings for this signal are described in Section 10.6, “Configuring the Port Busy Controls (DINe Card Only)” on page 331.

#### Pin 22—Data ready (strobe)

If a port is configured as **Strobed channel** (**Event word**) as highlighted in the example below, the system is ready to accept an event word from an external

device. When the strobe input on this pin goes active, the system captures the data word on the data pins (pins 1-16). To see how to interpret the data on these pins, see Section 10.4, “Configuring the Port Event Mode ” on page 325.




### Pin 20—Power output option, +5V / +3.3V

Pin 20 provides electrical power that you can use to run other equipment in your experiment. The voltage level on this pin is set to +5V in the factory. If you prefer to have +3.3V instead, you can set a jumper on the DINe card as described below.

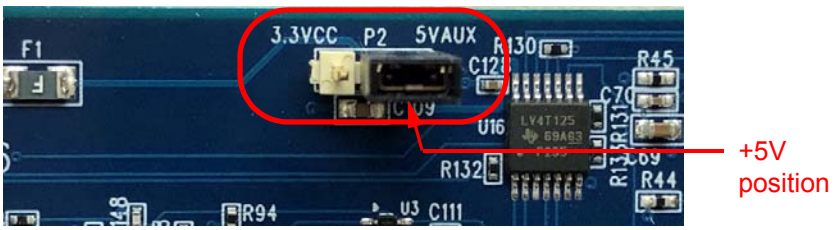
The upper limit on the current supported on this pin is 0.5mA at 0 to +5V (or 0 to +3.3V if the jumper is in the +3.3V position).

**IMPORTANT:** When using pin 20 for power, be sure to connect the ground of your external device to one of the grounding pins on the same DI port, that is, Pin 19, 21, 23 or 25.

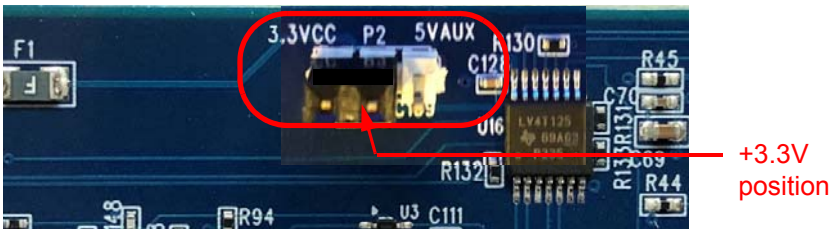
Use this procedure to move the jumper to the +3.3V position:

1. Stop data acquisition (see Section 3.10, “Stopping Data Acquisition” on page 103).
2. Save any open files as needed, then close PlexControl and Server.
3. Power down the host computer and then the eChassis.
4. Pull out the DINe card.

   Notice that there are three pins on the receptacle. In the +5V position (the factory default), the jumper is positioned over the middle and right pins.



5 To move the jumper to the +3.3V position, pull straight up on the jumper until it comes off the receptacle. Then place the jumper over the left and middle pins. (Push the jumper down securely onto the pins.)




6 Reinstall the DINe card.

7 Power up the eChassis and then the host computer.

8 Open Server and then PlexControl.

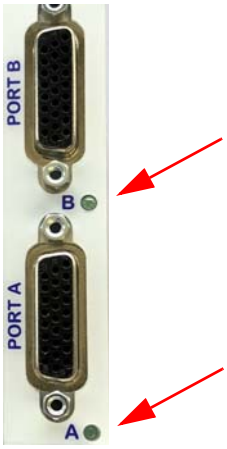
### LED Information—DINe Card

The green LED next to Port A (or Port B), flashes to indicate that activity is being detected on the applicable port.

- When the DINe card port is configured for **Individual events,** single events detected on pins 1 through 16 cause the LED to flash.

  In **Individual events** mode, strobe inputs are not recognized. Instead, the system interprets logic transitions on the input pins as individual events, which causes the LED to flash. Verify that you have configured the port for **Event word** if you want the port to receive strobe inputs (event words).

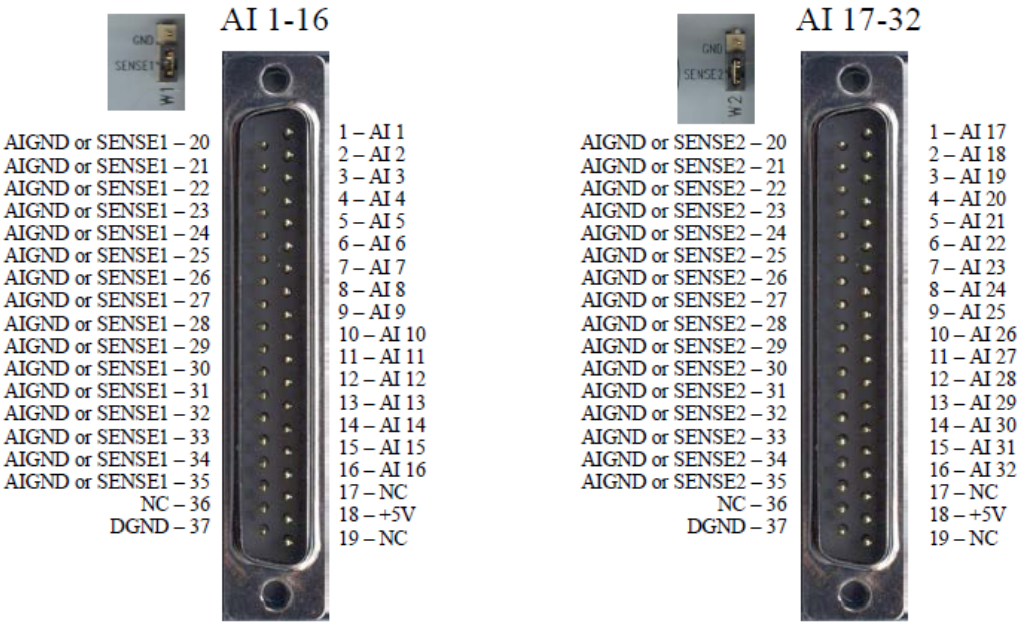
- When the DINe card port is configured for **Event word**, a strobe input causes the LED to flash. Single events (individual bit inputs on pins 1 through 16) have no effect on the LED.



## Auxiliary Analog Input Card

See Chapter 12, Auxiliary Analog Input for general information on using the AuxAI card with the OmniPlex System software.

The AuxAI card provides 32 analog inputs which are by default sampled at a rate of 1 kHz per channel. The 32 analog inputs (AI 1-32) are divided into two 16 channel groups (AI 1-16 and AI 17-32). Access to these inputs is provided through two 37 pin D-sub connectors as shown below. Alternately, AI 1-8 can be accessed through eight BNC connectors located in the center of the panel. Care should be taken to only connect AI 1-8 in one location, either on the 37 pin connector or on the BNC connector, but not both.




The remainder of this section contains information on grounding, input pull-downs, and other topics which can be skipped on a first reading.

For each channel group there is a common reference input (AISENSE1 for AI 1-16 or AISENSE2 for AI 17-32) that can also be accessed through the 37 pin D-sub connectors. The acquired signal is the difference between the channel input (AI n) and the AISENSE input for that group of channels. This differential recording helps reject common mode noise that can be picked up in the cabling between the signal source and the analog inputs and also helps reject artifacts due to fluctuations between the "ground" level measured at the signal source and the "ground" level measured at the analog input card (AIGND). Note however that the default configuration requires that the device providing the signals be "ground referenced." This means that both the AI n and AISENSE input signals coming from the device must remain within a limited range of AIGND (±11V absolute max). This will typically be the case when the signal source is an instrument that is powered from a wall outlet using a three prong plug, but may not be the case for instruments with special isolated outputs and will not be the

case for instruments that are battery powered and not connected to ground in any way.

In order to record signals from such “floating” instruments, it is necessary to provide them with a ground reference to the Auxiliary I/O panel. To accomplish this each D-sub connector has a jumper block associated with it that allows you to select one of two configurations SENSE (default) or GND. When the jumper is put in the GND position, the AISENSE input and connector pins 20-35 for that group of channels become connected to AIGND. One of these AIGND pins can then be connected to the “floating” instrument to ground it.

Removable 3.3 kΩ pull down resistor packs were added to the analog inputs in Revision A of the AuxAI breakout panel. In the absence of these (pre- Revision A) when a signal was connected to one analog input and adjacent analog inputs were left floating, the applied signal typically also appeared on the floating channels. This is a normal consequence of the multiplexed analog-to-digital converter in the Auxiliary I/O card. The multiplexer has a small amount of capacitance and during the sampling process this capacitance rapidly charges to the level of the applied signal. When the multiplexer switches to another channel it takes a tiny amount of “settling” time for the capacitance of the multiplexer to adjust to the signal level of the new channel. However, if the new channel is not connected to anything, there is no signal to change the value of the multiplexer capacitance and no path for the capacitance to discharge. Therefore the value sampled for the un-connected channel is approximately the same as it was for the previously sampled channel. The added pull down resistors provide a path to discharge the multiplexer capacitance and cause a value near zero to be read for un-connected channel instead of a value mirroring the previously sampled channel.

There is a consequence of having the pull down resistors in place. The pull down resistor forms a voltage divider with the output impedance of the signal source. For example, a typical function generator has an output impedance of 50 ohms, meaning the signal seen by the Auxiliary I/O card will be

(3.3 kohms) / (3.3 kohms + 50 ohms)

or 98.5% of the output value of the function generator. The signal will appear to be attenuated 1.5%. Sources with higher output impedance will experience greater levels of attenuation. If this attenuation is a concern, the yellow pull down resistor packs installed in the sockets next to the D-sub connectors may be removed. Note that a signal will then appear on un-connected channels as described above.

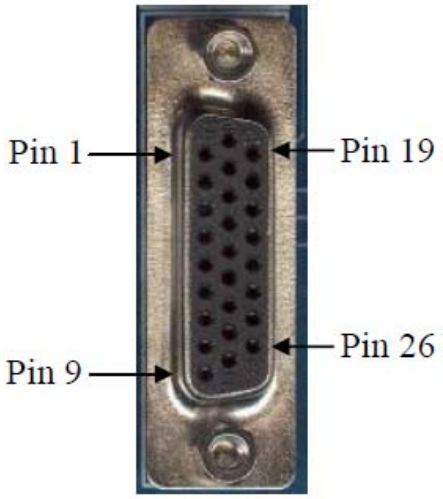
Both analog input D-sub connectors also provide access to power (+5V and DGND) that can be used to power external devices. The total combined current drawn from the +5V terminals should be kept under 1 A.
Revision A Auxiliary I/O panels incorporate resettable fuses that will cut off the power if too much current is drawn from the +5V terminals. The most likely scenario of this happening would be if the power and ground pins were

accidentally shorted. The resettable fuses (also called positive temperature coefficient devices or PTCs) will reset after the overload condition has been removed and the device has cooled down.

## AuxAI digital outputs

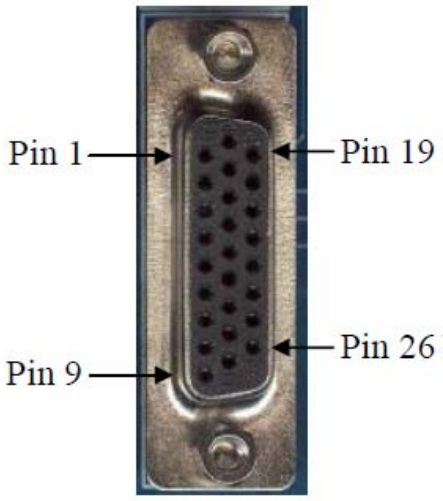
In addition to the 32 analog inputs, the AuxAI card also provides up to 32 digital outputs, depending on the model of AuxAI card installed. Two 26 pin HDDsub connectors provide access to the 32 digital outputs DO 32. These digital outputs can be controlled using the PlexDO utility included with the C/C++ and MATLAB® client development kits available from the Plexon website. In the PlexDO utility, DO 32 are referred to as bits 32.

### Connector labeled "DIGITAL OUT 1–16":




| Pin # | Function |
|---|---|
| 1 | DO1 |
| 2 | DO2 |
| 3 | DO3 |
| 4 | DO4 |
| 5 | DO5 |
| 6 | DO6 |
| 7 | DO7 |
| 8 | DO8 |
| 9 | DO9 |
| 10 | DO10 |
| 11 | DO11 |
| 12 | DO12 |
| 13 | DO13 |
| 14 | DO14 |
| 15 | DO15 |
| 16 | DO16 |
| 17 | NC |
| 18 | NC |
| 19 | DGround |
| 20 | NC |
| 21 | DGround |
| 22 | NC |
| 23 | DGround |
| 24 | NC |
| 25 | DGround |
| 26 | NC |

### Connector labeled “DIGITAL OUT 17–32”:




| Pin # | Function |
|---|---|
| 1 | DO17 |
| 2 | DO18 |
| 3 | DO19 |
| 4 | DO20 |
| 5 | DO21 |
| 6 | DO22 |
| 7 | DO23 |
| 8 | DO24 |
| 9 | DO25 |
| 10 | DO26 |
| 11 | DO27 |
| 12 | DO28 |
| 13 | DO29 |
| 14 | DO30 |
| 15 | DO31 |
| 16 | DO32 |
| 17 | NC |
| 18 | NC |
| 19 | DGround |
| 20 | NC |
| 21 | DGround |
| 22 | NC |
| 23 | DGround |
| 24 | NC |
| 25 | DGround |
| 26 | NC |

### Other AuxAI outputs

The four BNC connectors labeled AO 1, AO 2, Line 1 and Line 2 are not currently used by the OmniPlex System and are reserved for future use.

## Timing Control (TIM and TIMe) Board Front Panel Connections and LEDs

### BNC connectors

This information about the BNC connectors applies to both the TIM card in the basic chassis and the TIMe card in the eChassis.

TRIG OUT —Outputs a digital pulse when a spike is detected on the currently selected channel in the currently selected unit.

ANALOG OUT—Outputs an analog version of the spike filtered continuous signal (SPKC) for the currently selected channel in the currently selected unit. A typical use is monitoring the signal on an external oscilloscope.

**Note:** For audio monitoring, it is usually more convenient to use the audio output from the PC connected to the OmniPlex System, as described in Chapter 2, Startup (with DigiAmp Subsystem) and Chapter 3, Startup (with DHP Subsystem), as applicable.

### TIM card LEDs (left to right) in the basic OmniPlex chassis

This section explains how to interpret the LEDs on the front panel of the TIM card in the basic OmniPlex chassis. Note that there is no need to pay attention to these LEDs in normal operation, and this information is only included for informational and troubleshooting purposes.

**Note:** If you have an OmniPlex eChassis and a TIMe card, see TIMe card LEDs (left to right) in the OmniPlex eChassis on page A-73.

1. Power
2. Spike detected
3. System sync

The LEDs can be interpreted as described below for typical system states, where

+ ON (fully lit)
– OFF (not lit)
A Flashes every time a spike is detected on the currently selected channel in the currently selected unit
S Flashes once every 20 seconds to indicate a system timestamp check has occurred

Chassis turned on, but PC is off:

+ + +

Chassis and PC are both turned on;
Server may also be on, but data acquisition is not running:

+ - -

Data acquisition running:

+ A S

### TIMe card LEDs (left to right) in the OmniPlex eChassis

This section explains how to interpret the LEDs on the front panel of the TIMe card in the OmniPlex eChassis. Note that there is no need to pay attention to these LEDs in normal operation, and this information is only included for informational and troubleshooting purposes.

**Note:** If you have a basic OmniPlex chassis and a TIM card, see TIM card LEDs (left to right) in the basic OmniPlex chassis on page A-72.

**1** Power

**2** PCIe OK

**3** System sync

The LEDs can be interpreted as described below for typical system states, where

+ ON (fully lit)
– OFF (not lit)
S Blinks to indicate system sync

Chassis turned on, but PC is off:

+ - -

Chassis and PC are both turned on:
Server may also be on, but data acquisition is not running:

+ + -

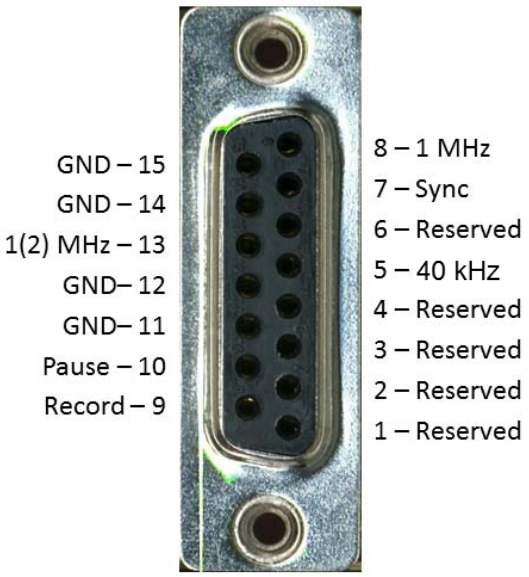
Data acquisition is running:

+ + S

### TIM I/O (D-sub) on TIM card and TIMe card

The TIM I/O multi-pin D-sub connector provides signals that are used to synchronize external devices (such as a CinePlex® video recording system) with the OmniPlex System. All signals are digital outputs.

**Note:** All outputs initially go to 5V when power to chassis is turned on.




| Pin | Signal |
|---|---|
| 5 | **40 kHz sampling clock**<br>The 40 kHz sampling clock is output only while data acquisition is running in the OmniPlex System. |
| 7 | **Sync pulse**<br>Goes high for ~1 µs when data acquisition is started in the OmniPlex System, then pulses high approximately every 20 seconds thereafter. When recording is started, the 20 second cycle is re-started such that a pulse occurs on this pin approximately 15 seconds into the recording and approximately every 20 seconds thereafter. |
| 8 | **1 MHz clock**<br>The line goes high when the chassis is turned on. The 1 MHz clock starts when the computer is turned on and runs continuously.<br>The clock signal is ~ 0 to 4 V. |
| 9 | **Record**<br>Goes high while recording to disk in PlexControl (even when recording is paused). |

| Pin | Signal |
|---|---|
| 10 | **Pause**<br>Goes high while recording to disk is paused in PlexControl. |
| 11, 12, 14, 15 | **GND**<br>All other pins are measured relative to ground. |
| 13 | **1 MHz clock (2 MHz on some systems)**<br>The line goes high when the chassis is turned on. The 1 MHz clock starts when the computer is turned on and runs continuously. The clock signal is ~ 0 to 4 V.<br>Originally, pin 13 output a 2 MHz clock that was used to synchronize a MAP system to the OmniPlex System. An issue was introduced in later firmware versions which results in this clock being output at 1 MHz rather than the correct 2 MHz. If in doubt, use an oscilloscope or frequency counter to verify the frequency. |

### DigiAmp Connections and Pinouts

This section describes the connections and pinouts on the front panel of the DigiAmp™ Amplifier.

Sig Com—Signal common is the local zero-voltage reference point of the DigiAmp unit.

Earth—Earth is a direct connection to Earth ground. In the United States, Earth ground connects to the third prong on the wall outlet, which is eventually tied to hard earth at the power service entrance to the building.

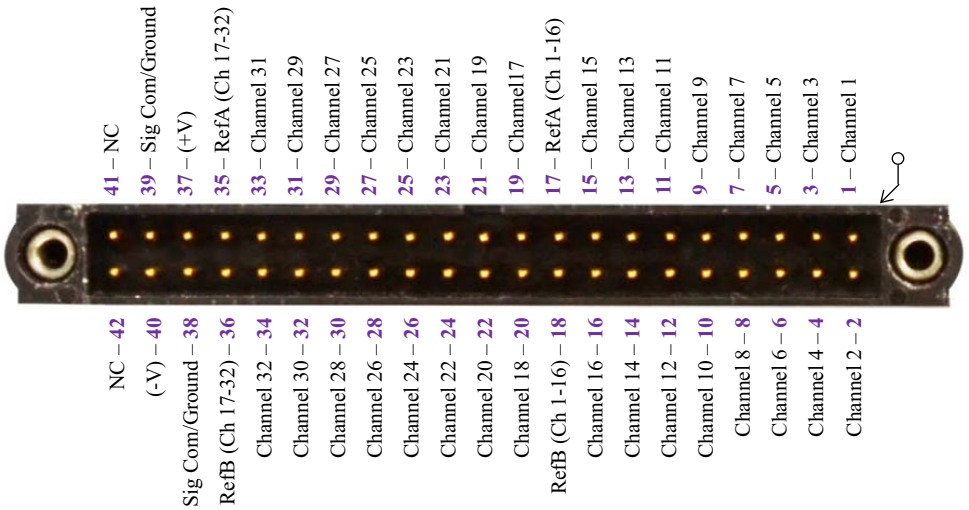
**Note:** Ambient noise in buildings and noise radiated by electronic equipment are very common, and they can interfere with the signals in your experiment. In some cases, to reduce noise problems, it may be helpful to connect the green ground wire (shown above and provided with each DigiAmp Amplifier) from either **Sig Com** or **Earth** to metal object(s) near the animal being studied, such as headposts or apparatus framing or plates. It is best to try connecting to **Sig Com** and observing the noise reduction effect, then connecting instead to **Earth** and observing the effect, then comparing the results. Use the connection that gives the best results (best noise reduction). In some cases, the best noise performance will be achieved without the green wire being connected at all.

Isolated/Grounded switch—The switch allows you to directly connect Sig Com to Earth ground or not. If Sig Com is not directly connected to Earth ground, the DigiAmp unit is isolated from Earth ground.

HST PWR—This switch turns the power supply to the headstages on or off.

The DigiAmp Amplifier can be supplied with up to eight 42-pin headstage connectors. Each connector has pins for 32 channels, four pins for reference signals (RefA and RefB), two pins for power (+V and –V) and two pins for DigiAmp signal common/ground (Sig Com/Ground). Two pins are reserved (not connected).

The diagram below shows the pinouts for Channels 1 to 32, which are on the first connector. Notice that there is one RefA pin for the first set of 16 channels (RefA Ch 1-16) and a different RefA pin for the second set of 16 channels (RefA Ch 17-32). Similarly, there is a RefB (Ch 1-16) and a RefB (Ch 17-32).




The functions of the pins are as follows:

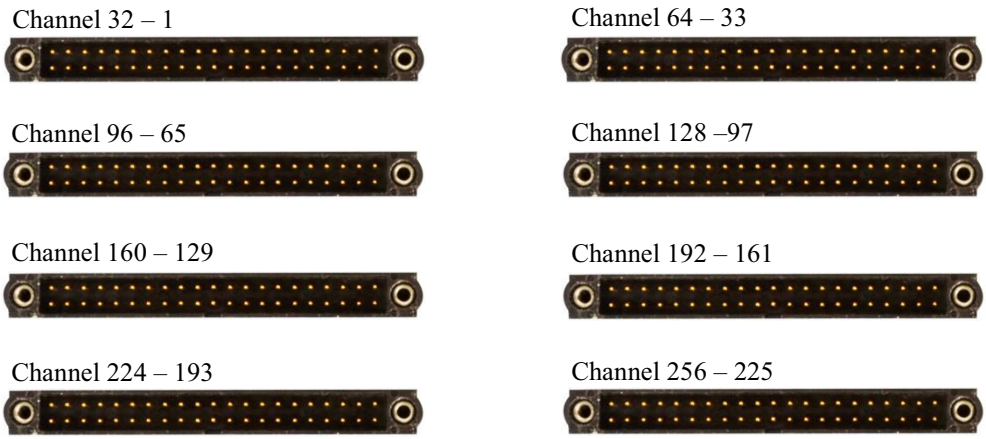
- RefA and RefB—Reference signal input provided by the headstage to the DigiAmp unit. You can select RefA or RefB through the **DigiAmp Device Settings** dialog box. (See Appendix C: DigiAmp Device Settings—Filtering, Referencing and Latency on page A-15). The resultant signal for a particular channel is determined by subtracting the RefA or RefB signal from the signal measured at the pin assigned to that channel. For example, the resultant signal for Channel 21 is the signal on pin 23 (Channel 21 is assigned to pin 23) minus the signal on pin 35 (if RefA is selected) or pin 36 (if RefB is selected).
- +V and –V—Power provided by the DigiAmp unit to headstages, +3.0V and –3.0V with respect to DigiAmp Sig Com/Ground.
- Sig Com/Ground—Signal common and ground. Pins labeled “Ground” on headstage cables and headstages should be connected to signal common.

Signal common in the DigiAmp Amplifier can be either isolated from Earth ground or connected to Earth ground. (See the information about the Isolated/ Grounded switch, above.)

- NC—No connection (not used).

There are two 42-pin connectors placed side by side on a board, and each board supports 64 channels. The DigiAmp can have up to four boards for a total of 256 channels.

The pinouts for all 256 channels on the eight 42-pin connectors follow the pattern shown in this diagram.



## MiniDigi Connections and Pinouts

This section describes the connections and pinouts on the front panel of the MiniDigi™ Amplifier.

Sig Com—Signal common is the local zero-voltage reference point of the MiniDigi unit.

Earth—Earth is a direct connection to Earth ground. In the United States, Earth ground connects to the third prong on the wall outlet, which is eventually tied to hard earth at the power service entrance to the building.

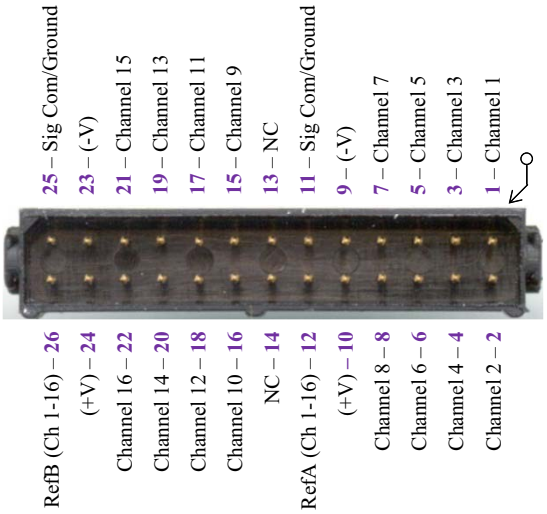
**Note:** Ambient noise in buildings and noise radiated by electronic equipment are very common, and they can interfere with the signals in your experiment. In some cases, to reduce noise problems, it may be helpful to connect the green ground wire (shown above and provided with each MiniDigi Amplifier) from either **Sig Com** or **Earth** to metal object(s) near the animal being studied, such as headposts or apparatus framing or plates. It is best to try connecting to **Sig Com** and observing the noise reduction effect, then connecting instead to **Earth** and observing the effect, then comparing the results. Use the connection that gives the best results (best noise reduction). In some cases, the best noise performance will be achieved without the green wire being connected at all.

Isolated/Grounded switch—The switch allows you to directly connect Sig Com to Earth ground or not. If Sig Com is not directly connected to Earth ground, the MiniDigi unit is isolated from Earth ground.

HST PWR—This switch turns the power supply to the headstages on or off.

The MiniDigi Amplifier can be supplied with up to four 26-pin headstage connectors. Each connector has pins for 16 channels, two pins for reference signals (RefA and RefB), four pins for power (+V and –V) and two pins for MiniDigi signal common/ground (Sig Com/Ground). Two pins are reserved (not connected).

The diagram below shows the pinouts for Channels 1 to 16, which are on the first connector.




The functions of the pins are as follows:

- RefA and RefB—Reference signal input provided by the headstage to the MiniDigi unit. You can select RefA or RefB through the **DigiAmp Device Settings** dialog box. (See Appendix C: DigiAmp Device Settings—Filtering, Referencing and Latency on page A-15). The resultant signal for a particular channel is determined by subtracting the RefA or RefB signal from the signal measured at the pin assigned to that channel. For example, the resultant signal for Channel 14 is the signal on pin 20 (Channel 14 is assigned to pin 20) minus the signal on pin 12 (if RefA is selected) or pin 26 (if RefB is selected).
- +V and –V—Power provided by the MiniDigi unit to headstages, +3.0V and –3.0V with respect to MiniDigi Sig Com/Ground.
- Sig Com/Ground—Signal common and ground. Pins labeled “Ground” on headstage cables and headstages should be connected to signal common. Signal common in the MiniDigi Amplifier can be either isolated from Earth

ground or connected to Earth ground. (See the information about the Isolated/ Grounded switch, above.)

- NC—No connection (not used).

There is one 26-pin connector on a board, and each board supports 16 channels. The MiniDigi unit can have up to four boards for a total of 64 channels.

The pinouts for all 64 channels on the four 26-pin connectors follow the pattern shown in this diagram.

Channel 16 – 1

Channel 32 – 17

Channel 48 – 33

Channel 64 – 49

### DHP Connections and Pinouts

This section describes the connections and pinouts for the Digital Headstage Processor (DHP) unit. The front panel is shown in the image below.

Sig Com—Signal common is the local zero-voltage reference point of the DHP unit.

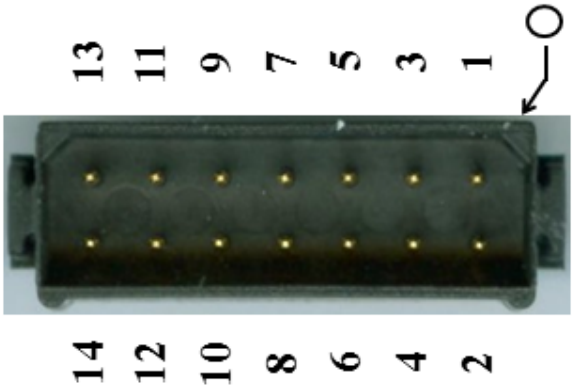
Earth—Earth is a direct connection to Earth ground. In the United States, Earth ground connects to the third prong on the wall outlet, which is eventually tied to hard earth at the power service entrance to the building.

**Note:** Ambient noise in buildings and noise radiated by electronic equipment are very common, and they can interfere with the signals in your experiment. In some cases, to reduce noise problems, it may be helpful to connect the green ground wire (shown above and provided with each DHP unit) from either **Sig Com** or **Earth** to metal object(s) near the animal being studied, such as headposts or apparatus framing or plates. It is best to try connecting to **Sig Com** and observing the noise reduction effect, then connecting instead to **Earth** and observing the effect, then comparing the results. Use the connection that gives the best results (best noise reduction). In some cases, the best noise performance will be achieved without the green wire being connected at all.

Isolated/Grounded switch—The switch allows you to directly connect Sig Com to Earth ground or not. If Sig Com is not directly connected to Earth ground, the DHP unit is isolated from Earth ground.

PWR—The DHP unit is powered through the blue cable, which connects the DHP to the DATA LINK card in the OmniPlex chassis. The DHP is powered on, and the PWR (power) light on the DHP unit is lit, when the OmniPlex Server application is running and a DHP topology is loaded. See also, Section 3.1, “Connections and Power-up” on page 62.

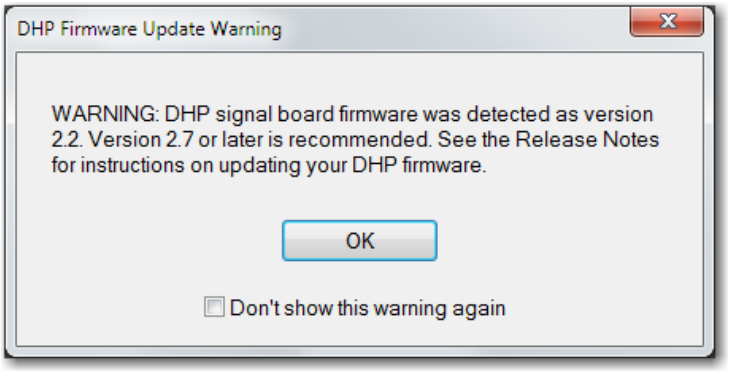
The diagram below shows the pinouts for the individual DHP connectors. Connections are made to Plexon 8, 16, 32 and 64 channel digital headstages through the appropriate Plexon digital headstage cables.




The DHP unit can be supplied with up to four signal cards, each of which contains four DHP connectors. Each signal card can accommodate up to 128 neural signal channels, so a full complement of four signal cards can handle up to 512 channels with appropriate configuration of the OmniPlex software.

## Appendix K: Firmware Upgrade for DHP Unit

If you start OmniPlex Server on a system whose DHP is not running the latest firmware version, you will see a warning similar to the following:




The warning will be displayed each time you start Server or load a new topology. Even if you select **Don't show this warning again**, a message will still be displayed in Server's message log window until you update your DHP firmware to the latest version. This Appendix explains why this firmware upgrade is important and where to find the procedure for the upgrade.

### Firmware upgrade for digital headstage communications integrity protection

Digital headstages have several advantages over analog headstages. One of the most important is that whereas analog headstage cables can pick up varying amounts of electrical noise from the surrounding environment, digital headstage cables are far more robust to interference, and in normal situations the digitized signal from the headstage is received without error at the Digital Headstage Processor (DHP). However, in rare cases, a strong burst of ambient noise can be so severe that it momentarily disrupts the digital communication link between the DHP and the headstage. For example, a large electrostatic discharge in a very dry room, or a switching transient caused by a motor or heater turning on or off, could in some cases cause such a disruption. A more common case would be electrical transients from headstage commutator brushes. Without the protection described in this section (below), a brief disruption of this type could lead to groups of channels becoming "stuck" in an incorrect state until data acquisition is stopped and restarted.

The OmniPlex System (Version 1.15 and later) includes updated firmware for the DHP which protects against disruptions by continuously refreshing the state of the headstages, so that any disruption is recovered from as soon as the interference ceases. The recovery time is typically on the order of 1 to 2 seconds, but can be longer if the headstage highpass filters are set to a very low cutoff frequency.

In order to update your system to support the enhanced protection functionality, you must, in addition to updating the OmniPlex software, update the firmware in

your DHP. To do this, first update the OmniPlex software using the installer in the usual way, then use the firmware upgrade procedure, below.

You are not required to update the DHP firmware to use OmniPlex software, but you must upgrade to Version 1.15 or later to use the upgraded firmware and enable the enhanced protection.

### Firmware upgrade for DHP and DIN/DINe card

Recommended firmware updates for both the DHP and the DIN (eChassis digital input card, also known as DINe) are included with Release 19 and above. The DHP firmware update (DADH 2.9) adds support for 128 channel headstages, while the DIN update (DIN 2.2) fixes a problem with the low-true logic option in Individual Events mode. Note that the DIN update is only needed by and applicable to the new eChassis systems; look for “DINe” on the front panel of the digital input card.

### Firmware upgrade procedure

For instructions on updating the DHP and/or DIN firmware, please refer to the following documentation:

C:\Program Files (x86)\Plexon Inc\Common Files\ffu\FFU_instructions.pdf

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# OmniPlex®

Neural Data Acquisition System

## User Guide

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