



Neurotechnology Research Systems

User Guide

Version 3.0 April 2009

Data Analysis Software **Offline Sorter**



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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.

Data Analysis Software

Offline Sorter

User Guide

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Chapter 1

Getting Started with Offline Sorter

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1.1 Installation

Before Offline Sorter can be used, the Offline Sorter program files, the Sentinel System Drivers, and the Sentinel parallel port or USB hardware key must be installed. The Plexon hardware key(s) are marked with the Plexon logo and they are included with the shipment of Offline Sorter.

1.1.1 Installing Offline Sorter

The Offline Sorter setup USB memory stick comes with two versions of Offline Sorter. OfflineSorter.exe is for 32-bit machines, and OfflineSorter64.exe is for 64-bit machines. Both versions will be installed on the computer, but the desktop icon initially defaults to the OfflineSorter.exe 32-bit version. The software may also be downloaded from the Plexon website.

Running Offline Sorter Setup

Note: Before installing Offline Sorter, exit all currently running applications.

- 1 Place the Offline Sorter setup USB stick in a USB slot on the computer.
- 2 Navigate to the `OFSt3Setup.exe` file on the CD and double-click the file.
The Offline Sorter Version 3 Setup screen appears.
- 3 Follow the prompts on the setup dialogs and complete the installation.
At the end of the installation process, the Sentinel System Driver Setup starts automatically.
- 4 Follow the Sentinel Driver Setup prompts and complete the installation of the Sentinel Drivers.

By default, the Setup program places the Offline Sorter program and the help files in `C:\Program Files\Plexon Inc\OfflineSorter`.

1.1.2 Installing Hardware Keys

USB hardware keys must be installed on the computer running Offline Sorter.

To install a USB key

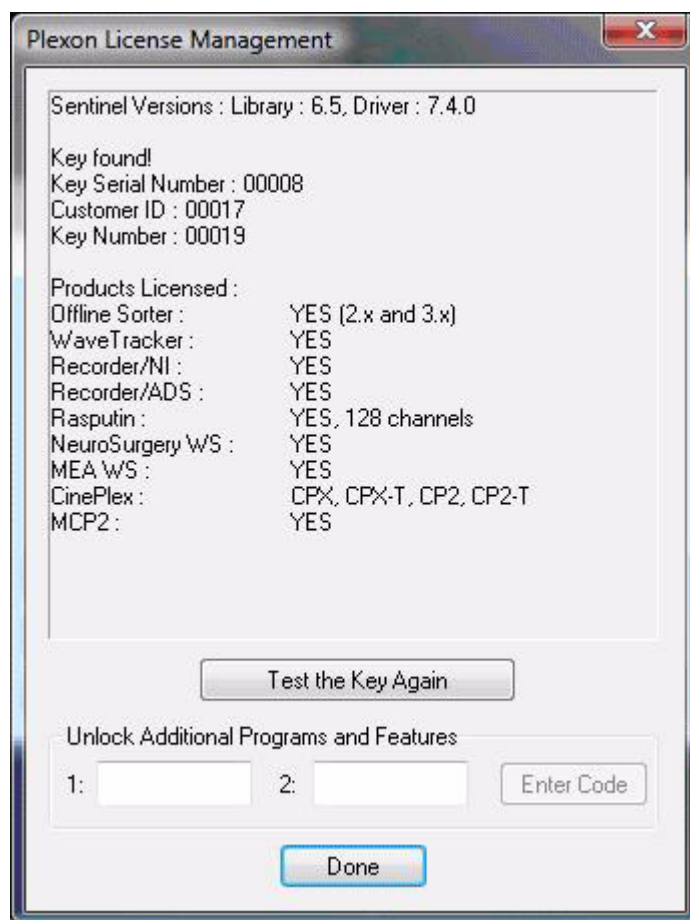
- Locate an available USB port on the computer and insert the USB key in the USB port.

1.2 Licensing

After the hardware key is installed, it requires no additional attention. However, Offline Sorter contains facilities for examining the licensing status of Offline Sorter and other Plexon products, and for performing remote upgrades to unlock additional features, or product licenses. Use the following procedure to check Plexon licenses.

Opening the License Management Window

- 1 From the main menu, select **Help | About Offline Sorter**, then press the **Licensing...** button to bring up the **Plexon License Management** window.



Note: In the main text area, the window contains a summary of the licensing status of the hardware key or keys. If Offline Sorter cannot read a hardware key, an explanation appears in the window. If Offline Sorter can read a hardware key, it interrogates it and displays the information from it. The *Key Serial Number*, *Customer ID*, and *Key Number* identify a particular key. This information must be provided to Plexon to unlock additional Plexon products. The *Products Licensed* section tells which Plexon products are currently unlocked by the hardware key.

Note: Offline Sorter 3.x is licensed separately from Offline Sorter 2.x. Contact Plexon for an upgrade from Offline Sorter 2.x to Offline Sorter 3.x, if needed.

- 2 Click **Test the Key Again** to re-interrogate the key and re-display the information. The **Unlock Additional Program and Features** section applies to remote upgrades. If a remote upgrade applies, Plexon provides instructions on how to use this feature.

Note: Multiple Plexon hardware keys can be installed on the computer. Offline Sorter can find and use a license on any of the keys attached to the computer. If

Plexon software detects multiple Plexon keys on the computer, additional **Next Key>>** and **<<Previous Key** buttons appear in the license window. These buttons can be used to step through the keys to examine each key individually.

1.3 Off-line Sorter Technical Support and Updates

1.3.1 Offline Sorter Technical Support

The email address for help with Offline Sorter is at support@plexoninc.com.

1.3.2 Offline Sorter Updates

The latest version of Offline Sorter may be downloaded from the Plexon website:

<http://www.plexoninc.com/support/softwaredownloads.html>

OFSv3Setup.exe is the standard Offline Sorter setup program. To install the latest version of Offline Sorter, download the program to the PC and run it from the hard drive.

1.3.3 Web Updates

If a previous version of Offline Sorter has been installed, use the **Help | Web Update** menu item to automatically check for updates at the Plexon web site, and to download and install them.

1.4 Demo Mode

If a valid Plexon hardware key has not been connected to the computer, Offline Sorter runs in **Demo Mode**. Offline Sorter opens a startup message box stating that it is running in Demo mode.

Plexon provides all Offline Sorter features in Demo mode, except the file saving, batch processing, and Export functionality.

Chapter 2

Overview

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2.1 Offline Sorter Uses

Offline Sorter can be used to:

- Inspect the data. Offline Sorter can display any action potential waveforms (spikes) in a variety of displays, including displays that show how the waveforms changed as a function of time during the experiment. The spike data can be shown alongside simultaneously-collected continuous and event data.
- Extract and sort action potential waveforms (spikes) from continuously recorded data, such as the data generated by Plexon programs like Recorder (DDT file) or other data acquisition systems such as:

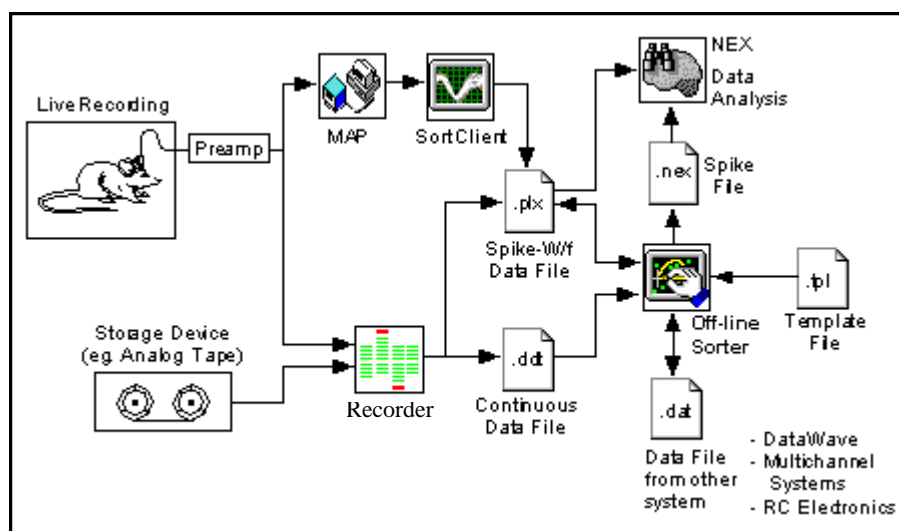
- Panasonic MED
- Data Translation

Offline Sorter detects spike waveforms within a continuous file using a threshold-crossing method. Thresholding for waveform detection can be applied to the:

- Raw voltage signal amplitude
- Energy of the signal
- Non-linear energy of the signal
- Re-sort action potential waveforms from previously sorted spike/waveform data generated either by the Plexon Multichannel Acquisition Processor (MAP) data acquisition and Sort Client system (PLX file), or some other data acquisition system from the following list:
 - CyberKinetics
 - CED Spike 2
 - DataWave
 - MultiChannel Systems
 - Neuralynx

The result of the sorting process is a set of timestamps that correspond to the spike times of the sorted units. Offline Sorter can save these spike trains in the form of a NEX datafile. If Offline Sorter is used to open a Spike/Waveform data file (e.g. a Plexon PLX file, or a data file from the DataWave or CyberKinetics systems), the resorted waveforms and spike times can be saved in a file of the same format, or in a new PLX file. The resulting spike files (PLX, NEX, etc.) can

then be analyzed with NeuroExplorer (NEX), MATLAB, or etc. A diagram of the typical analysis pathways and files related to the Offline Sorter follows.



2.2 Offline Sorter Inputs

Offline Sorter can **Open** or **Import** data files recorded from Plexon data acquisition hardware (PLX or DDT files), and files from various other data-acquisition hardware vendors. In general, these data files contain at least three types of data:

- **Continuously-recorded data:** With this type of data, an analog signal is digitized at a certain rate and every digitized sample is stored in the data file. Examples of this type of file are Plexon DDT files and “continuous” recording channels within a Plexon PLX file.
- **Spike Waveform data:** It is generally desirable to record the waveforms resulting from true neural firings at higher frequencies (e.g. 40 kHz). However, storing the digitized samples between the neural firings can lead to large data files. Therefore, neural data acquisition systems generally employ a thresholding or some other “triggering” mechanism to record only the digitized data in a narrow time window around what is likely to be a neural firing. These waveform segments are generally called “spikes”.
- **Event data:** Many data acquisition systems have the ability to record timestamped markers in the data stream when some external event happens. While these external event markers are somewhat tangential to Offline Sorter’s main purpose of sorting spikes, Offline Sorter can display them and the event markers can be used to select time periods of interest in the data.

Note: NEX file have event types that are time intervals. Offline Sorter does not recognize time intervals and will not save them if a new file is saved.

Offline Sorter can also read in batch command files to direct its processing and files of sorting parameters that can be applied to data files. For more information see [“5.19 Batch File Processing” on page 225](#).

2.3 Sorting Techniques

Basic to multi-neuron study is the ability to resolve the spike waveforms associated with the multiple individual neurons, which often are present on single or multiple electrodes. For reviews of spike sorting techniques, see Wheeler and Heetderks, 1982, Schmidt, 1984, Lewicki, 1998, Wheeler, 1999.

For many data acquisition systems, spike detection is done on-line, and Offline Sorter can read in and use the already-detected spike waveforms. For continuously-recorded data files, Offline Sorter can perform spike detection by using a threshold-crossing algorithm.

When spike waveforms are present, Offline Sorter helps to separate the waveforms collected from single or multiple electrodes into distinct waveform groups or classes that are associated with individual neurons. Each class of waveforms is called a unit or a cluster.

Offline Sorter currently offers the following spike-sorting algorithms, grouped into categories:

- Manual Methods:
 - Waveform Crossing
 - Contours
- Semi-Automatic Methods:
 - Template
 - Boxes
 - K-Means
 - Standard E-M (Expectation Maximization)
- Automatic Methods:
 - Valley-Seeking
 - T-Distribution E-M
 - Scanning methods

With manual spike sorting methods allow full control of the sorting process, and is possible to indicate manually which waveforms should be assigned to each unit. With the Waveform Crossing method, the units are manually selected by using the actual waveforms. Crossing a bundle of waveforms with the mouse pointer can specify the unit.

All sorting methods work in so-called “feature space”, except for the Waveform Crossing, Template, and Boxes sorting methods. Instead of using the entire raw waveform, sorting methods that work in feature space characterize the essence of the waveform by using a few calculated features. The feature space can be defined by selecting features from among several for each axis, which are

described under [“5.5 Features Available for Sorting” on page 191](#). The features available for assignment to the axes in feature space include:

- Projections onto principal components (PCA)
- Waveform heights at chosen times (“slices”)
- Peak height, valley height, peak-valley difference, widths
- Timestamp of the waveform
- For stereotrode and tetrode data, per-electrode and ratio-between-electrodes features

Thus the waveform can be represented by the point (x, y) in a 2D feature space, or (x, y, z) in a 3D feature space, where the x , y and z axes can be chosen from the available features.

In the 3D view of the feature space points, it is possible to navigate the eyepoint through the 3D space to get a better idea of what the data set looks like and to find a vantage point where clusters are best separated. After the desired perspective in 3D space is achieved, the sorting methods can be applied.

Using the Contours method, a cluster is specified by drawing an arbitrary shape in feature space (usually around a visible cluster). Offline Sorter assigns all waveforms inside the arbitrary shape to that cluster. When the Contours method is applied from the 3D view, it operates in a 2D projection of the 3D feature space that is a standard perspective projection based on the chosen viewpoint. That is, the 3D feature space is first transformed to a 2D feature space (“screen space”) by using a standard perspective projection, then the contour sorting algorithm runs in screen space. The other sorting algorithms can also be run in either 2D or 3D feature space.

The Semi-automatic sorting methods generally require specifying cluster centers (and therefore implicitly indicate the number of clusters), and then the algorithm assigns waveforms to the clusters.

The Template method requires picking an existing waveform to serve as the template for a unit. Offline Sorter then adds other waveforms to the unit based on their similarity (in the least-squares difference sense) to the template unit, with a user-defined tolerance.

The Boxes sorting method requires placing two boxes on the Waveforms view to define each unit. Each box thus specifies a region in time-voltage space. Any waveforms that intersect with both boxes for a unit are sorted into that unit. The boxes can be resized and moved to any position on the Waveforms view.

The K-Means method is a well-known iterative algorithm that assigns each waveform to one of the user-specified cluster centers, based on Euclidean distance in feature space. Then it recomputes the cluster centers, and it repeats the process until no more waveforms change units.

The Standard E-M (Std E-M) method is a variation of the Expectation Maximization algorithm. The Expectation Maximization algorithms in general fit a mixture of Gaussians to the point densities in feature space by varying the normal distribution parameters (means, covariances) to maximize a likelihood function. This algorithm uses the user-specified center points to start the search.

The Semi-automatic sorting methods are also capable of using an existing set of sorted units as a starting point, in what is referred to as “Continue” sorting. The algorithms can run using the centroids or template waveforms of existing clusters as the starting points, instead of manually specifying them.

The Automatic sorting methods are capable of automatically finding the optimal number of clusters, and of assigning waveforms to the clusters. Thus they require no user intervention to arrive at the initial clustering of the data, and they can operate on completely unsorted data.

The Valley-Seeking automatic method uses a valley-seeking algorithm that it applies to inter-point distances to automatically determine the number of clusters and the cluster memberships. The T-Distribution E-M (T-Dist E-M) method is another variation of the Expectation Maximization algorithm that fits a mixture of T-Distributions instead of Gaussians to the point densities in feature space. This algorithm is also capable of adjusting the number of clusters as it runs (by removing unfavorable clusters) to arrive at the optimum number of clusters.

The Scanning methods are a family of automatic sorting methods that attempt to find an optimal clustering by stepping a sorting parameter (a value that controls how the sorting is performed) through a range of values. One of the Sorting Quality Statistics described on [page 203](#) is chosen as the metric that defines what ‘optimal’ means. For example, Valley Seeking automatic sorting method has a sorting parameter called the Parzen Multiplier that affects how the clustering proceeds. The Valley Seek Scan sorting method will step the value of the Parzen Multiplier through a user-defined range of values and calculate the Sorting Quality Statistics for each step. The step that produces the best value of the chosen Sorting Quality Statistic is taken as the final sorting.

Offline Sorter provides many mechanisms for inspecting and manually adjusting the sorting results; it is possible to add or remove waveforms from clusters or invalidate them completely.

With Offline Sorter the waveforms can be resorted by using any of the clustering methods. After the units have been defined, the contours, templates and principal components can be saved to a TPL file and they can be used to sort waveforms in other data files.

For more information on the sorting algorithms, see [“5.6 Details of the Sorting Algorithms” on page 196](#).

References:

Lewicki, M.S., *A review of methods for spike sorting: the detection and classification of neural action potentials*, Network: Comput. Neural Syst., 9, R53-R78, 1998.

Schmidt, E.M., *Computer separation of multi-unit neuroelectric data: a review*, J. Neurosci. Meth., 12, 95-111, 1984.

Wheeler, B.C., *Automatic Discrimination of Single Units*, *Methods for Neural Ensemble Recordings*, ed. by M. Nicolelis, CRC Press, Boca Raton, 61-77, 1999.

Wheeler, B.C., and Heetderks, W.J., *A comparison of techniques for classification of multiple neural signals*, IEEE Trans. Biomed. Eng., 29, 752-759, 1982.

2.4 Offline Sorter Outputs

Offline Sorter provides a variety of ways to view and save the results of spike-sorting. The Offline Sorter **File** menu includes the following actions.

2.4.1 Printing Results

The 2D and 3D Clusters Views can be printed, as well as the Waveform Display and the various grid-based views. The [“3.16 Sorting Summary View” on page 79](#) provides a convenient summary of the sorted units on a channel, and can be printed or exported to a Microsoft PowerPoint* or an Adobe PDF* file. For more information, see [“5.21 Printing” on page 237](#).

2.4.2 Saving Results

For PLX, Datawave, CyberKinetics NEV, Neuralynx, and CED Spike-2 data files, Offline Sorter can save the unit assignments for each spike back into an output file of the same type. Offline Sorter preserves all records from the original file in the output file, except that it modifies the unit assignments for spike records to reflect the sorting that has been performed in Offline Sorter. For more information, see [“4.8.1 Saving Timestamps and Waveforms” on page 162](#).

2.4.2.1 Export to New .PLX:

For any of the data formats that Offline Sorter can read, it can write the spike data out as a PLX file. With this feature, Offline Sorter can effectively translate any format data file into a Plexon PLX file. For more information, see [“4.8.1 Saving Timestamps and Waveforms” on page 162](#).

2.4.2.2 Export to .NEX:

Offline Sorter can export the timestamps of sorted waveforms into a NEX file, for use with Neuroexplorer. Also Offline Sorter can export waveforms and continu-

ous data. For more information, see [“4.8.2 Exporting to a NEX File” on page 165](#).

**CAUTION****Exporting to PLX0 requires time and disc space**

Before exporting a data file to PLX0 format, make sure there is enough time and disc space. For complete information, see [“5.22.6 Using .PLX0 Files” on page 239](#).

2.4.2.3 Export to .PLX0:

Offline Sorter can save any data file to a special optimized PLX0 format, for use with Offline Sorter. The PLX0 format is more efficient and it speeds up future Offline Sorter access to that same data file. For more information, see [“5.22.6 Using .PLX0 Files” on page 239](#).

2.4.2.4 Exporting Per-Waveform Data

To accommodate later analyses of the data, Offline Sorter can write out selected information about each waveform into either a text or a MATLAB[®] file, or into an Excel[®] spreadsheet. For a given waveform, any of the calculated feature values can be exported, along with the unit assignment, timestamp, raw waveform voltage values, etc. For more information, see [“4.8.4 Exporting Information” on page 166](#).

2.4.2.5 Exporting Per-Channel Data

To accommodate later analyses of the data, Offline Sorter can write out selected information about each channel into either a text or a MATLAB file, or into an Excel spreadsheet. For a given channel, any of the sorting quality statistics can be exported, and also the unit counts, sorting criteria, etc. For more information, see [“4.8.4 Exporting Information” on page 166](#).

2.4.2.6 Exporting Per-Unit Data

To accommodate later analyses of the data, Offline Sorter can write out selected sorting information about each sorted unit into either a text or a MATLAB file, or into an Excel spreadsheet. For a given unit, it is possible to export items like cluster center positions, cluster sigmas, ISI percentages, etc. For more information, see [“4.8.4 Exporting Information” on page 166](#).

2.4.3 Reproducing Results

Offline Sorter provides several methods to apply or re-apply sorting criteria to data.

2.4.3.1 Export Sort Info to .TPL

The sorting criterion used in sorting channels can be written to a TPL file. The TPL file remembers information such as the features used for sorting, the sorting method, and any contours, templates, or parameters that control or affect the sorting. For more information, see [“5.13.1 Saving Templates” on page 214](#).

2.4.3.2 Sort Using .TPL file

Offline Sorter can read the TPL file and use the information to resort the current file. For more information, see [“5.13.2 Sort Using Saved Templates” on page 216](#).

2.4.3.3 Sort Using .NEX file

Offline Sorter can read a previously-saved .NEX file and apply the sorting embodied in the .NEX file to the currently loaded file. This only makes sense if the .NEX file was saved from the same file as is currently loaded. For more information, see [“4.8.2.1 Re-sorting from a NEX File” on page 166](#).

2.4.3.4 Update .EXP file with Sort Info

Offline Sorter can read a Plexon Sort Client experiment file (EXP file), and replaces the unit templates with the unit templates calculated from sorted units in the current file. For more information, see [“5.13.3 Save Templates to a Sort Client EXP File” on page 216](#).

2.4.3.5 Execute Batch Command File

Offline Sorter has a batch-processing facility that can perform sorting actions based on simple commands read from a text file. This facility is useful for automatically sorting large files, which can be time-consuming. For more information, see [“5.19 Batch File Processing” on page 225](#).

2.5 Terminology

In the discussion below, *continuous* and *digital* refer to types of data.

2.5.1 Continuous

In general, *continuous* is used throughout this manual to refer to any signal that was recorded continuously for as long as the data acquisition switch is turned on in Sort Client or Recorder. *Continuous* excludes spike waveform segments and digital event data. *Continuous* includes both continuous signals sampled at high frequencies (spike and wide-band signals, EMG, etc.) and continuous signals sampled at low frequencies (field potentials and other external signals such as x,y, eye position, etc.).

2.5.2 Digital

The term *digital* refers to digital events that Offline Sorter reads from the file. Digital events, in general, can also have a ‘strobe’ value for each event in addition to a timestamp. These are often called ‘Events’, as in the ‘Events View’.

2.5.3 Fast and Slow

The terms *fast* and *slow* describe sampling rates, when there is a need to distinguish between two rates.

2.5.4 Spike waveform segments

The term *spike waveform segments* describes the waveform segments around threshold crossings. These are more commonly called simply ‘spikes’ or ‘waveforms’.

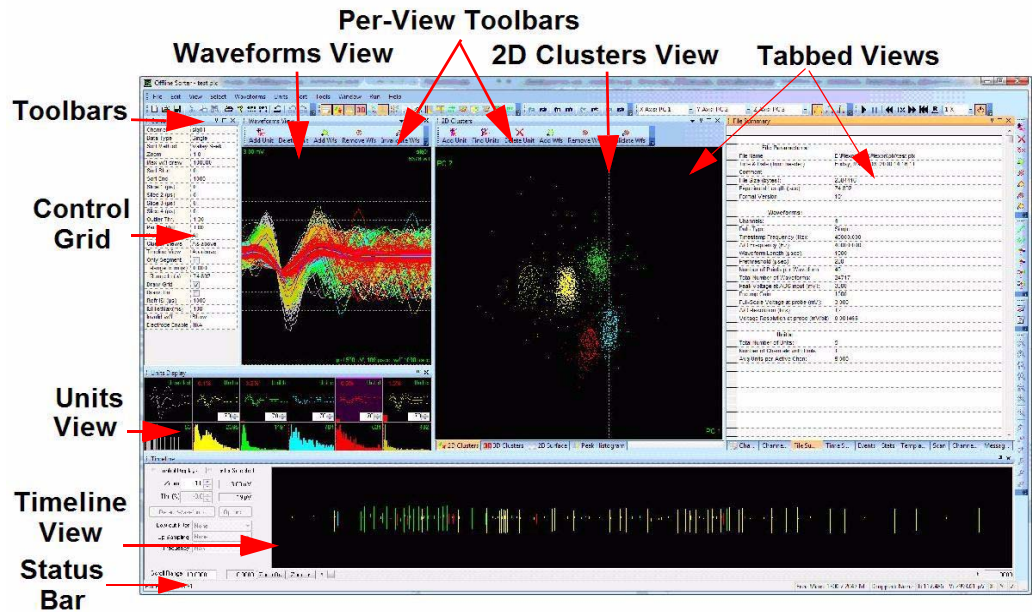
Chapter 3

Screen Elements and Displays

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3.1 Screen Elements Overview

Offline Sorter has a highly-customizable screen layout. By default, it contains the following screen elements.



3.1.1. Toolbars

Frequently-used menu items have equivalent buttons on the toolbars. Move the mouse pointer over a toolbar button to open a tooltip window that describes the function of that button. The toolbars can be moved and docked to the top or bottom of the screen. The contents of the toolbars can be rearranged and customized by using the **Toolbars** tab under **Window | Customize...** menu item.



HINT Keyboard Bindings

Common operations may be conveniently and quickly performed by binding a keyboard shortcut to any function available from the menus or toolbars. Use **Keyboard** tab under **Window | Customize...** menu item.

3.1.2 Status Bar

At the bottom of the screen is a status bar. Tool tips for the currently-selected toolbar or menu item appear at the left edge of the status bar. When Offline Sorter is performing a time-consuming operation, a progress bar will appear in the middle of the status bar. On the right side are text fields that change based on the state of the program, such as the approximate amount of memory that is still available to use. Of particular importance are the time, voltage, and feature space labels that display the values corresponding to the mouse position within some of the views.

3.1.3 Views

The bulk of the user interface of Offline Sorter consists of many independent ‘Views’ or windows that can be docked, floated, and placed into tabs (See [A.1 Plexon User Interface](#)). Each available view within Offline Sorter has a corresponding menu item under the ‘**View**’ main menu entry, and many of the common views also have a corresponding toolbar button.

Some of the Views within Offline Sorter have associated with them their own Toolbars. The presence of the per-View Toolbars is indicated by the small triangle in the title bar.



Pressing the triangle will toggle showing or hiding the per-View toolbar. When shown, the per_view toolbar can include both icons and text:



or just the icons:



Showing the text can be turned on or off by right-clicking on the toolbar and selecting the **Text Below Icons** item from the menu.

Note that the default layout depicted above does not include all of the Views that are available in Offline Sorter; see the View menu for the complete list of the Views available in Offline Sorter. The following is a brief description of each of the available views.

The **Control Grid** is used to select and set values for other important control parameters and viewing options. See [“3.2 Control Grid” on page 21](#).

The **Waveform View** shows the waveforms for the selected channel, which are all superimposed. The mouse may be used to add and remove waveforms from units in this display. For more information, see [“3.3 Waveform View” on page 25](#).

The **Units View** shows the templates (average waveforms) for each sorted unit, plus the remaining unsorted waveforms. Click in this view for the fastest way to select the current unit. The graphs at the bottom of the view display the histograms of interspike intervals. See [“3.4 Units View” on page 29](#).

The **Timeline View** shows the waveforms and/or continuous data as a function of time. The time range viewed can be expanded or reduced, and it can be repositioned within the file. The display can be used to specify digital filtering and upsampling, to select the threshold for waveform extraction, and to select individual waveforms for inspection. See [“3.5 Timeline View” on page 31](#).

The **Info Grid Views** are a collection of spreadsheet-like informational views that are available under the **View | Info Grid Views** sub-menu. The available **Info Grid Views** are:

- The **Channels** view contains counts of waveforms for each unit in each channel. The count of continuous samples is also shown.
- The **Channel Parameters** view contains channel-by-channel information, such as thresholds, gains, and filtering.
- The **File Summary** view contains the global waveform and sorting parameters and file information.
- The **Time Segments** view shows how the file has been subdivided into time segments.
- The **Sort Details** view shows the details about the sorting performed on each channel, like what features and sorting methods were used.
- The **Events** view lists the external events present in the data file. It allows events to be displayed with custom colors on the Timeline View.
- The **Stats** view contains multivariate ANOVA tests on the separation of unit clusters and other sorting quality-related statistical information.
- The **PCA** view displays the eigenvectors and values of the principal component analysis on the waveforms that is used to generate the principal component displays.
- The **Scan** view is an exploration tool that can automatically resort a channel numerous times while stepping the values of parameters. This allows one to get a feeling for the optimal values of the sorting parameters.
- The **Templates** view displays the mean template for each sorted unit.
- The **Adaptive Templates History** view is only relevant if sorting with adaptive templates was performed. Then it displays a time-stamped history of when the templates were changed, along with the numerical values for the new templates.
- The **Channel Map** view shows the mapping between ‘raw’ data channels from the data file and the channel number used by Offline Sorter. This mapping includes the ability to combine ‘raw’ channels to form stereotrodes and tetrodes.

-
- The **Messages** view displays a list of significant occurrences within Offline Sorter. It is useful for debugging problems or reminding oneself of what operations were previously executed.

2D Clusters shows the waveforms as points in 2D feature space. Each axis can be selected from any of the [“5.5 Features Available for Sorting” on page 191](#). This display can be used to select the clusters of waveforms. For more information, see [“3.7 Clusters Views” on page 43](#).

3D Clusters shows the waveforms as points in 3D feature space. Each axis can be selected from any of the [“5.5 Features Available for Sorting” on page 191](#). This display can be used to select the clusters of waveforms. For more information, see [“3.7 Clusters Views” on page 43](#).

Waveform Inspection view compares individual waveforms selected in the Timeline View with the average waveform (template) of each sorted unit. For more information, see [“3.11 Waveform Inspection View” on page 68](#).

Waveform Detection view is only available for files with continuously recorded signals (e.g. DDT files). It displays histograms of detected peak heights that allows a spike detection threshold to be set. For more information, see [“3.12 Waveform Detection View” on page 69](#).

Feature vs. Feature displays miniature 2D Clusters View windows for various combinations of features. It is possible to identify features quickly that are the most useful in the sorting of units. For more information, see [“3.15 Feature vs. Feature View” on page 75](#).

Sorting Summary displays a concise, printable summary of the sorting on a channel. The Sorting Summary View can also be exported to PowerPoint*. For more information, see [“3.16 Sorting Summary View” on page 79](#).

PCA Results graphically displays the information that can be found on the **PCA** view of the **Info Grid View**. Each of the PCA eigenvectors can be plotted, along with a bar graph of the eigenvalue magnitude. For more information, see [“3.17 PCA Results View” on page 83](#).

ISI Histograms shows the same ISI Histograms that are plotted in the Unit Display (see below), but the view can be printed and it offers several other display options, such as log scaling of the axes. For more information, see [“3.14 ISI Histograms View” on page 75](#).

Cross-Correlograms shows a matrix of cross-correlation plots between all of the units in the current channel. For more information, see [“3.18 Cross-Correlograms View” on page 84](#).

Rasters shows Raster Plots of the spike and event times for all channels, and the continuous data for the selected channel. For more information, see [“3.19 Rasters View” on page 85](#).

The **Surface View** shows the density of points in 2D feature space, plotted as a three dimensional surface plot. See [“3.8 Surface View” on page 61](#).

The **Clusters vs Time View** shows the points in 2D feature space, plotted against time on the third axis. This is useful to see how the waveforms changed as a function of time. See [“3.9 Clusters Vs Time View” on page 63](#).

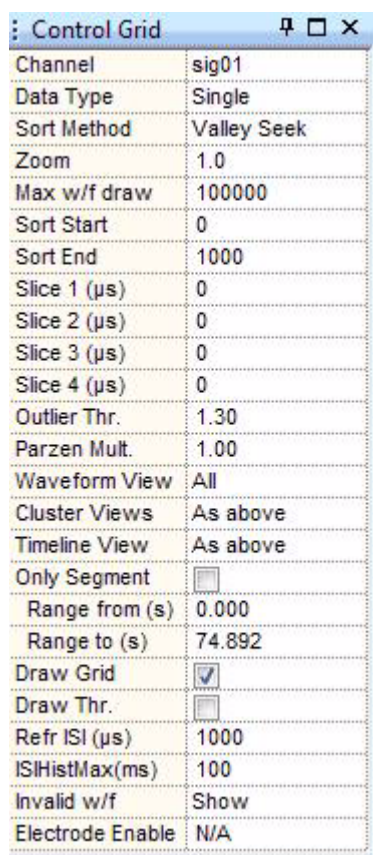
The **Sort Quality vs Segment Graph** shows how the sorting quality statistics changed as a function of time throughout the duration of the file. See [“3.10 Sort Quality vs Segment Graph View” on page 66](#).

The **Templates vs Time View** is only relevant if adaptive template sorting has been performed. It shows the template waveforms plotted against time, which shows how the waveforms changed as a function of time. See [page 135](#).

The **Continuous Frequency Spectrum View** is only relevant for channels that contain continuous data samples. It displays the results of a Fast Fourier Transform performed on a span of continuous data samples, showing the amount of energy at each frequency. See [“3.13 Continuous Frequency Spectrum View” on page 74](#).

The **Scan Graph View** shows the result of scanning a sorting parameter over a range of values. At each value a sort is performed and the sorting quality statistics are calculated. The **Scan Graph** shows the values of sorting quality statistics as a function of the sorting parameters value. See [“5.15 Scan Graph View” on page 220](#).

3.2 Control Grid



Control Grid	
Channel	sig01
Data Type	Single
Sort Method	Valley Seek
Zoom	1.0
Max w/f draw	100000
Sort Start	0
Sort End	1000
Slice 1 (µs)	0
Slice 2 (µs)	0
Slice 3 (µs)	0
Slice 4 (µs)	0
Outlier Thr.	1.30
Parzen Mult.	1.00
Waveform View	All
Cluster Views	As above
Timeline View	As above
Only Segment	<input type="checkbox"/>
Range from (s)	0.000
Range to (s)	74.892
Draw Grid	<input checked="" type="checkbox"/>
Draw Thr.	<input type="checkbox"/>
Refr ISI (µs)	1000
ISIHistMax(ms)	100
Invalid w/f	Show
Electrode Enable	N/A

Use the **Control Grid** to set the following parameters:

Channel – is the currently active channel. Click to show a drop-list of all the available channels. The channel can be selected by double-clicking it in the Info Grid View. For more information, see [“3.6 Info Grid Views” on page 34](#).

Data Type – is the electrode recording mode. **Single** indicates that each channel is viewed separately. **Stereotrode** indicates that channels are arranged in groups of two. **Tetrode** indicates that channels are arranged in groups of four. **Data Type** automatically selects the appropriate mode for Plexon PLX, Datawave UFF, and Neuralynx files. If **Data Type** is changed, Offline Sorter is forced to interpret the file as if it contained the selected data type. For more information, see [“5.3 Stereotrode and Tetrode Data” on page 186](#).

Sort Method – is the cluster selection method. Offline Sorter provides the following methods:

- Templates ([“4.5.4 Semi-Automatic Clustering using Templates” on page 132](#))
- Boxes (See [“4.5.5 Semi-Automatic Clustering using the Boxes Method” on page 135](#))
- K-Means ([“4.5.6 Semi-Automatic Clustering using K-Means” on page 137](#))

- Standard E-M ([“4.5.7 Semi-Automatic Clustering using Standard E-M ” on page 141](#))
- Valley-Seeking ([“4.5.8 Automatic Clustering using Valley-Seeking Method” on page 142](#))
- T-Distribution E-M ([“4.5.9 Automatic Clustering using T-Distribution E-M Method” on page 144](#))
- K-Means Scan (See [“4.5.10 Automatic Clustering Using the Scanning Methods” on page 145](#))
- Standard E-M Scan (See [“4.5.10 Automatic Clustering Using the Scanning Methods” on page 145](#))
- Valley Seeking Scan (See [“4.5.10 Automatic Clustering Using the Scanning Methods” on page 145](#))
- T-Distribution E-M Scan (See [“4.5.10 Automatic Clustering Using the Scanning Methods” on page 145](#))

When one of these options is selected, you set the current sorting mode is set, but a sorting operation is not actually initiated.

Zoom – is the vertical zoom factor in the Waveform View and the Timeline View. The up or down arrows can be used to increase or decrease the factor by steps, but any floating point value can be entered by typing the numbers. By default, the up-down arrows increase or decrease the factor by increments of 0.1 when the current zoom factor is small, and by increments of 1.0 when the zoom factor is higher. The value at which the 1.0 increment applies can be adjusted by typing the value in the **Zoom control steps by 1.0 starting at value:** box on the **Display** tab in the **Tools | Options** window.

Max w/f draw – Maximum-waveform-draw is the maximum number of waveforms drawn in the Waveform View and the Clusters Views. For example, if there are 30000 waveforms in the active channel, and **Max w/f draw** is set to 10000, then only every 3rd waveform is drawn. Change the **Max w/f draw** setting from the large default value only to improve drawing speed on slower computers when viewing channels with many waveforms. When the number of waveforms in a channel is greater than the **Max w/f draw** setting, the Waveform View shows the waveform count section highlighted in red, and there is a

divided-by indicator that shows the fraction of waveforms that are being displayed; e.g., for the preceding example, the divided-by indicator is 3.



CAUTION

Misleading Sort

It can be misleading to sort units on a channel where **Max w/f draw** is less than the number of waveforms. If Offline Sorter takes too long to redraw the display, it is generally safer to use the deferred drawing feature. See [“5.22.5 Deferring Waveform Drawing” on page 239](#).

In general, if the waveform count on a channel exceeds the **Max w/f draw** setting, do not select the **Use only currently visible waveforms** checkbox in the **Tools | Options General** tab; that selection can result in valid waveforms being unassigned when selecting units by using the waveform crossing or contour sorting methods.

Sort Start and **Sort End** – are the lower-bound and upper-bound of the time interval (See [“4.4.4 Adjusting the Sort Range” on page 118](#).) of the waveform that Offline Sorter uses to calculate principal components and feature values and in [“5.6 Details of the Sorting Algorithms” on page 196](#). The default value for Sort Start is zero. The default value for the Sort End is the total waveform length. Offline Sorter only uses the time points of the waveforms between Sort Start and Sort End in the template sorting algorithm, in the recalculation of the principal component scores, and in the calculation of feature vectors. For options in principal component calculations, see [“4.4.4 Adjusting the Sort Range” on page 118](#).

Slice 1, Slice 2, Slice 3, Slice 4 – are the time points in the waveform (slices in time) that Offline Sorter uses to calculate the Slice feature. A vertical line in the Waveform View represents the Slice time. It is possible to view the voltage of the waveforms at the Slice time point on any axis of the Clusters Views and it can be used in the sorting algorithms.

Outlier Thr. – Outlier Threshold can be used for several purposes. The value in this field is in units of standard deviations (sigmas). When Offline Sorter renders ellipses in the Clusters Views, this field controls where Offline Sorter draws the lines or surfaces. For the [4.5.6 Semi-Automatic Clustering using K-Means](#) sorting algorithm, and for the **Tools | Remove Outliers** menu selection, this field controls the removal of outliers. The K-Means clustering algorithm assigns every waveform to a cluster. To eliminate the outliers, Offline Sorter calculates the standard deviation σ of the distance from all points in the cluster to its center, for each cluster. Then, if the distance from a given point to the cluster center is more than $\sigma \times \text{Outlier_Threshold}$, Offline Sorter removes the point from the cluster.

Parzen Mult., D.O.F. Mult., or Beta – Only one of these values appears, which depends upon the sorting method currently selected. All the values control parameters in the sorting algorithms, as follows. The [4.5.8 Automatic Clustering using Valley-Seeking Method](#) algorithm works by counting neighbors that are within the Parzen radius times this **Parzen Mult.** multiplier around the point in feature space. If the value is decreased, it generally results in more numerous,

smaller clusters. The [4.5.9 Automatic Clustering using T-Distribution E-M Method](#) has a setting for the number of degrees of freedom (DOF) for the fitting procedures. Use of the theoretically-predicted value of this parameter results in poor sorting. The **D.O.F Mult.** value multiplies the theoretical DOF setting. Smaller values of this multiplier generally result in slow convergence to a larger number of clusters, larger values result in rapid convergence to a smaller number of clusters. The [4.5.7 Semi-Automatic Clustering using Standard E-M](#) algorithm depends upon an “annealing rate” parameter **Beta**. When **Beta** is 1, a completely standard E-M algorithm runs. When the value is other than one, a variant of the standard E-M algorithm runs.

Waveform View – specifies which waveforms Offline Sorter draws in the Waveform View (either **All** waveforms, **Selected Unit(s)**, **Unsorted**, or only the **Selected Waveform**). **Selected Unit(s)** permits a quick selection of units by clicking in the Units View.

Clusters Views – specifies which waveforms appear in the Clusters Views (either **All** waveforms, or **Same as above**—which displays the waveforms specified in the **Waveform View** parameter).

Timeline View – specifies which waveforms are shown in the Timeline View (either **All** waveforms, or **Same as above**—which displays the waveforms specified in the **Waveform View** parameter).

Only Segment – is an option that displays the waveforms for the currently selected time segment only. See [“3.6.4 Time Segments View” on page 40](#). The time range covered by the currently selected time segment is displayed in the **Range From** and **Range To** parameters that follow. By default, the program displays all the waveforms from a selected channel. Use this option to display only a subset of waveforms based on a time segment. It is also possible to set up how the data file is divided into time segments using the **Waveforms | Create Time Segments** menu command.

Draw Grid – draws a grid in the Waveform View.

Draw Thr. – Draw Threshold draws a horizontal dashed red line in the Waveform View to show the position of the threshold for the channel. For more information, see [“5.17 Re-thresholding Spike Data” on page 223](#).

Refractory Interspike Interval – is the minimum valid interspike interval. If there are intervals smaller than the refractory interval, Offline Sorter displays a red bar to the left of the template display in the Units View that corresponds to the proportion of spikes with ISIs less than the refractory interval (See [“3.4 Units View” on page 29](#)). Lines between spikes with ISIs less than the refractory interval are also drawn in the Clusters Views when that option is enabled. The ISI is specified in microseconds. For more information, see [“3.7 Clusters Views” on page 43](#).

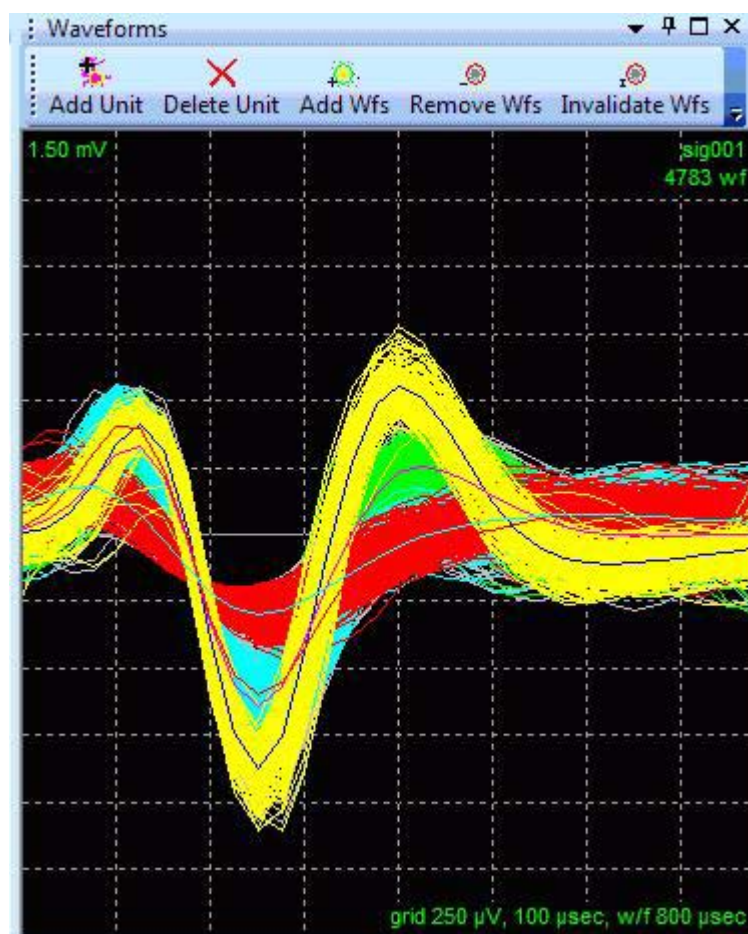
ISI Hist Max – Interspike Interval (ISI) Histogram Maximum is the maximum value of the X axis in the Interspike Interval Histogram in the [3.4 Units View](#), in milliseconds.

Invalid w/f – Invalid Waveforms is an option to **Show** or **Hide** the waveforms marked as invalid in the Waveform and Clusters Views. Offline Sorter retains this value between invocations.

Electrode Enable – is only relevant if stereotrode or tetrode data is being processed by Offline Sorter. Then this droplist will show checkboxes for each of the electrodes within the stereotrode or tetrode. Unchecking the checkboxes will cause data from that electrode to be ignored when calculating PCA and feature values.

3.3 Waveform View

The Waveform View shows the superimposed waveforms aligned, by default, at the threshold crossing times. Offline Sorter draws the waveforms assigned to units in the colors associated with those units. Offline Sorter also draws the template for each unit in a contrasting color.



Note that by default, the Waveforms View shows its toolbar, and the toolbar has the ‘**Text Below Icons**’ option checked (See [“A.2.2 Toolbars” on page 5](#)). Many of the common actions described below can be initiated by pressing a button in this toolbar.

Waveforms (See [“4.4.3 Waveform Alignment” on page 115.](#)) can be aligned at their minimum or maximum values by using the **Tools | Align Waveforms** or the **Waveforms | Align** menu commands.

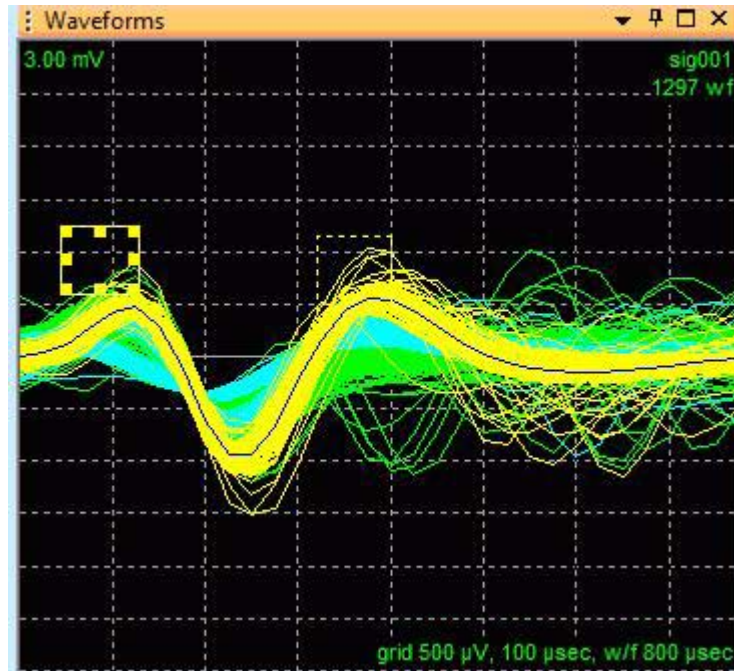
Among the operations commonly done from the Waveform View are:

- **Add Unit** - Unit clusters in this view can be created by using the mouse pointer to ‘cross’ waveform bundles; see [“4.5.3 Manual Clustering using the Waveform Crossing Method” on page 128.](#)
- **Delete Unit** - deletes the currently selected unit.
- **Add Wfs** - adds the crossed waveforms to the currently selected unit
- **Remove Wfs** - removes the crossed waveforms from the currently selected unit (i.e. change them to unsorted)
- **Invalidate Wfs** - marks the crossed waveforms as being invalid so that they are ignored. See [“4.6.3 Cleaning the Selection” on page 150.](#)

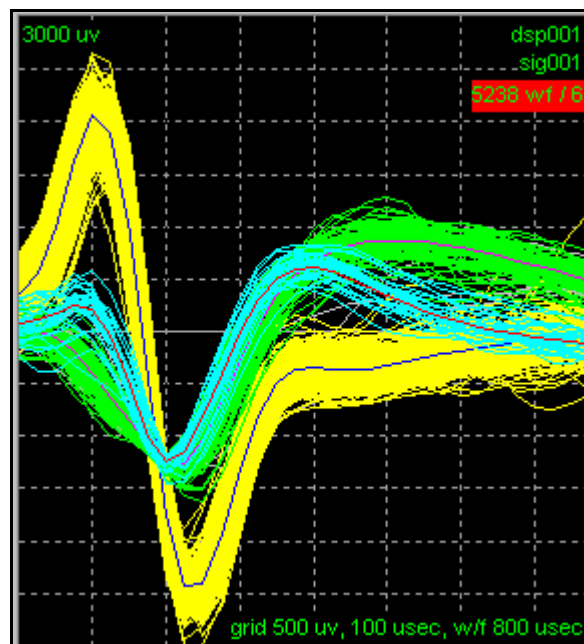
To control the temporal range and type of waveforms shown in the Waveform View, it is possible to use the scroll bar at the bottom of the [3.5 Timeline View](#) (when the **Control Displays** check box is checked) and the **Waveform View** parameter in the [3.2 Control Grid](#).

The **Draw Grid** option in the [3.2 Control Grid](#) determines if a reference grid appears in the Waveform View.

The Waveforms View will show the template waveform for each unit drawn as a single complementary-colored waveform. By default, the waveform view will show the template waveforms for all units, along with the sorting boxes for all units if in Boxes sorting mode. But by selecting **Show Templates and Boxes only for Selected Units** from the right-click menu, the Waveforms View can be made to show the templates (and boxes) for only the selected units. This can provide a less-cluttered view of the waveform data.



If the number of waveforms in a channel exceeds the **Max w/f draw** value in the [3.2 Control Grid](#), the Waveform View and the Cluster Displays draw only every N th waveform, where the divisor N is calculated to bring the number of drawn waveforms under the **Max w/f draw** limit. This ensures that drawing occurs with an acceptable speed on slower computer systems. When this “decimation” is in effect, Offline Sorter highlights the wf count text in red.



In the preceding screen shot, the **Max w/f draw** is set to 1000. The divisor N in this case is 6 as shown in the red highlighted section, which indicates that only every 6th waveform is being drawn.

When the **Control Displays** checkbox in the Timeline View is checked, decimation is disabled and all waveforms in the selected time interval are displayed.

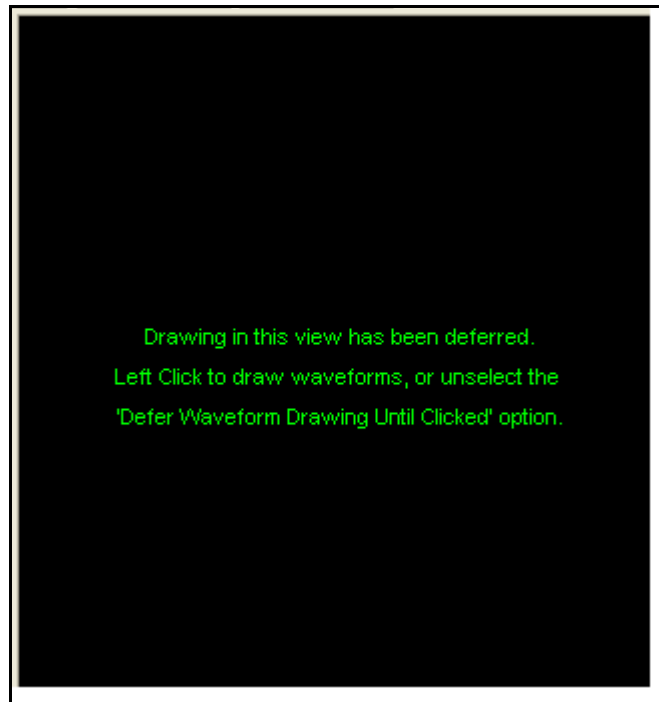


CAUTION

Sorting During Decimation

If sorting is performed while decimation is occurring, it can result in lost information. If possible, increase the **Max w/f draw** setting so that decimation does not occur. The **Use only currently visible waveforms** setting in the **Tools | Options | General** menu dialog controls whether waveforms that are not being drawn are included in waveform crossing and contour sorting.

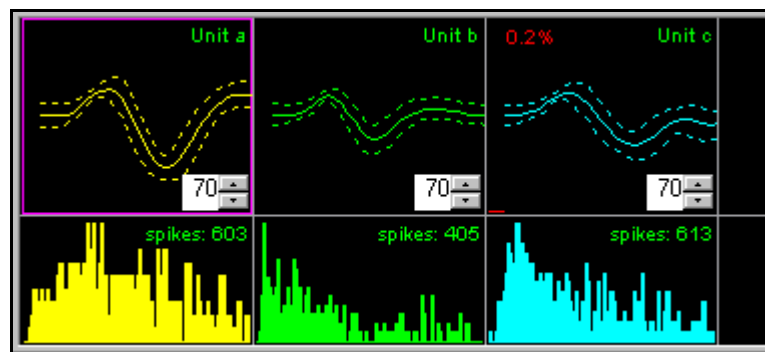
As an alternative to using the **Max w/f draw** feature to speed up the display when viewing large files, the Waveform View can be configured only to redraw “on demand” by using the **Defer Waveform Drawing Until Clicked** checkbox in the **Tools | Options | Display** dialog box. When this item is checked, the Waveform View displays the following view instead of drawing the waveforms.



Left-clicking in the Waveform View causes all the waveforms to be redrawn. After that, any operation that would normally cause the Waveform View to be drawn results in the above text instead. The **Defer Waveform Drawing Until Clicked** option also affects drawing in the Timeline View.

3.4 Units View

Offline sorter provides information about each of the currently-defined units in the Unit Display along with a display for the waveforms that are currently unsorted.



The top row of graphs of this display shows the template (average waveform) for the unit (solid line) as well as ± 3 standard deviations from the template (dashed lines). The edit controls can be used with the spin buttons to adjust the fit tolerances for Template sorting.

The default template Fit Tolerance (70 in the preceding screenshot) can be set by using the **Templates** tab on the **Tools | Options** menu panel. After a new value for the Fit Tolerance is set, one can elect to apply the new value to existing units, or to only use the new value when new units are defined. For additional information on templates, see [“5.6.2 Template Algorithm” on page 197](#).

The bottom row of graphs of this display shows the **Interspike Interval Histograms**. The X axis of each histogram runs from zero to **ISIHistMax**. The graphs also show the total number of spikes for each unit. Larger printable versions of these Interspike Interval Histograms can be displayed in the [“3.14 ISI Histograms View” on page 75](#).

The Unit Display shows the percentage of spikes with Interspike Intervals less than the **Refractory Interspike Interval** in red, which also includes a red bar in the upper portion of the display. The values of **ISIHistMax** and **Refractory Interspike Interval** can be changed in the [3.2 Control Grid](#). The **Refractory Interspike Interval** value can also be set by using the **Refractory Period** tab on the **Tools | Options** menu command.

3.4.1 Selecting Units

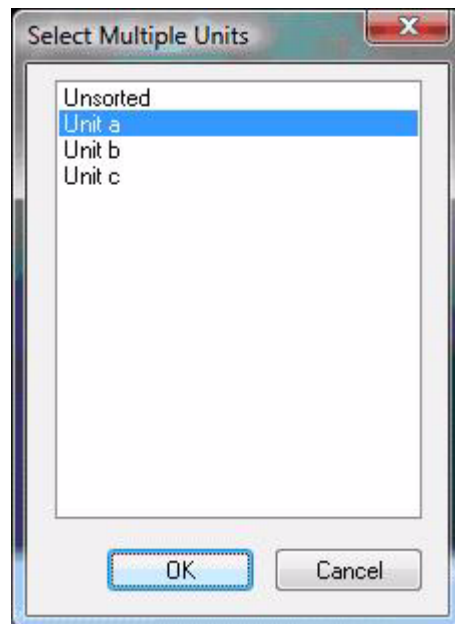
Units in the Units View can be selected by left-clicking on the corresponding template. Offline Sorter draws a solid highlighted box and a colored background on the box corresponding to the currently selected unit. For purposes of selecting units, the Unsorted “unit” behaves just like the actual sorted units. When the **Waveform View** setting in the Control Grid is **Sel.Unit(s)**, selecting units by clicking in the Units View is a convenient means to quickly see the differences and similarities between units.

Offline Sorter uses the concept of selecting a single primary unit and multiple secondary units. The primary unit is the unit that is affected by operations such as **Delete Selected Unit**, **Add Wfs**, and **Remove Wfs**. Offline Sorter shows the multiple secondary units with a dashed box highlighting the unit template, but with the default background color. When the **Waveform View** setting in the Control Grid is set to **Sel.Units(s)**, Offline Sorter also displays the waveforms for the multiple selections in the Waveform View, Clusters Views, and Timeline View. This is a convenient way to compare and contrast units. Also, certain operations like **Units | Combine Units** and **Swap Units** use multiple unit selections.

To select multiple units, follow the Windows standard for selecting multiple items:

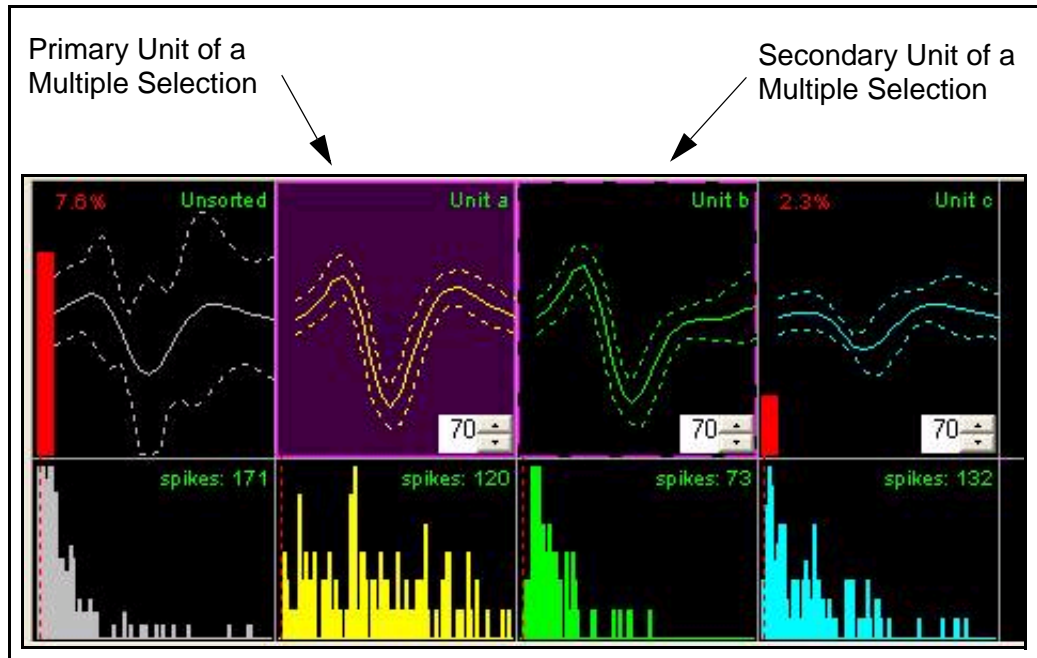
- To select a unit, click the unit.
- To select non-adjacent units, press and hold down **CTRL**, and then click each unit.
- To select adjacent units, click the first unit, press and hold down **SHIFT**, and then click the last unit.
- To cancel an existing multiple selection, click any unit.

Units can also be selected by using the **Units | Select Unit(s)** dialog box.



The right-click menu on the Units View provides easy access to some common functionality that is available from other main menu selections, such as the ability to delete the selected unit, delete all units, swap units, combine units, invalidate all waveforms in the selected unit, and to select multiple units using a dialog.

To set the colors assigned to each of the sorted units, select the **Colors** tab of the **Tools | Options** dialog box.

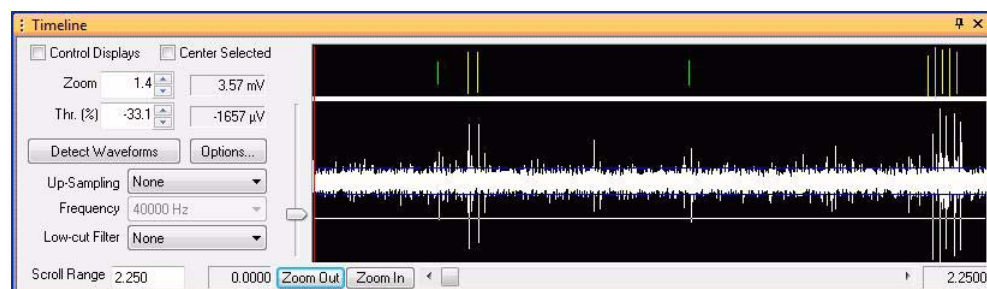


3.5 Timeline View

The Timeline View shows the data, either continuously digitized or waveform segments, in its proper temporal sequence.

This window can be used to:

- specify an optional digital filter and/or up-sample the data, select a threshold and extract waveform segments for continuously recorded data
- control the temporal range of waveforms shown in the Waveforms View and Clusters views
- select individual waveforms (left-mouse click on waveform) for viewing in the Waveforms View and Waveform Inspection View



There are two sections in the stripchart display (black background), the bottom section shows the continuous data and the top section shows the spikes for a channel. The two sections are separated by a moveable splitter. If there is no continuous data for a channel, the top section fills the entire stripchart section.

The Timeline View consists of the following components:

- **Waveform Segments stripchart display** - The top portion of the stripchart display shows waveform segments positioned according to their timestamps. If there is no continuous data present for the channel being viewed, then this waveform segments portion will fill the entire stripchart display vertically. Colors represent the unit assigned to each waveform segment. Individual waveforms can be selected for viewing in the Waveforms View and in the Waveform Inspection View by clicking on the waveform with the left mouse button.
- **Continuous stripchart display** – The bottom portion of the stripchart display is only present when there is continuous data for the channel. The voltage versus time trace of the continuous data is plotted.
- **Control Displays** – This checkbox restricts waveforms displayed in the Waveforms View and Clusters View to those contained in the **Scroll range** setting of the Timeline View.
- **Center Selected** – If checked, when a waveform is selected using the [3.7 Clusters Views](#) it causes the time range displayed to change such that the selected waveform is shown in the center of the window.
- **Zoom** – This item represents the zoom factor for the voltage display, which is the same as the **Zoom** parameter in the Control Grid. The voltage from zero to peak appears to the right of the **Zoom** control.
- **Thr.(%)** – This is the voltage threshold control for waveform extraction; the threshold voltage level is shown to the right of the **Threshold** control and it is represented by a solid horizontal line in the window display. The threshold can be adjusted by dragging the horizontal line up or down with the mouse. This item is enabled only if the data is continuous.
- **Detect Waveforms** – Click this button to extract waveform segments around threshold crossings, using the current waveform detection option settings. This item is enabled only if the data is continuous.
- **Options** – Click this button to display a dialog box for defining the waveform detection option settings, such as the Total Length, Prethreshold Period, Dead Time for the waveforms extracted, Signal to be thresholded (Raw, Energy, Nonlinear Energy), and the Energy Window Width. See [“4.2.6 Specifying the Waveform Detection Options” on page 99](#). This item is enabled only if the data is continuous.
- **Up-Sampling** - This item selects the methods to use for any up-sampling to be performed on the continuous data. See [“4.2.3 Specifying Up-Sampling” on page 94](#). This item is enabled only if the data is continuous.
- **Frequency** – This item displays the frequency of the continuous data. If up-sampling is being performed, the new up-sampled frequency is selected from

this droplist. See [3.3“4.2.3 Specifying Up-Sampling” on page 94](#). This item is enabled only if the data is continuous.

- **Low-cut filter** – This item selects the cutoff frequency for the 4th order low-cut Butterworth IIR filters. This item is enabled only if the data is continuous.
- **Scroll bar, Zoom Out, Zoom In** – Use these controls to change the limits of the scroll range for the Timeline View.
- **Scroll range** – The scroll window length and start time appear to the left of the scroll bar; the end time appears to the right.

3.5.1 Other Features of the Timeline View

Right-clicking on a waveform brings up a menu that enables one to:

- **Mark Waveform Valid:** if the selected waveform is invalid, mark it valid
- **Mark Waveform Invalid:** if the selected waveform is valid, invalidate it
- **Unsort Waveform:** remove the waveform from its currently assigned unit, making it unsorted
- **Add Waveform to Selected Unit:** assign the selected waveform to the current unit
- **Add Waveform to New Unit:** create a new unit, and assign the selected waveform to that unit
- **Use Waveform as Template for New Unit and Sort:** use the selected waveform as a template to define a new unit using the Templates sorting (See [“4.5.4 Semi-Automatic Clustering using Templates” on page 132.](#)).
- **Zoom Time Range to Selected Segment:** set the displayed time range to show the entire current time segment. See [“3.6.4 Time Segments View” on page 40.](#)
- **Show Time Segment Boundaries:** if checked, show vertical lines and labels at the boundaries between time segments
- **Show Zero Line:** if checked, shows a white line representing 0 volts
- **Show 3-Sigma Peak Heights Line:** if checked, shows blue dotted horizontal lines that represent \pm standard deviations of noise. The noise is calculated by setting the Threshold to \pm 3 standard deviations of the original signal and then removing waveform segments 250 msec before and 750 msec after the threshold crossings.
- **Show Dots at Sample Locations for Continuous Data:** when showing continuous data, the Timeline View connects the sample points with lines. When this option is checked, a point is drawn at the time/voltage coordinates of each continuous data sample

When the channel **Data Type** is **Stereotrode** or **Tetrode**, the display divides up to vertically stack 2 or 4 continuous traces or spike waveforms, as shown in the “[5.3 Stereotrode and Tetrode Data](#)” on page 186.

For both the waveform and continuous data displays, the status bar on the bottom of the screen shows the time and the voltage corresponding to the current mouse position within the display.

Free Mem: 190 / 510 M	Dropped: None	t: 0.11	V: 156.46 mV	X:	Y:	Z:
-----------------------	---------------	---------	--------------	----	----	----

3.6 Info Grid Views

The **Info Grid Views** consist of a family of similar views that display information in a spreadsheet format. All of the Info Grid Views are grouped into an ‘**Info Grid Views**’ sub-menu under the View menu.

3.6.1 Channels View

Channels								
	Name	Samples	Waveforms	Unsorted	Unit a	Unit b	Unit c	Unit d
>>1	sig001	0	6730	1651	3717	1362	-	-
2	sig002	0	6319	3039	3280	-	-	-
3	sig003	0	7386	1962	5424	-	-	-
4	sig004	0	6619	3576	3043	-	-	-
5	sig005	0	5992	743	3176	2073	-	-
6	sig006	0	4835	134	868	3833	-	-
7	sig007	0	10655	3813	1261	5581	-	-
8	sig008	0	5078	4852	226	-	-	-
9	sig009	0	2958	355	2603	-	-	-
10	sig010	0	5982	4294	1688	-	-	-
11	sig011	0	8798	5585	3213	-	-	-
12	sig012	0	7222	1377	890	2187	2768	-
13	sig013	0	6783	1788	4995	-	-	-
14	sig014	0	4007	266	1530	2211	-	-
15	sig015	0	20388	5131	8024	1243	5990	-
16	sig016	0	17166	12899	4267	-	-	-
17	sig017	0	5693	4472	1221	-	-	-
18	sig018	0	7156	5139	2017	-	-	-
19	sig019	0	2824	868	1956	-	-	-
20	sig020	0	12823	3870	8953	-	-	-
21	sig021	0	3911	582	482	2847	-	-
22	sig022	0	6707	286	1755	4666	-	-
23	sig023	0	5553	4540	1013	-	-	-



HINT

Viewing Individual Channels

Individual channels can be selected for viewing or sorting, or both, by double-clicking the channel # on the far left-hand side of the page.

The **Channels View** offers a quick overview of the contents and the sorting status of a file.

The **Channels View** contains a row for each channel in the file. The columns that display in the **Channels View** depend upon the file type that is open.

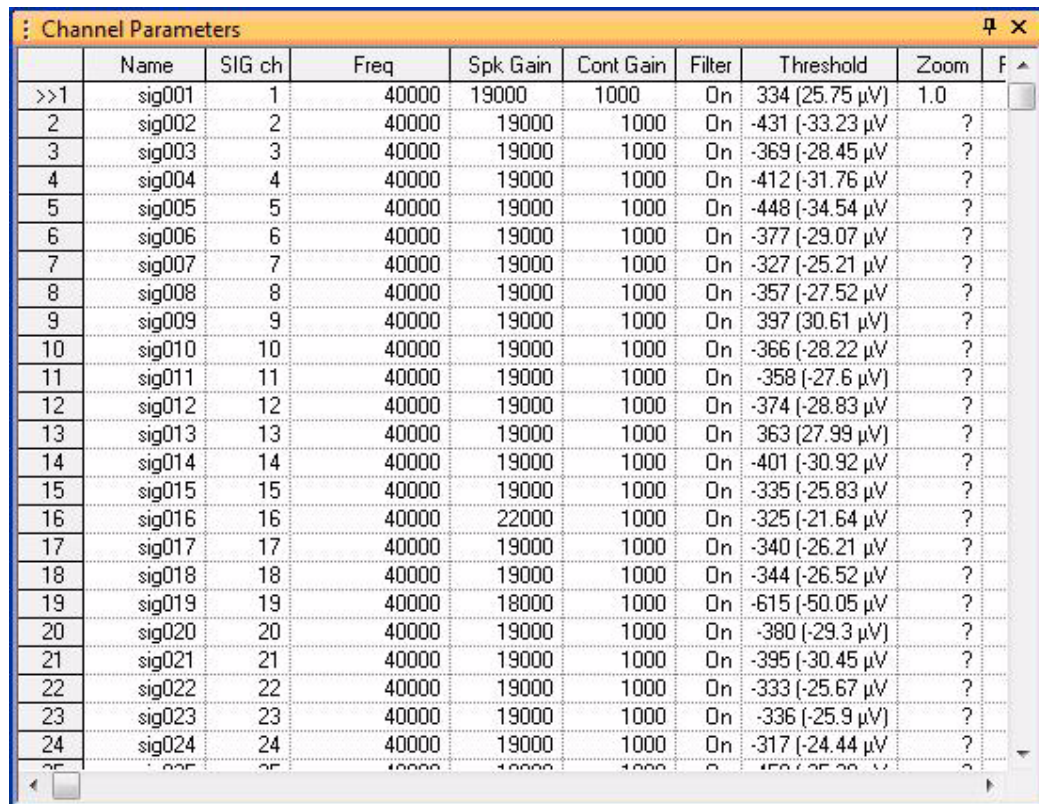
For data files with timestamped spikes (e.g. PLX files, as pictured earlier), the **Channels View** contains the following columns:

- **Name** – refers to the channel name. Channels can be renamed by clicking in this column and typing a new name.
- **Samples** – indicates the number of data points for continuous channels; if the channel has only spike waveforms, the number of data points can be 0.
- **Waveforms** – indicates the number of spikes in the channel; if the channel has only continuous data, the number of spikes can be 0.
- **Invalidated** – shows the number of invalidated waveforms in the channel.

Note: By default, this column is very narrow and it can appear to be blank. To see the column information, the column must be expanded by dragging the dividing lines on the header bar for the column.
- **Unsorted** – shows the number of spikes that have not been assigned to a unit.
- **Unit a....z** – shows the number of spikes assigned to each unit.

3.6.2 Channel Parameters View

The **Channel Parameters** (channel parameters) tab contains more detailed, per-channel information about the settings in effect when the data was acquired.



	Name	SIG ch	Freq	Spk Gain	Cont Gain	Filter	Threshold	Zoom	F
>>1	sig001	1	40000	19000	1000	On	334 (25.75 μ V)	1.0	
2	sig002	2	40000	19000	1000	On	-431 (-33.23 μ V)	?	
3	sig003	3	40000	19000	1000	On	-369 (-28.45 μ V)	?	
4	sig004	4	40000	19000	1000	On	-412 (-31.76 μ V)	?	
5	sig005	5	40000	19000	1000	On	-448 (-34.54 μ V)	?	
6	sig006	6	40000	19000	1000	On	-377 (-29.07 μ V)	?	
7	sig007	7	40000	19000	1000	On	-327 (-25.21 μ V)	?	
8	sig008	8	40000	19000	1000	On	-357 (-27.52 μ V)	?	
9	sig009	9	40000	19000	1000	On	397 (30.61 μ V)	?	
10	sig010	10	40000	19000	1000	On	-366 (-28.22 μ V)	?	
11	sig011	11	40000	19000	1000	On	-358 (-27.6 μ V)	?	
12	sig012	12	40000	19000	1000	On	-374 (-28.83 μ V)	?	
13	sig013	13	40000	19000	1000	On	363 (27.99 μ V)	?	
14	sig014	14	40000	19000	1000	On	-401 (-30.92 μ V)	?	
15	sig015	15	40000	19000	1000	On	-335 (-25.83 μ V)	?	
16	sig016	16	40000	22000	1000	On	-325 (-21.64 μ V)	?	
17	sig017	17	40000	19000	1000	On	-340 (-26.21 μ V)	?	
18	sig018	18	40000	19000	1000	On	-344 (-26.52 μ V)	?	
19	sig019	19	40000	18000	1000	On	-615 (-50.05 μ V)	?	
20	sig020	20	40000	19000	1000	On	-380 (-29.3 μ V)	?	
21	sig021	21	40000	19000	1000	On	-395 (-30.45 μ V)	?	
22	sig022	22	40000	19000	1000	On	-333 (-25.67 μ V)	?	
23	sig023	23	40000	19000	1000	On	-336 (-25.9 μ V)	?	
24	sig024	24	40000	19000	1000	On	-317 (-24.44 μ V)	?	

The **Channel Parameters View** can contain different items depending on the file type and the data in the file.

- **Name** – refers to the channel name. Channels can be renamed by clicking in this column and typing a new name.
- **SIG ch** – is for PLX files only and it shows the channel number on the SIG board that is associated with this channel.
- **Freq** – shows the digitization frequency for this channel
- **Spk Gain** - indicates the overall gain setting for the spike signal path, taking into account all preamp and final amplification gains.
- **Cont Gain** - indicates the overall gain setting for the continuous data path, taking into account all preamp and final amplification gains.

By right clicking in the Channel Parameter view and selecting '**Adjust Gains**', the gain values can be changed for a channel. See [“4.2.4 Adjusting Gains” on page 95](#).

- **Filter** – is for PLX files only and it indicates if the filter is switched on for the channel.

-
- **Threshold** – shows the threshold value in A/D counts and the associated voltage that was used to collect the waveforms. For continuously-recorded data files, the threshold for the currently selected channel can be changed by typing a new value.
 - **Zoom** – refers to the scaling factor that Offline Sorter uses to display the waveforms in the Waveform View and cluster display. The scaling factor for the currently selected channel can be changed by typing a new value.

3.6.3 File Summary View

The **File Summary View** contains various items of interest for the currently-loaded file, in a two-column spreadsheet format.

File Summary	
File Parameters:	
File Name:	E:\PlexonData\au101602a.plx
Time & Date (from header):	Wednesday, October 16, 2002 09:33:41
Comment:	
File Size (bytes):	67446880
Experiment Length (sec):	601.283
Format Version:	102
Waveforms:	
Channels:	96
Data Type:	Single
Timestamp Frequency (Hz):	40000.000
A/D Frequency (Hz):	40000.000
Waveform Length (µsec):	800
Prethreshold (µsec):	200
Number of Points per Waveform:	32
Total Number of Waveforms:	763463
Peak Voltage at ADC input (mV):	3000
Preamp Gain:	1000
Full-Scale Voltage at probe (mV):	3.000
A/D Resolution (bits):	12
Voltage Resolution at probe (mV/bit):	0.001465
Units:	
Total Number of Units:	124
Number of Channels with Units:	74
Avg Units per Active Chan:	1.676
Continuous Data:	
Channels:	64
Peak Voltage at ADC input (mV):	5000
Preamp Gain (for first cont. chan):	500
Full-Scale Voltage at probe (mV):	10.000
A/D Resolution (bits):	12
Voltage Resolution at probe (mV/bit):	0.004883
Events:	
Channels:	4
Total Count:	1474

File Parameters:

- **File Name** – shows the full path to the currently-loaded file on disk.

-
- **Time & Date (from header)** – shows the time and date that the data was collected, as the data acquisition software wrote the information into the file header. If the time and date is unavailable from the file header, the time & date of the file itself appears.
 - **Comment** – lists the comment field in the file header, if present.
 - **File Size (bytes)** – indicates the size of the currently-loaded file in bytes.
 - **Experiment Length (sec)** – is the total amount of data in seconds that is in the current file.
 - **Format Version** – shows (for some file formats) the version of the file format currently loaded.

Waveforms:

- **Channels** – shows the total number of spike channels in the file.
- **Data Type** – indicates whether the data type is Single, Stereotrode, or Tet-rode.
- **Timestamp Frequency (Hz)** – shows the frequency in Hz of the timestamp clock.
- **A/D Frequency (Hz)** – shows the frequency in Hz of the analog-to-digital (A/D) conversion while the data was collected.
- **Waveform Length (μsec)** – indicates the number of clock ticks (at the analog-to-digital frequency) of data for each spike waveform.
- **Prethreshold (μsec)** – indicates the number of clock ticks collected prior to the triggering event (threshold crossing).
- **Number of Points per Waveform** – indicates the number of samples taken per spike waveform (waveform length * A/D frequency).
- **Total Number of Waveforms** – indicates the total spikes (waveforms) in the file.
- **Peak Voltage at ADC input (mV)** - shows the voltage at the ADC input that will produce the maximum analog-to-digital converter count for spike channels.
- **Preamplifier Gain** - shows the total amount of gain in the preamps for spikes.
- **Full-Scale Voltage at probe (mV)** - shows the voltage at the probe that will produce the maximum analog-to-digital converter count for spike channels.
- **A/D Resolution (bits)** – is the resolution of the analog-to-digital (A/D) converter for the spike channels.
- **Voltage Resolution at probe (mV/bit)** - shows the amount of voltage at the probe that will produce a change of 1 analog-to-digital converter count, when all gains and ADC voltage ranges are taken into account.

Units:

- **Total Number of Units** – the total number of units on all channels for the current file.
- **Number of Channels with Units** – indicates the number of channels that have >0 units.
- **Avg Units per Active Chan** – is the value in **Total Number of Units** divided by the value in **Number of Channels with Units**.

Continuous Data:

- **Channels** – indicates the total number of slow continuous channels in the file.
- **Peak Voltage at ADC input (mV)** - shows the voltage at the ADC input that will produce the maximum analog-to-digital converter count for continuous channels.
- **Preamplifier Gain** - shows the total amount of gain in the preamps for the first continuous channel. Note that Offline Sorter allows each continuous data channel to have a different gain, and this only displays the value for the first continuous data channel. See the Channel Parameters View to see the gains for each continuous channel. This just gives a ‘typical’ value assuming that the gains for all continuous data channels are the same as the first channel.
- **Full-Scale Voltage at probe (mV)** - shows the voltage at the probe that will produce the maximum analog-to-digital converter count for the first continuous channels. Again, the gain in each channel may be different; this just shows a typical value.
- **A/D Resolution (bits)** – is the resolution of the analog-to-digital converter for the slow channels.
- **Voltage Resolution at probe (mV/bit)** - shows the amount of voltage at the probe that will produce a change of 1 analog-to-digital converter count, when all gains and ADC voltage ranges are taken into account. This again just shows a typical value based on the gain for the first continuous data channel.

Events:

- **Channels** – indicates the number of events with unique ID codes in the file
- **Total Count** – indicates the total number of events in the file, for all event channels

3.6.4 Time Segments View

Offline Sorter has the ability to divide a file into an arbitrary number of time segments. These time segments can represent periods when different experimental conditions were in effect, or they can just be evenly spaced within the file in order to see how conditions evolved as the experiment progressed. Offline Sorter can calculate sort quality parameters on a segment-by-segment basis, and can display

the time segment boundaries on various views. The Time Segments View shows the current division of the file into time segments in a tabular format, with a count of waveforms and the values of calculated sort quality measures for each time segment. See “3.9.1 Time Segments” on page 64.

Time Segments										
	Segment	Start	End	Waveforms	Unsorted	Unit a	Unit b	J3	Pseudo-F	D-B
>>0	Entire File	0.000000	601.283425	7386	0	5010	2376	1.096066	8093.354	0.316244
1	Segment 1	0.000000	60.128342	2601	0	2262	339	0.367893	956.1552	0.364722
2	Segment 2	60.128342	120.256685	1010	0	689	321	1.129458	1138.494	0.320715
3	Segment 3	120.256685	180.385027	694	0	467	227	1.018539	704.8292	0.312256
4	Segment 4	180.385027	240.513370	240	0	89	151	2.875035	684.2585	0.247577
5	Segment 5	240.513370	300.641712	265	0	67	198	2.254888	593.0356	0.278686
6	Segment 6	300.641712	360.770055	284	0	140	144	2.994277	844.3862	0.235718
7	Segment 7	360.770055	420.898397	297	0	123	174	2.000837	590.2469	0.291477
8	Segment 8	420.898397	481.026740	426	0	258	168	2.071340	878.2485	0.271106
9	Segment 9	481.026740	541.155082	487	0	259	228	1.712107	830.3721	0.292423
10	Segment 10	541.155082	601.283425	1082	0	656	426	1.385761	1496.622	0.321650

3.6.5 Sort Details View

The **Sort Details View** shows information about the channels that have been sorted during this session of Offline Sorter. Channels that have not yet been loaded during this session of Offline Sorter will have ‘?’s in their entries.

Sorting Details									
	Name	#Units	Sorted by	X	Y	Z	Sort Range		
1	sig001	2	Valley	PC 1	PC 2	PC 3	0 to 32		
2	sig002	2	Contours	PC 1	PC 2	PC 3	0 to 32		
>>3	sig003	2	T-Dist E-M	PC 1	PC 2	PC 3	0 to 32		
4	sig004	?	?	?	?	?	?		
5	sig005	?	?	?	?	?	?		
6	sig006	?	?	?	?	?	?		

The **X**, **Y**, and **Z** columns show which feature is selected for each dimension of feature space.

The **R** columns show the range covered by the feature values - the column width can be expanded by dragging the column header to see these values.

The **Sort Range** column indicates how much of the waveform width (in clock ticks) was used in the sorting.

The contents of this view is essentially what is written out to a TPL file. For more information, see “5.13.1 Saving Templates” on page 214.

3.6.6 Events View

The **Events View** displays the events that are present in the data file. Individual events can be selected to show in the Timeline View and the Rasters views. For more information, see “5.9 Examining Events” on page 207.

3.6.7 Stats View

The **Stats View** contains the results of statistical tests on the separation of selected unit clusters in 2D and 3D feature space for the current channel. The page is blank if a channel is not loaded. For more information, see [“4.7.2.1 Info Grid View Statistics” on page 157](#).

3.6.8 PCA View

The **PCA View** displays the eigenvectors and eigenvalues for the waveforms in the current channel. The first 8 eigenvalues from the PCA appear at the top of the display. The eigenvector corresponding to each of those eigenvalues appears in a column directly underneath the eigenvalue. This is a grid-based view that numerically shows the same information displayed in graphical form in the [“3.17 PCA Results View” on page 83](#).

3.6.9 Templates View

The **Templates View** displays a numerical representation of the template for each unit. These templates can be copied and pasted into other analysis programs. Note that the templates will not be displayed if Offline Sorter is running in Demo Mode.

3.6.10 Adaptive Templates History View

The Adaptive Templates History View is only relevant if an adaptive templates sort has been performed. It shows all the templates used for each unit, with the time that each template was put into effect. This is a tabular view of the same information that is displayed graphically in the Templates vs Time View. See [“4.5.4.2 Applying Adaptive Template Sorting” on page 133](#). Note that the templates will not be displayed if Offline Sorter is running in Demo Mode.

3.6.11 Scan View

The **Scan View** is a data exploration tool that allows multiple automatic and semi-automatic sortings on a channel while stepping the value of a sorting parameter over a range of values. This provides a feeling for how the sorting parameters affect the sorting quality. For more information, see [“5.14 Parameter Scans” on page 217](#).

3.6.12 Chan Map View

The **Chan Map View** shows how the raw channel numbers from the data files map to the Offline Sorter channel numbers. Each Offline Sorter channel has a line that shows which raw channel(s) are associated with it. For more information, see [“5.2 Channel Mapping” on page 181](#). The Chan Map View can be used to change the Data Type and to map and re-map raw channels in arbitrary ways.

3.6.13 Messages View

When Offline Sorter does some significant processing or otherwise reaches a milestone, it makes an entry into a Message Log to that effect. This Messages View displays the contents of the Messages Log. Consulting the Messages View

is useful if it becomes necessary to debug a problem with Plexon Support, and also as a reminder of what actions were taken recently.

The right-click menu in the Messages View contains convenient entries to select and copy the log to the Windows clipboard, to save the log to a text file, and to prepare an E-mail to support@plexoninc.com containing the contents of the log. Please only send this E-mail if directed to do so by Plexon support personnel.

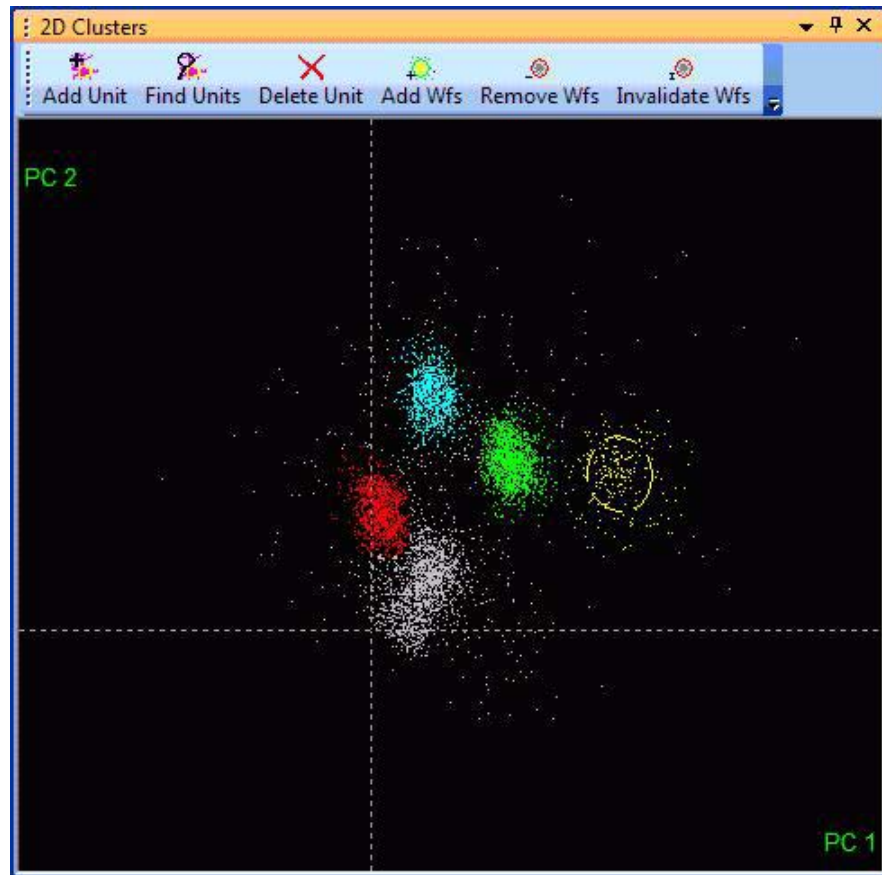
Note that there is an additional level of detail for log messages that by default do not show up in the message log. Select '**Show Debug Messages**' from the right-click menu to cause these more detailed messages to appear in the message log.

3.7 Clusters Views


Although they are treated as separate views in Offline Sorter, the 2D Clusters View and the 3D Clusters View share many features in common. Both Clusters Views show the waveforms in feature space. The 2D Clusters View shows the waveforms as points in a 2-dimensional feature space, and the 3D Clusters View displays points in a 3-dimensional feature space. Both the 2D and 3D Clusters Views can be active at the same time.

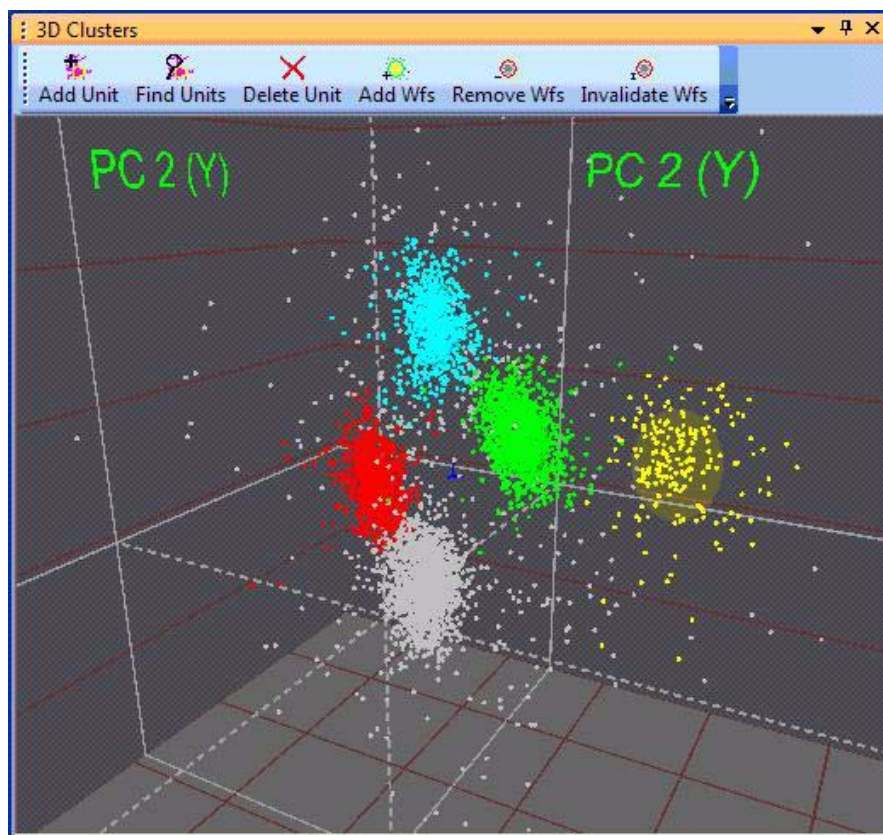
To display the 2D Clusters View

- Click the 2D Clusters toolbar button , or, select the **View | 2D Clusters** menu command.



To display the 3D Clusters View

- Press 3D Clusters toolbar button , or, select the **View | 3D Clusters** menu command.



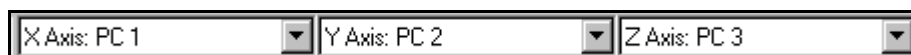
Features common to both the 2D and 3D views are discussed in the [3.7.1 Clusters View Common Functionality](#) section that follows. Features specific to either view are discussed in “[3.7.1.13 2D Clusters View Details](#)” on page 53 and in “[3.7.1.14 3D Clusters View Details](#)” on page 57.

3.7.1 Clusters View Common Functionality

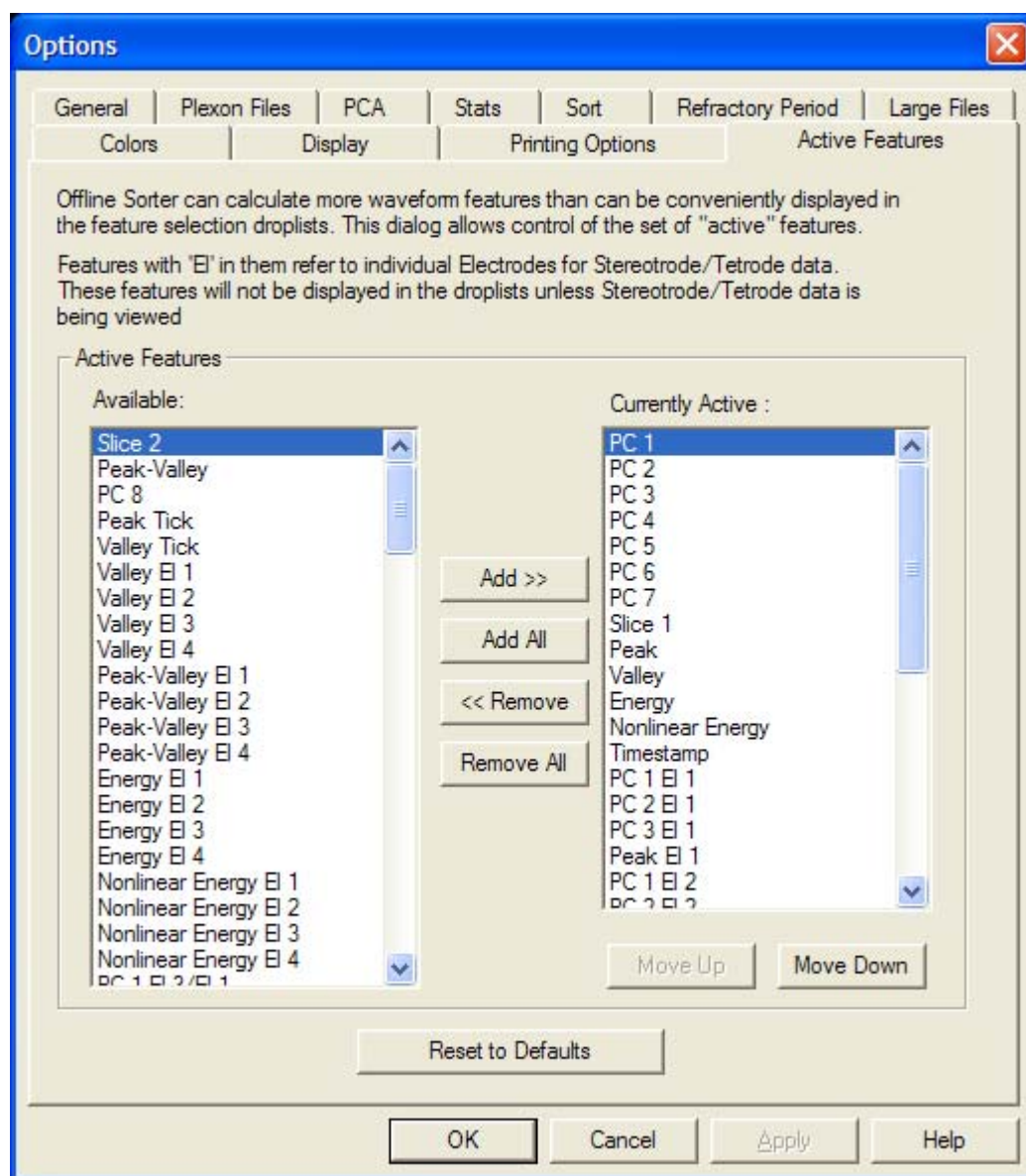
The following features are common to both the 2D Clusters View and the 3D Clusters View:

3.7.1.1 Axis Feature Selection

The variables shown on the **X Axis** and **Y Axis** (for the 2D Clusters View) and the **Z Axis** (for the 3D Clusters View) can be selected from among any of the “[5.5 Features Available for Sorting](#)” on page 191 by using the **X Axis**, **Y Axis**, and **Z Axis Drop-Down Feature Lists** on the **Toolbar**.



Only the currently “active” features show up in the drop-down feature lists. The currently active features in Offline Sorter are a subset of the [“5.5 Features Available for Sorting” on page 191](#). To control which features are active, use the **Active Features** tab in the **Tools | Options** dialog box.



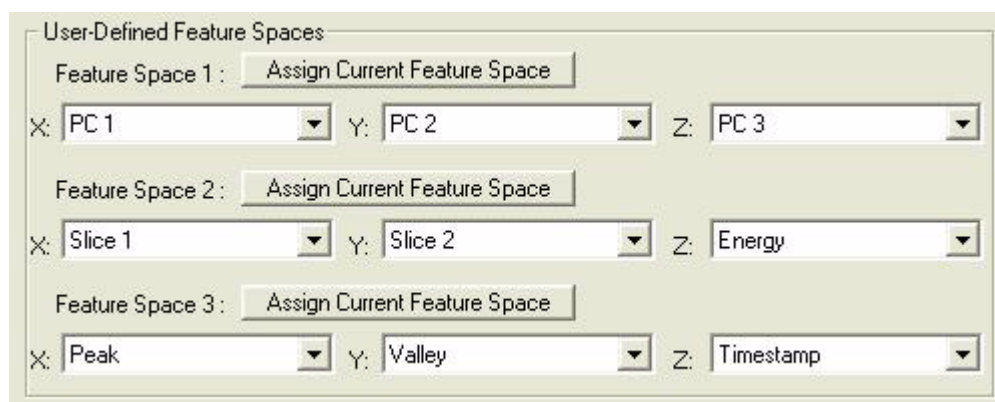
The currently active features appear in the right-hand list box, and the currently inactive (and thus available for activation) features appear in the left-hand list box. Select one or more features in either list box, then press the **Add >>** or **<< Remove** buttons to transfer features between list boxes. The order in which the active features show up in the drop-down feature lists is the same as the order in the **Currently Active** list box. Re-order the items in this list box by selecting one or more features and pressing the **Move Up** or **Move Down** buttons. Pressing the **Reset to Defaults** button reverts the set of active features to the as-shipped defaults.

Some of the features are relevant only for stereotrode or tetrode data; these features, although they can be active, do not show up in the drop-down feature lists when single-electrode data is being viewed in Offline Sorter.

3.7.1.2 User-Defined Feature Spaces

Offline Sorter can remember up to three Feature Space definitions that are commonly used, and it enables one quickly to put those pre-defined Feature Spaces into effect. For example, if there is a constant switching between a {PC1, PC2, PC3} feature space and a {Slice 1, Peak, Valley} feature space, Feature Space 1 can be defined to be {PC1, PC2, PC3} and Feature Space 2 to be {Slice 1, Peak, Valley} by using the **User-Defined Feature Spaces** section of the **Feature Calc** tab of the **Tools | Option** window. Then, one can use the toolbar buttons corresponding to each of the three user-defined feature spaces or the menu items under **Select | Feature Space Definitions** (which can be bound to keystrokes for faster selection) to flip between the feature spaces, without having to use the toolbar feature drop lists.

User-Defined Feature Spaces include three dimensions of a feature space. If a 2D feature space is used or displayed, only the first two dimensions are used.



To define a user-defined feature space, use the drop-lists to select the feature for each dimension. Or, if the Clusters View is already showing the desired feature space, click **Assign Current Feature Space**.

3.7.1.3 Waveform Highlighting

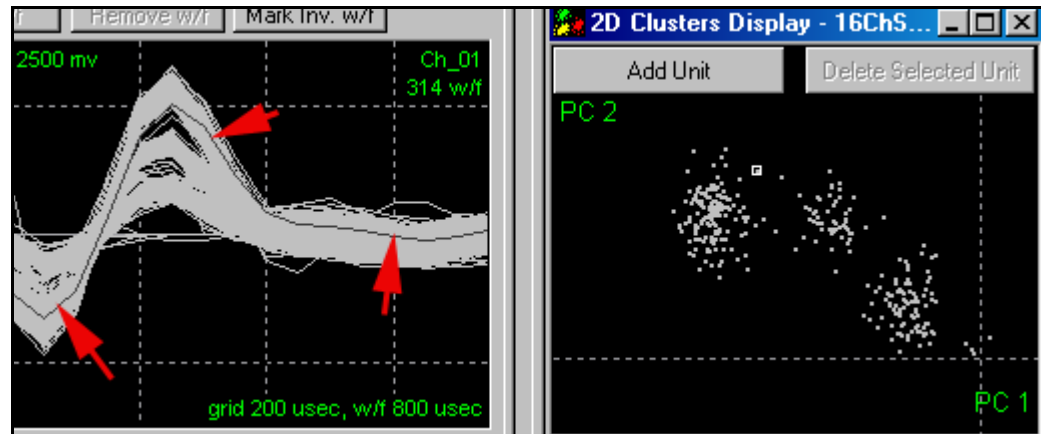
The Clusters Views allow viewing the waveform shape corresponding to any point in the display.

To view the waveform

- 1 Left-click in the 2D Clusters view.
- 2 Left-click while holding down the **ALT** key in the 3D Clusters View.

The pointer becomes crosshairs, the nearest point in the Clusters View is highlighted with a box, and the corresponding waveform is shown in the Waveform View to the left (**red arrows**). If the waveform is in the time range being dis-

played, the selected waveform is also highlighted with a white box in the Timeline View.



If the mouse pointer is moved while pressing the left button, the Waveform View and Timeline View select the waveform currently closest to the mouse pointer.

On the bottom status bar of the application, Offline Sorter displays the exact X, Y, and Z (if applicable) feature coordinates for the highlighted waveform

X: 606.9104 Y: -2395.08 Z: -2395.08

as well as the timestamp associated with the highlighted waveform.

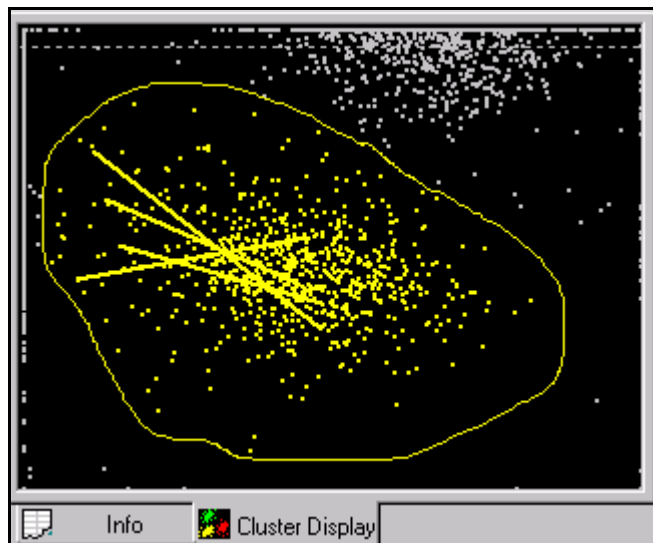
3.7.1.4 Controlling Which Waveforms Are Displayed

If the **Control Displays** checkbox is selected, the scroll bar at the bottom of the [3.5 Timeline View](#) can be used to control the temporal range of the waveforms shown in the Clusters View. The **Clusters Views** parameter in the [3.2 Control Grid](#) can be used to restrict which waveform types are displayed in the Clusters Views (either **All**, or only those shown in the Waveform View).

Unit clusters in feature space can be defined by using either the manual Contours method (See [“3.4 Units View” on page 29.](#)) or other sorting methods. Specific waveform points from a unit cluster can be added or removed by selecting the **Units | Add (Remove) Waveforms from the Selected Unit** menu commands. The Waveform View can be used to add or remove waveforms by crossing them. Also, one can select and add or remove individual waveforms from a unit in the Continuous Data Display by using the right-click menu.

3.7.1.5 Short ISI Spike Pairs Display

The Cluster Display shows the waveforms assigned to the same unit with inter-spike intervals less than the Refractory ISI (inter-spike interval) by drawing a line between the two corresponding dots in feature space.









One can turn on or turn off the drawing of lines between spikes with small ISIs by using either **Show Short ISI Lines** in the context menu or by using the **Tools | Options** menu command and selecting the **Refractory Period** tab. One can set the Refractory ISI either in the **Tools | Options | Refractory Period** tab or in the [3.2 Control Grid](#) using the **Refr ISI** edit box.

3.7.1.6 Cluster Positioning


One can expand or contract the cluster image in feature space along the

x (, ) and

y (, ) axes or move it

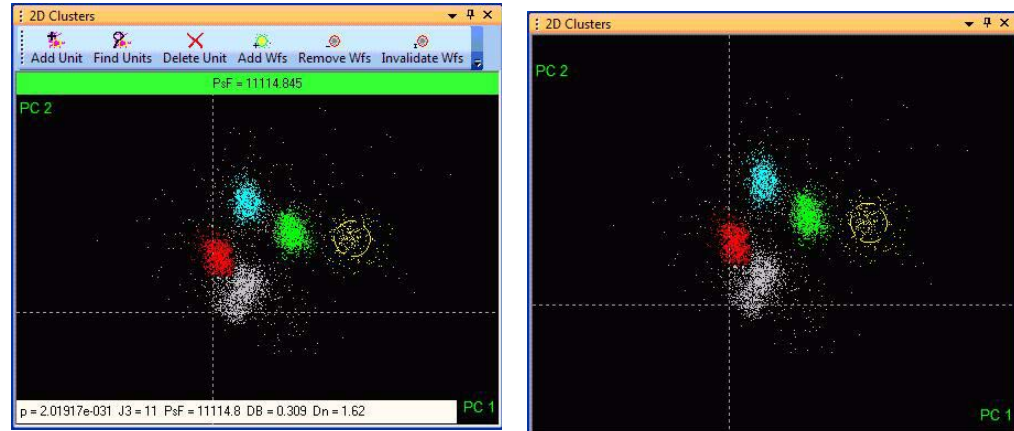
left , right , up , or down 

by using the corresponding toolbar buttons, or by using selections from the context menu. Also, one can right-click and move the mouse up-and-down to zoom the display in and out. The display can be panned by left-clicking, holding the SHIFT key down, and moving the mouse.

The zoom reset function ( in the toolbar, or **Reset Range and Zoom** in the context menu) resets the zoom and pan to the original range, which is calculated so as to include all the points.

3.7.1.7 Per-View Toolbars, Hints and Statistics Display

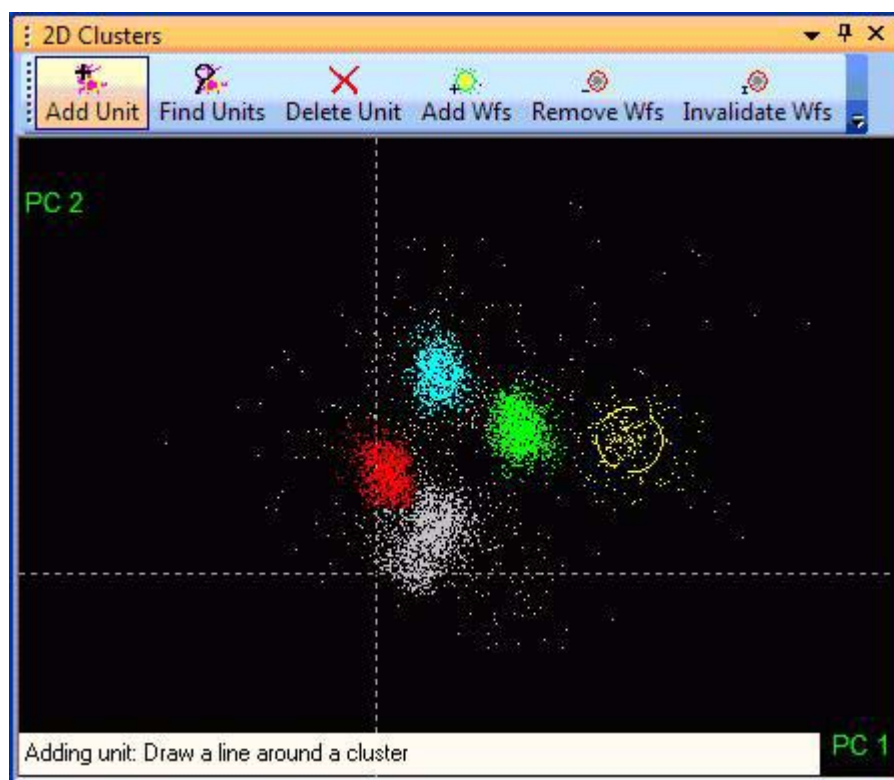
The buttons displayed on the top and the operational hints and statistics shown at the top and bottom of the Clusters Views can be *on* (following left) or *off* (following right).



To show or hide the toolbar, click on the triangle in the windows title bar, or right-click in the title bar and select the **Toggle Toolbar** menu option.

The text box at the top of the display shows the current value of a selected cluster quality metric, along with the percentage change of that metric that resulted from the last sorting-related change. If the last change improved the cluster quality, the box appears in green and if it degraded the cluster quality, the box appears in red. This can be very useful when deciding which unit to assign ‘borderline’ waveforms to. Any of the available cluster quality metrics described in [“5.7 Sorting Quality Statistics” on page 203](#) can be used for this display - the metric can be chosen in the **Stats** tab of the **Tools | Options** dialog box. To show or hide this text box, select the **Show Cluster Quality** menu option in the right-click menu.

The text box at the bottom of the screen will either show the values of all Sort Quality Metrics described in [“5.7 Sorting Quality Statistics” on page 203](#) (above), or an operational hint (below), depending on context.



Whether or not this text is displayed is controlled by the Show Hints and Show Statistics menu commands in the right-click menu.

If the **Show Ellipses** (or **Show Ellipsoids** on the 3D Clusters View) menu command is selected, ellipses/ellipsoids for each unit are shown on the display. The ellipses are calculated by finding the standard deviations along the principal axes. The ellipse or ellipsoid is scaled by the number of standard deviations specified by the **Outlier Threshold** in the Control Grid.

3.7.1.8 Colors

To set the colors assigned to each unit in the Clusters Views (and Units View), select **Tools | Options** menu command and select the **Colors** tab.

3.7.1.9 Point Size

To set the point size in the Clusters Views, select the **Display** tab under the **Tools | Options** dialog box.

3.7.1.10 Automatic Cluster Redraw

To update the Clusters View immediately when the **Slice 1**, **Slice 2**, **Slice 3**, or **Slice 4** values change, select the **Redraw Cluster Display Immediately when Slice Position Changes** in the **Display** tab under the **Tools | Options** dialog box.

3.7.1.11 Zooming and Panning

Both of the Clusters Views support similar mechanisms to zoom and pan in the other display. Hold down the right mouse button and move the mouse vertically to zoom the display in and out. To pan the display, hold down the `SHIFT` key, then hold down the left mouse button, and move the mouse in the direction that to pan the display. The pointer becomes a hand-shaped symbol when zooming or panning occurs.

Note: The displays can be zoomed and panned using toolbar buttons or context menu entries. Both zooming and panning can be reset by using the **Reset Zoom** toolbar button, or by using the **Reset Range and Zoom** context menu option. This sets the display to default such that all the points are visible in the field of view.

3.7.1.12 Context Menus

Both of the Clusters Views support context menus (commonly known as right-click menus), although the menu items available in each differ slightly. To bring up the context menu, hold down the `ALT` key and then click the right mouse button.

The menu items common to both the 2D and 3D Clusters views are:

- **Add Unit** – creates a new unit using Contour sorting, i.e. circling the waveforms in the new unit. This is the same as pressing the **Add Unit** button in the Per-View Toolbar.
- **Find Units** - executes the currently-selected Sort Method to find new units. This is the same as pressing the **Find Units** button in the Per-View Toolbar.
- **Clear Centers** - this command is only relevant if the K-Means or Std E-M Sort Methods are in effect, and some unit centers have been selected on the view (See [“4.5.6 Semi-Automatic Clustering using K-Means” on page 137](#) and [“4.5.7 Semi-Automatic Clustering using Standard E-M ” on page 141](#)). Selecting this menu item will clear the unit centers to allow the user to start again.
- **Delete Selected Unit** – removes the unit currently highlighted in the Units Display. This is the same as pressing the **Delete Unit** button in the Per-View Toolbar.
- **Delete All Units** – removes all units currently defined for the channel.

Note: The above two options are also available from the **Units** menu on the main menu bar.

- **Add Waveforms to the Selected Unit** - allows adding new waveforms to an existing unit by circling them. This is the same as pressing the **Add Wfs** button in the Per-View Toolbar.

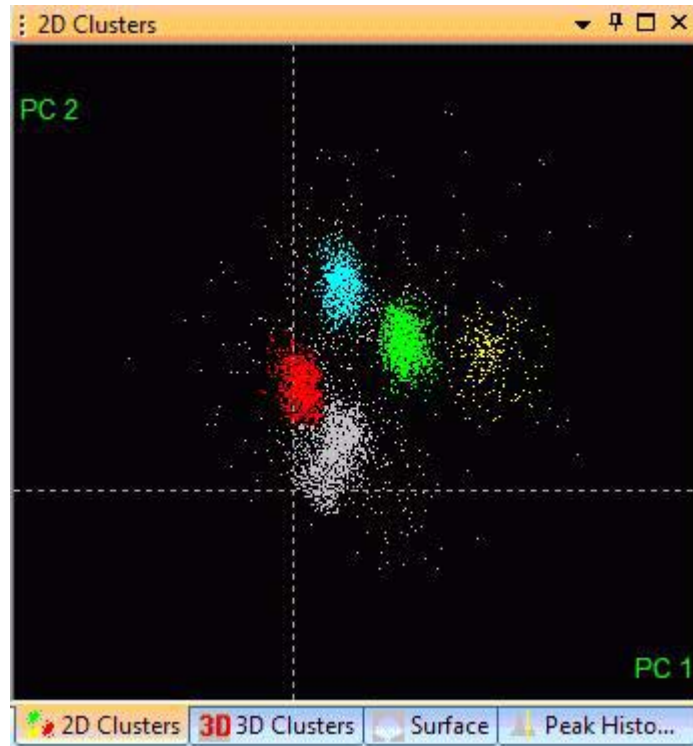
-
- **Remove Waveforms from the Selected Unit** - allows removing waveforms from an existing unit by circling them. This is the same as pressing the **Remove Wfs** button in the Per-View Toolbar.
 - **Invalidate Waveforms** - allows marking waveforms as being invalid by circling them. This is the same as pressing the **Invalidate Wfs** button in the Per-View Toolbar.
 - **Show Buttons** – shows the buttons as described in “[3.7.1.7 Per-View Toolbars, Hints and Statistics Display](#)” on page 50.
 - **Show Hints** – shows text hints as described in “[3.7.1.7 Per-View Toolbars, Hints and Statistics Display](#)” on page 50.
 - **Show Statistics** – shows the Sort Quality Statistics in the Hints area. When Hints are being shown, they use the same area of the screen.
 - **Show Cluster Quality** – shows the selected Sort Quality metric changes at the top of the display, as described earlier.
 - **Show Ellipses (Ellipsoids)**- shows the ellipses for units (ellipsoids for the 3D Clusters View) as described earlier.
 - **X Range, Y Range** – expands, contracts, or shifts the viewing range as described in [Zooming and Panning](#) earlier.
 - **Reset Range And Zoom** – resets the zooming and panning for the view to values calculated so that all the points are visible.

3.7.1.13 2D Clusters View Details

3.7.1.13.1 Showing Dots or a Density Histogram. In response to the selection made with the right-click menu, the 2D Clusters View can display the feature space points as individual dots, a density histogram, or both. Displaying as a density histogram builds a 2-dimensional histogram of the points, and maps the histogram bin contents to a gray-scale. The information that appears in the 2D view is the same information that appears in the Surface View in 3D format; for more information see “[3.8 Surface View](#)” on page 61.

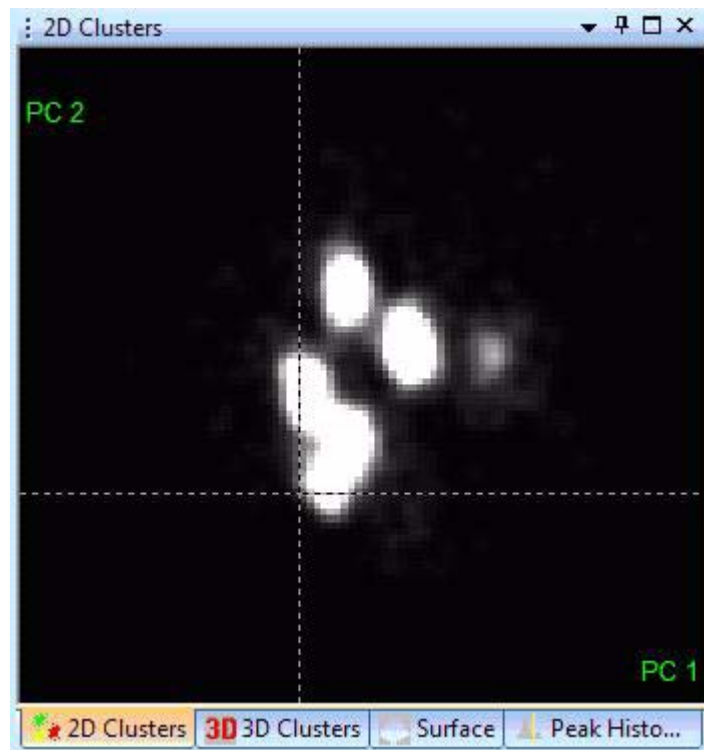
To display the feature space points as dots (the default)

- From the **2D Clusters** shortcut menu (ALT + right-click), select **Show Dots**.



To display the feature space points as a density histogram

- From the **2D Clusters** shortcut menu (ALT + right-click), select **Show a Density Histogram**.



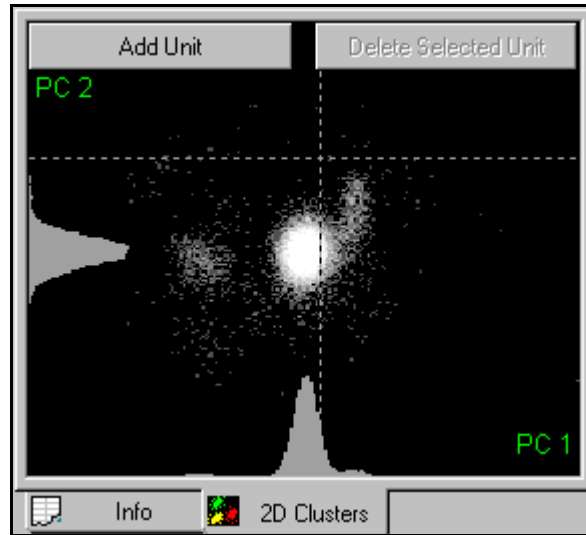
The density histogram can be displayed in linear or log scale. When the density histogram appears, Offline Sorter enables the **Density Histogram Settings** item on the right-click menu. Select this item to display a floating dialog box that can be used to set the contrast, enable/disable smoothing, control the bin size, and to choose between linear or log scaling for the density histogram color assignments. The **Number of Histogram Bins** parameter controls the number of bins per side that make up the density histogram. From the **Smoothing** drop-down list, an algorithm can be selected to smooth out the density histogram by combining and averaging the counts in adjacent bins. The **Histogram Count for Saturation** parameter controls the mapping between the dot density and the grey level scale. If **Histogram Count for Saturation** = 10, for instance, then all cells with more than 10 dots appear in white.



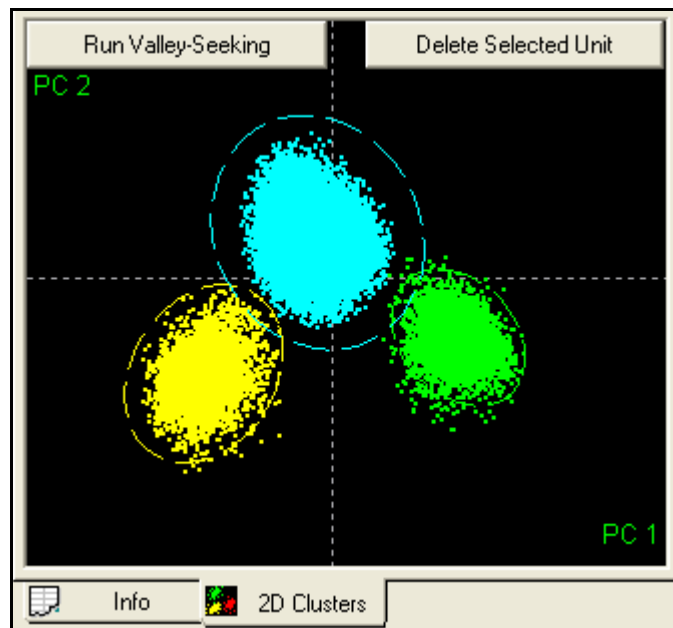
Both the dots and the density histogram can be viewed simultaneously in the 2D Clusters View by selecting the **Show Dots and Density Histograms** right-click (shortcut) menu.

3.7.1.13.2 Showing 1D Histograms. The 2D Clusters View can display histograms of the point distributions in each dimension by selecting the **Show 1D His-**

tograms right-click menu command. The histograms are displayed along the margins.

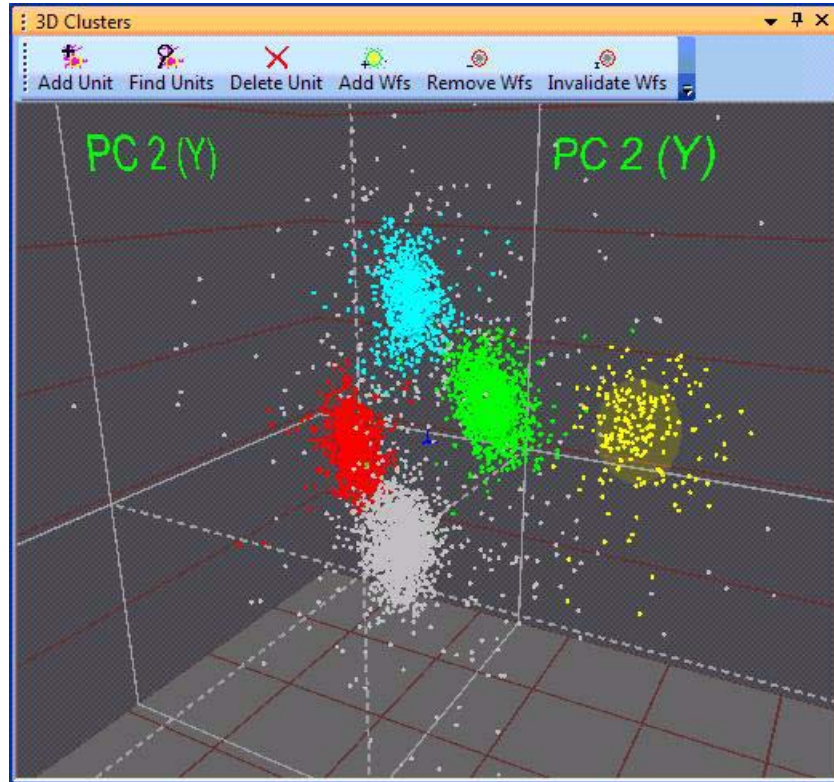


3.7.1.13.3 Showing Ellipses. The 2D Clusters View can display standard deviation ellipses around clusters by selecting the **Show Ellipses** right-click menu command, or by pressing the Ξ key while the 2D Clusters View has input focus. The ellipses are oriented along the principal axes of the cluster of points, with the lengths of the major and minor axes for the ellipse being the standard deviation (sigma) of the points along each axis scaled by the value of the **Outlier Threshold** in the Control Panel. The ellipses in the following illustration show the 4 sigma boundaries around each unit. Offline Sorter draws the ellipses with dashed lines, in the color associated with the unit.



3.7.1.14 3D Clusters View Details

The 3D Clusters View allows changing the eyepoint in 3D feature space via mouse and keyboard actions in order to better visualize the data, and to find the viewing angle where cluster separation is maximized.



For the 3D Clusters View, the +Z axis is initially into the screen. All the points are contained inside a box with grid lines to provide a frame of reference. The dashed grey lines represent the X, Y, and Z axis, and they intersect at (0,0,0) in feature space. The solid grey lines show the projection of the X, Y, and Z axes onto the reference box. The axes projections onto the box are labeled with the feature name represented by that axis.

Components in the 3D display such as the box wall, the box top and bottom, and the grid lines on the box can be shown or hidden using the **Display** tab under the **Tools | Options** dialog. Their colors can be set using **Colors** tab.

The grid lines provide only cues—they do not correspond to actual values of features. The number of grid lines drawn can be controlled in the **Tools | Options Display** tab.

3.7.1.14.1 Positioning the Eyepoint. The eyepoint can be positioned with the following procedure.

To rotate the eyepoint

- Click and hold the **left mouse button**. The pointer changes to a hand. Mouse movement in the left-right direction then rotates the eyepoint around the y

axis, and movement in the up-down direction rotates it about the x axis (pitch the view up-down).

To move the eyepoint nearer or farther away

- Click and hold the **right mouse button**. The pointer changes to a hand. Moving the mouse up moves the eyepoint farther away, and moving it down moves the eyepoint nearer.



HINT

Manipulating the Eyepoint

To get accustomed to manipulating the eyepoint, think of the hand grabbing onto and turning the point clouds.

By default, the eyepoint motion is constrained to not allow “upside down” eyepoints, which can be confusing. This constraint can be relaxed by unchecking the **Constrain view rotation so that +Y axis is always upwards** checkbox in the **Tools | Options | Display** tab.

To animate the eyepoint rotation

- The 3D Clusters View supports a mode of operation that enables the eyepoint for the 3D view to be continuously animated. This can be controlled by using the **Animate the 3D rotation after left mouse release** option in the **Tools | Options Display** tab. When this option is enabled, the eyepoint continues to move in whatever direction it was going when the mouse button is released. This is as if the left mouse button “throws” the eyepoint into an orbit around the look-at point. This option can quickly be toggled on or off by pressing the A key while the 3D Clusters View has focus.

3.7.1.14.2 The Look-At Point (the point of rotation in 3D). Wherever the eyepoint is positioned, the “direction of gaze” is always adjusted so that the eye is looking at a certain point in space. This Look-At point is marked on the 3D Clusters View with a small blue (by default) axis icon.



The display of the Look-At icon can be turned on and off using the **3D Look-At Icon** checkbox in the **Tools | Options Display** tab. The color of the Look-At icon can be changed in the **Tools | Options Colors** tab.

The look-at point can be changed in two ways:

- Manually positioning
- Positioning it to the center of gravity of clusters

To manually position the Look-At point

- 1 Hold down the **Shift key**, then click and hold the **left mouse button**.
- 2 The pointer changes to a hand. Mouse movement then translates the eyepoint in screen space. That is, moving the mouse in the left-right direction translates the Look-At point in the screen x direction, and moving the mouse up-down translates it in the screen y direction.
- 3 To move the Look-At point nearer or farther from the eye, hold down the **SHIFT** key, then click and hold the right mouse button. The pointer changes to a hand. Moving the mouse up and down translates the Look-At point in the screen z direction.



HINT

Moving the Look-at Point

To get accustomed to manually moving the Look-at Point, think of the hand grabbing and pulling the enclosing 3D grid box.

To position the Look-At point at cluster centers

- Press the **C** or **L** key to position the Look-At point at the center of gravity (CG) of the unit clusters, and make a selection by cycling through each assigned unit cluster with a key press. The center of gravity of all points, and the center of gravity of all unassigned points are also included in the cycle. For example, if a channel contains two 2 sorted units, pressing the **C** or **L** keys moves the Look-At point as follows:

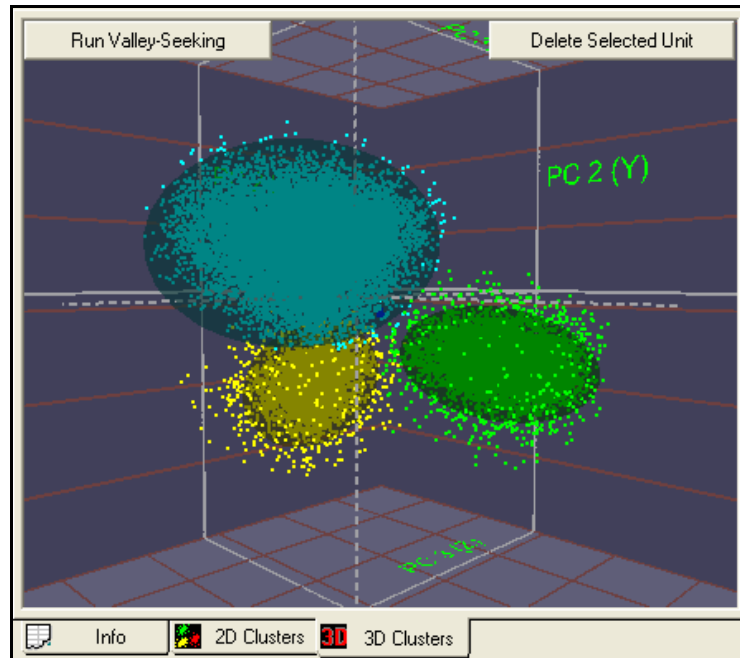
CG of All points -> CG of Unit a points -> CG of Unit b points -> CG of all unassigned points

Resetting the Eyepoint and Look-At points

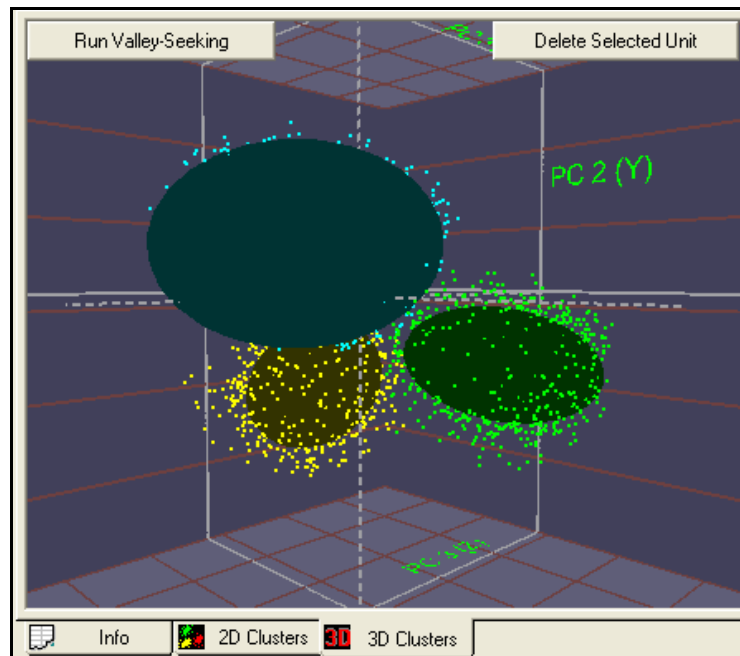
- Pressing the left and right mouse buttons simultaneously resets the eyepoint and Look-At points to their defaults (looking directly down the Z axis, with the Look-At point at the center of gravity of all points).

3.7.1.14.3 Showing Ellipsoids. The 3D Clusters View can display ellipsoids around clusters by selecting the **Show Ellipsoids** right-click menu command, or by pressing the **E** key while the 3D Clusters View has input focus. The ellipsoids are oriented along the principal axes of the cloud of points, with the lengths of the ellipsoid axes being the standard deviation (sigma) of the points along each axis scaled by the value of the Outlier Threshold in the Control Panel. For example, the ellipses shown below show the 3 sigma boundaries around each unit, because the Outlier Threshold was set to 3. Offline Sorter draws the ellipses semi-trans-

parently, in the color associated with the unit. Observe that the points both inside and outside the ellipsoid can be seen.



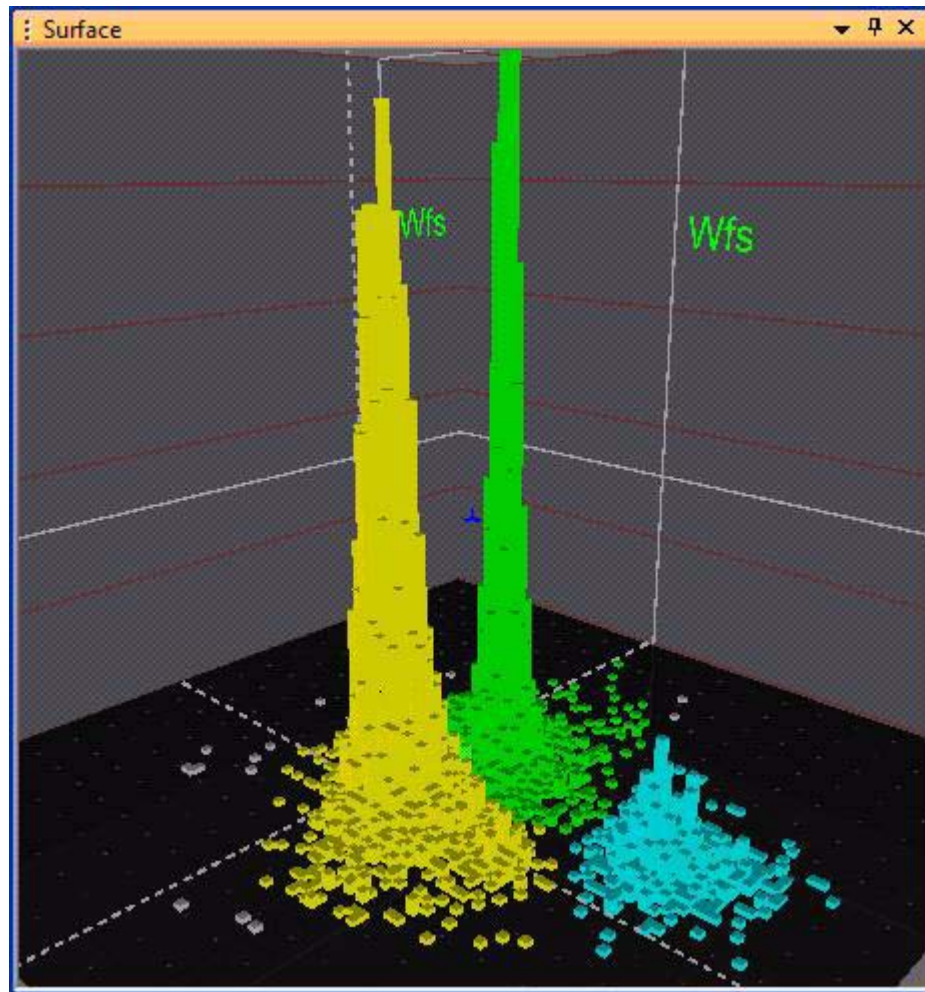
The transparency of the ellipsoid can be changed by pressing the \uparrow and \circ keys (for fade-in, fade-out) while the 3D Clusters View has input focus. The following screenshot is the same as the previous screenshot, except the \uparrow key has been repeatedly pressed until the ellipsoids became totally opaque.



Note: If enabling the ellipsoid display does not cause the ellipsoids to appear, press the \perp key repeatedly, as it is possible that the ellipsoids are there but are completely transparent.

3.8 Surface View

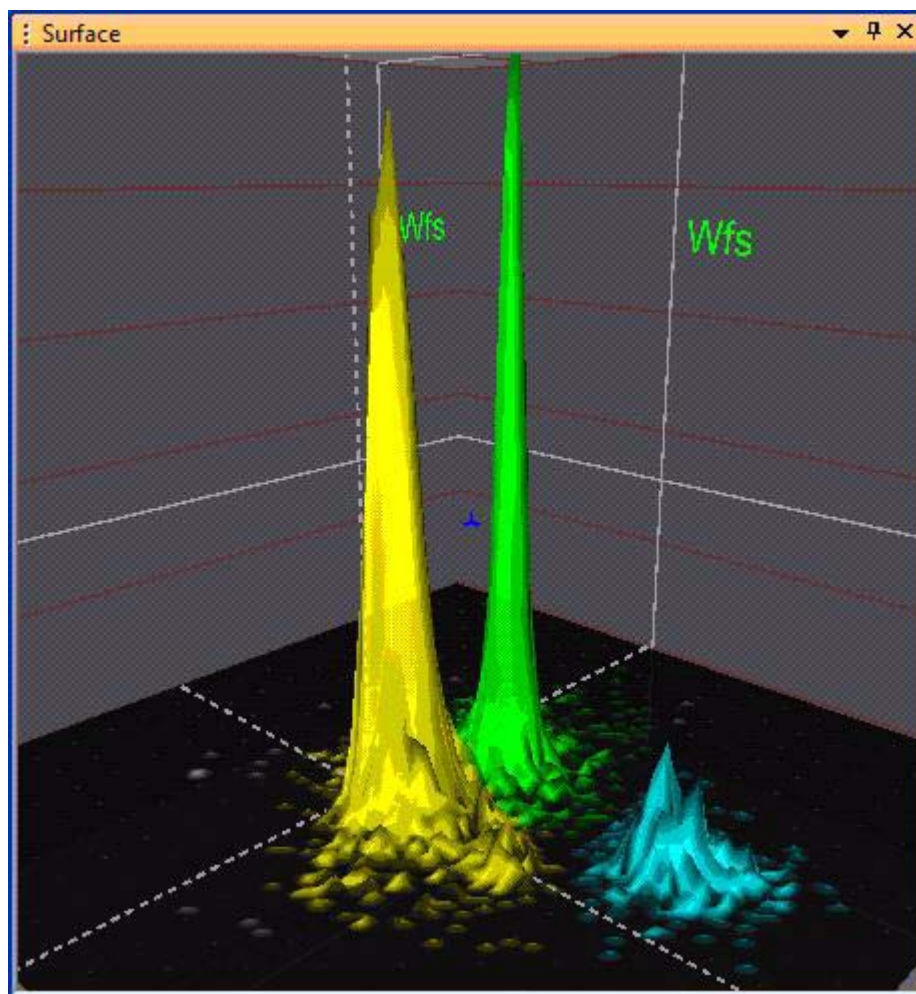
The Surface View displays the same density histogram described in “[3.7.1.13.1 Showing Dots or a Density Histogram](#)” on [page 53](#), except that the histogram appears as a 2D surface in a 3D view as shown in the following illustration.



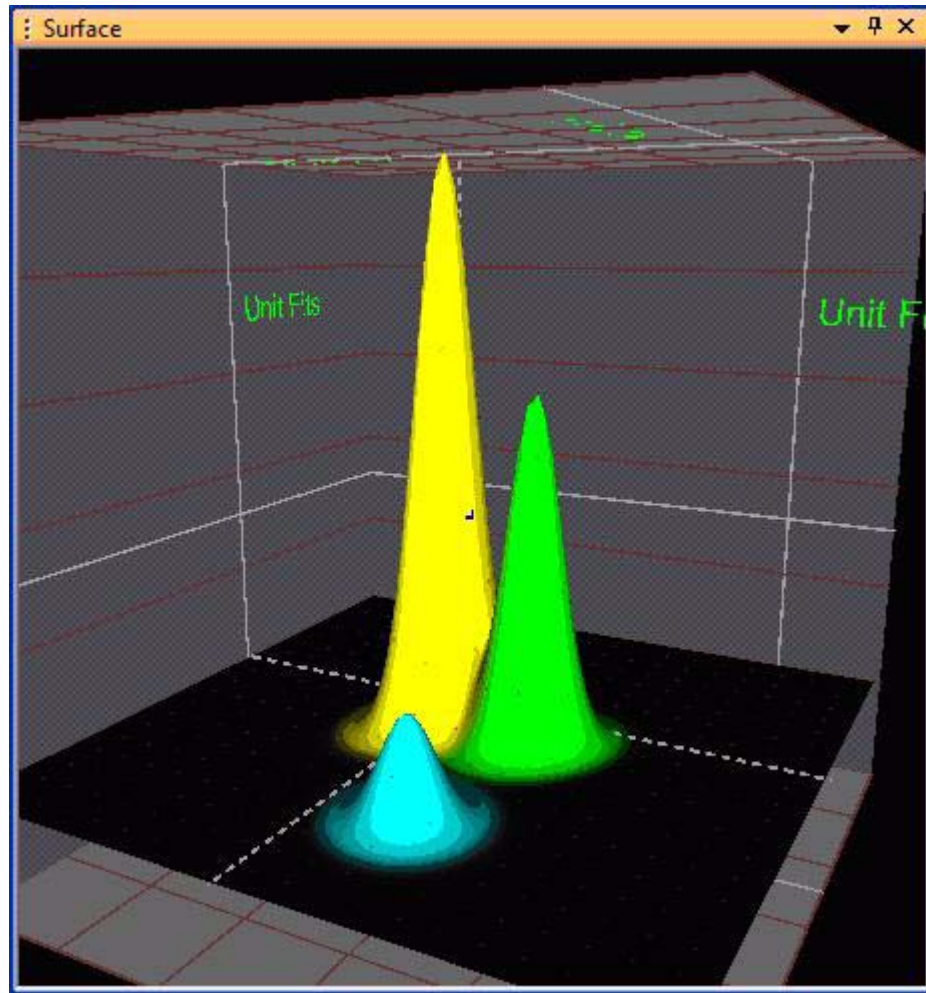
The control for the 3D viewpoint in the Surface View is the same as the control for the 3D Clusters View, which is described in the preceding section. To control the Density Histogram, the same **Density Histogram Settings** dialog box can be used, which appears on [page 53](#). To display the dialog box, from the shortcut (right-click) menu, select **Density Histogram Settings**, or press the **D** key.

The color of the surface above each histogram bin is a blend of the unit colors of the waveforms in that bin. For example, if 60% of the waveforms in a bin are sorted as Unit a, and 40% are sorted as Unit b, the color of the surface above that bin is 60% yellow (by default) blended with 40% green (by default).

By default, the surface view is displaying the data as a tower plot. The tower plot shows a ‘tower’ or elongated box for each bin, where the height of the tower derives from the number of counts in the bin. As shown in the following screen-shot, the Surface View can also display in a mode where the surface has a single vertex for each histogram bin. The number of counts in the bin determines the height of the vertex. Adjacent bins provide adjacent vertices that are connected together to form the surface. To toggle the tower plot mode, select **Show “Tower” Plots** from the right-click (shortcut) menu, or press the **T** key.



In addition to the surface formed by the contents of the Density Histogram, the Surface View can also show another surface by fitting 2-dimensional Gaussians to the existing units. The standard deviations of the points within the unit determine the size of the base of the Gaussian plot, and the height of the Gaussian plot is proportional to the number of points in the unit. To see the Unit Fits surfaces as shown in the following illustration, select **Show Gaussian Unit Fits** from the right-click (shortcut) menu.



To return the display to Density Histogram only, select **Show Density Histogram** from the right-click (shortcut) menu. To show both surfaces simultaneously with transparency, select **Show Both Density Histogram and Unit Fits**. To toggle the display through these settings, press the **F** key. When both surfaces appear together, the two surfaces can be faded by pressing the **I** or **O** key, or the **Contrast** slider in the **Density Histogram Settings** dialog box can be adjusted.

The Surface View can be used to perform Contour sorting. When a contour sort in the Surface View is done, think of the contour as being drawn on the base “counts = 0” plane. Any existing contours are projected onto the surface and plotted as lines.

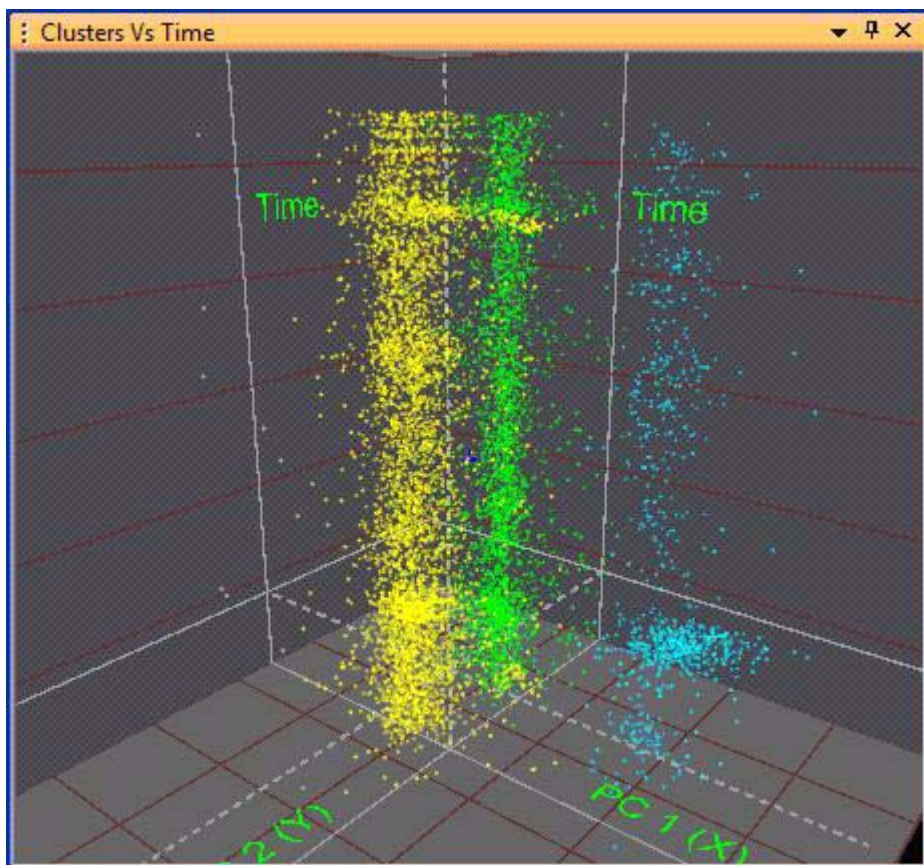
3.9 Clusters Vs Time View

It is useful to examine and understand the time-structure of when spikes occurred in the experiment. The Timeline View provides this functionality while showing the spikes in ‘waveform space’, but the Clusters Vs Time View shows this information in Feature Space. Examining the Clusters Vs Time View can indicate at a glance just how spikes are distributed within the data file (e.g. “Unit A was only

firing for the first half of the experiment”), or that waveform shapes changed as a function of time (e.g. “Unit A drifted away from the noise cluster as the experiment proceeded”).

To display the Clusters Vs Time View

- Select the **View | Clusters Vs Time View** menu command or press the  button on the toolbar.



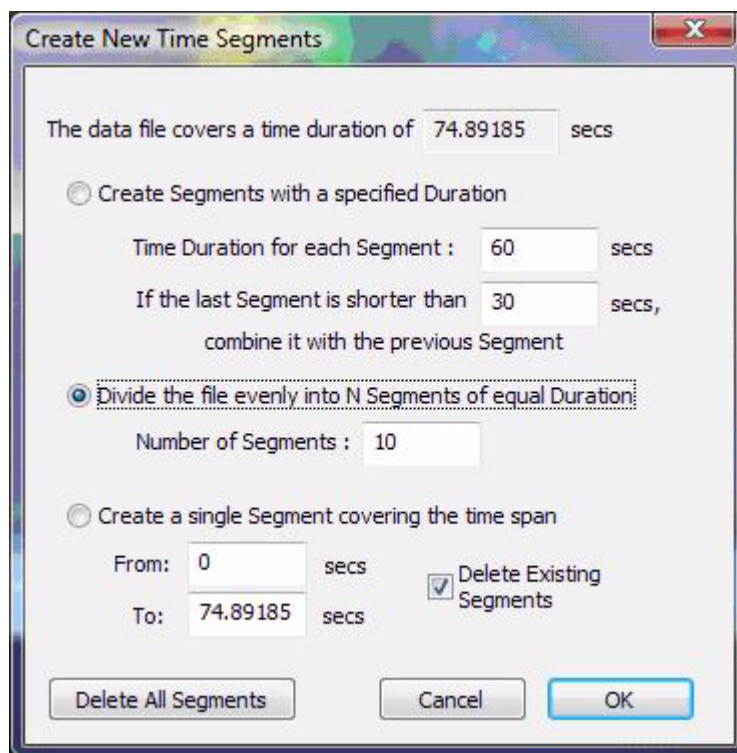
The Clusters Vs Time View shows the waveform points in 2D Feature Space, but time is plotted as the third axis, with the start of the file at the bottom and the end of the file at the top of the screen initially. Thus, a perfectly stable unit whose spikes occurred continuously during the experiment will show up as a continuous vertical column of waveform points.

The Cluster Vs Time View behaves identically to the 3D Clusters View as for how the view point can be rotated and zoomed. See [“3.7.1.14 3D Clusters View Details” on page 57](#). Also, all of the functionality to create and edit units using Contours is available in the Clusters Vs Time View.

3.9.1 Time Segments

As mentioned in [“3.6.4 Time Segments View” on page 40](#), Offline Sorter can divide up the time span covered by the experiment into an arbitrary number of time segments. By default, Offline Sorter will divide up the file into 10 evenly spaced time segments. To change how the file is divided into time segments,

select **Waveforms | Create Time Segments** from the main menu, or **Create New Segments** from the right-click menu in the Time Segments View. A dialog will appear that allows one to divide up the file into time segments in various ways:

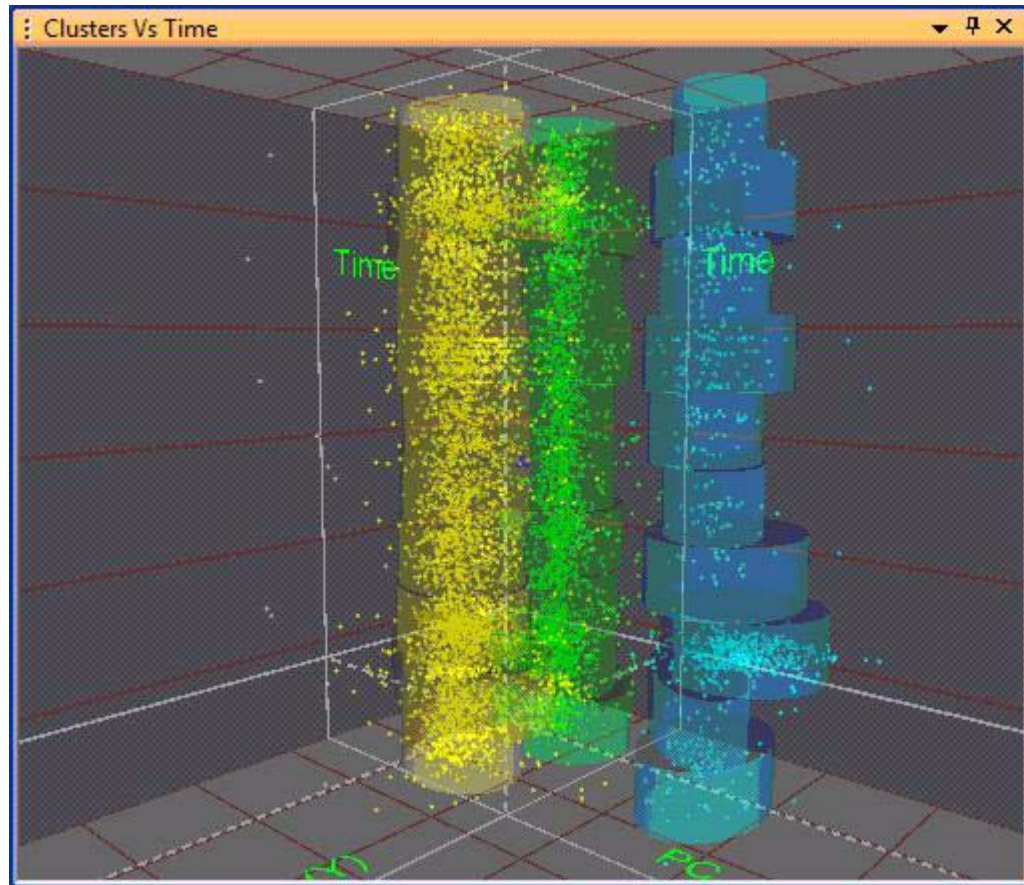


To divide up the file into time segments that have a specified duration, select the first radio button. With this method, there may be 'leftover' time at the end of the file, the method allows one to specify whether that leftover time should be combined with the last full-duration time segment in the file (if the amount of leftover time is less than the specified number of seconds), or if it should become a new (shorter) segment.

To divide up the file evenly into equal-duration time segments, select the second radio button and specify the number of segments.

A more complicated time segment structure can be created by selecting the third radio button. Arbitrary time segments can be entered, perhaps to represent different experimental conditions. It is possible to choose to preserve the previous segments and add an additional segment or to delete any existing segments.

However the time segments were specified, Offline Sorter calculates statistics and fits a 2D ellipse to the points in every segment. The Clusters Vs. Time view can plot these 2D ellipses as semi-transparent elliptical cylinders that cover the time range of the segment.



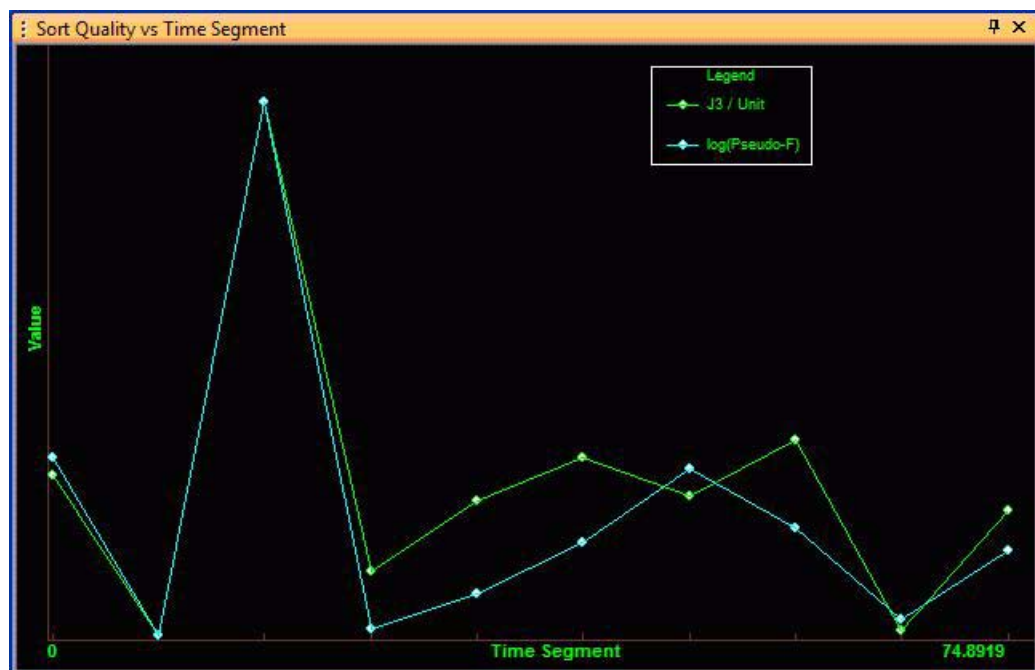
When plotted in this fashion, the elliptical cylinders for each unit stack up into a tube shape; these tubes are called **PCSTs (PCA Stability Tubes)**. These tubes can be shown or hidden by selecting **Show PCSTs** from the right-click menu, and they can be made more or less transparent by pressing the **I** (Fade In) or **O** (Fade Out) keys.

3.10 Sort Quality vs Segment Graph View

Offline Sorter can display a graph of selected sort quality metrics as a function of time segments. The values plotted are the same as those shown in tabular format in the [“3.6.4 Time Segments View” on page 40](#). This view can reveal episodes of poor sort quality over certain periods in the file.

To display the Sort Quality vs Time Segment View

- Select **View | Sort Quality vs Time Segment View** menu command, or press the  button on the toolbar.




The vertical axis has arbitrary scaling; each plotted sort quality metric is scaled independently so that the entire range of sort quality metric values fits vertically on the plot. The horizontal axis is each Time Segment. See [“5.15 Scan Graph View” on page 220](#) for details on how to customize the display by showing a different selection of sort quality metrics or plotting them with different colors or symbols.

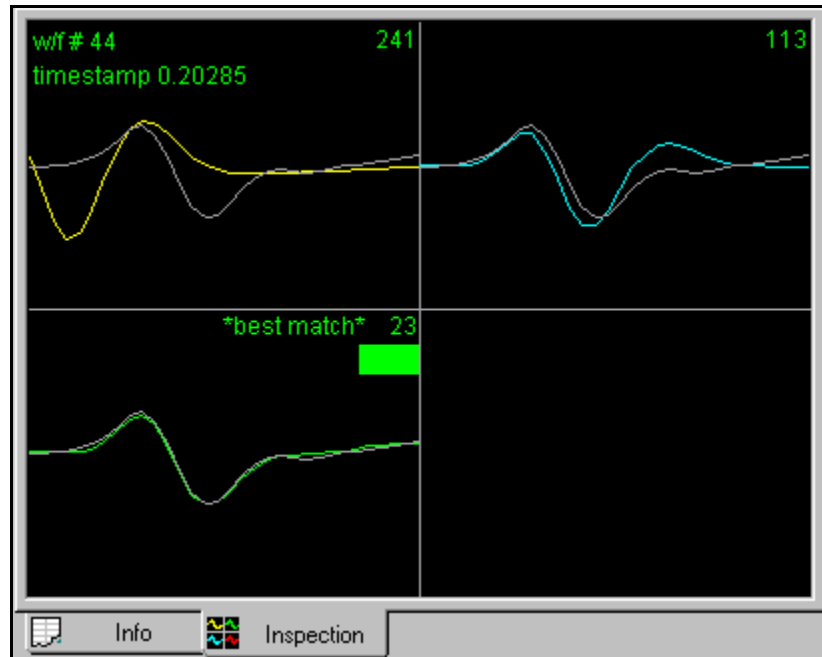
3.11 Waveform Inspection View

The Waveform Inspection view is used for comparing individual waveforms with the template (average waveform) for each sorted unit.

To display the Waveform Inspection View

- Select the **View | Waveform Inspection** menu command, or

click  on the toolbar.



The Waveform Inspection Window shows the average waveform (template) of each sorted unit in the appropriate unit color. The individual waveforms, which are selected either in the [3.5 Timeline View](#) (left-click on waveform) or in either of the [3.7 Clusters Views](#) (left-click on waveform's dot in feature space), are displayed in grey and positioned over each template for comparison.

Display Features:

- **colored waveforms** – template (average waveform) for each sorted unit
- **grey waveform** – waveform selected in the Timeline View or Clusters Views
- **w/f #** – number of the selected waveform in the temporal sequence of waveforms on the channel
- **timestamp** – time of occurrence of the selected waveform in seconds
- **dotted vertical lines** – sort start and sort stop times (if present); see [“4.4.4 Adjusting the Sort Range”](#) on page 118.
- **# in upper right-hand corner of each plot** – sum of squared difference between the selected waveform and the template

-
- ***best match***- displayed on the unit with the best match (least sum of squared difference) between the unit template and the selected waveform
 - **Colored bar** – displayed on the unit assignment of the selected unit (green bar above)

3.12 Waveform Detection View

The Waveform Detection view is only available when a channel containing continuously digitized data is opened. The view displays distributions of quantities (e.g. peak heights, energy, or non-linear energy) that can be used for thresholding the data for spike detection.

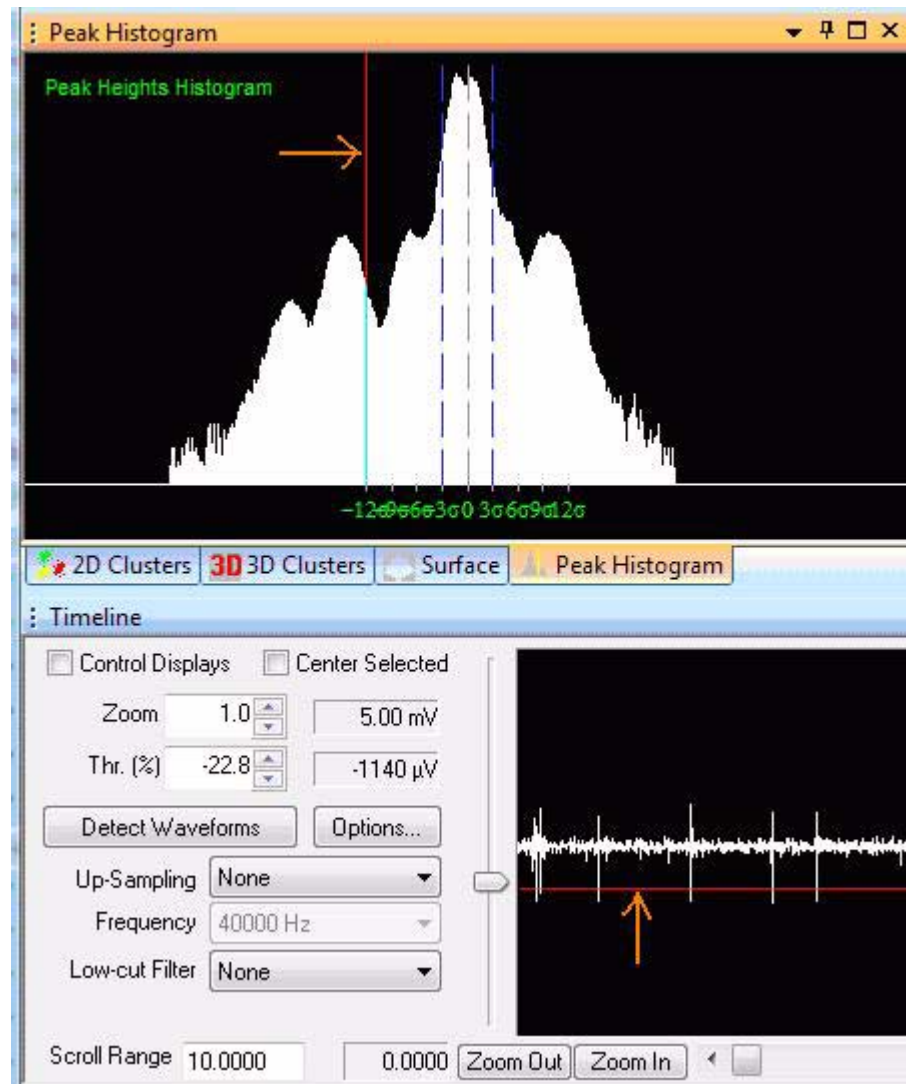
To display the Waveform Detection view

- Select the **View | Waveform Detection** menu command, or,

press the  button on the toolbar.

By default, the Waveform Detection view is selected when a continuous data channel is opened. The Waveform Detection view shows a histogram of the logarithm of the waveform peak heights. The dashed white line indicates zero, and

dashed blue lines indicate the 3 sigma noise level. The solid red line (marked with an orange arrow below) indicates the selected threshold.



3.12.1 Adjusting the Threshold for Waveform Detection

The threshold can be changed by clicking and dragging the solid red line, either on the Waveform Detection view or on the Timeline View. Thresholding can be done on either positive or negative amplitudes. Alternately, a threshold can be set by entering a value in the **Thr. (%)** edit box in the Timeline View, or by clicking on the associated spin control.

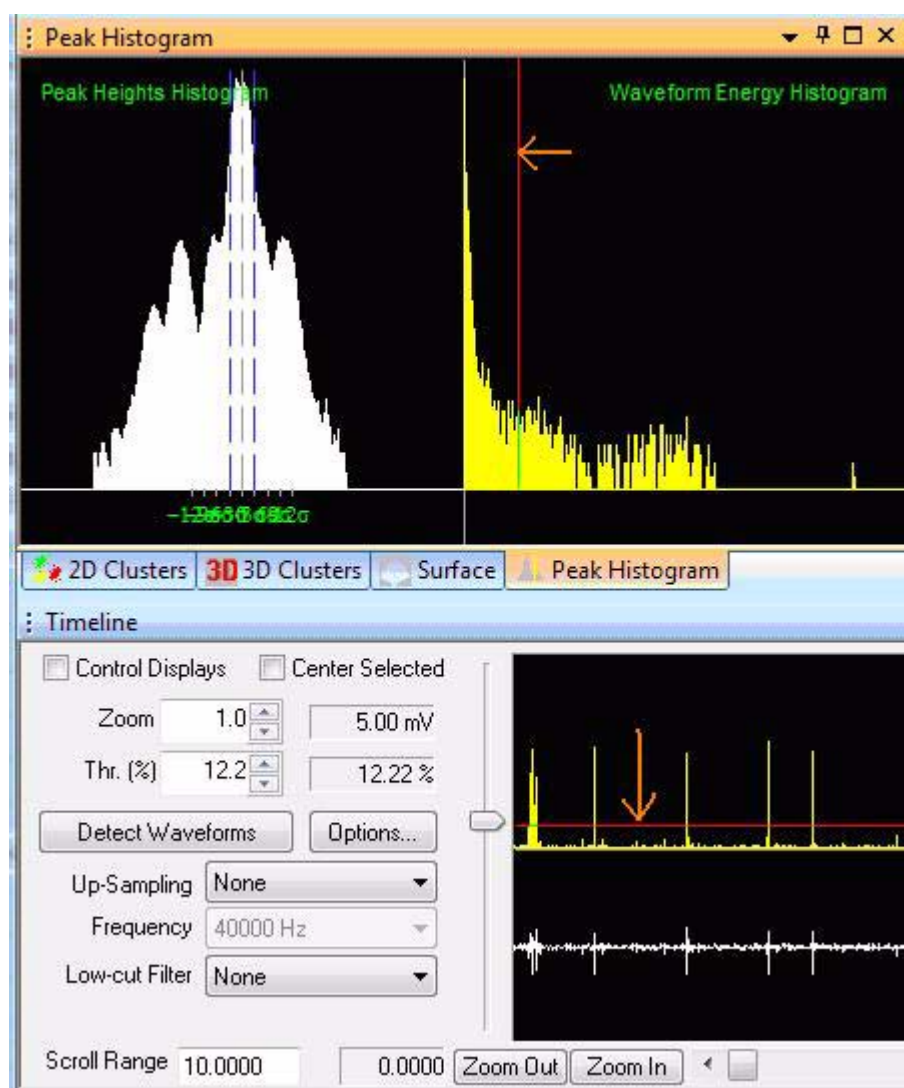
3.12.2 Changing the Detector

The waveform detection algorithm can use thresholds on quantities other than raw waveform amplitude. As explained in [“4.2.6 Specifying the Waveform Detection Options” on page 99](#), the **Detector and Waveform Options** menu dialog can be used to select the signal threshold to be the:

- Raw Voltage Signal (the default), or

- Energy of the Signal, or
- Nonlinear Energy of the Signal, or
- Signed Energy of the Signal

When a signal is selected other than **Raw Voltage** amplitude for use in spike detection, the Waveform Detection view and the Timeline View show both the selected parameter (top display) and the **Raw Voltage** (bottom display). Shown below are the Waveform Detection view and the Timeline View when the **Energy of the Signal** threshold has been selected.



The top histogram in the Waveform Detection view above shows the distribution of the logarithm of the signal energy, and the top trace in the Timeline View shows the waveform energy. The thresholding is set in the top histogram or trace.

Offline Sorter calculates the Energy of the Signal by taking a moving-window average of the square of the voltage amplitude. The width of the window used for the averaging is controlled in the **Detector and Waveform Options** dialog. Nonlinear Energy is akin to the product of the raw amplitude times the derivative of the signal. If the voltage is constant or zero, the Nonlinear Energy is zero. If the waveform is rapidly varying and has a large amplitude, the Nonlinear Energy is a maximum.

Mathematically, the energy and nonlinear energy of the signal at time point i is given by:

$$\text{Energy}(i) = \left(\frac{1}{\text{width}}\right) \sum v(j) v(j)$$

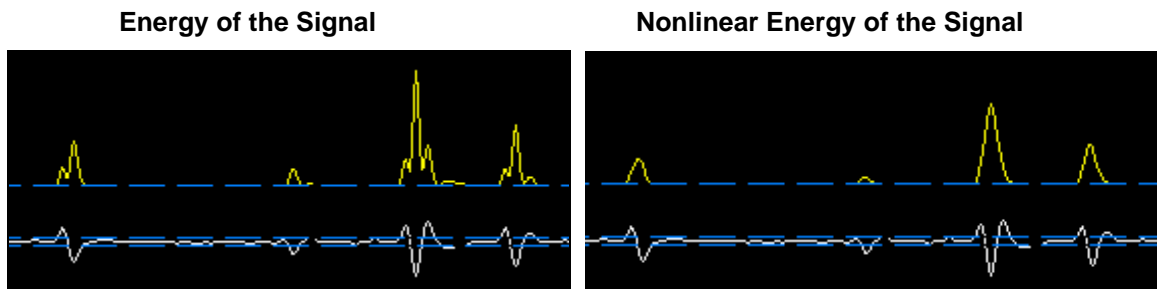
$$\text{Nonlinear Energy}(i) = \left(\frac{1}{\text{width}}\right) \sum [v(j) v(j) - v(j-1) v(j+1)]$$

where Σ is the sum over the energy window centered around i (i.e., the sum over j where

$$j = i - \frac{\text{width}}{2} \dots i + \frac{\text{width}}{2}$$

The Signed Energy of the Signal is the Energy of the Signal multiplied by +1 when the average amplitude of the raw signal in the energy window is positive, and multiplied by -1 when the average amplitude is negative. The Signed Energy enables the threshold to differentiate between energy due to large negative excursions and energy due to large positive excursions.

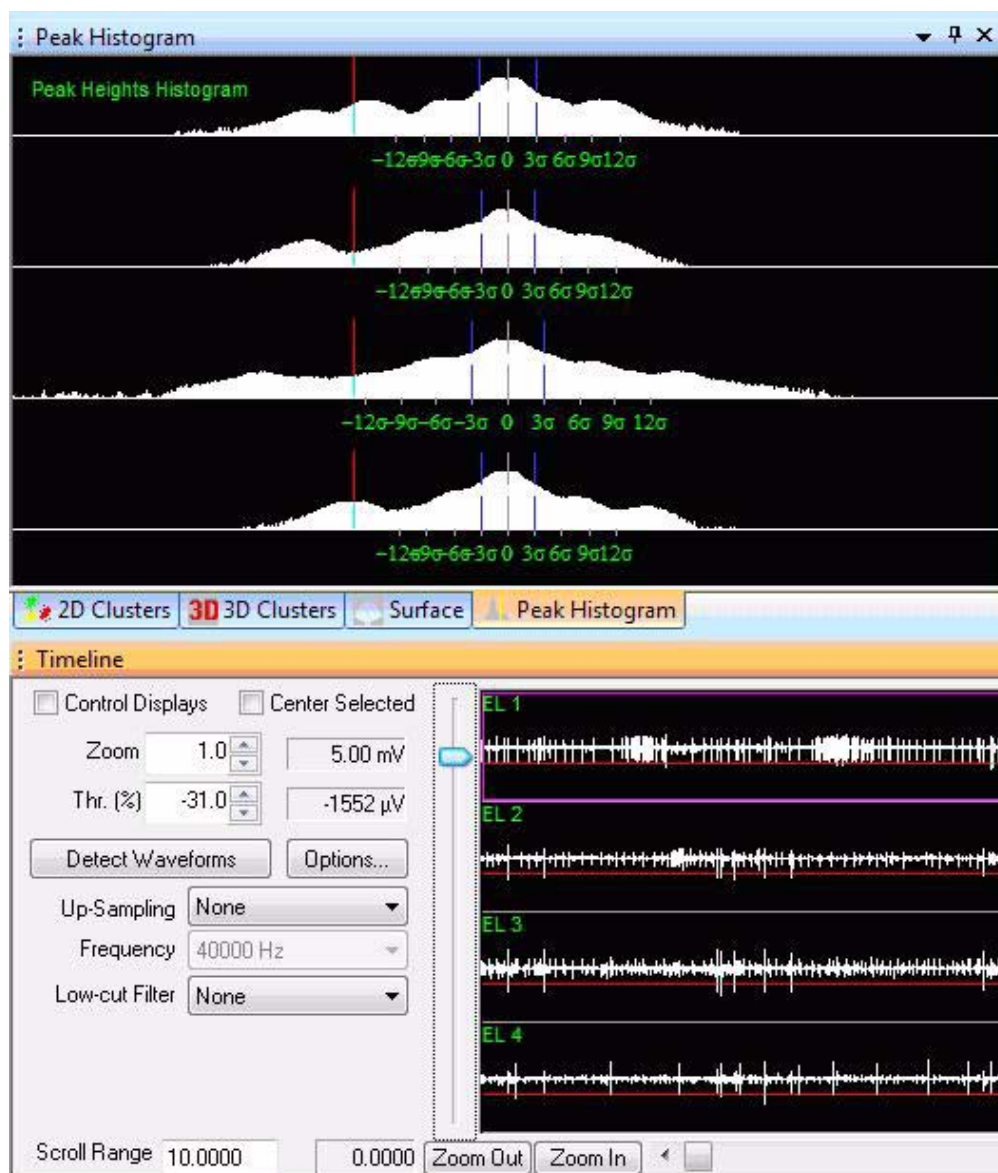
It is instructive to look closely at these calculated quantities for typical waveforms. The top yellow trace is the calculated quantity, and the bottom trace is the raw waveform amplitude.



3.12.3 Stereotrode and Tetrode Waveform Detection

Offline Sorter supports extracting stereotrode and tetrode waveforms from continuous data. To begin, configure the continuous channels as either stereotrode or tetrode channels. From the **Info Grid View**, select the **Chan Map View** and configure the channels described in [“5.2 Channel Mapping” on page 181](#).

When a stereotrode or tetrode channel loads, the **Waveform Detection** view and the **Timeline View** show 2 or 4 traces instead of just one. Observe this arrangement in the following illustration.




As before, the threshold in any view can be adjusted by clicking and dragging the threshold line, which is the red line in each of the preceding traces. By default, Offline Sorter constrains the threshold level to be the same on all electrodes within the stereotrode/tetrode. When the threshold is adjusted on one electrode, the threshold changes for all electrodes. However, to adjust the threshold for each electrode independently, click **Detector and Waveform Options** and in the **Waveform Detection Options** dialog box, click **For stereotrode/tetrode data, allow an independent threshold for each electrode**.

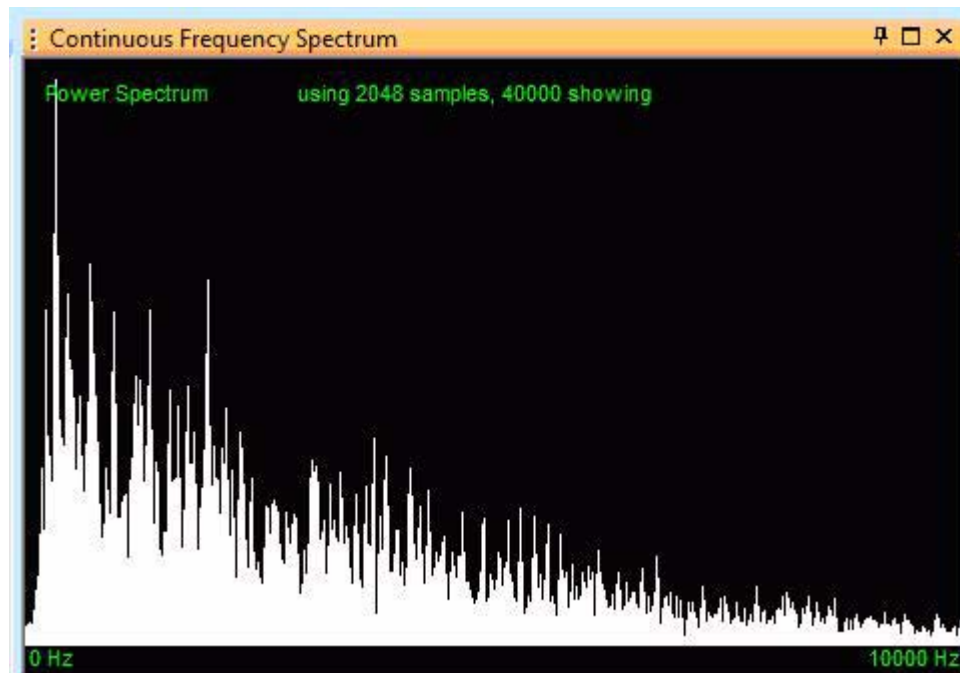
For waveform detection on stereotrode or tetrode continuous data, a threshold crossing on any electrode results in the capture of the waveform across all electrodes. As in the single electrode case, if the **Detector** is specified to be anything other than **Raw Signal**, each of the traces again divide into two halves, showing both the raw signal and quantity used for thresholding.

3.13 Continuous Frequency Spectrum View

If Continuous Data has been loaded into Offline Sorter, Offline Sorter can perform an FFT on the continuous data and display a frequency spectrum of the signal. This is not directly relevant to Offline Sorter's primary purpose of sorting spikes, but it can provide useful information, such as whether the continuous data is contaminated by 60 Hz noise.

To display the Continuous Frequency Spectrum View

- Select the **View | Continuous Frequency Spectrum View** menu command, or press the  button on the toolbar.



The display power spectrum is the result of running an FFT on the samples that are currently displayed in the Timeline View, starting at the left hand (earliest time) side. The FFT is run on a maximum of 2048 samples, so if the time range is such that many more samples are showing, the power spectrum will be calculated from the first 2048 points (as is the case in the above screenshot). The vertical scale is always set such that the tallest peak fills the display vertically. By default the frequency range displayed is 0 to one half of the sampling frequency for the continuous data. Using right-click menu items, the frequency range displayed can be expanded or shrunk, moved higher or lower, or reset to the initial range.

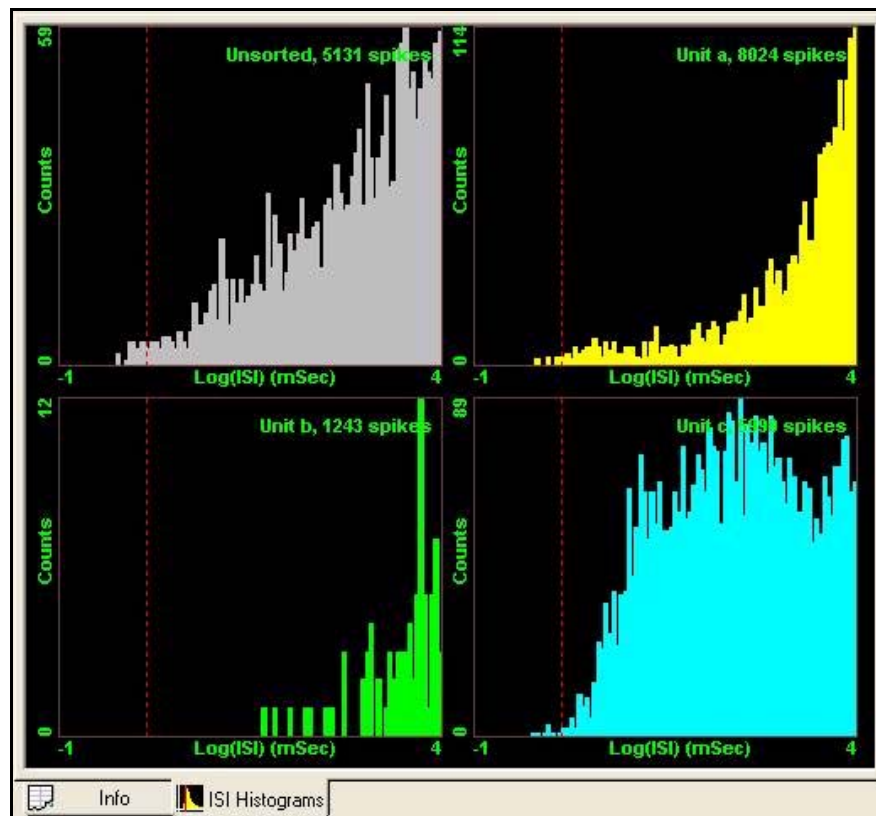
3.14 ISI Histograms View

The ISI Histograms View provides a larger, printable version of the Inter-spike Interval Histograms that appear in the Cluster Display. The range for the ISI Histograms is 0 (zero) to the ISIHistMax(ms) Control Panel setting.

To display the ISI Histograms view

- Select the **View | ISI Histograms** menu command, or,

press the  button on the toolbar.



There are several options for displaying the ISI Histograms from the right-click context menu. The view can be configured to show the ISI Histograms from all sorted units, from all sorted units plus an ISI Histogram for the unsorted waveforms, or only for the currently-selected unit. Additionally, one can display the Time axis or the Counts axis, or both, using a log scale.

3.15 Feature vs. Feature View

The Feature vs. Feature view can be quickly used to view the feature space point distributions for many different features. This can give a quick impression of which features might be most useful for sorting. Offline Sorter divides the screen into boxes, and each box contains a 2D Clusters View. Much of the functionality

from the 2D Clusters View is available in the Feature vs. Feature view, including the point selection, zooming, and contour sorting functionality.

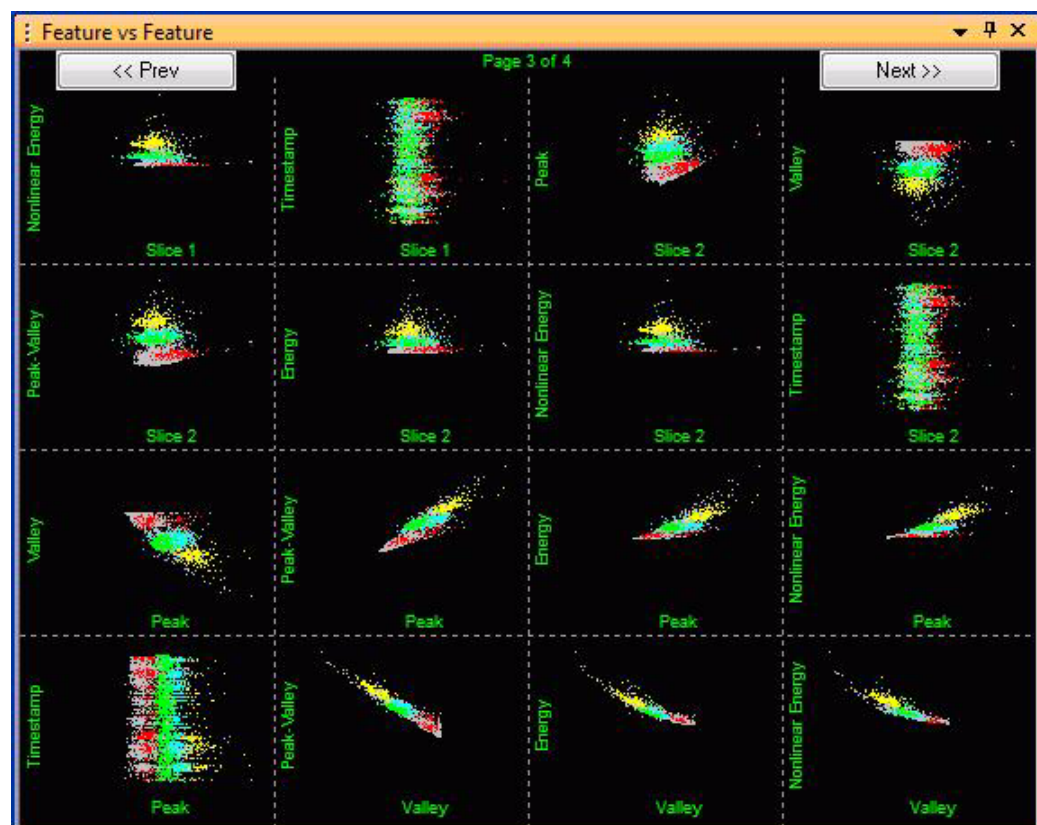
To display the Feature vs. Feature view

- Select the **View | Feature vs. Feature** menu command, or,

press the  button on the toolbar.

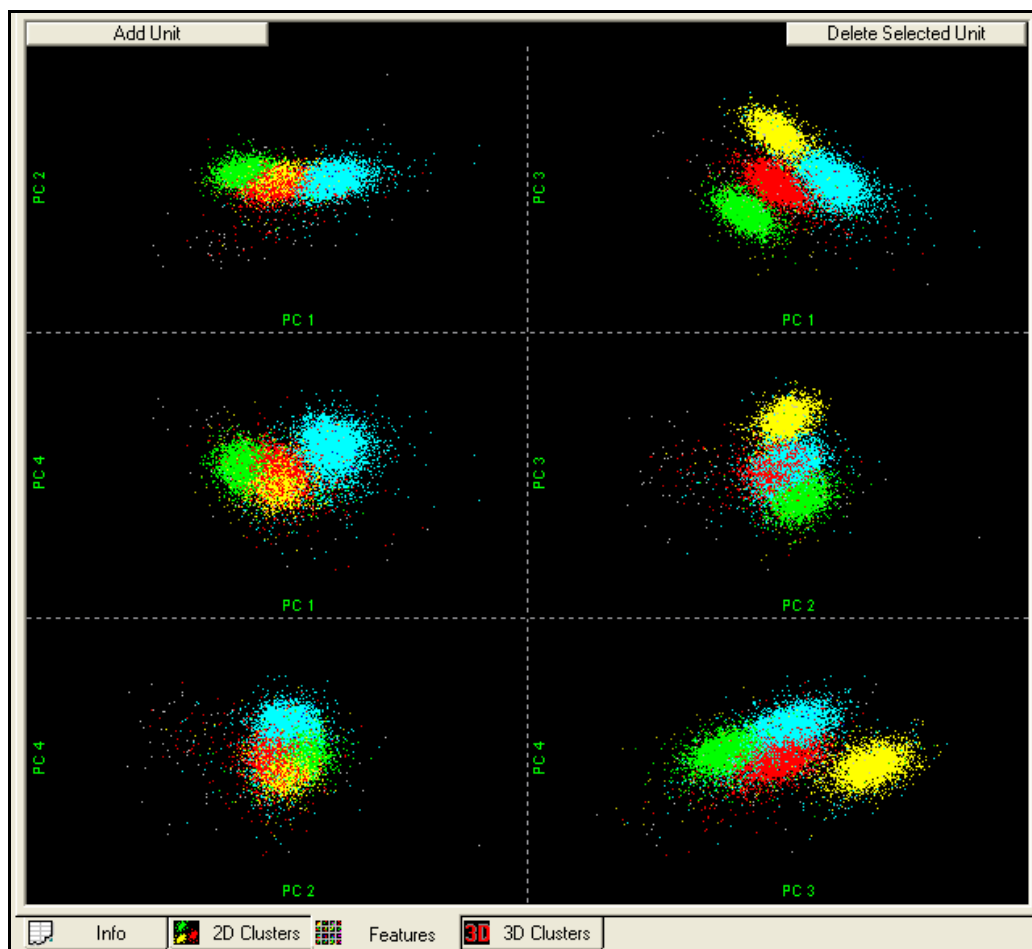
The Feature vs. Feature view can operate in one of several modes, which differ in the combinations of features that are shown in the plots. One can select different display combinations via the context menu that is available by holding down the ALT key and right-clicking in the view. The modes are:

- **Show All Active Feature Combinations:** shows every permutation of the currently Active Features. This is the default mode for non-Tetrode Data Types. Generally the number of permutations is large enough that several pages are required to show all of the plots. To step through the pages, use the **Next >>** and **<< Prev** buttons on the top of the screen, or the N (Next) or P (Previous) keys.



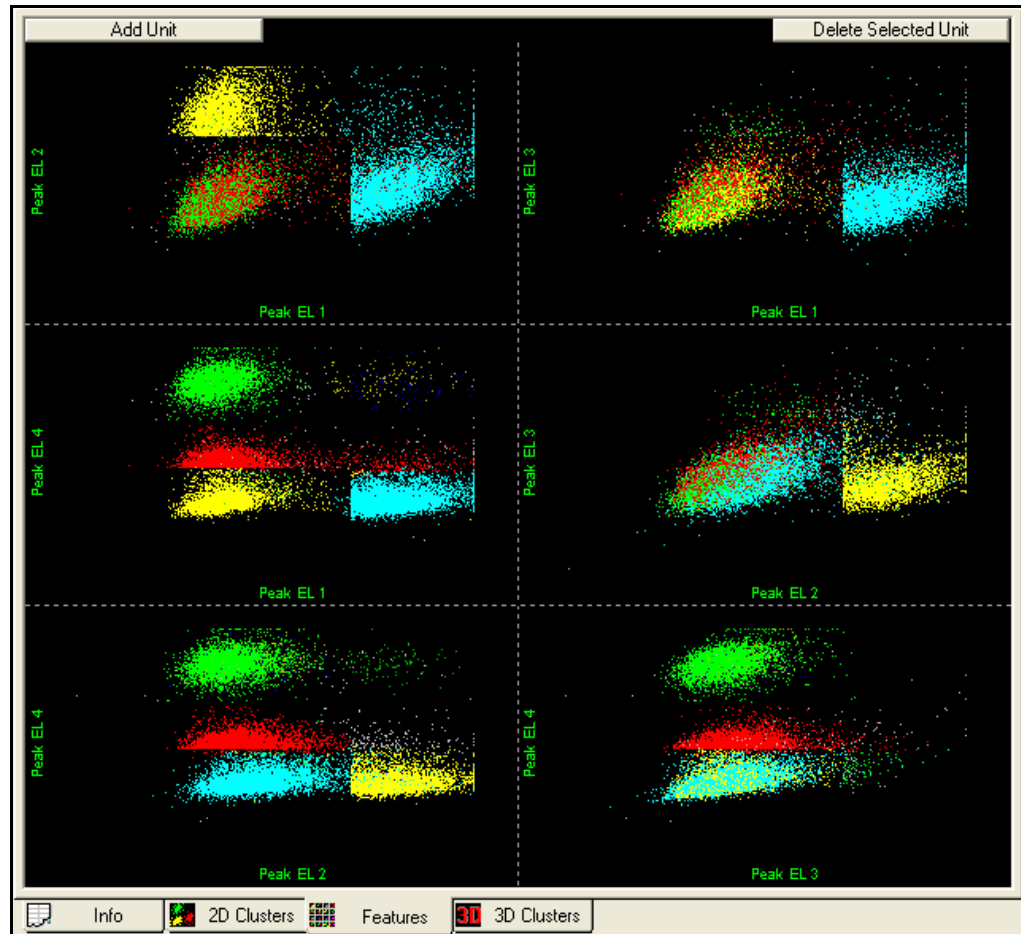
- **Show PC1-4 Combinations:** shows the 6 permutations of the first 4 Principal Component projections; PC1 vs. PC2, PC1 vs. PC3, PC1 vs. PC4, PC2 vs.

PC3, PC2 vs. PC4, and PC3 vs. PC4. In this mode, press the N or P keys to go to the next or previous channel (as in the 2D Clusters View).



- **Show Tetraode Electrode Combinations:** shows the 6 permutations of 4 tetraode electrodes, using a tetraode per-electrode feature value (Per-Electrode Peak Height by default). For example, this mode shows plots of Peak Height for Electrode 1 vs. Peak Height for Electrode 2, Peak Height for Electrode 1 vs. Peak Height for Electrode 3, and Peak Height for Electrode 3 vs. Peak Height for Electrode 4. This is the default mode for Tetraode Data Types. In

this mode, press the **N** or **P** keys to go to the next or previous channel (as in the 2D Clusters View).



To select which Per-Electrode feature is used for the Tetra electrode display mode, select that feature for the X Axis in the 2D Clusters View using the feature droplists in the toolbar. For example, if one selects PC 1 EL 1 for the X Axis of the 2D Clusters View, the Feature vs. Feature View operating in Tetra electrode display mode shows the plots PC1 EL 1 vs. PC1 EL 2, PC1 EL 1 vs. PC1 EL 3, and PC1 EL 3 vs. PC1 EL 4. If one selects a non-Per-Electrode feature for the X Axis in the 2D Clusters View, the tetra display uses Per-Electrode Peak Height by default.

3.15.1 Common Functionality

In general the plots in the Feature vs. Feature view behave the same as the 2D Clusters View plot, responding to the **Add/Remove Waveforms/Units** buttons and menu items, the **Waveform View** selection, and the zooming/panning toolbar buttons in the same way. For example, the following mouse functions from the 2D Clusters View are available:

- **Left-Click** selects the nearest waveform point, highlighting that point on all displays and in the waveform view.

-
- **Control-Left-Click** while in Contour sorting mode adds a new unit.
 - **Shift-Left-Click** pans the display.
 - **Right-Click** and hold zooms the display in and out.
 - **Alt-Right-Click** displays the context menu.

Additionally, **Double-click** any sub-box to bring up the full-size 2D Clusters View with the corresponding features on the X and Y axes.

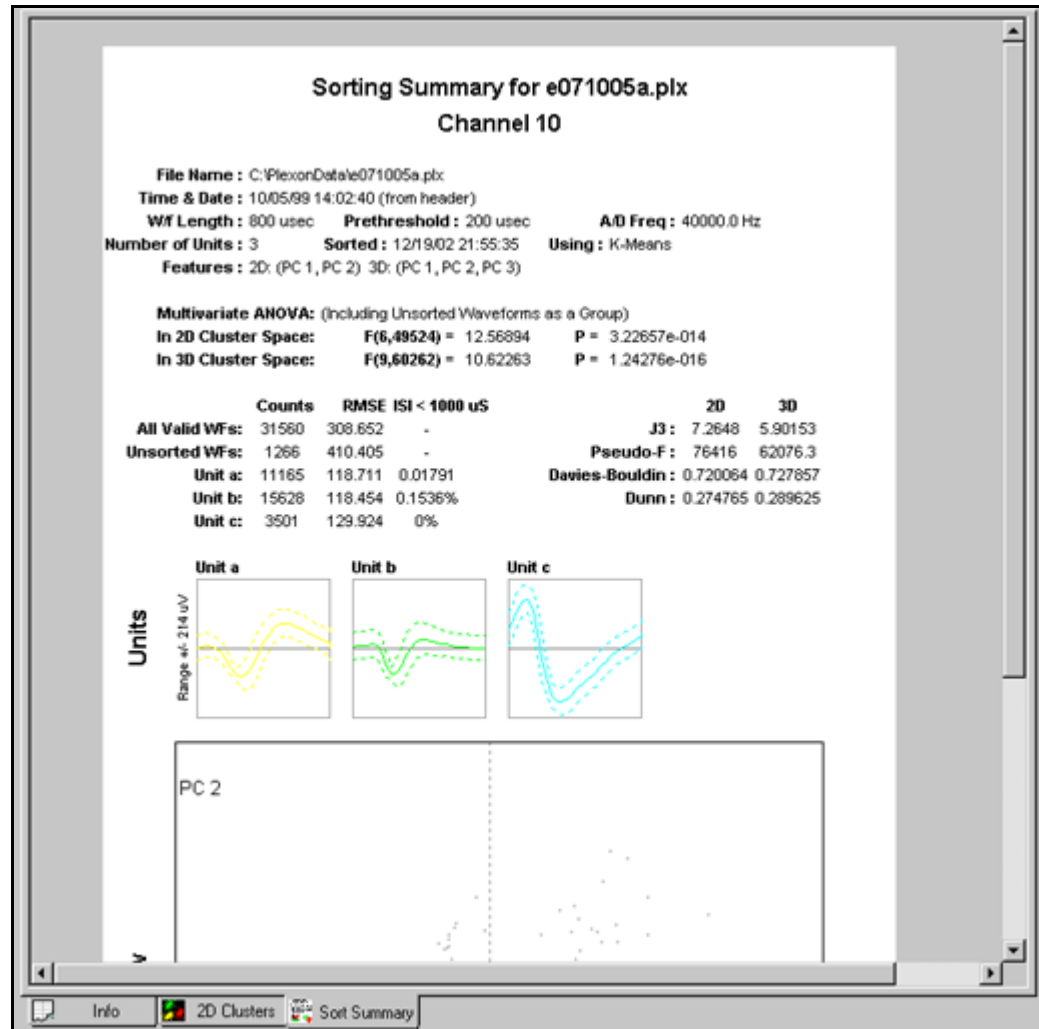
3.16 Sorting Summary View

The Sorting Summary view can be used to quickly get an overview of the results of sorting a channel. The Sorting Summary view is intended to be printable so that it can serve as a permanent record of the sorting. The contents of the Sorting Summary view can also be exported to PowerPoint. Much of the information on the Sorting Summary view can be found in other views or windows in Offline Sorter; the value of the Sorting Summary view is that it gathers the most important sorting information together in one view.

To display the Sorting Summary view

- Select the **View | Sorting Summary** menu command, or

press the  button on the toolbar.



The title on the top contains the channel number, and optionally the file name. To change whether the file name shows up in the title, select the **Show Filename in Title** entry in the right-click menu on the Sorting Summary view.

The other entries on the form include:

- **Filename:** the full path to the file
- **Time & Date:** the time and date that the data file was collected. Whenever possible, this time and date is obtained from the header records in the data file, so (**from header**) appears on this line. If the time and date cannot be obtained from the file header, it is taken from the time/date on the file itself, in which case (**from file**) appears on this line.
- **W/f Length:** the total length of the waveform, in microseconds
- **Prethreshold:** the time between the start of the waveform and the threshold crossing, in microseconds
- **A/D Freq:** the digitization rate, in Hertz

-
- **Number Of Units:** the number of units found on this channel
 - **Sorted:** the time & date that sorting was done. Currently this is always the current date.
 - **Using:** the name of the algorithm used for the sorting
 - **Features:** the features selected to define the features space. All the sorting quality statistics depend on the choice of features.
 - **Multivariate ANOVA:** the string in parenthesis indicates whether or not unsorted waveforms are treated as a unit in the ANOVA and the other statistics calculations. The next lines contain the ANOVA F and p-values, as explained in [“5.7 Sorting Quality Statistics” on page 203](#).

The next section contains a table with a row per unit, plus entries for all waveforms and for unsorted waveforms. The columns are:

- **Counts:** The total number of waveforms (in the **All Valid WFs** row), the number of unsorted (unassigned) waveforms (in the **Unsorted WFs** row), and the number of waveforms in each unit.
- **RMSE:** the summed root mean squared for all waveforms in the category. This is a sample-by-sample summation of the deviation from the average waveform, summed across all waveforms. This sum is then divided by the total number of ticks, then the square root is taken.

$$\text{RMSE} = \sqrt{(\sum \sum ((v(w, t) - \text{avg}(t))^2) / (N_w N_t))}$$

where

the sums run over N_w waveforms w and N_t ticks t in each waveform

$v(w, t)$ is the waveform amplitude at tick t for waveform w

$\text{avg}(t)$ is the average amplitude of all waveforms at tick t

Thus, this number is smaller for compact clusters. The Plexon MAP system calculates a similar summed squared difference for the real-time online template sorting unit assignment algorithm.

- **ISI Percentage:** lists the percentage of waveforms that occur within the Refractory ISI period (as set by from the Control Grid) of each other. Large percentages here for a unit can indicate that errors have been made in the sorting of that unit.

The next section to the right displays the 2D and 3D versions of each of the J3, Pseudo-F, Davies-Bouldin, and Dunn cluster validity metrics (See [“5.7 Sorting Quality Statistics” on page 203](#)). The feature space used for this sorting quality statistics displayed under **Features** above.

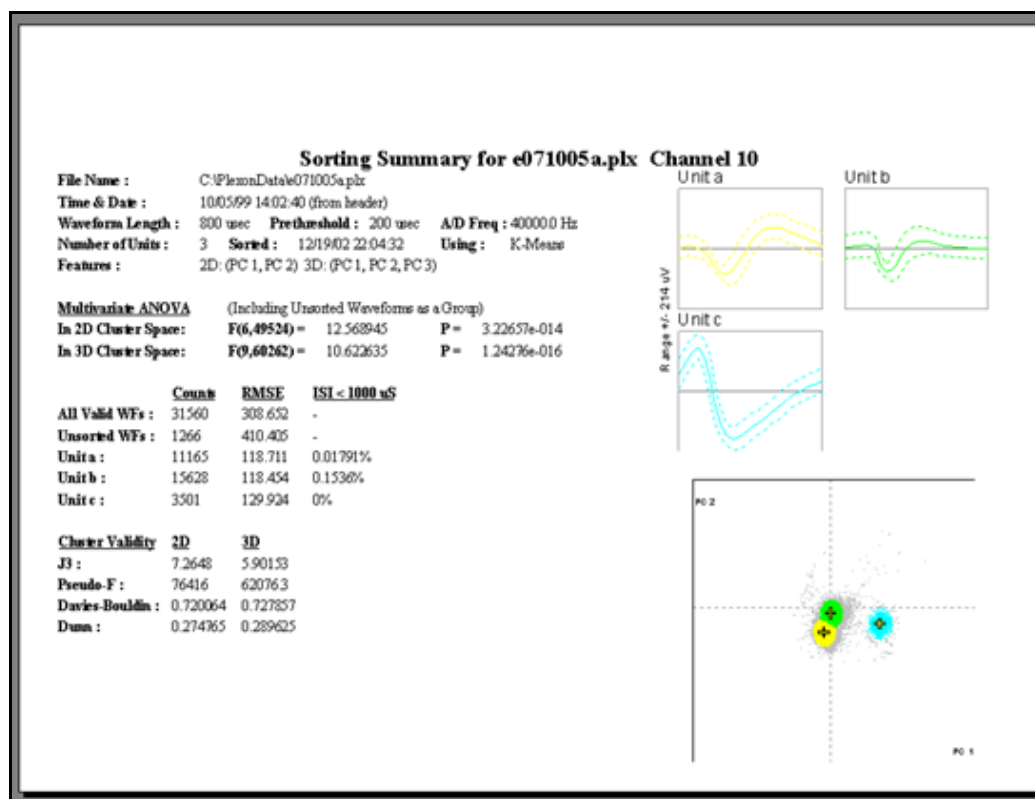
The next section displays the template (average) waveform for each of the units on the channel, as described in the “3.4 Units View” on page 29. The dashed line represents the 3-sigma deviation of the waveforms around the average.

Note: The colors displayed for the units are controlled by the printing colors, not the display colors. The printing colors can be set in the **Printing Options** tab of the **Tools | Options** dialog.

The bottom section is the 2D Clusters View. Again the colors displayed are controlled by the printing colors, not the display colors.

3.16.1 Output to PowerPoint

The Sorting Summary view can be sent to PowerPoint by selecting **Send To PowerPoint** in the right-click menu in the Sorting Summary view. For this feature to be available, PowerPoint must be installed on the computer.



When the **Send To PowerPoint** menu item is selected, PowerPoint opens (if it is not already open), and a new slide is added to the presentation. Sending another Sorting Summary view to PowerPoint results in another slide being added to the same presentation. PowerPoint does not save the presentation to disk—select **Save** from the PowerPoint menu to save the presentation.


3.16.2 Outputting for All Channels

The **File – Print All Sort Summaries** and **File – Send All Sort Summaries to PowerPoint** iterate through all channels and print or export the Sort Summary for each channel to a single PowerPoint file, or to a single print job.

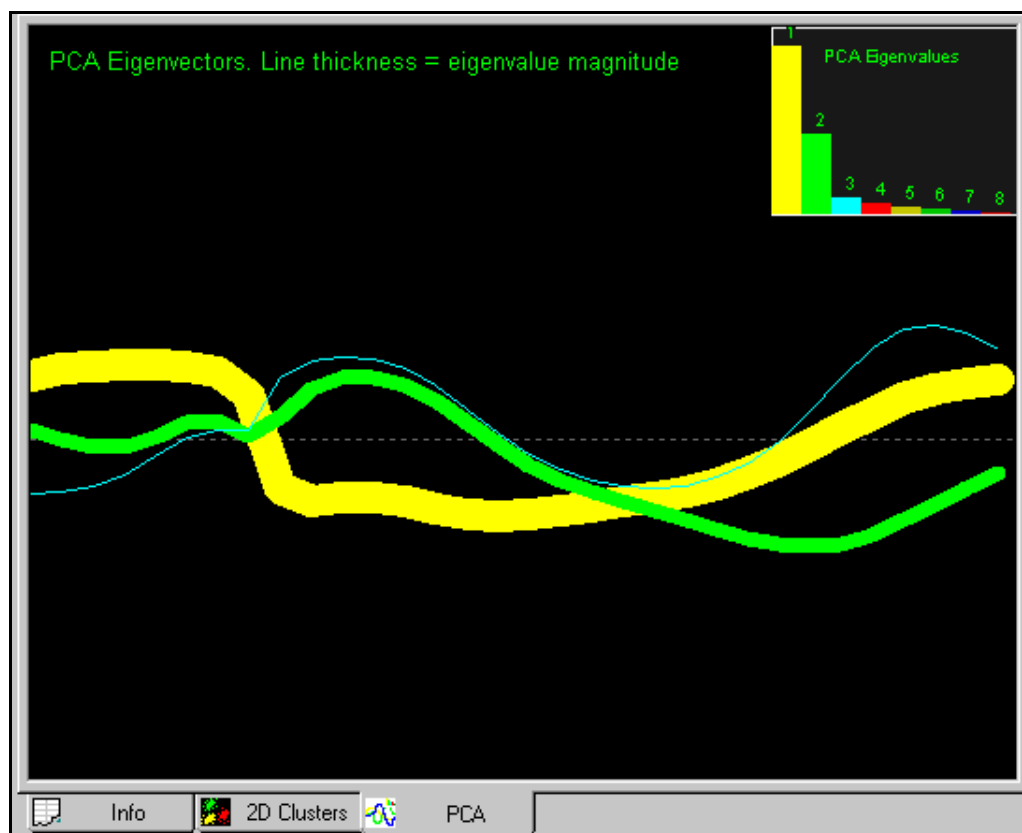
3.17 PCA Results View

The PCA Results View graphically displays the results of the Principal Components Analysis (PCA) that runs on the waveforms. The calculated principle component eigenvectors form a basis onto which waveforms can be projected in order to get the PC1-PC8 feature values. A bar graph of the eigenvalues associated with each eigenvector is also displayed. The same PCA information is displayed in table form in the PCA View of the [“3.6 Info Grid Views”](#) on page 34.

To display the PCA Results View,

- Select the **View | PCA Results** menu command, or press the  button on the toolbar.

Note: If this button does not appear on the toolbar, select **Toolbars tab** under **Windows | Customize.....**, and select the line labelled **View** in the list box in the **Toolbars** tab. Do not uncheck the checkbox—just select the line. Then click the **Reset** button.



By default, Offline Sorter plots the first 3 principal component eigenvectors in the main portion of the view. Each different color corresponds to an eigenvector/eigenvalue pair. Offline Sorter calculates a maximum of 8 principal components eigenvalues/eigenvectors. All 8 eigenvectors can be displayed by selecting **Show All 8 Eigenvectors** from the right-click menu.

The width of the line used to plot the eigenvector is proportional to the magnitude of the corresponding eigenvalue. A bar chart graph of the eigenvalue magnitudes is shown in the upper right corner by default. The graph can be moved by clicking on the interior of the plot and dragging it to a new location, and it can be resized by clicking on the border and dragging. The eigenvalue bars are scaled for display in the graph such that the first eigenvalue takes up 90% of the range. This graph is sometimes referred to as a “Scree Plot”.

The magnitude of an eigenvalue for a principal component eigenvector, relative to the total of all eigenvalues summed, represents the fraction of the total variance between waveforms that has been captured in that PCA dimension. In the above screenshot, it is clear that the first two principal components capture the vast majority of the variance. So, there is likely to be very little discriminatory power in the higher principal components PC3-PC8. The detailed shape of the PCA eigenvectors are not always straightforward to interpret. Generally, the first principal component (the yellow line) is largest in those sections of the waveform where the most variance is. The shapes of the higher principal component eigenvectors are constrained by the fact that all the eigenvectors must be orthogonal to each other, which makes their interpretation difficult. Note though that they approach zero at the trigger point (e.g. at a time value equal to the prethreshold into the waveform). All waveforms have an amplitude equal to the trigger threshold value at this point in the waveform, with essentially no variance. Thus, all the eigenvectors are zero at that point.

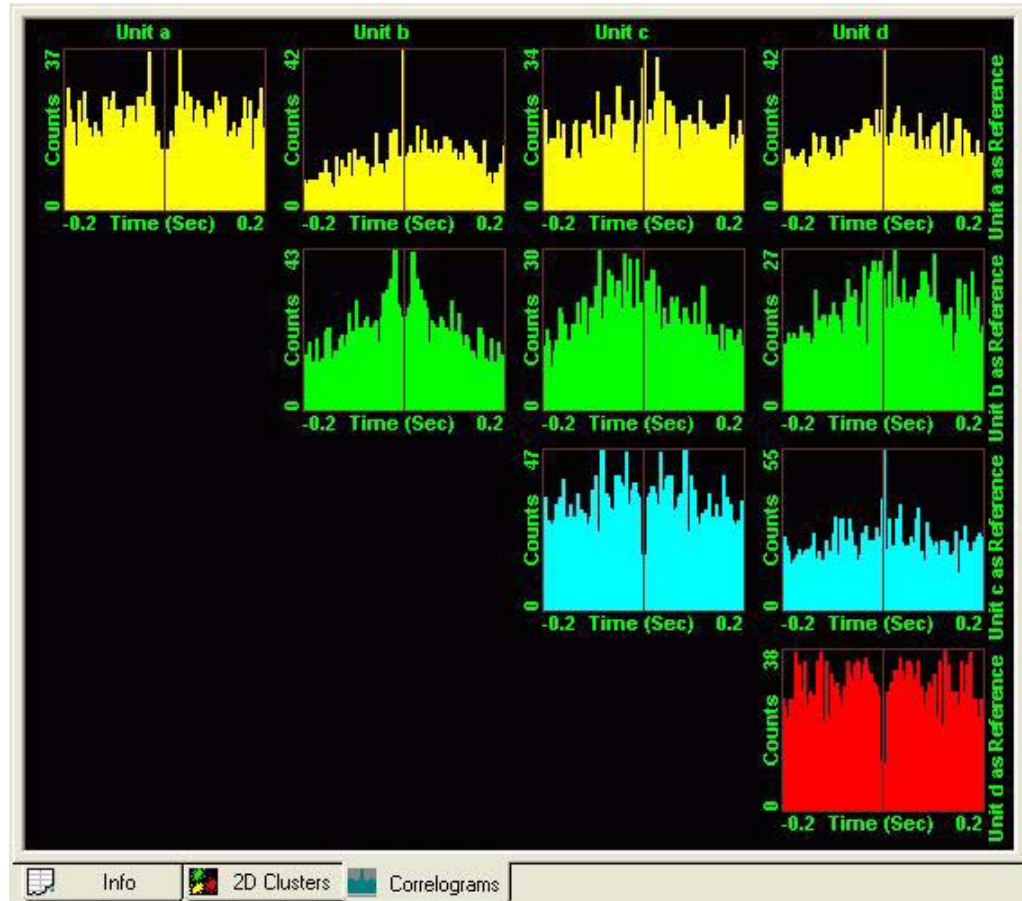
3.18 Cross-Correlograms View

The Cross-Correlograms View displays a half-matrix of cross-correlograms between all units (and optionally the unsorted waveforms as well) in a channel. The firing of each unit is used as a reference time (time zero) to form a correlogram of a different unit's firings, using an adjustable timescale. The cross-correlograms are arranged on a grid where the reference unit increases along the vertical axis and the plotted unit increases along the horizontal axis, with the redundant (mirror-image) correlograms omitted.

To display the Cross-Correlograms View,

- Select the **View | Cross-Correlograms** menu command, or,

press the  button on the toolbar.



The parameters for the Cross-Correlograms are set by using the right-click context menu. The view can be configured to show the cross-correlograms formed by permuting either: all sorted units, all sorted units plus the unsorted waveforms, or just the currently selected units. The labeling can be enabled or disabled, which is useful if there are large numbers of units. Also, whether or not the reference unit firing is counted in the correlogram can be selected. The timescale and bin widths used to build the correlograms can be set using the '**Binning**' section of the dialog that is displayed when '**Properties...**' is selected from the context menu.

3.19 Rasters View

The Rasters View displays color-coded tick marks that correspond to the times of unit firings, which is similar to the Timeline View, but the Rasters View does so for all channels simultaneously.

To display the Rasters View,

- Select the **View | Rasters** menu command, or,

press the  button on the toolbar.



In addition, the Rasters View can show the times when Events occur. The Events can appear either as tick marks on separate raster lines, or as lines overlaid across all rasters.

The Rasters View can only display the timestamps for channels that have been loaded into memory. When a channel is loaded, its timestamps are available for display. By default, the Rasters View only displays the timestamps for the channels that have been loaded. To load all timestamps for all channels into memory, select **Load All Rasters** from the right-click context menu. For some data files (including .PLX), the timestamps for all channels when the file loads can automatically be loaded by checking the **Pre-Load all Timestamps** checkbox in the **General** tab of the **Tools - Data Import Options** dialog. However, if this option is checked, Offline Sorter uses more system memory and it slightly increases the time it takes to load the data.

The Rasters View displays the same time range as the time range that appears in the Timeline View, and pressing the **Zoom In** or **Zoom Out** buttons or moving the time-range scroll bar on the Rasters View is equivalent to doing the same in the Timeline View. By default, the Rasters View displays all channels that have spikes. The number of channels displayed vertically can be changed by selecting **Zoom In To Show Fewer Channels** or **Zoom Out To Show More Channels** from the context menu, or by pressing the **↑** or **↓** keys. To scroll the display vertically, move the mouse wheel or left-click and drag the mouse in the label area on

the left. The display can be scrolled vertically by using the PAGE UP and PAGE DOWN or HOME and END keys. One can also grab the dividing line between the labels and the tick marks and move it by left-clicking and dragging the line.

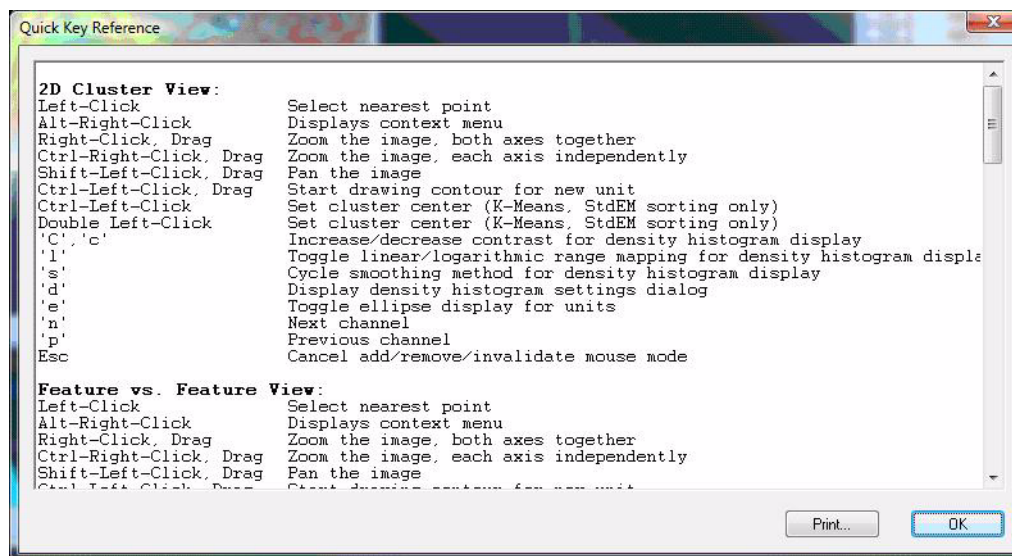
The Rasters View reflects the **Invalid w/f** setting in the Control Grid, however it does not reflect the **Waveform View** setting; the Rasters View always shows the tick marks for all units, and one can optionally show the unsorted waveforms by checking **Show Spike Marks For Unsorted** in the context menu. By default, each channel appears on a separate raster; however, if **Show All Units for a Channel on One Raster** is unchecked in the context menu, a line for each sorted unit can be shown (and possibly also the unsorted units, which are denoted by a “U” postfix, e.g. “sig002U” is the unsorted waveforms on the “sig002” channel).

By default, any Events that are selected to show, by clicking **Show** in the **Events** view of **Info Grid View**, are plotted each on its own raster lines, in the color selected in the **Events** view. The rasters for Events can be shown either on the top (default) or on the bottom of the spike timelines by checking or unchecking **Show Events on Top-most Rasters** the context menu. Alternatively, select **Show Events Across All Rasters** in the right-click context menu to plot the Events as vertical lines that cross all the raster lines. Also, one can select **Show All Events** in the context menu, which is equivalent to clicking **Show** for all Events.

Selecting **Show Time Segments Boundaries** in the right-click menu will plot vertical lines at the time positions where time segments begin and end, and selecting **Zoom Time Range To Selected Segment** will zoom and scroll the time range so that the currently selected time segment fills the time range.

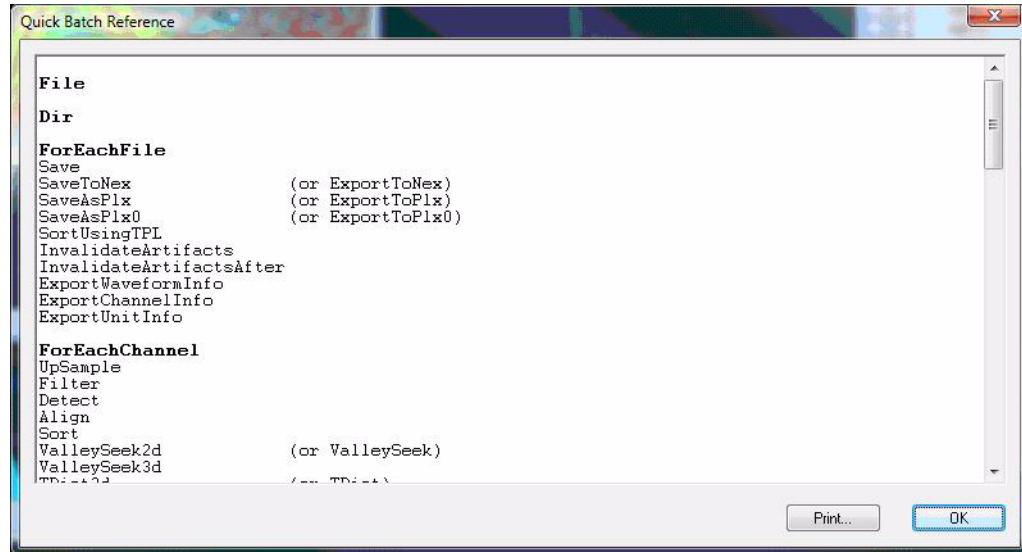
3.20 Help Files and Quick Key Reference

From the **Help** menu, one can click **Help Topics** to open the on-line version of this manual, and one can click **Quick Reference** to open the following window.



Scroll down to find the key combinations that apply to the view currently being used. To print the complete three-page reference, click **Print**. Click **OK** to close the window and return to Offline Sorter.

Similarly, clicking **Quick Batch Reference** will show the following window.



This window lists all Offline Sorter Batch commands with their arguments. See [“5.19 Batch File Processing” on page 225](#).

Chapter 4

Spike Sorting Step-by-Step

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4.1 Procedure Summary

The spike sorting process with Offline Sorter involves the following general procedures:

- Waveform Extraction (can be omitted, depending on type of data files)
- Preparation for Sorting
- Unit (Cluster) Selection
- Waveform Inspection and Unit Cleaning
- Saving Sorted Spike Times

Offline Sorter is capable of sorting action potential waveforms (spikes) in either continuously digitized data, or in previously sorted spike/waveform data files (re-sorting).

For sorting spikes in Continuously Recorded data

- 1 Open the continuously digitized data file. See [“4.2.1 Opening the Digitized Data Files” on page 91](#).
- 2 For each of the channels that contain spike data:
 - Select the data channel. See [“4.2.2 Selecting the Continuous Data Channel” on page 92](#).
 - Specify up-sampling if needed. See [“4.2.3 Specifying Up-Sampling” on page 94](#).
 - Check and adjust gains if needed. See [“4.2.4 Adjusting Gains” on page 95](#).
 - Specify the low-cut digital filter if needed. See [“4.2.5 Selecting the Low-Cut Digital Filter” on page 98](#).
 - Specify the waveform detection options. See [“4.2.6 Specifying the Waveform Detection Options” on page 99](#).
 - Set the threshold and extract the waveforms. See [“4.2.7 Extracting the Thresholded Waveforms” on page 102](#).
 - Go to [Step 3 on page 90](#).

For Re-sorting Spikes from Previously Sorted Waveform/spike Data files

- 1 Open the spike/waveform data file. See [“4.3.1 Opening the Spike/Waveform Data File” on page 103](#). Go to [Step 3 on page 90](#).
- 2 Convert Data Type if necessary. See [“5.3 Stereotrode and Tetrode Data” on page 186](#).
- 3 For each channel with spike waveforms:
 - Inspect the data. See [“4.4.1 Data Inspection” on page 106](#).


-
- Remove artifact waveforms. See [“4.4.2.1 Marking Artifact Waveforms as Invalid using the Waveform View”](#) on page 109.
 - If necessary, select intervals of valid data. See [“5.11 Interval Selection”](#) on page 211.
 - Check Waveform Alignment if necessary. See [“4.4.3 Waveform Alignment”](#) on page 115.
 - Adjust Sort Width if necessary. See [“4.4.4 Adjusting the Sort Range”](#) on page 118.
 - Select a Feature Space in which to perform sorting. See [“4.4.5 Selecting Features to Use For Sorting”](#) on page 119.
 - Select units using one of the supported spike sorting methods. See [“4.5 Spike Sorting”](#) on page 120.
- 4 For each sorted unit:
 - Clean the selection if necessary. See [“4.6.3 Cleaning the Selection”](#) on page 150.
 - Inspect individual waveforms if necessary. See [“4.7.1 Waveform Inspection”](#) on page 155.
 - 5 Save the timestamps as a NEX data file. See [“4.8.2 Exporting to a NEX File”](#) on page 165, or save the timestamps and waveforms in a data file. See [“4.8.1 Saving Timestamps and Waveforms”](#) on page 162.

4.2 Waveform Acquisition From Continuously Recorded Data

4.2.1 Opening the Digitized Data Files

Offline Sorter can sort waveforms from files containing continuously digitized data, such as that generated by the Plexon Recorder program (DDT file), other vendor’s files, or from other binary files that contain multiplexed A/D values recorded from several channels. For more information about reading and importing data files, see [“5.1 Importing Data Files”](#) on page 176.

To open a continuously digitized DDT data file

- press **File Open** toolbar button  , or,
select **File | Open** menu command.

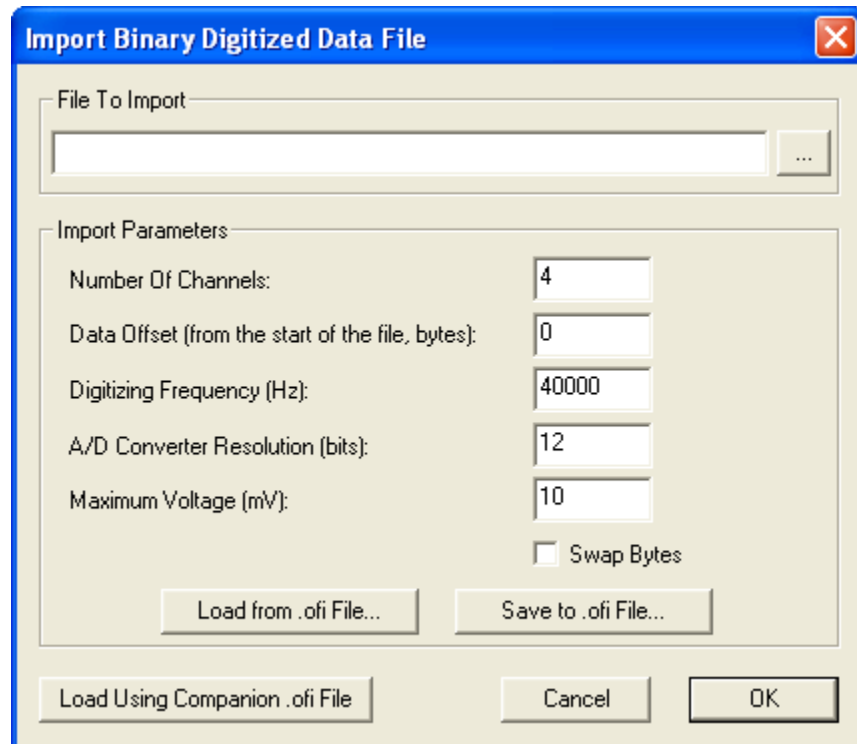
To open a continuously digitized data file from another vendor’s hardware

- Select **File | Import** menu command and select the vendor.

To open an arbitrary binary file with continuously digitized data (header plus multiplexed A/D values stored as 2-byte signed integers)

- 1 Select **File | Import | Binary file with Continuously Digitized Data**.

The *Import Binary Digitized Data File* dialog box opens.



- 2 Enter a file name and the following information: number of channels, data offset, digitization frequency, A/D conversion resolution, and maximum voltage. To perform byte swapping on the file, click **Swap Bytes**.

4.2.2 Selecting the Continuous Data Channel

By default, Offline Sorter will load the first data channel into memory immediately. Since loading the data channel from a large file can require considerable time, this feature can be disabled by unchecking the **Automatically Load First Channel after Opening a File** checkbox in the **General** tab of the **Tools | Data Import Options** dialog.

To select a particular data channel

- 1 Click on the **Channel** drop list box in the **Control Grid** (shows **None** in the illustration that follows).
- 2 Select the channel from the drop list.

Channel	None
Data Type	Single
Sort Method	Contours
Scale (%)	100
Max w/f draw	10000
Sort Start	0
Sort End	800
Slice 1	0
Slice 2	0
ClustHist Max	100000
Outlier Thr.	1.30
View w/f	All
Cluster View	All
Draw Grid	<input checked="" type="checkbox"/>
Refr ISI (us)	1000
ISIHistMax(ms)	100
Use Range	<input type="checkbox"/>
Range from (s)	0.000
Range to (s)	100.000
Invalid w/f	Show

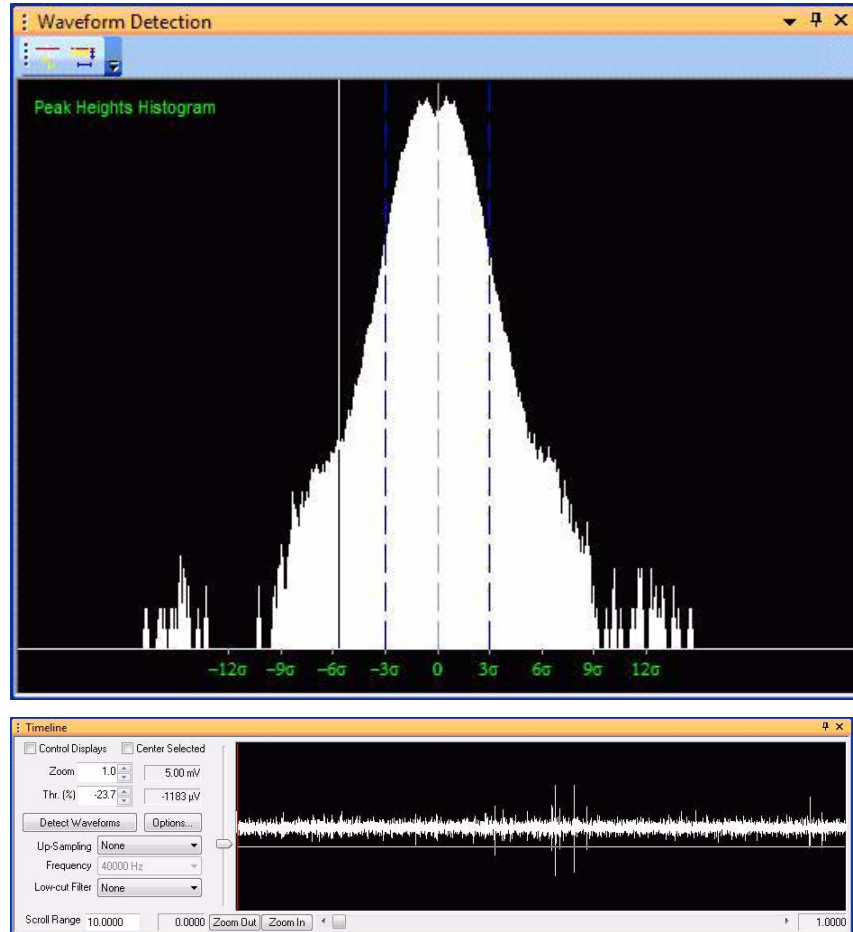
or in the **Channels** view of the Info Grid View, double-click on the row of the desired channel.

	Name	Type	Count	Unsorted	Unit a	Unit b
1	dsp001	Spikes	5376	5376	-	-
2	dsp002	Spikes	8956	8956	-	-
3	dsp003	Spikes	6414	6414	-	-
4	dsp004	Spikes	3971	3971	-	-

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The Timeline View will then show the channel's data. If it is not already showing, select **View | Waveform Detection**.



- 3 To move quickly to the next or previous channel in the list of channels, click **Select | Next Channel** or **Select | Previous Channel**. The following toolbar buttons can also be used:



- 4 Offline Sorter maintains a history of previously-opened channels. To revisit a previously-opened channel, click **Edit | Previous Channel in History** or click **Edit | Next Channel in History**. The following toolbar buttons can also be used.



The default setting is the Raw Signal detector, in which case the Waveform Detection view shows a histogram of the logarithm of the Peak Heights. The white lines on the displays indicate the current threshold setting.

4.2.3 Specifying Up-Sampling

Offline Sorter can increase the sampling frequency of continuous data channels by interpolating new samples between the original samples. This is useful in situations where continuous data is sampled at 20 KHz in the same file with spikes

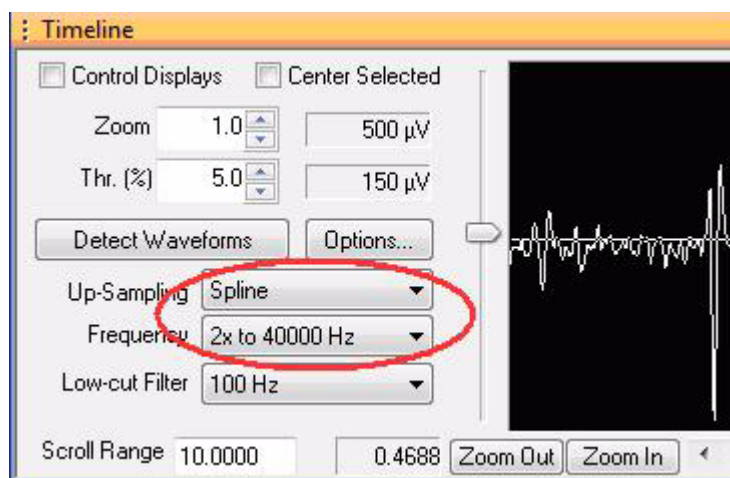
digitized at 40 KHz. Spikes cannot be extracted from the 20 KHz channels unless and until they are up-sampled to the spike frequency of 40 KHz.

Note that the new up-sampling frequency must be:

- 1 a multiple of the original sampling frequency, and
- 2 less than the spike frequency for the file.

Two different interpolation methods are available, Linear and Spline. Spline interpolation generally gives better results but is limited to doubling the original frequency of the continuous data. Linear interpolation is more flexible in that it can create any multiple of the original frequency, but the result is of somewhat lower quality.

To enable up-sampling, select either Spline or Linear from the Up-Sampling droplist in the Timeline View. The possible choices for the up-sampled frequency appear in the Frequency droplist. The data is immediately up-sampled as the Up-Sampling frequency is selected.



Note that it may not be apparent that the up-sampling was performed on the continuous data by looking at the Timeline View. It is instructive to select the **Show Dots at Sample Locations for Continuous Data** option in the right-click menu of the Timeline View, then changing the Frequency droplist. The number of dots will increase, as new sample locations appear between the previous ones.

4.2.4 Adjusting Gains

Offline Sorter utilizes the following ‘model’ for channel gains:

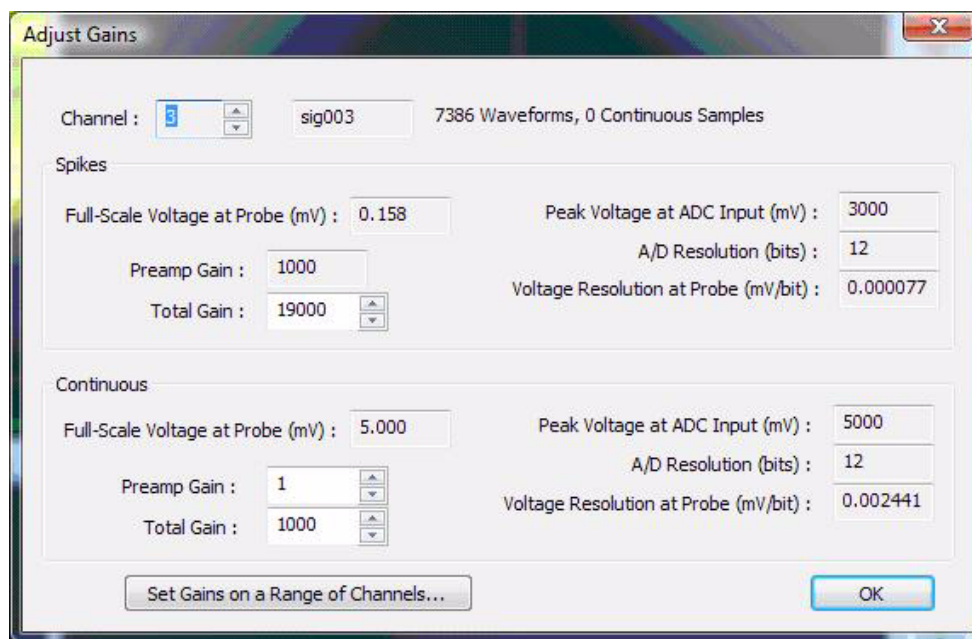
- Spike channels can have a single Spike Preamp Gain for all channels, and also a channel-specific Spike Gain. So the total gain for the spike signal path for a channel is the product of these gains.
- Continuous channels can have both a per-channel Continuous Preamp Gain and a per-channel Continuous Gain. So the total gain for the continuous signal path for a channel is the product of these gains.

- Any given channel can have both spike and continuous data present.

For a variety of reasons, gains can sometimes be mis-represented in data files. When this happens, voltages that Offline Sorter reports may be off by some constant factor. In many cases, this is not really a problem, as all the waveform shapes are still correctly represented and spike sorting depends only on relative voltage differences, not absolute voltages.

The File Summary Info Grid View shows an overview of the gain situation for a file, and the Channel Parameters View shows the individual gains for all channels. The individual gains can be adjusted using the Channel Parameters View, and in general, these gain changes will affect the interpretation of the currently-loaded data, and will be saved with an **'Export to .PLX'** operation.

There is also a **Tools | Adjust Gains** dialog that offers a convenient way to see the detailed gain situation for the current channel. This dialog can also be brought up from the right-click menu in the Channel Parameters View.



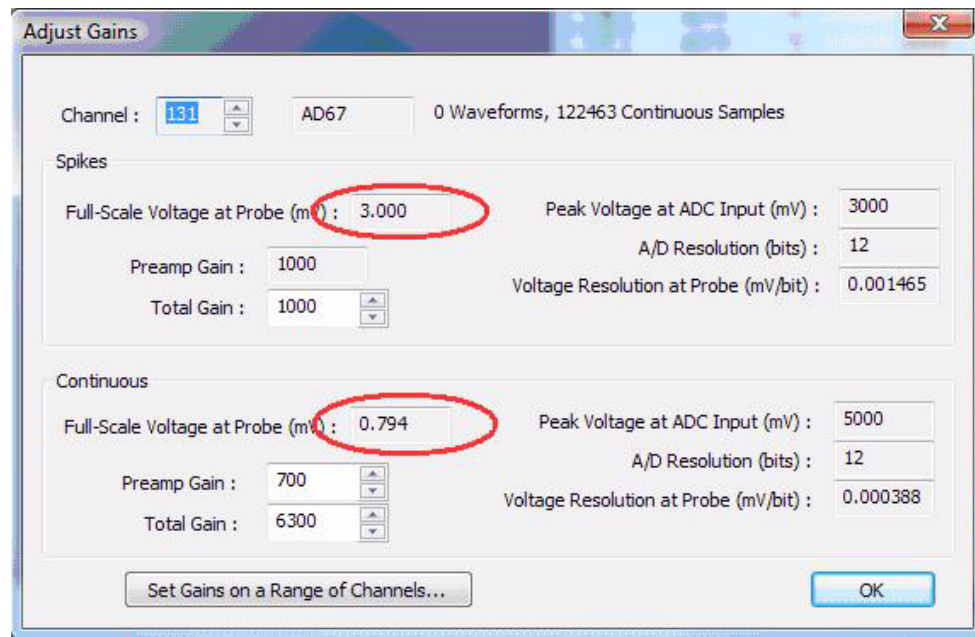
See descriptions of the gain-related items under the [“3.6.3 File Summary View” on page 38](#) and the [“3.6.2 Channel Parameters View” on page 36](#).

One situation in which having misrepresented gains can be problematic is for waveform extraction. It is recommended that before waveform extraction, the Adjust Gains dialog should be inspected for one or more channels. If the gain settings for the spike and continuous data are such that the Full-Scale Voltage at Probe value for spikes is significantly different that the value for continuous data, the result of the extraction could be sub-optimal in one of two ways:

- If the Full-Scale Voltage at Probe value is much greater for spikes than it is for continuous data, then the extracted spikes will be too small. That is, the

largest extracted spike will use only a small portion of the dynamic range available for spike waveforms. This means the resolution and detail is lost.

- If the Full-Scale Voltage at probe is much less for spikes than it is for continuous data, then the extracted spikes will be too large. That is, the extracted spikes are likely to exceed the maximum representable voltage for spikes and thus be cut-off or 'clipped'. This again results in information loss.



For example, in the above screenshot, the largest voltage value in any waveform that is present in the continuous data signal is 0.794 mV. But this voltage value represents about a quarter of the full-scale voltage range available for extracted spikes (3.0 mV). Thus, all extracted spikes will be small and some of the dynamic range for spikes will be wasted.

This situation can be changed by altering the gains until the Full-Scale Voltage at Probe values are more similar, but such that no clipping of the signal occurs.

Note: For versions of Offline Sorter prior to 3.0, waveform extraction was done in 'a/d count space', in that the threshold was applied to the raw a/d values, and the raw continuous a/d values became the raw spike a/d values verbatim. This would generally avoid the clipping and dynamic range under-utilization problems described above, but could make it nearly impossible to 'recover' actual voltage values in the extracted spike waveforms. Starting with Offline Sorter 3.0, the waveform extraction is now done in 'Voltage at the probe space', where if a waveform peak was 1.0 mV at the probe in the continuous data, the waveform peak will also be 1.0 mV at the probe in the extracted spike.

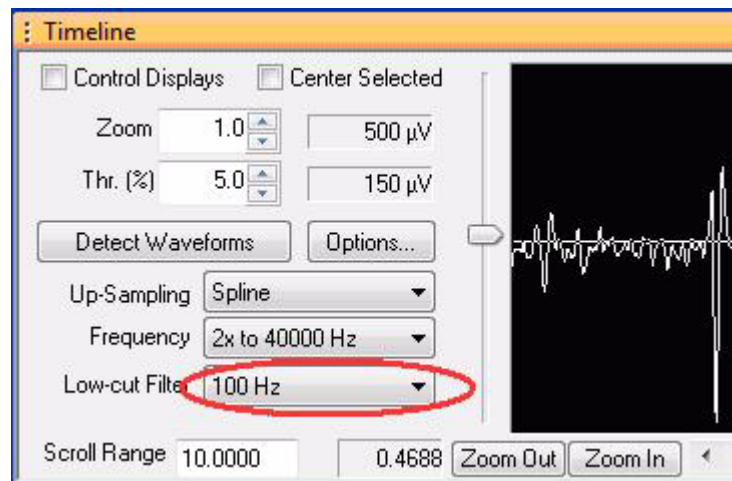
If the gain settings need to be changed for many channels, pressing the **Set Gains on a Range of Channels...** button will bring up a simple dialog where the range of channel numbers can be specified. If the **OK** button is pressed, those channels

will have their per-channel gain settings set to be identical to the channel being shown in the **Adjust Gains** dialog.

4.2.5 Selecting the Low-Cut Digital Filter

Offline Sorter can filter the signal from an active channel by using the low-cut digital filters. The filters available in the program are the 4th-order Butterworth IIR filters.

To select a filter, click the arrow of the **Low-cut filter** menu, which is highlighted in the following illustration, and then select the cut-off frequency value from the drop-down list.

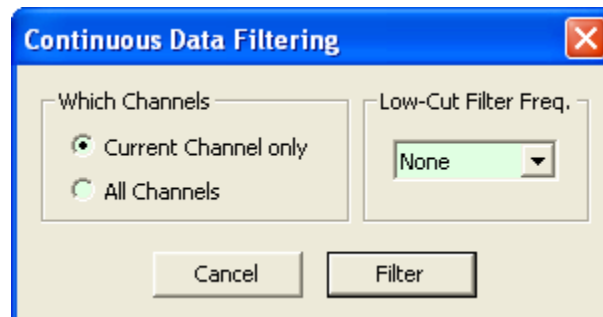


Note: Changing the filter causes the histograms and the waveform displays to be redrawn. In the **Display** tab of the **View | Options** window, if **Auto Zoom** is clicked, the zoom also adjusts.

Filtering All Channels

To apply the same low-cut filter to all channels, instead of just the current channel, from the **Waveforms** menu, select **Filter Continuous Data**.

*The **Continuous Data Filtering** dialog box opens.*



To apply the same filter to all channels, click **All Channels** and select a filter frequency from the **Low-Cut Filter Freq** area. Click **Filter** to apply the filter and close the dialog box.

4.2.6 Specifying the Waveform Detection Options

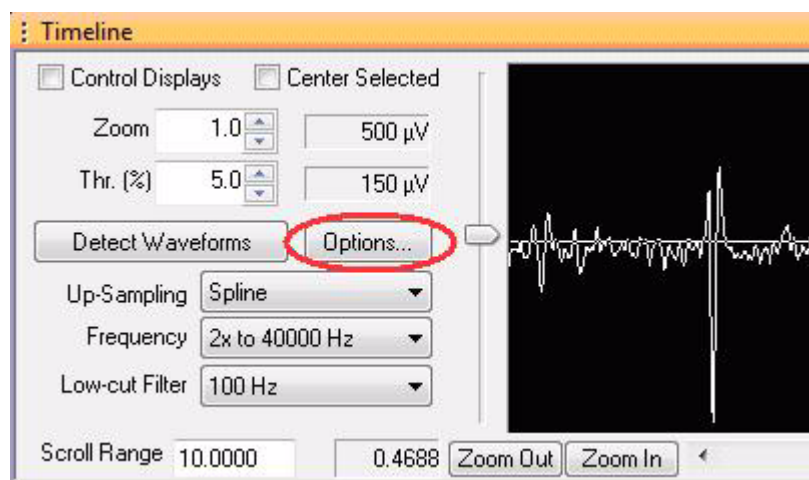
The **Total Waveform length** and **Prethreshold Period** parameters specify the time range of the waveform segments extracted from the continuous record.

The **Dead Time** is the minimum time between extracted waveform segments. For example, if the DeadTime = 1 msec and the recorded signal crosses the threshold at times $t = 5000.0$ msec and $t = 5000.8$ msec, then only one waveform segment aligned around the $t = 5000.0$ msec crossing is detected and extracted. If the DeadTime = 0.5 msec however, then two waveform segments are extracted: one aligned around the $t = 5000.0$ msec crossing and the other aligned around the $t = 5000.8$ msec crossing.

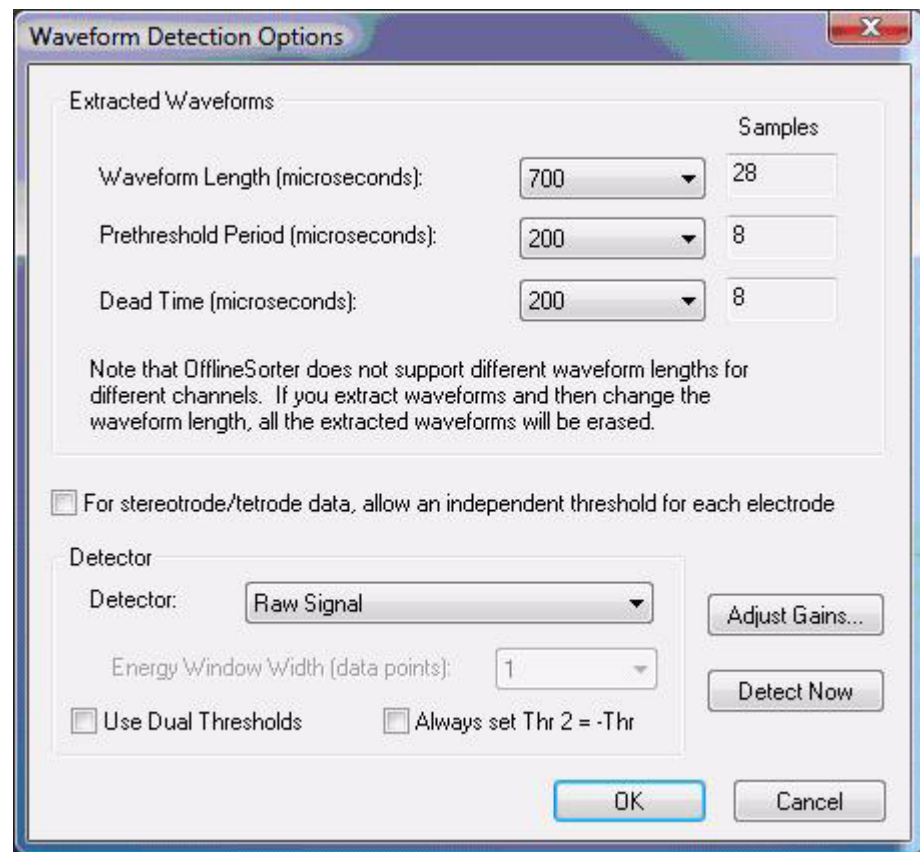
The **Detector** settings specify the quantity used in the threshold crossing algorithm for spike detection (See “3.12 Waveform Detection View” on page 69.). By default Offline Sorter uses the **Raw Signal** amplitude. If either **Energy of the Signal** or **Nonlinear Energy of the Signal** is selected the Energy Window Width can be specified.

To set the waveform detection parameters

- Select the **Detector and Waveform Options** button in the Timeline View.



The **Waveform Detection Options** window opens.



The **Waveform Options** window contains the following items:

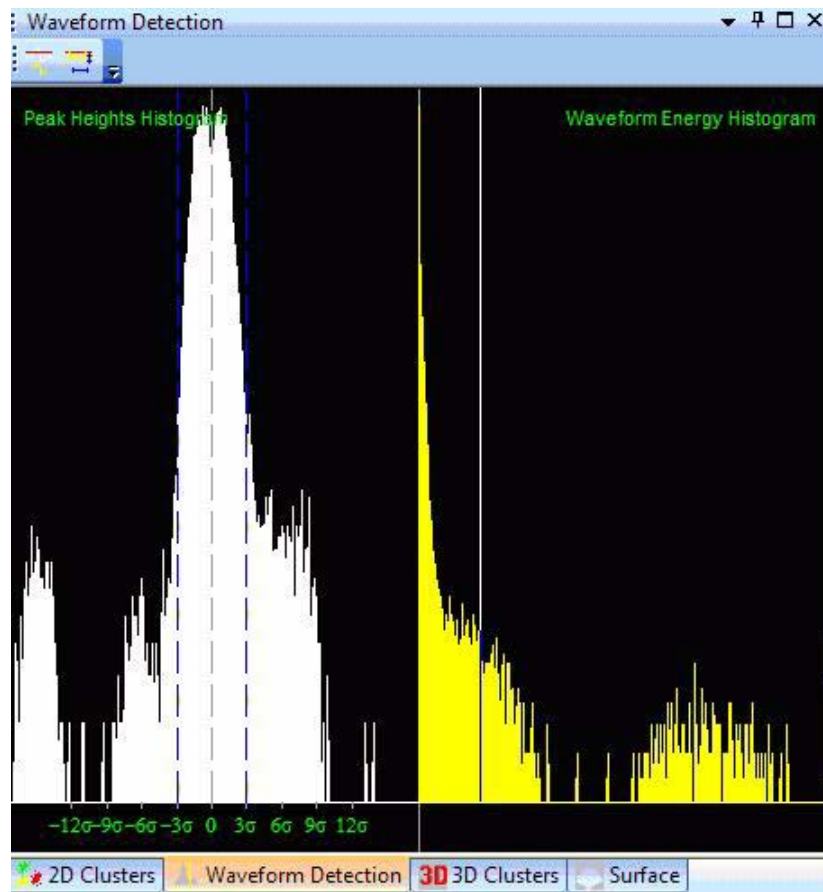
- **Total Waveform Length** – This item specifies the total length, in μsec and number of samples, of the thresholded waveforms that are extracted from the continuous data.
- **Prethreshold** – This item specifies the time, in μsec and number of samples, prior the threshold crossing included in the extracted waveforms.
- **Dead Time** – This item specifies the minimum time, in μsec and number of samples, between extracted waveform segments.
- **For stereotrode/tetrode data...** – This item provides a independent threshold setting for each electrode.
- **Detector** – The item indicates quantity used for thresholding, either **Raw Signal**, **Energy of the Signal**, or **Nonlinear Energy of the Signal**.
- **Energy Window Width** – This item specifies the width of the moving window over which the quantity is averaged (for energy and nonlinear energy detector options only).

- **Use Dual Thresholds** - This checkbox enables setting two different thresholds, with the waveform segments being taken if the signal exceeds either threshold (a logical OR of thresholds).
- **Always set Thr 2 =- Thr** - This checkbox always sets the second threshold to be the negative value of the first threshold, making the dual thresholds symmetric around zero.

Note: Using dual thresholds is not recommended except for unusual cases. A detailed understanding of waveform alignment issues is required to avoid being fooled, as dual thresholds can easily make a single unit appear as two distinct units depending on which part of the waveform crosses threshold first.

- **Adjust Gains** - This button brings up the Adjust Gains Tool, which allows for reconciling the voltage ranges between the continuous data and spikes. See [“4.2.4 Adjusting Gains” on page 95](#).
- **Detect Now** - This button will perform the waveform extraction, it is equivalent to pressing the Detect Waveforms button on the Timeline View.

If energy or nonlinear energy is selected as the signal to be thresholded in the **Detector** options menu, the quantity is displayed in the Timeline View and the histogram of the quantity (log axis) is displayed in the Waveform Detection view.



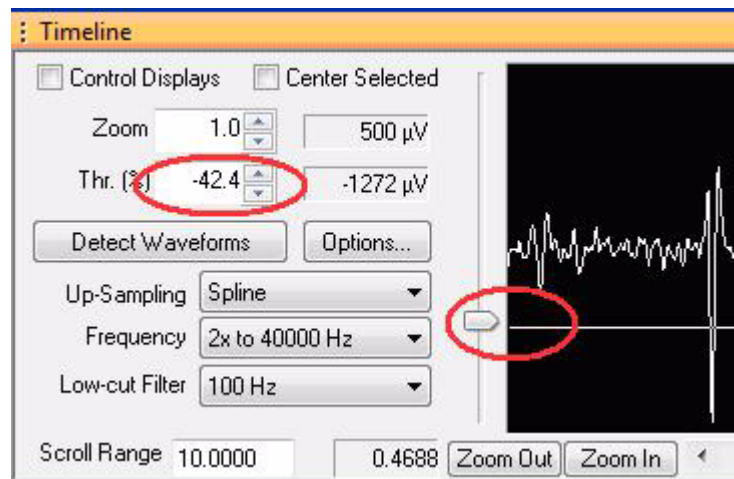
The threshold can then be adjusted to different energy/nonlinear energy levels or histogram values.

4.2.7 Extracting the Thresholded Waveforms

Offline sorter uses the following algorithm to extract the waveforms from continuously recorded signals:

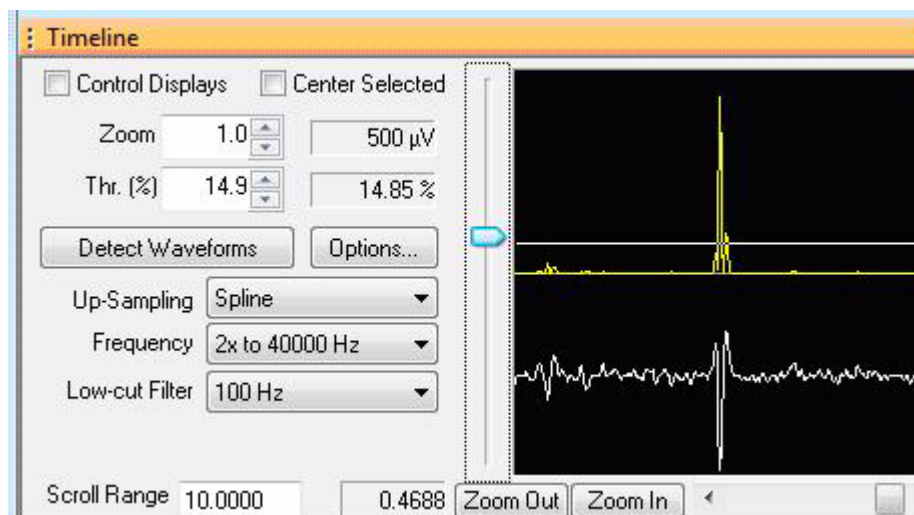
- If the threshold is *positive or zero*, the program looks for the moment when the signal crosses the threshold in the positive direction:
find the first t such that $\text{signal}[t-dt] < \text{threshold}$ and $\text{signal}[t] \geq \text{threshold}$
- If the threshold is *negative*, the program looks for the moment when the signal crosses the threshold in the negative direction:
find the first t such that $\text{signal}[t-dt] > \text{threshold}$ and $\text{signal}[t] \leq \text{threshold}$
- The time t then becomes the timestamp of the waveform. The program stores the specified number of data points before t and the number of points after t in the extracted waveform that is used later for sorting (See [“4.2.6 Specifying the Waveform Detection Options” on page 99](#) to see how to set the waveform length and the number of prethreshold points.).
- When the current waveform is extracted (that is, the specified number of data points after the threshold crossing is stored), the program moves ahead in time by the amount specified by the **Dead Time** parameter—see [“4.2.6 Specifying the Waveform Detection Options” on page 99](#)—and then it starts looking for the next waveform. This means that waveforms that cross the threshold less than the dead time after a detected spike are not detected.

To set the threshold, use the threshold edit control in the Timeline View or drag the horizontal threshold line with the mouse.



Alternately, dragging the vertical threshold line on the histogram in the Waveform Detection view to the left or right can set the threshold.

Click **Detect Waveforms** to extract the waveforms using the specified detector, total waveform length, prethreshold period, dead time, and threshold level.



One can automatically set a threshold based on the statistical properties of the peak heights and perform waveform detection on all channels (as opposed to doing a single channel at a time). For complete information, see [“5.4 Automatic Waveform Detection” on page 190](#).

4.3 Waveform Acquisition From Spike/Waveform Data

4.3.1 Opening the Spike/Waveform Data File

Offline Sorter can read native data files containing spike timestamps and spike waveform segments created by the Plexon MAP system (PLX) as well as other data acquisition systems (CyberKinetics, CED Spike 2, DataWave, Instrutech, MultiChannel Systems, Panasonic MED, Neuralynx, RC Electronics).

To open a data file

- Click the **File Open** toolbar button,



, or

select **File | Open** menu command.

To open a spike/waveform file from another vendor's hardware

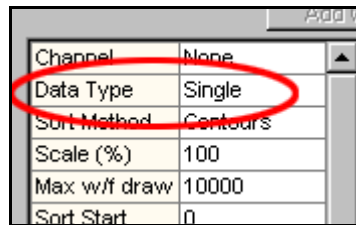
- Select **File | Import** menu command and select the vendor.

Once the data file is opened, the waveforms are viewable and ready for sorting.

4.3.2 Stereotrode and Tetrode Data Type Selection

Offline Sorter can sort files with signals from single electrodes, stereotrodes, or tetrodes (see also [“5.3 Stereotrode and Tetrode Data” on page 186](#)).

The **Data Type (Single/Stereotrode/Tetrode)** is displayed in the Control Grid.



For Plexon PLX files, DataWave UFF files, and Neuralynx files, Offline Sorter automatically detects stereotrode or tetrode data when the file is opened, and sets the **Data Type** accordingly. For all other file types, Offline Sorter initially assumes single-channel data.

If the data type displayed in the Control Grid is not appropriate for the data file opened, Offline Sorter can sometimes convert the data type. For a description of this procedure, see [“5.3.3 Converting Data Type” on page 188](#).

4.3.3 Selecting the Data Channel

By default, Offline Sorter will load the first data channel into memory immediately. Since loading the data channel from a large file can require considerable time, one can disable this feature by unchecking the **Automatically Load First Channel after Opening a File** checkbox in the **General** tab of the **Tools | Data Import Options** dialog.

To select a particular data channel

- 1 Click on the **Channel** drop list box in the Control Grid (shows **None** in the illustration that follows).
- 2 Select the channel from the drop list.

Channel	None
Data Type	Single
Sort Method	Contours
Scale (%)	100
Max w/f draw	10000
Sort Start	0
Sort End	800
Slice 1	0
Slice 2	0
ClustHist Max	100000
Outlier Thr.	1.30
View w/f	All
Cluster View	All
Draw Grid	<input checked="" type="checkbox"/>
Refr ISI (us)	1000
ISIHistMax(ms)	100
Use Range	<input type="checkbox"/>
Range from (s)	0.000
Range to (s)	100.000
Invalid w/f	Show

or in the **Channels** view of the Info Grid View, double-click anywhere on the row of the desired channel.

	Name	Type	Count	Unsorted	Unit a	Unit b
1	dsp001	Spikes	5376	5376	-	-
2	dsp002	Spikes	8956	8956	-	-
3	dsp003	Spikes	6414	6414	-	-
4	dsp004	Spikes	3971	3971	-	-

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The main views in Offline Sorter then show the channel's data.

- 3 To move quickly to the next or previous channel in the list of channels, click **Select | Next Channel** or **Select | Previous Channel**. The following toolbar buttons can also be used:



- 4 Offline Sorter maintains a history of previously-opened channels. To revisit a previously-opened channel, click **Edit | Previous Channel in History** or **Edit | Next Channel in History**. The following toolbar buttons can also be used.



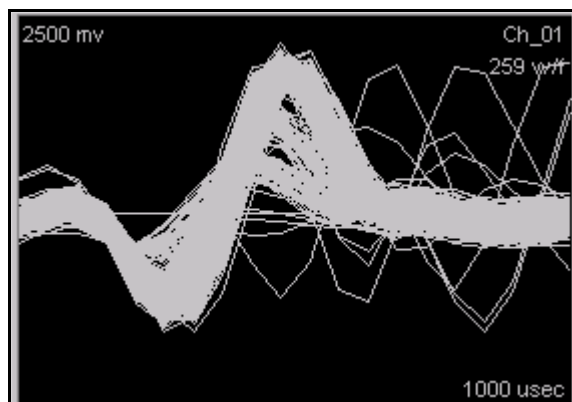
4.4 Preparation For Sorting

4.4.1 Data Inspection

After the segmented waveforms (spikes) are acquired, they are displayed in the Waveform View and the Clusters Views. Offline Sorter allows the user to quickly get an overview of the data.

4.4.1.1 Waveform View

The Waveform View shows the superimposed waveforms aligned at the threshold crossing times:



HINT

Controlling Which Waveforms Are Displayed

The scroll bar at the bottom of the [Timeline View](#) (use the **Control w/ form display** check box) and the **Waveform View** parameter in the [Control Grid](#) can be used to control which waveforms are displayed in the Waveform View. If one limits the waveforms displayed in this way it can often reveal structure that is hidden when all waveforms are drawn.

By default, Offline Sorter automatically determines the zoom factor for the Waveform View and the Timeline View. The zoom factor can be disabled by unchecking the **Auto Zoom** checkbox in the **Tools | Options | Display** dialog box. The auto-zoom calculation finds the peak amplitude present in any spike waveform, and sets the zoom to the largest integer value that displays the waveforms without clipping them. The Auto-zoom procedure can be performed at any time by selecting the **Waveforms | Auto-Zoom Waveform Displays** menu item.

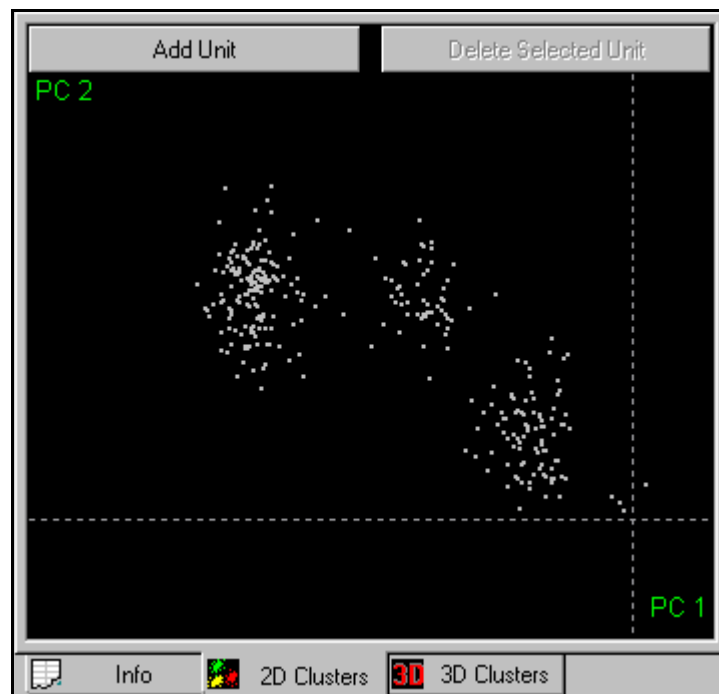
Offline Sorter reads spike waveforms from a file or detects them from continuous data, and stores them in memory. One can invert the waveforms stored in mem-

ory by using **Tools | Invert Waveforms** on the main menu. After an **Invert Waveforms** operation is done, all principal component and feature calculations use the inverted version of the waveform. If **Export to New .PLX** is selected, Offline Sorter writes the inverted version of the waveforms to the output file. However, if a normal **Save** or **Save As** operation is done, Offline Sorter does not save the inverted waveforms.

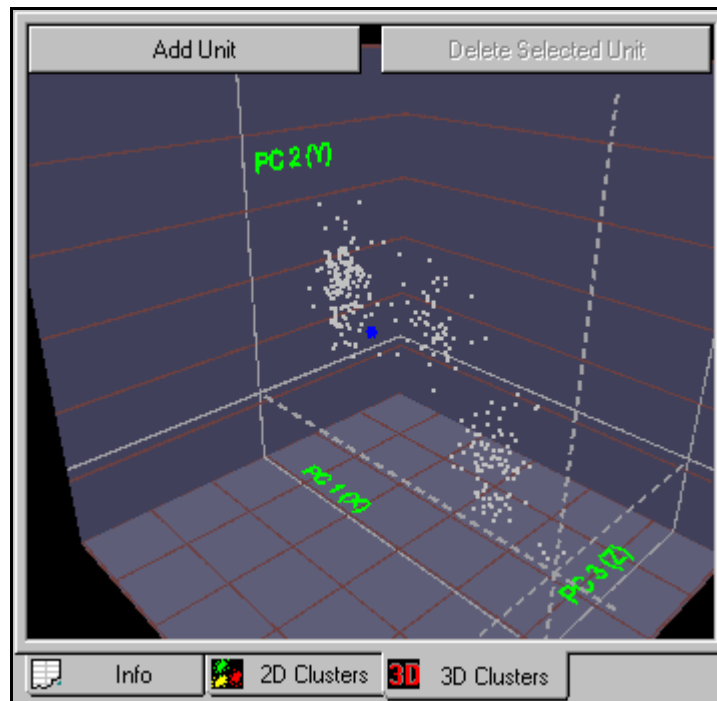
4.4.1.2 Clusters Views

The “[3.7 Clusters Views](#)” on [page 43](#) show the waveforms as points in feature space. One can choose to use either the 2D Clusters View or the 3D Clusters View, or both. The variables shown on the X, Y and Z axes can be chosen from a list of active features. For more information, see the “Axis Feature Selection” section under “[3.7.1 Clusters View Common Functionality](#)” on [page 45](#). By default, the Clusters Views show the PC1, PC2, and PC3 features on the X, Y, and Z axes.

2D Clusters View



3D Clusters View



4.4.1.3 Timeline View

The Timeline View shows the waveforms in their temporal sequence. This is useful for determining if the data comes in “bursts” or if there are gaps in the data. Also, the times of occurrences of external events can be superimposed on the Timeline View (See [“5.9 Examining Events” on page 207.](#)), which the spike activity surrounding those events to be seen.

4.4.2 Invalidating Waveforms

Offline Sorter contains a mechanism to mark waveforms as being “invalid”, so that they are not considered in subsequent operations such as spike sorting. There are several reasons to do this:

- 1 Waveforms with shapes uncharacteristic of neuronal action potentials, such as those caused by stimulus or movement artifacts, can be marked as invalid and removed before spike sorting is performed. One can remove these waveforms by following the procedure in the [“4.4.2.1 Marking Artifact Waveforms as Invalid using the Waveform View” on page 109.](#) In general, artifact waveforms are best invalidated, because they can significantly influence the principal components and the display ranges for the other views (invalidated waveforms are not used when auto-scaling and auto-zooming operations are performed by Offline Sorter).
- 2 Waveforms can be just “noise” that satisfied the threshold but that did not represent action potentials. The easiest way to invalidate these noise waveforms is to use the Clusters Views, as outlined in the [“4.4.2.2 Marking Noise Wave-](#)

forms as [Invalid Using the Clusters Views](#)” on page 110. This step is optional; one can also just leave these noise waveforms as unsorted.

- 3 Another reason to mark waveforms as invalid is that they are simply not of interest. For example, if the experimental animal was not exhibiting “interesting” behavior during certain time intervals, the waveforms collected from those intervals can be invalidated simply to get them out of the way. Offline Sorter contains a tool that enables selection of waveforms within some interval in time around an Event (or between pairs of Events) in the data file. For more details, see [“5.11 Interval Selection”](#) on page 211.

Note: Waveforms can be marked as invalid in either waveform space or in feature space.



CAUTION

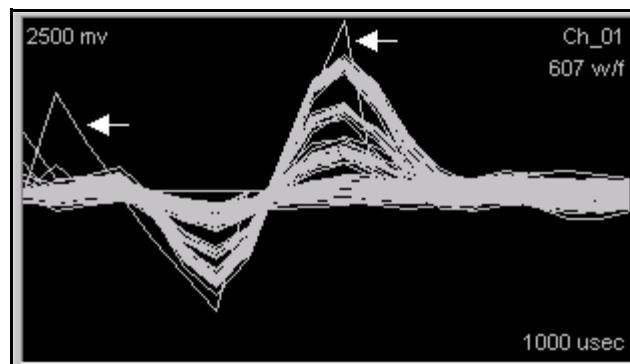
Invalidated waveforms not marked

Invalidated waveforms may not be marked as such in the output file for some file formats (notably PLX). When the file is read in again, the invalidated waveforms will be valid again. However, the **File | Export to New .PLX** feature offers an option to not write invalidated waveforms to the output file.

View

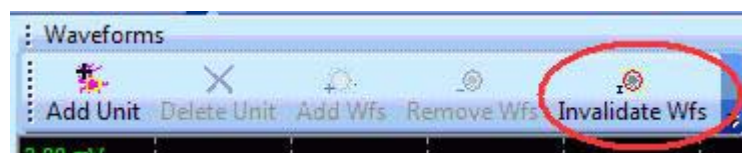
4.4.2.1 Marking Artifact Waveforms as Invalid using the Waveform

In the Waveform View shown below, two artifacts (arrows) can be clearly identified due to their sharp waveform shapes.




To mark the artifact waveforms as invalid and remove them from the display

- 1 Click **Invalidate Wfs.**

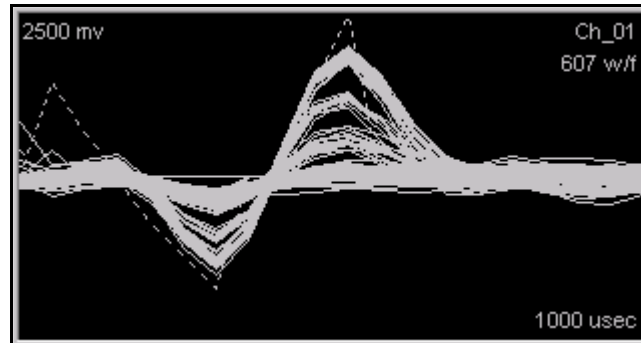


- 2 Move the pointer to the **Waveform View**.

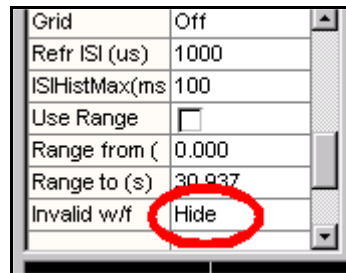
The pointer changes to a “drawing hand” .

- 3 Click the left mouse button and drag a line across the artifact waveforms.

The invalidated waveforms appear as dashed lines.



The invalid waveforms are ineligible for unit selection in any of the spike sorting methods. One can remove them from the Waveform View and the Clusters Views by setting the **Invalid w/f** parameter in the Control Grid to **Hide**.



One can undo the invalidation operation by using the undo mechanism, or by selecting the **Waveforms | Mark All Waveforms As Valid** menu command.

Views

4.4.2.2 Marking Noise Waveforms as Invalid Using the Clusters

Artifact waveforms generally appear as outlying points on the Clusters Views. One can mark the points as invalid by using a procedure similar to that described in this section.

Another reason to mark waveforms as invalid is because they are due to “noise” that does not represent a valid neural action potential. This section describes how to invalidate noise waveforms when the 2D Clusters View is showing PC1 vs. PC2. One can use the same procedure in the 3D Clusters View and in any feature space.

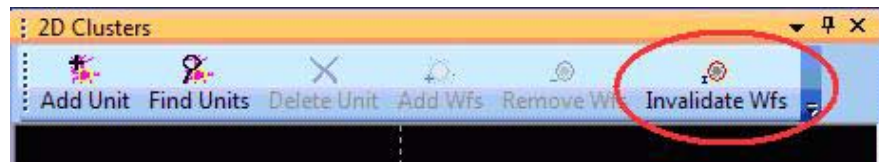
The principal components of the extracted waveforms represent linear combinations (linearly independent projections) of the waveforms which account for

maximal variance in the waveform data. Maximal variance implies maximal separation of the noise cluster from the unit clusters and the unit clusters from one another. For low amplitude noise, the noise cluster is centered close to the origin (0,0) in principal component space.


If the noise cluster is localized near zero and isolated, it can be removed from the principal component calculations so that the new principal components maximally separate the remaining unit clusters from one another. One can then select the maximally separated unit clusters by using the contour method.

To invalidate the noise waveforms near zero

- 1 Click **Invalidate Wfs.**

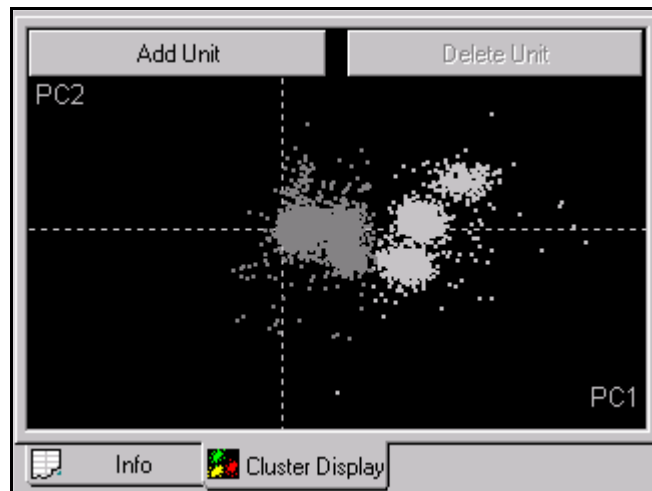


- 2 Move the mouse pointer to the Clusters View.

The pointer changes to a “drawing hand” .

- 3 Click the left mouse button and drag a line around the invalid “noise” waveforms near zero.

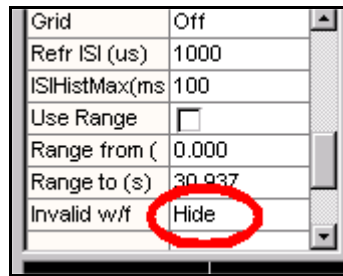
In the following 2D Clusters View, the noise cluster near the origin is invalidated (gray).



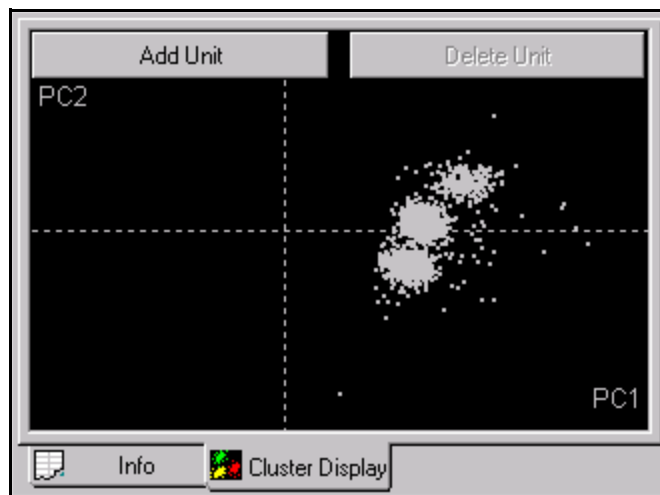
Note: One can also use the 3D Clusters View for the following procedure.

To remove the invalid waveforms from the display

- Set the **Invalid w/f** parameter in the Control Grid to **Hide**.

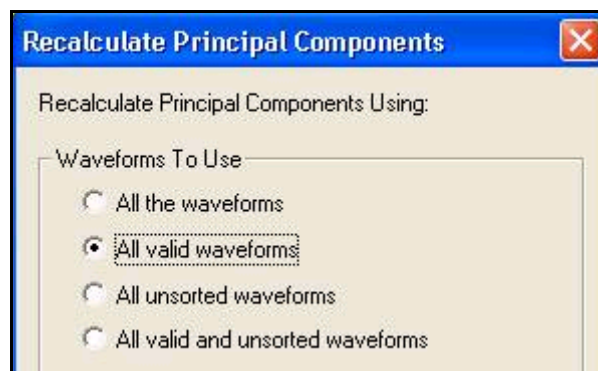


The invalid waveforms disappear from the display.

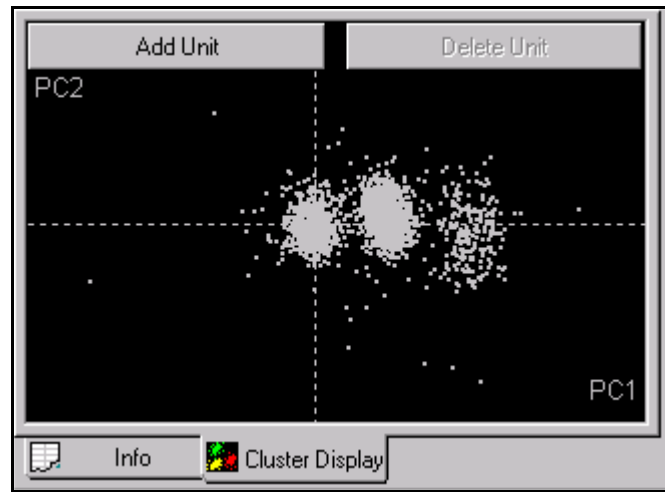


To recalculate the principal components using only the valid unit cluster data:

- Select the **Tools | Recalculate the Principal Components** menu command and rerun the principal component analysis using **All valid waveforms**.



The resulting 2D Clusters View can resemble the following one.



The three unit clusters (white dots) are now slightly better separated from one another than they were when the noise cluster was included in the PCA calculations.

Note: One can undo the invalidation operation by selecting the **Waveforms | Mark All Waveforms As Valid** menu command.

4.4.2.3 Invalidating All Except

The preceding invalidation procedures designated the waveforms to be *invalidated*. One can “invert” that process to designate the waveforms to be *retained* by using the **Waveforms | Invalidate All Except Selected Waveforms** setting. When this setting is selected, all waveforms that are not selected are invalidated. This selection applies in both the Waveform View (cross the waveforms to be *retained*) and in the Clusters Views (circle the waveforms that are to be *retained*).

4.4.2.4 Automatically Invalidating Artifacts

Offline Sorter includes a tool that automatically finds and invalidates artifacts that occur simultaneously (or nearly so) across several channels. The Invalidate Artifacts tool only uses spike times. The tool does not consider waveform shapes or amplitudes.

To use the Invalidate Artifacts tool, two criteria must be set:

- 1 a time width that ranges downstream from the first artifact threshold crossing point on any channel
- 2 a presence criterion that sets the number of channels on which a spike must be simultaneously present within the time range, to be identified as an artifact

When the tool determines that an artifact is present at time T on a sufficient number of channels, the tool invalidates all waveforms that are present on all channels between the times T_{\min} and T_{\max} , where T_{\min} and T_{\max} are the minimum and

maximum time stamps of the spikes in the artifact. The tool runs on all channels, not just the currently loaded channel.

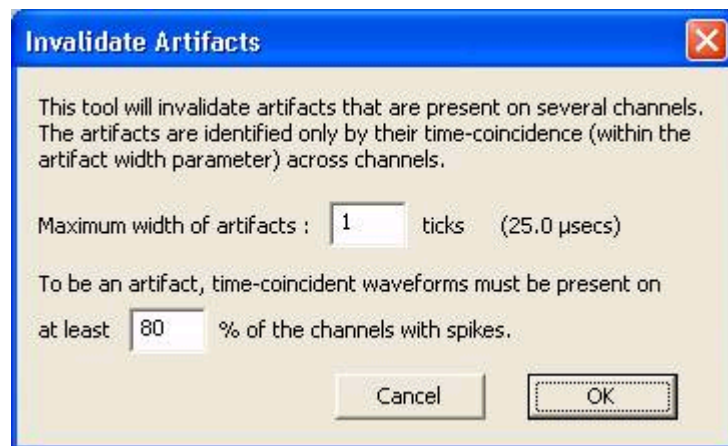
To be identified as artifacts, *simultaneous* spikes must be present on at least a critical number of channels. Set the presence criterion in percent multiplied by the number of channels with spikes. The Invalidate Artifacts tool completely ignores any channels that have 0 spikes in them and assumes they are inactive or disabled channels.

Because artifact waveforms can cross different thresholds at slightly different times, different threshold settings on different channels effect the timestamps of artifacts. The time width setting provides a way to bracket timestamps so that artifacts are still recognized with different thresholds. One can set the channel presence criterion to take into account the fraction of channels that contain high-quality, analyzable data, as opposed to occasional noise triggers.

Using automatic artifact invalidation

- 1 Click **File | Invalidate Cross-Channel Artifacts**.

*The **Invalidate Artifacts** window opens.*



- 2 In the **Maximum width of artifacts** box, type the maximum time width in ticks (units of 25.0 μ s) of the artifacts to be invalidated.

Note: For example, if the timestamps on two channels differ by this number of ticks or less, the tool considers the spikes on the two channels to be simultaneous. If enter 0 (zero) is entered, the tool only invalidates artifacts with identical timestamps.

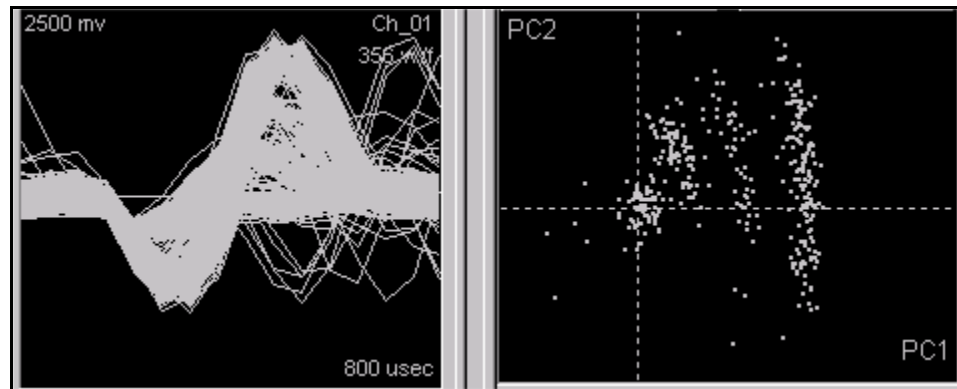
- 3 In the **% of the channels with spikes** box, type the channel presence criteria in percent. For example, in the illustration in Step 1, the tool invalidates

artifacts of the specified width and with simultaneous timestamps that are present on 80% of the channels with spikes.

- 4 To start the tool, click **OK**.

4.4.3 Waveform Alignment

After detection and extraction, the waveforms are initially aligned at the point of threshold crossing in the Waveform View (below left). The principal components are calculated using the threshold-aligned waveforms. One can select the projections onto the principal components in the 2D Clusters View, as follows in the right-side illustration.

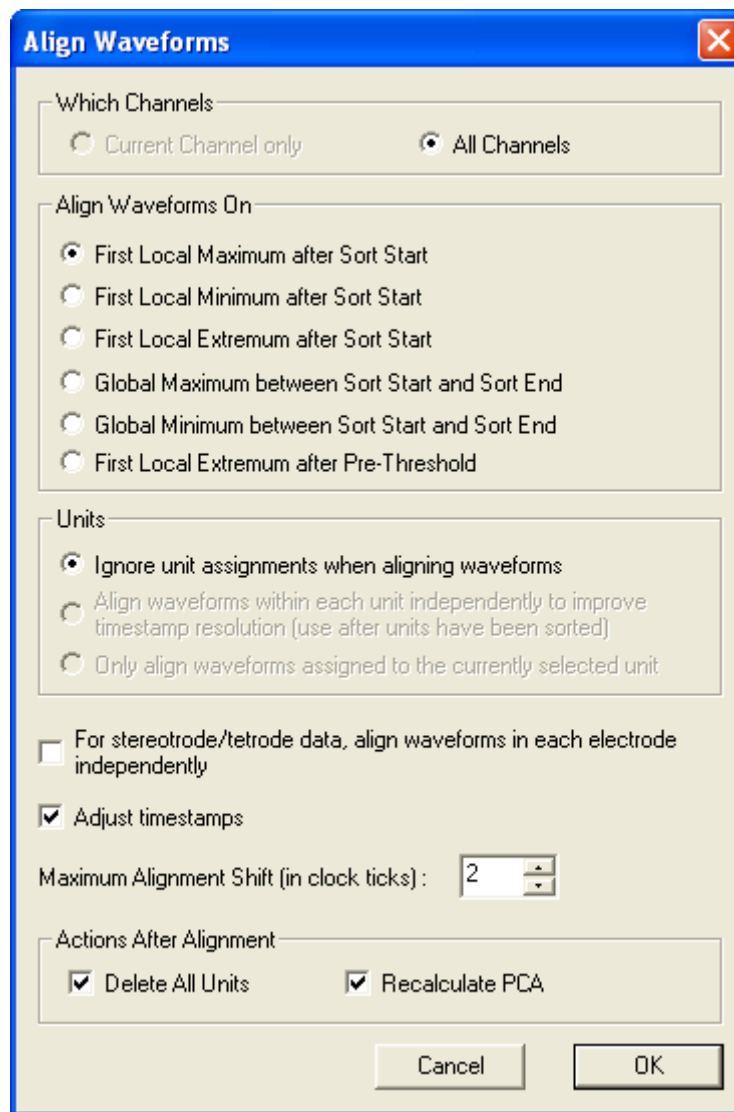


The variance within waveform groups can frequently be reduced, and the separation between waveform groups enhanced, by aligning the waveforms at their minimum or maximum values.

To set the alignment criteria

- 1 Select the **Waveforms | Align** menu command,
or,
select the **Tools | Align Waveforms** menu command.

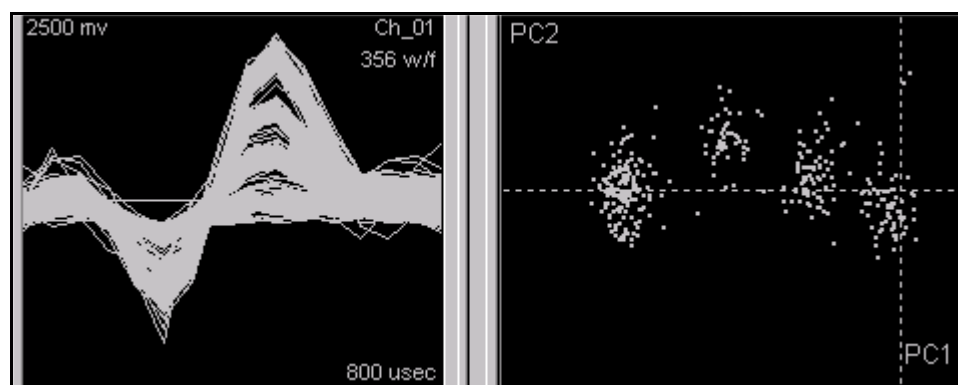
- 2 Then select the desired alignment method.



The image shows a software dialog box titled "Align Waveforms". It contains several sections for configuring waveform alignment. The "Which Channels" section has two radio buttons: "Current Channel only" and "All Channels", with "All Channels" selected. The "Align Waveforms On" section has six radio buttons: "First Local Maximum after Sort Start" (selected), "First Local Minimum after Sort Start", "First Local Extremum after Sort Start", "Global Maximum between Sort Start and Sort End", "Global Minimum between Sort Start and Sort End", and "First Local Extremum after Pre-Threshold". The "Units" section has three radio buttons: "Ignore unit assignments when aligning waveforms" (selected), "Align waveforms within each unit independently to improve timestamp resolution (use after units have been sorted)", and "Only align waveforms assigned to the currently selected unit". Below this is a checkbox "For stereotrode/tetrode data, align waveforms in each electrode independently" which is unchecked. Another checkbox "Adjust timestamps" is checked. A text field "Maximum Alignment Shift (in clock ticks):" contains the value "2". The "Actions After Alignment" section has two checkboxes: "Delete All Units" and "Recalculate PCA", both of which are checked. At the bottom are "Cancel" and "OK" buttons.

Under most circumstances, aligning around the **Global Maximum** or **Global Minimum** provides the best alignment of the waveforms. Optionally, all units can be deleted (the **Delete All Units** checkbox) and the PCA recalculated (**Recalculated PCA** checkbox) when the waveforms are realigned.

After alignment around the global minimum (as follows), note how the waveforms are more tightly clustered in waveform space and principal component space.



By default, unit assignments are ignored when performing alignment (the **Ignore unit assignments when aligning waveforms** radio button is checked). One can check the **Align waveforms within each unit independently** radio button after the units have been sorted. This actually performs N independent alignment operations, where N is the number of units. For each alignment, only the waveforms in a given unit are considered when choosing the alignment center for the operation. This has the effect of adjusting timestamps to correct for triggering jitter.



CAUTION

View reports with caution

Use the **Align waveforms within each unit independently** setting with caution! By its nature, it appears to “tighten up” the clusters. However, this is a bit misleading because it was done with a-priori knowledge of the clusters. After performing this operation, view the probabilities and F-statistics reports with caution.

The **Only align waveforms assigned to the currently selected unit** option is similar to the previous option in that it only performs the alignment on the current primary selected unit.

The **For stereotrode/tetrode data, align waveforms in each electrode independently** checkbox is only relevant when working with stereotrode/tetrode data. When unchecked, the concatenated waveform (See “[5.3 Stereotrode and Tetrode Data](#)” on page 186.) is aligned as a whole—the min or max of the entire concatenated waveform is found and the concatenated waveform is shifted as a whole. When checked, the alignment is instead performed per-electrode. For example, for tetrode data, four independent alignments are performed, one for each electrode. Note that the waveform in each electrode can be shifted a different amount.

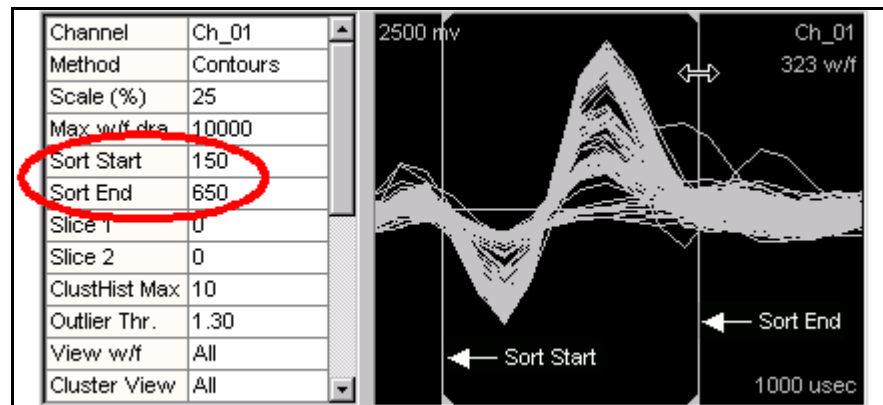
The **Adjust Timestamps** checkbox controls whether or not the timestamp of the waveform is shifted by the amount the waveform is shifted.

The **Maximum Alignment Shift** adjustment enables one to put a cap on the number of clock ticks that any waveform is shifted. It is seldom desirable to shift a waveform by more than a few clock ticks, because zeros are added onto the end of the waveform to backfill the shifted amount. Waveforms that would be shifted by more than the specified number of clock ticks are not shifted at all.

One can perform the same alignment operation on all channels, instead of just on the current channel. To do so, select **Waveforms | Align**. When the same **Align Waveforms** dialog box as shown in [Step 2 on page 116](#) appears, click **All Channels**. Now the alignment operation is applied to all of the spike channels in the file.

4.4.4 Adjusting the Sort Range

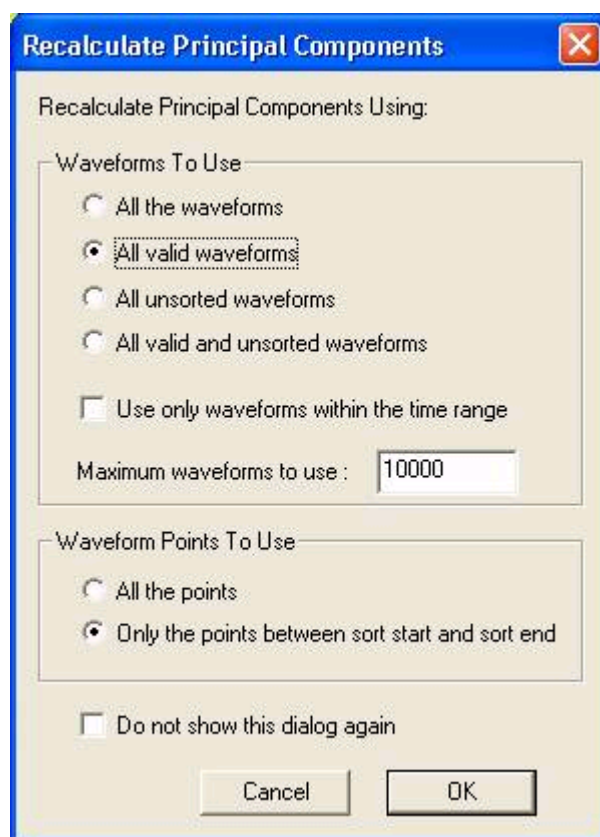
Often the extreme tail and the earliest samples of the waveforms are not useful in separating units from each other or separating the units from noise. To specify the range of samples within the waveform that is used to calculate the Principal Components (See [“5.5.1 Principal Components” on page 193.](#)) and used in feature calculation and template sorting, adjust the **Sort Start** and **Sort End** parameters in the Control Grid; see the following illustration to locate the parameters. When they are visible, one can adjust the **Sort Start** and **Sort End** times by dragging the corresponding vertical line with the mouse; see the double-ended arrow in the following illustration.



The **Sort Start** and **Sort End** time point is the lower and upper bound, respectively, of the time interval of the waveform used to calculate principal components, feature values, and for template sorting. The **Sort Start** and **Sort End** times are represented by vertical lines in the Waveform view; see the arrows in the preceding illustration. Small arrows on the top and bottom of the lines indicate whether it's the start or end. In the previous example, the total waveform length is 1000 msec, the **Sort Start** time is 150 msec, and the **Sort End** time is 650 msec.

Note: For stereotrode and tetrode data, the sort range is specified per-electrode, not for the concatenated waveform.

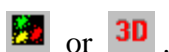
To recalculate the principal components using the time range between the **Sort Start** and **Sort End** time points, select the **Tools | Recalculate Principal Components** menu command and recalculate using **All the points** and **Only the points specified by sort start and sort end**.



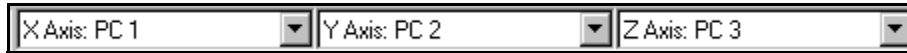
4.4.5 Selecting Features to Use For Sorting

For those sorting methods that use feature space (i.e. all methods except waveform crossing and templates), one must first decide which feature space to work in. One does this by selecting particular features for each of the 2 (for the 2D Clusters View) or 3 (for the 3D Clusters View) axes in feature space (See [“5.5 Features Available for Sorting” on page 191.](#)). Offline Sorter does all spike sorting and calculates all statistics in the feature space selected, so the objective is to find a combination of features that gives maximal cluster separation. The description of the [“3.15 Feature vs. Feature View” on page 75](#) can be useful in selecting which features to use.

First, select either the **2D Clusters View** or the **3D Clusters View** using the **View** menu command or the toolbar buttons



Then, select features for the X, Y, (and Z) axes from the drop-down lists on the toolbar:



Note: Only the “active” features appear in the drop-down lists, which are a subset of all features. Use the **Active Features** tab of the **Tools | Options** dialog box to select which features are active. For more information, see [“3.7.1 Clusters View Common Functionality” on page 45](#).

When switching channels within the currently-opened data file, the selected features can either be “remembered” on a per-channel basis, or the same feature space can be used across all channels. One can control this by the **Remember selected features for each channel** checkbox in the **Tools | Options | Feature Calc** dialog box.

If the sorting algorithms are used, remember that they work differently in 2D and 3D feature space. The feature space that the algorithms use depends on the active view when the sorting operation initiates. See [“5.6.1 Feature Space Used for Sorting” on page 196](#).

4.5 Spike Sorting

Finally, the waveforms can now be sorted into units. Offline Sorter offers several methods to accomplish this.

4.5.1 Manual, Semi-Automatic, and Automatic Clustering Methods

Offline Sorter offers manual, semi-automatic, and automatic methods to identify units or clusters. On a given channel, any or all of these methods can be used.

4.5.1.1 Manual Clustering Methods

With these methods, one can manually specify the unit assignment for each waveform.

- [“4.5.3 Manual Clustering using the Waveform Crossing Method” on page 128](#)
 - Waveforms are manually selected as belonging to a unit or cluster by crossing a bundle of waveforms in the Waveform View with the mouse.
- [“4.5.2 Manual Clustering Using Contours” on page 122](#)
 - Waveforms are manually selected in feature space in the Clusters Views by drawing an arbitrary shape around a visible cluster.

4.5.1.1.1 Semi-Automatic Clustering Methods. With these methods, the user specifies a number of units, and “starting” cluster centers, and the algorithms then assign waveforms to the units.

- [“4.5.4 Semi-Automatic Clustering using Templates” on page 132](#)

-
- The user selects an individual waveform to serve as the template for the unit, waveforms are then assigned to clusters using a template matching algorithm.
 - [“4.5.5 Semi-Automatic Clustering using the Boxes Method” on page 135.](#)
 - The user places a pair of boxes for each unit on the Waveforms View, and any waveforms that intersect both boxes are sorted into that unit.
 - [“4.5.6 Semi-Automatic Clustering using K-Means” on page 137](#)
 - The user selects tentative cluster centers in feature space in the Clusters Views and then runs the K-Means algorithm which assigns points to clusters automatically.
 - [“4.5.7 Semi-Automatic Clustering using Standard E-M ” on page 141](#)
 - The user selects tentative cluster centers in feature space in the Clusters Views and then runs the Standard E-M algorithm which assigns points to clusters automatically.

4.5.1.2 Automatic Clustering Methods

With these methods, the number of clusters is decided algorithmically, and the waveforms are assigned to the clusters automatically.

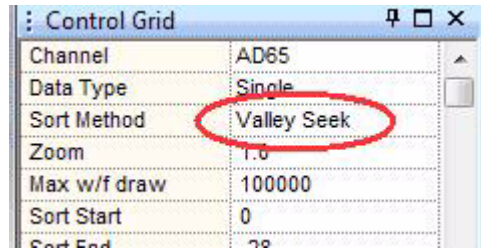
- [“4.5.8 Automatic Clustering using Valley-Seeking Method” on page 142](#)
 - The number of clusters and cluster membership is automatically determined via a valley-seeking algorithm operating on nearest-neighbor distances in feature space.
- [“4.5.9 Automatic Clustering using T-Distribution E-M Method” on page 144](#)
 - The number of clusters and cluster membership is automatically determined via an E-M algorithm based on the T-Distribution.
- [“4.5.10 Automatic Clustering Using the Scanning Methods” on page 145](#)
 - The number of clusters and cluster membership is automatically determined by optimizing a cluster quality metric during a scan over values of a sorting parameter.

For the sorting methods that work in feature space, any of the active features can be assigned to the 2 or 3 axes of feature space and used in the sorting algorithms. See [“4.4.5 Selecting Features to Use For Sorting” on page 119](#). In the examples that follow, the feature space axes are generally left to the defaults values (PC1, PC2, and PC3 if using the 3D Clusters View).

One can re-sort the waveforms after the units have been selected by using the manual or automatic clustering procedures.

4.5.1.3 Selecting the Sorting Method

This can be done either by selecting the **Sort Method** parameter in the Control Grid,



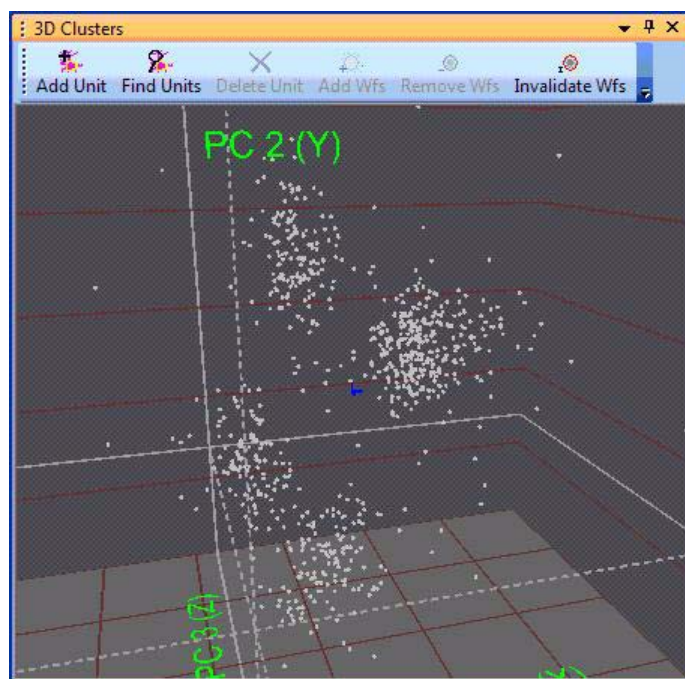
by using the **Sort | Change Sort Method** menu command to select the method, or by using the corresponding buttons on the Toolbar. In general, use only *one* of the sort methods that are described in the following sections.

4.5.2 Manual Clustering Using Contours

The Contours method of sorting is always available, no matter which Sort Method is selected in the Control Grid. (This is different from pre-3.0 versions of Offline Sorter.) Pressing the **Add Unit** button on the toolbar of the 2D or 3D Clusters Views will initiate contour sorting. Holding down the Control key while clicking and drawing in these views is also a shortcut to creating a new unit using the Contours method.

For details on how to use the Clusters Views, see [“3.7 Clusters Views” on page 43](#).

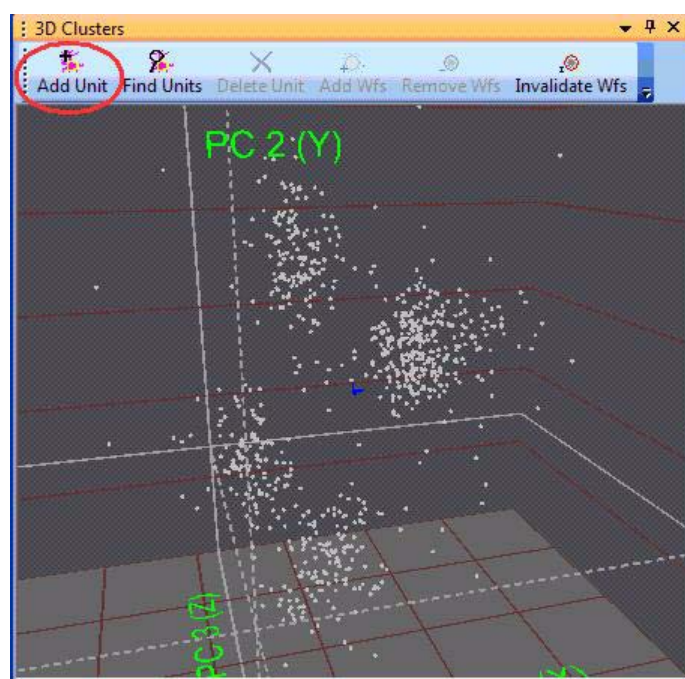
If the 3D Clusters View is being used, orient the eyepoint as described in “3.7.1.14 3D Clusters View Details” on page 57 so as to maximally separate the clusters.



To add a unit using the Contour sorting method

Note: Adding a unit is to identify a subset of waveforms that are presumably generated by the same neuron.

- 1 In the Clusters View, click **Add Unit**,




or, select the **Units | Add Unit** menu command,

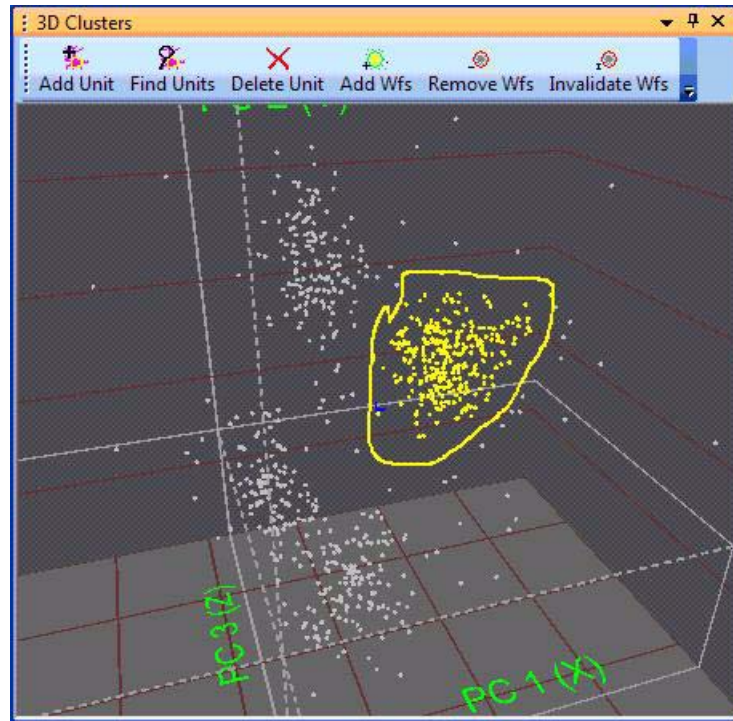
or, hold down the **ALT** key and right-click in the Clusters View and select the **Add Unit** menu command,

or, hold down the **CTRL** key.

- 2 Move the mouse pointer to the Clusters View.

The pointer changes to a “drawing hand” .

- 3 Click the left mouse button and drag a line around the cluster.



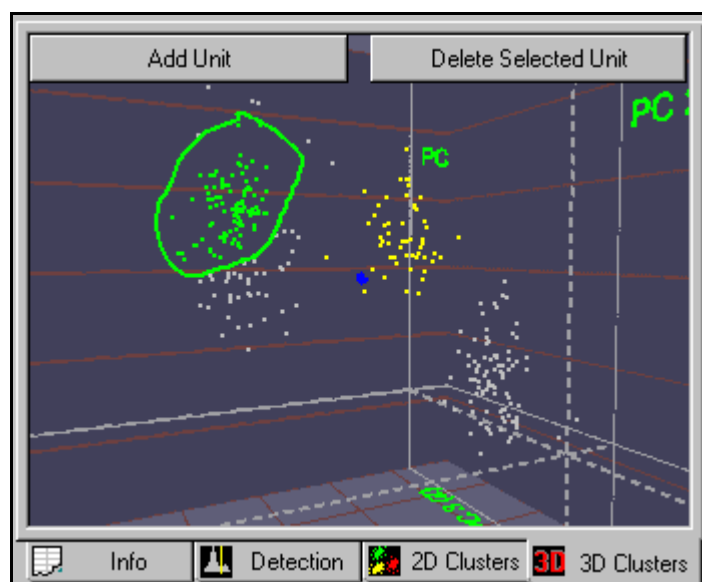
Note: In the 3D Clusters View, contours are drawn in “screen space”—the projection of the 3D feature space points onto the 2D screen. So the contour sorting operation is performed on a 2D projection of the 3D feature space. Up to 26 units can be sorted for each data channel.

4.5.2.1 Adjusting the Sorting

After any sorting (not just sorting performed using the Contours method), Offline Sorter can be used to adjust the sorting by adding or removing individual or groups of waveforms from clusters, or deleting units entirely.

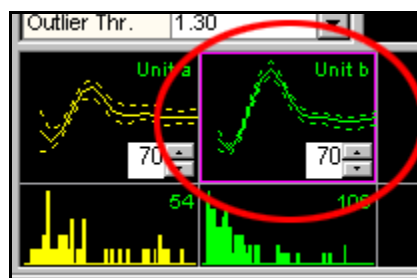
4.5.2.1.1 Adding Waveforms to Selected Units in the Clusters Views. Waveforms can be added to existing units. The following illustration shows an existing

unit, which is outlined in green. Use the procedure that follows to add additional waveforms to the unit.

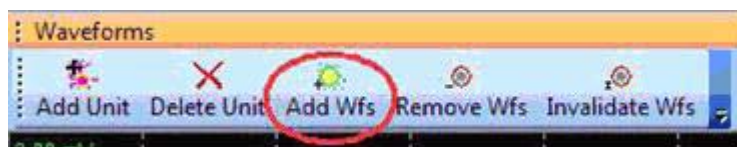


To Add Waveforms to a Unit

- 1 Select the unit by clicking it's template in the Units View.




- 2 Click **Add Wfs** above the Waveform View,

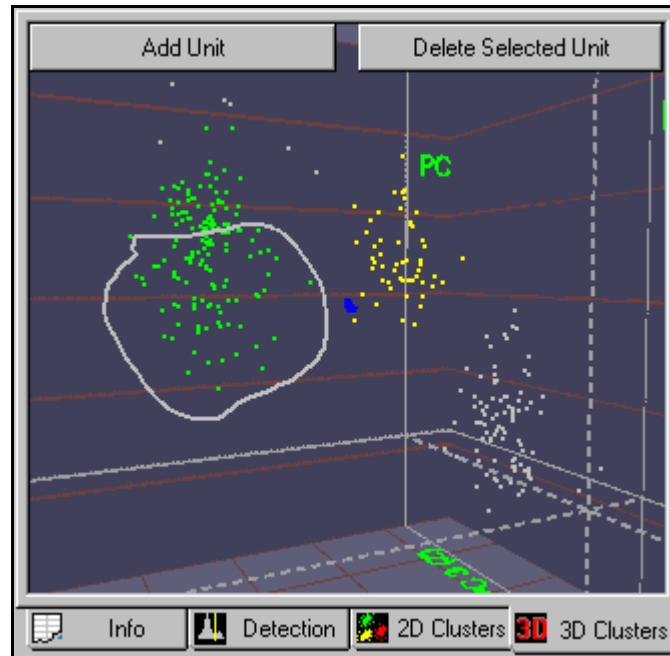


or, Select the **Units | Add Waveforms to the Selected Unit** menu command.

- 3 Move the mouse pointer to the Clusters View.

The pointer changes to a "drawing hand" .

- 4 Click the left mouse button and drag a line around the waveforms to add.
The circled waveforms are added to selected unit.

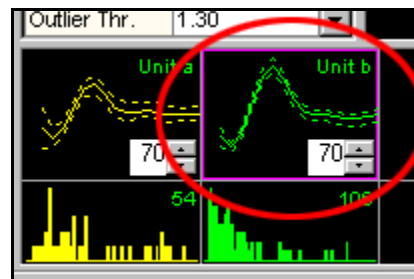


4.5.2.1.2 Removing Waveforms from Selected Units In The Clusters Views.

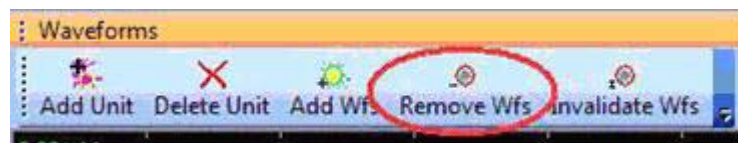
Waveforms can also be trimmed away from existing units.

To Remove Waveforms from a Unit

- 1 Select the unit by clicking it's template in the Units View.




- 2 Press the **Remove Wfs** button above the Waveform View,



or, select the **Units | Remove Waveforms from the Selected Unit** menu command.

- 3 Move the mouse pointer to the Clusters View.

The pointer changes to a “drawing hand” .

- 4 Click the left mouse button and drag a line around the waveforms to remove.

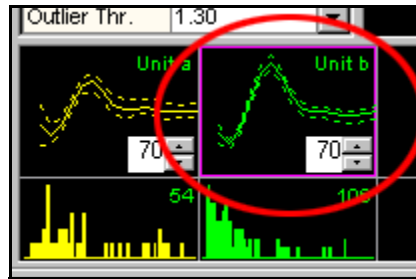
The circled waveforms are removed from the selected unit.

4.5.2.1.3 Retaining Waveforms In Selected Units In The Clusters Views.


Offline Sorter also includes a reciprocal function that retains the selected waveforms and removes all the unselected waveforms. This function is similar to previous removal function, except that all selected waveforms are retained and the rest are removed from the cluster.

To Remove All Unselected Waveforms from a Unit

- 1 Select the unit by clicking it's template in the Units View.



- 2 Select the **Units | Remove All Except Selected Waveforms from the Selected Unit** menu item, or click the corresponding toolbar button.
- 3 Move the mouse pointer to the Clusters View.

The pointer changes to a “drawing hand” .

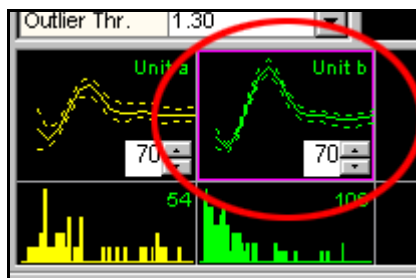
- 4 Click the left mouse button and drag a line around the waveforms to retain.

The uncircled waveforms are removed from the selected unit.

4.5.2.1.4 Deleting Units. Units can be removed entirely.

To Delete a Unit

- 1 Select the unit by clicking it's template in the Units View.



- 2 Click the **Delete Unit** button in the toolbar.



or, select the **Units | Delete Selected Unit** menu command,

or, right-click in the Clusters View and select the **Delete Selected Unit** menu command. (Use **ALT**+right-click in the 3D Clusters View).

The selected unit is deleted.

Note: Other units can shift down after the unit is deleted. For example, if Unit a and Unit b existed prior to deletion, when Unit a is deleted, Unit b becomes Unit a.

To delete all the sorted units

- Select the **Units | Delete All Units** menu command,
or, hold down the **ALT** key and right-click in the Clusters View and select the **Delete All Units** menu command.

4.5.3 Manual Clustering using the Waveform Crossing Method

The Waveform Crossing method of sorting is always available, no matter which Sort Method is selected in the Control Grid (This is different from pre-3.0 versions of Offline Sorter.). Pressing the Add Unit button on the toolbar of the Waveforms Views will initiate waveform crossing sorting. Holding down the Control key while clicking and drawing in the Waveforms View is also a shortcut to creating a new unit using the Waveform Crossing method.


To add a unit

That is, to identify a subset of waveforms that comprise a unit.

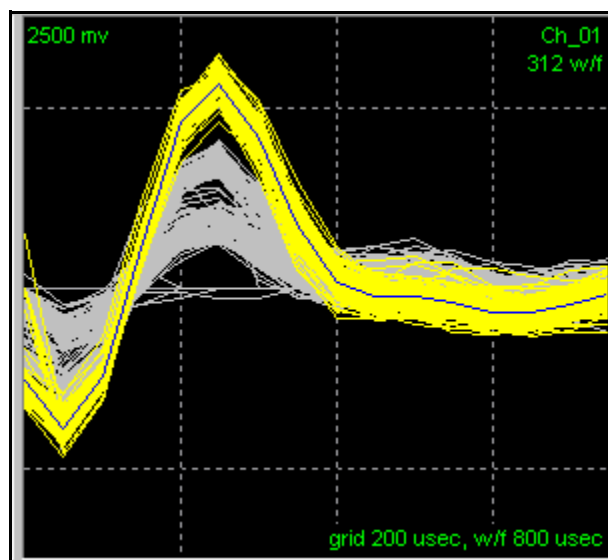
- 1 Click **Add Unit**.



Move the mouse pointer to the Waveform View.

The pointer changes to a "drawing hand" .

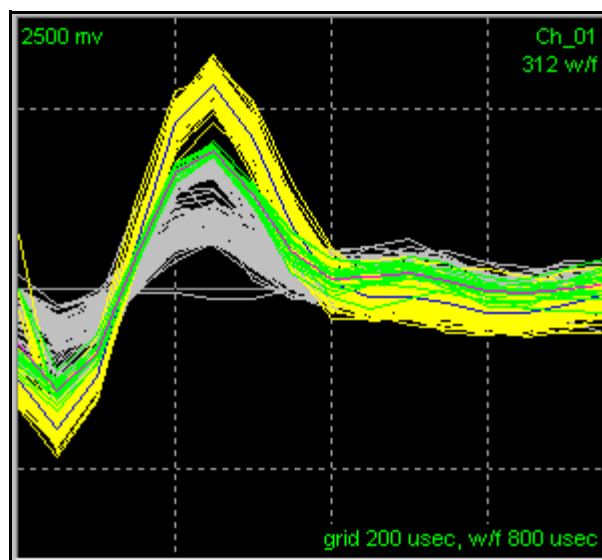
- 2 Click the left mouse button and drag a line across the bundle of waveforms.



Note: Up to 26 units can be sorted for each data channel.

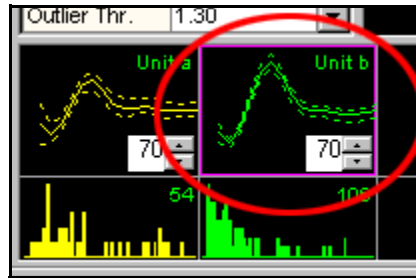
4.5.3.1 Adjusting the Sorting

4.5.3.1.1 Adding Waveforms to Selected Units in the Waveform View. Waveforms can be added to existing units by crossing waveforms in the Waveform View.



To Add Waveforms to a unit (green unit above)

- 1 Select the unit by clicking it's template in the Units View.




- 2 Click **Add WFs** above the Waveform View,



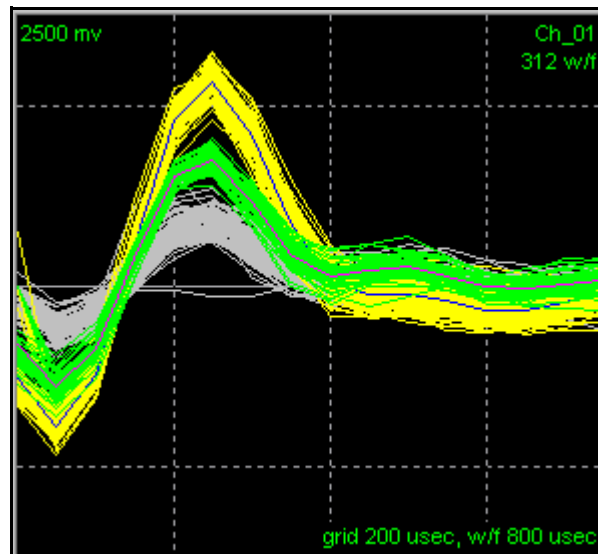
or, Select the **Units | Add Waveforms to the Selected Unit** menu command.

- 3 Move the mouse pointer into the Waveform View.

The pointer changes to a "drawing hand" .

- 4 Click the left mouse button and drag a line across the waveforms to add.

The crossed waveforms are added to selected unit.

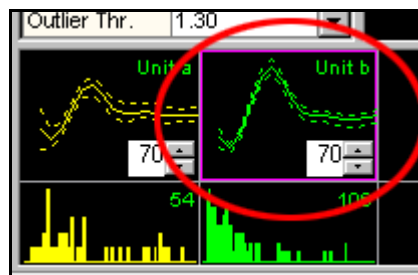


4.5.3.1.2 Removing Waveforms from Selected Units in the Waveform View.

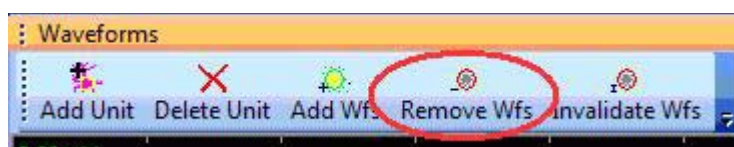
Waveforms can be trimmed away from existing units.

To Remove Waveforms from a Unit

- 1 Select the unit by clicking it's template in the Units View.




- 2 Click the **Remove Wfs** button above the Waveform View,



or, select the **Units | Remove Waveforms** from the Selected Unit menu command.

- 3 Move the mouse pointer into the Waveform View.

The pointer changes to a "drawing hand" .

- 4 Click the left mouse button and drag a line across the waveforms to remove.

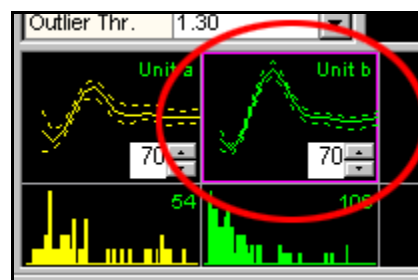
The crossed waveforms are removed from the selected unit.

For methods to display only the selected unit while removing waveforms, see ["4.6.3 Cleaning the Selection" on page 150](#).

4.5.3.1.3 Deleting Units. Use the following procedure to delete a unit.

To Delete a Unit

- 1 Select the unit by clicking it's template in the Units View.



- 2 Click the **Delete Unit** button in the toolbar,



The selected unit is deleted.

To delete all the sorted units

- Select the **Units | Delete All Units** menu command,
or, hold down the ALT key and right-click in the Clusters View and select the **Delete All Units** menu command.

4.5.4 Semi-Automatic Clustering using Templates

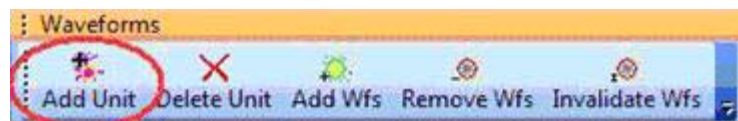
Select the templates method of sorting by selecting **Templates** in the **Sort Method** drop list in the Control Grid, or by selecting the **Sort | Change Sort Method | Use Templates From Designated Waveforms** menu item.

The Templates algorithm works in waveform space, not in feature space. The goal of the algorithm is to select an existing waveform to serve as the template waveform for a new unit. However, the Clusters Views (showing feature space) can be used to select the template waveform.

To add a unit using the Template sorting method

The goal is to select a waveform to be used as a unit template.


- 1 Press **Add Unit** button in the Clusters View,



or, select the **Units | Add Unit** menu command,

or, hold down the ALT key and right-click in the Clusters View and select the **Add Unit** menu command.

- 2 Move the mouse pointer to the Clusters View.

The pointer changes to a “drawing hand” ) and the display is in a mode that selects the waveform closest to the pointer position.

Note: For a discussion of waveform selection, see [“3.7.1 Clusters View Common Functionality” on page 45](#). The selected waveform appears in the Waveform display. If **Sel.Wave** is selected in the **Waveform View** entry in the Control Grid, it only shows the selected waveform, which can make it easier to select a template.

-
- 3 When the desired waveform is selected (shown with a box around it), click the left mouse button.

A new unit is added, and Template sorting is carried out using all of the templates.

To add a unit using the Template sorting method using the Timeline View

Alternately, the bottom Timeline View can be used to select a template waveform.

- 1 In the Timeline View, right-click and move the mouse to select the desired waveform.
- 2 Release the right mouse button
- 3 Select **Use Waveform As Template For New Unit And Sort** from the right-click (shortcut) menu.

4.5.4.1 Re-applying Template Sorting

Regardless of how clusters are created, one can perform template sorting on the clusters using the current unit templates and fit tolerances. To perform a template sort, select **Sort | Apply Template Sorting with Current Templates**, or click the **Run Template Sort** button



on the toolbar.

For a discussion of how Offline Sorter creates and maintains unit templates and fit tolerances, see [“5.12 Managing Unit Templates and Fit Tolerances” on page 213](#).

4.5.4.2 Applying Adaptive Template Sorting

Similarly, Offline Sorter can apply a variant of Template Sorting to the existing clusters where the template is modified as the template sorting proceeds through the file. With the right choice of the Adaptive Template Weight and Threshold parameters (See [“5.6.3 Adaptive Templates Algorithm” on page 198](#)), the templates can continually adjust to slow shifts in the unit waveforms (perhaps caused by a settling electrode) over the course of the experiment, thus tracking the units as they drift.

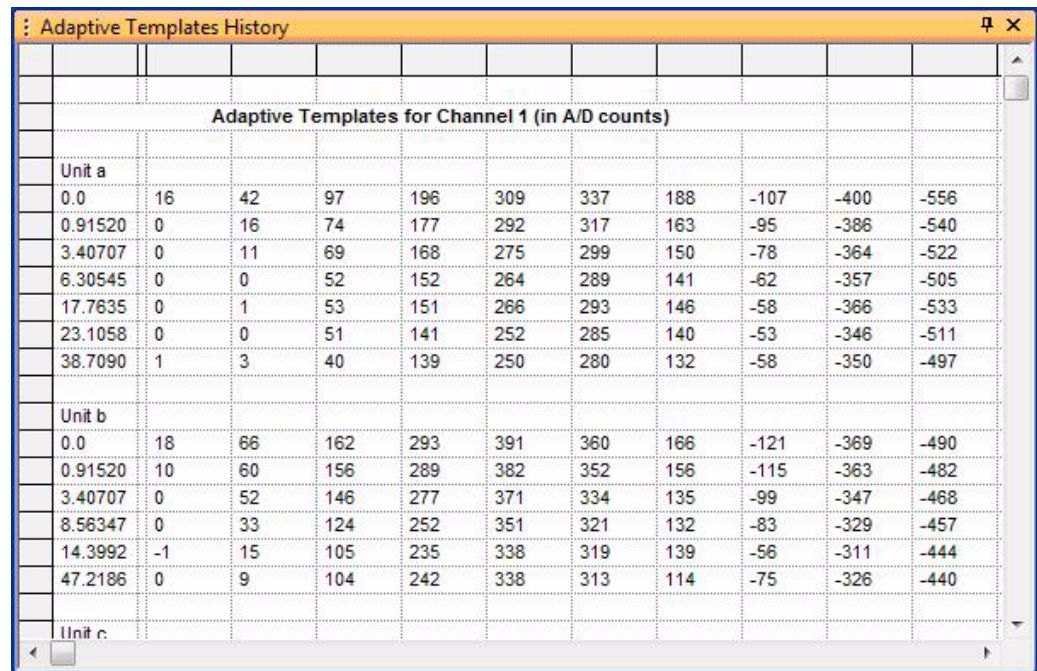
To initiate Adaptive Template Sorting, select **Sort | Apply Template Sorting with Adaptive Templates**, or click the



button on the toolbar.

After Adaptive Template Sorting has been applied to a channel, there are two views available in Offline Sorter that are designed to show how the templates for each unit evolved as a function of time.

Adaptive Templates History Info Grid View shows each template that was used during the adaptive template sort on each unit, along with the time at which each template was put into effect.

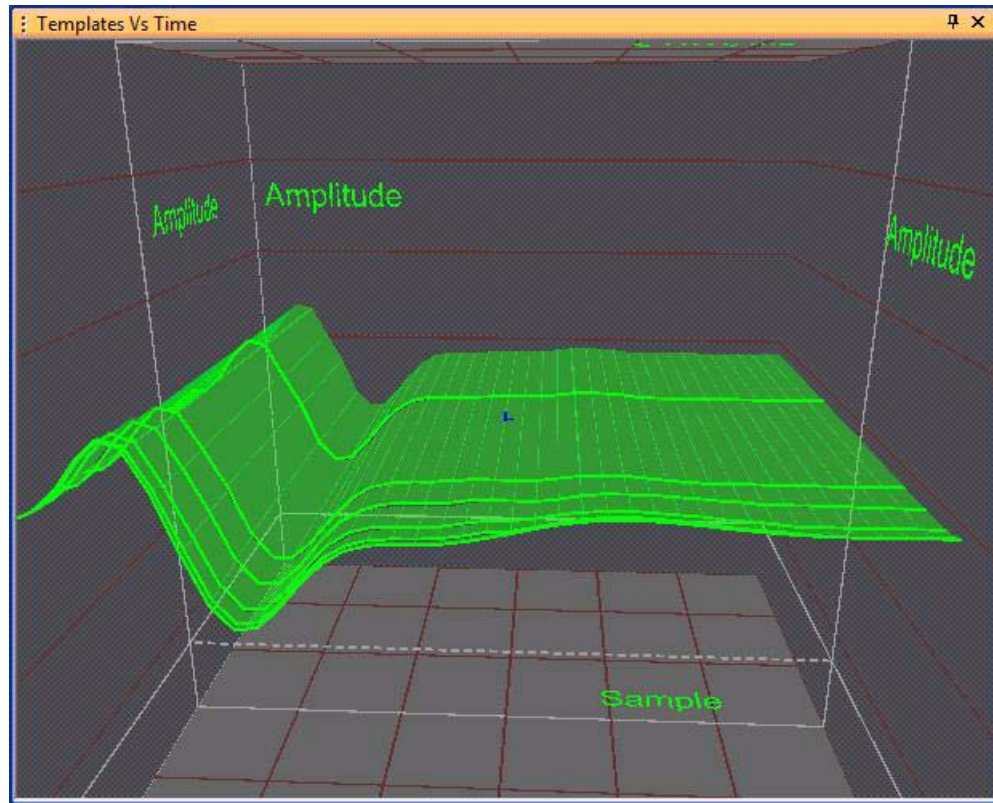


Adaptive Templates History											
Adaptive Templates for Channel 1 (in A/D counts)											
Unit a											
0.0	16	42	97	196	309	337	188	-107	-400	-556	
0.91520	0	16	74	177	292	317	163	-95	-386	-540	
3.40707	0	11	69	168	275	299	150	-78	-364	-522	
6.30545	0	0	52	152	264	289	141	-62	-357	-505	
17.7635	0	1	53	151	266	293	146	-58	-366	-533	
23.1058	0	0	51	141	252	285	140	-53	-346	-511	
38.7090	1	3	40	139	250	280	132	-58	-350	-497	
Unit b											
0.0	18	66	162	293	391	360	166	-121	-369	-490	
0.91520	10	60	156	289	382	352	156	-115	-363	-482	
3.40707	0	52	146	277	371	334	135	-99	-347	-468	
8.56347	0	33	124	252	351	321	132	-83	-329	-457	
14.3992	-1	15	105	235	338	319	139	-56	-311	-444	
47.2186	0	9	104	242	338	313	114	-75	-326	-440	
Unit c											

To display the Adaptive Templates History View, select **View | Info Grid Views | Adaptive Templates History** from the main menu.

The grid is divided into sections, one section for each unit. Within each section, each row corresponds to a new template that was put into effect during the adaptive template sorting. On each row, the first entry is the time (in seconds) at which the template was put into effect. The other N columns show each sample value for the template waveform, where N is the number of samples per waveform. By default the sample values are shown in raw a/d counts, but they can be displayed in microvolts by selecting **Show Templates in Microvolts** from the right-click context menu.

The Templates vs Time View shows a graphical depiction of the same information that is displayed in table form in the Adaptive Templates History view. In the Templates vs Time View, the template waveform amplitude is plotted versus the sample number within the waveform on the X and Y axis, but the Z axis represents time elapsed in the file; the start of the file is (initially) closest to the eye-point, and the end of the file is furthest away. Each template waveform is rendered as a solid line (as in the Waveform View) at the time when that template was put into effect, and a solid sheet is rendered with the same sample-by-sample shape as the template waveform, extending through time, until the next template was put into effect (or the file ends).



In the screenshot above, there were a total of six different templates used for this unit during the adaptive template sort of this channel. Most of the adaptive templates were put into effect near the start of the file (closer to the eyepoint), with only a single template change occurring during the second half of the file (away from the eyepoint).

The Templates Vs Time View shows the history of the templates for a single unit at a time. Selecting a different unit, either by clicking a unit in the Units View or using the **Select | Next Unit** or **Select | Previous Unit** menu items, will change the display to show the history of template waveforms for that unit.

The Templates Vs Time View behaves identically to the 3D Clusters View for manipulating the eyepoint, see [“3.7.1.14 3D Clusters View Details” on page 57](#). To show semi-transparent planes at the boundaries between Time Segments, select **Show Time Segment Boundary Planes** from the right-click menu.

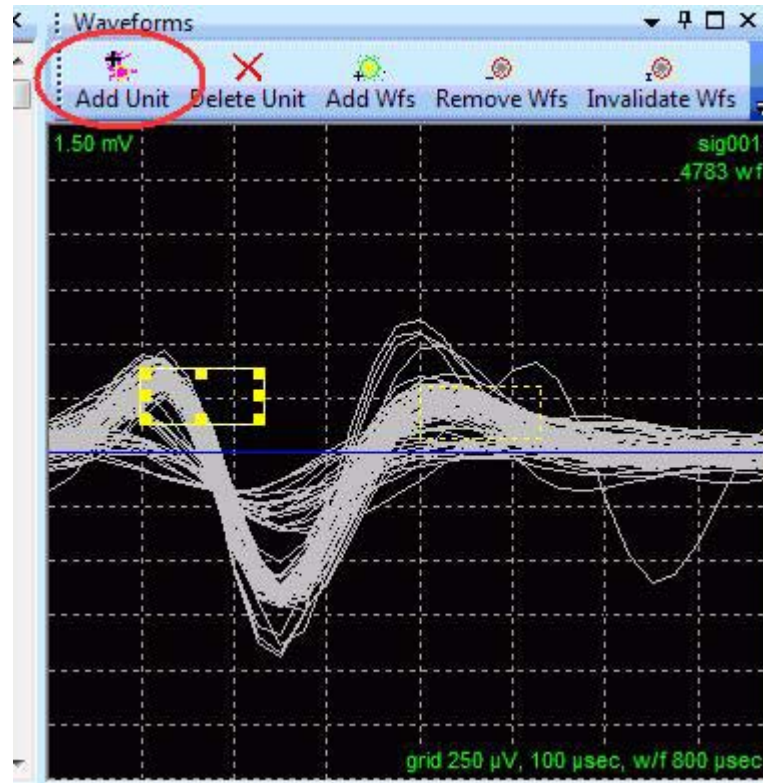
4.5.5 Semi-Automatic Clustering using the Boxes Method

Select the Boxes method of sorting by selecting **Boxes** in the **Sort Method** drop list in the Control Grid, or by selecting the **Sort | Change Sort Method | Use Boxes** menu item.

The Boxes algorithm works in waveform space, not in feature space. The goal of the algorithm is to set two boxes in time-voltage space on the Waveforms View that together define a unit.

To add a unit using the Boxes sorting method

- 1 Press the Add Unit button in the Waveforms view



or, select the **Units | Add Unit** menu command,

or, right-click in the Waveforms view and select the **Add Unit** menu command.

- 2 Move and resize the boxes so that only the desired waveforms pass through the boxes.

When the cursor is inside either box, it turns into a 4-sided arrow and dragging will move the box.

When the cursor is over one of the 8 resize handles, it turns into a 2-sided arrow. Then clicking and dragging will resize the box in the indicated direction.



- 3 When finished moving or resizing a box, the waveforms are sorted according to the new box positions.

Note: The Boxes sorting algorithm is “greedy”, in that a waveform is sorted into the first unit (starting with Unit a) for which it passes through both boxes. If a waveform passes through both boxes for several units, it is assigned the lowest-lettered unit.

4.5.6 Semi-Automatic Clustering using K-Means

Select the K-Means method of sorting by selecting **K-Means** in the **Sort Method** drop list in the Control Grid, or by selecting the **Sort | Change Sort Method | Use K-Means with Cluster Centroids** menu item.

The K-Means algorithm assigns waveforms to a pre-determined number of clusters (units) using an iterative algorithm that operates on the waveform points in feature space. For more details, see [“5.6.4 K-Means Algorithm” on page 199](#). The K-Means algorithm operates differently depending on which view it is initiated from and some options settings; see [“5.6.1 Feature Space Used for Sorting” on page 196](#).

There are two steps involved:

- 1 [“4.5.6.1 Selecting Initial Cluster Centroids” on page 138](#)
- 2 [“4.5.6.2 Running the K-Means Clustering Algorithm” on page 139](#)

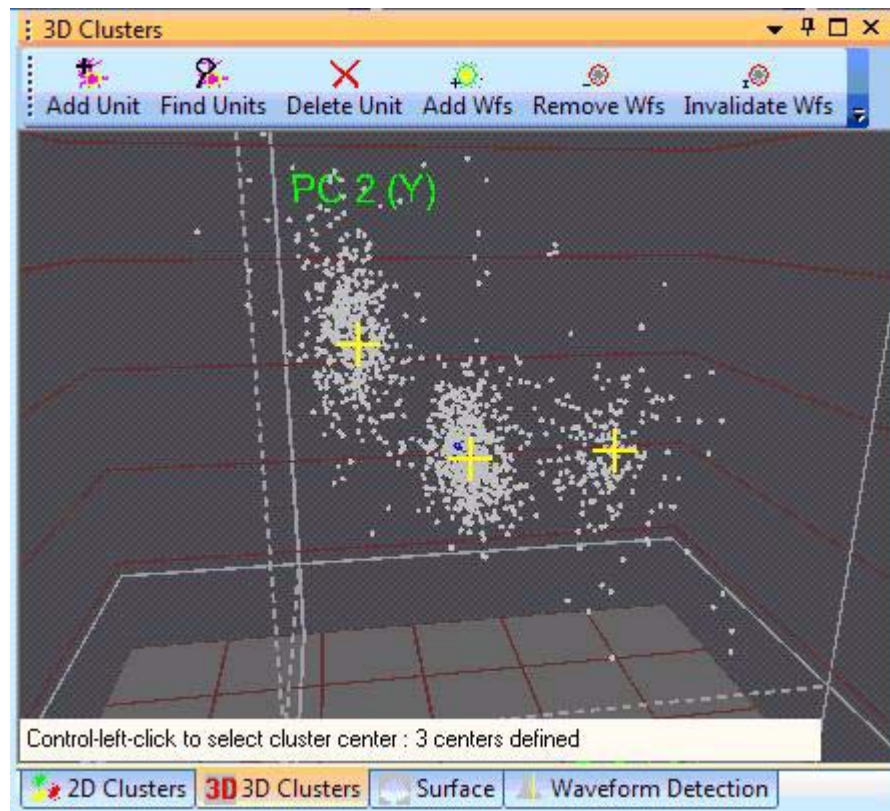
4.5.6.1 Selecting Initial Cluster Centroids

For the K-Means algorithm to work, it is necessary to specify:

- how many clusters there are
- the approximate cluster centroids

To define a cluster center

- 1 Move mouse pointer to the Clusters View.
- 2 Point to the center of the cluster.
- 3 Double-click the left mouse button, or hold down CTRL and single-click.



Note: The centroids cannot be moved or dragged. To adjust the centroids positions, remove all the centroids first and then select the new centroids.

Note: When using the 3D Clusters View, take care not to rotate the 3D viewpoint while selecting cluster centroids. Any rotation or movement of the eyepoint performs a “Clear All Centers” operation so that the cluster centers must be re-selected.

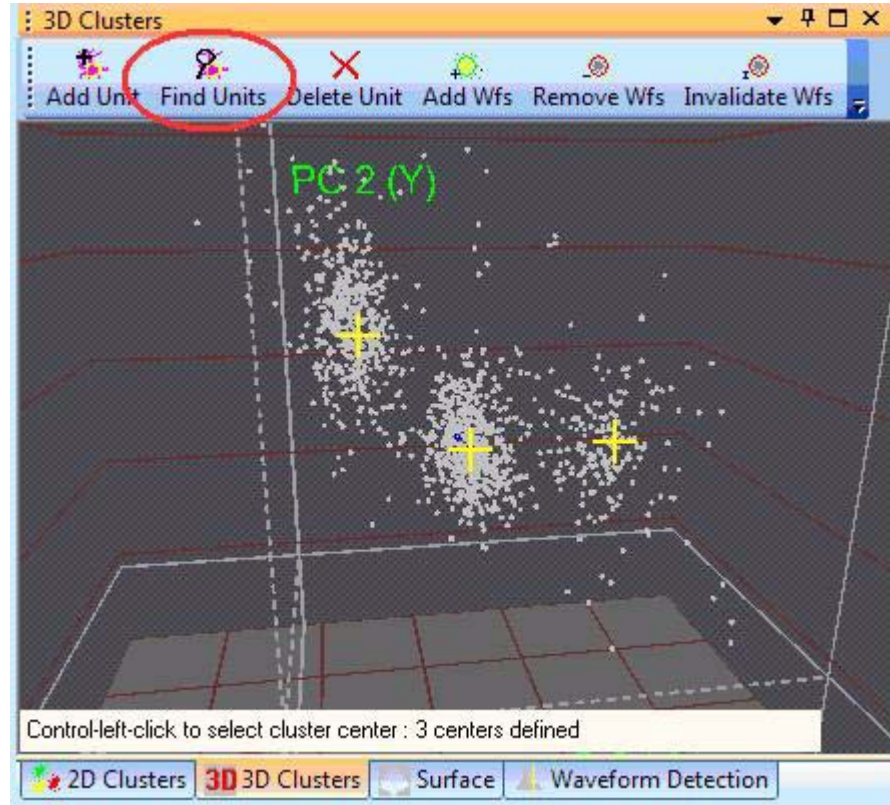
To remove the centroids, select **Clear Clusters** from the right-click menu.

4.5.6.2 Running the K-Means Clustering Algorithm

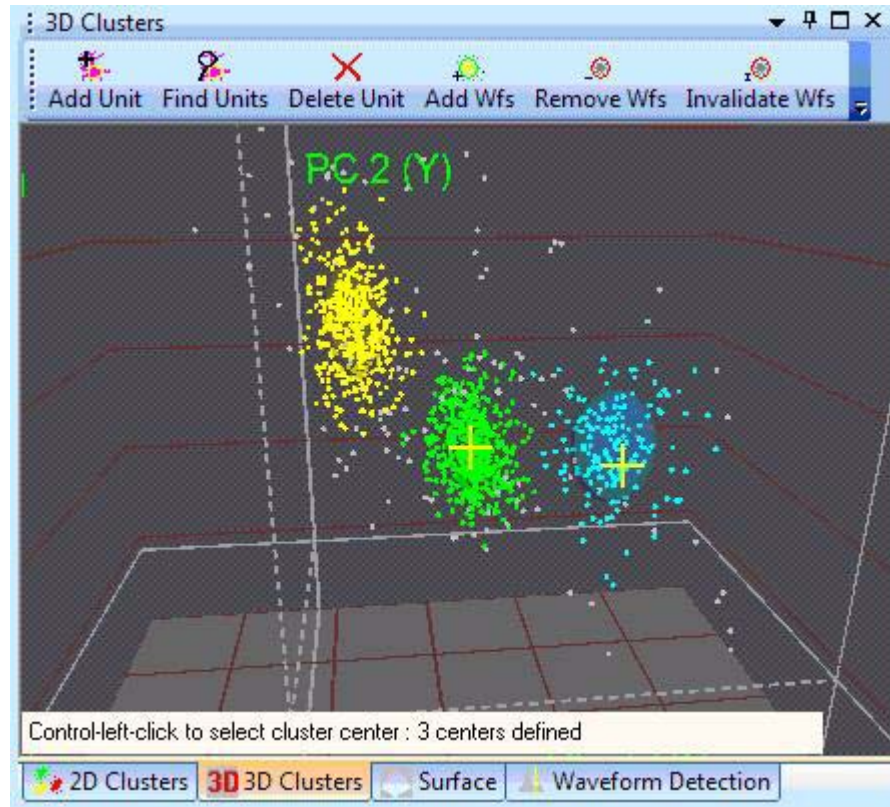
After the cluster centers have been specified, start the algorithm.

To run the K-Means clustering algorithm

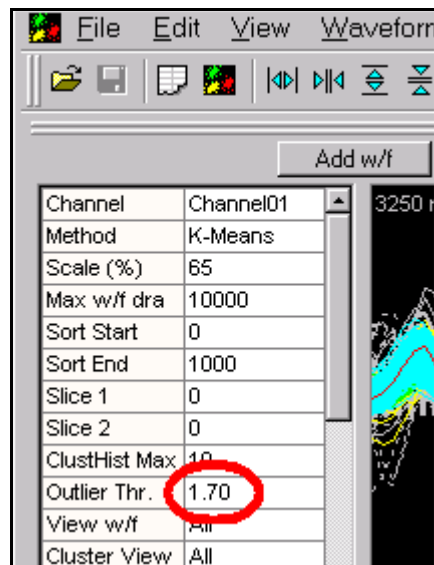
- Click **Find Units** in the toolbar, or select **Find Units** from the right-click menu.



When the algorithm completes, the points are sorted.



As part of its operation, the K-Means algorithm removes the outliers from the clusters. This is affected by the **Outlier Thr.** parameter, which can be adjusted by using the Control Grid:



To sort using the new Outlier Threshold, rerun the K-Means sorting algorithm.

One can “clean up” the clustering results after the K-Means sorting is complete by adding or removing waveforms from each unit:

- either in the Waveform View:
 - See [“4.5.3.1.1 Adding Waveforms to Selected Units in the Waveform View” on page 129](#) and
 - [“4.6.3 Cleaning the Selection” on page 150.](#)
- or in the Clusters Views:
 - See [“4.5.2.1.1 Adding Waveforms to Selected Units in the Clusters Views” on page 124](#) and
 - See [“4.5.2.1.2 Removing Waveforms from Selected Units In The Clusters Views” on page 126.](#)

4.5.7 Semi-Automatic Clustering using Standard E-M

Select the Standard E-M method of sorting by selecting **Std E-M** in the **Sort Method** drop list in the Control Grid, or by selecting the **Sort | Change Sort Method | Use Standard E-M Sorting Method** menu item.

The Standard E-M algorithm assigns waveforms to a pre-determined number of clusters (units) using an iterative algorithm operating on the waveform points in feature space. For more details, see [“5.6.5 Standard E-M Algorithm” on page 200](#). The Standard E-M algorithm operates differently depending on which view initiates it and which options are set; see [“5.6.1 Feature Space Used for Sorting” on page 196](#).

There are two steps involved:

- 1 Selecting initial cluster centers. This is done in exactly the same fashion as for the K-Means algorithm described above. See [“4.5.6.1 Selecting Initial Cluster Centroids” on page 138](#) for a step-by-step description.
- 2 [“4.5.7.1 Running the Standard E-M Clustering Algorithm” on page 141](#)

4.5.7.1 Running the Standard E-M Clustering Algorithm

After the cluster centers have been specified, start the algorithm.

To run the Standard E-M clustering algorithm

- Click **Find Units** in the toolbar, or select **Find Units** from the right-click menu.

If it is not cancelled, the algorithm runs until convergence, or until a specified maximum number of iterations has completed. This **Max Iterations** parameter is set in the **E-M Sorting** section of the **Sort** tab under the **Tools | Options** dialog box.

There is a parameter in the Control Grid labelled **Beta** that appears when the Standard E-M sorting method is selected. This parameter controls the conver-

gence properties of the algorithm; for details, see [“5.6.5 Standard E-M Algorithm” on page 200](#).

The clustering results can be “cleaned-up” after the sorting is completed by adding or removing waveforms from each unit:

- either in the Waveform View:
 - See [“4.5.3.1.1 Adding Waveforms to Selected Units in the Waveform View” on page 129](#) and
 - [“4.6.3 Cleaning the Selection” on page 150](#).
- or in the Clusters Views:
 - See [“4.5.2.1.1 Adding Waveforms to Selected Units in the Clusters Views” on page 124](#) and
 - [“4.5.2.1.2 Removing Waveforms from Selected Units In The Clusters Views” on page 126](#).

4.5.8 Automatic Clustering using Valley-Seeking Method

Select the Valley Seeking method of sorting by selecting **Valley Seek** in the **Sort Method** drop list in the Control Grid, or by selecting the **Sort | Change Sort Method | Use Valley-Seeking Sorting Method** menu item.

The Valley-Seeking algorithm uses the inter-point distances in feature space to assign the waveforms to an optimal number of clusters. For more details, see [“5.6.6 Valley Seeking Algorithm” on page 201](#). The Valley-Seeking algorithm operates differently depending on which view initiates it and which options have been set, see [“5.6.1 Feature Space Used for Sorting” on page 196](#).

Note: The Valley-Seeking algorithm scales as N^2 , where N is the number of points. So, for large number of points (more than a few thousand), the algorithm can use unreasonable amounts of memory and take a long time to complete.

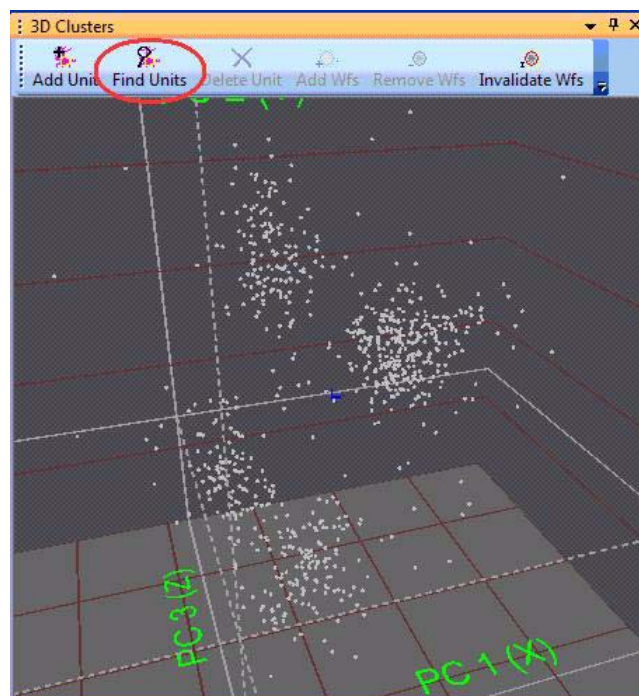
Offline Sorter can limit the number of points that are used as input to the Valley-Seeking algorithm. The **Sort** tab on the **Tools | Options** dialog box contains the **Limit Number of Waveforms used in Valley-Seeking sorting** checkbox that, when checked, limits the number of waveforms sorted to be the number in the associated edit box. Offline Sorter then uses every n th waveform in the valley seeking calculation, where n is the smallest integer such that the total number of waveforms used is less than the specified maximum. After the valley seeking algorithm sorts the waveforms, one can optionally operate on the unsorted waveforms (those that were skipped) by using the Assign Unsorted Waveforms tool. This is the same tool that is available from the **Tools | Assign Unsorted to Closest Unit** menu selection. See [“5.18 Assigning Unsorted Points to Closest Unit” on page 224](#). One can use the Assign Unsorted Waveforms tool to assign the waveforms to one of the clusters the valley seeking algorithm creates.

The outlier threshold specified in the Control Grid also affects how these skipped points are assigned to units. Finally, one can use the **Tools | Remove Outliers**

menu selection to optionally run following the sort, in order to remove outliers using the outlier threshold value. For more information, see [“4.6.3.1 Automatic Outlier Removal Tool”](#) on page 150.

To run the Valley-Seeking clustering program

- Click **Find Units** in the toolbar, or select **Find Units** from the right-click menu, or select **Sort | Sort Current Channel | Run Valley-Seeking Sorting**.



The algorithm attempts to determine the correct number of clusters and assign the waveforms to those clusters.

Note: Select **Sort | Stop Sorting** to stop the algorithm after the next iteration completes. The algorithm runs until convergence (unless it is cancelled), or until a maximum number of iterations completes.

There is a parameter in the Control Grid labelled **Parzen Mult.** that appears when selecting the Valley Seeking sorting method. This parameter controls the behavior of the algorithm; see [“5.6.6 Valley Seeking Algorithm”](#) on page 201 for more details.

One can “clean up” the automatic clustering results after sorting completes by adding or removing waveforms from each unit:

- either in the Waveform View
 - See [“4.5.3.1.1 Adding Waveforms to Selected Units in the Waveform View”](#) on page 129 and
 - [“4.6.3 Cleaning the Selection”](#) on page 150.
- or in the Clusters Views

- See [“4.5.2.1.1 Adding Waveforms to Selected Units in the Clusters Views”](#) on page 124 and
- [“4.5.2.1.2 Removing Waveforms from Selected Units In The Clusters Views”](#) on page 126.

4.5.9 Automatic Clustering using T-Distribution E-M Method

Select the T-Distribution E-M method of sorting by selecting **T-Dist E-M** in the **Sort Method** drop list in the Control Grid, or by selecting the **Sort | Change Sort Method | Use T-Distribution E-M Sorting Method** menu item.

The T-Distribution E-M algorithm is an iterative procedure that operates on the points in feature space to assign the waveforms to an optimal number of clusters. For more details, see [“5.6.7 T-Distribution E-M Algorithm”](#) on page 201. The T-Distribution E-M algorithm operates differently depending on which view initiates it and what options are set, see [“5.6.1 Feature Space Used for Sorting”](#) on page 196.

To run the T-Distribution E-M clustering algorithm

- Click **Find Units in the toolbar**, or select **Find Units** from the right-click menu, or select **Sort | Sort Current Channel | Run T-Dist E-M Sorting**.

If it is not cancelled, the algorithm run until convergence, or until a specified maximum number of iterations completes. One can set the **Max Iterations** parameter in the **E-M Sorting** section of the Sort page under the **Tools | Options** dialog box.

There is a parameter in the Control Grid labelled **D.O.F. Mult.** that appears when the T-Distribution E-M sorting method is selected. This parameter controls the convergence properties of the algorithm; for details see [“5.6.7 T-Distribution E-M Algorithm”](#) on page 201.

The first step of the T-Distribution sorting algorithm is actually to do a K-Means sort using a preset number of “seed” cluster centers. The E-M algorithm can then combine clusters together as the algorithm progresses to arrive at the optimal number of final clusters. One can set the number of seed clusters used initially in the **E-M Sorting** section of the **Sort** page under the **Tools | Options** dialog box. The T-Distribution sorting algorithm never produces more clusters than the initial number of seed clusters.

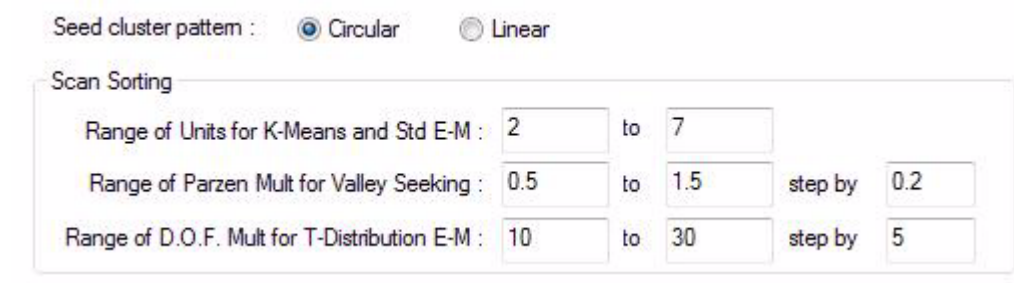
One can “clean up” the clustering results after the sorting is completed by adding or removing waveforms from each unit:

- either in the Waveform View
 - [“4.5.3.1.1 Adding Waveforms to Selected Units in the Waveform View”](#) on page 129 and
 - [“4.6.3 Cleaning the Selection”](#) on page 150.
- or in the Clusters Views

- See “4.5.2.1.1 Adding Waveforms to Selected Units in the Clusters Views” on page 124 and
- “4.5.2.1.2 Removing Waveforms from Selected Units In The Clusters Views” on page 126.

4.5.10 Automatic Clustering Using the Scanning Methods

Offline Sorter also offers a family of automatic sorting methods that are based on scanning sort parameters. At each value for the sort parameter, one of the other sorting methods is run, and the sort quality metrics are evaluated. The sorting that produced the best value of a specified sort quality metric is then chosen as the sorting for that channel. The options that control the behavior of the scanning sorting methods are set in the **Tools | Options | Sort** tab:



Seed cluster pattern : ☒ Circular ☐ Linear

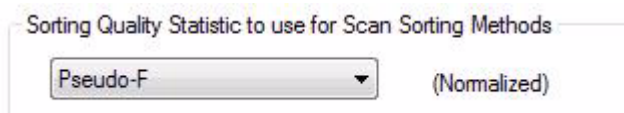
Scan Sorting

Range of Units for K-Means and Std E-M : 2 to 7

Range of Parzen Mult for Valley Seeking : 0.5 to 1.5 step by 0.2

Range of D.O.F. Mult for T-Distribution E-M : 10 to 30 step by 5

And, in the **Tools | Options | Stats** tab, the sort quality statistic that is used to determine the ‘best’ sorting within the scan can be set:



Sorting Quality Statistic to use for Scan Sorting Methods

Pseudo-F (Normalized)

There are four different scanning sorting methods that can be selected in the **Sort Method** drop list in the Control Grid, or by selecting the corresponding entry under the **Sort | Change Sort Method** menu item:

K-Means Scan - Scans over a range of the number of units, as specified in the **Tools | Options | Sort** tab. Since the K-Means method is a semi-automatic sorting method, Offline Sorter chooses the initial cluster centers using one of the two different methods. For either method, Offline Sorter calculates the ‘bounding box’ of all the waveforms in either 2D or 3D Feature Space. That is, it finds the smallest axis-aligned box that contains all of the waveform points. Then, depending on the **Seed cluster pattern** option in the **Tools | Options | Sort** tab, it determines the initial cluster centers as follows:

- **Circular**: the N-1 cluster centers are evenly spaced around the perimeter of a circle that fits into the bounding box. The final cluster center is placed at the exact center of the circle.

- **Linear:** the cluster centers are evenly spaced along a diagonal of the bounding box.

Std E-M Scan - is very similar to the K-Means scan in that it scans over the number of units, and the initial cluster centers are placed according to the **Seed cluster pattern**, but the Std E-M sorting algorithm is run at each step.

Valley Seek Scan - Scans over a range of Parzen Multipliers. The range is specified in the **Tools | Options | Sort** tab, as is the step increment value.

T-Dist E-M Scan - Scans over a range of D.O.F. Multipliers. The range is specified in the **Tools | Options | Sort** tab, as is the step increment value.

Note that the running one of these scanning sorting methods actually runs the same parameter scan that can be done using the Scan View. In fact, running one of these sorting methods will populate the Scan View and the Scan Graph View (see [“5.14 Parameter Scans” on page 217](#)) with the results of the scan.

4.6 Unit Cleaning

4.6.1 Controlling Waveforms Displayed

To inspect the sorting more closely, it is sometimes desirable to select which waveforms are displayed in the windows. There are two main ways of limiting the waveforms that display: units and time interval.

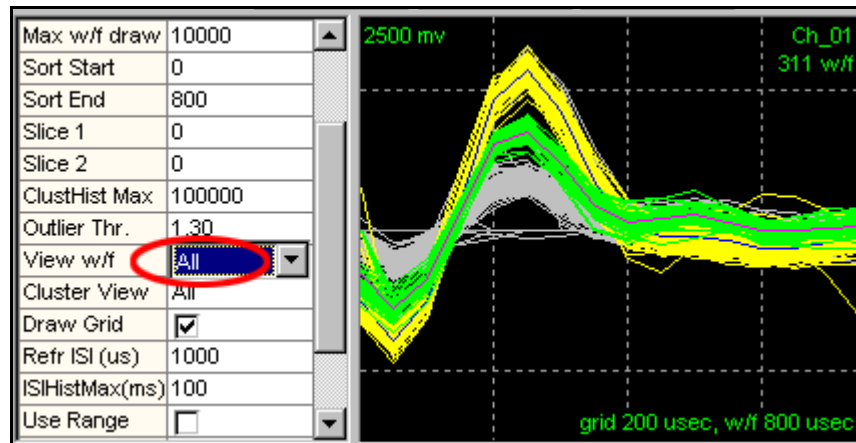
4.6.1.1 Controlling waveforms viewed by Units

Offline Sorter can display the waveforms from only selected unit(s) in the Waveform View and in the Clusters Views.

4.6.1.1.1 Controlling waveforms in the Waveform View by Units. One can change the type of waveforms viewed in the Waveform View by using the **Waveform View** parameter in the Control Grid, or by using the **Waveforms | View Only Currently Selected Unit(s)** menu entry.

To view waveforms from all units in the Waveform View

- Set the **Waveform View** parameter in the Control Grid to **All**.



or, unselect (make so that it is not checked) the **Waveforms | View Only Currently Selected Unit(s)** menu entry.

To view waveforms from specific units in the Waveform View

- 1 Set the **Waveform View** parameter in the Control Grid to **Sel.Unit(s)**, or select (make so that it is checked) the **Waveforms | Use Only Currently Selected Unit(s)** menu entry.
- 2 Select the unit by clicking on the unit's template in the Unit's Display.



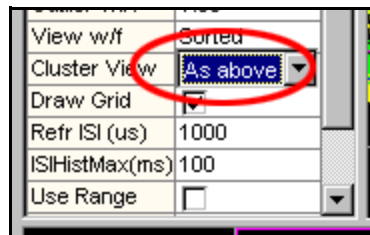
Note: To see the waveforms that are currently unsorted, select the unsorted "unit" in the display.

Note: One can select multiple units as described in “3.4 Units View” on page 29. Holding down the `CTL` key and left-clicking on a unit in the Units View selects the clicked unit, but leaves the previously-selected unit(s) in the multiply selected state (denoted by a dashed box drawn around the unit). Holding down the `SHIFT` key and left-clicking a unit selects that unit and keeps the previously selected unit(s) in the multiple-select state, and also multiple-selects all units between the previous and the clicked unit.

If **Sel. Wave** is selected from the **Waveform View** drop list, Offline Sorter displays only the waveform currently selected in the Timeline View, or the Clusters View.

If **Unsorted** is selected from the **Waveform View** drop list, Offline Sorter displays only the unsorted waveforms, regardless of what unit is selected. This selection can help to add unsorted waveforms into an existing unit; one can view only unsorted waveforms, yet still have the “target” unit selected. If this selection is chosen, use one of the following waveform selection options: **Use Only Visible Waveforms** or **Use Only Unsorted Waveforms**. For more information, see “4.6.2 Waveform Selection Options” on page 149.

4.6.1.1.2 Controlling waveforms in the Clusters Views by Units. By default, the type of waveforms shown in the Clusters Views matches those shown in the Waveform View because the **Clusters View** parameter in the **Control Grid** defaults to **As above**:



However, one can override the default by selecting **All** in the Control Grid, which forces all waveforms to be shown in the Clusters Views, regardless of what is showing in the Waveform View.

4.6.1.1.3 Controlling waveforms in the Timeline View by Units. By default, the type of waveforms shown in the Timeline View matches those shown in the Waveform View because the **Contin.View** parameter in the **Control Grid** defaults to **As above**. However, one can override the default by selecting **All** in the Control Grid, which forces all waveforms to be shown in the Clusters Views, regardless of what is showing in the Waveform View.

4.6.1.2 Controlling waveforms viewed by Time

4.6.1.2.1 Using the Timeline View. The **Control Displays** checkbox in the upper left portion of the **Timeline View** detailed on page 31, can be used to restrict the waveforms shown in the Clusters and Waveforms Views to those waveforms contained in the scroll region of the Timeline View. One can use this

feature to display “animations” of how things progressed during the course of the file—for details, see [“5.8 Playing a Movie of the data” on page 206](#).

4.6.1.2.2 By selecting a Time Range. The **Waveforms | Create Time Segments** menu entry brings up a dialog box that allows one to enter a time range that is relative to the beginning of the data file, or create any number of time segments within the file. If the **Waveforms | View Only Selected Time Segments** menu item is checked or the **Only Segment** checkbox in the Control Grid is checked, then the Clusters and Waveforms Views will show only waveforms inside the currently selected time segment.

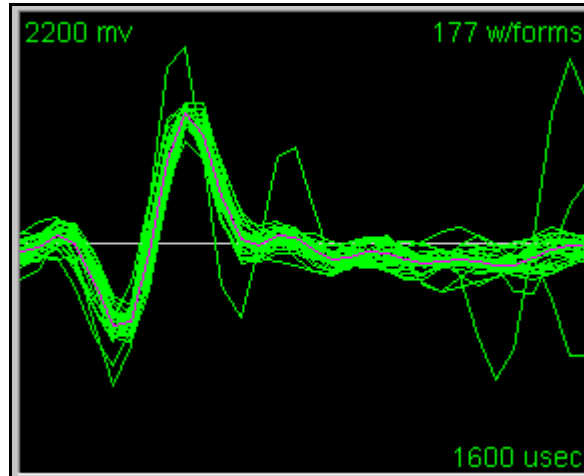
4.6.2 Waveform Selection Options

Use the **Operations Involving Waveforms** section in the **Tools | Options | General** dialog to determine which waveforms are included in waveform operations, such as sorting, selecting waveforms, adding waveforms, removing waveforms, etc. There are three options: either use all waveforms, or use only the currently visible waveforms, or use only the unsorted waveforms. If the **Use Only Currently Visible Waveforms** checkbox is checked, Offline Sorter restricts operations to affect only those waveforms that are currently visible in the Waveform View or Clusters Views (as appropriate). For methods for selecting which waveforms are made visible, see [“4.6.1 Controlling Waveforms Displayed” on page 146](#). If the **Use Only Unsorted Waveforms** checkbox is checked, Offline Sorter restricts operations to affect only those waveforms that are currently unsorted. One must use either the **Use Only Currently Visible Waveforms** option or the **Use Only Unsorted Waveforms** option; both options cannot be used together.

One can also use the **Waveforms | Use Only Currently Visible Waveforms** or **Waveforms | Use Only Unsorted Waveforms** menu options, or the corresponding toolbar buttons; they have the same effect as the checkboxes.

4.6.3 Cleaning the Selection

Offline Sorter also provides the tools to remove waveforms from a selected unit, that is, to label the waveforms as unsorted. For example, it may be desired to remove some of the outlying waveforms from this unit:



4.6.3.1 Automatic Outlier Removal Tool

The **Tools | Remove Outliers** menu selection provides a tool that automatically removes outliers from clusters, based on the **Outlier Thr.** setting in the Control Grid. This outlier removal works by considering the location of the points corresponding to the waveforms in the currently selected feature space. If the 3D Clusters View appears when the tool is selected, then 3D feature space is used, otherwise 2D feature space is used.

The outlier removal tool automatically does the following for each unit:

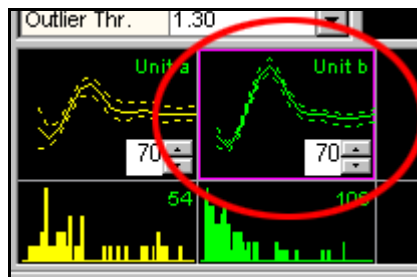
- Calculates the centroid and sigmas along the principal axis in the 2D or 3D feature space.
- For each point, calculates the Mahalanobis distance between the point and the centroid. The units of Mahalanobis distance are standard deviations.
- If this distance is greater than the **Outlier Thr.** setting, it sets the waveform point to unsorted.

4.6.3.2 Manual Waveform Removal

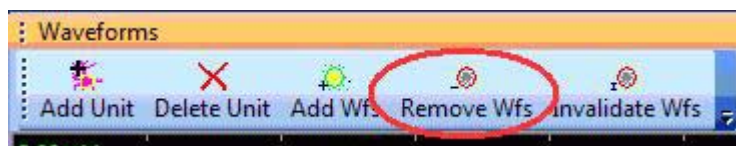
One can also manually remove any waveform (not just outliers) from a unit. This feature is easier to use if the Waveform view shows only the waveforms of a particular unit, not all the waveforms for the channel. To select which waveforms are shown in the Waveform view, use the **Waveform View** parameter in the Control Grid.

To remove some of the waveforms from a particular unit


- 1 In the Control Grid, change **Waveform View** parameter value to **Sel. Unit(s)** (Selected Units).
- 2 To select the unit, click at the unit's template display.



- 3 Click **Remove Wfs**.

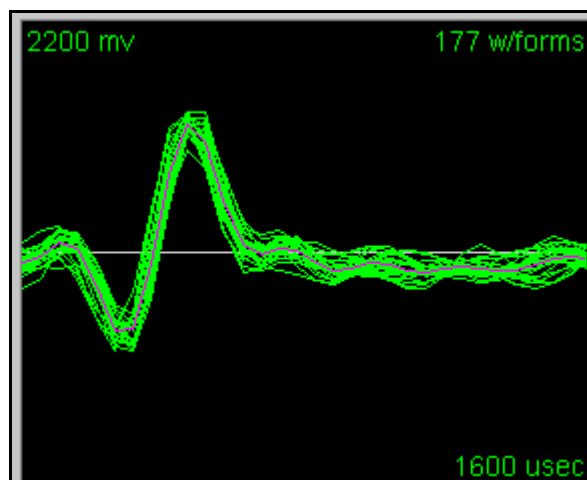


- 4 Move the mouse pointer to the Waveform view.

The pointer changes to a "drawing hand" .

- 5 Click the left mouse button and drag a straight line across the waveforms to be excluded from the unit.

After several iterations of clicking **Remove Wfs** and drawing a line, the unit can appear as follows:



Adding or Removing Waveforms using other views

Note: Waveforms can be added or removed from the selected unit by circling them in the Clusters Views:

- after pressing the **Add Wfs** or **Remove Wfs** buttons, or
- by selecting the **Units | Add Waveforms to the Selected Unit** or
- the **Units | Remove Waveforms from the Selected Unit**

- 1 After selecting one of these commands, move the pointer to the Clusters View.

Note: The pointer changes to a blue drawing hand



for adding waveforms and a red drawing hand



for removing waveforms.

- 2 Click the left mouse button and drag a line around the units to be added or removed.

Note: Waveforms can be un-sorted or added to the current view from the Timeline View. Right-click on a waveform to select it, and, from the pop-up menu, select **Unsort Waveform** or **Add Waveform to Selected Unit**.

4.6.3.3 Combining Units

Sometimes what initially appeared to be multiple units is really a single unit. The **Units | Combine Units** menu selection enables several units to be selected and combine them into a single unit. If multiple units are selected to combine in the Units View, these units also appear as selected units in the **Combine Units** dialog box.

4.6.3.4 Swapping Units

For comparing units across files, one can control which unit gets which unit label. One can use the **Units | Swap Units** menu selection to select two units to be swapped.

4.6.3.5 Invalidating Units

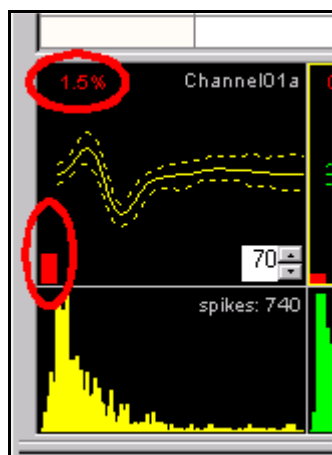
Occasionally, an entire unit's worth of waveforms can be "noise" or other undesirable artifacts. One can use the **Units | Invalidate All Waveforms in Selected Unit** or **Waveforms | Invalidate All Waveforms in Selected Unit** menu selection to invalidate all waveforms in the primary selected unit (See ["4.4.2 Invalidating Waveforms" on page 108.](#)).

4.6.4 Removing Spikes with Short Inter-spike Intervals

Most neurons have absolute refractory periods after generating an action potential. Effective isolation of a single unit within a cluster should therefore have relatively few spikes with inter-spike intervals (ISI) less than the **Refractory Interspike Interval**. One can set the value for the Refractory ISI in the Control Grid or through the **Refractory Period** tab on the **Tools | Options** dialog box.

One can use the Units View to inspect the interspike interval-related aspects of the current units. The top row of graphs of this display shows the template (average waveform) for the unit (solid line) as well as ± 3 standard deviations from the

template (dash lines). One can use the edit controls with spin buttons to adjust the tolerances for Template sorting.



The bottom row of graphs of this display shows the interspike interval histograms. The X axis of each histogram is from zero to **ISIHistMax**—a value that may also be set in the Control Grid. The total number of spikes for each unit is also shown.

The percentage of spikes with interspike intervals less than the **Refractory Interspike Interval** appears in red and a red bar indicator also appears in the upper portion of the Units View. One can change the value of **ISIHistMax** and **Refractory Interspike Interval** in the Control Grid. One can also set the Refractory Interspike Interval value by using the **Tools | Options | Refractory Period** tab.

Offline Sorter provides a means for visualizing which spikes in a cluster have inter-spike intervals less than the refractory period and for removing them.

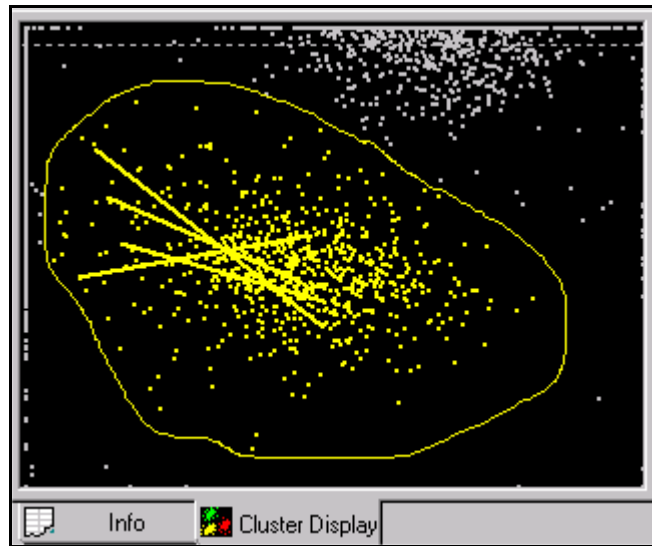
To Draw Lines in the Clusters View between spike pairs with interspike intervals less than the Refractory ISI

- 1 Select **Draw lines showing detected small interspike intervals** in the **Refractory Period** tab on the **Tools | Options** dialog box.
- 2 After zooming in to the cluster in question using the zoom

(, , , ) and move

(, , , ) control buttons on the toolbar,

the Clusters View might then look like this:



4.6.4.1 Automatic Short ISI Spike Removal

Offline Sorter provides a tool to automatically remove waveforms with ISIs below the refractory period. Selecting **Tools | Remove Short ISI Waveforms** from the main menu removes waveforms from the clusters according to the following 2D algorithm:

For each unit:

- 1 Calculate the center of gravity of points in that unit.
 - *This gives a point in 2D feature space that is near the center of the cluster.*
 - For each pair of waveforms with ISIs below the Refractory Period, calculate which member of the pair is furthest from the center of gravity point for the unit.
- 2 Remove that waveform from the unit.

Note: After this tool runs, short ISI waveform pairs can still exist if a particular waveform was within the refractory period of more than one other waveform. If needed, re-run the tool to get rid of all short ISI waveforms.

It is also possible to manually remove spikes with short ISIs from units. See [“5.10 Manual Short ISI Spike Removal” on page 209](#).

4.7 Examining the Sorting

Online Sorter offers many ways to inspect and examine the sorting done to a channel to be sure that it is of sufficient quality.

4.7.1 Waveform Inspection

After units have been sorted, individual waveforms can be compared with the template (average waveform) of each of the sorted units in the Waveform Inspection window.

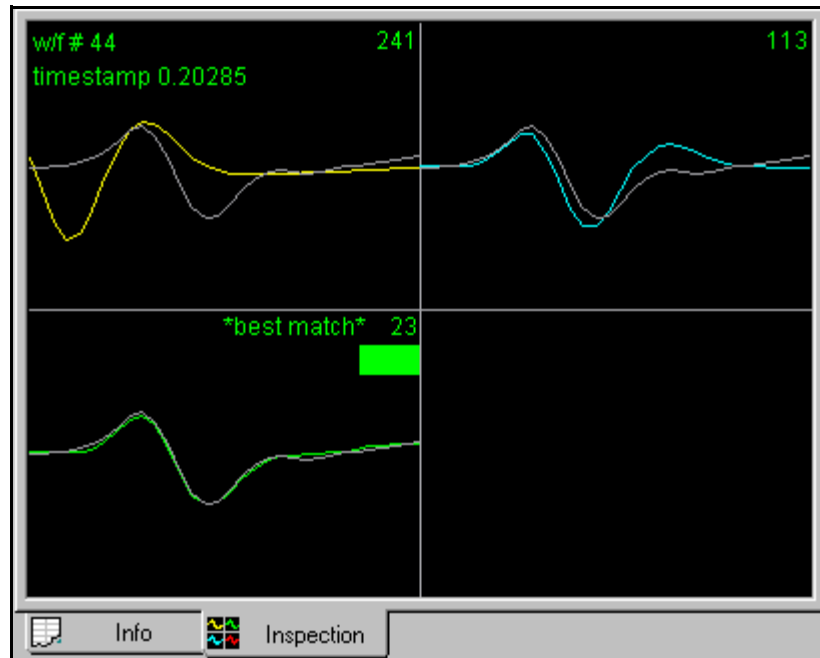
The waveform inspection process involves three steps:

- 1 [Activating the Waveform Inspection Window \(page 155\)](#)
- 2 Selecting a waveform, either by [Selecting Waveforms in the Timeline View \(page 155\)](#) or by [Selecting Waveforms in the Clusters Views \(page 156\)](#).
- 3 [Comparing the Waveform with the Templates \(page 156\)](#)

Activating the Waveform Inspection Window

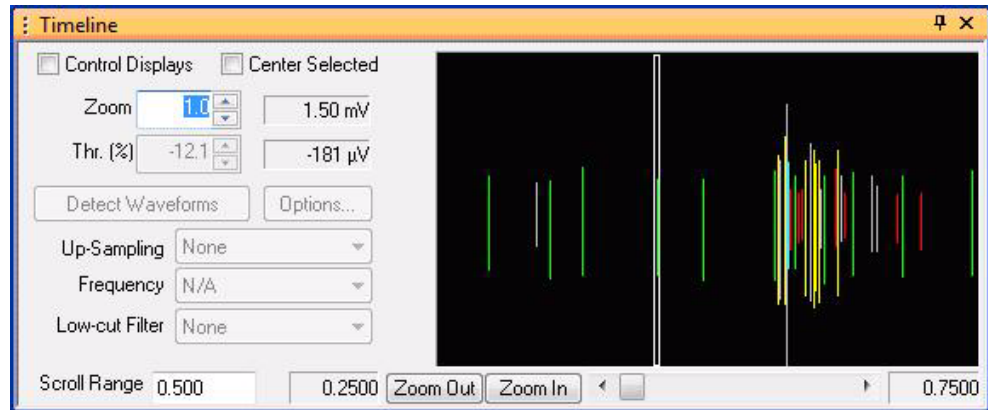
- Select the **View | Waveform Inspection Window** menu command, or,

press the  button on the toolbar.



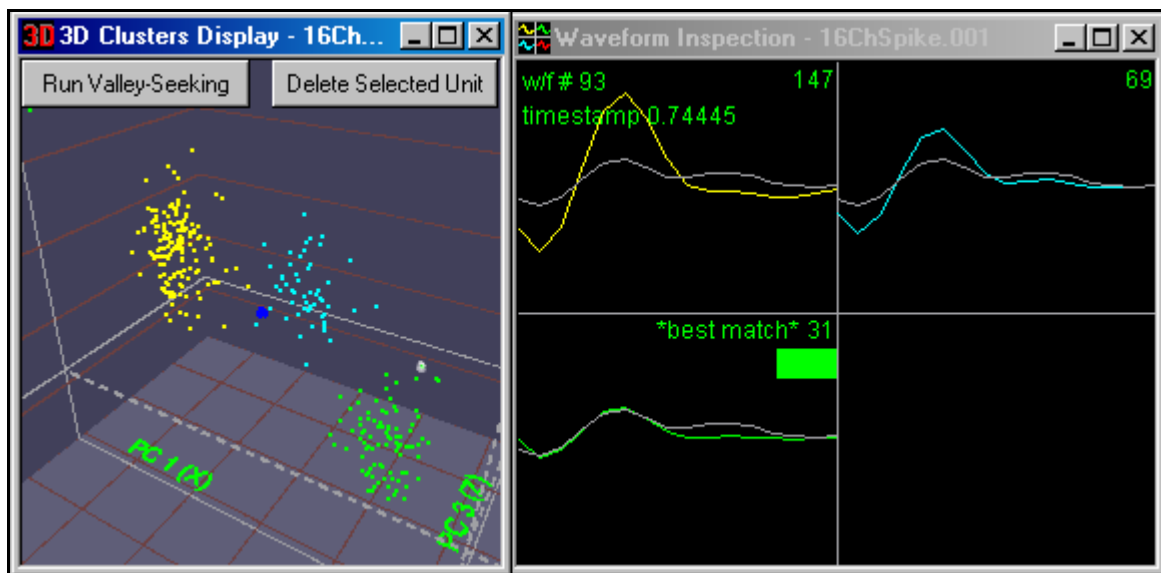
4.7.1.1 Selecting Waveforms in the Timeline View

Left-clicking in the Timeline View near a waveform will select that waveform, drawing a white rectangle around it.



The selected waveform is then viewable in the Waveform Inspection Window.

4.7.1.2 Selecting Waveforms in the Clusters Views



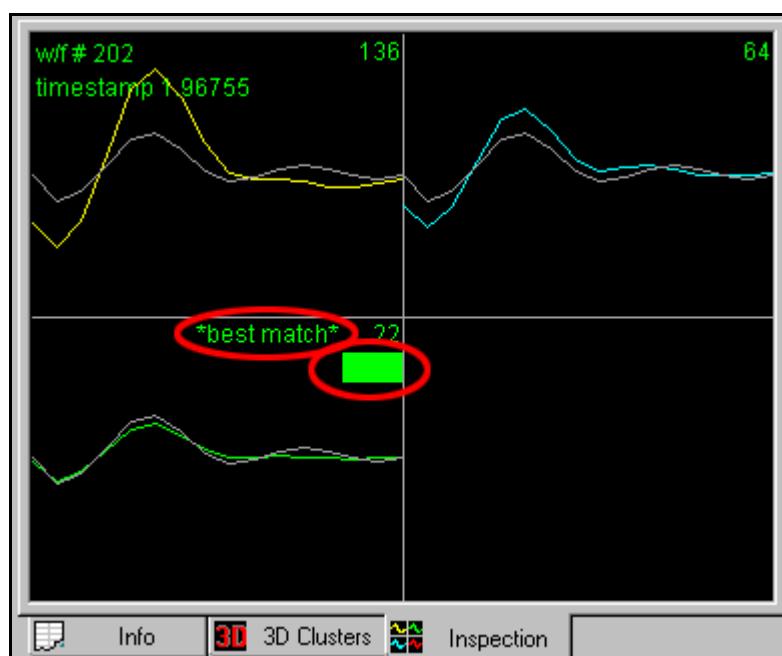
- To select a waveform, **left-click on the waveform's dot** in the Clusters View (hold down the **ALT** key before left-clicking if the 3D Clusters View is being used)

The selected waveform is then viewable in the Waveform Inspection window, as shown above.

4.7.1.3 Comparing the Waveform with the Templates

The waveform selected in the Timeline View or the Clusters Views (drawn in white in the Waveform Inspection window) is plotted against the average wave-

form (template) of each sorted unit (drawn in color) in the Waveform Inspection window:



The degree to which the selected waveform matches each of the sorted unit templates is given by the sum of squared difference between the templates and the waveform, shown in the upper right of each plot.

The Unit with the minimum sum of squared difference with the selected waveform is denoted with the symbol ***best match*** in the upper portion of the box (circled in previous illustration).

The Unit to which the waveform is assigned is illustrated by a **colored bar** (circled in previous illustration).

In the earlier example, the selected Waveform (# 202 on this channel) occurred at time $t = 1.96755$ seconds and is assigned to the green unit (Unit b).

Note: In most cases for correctly sorted units, the unit template (average waveform) that best matches the waveform (minimum sum-of-squared difference) should also be the unit to which the waveform is assigned. That is, the ***best match*** and the “colored bar” should be in the same template box.

4.7.2 Examining Sorting Statistics

There are two methods to examine sorting statistics. Each one is described in detail in this section.

4.7.2.1 Info Grid View Statistics

The **Stats** view under the **Info** tab contains more detailed statistical information about the sorting performed on a channel. The [Sorting Summary View](#) detailed

on [page 79](#) provides a convenient place to look to see a more concise summary of the most important sorting quality statistics.

Sorting Statistics for Channel 1									
Features : 2D: (PC 1, PC 2) 3D: (PC 1, PC 2, PC 3)									
Multivariate ANOVA: (Not Including Unsorted Waveforms as a Group)									
In 2D Cluster Space:		F(4,54724) =		11.67906		p =		1.75465e-009	
In 3D Cluster Space:		F(6,54722) =		8.104329		p =		8.8966e-009	
		2D	3D						
J3 :		3.84931	2.6387						
Pseudo-F :		189415	129844						
Davies-Bouldin :		0.306595	0.372886						
Dunn :		1.73042	1.38495						
Per-Unit Statistics									
	Counts	ISI < 100	Center (2D)	Sigmas (2D)	Center (3D)	Sigmas (3D)			
Unit a:	7366	0.3530	-568.5524, 554.481	324.8189, 226.2428	-568.5524, 554.4814, -218.42	345.5869, 226.2481, 163.0215			
Unit b:	37570	0.1544	-262.5755, -1160.0	280.8709, 218.1188	-262.5755, -1160.0490, -510.2	292.0075, 239.2599, 193.5820			
Unit c:	53482	0.2412	870.6649, -892.622	324.7222, 202.5226	870.6649, -892.6222, -501.00	345.0548, 271.4262, 179.9306			
Normality Statistics									
	Counts	Skew(2D)	Kurtosis(2D)	Skew(3D)	Kurtosis(3D)				
All Valid WFs:	98418	-1.1101, 17.4594	-1.1775, 4.4132	-1.1101, 17.4594, -0.1175	-1.1775, 4.4132, 0.1527				
Unsorted WFs:	0	0.0000, 0.0000	0.0000, 0.0000	0.0000, 0.0000, 0.0000	0.0000, 0.0000, 0.0000				
Unit a:	7366	-0.3921, -0.2196	1.6444, 0.0334	-0.3921, -0.2196, -0.3578	1.6444, 0.0334, 0.0735				
Unit b:	37570	-0.3803, 0.4460	0.0153, 1.3004	-0.3803, 0.4460, 0.4680	0.0153, 1.3004, 0.2264				
Unit c:	53482	-0.4105, -0.2450	-0.0598, 0.2803	-0.4105, -0.2450, 0.3551	-0.0598, 0.2803, 0.2417				
Pairwise Sort Quality									

4.7.2.1.1 Top Half of the Window. In the top half of the window, the **Features** line shows which features were used for each dimension of the 2D and 3D feature spaces. All sorting statistics are dependent on the feature space.

The **Multivariate ANOVA** section shows the p-value and the F Statistics (with the Hypothesis and Error degrees of freedom in parenthesis) from a 2D and 3D Multivariate Analysis of Variance (ANOVA) on the sorted units in the currently displayed in 2D and 3D feature space:

- **In 2D Cluster Space:** (first row) Parameters from Multivariate ANOVA test on clusters in 2D feature space
- **In 3D Cluster Space:** (second row) Parameters from Multivariate ANOVA test on clusters in 3D feature space

One can display the p-value, along with all the other sorting quality statistics, in the Clusters Views by selecting **Show Statistics** in the right-click menus in the Clusters Views.

4.7.2.1.2 Bottom Half of the Window. The bottom half of the window, lists the values of the [Sorting Quality Statistics](#), which are detailed on [page 203](#), for both the 2D and 3D feature spaces.

4.7.2.1.3 Per-Unit Statistics Section. The next section is a table that present the means and standard deviations of the multivariate normal distributions for the waveforms of each unit, for both the 2D and 3D feature spaces. The percentage of waveforms in each unit that do not meet the minimum ISI (interspike interval) period is also displayed.

4.7.2.1.4 Normality Statistics Section. The next section is a table that calculates the Skew and Kurtosis characteristics of the multivariate normal distribution for groups of waveforms (all valid waveforms, the unsorted waveforms, and the waveforms for each Unit), for both the 2D and 3D feature spaces. Large values of Skew and/or Kurtosis indicate that the data is not well-fit by a multivariate normal distribution. Some analyses have underlying assumptions of normally-distributed data (e.g. the Multivariate ANOVA analysis).

4.7.2.1.5 Pairwise Sort Quality Section. This section contains a number of tables, for the p-values and each of the [Sorting Quality Statistics](#), which are detailed on [page 203](#), for both 2D and 3D feature spaces. Each table has the units listed across both the rows and the columns; the value at the intersection of the i th row and the j th column represents the pairwise statistic calculated for Unit i and Unit j . The value is calculated as if only those two units existed in the channel, and only the waveforms assigned to those units existed. All other waveforms (in other units, or unsorted) are ignored.

Note: In each table, the “matrix” formed by the values is symmetric ($a(i, j) = a(j, i)$), and the diagonals (that correspond to the pairwise statistics calculated between some unit and itself) are meaningless and are set to 0.

The values in the tables in some sense indicate how well-sorted each pair of units is. A poor sort quality value between two units can indicate that the two units need to be combined into a single unit. Take care with the interpretation of these pairwise results, as they are a post-hoc test, and the probability is high that somewhere in the tables there are values that indicate exceptionally well-sorted units.

The calculation of these values can take a noticeable amount of time to complete. Because of this, the calculation is optional, and it is disabled by default. To enable it, check the **Calculate unit pairwise statistics after each change** checkbox in the **Tools | Options | Stats** dialog box.

4.7.2.2 Statistics Feedback While Sorting

On the 2D and 3D Clusters Views, there are mechanisms that can be used to get immediate feedback on the sorting quality while performing sorting. It is sometimes useful to know immediately if an action (e.g. removing outliers from a cluster) helped or hurt the sorting quality. If the action hurt the sort quality, it can immediately be undone by using the **Edit | Undo** menu selection.

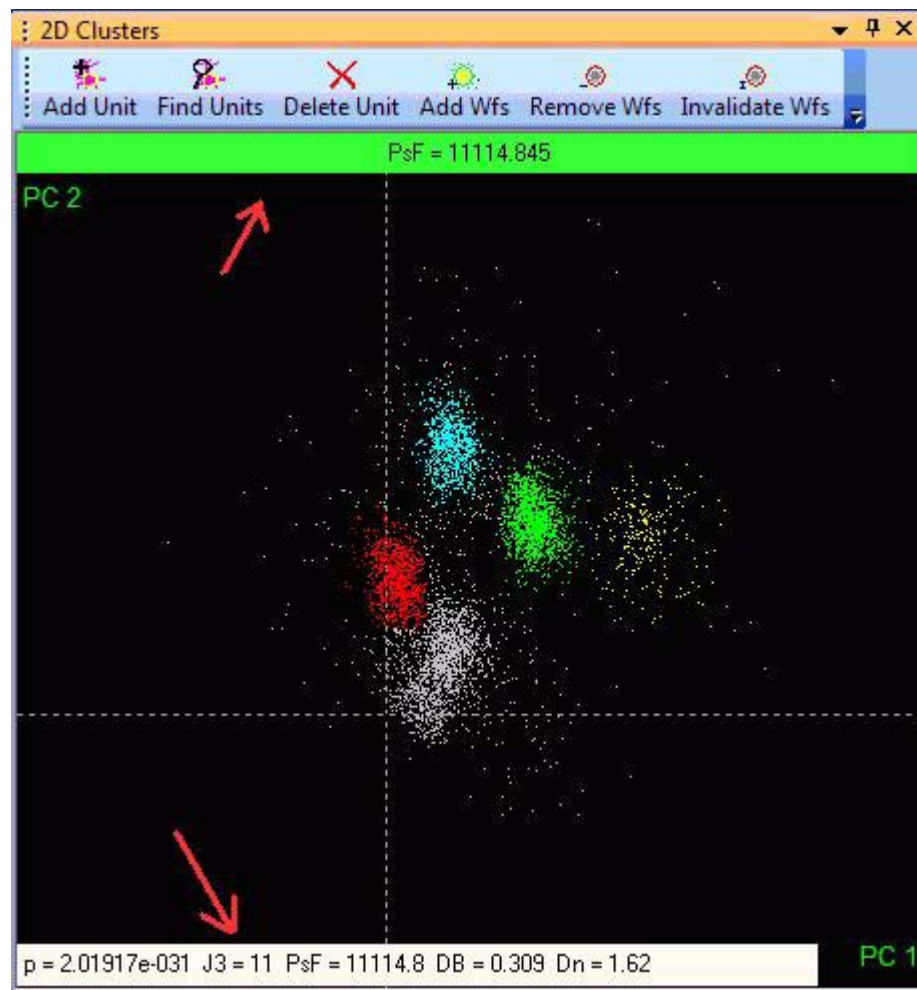
There are two similar ways to display the sort quality statistics on the Clusters Views. The descriptions follow.

One can display the absolute values of all [Sorting Quality Statistics](#), which are detailed on [page 203](#), in a white bar along the bottom of the view by selecting

Show Statistics from the right-click menu. The abbreviations appear in this line are:

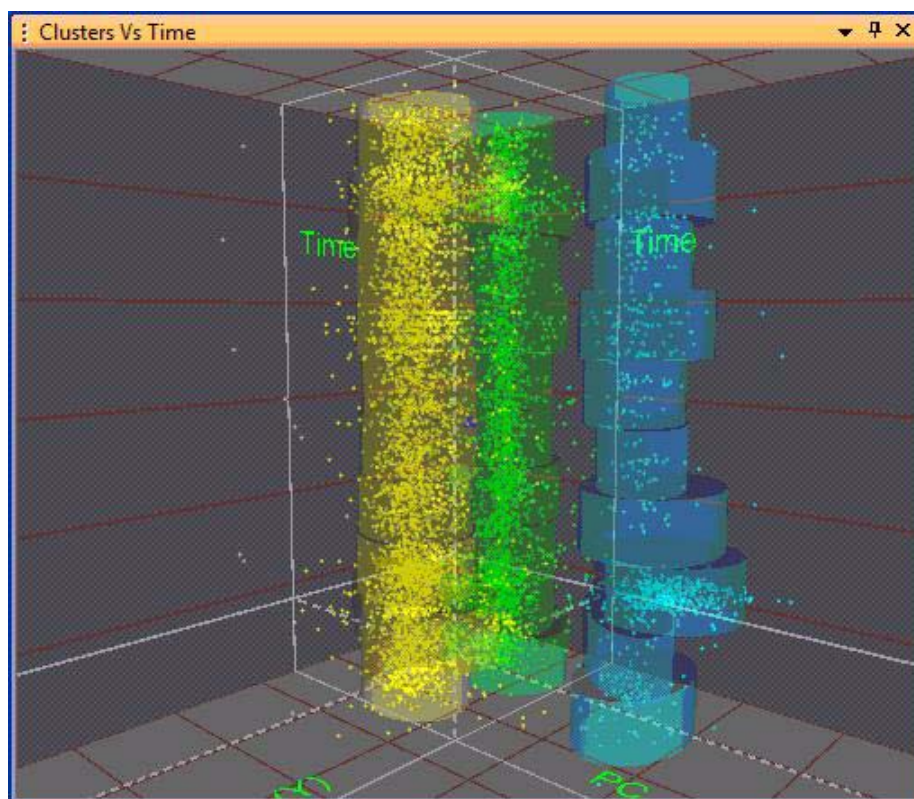
- p: the p-value from the multivariate ANOVA
- J3: the J3 statistic
- PsF: the Pseudo-F statistic
- DB: the Davies-Bouldin statistic
- Dn: the Dunn statistic

There are several available Sorting Quality Statistics that can be chosen for display at the top of the view in a green or red bar by selecting **Show Cluster Quality** from the right-click menu. One can choose the Sorting Quality Statistics to display in the top bar from the **Sorting Quality Statistic to use on the Clusters View Displays** droplist on the **Tools | Options | Stats** tab. The value of that statistic appears, along with the percentage change from its previous value that the last sorting-related action caused. If the action made the clustering better according to the statistic, the bar is green; if the sorting quality decreased, the bar is red.



4.7.3 Examining the Sorting vs Time

It is advisable to examine the time structure of the data using the Clusters Vs Time View, see [“3.9 Clusters Vs Time View” on page 63](#). This can reveal situations such as the waveforms occurring in bursts, units appearing or disappearing, etc.



4.7.4 Saving and Recalling Sortings

When examining the sorting on a channel, it is sometimes useful to play ‘what if’ games to experiment with sorting the channel using different sorting parameters or sorting methods. To facilitate this, Offline Sorter has features that can save the current sorting (i.e., the current unit assignment of every waveform) of a channel to a file that can be later recalled. By default, these files have a .SORT file extension. This allows a ‘bookmark’ to be set on what may be an acceptable sorting so that it may be recalled if none of the ‘what if’ scenarios improves the situation.

When saving or recalling a sorting, two options are available:

- One can save to or recall from an arbitrary file, where a user-supplied file name is specified, or
- One can save/recall from one of two pre-defined ‘slots’.

When saving to or restoring from a pre-defined slot, Offline Sorter actually generates a temporary filename and saves to that filename. But using a pre-defined

slot allows a single toolbar button to be pressed to save or recall a sorting, which is more convenient than selecting a file with the file selection dialog.

To save the sorting to an arbitrary file, select **Sort | Save Sorting to .SORT File | Save to File** and select the file using the standard file selection dialog. To restore a sorting from a previously-saved file, select **Sort | Restore Sorting from .SORT File | Restore from File**.

To save the sorting to a pre-defined slot, select **Sort | Save Sorting to .SORT File | Save Sorting 1 (or 2)**, or press one of the toolbar buttons



To recall the sorting that was previously saved to a pre-defined slot, select **Sort | Restore Sorting from .SORT File | Restore Sorting 1 (or 2)**, or press one of the toolbar buttons



Note: A .SORT file is valid only for the current channel, and only as long as the number of waveforms in the channel does not change. That is, changing the channel or re-extracting waveforms with a different threshold (thus resulting in a different number of spikes) will invalidate any previously-saved .SORT files.

4.8 Saving the Sorting Results

4.8.1 Saving Timestamps and Waveforms

If **Offline Sorter** is used to resort spikes from a file with previously sorted waveform/spike data (e.g. a Plexon PLX data file, Datawave, Cybernetics NEV, or CED Spike-2), the resorted waveforms and timestamps can be saved into another file of the same format. All records in the original file are preserved in the output file, except that the unit assignments for spike records are modified to reflect the sorting that has been performed in Offline Sorter.

To save the waveforms and re-sorted timestamps

- Select **File | Save As** menu command.

Note: One cannot overwrite the original file with this operation.

4.8.1.1 Overwrite/Backup Control on File-Saves

When files are saved using the **File | Save** menu option or the toolbar **Save** button, Offline Sorter provides control over saving files in terms of whether the data files are overwritten or backups are kept. One can control this behavior using the **File-Save Behavior** drop-down list in the **General** tab of the **Tools | Options** dialog box.

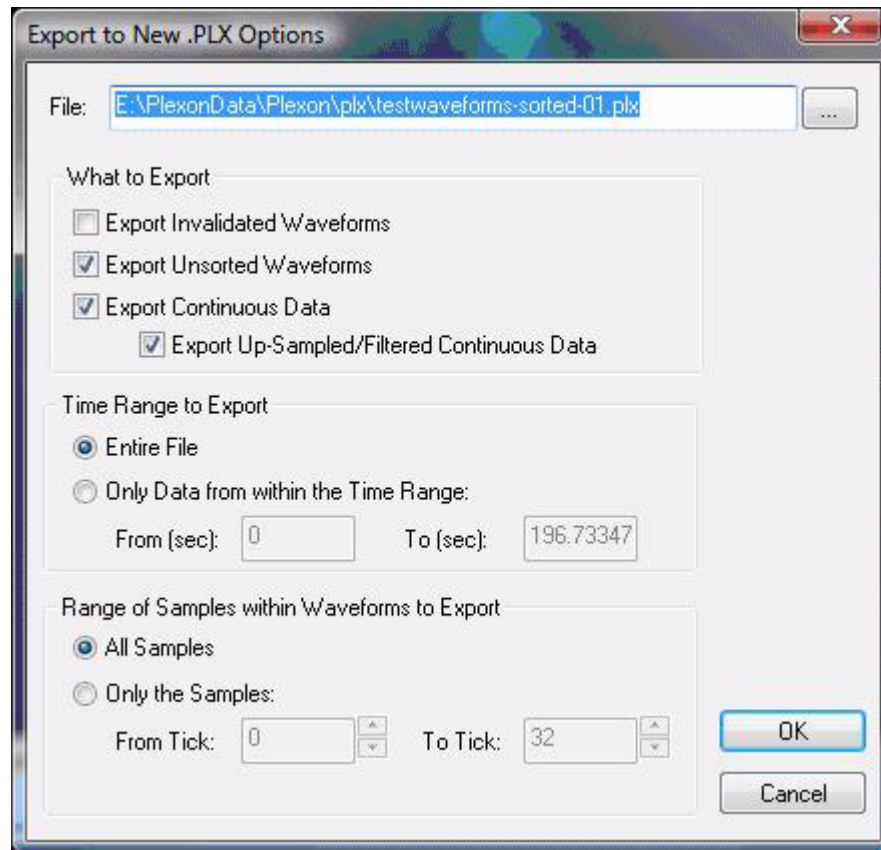
The options available are:

- **Preserve Original, prompt for new filename (defaults to filename-0n)** – This is the behavior available in older versions of Offline Sorter. The original data file is never altered, but Offline Sorter prompts for a new file name to write the sorting results into. During the prompt, a default file name appears that is constructed by appending numbers to the original filename until an unused filename has been made. The latest sorting results are in the filename-0n file with the largest n .
- **Overwrite Original, backup to filename-0n before each save** – With each save, Offline Sorter always overwrites the original file with the latest sorting results, but it saves the previous version of the file to a backup file. Offline Sorter constructs the backup file name by appending numbers to the original file name.
- **Overwrite Original, backup to filename-0n on first save only** – This item is the same as above, except that Offline Sorter makes the backup only on the first save. That is, the first time that a file is saved, Offline Sorter saves a backup of the original to a backup file whose filename it constructs by appending numbers to the original file name. Offline Sorter overwrites the original file. Subsequent saves overwrite the original file, but they do not save backups. With this option, the filename-0n backup file contains the data originally read into Offline Sorter, no matter how many times the file is saved.
- **Overwrite Original, backup to filename-0n and update each save** – On each save, Offline Sorter overwrites the original file and the backup file. The backup file contents are always “one save behind” the original file.
- **Overwrite Original, do not backup** – This item just overwrites the original file on each save and doesn't make backups. Plexon does not recommend this option unless the data files are backed up elsewhere.

4.8.1.2 Exporting to a New PLX File

Offline Sorter also supports a more flexible means to save timestamps and waveforms into a PLX file. No matter how the waveforms are read into Offline Sorter (from any supported file format, or from a DDT file after waveform extraction

has been performed), the waveforms can be saved into a PLX file. Select the **File | Export to New .PLX** menu command to bring up the following dialog box:



- **File** – Select the name of the output file. Click the **Browse** button to bring up a file browser.
- **Export Invalidated Waveforms** – If this item is selected, Offline Sorter still writes waveforms that have been invalidated to the output PLX file. If unselected (default), Offline Sorter does not write the invalidated waveforms to the file.
- **Export Unsorted Waveforms** – If this item is selected (the default), Offline Sorter writes out the waveforms that are not sorted. If unselected, Offline Sorter writes out only sorted waveforms.
- **Export Continuous Data** – Select this item to store continuous data channels in the .plx file. If this item is not selected, continuous channels are not stored in the .plx file, even if there are continuous channels in the current data.
- **Export Up-Sampled/Filtered Continuous Data** – Select this item to store the up-sampled (if relevant) and low-cut-filtered (if relevant) version of the continuous data. If this item is not selected, the 'raw' unprocessed continuous data is stored in the .plx file.
- **Time Range to Export** – If 'Entire File' is unselected (the default), Offline Sorter writes all waveforms out regardless of their timestamps. If 'Only Data

from within the Time Range’ is selected, Offline Sorter does not write waveforms with timestamps outside the specified range to the output file.

- **Range of Samples within Waveforms to Export** - The item enables shortened waveforms to be stored in the output file. For example, if the sort range is reduced, only the relevant portion of each waveform can be saved. If **‘All Samples’** (the default) is selected, the entire waveform is saved. If **‘Only the Samples’** is selected, only the samples in the specified range are written out for each waveform.

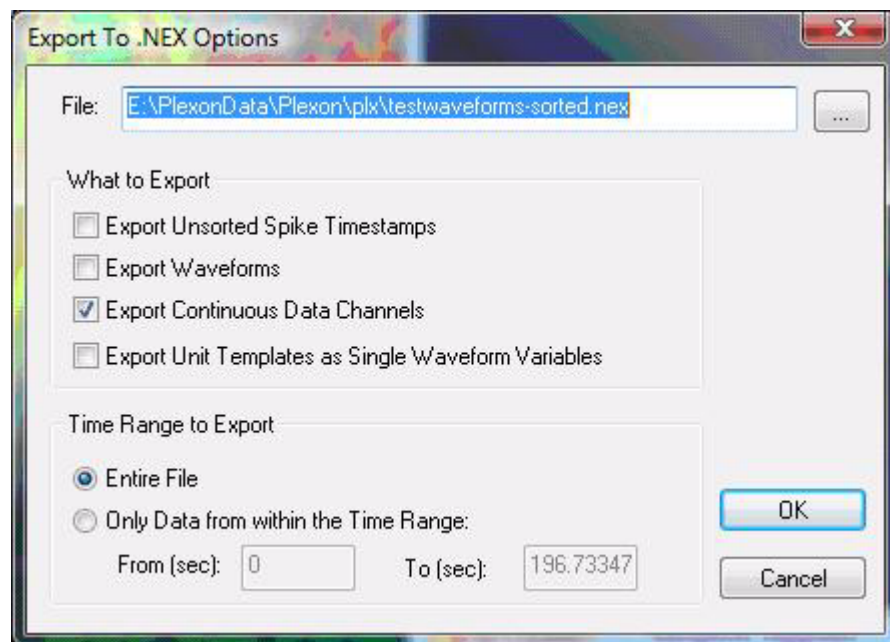
4.8.2 Exporting to a NEX File

The main result of the sorting process is a set of spike trains (arrays of timestamps) corresponding to the sorted units. One can analyze these spike trains with [NeuroExplorer \(NEX\)](#), which is a software package that is separately available from Plexon.

To save the timestamps and other data, such as continuous analog variables, in a NEX data file

- 1 Select **File | Export to NEX**.

*The **Export To .NEX Options** dialog box appears*



- 2 In the **What to Export** area, click the additional items to export. Spike timestamps for sorted units are always exported to the .nex file. One can add any of the following items:
 - **Export unsorted spike timestamps** – writes the timestamps for the unsorted waveforms as Nex variables. The Nex variables for unsorted timestamps have a ‘U’ appended to denote unsorted waveforms.

- **Export waveforms** – exports the waveforms for each unit as a new set of Nex waveform variables that are named as the timestamp variable name with “_wf” appended.
 - **Export continuous data channels** – exports any continuous data channels to the .nex file as Nex continuous variables.
 - **Export unit templates as single waveform variables** – creates a new Nex waveform variable for each unit. Each variable contains a single waveform at timestamp 0, which is the unit template.
- 3 Select a time range of the timestamps to save to the file, or accept the default setting where all timestamps are saved to the file.

One can optionally export a NEX waveform variable for each unit's template. The template waveforms have timestamp 0.

Also one can optionally write out the timestamps for the unsorted waveforms to the NEX file, as if it were a sorted unit. The NEX variable names that hold the unsorted waveform timestamps have a ‘U’ appended to denote Unsorted waveforms.

4.8.2.1 Re-sorting from a NEX File

Offline Sorter can also resort a file by using the unit assignments that are saved in a NEX file. This function is only useful if applied to the data file originally used to produce the NEX file. One can use this feature to facilitate retaining only the original, raw data file on disk, rather than retaining the raw data file plus possibly several versions of the data file sorted slightly differently.

To apply the sorting from a NEX file to the currently loaded file, select **Sort | Sort Using Saved .NEX File**. The operation works by matching timestamps from the Nex file to the waveform timestamps in the currently loaded file; there is also an option that can be used to control what happens to the waveforms that cannot be matched to timestamp from the NEX file. One can also multiply the timestamps from the Nex file by a factor to take into account the ‘timestamp compression’ that can happen due to NEX’s 31-bit timestamp limit.

4.8.3 Exporting to a .PLX0 file

Offline Sorter can also export the data loaded from any file as a .PLX0, which is a special, proprietary file format designed to make loading and saving operations much faster in Offline Sorter. For more information, see [“5.22.6 Using .PLX0 Files” on page 239](#).

Note: The .PLX0 file format is not appropriate for archiving data files. Plexon cannot guarantee that the .PLX0 file format will remain backwards-compatible in future releases of Offline Sorter.

4.8.4 Exporting Information

Offline Sorter has powerful and flexible facilities to export information for further analysis. Offline Sorter can export information to the following destinations:

- Text files: one can choose the delimiter between values.

-
- A MATLAB file: The data appears as a matrix variable when opening the file in MATLAB. This option is only appears MATLAB is installed on the same computer as Offline Sorter.
 - A Microsoft Excel spreadsheet: This option only appears if Microsoft Excel is installed on the same computer as Offline Sorter. During the information export, Excel opens and accepts the exported information into the first sheet on the spreadsheet. One can then save the spreadsheet from within Excel.

Offline Sorter can export three varieties of information:

- Per-Waveform information via the **File | Export Per-Waveform Data** menu selection: This item generates a line of information (a row) for each waveform that satisfies the export criteria. The kind of information items that can be exported per-waveform include the timestamp, unit assignment, and the values of any feature.
- Per-Channel information via the **File | Export Per-Channel Data** menu selection: This item generates a line of information for each channel in the file. The kind of information items that can be exported per-channel includes the numbers of waveforms and units, and the Sorting Quality Statistics.
- Per-Unit information via the **File | Export Per-Unit Data** menu selection: This item generates a line of information for each unit in the file. The kind of information items that can be exported per-channel include the numbers of waveforms, the cluster center positions and the sigmas and other normality statistics.

When exporting to a text file or to Excel, one can choose an option to export a row of column-header text to help in deciphering the exported data.

4.8.4.1 Exporting Per-Waveform Data

Selecting the **File | Export Per-Waveform Data** menu selection displays the following window:

The 'Export Per-Waveform Data' dialog box is shown with the following settings:

- Format:** ☒ Text file, ☐ Matlab file, ☐ Excel. Delimiter: Comma. ☐ Export a Row of Column Headers.
- Which Channels:** ☒ Current Channel only, ☐ All Channels into one file, ☐ All Channels, one file per channel.
- What Waveforms to Export:** ☐ Export Invalidated Waveforms, ☒ Export Unsorted Waveforms, ☐ Export Only Waveforms in the Time Range (From (sec): 0, To (sec): 196.733475). ☒ All Units, ☐ Only Unit (Unit a).
- What to Export for Each Waveform:**
 - Available:** Channel Name (Raw), Channel Name, Channel (Raw), Slice 1, Slice 2, Peak, Valley, Peak-Valley, Energy, Nonlinear Energy, PC 4, PC 5, PC 6, PC 7, PC 8.
 - Chosen:** Channel, Unit, Timestamp, PC 1, PC 2, PC 3.
 - Buttons: Add >>, Add All, << Remove, Remove All, Move Up, Move Down, Reset to Defaults.
- ☒ Append Raw Waveform A/D Data in units of ☒ microVolts, ☐ ADC Counts.
- What File(s) to Store the Exported Data in:** File: E:\PlexonData\Plexon\plx\testwaveforms-sorted.mat. Browse...
- Buttons: Export!, Cancel.

To export information

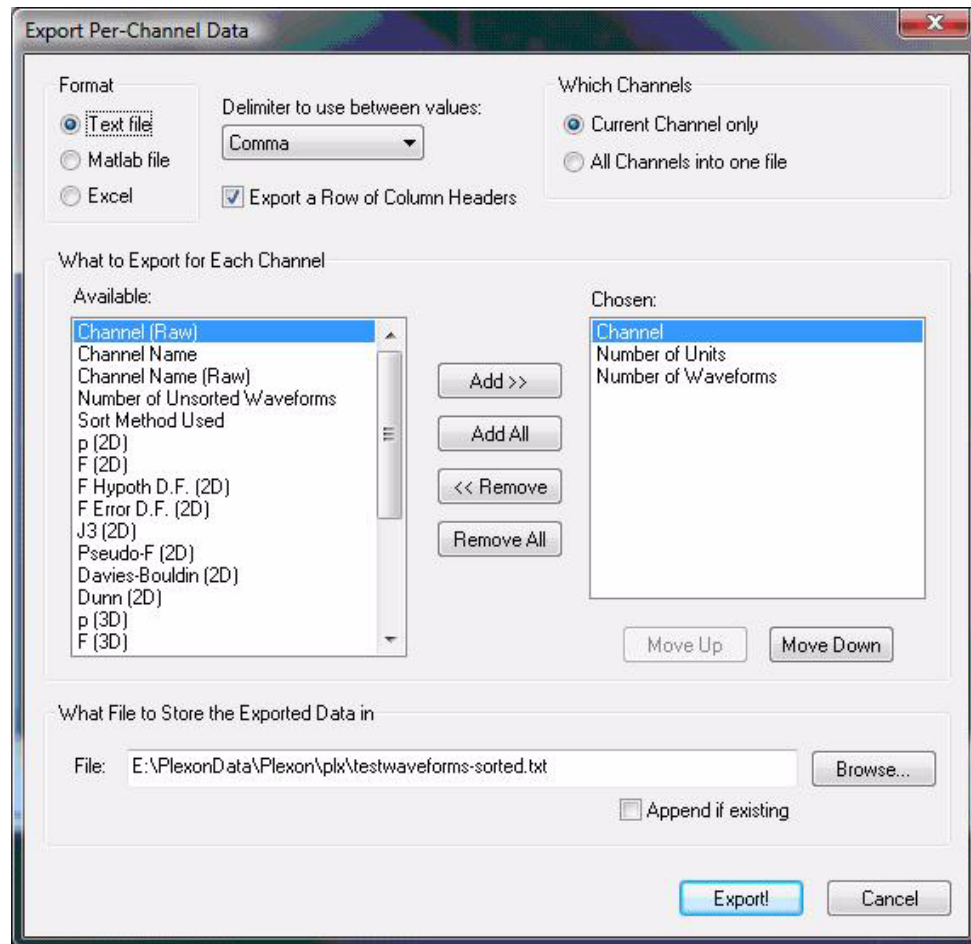
- 1 Select the **Format** of the export - either text file, MATLAB file (if MATLAB is available on the computer) or Microsoft Excel (if available on the computer).
- 2 For text export, choose which delimiter to use between values. Delimiter choices include comma, comma and space, space, or semicolon.
- 3 For Excel and text export, check the **Export a Row of Column Headers** to get a line of header text that labels each of the columns. This makes the exported file easier to read.
- 4 Select **Which Channels** to export. If a current channel has been selected in Offline Sorter when the **File | Export Per-Waveform Data** menu selection was selected, the default is the current channel only. Also, all channels can be exported to a single file. For text and MATLAB exports, another option is to do all channels, but generate one exported file per channel. In this case, a channel number is appended to the filename.
- 5 Select **What Waveforms to Export**. By default, Offline Sorter exports all valid waveforms. Check the **Export Invalidated Waveforms** to also include any waveforms that have been marked invalid in the exported information. Uncheck **Export Unsorted Waveforms** to cause any waveforms that have not been assigned to a unit to be ignored during the export. One can also define a time range for the waveforms to export using the **Export Only Waveforms in the Time Range** checkbox. Similarly, one exports only the waveforms sorted into a particular unit by selecting the **Only Unit** radio button.
- 6 Select **What to Export for Each Waveform**. This enables one to select what items appear in each column for each exported row of data. The **Available** list box on the left shows all the items that can be exported, and the **Chosen** list box on the right shows those that have been selected for export. Click on one or more items to highlight them and move items back and forth between the list boxes by then pressing the **Add>>** or **<<Remove** buttons. Use other convenience buttons to add all items, remove all items, or reset the chosen items to the “factory defaults”. The order that the items appear in the **Chosen** list box controls the order that they appear in the output file. One can change the order by selecting one or more items in the **Chosen** list box and pressing the **Move Up** or **Move Down** buttons. Offline Sorter remembers the chosen items, and their order, so that the next time information is exported, the same items are chosen automatically. Besides the obvious Channel, Unit, and Timestamp items, one can export the value of any of the Features Available for Sorting for each waveform.
- 7 In addition to, or instead of, the exported feature values, Offline Sorter can additionally export the raw A/D values associated with each waveform. To do so, click the **Append Raw Waveform A/D Data** checkbox. The A/D values appear in additional data columns after the exported feature columns. To export only the waveform A/D values, deselect all features from the **Chosen** list box and click the **Append Raw Waveform A/D Data** checkbox. The number of appended columns is equal to the number of points per waveform. The waveform A/D values can be exported in units of microvolts (the default), or in raw ADC counts, by clicking the radio buttons near the checkbox.
- 8 Select **What File(s) to Store the Exported Data in** by typing a filename or selecting one using the **Browse** button. For exports involving multiple

channels into multiple files, Offline Sorter appends a channel number to the filename entered.

- 9 Click the **Export!** button
- 10 For exports to Excel, save the spreadsheet using Microsoft Excel.

4.8.4.2 Exporting Per-Channel Data

Selecting the **File | Export Per-Channel Data** menu selection displays the following window:

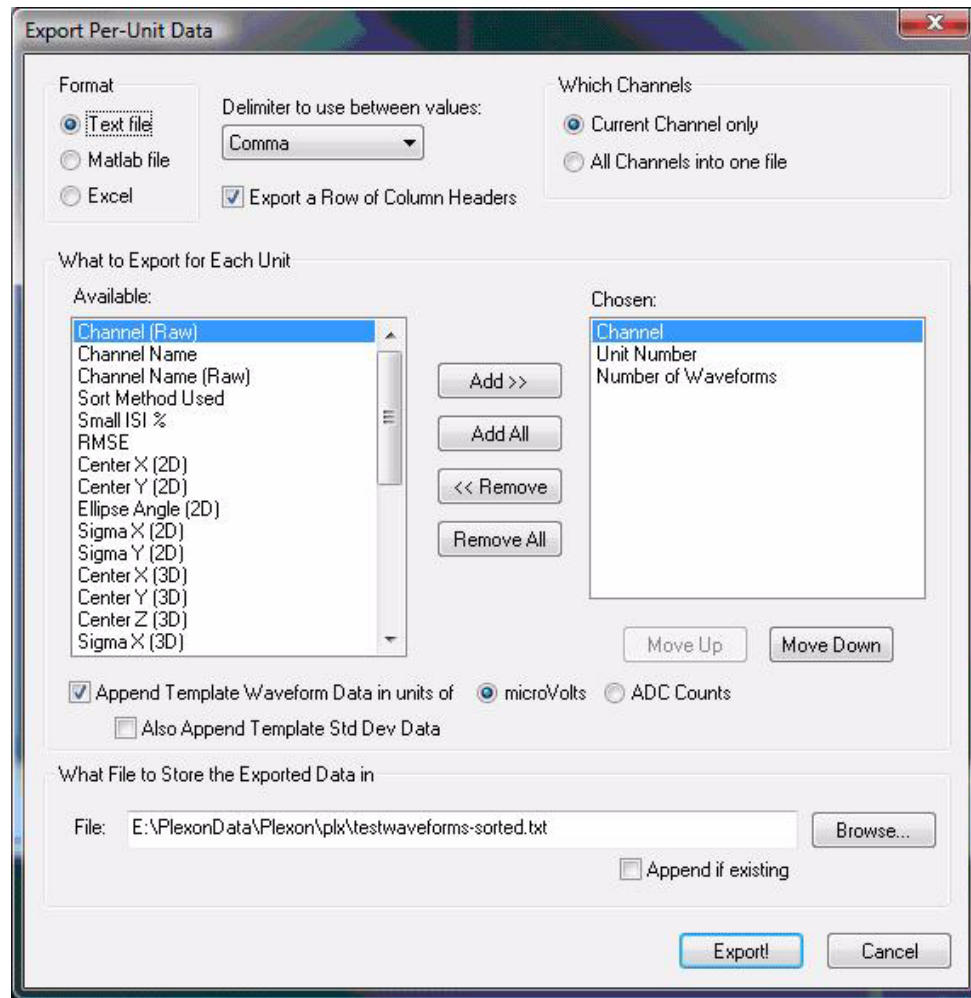


To perform an export

- 1 Select the **Format** for the exported information - either text file, MATLAB file (if MATLAB is available on the computer) or Microsoft Excel (if available on the computer).
- 2 For text export, choose a delimiter to use between values. Delimiter choices include comma, comma and space, space, or semicolon.
- 3 For Excel and text export, check **Export a Row of Column Headers** to get a header line of text that labels each of the columns. This makes reading the exported file easier.
- 4 Select **Which Channels** to export. If a current channel is selected in Offline Sorter when the **File | Export Per-Channel Data** menu selection was selected, the default is the **Current Channel only**. One can also export all channels to a single file.
- 5 Select **What to Export for Each Channel**. This enables selection what items appear in each column for each exported row of data. The **Available** list box on the left shows all the items that can be exported, and the **Chosen** list box on the right shows those that have been selected for export. Click on one or more items to highlight them and move items back and forth between the list boxes by then pressing the **Add>>** or **<<Remove** buttons. Use other convenience buttons to add all items, remove all items, or reset the chosen items to the “factory defaults”. The order that the items appear in the **Chosen** list box controls the order that they appear in the output file. One can change the order by selecting one or more items in the **Chosen** list box and pressing the **Move Up** or **Move Down** buttons. Offline Sorter remembers the chosen items, and their order, so that the next time information is exported, the same items are chosen automatically. The items available include the channel number, the number of units, waveforms, and unsorted waveforms in the channel, the sort method used to sort the channel, and the values of any of the Sorting Quality Statistics (both 2D and 3D). One can also export the features selected for each axis of feature space as a numerical code.
- 6 Select **What File to Store the Exported Data** in by typing a filename or selecting one by using the **Browse** button. To append the data onto the end of an existing file, click **Append if existing**.
- 7 Click **Export!**
- 8 For exports to Excel, save the spreadsheet using Microsoft Excel.

4.8.4.3 Exporting Per-Unit Data

Selecting the **File | Export Per-UnitData** menu selection displays the following dialog box:



To perform an export

- 1 Select the **Format** for the exported information - either text file, MATLAB file (if MATLAB is available on the computer) or Microsoft Excel (if available on the computer).
- 2 For text export, choose a delimiter to use between values. Delimiter choices include comma, comma and space, space, or semicolon.
- 3 For Excel and text export, check the **Export a Row of Column Headers** to get a header line of text that labels each of the columns. This makes reading the exported file easier.
- 4 Select **Which Channels** to export. If a current channel is selected in Offline Sorter when selecting **File | Export Per-Unit Data**, the default is **Current**

Channel only, which means Offline Sorter exports all units on the current channel. One can also export all units on all channels to a single file.

- 5 Select **What to Export for Each Channel**. This enables selection of what items appear in each column for each exported row of data. The **Available** list box on the left shows all the items that can be exported, and the **Chosen** list box on the right shows those that have been selected for export. Click on one or more items to highlight them and move items back and forth between the list boxes by then pressing the **Add>>** or **<<Remove** buttons. Use other convenience buttons to add all items, remove all items, or reset the chosen items to the “factory defaults”. The order that the items appear in the **Chosen** list box controls the order that they appear in the output file. One can change the order by selecting one or more items in the **Chosen** list box and pressing the **Move Up** or **Move Down** buttons. Offline Sorter remembers the chosen items, and their order, so that the next time information is exported, the same items are chosen automatically. The items available include the Channel number, unit number, number of waveforms, the values of the normality statistics (mean, sigma, skew and kurtosis) for both 2D and 3D. The numerical values are in units appropriate to the corresponding feature in the current feature space. One can also export the features selected for each axis of feature space as a numerical code.
- 6 In addition to, or instead of, the exported feature values, Offline Sorter can additionally export the raw A/D values associated with each waveform. To do so, click the **Append Raw Waveform A/D Data** checkbox. The A/D values appear in additional data columns after the exported feature columns. To export only the waveform A/D values, deselect all features from the **Chosen** list box and click the **Append Raw Waveform A/D Data** checkbox. The number of appended columns is equal to the number of points per waveform. The waveform A/D values can be exported in units of millivolts (the default), or in raw ADC counts, by clicking the radio buttons near the checkbox.
- 7 Select **What File to Store the Exported Data in** by typing a filename or selecting one using the **Browse** button. To append the data onto the end of an existing file, click **Append if existing**.
- 8 Click **Export!**
- 9 For exports to Excel, save the spreadsheet using Microsoft Excel.

Chapter 5

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5.1 Importing Data Files

The standard document types for Offline Sorter are the Plexon data file formats .PLX, .DDT, and the optimized .PLX0 format; see “5.22.6 Using .PLX0 Files” on page 239. Offline Sorter can also import data files from several other vendors, and also some generic raw data files via the **File | Import** menu selection.

Enabling the import of neural data from these different file formats into Offline Sorter is a formidable task. There are various limitations and factors to keep in mind for the different file formats, and sometimes different vendors have different concepts and assumptions that are difficult to represent in Offline Sorter.

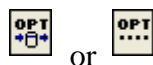
Offline Sorter is also capable of using Neuroshare DLLs (see www.neuroshare.org) to load other file formats. As explained on the Neuroshare website, the idea behind NeuroShare is for each vendor of neurophysiological data acquisition equipment to provide a Windows DLL that “knows” how to read and interpret the data file format created by that vendor’s data acquisition system. Applications such as Offline Sorter can write code to make use of the Neuroshare DLL to, in principle, read any file format for which the vendor has supplied a Neuroshare DLL.

The import of some formats is dependent on the presence of a Neuroshare DLL that is separately downloaded and installed on the computer. In general, Plexon cannot fix problems in the Neuroshare DLLs supplied by other vendors. During Offline Sorter testing, we observed that unfortunately there is much variation between the Neuroshare DLLs produced by different vendors. The Neuroshare loader in Offline Sorter tries to be as general and tolerant as possible, but in most cases the loading of the data has problems, e.g. the gains or reported voltage ranges are not correct, or the pre-threshold time is not available.

Note: Files loaded using a Neuroshare DLL cannot be saved via the Save or Save-As functionality. One must save these files using one of the “Export” functions. These files also do not react to the **Remove empty spike channels** setting, which is in the **Tools | Data Import Options** dialog box.

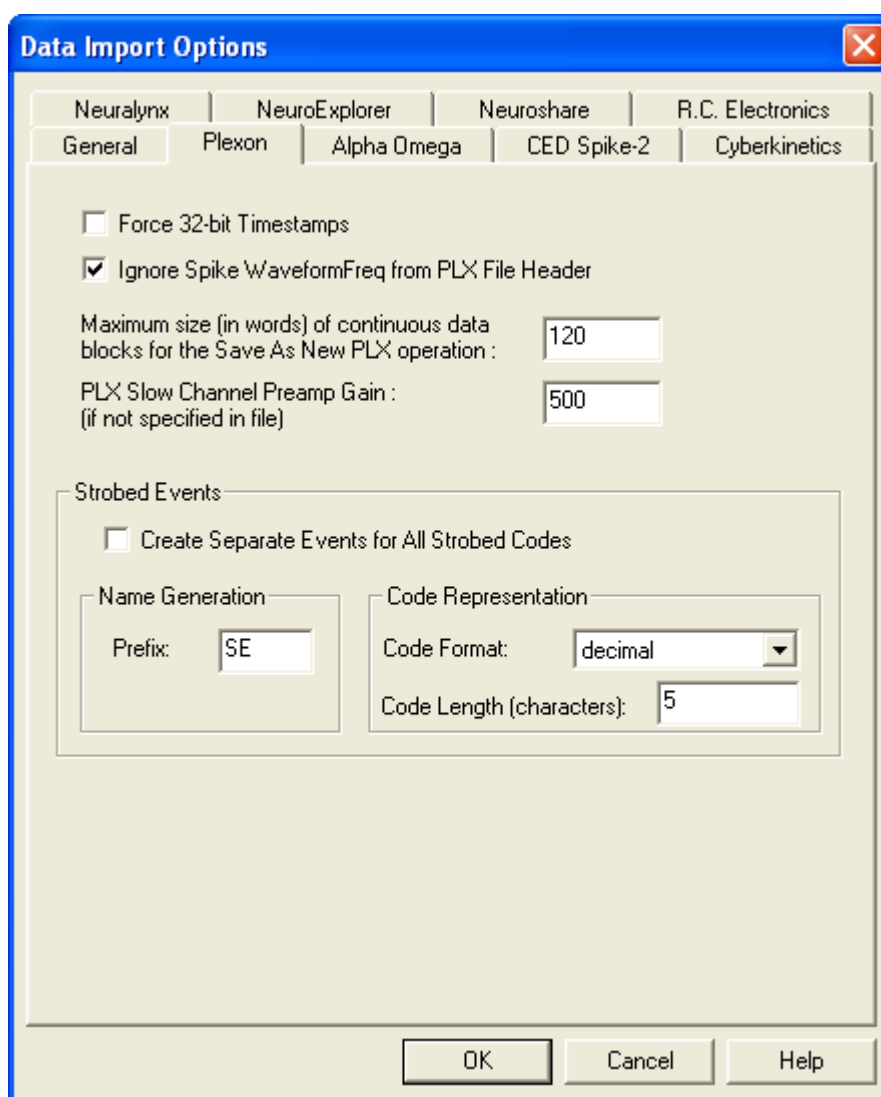
5.1.1 Data Import Options

The options that control the behavior of the loader for the different data file formats are grouped together as tabs in the **Tools | Data Import Options** dialog box. One can open the dialog box from the menu or click



and then, on the **General** tab, click **Data Import Options**.

The *Data Import Options* dialog box opens.



5.1.2 Other Vendor Format Import Notes

5.1.2.1 Alpha Omega

Import for Alpha Omega .MAP files is available only via the Neuroshare DLL available from Alpha Omega (see www.neuroshare.org). One must download this DLL file and use the **Alpha Omega** tab of the **Tools | Data Import Options** dialog box to set the name and location of this DLL.

As with most Neuroshare DLLs, there are some issues with handling gains for Alpha Omega files; the voltages displayed in Offline Sorter may not be accurate. There is also an indication (as of the time of this writing) that units beyond **Unit a** are mislabeled.

5.1.2.2 CED Spike-2

Import for CED Spike-2 .SMR files is available via either a “native” loader, or via CED’s Neuroshare DLL. To import the data via the Neuroshare DLL, one must download the Neuroshare DLL from CED (see www.neuroshare.org) and use the **CED Spike-2** tab on the **Tools | Data Import Options** dialog box to set the name and location of this DLL.

The **Save** and **Save As** operations are available for CED Spike-2 files to save the data back to a CED Spike-2 file, but only if the native CED loader is being used.

CED Spike-2 files that have a different number of samples per spike waveforms on different channels do not load properly into Offline Sorter – all spike channels must have the same number of samples per spike waveforms. Only single-electrode data types are supported by the native loader.

5.1.2.3 Cyberkinetics

Import for Cyberkinetics (also known as Black Rock Microsystems, formerly Bionic Systems) .NEV files is available via either a “native” loader, or via Cyberkinetics’ Neuroshare DLL. To import the data with the Neuroshare DLL, one must download this DLL file (see www.neuroshare.org) and use the **Cyberkinetics** tab in the **Tools | Data Import Options** dialog box to set the name and location of this DLL. Units marked with a -1 unit code (“Noise”) are imported as invalidated spikes.

5.1.2.4 Data Translations

Import for Data Translations .DCF files is available via a custom loader. This format contains only continuous data samples.

5.1.2.5 Datawave

Import for Datawave .UFF and Experimenters Workbench (EWB) files are available via a custom loader. The Non-EWB format supports all data types for spikes, continuous data, and events, the EWB format supports all data types for spikes and events.

For continuous data, there is a mismatch between the continuous sampling frequency reported in the header and what is actually seen in the data. It leads to gaps in the continuous data trace.

5.1.2.6 Instrutech

As of Offline Sorter version 2.8, support for this file format was dropped. If this causes great hardship, please contact support@plexoninc.com for assistance.

5.1.2.7 MultiChannel Systems

Support for MultiChannel Systems files depends on the MCStream.DLL COM object being installed and properly registered on the system. If it is not available on the system, a dialog box appears, which shows how to install it.

5.1.2.8 Neuralynx

Support for Neuralynx files is available via a native loader. By default, all files in the same directory as the file loaded have the Neuralynx file naming conventions applied. There is an option on the **Neuralynx** tab of the **Tools | Data Import Options** dialog box that restricts Offline Sorter to loading only the selected file, in which case there is only one channel available in Offline Sorter. The Neuralynx loader supports all data types for spikes, continuous data, and events.

5.1.2.9 NeuroExplorer

Import for NeuroExplorer .NEX files is available only via the Neuroshare DLL available from NeuroExplorer.com (see www.neuroshare.org). One must download this DLL file and use the **NeuroExplorer** tab of the **Tools | Data Import Options** dialog box to set the name and location of this DLL.

Only NeuroExplorer waveform variables are imported. Each Unit gets its own channel in Offline Sorter.

5.1.2.10 NeuroShare Native

Import for Neuroshare native .NSN files is available only via the Neuroshare DLL available from www.neuroshare.org. One must download this DLL file and use the **Neuroshare** tab of the **Tools | Data Import Options** dialog box to set the name and location of this DLL.

5.1.2.11 Alpha Omega

Import for Alpha Omega .MAP files is available only via the Neuroshare DLL available from Alpha Omega (see www.neuroshare.org). One must download this DLL file and use the **Alpha Omega** tab of the **Tools | Data Import Options** dialog box to set the name and location of this DLL.

5.1.2.12 Panasonic MED

Import for Panasonic .med files is available via a custom loader. This format contains only continuous data samples.

5.1.2.13 R.C. Electronics

As of Offline Sorter version 2.8, import for R.C. Electronics files is available only via the Neuroshare DLL available from R.C. Electronics (see www.neuroshare.org). One must download this DLL file and use the **R.C. Electronics** tab of the **Tools | Data Import Options** dialog box to set the name and location of this DLL.

Note: Prior to version 2.8, Offline Sorter contained a native loader for R.C. Electronics. This native loader is no longer supported.

5.1.3 Generic Binary Continuous File Import

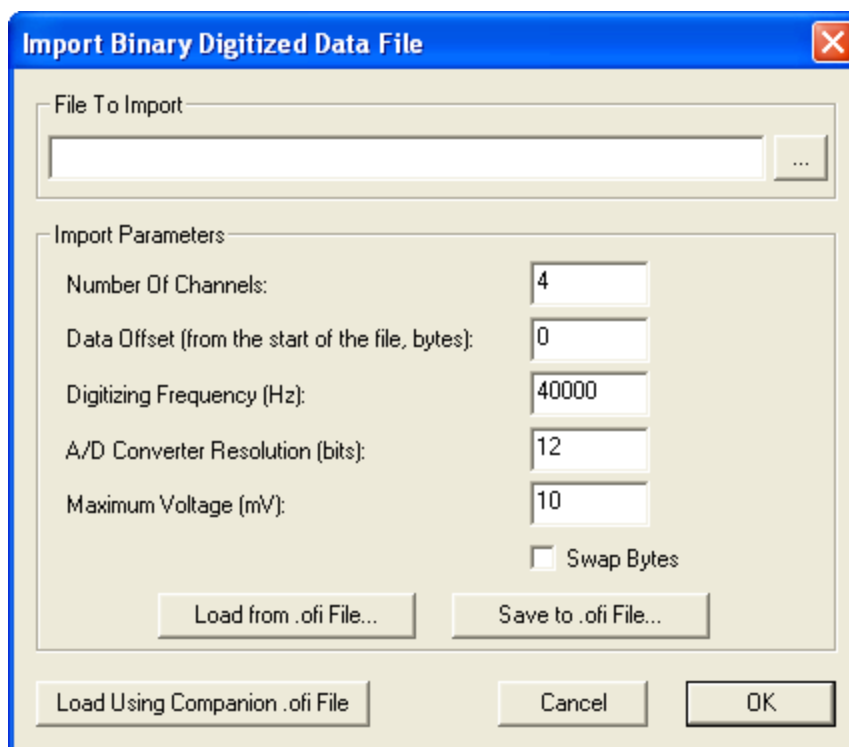
Offline Sorter can sometimes import a “raw” binary file that consists of continuously-digitized samples.

Note: The binary file is expected to conform to the following specifications:

- The data file can contain a fixed-size header block at the beginning of the file. The size of this header block must be known and entered into the dialog so that this part of the file can be skipped over. Zero is a valid size for the header.
- Once the continuously digitized data starts after the header, it cannot have any kind of “block structure” to it—it must contain just A/D values arranged in sweeps; each sweep contains a single A/D value from each channel.
- Each A/D value is stored in a signed 16-bit word, regardless of the A/D conversion resolution. If the **Swap Bytes** checkbox is checked, Offline Sorter interprets the 16-bit words in “big-endian” (MSB first) format instead of the default Intel-standard “little-endian” format.

To import a binary file, from the main menu select **File | Import | Binary File with Continuously Digitized Data**.

*The **Import Binary Digitized Data File** dialog box appears.*



Select a file, then enter the required information in the **Import Parameters** area. Click **OK** to close the dialog box and import the file.

Note: One can click **Save to .ofi File** to save the choices of Import Parameters to a file that by convention has an .ofi extension. Then the same Import Parameters can be restored by loading the file, using the **Load from .ofi File** button. If **Load Using Companion .ofi File** is clicked, Offline Sorter looks for an .ofi file

with the same base name (the filename minus the extension) and loads the binary file using the Import Parameters in the companion .ofi file.

The format of the .OFI file is a number of lines in the format “Parameter=Value”. The parameters correspond to the edit boxes in the above dialog, with the keywords “NChan”, “Offset”, “Freq”, “MaxMV”, “ResolutionBits”, and “Swap”, respectively. For example, a typical .OFI file could be:

- NChan=81
- Offset=0
- Freq=25000.000000
- MaxMV=10.000000
- ResolutionBits=16
- Swap=1

5.1.4 Generic Neuroshare Import

In addition to the known vendor formats that can be imported via a vendor-supplied Neuroshare DLL, Offline Sorter provides a mechanism to use any arbitrary Neuroshare DLL to load a file. To do this, first open the **Tools | Data Import Options** dialog box and click the **Neuroshare** tab and specify the location of the Neuroshare DLL on disk. Then, load any arbitrary data file using the selected Neuroshare DLL and the **File | Import | File via Neuroshare** menu item.

Note: For any files imported using a Neuroshare DLL, the Neuroshare Segment entities become spike channels, and the Analog entities become continuous channels.

5.2 Channel Mapping

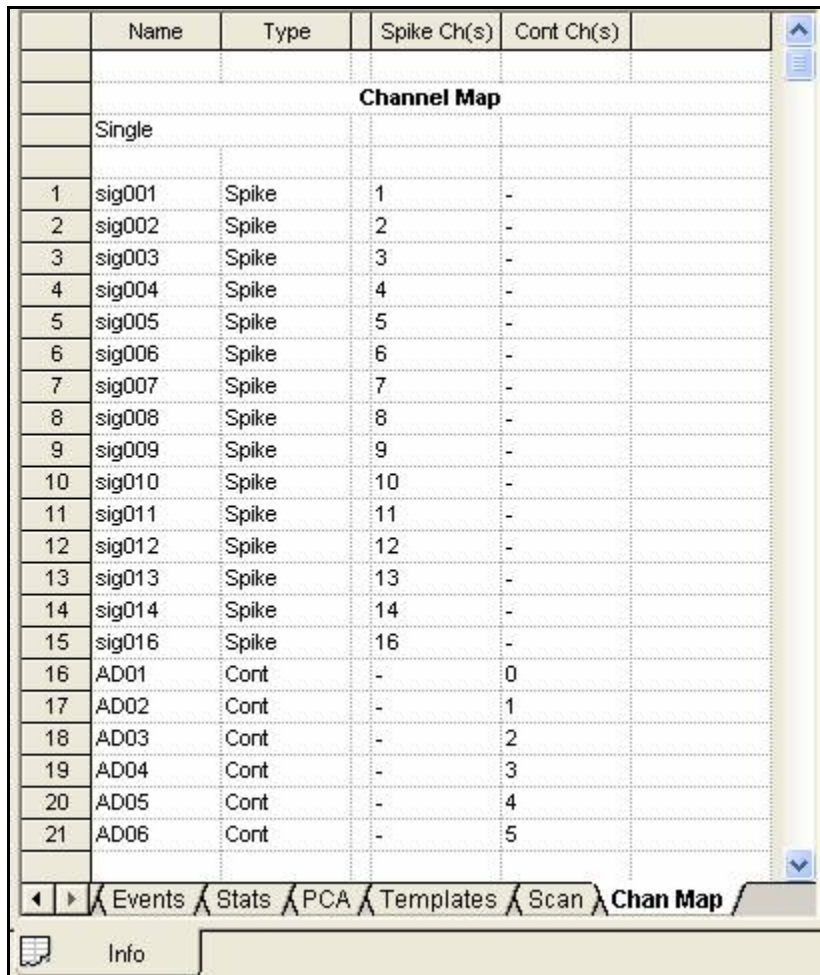
Starting with version 2.8 of Offline Sorter, the somewhat confusing concepts of Channel Types and Associated Channels were modified into an over-arching concept of Channel Mapping.

Offline Sorter has the concept of a “channel”, which has a name and a number. An Offline Sorter channel can potentially contain both spikes and continuous data. A channel in Offline Sorter also has a Data Type, which is either single-electrode, stereotrode, or tetrode.

Neural data files also have a concept of a “channel”, which generally also has a name and a number. We will refer to the channels in the neural data file as “Raw” channels. A Raw channel is always either a Spike channel or a Continuous channel, but not both.

The purpose of the **Info | Chan Map** tab is to display and allow control over how the Raw channels from the neural data file are assigned to Offline Sorter channels.

The format of the **Chan Map** tab has one line per Offline Sorter channel. The Raw channel number(s) that are associated with the Offline Sorter channels appear on the line, as shown in the following illustration:



	Name	Type	Spike Ch(s)	Cont Ch(s)
	Channel Map			
	Single			
1	sig001	Spike	1	-
2	sig002	Spike	2	-
3	sig003	Spike	3	-
4	sig004	Spike	4	-
5	sig005	Spike	5	-
6	sig006	Spike	6	-
7	sig007	Spike	7	-
8	sig008	Spike	8	-
9	sig009	Spike	9	-
10	sig010	Spike	10	-
11	sig011	Spike	11	-
12	sig012	Spike	12	-
13	sig013	Spike	13	-
14	sig014	Spike	14	-
15	sig016	Spike	16	-
16	AD01	Cont	-	0
17	AD02	Cont	-	1
18	AD03	Cont	-	2
19	AD04	Cont	-	3
20	AD05	Cont	-	4
21	AD06	Cont	-	5

The columns in the **Chan Map** tab are:

- **Name:** the name of the Offline Sorter channel
- **Type:** an informational display of whether the Offline Sorter channel contains spikes only (“Spike”), continuous data only (“Cont”), or both spikes and continuous data (“Spike+Cont”). This column is always read-only.
- **Spike Ch(s)** and **Cont Ch(s):** these columns represent the Raw spike/continuous channel numbers from the neural data file that are associated with this Offline Sorter channel. If the Data Type is Stereotrode or Tetrode, these columns display 2 or 4 Raw spike/continuous channel numbers. If the loaded file type supports channel re-mapping, these entries are drop-lists that allow selecting a different Raw channel number.

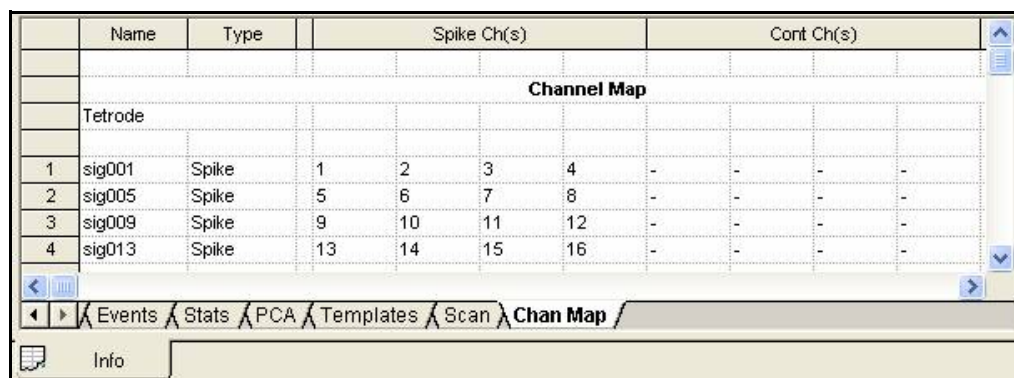
Note: Offline Sorter channel numbers always start numbering at 1. Usually Raw channel numbers also start numbering at 1, but occasionally some file formats

start numbering Raw channels at 0. In particular, the continuous channels from .plx files start numbering at 0.

There is always a current **Channel Map** in effect. If no channel re-mapping has been performed, a neural data file loads with a default channel mapping scheme.

5.2.1 Stereotrode and Tetraode Channel Mapping

The following screenshot shows a standard mapping of 16 Raw spike channels into 4 tetraode Offline Sorter channels.



	Name	Type	Spike Ch(s)				Cont Ch(s)			
Channel Map										
Tetrode										
1	sig001	Spike	1	2	3	4	-	-	-	-
2	sig005	Spike	5	6	7	8	-	-	-	-
3	sig009	Spike	9	10	11	12	-	-	-	-
4	sig013	Spike	13	14	15	16	-	-	-	-

For example, the tetraode channel named **sig009** consists of Raw spike channel 9 as electrode 1, Raw spike channel 10 as electrode 2, etc.

Note: The Neuralynx file format stores stereotrode/tetrode data such that a single Raw channel contains of the data for all electrodes in the stereotrode/tetrode data. In this case the **Chan Map** tab can be misleading.

5.2.2 Associated Spike and Continuous Channels

The following screenshot shows a file that contains four Offline Sorter channels, where each Offline Sorter channel has spikes from a Raw spike channel and continuous data from a Raw continuous channel. Moreover, the spikes were obtained by thresholding the continuous data. In this sense, the Raw spike and continuous

data channels are associated, because they both came from the same electronic probe.

	Name	Type	Spike Ch(s)	Cont Ch(s)
Channel Map				
	Single			
1	Channel01	Spike+Cont	1	1
2	Channel02	Spike+Cont	2	2
3	Channel03	Spike+Cont	3	3
4	Channel04	Spike+Cont	4	4

Note: One can have stereotrode/tetrode files with associated spike and continuous data.

For .plx files recorded with the Plexon MAP system, channel associations can be set up in the SortClient application and preserved in the acquired .plx files. These associations are restored when the .plx file is loaded into Offline Sorter.

5.2.3 Changing the Channel Map

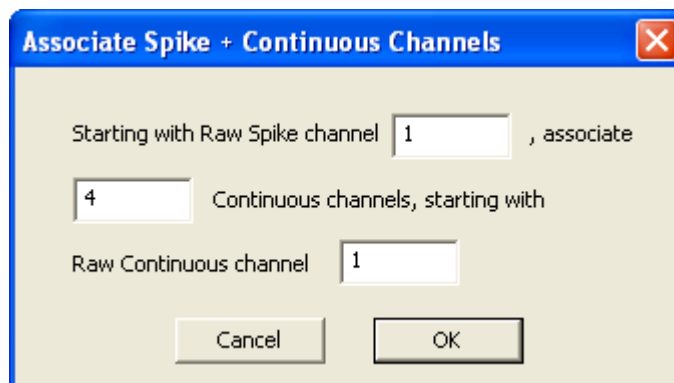
Changing the channel map or the Data Type is supported only for .PLX files and for the file formats that contain only continuous data (Plexon .DDT files, RC Electronics, Data Translations, Panasonic MED, and generic binary). For other file formats that contain spike data, a workaround for this limitation is to first Export the data file to .PLX format, then perform the desired channel mapping on that .PLX file.

The line just below the **Channel Map** label contains a drop-list to select the Data Type. Selecting a Data Type here is equivalent to selecting it from the control grid. One must set the desired Data Type before doing any other channel mapping. After changing the Data Type, Offline Sorter applies a straightforward channel map in which all the Raw spike channels are assigned in order.

If the currently-loaded file supports changing the channel map, then the entries under the **Spike Ch(s)** and **Cont Ch(s)** columns become drop-lists. To assign a different Raw channel to an Offline Sorter channel, select the Raw channel from the drop-list. As soon as any change to the channel map is made, an **Apply** button appears underneath the **Channel Map** label. The new channel map is not put into effect until the **Apply** button is clicked. Select **Revert to Current Channel Map** to revert any channel mapping changes to the original state.

Two common operations are included as right-click (shortcut) menu entries:

- **Associate Spike + Continuous Channels** – attempts to “match up” each continuous channel to an existing spike channel and opens the following dialog box:



One can enter values to “offset” the association relative to the start of the Spike or Continuous Raw channels. One can also associate only a subset of channels.

- **Disassociate All Spike + Continuous Channels** – breaks any existing associations and assigns one Offline Sorter channel to each Raw spike and to each Raw continuous channel.

Note: It is possible to make channel mappings and associations that cannot be saved in a .PLX file. Offline Sorter tries to display a warning when this is the case.

5.2.4 Loading a Channel Map from a File

One can set up a text file that describes the desired channel mapping, then apply that channel map in Offline Sorter. To do this manually, from the in the **Info | Chan Map** tab right-click (shortcut) menu, select **Load Channel Map from a File**. One can also apply the channel mapping file in a batch command file using the **Set ChanMap** filename command; see [“5.2 Channel Mapping” on page 181](#).

The format of the channel mapping file is as follows:

- The first line contains two numbers, separated by commas. The first number N is the number of Offline Sorter channels described in the mapping. The second number is the “trodalness” T of the mapping : 1 for single electrode, 2 for stereotrode, or 4 for tetrode. The example that follows represents 18 channels mapped as tetrodes (N,T=18,4).
- The next N lines describe the raw channels mapped to each Offline Sorter channel. Each line consists of the Offline Sorter channel number, which must be greater than or equal to 1, followed by a tab or space(s), then followed by a comma-separated list of T raw channel numbers. The position within the comma-separated list determines the specific electrode within a stereotrode or tetrode. In the following example, channel 4 has electrodes 13, 14, 15, and 16 as a tetrode.

Sample channel mapping file contents:

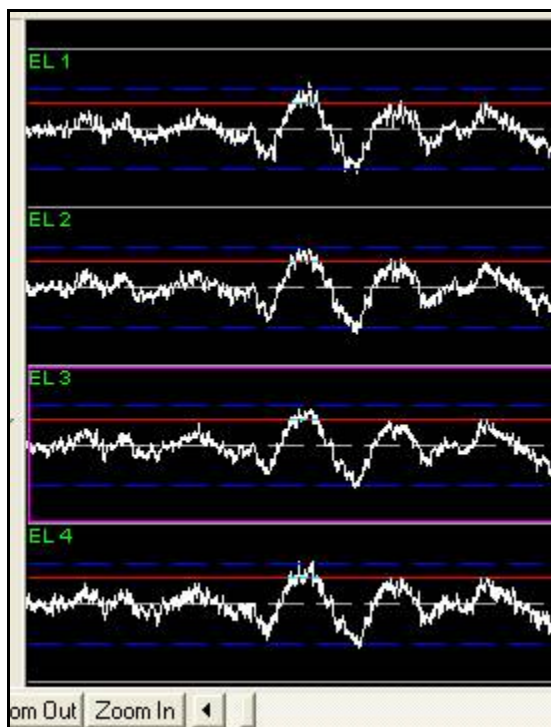
18,4	
1	1,2,3,4
2	5,6,7,8
3	9,10,11,12
4	13,14,15,16
5	17,18,19,20
6	21,22,23,24
7	28,29,30,31
8	32,33,34,35
9	36,37,38,39
10	40,41,42,43
11	44,45,46,47
12	48,49,50,51
13	55,56,57,58
14	59,60,61,62
15	63,64,65,66
16	67,68,69,70
17	71,72,73,74
18	75,76,77,78

5.3 Stereotrode and Tetrode Data

5.3.1 Extracting Stereotrode or Tetrode Spikes from Continuous Data

As outlined on [page 72](#), Offline Sorter can extract spikes from continuous data channels that are mapped to stereotrode/tetrode data using the **Chan Map** tab; see [“5.2.4 Loading a Channel Map from a File” on page 185](#). In this case, the

Continuous Data Display shows the 2 or 4 continuous data traces stacked vertically on top of each other:



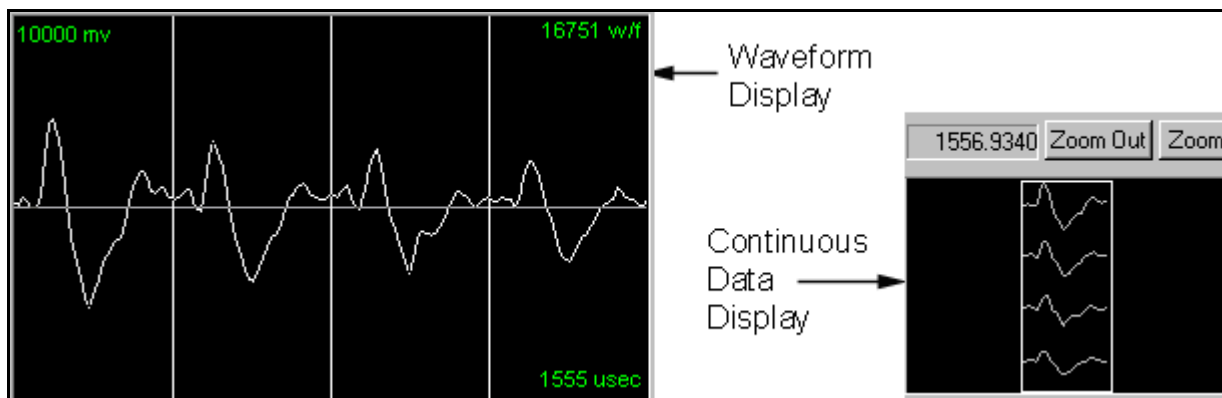
If a low-cut filter is applied, it applies to all traces in the stereotrode or tetrode channel. For waveform detection on stereotrode/tetrode continuous data, a threshold crossing on any electrode results in the capture of the waveform across all electrodes.

The threshold by default is the same for all electrodes in the channel. To adjust the threshold for each electrode independently, click **Detector and Waveform Options** to open the **Waveform Detection Options** dialog box, then click **For stereotrode/tetrode data, allow an independent threshold for each electrode**. In the preceding illustration, the purple highlighting box shows which electrode is current for the purpose of adjusting the independent threshold.

5.3.2 Sorting Stereotrode and Tetrode Spike Waveforms

By default, Offline Sorter analyzes stereotrode and tetrode spike data by concatenating the individual waveforms from each electrode in the tetrode or stereotrode group into a single waveform. The resulting concatenated waveform is twice (for stereotrode) or four times (for tetrode) as long as each individual waveform.

The Waveform Display shows the concatenated waveform with vertical white lines separating the waveforms from each individual electrode channel within the group. The Timeline View represents the waveform group arranged vertically, indicating that they were acquired at the same point in time.



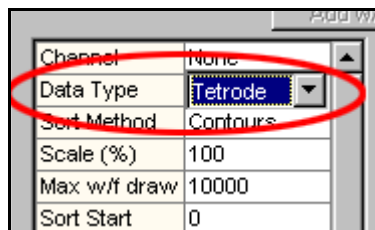
One can then apply all cluster display and sorting features to the concatenated waveforms. Offline Sorter includes sorting features that calculate values on the per-electrode waveforms instead of the concatenated waveforms (e.g. “Peak EL 1” is the peak value of the waveform on the first electrode only). Also, ratios can be formed from per-electrode features (e.g. “Peak EL 1 / EL 2” is the peak value on electrode 1 divided by the peak value on electrode 2).

Note: One can set the data type mode (**Single** electrode, **Stereotrode**, or **Tetrode**) by using the **Data Type** variable in the Control Grid. Changing the data type forces the file to be interpreted as containing data of that type. See the next section for details.

Stereotrode and tetrode data formats in Plexon PLX, DataWave UFF, and Neuralynx files are automatically detected when the file is opened.

5.3.3 Converting Data Type

An advanced feature in Offline Sorter enables one to force-interpret spike data to be any data type. For example, data files that were acquired as single-channel data can be treated as if they were stereotrode or tetrode data. To convert the data type, use the **Data Type** drop-list in the Control Grid,



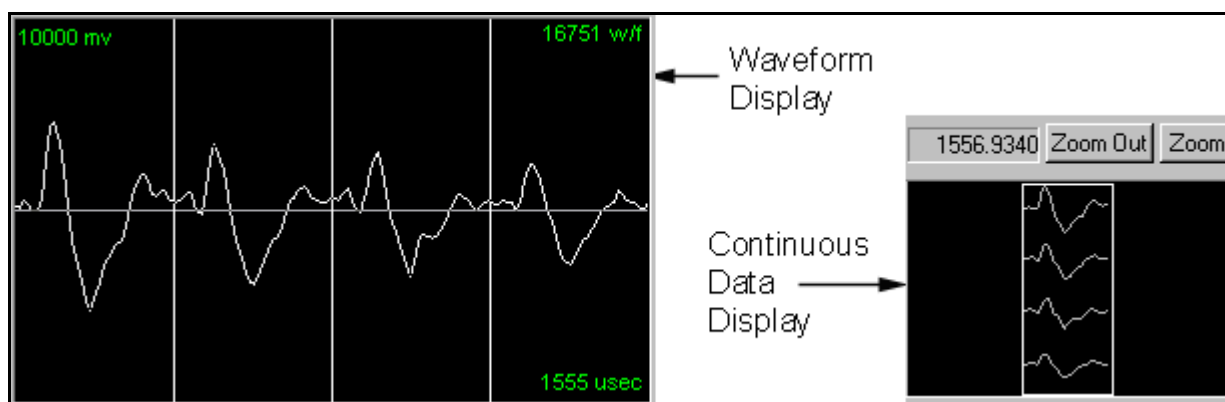
or, use the drop-down list in the **Chan Map** tab of the Info Grid View.

When a different data type is selected, Offline Sorter performs a conversion algorithm on the data, which results in the data being re-loaded and all sorted unit assignments and parameters to be lost.

The algorithm used to convert data type is as follows, using the conversion of single-channel data into tetrode data as an example:

- 1 Group the channels into groups of four (two for stereotrode data). If the original single-channel data file has N channels, there will be $N/4$ tetrode channels ($N/2$ for stereotrode) after the conversion.
- 2 Using the first channel in each group, step through each spike in the channel and obtain its timestamp. Find the corresponding spikes with the same timestamp on the other three channels in the group. Failure to find a spike on the other channels with the same timestamp results in a “dropped waveform”. A count of dropped waveforms appears on the status bar when a converted channel is opened.
- 3 The waveforms for the four time-coincident spikes are concatenated together to form the “expanded waveform” for the single spike on tetrode channel. If each single channel contains 800 microsecond-long waveforms, the four spikes on the four single channels become a single spike on the tetrode channel with a length of $4 \times 800 = 3200$ microseconds. Any dropped waveforms are represented as zeros in the corresponding position in the expanded waveform.

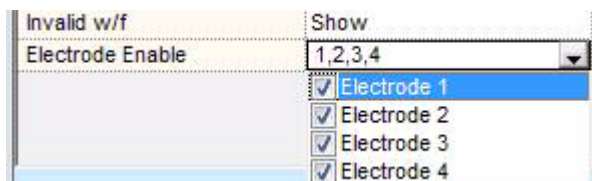
For tetrode data, the Waveform display shows the expanded waveform with vertical white bars separating the portions corresponding to each electrode. In the Continuous Data display, the waveforms for each electrode are stacked vertically to indicate their time-coincidence.



5.3.4 Disabling Electrodes

With Stereotrode/Tetrode data, an electrode (or wire) can break or otherwise just produce noise or artifacts. One can choose to “ignore” individual electrodes within a stereotrode/tetrode by clicking open the **Electrode Enable** drop list in the Control Grid and unchecking the checkboxes. The Electrode Enable entry in

the Control Grid will display 'N/A' if single-electrode data is loaded into Offline Sorter.



When an electrode is disabled, a red box with the word 'Disabled' will appear in the space where that electrode is being displayed in the waveform display. Disabled electrodes do not contribute to PCA calculations or to calculations of any other Feature values for the waveforms.

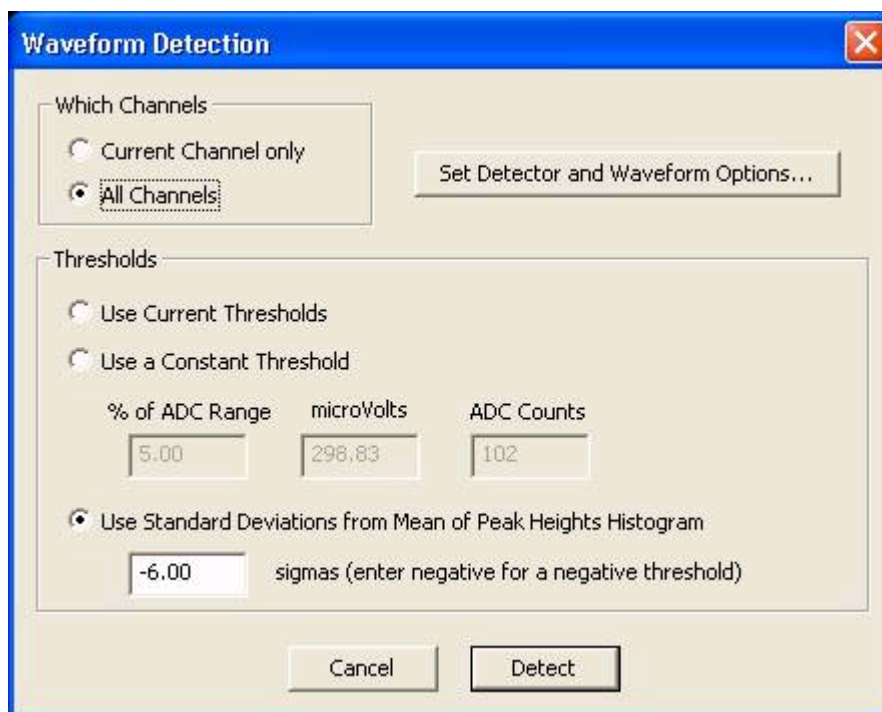
5.4 Automatic Waveform Detection

Offline Sorter can also perform waveform detection on all the spike continuous channels in a file. Use the following procedure to set up waveform detection.

To initiate waveform detection

- 1 Click **Waveforms | Detect**.

The **Waveform Detection** window appears.



- 2 In the **Which Channels** area, click a channel selection. To perform waveform detection on the current channel, click **Current Channel only**. To perform waveform detection on all channels, click **All Channels**.
- 3 To open the **Waveform Detection Options** window, click **Set Detector and Waveform Options**. For more information on detector and waveform options, see [“4.2.6 Specifying the Waveform Detection Options” on page 99](#).
- 4 In the **Thresholds** area, click one of the following detection thresholds:
 - **Use Current Thresholds** – This setting uses the thresholds that are currently set for each channel. One can view and edit the threshold settings for each channel in the **Info | Channel Parameters** tab.
 - **Use a Constant Threshold** – Type a constant threshold value into of the following boxes:

% of ADC Range – the percentage of the analog-to-digital converter (ADC) range indicated in the **Timeline View** (see [“3.5 Timeline View” on page 31](#))

microVolts – voltage threshold in μV

ADC Counts – the threshold in raw analog-to-digital conversion counts
 - **Use Standard Deviations from Mean of Peak Heights Histogram** – Type the number of standard deviations (sigmas) from the mean to set the threshold at that distance from the mean of the peak height histogram. A negative number sets the threshold to be less than the mean. A positive number sets the threshold to be greater than the mean.
- 5 To start the waveform detection process, click **Detect**.

5.5 Features Available for Sorting

One can choose each dimension of the 2D or 3D feature space used in Offline Sorter from the list of available features. Any of the following features can be made “active”, so that they appear in the X axis, Y axis and Z axis feature drop-lists in the toolbar. See “Axis Selection” under [“3.7.1 Clusters View Common Functionality” on page 45](#).

In the following,

$w(t) = [w(1), \dots, w(n)]$ is the waveform ($n = \text{number_of_points_in_waveform}$)

$p1(t) = [p1(1), \dots, p1(n)]$ is the first principal component vector

$p2(t) = [p2(1), \dots, p2(n)]$ is the second principal component vector

$p3(t) = [p3(1), \dots, p3(n)]$ is the third principal component vector

Σ represents a sum over t

The available features are:

- **PC1** – The waveform projection onto the first principal component

$$PC1 = \Sigma p1(t) * w(t)$$
- **PC2** – The waveform projection onto the second principal component

$$PC2 = \sum p2(t) * w(t)$$

- **PC3** – The waveform projection onto the third principal component

$$PC3 = \sum p3(t) * w(t)$$

- **PC4-PC8** – The waveform projections onto higher principal components

- **Slice 1** – The waveform height at a selected position in time. One can select the time position in the Control Grid or the Waveform display.

$$\text{Slice 1} = w(i) : \text{the waveform voltage at time } t=i$$

- **Slice 2** – The waveform height at a selected position in time. One can select the position in the Control Grid or the Waveform display.

$$\text{Slice 2} = w(j) : \text{the waveform voltage at time } t=j$$

- **Slice 3** – The waveform height at a selected position in time. One can select the position in the Control Grid or the Waveform display.

$$\text{Slice 3} = w(k) : \text{the waveform voltage at time } t=k$$

- **Slice 4** – The waveform height at a selected position in time. One can select the position in the Control Grid or the Waveform display.

$$\text{Slice 4} = w(l) : \text{the waveform voltage at time } t=l$$

- **Peak** – The maximum voltage amplitude across the entire waveform length

$$\text{Peak} = \max(w(t))$$

- **Valley** – The minimum voltage amplitude across the entire waveform length

$$\text{Valley} = \min(w(t))$$

- **Peak-Valley** – The difference between the maximum and minimum waveform amplitude

$$\text{Peak-Valley} = \max(w(t)) - \min(w(t))$$

- **Peak, Valley FWHM** – The “Full Width at Half Maximum” for the peak or valley of the waveform, expressed in milliseconds. The exact points where the waveform crosses the half max value are obtained by interpolation.

- **Peak, Valley Tick** – The clock tick (as measured from the beginning of the captured waveform) at which the peak or valley occurs. No interpolation or waveform fitting is done, so these values are “quantized” to integer values.

- **Area** – The total integrated area underneath the waveform

$$\text{Area} = \sum \text{abs}(v(t))$$

- **Energy** – The waveform energy, as it is calculated for spike detection. See [“3.12 Waveform Detection View” on page 69](#).

$$\text{Energy} = \left(\frac{1}{\text{width}} \right) \sum v(t) v(t)$$

- **Sqrt(Energy)** - The square root of the above-mentioned waveform energy.
- **Non-Linear Energy** – The non-linear waveform energy, as it is calculated for spike detection. See [“3.12 Waveform Detection View” on page 69](#).

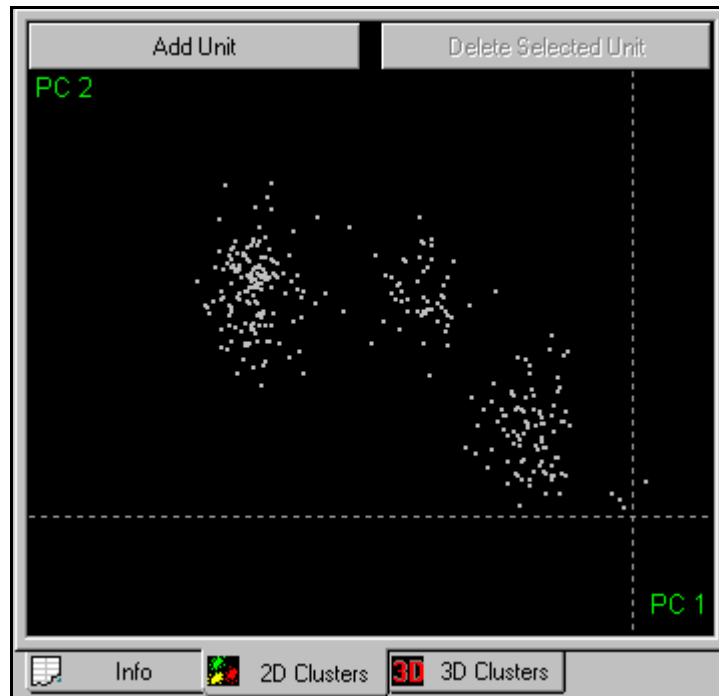
$$\text{Non-Linear Energy} = \left(\frac{1}{\text{width}}\right) \sum [v(t) \cdot v(t) - v(t-1) \cdot v(t+1)]$$

- **ISI(Previous), ISI(Next)** - For waveform *i*, ISI(Previous) is the amount of time (in mSecs) between the waveform *i*-1 and *i*, while ISI(Next) is the amount of time between waveform *i* and *i*+1. Both values are capped at 9999 mSecs to avoid large gaps from over-extending the range of values.
- **Timestamp** – The timestamp of the waveform in the file. Note: Selecting the timestamp as one of the dimensions in the Clusters View allows the viewing of the other feature(s) as a function of time. Discontinuities or drifts in time can indicate a change in the electrode position or the recording conditions.
- **Per-Electrode Features** – (only available for stereotrode or tetrode data) Most of the above features are available calculated per-electrode. For example, the feature “Peak EL 1” is the peak value of the waveform within the first electrode in a stereotrode (or tetrode). “Peak EL 2” is the peak value within the second electrode of the 'trode. For further discussion, see [“5.3 Stereotrode and Tetrode Data” on page 186](#).
- **Per-Electrode Ratio Features** – (only available for Stereotrode or Tetrode data) These features are formed by taking the ratio of Per-Electrode feature values. For example, the feature “Peak EL 1/EL 2” is “Peak EL 1” divided by “Peak EL 2”. Ratios with a denominator value of 0 are arbitrarily set to 0. For further discussion, see [“5.3 Stereotrode and Tetrode Data” on page 186](#).

5.5.1 Principal Components

The projections onto the first eight principal components can be plotted on any axis of the Clusters Views. By default, the principal component vectors are calculated using all the valid waveforms of the current channel and all time points in the waveform between Sort Start and Sort End. For more information, see [“4.4.4 Adjusting the Sort Range” on page 118](#).

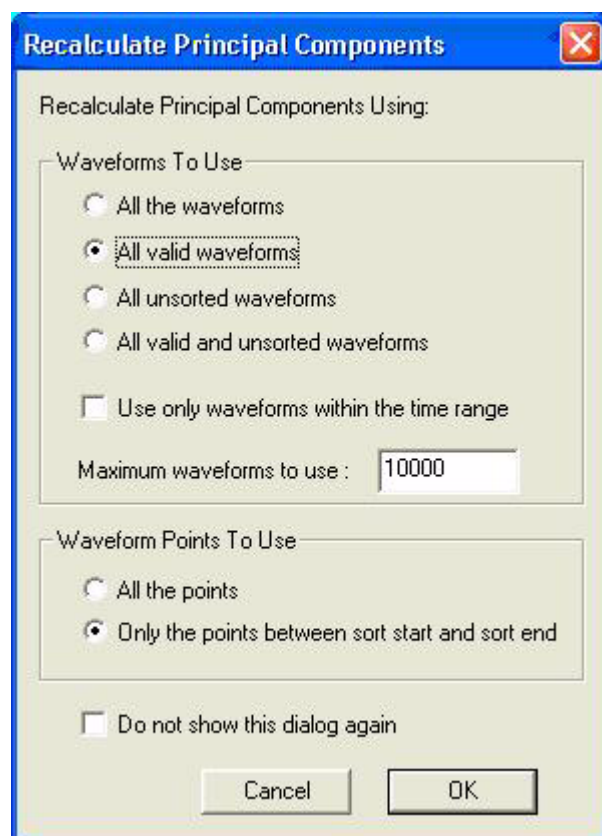
For example, waveforms projected onto the first two principal components appear as follows when viewed in the 2D Clusters view.



The dashed lines are the X and Y axes, representing the zeroes of PC1 and PC2. The points representing the lowest-amplitude waveforms have principal component projections near zero (i.e. near the intersection of the two axes).

One can recalculate the principal components using subsets of waveforms (e.g. only valid waveforms, only unsorted waveforms, only waveforms within a time range) or using only the points specified by the **Sort Start** and **Sort End** parameters in the Control Grid. The various options appear in the **Tools | Recalculate**

the **Principal Components** menu command, which displays the following window.



One can restrict the PCA Calculation to use only a certain number of waveforms. This is a useful feature for larger files, because the PCA calculation for channels containing large numbers of waveforms can be time-consuming. All of the options that are available in the **Recalculate Principal Components** window are also available under the **Feature Calc** tab of the **Tools | Options** window. Offline Sorter remembers all of the options that control the PCA calculation between invocations.

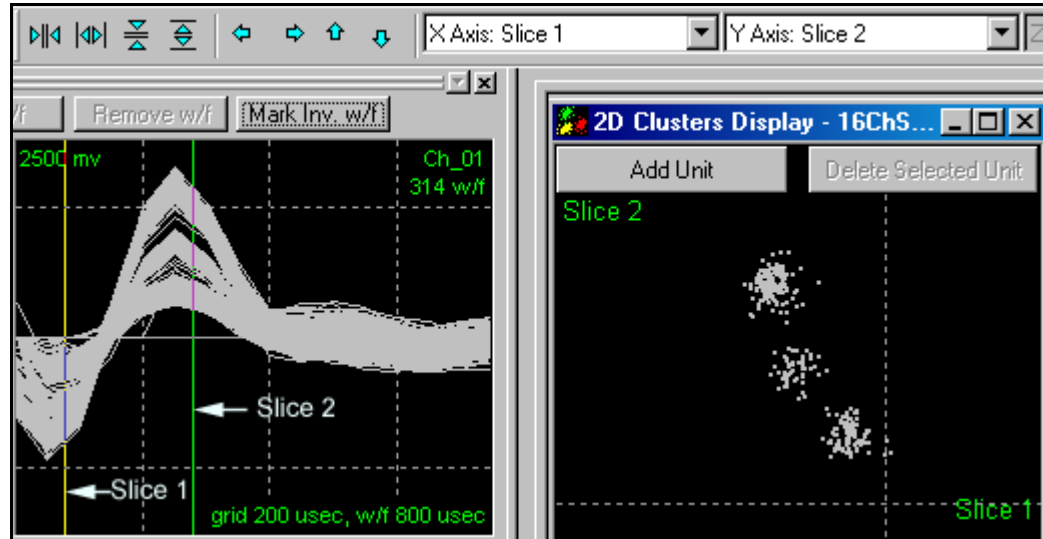
Click **Do not show this dialog again** to suppress the display of the **Principal Components** window, after selecting or clicking **Recalculate the Principal Components**. To re-enable the display click to clear the **Do not show the Recalculate Principal Components dialog** checkbox on the **Feature Calc** tab of the **Tools | Options** window.

5.5.2 Voltage at Specific Slices in Time

To view waveform voltages at specific points in time on the X, Y, or Z axis of the Clusters Views:

- Select **Slice 1**, **Slice 2**, **Slice 3**, or **Slice 4** for the X, Y, or Z axis using the **Drop-Down List Boxes** on the **Toolbar**.

- Adjust the time values for **Slice 1**, **Slice 2**, **Slice 3**, or **Slice 4** in the Control Grid. When visible, one can also drag the Slice 1, Slice 2, Slice 3, and Slice 4 lines into position with the mouse on the Waveform display.
- Right click the Clusters View and select **Redraw** in the pop-up menu.



- To update the Clusters View immediately when any of the slice n values are changed, select the **Redraw Cluster Display Immediately when Slice Position Changes** in the **Display** tab under the **Tools | Options** dialog box.
- By default, each channel “remembers” its slice positions independently. To have the same slice positions used across all channels, click **Use Same Slice Positions for all Channels** in the **Feature Calc** tab under the **Tools | Options** dialog box.

5.6 Details of the Sorting Algorithms

5.6.1 Feature Space Used for Sorting

The Semi-Automatic and Automatic sorting algorithms behave differently depending on how they are initiated:

- If the sorting is initiated from the 2D Clusters View, the Clusters vs Time View, or the Surface View (e.g. by pressing the **Find Units** button on the view-specific toolbar), the sorting is done in 2D feature space.
- If the sorting is initiated from the 3D Clusters View, the sorting is done in 3D feature space.
- If the sorting is initiated from the Sort menu on the main menu bar, then the radio buttons in the **When performing automatic sorting from menus, use** box in the **Tools | Options | Sort** tab controls whether the sorting is done in 2D or 3D feature space.

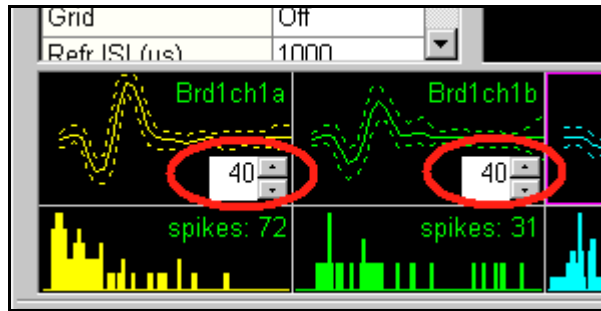
5.6.2 Template Algorithm

The Template matching sorting algorithm is designed to mimic the online real-time sorting performed by the Plexon MAP data-acquisition hardware.

For each waveform, the algorithm:

- 1 calculates the sum-of-squares differences from this waveform to all the templates:
- 2 finds the template with the minimum sum-of-squares difference
- 3 if the minimum difference is less than the Fit Tolerance for this template, the waveform is assigned to this template's unit.

One can adjust the Fit Tolerance (measured in units of 0.01% of the maximum A/D value) in the Units view:



Note: For Plexon MAP users: The MAP system hardware uses a slightly different scaling factor on the fit tolerance. To use Fit Tolerance values that will give identical behavior in Offline Sorter as they will on SortClient, check the **Use MAP-compatible Fit Tolerance** checkbox on the **Tools | Options | Templates** tab.

Let,

$$\text{waveform} = w = [w_1, \dots, w_N]$$
$$\text{template} = t = [t_1, \dots, t_N]$$

where

w_i and t_i values are in AD units (typically between -2047 and $+2048$)

N = # digitized data points

Note: For Plexon MAP users: The MAP system has 12-bin A/D conversion ($2^{12}=4096$). Therefore the AD values of waveform and template amplitudes are integers between -2047 and $+2048$.

The difference measure (d_w) between a waveform and a template is the square root of the mean sum of square error:

$$d_w = \sqrt{\frac{1}{M} \sum (w_i - t_i)^2}$$

where

M is the sort width for the current channel in terms of the number of data points

Σ represents a sum over i (the samples in the sort width)

For stereotrode and tetrode data, the sum is

$$S_u = \sqrt{\frac{1}{M} \sum \sum (w_i - t_i)^2}$$

where the first summation is over the electrodes.

The waveform is assigned to a template's unit if

$$\text{Template Fit Tolerance} < d_w$$

5.6.3 Adaptive Templates Algorithm

The adaptive template algorithm behavior is controlled by two parameters:

- the Weight
- the Update Threshold

which can be set on the **Tools | Options | Templates** tab:



To adapt the templates, Offline Sorter will process the spikes in the file in time-order. As it proceeds, it will maintain both the current template waveform being used for sorting $t(i)$, and an average waveform $s(i)$ for each unit, where i indexes the sample within the waveform. The average waveform $s(i)$ starts out equal to the initial template waveform $t(i)$ at the beginning of the file. For each spike waveform $w(i)$ that is encountered in the file and that is sorted into the unit under consideration (because it is within the fit tolerance of the current template $t(i)$), the adaptive template algorithm will add that spike waveform to the average waveform to get an updated average

$$s(i) = s(i)(1 - W) + w(i)W$$

using a weighting factor W that is the adaptive template Weight mentioned above.

Then the average waveform $s(i)$ is compared with the current template waveform $t(i)$ by calculating the sum-squared-difference between $s(i)$ and $t(i)$

$$d = \sum_{i=1}^N \frac{(s(i) - t(i))^2}{N}$$

where N is the number of samples in the waveform.

If the calculated quantity d is greater than the adaptive template threshold value, then a new template will be put into effect. The new template waveform $t(i)$ is set equal to the current average waveform $s(i)$. The timestamp of the waveform being processed when this occurs is recorded, as is the new $t(i)$, so that Offline Sorter can display a history of the template waveform changes made during adaptive template sorting.

Note that for small values of the adaptive template Weight W , the average waveform changes only very slowly when the waveforms trend away from the template, whereas for values of W near 1 the average waveform responds rapidly to shifting waveforms. Also, a small value of the adaptive template threshold will cause the algorithm to be eager to adopt new templates, whereas larger values will make the algorithm more reluctant to change templates.

5.6.4 K-Means Algorithm

The K-Means algorithm is described in any pattern recognition textbook. The algorithm runs in feature space, and requires starting positions for the clusters (and thus implicitly the number of clusters) to be specified before the algorithm begins.

Algorithm outline:

- 1 The user first sets initial cluster centers.
- 2 Each point is assigned to the cluster whose center is nearest, using a simple 2D or 3D Euclidean distance.
- 3 Cluster centers are then recalculated using the points assigned to each cluster, using a simple center-of-gravity method.

Steps 2 and 3 are repeated until the cluster centers no longer change.

When the K-Means algorithm converges, an outlier removal algorithm runs.

The standard deviation of the distances from each point to its cluster center computes. Any point that is more than a user-specified constant (OutlierThreshold)

times this standard deviation away from its cluster center is removed from the cluster.

In Offline Sorter, one can run the K-Means algorithm on unsorted data after the user has specified cluster centers, or it can be run to re-sort data, using the cluster centers of the existing clusters as a starting point. To run K-Means with existing centers, click **Sort** | **Continue Sort Current Channel** | **Continue Sort**.

References:

Webb, Andrew, *Statistical Pattern Recognition* Second Edition, John Wiley and Sons 2002
and many others...

5.6.5 Standard E-M Algorithm

The Expectation-Maximization algorithm is a widely-known iterative, parametric approach that fits a normal mixture model (a mixture of several Gaussians) to the available data points. It is so named because for each iteration the algorithm goes through two phases, an Expectation calculation followed by a Maximization phase. One can find the derivation of the equations for the algorithm in many sources, some of which are listed below.

The standard E-M algorithm requires the selection of the number of Gaussians to be fit (i.e. the number of units) and an initial starting assignment of points to clusters. In Offline Sorter, if a Continue Sort is being performed (See [“5.6.9.1 Continue Sorts” on page 203.](#)), the existing point assignments and number of clusters is used. If a normal Standard E-M sort is requested, the user is required to specify starting cluster centers, and a K-Means sort is used to get the initial assignment of points to clusters.

Offline Sorter actually implements a variant of the Standard E-M algorithm called REM-2, as discussed in the Sahani reference below. With this variant, a new parameter “beta” is introduced that influences the convergence of the E-M algorithm. In the Sahani reference, this beta parameter is systematically decreased as the algorithm progresses in an “annealing schedule”, which causes clusters to be “frozen out” as “phase transitions” are encountered at certain values of beta. When the beta value is set to 1.0, the REM-2 method becomes equivalent to the Standard E-M algorithm.

References:

Webb, Andrew, *Statistical Pattern Recognition* Second Edition, John Wiley & Sons 2002

Shahani, Maneesh *Latent Variable Models for Neural Data Analysis*, Doctoral Thesis, California Institute of Technology 1999

McLachlan and Peel, *Finite Mixture Models*, John Wiley & Sons 2000

5.6.6 Valley Seeking Algorithm

The valley seeking algorithm in Offline Sorter starts by calculating the number of neighbors that each point in feature space has. A neighbor is defined as a point that is within a certain critical distance of the target point.

The critical distance is given by

$$\text{Dist} = 0.25 * \text{sigma} * \text{Parzen Mult}$$

where sigma is the standard deviation of the distances of all points to the overall mean, and the Parzen Mult is a user-specified parameter so-named for its similarity to the Parzen density estimation kernel.

Note: Creating the neighbor counts inherently scales as N^2 , where N is the number of points. This fact limits the number of points that can be handled by this algorithm to several thousand before computing resources are overwhelmed.

The neighbor count for each point is then put into a list and the list is sorted so that the points with the most neighbors are at the top of the list.

Next, a set of seed points are found that are “local maxima” in that they have more neighbors than any of their neighbors do. The search for seed points starts at the top of the list sorted according to the number of neighbors, so the seed points are points near the centers of the densest clusters. Points with less than a certain minimum number of neighbors cannot become seed points.

Then, initial clusters are formed from each seed points and all of the seed point’s neighbors. Also, if any of the seed point’s neighbors are themselves seed points, their initial clusters are combined.

At this stage, the clusters consist of a circle (or sphere in 3D) of points centered around each density maximum with a radius of roughly the critical neighbor distance, with the other points unassigned.

The unassigned points are then assigned to clusters using a modified Fukunaga algorithm. This is an iterative algorithm; at each step, unassigned points are assigned to the cluster that the majority of its neighbors belong to. This continues until points stop being assigned or a maximum number of iterations is completed.

References:

Fukunaga, Keinosuke *Introduction to Statistical Pattern Recognition*, Chapter 11, Academic Press 1972

5.6.7 T-Distribution E-M Algorithm

A variant of the E-M algorithm by Shoham et al (see references that follow). This algorithm has two important features:

1) Instead of fitting a mixture of normal (Gaussian) distributions, T-Distributions are used instead. The T-Distribution is similar to a Gaussian, except that the tails

are “fatter”. This characteristic can enable E-M algorithms based on T-Distributions to better handle outliers in the data set.

2) The algorithm has built into it a way to automatically decide on the number of clusters. This feature is agglomerative in nature, as the algorithm starts with a large number of clusters and combines them together as the algorithm proceeds, so as to minimize a penalized likelihood function.

As discussed in the Shoham reference, the algorithm does not perform well when using the theoretical value for the number of parameters per component. Offline Sorter introduces a multiplier on this theoretical degrees of freedom value that can be adjusted. Empirically, values of around 20-30 produce the most pleasing clusters. Smaller values generally lead to too many clusters, larger values lead to too few. The Scan feature in Offline Sorter can perform sweeps over this sorting parameter, so its affect on sorting performance can be investigated.

The algorithm must be started with an existing clustering of the data, generally with too many clusters defined, as the algorithm can agglomerate clusters together as it proceeds. The initial clusters can either be taken from the currently-defined units (See [“5.6.9.1 Continue Sorts” on page 203.](#)), or they can be generated automatically. To automatically generate the starting configuration, the K-Means algorithm is used. One can set the number of cluster centroids used in this initial sort by using the **Tools | Options | Sort** dialog in the **Number of seed clusters for T-Distribution algorithm** edit box. The initial cluster centers given to the K-Means algorithm are evenly spaced along a diagonal line through the bounding box in feature space that contains all of the points to be sorted.

References:

Shoham, S., Fellows, M., and Normann, R. *Robust, automatic spike sorting using mixtures of multivariate t-Distributions*, Journal of Neuroscience Methods, 127(2), 111-122 2003

Figueiredo, Mario and Jain, Anil *Unsupervised Learning of Finite Mixture Models*, IEEE Transaction on Pattern Analysis and Machine Intelligence, vol. 25 pp.1150-1159, Sep 2003

5.6.8 Running Automatic Sorting on All Channels

As a convenience, Offline Sorter can automatically run the Valley-Seeking or T-Distribution E-M sorting algorithms sequentially on all channels in the current file. One initiate this operation by selecting the desired sorting algorithm from the **Sort | Sort All Channels** menu. The current values of all parameters that affect the sorting algorithms (the Parzen Multiplier, D.O.F. Multiplier, Outlier Threshold, waveform limiting options, etc.) are used to sort all channels. This is completely equivalent to manually loading each channel in turn and running the sorting on it. One can cancel the sorting operation by selecting **Sort | Stop Sorting** menu entry.

The **When performing automatic sorting on all channels** option in the **Tools | Options | Sort** dialog controls whether 2D or 3D feature space is used when sorting all channels.

5.6.9 Special Sorting

Normally, for the K-Means, Standard E-M, Valley-Seeking, and T-Distribution E-M sorting methods, initiating a Sort on a channel deletes all existing units and create new units according to the algorithm. However, other modes of operation are possible.

5.6.9.1 Continue Sorts

Some of the sorting algorithms require a set of starting units to be defined. One can normally do this by specifying cluster centers, or by running an initial K-Means sort. However, if units already exist, those units can themselves be used as the starting point for sorting. This is called “Continue sorting” for lack of a better name. One can use the K-Means, Standard E-M, T-Distribution E-M, and (trivially) the Template sorting algorithms for a Continue sort.

To initiate a Continue sort, select the desired sorting algorithm from the **Sort | Continue Sort** menu.

For Template sorting, a Continue sort is the default, in a manner of speaking. The nature of the Template sorting algorithm is such that all waveforms are sorted against all of the available units.

Also, one can do Continue sorts for all channels by selecting an algorithm from the **Sort | Continue Sort All Channels** menu.

5.6.9.2 Partial Sorts

When the sorting algorithms are run when not all the waveforms are visible in the Clusters Views (because the **Use Only Currently Visible Waveforms** option is in use and the waveforms being displayed is being limited by the **Waveform View** setting or by the **Control Displays** checkbox on the Timeline View), the behavior of the sorting algorithms changes. All existing units are preserved, and the sorting algorithm is only allowed to run on the currently visible waveforms. This generally results in some number of new units in addition to the originally present units. The final step in the sorting process is to remove any units that are empty (units with no waveforms in them).

One can also do Continue sorts when only some of the waveforms are visible.

5.7 Sorting Quality Statistics

There is no universally agreed upon way to measure sorting quality, so Offline Sorter can calculate and display a number of sort quality metrics. It can also calculate pair-wise cluster separation statistics, and also other supporting statistical data such as measures of normality of distributions.

The following sections describe the sort quality metrics and other statistics available in Offline Sorter. One can view these values on the [Sorting Summary View](#), which is detailed on [page 79](#), or on the **Stats** view of the [Info Grid Views](#), which is detailed on [page 34](#). Also, one can choose one of the sorting quality metrics to be displayed on the top of the [Clusters Views](#), which are detailed on [page 43](#), where it can be used to give immediate feedback on the effectiveness of sorting decisions. See “[4.7.2.2 Statistics Feedback While Sorting](#)” on [page 159](#).

5.7.1 MANOVA F and p-values

The degree to which the selected unit clusters are separated in the 2D and 3D Clusters Views is determined by a **Multivariate Analysis of Variance (MANOVA)** test. The Null hypothesis in the MANOVA is that all clusters actually come from the same underlying statistical distribution in 2D or 3D space. The p-value is the probability that an error is committed by rejecting the null hypothesis. Therefore, a small p-value indicates that each of the unit clusters has a statistically different location in 2D/3D space, and that the clusters are statistically well separated.

After clusters are selected in 2D or 3D cluster space, one can view the resulting F and p-values derived from the MANOVA test in two and three dimensions in the Stats Spreadsheet in the [Info Grid Views](#), which is detailed on [page 34](#), and in the Clusters View; see “[3.7.1 Clusters View Common Functionality](#)” on [page 45](#).

5.7.1.1 Some details on the MANOVA calculation

In the jargon of MANOVA, the MANOVA “number of groups” is our number of units, and the MANOVA “dependent variables” (DV) are our feature vectors (e.g. principal components). This analysis is that of a single “Factor” experiment, with the “Treatment” being the unit assignments.

The MANOVA F-statistic is calculated using a ratio of determinants to get Wilk's lambda, followed by Rao's method for converting Wilk's lambda into an F value. See “Applied Multivariate Statistics for the Social Sciences”, 2nd Edition by James Stevens, page 191 for more details.

An unweighted MANOVA is performed, in that all groups (regardless of the number of waveforms they contain) contribute equally to the F-statistic calculation, so as not to bias the MANOVA calculation toward rapidly firing units. See “Statistical Principles in Experimental Design”, 2nd Edition by B.J. Winer, page 232.

The conversion of the F-statistic to a p-value is done via a calculation of the incomplete beta function (See “Numerical Recipes in C”, section 6.2), assuming a single-tailed probability distribution.

Including or not including the unsorted units in the ANOVA calculation:

The **Include unsorted waveforms as a unit in the statistics calculations** checkbox in the **Stats** tab in the **Tools | Options** dialog box controls whether the unsorted waveforms are treated as a unit in the statistics calculation, or simply

ignored. The text in parenthesis on the **Multivariate ANOVA** line indicates the current setting for this option. For additional information, see [“5.7.1.1 Some details on the MANOVA calculation” on page 204](#).

Note: The multivariate ANOVA test requires a minimum of 2 clusters, one of which can be the unsorted waveforms—if they are included as a unit. Also, it does not make sense to run the ANOVA test if any unit is defined that contains zero waveforms (again, including the unsorted waveforms if they are included).

Interpretation of the Multivariate ANOVA test

This section includes information on the interpretation of the Multivariate ANOVA tests. The test results appear in the lower half of the **Stats** view.

Assumption: The distributions of points is normally distributed in 2D or 3D cluster space.

The Null hypothesis is: All unit clusters actually have the same underlying distribution in 2D or 3D space and have the same mean (i.e. do not represent distinct spike waveforms).

The p-value is: The probability of rejecting the null hypothesis (that all clusters are actually the same) if it were actually true.

Conclusion: The smaller the p-value, the more confident one can be in rejecting the null hypothesis and concluding that the clusters are in fact distinct and represent different units.

5.7.2 J3

J3 is a non-parametric measure of the quality of sorting (i.e. no normality assumptions are involved).

$J3 = J2/J1$, where $J1 = \sum \sum E(f(u, i) - m(u))$ 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 ($\bar{x} * x$). The summations are over units u , and over feature vectors (points) in each unit i .

$J2 = \sum N(u)E(m(u) - m)$ 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.

References:

Wheeler, Bruce C., *Automatic Discrimination of Single Units in Methods for Neural Ensemble Recordings*, ed. by Nicolelis, M., CRC Press, Boca Raton, 1999

5.7.3 Pseudo-F

The Pseudo-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.

$$\text{Pseudo-F} = (N-g) / (g-1) J3$$

where N is the total number of waveforms, and g is the total number of units.

5.7.4 Davies-Bouldin

The Davies-Bouldin validity metric is calculated as:

$$DB = \frac{1}{N} \sum_i \max_{j \neq i} \left[\frac{(d'(i) + d'(j))}{d(i, j)} \right]$$

where $d(i, j)$ is the Euclidean distance between the centroids of unit i and unit j , and $d'(i)$ is the average distance of each point in unit i to the centroid of unit i . The sum is over all N units, and the max is taken over all units $j \neq i$.

Because the inter-cluster distance $d(i, j)$ is in the denominator, the Davies-Bouldin validity statistic takes on small values for well-separated unit clusters. The Davies-Bouldin validity statistic is a non-parametric measure.

5.7.5 Dunn

The Dunn validity metric is calculated as:

$$\text{Dunn} = \min_i \left[\min_{j \neq i} \left[\frac{d(i, j)}{\max_k (d'(k))} \right] \right]$$

where $d(i, j)$ is the Euclidean distance between the centroids of unit i and unit j , and $d'(k)$ is the average distance of each point in unit k to the centroid of unit k . The outermost min is taken over all units i , the inner min is taken over all $j \neq i$, and the max is taken over all units k .

The Dunn validity index assumes a maximum value for clusters that are compact and well-separated.

5.8 Playing a Movie of the data

A useful technique with Offline Sorter is to “play back” the data in a file in time order. This is useful in determining whether waveform shapes are drifting as a function of time.

One can make Offline Sorter automatically advance time in the Timeline View by using the operations under the **View | Animate** menu. The animation functions are completely equivalent to changing the time range by using the scroll bars on the Timeline View. The animation operations include:

Play: starts animating the playback of the file, but defaults advancing time at the normal rate of 1 second per second

Pause: pauses the animation; select it again to un-pause

Stop: stops the animation

Slower: slows down the rate of animation by a factor of $\frac{1}{2}$

Faster: speeds up the rate of animation by a factor of 2

Reset to 1X Speed: resets to the normal animation rate (1 second per second)

Rewind: resets to the beginning of the file

Reverse: a toggle that makes the rate of time passage negative (e.g. time flows backwards)

Loop: when checked, the animation resets to the beginning of the file when the end of the file is reached

To make operation much easier, one can bind keystrokes to these keys by using the **Keyboard tab** under **Windows | Customize...** menu item. Also, there is a toolbar that includes icons for these functions, but this toolbar is not displayed by default. To display this toolbar, select **Toolbars tab** under **Windows | Customize...** and click **Animation** in the **Toolbars** list.

One can increase the usefulness of the animation features by configuring the Waveform and Clusters Views to only show the waveforms within the current time range, thus what they display changes while the animation is in progress. One can accomplish this with the **Control Displays** checkbox in the Timeline View. If these checkboxes are checked, the Clusters Views or the Waveform View, or both, show only those waveforms that appear in the Timeline View. One can use the **Zoom In** and **Zoom Out** buttons on the Timeline View to control how many waveforms are visible in each “frame” of the animation.

Several commercially available screen capture packages are capable of making MPEG movie files of any on-screen activity, including the Offline Sorter displays.

5.9 Examining Events

Although not strictly relevant to spike-sorting, Offline Sorter offers the ability to examine the events in data files. The **Events** view of the **Info Grid View** lists the events present in the file by name and channel number, with a **Count** of the num-

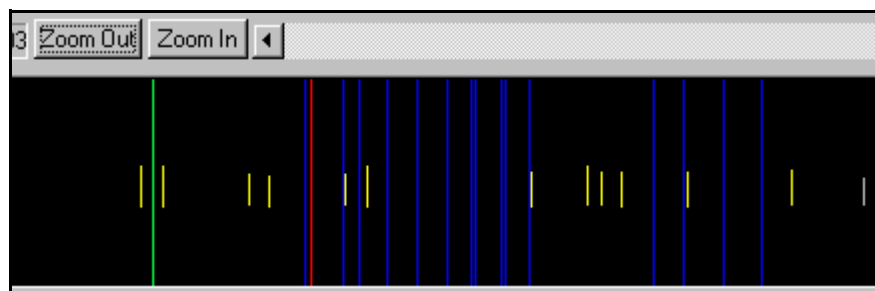
ber of times that events appears, along with a **Show** checkbox and a display color for each.

	Event Ch	Name	Count	Show	Display Color
1	3	FO	6	<input checked="" type="checkbox"/>	Dark Blue
2	4	VB	7	<input checked="" type="checkbox"/>	Teal
3	6	WN	7	<input checked="" type="checkbox"/>	Yellow
4	7	Lick	182	<input checked="" type="checkbox"/>	Blue
5	8	Water	10	<input type="checkbox"/>	Pink
6	258	Start	1	<input type="checkbox"/>	Red

Channels Chan Params Sort Details Global **Events**

Info 2D Clusters

When the **Show** checkbox is checked for an event, Offline Sorter plots each occurrence of that event as a line of the corresponding **Display Color** on the Timeline View and on the Rasters View, so that one can inspect the temporal relationships between events and nearby spikes. In the Timeline View, the full-height lines are the events, the short lines are spike waveforms.



One can quickly adjust the settings in the **Show** and **Display Color** columns by using **Set As Top Row** in the right-click context menu. The **Randomize Colors** entry in the context menu generates a random color for each different event.

Several of the data formats other than Plexon PLX files have an information type comparable to events (e.g. Datawave Markers). Wherever possible, these information records are translated into events when the data file is loaded.

Plexon PLX files also have the concept of strobed events. Strobed events have an event channel number of 257, but have an additional data fields that stores a data word. In Offline Sorter there is an option that controls the treatment of strobed events. If the **Create Separate Events for All Strobed Codes** option in the **Plexon Files** tab of the **Tools | Options** dialog box is unchecked, all strobed events simply show up as events with a channel of 257. When the **Create Separate Events for All Strobed Codes** option is checked, the strobed events are “expanded” into separate events for each possible data word value. The strobed events are given a name corresponding to the value of their associated data word,

the format of which can be controlled by using the **Name Generation Prefix** and the **Code Representation** options.

Note: These options also control how strobed events are exported into NEX files.

To accommodate downstream data analysis, Offline Sorter can create event occurrences when selected units fire. Click **Tools | Create Events at Unit Firing Times** to specify a Unit and an Event ID for the new event. One can display newly-created events in the Timeline View or the Raster Views, and one can save the events to a PLX file by using the **Export to New .PLX** option.

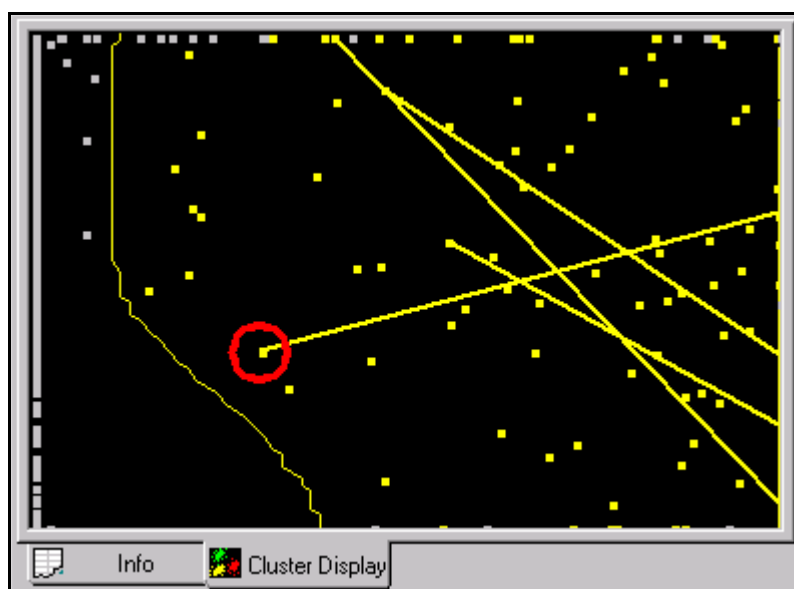
5.10 Manual Short ISI Spike Removal

If a relatively small percentage of spikes have ISIs less than the refractory period, one can manually remove the spike in each pair which is further away from the cluster center.

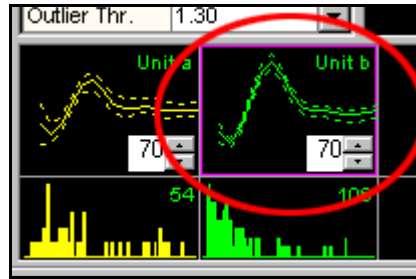
First, follow the procedure outlined in [“4.6.4 Removing Spikes with Short Inter-spike Intervals” on page 152](#) to get the lines drawn to connect pairs of spikes with short ISIs.


To remove a waveform from a selected unit

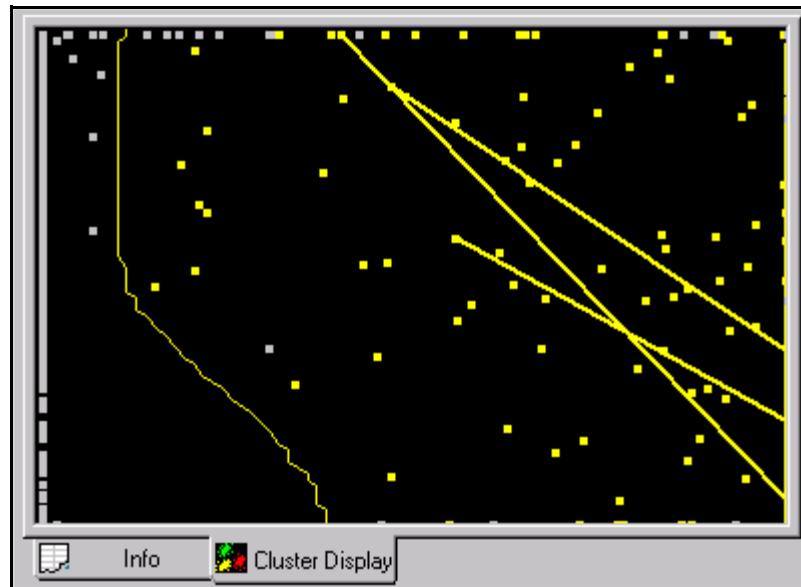
- 1 Zoom in to the waveform's dot (circled below) in the Clusters View using the toolbar control buttons.



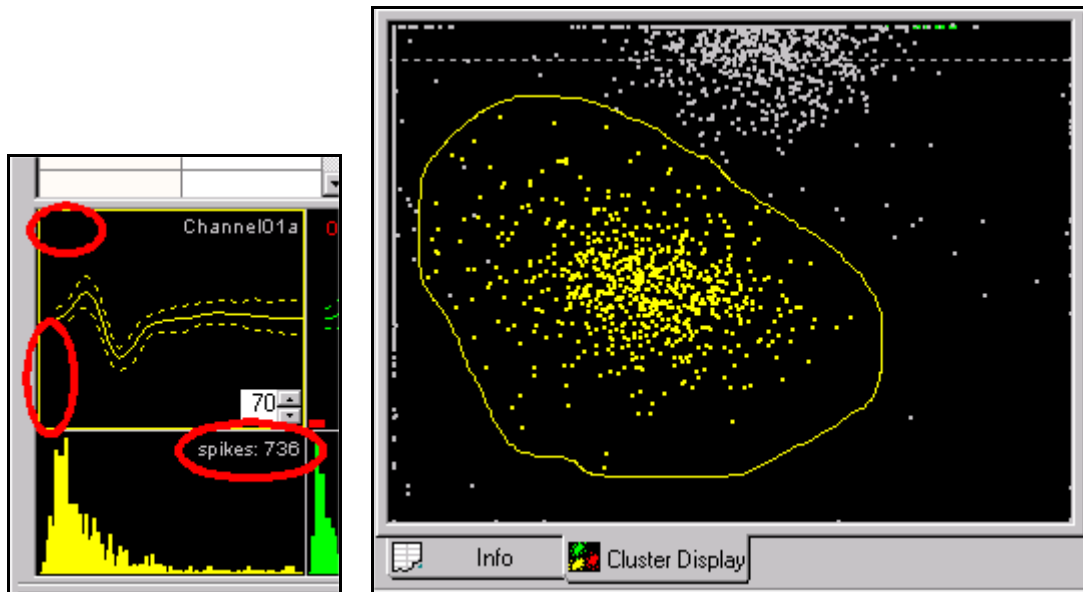
- 2 Select the unit: click at the unit's template display.



- 3 Select **Units | Remove Waveforms from the Selected Unit** menu command.
- 4 Move the mouse pointer to the Clusters View (note that the pointer changes to a “drawing hand” ).
- 5 Press the left mouse button and draw a line around the waveform's dot in feature space while pressing the left button.
- 6 Release the mouse button.



One can repeat this procedure until all the waveforms furthest away from the cluster center with ISIs less than the refractory ISI have been removed:



If a waveform is removed by accident, one can add it to the selected cluster again within the Clusters View.

To add units

- 1 Select the unit: click the unit's template display.
- 2 Select **Units | Add Waveforms to the Selected Unit** menu command.
- 3 Move the mouse pointer to the Clusters View.

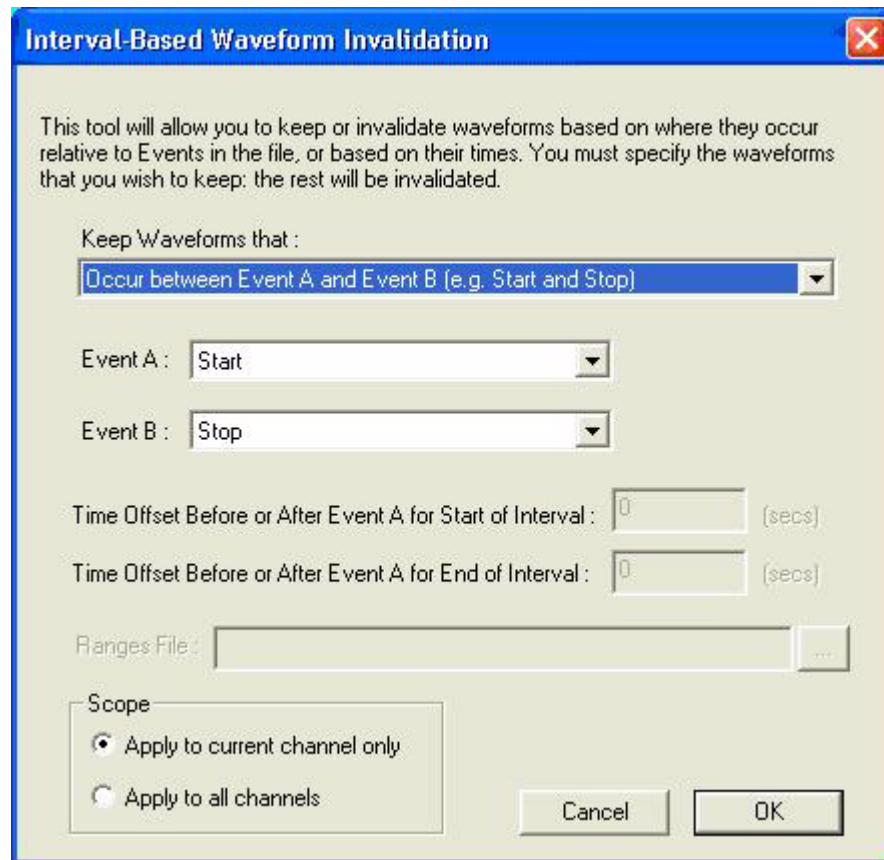
The pointer changes to a blue drawing hand  for adding waveforms.

- 4 Click the left mouse button and drag a line around the units to be added.

5.11 Interval Selection

A valid reason to mark waveforms invalid is that they are simply not of interest. For example, if the experimental animal was not exhibiting “interesting” behavior during certain time intervals, one can invalidate the waveforms collected from that interval simply to get them out of the way. Offline Sorter contains a tool that enables the selection of waveforms within some interval in time around an event (or between events) in the data file. Or more accurately, it enables one to invalidate those waveforms that do not meet the selection criteria (those that are not in

the interval). Select **Tools | Interval Selection** from the main menu to display this window:



First, select the desired action in the **Keep Waveforms that** droplist. The rest of the controls on the dialog either enable or disable as is appropriate for the droplist selection. The **Keep Waveforms that** droplist selections are:

- **Occur between Event A and Event B (e.g. Start and Stop)** – This selection enables both the Event A and Event B droplists, and shows a list of all the event names that occur in the data file. Pick the Event A that marks the beginning of the desired waveforms, and pick the Event B that marks the end. The waveforms that are not between Event A and Event B are invalidated.
- **Occur within a time span around Event A** – This selection enables the Event A droplist as well as the time offset edit boxes. Select the Event in the Event A droplist and enter the number of milliseconds before and after the event for which waveforms are to be kept.
- **Occur outside of a time span around Event A** – This selection is the logical opposite of the previous option. All waveforms are kept except those that occur within the specified time span.
- **Occur between a time offset from Event A and a time offset from Event B** – This selection enables both the Event A and Event B droplists, as

well as the offsets. This mode is like the first option, except the interval between the start and stop events extends by a time offset. The waveforms are kept if they occur between the time of Event A plus the first offset and the time of Event B plus the second offset.

- **Occur within an arbitrary range of timestamps** – With this selection, no events need to be selected, only the beginning and end of a time interval (as measured from the beginning of the file - timestamp 0) needs to be specified. All waveforms that occur *outside* of this time range are invalidated.
- **Occur outside of an arbitrary range of timestamps** – With this selection, no events need to be selected in this case, only the beginning and end of a time interval (as measured from the beginning of the file - timestamp 0) needs to be specified. All waveforms that occur *within* this time range are invalidated. This is the inverse of the above option.
- **Occur outside of time ranges read in from a file** – This item selects and reads a text file that contains ranges of times to invalidate. Each line contains a channel number followed by the start and end times for the invalidation range, which are separated by a space, tab, or comma. The times are specified in seconds, from the beginning of the file. A special value of -1 for the channel number is interpreted as meaning “apply to all channels”.

One can choose to invalidate just the current channel, or all channels in the file. One can make this selection by using the radio buttons under Scope in the dialog box. If Offline Sorter reads the ranges in from a text file, it determines the channels affected based on the contents of the Ranges file.

As soon as the **OK** button is pressed, the filtering takes place. One can undo the operation by using the **Undo** mechanism, or by selecting the **Waveforms | Mark All Waveforms Valid** menu selection.

5.12 Managing Unit Templates and Fit Tolerances

Depending on how Offline Sorter is used, the unit templates that appear in the Units View may or may not be of importance. Offline Sorter allows some degree of control over how and when these unit templates are updated and used.

Besides being displayed in the Units View, the unit templates also appear numerically in the **Templates** tab of Info Grid View.

By default, Offline Sorter always recalculates the unit template for a unit after any change to the sorting is made. Offline Sorter calculates the unit template for a unit by averaging together all of the waveforms in that unit. It will also recalculate the unit templates when a file is first loaded. However, this can be slightly confusing when loading a .plx file that was collected by the Plexon MAP system using template sorting. The recalculated unit templates in Offline Sorter may be slightly different than the unit templates used by the MAP system for online sorting.

One can disable the automatic recalculation of unit templates by clicking to clear the **Units | Automatically Recalculate Unit Templates after Each Change** menu item, or by clicking to clear the **Automatically Recalculate Unit Templates** checkbox on the **Sort** tab of the **Tools | Options** dialog box. In this case, the Unit Templates are only recalculated when the user explicitly requests it by selecting **Units | Calculate Unit Templates from Existing Units**, or clicks the **Recalculate Templates** button in **Templates** tab of the Info Grid View. Be aware that this can lead to confusing displays, because sometimes unit templates may not exist until they are explicitly calculated, and will show up as flat lines (for example, after performing a Valley-Seeking sort).

Note: Note that the unsorted 'unit' is not affected by the automatic recalculation setting – the unit template for the unsorted pseudo-unit is always recalculated after any sorting change.

Offline Sorter can also calculate a Fit Tolerance for each existing unit, by finding the smallest Fit Tolerance value that would result in all of the points in a unit to be sorted into that unit during a Template sort. By default, recalculating the Fit Tolerances is only done when the user explicitly requests it by selecting **Units | Calculate Fit Tolerances from Existing Units**, but it can also be done automatically after any change to the sorting is made by clicking to clear the **Units | Automatically Recalculate Fit Tolerances after Each Change** menu item, or by clicking to clear the **Automatically Recalculate Fit Tolerances** checkbox on the **Sort** tab in the **Tools | Options** dialog box.

If the option to automatically recalculate the Fit Tolerance is not selected, the initial fit tolerance given to a newly-created unit can be specified via the **Initial Template Fit Tolerance** setting on the **Sort** tab of the **View | Options** dialog box.

Note: Even immediately after the Unit Templates and the Fit Tolerances are calculated from the existing units, performing a Template sort may not accurately reproduce the current sorting.

5.13 Template Files

5.13.1 Saving Templates

As discussed above in the *Spike Sorting Step-by-Step* chapter, after selecting units by using the manual or automatic clustering methods, a template can be used to resort the spikes. One can save the following items in a TPL text file:

- templates
- contours
- tolerances
- sort widths
- feature selections
- principal components

-
- thresholds, filters, and detection parameters for continuous data files

One can then use the saved information to perform sorting on other data files or export it to other analysis programs. Sorting using saved templates is useful for files recorded in succession with the same number of channels, gain on each channel, and global sorting parameters (waveform length and prethreshold time).

If Contour sorting is used to sort a channel, Offline Sorter saves the contour information in the TPL file. Offline Sorter also saves the sort method used for the channel in the TPL file, so some channels can be resorted using templates and some with contours.

If waveform extraction has been performed on a channel with continuous data, Offline Sorter saves the extraction parameters in the TPL file. Likewise, if alignment was performed on the spikes, Offline Sorter saves the alignment parameters.

The **Sort Details** view of the **Info** tab shows what sorting has been performed on which channels using which sorting parameters, and it is a good indication of what will be written to the TPL file.

To save the information in a TPL file

- 1 Sort each channel using templates—see [“5.6.2 Template Algorithm” on page 197](#)—(adjust template tolerances, set the sort width, etc.) or using the Contours method.
- 2 Select **File | Save Sort Info to .tpl** menu command.

The TPL file contains information only for the channels that have been sorted.

Note: The only sorting methods that can be captured in a TPL file are Template and Contours. If a channel was sorted using another method (e.g. Valley-Seeking), one can approximately capture the results into a TPL file as a template sort. To do this, one needs to have Offline Sorter adjust the unit templates and fit to frames such that the results of the template sort approximate the desired sorting. For more information, see [“5.12 Managing Unit Templates and Fit Tolerances” on page 213](#).



CAUTION

Uncaptured Operations

One can perform sequences of operations on a channel that cannot be captured in a TPL file. Thus, the same operations are not exactly duplicated when a saved TPL file is re-applied. Examples of operations that are not saved in TPL files include:

- any operation that invalidates waveforms (e.g. removing outliers)
- multiple applications of alignments or sorting operations. Only the last operation is saved to the TPL file.
- manually adding or removing waveforms from clusters.

5.13.2 Sort Using Saved Templates

One can sort both continuous data files and spike/waveform data files using templates saved in TPL files. To sort a file using saved templates, first open the continuously digitized data file, or open the spike/waveform data file—see [“4.2.1 Opening the Digitized Data Files” on page 91](#).

To select the .tpl template file

- Select **File | Sort Using .TPL file** menu command, or,
select **Sort | Sort Using .TPL file** menu command.

The templates, tolerances, contours, feature selections, extraction and alignment parameters, and sort widths that were saved for each channel in the TPL file are then used by Offline Sorter to sort the current data file.

For continuously digitized data files, threshold, low-cut filter, detector type (as described in [“4.2.6 Specifying the Waveform Detection Options” on page 99](#)) settings are also saved in the TPL file for each channel and applied to the new file to extract the waveforms for sorting.

When a channel is sorted using a saved TPL file, the order of operations is:

- 1 extract the waveforms using the saved parameters (for continuous data channels only)
- 2 perform alignment on the spikes (if indicated by the saved TPL file)
- 3 perform either template or contour sorting on the spikes

5.13.3 Save Templates to a Sort Client EXP File

Note: This feature is only relevant for users of the Plexon MAP system.

The following scenario can occur: A Plexon MAP system collects data from an experimental animal, using the settings contained in a Sort Client experiment (EXP) file. Among other settings, the EXP file contains the templates for the units that the MAP system hardware sorts in real-time. After the data-taking session, Offline Sorter reads in the PLX file collected during the experiment and “cleans up” the real-time sorting done by the MAP system. This is sometimes necessary because units can appear or disappear and waveform characteristics can drift over the course of the experiment. Suppose that the same experimental animal is to be connected to the MAP system the next day for another session. It is preferable to use the cleaned-up unit templates in the MAP system instead of the original unit templates. With this feature of Offline Sorter, one can save the templates resulting from re-sorting done during a clean up session into a Sort Client EXP file for use by the MAP system.

The way in which this feature works is to read the original Sort Client EXP file, replace the unit templates in it with the new templates resulting from the sorting done within Offline Sorter, and re-write everything to a new EXP file. Offline Sorter saves all of the settings in the original EXP file into the new EXP file, except that it replaces the unit templates for some channels.

To use this feature, one must sort channels using the template method—see “5.6 Details of the Sorting Algorithms” on page 196. Note: *only unit templates on those channels sorted with the template method are saved to the EXP file* -- Offline Sorter ignores all channels not sorted or those sorted using a different method. Then select **Sort | Update an .EXP File with Sort Info** from the main menu. In the first file dialog that appears, select the original EXP used by Sort Client to collect the current PLX file. In the second dialog, select the name of a new EXP file (it must be different from the original). Offline Sorter then writes the EXP file. Then, load the new EXP file into Sort Client for the next data-taking session.

5.14 Parameter Scans

Offline Sorter contains a tool to explore different sorting scenarios by scanning the values for parameters that affect the sorting over a range of values, re-running the sorting and recording the Sorting Quality Statistics at each step.

Select the **Scan** tab under the **Info** tab to display the Parameter Scan grid view:

Parameter Scan for Channel 1							
Type:	Scan Parzen Radius Multiplier for Valley-Seeking						<input checked="" type="checkbox"/> Use 3D
							<input checked="" type="checkbox"/> Normalize
Start:	0.500000	End:	2.500000	Step By:	0.200000	<input type="button" value="Start Scan"/>	
Scan Val	#Units	J3	Pseudo-F	D-B	Dunn	p	Sorted %
0.5	5	1.48502	3.87599	-0.518975	0.940562	30.303	84.7585
0.7	4	1.29533	3.86284	-0.444593	1.07657	13.2002	88.3546
>> 0.9	5	1.12427	3.79867	-0.434567	0.986281	36.3264	93.686
1.1	5	1.00179	3.75751	-0.448395	0.9388	34.5066	95.6304
1.3	5	0.920033	3.72601	-0.480771	0.880151	32.0264	96.843
1.5	5	0.886345	3.71271	-0.487693	0.860754	30.4831	97.4911
1.7	4	0.657856	3.61304	-0.417802	0.950355	15.2479	97.8674
1.9	4	0.622359	3.59126	-0.420817	0.935777	13.4549	98.3901
2.1	2	0.465655	3.64331	-0.449213	1.01862	0.970401	98.7874
2.3	2	0.444975	3.62542	-0.455425	1.011	0.918988	99.2055
2.5	2	0.435953	3.61798	-0.457899	0.996926	0.889621	99.54

	Scan Val	#Units	
Best by J3:	0.5	5	<input type="button" value="Re-sort Using This Value"/>
Best by Pseudo-F:	0.5	5	<input type="button" value="Re-sort Using This Value"/>
Best by D-B:	1.7	4	<input type="button" value="Re-sort Using This Value"/>
Best by Dunn:	0.7	4	<input type="button" value="Re-sort Using This Value"/>
Best by p:	0.9	5	<input type="button" value="Re-sort Using This Value"/>

One can do several different types of scans, which can be selected from the **Type** droplist. For each type, click **Start Scan** to cause the procedure indicated by the Type droplist to be performed at each step of the parameter over the range specified by the **Start**, **End** and **Step By** values. At each step, the Sorting Quality Statistics are calculated and displayed in a table, along with the number of units and percentage of waveforms sorted. By default, Offline Sorter sorts and calculates

the Sorting Quality Statistics in 2D feature space, but the 3D feature space can be used instead by checking the **Use 3D** checkbox. When the scan completes, the “best” values of the parameter being scanned according to each of the Sorting Quality Statistics is displayed in a table. To re-sort the channel with any of these best values, click **Re-Sort Using This Value** on the line for the statistic. Also, double-click on any line in the scan table to re-run the procedure using that value of the scanned parameter.

For some of the sort quality metrics, smaller values are better, while for others, larger values are better. And, the magnitude of the numbers calculated varies widely, which makes it difficult to plot different metrics on the same graph (see the table below). To address these problems, Offline Sorter can also calculate and display a second, ‘normalized’ version of each sort quality metric. The normalized version of a sort quality metric is just a simple mathematical function on the original sort quality metric:

Original	Normalized	Comment
J3	J3/#Units	dividing by the number of units
PsuedoF	log(PseudoF)	taking the logarithm
Davies-Bouldin	-(Davies-Bouldin)	flipping the sign
Dunn	Dunn	no change
p-Value	-log(p-Value)	taking the negative logarithm

For the normalized sort quality metrics, larger numbers are always better, and the range of values is generally within an order of magnitude or so of unity.

To enable the calculation and use of the Normalized version of the sort quality metrics, check the **Normalize** checkbox.

Note: Other settings in Offline Sorter can also affect the results of the scan. For example, the **Include unsorted waveforms as a unit in the statistics calculations** in the **Tools | Options Stats** tab affects all the Sorting Quality Statistic values.

The available scan types are:

- Scan Parzen Radius Multiplier for Valley-Seeking: This scan type steps the Parzen Mult. parameter (as can be set via the Control Grid) for the [Automatic Clustering using Valley-Seeking Method \(page 142\)](#) sorting algorithm over the range specified by the **Start**, **End**, and **Step By** values, re-running the sorting each time.

Note: The **Limit Number of Waveforms used in Valley Seeking Sorting** setting in the **Tools | Options Sort** tab can affect the clustering.

- Scan number of clusters for K-Means: This scan type runs the [Semi-Automatic Clustering using K-Means \(page 137\)](#) sorting algorithm, scanning the number of clusters over the range specified by the **Start**, **End** and **Step By**

values. The initial cluster centers are automatically arranged along a diagonal line through the feature space origin for each step. This procedure is questionable for two reasons:

- The results of the K-Means algorithm can be sensitive to the initial cluster centers, and the algorithm used to place the centers is not the optimal choice for the center locations.
- The sorting quality statistics sometimes cannot be compared “apples-to-apples” across different numbers of clusters, which can cause the best values to be at the extremes of the scanned range.

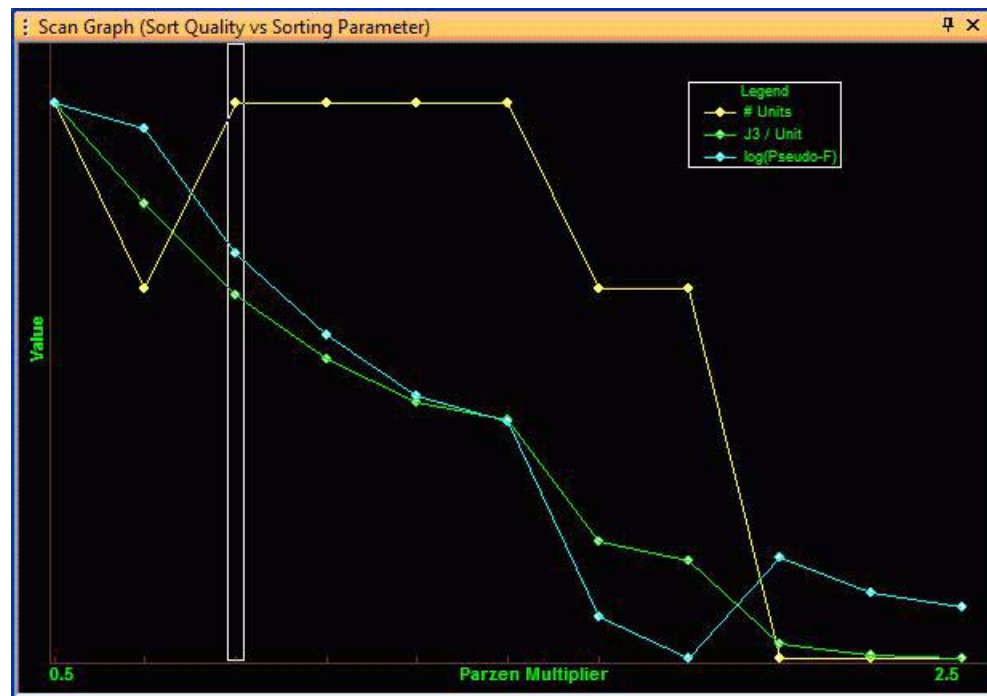
Note: The current setting of the **Outlier Threshold** in the Control Grid is used in the K-Means sorting at each step.

- Scan Outlier Threshold: This scan type runs the Remove Outliers tool (See [“4.6.3 Cleaning the Selection” on page 150.](#)), scanning the Outlier Threshold over the range specified by the **Start**, **End**, and **Step By** values. At each step, the sorting resets to the initial state, that is, the effects of the Remove Outliers tool are undone after each step. However, when one clicks **Re-Sort Using This Value** or double-clicks on a scan table line, remember to undo the action before it is repeated, or the Remove Outliers tool is applied to the results of the last operation, which is not the same initial condition that was in effect during the initial scan.
- Scan Waveform Limit for Valley-Seeking: This scan type runs the [Automatic Clustering using Valley-Seeking Method \(page 142\)](#) sorting algorithm while stepping the waveform limit over the range specified. The waveform limit, which can be set in the **Tools | Options Sort** dialog box, limits the number of points used by the Valley-Seeking sorting algorithm. A typical scan range for this type of scan might be 1000 to 10000 in steps of 1000. This is useful way to get a feeling as to how the number of points can affect the values of the sort quality metrics.
- Scan D.O.F. Multiplier for T-Dist E-M: This scan type runs the [Automatic Clustering using T-Distribution E-M Method \(page 144\)](#) sorting algorithm over the range of D.O.F. Multipliers specified. The D.O.F. Multiplier affects the sorting as described in [Details of the Sorting Algorithms \(page 196\)](#).
- Scan Number of Clusters for Std E-M: This scan type runs the [Semi-Automatic Clustering using Standard E-M \(page 141\)](#) sorting algorithm, which scans the number of clusters over the range specified. Offline Sorter automatically sets the cluster center positions by using the same procedure previously described for the “Scan number of clusters for K-Means”, and the same caveats apply.
- Number of Waveforms To Use In PCA: This scan type re-runs the PCA using only the first N waveforms, where N is the step value. Then Offline Sorter recomputes the sort-quality metrics. Although the unit assignments are not altered, re-running the PCA changes the positions of points in PCA-based

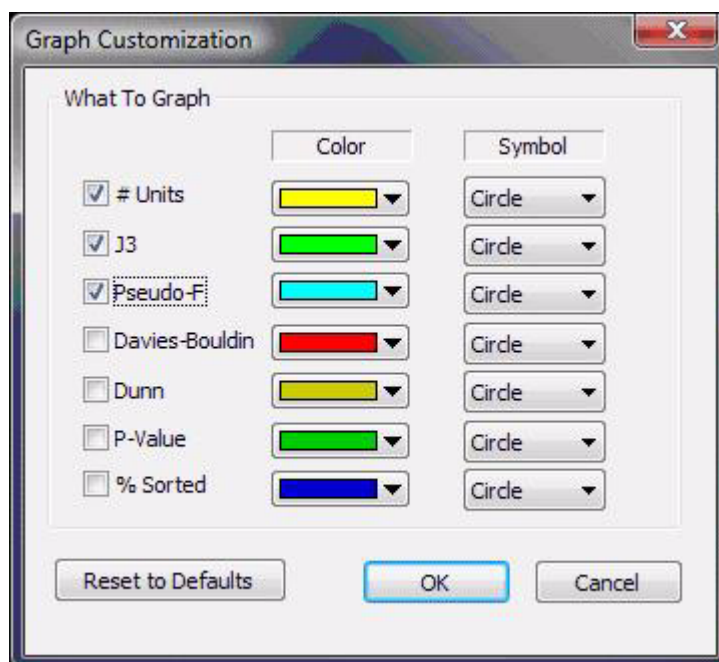
feature space, and so the sort quality metrics can change. This feature is useful to examine the sensitivity of sorting results to different PCA results.

5.15 Scan Graph View

Offline Sorter also offers a graphical view of the scan results. The Scan Graph View can be displayed by selecting **View | Scan Graph** from the main menu, or pressing the corresponding toolbar button.



The Scan Graph View shows a simple graph of some sort quality metrics versus the scanning parameter (the Parzen Multiplier in the above screenshot). The values of just one or of all sort quality metrics can be plotted on the Scan Graph View, this is controlled by selecting **Customize Display** from the right-click menu:



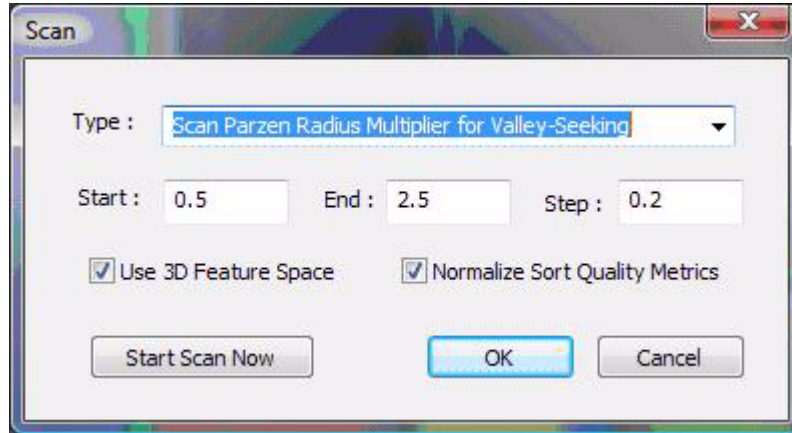
Checking the checkbox next to a sort quality metric will display that metric as a series of symbols connected by a line on the graph. The color of the symbols and line can be selected, as can the shape of the symbol. Pressing the **Reset to Defaults** button will reset all values to the factory default values.

Offline Sorter has the concept of a ‘sorting’, which is the unit assignment for every waveform in the channel. At each step of the parameter scan, Offline Sorter remembers the resultant sorting. Left-clicking in the Scan Graph View or on a result row in the Scan View will select the corresponding sorting and make it current, and all other views will change to show that sorting. The last selected sorting is shown by drawing a white rectangle in the Scan Graph View, and by showing a ‘>>’ in the row header in the Scan View. In effect, the selected sorting becomes the current sorting for the channel. Left-clicking in the Scan Graph View is a convenient way to compare quickly different sortings and select the most pleasing one as the final sorting of the channel.

The automatic sorting methods that utilize scanning (see [“4.5.10 Automatic Clustering Using the Scanning Methods” on page 145](#)) are in reality only doing parameter scans, and so will fill the Scan View and the Scan Graph View as they run.

Offline Sorter can also save and restore the results of a scan to and from files, see [“5.16 Saving and Restoring Scan Results” on page 222](#). To save or restore a set of scan results, select **Save Scan Results to a File** or **Restore Scan Results from a File** from the right-click menu of either the Scan View or the Scan Graph View.

A new scan can be set up and initiated by selecting **Scan Parameters** from the right-click menu on either the Scan Graph View or the Scan View.



This dialog is equivalent to the top portion of the Scan View, it sets the same values.

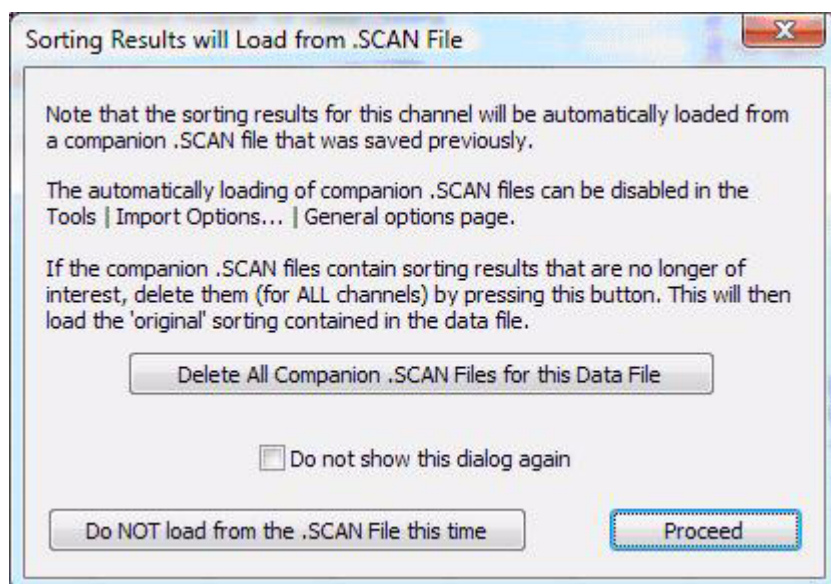
5.16 Saving and Restoring Scan Results

After a scan has been performed, the results of the entire scan (including the sorting done at each step) can be saved to a file and later recalled. To save the results of a scan to a file, select **Save Scan Results to a File** from the right-click menu on either the Scan View or the Scan Graph View. To recall a previously-saved scan, select **Restore Scan Results from a File**. When saving or restoring a scan, a filename in a standard format is suggested, with the suggested format being:

<filename>_chan<NNN>.scan

where <filename> is the name of the file currently being processed, and <NNN> is the current channel number.

A feature in Offline Sorter exists to load automatically a .SCAN file for a channel if it exists in the same directory as the data file, and is named according to the above format. When the companion .SCAN file is loaded, it makes the complete results of the scan available for inspection. To enable this feature, check the **'Automatically Load Companion .SCAN Files if they exist'** checkbox on the **Tools | Data Import Options | General** tab. If the **'Warn when loading sorting results from a .SCAN file'** checkbox is checked, the following dialog will appear whenever a companion .SCAN file is found for the channel being loaded:



The dialog contains a button to delete all .SCAN files that fit the above format and match the current filename. This function is also available from the **Sort | Delete All Companion .SORT Files** menu. If desired, loading the scan result from the .SCAN file can be skipped for this channel this one time (next time the channel is loaded it will again attempt to load the companion .SORT file). Checking the **‘Do not show this dialog again’** checkbox is equivalent to unchecking the **‘Warn when loading sorting results from a .SCAN file’** checkbox discussed above, and will cause companion .SCAN files to be loaded always.

As covered in [“5.19 Batch File Processing” on page 225](#), a sorting parameter scan can be done from a batch command file using the `ForEachFile Scan` and `ForEachFile SaveScanResults` batch commands. This feature in combination with the automatic loading of .SCAN files discussed above enables a convenient way of working with Offline Sorter, which might be termed ‘Supervised Automatic Sorting’. To use Supervised Automatic Sorting, one can set up a batch file to perform extensive sorting parameter scans on several large files and save the scan results to .SCAN files. This batch file can be run overnight, as it could take hours to complete. Then, upon arriving at work in the morning, one can simply open those files in Offline Sorter and the companion .SCAN files will be automatically loaded for each channel. One can then check each channel and use the Scan Graph View to select and inspect quickly each sorting within the scan. One can quickly pick the best sorting, and move on to the next channel (i.e. by pressing the N key). When the file is saved, the picks for the best sorting for each channel will be saved. This Supervised Automatic Sorting has several advantages: it combines the thoroughness of scanning through automatic sorting with the safety of having a human ‘in the loop’ to check sort quality, but the checking can be done very quickly without having to sit and wait for each scan to complete.

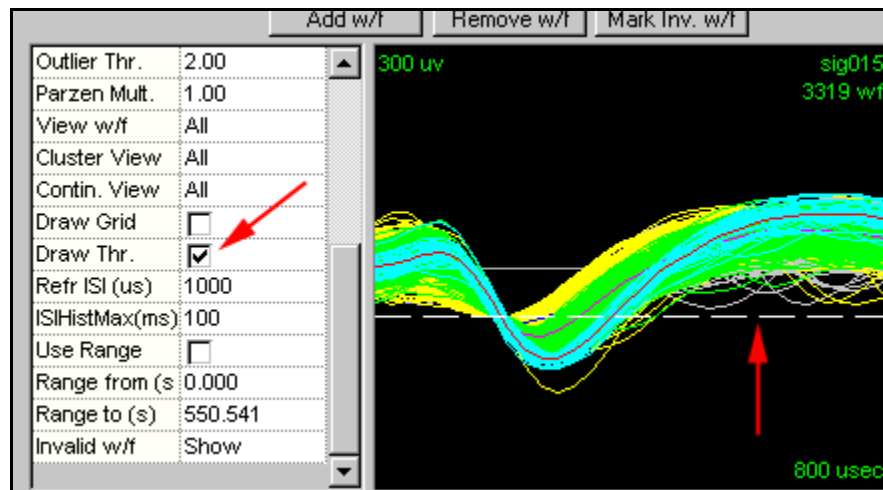
5.17 Re-thresholding Spike Data

In addition to the ability of Offline Sorter to threshold continuously-recorded data (e.g. DDT files), Offline Sorter can also simulate the effect of setting a different

voltage threshold for the detection of spike waveforms. All spike waveforms that do not cross the new threshold are marked as invalid. Obviously, setting a threshold lower than what was in effect when the file was created does not put more waveforms into the file.

The first step in running the re-thresholding tool is to make the threshold marker line (a dashed white horizontal line) appear on the Waveform View. There are two ways to accomplish this:

- Check the **Draw Thr.** checkbox on the control panel. This permanently enables the display of the threshold marker line on the Waveform display
- Select the **Tools | Re-Threshold Waveforms** menu item. This temporarily shows the threshold marker line. The line disappears after the re-thresholding operation completes.



When the threshold marker line appears, one can click-and-drag the line up or down to establish a new threshold. As soon as the drag is completed, the waveforms are re-thresholded. One can undo the effects of re-thresholding the data either with the **Undo** command or with the **Waveforms | Mark All Waveforms Valid** menu selection.

5.18 Assigning Unsorted Points to Closest Unit

After some operations (e.g. Valley-Seeking Sorting) it is desirable to assign unsorted waveforms membership in the nearest unit, subject to the Outlier Threshold. Select **Tools | Assign Unsorted To Closest Unit** to perform this operation.

The algorithm this tool uses is as follows:

- Calculate the centroid and standard deviations along each principal axis for each existing unit in the appropriate 2D or 3D feature space. If the 3D Clusters View was showing when the tool was selected, then 3D feature space is used, otherwise 2D feature space is used.

-
- Calculate the Mahalanobis distance from each unassigned waveform point to all the unit centroids, in order to find the closest unit. Note: The units of Mahalanobis distance is standard deviations.
 - If the distance to the closest unit is less than the Outlier Threshold for that unit, assign the waveform to that unit.

Note: One can invoke this tool automatically following a Valley-Seeking automatic sort. This is useful if the Limit Number of Waveforms used in Valley-Seeking Algorithm option is used, as this can leave points unassigned that clearly belong to units. One can set these options in the **Sort** tab of the **Tools | Options** dialog.

Note: Temporarily setting **Outlier Thr.** to a large number in the Control Grid before running this tool can force the algorithm to assign all unsorted points to a cluster, regardless of the distance of the point from the nearest cluster center.

5.19 Batch File Processing

Offline Sorter provides the ability to execute a subset of its commands via a script. By convention, batch file scripts for Offline Sorter have the file extension .ofb, but any file extension is legal. If a text file is selected with the **File | Execute Batch Command File** menu item, it results in that file being read and interpreted as batch commands.

For convenience, Offline Sorter offers the ability to edit a batch command file with the **File | Edit Batch Command File** menu selection. This command will remember the last-used batch command file and offer it as a suggestion in the File Open dialog. After a file is selected, Offline Sorter will launch a desired editor (notepad.exe by default) to edit the selected batch command file. To change the editor that is launched by this command, select **Tools | Options | General** and select the editor executable in the '**Editor to use for Batch Command Files**' section.

When a command file executes, Offline Sorter shows the progress in a dialog and also writes a log file with a .log extension appended to the original batch command file name. Offline Sorter also has a convenience command **File | View Log from Last Executed Batch Command File** that will quickly show the .log file from the last batch execution in the current editor.

Offline Sorter will recognize a command-line argument /b BatchFileName so that a batch command script can be executed from the command line or from a Windows .bat file.

For example, **OfflineSorter /b test.ofb** at the command prompt will start Offline Sorter, immediately execute the test.ofb batch command file, and then close Offline Sorter.

5.19.1 Batch File Commands

The format of the batch command files is one command per line. A command consists of a main keyword, followed by sub-keywords or arguments. A ‘//’ will comment out a line, and lines are not case-sensitive. Blank lines are ignored.

There are only six main keywords: **File**, **Dir**, **ForEachFile**, **ForEachChannel**, **Set**, and **Process**. But the **ForEachFile**, **ForEachChannel**, and **Set** commands have may sub-commands.

In general, an Offline Sorter batch file does the following things:

- pre-specify the files to process using the **File** or **Dir** commands.
- pre-specify the actions to be done on each channel of each file (e.g. do some sorting), and the actions to be done on a file-by-file basis (e.g. save the results) using the **ForEachChannel** and **ForEachFile** commands.
- pre-specify the values of other parameters that will affect how Offline Sorter performs the tasks (e.g. set the Parzen Multiplier for Valley Seeking Sorting) using the **Set** command.
- start all the processing with the **Process** command.

When running Offline Sorter, the **Help | Quick Batch Reference** menu item will display a convenient printable summary of the batch commands and their arguments. The batch command keywords are:

5.19.1.1 File

The **File** command queues a file for processing. The file is not actually processed until the **Process** command is encountered. Specify the full path to the files.

Syntax: **File** *filename*

e.g. **File** *c:\myfiles\test.plx* will queue the file test.plx for processing

5.19.1.2 Dir

The **Dir** command queues all of the files that match a template for processing. The files are not actually processed until the **Process** command is encountered. The template should include the full path to the files.

Syntax: **Dir** *template*

e.g. **Dir** *c:\myfiles*.plx* will queue all of the .plx files in the c:\myfiles directory for processing.

Note: It is not mandatory to specify any input data files using the File or Dir commands in a batch command file. When a batch file that does not specify any input data files is executed from within Offline Sorter, it will execute on the currently-open data file.

5.19.1.3 ForEachFile

The **ForEachFile** command specifies an action to be done for each file being processed. For some commands the action is performed before the channels are processed, for others it is only performed after all channels have been processed (See Notes below)

Syntax: **ForEachFile** *sub-command*

e.g. **ForEachFile** *ExportToNex*

Sub-commands for the ForEachFile command are:

- **Save** – saves the file. The saved file name is the same as the input file name with a numeric suffix added on to the original filename (same as **File | Save** menu option).
- **ExportToPlx** – saves the file as a new plx file (same as **File | Export to New .PLX** menu option), possibly with a numeric suffix
- **ExportToNex** – saves the timestamps etc. to a NEX file, possibly with a numeric suffix added on to the original filename
- **ExportToPlx0** – exports the current data to a .PLX0 file for optimized future access
- **SortUsingTpl** – applies a sorting template, which is specified by the **Set Tpl-FileName** command
- **InvalidateArtifacts** – applies cross-channel artifact removal the current file, using parameters specified by the **Set ArtifactXXX** commands.
- **InvalidateArtifactsAfter** - applies cross-channel artifact removal to the current file after the **ForEachChannel** commands are processed. See the Notes section below.
- **ExportWaveformInfo** - writes out selectable information about each waveform in all channels to either a text, matlab, or excel file. The information to be written and other controlling parameters must be specified in the **File | Export Per-Waveform Data** dialog.
- **ExportUnitInfo** - writes out selectable information about each unit in all channels to either a text, matlab, or excel file. The information to be written and other controlling parameters must be specified in the **File | Export Per-Unit Data** dialog.
- **ExportChannelInfo** - writes out selectable information about each channel to either a text, matlab, or excel file. The information to be written and other controlling parameters must be specified in the **File | Export Per-Channel Data** dialog.

5.19.1.4 ForEachChannel

The ForEachChannel command specifies an action to be done on each channel of each file.

Syntax: **ForEachChannel** *sub-command*

e.g. **ForEachChannel** *ValleySeek*

Sub-commands for the ForEachChannel command are:

- **Upsample** - performs interpolation to upsample each channel of continuous data, as configured with the **Set UpSampleXXX** commands.
- **Filter** – applies a low-cut filter to each channel of continuous data. The filter cutoff frequency is specified with the **Set FilterFreq** command.
- **Detect** – performs waveform detection for each continuous channel, as configured with the **Set DetectXXX** commands.
- **Align** – runs waveform alignment, using the currently set alignment parameters set via the **Set AlignXXX** commands.
- **DeleteAllUnits** – removes all existing units
- **Sort** - performs sorting that has been specified via the **Set SortType** command.
- **ValleySeek, ValleySeek2d** – runs valley seeking sorting on each channel, using 2D feature space
- **ValleySeek3d** – runs valley seeking sorting on each channel, using 3D feature space.
- **TDist, TDist2d** – runs T-Distribution E-M sorting on each channel, using 2D feature space.
- **TDist3d** – runs T-Distribution E-M sorting on each channel, using 3D feature space.
- **RemoveOutliers2d, RemoveOutlier3d** – after sorting, runs the Remove Outliers tool in either 2D or 3D feature space, using the currently set Outlier Threshold.
- **RemoveShortISI** – runs the Remove Short ISI tool, using the currently set Refractory Period.
- **AssignUnsorted2d, AssignUnsorted3d** – runs the Assign Unsorted tool in either 2D or 3D feature space, using the currently set Outlier Threshold.
- **KeepWfsBetweenEvents** – runs an interval filter that keeps only the waveforms between two specified events, with an optional time offset from each event.
- **KeepWfsInSpan** – runs an interval filter that keeps only the waveforms within a time range around a specified event.

- **KeepWfsOutsideSpan** - runs an interval filter that keeps only the waveforms that are outside of a time range around a specified event.
- **KeepWfsBetweenTimes** – runs an interval filter that keeps only the waveforms within a specified time range.
- **InvalidateWfsBetweenTimes** – runs an interval filter that invalidates the waveforms within a specified time range.
- **SortSummaryToPP** – after sorting, dumps the sort summary to PowerPoint for each channel.
- **LogStats2d, LogStats3d** – after sorting, dumps the 2D or 3D sort quality stats to the log file.
- **Scan** - performs a parameter scan as configured with the **Set ScanXXX** commands.
- **SaveScanResults** - saves the results of a previous **ForEachChannel Scan** command to a file that can be later inspected in Offline Sorter.

5.19.1.5 Set

The **Set** command changes a parameter, immediately.

Syntax: **Set** *parameterName* *parameterValue*

e.g **Set** *ParzenRadiusMult* 2.0

The available parameters are:

- **OptionsFromFile** - immediately sets all options to the values in the specified options file. The options file can be created using the **Tools | All Options | Save To File** menu command. Note that this can reset the values of any previous **Set** commands, so it generally should come before any other **Set** commands. This command is useful for making sure that all parameters are set to known values before executing a batch file.
- **ChanMap** – specifies a filename that contains a channel map to apply. Having this command present will force the named channel map file to be applied to each loaded file.
- **TplFileName** – sets the sorting template filename to use for ForEachFile SortUsingTpl.
- **DefaultFileType** – specifies the file type to assume when the data file extension does not match any known extension. The argument uses a numeric code from the following table to specify the file type:

Code	File Type
0	Plexon .PLX
1	Plexon .DDT
2	Plexon .PLX0

Code	File Type
3	reserved
4	Alpha Omega
5	CED Spike-2
6	Datawave
7	Data Translations
8	reserved
9	Multi-Channel Systems
10	Panasonic MED
11	Cyberkinetics
12	Neuralynx
13	Neuroexplorer .nex
14	Neuroshare Native .nsn
15	RC Electronics
16	generic binary
17	generic Neuroshare

- **ImportParameters** – specifies a filename for import parameters (.ofi file) to be used for binary file import.
- **FilterFreq** – sets the low-cut filtering frequency in Hz to use with ForEach-Channel Filter.
- **UpSampleType** - sets the type of interpolation to do for upsampling; 0=None, 1=Linear, 2=Spline.
- **UpSampleFactor** - sets the interpolation factor for upsampling (e.g. 2 means 2x interpolation).
- **UpSampleMinFreq, UpSampleMaxFreq** - can be used to select which continuous channels will be upsampled (e.g. if a file contains some continuous channels sampled at 1000 Hz and others sampled at 4000 Hz, setting a UpSampleMinFreq of 30000 will ensure that only the 40000 Hz channels are upsampled).
- **DetectNPW** – sets the number of points per waveform to use for **ForEach-Channel Detect**.
- **DetectNPre** – sets the number of pre-threshold samples to use for **ForEach-Channel Detect**.
- **DetectSigmas** – sets the detection threshold as a number of sigmas away from the mean of the Peak Height Histogram; the value can be negative for negative thresholds.
- **DetectDead** – sets the number of samples to ignore following a detection (the dead time) to use for **ForEachChannel Detect**.

-
- **AlignType** - determines how the waveforms will be aligned; 0=first local max, 1=first local min, 2=first local extremum, 3=global max, 4=global min.
 - **AlignMaxShift** – is the maximum amount a waveform will be shifted (in clock ticks).
 - **ArtifactWidth** – the number of samples used to detect coincidence for **ForEachFile InvalidateArtifacts**.
 - **ArtifactPercentage** – the percentage of channels that must have coincident spikes for **ForEachFile InvalidateArtifacts**.
 - **BetweenEvent1,BetweenEvent2** – event names to use for **KeepWfsBetweenEvents** interval filter.
 - **BetweenEvent1Offset,BetweenEvent2Offset** – time offsets in msec to use for **KeepWfsBetweenEvents** interval filter. Filter will invalidate waveforms outside of the time interval between (time of BetweenEvent1 + BetweenEvent1Offset) to (time of BetweenEvent2 + BetweenEvent2Offset).
 - **SpanEvent** – event name to use for **KeepWfsInSpan** interval filter.
 - **SpanEventStartOffset,SpanEventEndOffset** – time offsets in msec to use for **SpanEvent** interval filter. Filter will invalidate waveforms outside of the time interval (time of SpanEvent + SpanEventStartOffset) to (time of SpanEvent + SpanEventEndOffset).
 - **TimeStart,TimeEnd** – defines the time interval in msec to use for the **KeepWfsBetweenTimes** and **InvalidateWaveformsBetweenTimes** interval filters.
 - **SortType** - sets the sorting type to use for the **ForEachChannel Sort** command. The argument values are: **ValleySeek**, **TDist**, **ScanKMeans**, **ScanStdEM**, **ScanValleySeek**, **ScanTDist**.
 - **ParzenRadiusMult** – sets the parzen radius multiplier for valley seeking sorting to the specified value.
 - **DOFMult** – sets the degrees of freedom multiplier for T-Distribution E-M sorting to the specified value.
 - **OutlierThreshold** – sets the outlier threshold to the specified value.
 - **RefractoryPeriod** – sets the refractory period to the specified value, for the **RemoveShortISI** channel command.
 - **WaveformLimit** – sets the limit for the number of waveforms used in the valley-seeking sorting. For more details, see [“4.5.8 Automatic Clustering using Valley-Seeking Method” on page 142](#).
 - **FeatureX, FeatureY, FeatureZ** – set the feature for each dimension of feature space. See the following table for numerical values for the features.
 - **Slice1,Slice2,Slice3,Slice4** – set the slice position (in ticks) for the Slice n features.

- **ScanType** - sets the type of scan to perform for the **ForEachChannel Scan** command. The argument values are: **ParzenMult**, **DOFMult**, **KMEans**, **StdEM**, **OutlierThresh**, **WfLimit**, **PCAMax**.
- **ScanStart**, **ScanEnd**, **ScanStep** - sets the scan range for the **ForEachChannel Scan** command, and the amount to increment the scan parameter for each step.
- **ScanNormalize** - set to 1 to use normalize the sort quality metrics calculated during the scan and used to determine the 'best' sorting.
- **UseRangeStart**, **UseRangeEnd** – only consider the portion of the file with timestamps between the Start and End values, with the times specified in seconds. This range is used for sorting operations and for exporting to .plx and .nex files. Set either the start or the end value to a negative number to cancel a sort range and use the entire file.
- **SaveCont** – set to 1 to have ExportToPLX save the continuous data, set to 0 to save only the spike data.
- **SaveProcessedCont** - set to 1 to have ExportToPLX save the upsampled and filtered version of the continuous data, set to 0 to save the 'raw' continuous data.
- **SaveInvalidated**, **SaveUnsorted** - set to 1 to have ExportToPLX save invalidated or unsorted waveforms, set to 0 to ignore them.
- **SaveNexCont** - set to 1 to have ExportToNEX save the continuous data, set to 0 to ignore continuous data.
- **SaveNexProcessedCont** - set to 1 to have ExportToNEX save the unsampled and filtered version of the continuous data, set to 0 to save the 'raw' continuous data.
- **SaveNexWaveforms** - set to 1 to have ExportToNEX save waveforms, set to 0 to ignore them and just save the timestamps.
- **SaveNexUnsorted** - set to 1 to have ExportToNEX save unsorted waveforms, set to 0 to ignore them.
- **SaveNexUnitTemplates** - set to 1 to have ExportToNEX save the unit templates to a waveform variable in the exported .nex file.

5.19.1.6 Process

The **Process** command processes all queued files. Resets the queued files list.

Syntax: **Process**

5.19.2 Example Batch Command File

Here are the contents of example batch file, ofsbatch.ofb:

ForEachChannel *ValleySeek*

```

ForEachChannel SortSummaryToPP
ForEachChannel LogStats2d
//ForEachChannel LogStats3d
ForEachFile ExportToNex
Set FeatureX 0
Set FeatureY 1
Set ParzenRadiusMult 1.0
File C:\PlexonData\Plexon\plx\chan9threeunits3.plx
File C:\PlexonData\Plexon\plx\ev024.plx

Process
Set ParzenRadiusMult 2.0
File C:\PlexonData\Plexon\plx\chan9threeunits3.plx
File C:\PlexonData\Plexon\plx\ev024.plx

Process

```

The behavior of the example file is to:

- Set the feature space to be PC1 and PC2.
- Set the parzen radius multiplier to 1.0.
- Open chan9threeunits3.plx.

For each channel:

- Do 2D valley seeking sort, using PC1 and PC2.
- Send the sort summary to PowerPoint.
- Log the 2D sort quality stats to the log file.
- Save the timestamps to chan9threeunits3.nex.
- Open ev024.plx.

For each channel:

- Do 2D valley seeking sort, using PC1 and PC2.
- Send the sort summary to PowerPoint.
- Log the 2D sort quality stats to the log file.
- Save the timestamps to ev024.nex.
- Set the parzen radius multiplier to 2.0.

- Do the same thing as described above to both files, but this time using a parzen multiplier of 2.0.

5.19.3 Notes

- Executing a **Set** command via a batch file will generally affect a parameter that can also be set via the Offline Sorter graphical user interface. However, if a **Set** command changes a parameter, the new value of that parameter will only be in effect for the duration of the batch file execution. The new value will not be in effect the next time Offline Sorter is started.
- Any values not explicitly set by the batch commands obtain their values via the settings that were in effect the last time that Offline Sorter was run. Note that some parameters that affect batch operations can ONLY be ‘inherited’ in this fashion, since the Set commands to change all possible parameters do not exist in the batch syntax. This can lead to non-reproducible results, so it is good policy to do explicit **Sets** of all parameters that can affect the results in the batch file, or use the **Set OptionsFromFile** batch command to get all parameters into a known state.
- All processing done by batch commands is done in a certain order for each of the files being processed. Each of the following steps may or may not be specified in the batch commands, and some commands may not be relevant for a given channel. The order is:
 1. Apply a channel map
 2. Invalidate artifacts
 3. Apply a .tpl file
 4. Process each channel. The order of processing for each channel is:
 - a. Apply upsampling to continuous channels
 - b. Apply filtering to continuous channels
 - c. Detect waveforms for continuous channels
 - d. Invalidate waveforms (the **KeepWf** and **InvalidateWf** commands)
 - e. Perform alignment
 - f. Delete all units
 - g. Perform sorting
 - h Remove outliers
 - i. Remove short ISI waveforms
 - j. Assign unsorted waveforms to clusters
 - k. Dump sort summary to PowerPoint
 - l. Log stats
 - m. Perform parameter scans

5. Invalidate artifacts if the InvalidateArtifactsAfter command is used

6. All saving and export to file operations

- When importing a file from batch, Offline Sorter by default uses the loader implied by the file extension (e.g. the Cyberkinetics loader uses .nev files). If the file to be loaded does not have a recognized extension, Offline Sorter uses the loader corresponding to the currently selected default File Type. One can use the **Set DefaultFileType** command to set the default File Type.
- Numerical values to use in the Set FeatureXXX commands:
 - 0 PC 1
 - 1 PC 2
 - 2 PC 3
 - 3 Slice 1
 - 4 Slice 2
 - 5 Peak
 - 6 Valley
 - 7 Peak-Valley
 - 8 Energy
 - 9 Nonlinear Energy
 - 10 Timestamp
 - 11 PC 4
 - 12 PC 5
 - 13 PC 6
 - 14 PC 7
 - 15 PC 8
 - 16 Peak FWHM
 - 17 Valley FWHM
 - 18 Peak Tick
 - 19 Valley Tick
 - 20 Area under Waveform
 - 21 Slice 3
 - 22 Slice 4
 - 23 Peak to Valley Ticks
 - 24 Valley to Peak Ticks

- 25 Sqrt Energy
- 26 Previous ISI
- 27 Next ISI

5.20 Sample Batch Command Files

Offline Sorter ships with some sample batch command files, which are installed in the 'SampleBatchFiles' subdirectory of the main Offline Sorter installation directory.

The sample batch command files do not contain any File or Dir commands to specify the input data files. Thus, they all make use of the feature described above where they will operate on the currently loaded data file when they are executed from within Offline Sorter.

The general procedure for running the sample batch command files is as follows:

1. Load a data file using **File | Open** or **File | Import**. If there are not any data files available, sample data files can be downloaded from <http://www.plexoninc.com/downloads/sampledatafiles/SampleDataFiles.zip>.
2. Select **File | Execute Batch Command File**, navigate to the SampleBatchFiles directory (located at C:\Program Files\Plexon Inc\Offline Sorter 3\SampleBatchFiles for a default installation), and select the desired sample batch command file.
3. After the batch command file runs, inspect the results, for example by selecting **File | View Log from Last Executed Batch Command File**.

The sample files are:

5.20.1 SortAndSave.ofb

Performs Valley Seeking sorting using 2D feature space on all channels in a file, performs outlier removal, then saves the results. Note that this batch command file must be run on a data file that contains spikes, and it must be run on a type of data file that Offline Sorter can save natively (i.e. the **File | Save** and **File | Save As** menu items are available after loading the file). After the batch command files completes, the output should be in a file with the same name as the currently open file, with a number appended, e.g. if the current file is test.plx, the output file will be test-01.plx. There should also be a SortAndSave.ofb log file in the same directory as SortAndSave.ofb that records what happened during the batch command file execution.

5.20.2 RemArtifactsSortAndExport.ofb

Performs artifact invalidation, followed by T-Distribution E-M sorting using 3D feature space on all channels. The result is then exported to a new .PLX file such that the invalidated waveforms are not saved. Note that this batch command file must be run on a data file that contains spikes.

5.20.3 ScanKMeans.ofb

Performs a 2D K-Means Scan on all channels, and saves the scan results to .SCAN files. The .SCAN files can be loaded back into Offline Sorter to make the results available for inspection. Note that this batch command file must be run on a data file that contains spikes.

5.20.4 ScanValleySeeking.ofb

Performs a 3D Valley Seeking Scan on all channels, and saves the scan results to .SCAN files. The .SCAN files can be loaded back into Offline Sorter to make the results available for inspection. Note that this batch command file must be run on a data file that contains spikes.

5.20.5 FiltDetect.ofb

Performs low-cut filtering and waveform detection on a continuous data file, and exports the extracted spikes to a .PLX file. Note that this batch command file must be run on a data file that contains continuous data (e.g. a .DDT file).

5.20.6 SaveToNex.ofb

Performs low-cut filtering and waveform detection on a continuous data file, sorting, and artifact invalidation, then exports everything to a .NEX file. Note that the 'InvalidateArtifactsAfter' command is used in order to perform the artifact invalidation after the waveform extraction has been completed. Note that this batch command file must be run on a data file that contains continuous data (e.g. a .DDT file).

5.21 Printing

Offline Sorter can print any view from the Multi-Display Window, and also the Waveform View. Select **File | Print...** or **File | Print Preview...** to print or preview the currently active window in the Multi-Display Window.

The commands **File - Print All Sort Summaries** and **File - Send All Sort Summaries to PowerPoint** iterate through all channels and print or export the Sort Summary for each channel to a single PowerPoint file or a single print job.

If the computer has Adobe PDF Distiller installed, the **File - Print All Sort Summaries** offers a means to easily get the Sort Summaries for all channels in a file into a single PDF document. Simply select **Adobe PDF** as the printer in the **Print** dialog, and type the PDF file name.

The colors and the Clusters View point size for printing can be different from the values used for display. Select the values to use for printing in the **Printing Options** tab in the **Tools | Options** dialog box. By default, the printing colors are the same as the display colors, except that the background color is set to white. Some of the display colors do not print with high contrast on some printers.

The 2D Clusters View has an option to dump the display to a Windows metafile, which can then be imported into most graphics programs. To do so, from the right-click menu on the 2D Clusters View, select **Print to Windows Metafile**.

To capture the 3D Clusters View, use the built-in Windows screen capture method. With the 3D Clusters View showing, press ALT-PRINT SCREEN. Bring up a program such as Microsoft Paint, and select **Paste**. The image may have to be cropped to eliminate everything but the 3D Clusters View.

5.22 Large File Tips

The following are some settings and usage tips that can significantly speed up Offline Sorter when working with large PLX files.

5.22.1 Statistics Calculations

In general, Offline Sorter must recalculate sort quality statistics whenever any change is made to the sorting on a channel. This can take significant time, especially if unit pair-wise statistics are being calculated. The **Stats** tab of the **Tools | Options** panel contains checkboxes that enable or disable the calculation of the advanced sort-quality statistics, and also the unit pair-wise statistics. If the statistics are not of interest, uncheck these items to speed up sorting operations.

5.22.2 Buffer Sizes

On the **General** tab of the **Data Import Options** page, there are two **Buffer Size** settings to be aware of. In both cases, these buffer sizes are specified in megabytes. These values should always be less than the amount of RAM in the computer, or severe performance degradation can occur.

The **Reading Buffer Size for data files** is the size of the temporary cache Offline Sorter uses when reading data from files. Increasing this number generally speeds up reading larger files, but might slow down the reading of smaller files. However, if this value is too large, it can slow performance.

Buffer Size for Continuous Data specifies the size of the memory buffer that Offline Sorter uses for holding the data from continuous data channels. If it is not desired to work with continuous data channels, or if it is acceptable to wait for continuous data to be dynamically read in after a channel is initially loaded, set this to a small value.

5.22.3 Ignoring Continuous Data Channels

Plexon PLX files often contain a large amount of continuous (slow channel) data that can be a significant fraction of the total file size. By default, Offline Sorter reads in this continuous data when the file is opened.

If continuous channel data is not desired, instruct Offline Sorter to bypass reading the continuous data altogether by checking the **Disable loading continuous data**

channels checkbox under the **Continuous Data** section on the **General** tab of the **Tools | Data Import Options** dialog box.



CAUTION

Continuous Data Channels Quietly Ignored

When **Disable loading continuous data channels** setting is checked, continuous data channels are quietly ignored—Offline Sorter gives no indication that these continuous channels even exist in the data file, and they are not saved during a **Export to New .PLX** operation. Moreover, Offline Sorter retains this setting between startups. If this setting is checked, Offline Sorter ignores continuous channels without warning until the setting is unchecked.

When saving a NEX file, by default, Offline Sorter must read the continuous data channels in order to write them to the NEX file, which can be time-consuming. If the continuous data channels are not desired to be present in the NEX file, one can disable writing the continuous data channels by unchecking the **Export continuous data channels** checkbox in the **Export to NEX Options** dialog box.

5.22.4 Initial PCA Calculation

By default, Offline Sorter performs a PCA calculation on each channel after it is loaded into memory. However, this calculation is wasted if the sorting is to be done in a feature space that does not involve principal component projections (e.g. using peak heights to sort tetrode data). One can effectively turn off the PCA calculation by entering a value of 0 in the edit box under **Maximum Number of Waveforms to Use in the PCA Calculation** in the **Feature Calc** tab of the **Options** window.

5.22.5 Deferring Waveform Drawing

Sometimes, Offline Sorter's redrawing of the Waveform view and the Timeline View can be time-consuming. If the **Defer Waveform Drawing Until Clicked** checkbox in the **Display** tab of the **Options** dialog box is checked, the displays are not redrawn until the mouse is left-clicked in them. When viewing animated sorting progress, the views are temporarily put into deferred drawing mode, otherwise it slows down the animation too much.

5.22.6 Using .PLX0 Files

One can use an entirely differently approach to speed up Offline Sorter operations on large files. Any data file loaded into Offline Sorter can be exported as a special .PLX0 file using the **File | Export to .PLX0** menu selection. Subsequently load-

ing an exported .PLX0 file into Offline Sorter is generally many times faster than loading the original data file.

**HINT****Plan the work before converting to PLX0**

Converting a file to .PLX0 format can take significant time, and can require significant space on the hard drive. It generally only makes sense to convert a data file to .PLX0 format if it is desired to be working with that file several times, so that the cost of creating the .PLX0 file is “amortized”. One can create a Batch Command file to do the .PLX0 conversion overnight in order to have the fast-loading .PLX0 files available in the morning.

The .PLX0 file format is fast because it organizes the data by channel, with the data for each channel written to a separate file. The general information about a file and the digital events are stored in the .PLX0 file itself, with the data for each channel NNN being written to a file with extension .plxNNN (or .plxCNNN for continuous data). Do not attempt to open the per-channel .plxNNN or .plxCNNN files directly in Offline Sorter—open the .PLX0 instead. The .PLX0 file format should be considered solely as a tool to speed up Offline Sorter, not as a supported data file format. The .PLX0 file format is intentionally not documented and can change without notice in the future. Do not archive the data as .PLX0 files—use .PLX files instead.

Because of how the .PLX0 file is organized, it is not possible to use the **File | Save As** menu item on .PLX0 files, although **File | Save** works (and very quickly). Use **File | Export to .PLX0** instead of **File | Save As**.

Note: Formerly, the separate utility program PlxUtil was used to create .PLX0 files, but starting with Offline Sorter version 2.8.0, the ability to create .PLX0 files was built in to Offline Sorter itself. This allows any data file format that can be loaded into Offline Sorter to be converted into .PLX0 format. Older .PLX0 files created by PlxUtil will no longer work with Offline Sorter version 2.8.0 or later, and there is no guarantee that .PLX0 files written with one version of Offline Sorter will work with other versions. Again, do not archive the data .PLX0 files—use .PLX files instead.

Appendix A

Plexon User Interface

A.1 Plexon User Interface A-2

A.2 Screen Elements..... A-3

A.3 Standard Menu Items and Dialogs A-11

A.4 Customization A-19

A.1 Plexon User Interface

The **Plexon User Interface** embodies a standard look-and-feel for Plexon software products. To illustrate the underlying concepts behind the look-and-feel, this appendix uses screenshots from Plexon's **CinePlex Editor** (formerly called **CinePlex Markup**) and **CinePlex Studio** (formerly called **CinePlex Capture**) applications. Although there will be differences in menus and toolbars between different applications, how you use, handle, and operate the user interface components will be fundamentally the same across future generations of Plexon's software products. Included are:

- CinePlex Studio
- CinePlex Editor
- MAP 2
- Offline Sorter

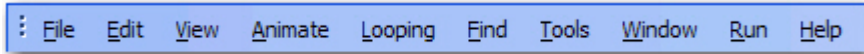
This appendix covers common functionality of the look-and-feel between different Plexon software applications. The discussion includes the following sections:

- **Screen Elements**
- **Standard Menu Items and Dialogs**
- **Customization**

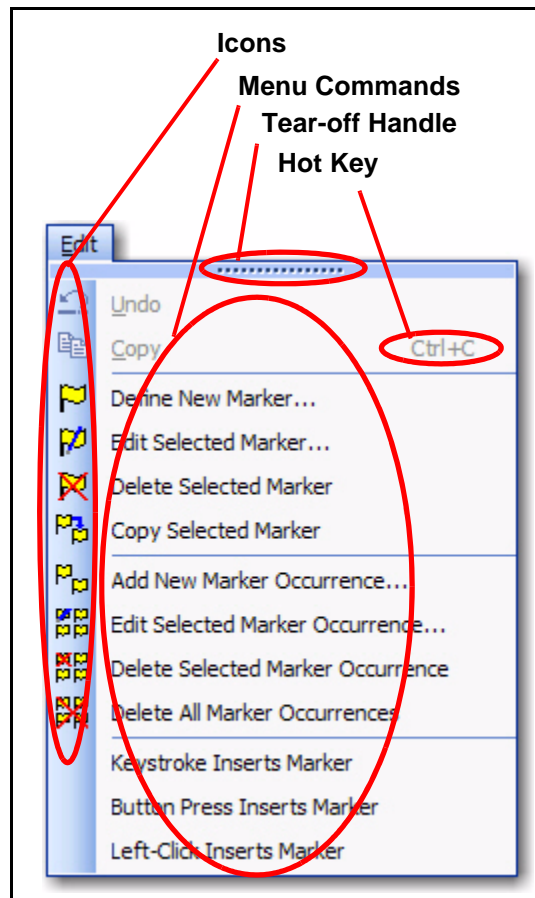
A.2 Screen Elements

A.2.1 Menus

The menu bar of the application contains the names of all of the menus for the application. Each menu name has a letter underlined which indicates the hot-key combination for that menu. To activate a particular menu you may click the left mouse button on the name or press and hold the **ALT** key while pressing the underlined letter. The illustration below is the main menu of **CinePlex Editor**.



Each menu of the **Plexon User Interface** contain menu commands and may also contain Icons, Hot Keys and a Tear-off Menu Handle as shown by the illustration below of the Edit menu of **CinePlex Editor**.

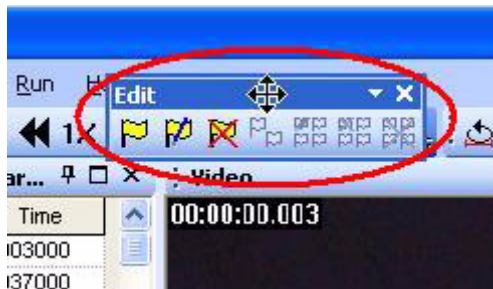
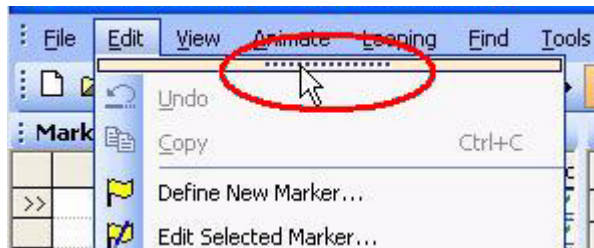


While all Plexon applications are shipped with their available menu commands organized into a set of menus, the contents of the menus can be customized and new menus can be created.

- **Icons** - Icons may be located immediately to the left of the menu commands. These icons will be displayed on the toolbar associated with the menu. In the

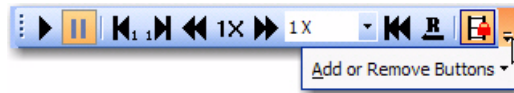
example menu, there are icons associated with all but the last three of the menu commands. The presence of an icon next to a menu command means that the icon is also a label on a toolbar button and will execute the same command when clicked as the menu command on the menu.

- **Hot Keys - Hot Keys** are key combinations that will cause a menu command to execute without having to click the command on the menu. Typically, hot keys will contain two keys that must be pressed simultaneously for the combination to execute. The area at the far right of the menu commands may display the hot key combinations for the menu commands. In the example menu, there is a hot key combination (CTRL + C) for the Copy command. Hot keys may be added or customized via the **Tools-Customize** dialog.
- **Tear-off Menu Handle** - The **Tear-off Menu Handle** is an area (the one containing the dots) at the top of the menu, present on many menus. The tear-off feature allows the quick creation of a toolbar that contains all of the commands in the menu that have command icons. You may hover the mouse over the tool to highlight it, drag the mouse to the toolbar area and place the menu as a toolbar there. The illustrations below show highlighting the **Tear-off Menu Handle** to begin dragging the toolbar, dragging the toolbar across the screen, and finally docking the toolbar in place.

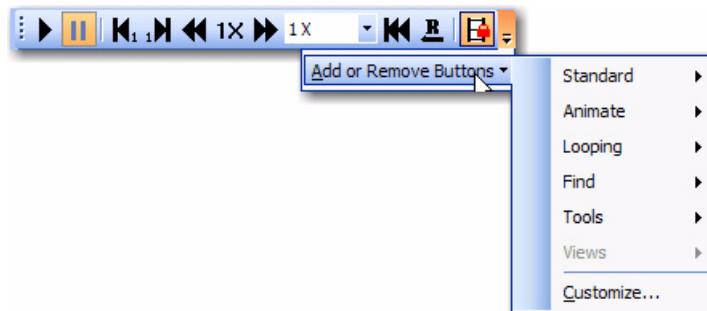


A.2.2 Toolbars

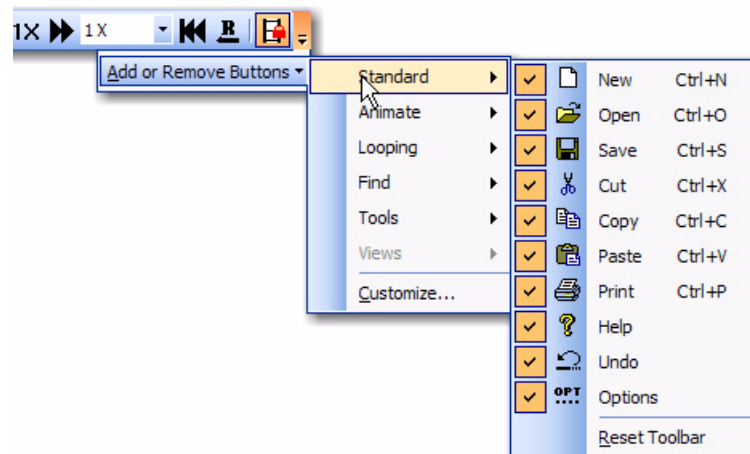
Each toolbar has a dropdown button at the right that will allow you to add or remove buttons from toolbars and will also allow you to access the **Customize** dialog box. When you click the dropdown button the **Add or Remove Buttons** dropdown menu will appear as shown below.



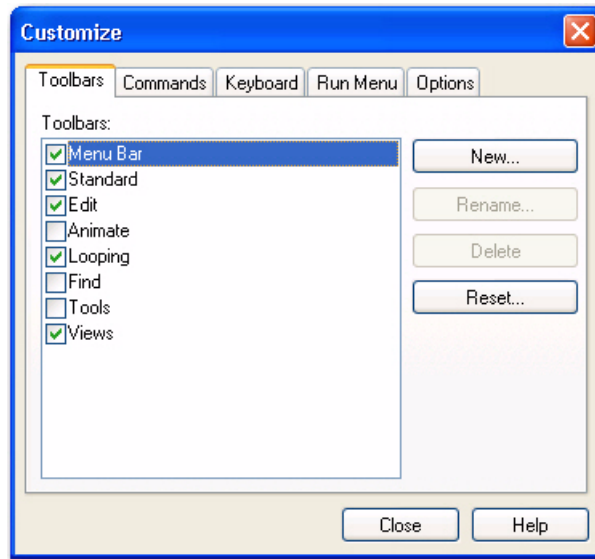
You will see that the dropdown menu also has a dropdown button. If you click that button you will see a menu list of the available toolbars and a menu command to allow access to the **Customize** dialog box.



Expanding one of the toolbar menus will display all of the available toolbar buttons for that particular toolbar and a checkbox beside each one that will indicate whether or not the button is visible on the toolbar. There is also an option to reset the toolbar back to its factory default setting.



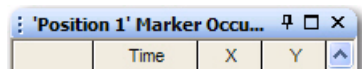
Clicking the **Customize** menu command will open the **Customize** dialog box. This dialog box is common to Plexon software applications but may have different content on the tabs according to the software application.



For details on using the **Customize** dialog box, see [“Customization” on page A-19](#)

A.2.3 Windows

The illustration below shows a typical window title bar. It contains (from left to right) a **Title**, a **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 **Auto Hide** button points in a vertical direction. If the window is rolled up, the **Auto Hide** button 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**



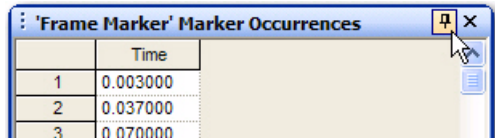
- The **Close** button closes the window.

Plexon software applications often display several windows simultaneously. These windows may be resized by using standard resizing methods, and you may also reposition windows by dragging and dropping and by using **Docking Stickers**. The illustration below shows the repositioning of a window (denoted by the blue transparent rectangle) and various **Docking Stickers** (with red circles around them). These **Docking Stickers** allow you to dock the window being moved in one of several ways described below.

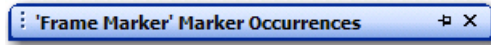


You may position a window by floating it, docking it at the desired docking sticker at a window edge or in a tab.

- **Floating a Window** - You may drag a window by the **Caption Bar** near the center of the screen and release it causing the window to float. Holding down the CTRL key while dragging will always float the window. Double-clicking on the **Caption Bar** will also float a window. Note that the size and position of the floating window is remembered.
 - **Rolled-up Windows** - Floating windows may be enabled for roll up by pressing the **Auto Hide** button. The window will roll up when the focus is changed to a different window. The first illustration below shows a floating window before rolling it up. The second illustration below shows the rolled-up window after the focus has changed.



	Time
1	0.003000
2	0.037000
3	0.070000



- **Docking a Window at the Application Frame** - When you begin to drag a window, a transparent blue rectangle appears to indicate the position of the window and the four **Docking Stickers** appear individually at each edge of the application frame to allow you to dock the window to the respective edge. To dock just move the mouse to the desired **Docking Sticker** and release the mouse button. The docked window will extend along the entire length of the edge to which it is docked. The illustration below shows all four screen **Docking Stickers**.



- **Docking a Window at a Window Edge** - When you move the window inside another window, the window **Docking Stickers** appear inside the window grouped together near the center of the window. Releasing the mouse button while it is over one of these stickers (except the center one) will dock the moving window to the respective edge of the window associated with the window **Docking Stickers**. The illustration below shows the window **Docking Stickers**. Note that the shading of these **Docking Stickers** is different than the shading of the **Docking Stickers** in the previous illustration.

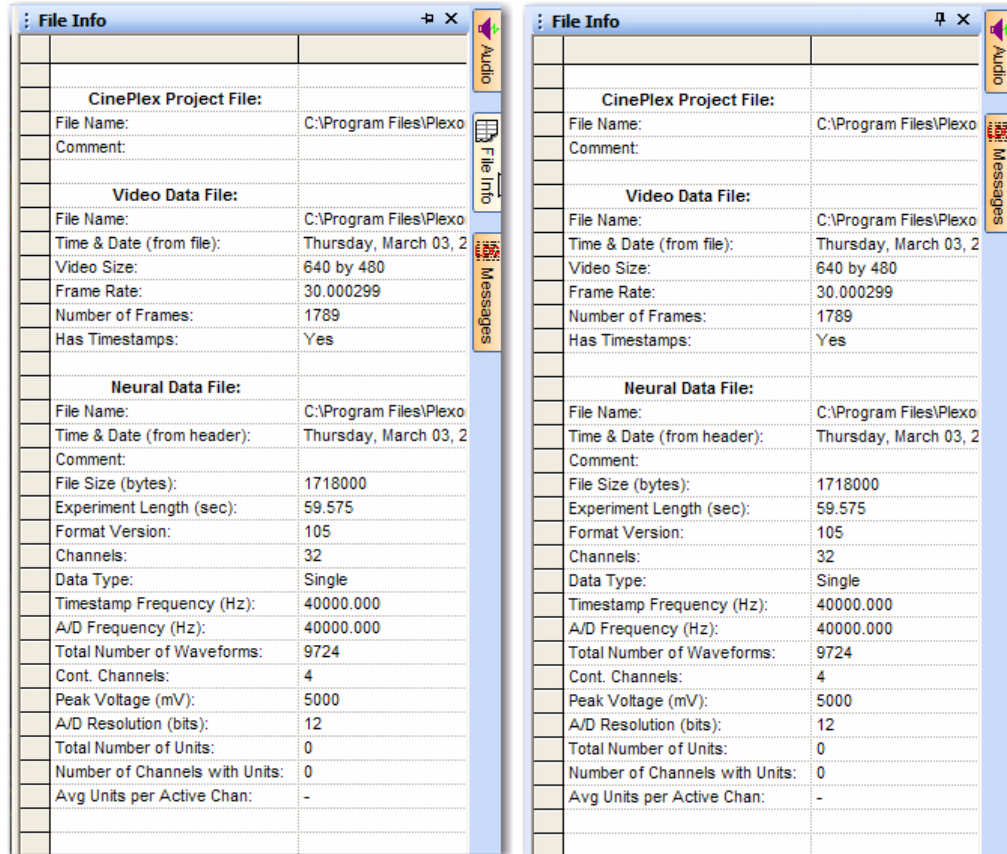


- **Placing Windows into Tabs** - Releasing the mouse button over the center **Docking Sticker** will allow the moving window to occupy the same space as the window beneath and will create tabs along the bottom for switching the view between the two windows. The illustration below shows the **Marker Occurrences** window without sidebar tabs on the left. On the right, a **Channels** window has been placed into the same space by using the center docking sticker. Note that there are two sidebar tabs at the bottom of the window.

'Strobed' Marker Occurrences			Channels				
	Time	Value	Name	Type	Count	Show	
>1	0.007375	21903	1	sig001	Spikes	178	<input checked="" type="checkbox"/>
2	0.009375	22972	2	sig002	Spikes	332	<input checked="" type="checkbox"/>
3	0.041375	21903	3	sig003	Spikes	3481	<input checked="" type="checkbox"/>
4	0.043375	22972	4	sig004	Spikes	4298	<input checked="" type="checkbox"/>
5	0.074375	21903	5	sig005	Spikes	332	<input checked="" type="checkbox"/>
6	0.076375	22972	6	sig006	Spikes	540	<input checked="" type="checkbox"/>
7	0.107375	21903	7	sig007	Spikes	343	<input checked="" type="checkbox"/>
8	0.109375	22972	8	sig008	Spikes	220	<input checked="" type="checkbox"/>
9	0.140375	21903	9	sig009	Spikes	0	<input type="checkbox"/>
10	0.142375	22975	10	sig010	Spikes	0	<input type="checkbox"/>
11	0.176375	21903	11	sig011	Spikes	0	<input type="checkbox"/>
12	0.178375	22975	12	sig012	Spikes	0	<input type="checkbox"/>
13	0.207375	21903	13	sig013	Spikes	0	<input type="checkbox"/>
14	0.209375	22975	14	sig014	Spikes	0	<input type="checkbox"/>
15	0.240375	21903	15	sig015	Spikes	0	<input type="checkbox"/>
16	0.242375	22975	16	sig016	Spikes	0	<input type="checkbox"/>
17	0.279375	21903	17	sig017	Spikes	0	<input type="checkbox"/>
18	0.281375	22975	18	sig018	Spikes	0	<input type="checkbox"/>
19	0.307375	21903	19	sig019	Spikes	0	<input type="checkbox"/>
20	0.309375	22975	20	sig020	Spikes	0	<input type="checkbox"/>
21	0.340375	21903	21	sig021	Spikes	0	<input type="checkbox"/>
22	0.342375	22975	22	sig022	Spikes	0	<input type="checkbox"/>
23	0.374375	21903	23	sig023	Spikes	0	<input type="checkbox"/>
24	0.376375	22975	24	sig024	Spikes	0	<input type="checkbox"/>
25	0.407375	21903	25	sig025	Spikes	0	<input type="checkbox"/>
26	0.409375	22975	26	sig026	Spikes	0	<input type="checkbox"/>
27	0.440375	21903	27	sig027	Spikes	0	<input type="checkbox"/>
28	0.442375	22974	28	sig028	Spikes	0	<input type="checkbox"/>
29	0.474375	21903	29	sig029	Spikes	0	<input type="checkbox"/>
30	0.476375	22974	30	sig030	Spikes	0	<input type="checkbox"/>
31	0.507375	21903	31	sig031	Spikes	0	<input type="checkbox"/>
32	0.509375	22972	32	sig032	Spikes	0	<input type="checkbox"/>
33	0.540375	21903	33	AD01	Continuous	59448	<input type="checkbox"/>
34	0.542375	22972	34	AD02	Continuous	59448	<input type="checkbox"/>
35	0.574375	21903	35	AD03	Continuous	59448	<input type="checkbox"/>
36	0.576375	22972	36	AD04	Continuous	59448	<input type="checkbox"/>
37	0.609375	21903					
38	0.611375	22972					
39	0.640375	21903					
40	0.642375	22972					
41	0.674375	21903					
42	0.676375	22972					
43	0.712375	21903					
44	0.714375	22972					
45	0.740375	21903					
46	0.742375	22972					
47	0.774375	21903					
48	0.776375	22972					
49	0.807375	21903					

- **Hidden Windows** - Docked windows that you do not need to access often can be hidden or “rolled up” by pressing the **Auto Hide** button. When hidden, the window is represented by a sidebar tab. Sidebar tabs may be located at the left, right, or bottom of the screen and indicate hidden windows. To show one of these windows, just hover the mouse over one of the tabs and the window will appear. The **File Info** window of **CinePlex Editor** opened by the sidebar tab is shown below on the left. If you click the **Auto Hide** control, the window will be pinned and

remain open after you move the mouse away from the sidebar tab as shown on the right. The **Auto Hide** button will also have changed direction and be pointed downward. Note also that the **File Info** sidebar tab is gone on the right illustration.

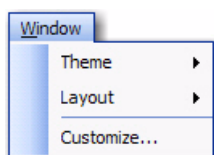


A.3 Standard Menu Items and Dialogs

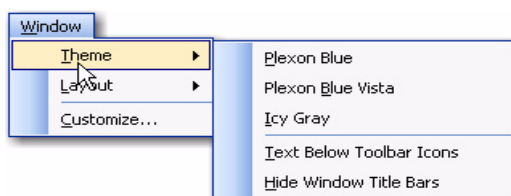
In Plexon software applications, some menu items may have the same functionality across several applications. These items are standard menu items and consist of the **Window** menu, the **Run** menu, and the **Help** menu.

A.3.1 Window Menu

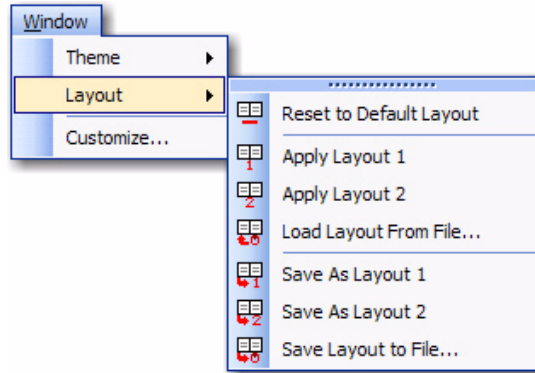
The **Window** menu contains three items: **Theme**, **Layout**, and **Customize**.



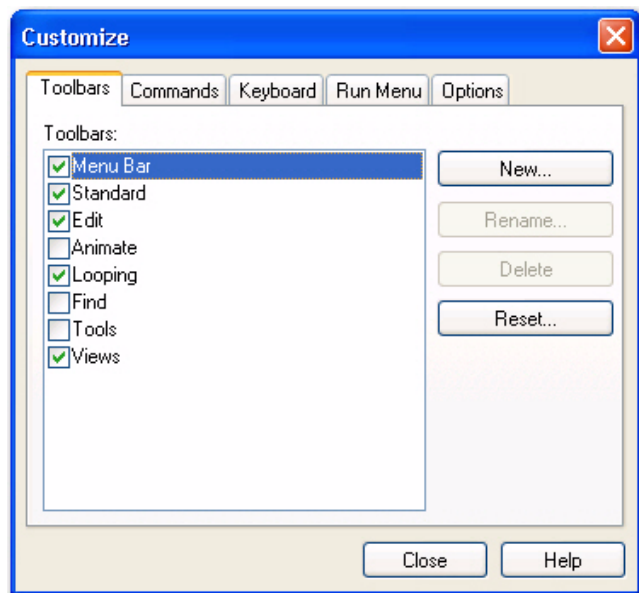
- **Theme** - Clicking on **Theme** displays a menu as shown below. A theme is a color scheme that is part of the look-and-feel of the user interface.



- **Theme Group** - The top group of items are theme toggle items that may be selected to apply to the user interface look-and-feel. Only one of the themes may be selected at a time.
 - **Text Below Toolbar Icons** - This item is a toggle item to show or not show text below the toolbar icons.
 - **Hide Window Title Bars** - This item is a toggle item to hide or show window title bars.
- **Layout** - Clicking on **Layout** displays a menu as shown below. A layout is the size and placement of the windows on the screen. It also remembers the number and placement of toolbars.



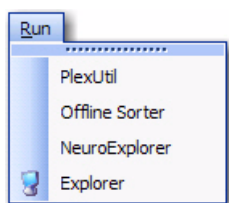
- **Reset to Default Layout** - Clicking on this item resets the layout to the factory default.
- **Load Layout Group** - Clicking on **Apply Layout 1** or **Apply Layout 2** applies one of the standard layouts to the user interface. Clicking on **Load Layout From File** will allow you to select a layout file to apply to the user interface.
- **Save Layout Group** - Clicking on **Save As Layout 1** or **Save As Layout 2** saves the current screen layout as one of the two standard layouts. You may also click **Save Layout to File** to save the current screen layout to a file that can be loaded by the **Load Layout From File** item.
- **Customize** - Clicking **Customize** displays the **Customize** dialog box as shown below



For details on using the **Customize** dialog box, see [“Customization” on page A-19](#).

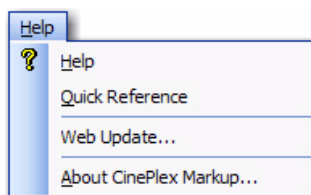
A.3.2 Run Menu

The purpose of the **Run** menu is to launch other applications quickly. The **Run** menu will be populated by default with some common Plexon applications. If you wish to be able to launch other applications from within the current application, you can add new entries to the **Run** menu. A sample **Run** menu is shown below. For additional information about customizing the **Run** menu, see [“Run Menu Customization”](#) on page A-22.

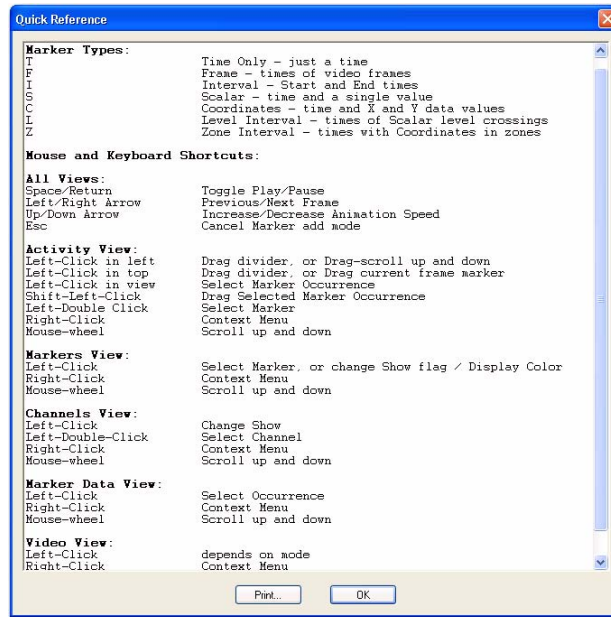


A.3.3 Help Menu

The **Help** menu contains four items: **Help**, **Quick Reference**, **Web Update**, and **About XXXXX** where **XXXXX** is the name of the application.

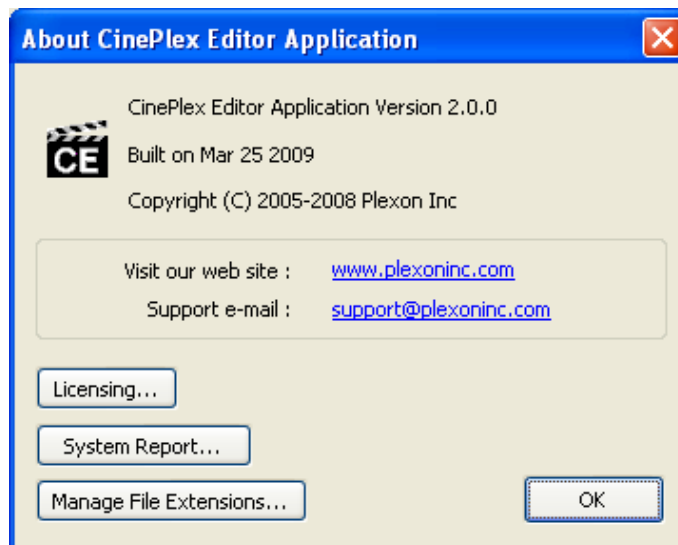


- **Help** - Clicking **Help** displays the User's Guide for the application.
- **Quick Reference** - Quick Reference may be missing in some applications. When present, clicking **Quick Reference** displays a dialog box similar to the one shown below. The purpose of the **Quick Reference** dialog box is to show keys and mouse buttons that control some of the commonly used functions in various views. You may print it by clicking **Print** or close it by clicking **OK**.

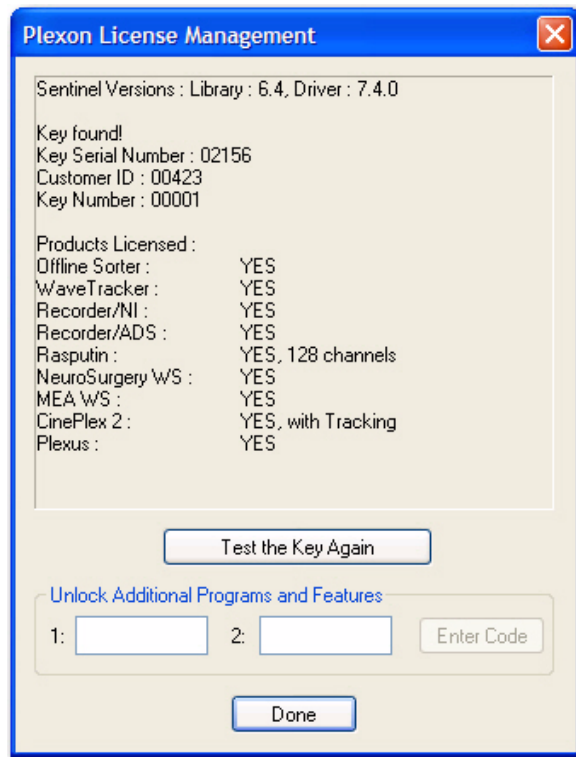


— **Print** – Click **Print** to print a copy of the Quick Reference.

- **Web Update** - This item allows you to update your software via the internet. Clicking **Web Update** will first check to see if an update is available. If an update is available, it will be downloaded and installed.
- **About** - Clicking the **About** item displays the **About** dialog box. The text of the **About** item varies according to the application. The **About** dialog contains the version number and build data of the application, links to the Plexon website and support e-mail, and buttons for **Licensing**, **System Report**, and **Manage File Extensions**.

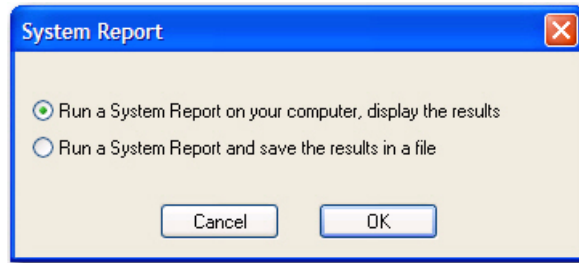


- **Licensing** - Clicking the **Licensing** button displays the **Plexon License Management** dialog box. The **Plexon License Management** window includes the complete licensing information for Plexon products. The window includes the following three areas: information, key testing, and code entry.

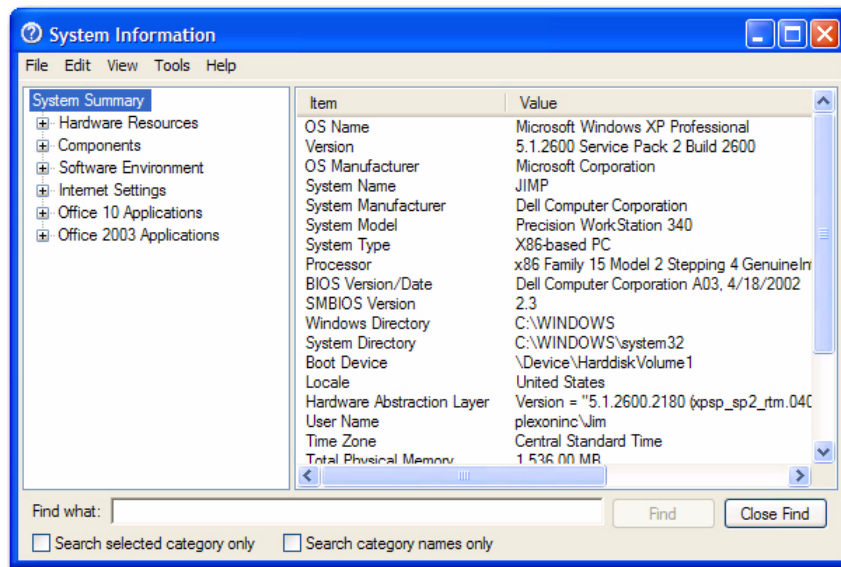


- The information area includes information on license keys and a list of all the Plexon products and their licensed status on this PC.
 - If you have moved or added a key, the **Test the Key Again** button provides a convenient tool to test license keys to ensure they function correctly.
 - If you have more than one key installed, the **Next Key>>** and **<<Prev Key** buttons appear. You may use these buttons to cycle through and test all keys.
 - The code entry area is used to enter the unlock codes for optional programs and features. If you have purchased optional items, instructions for entering codes and testing keys are included with Plexon installation programs.
- **System Report** - The purpose of the **System Report** button is to help Plexon Support diagnose problems by listing system information. Clicking the **System Report** button will first display a dialog box to

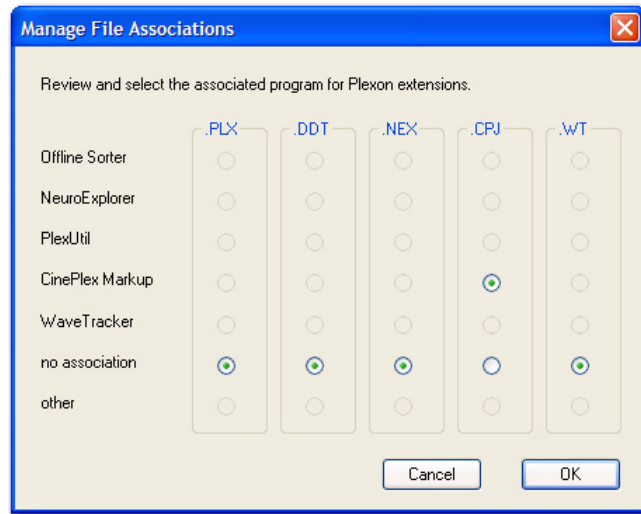
allow you to display the system report on the monitor or save the report to a file that can be sent via E-mail to Plexon Support.



- After selecting a choice, clicking **OK** will launch the standard Microsoft System Information tool. The illustration below shows the System Information report displayed on the monitor.



- **Manage File Extensions** - Clicking the **Manage File Extensions** button opens the **Manage File Associations** dialog box. The **Manage File Associations** dialog box allows you to review and select which file types are associated with Plexon software applications. For example, when the .plx file type is associated with **Offline Sorter**, double-clicking on a .plx file will open **Offline Sorter** and load that .plx file. If you have not installed some of the applications, their file extension radio buttons will be disabled.

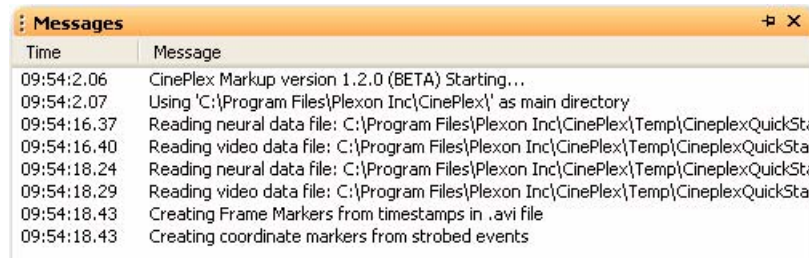


Note: On Windows Vista, the Manage File Associations dialog will only be able to update file associations if Offline Sorter is run with administrator privileges.

A.3.4 Messages Window

The **Messages** window displays a log of timestamps and associated application events that Plexon Support can use for troubleshooting purposes.

Note: The **Messages** window is not available for all applications.



Right-clicking the mouse in the **Messages** window will display a right-click menu with the following items:

- **Erase** - This item clears the window of all messages
- **Pause** - This item stops the logging of messages
- **Show Debug Messages** - This item is a toggle to show or hide debug messages
- **Select and Copy All** - This item allows you to copy all of the messages to another application - such as a word processor
- **Save Log to File** - This item allows you to save the messages to a log file.
- **Mail Log to Plexon** - This item allows you to send the message log to Plexon for troubleshooting purposes

A.3.5 Right-click Menus

Most windows have right-click menus that control their behavior and options. To open a right-click menu, place the cursor inside a window and click and release the right mouse button. The right-click menu appears where you click. To select a menu item, move the cursor over it and click the left mouse button.

A.3.6 Current Selections

In grid-based windows, the currently selected item always appears with a >> or > in the left column of the appropriate grid-based window.

A.3.7 . Undo

Plexon applications provide multiple *undo* levels. To undo an operation, on the **Edit** menu, click **Undo** or click the **Undo** button on the toolbar. You can undo operations that change the contents of the project file, but you cannot undo operations that change the user interface options or colors.

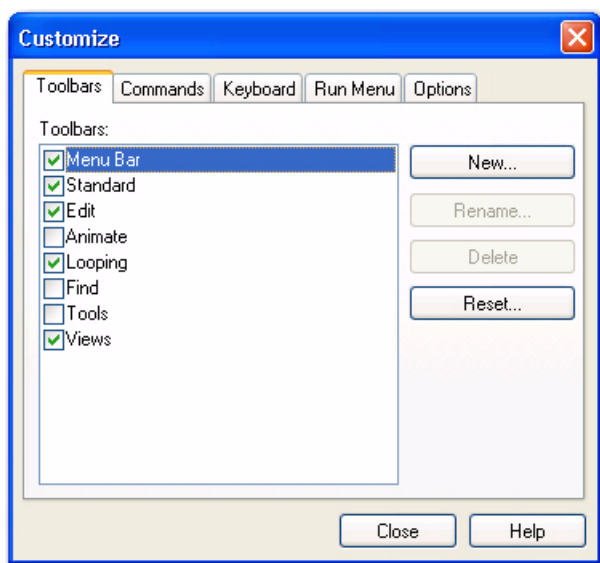
A.4 Customization

Although the menus and toolbars offer a rich set of commands and functions that should meet the needs of most Plexon customers, the **Customize** dialog box also allows you to customize several areas of the interface should the need ever arise. This section describes the uses of the **Customize** dialog box.

To open the **Customize** dialog box, from the **Tools** menu, select **Customize**. The **Customize** dialog box contains several tabs. The illustrations below show the **Customize** dialog box from **CinePlex Editor** and displays five tabs - **Toolbars**, **Commands**, **Keyboard**, **Run Menu**, and **Options**. Although the content of the tabs will vary according to the application, the functional operation of each tab is respectively the same across all Plexon software applications.

A.4.1 Toolbars Customization

An illustration of the **Toolbars** tab follows:



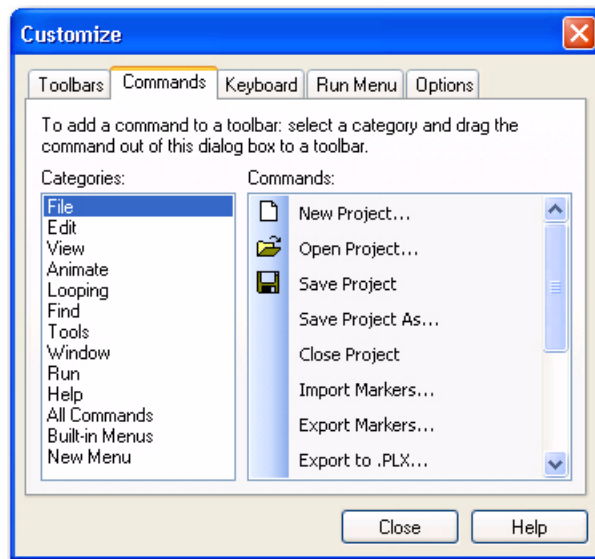
- **Toolbars** - This box contains a list of the toolbars for the application. Click a toolbar checkbox to have it appear in the main application window.
- **New** - This button opens the **New Toolbar** dialog box. You can use this feature to create a custom toolbar for commands you frequently use. Enter a toolbar name in the **Toolbar name** box.
- **Rename** - If you have selected a toolbar that you previously defined, click **Rename** to change the name of the toolbar. The **Rename Toolbar** dialog box displays.
- **Delete** - If you have defined a new toolbar and selected it, you can click **Delete** to remove that toolbar. There are no default values for new toolbars

you define. You cannot delete the standard toolbars. A confirmation dialog box displays.

- **Reset** - If you have selected a standard toolbar, click **Reset** to restore the toolbar to its default contents. If you have dragged new buttons to a toolbar, click **Reset** to restore the default version of the toolbar. A confirmation dialog box displays.

A.4.2 Commands Customization

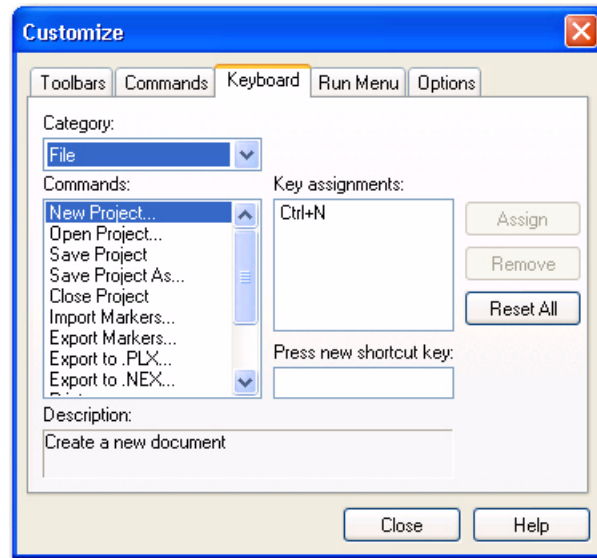
The **Commands** tab is used to customize which commands are available in toolbars. An illustration of the **Commands** tab follows:



- **Categories** - This is a list of all the toolbar categories. Select a toolbar category to see the buttons in the **Commands** area.
- **Commands** - This area shows all the buttons and the associated menu commands that belong to the category you select. You can select the command you want and drag it to the toolbar.

A.4.3 Keyboard Customization

The **Keyboard** tab allows you to bind keystrokes to commands. An illustration of the **Keyboard** tab follows:



- **Category** - This is a list of all the main menu headings. Select a menu heading to category to see the associated commands in the **Commands** area.
- **Commands** - This is a list of all the commands associated with the selected main menu heading in the **Category** area.
- **Key assignments** - This displays the current key assignment for the command selected in the **Commands** area.
- **Press new shortcut key** - This allows you to enter a shortcut key combination for the command selected in the **Commands** area.
- **Description** - This area displays a description of the currently selected command in the **Commands** area.
- **Assign** - This button assigns the shortcut in the **Press New Shortcut Key** area to the selected command in the **Commands** area. If the shortcut key is already assigned to another command, a confirmation dialog box displays to allow or cancel the reassignment.
- **Remove** - This button removes the selected shortcut key in the **Key Assignments** area from the selected command in the **Commands** area.
- **Reset All** - This button removes all custom key assignments. A confirmation dialog box displays to allow or cancel the operation.

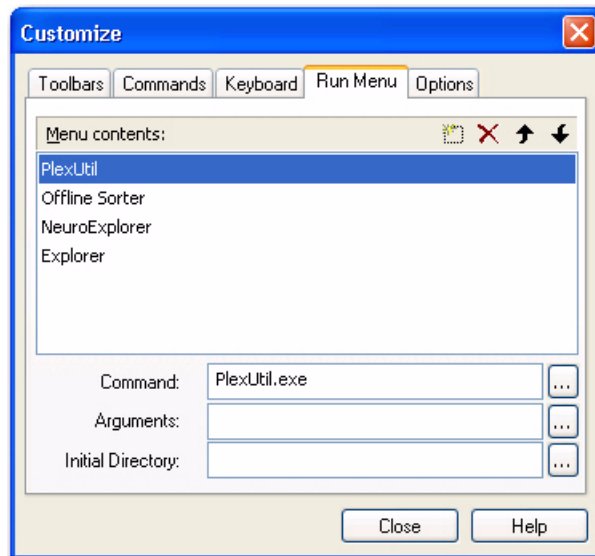
Procedure for Customizing Keystroke Shortcuts

- 1 From the **Window** menu, select **Customize**, and then click the **Keyboard** tab of the **Customize** dialog box.
- 2 Choose a category from the **Category** dropdown and from the **Commands** list, select the command that you want to bind to a keystroke shortcut.
- 3 If there is already a key assignment listed in the **Key assignments** area, you may remove it by clicking the **Remove** button if you wish. You may also just reassign a new key combination to the selected command. (See Step 5)
- 4 Click the mouse in the **Press new shortcut key** area.
- 5 Click the **CTRL** or **ALT** key and hold it down while clicking another key. The dual key combination will be displayed in the **Press new shortcut key** area. This combination will be the key assignment for the selected command.
- 6 Click the **Assign** button to assign the key combination to the selected command. If there already is a key assignment for the command, a confirmation box will display to allow you to confirm or cancel the reassignment.

Note: In CinePlex Editor do not bind menu operations to keystrokes that are associated with markers. Use only CTRL- or ALT- key combinations to bind to menu items, as these key combinations cannot be associated with markers.

A.4.4 Run Menu Customization

The **Run Menu** tab allows you to manage menu items on the **Run Menu**. An illustration of the **Run Menu** tab follows:



- **Menu contents** - This is a list of all the menu items in the **Run Menu**. Select an item to see its characteristics in the **Command**, **Arguments**, and **Initial Directory** areas.

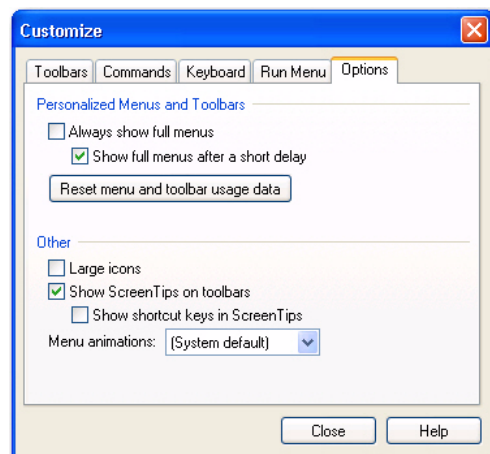
- **Buttons** - The toolbar buttons allow you to add a new menu item, delete a selected menu item, move the selected menu item upward in the list, and move the selected menu item downward in the list.
- **Command** - This area contains the name of the file that runs when the menu item is selected from the **Run Menu**. The browse button opens the standard **Open** dialog box filtered for executable files.
- **Arguments** - This is a list of all the arguments to be passed to the file in the **Command** area, if any. The browse button opens the standard **Open** dialog box filtered for all files. When applicable, the symbol “%1” can be used to denote the current document.
- **Initial Directory** - This contains the path of the file in the **Command** area. The path allows the system to find the file and execute it. The Browse button opens the standard **Browse For Folder** dialog box.

Procedure for Customizing the Run Menu

- 1 From the **Window** menu, select **Customize**, and click the **Run Menu** tab.
- 2 To add a menu item, click the **New** icon, enter the command in the **Command** text box, enter any arguments in the **Arguments** text box, and set the applications directory in the **Initial Directory** text box. In each case you may use the appropriate **Browse** button at the right of the text box. The **Browse** button for the command opens the standard **File Open** dialog box filtered for executable files. The **Browse** button for the arguments opens the standard **File Open** dialog box filtered for all files. Then **Browse** button for the initial directory opens the **Browse for Folder** dialog box.
- 3 To delete a menu item, select it and click the **Delete** button.
- 4 To reorder the menu items, select a menu item and use the **Up** and/or **Down** arrow buttons to reorder.

A.4.5 Options Customization

An illustration of the **Options** tab follows:



- **Personalized Menus and Toolbars** - This area contains two check boxes and a button. The application will hide infrequently used menu items, but you may customize the display of menu items using these two checkboxes. If you check the **Always show full menus** checkbox, the application will always show full menus and the **Show full menus after a short delay** checkbox will be disabled. If you do not check the **Always show full menus** checkbox, you will have the option of checking or clearing the **Show full menus after a short delay** checkbox. The **Reset menu and toolbar usage data** button will allow you to delete the record of commands you have used in the application and restore the default set of visible commands to the menus and toolbars. A confirmation dialog displays.
- **Other** - This area contains three checkboxes and a dropdown list. If you check the **Large icons** checkbox, the application will use large icons on the toolbar. If you check the **Show Screentips on toolbars** checkbox you will also have the option of checking the **Show shortcut keys in Screen Tips** checkbox. The **Menu animations** dropdown list allows you to select the type of animation to be used on menus that have animation.

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Data Analysis Software
Offline Sorter
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