PLEXON®
Neurotechnology Research Systems

FPAlign Utility Guide

Field Potential Time Alignment Tool v2.0 for
OmniPlex® Neural Data Acquisition System and
Multichannel Acquisition Processor (MAP) Data Acquisition System

FPAlign Utility Guide

Contents

Documentation History

Date	Version	Notes	Author
August 2013	FPATN0001a	- Minor edits. - Reformatted document to current branding guidelines.	Stacie Hyatt Yolanda Rowe
February 2012	v2.0	- Updated content.	Larry Spence
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Introduction

This guide provides an overview of the issue of filter-induced phase shifts (frequency-dependent timing delays) which can be induced by the analog and digital filters used in Plexon neural data acquisition systems, and the use of the FPAlign utility to correct for these effects in the field potential (FP) band. FPAlign can be used to correct FP timing delays in PLX files recorded with the following Plexon systems:

- OmniPlex® D Neural Data Acquisition System with DigiAmp™ or MiniDigi™ Digitizing Amplifiers (OmniPlex D)
- OmniPlex Neural Data Acquisition System with analog preamplifier (OmniPlex)
- Multichannel Acquisition Processor (MAP) Data Acquisition System and its PBX preamplifier

For the purposes of this document, all "OmniPlex" mentions refer to both the OmniPlex and OmniPlex D Systems, unless otherwise specified. Also, the terms "preamplifier" and "amplifier" are used interchangeably throughout this document.

Overview

Plexon neural preamplifiers incorporate per-channel analog filters for removing spectral content outside of the frequency ranges of interest. For the purposes of FPAlign, we are mainly interested in the field potential (FP) range, which extends from near-DC to 200 or 300Hz. The PBX uses dedicated FP boards with fixed-frequency, low-pass filters whose output is typically digitized by one or more NIDAQ cards at a rate of 1kHz per channel. The OmniPlex System does not use separate spike and FP boards, instead employing wideband preamplification with a low-pass cutoff at 8kHz. The wideband signal is then digitized at 40kHz, and subsequent software-programmable digital filters are used to extract the spike and FP bands, allowing more flexibility than the MAP system.

In both the PBX and OmniPlex amplifiers, there is also high-pass analog filtering in order to block extremely low frequencies and DC offsets. In the PBX, the FP boards have a fixed high-pass cutoff of around 0.7-3.3Hz (depending on which model of PBX is used). The OmniPlex preamplifier has a software-selectable cutoff of 0.05, 0.5, 3 or 100Hz, although the latter is useful when only spikes—and not FPs—are to be acquired. The PBX FP filters are Butterworths, either one or two poles for the high-pass, and one, two, or four poles for the low-pass. The analog wideband OmniPlex filters are Bessels, one pole for the high-pass and four pole for the low-pass. The OmniPlex software-programmable low-pass filters can be two to twelve poles, Butterworth, Bessel, or Elliptic, with a user-defined cutoff frequency; the default setting is a four pole Bessel, with the cutoff at 200Hz.

In addition, if a Plexon AC-coupled headstage is used, its AC coupling is implemented by an additional one pole high-pass filter with a fixed cutoff of either 0.33Hz (HST/8o50-G20) or 0.8Hz (HST/16V-G20, HST/32V-G20), preceding the preamp filters. Finally, the optional Plexon preamp breakout panel (PBOB), which can be inserted between the PBX output and the NIDAQ A/D card input(s), is AC-coupled, also using a one pole high-pass filter, with a cutoff of 0.7Hz. However, note that this AC-coupling is bypassed when the PBOB jumpers (rows of 16 jumpers labeled "DC COUPLING") are in place. Typically, either the AC-coupling in the headstage or the PBOB is used, but not both.

All these filters, like most filters, inherently produce phase shifts which vary with frequency. The phase shifts are equivalent to time delays (known as group delays) which likewise vary with frequency. In particular, when the high-pass filter cutoff is very low, the delays at low frequencies near the cutoff can be significant, and this can result in time misalignment with respect to higher-frequency signals such as spikes whose filtering does not exhibit significant time delays. One way of avoiding this problem is to make sure the low-end filter cutoff is a decade or more below the lowest field potential frequency of interest. In the case of the common 0.77Hz, two pole high-pass filter in the PBX, the filter will cause signals above 10Hz to be delayed (as measured by group delay) by less than three milliseconds, which is small compared to the period of the signal at that frequency. However, it is possible to post-process field potential recordings so that the time misalignment at lower frequencies is greatly reduced; this allows signals near the cutoff frequency to be used in subsequent analyses without large timing errors. The 0.05Hz high-pass setting in the OmniPlex amplifier was included in order to allow recording of very low frequency FPs without large phase shifts. However, in some situations, large amounts of near-DC at the recording electrode can be problematic, making it preferable to use a higher cutoff frequencies (e.g. the 0.5Hz or 3Hz settings) combined with post-processing to remove the filter-induced time misalignments.

About FPAlign

FPAlign is a utility program that "undoes" these filter-induced timing delays in field potential channels ("slow channels") in Plexon PLX recording files. The approach used is to apply a digital model of the analog filters that were used in the hardware and software filters for the original recording, but applying the filters to the recorded signal in time-reversed order, thereby cancelling most of the time delays caused by the original filters. This also has the effect of applying the magnitude (amplitude) characteristic of the filter a second time, effectively making the cutoff slopes twice as steep. For many applications, this is an advantage in that undesirable, out-of-band signals (such as spikes and very low frequency artifacts) are further attenuated. However, it should be noted that any analysis of the amplitude of the FPs should be performed on the original recorded data before applying FPAlign's processing. FPAlign never alters the input file. Rather, it writes the results of the phase correction to a new PLX file with the suffix "-aligned" appended to the original filename.

FPAlign cannot completely eliminate the filter-induced delays due to the non-zero tolerances of the components used in the analog filters. The residual delay corresponds to the difference between the ideal filter model and the actual filter response resulting from a particular set of real-world electronic components. However, even without measurement of individual headstages and preamps, the group delay can be reduced to where it is not an issue in most cases. On the other hand, the delays caused by the software "FP separator" low-pass filter in OmniPlex can be completely removed, since these are software filters and not subject to physical component tolerances like the preamp filters.

> **NOTE:** FPAlign can only work correctly when the preamp, headstage, PBOB, and (for OmniPlex) software filtering that were used at the time of recording are known, so that the appropriate reverse filtering can be applied. Do not use FPAlign if you are not sure what filters were used when the data was recorded.
>
> Plexon PBX preamps usually have a sticker on the underside of the blue aluminum enclosure indicating the filter characteristics for the spike and field potential boards. The individual preamp boards are also labeled with their filter characteristics. If there is any question of boards having been swapped or replaced, these board labels should also be examined. Please contact support@plexon.com for assistance.
>
> You must also determine the type of headstage that was used in recording, and whether AC coupling was enabled on the PBOB, if used. If you are unsure which model of headstage you have, please contact support@plexon.com for assistance. The OmniPlex amplifier is (as of this writing) produced with software-selectable high-pass cutoffs of 0.05, 0.5, 3 or 100Hz, so there is no need to examine stickers or circuit boards, but the user must keep track of which high-pass filter setting was selected at the time of recording. The default cutoff is 0.5Hz.

Using FPAlign

To run FPAlign, double click on FPAlign.exe or its desktop shortcut. Users on 64 bit versions of Windows® can also run


the 64 bit version, FPAlign64.exe.

The following FPAlign window appears. First, set the options which indicate the filter(s) which were in effect when the file was recorded. In most cases, the Preamp Filters section will be applicable, and depending on your system, you may also need to set options for Headstage Filters, Software FP Separator Filter, and/or AC-coupled PBOB. Each of these will be described below.
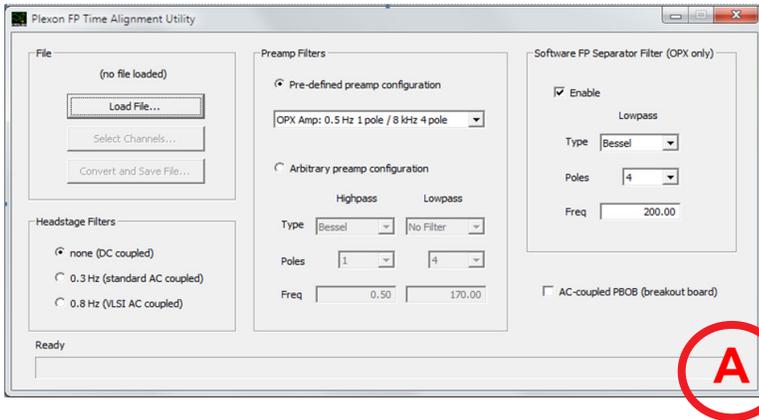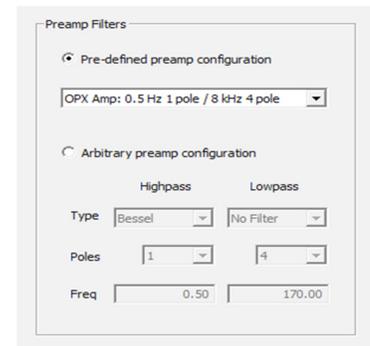
Headstage Filters

If the data was recorded with a DC-coupled headstage, select "none" to indicate that headstage correction is not necessary. Otherwise, select the applicable Plexon AC-coupled headstage. See the remarks in the Overview section regarding AC-coupled headstages. If you are unsure which headstage you are using, please contact support@plexon.com for assistance.
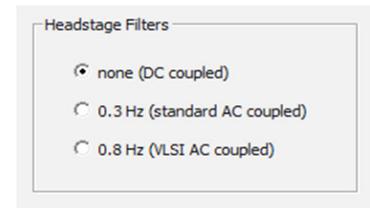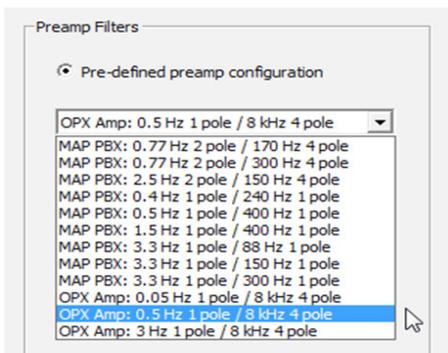
Preamp Filters

The Preamp Filters dialogue box to the right contains options for the high-pass (low-cut) and low-pass (high-cut) analog hardware filters present in a typical preamp. You can either select from a predefined list of Plexon preamps, or define the characteristics of an arbitrary (custom) preamp, for preamps not included in the list.

If you click the Pre-defined preamp configuration button in the dialogue box illustrated below, you may then use the drop-down list to select the desired preamp. Preamps used in the MAP (Harvey Box) and OmniPlex Systems are listed. The three entries for the OmniPlex amplifier represent three of the four software-selectable high-pass cutoff frequencies; the 100Hz setting is not displayed, since this setting is not used to record field potentials. Also, note that while the 8kHz anti-aliasing filter in the OmniPlex amplifier is listed, FPAlign does not apply any correction for it since its cutoff is well above the sampling rates typically used for field potentials. The OmniPlex Software low-pass filter which is described later takes the place of the hardware low-pass filters used in the MAP PBX preamps.

When you select one of the predefined configurations, these settings are also copied to the controls in the "Arbitrary preamp configuration" area seen in window A above, in case you wish to use one of the predefined configurations as a starting point for a custom configuration.

If you click the "Arbitrary preamp configuration" button as shown here, you may manually enter the low-pass and/or high-pass characteristics for preamps/filters which are not included in the predefined list. Each filter can be Butterworth, Bessel, or Elliptic (Cauer); one, two or four poles, and have a user-defined cutoff frequency. The high-pass and low-pass settings are completely independent. Following normal usage, FPAlign defines the cutoff frequency as the frequency where the amplitude response is reduced by 3dB. Selecting a filter type of "No Filter" for either filter means that no phase correction will be applied, and the remaining parameters for that filter will be ignored. Also, the distinction between Butterworth, Bessel, and Elliptic filters only comes into play at two or more poles. So, for a one pole filter, the filter type setting is ignored.

For non-Plexon preamps, you may need to contact the manufacturer to determine the filter characteristics so that you may specify them in FPAlign.

Note that, according to basic filtering theory, cutoff frequencies must be less than half the sampling rate, and a high-pass filter would typically have a cutoff well below this value (otherwise, most of the signal in the FP band would be attenuated). If your preamp has a low-pass filter whose cutoff is greater than half the sampling rate, it will have no effect on the digitized FP signals, and you should select No Filter in FPAlign; however, in such a case it is very likely that a software low-pass filter was used during acquisition, and you may correct for it using the Software FP Separator Filter options described later.

As a rough rule of thumb, for a Butterworth or Bessel one or two pole high-pass filter, FP components at greater than 10 times the cutoff frequency will not be significantly affected by phase shifts. If the OmniPlex Amp is used with the 0.05Hz high-pass cutoff selected, it is arguable whether it is even necessary to correct for the high-pass filter phase shifts, although the option is provided for those who desire to use it.

Although the term "preamp" has been used throughout, the actual preamplification (i.e. analog gain of the preamp or headstage) need not be specified, since it is irrelevant to FPAlign's phase correction processing.

Software FP Separator Filter (OmniPlex only)

If the FP data was acquired using an OmniPlex System, it was recorded using a wideband preamp and digitized at a 40kHz sampling rate, with subsequent online software low-pass filtering (default: four pole Bessel, 200Hz cutoff) and down-sampling to a "slow channel" rate, typically 1kHz. You can use the options in this section to completely correct for the phase shifts caused by this software low-pass filter.

The settings here are similar to those in the "Arbitrary preamp configuration" area seen in window A on page 5, except that the number of poles can be 2, 4, 6, 8, 10 or 12, corresponding to the settings supported by the OmniPlex FP Separator filter. You can see the parameters of this filter in OmniPlex by right-clicking on the FP Separator device shown in the OmniPlex Server topology diagram and selecting Device Options. You must stop data acquisition in OmniPlex before you can view and change the OmniPlex filter settings. The filter settings are saved by Server in its PXS file.

The software FP Separator does not apply to MAP Systems; make sure that Enable is unchecked when processing files recorded by MAP Systems.

AC-Coupled PBOB

As described in the Overview section, MAP Systems can optionally have a PBOB to allow easy access to NIDAQ input channels. If a PBOB with AC coupling enabled via jumpers was used during recording, select this option to correct for the PBOB phase shifts:

☐ AC-coupled PBOB (breakout board)

Loading a PLX File

Click the Load File button and specify the PLX file to be processed. Once you click Open in the File Open dialog, the filename is displayed. Only the file headers are loaded at this point, so even a very large file will appear to load quickly.
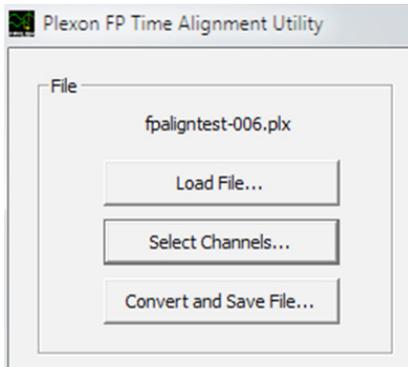

Selecting Channels to be Aligned

By default, every channel of continuous data in the file is assumed to contain field potentials that are to be time-aligned. However, if your file also contains non-FP continuous channels (such as continuous measurements of position or velocity, or field potentials which did not go through a Plexon field potential preamp), they must not be subjected to alignment processing, or they may be distorted or corrupted.

After loading a file, you may specify which channels are to be aligned by clicking the Select Channels button. The Select Channels dialog box will then appear and all the continuous channels in the file are listed with their names and digitizing rates. By default, all channels are selected (enabled for alignment), as long as all the digitizing rates are the same. If not, then all channels whose rate is the same as the first channel will be selected. You may change the default selection by clicking on individual channels to select or deselect them, and/or via the Select All, Deselect All, and Invert Selection buttons. Channels that are not selected will be copied to the output file unchanged. Click OK to accept the list of selected channels.

> **NOTE:** If you do not use the Select Channels dialog, every continuous channel will be selected for alignment.
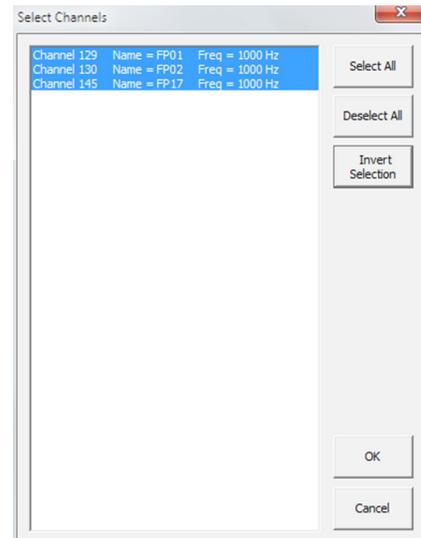
Aligning and Saving the File

You can now save a corrected, time-aligned version of the file by clicking Save File. Depending on file size and the field potential sampling rate used in the file, this can be a lengthy operation. The progress indicator at the bottom of the FPAlign window will show the following status messages during processing:

- Initializing filters...
- Reading data....
- Aligning channel 1...
- Writing channel 1...
- Aligning channel 2...
- Writing channel 2...

and so on. When processing is complete, a confirmation message is displayed. The output file name will be the same as the inupt file name, with "-aligned" automatically appended to it (e.g. myFP.plx, myFP-aligned.plx).
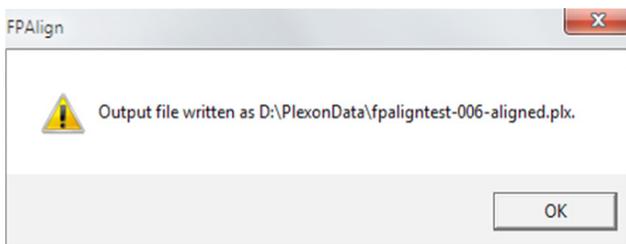
Batch Processing Multiple PLX Files

You may also run FPAlign in batch processing mode. To do so, simply select multiple files in the File Open dialog and click Open. FPAlign asks you to confirm batch processing as depicted in the illustration to the right.

When processing is completed, FPAlign will display a dialog box like the one below to to confirm the number of files converted.
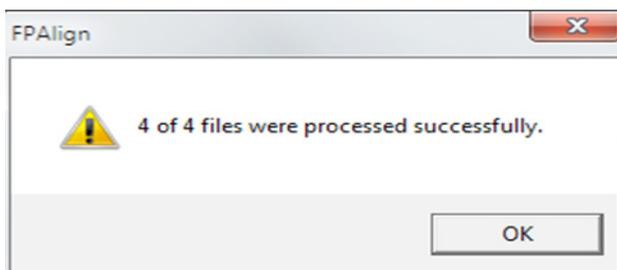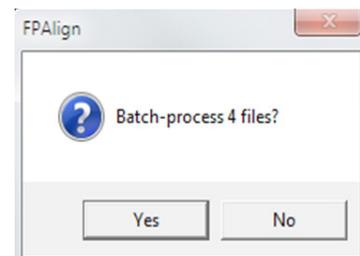
NOTE: Batch processing is only applicable when the same filter characteristics are shared by all the specified files, and when all the continuous channels in each file are to be aligned (i.e. there is currently no way to specify channel subsets for files in a batch).

Limitations

Currently, all the continuous channels in each PLX file are read into memory before being processed and written to the output file. If the input file is too large, the program may become slow or unstable. However, FPAlign64.exe is available for 64 bit versions of Windows and will utilize as much memory as is available.

Starting with version 1.4, processing of continuous channels containing gaps typically caused by pauses in recording is supported with the following caveats:

- FPAlign handles the gaps by zero-filling them as the file is read in, recreating a continuous data stream; note that the pause events are preserved in the output file and can be used to locate the data corresponding to the original input.

- Due to the finite settling time of the filters (see the preceding illustrations), there can be some "spill" of non-zero filtered data into what were originally gaps. Unless the gaps are very brief compared to the settling time of the filters, this effect can be ignored. In cases where the pauses are less than one to two seconds or so, you should consider recording continuously and marking the segments of interest (e.g. trials) with event markers, rather than actually pausing and un-pausing the recording.

- There is currently no way to interrupt processing once you have clicked Save File, so try using FPAlign on smaller files first, to get an idea of the amount of processing time required on your system.

- FPAlign uses SSE2 code to improve performance, so a Pentium® 4 or later processor is required.

About Plexon Inc

Plexon is a pioneer and leading innovator of custom, high-performance data acquisition, behavior and analysis solutions specifically designed for scientific research. We collaborate with and supply thousands of customers including the most prestigious neuroscience laboratories around the globe driving new frontiers in areas including basic science, brain-machine interfaces (BMI), neurodegenerative diseases, addictive behaviors and neuroprosthetics. Plexon offers integrated solutions for *in vivo* neurophysiology, optogenetics, and behavioral research – backed by its industry-leading commitment to quality and customer support. For more information, please visit www.plexon.com.

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