Webinar Q&A Report:

Online Neural Data Processing with Plexon OmniPlex: Referencing, Sorting and Online Manipulation

1. **Can you alter your referencing while recording, for example, if you notice that a unit is being reflected in every channel?**

   Yes, the referencing can be selected on-the-fly. Just be sure to keep track of what is being changed and watch the monitor channel closely. Any change in the reference will be applied at the time of the change. This means a change mid-recording will only impact the data after the change was made. Also, we strongly recommend recording the wideband data to ensure that an “original” version of the signal is available to work with offline.

2. **Do we need to upgrade the hardware (purchased 1-2 years ago) to get the new software referencing feature? Or is the software upgrade sufficient?**

   The referencing features depicted in the webinar became available in PlexControl (OmniPlex software) in version 1.16. Any supported OmniPlex hardware that is running with updated software will enable these features. The newest version of the OmniPlex software is available on the Plexon website here:

   http://www.plexon.com/products/omniplex-d-neural-data-acquisition-system-1

3. **Is same referencing useful (same channel) for spikes and FP?**

   The SPKC and FP CAR & CMR groups are completely independent. For example, a user can define one CAR group and three CMR groups on the SPKC signal, while defining two CAR groups and one CMR group on the FP channels. It is up to the user to decide if/when referencing the SPKC signal differently or the same as the FP signal is appropriate.

4. **Please could you explain again the difference between CAR and CMR? and CAR1/2/3**

   CMR is the Common Median Reference. The median of the signal, or signals, is calculated on a sample-by-sample basis and then subtracted from all of the channels to which the CMR is applied. CMR 1/2/3/4 allows the user to increase specificity by applying the calculated median to a subset of channels (e.g. CMR 2). For example, the user could apply CMR2 to channels 33-64, while using CMR1 to channels 65-128.

   CAR is the Common Average Reference. Each CAR allows the user to define a group of channels (e.g. channels 1-32) that are averaged together to serve as the reference for that CAR group.

5. **What's the difference of different CARs and CMRs? In which case CAR is preferred and in which case the other?**

   In short, CAR stands for Common *Average* Reference and CMR stands for Common *Median* Reference.

   We recommend all systems with fewer than 128 channels to use CMR. That said, the minimum number of channels within a CAR or CMR group (e.g. CAR3 or CMR2) should be 16, but preferably 32 or more. The reasoning behind that is to make sure there is a substantial enough data set for the average or median values to be reasonably representative of the channels to which the respective references are assigned.

   There must be enough channels in each referencing group that the spikes "average out" to near zero, and only the artifact remains in the reference. Be sure to look at the monitor channel (CARmon or CMRmon) to make sure that spikes are not visible.
   For systems with channel counts greater than 128, CAR can be used on any channel count and CMR should not be used on more than 128 channels at the same time.

6. **I implanted stereotrodes that I can use specifically as a reference. In the electrode interface board (EIB) they were threaded into the specific spots for ref (different from ground). So they are not in any of the 1-64 channels. How would I assign these as a reference in PlexControl? Thanks!**

   In PlexControl, it is possible to assign the reference of the headstage (i.e. the point of reference for the acquisition) to either the Ground input of the headstage or the Reference input of the headstage. The reference input of the headstage is sometimes referred to as the "true reference". The headstage referencing settings for OmniPlex are outlined in the OmniPlex User Guide available on the Plexon website. Appendix C: DigiAmp Device Settings outlines the setting for the OmniPlex DigiAmp system. Appendix D: DHP Device Settings outlines the settings for the OmniPlex DHP (digital headstage) system.

7. **What are the best strategies to deal with offline processing of large plx files created by OmniPlex?**

   Generally, large plx files are difficult to work with. The plx file format was developed many years ago. Back then large data files were tens of MB in size. With OmniPlex, it is possible to generate large files (i.e. 10's of GB). For this, it is recommended to use the pl2 file format instead of plx. OmniPlex can record to a pl2 format. Offline Sorter, NeuroExplorer, and Plexon's offline API's are all friendly to pl2. If you have a Plexon acquisition system, it is possible to convert plx files to pl2 files. The benefits of pl2 are outlined in the guide available on the Plexon website here:

   http://www.plexon.com/sites/default/files/downloads/PL2%20File%20System%20Overview.pdf

   If you are not yet using pl2 with OmniPlex, you should be, especially if you are generating large data files.

8. **How is the continuous field potential signal different from the wideband signal? Is it low pass filtered wideband?**

   Yes. The filtering for the wide-band signal is effectively the widest bandpass possible with OmniPlex. This will include "everything". The actual filtering value will vary depending on user settings and OmniPlex system vintage. From this signal, after acquisition but still online, the field potential is parsed. Within OmniPlex Server there is a field potential separator module, which will parse out the field potentials from the wide-band signal. Filtering for the field potential is typically from whatever the low end of the wide-band signal is (depends on OmniPlex system vintage) to 200Hz. The 200Hz lowpass cut-off is user programmable.

9. **How are you playing back recorded data online?**

   During the webinar, I was using a special headstage tester unit that allows for playing back eight channels of data. This is a new product from Plexon. It is similar to the headstage tester unit included with most Plexon system purchases, only with more channels. This new device interfaces to the PC via USB, and has eight channels of output. With this, I used a special .wav file I created of eight channels of previously recorded data. I used Matlab to create the .wav file. There are a number of utilities in Matlab for taking continuous data and converting it to an audio file.

10. **Is the wide-band signal filtered in any way?**

    Yes. The wide-band signal is the widest bandpass available in OmniPlex. It is filtered though, and effectively not raw. How/where the signal is filtered partly depends on the vintage of your OmniPlex system. The OmniPlex system used in the webinar was the digital headstage variety (OmniPlex DHP). This means the filtering for the wide-band signal will take place in the headstage itself (assuming a standard configuration). The user can change the settings of that filtering in the Digital HST Processor module in OmniPlex Server.

    Section 3 Startup (with DHP Subsystem) in the OmniPlex User Guide is a good place to start when looking for information about using OmniPlex Server with a DHP system. Here is a link:

    http://www.plexon.com/sites/default/files/downloads/OmniPlex%20User%20Guide%20v1_15.pdf

    Section 3.4 Step by Step: Specifying Digital Headstage Type is especially useful for getting started. Appendix D: DHP Device Settings – Filtering, Referencing, and Latency is useful for addressing details of filtering.

11. **Which Plexon version is this that you're showing?**

    During the webinar, I was using OmniPlex software version 16.2. This is the latest version available and is downloadable from the Plexon website here:

    http://www.plexon.com/products/omniplex-d-neural-data-acquisition-system-1

12. **Can the hardware reference be recorded and selected as Digital reference?**

    No. The hardware reference (i.e. whether the ground of the electrode, or a dedicated reference in the array) is not acquired. The hardware reference is used as the point of reference for the acquisition. The system is looking at whatever the hardware reference is set to as the point to measure the potential of all the other electrodes against. The reference is not recorded.

13. **Is it possible to select different thresholds for each channel?**

    Yes, thresholds can be set manually on a per-SPKC channel (per-continuous spike channel) basis. However, researchers often find it useful to use PlexControl's auto-thresholding feature. Auto-thresholding can be useful because it allows for setting thresholds in a consistent, well-defined manner. Auto-thresholding can also be convenient when working with an OmniPlex system which is running data acquisition on many channels. The OmniPlex User Guide:

    http://www.plexon.com/sites/default/files/downloads/OmniPlex%20User%20Guide%20v1_15.pdf

    provides details on both automatic and manual thresholding.

14. **Can you align by largest peak in Offline Sorter?**

    Yes, there is waveform alignment functionality in Offline Sorter software. Among numerous waveform alignment options, a user has the choice to align waveforms around a global maximum or a global minimum. Details of this functionality are available in the Offline Sorter User Guide:

    http://www.plexon.com/sites/default/files/downloads/Offline%20Sorter%20v4.3%20User%20Guide.pdf

15. **Can the thresholding mode be also set in Offline Sorter?**

    Yes, there are various thresholding options available within Offline Sorter. Offline Sorter is able to threshold continuously-recorded data, and Offline Sorter is also able to re-threshold on previously-detected spike waveforms.

    An interesting place to look in regards to thresholding is in the Waveform Detection options in Offline Sorter software ("Waveforms"-->"Detect").

16. **For automatic thresholding, does the presence of spontaneous spikes affect the threshold? I mean does it matter if the channel has noise only or if it includes spikes**?

    There is a feature called "use robust statistics to determine thresholds." This feature can help to minimize the influence that the presence of spontaneous spikes can have on auto-thresholding of the signal. Robust statistics can be useful because they are more resistant to the effect of outliers. In the context of auto-thresholding, spikes can be considered outliers, relative to the noise. Robust statistics are discussed further in the OmniPlex User Guide

    http://www.plexon.com/sites/default/files/downloads/OmniPlex%20User%20Guide%20v1_15.pdf

17. **If you reposition your electrode by going deeper into the tissue, do you have to repeat the auto thresholding?**

    If the recording conditions become significantly different as the electrode goes deeper into the tissue, in particular if the noise level changes, performing another auto-thresholding calculation on a new continuous spike snapshot can be useful.

18. **Just to make sure, increasing the length of the window would also prevent detection of a new waveform within the longer time window, right?**

    Setting the waveform length too short can make differentiating spikes with long action potentials difficult. This was the example used in the webinar. However, setting the waveform length too long can also have unintended results. Whenever a threshold crossing is detected, the entire waveform must resolve before another waveform is detected. This means a long waveform length with short action potentials can create one long waveform with multiple spikes. This can make spike sorting difficult. The waveform length setting should be set carefully. It is always possible to rethreshold data offline in Offline Sorter, so long as the SPKC (continuous spike signal) is saved.

    More discussion on waveform length settings and other considerations are also discussed in the OmniPlex User Guide

    http://www.plexon.com/sites/default/files/downloads/OmniPlex%20User%20Guide%20v1_15.pdf

19. Is there any export of the mean and standard error for the sorted spike amplitude and frequency?

No. There is no way to export the mean and standard error of the sorted spike amplitude and frequency. This would need to be calculated offline over the whole collected data set.

20. Can the parameters of the sorting be saved and reused next time? How to save the sorted data?

Yes, it is possible to save a configuration file in PlexControl. If you go to File | Save As, you will then be prompted to enter a filename for a PlexControl configuration file or pxc file. Once saved, you now have all of your sorting parameters, visualizations (window arrangements and contents), and properties from the properties spreadsheet saved for next time.

21. Can you use a combination of line sorting and sorting using PCA space?

Yes, so long as the sorting method that you use remains the same. For instance, in Template sorting mode you can both draw lines to calculate a template, or you can draw contours in feature space to calculate a template. However, drawing a line in Template sorting mode is different from drawing a line in Line sorting mode. In Template sorting mode, drawing a line or contour selects the waveforms used to calculate the template and fit tolerance, which then all incoming waveforms are compared against and either sorted as part of that unit or left unsorted. Drawing a line in Line sorting mode sorts all the waveforms that cross that line. In 2D Polygon mode, it is only the waveforms plotted into the contour that are sorted as part of that unit. It is not possible to combine Line sorting and 2D Polygon sorting mode online.

22. Can you eliminate misclassified waveforms by looking at Inter-stimulus interval?

Sorted waveforms that violate an inter-spike interval (ISI) cannot be automatically removed online in OmniPlex. This is possible in Offline Sorter, but not automatically possible in OmniPlex. However, PlexControl provides information on incoming waveforms that violate a certain minimum ISI threshold. Online, there is a small red bar that appears next to each defined unit known as the “short ISI” bar, along with a percentage. This percentage represents the number of incoming waveforms that violate the expected minimum ISI. The minimum ISI is the amount of time you expect a unit to be quiescent before firing another spike.

23. Can you take a snapshot or alter your sort method during recording?

Yes, it is possible to take a snapshot during recording. It is also possible to sort units while data is being recorded. However, you cannot change your sort method during recording. In order to change the sort method, you have to stop recording AND stop data acquisition. Only once both conditions are met can you change your sort method.

24. How can I make sure the same neuron is not being recorded in different channels with different amplitude?

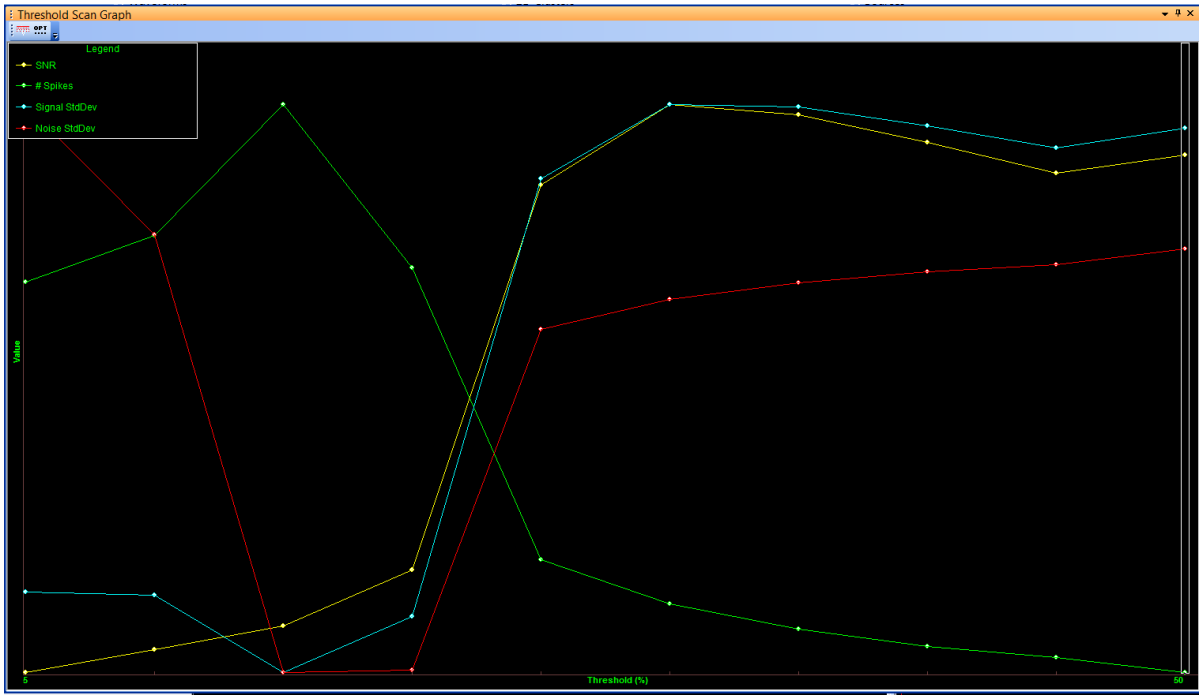
One way to do this is to look at a cross-correlogram of the units sorted online. This isn’t possible with OmniPlex, but it is possible to run NeuroExplorer online and look at data as it is being acquired. In NeuroExplorer it is possible to create online cross-correlograms on OmniPlex data as it is being acquired.

25. Is it possible save the sorting parameters for future recording session?

Yes, it is possible to save a configuration file in PlexControl. If you go to File | Save As, you will then be prompted to enter a filename for a PlexControl configuration file or pxc file. Once saved, you now have all of your sorting parameters, visualizations (window arrangements and contents), and properties from the properties spreadsheet saved for next time.

26. Does the software provide signal to noise ratio? If so how is it calculated and how is data saved?

While the PlexControl software does not provide a real time SNR, our Offline Sorter v4 software does provide an SNR in our "Threshold Scan Graph." This graph will calculate the SNR over multiple different thresholds (hence the "Scan") and plot the # of spike sorted against the SNR at different thresholds. A sample picture below:



27. If a tetrode is used, the same unit could be recorded on different channels: Is the software able to identify that?

No, instead the software concatenates the waveforms recorded from each electrode within a tetrode and uses this as more inputs to calculate a PCA space, allowing for the PCA to better differentiate and distinguish waveforms from different units.

One way to do look at the possibility of the same waveforms being recorded on multiple channels is to look at a cross-correlogram of the units sorted online. This isn't possible with OmniPlex, but it is possible to run NeuroExplorer online and look at data as it is being acquired. In NeuroExplorer it is possible to create online cross-correlograms on OmniPlex data as it is being acquired.

28. Is there any limit to how long you can sort the data online?

We interpreted this question two ways:

1. What's the longest single continuous file you can record from the system?
    - The limit to how long a continuously recorded file can be is 318 days. However, most applications (excluding the latest release of NeuroExplorer) are limited to Plexon file lengths around 29 hours.
    - If your goals is to record for days or weeks at a time, it is recommend to use the recording options to automatically start a new recording file every N hours, and try to keep each file under 2 GB in size. The file size will depend on what's recorded in the file and how many channels are being recorded.
2. How long of a snapshot can you take to sort the data?
    - The maximum recommended snapshot size is a function of the channel count. For a 16 or 32 channel system, it is recommended you can go up to collecting 1000 spikes and possibly more, but at 128 channels and up, it is not advisable to go beyond the default of 500 spikes. If changing between visualizations begins to cause hesitation or drops, the snapshot is too large.

29. If a waveform matches the sorting parameter for both units' a and b, does it prioritize to unit a?

Yes. Unit A is given priority over Unit B; Unit B over Unit C; etc.

30. You mention "well isolated data", does recording from different regions/cell types yield different recording quality? Or is that more of an experimental set up thing (i.e. noisy electrical devices)?

The answer is both. Recording from different regions will yield different recording quality results. For example, The Betz cells of the motor cortex fire larger, higher amplitude spikes compared to other brain regions. Similarly, electrical noise in the recording can impact the isolation of the signal of interest.

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