

# **NEURAL METRIC TOOL**

**USER GUIDE** 

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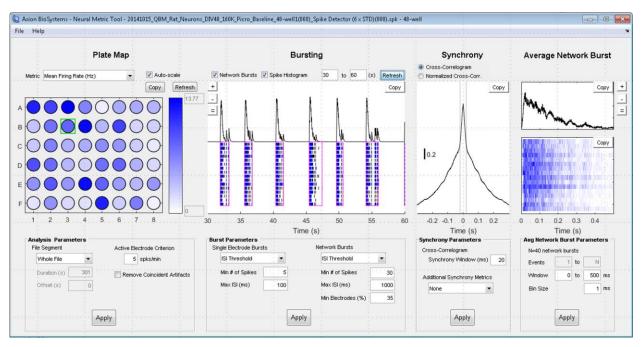
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#### 1. INTRODUCTION

The Neural Metric Tool calculates bursting and synchrony metrics similar to those computed by AxIS, along with additional metrics. It also offers advanced algorithms for burst detection, analysis of stimulation-evoked activity, plate map visualizations, raster plots, and synchrony cross-correlograms not available in AxIS. The Neural Metric Tool now performs these analyses on AxIS .spk files or on sorted spikes stored in .nex files from Plexon Offline Sorter<sup>TM</sup>. See Chapter 8 of the AxIS User Guide for a detailed description of spiking, bursting, synchrony, and network bursting.

#### 2. NEURAL METRIC TOOL OVERVIEW

The Neural Metric Tool is divided into four sections: Plate Map, Bursting, Synchrony, and Evoked with four corresponding analysis settings sections: Analysis Parameters, Bursting Parameters, Synchrony Parameters, and Stimulation Parameters. When no stimulation is present, the Evoked section is replaced by an Average Network Burst section and parameters. The Plate Map section displays results for the entire plate while Bursting, Synchrony, and Evoked or Average Network Burst sections display data only for the active well (highlighted by the green box). Adjusting the settings in a section and then clicking Apply will update the display sections above. Adjusting the settings in the Analysis Parameters section will recalculate all spiking, bursting, synchrony, and evoked activity endpoints and update all four display sections.



#### 2.1. Plate Map Display and Analysis Parameters

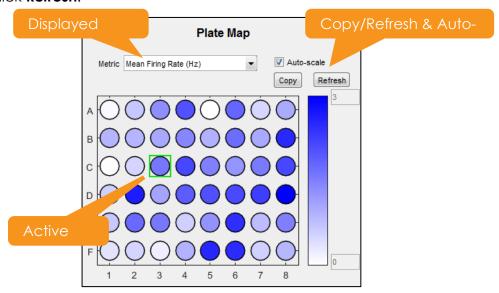


The **Plate Map** displays plate-wide trends and between well comparisons for the selected neural metric. Well shading indicates the value of the metric chosen in the dropdown menu located above the **Plate Map** display.

The active well displayed in the **Burst**, **Synchrony**, and **Evoked** or **Average Network Burst** sections is selected by clicking on the **Plate Map** and using the arrow keys to navigate through the wells.

To manually adjust the scale:

- 1. Deselect the Auto-scale checkbox.
- 2. Enter high and low values in the top and bottom fields, respectively to the right of the scale.
- 3. Click Refresh.



The **Analysis Parameters** section defines the analysis window, activity criteria, and coincident artifact removal. The analysis window is the file segment used to generate endpoint metrics. The activity criterion sets the threshold for electrode inclusion in the weighted mean firing rate. Coincident artifacts are artificial spikes detected on multiple electrodes at exactly the same time. They commonly occur during an electrical stimulus or some environmental interference like bumping the system or touching the media in a well.

To set the analysis window:

1. Select the segment type from the File Segment drop-down:

Segment Type	Description
Whole File	Analysis window is the entire duration of
	the file.

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Start of File	Analysis window begins at the start of the recording plus the <b>Offset</b> and continues for
	the <b>Duration</b> .
End of File	Analysis window begins at the end of the
	file minus the <b>Duration</b> and continues until
	the end.

2. Click the Apply button in the Analysis Parameters section.

To specify the activity criterion:

- 1. Type a minimum firing rate (spikes/min) in the **Active Electrode Criterion** (or **Active Unit Criterion**) field.
- 2. Click the **Apply** button in the **Analysis Parameters** section.

To remove coincident artifacts:

- 1. Click the **Remove Coincident Artifacts** checkbox to enable. To blank spikes after a coincident artifact see Section 2.6.
- 2. Click the **Apply** button in the **Analysis Parameters** section.

To blank stimulation artifacts in files containing **Electrical** or **Optical Stimulation Tags:** 

- 1. Click the **Remove Spikes Post-Stimulation Tag** checkbox.
- 2. Type the removal duration (ms) in the field. A removal duration of 2ms is recommended, increasing if needed, not to exceed 6 ms. Spikes detected during the removal duration after a tag will be removed.
- 3. Click the **Apply** button in the **Analysis Parameters** section.

To restore all analysis parameters to the default settings, select **File**  $\rightarrow$  **Restore Defaults**.

#### 2.2. Bursting Display and Parameters

The **Bursting** section displays a raster plot of the detected spikes on each electrode or sorted unit within the active well. Each tick indicates the time a spike occurred and each row indicates the electrode or unit. Blue ticks indicate the spikes are part of a single electrode or single unit burst while black ticks are not. Ticks included in network bursts are outlined by magenta rectangles. Above the raster is a filtered population spike time histogram, the total number of spikes occurring throughout the well at each time. When **Electrical** or **Optical Stimulation Tags** are present in the data file (See Chapter 4 of the Ax/S User Guide), black triangles appear at the bottom of the plot to indicate tag timing.

To show/hide network burst detection:

1. Select/Deselect the **Network Bursts** checkbox.



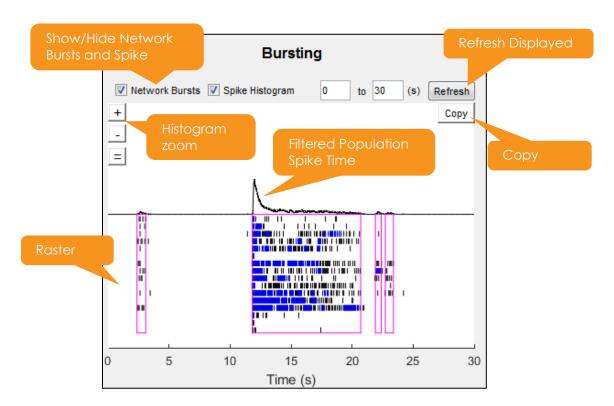
**Note**: Network bursting will still be calculated; the checkbox only affects the data display.

To show/hide the filtered population spike time histogram:

1. Select/Deselect the **Spike Histogram** checkbox. Zoom in, zoom out, and zoom reset (+, -, = respectively) are available for closer inspection of the histogram.

To change the time window displayed:

- Type the display start and end time into the fields above the plot.
   Note: This time window is for display purposes only. The analysis window is set in the Analysis Parameters section.
- 2. Click the **Refresh** button to the right of the time fields.



The **Burst Parameters** section defines single electrode or single unit and network bursting analysis parameters. **Single Electrode Bursts** (or **Single Unit Bursts**) can be identified using the same algorithms available in *AxIS*, inter-spike interval (ISI) threshold and Poisson surprise. **Network Bursts** can be identified using ISI threshold, Adaptive, or Envelope algorithms. While *AxIS* identifies network bursting using the ISI threshold method, the Adaptive and Envelope methods are only available in the *Neural Metric Tool*.

Many of the same metrics computed for burst detection on single electrodes or units are also computed for network bursts (See Section 3.5). The network burst metrics tend to be more robust, as they consider activity over the entire network. However, single

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electrode/unit burst metrics will be more accurate when bursting behavior is independent on each electrode or unit within a well. In this way, single electrode/unit burst metrics and network burst metrics are complementary, and their use will depend on the cell type and characteristics of the network activity.

To set the algorithm for burst detection:

- 1. Select ISI Threshold or Poisson Surprise from the Single Electrode Bursts (or Single Unit Bursts) drop-down list:
  - 1.1. ISI Threshold: Bursting is defined as at least N spikes on an electrode (or unit), each separated by an inter-spike interval (ISI) of no more than T seconds. The method is adapted from Chiappalone et al., 2005. Set the minimum number of spikes (N) and maximum time (T) between each spike using the Min # of Spikes and Max ISI (ms) fields, respectively.
  - 1.2. Poisson Surprise: Assumes the neurons are firing according to a Poisson distribution. It assesses a collection of spikes and determines how improbable a chance occurrence is according to a "surprise" threshold. The method is adapted from Legéndy & Salcman, 1985. In this way, the algorithm is adaptive to the mean firing rate on each electrode or unit. The Min Surprise field determines how sensitive burst detection is, such that a low Min Surprise will identify bursts more frequently.
- 2. Click **Apply** in the **Burst Parameters** section.

**Note**: To exclude electrodes or units that do not meet a minimum bursting rate see Section 2.6.

To set network burst detection:

- 1. Select ISI Threshold, Adaptive, or Envelope from the Network Bursts drop-down list:
  - 1.1. ISI Threshold: Defines a network burst as a collection of at least N spikes across all electrodes (or units) in the well, each separated by an inter-spike interval of no more than T seconds with at least X percent of electrodes (or units) participating in the burst. Adapted from Bakkum et al. 2013. Set the minimum number of spikes (N), maximum time between each spike (T), and minimum percent of electrodes (or units) (X) using the Min # of Spikes, Max ISI (ms), and Min Electrodes (%) (or Min Units (%)) fields, respectively.
  - 1.2. Adaptive: Defines a network burst the same as ISI Threshold. The maximum time between spikes (Max ISI) is set automatically on a well-by-well basis based on the mean firing rate of each well; wells with a higher mean firing rate have a lower Max ISI. In this way, the identification of network bursts is not biased by tonic activity in the well. Set the minimum number of spikes and minimum percent participating electrodes (or units) using the Min # of Spikes and Min Electrodes (%) (or Min Units (%)) fields, respectively.
  - 1.3. **Envelope**: Detects network bursts based on the filtered population spike time histogram, which is created by applying a Gaussian window to the binned spike



times across the well. The algorithm defines a network burst by identifying times when the histogram exceeds a threshold of N standard deviations above or below the mean. Bursts must be separated by T seconds and include at least X percent of electrodes (or units). Set the number of standard deviations (±N), minimum interburst interval (T), and percent of electrodes (or units) (X) with Threshold Factor, Min IBI (ms), and Min Electrodes (%) (or Min Units (%)), respectively. The boundaries of the network bursts are defined when the histogram falls back to near baseline. Burst Inclusion (%) defines how spikes near the boundaries are included, with higher values including more spikes.

**Note:** To exclude inactive or uncovered electrodes from Network Burst Calculations, use the drop down menu at the bottom

2. Click **Apply** in the **Burst Parameters** section.

Note: To exclude wells from network bursting calculations see Section 2.6.

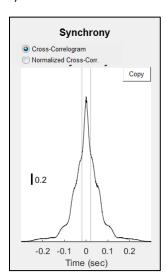
#### 2.3. Synchrony Display and Parameters

The **Synchrony** section displays either the standard or normalized well-wide cross-correlogram. A cross-correlogram describes how closely related two spike trains are. The well-wide cross-correlogram uses frequency domain methods (Halliday, Rosenber, Breeze & Conway 2006) to compute and pool the cross-correlogram across all unique pair-wise combinations of electrodes or units in a well. The normalized cross-correlogram normalizes the inter-electrode or inter-unit cross-correlations by their auto-correlations. This normalization reduces the effects of high mean firing rate or high spiking regularity on individual electrodes or units which can artificially increase correlations. The vertical gray lines indicate the size of the synchrony window.

To change the display between the standard and normalized cross-correlograms:

 Click either the Cross-Correlogram or Normalized Cross-Corr for standard cross-correlogram or normalized crosscorrelogram, respectively.

The **Synchrony Parameters** section defines synchrony analysis parameters. The **Synchrony Window (ms)** field is the window of time around zero that is used to compute the area under the cross-correlation and area under the normalized cross-correlation. A short synchrony window (e.g. 5 ms) quantifies synchrony on a millisecond timescale, while a long synchrony window (e.g. 100 ms) captures synchrony on slower timescales.



The Neural Metric Tool computes some synchrony metrics automatically for all Axion plates with more than 12 wells, but can also compute the Kreuz metric (derived from

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the Kreuz spike distance, Kreuz et al., 2013) and the Synchrony Index (based on Paiva, Park, and Principe, 2010).

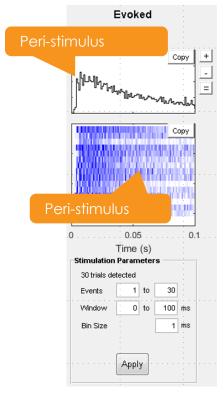
To calculate the Kreuz Metric and/or Synchrony Index:

- 1. Select Cross-Correlogram, Kreuz Metric, Synchrony Index, All, or None from the Additional Synchrony Metrics dropdown.
- 2. Click the **Apply** button in the **Synchrony Parameters** section.

#### 2.4. Evoked Display and Parameters

The **Evoked** section only displays data when **Electrical** or **Optical Stimulation Tags** are present in the recording (see Chapter 4 of the AxIS User Guide). The peri-stimulus histogram (top) displays the aggregate well-wide response averaged across the stimulus repeats for the active well. The peri-stimulus raster (bottom) displays the response of each active electrode or unit to the stimulus, binned by time, and averaged across the stimulus repeats. The darkness of the tick increases as spike number increases for that bin.

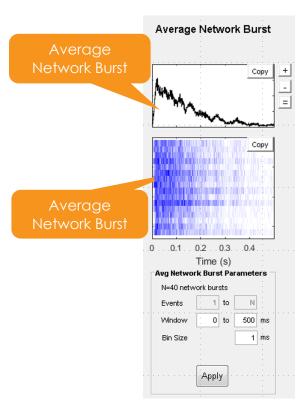
The **Stimulation Parameters** section defines evoked activity analysis parameters. Use the fields in the **Stimulation Parameters** section to specify which stimulus **Events** to analyze (set as a range of stimulus numbers), the time **Window** relative to these events to use for analysis, and the **Bin Size** used to aggregate spikes for the peri-stimulus raster and peri-stimulus histogram. The



zoom controls to the right allow the user to zoom the y-axis of the histogram and the color scale of the raster. To see the y-axis scale, use the **Copy** button.

## 2.5. Average Network Burst Display and Parameters

When no Electrical or Optical Stimulation Tags are present, the Evoked section is replaced by the Average Network Burst section that allows the user to consider the average network burst shape and the contribution of individual electrodes or units to network bursting. The average network burst histogram (top) displays the aggregate wellwide spike count averaged across the network bursts for the active well. The average network burst raster (bottom) displays the average contribution of each active electrode or unit to the network bursts. binned by time, and averaged across network bursts. The darkness of the tick increases as spike number increases for that bin. The zoom controls to the right allow the



user to zoom the y-axis of the histogram and the color scale of the raster.

#### 2.6. Advanced Options

The **Advanced** menu option under **File** provides options for spike blanking after coincident artifacts, setting burst activity criteria, selecting wells for network burst exclusion, and opening the LFP Exlorer.

To blank spikes after a coincident artifact:

- 1. Click File →Advanced →Artifact Blanking.
- 2. Type the blank duration (ms) in the **Blank** field in the **Analysis Parameters** section.
- 3. Click the **Apply** button in the **Analysis Parameters** section. Spikes detected during the blank duration after each coincident artifact will be removed.

To apply a minimum bursting criteria:

- 1. Click File → Advanced → Burst Criterion.
- 2. Type a minimum bursting rate (bursts/min) in the **Burst Electrode Criterion** (or **Burst Unit Criterion**) field in the **Burst Parameters** section.
- 3. Click the **Apply** button in the **Burst Parameters** section.

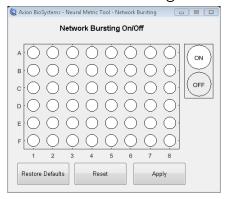
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**Note:** Electrodes or units with a burst rate below the minimum burst rate will be excluded from burst metrics for both Single Electrode (or Single Unit) Measurements and Well Averages.

To exclude wells from network burst calculations:

- Click File → Advanced → Turn off Network Bursts in select wells.
- 2. Click the well(s) in the **Network Bursting** window to disable detection of network bursts in those well(s). White wells are enabled, gray wells are disabled.
- 3. Click Apply to recalculate network bursting using only the enabled wells.

  Note: Click Reset to turn on all wells. Click Restore Defaults to restore all wells to the state they were in when the Network Bursting window was opened.



If the .spk file was recorded from a **Neural Event Detector** in AxIS, the **LFP Explorer** will become available. To view detected Local Field Potential Events (LFPs):

1. Click File →Advanced → LFP Explorer

**Note:** for instructions on operating the LFP Explorer, see section 3.5

#### 3. OPERATION

#### 3.1. AxIS Spike Files

The Neural Metric Tool most commonly uses **AxIS Spike** (.spk) files generated by AxIS. Data in .spk files are organized by well and electrodes, yielding both Well Average and Single Electrode metrics.

**Note:** Spike files generated for NETRI plates are not currently supported in the Neural Metric Tool. NETRI's UpLink software is designed to analyze data generated from these plates.

#### 3.1.1. Generate AxIS Spike File(s)

While plate map information is not used in the *Neural Metric Tool*, the information is preserved in the .csv output for use in other tools. Always enter complete plate maps



and experiment notes to an **AxIS Raw** file prior to beginning analysis (See Section 2.2 of the AxIS User Guide). Follow these steps to create **AxIS Spike** files in AxIS.

- 1. For a single file:
  - 1.1. Select File → Open Recording....
  - 1.2. Select the file for analysis and click **Open**.
- 2. For multiple files:
  - 2.1. Select File → New Batch Process....
  - 2.2. Click **Add** in the **Edit Batch Process Settings** dialog.

Note: Use Remove to remove any files from the Source File list.

- 2.3. Select the desired .raw files and click **Open**.
- 2.4. Select the analysis window using the **Segment Type** dropdown menu.
- 2.5. Click **OK**.
- Right-click on the file or batch process in the Streams window and select Configuration → Neural Offline → Spontaneous, Electrically Evoked, or Optically Evoked.

**Note:** Select **Spontaneous** for most applications, **Electrically Evoked** if electrical stimulation was used during the recording, or **Optically Evoked** if optical stimulation was used during the recording.

4. Right-click on the **Burst Detector** in the **Streams** window and select **Remove**. **Note:** This step will increase analysis speed but disable generation of file outputs

dependent on the Burst Detector and Neural Statistics Compiler. If using file outputs from these processors, skip this step.

- 5. Click on the **Experiment Setup Properties** module.
- 6. Select AxIS Spike from the Spike Detector dropdown.
- 7. Optional: Uncheck **Auto Name File** beside the selected file outputs to manually enter a file name. By default the name will be [SourceFile]\_[StreamName].
- 8. Click Record or Start Batch Process. The AxIS Spike files are saved to the AxIS Raw



file directory.

#### 3.1.2. Analyze a Single AxIS Spike File

To analyze a single **AxIS Spike** file in the Neural Metric Tool, follow these steps:

- 1. Click File →Load → Load AxIS Spike File (.spk)....
- 2. Select the file for analysis and click **Open**. Depending on the length of the recording, it can take several minutes to load the data.

**Note:** The window header now displays the loaded file name. The **Plate Map** section displays the selected metric across wells. The **Bursting**, **Synchrony**, and **Evoked** or **Average Network Burst** sections display a single well.

Adjust the analysis settings using the Analysis Parameters, Burst Parameters,
 Synchrony Parameters, and Evoked Parameters sections. See Section 2 for analysis setting options.

**Note**: To restore analysis settings to their default values, click **File** → **Restore Defaults**.

- 4. Export desired figures:
  - 4.1. Click the target well on the **Plate Map** to make it the active well.
  - 4.2. Adjust any display options, see Section 2 for display options.
  - 4.3. Click the **Copy** button beside the desired figure.
  - 4.4. Click **Save As** in the figure window.
  - 4.5. Type a file name, select a file extension, and click **Save**.

**Note**: Clicking **Copy** also copies the figure to the clipboard to directly paste into other software programs.

**Note**: Clicking **Copy** over the raster plot will export a low-resolution raster image by default for computational efficiency. If a full raster with all spikes represented is desired, right-click this **Copy** button and select **Full Resolution Copy**.

- 5. Export calculated metrics:
  - 5.1. Click File → Export → Export Recommended Metrics to CSV, Export Supplemental Metrics to CSV, or Export Metrics to Matlab File. See Section 3.5 for file output types.
  - 5.2. Type a file name and click **Save**.

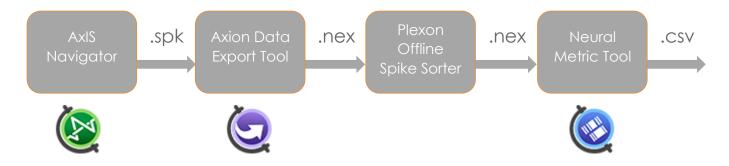
#### 3.2. Analyzing Sorted Spikes

The Neural Metric Tool can also analyze sorted spike data stored in .nex files generated in the Plexon Offline Sorter<sup>TM</sup>. Data in sorted .nex files are organized by units, localized to the well and electrode on which they were detected. Metrics are reported as both Well Average and Single Unit metrics.

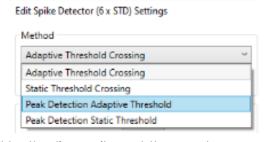
#### 3.2.1. Generate Sorted .nex File

Generating a sorted .nex file requires a few steps, as outlined in the pipeline below:





- 1. First, generate **AxIS Spike** file(s) in AxIS Navigator according to Section 3.1.1.
  - 1.1. Using a **Spike Detector** Method that aligns spikes at the peak, rather than at the threshold crossing, may improve spike sorting by grouping similar, but time-shifted spikes waveforms. Peak Detection Adaptive



- Threshold and Peak Detection Static Threshold both align spikes at the peak.
- 2. In the Axion Data Export Tool, select the **Export .spk to .nex** tab
  - 2.1. Click Load .spk file(s).
  - 2.2. Select one or more .spk files and click **Open**.
  - 2.3. Optional: Type a start and end time (in seconds) into the **Start Time** and **Stop Time** fields, respectively. Leave these fields empty to export the whole file(s).
  - 2.4. Click Export....
  - 2.5. A .nex file will be saved for each .spk file, in the same directory as the source .spk file.

**Note:** Although the Plexon Offline Sorter<sup>TM</sup> can read AxIS spike files, this conversion is important to preserve the metadata of the spike file. Loading the spike file directly into the Offline Sorter<sup>TM</sup> may result in corrupted spike times and other errors.

- 3. In the Plexon Offline Sorter<sup>TM</sup>, select **File**  $\rightarrow$  **Open**. Change the file drop-down to **NeuroExplorer** (\*.nex) and navigate to the location of the .nex file. Click **Open**.
  - 3.1. In the **Control Grid**, change the **Channel** drop-down to any channel of interest. The Channels are labeled as Well\_ElectrodeColumnElectrodeRow (e.g. A1\_11).
  - 3.2. The Sort menu offers multiple algorithms for spike sorting. See the Offline Sorter™ manual for details.
  - 3.3. For automatic sorting, select **Sort** → **Perform Automatic Sorting**.
  - 3.4. Under Which Channels, select **All Channels in Spike Source** to ensure that all channels are processed.
  - 3.5. Select the desired algorithm and click **Ok**.
  - 3.6. Optional: Once sorting is complete, use the Waveforms and Units menu to modify the sorting results.
  - 3.7. When satisfied with the sorting results, select **File → Export to .NEX** to save the sorted spike data to a .nex file for analysis in the Neural Metric Tool.

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#### 3.2.2. Analyze a Single Sorted .nex File

To analyze a single sorted .nex file in the Neural Metric Tool, follow these steps:

- 1. Click File →Load → Load Plexon Sorted Spikes File (.nex)
- 2. Select the file for analysis and click **Open**. Depending on the length of the recording, it can take several minutes to load the data.

**Note:** The window header now displays the loaded file name. The **Plate Map** section displays the selected metric across wells. The **Bursting**, **Synchrony**, and **Evoked** or **Average Network Burst** sections display a single well.

Adjust the analysis settings using the Analysis Parameters, Burst Parameters,
 Synchrony Parameters, and Evoked Parameters sections. See Section 2 for analysis setting options.

**Note**: To restore analysis settings to their default values, click **File** → **Restore Defaults**.

- 4. Export desired figures:
  - 4.1. Click the target well on the **Plate Map** to make it the active well.
  - 4.2. Adjust any display options, see Section 2 for display options.
  - 4.3. Click the **Copy** button beside the desired figure.
  - 4.4. Click **Save As** in the figure window.
  - 4.5. Type a file name, select a file extension, and click **Save**.

**Note**: Clicking **Copy** also copies the figure to the clipboard to directly paste into other software programs.

- 5. Export calculated metrics:
  - 5.1. Click File → Export → Export Recommended Metrics to CSV, Export Supplemental Metrics to CSV, or Export Metrics to Matlab File. See Section 3.5 for file output types.
  - 5.2. Type a file name and click **Save**.

#### 3.3. Analyze Multiple Files

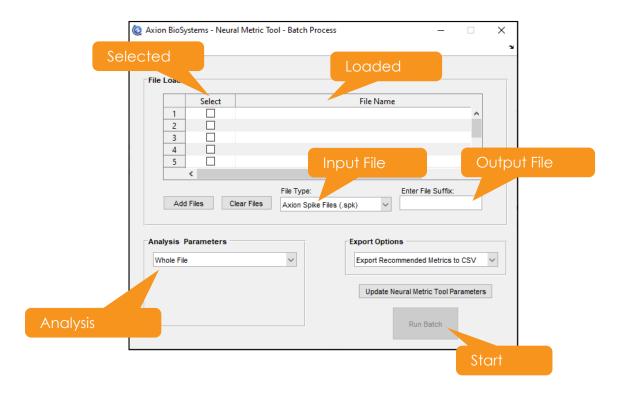
To analyze multiple **AxIS Spike (.spk)** or sorted **.nex** files in the *Neural Metric Tool*, follow these steps to **Batch Process Multiple Files**:

 Adjust the analysis settings using the Analysis Parameters, Burst Parameters, Synchrony Parameters, and Evoked Parameters sections. See Section 2 for analysis setting options.

**Note**: Analysis settings may be altered in the **Neural Metric Tool** window at any time. Click **Update Neural Metric Tool Parameters** in the **Batch Process** window to analyze with updated settings.

- 2. Click File → Batch → Batch Process Multiple Files.
- 3. Select either AxIS Spike Files (.spk) or Plexon Sorted Spikes Files (.nex) from the File Type drop-down menu.
- 4. Click Add Files in the Batch Process window.





5. Select the files to analyze and click **Open**.

**Note**: To exclude files from analysis after opening, deselect it from the table in the **Batch Process** window using the **Select** checkbox. Click **Clear Files** to remove all files from the batch process.

6. Select the analysis window from the **Analysis Parameters** drop-down menu.

Segment Type	Description
Whole File	Analysis window is the entire duration of the file.
Start of File	Analysis window begins at the start of the recording plus the <b>Offset</b> and continues for the <b>Duration</b> .
End of File	Analysis window begins at the end of the file minus the <b>Duration</b> and continues until the end.

- Select the file output (Export Recommended Metrics to CSV, Export Supplemental Metrics to CSV, or Export Metrics to Matlab File) from the Export Options drop-down menu.
- 8. Optional: Type a file name suffix to append to output files in the **Enter File Suffix** field.
- 9. Click Run Batch.

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**Note**: Exported neural metric files will be saved to the path of the source .spk or .nex file and automatically named [source file name] [\_neuralMetrics] [\_suffix] [.file extension].

#### 3.4. Analyze Multiple Time Segments

To analyze multiple time segments from one or more **AxIS Spike** or sorted .nex files in the *Neural Metric Tool*, follow these steps to **Batch Process Multiple Files and Time Segments**:

 Adjust the analysis settings using the Analysis Parameters, Burst Parameters, Synchrony Parameters, and Evoked Parameters sections. See Section 2 for analysis setting options.

**Note**: Analysis settings may be altered in the **Neural Metric Tool** window at any time. Click **Update Neural Metric Tool Parameters** in the **Batch Process** window to analyze with updated settings.

- 2. Click File → Batch → Batch Process Multiple Files and Time Segments.
- 3. Select either AxIS Spike Files (.spk) or Plexon Sorted Spikes Files (.nex) from the File Type drop-down menu.
- 4. Click Add Files in the Batch Process window.
- 5. Select the files to analyze and click **Open**.

**Note**: To exclude files from analysis after opening, deselect it from the **File Load** table in the **Batch Process** window using the **Select** checkbox. Click **Clear Files** to remove all files from the batch process.

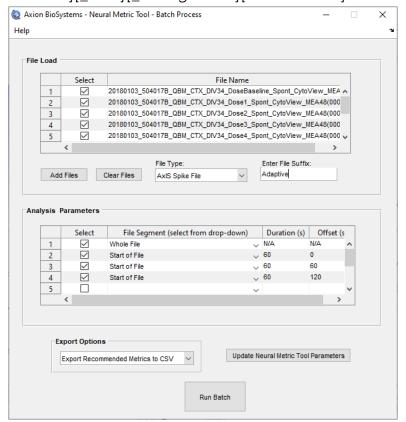
6. For each time segment, select the desired file segment from the drop-down menu in that row. Be sure to mark the checkbox next to the file segments to be included in the batch. Each selected segment will be processed for all selected files.

Segment Type	Description
Whole File	Analysis window is the entire duration of
	the file. Duration and Offset will default to
	N/A.
Start of File	Analysis window begins at the start of the
	recording plus the Offset and continues for
	the <b>Duration</b> .
End of File	Analysis window begins at the end of the
	file minus the <b>Duration</b> and continues until
	the end.

- Select the file output (Export Recommended Metrics to CSV, Export Supplemental Metrics to CSV, or Export Metrics to Matlab File) from the Export Options drop-down menu.
- 8. Optional: Type a file name suffix to append to output files in the **Enter File Suffix** field.
- 9. Click Run Batch.



**Note**: Exported neural metric files will be saved to the path of the source .spk or .nex file and automatically named [source file name][\_neuralMetrics][\_suffix][\_FileSegment#][.file extension].



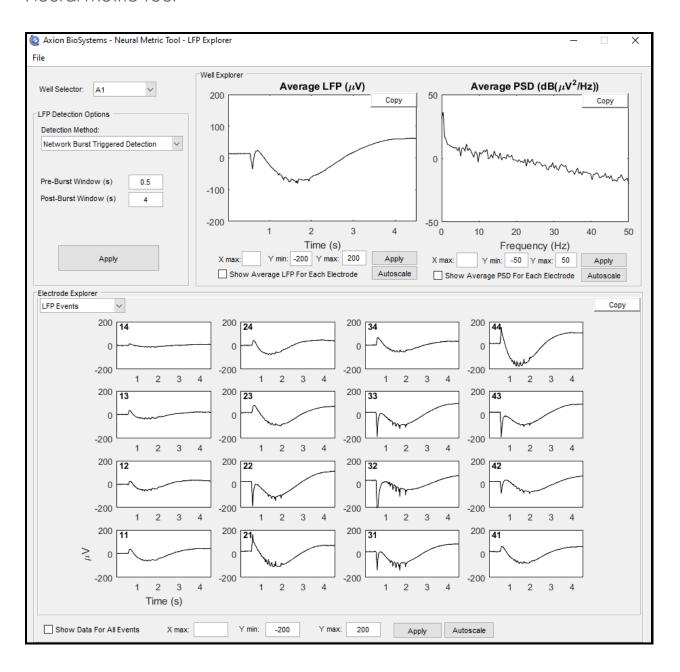
#### 3.5. The LFP Explorer

If the .spk file was recorded from a **Neural Event Detector** in AxIS, the **LFP Explorer** will become available. To view detected LFPs:

- 1. Click File →Advanced → LFP Explorer.
- 2. Choose an LFP Detection Method and click **Apply**

AxIS Neural Event Detection will pull detected LFPs from the **Neural Event Detector** file. Network Burst Triggered Detection requires a **Broadband Processor** .raw file, which can be obtained at the same time as the **Neural Event Detector** .spk file from AxIS Navigator. This detection method will pull data from the low frequency trace in the **Broadband Processor** file around Network Bursts detected by the **Neural Metric Tool**. **Pre-Burst Window** and **Post-Burst Window** are used to define the window around the beginning of network bursts from which low frequency signals are extracted and are only available when this detection method is selected.

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The **Well Explorer** panel displays the average LFP event and the average power spectral density for the selected well. The averages are performed across events and electrodes. To overlay the average LFP/PSD per electrode, use the checkboxes at the bottom of the **Well Explorer**. Use the **Well Selector** to browse LFPs detected in different wells.

The **Electrode Explorer** displays the average LFP Event on each electrode in the selected well. **Show Data For All Events** will display an overlaid plot of all LFPs detected on that electrode for the time segment chosen. To view the power spectral density (PSD) of each of these events, use the **Electrode Explorer Drop Down Menu**.

The **Copy** buttons export the figure and copy to the clipboard.



**Note:** The main window of the Neural Metric Tool will not allow for reprocessing until the LFP Explorer is closed

There are two options for exporting LFP waveforms. Within the LFP Explorer, click **File** → **Export** to access export options.

**Export LFP Waveforms to .MAT** will produce a Matlab file containing all LFP waveforms, all power spectral densities (in  $dB(\mu V^2/Hz)$ ), and the associated time and frequency vectors, respectively.

**Export LFP Waveforms to .CSV** will produce a .csv file which contains average LFPs and PSDs for each electrode and each well.

LFP Metrics can only be exported directly from the LFP Explorer and LFP Analysis cannot presently be batch processed. To export all neural metrics, including LFP metrics, click File →Export→Export All Metrics to CSV

#### 4. OUTPUT

The Neural Metric Tool can export calculated metrics to a .csv file or a .mat file. To export this data, click File → Export and select one of the export options. The resulting .csv file can be opened with any software that can read a delimited text file, such as Microsoft Excel. The Neural Metric Tool output mirrors the output of the Advanced Metrics output generated by the Neural Statistics Compiler in AxIS (See Section 8.4 of the AxIS User Guide). Unlike the Advanced Metrics output, treatment group averages are not included in the Neural Metric Tool output.

The output metrics of Export Recommended Metrics to CSV (recommended) and Export Supplemental Metrics to CSV (supplemental) are listed in the table below and defined in the following sections. In general, recommended metrics contain the mean values, whereas supplemental metrics contain mean and median values. A few additional non-median metrics are included in supplemental metrics as well. Export Metrics to Matlab File exports the supplemental metrics to a .mat file for further custom analysis. Note that the listed Electrode-based metrics are provided as Unit-based metrics when analyzing sorted .nex files (e.g. Number of Spikes per Network Burst per Channel becomes Number of Spikes per Network Burst per Unit).

Recommended Metrics	Supplemental Metrics
Activity Metrics	Activity Metrics
Number of Spikes	Number of Spikes
Mean Firing Rate	Mean Firing Rate
ISI Coefficient of Variation	ISI Coefficient of Variation
Number of Active Electrodes	Network ISI Coefficient of Variation
Weighted Mean Firing Rate	Fano Factor
	Number of Active Electrodes
	Weighted Mean Firing Rate
Electrode Burst Metrics	Electrode Burst Metrics

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Number of Bursts	Number of Bursts
Number of Bursting Electrodes	Number of Bursting Electrodes
Burst Duration	Burst Duration (Mean and Median)
Number of Spikes per Burst	Number of Spikes per Burst (Mean and Median)
Mean ISI within Burst	Mean ISI within Burst
Median ISI within Burst	Median ISI within Burst
Median/Mean ISI within Burst	Median/Mean ISI within Burst
Inter-Burst Interval	Inter-Burst Interval (Mean and Median)
Burst Frequency	Burst Frequency Normalized Duration IQR
IBI Coefficient of Variation	
Burst Percentage	IBI Coefficient of Variation
	Burst Percentage
Network Burst Metrics	Network Burst Metrics
Number of Network Bursts	Network Bursts Ignored Flag
Network Burst Frequency	Number of Network Bursts
Network Burst Duration	Network Burst Frequency
Number of Spikes per Network Burst	Network Burst Duration (Mean and Median)
Mean ISI within Network Burst	Number of Spikes per Network Burst (Mean and Median)
Median ISI within Network Burst	Mean ISI within Network Burst
Median/Mean ISI within Network Burst	Median ISI within Network Burst
Number of Elecs Participating in Burst	Median/Mean ISI within Network Burst
Number of Spikes per Network Burst per Channel	ISI CoV within Network Burst
	Number of Elecs Participating in Burst (Mean and
Network Burst Percentage	Median)
Network IBI Coefficient of Variation	Number of Spikes per Network Burst per Channel (Mean and Median)
Network Normalized Duration IQR	Network Burst Percentage
	Network IBI Coefficient of Variation
	Network Normalized Duration IQR
Synchrony Metrics	Synchrony Metrics
Area Under Normalized Cross-Correlation	Area Under Normalized Cross-Correlation
Area Under Cross-Correlation	Area Under Cross-Correlation
Full Width at Half Height of Normalized Cross-	Full Width at Half Height of Normalized Cross-
Correlation	Correlation
Full Width at Half Height of Cross-Correlation	Full Width at Half Height of Cross-Correlation
Synchrony Index	Synchrony Index
Kreuz SPIKE Distance	Kreuz SPIKE Distance
Evoked Metrics	Evoked Metrics
Number of Trials	Number of Trials
Evoked Spike Count	Evoked Spike Count
Evoked Response Probability	Evoked Response Probability
Evoked First Spike Latency	Evoked First Spike Latency
Evoked Jitter	Evoked Jitter
	Evoked CoV2
	Evoked Fano Factor
Avg Network Burst Metrics	Avg Network Burst Metrics
Start Electrode	Start Electrode
Percent Bursts with Start Electrode	Percent Bursts with Start Electrode
Burst Peak (Max Spikes per sec)	Burst Peak (Max Spikes per sec)



Time to Burst Peak (ms)	Time to Burst Peak (ms)
	Avg Network Burst CoV2
	Avg Network Burst Fano Factor
Viability Metrics*	Viability Metrics*
Resistance - Avg (kOhms)	Resistance - Avg (kOhms)
Resistance - Std (kOhms)	Resistance - Std (kOhms)
Number of Covered Electrodes	Resistance - Median (kOhms)
Weighted Mean Resistance (kOhms)	Resistance - MAD (kOhms)
	Number of Covered Electrodes
	Weighted Mean Resistance (kOhms)
LFP Metrics	LFP Metrics
	Number of LFPs
	Number of LFPs Per Electrode
	LFP Rate (Hz)
	LFP Amplitude (μV)
	LFP Rectified Area (µVs)
	LFP Low (0-1 Hz) Power ( $\mu$ V <sup>2</sup> )
	LFP Delta (1-4 Hz) Power (µV²)
	LFP Theta (4-8 Hz) Power (µV²)
	LFP Alpha (8-14 Hz) Power (μV²)
	LFP Beta (14-30 Hz) Power (µV²)
	LFP Gamma (30-50 Hz) Power (µV²)
	LFP Total Power (μV²)
	LFP Low (0-1 Hz) Relative Power
	LFP Delta (1-4 Hz) Relative Power
	LFP Theta (4-8 Hz) Relative Power
	LFP Alpha (8-14 Hz) Relative Power
	LFP Beta (14-30 Hz) Relative Power
	LFP Gamma (30-50 Hz) Relative Power

<sup>\*</sup>Viability metrics are only available if recorded in AxIS Navigator with the MEA Viability Module.

## 4.1. Activity Metrics

Metric	Definition
Number of Spikes	Total number of spikes over the duration of the analysis.
Mean Firing Rate	Total number of spikes divided by the duration of the analysis, in Hz.
	The coefficient of variation (standard deviation/mean) of the inter-spike interval, the time between spikes, for electrodes (or units) with activity greater than the minimum spike rate ("active electrodes" or "active units"). This metric is a measure of spike regularity and captures the distribution of spiking such that 0 indicates spikes perfectly distributed and > 1 indicates bursting; as bursting becomes clearly

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Network ISI Coefficient of Variation	distinguished from quiescence, the standard deviation of the ISI increases because spikes inside a burst have small ISIs, while the ISIs between the last spike in one burst and the first spike in the next are large.  Coefficient of variation (standard deviation/mean) of the inter-spike interval for all spikes in a well. This is a measure of spike regularity across the network. This metric captures the distribution of spiking such that 0 indicates spikes perfectly distributed and > 1 indicates network bursting.
Fano Factor	Fano factor is a measure of spike count variability over time. Fano Factor is calculated as the variance of spike counts across 6 second bins divided by the mean of the spike counts in 6 second bins (Becchetti et al 2012). The Fano Factor is computed for each active electrode or unit independently, and then averaged across active electrodes or units to compute a well-wide Fano Factor. Active electrodes or units are defined as those with activity greater than the minimum spike rate. An evoked fano factor is also provided, see Section 4.5.
Number of Active	Number of electrodes (or units) with activity greater than the
Electrodes	minimum spike rate set in the <b>Analysis Parameters</b> .
Weighted Mean Firing Rate	The mean firing rate based on only electrodes (or units) with activity greater than minimum spike rate ("active electrodes" or "active units").

#### 4.2. Electrode or Unit Burst Metrics

The "Measurement" values describe single electrode or single unit bursting on each electrode. Where applicable, a given metric is pooled across bursts according to the statistic after the dash. For example:

- Burst Duration Avg is the average burst duration across bursts on that electrode (or unit)
- Burst Duration Std is the standard deviation of burst durations across bursts on that electrode (or unit)
- Burst Duration Median is the median burst duration across all bursts on that electrode (or unit)
- Burst Duration MAD is the median absolute deviation of burst durations across bursts on that electrode (or unit)

The "Well Averages" are well-wide statistics where values are first computed as an average for each electrode (or unit) and then pooled across electrodes (or units) according to the statistic after the dash. For example:



- Burst Duration Avg is the average across electrode (or unit) average burst durations
- Burst Duration Std is the standard deviation across electrode (or unit) average burst durations
- Burst Duration (Median) Avg is the average across electrode (or unit) median burst durations
- Burst Duration (Median) Std is the standard deviation across electrode (or unit) median burst durations

For more detailed explanations of each metric and its variations, refer to the metric definitions list which can be accessed in the *Neural Metric Tool* menu under **Help** > **Neural Metric Definitions**.

Metric	Definition
Number of Bursts	Total number of single-electrode or single-unit bursts over the duration of the analysis. For a well, the total number of electrode (or unit) bursts across all electrodes (or units) in a well is reported.
Number of Bursting Electrodes	Total number of electrodes (or units) within the well with bursts/minute greater than the burst electrode criterion (or burst unit criterion).
Burst Duration	Average time from the first spike to last spike in a single electrode (or single unit) burst. For an electrode (or unit), the average duration across bursts is reported. For a well, the average across electrode (or unit) averages is reported. Longer bursts indicate more excitation, less inhibition, as it takes longer to shut down a burst.
Number of Spikes per Burst	Average number of spikes in a single electrode or single unit burst. For an electrode (or unit), the average across bursts is reported. For a well, the average across electrode (or unit) averages is reported.
Mean ISI within Burst	Mean inter-spike interval, time between spikes, for spikes in a single-electrode or single unit burst. For an electrode (or unit), the average across burst means is reported. For a well, the average across electrode (or unit) averages is reported. This is a measure of burst intensity; smaller values mean more intense bursts.
Median ISI within Burst	Median inter-spike interval, time between spikes, for spikes in a single electrode (or single unit) burst. For an electrode (or unit), the average across burst median ISIs is reported. For a well, the average across electrode (or unit) averages is reported.
Median/Mean ISI within Burst	The median/mean inter-spike interval (ISI) within single electrode (or single unit) bursts. Values close to 1 indicate

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	the distribution of ISIs within bursts is symmetric. For an electrode (or unit), the average across burst median/mean ISIs is reported. For a well, the average across electrode (or unit) average median/mean ISIs is reported.
Inter-Burst Interval	Average time between the starts of single electrode (or single unit) bursts. For an electrode (or unit), the average across bursts is reported. For a well, the average across electrode (or unit) averages is reported.
Burst Frequency	Total number of single electrode (or single unit) bursts divided by the duration of the analysis, in Hz. For a well, the average across electrode (or unit) burst frequencies is reported.
Normalized Duration IQR	Interquartile range of single electrode (or single unit) burst durations. This metric provides a measure of single electrode (or single unit) burst duration regularity. If the middle 50% of bursts are close to the same duration, this value will be small, whereas, if the bursts vary widely in duration, this range will be large. For a well, the average across electrode (or unit) normalized duration IQRs is reported.
IBI Coefficient of Variation	The coefficient of variation (standard deviation/mean) of the inter-burst interval, the time between single electrode (or single unit) bursts. This is a measure of burst regularity. For a well, the average across electrode (or unit) IBI CoVs is reported.
Burst Percentage	The number of spikes in single electrode (or single unit) bursts divided by the total number of spikes, multiplied by 100. For a well, the average across electrode (or unit) burst percentages is reported.

#### 4.3. Network Burst Metrics

Network bursts are detected as near synchronous spiking across electrodes (or units) in a well. All of these metrics apply to bursts across the well (a.k.a. across the network). For "Well Averages," averages, standard deviations, medians, and median absolute deviations (MADs) values for the metrics are computed across network bursts.

Metric	Definition								
Network Bursts Ignored	flag to indicate whether network bursts were ignored in th								
Flag	ell. Network bursts ignored = 1, network bursts included = 0.								
Number of Network Bursts	Total number of network bursts over the duration of the								
	analysis.								
Network Burst Frequency	Total number of network bursts divided by the duration of								
	the analysis, in Hz.								



Network Burst Duration	Average time from the first spike to last spike in a network burst. Longer bursts indicate more excitation, less inhibition, as it takes longer to shut down a burst.
Number of Spikes per Network Burst	Average number of spikes in a network burst.
Mean ISI within Network Burst	Average of the mean ISIs within network bursts.
Burst	Average of the median ISIs within network bursts.
Median/Mean ISI within Network Burst	Average of the median/mean ISI within network bursts. Values close to 1 indicate the distribution of ISIs within bursts is symmetric.
ISI CoV within Network Burst	Average across network bursts of the ISI CoV (standard deviation/mean of the inter-spike interval) within network bursts.
Number of Electrodes Participating in Burst	Average number of electrodes (or units) with activity during a network burst.
Number of Spikes per Network Burst per Channel	Average across network bursts of the number of spikes in the network burst divided by the number of electrodes (or units) participating in that burst.
Network Burst Percentage	The number of spikes in network bursts divided by the total number of spikes, multiplied by 100.
Variation	The coefficient of variation (standard deviation/mean) for the inter-network burst interval, the time between network bursts. This is a measure of network burst rhythmicity; bursts occurring at regular intervals have a small coefficient of variation, whereas sporadic bursting has a larger coefficient of variation
Network Normalized Duration IQR	Interquartile range of network burst durations. This metric provides a measure of network burst duration regularity. If the middle 50% of network bursts are close to the same duration, this value will be small; if the network bursts vary widely in duration, this range will be large.

## 4.4. Synchrony Metrics

Metric	Definition
Cross-Correlation	Area under the well-wide pooled inter-electrode (or inter- unit) cross-correlation normalized to the auto-correlations. Higher areas indicate greater synchrony.
	Area under the well-wide pooled inter-electrode (or inter-unit) cross-correlation.

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Full Width at Half Height of Normalized Cross- Correlation	Distance along the x-axis (phase lag) from left half height to right half height (probability) of the normalized cross-correlogram. This is a measure of network synchrony; higher half widths indicate a wider correlogram (less synchrony) whereas lower half widths indicate a taller correlogram							
Full Width at Half Height of Cross-Correlation	(greater synchrony).  Distance along the x-axis (phase lag) from left half height to right half height (probability) of the cross-correlogram. High full widths indicate a wider correlogram (less synchrony) whereas lower full widths indicate a taller correlogram (greater synchrony).							
Synchrony Index	A unitless measure of synchrony between 0 and 1 (Paiva et al 2010). Values closer to 1 indicate higher synchrony.							
Kreuz SPIKE Distance	1-Kreuz SPIKE distance (Kreuz et al 2013) such that 1 is perfect synchrony and 0 is perfect asynchrony. It is computed with only one previous and one subsequent spike for each reference spike. The time window for each computation varies with local firing rate of each spike train. It tracks changes in instantaneous clustering without being skewed by individual electrode (or unit) inter-spike interval.							

## 4.5. Evoked Metrics

Metric	Definition
Number of Trials	Number of stimulation events used for analysis.
Evoked Spike Count	Average across trials of the number of spikes detected across active electrodes (or active units) in the well during the time window specified by the <b>Stimulation Parameters</b> section. This is a measure of the magnitude of stimulus response.
Evoked Response Probability	Probability of finding at least one spike in the well during the time window specified by the <b>Stimulation Parameters</b> section. Computed as the number of stimulation events that evoked a response divided by the total number of stimulation events. This is a measure of the responsivity to the stimulus.
Evoked First Spike Latency	The average across trials of the time between the stimulation event and the first post-stimulus spike detected in the well.
Evoked Jitter	The standard deviation across trials of the time between the stimulation event and the first post-stimulus spike detected in the well. This is a measure of response consistency; lower values indicate more consistent responses.



Evoked CoV <sup>2</sup>	The square of the coefficient of variation (standard deviation/mean) of the inter-spike interval during the evoked response window, averaged across trials. This metric is a measure of spike timing regularity during the evoked response.
Evoked Fano Factor	Fano Factor is measure of spike count variability across trials, or, in other words, the variability of the evoked response magnitude. Fano Factor is calculated as the variance of spike count across trials divided by the mean of the spike count across trials (Becchetti et al 2012) and is calculated for each active electrode (or active unit). Spikes are counted for the evoked response time window entered in the Stimulation Parameters (see Section 2.4). For the well-wide evoked Fano Factor, the Fano Factor is computed based on the pooled spikes from all active electrodes (or active units) in the well during the evoked response time window.

## 4.6. Average Network Burst Metrics

Metric	Definition									
Start Electrode	The electrode (or unit) that most commonly contributes the									
	first spike to the network burst.									
Percent Bursts with Start	The percent of network bursts that start with a spike from the									
Electrode	Start Electrode (or Start Unit).									
Burst Peak (Max Spikes	The maximum number of spikes per second in the average									
per sec)	network burst. This value is equal to the peak of the Average									
	Network Burst Histogram divided by the histogram bin size to									
	yield spikes per sec (Hz).									
Time to Burst Peak (ms)	The time from the start to the peak of the average network									
	burst. This value is equal to the time to the peak of the									
	Average Network Burst Histogram.									
Avg Network Burst CoV <sup>2</sup>	The square of the coefficient of variation (standard									
	deviation/mean) of the inter-spike interval within the									
	average network burst time window. This metric is a measure									
	of spike timing regularity during network bursts.									
Avg Network Burst Fano	Fano Factor is calculated as the variance of spike count									
Factor	across network bursts divided by the mean of the spike									
	count across network bursts (Becchetti et al 2012) and is									
	calculated for each active electrode (or active unit). Spikes									
	are counted for the average network burst time window									
	entered in the Average Network Burst Parameters (see									
	Section 2.5). Fano Factor is a measure of spike count									
	variability across network bursts.									

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#### 4.7. Viability Metrics

MEA Viability is measured using the Maestro's impedance technology and MEA Viability Software Module. To measure viability, a very small electrical signal is delivered to the microelectrodes. When the electrode is uncovered (no cells), the electrical signal passes easily through the uncovered electrode to the media and a low impedance is measured. When intact cells are covering to the electrode, a higher impedance is measured.

Impedance-based MEA Viability is particularly sensitive to the integrity of the attached cell membranes, such that when cell membrane integrity is disrupted by cell death (e.g., lysis, necrosis), impedance decreases as the electrical signal more easily passes through the cell-electrode interface. Viability is reported as the resistance at 41.5kHz, a component of impedance that is highly correlated with viable cell coverage.

Metric	Definition
Resistance (kOhms)	Resistance is a measure of viable cell coverage over the electrode. Higher values indicate more intact cells are attached to the electrode. For a well, the average across electrodes is reported. (See the MEA Viability chapter of the AxIS Navigator user guide for more details.)
Number of Covered Electrodes	Total number of covered electrodes within the well. Covered electrodes are defined as electrodes with resistance greater than the Covered Electrode Threshold (default 18 kOhms). Uncovered CytoView MEA microelectrodes in media exhibit a resistance of 8-12 kOhms. Following neuronal cell death, debris may leave a slightly higher uncovered resistance of 15-18 kOhms.
Weighted Mean Resistance (kOhms)	The mean resistance across covered electrodes only (resistance greater than the Covered Electrode Threshold).

#### 4.8. LFP Metrics

LFP Metrics are only available for files recorded in Neural Broadband and may be exported from the LFP Explorer window.

Metric	Definition
Number of LFPs	Total number of LFPs over the duration of the analysis.
Number of LFPs Per	Total number of LFPs detected on a single electrode over
Electrode	the duration of the analysis. For a well, the average number
	of detected LFPs across all electrodes in a well is reported.
LFP Rate (Hz)	Total number of LFPs divided by the duration of the analysis,
ETT Rate (TIZ)	in Hz.
LFP Amplitude (µV)	Peak-to-peak amplitude of LFPs averaged across electrodes.
	Electrodes with no detected LFPs report an amplitude of 0.



LFP Rectified Area (µVs)	Area under the curve of rectified LFP waveforms averaged across electrodes. Electrodes with no detected LFPs do not contribute.
LFP Low (0-1 Hz) Power (µV2)	Power spectral density is averaged across detected LFPs for each electrode and then averaged across electrodes for a representative power spectral density curve for each well. Power is computed through integration of the per-well power spectral density curve from 0 – 1 Hz.
LFP Delta (1-4 Hz) Power (µV2)	Power spectral density is averaged across detected LFPs for each electrode and then averaged across electrodes for a representative power spectral density curve for each well. Power is computed through integration of the per-well power spectral density curve from 1–4 Hz.
LFP Theta (4-8 Hz) Power (µV2)	Power spectral density is averaged across detected LFPs for each electrode and then averaged across electrodes for a representative power spectral density curve for each well. Power is computed through integration of the per-well power spectral density curve from 4 – 8 Hz.
LFP Alpha (8-14 Hz) Power (µV2)	Power spectral density is averaged across detected LFPs for each electrode and then averaged across electrodes for a representative power spectral density curve for each well. Power is computed through integration of the per-well power spectral density curve from 8 – 14 Hz.
LFP Beta (14-30 Hz) Power (µV2)	Power spectral density is averaged across detected LFPs for each electrode and then averaged across electrodes for a representative power spectral density curve for each well. Power is computed through integration of the per-well power spectral density curve from 14 – 30 Hz.
LFP Gamma (30-50 Hz) Power (µV2)	Power spectral density is averaged across detected LFPs for each electrode and then averaged across electrodes for a representative power spectral density curve for each well. Power is computed through integration of the per-well power spectral density curve from 30 – 50 Hz.
LFP Total Power (µV2)	Power spectral density is averaged across detected LFPs for each electrode and then averaged across electrodes for a representative power spectral density curve for each well. Power is computed through integration of the per-well power spectral density curve from 0 – 50 Hz.
LFP Low (0-1 Hz) Relative Power	Ratio of Low Power to Total Power
LFP Delta (1-4 Hz) Relative Power	Ratio of Delta Power to Total Power
LFP Theta (4-8 Hz) Relative Power	Ratio of Theta Power to Total Power

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LFP Alpha (8-14 Hz)	Ratio of Alpha Power to Total Power
Relative Power	
LFP Beta (14-30 Hz)	Ratio of Beta Power to Total Power
Relative Power	
LFP Gamma (30-50 Hz)	Ratio of Gamma Power to Total Power
Relative Power	

#### 4.9. Export Evoked Data

The Neural Metric Tool can also export the peri-stimulus histogram and raster data. To export this data, click **File** → **Export** → **Export Evoked Data to CSV**. In the resulting .csv file, the data under Well Averages – PSTH represents the average number of spikes on active electrodes (or active units) in the well per trial for each histogram bin and is the data used to create the Peri-stimulus histogram for each well in the **Evoked** display (top plot). The data under Electrode – PSTH (or Unit – PSTH) represents the average number of spikes on the active electrode (or active unit) per trial for each histogram bin and is the data used for the Peri-stimulus raster in the **Evoked** display (bottom plot). The .csv file can be opened with any software that can read a delimited text file, such as *Microsoft Excel*.

Well Averages - PSTH	A1	A2	A3	A4	A5	A6	A7	A8	B1	B2	B3	B4	B5	B6
Treatment ID	DMSO	Picrotoxin	Picrotoxir	Picrotoxin	Picrotoxin	Picrotoxir	DMSO	Picrotoxir	DMSO	Picrotoxin	DMSO	Picrotoxin	Picrotoxir	Picrotoxin
	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0.033333	0.266667	0	0	0.2	0.133333	0.133333
	0	0	0	0	0	0	0	0	0.033333	0	0	0	0.066667	0.066667
	0	0	0	0	0	0	0	0.033333	0.166667	0	0	0.033333	0	0
	0	0	0	0	0	0	0	0	0.133333	0	0	0.033333	0.133333	0.1
	0	0	0	0	0	0	0	0	0.066667	0	0	0	0.066667	0.033333
	0	0	0	0	0	0	0	0.033333	0	0	0	0.033333	0.1	0.066667
	0	0	0	0	0	0	0	0	0	0	0	0.033333	0.066667	0.033333
	0	0	0	0	0	0	0	0	0.033333	0	0	0.033333	0.2	0.033333
	0.1	0	0	0	0	0	0.033333	0.033333	0.1	0	0.1	0.066667	0.233333	0.1
	0.1	0	0	0	0	0	0	0	0.033333	0	0.066667	0.133333	0.366667	0.033333
	0.166667	0	0	0	0	0	0	0.033333	0	0	0.066667	0.166667	0.3	0.066667
	0.133333	0	0	0	0	0	0.066667	0	0	0	0.133333	0.233333	0.366667	0.166667
	0.2	0	0	0	0	0	0.233333	0	0	0	0.2	0.266667	0.3	0.166667
	0	0	0	0	0	0	0	0	0	0	0.2	0.333333	0.2	0.333333
	0.166667	0	0	0	0	0	0.066667	0	0	0	0.166667	0.566667	0.533333	0.3
	0.3	0	0	0	0	0	0.066667	0.066667	0.033333	0	0.133333	0.266667	0.433333	0.3

#### 4.10. Export Average Network Burst Data

The Neural Metric Tool can also export the average network burst histogram and raster data. To export this data, click File → Export → Export Average Network Burst Data to CSV. In the resulting .csv file, the data under Well Averages – PSTH represents the average number of spikes on active electrodes (or active units) in the well per network burst for each histogram bin and is the data used to create the average network burst histogram for each well in the Average Network Burst display (top plot). The data under Electrode – PSTH (or Unit – PSTH) represents the average number of spikes on the active electrode (or active unit) per network burst for each histogram bin and is the data used for the average network burst raster in the Average Network Burst display (bottom plot). The .csv file can be opened with any software that can read a delimited text file, such as Microsoft Excel.

#### 4.11. Export Network Burst List

The Neural Metric Tool can also export a Network Burst List to csv. To export this csv, click File → Export → Export Network Burst List. In the resulting .csv file, the network bursts are listed by well, and the Time (s), Duration, Size, and Number of Electrodes (or Number of Units) are listed for each detected network burst in each well. Time (s) indicates the start time of the network burst, Duration (s) indicates the duration of the network burst, Size (spikes) indicates the number of spikes in the network burst, and Number of Electrodes (or Units) indicates the number of electrodes (or units) contributing spike(s) to the network burst.

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