



AXION
BIOSYSTEMS

AXIS NAVIGATOR 1.3 USER GUIDE

**SOFTWARE MANUAL FOR THE
MAESTRO™ MEA SYSTEMS**

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CHAPTER 1. INTRODUCTION

Axion Integrated Studio (AxIS) Navigator is a multipurpose software for data acquisition and analysis with the Maestro Pro and Maestro Edge microelectrode array (MEA) systems. This manual provides a basic overview of *AxIS Navigator* and instructions for data acquisition, stimulation, and analysis. For step-by-step instructions for data acquisition and analysis, see Sections 3.3 and 6.5. For operation of the Maestro Pro, Maestro Edge, Lumos, or APEX, refer to their respective manuals. For cell culturing protocols, application notes, posters, and publications, visit www.axionbiosystems.com. Sample data files can be found on the Maestro computer at Libraries/Documents/Public Documents/Axion BioSystems/Manuals and Documentation/Sample Data.

Throughout this manual, the term “Maestro” will be used to refer to both the Maestro Pro and Maestro Edge systems, since the majority of functionality in *AxIS Navigator* is shared by both platforms. Images in this document are for illustrative purposes and represent a variety of different plate types available for use with the Maestro Pro system. The only plate available for use with the Maestro Edge system is the CytoView MEA 24 plate.

Additional analysis tools are available to supplement *AxIS Navigator* and provide application-specific analysis support (see Appendix A). Tools are available for download through the ShareFile system. Contact support@axionbio.com for ShareFile access.

1.1. NEW AXIS NAVIGATOR FEATURES

AxIS Navigator 1.3 enables the induction, acquisition, and analysis of Local Extracellular Action Potential (LEAP) signals using the Maestro MEA Systems. A LEAP signal is an extracellular action potential waveform that provides a direct mapping from field potential to action potential morphology. The LEAP signal is much larger than the field potential, and arrhythmic events are clearer in the LEAP signal, improving automated analysis. For more information about LEAP, see Chapter 5.

In addition to LEAP, *AxIS Navigator 1.3* includes an array of subtle changes in an effort to continually improve the simplicity and usability of the Maestro MEA Systems.

1.2. STANDALONE TOOL UPDATES

Axion BioSystems provides a variety of standalone tools for processing data and producing figures. Guides for these tools can be found in the Appendix of this User Guide. The following list highlights changes to the standalone tools with this release.

CiPA Analysis Tool:

- When a LEAP file is loaded, the LEAP waveform is displayed and new LEAP metrics are computed
- Automatic classification of arrhythmia for LEAP signals
- Improved FPD detection
- Consolidated inspection window
- Option to lower the beat detection threshold

AxIS Metric Plotting Tool:

- Now compatible with Well Endpoint .csv files saved from the CiPA Analysis Tool
- Larger fonts on exported figures

1.3. TECHNICAL SUPPORT

For additional *AxIS Navigator* support and assistance, please contact your authorized Axion BioSystems distributor or Axion BioSystems directly.

Axion BioSystems contact information:

Phone: 1 (404) 477-2557

Email: support@axionbio.com

Please have the following information available when requesting technical assistance:

- Description of the problem
- What was happening at the time of the error
- What you have tried so far to solve the problem
- A copy of Axion Support Bundle (generated from the **Help** menu)
- Screen shots of any errors

CHAPTER 2. AXIS NAVIGATOR OVERVIEW

The *AxIS Navigator* user interface is designed to provide easy access to all experimental controls. The top **Menu bar** controls file loading, saving, and display. Most of the settings for data acquisition, analysis and display are found in the left panel. The left panel contains the current plate information (**Active Plate**), the **File Play** and **Display** controls, and the data stream and data processors under **Streams**. The **Control Bar** along the bottom contains functional and data visualization modules. The active window displays the module selected from the **Control Bar** below. The **Status Bar** at the bottom of the active window displays file status, play and record times, and timestamp notes.



2.1. MENU BAR

The **Menu** bar has four options:

Menu	Option	Description
File	Open Recording(s)...	Loads one or more .raw files into the Streams window.
	New Batch Process...	Opens the batch processing dialog. See Section 6.6.
	Open Stim Waveform	Loads a stimulation waveform file (.sswf) into the Stimulation Studio module. See Chapter 4.
	Save Stim Waveform	Saves the current stimulation waveform from Stimulation Studio . See Chapter 4.
	Exit	Closes <i>AxIS Navigator</i> .
View	Show All Modules	Displays all modules on the Control Bar . See Section 2.6.
	Module Name	Displays the selected Control Bar module in the active window.

Tools	Enable Remote Control	Allows AxIS Navigator to receive commands from other Axion tools.
	Motors → Ignore Safety Limits...	Allows direct control over the motors in the door and plate holder and bypasses safety controls. Only select this option with direct guidance from Axion BioSystems. Warning: Ignoring safety limits may result in mechanical damage or improper operation.
	Motors → Recalibrate Motors...	Recalibrates Maestro motors in the door and plate holder. Only select this option with direct guidance from Axion BioSystems.
Help	Restore Firmware	Reinstalls or updates the firmware on the Maestro (and Lumos if attached) to ensure compatibility with AxIS Navigator. This should be done after every software upgrade.
	User Guide	Opens the AxIS Navigator user guide.
	Create Support Bundle	Creates an error log report for email to Axion Support team.
	About	Displays AxIS Navigator version number and information.

2.2. ACTIVE PLATE

The **Active Plate** displays the current plate configuration. The plate configuration determines how electrodes are mapped to the wells and is critical for proper analysis. The **Active Plate** interface can be used to select wells or electrodes for recording and store information about well contents.

The **Active Plate** will automatically be set to the correct plate type when a plate is docked in a Maestro MEA system.

Maestro plate types include:

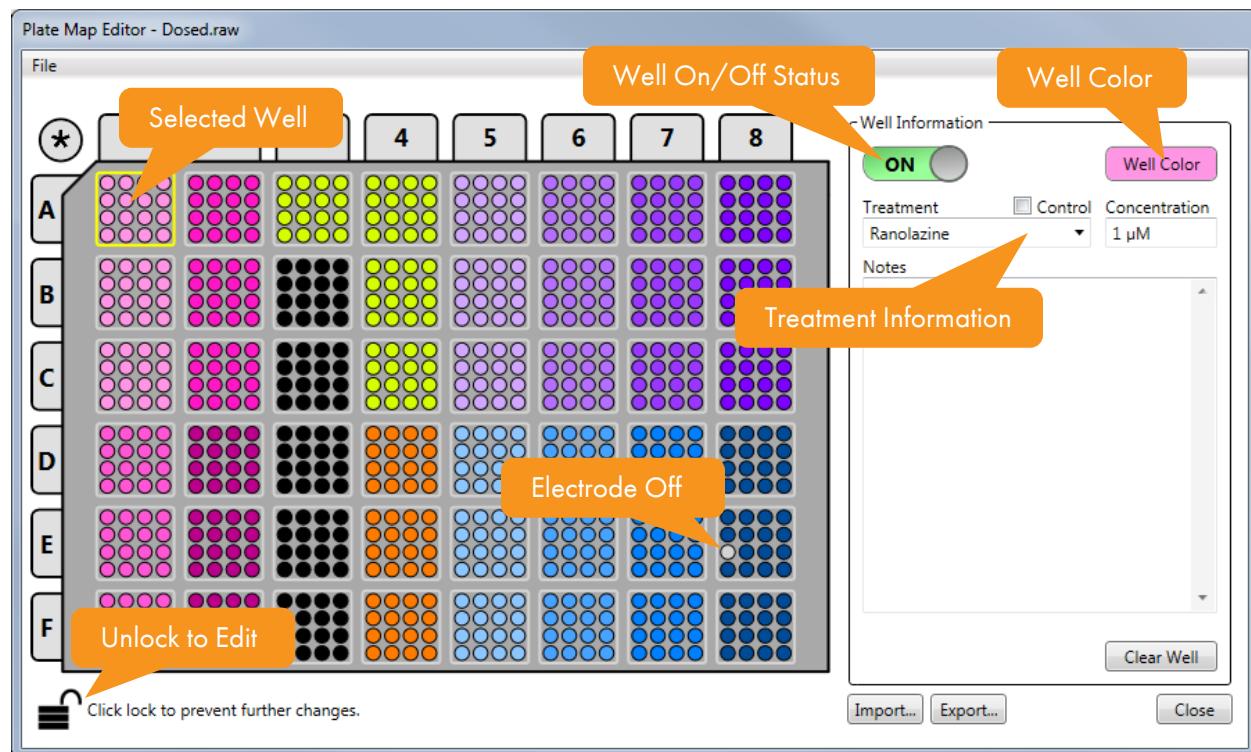


Option	Plate name	System Compatibility	Part number
Classic MEA 48 (incl. AccuSpot)	Classic MEA 48 AccuSpot MEA 48	Maestro Pro	M768-KAP-48 M768-KAP-48A
Classic MEA 48 (E-Stim+)	E-Stim+ Classic MEA 48	Maestro Pro	M768-KAP-48S
Classic MEA 96	Classic MEA 96	Maestro Pro	M768-KAP-96
CytoView MEA 12	CytoView MEA 12	Maestro Pro	M768-GL1-30Pt200 M768-GL1-30Au200
CytoView MEA 24	CytoView MEA 24	Maestro Pro Maestro Edge	M384-tMEA-24W
CytoView MEA 48	CytoView MEA 48	Maestro Pro	M768-tMEA-48B M768-tMEA-48W
Lumos MEA 48	Lumos MEA 48	Maestro Pro	M768-tMEA-48OPT

2.2.1. Plate Map Editor

Double-click the **Active Plate** to open the **Plate Map Editor**. The **Plate Map Editor** is used to select wells or electrodes for recording and store information about well contents. The editor has a representation of the plate where each well consists of a square of circle electrodes. The right section contains the well information of the currently selected well(s), including the **Treatment**, **Concentration**, **Well Color**, **Notes**, and whether the well is on or off for recording. **Import** and **Export** buttons allow the user to load previously created plate maps or export the current map for future use. The **Lock** icon prevents the information from being edited. When editing the plate map associated with the live Maestro stream, the Plate Map Editor is always unlocked. For a previously recorded raw file, click the lock on the lower left to make changes. Information entered in the editor is stored with the recording and used in data analysis.

Note: All changes to the plate map are automatically saved in the previously recorded file.



2.2.2. Enabling or Disabling Wells and Electrodes

Once selected, wells can be turned on or off with the toggle switch under **Well Information**. Disabled wells appear black in the Plate Map Editor. An electrode may be turned on or off by double-clicking on it. Disabled electrodes are connected to ground and appear gray in the Plate Map Editor. Turning off a well or electrode will prevent it from being recorded or analyzed, reducing the file size.

2.2.3. Entering Well Information

Click on a well to select it. Multiple wells can be selected by clicking on the column or row label to highlight the column or row, respectively; clicking the * in the upper left corner to highlight the entire plate; holding the **Ctrl** key and clicking on the desired wells; selecting two wells while holding the **Shift** key to select all of the wells between them; or by click-and-drag selecting wells in a region.

Note: A plate map can only be edited when it is unlocked using the **Lock** button.

To assign well information:

1. Select the desired well(s).
2. Type a name in the **Treatment** field and press enter or click on the green check mark that appears. Do not use commas in the Treatment name. Use the control checkbox to indicate if this well is a control well.
3. Type the concentration in the **Concentration** field, including units (a space between the units and concentration is optional), and press enter or click on the green check mark that appears. Do not use commas, dashes, or special characters in the Concentration field. If concentration is not applicable, leave the field blank.

Note: AxIS Navigator will convert "uM" or "um" to " μM " or " μm ", respectively.

4. Enter any additional information in the **Notes** field.

Note: When selecting multiple wells to edit, each field operates independently. If only the Treatment is entered, Concentration information will remain unchanged for those wells, and vice versa. If a field is empty, it will not be cleared, rather it will remain unchanged.

To copy well information between wells:

1. Select the desired well(s).
2. Press **Ctrl-C** or right-click → **Copy Well**.
3. Select the destination well(s)
4. Press **Ctrl-V** or right-click → **Paste Well**

Note: Multiple wells can be copied and pasted at the same time, as long as the selected wells have the same shape (e.g. copy/pasting a full row to a different row).

To clear well information from wells:

1. Select the desired well(s).
2. Click **Clear Well**.

2.2.4. Setting Well Color

To set well color manually:

1. Select the well(s).

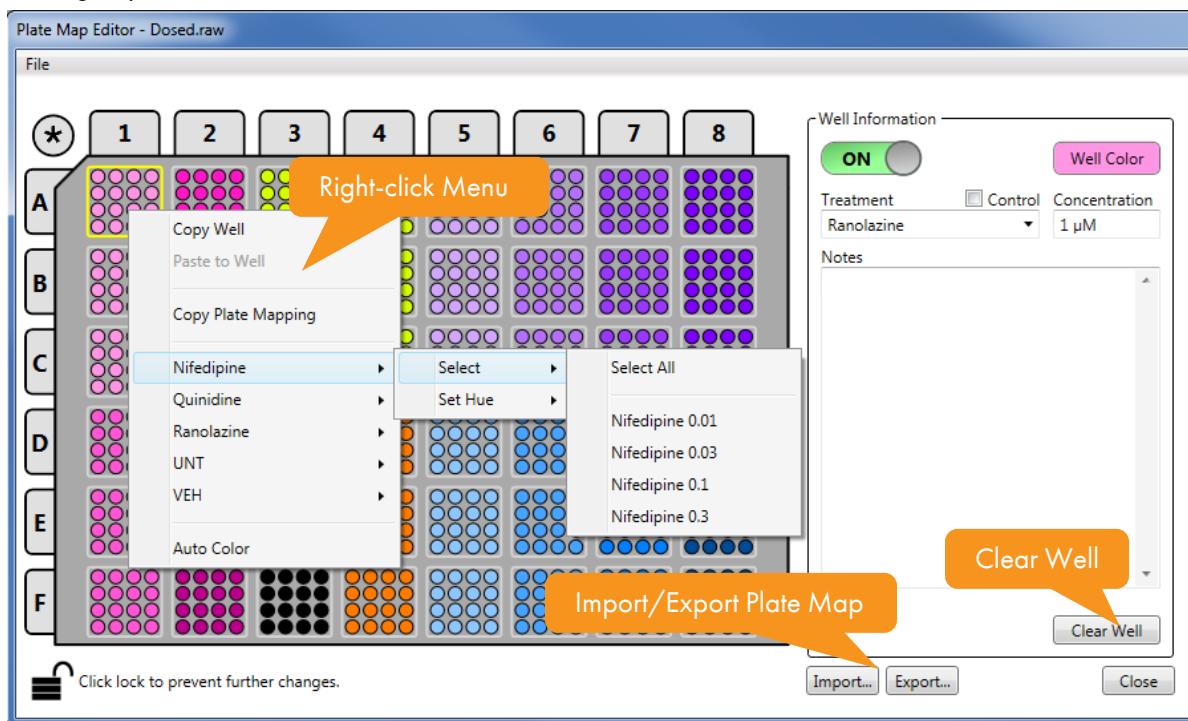
- Click on **Well Color** in the upper right corner of **Well Information** and choose a color.

To set well color automatically:

- Right-click on any well.
- Select **Auto Color**. Each treatment will receive a different hue and each concentration of a treatment will receive a different shade.

To change the hue of a treatment group:

- Right-click on any well.
- Select the treatment group from the menu.
- Select **Set Hue** and choose the hue from the options available. Each concentration in that treatment group will receive a different shade of the selected hue.



2.2.5. Importing and Exporting Plate Maps

Plate maps may be imported or exported using the **Import** and **Export** buttons in the **Plate Map Editor**. Plate maps can also be copied and pasted to and from *Microsoft Excel* (Section 2.2.6).

To export a plate map using the export button:

- Click the **Export** button in the **Plate Map Editor**.
- Enter a name for the plate map and click **Save**. The file will be saved with a .platemap extension.

To import a plate map using the import button:

1. Click the **Import** button in the **Plate Map Editor**.
2. Select the desired .platemap, .raw, or .spk file and click **Open**.

Note: Selecting a previous recording (.raw file) or Spike file (.spk) will load its plate map into the current file.

2.2.6. Creating a Plate Map in Excel

Axis Navigator recognizes two text formats which may be imported from or exported to Microsoft Excel.

In format 1, each row corresponds to a different piece of plate map data and each column represents a well. The columns are labeled with the well ID where well A1 is row A, column 1. The plate map data includes:

Option	Description														
Active	Whether or not the well is enabled. (TRUE or FALSE)														
Well Coloring	Color code for the Well Color														
Control	Whether or not Control field is selected (TRUE or FALSE)														
Treatment	String containing the Treatment field														
Concentration	String containing the Concentration field														
Additional Information	String containing the Notes field.														
Well	A1	A2	A3	A4	A5	A6	A7	A8	B1	B2	B3	B4	B5	B6	B7
Active	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	FALSE	TRUE	TRUE	TRUE	TRUE
Well Coloring	#FF96E4	#FF16C4	#D4FF00	#D4FF00	#D1A6FF	#B46FFF	#9838FF	#7C01FF	#FF96E4	#FF16C4		#D4FF00	#D1A6FF	#B46FFF	#9838FF
Control	FALSE	FALSE	TRUE	TRUE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	FALSE	FALSE	FALSE
Treatment	Ranolazin	Ranolazin	VEH	VEH	Quinidine	Quinidine	Quinidine	Quinidine	Ranolazin	Ranolazine	VEH	Quinidine	Quinidine	Quinidine	
Concentration	1 µM	10 µM			0.3 µM	1 µM	3 µM	10 µM	1 µM	10 µM			0.3 µM	1 µM	3 µM
Additional Information															

To copy the plate map in Axis Navigator and paste to Excel/in format 1:

1. Right-click on one or more wells in the **Plate Map Editor**.
2. Select **Copy Wells**.
3. Click on any cell in Excel/and press **Ctrl+V**.

To copy the plate map in Excel/and paste in Axis Navigator.

1. Highlight the desired wells in Excel/, including the Well Number.
2. Press **Ctrl+C**.
3. Select the corresponding wells in the **Plate Map Editor**.
4. Right-click on one of the wells and select **Paste to Well(s)**.

In format 2, each cell represents a well on the plate and contains information in the format of "Treatment [Concentration]". The color of the cell corresponds to the Well Color. Rows are labeled A, B, C... and columns are labeled 1, 2, 3....

	1	2	3	4	5	6	7	8
A	Ranolazine [1 µM]	Ranolazine [10 µM]	VEH	VEH	Quinidine [0.3 µM]	Quinidine [1 µM]	Quinidine [3 µM]	Quinidine [10 µM]
B	Ranolazine [1 µM]	Ranolazine [10 µM]		VEH	Quinidine [0.3 µM]	Quinidine [1 µM]	Quinidine [3 µM]	Quinidine [10 µM]
C	Ranolazine [1 µM]	Ranolazine [10 µM]		VEH	Quinidine [0.3 µM]	Quinidine [1 µM]	Quinidine [3 µM]	Quinidine [10 µM]
D	Ranolazine [3 µM]	Ranolazine [30 µM]		UNT	Nifedipine [0.01]	Nifedipine [0.03]	Nifedipine [0.1]	Nifedipine [0.3]
E	Ranolazine [3 µM]	Ranolazine [30 µM]		UNT	Nifedipine [0.01]	Nifedipine [0.03]	Nifedipine [0.1]	Nifedipine [0.3]
F	Ranolazine [3 µM]	Ranolazine [30 µM]		UNT	Nifedipine [0.01]	Nifedipine [0.03]	Nifedipine [0.1]	Nifedipine [0.3]

To copy the plate map in *AxIS Navigator* and paste to *Excel*/in format 2:

1. Right-click on any well in the **Plate Map Editor**.
2. Select **Copy Plate Mapping**.
3. Click on any cell in *Excel*/and press **Ctrl+V**.

To copy the plate map in *Excel*/and paste in *AxIS Navigator*:

1. Highlight the entire plate map in *Excel*, including the row and column headers.
2. Press **Ctrl+C**.
3. Select all wells in the **Plate Map Editor**.
4. Right-click on one of the wells and select **Paste to Well(s)**.

2.3. STREAMS

The **Streams** window displays all currently loaded data streams. A data stream contains the live or recorded continuous voltage data from a Maestro and all associated data processors. See Section 2.3.1 for more information on data processors. A stream is organized in a hierarchy beginning with the continuous voltage data and then passing through the data processors in sequence. Combining processors into different configurations provides customizable stream flows for different applications. Axion provides preset processor configurations for specific cardiac and neural applications (Sections 3.2.2 and 6.1). Customized configurations can be manually constructed by placing individual data processors on the stream (Section 2.3.1) and saved or loaded by right-clicking on the stream.

The first stream represents the Maestro continuous voltage data. When the Maestro is connected, the stream will be called **Maestro Pro** or **Maestro Edge**, depending which Maestro MEA system is used. If no Maestro is connected, the stream will be inactive and say "No Device Connected".

Only active streams may be viewed or analyzed in *AxIS Navigator* and only one stream may be active at a time. The active stream is indicated by a colored square and bold black text. Inactive streams have gray squares and gray text.

To activate a data stream:

1. Right-click on the continuous voltage data name.
2. Select **Select for Play/Rec**.

To load a new data stream:

1. Click **File → Open Recording(s)....**

2. Navigate to the desired recording (.raw file) and click **Open**.

To remove a data stream or batch process:

1. Right-click on the continuous voltage data name.
2. Select **Remove**.

To remove all data streams in the **Streams** window:

1. Right-click on white space in the **Streams** window.
2. Select **Remove All Files**.

2.3.1. Data Processors

Data processors are added to data streams to alter or analyze the data. They all require input from a continuous data stream or another data processor, and output an altered continuous data stream, metric(s), and/or an output file. Information from some data processors is visualized using the various *AxIS Navigator* modules. Some data processors and settings are disabled during live data streams and some may not be modified unless streaming has stopped.

Preset configurations containing combinations of data processors recommended for most applications are provided in *AxIS Navigator*. See Sections 3.2.2 and 6.1 for more information about these recommended default stream configurations.

To add a data processor:

1. Right-click on the stream in the **Streams** window.
2. Select **Add Processing** and select the desired data processor.
Note: Some data processors are dependent on others and may only be added below the requisite processor.
3. Enter the desired settings and click **OK**.

To remove a data processor:

1. Right-click on the processor in the **Streams** window.
2. Select **Remove**.

Note: Removing a data processor will remove all processors below it on the same continuous data stream.

To access the settings of a data processor:

1. Right-click on the processor in the **Streams** window.
2. Select **Settings**.
-- Or --
1. Double-click on the processor in the **Streams** window.

To change the visibility of a data processor:

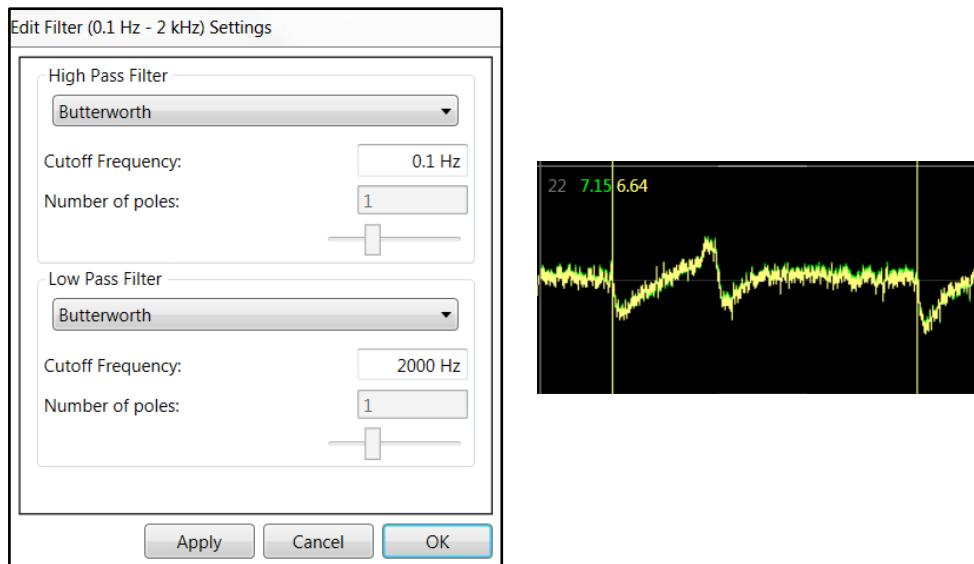
1. Right-click on the processor's stream in the **Streams** window.
2. Select **Plot → Show**. If enabled, the processor will show in dependent *AxIS Navigator* modules.

To change the color of a data processor:

1. Right-click on the processor in the **Streams** window.
2. Select **Plot → Change Trace Color**.
3. Select the desired color and click **Enter**.

2.3.2. Digital Filter

The **Digital Filter** processor applies a digital Butterworth bandpass filter to any continuous data stream to further reduce noise. This filter is applied on top of hardware-based filters selected in the **Maestro Pro/Edge Settings** (Section 3.2.1) during a recording. The output of this data processor is a filtered continuous data stream (.raw file), and data processors placed below the **Digital Filter** will act on the filtered data stream.



The digital filter has a **High Pass Filter**, the frequency components of the signal must be above the cutoff frequency to pass through, and a **Low Pass Filter**, the frequency components of the signal must be below the cutoff frequency to pass through. To adjust either, enter the new value into the respective **Cutoff Frequency** text field.

2.3.3. Artifact Eliminator

The **Artifact Eliminator** removes artifact in raw signals resulting from electrical stimulation. The removal includes blanking of the large initial impulse as well as removal of residual artifact based on the similarity of its profile across electrodes. The output of this data processor is a continuous data stream (.raw file) with the artifact removed. Data processors placed below the **Artifact Eliminator** will act on that output.

2.3.4. Stimulation Inspector

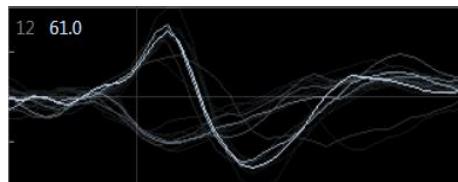
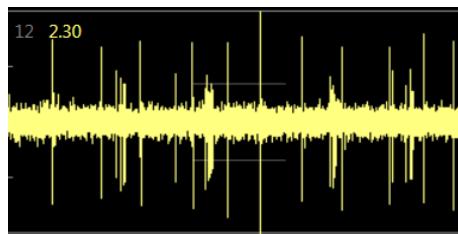
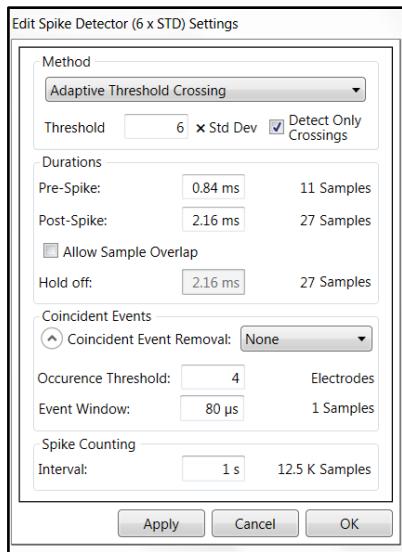
The **Stimulation Inspector** provides a close look at data near an **Electrical Stimulation Tag** to evaluate artifact elimination and cellular response to the stimulation. The **Stimulation Inspector** plots the continuous voltage data just before and after an **Electrical Stimulation Tag** in the **Spike Plots** module. Note the **Stimulation Inspector** is for visualization only and does not produce an output file.



While using the **Stimulation Inspector** data processor, the **Electrical Stimulation Tag** will appear as a triangle and vertical line at time zero on the spike plot. The time displayed before and after the tag may be changed in the **Stimulation Inspector** settings by the **Pre-Tag** and **Post-Tag** fields, respectively. The waveform plots overlay with the brightest trace representing the most recently detected stimulation event.

2.3.5. Spike Detector

The **Spike Detector** detects threshold crossings in the continuous data stream. These crossings are referred to as "spikes" and are plotted in the **Spike Plots** module as spike waveform and raster plots. The **Spike Detector** processor is the base processor for neural data visualization and analysis. It identifies the spike timing and location and is the source for the **Spike Plots** and **Activity Map** modules. **Burst Detector** data processors (Section 2.3.6) may only be placed below a **Spike Detector**. The **Spike Detector** produces several outputs including **AxIS Spike** (.spk), **Spike Count** (.csv), and **Spike List** (.csv) files. See Section 6.2 for more information about these output formats.



Setting a proper detection threshold is crucial for accurate neural data analysis. Lower thresholds increase the incidence of false-positives (small noise events misidentified as spikes); higher thresholds may not detect smaller amplitude action potentials. Axion recommends an adaptive threshold of 6 x standard deviations to minimize both false-positives and missed detections.

The **Spike Detector** has four possible threshold detection methods selected by the **Methods** drop-down menu:

- Adaptive Threshold** (*Recommended*): Threshold is set on a per electrode basis, as a multiple of the noise of the continuous data, each electrode threshold is specific to that electrode. The standard deviation multiple can be set in the **Threshold** field. **Detect Only Crossings** requires the signal to return below threshold before detecting an additional spike. The spike time is marked at the maximum slope of the spike voltage waveform.
- Static Threshold**: Threshold is set on a plate-wide basis, all electrode thresholds are the same. Spikes are detected as any event greater than the value defined in the **Threshold** field. **Detect Only Crossings** requires the signal to return below threshold before detecting an additional spike. The spike time is marked at the maximum slope of the spike voltage waveform.
- Peak Detection Adaptive Threshold**: Functions the same as the **Adaptive Threshold** above, but the spike time is marked at the peak of the spike voltage waveform, as defined by the **Peak Detection** drop-down menu.
- Peak Detection Static Threshold**: Functions the same as the **Static Threshold** above, but the spike time is marked at the peak of the spike voltage waveform, as defined by the **Peak Detection** drop-down menu.

The **Peak Detection** drop-down menu options are:

Option	Description
Positive Inflection	Marks the first peak with a positive voltage
Negative Inflection	Marks the first peak with a negative voltage
First Peak	Marks the first peak regardless of polarity (positive or negative)
Maximum Amplitude	Marks the peak with the largest amplitude

The **Durations** dialog box sets the display in the **Spike Plots** module. Use the **Pre-Spike** and **Post-Spike** fields to specify how much time before and after each spike crossing will be displayed on the spike waveform plot and saved in an AxIS Spike file. The spike time, determined by the **Method** drop-down menu, is plotted as zero.

The **Coincident Events** dialog box removes coincident artifacts from analysis results. Coincident artifacts are artificial spikes detected on multiple electrodes at exactly the same time. They may occur during an electrical stimulus or due to environmental interference like bumping the system or touching the media in a well. *Ax/S Navigator* identifies a coincident artifact as spikes occurring on a minimum number of electrodes (**Occurrence Threshold**) over a maximum length of time (**Event Window**).

The **Coincident Event Removal** drop-down menu specifies how *Ax/S Navigator* will search for coincident spikes across the plate. Select from:

1. **None**: No search will be performed.
2. **Well**: Search will look for coincidence across electrodes in a well.
3. **Plate**: Search will look for coincidence across electrodes in any well on the plate.
4. **Chip**: Search will look for coincidence across electrodes that share a hardware circuitry connection.

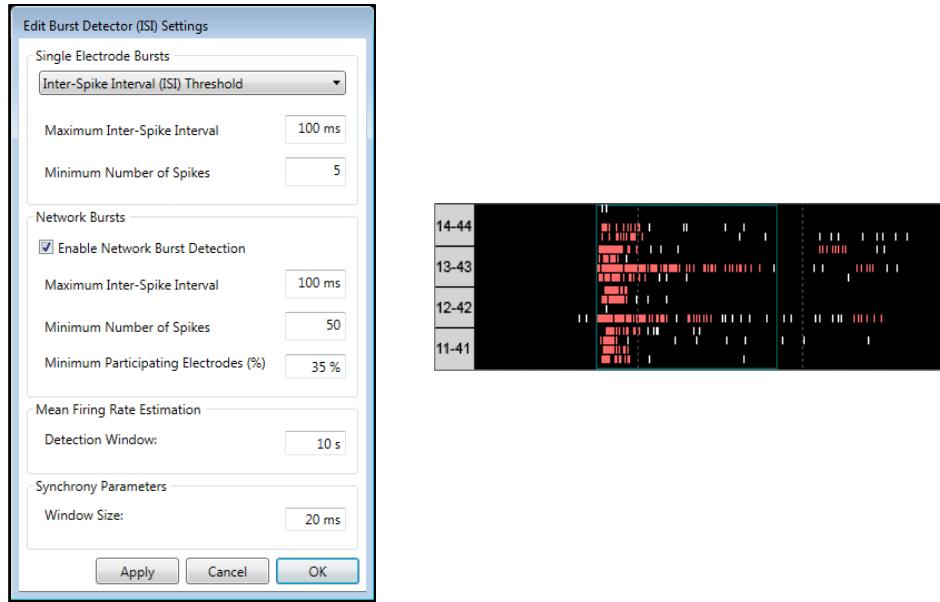
For example, if **Coincident Event Removal** is set to **Well** with **Occurrence Threshold** equal to 4 electrodes and **Event Window** equal to 80 µs, spikes that occur on at least 4 electrodes in the same well within 80 µs of each other will be ignored.

The **Spike Counting** box sets the bin time for counting spikes as defined by the **Interval** field. The bin time is used by the **Activity Map** and **Statistics Graphing** modules and the **Spike Count** files.

2.3.6. Burst Detector

The **Burst Detector** is a neural data processor that analyzes spike timing patterns to identify bursts on individual electrodes (single-electrode bursts) and across multiple electrodes (network bursts). See Section 8.3 for more information about neural bursting. Because it requires spike data, a **Burst Detector** can only be placed after a **Spike Detector**. A **Neural Statistics Compiler** data processor (Section 2.3.9) may only be placed below a **Burst Detector**. The **Burst Detector** has two outputs: **Electrode Burst List (.csv)** and **Network Burst List (.csv)**.

Burst Detector results are displayed in the raster plot in the **Spike Plots** module. Spikes belonging to single-electrode bursts are plotted on the raster in the same color as the **Burst Detector** plot color. Network bursts are indicated by a box of the contrasting color.



The **Burst Detector** has two single-electrode burst detection methods, selectable in the **Single Electrode Bursts** drop-down menu:

1. **Inter-Spike Interval (ISI) Threshold:** An electrode burst is defined as at least N spikes on an electrode, each separated by an inter-spike interval (ISI) of no more than T seconds. The method is adapted from Chiappalone et al., 2005. The minimum number of spikes and maximum time between each spike are set using the **Minimum Number of Spikes** and **Maximum Inter-Spike Interval** fields, respectively.
2. **Poisson Surprise:** This algorithm assumes the neurons are firing according to a Poisson distribution. A collection of spikes is compared to the probability that this collection of spikes would occur by chance. If the collection of spikes exceeds the “surprise” threshold, then it is considered an electrode burst. The **Minimum Surprise** field determines how sensitive burst detection is. A higher threshold makes the burst detector less likely to detect an electrode burst, while a lower threshold is more lenient and more likely to accept a less “surprising” collection of spikes as burst. The method is adapted from Legéndy & Salcman, 1985. In this way, the algorithm is adaptive to the mean firing rate on each electrode, computed dynamically to enable real-time detection and visualization of bursts.

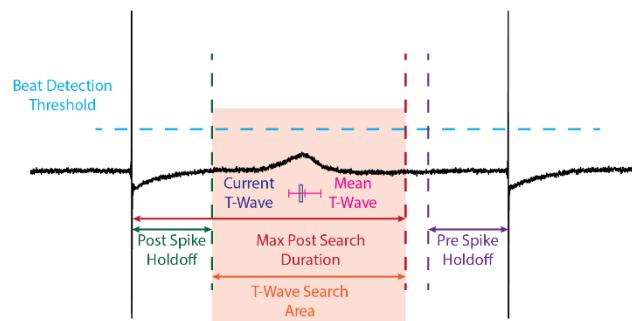
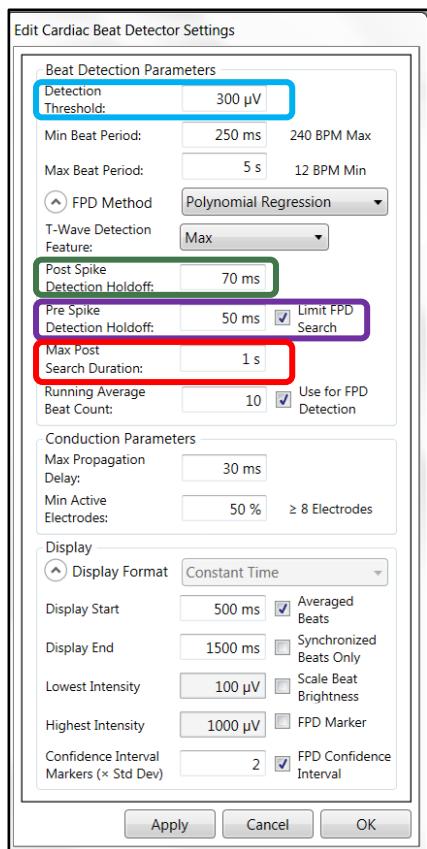
Use the checkbox in the **Network Bursts** section to **Enable Network Burst Detection**. As in the single-electrode burst definition, the network burst algorithm relies on defining a network burst as a collection of N spikes, each separated by an inter-spike interval of no more than T seconds, however the spikes are detected across the entire well instead of being limited to a single electrode. In addition to the above criteria, a minimum number of electrodes (E) must contribute to the network burst. In the **Network Bursts** box, set N spikes with **Minimum Number of Spikes**, T seconds with **Maximum Inter-Spike Interval**, and E electrodes as a percentage of the total electrodes with **Minimum Participating Electrodes (%)**.

The **Detection Window** field in the **Mean Firing Rate Estimation** box sets the duration of the sliding window used to calculate the mean firing rate for Poisson Surprise burst detection.

The **Window Size** field in the **Synchrony Parameters** box is the window of time around zero phase-lag used to compute the area under the cross-correlation and area under the normalized cross-correlation synchrony metrics provided by *AxIS Navigator*. See Section 8.3 for further explanation of synchrony.

2.3.7. Cardiac Beat Detector

The **Cardiac Beat Detector** processor detects threshold crossings in the continuous data stream to identify “beats” and calculates associated cardiac endpoints. The beats are displayed in the **Cardiac Beats Plots** module as cardiac waveform, conduction, and beat period plots. The **Cardiac Beat Detector** is the base processor for cardiac data visualization and analysis. It identifies the beat timing, propagation, amplitude, and duration and is used as a source for the **Cardiac Beat Plots** and **Activity Map** modules. The **Cardiac Statistics Compiler** data processor (Section 2.3.8) may only be placed below a **Cardiac Beat Detector**. The **Cardiac Beat Detector** has two outputs: **Electrode Beat List (.csv)** and **Well Beat List (.csv)**. See Chapter 7 for more information about cardiac analysis endpoints.



The **Cardiac Beat Detector** settings are divided into three sections: **Beat Detection Parameters**, **Conduction Parameters**, and **Display**.

Setting a proper detection threshold is crucial for accurate cardiac data analysis. Beat detection is controlled by three fields:

1. **Detection Threshold:** Sets the threshold for detecting a beat for field potential signals. When the continuous voltage data exceeds this threshold, the algorithm begins searching for a beat that meets the **Min Beat Period** and **Max Beat Period** parameters. If a beat is identified, the beat time is marked as the point of maximum slope of the depolarization spike. The threshold should be lower than the initial depolarization spike amplitude and higher than any other feature of the cardiac waveform (300-600 µV is recommended).

Note: A separate algorithm is used to detect beats for LEAP signals acquired using Cardiac: LEAP Acquisition Settings. Changing the Detection Threshold will not influence the detection of LEAP beats.

2. **Min Beat Period:** The minimum time between two threshold crossings to be considered a beat.
3. **Max Beat Period:** The maximum time between two threshold crossings to be considered a beat.

For field potential signals, Field Potential Duration (FPD) is calculated as the time between the depolarization and repolarization, noted by the beat time and the repolarization peak or t-wave, respectively. Due to processor constraints, FPD detection is disabled during data acquisition. To identify the t-wave, there are three methods selectable in the **FPD Method** drop-down menu:

1. **Polynomial Regression (Recommended):** Performs a polynomial regression to identify a peak or trough between two time points. The search window starts at the **Post Spike Detection Holdoff**, the time after the current depolarization spike. The window ends with either the **Pre Spike Detection Holdoff**, the time before the next depolarization spike, or the **Max Post Search Duration**, a fixed maximum time after the current depolarization spike. The **T-wave Detection Feature** drop-down determines whether the regression searches for a peak (**Max**), trough (**Min**), or either (**Auto (Max/Min)**).
2. **Inflection Search:** Segments the search window to identify the region containing the t-wave and then performs a polynomial regression on that segment to determine the exact location. To identify the t-wave segment, the algorithm searches for a region that crosses a threshold X times the noise as set by **Detection Threshold**. After identifying the segment, a polynomial regression is performed on a window of size **Regression Window Size**. The search window starts with the **Post Spike Detection Holdoff**, the time after the current depolarization spike, and ends with the **Pre Spike Detection Holdoff**, the time before the next depolarization spike. The number of segments is set by the **Beat Segmentation** field. The **T-wave Detection Feature** drop-down determines whether the regression searches for a peak (**Max**), trough (**Min**), either (**Auto (Max/Min)**), or the maximum slope of the waveform (**dV/dt**).
3. **Zero Crossing:** Performs a polynomial regression to identify a zero crossing between two time points. The search window starts with the **Post Spike Detection Holdoff**, the time after the current depolarization spike. The window ends with either the **Pre Spike Detection Holdoff**, the time before the next depolarization spike, or the **Max Post Search Duration**, a fixed maximum time after the current depolarization spike. The first zero crossing of this regression is chosen as the t-wave location.

Averaging multiple cardiac waveforms across beats can reduce noise and increase t-wave resolution and recognition by *Ax/S Navigator*. The cardiac waveform displayed in the **Cardiac Beat Plots** module is the average of the current beat and the previous N-1 beats as set by the **Running Average Beat Count**. Use the averaged beat for FPD detection by enabling the **Use for FPD Detection** checkbox.

The **Conduction Parameters** section identifies well beats, also called synchronized beats. A synchronized beat is a depolarization spike detected on a minimum number of electrodes within a well in a certain time window. The synchronized beat time is marked as the beat time of the first participating electrode. The minimum percentage of participating electrodes is set by **Min Active Electrodes**. The time frame is set by **Max Propagation Delay**. Synchronized beats are displayed on the **Conduction** plot in the **Cardiac Beat Plots** module.

The **Display** section controls the data visualization in the **Cardiac Beat Plots** module. It has the following settings:

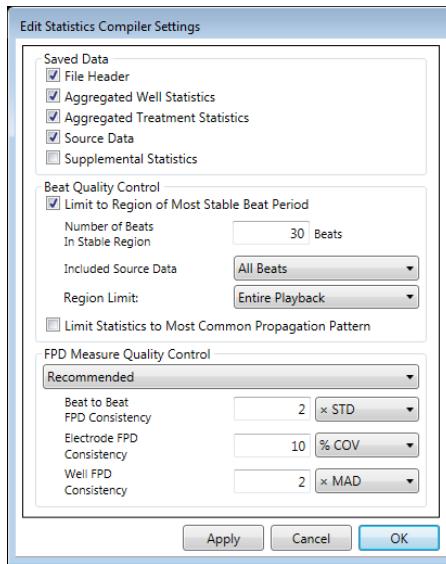
1. **Display Start**: The amount of time displayed before the depolarization spike.
2. **Display End**: The amount of time displayed after the depolarization spike.
3. **Averaged Beats** checkbox: If enabled, displays the averaged beat waveform plot instead of the instantaneous beat waveform plot for each electrode. The number of beats used to create the average is specified in the **Running Average Beat Count** field in **Beat Detection Parameters**.
4. **Synchronized Beats Only** checkbox: If enabled, displays only beats that qualify as synchronized beats.
5. **Scale Beat Brightness** checkbox: If enabled, beats with large amplitude depolarization spikes are displayed brighter than beats with low amplitude depolarization spikes.
6. **Lowest Intensity**: If **Scale Beat Brightness** is enabled, sets the amplitude of low amplitude depolarization spikes.
7. **Highest Intensity**: If **Scale Beat Brightness** is enabled, sets the amplitude of large amplitude depolarization spikes.
8. **FPD Marker** checkbox: If enabled, displays a rectangle marking the current T-wave location on each field potential, providing a visual verification of the FPD detection accuracy.
9. **FPD Confidence Interval** checkbox: If enabled, displays a white whisker plot on the field potentials in the cardiac waveform plot indicating the mean T-wave location and the confidence interval as set by **Confidence Interval Markers (x Std Dev)**.
10. **Confidence Interval Markers (x Std Dev)**: Sets the width of the confidence interval displayed as a multiple of the standard deviation.

The checkbox controls can also be set by right-clicking on the beat waveform plots in the **Cardiac Beat Plots** module, and selecting/deselecting the display options.

2.3.8. Cardiac Statistics Compiler

The **Cardiac Statistics Compiler** (displayed as **Statistics Compiler** in the **Streams** window) uses the outputs of a **Cardiac Beat Detector** to calculate a variety of cardiac endpoints. These metrics are calculated for individual

electrodes, well-wide averages, and treatment group averages. Treatment groups are defined by the **Plate Map Editor** in the **Active Plate** interface. The **Cardiac Statistics Compiler** can only be used after a **Cardiac Beat Detector** and only on recorded data. It has one output file, **Advanced Metrics (.csv)**. See Section 6.2 for more information on file types.



The settings window has three sections, **Saved Data**, **Beat Quality Control**, and **FPD Measure Quality Control**. The **Saved Data** section identifies what information is output to the **Advanced Metrics** file. The options include:

1. **File Header:** If enabled, output file will start with a list of all experiment acquisition and analysis settings.
2. **Aggregated Well Statistics:** If enabled, output file will include well-wide averages in addition to individual electrode metrics.
3. **Aggregated Treatment Statistics:** If enabled, output file will include treatment group averages as defined by the **Plate Map Editor**.
4. **Source Data:** If enabled, output file will include lists of individual beat statistics for both synchronized beats and electrode beats.
5. **Supplemental Statistics:** If enabled, additional cardiac endpoints are included in the file. Examples include median and median absolute deviation (MAD) for each metric and additional conduction velocity metrics.

The **Beat Quality Control** section limits which beats are averaged and reported. The options include:

1. **Limit to Region of Most Stable Beat Period:** If enabled, metrics will be calculated from the most stable well beats, calculated as N consecutive well beats with the lowest beat period standard deviation. If fewer than N beats are detected in the file, all detected beats will be used to calculate metrics.
2. **Number of Beats in Stable Region:** Defines the number of consecutive well beats used for the **Limit to Region of Most Stable Beat Period** checkbox.
3. **Included Source Data:** Specifies whether to output the source data for all well beats in the recording (**All Beats**) or for the most stable well beats only (**Most Stable Region Only**).

Note: All Beats is required for the CiPA Analysis Tool.

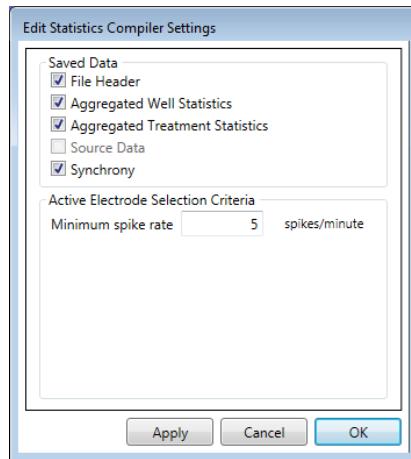
4. **Region Limit:** Sets the time window of the recording used to search for the most stable beats. Specify the time window by selecting **Entire Playback**, **Start of Playback**, or **End of Playback**, to choose the entire recording, the beginning with or without an offset, or the end of the recording, respectively.
5. **Limit Statistics to Most Common Propagation Pattern** checkbox: If enabled, metrics will be calculated from well beats that follow the most common propagation pattern.

The **FPD Measure Quality Control** section removes FPD measurements from beats and electrodes that do not meet user-specified statistical bounds, improving the accuracy of electrode and well-wide FPD averages. The number of remaining electrodes that are used for the well FPD calculation after the criteria are applied is output as "Total FPD Electrodes". The user-specified statistical bounds are applied in the following order:

1. **Beat to Beat FPD Consistency:** Individual beat metrics are compared to the mean or median of all beats on that electrode. FPD values for individual beats are removed when they exceed the specified number of standard deviations (\times STD) or median absolute deviations (\times MAD) from the mean or median, respectively.
2. **Electrode FPD Consistency:** Electrodes with large beat-to-beat variability in FPD are removed. Consistency is based on the coefficient of variation (CoV) of the FPD, with limits set as a percentage ([standard deviation/mean] * 100).
3. **Well FPD Consistency:** Electrodes with an FPD mean that exceeds the specified number of standard deviations (\times STD) or median absolute deviations (\times MAD) from the well mean or median are excluded from the Well Average FPD.

2.3.9. Neural Statistics Compiler

The **Neural Statistics Compiler** (displayed as **Statistics Compiler** in the **Streams** window) uses the outputs of a **Spike Detector** and **Burst Detector** processor to calculate a variety of spike, burst, and synchrony metrics. These metrics are calculated for individual electrodes, well-wide averages, and treatment group averages. Treatment groups are defined by the **Plate Map Editor** in the **Active Plate** interface. The **Neural Statistics Compiler** can only be used after a **Burst Detector** and only on previously recorded data. It has one output file, **Advanced Metrics (.csv)**. See Section 6.2 for more information on file types.



The settings window identifies what information is output to the **Advanced Metrics** file. The options include:

1. **File Header:** If enabled, output file will start with a list of all experiment acquisition and analysis settings.
2. **Aggregated Well Statistics:** If enabled, output file will include well-wide averages in addition to individual electrode values.
3. **Aggregated Treatment Statistics:** If enabled, output file will include treatment group averages as defined by the **Plate Map Editor**.
4. **Synchrony:** If enabled, output file will include synchrony metrics.

Active Electrode Selection Criteria: Identifies active electrodes defined as having a mean firing rate greater than the value defined in the **Minimum spike rate** field. The output file reports the number of active electrodes in each well, as well as the weighted mean firing rate, the mean firing rate averaged across only the active electrodes.

2.4. FILE PLAY AND DISPLAY CONTROLS

The **File Play** and **Display** controls activate the current stream and control data display in the active window.

The **File Play** options are:

Control	Icon	Description
Load/Eject Plate		Engages or disengages a plate from the Maestro. The Maestro door automatically closes when an MEA plate is docked and opens when an MEA plate is undocked. Right-click the Load/Eject button and select Keep Door Open to keep the Maestro door open regardless of plate presence.
Stop		Stops the current stream. A subsequent Play or Record command will resume streaming a .raw file from the start. A live stream will resume starting with a voltage offset.

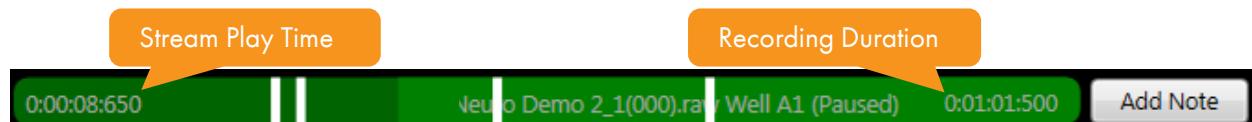
Pause		Pauses the current stream. A subsequent Play or Record command will resume a .raw file at the time it was paused. A live stream will resume without a voltage offset.
Play		Starts the current stream without saving. Data will be displayed in the Control Bar modules.
Record		Records and plays the current stream. Data will be displayed in the Control Bar modules and outputs selected in Experiment Setup Properties will be saved.

The **Display** controls scale data displayed in the active window and, in certain modules, provides a drop-down menu to select the type of data displayed. Use the **Source** drop-down to select the type of data displayed. Use the zoom in (), zoom out (, and reset scale () controls to change the voltage and time scales. The scale is indicated above the control as X units per division. The divisions are noted along the edges of the active window.



2.5. STATUS BAR

The **Status Bar** at the bottom of the active window displays the file status of the current data stream. The time a stream has played for is indicated on the left. The duration of a recording is indicated on the right. The text in the middle of the bar includes the Stream name, play status, well, and treatment information if present in the Active Plate. Click on the status bar to move to a particular time in the recording. The color of the status bar indicates the current file status as outlined in the table below.



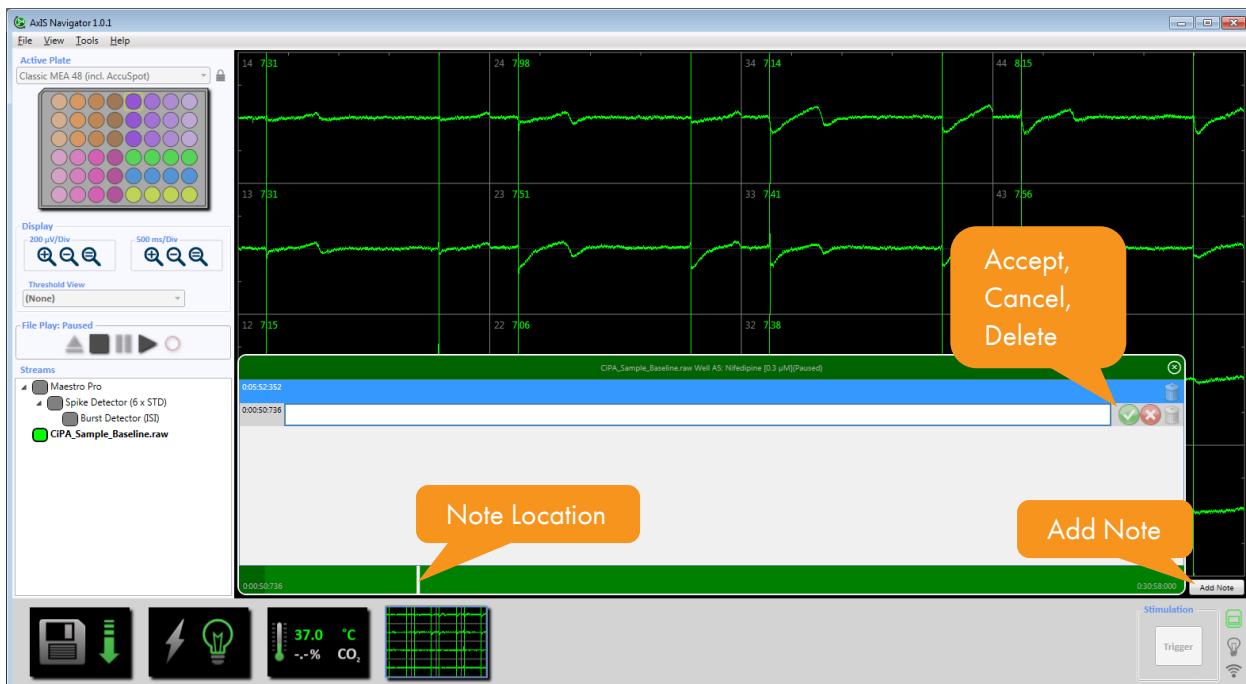
Color	File Status
Light Gray	Current stream is inactive, or live stream is active and playing
Blue Gray	Offset correction in progress.
Green	Current stream is a recording. Dark green indicates time before the currently displayed data.
Red	Current stream is recording. Output files specified in Experiment Setup Properties are being saved.

2.5.1. Adding Timestamp Notes

It is possible to add notes with a timestamp to a recording. Notes can mark important events for further review during analysis. Notes may be added while the file is playing, paused, or recording. A line will appear in the Status Bar at the time of the notes addition. When selected or scrolled over, the note will be displayed.

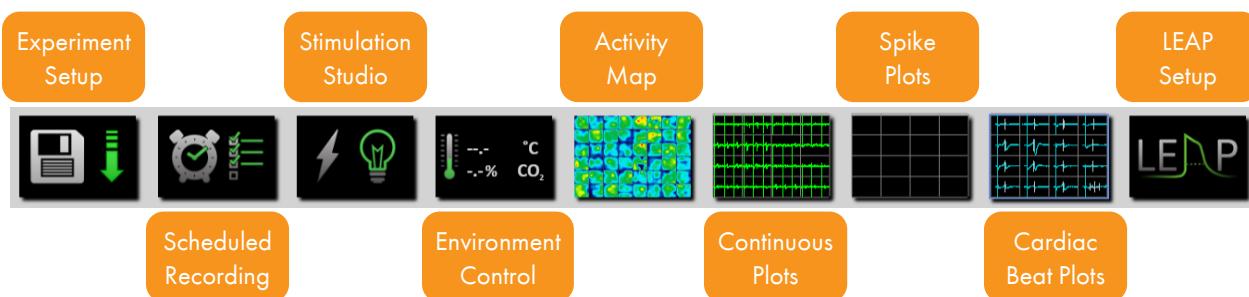
To add a timestamp note:

1. Click **Add Note** located to the right of the **Status Bar** at the desired file time.
2. Type the desired text and click **Accept** (green checkmark).
3. Click the **X** in the top right corner to close the **Notes** panel.



2.6. CONTROL BAR

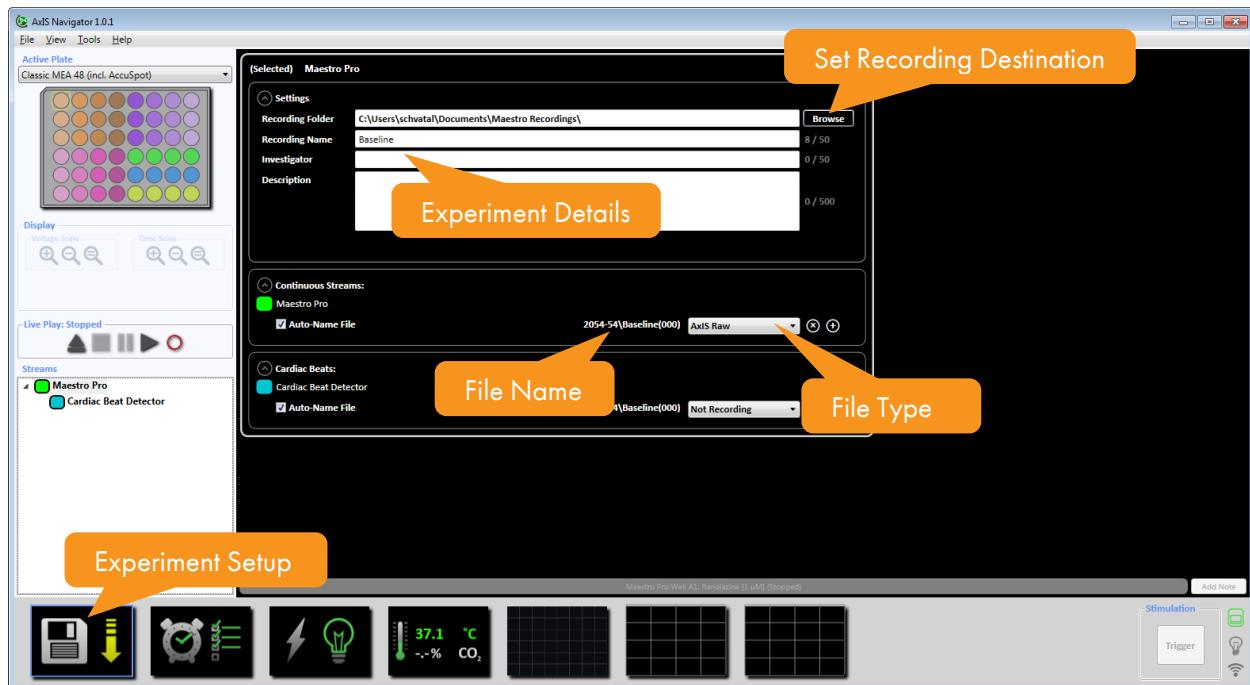
The **Control Bar** interface switches the module displayed in the active window. By default, only modules used by the current configuration in the **Streams** window are displayed. To display all **Control Bar** modules, select **Show all Modules** under the **View** menu. To switch the active module, click on the thumbnail.



Name	Description	Dependency
Experiment Setup Properties	File information controls. Sets save location, experiment notes, recording names, and file types.	Always visible
Scheduled Recording Setup	Automated recording and stimulation controls.	Live data only
Stimulation Studio	Stimulation definition controls.	Always visible
Environmental Control	Temperature and CO ₂ controls.	Live data only
Activity Map	Plate-wide visualization of spike or beat rate and amplitude.	Spike Detector Cardiac Beat Detector
Continuous Waveform Plots	Displays a raw and/or filtered voltage over time plot for every electrode in a selected well.	Data stream active
Spike Plots	Displays spike waveforms for every electrode in a selected well and a well raster plot.	Spike Detector
Cardiac Beat Plots	Displays cardiac waveforms, conduction maps, and beat period plots for every electrode in a selected well.	Cardiac Beat Detector
LEAP Setup	Select wells and electrodes for LEAP induction, or add/remove LEAP tags in recorded raw files.	LEAP Acquisition Settings or LEAP tags present in file

2.6.1. Experiment Setup Properties

The **Experiment Setup Properties** module specifies the recording destination, experiment description, and the file names and types to record. Available recording file types (see Section 6.2) are determined by the data processors present in the **Streams** window.



The **Recording Name**, **Investigator**, and **Description** fields are optional fields for the user to enter information about the experiment. This information will be saved with the file and appear on the analysis output files. They may be entered or updated at any time. **Recording Name** and **Investigator** and can be applied to file names as macros (Section 6.2.1).

To update the **Recording Name**, **Investigator**, or **Description** fields on a previously recorded file:

1. Click on the **Experiment Setup Properties** module in the Control Bar.
2. Click on the field and enter the new information
3. Click **Write to File**.

Note: To undo changes prior to clicking Write to File, click Reset.

To set the file save location:

1. Click on the **Experiment Setup Properties** module in the Control Bar.
2. Click the **Browse...** button.
3. Navigate to and select the destination folder.

Note: When analyzing previously recorded data, analysis files generated are saved to the folder of the original data. This destination cannot be changed in AxIS Navigator.

2.6.2. Scheduled Recording Setup

The **Scheduled Recording Setup** module automates data recording and stimulation. The module is divided into two sections: **Settings** and **Status**.



The **Settings** dialog controls how and when a file is recorded.

Field	Description	Options
Record Every	The interval between the start of each recording	Seconds, Minutes, Hours, or Days
Record For	The duration of each recording	Seconds, Minutes, Hours, or Days
Starting	The start time of the first recording.	Immediately – begin when Start Schedule is clicked. At – begin at a specified time (<i>mm/dd/yyyy hh:mm:ss</i>). <i>Note:</i> 24 hour time or AM/PM may be used. If starting Immediately , play the stream first to allow voltage offset to complete, then Start Schedule .
Execute	Number of repeats	Until Stopped – continue making recordings at set interval until Stop Schedule is clicked. Once – Only create one recording. Until – continue at set interval until a specified time. Exactly – repeat at set interval for X times.
Auto Stimulate	Starts the currently active stimulation protocol during a scheduled recording. <i>Note:</i> Only available if stimulation protocol is set to Once in Stimulation Studio.	From Recording Start – When to start the stimulation protocol after the start of a recording if Auto Stimulate is selected.

After specifying the **Settings**, click **Start Schedule** to begin the scheduled recording protocol.

The example below shows the recording and stimulation times for a **Scheduled Recording Setup** as follows:

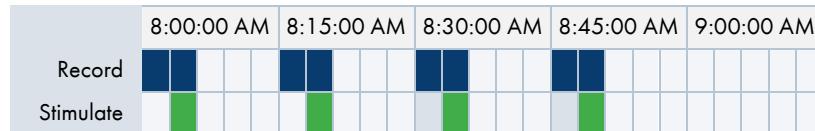
Record Every: 15 Minutes

Record For: 6 Minutes

Starting: At 8:00 AM

Execute: Exactly 4 times

Auto Stimulate: Enabled, 3 Min From Recording Start

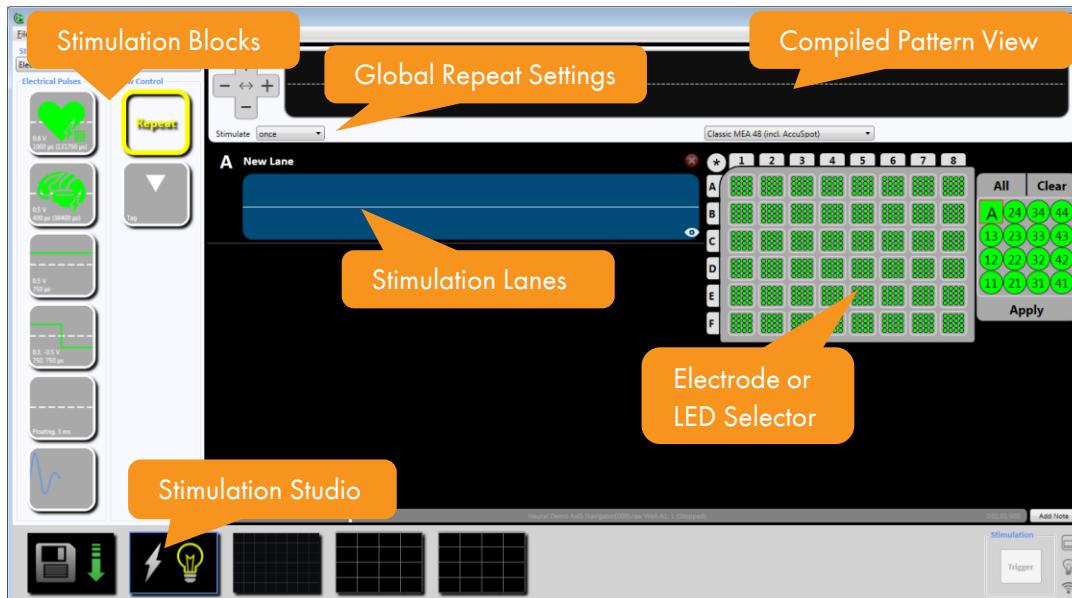


The Status panel provides feedback about the recording schedule.

Field	Description
Running Status	Current recording status. Either: Recording – a scheduled recording is in progress. Waiting to Record – another recording is scheduled to begin Waiting to Trigger – a scheduled recording is in progress and a stimulation is scheduled to begin.
Next Recording	Start time of the next scheduled recording
Previous Recording	Start time of the last completed recording

2.6.3. Stimulation Studio

The **Stimulation Studio** module designs both electrical and optical stimulation protocols. The module has four key components: the stimulation blocks, stimulation lanes, global repeat settings, compiled pattern view, and electrode or LED selector. See Chapter 4 for more information about building stimulation waveforms, selecting stimulation electrodes or LEDs, and applying a stimulus.



2.6.4. Environmental Control

The **Environment Control** module displays current temperature and CO₂ levels. The **Environmental Control** thumbnail displays the current temperature and CO₂ status. The color of the text indicates the current status.

Name	Status	Description
	Gray Text	Maestro is connected and the heater or CO ₂ control is off.
	Blue Text	Maestro is connected, heater or CO ₂ control is on, and temperature or CO ₂ is slightly below the set point.
	Green Text	Maestro is connected, heater or CO ₂ control is on, and temperature or CO ₂ is at the set point.
	Yellow Text	Temperature or CO ₂ is slightly above the set point.
	Red Text	Temperature or CO ₂ is greatly above the set point.

The screen on the Maestro Pro also displays temperature and CO₂ status.

When the Maestro is turned on, the heater control is automatically turned on and the temperature is set to 37°C. When a plate is docked in the Maestro, CO₂ control is automatically turned on and the CO₂ concentration is set to 5%.

To set a custom temperature or CO₂ concentration, or to use a custom gas mix, navigate to the **Environment Control** module using the **Control Bar**. The active window is shown below:



The temperature and CO₂ plots display the current set point as a dashed line and the current temperature or CO₂ as a solid line. To the right of the temperature plot are the heater controls, including the **Heater Control** switch and **Temperature (°C)** set point. The CO₂ control box contains the **CO₂ Control** switch, the **Gas Source**, and the **Concentration (%)** set point if 100% CO₂ is used.

A warning will display if the temperature is more than 1 °C away from the set point or the CO₂ concentration is more than 1% away from the set point for longer than 5 minutes.

To set the temperature to a custom temperature:

1. Click on the **Environmental Control** module in the **Control Bar**.
2. Set the desired temperature in the **Temperature (°C)** field (range Ambient + 5 °C to 46 °C).

Note: Temperatures above 37 °C may be detrimental to cells.

Like all incubators or environmental controllers, the system performs best when left in a closed and undisturbed state. For optimal environmental control, leave the **Heater Control** on and the Maestro Door closed when not in use. The **Heater Control** is automatically turned off when the Maestro is turned off.

To set the CO₂ concentration to a custom concentration:

1. Click on the **Environmental Control** module in the **Control Bar**.
2. Select **100% CO₂** from the **Gas Source** drop-down menu.
3. Set the desired CO₂ concentration in the **Concentration (%)** field.

To use pre-mixed gas rather than 100% CO₂:

1. Click on the **Environmental Control** module in the **Control Bar**.
2. Select **Custom Mix** from the **Gas Source** drop-down menu.
3. Use an inline flow meter to control the flow of gas into the Maestro.

To stop controlling temperature or CO₂:

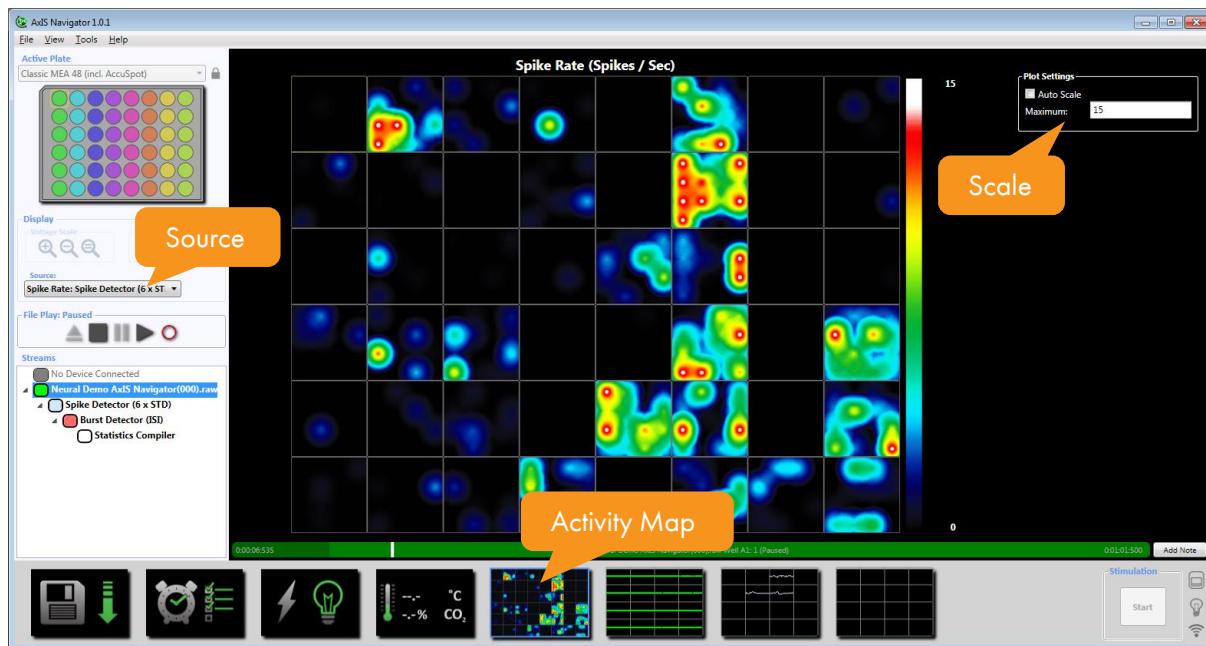
- Click the **Heater Control** or **CO₂ Control** switch to **Off**.

If **Auto Enable Gas** is selected, CO₂ is on only when an Axion MEA plate is docked and the door is closed. If **Auto Enable Gas** is not selected, CO₂ is manually controlled by the **CO₂ Control** switch (as long as the door is closed). **Note:** If the MEA plate barcode is not recognized, gas will not be automatically enabled.

When the door is opened, CO₂ Control is automatically turned off, regardless of **Auto Enable Gas** setting. The CO₂ concentration cannot be maintained when the door is open. The CO₂ Control is automatically turned off when the Maestro is turned off to conserve gas.

2.6.5. Activity Map

The **Activity Map** module displays a heat map of activity across the entire plate, providing an intuitive way to visualize differences between wells/conditions.



The metric displayed in the map is selected by the **Source** drop-down menu. The options available depend on the data processors in the **Streams** window as listed below:

Data Processor	Source Metric
Cardiac Beat Detector	Spike Amplitude (μ V) Beat Rate (beats/minute)
Spike Detector	Spike Amplitude (μ V) Spike Rate (Spikes/second)

The refresh rate of the **Activity Map** is set by the **Interval** setting in the **Spike Detector** (See Section 2.3.5). Small intervals increase the refresh rate, providing real-time feedback. Larger intervals will display a persistent signal integrated over many spikes.

Color corresponds to the magnitude; red or white areas have the highest magnitudes while blue and black areas have the lowest. The **Activity Map** scale is located to the right. The scale auto-adjusts by default but may be set to a fixed value in the **Plot Settings** dialog.

To manually adjust the **Activity Map** scale:

1. Click on the **Activity Map** module in the **Control Bar**.
2. Deselect **Auto Scale**, if enabled.
3. Enter the desired scale maximum into the **Maximum** field.
4. Click Enter.

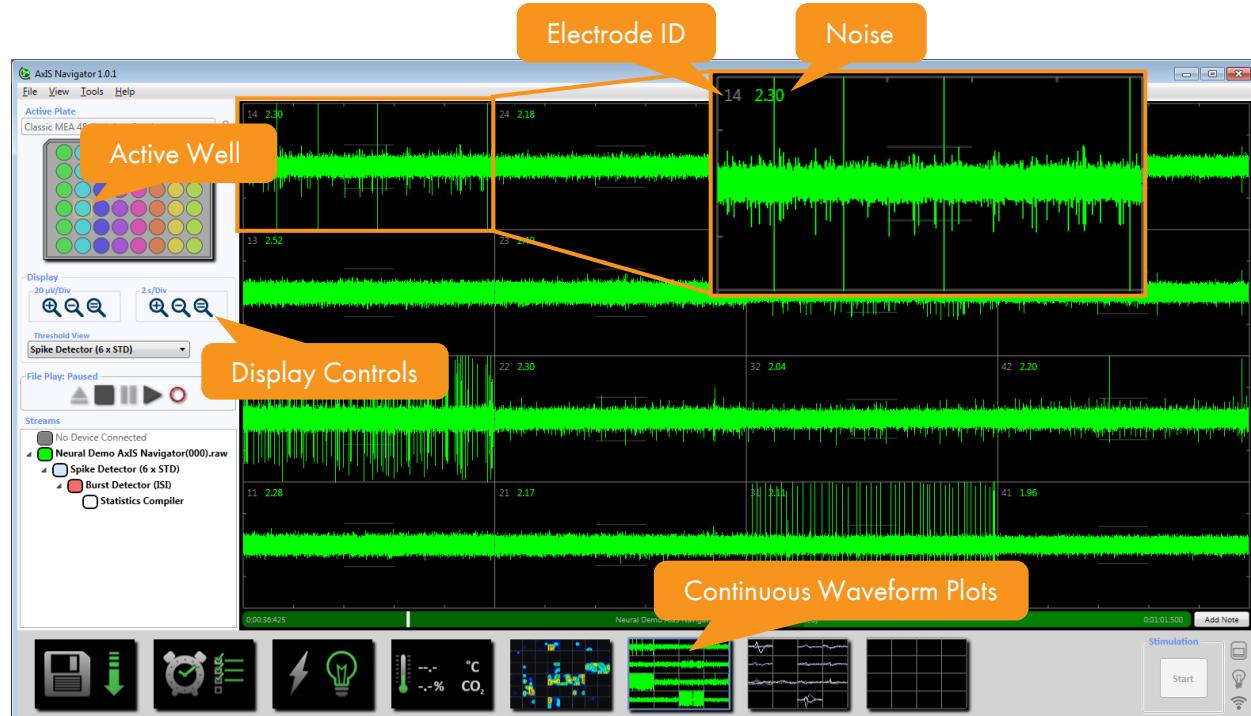
To copy the **Activity Map**:

1. Click on the **Activity Map** module in the **Control Bar**.
2. Right-click on the **Activity Map** and select **Copy**.

Selecting a well in the **Activity Map** will change the active well displayed in the **Continuous Waveform Plots**, **Spike Plots**, and **Cardiac Beat Plots** modules.

2.6.6. Continuous Waveform Plots

The **Continuous Waveform Plots** module displays continuous voltage data for each electrode of the active well. Select the active well by clicking on the desired well on the **Active Plate** or **Activity Map**.



The electrode ID consists of two digits, the column and row, and is displayed in grey text in the upper left corner of each plot. Adjacent to the electrode ID is the electrode noise level, displayed in μV . If the file contains LEAP tags, an inverted grey triangle is displayed in the upper right corner of the plot for electrodes that are tagged, indicating those electrodes were selected for LEAP induction.



The x and y scales are adjusted in the **Display** controls. Division marks along the outside of the active window indicate the zoom level chosen in the **Display** controls section. See Section 2.4 for more information about the **Display** controls. By default, electrodes are laid out in the active window by physical location but may be viewed as a vertical stack.

To view the electrodes in a vertical stack:

1. Click on the **Continuous Waveform Plots** module in the **Control Bar**.
2. Right-click on the active window and select **Stack Plots**.

*Note: The option to **Stack Plots** is not available when using CytoView MEA 12 plates.*

The continuous waveform plot has two copy options. To copy a single electrode, right-click on the electrode and select **Copy Electrode Waveform**. To copy the entire well plot, right-click anywhere on the plot and select **Copy Well Plot**.

A blank plot indicates an electrode has been turned off. Right click on an electrode plot and select **Disable Electrode** to turn it off or on, or use the **Plate Map Editor** (Section 2.2.2).

AxIS Navigator enhances the visualization of the continuous raw voltage data to highlight the important features of the signals. The plotting enhancements affect the data display only – the full, original, raw continuous voltage data is used for all analysis. The enhanced plotting options are described in the table below.

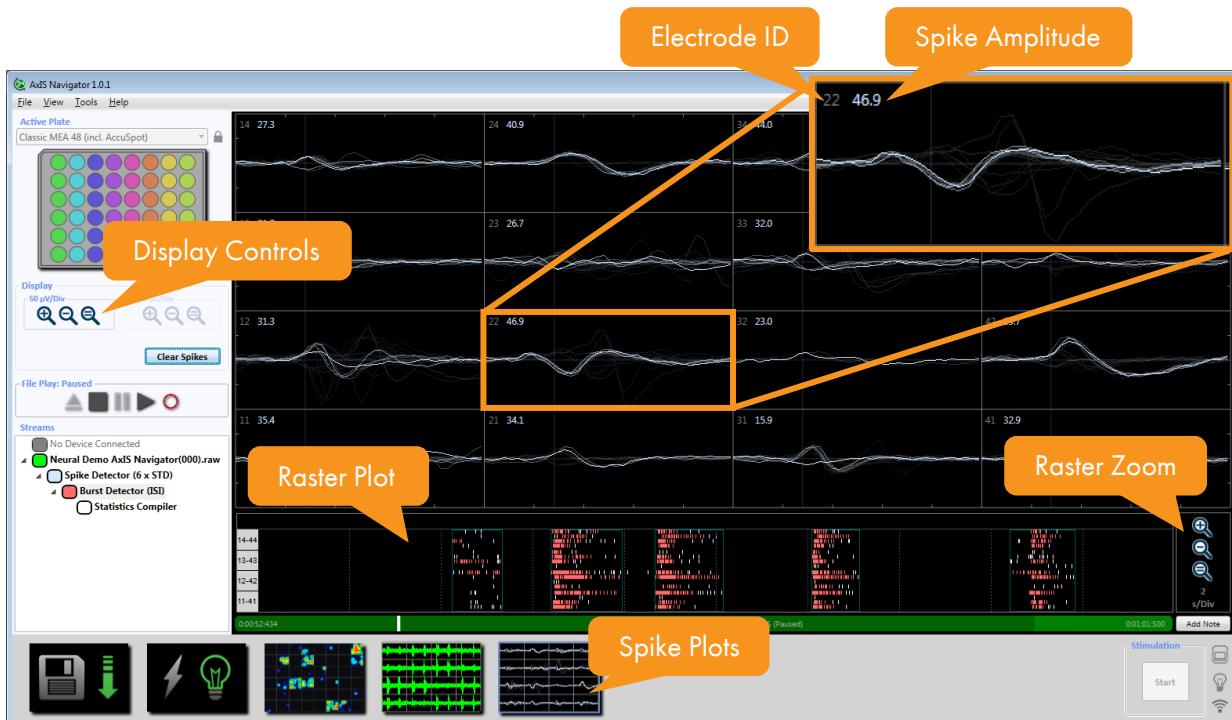
Name	Description	Dependency
Enhance for Neural	Subthreshold samples are filtered to highlight detected neural spikes.	Neural Configurations
Enhance for Cardiac	Field potential depolarization spikes are plotted at full scale, and signals between the spikes are filtered.	Cardiac Configurations
Enhance for LEAP	Applies a filter that removes the field potential components of the signal to highlight the LEAP signals.	Cardiac → LEAP Configurations

Enhanced plotting is selected by default. To disable or enable enhanced plotting:

1. Right-click on the stream in the **Streams** section.
2. Select **Plot → Enhance for Neural, Enhance for Cardiac, or Enhance for LEAP**.

2.6.7. Spike Plots

The **Spike Plots** module uses output from a **Spike Detector** and **Burst Detector** (See Sections 2.3.5 and 2.3.6, respectively) to display the spike voltage waveforms and raster plot of the active well. Like the **Continuous Waveform Plots**, each electrode of the active well is represented in the active window of the **Spike Plots** display. The gray electrode ID corresponds to the electrode ID in the **Continuous Waveform Plots** and the adjacent number is the most recent spike amplitude.



The waveform plots overlay each spike with the brightest trace representing the most recently detected spike on that electrode. Previously detected spikes fade to black. Empty black panels indicate no spikes have been detected on those electrodes. Spike detection and display settings can be adjusted in the Spike Detector Settings (See Section 2.3.5).

The spike waveform plots will display a maximum of 20 spikes overlaid at once. To specify how often spike waveforms are removed from the plot, right-click on the plot and select **Waveform Clearing**. Options include:

After 1 Minute	Spike waveforms are cleared 1 minute after they are detected.
At End Of Raster	Spike waveforms are cleared after they no longer display in the raster plot. Thus, the number of spikes displayed depends on the zoom level of the raster plot.
Manual	Spike waveforms are not cleared unless Clear Spikes is selected.

In all cases, if more than 20 spikes are detected on a given electrode, the oldest waveforms are cleared as new waveforms are plotted on top.

The x and y scale of the waveform plots are adjusted in the **Display** controls. Division marks along the outside of the active window indicate the zoom level chosen in the **Display** controls section. See Section 2.4 for more information about the **Display** controls. By default, electrodes are laid out in the active window by physical location but may be viewed as a vertical stack.

To view the electrodes in a vertical stack:

1. Click on the **Spike Plots** module in the **Control Bar**.
2. Right-click on the active window and select **Stack Plots**.

*Note: The option to **Stack Plots** is not available when using CytoView MEA 12 plates.*

The spike waveform plot has two copy options. To copy the entire well image, right-click anywhere on the plot and select **Copy Well Plot**. To copy a single electrode, right-click on the electrode and select **Copy Electrode Waveform**. This copies both the electrode image as well as the time and voltage values used to generate the image. To paste the electrode image, right-click and select **Paste Special** and choose the **Image** option. When pasting the data samples, the first column contains the time in seconds, and the remaining columns list the voltage values associated with each time sample for the displayed spikes. Each column contains the data samples for a single spike waveform, up to the maximum of 20 spikes displayed at any given time, with the most recent spike data in the last column.

Time (s)	Spike 0 (V	Spike 1 (V	Spike 2 (V	Spike 3 (V	Spike 4 (V	Spike 5 (V	Spike 6 (V	Spike 7 (V	Spike 8 (V	Spike 9 (V
-0.00088	-5.50E-07	1.77E-06	2.33E-07	-1.33E-07	5.59E-07	5.95E-08	-1.03E-06	-6.78E-07	-5.81E-07	-8.33E-07
-0.0008	2.75E-07	2.76E-06	2.04E-06	5.40E-07	-2.26E-06	-2.66E-07	-9.95E-07	-8.19E-07	-1.12E-06	-3.58E-07
-0.00072	-7.18E-08	2.99E-06	3.72E-06	1.88E-06	-2.69E-06	-5.65E-07	-1.07E-06	-2.39E-06	5.24E-07	-9.11E-07
-0.00064	-7.80E-07	1.43E-06	3.94E-06	2.41E-06	-7.28E-07	-1.13E-06	-1.36E-06	-2.05E-06	2.50E-07	4.16E-07
-0.00056	-4.33E-07	2.11E-07	2.05E-06	2.16E-07	3.69E-07	-3.25E-06	-3.59E-07	5.97E-07	-6.03E-07	2.15E-06
-0.00048	1.15E-07	-1.78E-07	5.80E-08	-9.39E-07	1.77E-06	-4.77E-06	-6.30E-08	2.16E-06	-3.15E-07	9.23E-07
-0.0004	-1.13E-06	-2.04E-06	-9.17E-07	-1.69E-08	1.47E-06	-3.30E-06	-1.11E-06	4.34E-07	3.75E-08	-1.86E-06

The raster plot at the bottom shows a running display of marks indicating the time each spike occurred with each row on the plot displaying the spikes from a single electrode. Single-electrode bursts are displayed in the color indicated by the **Burst Detector** while network bursts are indicated by a box of the contrasting color. Right-click to enable/disable display of network bursts. The time scale is controlled by the zoom controls on the right, allowing monitoring of spike history over long timescales.

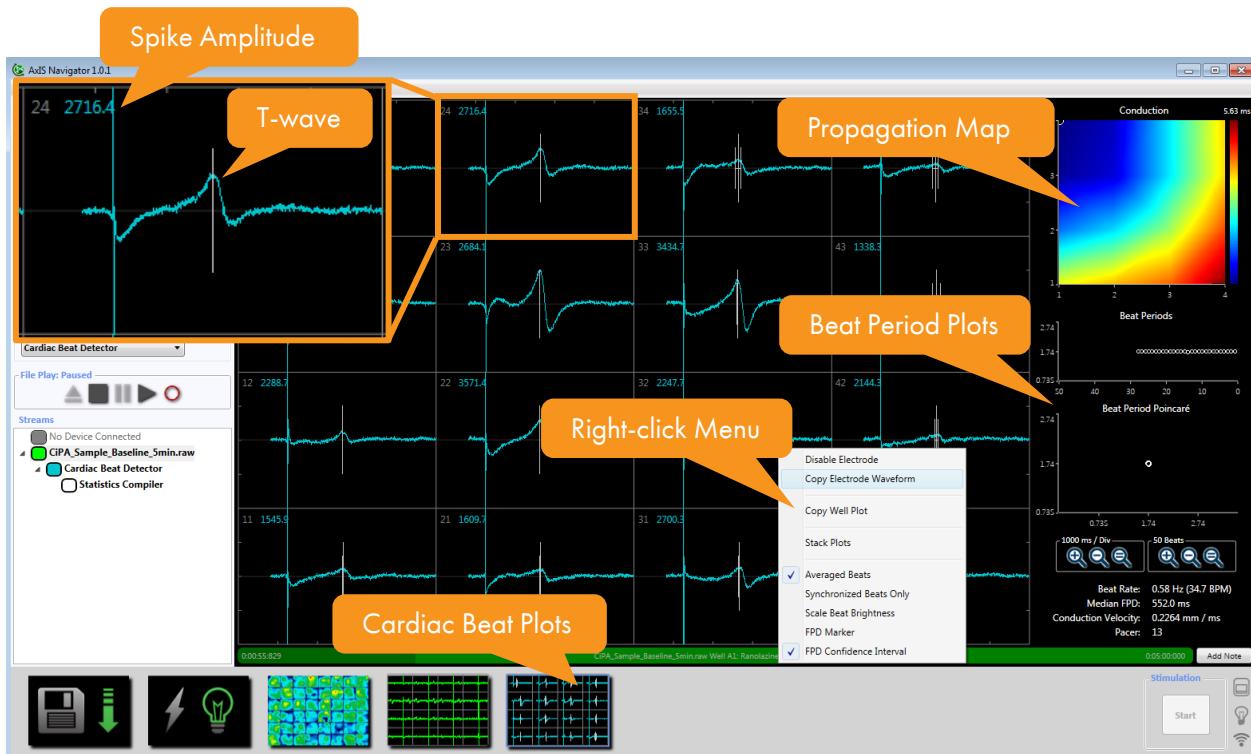
The raster plot can be copied as an image. To copy the raster plot, right-click on the raster plot and select **Copy**.

When electrical or optical stimulation is used, the stimulation protocol can be designed such that *AxIS Navigator* saves stimulation time tags to the .raw file. Stimulation tags contain information about the timing of the stimulus (see Chapter 4 for more details about stimulation). After starting the stimulation or when replaying a file containing stimulation tags, inverted white triangles will display above the raster plot indicating the time the stimulation was applied.



2.6.8. Cardiac Beat Plots

The **Cardiac Beat Plots** module uses output from a **Cardiac Beat Detector** (See Section 2.3.7) to display the cardiac voltage waveforms, conduction maps, and beat period plots of the active well. Like the **Continuous Waveform Plots**, each electrode of the active well is represented in the active window of the **Cardiac Beat Plots** display. The gray electrode ID corresponds to the electrode ID in the **Continuous Waveform Plots** and the adjacent number is the average amplitude of the depolarization spike, displayed in μV .



By default the average waveform of the last 10 beats are plotted. This may be changed either in the **Beat Detector Settings** (section 2.3.7) or by right-clicking on the plot and selecting/deselecting **Averaged Beats**. The field potential duration (FPD) detection is indicated by the blue rectangle and white whisker plot. It is disabled during data acquisition to limit processor workload.

The x and y scale is adjusted in the **Display** controls. Division marks along the outside of the active window indicate the zoom level chosen in the **Display** controls section. See Section 2.4 for more information about the **Display** controls. By default, electrodes are laid out in the active window by physical location but may be viewed as a vertical stack.

To view the electrodes in a vertical stack:

1. Click on the **Cardiac Beat Plots** module in the **Control Bar**.
2. Right-click on the active window and select **Stack Plots**.

*Note: The option to **Stack Plots** is not available when using CytoView MEA 12 plates.*

The beat waveform plots have two copy options. To copy a single electrode, right-click on the electrode and select **Copy Electrode Waveform**. This copies both the electrode image as well as the time and voltage values used to generate the image. When pasting the data samples, the first column contains the time samples in seconds and the second column contains the voltage samples corresponding to the selected beat. To paste the electrode image, right-click to select Paste Special and choose the image option. To copy the entire well image, right-click anywhere on the plot and select **Copy Well Plot**.

When LEAP is induced, *Ax/S Navigator* saves LEAP tags to the .raw file indicating which electrodes were selected for LEAP induction (See Chapter 5 for more details about LEAP). After inducing LEAP or when replaying a file containing LEAP tags, an inverted grey triangle is displayed in the upper right corner of the plot for electrodes that were selected for LEAP induction. An inverted green triangle is displayed for the subset of LEAP electrodes for which *Ax/S Navigator* identifies LEAP beats.



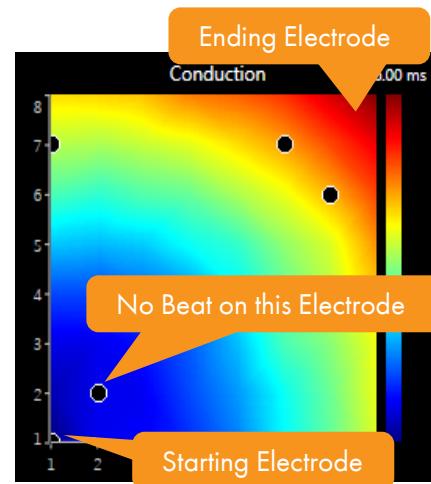
Note: LEAP beats are only identified if the file is recorded using **Cardiac: LEAP Acquisition Settings**. When other cardiac acquisition settings are used, field potential beats are not identified on electrodes selected for LEAP induction.

To the right of the cardiac waveforms are three plots: the **Conduction** plot, **Beat Periods** plot, and **Beat Period Poincaré** plot.

Right-click on any of the plots and select **Copy** to copy to the clipboard.

The **Conduction** Plot illustrates the propagation delay at each electrode from short (blue) to long (red). The well beat originates in the blue region and terminates in the red. The electrode column and row numbers are displayed on the x- and y-axis, respectively.

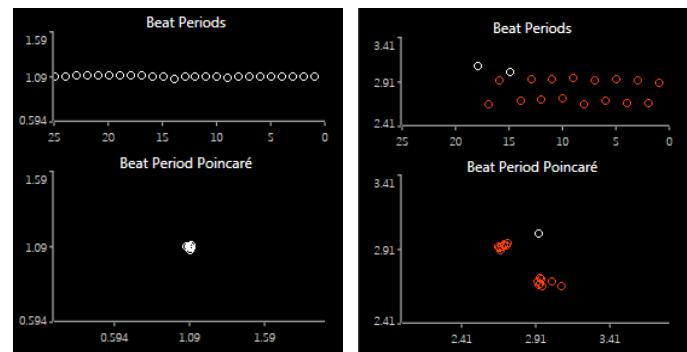
Right-clicking on the **Conduction** plot presents four display options:



Option	Description
Auto Scale	Sets the scale of the plot. Disable to manually adjust the top of the scale with Max Delay
Animate Conduction	Animates the plot with each beat. Disable to view a static plot that updates with each beat.
Extrapolate Missing Electrodes	Extrapolates values for electrodes that did not detect a depolarization spike.
Show Missing Electrodes	Extrapolated electrodes will be marked with a black circle.

The **Beat Periods** plot displays beat period (in seconds) on the y-axis, plotted against beat number on the x-axis. The **Beat Period**

Poincaré plot displays beat period (in seconds) on the y-axis, plotted against the previous beat period on the x-axis (BP_{t+1} vs. BP_t). Beats that exhibit a greater than 5% deviation from the previous beat are displayed as red, indicative of arrhythmia.

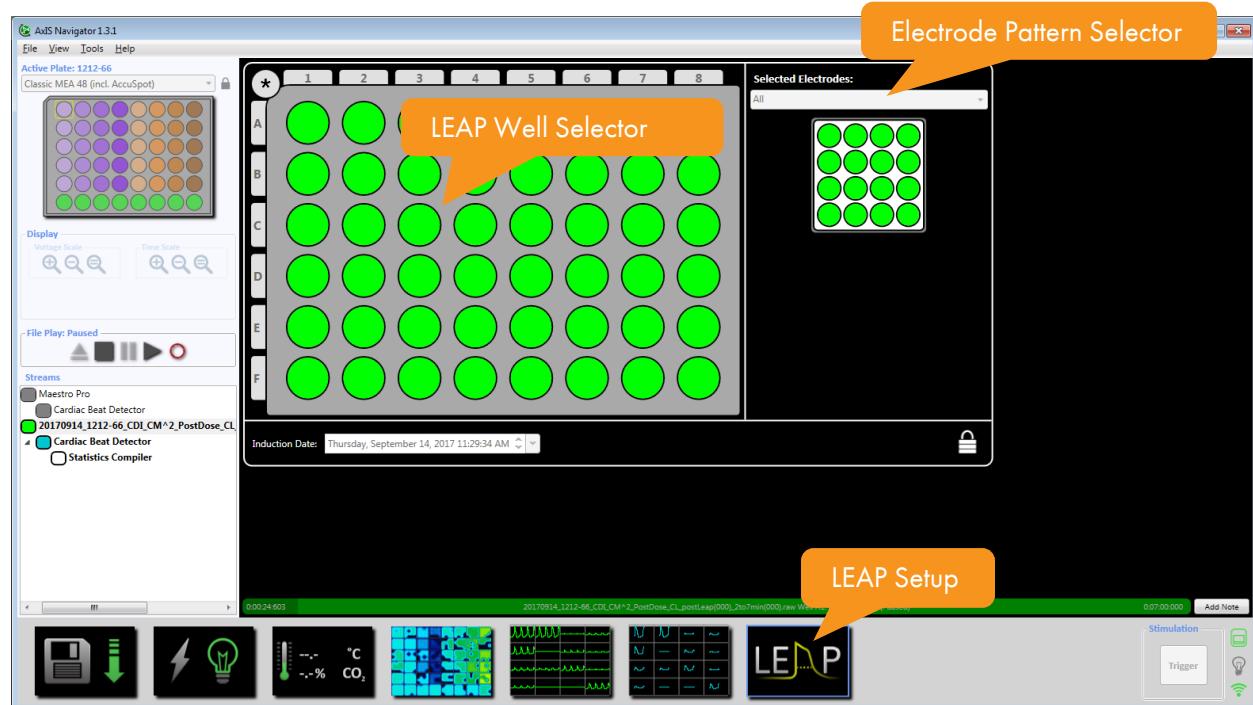


Below the **Beat Period Poincaré** plot are display controls for both plots. Use the zoom in (+), zoom out (-), and reset scale (E) controls to change time scales. The scale is indicated above the control as X units per division and N beats.



2.6.9. LEAP Setup

The **LEAP Setup** module is used to design a Local Extracellular Action Potential (LEAP) induction plate map and start LEAP induction to acquire extracellular action potential waveforms from cardiomyocytes. This module includes a LEAP Induction Electrode Pattern Selector and a LEAP Well Selector. See Chapter 5 for more information about LEAP.



CHAPTER 3. DATA ACQUISITION

The Maestro Pro and Maestro Edge stream data from all electrodes simultaneously. *Ax/S Navigator* can record the continuous voltage data from the entire plate at once, and the user may view the data stream with various data processors applied. Built-in environmental controls ensure the culture is kept under optimal conditions while recording. This chapter reviews how to acquire data from the Maestro systems using *Ax/S Navigator*. Section 3.3 contains a step-by-step tutorial for data acquisition.

3.1. AXIS NAVIGATOR COMMUNICATION WITH THE MAESTRO

Ax/S Navigator communicates with the Maestro through an Ethernet connection. Once the system is connected, turn on the Maestro using the button on the side and open *Ax/S Navigator* to begin system initialization. Startup can take a few minutes.

The status lights in the bottom right corner of *Ax/S Navigator* indicate the system status and connectivity. There are three status lights: **MEA Status**, **Lumos Status**, and **Remote Control Status**.

Maestro Connection Status	
	Device not detected
	Blinking. Connected to Device, and Device is booting up
	Connected to Device, no MEA plate detected
	Connected to Device, MEA plate detected
	Error
Lumos Connection Status	
	Lumos not detected
	Blinking. Connected to Lumos, and Lumos is booting up
	Connected to Lumos, and Lumos is on the stand
	Connected to Lumos, and Lumos is on the Maestro
	Error
Remote Control Status	
	Remote Control is not enabled
	Remote Control is enabled

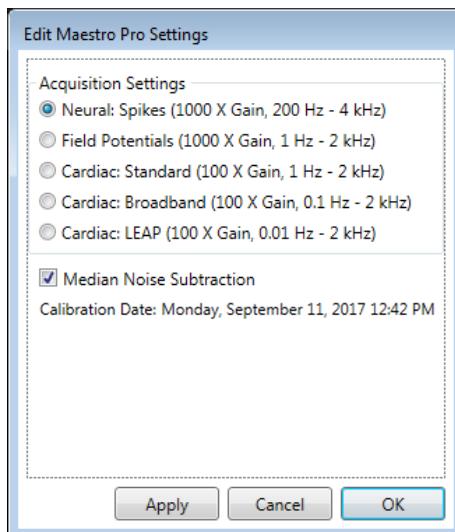
3.2. CONFIGURING THE MAESTRO FOR ACQUISITION

3.2.1. Configuring the Hardware

The Maestro must be configured prior to recording a continuous voltage stream. The hardware bandwidth and gain must be set properly for particular applications. These settings affect how the raw continuous voltage data is recorded, and cannot be changed after data is recorded. Software filtering added by a **Digital Filter** data processor may be changed after recording (Section 2.3.2).

Axion provides preset Neural or Cardiac recording configurations that will automatically configure the recommended hardware settings (Section 3.2.2).

With a device connected, use the **Settings** dialog from the **Maestro** stream to adjust the analog acquisition settings. To access the **Settings** dialog, double-click on the **Maestro Pro** or **Maestro Edge** stream, or right-click and select **Settings**.



The **Maestro Settings** contain the following options:

- Acquisition Settings:** Configure the hardware gain and bandwidth. There are 5 options for various neural and cardiac applications:

Option	Setting	Description
Neural: Spikes (default neural)	Gain: 1000X Bandwidth: 200-4000 Hz	High gain with a neural bandwidth. Recommended for most neural applications.
Field Potentials	Gain: 1000X Bandwidth: 1-2000 Hz	High gain with lower frequency bandwidth.
Cardiac: Standard (default cardiac)	Gain: 100X Bandwidth: 1-2000 Hz	Low gain with a cardiac bandwidth. Recommended for most cardiac applications.

Cardiac: Broadband	Gain: 100X Bandwidth: 0.1-2000 Hz	Low gain with a wide cardiac bandwidth. Use if a wider bandwidth is desired.
Cardiac: LEAP	Gain: 100X Bandwidth: 0.01-2000 Hz	Low gain with wide bandwidth to collect ultra-low frequency content. Use for LEAP induction and signal acquisition.

2. **Median Noise Subtraction** option: If enabled, subtracts the median signal from groups of electrodes. Grouping is performed based on the system's electronic architecture. This reduces the noise common to the electrode groups, improving the detection of low amplitude signals. Median noise subtraction is recommended for neural recording. When the neural option is selected in **Acquisition Settings**, median noise subtraction is enabled. It is disabled when a cardiac option is selected.

3.2.2. Stream Configurations

In addition to hardware configuration, it is possible to add data processors for easy data visualization during a recording. These additional data processors do not impact the raw continuous voltage data recorded and are purely for visualization.

A stream configuration sets the hardware configuration, adds data processors, and applies all the settings for data visualization and acquisition.

AxIS Navigator comes with a variety of preset configurations. Use the Real-Time configurations for data acquisition:

Configuration	Processing Applied	Description
Cardiac Real-Time		
Spontaneous	Cardiac Beat Detector	Generates activity map, cardiac waveforms, beat rate and conduction information. FPD detection disabled.
Electrically Paced	Artifact Eliminator Cardiac Beat Detector	Generates activity map, cardiac waveforms, beat rate and conduction information. Optimized for reducing stimulus artifacts in pacing experiments. FPD detection disabled.
Optically Paced	Cardiac Beat Detector	Generates activity map, cardiac waveforms, beat rate and conduction information. FPD detection disabled.
LEAP	Cardiac Beat Detector	Generates activity map, cardiac waveforms, beat rate and conduction information. Optimized for acquiring LEAP signals.
Neural Real-Time		
Spontaneous	Spike Detector Burst Detector	Generates activity map, spike waveforms, and raster plot. Network burst detection disabled.

Electrically Evoked	Artifact Eliminator Spike Detector Burst Detector	Generates activity map, spike waveforms, and raster plot. Optimized for reducing stimulus artifacts in electrically-evoked experiments. Network burst detection disabled.
Optically Evoked	Spike Detector Burst Detector	Generates activity map, spike waveforms, and raster plot. Network burst detection disabled.

To apply a configuration:

1. Right-click on the data stream.
2. Select **Configuration** and navigate to the desired configuration. Click on the configuration.
Note: A configuration adds data processors but also sets hardware configurations. To record with non-default acquisition settings, apply the configuration first and then manually change the acquisition settings. See Section 3.2.1.

To save a custom configuration:

1. Set up a data stream with desired hardware configurations, data processors, and selected file outputs.
2. Right-click on the data stream.
3. Select **Configuration → Save**.
4. Type a file name (.datastreams extension) in the **Save Data Stream Configuration** dialog and click **Save**.

To load a custom configuration:

1. Right-click on the data stream.
2. Select **Configuration → Load**.
3. Navigate to the .datastreams configuration file in the **Open Data Stream Configuration** dialog.
4. Click **Open**.

3.3. LIVE DATA ACQUISITION TUTORIAL

While most data processor and plate layout settings can be modified after a recording has taken place there are four key settings that must be correct prior to recording:

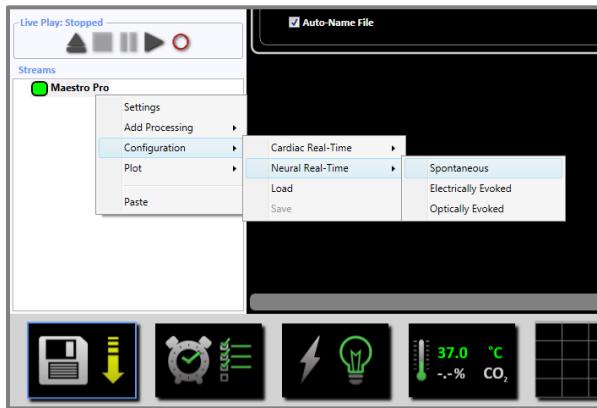
1. The Maestro must be communicating with *AxIS Navigator* (Section 3.1).
2. The environment control must be on and equilibrated (Section 2.6.4).
3. The hardware configuration must be set (Section 3.2).
4. Any stimulus applied must be configured before starting the stimulation or a scheduled recording with auto-stimulate (Chapter 4).

3.3.1. Setting Up for a Recording

1. Turn on the Maestro with the button on the left side.
2. Open *AxIS Navigator*.
3. Allow the system approximately 1 minute to connect as indicated by the Maestro status light (See Section 3.1 for more information on Status Lights).

Note: The data stream will say "Maestro Pro" or "Maestro Edge" when connected and "No Device Connected" when it is not or while it is initiating.
4. Allow the temperature to stabilize as indicated by the Environmental Control thumbnail.

Note: The Maestro Pro also displays temperature and CO₂ status on the touch screen.
5. Right-click on the Maestro Pro or Edge stream and select Configuration → Cardiac Real-Time or Neural Real-Time → Spontaneous, Electrically Evoked/Paced, Optically Evoked/Paced, or LEAP (cardiac only) to apply the desired configuration. See Section 3.2 for more information on hardware and software configurations.



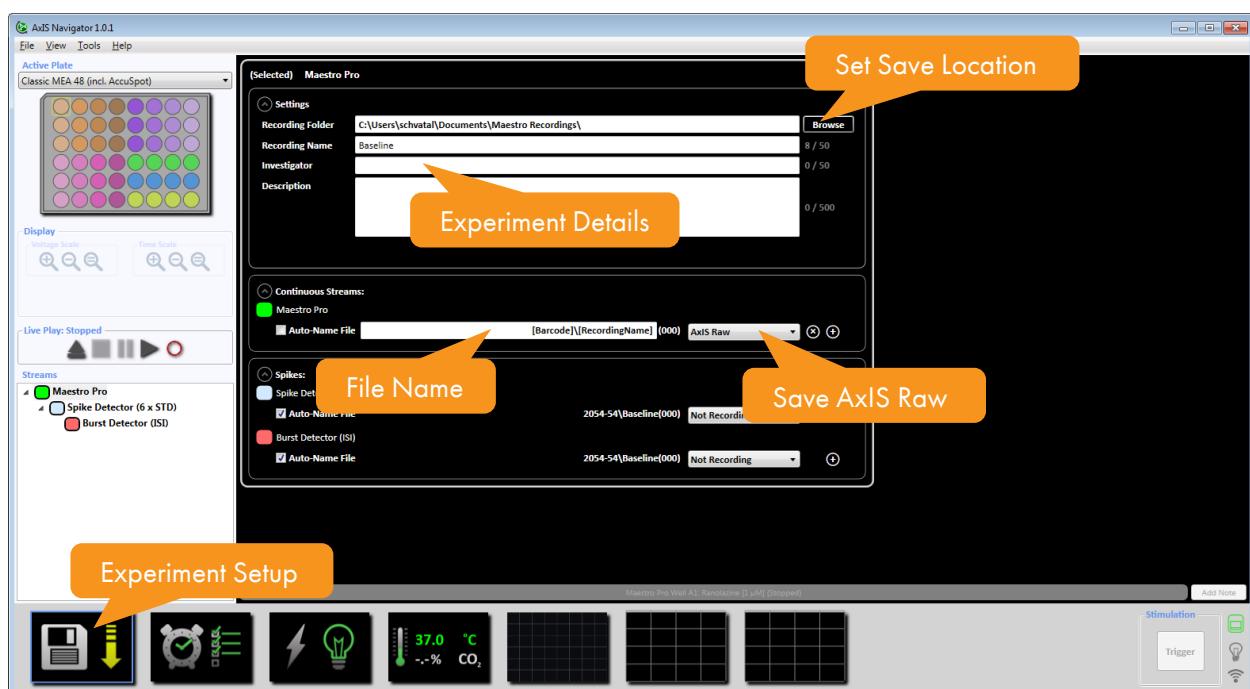
6. Click the **Experiment Setup Properties** module. See Section 2.6.1 for more information on the **Experiment Setup Properties** module.
7. Click **Browse** to set the save location in the **Recording Folder** field.
8. Enter a name for the recording in the **Recording Name** field. Optional: Enter the **Investigator** and a **Description** in the respective fields.

9. Select **AxIS Raw** from the **Maestro Continuous Stream** drop-down to save the continuous waveform data output. Leave all other streams set to Not Recording. See Section 6.2 for more information on output file types.

Note: The **AxIS Raw** file will automatically be selected for recording when one of the built-in Real-Time Configurations is selected.

10. Optional: Uncheck **Auto Name File** below the **Maestro** menu and enter a file name. By default, a folder will be created according to the MEA plate barcode, and the file name will be the **Recording Name** specified above. Auto-naming macros are available to add descriptive information to file names. See Section 6.2.1 for more information on naming output files.

Note: File names are appended with a number starting with 000. Recording a file with the same name will increase the number appended to the end so no two files share a name.



3.3.2. Recording from the Maestro Manually

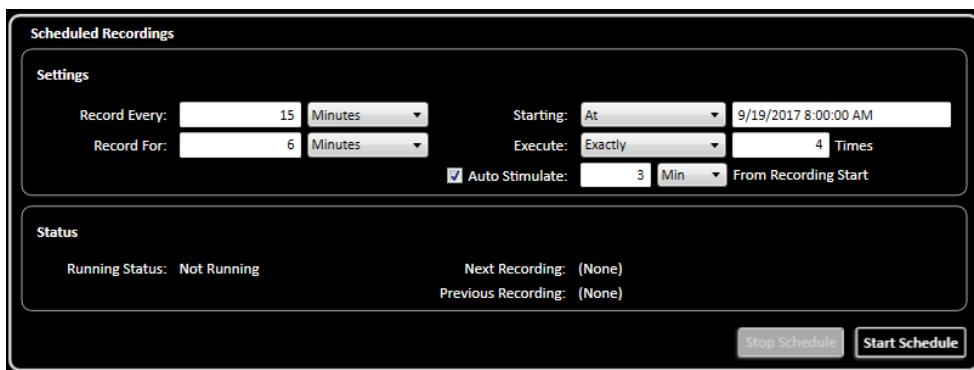
- Place the MEA plate into the MEA chamber and press the button on the Maestro to engage the MEA and close the door. The Maestro will automatically begin controlling CO₂.
 - Allow the MEA to equilibrate while docked in the Maestro for 10-20 minutes prior to recording.
 - Double click on the **Active Plate** figure to enter a plate map.
- Note:** Plate maps may be imported from a .platemap file or a previously recorded .raw file.
- Click **Play** to view the raw data and begin the voltage offset correction, indicated on the gray status bar. The voltage offset correction runs each time *AxIS Navigator* begins measuring data from the stopped state.

Note: Playback begins automatically after LEAP induction.

5. Wait for the voltage offset correction to complete (~30 sec in neural settings and ~1 min in cardiac settings).
6. Click the **Record** button to begin recording.
7. Click the **Record** or **Stop** button to stop recording.

3.3.3. Recording from the Maestro using Scheduled Recordings

1. Repeat Steps 1-5 from the previous section.
2. Click on the **Scheduled Recording Setup** module. See Section 2.6.2.
3. Enter the desired file duration in the **Record For** field.
4. Enter when to begin recording in the **Starting** field.
5. Enter how many times to create a recording in the **Execute** field. For a single recording, select **Once**.
6. If recording more than one file, enter the time between the start of each recording in the **Record Every** field.
7. Click **Start Schedule** to begin recording.
8. Click **Stop Schedule** to stop recording after initiating a scheduled recording.



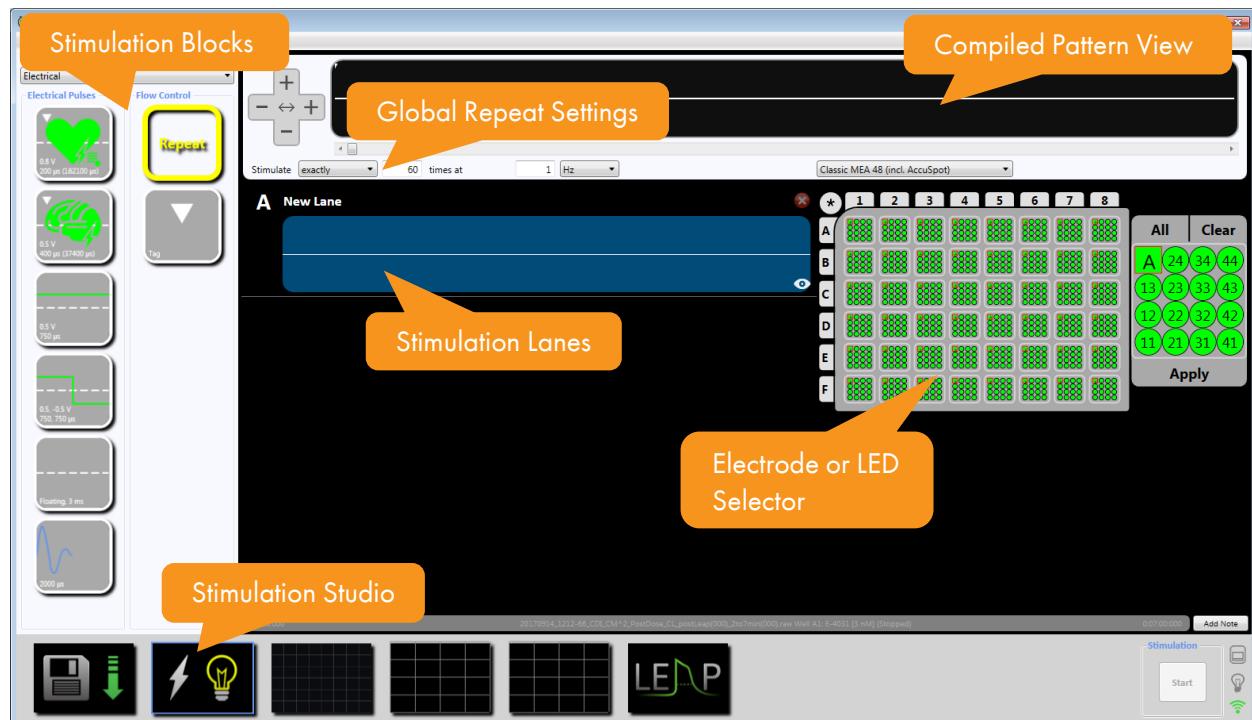
CHAPTER 4. STIMULATION

Stimulation offers a level of control over cell cultures to evoke specific activity or improve consistency between wells and plates.

The **Stimulation Studio** module is used to design both electrical and optical stimulation protocols. **Stimulation Studio** provides a variety of drag and drop blocks that can be used to build custom stimulation waveforms.

The **Stimulation Studio** has five major components:

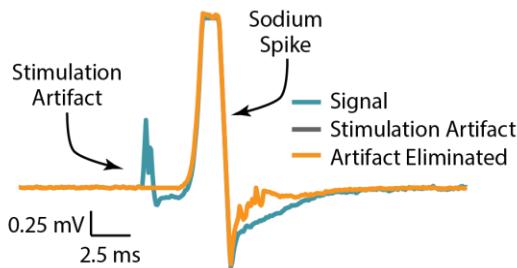
1. **Stimulation Blocks:** Predefined functions representing the basic elements of a stimulus protocol.
2. **Stimulation Lanes:** Displays the currently assigned stimulation blocks.
3. **Electrode or LED Selector:** Assigns the protocol from the selected stimulation lane to specific electrodes or LEDs.
4. **Compiled Pattern View:** Displays a composite of all stimulation lanes in a time/intensity plot.
5. **Global Repeat Settings:** Specifies how many times, and how often stimulation waveforms repeat. These settings apply globally to all lanes.



4.1. ELECTRICAL STIMULATION

Using electrical stimuli applies a voltage or current waveform directly to the cells on the stimulation electrode. It may be used to stimulate cells in a specific region of a well in their native state, without needing to biologically modify the cells in any way.

Because the MEA system records an electrical signal (voltage), electrical stimulation results in a stimulus artifact. This artifact is caused by both charge build-up on the electrode and saturation within the amplifiers. As a result, *AxIS Navigator* recording is not accurate during the stimulation. An example of a stimulation artifact is shown below in blue. The artifact is an initial biphasic waveform, followed by a slow recovery to baseline. In this case the stimulus triggered a depolarization of the cell culture, referred to as a "capture".



AxIS Navigator uses proprietary techniques to minimize stimulation artifacts. Built-in **Neural Stimulation** and **Cardiac Pacing** blocks are provided. These blocks disable the amplifiers when the stimulus is active and rapidly adjust filter settings to manage the stimulation artifact and drive the electrode voltage back to pre-stimulation levels as quickly as possible. This maximizes the fidelity of voltage recording. *AxIS Navigator* also manages stimulation artifact through built-in configurations, **Electrically Paced** for cardiac and **Electrically Evoked** for neural. Both real-time and offline versions of these configurations are available for data acquisition and analysis, respectively. Both configurations feature the **Artifact Eliminator** data processor, to minimize the stimulation artifact (Section 2.3.3).

For data acquisition with electrical stimulation:

1. Build the stimulation protocol according to Section 4.1.2.
2. Start recording data according to Section 3.3, selecting the **Cardiac Real-Time**→**Electrically Paced** or **Neural Real-Time**→**Electrically Evoked** configuration.
3. Click the **Trigger** button in the bottom right corner to begin the stimulation protocol manually. Click again to stop.

Note: If **Once** is selected in the **Global Repeat Settings**, the stimulation protocol may be started automatically by the **Scheduled Recording Setup** module. See Section 2.6.2.

For data analysis with electrical stimulation:

1. Analyze data according to Section 6.5, selecting the **Cardiac Offline**→**Electrically Paced** or **Neural Offline**→**Electrically Evoked** configuration.

Note: See the 'Neural Metric Tool' for evoked activity analysis options for neural analysis (Section B.2.4).

4.1.1. Electrical Stimulation Blocks

The electrical stimulation blocks are:

Block	Icon	Description
Cardiac Pacing Stimulation		<i>Recommended stimulation block for cardiac applications.</i> Applies a Biphasic Stimulation pulse (Voltage Stimulation mode) with artifact elimination routine optimized for cardiac stimulation. Set duration with Stimulus Duration in Pulse Settings dialog. Forces the voltage output to match Voltage as long as Max Current has not been reached. Use Stimulation Paddles for E-Stim+ Classic MEA 48 and CytoView MEA 24 plates and Microelectrode for all other MEAs.
Neural Stimulation with Artifact Elimination		<i>Recommended stimulation block for neural applications.</i> Applies a Biphasic Stimulation pulse (Voltage Stimulation mode) with artifact elimination routine optimized for neural stimulation. Set duration with Stimulus Duration in Pulse Settings dialog. Forces the voltage output to match Voltage as long as Max Current has not been reached.
Monophasic Stimulation		Applies a single phase pulse of a set current or voltage for a set duration. Use Stimulus Duration to set the duration. Current Stimulation: Forces the current output to match Current as long as Max Voltage has not been reached. Voltage Stimulation: Forces the voltage output to match Voltage as long as Max Current has not been reached.
Biphasic Stimulation		Applies a dual phase pulse of a set current or voltage for a set duration. Use Stimulus Duration to set the durations. Current Stimulation: Forces the current output to match Current as long as Max Voltage has not been reached. Voltage Stimulation: Forces the voltage output to match Voltage as long as Max Current has not been reached.
Delay		Applies a wait period. Set the duration using the Duration field.
Stimulation Artifact Eliminator		Discharges the electrode to pre-stimulus voltage. Length of discharge set by Discharge Duration . The transition from the stimulating to non-stimulating state is controlled by the time constant Soft Switch . Discharge Strength sets the maximum current used to "pull" charge off the electrode and to ground.
Loop Container		Repeats any blocks contained within the loop a set number of times. Double-click the repeat number (xN) in the top right corner of the block to set the number of times.

Electrical Stimulation Tag		<p>Set the location of a "tag" or time stamped note to indicate a stimulation occurred. Tags are useful for visualization and analysis of evoked activity. Only one tag block can be set per stimulation protocol, so tags cannot be assigned to multiple lanes of stimulation.</p> <p>Note: If no tag is manually set, a tag will be automatically generated at each repetition of Lane A. To prevent excessive size of output files, limit the tag rate to 50 Hz.</p>
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4.1.2. Stimulation Protocol Generation

A stimulation protocol should have four goals:

1. Maximize the efficacy, or capture, of the stimulus.
2. Minimize the damage to the cells.
3. Minimize the damage to the electrodes.
4. Minimize the stimulation artifact.

Cell damage can result from high charge injection or charge density, while damage to the electrodes is caused by electrolysis. For microelectrodes of this size, electrode damage is more likely than cell damage. These risks and the stimulus artifact increases with the length of stimulation, but can be significantly reduced by using a charge-balanced stimulus shape (biphasic) and the **Stimulation Artifact Eliminator** block. The recommended **Neural Stimulation** block and **Cardiac Pacing Stimulation** block contain these features. In general, choosing the smallest charge-balanced, biphasic, voltage-controlled pulse with an effective capture rate is recommended.

(Refer to Wagenaar et al. for an in depth discussion of stimulus waveform shape: Wagenaar, DA, Pine, J, and Potter, SM. Effective parameters for stimulation of dissociated cultures using multi-electrode arrays. J. Neurosci. Methods. 138(1-2), 27-37 (2004))

To evoke activity from neuronal cultures, the **Neural Stimulation Block** is recommended. This block contains an **Electrical Stimulation Tag**, a **Biphasic Stimulation**, and a **Stimulation Artifact Eliminator** optimized for neural cultures. The most important parameter for modulating the stimulus efficacy is the **Stimulus Duration**. A typical value is 0.2-4 ms for standard electrodes, or 0.2-1 ms if using the dedicated stimulation electrode on an E-Stim+ Classic MEA48 plate. Use a duration less than 0.5 ms for the best artifact elimination.

To evoke activity from cardiac cultures, the **Cardiac Stimulation Block** is recommended. This block contains an **Electrical Stimulation Tag**, a **Biphasic Stimulation**, and a **Stimulation Artifact Eliminator** optimized for cardiac cultures. The most important parameters for modulating the stimulus efficacy are the **Stimulus Duration** and the number of stimulating electrodes. Using an E-Stim+ Classic MEA 48 plate, only the stimulation paddle is used with a duration of 0.2-1 ms. For microelectrode stimulation, a duration of 1-4 ms is typically used, stimulating with 2-4 adjacent electrodes. It is recommended to start with the shortest duration and fewest electrodes and increase until consistent capture is achieved (See recommended workflow in Section 4.1.3).

Note: The Neural Stimulation and Cardiac Pacing Stimulation blocks already contain a stimulation time tag. Do not place an Electrical Stimulation Tag block directly in front of these blocks, or the built-in artifact elimination will not work.

It is good practice to save a stimulation protocol for future reference after the pattern is completed and the electrodes are selected. Save stimulation protocols using **File → Save Stim Waveform**. Open with **File → Open Stim Waveform**.

To build an electrical stimulation pattern:

1. Select **Electrical** in the **Stimulation Type** drop-down menu.
2. Click and drag a block into the desired stimulation lane. A gray bar appears in the lane indicating where a block will be dropped.
3. Double-click a block in a stimulation lane to change its settings.

Note: Use the shortest duration stimulus that is effective in order to minimize the stimulation artifact.

To copy a block from a stimulation lane:

1. Right-click on the block and select **Copy Pulse**.
2. Right-click on the stimulation lane and choose **Paste Pulse**.

To delete a block from a stimulation lane:

1. Right-click on the block and select **Delete Pulse**.

Note: A Loop Container provides the option of deleting the loop and its contents or just the loop.

To clear a stimulation lane:

1. Right-click on the lane and select **Clear**.

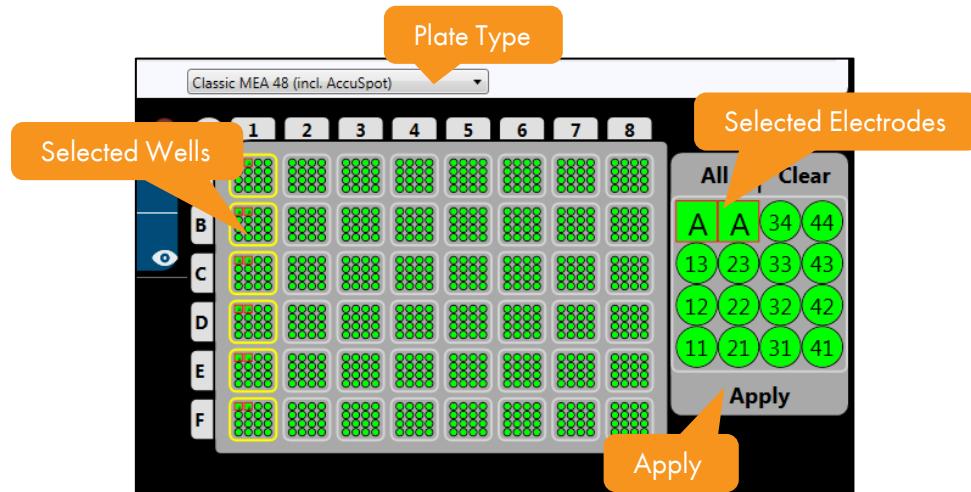
To specify how often to apply the stimulation:

1. Select an option from the **Stimulate** drop-down menu in the **Global Repeat Settings** bar

To assign a stimulation lane to one or more electrodes:

1. Select the plate type from the drop-down menu above the Electrode Selector in **Stimulation Studio**.
 2. Click on the desired stimulation electrodes in the electrode selector to the right of the plate map. Selected electrodes will display an "A" and are highlighted with a red square. Click **All** to assign all electrodes, or **Clear** to un-assign all electrodes.
- Note:** E-Stim+ Classic MEA 48 plates automatically assign electrode 41 (the dedicated stimulation electrode) when selected.
3. Click on the desired wells that should receive the stimulation pattern. Selection is the same as the **Plate Map Editor** in **Active Plate** (See Section 2.2.3).
 4. Click **Apply**. The stimulation electrodes in the electrode selector will be applied to the wells in the plate map.

Note: To remove stimulation electrodes from a well, "apply" the electrode selector to that well with no stimulation electrodes selected.

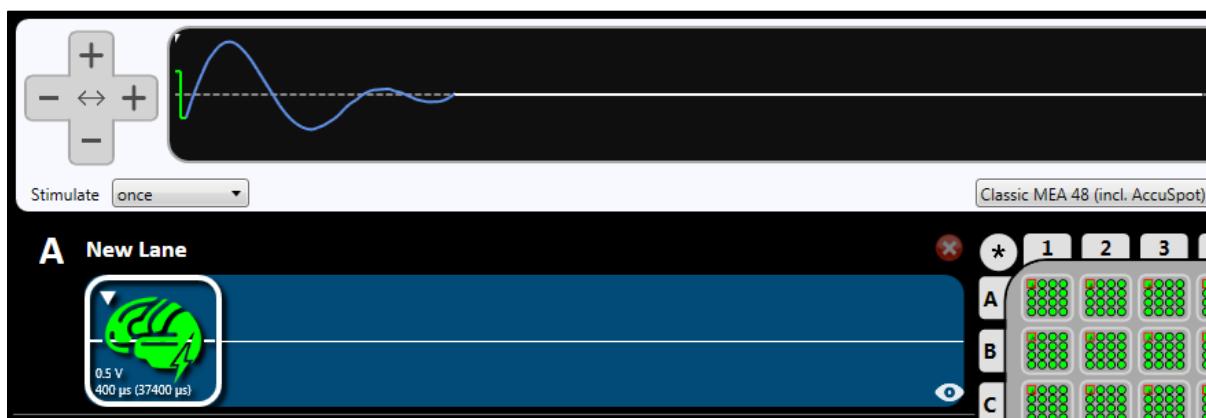


4.1.3. Example Neural Stimulation Patterns

To build the recommended neural stimulation protocol:

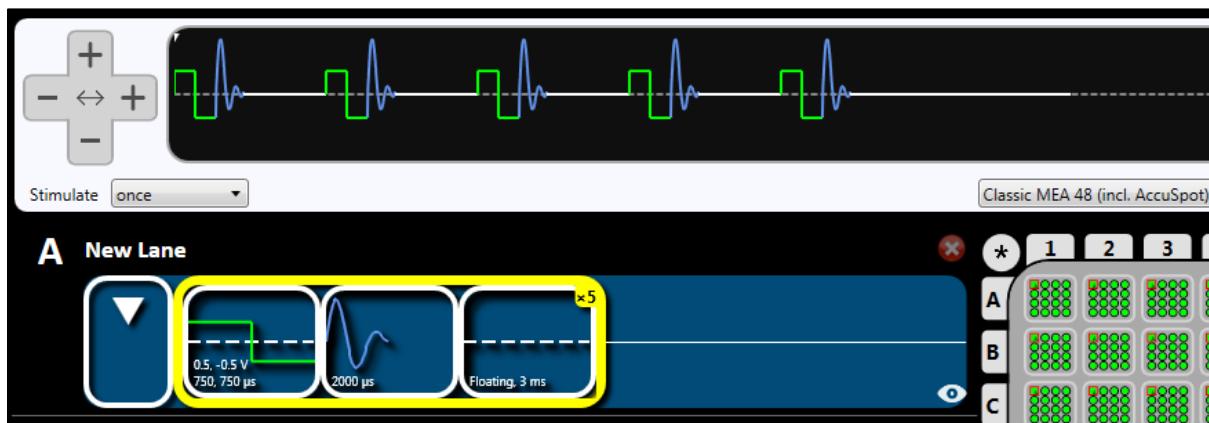
1. Select **Electrical** in the **Stimulation Type** drop-down menu.
2. Click and drag the **Neural Stimulation with Artifact Elimination** block into Lane A.
3. Optional: Double-click the block to change its settings.
4. Select an option from the **Stimulate** drop-down menu in the **Global Repeat Settings** bar.

Note: The **Neural Stimulation** block already contains a stimulation time tag. Do not add an **Electrical Stimulation Tag** block.



To build the custom neural stimulation protocol shown below:

1. Select **Electrical** in the **Stimulation Type** drop-down menu.
2. Click and drag an **Electrical Stimulation Tag** block into Lane A.
3. Click and drag a **Loop Container** into Lane A.
4. Double-click the x2 in the upper right corner of the **Loop Container** and change it to x5.
5. Click and drag the **Biphasic Stimulation** block into the **Loop Container**.
6. Click and drag the **Stimulation Artifact Eliminator** block into the **Loop Container** behind the **Biphasic Stimulation** block.
7. Click and drag the **Delay** block into the **Loop Container** behind the **Stimulation Artifact Eliminator** block.
8. Select **Once** from the **Stimulate** drop-down menu in the **Global Repeat Settings** bar.



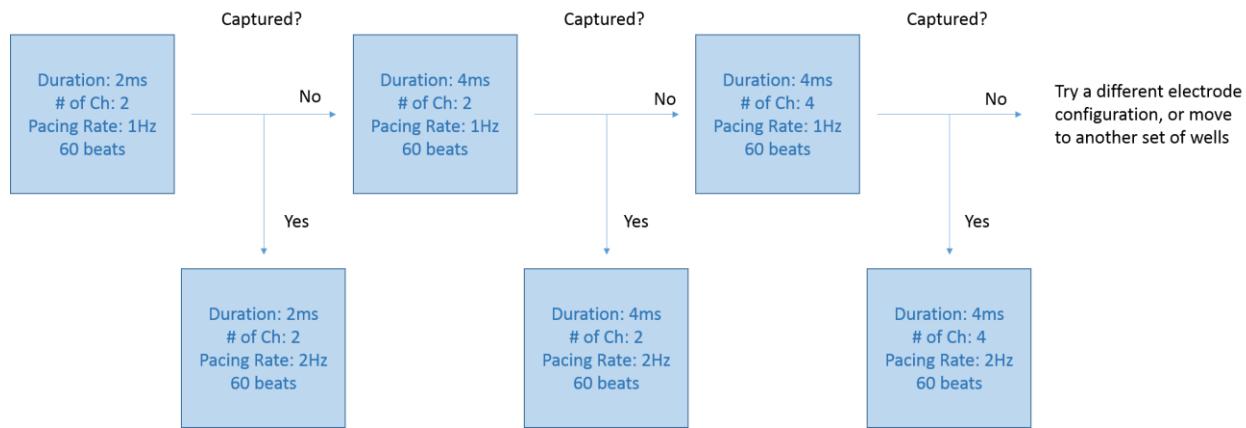
Note: Although the custom stimulation protocol described above contains a **Stimulation Artifact Eliminator** block, custom electrical stimulation waveforms will have larger artifacts because the **Artifact Eliminator Data Processor** (Section 2.3.3) only works with the **Neural Stimulation** and the **Cardiac Pacing Stimulation** blocks in Stimulation Studio. Axion recommends using the **Cardiac Pacing Stimulation** block to pace cardiomyocytes and the **Neural Stimulation** block for all other electrical stimulation applications.

4.1.4. Cardiac Pacing Stimulus Design Workflow

A few quick test stimuli are recommended to determine the appropriate stimulus for reliably capturing the wells of interest before beginning the full stimulation protocol.

For Microelectrode stimulation, the suggested workflow is shown below. Proceed until successful capture is achieved at the desired maximum frequency. In general, the required stimulus depends on cell type, plating density/uniformity, and culture viability. Choose the lowest level stimuli that achieves complete capture at the highest pacing rate.

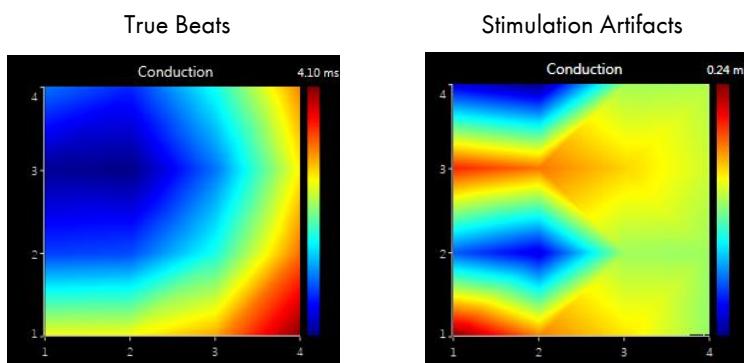
Note: It is not possible to pace slower than the spontaneous frequency of the cardiac culture. Be sure to choose the pacing rate with both the control frequency and the predicted pharmaceutical effects in mind. When using treatments that will prolong the beat period, choose a slow pacing rate; when shortening the beat period, choose a faster pacing rate.



Stimulating using the dedicated stimulation electrode (**Stimulation Paddles**) on the E-Stim+ MEA Plate is generally more effective at capturing the network than stimulating through the microelectrodes. If the default parameters are not sufficient to capture the culture, change the stimulation by:

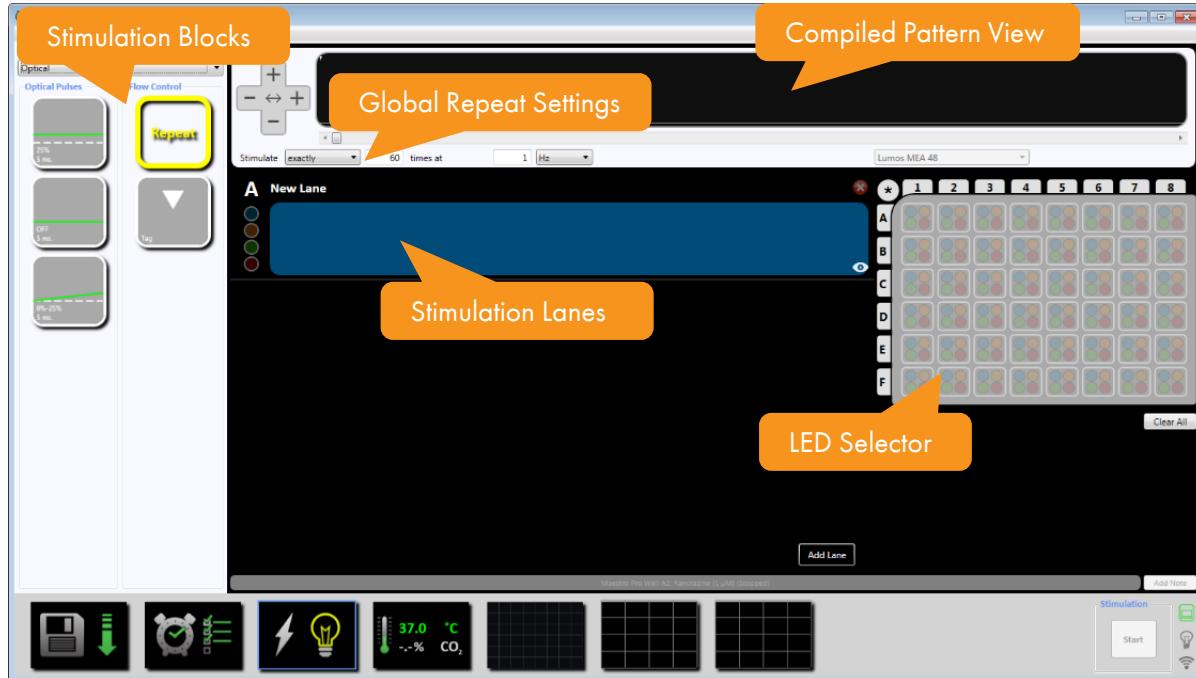
1. Increasing the duration to 0.5 ms, and decreasing the amplitude to 20 μ A.
2. If the stimulation still does not capture the culture, increase the duration and/or amplitude in small increments (0.1 ms and/or 10 μ A), not to exceed 1ms and 100 μ A. Faster pacing frequencies may require larger stimulation parameters than slower pacing frequencies.
3. If the stimulation still does not capture the culture, change the pacing frequency to a frequency closer to the culture's spontaneous beat rate.

As the duration and amplitude of stimulation are increased, stimulation artifact may begin to increase. It is important to make sure the beats detected by *AxIS Navigator* are true beats and not stimulation artifact. Check the **Conduction Plot** in the **Cardiac Beat Plots** module to make sure the beat propagation is in the physiological range 2-10 ms. If the conduction plot shows propagation less than 1.5 ms along with an unusual pattern, the **Cardiac Beat Detector** is most likely detecting simultaneous stimulation artifact rather than propagation across the syncytium. Alternatively, use the **Stimulation Inspector** (Section 2.3.4) to determine the size of the stimulation artifacts. Increase the **Beat Detection Threshold** to prevent detection of stimulation artifacts as beats.



4.2. OPTICAL STIMULATION

Axion designed the Lumos™ optical stimulator for artifact-free light stimulation. Cell cultures must be engineered with opsins in order to become light-sensitive. When integrated with *AxIS Navigator*, the Lumos can independently stimulate each well with up to four wavelengths of light. The optical stimulation interface in **Stimulation Studio** is laid out similarly to the electrical stimulation interface.



For data acquisition with optical stimulation:

1. Build the stimulation protocol according to Section 4.2.2.
2. Record data according to Section 3.3, selecting the **Cardiac Real-Time Optically Paced or Neural Real-Time Optically Evoked** configuration.
3. Click the **Start** button to begin the stimulation protocol manually. Click again to stop.

Note: If Once is selected in the Global Repeat Settings, the stimulation protocol may be started automatically by the Scheduled Recording Setup module (Section 2.6.2).

For data analysis with optical stimulation:

1. Analyze data according to Section 6.5, selecting the **Cardiac Offline Optically Paced or Neural Offline Optically Evoked** configuration.

Note: See the 'Neural Metric Tool' for evoked activity analysis options for neural analysis (Section B.2.4).

4.2.1. Optical Stimulation Blocks

The optical stimulation blocks are:

Block	Icon	Description
Optical Pulse Stimulation (On)		Applies a constant intensity of light for a set duration. Set intensity and duration with Intensity and Stimulus Duration in the Pulse Settings dialog.
Optical Pulse Stimulation (Off)		Applies a set duration of no light. Use as a wait period. Set duration with Stimulus Duration in Pulse Settings dialog.
Optical Ramp Stimulation		Applies a linearly increasing intensity for the duration of the pulse. Set the starting and ending intensity and duration with Starting Intensity , Ending Intensity , and Ramp Duration in the Ramp Settings dialog.
Loop Container		Repeats any blocks contained within a set number of times. Double-click the repeat number (xN) in the top right corner of the block to set the number of times.
Optical Stimulation Tag		Set the location of a "tag" or time stamped note to indicate a stimulation occurred. Tags are useful for visualization and analysis of evoked activity. Only one tag block can be set per stimulation protocol, so tags cannot be assigned to multiple lanes of stimulation. <i>Note: If no tag is manually set, a tag will be automatically generated at each repetition of Lane A. To prevent excessive size of output files, limit the tag rate to 50 Hz.</i>

4.2.2. Stimulation Protocol Generation

Stimulation Studio allows the user to generate multiple "lanes" of optical stimulation patterns. Each stimulation lane may be assigned only one wavelength but multiple stimulation lanes may be assigned the same wavelength. Each well may be assigned any combination of wavelengths, but no two stimulation lanes of the same wavelength may be assigned to the same well.

Warning: Running LEDs at high output intensity settings with high duty cycles for extended periods of time will result in significant build-up of radiated heat. Take special care not to overheat objects lying directly underneath the Lumos, such as a culture plate, the Maestro, or the Lumos stand. If elevated temperatures are

maintained, AxIS Navigator will turn the Lumos off, but it's possible to reach temperatures that could be damaging to cell cultures before then.

It is good practice to save a stimulation protocol for future reference, after the pattern is completed and the wells are selected. Save stimulation protocols using **File → Save Stim Waveform**. Open with **File → Open Stim Waveform**.

To build an optical stimulation pattern:

1. Select **Optical** in the **Stimulation Type** drop-down menu.
2. Select the MEA plate type from the drop-down menu above the plate map.
3. Click and drag a block into the desired stimulation lane. A gray bar appears in the lane indicating where a block will be dropped.
4. Double-click a block in a stimulation lane to change its settings.
5. Click on the desired wavelength for the lane.
6. (optional) Type a name for the lane in the space beside the lane letter.

To copy a block from a stimulation lane:

3. Right-click on the block and select **Copy Pulse**.
4. Right-click on the stimulation lane and choose **Paste Pulse**.

To delete a block from a stimulation lane:

2. Right-click on the block and select **Delete Pulse**.

Note: A Loop Container provides the option of deleting the loop and its contents or just the loop.

To add new stimulation lanes:

1. Click the **Add Lane** button.

To copy a stimulation lane:

1. Right-click on the lane and select **Copy Lane**.
2. Right-click on a lane and select **Paste Lane** to create an identical new lane.
3. Right-click on a lane and **Select Paste Lane Contents** to paste the contents of the copied lane to the current lane.

To clear a stimulation lane:

2. Right-click on the lane and select **Clear**.

To delete a stimulation lane:

1. Click on the red X (✖) in the top right corner of the lane.

To hide a stimulation lane:

- Click on the eye icon (👁) in the bottom right corner of the stimulation lane.

Note: Hiding a lane will only prevent it from being seen in the compiled pattern view, not disable its activity during stimulation.

To specify how often to apply the stimulation:

- Select an option from the **Stimulate** drop-down menu in the **Global Repeat Settings** bar

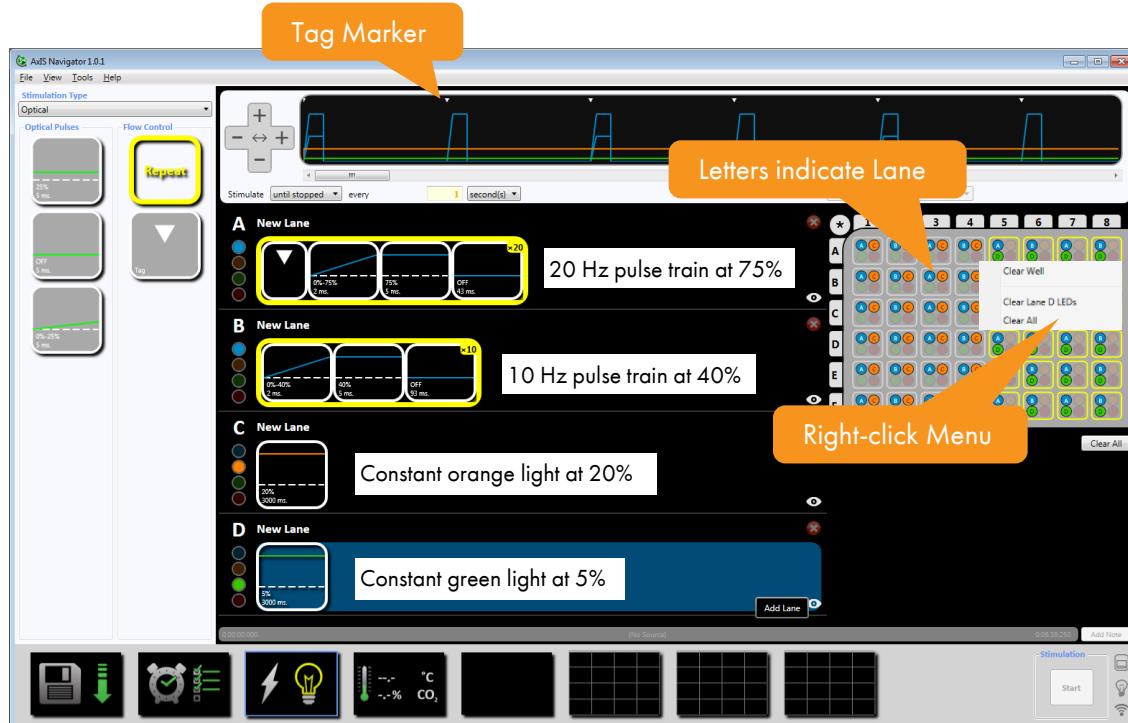
Note: The **Global Repeat Settings** bar applies to all stimulation lanes defined in the stimulus. To set a distinct frequency or number of repetitions across different lanes, use the repeat block in each individual lane.

To assign a stimulation lane to one or more wells:

- Click on the stimulation lane to select it.
- Click on the desired well(s) in the plate map in **Stimulation Studio** module to apply the lane to that well. Selection is the same as the **Plate Map Editor** in **Active Plate** (See Section 2.2.3).

Note: When selected the lane color will become active with the lane letter inside.

Multiple stimulation lanes of the same wavelength can be defined, but each well may only be assigned a single lane for each wavelength. Multiple stimulation lanes, each having a unique color, can be applied to the same collection of wells. Right-click on the **LED Selector** to quickly clear previous well assignments.



4.2.3. Example Stimulation Pattern

A basic example of a periodic blue light stimulus is shown below. The pulse block is set to 5 ms duration at 25% intensity. The lane is designated for the blue LED and is assigned to each well of the plate. The global repeat bar specifies a 1 Hz frequency. This stimulation could be used to pace a spontaneously beating cardiac culture with a periodic blue light stimulus.



Use the following steps to recreate the example stimulus above:

1. Select **Optical** from the **Stimulation Type** drop-down menu.
2. Click and drag an **Optical Stimulation Tag** block into Lane A.
3. Click and drag an **Optical Ramp Stimulation** block into Lane A.

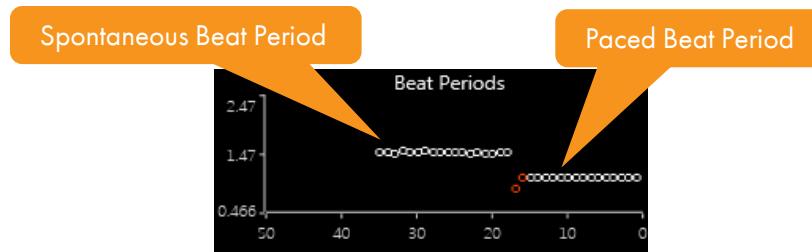
Note: Using a ramp is recommended, but not required. Although optical stimulation artifacts are rare, using a ramp before the pulse helps minimize any potential artifacts. The Ending Intensity of the ramp should match the pulse intensity.

4. Double-click the **Optical Ramp Stimulation** block to set the **Ending Intensity** to 25% and **Ramp Duration** to 2 ms.
5. Click and drag an **Optical Pulse Stimulation** block into Lane A.
6. Double-click the **Optical Pulse Stimulation** block to set the **Intensity** to 25% and **Stimulus Duration** to 5 ms.
7. Click the blue button to the left of Lane A to specify the wavelength as 475 nm (blue).
8. Click on the * in the upper left corner of the plate map to assign the stimulus pattern in Lane A to all wells in the plate.
9. Select **until stopped** in the **Stimulate** drop-down. Set **every** to **1 second(s)**.

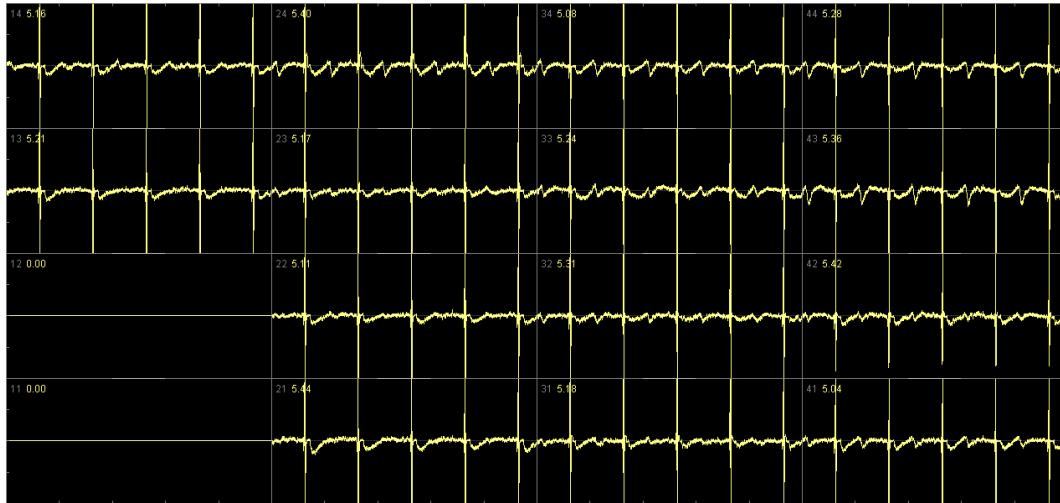
4.3. EVALUATING CARDIAC STIMULATION CAPTURE

Effective capture means each stimulation pulse elicits a beat in each of the stimulated wells. This is the most critical factor when determining stimulation parameters. If a stimulus does not capture the majority of wells, a stronger stimulus should be used (see Section 4.1.4 for recommendations). Generally, the “weakest” stimulus that consistently captures each well should be used. Stronger stimuli can affect the ability to record the sodium spike, due to the larger stimulation artifact. The **Beat Periods** plot in the **Cardiac Beat Plots** module, the **Continuous Waveform Plots** module, and the **Activity Map** module are all useful in diagnosing whether a well has been captured.

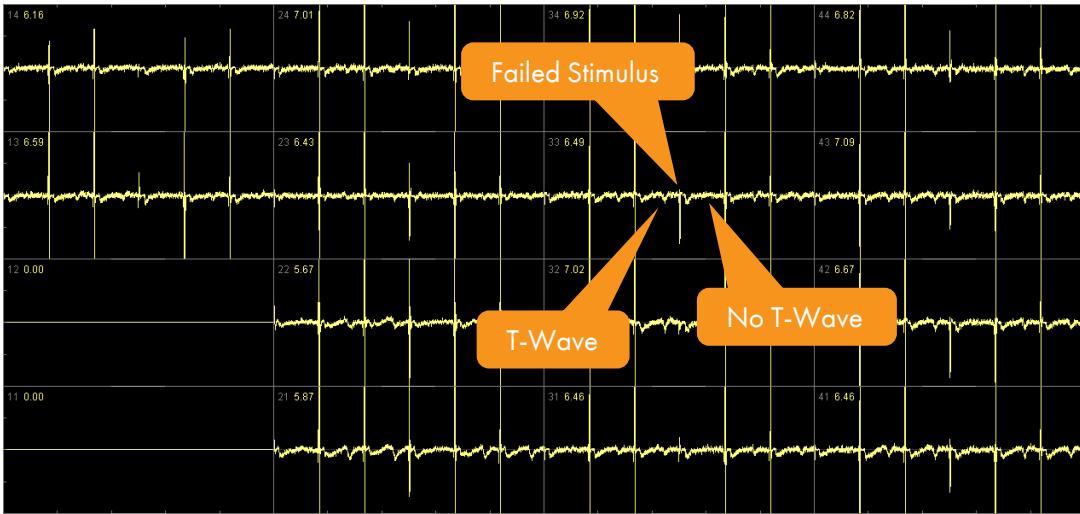
View the **Beat Periods** plot to see if the beat rate matches the stimulus rate.



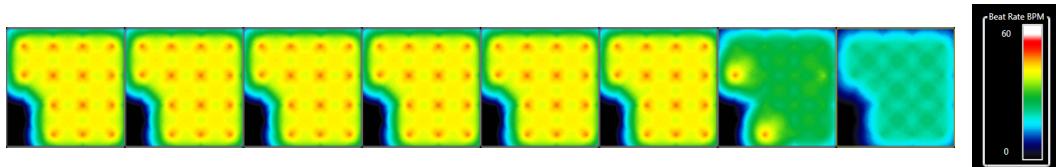
Capture is evident in the **Continuous Waveform Plots** module by looking for the depolarization spike and T-wave for each beat. When a depolarization spike and T-wave follow each stimulation artifact, capture is successful.



The figure below shows a partial capture in the **Continuous Waveform Plots** module. Most, but not all, stimuli show a corresponding cardiac beat.

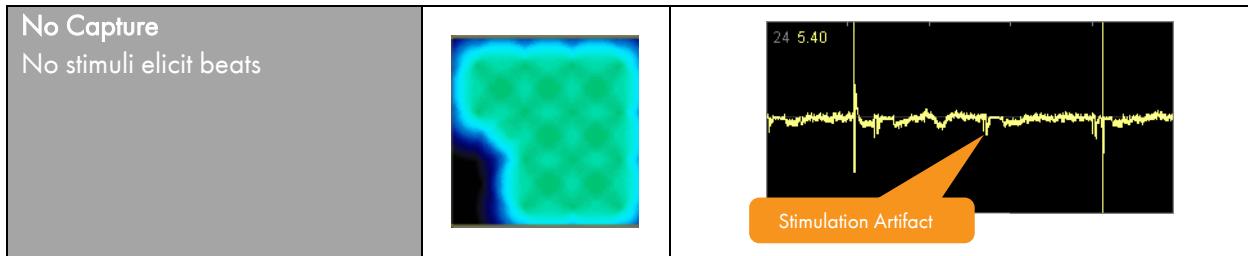


Capture can be assessed using the **Activity Map** with the source set to **Beat Rate**. Set the scale to the target paced beat rate. When the beat rate of each electrode is equal to the pacing rate, the well has been successfully paced.



The following table illustrates how various capture conditions appear in the **Activity Map** and **Continuous Waveform Plots** modules:

Status	Activity Map	Continuous Waveform Plot
Full Capture All stimuli elicit beats		 Callouts: 'Evoked Beat' (top), 'T-Wave' (right), 'Stimulation Artifact' (bottom left).
Partial Capture Some stimuli elicit beats		 Callouts: 'Evoked Beat' (top), 'T-Wave' (right), 'Stimulation Artifact' (bottom left), 'No T-Wave' (bottom right).

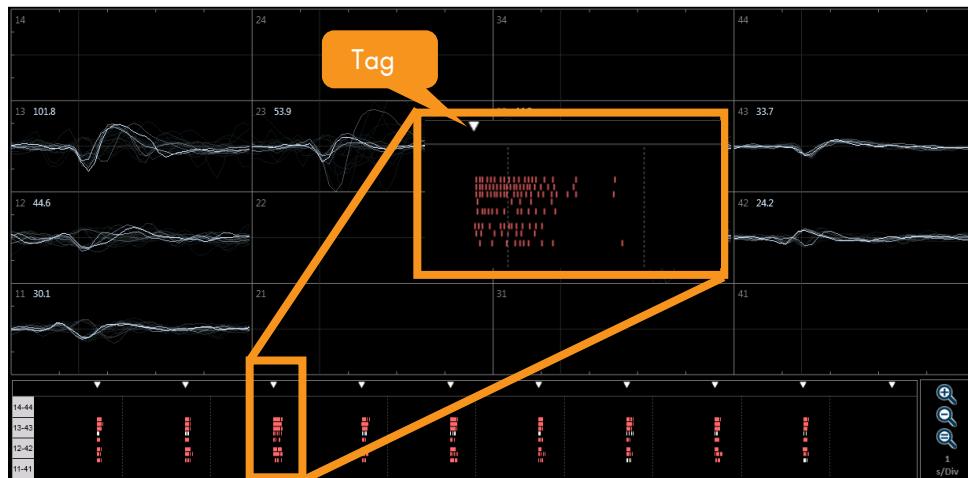


In order to use the **Beat Periods** plot and **Activity Map** to evaluate capture, ensure the **Beat Detector** is capturing true beats, not stimulation artifact (Section 2.3.7).

4.4. EVALUATING NEURAL STIMULATION CAPTURE

Neural stimulation capture can be viewed in the **Spike Plots** module with a combination of **Electrical** or **Optical Stimulation Tags** and the **Stimulation Inspector** data processor.

The stimulation tags synchronize the recorded electrophysiological activity with the stimulation pattern. The timing of each stimulation is displayed alongside the recorded electrophysiological data, providing instant feedback on a successful stimulus. The tags are displayed in the raster plot as inverted white triangles, corresponding to the tags defined in **Stimulation Studio**.



The **Stimulation Inspector** data processor plots the continuous voltage data from before and after an **Electrical** or **Optical Stimulation Tag** to the **Spike Plots** module. The waveform plots overlay with the brightest trace representing the most recent tag. This provides a close look at data near a **Tag** to evaluate artifact elimination and cell response to the stimulation. See Section 2.3.4 for more information on the **Stimulation Inspector**.

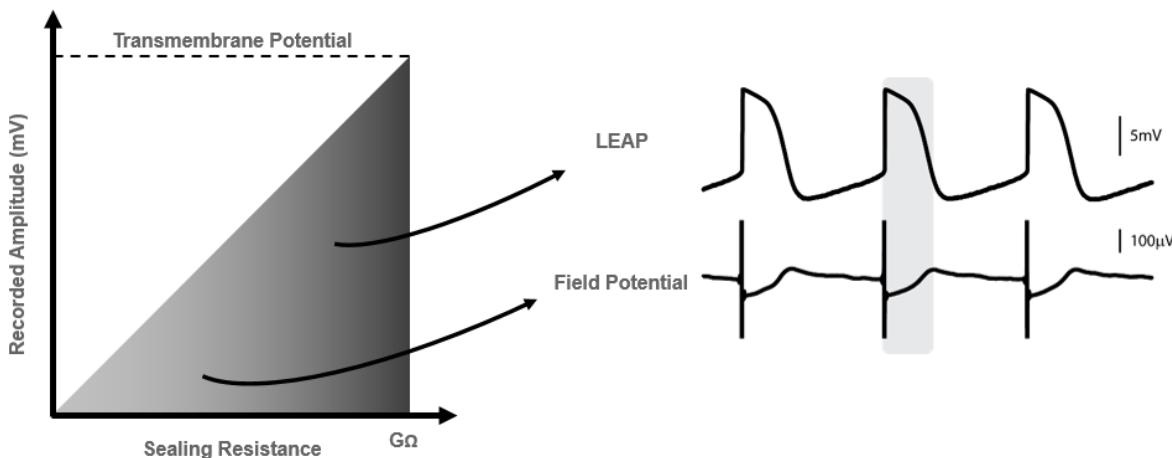


CHAPTER 5. LOCAL EXTRACELLULAR ACTION POTENTIAL (LEAP)

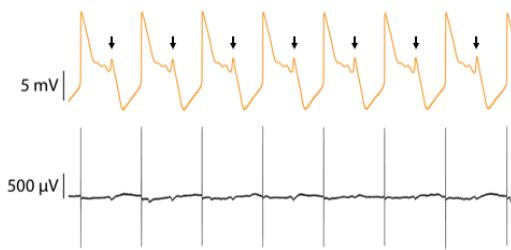
The Local Extracellular Action Potential (LEAP) assay allows acquisition of extracellular action potential waveforms from cardiomyocytes on Axion's MEA plates. Acquiring LEAP signals requires a proprietary induction procedure that increases the coupling between the cells and the electrodes. Unlike traditional patch clamp or voltage sensitive dye techniques, LEAP signals are stable for 10-20 minutes after induction and can be recorded from intact syncytia without altering spontaneous activity, compromising membrane integrity, or requiring addition of labels.

5.1. LEAP BACKGROUND

In a typical cardiomyocyte-MEA assay, field potential signals are detected from cells near the electrodes, which are the result of the cardiac action potential propagating across the array, similar to the way in which the ECG arises from propagation of the cardiac action potential across the heart. The field potential signal has always been qualitatively similar to the ECG waveform. However, unlike a surface ECG, the cardiomyocyte syncytium is in direct contact with the electrode. The LEAP assay uses proprietary techniques to enhance the cell-electrode coupling, enabling the electrodes to detect a Local Extracellular Action Potential (LEAP), which closely approximates the waveform of an intracellular action potential.



The LEAP signal provides a direct mapping from field potential to action potential morphology. The LEAP signal is much larger than the field potential, and arrhythmic events are easily seen on the extracellular action potential waveform, which improves the accuracy and automation of analysis along with automatic early after-depolarization (EAD) detection, classification, and counting. The image below shows a clear EAD on each beat in the LEAP signal, with the smaller corresponding field potential deflections below.



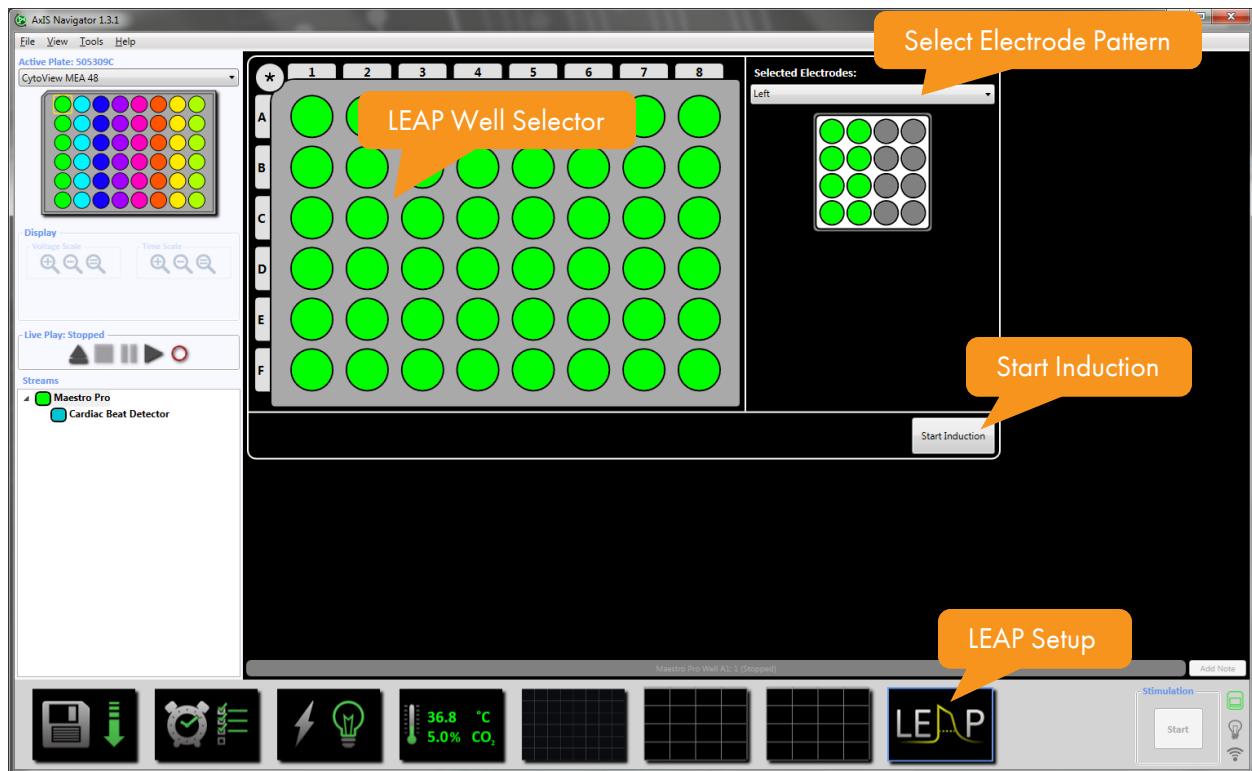
The signal detected on each electrode always contains contributions from both the field potential and LEAP; the LEAP assay is simply a way of enhancing the cell-electrode coupling such that the relative contribution of the LEAP signal is much larger. As such, a variety of waveforms may be observed after inducing LEAP, as described in the table below:

Waveform	Description
	Large LEAP signal with undetectable field potential contribution, typically 5-10 mV in amplitude.
	Strong LEAP signal, but note the field potential is large enough to still show above the peak of the LEAP signal. These LEAP signals are typically 500 μV to 5 mV. With Enhance for LEAP on, the field potential component is removed enabling the user to view only the LEAP signal.
	No LEAP signal; LEAP induction was not successful and a field potential signal remains.

Note: AxIS Navigator displays LEAP signals using the **Enhance for LEAP** option (Section 2.6.6), which automatically separates the LEAP and field potential and removes the field potential component of the signal, allowing the user to only view the LEAP signals. This enhancement is purely visual and does not affect the data analysis in AxIS Navigator or metric computation in the CiPA Analysis Tool.

5.2. DESIGNING A LEAP INDUCTION PLATE MAP

The **LEAP Setup** module is used to design a LEAP induction plate map, which specifies the wells and electrodes for LEAP induction.



Select a pattern of electrodes for LEAP induction using the **Selected Electrodes**: drop-down menu on the right. Electrodes in green will be used for LEAP induction, while electrodes in grey will not.

Click on a well in the LEAP Well Selector to select it. Multiple wells can be selected by clicking on the column or row label to highlight the column or row, respectively; clicking the * in the upper left corner to select the entire plate; holding the **Ctrl** key and clicking on the desired wells; selecting two wells while holding the **Shift** key to select all of the wells between them; or by click-and-drag selecting wells in a region.

LEAP is compatible with all plate types. However, due to differences in electrode geometry and material, the optimal LEAP induction protocol differs between plate types. For Classic MEA plates, choose the electrode pattern that is best suited for the experiment design – select **All** electrodes for maximal LEAP yield. With CytoView MEA plates, the pre-configured grid patterns in AxIS Navigator are recommended, such that LEAP induction occurs on every other electrode in a well.

To record LEAP signals and field potentials simultaneously, select a pattern of electrodes that leaves some electrodes un-induced for field potential recordings.

5.3. LEAP ACQUISITION AND ANALYSIS TUTORIAL

To acquire LEAP signals:

1. Set up the Maestro for data acquisition as described in section 3.3.
2. Right-click on the **Maestro Pro** or **Maestro Edge** stream and select **Configuration → Cardiac Real-Time → LEAP**.

Note: Applying the LEAP configuration will cause the LEAP Setup module to appear.

3. Design a LEAP induction plate map in the LEAP Setup module by selecting a pattern of electrodes using the drop-down menu on the right to be applied to the wells selected using the LEAP Well Selector in the center (Section 5.2).
4. Click **Start Induction** to initiate LEAP induction.

Note: LEAP induction takes ~10 minutes, during which file playback and recording are disabled. When LEAP induction is complete, playback will begin automatically.

5. Press **Record** to save a .raw file with the LEAP signals.

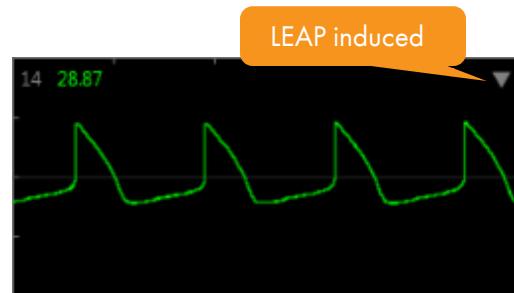
WARNING: LEAP signals last from 20 min up to a few hours, so be sure to press record after induction to save the LEAP data.

To analyze LEAP signals:

1. Follow the steps for data analysis as described in Section 6.5, using **Configuration → Cardiac Offline → LEAP** to save a Cardiac Statistics Compiler Advanced Metrics .csv file.
2. Load the .raw and .csv files into the CiPA Analysis Tool to generate LEAP metrics, such as LEAP Duration (LPD) 30%, 50%, and 90% repolarization. See Appendix C for more information about the CiPA Analysis Tool.

The **Cardiac Offline → LEAP** analysis configuration is used for analysis even when only a subset of electrodes were selected for LEAP induction. The same Cardiac Beat Detector analyzes both LEAP signals and field potential signals, depending on where LEAP is induced.

After inducing LEAP, tags are added to the .raw file indicating which electrodes were selected for LEAP induction. In the **Continuous Waveform Plots**, an inverted grey triangle in the upper right corner indicates LEAP was induced on that electrode.



The **Cardiac Beat Detector** applies a LEAP beat detection algorithm to data acquired using **Cardiac:LEAP Acquisition Settings** for electrodes that are LEAP induced/tagged or have spontaneous LEAP waveforms. It applies the field potential beat detection criteria (Section 2.3.7) to all other electrodes. For data recorded using **Cardiac: Spontaneous** or **Cardiac: Broadband Acquisition Settings**, the field potential beat detection criteria is applied to all electrodes except those selected for LEAP induction. In the **Cardiac Beat Plots**, an inverted green triangle indicates LEAP was induced on the electrode and the beats on that electrode are detected as



LEAP beats. An inverted grey triangle in the upper right corner indicates LEAP was induced on the electrode, but no LEAP beats are detected.

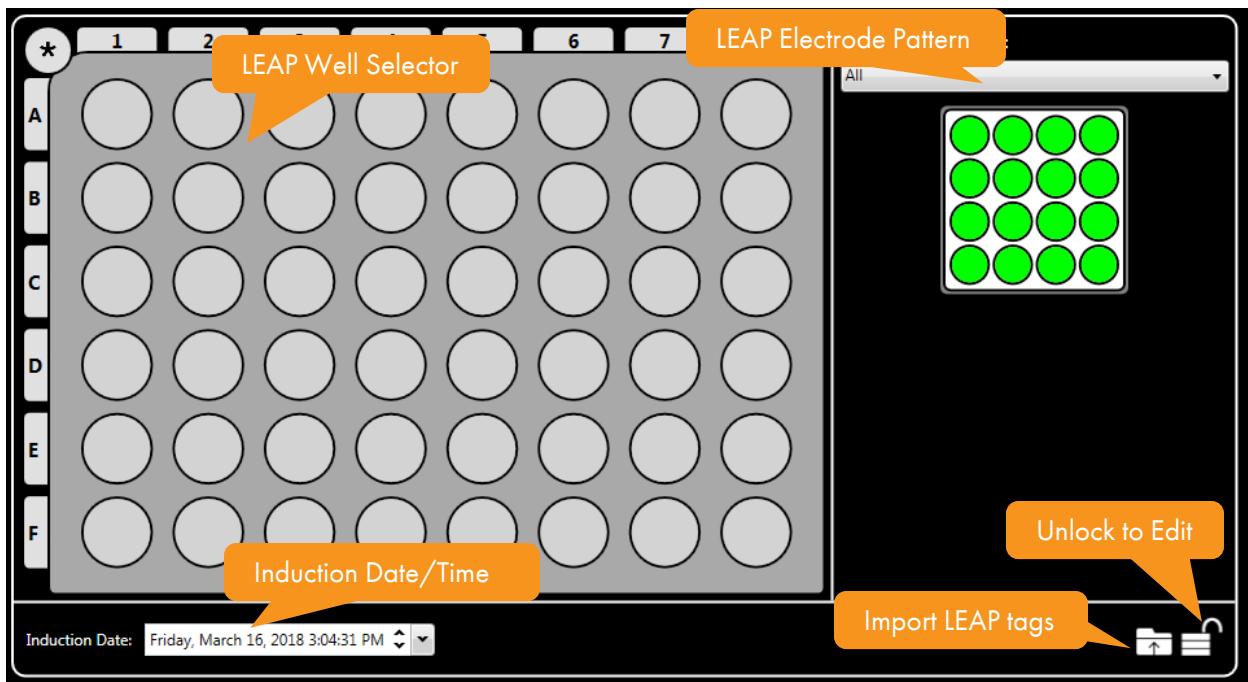
5.3.1. Adding or Removing LEAP Tags from Files

LEAP is not a mathematical change to the acquired signal, so previously recorded field potentials cannot be transformed into LEAP signals. LEAP induction is required to increase cell-electrode coupling and change the acquired signal.

In some instances, it may be desirable to add or remove LEAP tags from LEAP data in order to change the way the data is analyzed in the **Cardiac Beat Detector**. LEAP tags are initially added to the .raw file to indicate which electrodes were used for LEAP induction. Once a new plate is inserted into the Maestro, recordings will no longer retain the LEAP tags. For example, if a LEAP recording is performed at 10:00 am, then the plate is returned to the incubator while other plates are recorded, a subsequent recording at 12:00 pm will not contain the LEAP tags required for analysis of LEAP signals.

To add LEAP tags to a .raw file:

1. Load the .raw file into *AxiS Navigator* by selecting **File → Open Recording(s)...**
2. Click the thumbnail to select the **LEAP Setup** module.
3. Click the **Lock** icon to unlock the LEAP induction plate map for editing.
4. Click the folder icon to import a LEAP induction plate map from a different (e.g. earlier) .raw file.
– OR –
5. Select the wells where LEAP was induced from the LEAP well selector in the center and the LEAP electrode pattern from the **Selected Electrodes:** drop-down menu.
6. (Optional) Use the **Induction Date:** menu to specify the original date and time of LEAP induction.
7. Click the lock to prevent further changes to the LEAP induction plate map.



Conversely, to analyze field potentials from electrodes that were initially selected for LEAP induction, it is possible to remove the LEAP tags from the .raw file.

Note: If a grid of electrodes was used for LEAP, the **Cardiac Beat Detector** automatically applies LEAP beat detection to LEAP induced/tagged electrodes and field potential beat detection to all other electrodes. It is not necessary to remove LEAP tags from the .raw file unless you want to analyze field potentials from electrodes that were initially tagged as LEAP induced.

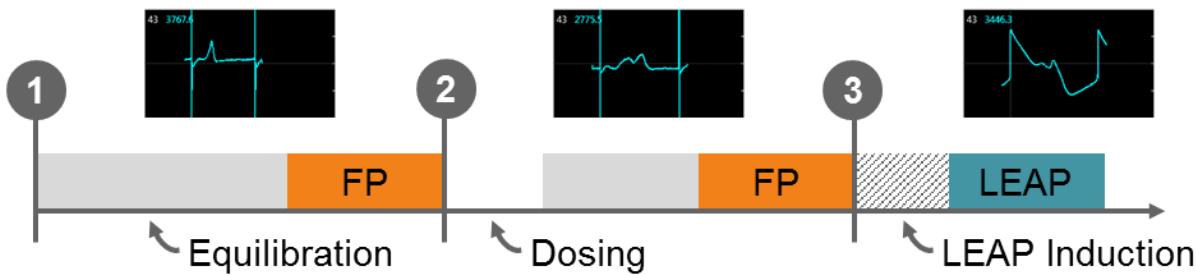
To remove LEAP tags from a .raw file:

1. Load the .raw file into *AxIS Navigator* by selecting **File → Open Recording(s)...**
2. Click the thumbnail to select the **LEAP Setup** module.
3. Click the **Lock** icon to unlock the LEAP induction plate map for editing.
4. Click the * in the upper left corner to de-select all wells in the LEAP Well Selector, or deselect a subset of wells by clicking on a single well or using the *, row label, column label, **Ctrl** or **Shift** keys.
5. Click the lock to prevent further changes to the LEAP induction plate map.

Note: There is an option in the CiPA Analysis Tool to ignore the LEAP tags and analyze field potentials only, so it is not necessary to remove the LEAP tags from the .raw file in *AxIS Navigator* in order to analyze field potentials in the CiPA Analysis Tool. Please see Appendix C for details.

5.4. EXAMPLE LEAP EXPERIMENT DESIGN

The LEAP assay provides complementary information to the Maestro field potential assay, such that it is helpful to measure both signals from the same plate. An example experiment design is illustrated below with (1) a baseline field potential (FP) recording, (2) a post-dose FP recording, and (3) a post-dose LEAP recording following LEAP induction. In this experiment design, the LEAP signal enables automated EAD detection and quantification of action potential morphology. For applications in characterizing stem cell derived cardiomyocytes, the user may elect to skip (2), and instead record activity at multiple stages in development.



The workflow for this experiment is described below:

1. Place the MEA plate in the Maestro and allow the cells to equilibrate for 10-20 minutes.
2. Select Configuration → Cardiac Real-Time → Spontaneous.
3. Record a baseline .raw file (5-30 minute recording is recommended).
4. Add compounds to the MEA plate.
5. Return the MEA plate to the Maestro and record a post-dose field potential recording (5-30 minutes recording is recommended, at post-dose time point of interest).
6. Select Configuration → Cardiac Real-Time → LEAP.
7. Induce LEAP.
8. Immediately record a post-dose LEAP recording.

5.5. FAQ

1. How does LEAP work?

The LEAP signal arises from an increased coupling of the cardiomyocytes to the electrodes on an Axion MEA plate following the LEAP induction process. The strength and shape of the LEAP waveform is determined by the cell-electrode coupling and the action potential morphology of the cells local to the electrode.

2. Is LEAP mathematical magic?

No. As stated in FAQ #1, LEAP is actually a change in the signal recorded by the electrodes resulting from an increase in cell-electrode coupling, rather than a result of signal manipulation. However, AxIS Navigator does employ optional filtering techniques ("Enhance for LEAP") to suppress the underlying field potential that remains from nearby cells for optimal visualization of LEAP signals.

3. Can LEAP be applied to old data?

No. LEAP induction is required to increase cell-electrode coupling and, thus, change the acquired signal.

4. Is LEAP compatible with all plate types?

Yes. However, due to differences in electrode geometry and material, the optimal LEAP induction protocol differs between plate types. For Classic MEA plates, choose the electrode pattern that is best suited for your experiment design. With CytoView MEA plates, we recommend using the pre-configured grid patterns in Axis Navigator, such that LEAP induction occurs on every other electrode in a well.

5. Does LEAP work for all cell types?

LEAP signals have been successfully recorded from most commercially-available iPSC-derived cardiomyocytes and multiple “home-grown” cardiomyocyte lines. Cells with beat period < 1s may have reduced LEAP yield, as the frequent contractions release the syncytium from the increased cell-electrode coupling. For these cells, we suggest performing LEAP induction at a slightly lower temperature (~ 35°C) to reduce the beat period. LEAP is not compatible with neuronal assays.

6. Can I perform LEAP on the original Maestro?

No. The BioCore v4 chip in the Maestro Pro and Edge enables LEAP induction and signal acquisition.

7. Will LEAP work with poor cell coverage?

Weak field potential signals indicate poor cell coverage over the electrode(s). Without sufficient cell coverage, it is difficult to increase cell-electrode coupling and thus LEAP yield may be limited. In some cases, LEAP induction may boost signal strength for experiments with low amplitude field potential signals.

8. Why do LEAP amplitudes vary?

LEAP amplitudes represent the degree of cell-electrode coupling achieved by LEAP induction, with larger signals representing stronger coupling.

9. How long does LEAP last?

LEAP signals are typically stable for 10-20 minutes, but may last for hours. LEAP signals will decay over long periods of time as the mechanical beating of the cells returns the cell-electrode coupling to field potential levels. So, be sure to record your data immediately after LEAP induction to avoid LEAP data loss.

10. Can LEAP be repeated on the same plate?

LEAP can be repeated on the same plate, but typically not on the same electrode(s). LEAP induction is more effective the first time and efficacy decreases with subsequent induction.

11. Why do some LEAPs have a superimposed field potential feature?

Axon's MEA plates are always sampling the field potential signal from cells near the electrode. LEAP induction increases the cell-electrode coupling, providing the LEAP signal from only the cells directly attached to the electrode. So, it is possible to see features of the field potential signal and LEAP signal on the same electrode. The optional “Enhance for LEAP” feature (see FAQ #2) reduces the field potential component of the signal recorded at the electrode for visual purposes only.

12. Is LEAP compatible with cardiomyocyte pacing?

Yes. The standard pacing tools available in Axis Navigator may be used with LEAP and field potential signals.

13. Will I see a LEAP signal on every electrode?

Even if every electrode is selected for LEAP induction, it is unlikely that you will see a LEAP signal on every electrode. LEAP yield depends on a variety of factors, including cell coverage, cell density, beat rate, and cell type. In general, we expect to see LEAP signals in 80-100% of wells when all electrodes are selected for induction.

14. Can LEAP signals be compared across electrodes in a well and between wells?

Yes. As with any assay, the user should quantify the degree to which biological variability may impact certain comparisons. For example, minor differences in LEAP signal shape on neighboring electrodes may be due to the distinct populations of cardiomyocytes and the cell-electrode coupling present on each electrode. Good well-to-well reliability of cardiomyocyte electrophysiology can easily support comparison of LEAP signals across wells.

15. Is LEAP the same as patch clamp?

No. The patch clamp technique relies on perforating the cell membrane to gain access to the transmembrane voltage and allow interrogation with current or voltage clamp protocols. Also, patch clamp typically requires isolating single cells. LEAP instead measures an extracellular action potential without disrupting the cell membrane from a local collection of cells within an intact, functional syncytium.

CHAPTER 6. DATA ANALYSIS

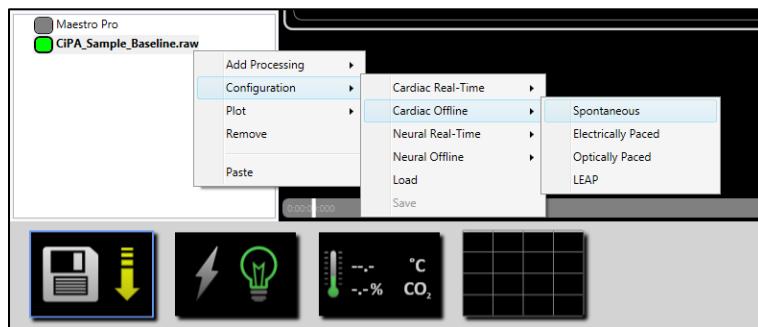
From a functional standpoint, data analysis in *AxIS Navigator* is similar to data acquisition. A previously recorded .raw file (continuous voltage data) is loaded into the **Streams** window, it passes through various data processors, and output files are generated while *AxIS Navigator* "records". Instead of recording the continuous voltage data from a **Maestro Pro** or **Edge** stream, file outputs from the data processors with various endpoint measurements are generated. This chapter reviews how to analyze data from the Maestro using *AxIS Navigator*. Sections 6.5 and 6.6 contain step-by-step tutorials for analyzing a single file and multiple files, respectively.

6.1. STREAM CONFIGURATIONS

Similar to data acquisition, *AxIS Navigator* comes with a variety of preset configurations for data analysis. Use the Offline configurations when working with previously recorded data.

To apply an analysis configuration:

1. Right-click on the data stream.
2. Select **Configuration** and navigate to the desired configuration. Click on the configuration.



Offline analysis configurations available in *AxIS Navigator* include:

Configuration	Processing Applied	Description
Cardiac Offline		
Spontaneous	Cardiac Beat Detector Statistics Compiler	Generates activity map, cardiac waveforms, beat rate, and conduction information. FPD detection is enabled. Generates a variety of cardiac beating endpoints.
Electrically Paced	Artifact Eliminator Cardiac Beat Detector Statistics Compiler	Generates activity map, cardiac waveforms, beat rate, and conduction information. Optimized for reducing stimulus artifacts in pacing experiments. FPD detection is enabled. Generates a variety of cardiac beating endpoints.

Optically Paced	Cardiac Beat Detector Statistics Compiler	Generates activity map, cardiac waveforms, beat rate, and conduction information. FPD detection is enabled. Generates a variety of cardiac beating endpoints.
LEAP	Cardiac Beat Detector Statistics Compiler	Generates activity map, cardiac waveforms, beat rate, and conduction information. Optimized for analyzing LEAP signals. Generates a variety of cardiac beating endpoints.
Neural Offline		
Spontaneous	Spike Detector Burst Detector Statistics Compiler	Generates activity map, spike waveforms, and raster plot. Network burst detection is enabled. Generates a variety of spiking, bursting, and synchrony endpoints.
Electrically Evoked	Artifact Eliminator Spike Detector Burst Detector Statistics Compiler	Generates activity map, spike waveforms, and raster plot. Optimized for reducing stimulus artifacts in electrically-evoked experiments. Network burst detection is enabled. Generates a variety of spiking, bursting, and synchrony endpoints.
Optically Evoked	Spike Detector Burst Detector Statistics Compiler	Generates activity map, spike waveforms, and raster plot. Network burst detection is enabled. Generates a variety of spiking, bursting, and synchrony endpoints.

Custom analysis configurations can be designed by adding data processors to the data stream (Section 2.3.1) or by modifying processor settings. For instructions on saving and loading custom stream configurations, see Section 3.2.2.

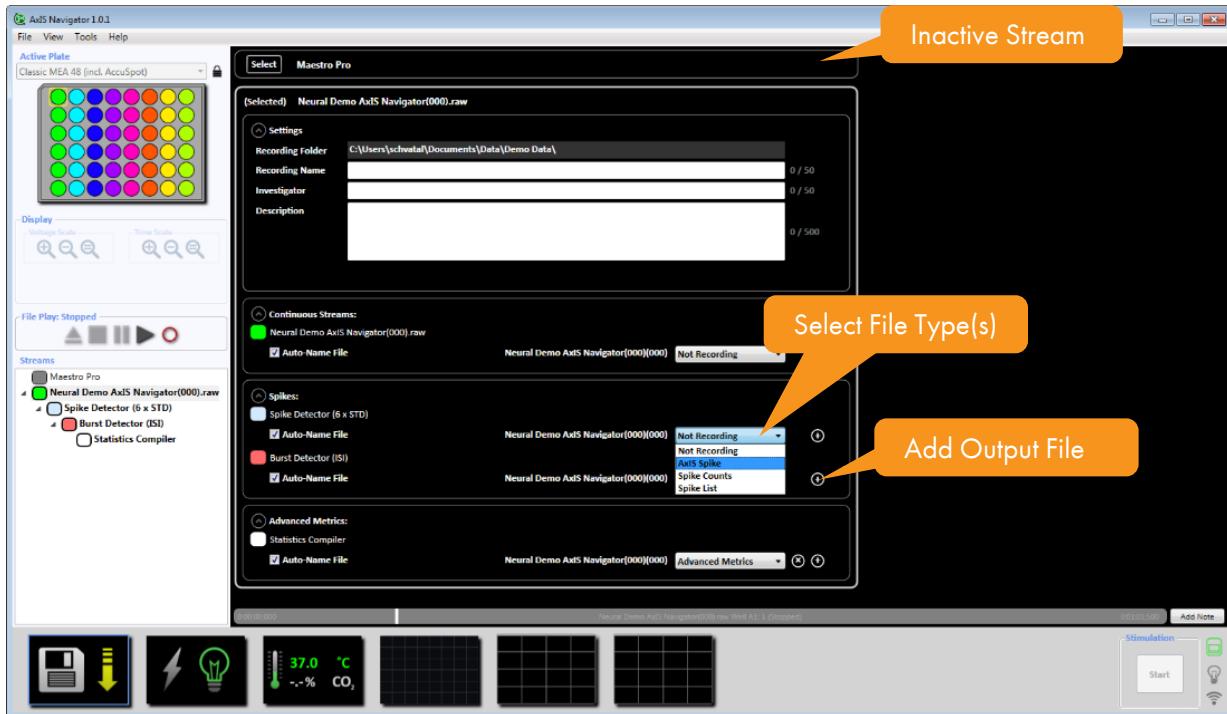
6.2. OUTPUT FILE TYPES

The **AxIS Raw (.raw)** file is the most fundamental *AxIS Navigator* file type; it contains the continuous voltage data. Any downstream processing can be recreated when the .raw file is loaded into *AxIS Navigator*. In addition to the .raw file, *AxIS Navigator* can generate a variety of file outputs from the various data processors.

Data Processor	Output Name	Description
Continuous Streams		
Maestro Pro/Edge	AxIS Raw (.raw)	<u>Primary format for data acquisition.</u> Continuous voltage data from all enabled electrodes before any digital processing.
Digital Filter	AxIS Raw (.raw)	Continuous voltage data from all enabled electrodes after the application of a Digital Filter .
Artifact Eliminator	AxIS Raw (.raw)	Continuous voltage data from all enabled electrodes after the application of an Artifact Eliminator .
Spikes		

Spike Detector	AxIS Spike (.spk)	Spike voltage waveforms with time and electrode for all spikes detected by the Spike Detector . Required output file for Neural Metric Tool.
	Spike Counts (.csv)	Table listing the number of spikes detected in a given time period for each electrode. Spike counting interval is set in the Spike Detector Settings .
	Spike List (.csv)	Table listing time, electrode, and amplitude of all spikes detected by the Spike Detector .
Burst Detector	Electrode Burst List (.csv)	Table listing the individual electrode bursts identified by the Burst Detector with descriptive information including time, electrode, number of spikes, and duration.
	Network Burst List (.csv)	Table of the network bursts identified by the Burst Detector with descriptive information including time, well, number of spikes, duration, number of electrodes, and spikes per electrode.
Cardiac Beats		
Cardiac Beat Detector	Electrode Beat List (.csv)	Table of individual electrode beats with time, electrode, depolarization amplitude, depolarization slope, beat period, field potential duration, well beat number, and delay from well beat time.
	Well Beat List (.csv)	Table of well beats with well, time, number of electrodes, starting electrode, ending electrode, beat period, well beat number, conduction velocity, and maximum delay from well beat time.
Advanced Statistics		
Cardiac Statistics Compiler	Advanced Metrics (.csv)	Primary output for data analysis. Tables containing group, well, and electrode endpoint metrics averaged over the duration of the analysis window. Includes depolarization amplitude, slope, beat period, beating irregularity, field potential duration, and conduction velocity metrics. Required output file for CiPA Analysis Tool and/or AxIS Metric Plotting Tool. See Section 7.3 for a list of included endpoints.
Neural Statistics Compiler	Advanced Metrics (.csv)	Primary output for data analysis. Tables containing group, well, and electrode endpoint metrics averaged over the duration of the analysis window. Includes spike, burst, and synchrony metrics. Required output file for AxIS Metric Plotting Tool. See Section 8.4 for a list of included endpoints.

The **Experiment Setup Properties** module defines the output file types that will be saved. Select the desired output from the drop-down menus in each section. More than one output may be selected from any drop-down menu. Any stream that should not be recorded must be set to **Not Recording**. Axion recommends saving an **AxIS Raw** file from the Maestro stream during data acquisition. Generally, it is not necessary to save any other **AxIS Raw** files during acquisition nor analysis. Preset **Real-Time** and **Offline** stream configurations specify the recommended output files to save.



To select multiple outputs from a single drop-down in **Experiment Setup Properties**:

1. Select the first output from the drop-down.
2. Click the **Add** button (\oplus).
3. Select the next output.
4. Repeat Steps 1-3 as needed.

Note: To remove an output, click the **Remove** button (\ominus) or select **Not Recording** from the drop-down menu.

AxIS Navigator supports the use of third party software, including *NeuroExplorer*[®], *Microsoft Excel*[®], *Offline Sorter*[®], *MATLAB*[®], *Spotfire*[®], and any software that can import comma-separated value (.csv) data. To use these software packages, *AxIS Navigator* data must be recorded in the appropriate file types. These files will contain different information depending on the application. **AxIS Raw** files can be processed in *AxIS Navigator*, *MATLAB*, or *NeuroExplorer*. **AxIS Spike** files can be processed in *MATLAB*, *NeuroExplorer*, and *Offline Sorter*. **Spike List**, **Spike Count**, **Burst List**, **Beat List**, and **Advanced Metrics** files can be processed in *Excel*.

6.2.1. Recorded File Names

AxIS Navigator automatically names files based on the continuous data stream used to generate the output. *AxIS Navigator* will append a 3-digit number to the end of every file name (manually or automatically named) to prevent identical file name conflicts. The number starts at 000 and increases by 1 for each file recorded to the same location with the same file name. As an example: an **Advanced**

Metrics file generated from a .raw file named "Compound_A.raw" will be named "Compound_A(000).csv".

Experiment properties such as date, duration, or stream source can be auto-generated in the file name using macros. A file name may be a mixture of macros and user entered text. For example, the file described above could be automatically named "Compound A_Statistics Compiler(000)" by entering macros [SourceFile]_[StreamName] in the file name field.

Available macros are listed in the table below:

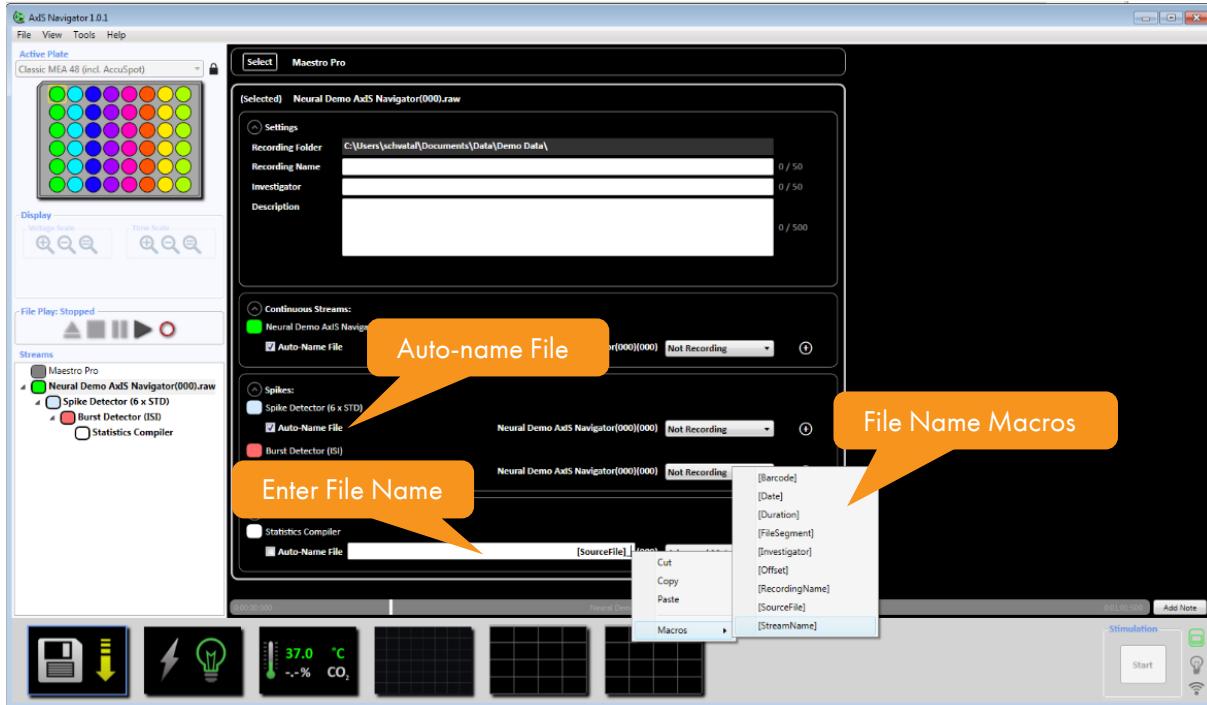
Macro	Description	Example
File path (example, C:\)	Places the output files in the specified folder instead of the source file folder. Available for batch processing only.	Places file in the designated folder
[Barcode]	The barcode of the plate used for the recording.	1166-3
[Date]	Date the output file is created. (yyyymmdd)	20161210
[Duration]	Length of the output file.	1m0s
[FileSegment]	Portion of the file was analyzed.	Start of File
[Investigator]	Investigator as entered in the Experiment Setup Properties module.	SC
[Offset]	Time from the start of the file processing began.	10s
[RecordingName]	Recording Name as entered in the Experiment Setup Properties module.	Experiment1
[SourceFile]	Original file name of the source .raw file.	Compound_A
[StreamName]	Name of the data processor generating the output file.	Statistics Compiler

To manually name a file:

1. Deselect the **Auto-Name File** checkbox in the **Experiment Setup Properties** module.

To add a macro to the file name:

1. Type the macro command from the above table into the **File Name** field.
– Or –
1. Right-click the **File Name** field.
2. Select **Macros** and choose the macro from the menu.



The **File Name** field can create folders that do not currently exist. Use '\' to create new paths for output files. For example, the entry [SourceFile]\[StreamName] for the previous example will name the output file "Statistics Compiler(000).csv" and place it in a new folder called "Compound A", created in the folder containing the "Compound A.raw" file.

6.3. PREPARING FILES FOR ANALYSIS

Before beginning an analysis ensure all files are appropriately named and contain accurate plate maps, notes, and descriptions. These notes will be kept in the analysis outputs. All output files are automatically stored in the same folder as the .raw files so organize the .raw files in appropriate folders before beginning.

6.4. SELECTING THE ANALYSIS WINDOW

While performing an analysis, *Ax/S Navigator* will stream through the continuous voltage data. *Ax/S Navigator* will process the entire file or a segment of the file specified in the Stream Settings. A portion of the file can be analyzed manually by starting and stopping the data stream and recording the region of interest using the **Play**, **Pause**, **Stop**, and **Record** buttons in the **Display Controls** (See Section 2.4).

To set the analysis window automatically:

1. Click **Stop** if *Ax/S Navigator* is not already in a stopped state.
2. Right-click the file in the **Streams** window.
3. Select **Settings**.

4. Set the analysis window using the **Segment Type** dialog.

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 Offset and continues for the Duration .
End of File	Analysis window begins at the end of the file minus the Duration and continues until the end.

5. Click **OK**.

Check the **Loop Playback** box to automatically replay the file from the beginning when the end has been reached. This is useful when examining short files, but is not recommended while recording analysis files.

6.4.1. Re-recording Segments of .raw Files

AxIS Navigator provides a single measurement for each metric that is a mean over the entire analysis duration. To split a raw file into segments to obtain a time course, for example, or to archive only a portion of the file, it is possible to record shorter .raw files from a .raw file.

1. Click **File → Open Recording...**
2. Select the file for analysis and click **Open**.
3. Set the analysis window. See Section 6.4.
4. Click the **Experiment Setup Properties** module.
5. Select **AxIS Raw** from the **Maestro Pro** or **Maestro Edge** drop-down.
6. Optional: Uncheck **Auto Name File** to manually enter a file name. By default, the name will be **[SourceFile]**. See Section 6.2.1 for more information on naming output files.
7. Click **Record**.

To generate a time course from a single .raw file:

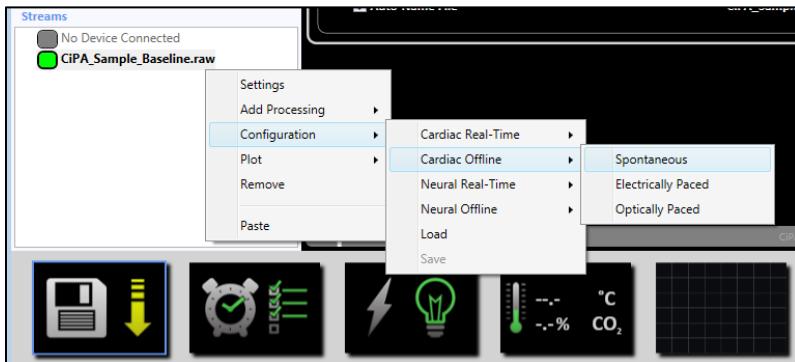
Select an analysis window and run an analysis according to Section 6.5. Repeat for each analysis window.

– Or –

Generate new .raw files for each analysis window and run a batch process according to Section 6.6.

6.5. DATA ANALYSIS TUTORIAL

1. Click **File** → **Open Recording...**
2. Select the file for analysis and click **Open**.
3. Prepare the file for analysis. See Section 6.3.
4. Set the analysis window. See Section 6.4.
5. Right-click on the file in the **Streams** window and select **Configuration** → **Cardiac Offline** or **Neural Offline** → **Spontaneous, Electrically Evoked/Paced, Optically Evoked/Paced, or LEAP** (cardiac only) to apply a configuration. See Section 6.1 for software configurations.



6. Click the **Experiment Setup Properties** module. See Section 2.6.1 for more information on the **Experiment Setup Properties** module.
7. Verify **Advanced Metrics** is selected in the **Statistics Compiler** drop-down. Select any additional desired file outputs. It is not necessary to record the .raw data again. See Section 6.2 for more information on output file types.
8. Optional: Uncheck **Auto Name File** beside the selected file outputs to manually enter a file name. By default, the name will be [SourceFile]. Auto-naming macros are available to add descriptive information to file names. See Section 6.2.1 for more information on naming output files.
9. Click **Play** to view the data.
10. Assess the analysis settings. See Chapter 7 for cardiac activity, Chapter 8 for neural activity.
11. Right-click on the file in the **Streams** window and select **Settings**.
12. Click **Accelerate Playback** to enable. **Accelerate Playback** plays the file and performs analysis faster than real time.
13. Click **OK**.
14. Click **Record** to run the analysis.

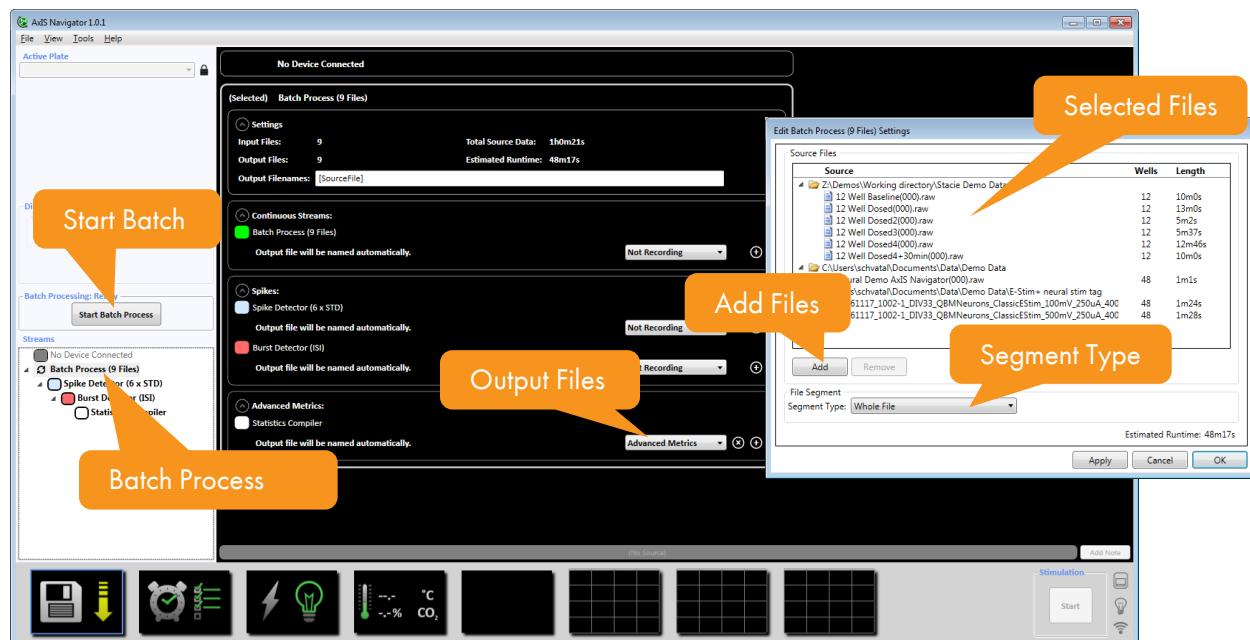
Note: If setting the analysis window manually, start and stop the recording at the desired times.

6.6. ANALYSIS OF MULTIPLE FILES (BATCH PROCESSING)

It is possible to analyze multiple files sequentially using identical analysis settings with batch processing. In this mode analysis can be done without having to queue each file individually and wait for the processing to finish.

A batch process appears as a stream in the **Streams** window. The **Batch Process** stream acts like a traditional stream; analysis configurations may be applied to the batch process in the same manner as a single file, and all files in the batch process will be processed according to the stream configuration.

When a batch process is the active stream, the **Play** button becomes a **Start Batch Process** button.



To begin a batch process:

1. Click **File** → **New Batch Process...**
2. Click **Add** in the **Edit Batch Process Settings** dialog.
*Note: Use **Remove** to remove any files from the **Source File** list.*
3. Select the desired .raw files and click **Open**.
4. Select the analysis window using the **Segment Type** drop-down menu. See Section 6.4 for setting an analysis window with the **Segment Type** dialog.
Note: An orange file indicates the segment to be analyzed is longer than the total length of the file. Analysis will stop when the file end is reached. If the file is so short that nothing will be analyzed, the file entry turns red.
5. Click **OK**.
6. Right-click on the batch process in the **Streams** window and select **Configuration** → **Cardiac Offline** or **Neural Offline** → **Spontaneous, Electrically Evoked/Paced, Optically**

Evoked/Paced, or LEAP (cardiac only) to apply a configuration. See Section 6.1 for more information about analysis configurations.

7. Click the **Experiment Setup Properties** module. See Section 2.6.1 for more information on the **Experiment Setup Properties** module.
8. Verify **Advanced Metrics** is selected in the **Statistics Compiler** drop-down menu. Select any additional desired file outputs. It is not necessary to record the .raw data again. See Section 6.2 for more information on output file types.
Note: File names are automatically generated as [SourceFile] appended with a number starting with 000, and output files are saved to the same directory as the source .raw file each was generated from.
9. Click **Start Batch Process**.

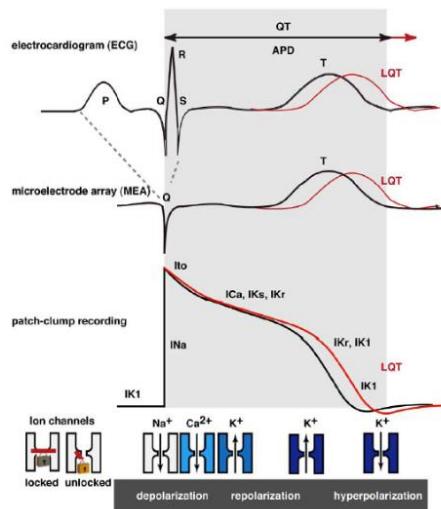
A white status bar will appear at the top of the active window while a batch process is running. The status bar will list the progress of the current file (**File**) and the entire batch process (**Overall**).



CHAPTER 7. CARDIAC DATA ANALYSIS

7.1. CARDIAC ACTIVITY INTRODUCTION

A cardiac action potential, or “beat”, consists of three phases, the depolarization, plateau, and repolarization. These phases are governed by the balance of ionic currents during each phase. Measured extracellularly on an MEA, these beats exhibit features similar to those present in an electrocardiogram (ECG).



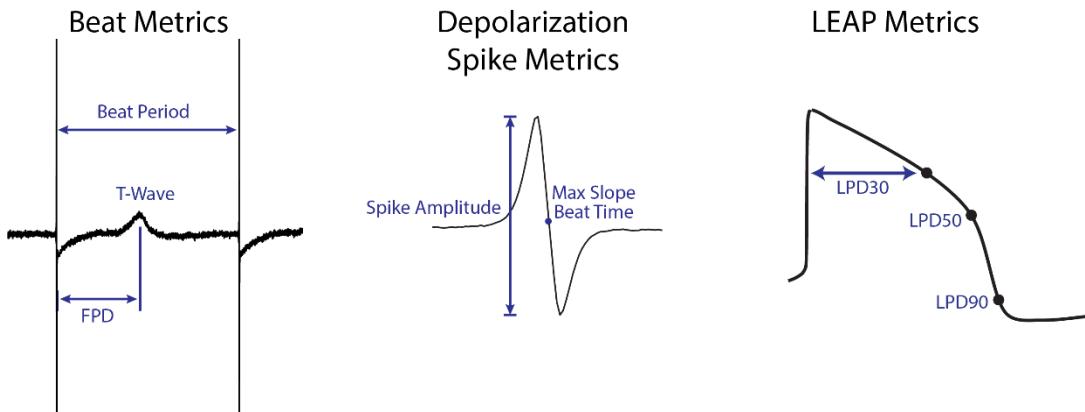
Asai et al., 2010. Current Stem Cell Research & Therapy.

The initiation of a beat starts with the depolarization phase which corresponds to the QRS complex in the ECG and the depolarization spike in the MEA field potential recording. The T-wave in the ECG and the corresponding peak in the MEA field potential represent repolarization, when the voltage returns to baseline. For this document, the repolarization peak in an MEA will be referred to as the T-wave. A peak corresponding to the P-wave is not present in an MEA field potential recording. In an ECG, the P-wave represents the depolarization of the atria while the QRS complex and T-wave represent the depolarization and repolarization of the ventricles. A cardiac culture typically lacks the structural compartmentalization of an intact heart and only has a single depolarization and repolarization phase.

The LEAP assay uses proprietary techniques to enhance the coupling between the cells and electrodes, enabling the detection of a LEAP signal which closely approximates the shape of a transmembrane action potential from a patch-clamp recording. See Chapter 5 for more information about LEAP.

Under basal conditions a typical cardiac culture should beat rhythmically, with each cardiac waveform identical to the last. From the cardiac beat waveforms numerous metrics can be evaluated to assess beat rhythmicity and waveform stability. The time between depolarization spikes constitutes the beat period and can be used to assess the beat rate and rhythmicity.

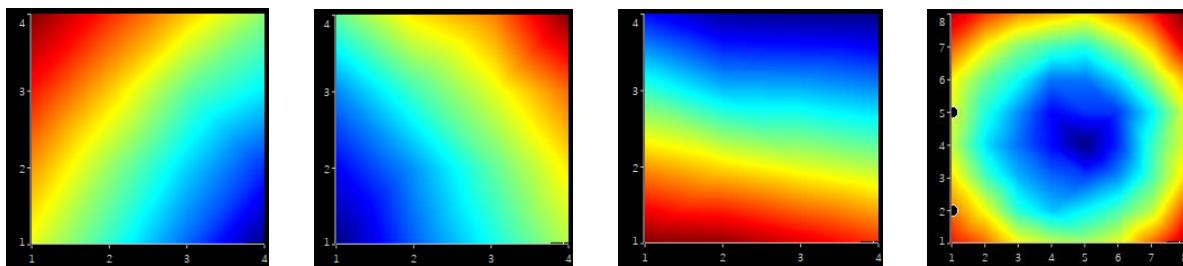
The time between the depolarization and repolarization is known as the field potential duration (FPD) for field potential signals and LEAP duration (LPD) for LEAP signals. These measures correspond to the QT interval in an ECG. Disrupting the balance of ion channels governing the plateau and repolarization phases can alter the FPD/LPD. Prolongation in the FPD/LPD is assessed as a potential indicator of pro-arrhythmia.



The two-dimensional structure of the MEA allows cardiac signal propagation to be measured and quantified by comparing the depolarization timing between electrodes. In culture, cardiomyocytes form an electrically coupled syncytium of cells that beat synchronously. When a cardiomyocyte in that syncytium depolarizes, it causes neighboring cardiomyocytes to depolarize, cascading throughout the culture. *In vitro* cardiac beat conduction is analyzed by tracking the beat time across the array of electrodes and calculating the delay from where the beat originated.

AxIS Navigator will classify well beats by common starting and ending locations. In 1- and 12-well plates (64 electrodes per well), a loosely defined nearest neighbors grid of electrodes is considered a common starting point; the most frequent starting electrode and any neighboring electrodes (vertically, horizontally, and diagonally) are considered a common starting location. For 48-well plates, a stricter nearest neighbors approach is used to define a common starting point; the most common starting electrode and neighboring electrodes vertically and horizontally are considered a common starting location. For 96-well plates, only beats beginning and ending at the exact same electrode are considered common. Each beat is assigned a Propagation Classification ID, wherein 1 represents the most common propagation pattern detected in the well, 2 is the next most common, and so on.

The figures below show four examples of propagation. The beat begins where the plot is blue (shortest delay from the beat origin) and ends where the plot is red (longest delay from the beat origin). The stability of beat propagation patterns and conduction velocity can be used to assess culture health, pacemaker stability, and evaluate compound effects. Slowing or disrupting beat propagation can lead to arrhythmia *in vivo*.



7.2. IDENTIFYING CARDIAC DEPOLARIZATION AND REPOLARIZATION

7.2.1. Cardiac Beat Detection

Cardiac beat detection is based on the depolarization spike for cardiac field potentials. The **Detection Threshold** of the **Cardiac Beat Detector** data processor, should be set lower than the depolarization spike but greater than any other waveform feature. Since *Ax/S Navigator* calculates endpoints based on well beats, the detection threshold only needs to be accurate on some electrodes, as set by **Min Active Electrodes** in the **Cardiac Beat Detector**. If *Ax/S Navigator* identifies an arrhythmia, ensure the threshold is properly set so only the depolarization spike, and every depolarization spike, crosses the threshold. The threshold can be viewed from the **Continuous Waveform Plots** module as horizontal gray lines above and below the data trace for each electrode.

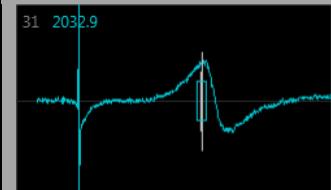
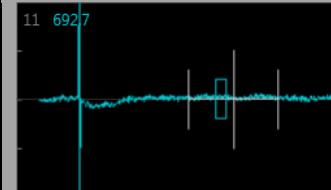


7.2.2. Field Potential T-Wave Identification

For cardiac field potential signals, the T-wave is often much less pronounced than the depolarization spike and can vary considerably in size, shape, and polarity from one electrode to the next. Use the **Cardiac Beat Plots** module to assess T-wave detection accuracy as indicated by the white whisker plot.

Characteristics of a cardiac waveform from a synchronous cardiac culture can be represented by a subset of the electrodes in each well, making it possible to eliminate electrodes containing noisy or anomalous data. This is particularly useful for FPD measurements. T-wave locations, and therefore FPD measurements, should

be similar between electrodes; however, physical culture differences across the well can cause some electrodes to display prominent T-waves and others to display none at all. For electrodes where the T-wave is small or absent, *AxIS Navigator* may generate FPD values showing high beat-to-beat variability (as indicated by a large **FPD Confidence Interval** displayed in the **Cardiac Beat Plots**) because of poor detection performance.

Waveform	Description	
	The T-wave has been properly and consistently detected. It shows a very small standard deviation.	Good Detection
	The trace shows no visible T-wave, therefore detection is unreliable, resulting in a large standard deviation.	No T-wave Present
	The detection algorithm is alternately picking the correct T-wave and a feature earlier in the beat. The mean reflects the average of these two humps and the standard deviation is very large.	Missed Detection

To optimize T-wave detection:

1. Adjust the **Post Spike Detection Holdoff**, **Pre Spike Detection Holdoff**, and **Max Post Search Duration** values. This sets the search range. If multiple peaks are present, *AxIS Navigator* will preferentially mark the first peak. Adjust the search range to bracket the preferred peak.
2. Adjust the **T-Wave Detection Feature** to match the majority of T-waves. **Max** for upward deflecting peaks, **Min** for downward deflecting T-waves.
3. Set the **FPD Measure Quality Control** settings to remove poorly detected electrodes. Arrhythmic wells will have low beat to beat consistency and electrode FPD consistency.
4. Optional: If there are many electrodes that identify the wrong peak but have a high beat to beat consistency, as indicated by the whisker plot, disable those electrodes.

AxIS Navigator will assess field potential duration for all electrodes in a plate by the same criteria. When analyzing plates with significantly different repolarization timing across wells, it may be necessary to perform the analysis multiple times with settings optimized for subsets of wells with similar waveforms. For data sets with highly variable or hard to analyze T-waves, use the *CiPA Analysis Tool* (Appendix C).

7.3. CARDIAC STATISTICS COMPILER ENDPOINTS

Ax/S Navigator identifies cardiac beats with the **Cardiac Beat Detector** data processor (See Chapter 7 and Section 2.3.7). It then calculates beat rate, waveform, and conduction metrics with the **Cardiac Statistics Compiler** module and outputs an **Advanced Metrics** file with the results (See Section 2.3.8). The **Advanced Metrics** endpoints are listed in the table below.

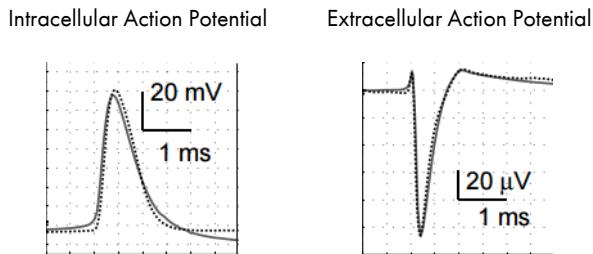
Measurement	Description
Starting Time	The start time of the analysis window. If Limit to Region of Most Stable Beat Period is enabled, the start time of the most stable beat period.
Ending Time	The end time of the analysis window. If Limit to Region of Most Stable Beat Period is enabled, the end time of the most stable beat period.
Number of Beats	Total number of well beats over the duration of the analysis.
Starting Electrode	Electrode detected first during a well beat. The starting electrode of the most common propagation pattern is listed in Well Averages.
Ending Electrode	Electrode detected last during a well beat. The ending electrode of the most common propagation pattern is listed in Well Averages.
Propagation Consistency	The number of well beats that had the most common propagation pattern divided by the total number of well beats.
Total Active Electrodes	The number of electrodes that detected beats.
Total FPD Electrodes	The number of electrodes remaining after FPD quality control parameters from the Cardiac Statistics Compiler are applied.
Beat Period	The time between successive depolarization spikes, in seconds.
Beat Period Irregularity	The coefficient of variation (standard deviation/mean) of the beat period multiplied by 100.
Spike Slope	The maximum change in voltage over time (dV/dt) of the depolarization spike, in V/s.
Spike Amplitude*	The peak to peak (positive plus negative) amplitude of the depolarization spike, in mV.
FPD*	The time from the depolarization spike to the peak of the T-wave, in ms.
FPD COV*	The coefficient of variation (standard deviation/mean) of the FPD multiplied by 100.
Conduction Velocity	Speed of depolarization spike propagation across the culture. The propagation delay of each electrode is plotted against its distance from the beat origin. A best fit line is created from these delays, and the conduction velocity is the reciprocal of its slope.
Max Delay	The time between depolarization spike detection at the starting and ending electrode.

*These metrics are available for field potential signals only (not computed for LEAP electrodes).

CHAPTER 8. NEURAL DATA ANALYSIS

8.1. NEURAL ACTIVITY INTRODUCTION

Neural cultures form intricate networks of cells that produce complex patterns of activity. A neural action potential consists of depolarization and repolarization phases governed by the balance of ionic currents during each phase. Examples of neuronal action potentials measured intracellularly and extracellularly are shown in the figure below.



Gold, et al., 2006. On the Origin of the Extracellular Action Potential Waveform: A Modeling Study. *J Neurophysiol*, 95:3113-3128.

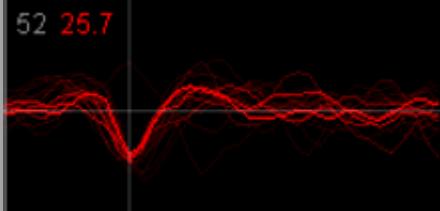
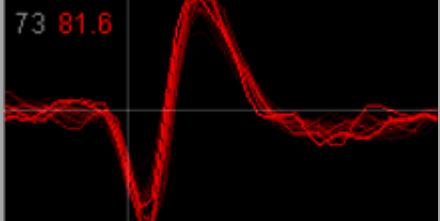
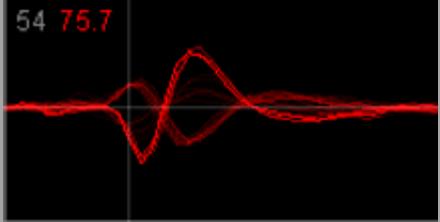
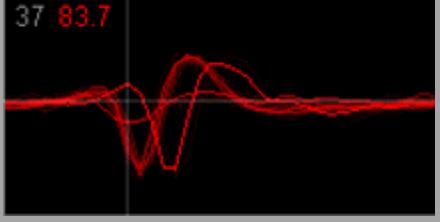
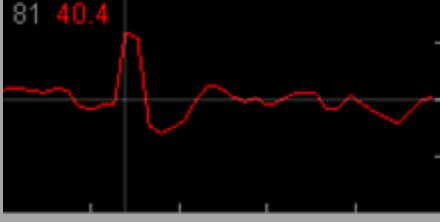
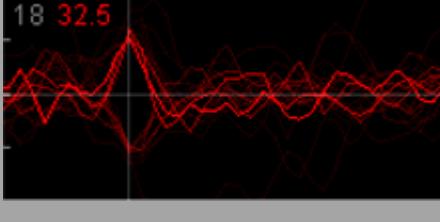
The first step to quantifying neural activity using an MEA is to identify individual neuronal action potentials or “spikes”. The spike shape depends upon the cells’ proximity to the electrode. Neuronal activity on an MEA is typically studied by quantifying the spike timing and how coordinated the spikes are across the culture.

8.2. IDENTIFYING NEURAL SPIKES

It is important to ensure the spikes detected are physiological. In general, physiological spikes will show a consistent shape that happens repeatedly, while electrical noise will not. A single electrode however will measure activity from all nearby neurons so more than one consistent spike waveform may be present. Spikes can have non-standard shapes based on a neuron’s position with respect to the recording ground. Spikes from multiple cells can sum together to create multiple peaks.

A quick check of the duration and amplitude of a spike can be helpful. Mammalian neurons produce action potential widths of approximately 1-2 ms. Any signals significantly shorter should be considered carefully. Amplitudes can vary greatly, but peak amplitudes typically range from 20 μ V and 150 μ V. Very large amplitudes should be reviewed skeptically.

The following figure provides a series of examples of physiological spikes and electrical noise. These spikes are easiest to evaluate within the **Spike Plots** module (Section 2.6.7).

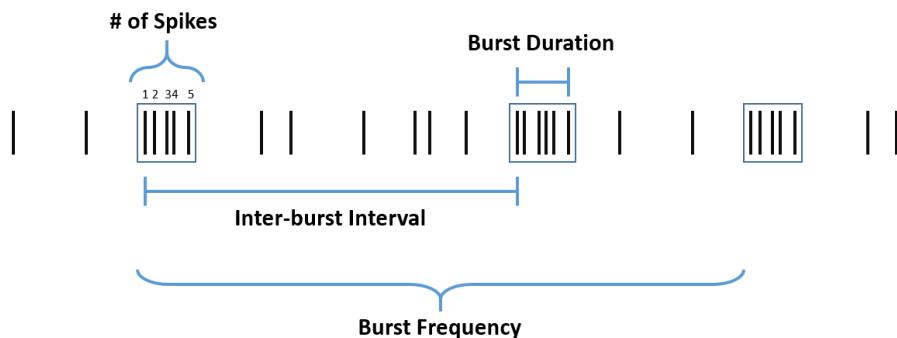
Waveform	Description	Physiological/Noise
	Consistent, repetitive monophasic waveform.	Physiological
	Consistent, repetitive biphasic waveform.	Physiological
	Multiple waveforms, but each waveform is consistent.	Physiological. Different neurons detected by the same electrode.
	Waveforms are the same shape but out of phase.	Physiological. Same neuron, detection is near threshold causing different times in the waveform to be marked as the start.
	Single occurrence.	Noise.
	The same shape in both positive and negative directions. Unstable voltage baseline before and after the spike.	Noise.

8.3. TYPES OF NEURONAL ACTIVITY

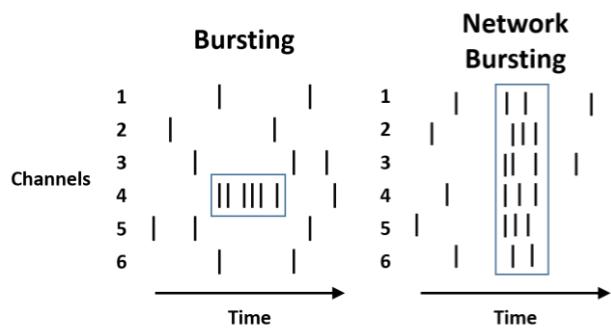
Beginning with continuous voltage data (top) from a single electrode, *AxIS Navigator* identifies neuronal action potentials, or “spikes”. Plotting just the spike time and location will generate a raster plot (below).



Neuronal activity on a single electrode may be quantified in a variety of ways. Firing may be random or rhythmic, fast or slow, occur as single spikes or clusters. A cluster of spikes is called a burst. *AxIS Navigator* refers to bursting on a single electrode as single-electrode bursting. From a burst, additional parameters may be quantified such as burst frequency and duration.



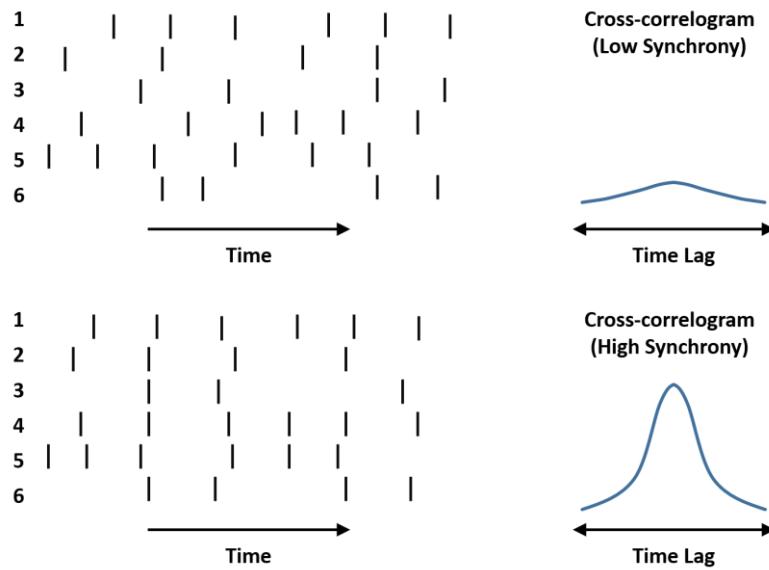
Having multiple electrodes in an array recording allows examination of network-wide coordinated activity. A network burst is a coordinated cluster of spiking across multiple electrodes. The distance between two electrodes is such that no two electrodes will detect firing from the same neuron. Coordinated activity such as a network burst therefore represents synaptic communication between neurons.



The level of coordinated or simultaneous spiking between electrodes is referred to as the culture's synchrony. Synchrony between two electrodes may be quantified using a cross-correlogram. The cross-correlogram assesses the probability of a spike occurring on electrode A at times relative to a spike on electrode B. This probability is summed across all spikes in electrode B to produce the cross-correlogram. For example, if both electrodes always fire together, the cross-correlogram would have a sharp peak at time 0.

Measuring the area under the cross-correlogram around zero is an effective way to quantify synchrony. A short synchrony window (e.g. 5 ms) quantifies synchrony on a millisecond timescale, while a long synchrony window (e.g. 50 ms) captures synchronous activity on slower timescales.

AxIS Navigator uses frequency domain methods (Halliday, Rosenber, Breeze & Conway 2006) to compute and pool the cross-correlogram across all unique pair-wise combinations of electrodes in a well. A normalized pooled cross-correlogram is generated by removing remove autocorrelations, each electrode's cross-correlation with itself. An example of a pooled cross-correlogram is given below. When spiking occurs at similar times between all electrodes, synchrony is high.



8.4. NEURAL STATISTICS COMPILER ENDPOINTS

Ax/S Navigator identifies spikes and bursting with the **Spike Detector** and **Burst Detector** data processors (See Sections 2.3.5 and 2.3.6). It then calculates spiking, bursting, and synchrony metrics with the **Neural Statistics Compiler** module and outputs an **Advanced Metrics** file with the results (See Section 2.3.9). The activity can be broadly grouped into four categories: spiking, single-electrode bursting, network bursting, and synchrony. The **Advanced Metrics** endpoints are listed in the table below.

Measurement	Description
Spiking	
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.
ISI Coefficient of Variation	The coefficient of variation (standard deviation/mean) of the inter-spike interval, the time between spikes. This is a measure of spike regularity.
Number of Active Electrodes	Number of electrodes with activity greater than the minimum spike rate set in the Neural Statistics Compiler .
Weighted Mean Firing Rate	The mean firing rate based on only electrodes with activity greater than minimum spike rate set by the Neural Statistics Compiler .
Single-Electrode Bursting	
Number of Bursts	Total number of single-electrode bursts over the duration of the analysis.
Number of Bursting Electrodes	Total number of electrodes in the well with single-electrode bursts.
Burst Duration	Average time from the first spike to last spike in a single-electrode burst.
Number of Spikes per Burst	Average number of spikes in a single-electrode burst.
Mean ISI within Burst	Average inter-spike interval, time between spikes, for spikes in a single-electrode burst. 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 burst.
Inter-Burst Interval	Average time between the start of single-electrode bursts.
Burst Frequency	Total number of single-electrode bursts divided by the duration of the analysis, in Hz.
Normalized Duration IQR	Interquartile range of single-electrode burst durations. This metric provides a measure of single-electrode burst duration regularity. If the middle 50% of single-electrode bursts are approximately the same duration, this value will be small, whereas, if the single-electrode bursts vary widely in duration, this range will be large.
IBI Coefficient of Variation	The coefficient of variation (standard deviation/mean) of the inter-burst interval, the time between single-electrode bursts. This is a measure of single-electrode burst regularity.

Burst Percentage	The number of spikes in single-electrode bursts divided by the total number of spikes, multiplied by 100.
Network Bursting	
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.
Number of Spikes per Network Burst	Average number of spikes in a network burst.
Number of Elecs Participating in Burst	Average number of electrodes with activity during a network burst.
Number of Spikes per Network Burst per Channel	Average number of spikes per burst divided by the number of electrodes participating in that burst.
Network Burst Percentage	The number of spikes in network bursts divided by the total number of spikes, multiplied by 100.
Network IBI Coefficient of Variation	The coefficient of variation (standard deviation/average) for the inter-network burst interval, the time between network bursts. This is a measure of network burst regularity.
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 approximately the same duration, this value will be small, whereas, if the network bursts vary widely in duration, this range will be large.
Synchrony	
Area Under Normalized Cross-Correlation	Area under the well-wide pooled inter-electrode cross-correlation normalized to the auto-correlations. Higher areas indicate greater synchrony.
Area Under Cross-Correlation	Area under the well-wide pooled inter-electrode cross-correlation.
Full Width at Half Height of Cross-Correlation	Distance along the x-axis (phase lag) from left half height to right half height (probability) of the cross-correlogram. Higher 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.

APPENDIX A. AXION STANDALONE TOOLS

Axion provides a number of standalone tools that enable data post-processing and visualization. Providing these tools independent of *AxIS Navigator* allows Axion to rapidly respond to customer requests and provide additional features that are not within the scope of the existing *AxIS Navigator* software. These tools use the *MATLAB Compiler Runtime* package (MCR), which is automatically downloaded during the installation process.

The Axion Standalone tools will be preinstalled on the Maestro computer and can be accessed from the icons on the desktop. The files required to re-install or upgrade the Axion Standalone tools can be downloaded from Axion's download site at <https://axionbiosystems.sharefile.com>. Access to this site requires a log in and password. This should have been provided when the Maestro was purchased. For account information, email support@axionbio.com. Log in to the download site and navigate to the Software folder to find the standalone tool installation files.

Download the executable installation file for the desired tool. Double-click this file to begin installation and follow the onscreen instructions. Most standalone tools work using the MATLAB Compiler Runtime program. The installer will detect if the correct version of MCR is present on the computer and will download and install the correct version, as needed. When the installation is complete, the standalone tool will appear in the start menu and, if selected during installation, a shortcut will appear on the desktop.

To install the tool on a computer that does not have internet access, contact support@axionbio.com.

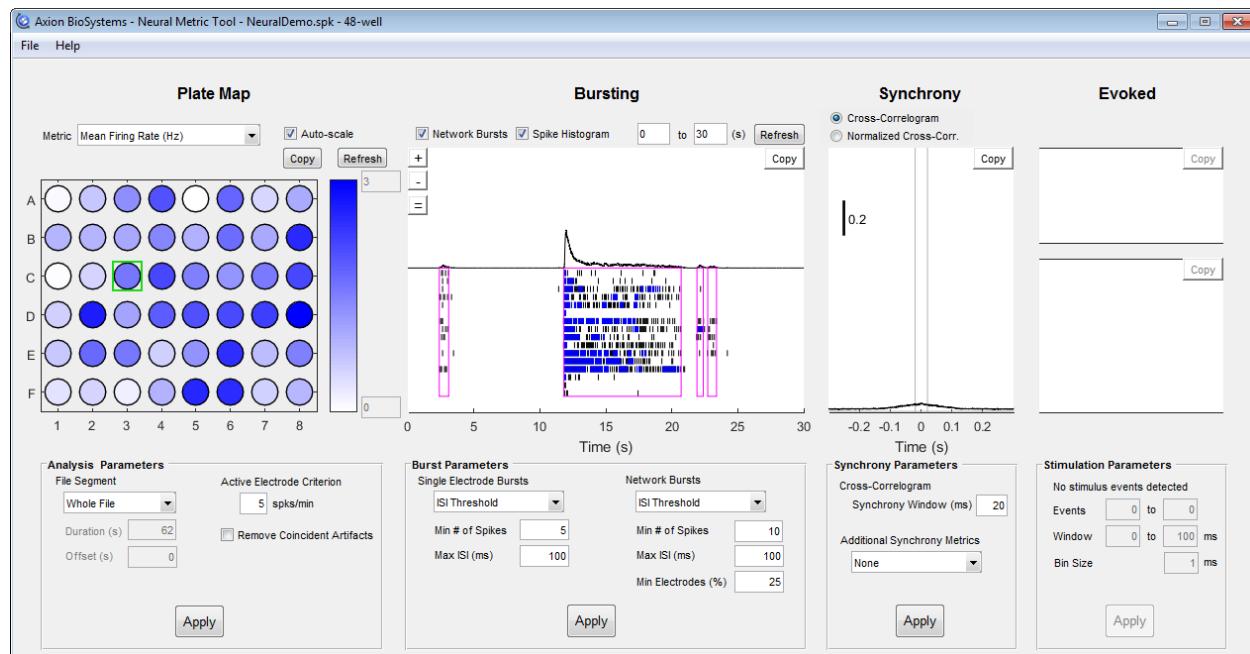
APPENDIX B. NEURAL METRIC TOOL

B.1. INTRODUCTION

The *Neural Metric Tool* calculates bursting and synchrony metrics, similar to those computed by *AxIS Navigator*, along with additional metrics. It also offers advanced algorithms for burst detection, stimulation-evoked activity analysis, and generates plate map visualizations, raster plots, and synchrony cross-correlograms not available in *AxIS Navigator*. See Chapter 8 for a detailed description of spiking, bursting, synchrony, and network bursting.

B.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 **Evoked Parameters**. The **Plate Map** section displays results for the entire plate while **Bursting**, **Synchrony**, and **Evoked** 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.



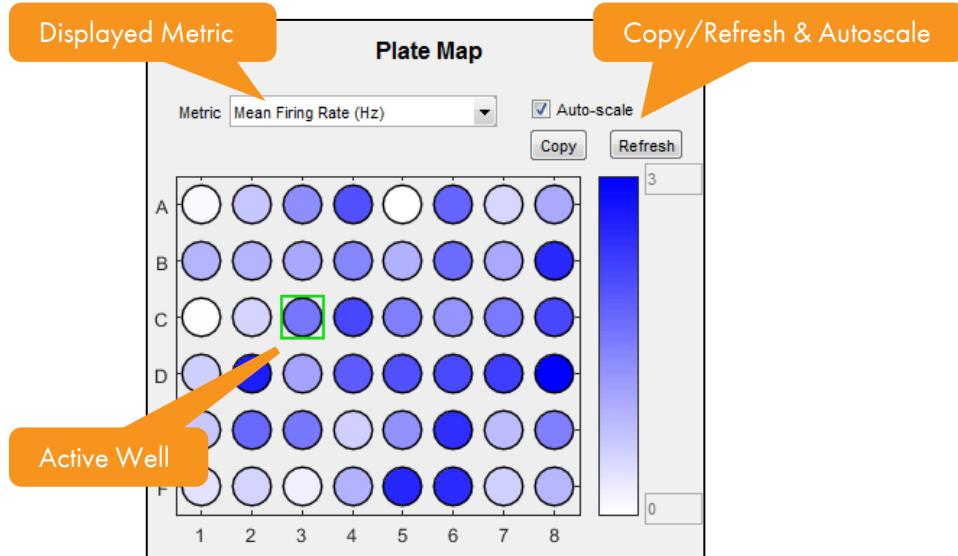
B.2.1. Plate Map Display and Analysis Parameters

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** sections is selected by clicking on the **Plate Map**. Use 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 removes coincident artifacts. 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.
Start of File	Analysis window begins at the start of the recording plus the Offset and continues for the Duration .
End of File	Analysis window begins at the end of the file minus the Duration 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** 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 B.2.5.
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 2 ms 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 → Restore Defaults**.

B.2.2. Bursting Display and Parameters

The **Bursting** section displays a raster plot of the detected spikes on each electrode within the active well. Each tick indicates the time a spike occurred and each row indicates the electrode. Blue ticks indicate the spikes are part of a single-electrode 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), 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:

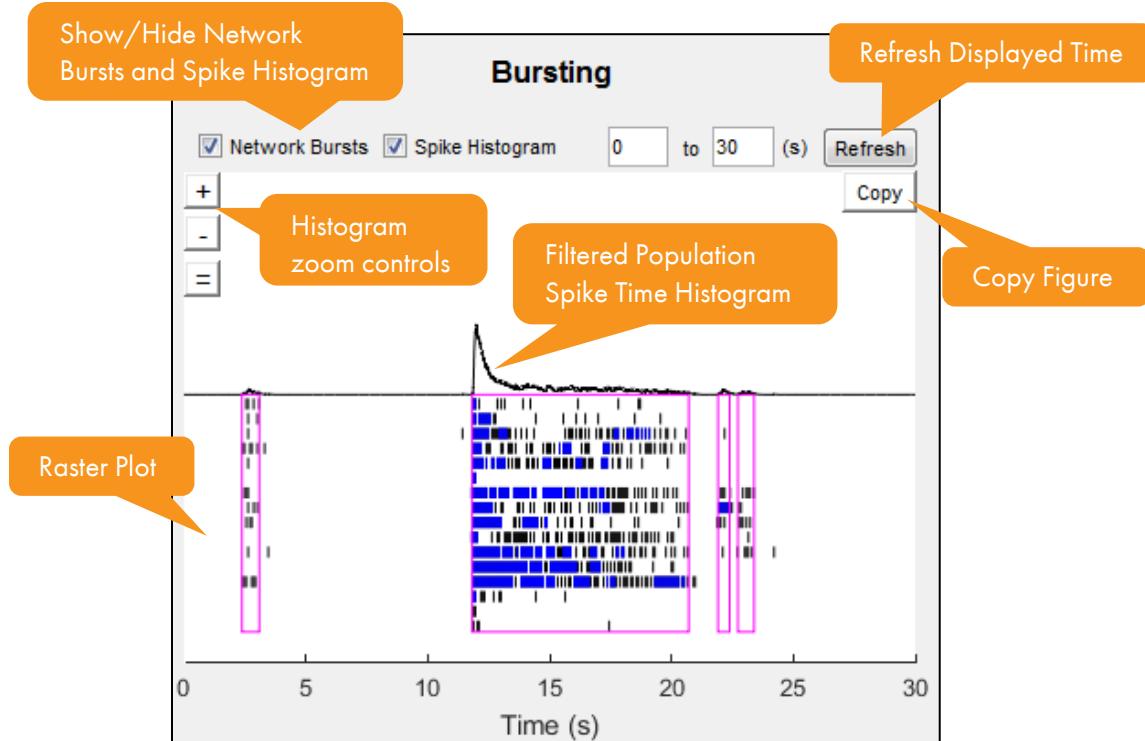
1. Select/Deselect the **Spike Histogram** checkbox. Zoom in, zoom out, and zoom reset (+, -, = respectively) are available for closer inspection of the histogram.

To change the time window displayed:

1. 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 and network bursting analysis parameters. **Single Electrode Bursts** can be identified using the same algorithms available in *Ax/S Navigator*, inter-spike interval (ISI) threshold and Poisson surprise. **Network Bursts** can be identified using ISI threshold, Adaptive, or Envelope algorithms. While *Ax/S Navigator* 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 are also computed for network bursts (See Section B.4). The network burst metrics tend to be more robust, as they consider activity over the entire network, but single electrode burst metrics will be more accurate when bursting behavior is independent across electrodes within a well. In this way, single electrode burst metrics and network burst metrics are complementary, and their use will depend on the cell type/source and characteristics of the network activity.

To set single electrode burst detection:

1. Select **ISI Threshold** or **Poisson Surprise** from the **Single Electrode Bursts** drop-down list:
 - 1.1. **ISI Threshold**: Bursting is defined as at least N spikes on an electrode, 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 it is a chance occurrence 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. The **Min Surprise** field

determines how sensitive burst detection is, such that a low **Min Surprise** will identify bursts more frequently.

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

Note: To exclude electrodes that don't meet a minimum bursting rate see Section B.2.5.

To set network burst detection:

- Select **ISI Threshold**, **Adaptive**, or **Envelope** from the **Network Bursts** drop-down list:
 - ISI Threshold**: Defines a network burst as a collection of at least N spikes across all electrodes in the well, each separated by an inter-spike interval of no more than T seconds with at least X percent of electrodes 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 electrodes (X) using the **Min # of Spikes**, **Max ISI (ms)**, and **Min Electrodes (%)** fields, respectively.
 - 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 electrodes using the **Min # of Spikes** and **Min Electrodes (%)** fields, respectively.
 - 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. Set the number of standard deviations ($\pm N$), minimum interburst interval (T), and percent of electrodes (X) with **Threshold Factor**, **Min IBI (ms)**, and **Min Electrodes (%)**, 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.
- Click **Apply** in the **Burst Parameters** section.

Note: To exclude wells from network bursting calculations see Section B.2.5.

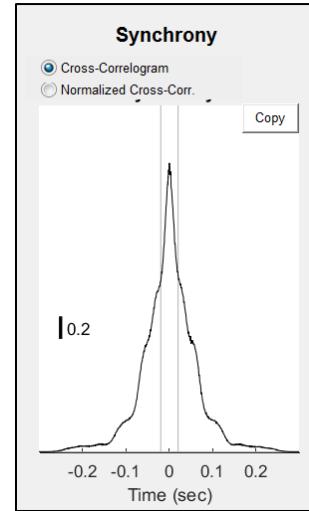
B.2.3. Synchrony Display and Parameters

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 in a well. The normalized cross-correlogram normalizes the inter-electrode cross-correlations by their auto-correlations. This normalization reduces the effects of single electrode high mean firing rate and/or high spiking regularity 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:

1. Click either the **Cross-Correlogram** or **Normalized Cross-Corr** for standard cross-correlogram or normalized cross-correlogram, 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 synchronous activity on slower timescales.



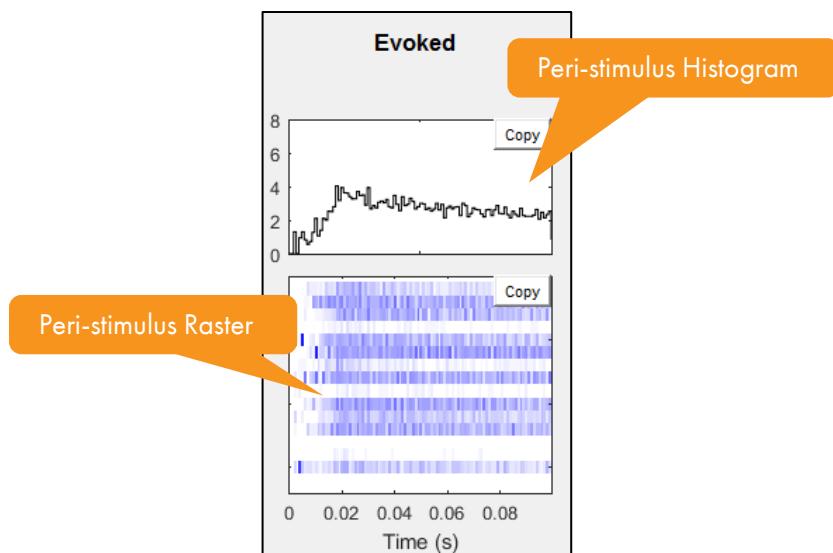
The *Neural Metric Tool* computes some synchrony metrics automatically (See Section B.4), but can also compute the Kreuz metric (derived from the Kreuz spike distance, Kreuz et al., 2013) and the Synchrony Index (based on Paiva, Park, and Principe, 2010).

To calculate the Kreuz Metric and Synchrony Index:

1. Select **Kreuz Metric**, **Synchrony Index**, or **All** from the **Additional Synchrony Metrics** dropdown.
2. Click the **Apply** button in the **Synchrony Parameters** section.

B.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). 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 electrode to the stimulus, binned by time, and averaged across the stimulus repeats. Tick darkness 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.

B.2.5. Advanced Options

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

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:

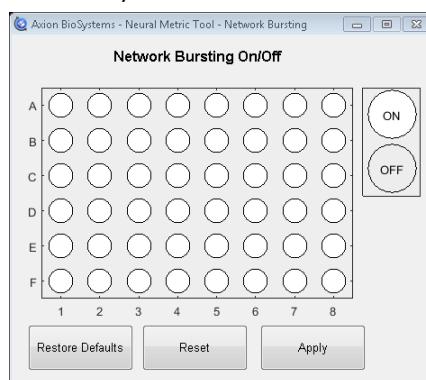
1. Click **File** → **Advanced** → **Burst Criterion**.
2. Type a minimum bursting rate (bursts/min) in the **Burst Electrode Criterion** field in the **Burst Parameters** section.
3. Click the **Apply** button in the **Burst Parameters** section.

Note: Electrodes with a burst rate below the minimum burst rate will be excluded from electrode burst metrics for both Single Electrode Measurements and Well Averages.

To exclude wells from network burst calculations:

1. 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, grey 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 their respective state when the Network Bursting window was opened.*



B.3. OPERATION

B.3.1. Generate AxIS Spike file

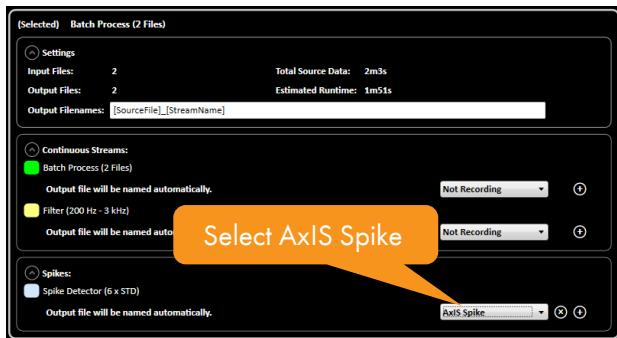
The *Neural Metric Tool* uses **AxIS Spike** (.spk) files generated by *AxIS Navigator*. While plate map information is not used in the *Neural Metric Tool*, the information is preserved in the .csv output for use in other tools. Always enter complete plate maps and experiment notes in an **AxIS Raw** file prior to beginning analysis (See Section 2.2.1). Follow these steps to create **AxIS Spike** files in *AxIS Navigator*.

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. See Section 6.4 for setting an analysis window with the **Segment Type** dialog.
 - 2.5. Click **OK**.
3. 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].
8. Click **Record** or **Start Batch Process**. The **AxIS Spike** files are saved to the **AxIS Raw** file directory.



B.3.2. 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 Axion Spike File....**
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** sections display a single well. The **Evoked** section will only display data if electrical or optical stimulation tags are present in the file (see Chapter 4).

3. Adjust the analysis settings using the **Analysis Parameters**, **Burst Parameters**, **Synchrony Parameters**, and **Evoked Parameters** sections. See Section B.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 B.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**.
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 B.4 for file output types.
 - 5.2. Type a file name and click **Save**.

B.3.3. Analyze multiple AxIS Spike files

To analyze multiple AxIS Spike files in the *Neural Metric Tool*, follow these steps to **Batch Process Multiple Files**:

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

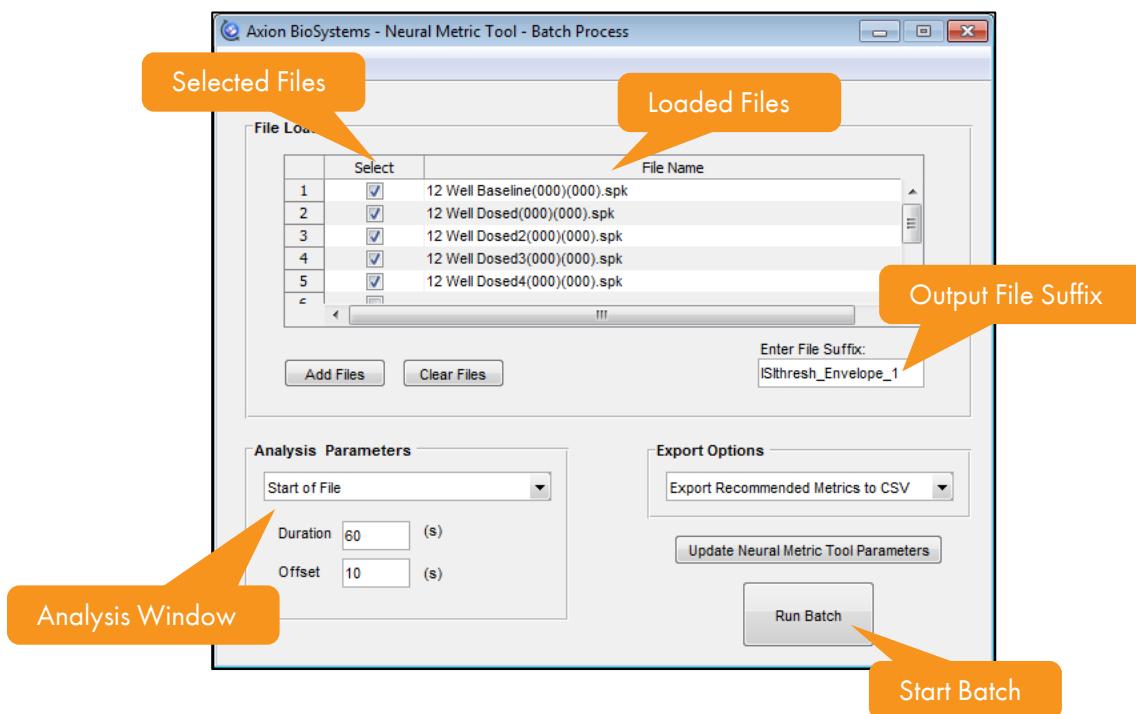
Note: Analysis settings may be altered in the *Neural Metric Tool* window at any time. Click **Update Neural Metric Tool Parameters** in the **Batch Process** window to analyze with updated settings.

2. Click **File → Batch process multiple files**.
3. Click **Add Files** in the **Batch Process** window.
4. Select the AxIS Spike files to analyze and click **Open**.
5. 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 Offset and continues for the Duration .
End of File	Analysis window begins at the end of the file minus the Duration and continues until the end.

6. 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.
7. Optional: Type a file name suffix to append to output files in the **Enter File Suffix** field.
8. Click **Run Batch**.

Note: Exported neural metric files will be saved to the path of the source spike file and automatically named [spike file name]_[neuralMetrics]_[suffix].[file extension].



B.3.4. Analyze multiple time segments

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

1. Adjust the analysis settings using the **Analysis Parameters**, **Burst Parameters**, **Synchrony Parameters**, and **Evoked Parameters** sections. See Section B.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 Process Multiple Files and Time Segments**.
3. Click **Add Files** in the **Batch Process** window.

- Select the Axis Spike files to analyze and click Open.

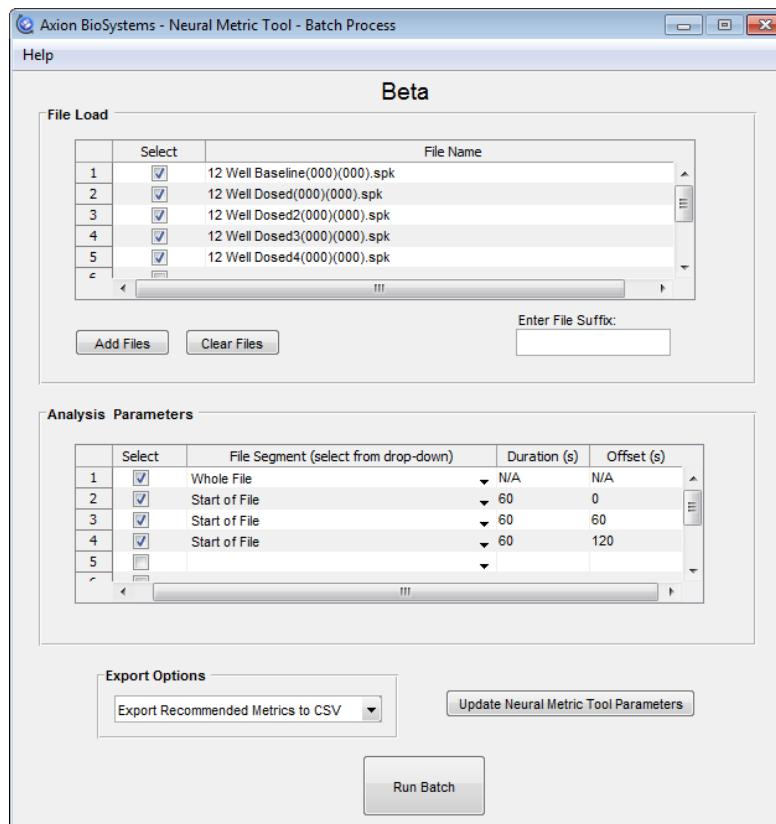
Note: To exclude files from analysis after opening, deselect it from the **File Load** table in the **Batch Process** window using the **Select** checkbox. Click **Clear Files** to remove all files from the batch process.

- 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 Duration .
End of File	Analysis window begins at the end of the file minus the Duration 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.
- Optional: Type a file name suffix to append to output files in the **Enter File Suffix** field.
- Click **Run Batch**.

Note: Exported neural metric files will be saved to the path of the source spike file and automatically named [spike file name]_[neuralMetrics]_[suffix]_[FileSegment#].[file extension].



B.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 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 *Ax/S Navigator* (See Section 8.4). 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. In general recommended metrics contain the mean values while supplemental metrics contain mean and median values. A few additional non-median metrics are included in supplemental metrics. **Export Metrics to Matlab File** exports the supplemental metrics to a .mat file for further custom analysis.

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*	Number of Active Electrodes*
Electrode Burst Metrics	Electrode Burst Metrics
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 ISI within Burst*
Inter-Burst Interval*	Median/Mean ISI within Burst
Burst Frequency*	Inter-Burst Interval (Mean and Median)*
IBI Coefficient of Variation*	Burst Frequency*
Burst Percentage*	Normalized Duration IQR*
Network Burst Metrics	IBI Coefficient of Variation*
Number of Network Bursts*	Burst Percentage*
Network Burst Frequency*	Network Burst Metrics
Network Burst Duration*	Network Bursts Ignored Flag
Number of Spikes per Network Burst*	Number of Network Bursts*
Mean ISI within Network Burst	Network Burst Frequency*
Median ISI within Network Burst	Network Burst Duration (Mean and Median)*
Median/Mean ISI within Network Burst	Number of Spikes per Network Burst (Mean and Median)*
Number of Elecs Participating in Burst*	Mean ISI within Network Burst
Number of Spikes per Network Burst per Channel*	Median ISI within Network Burst
Network Burst Percentage*	Median/Mean ISI within Network Burst
Network IBI Coefficient of Variation*	ISI CoV within Network Burst
Network Normalized Duration IQR*	Number of Elecs Participating in Burst (Mean and Median)*
Synchrony Metrics	Number of Spikes per Network Burst per Channel (Mean and Median)*
Area Under Normalized Cross-Correlation*	Network Burst Percentage*
Area Under Cross-Correlation*	Network IBI Coefficient of Variation*

Full Width at Half Height of Normalized Cross-Correlation	Network Normalized Duration IQR *
Full Width at Half Height of Cross-Correlation *	Synchrony Metrics
Synchrony Index *	Area Under Normalized Cross-Correlation *
Kreuz SPIKE Distance	Area Under Cross-Correlation *
Evoked Metrics	Full Width at Half Height of Normalized Cross-Correlation
Number of Trials	Full Width at Half Height of Cross-Correlation *
Evoked Spike Count	Synchrony Index *
Evoked Response Probability	Kreuz SPIKE Distance
Evoked First Spike Latency	Evoked Metrics
Evoked Jitter	Number of Trials
	Evoked Spike Count
	Evoked Response Probability
	Evoked First Spike Latency
	Evoked Jitter

Metrics marked with an asterisk (*) in the table above are defined in Section 8.4. Additional metrics provided by the *Neural Metric Tool* are defined below.

Metric	Definition
Median/Mean ISI within Burst	Average across electrode bursts of the median/mean inter-spike interval (ISI) within electrode bursts. Values close to 1 indicate the distribution of ISIs within bursts is symmetric.
Mean ISI within Network Burst	Average across network bursts of the mean ISIs within network bursts.
Median ISI within Network Burst	Average across network bursts of the median ISIs within network bursts.
Median/Mean ISI within Network Burst	Average across network bursts of the median/mean ISI within network bursts. Values close to 1 indicate the distribution of ISIs within bursts is symmetric.
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 (greater 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 inter-spike interval.
Number of Trials	Number of stimulation events used for analysis.
Evoked Spike Count	Average across trials of the number of spikes detected across all electrodes in the well during the time window specified by the Stimulation Parameters 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 Stimulation Parameters section. Computed as the number of stimulation events that evoked a response divided by the total number of stimulation events. A measure of the response 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.
Network ISI Coefficient of Variation	Coefficient of variation (standard deviation/mean) of the inter-spike interval for all spikes on all electrodes in a well. A measure of spike regularity. This metric captures the distribution of spiking such that 0 indicates spikes perfectly distributed and > 1 indicates network bursting; as network bursting becomes clearly distinguished from quiescence across the network, the standard deviation of the ISI grows because spikes inside a burst have low ISI but there are large ISIs between the last spike in one burst and the first spike in the next burst.

Network Bursts Ignored Flag	A flag to indicate whether network bursts are ignored in this well. Network bursts ignored = 1, network bursts included = 0.
ISI CoV within Network Burst	Average across network bursts of the ISI CoV (standard deviation/mean of the inter-spike interval) within network bursts.

B.4.1. 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 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 represents the average number of spikes on the active electrode 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*.

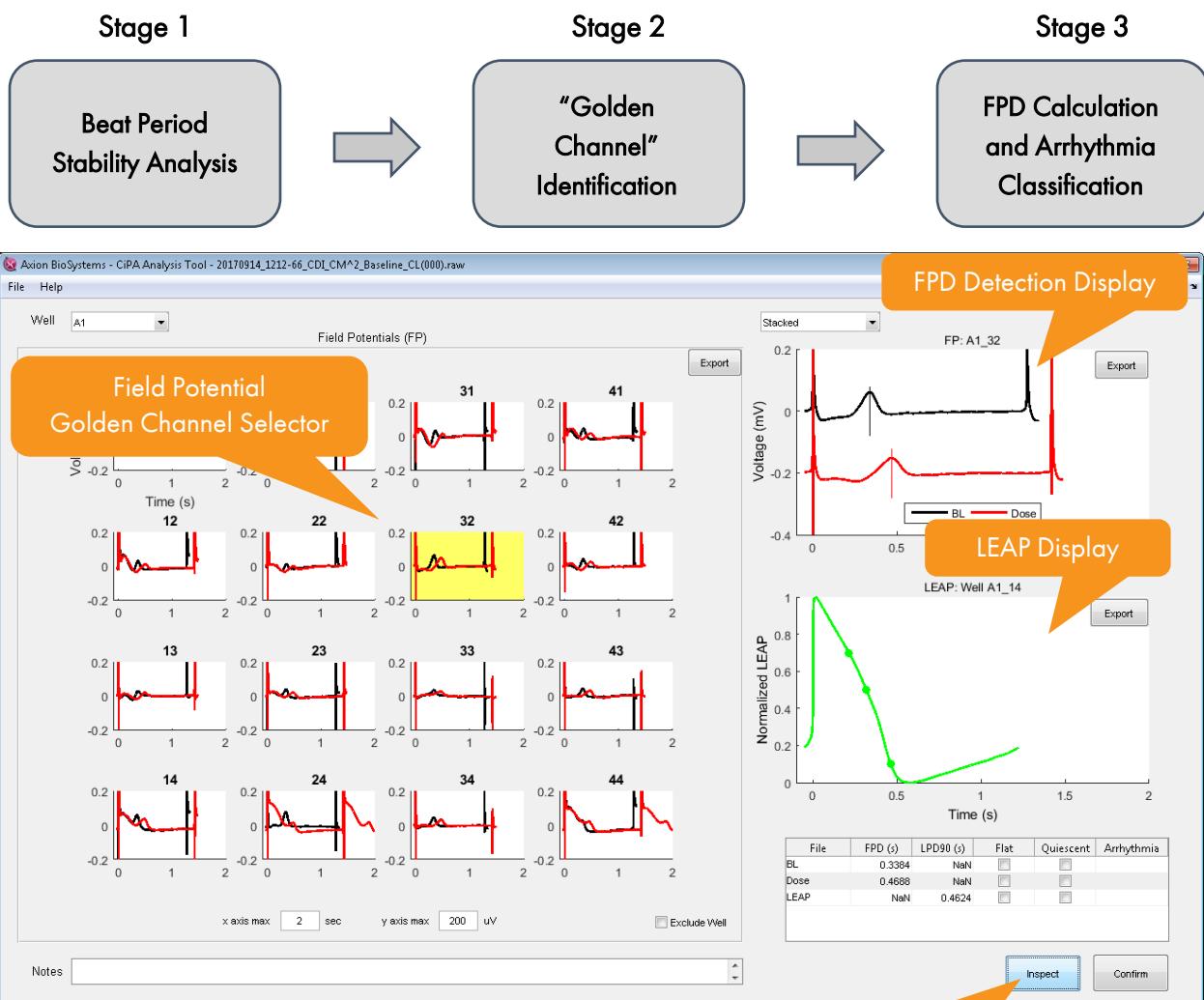
Well Averages - PSTH	A1	A2	A3	A4	A5	A6	A7	A8	B1	B2	B3	B4	B5	B6
Treatment ID	DMSO	Picrotoxin	Picrotoxin	Picrotoxin	Picrotoxin	Picrotoxin	DMSO	Picrotoxin	DMSO	Picrotoxin	DMSO	Picrotoxin	Picrotoxin	Picrotoxin
	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0.033333	0.266667	0	0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

APPENDIX C. CiPA ANALYSIS TOOL

C.1. INTRODUCTION

The *CiPA Analysis Tool* is a comprehensive cardiac analysis software designed for any cardiac application requiring precise assessment of field potential duration (FPD) and arrhythmia. The *CiPA Analysis Tool* now also uses LEAP signals, when available, for automated arrhythmia detection and classification.

The *CiPA Analysis Tool* operates according to the workflow described below. *AxIS Navigator* identifies all beats and a stable period of beating for each well in Stage 1. In Stage 2, a “Golden Channel” for field potential signals is identified, which is an electrode that has a trackable repolarization feature across all conditions. When LEAP signals are available, the best LEAP in each well is automatically selected and used to compute LEAP metrics and provide advanced arrhythmia inspection. Finally, FPD is calculated and arrhythmic events are identified and classified. The user reviews the results of the automated algorithm and has the option to manually correct the repolarization timing measurements.



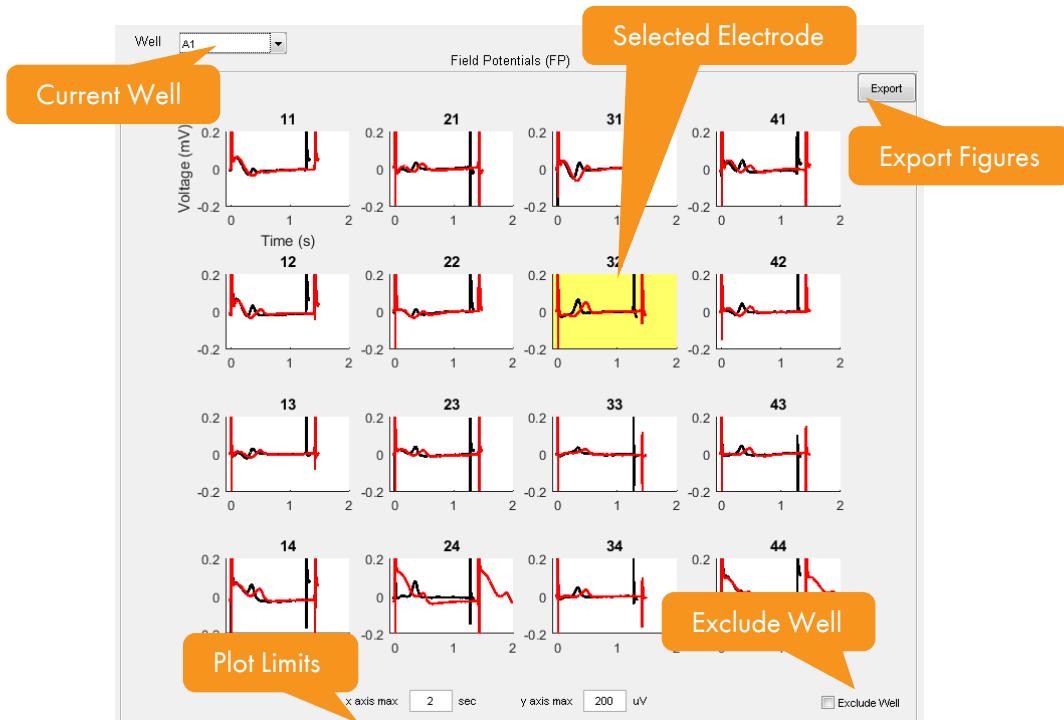
Access Arrhythmia Inspector

C.2. CiPA ANALYSIS TOOL OVERVIEW

The CiPA Analysis Tool has three main sections: the **Golden Channel Selector**, **FPD Detection Display**, and the **Arrhythmia Inspector**. The **Golden Channel Selector** is on the left side of the main screen, and the **FPD Detection Display** is on the right. The **Arrhythmia Inspector** is accessible through the Inspect button at the bottom. When a LEAP file is present, the **LEAP Display** appears below the FPD Detection Display. The LEAP Display shows the best LEAP signal for each file in that well.

C.2.1. Golden Channel Selector

The **Golden Channel Selector** displays an overlay of the field potentials for all files and is used to select the electrode for FPD analysis. The waveforms from the selected electrode are displayed in **FPD Detection** window. A “Golden Channel” is an electrode with a clear, consistent beat waveform. The T-wave should be identifiable across files and its timing representative of the electrodes in the well. Note that LEAP signal waveforms are not displayed in the Golden Channel Selector Window as the best LEAP signal is automatically detected for each well and file.



The beat waveforms from each file are displayed on a single plot for each electrode in the left panel. The waveform is an average of 5 beats from the stable region identified by *AxIS Navigator*. The tool selects a “Golden Channel” as the electrode with the largest T-wave in the baseline file and highlights the electrode in yellow.

To select a different “Golden Channel” electrode, click on the plot of another electrode. Use the **x axis max** and **y axis max** fields to adjust the x- and y-axes for all waveforms plots.

Click **Export** to generate two additional plots for the selected electrode. The first is a larger, interactive copy of the plot shown in the tool display. The second is an interactive plot showing the individual beats used to compute the displayed average beat for all conditions. These plots aid in selection of the "Golden Channel".

Use the **Exclude Well** checkbox to exclude a well from analysis. The endpoints from this well will be excluded from output figures and .csv reports (Section C.4).

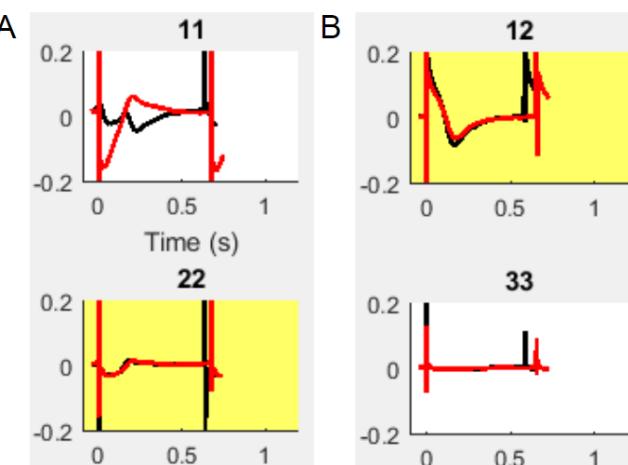
Select a "Golden Channel" according to the following criteria:

1. The T-wave is present on each file.
2. The T-wave time is comparable to other electrodes.
3. A T-wave feature is similar between files.

Note: The FPD, amplitude, or beat period may increase or decrease between files but features like the T-wave polarity should remain constant.

Example A: Electrode 11 has a larger T-wave in the baseline file (black) than electrode 22, and thus would be chosen by default. The comparison file (red) T-wave is quite different in electrode 11, while it is consistent and trackable for electrode 22. For this reason, electrode 22 should be chosen as the "Golden Channel".

Example B: Electrode 12 with a negative T-wave provides the most reliable feature tracking between files, while electrode 33 shows a small difficult to detect T-wave. In this case, electrode 12 should be chosen as the "Golden Channel".



If the average beat waveform is missing from several electrodes that detected beats in *Axis Navigator*, especially if the beats were low amplitude, there is an **Advanced** menu option to change the beat detection threshold used in the CiPA Analysis Tool.

To change the beat detection threshold:

1. Click **File → Advanced → Change Detection Threshold**
2. Type the **Beat Detection Threshold** as a multiple of the standard deviation on each electrode
3. Click **Apply**. Average beats will be calculated again using the new detection threshold.

To restore the beat detection threshold to the original settings (7 x std):

1. Click **File → Advanced → Change Detection Threshold**
2. Click **Restore Default**
3. Click **Apply**. Average beats will be calculated again using the original detection threshold.

C.2.2. FPD Detection Display

The **FPD Detection Display** is used to verify FPD measurements. It shows the beat waveform across all files for the electrode selected by the **Golden Channel Selector**. The tool will automatically compute the peak of the T-wave for each file and display it as a vertical line on the beat waveform. The FPD value is displayed in the summary table beneath the plot.

If the tool is not confident in the FPD placement, a warning triangle will appear and the vertical line marking the FPD will be dashed. If no FPD is detected for a file, a warning triangle will appear and there will be no vertical line.



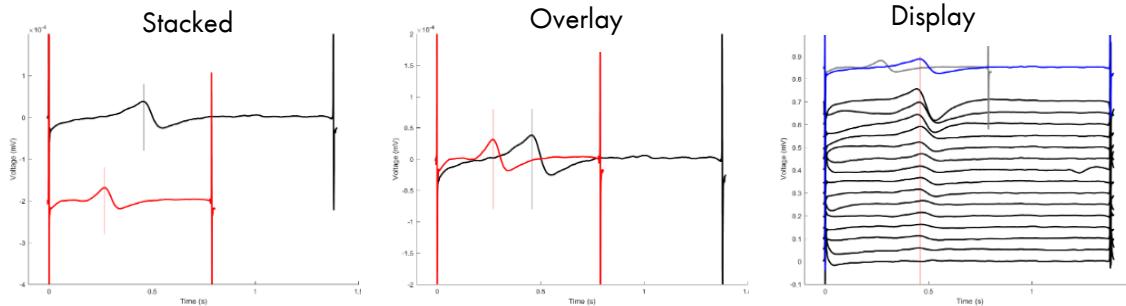
If the incorrect T-wave is identified for any file, click on the correct location on the beat waveform. The tool will automatically determine a new peak in the local region around the click.

Press **Confirm** to accept the FPD measurements and arrhythmia classifications (if applicable, see Section C.2.4) and proceed to the next well. Prior to confirming a well, if a new “Golden Channel” is selected, the tool will recalculate the FPD based on the new selected channel. After a well has been confirmed, the selected FPD values are retained for the well, regardless of which electrode is selected.

The drop-down menu below the plot specifies how data is displayed in the plot. All of the displays allow click-correct of the FPD. The plot display options are:

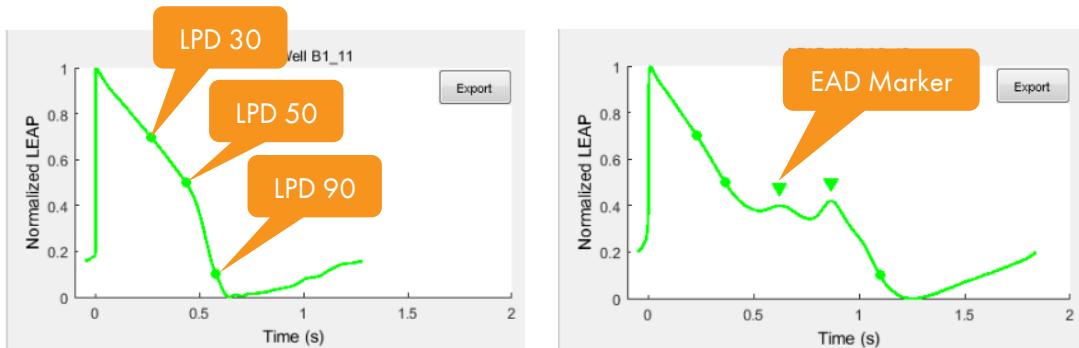
1. **Stacked** (default): The beat waveforms from each file are spaced vertically in the display. The T-wave peak in each file is marked by a vertical line.
2. **Overlay**: The beat waveforms from each file are plotted on top of one another. The T-wave peak in each file is marked by a vertical line.

3. Display name (example: Baseline, Dosed, etc.): The beat waveforms for a single file from all electrodes spaced vertically. The selected electrode in the **Golden Channel Selector** is displayed in blue at the top. Behind the blue trace, the selected electrode from the other files are displayed in gray. The “Golden Channel” T-wave peak is marked by a vertical line that crosses all electrodes.



C.2.3. LEAP Display

The LEAP Display shows an overlay of the LEAP waveforms for each LEAP file. The tool automatically selects the best LEAP in the well for each LEAP file. The circles along the trace indicate the LEAP Potential Duration (LPD) at 30%, 50%, and 90% of voltage repolarization, respectively. The LEAP signal also enables automated EAD detection and classification. If an EAD(s) is detected, the EAD(s) will be marked by a colored triangle above the feature. The EAD class will be indicated in the table below.

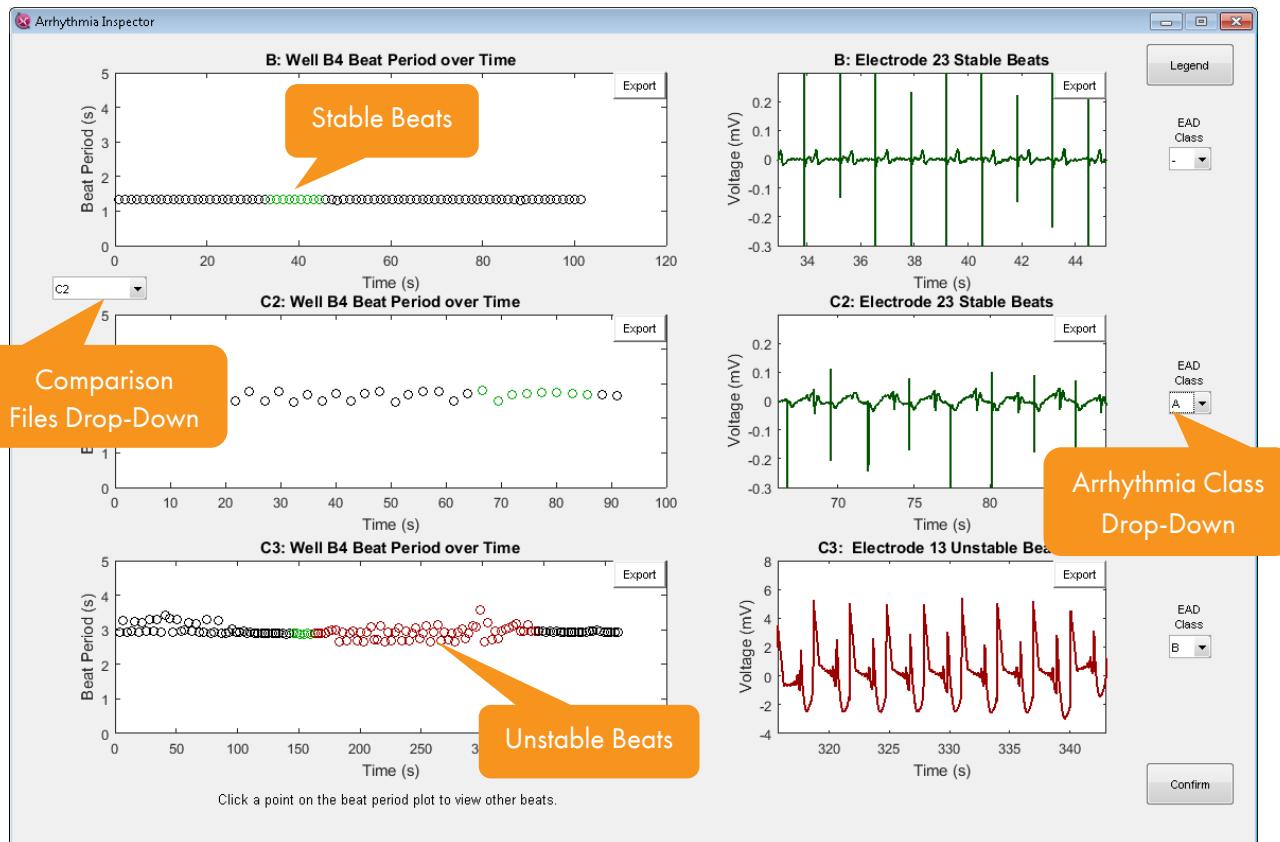


To exclude LEAP analysis and only perform field potential analysis on the remaining field potentials in a LEAP file, there is an **Advanced** menu option to change to Field Potentials Only Mode in CiPA Analysis Tool. To exclude LEAPs and analyze field potentials only, click **File → Advanced → Field Potentials Only Mode**.

C.2.4. Arrhythmia Inspector

The **Arrhythmia Inspector** displays beat period and continuous voltage plots for the Baseline, Comparison, and LEAP files (if present). It displays regions of instability and allows for the verification and classification of arrhythmic events.

Clicking the Inspect Button at the bottom of the main window will launch the **Arrhythmia Inspector**. If arrhythmic events are detected in any file, the Inspect button will be red. For field potential files, arrhythmias are detected based on an algorithm sensitive to beat period instability from beat to beat. In these cases, the tool will automatically select arrhythmia type A for comparison files. For LEAP files, arrhythmias are detected based on both beat period instability and repolarization irregularities in the LEAP beat waveforms. Both stable beats and unstable beats are inspected for arrhythmias or EADs. The related metrics, such as the Percentage of beats with EADs will default to the stable beats value to represent steady-state. However, if no EADs are present in the stable beats and EADs are present in the unstable beats, unstable beat values are reported.



Note: It is possible to have unstable beating when no arrhythmia is present, for example due to a drift in beat period. Likewise it is possible to have stable beating when arrhythmic events are present, particularly if every beat contains the EAD feature. Thus, the red button should only be used as a guide and all arrhythmic events should be visually verified and classified. LEAP signals are very useful for verifying EAD presence and type.

The left plots display **Beat Period over Time** for the Baseline, Comparison, and LEAP files (if present). The beat period plots display all beats included in the .csv file from Ax/S Navigator. Unstable beats are displayed as red, while the most stable beats (the stable beating region identified by Ax/S Navigator) are displayed as green.

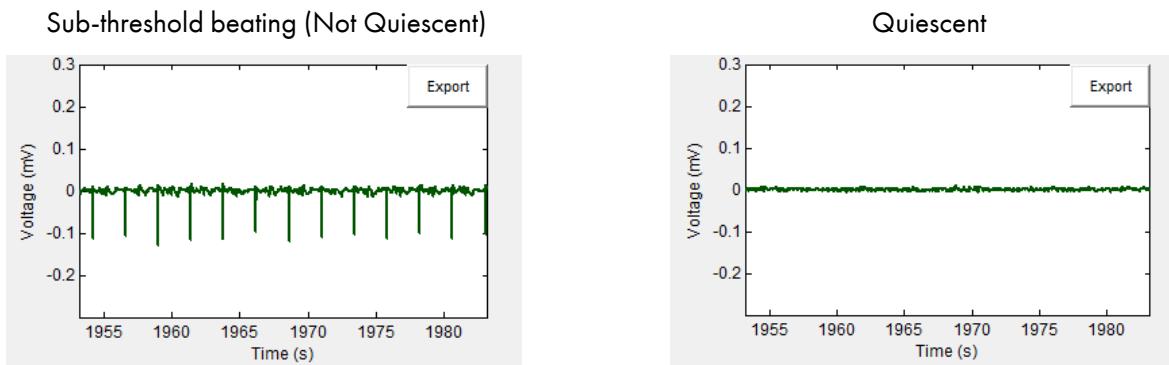
The right plots display representative **Continuous Voltage Traces** from the electrode selected by the **Golden Channel Selector** and/or the best LEAP signal. If unstable beats are present, the unstable beats will display by

default. Otherwise, the most stable beats will display. To view continuous voltage from other beats, click on a beat in the beat period plots. Clicking on a black beat will require extra time as the tool loads new data from the raw file. Use the continuous voltage data plots to check for arrhythmic events or quiescence.

The drop-down menus to the right of the Continuous Voltage Traces display the arrhythmia classification for that file. A dash indicates that no arrhythmias were detected, while the values A through D correspond to particular arrhythmia classifications, as defined by the CiPA Myocyte Committee. Click **Legend** to view an example of each arrhythmia classification. To change the arrhythmia classification for a file, simply change the drop-down selection. Once all files have been inspected and classes updated as needed, you must press **Confirm** to save these selections and return to the main window. Closing the Arrhythmia Inspector window will discard any changes.

If more than one comparison file is loaded, you can view data from the other files using the drop-down menus to the left. Note that any changes to the arrhythmia classifications for a file are stored as you toggle through the other comparison files.

If depolarization spike amplitudes were lower than the detection threshold in *AxIS Navigator*, the inspection button on the main figure will change to bold and the well will be marked **Quiescent** (i.e. not beating). View the continuous voltage plots in the **Arrhythmia Inspector** and if small amplitude beats are present in the raw data but were too small to be detected by *AxIS Navigator*, leave the **Quiescent** box on the main window unchecked. If no beats are present, check the **Quiescent** box to label this well/condition as quiescent in the output .csv file.



Note: Raw data is down-sampled by 10x in the CiPA Analysis Tool to speed computation and save time. Amplitudes displayed in the continuous voltage plots may not be accurate. The down-sampling only affects the visualization of these plots. Amplitudes reported in the output .csv files and figures are calculated by AxIS Navigator, which uses the complete dataset.

C.3. OPERATION

C.3.1. Process Recordings in AxIS Navigator

Continuous recording is required to take advantage of the full utility of the analysis tools. The *CiPA Analysis Tool* utilizes the **Advanced Metrics**.csv output from the **Cardiac Statistics Compiler** in *AxIS Navigator*. Follow these steps to process the data using *AxIS Navigator*:

1. Enter the plate map information into the baseline .raw file if it is not already present. See Section 2.2 for additional information about plate maps.
2. Click **File** → **New Batch Process...**
3. Click **Add** in the **Edit Batch Process Settings** dialog.
4. Select the desired .raw files and click **Open**.
5. Select **Whole File** in the **Segment Type** drop-down menu.
6. Click **OK**.
7. Right-click on the batch process in the **Streams** window and select **Configuration** → **Cardiac Offline** → **Spontaneous** or **LEAP**.
8. Double-click the **Cardiac Beat Detector** to open the settings and modify them if desired. Recommended settings:

Detection Threshold = 300 μ V

Min Beat Period = 250 ms

Max Beat Period = 10 s

FPD Method: Polynomial Regression

Note: It is not necessary to optimize FPD detection settings in AxIS Navigator because the CiPA Analysis Tool allows adjustment of FPD if required. However, the FPD reported by AxIS Navigator is used as an initial condition in the CiPA Analysis Tool.

Note: LEAP signals are processed using an automated algorithm that is independent of the field potential Detection Threshold.

9. Double-click the **Cardiac Statistics Compiler** to open the settings.

Recommended Settings:

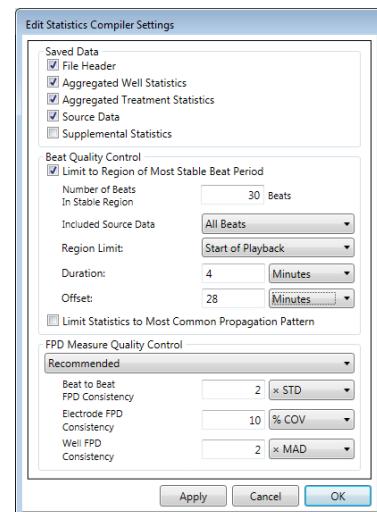
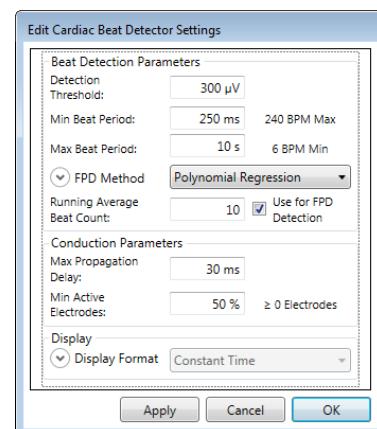
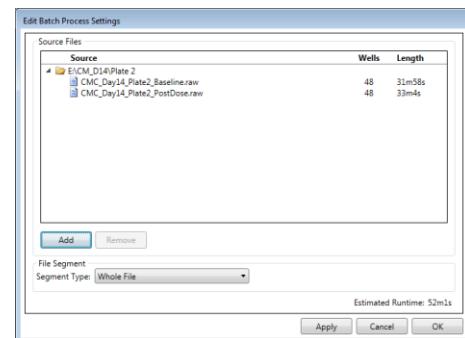
Limit to Region of Most Stable Beat Period = Enabled

Included Source Data = All Beats

Set the analysis window using the **Region Limit**, **Duration**, and **Offset** fields.

10. Confirm **Advanced Metrics** is selected from the **Statistics Compiler** drop-down menu in **Experiment Setup Properties**.

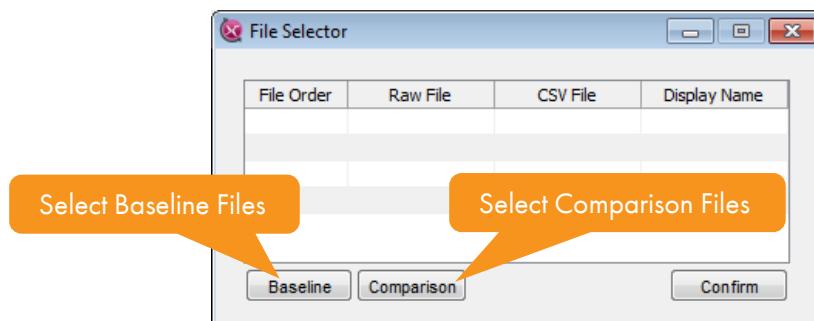
11. Click **Start Batch Process** to run the batch process and save the .csv files. The files are saved to the same directory as the .raw files.



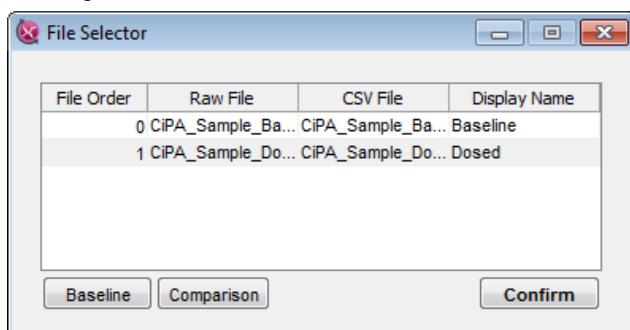
C.3.2. Analyze Data in the CiPA Analysis Tool

Loading a new experiment will take several minutes. The software computes an average beat waveform for every electrode in every file loaded. Status bars will display loading progress. After the data has been loaded and processed, new .mat files are created corresponding to each .raw file selected for analysis. Do not delete these .mat files, as they allow much quicker loading in the future.

1. Ensure the .raw files and associated .csv files are in the same directory.
2. Click **File → Load → New Experiment**. This opens a new **File Selector** window.



3. Click **Baseline** to import a baseline file that will be used as the reference point for the comparison files.
4. Navigate to the directory where the files are saved, hold **Ctrl** and select both the baseline .raw and .csv files.
5. Click **Comparison** to import up to four files to compare to the baseline file.
6. Navigate to the directory where the files are saved, hold **Ctrl** and select a .raw file and then a .csv file for each comparison file.
7. Optional: Adjust the file order. Comparison files will be loaded in alphabetical order.
 - 7.1. Type numbers into the **File Order** column. Files will be displayed in order from least to greatest.
8. Optional: Adjust the display name.
 - 8.1. The default display name for the baseline file is "B", and the comparison files are "C1", "C2", "C3", etc.
 - 8.2. Type a new name into the **Display Name** column. Names longer than 5-8 characters may not display properly on exported figures.
9. Click **Confirm** to apply the changes.



10. Optional: Load a new plate map if there is no plate map in the file or to use an alternate plate map. If there is a plate map in the baseline .csv file it will automatically be used for analysis.
 - 10.1. Click **File → Load → New Plate Map**.
 - 10.2. Navigate to and select a .platemap file or .csv file and click **Open**. For more information about generating plate maps in *AxIS Navigator* and saving .platemap files, see section 2.2.
11. Select a “Golden Channel” (highlighted in yellow) in the **Golden Channel Selector**. See Section C.2.1.
12. Verify the T-wave peak detection in the **FPD Detection Display**. Section C.2.2.
 - 12.1. Click the T-wave peak on the beat waveform to correct any misidentified T-waves.
13. Inspect for arrhythmia in the **Arrhythmia Inspector**. See Section C.2.4.
 - 13.1. Click on the **Inspect** button in the bottom right.
 - 13.2. Change arrhythmia classification as needed.
 - 13.3. Update the quiescent checkboxes on the main window as needed.
 - 13.4. Click **Confirm**.
14. Click **Confirm** to proceed to the next well.
15. Repeat steps 11-14 for each well.

Note: If a well was skipped or confirmed prematurely, select it in the Well drop-down menu above the Golden Channel Selector.
16. Click **File → Save Endpoints to .mat**
17. Type a file name for the .mat file and click **Save**.

Note: An incomplete analysis can be saved and exited at any time for future completion. The saved .mat file contains the file names, display names, Golden Channel selections, FPD selections, and arrhythmia classifications.
18. Click the **Export** button near any figure to copy the image to the clipboard and save the figure as a variety of file types.
19. Click **File → Export → Export Figures, Export Plot Source Data to CSV, Export Well Endpoints to CSV, or Export CiPA CSV**, to create file outputs. See Section 0 for more information about the *CiPA Analysis Tool* outputs.

C.3.3. Load a saved experiment

Previously loaded experiments may be reloaded for continued analysis.

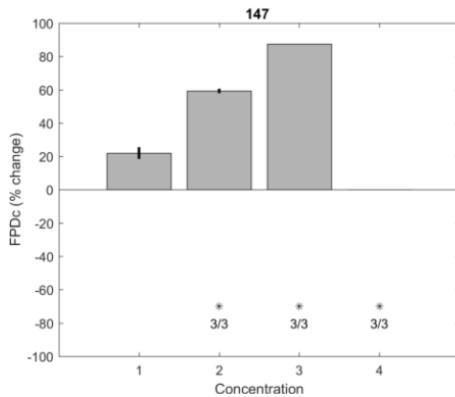
1. Click **File → Load → Saved Experiment**.

Navigate to and select the .mat file saved previously. If the name of any .raw or .csv files associated with the saved experiment have changed, the .mat file will not load properly.

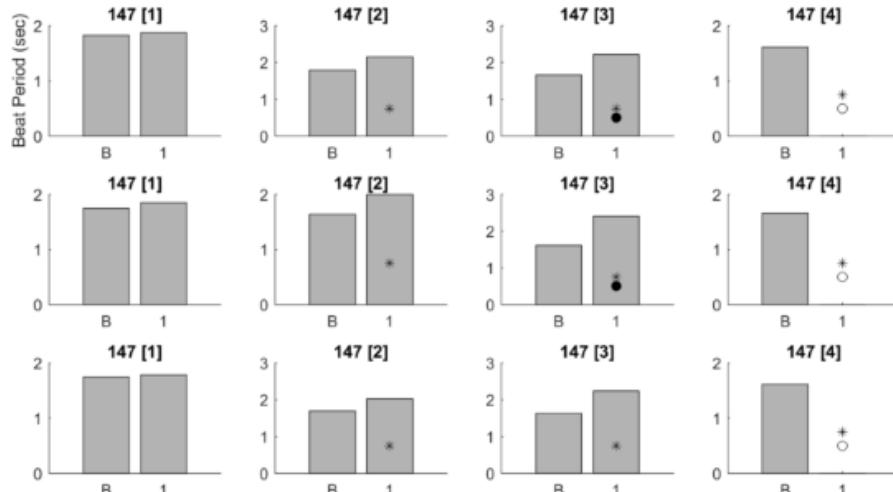
C.4. OUTPUTS

C.4.1. Export Figures

Export Figures creates bar plots for FPD, FPDc (FPD corrected for Beat Rate using Fridericia's correction), Beat Period, and Spike Amplitude. The endpoints for each well are first computed as a percentage change from the baseline file, and replicate wells are averaged to generate the dose-response. The error bars show the standard deviation across replicate wells. The number of wells containing arrhythmic events for each condition are indicated on the plots by an asterisk and a fraction indicating the number of arrhythmic wells out of the total number of wells in that condition. If there is only one comparison file, each treatment from the plate map will be plotted on a separate plot with concentration on the x-axis. If there are multiple comparison files, each group (treatment + concentration) from the plate map will be plotted on a separate plot with comparison files on the x-axis.



The figures also include summary plate map plots for each metric and Beat Period CoV. On these plots, * indicates arrhythmia in that well, ○ indicates no beats were detected in *AxIS Navigator*, and ● indicates beating was detected, but no FPD was identified (usually due to a flat T-wave or arrhythmic event).



C.4.2. Export Plot Source Data to CSV

Export Plot Source Data to CSV exports the data used to generate the replicate-averaged dose-response plots shown in the exported figures as a .csv file. For each endpoint, the average and standard deviation across replicates of the percent change from baseline are provided for each comparison file, as well as the individual replicate values that went into the average.

FPDc (% change)																
	144 [2]	144 [3]	144 [4]	145 [3]	145 [4]	147 [1]	147 [2]	147 [3]	147 [4]	148 [1]	148 [2]	148 [3]	148 [4]	DMSO		
Dosed Mean	3.705203	14.58947	59.84818	13.73555		22.07088	59.34157	87.49434		68.16613	161.1652	217.8626	267.5429	0.580577		
Dosed Std Dev	2.286244	2.633928	14.87951	0		3.515705	1.348871	0		16.50948	28.04275	47.84038	0	0.918771		
Dosed Replicates	1.575381	11.86604	48.81883	13.73555		20.61279	60.51598			58.35353	141.336	184.0344		1.641121		
	3.419262	14.77734	53.95388			26.08103	59.64037			58.91801		251.6909	267.5429	0.026299		
	6.120967	17.12444	76.77183			19.51882	57.86835	87.49434		87.22685	180.9945			0.074312		

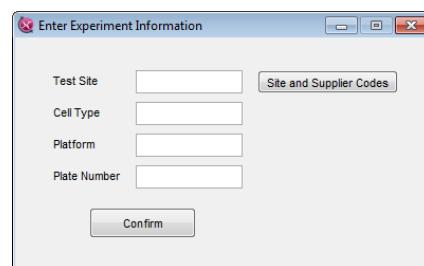
C.4.3. Export Well Endpoints to CSV

Export Well Endpoints to CSV exports the cardiac metrics for each well to a .csv file. For each file, a block of statistics are provided, organized according to well. The endpoints from all analyzed files are saved to a single .csv file.

Axis File	CIPA_Sample_Baseline.raw																
File Type	48-well																
Measurement	A1	A2	A3	A4	A5	A6	A7	A8	B1	B2	B3	B4	B5	B6	B7	B8	C1
Treatment/ID	Ranolazin	Ranolazin	Ranolazin	Ranolazin	Nifedipin	Nifedipin	Nifedipin	Nifedipin	Ranolazin	Ranolazin	Ranolazin	Ranolazin	Nifedipin	Nifedipin	Nifedipin	Nifedipin	Ranolazin
Start Time	1762.899	1700.623	1765.106	1761.737	1784.756	1805.395	1797.655	1710.888	1740.743	1695.55	1790.193	1704.294	1789.747	1695.306	1644.105	1734.862	1785.935
End Time	1812.703	1746.256	1807.869	1803.37	1826.712	1844.289	1837.545	1752.625	1785.725	1737.162	1830.501	1744.471	1829.939	1734.823	1684.034	1775.875	1829.384
Golden Channel	23	44	32	34	34	34	34	23	23	24	34	43	24	21	43	13	32
Beat Period (s)	1.71715	1.573415	1.474374	1.435473	1.39836	1.340862	1.375367	1.439059	1.49906	1.434634	1.38985	1.385281	1.385758	1.362357	1.376724	1.414068	1.498038
Beat Period CoV (%)	0.090851	0.108507	0.077176	0.201566	0.091718	0.076696	0.088444	0.085489	0.080531	0.089617	0.116763	0.122287	0.083262	0.100346	0.094175	0.077922	0.098375
FPD (ms)	552	586.4	580	549.6	436	415.2	440	552.8	475.2	520.8	512	518.4	436	406.4	432.8	448	469.6
FPDc (Fridericia ms)	460.9669	504.173	509.5954	487.2087	389.8942	376.5268	395.6518	489.6381	415.2122	461.7681	458.79	465.0351	391.0726	366.598	389.0495	399.1263	410.4124
Spike Amplitude (mV)	1.696184	1.496748	1.599644	1.701525	1.743256	1.894418	1.736429	1.880765	1.376033	2.258588	0.746716	1.231414	1.053235	2.101076	1.499118	1.855675	1.612399
Spike Amplitude CoV	0.024451	0.035747	0.041912	0.076879	0.040309	0.032606	0.030675	0.021791	0.022322	0.010612	0.063284	0.031622	0.060798	0.014731	0.04633	0.038764	0.020108
Spike Slope (V/s)	-3.80246	-3.32981	-3.53893	-3.70284	-3.84249	-4.00332	-3.88105	-4.24667	-3.10478	-4.8968	-2.00229	-2.65012	-2.58619	-4.48942	-3.33567	-4.14377	-3.70026
Spike Slope CoV	-0.0243	-0.03569	-0.03864	-0.11757	-0.04067	-0.02802	-0.02837	-0.02162	0.267089	-0.01084	-0.05055	-0.51149	-0.06033	-0.10393	-0.04361	-0.03555	-0.02043

C.4.4. Export CiPA CSV

Export CiPA CSV exports the cardiac metrics for each well to a .csv file formatted to the reporting requirements as defined by the CiPA Myocyte Committee. Enter experiment information in the dialog box that appears. Type the test site code, cell type code, platform code, and plate number into the respective fields, and click **Confirm**.



Endpoints are saved in a row-based format. The endpoints from all analyzed files are saved to a single .csv file.

Filename	Test Site	Cell Type	Platform	Plate Num	Well Row	Well Col	Concentr	Compoun	Dosing	Cc	Beat Peric	Beat Peric	FPD (ms)	Spike Am	Arrhythm	Number o	Start Tim	End Tim	Electrode	Notes
CIPA_Sam AXN	CDI	AXN		1	A	1	1 μM	Ranolazin	0	1717.15	0.090851	552	1.696184	0	30	1762.899	1812.703	23		
CIPA_Sam AXN	CDI	AXN		1	A	2	3 μM	Ranolazin	0	1573.415	0.108507	586.4	1.496748	0	30	1700.623	1746.256	44		
CIPA_Sam AXN	CDI	AXN		1	A	3	10 μM	Ranolazin	0	1474.374	0.077176	580	1.599644	0	30	1765.106	1807.869	32		
CIPA_Sam AXN	CDI	AXN		1	A	4	30 μM	Ranolazin	0	1435.473	0.201566	549.6	1.701525	0	30	1761.737	1803.37	34		
CIPA_Sam AXN	CDI	AXN		1	A	5	0.3 μM	Nifedipin	0	1398.36	0.091718	436	1.743256	0	30	1784.756	1826.712	34		
CIPA_Sam AXN	CDI	AXN		1	A	6	0.1 μM	Nifedipin	0	1340.862	0.076696	415.2	1.894418	0	30	1805.395	1844.289	34		
CIPA_Sam AXN	CDI	AXN		1	A	7	0.03 μM	Nifedipin	0	1375.367	0.088444	440	1.736429	0	30	1797.655	1837.545	23		
CIPA_Sam AXN	CDI	AXN		1	A	8	0.01 μM	Nifedipin	0	1439.059	0.085489	552.8	1.880765	0	30	1710.888	1752.625	23		
CIPA_Sam AXN	CDI	AXN		1	B	1	1 μM	Ranolazin	0	1499.06	0.080531	475.2	1.376033	0	30	1740.743	1785.725	24		
CIPA_Sam AXN	CDI	AXN		1	B	2	3 μM	Ranolazin	0	1434.634	0.089617	520.8	2.258588	0	30	1695.55	1737.162	34		
CIPA_Sam AXN	CDI	AXN		1	B	3	10 μM	Ranolazin	0	1389.85	0.116763	512	0.746716	0	30	1790.193	1830.501	43		
CIPA_Sam AXN	CDI	AXN		1	B	4	30 μM	Ranolazin	0	1385.281	0.122287	432.8	1.231414	0	30	1704.294	1744.471	24		

APPENDIX D. AXIS METRIC PLOTTING TOOL

D.1. INTRODUCTION

The *AxIS Metric Plotting Tool* allows rapid visualization of experiment results and organization of endpoints according to treatment condition. This tool imports all endpoints generated by *AxIS Navigator*, along with a plate map, and calculates % change relative to baseline for each well, averages replicates, and generates plots comparing treatment conditions or time points. The tool allows for viewing of different endpoints with a single click, facilitating data exploration.

D.2. OPERATION

D.2.1. Load files for analysis

1. Import an **Advanced Metrics** (*AxIS Navigator*), **Neural Metrics** (*Neural Metric Tool*), or **Well Endpoints** (*CiPA Analysis Tool*) .csv file that will be used as the reference point, or baseline, for the comparison files:
 - 1.1. Click **Baseline....**
 - 1.2. Select the baseline .csv file and click **Open**.

Note: If a Well Endpoints file is selected, skip to Step 3. The file already contains both baseline and comparison metrics.
2. Import files to compare to the baseline file:
 - 2.1. Click **Comparison....**
 - 2.2. Select the comparison .csv file(s) and click **Open**.

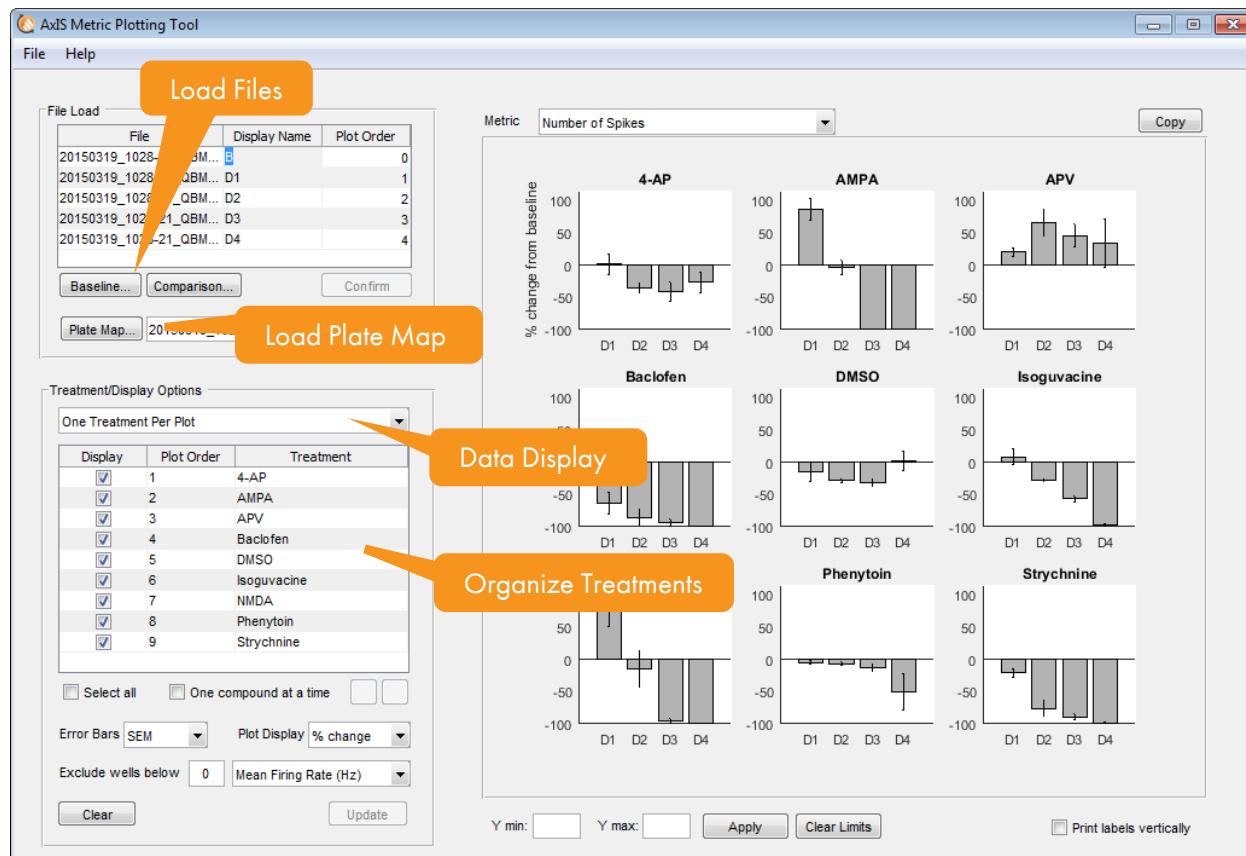
Note: Hold Ctrl to select multiple files. Comparison files will be loaded in alphabetical order.
3. Optional: Load a plate map.
 - 3.1. Click **Plate Map....**
 - 3.2. Select an **Advanced Metrics** .csv file or a .platemap file exported from *AxIS Navigator*.

Note: If no plate map is selected, the plate map from the baseline file will be used.
4. Optional: Type numbers in the **Plot Order** column of the **File Load** table to change the order of the comparison files. Baseline is 0, comparison files start at 1. To see the complete file names, hold the cursor over the table and the full file names will appear in the tooltip.
5. Optional: Type names for each file in the **Display Name** column of the **File Load** table. The name will appear on plots in place of the file name.
6. Click **Confirm** to apply the changes.

*Note: File display name and plot order may be changed at any time. Click **Confirm** to update plots after any changes.*

File Load		
File	Display Name	Plot Order
20150319_1028-21_QBM...		0
20150319_1028-21_QBM...	D1	1
20150319_1028-21_QBM...	D2	2
20150319_1028-21_QBM...	D3	3
20150319_1028-21_QBM...	D4	4

20150319_1028-21_QBM_Rat_Neurons_DIV



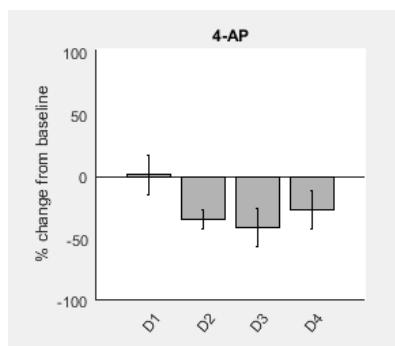
Click **Clear** to remove all files and data from the *AxIS Metric Plotting Tool*.

D.2.2. Adjust data display settings

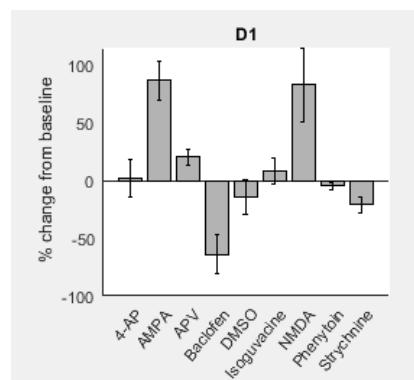
The lower left section of the *AxIS Metric Plotting Tool*/contains options to specify which data is displayed.

1. Select either **One Treatment Per Plot** or **One File Per Plot** in the **Treatment/Display Options** dropdown.
When **One Treatment Per Plot** is selected, each plot contains a different treatment, with each file on the x-axis. When **One File Per Plot** is selected, each plot contains a different file, with each treatment on the x-axis.

One Treatment Per Plot



One File Per Plot



- Click the checkboxes under the **Display** column in the **Treatment/Display Options** table to select or deselect treatments to plot.

Note: Click **Select All** to display all the treatments or **One compound at a time** to display all the concentrations of a single compound at a time. Scroll through compounds with the arrows to the right.

- Optional: Type numbers in the **Plot Order** column of the **Treatment/Display Options** table to change the order treatments are displayed starting at 1.
- Select SEM or STD from the **Error Bars** dropdown to display standard error of the mean or standard deviation error bars, respectively.
- Select % change or raw values from the **Plot Display** dropdown to display data normalized to baseline values (baseline/comparison x 100) or raw baseline and comparison values.

Treatment/Display Options		
One Treatment Per Plot		
Display	Plot Order	Treatment
<input checked="" type="checkbox"/>	2	AMPA
<input checked="" type="checkbox"/>	3	APV
<input checked="" type="checkbox"/>	4	Baclofen
<input type="checkbox"/>	5	Bicuculline
<input type="checkbox"/>	6	Carbamazepine
<input type="checkbox"/>	7	D-serine
<input checked="" type="checkbox"/>	8	DMSO
<input type="checkbox"/>	9	Ibuprofen

Select all One compound at a time

Error Bars: SEM Plot Display: % change

Exclude wells below: 0 Mean Firing Rate (Hz)

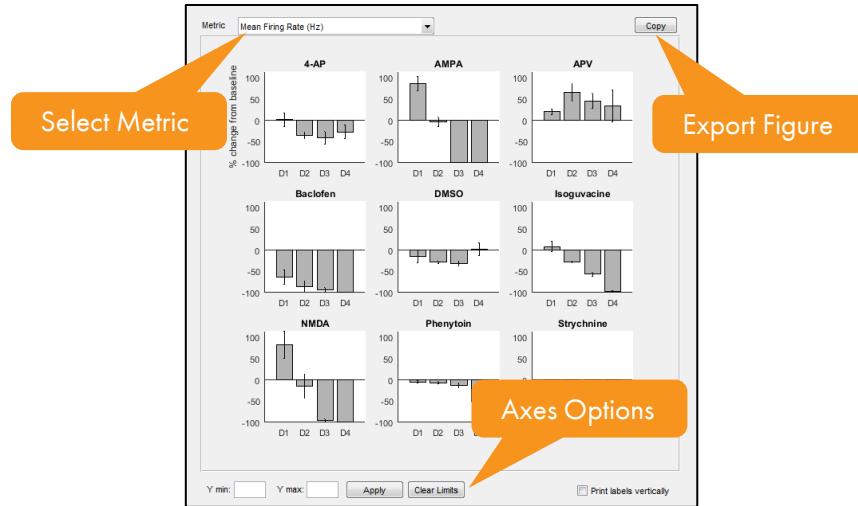
Clear Update

- If % change is selected, each well is normalized to its own baseline value, and the average % change across replicate wells is displayed.
- Optional: Exclude wells that do not meet a minimum activity criterion. Use the well exclusion dropdown to select the exclusion parameter and type the minimum value into the **Exclude wells below** field. Exclusion parameters include:
 - For neural files: Mean Firing Rate (Hz), Number of Active Electrodes, Burst Frequency – Avg (Hz), Burst Percentage - Avg, Number of Network Bursts, Network Burst Frequency (Hz), or Network Burst Percentage.
 - For cardiac files from AxIS Navigator: Number of Beats or Total Active Electrodes.
 - For cardiac files from the CiPA Analysis Tool: Spike Amplitude (mV).
- Click **Update** to apply the changes.

Note: Data display settings may be changed at any time. Click **Update** to update plots after any changes.

D.2.3. Inspect plots

- Select an endpoint to view from the **Metric** dropdown.
- Note:** When the dropdown is selected, endpoints may be scrolled through using the arrow keys.
- Optional: Adjust the y-axis using the **Y min** and **Y max** fields. Click **Apply** to rescale and **Clear Limits** to auto-scale.



D.2.4. Export Figures and Files

1. To export figures:
 - 1.1. Click **Copy** to export the currently displayed figures.
 - 1.2. Click **File → Save As...** in the figure window.
 - 1.3. Select a file type from the **Save as type** drop-down menu.
 - 1.4. Type a file name and click **Save**.

*Note: Upon clicking **Copy**, the image is also copied to the clipboard and can be pasted directly into a document or presentation.*
2. To export a .csv file do one of the following:
 - 2.1. Click **File → Export → Export Supplemental Metrics to CSV** to export all endpoints in a multi-table format. Type a file name and click **Save**.
 - 2.2. Click **File → Export → Export Recommended Metrics to CSV** to export selected endpoints in a multi-table format. Type a file name and click **Save**.
 - 2.3. Click **File → Export → Export Statistics Format** to export all endpoints as a machine-readable table. Type a file name and click **Save**.

D.3. OUTPUT

The output .csv files can be opened with any software that can read text files, such as *Microsoft Excel*. The output lists the files and settings used for analysis, followed by group means, standard deviations or standard error of the means (as selected in the software), and replicates. Results are given as %change or raw values as selected in the tool. The data is grouped by metric according to the plot layouts with each column representing a group on the plot. If **One File Per Plot** is selected, each column is a different treatment condition and each file gets a separate table. If **One Treatment Per Plot** is selected, each column is a different file and each treatment gets a separate table. The example below shows a .csv organized by **One Treatment Per Plot**.

14	Mean Firing Rate (Hz)								
15		C1	C2	C3	C4				
16	4-AP Mean	1.584345	-34.8585	-41.3568	-27.0856				
17	4-AP Std	27.99792	13.21044	26.42681	26.63974				
18	4-AP Replicates	-1.77497	-30.2407	-44.5629	-34.2179				
19		31.11036	-24.5768	-13.4733	2.394289				
20		-24.5824	-49.758	-66.0343	-49.4333				
21									
22	AMPA Mean	86.70941	-4.32466	-99.9979	-100				
23	AMPA Std	29.45765	19.95803	0.003715	0				
24	AMPA Replicates	83.80588	-10.0597	-100	-100				
25		117.5113	-20.7873	-100	-100				
26		58.81104	17.873	-99.9936	-100				
27									
28	APV Mean	20.06372	65.27455	45.82126	33.82989				
29	APV Std	12.0001	35.44409	29.52232	65.71324				
30	APV Replicates	6.59525	26.59741	25.58026	23.989				
31		23.97794	96.20376	32.18689	-26.4079				
32		29.61798	73.02247	79.69663	103.9086				
33									

Export Supplemental Metrics to CSV exports all metrics from the starting .csv files(s) to the new output format. To select only the most commonly metrics use **Export Recommended Metrics to CSV**. The metrics exported are listed in the table below:

Recommended Metrics		Cardiac (AxIS Navigator files)		Cardiac (CiPA Analysis Tool file)	
Neural (AxIS or Neural Metric Tool files)		Cardiac (AxIS Navigator files)		Cardiac (CiPA Analysis Tool file)	
Mean Firing Rate (Hz)		Beat Period Mean (s)		Beat Period (s)	
Number of Active Electrodes		FPD mean (ms)		Beat Period CoV	
Number of Bursting Electrodes		Spike Slope Mean (V/s)		FPD (ms)	
Burst Frequency – Avg (Hz)		Spike Amplitude Mean (mV)		FPDc (Fridericia, ms)	
Burst Duration – Avg (s)		Conduction Velocity Mean (mm/ms)		LPD30	
Normalized Duration IQR – Avg				LPD50	
IBI Coefficient of Variation – Avg				LPD90	
Burst Percentage – Avg				Spike Amplitude (mV)	
Network Burst Frequency (Hz)				Spike Amplitude CoV	
Network Burst Duration – Avg (sec)				Spike Slope (V/s)	
Network Burst Percentage				Spike Slope CoV	
Network IBI Coefficient of Variation				Conduction Velocity (mm/ms)	
Network Normalized Duration IQR				Percent Beats with EADs	
Area Under Normalized Cross-Correlation					

Export Statistics Format exports the data into a machine-readable format for use with statistical software programs. It generates a table with wells in rows and well information and endpoints in columns. All files are included in a single table.

Filename	Baseline	Display N	Plate Num	Well Row	Well Col	Treatment	Concentration	Number o	Mean Firing	ISI Coeffic	Number o	Burst Dur	Burst Dur	Number o	Mean ISI v	Mean ISI v	Median IS	Median IS	Inter-Bur
20150319_20150319_C1		1 A		1	Phenytoin	-7.33172	-7.33172	-7.0347	-13.8917	0	4.440868	6.006175	14.51754	15.16203	-10.0735	-10.1192	-10.5155	-34.4775	4.245423
20150319_20150319_C1		1 A		2		-71.8605	-71.8605	59.18748	-73.1171	0	9.091404	27.3574	1.604716	10.49781	5.321496	10.49338	11.64154	39.16877	161.6054
20150319_20150319_C1		1 A		3		40.16849	40.16849	-41.3501	45.53055	0	-14.0921	-5.67547	-2.8634	-2.202	-10.7834	-11.6519	-3.57663	-20.5135	-18.1709
20150319_20150319_C1		1 A		4		10.80638	10.80638	0.707687	5.423172	0	-0.62766	8.262858	3.83043	10.49245	-6.86651	-10.15	-6.22076	-35.1196	3.255559
20150319_20150319_C1		1 A		5		-42.479	-42.479	-27.9012	-51.5018	0	12.43182	57.63236	16.33838	15.6105	9.521095	78.54176	27.50323	167.3253	116.9961
20150319_20150319_C1		1 A		6	APV	6.59525	6.59525	-29.2694	-13.0323	0	19.61427	26.39418	22.65452	27.02681	6.428241	38.94856	4.137502	-8.12407	61.99387
20150319_20150319_C1		1 A		7	Baclofen	-36.5626	-36.5626	332.1955	-25.9259	-8.33333	-9.11812	-13.991	-15.251	-37.7186	-36.79553	-44.9449	-10.0381	-70.1472	8.048471
20150319_20150319_C1		1 A		8	Isoguvacine	25.5549	25.5549	-5.03269	33.18386	7.692308	-4.65361	-8.86182	-9.76578	3.096212	22.34417	157.4946	51.84581	477.2304	-25.7619
20150319_20150319_C1		1 B		1	Phenytoin	1.501203	1.501203	-6.92958	-18.2594	0	22.46294	52.23125	27.89722	55.28817	-0.20639	-14.2092	-3.32538	-11.1349	-32.0623
20150319_20150319_C1		1 B		2		-8.76316	-8.76316	0.71097	-13.9165	0	-3.86609	82.83363	11.45508	28.35737	-18.1818	-39.2775	-20.5669	-42.1233	-40.6078
20150319_20150319_C1		1 B		3		-49.0104	-49.0104	-16.6905	-50.2564	0	-1.68252	13.85347	4.028786	14.8686	-3.78044	-0.36867	2.420693	48.56947	-18.2559
20150319_20150319_C1		1 B		4		70.47692	70.47692	-19.117	55.75221	7.142857	4.453483	60.95116	6.386389	30.65822	-0.95906	-12.9409	-14.222	-31.2843	-32.9384
20150319_20150319_C1		1 B		5		-2.77363	-2.77363	7.434312	-0.64279	0	-8.92601	20.43937	-2.66356	-3.61757	-5.19432	-0.26826	0.49064	-1.31557	2.633151
20150319_20150319_C1		1 B		6	APV	23.97794	23.97794	4.099127	22.75132	12.5	-26.9661	-2.60165	-3.18554	9.303117	-25.2335	-17.6351	-17.7572	-13.0461	-23.9744
20150319_20150319_C1		1 B		7	Baclofen	-61.2723	-61.2723	-51.0704	-50.5447	0	-36.9822	6.064477	-26.6832	-39.4823	-24.6694	-40.2529	-4.59814	-61.3002	174.4232
20150319_20150319_C1		1 B		8	Isoguvacine	-14.4483	-14.4483	-0.07544	-20.9589	0	19.2502	19.47558	6.415985	2.102892	10.75056	17.4813	-11.4367	-34.0934	112.3934
20150319_20150319_C1		1 C		1	Phenytoin	-8.63355	-8.63355	-2.31521	-4.66067	0	-1.1366	-6.19691	-1.68726	-1.36314	4.524198	61.95048	3.574971	31.43487	-50.494
20150319_20150319_C1		1 C		2		-31.278	-31.278	-1.9387	-35.122	0	5.962862	81.58107	5.042863	-16.4965	-6.37375	4.803368	-3.72677	0.984369	57.98714

APPENDIX E. AXION DATA EXPORT TOOL

E.1. INTRODUCTION

The .raw file format, the continuous voltage data, can only be used within *AxIS Navigator*. The *Axion Data Export Tool* is used to convert .raw files into comma-separated value (.csv) files for use with 3rd party software. For 3rd party software that use other *AxIS Navigator* file output types; such as **AxIS Spike**, **Spike List**, or **Beat List**, it is not necessary to use the *Axion Data Export Tool*.

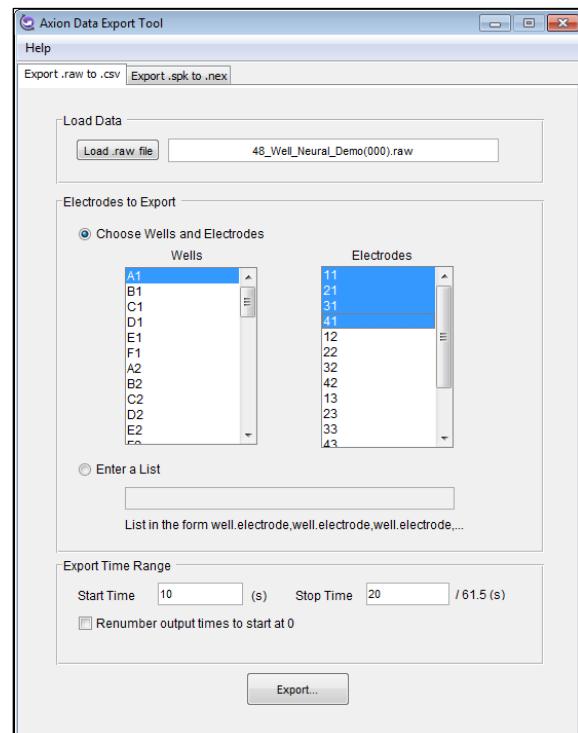
It is important to note that the .csv file generated by *Axion Data Export Tool* is significantly larger than the .raw file. Limit the data selected for export to only a few electrodes at a time and only export short periods of time (30 to 60 seconds maximum).

E.2. OPERATION

Start the *Axion Data Export Tool* by double-clicking the icon on the desktop or in the Start menu.

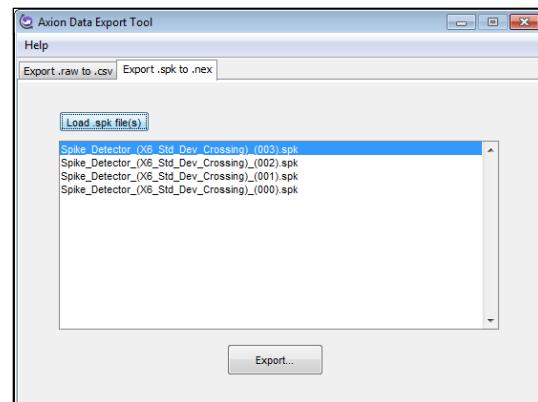
E.2.1. Export .raw to .csv

1. Select the **Export .raw to .csv** tab
2. Click **Load .raw file**.
3. Select a .raw file and click **Open**.
3. **Note:** The **Wells** and **Electrodes** selection boxes now display the available wells and electrodes.
4. Select either **Choose Wells and Electrodes** or **Enter a List**.
 - 4.1. If **Choose Wells and Electrodes** is selected: Select the desired wells and electrodes from the **Wells** and **Electrodes** lists.
 - 4.2. If **Enter a List** is selected: Type a list of the desired electrodes in the field below. Use the format [well.electrode] separated by commas. Example: A1.55, A2.83, B2.11.
5. Type the start and end time (in seconds) into the **Start Time** and **Stop Time** fields, respectively.
6. Optional: Click **Renumber output times to start at 0** to offset the exported timestamps to begin at zero seconds. If this box is not checked, the exported times will match the times in the original data.
7. Click **Export....**
8. Enter a file name and click **Save** to begin file export.



E.2.2. Export .spk to .nex

1. Select the **Export .spk to .nex** tab
2. Click **Load .spk file(s)**.
3. Select one or more .spk files and click **Open**.
4. Click **Export....**
4. **Note:** A .nex file will be saved for each .spk file, in the same directory as the source .spk file.



E.3. OUTPUT

When converting .raw to .csv, the resulting .csv file can be opened with any software that can read a text file, such as *Microsoft Excel*. The first column contains the timestamps of the data in that row. Each of the remaining columns contains the voltage measurements for a single electrode at the time indicated in the first column. The exported file is significantly larger than the original .raw file and much larger than most common spreadsheet files. Some programs limit the number of rows and columns that a file can contain. Keep these limits in mind when choosing the number of electrodes and the time points to export. It is usually best to choose the desired times, wells, and electrodes by viewing the data in *AxIS Navigator* first.

The screenshot shows a Microsoft Excel spreadsheet titled 'DataExportT'. The top menu bar includes FILE, HOME, INSERT, PAGE LAYOUT, FORMULAS, DATA, and RE^I. The main area shows a table with 15 rows and 7 columns. The first column is labeled 'Time (s)' and contains numerical values. The second column is labeled 'A1 11' and the third column is labeled 'A1 21', and so on up to 'A1 41'. The data consists of approximately 15 rows of timestamped voltage measurements for four electrodes.

Time (s)	A1 11	A1 21	A1 31	A1 41	F	G
2	10	-3.64E-06	2.54E-06	8.45E-07	1.50E-06	
3	10.00008	-2.80E-06	2.34E-06	3.90E-07	2.28E-06	
4	10.00016	-2.60E-06	1.24E-06	6.50E-08	-1.82E-06	
5	10.00024	0.00E+00	-1.17E-06	1.82E-06	-3.58E-06	
6	10.00032	-2.73E-06	-1.30E-06	-6.50E-08	-2.41E-06	
7	10.0004	-1.82E-06	-9.10E-07	1.24E-06	-1.37E-06	
8	10.00048	3.90E-07	2.28E-06	3.51E-06	8.45E-07	
9	10.00056	-2.60E-07	1.56E-06	3.06E-06	2.60E-07	
10	10.00064	1.17E-06	4.55E-07	3.97E-06	1.24E-06	
11	10.00072	2.86E-06	-8.45E-07	4.88E-06	1.17E-06	
12	10.0008	2.15E-06	-1.37E-06	4.10E-06	-1.37E-06	
13	10.00088	1.24E-06	-3.19E-06	2.08E-06	6.50E-08	
14	10.00096	1.17E-06	-2.86E-06	9.10E-07	1.24E-06	
15	10.00104	-9.75E-07	-5.01E-06	-6.50E-07	-2.60E-06	

When converting .spk to .nex, the resulting .nex file(s) can be opened with any software that can read a .nex file, such as *NeuroExplorer®* and *Offline Sorter™*.