AXION BIOSYSTEMS

AXIS NAVIGATOR 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 9.5. 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/resources.

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 (Section 2.1.1).

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 3.10 includes a variety of improvements in an effort to continually improve the usability of the Maestro systems.

1.2. 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 **panels** that support various analysis functions and data visualization. The active window displays the window 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

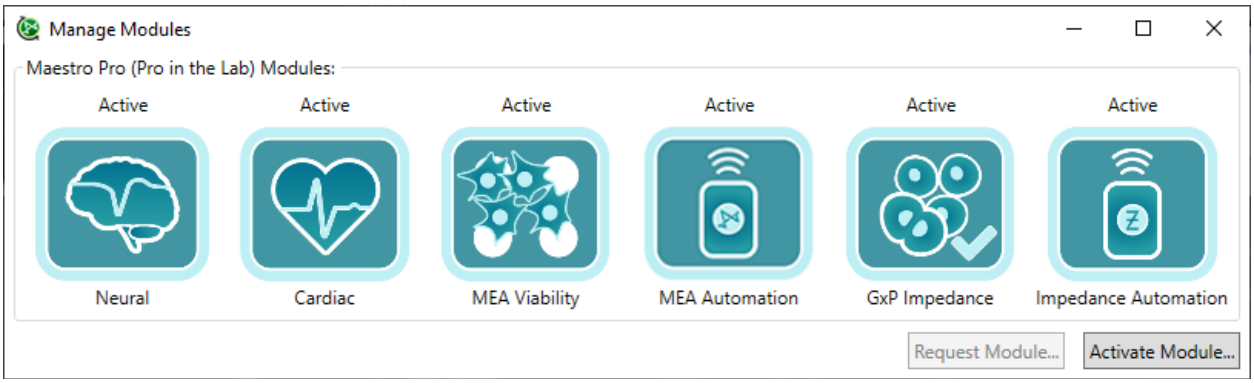
The **Menu** bar has four options:

Menu	Option	Description
File	Open Recording(s)...	Loads one or more .raw files into the **Streams pane.**
	New Batch Process...	Opens the batch processing dialog. See Section 9.6.
	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 *AxIS Navigator*.

Tools	Maestros	Allows users to **Locate** and **Select** a specific Maestro when more than one Maestro is connected to the same computer. Selecting **Locate** will cause the power button for the selected system to flash through a rainbow of colors. Clicking **Select** will choose that Maestro system for data collection in the software. Only Maestro Edge/Pro systems will be available in the list.
	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.
	Graphics → Disable Hardware Acceleration	Allows user to **Disable Hardware Acceleration** for *AxIS Navigator*. Users should contact support@axionbio.com before selecting this option.
	Reset AxIS Navigator	Restores AxIS Navigator to default settings. Removes all loaded files and configurations.
	Accelerate Playback	When replaying a previously recorded .raw file, **Accelerate Playback** plays the file back faster than real time.
	Accelerate Recording	When recording new analysis files from a previously recorded .raw file, **Accelerate Recording** plays the file back and performs analysis faster than real time.
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. If multiple Maestros are connected, users can select all or a specific Maestro system to generate an error log report.
	About	Displays *AxIS Navigator* version number and information.

2.1.1. Software Module Activation

The **Manage Modules…** option under the **Help** menu opens a window that displays all of the software modules available, each identified with a name, icon, and active status. Active software modules will be shown in blue, while inactive modules are grayed out. This option is only enabled if the Maestro is connected to *AxIS Navigator* (see Section 3.1).

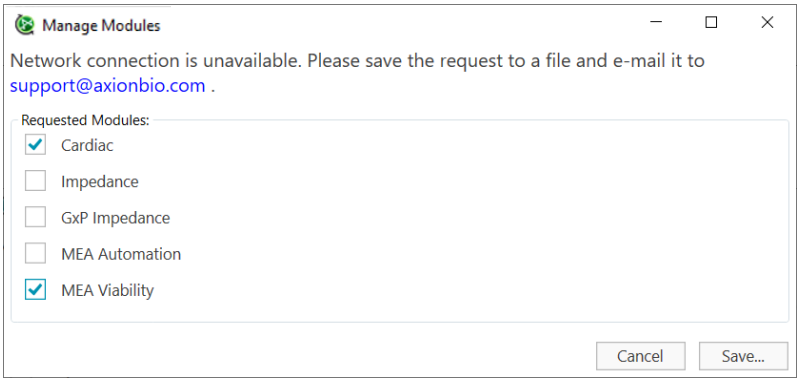


Available software modules are listed below:

Software Module	Description	Axion System	Software
Neural	Measure electrical network behavior of neurons, including: activity, synchrony, and network oscillations, label-free.	Maestro Pro Maestro Edge	*AxIS Navigator*
Cardiac	Record the 4 key measures of cardiac performance: action potential, field potential, propagation, and contractility.	Maestro Pro Maestro Edge	*AxIS Navigator*
MEA Automation	An application programming interface (API) that can be used for interfacing with liquid handling platforms or other systems to automate MEA experiments.	Maestro Pro Maestro Edge	*AxIS Navigator*
MEA Viability	Use impedance technology on MEA plates to track cell viability and coverage in each well from the same microelectrodes used for functional activity measurements.	Maestro Pro Maestro Edge	*AxIS Navigator*
Impedance	Track cell proliferation, viability, barrier function, immune cell-mediated killing, viral cytopathic effects, and more.	Maestro Pro Maestro Edge Maestro Z Maestro ZHT	*AxIS Z*
GxP Impedance	Achieve FDA 21 CFR Part 11 compliance in GMP/GLP labs with this version of the Impedance Software Module.	Maestro Pro Maestro Edge Maestro Z Maestro ZHT	*AxIS Z*
Impedance Automation	An application programming interface (API) that can be used for interfacing with liquid handling platforms or other systems to automate impedance experiments.	Maestro Pro Maestro Edge Maestro Z Maestro ZHT	*AxIS Z*

To request a module activation key(s) for an inactive software module(s):

1. Navigate to the **Manage Modules** window from the **Help** menu in *AxIS Navigator*.
2. Click **Request Module...**
3. If the Maestro computer has an active internet connection, the software will open Axion's licensing web page and auto-fill **Device ID** and **Serial Number** fields.
   3.2. If the Maestro computer is offline, the **Requested Modules** window will open. Select the box(es) next to the requested software modules.
   ***Note**: Software modules already active or requested will be checked and disabled.*
   3.3. Click **Save...** . The software will save the Device ID and Serial Number to a text file
   3.4. Email the text file to support@axionbio.com.

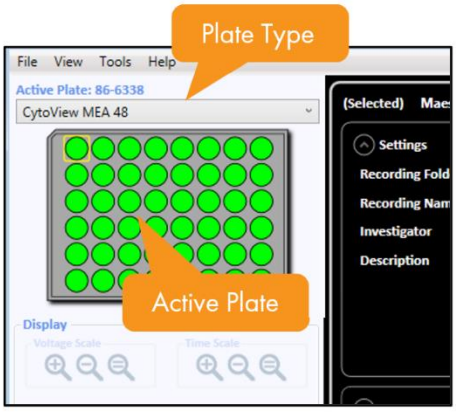


To activate a software module:

1. Navigate to the **Manage Modules** window in the **Help** menu in *AxIS Navigator*.
2. Click **Activate Module...**
3. Navigate to the folder with module activation key (.xml file).
   ***Note**: Axion Biosystems will send users module activation keys for purchased software modules.*
4. Select the module activation key and click **Open.**
5. The module activation key will be stored on the Maestro system and the corresponding software module will be displayed as active in the **Manage Modules** window.

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. The drop-down menu will populate with new plate types as new plates are used. To view all available plate types, select **View → Show All Plate Types**.

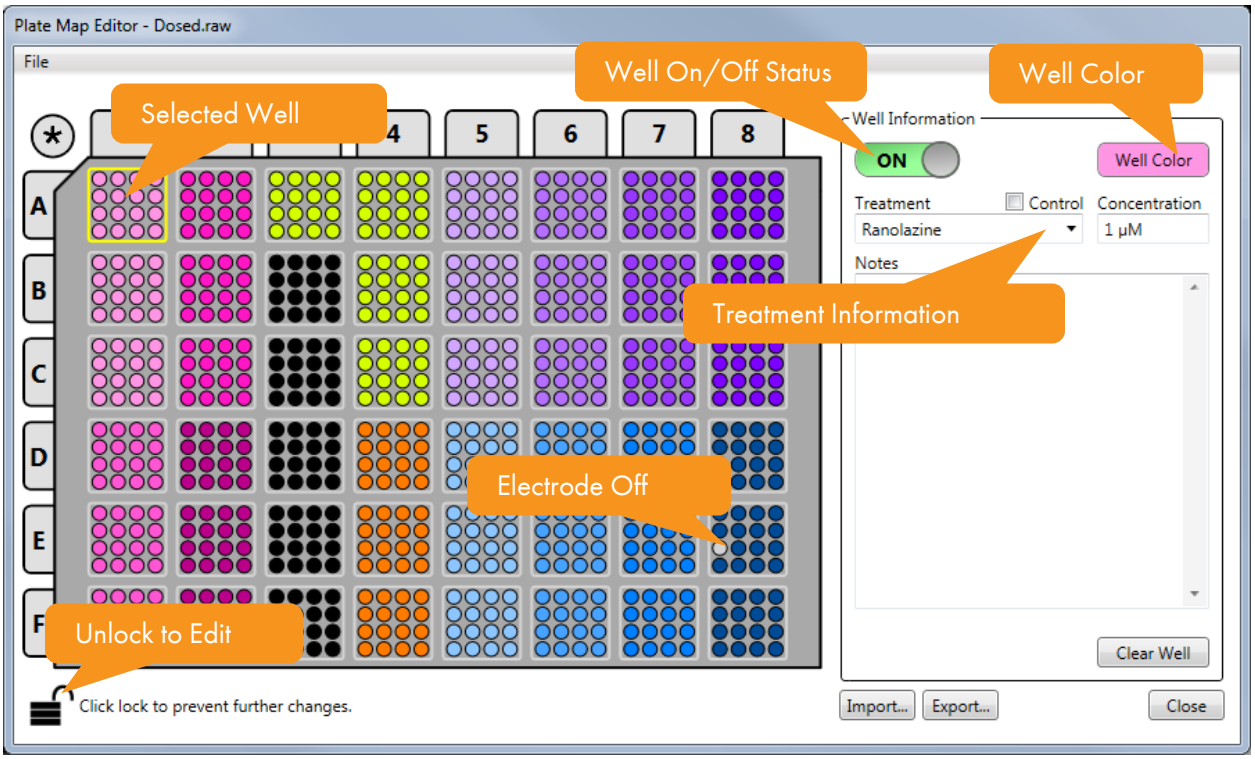
Maestro MEA plate types include:

Plate Type	System Compatibility	Part number
Smart Plate – Pro/Edge	Maestro Pro and Maestro Edge	N/A
BioCircuit MEA 24	Maestro Pro and Maestro Edge	M384-BIO-24
BioCircuit MEA 48	Maestro Pro	M768-BIO-48
BioCircuit MEA 96	Maestro Pro	M768-BIO-96
CytoView MEA 6	Maestro Pro and Maestro Edge	M384-tMEA-6B M384-tMEA-6W
CytoView MEA 24	Maestro Pro and Maestro Edge	M384-tMEA-24W
CytoView MEA 48	Maestro Pro	M768-tMEA-48B M768-tMEA-48W
CytoView MEA 96	Maestro Pro	M768-tMEA-96B M768-tMEA-96W
Lumos MEA 24	Maestro Pro and Maestro Edge	M384-tMEA-24OPT
Lumos MEA 48	Maestro Pro	M768-tMEA-48OPT
Lumos MEA 96	Maestro Pro	M768-tMEA-96OPT
NETRI DuaLink MEA – Pro/Edge	Maestro Pro and Maestro Edge	Product sold by NETRI, contact NETRI for more information
NETRI DuaLink Shift MEA – Pro/Edge	Maestro Pro and Maestro Edge	Product sold by NETRI, contact NETRI for more information
NETRI TriaLink MEA – Pro/Edge	Maestro Pro and Maestro Edge	Product sold by NETRI, contact NETRI for more information

***Note**: Contractility and Neural Broadband require CytoView or Lumos MEA plates.*

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.*



2.2.1. 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.

2.2.2. 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 any field is empty, it will not be cleared; instead 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**.

2.2.3. Setting Well Color

To set well color manually:

1. Select the well(s).
2. Click on **Well Color** in the upper right corner of **Well Information** and choose a color.

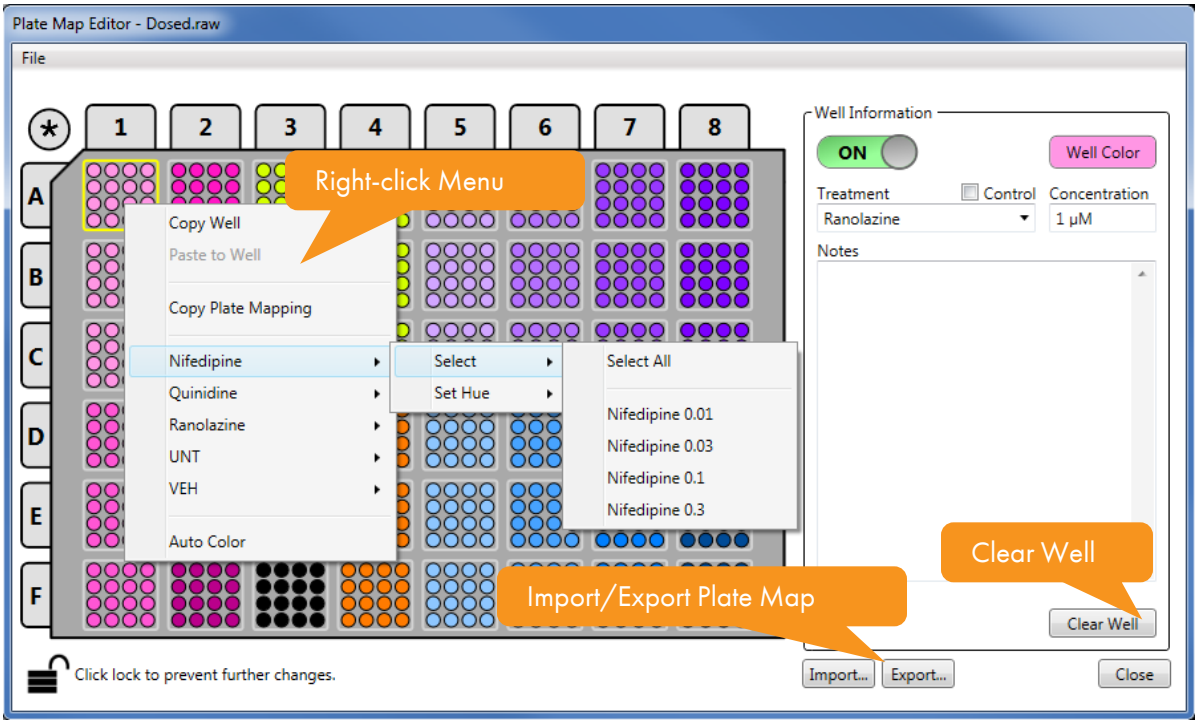
To set well color automatically:

1. Right-click on any well.

2. 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:

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



2.2.4. 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 2.2.5).

To export a plate map using the export button:

1. Click the **Export** button in the **Plate Map Editor**.
2. 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.*

2.2.5. 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	Hexadecimal 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
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** pane 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 9.1). 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 ("Name of Maestro Pro")** or **Maestro Edge ("Name of Maestro Edge")**, depending on which Maestro MEA system is used. If no Maestro is connected, the stream will be inactive and say "No Device Connected". The default Maestro name is "Maestro-XXX", where XXX is the last 3 digits of the Maestro serial number.

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** pane:

1. Right-click on white space in the **Streams** pane.
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* pane. 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 9.1 for more information about these recommended default stream configurations.

To add a data processor:

1. Right-click on the stream in the **Streams** pane.
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** pane.
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** pane.
2. Select **Settings**.

-- Or --

1. Double-click on the processor in the **Streams** pane.

To change the visibility of a data processor:

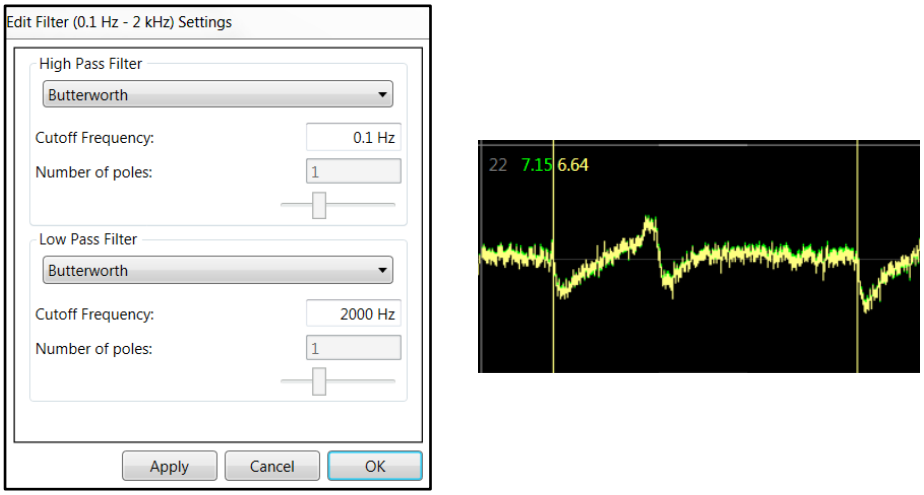
1. Right-click on the processor's stream in the **Streams** pane.
2. Select **Plot → Show**. If enabled, the processor will show in dependent *AxIS Navigator* panels.

To change the color of a data processor:

1. Right-click on the processor in the **Streams** pane.
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 voltage 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 voltage 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.

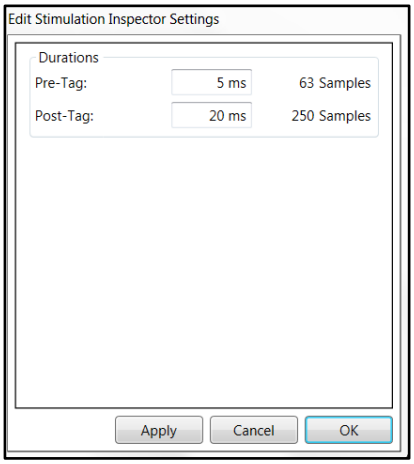
***Note**: The Digital Filter operates on voltage data, as is recorded in Neural configurations, Cardiac Field Potentials, and Cardiac LEAP configurations. It should not be applied to cardiac contractility signals.*

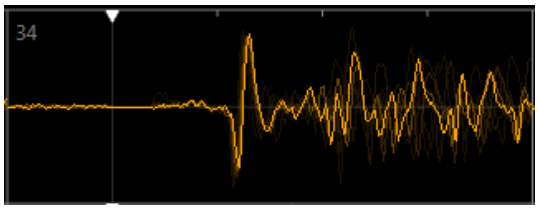
2.3.3. Artifact Eliminator

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 voltage data near an **Electrical or Optical 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 or Optical Stimulation Tag** in the **Spike Plots** panel. Note the **Stimulation Inspector** is for visualization only and does not produce an output file.





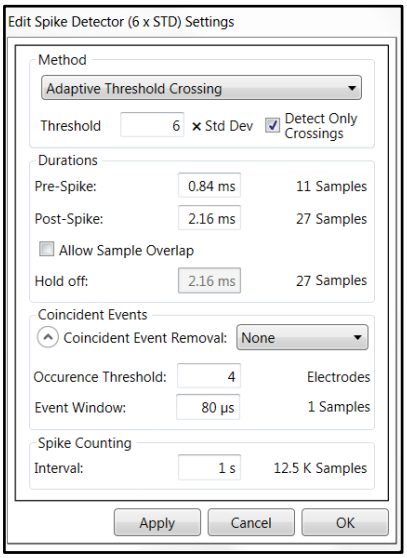
While using the **Stimulation Inspector** data processor, the **Electrical or Optical Stimulation Tag** will appear as a triangle and vertical line at time zero on the spike plot. The time displayed before and after the tag may be changed in the **Stimulation Inspector** settings by the **Pre-Tag** and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

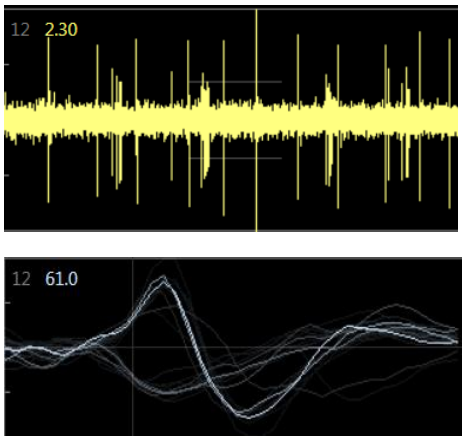
***Note**: The **Stimulation Inspector** displays voltage data, therefore is useful for evaluating capture in Neural Evoked configurations as well as Cardiac Field Potentials Paced and Cardiac LEAP Paced configurations. It should not be applied to cardiac contractility signals.*

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** panel 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** panels. **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 9.2 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:

1. **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.
2. **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.
3. **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.
4. **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** panel. 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 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. *AxIS 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 *AxIS 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.

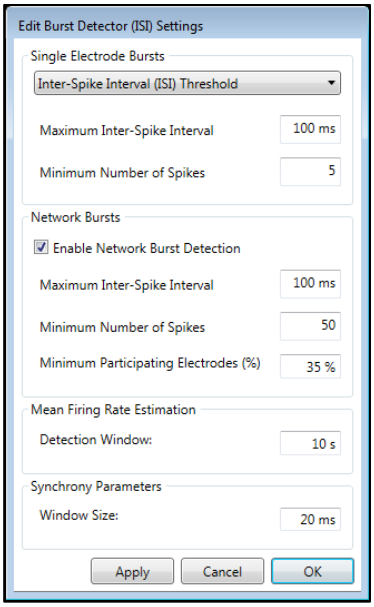
For example, if **Coincident Event Removal** is set to **Well** with **Occurrence Threshold** equal to 4 electrodes and **Event Window** equal to 80 µs, spikes that occur on at least 4 electrodes in the same well within 80 µs of each other will be ignored.

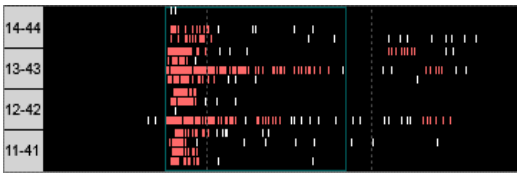
The **Spike Counting** box sets the bin time for counting spikes as defined by the **Interval** field. The bin time is used by the **Activity Map** panel and the **Spike Count** files.

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 11.3 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 2.3.1111) 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** panel. 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**. The network burst algorithm relies on defining a network burst as a collection of N spikes detected across the entire well (not limited to a single electrode), each separated by an inter-spike interval of no more than T seconds. 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 (%)**.

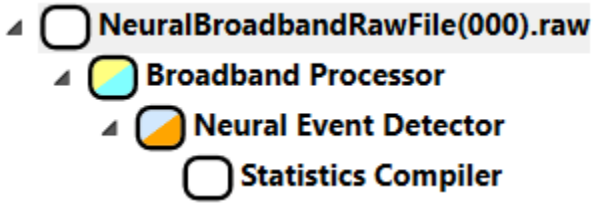
The **Detection Window** field in the **Mean Firing Rate Estimation** box sets the duration of the sliding window used to calculate the mean firing rate for Poisson Surprise burst detection.

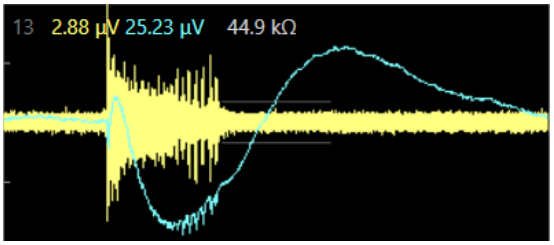
The **Window Size** field in the **Synchrony Parameters** box is the window of time around zero phase-lag used to compute the area under the cross-correlation and area under the normalized cross-correlation synchrony metrics provided by *AxIS Navigator*. See Section 11.3 for further explanation of synchrony.

2.3.7. Broadband Processor

The **Broadband Processor** is a specialized node which is used to divide broadband neural signals collected in **Neural Broadband** mode into two data streams:

1. High frequency (200 Hz - 5000 Hz) **Spike Trace**
2. Low frequency (1 Hz – 50 Hz) Local Field Potential (**LFP Trace**) which is downsampled to a rate of 100 samples/sec





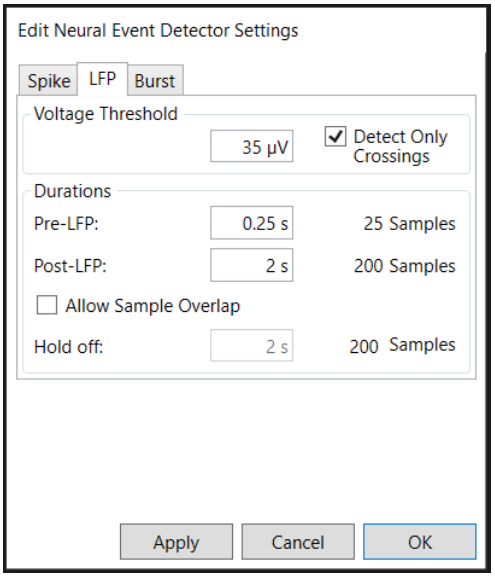
The **Broadband Processor** can only be attached to streams collected in **Neural Broadband** mode. This data processor outputs 2 filtered continuous voltage data streams which will be saved to the same .raw file.

The only data processor which may be attached to the **Broadband Processor** is the **Neural Event Detector**, which is designed to operate on the dual voltage streams.

2.3.8. Neural Event Detector

The **Neural Event Detector**, only available for attachment to the **Broadband Processor,** combines the functionality of the **Spike Detector**, the **Burst Detector,** and an **LFP Detector.** The **Spike Detector** and **Burst Detector** within the **Neural Event Detector** operate on the high frequency **Spike Trace** within the **Broadband Processor** and contains the same settings as the standalone **Spike Detector** and standalone **Burst Detector**.

Like the standalone **Spike Detector** and **Burst Detector**, the **Neural Event Detector** can output **Spike Count** (.csv), **Spike List** (.csv), **Electrode Burst List** (.csv), and **Network Burst List** (.csv) files. Unlike the standalone detectors, it is also capable of outputting **LFP Event List** (.csv) and **AxIS Event** (.spk) files.



The **LFP Detector** within the **Neural Event Detector** detects threshold crossings of the low frequency **LFP Trace** from the **Broadband Processor** it is attached to. These slow, discrete voltage deflections are referred to as LFPs and are plotted in the **LFP Plots** panel. The **LFP Detector** has settings similar to the **Spike Detector.**

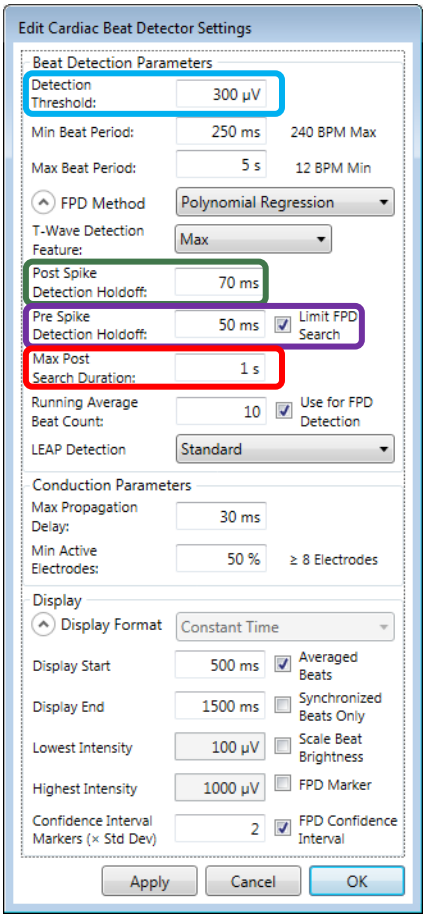
Voltage Threshold: Threshold is set on a plate-wide basis, all electrode thresholds are the same. LFPs are detected as any event greater than the value defined in the **Threshold** field. **Detect Only Crossings** requires the signal to return below threshold before detecting an additional LFP. The LFP time is marked at the time of initial threshold crossing.

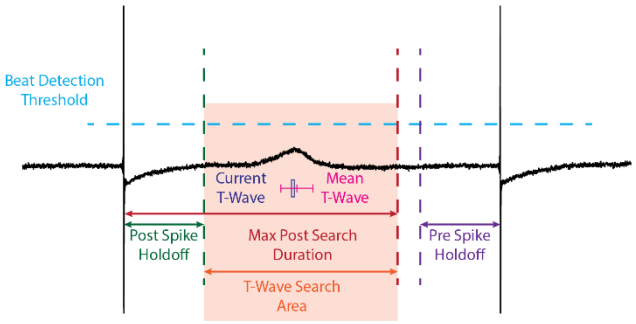
The **Durations** dialog box sets the display in the **LFP Plots** panel. Use the **Pre-LFP** and **Post-LFP** fields to specify how much time before and after each LFP crossing will be displayed on the LFP waveform plot and saved in an **AxIS Event** (.spk) file alongside spike times. The time of initial threshold crossing is plotted as zero.

Setting a proper detection threshold and duration is crucial for accurate LFP collection. Lower thresholds increase the incidence of false-positives (drift or noise events misidentified as LFPs); higher thresholds may not detect smaller amplitude LFPs. Depending on cell type or maturity, LFPs may have larger or smaller voltage deflections and longer or shorter durations. These settings will require tuning on an application specific basis.

2.3.9. 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** panel 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** panels. The **Cardiac Statistics Compiler** data processor (Section 2.3.1010) 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 1010 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 the following 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 and contractility signals acquired using Cardiac: Contractility Acquisition Settings**. Changing the **Detection Threshold** will not influence the detection of LEAP or contractility 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.
4. **LEAP Detection**: Sets the sensitivity for detecting LEAP signals. If small LEAP signals are not detected using the **Standard** algorithm, select **High Sensitivity** from the drop-down menu.

*Note: **High Sensitivity** LEAP detection can label signals as LEAP that are not true LEAP signals. Use with caution.*

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 *AxIS Navigator*. The cardiac waveform displayed in the **Cardiac Beat Plots** panel 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.

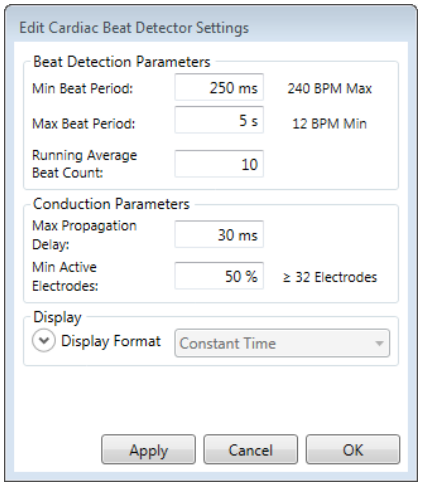
The **Conduction Parameters** section identifies well beats, also called synchronized beats. A synchronized beat is a depolarization spike detected on a minimum number of electrodes within a well in a certain time window. The synchronized beat time is marked as the beat time of the first participating 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** panel.

The **Display** section controls the data visualization in the **Cardiac Beat Plots** panel. 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.

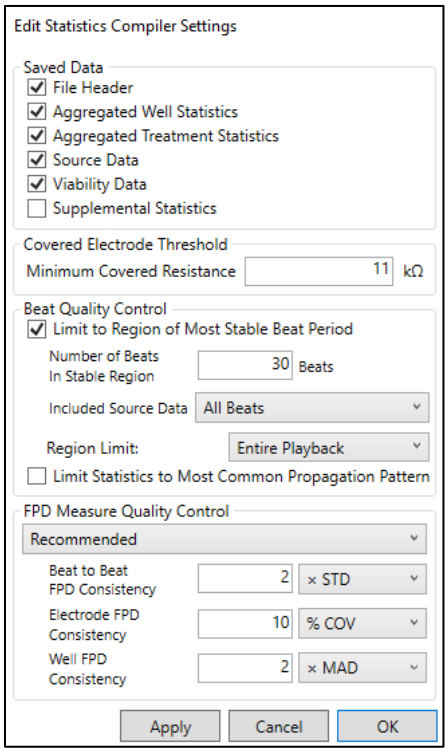
The checkbox controls can also be set by right-clicking on the beat waveform plots in the **Cardiac Beat Plots** panel, and selecting/deselecting the display options.

***Note**: When **Cardiac: Contractility Acquisition Settings** are used, only relevant parameters are displayed in the Cardiac Beat Detector Settings, as shown below.*



2.3.10. Cardiac Statistics Compiler

The **Cardiac Statistics Compiler** (displayed as **Statistics Compiler** in the **Streams pane**) 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 9.2 for more information on file types.



The settings window has four sections, **Saved Data**, **Covered Electrode Threshold**, **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 **Covered Electrode Threshold** section defines a threshold for identifying electrodes with sufficient cell coverage. Electrodes with a resistance greater greater than the value specified in the **Minimum Covered Resistance** field are considered Covered Electrodes. In general, fully uncovered CytoView microelectrodes with media only in the well have a resistance of 8 – 12 kΩ. (See FAQs in Section 8.3 for more detail). The output file reports the resistance of each electrode, the number of covered electrodes in each well, as well as the weighted mean resistance, the mean resistance across only the covered electrodes in a well.

***Note**: This section is only available with the MEA Viability Software Module. See Chapter 7 for more information.*

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 Cardiac 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:

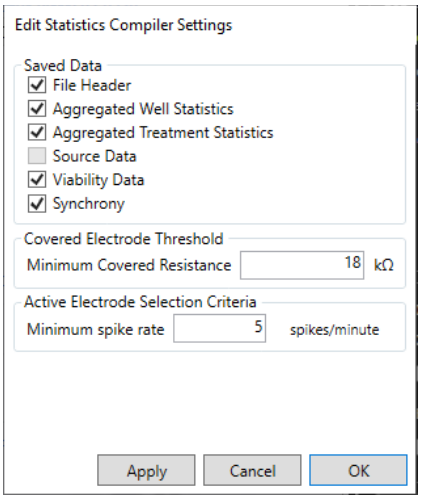
1. **Beat to Beat FPD Consistency:** Individual beat metrics are compared to the mean or median of all beats on that electrode. FPD values for individual beats are removed when they exceed the specified number of standard deviations (**x STD**) or median absolute deviations (**x MAD**) from the mean 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 (**x STD**) or median absolute deviations (**x MAD**) from the well mean or median are excluded from the Well Average FPD.

***Note**: When **Cardiac: Contractility Acquisition Settings** are used, there is not an **FPD Measure Quality Control** section in the **Statistics Compiler** Settings since those settings are not relevant for contractility.*

2.3.11. Neural Statistics Compiler

The **Neural Statistics Compiler** (displayed as **Statistics Compiler** in the **Streams** pane) 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** or a **Neural Event Detector** and only on previously recorded data. It has one output file, **Advanced Metrics** (.csv). See Section 9.2 for more information on file types.



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 values.
3. **Aggregated Treatment Statistics**: If enabled, output file will include treatment group averages as defined by the **Plate Map Editor**.
4. **Viability Data**: If enabled, output file will include viability metrics.
5. **Synchrony**: If enabled, output file will include synchrony metrics.

The **Covered Electrode Threshold** section defines a threshold for identifying electrodes with sufficient cell coverage. Electrodes with a resistance greater than the value specified in the **Minimum Covered Resistance**

field are considered Covered Electrodes. In general, fully uncovered CytoView microelectrodes with media only in the well have a resistance of 8 – 12 kΩ. (See FAQs in Section 8.3 for more detail). The output file reports the resistance of each electrode, the number of covered electrodes in each well, as well as the weighted mean resistance, or the mean resistance across only the covered electrodes in a well.

***Note**: This section is only available with the MEA Viability Software Module. See Chapter 7 for more information.*

The Active Electrode Selection Criteria section 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** panels. When playing back a file, right-click the Play button and select **Accelerate Playback** to replay the file faster than real time.
Record		Records and plays the current stream. Data will be displayed in the **Control Bar** panels and outputs selected in **Experiment Setup Properties** will be saved. When playing back a file, right-click the Record button and select **Accelerate Recording** to replay the file and save the analysis file(s) faster than real time.

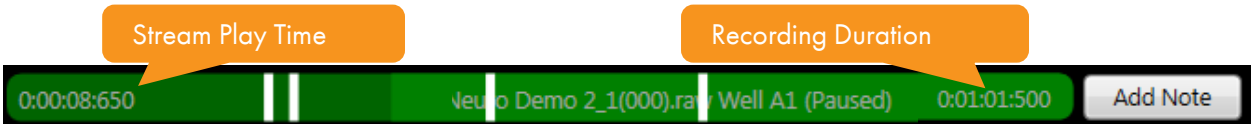
The **Display** controls scale data displayed in the active window and, in certain panels, provides a drop-down menu to select the type of data displayed. Use the **Source** drop-down to select the type of data displayed. Use the zoom in (), zoom out (), and reset scale () controls to change the voltage 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.

For **Cardiac: Contractility,** the **Display** controls are used to change the contractility (expressed as a percent change in impedance) and time scales.

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. When the active stream is a previously recorded file, the status bar represents the time within the file. 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.

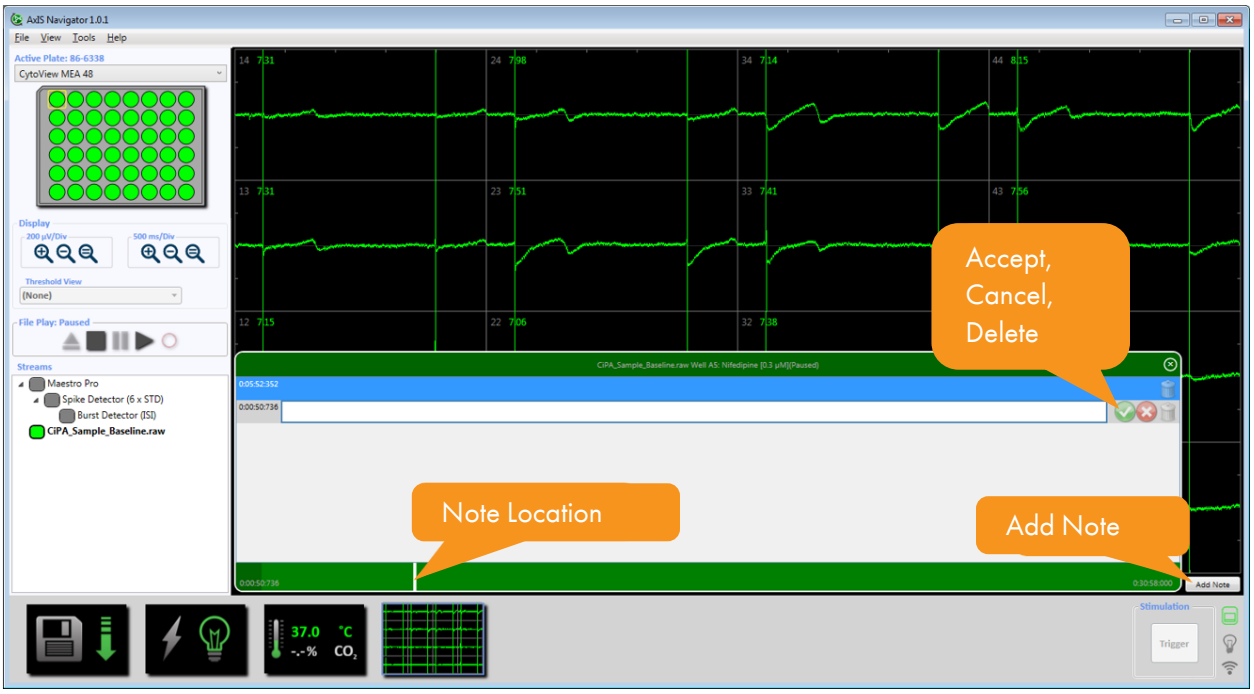
2.5.1. Adding Timestamp Notes

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:

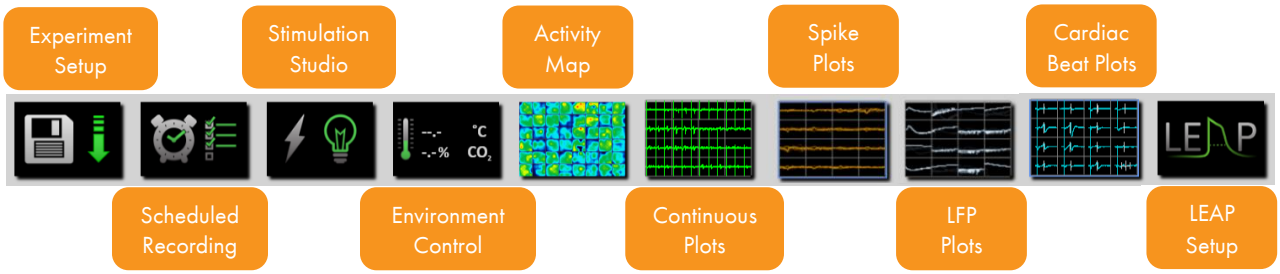
1. Click **Add Note** located to the right of the **Status Bar** at the desired file time.

2. Type the desired text and click **Accept** (green checkmark).
3. Click the ⓧ in the top right corner to close the **Notes** panel.



2.6. CONTROL BAR

The **Control Bar** interface switches the panel displayed in the active window. By default, only panels used by the current configuration in the **Streams** pane are displayed. To switch the active panel, click on the thumbnail or select the panel from the **View** menu.

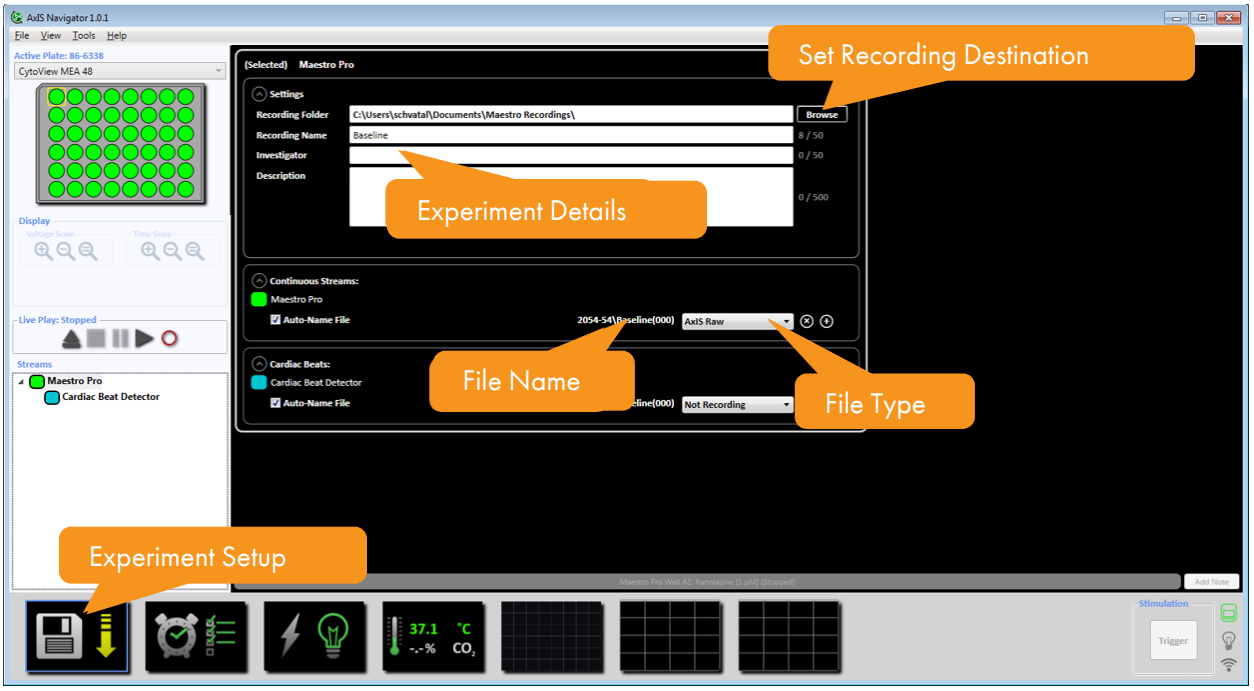


Name	Description	Dependency
Experiment Setup Properties	File information controls. Sets save location, experiment notes, recording names, and file types.	Always visible
Scheduled Recording Setup	Automated recording and stimulation controls.	Live data only
Stimulation Studio	Stimulation definition controls.	Always visible

Environmental Control	Temperature and CO_2 controls.	Live data only
Activity Map	Plate-wide visualization of spike rate, beat rate and amplitude, LFP amplitude, or viability.	Spike Detector Neural Event 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 Neural Event Detector
LFP Plots	Displays LFP waveforms for every electrode in a selected well.	Neural Event 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** panel specifies the recording destination, experiment description, and the file names and types to record. Available recording file types (see Section 9.2) are determined by the data processors present in the **Streams** pane.



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 9.2.1).

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

1. Click on the **Experiment Setup Properties** panel 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** panel 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** panel automates data recording and stimulation. The panel is divided into two sections: **Settings** and **Status**.

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

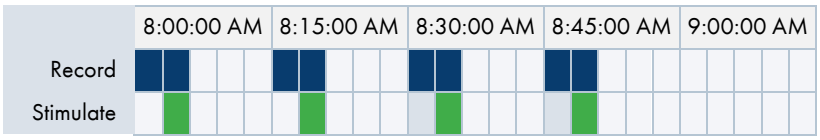
Field	Description	Options
Record Every[1]	The interval between the start of each recording	**Seconds**, **Minutes**, **Hours**, or **Days**
Record For	The duration of each recording	**Seconds**, **Minutes**, **Hours**, or **Days**
Starting	The start time of the first recording	**Immediately** – begin when **Start Schedule** is clicked. **At** – begin at a specified time *(mm/dd/yyyy hh:mm:ss).* *Note: 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 ***Note**: 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.

[1]The value for **Record Every** cannot be set to 0. To take one scheduled recording, select "Once" from the **Execute** drop down.

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

***Note**: For Viability Configurations, a message below Record For will indicate, "Viability will be acquired at the start of each file." Users must design their scheduled recordings to account for the viability measurement (~1 min) that is taken at the start of each scheduled time point (see Section 8.2).*

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

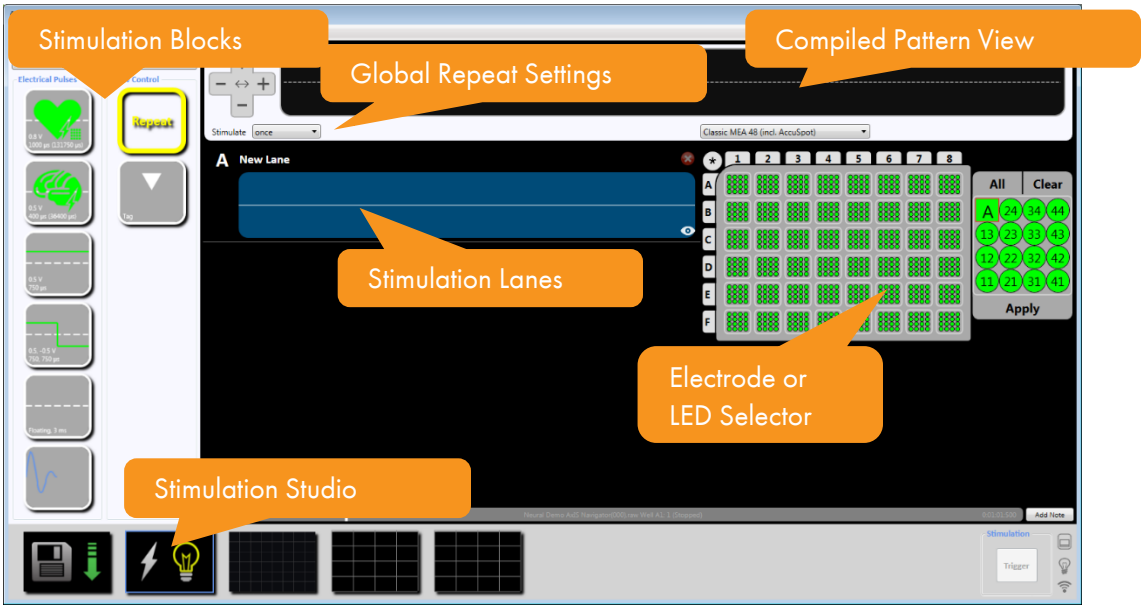


The **Status** panel provides feedback about the recording schedule.

Field	Description
Running Status	Current recording status: **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

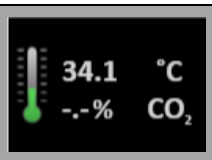
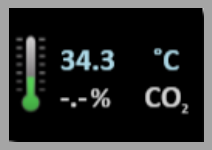
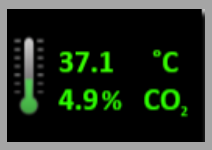
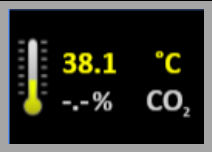
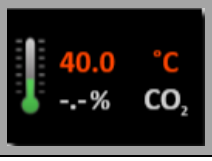
The **Stimulation Studio** panel designs both electrical and optical stimulation protocols. The panel has four key components: the stimulation blocks, stimulation lanes, global repeat settings, compiled pattern view, and electrode or LED selector. See Chapter 4 for more information about building stimulation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



2.6.4. Environmental Control

The **Environment Control** panel displays current temperature and CO_2 levels. The **Environmental Control** thumbnail displays the current temperature and CO_2 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 CO_2 status.

When a valid plate is docked in the Maestro, heater control is automatically turned on with the temperature set to 37°C, and CO_2 control is automatically turned on with the CO_2 concentration set to 5%.

To set a custom temperature or CO_2 concentration, or to use a custom gas mix, navigate to the **Environment Control** panel using the **Control Bar**. The active window is shown below:

The temperature and CO_2 plots display the current set point as a dashed line and the current temperature or CO_2 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 CO_2 control box contains the **CO_2 Control** switch, the **Gas Source**, and the **Concentration (%)** set point if 100% CO_2 is used.

A warning will display if the temperature is more than 1 °C away from the set point or the CO_2 concentration is more than 1% away from the set point for longer than 5 minutes.

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 or when a plate is undocked.

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

To set the temperature to a custom temperature:

1. Click on the **Environmental Control** panel 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.*

Legacy Heater Control mimics the original Maestro's heater control scheme, in which heat is only applied from below the plate. ***Note**: This setting may be well suited for some cell types, but condensation may arise over time in the absence of door heating.*

To use **Legacy Heater Control** settings:

1. Click the gear in the **Heater Control** settings.
2. Check the box next to the **Legacy Heater Control.**

If **Auto Enable Gas** is selected, CO_2 is on only when an Axion MEA plate is docked and the door is closed. If **Auto Enable Gas** is not selected, CO_2 is manually controlled by the **CO_2 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, **CO_2 Control** is automatically turned off, regardless of **Auto Enable Gas** setting. The CO_2 concentration cannot be maintained when the door is open. The **CO_2 Control** is automatically turned off when the Maestro is turned off to conserve gas. To set the CO_2 concentration to a custom concentration:

1. Click on the **Environmental Control** panel in the **Control Bar**.
2. Select **100% CO_2** from the **Gas Source** drop-down menu.
3. Set the desired CO_2 concentration in the **Concentration (%)** field.

To use pre-mixed gas rather than 100% CO_2:

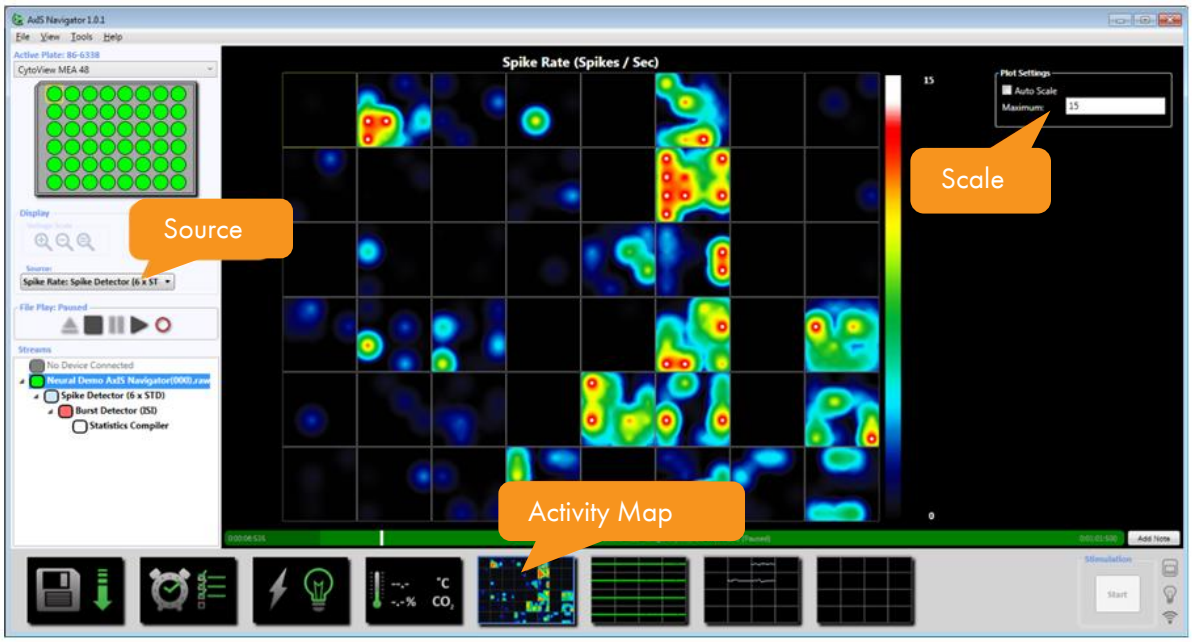
1. Click on the **Environmental Control** panel in the **Control Bar**.
2. Select **Custom Mix** from the **Gas Source** drop-down menu.
3. Use an inline flow meter to control the flow of gas into the Maestro.

To stop controlling temperature or CO_2:

1. Click the **Heater Control** or **CO_2 Control** switch to **Off**.

2.6.5. Activity Map

The **Activity Map** panel 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** pane as listed below:

Data Processor	Source Metric
Cardiac Beat Detector	Beat Rate (BPM) – All Cardiac Acquisition Settings Spike Amplitude (µV) – Cardiac: Standard, Broadband, and LEAP Beat Amplitude (%) – Cardiac: Contractility Viability (Resistance, kΩ)[1] – All Cardiac + Viability Acquisition Settings
Spike Detector	Spike Amplitude (µV) – All Neural Acquisition Settings Spike Rate (Spikes/Sec) – All Neural Acquisition Settings Viability (Resistance, kΩ)[1] – All Neural + Viability Acquisition Settings
Neural Event Detector	Spike Amplitude (µV) – Neural: Broadband

	Spike Rate (Spikes/Sec) – Neural: Broadband LFP Amplitude (µV) – Neural: Broadband Viability (Resistance, kΩ)[1] – Neural: Broadband+ Viability Acquisition Settings

[1]Only available with the MEA Viability software module

For non-viability metrics, 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. When set to **Viability**, the **Activity Map** displays the resistance values at the time listed at the top of the map (single time point).

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. For Viability, yellow areas indicate high levels of viable cell coverage, whereas dark blue and black indicate less cell coverage.

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

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

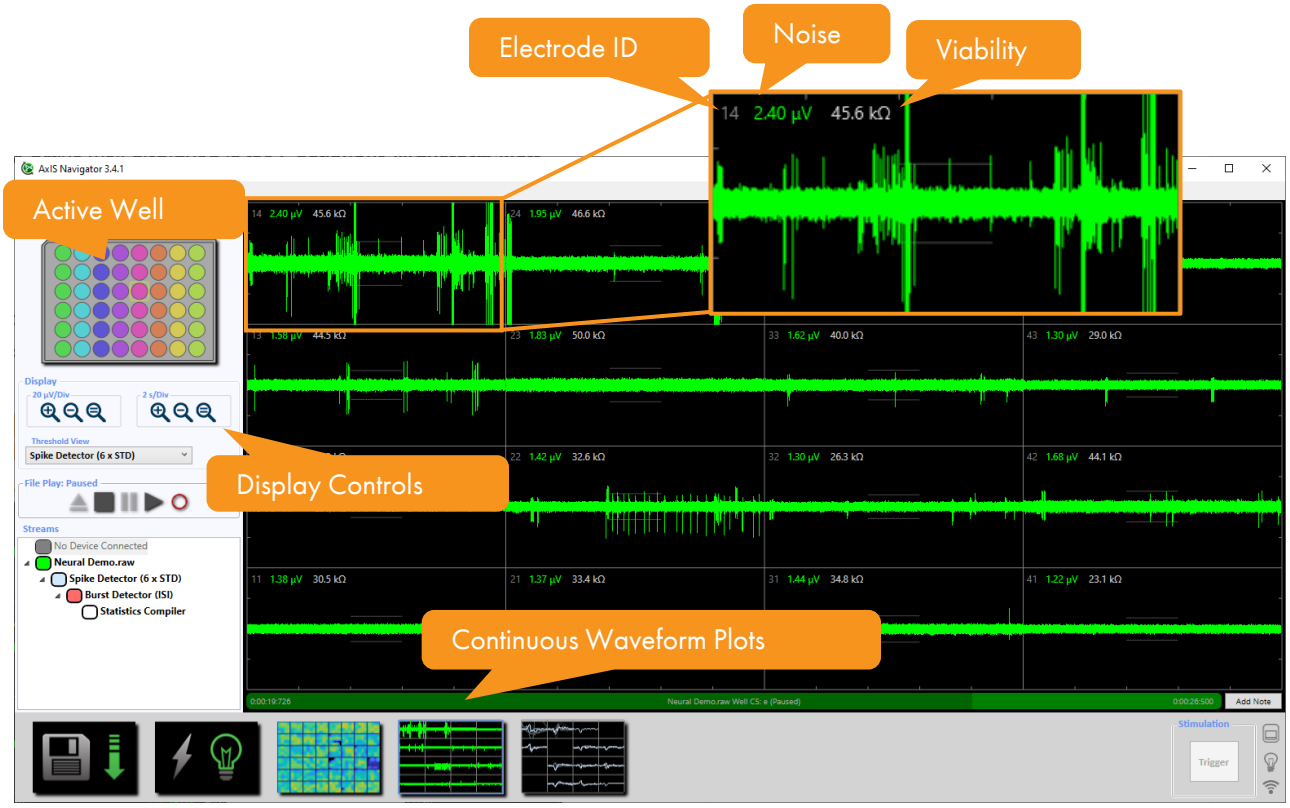
To copy the **Activity Map**:

1. Click on the **Activity Map** panel 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** panels.

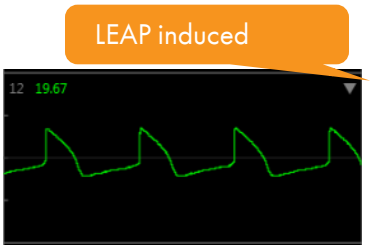
2.6.6. Continuous Waveform Plots

The **Continuous Waveform Plots** panel displays continuous voltage data (for most Acquisition Settings) or contractility data (for **Cardiac: Contractility** Acquisition Settings) for each electrode of the active well. Select the active well by clicking on the desired well on the **Active Plate** or **Activity Map**.

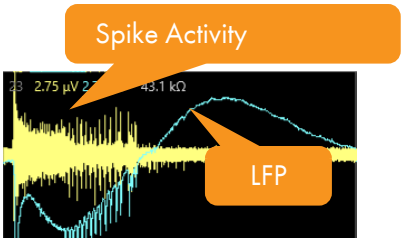


The electrode ID consists of two digits, the column and row, and is displayed in gray text in the upper left corner of each plot. Adjacent to the electrode ID is the electrode noise level, displayed in µV, whenever voltage data is displayed. Right click on the electrode and select **Hide RMS** to hide noise values. If a Viability configuration (only available with the MEA Viability Software Module) was used, the cell-electrode Resistance value is displayed in kΩ adjacent to the noise value. Right-click on the electrode and select **Hide Viability** to hide resistance values. If -- kΩ is displayed, the electrode impedance is out-of-range and resistance cannot be measured. See Chapter 7 for more information.

If the file contains LEAP tags, an inverted gray triangle is displayed in the upper right corner of the plot for electrodes that are tagged, indicating those electrodes were selected for LEAP induction.



If the file is recorded in the Neural → Broadband Spontaneous configuration, both the spike and LFP waveforms will be shown in the Continuous Waveform Plot.



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** panel 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 6 or NETRI NeuroFluidics™ MEA plates.*

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 2.2.1).

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**.

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[1]	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

[1]**Enhance for Neural** is only available for the high-frequency **Spike Trace** in the Neural → Broadband Spontaneous configuration.

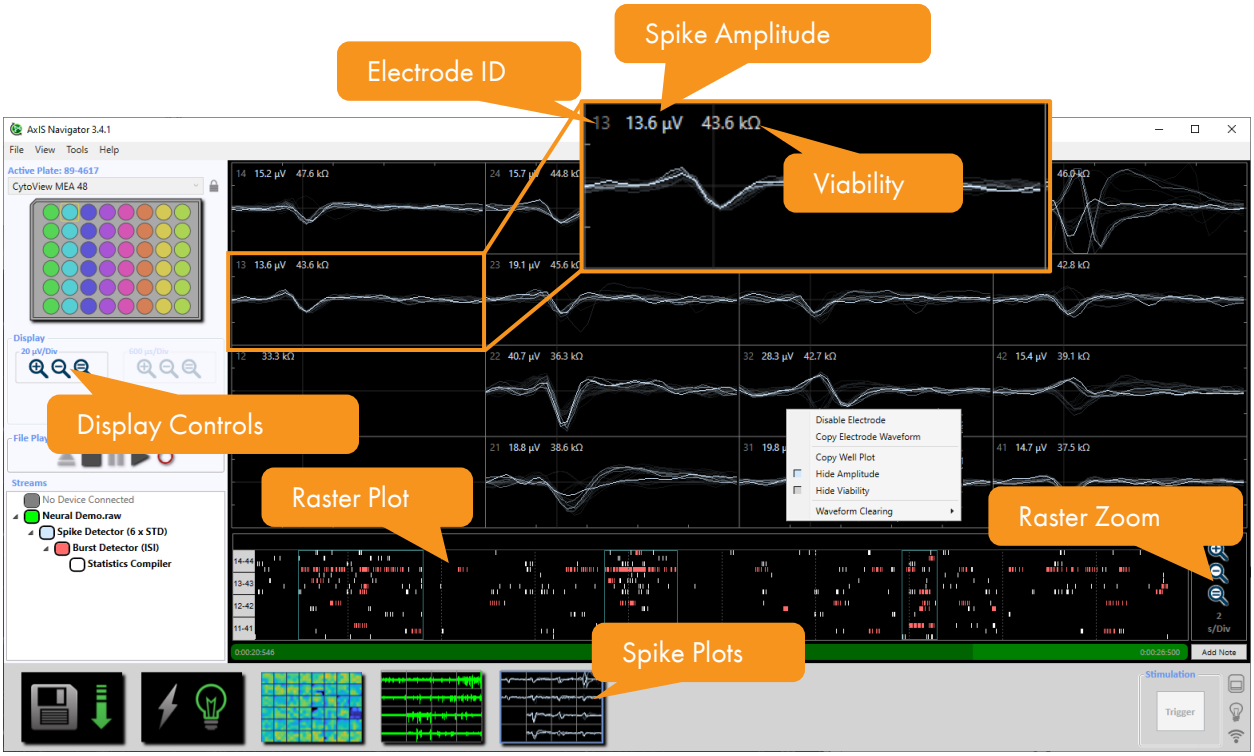
Enhanced plotting is selected by default. To disable or enable enhanced plotting:

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** panel 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, displayed in µV. Right click on the electrode and select **Hide Amplitude** to hide amplitude values. If a Viability configuration (only available with the MEA Viability Software Module) was used, the cell-electrode Resistance value is displayed in kΩ adjacent to the amplitude value. Right-click on the electrode and select **Hide Viability** to hide resistance values. If -- kΩ is displayed, the electrode impedance is out-of-range and resistance cannot be measured. See Chapter 7 for more information.

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** panel 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 6 plates.*

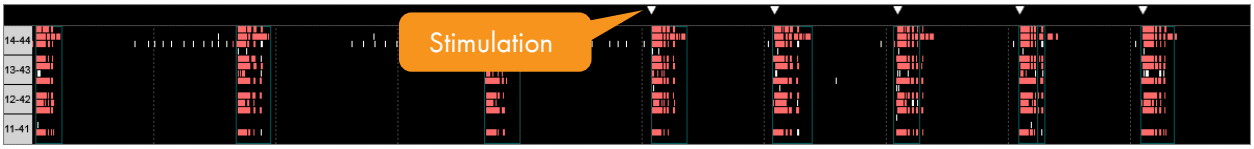
The spike waveform plot has two copy options. To copy the entire well image, right-click anywhere on the plot and select **Copy Well Plot**. To copy a single electrode, right-click on the electrode and select **Copy Electrode Waveform**. This copies both the electrode image as well as the time and voltage values used to generate the image. To paste the electrode image, right-click to 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**.

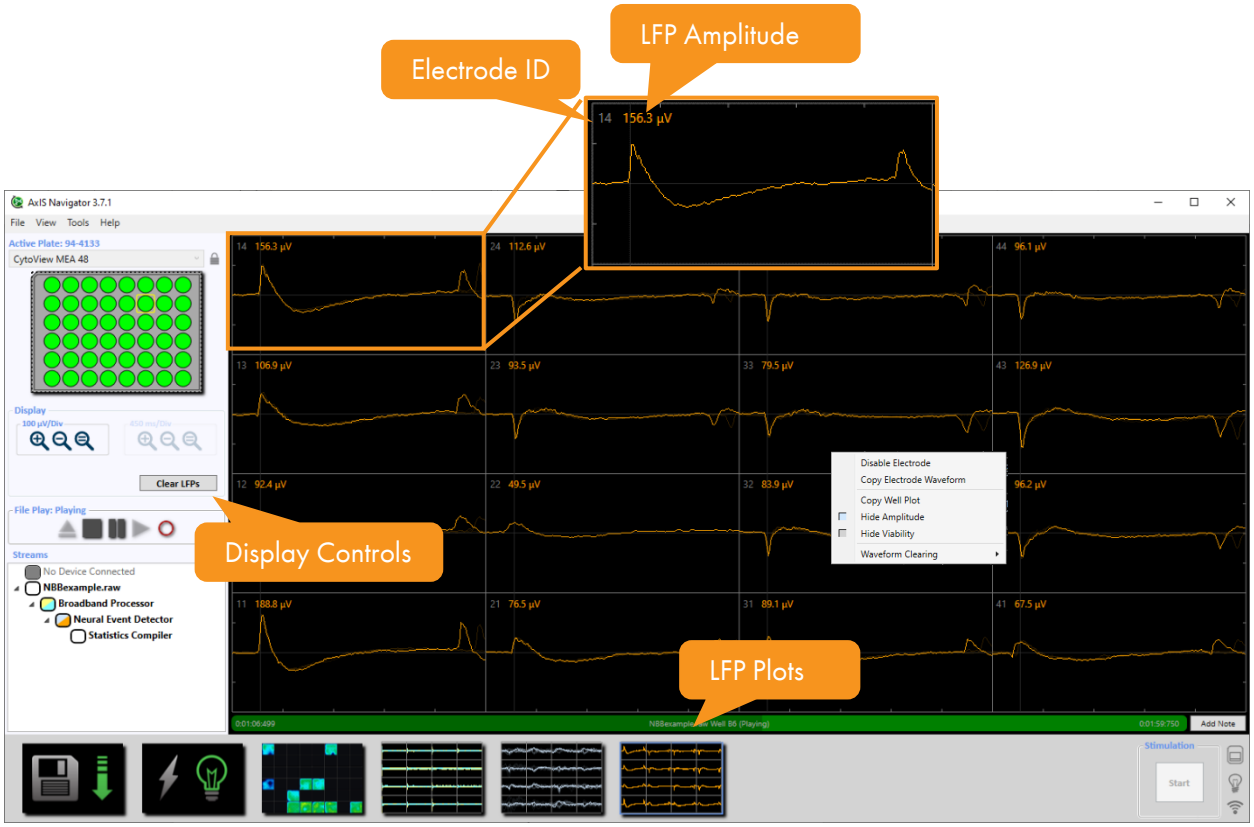
When electrical or optical stimulation is used, the stimulation protocol can be designed such that *AxIS Navigator* saves stimulation time tags to the .raw file. Stimulation tags contain information about the timing of the stimulus (see Chapter 4 for more details about stimulation). After starting the 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. LFP Plots

The **LFP Plots** panel uses output from a **Broadband Processor** and **Neural Event Detector** (See Sections 2.3.5 and 2.3.8, respectively) to display the LFP waveforms. Like the **Continuous Waveform Plots**, each electrode of the active well is represented in the active window of the **LFP Plots** display. The gray electrode ID corresponds to the electrode ID in the **Continuous Waveform Plots** and the adjacent number is the most recent LFP amplitude, displayed in µV. Right click on the electrode and select **Hide Amplitude** to hide amplitude values. If a Viability configuration (only available with the MEA Viability Software Module) was used, the cell-electrode Resistance value is displayed in kΩ adjacent to the amplitude value. Right-click on the electrode and select **Hide Viability** to hide resistance values. If -- kΩ is displayed, the electrode impedance is out-of-range and resistance cannot be measured. See Chapter 7 for more information.

The waveform plots overlay each LFP with the brightest trace representing the most recently detected LFP on that electrode. Previously detected LFPs fade to black. Empty black panels indicate no LFPs have been detected on those electrodes. LFP detection and display settings can be adjusted in the **Neural Event Detector Settings** (See Section 2.3.8).



The LFP waveform plots will display a maximum of 7 LFPs overlaid at once. To specify how often LFP waveforms are removed from the plot, right-click on the plot and select **Waveform Clearing**. Options include:

After 1 Minute	LFP waveforms are cleared 1 minute after they are detected.
Manual	LFP waveforms are not cleared unless **Clear LFPs** is selected.

In all cases, if more than 7 LFPs 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 **LFP Plots** panel 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 6 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 to 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 LFPs. Each column contains the data samples for a single LFP waveform, up to the maximum of 7 LFPs displayed at any given time, with the most recent LFP data in the last column.

Time (s)	LFP 0 (V)	LFP 1 (V)	LFP 2 (V)	LFP 3 (V)	LFP 4 (V)	LFP 5 (V)
-0.25	-6.86E-06	2.61E-05	3.66E-05	8.12E-06	-5.02E-05	2.58E-05
-0.24	-6.91E-06	2.68E-05	3.62E-05	7.84E-06	-5.78E-05	2.61E-05
-0.23	-7.02E-06	2.64E-05	3.59E-05	7.51E-06	-7.06E-05	2.66E-05
-0.22	-7.79E-06	2.70E-05	3.53E-05	7.79E-06	-7.04E-05	2.61E-05

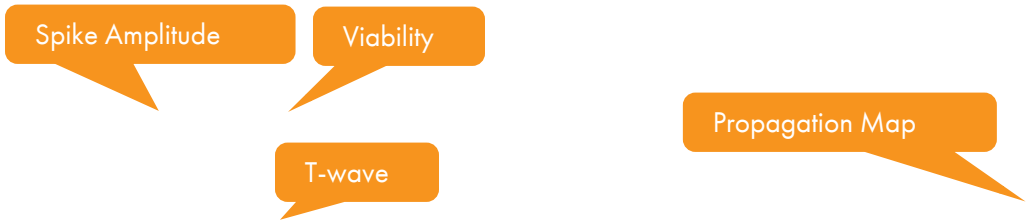
2.6.9. Cardiac Beat Plots

The **Cardiac Beat Plots** panel uses output from a **Cardiac Beat Detector** (See Section 2.3.7) to display cardiac voltage or contractility waveforms, conduction maps, beat period plots, and other relevant figures for the active well. The **Cardiac Beat Plots** panel displays different figures depending on the **Acquisition Settings** used for recording. Each variation is described below.

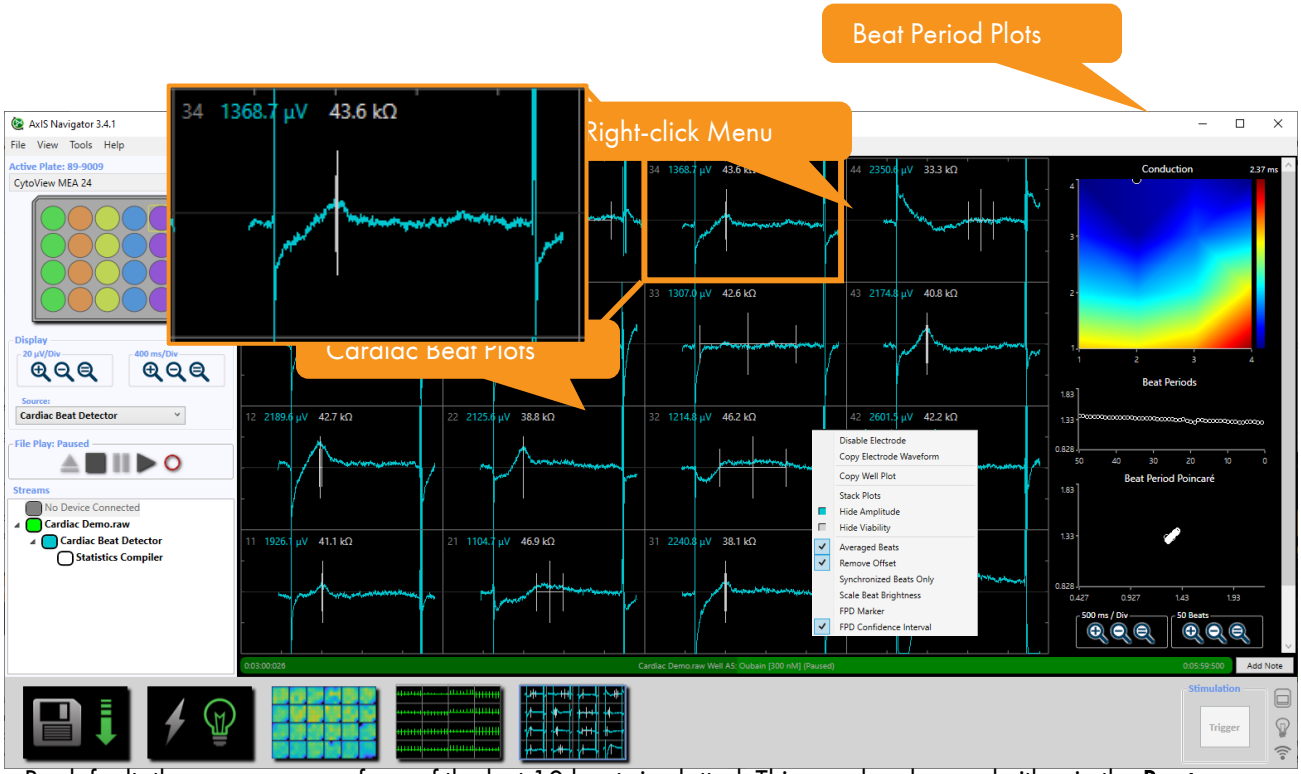
Cardiac: Standard, Broadband, and LEAP (voltage data)

For voltage data, the **Cardiac Beat Plots** panel displays 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. The amplitude value can be hidden by right-clicking on the plot and selecting **Hide Amplitude**. If a Viability configuration (only available with the **MEA Viability Software Module**) was used, the cell-electrode Resistance value is displayed in kΩ adjacent to the amplitude value. Right-click on the electrode and select **Hide Viability** to hide resistance values. If -- kΩ is displayed, the electrode impedance is out-of-range and resistance cannot be measured. See Chapter 7 for more information.

Warning: *Measuring MEA Viability shortly after LEAP induction may result in aberrant values. For best results,*



MEA Viability measurements should be made prior to LEAP induction.



By default, the average waveform of the last 10 beats is 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**.

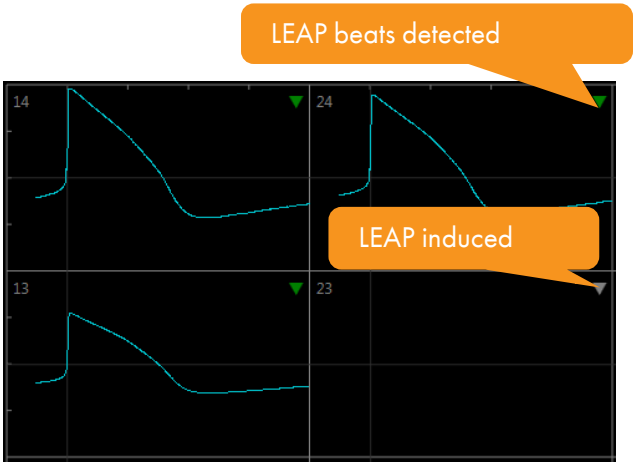
The field potential duration (FPD) detection is indicated by the blue rectangle and white whisker plot. It is disabled during data acquisition to limit processor workload.

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:

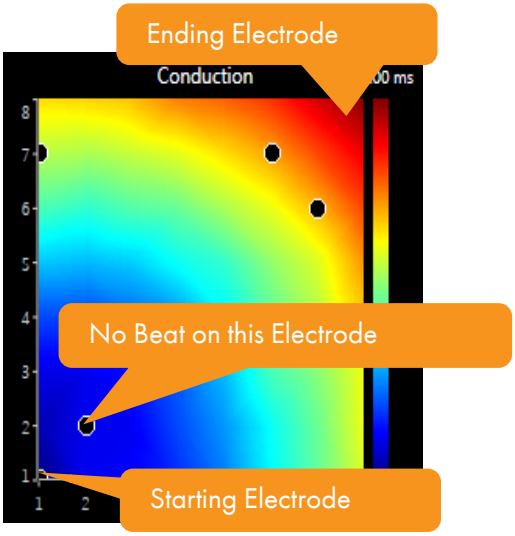
1. Click on the **Cardiac Beat Plots** panel in the **Control Bar**.
2. Right-click on the active window and select **Stack Plots**.
   ***Note**: The option to **Stack Plots** is not available when using CytoView MEA 6plates.*

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, *AxIS 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 gray 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 *AxIS 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.

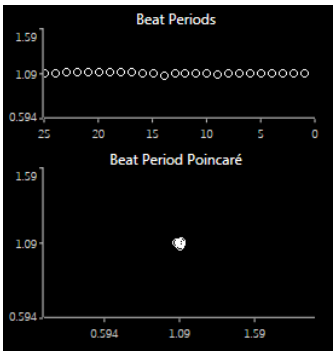
Right-click on any of the plots and select **Copy** to copy to the clipboard.

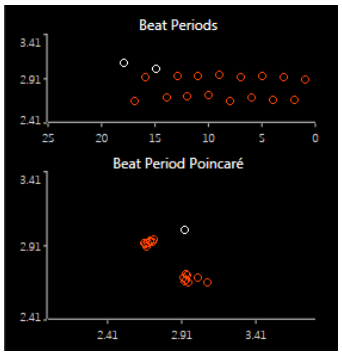
The **Conduction** Plot illustrates the propagation delay at each electrode from short (blue) to long (red). The well beat originates in the blue region and terminates in the red. The electrode column and row numbers are displayed on the x- and y-axis, respectively.

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

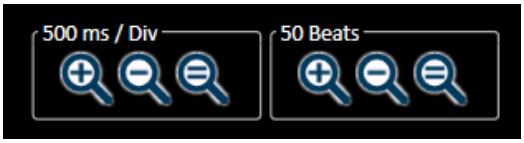
Option	Description
Auto Scale	Sets the scale of the plot. Disable to manually adjust the top of the scale with **Max Delay.**
Animate Conduction	Animates the plot with each beat. Disable to view a static plot that updates with each beat.
Extrapolate Missing Electrodes	Extrapolates values for electrodes 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 () controls to change time scales. The scale is indicated above the control as X units per division and N beats.

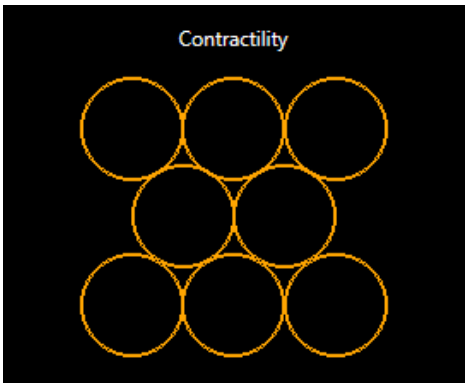


Cardiac: Contractility (contractility data)

For contractility data, the **Cardiac Beat Plots** panel displays the cardiac contractility waveforms, conduction map, contractility map, and beat periods plot 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 peak-to-peak amplitude as a percent change from baseline impedance over that electrode. Right-click on the electrode and select **Hide Amplitude** to hide the amplitude values. If a Viability configuration (only available with the MEA Viability Software Module) was used, the cell-electrode Resistance value is displayed in kΩ adjacent to the amplitude value. Right-click on the electrode and select **Hide Viability** to hide resistance values. If -- kΩ is displayed, the electrode impedance is out-of-range and resistance cannot be measured. See Chapter 7 for more information.

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 contractility 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 contractility samples in % (contractility is reported as a percent change from baseline impedance) 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**.

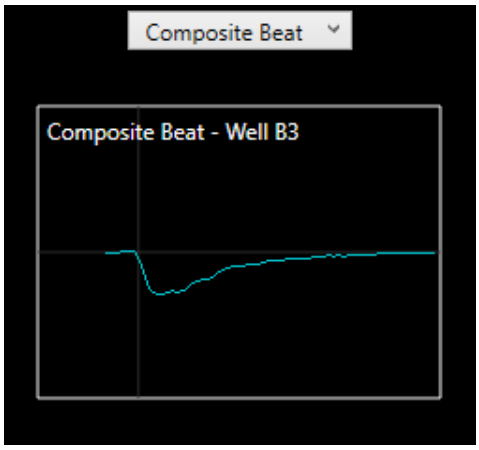
***Note**: Contractility beats are only shown if the file is recorded using **Cardiac: Contractility Acquisition Settings**. When other cardiac acquisition settings are used, field potential beats or LEAP beats are identified.*



To the right of the cardiac waveforms are three plots: the **Composite Beat** plot/**Conduction** map, **Contractility** map, and **Beat Periods** plot.

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

Using the drop-down menu above the plot, the **Conduction** map can be switched to show the **Composite Beat** plot. The **Composite Beat** plot displays the composite contractility waveform, computed by averaging the contractility waveforms recorded on the individual electrodes in the selected well.



Note: *The **Composite Beat** can include both contracting cells (positive waveform deflection) and stretching cells (negative waveform deflection) and is influenced by the spatial arrangement of cells over the electrode array. The average **Beat Amplitude** metric calculated during analysis (see Section 10.3) provides the average absolute peak-to-peak amplitude for all electrodes.*

The **Contractility** map displays a real-time representation of fluctuations in the shape of the cell(s) over the electrodes. Each circle represents an individual electrode for a selected well and changes in size according to its corresponding contractility signal. As the circle gets smaller, that represents nearby cells contracting, whereas a circle increasing in size represents nearby cells stretching. See Section 6.1 for more information on contractility waveform shapes.

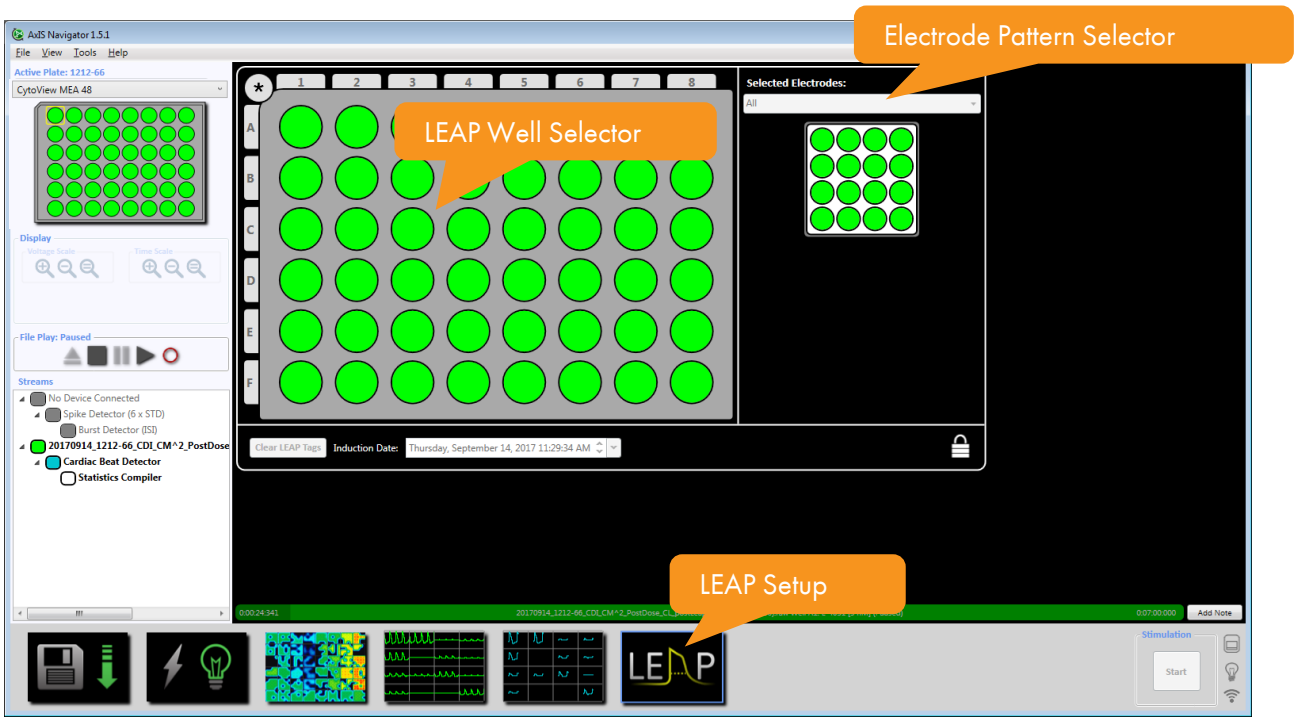
The **Beat Periods** plot displays beat period (in seconds) on the y-axis, plotted against beat number on the x-axis. Beats that exhibit a greater than 5% deviation from the previous beat are displayed as red, indicative of a possible arrhythmia.

2.6.10. LEAP Setup

The **LEAP Setup** panel 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 panel 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. *AxIS 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 *AxIS Navigator*. Section 3.3 contains a step-by-step tutorial for data acquisition.



3.1. AXIS NAVIGATOR COMMUNICATION WITH THE MAESTRO

AxIS 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 *AxIS Navigator* to begin system initialization. Startup can take a few minutes.

The status lights in the bottom right corner of *AxIS 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

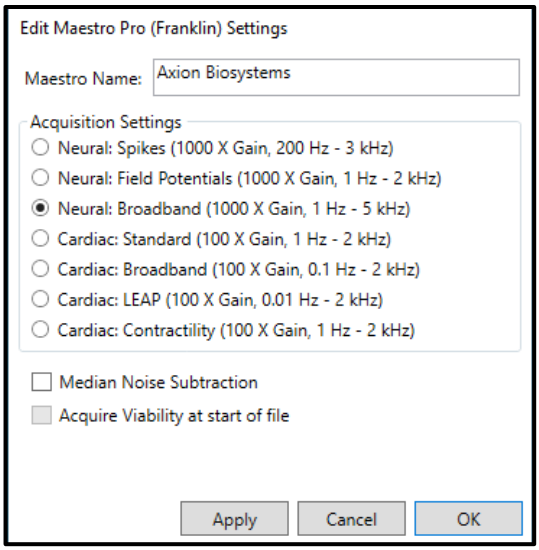
3.2. CONFIGURING THE MAESTRO FOR ACQUISITION

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 and 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:

1. **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: 200-3000 Hz	High gain with a neural bandwidth. Recommended for most neural applications.
Neural: Field Potentials	Gain: 1000X Bandwidth: 1-2000 Hz	High gain with lower frequency bandwidth.
Neural: Broadband	Gain: 1000x Bandwidth: 1 – 5000 Hz	High gain with wide bandwidth. Recommended for LFP applications.
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.
Cardiac: Contractility	Gain: 100x Bandwidth: 1– 2000 Hz	Low gain with a cardiac bandwidth. Use for contractility 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 when recording in Neural: Spikes or Neural Field Potentials, but not Neural:

Broadband. In highly active cultures, median subtraction in Neural: Broadband can sometimes create mathematical artifacts which appear to be good signals. When an appropriate neural option is selected in **Acquisition Settings**, median noise subtraction is enabled. It is disabled when a cardiac option is selected.

3. **Acquire Viability at start of file** option: If enabled, a viability measurement will be taken at the start of a file when the **Play** or **Record** button is pressed. To learn more about the **MEA Viability Software Module**, see Chapter 7.

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 acquisition and visualization.

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

Configuration	Processing Applied	Description
Cardiac Real-Time		
Field Potentials	Cardiac Beat Detector	Generates activity map, cardiac waveforms, beat rate, and conduction information. FPD detection disabled.
Field Potentials 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.
LEAP	Cardiac Beat Detector	Generates activity map, cardiac waveforms, beat rate, and conduction information. Optimized for acquiring LEAP signals.
LEAP 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.
Contractility	Cardiac Beat Detector	Generates activity map, cardiac contractility waveforms, beat rate, and conduction information.
Contractility Paced	Artifact Eliminator Cardiac Beat Detector	Generates activity map, cardiac contractility waveforms, beat rate, and conduction information. Optimized for reducing stimulus artifacts in pacing experiments.
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.
Field Potentials Spontaneous	Filter (1Hz – 200 Hz) Filter (200 Hz – 2 kHz) Spike Detector Burst Detector	Generates activity map, spike waveforms, and raster plot. Network burst detection disabled. Applied filters are optimized to separate low (LFP) and high (spikes) frequency components of the signal for spike detection. (Not recommended for electrical stimulation)
Broadband Spontaneous	Broadband Processor Neural Event Detector	Generates activity map, spike and LFP waveforms, and raster plot. (Not recommended for electrical stimulation)

Note: *In the* ***Acquisition Settings****, Neural:* ***Field Potentials*** *or Neural:* ***Broadband*** *is generally selected to record lower frequencies for neural applications, while the cardiac* ***Field Potentials Configuration*** *is applied to record field potential data for cardiac applications. All of the Neural and Cardiac Real-time configurations can be used with the* ***MEA Viability Software Module*** *to allow users to measure impedance data and MEA data from the same electrodes. To learn more about the* ***MEA Viability Software Module****, please see Chapter 8.*

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

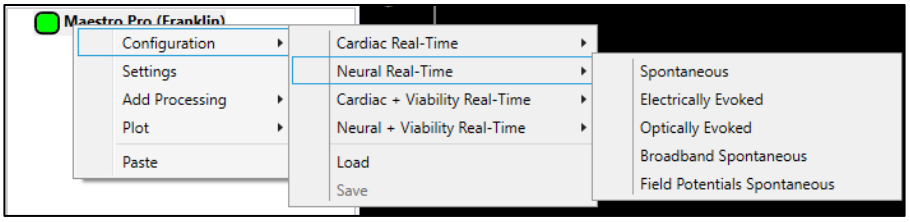
1. Turn on the Maestro with the button on the left side.
2. Open *AxIS Navigator.*
3. Allow the system approximately 1 minute to connect as indicated by the Maestro status light (See Section 3.1 for more information on Status Lights).

   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 CO_2 status on the touch screen.*
5. Right-click on the **Maestro Pro** or **Edge** stream and select **Configuration** to apply the desired configuration as outlined below. See Section 3.2 for more information on hardware and software configurations.

   Note*: Configuration options will depend on which software module(s) is active.*

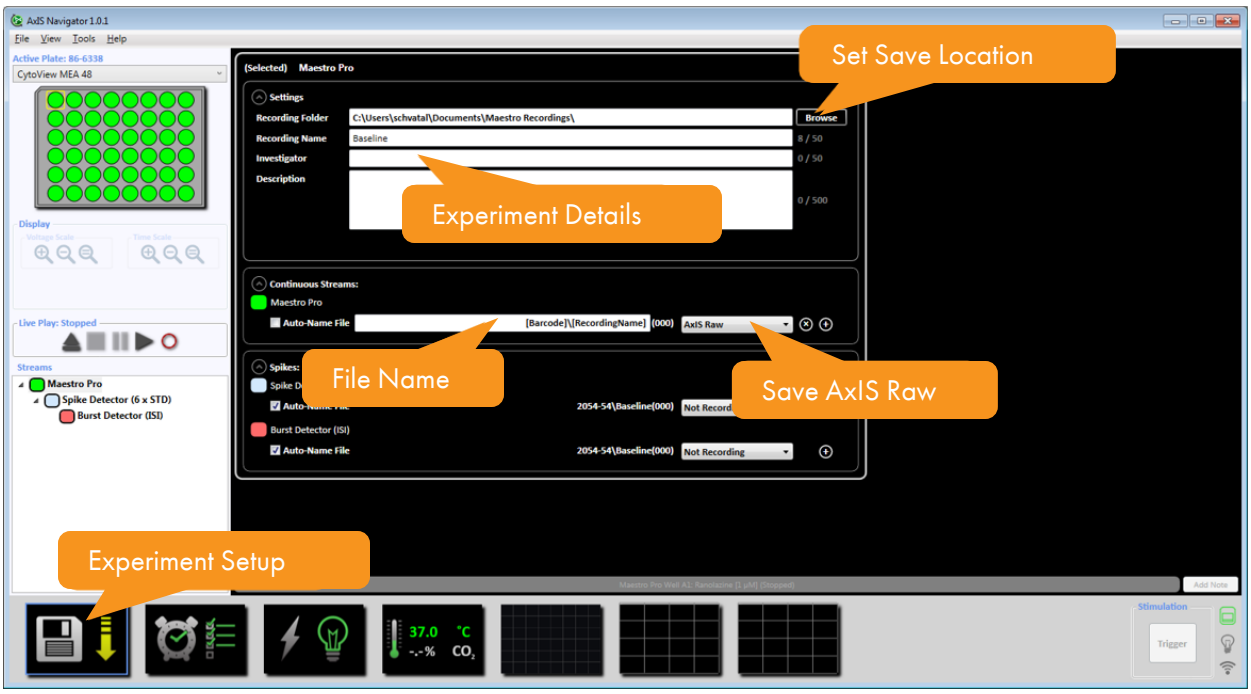
   a. **Cardiac Real-Time → Field Potentials, LEAP,** or **Contractility** with the option of **Paced** or **Viability** if needed.
   b. **Neural Real-Time → Spontaneous, Electrically Evoked, Optically Evoked, Field Potentials Spontaneous,** and **Broadband Spontaneous** with the option of **Viability** if needed.



6. Click the **Experiment Setup Properties** panel. See Section 2.6.1 for more information on the **Experiment Setup Properties** panel.
7. Optional: Click **Browse** to set the save location in the **Recording Folder** field. By default, files will be saved to the Maestro Recordings folder.
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 9.2 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 9.2.1 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.*



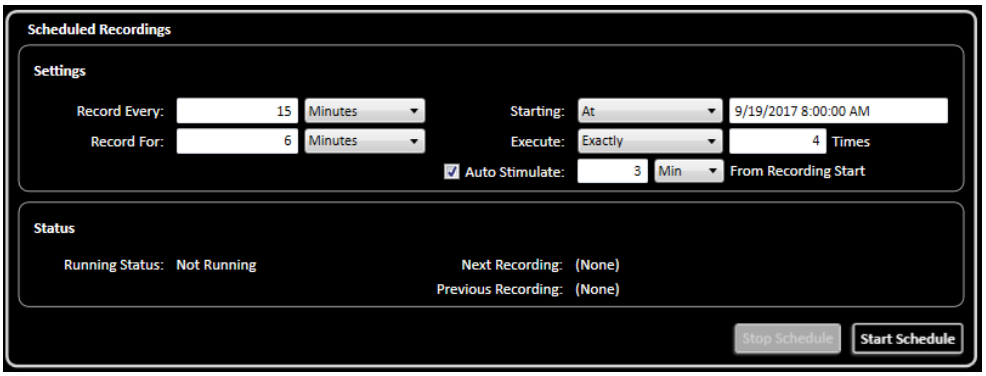
3.3.2. Recording a Single File from the Maestro

1. 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 CO_2.
2. Allow the MEA to equilibrate while docked in the Maestro for 10-20 minutes prior to recording.
3. 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.*
4. 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 or Viability acquisition*
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** panel. 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. ***Note**: For Viability Configurations, a message below Record For will indicate, "Viability will be acquired at the start of each file." Users must design their scheduled recordings to account for the viability measurement (~1 min) that is taken at the start of each scheduled time point (see Section 8.2).*
7. Click **Start Schedule** to begin recording.
8. Click **Stop Schedule** to stop recording after initiating a scheduled recording.



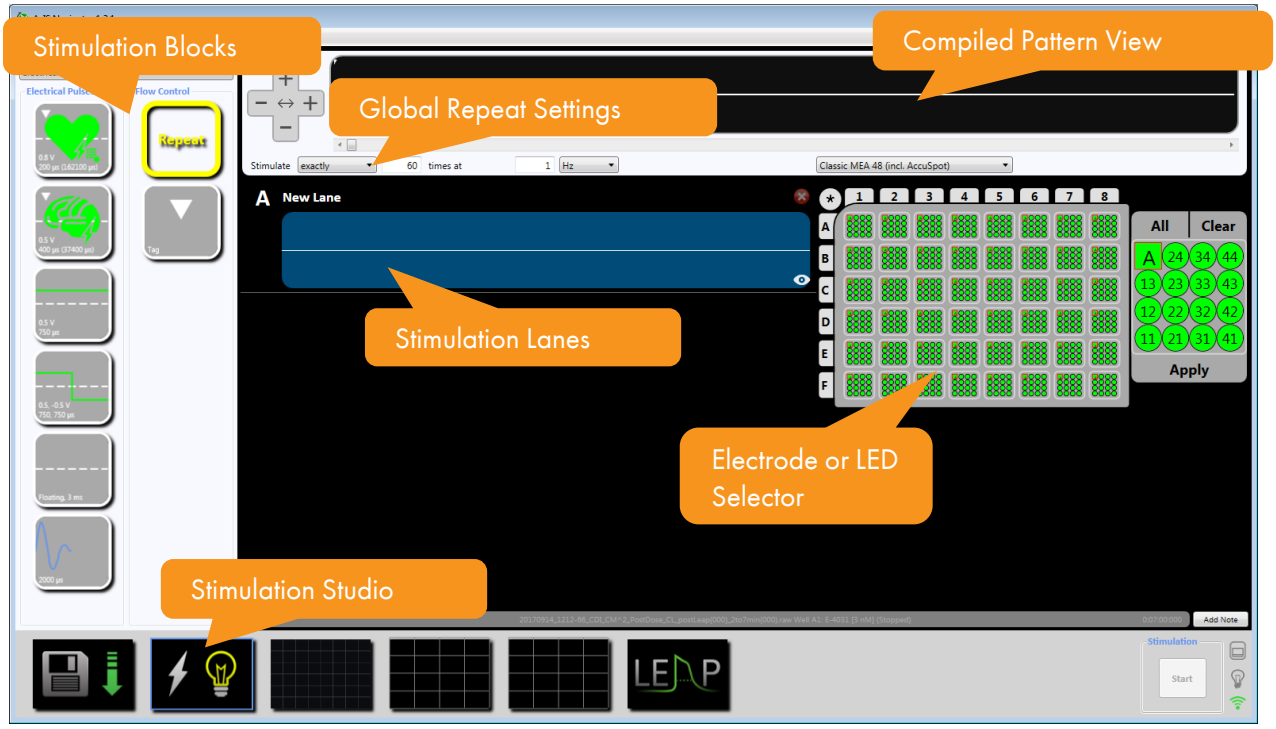
CHAPTER 4. STIMULATION

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

The **Stimulation Studio** panel 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:

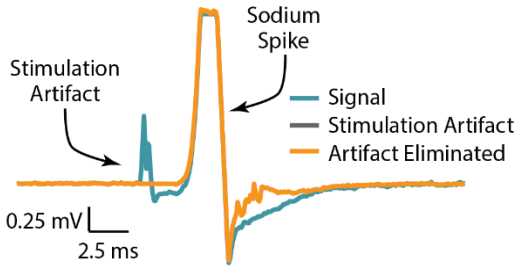
1. **Stimulation Blocks**: Predefined functions representing the basic elements of a stimulus protocol.
2. **Stimulation Lanes**: Displays the currently assigned stimulation blocks.
3. **Electrode or LED Selector**: Assigns the protocol from the selected stimulation lane to specific electrodes or LEDs.
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

Electrical stimulation applies a voltage or current waveform directly to the cells via electrodes in the MEA plate. 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** ("brain block") and **Cardiac Pacing** ("heart block") blocks are provided to optimize artifact elimination at the hardware level. 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 at the software level 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 appearance stimulation artifact during live streaming and file playback (Section 2.3.3).

Note: *The* ***Artifact Eliminator*** *relies on cues from the* ***Neural Stimulation*** *and* ***Cardiac Pacing*** *blocks to properly remove artifact. Applying the Artifact Eliminator with custom stimulations will have no effect.*

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, by selecting the desired configuration as outlined below:
    a. **Cardiac Real-Time → Field Potentials Paced, LEAP Paced,** or **Contractility Paced with the option of Viability if needed.**
    b. **Neural Real-Time → Electrically Evoked** with the option of **Viability** if needed.

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*** *panel. See Section 2.6.2.*

For data analysis with electrical stimulation:

1. Analyze data according to Section 9.5, selecting the **Field Potentials Paced, LEAP Paced, or Contractility Paced** or **Electrically Evoked** configuration.
   Note*: See the 'Neural Metric Tool' for evoked activity analysis options for neural analysis.*

4.1.1. Electrical Stimulation Blocks

The electrical stimulation blocks are:

Block	Icon	Description
Cardiac Pacing Stimulation		*Recommended stimulation block for cardiac applications for use with Cardiac* ***Electrically Paced*** *configuration.* 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, CytoView MEA 24 plates and **Microelectrode** for all other MEAs. ***Note****: This block already contains a stimulation time tag. Do not use an additional* ***Electrical Stimulation Tag*** *block with this block.*
Neural Stimulation with Artifact Elimination		*Recommended stimulation block for neural applications for use with Neural* ***Electrically Evoked*** *configuration (not recommended with Neural* ***Field Potentials Spontaneous*** *or* ***Broadband Spontaneous****).* 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. ***Note****: This block already contains a stimulation time tag. Do not use an additional* ***Electrical Stimulation Tag*** *block with this block.*
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.

		Note: *This stimulation will have a larger artifact because the* ***Artifact Eliminator*** *Data Processor (Section 2.3.3) only works with the* ***Neural Stimulation*** *and* ***Cardiac Pacing Stimulation*** *blocks.*
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. ***Note:*** *This stimulation will have a larger artifact because the* ***Artifact Eliminator*** *Data Processor (Section 2.3.3) only works with the* ***Neural Stimulation*** *and the* ***Cardiac Pacing Stimulation*** *blocks.*
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. While this block applies discharge, it does not enable software-based artifact elimination via the **Artifact Eliminator** Data Processor.
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		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. ***Note****: If there is no tag in the stimulation protocol, 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.*

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 increase 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. When using E-Stim+ Classic MEA 48, CytoView MEA 24, or BioCircuit MEA 24 plates, the stimulation paddle is typically used with a duration of 0.2-1 ms. For all other plates, microelectrode stimulation with 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:

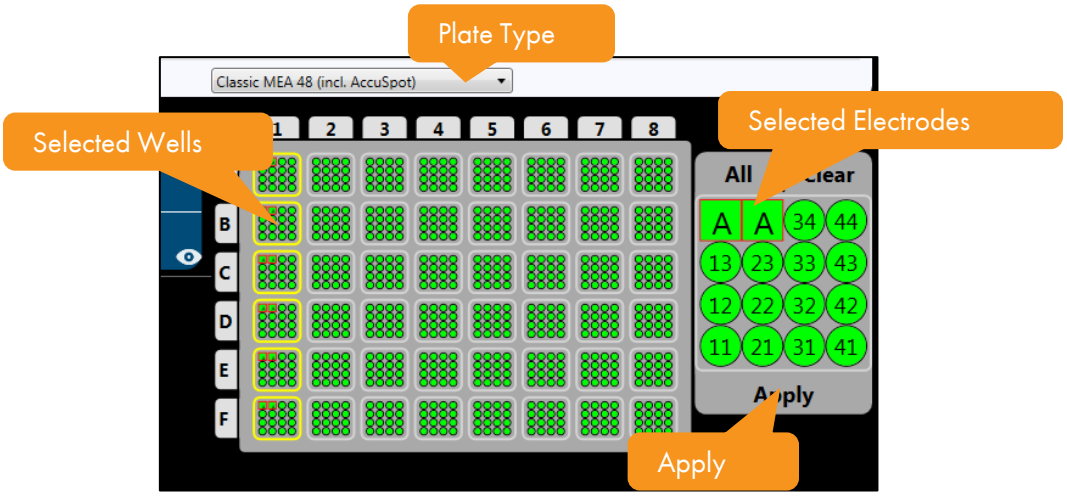
1. Right-click on the lane and select **Clear**.

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, CytoView MEA 24, and BioCircuit MEA 24 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 2.2.2).
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.*

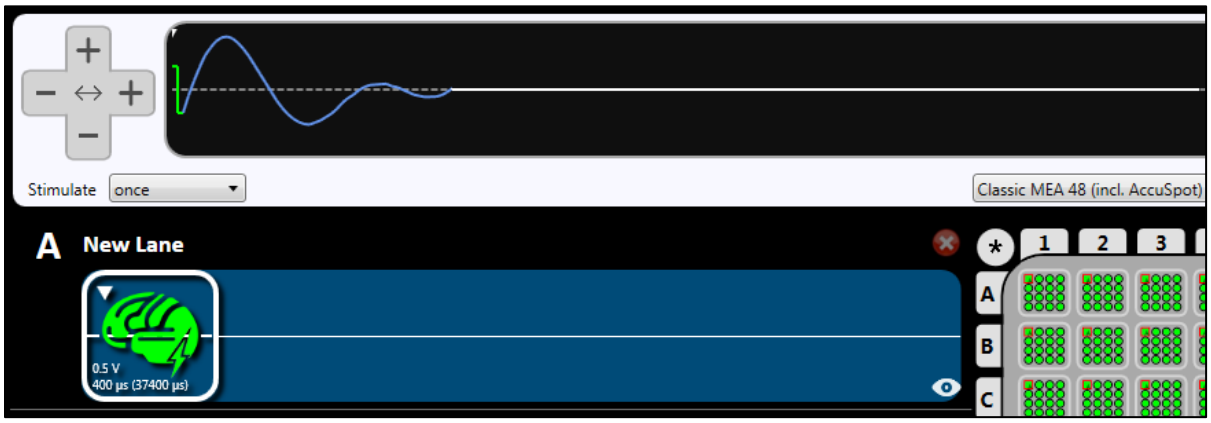


4.1.3. Example Neural Stimulation Patterns

To build the recommended neural stimulation protocol:

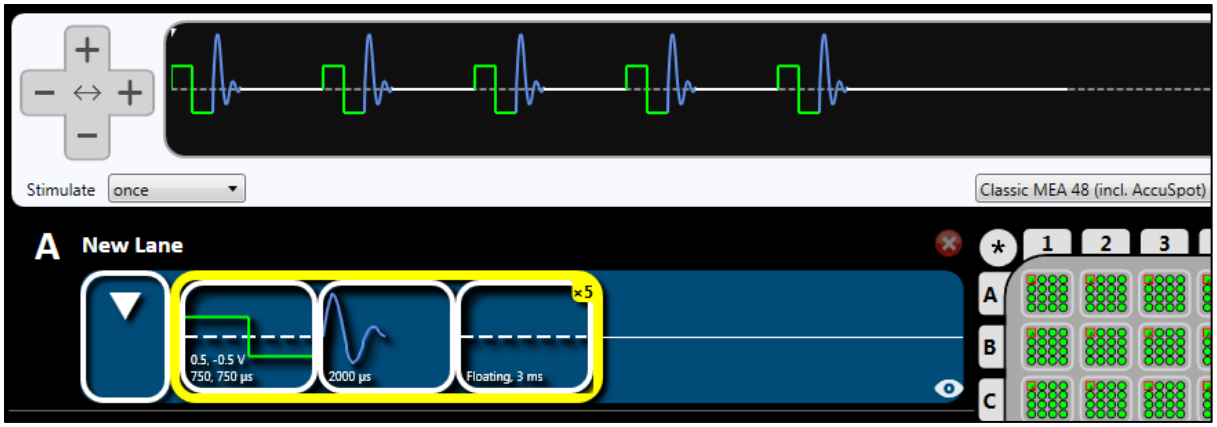
1. Select **Electrical** in the **Stimulation Type** drop-down menu.
2. Click and drag the **Neural Stimulation with Artifact Elimination** block into Lane A.
3. Optional: Double-click the block to change its settings.
4. Select an option from the **Stimulate** drop-down menu in the **Global Repeat Settings**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



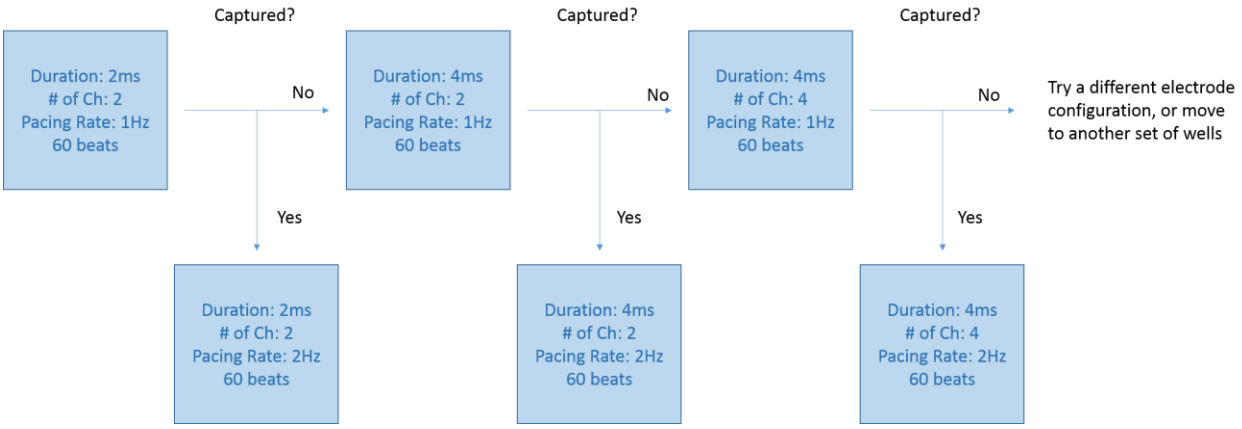
Note: Although the custom stimulation protocol described above contains a ***Stimulation Artifact Eliminator*** *block, custom electrical stimulation waveforms will have larger artifacts because the* ***Artifact Eliminator*** *Data Processor (Section 2.3.3) only works with the* ***Neural Stimulation*** *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.*



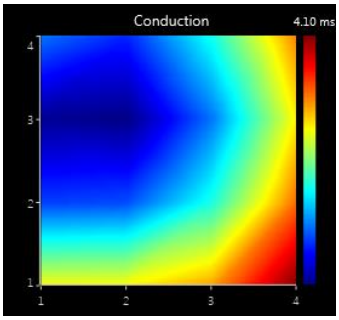
Stimulating using the dedicated stimulation electrode (**Stimulation Paddles**) on the CytoView MEA 24, E-Stim+ Classic MEA 48, and BioCircuit MEA 24 plates is generally more effective at capturing the network than stimulating through the microelectrodes. If the default parameters are not sufficient to capture the culture, change the stimulation by:

1. Increase the duration and/or amplitude in small increments (0.1 ms and/or 10 µA), not to exceed 1ms and 100 µA

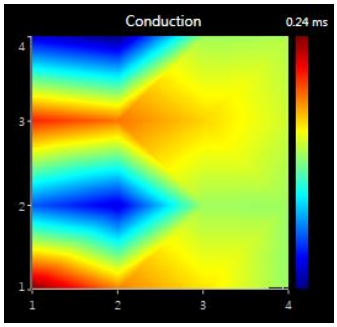
   ***Note**: Do not exceed 30 µA for BioCircuit MEA 24 Plates.*
2. Faster pacing frequencies may require larger stimulation parameters than slower pacing frequencies. If the stimulation still does not capture the culture, change the pacing frequency to a frequency closer to the culture's spontaneous beat 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** panel 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.

True Beats

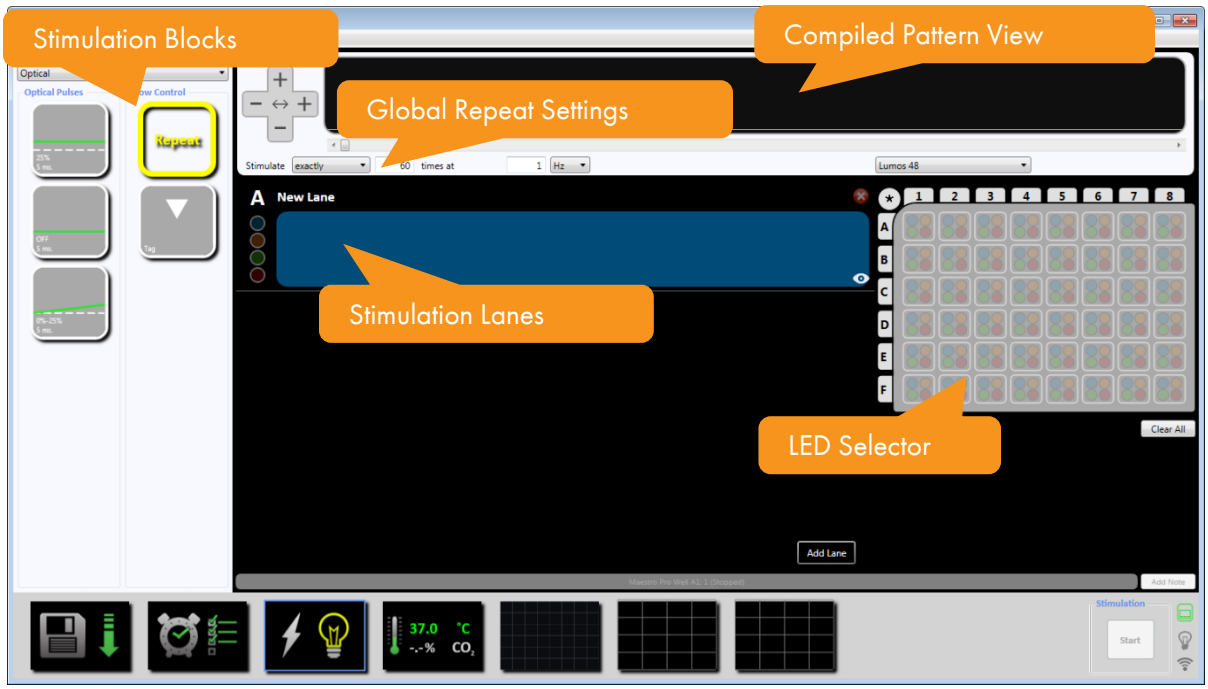


Stimulation Artifacts



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:

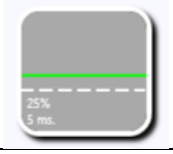
1. Build the stimulation protocol according to Section 4.2.2.
2. Start recording data according to Section 3.3, by selecting the desired configuration as outlined below:
    a. **Cardiac Real-Time → Field Potentials Paced, LEAP Paced,** or **Contractility Paced** plus **Viability** if needed.
    b. **Neural Real-Time → Optically Evoked** plus **Viability** if needed.
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** panel(Section 2.6.2).*

For data analysis with optical stimulation:

1. Analyze data according to Section 9.5, selecting the **Field Potentials Paced, LEAP Paced,** or **Contractility Paced** or **Optically Evoked** configuration.
    Note: *See the 'Neural Metric Tool' for evoked activity analysis options for neural analysis.*

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. ***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.*

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 Lumos model 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:

1. 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:

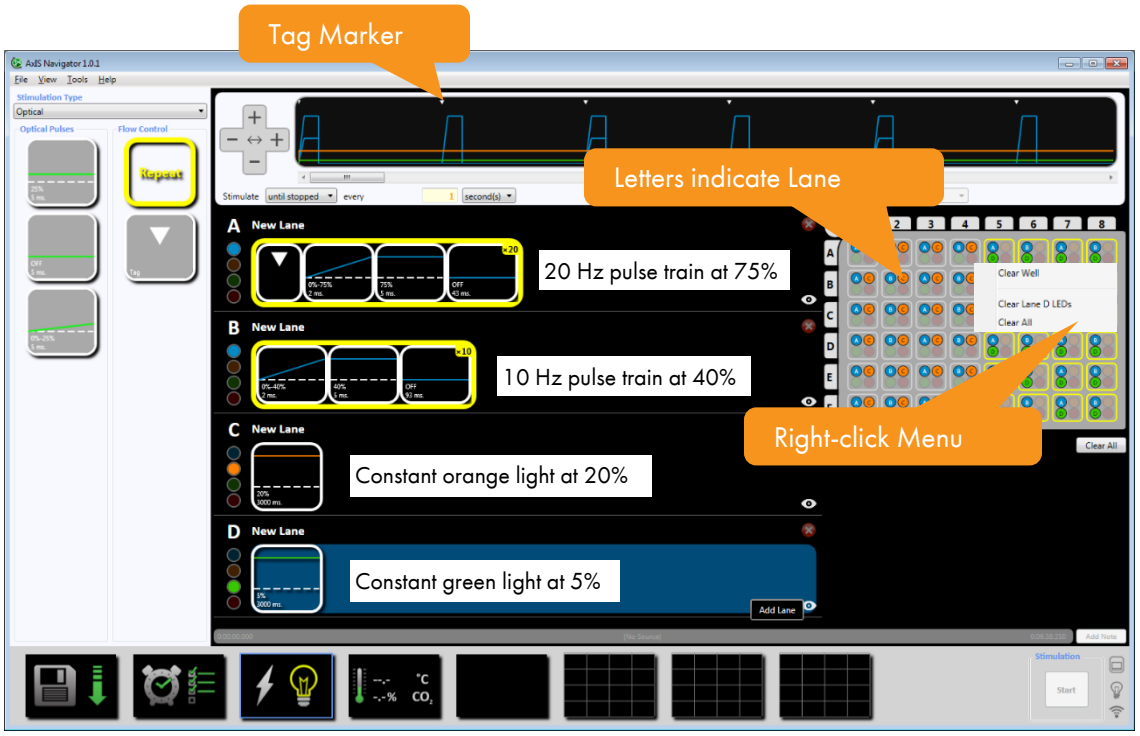
1. Select an option from the **Stimulate** drop-down menu in the **Global Repeat Settings** bar.

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:

1. Click on the stimulation lane to select it.
2. Click on the desired well(s) in the plate map in **Stimulation Studio** to apply the lane to that well. Selection is the same as the **Plate Map Editor** in **Active Plate** (See Section 2.2.2).
   Note: *When selected the lane color will become active with the lane letter inside.*

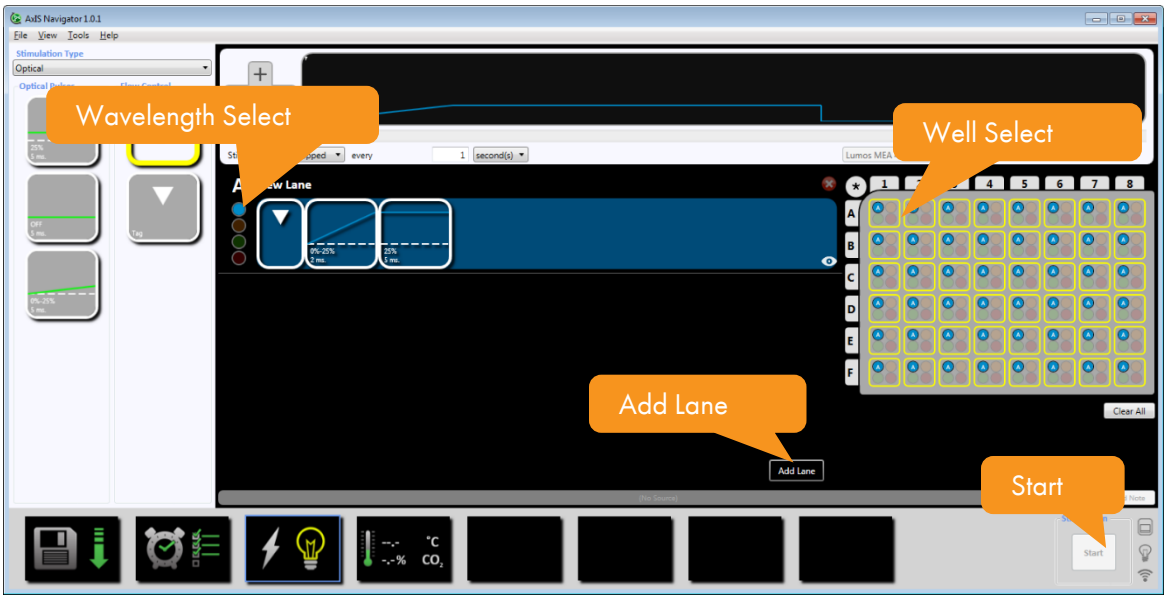
Multiple stimulation lanes of the same wavelength can be defined, but each well may only be assigned a single lane for each wavelength. Multiple stimulation lanes, each having a unique color, can be applied to the same collection of wells. Right-click on the **LED Selector** to quickly clear previous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

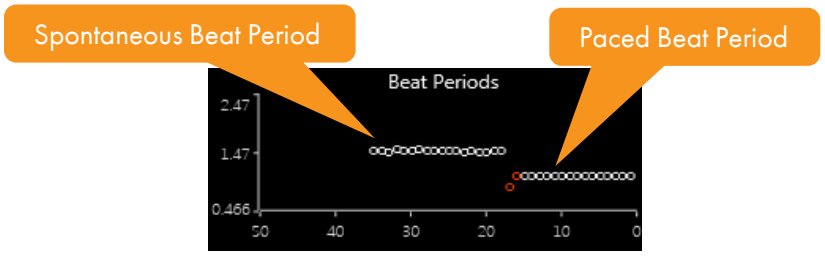
4.3. EVALUATING CARDIAC STIMULATION CAPTURE

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.

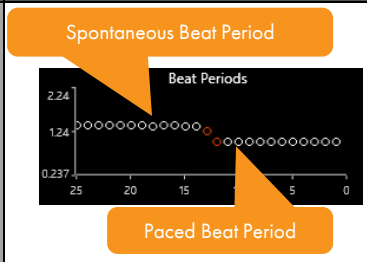
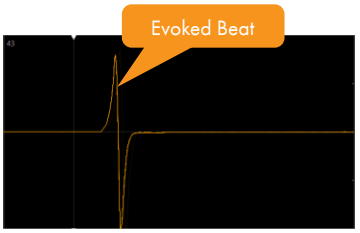
4.3.1. Field Potentials and LEAP Beat Capture

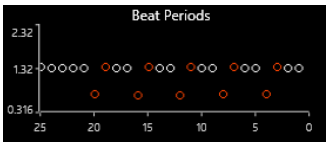
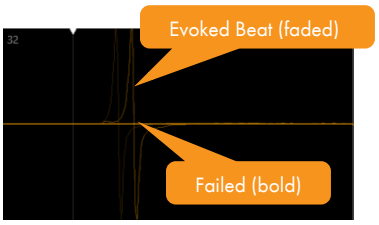
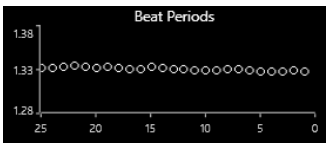
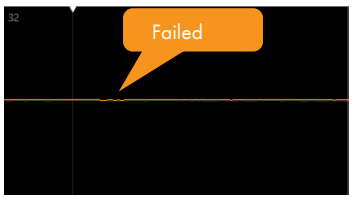
For voltage recordings using **Cardiac: Field Potentials** or **Cardiac: LEAP Acquisition Settings**, the **Beat Periods** plot in the **Cardiac Beat Plots** panel, the **Continuous Waveform Plots** panel, and the **Activity Map** panel are all useful in diagnosing whether a well has been captured.

In the **Beat Periods** plot, the spontaneous beat period will change to match the paced beat period.



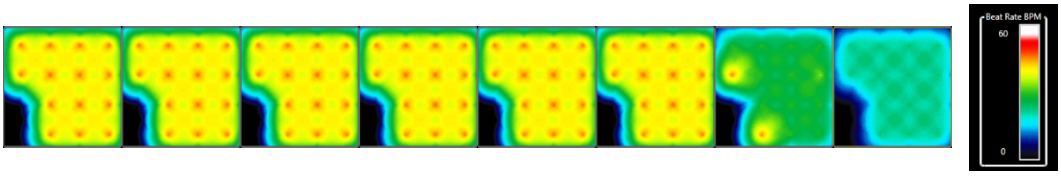
Capture can also be evaluated using the **Stimulation Inspector**. To add a stimulation inspector, right click on the Artifact Eliminator and select **Add Processing → Stimulation Inspector** (shown in orange in table below). The white triangle at the top indicates the timing of the stimulus. An evoked beat typically follows 2-5 ms after the stimulus. The following table illustrates how various capture conditions appear in the **Beat Period Plot** and **Stimulation Inspector** panel for pacing at 1 Hz:

Status	Beat Period Plot	Stimulation Inspector on Artifact Eliminator
Full Capture All stimuli elicit beats		

Partial Capture Some stimuli elicit beats		
No Capture No stimuli elicit beats		

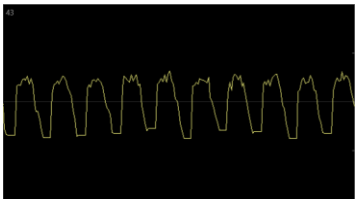
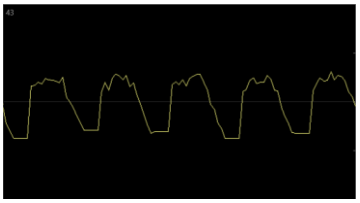
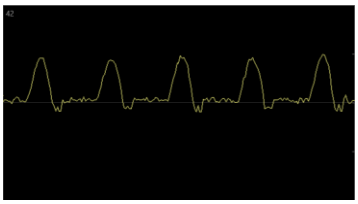
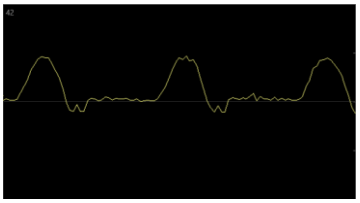
***Note**: The **Stimulation Inspector** displays voltage data, therefore is useful for evaluating capture in Cardiac Field Potentials Paced and Cardiac LEAP Paced configurations. It should not be applied to cardiac contractility signals.*

Capture can also 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. 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).

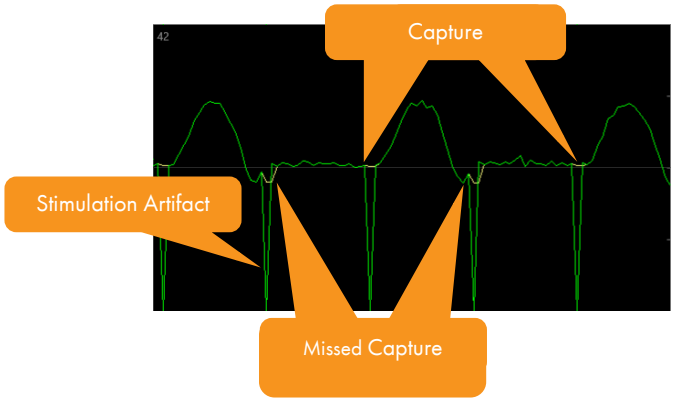


4.3.2. Contractility Beat Capture

Successful capture for contractility can be assessed in the **Continuous Waveform Plots** panel. Set the **Time Scale** to a value where the pacing rate can be evaluated by counting the number of evoked beats. If the number of beats doesn't agree with the stimulation rate, effective capture is not being achieved. The following table illustrates how various capture conditions for contractility appear in the **Continuous Waveform Plots** panel for pacing at 2 Hz:

Status	Scale set to 1s/div	Scale set to 500ms/div
Full Capture All stimuli elicit beats	2 contractility beats per div; 10 beats total in 5 sec window 	1 contractility beat per div; 5 beats total in 2.5 sec window 
Partial Capture Some stimuli elicit beats		

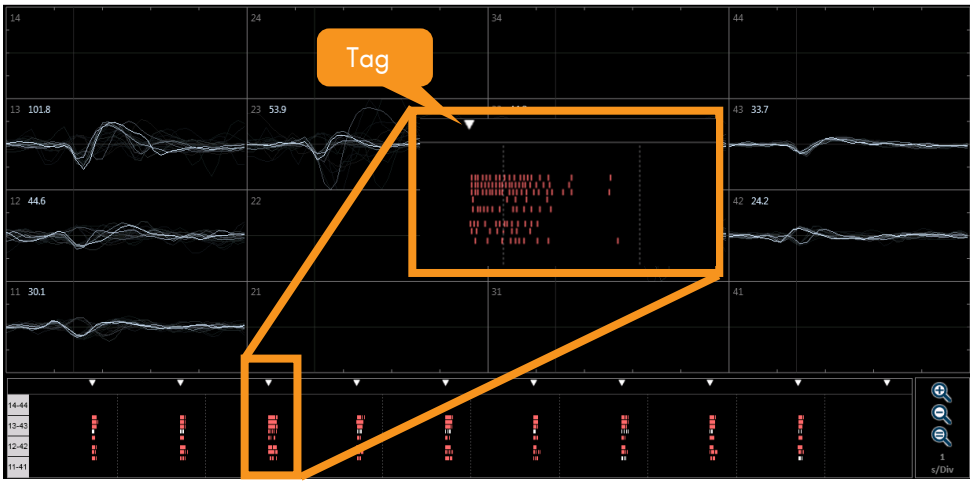
Alternatively, the Maestro trace can be plotted simultaneously with the Artifact Eliminator trace to show the stimulation artifact with respect to the contractility beat. In the example below, it can be easily seen that only some of the stimuli are evoking a beat, which is partial capture.



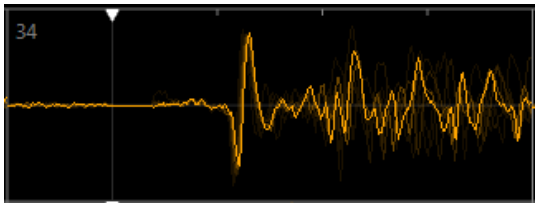
4.4. EVALUATING NEURAL STIMULATION CAPTURE

Neural stimulation capture can be viewed in the **Spike Plots** panel 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** panel. 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**.

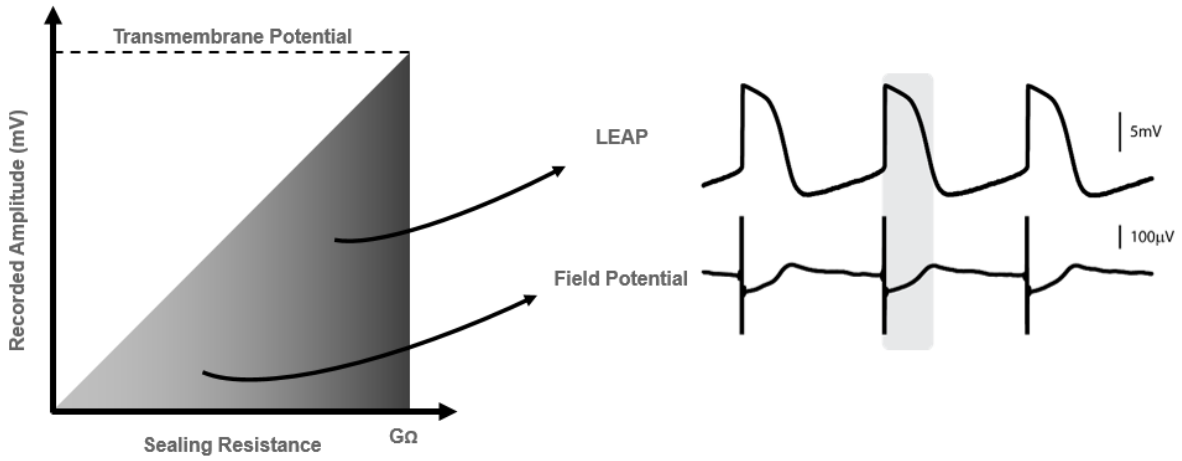


CHAPTER 5. LOCAL EXTRACELLULAR ACTION POTENTIAL (LEAP)

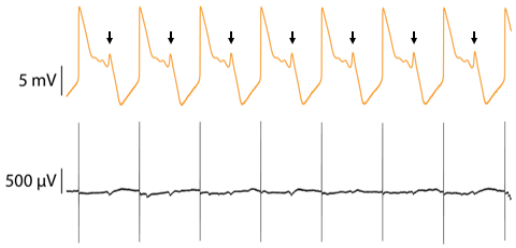
The Local Extracellular Action Potential (LEAP) assay allows acquisition of extracellular action potential waveforms from cardiomyocytes on Axion's MEA plates. Acquiring LEAP signals requires a proprietary induction procedure that increases the coupling between the cells and the electrodes. Unlike traditional 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

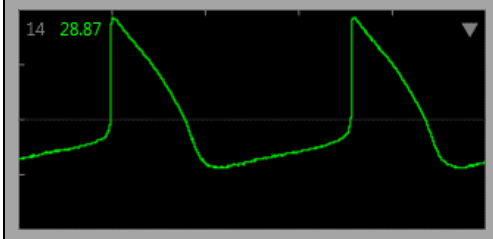
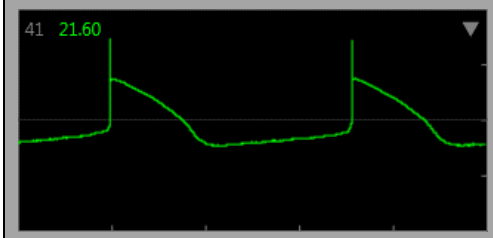
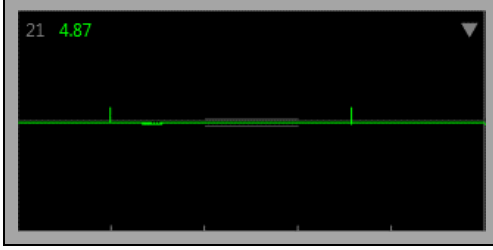
In a typical cardiomyocyte-MEA assay, field potential signals are detected from cells near the electrodes, which are the result of the cardiac action potential propagating across the array, similar to the way in which the ECG arises from propagation of the cardiac action potential across the heart. The 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.



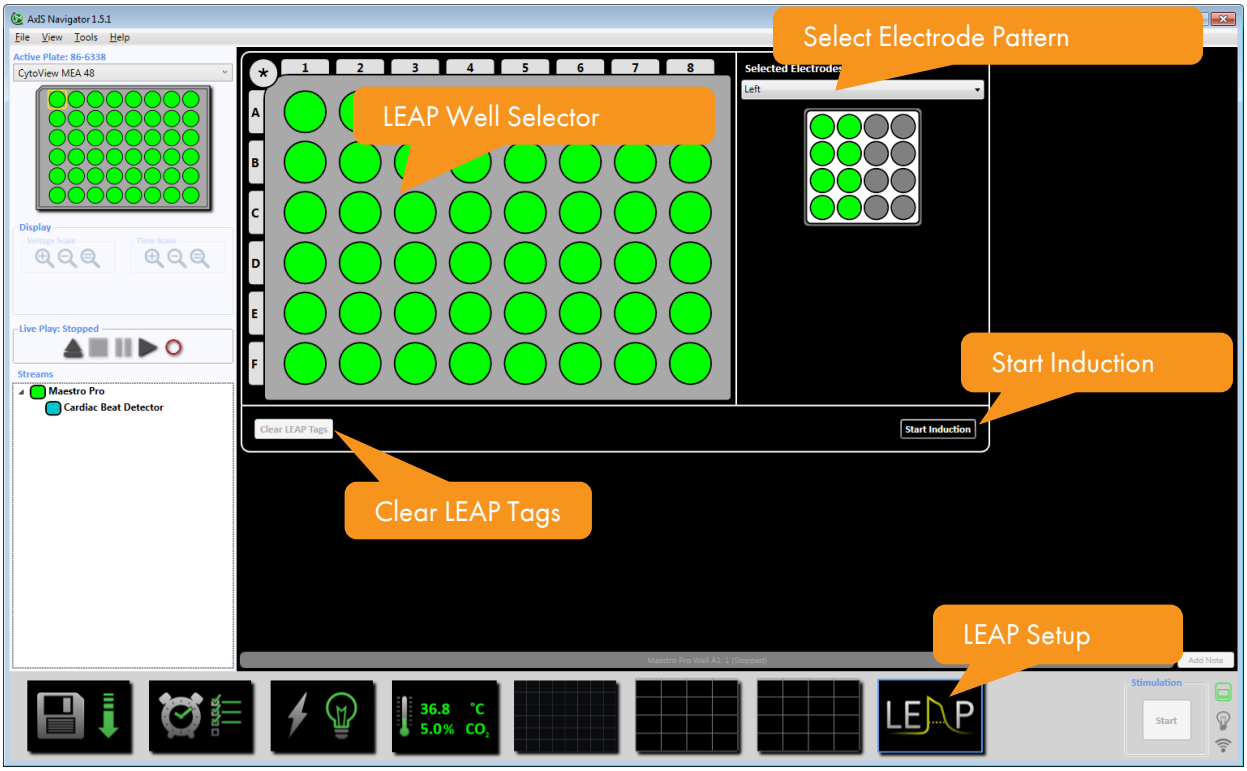
The signal detected on each electrode always contains contributions from both the field potential and LEAP; the LEAP assay is simply a way of enhancing the cell-electrode coupling such that the relative contribution of the LEAP signal is much larger. As such, a variety of waveforms may be observed after 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* Cardiac Analysis Tool.

5.2. DESIGNING A LEAP INDUCTION PLATE MAP

The **LEAP Setup** panel 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 gray 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: *Use the **Cardiac Real-Time → LEAP Paced** configuration when pacing electrically or optically. Applying the LEAP configuration will cause the **LEAP Setup panel** to appear.*
3. Design a LEAP induction plate map in the **LEAP Setup** panel 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.*

After inducing LEAP, tags are added to the .raw file indicating which electrodes were selected for LEAP induction. LEAP tags are retained and included in all subsequent recordings using any Cardiac **Acquisition Settings** so that the LEAP signals are analyzed correctly. To remove LEAP tags from subsequent recordings, select **Clear LEAP Tags** in the **LEAP Setup** panel before recording the next .raw file.

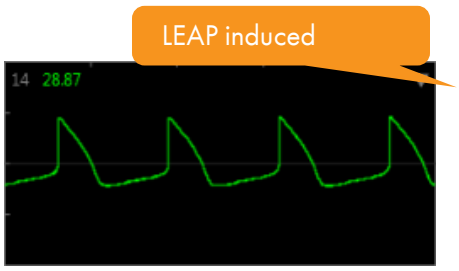
Note*: LEAP tags are cleared automatically when a new plate is docked in the Maestro.*

To analyze LEAP signals:

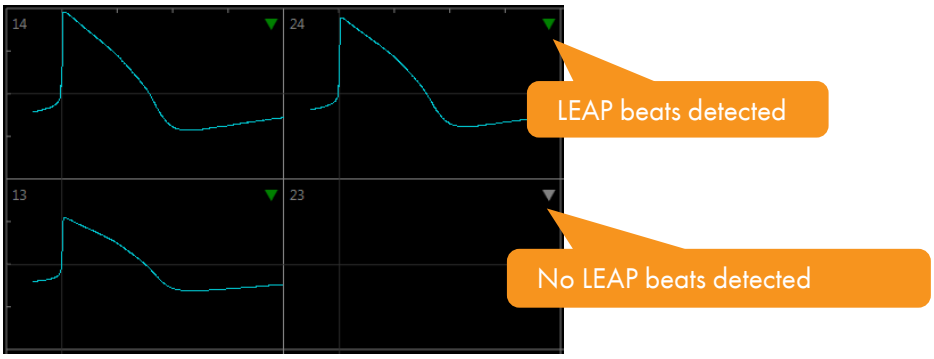
1. Follow the steps for data analysis as described in Section 9.5, using **Configuration → LEAP** to save a Cardiac Statistics Compiler **Advanced Metrics** .csv file.
2. Load the .raw and .csv files into the Cardiac Analysis Tool to generate LEAP metrics, such as Action Potential Duration (APD) 30%, 50%, and 90% repolarization.

The **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.

In the **Continuous Waveform Plots**, an inverted gray 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 gray triangle in the upper right corner indicates LEAP was induced on the electrode, but no LEAP beats are detected.



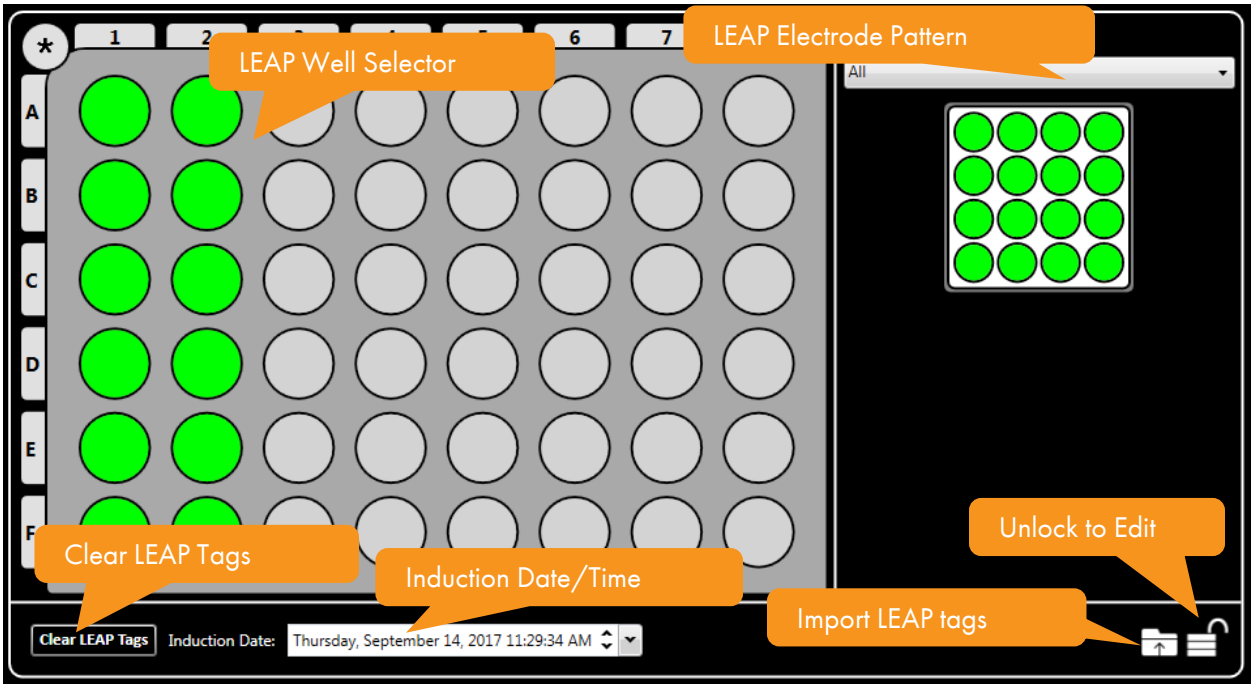
5.3.1. Adding or Removing LEAP Tags from Files

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 previously recorded 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, so it may be desirable to add LEAP tags for analysis.

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** panel.
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.*

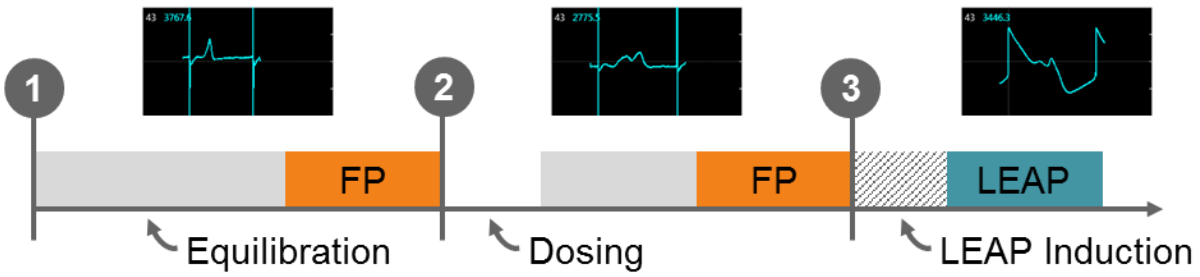
To remove LEAP tags from a .raw file:

1. Load the .raw file into *AxIS Navigator* by selecting **File → Open Recording(s)...**.
2. Click the thumbnail to select the **LEAP Setup** panel.
3. Click the **Lock** icon to unlock the LEAP induction plate map for editing.
4. Click **Clear LEAP Tags** to deselect 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* Cardiac 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* Cardiac Analysis Tool*. Please see the* Cardiac Analysis Tool *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 minute 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?**
    Axion'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?

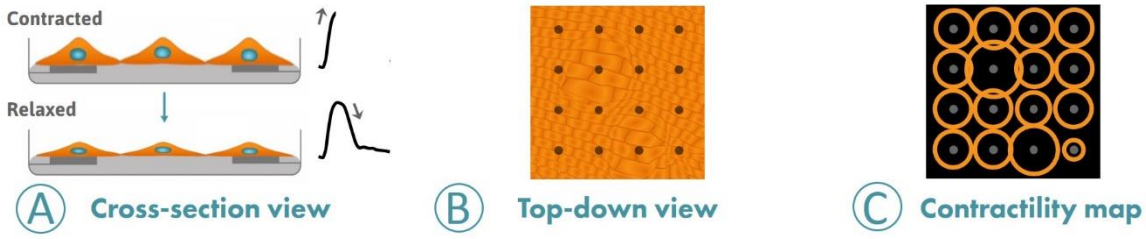
    Yes. As with any assay, the user should quantify the degree to which biological variability may impact certain comparisons. For example, minor differences in LEAP signal shape on neighboring electrodes may be due to the distinct populations of cardiomyocytes and the cell-electrode coupling present on 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.

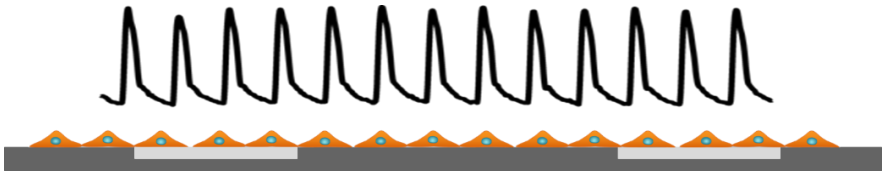
CHAPTER 6. CONTRACTILITY

When cardiomyocytes are plated over an array of microelectrodes, they form a spontaneously beating syncytium. Every beat is characterized by a propagating electrical impulse, measured by the field potential or action potential, that triggers a mechanical contraction of the heart muscle. As the cells contract and relax over an array of electrodes, cells change shape and thus change the coverage over the electrode. These rhythmic changes with each beat can be detected by impedance measurement technology, and represent the contractility of the cardiomyocytes. The Maestro offers high resolution contractility from an array of microelectrodes, providing a rich detailed map of contraction across the entire syncytium.

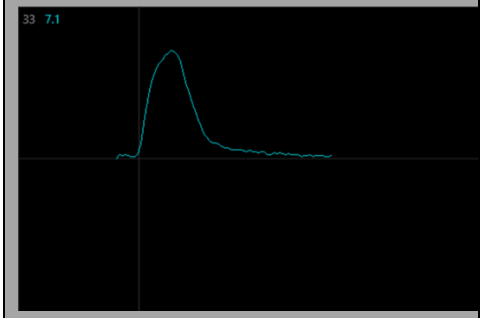
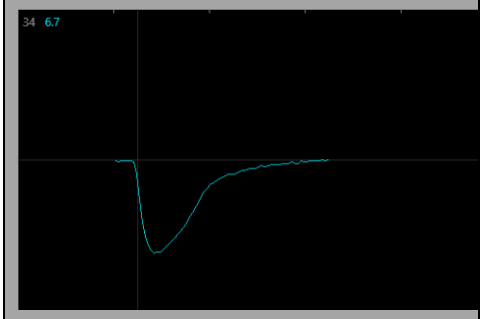
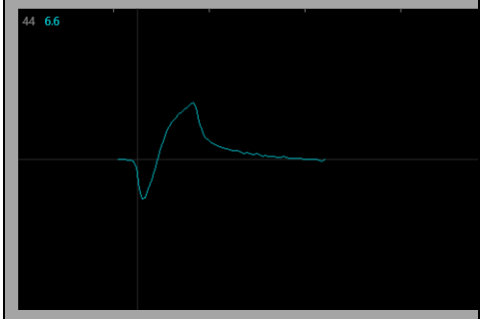
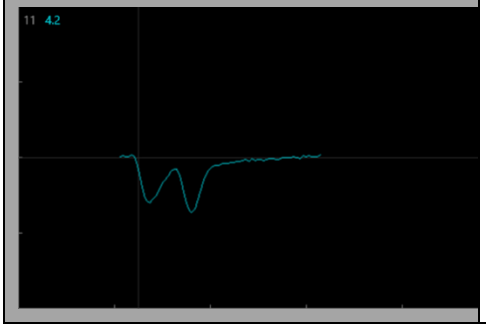


6.1. CONTRACTILITY BACKGROUND

Impedance describes the barrier to current flow out of the microelectrodes. Impedance varies with cell coverage, cell shape, and with the contraction and relaxation of the cells over the electrode. Contractility is the use of impedance technology to measure the small rhythmic changes in cardiomyocyte shape that occur with each beat.



The contractility signal detected on each electrode represents the local mechanical contraction and relaxation of the syncytium. As the electrical impulse (field potential) propagates and the cells contract and relax, they pull against each other and the attachment sites in the well. Thus, within a syncytium, some cells are contracting while others are relaxing, resulting in positive, negative, and biphasic contractility signals depending on the location in the culture. Example waveforms are illustrated below:

Waveform	Description
	Positive contractility waveform represents an increase in impedance on the electrode, likely caused by contraction and relaxation of the cardiomyocytes over the electrode
	Negative contractility waveform represents a decrease in impedance on the electrode, which may be the result of local cardiomyocytes being stretched by the contraction of neighboring cardiomyocytes
	Biphasic contractility waveform represents both a decrease and increase in impedance on the electrode, which may be due to cardiomyocytes being stretched and then contracting over the electrode
	Triphasic contractility waveform represents both a decrease and increase in impedance on the electrode, which may be the result of cardiomyocytes being stretched by neighboring cardiomyocytes before and after the peak contraction of the local cardiomyocytes

6.2. CONTRACTILITY ACQUISITION AND ANALYSIS TUTORIAL

To acquire contractility signals:

Follow the steps for data acquisition as described in Section 3.3, selecting **Configuration → Cardiac Real-Time → Contractility** to appropriately configure the hardware and software to record contractility signals.

To analyze Contractility signals:

Follow the steps for data analysis as described in Section 9.5, using **Configuration → Contractility** to save a Cardiac Statistics Compiler **Advanced Metrics** .csv file. LEAP and field potential signals may be recorded in series with contractility signals, by selecting a different **Cardiac Real-Time** configuration.

For more in-depth analysis of Contractility signals, .raw Contractility files can be imported and analyzed with the **AxIS Metric Plotting Tool**. This can be useful if field potential amplitudes are weak (<250 µV) or to provide additional Contractility metrics, such as Beat Width and Composite Beat Amplitude.

6.3. FAQ

1. How does contractility work?
   Contractility uses impedance technology to measure the beating of cardiomyocytes. As the cells contract and relax over an array of electrodes, the cells change shape and, thus, change the coverage over the electrodes. These changes are detected as rhythmic changes in impedance and represent the contractility of the cardiomyocytes.
2. How is contractility different on the Maestro?
   The Maestro offers high resolution array-based contractility. The Maestro samples from an array of microelectrodes to provide local measures of cardiomyocyte contractility with high spatial resolution from all across the culture. AxIS Navigator provides both a local contractility Beat Amplitude on each electrode, as well as well-wide composite contractility endpoints, including Composite Beat Amplitude, Beat Period, and Excitation-Contraction Delay, to describe average contractility behavior in each well. In contrast, most electrode-based impedance systems rely on 1-2 larger electrodes to provide a single measure from each well.
3. Why do contractility shapes and amplitudes vary?
   At any given moment, some regions of the syncytium are mechanically contracting, while others are being stretched. Array-based contractility allows the user to identify how contractility varies across the syncytium and to track these local changes in contractility. In addition, these mechanical patterns can be represented as a Contractility Map, where the relative size of each orange circle in the array indicates whether the local cells are contracting or being stretched.
4. Will contractility work with poor cell coverage?
   Yes. Array-based contractility is robust against poor cell coverage and allows the user to track local changes in contractility even if the other electrodes in the well are not well covered.
5. Can contractility be measured from 3D cardiac constructs?
   Yes. Array-based contractility enables advanced applications, such as measuring the contractility from several 3D spheroids in the same well. A large electrode, as used by other systems, would smear these signals or not detect them at all.
6. Is contractility compatible with all plate types?
   No. Contractility is compatible with premium CytoView MEA plates, which offer low impedance PEDOT microelectrodes. Contractility is not compatible with higher impedance Classic and BioCircuit MEA plates.

7. **Does contractility work for all cell types?**
   Contractility has been evaluated with most commercially-available iPSC-derived cardiomyocytes and multiple "home-grown" cardiomyocyte lines. Contractility requires cells to be mechanically active and cover at least one electrode.
8. **Can I record contractility on the original Maestro?**
   No. The Maestro Pro or Edge are required for contractility.
9. **Is contractility compatible with pacing?**
   Yes. The standard pacing tools available in AxIS Navigator may be used with contractility. For best results, use the **Contractility Paced** configuration and a dedicated stimulation electrode (paddle).
10. **Can contractility be measured simultaneously with field potentials?**
    The Maestro contractility assay provides excitation tracking based on the underlying field potential, measured from the same electrodes. Endpoints from the field potential related to excitation, such as Beat Period and Conduction, are tracked simultaneously with contractility endpoints. However, contractility does not allow for t-wave detection for field potential duration.
11. **Why do I see contractility signals in the Continuous Waveform Plots but not in the Cardiac Beat Plots?**
    Contractility uses the highly precise excitation timing from the underlying field potential to segment contractility beats. If the field potential amplitudes are too weak (< 250 µV), contractility beats will not be detected. If contractility beats are not detected, you can verify the field potential amplitude by applying the Field Potential Configuration (right click on the Maestro and select Configuration → Cardiac Offline → Field Potential). For commercially available cardiomyocytes, field potential amplitude increases as the cells attach to the electrodes, typically exceeding the detection threshold by DIV 3.
12. **Is contractility compatible with LEAP?**
    Contractility and LEAP can be recorded in the same experiment, but not simultaneously. For best results, contractility measurements should be made prior to LEAP induction, as LEAP changes the cell-electrode coupling.

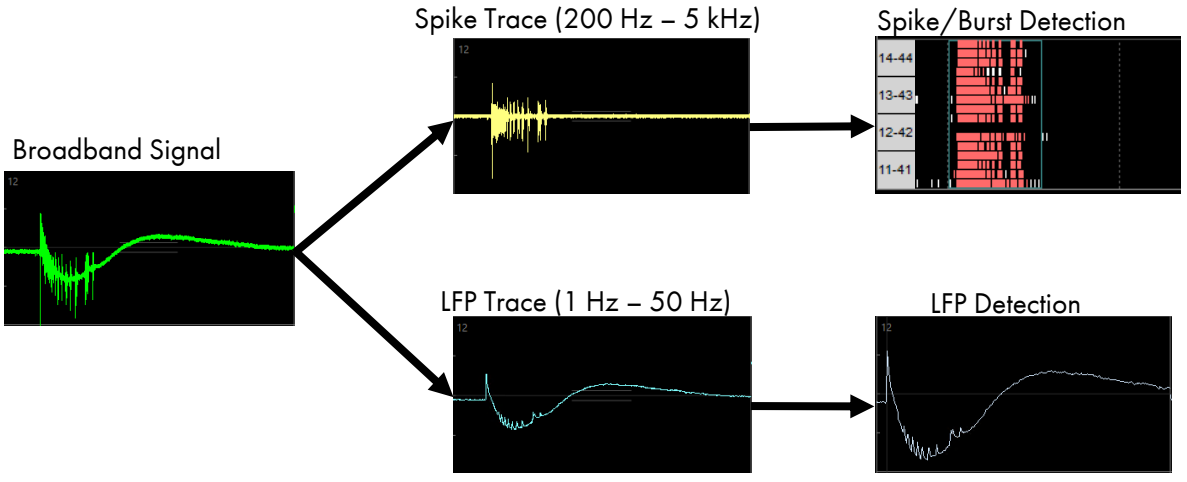
CHAPTER 7. NEURAL BROADBAND AND LOCAL FIELD POTENTIALS

Local field potential (LFP), broadly, refers to the electrical potential measured in the extracellular space near a neuron. Variation in local field potential is thought to largely be driven by a spatial summation of post-synaptic potentials. Slow oscillations of local field potentials in intact brains are often colloquially referred to as brain waves, and the frequency content of these waves is associated with distinct behavioral states *in vivo* such as focus or sleep. These low frequency signals may also be observed *in vitro,* where the oscillatory patterns and frequency content of discrete LFP events (hereafter referred to as LFPs) may provide insight into the excitatory-inhibitory balance of the network and the evolving complexity of the network during maturation. With the **Neural: Broadband** mode, the Maestro makes it easy to gather low frequency LFPs and perform high fidelity spike time identification simultaneously, providing another set of metrics for investigators characterizing *in vitro* neuronal models.

7.1. NEURAL BROADBAND MODE

While **Configuration → Neural Real-Time → Spontaneous** has the best signal to noise ratio for spike detection, users who wish to capture low frequency LFPs alongside spikes may use the **Broadband Spontaneous** configuration. **Neural: Broadband** mode allows the Maestro to record continuous voltage data across a wide band of frequencies (1 Hz – 5 kHz) for the simultaneous recording of spikes and LFPs. The Broadband Processor allows the user to easily separate the broadband data into its high frequency content for spike detection and low frequency content for LFP detection and possible spectral analysis in post processing. The **Broadband Processor** produces an AxIS .raw file containing two continuous voltage datasets. The high frequency dataset (200 Hz – 5 kHz) is produced by a 2nd order digital Butterworth bandpass filter, whereas the low frequency dataset (0.1 Hz – 50 Hz) is produced by a median filter and downsampler at 100 Hz, yielding an effective cut-off frequency of 50 Hz.

The Neural Event Detector operates on the Broadband Processor, allowing the user to detect spikes, LFPs, and network bursts with one stream. The resulting AxIS Events .spk file contains two datasets, one with Spike times, locations (well and electrode), and waveforms and one with LFP times, locations (well and electrode), and waveforms.

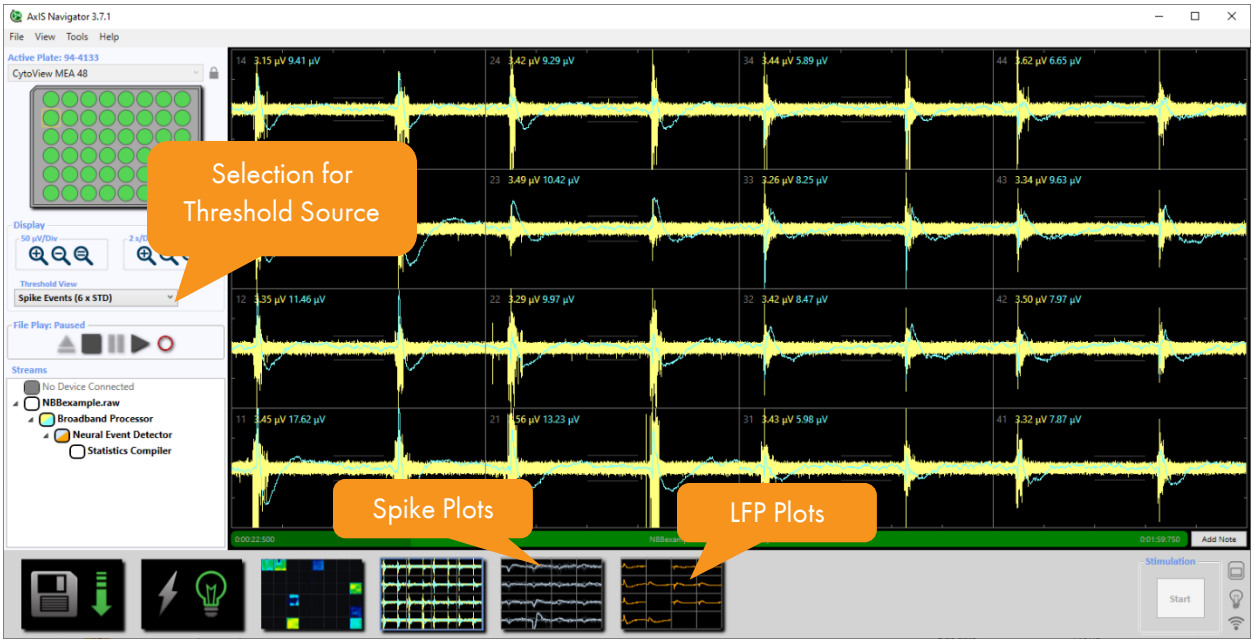


7.2. NEURAL BROADBAND ACQUISITION AND ANALYSIS TUTORIAL

To acquire local field potentials and spikes:

Follow the steps for data acquisition as described in Section 3.3, selecting **Configuration → Neural Real-Time → Broadband Spontaneous** to appropriately configure the hardware and software to record broadband neural signals.

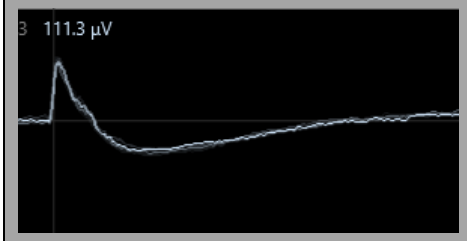
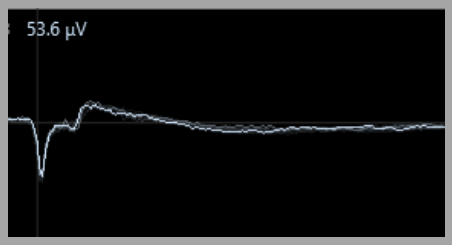
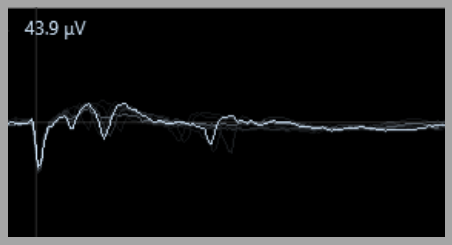
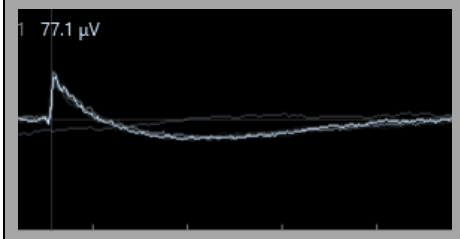
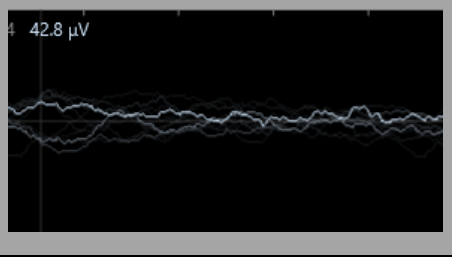
Press the **Record** button to begin collection.



To analyze local field potentials alongside spike times:

Follow the steps for data analysis as described in Section 9.5, using **Configuration → Neural Real-Time → Broadband Spontaneous** to save a Neural Statistics Compiler **Advanced Metrics** .csv file. It is recommended here to also save an **AxIS Events** (.spk) file and a **Broadband Processor** (.raw) file for use with the Neural Metric Tool.

To detect LFPs, the **Neural Event Detector** uses a threshold method which identifies large deflections in the low frequency component of the broadband signal. Since cell type, culture maturity, culture structure can all play a large role in the size and shape of the LFPs, Axion recommends modifying the LFP detection settings within the **Neural Event Detector** to fit each application (see Section 2.3.8).

Waveform	Description	Physiological/Noise
	Consistent, repetitive biphasic waveform.	Physiological
	Consistent, repetitive biphasic waveform.	Physiological
	Higher complexity event, with consistent initiation and multiple activity peaks.	Physiological – more complex network activity, which may reflect increased maturation
	Alternation between biphasic waveform with clear peak and flat waveform.	Physiological – the LFP events are being detected on the positive and negative portions. Considering raising the LFP detection threshold.
	Inconsistent waveforms without clear peaks in activity.	Noise – this electrode may not have cell coverage or in range MEA viability measurement.

7.3. FAQ

1. What is a local field potential (LFP)?

   The local field potential (LFP) is the electrical potential measured in the extracellular space near one or more neurons. Variation in local field potential is thought to largely be driven by a spatial summation of

post-synaptic potentials. As such, the local field potential may present as discrete events coupled with network spiking activity or as ongoing low frequency oscillations.

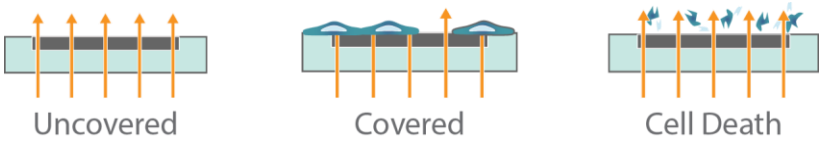
2. How is Neural Broadband mode different from Neural Spikes mode?
   The Neural Spikes mode is designed to isolate, visualize, and quantify the action potential spiking activity of neuronal populations. The Neural Broadband mode retains the same biological activity from Neural Spikes mode, and supplements with additional information at low and high frequencies. The Broadband Processor separates this information into spiking activity (200 Hz – 5 kHz) and local field potentials (1Hz – 50 Hz).
3. Will I detect the same number of spikes in the Neural Broadband and Neural Spikes modes?
   The Neural Broadband modes does include additional low and high frequency content relative to Neural Spikes mode, which can raise the spike detection threshold. This could result in a modest decrease in the number of spikes detected in Neural Broadband relative to Neural Spikes modes when the adaptive threshold crossing method is used for spike detection.
4. Why do LFP shapes and amplitudes vary?
   The local field potential is believed to represent the spatial summation of post-synaptic potentials across a small population of neurons nearby the electrode. As such, the shape, polarity, and amplitude of an LFP event will result from the involvement of the local neuronal population in the overall network activity in the well. In addition, the type of neurons, and their density, can impact the shape and amplitude of an LFP.
5. Will LFP work with poor cell coverage?
   Sufficient cell coverage over the electrodes is required to obtain a strong LFP signal. Perhaps more importantly, large LFP events are typically associated with synchrony and network bursts. Therefore, optimized culture conditions for LFPs should match the optimized conditions for spiking activity.
6. Can LFP be measured from 3D neuronal models?
   Yes, LFP signals have been acquired and characterized from 3D neuronal models with the Maestro Pro and Edge. The Neural Broadband mode and associated analysis further enable these applications.
7. Is Neural Broadband compatible with all plate types?
   No. Neural Broadband mode is compatible with CytoView MEA plates, which offer low impedance PEDOT microelectrodes. Neural Broadband mode is not compatible with higher impedance BioCircuit MEA plates.
8. Does Neural Broadband work for all cell types?
   Neural Broadband mode has been evaluated with rodent cortical neurons and commercially-available iPSC-derived neurons.
9. Can I record LFP activity on the original Maestro?
   No. The Maestro Pro or Edge are required for Neural Broadband mode.
10. Is Neural Broadband mode compatible with electrical stimulation?
    No. The standard electrical stimulation tools available in AxIS Navigator may not be used with Neural Broadband at this time.
11. What is value of low frequency LFP signals in neuronal assays?
    The LFP provides an additional avenue to quantify neuronal activity. In most cases, low frequency LFP signals will complement existing analysis of neuronal function via spiking activity. In some cases, low frequency LFP signals will provide new information not easily detectable in spiking activity.

CHAPTER 8. MEA VIABILITY

Cell structure and function are both vital to characterizing cell behavior in health and disease. With the **MEA Viability Software Module**, you can measure cell coverage and viability from the same microelectrodes used to characterize the electrical function of neural and cardiac networks. The **MEA Viability Software Module** uses the Maestro's impedance technology to assess the cells over each microelectrode, providing a high-resolution map of coverage and viability in each well. Since impedance is non-destructive and label-free, the measurement can be repeated many times to track changes over chronic time courses.

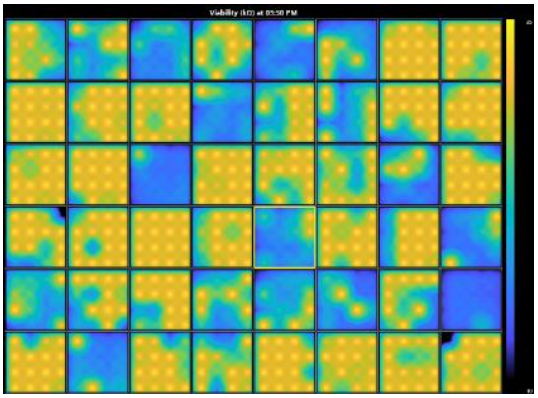
8.1. MEA VIABILITY BACKGROUND

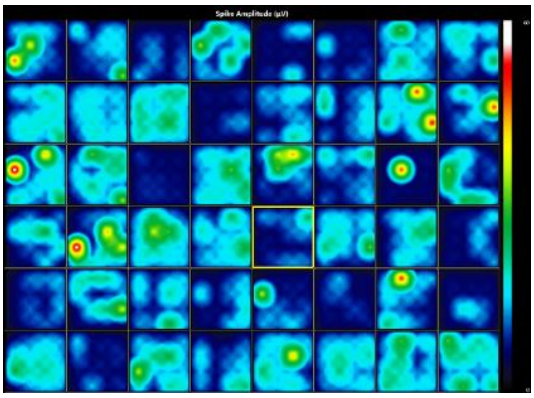
To measure viability, a small electrical signal is delivered to the microelectrodes. How easily the signal passes through the cell-electrode interface to the media depends on the number of cells attached to the electrode and the cell membrane integrity. Thus, impedance can be used to measure cell density, coverage, and viability. When the electrode is uncovered, the electrical signal passes easily through to the media, and a low impedance value is measured. When viable cells are covering the electrode, a higher impedance is measured. MEA Viability is reported as the resistance at 41.5kHz, a component of impedance that is highly correlated with cell density and coverage.



In contrast to viability assays that measure metabolic activity in cells, impedance-based MEA Viability is particularly sensitive to the structural integrity of the attached cell membranes, similar to traditional lactate dehydrogenase (LDH) and trypan blue exclusion assays[1]. When the cell membrane integrity is disrupted (e.g., lysis, necrosis), impedance decreases as the electrical signal more easily passes through the cell-electrode interface. Impedance may also detect early changes in cell morphology after dosing with specific drugs or compounds, providing users a way to predict declining cell health (see FAQ below). For some forms of apoptosis, the cells may initially shrink, or become pyknotic, before a disruption to membrane integrity occurs, thus delaying detection by MEA Viability.

In *AxIS Navigator*, MEA Viability can be visualized in the **Activity Map** panel. In the example below, both viable cell coverage (left) and spiking (right) were measured from the same hiPSC-derived neurons on a CytoView MEA 48-well plate using the MEA Viability Module:

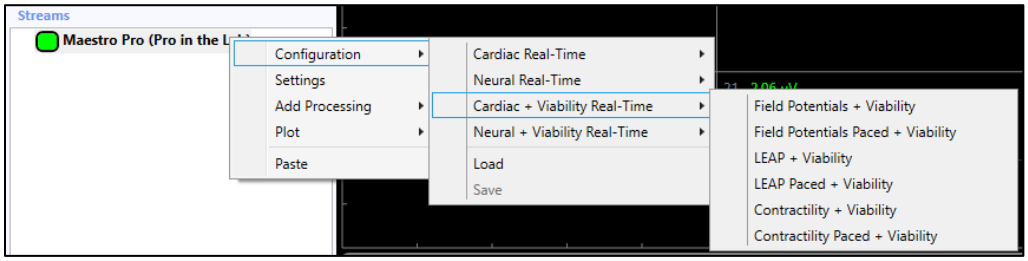




8.2. MEA VIABILITY ACQUISITION AND ANALYSIS TUTORIAL

To acquire impedance-based MEA viability:

1. Set up the Maestro for data acquisition as described in section 3.3. Right-click on the **Maestro Pro** or **Maestro Edge** stream and select **Configuration** to choose either a **Cardiac + Viability Real-Time** or **Neural + Viability Real-Time** configuration.



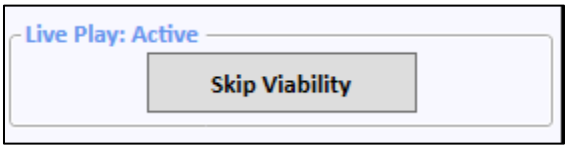
2. Press **Play** or **Record** to begin acquiring **Viability**. A banner will indicate that the system is "Acquiring viability..." while impedance is measured. Once complete (approx. 1 minute), the banner will indicate "Viability successfully acquired." The Maestro will then transition to the requested Cardiac or Neural acquisition settings.

***Note:** Only **Record** will save the Viability data (i.e. Resistance at 41.5 kHz for each electrode) in a .raw file for future viewing and analysis.*

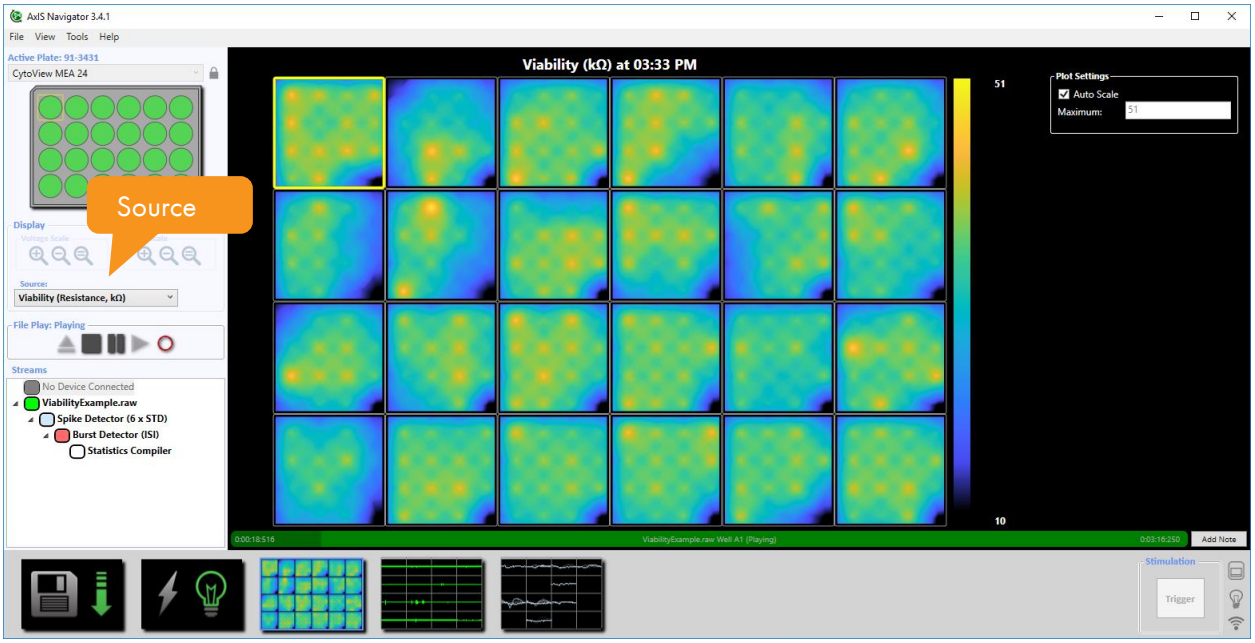
To skip impedance-based MEA Viability acquisition when a viability configuration is applied:

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** to choose either a **Cardiac + Viability Real-Time** or **Neural + Viability Real-Time** configuration.
3. Press **Play** or **Record** to begin acquiring **Viability**. A banner will indicate that the system is "Acquiring viability..." while impedance is measured.

4. Press the **Skip Viability** option that replaces the play controls. A banner will indicate that the system is "Viability is being skipped...". The Maestro will then transition to the requested Cardiac or Neural analog acquisition settings.

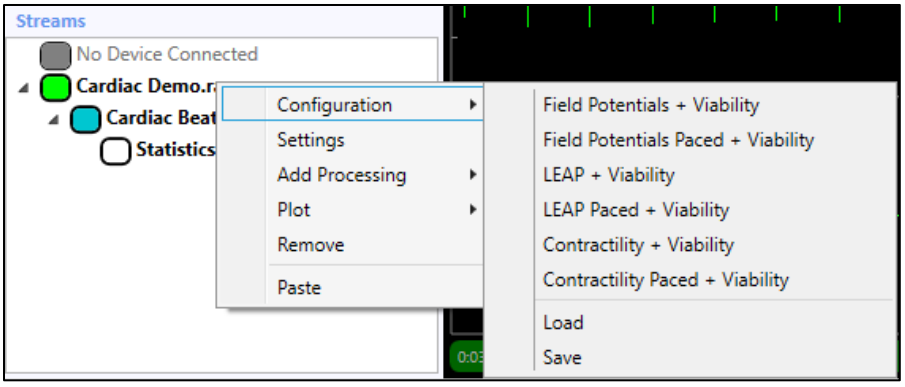


After acquiring Viability, resistance values in kiloohms (kΩ) can be visualized in the **Activity Map** by selecting **Viability (Resistance, kΩ)** in the **Source** drop-down above the play controls.



To analyze impedance-based MEA Viability:

1. Follow the steps for data analysis as described in Section 9.5.
2. Right-click on the file in the **Streams** pane and select **Configuration** to apply the desired configuration as outlined below. See Section 9.1 for more information about analysis configurations.
    a. For Cardiac files, select **Configuration → Field Potentials + Viability, LEAP +Viability, or Contractility + Viability** with the option of **Paced** if needed.
    b. For Neural files, select **Configuration → Spontaneous + Viability, Electrically Evoked + Viability, Optically Evoked + Viability, Field Potentials Spontaneous + Viability,** or **Broadband Spontaneous + Viability**.



3. Save the **Statistics Compiler Advanced Metrics** .csv file, which will contain both cardiac/neural and viability metrics, such as Resistance, Number of Covered Electrodes, and Weighted Mean Resistance.
4. Load the .csv files from multiple time points into the AxIS Metric Plotting Tool to track changes in viability metrics over time.

8.3. FAQ

1. **How does MEA Viability work?**
   MEA Viability uses impedance technology to assess the cells covering each microelectrode. A very small electrical signal is delivered to the microelectrodes, and impedance measures the resistance to signal flow through the cell-electrode interface to the media. Impedance depends on the number of the cells attached to the electrode, the conformation of those cells, and the integrity of their cell membranes. Thus, impedance can be used to measure both cell coverage and structural integrity. MEA Viability is reported as Resistance at 41.5 kHz in units of kiloohms (kΩ).
2. **What is the expected range of resistance values?**
   Covered electrodes are defined as electrodes with resistances greater than the Covered Electrode Threshold. Uncovered CytoView MEA microelectrodes in media exhibit a resistance of 8-12 kΩ. Following cell death, debris may leave a slightly higher uncovered resistance of 15-18 kΩ. Users may adjust the Covered Electrode Threshold in the Statistics Compiler according to internal assay validation.
3. **Why does the resistance of covered electrodes vary across a well?**
   The absolute resistance value may vary across electrodes due to the number of cells present on each electrode, their position relative to the electrode, and the strength of cell attachment. While differences in resistance between electrodes can be used to distinguish covered from uncovered electrodes, MEA viability is most sensitive to changes over a single electrode. When cells die, they often deteriorate and detach causing a reduction in resistance. Thus, changes in impedance can be used to track cell viability on each electrode over the course of your experiment, resolving even subtle changes (as small as 0.5%) in response to perturbations.
4. **Why does resistance sometimes increase within a few hours of dosing with some cytotoxic compounds?**

An increase in resistance after dosing (2-8 hrs) is reflective of changes in cell morphology, and may be an early indicator of poor cell health. An increase may precede later decreases in impedance as the structural integrity of the membrane is disrupted by secondary necrosis or lysis.

5. **Is MEA Viability compatible with all plate types?**
   No. MEA Viability is compatible with CytoView MEA plates, which offer low impedance PEDOT-coated microelectrodes. MEA Viability is not compatible with BioCircuit MEA plates.
6. **Does MEA Viability work for all cell types?**
   MEA Viability has been evaluated with commercially available iPSC-derived cardiomyocytes and multiple primary and iPSC-derived neuron models. Impedance-based MEA Viability is highly sensitive to cell density and coverage for all cell types. Impedance is also highly sensitive to the structural integrity of cell membranes. For some forms of neuronal apoptosis, the cells may initially shrink or become pyknotic before a disruption to membrane integrity occurs, thus slightly delaying detection of cell death.
7. **Can I record MEA Viability on the original Maestro?**
   No. The Maestro Pro or Edge, along with the MEA Viability Software Module, are required for MEA Viability.
8. **I already have a Maestro Pro/Edge. Can I add the MEA Viability Module?**
   Yes. Your sales representative can provide additional information regarding the purchase of the MEA Viability Module. For Maestro Pro or Edge systems manufactured before July 2020, a hardware upgrade will be required in addition to the MEA Viability Module software update. This upgrade will be performed at Axion headquarters in Atlanta, GA.
9. **How does MEA Viability differ from contractility?**
   Both Contractility and MEA Viability use impedance technology, but at different frequencies to enhance sensitivity to specific aspects of cell health and behavior. Contractility measures impedance at 3.125 kHz and is sensitive to movement (e.g., contraction and relaxation) of beating cells over the microelectrodes. MEA Viability measures resistance at 41.5 kHz, which is highly correlated with the number of viable cells attached to the microelectrodes.
10. **Is MEA Viability compatible with LEAP?**
    MEA Viability and LEAP can be recorded in the same experiment, but not simultaneously. For best results, MEA Viability measurements should be made prior to LEAP induction.
11. **How does MEA viability with the Maestro platform compare to other common cell viability assays?**
    Cell death is a complex process involving several potential modalities, such as apoptosis and necrosis. Viability assays typically measure one feature of cell death, such as reduced metabolic function, caspase activity, cell morphology, or plasma membrane integrity[2,3]. For example, resazurin (a.k.a., alamar blue) and MTT assays rely on mitochondrial reduction to distinguish metabolically active cells from dying cells, whereas trypan blue exclusion and LDH rely on membrane integrity to distinguish viable (intact) and fully dead (disrupted) cells. Metabolism based assays tend to indicate cell death early in the apoptotic process, but can be confounded by other changes in metabolism. Exclusion assays require membrane disruption, which often comes later in apoptotic processes in the form of post-apoptotic necrosis, but indicate full cell death. Impedance-based MEA Viability is most similar to trypan blue and LDH assays, as impedance measures the ability of intact cellular membranes to impede current flow. Unlike many label-dependent viability assays, impedance is non-destructive and label-free. Thus, MEA Viability can be

repeated many times over chronic time scales for multiplexed tracking of cellular function, coverage, and viability.

[1] Orrenius, S., Nicotera, P. & Zhivotovsky, B. Cell death mechanisms and their implications in toxicology. Toxicol. Sci. 119, 3–19 (2011).

[2] Gerber, L., Melis, L. V. J., Kleef, R. G. D. M., Groot, A. & Westerink, R. H. S. Culture of Rat Primary Cortical Cells for Microelectrode Array (MEA) Recordings to Screen for Acute and Developmental Neurotoxicity. *Curr. Protoc.* **1**, 1–35 (2021).

[3] Druwe, I., Freudenrich, T. M., Wallace, K., Shafer, T. J. & Mundy, W. R. Sensitivity of neuroprogenitor cells to chemical-induced apoptosis using a multiplexed assay suitable for high-throughput screening. Toxicology 333, 14–24 (2015).

CHAPTER 9. DATA ANALYSIS

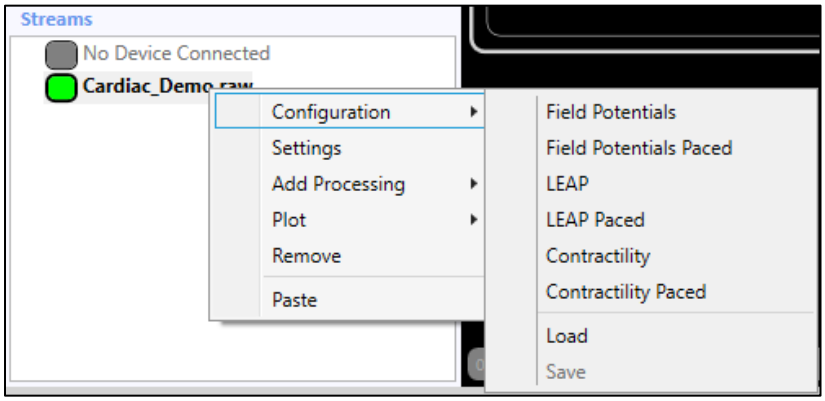
From a functional standpoint, data analysis in *AxIS Navigator* is similar to data acquisition. A previously recorded .raw file (continuous voltage data) is loaded into the **Streams** pane, it passes through various data processors, and output files are generated while *AxIS Navigator* "records". Instead 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 9.5 and 9.6 contain step-by-step tutorials for analyzing a single file and multiple files, respectively.

9.1. STREAM CONFIGURATIONS

Similar to data acquisition, *AxIS Navigator* comes with a variety of preset configurations for data analysis.

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.



Analysis configurations available in *AxIS Navigator* include:

Configuration	Processing Applied	Description
Cardiac		
Field Potentials	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.
Field Potentials 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.
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.
LEAP 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. Generates a variety of cardiac beating endpoints.
Contractility	Cardiac Beat Detector Statistics Compiler	Generates activity map, contractility waveforms, beat rate, and conduction information. Optimized for analyzing contractility signals. Generates a variety of cardiac beating endpoints.
Contractility Paced	Artifact Eliminator Cardiac Beat Detector Statistics Compiler	Generates activity map, contractility waveforms, beat rate, and conduction information. Optimized for reducing stimulus artifacts in pacing experiments. Generates a variety of cardiac beating endpoints.
Neural		
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.
Field Potential Spontaneous	Filter (1Hz – 200 Hz) Filter (200 Hz – 2 kHz) Spike Detector Burst Detector	Generates activity map, spike waveforms, and raster plot. Network burst detection disabled. Applied filters are optimized to separate low (LFP) and high (spikes) frequency components of the signal for spike detection.
Broadband Spontaneous	Broadband Processor Neural Event Detector	Generates activity map, spike and LFP waveforms, and raster plot. Network burst detection is enabled. Generates a variety of spiking, bursting, synchrony, and LFP endpoints.

***Note:** Configurations options will be filtered based on the Acquisition mode used to record the raw file.*

All of the Neural and Cardiac configurations can be used with the **MEA Viability Software Module** to allow users to measure impedance data and MEA data simultaneously. To learn more about the **MEA Viability Software Module**, please see Chapter 7.

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.

9.2. 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.

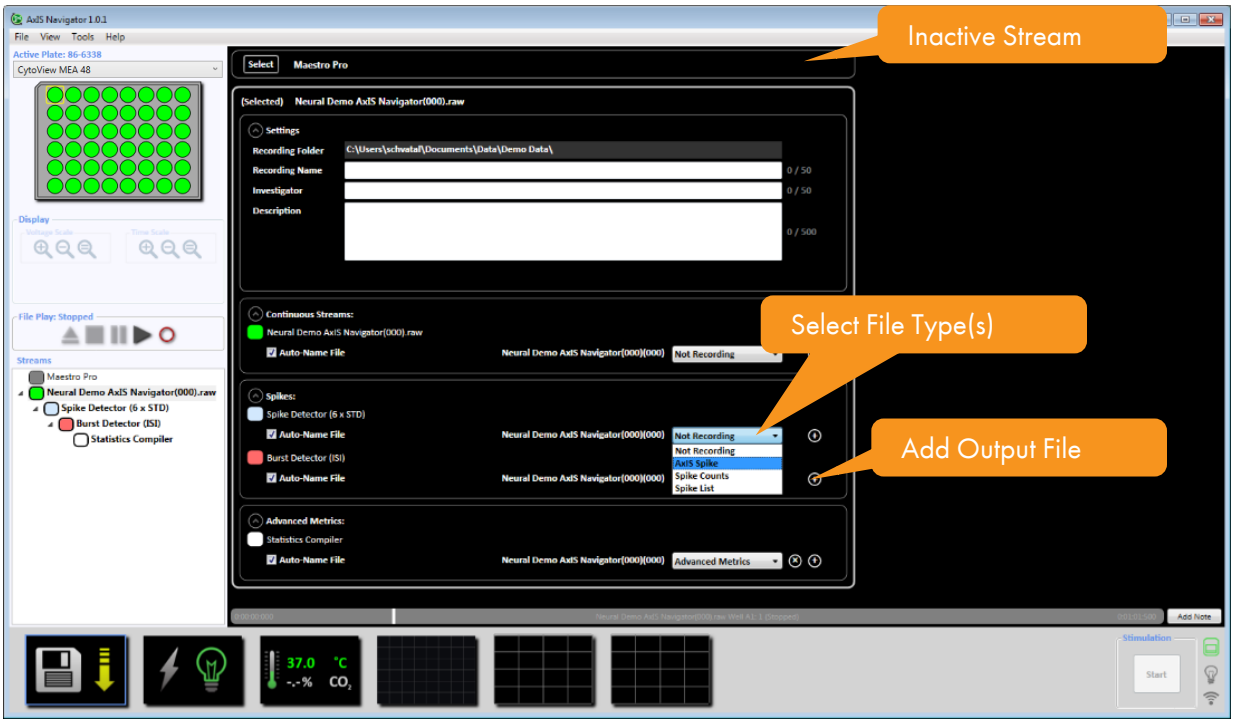
Data Processor	Output Name	Description
Continuous Streams		
Maestro Pro/Edge	AxIS Raw (.raw)	Primary format for data acquisition. Continuous voltage data from all enabled electrodes before any digital processing. MEA Viability is also stored in the .raw file when requested.
	Environmental Data (.csv)	Table listing temperature and CO_2 concentration throughout the recording.
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**.
Broadband Processor	AxIS Raw (.raw)	Contains two streams of continuous voltage data, one with high frequency spiking (200 Hz – 5 kHz) and one with low frequency local field potentials (1 Hz – 50 Hz). Playback not available in AxIS Navigator.
Neural Events		
Spike Detector	AxIS Spike (.spk)	Spike times and voltage waveforms organized by electrode for all spikes detected by the **Spike Detector**. MEA Viability is also stored in the .spk file when requested. 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.
Neural Event Detector	AxIS Events (.spk)	Spike times, LFP times, and voltage waveforms organized by electrode for all spikes and LFPs detected by the **Neural Event Detector.** MEA Viability is also stored in the .spk file when requested. 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 **Neural Event Detector Settings**.
	Spike List (.csv)	Table listing time, electrode, and amplitude of all spikes detected by the **Neural Event Detector**.
	Electrode Burst List (.csv)	Table listing the individual electrode bursts identified by the **Neural Event Detector** with descriptive information including time, electrode, number of spikes, and duration.
	Network Burst List (.csv)	Table of the network bursts identified by the **Neural Event Detector** with descriptive information including time, well, number of spikes, duration, number of electrodes, and spikes per electrode.
	LFP Event List (.csv)	Table listing time, electrode, and amplitude of all LFPs detected by the **Neural Event Detector**.
Cardiac Beats		
Cardiac Beat Detector	Electrode Beat List (.csv)	Table of individual electrode beats and corresponding endpoints, which may include beat time, field potential spike time, electrode, depolarization amplitude, depolarization slope, beat period, field potential duration, beat amplitude, excitation-contraction delay, well beat number, and delay from well beat time, depending on the **Acquisition Setting**.
	Well Beat List (.csv)	Table of well beats and corresponding endpoints, which may include well, time, number of active electrodes, starting electrode, ending electrode, beat period, well beat number, conduction velocity, maximum delay from well beat time, composite beat amplitude, and composite excitation-contraction delay, depending on the **Acquisition Setting**.
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. Depending on the **Acquisition Setting**, may include depolarization amplitude, slope, beat period, beating irregularity, field potential duration, conduction velocity metrics, beat amplitude, excitation-contraction delay, and viability metrics. Required output file for Cardiac Analysis Tool and/or AxIS Metric Plotting Tool. See Section 10.3 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. Depending on the **Acquisition Setting**, output metrics may include spike, burst, synchrony metrics, and viability metrics. Required output file for AxIS Metric Plotting Tool. See Section 11.4 for a list of included endpoints.

Note: *Viability metrics are only available with the* ***MEA Viability Software Module****. To learn more about the* ***MEA Viability Software Module****, please see Chapter 7.*

The **Experiment Setup Properties** panel 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 only 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 to 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 (⊕).
3. Select the next output.
4. Repeat Steps 1-3 as needed.
   Note: *To remove an output, click the* ***Remove*** *button (⊗) or select* ***Not Recording*** *from the drop-down menu.*

AxIS Navigator supports the use of third party software, including *NeuroExplorer®*, *Microsoft Excel®*, *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* and *NeuroExplorer. For NeuroExplorer, conversion to a .nex file using the Axion Data Export Tool is recommended to preserve platemap, stimulation, and other data and to avoid errors.* **Spike List**, **Spike Count**, **Burst List**, **Beat List**, and **Advanced Metrics** files can be processed in *Excel*.

9.2.1. 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** panel.	SC
[Offset]	Time from the start of the file processing began.	10s
[RecordingName]	**Recording Name** as entered in the **Experiment Setup Properties** panel.	Experiment1
[SourceFile]	Original file name of the source .raw file.	Compound_A
[StreamName]	Name of the data processor generating the output file.	Statistics Compiler
[PlateType]	The name of the MEA plate used for the recording.	CytoView MEA 96

To manually name a file:

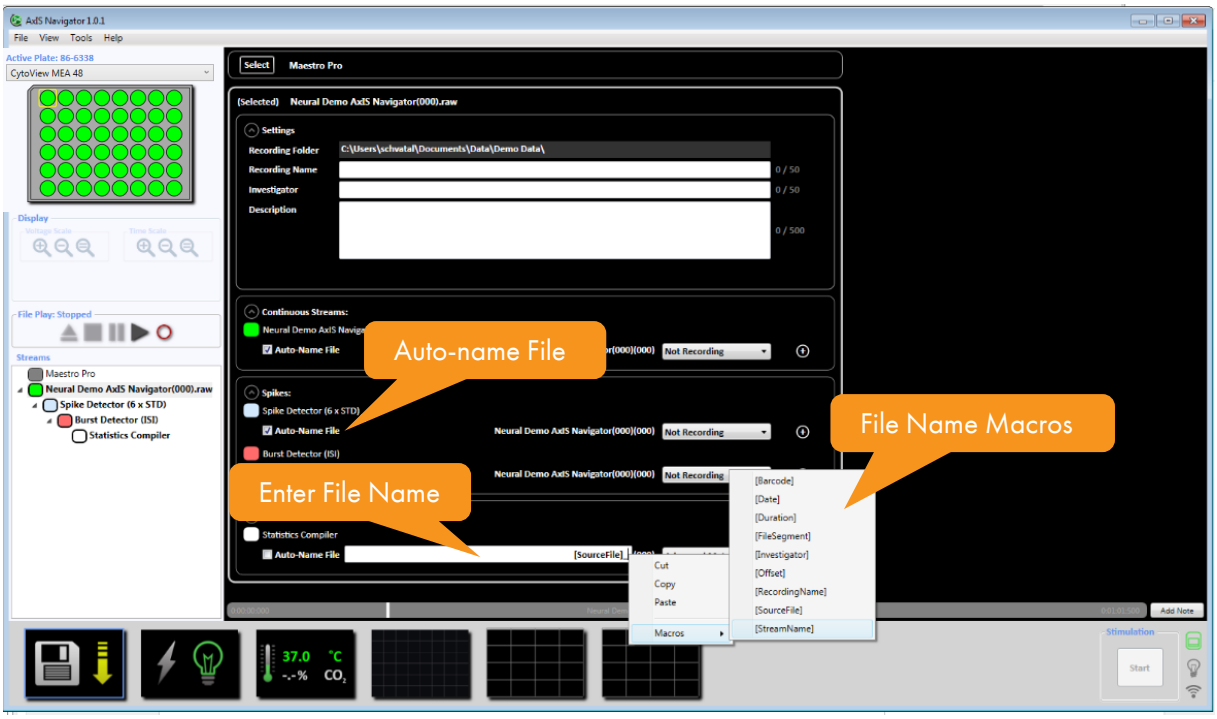
1. Deselect the **Auto-Name File** checkbox in the **Experiment Setup Properties** panel.

To add a macro to the file name:

1. Type the macro command from the above table into the **File Name** field.

– Or –

2. Right-click the **File Name** field.
3. 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.

Note: Output files from the **Broadband Processor** and the **Neural Event Detector** default to the following macro structure: [SourceFile]_[StreamName].

9.3. 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.

9.4. SELECTING THE ANALYSIS WINDOW

While performing an analysis, *AxIS Navigator* will stream through the continuous voltage data. *AxIS 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 *AxIS Navigator* is not already in a stopped state.
2. Right-click the file in the **Streams** pane.
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 recommend while recording analysis files.

9.4.1. 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 9.4.
4. Click the **Experiment Setup Properties** panel.
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 9.2.1 for more information on naming output files.
7. Click **Record**.

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

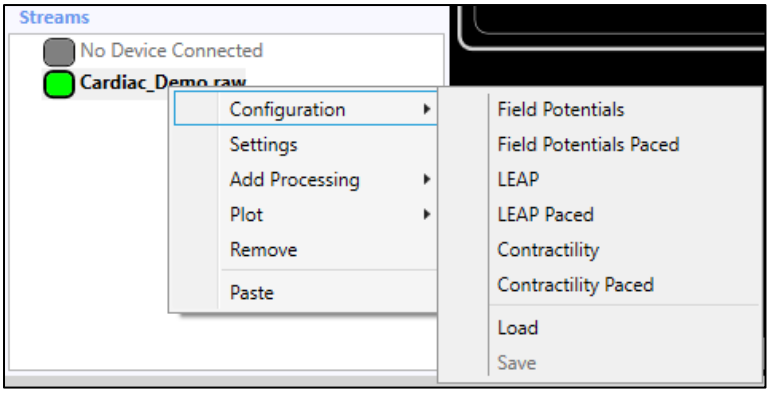
Select an analysis window and run an analysis according to Section 9.5. Repeat for each analysis window.

– Or –

Generate new .raw files for each analysis window and run a batch process according to Section 9.6.

9.5. DATA ANALYSIS TUTORIAL

5. Click **File → Open Recording...**.
6. Select the file for analysis and click **Open**.
7. Prepare the file for analysis. See Section 9.3.
8. Set the analysis window. See Section 9.4.
9. Right-click on the file in the **Streams** pane and select **Configuration** to apply the desired configuration as outlined below. See Section 9.1 for more information about analysis configurations.
    a. For Cardiac files, select **Configuration → Field Potentials, LEAP,** or **Contractility** with the option of **Paced** or **Viability** if needed.
    b. For Neural files, select **Configuration → Spontaneous, Electrically Evoked, Optically Evoked, Field Potentials Spontaneous,** or **Broadband Spontaneous** with the option of **Viability** if needed.



6. Click the **Experiment Setup Properties** panel. See Section 2.6.1 for more information on the **Experiment Setup Properties** panel.
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 9.2 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 9.2.1 for more information on naming output files.
9. Click **Play** to view the data.
10. Assess the analysis settings. See 8 for cardiac activity, 9 for neural activity.

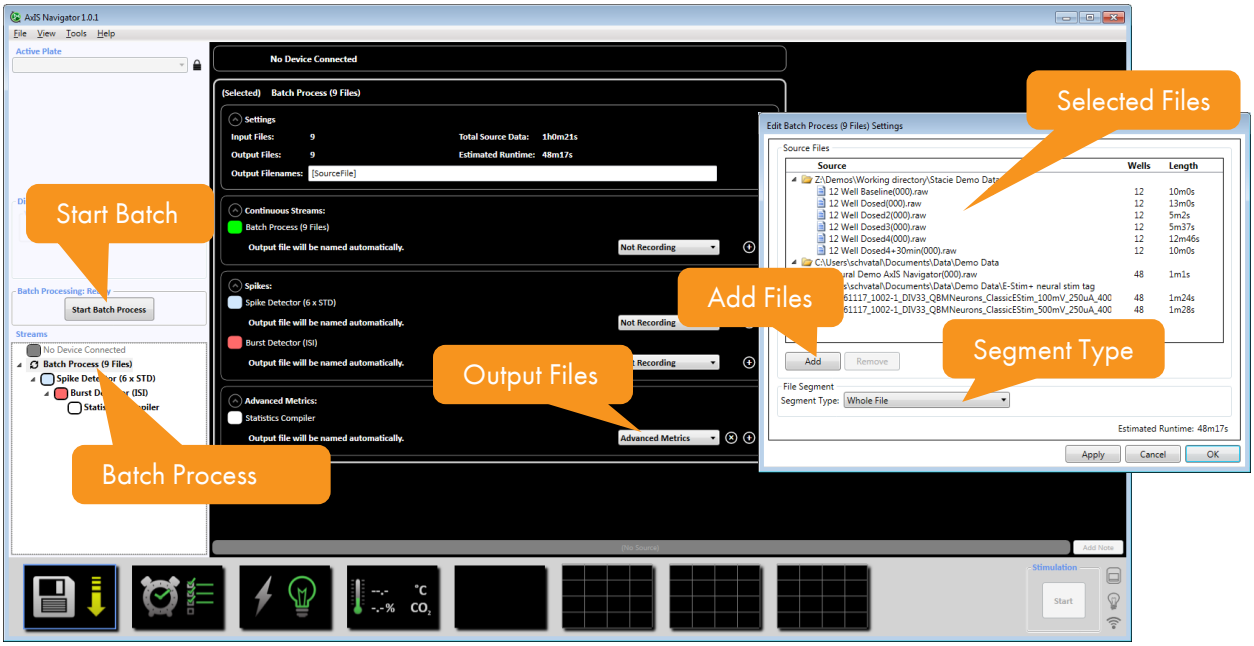
11. The **Accelerate Playback** and **Accelerate Recording** options in the **Tools** menu play and process files faster than real time, respectively, for quicker analysis and can be toggled on/off, if desired.
12. Click **Record** to run the analysis.

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

9.6. 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** pane. 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.

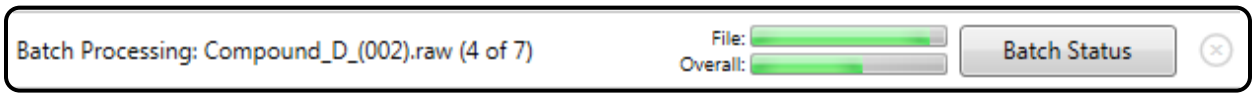
   Note*: Use* ***Remove*** *to remove any files from the* ***Source File*** *list.*
3. Select the desired .raw files and click **Open**.
4. Select the analysis window using the **Segment Type** drop-down menu. See Section 9.4 for setting an analysis window with the **Segment Type** dialog.

***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** pane and select **Configuration** to apply the desired configuration as outlined below. See Section 9.1 for more information about analysis configurations.
    a. For Cardiac files, select Configuration → **Field Potentials, LEAP, or Contractility** with the option of **Paced** or **Viability** if needed.
    b. For Neural files, select Configuration→ **Spontaneous, Electrically Evoked, Optically Evoked, Field Potentials Spontaneous,** or **Broadband Spontaneous** with the option of **Viability** if needed.
7. Click the **Experiment Setup Properties** panel. See Section 2.6.1 for more information on the **Experiment Setup Properties** panel.
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 9.2 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 each source .raw file was generated from.*
9. Click **Start Batch Process**.

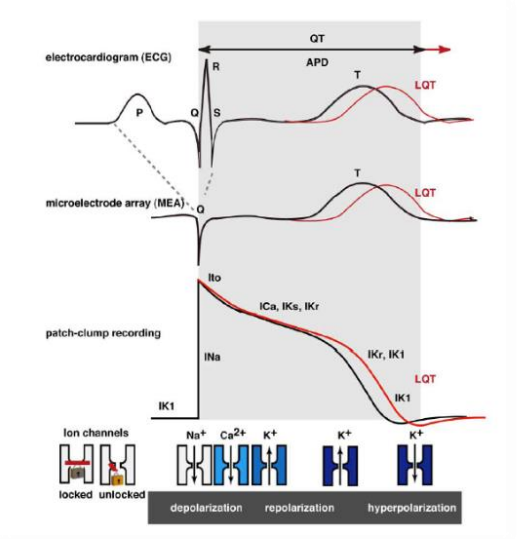
A white status bar will appear at the top of the active window while a batch process is running. The status bar will list the progress of the current file (**File**) and the entire batch process (**Overall**).



CHAPTER 10. CARDIAC DATA ANALYSIS

10.1. 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).



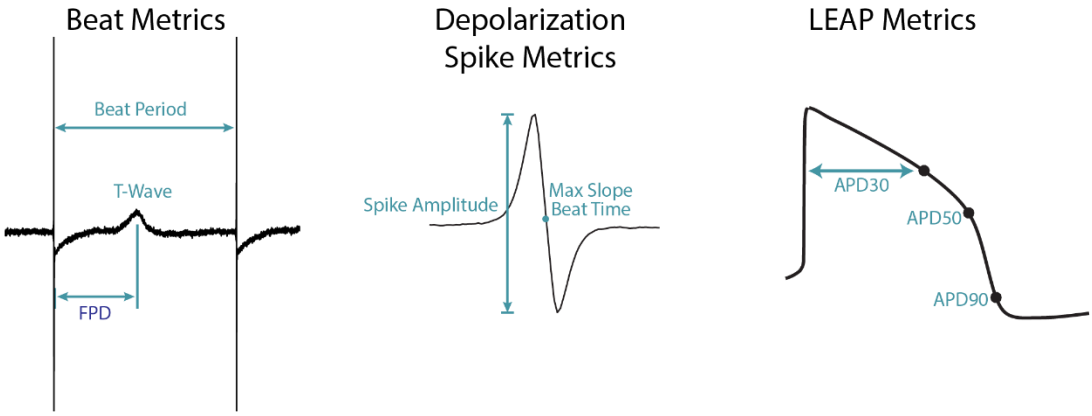
Asai et al., 2010. Current Stem Cell Research & Therapy.

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.

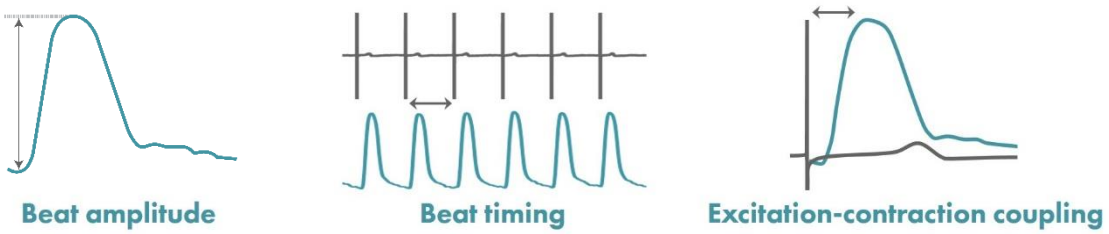
The LEAP assay uses proprietary techniques to enhance the coupling between the cells and electrodes, enabling the detection of a LEAP signal which closely approximates the shape of a transmembrane action potential from a patch-clamp recording. See Chapter 5 for more information about LEAP.

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, or action potential duration (APD) 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/APD. Prolongation in the FPD/APD is assessed as a potential indicator of pro-arrhythmia.



In the Maestro, contractility is measured from each electrode, providing local measurements of cardiomyocyte contractility in high spatial resolution across the culture. AxIS Navigator provides a contractility Beat Amplitude on each electrode, allowing you to track local changes in beating. In addition, well-wide composite contractility metrics, including Composite Beat Amplitude, Beat Period, and Excitation-Contraction Delay, describe the average contractility behavior across the well. These metrics are defined in Section 10.3.

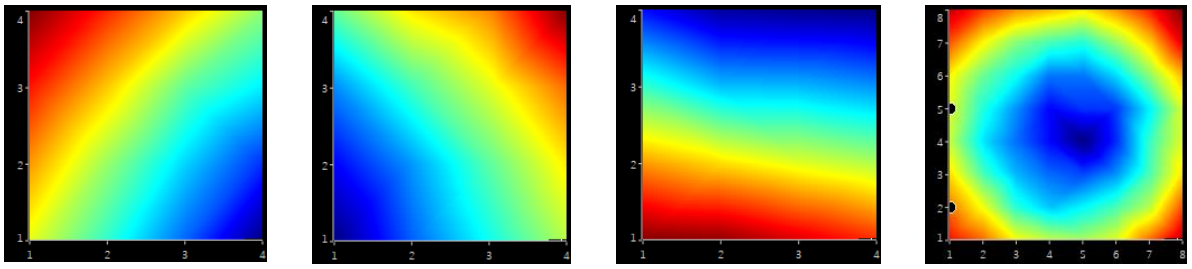


The two-dimensional structure of the MEA allows cardiac signal propagation to be measured and quantified by comparing the depolarization timing between electrodes. In culture, cardiomyocytes form an electrically coupled syncytium of cells that beat synchronously. When a cardiomyocyte in that syncytium 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.

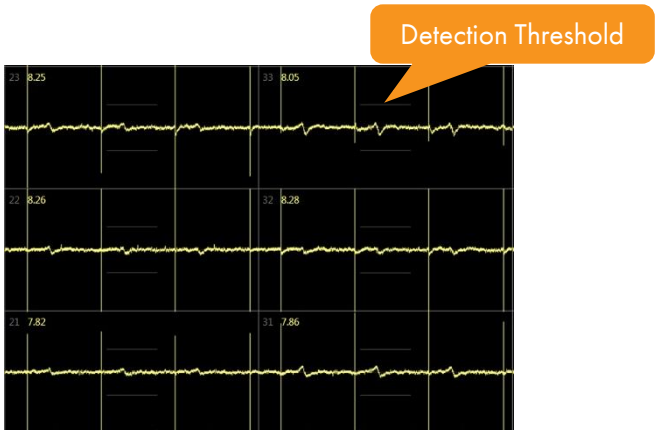
The figures below show four examples of propagation. The beat begins where the plot is blue (shortest delay from the beat origin) and ends where the plot is red (longest delay from the beat origin). The stability of beat propagation patterns and conduction velocity can be used to assess culture health,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



10.2. IDENTIFYING CARDIAC DEPOLARIZATION AND REPOLARIZATION

10.2.1. Cardiac Beat Detection

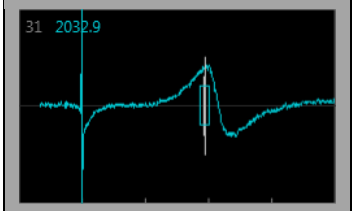
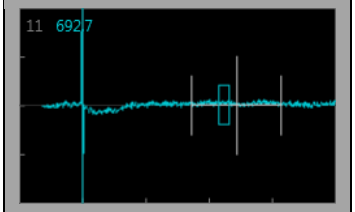
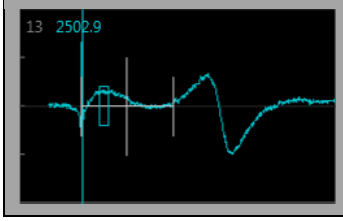
Cardiac beat detection is based on the depolarization spike for cardiac field potentials. The **Detection Threshold** of the **Cardiac Beat Detector** data processor should be set lower than the depolarization spike but greater than any other waveform feature. Since *AxIS Navigator* calculates endpoints 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 *AxIS 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** panel as horizontal gray lines above and below the data trace for each electrode.



10.2.2. Cardiac 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** panel 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 *Cardiac Analysis Tool*.

10.3. CARDIAC STATISTICS COMPILER ENDPOINTS

AxIS Navigator identifies cardiac beats with the **Cardiac Beat Detector** data processor (See Chapter 108 and Section 2.3.7). It then calculates beat rate, waveform, and conduction metrics with the **Cardiac Statistics Compiler** panel and outputs an **Advanced Metrics** file with the results (See Section 2.3.10). 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[1]	The peak to peak (positive plus negative) amplitude of the depolarization spike, in mV.
FPD[2]	The time from the depolarization spike to the peak of the T-wave, in ms.

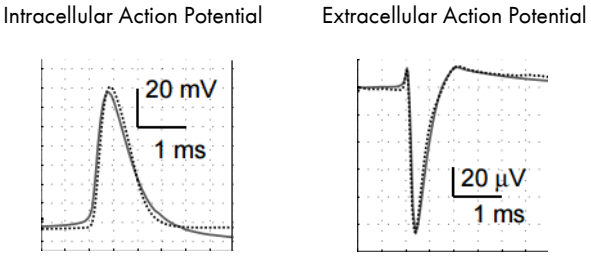
FPD COV[2]	The coefficient of variation (standard deviation/mean) of the FPD multiplied by 100.
Conduction Velocity	Speed of depolarization spike propagation across the culture. The propagation delay of each electrode is plotted against its distance from the beat origin. A best fit line is created from these delays, and the conduction velocity is the reciprocal of its slope.
2-point Conduction Velocity	Speed of depolarization spike propagation across the culture. The 2-point Conduction Velocity is calculated as the distance between the starting and ending electrode divided by the time between spike detection at the starting and ending electrode (i.e. Max Delay).
Max Delay	The time between depolarization spike detection at the starting and ending electrode.
Beat Amplitude[3]	The peak to peak (positive plus negative) amplitude of the contractility beat, in % change in impedance from baseline.
Excitation-Contraction Delay[3]	The time between the depolarization spike and the peak of the contraction.
Resistance[4]	Resistance is a measure of viable cell coverage over the electrode. Higher values indicate more intact cells are attached to the electrode. For a well, the average across electrodes is reported.
Number of Covered Electrodes[4]	Total number of covered electrodes within the well. Covered electrodes are defined as electrodes with resistance greater than the Covered Electrode Threshold defined in the Statistics Compiler settings (default 18 kOhms). Uncovered CytoView MEA microelectrodes in media exhibit a resistance of 8-12 kOhms. Following neuronal cell death, debris may leave a slightly higher uncovered resistance of 15-18 kOhms.
Weighted Mean Resistance[4]	The mean resistance across covered electrodes only (resistance greater than the Covered Electrode Threshold).

[1]This metric is available for field potential and contractility signals only (not computed for LEAP).
[2]These metrics are available for field potential signals only (not computed for LEAP or contractility).
[3]These metrics are available for contractility signals only (not computed for field potentials or LEAP).
[4]These metrics are only available with **MEA Viability Module**.
See Sections A.2 and A.3 for additional cardiac metrics available from the Cardiac Analysis Tool and AxIS Metric Plotting Tool.

CHAPTER 11. NEURAL DATA ANALYSIS

11.1. 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.

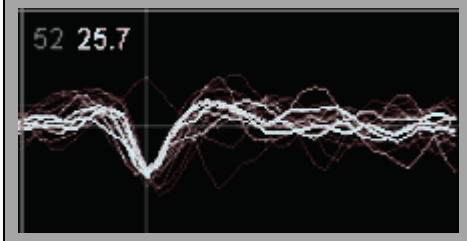
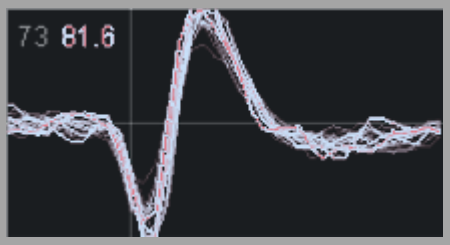
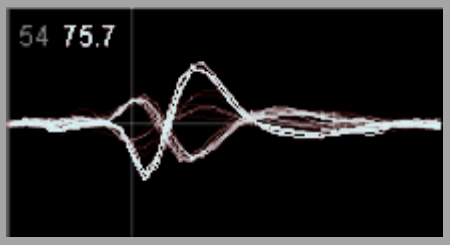
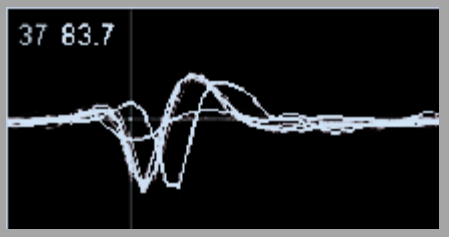
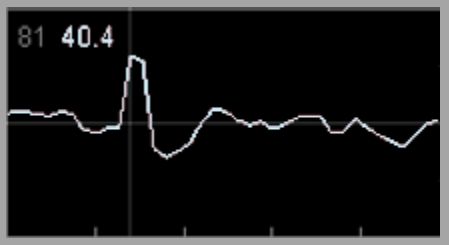
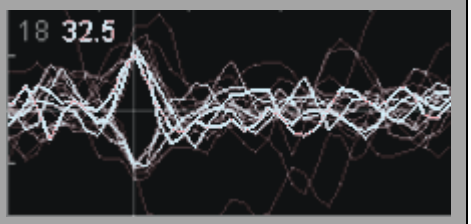
The first step to quantifying neural activity using an MEA is to identify individual neuronal action potentials or "spikes". The spike shape depends upon the cells' proximity to the electrode. Neuronal activity on an MEA is typically studied by quantifying the spike timing and how coordinated the spikes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

11.2. 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.

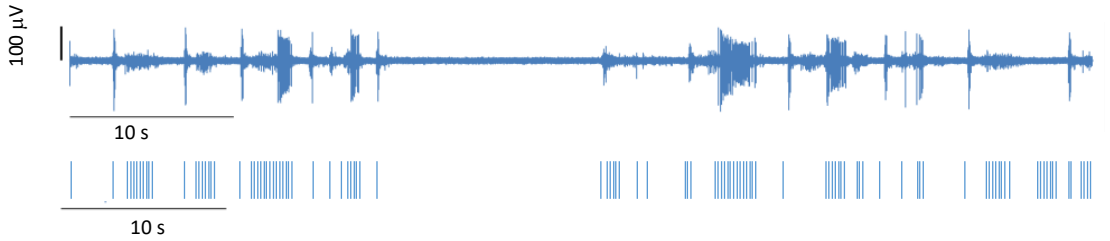
A quick check of the duration and amplitude of a spike can be helpful. Mammalian neurons produce action potential widths of approximately 1-2 ms. Any signals significantly shorter should be considered carefully. Amplitudes can vary greatly, but peak amplitudes typically range from 20 µV and 150 µV. Very 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** panel (Section 2.6.7).

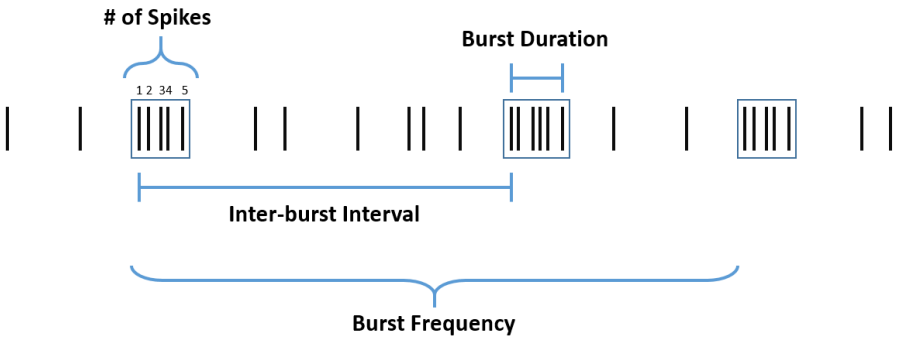
Waveform	Description	Physiological/Noise
	Consistent, repetitive monophasic waveform.	Physiological
	Consistent, repetitive biphasic waveform.	Physiological
	Multiple waveforms, but each waveform is consistent.	Physiological - different neurons detected by the same electrode
	Waveforms are the same shape 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

11.3. TYPES OF NEURONAL ACTIVITY

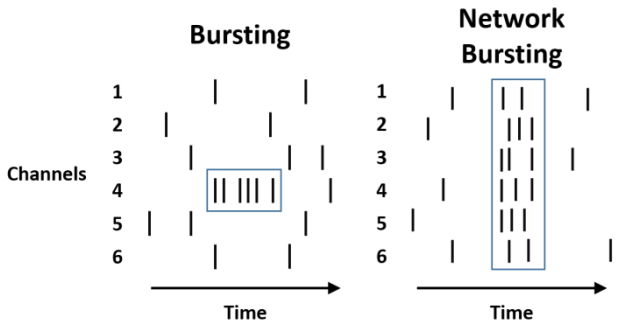
Beginning with continuous voltage data (top) from a single electrode, *AxIS Navigator* identifies neuronal action potentials, or "spikes". Plotting just the spike time and location will generate a raster plot (below).



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.



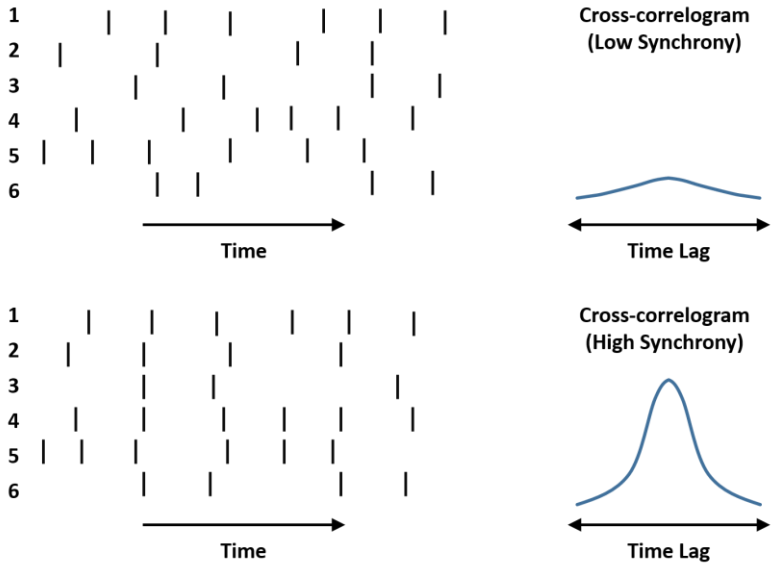
Having multiple electrodes in an array recording allows examination of network-wide coordinated activity. A network burst is a coordinated cluster of spiking across multiple electrodes. The distance between two electrodes is such that no two electrodes will detect firing from the same neuron. Coordinated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



While spikes represent a direct measure of neural activity, LFPs are an indirect measure of activity. In monolayer neural cultures, noticeable LFP events tend to be phase-locked with network bursting, and higher levels of synchrony within bursts tend to result in larger LFPs. In addition, quantifying features of the LFP shape such as amplitude and duration, a common method of analyzing LFPs is to take the power spectral density and compute the relative power of the signal in different frequency bands.

11.4. NEURAL STATISTICS COMPILER ENDPOINTS

AxIS Navigator identifies spikes and bursting with the **Spike Detector** and **Burst Detector** or the **Neural Event 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** panel and outputs an **Advanced Metrics** file with the results (See Section 2.3.11). 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.
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. This is a measure of spike regularity across the network. This metric captures the distribution of spiking such that 0 indicates spikes perfectly distributed and > 1 indicates network bursting.
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 Electrodes Participating in a 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.
Viability	
Resistance[1]	Resistance is a measure of viable cell coverage over the electrode. Higher values indicate more intact cells are attached to the electrode. For a well, the average across electrodes is reported.
Number of Covered Electrodes[1]	Total number of covered electrodes within the well. Covered electrodes are defined as electrodes with resistance greater than the Covered Electrode Threshold defined in the Statistics Compiler settings. Uncovered CytoView MEA microelectrodes in media exhibit a resistance of 8-12 kOhms. Following neuronal cell death, debris may leave a slightly higher uncovered resistance of 15-18 kOhms.
Weighted Mean Resistance[1]	The mean resistance across covered electrodes only (resistance greater than the Covered Electrode Threshold).
LFPs	
Number of LFPs[2]	Total number of LFPs detected across all electrodes in a well.
Number of LFPs per electrode[2]	Mean number of LFPs per electrode in each well. Standard deviation also reported.
LFP Rate[2]	For each well, reports the mean and standard deviation of the rate of LFP occurrence in Hz.
Amplitude[2]	For each well, reports the mean and standard deviation of the peak-to-peak amplitude of detected LFPs in µV.

[1]These metrics are only available with **MEA Viability Module**.
[2]These metrics are only available from a **Statistics Compiler** which has been attached to a **Neural Event Detector**

See Section A.1 for additional neural metrics available from the Neural Metric Tool.

APPENDIX A. AXION STANDALONE TOOLS

Axion provides a number of standalone analysis 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.

A.1. NEURAL METRIC TOOL

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, an LFP visualization tool with an additional method for extracting LFPs, and generates plate map visualizations, raster plots, and synchrony cross-correlograms not available in *AxIS Navigator*. See Chapter 11 for a detailed description of spiking, bursting, synchrony, and network bursting.

Note: *Files generated by NETRI NeuroFluidics™ MEA plates are not supported in the Neural Metric Tool.*

A.2. CARDIAC ANALYSIS TOOL

The *Cardiac Analysis Tool* (previously *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 *Cardiac Analysis Tool* also analyzes LEAP signals for automated arrhythmia detection and classification and generation of LEAP endpoints (including APD90). The tool automatically generates a PDF report containing experimental results for field potentials and LEAP.

A.3. AXIS METRIC PLOTTING TOOL

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. For neural files, the tool automatically generates a PDF report containing experiment results. In addition to these, the AxIS Metric Plotting Tool analyzes contractility raw signals for beat detection and generation of additional endpoints, such as Beat Width and Composite Beat Amplitude.

A.4. AXION DATA EXPORT TOOL

The *Axion Data Export Tool* is used to export .raw data into a .csv file, and convert .spk files into .nex files for use with 3rd party software.