
# CARDIAC ANALYSIS TOOL

## USER GUIDE

Version 3
August 2021

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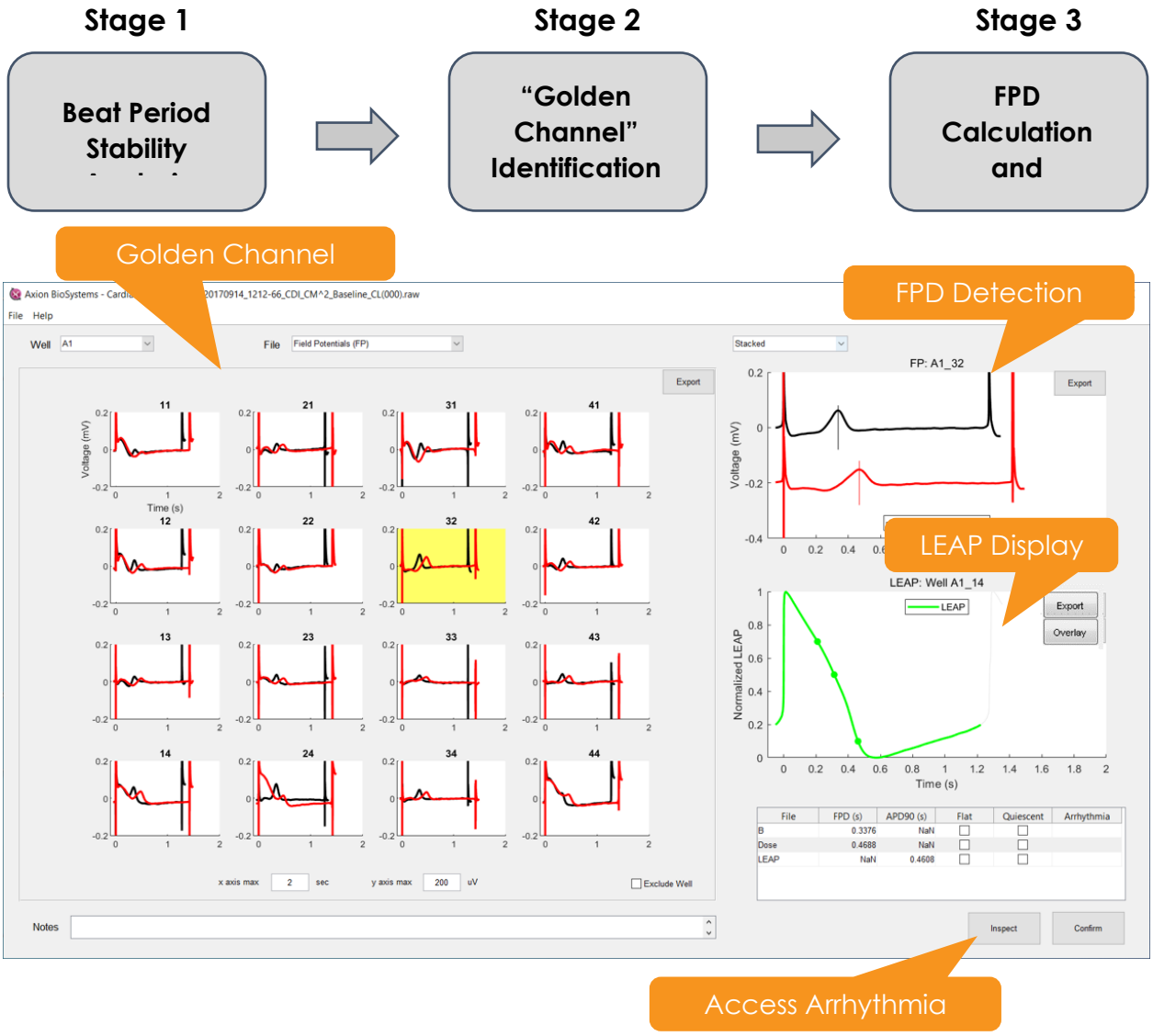
# 1. INTRODUCTION

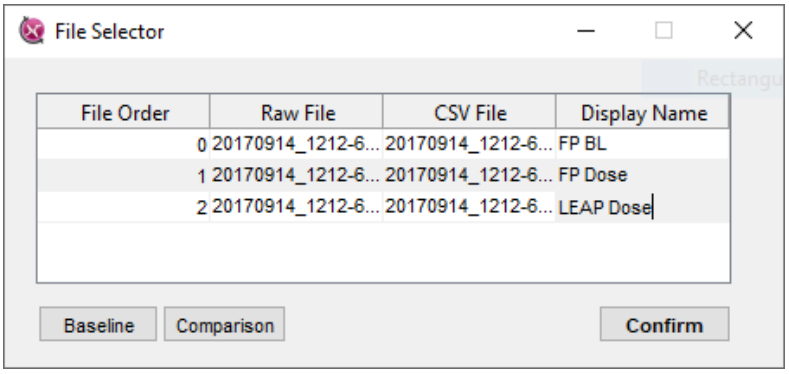
The *Cardiac Analysis Tool* is a comprehensive cardiac analysis software designed for any cardiac application requiring precise assessment of field potential duration (FPD) and arrhythmia. The *Cardiac Analysis Tool* also provides LEAP signal analysis for characterization of action potential morphology and automated arrhythmia detection and classification. The *Cardiac Analysis Tool* is not compatible with Contractility files.

The *Cardiac Analysis Tool* operates according to the workflow below. *AxIS*/*AxIS Navigator* identifies all beats and a stable period of beating for each well in Stage 1. In Stage 2, a "Golden Channel" is identified. The "Golden Channel" is an electrode with trackable repolarization and that best represents the electrodes in the well. The tool automatically selects a Golden Channel for the field potentials and for each LEAP file. In Stage 3, FPD and/or LEAP endpoints are calculated and arrhythmic events are identified and classified. The user reviews the results of the automated algorithm and

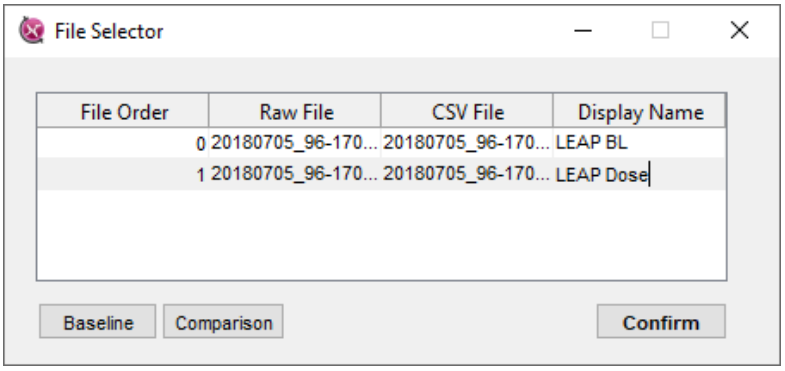


has the option to manually correct the "Golden Channel" selections and FPD repolarization timing.

Field potential and LEAP files can be analyzed at the same time. For example, if you record a Baseline field potentials file, followed by a dosed field potential file and dosed LEAP file, all three files may be analyzed together.




However, if comparing multiple LEAP files (e.g. Baseline and dosed), it may be beneficial to analyze field potential and LEAP files separately, allowing you to specifically identify the Baseline file for each specific file type. In this case, you may load a Baseline field potentials file with Comparison (e.g. dosed) field potential file(s) for one analysis. Then, load a Baseline LEAP file with Comparison (e.g. dosed) LEAP file(s) for separate analysis.

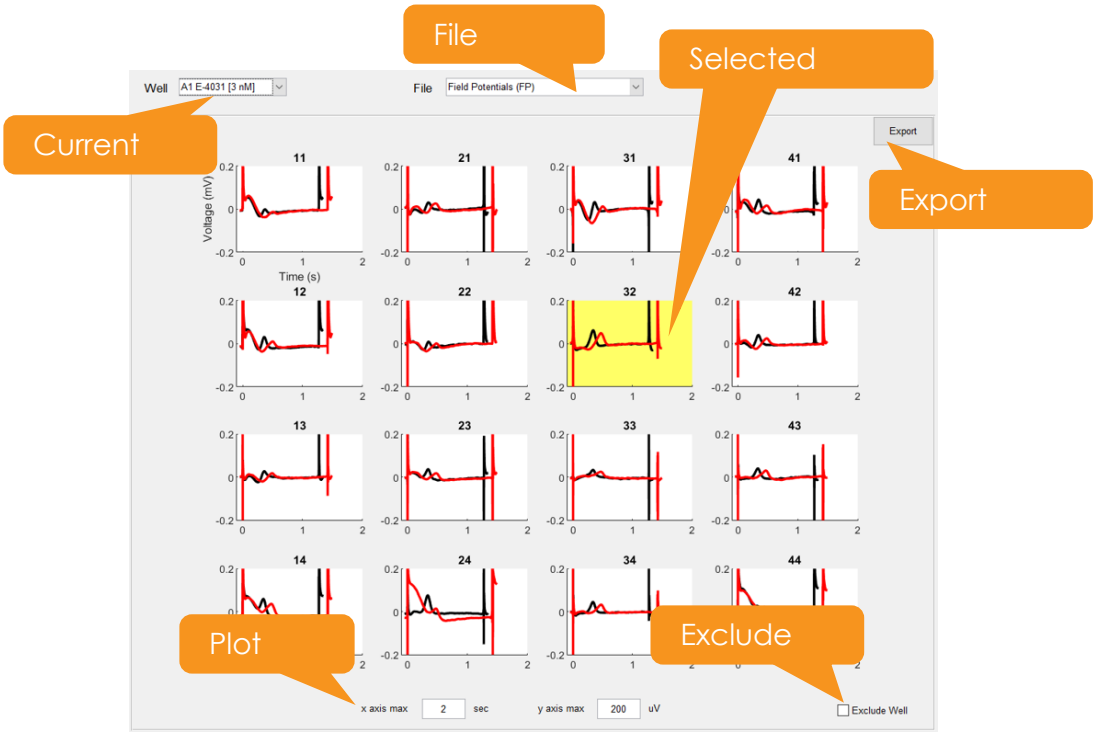


# 2. CARDIAC ANALYSIS TOOL OVERVIEW

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

## 2.1. Golden Channel Selector

The **Golden Channel Selector** displays the field potential or LEAP waveforms for the file(s) selected in the **File Selector** and is used to select the "Golden Channel" electrode for endpoint analysis. The selected "Golden Channel" is highlighted in yellow. The beat waveforms for each electrode are an average of 5 beats from the stable region




identified by *AxIS*.

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

To change the axis limits, use the **x axis max** and **y axis max** fields to adjust the x- and y-axes for all waveform plots. Note that LEAP waveforms are amplitude normalized on the y-axis and plotted from 0 to 1.

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

### 1.1.1. Field Potential Golden Channel

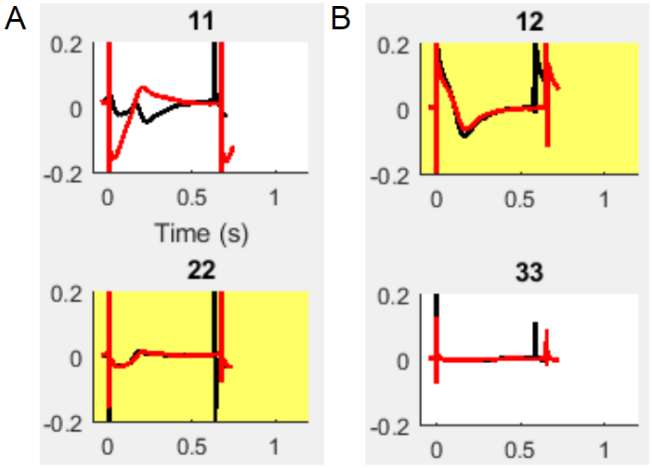
For field potentials, the "Golden Channel" (GC) is an electrode with a clear, consistent beat waveform, and the GC applies to all field potential files. The T-wave should be identifiable across files and its timing representative of the electrodes in the well. The tool automatically selects the FP GC as the electrode with the largest T-wave in the baseline file and highlights the electrode in yellow. The GC can be changed by first selecting **Field Potentials (FP)** in the **File Selector** and then clicking on a new GC. The FP waveforms from the selected GC electrode are displayed in **FPD Detection** window.

Select a field potential GC according to the following criteria:

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

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

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




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

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

To change the beat detection threshold:

1. Click **File → Advanced → Change Detection Threshold.**
2. Type the **Beat Detection Threshold** as a multiple of the standard deviation on each electrode.

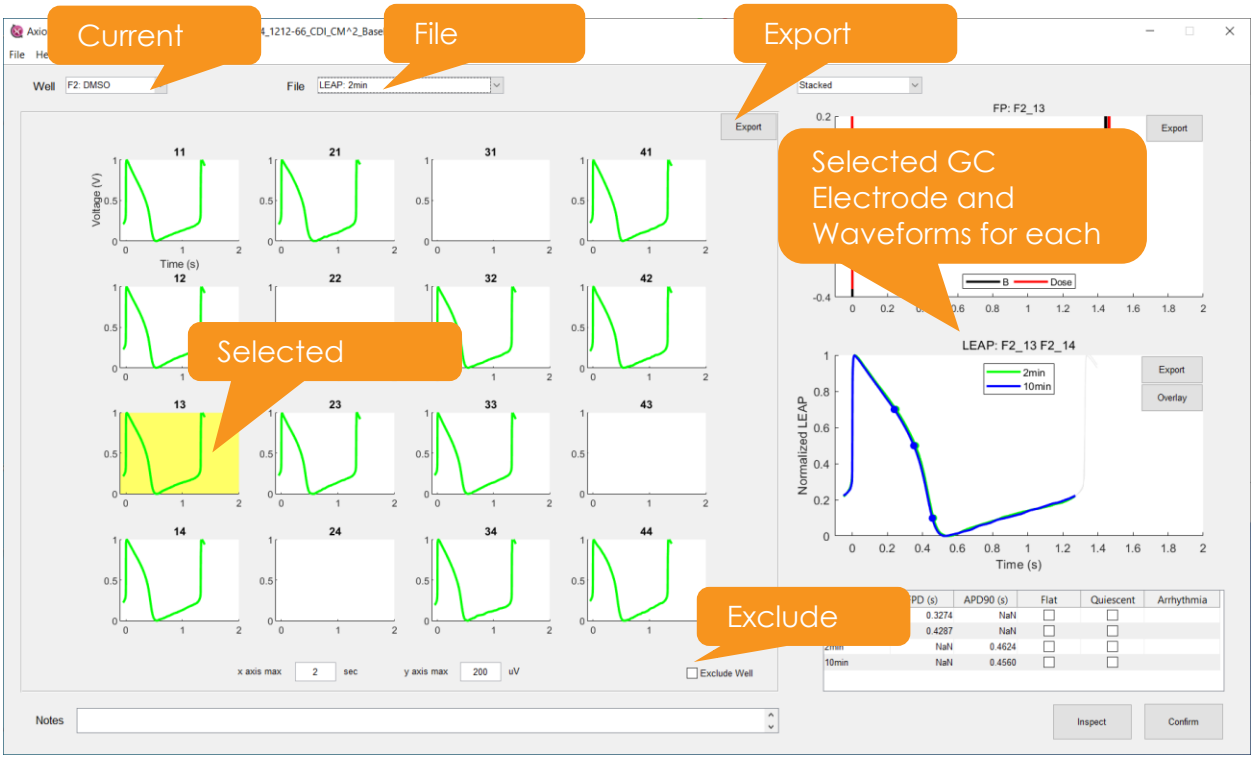
3. Click **Apply**. Average beats will be calculated again using the new detection threshold.

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

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

### 1.1.2. LEAP Golden Channel

For LEAP, the GC is file specific, as it is recommended that different electrodes are LEAPed for each LEAP induction. The largest and best LEAP signal is automatically detected for each LEAP file in each well. The LEAP GC can be changed by first selecting the LEAP file in the File Selector and then clicking on a new LEAP GC for that




file. The LEAP waveforms from the selected GCs are displayed in the **LEAP Display** window.

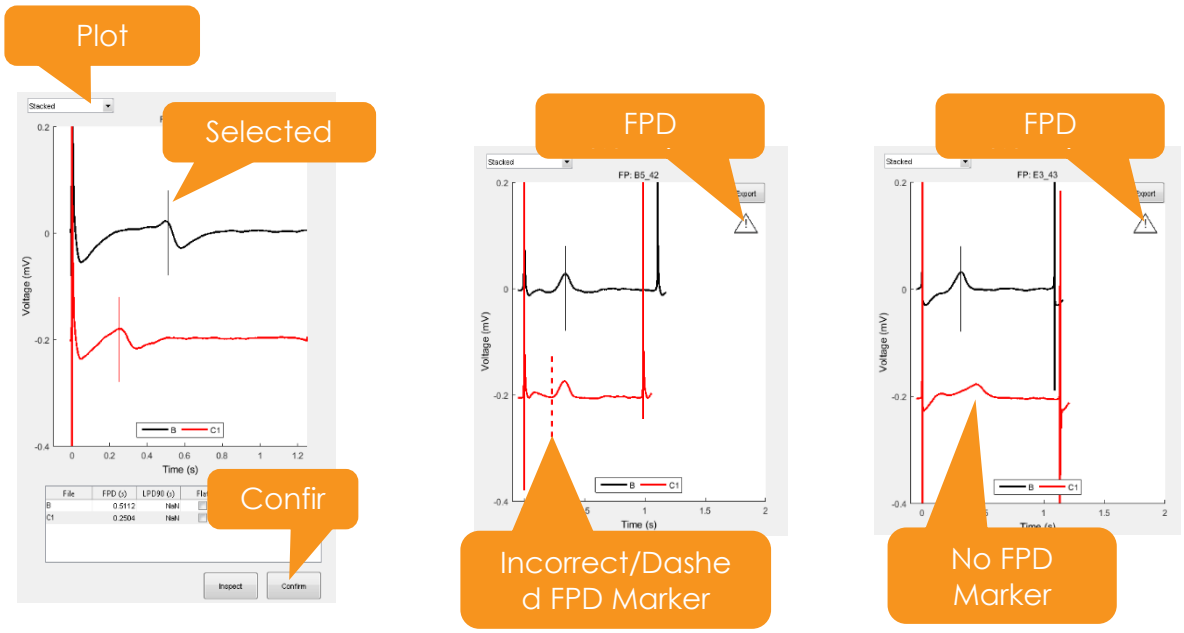
If the average beat waveform is missing from several LEAP electrodes that detected LEAP beats in AxIS, there is an **Advanced** menu option to **Use AxIS Beat Detection**. In this case, beat timings are based on AxIS well beats. To change the beat detection method, click **File → Advanced → Use AxIS Beat Detection**. When the current beat

detection method is AxIS Beat Detection, click **File → Advanced → Use Standard Beat Detection** to revert to the standard Cardiac Analysis Tool beat detection algorithm. In addition, if LEAP beats are particularly small (< 1 mV), refer to the AxIS Navigator User Guide for **High Sensitivity** LEAP Detection.

## 2.2. FPD Detection Display

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

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

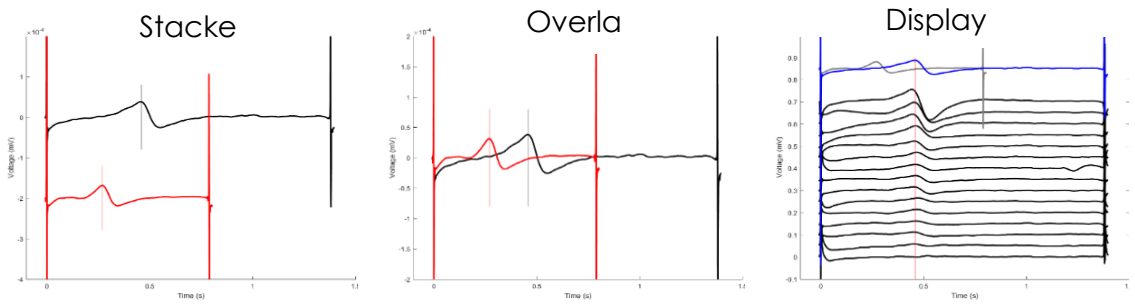



If the incorrect T-wave is identified for any file, click on the correct location on the beat waveform. The tool will automatically determine a new peak or trough in the local region around the click.

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

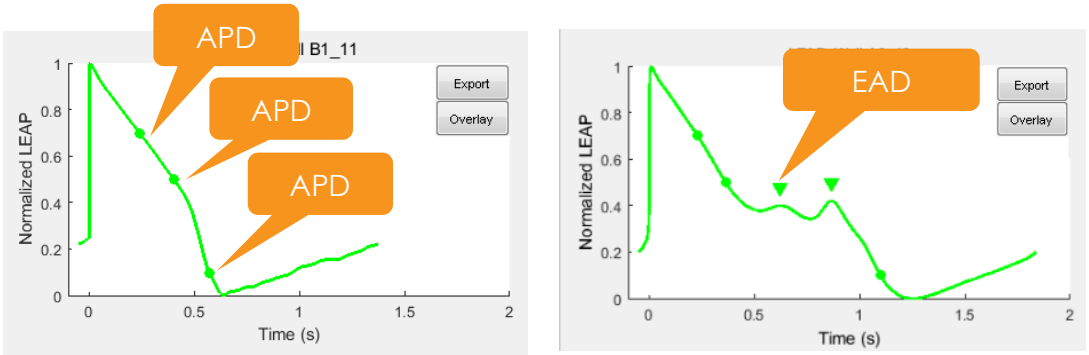
1. **Stacked** (default): The beat waveforms from each file are spaced vertically in the display. The T-wave peak in each file is marked by a vertical line.
2. **Overlay**: The beat waveforms from each file are plotted on top of one another. The T-wave peak in each file is marked by a vertical line.

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



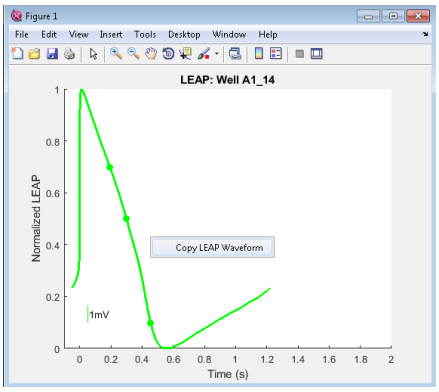

## 2.3. LEAP Display

The LEAP Display shows an overlay of the LEAP waveforms for each well, normalized to its maximum value. The tool automatically selects the best LEAP in each well. If a different GC is selected in the **Golden Channel Selector**, the **LEAP Display** will automatically update. The circles along the trace indicate the LEAP Potential Duration (APD) at 30%, 50%, and 90% of voltage repolarization, respectively. The LEAP signal also enables automated EAD detection and classification. If an EAD(s) is detected, the




EAD(s) will be marked by a colored triangle above the feature. The EAD class will be indicated in the table below.

Click **Export** to export a copy of the LEAP display to an exported figure and to the clipboard. Additionally, to copy the LEAP waveform data, right click in the exported figure window and select **Copy LEAP Waveform**. The waveform data is copied to the clipboard and can be pasted in a program like Excel for plotting. Click **Overlay** to copy the LEAP waveform to an overlay figure. Additional wells can be added to the overlay figure as desired using the Overlay button.

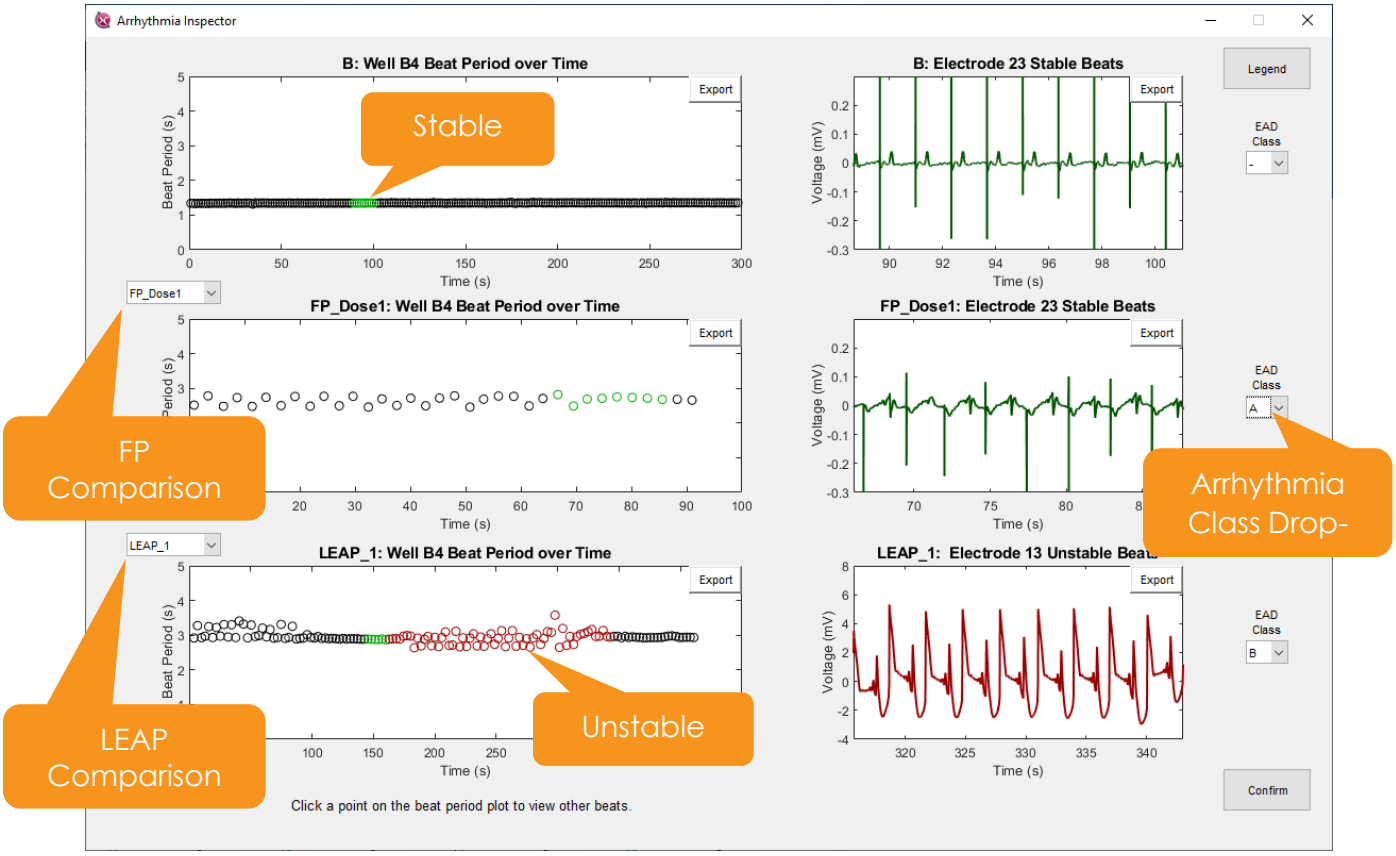



To exclude LEAP analysis and only perform field potential analysis on all channels in a LEAP file, there is an **Advanced** menu option to change to **Field Potentials Only Mode** in Cardiac Analysis Tool. To exclude LEAPs and analyze field potentials only, click **File → Advanced → Field Potentials Only Mode**. Note that even LEAP tagged channels will now be processed as field potentials.

## 2.4. Arrhythmia Inspector

The **Arrhythmia Inspector** displays beat period and continuous voltage plots for the Baseline, Comparison, and LEAP files (if present). It displays regions of instability and allows for the verification and classification of arrhythmic events. Clicking the Inspect Button at the bottom of the main window will launch the **Arrhythmia Inspector**. If arrhythmic events are detected in any file, the Inspect button will be red.

For field potential files, arrhythmias are detected based on an algorithm sensitive to beat period instability from beat to beat. In these cases, the tool will automatically select arrhythmia type A for comparison files. If more than one comparison file is loaded, a drop-down will appear for selecting which field potential file to inspect. For LEAP files, arrhythmias are detected based on both beat period instability and repolarization irregularities in the LEAP beat waveforms. Both stable beats and unstable beats are inspected for arrhythmias or EADs. The related metrics, such as the Percentage of beats with EADs will default to the stable beats value to represent




steady-state. However, if no EADs are present in the stable beats and EADs are present in the unstable beats, unstable beat values are reported.

***Note**: It is possible to have unstable beating when no arrhythmia is present, for example due to a drift in beat period. Likewise it is possible to have stable beating when*

*arrhythmic events are present, particularly if every beat contains the EAD feature. Thus, the red button should only be used as a guide and all arrhythmic events should be visually verified and classified. LEAP signals are very useful for verifying EAD presence and type.*

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

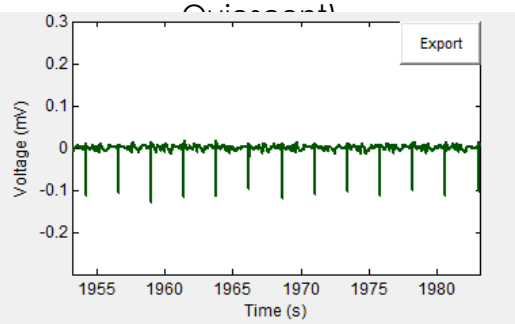
The right plots display representative continuous voltage traces from the electrode(s) selected by the **Golden Channel Selector** for FP and LEAP files. If unstable beats are present, the unstable beats (red) will display by default. Otherwise, the most stable beats (green) will display. To view continuous voltage from other beats, click on a beat in the beat period plots. Clicking on a black beat will require extra time as the tool loads new data from the raw file. Use the continuous voltage data plots to check for arrhythmic events or quiescence.

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

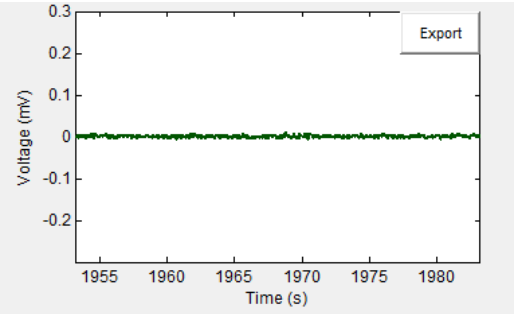
If more than one comparison file is loaded, you can view data from the other files using the drop-down menus to the left. Note that any changes to the arrhythmia classifications for a file are stored as you toggle through the other comparison files.

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

Sub-threshold beating (Not Quiescent)




Quiescent



beats are present, check the **Quiescent** box to label this well/condition as quiescent in the output .csv file.

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

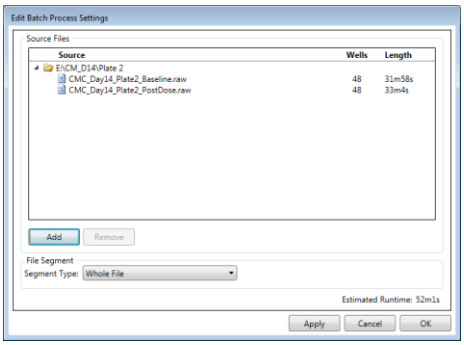
## 2.5. Confirm

Importantly, press **Confirm** to accept the GC selections, FPD markings, and arrhythmia classifications (if applicable, see Section 2.3) and proceed to the next well. Prior to confirming a well, if a new GC is selected, the tool will recalculate the FPD and LEAP Rise Time, Triangulation, and APD based on the new selected channel. After a well has been confirmed, the selected GC and associated endpoints are retained for the well.

# 3. OPERATION

## 3.1. Process Recordings in AxIS

Continuous recording is required to take advantage of the full utility of the analysis tools. The *Cardiac Analysis Tool* utilizes the **Advanced Metrics** .csv output from the **Cardiac Statistics Compiler** in *AxIS*. Follow these steps to process the data using *AxIS 2.3*, *AxIS 2.4*, or *AxIS Navigator*:

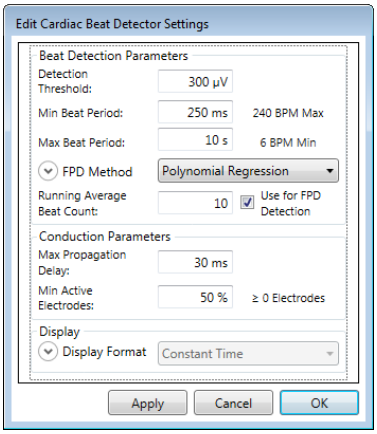



1. Enter the plate map information into the baseline .raw file if it is not already present. See Section 2.2 of the *AxIS* User Guide for additional information about plate maps.
2. Click **File → New Batch Process...**.
3. Click **Add** in the **Edit Batch Process Settings** dialog.
4. Select the desired .raw files and click **Open**.
5. Select **Whole File** in the **Segment Type** drop-down menu.

   ***Warning:*** *When analyzing a file segment, only record the .csv and do not re-record the .raw file. This will result in a mismatch of the beat times and .raw file time.*

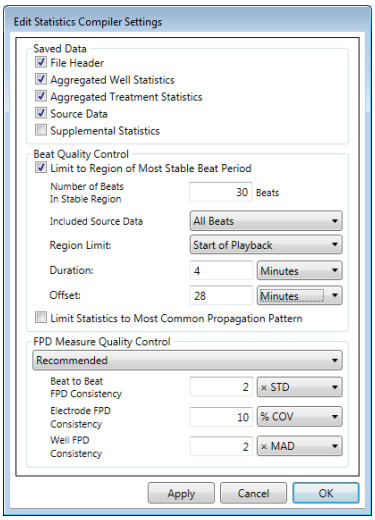
6. Click **OK**.
7. Right-click on the batch process in the **Streams** window and select **Configuration → Cardiac Offline → Spontaneous** or **LEAP**.
8. Double-click the **Cardiac Beat Detector** to open the settings and modify them if desired. Recommended settings:

   **Detection Threshold** = 300 µV
   **Min Beat Period** = 250 ms
   **Max Beat Period** = 10 s
   **FPD Method**: **Polynomial Regression**



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

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

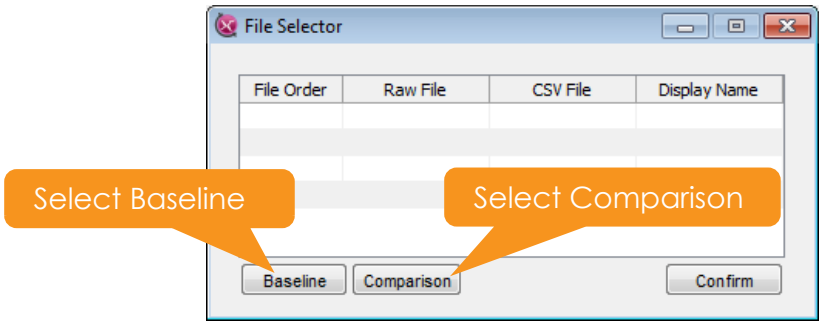



9. Double-click the **Cardiac Statistics Compiler** to open the settings. Recommended Settings:
   **Limit to Region of Most Stable Beat Period** = Enabled
   **Included Source Data** = **All Beats**
   Set the analysis window using the **Region Limit**, **Duration**, and **Offset** fields.
10. Confirm **Advanced Metrics** is selected from the **Statistics Compiler** drop-down menu in **Experiment Setup Properties**.
11. Click **Start Batch Process** to run the batch process and save the .csv files. The files are saved to the same directory as the .raw files.

## 3.2. Analyze Data in the Cardiac Analysis Tool

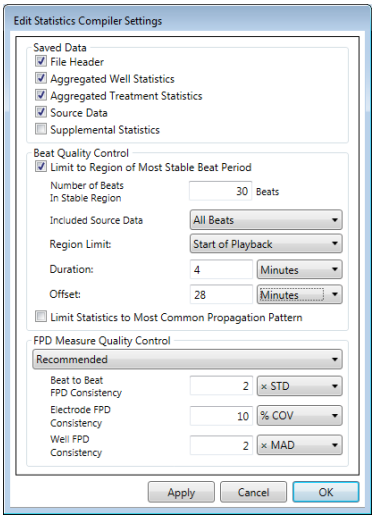
Loading a new experiment will take several minutes. The software computes an average beat waveform for every electrode in every file loaded. Status bars will display loading progress. After the data has been loaded and processed, new .mat files are created corresponding to each .raw file selected for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

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

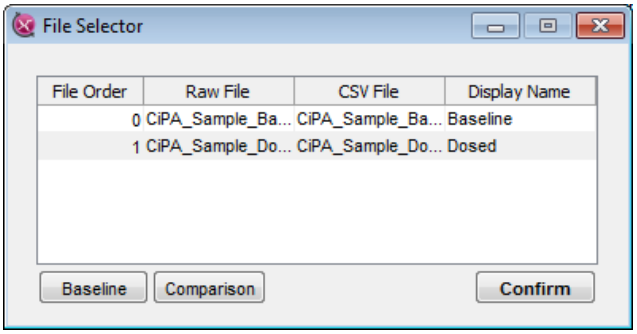



3. Click **Baseline** to import a baseline file that will be used as the reference point for the comparison files.

***Note**: Field potential and LEAP files can be analyzed together. For example, if you record a Bassline field potentials file, followed by a dosed field potential file and dosed LEAP file, all three files may be analyzed together. However, if comparing multiple LEAP files (e.g. Baseline and dosed), it may be beneficial to analyze field potential and LEAP files separately, allowing you to specifically identify the Baseline file for a specific file type.*




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




10. Optional: Load a new plate map if there is no plate map in the file or to use an alternate plate map. If there is a plate map in the baseline .csv file it will automatically be used for analysis.
    10.1. Click **File → Load → New Plate Map**.
    10.2. Navigate to and select a .platemap file or .csv file and click **Open**. For more information about generating plate maps in *AxIS* and saving .platemap files, see section 2.2 of the *AxIS* User Guide.

11. To modify the automatic "Golden Channel" selections for a file(s), select either Field Potential (FP) or a LEAP file from the **File Selector**. Then, select a new "Golden Channel" (highlighted in yellow) in the **Golden Channel Selector**. See Section 2.1.
12. Verify the T-wave peak detection in the **FPD Detection Display**. Section 2.2.
    12.1. Click the T-wave peak on the beat waveform to correct any misidentified T-waves.
13. Inspect for arrhythmia in the **Arrhythmia Inspector**. See Section 2.3.
    13.1. Click on the Inspect button in the bottom right.
    13.2. Change arrhythmia classification as needed.
    13.3. Update the quiescent checkboxes on the main window as needed.
    13.4. Click **Confirm**.
14. Click **Confirm** to proceed to the next well.
15. Repeat steps 11-14 for each well.

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

    ***Note**: An incomplete analysis can be saved and exited at any time for future completion. The saved .mat file contains the file names, display names, Golden Channel selections, FPD selections, and arrhythmia classifications.*
18. Click the **Export** button near any figure to copy the image to the clipboard and save the figure as a variety of file types.
19. Click **File → Export → Export Well Endpoints to CSV (Field Potential Endpoints or LEAP Endpoints)**, **Export Report (Field Potential Report or LEAP Report), Export Figures**, **Export Plot Source Data to CSV**, or **Export CiPA CSV**, to create file outputs. See Section 4 for more information about the *Cardiac Analysis Tool* outputs.

## 3.3. Load a saved experiment

Previously loaded experiments may be reloaded for continued analysis.

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

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

# 4. OUTPUTS

## 4.1. Export Well Endpoints to CSV

**Export Well Endpoints to CSV** exports the cardiac endpoints for each well to a .csv file. For each file, a block of statistics is provided, organized according to well. The endpoints from all analyzed files are saved to a single .csv file. Endpoints can be exported for all loaded Field Potential files or all loaded LEAP files.

| AxIS File | CiPA_Sample_Baseline.raw | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| File Type | 48-well | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | |
| Measurement | A1 | A2 | A3 | A4 | A5 | A6 | A7 | A8 | B1 | B2 | B3 | B4 | B5 | B6 | B7 | B8 | C1 |
| Treatment/ID | Ranolazin | Ranolazin | Ranolazin | Ranolazin | Nifedipin | Nifedipin | Nifedipin | Nifedipin | Ranolazin | Ranolazin | Ranolazin | Ranolazin | Nifedipin | Nifedipin | Nifedipin | Nifedipin | Ranolazin |
| Start Time | 1762.899 | 1700.623 | 1765.106 | 1761.737 | 1784.756 | 1805.395 | 1797.655 | 1710.888 | 1740.743 | 1695.55 | 1790.193 | 1704.294 | 1789.747 | 1695.306 | 1644.105 | 1734.862 | 1785.935 |
| End Time | 1812.703 | 1746.256 | 1807.869 | 1803.37 | 1826.712 | 1844.289 | 1837.545 | 1752.625 | 1785.725 | 1737.162 | 1830.501 | 1744.471 | 1829.939 | 1734.823 | 1684.034 | 1775.875 | 1829.384 |
| Golden Channel | 23 | 44 | 32 | 34 | 34 | 34 | 23 | 23 | 24 | 34 | 43 | 24 | 21 | 43 | 13 | 32 | 32 |
| Beat Period (s) | 1.71715 | 1.573415 | 1.474374 | 1.435473 | 1.39836 | 1.340862 | 1.375367 | 1.439059 | 1.49906 | 1.434634 | 1.38985 | 1.385281 | 1.385758 | 1.362357 | 1.376724 | 1.414068 | 1.498038 |
| Beat Period CoV (%) | 0.090851 | 0.108507 | 0.077176 | 0.201566 | 0.091718 | 0.076696 | 0.088444 | 0.085489 | 0.080531 | 0.089617 | 0.116763 | 0.122287 | 0.083262 | 0.100346 | 0.094175 | 0.077922 | 0.098375 |
| FPD (ms) | 552 | 586.4 | 580 | 549.6 | 436 | 415.2 | 440 | 552.8 | 475.2 | 520.8 | 512 | 518.4 | 436 | 406.4 | 432.8 | 448 | 469.6 |
| FPDc (Fridericia ms) | 460.9669 | 504.173 | 509.5954 | 487.2087 | 389.8942 | 376.5268 | 395.6518 | 489.6381 | 415.2122 | 461.7681 | 458.79 | 465.0351 | 391.0726 | 366.598 | 389.0495 | 399.1363 | 410.4124 |
| Spike Amplitude (mV) | 1.696184 | 1.496748 | 1.599644 | 1.701525 | 1.743256 | 1.894418 | 1.736429 | 1.880765 | 1.376033 | 2.258588 | 0.746716 | 1.231414 | 1.053235 | 2.101076 | 1.499118 | 1.855675 | 1.612399 |
| Spike Amplitude CoV | 0.024451 | 0.035747 | 0.041912 | 0.076879 | 0.043059 | 0.032606 | 0.030675 | 0.021791 | 0.022322 | 0.010612 | 0.063284 | 0.031622 | 0.060798 | 0.014731 | 0.04633 | 0.038764 | 0.020108 |
| Spike Slope (V/s) | -3.80246 | -3.32981 | -3.53893 | -3.70284 | -3.84249 | -4.00332 | -3.88105 | -4.24667 | -3.10478 | -4.8968 | -2.00229 | -2.65012 | -2.58619 | -4.48942 | -3.33567 | -4.14377 | -3.70026 |
| Spike Slope CoV | -0.0243 | -0.03569 | -0.03864 | -0.11757 | -0.04067 | -0.02802 | -0.02837 | -0.02162 | 0.267089 | -0.01084 | -0.05055 | -0.51149 | -0.06033 | -0.01393 | -0.04361 | -0.03555 | -0.02043 |

The available cardiac endpoints are defined in the table below.

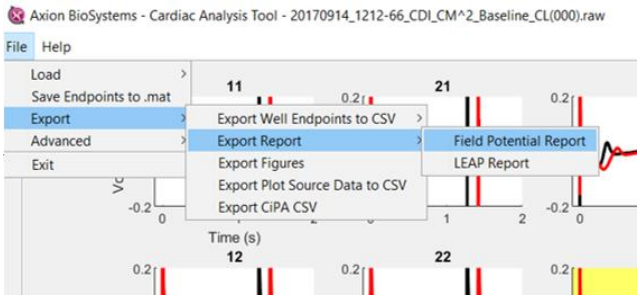
| Measurement | Description |
|---|---|
| **Starting Time** | The start time of the analysis window. |
| **Ending Time** | The end time of the analysis window. |
| **Beat Period** | The time between successive depolarizations (i.e. beats), in seconds. |
| **Beat Period CoV** | The coefficient of variation (standard deviation/mean) of the beat period multiplied by 100. |
| **Conduction Velocity** | Speed of depolarization 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. |
| **Field Potential Only Endpoints:** | |
| **FPD** | The time from the depolarization spike to the peak of the T-wave, in ms. |
| **FPDc** | The FPD corrected for beat rate according to Fridericia's method, in ms. FPDc = (FPD in ms)/(BP in s)$^{1/3}$ |
| **Spike Amplitude** | The peak to peak (positive plus negative) amplitude of the depolarization spike, in mV. |
| **Spike Amplitude CoV** | The coefficient of variation (standard deviation/mean) of the spike amplitude multiplied by 100. |
| **Spike Slope** | The maximum change in voltage over time (dV/dt) of the depolarization spike, in V/s. |

| | |
|---|---|
| **Spike Slope CoV** | The coefficient of variation (standard deviation/mean) of the spike slope multiplied by 100. |
| **LEAP Only Endpoints:** | |
| **LEAP Flag** | This flag indicates whether at least one LEAP was detected and included in a well (1) or not (0). |
| **APD30, 50, 90** | The duration of the action potential from beat start to 30%, 50%, or 90% voltage repolarization (APD30, APD50, APD90, respectively), where full repolarization is defined from peak to trough. APD is reported in seconds. |
| **APDc** | The APD90 corrected for beat rate according to Fridericia's method, in seconds. APD90c = (APD in s)/(BP in s)$^{1/3}$ |
| **Triangulation Ratio** | The ratio of APD50 to APD90, such that increased triangulation results in a decreased triangulation ratio. |
| **Rise Time** | Rise time is measured as the time between the take-off point to the peak of the LEAP, in ms. |
| **Percent Beats with EADs** | The percentage of analyzed beats with at least one early after depolarization. |
| **Viability Endpoints:** | |
| **Resistance (kOhms)** | Resistance is a measure of viable cell coverage over the electrode. Higher values indicate more intact cells are attached to the electrode. For a well, the average across electrodes is reported. (See the MEA Viability chapter of the AxIS Navigator user guide for more details.) |
| **Number of Covered Electrodes** | Total number of covered electrodes within the well. Covered electrodes are defined as electrodes with resistance greater than the Covered Electrode Threshold (default 11 kOhms). Uncovered CytoView MEA microelectrodes in media exhibit a resistance of 8-12 kOhms. |
| **Weighted Mean Resistance (kOhms)** | The mean resistance across covered electrodes only (resistance greater than the Covered Electrode Threshold). |

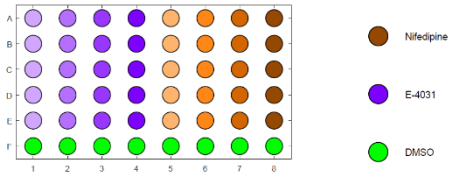
*Viability metrics are only available if recorded in AxIS Navigator with the MEA Viability Module.

## 4.2. Export Report

**Export Report** creates a PDF report with analysis of baseline variability, outlier wells, sample waveforms, and dose-response bar plots for each compound or condition. Reports can be created for Field Potential files (requires at least 2 files) or LEAP files. Example report elements are shown below.




**Chapter 1. Experiment Information**

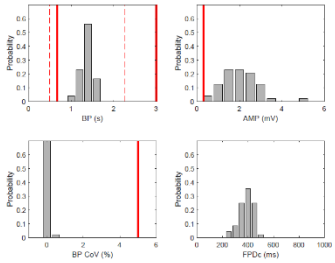



| | |
|---|---|
| Experiment Date | 9-14-2017 |
| Investigator | |
| Description | |
| File Path | F:\1212-66_CDI_CM2\M2_Dose_LEAP\ |
| Baseline File | 20170914_1212-66_CDI_CM^2_Baseline_CL(000).raw |
| Comparison File | 20170914_1212-66_CDI_CM^2_PostDose_CL(000).raw |
| Comparison File | 20170914_1212-66_CDI_CM^2_PostDose_CL_postLeap(000)_2to7min(000).raw |

**Chapter 2. Baseline Well-to-Well Reliability**

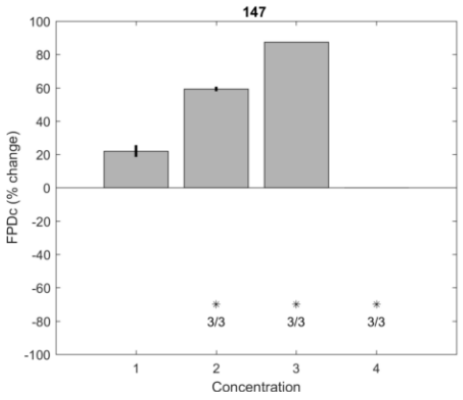
**2.1. Figures**

These figures present the histogram across wells for Beat Period (BP), Beat Period Coefficient of Variation (BP CoV), Spike Amplitude (AMP) and Fridericia Rate-corrected Field Potential Duration (FPDc) at Baseline. Red lines indicate absolute cut-offs for HESI defined CiPA Phase II inclusion criteria, and dashed red lines indicate cut-offs relative to the mean and standard deviation of the data set. Wells that fail these criteria are listed in the section below.

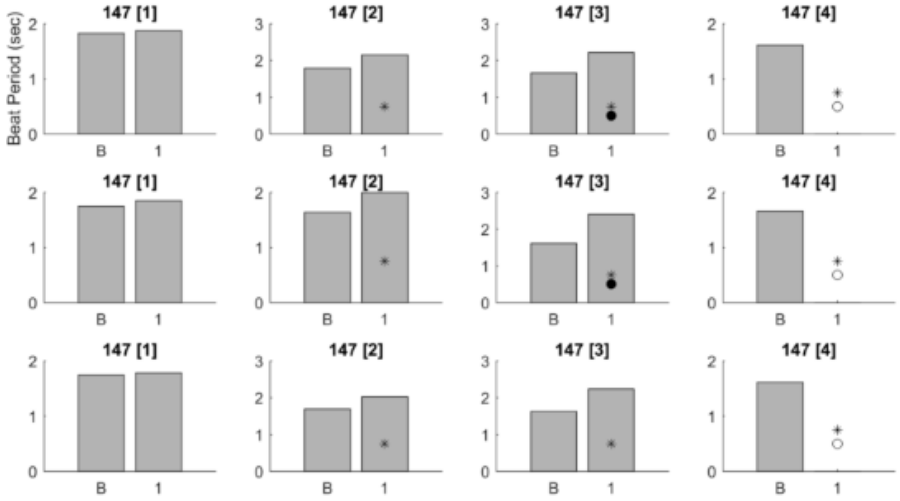



## 4.3. Export Figures

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




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



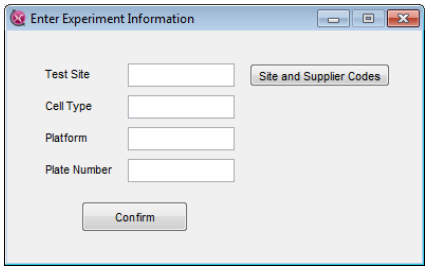

## 4.4. Export Plot Source Data to CSV

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

| FPDc (% change) | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 144 [2] | 144 [3] | 144 [4] | 145 [3] | 145 [4] | 147 [1] | 147 [2] | 147 [3] | 147 [4] | 148 [1] | 148 [2] | 148 [3] | 148 [4] | DMSO |
| Dosed Mean | 3.705203 | 14.58947 | 59.84818 | 13.73555 | | 22.07088 | 59.34157 | 87.49434 | | 68.16613 | 161.1652 | 217.8626 | 267.5429 | 0.580577 |
| Dosed Std Dev | 2.286244 | 2.633928 | 14.87951 | 0 | | 3.515705 | 1.348871 | 0 | | 16.50948 | 28.04275 | 47.84038 | 0 | 0.918771 |
| Dosed Replicates | 1.575381 | 11.86664 | 48.81883 | 13.73555 | | 20.61279 | 60.51598 | | | 58.35353 | 141.336 | 184.0344 | | 1.641121 |
| | 3.419262 | 14.77734 | 53.95388 | | | 26.08103 | 59.64037 | | | 58.91801 | | 251.6909 | 267.5429 | 0.026299 |
| | 6.120967 | 17.12444 | 76.77183 | | | 19.51882 | 57.86835 | 87.49434 | | 87.22685 | 180.9945 | | | 0.074312 |

## 4.5. Export CiPA CSV

**Export CiPA CSV** exports the cardiac metrics for each well to a .csv file formatted to the reporting requirements as defined by the CiPA Myocyte Committee. Enter experiment information in the dialog box that appears. Type the test site code, cell type code, platform code, and plate number into the respective fields, and click **Confirm**. Endpoints are saved in a row-based format. The endpoints from all analyzed files are saved to a single .csv file.




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