



NemAnalysis Software

User Guide

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NemAnalysis detects and quantifies pharyngeal pumps in ScreenChip recordings. In NemAnalysis, a **pharyngeal pump is defined as** an event with a distinct positive excitation event (E spike) followed shortly by a distinct negative relaxation event (R spike).

The number of pumps, pump durations, pump amplitudes and inter-pump intervals can then be exported and compared for easy statistical analysis.

Minimum Computer Requirements

1. Windows 10 64-bit OS or Mac OS X 10.11
2. 4 GB memory
3. 2.6Hz Intel Celeron Processor
4. Minimum 1440 x 900 display resolution

Installation

1. Open the NemAnalysis installation file on your computer. For Windows this will be an .exe file. For Mac OS X this will be a .pkg file.
2. For Windows Users:
Follow the on-screen directions to complete installation. A NemAnalysis icon will be placed on your desktop. Double-click this icon to run the program.
3. For Mac Users:
Double-click on the NemAnalysis .dmg file. This will open a small window, where you can drag the icon to your Applications folder. This will install the program. To run the program, double-click on the new NemAnalysis icon in the Applications folder.
 - If your Mac gives you a '**Unidentified Developer**' error, you may need to change your Mac security settings. Go to the Apple menu at the top left of the screen and select System Preferences. Select Security & Privacy from the main menu. Then, select the General tab. In the 'Allow Apps downloaded from:' section, there will be a notice about NemAnalysis. Click '**Open Anyway**' to run the program.





Quick Start

1. Double-click on the NemAnalysis icon to start the program. The first time the program runs, a welcome message is shown. Select **OK** in this dialog to select a folder where your ScreenChip recordings are saved. Click **Select Folder** to load the files into NemAnalysis.
 - To change folders, click the blue **Select Folder** button or choose **Select Folder** from the **File** Menu. The five most recently selected folders are listed in the Selected Folder Dropdown and can be selected again through the Dropdown for convenience.
 - If you do not have any ScreenChip recordings, a demo file is included. Select this by choosing **Open Demo File** from the **File** Menu.
2. The ScreenChip recordings are listed in the File Navigation Panel, to the left. Select the first file in the list to automatically analyze it. Information about the analysis status is shown on the bottom of the window, in the blue Status Bar.
3. For accurate analysis and display, it is essential that the orientation of the worm is correct. The waveform plot displays normal polarity by default, which means that E spikes are positive and R spikes are negative. Select the **View Recording Notes** button at the top of the screen and verify that the Orientation field accurately reflects the recording. If the field is blank, the program will default to assume the worm is head-first, with positive E spikes and negative R spikes.
 - If the orientation is not correct, select **Unlock** to make the fields editable. Change the text in the orientation field to either 'H' (head-first) or 'T' (tail-first) and then select **Save**. Close the window by clicking the **Close** button. The display will refresh using the new setting. All subsequent analysis will now use this new setting. To reanalyze a file, select the **Customize Analysis** button. Click **Re-analyze** and then **Close**.



5. Once analysis is complete, information about the analyzed recording is displayed both within the Waveform and Panels to the left under the file navigation.
 - Within the Waveform Plot:
 - Yellow pump indicators show the beginning, end and duration of each pump.
 - Blue circles indicate E spikes identified by the current analysis settings.
 - Green circles indicate R spikes identified by the current analysis settings.
 - Mean pump duration and frequency are on the upper-right side of the plot.
 - From “View” select “Show Pump Statistics” to display information about the recording using the current analysis settings.

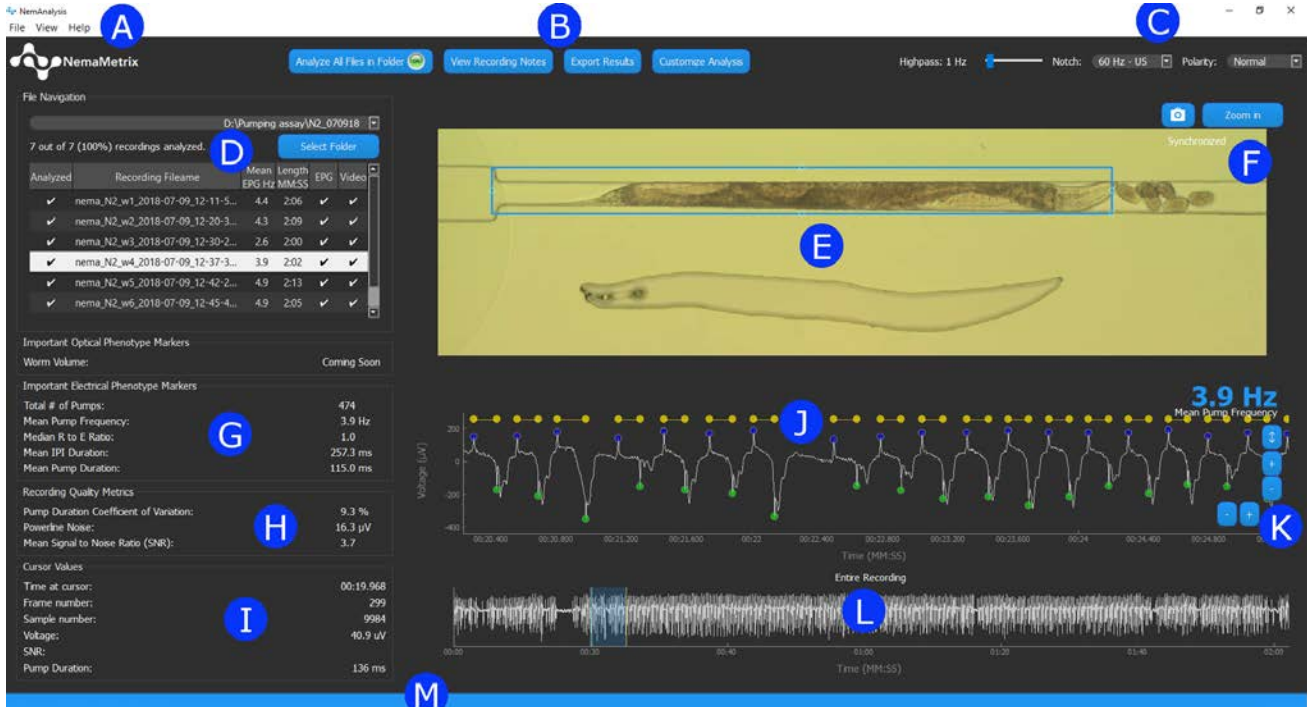
6. There are several ways to navigate through the recording:
 - The mouse can be used to navigate: the left button will pan across the recording, while the right mouse button will scale the recording.
 - The Scrolling Plot below the Waveform Plot always shows the whole length of the recording. It can be used to scroll across the plot. It also gives context about the portion of the recording shown in the Waveform Plot in relation to the full recording.
 - Use the buttons in the bottom left of the Waveform Plot to scale the recording. The fit button will fit the amplitude of the current selection within the Waveform Plot.

7. Once the file is analyzed, select **Export Results** to export the data to a spreadsheet.
 - The **Browse** button will allow you to select a location for the spreadsheet. You can choose to export results for only one file or all analyzed files in the folder. Click **Save** to export the results.

8. Congratulations! You have now analyzed and exported your first ScreenChip file!



Reference Guide



Main Features

- A: Main Menu
- B: Main Button Panel
- C: Display Panel
- D: File Navigation Panel
- E: Camera Panel
- F: Camera Panel Buttons
- G: Important Electrical Phenotype Markers
- H: Recording Quality Metrics
- I: Cursor Values
- J: Waveform Plot
- K: Plot Scaling Buttons
- L: Scrolling Plot
- M: Status Bar



A. Main Menu

File Menu

1. **Select Folder:** Use this menu item to select a new folder containing ScreenChip recordings.
2. **Show Selected Folder:** Selecting this menu item will open the most recently selected folder in a new file system window.
3. **Delete Results in Selected Folder:** This menu item will delete all results files associated with the ScreenChip files in the current folder, reverting the files to an “unanalyzed” state. A warning is shown and must be verified before the files are deleted.
4. **Open Demo File:** This menu item will load the included demo file.
5. **Export Plots:** This menu item will open a export plots dialog that will allow for video display and EPG trace to be exported.

View Menu

Mean Recording Values

1. **Show Mean EPG:** Checking this menu option will show the blue mean EPG value in the upper-right corner of the Waveform plot and unchecking it will hide the value.
2. **Show Amplitude:** Checking this menu option will show the white mean amplitude value in the upper-left corner of the Waveform plot and unchecking it will hide the value.
3. **Show Offset:** Checking this menu option will show the white mean offset value in the upper-left corner of the Waveform plot and unchecking it will hide the value.

Analysis Symbols

4. **Show Pump Indicators (P):** If checked, yellow pump indicators are shown. Hide the indicators by unchecking this menu option. This option can also be changed using the P key.
5. **Show E Spike Indicators (E):** If checked, blue E spike indicators are shown. Hide the indicators by unchecking this menu option. This option can also be changed using the E key.



6. **Show R Spike Indicators (R):** If checked, green R spike indicators are shown. Hide the indicators by unchecking this menu option. This option can also be changed using the R key.
7. **Show Candidate Spike Indicators (U):** During analysis, some identified E and R spikes may not qualify for the final result. To see the unused E and R spikes, check this menu option. Hide the unused indicators by unchecking the menu option. This option can also be changed using the U key.
8. **Show Cursor (C):** Check this option to show a blue cursor line over the Waveform Plot where the mouse is. This option can also be changed using the C key. The Cursor Stats Panel will be shown beneath the “Recording Quality Metrics” Panel. The cursor panel lists information about the sample at the position of the cursor line, including:
 - a. Time at cursor position
 - b. Frame number of cursor position
 - c. Voltage of the data at the cursor position
 - d. The signal-to-noise ratio (SNR) at the cursor position
 - e. If the cursor is over a pump, the pump’s duration
9. **Show Noise Envelope (N):** Check this option to show a red line across the data that represents the amplitude of the noise. This is used to calculate signal-to-noise ratios (SNR) throughout NemaAnalysis. Uncheck the menu option to hide the noise envelope. This option can also be changed using the N key.

Recording Distribution Plots

11. **Show Spectral Analysis:** Selecting this menu item will open a new Spectral Analysis window. This window displays the power spectrum of the entire recording, with Frequency in Hz along the x-axis and power on the y-axis.
12. **Show Amplitude Distribution:** Selecting this menu item will open a new Amplitude Distribution window. This window displays the amplitude of every sample in the recording, with amplitude in μV along the x-axis and percentage of points at that amplitude on the y-axis.
13. **Show Pump Statistics:** Selecting this menu item will open a new window displaying four Analysis Subplots displaying information about the recording using the current analysis settings. See the Reference Guide below for more information about these plots.





Help Menu

1. **About NemAnalysis**: This menu item opens a window that displays information about the software version, build date and copyright.
 - On the **Mac**, this menu option will be under the NemAnalysis menu
2. **NemAnalysis User Guide**: Selecting this menu item will open this document, the NemAnalysis User Guide.
3. **NemAnalysis Tutorial Video**: Selecting this menu item will open a tutorial video on YouTube.

B. Main Button Panel

Analyze All Files in Folder

Selecting this button will turn on automatic analysis. The default analysis settings will be used to automatically analyze all valid NemAcquire files in the current directory. The automatic analysis continues until all files in the current folder have been analyzed. To stop automatic analysis, simply click the button again. The status of the automatic analysis is indicated by the light on the button: a green light indicates that automatic analysis is on.

View Recording Notes

This button opens a new window that displays the notes entered when the recording was taken with NemAcquire. The previous or next file can be easily selected from within this window by clicking the Previous File or Next File buttons. The current file name is displayed at the top of the window.

By default, the recording note fields are locked. To edit any value, select Unlock and then edit the field of your choice. The Revert button can be used to acquire the old data if a field has been edited. Once the field is edited, select the Save button to finalize the change.

- **The Orientation field**
It is essential to have the correct value in the Orientation field for accurate analysis results. The Orientation indicates the direction of the worm in the chip during the recording. Usually, this value is entered during recording time. A blank value defaults to a 'head-first' orientation.

If this value needs to be updated, open the Experiment Notes window and select Unlock. In the Orientation field, type:

- 'H', 'head', or 'head-first' for head-first worms. You can also leave the field blank for head-first worms, because this is the default value.





- 'T', 'tail', or 'tail-first' for tail-first worms. A value must be entered for tail-first worms, otherwise analysis results will not be accurate.

Export Results

Selecting this button will open the Export Results window, which will export the analysis results to an excel spreadsheet. The name of the file and the location where the file is saved can both be customized. There are two export types:

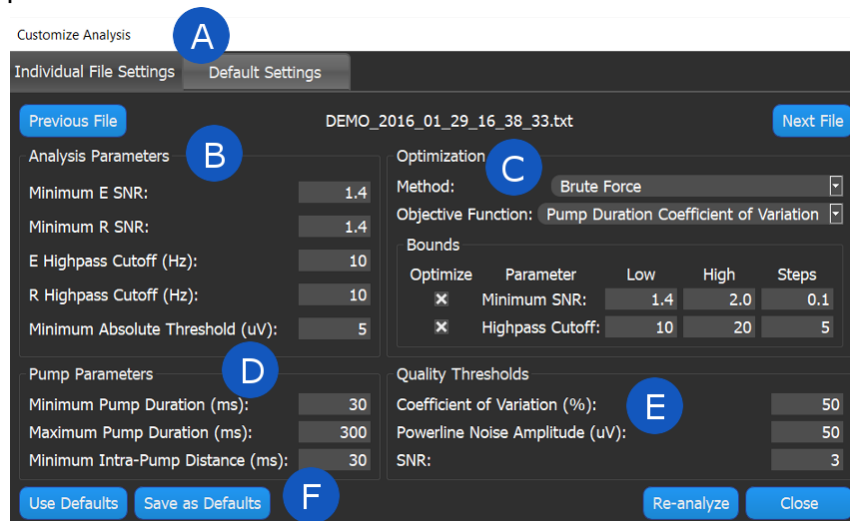
- Export All Files in Currently Selected Folder: each analyzed file in the selected folder will be exported to a row in the same excel file. This will only export analyzed files, and the exported document will not include unanalyzed files.
- Export One Selected File Only: selecting this option will only export the currently selected file to the excel spreadsheet.

There are two additional options for the exported file:

- Save Spike Times: If this option is checked, the exported file will include a second worksheet that includes the times (in milliseconds) of each E and R event in the recording.
- Show Exported File: Checking this option will open the exported results file immediately after pressing Save.

Customize Analysis

The analysis settings can be customized by selecting the Customize Analysis button, which will open a new window.



Features

- A: Settings Tabs
- B: Analysis Parameters
- C: Optimization Settings

- D: Pump Parameters
- E: Quality Thresholds
- F: Main Buttons

A. Settings Tabs



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The settings tabs can be used to switch between Single File Settings and Default Settings. Changes made in the Individual Settings tab will only affect the currently selected file. This file is indicated at the top of the window. Changes made in the Default Settings tab will affect any recordings analyzed in the future, until the defaults are changed again.

B. Analysis Parameters

The Analysis Parameters box contains fields displaying the currently applied analysis settings. After optimization, the ideal values will be listed in these fields. These fields can also be edited directly to change the current analysis results. Each field is described below:

- **Minimum E SNR**: This indicates the minimum signal-to-noise ratio (SNR) value that a point must have to qualify as an 'excitation spike' or E spike. Any point below this value will not qualify as an E spike candidate.
- **Minimum R SNR**: Same as above, but used to determine 'relaxation spike' or R spike candidates.
- **E Highpass Cutoff (Hz)**: The highpass cutoff frequency (in Hz) used to filter the original signal and obtain E spike candidates. This filter is used for the analysis and does not affect the highpass display filter seen on the main window. The filter applied to the analyzed data is a second order highpass Bessel filter, with a sampling rate of 500 Hz. The filter is applied twice: once forward and once backwards.
- **R Highpass Cutoff (Hz)**: Same as above, but used to obtain 'relaxation spike' or R spike candidates.
- **Minimum Absolute Threshold (uV)**: For any point to be considered an E or R spike candidate, it must be above this value in microvolts. Any value below this threshold will be considered noise.

To fine tune the settings in this panel and apply them to the selected recording, do the following:

1. Select "None – Use Analysis Parameters Left" from the Optimization Method box in Optimization Settings (D in the image above).
2. Change any value in the Analysis Parameter fields.
3. Select the Re-analyze button to apply these analysis results to the selected file.





Optimization Settings

NemAnalysis uses an optimization method to find the ideal settings for any given recording. The optimization routine will use all combinations of the bounds settings and then return the most successful combination of the settings. These settings are listed in the Analysis Parameters fields when analysis is complete.

- **Method:** “Brute Force” is the only current optimization method. This method takes all combinations of the bounds set by the user and uses the most ideal settings to produce the final analysis results.
 - The optimization routine can be turned off by using this dropdown menu and selecting the “None – Use Analysis Parameters Left” option. It can be turned back on by selecting the “Brute Force” option from the same dropdown menu.
- **Objective Function:** This option defines the ideal settings of the optimization routine. Currently there is only one objective function setting – “Pump Duration Coefficient of Variation”. This indicates that the optimization routine will select the settings that result in the most uniform pump durations and adhere to the current Pump Parameters (C, in the image above).
- **Bounds:** The bounds settings allow the user to define a range for the SNR and highpass settings. In each range, the user can also customize the step size within the range. For example, the factory defaults for NemAnalysis define a Minimum SNR range from 1.4 (Low) to 2.2 (High), with a Step size of 0.1. When the optimization routine runs, it will check the pumps resulting from an SNR setting of 1.4, 1.5, 1.6 ... and continue until it tests an SNR value of 2.2.
 - **Minimum SNR:** The signal-to-noise ratio for determining E and R spike candidates.
 - **Highpass Cutoff:** The highpass signal cutoff frequency used to filter the signal. This applies a second-order highpass Bessel filter with a sampling rate of 500 Hz. The filter is applied twice, once forward and once backwards.
 - **Notice:** because the optimization method is brute force, the analysis time is determined by the number of settings combinations. Thus, the analysis time will increase if:
 - The range for either bounds setting increases
 - The Step size for either bounds setting decreases

Each combination of SNR and Highpass cutoff settings is considered an analysis ‘iteration’. The number of iterations for the current optimization method can be seen in the blue status bar at the bottom of the main screen (J in the main Reference Guide image), under the





statistic “Current Iteration”. This will display the current analysis iteration and the total number of iterations.

Pump Parameters

The Pump Parameters will change how the analysis process defines pumps. These settings are not changed by the optimization process, but will strongly affect the analysis. Each field is defined below:

- **Minimum Pump Duration (ms)**: This sets the minimum pump length. The distance between an E spike and an R spike must be at least this duration in milliseconds to be considered a pump.
- **Maximum Pump Duration (ms)**: The distance between an E spike and an R spike must not exceed this duration in milliseconds to be considered a pump.
- **Minimum Intra-Pump Distance (ms)**: This indicates the time between one pump and the next. More specifically, this is the time between the R spike of the previous pump and the E spike of the next neighboring pump. For the pumps to be valid, there must be a minimum of this amount of time, in milliseconds.

Quality Thresholds

The Quality thresholds can indicate recordings that may be difficult to analyze automatically. These values set the threshold for the “Recording Quality Metrics” Panel in the lower left corner of the main window (see I in the main Reference Guide image). If a value is outside of the specified threshold, the associated statistic will be displayed in the “Recording Quality Metrics” Panel in red text. These settings do not affect analysis, but instead indicate the quality of the recording.

- **Pump Duration Coefficient of Variation (%)**: This field is used to set the threshold for the acceptable amount of variation in pump duration. If the pump durations in an analyzed recording exceed this threshold, the Pump Duration Coefficient of Variation statistic in the “Recording Quality Metrics” will be displayed in red. A higher coefficient indicates less uniform pump durations.
- **Powerline Noise Amplitude (uV)**: The value in this field indicates the acceptable threshold for average Powerline (60 or 50 Hz) noise present in the recording. If the average amplitude of the powerline noise exceeds this value in microvolts, the Powerline Noise statistic in the “Recording Quality Metrics” panel will be displayed in red.





- **Mean Signal to Noise Ratio (SNR)**: This field represents the acceptable threshold for the average signal-to-noise ratio (SNR) in the recording. The SNR is the ratio between the amplitude of signal points (E and R spikes) and the amplitude of the baseline noise. This field indicates the threshold for the average SNR throughout the recording. If the recording has an average SNR value below this threshold, the Mean Signal to Noise Ratio (SNR) statistic in the “Recording Quality Metrics” Panel will displayed in red.

Main Buttons

Individual File Settings Tab

- **Use Defaults**: Selecting this button will reset all fields in the Individual Settings Tab to match the settings in the Default Settings tab. Any reset fields are displayed in blue.
- **Save as Defaults**: Selecting this button will make the current setting in the Individual File Settings tab the new Default settings. This copies all values in the Individual File Settings fields to the Default Settings fields. Any future recording will be automatically analyzed with these settings. All updated fields will be displayed in blue. A confirmation message will be shown before the change is applied.
- **Re-analyze**: Pressing this button will apply the current analysis settings to the selected recording. Any changes to the Analysis Parameters will not be applied if the Brute Force optimization method is selected. To fine-tune the Analysis Parameters settings and apply them, change the optimization method to “None – Use Analysis Parameters Left”. For more detail, see the Optimization Settings section. A confirmation message will be shown before the recording is reanalyzed.

Default Settings Tab

- **Reset to Factory Defaults**: Selecting this button will reset all fields in the Default Settings Tab to match the original default settings for NemAnalysis. All reset fields will be displayed in blue.
- **Save**: Selecting this button will save the current Default Settings. Any future recording will be automatically analyzed with these settings.



C. Display Panel

All Display Panel settings are for **display only**. These settings do not affect analysis settings or the exported results.

Highpass Filter

Moving the highpass slider changes the highpass filter cutoff frequency. The highpass cutoff frequency is shown just to the left of the slider. Moving the slider entirely left will turn off the highpass filter completely, and any drift in the recording will be shown in the main graph. The applied filter is a second order Bessel highpass filter with a sampling-rate of 500 Hz. This filter is applied to the signal twice: once forward and once backwards.

Notch Filter

There are options to apply a Notch filter to remove powerline noise from the recording (display only). The notch filter is a second order Bessel bandpass filter and it can block either 50Hz or 60Hz powerline noise. This filter is applied to the signal twice: once forward and once backwards. The Notch filter dropdown menu has four different options:

- Auto: The power spectrum of the recording is analyzed to find the most prevalent powerline frequency. A notch filter is applied to block the most prevalent frequency.
- 60 Hz – US: A 60Hz notch filter is applied. This is the powerline frequency in the US.
- 50 Hz – EU: A 50Hz notch filter is applied. This is the powerline frequency in Europe.
- None: The notch filter is disabled.

Polarity

The polarity dropdown can be used to invert the recording across the x-axis. This option may be useful when determining a worm's orientation.



D. File Navigation Panel

The File Navigation Panel is used to select recording folders and navigate from one recording to another. The currently selected folder is listed in the drop-down menu at the top of the Panel. The five most recent folders are listed in the drop-down, for ease-of-access.

Select Folder: Pressing this button opens a new window where a folder containing NemAcquire recordings can be selected.

After selecting a folder, all valid NemAcquire recordings will be listed in the File Navigation Panel beneath the Select Recording button. Selecting a file will start the analysis process. The Analyzed column on the far left will be blank if a file is unanalyzed, show a status symbol if the file is being currently analyzed, and show a checkmark once analysis is complete. The average EPG frequency and the length of the recording are both shown in the far-right columns in the File Navigation Panel.

Finally, text under the folder drop-down menu indicates how many recordings are in the currently selected folder, and what percentage of these recordings have been analyzed.

E. Other panels

❖ Camera Panel

If a camera is connected the image will be displayed here, the image can be zoomed in on by adjusting the ROI (region of interest), ROI is persistent after closing application.

❖ Camera Buttons

The zoom in/out button will zoom in on the ROI.

The Camera Icon will save a still of the video in the selected recordings folder.

❖ Important Electrical Phenotype Markers

The “Important Electrical Phenotype Markers” Panel is located under the File Navigation Panel on the left side of the main screen. Each of these is described below:

- **Total # of Pumps:** The number of pumps identified in the current recording.
- **Mean Pump Frequency:** The average rate of pumping throughout the recording. This is also displayed in blue text on the Waveform Plot.





- **Mean IPI Duration:** IPI stands for 'Interpump Interval'. This is the interval between one E spike and the next neighboring E spike. This statistic indicates the average IPI duration in the current recording.
- **IPI Duration Standard Deviation:** The standard deviation of the IPI durations identified in the current recording.
- **Mean Pump Duration:** The average pump duration in the current recording. Pump duration is considered the interval from a pump's E spike to the same pump's R spike. This is also displayed in blue text on the Waveform Plot.

❖ Recording Quality Metrics

The "Recording Quality Metrics" Panel is located under the "Important Electrical Phenotype Markers" Panel on the left side of the main screen. Each of these is described below:

- **Pump Duration Coefficient of Variation:** The standard deviation of the pump durations divided by the mean pump duration. A lower coefficient of variation indicates more uniformity in pump length.
- **Powerline Noise:** The mean amplitude of the powerline noise in the recording. This value reflects the recording, and does not change if the display Notch filter is enabled or disabled.
- **Mean Signal to Noise Ratio (SNR):** The average signal-to-noise ratio in the recording. The SNR is computed by comparing the amplitude of the E and R spikes to the baseline noise envelope. This value is dependent on analysis settings.

❖ Cursor Values

The "Cursor Values" Panel is located under the "Recording Quality Metrics" Panel on the left side of the main screen and will only be visible after pressing "c". Each of these is described below:

- **Time at cursor:** The time at the position of the cursor (blueline) in the waveform plot.
- **Frame number:** The frame index of the video being played.
- **Sample number:** The sample number of the EPG waveform.
- **Voltage:** Voltage of the EPG sample.
- **SNR:** Signal to noise ratio of the EPG sample.
- **Pump Duration:** The duration the selected pump in ms.

❖ Waveform Plot

The Waveform Plot displays the recorded waveform, as well as the indicators for E spike, R spikes and pump indicators. You can navigate across the plot in a variety of ways:





- Hold the left mouse button and drag to pan across the recording.
- Hold the right mouse button and drag to scale the recording
- Use the left and right arrow keys on the keyboard to pan left and right across the recording.
- Use the scrolling buttons (F, in the Main Reference Guide image above).
- Use the scrolling plot (H, in the Main Reference Guide image above).

❖ **Plot Scaling Buttons**

The scrolling buttons can be used to scale the waveform displayed in the Waveform Plot. The x-axis buttons will double or halve the current view along the x-axis, while the y-axis buttons double or halve the view along the y-axis. Pressing the fit-Y (↕) button, the graph will shift to fit the amplitude of the waveform on the screen.

❖ **Scrolling Plot**

The scrolling plot is an overview of the entire waveform plot and can be used to select a specific time period of the EPG waveform plot. Drag the highlighted area left or right to change times.

❖ **Status Bar**

The status bar reflects the latest status of the current recording.

