

zERG (Zebrafish ECG Reading GUI)

User Guide

Code version: 1.1

Guide version: 1.1 (06/23/2021)

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Description

zERG (**Z**ebrafish **E**CG **R**eading **G**UI) is a Matlab-based Graphic-User Interface (GUI) that allows for the analysis of electrocardiogram (ECG) traces collected from zebrafish. Standard ECG analysis software is limited in terms of analyzing traces where the P wave exceeds the R wave due to incorrect wave assignment. zERG circumvents this and is easily able to identify the ECG waves in such cases to produce the correct average ECG trace, from which standard ECG measurements such as heart rate, intervals, and wave amplitudes can be calculated. All results are then exported in a .txt file for downstream analyses.

Currently, input files are limited to .mat files exported from Labchart (ADIInstruments) or a .txt file containing voltage measurements and the corresponding times. Future versions will expand on additional file formats that can be read and used for ECG analysis.

To run zERG, users need to download both zERG.m and zERG.fig and place both files within the same folder. Traces do not need to be in the same file as the .m and .fig files; zERG will automatically ask for the location of the traces to be analyzed.

zERG was developed and has been tested to run on Matlab version 9.7 and GUIDE version 2.5. The following add-ons are required for use: Signal Processing Toolbox version 8.3 and Image Processing Toolbox version 11.0. For help downloading Matlab add-ons, please visit: https://www.mathworks.com/help/matlab/matlab_env/get-add-ons.html.

As of manual version 1.0, this document will detail trace the main ECG trace analysis features. Subsequent releases will detail additional features such as the noise remover function and identifying the minima in an ECG trace.

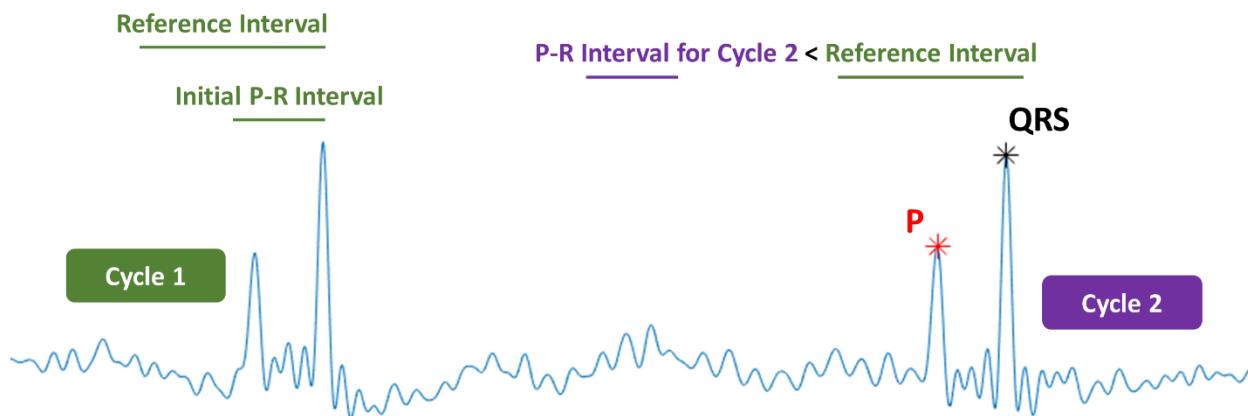
Guide Edits

Version 1.0

Version 1.1 – deleted extraneous text; added section detailing version edits

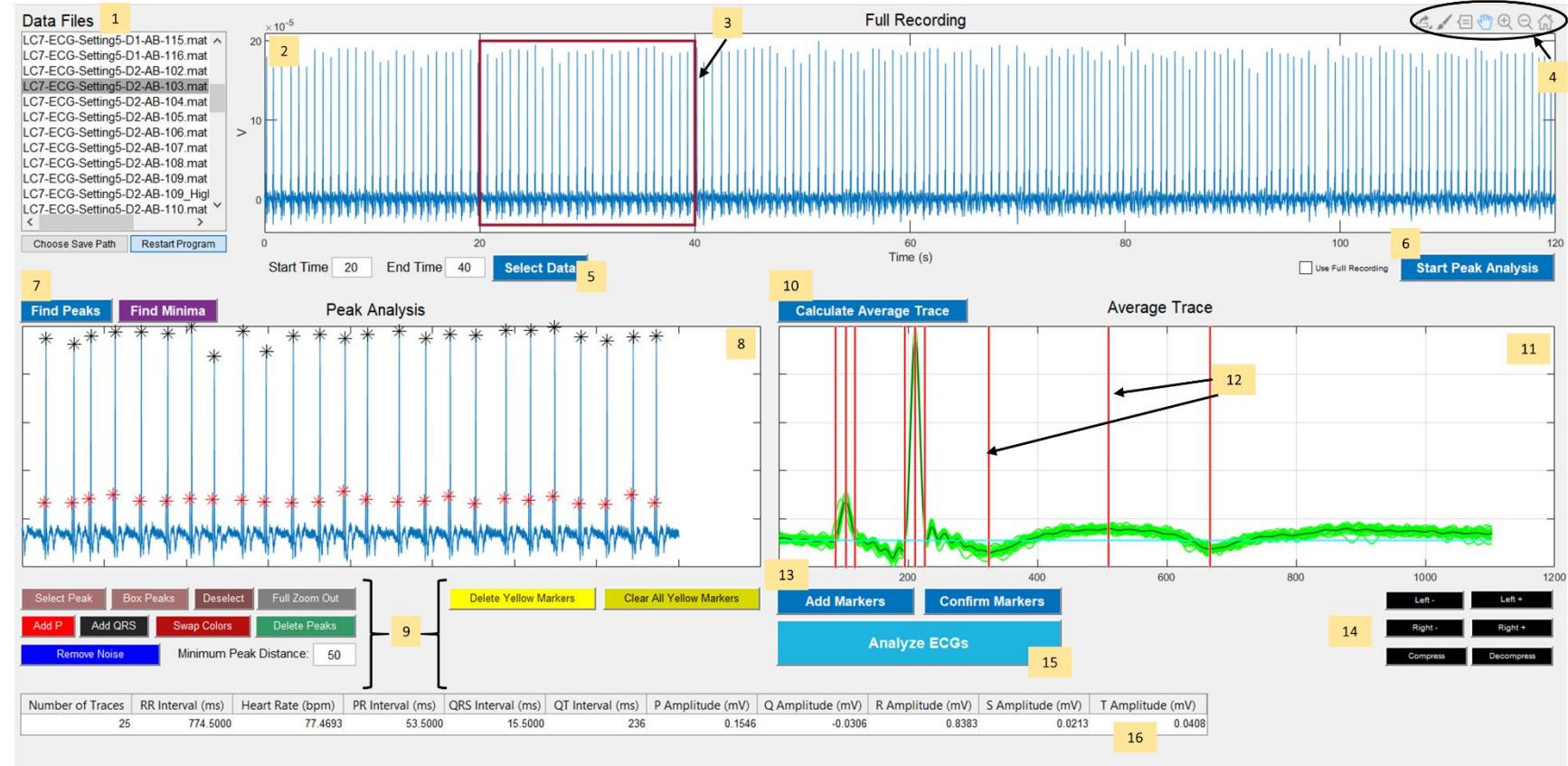
Analysis Overview

ECG voltage measurements (either in .mat or .txt file formats) are first imported into zERG. Data for peak analysis is then selected; users can choose to analyze the entire trace or only a specific segment of the trace. To begin peak analysis, the user is asked to set the wave amplitude threshold. Any maxima above the user-defined wave amplitude threshold and within an initial user-defined minimum peak distance are considered as peaks. To classify these peaks as either P waves (red *) or QRS complexes (black *), the following calculations are considered. In a given cycle, the interval between the P wave and the R wave is smaller than the interval from the R wave to the next R wave. The reference interval for identification of all P wave and QRS complexes within the trace is considered as two times the first P-wave-to-R-wave distance. That is, if the interval between any two given peaks is within this reference interval, the peaks are identified as a single cycle comprised of 1 P wave and 1 QRS complex, regardless of wave amplitude.



If the program detects consecutive red or black peaks, it will label both peaks yellow to allow for error correction. The user can then manually choose to add and/or delete P waves and/or QRS complexes. Once all waves are marked correctly, choosing the *Calculate Average Trace* button will plot an average trace and allow for the user to click on *Add Markers* in order to note where the wave markers are on the average trace. For traces with unusual QRS morphology where alignment by the R wave results in a poor average trace (such as QS, rS, qr, etc.), the user can choose to align by the minima (but only if minima have been identified within the *Peak Analysis* portion of the GUI). Once wave markers are appropriately set and confirmed, the user selects *Analyze ECGs* to obtain a table with the ECG metrics as well as an output .txt file and several average trace plots. The yellow boxes show an example of an ECG trace where the P wave amplitude is larger than the R amplitude and how zERG handles such traces.

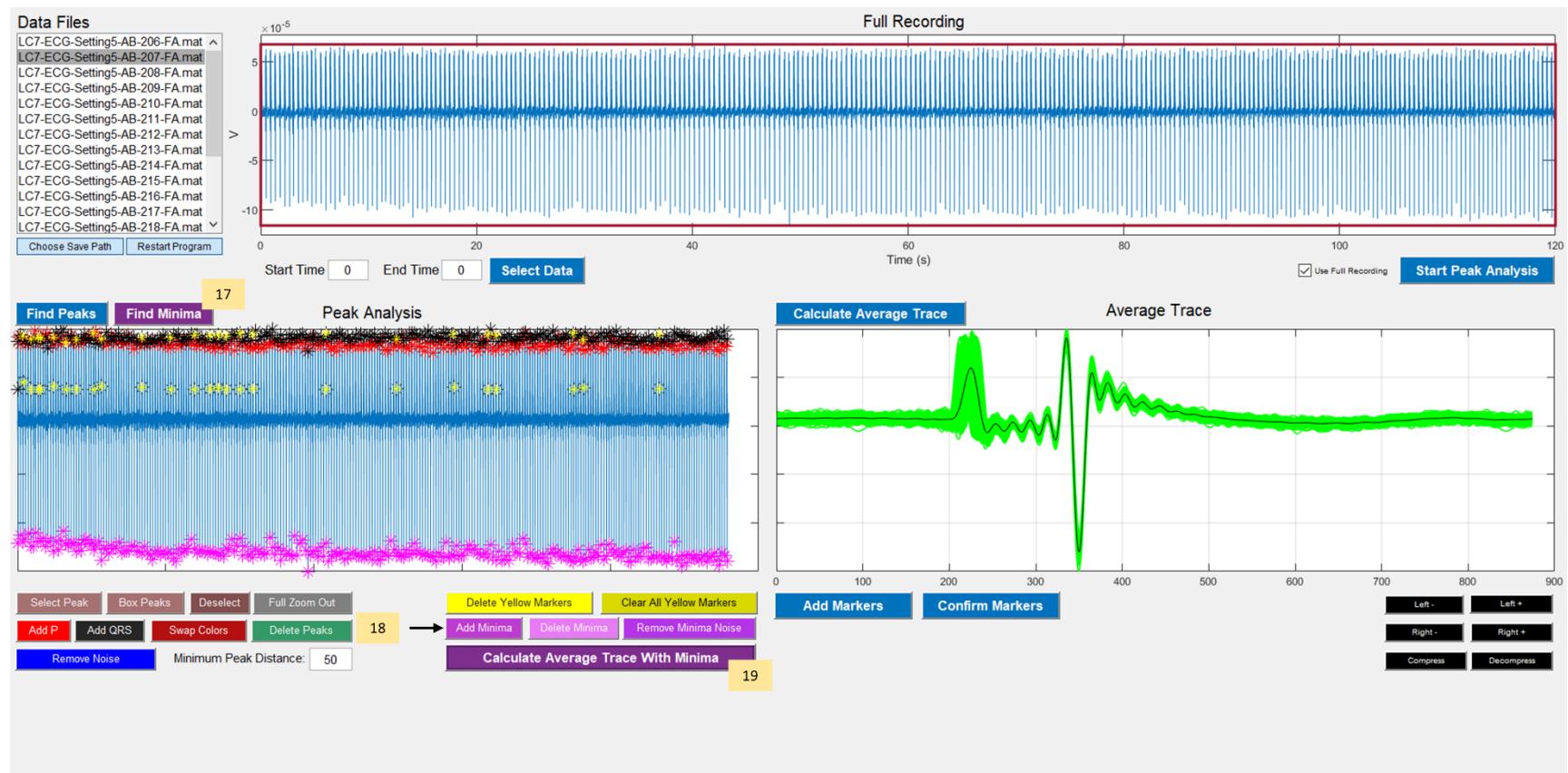
GUI Overview



- 1: Data Files - List of .mat or .txt files in folder
- 2: Full Recording - Plot of trace (highlighted gray under Data Files)
- 3: Red Rectangle - Selected portion of trace for Peak Analysis
- 4: Matlab Tool Bar
- 5: Select Data – Choose potion of trace for Peak Analysis
- 6: Begin Peak Analysis of selected data
- 7: Find Peaks - Identify P and QRS complexes
- 8: Peak Analysis – Plot of trace (P - *; QRS - *)

- 9: Edit peak identification initially done through Peak Analysis
- 10: Calculate Average Trace – Align peak to generate an average trace
- 11: Average Trace plot of selected data
- 12: Wave markers after confirmation
- 13: Add Markers – add markers to Average Trace
- 14: Edit Average Trace window
- 15: Analyze ECGs – output measurements and plots
- 16: Table listing ECG measurements

Options to Align Trace Using Minima

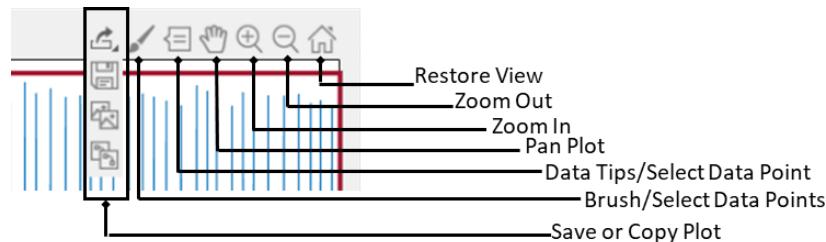


17: Find Minima – Identify minima in ECG trace

18: Edit minima identification initially done through *Find Minima*

19: Calculate Average Trace With Minima – Align ECG traces by the identified minima to generate an average trace

Matlab Tool Bar



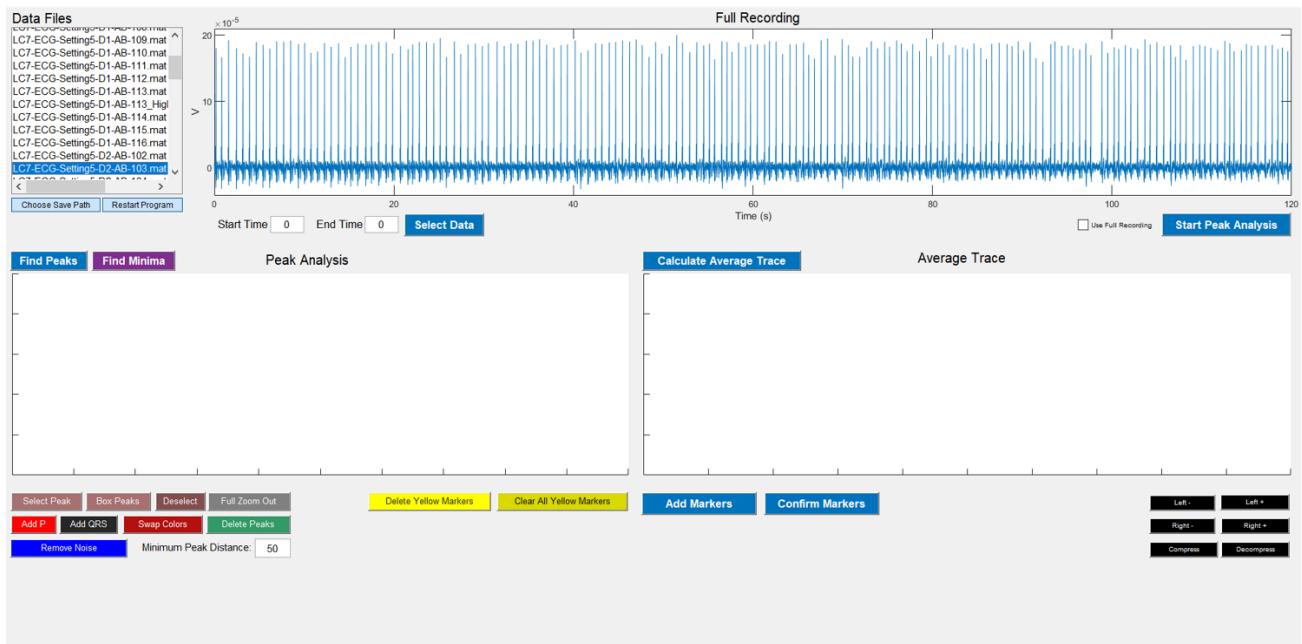
This tool bar is available for every plot within zERG and can be accessed anytime by hovering over the top right-hand corner of each plot. For additional documentation, please visit the Matlab website:

https://www.mathworks.com/?s_tid=gn_logo

Trace Analysis

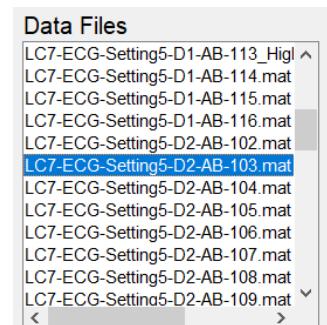
Loading and Reading ECG Recording

1. Ensure that both zERG.m and zERG.fig are saved within the same directory. .mat and/or .txt files that contain the trace data do *not* need to be in the same directory as the .m and .fig files that are required to run the GUI.
2. To run, open zERG.m in Matlab. Navigate to the *Editor* tab and select *Run*.
3. A file explorer will open. Select the directory containing the .mat and/or .txt files you want to analyze with zERG. Once the directory is selected, the GUI window will open:



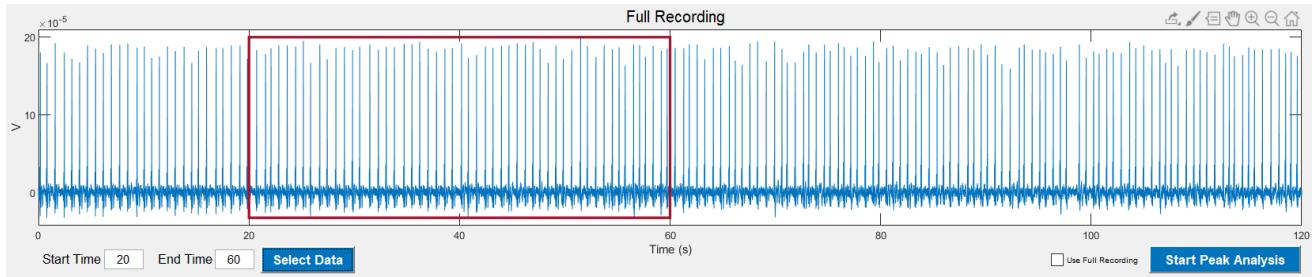
If the wrong directory is selected or if there are any issues that occur throughout the analysis process, select *Restart Program* to automatically close and re-open zERG. All previously created objects in the workspace will be cleared.

4. Select *Choose Save Path* to choose the directory all results files should be saved in. If not chosen, the default directory is the one folder selected in the previous step.
5. **Data Files** lists all of the .mat and/or .txt files in the directory chosen in Step 3. zERG can only analyze one file at a time. The selected file for analysis will be highlighted in blue.

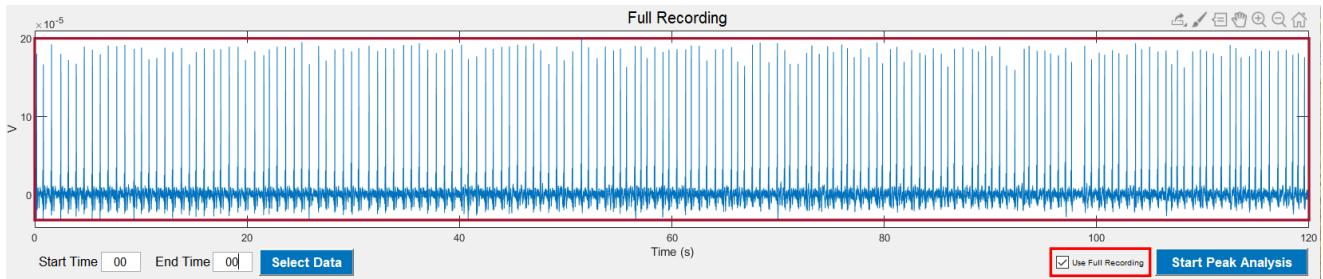


Peak Analysis

1. The ECG recording for the selected .mat or .txt file will be plotted within the **Full Recording** window. Select the data range you want to analyze from the **Full Recording** trace at the top of the screen by inputting the start and end times, in seconds, into the corresponding text boxes, and pressing *Select Data*. A red rectangle will appear to indicate the range selected.

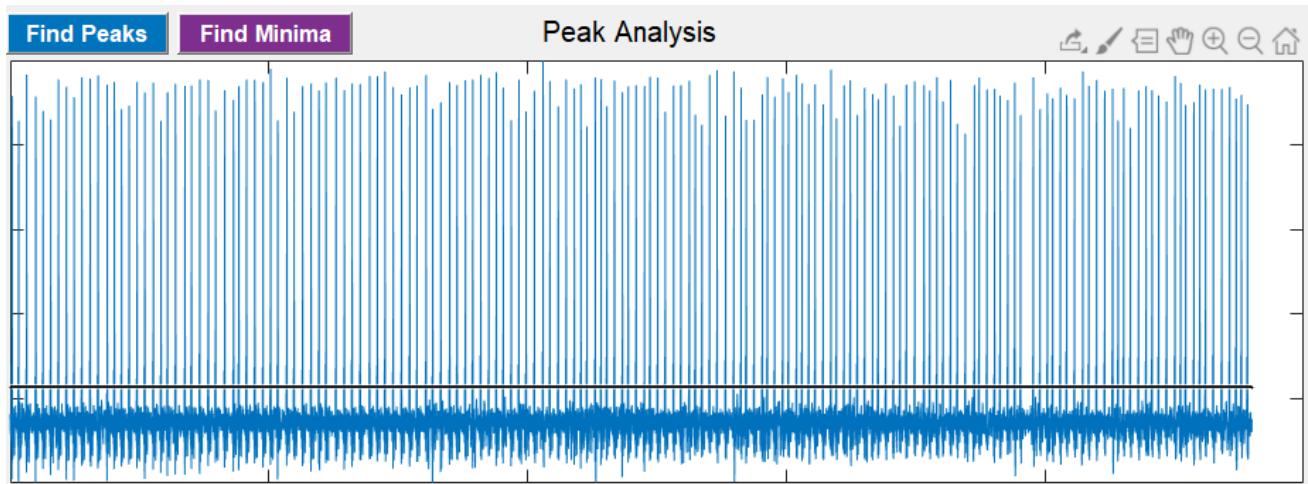


If you simply want to analyze the full recording contained in the selected .mat or .txt file, check the *Use Full Recording* checkbox.



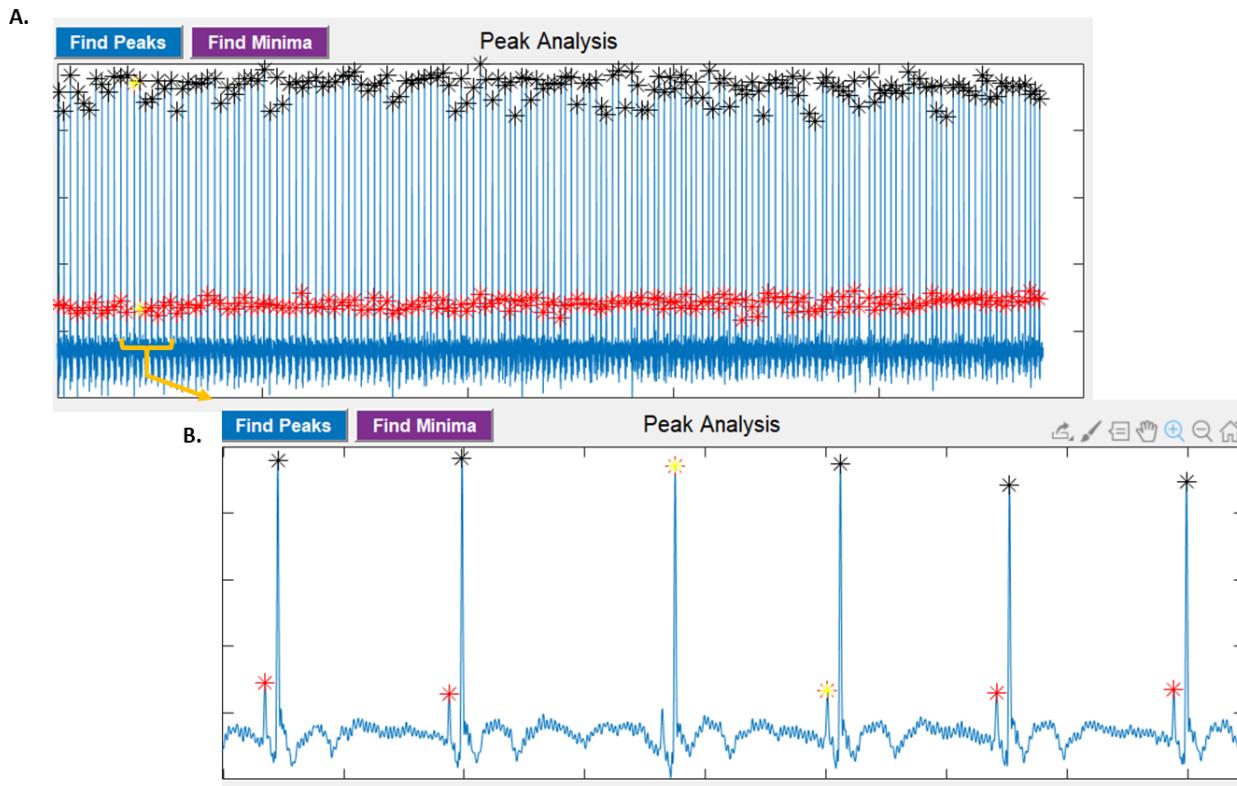
When finished, click the *Start Peak Analysis* button.

2. The portion of the trace you want to analyze has now been plotted in the bottom left window (**Peak Analysis**). Drag the **black line** (threshold) so that all peaks in the trace lie above it.



Once the threshold has been appropriately set, click the *Find Peaks* button.

3. *Find Peaks* will find all peaks in the program, then pair peaks which are close together according to a reference interval (see the figure in the [Analysis Overview](#) section). The left peak in each pair will be labelled as a P wave (**red**), while the right peak in each pair will be labelled as the R wave (**black**). Peaks which could not be paired, or peaks which appear next to another peak of the same color, are labelled **yellow** to indicate a potential error.



(A) Identification of P and R waves after using *Find Peaks*. (B) Zoomed in view of trace section where two waves have been labelled with yellow markers, indicating a potential error in peak wave identification.

4. *Find Peaks* relies on the ‘findpeaks’ function within Matlab. Several parameters are required for the accurate identification of peaks including the minimum peak height (the threshold set by *Start Peak Analysis*) and the minimum interval between two peaks. This latter parameter can be adjusted directly within zERG by adjusting the *Minimum Peak Distance* text box.



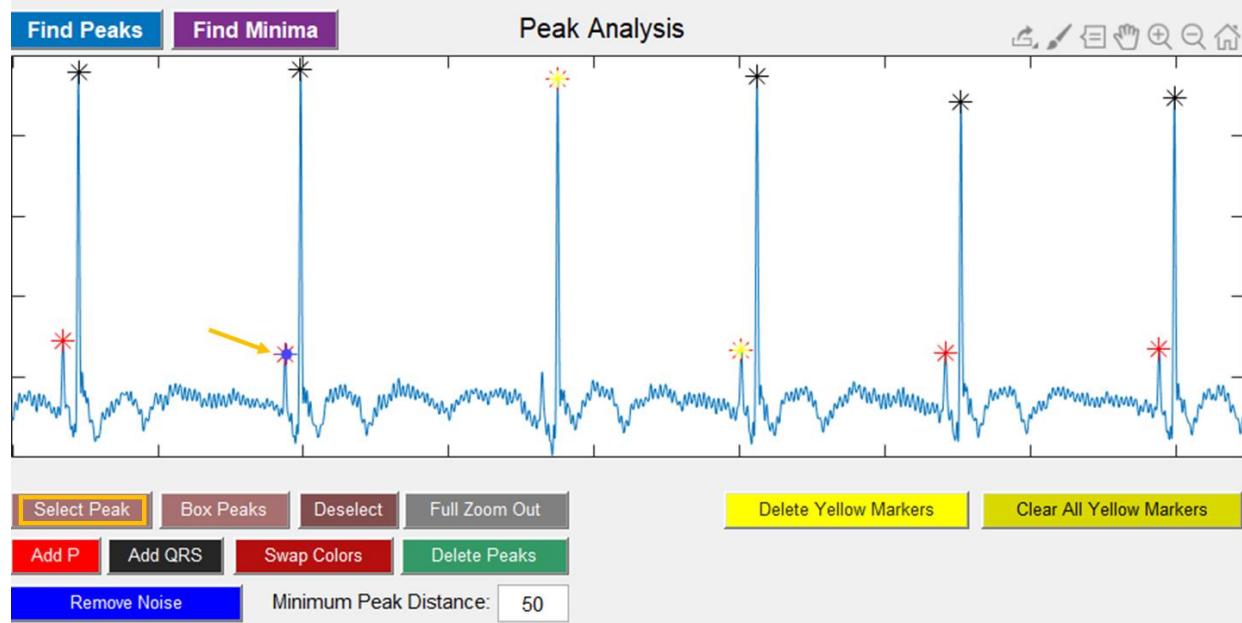
The starting distance is set at 50. This distance can be changed and updated results can be viewed immediately by pressing *Find Peaks*. Adjusting this value may improve peak identification, with larger values leading towards fewer peaks being identified.

Editing Peak Analysis

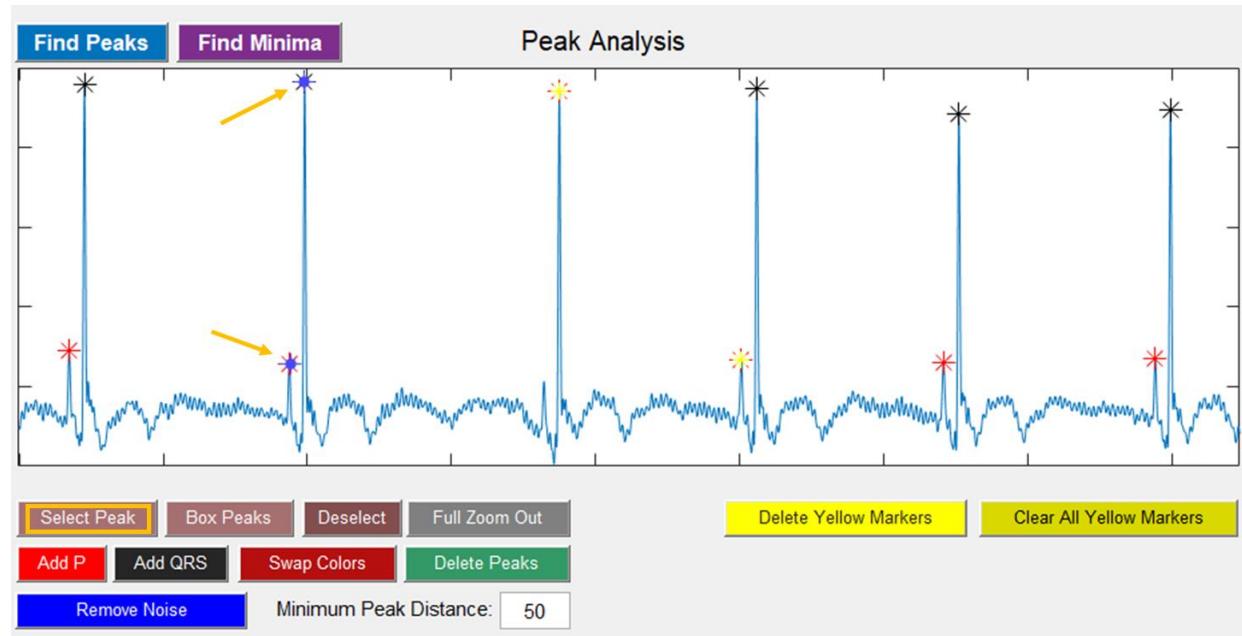
This section highlights options available to the user to manually edit the labeling performed by *Find Peaks*. Successful completion of this section will result in a trace where all P and R waves are appropriately labeled. The next step entails generating an average trace to acquire ECG measurements.

Individual Peak Selection

Individually select a peak by clicking *Select Peak* and choosing the peak in the recording within the **Peak Analysis** window. A **blue** dot will appear over the selected peak.

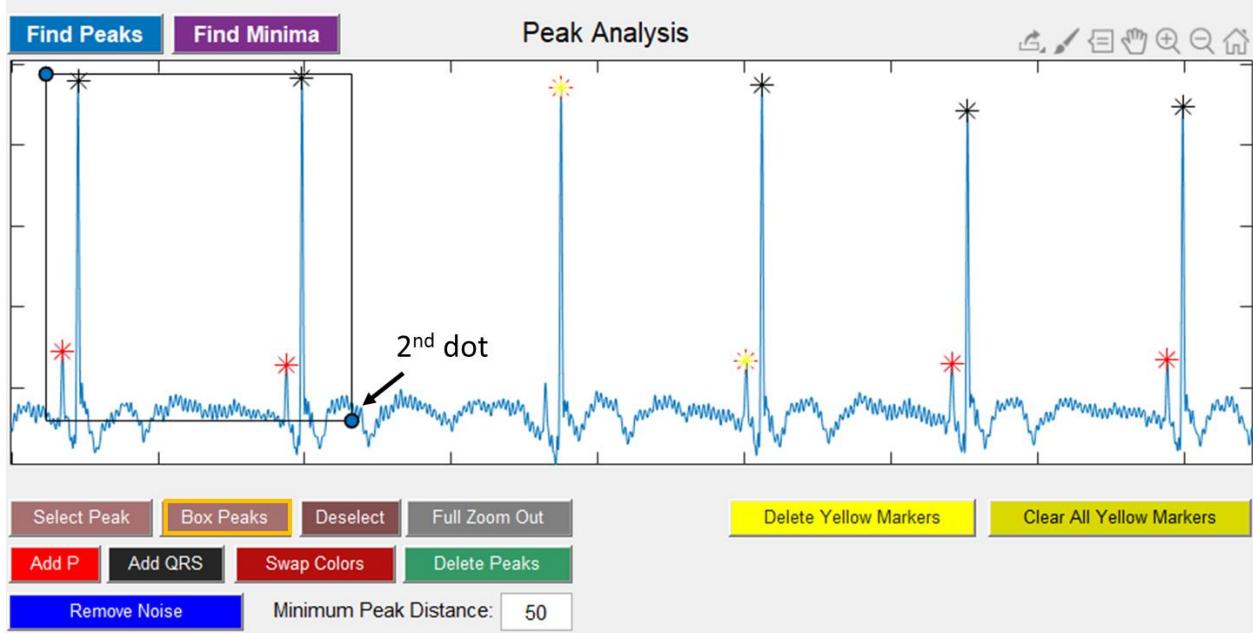
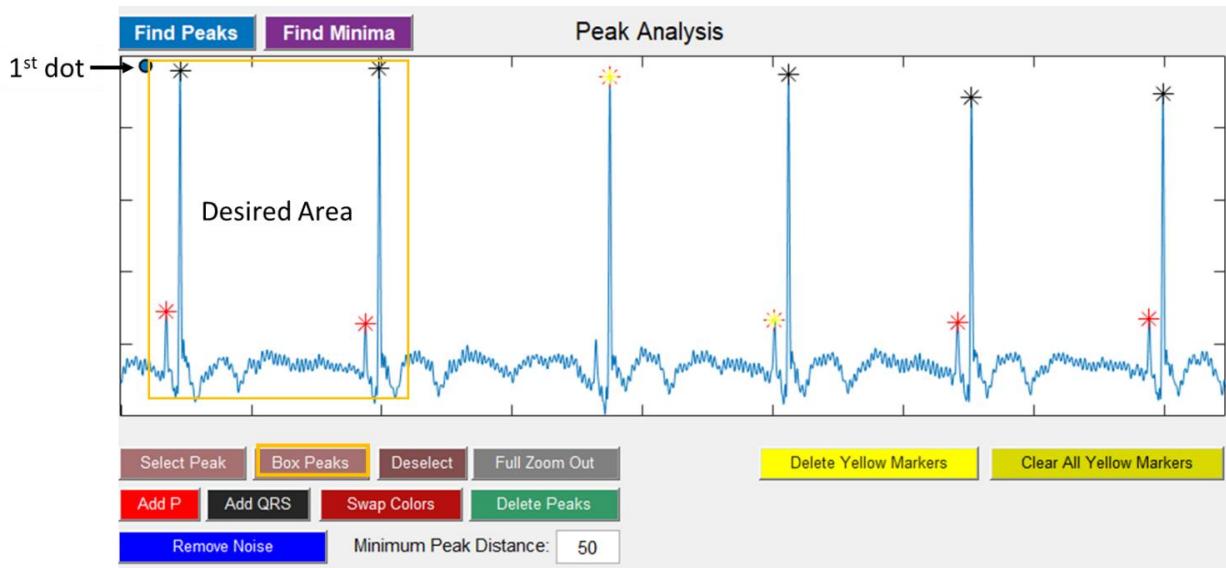


Multiple peaks may be selected using *Select Peak* if desired.



Grouped Peak Selection

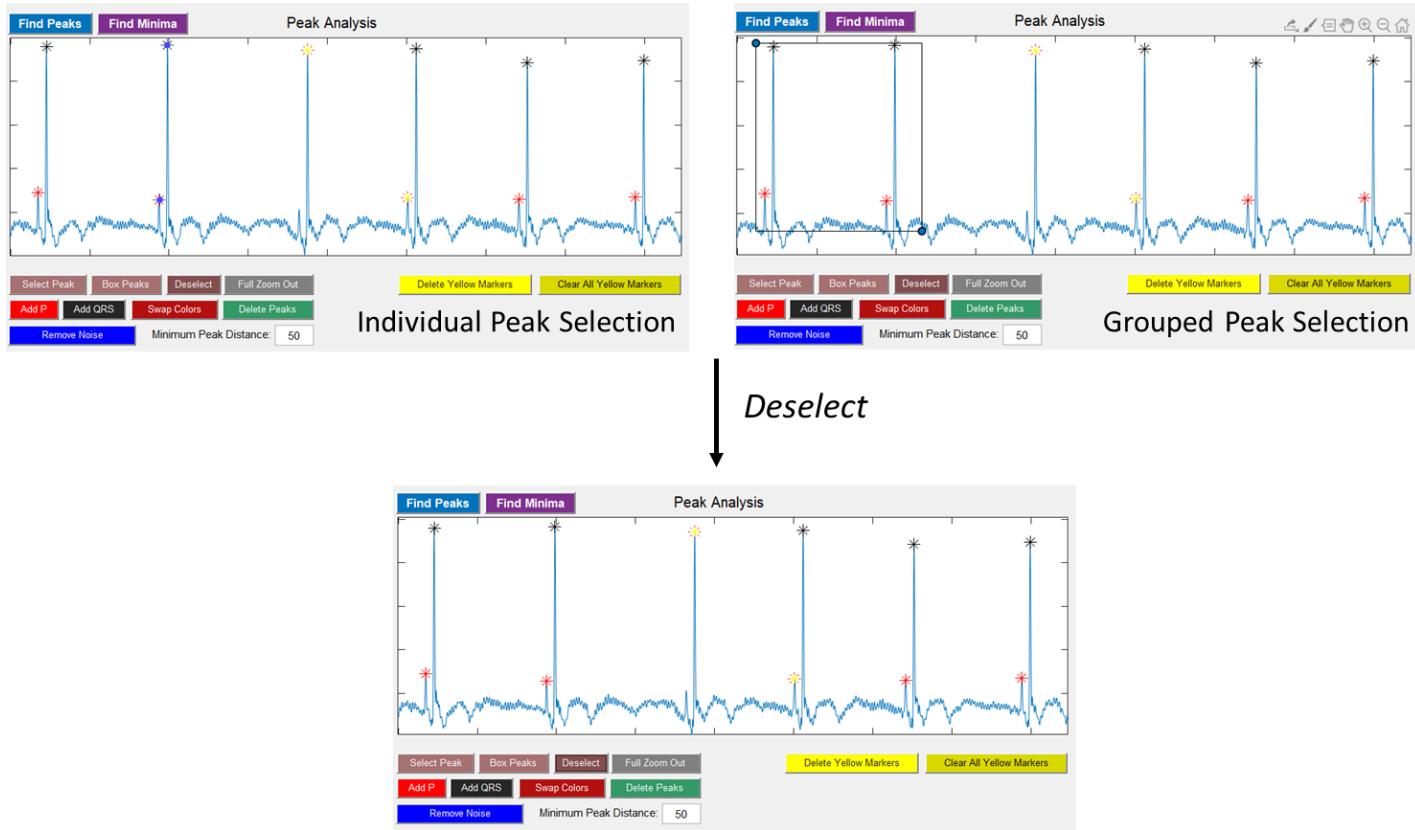
A group of peaks may be selected at once using *Box Peaks*. Place a blue dot at one corner of the rectangle you would like to draw, and the other marker at the corner diagonal from the first marker. All peaks within the black rectangle will be selected.



We recommend that only one mode of peak selection be used at a time i.e. it is not possible to select and edit peaks using both individual selection and grouped selection.

Peak Deselection

Clicking **Deselect** in either individual or grouped peak selection will remove the individual **blue** dot(s) or the entire **black** rectangle.



Note that this option does not remove the peak calling as previously done by *Find Peaks*; all **red**, **black** and **yellow** markers remain.

Peak Deletion

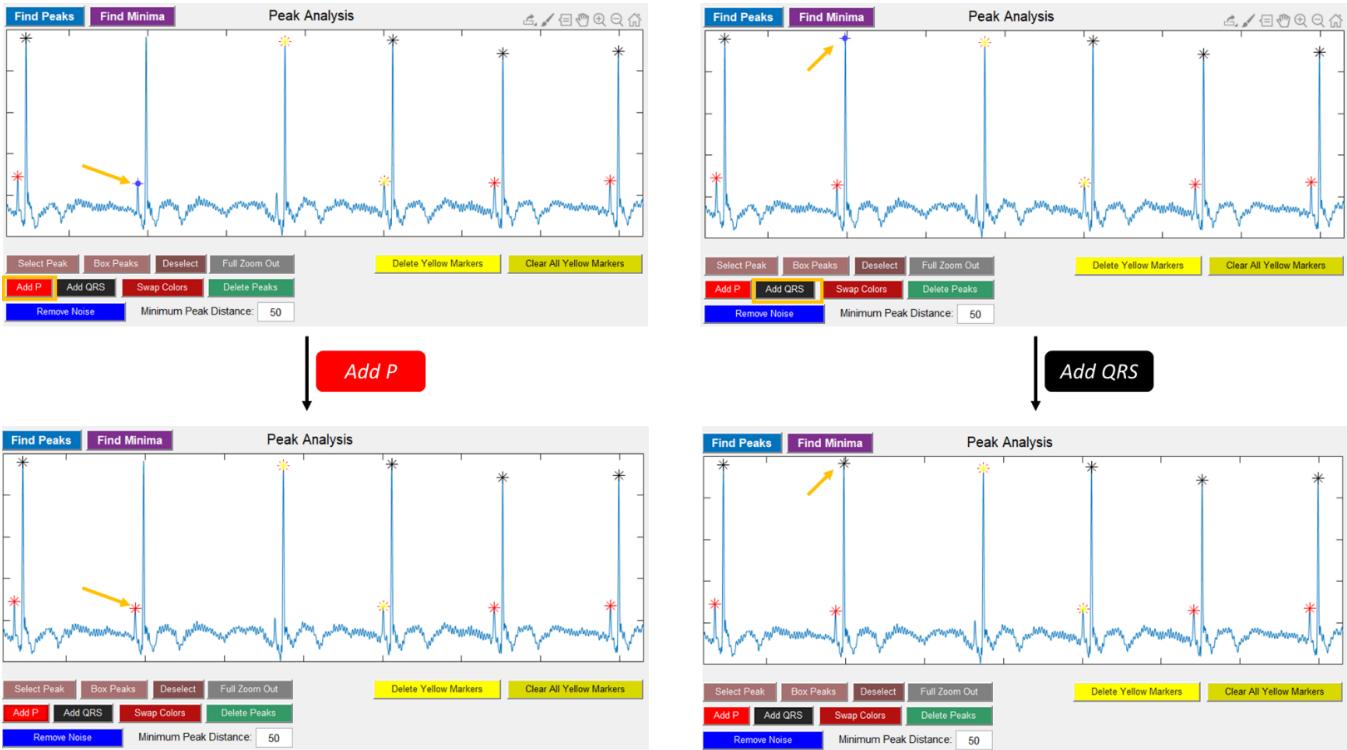
After the peak(s) are selected, click *Delete Peaks* to remove the red or black marker indicator.



Deletion of these peaks mean that the unmarked cycle(s) (P wave-QRS Complex-T wave) will **not** be included in the average trace calculation and all downstream measurements obtained. More than one peak may be deleted at a time; this can be achieved by individually selecting the peaks or by using *Box Peaks* to draw the rectangle where all peaks called within the rectangle will be deleted.

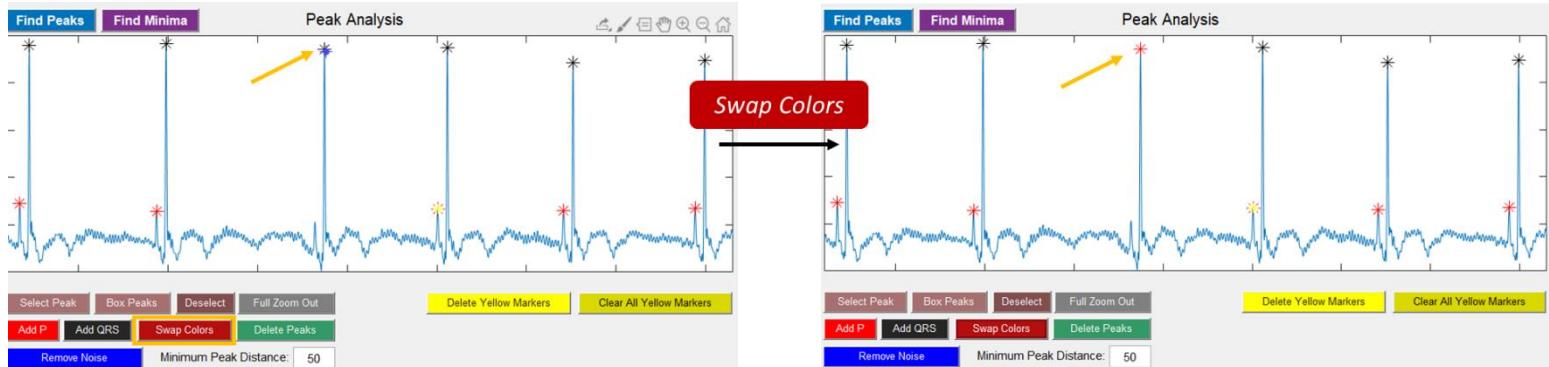
Peak Addition

Box Peaks is incompatible with peak addition. To add a **P wave**, select the unmarked peak with *Select Peak* and then click **Add P**. To add an **R wave/QRS complex**, select the unmarked peak and then click **Add QRS**. Multiple waves of the same type may be added by adding multiple blue dots using *Select Peak*.



Swapping Peak Color

In cases where *Find Peaks* has correctly identified a peak but incorrectly labeled the wave (i.e. a QRS complex is labeled with a **red** marker instead of a **black** marker), it can be more convenient to use **Swap Colors** to switch the wave label instead of deleting the marker, selecting the peak and adding the appropriate marker using the steps outlined under [Peak Addition](#). More than one peak may be swapped at the same time (i.e. two selected peaks with **red** markers can be swapped to two **black** markers or one **red**, one **black** can be swapped to their corresponding colors (black and red, respectively)).



Removing Yellow Markers

Individual yellow markers may be deleted by clicking *Select Peak*, placing a blue dot on the yellow marker of interest, and selecting *Delete Yellow Markers*. To remove all yellow markers within a trace, click *Clear All Yellow Markers*.

Yellow markers can only be added to a trace through the [Noise Remover](#) method.



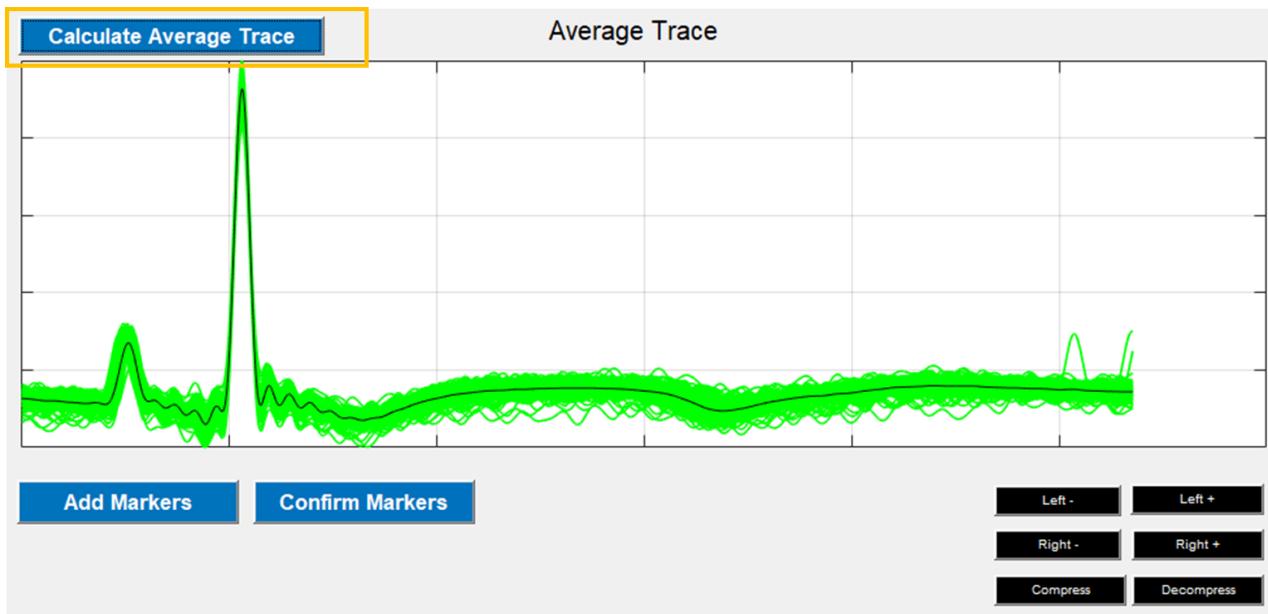
Resetting Peak Analysis

At any time during the process of **Peak Analysis**, a new amplitude threshold can be set by selecting *Start Peak Analysis*. This updated threshold will be used for a new *Find Peaks* calculation. The data selected for analysis will remain the same unless the user chooses to enter a different *Start Time* and *End Time* OR chooses to analyze the entire recording in the .mat or .txt file.

In general, any function with zERG may be stopped by pressing CTRL+C in the command window within Matlab.

Calculating Average Trace

After all peaks are appropriately labeled, an average trace may be calculated to begin acquiring the ECG measurements. Click *Calculate Average Trace* create a compiled average trace in the bottom right window: **Average Trace**. Alignment of the average trace is by the R wave. For traces with unusual QRS morphology, alignment by a minima may enhance the quality of the average trace. See [Alignment By Minima](#) for a guide on how to identify minima within the recording.



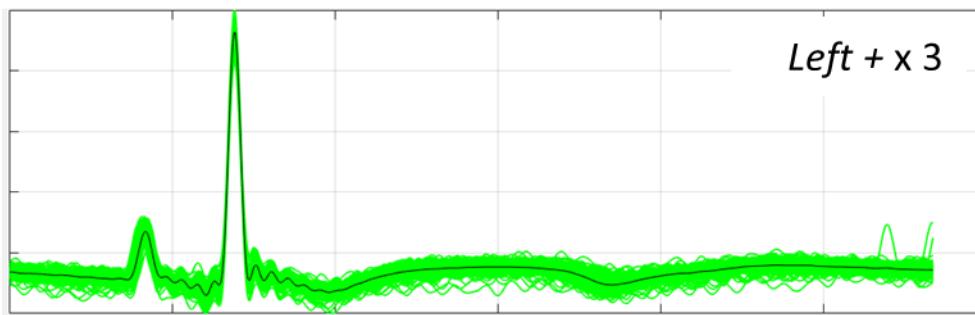
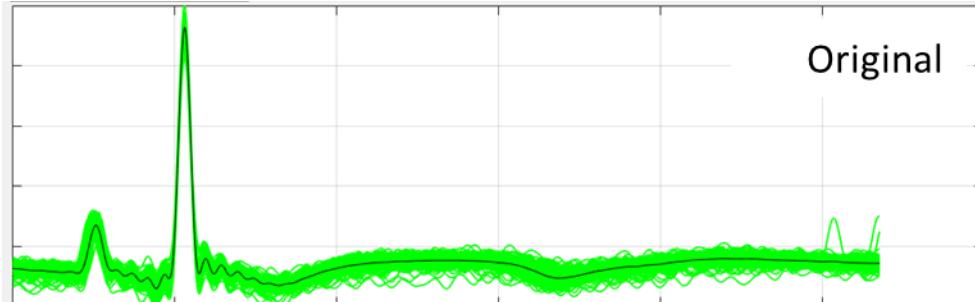
— Each green line represents one trace cycle (P wave-QRS complex) labeled through the **Peak Analysis** process. Here, all cycles are plotted on top of each other to generate a compiled trace to show the overall ECG wave morphology.

— Average trace calculated by averaging the voltage measurements of all green traces

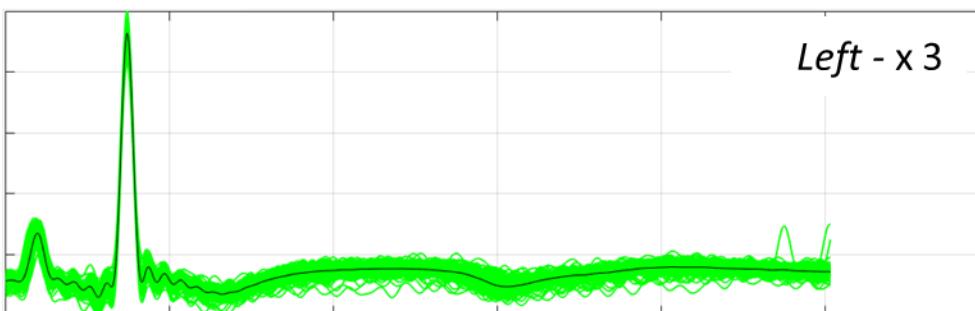
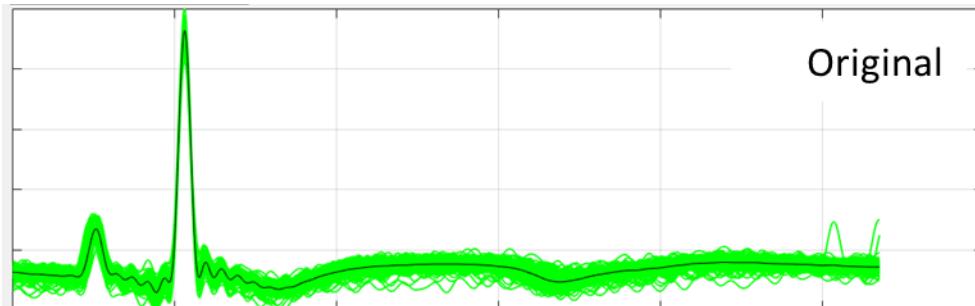
Adjusting Average Trace Plot Window

To adjust the plot window, use the black buttons in the bottom right corner under **Average Trace**.

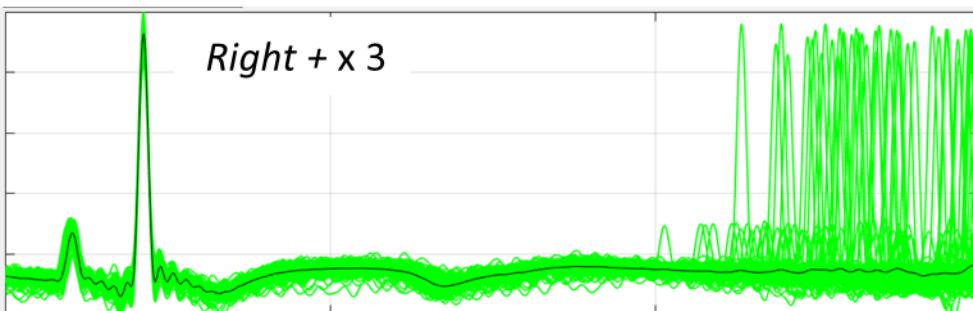
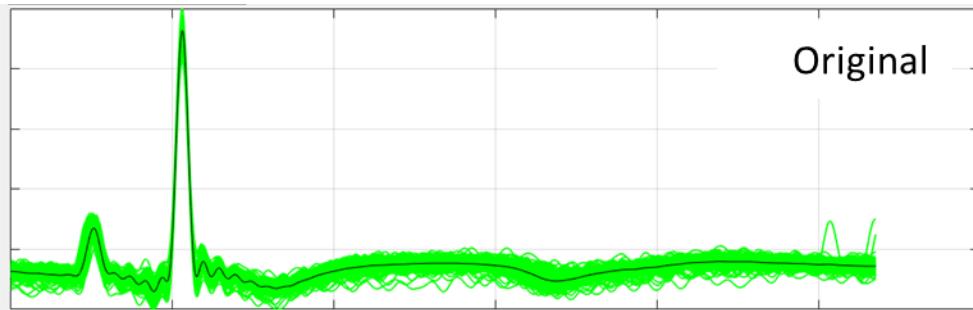
Left + adds to the window on the left:



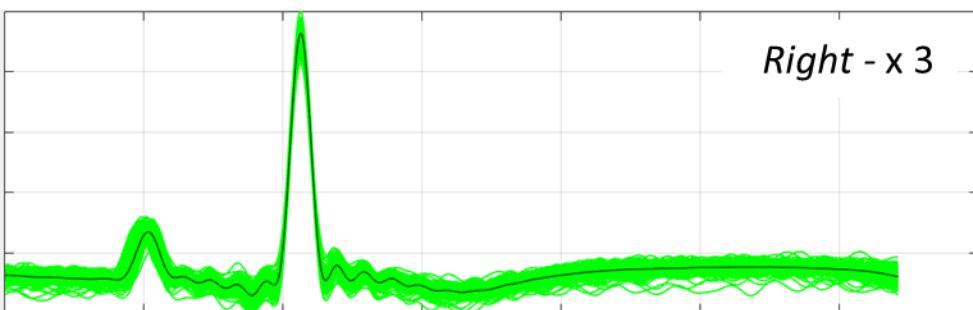
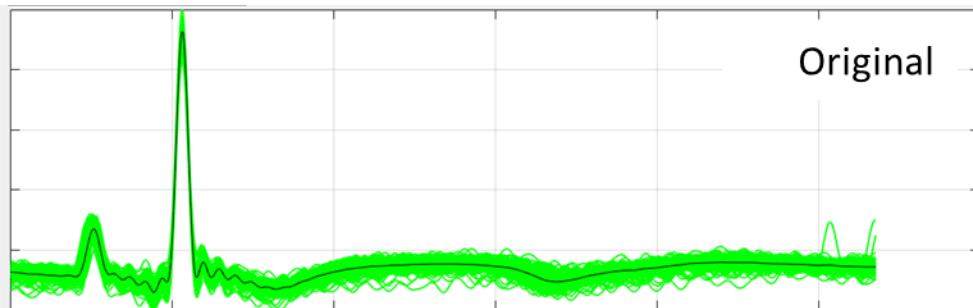
Left - removes from the window on the left:



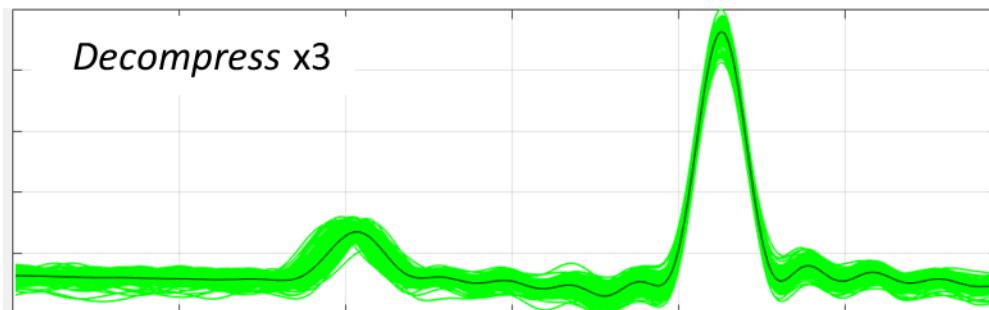
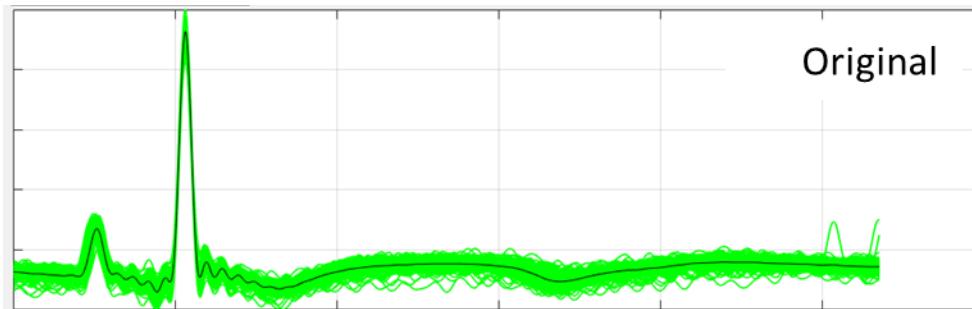
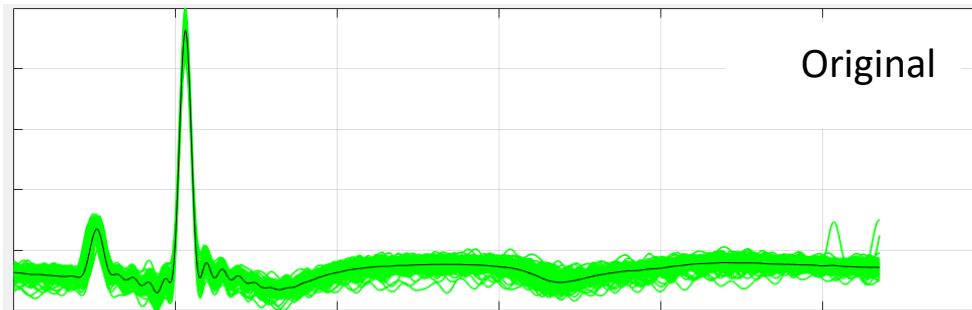
Right + adds to the window on the right:



Right - removes from the window on the right:



Compress and *Decompress* buttons compress and decompress the window along the x-axis:

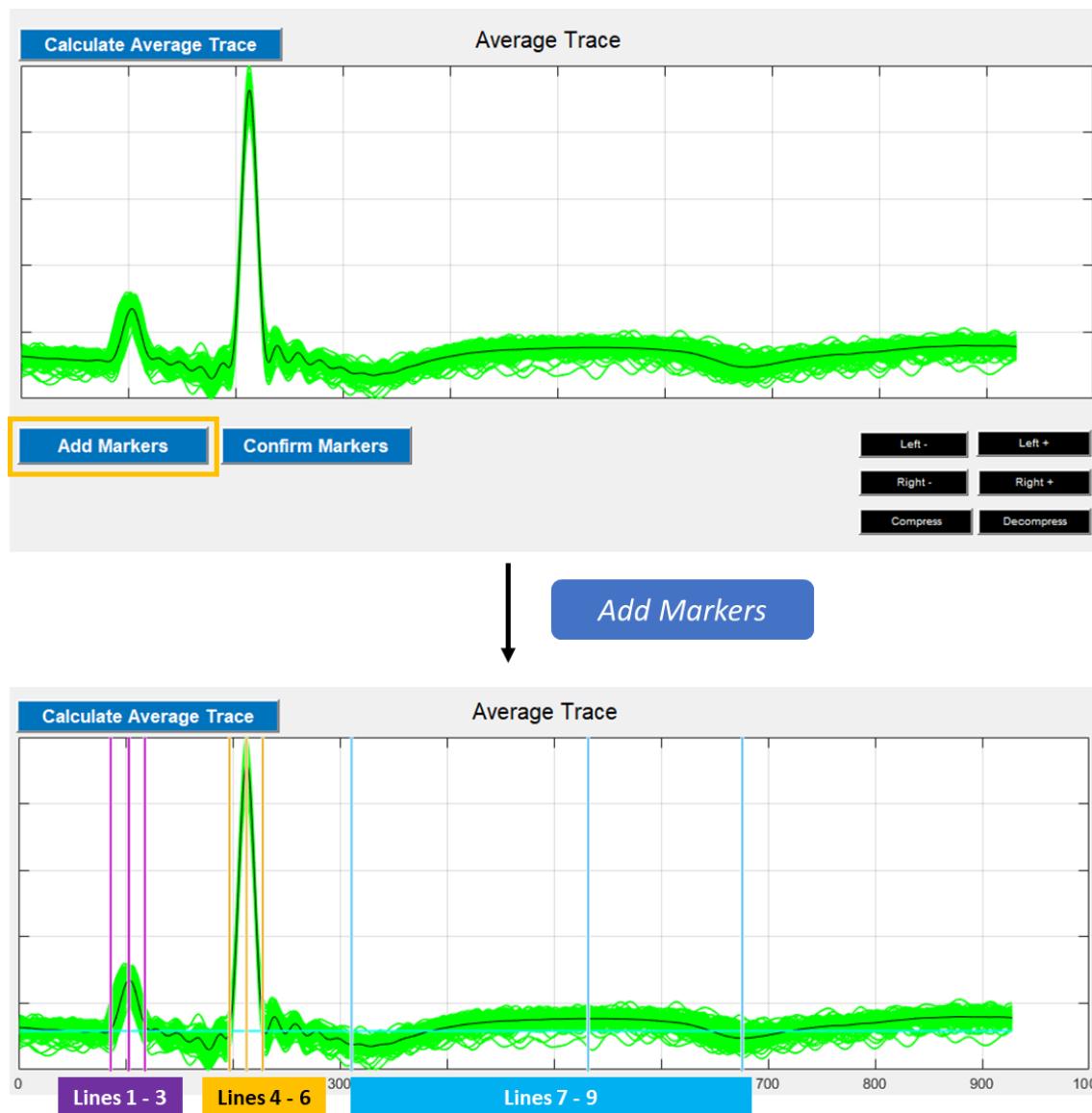


Wave markers **will** be erased if the plot window is adjusted after wave markers are added. Therefore, we recommend that the plot window be adjusted prior to marker addition.

Adding Wave Markers

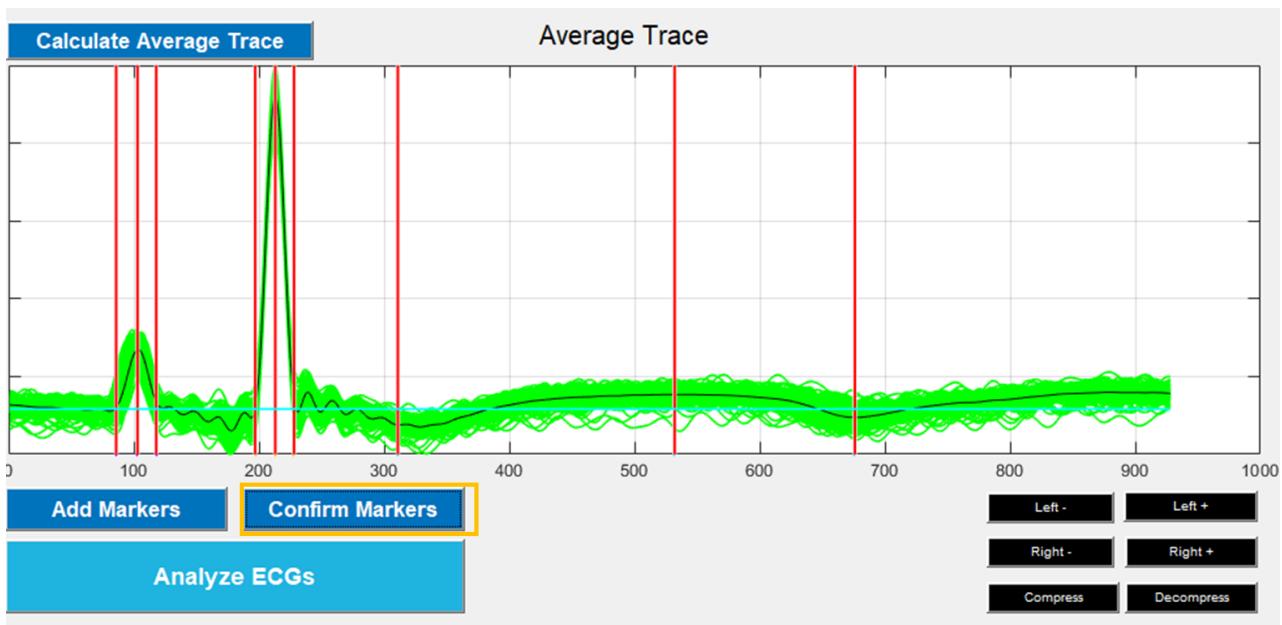
When the **Average Trace** window is appropriately adjusted, click the *Add Markers* button to begin wave identification on the average trace. Markers representing the following ECG features will appear (left to right): the start of the P wave (1), the peak of the P wave (2), the end of the P wave (3), the start of the QRS complex (4), the peak of the QRS complex (5), the end of the QRS complex (6), the start of the T wave (7), the peak of the T wave (8), and the end of the T wave (9).

Although zERG automatically calculates the optimal positions of these markers, the user may individually move them for better placement. Markers are sorted by position on the x-axis before ECG measurements are calculated so markers can be interchangeable (i.e. line 1 can be used to mark the peak of the P wave or line 2 can be used to mark the peak of the QRS complex). All measurements are calculated from the intervals as calculated solely based on the average trace. An isoelectric line calculated as the median of all points before the start of the QRS complex is plotted for convenience.



Confirming Wave Markers

Once the markers have been appropriately set, click *Confirm Markers*. All markers should turn red and the *Analyze ECGs* button will appear.

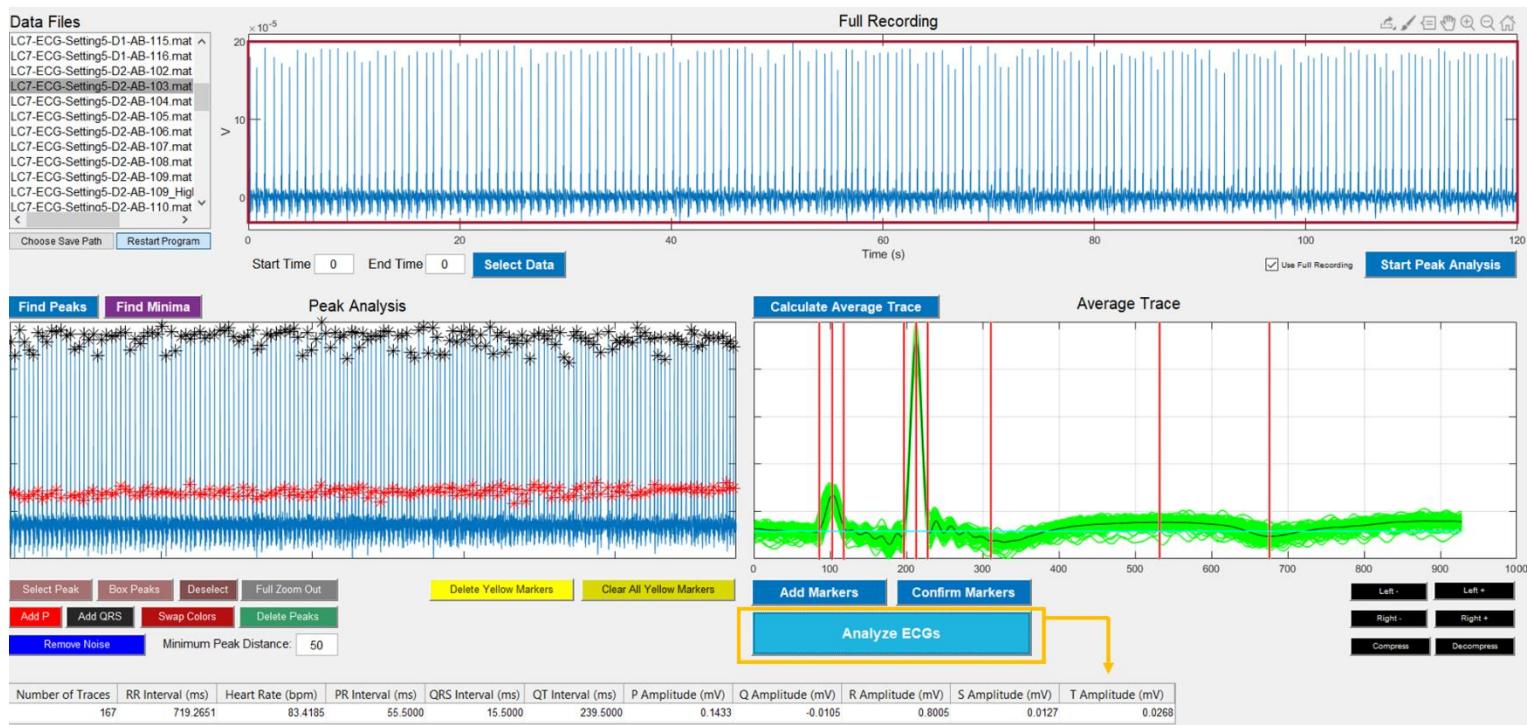


Markers are locked in at this point in the analysis. If the position of the markers need to be changed, select *Calculate Average Trace* then *Add Markers* to change the position of the markers.

Obtain ECG Measurements

Select *Analyze ECGs* to obtain ECG measurements for the analysis. A table will appear within the GUI to output the following information:

- Number of individual traces considered in the compiled average trace
- RR interval (ms)
- Heart rate (bpm)
- PR interval (ms)
- QRS interval (ms)
- QT interval (ms)
- P amplitude (mV)
- Q Amplitude (mV)
- R Amplitude (mV)
- S Amplitude (mV)
- T amplitude (mV)



The information presented in the GUI is also saved within a .txt file with the following name scheme: *filename_Results.txt*, where *filename* is the name of the .mat or .txt file containing the ECG voltage data. The file will be saved in the directory chosen upon clicking *Choose Save Path*. If no save path is chosen, the default directory will be the one where the .mat and/or .txt files are located.

Results Files

Several plots are automatically generated and stored within the directory chosen upon clicking *Choose Save Path*. If no save path is chosen, the default directory will be the one where the .mat and/or .txt files are located. The plots are saved following the naming scheme *filename_FigureX.tif*, where *filename* is the name of the .mat or .txt file containing the ECG voltage data and FigureX refers to the following:

Figure 1: overall average trace without the compiled traces, confirmed wave markers present

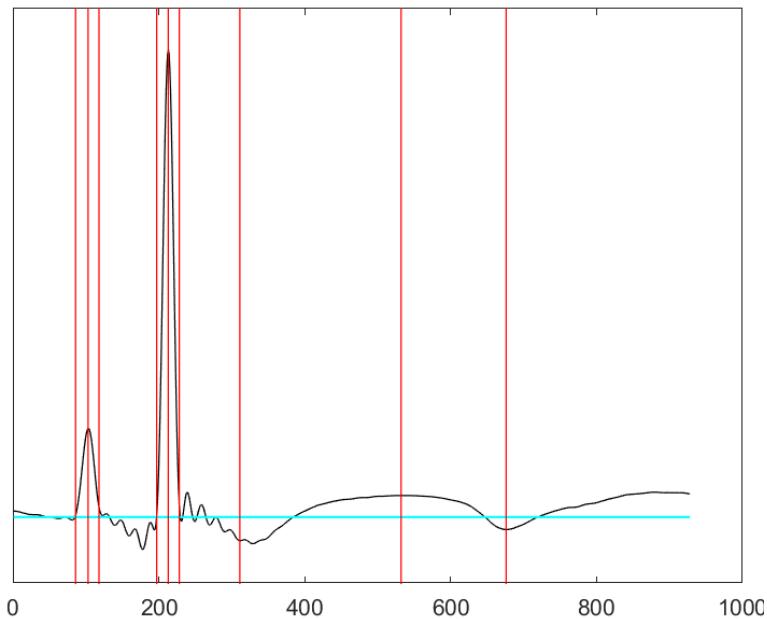


Figure 2: Average trace as observed within zERG after selecting *Calculate Average Trace*

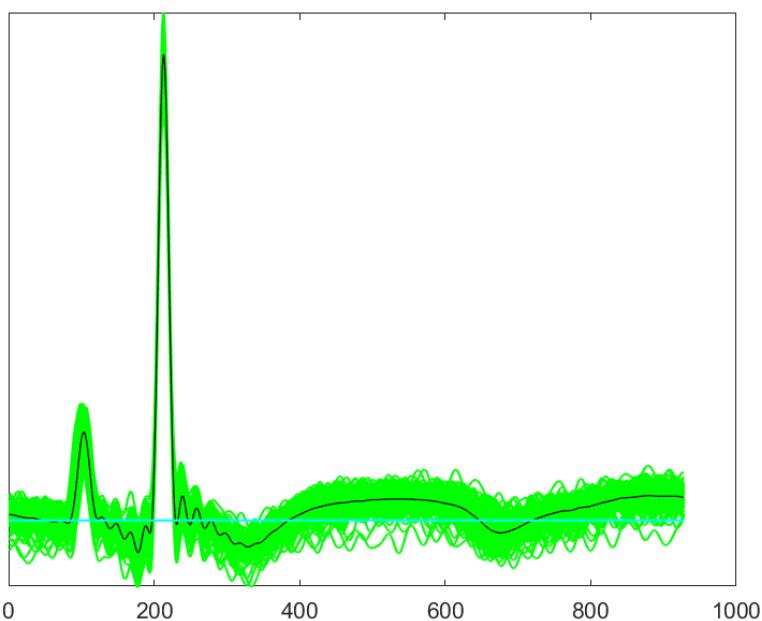


Figure 3: Peak Analysis plot of the selected data for analysis, P waves and R waves are labeled

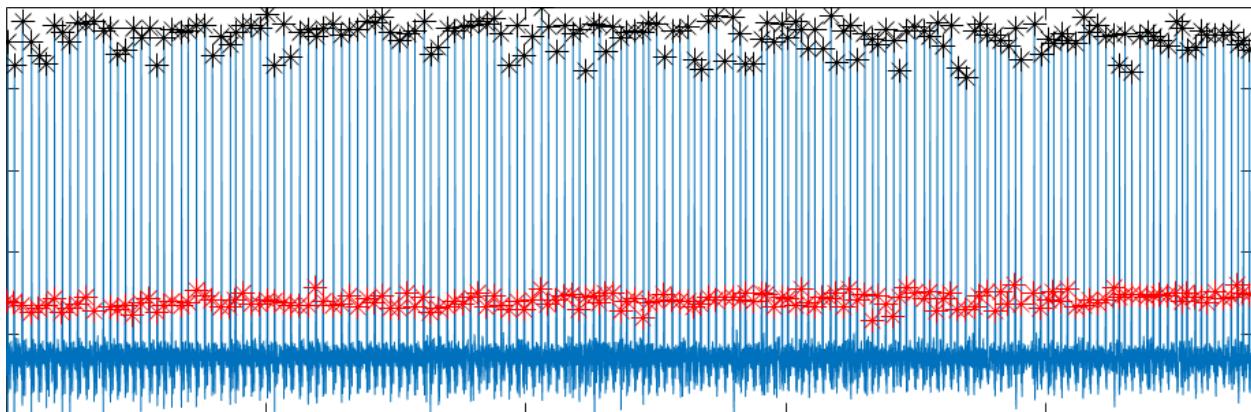
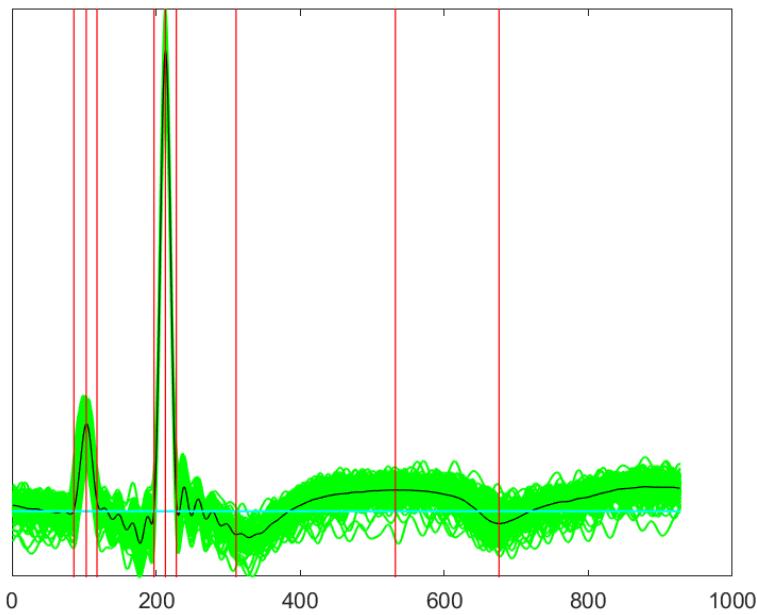


Figure 4: Final average trace, with confirmed markers



[Continue Trace Analysis](#)

To continue analysis within the same directory, sampling select the next file in the **Data Files** list. All parameters created to analyze the previous trace will be erased. As such, be sure to obtain all necessary data before selecting another file for analysis! Future iterations of zERG will contain options to save all outputs into a .mat file to allow for easy reload and plotting of data.