

zERG (Zebrafish ECG Reading GUI) User Guide

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Table of Contents

Introduction	3
How to Run.....	3
Software Requirement	3
Citation	3
Contact	3
Analysis Overview.....	4
GUI Overview	6
Options to Align Trace Using Minima	7
MATLAB Toolbar.....	7
Version 1.2 Overview	8
Quick Start.....	9
Trace Analysis.....	10
Loading and Reading ECG Recording.....	10
Peak Analysis	11
Editing Peak Analysis	13
Navigating the Peak Analysis Module	13
Individual Peak Selection.....	17
Grouped Peak Selection.....	18
Peak Deselection	19
Peak Deletion	20
Peak Addition	21
Swapping Peak Color.....	21
Removing Yellow Markers.....	22

Resetting Peak Analysis.....	22
Calculating Average Trace	23
Adjusting Average Trace Plot Window	24
Adding Wave Markers	27
Confirming Wave Markers.....	28
Obtain ECG Measurements.....	29
Results Files.....	30
Continue Trace Analysis	31
Peak Noise-Remover.....	32
Route A: If the User Answered YES to the First Prompt	33
Route B: If the User Answered NO to the First Prompt.....	34
Editing During Peak Noise-Remover.....	35
Route A Editing	35
Route B Editing	36
Trace Analysis Using Minima	37
Minima Editing	39
Compatibility with Peak Functions.....	39
Minima Noise-Remover	40
Editing During Minima Noise-Remover.....	41
New Features.....	43
Converting Data into MATLAB Format	43
Saving Progress.....	43
Loading Save States	43
Noise Remover Pop-Up Frequency.....	45
Tips & Troubleshooting Guide	46
ECG Calculations.....	47
Glossary.....	48

Introduction

[zERG](#) (Zebrafish ECG Reading GUI) is a MATLAB-based Graphic-User Interface (GUI) that allows for the analysis of electrocardiogram (ECG) traces collected from zebrafish. Commercial ECG analysis software like Labchart (ADIInstruments) is limited in terms of analyzing traces where the P wave amplitude exceeds the R wave amplitude. In these traces, incorrect wave assignment occurs: the QRS complex is marked as the P wave and unfortunately, this assignment is unable to be edited by the user. [zERG](#) circumvents this limitation and is 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 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.

How to Run

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.

Software Requirement

[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.

Citation

Duong T, Rose R, Blazeski A, Fine N, Woods CE, Thole JF, Sotoodehnia N, Soliman EZ, Tung L, McCallion AS, Arking DE. Development and optimization of an in vivo electrocardiogram recording method and analysis program for adult zebrafish. *Dis Model Mech*. 2021 Aug 1;14(8): dmm048827. <https://doi.org/10.1242/dmm.048827>. Epub 2021 Aug 11. PMID: 34378773.

Contact

Materials related to [zERG](#) can be found at: <https://github.com/tvyduo/zERG>.

Please contact ThuyVy Duong at tvyduo@gmail.com for questions and/or assistance. Feedback and suggestions for additional features are welcomed!

Analysis Overview

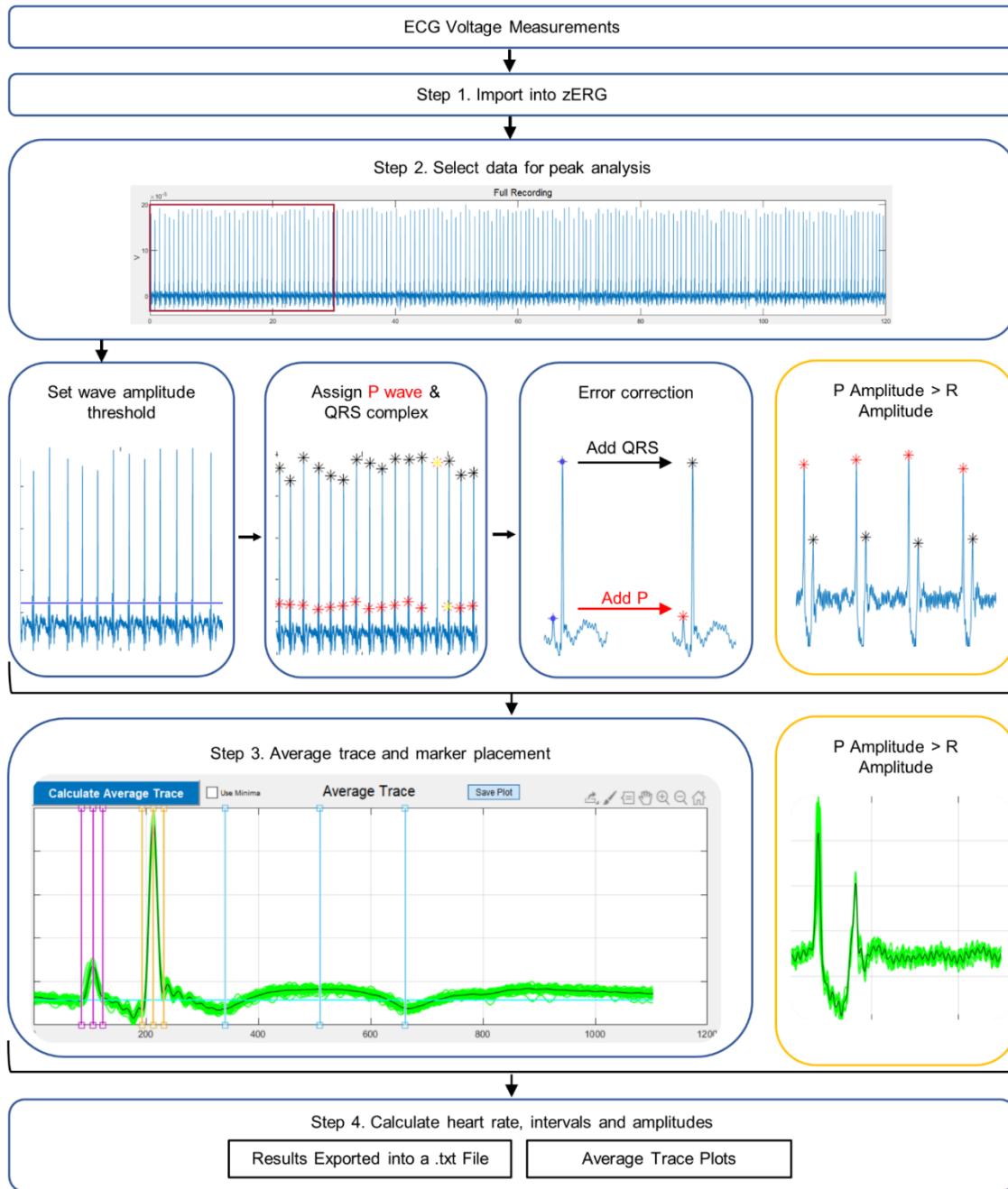


Figure 1: Figure 2 in Duong et. al. (2021), a flowchart depicting zERG analysis pipeline

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.

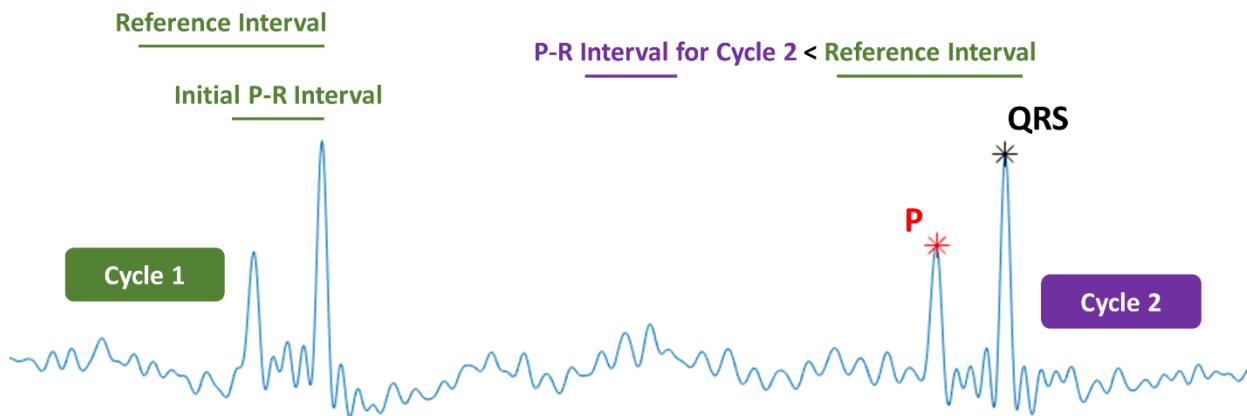
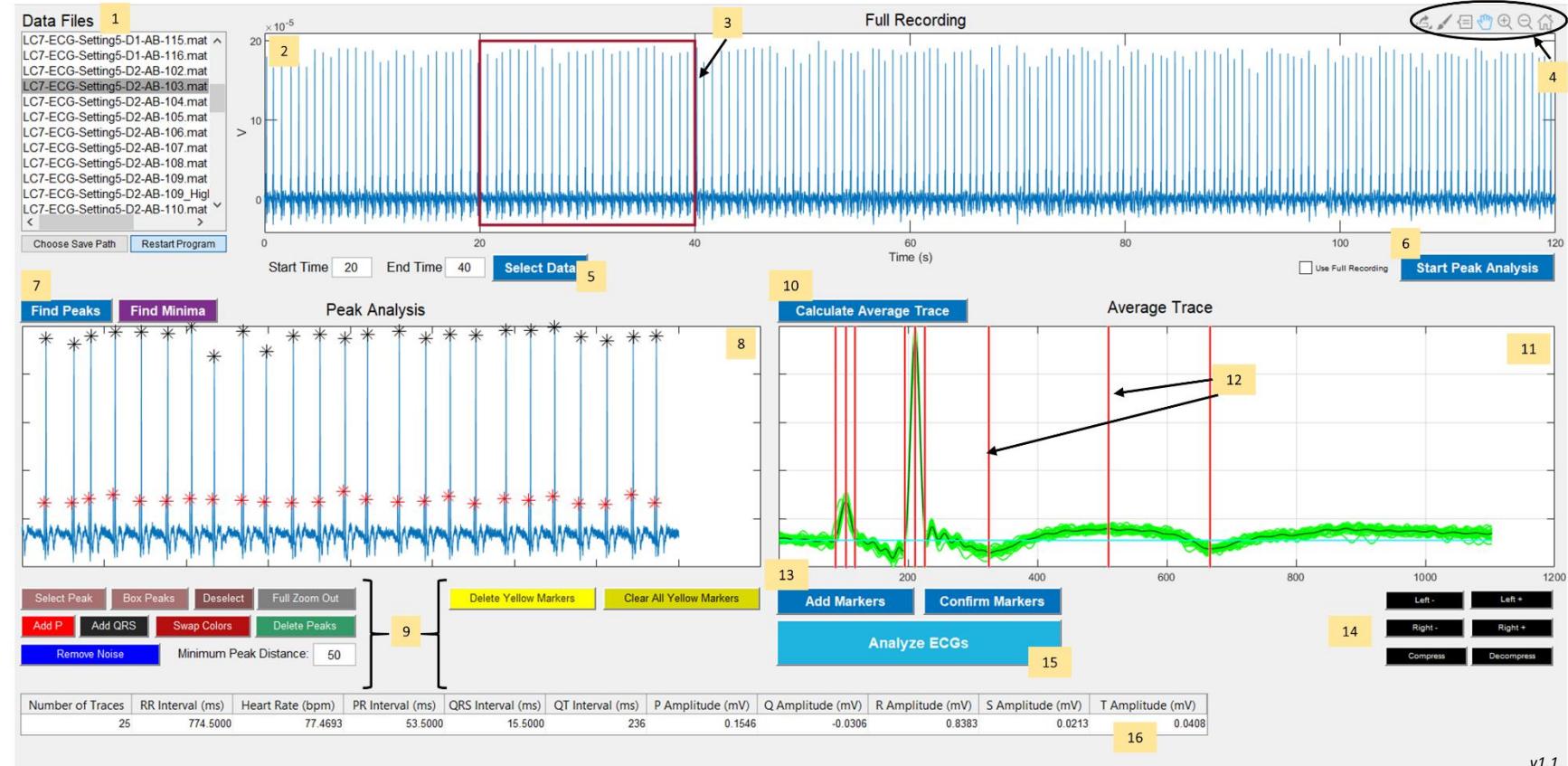


Figure 2: Explanation of P wave & QRS complex Identification using Reference Intervals

If zERG 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, 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 in the flowchart show an example of an ECG trace where the P wave amplitude is larger than the R amplitude and how zERG handles such traces.

zERG also contains a noise-remover function to more quickly isolate P wave and QRS pairings. After answering a series of questions regarding trace characteristic ("Is the R amplitude greater than the P amplitude?", "Does this pattern occur throughout the entire trace or is there a mixture?"), the noise-remover automatically calls peaks using a combination of the minimum height of the taller peak, the interval between the P wave peak and R wave, and the interval of the R wave to the next P wave peak but will ask for user input if it cannot determine the appropriate peak calling. Incorrect peaks and places where the user stepped in are labeled for user review once the function is complete.

GUI Overview



- 1: **Data Files** - List of .mat or .txt files in folder
- 2: **Full Recording** - Plot of trace (highlighted gray under Data Files box)
- 3: Red Rectangle - Selected portion of trace for Peak Analysis
- 4: **MATLAB Tool Bar**
- 5: **Select Data** - Choose portion 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

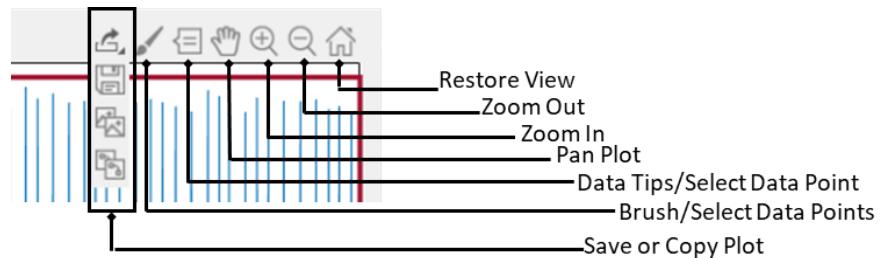


17: *Find Minima* – Identify minima in ECG trace

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

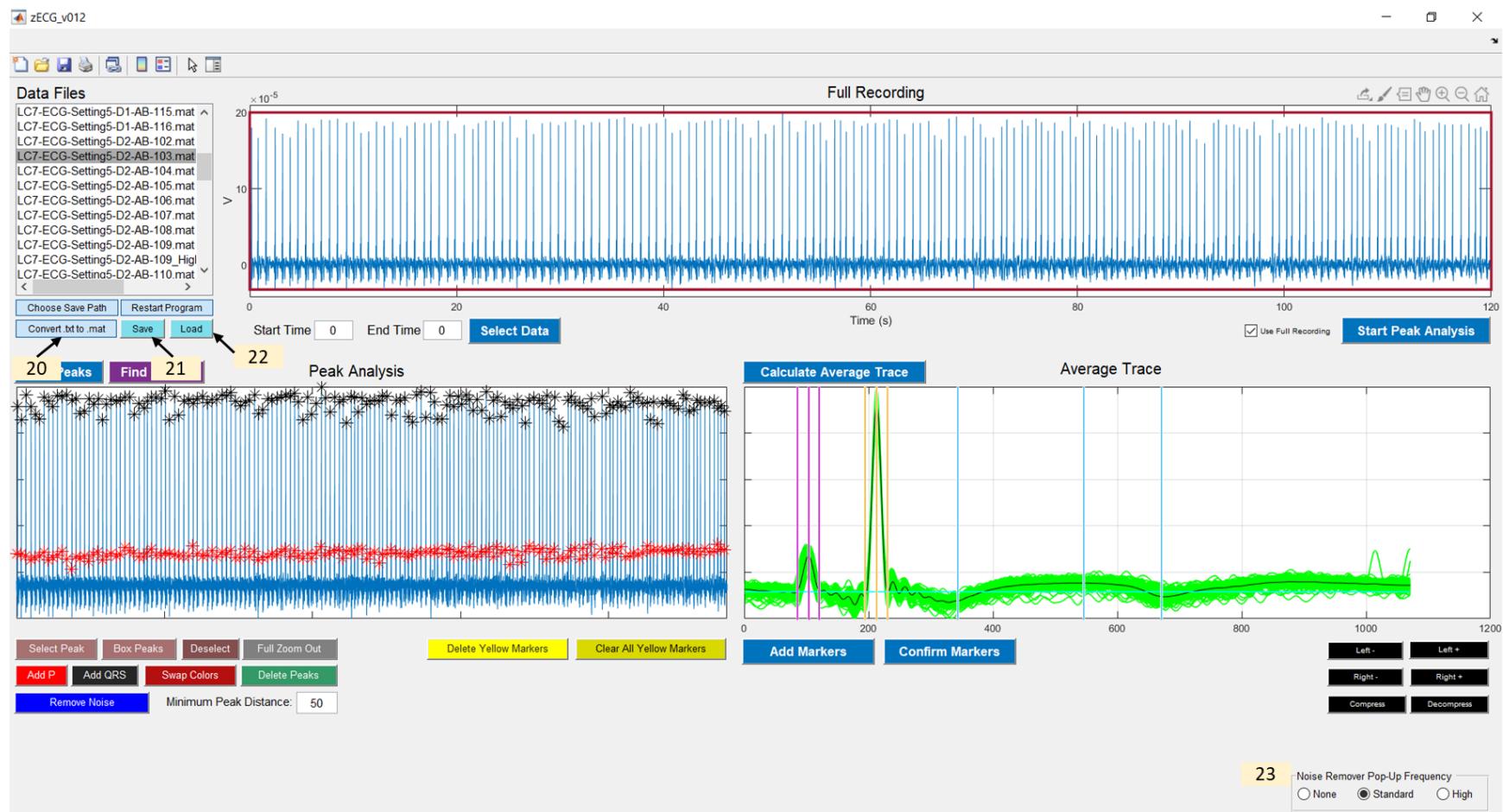
19: *Calculate Average Trace With Minima* – Align ECG traces by the identified minima to generate an average trace

MATLAB Toolbar



This toolbar 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

Version 1.2 Overview



20: **Convert .txt to .mat** - Convert imported .txt into .mat format
22: **Load** - Load *ss.mat file back into zERG

21: **Save** - Save trace analysis progress (*.ss.mat file)
23: **Noise Remover Pop-Up Frequency** - Change whether instructions appear only as pop-ups, only within the console, or a combination of both during the noise-remover functions

Quick Start

1. Download `zERG.m` and `zERG.fig`. Save both files into the same directory.
2. Open MATLAB and run `zERG.m`.
3. Once the file explorer window appears, select the folder containing the ECG traces.
4. The list of ECG traces will appear under `Data Files`; highlight the trace to be analyzed.
 - a. Click `Choose Save Path` to change the save path where results are saved
5. Check the `Use Full Recording` box if analyzing the entire ECG trace. If only analyzing a segment of trace, enter the start and end time of the segment; a rectangular box will appear around the selected area to be analyzed after clicking `Select Data`.
6. Click `Start Peak Analysis`; this will plot the data selected in the `Peak Analysis` box.
7. Click and drag the black line that in the `Peak Analysis` box until all peaks are above it.
8. After the threshold has been set, click `Find Peaks`. P waves will be designated by red markers (*) while QRS complexes will be denoted by black markers (*).
9. If necessary, edit the peak assignment using the manual edit buttons (`Add P`, `Add QRS`, `Swap Colors`, `Delete Peaks`) or semi-automatic method using the noise-remover (prompts will appear either as pop-ups or within the console; to change this, select the appropriate option under the `Noise-Remover Pop-Up Frequency` box).
 - a. Alignment via minima within the trace requires minima identification using `Find Minima` before `Calculate Average Trace With Minima` can be selected
10. After all peaks are marked and identified correctly, click `Calculate Average Trace`. Use the black buttons to change the `Average Trace` window.
11. Click `Add Markers`.
12. Manually move the lines to denote the start, peak, and end of the P wave, QRS complex, and T wave.
13. Once all lines are set appropriately, click `Confirm Markers` (markers will turn red).
14. Click `Analyze ECGs` – a results table will appear on the GUI containing ECG measurements. The results are exported into a `.txt`. Four figures are also automatically created and saved within the chosen save path.

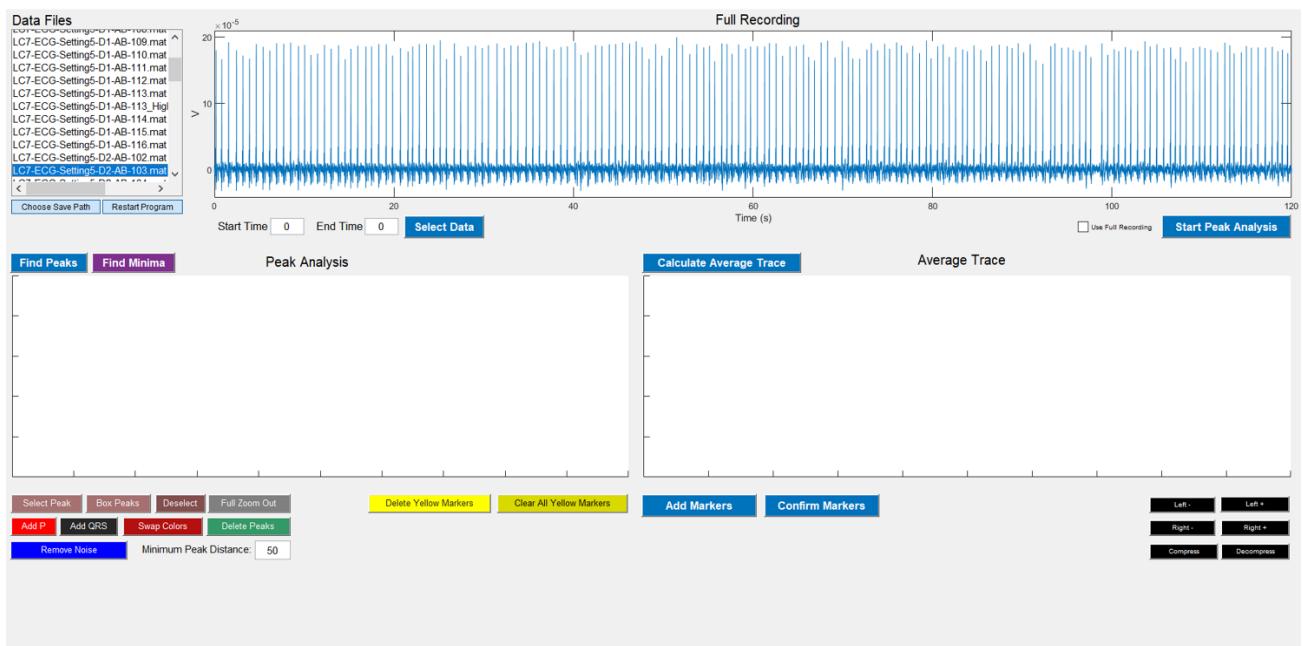
To save trace analysis progress, click `Save` and provide a file name; the output file will end in `*.ss.mat`. The file must be saved within the same folder where the ECG traces are stored.

To load the save state file back into `zERG`, click `Load` and select the folder where the save state is located. All values and analyses are reloaded (with the exception of the unconfirmed `Average Trace` markers).

Trace Analysis

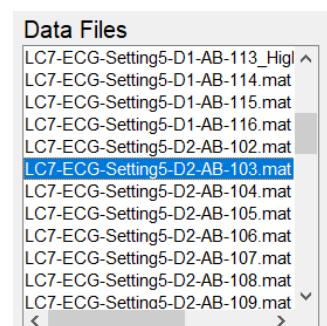
Loading and Reading ECG Recording

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 to analyze with `zERG`. Once the directory is selected, the GUI window will open:



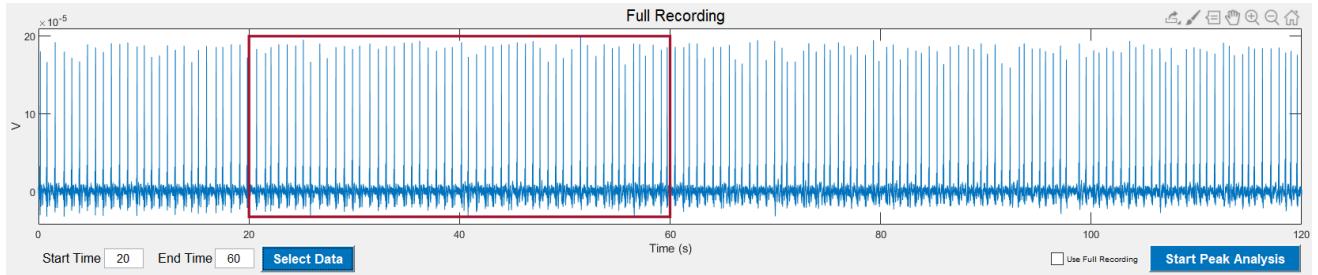
If the wrong directory is selected or if there are any issues that occur throughout the analysis process, select **Restart Program** to automatically close and re-open `zERG`. All previously created objects in the workspace will be cleared.

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.

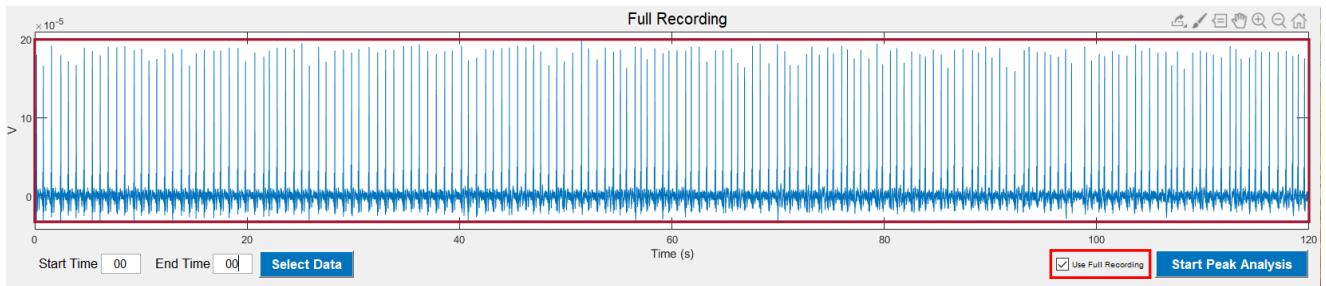


Peak Analysis

1. The ECG recording for the file will be plotted within the **Full Recording** window. Select the range 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.

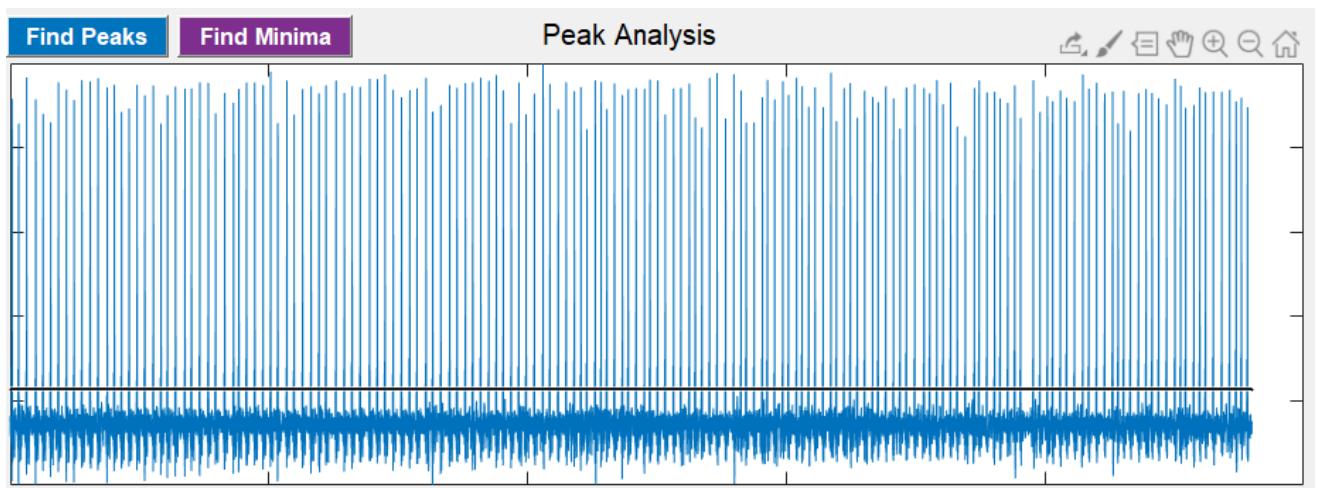


To analyze the full recording contained in the file, check the **Use Full Recording** box.



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

2. The portion of the trace 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 **Find Peaks**.

3. **Find Peaks** will find all peaks, then pair peaks which are close together according to a reference interval (see Figure 1 in [Analysis Overview](#)). 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.

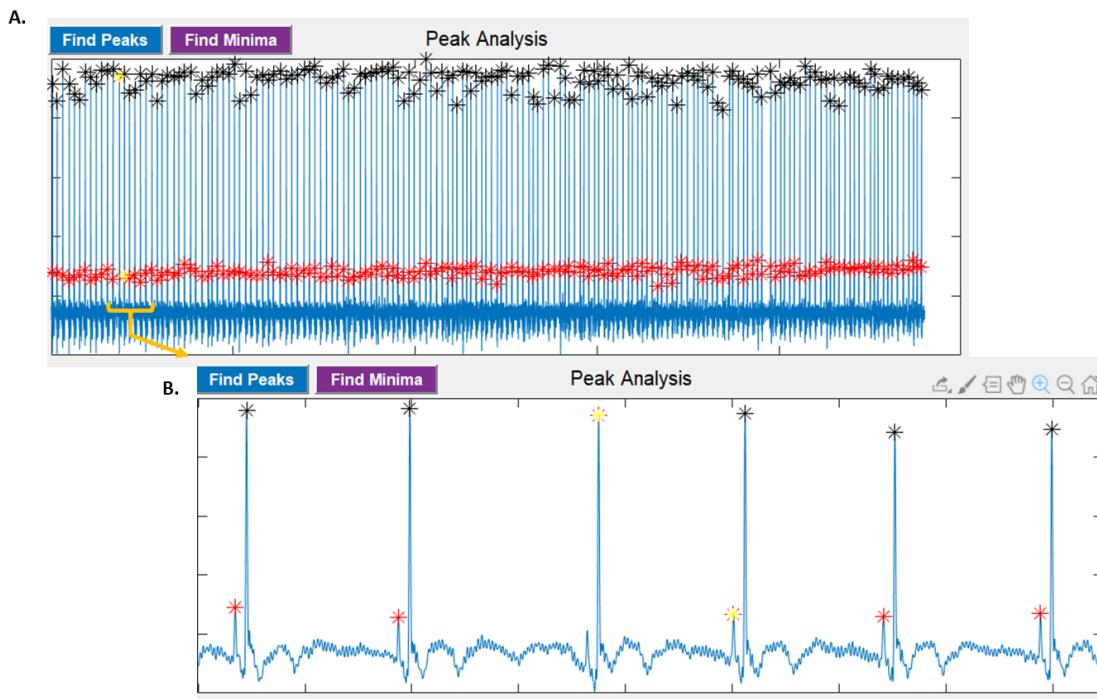


Figure 3: Find Peaks Identifies ECG Trace Features. A) Identification of P and R waves after using **Find Peaks**. B) Zoomed in view of trace section where two waves have been labelled with yellow markers, indicating a potential error in peak wave identification.

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 to manually edit the labeling performed by [Find Peaks](#). Successful completion will result in a trace where all P and R waves are appropriately labeled.

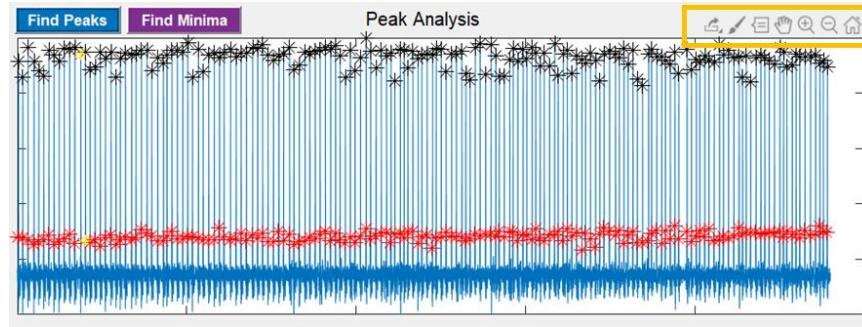
Navigating the Peak Analysis Module

This section details useful functions to navigate the trace in the [Peak Analysis](#) box.

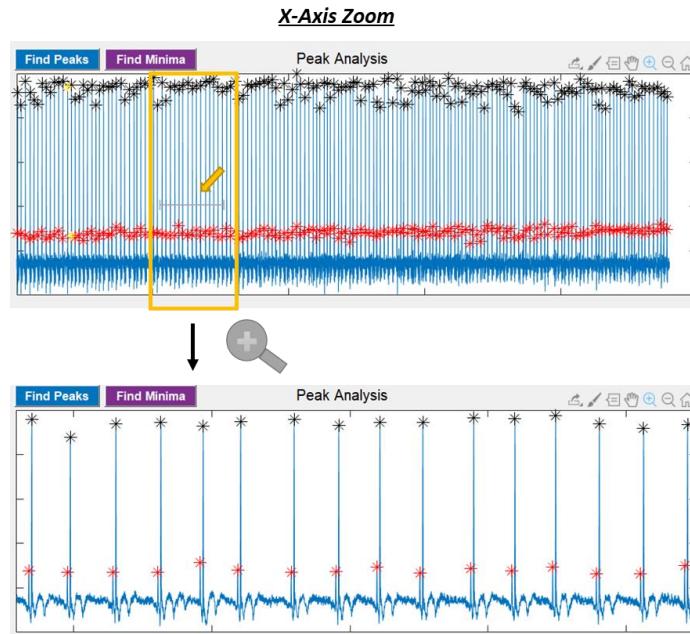
Zoom In

Users can zoom in on the trace within the [Peak Analysis](#) box by using the [MATLAB Toolbar](#), which can be found by hovering over the top right-hand corner of the [Peak Analysis](#) box.

Below is the 'Home View' for which the following examples will be based off of. This view is originally plotted after proceeding with the initial steps of the [Peak Analysis](#) function.



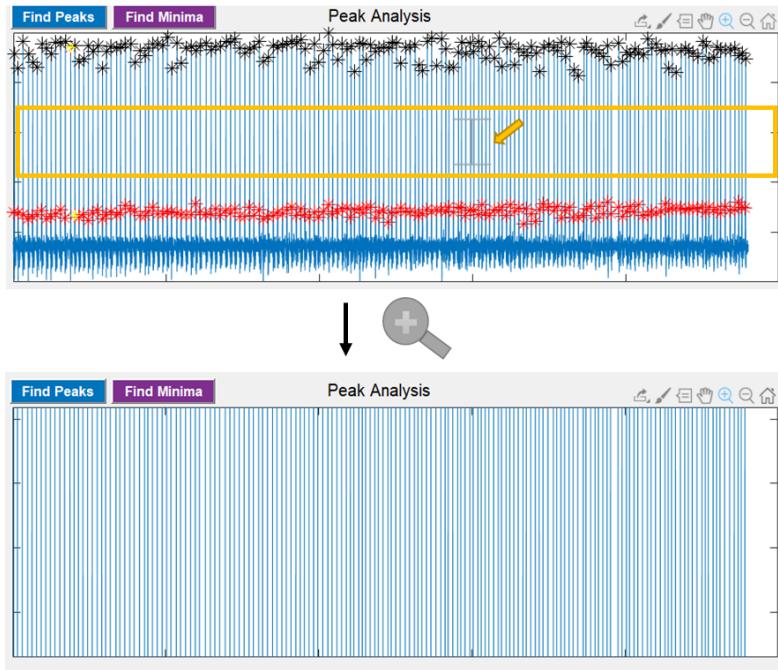
To **zoom in on the X-axis**, click on (it will turn blue once selected). Select the first point of the section that to zoom in on and then draw a horizontal line to a second point such that the faint, gray horizontal line covers the section to zoom in on.



We recommend deselecting the zoom in function before performing any zERG functions to the zoomed in trace (this can be done simply by clicking on).

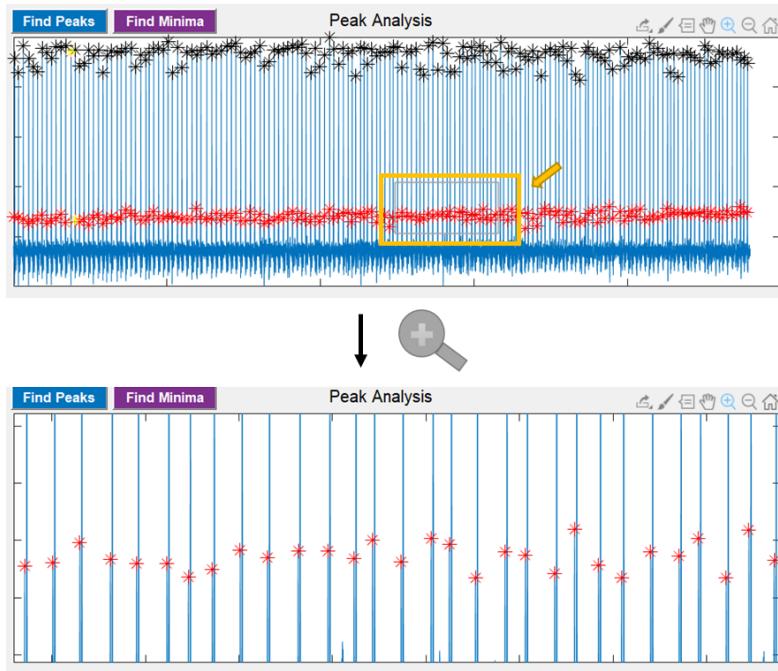
To **zoom in on the Y-axis**, select the first point of the section that will be zoomed in on and then draw a vertical line to a second point such that the faint, gray line covers the section that will be zoomed in on.

Y-Axis Zoom



Both axes can be zoomed in on at the same time, that is, a zoomed "box" can be selected. To generate the box, select a point then drag and draw a box.

Boxed Zoom



Zoom Out

Users can zoom out on any portion of the trace within the **Peak Analysis** box by using the **MATLAB Toolbar**.

To **zoom out**, click on  (it will turn blue  once selected). Then click any point within the **Peak Analysis** box. Each click will zoom out one level until the trace returns to the 'Home View'.

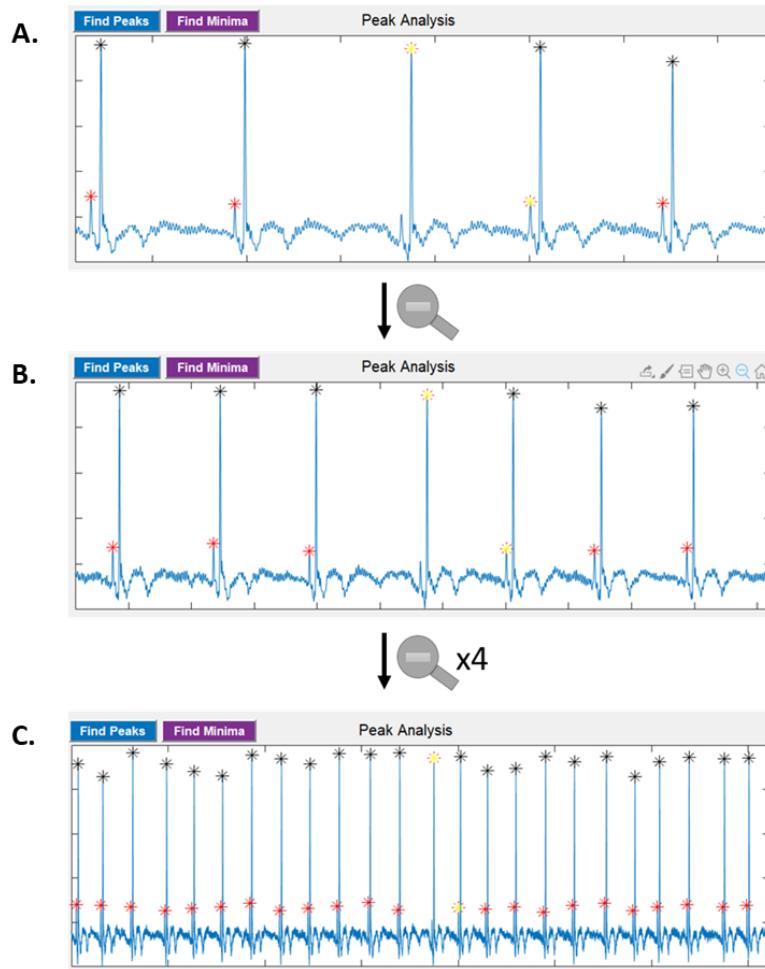


Figure 4: Zooming out within an ECG trace. (A) is the portion of the trace that has been zoomed in. After one click of the 'zoom out' button, the view changes to what is in (B). After four additional clicks of the 'zoom out' button, the view changes to what is in (C).

Restore View

The amount of trace that is zoomed out with each click is small and several clicks may be needed to return to the 'Home View' (in the above example, 8 clicks were needed). To quickly return to the 'Home View' without needing to consecutively click the **Zoom Out** button, select the home icon  in the MATLAB Toolbar.

Zoom Functions During Peak Editing

The **Zoom In** button is compatible with all peak editing functions (described in the next sections). Users are encouraged to use this button to zoom in on a trace to allow for improved peak selection accuracy. The button is also compatible with both noise-remover functions (described in the [Peak Noise-Remover](#) and [Minima Noise-Remover](#) sections).

The **Zoom Out** button is compatible with all of the peak editing functions only up to a certain level/number of clicks. The button **cannot** be used to navigate back to the 'Home View.' Use of this button is recommended to zoom out to 1-2 levels only. The button, however, is compatible with both noise-remover functions with no limitations.

The **Restore View** button is incompatible with all peak editing functions. If, for example, the button is selected after zooming in to add a P wave marker, the view **will not** change. The incompatibility remains for the entire editing session; the button will be compatible again if a different trace is selected or if the user begins the [Peak Analysis](#) procedure again from the beginning (i.e., from when [Start Peak Analysis](#) is clicked). To quickly return to the 'Home View' during or after peak editing, click on [Full Zoom Out](#) under the [Peak Analysis](#) plot. The **Restore View** and **Full Zoom Out** buttons are compatible with both noise-remover functions, however.

Scrolling through the ECG Trace

Selecting the **Pan** () button in the [MATLAB Toolbar](#) will allow the user to click, hold and drag the trace. Movement on the X- and Y-axes is possible but can be limited to only one direction. To do this, ensure  is selected (it will turn blue  once selected), right-click anywhere in the plot area and select either [Horizontal Pan](#) or [Vertical Pan](#) to restrict movement only to the X- or Y-axis, respectively. [Unconstrained Pan](#) allows for movement on both axes.

The navigational arrows on a keyboard can be also used to scroll through the ECG trace. The trace will move in the direction of the arrow. If the left arrow is pressed, the trace will move from left to right (towards the end of the trace). If the right arrow is pressed, the trace will move from right to left (towards the beginning of the trace).

Note that the [Restore View](#) option that appears after right-clicking the plot with the **Pan** button selected has the same constraints as the **Restore View** button within the Toolbar in regards to usage during and after peak editing.

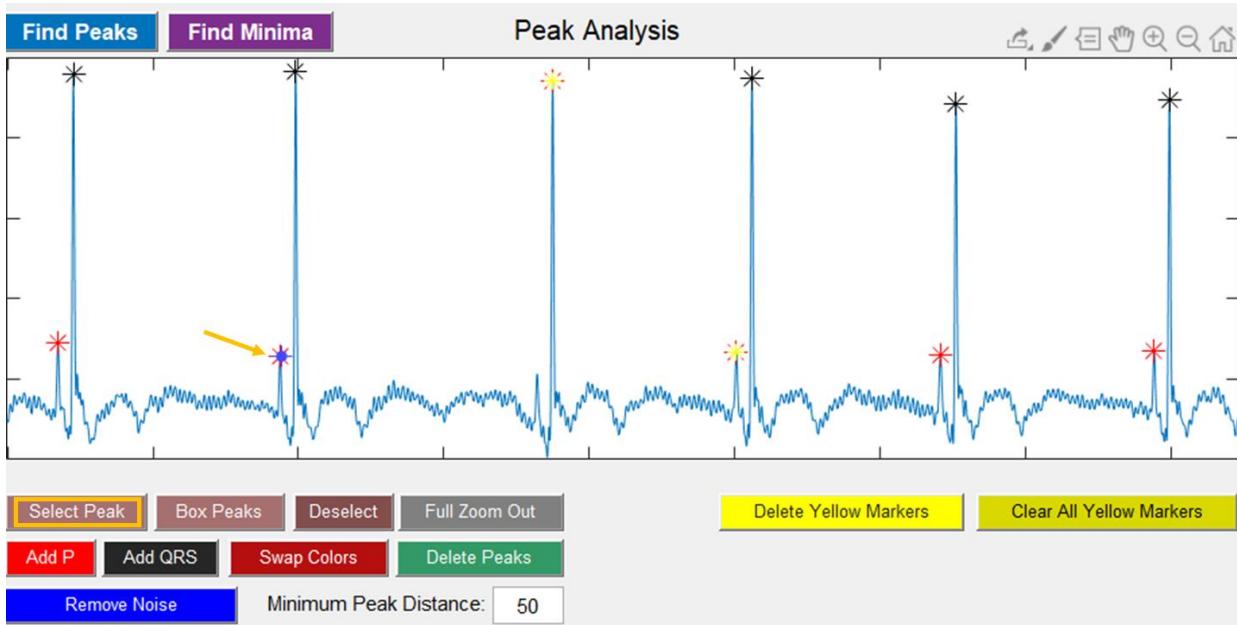
MATLAB Toolbar Usage for Other Plots

All [MATLAB Toolbar](#) functions can be used for the three plot boxes ([Full Recording](#), [Peak Analysis](#), [Average Trace](#)). The Toolbar is located in the top right-hand corner directly outside of the three boxes and can be found by hovering over that area. No limitations exist for the [zoom](#) and [Restore View](#) buttons for the plots within the [Full Recording](#) and [Average Trace](#) boxes.

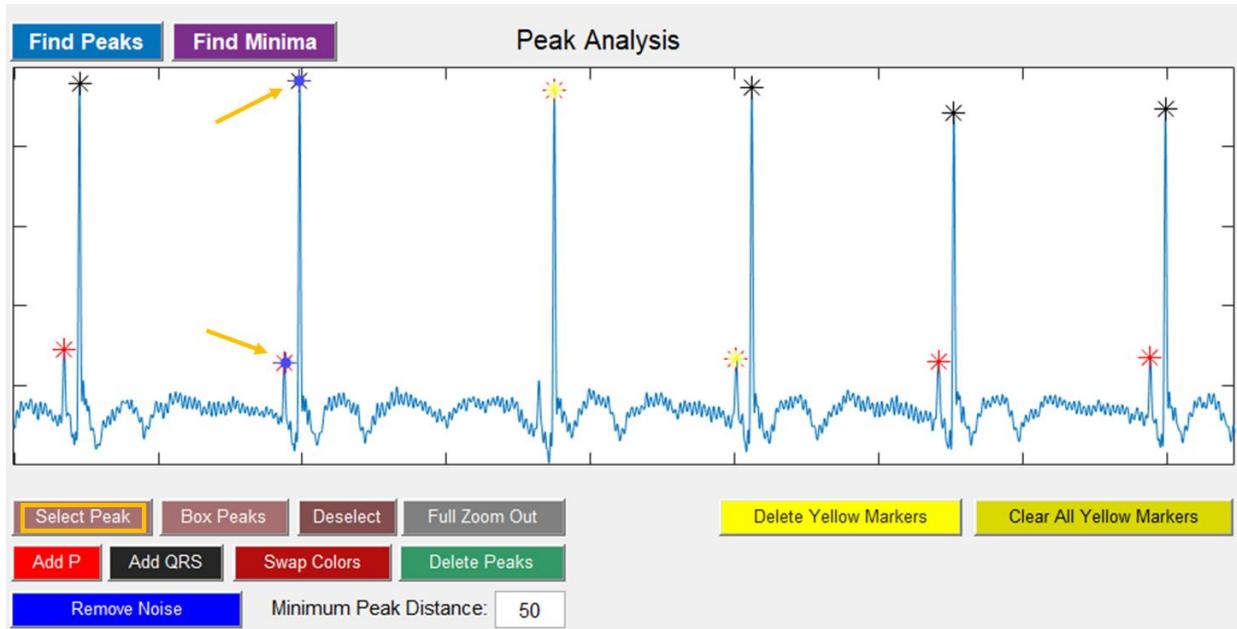
The [Save](#) and [Data Tips](#) buttons can be used within [zERG](#). It is recommended to not use the [Brush>Select](#) data tool as accidental trace data deletion can occur if data is selected followed by the execution of the 'Delete' button. If this occurs, the data will only reappear if it is replotted by beginning the [Peak Analysis](#) protocol again (i.e., selecting [Start Peak Analysis](#)).

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 to be drawn, and the other marker at the corner diagonal from the first marker. All peaks within the **black** rectangle will be selected.

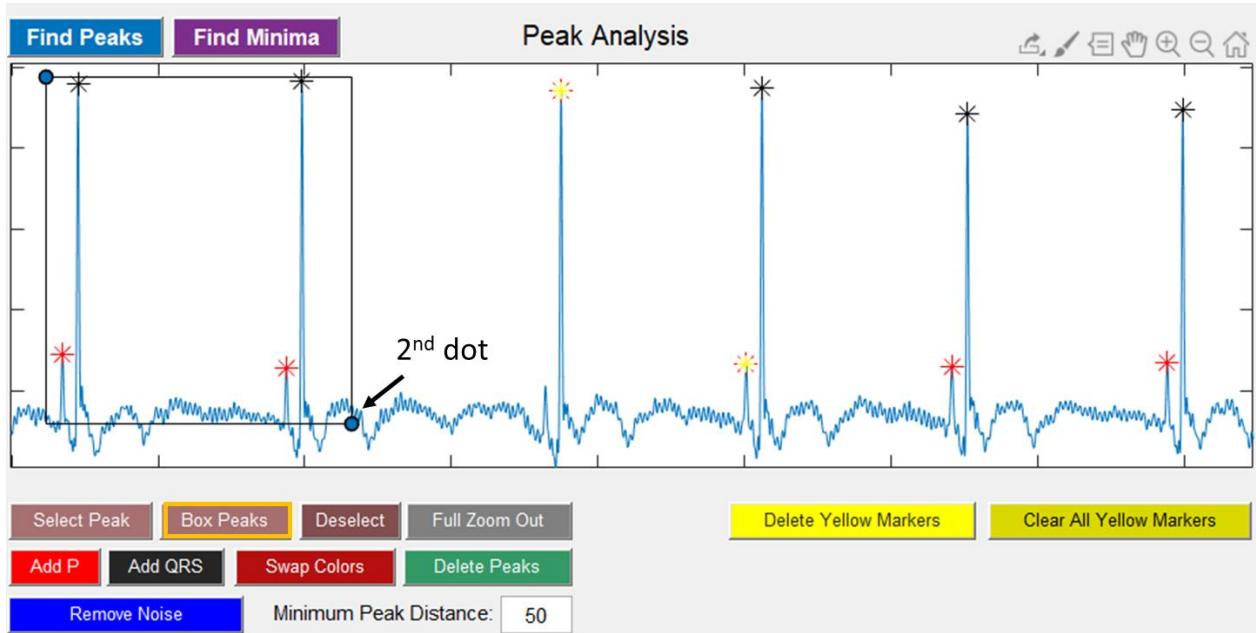
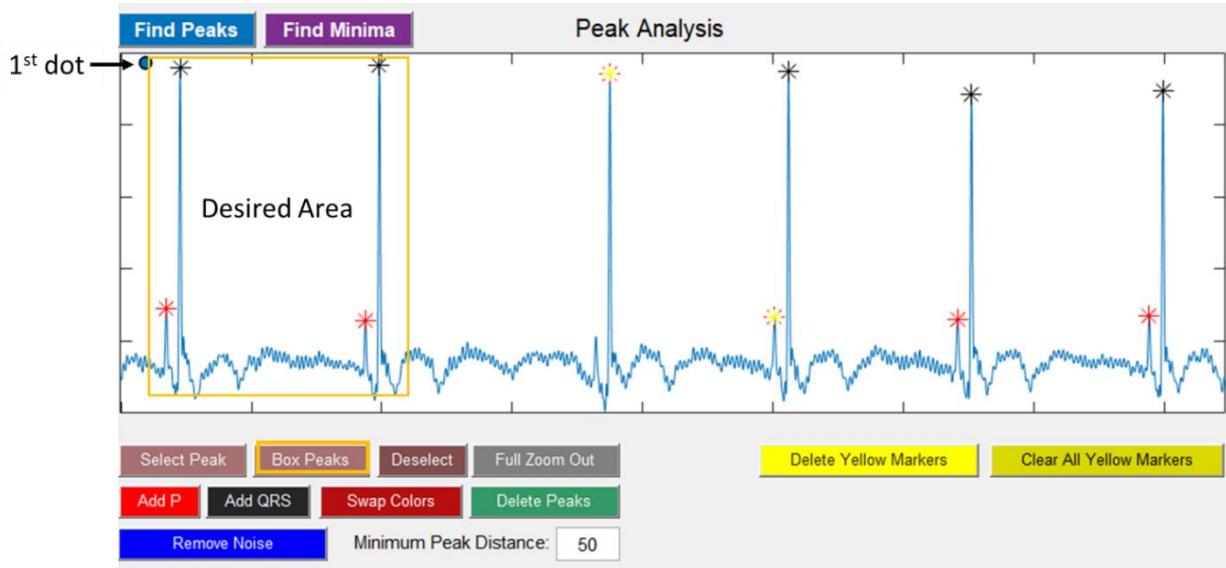


Figure 5: Selecting multiple peaks using **Box Peaks**. (Top) Selection of the first point of the rectangle. (Bottom) Selection of the second point of the rectangle. All peaks within the rectangle are selected.

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.

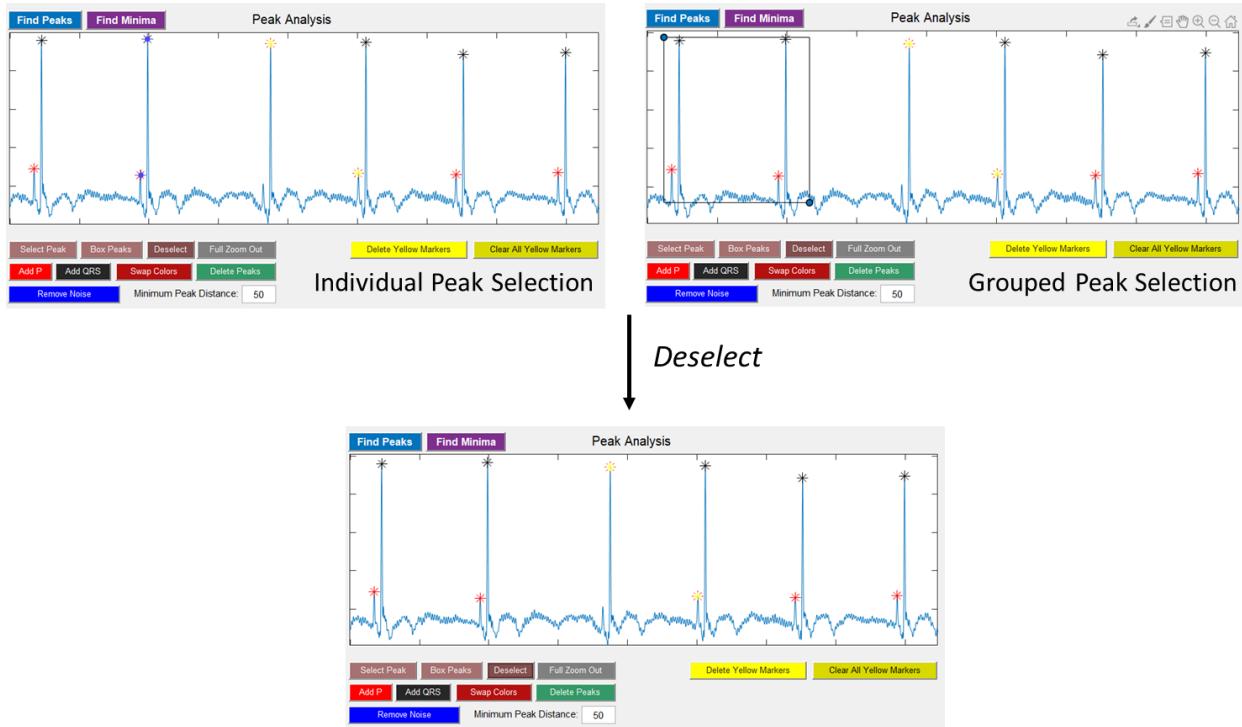


Figure 6: Deselecting peaks in an ECG trace. (Topleft) Both peaks in the 2nd ECG cycle are individually selected using **Select Peak**. (Topright) The first two ECG cycles are boxed using **Box Peaks**. (Bottom) Clicking **Deselect** will clear the blue dots or black rectangle.

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**.

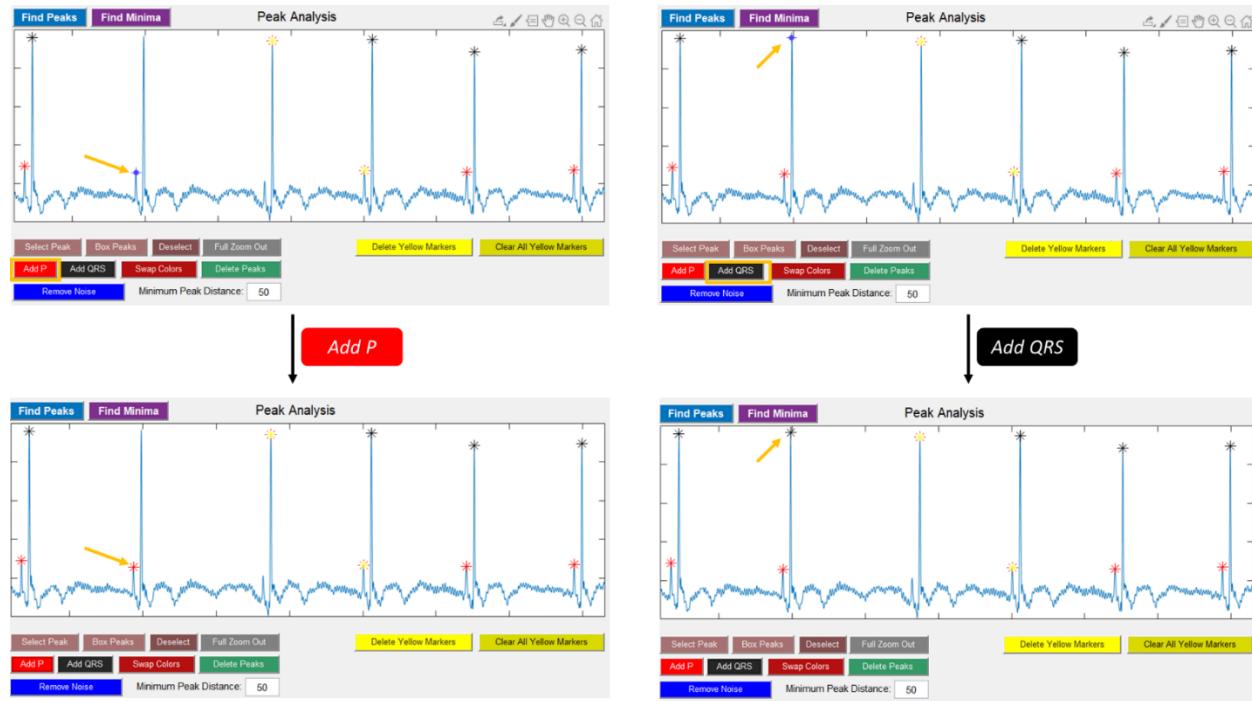
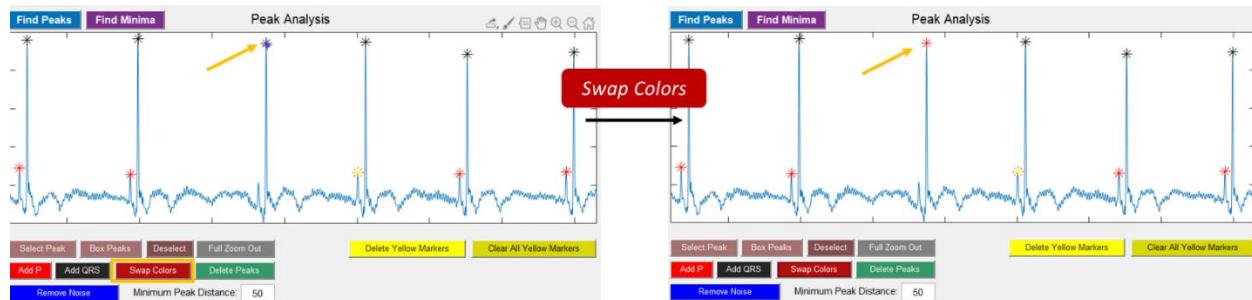


Figure 6: Adding a P wave (left) or QRS (right) to the trace

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.



Figure 8: Deleting an individual yellow marker or clearing all yellow markers

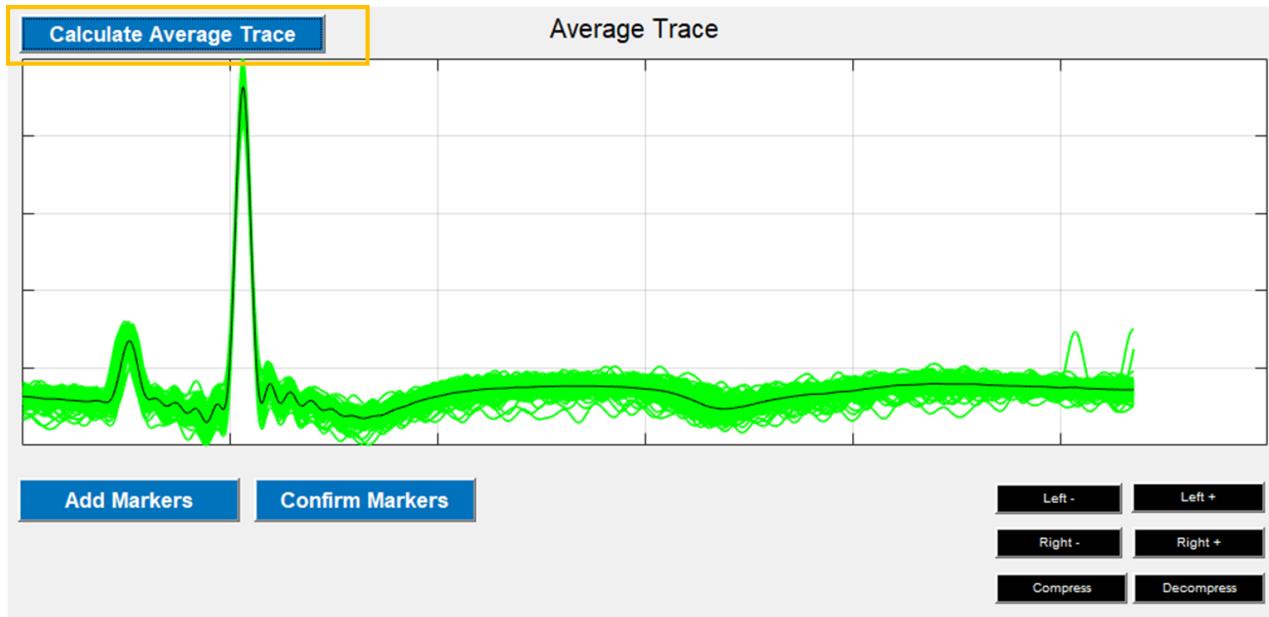
Resetting Peak Analysis

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** to 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 minima may enhance the quality of the average trace. See [Trace Analysis Using Minima](#) on how to identify minima within the recording.



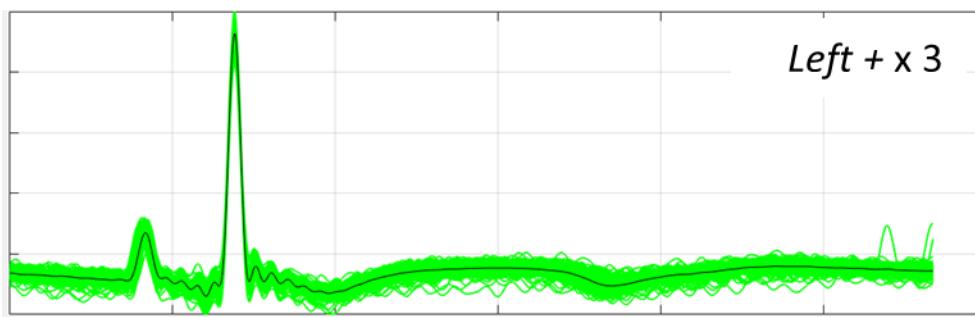
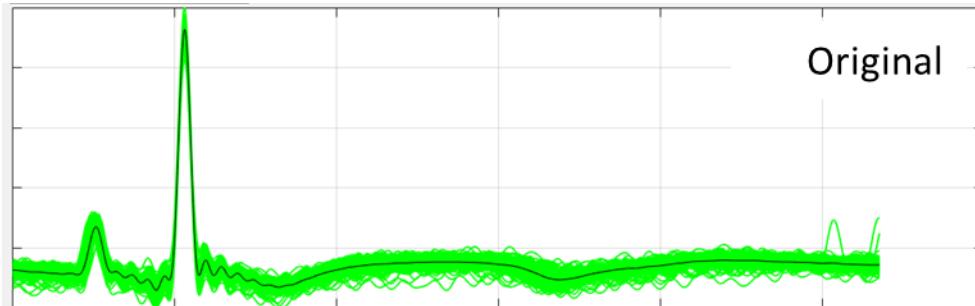
— Each green line represents one trace cycle (P wave-QRS complex) labeled through the **Peak Analysis** process. Here, all cycles are plotted on top of each other to generate a compiled trace to show the overall ECG wave morphology.

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

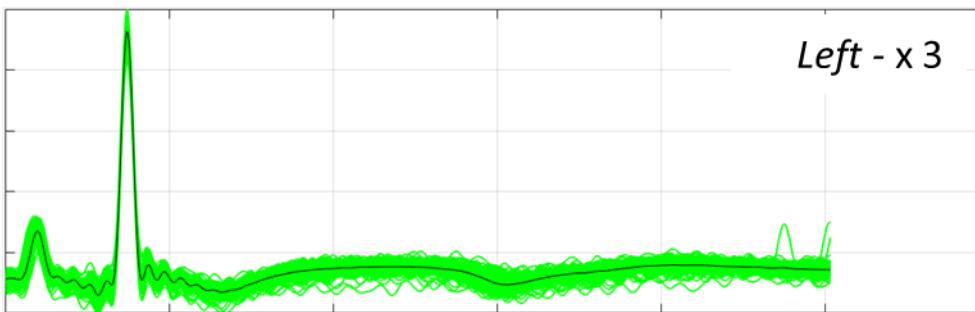
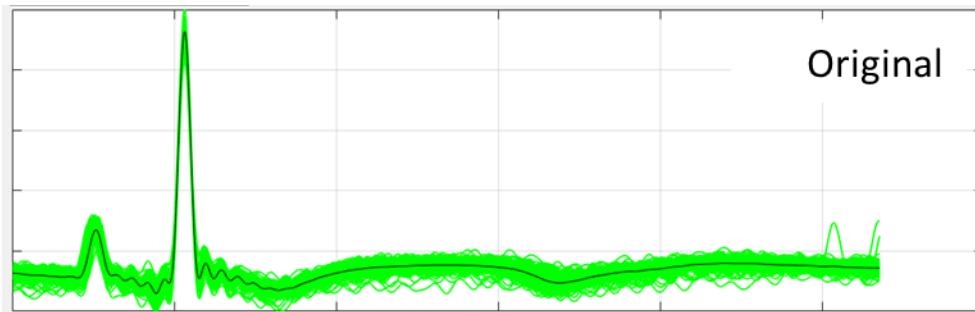
Adjusting Average Trace Plot Window

To adjust the plot window, use the black buttons in the bottom right corner under [Average Trace](#).

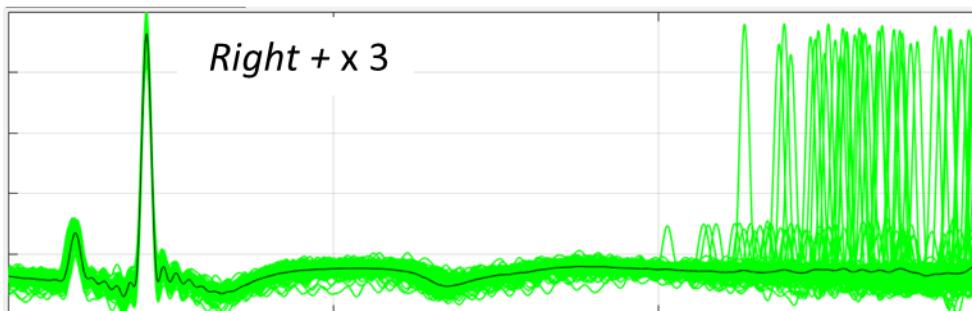
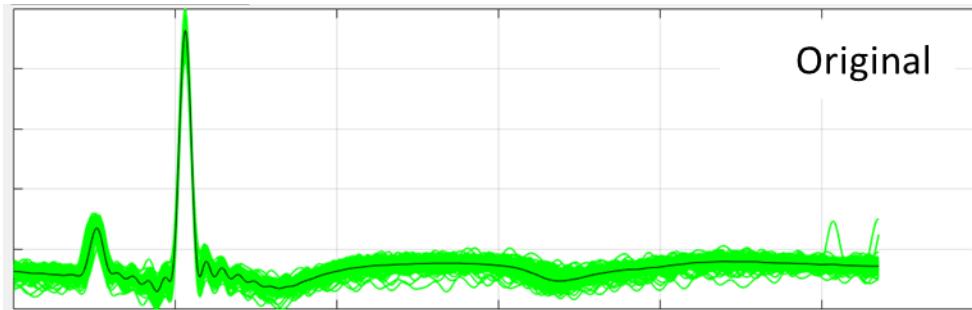
Left + adds to the window on the left:



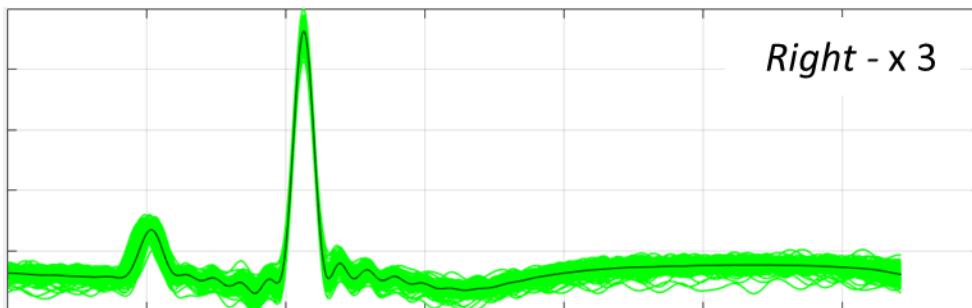
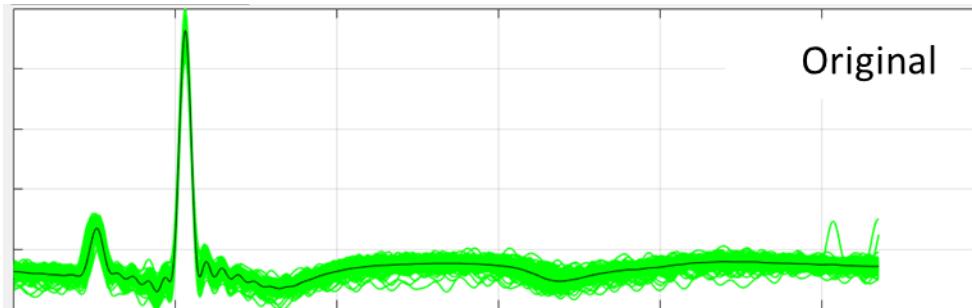
Left - removes from the window on the left:



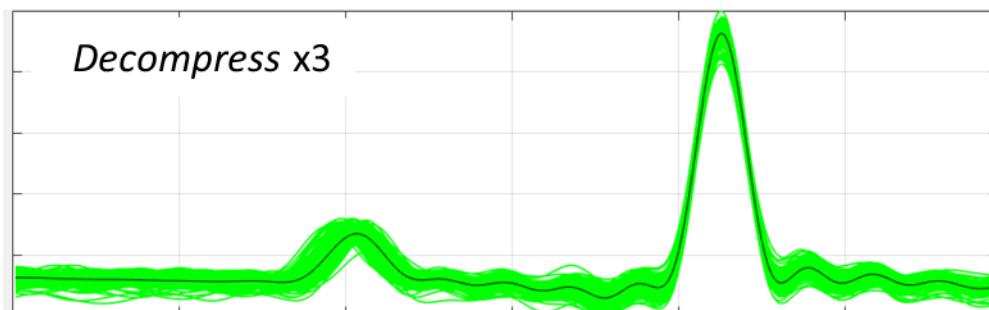
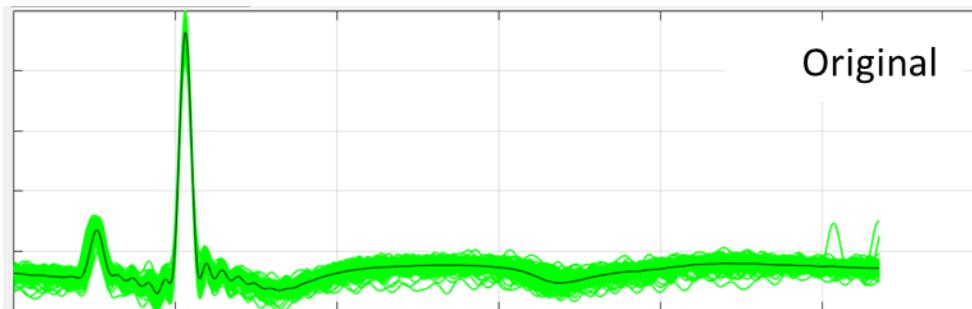
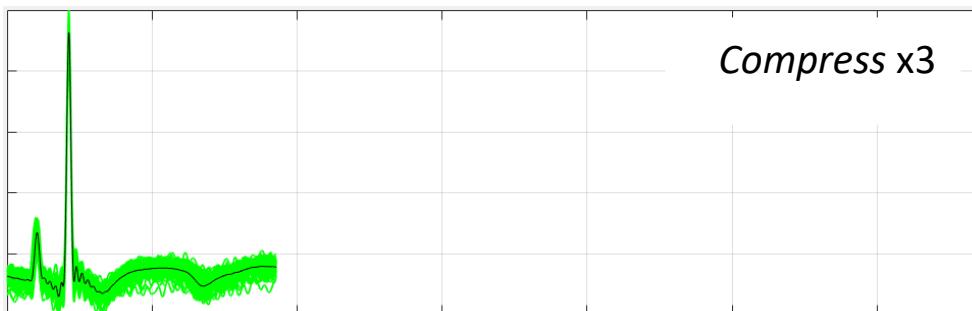
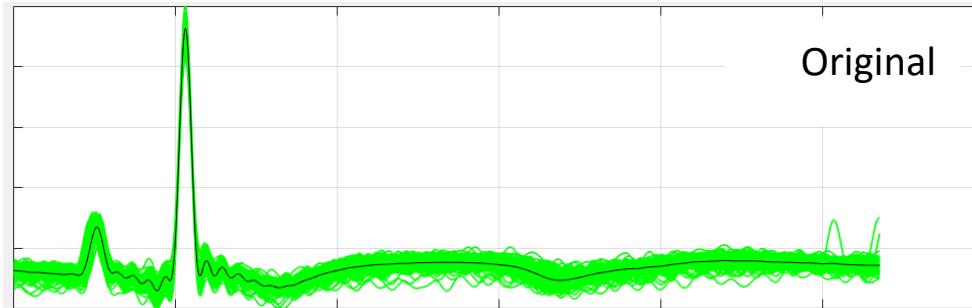
Right + adds to the window on the right:



Right - removes from the window on the right:



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

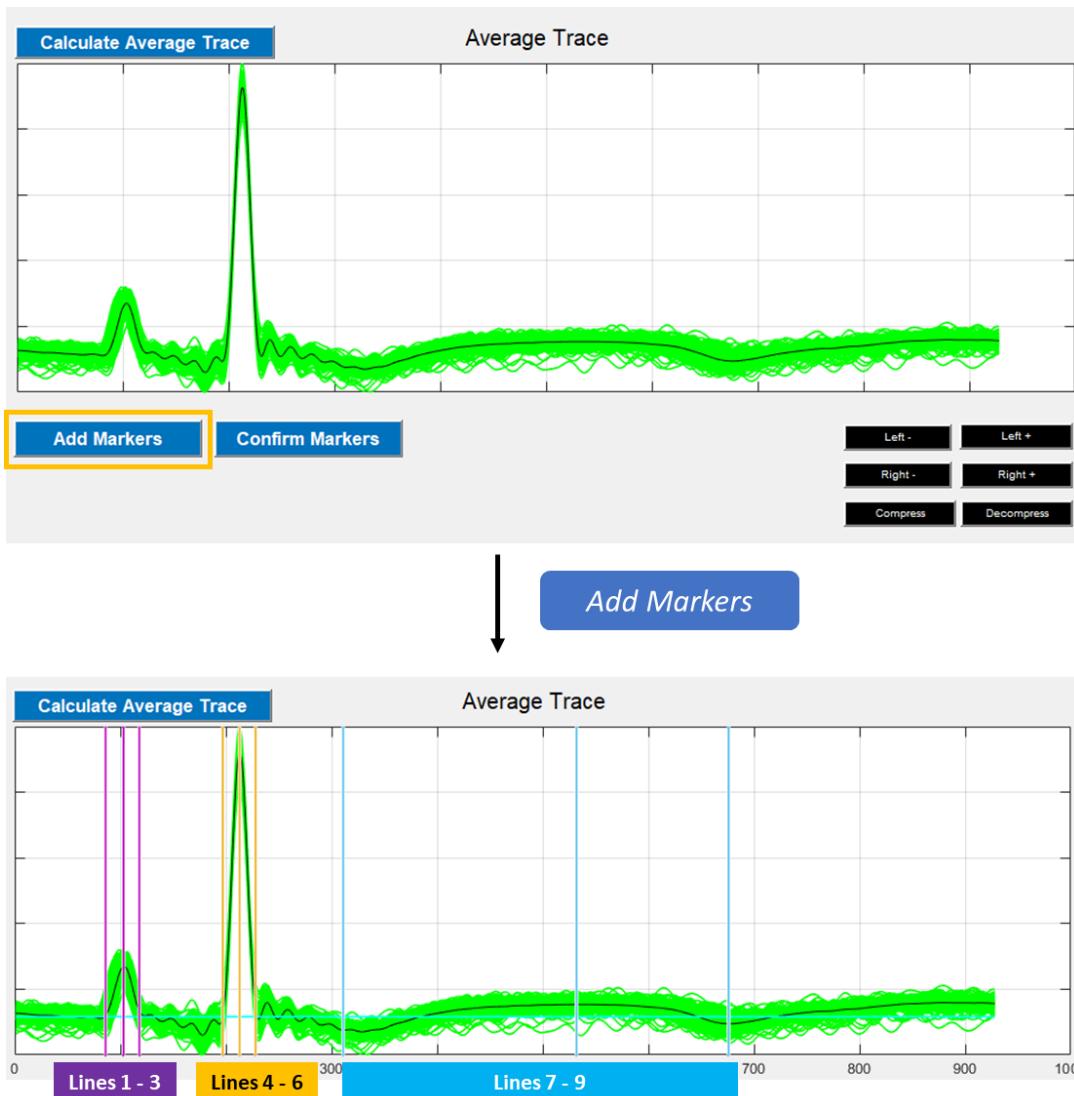


Wave markers will be erased if the plot window is adjusted after wave markers are added. Therefore, we recommend that the plot window be adjusted prior to marker addition.

Adding Wave Markers

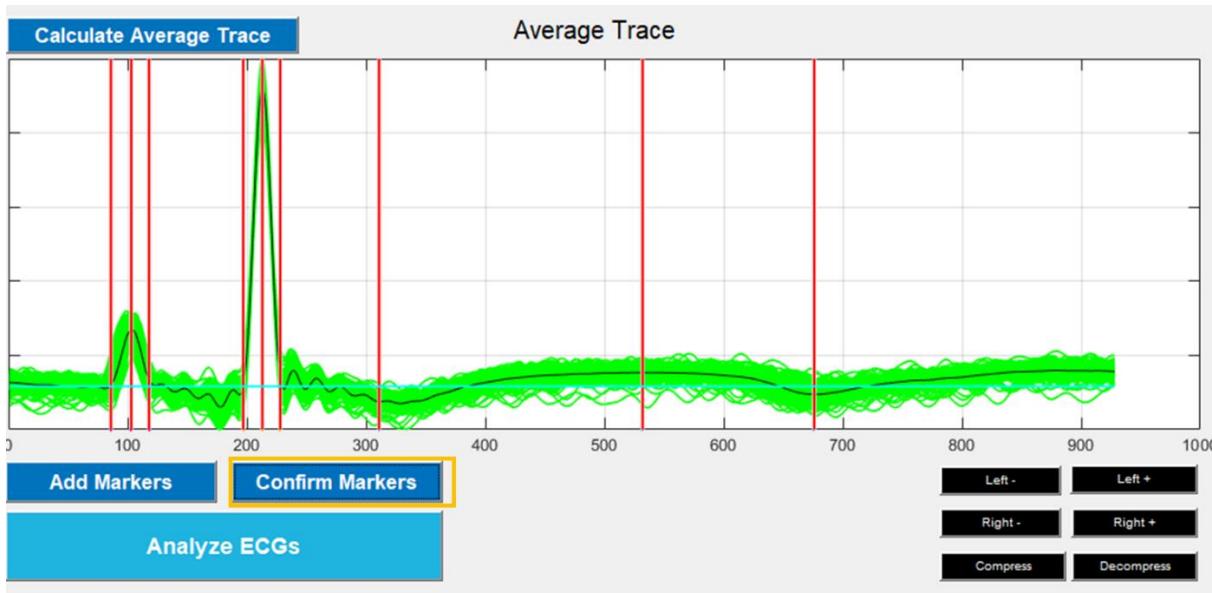
When the **Average Trace** window is appropriately adjusted, click the **Add Markers** button to begin wave identification on the average trace. Markers representing the following ECG features will appear (left to right): the start of the P wave (1), the peak of the P wave (2), the end of the P wave (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. If the position of the markers needs 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 measurements for the analysis. A table will appear in the GUI to output the following measurements:

- 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

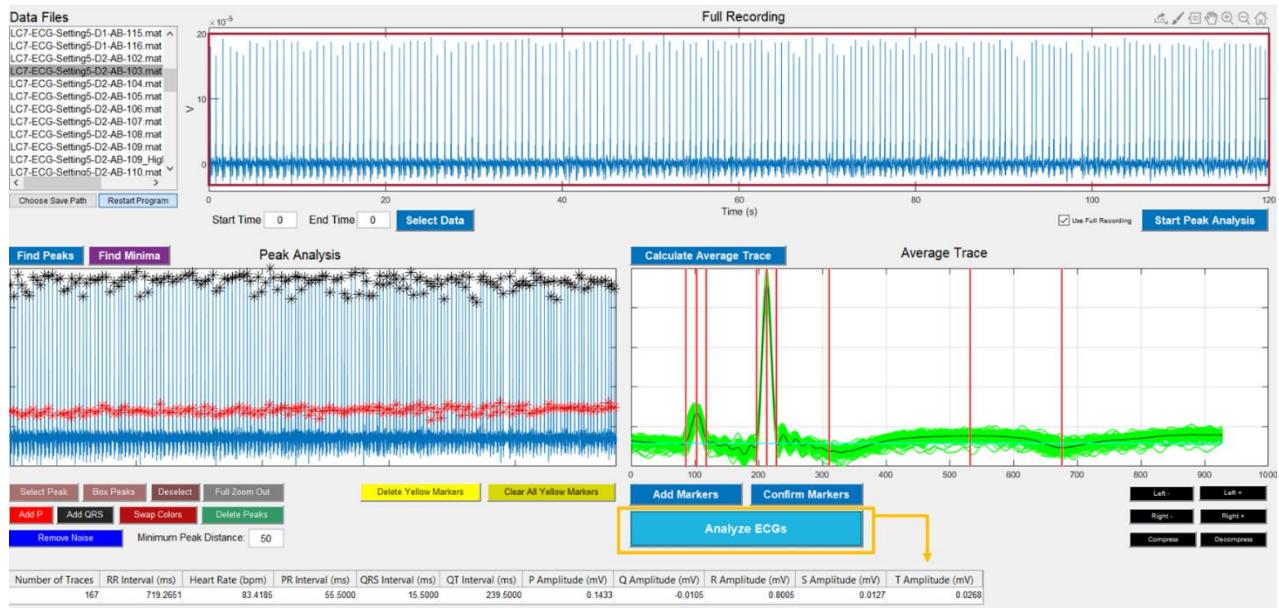


Figure 9: Appearance of table within GUI containing ECG measurements after selecting Analyze ECGs

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 .txt file will also contain the analysis date and name of the trace that was analyzed. 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

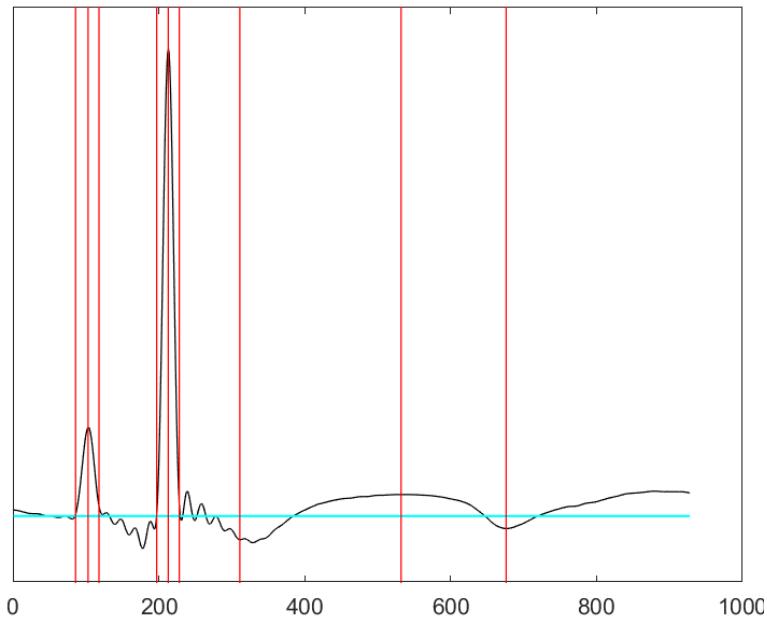


Figure 2: Average trace as observed within [zERG](#) after selecting [Calculate Average Trace](#)

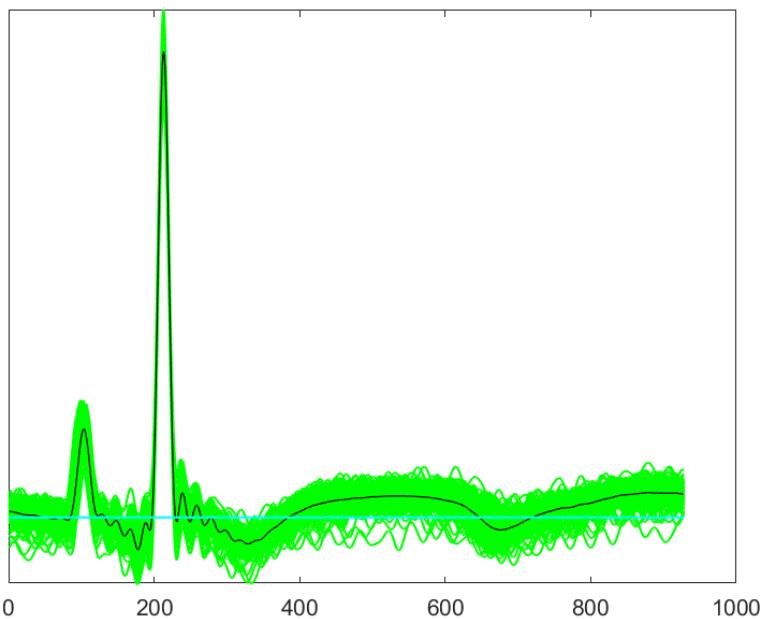


Figure 3: Peak Analysis plot of the selected data for analysis, P waves and R waves are labeled

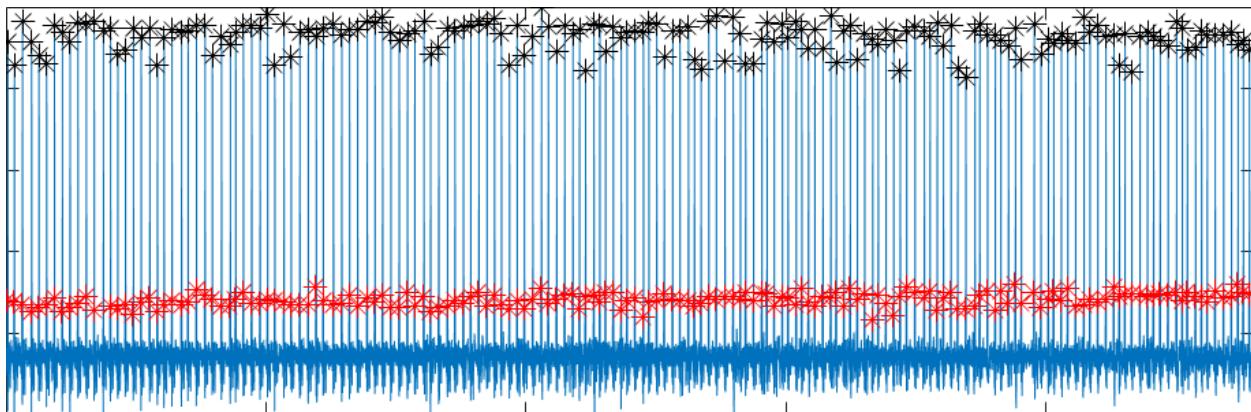
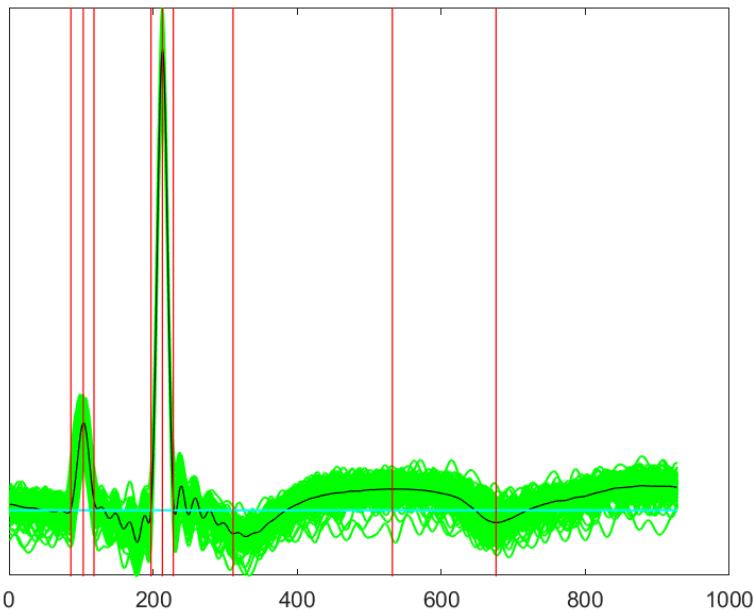


Figure 4: Final average trace, with confirmed markers



Continue Trace Analysis

To continue analysis within the same directory, simply 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! Use the save/load feature to save the trace analysis and reload it at a later time (described in the [New Features](#) section).

Peak Noise-Remover

For particularly noisy traces (i.e., traces with a substantial number of artifacts), the user can elect to use the noise-remover to more efficiently identify P/QRS pairings. For example, in the below trace, due to the variable wave amplitude, the height threshold selection causes a substantial number of yellow markers to be added.

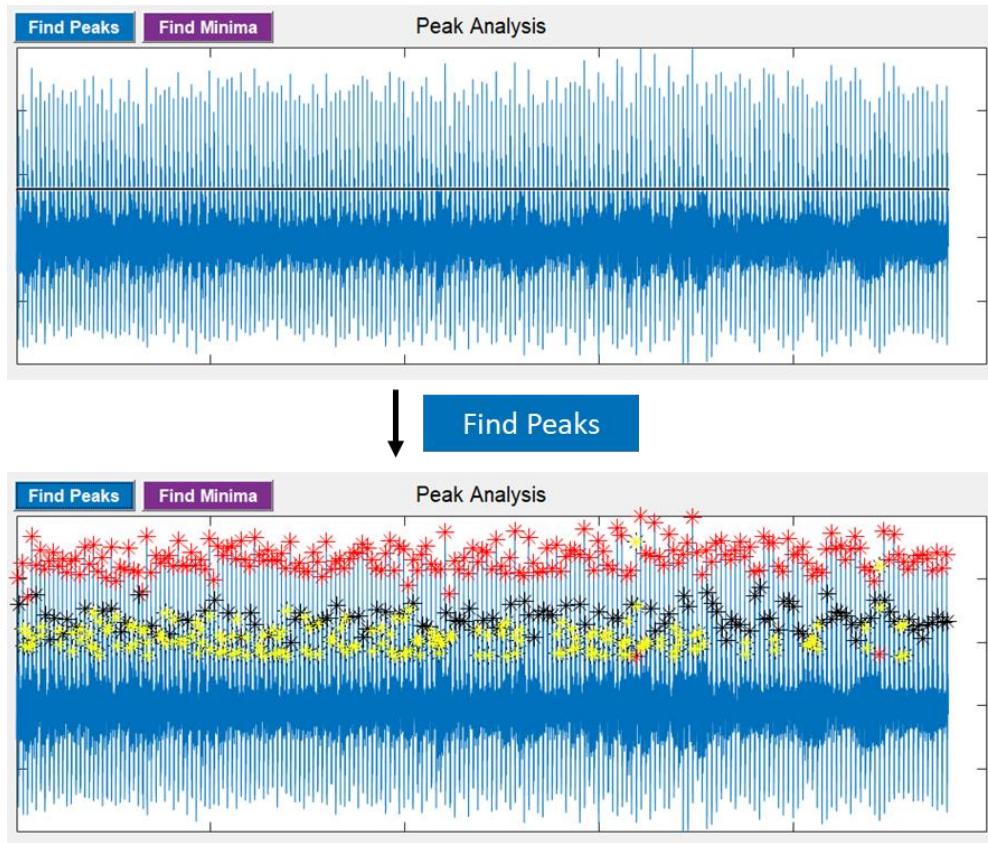


Figure 10: Marker identification after clicking Find Peaks

If there are only a few misplaced or added peaks, manual editing is recommended. Alternatively, the user can use a larger **Minimum Peak Distance**. In cases where a substantial number of edits need to be made, the noise-remover could decrease editing time by performing semi-automatic P/QRS identification.

To use the noise-remover:

1. Select **Remove Noise**. All already identified P waves and R waves are re-colored **cyan**, and minima (if present) are removed from the plot (they will be added back later).
2. Prompts then appear in a mix of pop-ups and the command window, depending on the pop-up frequency selected by the user. The first prompt will first ask the user: between the P and R wave, is there a pattern such that one wave amplitude exceeds

the other wave's amplitude for the entire duration of the trace to be analyzed? The answer is crucial to the method of wave identification that will be used.

- If YES: the Y-coordinate of the lowest P and R wave peaks will be used as thresholds to identify the rest of the P and R waves.
- If NO: single P/QRS pairings will be identified by the user before zERG uses these initial identifications to perform fill in the pairings present in the rest of the trace semi-automatically

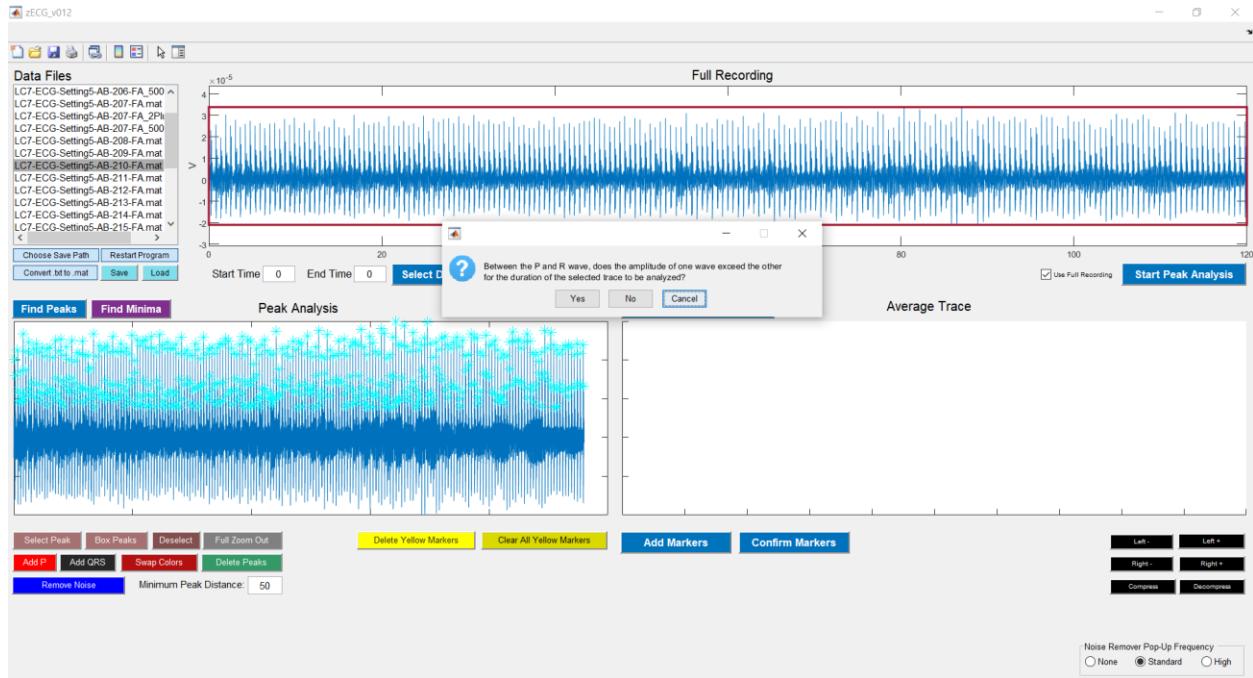
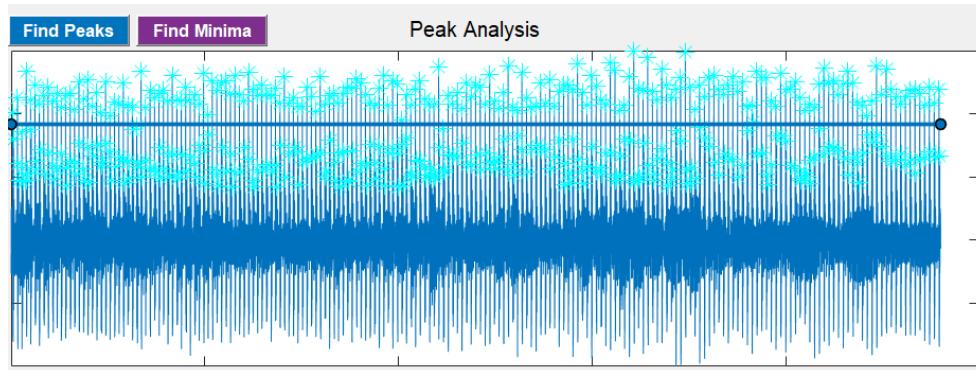


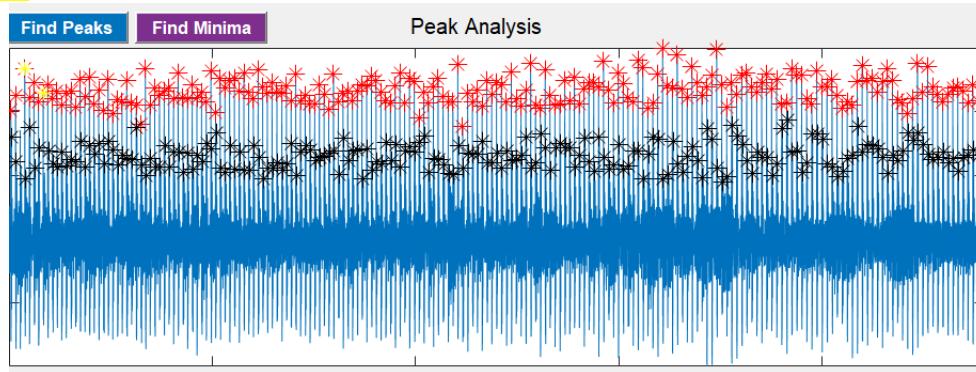
Figure 11: First prompt that appears after selecting Remove Noise

Route A: If the User Answered YES to the First Prompt

- Select which wave has the greater amplitude
- Then, select the height of the wave answered in the above step with the lowest amplitude. The screenshot below indicates the height (blue line) that should be selected for the above trace.



5. Select the first few waves in the trace. Directions in the console or pop-ups will indicate as to which type of wave (P or R) the user should be selecting.
6. Once all of the P or R waves have been identified, the user is asked if they would like to edit the corresponding wave (please see [noise-remover editing section](#)).
7. If no edits need to be made, step 5 then occurs for the wave with the lower amplitude.
8. After all waves are marked, the user is asked a final time if additional waves or markers should be added.
9. If not, the user can elect to finish the noise-remover. Note that all **cyan markers** disappear (but will reappear if the user needs to repeat the noise-remover; **yellow markers** remain).



Route B: If the User Answered NO to the First Prompt

3. Select the first few waves in the trace. Directions in the console or pop-ups will indicate as to which type of wave (P or R) the user should be selecting.
- zERG** relies heavily on these first few wave selections, so if they are incredibly unrepresentative (say, an RR gap triple the average), then the user should select later wave pairings as the initial waves, and return to editing the beginning of the trace once the initial run of the noise remover is complete (the user has the option to edit selections while in the noise-remover function). As the user selects these waves, **zERG** recolors the cyan points appropriately.
4. Once **zERG** has enough information (usually after the first two to four waves), it automates the process, filling in P and R waves red and black while filtering out noise.
 5. After all waves are marked, the user is asked if additional waves or markers should be added (please see below for instructions on how to edit within the noise-remover).
 6. If no edits need to be made, the user can elect to finish the noise-remover. Note that all **cyan markers** disappear (but will reappear if the user needs to repeat the noise-remover); **yellow markers** remain.

For both route A and B, the noise remover leaves the cyan markers on the points that it suspects of being noise until it is finished, so that if the noise remover made a mistake, the user can easily fix it when the option to edit selections appears. Additionally, the noise remover will occasionally deposit a yellow marker on a wave it suspects of having mislabeled.

Sometimes, zERG will not have enough information to continue automatically with the process, and will ask the user to step in until it feels confident enough to continue without the user's help. This will happen more frequently on noisier traces. If the user is stepping in very frequently, then, as suggested above, it may be worthwhile for the user to try starting over with peaks selected using a larger Minimum Peak Distance, as this itself can often be an efficient way to filter out noise.

However, the trace is sometimes just too noisy, and can only be filtered effectively with frequent assistance from the user. Importantly, if the user is asked to step in and notices that certain waves earlier were marked as noise, the user should not go back and select these when prompted to select P or R waves; the user should only be selecting waves to the right of the rightmost filled-in P/R wave pair. Missed waves can be colored appropriately in the editing step.

Editing During Peak Noise-Remover

Wave marker additions, normally a tedious task, can be done easily using the cyan markers which zERG placed on the graph earlier during the noise-removal process. The user can make edits as prompted until the user tells the program that editing is finished.

At after all editing is complete, the noise removal is complete, and all cyan markers are removed. Red, black, and yellow markers remain, while minima (if selected) are added back to the plot; all cyan markers are removed. When editing is done halfway through, noise removal simply continues.

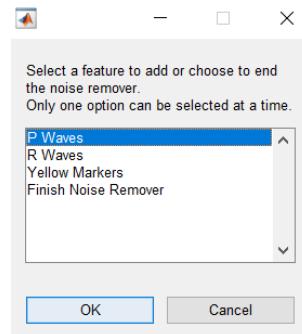
Route A Editing

1. After adding all P waves, the user is asked if they would like to edit the P waves.
 - a. Selecting No will move to R wave identification and subsequent editing
2. Select Yes to edit the P waves. Currently, only P waves already labeled may be added.
3. Enter the number of P waves to be added (currently, this ranges from 1-5).
4. Select the appropriate number of P waves to add.
5. Continue to add P waves until all desired P waves are added.
6. Proceed through R wave identification.

7. After all R waves are identified, the following prompt will appear



8. Selecting Yes will trigger a 2nd prompt to appear, allowing the user to select which feature they would like to add.

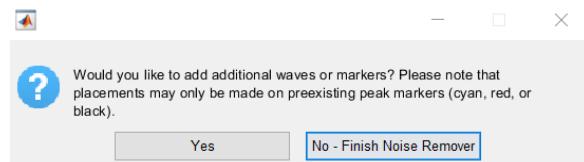


9. After selecting the feature, follow steps 4-6 to add that feature.

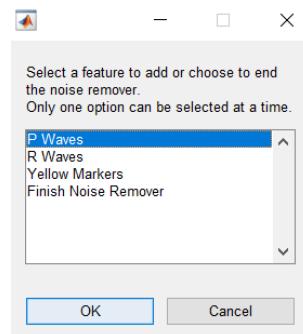
10. When all features have been added, select No – Finish Noise Remover to end the noise remover function.

Route B Editing

1. After all P and R waves are identified, the following prompt will appear



2. Selecting Yes will trigger a 2nd prompt to appear, allowing the user to select which feature they would like to add.



3. After selecting the feature, follow steps 4-6 under the [Route A Editing Section](#).
4. When all features have been added, select No - Finish Noise Remover to end the noise remover function.

Trace Analysis Using Minima

Standard trace analysis may not be appropriate for traces with unusual wave form. For example, using the trace analysis protocol for the trace below (without manual editing) would result in an incorrectly aligned average trace.

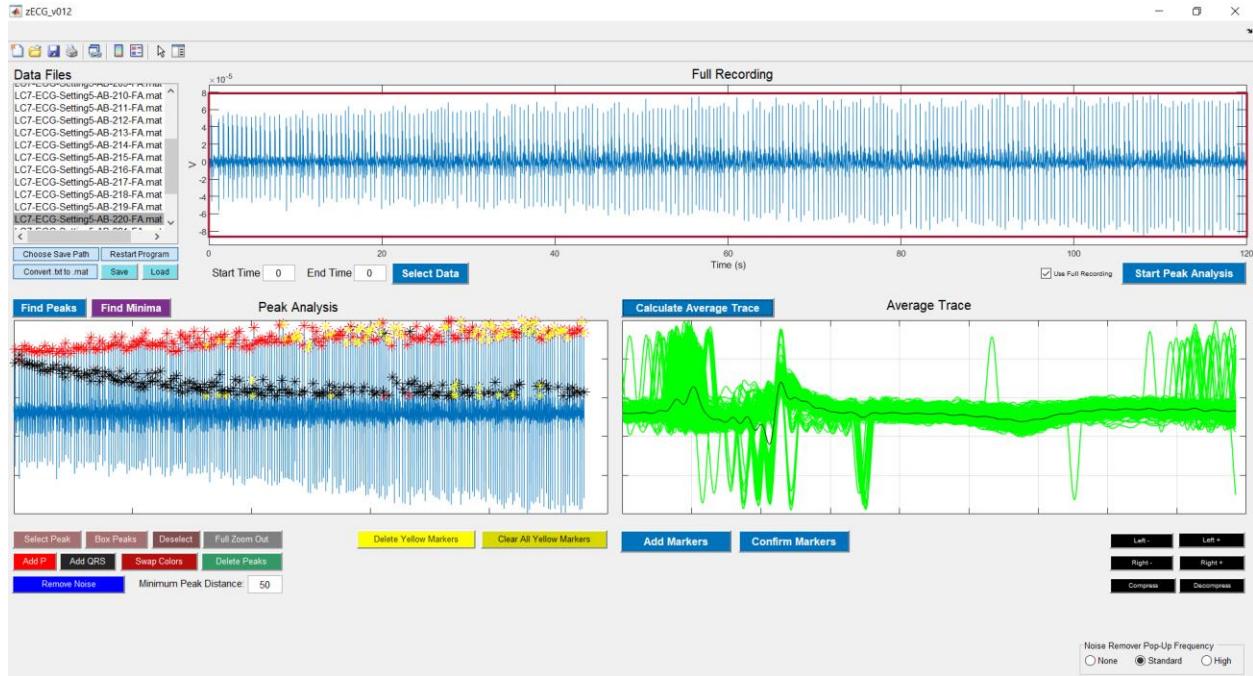


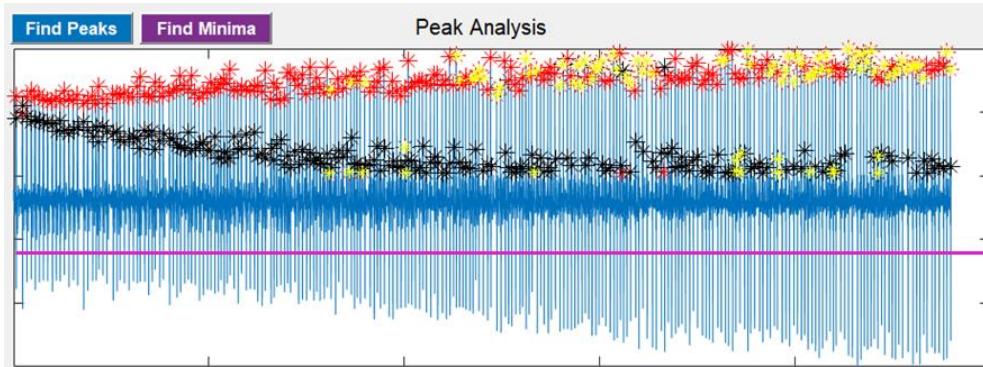
Figure 12: Improper average trace generation after using Find Peaks

To properly align this trace, the user could manually proceed with peak editing to align by the QRS. Another option is to use the minima trace analysis functions, which will find minima within the ECG trace and then align each trace cycle using the minima. The minima are used for alignment and RR interval and heart rate calculations, as the black QRS markers may not necessarily be present in the [Peak Analysis](#) steps. The red P wave markers are obsolete in this scenario as well.

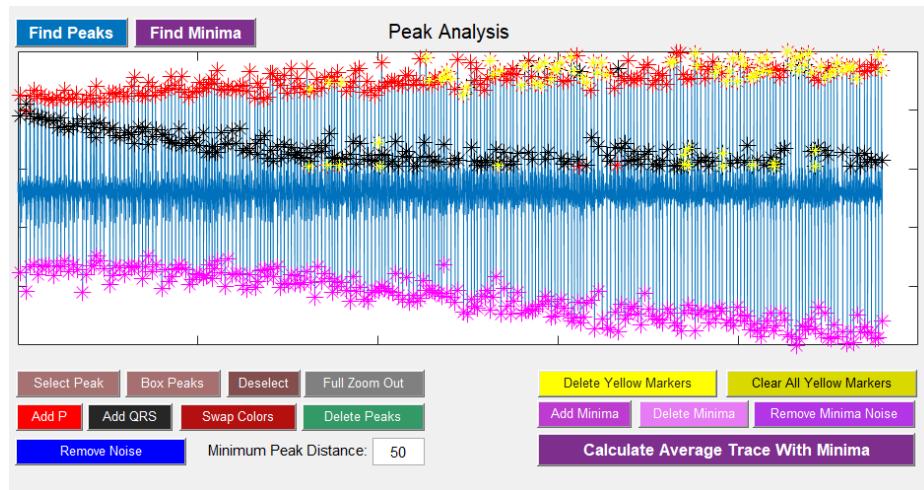
To align a trace by minima:

1. Proceed with [Peak Analysis](#) such that red and black markers are noted on the trace (it is fine for them to be incorrectly placed).
2. Select [Find Minima](#). A prompt will appear (either in the console or in a pop-up, depending on the pop-up frequency selected by the user) asking for the user to Select

the height of the highest minima. The user should select the point such that all minima fall below the Y-coordinate of the point they selected. The screenshot below shows the line along which the user should have picked a point to satisfy the criteria of the prompt.



- Once the point is selected, all minima below that point will be marked with a magenta marker (*). Peak editing and average trace alignment functions involving the minima will now be visible.



- To generate an average trace aligned by the minima, select **Calculate Average Trace with Minima**. The screenshot below shows the new and properly aligned average trace. Markers can then be added using the same protocol as previously described.



Figure 13: Proper average trace generation after using Find Minima

Minima Editing

Similar to peak editing, minima can be added or deleted.

To add minima, use the [Select Peak](#) button to select the point where the minima should be added then click [Add Minima](#). To delete minima, use the [Select Peak](#) button to select the marked minima (*) to be deleted then click [Delete Minima](#).

Compatibility with Peak Functions

Although they are named as [Select Peak](#) and [Box Peaks](#), these functions are compatible with the minima features. [Deselect](#) and [Full Zoom Out](#) are also compatible, as well as changing the [Minimum Peak Distance](#). All zoom functions in the [MATLAB toolbar](#) are compatible, with the same exceptions (i.e., [Reset View](#) cannot be used after editing minima). The two yellow markers related functions are also compatible; note that [Clear All Yellow Markers](#) will clear all yellow markers created during both peak trace analysis and minima trace analysis.

Average trace alignment by P/QRS pairings and minima at the same time is not possible. Alignment can be toggled between the two, however. For example, if the user finished identifying minima and performed the average trace calculation with the minima but would now like to revert to alignment by the P/QRS pairings, the user should finish the peak editing and then select [Calculate Average Trace](#) as the standard zERG protocol.

Minima Noise-Remover

This functions similarly to the peak noise-remover previously described. It can be used to more efficiently isolate minima, particularly in traces with a substantial amount of noise and artifacts or when there are minima of different height that require the placement of the line to be higher (causing multiple minima to be picked, like the example below).

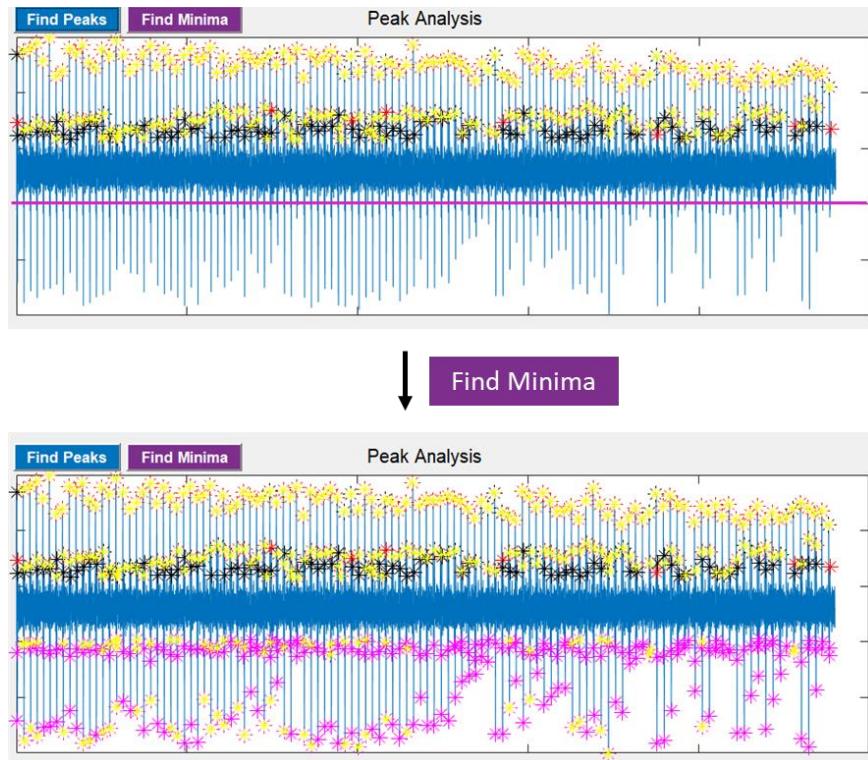


Figure 14: Minima identification after clicking Find Minima

If there are only a few misplaced or added minima, manual editing is recommended. Alternatively, the user can use a larger **Minimum Peak Distance**; often, a much larger **Minimum Peak Distance** is needed to find minima effectively than it is needed to find peaks effectively.

In cases where a substantial number of edits need to be made, the noise-remover could decrease editing time by performing semi-automatic minima identification. To use the minima noise-remover:

1. Find minima then click **Remove Minima Noise**. All potential minima will then be noted with a cyan marker (*). All identified P and R waves (if present in the trace) are removed from the plot but will be added back once noise-remover is finished.
2. Select the first minima in the trace. The minima selected will turn magenta (*).
3. Then, select the second minima. The minima selected will turn magenta (*).

4. Follow the prompts (either as pop-ups or in the terminal) and continue to select minima until all minima in the trace has been selected, using the zooming functions to more accurately select the minima, and/or has been automatically selected by zERG. Note that if the view was zoomed in before the last minima is picked, the view will be automatically zoomed back out after the last minimum is picked.
5. A prompt will appear asking if the user would like to edit any minima. To end the noise-remover function without making additional edits, select No - Finish Noise Remover. Select Yes if additional minima need to be added. If the user chooses to end the noise-remover, note that all **cyan markers** disappear (but will reappear if the user needs to repeat the noise-remover); **yellow markers** remain.

Editing During Minima Noise-Remover

While using the noise-remover, any minimum that fall outside the parameters in which a minimum has been defined to be found in (i.e., smaller interval between minima calculated, etc.) will be marked with a **yellow marker** for user review and editing.

It is important that the user does not select a minimum that is behind the point in the trace where the noise-remover is currently attempting to find minima, similar to the peak noise-remover limitation. Doing so will cause the function to operate incorrectly.

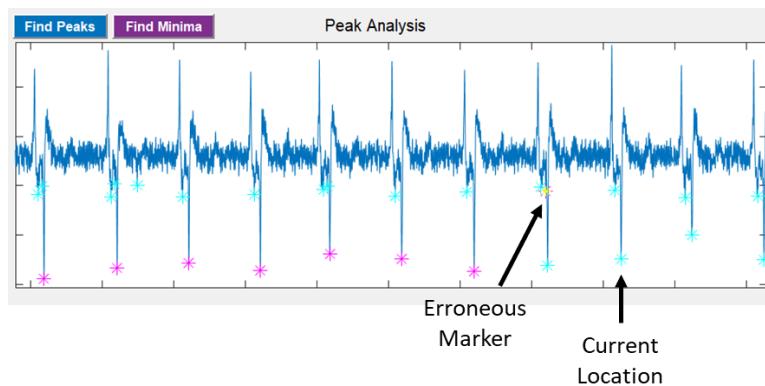
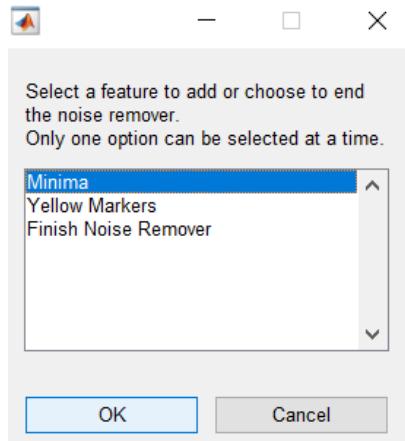


Figure 15: Users should proceed forward to the minimum at the current location during the noise-remover process and not edit the erroneous marker

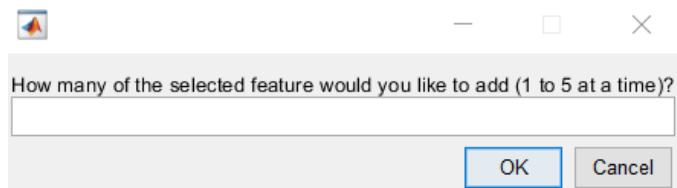
Missed minima can be added after the noise-remover finishes. **Only possible minima that have been identified by the noise-remover (those marked cyan) can be added.** If minima outside of this pool need to be added, use the minima editing functions described in the Trace Analysis Using Minima section.

To begin adding minima, select Yes at the prompt listed above in step 5.

1. A prompt will appear listing the features that can be edited. Select the appropriate feature to edit or select Finish Noise Remover to end the operation. Currently, only adding a feature is supported.



2. Only 1-5 markers can be edited at an individual time. Enter the number of markers to be edited.



3. Select the point(s) at which the selected feature will be added. After the appropriate number of minima are added, the prompt in Step 1 will appear again.

Note that the addition of a **yellow marker** to an already selected/**magenta** minima does not remove the **magenta marker**; the yellow marker is overlaid on the magenta marker. Additionally, the yellow marker can only be added to existing **magenta markers** (and not the light blue markers).

4. Continue to add the selected feature as needed. Once complete, select Finish Noise Remover to end the process. All **cyan markers** will disappear; only **yellow** and the **magenta** selected minima markers remain (in addition to any **red/black/yellow** markers that were in place before the start of the minima noise-remover).
5. If additional edits need to be made, use the [editing minima functions](#).

New Features

Beginning in [zERG](#) 1.2, additional features have been added. They are described below.

Converting Data into MATLAB Format

In cases where users only have the ECG traces in the form of a .txt file, this function allows the .txt file to be converted and stored into the .mat format, which can be used in [zERG](#) for data analysis. This is not required and is more of a convenience for the user to be able to store data in multiple formats.

The user must input the sample and tick rate for the trace. These values are important in calculating the interval measures.

The new .mat file is named using the same naming as the .txt file but a designation is made to indicate that the file was converted from .txt (i.e., *_fromtxt.mat). The function checks for the existence of files with the same naming as the to-be-saved .mat file and warns the user about overwriting files. For example, if there exists a .mat file named *ABC.mat* within the *DEF* directory and the user is trying to convert *ABC.txt* to *ABC_fromtxt.mat*, the function will warn the user that *ABC.mat* exists.

Note that this check only occurs in the directory where the traces listed in [Data Files](#) are stored. The check does not occur in the directory that has been designated as the save path.

Saving Progress

Users can save their trace analysis process. The data is stored as a .mat file (file extension: *.ss.mat) and can be loaded back into [zERG](#) at a later time for further analysis.

After clicking [Save](#), users provide a filename. Saving the file in a different directory (i.e., by changing the save path) is not compatible with this [Save](#) function. The function requires the original ECG trace in order to appropriately reload all data. Similar to the conversion function, this function checks for the existence of files with the same naming as the to-be-saved .mat file and warns the user about overwriting files.

Loading Save States

Users can load the save states into [zERG](#) to continue with their trace analysis. Upon clicking [Load](#), users are prompted to select the directory where the save state and the original ECG trace are located. A prompt will appear listing all the save states within the selected directory. Select the save state to be loaded. All data at the point of saving will be reloaded, with the exception of unconfirmed average trace markers. If ECG trait values have been calculated, the values will reload in the results table.

Within the [Data Files](#) box, the name of the loaded save stage will be that of the original ECG trace. For example, the screenshot below illustrates the save state in which the analysis

process for trace LC7-ECG-Setting5-D2-AB-106.mat was saved in, and the name of the save state file (D2-AB-106_Initial.ss.mat).

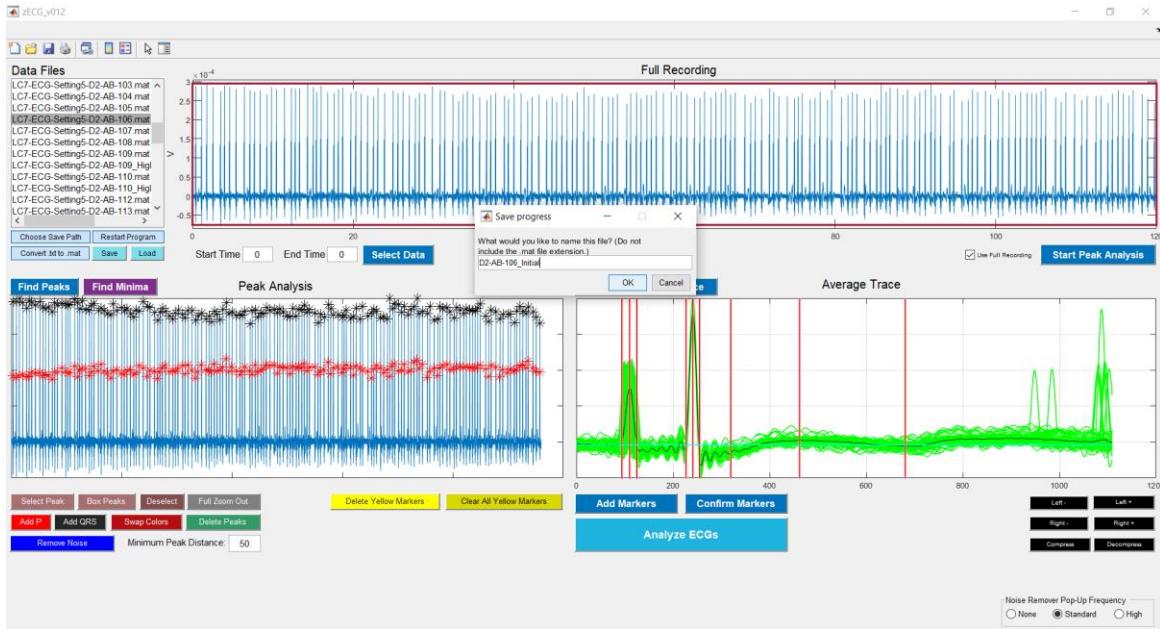


Figure 16: Prompt that appears after Save is clicked, asking the user for to name the save state

In the image below, analysis has proceeded to another trace (highlighted in the Data Files box). We seek to load the trace analysis progress for trace LC7-ECG-Setting5-D2-AB-106.mat as shown above.

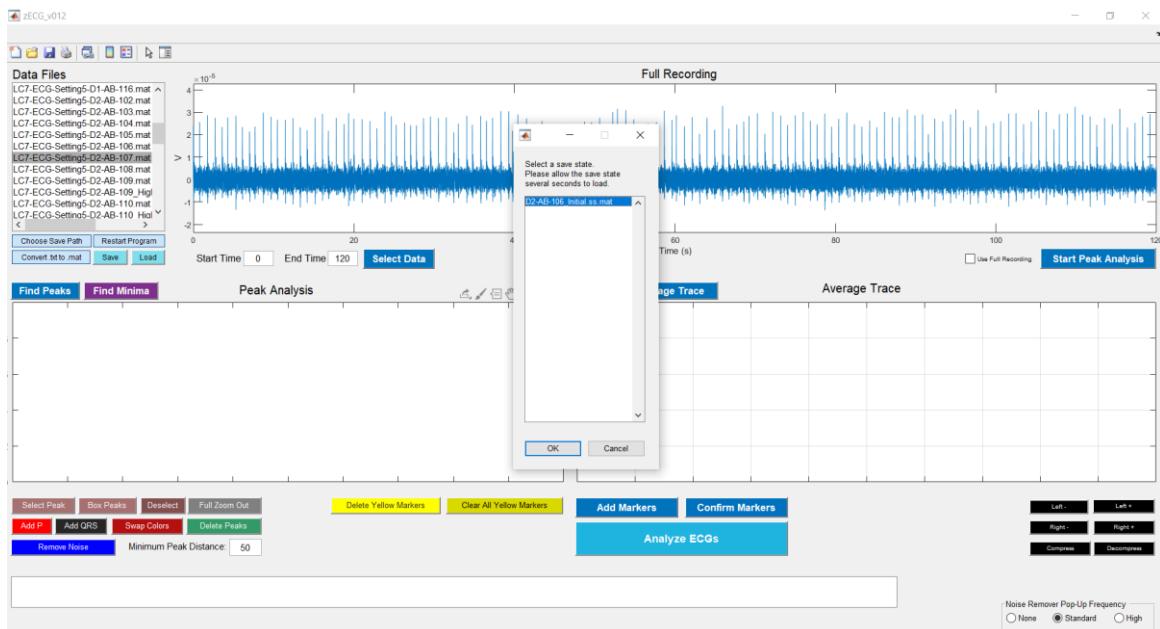
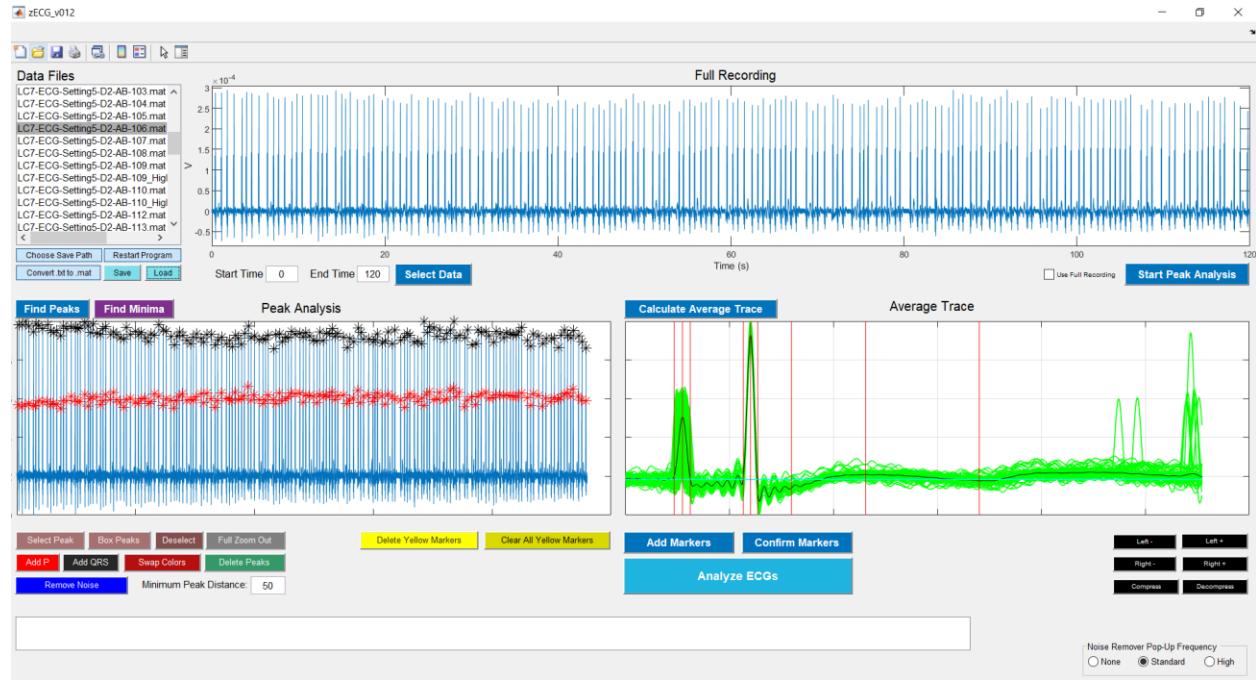


Figure 17: Prompt that appears after Load is clicked, and a folder has been selected. All .ss.mat files within the folder are shown in the pop-up.

After selecting OK, the zERG GUI returns to the save state within the D2-AB-106.ss.mat file. Note that the trace LC7-ECG-Setting5-D2-AB-106.mat is now highlighted in the Data Files box.



Noise Remover Pop-Up Frequency

To adapt to user proficiency level, this was added to allow user to change how prompts are given within the two noise-remover functions. Regardless of the pop-up frequency, the prompt will be the same. If None is selected, all prompts appear in the MATLAB console and users must respond by typing their responses. If Standard is selected, a mix of pop-ups and console prompts will appear. If High is selected, only pop-ups will appear.

We recommend new users to zERG to select High and then to change the frequency as they become more proficient with the program.

Tips & Troubleshooting Guide

Below are some common issues the user may run into, and how to fix them.

Issue	Suggested Solution(s)
GUI unable to start	Ensure that both the .m and .fig files are in the same folder.
Too many peaks or minima marked	The user should raise the Minimum Peak Distance . If this is insufficient, or removes too many peaks, the user should try using the noise remover.
Difficulty adding individual peaks or minima	The algorithm for adding peaks requires much more precision than the one for removing; additionally, only closeness to the peak along the x-axis is factored in. So, the user should try zooming in further along the x-axis.
Noise remover or minima noise remover ineffective*	If Minimum Peak Distance is already optimized, the user will have to change the RemoveNoiseButton_Callback function in the MATLAB code. Specifically, tweaking any of the constants and constant multipliers (variables named in all caps) may help the user. But changes should be saved to a new .mat file, as the default values are well calibrated for moderately messy traces. A basic understanding of how the noise remover operates will be necessary to change these values effectively.
Abnormal average trace	The user should ensure all QRS peaks (or minima, depending on which version of the average trace is in use) are labelled correctly. If the average trace is still shaped abnormally, the user should experiment with expanding/contracting and compressing/decompressing the window in several ways.
Markers not appearing after Add Markers button pressed*	The user should experiment with different window sizes, with the default compression. If this is still not working, there may be an issue with the default bounds of marker placement. Given a basic understanding of how these markers are added by the MATLAB code, the user can find and edit these variables in the FindAvgPeaksbutton_Callback function.
Weird values after ECG analyzed	Since several of the markers that the user places are identical, it is very easy for the user to mix up wave start, peak, and end markers. The user should add these markers again more carefully, ensuring that they remain in the appropriate order (the one in which they started).
Function frozen or function is working in an unexpected way	Navigate to the console and type CTRL + C to cancel any commands or restart the program

*Advanced troubleshooting (changing the MATLAB code) may be required

ECG Calculations

After the user finalizes the average trace markers, the **Analyze ECGs** button appears. Upon pressing this button, the following calculations are performed and returned to the user:

- **RR Interval (ms)**: calculated based on the average gap between QRS waves. If the average trace is based on the minima, the RR interval is calculated from the average gap between minima.
- **Heartrate (bpm)**: 60 divided by the average RR interval, multiplied by 1000.
- **PR Interval (ms)**: calculated based on the gap between the start of the P wave and the start of the QRS wave marked in the average trace.
- **QRS Interval (ms)**: calculated based on the gap between the start of the QRS wave and the end of the QRS wave marked in the average trace.
- **QT Interval (ms)**: calculated based on the gap between the start of the QRS wave and the end of the T wave marked in the average trace.
- **P Amplitude (mV)**: calculated based on the height difference between the peak of the P wave and the isoelectric line in the average trace.
- **Q Amplitude (mV)**: calculated based on the height difference between the start of the QRS wave and the isoelectric line in the average trace.
- **R Amplitude (mV)**: calculated based on the height difference between the peak of the QRS wave and the isoelectric line in the average trace.
- **S Amplitude (mV)**: calculated based on the height difference between the end of the QRS wave and the isoelectric line in the average trace.
- **T Amplitude (mV)**: calculated based on the height difference between the peak of the T wave and the isoelectric line in the average trace.

Glossary

Term	Category	Explanation
*.ss.mat	Output file	Extension for save state file
*_fromtxt.mat	Output file	Extension for .txt files converted to .mat
Add Markers	Button	Adds markers to the average trace
Add Minima	Button	Adds a minimum to the nearest peak near the selected point
Add P	Button	Adds a P wave marker to the nearest peak near the selected point
Add QRS	Button	Adds a QRS wave marker to the nearest peak near the selected point
Align By Minima	Feature/Concept	To align the average trace by minima, use Find Minima then the Calculate Average Trace with Minima button
Analyze ECGs	Button	Given correct markers on the average trace, completes ECG analysis
Average Trace	Plot	A plot of the compiled ECG cycles within the entire trace
Black Markers	Marker	Indicates the peak of a QRS wave
Box Peaks	Button	Allows the user to select a region of peaks, minima, or markers
Calculate Average Trace	Button	Plots the Average Trace on the Average Trace plot
Choose Save Path	Button	Allows the user to select a folder to which to direct the output (default is the current folder where traces are stored)
Clear All Yellow Markers	Button	Clears all yellow markers
Compress	Button	Compresses the average trace

Term	Category	Explanation
Confirm Markers	Button	Locks in the current marker locations; unlocks the Analyze ECGs button
Convert .txt to .mat	Button	Convert imported .txt traces to .mat format
Cyan Markers	Marker	Marks the pool of P waves, R waves or minima within the noise-remover functions
Data Files	Drop-down menu	The user selects the file to analyze from this menu
Decompress	Button	Decompresses the average trace
Delete Minima	Button	Deletes the minima nearest to the selected points, or all minima in the boxed region
Delete Peaks	Button	Deletes the peaks nearest to the selected points, or all peaks in the boxed region
Delete Yellow Markers	Button	Deletes the yellow markers nearest to the selected points, or all yellow markers in the boxed region
Deselect	Button	Deselects any selected peaks
End Time	Textbox	Specifies the desired end time from the Full Recording plot to be used in the peak analysis plot
Figure 1	Results Figure	Overall average trace without the compiled traces, confirmed wave markers present
Figure 2	Results Figure	Average trace as observed within zERG after selecting Calculate Average Trace
Figure 3	Results Figure	Peak Analysis plot of the selected data for analysis, P waves and R waves are labeled
Figure 4	Results Figure	Final average trace, with confirmed markers
Find Minima	Button	Find minima in the ECG for average trace alignment by the minimum
Find Peaks	Button	Finds P/QRS pairings in the ECG trace
Full Recording	Plot	Plots the entire recording in the file

Term	Category	Explanation
Full Zoom Out	Button	Zooms out the Peak Analysis plot fully
Heart Rate	Measurement	Calculated from the average RR interval
Home View	Feature/Concept	Furthest zoomed out view in Peak Analysis
Isoelectric Line	Feature/Concept	Median of all points before the start of the QRS complex
Left -	Button	Shrink the left window of the Average Trace plot
Left +	Button	Increase the left window of the Average Trace plot
Load	Button	Load the save state files
MATLAB toolbar	Button	Toolbar appended to each plot, with zoom functions, etc.
Minimum Peak Distance	Textbox	Peaks and Minima found automatically in the Peak Analysis plot must have at least this amount of space between them.
Noise-remover pop-up frequency	Button	Changes the frequency at which prompts appear in the console or as pop-ups
Number of Traces	Measurement	Number of individual P-QRS cycles measured in the trace
P Amplitude	Measurement	Amplitude of the P wave, measured from the average trace
Peak Analysis	Plot	Allows for analysis of the region selected from the Full Recording plot
Magenta Markers	Marker	Indicates minima
PR Interval	Measurement	Interval from the start of the P wave to the start of the QRS, measured from the average trace
Q Amplitude	Measurement	Amplitude of the Q wave, calculated from the average trace
QRS Interval	Measurement	Interval from the Q wave to the S wave, measured from the average trace
QT Interval	Measurement	Interval from the Q wave to the end of the T wave, measured from the average trace

Term	Category	Explanation
R Amplitude	Measurement	Amplitude of the R wave, calculated from the average trace
Red Markers	Marker	Indicates the peak of a P wave
Remove Minima Noise	Button	A minima noise remover for traces with too many false minima marked
Remove Noise	Button	A noise remover for traces with too many false peaks marked
Restart Program	Button	Restarts zERG
Right -	Button	Shrink the right window of the Average Trace plot
Right +	Button	Increase the right window of the Average Trace plot
RR Interval	Measurement	Average of the interval between consecutive R waves
S Amplitude	Measurement	Amplitude of the S wave, calculated from the average trace
Save	Button	Save the trace analysis progress as a *.ss.mat file
Select Data	Button	Selects the data specified in the Start and End textboxes from the full recording plot
Select Peak	Button	Allows for selection of a point in the Peak Analysis plot
Start Peak Analysis	Button	Renders the initial Peak Analysis plot based on the selected region in the full recording plot; adds a moveable lower bound for peaks
Start Time	Textbox	Specifies the desired start time from the Full Recording plot to be used in the peak analysis plot
Swap Colors	Button	Swaps the colors of the peaks nearest to the selected points, or all peaks in the boxed region
T Amplitude	Measurement	Amplitude of the T wave; calculated based on the average trace
Use Full Recording	Checkbox	Selects the entire recording from the Full Recording plot to analyze