hiPSC Arrhythmia Detection Shiny App Manual

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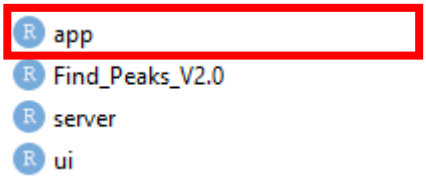
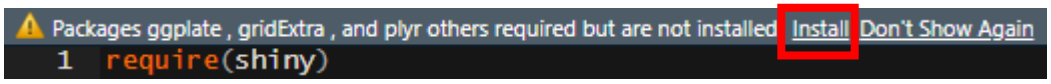
Running the Shiny App

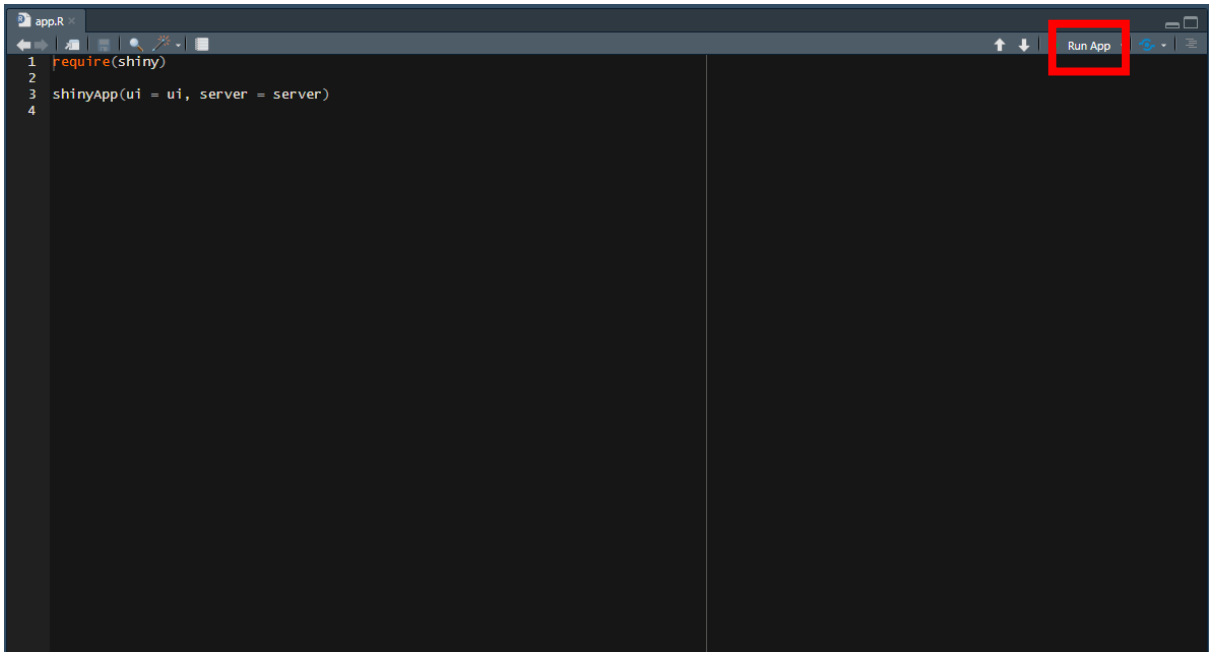
There are 4 essential R files you need in the desired folder before running the Shiny App which can be downloaded from GitHub/Ben.



Open the app R file.



Most likely the library packages will need to be installed, this is only needed for the first time the script is open. If this option appears click the “Instal” button highlighted to install required library packages.

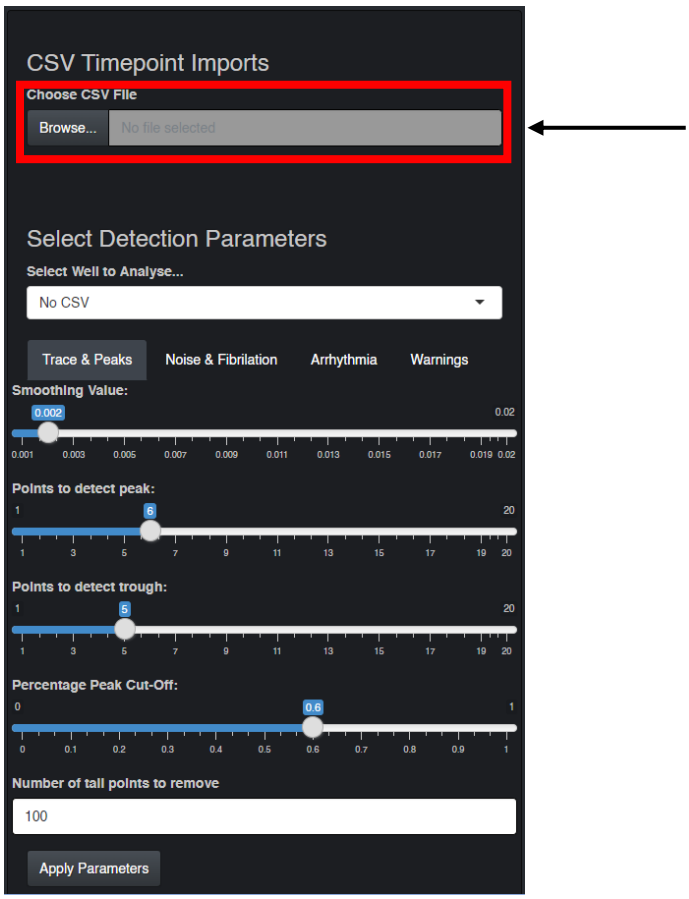


Once installed, click ‘Run App’ as highlighted above to open the app

Walkthrough Step by Step

1) Open the Shiny App as guided in 'Running the Shiny App' section.
2) Import the desired CSV to analyse.
   a) Note make sure the CSV follows the correct layout as described in CSV Import
   b) Wait for the Importing Datasets… bar to finish
3) Select a well to examine and click 'Apply Parameters'.
   a) Ideally choose a well with a good trace to set initial parameters against
4) Click on the 'Trace' tab that has appeared to view the trace.
5) Set the parameters as you want to fit the experiment best, re-clicking "Apply Parameters" after each adjustment and analysing several traces till content with settings.
   a) Parameter detailed guide can be found here.
6) Go to the 'Full Analysis' tab
7) Click 'Perform Full Analysis of CSV' button.
   a) Depending on the size of the CSV this may take a while but is tracked in real time
   b) Don't change parameters or click anything else while this is running.
8) When analysis is complete a table will appear with the summary of the results
9) You can then either…
   a) Rename results and click "Export Results"
   b) Or Import a plate plan, then rename Results and click "Export Results"
10) The results are exported as the desired name immediately to your working directory.
11) Using the 'Plate View' tab you can see what the Wells we scored (currently only 384 well)
   a) This most likely wont work if the samples in the Wells column have non-standard names.
12) The parameters can always be changed and the Full Analysis reran with the new parameters
   a) **IMPORTANT NOTE**: Once you have imported the plate plan and clicked export results, any new rerun will automatically merge the plate plan and export as the chosen name. Overwriting the previous file, or crashing if that file is open.
   b) If you import a new CSV this does not occur, only when rerunning after clicking the "Export Results" button.

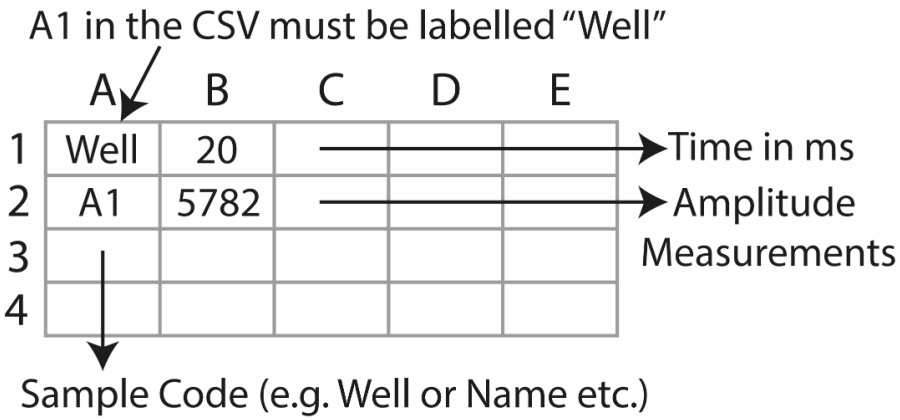
CSV Import



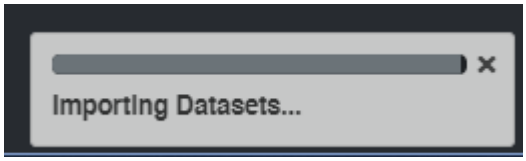
The initial screen upon opening the app only provides these above options.

To start the analysis first upload the CSV file by clicking the 'Browse…' button under 'Choose CSV File'.

The CSV you upload must have the below set up to be read correctly.

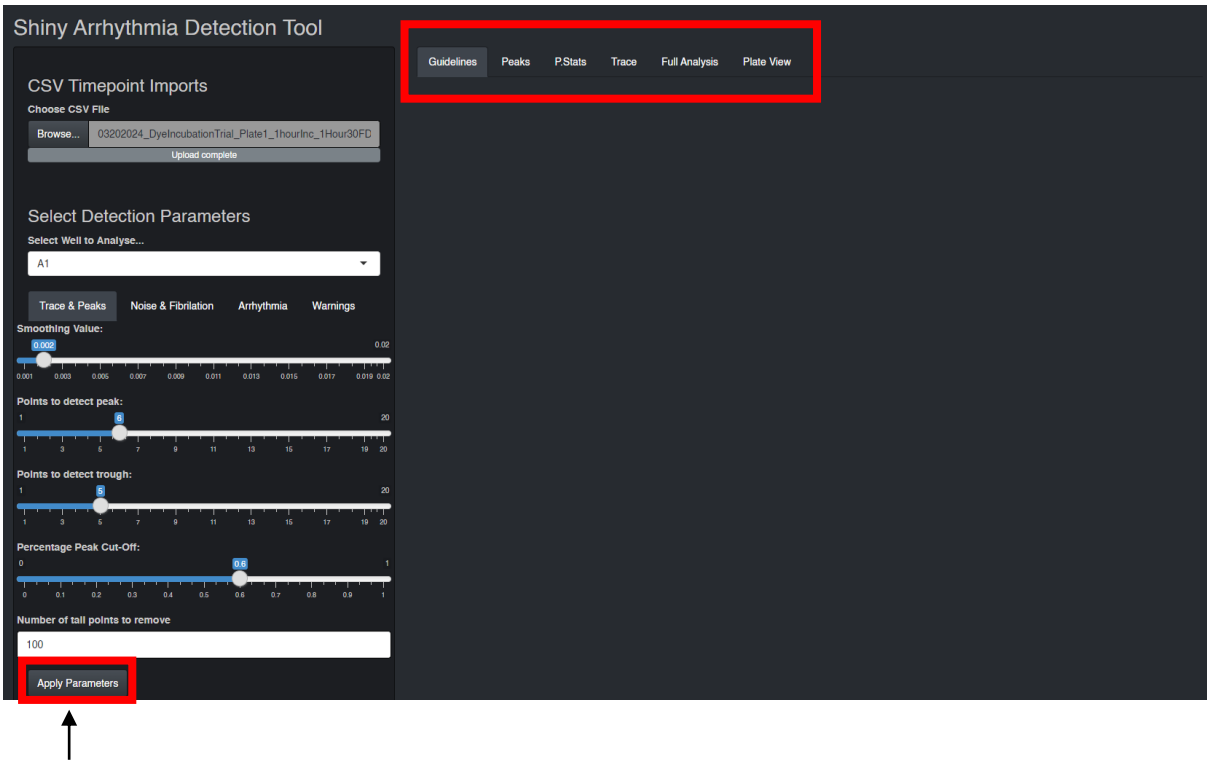


A box will appear in the bottom right of the screen when importing and gives your real time CSV importing %.



Note: Depending on the size of the CSV this could take up to a minute.

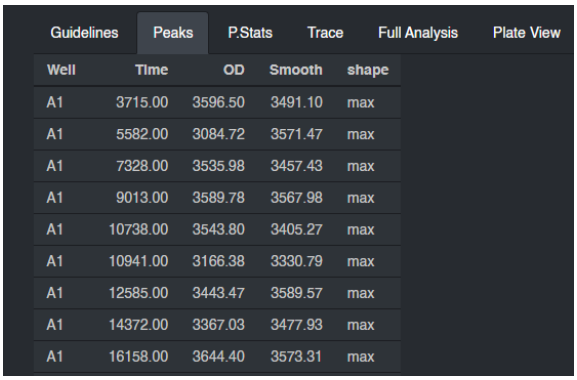
Once the CSV has been imported several new tabs will now be available.



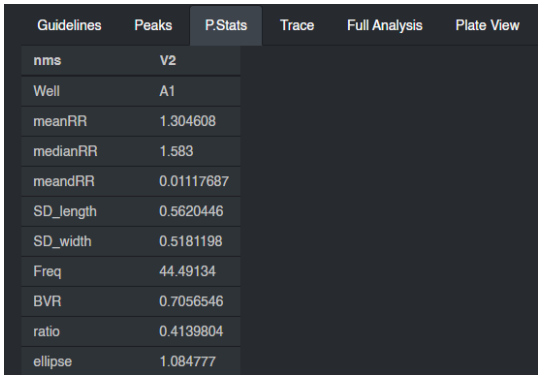
Although, these tabs will have no output until the “Apply Parameters” button is pressed.

When “Apply Parameters” is pressed, the Arrhythmia detection will run through the Well chosen in the drop down menu (e.g. A1) using the current selected parameters.

You can now view the Peaks and Troughs that are detected (max = peak, min = trough) in the “Peaks” tab, or the stats generated during the analysis of the trace in the “P.Stats” tab.



Well	Time	OD	Smooth	shape
A1	3715.00	3596.50	3491.10	max
A1	5582.00	3084.72	3571.47	max
A1	7328.00	3535.98	3457.43	max
A1	9013.00	3589.78	3567.98	max
A1	10738.00	3543.80	3405.27	max
A1	10941.00	3166.38	3330.79	max
A1	12585.00	3443.47	3589.57	max
A1	14372.00	3367.03	3477.93	max
A1	16158.00	3644.40	3573.31	max



nms	V2
Well	A1
meanRR	1.304608
medianRR	1.583
meandRR	0.01117687
SD_length	0.5620446
SD_width	0.5181198
Freq	44.49134
BVR	0.7056546
ratio	0.4139804
ellipse	1.084777

See more information on the parameter options below.

Understanding the Parameters

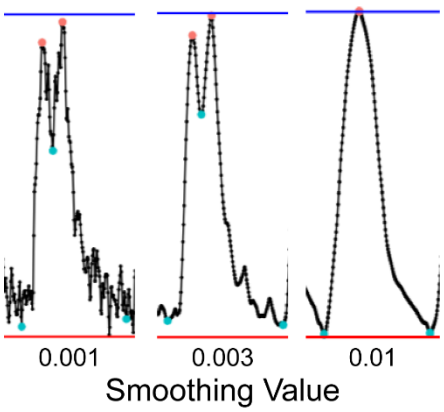
This arrhythmia detection tool is very dependent on the correct set-up to reliably offer accurate results. While adjusting these parameters be sure to analyse a clear trace and "Apply Parameters" often to monitor changes to your liking.

Trace & Peaks

Here I will break down the 'Trace & Peaks' tab. These parameters will determine the peaks and troughs that are found through your traces and are the essential first step to accurate arrhythmia detection.

Smoothing

Smoothing helps with removal of fluctuating baseline and highlights strong arrhythmias more clearly, this also allows for more accurate peak/trough detection and noise thresholding.



Although, too much smoothing can cover arrhythmias as can be seen in the image to the left.

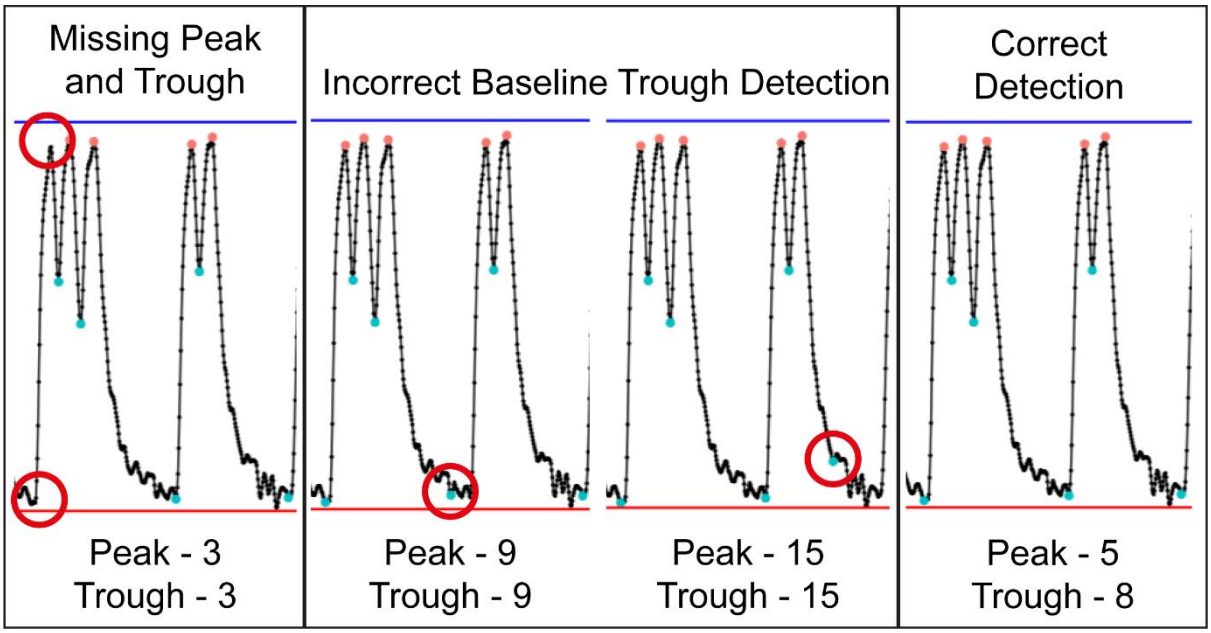
Smoothing 0.001 is pretty much the original amplitude readings. I usually use between 0.002 and 0.004 smoothing depending on the level of fluctuation and amplitude.

Nearing 0.01 smoothing will hide even strong clear arrhythmias.

Note: When changing smoothing and applying the graph refreshes.

Points to detect Peak & Trough

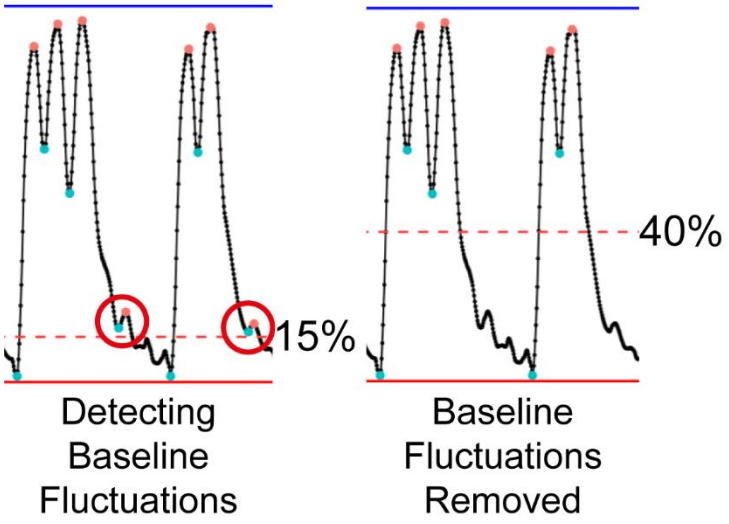
This parameter selects the highest (Peak) or lowest (Trough) point in the user selected range of points. If this is set incorrectly peaks/troughs will be missed, the baseline will be altered as seen in the figure below.



Often, I aim to find a trace where I see one of the above errors occurring, make small adjustments to the Peaks/Troughs to find optimal condition and then check with several other traces before running full analysis. Usually A higher trough and lower peak value is optimal.

Percentage Peak Cut-Off

This sets a threshold per trace where no Peaks can be detected below. This is essential for getting a clean baseline reading where the final point before the contraction is a trough, and reduces the incorrect reading of baseline fluctuations as peaks. This is also a visible toggle on the 'Trace' tab labelled "% Peak Cut Off" shown by an orange dashed line.

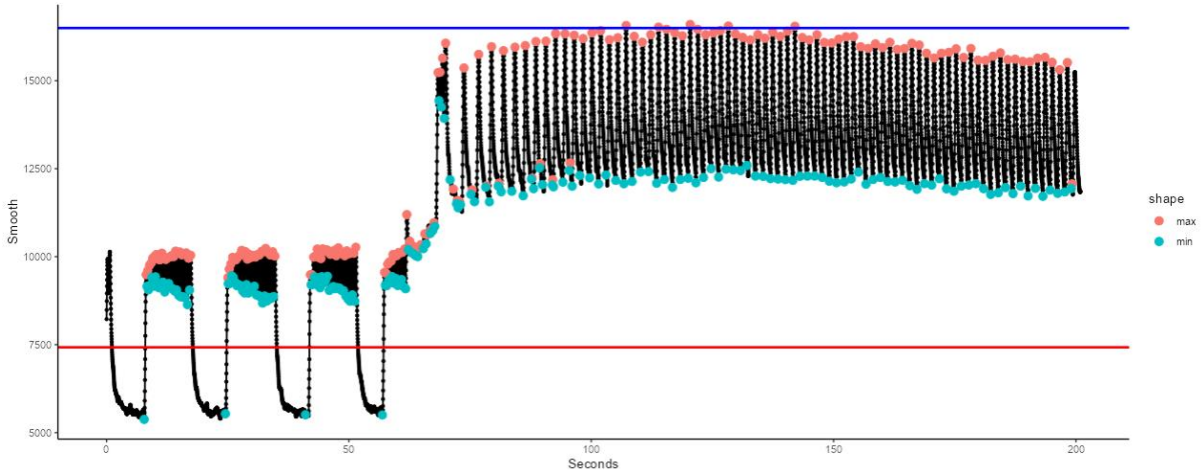


Adjust this threshold to be lower if you are intending on picking up smaller amplitude peaks.

Number of tail points to remove

This simply removes X number of points from the end of the trace before analysis. Sometimes traces have a weird drop-off or rise that will alter total baseline/trace height. I recommend trimming the traces slightly regardless to get a more accurate baseline.

This is also essential to do if you add a compound mid trace that causes a large change in the baseline level recorded (see image below).



Noise & Fibrillation

Noise Amplitude Cutoff

Here we set the Noise Amplitude Cutoff, this is the threshold amplitude a trace must pass to not be voided. **This value is also used for determining the minimum amplitude required to detect fibrillation and warning systems so is important you set this accurately for the experiment**.

A trace must exceed an Amplitude above the Noise value x 1.5 to be fibrillation

Any trace with an amplitude under Noise value x 1.5 is flagged as a warning.

E.g. The Noise value is set to 1000

- Any traces that have an amplitude below 1000 are VOID

- The trace must have an amplitude of >1500 to pass the first check in determining fibrillation

- If a trace has an amplitude between 1000 and 1500 a Warning is flagged TRUE

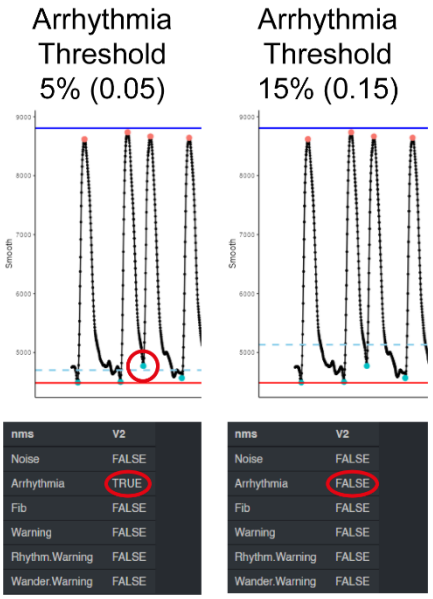
Fibrillation Peak to Peak detection

This parameter is simply set as if the user defined preference is less that the meanRR (see P.Stats for more info) the trace can be considered fibrillation. Since rapid arrhythmic contractions can be considered fibrillation there is a chance the trace can be considered as fibrillation when just arrhythmic. There is an extra step in the IF statement to reduce the chance of this and to stop rapid fibrillation traces flagging as arrhythmias due to changes in baseline. If you are not interested in tracking fibrillation you can set the fibrillation value low.

Arrhythmia

Flag Troughs Above This Threshold

This is the main detector for arrhythmias, and simply put, is there a trough found above %X of the trace amplitude. To visualise this, checking the 'Arrhythmia Threshold' box on the trace will show this threshold.



In the figure, the script will flag the trace as arrhythmic if the threshold is set to 5% detection, while not if set higher. This is a personal choice to how strict you wish to be for arrhythmia detection. If the arrhythmias you are expecting are ectopic beats then there is a separate detection for that (see Irregular Rhythm Warning).

Note: the more smoothing the lower the background therefore more strict you can be without baseline fluctuation flagging as arrhythmias.

Trough SD Percent Fluctuation

This is a backup arrhythmia detection system where by the standard deviation of the troughs is calculated as a percentage of the amplitude of the trace. E.g. if the setting is set to 10, IF the SD of the troughs is higher that 10% of the trace amplitude then it is flagged as an arrhythmia.

Warnings

There are several warnings that have been implemented in this script, these are to mitigate incorrect prediction of the traces and flag a trace for potential user interpretation. It is up to the user to determine if they want to analyse warning traces manually, ignore the warning or simply void these traces.

Warning

The first warning is to simply flag up the trace is close to the chosen void parameters so could be incorrectly predicted.

This warning will flag if the trace has an amplitude of 1.5 times the selected noise value:

E.g. – The Noise value is set to 1000

- A trace has an amplitude of 1400 Warning is flagged TRUE

OR

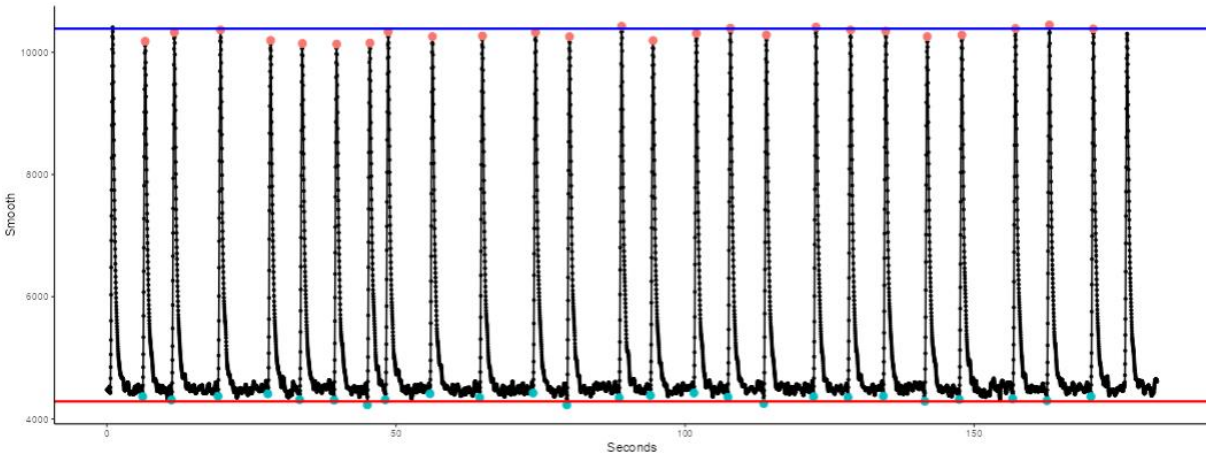
This warning will flag if the trace has an amplitude less than 2 times the noise value and a meanRR under 1:

E.g. – The Noise value is set to 1000

- A trace has an amplitude of 1800 and a meanRR of 0.6, a Warning is flagged TRUE

Rhythm Warning: SD distance between peaks

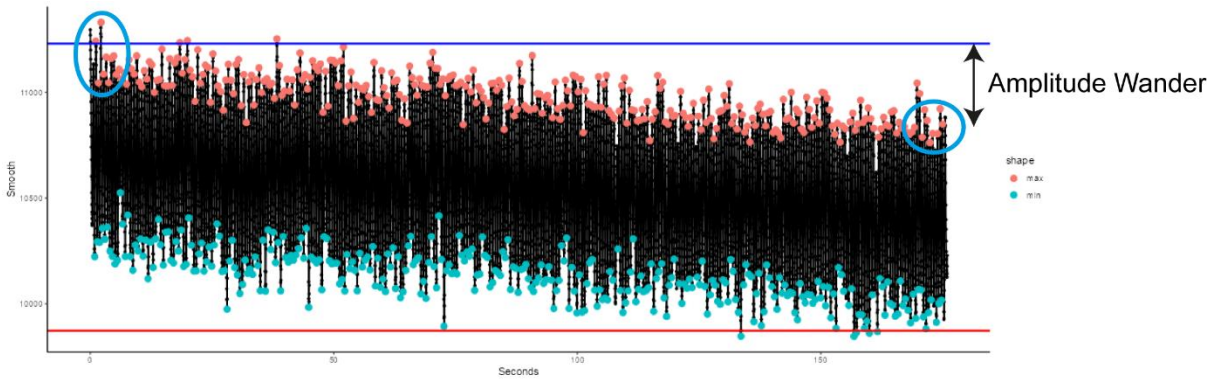
Here is the option you can use to detect ectopic beats or irregular contraction patterns. The system simply flags a Rhythm Warning if the SD_length (see P.Stats for more info) is more than the selected value. In this situation, the lower value is more sensitive, while a higher value is less sensitive. For example the trace below will flag a warning if the setting is set to 1.2, while not at 1.6.



It is important to note again, this is a personal preference. If the trace involves paced cardiomyocytes then this irregular rhythm is indicative of an arrhythmia and the setting could be set to a strict value to detect the irregularities. While un-paced hiPSC cardiomyocytes may already have a baseline irregular rhythm, in which case you cannot claim an irregular rhythm is arrhythmic.

Wander Warning: Trace Wandering Warning

This final warning was simply put in to flag the user that there is a substantial change in the amplitude from the start of the trace to the end of the trace. Simply put, if the first 5 peaks are more that X% amplitude apart from the last 5 peaks, a Wander.Warning is flagged TRUE. This is especially important as this will most likely cause false positive arrhythmia predictions, the image below is flagged as Arrhythmia TRUE, since the baseline wanders substantially.

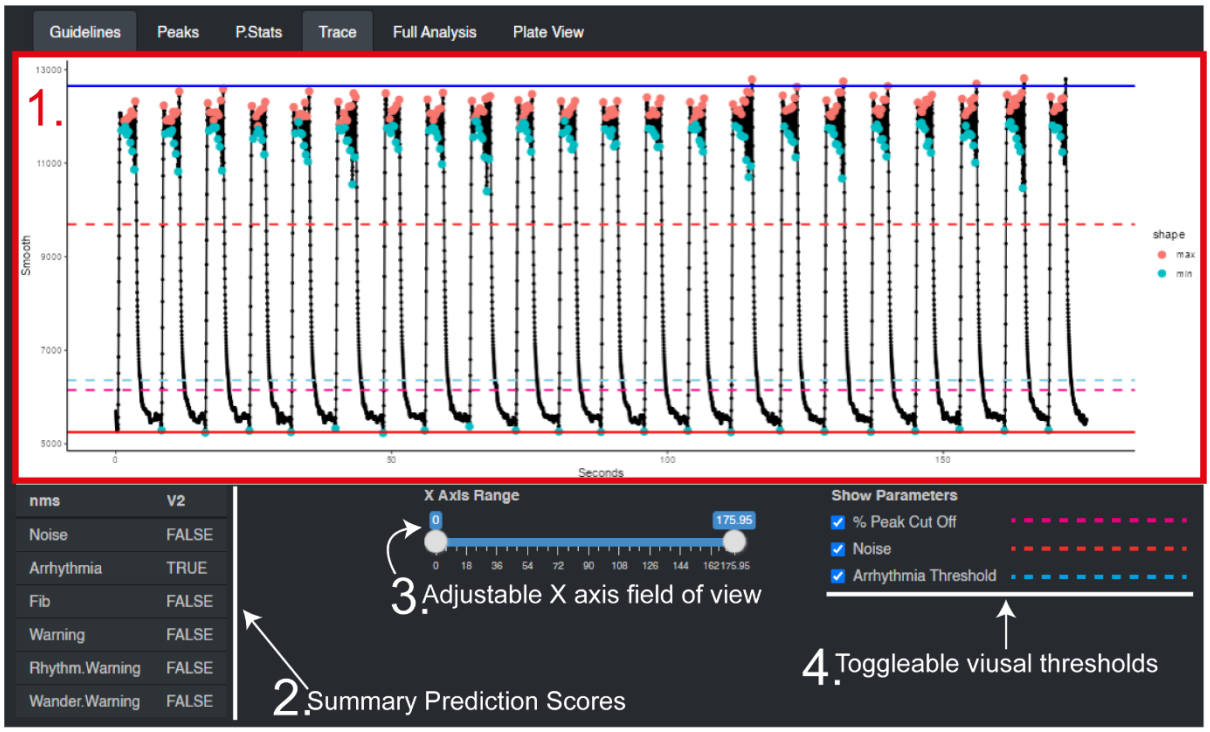


P.Stats Tab

nms	V2	Explanation
Well	A1	Well code pre-designated in the CSV
meanRR	1.304608	The Mean Peak to Peak time of the trace
medianRR	1.583	The Median Peak to Peak time of the trace
meandRR	0.01117687	The mean Peak Lag time - Peak lead time
SD_length	0.5620446	Standard deviation of the Lead Peak time
SD_width	0.5181198	Standard deviation of the meandRR
Freq	44.49134	Number of peaks per minute detected
BVR	0.7056546	Sum of meandRR
ratio	0.4139804	Mean SD_Length, SD_Width / meanRR
ellipse	1.084777	SD_Length / SD_Width
minTroughAmp	2404.339	Amplitude of the Minimum Trough detected
maxTroughAmp	3705.839	Amplitude of the Maximum Trough detected
diffTroughAmp	1301.5	maxTroughAmp - minTroughAmp
sdTroughAmp	280.1811	Standard deviation of all troughs detected
lowSmooth	2468.069	Mean of the 10 lowest amplitudes
hiSmooth	3781.415	Mean of the 10 highest amplitudes
Amplitude	1313.346	hiSmooth - lowSmooth
Wander	448.5912	Mean amplitude of first 5 peaks – last 5 peaks
userAmpDetectP	2862.073	User defined % of trace where any troughs above this threshold will be classed as arrhythmias
FibArrhythDetect	3256.077	Doubles % classed in above detection for means of classifying arrhythmias found within fibrillation
sdPercentDetect	21.33338	% Standard deviation of trough variation to flag arrhythmia

Trace Tab

Once a CSV has been imported, a well selected and the parameter applied the Trace tab should render a graph as seen below. This is a simple way to view traces are being analysed correctly and if the chosen parameters are given the expected outcome result.



1. Main graph render plot. The calculated baseline is shown as a solid red bar, while calculated amplitude is the solid blue bar. Peaks are represented by orange dots, with troughs highlighted as blue dots.

Every time 'Apply Parameters' is pressed the graph will update with the new parameters. When showing parameters with toggle switches (4) the graph will update in real-time as the sliders are changed.

2. Summary Prediction Scores. This is the predicted outcome of the trace based off the chosen parameters, in the example image Arrhythmia is flagged as TRUE, while everything else is FALSE. This will also update as 'Apply Parameters' is pressed.

3. Adjustable X Axis. Here the trace can be "Zoomed in on" by reducing the visible X axis range. Helpful for checking all peaks/troughs are correctly highlighted or where smoothing covers a small arrhythmia etc.

The graph will maintain the zoomed in view when applying new parameters, except when the Smoothing Value or Number of Tail Points to Remove, is altered.

4. Toggleable visual thresholds. Here the parameters for %Peak cut off (pink), Noise (Red) and Arrhythmia Threshold (Blue) will overlay on the graph if selected to give the user a visual concept of where certain cut-off values are.

Full Analysis, Plate Plan Import & Exporting Results

Here you can perform the full analysis of the CSV. When clicking 'Perform Full Analysis of CSV' the program will apply the current parameters to the entire CSV and search for arrhythmias. During this process the loading bar will slowly fill (CSV size dependant) displaying either "Applying Smoothing…", "Finding Peaks & Searching for Arrhythmias…" or "Analysis Complete".

I would advise against changing any parameters or clicking any other buttons while it is running as this may cause errors. The full analysis can always be re-run after if there is a parameter you wish to change.

Once the analysis is complete a table of results will be auto generated summarising the results from the CSV (see below).

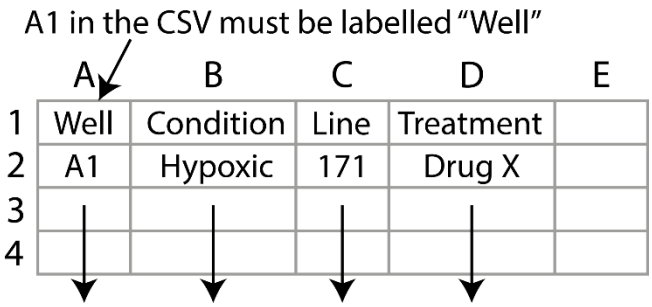
Arrhythmias_Detected	No_Arrhythmia	Noise	Fibriliation	Warning	Rhythm_Warning	Wandering_Trace
292	0	92	0	9	0	8

Importing Plate Plan (Optional)

Once the full analysis is complete you can attach a separate CSV containing the details of the experiment, once imported this will automatically be stitched to the results of your "Full Analysis".

The plate plan must be a CSV, and have the same well names as given in the main CSV for correct stitching and cell A1 must be labelled "Well" again.

The column titles in B onwards can be named as you wish and the content will be auto-stitched to the results.

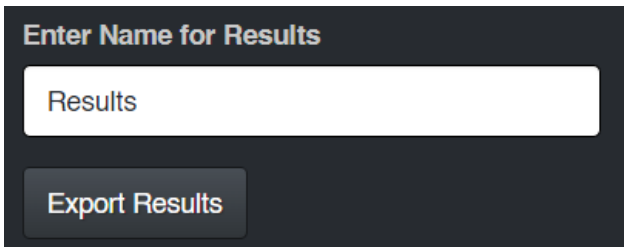


There are 3 column titles that if used in the plate plan will be visible on the "Plate View" layout as an extra option to view. These columns are "Condition", "Line" and "Treatment".

Exporting Results

Here you can export a CSV of results that will contain the Well code taken from the input CSV, any attached plate plan, all the p.stats values and the TRUE FALSE values for Noise, Arrhythmia, Fibrillation and Warnings.

To export the results click the 'Export Results' button, this will automatically export a CSV with the name as given in the text box above (e.g. Results in the image) to your working directory (usually where you have saved the R files and are running this app).



Important notes:

If there is a CSV with the same name in the working directory this will be overwritten without prompts! If the CSV with the same name is open at the time the results are exported, the app will crash.

There is a bug, where once 'Export Results' is clicked, any rerun of the full analysis will be automatically exported with the name in the text box and with any attached plate plan.

Therefore if you export your results and wish to rerun analysis with new parameters, make sure the original CSV output is closed and, either change the save name to something new or allow the app to overwrite the current CSV output.

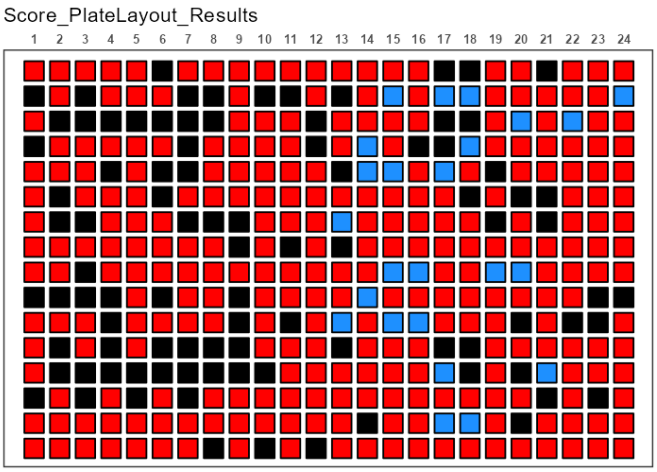
When a new CSV is imported as per CSV Import this bug is reset and the results CSV will NOT be automatically exported.

Plate Viewer

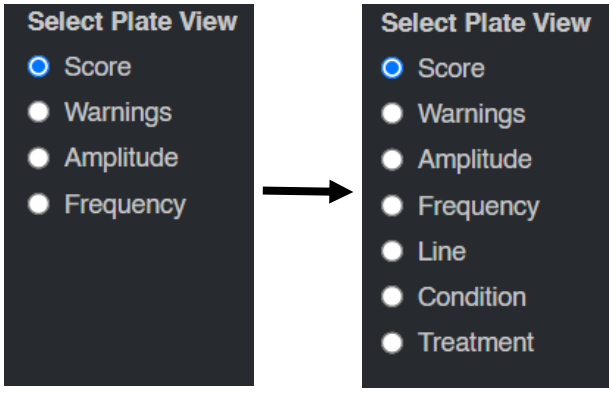
The final tab available is the "Plate View" tab where you can visualise the results of the full analysis in plate format. *Note, this is still a work in progress so may not be identical as described here.*

The first view that is visible will be the 'Score' view.

With this layout several conditions have been scored to provide a colour depending on the result (see below)

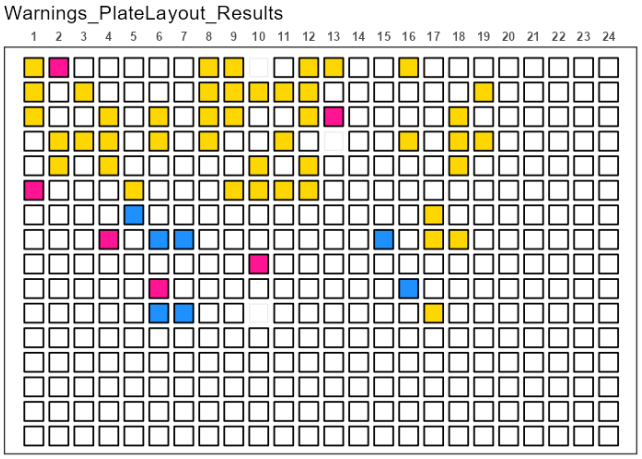


Score Legend	Colour
Noise	Black
No Arrhythmia	Blue
Fibrillation	Gold
Arrhythmia	Red
Other	Pink



Using the Select Plate View radio buttons you can change the Plate View to either Warnings, Amplitude or Frequency. If you have imported a plate plan following the guides recommended colums (here) then 3 extra options will be availible for view (see below).

If the Warnings view is selected all detected warnings will be flagged following the below key.



Warning Legend	Colour
No Warning	White
Warning	Pink
Rhythm Warning	Gold
Wander Warning	Blue
Multiple Warnings	Red

You can also change the plate size to 96 using the drop down menu, 384 well is selected as standard.

Important Notes

The trace that is input must have ideally **12** contractions. The first and last peaks are removed, then the top 10 peaks and troughs are taken to calculate the average baseline and amplitude.

If there are less than **5** readable contractions the script will be unable to generate a Trace or P.stats and upon reading results will just be met with N/A.

If there are between **5 and 10** readable contractions the script will average the "highest and lowest" detected peaks and troughs, which will result in incorrect baselines and amplitudes calculated. There is also currently no warning for this!

If you reload the Shiny App, the parameter settings will also reload to the default, so best to keep note of them (was planning on making a parameter export/import function) if wanting them to be the same. Although, you can import a new CSV file and the parameters will not change from the ones used for the previous CSV.

This app will provide predictions based on user input choices and therefore should be taken as such, a predicted outcome. The app is designed for hiPSC cardiomyocyte calcium traces and therefore is not designed to be used for other applications.