# **EasieRR User's Guide**



EasieRR is a stand-alone software optimized for analysing ECG in non-restrained animals. EasieRR is free, open-source and available for Microsoft Windows operating systems (Version 7 and upwards).

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#### **Find EasieRR**

EasieRR figshare account EasieRR GitHub repository

#### Cite

Please acknowledge and cite the use of this software and its authors when results are used in publications or published elsewhere:

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# Legal

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# **Install EasieRR**

The latest version of EasieRR can be installed on the EasieRR figshare account. The latest and all previous versions of EasieRR are available in the Github repository.

# About EasieRR

EasieRR is an open-source software developed to assist researchers in the use of heart rate parameters and their processing and analysis. The intuitive graphical user interface (GUI) facilitates manual detection and correction of artefacts and the lean program structure eases analysis of time-domain and non-linear estimates of heart rate variability.

# Features

# a. Main features

- Open-source software
- Intuitive GUI
- Automated and manual peak detection for inter beat interval extraction in ECG data
- Easy artefact detection by means of tachogram and Poincaré plots
- Manual and transparent artefact processing in ECG data
- Analysis in time-domain and non-linear domain

# b. Customized analysis for different species

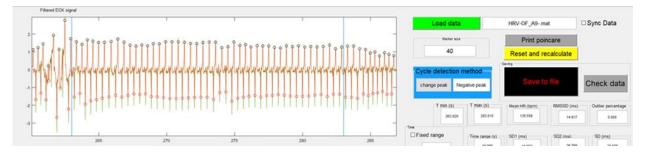
- Allows to set refractory time
- Analyses raw ECG data from txt format and csv format

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- Default settings for small ruminants and pigs, but customizable for other species

🚺 loadpopup3	- 🗆 X
Set sampling frequency 250	Set refractory time 200
Load Biopac TXT file	Ch
Load BioPac *.txt and cont	
Load CSV file	
Load CSV and co	ontinue
Load previously analysed o	lata and continue
Peak prominence Low cutoff 0.5500 1	High cutoff 20

#### c. Detection of peaks in ECG data and extraction of inter beat intervals

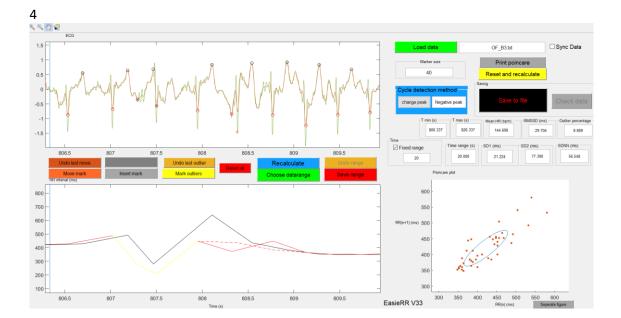
- Customized filtering of data (high and low pass)
- Automatic R-peak detection utilizing a peak prominence algorithm
- Two options for peak detection: positive peaks and negative peaks, depending on data quality



#### d. Detection and processing of artefacts

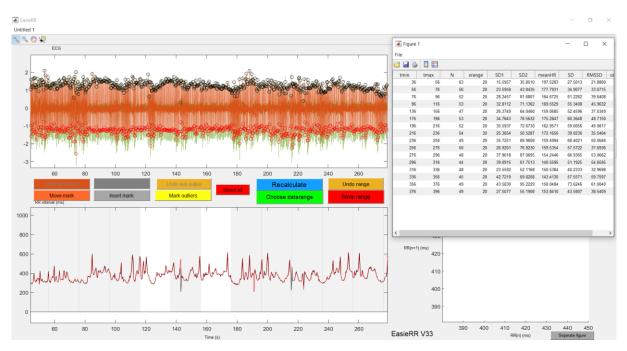
Allows for manual correction of artefacts: deleting spuriously detected peaks, selecting missed peaks or deselecting spurious detections.

- Displays corrections instantaneously in tachogram
- Visualization of data points in Poincaré plot to facilitate detection of artefacts



# e. Analysis of heart rate variability (HRV) estimates in time and frequency domain

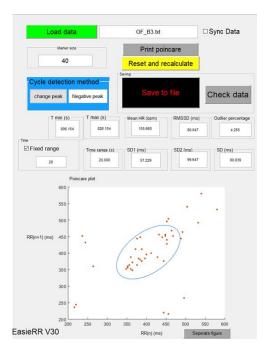
- Computes HRV estimates in time and non-linear domain
- Displays HRV statistics and Poincaré plots as soon as data sequences are selected
- Allows selection and subsequent analysis and storage of several sequences in a row



#### f. Storage of analysis and HRV statistics

- Stores summary of HRV estimates of analysed sequences to the disk
- Stores Poincaré plots and RR time probability histogram to disk
- Allows re-loading of previously analysed data

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# g. Synchronization with behavioural data

- Offers the possibility to choose synchronization option when data is imported
- Facilitates comparison of ECG data with behavioural data from videos

# **Computational background**

EasieRR has been programmed using MATLAB 2018b (Mathworks, Natick, MA, USA). and compiled as a stand-alone application with the MATLAB compiler 7.0 for Microsoft Windows operating systems (Version 7 and upwards). The appropriate runtime is included in the EasieRR installer.

#### Filter

EasieRR applies by default a band pass filter (4. Order 0 degree Butterworth filter with low frequency cut-off at 1 Hz and high frequency cut-off at 20 Hz), hence removing high frequency peaks in the signal as well as removing any DC offset. Default settings can be easily customized and adapted for every species. If peak detection is not accurate enough with default settings, try adjusting lower and/or higher cut-off frequency.

# Peak detection

The peak detection used for heart cycle determination and inter beat interval extraction in EasieRR is based on a peak prominence algorithm: The height of the potential peak to be detected must be at least a certain user-determined value (default: 0.55) times the width of the peak at half the max. height. Combined with the band pass filter and a predetermined minimum timespan (default: 300ms) between inter beat intervals (=heart refractory time of the species used), this method allows for robust peak detection.

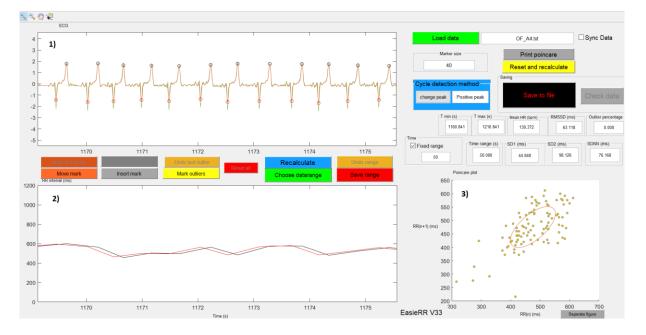
#### 6 Artefact correction

Artefact correction in EasieRR is based on a deletion algorithm proposed by Lippmann and colleagues (1994). Artefacts can be manually corrected by deleting spuriously detected peaks, selecting missed peaks or deselecting spurious detections.

# **Graphical user interface**

#### Overview

The GUI is split into three separate interactive windows: an upper window displaying the ECG signal (1) which allows to visually inspect QRS complexes and to correct artefacts. The lower left window shows the corresponding tachogram (2) and the lower right window a Poincaré plot (3) facilitating the efficient location of artefacts.



#### EasieRR Buttons: Description and Function

The EasieRR GUI is operated via a range of buttons varying in colour. Descriptions as well as function of every single button can be found in the table below. Colours in the table match the button colours in the GUI.

Button Description	Button Function
Choose data range	Selects the data range to be analysed
Move mark	Moves mark of detected data point from one location to new location
Undo last move	Reverses the last movement
Insert mark	Inserts a new mark on an undetected data point
Undo insertion	Reverses last insertion
Mark outlier	Marks a data point as an outlier to be deleted

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Undo last outlier	Reverses last deletion
Recalculate	Update HRV estimates after artefact correction or new selection of
	data range
Reset all	Resets all artefact corrections within the current selection
Save range	Saves currently selected data range and HRV parameters into list of
	data ranges (as shown when pressing "Check data")
Undo range	Delete last data range in list of data ranges
Save to File	Saves data from all previously saved sequences to a file
Load data	Loads ECG data in txt format
Print Poincaré	Reloads previous matlab file and plots poincaré plots and histogram
Reset and	Resets all parameters to the starting point
recalculate	
Check data	Displays data of all previously saved data ranges
Change peak	Switches between positive and negative peak cycle detection
Separate figure	Plots Poincaré plot as separate figure
Zoom in/out tool	Zooms in/out of ECG and tachogram
Hand tool	Moves displayed section of ECG and tachogram
Sync box	Asks for xls file to synchronize with ECG txt file when ticked
Fixed range box	Use a set time duration for data range

# Workflow

#### Input data preparation

If saving in BIOPAC

• Save file as txt: use header, comma or tab as delimiter, DOS as line ending. If saving in any other software:

• Save as csv: include only data points, no headers.

#### ECG analysis

- 1. Start EasieRR and a dialogue box will pop up.
- 2. Use default settings or set the sampling frequency, refractory time, peak prominence for peak detection as well as the frequency for the low cut-off and high cut-off filter and the channel containing the raw ECG data. As this software is reading in txt or csv format it is important to set the sampling frequency used during the experiment.
- 3. Load a new data file (formatted as mentioned above) or a previously analysed file by using the appropriate button on the loading screen
- 4. To increase the size of the markers for peak detection change the value in the marker size window.

- 5. To zoom into press the ' <sup>Q</sup> ' button, position the cursor on area of interest on ECG or tachogram, left click and hold to select the area for enlarged viewing.
- 6. To zoom out press the ' Q' button, position the cursor on area of interest on ECG or tachogram and left click
- 7. Select the time range to be analysed by pressing the 'choose data range' button and click on the preferred start and end of the range in the ECG or tachogram panel. In case several ranges of the same length should be analysed, tick the 'Fixed range' button and choose a time span in seconds. Now only the start of the range to be analysed has to be marked after pressing the 'choose range' button.
- 8. Examine data by means of ECG, tachogram and poincaré plot.
- Correct misdetected peaks by left clicking them with the mouse in the ECG window. Detected peaks are marked with a little circle (black=positive peak, red=negative peak).
  - 9.1. To mark the location of a missed peak: press 'insert mark' button and mark the missed peak on the ECG signal by left clicking it. A new peak will be marked with a black or red '+' (depending on if positive or negative peaks were chosen) in the ECG. After pressing the 'recalculate' button the edits to the tachogram will be displayed as a black dotted line.
  - 9.2. to move the mark of a misdetected peak: press the 'move mark' button and 1) select misdetected peak location by left clicking it, 2) correct peak detection by left clicking correct peak (needs to be within two intervals of the original peak). A corrected misdetected peak will now display a red or black 'x' (depending on if positive or negative peaks were chosen) in the original circle in the ECG. The new mark will show as a plus sign. After pressing the 'recalculate' button the edits to the tachogram will be displayed as a red dotted line.
  - 9.3. To delete artefact: press 'mark outlier' button and mark the outlier you want to delete by left clicking it. A deleted peak will always be marked with a 'x' in the original circle in the ECG and after pressing the 'recalculate' button the edits to the tachogram will be displayed as a yellow line.
  - 9.4. To undo any of the above corrections press the 'undo last move', 'undo insertion' or 'undo last outlier' buttons and then update the ECH and tachogram by pressing the 'recalculate' button.

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- 10. Save every data range individually after editing is finished by pressing the 'save range' button and view HRV statistics for all ranges analysed using the 'check data' button.
- 11. If the last data range created is not correct, pressing "Undo range" will erase the last range from the list. This can be done as many times as there are ranges in the list.
- 12. Proceed by choosing next data range and repeat steps 4-7.
- 13. Save all previously analysed data ranges using the 'save to file' button. Results will be saved in mat format and two files in xlxs format: (1) a file with a single line per data range for manual analysis and (2) a file with two lines per data range, optimized for import into The Observer<sup>®</sup>.
- 14. To review previously analysed data press the 'reload data' button and choose the mat file containing all relevant data for plotting the analysed data ranges and Poincaré plots.

# Synchronization with behavioural data

HRV data can also be synchronized with behavior via import into common software for behavioral video analysis (i.e. The Observer<sup>®</sup>). This is useful if video and ECG recording have not been synchronized before analysis or if ECG data files have not been cropped to fit video length. In this case it is necessary to establish a common point in time observable both in the video and in the ECG recordings, T<sub>0</sub>. This can be whatever time point most convenient for the current experiment, but it has to be clearly observable in both the ECG data as well as in the video.

- 1. Prepare an additional file in xls format arranged as follows:
- First column: exact filename used for the ECG txt files.
- Second column: latency (in sec) from start of ECG recording to T<sub>0</sub>
- Third column: latency (s) from video start to T<sub>0</sub>
- If comma is used as decimal separator, use time measurements in seconds without decimals.
- 2. Open EasieRR and tick 'Sync' box before loading a new data file.
- Press the 'Load data' button. The program will ask you to 1.) Choose the ECG data file (in txt format) to be analysed and 2.) to choose the file (in xls format) prepared for synchronization as mentioned above.
- 4. The data will be displayed as ECG and tachogram with a common start of video and ECG at  $T_0$  also marked with a blue vertical line in the ECG panel.
- 5. Proceed with steps 4-12 (described in the workflow for ECG analysis above).