



For Better Heart Rate Variability Analysis

Kubios HRV

(ver. 3.0)

USER'S GUIDE

- ✓ HRV Standard
- ✓ HRV Premium

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Abbreviations

LIST OF ABBREVIATIONS

ACQ	AcqKnowledge data file format (Biopac Inc.)
ANS	Autonomic nervous system
ApEn	Approximate Entropy
AR	Autoregressive (model or process)
ASCII	Text file using the ASCII character set, which is the most common format for English-language text files
CSV	Comma separated value (file format)
DFA	Detrended fluctuation analysis
ECG	Electrocardiogram
EDF	European data format (file format)
EDR	ECG derived respiration
FFT	Fast Fourier transform
GDF	General data format (file format)
GUI	Graphical user interface
HF	High frequency (refers to HRV frequency band, by default 0.15-0.4 Hz)
HR	Heart rate
HRV	Heart rate variability
IBI	Inter-beat-interval (same as RR interval)
LF	Low frequency (refers to HRV frequency band, by default 0.04-0.15 Hz)
MAT	MATLAB data file format (Mathworks Inc.)
MSE	Multiscale entropy
NNxx	Number of successive RR interval pairs that differ more than xx msec
PDF	Portable document format (file format)
pNNxx	Relative number of successive RR interval pairs that differ more than xx msec
PNS	Parasympathetic nervous system
QRS	QRS complex of electrocardiogram
RMSSD	Root mean square of successive RR interval differences
RPA	Recurrence plot analysis
RR	Time interval between successive ECG R-waves (RR interval, same as IBI)
SampEn	Sample entropy
ShanEn	Shannon entropy
SDANN	Standard deviation of the averages of RR intervals in 5-min segments
SDNN	Standard deviation of normal-to-normal RR intervals
SDNNI	Mean of the standard deviations of RR intervals in 5-min segments
SNS	Sympathetic nervous system
SPSS	Statistical analysis software package(IBM Corp.)
TINN	Triangular interpolation of normal-to-normal intervals
VLF	Very low frequency (refers to HRV frequency band, by default 0-0.04 Hz)

Chapter 1

Overview

1.1 About Kubios HRV

Kubios HRV is an advanced tool for studying the variability of heart beat intervals. Due to its wide variety of different analysis options and the easy-to-use interface, the software is suitable for researchers and clinicians with varying premises. The software is suitable for clinical and public health researchers, professionals working on human or animal well-being, or sports enthusiasts; for anybody who want to perform detailed analyses on heart rate variability, e.g. to examine autonomic nervous system function. The software is mainly designed for the analysis of human HRV, but it can also be used for animal research by taking into account necessary differences in analysis settings.

The first versions of the Kubios HRV were developed as part of academic research work carried out at the Department of Applied Physics, University of Eastern Finland, Kuopio, Finland. The first version of the software was released in 2004 and is described in [34]

Niskanen J-P, Tarvainen MP, Ranta-aho PO, and Karjalainen PA. Software for advanced HRV analysis. *Comp Meth Programs Biomed*, 76(1):73-81, 2004.

The last version of Kubios HRV developed at University of Eastern Finland (version 2.2) was released in May 2014 and is described in [49]

Tarvainen MP, Niskanen J-P, Lipponen JA, Ranta-aho PO, and Karjalainen PA. Kubios HRV – Heart rate variability analysis software. *Comp Meth Programs Biomed*, 113(1):210-220, 2014.

Kubios Oy (limited company) is a medical technology company focusing on software and algorithms development for medical signal analysis. The company was founded in 2016 by the developers of Kubios HRV software and will be responsible for further development and distribution of the software. Kubios HRV is the most popular HRV analysis software for scientific research, being used in over 800 scientific studies by now. In addition, Kubios HRV has gained popularity among athletes and people who are motivated in monitoring their well-being.

The first commercial version of Kubios HRV (version 3.0) was released in January 2017. Kubios HRV is available as two alternative products: Kubios HRV Standard and Kubios HRV Premium. Both versions include all the commonly used time- and frequency-domain variables of HRV as well as a few most commonly utilised nonlinear analysis methods. The differences between Standard and Premium versions are summarised in the following (for details, see Table 1.1):

Kubios HRV Standard: Freeware HRV analysis software for non-commercial research and personal use. Supports HR data from most common HR monitor manufacturers and computes all commonly used time- and frequency-domain HRV parameters. Software is operated through an easy-to-use GUI and analysis results can be saved as PDF report or text file.

Kubios HRV Premium: Versatile HRV analysis software for scientific research and professional use. Supports wide range of ECG and HR data and computes all commonly used time-domain, frequency-domain and nonlinear HRV parameters. In addition, Kubios HRV Premium includes time-varying analysis options and support for group analyses. Software is operated through an easy-to-use GUI and analysis results can be saved as PDF, CSV text file or MATLAB MAT file.

Table 1.1: Summary of Kubios HRV Standard and Kubios HRV Premium features

FEATURES	HRV Standard	HRV Premium
Data support <ul style="list-style-type: none"> • Inter-beat-interval (IBI) data from HR monitors (Polar, Suunto & Garmin) or text file • Electrocardiogram (ECG) data in EDF/EDF+, GDF, Biopac ACQ3 or text file formats • Support for ActiHeart & ActiWave Cardio, eMotion Faros, and Zephyr BioHarness data files 	✓ — —	✓ ✓ ✓
Pre-processing <ul style="list-style-type: none"> • QRS detector for accurate detection of beat-to-beat RR intervals from ECG data • Artefact correction methods: Threshold based RR correction algorithm / Automatic RR correction algorithm / ECG based R-wave correction • Trend removal from RR interval time series 	— ✓ / — / — ✓	✓ ✓ / ✓ / ✓ ✓
Analysis options <ul style="list-style-type: none"> • Time-domain parameters: Mean RR and HR, min/max HR, SDNN, RMSSD, pNN50, HRV triangular index, TINN etc. • Frequency-domain parameters: VLF, LF and HF band powers (in absolute, relative and normalised units), peak frequencies and LF/HF ratio • Spectrum estimation methods: Welch's periodogram / Lomb-Scargle periodogram / AR spectrum estimate • Basic nonlinear parameters: Poincaré plot, approximate entropy (ApEn), sample entropy (SampEn) and detrended fluctuation analysis (DFA) • Additional nonlinear parameters: correlation dimension (D_2), recurrence plot analysis (RPA), multiscale entropy (MSE) • ECG derived respiration for accurate respiratory sinus arrhythmia (RSA) analysis • Time-varying analysis: time trends for time-domain and frequency-domain parameters, spectrogram and Kalman smoother based time-varying spectrum estimates 	✓ ✓ ✓ / — / ✓ ✓ — — —	✓ ✓ ✓ / ✓ / ✓ ✓ ✓ ✓ ✓
Reports and results export <ul style="list-style-type: none"> • HRV reports (PDF reports) including: General time-domain, frequency-domain and nonlinear results / Time-varying analysis results • ECG print (PDF report) showing the raw ECG trace for selected time period • HRV analysis results export options: PDF file / Text file / MATLAB MAT file • "SPSS friendly" batch file export (ideal for group analyses or repeated measurements) 	✓ / — — ✓ / ✓ / — —	✓ / ✓ ✓ ✓ / ✓ / ✓ ✓

1.2 Release notes

Since this is the first commercial release of Kubios HRV, launching the Kubios HRV Standard and Kubios HRV Premium versions, we do not describe here details of the version history. Instead we summarise here the new features available in the Standard and Premium versions (ver. 3.0) compared to the previous release (ver. 2.2) published in [49].

Main new features of Kubios HRV Standard and Kubios HRV Premium ver. 3.0 (compared to ver. 2.2) are (*indicates features available only in the Premium version):

- **Data support:** 1) Polar IBI file support updated; 2) Custom ASCII file import support updated; 2*) ActiHeart and ActiWave Cardio data support added; 3*) Zephyr BioHarness data support added; 4*) annotations support for EDF+ files updated.
- **Pre-processing:** 1*) An automatic RR artefact correction method was added. The new method provides enhanced artefact and ectopic beat detection performance and does not require a manual selection of optimal threshold value.
- **Time-domain HRV parameters:** 1) Computation of minimum and maximum HR as average of 5 beats (default value, can be changed in preferences) was added; 2) NN50 and pNN50 parameters renamed as NNxx and pNNxx (default threshold is 50 ms, can be changed in preferences).



- **Frequency-domain HRV parameters:** 1*) Lomb-Scargle periodogram was added as an alternative for Welch's periodogram (selection can be made in preferences); 2) VLF, LF and HF band powers are now given also in natural logarithm scale; 3*) warning given if ECG derived respiration is outside the defined HF band.
- **Nonlinear HRV parameters:** 1) Poincaré plot ratio SD2/SD1 was added.
- **Time-varying analysis*:** Time-varying analysis methods added to HRV Premium. These methods include time trends for time-domain and frequency-domain parameters as well as for a limited number of nonlinear analysis parameters. Changes in time-frequency information of HRV data are assessed by spectrogram and a parametric Kalman smoother based time-varying spectrum estimate.
- **Reports and results export:** 1*) "SPSS friendly" batch file export was added; 2) Time-varying analysis results added to HRV report (additional page included); 3) Handling of report figures was updated (HRV Report and *ECG print). All report pages open in the same window, where the user can view the report pages, print selected pages or save pages to PDF file.
- **GUI and usability:** 1) Visual layout of the software was changed; 2) HR (instead of RR) data visualisation can be selected in the GUI. In addition, several usability modifications to improve user experience.

1.3 System requirements

Kubios HRV (ver. 3.0) was developed using MATLAB® 2016b¹ (The MathWorks, Inc.) and was compiled to a standalone application with the Matlab Compiler. As a result, the MATLAB Runtime R2016b (ver. 9.1) is required for running Kubios HRV. System requirements for running Kubios HRV are similar to those requirements for Matlab (see <https://www.mathworks.com/support/sysreq/>). Only 64-bit operating systems are supported.

Windows 7 SP1, 8, 8.1 or 10 (64 bit version) operating system with 2 GB of RAM, 3-5 GB of disk space, screen resolution of 1024×768 or higher, and the MATLAB Runtime R2016b (ver. 9.1) installation.

Mac OS X operating system with 2 GB of RAM, 3-5 GB of disk space, screen resolution of 1024×768 or higher, and the MATLAB Runtime R2016b (ver. 9.1) installation

1.4 Installation

In order to run Kubios HRV, you need to install Kubios HRV Standard or Kubios HRV Premium and MATLAB Runtime R2016b (ver. 9.1) on your computer. The first time you launch Kubios HRV, you will be prompted to activate your license on the current computer. This can be done automatically over the internet or manually if internet connection is not available. You will receive your personal license key as well as the links for downloading the necessary installers after completing registration or purchase at <http://www.kubios.com/>. A short description of the installation process at different operating systems is given below.

Windows: Make sure that you have administrative privileges (you will need them to install Kubios HRV). In order to install Kubios HRV on a Windows computer, you need to first install the MATLAB Runtime R2016b (ver. 9.1) on your computer. After you have installed the MATLAB Runtime, run the Kubios HRV installer file and follow the instructions given in the setup wizard to complete installation. You can launch Kubios HRV by using the Desktop icon (if created) or by selecting it from the Start Menu. Please note that Kubios HRV also starts the MATLAB Runtime and may take some time especially with older computers. The first time you start Kubios HRV, you also need to activate the software using your personal license key.

Mac OS: The macOS version of Kubios HRV will be released soon...

¹MATLAB®. ©1984-2016 The Mathworks, Inc.

1.5 Uninstallation

Windows: The software can be uninstalled using the "Windows Settings > System > Apps & Features" (Windows 10) or "Control Panel > Programs and Features" (Windows 8 and 7). However, the uninstaller does not remove your preferences settings or license file. These have to be deleted manually and can be found in the following folders:

Kubios HRV Standard

C:\Users\<username>\AppData\Roaming\Kubios\KubiosHRVStandard

C:\ProgramData\Kubios\KubiosHRVStandard

Kubios HRV Premium

C:\Users\<username>\AppData\Roaming\Kubios\KubiosHRVPremium

C:\ProgramData\Kubios\KubiosHRVPremium

Mac OS: Move the installed applications (the MATLAB Runtime and Kubios HRV application) to trash.

1.6 Software home page

The Kubios HRV home page on the web can be found at

<http://www.kubios.com/>

where you can find current information on the software and download possible updates and related material. **If you have any trouble or questions regarding the software, please check first if your particular problem or question has an answer in the FAQ/troubleshooting section at the software homepage!** You can also follow Kubios HRV on Facebook (www.facebook.com/kubios).

1.7 Structure of this guide

The aim of this guide is to help the user to get started with Kubios HRV. It should not, however, be thought of as being an easy to follow step by step manual, but more like a reference material from which you can probably find answers to your problems related to HRV analysis or usability of the software. The structure of this guide is as follows.

After the overview chapter, from where you will find useful information about the system requirements and installation, an introduction to heart rate variability is given in Chapter 2. This chapter starts with a short discussion on the control systems of heart rate after which the extraction of heart beat intervals is discussed and the derivation of HRV time series is described.

In Chapter 3, the description of the features and usage of the software is given. First, the input data formats supported by the software are described and then the user interface through which the software is operated is described. Then, different options for saving the analysis results are described and, finally, instructions on how to set up the preference values for the analysis options are given. So if you want to learn how to use all the functionalities of the software, this is the chapter to read.

In Chapter 4, two sample runs of the software are presented. The first sample run describes how to analyse the lying and standing periods of the orthostatic test measurement (distributed along this software) separately as stationary segments. The second sample run, on the other hand, describes the time-varying analysis procedure of the same measurement.

Finally, Chapter 5 describes in detail the pre-processing and analysis methods included in the software. This last chapter is useful for better understanding how the pre-processing steps (artifact correction and baseline trend removal) function and how they effect HRV analysis results. The description of the analysis methods included in the software is divided into time-domain, frequency-domain, nonlinear and time-varying categories. For most of the methods, exact formulas for the different variables are given and possible parameter selections are pointed out.

Chapter 2

Heart rate variability

Heart rate variability (HRV) describes the variations between consecutive inter-beat-intervals or IBIs. Both sympathetic and parasympathetic branches of the ANS are involved in the regulation of heart rate (HR). Sympathetic nervous system (SNS) activity increases HR and decreases HRV, whereas parasympathetic nervous system (PNS) activity decreases HR and increases HRV [5]. The control of the autonomic output involves several interconnected areas of central nervous system, which form the so-called central autonomic network. In addition to this central control, arterial baroreceptor reflex as well as respiration are known to induce quick changes in heart rate. The baro reflex is based on baroreceptors which are located on the walls of some large vessels and can sense the stretching of vessel walls caused by pressure increase. Both sympathetic and parasympathetic activity are influenced by baroreceptor stimulation through a specific baroreflex arc, Fig. 2.1.

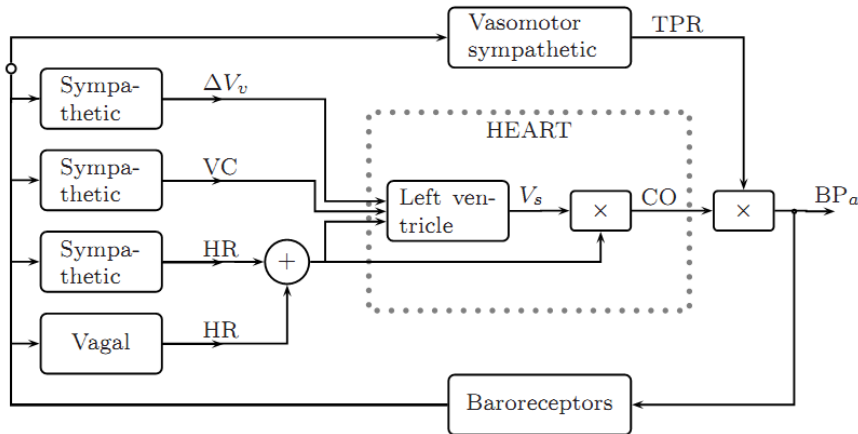


Figure 2.1: The four baroreflex pathways (redrawn from [45]). Variation in venous volume (ΔV_v), left ventricular contractility (VC), sympathetic and parasympathetic (vagal) control of heart rate (HR), stroke volume (V_s), cardiac output (CO), total peripheral resistance (TPR), and arterial blood pressure (BP_a).

Typically, the most conspicuous oscillatory component of HRV is the respiratory sinus arrhythmia (RSA), where the vagus nerve stimulation is being cut-off during inhalation, and thus, HR increases during inhalation and decreases during exhalation. This high frequency (HF) component of HRV is thus centered at respiratory frequency and is considered to range from 0.15 to 0.4 Hz. Another conspicuous component of HRV is the low frequency (LF) component ranging from 0.04 to 0.15 Hz. The HF component is mediated almost solely by the PNS activity, whereas the LF component is mediated by both SNS and PNS activities and is also affected by baroreflex activity [51, 5, 22]. The origin of the LF oscillations is however considered to be dominated by SNS and the normalized power of the LF component could be used to assess sympathetic efferent activity [35, 15]. The fluctuations below 0.04 Hz, on the other hand, have not been studied as much as the higher frequencies. These frequencies are commonly divided into *very low frequency* (VLF, 0.003-0.04 Hz) and *ultra low frequency* (ULF, 0-0.003 Hz) bands, but in case of short-term recordings the ULF band is generally omitted [51]. These lowest frequency

rhythms are characteristic for HRV signals and have been related to, e.g., humoral factors such as the thermoregulatory processes and renin-angiotensin system [5].

Heart rate variability (HRV) is a commonly used tool when trying to assess the functioning of cardiac autonomic regulation. It has been used in multitude of studies, related to cardiovascular research and different human wellbeing applications, as an indirect tool to evaluate the functioning and balance of the autonomic nervous system (ANS) [51].

One of the main clinical scenarios where HRV has been found valuable include the risk stratification of sudden cardiac death after acute myocardial infarction [51, 1, 22, 42]. In addition, decreased HRV is generally accepted to provide an early warning sign of diabetic cardiovascular autonomic neuropathy [51, 1], the most significant decrease in HRV being found within the first 5-10 years of diabetes [55, 48]. Besides these two main clinical scenarios, HRV has been studied with relation to several cardiovascular diseases, renal failure, physical exercise, occupational and psychosocial stress, gender, age, drugs, alcohol, smoking and sleep [54, 27, 51, 43, 2, 1].

The term HRV refers, in general, to changes in heart beat interval which is a reciprocal of the heart rate. This is also the case here. The starting point for HRV analysis is the ECG recording from which the HRV time series can be extracted. In the formulation of the HRV time series, a fundamental issue is the determination of heart beat period.

2.1 Heart beat period and QRS detection

The aim in HRV analysis is to examine the sinus rhythm modulated by the autonomic nervous system. Therefore, one should technically detect the occurrence times of the SA-node action potentials. This is, however, practically impossible and, thus, the fiducial points for the heart beat is usually determined from the ECG recording. The nearest observable activity in the ECG compared to SA-node firing is the P-wave resulting from atrial depolarization (see Fig. 2.2) and, thus, the heart beat period is generally defined as the time difference between two successive P-waves. The signal-to-noise ratio of the P-wave is, however, clearly lower than that of the strong QRS complex which results primarily from ventricular depolarization. Therefore, the heart beat period is commonly evaluated as the time difference between the easily detectable QRS complexes.

A typical QRS detector consists of a preprocessing part followed by a decision rule. Several different QRS detectors have been proposed within last decades [52, 36, 37, 20, 14].

The accuracy of the R-wave occurrence time estimates is often required to be 1–2 ms and, thus, the sampling frequency of the ECG should be at least 500–1000 Hz [51]. If the sampling frequency of the ECG is less than 500 Hz, the errors in R-wave occurrence times can cause critical distortion to HRV analysis results, especially to spectrum estimates [32]. The distortion of the spectrum is even bigger if the overall variability in heart rate is small [40]. The estimation accuracy can however be improved by interpolating the QRS complex e.g. by using a cubic spline interpolation [11] or some model based approach [6]. Kubios HRV uses interpolation to improve the detection accuracy.

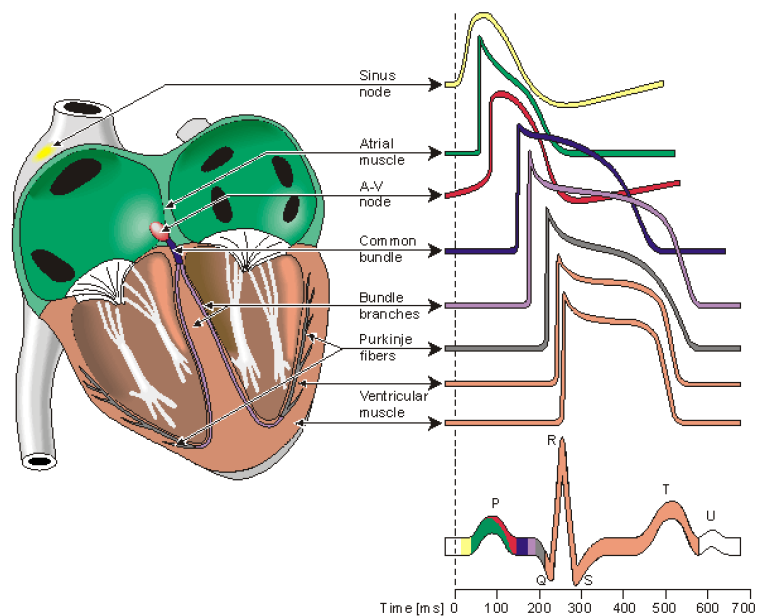


Figure 2.2: Electrophysiology of the heart (redrawn from [28]). The different waveforms for each of the specialized cells found in the heart are shown. The latency shown approximates that normally found in the healthy heart.

2.2 Derivation of HRV time series

After the QRS complex occurrence times have been estimated, the HRV time series can be derived. The inter-beat intervals or RR intervals are obtained as differences between successive R-wave occurrence times. That is, the n 'th RR interval is obtained as the difference between the R-wave occurrence times $RR_n = t_n - t_{n-1}$. In some context, normal-to-normal (NN) may also be used when referring to these intervals indicating strictly intervals between successive QRS complexes resulting from SA-node depolarization [51]. In practice, the NN and RR intervals appear to be the same and, thus, the term RR is preferred here.

The time series constructed from all available RR intervals is, clearly, not equidistantly sampled, but has to be presented as a function of time, i.e. as values (t_n, RR_n) . This fact has to be taken into account before frequency-domain analysis. In general, three different approaches have been used to get around this issue [51]. The simplest approach that have been adopted in, e.g., [4] is to assume equidistant sampling and calculate the spectrum directly from the RR interval tachogram (RR intervals as a function of beat number), see the left panel of Fig. 2.3. This assumption can, however, cause distortion into the spectrum [30]. This distortion becomes substantial when the variability is large in comparison with the mean level. Furthermore, the spectrum can not be considered to be a function of frequency but rather of cycles per beat [12]. Another common approach, adopted in this software, is to use interpolation methods for converting the non-equidistantly sampled RR interval time series (also called the interval function) to equidistantly sampled [51], see the right panel of Fig. 2.3. One choice for the interpolation method is the cubic spline interpolation [30]. After interpolation, regular spectrum estimation methods can be applied. The third general approach is to apply methodology, which are designed for analysing non-equidistantly sampled data. Such a method is for example the Lomb-Scargle periodogram, which computes the periodogram spectrum estimate for non-equidistantly sampled data [13].

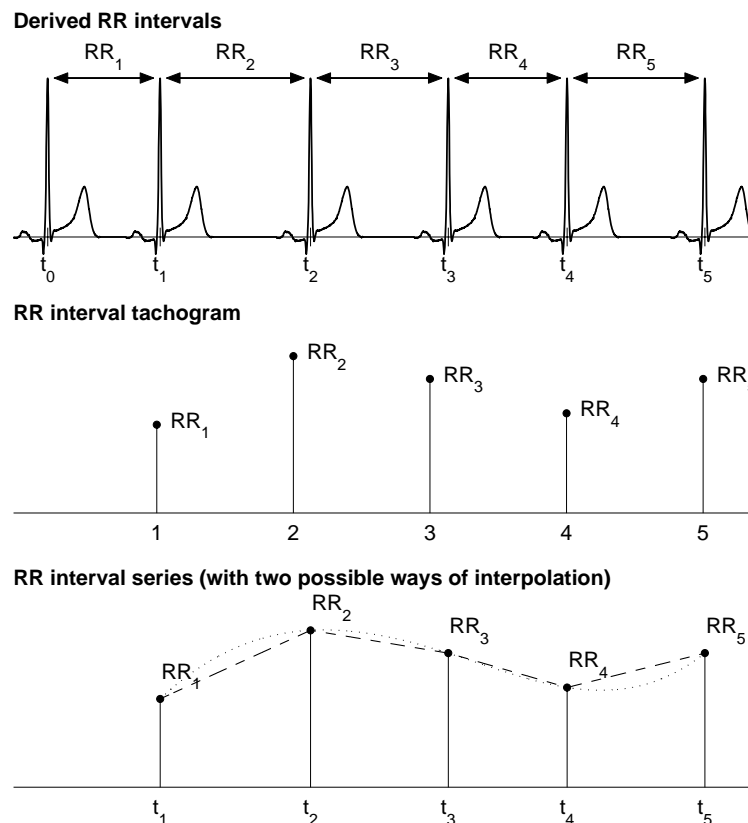


Figure 2.3: Derivation of two HRV signals from ECG: the interval tachogram (middle panel) and interpolated RR interval series (bottom panel).

Chapter 3

Software description

This chapter describes the different features and functionality of Kubios HRV, covering both the Standard and Premium versions of the software. Features available only in the Premium version are indicated in the text and an overview of differences between these versions is given in Table 1.1.

3.1 Input data formats

Kubios HRV supports the following data formats:

1. Polar HRM files (Polar Electro Ltd.)	(*.hrm)
2. Suunto SDF, STE and XML files (Suunto Ltd.)	(*.sdf,*.ste,*.xml)
3. Garmin FIT files (Garmin Ltd.)	(*.fit)
4. RR interval text files	(*.txt,*.dat,*.csv)
5. Custom text data files (only RR data)	(*.txt,*.dat,*.csv)
Available only in Premium version:	
6. ECG data text files	(*.txt,*.dat,*.csv)
7. Custom text data files (RR and ECG data)	(*.txt,*.dat,*.csv)
8. Biopac ACQ3 files (Biopac Systems Inc.)	(*.acq)
9. European data format (EDF and EDF+) files	(*.edf)
10. General data format (GDF) files	(*.gdf)
11. ISHNE Holter ECG data format files	(*.ecg)
12. Zephyr BioHarness ECG and RR data files	(*.csv)
13. Kubios HRV Matlab MAT files	(*.mat)

Kubios HRV supports the following RR interval file formats. First of all, data of three commonly used heart rate monitor manufacturers are supported. These are POLAR HRM and text files, SUUNTO SDF/STE/XML, and GARMIN FIT files. When analysing data of these devices it should however be noted that the **RR intervals must be measured/stored in beat-to-beat!** If only averaged data (e.g. HR values at every 5 seconds) are stored, one can not perform HRV analyses. In addition to Polar, Suunto and Garmin file formats, a support for plain RR interval text files (ASCII files) is provided. The input text file can include RR interval values in one or two column format. That is, the RR interval values can be given as

Type 1	Type 2	
0.759	0.759	0.759
0.690	1.449	0.690
0.702	2.151	0.702
0.712	2.863	0.712
0.773	3.636	0.773
⋮	⋮	⋮

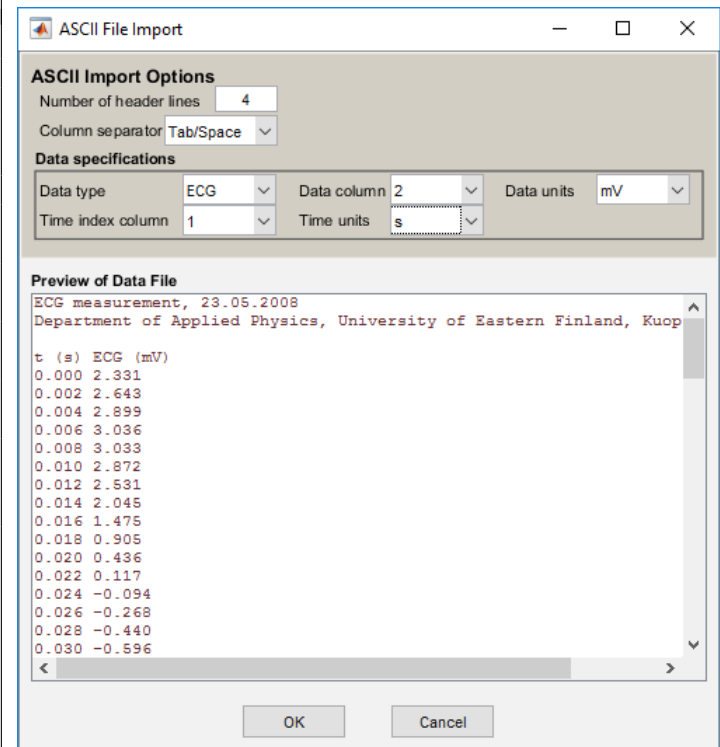
So in the second type of input, the first column includes the time indexes of R wave detections (zero time for the first detection) and second column the RR interval values. The RR interval values above are given in seconds, but millisecond values can also be given.

Similarly, ECG data can be given as input in text file formatted as shown below

Type 1	Type 2	
-0.173	0	-0.173
-0.119	0.002	-0.119
-0.025	0.004	-0.025
0.091	0.006	0.091
0.218	0.008	0.218
⋮	⋮	⋮

where the first column on the second format type is the time scale in seconds for the ECG data. The sampling rate of this example file is, thus, 500 Hz (samples per second). If ECG data is given according to the first type, user is prompted to enter the sampling rate manually.

In addition to above text file formats, a custom text file option is also provided. Using this option you can import text files including header lines and/or several data columns. Once you have selected an input file, an interface for importing the file into Kubios HRV is opened. This interface and the options that you need to set according to your data file are shown in Fig. 3.1.



Options to be set based on the data file:

- Number of header lines
- Column separator (tab/space, comma, or semicolon)
- Data type (ECG or RR)
- Data column (the ordinal number of data column)
- Data units (μ V, V or mV for ECG / ms or s for RR)
- Time index column (the ordinal number of time indexes)
- Time units (units of time indexes in ms, s or date/time format)
- ECG sampling rate in Hz (if no time index column is defined for ECG)

Once you have specified the above values for your file, press OK to proceed to opening the file.

Figure 3.1: The interface for importing custom text data files into Kubios HRV.



Figure 3.2: The graphical user interface of Kubios HRV analysis software.

from a corrupted RR interval series. The user can select between two methods: 1) Automatic correction (available only in Premium) and 2) Threshold correction. The automatic correction is a robust algorithm for detecting artefacts (missed beat detections, misplaced beats etc.) and ectopic beats (e.g. premature ventricular beats). The threshold correction simply compares every beat interval against a local mean RR, and identifies the beat as artefact if it exceeds the specified threshold. The threshold should be selected individually, because normal variability in RR intervals can be quite different between individuals, and therefore, a fixed threshold could over-correct the RR data. For details on these correction algorithms, see Sections 5.1.1.

If your RR data includes artifacts and you select a correction method, Kubios HRV displays on the RR data axis the corrections to be made on the RR intervals as shown in Fig. 3.4. In this case, the RR interval data includes two clear artifacts: 1) a simulated ectopic beat at 10:13:40 (short interval followed by longer interval) and 2) a simulated missed beat detection at 10:15:30. In order to remove these artifacts the automatic correction was selected. From the RR data axis it can be seen that the two artifacts (consisting of three erroneous beat intervals) would be corrected by the method and none of the normal RR intervals are affected (see the plot on the RR data axis and the summary of the right side of the axis). To make the corrections press the Apply button. A piecewise cubic spline interpolation method is used in the corrections. You can reverse the correction by pressing the Undo button or by selecting none as the correction level.

The importance of artifact correction is highlighted in Figs. 3.4 B-C. As can be seen, having only two artifacts within the 5-min segment that is analysed, has a significant effect on the time-domain HRV parameters, especially to SDNN, RMSSD and TINN. Thus, even single artifacts should always be taken care of prior to HRV analysis.

It should however be noted that artifact correction generates artificial values (when replacing the

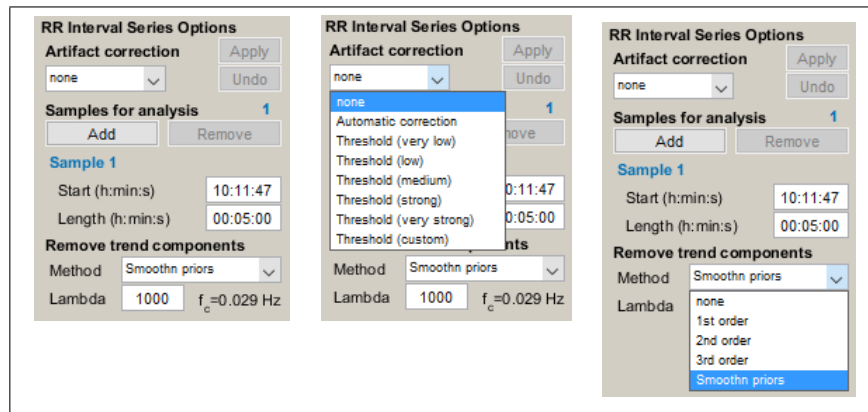


Figure 3.3: The RR interval series options segment of the user interface.

identified artifacts with interpolated values) into the RR interval data. Thus the number of corrected beats should not be too high (preferably <5%) not to cause significant distortion to analysis results. Also, if ECG is measured, you should first try to correct the RR intervals by editing the R-wave detections shown in the ECG data axis as described in Section 3.2.2.

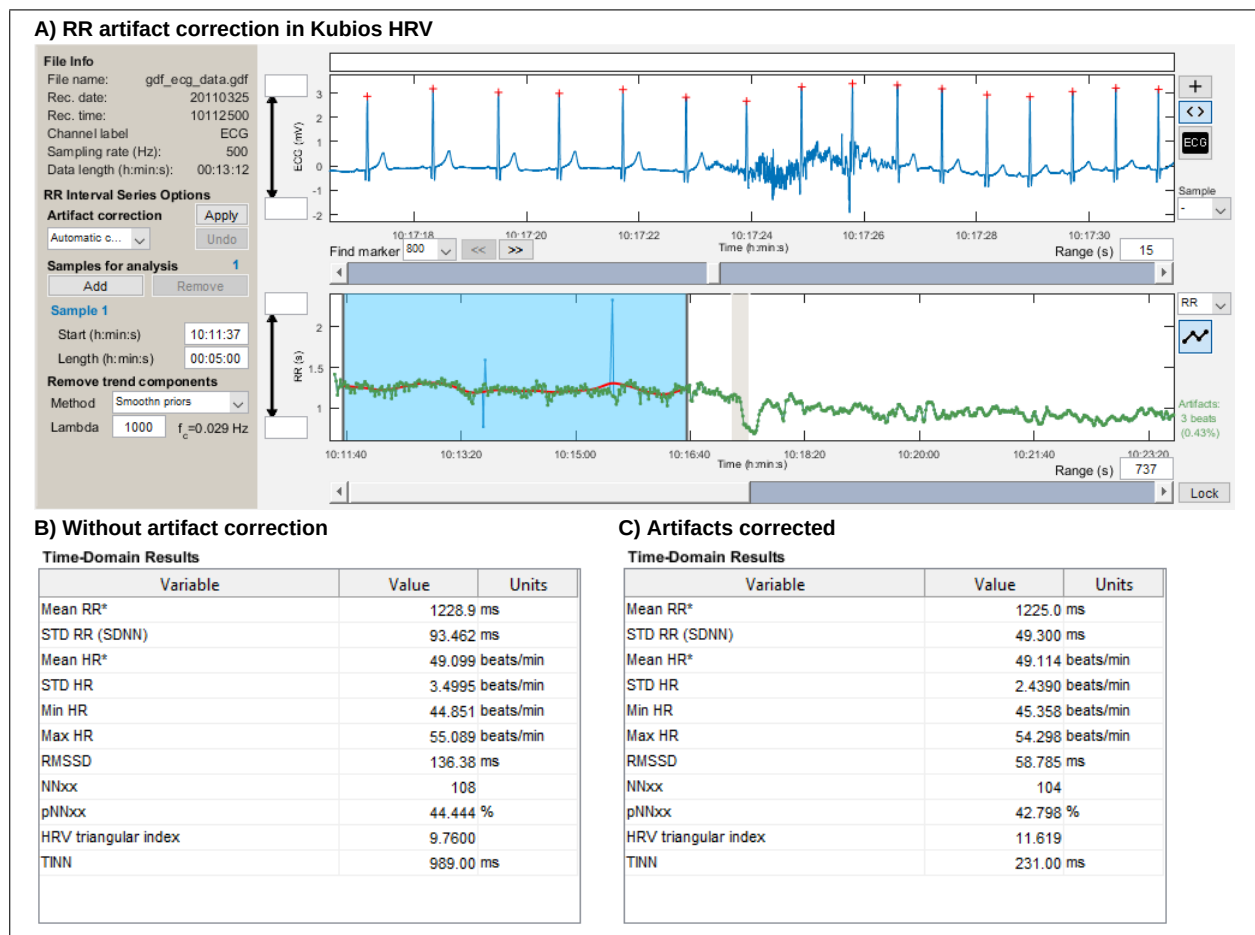


Figure 3.4: RR interval artifact correction. A) The artifact corrected series is visualized on top of the raw RR interval series, summary of corrected beats within the recording is given on the right side of RR data axis. Time-domain analysis results B) before artifact correction and C) after the artifacts have been corrected.

In the *Samples for analysis* options, the time period(s) of the RR interval data to be analysed can

be modified by adding or removing samples and by changing the start time or length of the sample. If more than one sample is selected, the analysis can be done either for the single samples separately or by merging the samples into one longer sample before analysis. This selection is visible under the RR series axis when multiple samples are selected. The starting point and length of the samples can also be changed by moving/resizing the patch over the RR data axis as described in Section 3.2.2. This section also describes how to add/remove samples to/from RR series axes.

Sometimes the RR interval time series includes a disturbing low frequency baseline trend component. Detrending options can be used to remove this kind of trend components. Detrending options include removal of the first, second, or third order linear trend or the trend can be removed using a method called smoothness priors which was presented in [50]. In the smoothness priors method, the smoothness of the removed trend can be adjusted by editing the Lambda value. The smoothness priors method is basically a time-varying high pass filter and its cut-off frequency can be adjusted with the Lambda parameter (the bigger the value of Lambda the smoother is the removed trend). The estimated cut-off frequency for the given Lambda value is presented next to the Lambda value edit box. The trend to be removed from the RR interval data is shown as a red line over the analysed RR data sample.

3.2.2 Data browser

The data browser segment shown in Fig. 3.5 displays the measured ECG signal and the extracted RR interval series. It should be noted that if RR interval data is given as input, the ECG axis will not be displayed and the RR series axis will be bigger in size. The ECG and RR interval data can be scrolled with the two sliders. The position of the ECG axis is displayed as a grey patch in the RR axis. This patch can also be moved with the left mouse button. The range of both axes can be changed by editing the Range values and also the Y-limits of the axes can be manually changed by editing the edit boxes on the left hand side of the axes. The ECG and RR interval axes can also be scrolled together by locking the axes by pressing the “Lock” button on the bottom right corner.

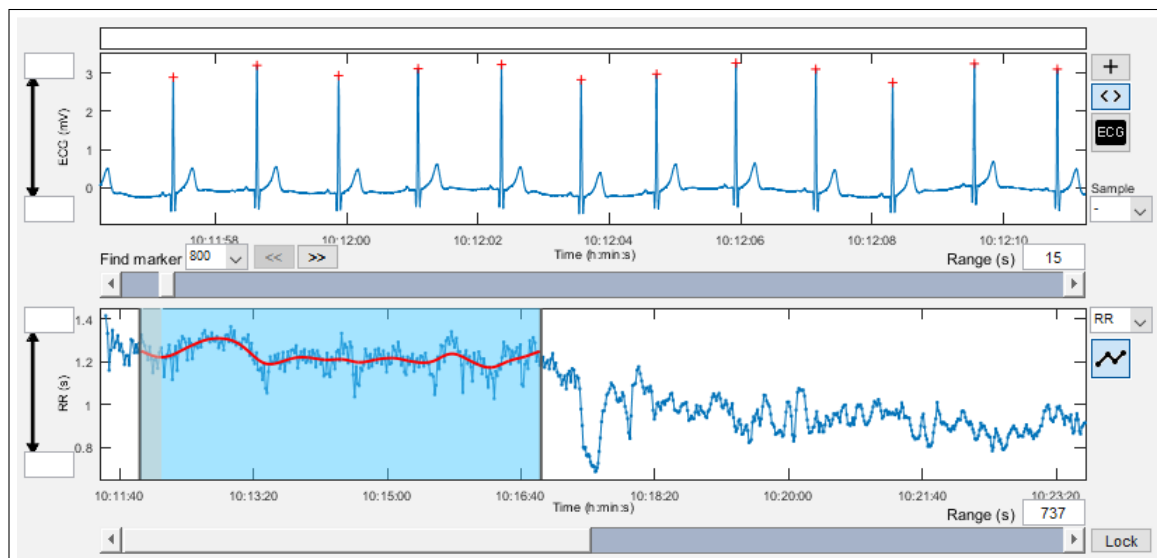


Figure 3.5: The data browser segment of the user interface.

In addition to the visualization of the ECG and RR data, the main function of this segment is to enable RR artifact correction, which can be done in two ways. If only RR data is available, the artifact corrections described in Section 3.2.1 are displayed in the RR axis. If the ECG is available, RR intervals can be corrected by editing the misdetracted R-wave as follows. Each detected R-wave is marked in the ECG axis with a “+” mark. Each mark can be moved or removed by right clicking it with the mouse. In addition, new R-peak markers can be added by either right clicking some other marker and selecting “Add”, or by pressing the (+) button on the right hand side of the ECG axis. Moved or added R-peak markers are by default snapped to closest ECG maximum, but manual positioning can also be achieved by pressing the (<>) button on the right hand side of the ECG axis. The changes made in R-wave markers will be automatically updated to RR interval series.

The selected sample(s) (light blue patches over the RR data axis) can be modified with mouse as follows. Each sample can be moved by grabbing it from the middle with the left mouse button and resized by grabbing it from the left or right edge. You can also add a new sample to a specific location in the RR data axis by right clicking on the RR axis. The new sample will start from the clicked time instant and the length of the new sample is by default equal to the previous sample. After right clicking on the RR axis, a small popup window opens in which the sample start time and length can be accepted/modified. When more than one analysis sample has been generated, any sample can be removed by right clicking it with the mouse.

In addition, the data browser segment includes buttons for displaying a printout of the ECG recording (ECG) and moving the ECG axis view to the beginning of a selected sample (on the right hand side of the ECG axis), scrolling the markers of the recording session (below the ECG axis), and changing the RR data type (RR or HR) and display mode (M) (on the right hand side of the RR axis). An example of the ECG printout is shown in Fig. 3.6. When clicking on the button for displaying a printout of the ECG recording, a popup window will appear in which you can select the range for the ECG to be printed (e.g. the whole recording or the range of the analysed sample). In addition, you can adjust "print speed" in mm/sec of the ECG in this popup window. Once you have defined the range for ECG printout and clicked the OK button, the ECG signal is displayed in a preview window where it can be easily printed or saved into a PDF file (see Section 3.3.2 for details on the preview window functionalities).

3.2.3 Results view

The results for the selected RR interval sample are displayed in the results view segment (see Fig. 3.2). The results are divided into time-domain, frequency-domain, nonlinear and time-varying (available only in Premium) results. The results of each section are displayed by pressing the corresponding button on the top of the results view segment. The results are by default updated automatically whenever analysis samples or analysis settings are changed. The processing time for computing all the analysis results depends on the length and number of analysis samples that you have generated. If the updating of the results takes too much time, you can disable the automatic update by unchecking the "Auto-refresh results" check box on top of the results view segment. When unchecked, you can do all the changes to the analysis samples and settings at once, and then press the "Refresh" button when you want to update the results.

Time-domain results view

The time-domain results view shown in Fig. 3.7 A) displays the time-domain HRV parameters in a table and the RR interval and HR histograms in the two axes. Most of the results are calculated from the detrended RR interval data (if detrending is applied), but there are two obvious exceptions to this (i.e. mean RR and mean HR), which are marked with the * symbol. In the edit boxes below the histograms, you can define fixed lower and upper limits for RR and HR values. These limits saved in software preferences, so you only need to enter them once. These limits have effect on how the RR and HR histograms are displayed, not only in the results view segment but also in the report figure described in Section 3.3.2. If you leave the edit boxes empty, the histograms are auto-scaled according to the minimum and maximum values in the data.

Frequency-domain results view

The frequency-domain results view shown in Fig. 3.7 B) displays the results for both FFT and AR spectrum estimation methods. Both methods are applied to the detrended RR series. The spectra of the two methods are presented in the two axes (FFT spectrum on the left and AR spectrum on the right). In Premium version, user can choose in software preferences (see. Section 3.4) to use Lomb-Scargle periodogram instead of FFT based Welch's periodogram. The frequency axes of the spectra are fixed to range from 0 Hz to the upper limit of HF band plus 0.1 Hz. Thus, for the default frequency band settings the frequency axis range is 0–0.5 Hz. The results for both spectra are displayed in the table on the left. If ECG is measured, an estimate of the respiration frequency is also computed. This estimate, i.e. electrocardiogram derived respiration (EDR) is shown as a vertical line in both spectrum estimates. The EDR value is also shown below the spectrum Y-limit options.

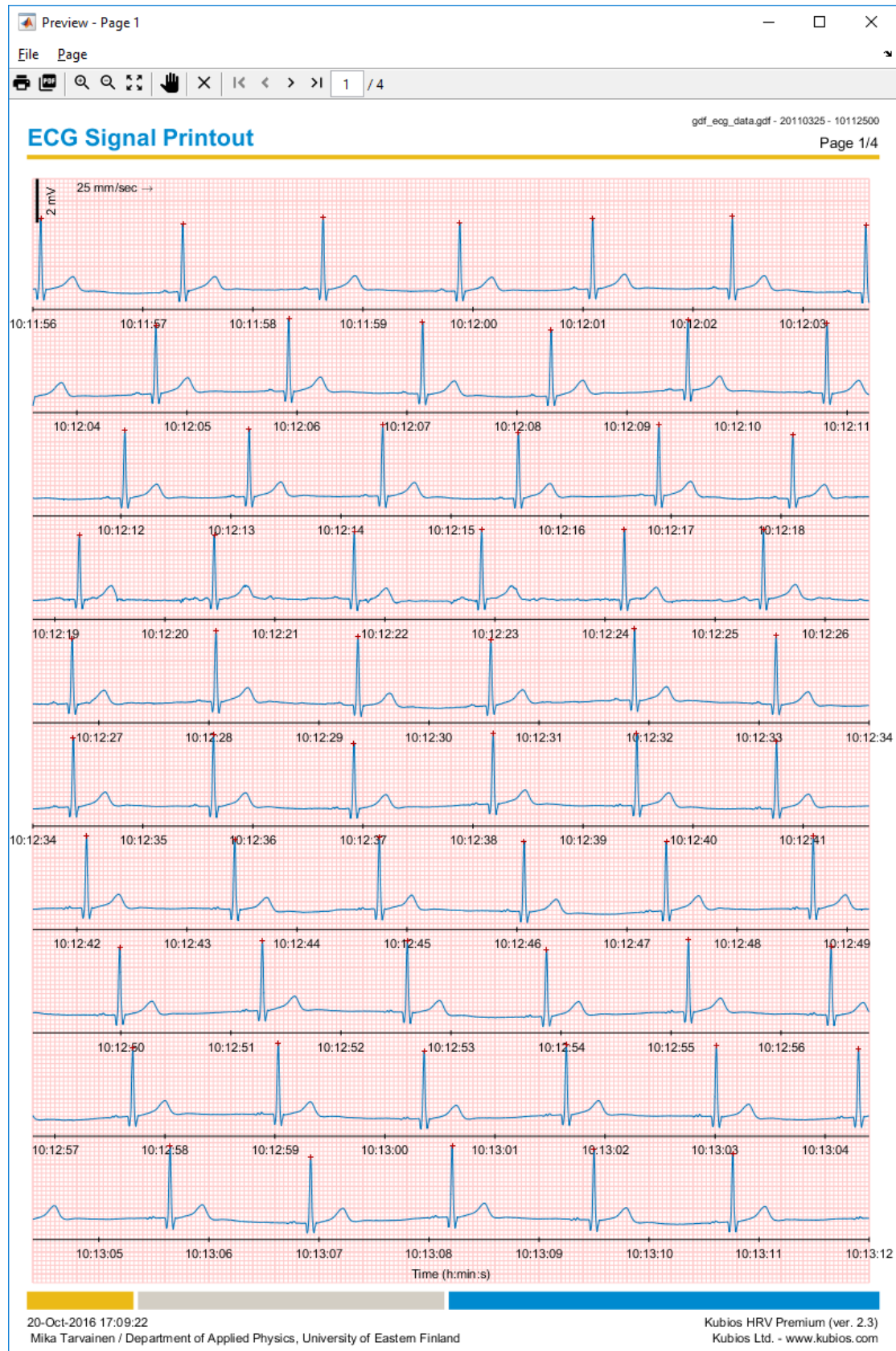


Figure 3.6: The printout of the ECG signal generated by the software.

The frequency-domain results view includes the following settings. The power axes limits, can be adjusted with the options below the spectrum axes. The power axes can be selected to have either

(VLF), low frequency (LF), and high frequency (HF) bands limits. The default values for the bands are VLF: 0–0.04 Hz, LF: 0.04–0.15 Hz, and HF: 0.15–0.4 Hz according to [51]. The default values for the bands can be restored by pressing the Defaults button. Adjustments to the frequency bands here apply only for the current session, if you want to change these settings permanently, you need to do it by editing software preferences (see Section 3.4).

Nonlinear results view







The nonlinear results view shown in Fig. 3.7 C) displays all the calculated nonlinear variables in one table. The Poincaré plot and the DFA results are also presented graphically in the two axes. In the Poincaré plot (left hand axis), the successive RR intervals are plotted as blue circles and the SD1 and SD2 variables obtained from the ellipse fitting technique are presented (for details see Section 5.2.3). In the DFA plot (right hand axis), the detrended fluctuations $F(n)$ are presented as a function of n in a log-log scale and the slopes for the short term and long term fluctuations α_1 and α_2 , respectively, are indicated (for details see Section 5.2.3).



Time-varying results view

The time-varying results view shown in Fig. 3.7 D) displays the time-varying trend of the selected variable. The variable is selected using the two dropdown buttons on the top left corner of the view. Selectable variables are divided into time-domain, frequency-domain, and nonlinear categories. The trend of the selected variable will appear immediately in the axis. Trend lines can be plotted with or without background grid and markers (checkboxes above the axis). When the time-varying spectrum is selected for view, a color bar indicating the power values is also shown on the right. The color map of the spectrum can be changed with the Color map dropdown button. The adjustable options for the time-varying analysis include the window width and grid interval for the moving window, which is used to calculate the results. In addition, the time-varying spectrum can be estimated using either the spectrogram method or the Kalman smoother method. The latter one is a parametric approach where the time-varying AR parameters are solved with a Kalman smoother algorithm. The adaptation speed of the algorithm can be adjusted manually by changing the Adaptation coeff. value. For bigger values of this coefficient the algorithm adapts faster to local changes in the signal with the expense of increased variance. The default value for the adaptation coefficient is 0.0001.

3.2.4 Menus and toolbar buttons

The user menus and toolbar buttons are located on the upper left hand corner of the user interface. There are all together three user menus and eight toolbar buttons. The toolbar button icons and their actions are given below

-  **Open new data file** button is for opening a new data file for analysis. If the results of the current analysis have not been saved, user is prompted to do so.
-  **Save results** button is for saving the analysis results. The results can be saved in ASCII, PDF, and MATLAB MAT file format (see Section 3.3 for details).
-  **Append results to "SPSS friendly" batch file** (available only in Premium) button is for adding the current analysis session results into an existing (or creating a new) "SPSS friendly" batch file (see Section 3.3 for details).
-  **Print results** button is for printing the current results without opening report sheet windows.
-  **Report sheet** button opens the report sheet preview window which include all the analysis results (see Section 3.3.2 for details).
-  **Edit preferences** button opens a preferences window in which you can, e.g., change the default values for analysis options (see Section 3.4 for details).

-  **About Kubios HRV** button opens the about dialog of the software, which includes the version number and contact information. Also the Kubios HRV End User License Agreement can be viewed in the about dialog.
-  **Close file** button closes the current data file. If the results of the current analysis have not been saved, user is prompted to do so.

All the above actions are also available on the user menus. The File menu includes Open, Save Results, Save Results As, Append Results to "SPSS friendly" Batch File, Print Results, Edit Preferences, Close, and Quit commands. The Open, Save Results, Append Results to "SPSS friendly" Batch File, Edit Preferences, and Close commands work exactly as the corresponding toolbar buttons. The difference between the Save and Save As commands is that when the results have already been saved, the Save command automatically overwrites these results, whereas the Save As command asks the user for a new file name. The Quit command of the File menu is for exiting from the software. The View menu includes Markers menu and Report sheet command. The latter works as the corresponding toolbar button. The Markers menu, on the other hand, is for displaying possible stimuli or event markers presented in the experimental procedure and stored in the data file. If no markers are found from the data file the Markers menu will be disabled. Finally, the Help menu includes links to Kubios Home and Kubios Support pages, link to contact us by e-mail, and the About Kubios HRV (opening the same about dialog as the corresponding toolbar button) commands.

3.3 Saving the results

The analysis results can be saved by selecting Save Results or Save Results As from the File menu or by pressing the save button on the toolbar. This will open a file save dialog in which the saving type can be selected. There are three different types in which the results can be saved. That is, the results can be written in an ASCII text file for further inspection, the report sheets generated from the results can be saved in a PDF-file, and the results can be saved in a MATLAB MAT-file (available only in Premium). In addition to these, Kubios HRV Premium includes the Append to "SPSS friendly" batch file option to save session results into existing batch file (ideal for saving group results).

3.3.1 ASCII text file

When the ASCII text file is selected for the saving type, the numeric results of the analysis will be written in an ASCII text file. The resulting text file includes the following information in the enumerated order.

1. Software, user, and data file informations
2. Used analysis parameters
3. Samples selected for analysis
4. Time-domain results
5. Frequency-domain results
6. Nonlinear results
7. Time-varying results
8. RR interval data and spectrum estimates

The columns of the file are separated with comma or semicolon (can be adjusted in software preferences) so that the results could easily be imported to, e.g., spreadsheet programs such as the Microsoft Excel® for further inspection.












3.3.2 Report sheet

The report sheets include all the analysis results for the current session. The report sheets open in a preview window where they can be easily printed or saved into a PDF file. Kubios HRV Premium produces two report pages for every analysis sample. The first page includes all the time-domain, frequency-domain, and nonlinear analysis results (see Figs. A.2 and A.3). The second page (available only in Premium) includes time-varying analysis results (see Fig. A.6). The RR interval data and the sample

selected for analysis are presented on the two axes on top of both report pages and the analysis results below them. If multiple analysis samples have been selected, report pages are generated for each sample.

When Save Results have been selected, the report sheet(s) can be saved in a single PDF-file by selecting Report figure as the saving type in the save dialog. In this case, the report sheet(s) will not be displayed, but just saved in the selected PDF-file. If you wish to view the report sheet(s) and/or to export it into some other file format choose Report sheet from the View menu or just press the corresponding toolbar button. This will open the report sheet windows for view.

The report sheet window includes 11 toolbar buttons and File and Page menus on the upper left hand corners of the windows. The toolbar button icons and their actions are given below

-  **Print** button opens a print dialog from which the report sheet(s) can be sent to the selected printer.
-  **Save all pages as PDF-file** button is for saving all report sheets into a single PDF-file.
-  **Zoom in** button if for zooming in (magnifying) the report sheet.
-  **Zoom out** button is for zooming out the report sheet.
-  **Reset to original size** button can be used to restore the original zoom level. This also resets the size of the corresponding report sheet window to its original size.
-  **Move visible area** button is for moving the visible area of the zoomed report sheet in the report window (just grab the sheet with mouse and drag it to the desired direction).
-  **Close** button is for closing the report sheet.
-  **Go to first page** button is for displaying the first report sheet page in the preview.
-  **Go to previous page** button is for displaying the previous report sheet page in the preview.
-  **Go to next page** button is for displaying the next report sheet page in the preview.
-  **Go to last page** button is for displaying the last report sheet page in the preview.

The File menu includes Save All Pages as PDF, Print Current Page, Print All Pages, and Close commands. The Save All Pages as PDF, Print All Pages, and Close commands are also given as toolbar buttons described above. The Page menu includes commands for changing the page that is displayed in the preview window (First page, Previous page, Next page, Last page), which are all also given as toolbar buttons.

3.3.3 Matlab MAT-file (available in Premium)

In addition to saving the numeric results into an ASCII text file or saving the report sheet(s) in a PDF-file, the analysis results can also be saved in a MATLAB MAT-file (compatible with MATLAB® 2016bR2006b or later). The MAT-file include all the analysis results and analysis parameters, exactly as they where when you saved the results. In addition, these files include the raw data (ECG or RR data). This saving option has two purposes:

1. The main purpose of the MAT-file is that by opening the MAT-file in Kubios HRV, you can return to the previously performed analyses session as it was (all settings and analysis samples are presented as they were) when the analysis was originally performed. Thus, the MAT file makes it easy for you to change something in the analysis (e.g. add a new analysis sample or change some settings) and re-analyse the data. Thus, we recommend that you save the analysis results always as a MAT-file, just in case if something needs to be done differently.

2. In addition, the MAT files are useful for anyone working with MATLAB (further analysis or processing can be performed easily by loading the MAT-files into MATLAB).

The MAT files include a single structured array variable named `Res`. The `Res` variable includes the numeric results as well as the RR interval data and all the analysis options. The `Res` structure includes four fields which are shortly described as follows

`f_name`: File name of the analysed data file
`f_path`: Full path for the analysed data file
`CNT`: Basic information of the data file (the field name refers to Neuroscan CNT-file for historical reasons)
`HRV`: Used analysis options, RR interval data, and all analysis results.

The `HRV` field is the most essential one of these fields. The `HRV` field includes six fields the contents of which are shortly described as follows

`Param`: The analysis options used in the calculation of the results
`Data`: The RR interval data
`Statistics`: Time-domain analysis results
`Frequency`: Frequency-domain analysis results
`NonLinear`: Nonlinear analysis results
`TimeVar`: Time-varying analysis results

The variable names of the different fields are more or less self-descriptive and are not documented here.

3.3.4 "SPSS friendly" batch file (available in Premium)

In Kubios HRV Premium, you can also save the analysis results into a "SPSS friendly" batch file. This saving options is ideal for saving group results, for example if you need to analyse HRV data of several subjects and want to be able to have the group results easily available for statistical testing e.g. in MS Excel or SPSS. Alternatively, you can use the batch file saving option for saving HRV results of repeated personal recordings, e.g. to monitor training effect or daily stress levels. Kubios uses Comma Separated Values (CSV) file format for the batch file, which can be easily imported into many spreadsheet and statistical software packages (MS Excel, SPSS).

The Append to "SPSS friendly" batch file functions as follows:

1. When saving the analysis results of the first subject (i.e. when you want to initialise a new CSV file), select the destination and file name for the new CSV file from the file dialog. In this case, Kubios HRV will initialise the CSV file by writing the column labels and add the analysis results into the first row below the column labels.
2. When saving the analysis results of other subjects, simply select the previously saved CSV file. In this case, Kubios HRV will add the results of the current analysis session into the last row of the file.

The structure of the "SPSS friendly" batch file is presented in Fig. 3.8. Every row of the batch file consists of the file name string and used analysis parameters values ([1x20] array); followed by the following information for every analysis sample: sample info consisting of sample onset/offset time and artifact correction statistics ([1x2] array), and HRV analysis results ([1x79] array). For more details on the different HRV analysis variables please see Table A.1.

3.4 Setting up the preferences

Kubios HRV includes several settings related to how the ECG or RR interval data is processed and analysed. The default values for these settings are designed to be more or less suitable for short-term (normal human) HRV recordings, but may sometimes need to be redefined. Some of these settings can be adjusted in the user interface to apply for the current analysis session, but in order to make permanent changes into these settings you need to edit them at software preferences. Preferences can be edited by selecting Edit Preferences from the File menu or by pressing the corresponding toolbar button. This will open the preferences window in which the preference values can be redefined. The preferences are divided into four categories: 1) User information, 2) Input data & pre-processing, 3) Analysis options (divided into time/frequency-domain, nonlinear and time-varying analysis preferences), and 4) Report settings.

In the **User information** settings shown in Fig. 3.9 you can set up your personal contact information (Name, Department, and Organization). This information will only be included in the bottom left corner of the report sheet and in the beginning of the ASCII text file including the analysis results. That is, the user information is meant just for indicating the person/organisation that has carried out the analysis.

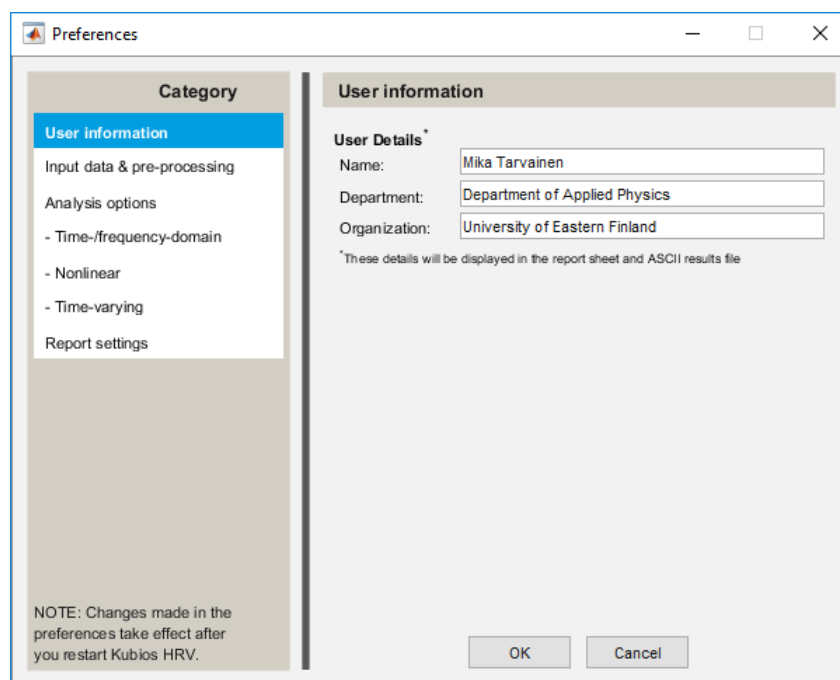


Figure 3.9: Set up preferences window of the software – User information settings.

From the **Input data & pre-processing** settings shown in Fig. 3.10, you can change the default input data type into any of the file formats mentioned in Section 3.1. The selected input data type is used as the default data type every time a new data file is opened, so you can save one additional click by defining the data type you usually work on as the default. In the QRS detection settings, you can force Kubios to look for the R-waves either from positive or negative amplitudes, or let Kubios to decide (R-wave polarity=Automatic). Also, you can manually fix the prior guess for the average RR interval (used by the QRS detector as initial value), or let Kubios try to estimate it automatically. By default the QRS detection settings are set to automatic and there is no reason to change them unless you are experiencing problems in R-wave detection. Problems may arise if you are trying to analyse something else than normal human ECG data. For example, in small animal studies, the prior guess for the RR interval might need to be fixed manually (because the mean RR interval is considerably shorter compared to normal human recordings). In addition, the interpolation rate (used for forming an equidistantly sampled time series from the non-equidistantly sampled RR interval data, which is required for FFT and AR based spectrum estimation) and the detrending method can be set here. As the interpolation method a piecewise cubic spline interpolation is used and the default rate is 4 Hz. For short-term HRV analysis, we recommend the smoothness priors detrending method (with a smoothing parameter, which gives a cutoff frequency

below the LF band), which is described in Section 5.1 and in reference [50].

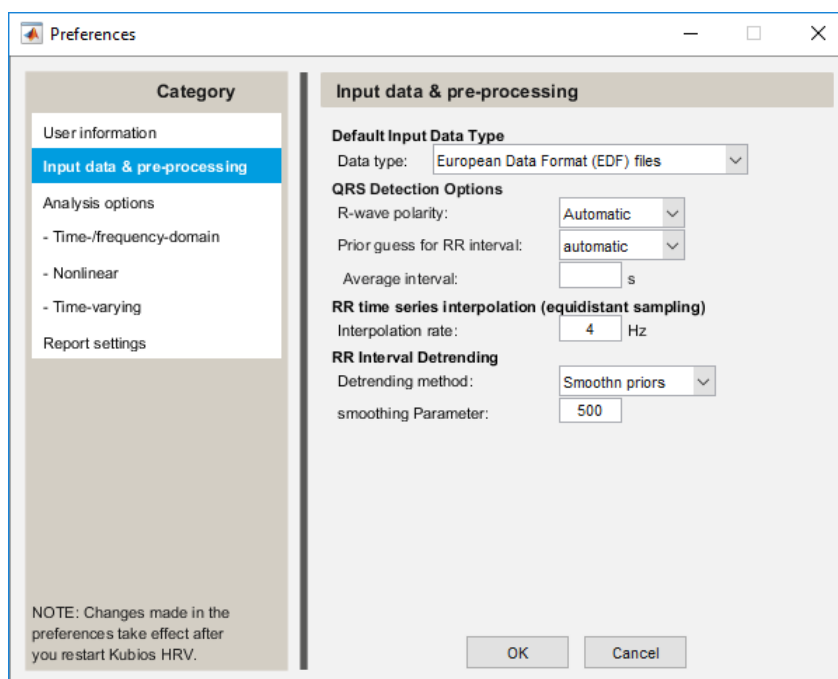


Figure 3.10: Set up preferences window of the software – Input data & pre-processing settings.

The **Analysis options** settings include some general analysis options, and detailed settings of different analysis methods under three sections: 1) Time/frequency-domain, 2) Nonlinear and 3) Time-varying. The general analysis settings shown in Fig. 3.11 includes selection of analysis to be performed: 1) General analysis (all time-domain, frequency-domain and nonlinear analysis for selected stationary samples), and 2) Time-varying analysis. Only selected analysis will be performed and thus un-checking the unnecessary analysis type will speed up the computations. Using the settings for RR interval samples, you can define how many analysis samples are generated by default and what is the length of these samples. In case of several samples, you can choose the analysis type between Single samples (in this case, Kubios will perform analysis for every sample separately) and Merge samples (the samples are merged into one longer sample for which analysis is then performed). Finally, the Update mode can be changed between Automatic (analysis results are refreshed automatically) and Manual (you need to refresh results manually).

The time and frequency-domain analysis settings are shown in Fig. 3.12. For time-domain analysis methods, you can adjust the window width of the moving average filter (default 5 beats), which is used to extract minimum and maximum HR values. Also, you can adjust the threshold used in the computation of NNxx and pNNxx parameters (default 50 ms → NN50 and pNN50). Under HRV frequency bands, the very low frequency (VLF), low frequency (LF), and high frequency (HF) bands of HRV frequency-domain analysis can be adjusted. The default values for these frequency bands are VLF: 0–0.04 Hz, LF: 0.04–0.15 Hz, and HF: 0.15–0.4 Hz according to [51]. The rest of the settings relate to spectrum estimation methods. The points in frequency-domain is given as points/Hz and corresponds by default to the window width of the FFT spectrum. If spectrum interpolation is desired the points in frequency-domain can be increased. The spectrum for the selected RR interval sample is calculated both with Welch's periodogram method (FFT spectrum) and with an autoregressive modeling based method (AR spectrum). In the Welch's periodogram method, the used window width and window overlap can be adjusted by editing the corresponding value. The default value for window width is 300 seconds and the default overlap is 50 % (corresponding to 150 seconds), which produce three overlapping windows for a 10-min (600 sec) analysis sample. As an alternative to FFT spectrum, you can select to use the Lomb-Scargle periodogram, which does not assume equidistant sampling and has been recommended for HRV spectral analysis in some studies. The default smoothing window for this spectrum estimate is 0.02 Hz. For the AR spectrum, there are two options that can be selected. First, the order of the used AR model can be selected. The default value for the model order is 16. The second option is whether

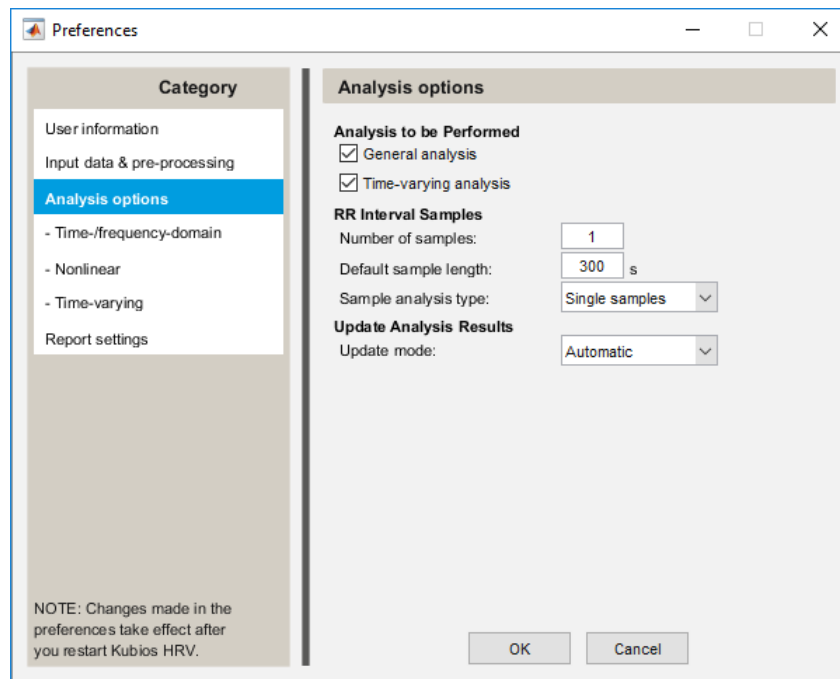


Figure 3.11: Set up preferences window of the software – Analysis options settings.

or not to use spectral factorization in the AR spectrum estimation. In the factorization the AR spectrum is divided into separate components and the power estimates of each component are used for the band powers. Spectral factorisation has been shown to provide some advantage especially when e.g. the HF component is partially overlapping with the LF band [46], but AR spectrum without factorisation is probably more robust estimate of spectrum.

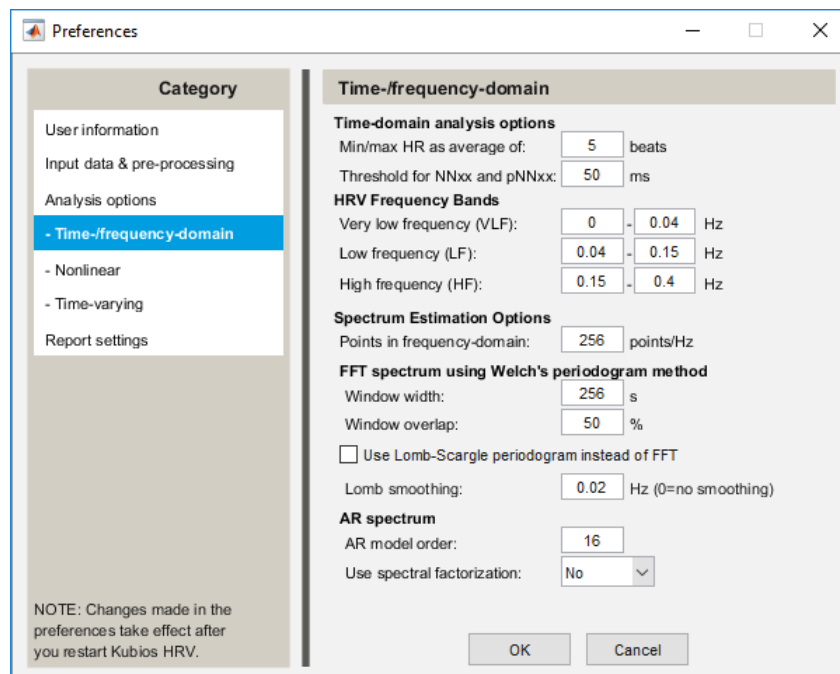


Figure 3.12: Set up preferences window of the software – Analysis options: time-/frequency-domain methods.

The nonlinear analysis settings are shown in Fig. 3.13. The embedding dimension m (default 2 beats) and the tolerance value r (default 0.2 times SD) used in for the computation of Approximate entropy

(ApEn) and Sample entropy (SampEn) can be modified. Note that the tolerance value is adjusted in relation to the standard deviation of the RR interval data. Next, limits of the short-term (N1) and long-term fluctuations used in the Detrended fluctuation analysis (DFA) can be modified (defaults 4-12 and 13-64 beats, respectively). Finally, the embedding dimension (default 10 beats) used both in the computation of the Correlation dimension (D_2) and in the Recurrence plot analysis (RPA), and the threshold level (default $\sqrt{10}$) used in RPA, can be modified. For more information on the meaning of these different options see Section 5.2.3.

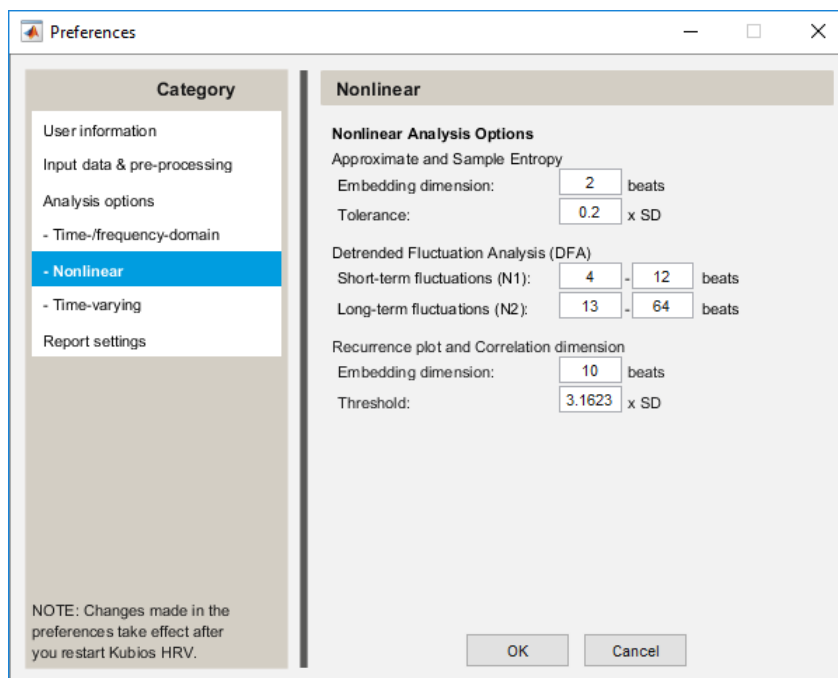


Figure 3.13: Set up preferences window of the software – Analysis options: nonlinear methods.

The time-varying analysis settings are shown in Fig. 3.14. You can adjust the width (default 60 sec) and grid interval (default 10 sec) of the moving window used for time-varying analysis. The grid interval is the time interval by which the window is moved at every step, and analysis results are available at these intervals. For example, you want to perform time-varying analysis at 10-min non-overlapping segments for the whole duration of recording, you need to select the whole recording as an analysis sample and then define the window width and grid interval both to 600 seconds. For the time-varying spectrum estimation there are two options: 1) the well known spectrogram (default) and 2) a Kalman smoother spectrum estimate proposed in [46].

The **Report settings** shown in Fig. 3.15 includes the following options. The contents of the results to be exported can be selected by checking the General and/or Time-varying results options. If either one of these is unchecked, only the selected results will be exported (in PDF reports or the other export file formats). Concerning the ASCII text file as well as the "SPSS friendly" CSV batch file, the field delimiter and decimal point used when saving the results can be selected (the default values being comma "," for field delimiter and dot "." for decimal separator). The paper size of the report sheet can be changed between A4 (210×297 mm) and Letter (8.5×11 inch) size. The default paper size is A4. The report sheet settings include also three options regarding how the time-varying analysis results are presented: 1) select two time-domain parameters to be displayed in report sheet (STD RR, STD HR, RMSSD, NNxx, pNNxx, HRV triangular index, TINN), 2) select VLF, LF and HF band power units (ms^2 or log) and presentation of relative band powers (n.u., % or LF/HF ratio), and 3) select two nonlinear parameters to be displayed in report sheet (SD1, SD2, SD2/SD1, ApEn, SampEn, DFA α_1 , DFA α_2).

All modifications for the preferences are saved by pressing the OK button. Note that the OK button saves the preferences, but they will be applied only in the next session. A session is considered to be ended when the program is restarted or Close file is selected. If, on the other hand, a new file is opened (without first closing the previous file) preferences will not be applied, but the local settings (changes made in the user interface) are applied for the new file as well.

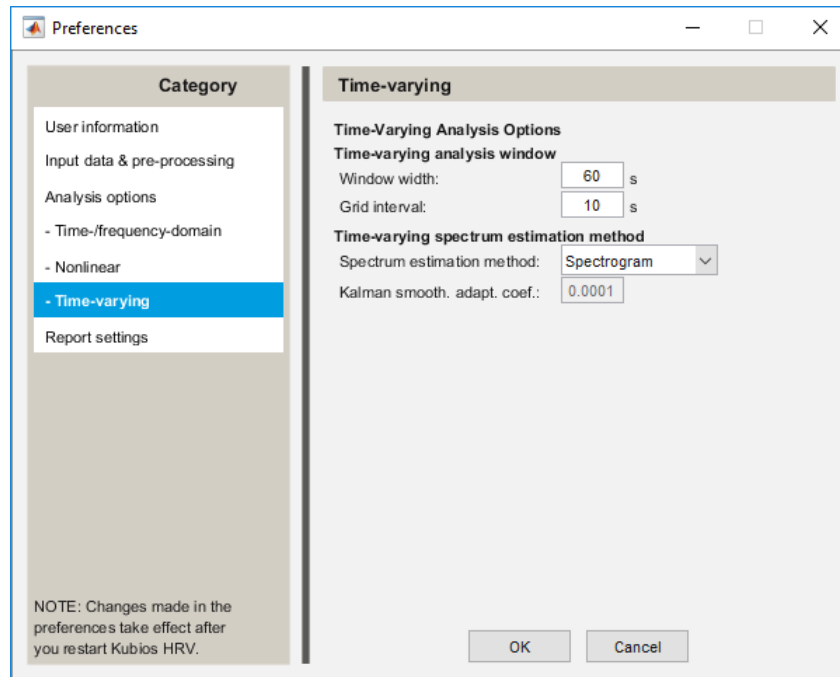


Figure 3.14: Set up preferences window of the software – Analysis options: time-varying methods.

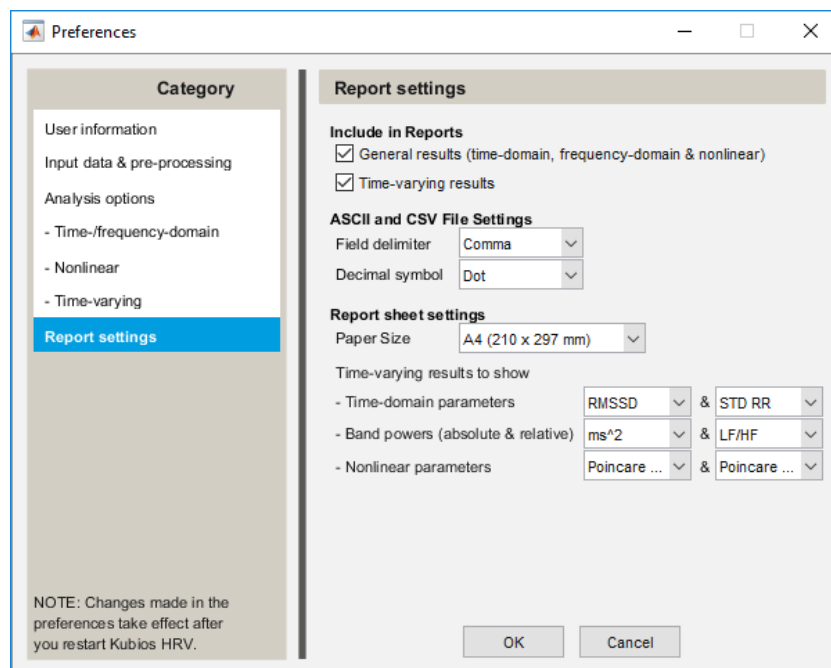


Figure 3.15: Set up preferences window of the software: Report settings category.

In addition to the actual analysis options, there are various other editable options which have mainly influence on the usability of the software. Such options are e.g. the Range and Y-limit values of the data axis and various visualization options. The values of these options are preserved in memory and any changes made to them will be applied in the future sessions. Also the preference directory paths from where the data file is searched for and in which the results are saved are preserved in memory. The last nine opened data files will also appear in the File menu of the user interface and can be reopened from there.

All the preferences and preserved options used by Kubios HRV are saved in user specific folders¹.

Windows 7,8 or 10:

HRV Standard: C:\Users\\AppData\Roaming\Kubios\KubiosHRVStandard

HRV Premium: C:\Users\\AppData\Roaming\Kubios\KubiosHRVPremium

Mac OSX:

HRV Standard: ~/Library/Preferences/Kubios/KubiosHRVStandard

HRV Premium: ~/Library/Preferences/Kubios/KubiosHRVPremium

where <username> is the name of your user profile. The folder will include a file named KubiosHRVprefs.mat, which includes all the preferences for the analysis options, user information and user interface usability. The file is created when Kubios HRV is started for the first time and it will be updated whenever the preference values are edited/updated. The original settings of the preferences can be restored by deleting this preference file. Also, if the preference file gets somehow corrupted, the preference file will be rewritten using the default values.

¹Note that the AppData folder in Windows 7 or higher is hidden by default and are not visible in the Windows File Explorer if the "Show hidden files and folders" is not selected from the "Folder Options" section of the File Explorer.

Chapter 4

Sample run

In this chapter, we present a sample run of the software. The sample run is made for the GDF data file (`gdf_ecg_data.gdf`) distributed with this software. The sample data is measured from a healthy young male during an orthostatic test. The change in the posture is known to be reflected in the low frequency and high frequency HRV in an opposite way. That is, when subject stands up after lying for few minutes a strong decrease in the HF power and a more gradual increase in LF power are observed. In addition, a strong increase in heart rate is observed immediately after standing up, which aims to compensate the sudden decrease in blood pressure. In the Sample run 1 ([4.1](#)), the data file is analysed by considering the lying and standing periods separately. Alternatively, the file could also be analysed with the time-varying methods as a whole as shown in Sample run 2 ([4.2](#)).

4.1 Sample run 1 - Stationary analysis

In this sample run, we show how to make the time-domain, frequency-domain, and nonlinear analysis, for the lying and standing periods of the orthostatic measurement separately. This task can be easily accomplished in a single session. First, start the software and open the data file into the user interface. At this point, you can edit any of the analysis options to fit your demands. If you need to analyse several data files with the same options, you should modify analysis options at the software preferences. For example, if you are using the Premium version, you can choose to perform only stationary analysis on the selected samples, and thus you might want to turn off the time-varying analysis from the software preferences.

When you open a data file in Kubios, you will by default have one pre-defined analysis sample (illustrated with the light-blue patch over the RR time series axis). In this case, we modify this sample to be 300 seconds long (5-min) and place it over the supine period. Next, we add a second analysis sample for analysing the standing period. This can be done by right clicking the RR axis at the point where you want the second sample to start, then pressing Yes to the Add sample popup window, and OK to verify the sample properties. If necessary you can move the two samples or change their length by grabbing them with the left mouse button from the center or edge of the sample. Alternatively, the ranges of the samples can be changed by editing the Start and Length values in RR interval series options segment (see [Section 3.2.2](#)). The sample selections that we made for this sample run are illustrated in [Fig. A.1](#). Note that the Sample analysis type option under the RR axis is set for Single samples. Then, analysis results are calculated for both samples separately. If, on the other hand, Merge samples is selected, then the two samples are first merged into one sample and the analysis results are calculated for this merged sample.

Since we are now only interested in the changes in LF and HF bands, we wish to remove the lowest frequency trend components from the RR series. These trend components affect on the time and frequency-domain variables and, thus, by removing the trend from the data enables these variables to better describe the LF and HF variability which we are interested of. We select to remove the trend with the smoothness priors based method. Once the detrending method is selected red lines appear over the RR interval data indicating the removed trend components. The smoothness of the removed trend in the smoothness priors method can be adjusted by changing the Lambda value. The smoothness priors detrending method can be compared to a high-pass filter in which the cutoff frequency is determined by

the lambda value (bigger lambda corresponds to lower cutoff). The estimated cutoff frequency of the detrending method is also shown next to the Lambda value. Since we are now interested in LF and HF frequencies, we wish to make sure that the detrending does not remove those frequencies. This can be easily done by changing the Lambda value in such a way that the cutoff frequency is below 0.04 Hz. The effect of detrending can also be verified by inspecting how it changes the FFT spectrum. Here, we set the Lambda value to 500.

The time-domain, frequency-domain, and nonlinear analysis results for the selected samples can then be viewed in the results view segment. Just make sure that the results have been updated (check that the Auto-refresh is checked on top of the results view segment). Press the Time-domain, Frequency-domain, or Nonlinear buttons to view the corresponding results. Note that the results are shown only for one of the samples at a time. To take a look at the results of the other sample press the \ll or \gg button on the top right corner of the results view segment (the text on the left changes to indicate which sample's results are shown, this sample will also be highlighted in the RR series axis). Note that you can force a common Y-limit for the spectra of both samples by setting a common Y-limit value manually in the frequency-domain results view.

The analysis results are now ready to be saved, and we will save the results in all possible formats here. To do this we select Save Results from the File menu or just by pressing the save button on the toolbar. Then select Save all (*.txt,*.mat,*.pdf) (note that the *.mat option is available only in Premium version) as the save type and enter a file name. You do not need to give any extension to the file name. The numeric results of the analysis will be saved in the *.txt text file, in the *.mat MATLAB file and the report sheets in the *.pdf file. The generated PDF-file will now include two pages, one for the results of the first RR interval sample (the lying period) and one for the second sample (standing period). These report sheet pages are shown in Figs. A.2 and A.3. Note: if you are using the Premium version and time-varying analysis is not turned off, you will have altogether four report sheet pages (two extra pages for the time-varying analysis results for the two samples).

In the text file, the results for the two samples are presented side by side as can be seen from Fig. A.4. The structure of the text file is described shortly in Section 3.3.1, but the content is or less self descriptive. The saved MATLAB MAT-file includes all the analysis results as well as measurement data and analysis options as described in Section 3.3.3. It is recommended to always save the analysis results in the MAT-file (even if you don't have MATLAB), because the MAT-file can be later re-opened in Kubios. When opening a Kubios HRV exported MAT-file, the analysis session saved in the file will be opened as it was when the MAT-file was saved and you can easily check or re-analyse the data.

Finally, the analysis results can also be saved into a "SPSS friendly" batch file as described in Section 3.3.4. This format is useful if you are analysing HRV recordings of several subjects, or alternatively, repeated personal recordings for monitoring e.g. daily changes. Kubios uses Comma Separated Values (CSV) file format for the batch file, which can be easily imported into many spreadsheet and statistical software packages (MS Excel, SPSS).

4.2 Sample run 2 - Time-varying analysis

In the second sample run, we show how to make time-varying analysis for the whole orthostatic HRV measurement. First of all, we need to enable the time-varying analysis from the preferences. To do this select Edit preferences from the File menu and check the Time-varying analysis from Analysis options category of the preferences window. If you do not want to view any of the time-domain, frequency-domain, or nonlinear analysis results and nor do you wish to include any of them in the report sheets or results file, you should at the same time uncheck the general analysis from the preferences. Then press the OK button, and the time-varying analysis will be enabled in the user interface.

For the time-varying analysis we wish to include the whole measurement period and, thus, we set the Range of the RR interval sample to cover the whole recording (starting at 10:11:33, length 00:12:09). This can be done using the Start and Length edit boxes or by resizing the sample patch with the mouse. For the same reasons as in the first sample run we use again the smoothness priors detrending method with the Lambda value of 500.

The time-varying analysis results can then be viewed in the results view segment (press the Time-varying button). The variable in view can be selected from the two pop up buttons on the left hand corner of the results view. For example, in Fig. A.5 the time-varying spectrum from frequency-domain variables list has been for view. All time-domain and frequency-domain variables as well as most of

the nonlinear variables are included in time-varying parameters. The parameters are calculated using a moving window, the width and time shift of which can be changed by editing the Window width and Grid interval values. Here we have used a 60 second window and a 10 second grid interval. The frequency-domain variables, are obtained from a time-varying spectrum estimate for which there are two different methods available. These are the spectrogram and the Kalman smoother methods. The spectrogram method is simply a moving window Fourier transformation method. The same moving window settings as described above are used for computing the spectrogram. The Kalman smoother method, on the other hand, is based on time-varying AR modelling and does not utilize the same kind of moving window as the spectrogram. Thus, the window width value does not apply to the frequency-domain variables if the Kalman smoother is used for spectrum estimation. The Grid interval is, however, utilized for the Kalman smoother method as well.

The differences between the spectrogram and Kalman smoother methods have been discussed e.g. in [47, 46]. In brief, it can be said that the Kalman smoother is computationally more complex but yields better resolution than the spectrogram. The spectrogram is also more robust and requires only the moving window settings to be defined. The Kalman smoother methods, on the other hand, requires the fixing of both the adaptation coefficient and the AR mode order. Note also that if ECG is measured, values of EDR (electrocardiogram derived respiration) are illustrated on top of the time-varying spectrum.

The results of the time-varying analysis can be saved as in the first sample run. If the general analysis was disabled from the preferences window, only time-varying results will be included in the text file, MAT-file and PDF file exports. In the PDF-file the time-varying results are presented in one page shown in Fig. A.6. Time-varying results are written in the text file as illustrated in Fig. A.7. The MATLAB MAT-file will include the `TimeVar` field, under which you can find the time trends of all computed HRV parameters.

Chapter 5

Pre-processing and analysis methods

5.1 Pre-processing of RR interval time series

Any artifact in the RR interval time series may interfere the analysis of these signals. The artifacts within HRV signals can be divided into technical and physiological artifacts. The technical artifacts can include missing or additional QRS complex detections and errors in R-wave occurrence times. These artifacts may be due to measurement artifacts or the computational algorithm. The physiological artifacts, on the other hand, include ectopic beats and arrhythmic events. In order to avoid the interference of such artifacts, the ECG recording and the corresponding event series should always be manually checked for artifacts and only artifact-free sections should be included in the analysis [51]. Alternatively, if the amount of artifact-free data is insufficient, proper interpolation methods can be used to reduce these artifacts, see, e.g., [24, 25, 31]. Kubios HRV includes two methods for correcting any artefacts and ectopic beats present in the RR interval data, which are described in Section 5.1.1.

Another common feature that can alter the analysis significantly are the slow linear or more complex trends within the analysed time series. Such slow nonstationarities are characteristic for HRV signals and should be considered before the analysis. The origins of nonstationarities in HRV are discussed, e.g., in [5]. Two kinds of methods have been used to get around the nonstationarity problem. In [57], it was suggested that HRV data should be systematically tested for nonstationarities and that only stationary segments should be analysed. Representativeness of these segments in comparison with the whole HRV signal was, however, questioned in [18]. Other methods try to remove the slow nonstationary trends from the HRV signal before analysis. The detrending is usually based on first order [26, 33] or higher order polynomial [41, 33] models. In addition, Kubios HRV software includes an advanced detrending procedure originally presented in [50]. This approach is based on smoothness priors regularization and is described in Section 5.1.2.

5.1.1 Artefact correction methods

Kubios HRV includes two alternative methods: 1) Threshold based correction, in which the artefacts and ectopic beats are simply corrected by comparing every RR interval value against a local average interval; 2) Automatic correction, in which artefacts are detected from a time series consisting of differences between successive RR intervals. Details of these correction method are given below. In addition to these RR interval based correction methods, the R-wave detections can be corrected manually when ECG data is used for analysis (see Section 3.2).

Threshold based artefact correction algorithm

The threshold based artefact correction algorithm compares every RR interval value against a local average interval. The local average is obtained by median filtering the RR interval time series, and thus, the local average is not affected by single outliers in RR interval time series. If an RR interval differs from the locale average more than a specified threshold value, the interval is identified as an artefact and is marked for correction. The threshold value can be selected from:

- Very low: 0.45 sec (threshold in seconds)

- Low: 0.35 sec
- Medium: 0.25 sec
- Strong: 0.15 sec
- Very strong: 0.05 sec
- Custom, for setting a custom threshold in seconds

For example, the “Medium” correction level will identify all RR intervals that are larger/smaller than 0.25 seconds compared to the local average. The correction is made by replacing the identified artefacts with interpolated values using a cubic spline interpolation.

It should be noted, that Kubios HRV adjusts these threshold with mean heart rate. That is, thresholds shown above are for 60 beats/min heart rate, but for higher heart rates the thresholds are smaller (because the variability is expected to decrease when HR increases) and vice versa for lower heart rates.

Because the artefacts are identified by simple thresholding, this correction method should not be duplicated between subjects because normal variability is highly individual. Instead, the correction level should be adjusted individually as follows. First, identify if there are any artefacts in your data that should be corrected. If there are artefacts, then select the lowest possible correction level, which identifies the artefacts but does not identify too many normal RR intervals as artefacts.

Automatic artefact correction algorithm

In automatic correction algorithm artefacts are detected from dRR series, which is a time series consisting of differences between successive RR intervals. The dRR series provides a robust way to separate ectopic and misplaced beats from the normal sinus rhythm. To separate ectopic and normal beats, time varying threshold (Th) is used. To ensure adaptation to different HRV levels, the threshold is estimated from the time varying distribution of the dRR series. For each beat, quartile deviation of the 90 surrounding beats is calculated and multiplied by factor 5.2. Beats within this range cover 99.95% of all beats if RR series is normally distributed. However, RR interval series is not often normally distributed, and thus, also some of the normal beats exceed the threshold. Therefore, decision algorithm is needed to detect artefact beats.

Ectopic beats form negative positive negative (NPN) or positive negative positive (PNP) patterns to the dRR series. Similarly long beats form positive negative (PN) and short beats negative positive (NP) patterns to the dRR series. Only dRR segments containing these patterns are classified as artefact beats. Missed or extra beats are detected by comparing current RR value with median of the surrounding 10 RR interval values ($medRR$). A missed beat is detected if current RR interval ($RR(i)$) satisfies condition

$$\left| \frac{RR(i)}{2} - medRR(i) \right| < 2Th \quad (5.1)$$

and an extra beat is detected if two successive RR intervals ($RR(i)$ and $RR(i+1)$) satisfies condition

$$|RR(i) + RR(i+1) - medRR(i)| < 2Th. \quad (5.2)$$

Correction of detected artefacts Detected ectopic beats are corrected by replacing corrupted RR times by interpolated RR values. Similarly too long and short beats are corrected by interpolating new values to the RR time series. Missed beats are corrected by adding new R-wave occurrence time and extra beats are simply corrected by removing extra R-wave detection and recalculating RR interval series.

5.1.2 Smoothness priors based detrending approach

The theory behind the smoothness priors detrending methods included in Kubios HRV is described below. For more details, see [50].

Let $z \in \mathbb{R}^N$ denote the RR interval time series which can be considered to consist of two components

$$z = z_{\text{stat}} + z_{\text{trend}} \quad (5.3)$$

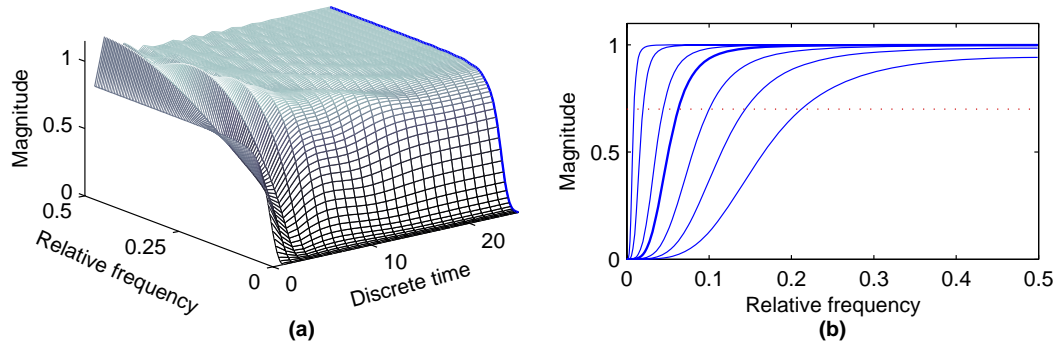


Figure 5.1: a) Time-varying frequency response of \mathcal{L} ($N - 1 = 50$ and $\lambda = 10$). Only the first half of the frequency response is presented, since the other half is identical. b) Frequency responses, obtained from the middle row of \mathcal{L} (cf. bold lines), for $\lambda = 1, 2, 4, 10, 20, 100$, and 500 . The corresponding cut-off frequencies are 0.213, 0.145, 0.101, 0.063, 0.045, 0.021 and 0.010 times the sampling frequency.

or, correspondingly, the mean HR (\overline{HR}). In addition, several variables that measure the variability within the RR series exist. The standard deviation of RR intervals (SDNN) is defined as

$$SDNN = \sqrt{\frac{1}{N-1} \sum_{j=1}^N (RR_j - \overline{RR})^2} \quad (5.9)$$

where RR_j denotes the value of j 'th RR interval and N is the total number of successive intervals. The SDNN reflects the overall (both short-term and long-term) variation within the RR interval series, whereas the standard deviation of successive RR interval differences (SDSD) given by

$$SDSD = \sqrt{E\{\Delta RR_j^2\} - E\{\Delta RR_j\}^2} \quad (5.10)$$

can be used as a measure of the short-term variability. For stationary RR series $E\{\Delta RR_j\} = E\{RR_{j+1}\} - E\{RR_j\} = 0$ and SDSD equals the root mean square of successive differences (RMSSD) given by

$$RMSSD = \sqrt{\frac{1}{N-1} \sum_{j=1}^{N-1} (RR_{j+1} - RR_j)^2}. \quad (5.11)$$

Another measure calculated from successive RR interval differences is the NN50 which is the number of successive intervals differing more than 50 ms or the corresponding relative amount

$$pNN50 = \frac{NN50}{N-1} \times 100\%. \quad (5.12)$$

In addition to the above statistical measures, there are some geometric measures that are calculated from the RR interval histogram. The HRV triangular index is obtained as the integral of the histogram (i.e. total number of RR intervals) divided by the height of the histogram which depends on the selected bin width. In order to obtain comparable results, a bin width of 1/128 seconds is recommended [51]. Another geometric measure is the TINN which is the baseline width of the RR histogram evaluated through triangular interpolation, see [51] for details.

5.2.2 Frequency-domain methods

In the frequency-domain methods, a *power spectrum density* (PSD) estimate is calculated for the RR interval series. The regular PSD estimators implicitly assume equidistant sampling and, thus, the RR interval series is converted to equidistantly sampled series by interpolation methods prior to PSD estimation. In the software a cubic spline interpolation method is used. In HRV analysis, the PSD estimation is generally carried out using either FFT based methods or parametric AR modeling based methods. For details on these methods see, e.g., [29]. The advantage of FFT based methods is the simplicity of

implementation, while the AR spectrum yields improved resolution especially for short samples. Another property of AR spectrum that has made it popular in HRV analysis is that it can be factorized into separate spectral components. The disadvantages of the AR spectrum are the complexity of model order selection and the contingency of negative components in the spectral factorization. Nevertheless, it may be advantageous to calculate the spectrum with both methods to have comparable results.

In this software, the HRV spectrum is calculated with FFT based Welch's periodogram method and with the AR method. Spectrum factorization in AR method is optional. In the Welch's periodogram method the HRV sample is divided into overlapping segments. The spectrum is then obtained by averaging the spectra of these segments. This method decreases the variance of the FFT spectrum.

Kubios HRV includes also the Lomb-Scargle periodogram [13], which differs from the Welch's periodogram in the sense that it does not assume equidistant sampling and is thus computed directly from the non-interpolated RR interval time series. The variance of the Lomb-Scargle periodogram is decreased by smoothing the periodogram using MA-filtering (the window width of the MA-filter can be adjusted in software preferences).

The generalized frequency bands in case of short-term HRV recordings are the very low frequency (VLF, 0–0.04 Hz), low frequency (LF, 0.04–0.15 Hz), and high frequency (HF, 0.15–0.4 Hz). The frequency-domain measures extracted from the PSD estimate for each frequency band include absolute and relative powers of VLF, LF, and HF bands, LF and HF band powers in normalized units, the LF/HF power ratio, and peak frequencies for each band (see Table A.1). In the case of FFT spectrum, absolute power values for each frequency band are obtained by simply integrating the spectrum over the band limits. In the case of AR spectrum, on the other hand, if factorization is enabled distinct spectral components emerge for each frequency band with a proper selection of the model order and the absolute power values are obtained directly as the powers of these components. If factorization is disabled the AR spectrum powers are calculated as for the FFT spectrum. The band powers in relative and normalized units are obtained from the absolute values as described in Table A.1.

5.2.3 Nonlinear methods

Considering the complex control systems of the heart it is reasonable to assume that nonlinear mechanisms are involved in the genesis of HRV. The nonlinear properties of HRV have been analysed using measures such as Poincaré plot [7, 8], approximate and sample entropy [44, 16], detrended fluctuation analysis [38, 39], correlation dimension [19, 21], and recurrence plots [56, 53, 58]. During the last years, the number of studies utilizing such methods have increased substantially. The downside of these methods is still, however, the difficulty of physiological interpretation of the results.

Poincaré plot

One commonly used nonlinear method that is simple to interpret is the so-called Poincaré plot. It is a graphical representation of the correlation between successive RR intervals, i.e. plot of RR_{j+1} as a function of RR_j as described in Fig. 5.2. The shape of the plot is the essential feature. A common approach to parameterize the shape is to fit an ellipse to the plot as shown in Fig. 5.2. The ellipse is oriented according to the line-of-identity ($RR_j = RR_{j+1}$) [7]. The standard deviation of the points perpendicular to the line-of-identity denoted by SD1 describes short-term variability which is mainly caused by RSA. It can be shown that SD1 is related to the time-domain measure SDSD according to [7]

$$SD1^2 = \frac{1}{2} SDSD^2. \quad (5.13)$$

The standard deviation along the line-of-identity denoted by SD2, on the other hand, describes long-term variability and has been shown to be related to time-domain measures SDNN and SDSD by [7]

$$SD2^2 = 2 SDNN^2 - \frac{1}{2} SDSD^2. \quad (5.14)$$

The standard Poincaré plot can be considered to be of the first order. The second order plot would be a three dimensional plot of values $(RR_j, RR_{j+1}, RR_{j+2})$. In addition, the lag can be bigger than 1, e.g., the plot (RR_j, RR_{j+2}) .

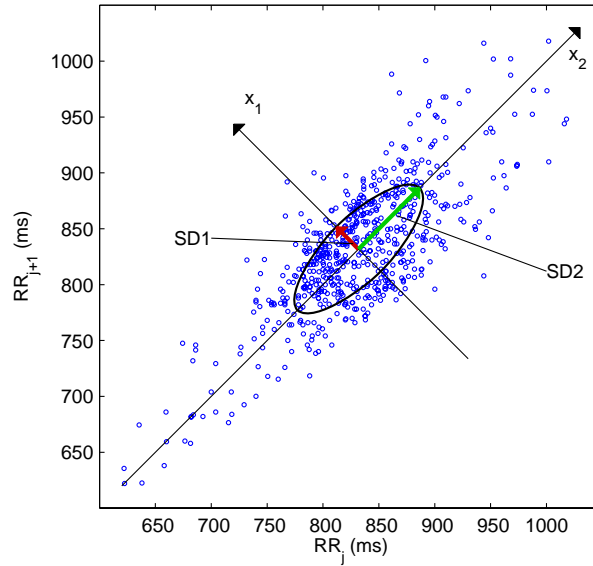


Figure 5.2: Poincaré plot analysis with the ellipse fitting procedure. SD1 and SD2 are the standard deviations in the directions x_1 and x_2 , where x_2 is the line-of-identity for which $RR_j = RR_{j+1}$.

Approximate entropy

Approximate entropy (ApEn) measures the complexity or irregularity of the signal [16, 44]. Large values of ApEn indicate high irregularity and smaller values of ApEn more regular signal. The ApEn is computed as follows.

First, a set of length m vectors u_j is formed

$$u_j = (RR_j, RR_{j+1}, \dots, RR_{j+m-1}), \quad j = 1, 2, \dots, N - m + 1 \quad (5.15)$$

where m is called the embedding dimension and N is the number of measured RR intervals. The distance between these vectors is defined as the maximum absolute difference between the corresponding elements, i.e.,

$$d(u_j, u_k) = \max \{ |RR_{j+n} - RR_{k+n}| \mid n = 0, \dots, m-1 \}. \quad (5.16)$$

Next, for each u_j the relative number of vectors u_k for which $d(u_j, u_k) \leq r$ is calculated. This index is denoted with $C_j^m(r)$ and can be written in the form

$$C_j^m(r) = \frac{\text{nbr of } \{u_k \mid d(u_j, u_k) \leq r\}}{N - m + 1} \quad \forall k. \quad (5.17)$$

Due to the normalization, the value of $C_j^m(r)$ is always smaller or equal to 1. Note that the value is, however, at least $1/(N - m + 1)$ since u_j is also included in the count. Then, take the natural logarithm of each $C_j^m(r)$ and average over j to yield

$$\Phi^m(r) = \frac{1}{N - m + 1} \sum_{j=1}^{N-m+1} \ln C_j^m(r). \quad (5.18)$$

Finally, the approximate entropy is obtained as

$$\text{ApEn}(m, r, N) = \Phi^m(r) - \Phi^{m+1}(r). \quad (5.19)$$

Thus, the value of the estimate ApEn depends on three parameters, the length m of the vectors u_j , the tolerance value r , and the data length N . In this software the default value of m is set to be $m = 2$. The length N of the data also affects ApEn. When N is increased the ApEn approaches its asymptotic value. The tolerance r has a strong effect on ApEn and it should be selected as a fraction of the standard deviation of the data (SDNN). This selection enables the comparison of different data types. A common selection for r is $r = 0.2SDNN$, which is also the default value in this software.

Sample entropy

Sample entropy (SampEn) is similar to ApEn, but there are two important differences in its calculation [44, 23]. For ApEn, in the calculation of the number of vectors u_k for which $d(u_j, u_k) \leq r$ also the vector u_j itself is included. This ensures that $C_j^m(r)$ is always larger than 0 and the logarithm can be applied, but at the same time it makes ApEn to be biased. In sample entropy the self-comparison of u_j is eliminated by calculating $C_j^m(r)$ as

$$C_j^m(r) = \frac{\text{nbr of } \{u_k \mid d(u_j, u_k) \leq r\}}{N - m} \quad \forall k \neq j. \quad (5.20)$$

Now the value of $C_j^m(r)$ will be between 0 and 1. Next, the values of $C_j^m(r)$ are averaged to yield

$$C^m(r) = \frac{1}{N - m + 1} \sum_{j=1}^{N-m+1} C_j^m(r) \quad (5.21)$$

and the sample entropy is obtained as

$$\text{SampEn}(m, r, N) = \ln(C^m(r)/C^{m+1}(r)). \quad (5.22)$$

The default values set for the embedding dimension m and for the tolerance parameter r in the software are the same as those for the approximate entropy calculation. Both ApEn and SampEn are estimates for the negative natural logarithm of the conditional probability that a data of length N , having repeated itself within a tolerance r for m points, will also repeat itself for $m + 1$ points. SampEn was designed to reduce the bias of ApEn and has a closer agreement with the theory for data with known probabilistic content [23].

Multiscale entropy (MSE)

Multiscale entropy (MSE) is an extension of SampEn in the sense that it incorporates two procedures [9]

1. A *course-graining* process is applied to the RR interval time series. Multiple course-grained time series are constructed for the time series by averaging the data points within non-overlapping windows of increasing length τ , where τ represents the scale factor and is selected to range between $\tau = 1, 2, \dots, 20$. The length of each course-grained time series is N/τ , where N is the number of RR intervals in the data. For scale $\tau = 1$, the course-grained time series is simply the original beat-to-beat RR interval time series.
2. SampEn is calculated for each course-grained time series. SampEn as a function of the scale factor produces the MSE. MSE for scale factor $\tau = 1$ returns standard SampEn (computed from the original data points).

Detrended fluctuation analysis

Detrended fluctuation analysis (DFA) measures the correlation within the signal. The correlation is extracted for different time scales as follows [38]. First, the RR interval time series is integrated

$$y(k) = \sum_{j=1}^k (RR_j - \overline{RR}), \quad k = 1, \dots, N \quad (5.23)$$

where \overline{RR} is the average RR interval. Next, the integrated series is divided into segments of equal length n . Within each segment, a least squares line is fitted into the data. Let $y_n(k)$ denote these regression lines. Next the integrated series $y(k)$ is detrended by subtracting the local trend within each segment and the root-mean-square fluctuation of this integrated and detrended time series is calculated by

$$F(n) = \sqrt{\frac{1}{N} \sum_{k=1}^N (y(k) - y_n(k))^2}. \quad (5.24)$$

This computation is repeated over different segment lengths to yield the index $F(n)$ as a function of segment length n . Typically $F(n)$ increases with segment length. A linear relationship on a double log

graph indicates presence of fractal scaling and the fluctuations can be characterized by scaling exponent α (the slope of the regression line relating $\log F(n)$ to $\log n$). Different values of α indicate the following

- $\alpha = 1.5$: Brown noise (integral of white noise)
- $1 < \alpha < 1.5$: Different kinds of noise
- $\alpha = 1$: $1/f$ noise
- $0.5 < \alpha < 1$: Large values are likely to be followed by large value and vice versa
- $\alpha = 0.5$: white noise
- $0 < \alpha < 0.5$: Large value is likely to be followed by small value and vice versa

Typically, in DFA the correlations are divided into short-term and long-term fluctuations. In the software, the short-term fluctuations are characterized by the slope α_1 obtained from the $(\log n, \log F(n))$ graph within range $4 \leq n \leq 16$ (default values). Correspondingly, the slope α_2 obtained by default from the range $16 \leq n \leq 64$ characterizes long-term fluctuations, see Fig. 5.3.

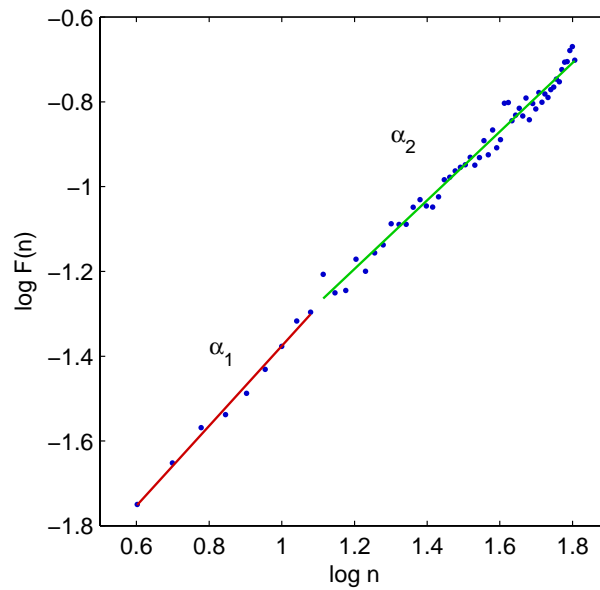


Figure 5.3: Detrended fluctuation analysis. A double log plot of the index $F(n)$ as a function of segment length n . α_1 and α_2 are the short term and long term fluctuation slopes, respectively.

Correlation dimension

Another method for measuring the complexity or strangeness of the time series is the correlation dimension which was proposed in [17]. The correlation dimension is expected to give information on the minimum number of dynamic variables needed to model the underlying system and it can be obtained as follows.

Similarly as in the calculation of approximate and sample entropies, form length m vectors u_j

$$u_j = (RR_j, RR_{j+1}, \dots, RR_{j+m-1}), \quad j = 1, 2, \dots, N - m + 1 \quad (5.25)$$

and calculate the number of vectors u_k for which $d(u_j, u_k) \leq r$, that is

$$C_j^m(r) = \frac{\text{nbr of } \{u_k \mid d(u_j, u_k) \leq r\}}{N - m + 1} \quad \forall k \quad (5.26)$$

where the distance function $d(u_j, u_k)$ is now defined as

$$d(u_j, u_k) = \sqrt{\sum_{l=1}^m (u_j(l) - u_k(l))^2}. \quad (5.27)$$

Next, an average of the term $C_j^m(r)$ is taken

$$C^m(r) = \frac{1}{N - m + 1} \sum_{j=1}^{N-m+1} C_j^m(r) \quad (5.28)$$

which is the so-called correlation integral. The correlation dimension D_2 is defined as the limit value

$$D_2(m) = \lim_{r \rightarrow 0} \lim_{N \rightarrow \infty} \frac{\log C^m(r)}{\log r}. \quad (5.29)$$

In practice this limit value is approximated by the slope of the regression curve $(\log r, \log C^m(r))$ [21]. The slope is calculated from the linear part of the log-log plot, see Fig. 5.4. The slope of the regression curves tend to saturate on the finite value of D_2 when m is increased. In the software, a default value of $m = 10$ was selected for the embedding.

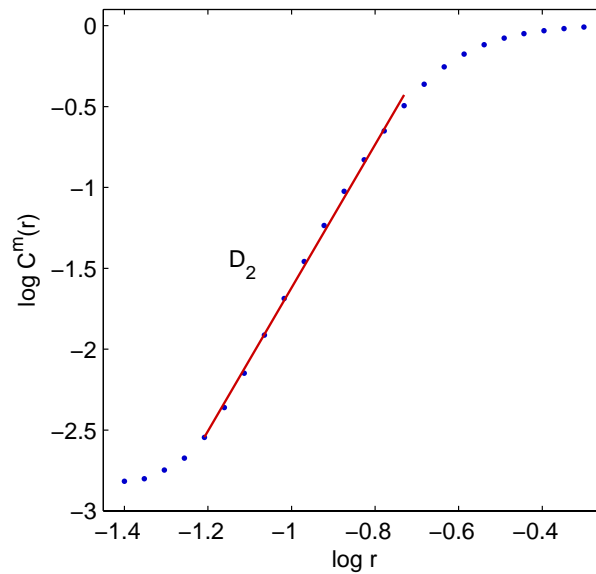


Figure 5.4: Approximation of the correlation dimension D_2 from the $(\log r, \log C^m(r))$ plot.

Recurrence plot analysis

Yet another approach, included in the software, for analyzing the complexity of the time series is the so-called *recurrence plot* (RP) analysis. In this approach, vectors

$$u_j = (RR_j, RR_{j+\tau}, \dots, RR_{j+(m-1)\tau}), \quad j = 1, 2, \dots, N - (m-1)\tau \quad (5.30)$$

where m is the embedding dimension and τ the embedding lag. The vectors u_j then represent the RR interval time series as a trajectory in m dimensional space. A recurrence plot is a symmetrical $[N - (m-1)\tau] \times [N - (m-1)\tau]$ matrix of zeros and ones. The element in the j 'th row and k 'th column of the RP matrix, i.e. $RP(j, k)$, is 1 if the point u_j on the trajectory is close to point u_k . That is

$$RP(j, k) = \begin{cases} 1, & d(u_j - u_k) \leq r \\ 0, & \text{otherwise} \end{cases} \quad (5.31)$$

where $d(u_j, u_k)$ is the Euclidean distance given in (5.27) and r is a fixed threshold. The structure of the RP matrix usually shows short line segments of ones parallel to the main diagonal. The lengths of these diagonal lines describe the duration of which the two points are close to each other. An example RP for HRV time series is presented in Fig. 5.5. Methods for quantifying recurrence plots were proposed in [56]. The methods included in this software are introduced below.

In the software the following selections were made. The embedding dimension and lag were selected to be $m = 10$ (default value) and $\tau = 1$ (fixed), respectively. The threshold distance r was selected to

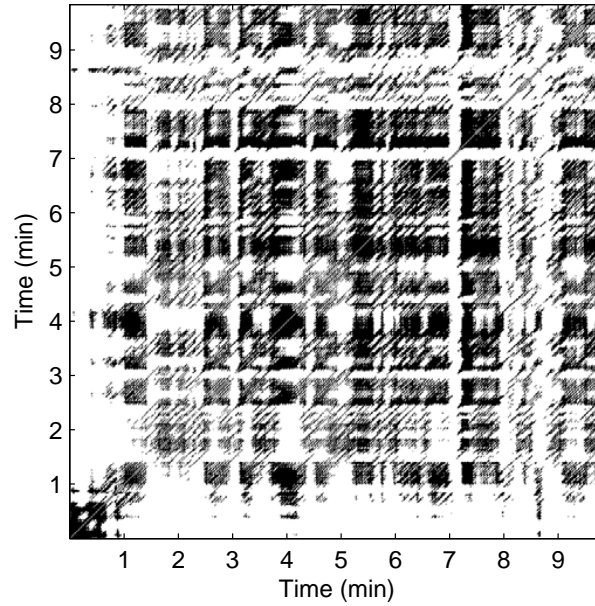


Figure 5.5: Recurrence plot matrix for HRV time series (black = 1 and white = 0).

be \sqrt{m} SD (default value), where SD is the standard deviation of the RR time series. The selection are similar to those made in [10].

The first quantitative measure of RP is the *recurrence rate* (REC) which is simply the ratio of ones and zeros in the RP matrix. The number of elements in the RP matrix for $\tau = 1$ is equal to $N - m + 1$ and the recurrence rate is simply given as

$$\text{REC} = \frac{1}{(N - m + 1)^2} \sum_{j,k=1}^{N-m+1} \text{RP}(j, k). \quad (5.32)$$

The recurrence rate can also be calculated separately for each diagonal parallel to the line-of-identity (main diagonal). The trend of REC as a function of the time distance between these diagonals and the line-of-identity describes the fading of the recurrences for points further away.

The rest of the RP measures consider the lengths of the diagonal lines. A threshold $l_{\min} = 2$ is used for excluding the diagonal lines formed by tangential motion of the trajectory. The maximum line length is denoted l_{\max} and its inverse, the divergence,

$$\text{DIV} = \frac{1}{l_{\max}} \quad (5.33)$$

has been shown to correlate with the largest positive Lyapunov exponent [53]. The average diagonal line length, on the other hand, is obtained as

$$l_{\text{mean}} = \frac{\sum_{l=l_{\min}}^{l_{\max}} l N_l}{\sum_{l=l_{\min}}^{l_{\max}} N_l} \quad (5.34)$$

where N_l is the number of length l lines. The determinism of the time series is measured by the variable

$$\text{DET} = \frac{\sum_{l=l_{\min}}^{l_{\max}} l N_l}{\sum_{j,k=1}^{N-m+1} \text{RP}(j, k)}. \quad (5.35)$$

Finally, the Shannon information entropy of the line length distribution is defined as

$$\text{ShanEn} = - \sum_{l=l_{\min}}^{l_{\max}} n_l \ln n_l \quad (5.36)$$

where n_l is the number of length l lines divided by the total number of lines, that is

$$n_l = \frac{N_l}{\sum_{l'=l_{\min}}^{l_{\max}} N_{l'}}. \quad (5.37)$$

5.2.4 Time-varying methods

The time-varying methods of the software include the trends of the time-domain measures \overline{RR} , SDNN, \overline{HR} , SD of HR, RMSSD, NN50, and pNN50. For frequency-domain measures the trends are obtained for VLF, LF, and HF peak frequencies, VLF, LF, and HF band powers, and LF/HF ratio. In addition, trends are calculated for the nonlinear measures ApEn and SampEn. The trends for the time-domain and nonlinear measures are obtained by using a moving window, the length and shift of which can be changed.

The trends of the frequency-domain measures are computed based on a time-varying spectrum estimate. The time-varying spectrum is estimated either by using the moving window FFT, which is also known as the spectrogram method, or with the Kalman smoother algorithm. The Kalman smoother algorithm is an iterative algorithm for estimating the parameters of a time-varying model. In the software, a time-varying AR model is used to model the HRV signal. The adaptation of the Kalman smoother algorithm affecting on the resolution of the spectrum can also be altered. For details on the Kalman smoother spectrum estimation see [46].

Appendix A

Appendices

A.2 Kubios HRV sample run figures

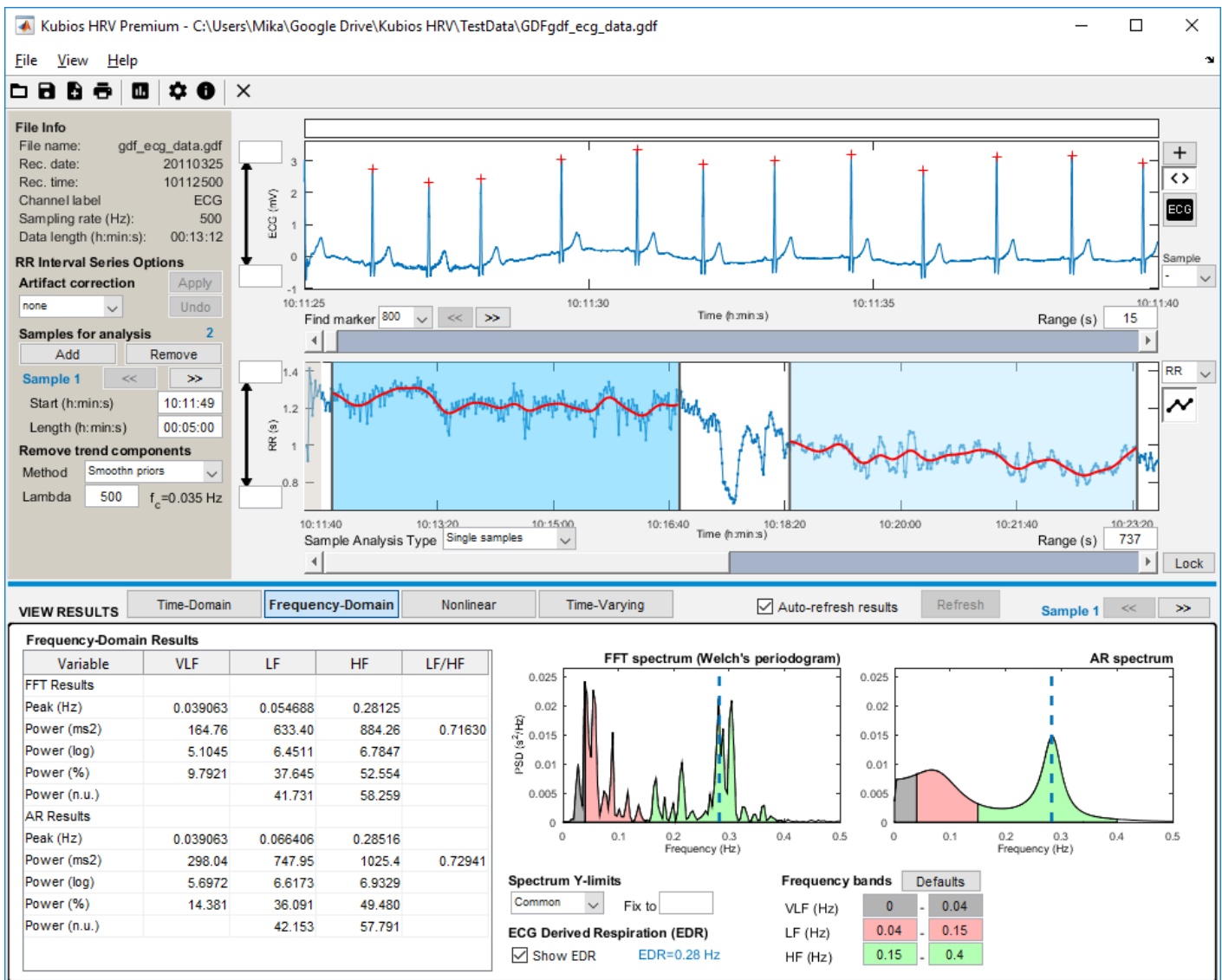


Figure A.1: Analysis of lying (supine rest) and standing periods of an orthostatic test (healthy young male) using Kubios HRV Premium (Sample run 1).

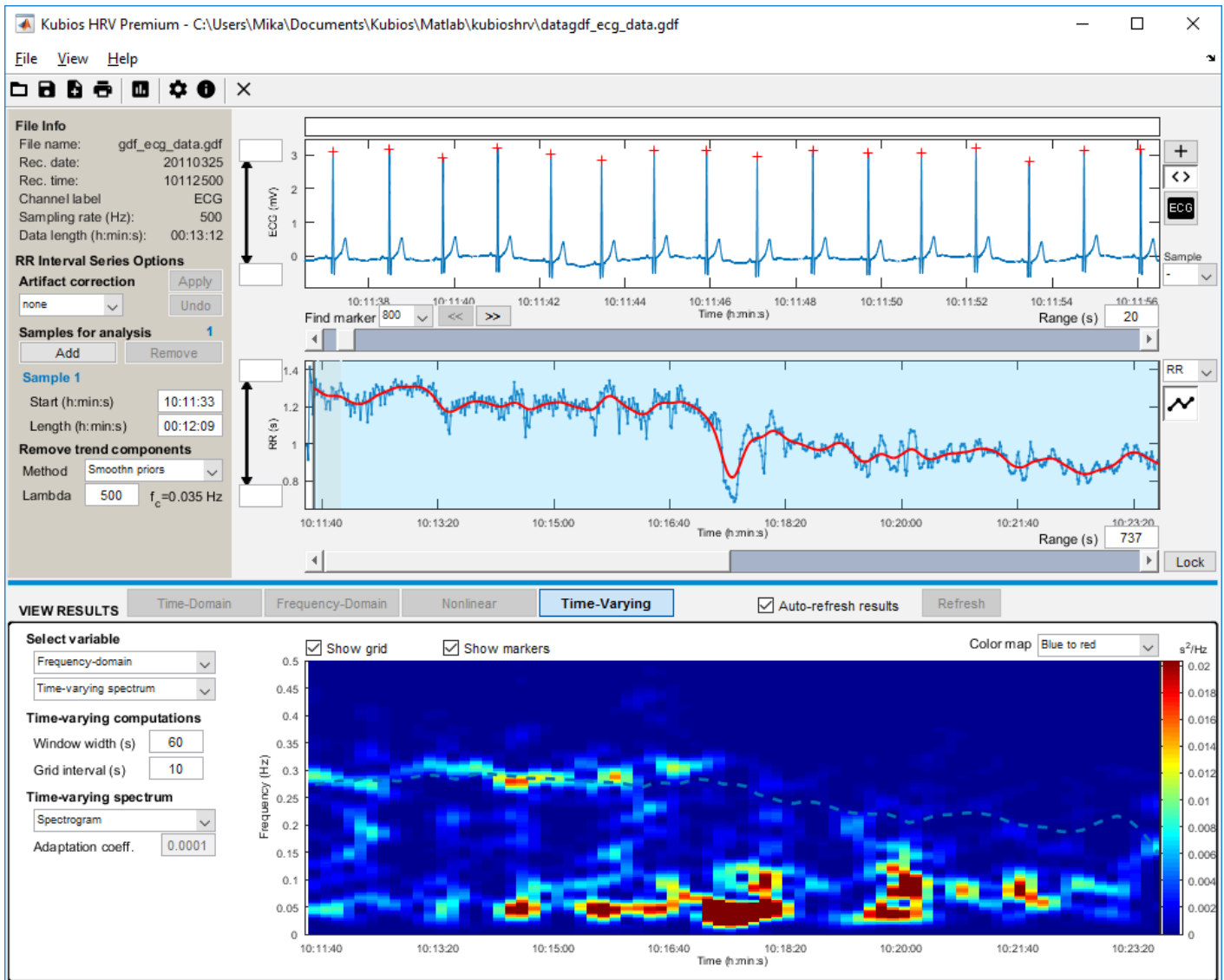


Figure A.5: Analysis of the orthostatic test (6-min supine rest followed by 6-min standing; healthy young male) using time-varying analysis options of Kubios HRV Premium (Sample run 2).

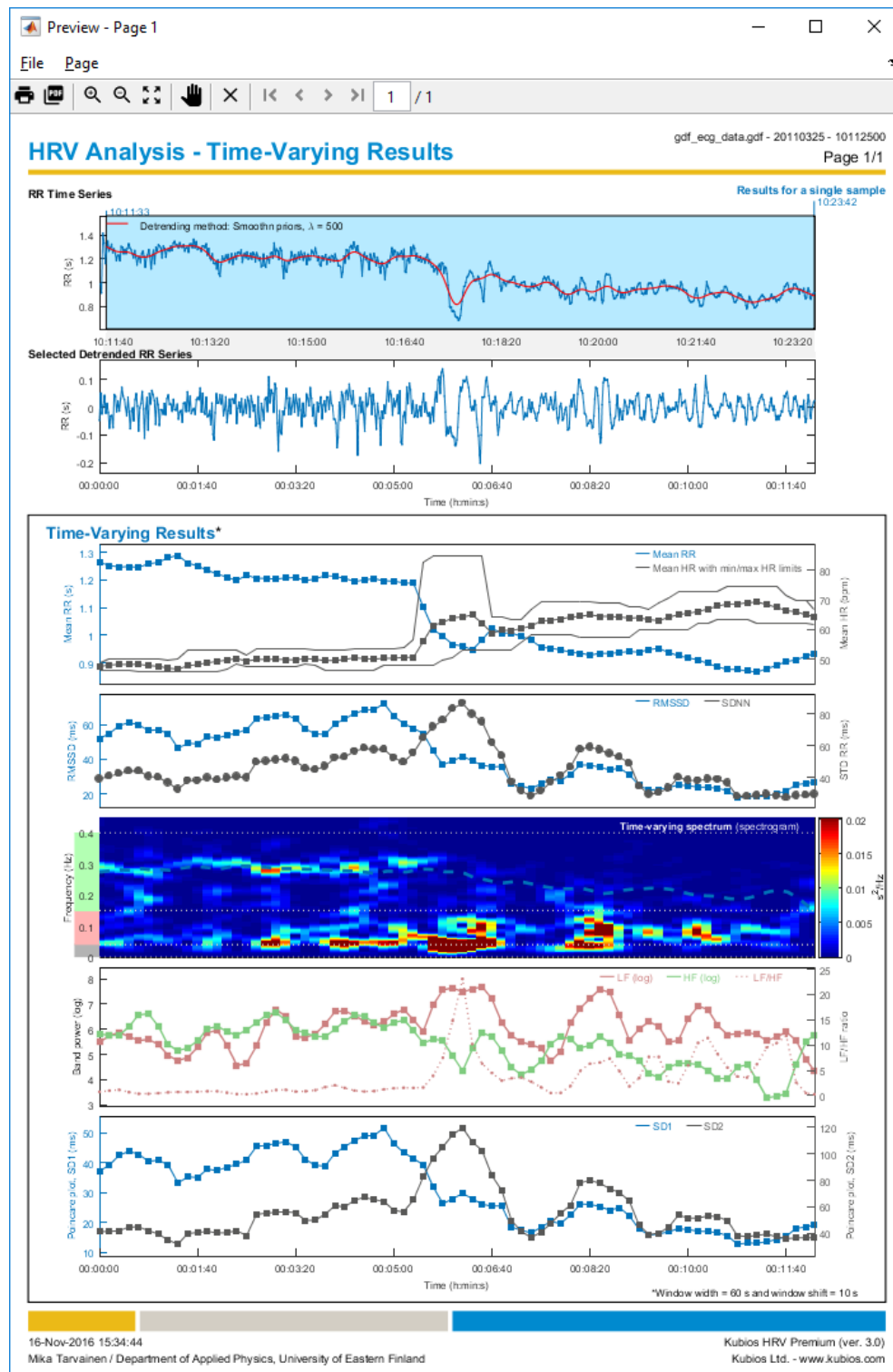


Figure A.6: Report sheet with time-varying results for the orthostatic test (6-min supine rest followed by 6-min standing; healthy young male) using time-varying analysis options of Kubios HRV Premium (Sample run 2).

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