frequency band with its very-low, low and high frequency over all recorded positions; units [ms^2]) representing the total averaged area under all consecutive spectral curves within the short-term recording is calculated

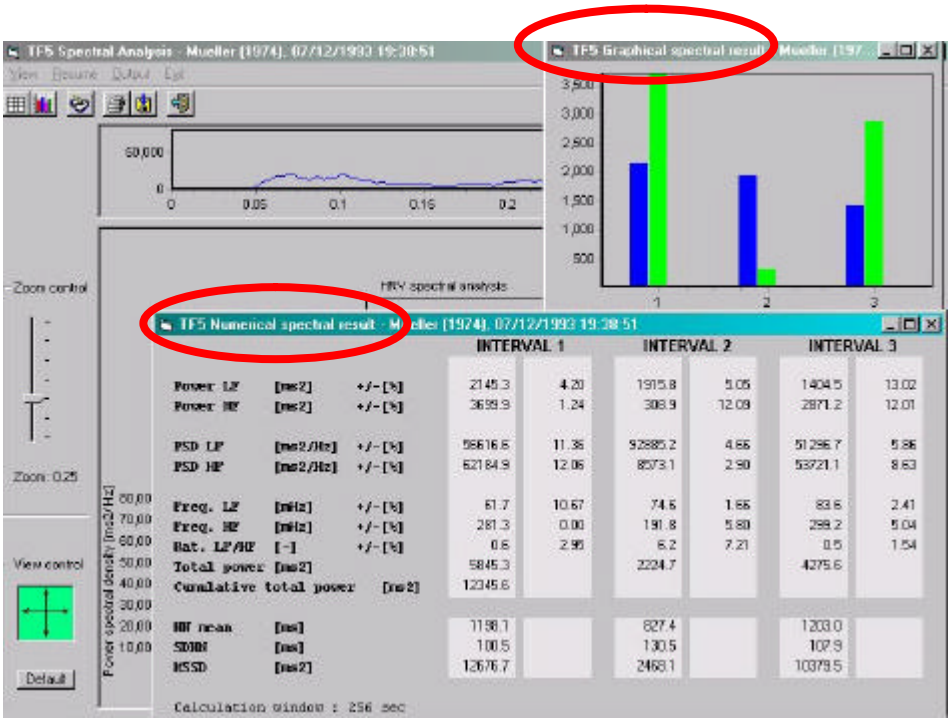
- **Standard deviations** (units [%]): calculated for each parameter, as this information is necessary for assessment of the **'stationarity'** of the examination. We recommend excluding any findings having more than 20-30% relative deviation in any of the time segments recorded. In those cases, the examinations should be repeated

Additionally, calculations of ***time-domain*** parameters are made, as well:

- **Mean R-R** interval (NN mean)
- **Standard deviation** of mean R-R interval (SDNN)
- **MSSD** (Mean of the squared differences between two adjacent normal R-R intervals over given recording period)

It is possible to display the **results in three different display options**, as:

(1) Three-dimensional running spectra graph (see Chapter 7.4. How to Perform the Analysis)
(2) Statistical table ('*Numerical spectral results'*)
(3) Graphical form ('*Graphical spectral results*').



Practical example: *The figure above shows results options (2) and (3), based on normal cardiac autonomic control as obtained during modified orthostatic load: the numerical data displayed correspond with the above listed main outcome variables, the graphical output displays absolute values of spectral powers LF & HF in (three) examination intervals. It documents predominance of HF band (right column, in green) in supine -- intervals 1 and 3 -- and predominance of LF band (left column, in blue) in standing positions -- interval 2.*

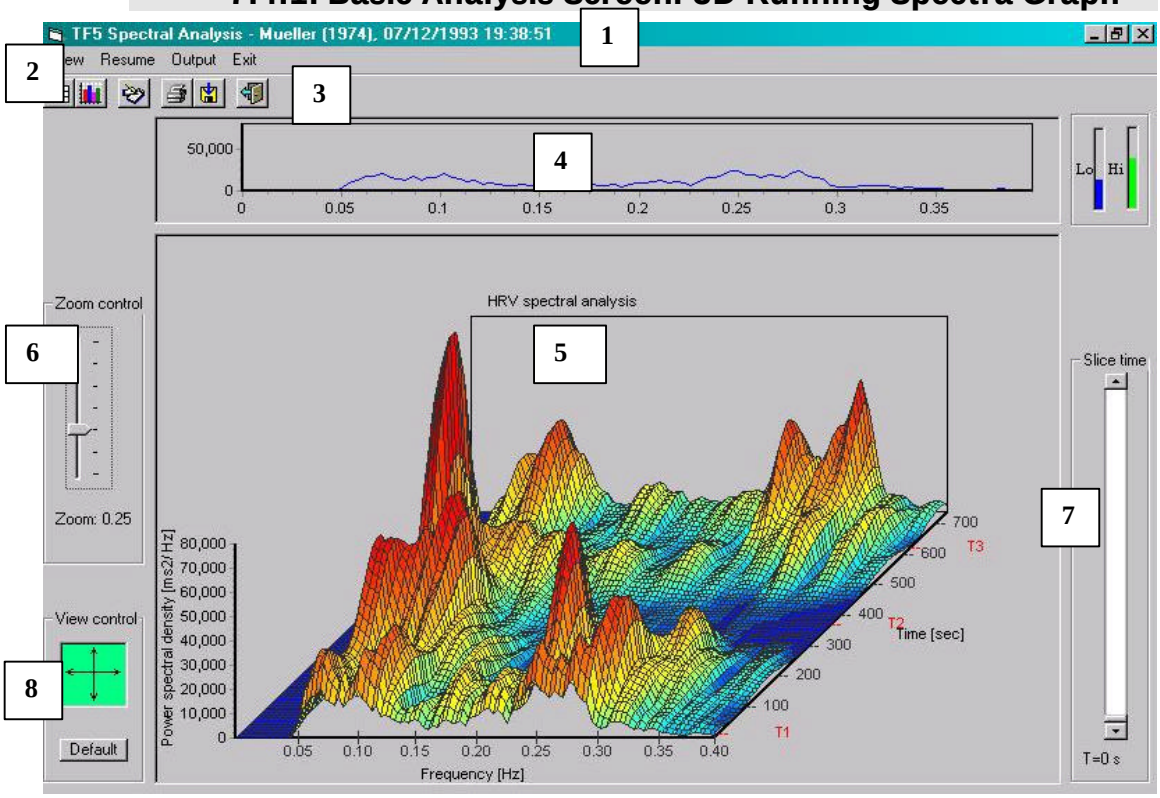
7.4. Analysis. How to Perform the Analysis

First select the subject for the analysis from the **Subjects listing** in the basic operation screen (see Chapter 5.4. Database: How to Find a Subject).

Optionally, then open the **Browser** – click on icon  -- and check the consistency of the R-R data, particularly with respect to artefacts. If artefact found, process it properly – see the Chapter 6.3.2. How to find and process artefacts. Bear in mind, please, that even one artefact might totally destroy the quality of information received over the total 15 minutes measurement.

However, you can also directly progress to the 3D analysis: click on icon Frequency domain analysis  **or** press CTRL + F together **or** in the main program menu open Analysis -> Frequency domain. Following **Basic analysis screen** is opened:

7.4.1. Basic Analysis Screen. 3D Running Spectra Graph



Practical example *shows a typical pattern obtained in a subject with no detected disturbances in cardiovascular autonomic control during a modified orthostatic test (lying down [T1] - standing up [T2] - lying down [T3], calculation for every position is based on 256 artefact-free data points; note that this display includes only low and high-frequency bands /range adjustable in the Engineering->Program Setup/). The graphics shows clearly a predominance of HF band (0.15-0.40 Hz) during supine positions (marked as T1 and T3) while LF band (0.05-0.15 Hz) gets 'activated' during standing-up (marked as T2). The numbers 1-8 are related to its detailed description, see below.*

The standard **Basic analysis screen** of the measurements includes:

(1) Windows heading includes subject's last name, year of birth and recording time details of the record analysed.

(2) Below, a line with Program sub-menu commands include options **View** (Statistical table or Graphical output), **Resume** (allows to write comments on the results), **Output** (Printing or Export of the results in text format) and **Exit**.

(3) **Icons** enabling quick access to the most of above functions.

(4) **Two-dimensional spectral graph** that shows single spectral curve created at specific time point – see the 'slider' at the right-hand side of the output screen. An instantaneous sympathovagal balance 'online' chart (is displayed right along the 2D spectral curve (blue & green bars).

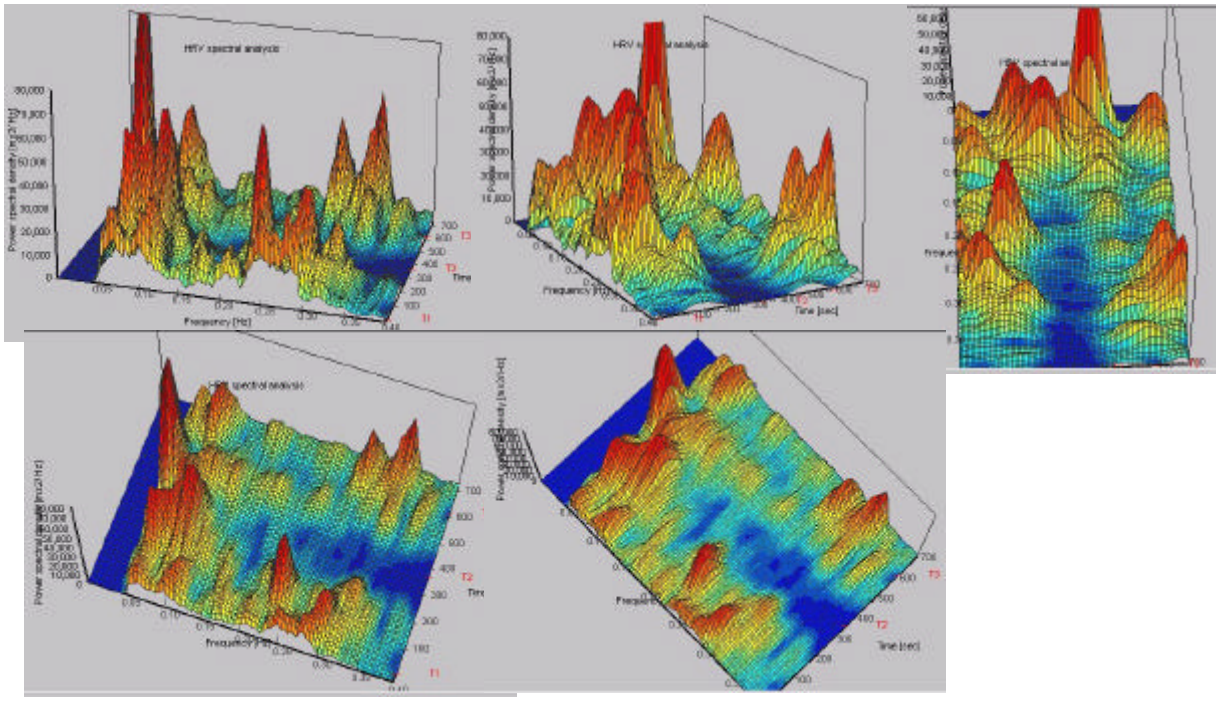
(5) The biggest part of the screen covers **three-dimensional running spectra graph** showing the behaviour of the autonomic cardiac control system during the whole examination. Note, please, that while this display shows the complete record including the changes of position etc., the final statistical results are based only on 0 - 300 seconds periods from pressing the marker in each position (see markers T1, T2 and T3).

(6) **Zoom control** slider allows to adjust the size of the displayed graph.

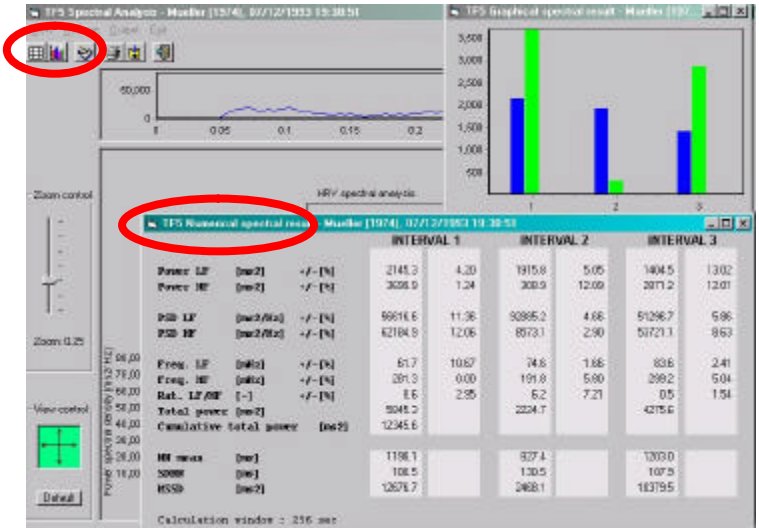
(7) **Time control** slider allows to 'slide' the graph while showing instantaneous spectral curve at specific time point ('slice time'). This interval is displayed in [seconds] from the start of measurements (and *not* from pressing any of the markers!) below the slider.

(8) **View control** pointer at the left lower corner below allows to adjust the view angle of the 3D graph in order to enable better overview over the results.

Practical example: *Below you can find some examples of 3D display manipulation using the '**View control**' option, in order to obtain an optimum graphical overview of the autonomic control during the modified orthostatic test.*



7.4.2. Basic Analysis Screen. Statistical Table



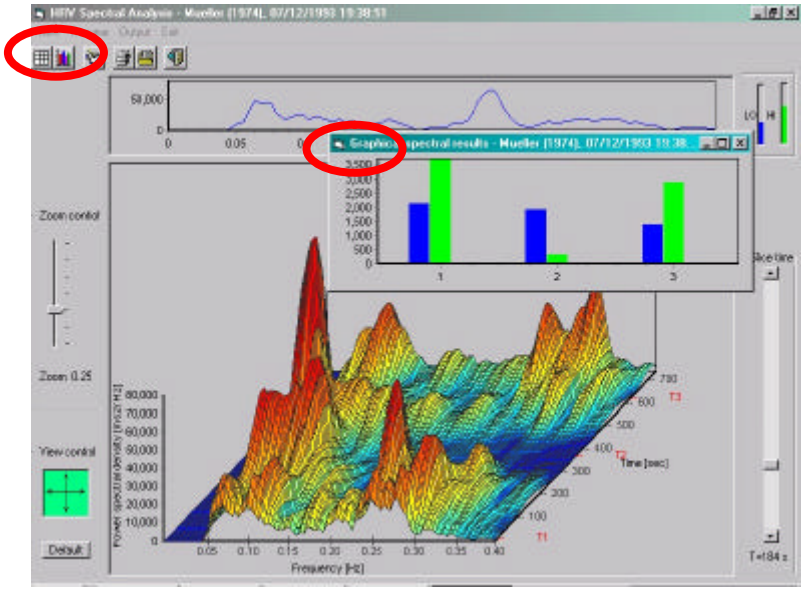
The results can be obtained in form of the **Numerical spectral results** table that is reached by clicking on the first icon in the Submenu commands line.

The table is *vertically* divided into three sections covering 3 measurement intervals (supine/ standing/ supine). *Horizontally,* groups of parameters are shown where the most important information includes the (**Spectral**) **Power** variable, displaying power within specific frequency bands.

Above in the **Statistical table** you can see results of short-term HRV spectral analysis as obtained in a normal subject (see also Chapter 7.3. HRV Basics):

- Variables **Power LF** and **HF** represent low and high frequency bands here.
- Variables **PSD** mean **Power Spectral Density**, in both frequency bands.
- **Freq.** shows the mean frequency within the specific bands.
- **Rat. LF/HF** displays interconnections between low and high frequency band spectra in all positions separately. Some authors declare this variable as 'sympathovagal balance'.
- **Total power** sums the LF and HF powers.
- **Cumulative total power** shows sum of three total power variables above.
- **NN mean** shows a mean value of R-R intervals within the position.
- **SDNN** standard deviation of mean R-R interval.
- **MSSD** shows Mean of the squared differences between two adjacent normal R-R intervals over recording position.

7.4.3. Basic Analysis Screen. Graphical Table

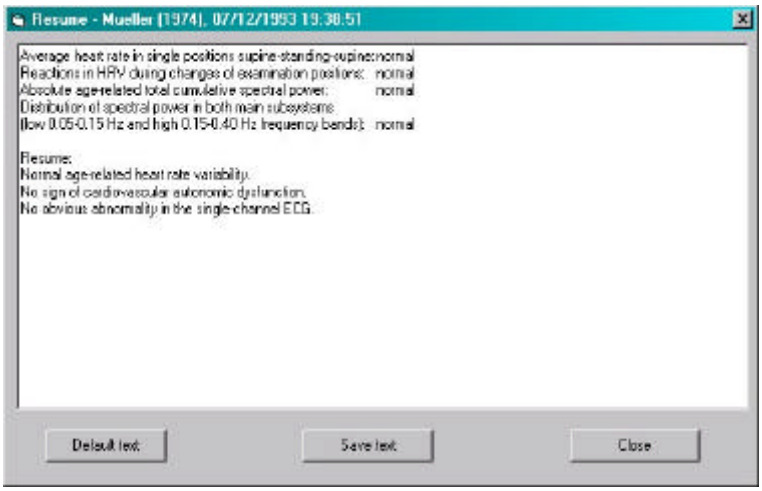


Also, there is a possibility to obtain a quick overview of the results in a graphical form (**Graphical spectral results** table), by clicking on the first icon in the Submenu commands line.

The chart shows distribution of LF and HF spectral powers related to each examination interval, here shown as 1, 2, 3. On the left-hand axis absolute value of the parameters can be found (unit [ms^2]).

Important notice:
Please note that for the statistics, only those 300 seconds' time intervals marked previously in the measurement session are included. Check the Browser, please, where the start of *statistical* intervals is marked in green. This can sometimes 'optically' diverge from the *graphically* displayed results.

7.4.4. Basic Analysis Screen. Resume



For reporting, comments on the HRV analysis results prepared by medical professional might be necessary. For this reason, an option **Resume** allows to enter and store record-specific texts related to any part of the examination procedure.
Click on icon or in the program submenu open the Resume option. In the window, type in your texts. Alternatively, you can use pre-defined text patterns as prepared in the Main program menu, **Engineering** -> **Program setup** menu -> **Default resume** option.
When finished, please be sure to save the created texts and close the window. This Resume text appears in the printed Report, as described below.

7.4.5. Basic Analysis Screen. Export of Numerical Results

For various reasons, the export of results in digital form might be necessary. In such case click, please, on the icon **or** open in the Program submenu the option **Output** -> **Data export**. A text table 'export.txt' (located in the TF5 program directory or as adjusted in the **Engineering** -> **Program Setup** -> **Export/Send** menu) is then created which includes consecutively listed following variables as originally displayed in the **Numerical spectral results** table, separated by ';':

*id; name; date; Power LF **1** [ms2]; Power LF 1 variance [%]; Power HF 1 [ms2]; Power HF 1 variance [%]; PSD LF 1 [ms2/Hz]; PSD LF 1 variance [%]; PSD HF 1 [ms2/Hz]; PSD HF 1 variance [%]; Freq. LF 1 [mHz]; Freq. LF 1 variance [%]; Freq. HF 1 [mHz]; Freq. HF 1 variance [%]; Rat. LF/HF 1 [-]; Rat. LF/HF 1 variance [%]; R-R inter. 1 [ms]; R-R inter. 1 variance [%]; Total power 1 [ms2]; Cumulative total power [ms2];*

*Power LF **2** [ms2]; Power LF 2 variance [%]; Power HF 2 [ms2]; Power HF 2 variance [%]; PSD LF 2 [ms2/Hz]; PSD LF 2 variance [%]; PSD HF 2 [ms2/Hz]; PSD HF 2 variance [%]; Freq. LF 2 [mHz]; Freq. LF 2 variance [%]; Freq. HF 2 [mHz]; Freq. HF 2 variance [%]; Rat. LF/HF 2 [-]; Rat. LF/HF 2 variance [%]; R-R inter. 2 [ms]; R-R inter. 2 variance [%]; Total power 2 [ms2];*

*Power LF **3** [ms2]; Power LF 3 variance [%]; Power HF 3 [ms2]; Power HF 3 variance [%]; PSD LF 3 [ms2/Hz]; PSD LF 3 variance [%]; PSD HF 3 [ms2/Hz]; PSD*

HF 3 variance [%]; Freq. LF 3 [mHz]; Freq. LF 3 variance [%]; Freq. HF 3 [mHz]; Freq. HF 3 variance [%]; Rat. LF/HF 3 [-]; Rat. LF/HF 3 variance [%]; R-R inter. 3 [ms]; R-R inter. 3 variance [%]; Total power 3 [ms2];
The recently edited results are consecutively added to the previous one into one text file, line by line. I.e., whenever numerical results data of any subject are exported, these numbers appear in the 'export.txt' file, in the next line.

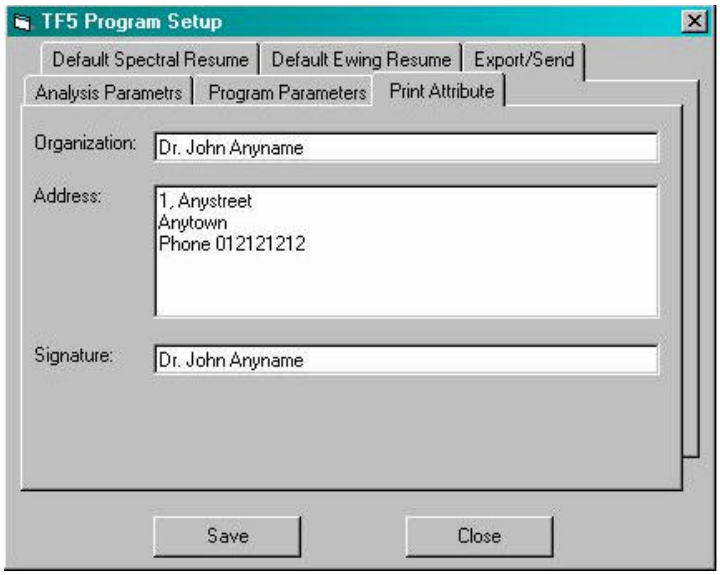
7.5. Analysis. Medical Report and How to Obtain It

Detailed medical reports including the graphical and numerical information obtained during the HRV measurements, completed by personal data details and summary prepared by the examiner/reviewer can be printed from the Basic analysis screen session after pressing the Print icon **or** by following in the Program submenu the option Output -> Print protocol **or** by pressing CTRL + P.

Medical report includes following main sections:

Page 1 of the Report

(1) Organisation / Reporting physician details (adjustable in the Main program menu -> **Engineering** -> **Program Setup** -> **Print Attribute**)



(2) Subject's personal data

(3) Record technical information including number of artefacts and ECG records

(4) Written resume – as/if prepared by reporting physician (text can be entered in the Basic analysis screen -> **Resume**) -- followed by the date of analysis, printing and name of reporting physician.

(5) Below, at the bottom line technical data on current system installation are included.

Page 2 of the Report

(6) Graphical 3D running spectra output including the last name, year of birth and recording details in the figure heading. Note that the printout is 'interactive' as it corresponds with the current view angle of 3D graphical output as just adjusted.

(7) Statistical table including all variables originally displayed in the Numerical spectral results

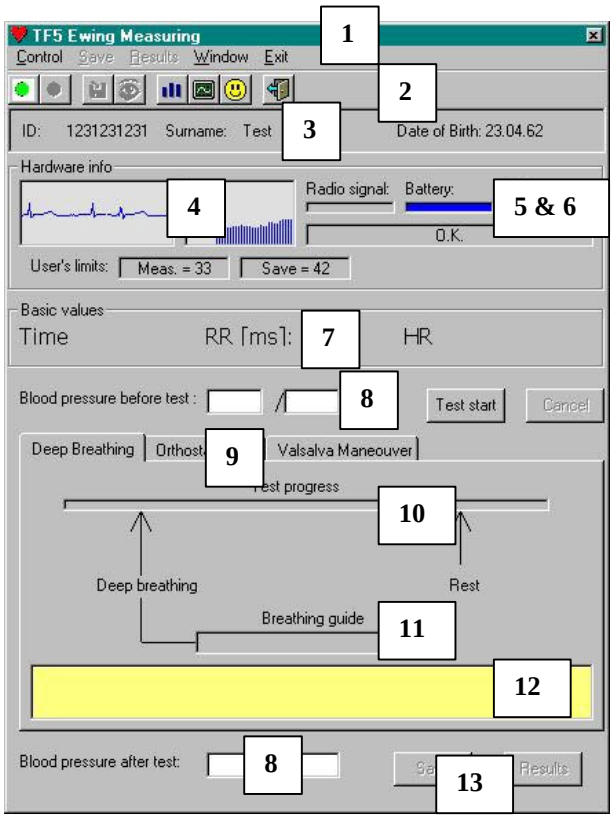
8. Battery of Short Cardiovascular Reflex Tests as Proposed by Ewing (so-called 'Ewing battery')

8.1. Ewing Battery: Introduction

This battery of cardiovascular bedside reflex tests consists of three/four functional tests of heart rate variability, for details see below. The obtained results are then analysed in time-domain as proposed by DJ Ewing *(e.g., see DJ Ewing et al: The Value of Cardiovascular Autonomic Function Tests: 10 Years Experience in Diabetes. Diabetes Care 1985, Vol.8, No.5; 491-498)*. The tests have been suggested to be suitable for a clinical routine screening ('gold standard'), although they predominantly represent vagal function information, are semi-quantitative only, and probably do not detect sufficiently those early stages of autonomic dysfunction that usually are modifiable by various interventions.

8.2. How to Perform the Ewing's Test Battery

In the basic operation screen, click on icon Measuring  (**or** press CTRL + E **or** open Measuring option in the main program menu and select Ewing's Test).
New screen **Ewing's test measuring** is displayed with following main sessions:



1. **Commands menu** including submenu items Control, Save, Results, Window and Exit.
2. **Icons menu** corresponding with the above, including additionally Start and Stop, Save, Browse the results, Display/Hide specific windows, for details see below.
3. **Subject identification** with ID, Surname and Date of birth items.
4. **Hardware info** shows the current quality of data transfer/ECG in *left* window and progress of heart rate over recent time in the *right* window.
5. **Radio signal** quality indicator, and **Battery** status show current state of these items.
6. **Alert window** below the radio signal and battery status indicators informs you of system configuration and of possible errors (e.g., alert 'Receiver switched off…'). A strip with information "OK" shows normal setting, strip blinking in orange warns of errors and possible cause of the problem encountered is displayed. Furthermore, pre-defined user's limits – in accordance with pre-set/prepaid service – and currently available number of TF5 test actions are shown.

7. **Blood pressure** values can be *optionally* entered before and after the Valsalva maneuver and Deep breathing tests. These BP values are, however, *required* for correct consideration of results during the orthostatic test.
8. **Basic values** window delivers exact numeric information on instantaneous R-R data: Time elapsed since pressing 'Test start' button in [min:sec], Length of R-R interval in [ms] and Heart rate in [beats per minute].
9. **Test selection menu** enables to choose among the tests: deep breathing, Valsalva or orthostatic load.
10. **Test progress bar** is an optical hint showing progress of the test in form of constantly growing 'line' / small squares.
11. **Breathing guide bar** is used for guiding/support of correct & reproducible deep breathing pattern.
12. **Help alerts** can be seen in well readable big letters in a yellow bar as indicated above. Commands shown here can be used as guides for correct/standardised performing the tests. Additionally, corresponding voice commands can be heard paralelly.
13. **Measuring control buttons** at the bottom of the window control program functions such as Save and Results. Compared to the spectral analysis section, here is no need to use Stop buttons as the test ends automatically in accordance with standardised test protocol. An alert/help & voice messages inform about the end of each test.

In the Test selection menu (section 9, see above) in the measuring screen click on appropriate button, that allows you to perform one of the three reflex tests:

(1) **Deep breathing** - heart rate changes during four deep breathing cycles are recorded, each of them consisting of 5 sec. inspiration and 5 sec. expiration. After four breathing cycles are completed, test ends automatically.

(2) **Valsalva manoeuvre** - tests heart rate changes following a deep inspiration and 15-seconds expiration against 40 mmHg pressure. As reported, it is recommended to perform this test three times in one measurement session (or to perform one "test" measurement at least before the recording, in order to demonstrate the exact test procedure for the patient).

(3) **Orthostatic test** - changes of heart rate and blood pressure (separately, using a common BP measurement device) during supine position, and, during the first 30 seconds, HR changes and/or some 2 - 5 minutes BP changes after standing up are recorded.

To perform any of the individual tests, see the following description. The help function at the bottom of the screen offers information and hints as to how to proceed. After starting the test, the elapsed time interval since the beginning of the test, R-R interval length and the instantaneous heart rate are displayed.

Generally, before starting the first test, you have to **choose / add a new test subject** in the program database. For details, refer to the Chapter 'How to find a subject' or 'How to add a new subject', please. Afterwards, in the **Main menu** progress to **Measuring** -> **Ewing's Test** (alternatively press CTRL+E together or click on the icon). If the transmitter is already correctly positioned on the subjects's chest and it is switched 'on', you will already have an option to check the quality of transmitted data in the hardware info windows (section 4, see above). This option is identical with the function in the spectrum measuring screen. If the displays are satisfactory, progress to the first Ewing's test: