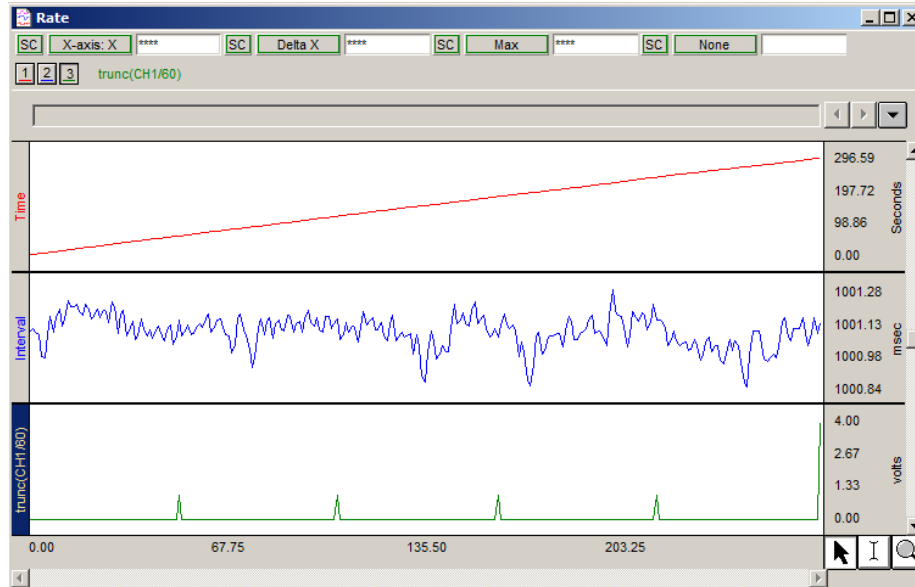


## BSL PRO Lesson H32: Heart Rate Variability Analysis



This *PRO* Lesson describes basic and advanced heart rate variability measurements and details hardware and software setup of the BSL *PRO* software to record ECG. All data collection and analysis is done via the BIOPAC MP36/MP35/MP30/MP45 data acquisition unit and the Biopac Student Lab *PRO* software.

### Objective

This *PRO* Lesson will explain [statistical measures](#), [geometric measures](#), and [spectral analysis](#) in heart rate variability studies.

<b>Statistical Measures:</b>	NN50 count, PNN50, RMSDD, SDANN, SDDSD
<b>Geometric Measures:</b>	HRV triangular index, TINN
<b>Spectral Analysis:</b>	5-min total power ( $\text{ms}^2$ ), HF ( $\text{ms}^2$ ), LF ( $\text{ms}^2$ ), HLF norm (n.u.), LF norm (n.u.), LF.HF ratio, VLF ( $\text{ms}^2$ )

### Definitions

<a href="#">5-min total power (<math>\text{ms}^2</math>)</a>	Variance of NN intervals over the temporal segment (5 minutes).
<a href="#">HF (<math>\text{ms}^2</math>)</a>	Power in high frequency range (0.15-0.4 Hz)
<a href="#">HF norm (n.u.)</a>	HF/(Total Power - VLF) x 100
<a href="#">HRV triangular index</a>	Integral of the density distribution divided by the maximum of the density distribution.

<a href="#"><u>LF norm (n.u.)</u></a>	LF/(Total Power - VLF) X 100
<a href="#"><u>LF/HF ratio</u></a>	LF(ms <sup>2</sup> )/HF(ms <sup>2</sup> )
<a href="#"><u>NN</u></a>	Normal to normal R-R: value of R-R intervals for normal beat. Obtained in real time or post-processing using the Rate function.
<a href="#"><u>NN50 count</u></a>	Number of pairs of adjacent NN intervals differing by more than 50 ms.
<a href="#"><u>pNN50</u></a>	NN50 count divided by the total number of all NN intervals.
<a href="#"><u>RMSSD</u></a>	Square root of the mean squared differences of successive NN intervals.
<a href="#"><u>SDANN</u></a>	Standard deviation of the average NN interval calculated over short periods: usually 5 minutes. (This lesson is written for 1 minute intervals)
<a href="#"><u>SDSD</u></a>	Standard deviation of differences between adjacent NN.
<a href="#"><u>TINN</u></a>	Baseline width of the distribution measured as a base of a triangle, approximating the NN interval distribution HRV.

For a comprehensive overview, see [Guidelines: Heart Rate Variability](#), *European Heart Journal* (1996) **17**, 354-381.

## Equipment

- BIOPAC electrode lead set (SS2L)
- BIOPAC Disposable Electrodes (EL503)—three per subject
- Electrode gel and abrasive pad (BIOPAC GEL1 and ELPAD)
- Computer running Windows 7/Vista/XP or Mac OS X
- Biopac Student Lab PRO software
- BIOPAC Data Acquisition Unit (MP36/MP35/MP30/MP45)

## Setup

### Hardware

1. Make sure that the MP unit is off.
2. Turn on the computer.
3. Plug the Electrode Lead (SS2L) into CH 2 on the front of the MP unit.

### Software

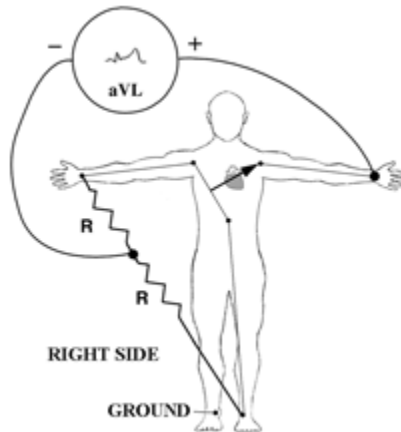
1. Launch the **BSL PRO** software.
2. Open the *Heart Rate Variability* template file by choosing **File menu > Open > choose Files of type: Graph Template (\*.GTL) > File Name: [H32 Heart Rate Variability.gtl](#)**.

**OR:** In BSL 3.7.7 and higher: Launch the Startup Wizard, choose **Record a lesson > BSL PRO tab > H32 Heart Rate Variability.gtl**.

### Calibration

- No calibration required.

## Subject



**NOTE:** A **Lead II** configuration with electrodes placed directly on the torso will reduce artifact.

1. Remove all substantial metal jewelry from the Subject and make sure that the Subject is not touching any metal (metal pipes, chairs etc.).
2. For best results, lightly abrade the skin with abrasive pads, and put a spot of gel on the electrode contact area.
3. Place three electrodes on the Subject as the table indicates.

Electrode	Position	Lead Color
Right arm	Centered on the anterior wrist (same side as the palm of the hand), about 0.5-1.0" down from the palm	<b>White</b>
Right leg	Medial, just above the ankle, flat on skin (not over bone)	<b>Black</b>
Left leg	Medial, just above the ankle, flat on skin (not over bone)	<b>Red</b>

1. Attach the electrode leads by color as shown in the table.
  - The pinch connectors work like small clothes pins; however, they will attach to the electrode only on one side. You may have to rotate the pin to make sure the metal on the inside of the clip is connected, touching and clamped onto the electrode at the base of the nipple.
  - Clip connector cables to Subject's clothing, or place so that there is no strain on the electrode clips or the cable wires at any point in the setup.
5. Turn on the MP unit (assuming the AC100A power adapter has already been connected).
6. Wait 5 minutes after the electrodes have been attached to the skin to begin recording (this gives the gel time to settle and maximize conductivity).

## Recording

- The optimum setup is **Subject** relaxed and in a supine position.
- Record 5 minutes of continuous ECG.
  - The template is set for 30 minutes of recording, but for the purposes of this lesson, 5 minutes is used.

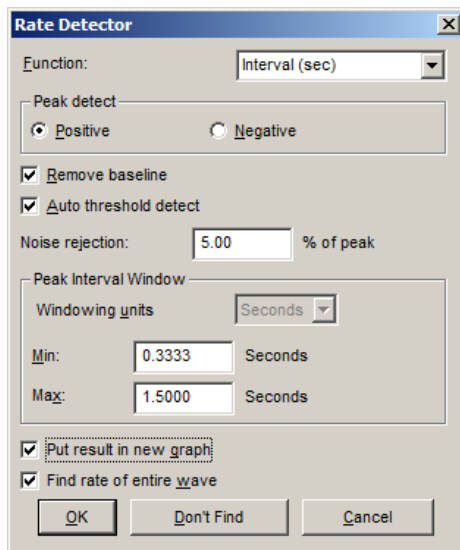
## Analysis

### Statistical Measures

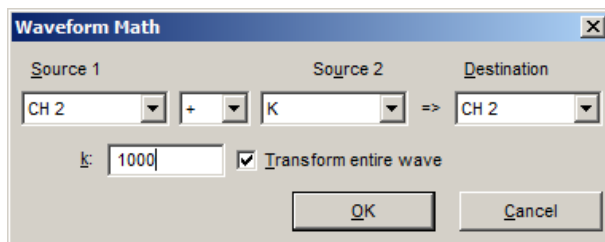
SDANN will be calculated.

#### R-R Interval

- If you used the template for recording setup, R-R interval was calculated in real-time, but you still need to perform this post-processing procedure.
1. Select the ECG or filtered ECG channel.
  2. Click **Transform > Find Rate**. (**Analysis > Find Rate** in BSL 4 and higher)
    - a. Set the Function to Interval (sec).
    - b. Select the option to “Put result in a new graph.”
    - c. Click OK to generate an X/Y plot of RR intervals against time.

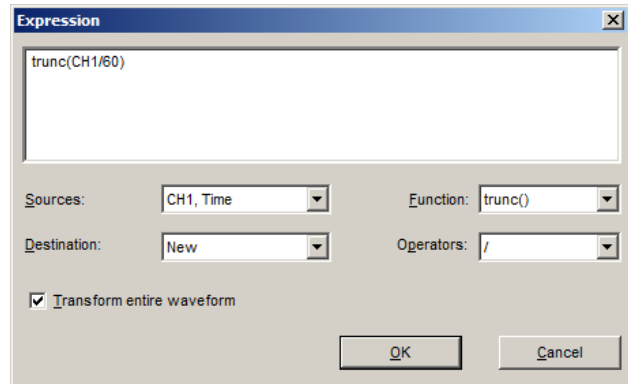


3. Switch to Chart mode (click on the second icon from the left on the tool bar).
4. To convert R-R Interval from seconds to msec, click **Transform > Waveform Math**.



- a. Enter “CH2 \* K” to multiply the channel by a constant.
  - b. Set K, Constant = 1000.
  - c. Click OK.
  - d. Double-click on the word “seconds” on the vertical scale of the R-R Interval Channel
  - e. Change the units in the dialog from seconds to milliseconds (or msec).
  - f. Click OK.
5. To return results at specific intervals, select the Time channel and then **Transform > Expression**.

- a. Select the Sources and Functions/operators to generate the function “**Trunc(CH1/60).**”
    - o For different intervals, change the divisor value from 60 (which equals 1 minute).
  - b. Set the Destination to **New**.
  - c. Click **OK**.
6. To obtain a spike every 1 minute, select the Trunc channel (CH 3) and then **Transform > Difference**.
- a. Interval: **1**.
  - b. Click **OK**.
  - c. Autoscale vertically and horizontally.

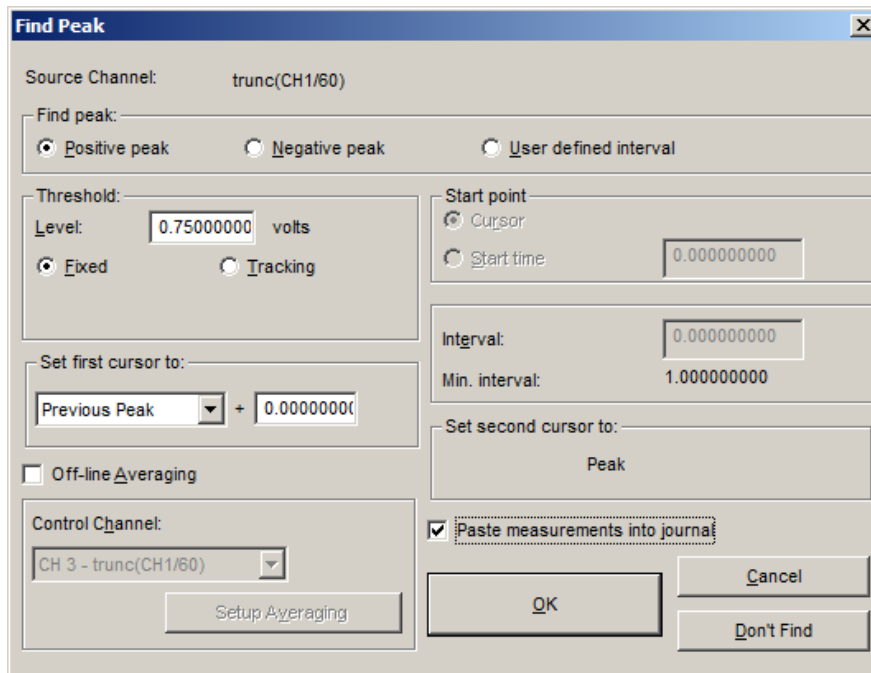


The resulting graph should resemble this one:

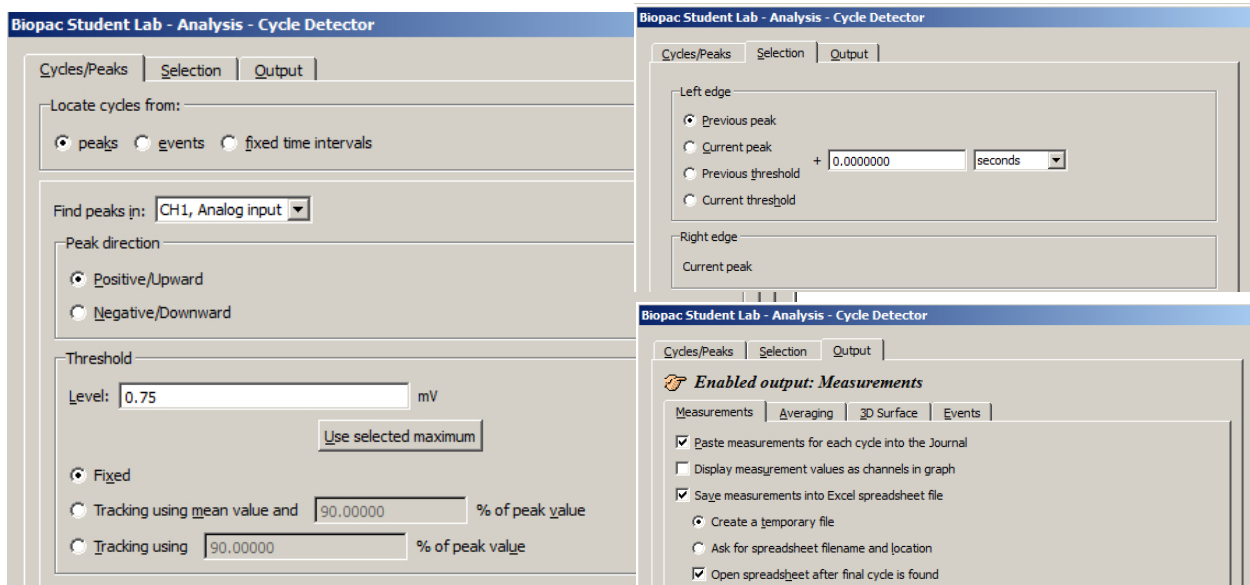


**Time Spike Graph**

7. **IMPORTANT:** Go to **File > Preferences > Journal** and deselect all of the “Measurement paste options.”
8. Set a measurement for **Mean** on the R-R Intervals channel (CH2). Set all other measurements to ‘None’.
9. Select the Trunc channel (CH 3) and highlight the first segment of recording, including the peak.
10. Click **Transform > Find Peak**. (**Analysis > Find Cycle** in BSL 4 and higher)
  - a. Set Find Peak: Pos. Peak.
  - b. Set Threshold Level: 0.75 V Fixed.
  - c. Set first cursor to “previous peak + 0 sec.”
  - d. Select “Paste measurements into journal”.
  - e. Click **Don't Find**.



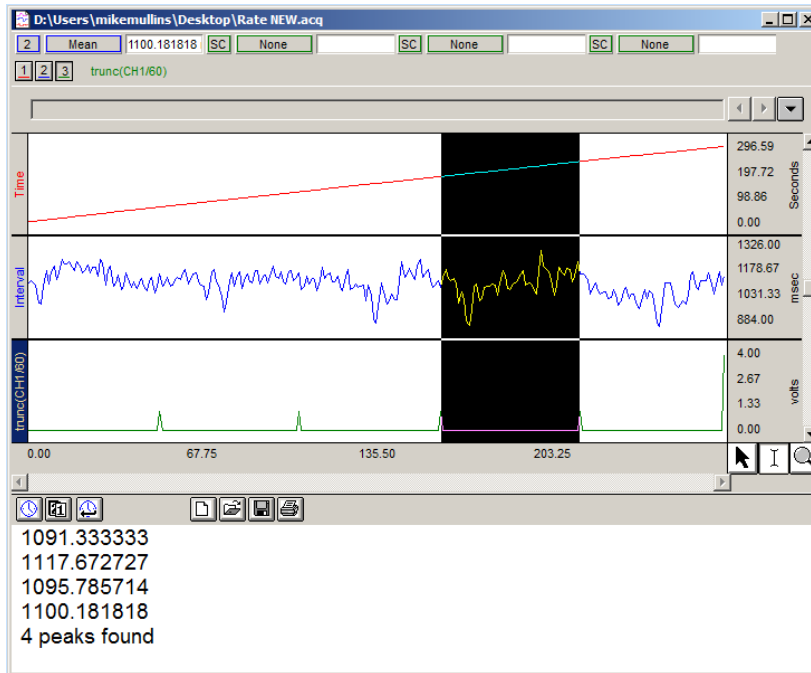
Above: Find Peak setup dialog (BSL 3.7x)



Above: Find Cycle setup tabs and dialogs (BSL 4)

11. Show the Journal by clicking on the 'Journal' toolbar button.
12. Move the cursor to the left of the first peak and choose **Transform > Find All Peaks**. (**Analysis > Find All Cycles** in BSL 4 and higher. In BSL 4, choose the **Output** tab to set Paste option).

You should now have the measurements pasted into the journal:

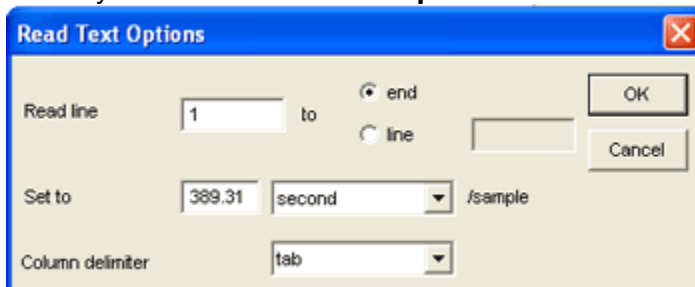


13. Make a note of the last line, “# peaks found” and then delete the last line.
  - o You will use this # in step 16, but you need to delete the line from the journal so it will not be plotted later.
14. Save the journal by clicking the disk icon.
15. Click: **File > Open**.



16. Change “Files of type” to **.TXT**.  
**In BSL 4:** Copy and paste the Journal data to a Notepad \*.TXT file and open this file in BSL. (**File > Open** as **> Text** (\*.txt, \*.csv), **OR** output the Find Cycle results to an Excel file.

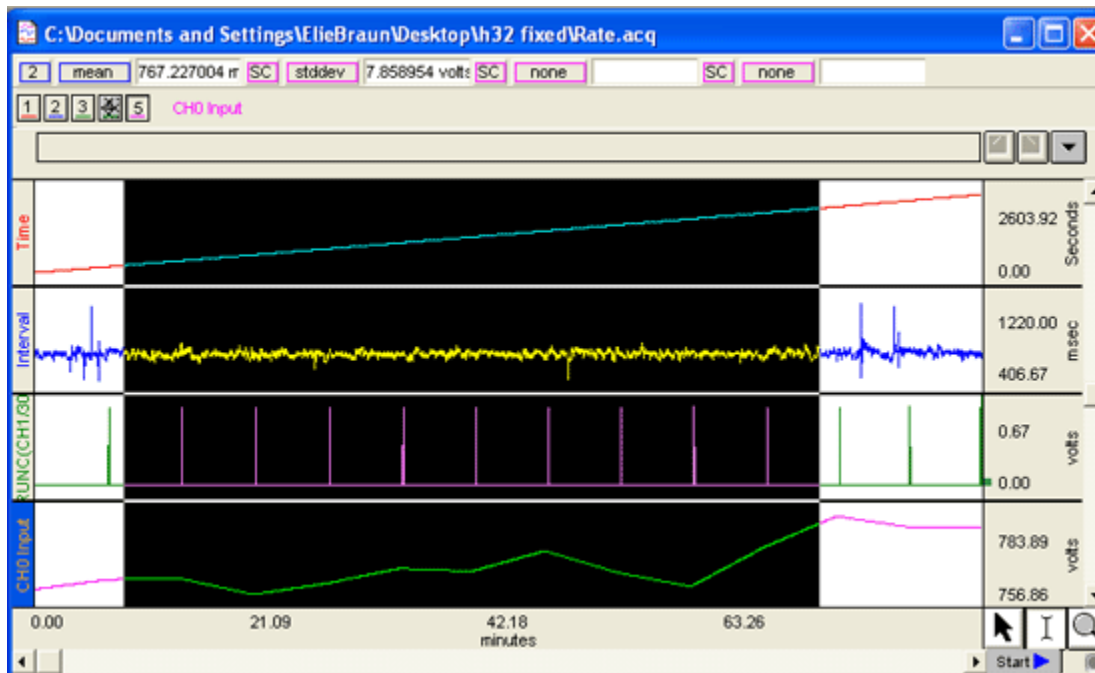
Select your file and then click **Options**. .



- a) Enter a **Set to** value equal to total length of data (in seconds) / number of peaks found.
- b) Click **OK**.

17. Click **Open**. Waveform will appear in new window.
  - o You might have to Autoscale vertically and horizontally to see it.
18. Click **Edit > Select All**.
19. Click **Edit > Copy** to transfer this waveform to your previous window.
20. Click in the previous graph window and click **Edit > Insert Waveform**.

The waveform should now be inserted below the previous three waveforms:



21. Select the Mean channel and highlight the data.
22. Set a measurement for **Stddev** and review the result.

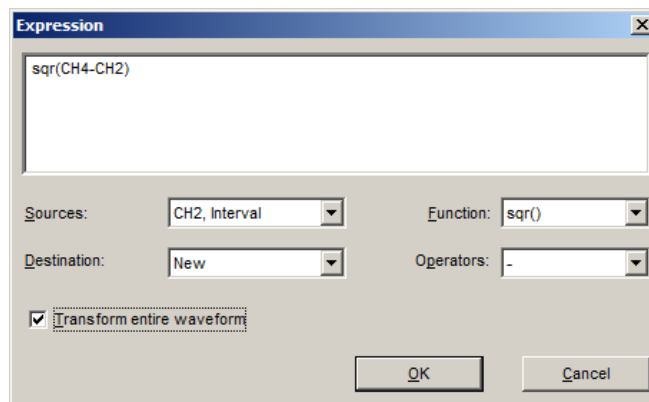
## b) RMSSD

1. Select the Interval channel (from the 'Rate' graph described above) and choose **Edit > Duplicate waveform**. (For easier viewing, hide CH1 and CH3 as they are not needed for this analysis, Ctrl+click the channel button).
2. Zoom in on the first section of your data set.
3. Set a measurement for **Delta s** for the selected channel (SC).
4. Select the duplicate Interval channel.
5. Highlight a section of **3** samples (Delta s = 3).
6. Use **Edit > Cut** to cut the segment.
7. Select the original Interval channel and highlight a segment of **2** samples (Delta s = 2).
8. Choose **Edit > Cut** to cut the segment.

**NOTE:** Select the entire graph and check the Delta S measurement to verify that you have a one sample difference between the original and duplicate RR channels.

You should now have two RR waveforms separated in time by one beat. Use **Transform > Expression** to take the square of the difference between the two channels.

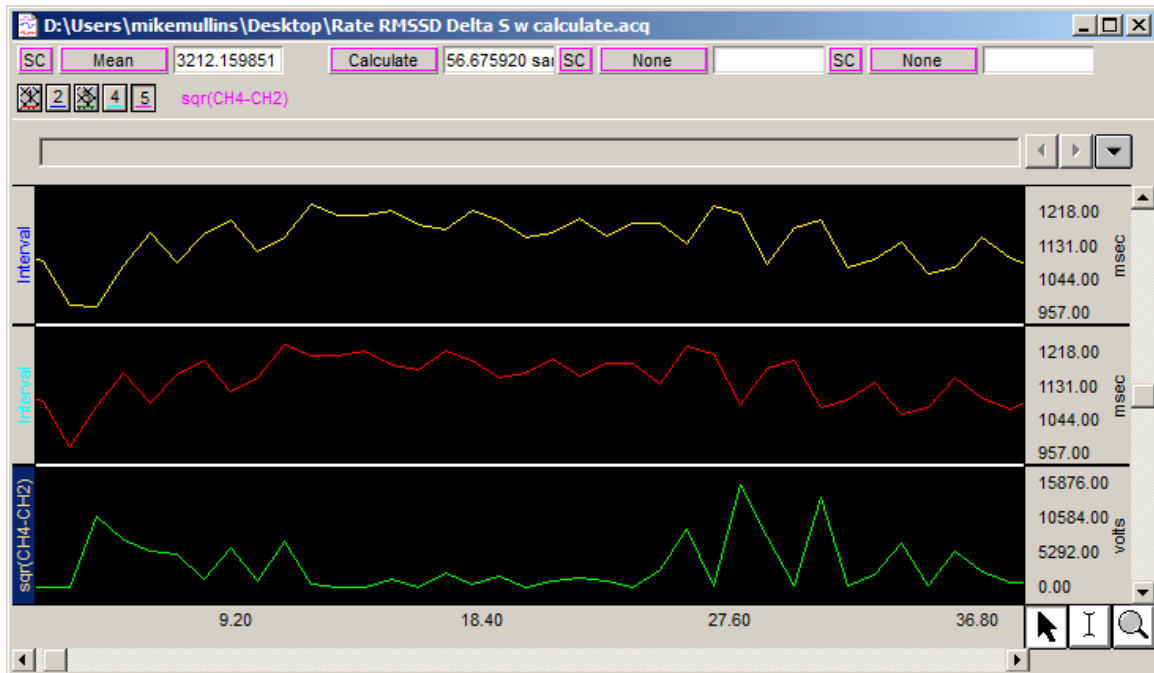
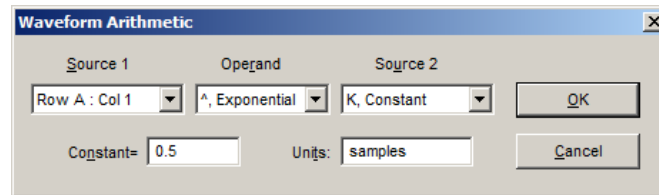
9. Click **Transform > Expression**.
  - a. Equation:  $\text{sqr}(\text{CH4}-\text{CH2})$  (This may not necessarily be CH4 and CH2 for your experiment. Use your own corresponding channel numbers.)
  - b. Destination: New.
  - c. Click **OK**.



10. Select the resultant waveform and choose **Edit > Select All**.
11. Set a measurement for **Mean** in the first measurement box to obtain the mean of the differences.
12. Set a measurement for **Calculate** (second measurement box) to extract the square root of the mean.



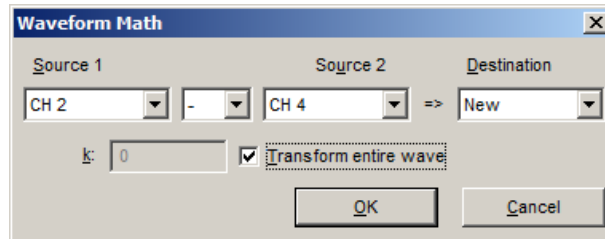
13. Right-click on the **Calculate** measurement button to open the Waveform Arithmetic dialog.
  - a. Enter "**Row A: Col 1 ^, Exponential K, Constant**".
  - b. Set K, Constant = **0.5**.
  - c. Click **OK** and review the calculated result.



### c) SDSD

Use Waveform Math to take the difference of the previously calculated R-R Interval channels.

1. Select Channel 2 and click **Transform > Waveform math**.
  - a. Enter "**CH 2 - CH 4**" (If your channels numbers are different than in this example, use your corresponding channel numbers)
  - b. Destination: **New**.
  - c. Click **OK** and review the calculated result.

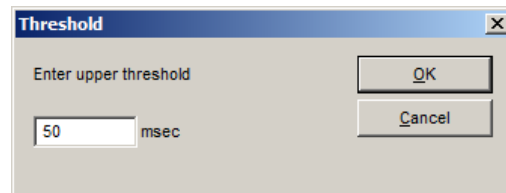
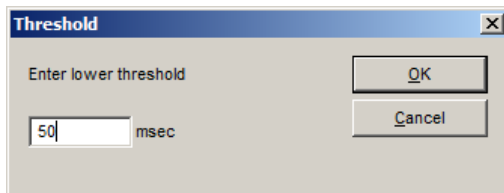


2. Set a measurement for **Stddev** on the resultant channel (re-label as SDSD) and review the result.

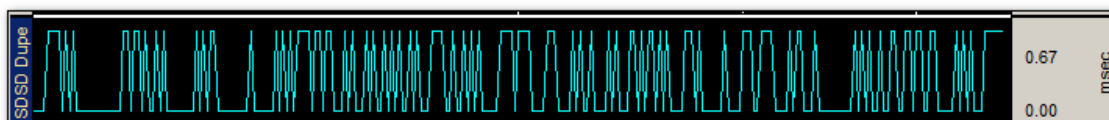
### d) NN50 count

1. Select the SDSD waveform (obtained in c).
2. Click **Edit > Duplicate waveform**.
3. Click **Edit > Select All**.
4. Click **Transform > Math Functions > Abs**.
5. Click **Transform > Math Functions > Threshold**.
  - a. Lower threshold: 50 msec.
  - b. Click **OK**.
  - c. Upper threshold: 50 msec.
  - d. Click **OK**.
  - e. Choose **Display > Autoscale waveforms**.

This function will return a value of 1 when the difference is higher than 50 msec. and zero otherwise.



6. To obtain the number of NN50:
  - a. Choose **Edit > Select All**.
  - b. Set a measurement for **Area**.
  - c. Set a measurement for **Calculate**.
    - i. **Row A. Col 1 x Constant**.
    - ii. K, constant = **1000**.
    - iii. Click **OK** and review the calculated result.



**e) PNN50**

Each beat corresponds to one point or one sample, so you can obtain a percentage by taking the NN50 Count and dividing it by the number of samples, and then multiplying by 100.

1. Set a measurement to **samples**.
2. Set a measurement to **Calculate**.
  - a. **K / Row (Samples)**.
  - b. K = **NN50 count** (as determined in Step D6).
  - c. Click **OK** and review the calculated result.
3. Manually multiply the result by 100 to get a percentage.

**Geometric measures**

Geometric measures are most applicable to long term recordings (24 hours preferred), where the histogram follows a normal distribution. A brief theoretical explanation of two geometrical measures follows, but the user is encouraged to conduct further research on the measures, beginning with the comprehensive overview provided in [Guidelines: Heart Rate Variability](#), *European Heart Journal* (1996) **17**, 354-381.

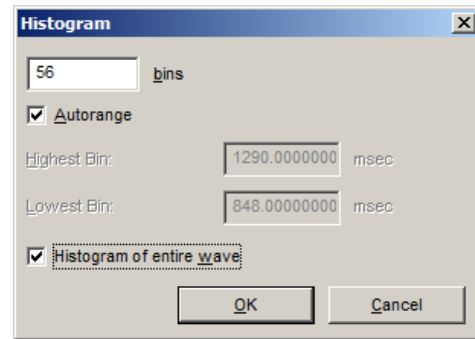
**a) HRV triangular index:**

Using a discrete scale, the measurement is approximated by:

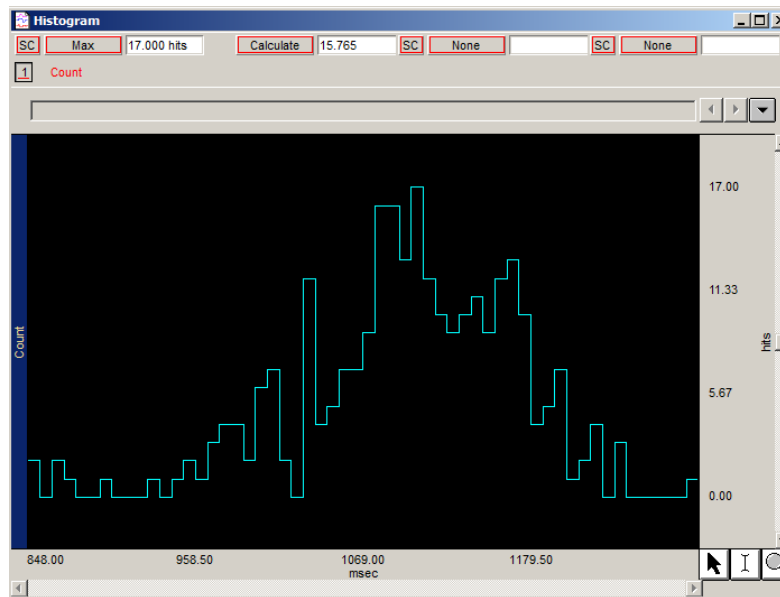
**(Total number of NN intervals) / (Height of the histogram of all NN intervals as measured on a discrete scale)**

1. From the raw ECG waveform, click **Transform > Find Rate**. (**Analysis > Find Rate** in BSL 4 and higher).
  - a. Function: Interval: Sec.
  - b. Click to put result in a new graph.
2. Change the display to **Chart** mode.
3. **Autoscale** the result horizontally.
4. Select the R-R Interval channel.
5. To convert R-R Interval from seconds to msec, click **Transform > Waveform Math**.
  - a. Enter "**CH2 x K, Constant**"
  - b. Set K, Constant = **1000**.
  - c. Click **OK**.
  - d. Double-click on 'seconds' on the vertical scale bar of the waveform.
  - e. In the dialog box, change the units from seconds to milliseconds (or msec).
  - f. Click OK.
6. Select the first few data points (which are artifacts) and choose **Edit > Cut**.
7. Set a measurement for **P-P**.
8. Set a measurement for **Calculate**.
  - a. Enter "**Row (P-P) / K, Constant**."
  - b. Set K, Constant = **7.8125**  
**NOTE:** Most experience has been obtained using bin lengths of approximately 8 ms (precisely 7.8125 ms = 1/128 seconds) which corresponds to the precision of the current commercial equipment.
  - c. Set units to "msec".
    - This gives you an approximation of the number of bins to use.
9. Set a measurement to **Samples** and choose **Edit > Select All** to determine the total number of points.

10. Click **Transform > Histogram**.
  - a. For the bins value, enter the **Calculate** result yielded in Step 8.
  - b. Select **Autorange**.
  - c. Click **OK**.
11. Click on the histogram and choose **Edit > Select All**.
12. Set a measurement to **max**.
13. Set a measurement to **calculate**.
14. Enter **“K, Constant / Row (max)”**.
15. Set **K, Constant = total number of samples** (as determined by the **Samples** measurement in Step 9).
16. Click **OK** and review the calculated result.



Measure the height of the Histogram. That is the HRV index.

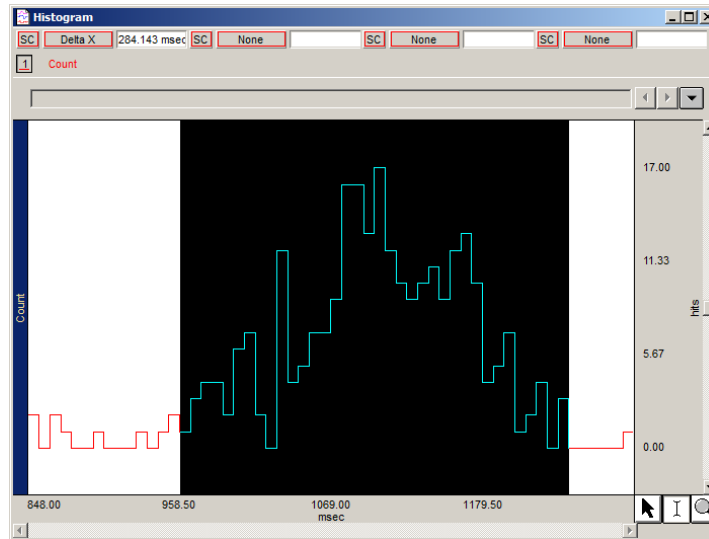


**In this example, 268 samples were taken; the number of hits in the modal bin was 17 and the triangular index is 15.765.**

#### **b) TINN** *Triangular interpolation of NN*

This measurement provides the baseline width of the distribution measured as a base of a triangle, approximating the NN interval distribution HRV.

1. Select the Histogram.
2. Set a measurement for **Delta X**.
3. Highlight the area between the point of initial increase on the bell curve distribution and the point of end of decline (return to baseline).
4. Review the **Delta X** result.



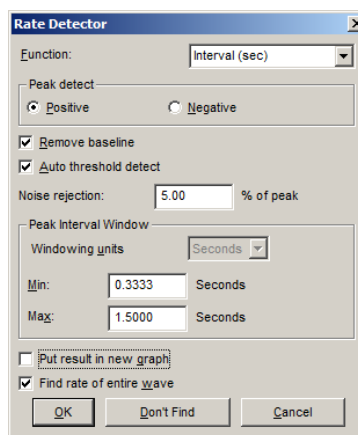
On this histogram, TINN can be approximated as the difference between the start and the end of the highlighted segment, i.e., 284 msec (delta x result).

### Spectral Analysis

Spectral analysis is typically done over either a short period of time (five minutes) or periods of 24 hours. We will cover short term analysis.

To calculate R-R Intervals post-processing:

1. Select the ECG channel.
2. Click **Transform > Find Rate**. (**Analysis > Find Rate** in BSL 4 and higher)
  - a. Function: Interval (sec).
  - b. Deselect "Put result in a new graph".
    - Contrary to the measurements described above, the R-R Interval is taken directly from the main recording and not on a new graph.



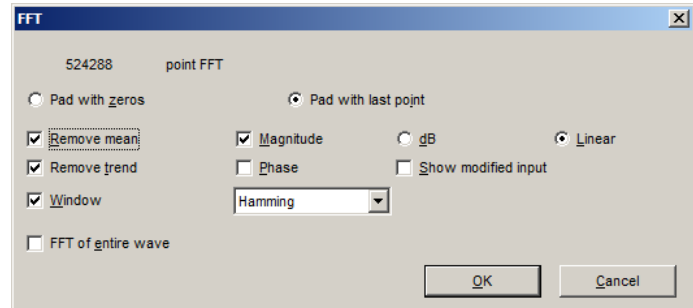
3. To convert the R-R Intervals from seconds to msec, click **Transform > Waveform Math**.
  - a. Enter "**CH R-R x K, constant.**"
  - b. Set K, Constant = **1000**.
  - c. Click "**Scaling**" and change the units to **msec**.
  - d. **Autoscale** the waveform.

**a) 5 min total power (ms<sup>2</sup>):**

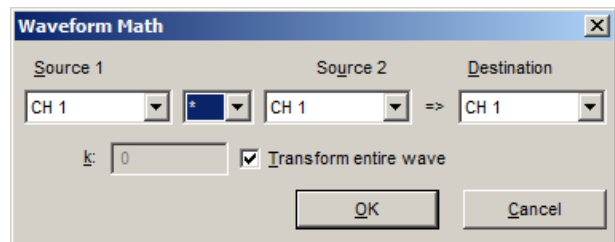
1. Highlight all of the recording (delta t = 300 sec).
2. Set a measurement to **Stddev** on the R-R Interval channels.
3. Set a measurement to **calculate**:
  - a. Enter "Row (Stddev) x Row (Stddev)" to square the standard deviation to obtain variance.
  - b. Click OK and review the calculated result.

Before doing the next measurements, you need to do an FFT on the selected R-R Interval.

4. Click **Transform > FFT**.
  - a. Select **remove mean**.
  - b. Select **remove trend**.
  - c. Select **linear**.
  - d. Select **pad with end point**.
  - e. Set Window = **Hamming**.
  - f. Click **OK** to generate an FFT graph.

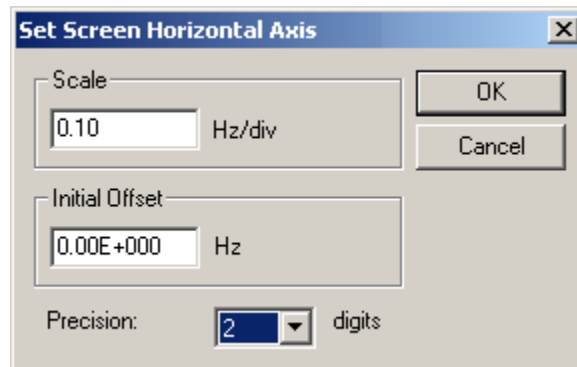


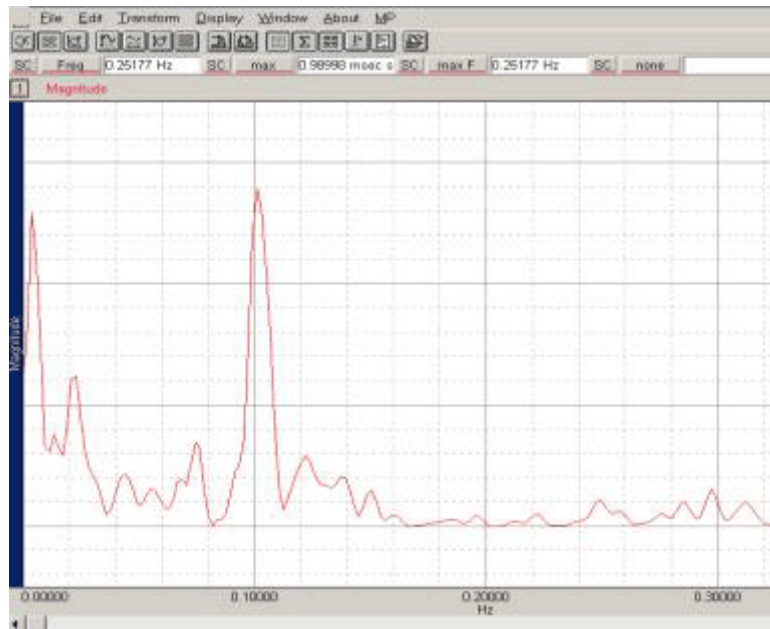
5. Select the FFT graph and click **Transform > Waveform math**.
  - a. Enter "**CH1 \* CH1**" to square the FFT.
  - b. Set Destination = **CH1**.
  - c. Click **Scaling** to change the units to **msec square**; short term spectral analysis results are often reported in msec<sup>2</sup>
  - d. Click **OK**.



To look at the lower frequency range:

6. Click on the **horizontal** scale.
  - a. Enter **0.10** Hz/div.
  - b. Click **OK**.

**b) VLF (ms<sup>2</sup>) Power in the very low frequency range, < 0.04 Hz**



1. Set the measurements **X-axis: F** and **F@Max** to obtain the value of maximum power at the maximum frequency.
2. Highlight a segment from zero to about 0.04 Hz (use X-axis: F results as a selection guide, and use Integral measurement to calculate the total power from 0 Hz to 0.04 Hz).
3. Report the results for power (X-axis) and the frequency (F@Max) at which it was found.

**c) LF ( $ms^2$ )** Power in low frequency range, 0.04 to 0.15 Hz

1. Same as for **VLF ( $ms^2$ )** obtained in b), but highlight the segment between 0.04 to 0.15 Hz (use Freq result as a selection guide).
2. Report the results for power (X-axis) and the frequency (F@Max) at which it was found.

**d) HF ( $ms^2$ )** Power in the high frequency range, 0.15-0.4 Hz

1. Same as for **VLF ( $ms^2$ )** obtained in b), but highlight the segment between 0.15 to 0.4 Hz (use Freq result as a selection guide).
2. Report the results for power (X-axis) and the frequency (F@Max) at which it was found.

**e) LF norm (n.u.)** Low frequency power in normalized units

1. Use results from previous steps to manually calculate:  

$$\frac{LF}{(Total\ Power - VLF)} \times 100$$

$$c / (a - b) \times 100$$

**f) HF norm (n.u.)** High frequency power in normalized units

1. Use results from previous steps to manually calculate:  

$$\frac{HF}{(Total\ Power - VLF)} \times 100$$

$$d / (a - b) \times 100$$

**g) LF/HF ratio**

1. Use results from previous steps to manually calculate:  

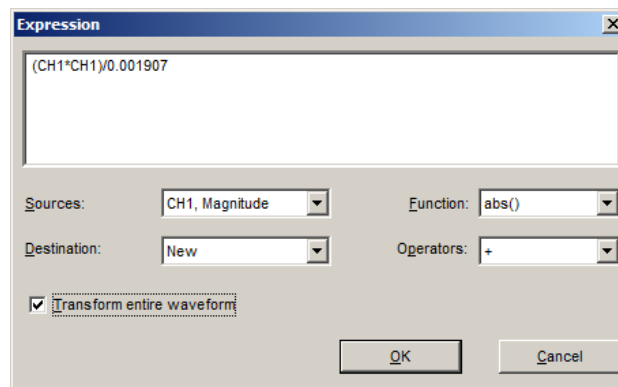
$$\frac{LF (ms^2)}{HF (ms^2)}$$

$$e / f$$

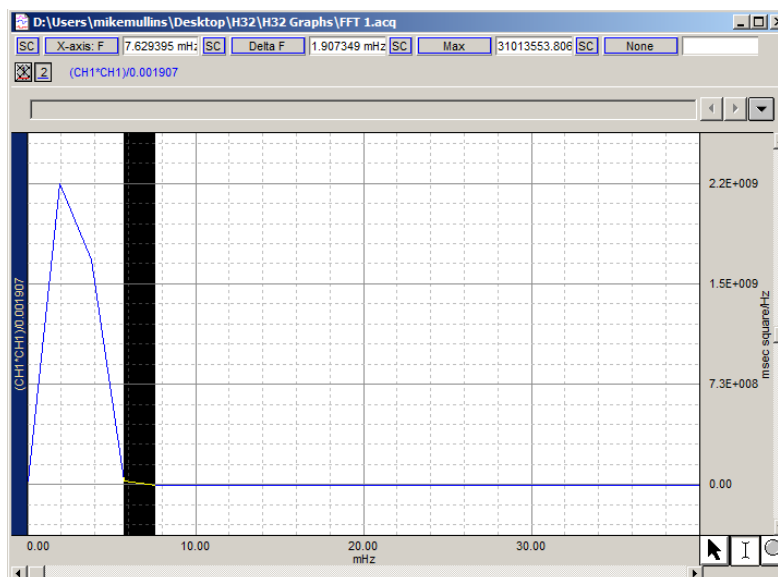
**Power spectral density plot:**

For a power spectral density plot, divide the square of the FFT by the spectral bandwidth (i.e. frequency increment of the FFT). The frequency increment will vary depending on the number of R-R Intervals used and on the sampling frequency of the raw data. To measure it:

1. Click the **horizontal scale** of the FFT.
2. Change the time to **0.01**.
3. Right-click or choose **Display > Show > Dot Plot**.
4. Highlight a segment of data that contains only two (2) points.
5. Set a measurement to **delta F** to obtain the frequency increment.
6. Click **Transform > Expression**.
  - a) Enter "**(CH1\*CH1)/(delta F frequency increment)**" to square the waveform and divide it by the frequency increment, (in this case 0.001907).
  - b) Set Destination to **New**.
  - c) Select "Transform entire waveform."
  - d) Click "**Scaling**" and change volts to "msec square/Hz" or "sec square/Hz" to acquire the correct units.



The resulting graph should be similar to:



**Power Spectral Density Graph**