Article

On the estimation of Ultra-Short-Term Heart Rate Variability

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Abstract: Heart rate variability (HRV) is commonly intended as the variation in the heart rate (HR) and it is evaluated in the time and frequency domains with various well known methods. In the present paper, we first consider an abstract model in which the HR is the instantaneous frequency of an otherwise periodic signal such as the electrocardiogram (ECG). Thus the ECG is assumed as a frequency modulated signal, or carrier signal, where HRV or $HRV(t)$ is a supposed time-domain signal which is frequency modulating the carrier ECG signal around its average frequency. Hence we describe an algorithm able to frequency demodulate the ECG signal to extract a continuous signal $HRV(t)$ with possibly enough time resolution to analyse fast time-domain variations in the instantaneous HR. After exhaustive testing of the method on simulated frequency modulated sinusoidal signals, we have applied the procedure on actual ECG tracings. The purpose of the work is to eventually use the heart as a kind of biological sensor of the fast activity of the autonomous nervous system (ANS) to study the ANS ultra-short-term event-evoked responses. A few preliminary, not clinical, real examples are also given.

Keywords: heart rate variability; vagus nerve response; vagus nerve event evoked responses; Valsalva manoeuvre; signal analysis

1. Introduction

In this paper we first tackled the problem of analyzing the variation of the heart rate (HR) in the framework of the general theory of frequency modulated signals. The second problem we faced was that of recovering the continuous frequency modulating signal from the modulated signal at hand. Both the problems above are very well solved for applications like frequency modulated (FM) radio broadcasting but they are very tricky being put to the physical limits when considered for the heart rate and, in this particular case, when a large bandwidth of the modulated signal is needed to preserve.

On the contrary to commonly known HRV analysis methods, we are looking for an algorithm able to detect very fast variations in the heart rate or variations intervening in a few heart cycles.

Comparing the frequency band occupation of a radio FM signal with the frequency occupation of the ECG signal (simplifying the ECG to having a sinusoidal shape to avoid taking into account also the particular waveform of the ECG) may be very much instructive to highlight the originality and importance of this study.

FM radio broadcasting is very popular (although now made obsolete with the introduction of the digital audio broadcasting or DAB). In the famous 88-108 MHz radio band, many FM stations are allocated with a spacing of 200 KHz and a maximum modulation deviation of ± 75 KHz is permitted for sending stereo audio signals in a band

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from tens of Hz to 50 KHz (considering also stereo multiplexing). It is very well known that the intrinsic non-linearity of the frequency modulation process makes the calculation of the occupied band of the modulated signal very difficult to assess. The popular Carson bandwidth rule [1] gives an estimation of the bandwidth (Carson Bandwidth or CB) of a signal frequency modulated by another signal having a maximum frequency f_m with a maximum permitted frequency deviation of the carrier Δf from the non modulated carrier frequency:

$$CB = 2 \cdot (f_m + \Delta f) \quad (1)$$

For a FM radio emission with $f_m = 53KHz$ and $\Delta f = 75KHz$ we have $CB = 256KHz$ which is even a bit larger than the allocated band for each FM radio channel. By the way the occupied band is just 0.26 percent of the carrier (at center band of 98 MHz).

The same calculation for the frequency modulated heart rate is quite astonishing: first we may assume that the possible maximum deviation of the heart rate from the normal heart rate would be in the order of 40 beats per minute (bpm) or 0.7 Hz, meaning that we might expect, for example, for the heart rate running at 70 bpm (1.17 Hz) to go down to 30 bpm (0.5 Hz) or up to 110 bpm (1.8 Hz) (this is just an assumption with approximate figures); second the maximum frequency of the variation might be assumed to be a bit larger than the breath frequency which is a well known modulator of the heart rate, say 15 events per minute (0.25 Hz). This is just for the sake of having some acceptable figures to let us compute the CB. Thus for the heart rate, normally running at 70 bpm (1.17 Hz), we have $f_m = 0.25Hz$ and $\Delta f = 0.7Hz$ which gives $CB = 1.9Hz$ so explaining the intrinsic difficulty or even impossibility of a correct estimation of the HRV, as the signal modulating the heart rate has a larger bandwidth than the carrier itself ! In the radio jargon the heart rate would be classified as an Ultra Wide Band (UWB) emission ! Indeed, in the calculation above we considered just maximum values, but it is really uncommon that the breath frequency induced HRV have a shift of 40 bpm, by the way the problem remains as the CB of the heart rate signal is still comparable to the mean heart rate itself.

The concepts previously highlighted make the use of standard frequency de-modulators commonly used in radio technology impossible. Indeed HRV has been always evaluated by the computing of the actual time delays of arrival of each individual heart beat or instantaneous heart period. Much the same strategy used in radio detection of UWB pulses.

There are two problems to underline. First is that, no matter the demodulation process to estimate HRV, we will never obtain a continuous heart rate modulation signal as the instantaneous heart rate is not available at each instant of time but only at each heart beat (by computing the inverse of the instantaneous heart period). Thus the heart rate variability signal, which we may surmise a signal continuous in nature, is not available in a continuous form (like the blood pressure signal for example) for us to acquire. Second, it is true that we are anyway digitally acquiring biological signals in a sampled form but the sampling frequency is chosen by the experimenter while for HRV signal we should obey to its own sampling frequency (which is the heart period). But the heart period or frequency is exactly what we are looking for ! So the HRV signal is in general a non continuous signal available at its own sampling frequency which is by the way the signal itself !

The authors think that there is no need here to review once again all the HRV acquisition and analyzing techniques developed so far. All these algorithms can be roughly divided into long term and short term HRV analyzing methods depending on the time length of the epoch of the ECG to study. The results can also be divided into time or frequency analysis. The readers well acquainted in this field know the story very well. For the other interested readers the reference [2] is warmly suggested.

Nevertheless, to discuss the current state of the research field special attention should be devoted to the papers dealing with the problem of HRV signal resampling. Of special interest for our work is the paper [3] where the effects of resampling frequency of RR

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interval and length of the epoch of analysis are considered toward the evaluation of the autonomic nerve system.

Fast variations of acceleration and deceleration of heart rate were also analyzed in [4] where interest was given to the preprocessing of unevenly sampled RR interval signals by the use of interpolation and resampling.

A special mention for comparing HRV analysis merthods is the Lomb-Scargle periodogram [5,6] which can be used for a reliable estimation of the frequency spectrum of unevenly sampled signals like RR series. Lomb-Scargle periodogram is more common in the astronomy community but it is, quite unfortunately, less popular in the HRV research community mostly because the mathematical difficulties in understanding it.

The problem of resampling and actual final sampling rate of HRV signal has been studied also in [7] where another time domain measure of HRV has been introduced. Errors and effects introduced in the resampling process of HRV were also studied in [8-9].

The present paper introduces yet another method for obtaining a resampled and clean HRV signal out of a sequence of RR intervals with the aim of preserving the bandwidth of the frequency modulating signal even on short or ultra-short time epochs.

2. A model of the heart rate signal on which to test the new algorithm

The central assumption put forward in this paper is that any heart-activity-related biomedical signal (electrocardiogram ECG, phonocardiogram PCG, photopletysmogram PPG, etc.) is a frequency modulated signal being its instantaneous frequency known as the instantaneous heart rate. Considering the ECG, as the instantaneous frequency of it is time varying, then it is a time signal. The purpose of this paper is to describe a method for the frequency demodulation of the ECG to obtain an estimation of the instantaneous heart rate signal. The carrier signal is sinusoidal in conventional frequency modulated signals like those used in radio communications. In the case here, the heart-activity-related biomedical signal is not sinusoidally shaped. Indeed the ECG is not sinusoidal, although we may consider it as a periodical signal as well, made of repetitions of the well-known P-Q-R-S-T waves (instead of sinusoidally shaped periods). Now, no matter the actual frequency content of the biomedical signal, which depends on its wave shape, we consider the signal with its periodism as the carrier of a frequency modulating signal in the time domain, which we name the heart rate signal. The heart rate signal is not directly available for acquisition from the real world. Still, we know that the heart rate signal is visible as the instantaneous frequency of other signals generated synchronously with the heart activity, being the electrocardiogram one of those. As the ECG has a large bandwidth between other heart-activity-related signals and it is simple to process for extracting the heart rate, then the heart rate is commonly inferred from the frequency of the ECG events as its jitter incertitude is at the minimum. Jitter may be defined as the deviation from the true periodicity of a presumably periodic signal. As the ECG is, by its nature, not periodic, then it is evident that the jitter, in assessing the true instant of time at which the R-wave in the ECG arrives, will be made of two components: one component is the natural (physiological) wandering of the heart rate (the scope of our research) and the second component is the instrumental error in detecting the time of the R-wave (instant of the heart beat event). The first component is due to the heart rate variability signal (also made of two components: the real wandering of the cardiac physiological pace-maker, which generates P-waves, summed to the wandering of the transmission delay of the impulse from the physiological pace-maker through the specific heart conduction tissues to the ventricles, which generate the R-waves) and the second component is an unavoidable, although it can be minimized, instrumental error. Indeed, ECG is used because its large bandwidth so that on this signal the instrumental error can be kept at a minimum compared to other heart-activity-related biological signals. At the end of the preliminary description, we will use the ECG signal to infer the shape of the heart rate signal and we will always assume the instrumental jitter as zero. First we consider, obviously, the ECG

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as the time domain signal produced by the ventricles' electrical activation: $ECG(t)$. To better describe our model, and without loss of generality, we may initially consider a sinusoidal signal as a classical kind of a periodical signal that is clearly simpler to manage than the ECG itself. Trivially, a sinusoidal period has the shape of a sinusoid while an ECG period has the well known shape of the P-Q-R-S-T waves complex: in a sinusoidal signal we have repetitions of sinusoids and in a ECG signal we have repetitions of P-Q-R-S-T complexes. So, let's first consider a sinusoidal signal instead of an ECG signal. As we are interested in the wandering of the frequency of repetitions, the shape of what repeats itself is meaningless. To help correctly understand the matter, we start from Fig. 1.

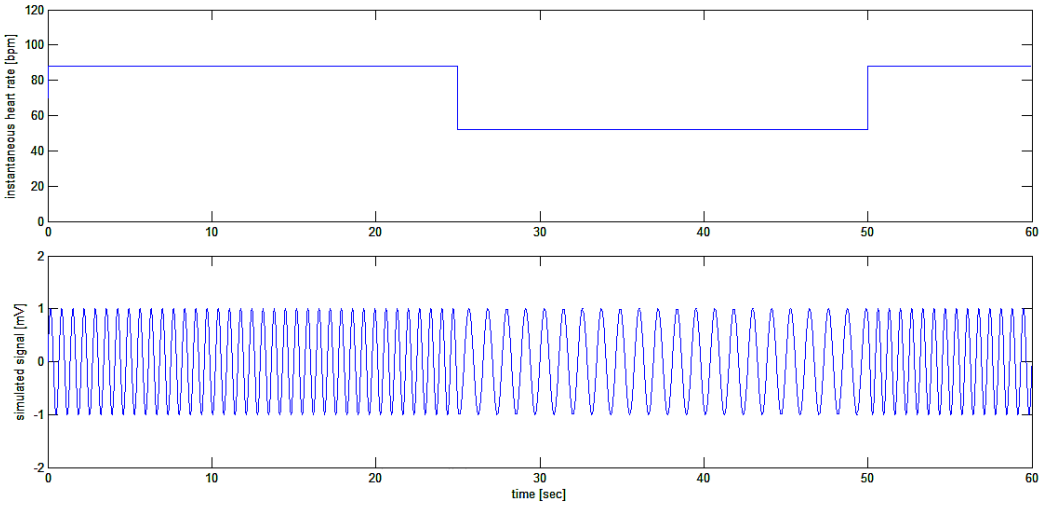

Figure 1. Upper tracing: the simulation of an instantaneous heart rate signal, or $HR(t)$, composed of a constant signal with an amplitude of 70 bpm summed to a square-wave signal having a peak-to-peak amplitude of 36 bpm and a frequency of 0.02 Hz; the resulting signal is a square-wave signal having an average value of 70 bpm alternating between 88 bpm and 52 bpm every 25 seconds. Lower tracing: the $HR(t)$ signal imposes the frequency of a sinusoidal signal creating our "sinusoidal ECG" signal whose frequency is, at each instant of time, precisely that shown in the upper tracing. Please note that the amplitude of the $HR(t)$ signal is purposely given in beats per minute (bpm) while the units of the simulated sinusoidal signal may be arbitrary. Please note that at each instant of time the simulated sinusoidal signal frequency is exactly the value of the square-wave modulating signal $HR(t)$.

Software implementation of the simulation in Fig. 1 is relatively straightforward as the frequency varying sinusoidal signal is composed of epochs in which its frequency is constant (in the example, there are epochs in which frequency is 88 bpm alternating with epochs at 52 bpm). But this situation is straight forward and it is not what we might expect in general in the frequency wandering of the ECG: indeed, we will expect a continuous variation of the instantaneous frequency of the ECG. Let's put it in formulas. The sinusoidal signal that we will use must show a continuous frequency wandering behaviour, as we might see in the ECG. We continue using a sinusoidal signal instead of an ECG signal. From secondary school, the sinus function is presented as:$sin(2 \cdot \omega \cdot t) = sin(2 \cdot \pi \cdot f \cdot t)$, which is strictly an accurate periodical signal of period$2 \cdot \pi$. For the scope of introducing frequency wandering in the formula, we have to re-learn what a sinus function is all about: given a time domain function $HR(t)$ representing the instantaneous value of the frequency of the sinus signal, or heart rate, then this can be represented in formula as:

$$sin\left(2 \cdot \pi \cdot \left(\int HR\,(t) \cdot dt\right)\right) \tag{2}$$

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Should $HR(t)$ be reduced to a constant f not depending on time, then the formula above will become the well known formula for the actual periodic (not frequency wandering) sinus function:

$$sin\left(2 \cdot \pi \cdot \left(\int HR\,(t) \cdot dt\right)\right) = sin\left(2 \cdot \pi \cdot \left(\int f \cdot dt\right)\right) = sin(2 \cdot \pi \cdot f \cdot t) \quad (3)$$

Of course, as the frequency of our "heart sinusoidal" signal is not constant, we should keep the first representation as in (2).

Following the example in Fig. 1, we say that the function $HR(t)$ is a time-domain signal whose amplitude is the instantaneous heart rate or the instantaneous frequency of the ECG signal. As a matter of fact, we consider the signal $HR(t)$ as composed of two parts, one constant and another variable in time such as:

$$HR(t) = \bar{f}_0 + HRV(t) \quad (4)$$

where $\bar{f}_0$ is the constant average value of $HR(t)$ and the function $HRV(t)$ is the variable component or, more appropriately, the heart rate variability signal we are looking for. In the example simulated in Fig. 1 we used the following values:

$\bar{f}_0 = 70$ bpm, and $HRV(t) = 18 \cdot sign\big(sin(2 \cdot \pi \cdot f_t \cdot t)\big)$ bpm

where $sign(t)$ is the sign function and f_t is the frequency of variation of the instantaneous signal frequency (0.02 Hz).

In a more general case, we will define $\bar{f}_0$ as the average (constant) heart rate during a given (short) epoch of signal acquisition and we will define $HRV(t)$ as the wandering frequency signal, or the heart rate variability signal, whose amplitude is the instantaneous shift of the heart rate from the average heart rate (frequency modulation).

We can state the problem of recording the $HRV(t)$ signal as the method by which this signal can be inferred from the quasi-periodic signal $ECG(t)$.

As we have already noted, the $HRV(t)$ signal is not directly available in nature. We may only infer this signal by measuring the frequency of the ECG signal, i.e., the frequency of R-waves in it. Unfortunately each measure of frequency implies an integration such as counting (integrating) the number of appearances of certain events over a given period of time. At the limit, looking for the fastest way of estimating the instantaneous frequency of the ECG or the instantaneous heart rate, we may measure the time delay between two events (period) and then calculate the frequency as the inverse of this measure. Of course, this means we may only infer the frequency of the ECG (heart rate) at each heart beat and not between two heart beats. Now it appears that the heart rate is, in nature, a sampled signal (we may only get one sample of it at each detected R-wave or heart beat) as we will never know the actual instantaneous heart rate value in the time between two heart beats. By the way we still want to consider the signal $HR(t)$ as a continuous in time signal implicitly.

The need to obtain a "continuous", or "instantaneous", $HR(t)$ signal comes from the interest in analysing very short or even ultra-short-term heart rate variations in the time domain instead of considering cyclical variations on very long epochs of time.

Thus we may restate our problem as it follows: we are looking for a reliable, robust and possibly simple method able to estimate the continuous $HR(t)$ thus $HRV(t)$ signal from the $ECG(t)$ signal at hand.

Before going further into the tricky algorithm, let's look at Fig. 2 where we try to connect the mathematics and signal theory discussed so far with the neurophysiological nature of things.

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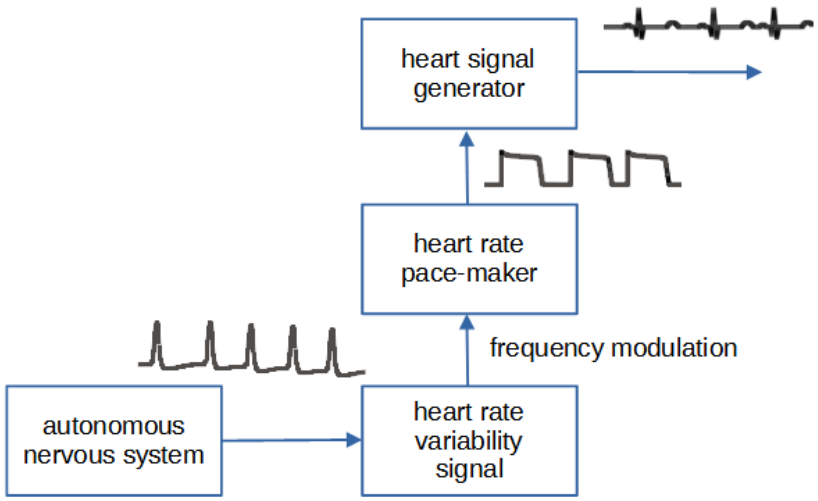

Figure 2. Abstract model of the heart rate variability signal as a heart rate frequency modulator.

The model in Fig. 2 is quite abstract because, in this work, we don't need to understand the physiological mechanism at the base of the heart rate and its time wandering. Nevertheless, we may remember that the autonomous nervous system, besides other actors in the process, may interact with the physiological pacemaker of the heart (the sinus node) by modifying the heart rate which we might suppose, otherwise, as constant. Thus we may suppose (here just for clarity sake as, again, the complete explication of the phenomenon is out of the scope of this paper) that the autonomous nervous system is generating the $HRV(t)$ signal (codified in the sequence of neural spikes) which will modulate the rate of the pacemaker generating the $HR(t)$ signal which will eventually impose the instantaneous frequency of the $ECG(t)$ signal.

3. Algorithm for the estimation of the instantaneous heart rate signal from the ECG

As already stated, the presented algorithm aims to obtain a signal representative of the "instantaneous" heart rate. This signal should be provided with a sampling rate even higher than the heart rate itself.

There are two standard and straightforward ways of extracting the heart rate signal from the ECG signal [2]. Both methods start with the measuring each heart period and convert it to frequency by calculating the inverse. The collection of measures will normally be unevenly spread in time as the time delay between a measure and the following is just equal to each heart period which is varying. We may say that the $HR(t)$ signal is available only in an unevenly sampled fashion where the actual sampling is the signal itself ! Thus, to create an evenly sampled signal from the set of measures, the first possibility is to distribute each sample measure on a reconstructed sampling frequency equal to the average heart rate in the epoch to be analyzed. The second possibility is that of leaving the measures at the instants of time where they were taken and interpolating them on an evenly distributed instants of time. Same interpolation is to be done also when using the first method if a more significant number of points is required. This problem is commonly known as resampling.

Both methods will provide a $HR(t)$ signal suitable for subsequent analysis in the frequency domain but relatively poor for direct study in the time domain. Indeed, time-domain studies of $HR(t)$ are never done on the waveform of $HR(t)$ but, more often, on indexes extracted from it [2].

We aim to obtain a kind of $HR(t)$ signal that might be directly analysed for the study of fast transitory events appearing in the signal itself. Indeed the final goal, and the advantage of the algorithm which is about to be described, is that of catching fast

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variations in the heart rate, which might be related to sudden correlated variations of the activity of the autonomous nervous system.

Thus the problem we want to tackle is estimating the signal $HRV(t)$, or $HR(t)$, from $ECG(t)$. At first we have to recognize that nor $HRV(t)$ neither $HR(t)$ can be directly measured with appreciable time resolution ! Indeed, even supposing that we have a reliable R-wave detector on the signal $ECG(t)$, we could just measure $HR(t)$ only at each nth heart beat occurring at each instant of time t_n. We might note that the $HR(t)$ signal is the only biological signal which is only naturally available in sampled form; moreover, as written above, its sampling instants of time are the signal itself!

Thus in the proposed method, we build a new signal $SR(t)$ as the cumulative sum of the heart beats (or complete $ECG(t)$ cycles). We define a suitable evenly spaced sampling time t_s for the $SR(t)$ signal. This means that the $SR(t)$ will be created as a signal sampled at a frequency $f_s = \frac{1}{t_s}$. We may chose a convenient sampling frequency for f_s, often even higher than the actual heart rate and for sure higher than the frequency content of $HR(t)$, and maybe we may even use the same sampling frequency at which the signal $ECG(t)$ itself has been acquired. Then, at each time instant of sampling t_i, we apply the following algorithm:

if a heart beat has occurred at an instant of time t_n where $t_{i-1} < t_n \leqslant t_i$ then $SR(t_i) = SR(t_{i-1}) + 1$ otherwise $SR(t_i) = SR(t_{i-1})$.

This means that $SR(t)$ will be built as the running count function of the detected heart beats. Obviously it will be a not-descending function of time, it will be sampled at the frequency f_s with a [instantaneous] slope equal to $HR(t)$ beats per minute. Indeed we used the word "instantaneous" in brackets because the signal $SR(t)$ is a sampled staircase signal which remains constant during many samples between each heart beat and the following. Please note that the signal $SR(t)$ rises as faster as higher will be the signal $HR(t)$: indeed, supposing $HR(t)$ be constant at 70 bpm, then $SR(t)$ will obviously grow at a rate of 70 units per minute.

Thus, we might evaluate $HR(t)$ as the first derivative of $SR(t)$ or

$$HR(t) = \frac{\partial SR(t)}{\partial t} \text{ in bpm.} \tag{5}$$

As $HR(t) = HRV(t) + \bar{f}_0$ we may subtract the signal's average (mean heart rate) to obtain $HRV(t) = HR(t) - f_0$ in bpm.

Apart from a series of difficulties which we shall discuss in the following, it is worth noting that the $HRV(t)$ signal so estimated is in principle known with a remarkable time resolution (it is sampled at a very high sampling frequency) hence it is helpful for ultra short term heart rate variability (USTHRV) studies even to detect fast and short lived heart rate variations (time domain HRV studies).

It must be noted that this proposed method is better than the standard method based on interpolation of the inverse of individual heart periods. By the way, here the interpolation is still used for computing the derivative of the signal and it is also done through low pass filtering as described in the following. Nevertheless, the obtained signal still maintains a wide frequency content that shows fast heart rate variations.

4. Simulating a frequency wandering signal on which to test the performances of the algorithm

To describe the performances of the method described in the previous paragraph, we will initially use simulated signals for which we exactly know the $HR(t)$ signal used for simulating those. Eventually, we will apply the method on real ECG biological signals as in next paragraph 6.

So we need a reliable simulator for creating an artificial and perfectly known frequency wandering or frequency modulated signal, we will need to test the algorithm

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for acquiring a continuous recording of the $HR(t)$ signal. It is clear that as the algorithm's output we should obtain what we expect, namely the known modulation signal we used in the simulation.

The simulator will produce a sinus shaped signal instead of an ECG shaped signal as there is no loss of generality. Just the periods in the sinusoidal signal will be detected by positive zero-crossings of the signal (inflections crossing the baseline from a negative value to a positive value) while, in the actual real application, the periods on a real ECG signal will be detected by the peaks of R-waves. In spite of using a sinus waveform, we will call the simulated signal as $ECG(t)$ as well. For the $HRV(t)$ signal we will use various wave-shapes, from a square waveform as already shown in Fig. 1, to a mix of superposed sinusoidal signals at different frequencies.

Initially we restate:

$$HR(t) = \bar{f}_0 + HRV(t) \tag{6}$$

then from (1):

$$ECG(t) = sin\left(2 \cdot \pi \cdot \left(\int \left(\bar{f}_0 + HRV(t)\right) \cdot dt\right)\right) \tag{7a}$$

$$ECG(t) = sin\left(2 \cdot \pi \cdot \bar{f}_0 \cdot t + 2 \cdot \pi \cdot \left(\int HRV\,(t) \cdot dt\right)\right) \tag{7b}$$

This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation, as well as the experimental conclusions that can be drawn.

4.1. Simulating a square wave-form which is frequency modulating the sinusoidal carrier

In a first simulation we will consider:

$\bar{f}_0$ (Hz) or $60 \cdot \bar{f}_0$ (bpm) as the average heart rate and

$$HRV(t) = f_s \cdot sign\big(sin(2 \cdot \pi \cdot f_{HRV} \cdot t)\big) \tag{8}$$

as a heart rate variability square wave periodic signal producing a frequency shift $\pm f_s$ at a frequency f_{HRV} on the carrier signal $ECG(t)$. This simulation is much similar to what is already presented in Fig. 1 before.

As an example, we will take the following values for the various parameters:

$\bar{f}_0 = 1.17$ Hz or 70.2 bpm, $f_s = 0.12$ producing a frequency shift of $\pm 60 \cdot 0.12 = \pm 7.2$ bpm and $f_{HRV} = 0.02$ Hz for a frequency shift period of 50 seconds.

With the indicated parameters, the simulated ECG signal will have the following expression (we use the subscript on the ECG signal for indicating the various simulated signals, which will then be used for testing the algorithm and $sign(\ \ )$ is again the sign function):

$$ECG_1(t) = sin\left(2 \cdot \pi \cdot \left(\int \left(f_0 + f_s \cdot sign\big(sin(2 \cdot \pi \cdot f_{HRV} \cdot t)\big)\right) \cdot dt\right)\right) \tag{9}$$

$$ECG_1(t) = sin\left(2 \cdot \pi \cdot \left(\int \left(1.17 + 0.12 \cdot sign\big(sin(2 \cdot \pi \cdot 0.02 \cdot t)\big)\right) \cdot dt\right)\right) \tag{10}$$

The signal $ECG_1(t)$ is a sinusoidal signal suddenly changing its frequency every 25 seconds from $70.2 - 7.2 = 63$ bpm to $70.2 + 7.2 = 77.4$ bpm and vice-versa as in Fig. 3.

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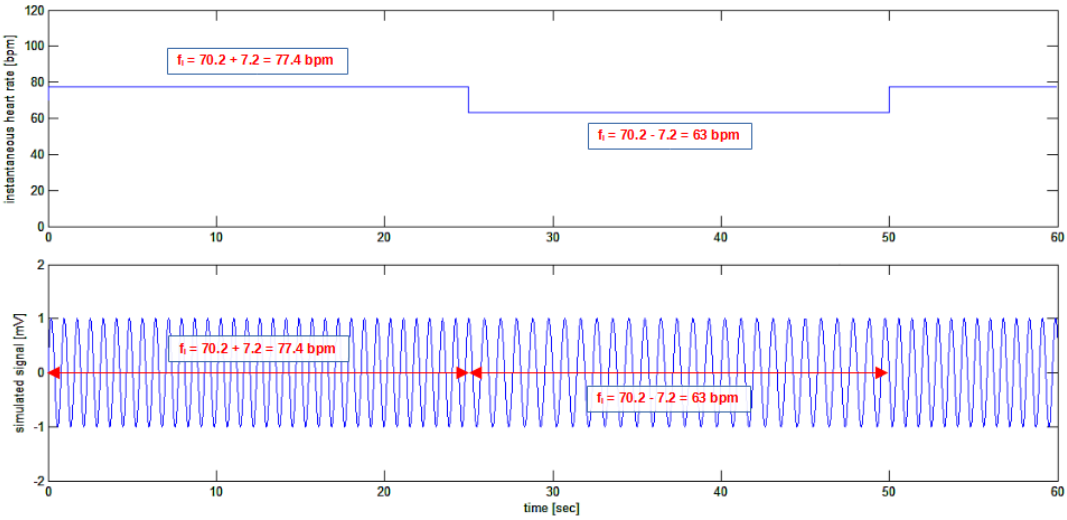

Figure 3. Simulation of test signal $ECG_1(t)$: a sinusoidal signal (bottom tracing) frequency modulated with a square wave (upper tracing).

4.2. Simulating a sinusoidal wave-form which is frequency modulating the sinusoidal carrier

In a second simulation we will consider:

$\bar{f}_0$ (Hz) or $60 \cdot \bar{f}_0$ (bpm) as the average heart rate and

$$HRV(t) = f_s \cdot sin(2 \cdot \pi \cdot f_{HRV} \cdot t) \tag{11}$$

as a heart rate variability sinusoidal wave periodic signal producing a frequency shift $\pm f_s$ at a frequency f_{HRV}.

As an example we will take the following values for the various parameters:

$\bar{f}_0 = 1.17$ Hz or 70.2 bpm

$f_s = 0.32$ producing a frequency shift of $\pm 60 \cdot 0.32 = \pm 19.2$ bpm

$f_{HRV} = 0.12$ Hz producing the continuous, sinusoidal varying, frequency shift with a period of 8.33 seconds.

With the indicated parameters the simulated ECG signal will have the following expression:

$$ECG_2(t) = sin\left(2 \cdot \pi \cdot \left(\int \left(\bar{f}_0 + f_s \cdot sin(2 \cdot \pi \cdot f_{HRV} \cdot t)\right) \cdot dt\right)\right) \tag{12}$$

$$ECG_2(t) = sin\left(2 \cdot \pi \cdot \left(\int \left(1.17 + 0.32 \cdot sin(2 \cdot \pi \cdot 0.12 \cdot t)\right) \cdot dt\right)\right) \tag{13}$$

and, after solving the integral,

$$ECG_2(t) = sin\left(2 \cdot \pi \cdot 1.17 \cdot t - \frac{0.32}{2 \cdot \pi \cdot 0.12} \cdot cos(2 \cdot \pi \cdot 0.12 \cdot t)\right) \tag{14}$$

finally:

$$ECG_2(t) = sin\left(7.3513 \cdot t - 0.4244 \cdot cos(0.7539 \cdot t)\right) \tag{15}$$

The signal $ECG_2(t)$, so obtained, is a sinusoidal signal with a sinusoidal periodic varying frequency from a minimum of $70.2 - 19.2 = 51$ bpm to a maximum of $70.2 + 19.2 = 89.4$ bpm as in Fig. 4.

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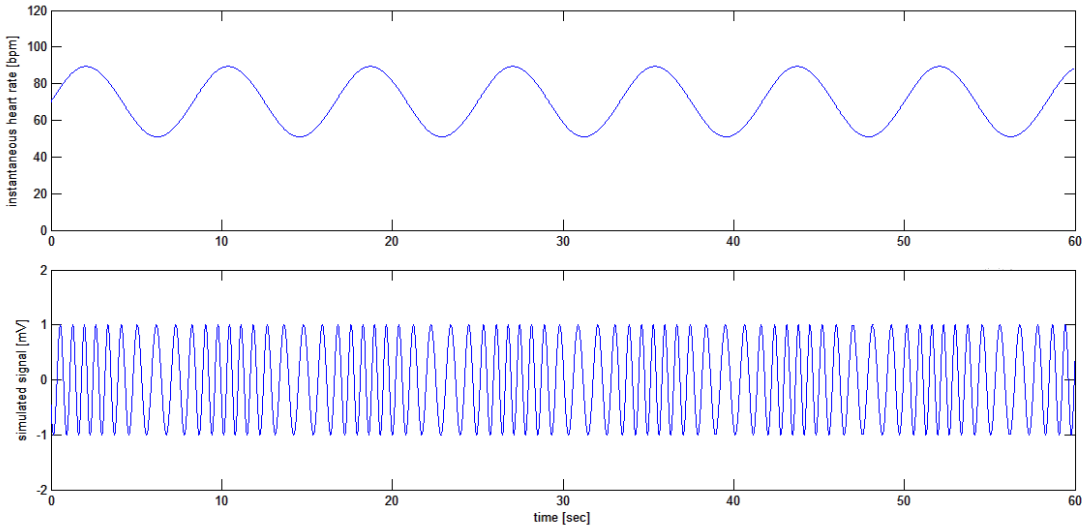

Figure 4. Simulation of test signal $ECG_2(t)$: a sinusoidal signal (lower tracing) frequency modulated with a sinusoidal wave (upper tracing).

4.3. Simulating a mix of sinusoidal wave-forms which are frequency modulating the sinusoidal carrier

In a third simulation we will consider:

$\bar{f}_0$ (Hz) or $60 \cdot \bar{f}_0$ (bpm) as the average heart rate and

$$HRV(t) = f_{s1} \cdot sin(2 \cdot \pi \cdot f_{HRV1} \cdot t) + f_{s2} \cdot sin(2 \cdot \pi \cdot f_{HRV2} \cdot t) \quad (16)$$

as a heart rate variability signal produced by the sum of two sinusoidal waves with two different amplitudes and two different frequencies.

As an example we will take the following values for the various parameters:

$\bar{f}_0 = 1.17$ Hz or 70.2 bpm, $f_{s1} = 0.06$ and $f_{s2} = 0.12$ as modulation width of the two modulating signals, $f_{HRV1} = 0.19$ Hz and $f_{HRV2} = 0.32$ Hz as the modulation frequencies of the two modulating signals.

With the indicated parameters the simulated ECG signal will have the following expression:

$$ECG_3(t) = sin\left(2 \cdot \pi \cdot \left(\int \left(\bar{f}_0 + f_{s1} \cdot sin(2 \cdot \pi \cdot f_{HRV1} \cdot t) + f_{s2} \cdot sin(2 \cdot \pi \cdot f_{HRV2} \cdot t)\right) \cdot dt\right)\right) \quad (17)$$

$$ECG_3(t) = sin\left(2 \cdot \pi \cdot \left(\int \left(1.17 + 0.06 \cdot sin(2 \cdot \pi \cdot 0.19 \cdot t) + 0.12 \cdot sin(2 \cdot \pi \cdot 0.32 \cdot t)\right) \cdot dt\right)\right) \quad (18)$$

and, solving the integral:

$$ECG_3(t) = sin\left(2 \cdot \pi \cdot 1.17 \cdot t - \frac{0.06}{2 \cdot \pi \cdot 0.19} cos(2 \cdot \pi \cdot 0.19 \cdot t) - \frac{0.12}{2 \cdot \pi \cdot 0.32} cos(2 \cdot \pi \cdot 0.32 \cdot t)\right) \quad (19)$$

finally:

$$ECG_3(t) = sin\left(7.3513 \cdot t - 0.0502 \cdot cos(1.1938 \cdot t) - 0.0597 \cdot cos(2.0106 \cdot t)\right) \quad (20)$$

thus the signal$ECG_3(t)$is a sinusoidal signal with a sinusoidal periodic varying of its frequency given by the sum of the two sinusoidal frequency modulating signals as in Fig. 5.

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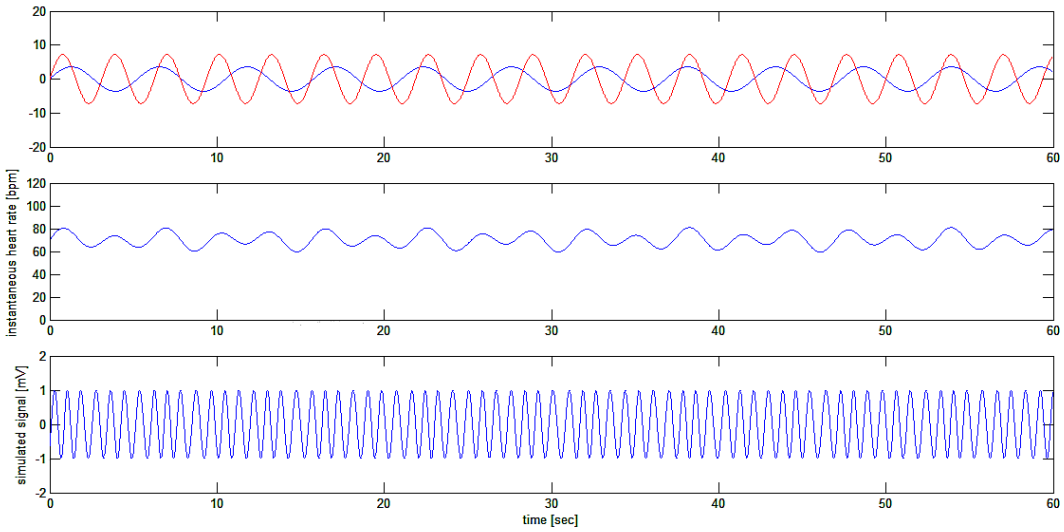

Figure 5. Simulation of test signal $ECG_3(t)$: a sinusoidal signal (bottom tracing) which is frequency modulated with the sum of two sinusoidal waves (shown separately on upper tracing) producing a final modulating signal around the average frequency of 70.2 bpm (middle tracing).

The three signals: $ECG_1(t)$, $ECG_2(t)$ and $ECG_3(t)$ will then be used to test the algorithm for the detection of $HR(t)$ hence $HRV(t)$.

5. Analysis of simulated signals epochs and detailing of the algorithm

The signals simulated and described in chapter 4 will now be analysed with the algorithm to recover the modulating signal from the frequency modulated one. At the same time, a deeper insight will be given into the details of practical implementation of the algorithm described in chapter 3.

It may be useful to recall that the task will be that of considering the ECG signal as a frequency modulated (FM) signal recovering the modulation signal like the audio in any FM radio. But in this case the difference in the frequency of the carrier and that of the modulating signal is not as large as in FM radio transmissions as presented in the introduction.

The first step in FM demodulation of the ECG signal is detecting the signal cycles. In case the simulated ECG signal is used (sinusoidal shape), positive zero-crossings are detected, while in the case of a real ECG signal, the peaks of R wave are detected. As in this chapter we will be concerned about simulated signals, positive zero-crossings will be used. A positive zero-crossing event in the signal is detected at the instant when the signal is crossing the zero line travelling from a negative value to a positive value (positive first derivative). As the signal is artificially generated without any noise component, the following very simple MATLAB function has been written for zero-crossing detection:

```
function pf = zerofind(y)
% zerofind function
%
% This function finds all the positive zero-crossings in the series
%   of values passed by y which is a row vector with values to explore for
%   zero-crossings
% the function returns a two columns matrix
%     - first column contains the indexes where zeroes were found,
%     - second columns contains values of y vector at those indexes
% size(pf,1) is the number of zeroes found
%
pf=[];
tdold=0;
iold=1;
for i=2:size(y,2)
    if (y(i-1)<0) && (y(i)>0)
```

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```
        td=i-iold;
        if (td-tdold)>0
            pf=[pf; i-1 y(i)];
            tdold=td;
        end
    end
end
```

In the following we will use the first simulated signal as described in subsection 4.1 above. Using the previous `zerofind(y)` function, the zeroes in the signal are found and, after this zero-crossing detection, the $SR(t)$ signal is created by the cumulative sum of the zero-crossing events in time as in Fig. 6.

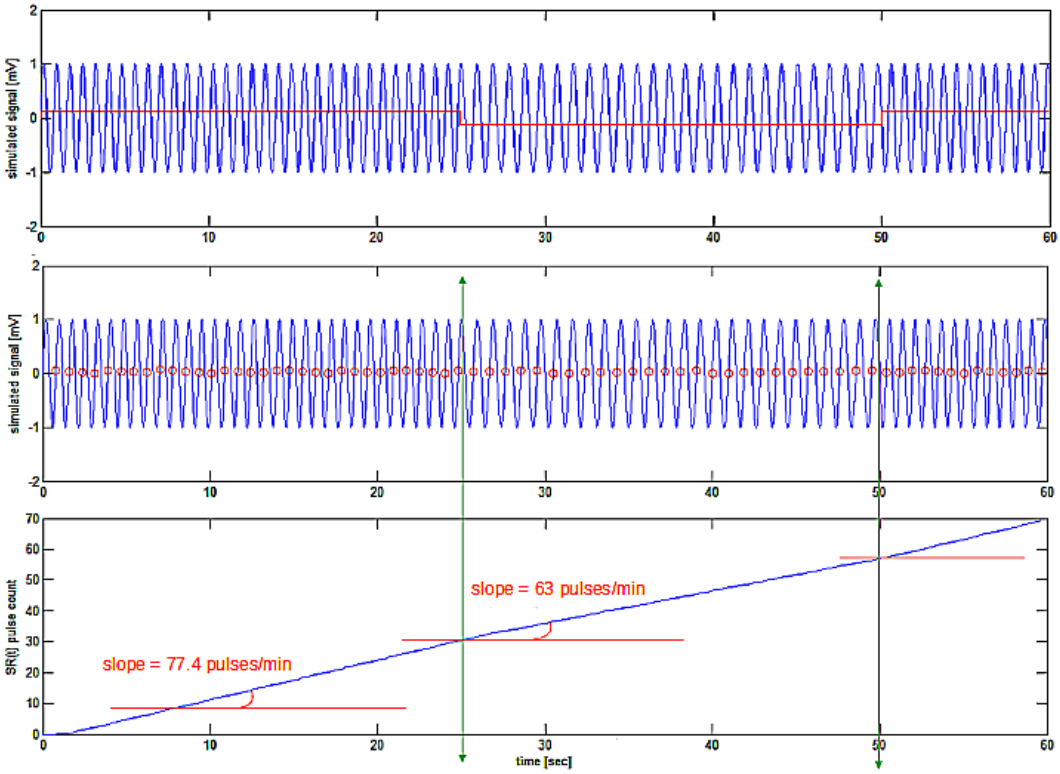

Figure 6. Upper tracing shows frequency modulated signal with the square-wave modulating signal superposed in red. Zero-crossing detection points (red) on the signal $ECG_1(t)$ are shown in the middle tracing. Bottom tracing shows the created SR(t) signal with a slope depending on the local instantaneous frequency of the simulated $ECG_1(t)$ signal.

The method for obtaining the cumulative sum signal implies counting the number of cycles detected along time using the following MATLAB script:

```
% events cumulative summing (or counting of complete cycles)
% vector peaks is the output of the zerofind function
%
sumecg=[0]; % initialize the cumulative sum count signal
j=1;        % initialize index on the peaks matrix
for i=2:(size(ecg,2)-1) % running over the ecg values
    if i>peaks(j,1) % present index just passed event position
        % event found, increment cumulative counter and add
        sumecg=[sumecg sumecg(size(sumecg,2))+1];
        if i<peaks(size(peaks,1),1) % go on if event not found
            j=j+1;
        else
            break; % exit for
        end
    else
        % no event found, no increment cumulative counter and add
        sumecg=[sumecg sumecg(size(sumecg,2))];
    end
end
```

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In the above MATLAB script, the `ecg` vector is the simulated frequency modulated signal (or the sampled ECG epoch), the same vector passed to the `zerofind` function above.

The `sumecg` vector so obtained merits some discussion to be clearly understood. As already described, it is the summing count of the cycles (zero crossings in the simulated signal or R-waves detected in the ECG epoch). In Fig. 6 apparently, it is shown as a rising straight line. But it must be noted that the higher the number of cycles (or R-waves) detected per unit time, the steeper will be the slope of the `sumecg` vector (which is, by the way, the $SR(t)$ signal), so it is not really a straight line! This means that the first derivative of the `sumecg` vector or the $SR(t)$ signal, should give the instantaneous signal frequency (or heart rate).

The obtained signal is not directly helpful in estimating its first derivative because it is composed of a series of steps of one count for each cycle (or heart beat) found. Numerical first derivative is quite a tricky procedure to obtain a sufficiently smooth and low noise output signal! We used the following steps:

a) initially, a low pass filter is applied to the $SR(t)$ signal with a moving average 256 points Kaiser-Bessel weighted filter whose Bode plot is shown in Fig. 7 (corresponding, at the sampling frequency used of 128 Hz, to 2 seconds epoch and giving a filter delay of 1 second[1])

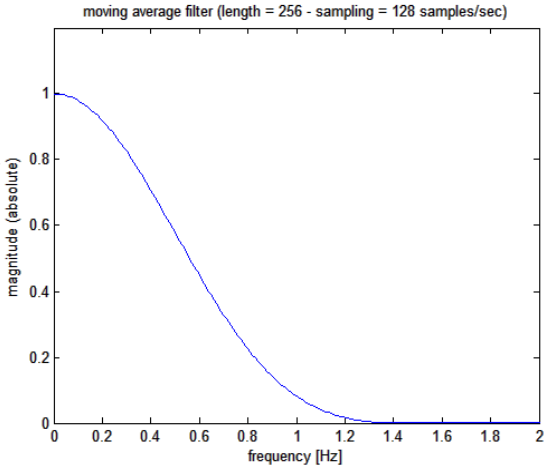

Figure 7. Magnitude Bode plot of the moving average filter applied to the cumulative sum signal.

b) then downsample the $SR(t)$ signal to a sampling frequency of 8 Hz as the maximum bandwidth of the HRV signal is expected to be around 0.5 Hz at most (30 cycles/minute)

c) eventually filter the downsampled $SR(t)$ signal with a differentiating noise-robust filter to obtain the first derivative $HR(t)$ or the instantaneous heart rate signal; signal differentiation by FIR filtering is a classical procedure but we followed the original and robust method proposed by Holoborodko [10] using an 11-order one-sided noise-robust differentiator whose Bode plot is shown in Fig. 8; a filter delay of 625 ms is to be expected. This filter has a linear characteristic at low frequencies (implying a multiplication by the s-variable in the Laplace domain), while at higher frequencies, it becomes a low pass.

[1] Just to recall a basic signal theory concept: the time delay t_d for a FIR filter having N taps (points or coefficients) at a sampling frequency f_s is equal to $t_d = (N-1)/(2 \cdot f_s)$.

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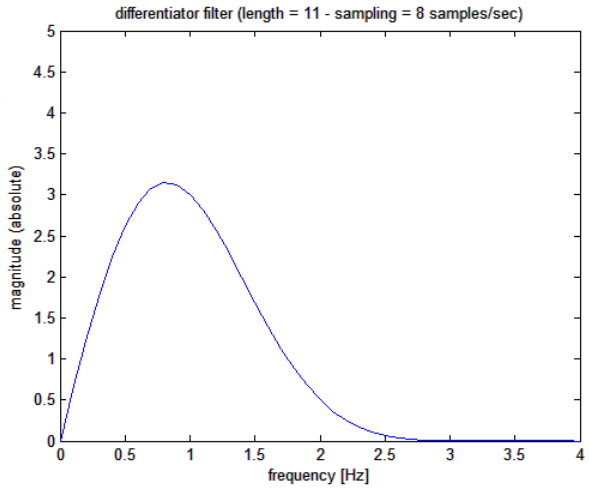

Figure 8. Magnitude Bode plot of the Holoborodko's differentiating filter applied to the cumulative sum signal. Please note the differentiating behavior at low frequencies thanks to the almost linear line of the graph (output linearly proportional of the frequency of the input signal).

d) a low pass filter is then applied to the $HR(t)$ signal with a moving average Kaiser-Bessel weighted filter with 16 points whose Bode plot is shown in Fig. 9 (corresponding, at the sampling frequency of 8 Hz, to approximately 2 seconds and giving a filter delay of 937,5 ms); following the differentiating filter, which provides the required first derivative, this further moving average filter is used to clean the output from residuals of original R-wave detection points (the original carrier frequency).

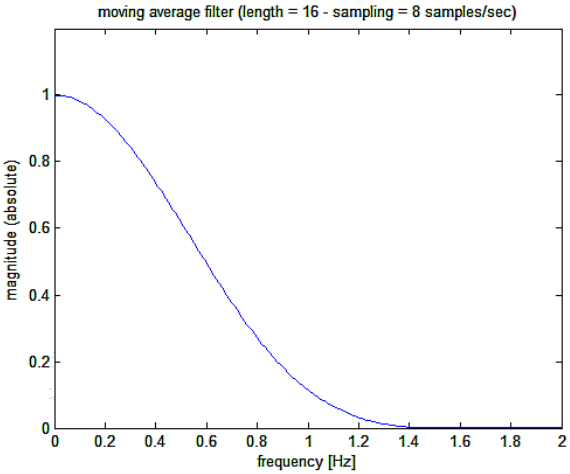

Figure 9. Magnitude Bode plot of the moving average filter applied to the recovered first derivative signal or the heart rate signal.

e) eventually, the average value of it is subtracted from $HR(t)$ so to obtain the final$HRV(t)$ signal or heart rate variation signal.

Eventually, the heart rate signal can be plotted as in Fig. 10.

We can now apply the method for demodulating a frequency modulated signal to the other simulated signals described in subsections 4.2 and 4.3.

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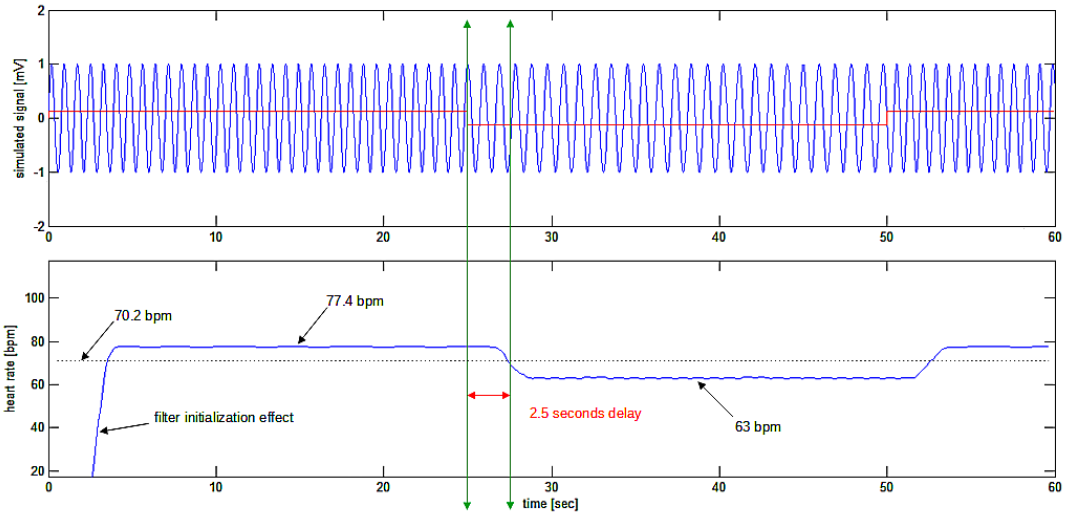

Figure 10. Bottom tracing: the recovered heart rate signal, at last! The 2.5 seconds delay of the recovered signal corresponds to the delays expected from the sequence of FIR filters.

Fig. 11 presents a simulated frequency modulated sinus according to the simulation presented in subsection 4.2. The sinusoidal signal has an average frequency of 70.2 cycles per minute (1.17 Hz), modulated by a 0.12 Hz (7.2 cycles per minute) sinusoidal signal with a modulation depth of $\pm$19.2 bpm.

The method performs very well as the modulating signal is reconstructed and the instantaneous carrier's frequency is detected. Distortions in the valleys of the reconstructed modulation signal are due to a too large modulation depth, thus the carrier frequency appears in the output signal. This is not usually to be expected in real situations using a natural ECG signal.

The beginning of the reconstructed signal is affected by initialization transition period of reconstructing filters or edge effect.

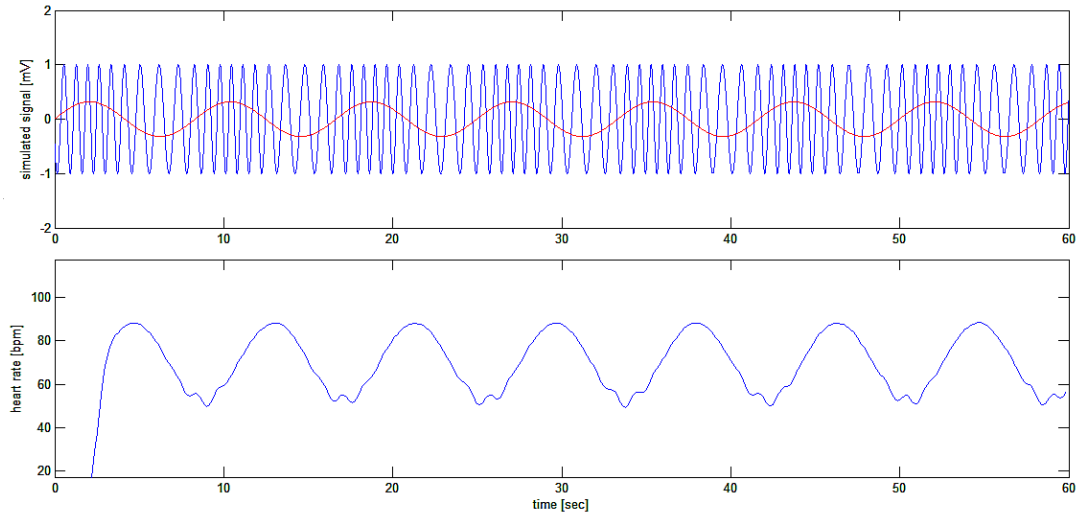

Figure 11. Upper tracing: frequency modulated signal and modulating signal. Bottom tracing: the recovered heart rate signal. Carrier residuals appears because of huge modulation depth.

Before analyzing our method's performances on actual ECG signals, we present the algorithm's performances on a more complex modulating structure. The sum of two sinusoidal signals is used to frequency modulate the sinusoidal carrier as described in subsection 4.3.

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The sinusoidal carrier with an average frequency of 70.2 bpm is frequency modulated by two sinusoidal signals respectively at 0.19 Hz (11.4 cycles per minute) and 0.32 Hz (19.2 cycles per minute) with modulation depths respectively of 3.6 and 7.2 cycles per minute. Results are quite good indeed.

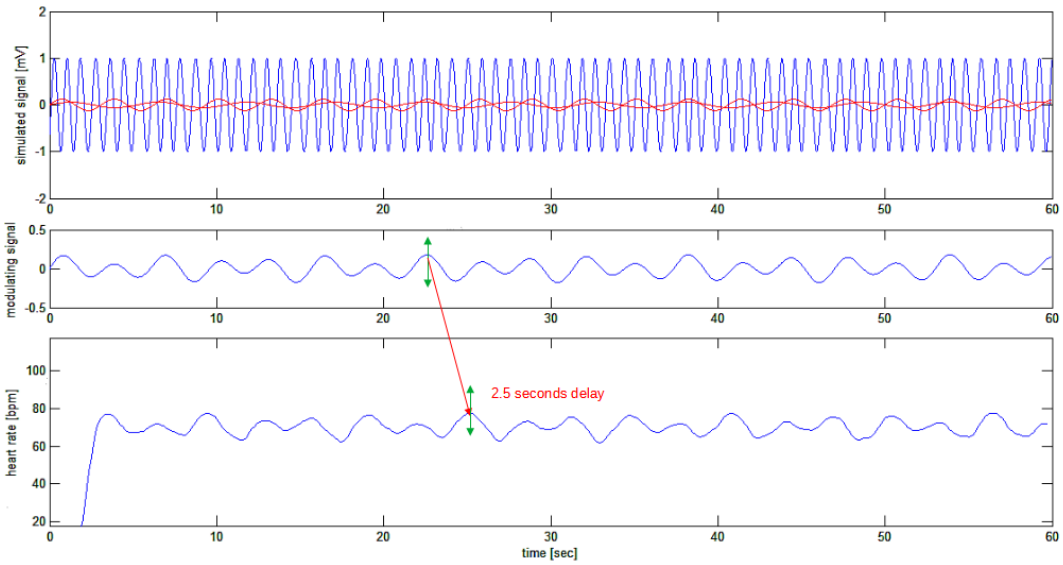

Figure 12. Upper tracing: frequency modulated signal and modulating signal. Bottom tracing: the recovered heart rate signal. Middle tracing represents the modulating waveform (cfr. Fig. 5).

In an attempt to verify to quality of modulation recovery (i.e. the heart rate variability signal) from the simulated heart rate signal, we computed the fast fourier transform (FFT) of the original modulating signal and of the recovered modulation3. Signal. The FFT was computed on the third simulation as shown in Fig. 5 above thus on the middle and bottom tracings of Fig. 12 above. Results are plotted in Fig. 13.

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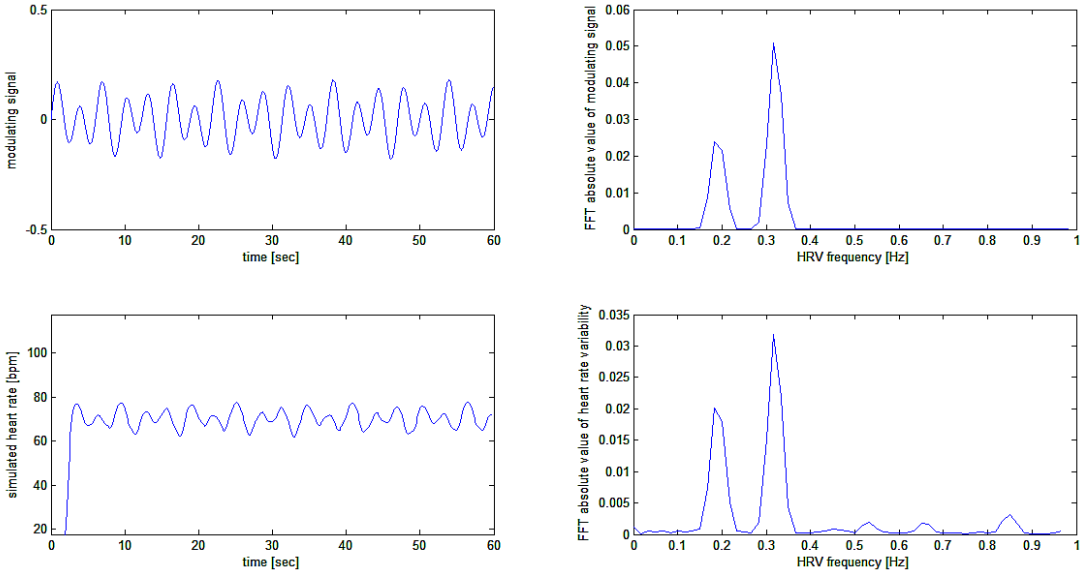

Figure 13. Comparison of modulating and recovered simulation signals in the time and frequency domains. HRV frequencies are perfectly recovered. Different amplitudes of signals in the time domain come from changing the scale from beats per second to beats per minute. Different amplitudes in the frequency domain are also due to the windowing effect (a Blackman-Harris window was used before FFT calculation).

6. Analysis of real ECG signals epochs for appreciation of ultra-short-term HRV

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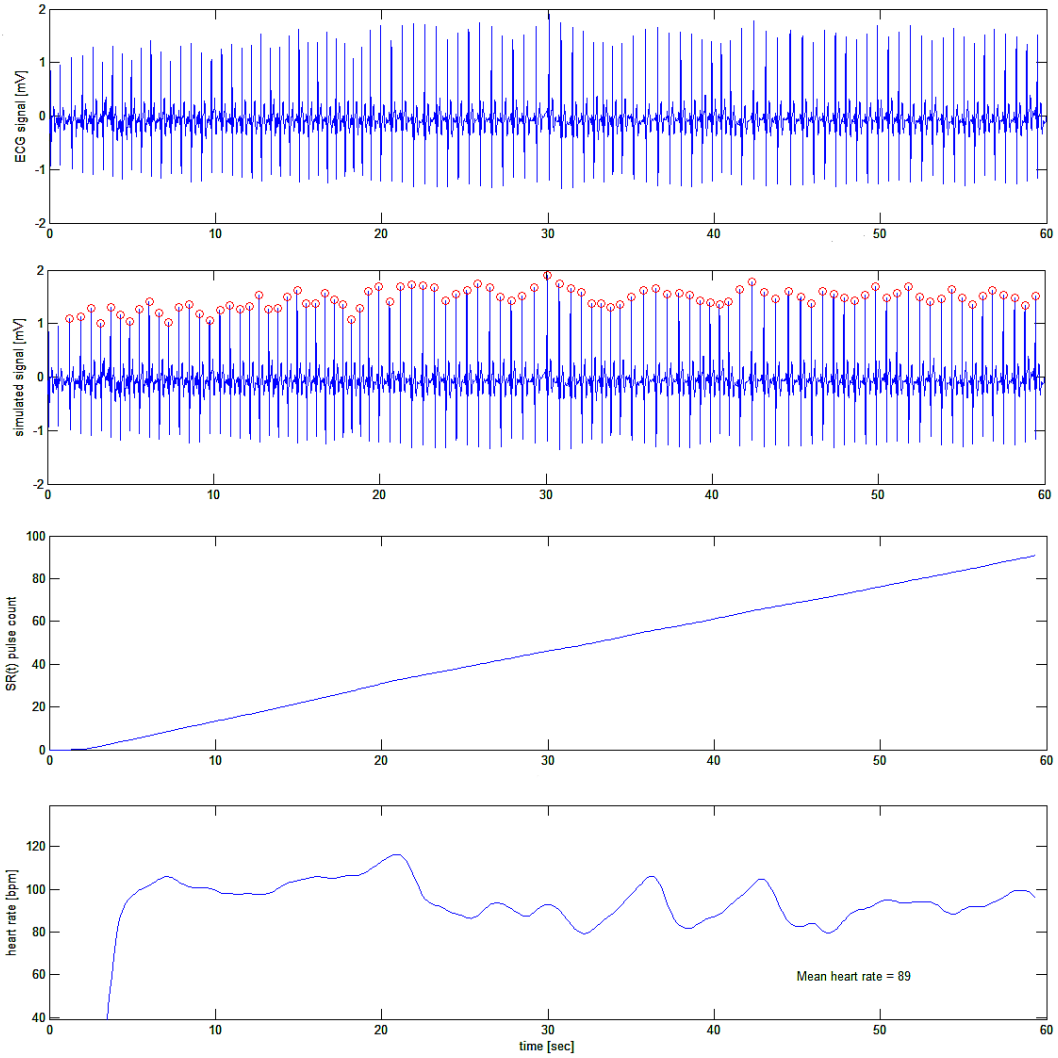

Figure 14. First test of the algorithm on a real ECG tracing (top tracing). In the second tracing from the top, ECG R waves are indicated as detected. In the third tracing the cumulative summing signal is shown and in the bottom trace, the filtered first derivative of the latter is plotted showing fast variations of the heart rate during the subject's activity.

As it was not the purpose of the present work that of analysing ECG signals for HRV estimation, but only to explain a method for doing so, nevertheless we initially tested the algorithm on a few epochs of our own ECG (that of one of the authors). No human subject was used for acquiring ECG tracings but the authors themselves just to show the application of the method on real tracings.

In Fig. 14 the ECG of one of the authors (H.K.) was taken while he was busy in an emotional computer game. The idea was to acquire the fast variations of heart rate (if any) during the cognitive and emotional effort of computer gaming. Again, we were only interested in the ECG tracing *per se* and no interest was given, at this stage of the research, to the meaning of HRV signals and correlation with the activity of the subject. Further papers will take care of this by using the present algorithm.

There are many different and more or less robust and efficient algorithms for the real-time detection of the R-wave []. In this work, we programmed our own based on an adaptive amplitude and time thresholds. The resulting peak finder R-wave detector function was written in MATLAB as follows:

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```
function pf = RWaveFind(y,yth,tth)
%
% RWAVEFIND Find all the peaks in the values passed on y
%    RWAVEFIND (y, yth, tth)
%    where each valid peak found must have a value above yth and
%    must be more distant than tth indexes from last found peak
%    returns a two columns matrix
%      first column are indexes where peaks were found
%      second column are amplitudes of peaks found
%
pf=[];   % start with an empty output matrix
iold=1;  % sampling index where last peak found
for i=2:(size(y,2)-1) % explore all samples on the input vector
    if (y(i-1)<y(i)) && (y(i+1)<y(i)) && (y(i)>yth)
                                % test if current sample is a peak
        td=i-iold;   % calculate time delay from last peak found
        if td>tth    % test if new time delay too short
            pf=[pf; i y(i)]; % add time delay to output vector
            iold=i;          % keep memory of new peak found
            yth=0.5*y(i);    % threshold adapting to half last
        end
    end
end
```

6.1. Another real world test example: HRV in the Valsalva manoeuvre

As the underlying scope of our research is that of implementing a method for assessing vagus nerve activity using the heart (heart rate) as a sort of "vagus sensor" with the final ambitious goal of recording "vagus nerve event related activity", we just tried to preliminary use the method on one of the well-known vagal manoeuvres like the Valsalva manoeuvre. We would like to stress once more the fact that we didn't perform clinical research on humans, we just used the newly developed algorithm on our own ECG tracings (the ones of the authors) to look at a real HRV signal out of the simulations which permitted us to test the method "*in vitro*" as explained before.

Methods to stimulate the vagus nerve are well known and clinically assessed. They all go under the name of vagal manoeuvres. Most clinically useful are: the Valsalva manoeuvre, the carotid sinus massage, the cold water immersion, and the eyeball pressure. Effects of these manoeuvres are different, but any of them provokes an increase or decrease in heart rate, which is often mediated by a concurrent vagal stimulation.

Valsalva manoeuvre was chosen as the simplest to qualitatively test our method. It is performed doing a forced expiratory effort against a closed airway and is considered a very low-risk procedure [12].

Using a custom made two channels ECG [13], we recorded the ECG tracings. The second channel was used for recording marks for the different phases of the Valsalva manoeuvre, namely exact beginning and ending times of forceful attempt of exhalation. Then we computed the ECG tracing so obtained using our algorithm and the results are given in Fig. 15.

In Fig. 15 a typical tracing is shown where the resting heart rate of the subject (one of the authors: H.K.) is 108 bpm. At the end of the force expiratory effort, the heart rate went up to 143 bpm (or 35 bpm above resting value). During the recovery phase the heart rate went down to 89 bpm in just 7 seconds (or 54 bpm down with a negative slew rate of 8 bpm per second) before restoring the resting heart rate to 108 bpm.

Indeed a very interesting test for our method ! It can detect fast heart rate variations with fine detail.

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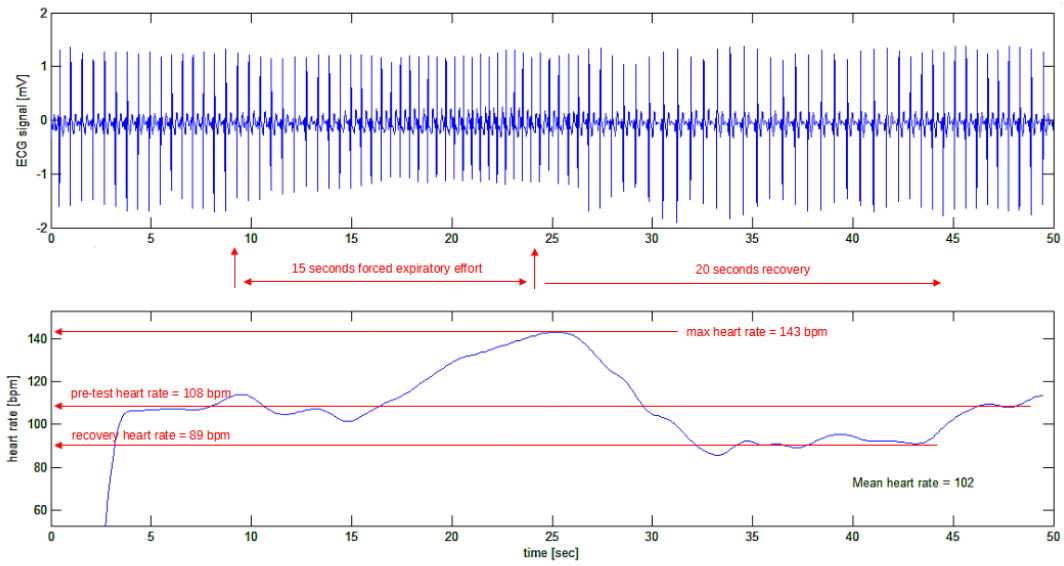

Figure 15. Test of the algorithm on a real ECG tracing (top tracing) during the Valsalva manoeuvre.

7. Discussion

We have already highlighted the proposed method for HRV signal detection and tested the method extensively on simulated signal and real ECG tracings. In this place a particular attention should be put on the comparison of our method with others, more conventional, methods of HRV detection. We affirm the superiority of our method as it avoids completely any suspicious activity of resampling or interpolation on the RR periodogram. By the way a form of filtered interpolation is still to be detected in the use of low pass filtering before and after diffentiation of the cumulative sum signal.

Nevertheless the method appeared to provide a very clean HRV signal which keep its frequency content even at high frequencies.

The method might also be used on other frequency modulated biomedical signals to extract a reliable modulation signal embedded into them.

Author Contributions: Literature search: All authors; Study design and conceptualization: Enrico M. Staderini and Harish Kambampati; Methodology and software: Enrico M. Staderini and Amith K. Ramakrishnaiah; Validation: Stefano Mugnaini, Sandro Gentili and Andrea Magrini; Writing and original draft preparation: Enrico M. Staderini; Review and editing: Harish Kambampati and Amith K. Ramakrishnaiah; Tables and figures: Enrico M. Staderini; Supervision and project administration: Enrico M. Staderini and Sandro Gentili (Coordinator of Prevention and Rehabilitation Occupational Medicine Laboratory); Funding acquisition and supervision: Andrea Magrini. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement: Aactually this article is not describing a study involving humans as the only real ECG tracings were obtained from the authors themselves for the purpose of rapidly having a signal at hand for testing the algorithm. Not having been real patients in the study, that's why the consent was waived also considering it as not applicable.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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