APPLICATION FOR OFF-LINE ANALYSIS OF BIOSIGNALS MEASURED DURING STIMULATIONS ON ISOLATED ANIMAL HEARTS

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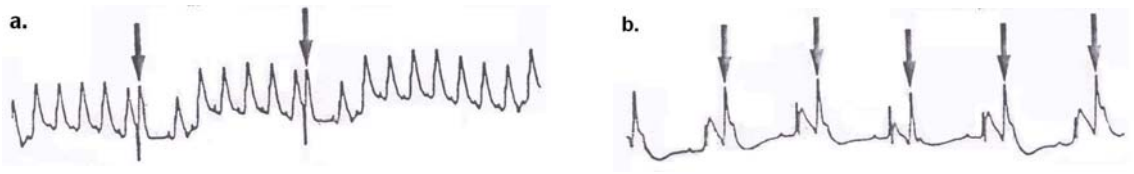
Abstract

Isolated perfused animal heart in Langendorff setup is widely used for electrophysical experiments and most of them are oriented on "in vitro" study of effects induced by experimentally designed drugs on organ level. For measuring and subsequent off-line analysis of biosignals originated from stimulation experiments of rat's hearts we designed measuring system BioLabF. This paper presents a solution of software for analysis of data from that type of experiments. The three types of signals (electrogram - Ecg, left ventricular blood pressure and drug dossage) were measured on experimental rats (wistar breed) during their electrical and pharmacological stimulations. The application program is focused mainly on semi-automatic off-line analysis of selected parts of biosignals and is devoted especially to detection of QRS complexes pertinent to sinus rhythm, other typical rhythms defined by the Lambeth conventions and QRS complexes originating from extrasystoles in ventricular electrograms. For this purpose the algorithm based on the original Pan-Tompkins algorithm was designed and its ability to detect QRS complexes in data from animal experiments was evaluated. Designed algorithm with other signal processing routines was incorporated into a GUI based software developed in MATLAB. Developed software enables the user to compute important parameters from signals and transfer them directly to an Excel sheet and thus speed-up the analysis of data originated from long term experiments (cca. 1.5 hour).

1 Introduction

Isolated perfused animal heart in Langendorff setup is widely used for electrophysical experiments and most of them are oriented on "in vitro" study of effects induced by experimentally designed drugs on organ level [1]. In the previous work we designed measuring system and software for off-line analysis of biosignals measured during stimulation experiments of rat's hearts at the Institue of Experimental Pharmacology [2]. The main purpose of these experiments is to study the mechanism of spontaneous ventricular defibrillation in mammals and to facilitate its occurrence [3]. Large part of experiments is directly oriented to study of hypothesis about anti-fibrillation effect of omega-3 essential fatty acids (namely docosahexaenoic and elcosahexaenoic acids) [4].

One of the main problem related to analysis of this type of signals is to accurately measure the frequency of native rhythm, especially when the Ecg signals are obcured by presence of incidental extrasystoles or others events. Furthermore, when the external stimulation is presence during measurement of Ecg signals, the detection of QRS complexes and computation frequency of various rhythm is to become a serious problem. For illustration of the problem, the typical templates of Ecg signals recorded on rat's isolated hearts according to Lambeth conventions [5] are depicted in Fig. 1.



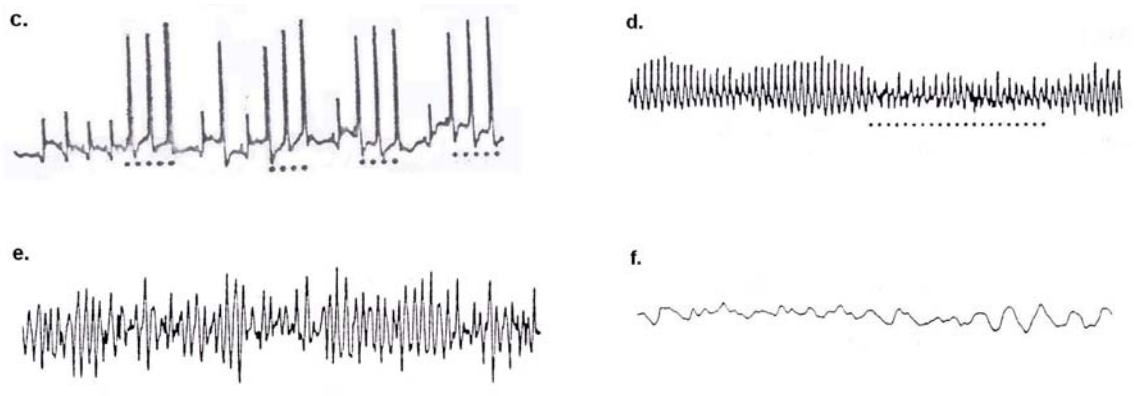


Figure 1: The templates of typical Ecg signals measured on rat's isolated heart: a. Ventricular premature beats (VPB) indicated by arrows; b. Bigeminy characterized by minimum sequence: P, QRS, VPB, P, QRS, VPB indicated by arrows; c. Salvos (are underlined) d. Non-sustained ventricular fibrillation (NSVF) in a background of ventricular tachycardia (VT); e, Ventricular fibrillation - first type; f, Ventricular fibrillation - second type.

2 Software for analysis of biosignals

2.1 Algorithm for detection of QRS complexes in the Ecg signals

Various techniques for detection of QRS complexes in time domain were designed for ECG signals measured on human body but we could state that they are not optimal for detection of events in signals measured on isolated hearts under various types of external stimulation (pharmacological, electrical, etc.) in generally. Our approach is based on the original Pan-Tompkins algorithm for detection of QRS complexes in human ECG signal during measurement [6]. Typical Ecg signal measured on rat's isolated heart pertinent to sinus rhythm and situation when the Ecg signal is obscured by presence of incidental extrasystoles are depicted in Fig 2.a and Fig 2.b respectively.

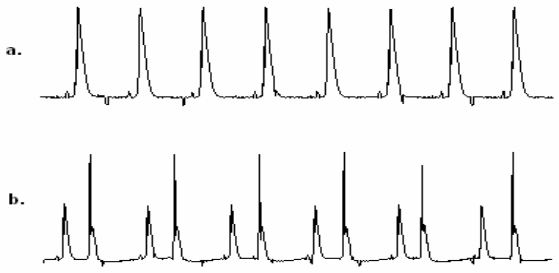


Figure 2: Ventricular ECG signal measured from isolated heart of rat: a. ECG with normal rhythm, b. ECG with extrasystoles

The algorithm is based on analysis of the slope, amplitude and width of QRS complexes (see Fig. 3.). It is composed from preprocessing operations (bandpass filtration, differentiation, squaring and moving integration). Bandpass filter has low and up cuttof frequency f_d = 1 Hz, f_h = 30 Hz and effectively suppresses power-line interference, if present. Derivative operator approximates the ideal *d/dt* operator up to 30 Hz. The derivative procedure suppresses the low frequency components of the P and T wave and provides large gain to the high- frequency components arising from the high slopes of the QRS complexes. Because output from derivation operation could exhibit multiple peaks within duration of a single QRS complex the moving integrator is used to perform smoothing of previous output. The moving integrator is implemented by equation:

$$y(n) = \frac{1}{N}[x(n-(N-1)) + x(n-(N-2)) + ... + x(n)] \quad (1)$$

The width of window (N) was set to fixed value of 60 found to be suitable for sampling frequency 1000 Hz, but in the next version of software, user will be permitted set width of window to value which is more appropriate to shape of QRS complex in particular type of analyzed Ecg signal, according to schema depicted in Fig. 4.

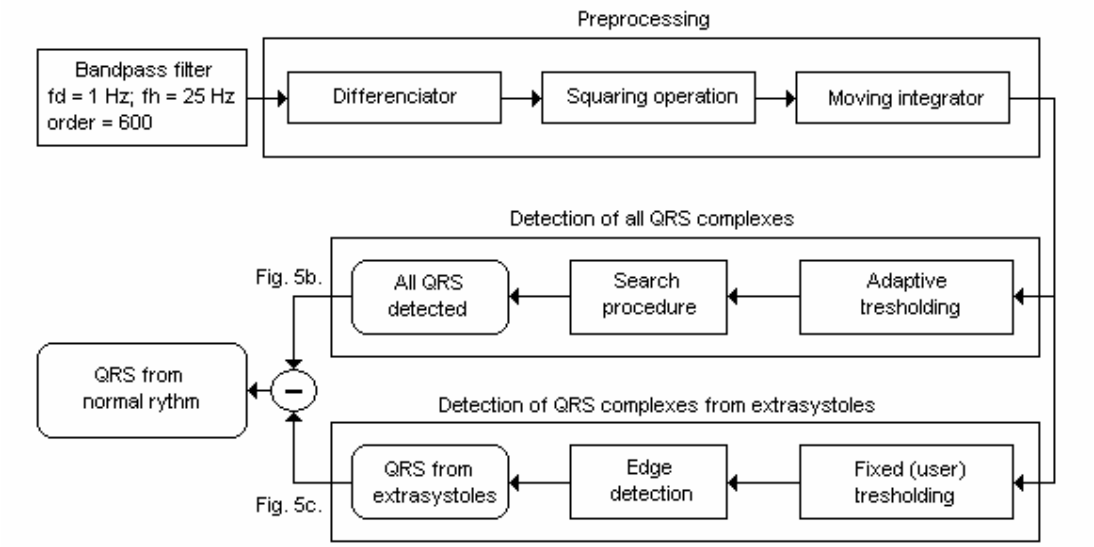


Figure 3: Algorithm for separate detection of QRS complexes from normal rhythm and from extrasystoles.

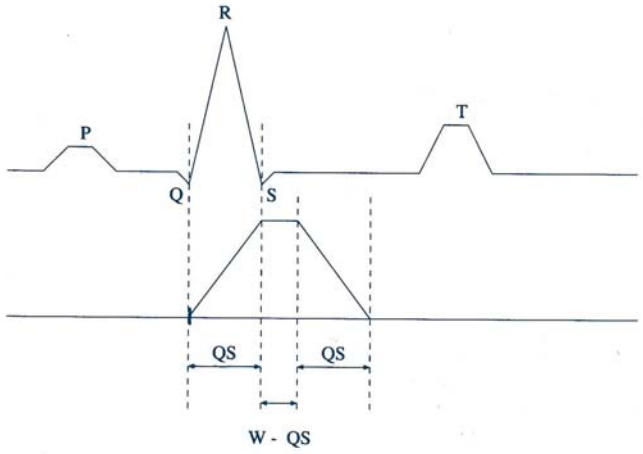


Figure 4: The relationship of a QRS complex to the moving-window integrator output. Upper plot: schematic ECG signal. Lower plot: Output from the moving window integrator. QS: QRS complex width. W: width of the integrator window, given as $N / f_{sampling}$.

Results from preprocessing operations is then processed in two separate branch. In the upper branch, the adaptive thresholding and search procedures are applied. The result from this branch is impulse function shown in Fig. 5.b. and represents the mixture of impulses originate from QRS complexes of sinus rhythm and from extrasystoles. In the lower branch, the fixed amplitude thresholding with user defined value of threshold is applied and then edge detection of obtained square function gives the impulse function depicted in Fig. 5.c. Each impulse represents one extrasystole. If we analyze both

impulse functions simultaneously and consider occurrences of impulses, we can clearly distinguish between QRS complexes originate in sinus rhythm and originate in extrasystoles. After elimination of impulses which represent the extrasystoles, we obtain the impulse function corresponding to sinus rhythm and we can calculate R-R interval and also R-R variability in selected part of analyzed Ecg signal.

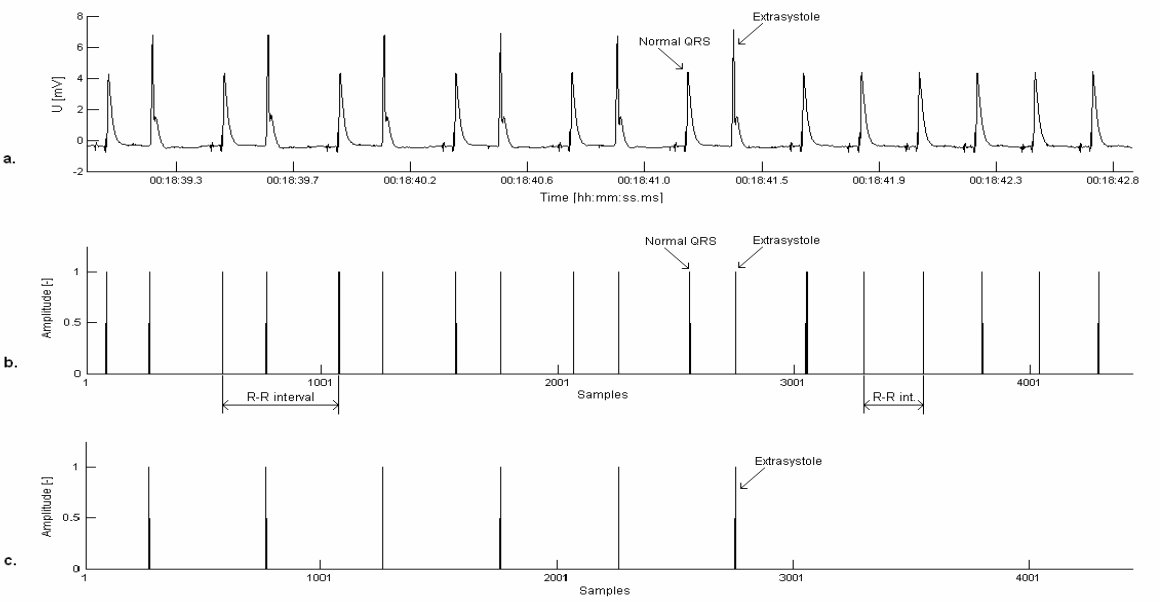


Figure 5: Proposed algorithm applied to Ecg signal: a: analyzed input Ecg signal, b: output from the upper branch of algorithm depicted in Fig. 3. - impulse function representing mixture of impulses from normal QRS and from extrasystoles, c: output from the lower branch of algorithm depicted in Fig. 3. - impulse function representing impulses from extrasystoles only.

2.2 Evaluation of detectability of designed algorithm

The detectability has been evaluated on typical types of Ecg signals (templates depicted in Figures 1.a. to 1.f.) and normal sinus rhythm signal with presence of extrasystoles (depicted on Figure 2.b.) according to equation:

$$dct = \frac{n_{aQRS}}{n_{dQRS}}.100 \quad [\%] \tag{2}$$

where dct is detectability factor, n_{aQRS} is number of all QRS complexes, n_{dQRS} is number of positive detected QRS complexes in analyzed Ecg signal.

Table 1: Results of detectability of designed algorithm for various Ecg signals.

Type of events	*Depicted on*	*dct [%]*
Ventricular premature beats (VPB)	Fig 1.a.	100 %
Bigeminy	Fig 1.b.	100 %
Salvos	Fig 1.c.	100 %
Non-sustained ventricular fibrillation (NSVF)	Fig 1.d.	80%
Ventricular fibrillation - first type	Fig 1.e.	60%
Ventricular fibrillation - second type	Fig 1.f.	no detectable
Sinus rhythm with extrasystoles	Fig 2.b.	99 %

2.3 Architecture of developed software

Because two type of measurement on isolated animal hearts can be done with BioLabF measuring system (first type: Ecg, left ventricular pressure and drug dossage; second type: Ecg, left ventricular pressure and left ventricular perfusion pressure of drug are measured during experiment) raw data from measurement can be loaded to application software with various preprocessing operations (filtration of 50 Hz if presence, computation of pressure signals in requested units, etc.). For more detail see the Fig. 6. with bock diagram of developed software. After loading of data the visualized signals can be analysed directly in the time domain (example of user interface with loaded signals from experiments is depicted in Fig. 8.) or also in time-frequency domain (depicted in Fig. 9.) after computation of spectrum. To simplify and speed up the off-line analysis of measured signals we designed an automation analysis framework. It is based on cursors (controlled by the user) that can be used to select in every channel (Ecg, pressure signals or drug dosage signals) requested part of the signal for analysis. Up to 28 parameters which characterized of hemodynamics of isolated animal heart are computed for each group of signals. The most important of them are:

frequency of sinus rhythm, frequency of extrasystoles, drug dossage, minimum and maximum values of pressure's derivations, time tension index (TTI), etc.

Computed parameters from analyzed signals can be displayed on the screen and requested group of such parameters can be immediately transferred to an opened Excel sheet. Example of window with automatically computed parameters from segment of signals, selected by two cursors depicted in Fig. 8., together with Excel sheet after transfer of requested parameters are depiced in Fig. 7. After the analysis is finished, user can select another part of signals and repeat computation of parameters and their transfer to following column of the Excel sheet. By this procedure a complete analysis of signals from particular experiment and following statistical evaluation of the data in Excel can be done in a very simply way.

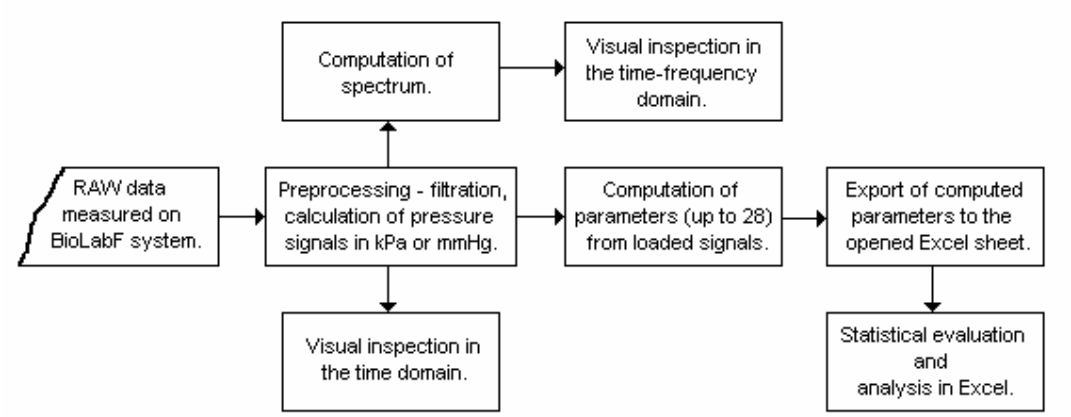


Figure 6: Block schema of software for off-line analysis of biosignals developed in MATLAB.

The presented software was debugged and after compilation it was deployed as a stand-alone application to target laptop (with WinXP OS installed) at the Institute of Experimental Pharmacology of SAS. Measuring system BioLabF together with developed software is full-value instrument to realization of stimulation experiments on isolated animal hearts in Langendorff experimental setup.

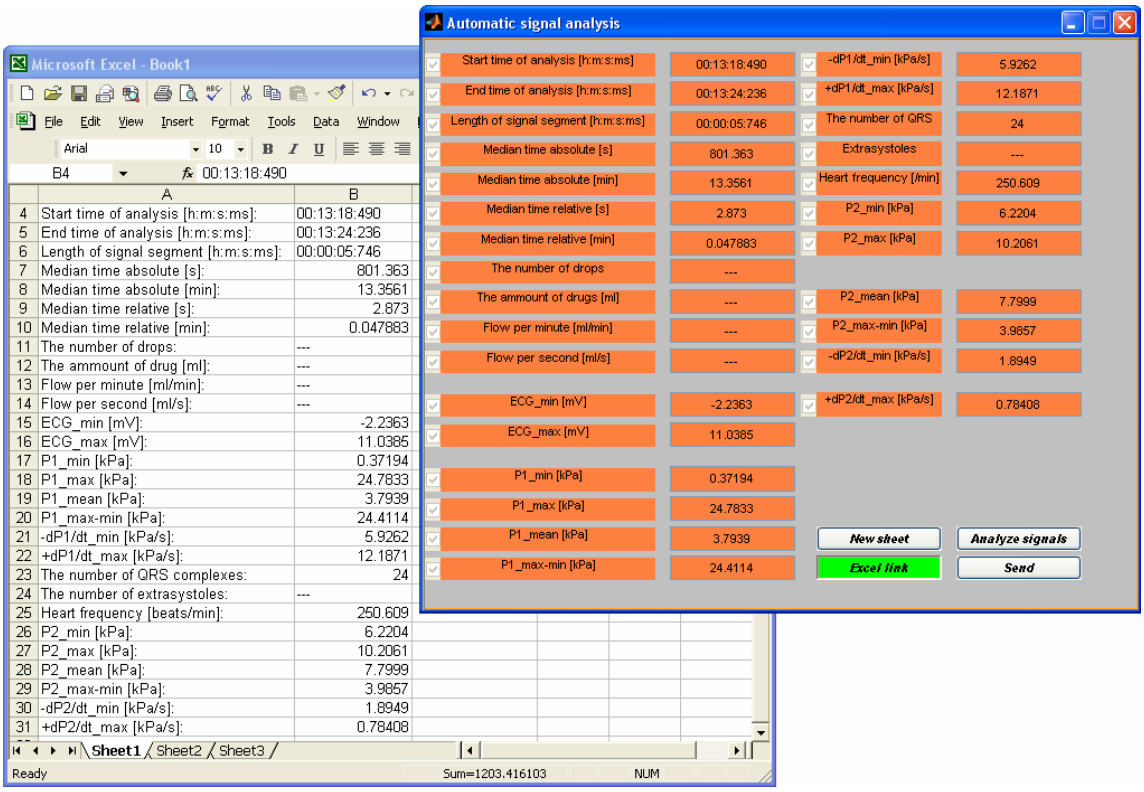


Figure 7: Window with parameters computed from segment of loaded signals selected by two cursors (blue and red) in Fig. 8 and its transfer to opened Excel sheet.

3 Results

Evaluation of detectability shown that the designed algorithm is able to detect the QRS complexes in most of the typical types of Ecg signals measured on isolated animal heart with 100 % value of detectability factor (dct). The algorithm detected QRS complex reliably in ventricular premature beats (Fig 1.a)., bigeminy (Fig 1.b.) and salvos (Fig 1.c.). In the case when the Ecg signals became in more and more uncoordinated manner in which individual QRS deflections could not longer be distinguished from one another (implying morphological instability), detectability factor of such QRS complexes decreased and frequency could not longer be measured correctly. This situation occurred when the Ecg signals with non-sustained ventricular fibrillation (NSVF, Fig 1.d.) and with first type of ventricular fibrillation (Fig 1.e.) was analyzed. In the case of second type of ventricular fibrillation (Fig 1.f.), the algorithm failed.

The measuring system and presented software is now intensively being used in the Institute of Experimental Pharmacology for long-term biophysical experiments on small isolated animal hearts. Figure 6 depicts the record from latest pharmacological experiment with omega-3 essential fatty acid (specifically docosahexaenoic acid - DHA). The isolated rat's heart was firstly electrically stimulated by contact electrode (not show on the picture) to induce ventricular fibrillation. Ventricular fibrillation last 2 minutes total (on the figure we see only last 5 seconds of fibrillation). After 1 minute and 14 second of the fibrillation, the bolus of DHA was applied which caused restoration of sinus rhythm (after 46 sec. from start of bolus). Transition to sinus rhythm last cca. 1 sec. and 680 msec (see figure 6).

For more detailed representation and study of dynamic effect induced by external drug stimulation, the time-frequency representation seems to be much more suitable. For this purpose we developed spectral analysis module which is implemented in software. It enables to compute Short Time Fourier Transform (STFT) of selected part of Ecg signals with user defined input parameters (number of FFT points, window type, etc.) and represent the result in 2D (spectrogram) or 3D (CSA – compressed spectral array) image. Figure 7 depicts the same Ecg signal as on previous picture along

with spectrogram computed by STFT algorithm. It is clear that we recognize ventricular fibrillation, transient component and return to the sinus rhythm, but it is surprised that ventricular fibrillation is running in coordinated manner with its own several frequencies what is not visible in the Ecg signal itself.

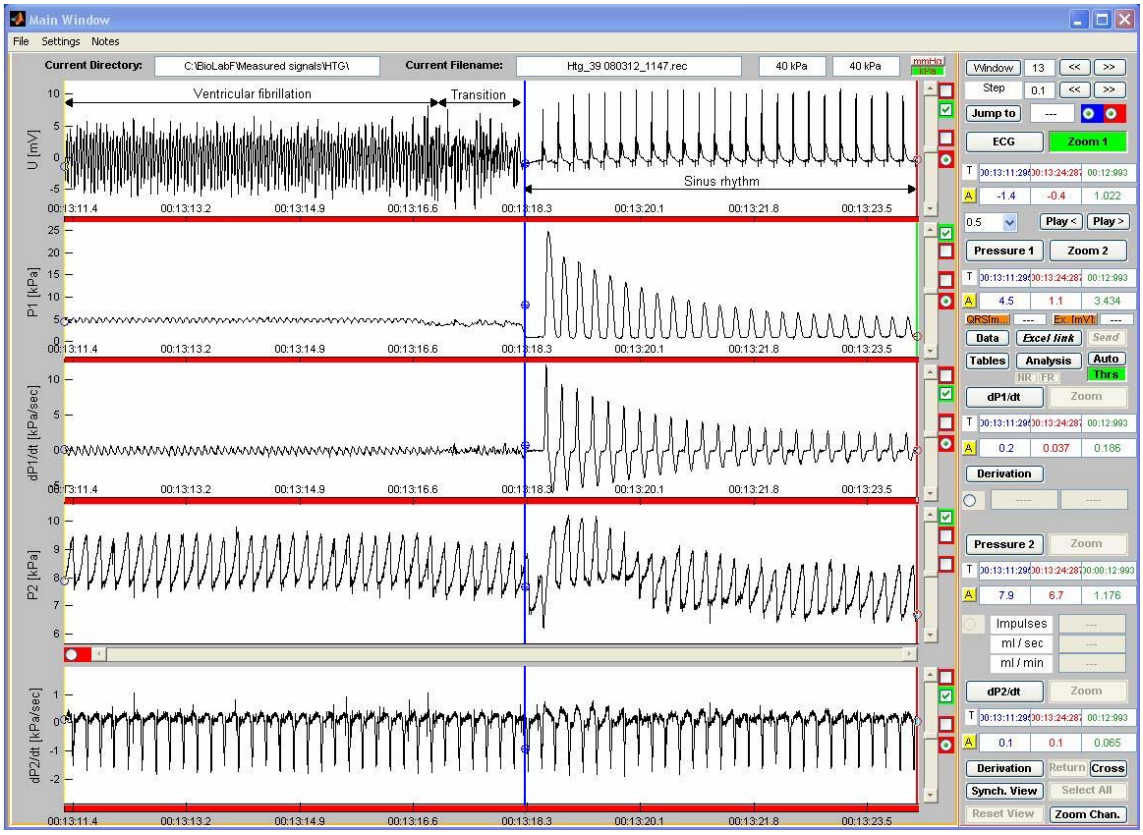


Figure 8: The user interface of developed software for biosignal analysis with data loaded from pharmacological experiment. From up to down: ventricullar Ecg signal (firstly electrically induced fibrillation - last 5 sec. depicted only, then applied bolus of DHA, transient segment and restoration of the sinus rhythm finally), left ventricular perfusion pressure (in kPa), derivation of left ventricular perfusion pressure (in kPa/sec), right ventricullar pressure (in kPa), its derivation (in kPa/sec). Length of window is 13 sec.

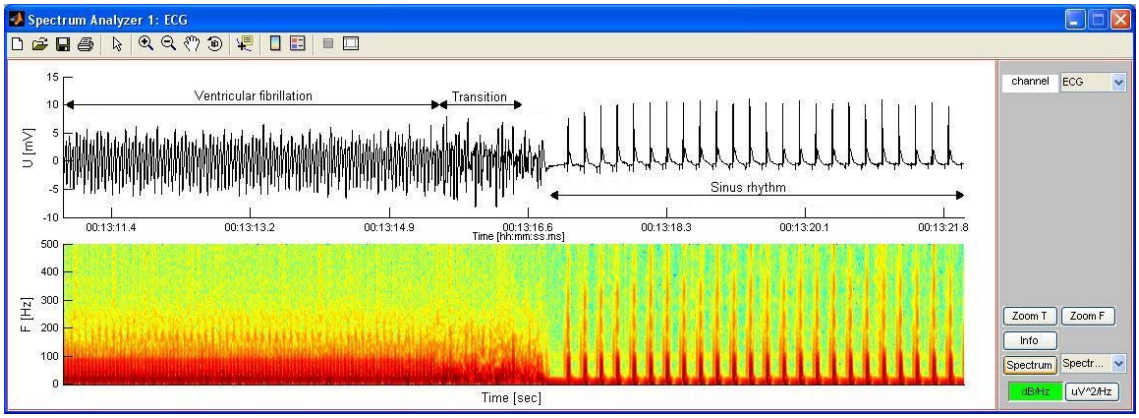


Figure 9: The user interface of spectral analysis modul. From up to down: Ecg signal (electrically induced ventricular fibrillation - last 5 sec. duration is depicted only, then transient segment and restored sinus rythm). Length of window is 13 sec.; Spectrogram computed by Short Time Fourier Transform algorithm (number of FFT point 512, window length 64 points, window overlap 95 % , Hamming window).

4 Conclusions

The presented algorithm is able to correctly detects the most common types of events in the Ecg signals according to guidelines defined by Lambeth conventions [5]. Moreover, the algorithm is able to distinguish between the QRS complexes pertinent to sinus rhythm and QRS complexes originate from extrasystoles but the detectability in this case is strongly dependent of the threshold set by the user. Problems with detectability arised also in the case of ventricular fibrillations where the algorithm failed and thus it is not able to analyse this type of events.

From analysis of spectrogram it is clear that the time-frequency transforms have a potential to study of the effects induced by drugs stimulation in advanced manner. In addition, from this type of representation of the Ecg signals we can clearly identify segment of ventricular fibrillation and sinus rhythm along with the activity of atria (manifested as P wave) and ventricle (manifested as QRS complex). We can expect, that the various type of fibrillation (induced by different types of tested pharmacological substances) will created different and more complex shapes in spectrogram image. Mallat [7] showed that complicated formations and shapes in spectrogram image could be analytically described by ridges and modulus maxima techniques. This knowledge is a base of a new method for unique identification, description and classification of various phenomena (namely fibrillations, QRS complexes, etc.) in Ecg signals which is currently being developed. The method of morphological analysis of electrograms will be based on wavelet transform and we believe that itl have a potential to reveal new information about heart function.

Acknowledgements

We thank to Dr. Narcisa Tribulova and Dr. Vladimir Knezl from Institue of Experimental Pharmacology for their valuable comments as active users of the designed system. The work described in the paper was supported in part by ESF project Meditech (SORO/JPD-26/2005)") and project ESF 2004/4-016.

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