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Simple and Efficient Excitation Signals for Fast Impedance Spectroscopy

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***Abstract*—This paper deals with using of simple but energy efficient binary multififrequency excitation waveforms for fast bioimpedance spectroscopy. Binary waveforms can not only be generated in a simple way, but also the amount of useful excitation energy in these exceeds the energy of comparable sine wave based signals. This type of signals allows also versatile adapting (matching) of the shapes of the spectra of the object and excitation signal.**

***Index Terms*—Bio-impedance, fast impedance spectroscopy, excitation signal, binary excitation waveform, binary multifrequency signal, cytometry.**

I. INTRODUCTION

Using of simple binary waveforms in fast bioimpedance spectroscopy is discussed in the paper. The use of excitation waveforms discussed here is not limited for biological objects, however, bioimpedance spectroscopy is a common field where fast and sensitive measurements are required. Electrical bio-impedance spectra are used to characterize the structure of tissues and cell cultures [1]. Also single cells can be detected and their behaviour characterized even if the parameters of which are changing fast, e.g. when the cells are flowing within microfluidic channels [2] or nerve cells are fired. Only milliseconds can be allowed to avoid significant dynamic errors in last case.

The signal-to-noise ratio (SNR) of the measured response signal depends directly on the energy of the excitation signal, more exactly, is proportional to the RMS value of the signal. However, the level of the excitation signal may be low due to nonlinearities of both, object and interfacial double layers. In the case of measuring biological objects the most important factors are safety limit and distortions originated by nonlinearities of an object [3] and interfacial double-layers. To avoid the nonlinearities which are due to double-layers, the voltage drop on the electrodes should be kept below ±50 mV [4]. Since shorter signals carry less energy this also reduces the SNR performance.

Classically multi-frequency sine waves or sine wave based chirps are used in fast impedance measurements [1], [5]–[7]. However, it is found out that some binary waveforms can not only be generated in a simple way, but also the amount of useful excitation energy in these exceeds the energy of comparable sine wave based signals [5], [7]–[9]. Energy of the binary waveforms can be concentrated onto selected separate frequencies. At the same time, not only the frequencies of selected signal components but also their levels are controllable. We can optimize the binary excitation waveform depending on the predicted shape of the frequency response of impedance under study [5]–[8].

Bio-impedance spectra have a tendency to decrease at higher frequencies [1]. For example, it is nearly inverse proportional to the frequency in the case of single cell spectroscopy. Pre-emphasizing of higher frequency components in the excitation signal spectrum allows compensating a lower level of the response signal in this area. However, an optimal solution depends also on some other factors, which are discussed below.

II. IMPEDANCE MEASUREMENT ARCHITECTURE

A simplified architecture of the bio-impedance spectroscopy system is presented in Fig. 1. An excitation signal source (generator of excitation waveform) has inputs for timing and duration control (from t_1 to t_2) and for frequency range setting (from f_1 to f_n). The binary signal from this generator is used as an input excitation for the complex impedance $\dot{Z}$ under study, as well as a reference signal source. For the differential measurement the reference impedance $\dot{Z}_{ref}$ is placed in the reference signal path. In the signal conditioning block, amplification, filtering and signal level matching for the signal processing block is performed. Discrete or fast Fourier transforms (DFT or FFT) are used for obtaining the amplitude and phase spectra $|\dot{Z}(f)|$ and $\Phi(f)$ of the impedance $\dot{Z}$.

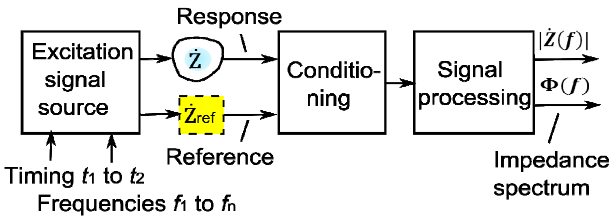

Fig. 1. A simplified architecture of the spectroscopy system designed for measurement and analysis of complex bio-impedance $\dot{Z}$.

To measure unknown impedance, we can use one of two simple approaches: either we apply a known voltage

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excitation across the object and then measure the current flowing through it, or we inject a known current injection into that object and measure the voltage across it. We can also measure both values, current and voltage simultaneously. There are also other methods, like bridge balancing etc, but these are not described here.

The impedance of an object can be characterized by its electrical equivalent, which, on the other hand, can be represented as the frequency-dependent complex vector

$$\dot{Z}(j\omega) = \mathrm{Re}(\dot{Z}(j\omega)) + j\,\mathrm{Im}(\dot{Z}(j\omega)) = \\ = |\dot{Z}(\omega)|\exp(j\Phi_z(\omega)), \tag{1}$$

where $\omega=2\pi f$, $|\dot{Z}(\omega)|=(\mathrm{Re}(\dot{Z}(j\omega))^2+\mathrm{Im}(\dot{Z}(j\omega))^2)^{½}$, and $\Phi(\omega)=\mathrm{arctg}(\mathrm{Im}(\dot{Z}(j\omega))/\mathrm{Re}(\dot{Z}(j\omega)))$. Using the current excitation I_{exc} through the object and measuring the response voltage V_{z}, we can find out the impedance spectrum of it as

$$\dot{Z}(j\,\omega) = \mathbf{F}\,(V_z(t)) / \mathbf{F}\,(I_{exc}(t))\,. \tag{2}$$

In case of using voltage excitation source V_{exc} the impedance spectrum can be found in a similar way

$$\dot{Z}(j\omega) = \mathbf{F}\,(V_{exc}(t)) / \mathbf{F}\,(I_z(t))\,. \tag{3}$$

III. CURRENT SOURCE VERSUS VOLTAGE SOURCE

For bioimpedance measurements, excitation with a current source is usually considered to be preferable over sourcing of voltage. Since the biological objects are sensitive to electric fields, the current flow becomes nonlinear with respect to voltage. Current source will produce negative feedback reducing this nonlinearity [3]. Moreover, the current density on the electrodes is well controlled, and the voltage drop across the electrodes can be predicted, which opens a possibility for correcting of electrode stray effects [3].

Despite of these advantages of the current source excitation method, more parameters should be taken into account to estimate the overall performance of different methods and their practical realizations.

Real current sources have limitations, especially at the high frequencies, where the stray capacitances degrade significantly the performance of current sources. Active current sources work reasonably well up to some MHz. As it is shown in [9] a simple resistor has been used as a current source, while not comparable at lower frequencies, much better performance has been reached potentially at higher frequencies. Disadvantage of active current sources (in comparison to resistor) is also their higher noise level.

There is one more important difference in using of current or voltage excitation sources. Typical magnitude spectrum of the impedance of a single cell surrounded by saline suspension is shown in Fig. 3. In the case of using current source it should have a rising shape of spectrum for the pre-emphasizing of higher frequency components and a falling shape of the spectrum at lower frequency area to protect the object from over voltages.

In the case of using voltage excitation source, the pre-emphasizing of higher frequency components is not necessary at all, since the current is increasing itself when the impedance is decreasing. As it is illustrated below, the waveforms with falling spectra are more energy efficient and easier to design, then the waveforms with rising spectra. One more positive effect of using voltage source is that the rise of impedance in the lower frequency area of frequency range does not produce any excessive voltage.

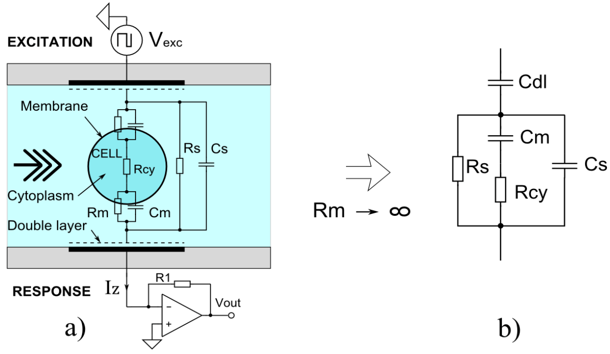


Fig. 2. Simplified electrical model of a single cell in suspension between the electrodes (a) and its equivalent circuit (b).

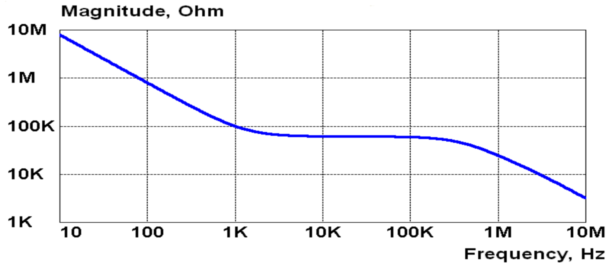


Fig. 3. A magnitude spectrum of the impedance of a single cell in saline suspension shown in Fig. 2. Cdl = 2nF, Cm= 1 pF, Cs = 5pF, Rs = 60 kΩ and Rcy = 100 kΩ.

Please notice that rise of impedance in this area is caused by the polarization of electrodes (double layer effect) and does not provide useful information about the properties of object.

In some cases, e.g. in the channel of the microfluidic system with a low concentrations of cells and sufficient channel height to cell diameter ratio, the current nonlinearities with respect to voltage are small, and a voltage source can be used instead of the more complicated and noisier current source.

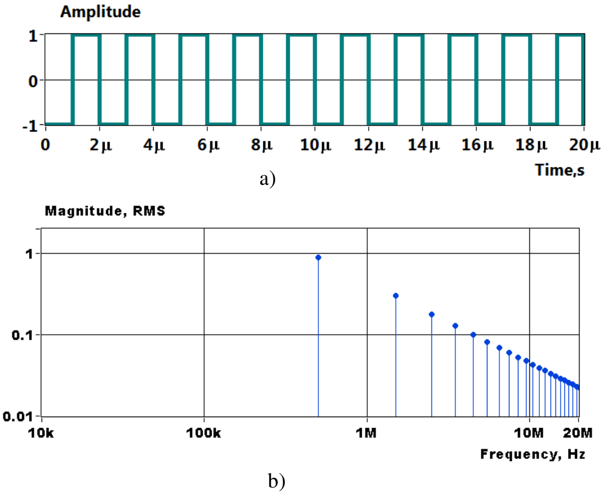
IV. SIMPLE ENERGY EFFICIENT BINARY WAVEFORMS

Energy content of the optimized binary waveforms exceeds the energy of comparable sine wave based signals [5]–[8]. However, as it is shown in [8] the mean RMS magnitudes of frequency components $V_{\mathrm{rms(i)b}}$ is decreasing proportionally to the square root of $1/n$, where n is a number of all frequency components

$$V_{rms(i)b} = \frac{4A_N}{\pi\sqrt{2}}\sqrt{\frac{1}{n}}. \tag{4}$$

It means, that the binary waveform with 4 frequency components (bins of spectrum) have two times more energy in each spectral bin than the binary waveform with 8 frequency bins and same duration. Benefit of the excitation signal with more frequency bins is that it gives more detailed shape of the spectrum of the response signal or covers a wider frequency range in the same timeframe. For the better SNR performance some minimum number of frequency components should be used according to the nature of object and level of additive noise accompanying the response signal. If the spectrum of the response signal is smooth and characteristic changes fall into 1-2 frequency decades, as illustrated in Fig. 3, the waveform with 5 to 10 frequency components is sufficient.

The simplest binary waveform which almost fulfills the requirements described above in the case of using voltage excitation, is the simple rectangular signal (meander). The spectrum of this waveform contains odd harmonics (1^{st}, 3^{rd}, 5^{th}, 7^{th}, 9^{th}, etc) which are declining with frequency by 1/f rule (Fig. 4, (b)). Energy content of the first 4 frequency bins compared to the total energy of the signals is 95 % and 98,6 % for the first 15 bins.



a)

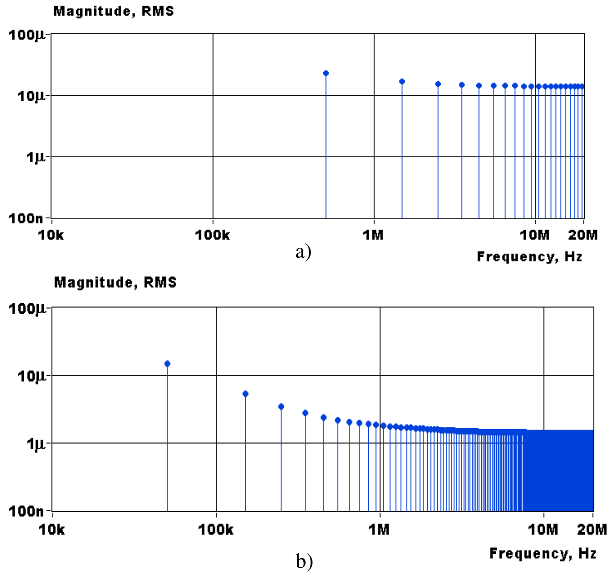
b)

Fig. 4. Waveform and magnitude spectrum of the rectangular signal.

If the duration of the cycle of the waveform is chosen so that the first frequency bin of the magnitude spectrum is placed on the knee of the impedance spectrum curve (see Fig. 3), then the spectrum of the response current is almost flat (as is illustrated in Fig. 5, (a)). Shifting the first frequency bin lower does not give flat spectrum anymore as it shown in Fig. 5, (b). RMS magnitudes of the response current are also significantly lower (except the first frequency bin).

Effective solution for covering of lower frequency area is using of specially designed binary waveforms which have flat spectrum in this area. A binary waveform which is shown in Fig. 6, has a flat spectrum of first 4 frequency bins (1st, 3rd, 5th, 7th harmonics) and allows getting magnitudes of the response current near to 10 µA. Please notice, that rectangular waveform with a longer period (Fig. 5, (b)) provides lower magnitudes of the response current in this area.

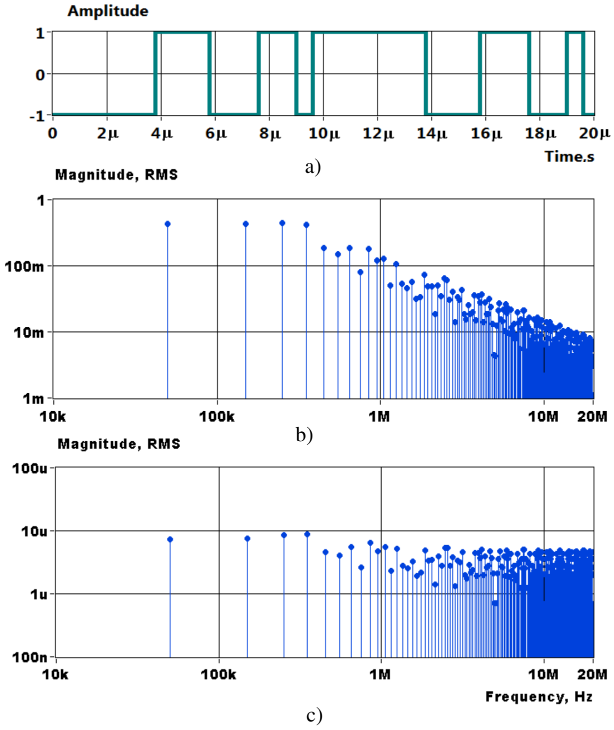
Though the description of the methods for designing of binary multifrequency waveforms is out of the scope of this paper, however, it must be noted that such type of excitation signals allow versatile adapting the shape of the excitation spectrum to the spectrum of the object to be measured.



a)

b)

Fig. 5. Magnitude spectrum of the response current for the different durations of the cycle of rectangular excitation voltage: a – 2 µs and b – 20 µs.



a)

b)

c)

Fig. 6. Excitation waveform – a, magnitude spectrum of the excitation voltage – b, and corresponding magnitude spectrum of the response current – c.

Two simple waveforms illustrated in Fig. 4 and 6 cover the frequency range over 3 decades in a short timeframe. However, to keep the overall spectrum undistorted, both excitation signals must be applied one by one sequentially. Required interval between the two parts of the signals depends on the characteristics of an object, more specifically on relaxation time-constants formed by the capacitances and resistances of its equivalent circuit (Fig. 2). In current case the biggest influence has a pair of R_s and C_s, relaxation time-constant τ_s of which equals as

$$\tau_s = 2\pi R_s C_s. \quad (5)$$

As a rule of thumb, time-interval of 3τ is sufficient for avoiding cross-disturbances (τ_s is about 10 µs in our case). Since the durations of the excitation pulses are less than τ_s this time interval could be less than 3 τ_s. In practice this time-constant is also decreased by output resistance of the excitation source and usually more than one period of excitation signals is used for improving SNR by averaging. In the last case a short time interval between the two signal portions becomes inconsiderable.

V. CONCLUSIONS

Simple binary waveforms can not only be generated in a simple way, but also the amount of useful excitation energy in these exceeds the energy of comparable sine wave based signals. This type of signals allows also versatile adapting (matching) of the shapes of the spectra of the object and excitation signal.

Using of voltage excitation sources rather than current ones allows us to use simple and energy efficient rectangular signals and also simple matching with a bio-impedance spectra becomes achievable.

Using a waveform with two separate parts allows to maximize the RMS magnitudes of response signals and to improve the signal-to-noise-ratio of measurements. The drawback is that a time delay is necessary between the two signal parts. Despite of this delay more than three frequency decades beginning from 50 kHz can be covered during less than 100 µs.

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