

Adaptive signal sampling for high throughput, broadband impedance spectroscopy

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Abstract. High throughput applications using broadband impedance spectroscopy as method for label free detection are limited by measurement speed and the enormous amount of raw data. Although often ignored, the fastest method for determining the passive electrical properties is the processing of the answer after step excitation. Sampling with constant time interval is common but not feasible for real time applications. Only adaptive sampling ensures high bandwidth of the measurement but still acceptable data volume. Exponentially decaying relaxations are typical answers of biological objects to step functions. Sampling of these signals using gradually increasing integration times does not violate the sampling theorem and allows comparatively simple data processing.

Keywords: impedance, dynamic, adaptive sampling, time domain

1. Introduction

Electrical impedance measurement is a method for nondestructive and label free characterization of any kind of material. The impedance of biological material has manifold use, e.g. for quality assessment, tissue discrimination or monitoring of growth processes [Grimnes et al. 2014]. While applications in former time focused on tissue and cell suspensions, today increasingly single cells or cell agglomerates are characterized by their electrical properties [Wolf et al. 2008]. The great advantage of electrical measurements is the label free method and the comparatively cheap instrumentation.

The electrical impedance of biological material exhibits three well distinguishable dispersion regions [Grimnes et al. 2014]: The α -dispersion arising from lateral movement of ions along cell membranes with a characteristic frequency below 100 Hz, the β -dispersion in a frequency range between 10 kHz and 10 MHz based on interfacial polarization of membrane structures and δ -dispersion which is governed by orientation polarization of dipoles (Debye-relaxation) with the absorption peak above 100 MHz. A drawback of impedance measurements below 1 GHz is the low selectivity compared to other methods.

While impedance measurement in early years were often done in narrow frequency band (e.g. only one dispersion region), today broadband measurements over more than 6 decades can increase the selectivity considerably by assessment of more than one dispersion region. For instance, a cell suspension can be characterized not only by the polarization arising from cell membranes but also by the conductivity of the suspension medium at very low frequency. Additionally, the density of water dipoles is manifested as absorption within the GHz-range [Sachs et al. 2007].

Besides suitable electrode configuration for broadband measurement, also the instrumentation needs special attention. A key feature of most biological material is the considerable drop in impedance magnitude over several orders of magnitude between 10 Hz and 10 GHz. The classical way in bio-impedance measurement, a frequency sweep, requires a measurement time ranging from a few seconds (abandonment of low frequencies, small number of discrete frequencies, short integration time) up to several minutes. Moreover, most instrumentation is not suitable for such broadband measurements.

A way out is the electrical characterization in time domain by excitation with broad bandwidth signal and monitoring the response [4;5]. Digitizing of the excitation and the response is compatible with modern technology and makes economic instrumentation available. However, measurement over several decades becomes demanding. The lower cutoff is given by the duration of the excitation signal. For instance, if the lowest frequency is 100 Hz, the signal length should be at least 10 ms. If a

measurement under steady state condition is considered, at least 100 ms are necessary (10 periods). In order to fulfill the Kotelnikov's sampling criteria, the sampling frequency should be at least twice the upper cutoff frequency. Let's consider a frequency band between 100 Hz and 10 MHz (5 decades), sampling should be done with minimally 20 MS/s over 10 ms. This results in a vector length of 200.000 samples for each channel (excitation and response). This however, is the best case. Given unavoidable noise, a higher sampling frequency would be desirable. Although, this is still feasible for single measurements, high throughput monitoring (e.g. 100 cells / s) on this basis is either impossible or requires extensive computation power. The practical solution today is the use of less bandwidth, for instance between 10 kHz and 1 MHz where a time requirement for a single measurement can be reduced down to 1 ms. Frequently chosen excitation signals are the Chirp function [Min et al. 2008], Multi-sinus [Bragos et al. 2001] or maximum length sequences [Sachs et al. 2007].

Using fast Fourier transformation and sufficient computing power, real time monitoring is possible, but only for narrow frequency range which limits the all over selectivity. The use of adaptive sampling, e.g. high sampling rate where the signal changes fast and reduction of the sampling for slow parts can considerably reduce the data volume without comprising accuracy of the method. The processing of these data requires algorithms other than fast Fourier transform (FFT) but can be done as fast as well. However, the signals generally favored for transformation into frequency domain are not suitable for adaptive sampling. For this purpose, functions like Dirac-function, step function or ramps are a better choice because of the typical response of biological material which is the superposition of exponentials. Creation of even nearly Dirac-functions is demanding and ramp function lack energy within the high frequency range. This makes step function a primary choice for excitation.

2. Electrical Characterization in Frequency and Time Domain

The electrical characterization of biological material in frequency domain is well established [Grimnes et al. 2014]. A great number of devices, network analyzers, gain-phase analyzers or just phase sensitive voltmeters is widely available and the measurement by sweeping the frequency through the frequency band of interest is widely understood. A well-recognized advantage of measurements in frequency domain is the use of selective amplifiers like lock-in-amplifiers which greatly reduces stochastic noise. The sometimes long measurement time is in most cases not critical. Taking all this together, this method is today the golden standard for bio-impedance measurement.

While the impedance as material property is a function of frequency, the response to a broadband signal is a voltage or current as function of time. Strictly speaking, impedance does not exist in time domain. However, characterization of material with time variant impedance by means of impedance measurement as function of time should be clearly distinguished from electrical material characterization in time domain.

If a system is time invariant and linear with respect to current/voltage characteristics, time and frequency domain can be converted into each other using several kind of transformation where the most popular one, the Fourier transformation is limited to steady state condition. Laplace transformation is required for transient behavior. A popular method for fast impedance measurements is the measurement in time domain with transformation into frequency domain where all subsequent processing like model fit or other kind of data interpretation are done in frequency domain as well.

It is however feasible to characterize a material directly in time domain. The impedance as characteristic material property in frequency domain corresponds to a system of differential equations in time domain [Wunsch, 1970]. A particular solution of these equations for stimuli like Dirac-function or step function as boundary condition is a sum of exponentials, either as current or voltage trace in time.

From the impedance spectrum distinguishable dispersions characterized by the circumference and the characteristic frequency can be derived. A particular frequency dispersion, characterized by its characteristic frequency (ω_0) and its circumference ($R_0 - R_\infty$) corresponds to a relaxation in time domain described by the time constant (τ) and a relaxation strength ($U_\infty - U_0$) (see Fig.2). The relation between time and frequency domain is shown (Fig.1) at the commonly used equivalent circuit for biological material with a resistor R_a for the extracellular electrolytes and the RC-combination for the cell surrounded by an insulating membrane modeled by C.

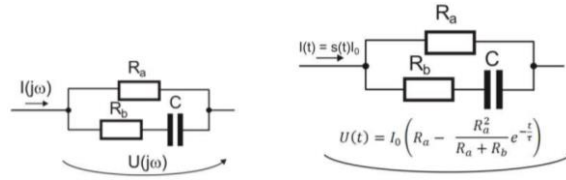


Figure 1 Current and voltage in frequency and time domain. The impedance (left) is $Z(j\omega) = U(j\omega) / I(j\omega)$. A transient stimulus in time domain (current step, $s(t)I_0$) applied at t_0 without any signal history ($I_{t<0} = 0$) yields an exponentially relaxing voltage depending on the impedance of the material.

The locus diagram of the impedance for a circuit in Fig.1 is a half circle (Fig.2) while the voltage response to a current step is an exponential function.

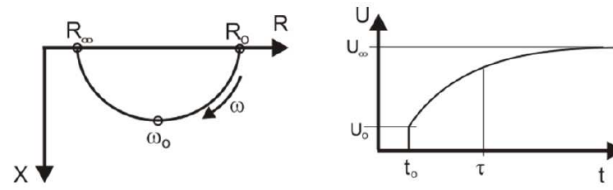


Figure 2 Representation of electrical properties in frequency domain (impedance, left) and time domain (relaxation, right)

3. Processing Data in Time Domain

The performance of using the step response either for calculation of the impedance or for assessment of electrical properties directly in time domain depends on the sampling regime. In general, according to the sampling theorem, the highest assessable frequency depends on the sample rate. If the sample rate is too small with respect to higher frequency compounds within the signal, aliasing can significantly disturb the result. The optimum would be a signal filtered with a cutoff at the highest desired frequency and sampled with twice as the cutoff frequency. Moreover, in order to have acceptable quantization noise, high ADC-resolution (digital/analog converter) is essential.

Fourier transformation using this kind of data to calculate the impedance spectrum yields often disappointing results. Especially at high frequency, incredible noise appears due to the low signal energy. Algorithms for smoothing the spectrum are not considered here since the focus lies on the main problem, the big data volume. For the assessment of all three relaxations (α, β, γ) corresponding to the frequency dispersion, long sample vectors (e.g. 20 million samples) are necessary. It is not a question that modern technology is able to handle such big data. However, it becomes important when continuous monitoring in high throughput applications is desired with real time processing of the data. A relatively fast algorithm lies in the partial fit of relaxations yielding a time spectrum as shown in Fig.3 for KCl-solution contacted by microelectrodes (5 μm distance).

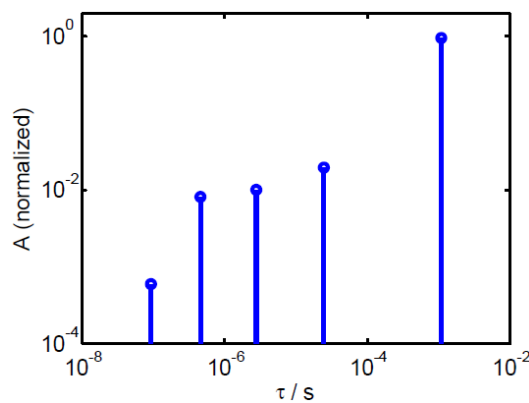


Figure 3 Time-spectrum calculated from the voltage across the electrodes

The relaxation strength has been normalized to the sum of all relaxation strengths. There is one dominating relaxation arising mostly from the interface electrode-electrolyte. Due to the fit procedure, only ideal relaxations were calculated. More sophisticated algorithms use distribution of time constants which can also account for constant phase elements describing for instance the impedance of the electrode interface. Given the nature of relaxation process, fast sampling is not necessary throughout the signal. Breaking the signal into windows, high frequency compounds appear only immediately after the current step. It should be noted that this consideration does not violate the steady state condition of a Fourier transformed signal, where time information is lost and any frequency component has an invariant amplitude within $-\infty < t < \infty$. With respect to fig.4, a marked difference to general approach is that only a window of a transient response is considered and not a steady state signal. Having a high sampling frequency at the beginning is essential while with longer time after the step no further high frequency information appear. Therefore, the sampling rate can gradually cease (Fig.5) without losing information. An often underestimated problem is the high frequency noise present even at the slowly changing part of the signal. This would require an adaptive anti-aliasing filter as well. Current solution use averaging between 2 sampling points which yields the low pass filtering with a cutoff frequency which is half the sample rate. This fulfills the sampling theorem and achieves adaptive anti-aliasing filtering.

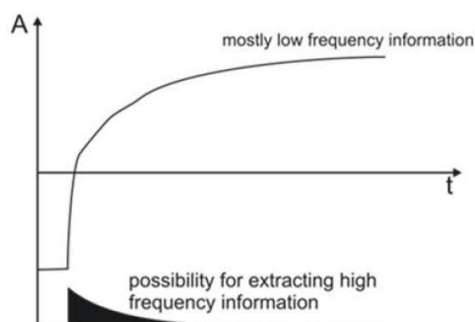


Figure 4 Illustration of the step response (e.g. voltage) with respect to the possibility for extracting high frequency information.

With longer time after the step, the high frequency compounds diminish. Using 10 sample points per decade requires 60 points for a bandwidth ranging over 6 orders of magnitude, for instance from 100 Hz up to 100 MHz. Since applications seek a characterization of the material, calculation of the impedance spectrum is not necessary. A simple algorithm uses three sample points for calculation of time constant and relaxation strength. A requirement is that the three sample points are evenly spaced as shown in Table 1.

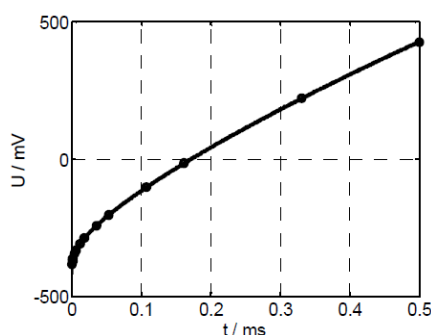


Figure 5 Non-uniformly spaced sampling points (dots) of a voltage response from micro electrodes in KCL-solution.

Starting from the end of the signal $U = f(t)$ (0.5 ms in Fig.5), the largest time constant ($i=1$) and the relaxation strength can be calculated as:

$$\tau_i = \frac{t_i - t_{i-1}}{\ln \frac{(U_{i-1} - U_i)}{(U_i - U_{i+1})}} \quad a_i = \frac{(U_{i-1} - U_i)}{e^{\frac{-t_{i-1}}{\tau_i}} - e^{\frac{-t_i}{\tau_i}}} \quad (1)$$

The dc-offset for this segment is:
$$c_i = U_{i-1} - a_i e^{\frac{-t_{i-1}}{\tau_i}} \quad (2)$$

After subtraction of this relaxation from the vector, the next points are used for calculation of the next relaxation until the relaxation strength fall behind a pre-defined minimum. Such result is represented in fig.3. The same algorithm can be used for transformation into frequency domain, by adding the integrals of all the segments. A particular coefficient has the form of:

$$A(\omega_k) = \sum_i^N \int_{t_{i-1}}^{t_{i+1}} (a_i e^{\frac{-t}{\tau_i}} + c_i) e^{-j\omega_k t} dt \quad i = 2, 4, 6, \dots, N-1 \quad (3)$$

It should be noted that the analytical solution for the integral should be used rather than a numerical approach. The simplest way for transformation bases on the equivalent circuit in Fig.6.

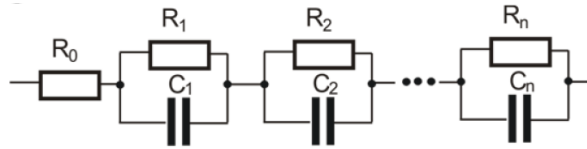


Figure 6 Equivalent circuit for series of relaxing structures

This yields directly the impedance spectrum

$$Z = \frac{1}{I_0} \left(a_0 + \sum_{i=1}^n \frac{a_i}{1 + j\omega\tau_i} \right) = R_0 + \sum_{i=1}^n \frac{R_i}{1 + j\omega R_i C_i} \quad (4)$$

with distinguished dispersions represented by the RC-combinations. a_0 is the dc-part and is calculated as sum of all c-coefficients $a_0 = \sum_{i=1}^n c_i$. I_0 is the magnitude of the current step. If the biophysical model requires parallel and serial arrangement of relaxing structures, a rearrangement of the equivalent circuit is feasible.

Table 1 Example for a sampling time vector with gradually increasing spacing but two subsequent sampling intervals being equal.

sample point	t / μ s	$\Delta t_1 = t_n - t_{n-1}$	$\Delta t_2 = t_{n+1} - t_n$
1	0.67		
2	1.33	0.67	0.67
3	2		
4	4	2	2
5	6		
6	12	6	6
7	18		
8	36	18	18
9	54		
10	108	54	54
11	162		
12	331	168	168
13	500		

The algorithm used here assumes a nearly ideal current step with fast rise time, no overshoot and flat throughout the measured period. Current sources, especially made for this purpose are described in [Pliquett, 2011].

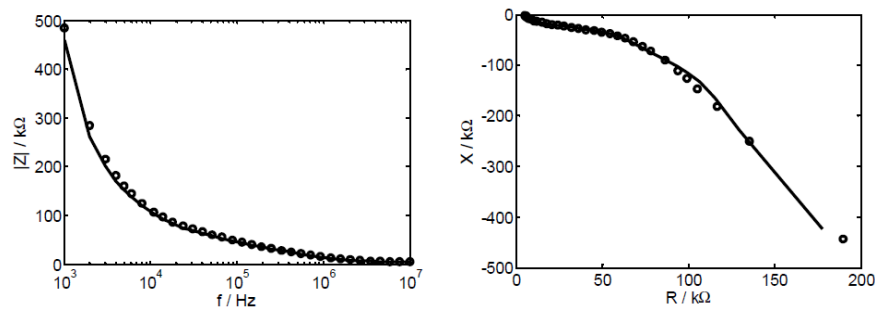


Figure 7 Comparison of a reference measurement (multisine, dots) with the impedance directly calculated from time domain (line) for an electrode distance of $5 \mu m$. The right panel shows the Wessel-plot for both measurements.

A similar algorithm is possible for voltage controlled excitation. With some changes, using transformation of both, current and the voltage, signals of less quality (overshoot, bad quality of slope, value arte $t=0$ not constant) can be used. Moreover, methods for handling constant phase elements or parasitic elements like parallel capacitors exist but are behind the scope of this paper. The comparison between the signal measured and processed in the described way and the result of a multisine approach at the same object is shown in Fig.7.

4. Conclusions

Impedance measurements for high throughput application are not only critical with respect to the measurement speed but also with respect to the data volume acquired. Gradually decreasing of sample rate together with adaptive filtering, accomplished by variable integration time during the sampling process of step response, results in great data reduction without comprising measurement precision.

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