Multifrequency simultaneous bioimpedance measurements using multitone burst signals for dynamic tissue characterization.

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Abstract. In this paper we present the keypoints to perform multifrequency simultaneous bioimpedance measurements using multitone signals. Concerning the frequency distribution, tones are spread over 1kHz to 1MHz range using a custom frequency distribution which we called Bilateral Quasi Logarithmic (BQL). BQL concentrates a higher number of tones around the impedance relaxation and contains a frequency plan algorithm. It minimizes the intermodulation effects due to non-linearities behaviours of the DUT and electrodes by slightly shifting the original tones in order to guarantee a guard bandwith. Regarding the multitone phase distribution, a Genetic Algorithm (GA) has been developed to minimize multitone Crest Factor (CF). This allow us to maximize the resultant Signal to Noise Ratio (SNR) of the acquisition system. This paper also presents the relation between parameters such as sampling frequency and ADC bits with the SNR and the effect in the overall amplitude and phase error when using multitone signals as excitation waveforms. Finally, we present characterization results from a measurement system based on a modular PXI architecture.

1. Introduction
Multitone signals are useful for fast impedance spectroscopy characterization of biological systems whose electrical properties change along within time. Using this technique, is possible to obtain the impedance spectrum free of the errors induced by asynchronous undersampling of the mechanical modulation of the biological system under test. Essentially, a multitone is composed by the sum of M tones, each one with its own amplitude and phase (eq.1).

\[ x[n] = \sum_{m=0}^{M-1} a_m \cos \left( 2\pi \frac{f_m}{f_s} n + \phi_m \right), \quad n = 0, ..., N - 1 \] (1)

1.1. Measures of Signal Quality: Crest Factor
When comparing signals it is necessary to have a quality measure in the form of a performance index. A classical index is the Crest Factor (CF), which gives an idea of signal’s compactness but there are other options like Performance Index for Perturbation Signals (PIPS) [1] or Time Factor (TF) [2]. Thus, CF of a function \( x(t) \) is defined as the ratio of its peak value (also known
as Chebyshev norm) and its Root Mean Square value (RMS) \([3]\) is given by:

\[
CF(x) = \frac{l_\infty(x)}{l_2(x)} = \frac{\max_{n \in [0,N-1]} |x[n]|}{\sqrt{\frac{1}{N} \sum_{n=0}^{N-1} |x[n]|}} \tag{2}
\]

The classical ways to minimize CF use heuristic approaches \([4], [5]\). Other approaches consisted on analytical methods. They use different iterative methods \([6], [7]\) or employ natural selection and evolutionary-inspired operators like Genetic Algorithms (GA) \([8], [9]\).

### 1.2. Frequency distribution

Typically, multitone frequencies are either distributed logarithmically or equally spaced \([10]\). However, both have important disadvantages when measuring impedance. In one hand, equidistant frequency distribution needs a high number of tones to cover a wide frequency range and avoid losing spectral resolution. Moreover, intermodulation products will appear at the same measurement frequencies if the global system presents non-linearities, provoking errors in the amplitude and phase determination. In the case of logarithmic distribution there is a big number of tones at low and high frequencies content where the relaxation do not include much information.

One of the main issues is how to arrange multitone frequencies to minimize intermodulation products effects. One option is generate each tone as a result of a fundamental frequency multiplied by a sequence values. This solution is based on special sets of multipliers in order to accomplish as many as possible disjunct distortion signal frequencies and to test signal frequencies is presented in \([11]\). In our case, BQL algorithm algorithm combines exponential and logarithmic functions to concentrate more frequency components close to the relaxation slope band. Moreover, it spreads the remaining tones at low and high frequencies (fig.1) \([9]\) and also redistributes original tones taking into account where the intermodulation frequencies would appear.

![BQL Frequency Distribution](image1)

![Multitone Power Spectral Density](image2)

**Figure 1.** Example of BQL fundamental harmonics to characterize impedance relaxation around 50kHz.

### 2. Precision of Transfer Function Measurement using Multitone Signals

In single frequency impedance measurements is well known the relationship between the SNR with parameters such as ADC bits or sampling frequency. In the case of multitone measurements
there are more hidden parameters that have to be taken into account. In fact, the SNR of an ideal b-bit AD converter measured over the Nyquist bandwidth \((DC - F_s/2)\) when using a multitone input signal as excitation is decreased due to the Crest Factor and the number of tones \(M\) according to eq.3:

\[
SNR_{\text{multitone}} = 6.02b + 1.76 - 20\log_{10} \left(\frac{CF}{\sqrt{2}}\right) - 10\log_{10}(M) \quad [dB]
\] (3)

Finally, has been shown in [12] the relationship between SNR when using multitone and the magnitude and phase measurement accuracy:

\[
\epsilon_l = 20\log_{10} \left(1 + 10^{-\frac{SNR_{\text{multitone}}}{20}}\right) [dB] \quad \epsilon_\theta = \frac{180}{\pi}10^{-\frac{SNR_{\text{multitone}}}{20}} [\circ]
\] (4)

where \(\epsilon_l\) and \(\epsilon_\theta\) are magnitude and phase accuracy. Eq.4 allows to find the minimum system that satisfies quality measurement requirements in terms of SNR or accuracy using multitone signals only with the a priori information of Crest Factor, number of bits and number of tones.

3. System characterization

The measurement system has been build around a PXI architecture from National Instruments. The system includes an embedded controller PXIe-8130, 2 channel digitizer card PXIe-5122 (100Ms/s, 64MB/channel, 14bits) and an arbitrary waveform card PXI-5422 (200Ms/s, 32MB, 16 bits). The system has been characterized measuring resistors and RC networks with a custom 4-wire wideband front-end. The set up has been carried out performing 100 measurements (2ms burst length) of a set of resistors (1% tolerance). Multitone signal was designed with 21 tones spread from 5kHz to 1.313MHz, 1mA of amplitude current and crest factor 2.7. Theoretical SNR obtained with this system parameters is 67.2dB which corresponds to a measurement error of 0.0038dB and 0.023\(^\circ\) in magnitude and phase respectively. Magnitude and phase accuracy has been validated with the standard desviation obtained from the measurements. Also a preliminary dynamic scenario have been characterized based on yeast cell suspension settlement (fig.2) over interdigitated 4-electrode located at the bottom of a small bioreactor [13]. Multitone parameters used were the same as described before. System performed 100 measurements (10ms) every 5 minutes up to 12 hours, with a a total amount of 14400 measurements.

![Figure 2. Time evolution of Cole-Cole arcs and cell growth estimator’s described in [14].](image)

At this moment, our work is focused on characterizing human stem cells for tissue engineered applications. The goal is to characterize stem cells differentiation into cardyomiogenic cells when applying electrical and mechanical stimuli in regenerative medicine.
4. Conclusions
One of the multitone signal strengths is that it is very rapid for those systems that have a short settling time and are not plagued by significant noise. In addition, SNR can be increased by applying more energy in the desired spectral components in applications where the total amount of energy applied is not restricted. In applications where the energy is limited, a single multitone shot characterization obtains greater SNR than using Pseudo-Random Binary Sequences (PRBS). This is due the fact that the energy is focused on the measurement frequencies and it is not spread in the whole frequency band. One of the weaknesses of multitone signals is the crest factor. However, it is possible to minimize the impact of the crest factor over the measurement quality using common algorithms proposed in the literature.

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5. References
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