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Electrical Impedance Spectroscopy cell monitoring in a miniaturized bioreactor

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Abstract- Electrical impedance spectroscopy measurement of biologic materials provides information about viable cell density as well as cell size and shape homogeneity. Several experimental and even commercial biomass density probes have been developed both for cell suspensions and monolayer cell cultures in the last twenty years. This communication describes the development of a set of electrodes and circuits designed to add viable biomass density measurement capability to a set of single-use miniaturized bioreactors. Two applications: adherent animal cultures and generic cell suspension cultures drive to the need of providing two electrode sets and two measurement systems with different frequency ranges.

Keywords: Electrical Impedance Spectroscopy, biomass monitoring, bioreactor, front-end

I. Introduction

Electrical Impedance Spectroscopy (EIS) allows non-invasive and non-destructive characterization of biological materials. One of their applications is to monitor or to characterize cell suspensions in a bioreactor or cellular monolayers growing on the bottom of a vessel (petri dish or bioreactor) [1,2]. In both cases, the goal is to monitor the temporal evolution (growth) and changes in response to stimuli (drug tests, effects of growth factors or medium composition in cell differentiation ...). The fact that EIS is a "label-free" technique is a clear advantage over other characterization techniques which require the preparation of multiple replicas of each experiment, given that the preparation and dying processes are usually destructive. In contrast, EIS is less specific than those techniques.

Biomass monitoring in cell suspensions in desktop or industrial bioreactors has overcome the research phase and several commercial probes are available (Aber, Fogale). Both measure the suspension capacitance. For monolayer cultures, the developments of recent years converge to the use of interdigitated microelectrodes (IME) [3] located in the bottom of the vessel. This technique has been used in tissue engineering applications to measure cell growth [4] and even differentiation [5] and also has commercial implementations (Roche Diagnostics, Applied Biophysics ECIS).

Hexascreen Culture Technologies (HCT) is a start-up company which develops and manufactures single use miniaturized bioreactors [6], using optical probesto monitor cell growth, pH and pO2. The Current developments include the use of EIS for cell monitoring in the frame of a project with the Electronic and Biomedical Instrumentation group of the Electronics Engineering Department of UPC.

II. System overview

HCT mini-bioreactors (10-25 ml) cover the gap between the multiwell plates and the benchtop bioreactors, with the aim of providing realistic screening capabilities in the design of cell culture conditions.

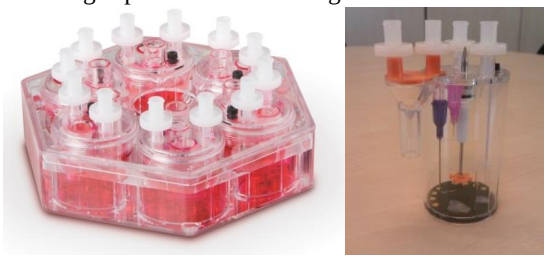

Figure 1. HCT single-use mini-bioreactors for cell culture optimization, Hexabatch (left) and Monoscreen-Fedbatch (right).

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The mini-bioreactors mimic the stirring and aeration conditions of bioreactors while maintaining the single use character of multiwall plates. Figure 1 shows two different mini-bioreactors which include stirring, aeration and optical measurement of biomass density, pH and dissolved oxygen. The left one is the Hexabatch model, which includes 6 mini-bioreactors and which operates en batch mode and the right one is the Monoscreen-Fedbatch model, which allows fed-batch operation with medium addition and sterile sample extraction. They are conceived as a tool to accelerate experimentation in cell culture optimization. The current project aims to complement the optical biomass density measurement with viable biomass measurement using EIS trough electrodes embedded in the bioreactor structure. The electrode sets allow measuring cell growth both in monolayer and cell suspension cultures and both using 2-electrode and 4-electrode configurations. This capability is useful to discriminate between cell density and cell adhesion, which masks cell density increase after cell confluence is reached when using only two electrodes [7]. The needed frequency range is also different in both applications. With adherent animal cells, 1kHz – 200 kHz is enough, given that the impedance relaxation occurs at low frequency due to the sealing resistance between cells and substrate and the big cell sizes. On the other hand, with yeast and bacteria suspensions, a higher frequency range (100 kHz – 20 MHz) is needed. To cope with this requirement, two measurement systems have been developed.

III. Developed measurement systems

A. System for cultures of cells in suspension

Figure 2 shows the block diagram of the high frequency version, intended for cultures of cells in suspension, like bacteria and yeast. It includes a two channels direct digital synthetiser which provides the current injection frequency and a four times higher reference oscillator frequency to allow performing the 0º and 90º coherent demodulation. The demodulator has four channels to provide both components of the measured voltage and current signals. The four output filters have a passive-active cascade structure. A wideband front-end which includes a current source, a buffered differential amplifier and a transimpedance amplifier to measure the injected current is built in a separate small board to allow placing it close to the electrodes to reduce the system input capacitances

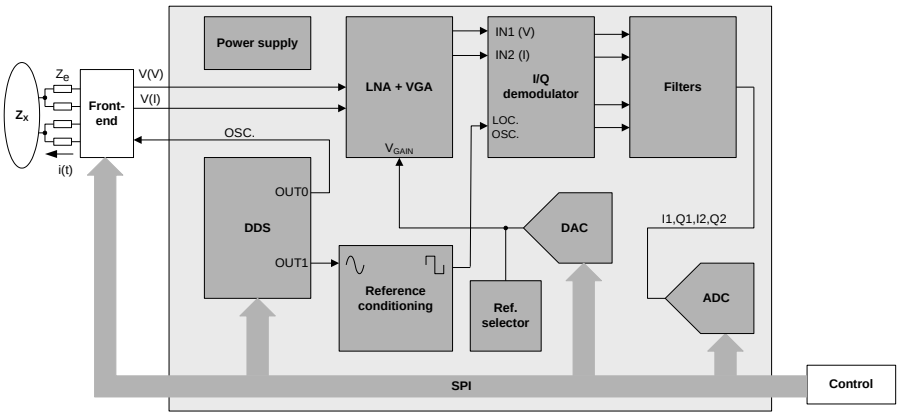

Figure 2. Block diagram of the 100 kHz – 20 MHz impedance spectrometer

Figure 3 shows the 4-layer manufactured board which is the core of the high frequency impedance spectrometer system. The communication with an external controller is performed by the SPI serial bus. The higher frequency capability for yeast and bacteria suspensions is featured by a system based on the AD8333 I/Q dual-channel demodulator from Analog Devices which includes two demodulation channels in-phase and quadrature and the references (0º and 90º) circuit generation through a unique clock frequency of four times the excitation signal frequency. The impedance excitation signal (fo) and the local oscillator signal (4fo) are generated by a two-channel DDS. These two signals have the same reference clock so they´re inherently synchronized. The current and the voltage developed across the unknown impedance are conditioned before the coherent demodulation using a low noise amplifier with programmable gain. The local oscillator signal (4fo) presented by the DDS is conditioned to square signal before drive the LO demodulator input using a low phase noise clock distribution IC for improving low frequency response. The in-phase and quadrature outputs for voltage and current of the demodulator are low-pass filtered after be presented to the ADC converter. The acquisition can be performed internally using the on-board ADC or externally given that the filter outputs are provided to the board connector.

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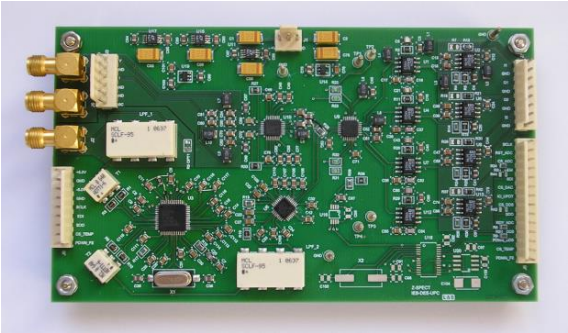

Figure 3. Yeast and bacteria suspension impedance measurement system

B. System for adherent animal cells

The low frequency system, which is much simpler and has a lower cost, is based on the system-on-chip AD5933 from Analog Devices, which already includes the DDS and a digital demodulator. Figure 4 shows its block diagram, together with the external front-end simplified schematic. The on-chip DDS allows selecting the frequency sweep and the excitation amplitude for the impedance characterization, both programmed by the user. The magnitude and the phase angle of the impedance Z(ω) are calculated through the real (R) and imaginary (I) components returned by the DFT at each frequency which can be read from de I2C serial interface.

The internal transimpedance amplifier is intended to measure the current over Z(ω) with a transimpedance given by the external feedback resistor RFB and the internal gain programmed by the user. Its output voltage is low-pass filtered and presented to the ADC whose digital outputs are used to perform the DFT. The system needs a previous calibration performed with a known impedance for the gain factor calculation.

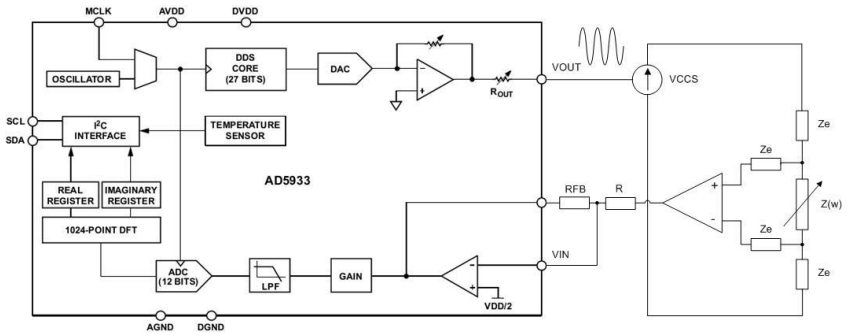

Figure 4. Block diagram of the 1 kHz – 200 kHz impedance spectrometer

The original AD5933 has a 2-wire measuring structure. It applies a voltage to one end of the unknown impedance and measures the resulting current by driving it to the internal transimpedance amplifier. To adapt this structure to a 4-wire scheme, we have used the AD5933 output voltage to control a floating current source which applies a constant-amplitude current to the injecting electrodes. The resulting differential voltage is detected in two additional electrodes, then converted to a unipolar voltage through a differential amplifier and then converted to current through a resistor to allow measuring it with the AD5933 internal transimpedance amplifier. This way, the ratio between applied voltage and measured current at the injected frequencies which is determined using digital demodulation, has admittance dimensions instead of impedance. The AD5933 operates with an unipolar power supply and all signals have a Vcc/2 DC voltage shift. This shift cannot be transmitted to the electrodes, thus, additional circuits to block and restore the DC shift have been designed.

C. Electrode sets

The electrical connection between the embedded electrodes and the electronic hardware is performed using spring probes. Several electrode configurations have been manufactured in order to validate the simulations performed to determine the best placement of the electrodes.

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IV. System characterization and preliminary results

The high frequency board has been characterized including accuracy, linearity error and temperature drifts. Also their subsystems have been characterized. One of the key parts is the set of four low-pass filters placed at the current-mode outputs of the demodulator. The active filter at 100 Hz based on a high open loop gain opamp (AD8021) was not able to keep a monotonously decreasing frequency response above 30 kHz. A previous stage with a low-pass cut-off frequency at 90 kHz based on a fast opamp (AD8065) allowed us to obtain a rejection below -90 dB up to 10 MHz (figure 5). A HP4192A in frequency-response analyser mode has been used to obtain this measurement.

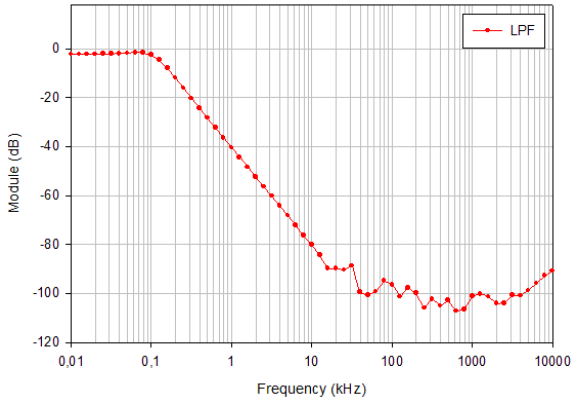

Figure 5. Low-pass output filters frequency response

Figure 6 displays the uncalibrated results obtained when measuring a simulated growth obtained by leaving settle a yeast culture using a discrete set of four electrodes placed at the bottom of a Monoscreen-Fedbatch minibioreactor along 3 and 1/2 hours. As it can be seen, the main impedance relaxation mechanism can be observed around 1-2 MHz.

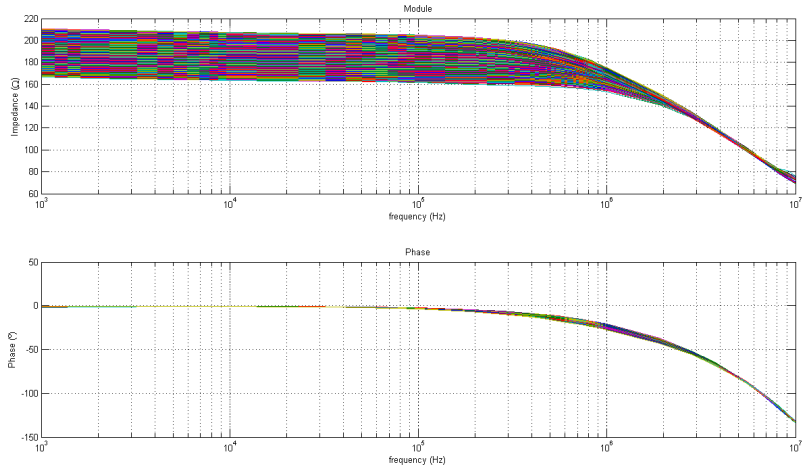

Figure 6. Impedance of a yeast sedimentation using electrodes placed at the bioreactor bottom

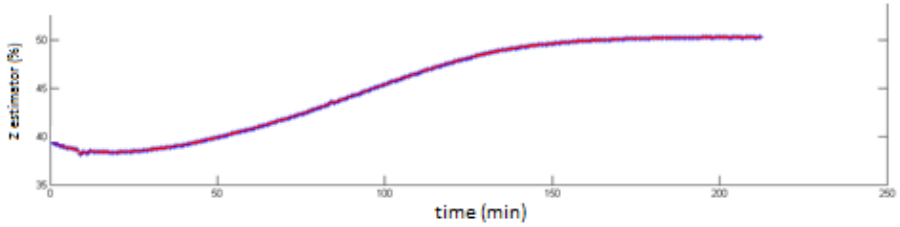

Figure 7. Impedance magnitude relative variation below and above the relaxation as biomass growth estimator

V. Conclusions

Two EIS measurement systems suitable for two kinds of cell cultures have been designed and built. A low frequency system (1 kHz – 200 kHz) will allow measuring adherent animal cell cultures at low cost while a higher frequency system (100 kHz – 20 MHz) would allow measuring bacteria and yeast cell cultures in suspension. Preliminary measurements using the settlement of a yeast cell culture show the feasibility of applying this solution in single use bioreactors.

Acknowledgements

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