*"Learn from yesterday, live for today, hope for tomorrow. The important thing is to not stop questioning"*
Albert Einstein



Texas Instruments European Analog Design Contest 2013 Project Report

# Cell-Culture Real-Time Monitoring System

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## TI Part Number & URL

**3 OPA4227 ,1 BQ24005, 1 LM2611, 1 TLV3501, 1 MPC507, 1 MSP430F5659, 1 LM2841, 1 LMH6503, 1 TPS7133**
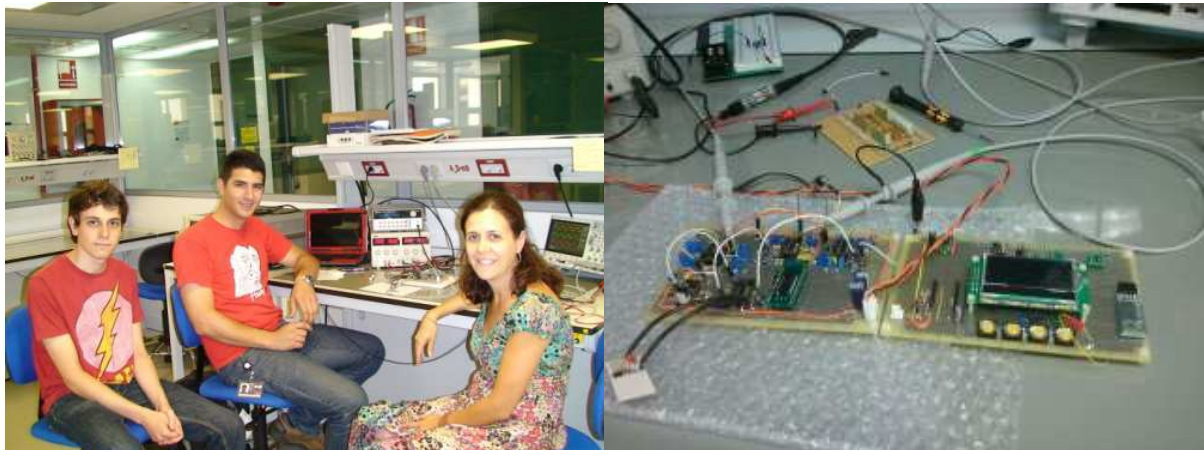
**Project abstract:** In this work, a way to cell-culture real-time monitoring system by means of the Oscillation-Based Test (OBT) methodology [1]is proposed and implemented with Texas Instrument (TI) components. The idea is inspired in previous works from the authors [1] in the area of testing analogue integrated circuits and deals with solving some critical points in this kind of biological measurements. The use of the cell-microelectrode model proposed in [2]confirms the feasibility of the approach. A simple topology based on a non-linear element embedded in a feedback loop is employed for converting the Cell-Culture Under Test (CCUT) into a suitable "biological" oscillator. Then, the oscillator parameters (frequency, amplitude, phase, etc…) are used as empirical markers to carry out an appropriate interpretation in terms of cell size identification, cell counting, cell growth, growth rhythm, etc. We use the Describing Function (DF) approach for the involved mathematical calculations required to analyse the "biological circuit", thus predicting the frequency and amplitude of the oscillations. The precise values of oscillation parameters are closely related to the cell-electrode area overlap in the cell-culture. In order to establish the accuracy of these theoretical predictions, the oscillators have been previously implemented and validated by simulations and, finally, empirical results have been achieved from an experimental demonstrator employing TI modules and elements.

***Keywords—***Cancer cell-culture proliferation monitoring, impedance sensing; signal conditioning circuits; microelectrode; Oscilation Based Test (OBT).

## 1. INTRODUCTION AND MOTIVATION

Characterizing in detail the number of cells in a culture at a specific time as well as measuring the cell proliferation rate have deep implications in biomedicine, both at a technical and at a biological level. Indeed, the density of cells in the plate will affect the success of many different techniques, including transfection, infection, reaction to many chemicals, etc. Cells distributed too sparsely will have problems growing and will react strangely to all the aforementioned treatments.

More important from a biological point of view, cell proliferation is a potent tool to define a cell. Excessive proliferation is the hallmark of cancer cells, and any physical or chemical treatments that slow down cell growth may lead to potential oncogenic therapies. Lack of proliferation normally represents excessive cell death, either due to extrinsic or intrinsic mechanisms, or the activation of cellular aging pathways (cellular senescence). Thus, understanding the processes that slow down or accelerate the proliferation of a culture is a potent research tool.

However, despite its importance, biologists cannot monitor cell proliferation in detail. They face two important technical problems. First, protocols to estimate the number of cells in a plate are heavily invasive, and profoundly affect the biology of the cells. In most cases the cells cannot longer be used for subsequent experiments. Secondly, scientists can only take snapshots of the culture at specific points. Thus, for most researchers using mammalian cells the only alternative is to set up parallel experiments and reconstitute the progression of a culture using them. In many cases this will be retrospective analysis, and understanding how cells have behaved can only be done at the end of the experiment.

For all those reasons, *any new technical approach that facilitate monitoring cell progression, especially if promising real-time on-line monitoring, will have an immediate impact in the biological sciences*; more in particular when focusing on reducing the negative effects over the cells and allowing continuous measuring of the number of cells on a plate. Then, it should be worthwhile to develop measurement techniques allowing researchers to monitor the evolution of their experiments on-line, yet requiring a simple set-up.

This work aims to provide living cell essays with a simple on-line measurement facility. The core idea is the proposal of a strategy for the conversion of the Cell-Culture Under Test, CCUT into an oscillator, whose characteristic parameters (frequency, amplitude, phase, etc…) are related to the cell-culture evolution and can be easily determined. In this preliminary approach, we use a simple second-order band-pass filter followed by the bio-impedance of the cell culture and a simple comparator to establish the oscillations. The oscillating conditions are theoretically analysed using the Describing-Function (DF) method and initially validated through simulations. Both simulation and experimental results are presented corresponding to a prototype built using TI components.

## 2. THEORETICAL BACKGROUND

Before starting any circuit description, some fundamental issues regarding the operation method as well as the theoretical basis of the proposed approach have to be clarified.
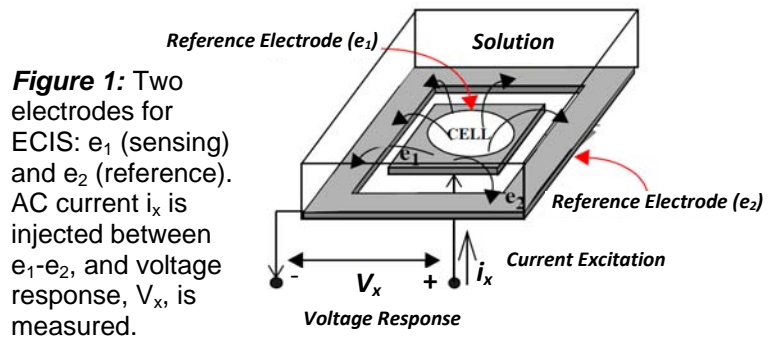
### 2.1 Impedance Spectroscopy Principles

Electrical Cell-substrate Impedance Spectroscopy (ECIS) was described by Giaever and Keese in 1986 [3] as an interesting alternative for experimentation in Cell Culture assays, allowing researchers to explore the dynamical aspects of cell behaviour. Many other works have considered the potential advantages for cell

culture real-time monitoring [4, 5], although the success in reducing economic cost and human effort has been limited.

Impedance Spectroscopy (IS) measurements require a two-electrode setup, and an AC signal source for excitation (normally a current), $i_x$ at several frequencies (see Figure 1). From system voltage response, $V_x$, the impedance (magnitude and phase) of the two-electrode plus cell culture system at a given frequency is calculated. Impedance changes are due to cell adhesion to the bottom surface, where cell culture is growing, and consequently, increase with the number of cells attached to the bottom. The measured impedance depends on the electrode technology and geometry, the electrical cell-electrode model [2, 6-7] and the working frequency. Electrical measurements must consider the electrical model of the entire system to extract the useful information.

Circuit approaches to perform impedance measurements have been proposed elsewhere [3, 5, 8], considering the need of providing an excitation (normally current) at a given frequency and measuring the obtained response.



**Figure 1:** Two electrodes for ECIS: $e_1$ (sensing) and $e_2$ (reference). AC current $i_x$ is injected between $e_1$-$e_2$, and voltage response, $V_x$, is measured.

However, those circuits available in the literature cannot be extended to provide real-time measurements due to the external components they require, especially the input source and its control circuitry. Before explaining our proposal, the model for the underlying bio-electronic measurement system has to be detailed.

## 2.2 Cell-Electrode Model

The impedance under test of a two-electrode system in Figure 2 (a) has been already estimated [2, 6-7]. Particularly, in this work, it has been considered a squared gold microelectrode of 50 $\mu$m side, which can be total or partially covered by cells in the culture. The fill factor (*ff*) parameter represents the amount of the electrode´s area (A) covered by cells (Ac). Our objective is, using the electrical model for this system in Figure 2 (b), to obtain the cell-to-electrode area overlap (Ac) from measurements performed with the proposed circuitry.

The magnitude and phase response for $Z_{cell-electrode}(s)$ can be obtained from the set-up in Figure 2. The $R_{in}$ resistor maintains the current flowing across the cell-electrode in adequate signal levels (1-20$\mu$A for cell protection, and 10-50mV for electrode modelling constrains [7]).

## 2.3. The proposed method: using Oscillation-Based Test (OBT)

Our proposal is based on converting the "biological circuit" (formed by the cell culture, the electrodes and a few added electronic components) into an oscillator. Since a modification in the biological part (for instance a change in the number of cells) will produce changes in either the frequency or the amplitude (or both) of oscillations, that alteration will become observable.

To apply OBT to $Z_{cell-electrode}$ measurements, the CCUT (from now on we will use this for Cell-Culture Under Test) will be transformed into a robust oscillator, adding some extra components. In order to force oscillations, a positive feedback loop has to be implemented. From the point of view of OBT application, it is particularly important to accurately predict the parameters of oscillation (frequency and amplitude),

analytically or by means of simulations [1, 9-10]. It is also necessary to avoid the dependence of these parameters on the saturation characteristics of the active elements, like occurs in common oscillators. A solution to this problem is to use a non-linear element (a simple comparator) closing a feedback loop to guarantee self-maintaining oscillations [1, 9-10]. This non-linear element also provides a precise control of the oscillation amplitude. On the other hand, we need to guarantee that this system complies with a (relatively) simple set of oscillation conditions, which can give information on the oscillation parameters. A simple way to get this is to use a band-pass filter in the loop, as proposed in the general circuit block in Figure 3.

**Figure 2:** Bio-electronic measurement system. (a) Electrical model of an electrode. (b) Electrical model of an electrode partially covered by cells (ff). (c) Circuit for cell-electrode impedance testing. Values for a 50x50 $\mu m^2$ electrode size are: $R_s$=5.4 k$\Omega$, $Z(\omega)$ = $C_l \| R_p$, with $C_l$= 0.37nF and $R_p$= 25M$\Omega$. $R_{gap}$=75k$\Omega$.
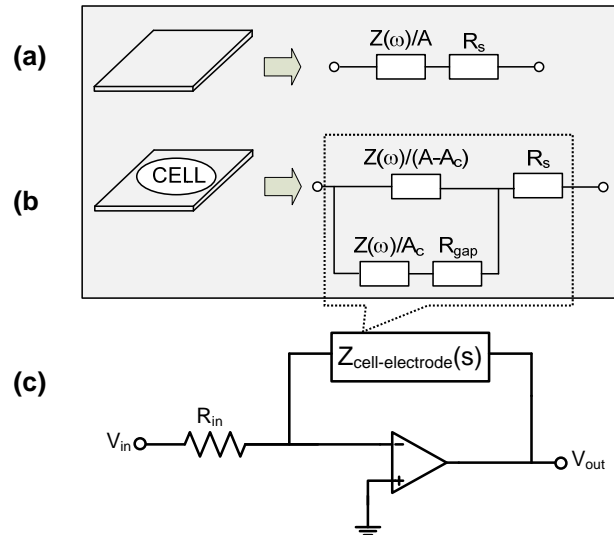


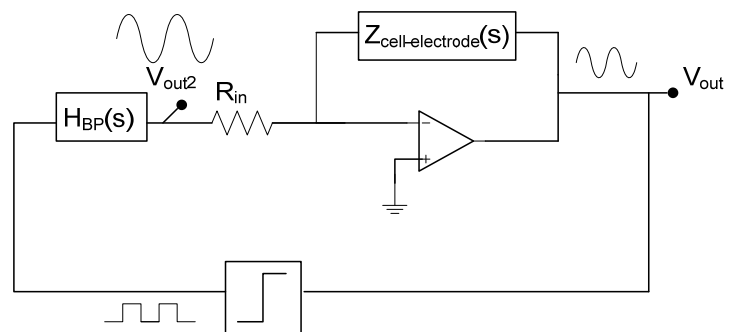For the sake of simplicity, let us now set up the case of a second-order band-pass filter and a comparator with saturation levels ±Vref. This closed-loop system verifies the required premises: the system is autonomous, the nonlinearity is separable and frequency-independent, and the linear transfer function contains enough low-pass filtering to neglect the higher harmonics at the comparator output. Furthermore, the equations ruling the oscillation conditions can be handled easily.

Choosing adequately the band-pass filter, the closed-loop system of Figure 4 can be forced to oscillate and its first-order Describing-Function equation [1], N(a)+1/H(s)=0, has an oscillatory solution ($\omega_{osc}$, $a_{osc}$), being $\omega_{osc}$ the oscillation frequency and $a_{osc}$ the oscillation amplitude. The DF function will be in this case N(a), while H(s) is the transfer function of the open-loop system.

**Figure 3:** Block diagram of OBT implementation measurement set-up.



With the aim to validate these results, the proposed biological oscillator was firstly implemented in Simulink. A comparison between theoretical predictions and simulation results led us to confirm that both are very similar. It can be seen that the adopted method predicts the oscillation parameters with enough accuracy for this application. As an example, the obtained waveforms are shown in Figure 5 for the case *ff*=0.9. The oscillation frequency obtained is 7.93 kHz, and the amplitude in

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$V_{out2}$, 447.60mV. It is represented also the frequency spectrum for both proposed output voltages, showing an excellent linear performance. Table 1 summarizes the oscillation parameters measured for 0.1, 0.5 and 0.9 filled factors, in agreement with it was derived from theory.
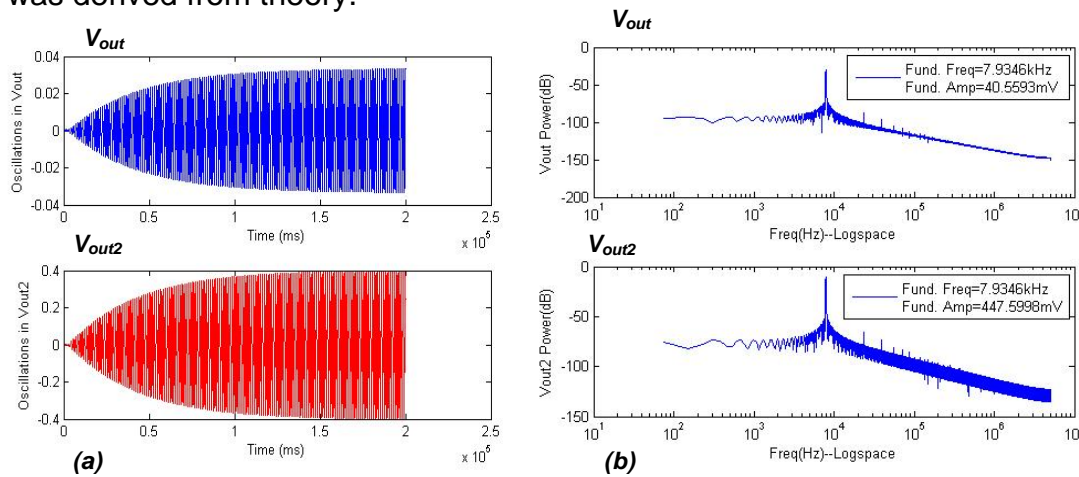


**Figure 4:** (a) $V_{out}$ and $V_{out2}$ oscillation waveforms for ff = 0.9. (b) $V_{out}$ and $V_{out2}$ frequency spectrum.

| ff | $f_{osc}$ [kHz] | $V_{out}$ [mV] | $V_{out2}$ [mV] |
|-----|------|-------|--------|
| 0.1 | 7.55 | 3.27 | 57.02 |
| 0.5 | 7.78 | 9.48 | 155.04 |
| 0.9 | 7.93 | 40.56 | 447.60 |

**Table 1:** Simulated oscillation parameters: frequencies and amplitudes.

The reasons making attractive this approach are: its concept is very simple, it avoids the need of expensive and complex equipment for stimuli generation (in fact, no external inputs are needed), and the measurements to perform are relatively simple [1] and it can be carried out in a very short time[1]. These features open the door to extend the concept for real-time monitoring.

## 3. ACTUAL  OBT IMPLEMENTATION USING TI COMPONENTS

### 3.1. Complete System:
The core system (oscillator for measuring) schematic was previously shown in Figure 3. As was said, it is composed of three main blocks: a band pass filter (BP), the bio-impedance block and a comparator. Providing a start-up mechanism would be also needed.

### 3.2. Band-Pass Filter:
A comprehensive theoretical study of our "biological" oscillator (when the particular case of a squared gold microelectrode of 50 $\mu$m side is considered, which can be totally or partially covered by cells in the culture) reveals that the optimum frequency for the band pass filter in order to achieve a successful OBT approach is around 8kHz (see previous section). However, due to the nature of different kinds of electrodes and different cells, a tunable filter has been implemented. A state-variable filter has been chosen due to the fact that resonance frequency $\omega_0$ and quality factor Q can be adjusted independently in this topology. Figure 5 shows the complete filter design implementation (using OPA4227).
With the help of a MATLAB script the possible values of resistance can be obtained

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[1] Reference [1] shows how just a counter can be used to carry out the necessary measurements.

and therefore the values of $\omega_0$ (once selected the values of $R_m$ and C). These values have been chosen to obtain a frequency range from 6.5kHz to 13kHz and almost linear variation on the full range. This leads to $R_m=10k\Omega$ and C=1.2nF.
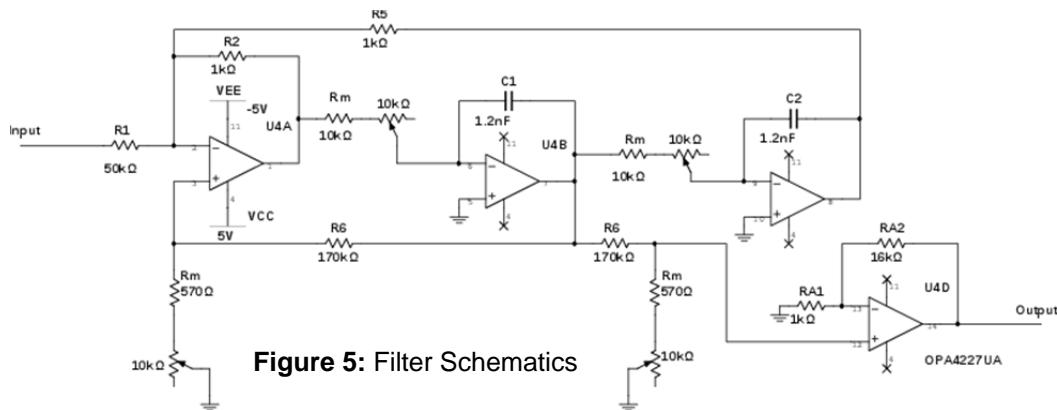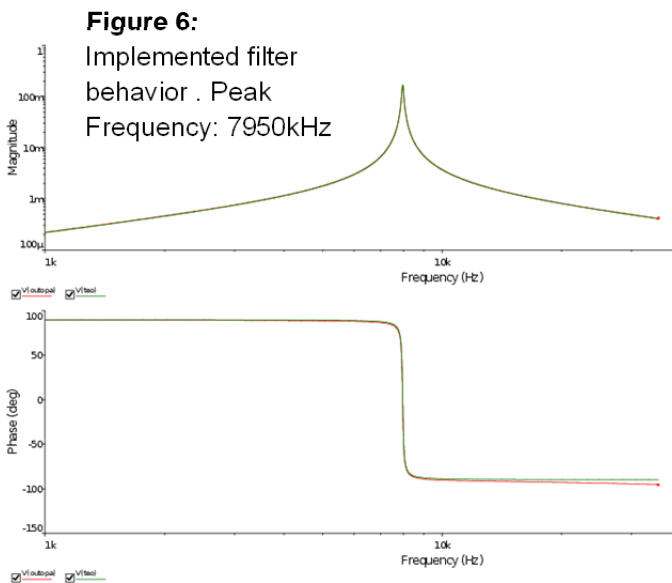


**Figure 5:** Filter Schematics



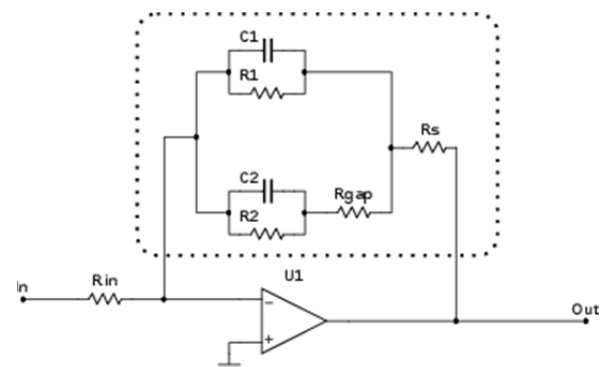**Figure 6:** Implemented filter behavior . Peak Frequency: 7950kHz

The precise behaviour of the filter has been tested using MULTISIM and TINA simulations. Figure 6 shows an evaluation between the theoretical (green) and the implemented (red) filter for the selected operation frequency.

### 3.3. Bio-impedance Emulator

With the help of the proposed bioimpedance model (see Figure 2), a simulator has been built for testing purposes. This circuit allows us to test the complete system and check that voltage and current applied to the cultures are within the specifications ($\mu$V; pA). The schematic proposed is shown in Figure 7 inside the dotted box.

The resistor $R_{in}$ and the Opamp (OPA4227) are part of the excitation circuit. The values of $R_{gap}$ and $R_s$ are fixed by the employed cell-electrode, the values of $C_1$, $C_2$, $R_1$ and $R_2$ change with the occupation level.

The connections to bio-impedance have been multiplexed to measure up to 8 electrodes at the same time.

**Figure 7:** Bioimpedance simulator



### 3.4. Comparator

Figure 8 shows the comparator used in this work. It is composed of three blocks, the comparator itself, a high pass filter and a non-inverting amplifier. The output signal from the bioimpedance is first filtered to reduce low frequency noise (mainly 50Hz noise) with a High-pass Sallen-Key filter tuned at 1kHz and variable Q to allow for a

precise selection of the phase shift at Band-pass filter resonance frequency. Then, the signal is amplified by a non-inverting x20 amplifier and passes to the comparator where thanks to the 6mV of hysteresis of the TLV3501 noise rejection is achieved. Also, its fast response, guarantees low phase shift. Moreover, thanks to its *enable* input we can maintain the system in standby while changing the bio-impedance or while the system is in normal mode.
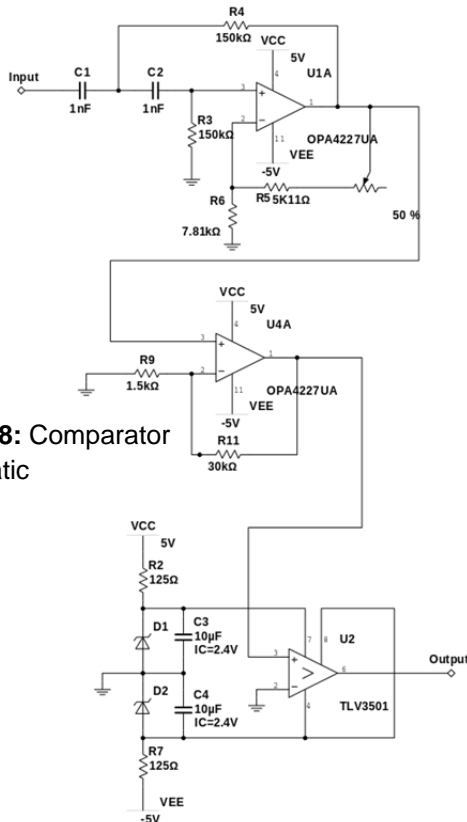
**Figure 8:** Comparator schematic

### 3.5. Automatic Gain Controller

This element was not included in the preliminary system but added later because of its advantages guaranteeing constant output voltage for different cell occupations. The AGC (OPA4227 and LMH6503) allows us to use higher voltage level through the bioimpedance at low occupation level and lower voltage level at higher occupation. This makes the system less sensitive to noise and solves problems that we may face with signal around the tens of millivolts obtained with lower level of occupations. With the use of the AGC the information about the occupation level resides in the voltage applied to the variable gain amplifier. The AGC system is composed of three blocks: a

**Figure 9:** AGC schematic.

variable gain amplifier, a precision inverting rectifier and an inverting amplifier (Figure 9).

### 3.6. Digital Part

The digital part involves a MSP430F5659 micro-controller responsible for activating the bio-oscillator, choosing the multiplexed output

and interacting with users. Its 12bits ADC provides enough accuracy to measure the AGC gain whereas the timer is used to measure the oscillation frequency. After that, the MCU calculates the fill-factor and send it to a Bluetooth module which can be connected to any computer or mobile phone via Bluetooth to display information about the fill-factor, temperature, humidity, time... Configuring the bio-oscillator is also possible from Bluetooth devices. A LCD display is also provided to show the information in case of no Bluetooth device available. A buzzer and an USB port complete the interface and connectivity of our system.
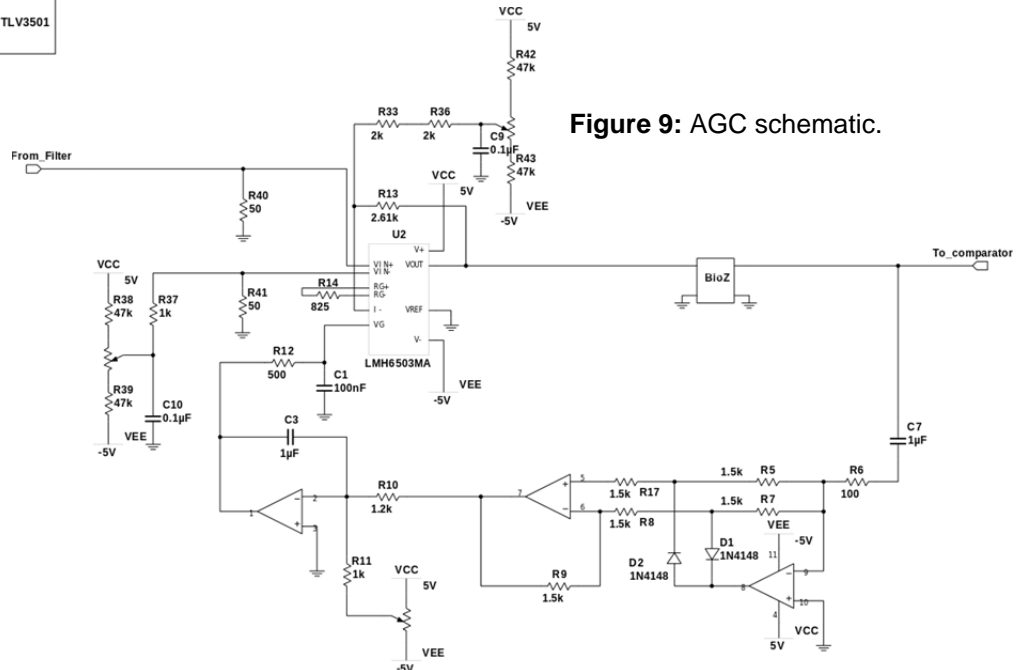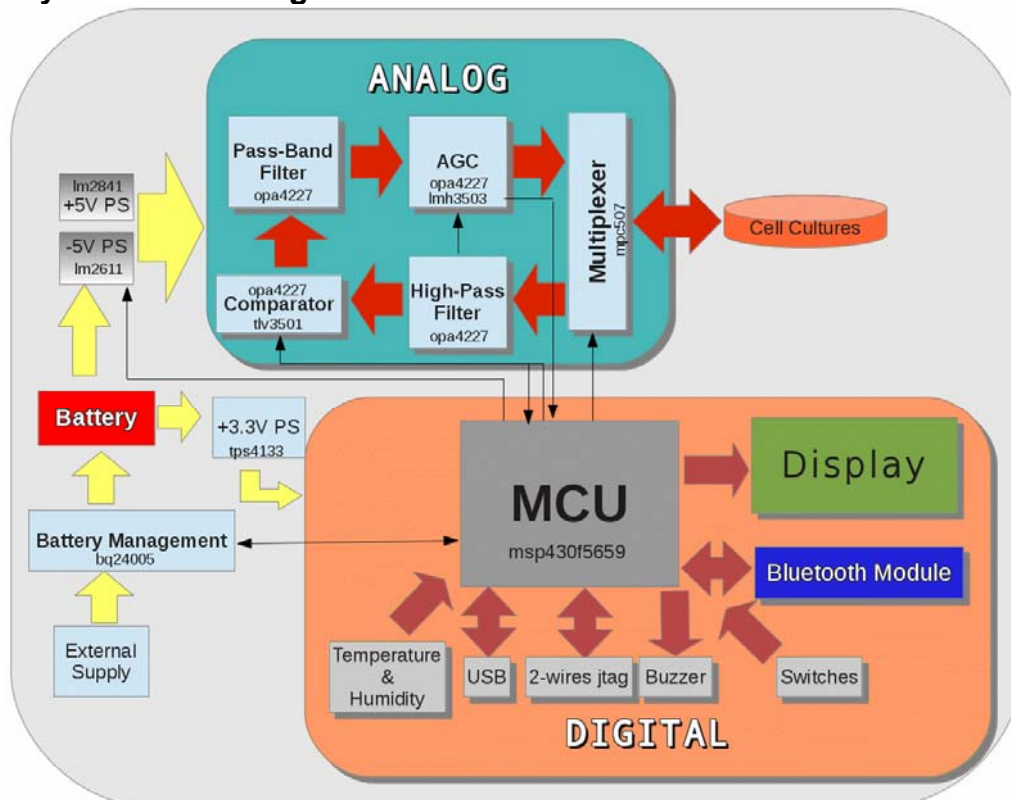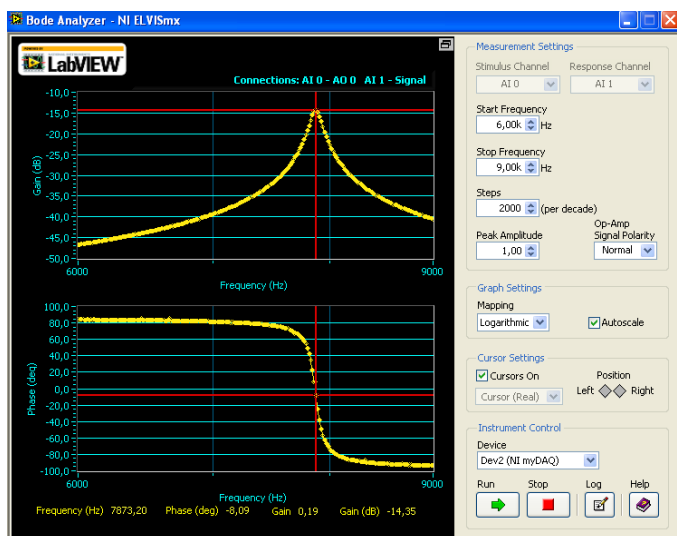
## 4. Full-System Block-Diagram



## 5. Full-System experimental results

In order to prove the experimental circuit, empirical data corresponding to the whole system are displayed.



**Figure 10:** Band-pass filter experimental data accomplished with NI MyDAQ. The peak frequency is just where was designed by simulation (7873,20Hz)

Figures 11, 12 and 13 show the oscillation results achieved for occupation levels of 10%, 50% and 100%, respectively.

On the other hand, Figure 14 shows the AGC experimental performance. The oscillation signal at the bioimpedance input (signals in red) is always maintained at 60mV while the filter output (in yellow) changes according to the cell occupation level. The closed-loop feedback system automatically changes the reference voltage in the comparator input (signals in green) and this value gives the information about the cell-culture occupation level which is shown by the digital display.
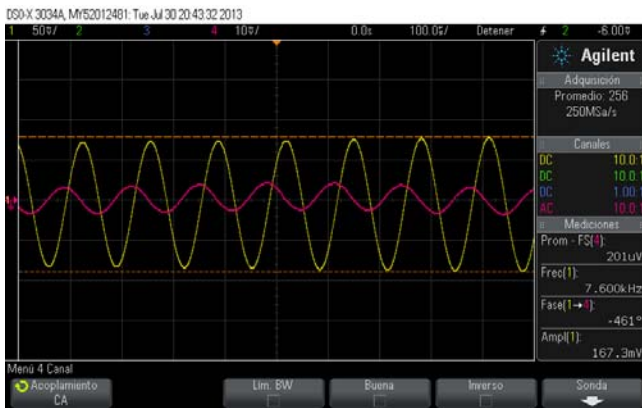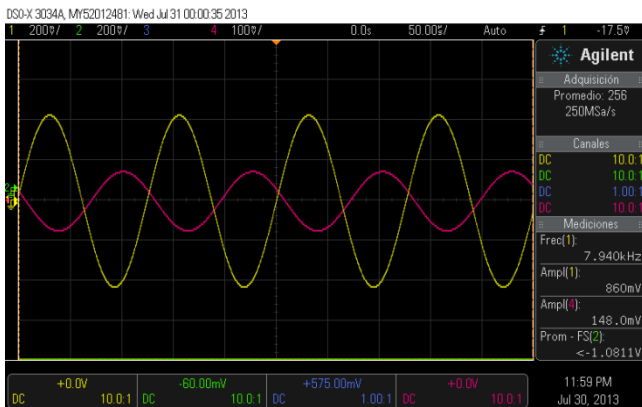
**Figure 11:** Experimental oscillations (10% occupation level, in yellow: $V_{out2}$ amplitude:167mV, frequency: 7.600kHz, very similar to the theoretical data (see *Table 1*)).
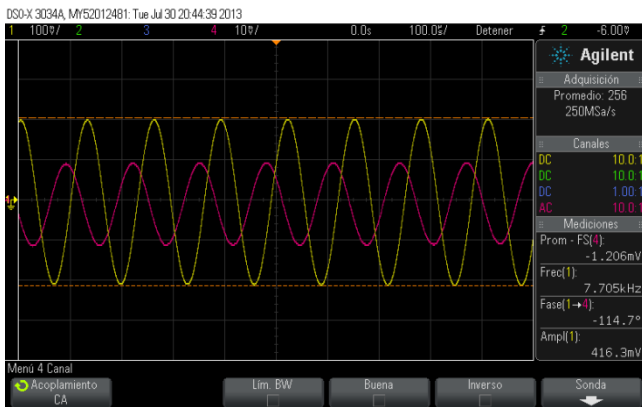


**Figure 12:** Experimental oscillations (50% occupation level, in yellow: $V_{out2}$ amplitude:416.3mV, frequency: 7.705kHz, very similar to the theoretical data (see *Table 1*)).



**Figure 13:** Experimental oscillations (100% occupation level, in yellow: $V_{out2}$ amplitude:860mV, frequency: 7.940kHz, very similar to the theoretical data (see *Table 1*)).



**Figure 14:** Experimental oscillations with the AGC. (a) 100% occupation level. (b) 10% occupation level.

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Albert Einstein

## 5. CONCLUSIONS:

This project shows that low-cost, real-time monitoring of biological cell growth is feasible. *A straightforward idea has been presented with a great potential in the fields of medicine and biology*. OBT has been broadly explored by our research group in the context of testing analogue and mixed-signal integrated circuits, but this work is the first attempt to extend the concept to biological measurements, what seems very promising indeed.

## 6. FUTURE WORK:

Future work will focus on applying this proposed OBT technique in vivo in a lab at the Andalusian Center for Molecular Biology and Regenerative Medicine (CABIMER), a research institute next door to ours, in order to validate it in a real scenario. Some of the CABIMER researchers have shown their interest and offered their facilities to include our system into their actual experiments.

## 7. ACKNOWLEDGEMENTS:

## References:

[1] G. Huertas et al. Oscillation-Based Test in Mixed-Signal Circuits (Frontiers in Electronic Testing). Springer. 2006.

[2] P. Daza, A. Olmo, D. Cañete, and A. Yúfera. Monitoring Living Cell Assays with Bio-Impedance Sensors. Sensors and Actuators B: Chemical. Elsevier, pp: 605-610: vol.176. January. 2013.

[3] I. Giaever and C. R. Keese, "Use of Electric Fields to Monitor the Dynamical Aspect of Cell Behaviour in Tissue Cultures," IEEE Trans. on Biomedical Engineering, vol. BME-33, nº 2, pp: 242-247. 1986.

[4] A. Manickam, A. Chevalier, M. McDermott, A. D. Ellington, and A. Hassibi, "A CMOS Electrochemical Impedance Spectroscopy (EIS) Biosensor Array," IEEE Transactions on Biomedical Circuits and Systems, vol 4, nº 6. pp: 379-390. 2010.

[5] A. Yúfera and A. Rueda. Design of a CMOS closed-loop system with applications to bio-impedance measurements. Microelectronics Journal. Elsevier. vol. 41, pp: 231-239. Apr. 2010.

[6] X. Huang et al., Simulation of Microelectrode Impedance Changes Due to Cell Growth. IEEE Sensors J., vol.4, nº 5, pp: 576-583. 2004.

[7] D. A. Borkholder. Cell-Based Biosensors Using Microelec-trodes. PhD Thesis, Stanford University. 1998.

[8] A. Yúfera et al., A Tissue Impedance Measurement Chip for Myocardial Ischemia Detection. IEEE Transaction on Circuits and Systems: Part I. vol.52, nº 12 pp: 2620-2628. 2005.

[9] J. Gibson. Nonlinear Automatic Control. McGraw-Hill. Japan, 1963.

[10] P. Fleischer, A. Ganesan and K. Laker, A Switched Capaci-tor Oscillator with Precision Amplitude Control and Guar-anteed Start-Up, IEEE Journal of Solid-State Circuits, Vol. SC-20, No. 2, April 1985.