Bioelectric Signal Measuring System

A Guadarrama-Santana[1], L Pólo-Parada[2], A García-Valenzuela[1]

[1] Centro de Ciencias Aplicadas y Desarrollo Tecnológico
Universidad Nacional Autónoma de México
Ciudad Universitaria, Apartado Postal 70-186, Distrito Federal, 04510, México
[2] Department of Medical Pharmacology and Physiology, University of Missouri
John M. Dalton Cardiovascular Research Center
134 Research Park Drive Columbia, Missouri 65211, USA

E-mail: asur.guadarrama@ccadet.unam.mx

Abstract.
We describe a low noise measuring system based on interdigitated electrodes for sensing bioelectrical signals. The system registers differential voltage measurements in order of microvolts. The base noise during measurements was in nanovolts and thus, the sensing signals presented a very good signal to noise ratio. An excitation voltage of $1V_{rms}$ with 10KHz frequency was applied to an interdigitated capacitive sensor without a material under test and to a mirror device simultaneously. The output signals of both devices was then subtracted in order to obtain an initial reference value near cero volts and reduce parasitic capacitances due to the electronics, wiring and system hardware as well. The response of the measuring system was characterized by monitoring temporal bioelectrical signals in real time of biological materials such as embryo chicken heart cells and bovine suprarenal gland cells.

1. Introduction

Interdigitated Capacitance Sensors (IDCS) have been used in different fields of industry as well as in physical, chemical, biochemical and medical research. They offer practical advantages for materials characterization by means of measuring the electrical properties of a Material Under Test (MUT) such as impedance and capacitance due to the frequency dependent dielectric function. An important advantage of plane sensors is that only one surface of the MUT can be directly in contact with the electrodes allowing the sensing of the electric field perturbations on the MUT [1]. The dynamic range and sensitivity of the sensor can be improved depending on the number and dimensions of the electrodes that form the Interdigitated Capacitor (IDC), the electric field applied to it, the substrate and the MUT thickness principally. Finite Element Method is a very useful tool to obtain a good approximation of the physical parameters mentioned above [2]. These devices can sense temporal changes of the electric field in the MUT due to physical or chemical variables affecting biological samples. Temporal variations of the dielectric function of a MUT are manifested as perturbations to an electric field applied to it and can be sensed as temporal electric signals. This sensing principle makes it suitable for bio-sensor applications [3].

Bio-sensors are used to detect and analyze temporal cellular response to electrical or bio-chemical conditions. The resulting bio-signals can be sensed as voltage or current signals by means of impedance changes of the cell or biological tissue. The cell impedance technique is used to sense and monitor the behavior and growth of cell population when cells are in touch with conductive electrodes deposited on a plain substrate [4]. Measurements with many cells use superficial electrodes as sensors, the electric field generated by the action of many cells distributed in the neighbourhood of the electrode constitute the bioelectric signal. The electric field traverses the biological substance, so the electric potential difference can be sensed in suitable locations across the biological tissue, eliminating the need of invading the system. Bioelectrical signals require a relatively simple transducer for its registering, as the case of the electrocardiogram ECG, electroencephalogram EEG, electromyography EMG and others. With these techniques, information can be obtained only in a collective way to a given physiological condition on an electrode array pattern, usually plane or coplanar electrodes.

Patch-Clamp commonly used in Electro-Physiology is another measuring technique used to characterize a single cell response that cannot be made in a collective way [5]. Ion currents that flow through the ion channels of the cellular membrane due to physiological conditions are sensed with this technique. Patch-Clamp technique is suitable for studying a single cell response to a determined stimulus (electrical, optical, chemical or spontaneous) [6,7]. Other bioelectrical signals are generated by nerve and muscular cells. Their source is the membrane potential, which under certain conditions can be excited to generate it. Single cell measurements use specific microelectrodes as sensors and the same excited electric potential is the bioelectrical signal. With the measuring system proposed microelectrodes could be used as sensors, for electrophysiology local measuring techniques. Although, measuring work must be done in order to characterize the system response for a particular electrophysiology application.

IDCSs can detect small electric field perturbations present near its sensing surface due to small bioelectrical signals of some biological sample as in signal measuring techniques mentioned above. These electrical signals can be very small and be immersed in surrounding noise. In common situations, noise becomes a real problem to the point of impeding electric signal registering. Therefore, a low noise measuring system is required for bioelectric other sensing techniques in order to obtain a convenient SNR like those obtained with electrophysiology techniques [8].

The low noise measuring system proposed here is based on interdigitated capacitive sensors, a home- made electronic conditioning stage and a digital Lock-in amplifier. The measuring system allows to reduce noise and parasitic capacitances present in the system hardware and secondary glass substrates where the biological samples are placed. The measuring system detects electric field perturbations on the sensitive surface of the sensing device, the perturbations are registered as differential voltage measuring due to the impedance of the biological sample and its bioelectrical activity with a very suitable SNR with nanovolts base noise.

The response and sensitivity of the system were obtained characterizing several measurements of bioelectrical activity from different cell samples. The experiments proposed are related to the study of the release of neurotransmitters of adrenal medulla cells which can be determined with an independent bioelectrical measurement to capacitance changes in the cell that represent the fusion of the vesicles. Also, cardiac cells were used to study the evaluation of the possible changes in capacitance due to spontaneous contractions, which are reflected in the biosignal activity.

The results obtained indicate that it is possible to apply this measuring system in the electrophysiology field.

2. Low Noise Capacitive Measuring System

The low noise capacitive measuring system proposed is based on interdigitated capacitive sensors, an electronic conditioning stage, an analog to digital converter (ADC) and a digital Lock-in amplifier, figure 1.

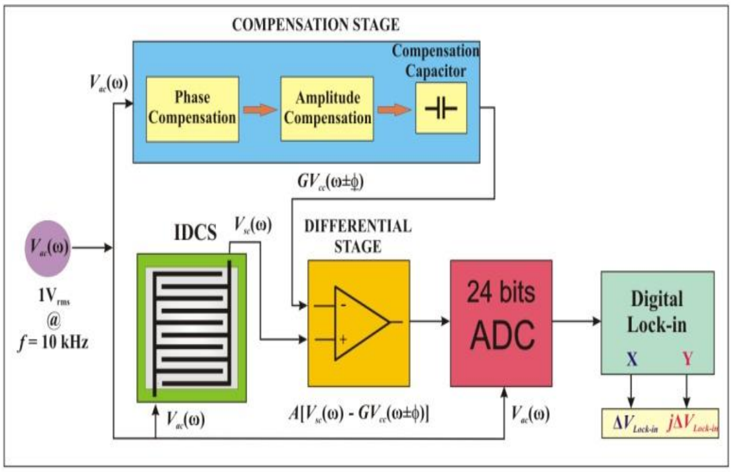


Figure 1. Block diagram of the Low Noise Capacitive Measuring System.

The sensing method proposed is based on phase changes of a sine excitation voltage $V_{ac}(\omega)$ due to electric field perturbations of biological samples placed over 1cm^2 sensitive area of the interdigitated capacitive sensor (IDCS). The excitation voltage $V_{ac}(\omega)$ with amplitude and frequency used in the sensing device were 1V$_{rms}$ and 10 kHz respectively resulting a voltage signal $V_{sc}(\omega)$. The same excitation voltage $V_{ac}(\omega)$ was applied simultaneously to a compensation capacitor (mirror device) as well.

The resulting $GV_{cc}(\omega\pm\varphi)$ signal from the mirror device was compensated in amplitude and phase in order to equalize it with $V_{sc}(\omega)$ obtaining an initial minimal reference voltage from a differential stage. The differential signal can be amplified up to a maximum gain factor of A=1x10^6 to obtain a suitable voltage for digitalizing it with the 24 bits ADC.
The sensitivity primary limit of the digital Lock-in is in its dynamic range, which in order is determined principally for the National Instruments 4431 ADC. In this case the dynamic range was 100 dB and 102.4kS/s sample rate. The differential output signal $A[GV_{cc}(\omega\pm\varphi) - V_{sc}(\omega)]$ is digitalized by the ADC with approximately 1μV minimum voltage level. This signal is processed by the digital Lock-in stage implemented with LabView to the reference frequency mentioned before.
The signal goes through phase detection and digital filter stages in order to obtain a very narrow width band around the reference central frequency to reduce surrounding noise to a minimum level.
The registered data are displayed in real time in graphs generated with the same software as a part of a virtual instrument. Besides, statistics data are obtained which are necessary to calculate the base noise of the system in the width band determined.
Data samples in study can also be stored to be analyzed in its real and complex resulting components. The reference base noise obtained with the system was in nanovolts range with ambient conditions.

3. Experimental

Experimental measurements were made with the system proposed in order to characterize its sensitivity and resolution response. When a MUT is placed on the sensitive surface of the capacitive sensor impedance and current are generated when a potential is applied across to it.
Thus, current and voltage across the MUT can be sensed. When an ac potential is applied to the MUT a dissipative current due to the resistive impedance and a displacement current due to the capacitive impedance are generated [9].
Performing an admittance analysis of the system the capacitance as a function of the displacement current across the material under test can be obtained. This would be useful for impedance characterization of biological samples, but it is out of the scope of this work.

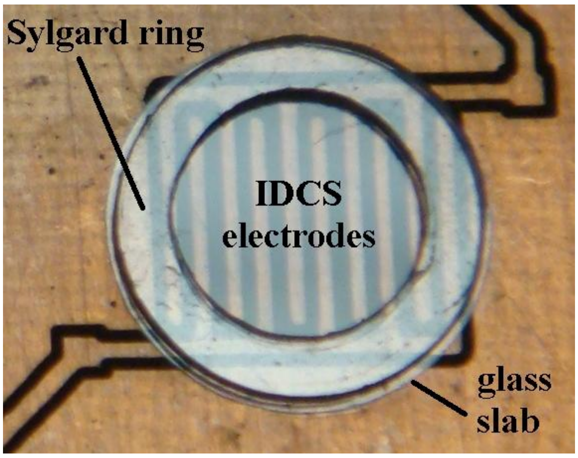
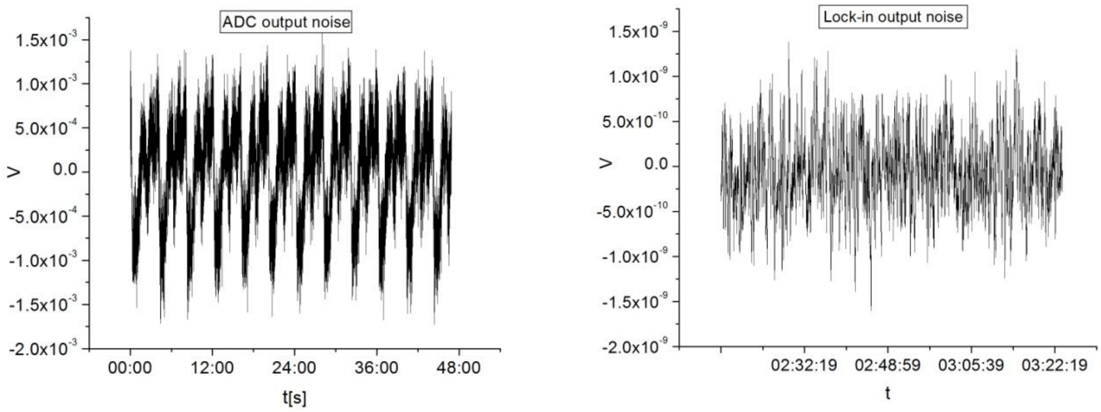


Figure 2. Small Petri dishes formed with circular glass slabs and Sylgard rings placed over $1cm^2$ sensitive surface of the Interdigitated Capacitive Sensor (IDCS).

Small Petri dishes were fabricated with 100 µm thick glass substrates and 1cm diameter placed over $1cm^2$ IDCS sensitive surface with 50µm wide and 50µm gaps between electrodes. The edges of the circular glass slabs were surrounded with Sylgard rings in which biological samples immersed in culture medium were deposited in order to sense its bioelectrical signals in diverse experiments, figure 2.

Before performing any measurements a minimal reference voltage was set in the electronic conditioning stage, a $3mV_{p-p}$ noise voltage was present at the 24 bit ADC output. After the digital Lock-in stage the noise output voltage was reduced to $3nV_{p-p}$. This value was the system base noise as shown in figure 3.

Experimentally, the real and imaginary components of the voltage signal were registered by the digital Lock-in stage.



(a) (b)

Figure 3. a) $3mV_{p-p}$ ADC output base noise, b) $3nV_{p-p}$ digital Lock-in output base noise.

The differential stage is based on an instrumentation amplifier that has an offset voltage of 50µV max. The sensing signal from the sensing device and a reference signal compensated in amplitude and phase enter the differential stage in order to get a minimum offset reference signal before place any biological sample, as can be seen in figure 3b.

We will refer to the sensitivity as the smallest signal change that the measuring system can detect. In this case the sensitivity is twice the Lock-in stage output base noise.

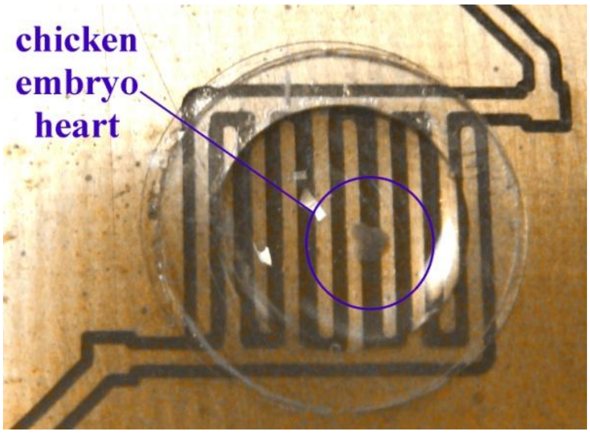


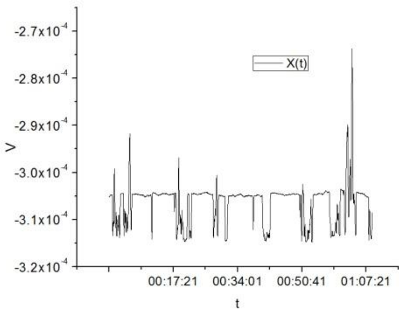
Figure 4. Chicken embryo heart immersed in 70µl culture medium over an Interdigitated Capacitive Sensor with 1cm^2 sensitive surface.

Different biological samples were monitored in order to register their bioelectrical signals with the measuring system proposed. First, a chicken embryo heart of around 100 microns in diameter immersed in 70 µl culture medium was placed in the Petri dish over the sensing device as it is shown in figure 4.

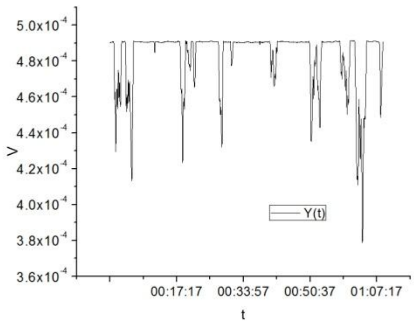
Chicken embryo heart cells and bovine suprarenal cells were also monitored following the same methodology.

4. Results

The measuring system proposed was able to register temporal bioelectrical signals of the chicken embryo heart, as it can be seen in figure 5. Electrical activity is represented as amplitude voltage variations over an offset around -0.305 mV for X(t) and 0.489 mV for Y(t) corresponding to the real and imaginary components of the signal respectively. The signals show certain periodicity and 6µV minimum variation showing SNR with respect 3nV$_{p-p}$ system base noise despite the offset presented in the graphs.



(a)



(b)

Figure 5. Signals registered from a chicken embryo heart, a) for X(t) and b) for Y(t).

The same measurement methodology was applied with chicken embryo heart cells of around 10-12 µm in diameter immersed in 50 µl culture medium, used as a second biological sample. Temporal bioelectrical signals registered are shown in figure 6. The graphs show an offset voltage around 5µV for X(t) and 3.6µV for Y(t) and maximum signal variation around 1µV showing a good signal to noise ratio with respect 3nV$_{p-p}$ system base noise.

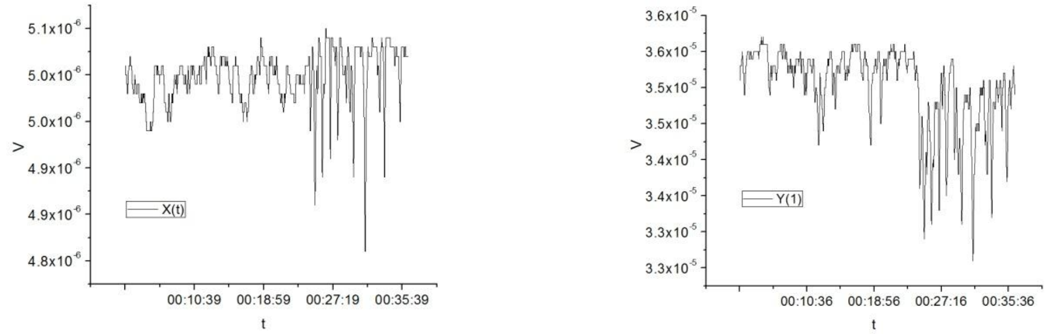
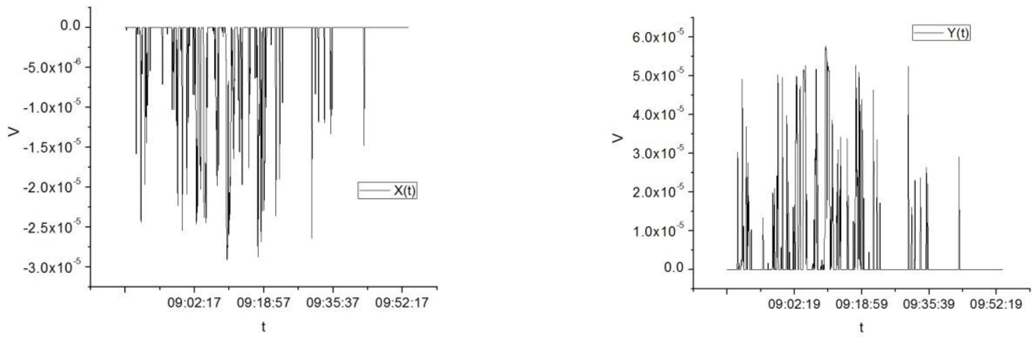


Figure. 6. Signals registered from a bunch of chicken embryo hart cells, a) for X(t) and b) for Y(t).
t is in a h:m:s format.

Finally, bovine suprarenal gland cells of about 14-16 µm in diameter immersed in 50 µl culture medium were used as a third biological sample following the same measurement methodology. The resulting bioelectrical signals registered are shown in figure 7.



(a) (b)

Fig. 7. Bioelectrical signals registered from a bunch of suprarenal gland cells, a) for X(t) and b) for Y(t). t is in a h:m:s format.

In this case the graphs do not show a significant offset voltage as in the cases above, this is the main difference between bioelectrical signals of chicken embryo hart cells and bovine suprarenal gland cells. Clearly it can be noticed the minimal offset voltage of the signal and the maximum signal variation registered, around -3µV for X(t) and 6µV for Y(t) showing a good SNR, with respect $3nV_{p-p}$ system base noise.

Graphs obtained showed bioelectrical signals from some nanovolts with SNR up to 100 as in the case of chicken embryo heart cells, figure 6a, and up to 10000 as in the cases of chicken embryo hart and bovine suprarenal gland cells, figures 5a and 7a respectively.

The offset shown in figures 5 and 6 for chicken heart embryo and chicken heart embryo cells could be due to the culture cell and the same biological samples as well. This is not the case with suprarenal gland cells where offset seems to be lower. In all cases bioelectrical activity is well defined with a good SNR.

5. Conclusions

The measurements obtained with the system proposed in this work are useful to estimate its sensitivity and response to different kind of bioelectric signals with no controlled ambient conditions. The measuring system presented very good stability with a base noise around 3nV peak-peak, this characteristic allowed us to register biosignals for cellular electrophysiology applications. The results obtained with the proposed measuring system show that capacitance sensors can be used for bioelectrical signal characterization from different kind of cells. The analog bioelectrical signals were electronically conditioned, digitalized and registered in real time in order to monitor biologic material for its analysis. Up to three samples can be monitored in real time with the measuring system due to the characteristics of the ADC. The measuring technique is practical because only it is necessary to change the small Petri dish with the biological sample of interest using the same capacitive sensor. Finally, this measuring system can offer additional information compared with established measuring techniques used in electrophysiology as the Patch Clamp technique [5]. One possible application for this measuring technique is to characterize the bioelectrical response of the cells to different drugs in order to determine the effectiveness of a particular treatment by comparing bioelectrical signals activity. Even though, more measurements must be done in order to characterize different bioelectric signals for different cells in determined conditions, the present work shows the potential of the measuring system proposed and IDCS for applications in electrophysiology research.

6. References

[1] Starzky F 2008 Parametrisation of interdigit comb capacitor for dielectric impedance spectroscopy, Archives of Materials Science and Engineering, Vol. **34**, Issue 1, 31-34.
[2] Hu X and Wuqiang 2010 Planar capacitive sensors – designs and applications, Sensor Review, **301** 24-39.
[3] Ibrahim M, Claudel J, Kourtiche D and Nadi M 2013 Geometric parameters optimization of planar interdigitated electrodes for bioimpedance spectroscopy, Journal of Electrical Bioimpedance, vol. **4** pp. 13-22.
[4] Berggren C, Bjarnason B and Johanson G 2001 Review Capacitive Biosensors, Electroanalysis, 13, No. **3** 173-180.
[5] Zhao Y, Inayat S, Dikin D A, Singer J H, Ruoff R S, and Troy J B 2009 Proc. IMechE Vol. **222** Part N: J Nanoengineering and Nanosystems.
[6] Asphahani P, Wang K, Thein M, Veiseh O, Young S, Xu J and Zhang M 2011 Phys Biol 015006 (11pp).
[7] Shapiro M G, Homma K, Villarreal S, Richter C P and Bezanilla F 2012 nature communications DOI:10.1038/ncomms1742.
[8] Koester P J, Mortiz Buehler S, Stubbe M, Tautorat C, Niendrof M, Baumann W and Gimsa J 2010 Modular glass chip system measuring the electric activity and adhesión of neuronal cells-application and drug testing with sodium valproic acid, Lab Chip **10**, 1579-1586.
[9] Baxter L K 1997 Capacitive Sensors Design and Applications, IEEE Series on Electronics Technology, ISBN o-7803-1130-2.

7. Acknowledgments

Authors wishing to thank the Dirección General de Asuntos del Personal Academico, Universidad Nacional Autonoma de Mexico for financial support of AGS's postdoctoral work