**Proceedings of the 10th World Congress on Biosensors, 14 – 16, May 2008, Shanghai, China**

# Impedimetric discrimination of cell types for use in a whole cell biosensor

Zhiyan Wang, Janice Kiely,Mokhtar Nibouche, Richard Luxton

Faculty of Applied Science, University of the West of England, Bristol, UK

***Abstract*** -A new method has been presented of cell type discrimination using impedance measurement. Three types of cell ECV304, Caco-2 and Jurkat grown in culture plates have been used to investigate the different method of impedance analysis. One particular method using short time Fourier transforms enable cells to be characterized. The method employs joint time-frequency representation to investigate the signal spectral power density changes. In the paper results have been presented of time-frequency analysis of three types of cell, the data also shows that the spectrogram of the different cells changes with the time. The results of this investigation demonstrate that cells can be used in a whole cell biosensor and will have characteristic spectrograms, derived from time-frequency analysis. These can be monitored over time, show changes associated with cell growth. This technique can used to monitor cell change associated with applied physiological factors such as different signals.

**Keywords: Impedance measurement; STFT, Spectrogram**

## 1 INTRODUCTION

The traditional biochemical methods in vivo experiments have lot of drawbacks such as the time consuming of the procedure and the difficulty of research monitor. The impedance measurement using the micro electrode has becomes a necessary tool to determine the properties of live cells. The impedance, Z arises from the response of the different types of cells can be considered as a transfer function relate to the output voltage and input current, it is a complex quantity with real and imaginary component given by*:* $Z=R + j X$ Where $R$ is the resistance, $X$ is the reactance. Giaever and Keese [1, 2, 3] first developed the method, referred to as electric-substrate impedance sensing (ECIS) for real time monitor the cell motion and morphological changes. This method uses a small electrode that is deposited on the bottom of tissue culture wells and immersed in the culture medium. The measured impedance will change due to the cell attachment and spreading within the acceptable current flow. ECIS has contributed many applications in the cell biology research [4, 5, 6]. RT-CES system is the another famous impedance measurement system from ACEA bioscience [7,8,9,10], the company developed a cell sensor array technology where electrodes are integrated in the bottom of the wells of 16 or 96 micro titer plates , the device station receives the plates and enable to switch any one of wells to the sensor analyzer for impedance measurement.

Data analysis is valuable method for biologist to deep understanding the experiment results, the traditional statistical method may not give a suitable investigation of impedance measurement. Gary [11] used a powerful approach to fit the impedance raw data with a polynomial approximation, he transformed the frequencies into a domain between -1 to 1 using a logarithmic transformation, after calculating by a Legendre polynomials, the response at each time points could be a characterized by 7 coefficients. In this paper, the impedance of cells was first measured by using an 8 plate impedance analyzer, and then a reliable method for the interpretation of the impedance data based on the short time Fourier transformation (STFT) [12] was developed. It uses joint time-frequency representation transform a one dimensional time signal into two dimensional representations into the time-frequency plane, which make the original signal easy to identify. The method is capable of processing a large variety of data sets from in vivo experiments and may give a different set of information than the traditional data analysis method.

## 2 MATERIMALS AND METHODS

### *2.1 Cell Culture*

Three different cell types were studied in this project, and endothelial cell type ECV 304, and epithelial cell type Caco-2 and a T cell Jurkat. They were obtained from European Collection of Animal Cell Culture (ECACC) and seeded in plates. The cultures were exposed to an agent at $37^0C$ in a humidified atmosphere containing 5% $CO_2$ after the desired exposure period 24 hours.

### *2.2 Impedance Measurement*

The measurement instrument is fabricated by Kaiku Ltd Manchester, which is an 8-plate impedance analyzer. It can provide the real time cell culture information by generating an impedance spectrum characteristic of a specify type of cell. The system can process 8 wells namely, $A_1, B_1, C_1, D_1$ and $A_2, B_2, C_2, D_2$. After the first measurement is done on well $A_1$ , the control box

automatically switches in sequence among well $A_2$, $B_1$, $B_2$ until to well $D_2$, an *A.C* current is applied to each electrode in the frequency range 400 kHz to 1395 kHz. The system uses an array of 8 electrode of 22.1 mm diameter on a printed circuit board (PCB), on which the plastic well plate is placed. The output is represents the reactance changes in impedance of cells. The software CChange is responsible for the communication and control the well sensor between the analyzer and personal computer, the software MATLAB with the time-frequency toolbox (The Mathworks, Inc.) was performed for off-line data analysis.

### *2.3 Data Analysis*

As a useful tool, the STFT has been used in the wide range of research area, Soares [13] proposed this method to study the properties analysis of ocean wind waves, Mario [14] illustrates the usefulness of the STFT algorithm in the analysis of gastrointestinal contractile signal, the outcomes showed that androgen treatment brings about a gradual increase in the energy of characteristic frequency component of control ileal tissues. Dong [15] demonstrate the advantage of using joint time-frequency analysis of horn vibration for monitoring the wire bonding, the sensed signal of a typical bond doesn't reveal any more information, the spectrogram shows the time-frequency distribution of same signal, corresponding to the main harmonics, which describes the to the change of the vibration. Another application of time frequency analysis is air-coupled ultrasonic test to concrete [16], by comparing the spectrogram results of the ultrasonic chirp pulse, researcher can discriminate three types of concrete samples in various mixes. The application of this method has also been presented for the investigation of electrochemical noise measurement and electrochemical oscillations [17, 18].

The STFT is the extension of Fourier transform, defined as:

$$STFT(t,\omega) = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{\infty} x(\tau) h(t-\tau) e^{-j\omega t} d\tau \quad (1)$$

where $h(t-\tau)$ is the window function in time. There are several types of window functions which are widely used in spectral analysis, such as the rectangular window, Hamming window, Hann window and Gauss window. The hamming window is chosen to achieve sidelobe suppression in this study; the coefficients of a Hamming window are computed from the following equation:

$$h(n) = 0.54 - 0.46\cos\left(\frac{2\pi n}{N-1}\right) \quad (2)$$

Where N represents the length of window function, short window length brings the good time resolution but the poor frequency resolution; on the other hand, long window length brings the good frequency resolution, but the poor time resolution.

A quadratic form is known as spectrogram, and measures the spectral energy density of the signal along the frequency direction at a given time, is defined as:

$$SPEC(t,\omega) = |STFT(t,\omega)|^2 \quad (3)$$

## 3 RESULTS AND DISCUSSIONS

From the figure 2, the data records of impedance change from three types of cells are shown. Clearly the analyzed signals from time domain are difficult to interpret. STFT is used to transform the original three signals into time-frequency analysis. The reasonable window length was decided in order to balance the appropriate time and frequency resolution.

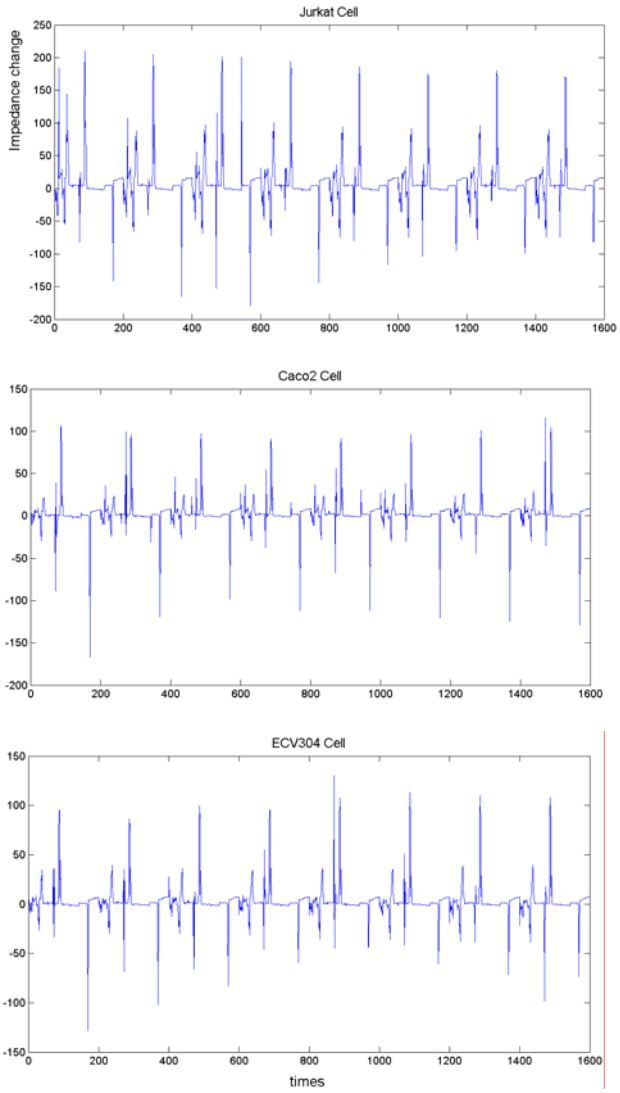


Figure 2 impedance changes of three types of cells, Jurkat cell, caco2 cell and ECV304 cell after four days exposure

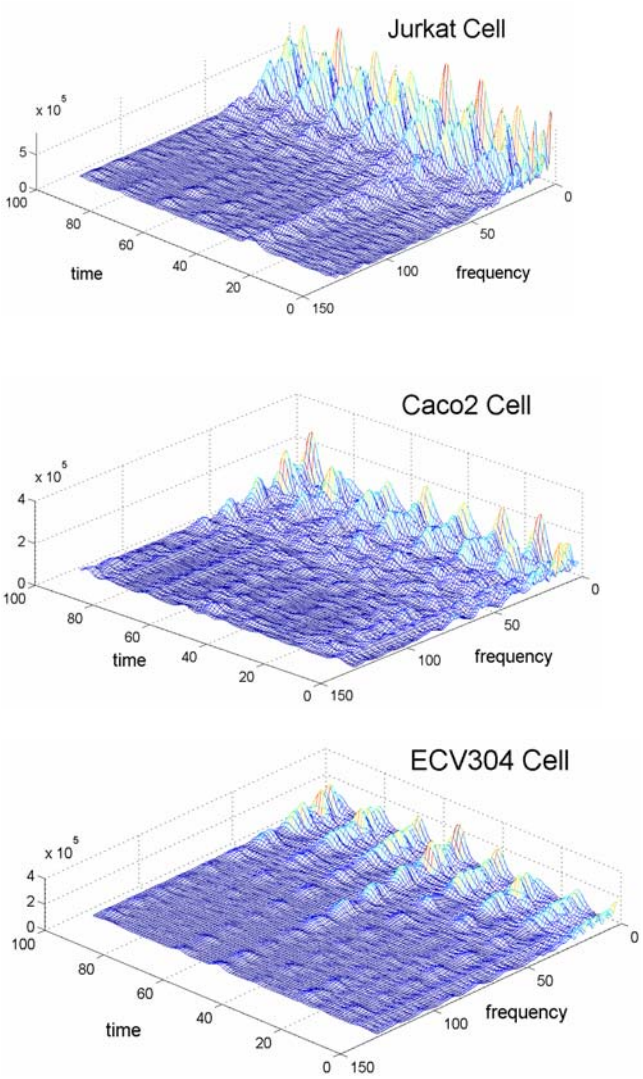


Figure 3 STFT spectrograms of three types of cells from Jurkat cell, caco2 cell and ECV304 cell after four days exposure

The spectrogram results were shown in figure 3, most of signal energy is located in the low frequency region, particularly below 50 Hz. Based on the Parseval's theory, the total energy of contained in waveform summed across all the time domain is equal to the total energy of waveform's Fourier transform summed across all of its frequency components, it is obvious to explain the energy values in Jurkat cell is larger than the energy values of the other cells, because impedance changes of Jurkat cell in time domain is larger than the others, there is high frequency components emerged at 25 seconds in Jurkat cell spectrogram, the spectrograms of Caco2 and ECV304 cells are similar in terms of energy distributions in the lower frequency region. Nonetheless, in the higher frequency region the energy distribution in ECV304 is smoother than Caco2 but shows slight variations in the time domain. For example, in ECV304 cell spectrogram, within the regular time intervals the energy level rises with similar pattern. In addition, at the 50 seconds time, the energy level has the visible shape in the frequency of 80 Hz compare with Caco2 at the same time. The difference of spectrogram correspond to the electrical properties of each type of cells, such as the conductivity, permittivity…because the impedance was measured in the frequency region 400 kHz to 1395kHz, the cell membranes act as short circuits composed of resisters and capacitors allowing current to flow through both intracellular and intercellular fluids, different type of cell will have different reaction.

Another investigation was focus on the spectrogram were changed over the time associated with the cell growth, as shown in figure 3, 4, the spectrograms of ECV304 cell have different energy shapes after four day exposure and eight day exposure, the visible shape of energy of high frequency components at time 50 seconds is disappeared at the spectrogram result shown in figure 4.

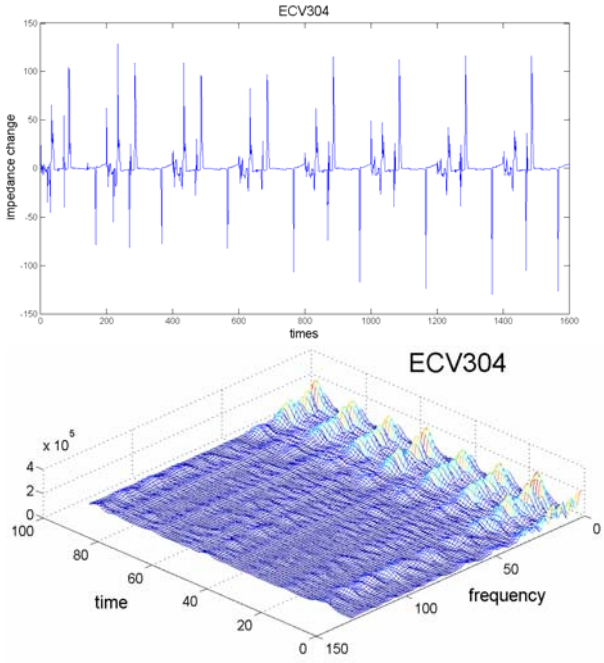


Figure 4 the impedance change and STFT spectrogram of ECV304 after eight days exposure

## 4 CONCLUSIONS

After impedance measurement from current system, the STFT can be used as criteria for discriminating the different type of cells, can be monitored over time, show changes associated with cell growth. Furthermore, STFT could be the appropriate way to extracting important feature from impedance results.

## 5 ACKNOWLEDGMENT

This work is supported by the EPSRC, some of impedance measurement data is supplied by the Nines Martinez-Vargas in the project of “Real-time Monitoring of Cellular Toxicity Using Electrical Impedance Measurements”

## REFERENCES

[1] Keese,C.R. and Giaever,I (1994). A biosensor that monitors cell morphology with electrical fields. *IEEE Engineering in Medicine and Biology magazing*, June/July,pp. 402-408

[2] Keese, C.R.,Bhawe,K., Wegener, J. and Giaever,I (2002). Real-time impedance assay to follow the invasive activies of metastatic cells in culture.*BieTechniques,33* (4),pp.842-4,846,848-50.

[3] Keese,C.R. and Giaever,I (1991). Micromotion of mammalian cells measured electrically. *Proc.Natl.Acad.Sci.USA*,vol 88, pp 7896-7900

[4] Caide Xiao, Bernard Lachance, Geoffrey sunahara, and John H. T. Luong (2002*).*Assessment of cytotoxicity using electric cell-substrate impedance sensing: concenration and time response function approach. *Anal. Chem*, vol 74, pp 5748-5753

[5] Caide,Xiao and John,H.T.Liong(2003). On-line monitoring of cell growth and cytoxicity using electric cell-substrate impedance sensing (ECIS).*Biotechnol.Prog*, vol 19,pp 1000-1005.

[6] Joachim, Wehener,C R.Keese, and Giaever,I(2002) Recovery of adherent cells after in situ electroporation monitored electrically. *Biotechniques* vol 33,pp 348-357

[7] Yama Abassi and Josephine M. Atienza. Dynamic monitoring of cell adhension and spreading using the RT-CES system.

[8] Yama Abassi and Jenny Zhu. Label-free assay for NK cell-mediated cytolysis on the RT-CES system

[9] Josephine M.Atienza,Jenny Zhu (2005) Dynamic monitoring of cell adhension and spreading on microelectronic sensor arrays. *Biomol Screen.*

[10] Naichen Yu, Josephine M. Atienza, Jerome Bernard (2005) Real-time monitoring of morphological changes in living cells by electronic cell sensor arrays: an approach to study G protein-coupled receptors.*Analytical Chemistry*

[11] Gary J. ciambrone,Vivian F. Liu, Deborah C. Lin (2004) Cellular dielectric spectroscopy: a powerful new appreach to label-free cellular analysis. *Biomol Screen* pp 467-480

[12] D.Gabor, theory of communications, (1946)

[13] C. Guedes Soares, Z. Cherneva (2005) Spectrogram analysis of the time –frequency characterisitics of ocean wind waves. *Ocean Engineering*, vol 32, pp 1643-1663

[14] Mario Diaz (2007). Applications of Fourier linear spectral analysis to the characterization of smooth muscle contractile signals. *Biochem. Biophys. Methods*.

[15] Dong Zhang and Shih Fu Ling (2003) Monitoring wire bonding via time-frequency analysis of horn vibration. *IEEE transactions on Electronics Packing Manufacturing*, vol 26,pp 216-220

[16] James R.Berriman, David A.Hutchins,(2006).*IEEE Transactions on Ultrasonics,Ferroelectrics, and Frequency Control*, vol 53,pp768-776

[17] K. Darowicki, A. Zielinski, A. Krakowiak. (2003). Anlysis of spontaneous electrochemical oscillations by energy distribution technique. *Electrochimical Acta*, vol 48 ,pp 1559-1566

[18] K. Darowicki, A. Zielinski. (2001). Joint time-frequency analysis of electrochemical noise. *Electroanalytical Chemistry*, vol 504,pp 201-207