Electrical Impedance Spectroscopy for Single-Cell Analysis

Zurich Instruments

Application Note
Applications: Bio-engineering / Microfluidics
Products: HF2IS, HF2IS-MFK, HF2CA

Release date: January 2010

Summary

This application note reports on a differential electrical impedance spectroscopy system for single-cell detection, discrimination and analysis in microfluidic systems. The setup allows for acquisition of electrical impedance spectroscopy data of biological cells with high fidelity. Signal waveforms of the impedance spectroscopy have been explained and verified both theoretically and in simulations.

Here, a 2-stage microfluidic system comprising a flow-through differential impedance spectroscopy stage in combination with an AC dielectrophoresis sorting stage is presented. Such a setup implements a robust cell characterization and sorting system realized for a fraction of the cost compared to established optical measurement technologies.

Application

Description

Electrical impedance spectroscopy (EIS) is a method to analyze the electrical properties of dielectric materials typically biological, and molecular objects. It is a non-invasive, label-free method that provides quantitative information on the electrical properties of, e.g., the cell membrane capacitance, the cell membrane conductivity and the cytoplasmic conductivity. The method is applicable, e.g., in diagnostics including cell counting and characterization in hematology, in biology including sample preparation procedures like cell separation. The main alternative to the presented impedance-based method is called FACS and relies on fluorescent labels and fluorescent-activated cells to achieve precise screening and sorting of single cells.

The popularity of EIS is growing in different fields of application due to improvements in measurement technology and reduced costs versus optical systems. Engineers stress that the entirely electrical analysis method allows for compact and robust laboratory setups, while

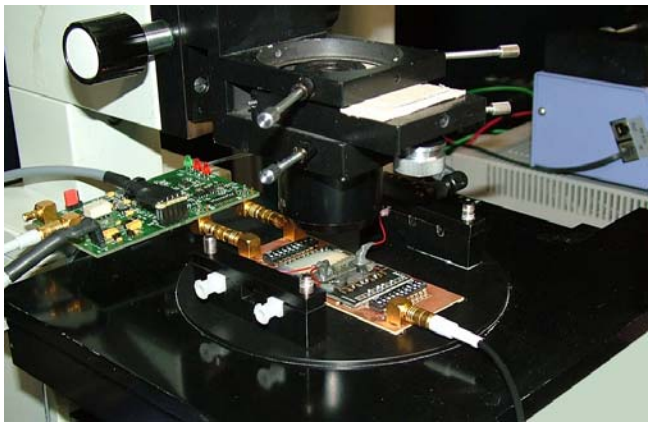


Figure 1. Microfluidic setup using electrical impedance spectroscopy.

biologists profit from the label-free analysis, which simplifies the sample preparation and avoids sample modification.

Setup Description and System Requirements

Figure 2 shows the principle setup of the described microfluidic system. The two electrodes E1 and E2 are placed at the top, and the two electrodes E3 and E4 are placed at the bottom of the channel in a symmetrical arrangement. The top electrodes E1 and E2 are stimulated with an alternating electrical signal, and a response current is measured from the electrodes E3 and E4. As a particle passes the electrode area from left to right, it first changes the electrical current flowing through E3, then it changes the current flowing through E4. The signal of interest is the differential current I1-I2 measured at the electrodes E3 and E4. The transient curve of the current depends on the frequency, the electrical properties of the medium, and the cell. The expected signal is symmetrical and bipolar as illustrated in Figure 3. The characteristic of measuring differential currents with this four-electrode arrangement is, that the dynamic range is increased and that common-mode signals are rejected.

The HF2CA current amplifier acts as active probe close to the setup reducing interferences and signal losses. This preamplifier and converts the currents into voltages by means of two precision resistors, shunted to ground, and a high-speed differential instrumentation amplifier. The two resistors and the measurement electrodes are arranged in a Wheatstone configuration. The channel impedance can be reconstructed from the voltage drop across the shunt resistors. This configuration is preferred over a transimpedance amplifier for its improved stability when working with electrodes in liquid phase at high frequencies of up to 50 MHz.

The HF2IS impedance spectroscope generates the electrical stimulation signal, which is a sum of several sinusoidals at different frequencies and different amplitudes. Further the HF2IS concurrently demodulates and filters the measured differential signal at the stimulated frequencies.

The information derived from the measured impedance signals can be used to characterize, recognize, and count the cells that pass by the arrangement of electrodes E1 to E4. Subsequently it is possible to use, e.g., dielectrophoresis to sort the cells into different bins. This is done by applying an AC voltage signal to the electrodes E5 and E6. Depending on the frequency, an attractive or an repellent force is exerted on the cells, which are hence directed into the desired channel. The HF2IS has the unique capability to perform all required actions including impedance spectroscopy, decision making and dielectrophoresis voltage generation in real-time and to thus perform cell sorting.

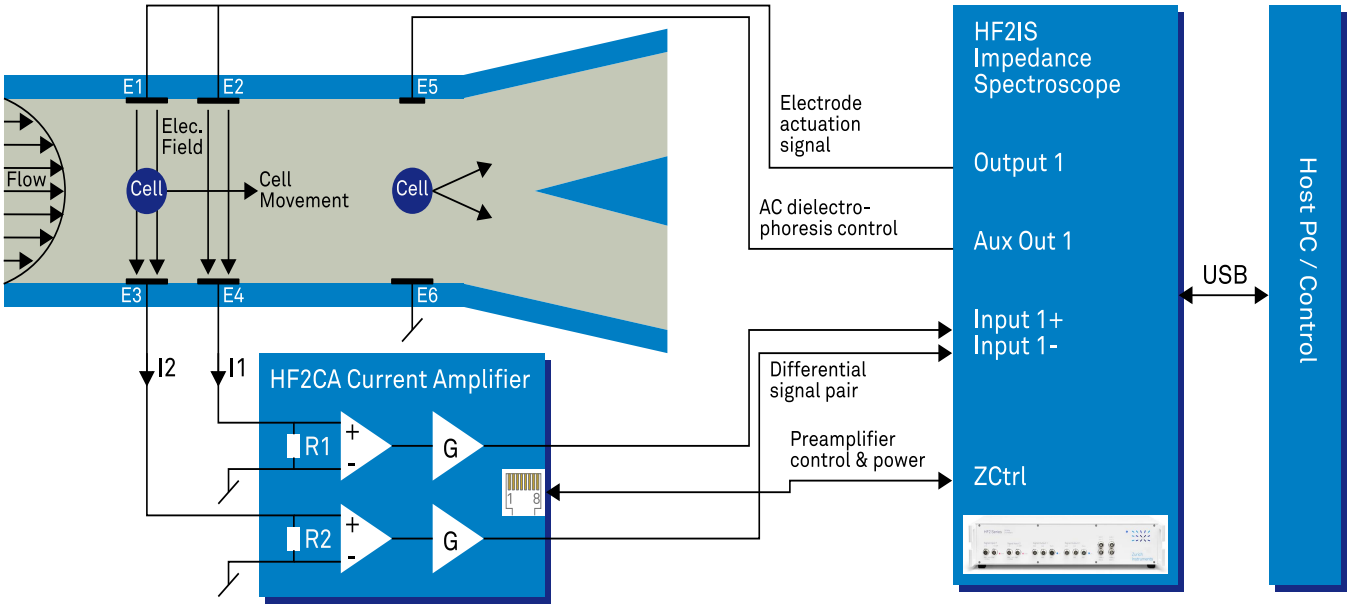


Figure 2. Schematic view of microfluidic system for single-cell analysis.

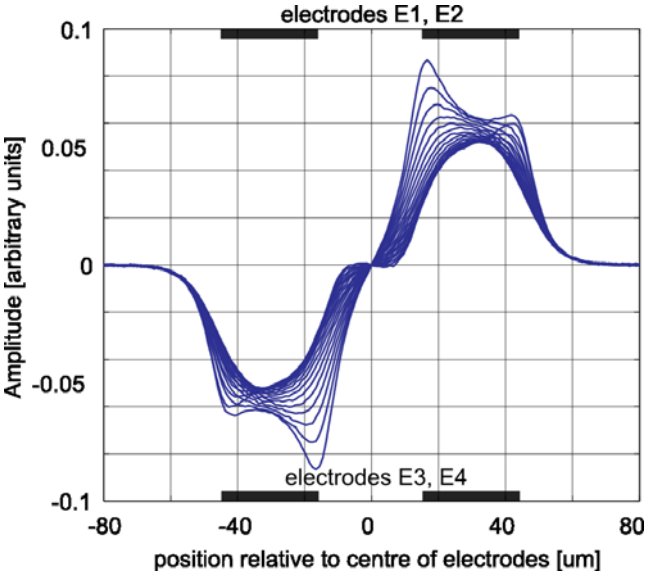


Figure 3. Simulated current waveforms for different positions relative to the centre of the channel.

Simulations of Cells Measurement

In order to extract the information contained in the impedance spectroscopic measurements, it is required to have a model system that explains the measured waveforms. Finite-element simulations, comprising the channel with electrodes, the liquid, and a sample particle, have been performed to find an electrode layout with maximum signal-to-noise ratio. The calculated waveform templates (Figure 3) are then fitted to the measured data, so that high-quality data can be obtained [3].

Achievements

The presented waveforms have been experimentally confirmed in publications [3], which also showed how electrode size and position are optimized.

Figure 4 shows a differential electrical impedance spectroscopy data acquisition measured on yeast cells at 8 frequencies simultaneously. The plot clearly shows how amplitude and phase differ at different frequencies. An important part of the information required to subsequently characterize and classify the cells is carried in these differences. This underlines the importance and benefits of multi-frequency measurements.

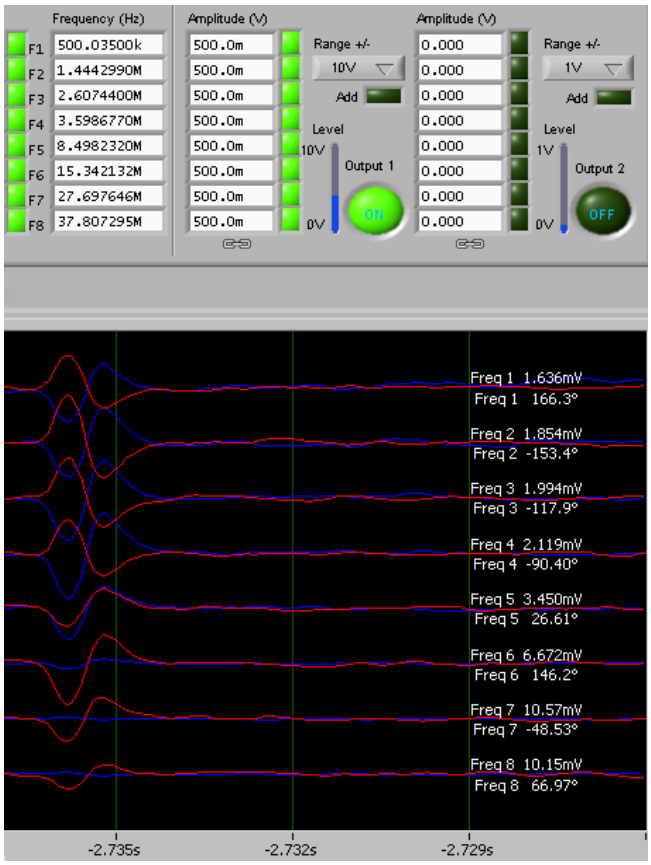


Figure 4. Real-time differential EIS.

Conclusions and User Benefits

Electrical impedance spectroscopy allows to reliably analyze the characteristics of biological cells depending on signal amplitude and phase. Current measurements can be correlated to previously calibrated data to discriminate cells of 2 or more populations.

The HF2IS impedance spectroscope distinguishes itself versus lock-in amplifiers and other impedance analyzers because of the following reasons:

- dedicated for fast dynamic signals: very low time constants (below µs) and high sample rate to the host computer allow to capture very fast dynamic events
- 2 integrated signal generators in the frequency range from µHz to 50 MHz, whereas some high-frequency lock-in amplifiers do not feature any signal outputs
- integrated multi-frequency capability boosts the pattern recognition capability of the cell detection unit

- integrated microprocessor to run user-specific code written in C for control of, i.e., AC dielectrophoresis

Scientists using the HF2IS impedance spectroscope profit from the accuracy of a digital instrument covering a frequency range that used to be measurable only with analog instruments.

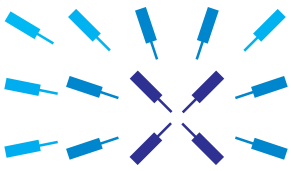
References and Further Reading

[1] Gawad, Holmes, Benazzi, Renaud, Morgan, Impedance Spectroscopy and Optical Analysis of Single Biological Cells and Organisms in Microsystems, Springer, 2008.

[2] Sun, van Berkel, Green, Morgan, Digital signal processing methods for impedance microfluidic cytometry, Springer, 2009.

[3] Lanz, Hafizovic, Rothe, Streichan, Goedecke, Heer, Hierlemann, Differential Impedance Spectrometer and Vision System for Analysis of Single Cells, Proceedings Transducers, 2009.

[4] Mernier, Piacentini, Tornay, Buffi, and Renaud, EPFL, Switzerland, Cell Viability Assessment by Flow Cytometry using Yeast as Cell Model, Sensors and Actuators B: Chemical, doi:10.1016/j.snb.2009.11.066, 2009.



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Headquartered in Zurich, Switzerland, technology leader Zurich Instruments (ZI) designs and manufactures premium dynamic signal analysis instruments for advanced scientific research and leading industrial applications. As technology spin-off from Swiss-based ETH, Zurich Instruments was established in 2008. Products include lock-in amplifiers, instruments for electrical impedance spectroscopy, and a competitive range of application specific pre-amplifiers.