DIFFERENTIAL IMPEDANCE SPECTROMETER AND VISION SYSTEM FOR ANALYSIS OF SINGLE CELLS

T. Lanz, S. Hafizovic, J. Rothe, R. Streichan, N. Goedecke, F. Heer, A. Hierlemann*
ETH Zurich, Department of Biosystems Science and Engineering,
Mattenstrasse 26, 4058 Basel, Switzerland

ABSTRACT

This paper reports on a differential impedance spectroscopy system for single-cell analysis, which also features an optical setup with machine-vision that simultaneously provides optical data for a direct comparison. Such a combined setup allows for benchmarking the differential impedance spectroscopy against the optical data in a quantitative way. Here we present simultaneously acquired optical and impedance-spectroscopic data of yeast cells and polystyrene beads. Signal simulations have been performed and fitted to the measured data to increase the quality of the results and to explain the recorded signal shapes.

INTRODUCTION

Electrochemical impedance spectroscopy is a method to analyze the electrical properties of chemical and biological objects at different frequencies. It is a non-invasive, label-free method and provides quantitative information on, e.g., cellular electrical properties such as membrane capacitance, membrane resistance and cytoplasmic resistance [1-3]. This method can be applied to, e.g., diagnostics including cell counting and cell separation in hematology (e.g., blood cells or leukocytes) [4-6]. An alternative to electrical methods is the use of fluorescence labels in combination with fluorescent activated cells (FACS), which allows for precise screening and sorting of single cells.

The most common techniques to investigate electrical cell properties include the analysis of cell suspensions, patch clamping and, more recently, flow-through impedance spectroscopy. The investigation of cell suspensions provides average electrical parameters and does not give any information on single cell characteristics or on distribution statistics. Patch clamp can be used for single cells but it is time-consuming and invasive. Impedance spectroscopy in flow-through microsystems is suitable to continuously and non-invasively monitor the dielectric properties of single cells.

In this paper we present a micro fluidic system for flow-through differential impedance spectroscopy combined with a vision system as illustrated in Figure 1 and Figure 2. The vision system comprises a camera that is capable of recording from cells as they flow through the micro channel at 75 frames per second. The combination of electrochemical impedance spectroscopy with such a vision system has two advantages: (i) It allows for the calibration of the electrical impedance data by using the geometric size of the cell recorded by the camera, and (ii) the information content is increased by having an additional parameter. For example in hematology fluorescence measurements are more and more complemented with spectral impedance data.

Functional Principle

Figure 1 shows the principle of differential impedance spectroscopy in a microfluidic channel. 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 excited with a sinusoidal voltage, and the differential current is recorded 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 expected signal is symmetrical and bipolar as illustrated in Figure 1 and Figure 6. The characteristic transient curve of the current

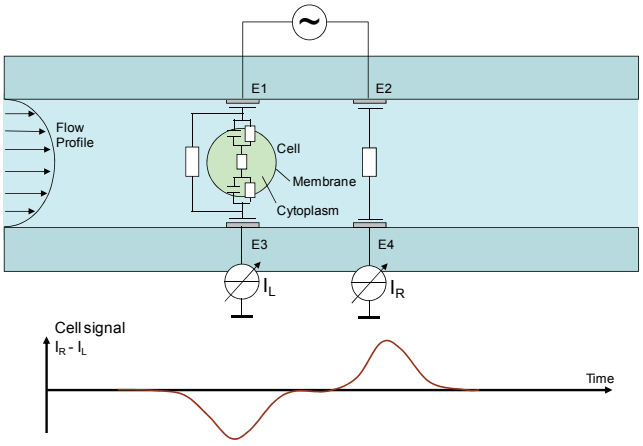

Figure 1: Functional principle: Two pairs of opposed electrodes are integrated in the channel, and electrodes E1 and E2 are stimulated. When a cell passes the electrode area, the channel impedance and, consequently, the current, measured at electrodes E3 and E4, changes as illustrated at the bottom.

depends on the frequency and the electrical properties of the medium and the cell. The advantage of measuring differential currents with this four-electrode arrangement is, that the dynamic range is increased and that common-mode signals are rejected.

SETUP AND FABRICATION

A block schematic of the complete measurement setup is shown in Figure 2. The lock-in amplifier (HF2, Zurich Instruments, www.zhinst.com) generates the stimulation signal, which is a superposition of up to 8 sinusoidals at different frequencies. A custom-built preamplifier converts the current to a voltage and amplifies the signal close to the setup, which reduces signal interference and signal losses. The lock-in amplifier then concurrently demodulates and filters the signal at several frequencies.

The preamplifier is realized by 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 [4]. The channel impedance can be reconstructed from the voltage drop across the shunt resistors. Compared to a transimpedance amplifier, this configuration shows improved stability, when working with electrodes in liquid phase at high frequencies of up to 50 MHz.

In the optical setup a collimator aperture in the light beam ensures parallel light. This increases the depth of focus, which is required for sharp images of the cells. A camera (MV-D1024E-80, Photonfocus) captures particles in the area of the electrodes. The optical recordings are annotated with timestamps, packaged with the data from the lock-in amplifier and saved on the computer. LabView and its Vision

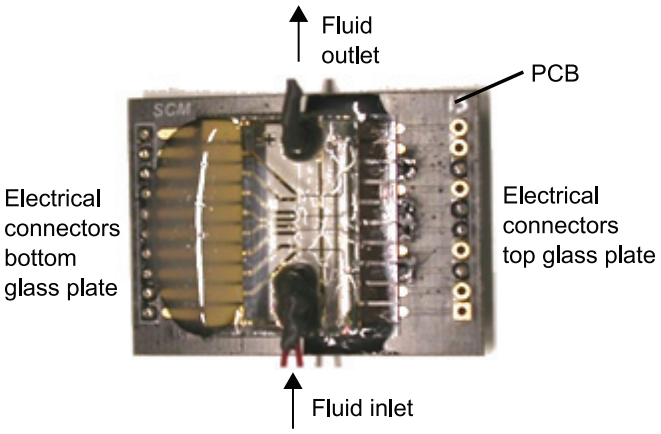

Figure 3: Picture of the measurement microchip, consisting of two sandwiched glass plates mounted on a printed circuit board.

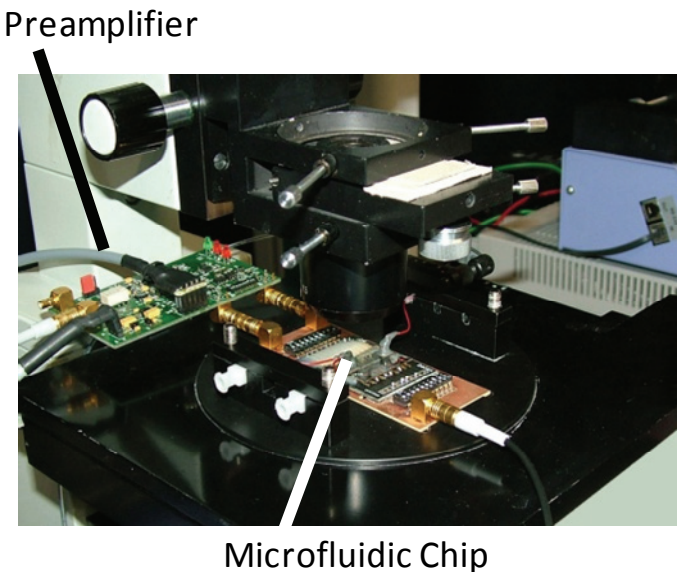

Figure 4: Picture of the measurement setup with microscope, microchip holder, and preamplifier.

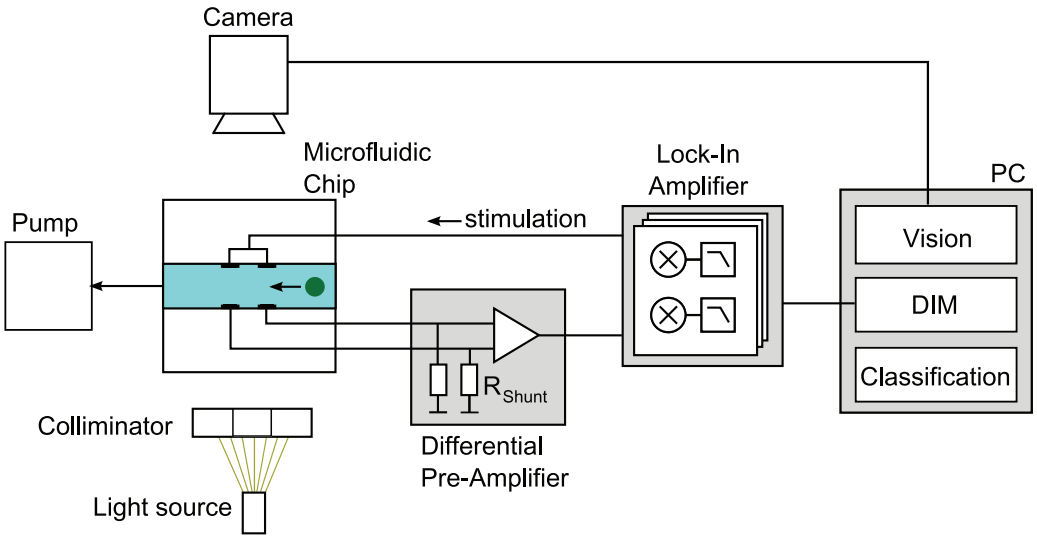

Figure 2: Block schematic of the measurement setup.

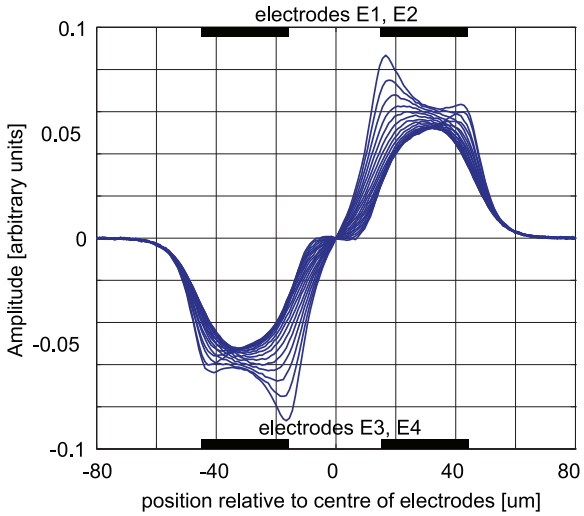

Figure 5: Signals induced by cell trajectories at different vertical levels (-8 µm to 8 µm displaced from center) as calculated by finite element simulations. The x-axis is the position of the particle relative to the center of the electrodes; the y-axis is the measured signal amplitude change. These signals are used as templates for the measurement data.

Development Module, Version 8.5, are used for recording the optical and impedance data. In a second step, the video data are analyzed. First, the background image is subtracted from each frame in order to increase the contrast. Then, the frame is smoothed, and a threshold is applied to generate a 2-color image, from which the particle size is extracted. The electrodes are also captured in the video and provide a reference scale for the particle size.

Microchip Fabrication

The microfluidic device, shown in Figure 3, consists of two glass plates with 200 nm thick gold electrodes. A 15 µm thick SU-8 layer on each plate is patterned by lithography to yield the microfluidic structures. The two plates with the SU-8 layers are assembled on top of each other, this way realizing the fluidic channels (30 x 50 μm^2) [7]. The glass plates are then bonded onto a small PCB, which is connected to a second PCB, placed near the microscope. Figure 4 shows a picture of the complete measurement setup.

Sample Preparation

The yeast cells (Saccharomyces cerevisiae, FY4 strain) are removed from the agarose culture plate and put into the suspension medium. They are separated using a vortexer. To wash the cells they are centrifuged and re-suspended three times. The final sample concentration is about $5 \cdot 10^6$ cells per ml.

Simulation Templates

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 for two purposes: (i) to find an electrode layout with maximum signal-to-noise ratio, and (ii) to yield electrical-signal waveform templates for different cell trajectories. The calculated waveform templates (Figure 5) are then fitted to the measured data, so that high-quality data for signal amplitude, cell speed, and the vertical height (z) of the cell trajectory can be obtained.

RESULTS

Figure 6 shows measurements of 4-µm-diameter polystyrene beads suspended in PBS buffer solution at 700 kHz. The simulation templates are fitted to the data of the impedance measurements in order to extract the size information of the particles. The fit parameters for the templates include signal amplitude, vertical offset, and flow velocity.

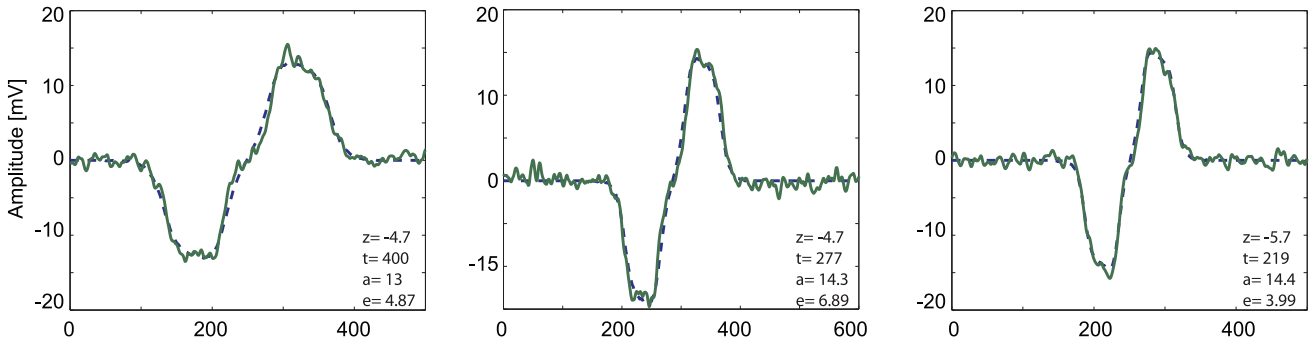

Figure 6: Three examples of transient signals of 4-µm polystyrene beads passing the measurement area. The solid line is the measured signal, and the dashed line is a template fitted to the measured data. The fitting parameters include amplitude, 'a', vertical offset, 'z', given in µm with respect to the center of the channel, and time stretch, 't', which relates to the speed of the cell. 'e' denotes the fitting error.

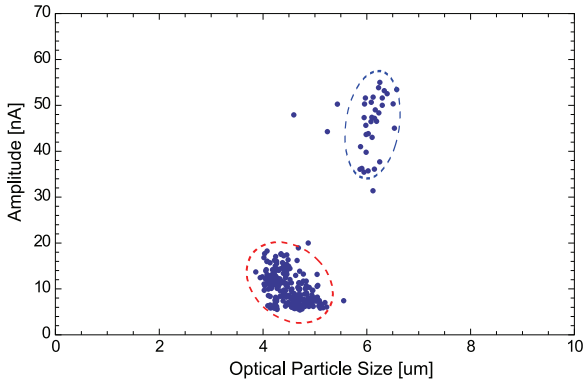

Figure 7: Comparison of the differential impedance measurements (y-axis) to optically measured particle sizes (x-axis). The impedance data were measured at 1.5MHz.

Figure 7 Differential impedance measurements of polystyrene beads (4 µm and 6 µm diameter) at 1.5 MHz are compared to optical measurements in Figure 7. The two populations can be clearly separated.

Figure 8 shows the comparison of differential impedance measurements at 3.2 MHz to optical measurements of yeast cells. Cell size is the dominant factor that determines the measured impedance signal at this frequency. The figure illustrates that the signal phase linearly correlates with the cell diameter. The outliers in the lower left are due to insufficient optical contrast of smaller cells.

CONCLUSION

The combination of differential impedance spectroscopy methods with an optical reference system enables to directly correlate the performance of impedance spectroscopy to optical data on large sample quantities, which is a prerequisite for establishing impedance spectroscopy as an analysis tool in cell handling, sorting, and cellular analysis.

In the future, we will increase the information content of the measurements by increasing both, the frequency range, and the number of frequencies available from the lock-in amplifier. This will lead to a cell analysis tool providing impedance spectroscopy at simultaneously 8 frequencies in a range of 100 kHz to 20 MHz along with optical measurement of the cells.

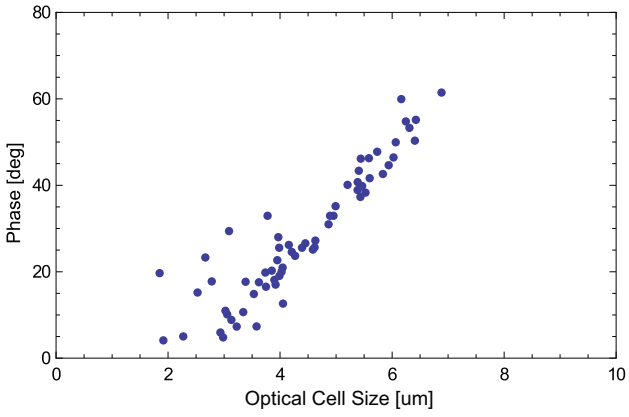

Figure 8: Optical versus impedance measurement data of yeast cells.

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CONTACT

*S. Hafizovic, tel: +41 61 633 78 48; sadik.hafizovic@bsse.ethz.ch.