Amphasys AG • Technopark Lucerne
Platz 4 • CH-6039 Root D4
www.amphasys.com

Amphasys – Impedance Microflow Cytometry – The Technology

Introduction

Impedance-based single cell analysis systems, also known as Coulter counters, represent a well-established method for counting and sizing any kind of cells and particles. This technology, however, was until recently not suitable for cell characterization applications, for which advanced and powerful fluorescence-based cell analysis and sorting devices (FACS) provided the gold standard in research and clinical laboratories. The advent of microtechnology in life sciences provides nowadays new possibilities to improve the sensitivity of electrical detection methods, revitalising the perspective of a simpler and label-free cell analysis approach. Here we describe the technology of this novel impedance-based microflow cytometer (Fig. 1), which will conquer application fields that were so far unachievable.

Fig. 1 Impedance Microflow Cytometer Ampha Z_{30}.

Fig. 2 Impedance micro-fluidic chip in holder frame.

Working principle

The heart of the cytometer consists of a microfluidic chip supplied with microelectrodes (Fig. 2). In this chip we measure changes of the electrical resistance of a fluidic medium when particles or cells pass through the applied electric field (Fig. 3). The high sensitivity of the device is achieved by adjusting the channel dimensions close to the size of the measured cell type, increasing thereby the ratio between cell and detection volume. Thus, for the various cell types chips with different channel dimensions are available for optimal sensitivity. The main difference from the Coulter principle is that we are measuring with AC (alternating current) over a broad frequency range, while Coulter works with DC (direct current) or with AC at very low frequencies.

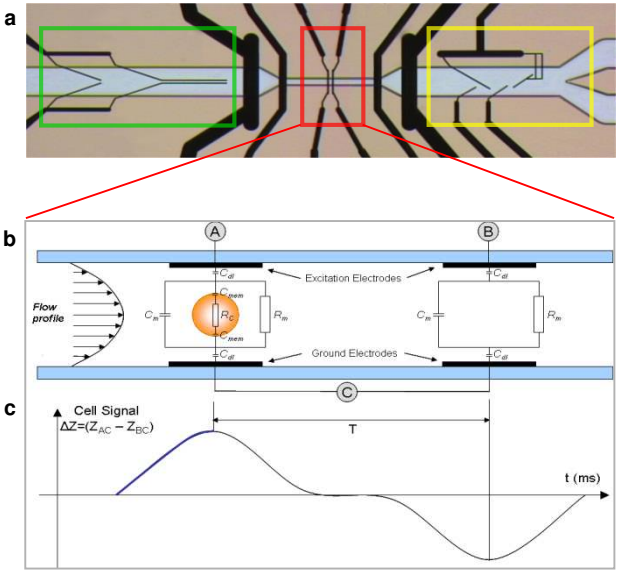

Fig. 3 (a) Schematic top view of the impedance chip. Cells are first focused by dielectrophoresis (DEP) in the green framed focusing region (channel dimensions are 20 x 200 µm). For many cell types focusing is even not required, since (larger) cells may align themselves in the measurement channel through the constriction. Impedance changes are determined in the red bordered detection area (channel dimensions can vary from 10 x 10 µm to 40 x 40 µm). In order to avoid channel clogging, samples need to be filtered according to the channel dimensions of the used chip. Cells could be sorted in the yellow marked sorting area again using DEP (functionality not yet implemented) or another type of sorting technology. (b) Side view of detection area. Cells move under pressure-driven flow and pass successively the two electric field regions, modifying this way the current through each detection volume. (c) Impedance signal resulting when a cell passes through the electric field established by the two electrode pairs. The differential measurement enables to determine the particle speed T and thus the cell concentration. Usually, during a measurement, a flow rate of 1 to 10 µl/min is applied consenting cell concentrations in the range of 10^4 – 10^7 cells/ml.

Dielectric properties of cells

If an AC electric field is applied, biological cells become polarized as a result of charge accumulation at the limits between the insulating plasma membrane and the aqueous medium. This interfacial polarization goes along with the formation of dipoles, which are characterized by a specific time constant and are therefore dependent on the frequency of the applied AC. At low AC frequencies, maximum polarization can easily be achieved in a short time, while at higher frequencies, the rate is too high for the dipoles to attain full polarization. This phenomenon has an important impact when measuring the impedance of a cell suspension or of a single cell and can be exploited to characterize the measured cells (Fig. 4) by means of their volume, membrane capacitance and cytoplasm conductivity. These parameters are important indicators of physiological cell conditions.

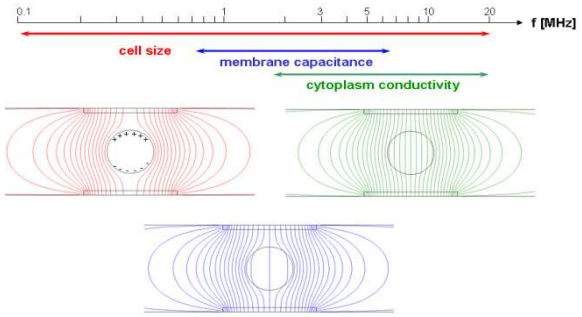

Fig. 4 Since the insulating properties of cell membrane represent a substantial obstacle to current flow at low frequencies (below 500 kHz), the cell is principally non-conducting, reflecting essentially the Coulter volume measurement. However, at intermediate frequencies (around 500 kHz to 6 MHz) the plasma membrane polarization decreases leading to a decrease of the capacitance of the suspension, an effect known as beta-dispersion or dielectric relaxation. Measurements in this range normally provide information about the electrical properties of the plasma membrane. At high frequencies (6 – 20 MHz), a polarization of the plasma membrane is almost non-existent. Under these conditions, the cell membrane does not represent a barrier to the current, and the measurements rather provide information about the cytoplasmic conductivity.

Data acquisition and analysis

Impedance is a complex quantity and extends the concept of DC resistance to AC circuits. It describes not only the relative amplitudes of the voltage and current, but also the relative phases. These values depend on the cell type, cell status and the applied AC frequency. The presented microflow cytometer prototype allows for simultaneous impedance measurement at four different frequencies and provides therefore, like FACS analyses, a multiparametric measurement of single cells. The obtained data (real & imaginary part, absolute amplitude, phase, cell velocity, etc.) are converted into the standard FCS3 format and can thus be analyzed with commercially available flow cytometry software.

The performance of the impedance microflow cytometer is limited by the sample flow rate and cell concentration (see also Fig. 3) and also depend on the application. For counting purposes, event rates of about 5000/sec can be presently attained. Since cell characterization applications require more data, these analyses can run at rates of about 1000 events/sec.

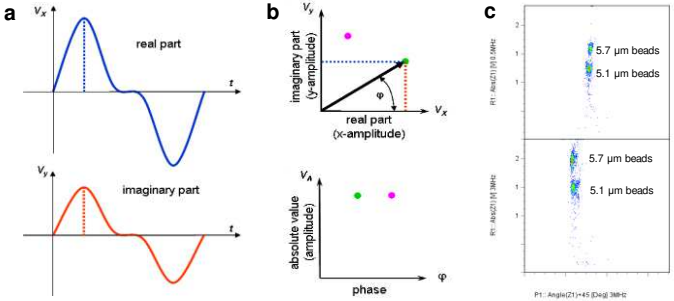

Fig. 5 (a) The measured impedance signal consists of a real and an imaginary part that are resolved in two different channels. (b) The corresponding values can be plotted as real part vs. imaginary part or as phase angle vs. absolute amplitude. (c) Phase vs. absolute amplitude plot of a 5.1 and 5.7 µm polystyrene beads mixture at 0.5 (upper graph) and 3.0 MHz (lower graph). As expected, particle counting and size discrimination is easily possible with impedance microflow cytometry.