ENHANCEMENT OF A LABEL-FREE DIELECTROPHORETIC CELL SORTER WITH AN INTEGRATED IMPEDANCE DETECTION SYSTEM

M. Carminati[1*], M.D. Vahey[2], A. Rottigni[1], G. Ferrari[1], J. Voldman[2] and M. Sampietro[1]
[1]*Politecnico di Milano, ITALY*
[2]*Massachusetts Institute of Technology, USA*

ABSTRACT

We present a miniaturized CMOS impedance detector integrated within a dielectrophoretic microfluidic device for label-free separation, enabling the sorting and counting of particle suspensions in a fully electrical manner. This approach makes possible a truly portable system for cell sorting and analysis. Towards this end, the design of ad hoc coplanar electrodes for impedance sensing, supported by numerical simulations, is illustrated. The validation of the system with 10μm polystyrene beads is reported. A USB-controlled single-chip lock-in demodulator allows high-throughput counting of resistive peaks of 1% amplitude, with a signal-to-noise ratio >10 and with a time resolution of 200μs.

KEYWORDS: Flow Cytometry, Intrinsic Cell Separation, Lock-In Impedance Detection.

INTRODUCTION

Miniaturization, integration, parallelization and portability have become dominant paradigms in the development of point-of-care micro-analytical systems. Traditionally, the portability made possible by microfluidic technology has been undermined by the need to couple compact microfluidic devices to bulky, non-portable instruments in order for the devices to operate. Within this context, we present a novel microfluidic system that enables label-free, high-throughput, fully-electrical particle sorting, separation and counting. It is based on the original combination of two consolidated AC techniques that complementarily probe the intrinsic passive electrical properties of cells: dielectrophoresis and impedance sensing.

To achieve this goal, we have integrated coplanar impedance sensing electrodes at the outlets of an *isodielectric separation* (IDS) device [1], enabling compact, high-speed and real-time electronic monitoring of particle separations without the need for a microscope (Figure 1). In IDS, cells and particles are resolved according to differences in their electrical properties (i.e. conductivity and permittivity) by using dielectrophoresis to deflect them to their equilibrium positions in an electrical conductivity gradient. Using impedance sensing to count the number of cells or particles, of diameter ranging from 5 to 15μm, flowing through different outlets of the device enables one to perform separations - and monitor their progress - in a purely electrical manner.

THEORY: DESIGN OF ELECTRODES

For compatibility with devices fabricated using glass substrates and PDMS channels, a coplanar configuration is mandatory. Transversal sensing electrodes are preferred to longitudinal ones, for easier alignment of the electrodes with the channel. Electrodes have been designed in order to maximize detection sensitivity and extend the operating frequency range for a channel of 40μm width and 20μm height. Conformal mapping [2] provides analytical expressions for quick electrode sizing. However, as numerical simulations were necessary for estimating the impedance variation produced by the a cell passing over the electrodes, they have been also employed for optimal electrode sizing. Initial coarse sizing (the vertical extension of the electric field is roughly equal to the electrode gap) has been refined by finite element simulations (Comsol). The symmetry of the geometry has been leveraged to improve accuracy and save memory, leading to the quarter model shown in Figure 2a. For a 15μm cell located in the middle of the electrode gap, against the channel ceiling, optimal inter-electrode gap resulted of 12μm (Figure 2b) that corresponds to a 1.2% resistance increase (R ~50kΩ) and analogously to a geometrical capacitance decrease (ΔC/C = -1.1%). For larger gaps, the sensitive volume increases and the volume fraction, affected by the cell, decreases, while the total resistance increases, leading to a smaller ΔR/R. At the same time, for shorter gaps, the sensitive height is lowered under the cell.

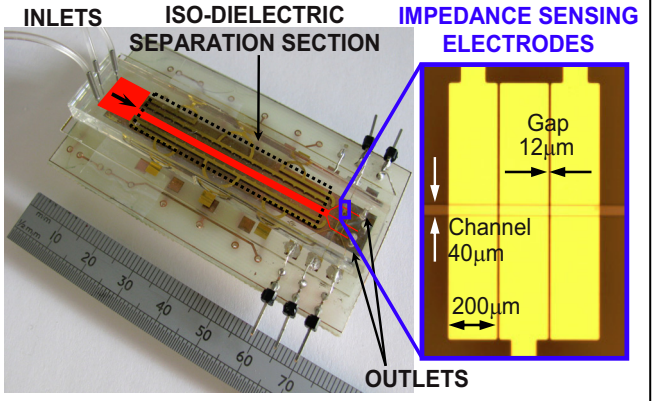

Figure 1: Assembled device combining electrokinetic separation and impedance flow cytometry. Coplanar gold microelectrodes are patterned on Pyrex glass substrates. The 20μm deep and 40μm wide channels are fabricated in PDMS with standard replica molding techniques from SU-8 patterned silicon masters. A third electrode for differential sensing is also present.

As visible in the simulation, only the first ~10μm of the metal length contribute to impedance sensing, due to the edge effect of the electric field. In fact, along the edges of the electrodes the field intensity is more than ten times larger that the average field. Simulations show that above 50μm the sensitivity in no longer influenced by the electrode length l. Thus, large electrodes can be

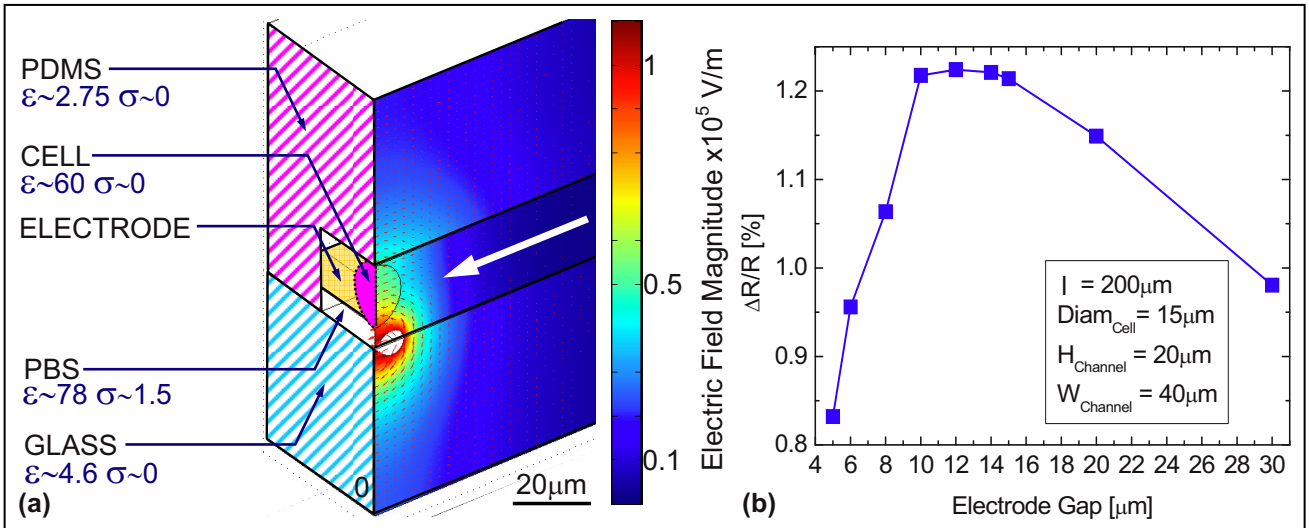

Figure 2: (a) Quarter model employed in FEM simulations of the symmetric channel geometry. The electric field magnitude in the middle transversal section is shown, highlighting edge effects. Relative permittivity ε and conductivity σ of the different domains are indicated. (b) The normalized resistance variation ΔR/R upon volume fraction modulation due to passing cells (5-15μm target diameters) depends on electrode gap (setting the field vertical extension) and reaches a maximum of 1.2% for a gap of 12μm. Sensitivity scales with cell volume and vertical position.

realized for increasing the double layer capacitance and consequently shifting to lower frequencies the beginning of resistive plateau of the bulk solution. A length of 200μm has been selected corresponding to a minimum frequency of ~10kHz, comparable with that obtained with platinum black treatment of smaller electrodes [3]. Simulations also confirmed that the sensitivity scales with cell volume (0.03% for 5μm against the top) and with the cell vertical position, doubling from the top to the middle height.

EXPERIMENTAL: IMPEDANCE DETECTION UNIT

A compact ad-hoc impedance detector has been designed and tested with the fabricated microfluidic device. As pictured in Figure 3, to measure impedance, a probe sinusoidal voltage (only few tens of mV) is applied to one electrode. The current flowing between the electrodes is amplified by a transimpedance stage (100kΩ) and synchronously demodulated with a phase-sensitive detector (by multiplying the signal by the same excitation signal and low-pass filtering the in-phase component). The core of the unit is a custom single-chip lock-in demodulator coupled with a 10MHz 20bit sigma-delta analog-to-digital converter [4]. The multiplier is realized with passive switches. The single-bit sigma-delta modulator has a standard 2nd order topology. The real (resistive) and imaginary (capacitive) part of impedance can be alternatively probed up to 1MHz. The chip has been fabricated in standard 0.35μm CMOS process and occupies an area of 0.5mm^2. A 3V supply voltage is provided by batteries. A field-programmable gate array (FPGA) filters the output digital stream (through a selectable decimation factor) and provides a direct USB interface for laptop data acquisition.

RESULTS AND DISCUSSION

The behavior of fabricated electrodes has been preliminarily characterized with impedance spectroscopy, before and after bonding the glass substrate to the PDMS. Vertical (versus a macroscopic platinum external counter electrode) and coplanar configurations have been compared for both 200μm and 50μm long electrodes.

It has been experimentally verified that for the calculation of the double layer capacitance (~0.1pF/μm^2), the whole geometric area has to be considered, irrespective of the edge effects. This can be explained considering that the local electrochemical interfacial field is ~100MV/m (~200mV/2nm), while the external field is ~0.1MV/m.

Very interestingly, it has been then observed that the vertical confinement of the resistive paths, due to the presence of the microchannel, produces a smooth transition (with a slope of ~10dB/decade) between the capacitive region and the resistive plateau.

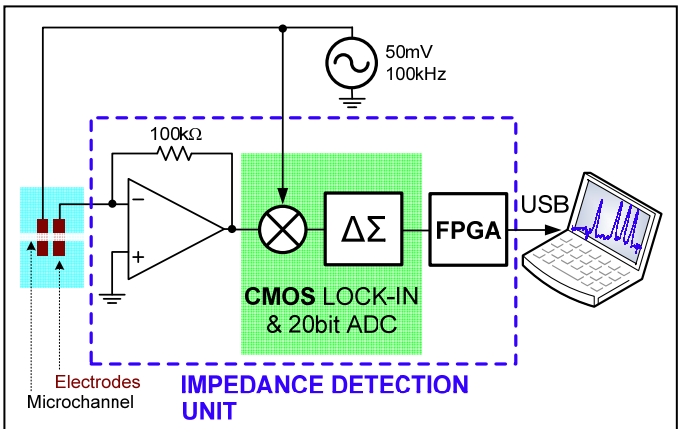

Figure 3: Compact custom impedance detection circuit based on a transimpedance amplifier, a single-chip lock-in demodulator and a 10MHz 20bit sigma-delta converter. Digital data are filtered by a FPGA and recorded via a USB interface.

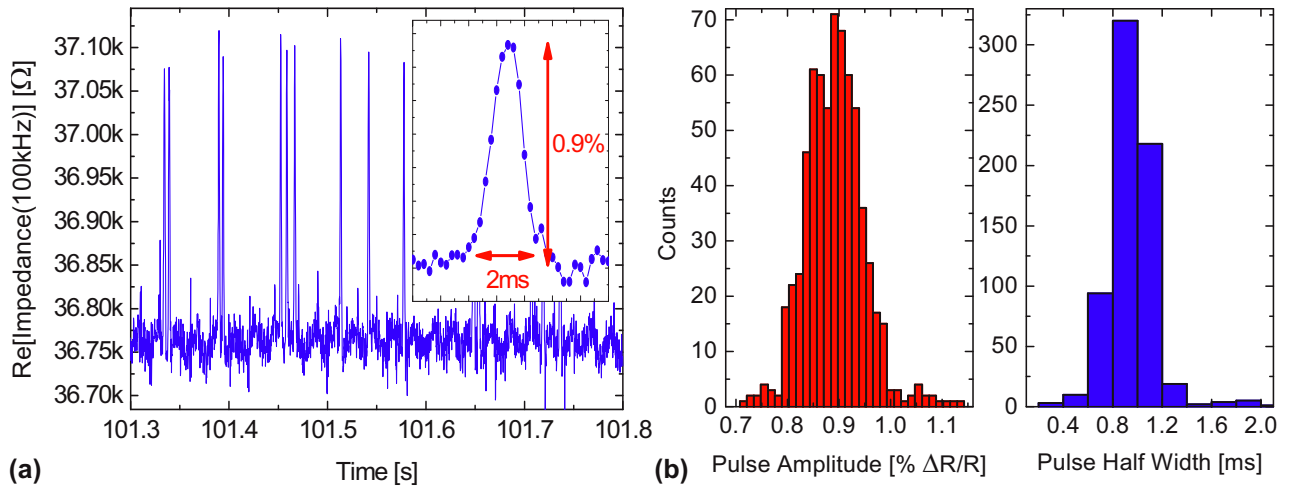

Figure 4: (a) Counting 10μm polystyrene beads diluted in PBS, flowing in the output channel at 50beads/s. The excitation signal is a 50mV sinusoid at 100.1kHz. A 5kHz sampling frequency provides sub-ms time resolution and a SNR=15 that allows low-noise threshold detection of single pulses (~340Ω = 0.9% ΔR/R). (b) Statistical analysis of a sequence of 677 beads. A dispersion of 10% in pulse amplitude is due to the spread in vertical position, while duration distribution is sharply peaked around 2ms and shows the 200μs acquisition temporal quantization.

By choosing an operating frequency of 100.1kHz and an excitation amplitude of 50mV, we have demonstrated particle counting using 10μm polystyrene beads, as shown in Figure 4. The beads are dispersed in PBS and injected in the device with a syringe pump at ~50beads/s. A sampling rate of 5kHz provides a 200μs temporal resolution that is adequate to capture each pulse with 10 samples (inset of Figure 4a). Increasing the rate up to 20kHz enables a maximum throughput of ~1000particles/s, comparable with the performance of state-of-the-art high-throughput systems [5]. Although electromagnetic shielding was not complete, in order to provide access to the microscope objective for optical cross-check of particle passage, a signal-to-noise ratio (SNR) of 15 has been measured. It allows implementing automatic counting by simple threshold detection. An example of off-line peak statistics performed in Matlab (Mathworks) is reported in Figure 4b. Peak amplitude (0.9±0.1%) is in agreement with the simulated values and pulse duration (2±0.2ms) is consistent with the input flow-rate (1μl/min). A third electrode for differential measurements is present, but slow thermal and mechanical drifts have been straightforwardly eliminated with a 300ms digital high-pass filter.

CONCLUSION

The complete compatibility of the impedimetric flow-cytometer circuit with standard CMOS microtechnology offers two key advantages: extreme miniaturization (and thus real portability) and parallelization. A bulky bench-top lock-in amplifier has been miniaturized into a millimeter-sized chip. Several detection channels can be integrated in parallel, one for each outlet channel. Furthermore, it is envisioned that multiple lock-in's, contemporaneously locked on different frequencies, might be integrated to perform further particle discrimination, provided a bandwidth extension, as pioneered by Renaud and Morgan [5]. The feasibility of a new low-cost miniaturized fully-electronic single-particle sorting and analysis device has been demonstrated.

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CONTACT

*M. Carminati, tel: +39-02-2399-3773; carminati@elet.polimi.it