

Microfabricated Multi-Frequency Particle Impedance Characterization System

C. K. Fuller, J. Hamilton, H. Ackler, P. Krulevitch, B. Boser, A. Eldredge, F. Becker, J. Yang, P. Gascoyne

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MICROFABRICATED MULTI-FREQUENCY PARTICLE IMPEDANCE CHARACTERIZATION SYSTEM

Chris K. Fuller¹, Julie Hamilton¹, Harold Ackler¹, Peter Krulevitch¹, Bernhard Boser², Adam Eldredge², Frederick Becker³, Jun Yang³, Peter Gascoyne³

1. Lawrence Livermore National Laboratory, Center for MicroTechnology
2. University of California at Berkeley, Berkeley Sensor & Actuator Center
3. University of Texas MD Anderson Cancer Center
(contact: fuller14@llnl.gov)

Abstract

We have developed a microfabricated flow-through impedance characterization system capable of performing AC, multi-frequency measurements on cells and other particles. The sensor measures both the resistive and reactive impedance of passing particles, at rates of up to 100 particles per second. Its operational bandwidth approaches 10 MHz with a signal-to-noise ratio of approximately 40 dB. Particle impedance is measured at three or more frequencies simultaneously, enabling the derivation of multiple particle parameters. This constitutes an improvement to the well-established technique of DC particle sizing *via* the Coulter Principle. Human peripheral blood granulocyte radius, membrane capacitance, and cytoplasmic conductivity were measured ($r = 4.1 \mu\text{m}$, $C_{\text{mem}} = 0.9 \mu\text{F}/\text{cm}^2$, $\sigma_{\text{int}} = 0.66 \text{ S}/\text{m}$) and were found to be consistent with published values.

Keywords: Coulter counter, cell impedance, cell capacitance, electrorotation, MEMS

1. Introduction to Particle Impedance Characterization

The Coulter counter, a commercial instrument which uses DC impedance (resistance) measurements to determine the volume of small particles in suspension, has existed for over 40 years. Instruments that combine Coulter Principle and single high frequency impedance measurements to determine the cellular cytoplasmic conductivity are available for blood cell analysis. However, such two-parameter measurements are incapable of distinguishing between some particle subpopulations of interest (e.g. granulocytes and monocytes, or spores and background particles). What is desired for a host of hematology, pharmacology, forensic, and counter biological warfare applications is a system that can perform broad-band impedance characterizations of particles to enable superior particle differentiation *via* features in their impedance spectra. Recent research has shown that cellular parameters vary with such physiologic alterations as apoptosis, malarial infection, cell differentiation, and exposure to toxins.

A method often employed for broad-band electrical characterization of particles is electrorotation (ROT), whereby a rotating electric field is used to generate a torque on a particle [1]. The magnitude and direction of the torque, and the resulting particle rotation, depend on the particle's dielectric properties. By measuring a particle's rotation rate as a function of excitation frequency, its conductivity, membrane capacitance, membrane resistance, and other properties can be determined. Although the technique is precise, it requires significant measurement time; even automated ROT systems take several minutes per particle [2].

A faster approach uses a pulse-FFT measurement scheme [3]. As particles pass through a sensor orifice, a broadband electrical impulse is used to excite them. The particle's impedance signature is then generated using the Fast Fourier Transform (FFT) of the sensor's impulse response. This technique produces a quasi-continuous measure of the particle's impedance spectra, but since the energy of the excitation signal is spread across a band of frequencies, the signal-to-noise ratio (SNR) is inherently worse than in a discrete frequency system.

Another approach involves the use of tuned oscillators to perform simultaneous impedance measurements at multiple, discrete frequencies which are chosen to capture the important transitions in the particle's impedance spectra [4]. Since the system's signal power is concentrated at the measurement frequencies, this technique produces a better signal to noise ratio than the pulse-FFT approach. However, changes in the operating frequencies or solution conductivity may cause the oscillator circuit to become de-tuned. Such a system is most useful for measurements involving predetermined particle types under specific operating conditions.

2. System Architecture

The system presented here combines the advantages of microfabrication technology (low cost, scalability, size) with a new circuit architecture and high-speed data processing to overcome the limitations of existing impedance characterization schemes. It consists of a microfabricated device (Fig. 1) with two identical microchannel sensors: one channel for sensing particles as they flow through the device and the other for use as an electrical reference. The microfluidic chip is mounted on a circuit board (Fig. 1) containing electronics that simultaneously measure resistive and reactive changes in the differential impedance of the microchannel sensors. The system operates at multiple, software programmable frequencies from 100 kHz to 10 MHz and can accommodate solution conductivities of between 50 and 250 mS/m. Unique packaging (Fig. 1) allows the sensor, circuit board, and fluidic interconnects to be assembled easily and within a small footprint.

Microfabrication techniques enable the construction of specific sensor geometries which maximize the operational bandwidth by reducing the effects of the sensor's parallel plate and double-layer capacitances. The microchannels are isotropically etched 20 μm into a glass substrate, and electrodeposited photoresist is used in a lift-off process to define 3D platinum electrodes in the channel bottoms [5]. The opposing electrodes are patterned onto a second glass substrate that is ultrasonically drilled to produce electrical and fluidic *vias*. These two glass plates are aligned and anodically bonded, sealing the electrodes inside the channels. The resulting active sensing region is approximately 70 μm wide and 70 μm long with 20 μm of separation between the electrodes.

The system uses a homodyne circuit architecture which mixes the resistive and reactive components of particle impedance to DC for detection. It accommodates various operating conditions and measurement frequencies with a simple gain adjustment; the tuning of individual frequency channels is not required. Moreover, system and sensor-induced gain and phase shifts are easily normalized using polystyrene calibration beads. During testing, particles suspended in the carrier solution flow through the sense microchannel, while particle-free carrier solution flows through the reference channel. The sense and reference electrode sets are driven out of phase using a signal formed from a summation of the measurement frequencies. The currents through each electrode set are combined, amplified, mixed, filtered, digitized, and transferred to a computer for analysis. As particles enter the sensor region, the computer stores the maximum changes in the difference currents for the in-

phase and quadrature-phase components at each frequency. These changes are proportional to the real and imaginary parts of the particle's impedance, from which the desired parameters are derived.

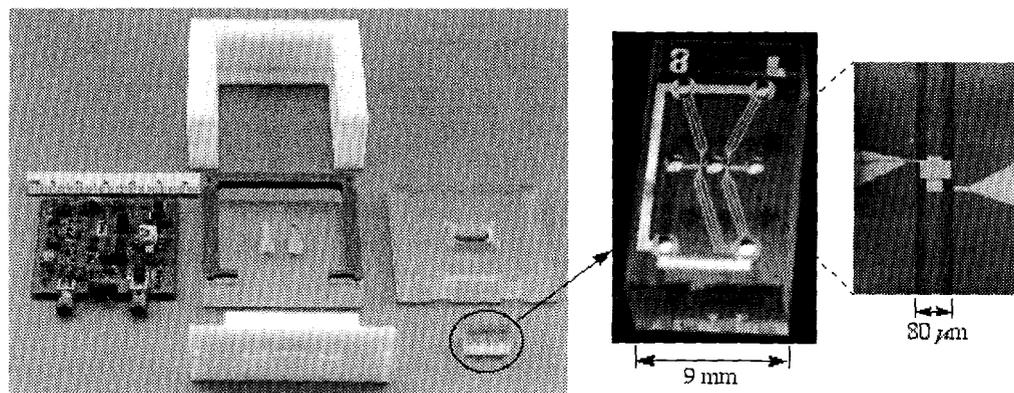


Figure 1: Detection electronics, device package, and the microfabricated sensor (left). Close-up of the sensor with both the sense and reference channels visible (center). A microscope view of the microfabricated channel and parallel plate sensor electrodes (right).

The first system prototype uses discrete mixers, filters, and direct digital signal synthesis circuitry to measure complex impedance at three frequencies, and it achieves an SNR of approximately 40 dB. A mixed digital and analog application-specific integrated circuit (ASIC) that will expand the number of possible simultaneous measurement frequencies to eight and improve the SNR has been fabricated and is undergoing testing. Eight frequencies will enable the use of more complex particle models and allow conclusions to be drawn about additional particle parameters, including cell membrane conductivity and internal permittivity (which reflects endoplasmic reticulum and nuclear structure). For both systems, the maximum measurement rate is limited by the analysis software to less than one hundred particles per second, but by incorporating the peak picking and filtering algorithms in hardware, measurement rates exceeding one thousand particles per second are readily attainable.

3. Results and Discussion

Initial device testing revealed differences in the measured and expected volume distributions of calibration beads. This discrepancy was attributed to the settling of beads in the microchannel; at low flow rates the beads fall from suspension and encounter the fringing field at the edge of the sensor electrode. For flow rates in excess of 1 ml/hr, hydrodynamic focusing positions the particles towards the channel's center, away from the inhomogeneous field, and the measured particle sizes closely match the predicted distribution based on the manufacturer's specifications (Fig. 2).

Preliminary tests using human peripheral blood granulocytes in a 50 mS/m buffer solution are shown in Fig. 3. The mean radius of 4.1 μm , membrane capacitance of 0.9 $\mu\text{F}/\text{cm}^2$, and cytoplasmic conductivity of 0.66 S/m derived from impedance measurements compare favorably with accepted mean values from ROT experiments ($r = 4.7 \mu\text{m}$ and $C_{\text{mem}} = 1.1 \mu\text{F}/\text{cm}^2$, $\sigma_{\text{int}} = 0.60 \text{ S/m}$) [6]. In addition, the system successfully measured the expected difference in cytoplasmic conductivity between paraformaldehyde fixed and unfixed granulocytes.

4. Conclusion

A system has been demonstrated that accurately measures resistive and reactive particle impedance in a flow-through system. Its wide bandwidth and easily adjustable operating frequencies enable characterizations of various particle types under different operating conditions. The volume, cytoplasmic conductivity, and membrane capacitance of human peripheral blood granulocytes were successfully measured. It is anticipated that this system will be capable of differentiating sub-populations of cells and biological particles that are indistinguishable with existing flow-through electrical particle characterization techniques.

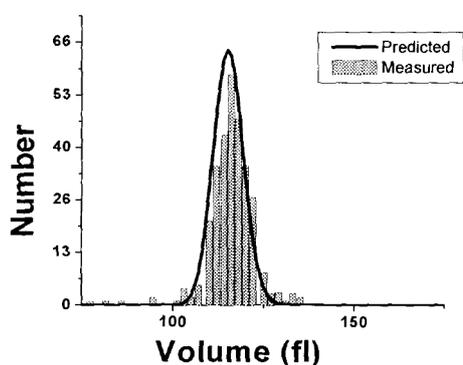


Figure 2: Predicted and measured volume distributions for calibration beads.

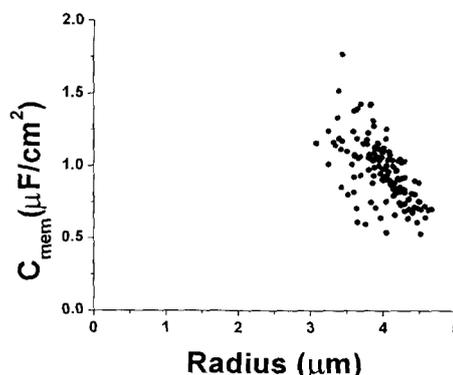


Figure 3: Cellular parameters derived from preliminary measurements.

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