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Bandwidth Compensation for High Resolution Impedance Spectroscopy

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Abstract

This paper reports on a microfluidic differential impedance cytometer, which uses a bandwidth compensation technique together with a small detection volume and multi-frequency analysis to achieve an increased sensitivity. The bandwidth compensation technique allows for measurements within small bandwidths by accounting for the increased signal amplitude dependence on the particle speed. We demonstrate detection and clear baseline discrimination of polystyrene beads with diameters of 1 μm and 2 μm and the discrimination of 5 μm beads from yeast cells of similar size. We show that using multiple frequencies in parallel significantly improves the discrimination performance of the cytometer.

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Nomenclature

CML Carboxylate-modified latex

PBS Phosphate buffered saline

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1. Introduction

Electrochemical impedance spectroscopy is a powerful method for label-free flow-through detection and analysis of individual biological cells in a lab-on-a-chip setup [1]. Particles are dispersed in a liquid, which flows through a microfluidic channel with two pairs of planar electrodes patterned on top and bottom. An AC voltage is applied to the electrodes causing a current to flow between them. The current change upon passage of a particle between the electrodes is measured differentially and analyzed to determine the cellular dielectric properties. The dielectric characteristics provide quantitative information on membrane capacitance, membrane resistance and cytoplasmic resistance [2-3].

A number of factors influence the sensitivity and specificity of such a system. The volume fraction of the particle, defined as the ratio of particle volume to that of the electrical field, influences the magnitude of the current change and should be maximized. The relative position of the particle influences its speed, due to the parabolic flow profile in the channel, as well as the shape of the detected signal [4]. The signals are measured using an impedance spectroscopy, which acts like a band-pass filter centered at the AC frequency. A smaller bandwidth—or alternatively a larger time constant—rejects noise, but makes the filter slow to react, causing a reduction in measured amplitude for faster moving particles.

In this work we present a technique for compensating for the influence of particle speed on the amplitude of the measured signal, due to the bandwidth of the impedance spectroscopy. This enables measurements at a lower bandwidth for a given flow rate, which increases the signal-to-noise ratio and therefore the sensitivity. We combine this with a new microfluidic device with reduced channel dimensions to greatly increase the sensitivity in comparison to existing systems [5].

2. Method

The microfluidic device consists of two glass plates with 200-nm-thick and 20- μm -wide platinum electrodes. The two plates are bonded face-to-face using a 14- μm -thick lithographically structured dry film resist as spacer that defines the microfluidic structure. The final channels have a reduced cross-section of $14 \times 20 \mu\text{m}^2$.

Fig. 1 (a) shows a block schematic of the measurement setup. The impedance spectroscopy contains exponential moving average filters with configurable order and bandwidth. Fig. 1 (b) shows the results of processing an ideal signal from a particle passing the electrodes by using a model of the impedance spectroscopy filter with different bandwidths. This illustrates how a smaller bandwidth can lead to a reduction in amplitude. The transit time is the time it takes a particle to move between the electrode pairs.

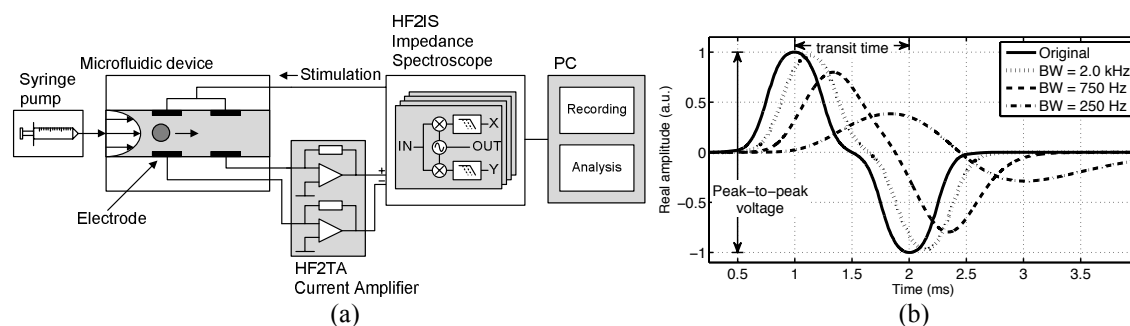


Fig. 1. (a) Block schematic of the measurement setup using an HF2IS Impedance Spectroscopy and an HF2TA Current Amplifier (both Zurich Instruments AG, Switzerland) for converting currents to voltages; (b) Response of a particle passing the electrodes (Original) and filtered response for three different bandwidths (BW).

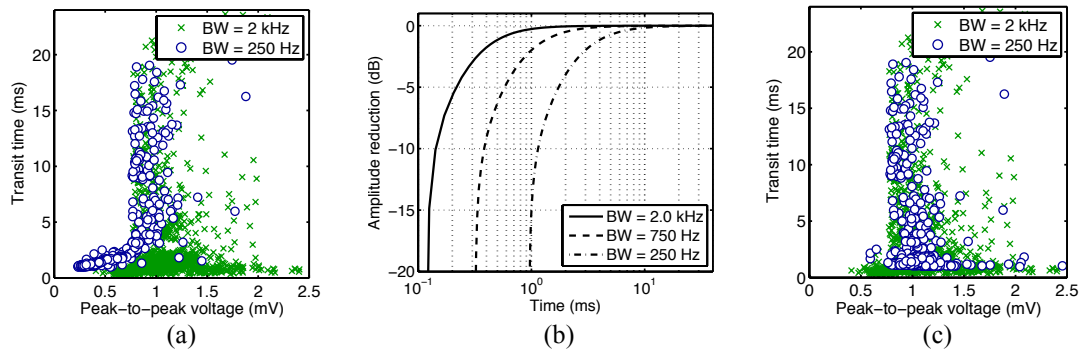


Fig. 2. (a) Differential impedance measurement of carboxylate modified latex (CML) beads featuring a diameter of $5\ \mu\text{m}$ recorded at $3.8\ \text{MHz}$ using two different bandwidths; (b) Influence of the HF2IS Impedance Spectroscopy setup, which was configured with different bandwidths, on the measured signal as a function of transit time; (c) Data from (a) after applying the bandwidth compensation technique.

The effect is further illustrated in Fig. 2 (a), which shows a scatter plot of the detected peak-to-peak amplitudes from two measurements of $5\ \mu\text{m}$ beads made with different bandwidths. The amplitude of the faster moving particles shows a skew when measured with a low bandwidth of $250\ \text{Hz}$. Fig. 2 (b) shows the simulated amplitude reduction for different bandwidths and particle speeds. These curves are used to compensate the amplitude of the detected particles, the result of which is shown in Fig. 2 (c), thereby achieving an improvement in accuracy of more than 60% compared to a large-bandwidth measurement.

3. Experimental results

Fig. 3 shows that the combination of bandwidth compensation and small channel dimensions makes it possible to determine the size of objects with unprecedented accuracy in comparison to using only, e.g., a 2-D hydrodynamic focusing stage in a larger channel [6]. The histogram in Fig. 3 (b) has been fitted with Gaussian curves. The relationship between the centers of the two main peaks show a cubic dependence on the mean diameter of the beads ($20.5\ \mu\text{V} \cdot 2^3 / 1.1^3 \approx 123\ \mu\text{V}$), meaning that the amplitude is proportional to the volume of the detected object.

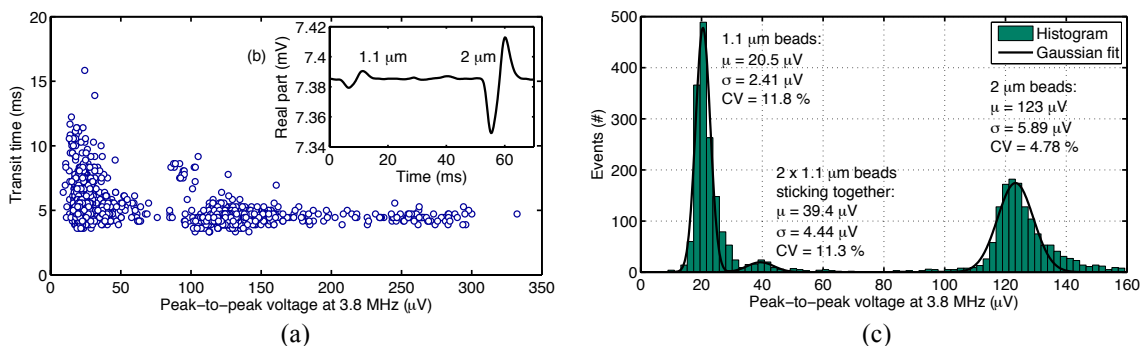


Fig. 3. (a) Differential impedance measurement of CML beads with diameters of $1.1\ \mu\text{m}$ and $2\ \mu\text{m}$ suspended in PBS, recorded at $3.8\ \text{MHz}$ and a bandwidth of $100\ \text{Hz}$. (b) Real part of the electrical impedance of two individual events. (c) Histogram of the same measurement fitted with Gaussian curves showing the mean (μ), standard deviation (σ) and coefficient of variation (CV) for the populations.

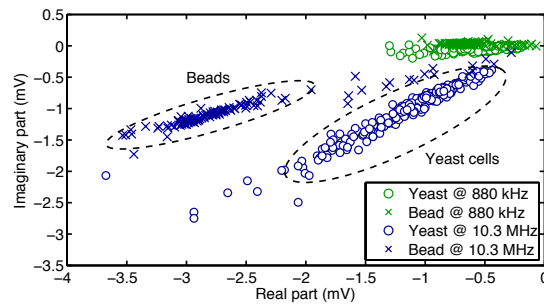


Fig. 4. Scatter plot of real and imaginary parts of the impedance signals of a mixture of 5- μ m-size beads and yeast cells measured simultaneously at 880 kHz and 10.3 MHz; data from all measured frequencies was used for clustering.

Fig. 4 demonstrates discrimination of 5- μ m-sized particles and yeast cells of similar size (*S. cerevisiae*, BY4741 strain) by using multiple frequencies together with the bandwidth compensation technique. At 880 kHz, particles and cells are indistinguishable from each other, but at 10.3 MHz, the capacitive nature of the membrane of the cells starts to play a role, giving rise to a more capacitive (imaginary part) and less resistive (real part) response. A clear discrimination becomes possible, allowing the use of standard clustering methods for classifying the data.

4. Conclusion

We have developed a bandwidth compensation technique, which increases sensitivity by allowing measurements to be performed with a low bandwidth. Clear baseline discrimination of 1 μ m and 2 μ m beads has been demonstrated by combining the technique with a microfluidic device with reduced channel dimensions. We have also shown that discrimination between 5 μ m beads and yeast cells is possible by measuring at several frequencies in parallel and clustering the resulting multidimensional data set.

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