CODED ELECTRODES FOR LOW SIGNAL-NOISE RATIO SINGLE CELL DETECTION IN FLOW-THROUGH IMPEDANCE SPECTROSCOPY

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ABSTRACT

We introduce a novel way of improving the signal-to-noise ratio of microfluidic flow-through impedance spectroscopy (FTIS) by increasing the number of electrodes and arranging them according to a maximum-length-sequence pattern. Our results show this concept of coded electrodes will be particularly suitable for systems where high sensitivity is required and which are dominated by noise that is uncorrelated with the electrode area.

KEYWORDS: flow-through impedance spectroscopy, correlation, signal-to-noise ratio, matched filter

INTRODUCTION

Microfluidic single cell flow-through impedance spectroscopy (FTIS) has demonstrated that it can successfully discriminate between different cell populations such as for white blood cells [1]. Measurements on platelets, with a typical diameter of 2-4 µm, result in a low signal-to-noise ratio (SNR). There are three primary sources of noise: (a) electronic noise from the channel impedance between the electrodes, (b) noise from the amplification electronics [2] and (c) noise from background particle debris as they occur as a result of lysing the red blood cells to enable measurements on the white blood cells [1].

THEORY

Figure 1 shows a schematic cross-section of a FTIS chip with input electrodes placed on top and output electrodes on the bottom. As a cell passes each electrode pair, it causes a change in impedance with respect to the other undisturbed electrodes. Dependent on the AC signal frequency, this change is related to the cell's size, membrane capacitance, and cytoplasm conductivity and can be described as a raised cosine shaped pulse. The orientation of the pulse (positive/negative) depends on how each particular output electrode is connected to the differential amplifier. In the special case when the number of electrodes, K, is limited to 2, the resulting chip design is identical to previous designs as used in [1,2,3].

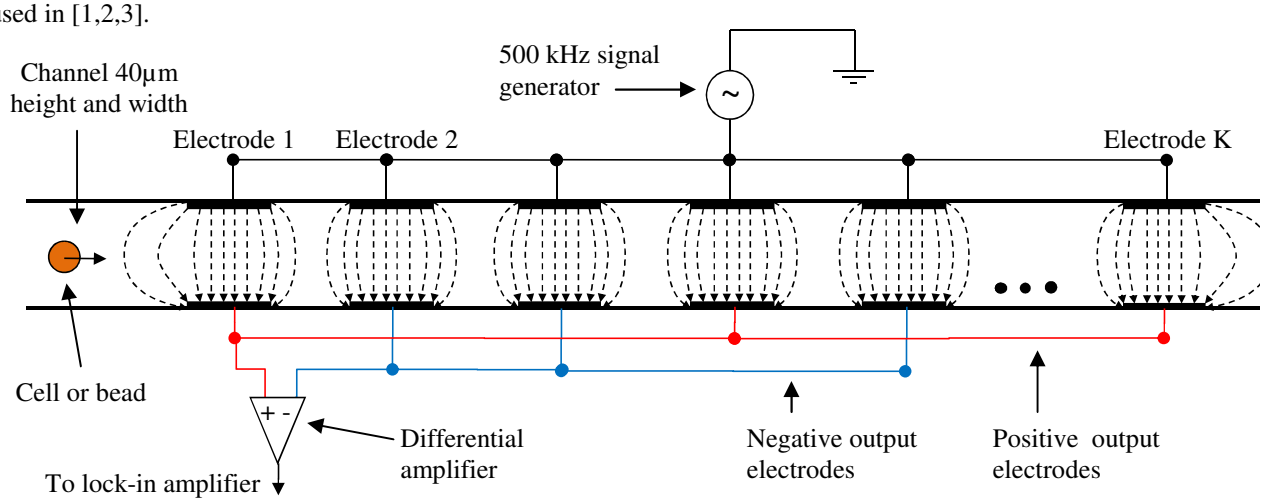

Figure 1: Cross section of the microfluidic FTIS chip with input and output electrodes placed on top and bottom respectively. As a cell, flowing in the microfluidic channel, passes the different electrode pairs (top/bottom) it causes a raised- cosine pulse shaped current change. The orientation of the pulses (positive/negative) depends on how that particular electrode is connected to the differential amplifier. For each cell flowing through the sensor a code like signal is therefore generated which shape (or code) is determined by the electrode connections to the amplifier.

When the number of electrodes is increased by a factor of N, while the excitation voltage is kept constant, the signal energy is increased by a factor of N. In most cases the dominant noise source is noise from the external amplification electronics [2], which remains unaffected by the increased electrode area. As such, the SNR increases proportional to the number of electrodes N.

A potential impact of the increased electrode count is a lower temporal resolution as more particles will be in the detection volume simultaneously. To overcome this effect, the electrodes are arranged according to a maximum-length sequence (MLS) code pattern. MLS codes have a sharp autocorrelation and are spectrally flat with the exception of the DC term. Since MLS codes have odd lengths, a single extra balancing bit is added at the end to prevent a bias current from an uneven number of electrode pairs.

The robustness of the detection principle is verified with a simulation to see whether particles can be detected with a low SNR. For this purpose, a 16 bit code sequence [+,-,+,-,-,-,-+,+,+,-,+,+,-,-,+] is used. As can be seen in Figure 2, even when the simulated 16 bit signal of a single bead is apparently masked by noise, the bead's position in time and the original signal amplitude can be successfully identified as a result of the sharp autocorrelation of the 16 bit code sequence.

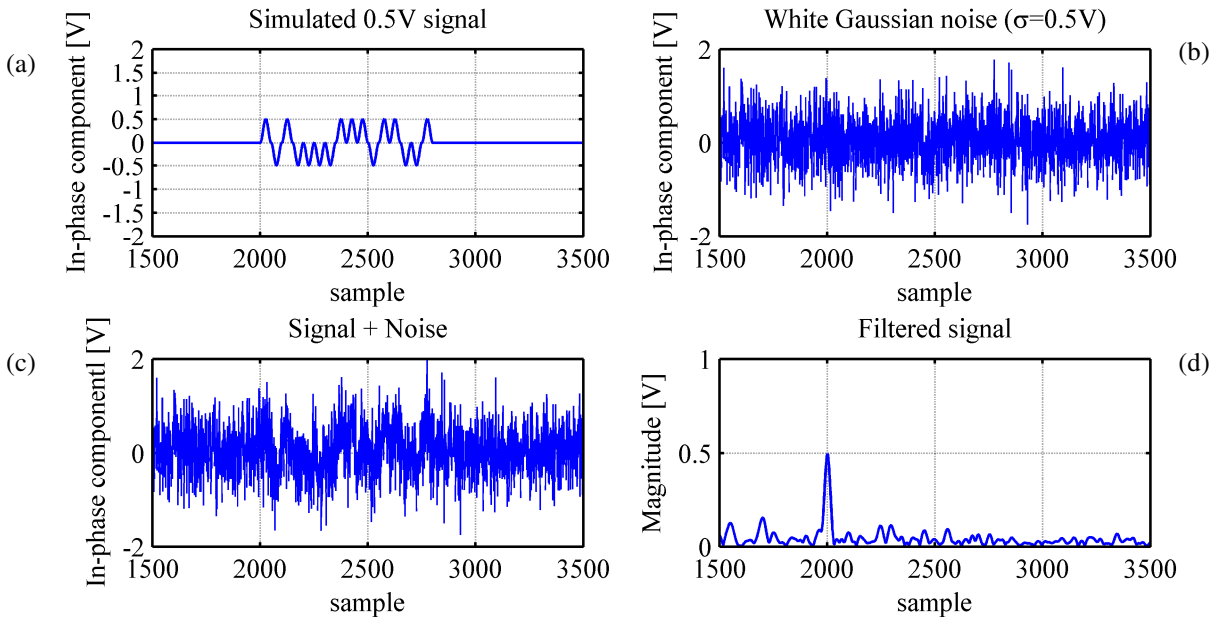

Figure 2: (a) Simulated in-phase signal of a single bead in a 16 bit chip with an amplitude of 0.5V. (b) White Gaussian noise signal with μ=0V and σ=0.5V. (c) The simulated signal with the noise added and (d) the result of this noisy signal after being filtered with the matched filter. Although the signal is clearly masked by noise it is possible to successfully retrieve it.

EXPERIMENTAL

Together with Philips MiPlaza, a 16 bit chip was designed and manufactured, of which a close-up of the electrode section can be seen in Figure 3. The chip is connected via a SR844 lock-in amplifier (Stanford Research Systems, Sunnyvale, CA) and a PCIe-6251 data acquisition card (National Instruments, Austin, TX) to a desktop PC. The sample consisted of 5 μm NIST Microbeads (Polysciences, Warrington, PA) diluted to a concentration of 10^5 particles per mL in pocH-pack D diluent (Sysmex, Japan). A 500 kHz sine wave was used as excitation frequency and data was sampled at a frequency of 110 kHz.

To detect the presence of a particle in the signal, a matched filter is applied. As a template for the filter a series of raised cosine pulses was used where each pulse is oriented according to the original MLS code sequence. Next, an array of matched filters was created, each scaled in length to represent particles travelling at different speeds as a result of the parabolic flow profile in the channel. Since each filter is normalized with respect to energy, the output of the matched filter provides the signal amplitude as function of particle speed and time. A streaming min-max filter [4] is then used to detect local maxima in this function. Each maximum represents a potential particle with properties {signal amplitude, speed, time}. All signal processing was performed in MATLAB (MathWorks, Natick, MA).

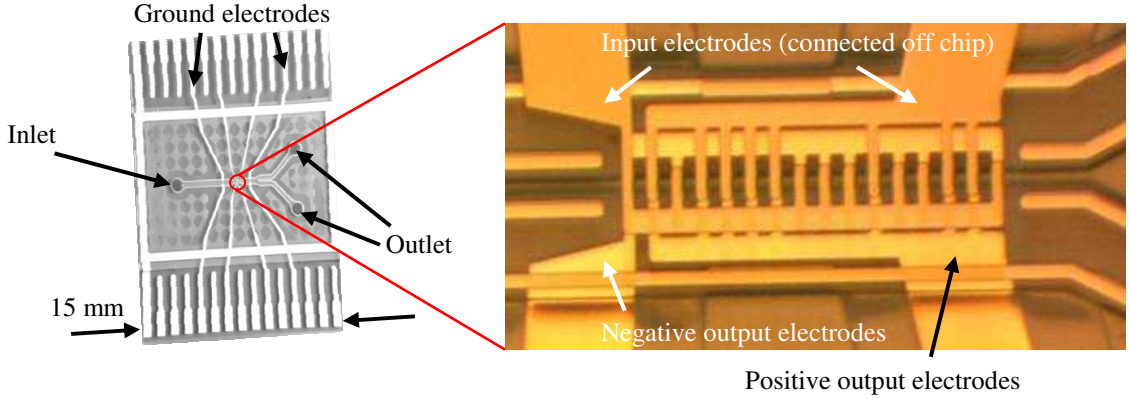

Figure 3: Top down view of a general microfluidic FTIS chip with close-up view of a 16-bit coded electrode section. The electrodes have a 70 μm spacing and a MLS code sequence: [+,-,+,-,-,-,-+,+,+,-,+,+,-,-,+].

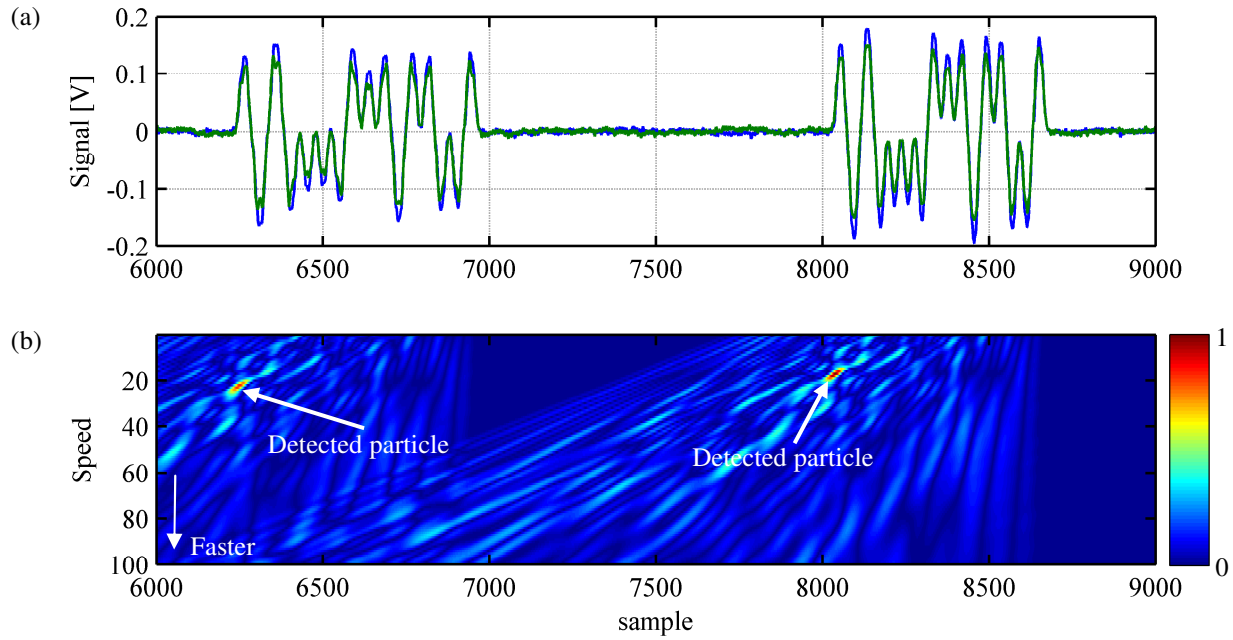

Figure 4: (a) In-phase (blue) and out of phase (green) signals from a 5 µm polystyrene bead experiment at 500 kHz. (b) Matched filter output showing normalised particle amplitude (colour intensity) as a function of time and particle speed.

RESULTS AND DISCUSSION

Figure 4a shows a brief trace of a coded electrode chip experiment as seen at the lock-in amplifier output. It can be clearly seen that each of the two 5 µm beads passing the electrode structure generates a sequence of 16 pulses according to the designated MLS code sequence.

Figure 4b shows the same signal enhanced with the matched filter array. The filter has transformed the two long signals into two relatively small areas of high intensity (signal amplitude) due to the sharp autocorrelation of the code sequence. As such, the analysis can cope with (partially) overlapping signals, which happens when multiple particles are in the sensing volume at the same time.

The principle of coded electrodes works well when noise sources are uncorrelated with the increased electrode surface area of the coded chips. This is the case when the microfluidic device is used as part of a complete blood count, where the dominant noise sources are either the amplification noise or the background debris particle noise as a result of a red blood cell lysis.

CONCLUSION

Our simulation results show that the coded electrodes are able to detect signals completely masked by white Gaussian noise. The experimental results from the 16 bit MLS coded chip confirm that the coded electrode principle works on a microfluidic chip. It will be particularly suitable for systems where high sensitivity is required and which are dominated by noise uncorrelated with the electrode area.

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