On-Chip Detection of Beads with a New Electrical Impedance Sensor

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***Abstract*— Electrical impedance measurements in microfluidic chips are used for single cell analysis. Parallel electrodes are more suited than planar ones since the electrical field distribution is more homogenous. Previous studies showed methods to make parallel electrodes by incorporating an additional layer between two glass wafers, making electrical connections to both sides needed. Also alignment of electrodes is necessary, making the fabrication of parallel electrodes more elaborate. Therefore a new, simpler fabrication method is developed for the fabrication of parallel electrode chips by incorporating a floating electrode in the microchannel just by adding one step in the fabrication process. In this way, only one side of the chip contains electrical connections. Finally, electrical impedance measurements with 3 and 6 µm polystyrene beads were done. All beads were detected and we have shown that it is possible to distinguish the two beads sizes from each other with a confidence level of 95%, based on the relative change in the electrical impedance.**

I. Introduction

Electrical impedance measurements with planar electrodes were already used to determine the spermatozoa concentration in semen [1]. The deviations in the impedance change resulting from the passing of particles and cells were relatively large, partly due to the planar electrode configuration. According to simulation of Gawad et al. [2], parallel electrodes give rise to a larger change in the response due to the more homogeneous electrical field distribution. Parallel electrodes in microfluidic chips have already been reported [3,4]. However, these electrodes are mostly realized by incorporating an additional layer (for instance polydimethylsiloxane [3] and polyimide [4,5]) between two glass wafers each containing electrodes. This fabrication process for parallel electrodes is more elaborate, since electrical connections to both sides of the microchannel have to be realized and alignment of the electrodes are necessary. Therefore a new, simpler fabrication method is developed for the fabrication of parallel electrode by incorporating a floating electrode in the microchannel.

II. Chip Design

A. Floating electrode

The concept of the floating electrode configuration is shown in Fig. 1a actually consisting consists of three electrodes: two active electrodes and one floating electrode. The floating electrode is just deposited on the other side of the channel than the active electrodes and it is not connected (floating) during an electrical impedance measurement. Due to this, no electrical connections to both sides of the chip are necessary, but only electrical connections with the active electrodes have to be made. In order to operate the chip with the floating electrode properly, the chip consists of two electrode pairs that are situated in two microfluidic channels (see Fig. 1b). The main channel is separated from the side channel by a filter, since otherwise particles in the main channel could accidently enter the side channel and disturb the electrical impedance measurement in the main channel.

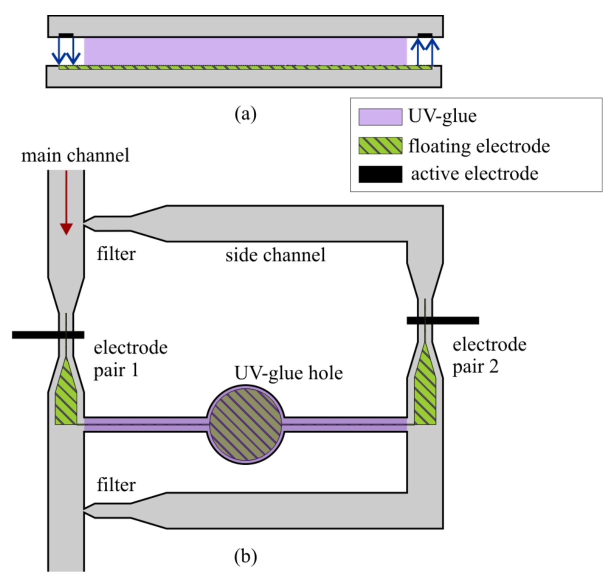

Figure 1. Schematic representation of the concept of the floating electrode (a) and the complete chip (b).

Furthermore an interconnecting channel between the main and side channel is necessary to host the floating electrode. This fluidic connection is also not desirable and therefore the channel is blocked by a barrier, which is in this case UV-glue. In this design no additional layer between the glass layers is necessary and for electrical impedance measurements only electrical connections to the active electrodes at one side of the microfluidic chip need to be made.

B. Fabrication process

The new fabrication process is developed for the fabrication of parallel electrodes in a microchannel is shown in Fig. 2. Compared to our fabrication process for planar electrodes [1], only one additional processing step is necessary. Furthermore the fabrication process contains no critical alignment of the electrodes and no other material than glass is needed for the realization of the microfluidic channel.

First a glass wafer is covered with chromium and gold layers, followed by photoresist (a1). By means of a standard photolithographic process and wet etching, the microchannel is realized (a2). The floating electrode is realized by sputtering the platinum through a shadow mask (a3). Finally, access holes are powder blasted from the backside (a4). Glass wafer 2 contains both active electrodes that are connected with the measurement system. Both electrodes are recessed using standard photolithography, such that proper bonding to glass wafer 1 is possible (b1+b2+b3). In the last step, both glass wafers are direct bonded using fusion bonding (c) and subsequently diced into separate chips.

With the described fabrication process, two parallel electrode pairs are formed in our chip. At both parallel electrode pairs, the microchannel has a width and height of 38 µm and 18 µm respectively. The active electrode spans the microchannel and has a width of 20 µm. The two electrode pairs are separated from each other by means of a filter and an obstruction, since otherwise particles could be simultaneously detected. UV-glue (Loctite 325) was used to block the interconnecting channel between the main and side channel. A drop of UV-glue was inserted in the UV-glue hole and due to capillary forces the glue spread through the interconnecting channel until a UV-source cured the glue.

III. METHOD

A. Characterisation of the chip

First the frequency behavior of the microfluidic chip was characterized using the HP impedance/gainphase analyzer type HP4194A to ensure that the electrical impedance measurements were done at a frequency within the resistive plateau [1]. For this purpose the microfluidic chip was filled with the background electrolyte and a Bode plot from 100 Hz to 40 MHz was made.

B. Electrical impedance measurements

After the characterization of the microfluidic chip, the chip was put into a homemade chip holder, enabling reliable electrical and fluidic connections. A syringe pump (Harvard PHD2000) was used to pump the fluid through the chip with a flow rate of 0.02 µL/min. Electrical impedance measurements were done at a frequency of 600 kHz using a HF2IS impedance spectroscope in combination with the HF2CA current amplifier (both Zurich Instruments, Zurich, Switzerland). The chip with chip holder was put on an inverted microscope, enabling visual inspection during the measurements. First a suspension of 6 µm polystyrene beads diluted in washing medium was guided along the electrode pair. The measured signal was analyzed using Matlab (R2007B, version 7.5.0.342, 2007, the Mathworks Inc.). In the Matlab program, the signal was converted to an electrical impedance signal, followed by detection of the peaks and calculation of the peak heights.

Next the same measurement was performed for a suspension of 3 µm polystyrene beads. At the measurement frequency of 600 kHz, the measured electrical impedance change is related to the size of the particle [2,4]. Therefore a smaller change in electrical impedance signal is expected when a smaller particle passes the electrode pair.

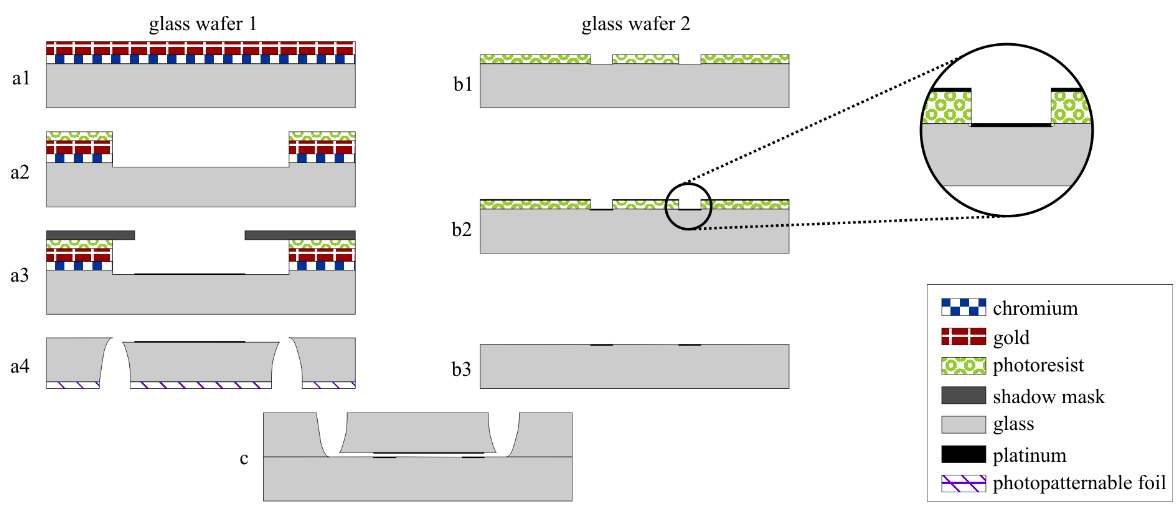

Figure 2. Fabrication process of the microfluidic chip. Glass wafer 1 contains the floating electrode in the microchannel. The electrodes at the other side are made on glass wafer 2. The extra step in comparison with the fabrication of a planar electrode configuration is step a3.

C. Samples

Two diameters of polystyrene beads were used during the experiments. These are Polybead Polystyrene Blue Dyed beads with a diameter of 6 µm and Polybead Polystyrene Red Dyed beads with a diameter of 3 µm, both obtained from Polysciences Inc (Warrington, Pennsylvania USA). The beads were suspended in Ferticult^{TM} Flushing medium (chemically balanced salt solution, HEPES buffered with 0.4% HSA, purchased from Fertipro NV (Beernem, Belgium)) with a specific electrical conductivity of 1.4 S/m.

IV. RESULTS AND DISCUSSION

A. Blocking of the interconnecting channel

The results of the blocking of the interconnecting channel with UV-glue are shown in Fig. 3. In this figure a slightly different chip design was used, that did not yet contain any electrodes, but the dimensions of the channels are the same as in the microfluidic chip used for the electrical impedance measurements. After adding a drop of the UV-glue in the inlet, the glue was cured with a UV-source (365 nm, ELC-403, Electro-lite corporation). Clearly, the blocking of the fluidic connection between both channels can be seen.

B. Frequency characteristics of the microfluidic chip

After blocking the fluidic connection, the frequency behavior of the chip filled with washing medium was determined. In Fig. 4 the results of the frequency characterization of the microfluidic chip are shown. For the detection of beads in the microchannel, a measurement frequency of 600 kHz was chosen. This frequency is suited for the electrical impedance measurements, since it is at the resistive plateau of the Bode plot. At this frequency the double layer capacitance, that dominates the Bode plot at lower frequencies, has a negligible influence on the measurements. In addition, the parasitic capacitances, dominating the high frequency part of the Bode plot, can also been neglected.

C. Detection of polystyrene beads

All polystyrene beads caused a change in the electrical impedance signal as was observed with visual inspection. A typical electrical impedance signal is shown in Fig. 5. Four 6 µm polystyrene beads passed the electrode pair, clearly causing a change in the electrical impedance. The peak height of each change was calculated using a Matlab program. In total the peak heights of 95 polystyrene beads with diameter of 6 µm were calculated (461 ± 124 Ω) and none of the beads went into the side channel.

In the next experiment a suspension with 3 µm polystyrene beads was put in the microfluidic chip. The peak heights calculated from the electrical impedance change of these beads were 56 ± 13 Ω (n=105). The average peak height is smaller for the 3 µm beads than 6 µm beads as expected by their size. In Fig. 6 the 95% confidence intervals of the relative impedance changes when 3 and 6 µm beads passed a parallel electrode pair are shown. The intervals do not overlap,

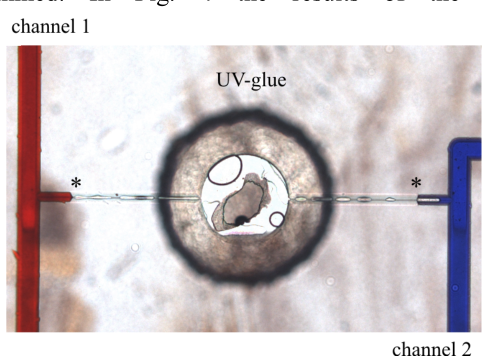

Figure 3. The results of the UV-glue in a different, but comparable chip. Channel 1 (red) and channel (2) are not in contact with each other. The asterisks denote the borders between the UV-glue and the fluid in both channels.

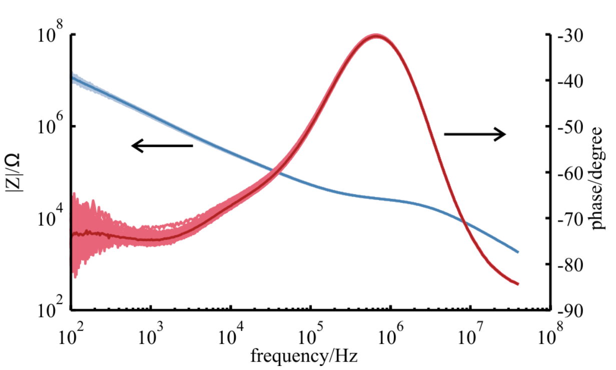

Figure 4. The Bode plot for the characterisation of the chip. The blue line is the measured impedance and the red line indicates the phase.

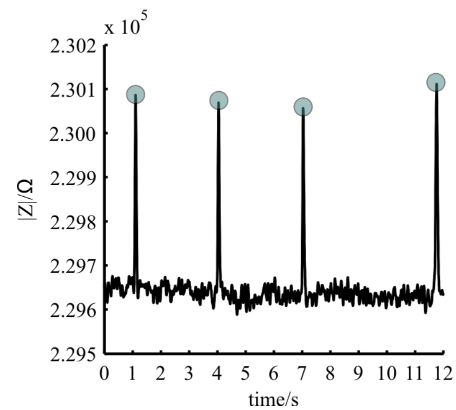

Figure 5. Typical example of an electrical impedance measurement where four 6 µm beads passed the electrode pair

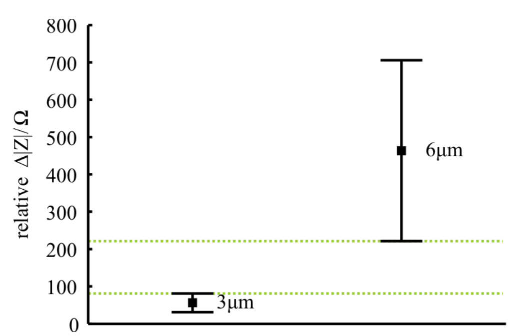

Figure 6. The 95% confidence intervals of the relative impedance changes when 3µm and 6µm beads passed the electrodes

so it is possible to distinguish between the beads sizes based on the relative impedance change.

V. Conclusions

The new fabrication method for the parallel electrodes configuration using a floating electrode is suitable for electrical impedance measurements. It was possible to count and distinguish 3 μm and 6 μm polystyrene beads with the novel parallel electrode configuration. Further investigation is focused on increasing the sensitivity of the electrical impedance measurement and improving the overall chip design.

Acknowledgment

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