Picoliter Droplet Dispenser with Integrated Impedance Detector for Single-cell Printing

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Abstract:
We present the fabrication and first experimental results of a microfluidic droplet dispenser with **electrical impedance detection of single cells**. The microdispenser features microchannels, two sets of parallel facing electrode pairs and an **on-demand droplet generator**. It has been fabricated by sandwiching dry film photopolymer (TMMF) between silicon and Pyrex substrates. The channels and dosage chamber are patterned in TMMF, the platinum electrodes are on the fluid-contacting sides of the silicon and Pyrex substrates. The presented fabrication is achievable at full-wafer level, offering **low temperature (75 °C) silicon to glass bonding** using adhesive TMMF. Experimental characterization for the electrical impedance detection using single micro beads (10 µm diameter) suspended in standard saline (PBS) has shown that the sensor is capable to detect single particles as they pass the electrode pairs. Real-time data processing is able to detect particles, calculate their velocities and issue a trigger to dispense the detected particle encapsulated in a microdroplet successfully.

Keywords: Cell printing, Impedance spectroscopy, Non-contact microdosage

Introduction

Single cell analysis is an important method for biology and life sciences ([1] and references therein), and there is a need for technical instruments to separate cells. Conventional cell studies are performed on large cell populations and inherently yield averaged and possibly misleading results. Cell heterogeneity can only be studied when analyzing cells individually. The development of technical instruments to separate and sort single cells efficiently will revolutionize single cell analysis and reveal new insights in cell biology. Such technologies are expected to will find various applications in pharmaceutical, cancer and stem cell research, cell line development, tissue engineering and diagnostics.
Drop-on demand printing is particularly suited for single cell separation because it is virtually independent of the substrate and cell deposition positions can be chosen freely. This allows the use of both standard laboratory substrates (micro well plate, glass slides etc.) and custom-made substrates on the same instrument.
InkJet printing technologies have been widely used to print viable biological cells [2]. For single cell applications it is crucial to avoid empty droplets and droplets with multiple cells. Therefore, cell detection in the printhead is necessary before printing, which is unavailable in standard InkJet printers.
In this work, we combine drop-on-demand printing [3] and electrical impedance particle detection (see [4] for a review). Previous works describe a similar approach with different dispenser, fabrication and electrode configuration. Nevertheless, reproducible single particle printing could not be demonstrated so far [5]. The combination of flow-through impedance particle detection and printing of cells has the potential to become a unique label-free method for sorting and positioning of single cells.

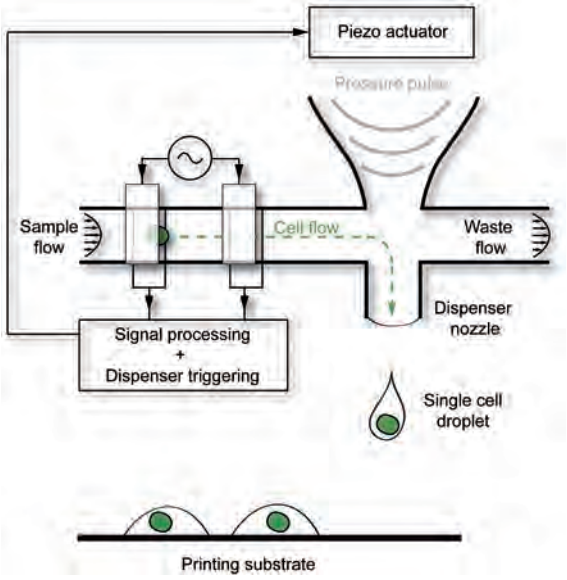

Fig. 1: *Schematic of single cell dispenser with integrated impedance detector. Cells passing the electrodes get detected and a piezo actuator generates microdroplets at the nozzle that encapsulate the detected individual cells or particles.*

Chip Design and Fabrication

The microfluidic dispenser chip includes three functional components: cell focussing channels (not shown), cell detection electrodes and a droplet generator.

The mode of operation has three basic steps. First cells or particles enter the sample inlet of the chip and are hydrodynamically focussed in two dimensions by a sheath flow. Subsequently, a stream of individual particles passes the sensing electrodes and, due to differential impedance measurement, each cell produces a double peak. A real-time processing algorithm detects both peaks, calculates the particle velocity, and sends a trigger signal to the dispenser when the particle is expected to arrive at the nozzle (Fig. 1).

In conventional flow-through impedance setups, cell detection is performed with co-planar electrodes (Fig 2 a)). This design causes inhomogeneous electrical fields and the particle position in vertical direction influences the measurement signal strongly. Here, to avoid such shortcomings differential measurement of the channel impedance is achieved via facing electrodes. Each electrode pair has one electrode at the top and one on the bottom of the channel (Fig 2 b)). This reduces the dependence on the vertical particle position of the signal.

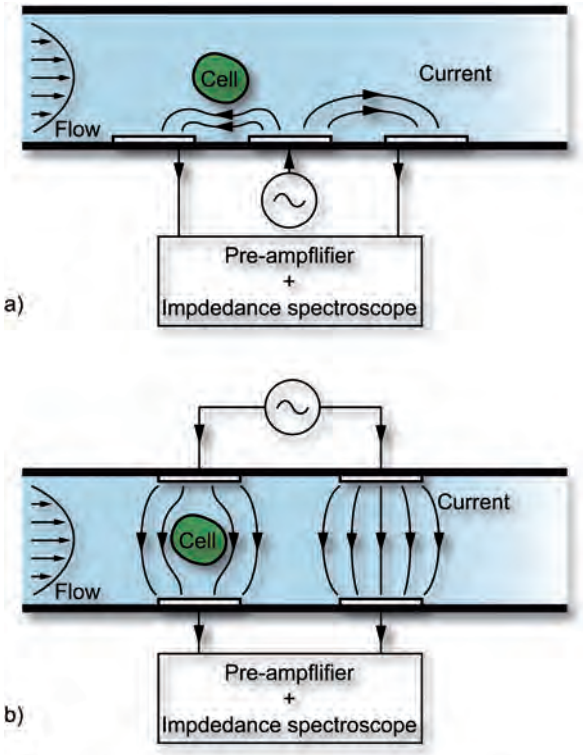

Fig. 2: *a) Schematic of impedance flow cytometry with parallel facing electrodes (similar to* [6]*). b) Schematic of impedance flow cytometry with co-planar electrodes (similar to* [7]*).*

The chip fabrication includes electrode patterning on Pyrex and silicon wafers, TMMF dry resist lamination on the Pyrex, photolithographic structuring of the fluidics and electrodes as well as thermal bonding (Fig. 3).

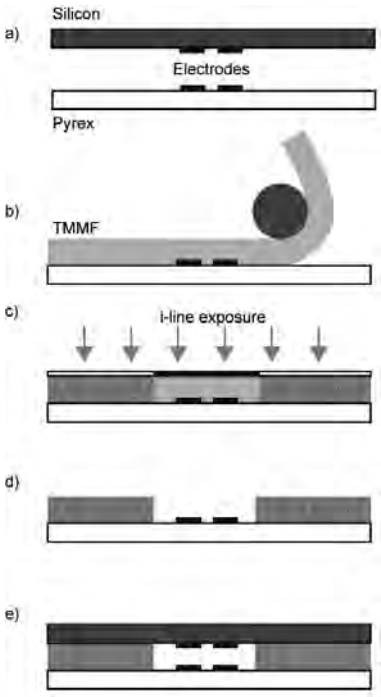

Fig. 3: *Fabrication process: (a) Electrode patterning, (b) TMMF lamination, (c) I-line exposure and (d) Development, (e) Glass to silicon bonding.*

The fabrication process for the Pyrex wafer consists of two steps. The first step is evaporation of platinum electrodes and to open hydraulic access holes by etching as described in our previous work [8]. Subsequently a TMMF layer with 55 µm thickness is laminated onto the Pyrex using hot roll lamination (60 °C, 1 m/min, 0.1 MPa). The fluidic channels are formed photolithographically using contact i-line exposure (150 mJ/cm^2). After exposure, the Pyrex wafer is baked on a hotplate for 2 minutes at 70 °C. Then the TMMF is developed in propylene glycol monomethyl ether acetate (PGMEA, SU-8 developer) and rinsed with isopropyl alcohol (IPA), deionised water and spin-dried. The fabrication of the silicon wafer steps consist of deposition of a passivation layer deposition (SiO_2) and platinum electrode patterning. First, a 500 nm SiO_2 is grown on the silicon wafer by thermal oxidation. Subsequently, a platinum layer (300 nm) is sputtered and the electrodes and bond pads are formed by a lift-off process. The facing electrode configuration is realized by bonding the glass/TMMF stack to the

silicon wafer (75 °C, 60 N/cm^2, 50 minutes). Finally, dicing separates the chips (Fig. 4) and exposes the dispenser nozzle.

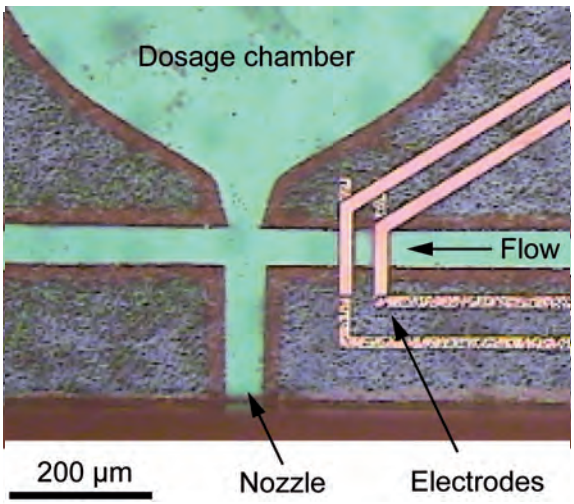

Fig. 4: *Micrograph of the dispenser chip. The electrodes are used for cell/particle detection. A pressure pulse from the dosage chamber expels cell/particles through the nozzle.*

Printing Setup

The chip is embedded into a dispenser module (P18, Biofluidix, Germany). A piezo stack actuator deflects the silicon membrane of the chip. Membrane deflection reduces the volume of the dosage chamber; liquid exits the nozzle and forms a droplet (Fig. 5). The chip is clamped with two custom made PMMA holders to the actuator housing. These holders serve as mechanical fixture and support the electrical and fluidic interconnects. Fluid flow is driven by the hydrostatic pressure difference of external reservoirs.

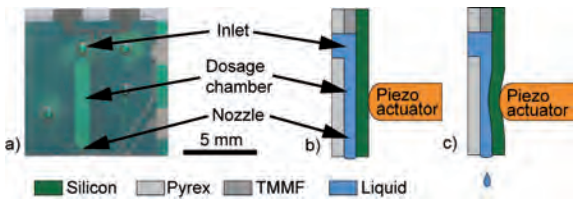

Fig. 5: *Schematic of single cell dispenser showing: a) a fabricated chip, b) side view of the chip and c) its deflected membrane. A piezo stack actuator deflects a silicon membrane, the dosage chamber volume is reduced and a droplet expelled.*

External electrical interfacing from the chip to the measuring device is accomplished via spring loaded connector embedded in the chip holders. The electrodes on the Pyrex side are connected to the signal output of an impedance spectroscope (HF2IS, Zurich Instruments, Switzerland). The sensing electrodes on the silicon side are connected to a pre-amplifier (HF2CA, Zurich Instruments), which amplifies the signals from both electrodes before they are analysed by the impedance spectroscope. A real-time processing algorithm running within the impedance spectroscope detects double peaks, calculates the particle velocity and issues a dispensing trigger when the particle is expected to be at the nozzle. Only droplets containing cells are dispensed. (Fig 1, Fig. 4).

Experimental Results

The dispensing process takes place by defining appropriate voltages to drive the actuator. The actuator extension is proportional to the voltage applied to it. Fig. 6 shows stroboscopic images of droplets at actuator extensions of 4 µm, 12 µm and 18 µm.

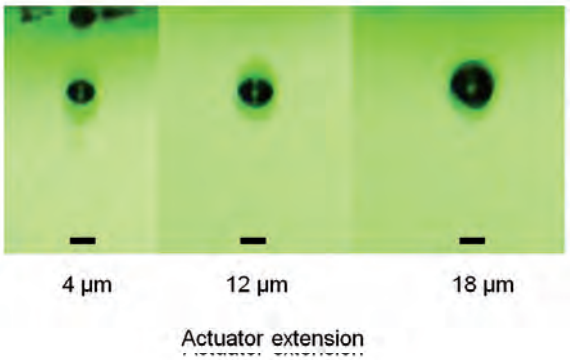

Fig. 6: *Droplets at different actuator extensions. Scale bar is 40 µm.*

To evaluate the impedance sensing performance 10 µm polysterene beads (GKisker, Germany) have been suspended in PBS. The beads serve as model for biological cells. This bead solution was flown through the chip. A typical double peak caused by a 10 µm diameter is shown in Fig. 7. Droplets are dispensed on a glass slide, where they immediately dried. A dried PBS droplet with a single 10 µm bead is depicted in Fig. 8.

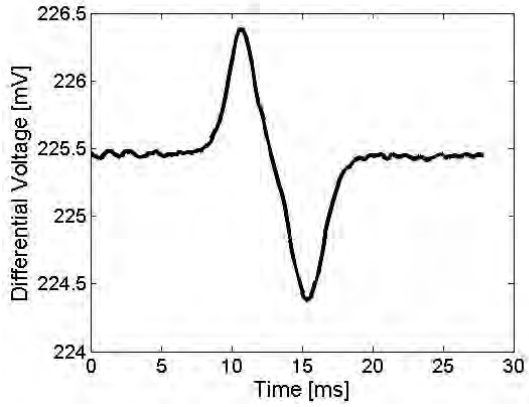

Fig. 7: *Double peak of single 10 µm diameter polysterene bead at an excitation frequency of 500 kHz. Peak-to-peak voltage is 2.0 mV with an transit time of 4.8 ms. The first peak defines when the bead is at the first electrode pair and the second (negative) peak, when the bead is at the second electrode pair.*

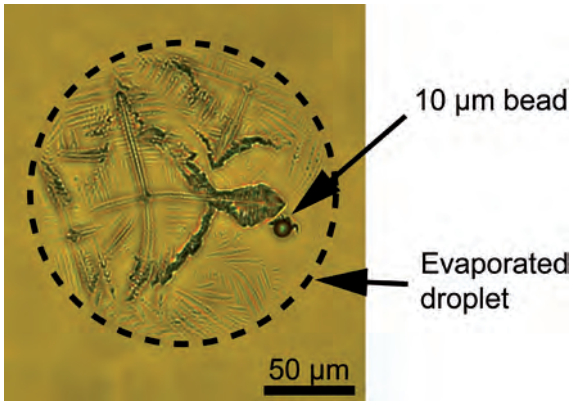

Fig. 8: *Micrograph of 10 µm diameter polysterene bead. The PBS droplet dried and salt remained around the bead.*

Conclusion

Single cell printing is a flexible method to separate cells on virtually any substrate. The presented chip combines real-time impedance-based single cell detection, velocity measurement and, finally, single particle dispensing. In the future, possibilities to differentiate between impedance spectra of different particles/cells will be evaluated as a method for label-free single cell sorting. Furthermore, the flow system and dispensing parameters need optimization to improve the single particle printing yield.

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References

[1] N. de Souza, Nature Methods, 8 (2001), 307-307
[2] W. C. Wilson, T. Boland, The anatomical record. Part A, 272 (2003), 491-496
[3] A. Yusof et al., Lab on a chip,11 (2011), 2447-2454
[4] K. C. Cheung et al., Cytometry. Part A, 77 (2010), 648-66
[5] R. Tornay et al., Proc. of TRANSDUCERS, (2007), 695-698
[6] S. Gawad, et al., The Review of scientific instruments, 78 (2001), 054301 1-7
[7] S. Gawad et al., Lab on a chip, 1 (2001), 76-82
[8] A. Yusof et al., Proc. Eurosensors XXV, (2011), 827-830