TOWARDS A MICROFLUIDIC DISPENSER CHIP FOR PRINTING OF SINGLE CELLS

A. Yusof[1], R. Zengerle[1,2], and P. Koltay[1]
[1]University of Freiburg - IMTEK, Germany
[2]Centre for Biological Signaling Studies (BIOSS), Freiburg, Germany

ABSTRACT

We present a microfluidic dispenser chip for printing of single-particles or cells onto a substrate. The dispenser chip, fabricated in silicon/glass technology features hydrodynamic flow focusing to transport single particles in a fluid channel and a drop-on-demand dispensing mechanism to eject single particles confined in a free flying droplet of about 200 pl volume. A computer vision-based approach is employed to detect single particles prior to dispensing. We have evaluated the system experimentally by successfully depositing single polystyrene-beads on glass slides with a yield of 92%.

INTRODUCTION

The need for suitable tools to separate cells into individual functional unit is in great demand due to the facts that more specific and predictive information can be obtained by analyzing cells individually. The ability to separate cells and locate them individually onto a substrate for further analysis enables many novel cellular based applications especially in drug discovery, basic cell biology or system biology. To date, flow cytometry and fluorescence activated cell sorting (FACS) are among the most efficient methods to separate and manipulate individual cells at high throughput. However, flow cytometry suffers from the limitation that it does not enable repeatable interrogation of single cells over the course of time and in a defined space. Therefore, often alternative strategies are applied which first confine single cells at retrievable positions and then perform the analysis.

There are several well known techniques to confine single cells onto a substrate with controlled spatial localization like micro-well trapping [1] and soft lithographic [2] techniques. Another interesting approach exploits a non-contact printing technique to locate cells onto substrate by using a modified inkjet printer [3] or laser directed methods [4]. To print cells with a drop-on-demand inkjet, the cell suspension has to replace the ink in a customized bubble jet printer. Interestingly, certain cell types can be successfully patterned or printed onto "bio paper" with no significant signs of deterioration on the printed cells. This remarkable finding has motivated several research groups to initiate cell printing techniques for applications in artificial tissue or organ engineering. Unfortunately, since the number of cells contain in each printed spot is random, the feasibility to use such a non-contact technique for printing single cells onto a substrate at larger scale format has remained a technological challenge.

A recently reported technique by using a focused cell flow and impedance measurements for particle detection as well as drop-on-demand dispensing for non contact cell printing [5] constitutes a fundamental approach towards development of devices for non-contact single cell printing. Though, a fully functional device has been described, printing of single particles or single cells onto substrates with significant yield has not been reported yet [6]. In this work, we report on a similar microfluidic solution for printing of single cells. We have evaluated the system using polystyrene beads as model for biological cells and successfully demonstrated single-particle printing onto a glass substrate.

WORKING PRINCIPLE

The dispenser chip as shown in figure 1 fabricated by standard MEMS processing technology features three main elements: (1) a hydrodynamic flow focusing section to make the particles flow in single file, which are provided to (2) a fluidic crossing that is amenable for particle detection by automatic image processing and (3) a piezo driven drop-on-demand dispensing device connected to the crossing to eject single particles detected within the crossing.

The heart of this dispenser chip rests upon the mechanism to generate droplets of sub-micron volume range presented at the IEEE-MEMS in 1998. Figure 1b shows a schematic diagram of the positive displacement droplet generation mechanism. Details of the dispensing method as well as the droplet characterization and measurement approach can be found in [7].

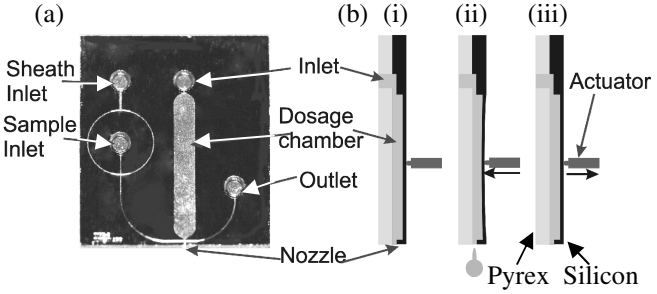

Figure 1 (a) Fabricated chip with main components nomenclature and (b) schematic sketch of the on-demand dispensing mechanism with (i) capillary priming, (ii) droplet ejection by volume displacement and (iii) liquid refill.

We employed hydrodynamic flow focusing to pinch the flow of the particle suspension to focus them in the central channel. Whenever a cell or particle suspension is supplied through the sample channel, the two coaxial sheath liquids

can be applied to squeeze the sample liquid flow. The thinning of the sample fluid depends on the flow rate ratio between the sample and the perpendicular sheath flow. An appropriate ratio between sheath and central sample flow causes the particles passing through the focusing channel in single file.

Whenever a single particle arrives at the crossing between flow channel and dispenser nozzle, the actuation of the piezo creates a volume displacement inside the chip which causes a liquid droplet to be expelled from the nozzle. The droplet contains exactly one single particle if the flow of particles and the dispensing are precisely synchronized. Therefore, single particles approaching the nozzle are detected through a computer vision system that serves to trigger the droplet dispensing at the right time.

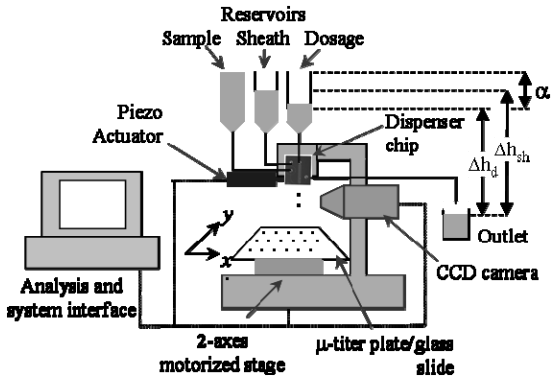

Figure 2 shows the schematic diagram for the experimental set-up to perform automated single-particle printing.

Assembling the dispenser chip on a precision translational motorized stage with control software as depicted in figure 2, enables to print single beads on predefined locations on a substrate. To facilitate automated positioning of the deposited droplet onto substrate, a user-defined programmable coordinate definition was included on the tailor made graphical user interface (GUI).

MATERIALS AND METHODS

Fabrication: The fabrication steps for the silicon / glass dispenser chip are summarized in the figure 3. The fluid channels were etched into a silicon wafer by first spin coating with photoresist, lithographic structuring and finally an anisotropic etch of 40 um depth via the deep-reactive ion etching (DRIE) process. The hydraulic access holes were formed in the Pyrex wafer through a wet etching process. The Pyrex wafer was first coated on both sides with a polysilicon (p-Si) masking layer via a LPCVD process. The holes pattern was formed on either side of the p-Si coated layer via photolithography and DRIE to selectively remove the p-Si layer. The through holes were then formed isotropically through wet etching process using concentrated hydrofluoric acid (49%). Both wafers (silicon and pyrex) were bonded anodically and finally diced. The dicing process finally created the nozzle at one side of the chip.

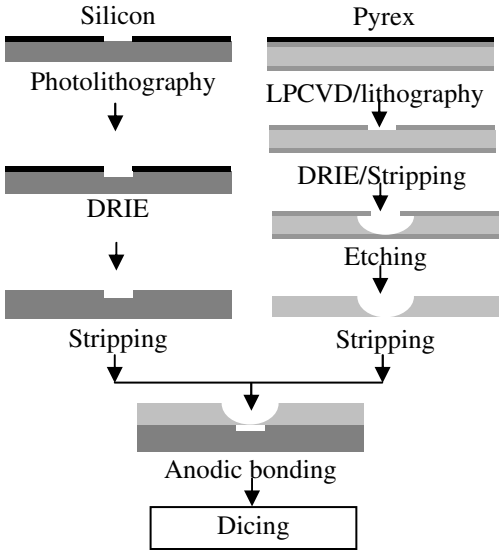

Figure 3. The fabrication process flow for the dispenser chip fabricated in silicon and Pyrex.

Liquid driven system: The liquid flow to supply cell sample and buffer was realized by a hydrostatic pressure difference. It was controlled by height adjustable reservoirs as shown schematically in figure 2. Prior to the experimental work, all the reservoir positions were calibrated to achieve an equilibrium situation where liquid flow within all fluidic channels remained stationary. Calibration was done by mounting a flow meter at the outlet channel and adjusting each reservoir until the equilibrium position was reached (indicated by no flow reading through the flow meter). Upon achieving the equilibrium position, only sheath and sample reservoir were varied to obtain fluid flow at different flow rate.

Flow focusing: A fluorescein dye was used as sample liquid supplied to the sample inlet channel and phosphate buffer saline (PBS) was supplied to the sheath inlet channel. The sample reservoir was fixed at a height that provides a flow rate at 1μl/min (calibration data not shown). The flow ratio between sample and sheath flow was obtained by varying only the height of sheath reservoir. The formation of focused fluorescent liquid streams was observed using a CCD camera equipped with fluorescent filter (λemission: 490 nm ; λexcitation: 525 nm). The image of the focused stream was captured and analyzed off-line using open source image processing software (ImageJ) to measure the stream width.

Beads suspension: Polystyrene microbeads (poly-beads) (Gerlinde Kisker, Germany) with average diameter of 10 μm were diluted in PBS to form a buffer suspension at a concentration of 20 x 10^5 beads/ml. Before supplied to the reservoir, the suspension was sonicated to obtain a homogeneous mixture.

RESULTS AND DISCUSSIONS

Flow focusing calibration

We optimized the focused stream formation by varying the flow rate ratio of the sheath and sample flow (α = sheath flow/sample flow). The ultimate aim is to estimate the focused stream width at a given flow ratio. Therefore, in order to obtain a single particle travelling in the centre plane of the channel, the focused steam width should be of similar size than the particle's diameter. Figure 4 shows the pinching effect of fluorescence dye after being squeezed by two side o by the PBS sheath flow at varying flow ratios.

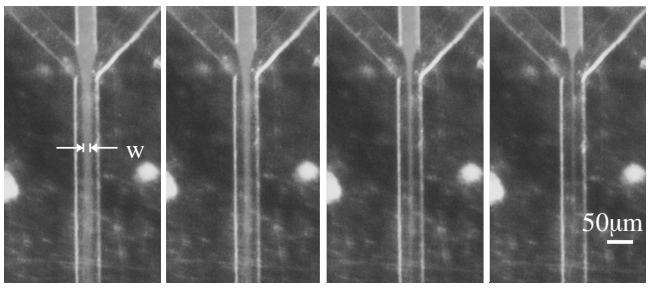

Figure 4. Hydrodynamic flow focusing calibration at different sheath and sample flow ratio (α) to obtain estimates for the focused stream width (w). (a) α = 2.4, w = 18.7μm, (b) α = 2.5, w = 14.6μm, (c) α = 3.3, w = 10.4 μm, (d) α = 4.0, w = 6.2μm.

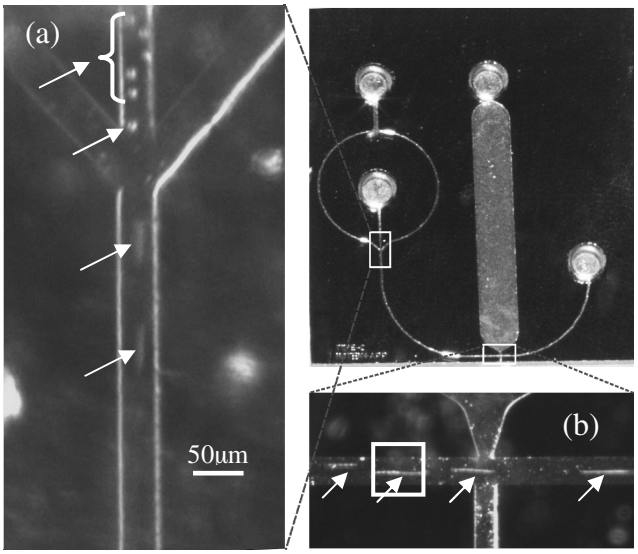

Figure 5. Individual poly-beads flowing in single file along the fluid channel at (a) the flow focusing section and (b) the nozzle section. The slower shutter time of the CCD camera causes the images of the single beads to appear as streak line. The square sector in (b) is the sensing area for the computer vision based single particle detection.

To vizualize the particle flow after being focused hydrodynamically, the sample flow was supplied with poly-beads suspension while PBS solution was maintained as sheath flow. The flow ratio was adjusted to 3.3 which is equivalent to a focused stream width of 10 um (refer to fig. 4 b). Figure 5 shows micrographs of poly-beads flowing along the channel at different sectional view of the microchip. Obviously, poly-beads have been successfully made to travel single file along the flow path.

Single particle detection

The mechanism to detect single particles prior to dispensing was employed by a computer vision system. The CCD camera, which streamed images at maximum frame rates of 30 fps was furnished with a motion detection algorithm to automatically detect the particles within the region of interest (ROI). Details of the detection algorithm can be found elsewhere [8]. The ROI was defined by square sector (cf. figure 5) and located at a distance Δx from the nozzle junction (cf. figure 6).

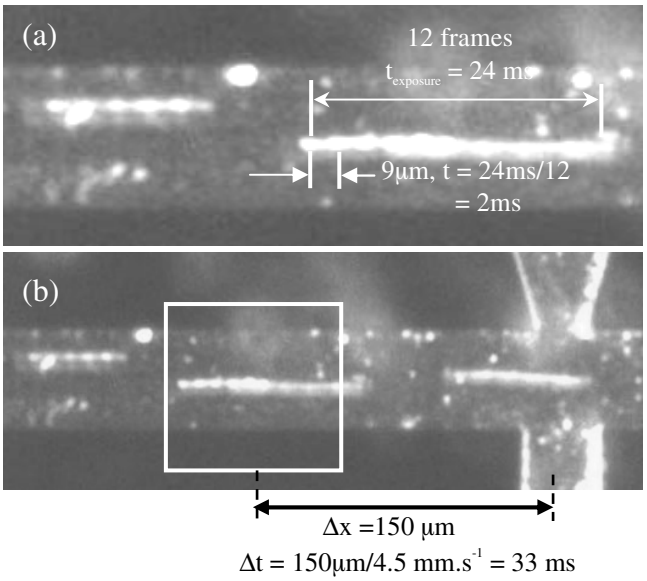

Figure 6 Single polystyrene bead flow captured at shutter time of 24 ms resp. 2 ms. The particle velocity was estimated to be v=4.5 mm/s. The white square area (ROI) is located at Δx=150 μm and a delay time of Δt=Δx/v=33 ms was used to trigger the droplet ejection to expel the particle.

Whenever a single particle arrives within the ROI, the algorithm will process the image and provide a decision for triggering the dispensing event. The necessary condition for synchronizing the arrival of the single particle at the crossing next to the nozzle and the triggering of the droplet can be estimated from the particle velocity v and the distance Δx. Using single images as shown in fig. 6a, the estimated particle velocity can be determined. Since the distance Δx is known, the delay time Δt for ejecting the particle can be calculated as Δt = Δx/v. A corresponding delay timer was implemented within the software to ensure

that the dispensing is triggered at the moment when the single particle is at the crossing closest to the nozzle.

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Single polystyrene bead printing

Automated single particle printing on a glass substrate was performed upon proper definition of the actuator's driving parameters to generate a droplet and the right flow rate ratio to obtain continuous single particles flow along the fluid channel. Deposition of single particles was realized on a glass slide like shown in figure 7 by appropriate coordinate positioning to arrange the droplets in an array.

Previous work [6] has demonstrated a similar device made from PDMS and the attempt to deposit single latex beads on a glass cover-slip. However, no printing of single particles was reported. Probably, this was prevented by a poor synchronization of the particle detection with the droplet generation, which could be related to the relatively slow response time of the pneumatic dispenser used [6]. With the described piezo driven droplet generator and the optical detection, we demonstrated for the first time controlled dispensing of single particles with such a "cross-flow method". With the described chip a constant single-particle flow could be achieved and small droplets with volumes of about 200 pl could be generated. The applied simple optical detection system and algorithm resulted in a reasonable high yield of 92% of the printed droplets on the substrate containing exactly one single particle. Future work will include to evaluate the system with real living cells and to observe the post printing viability of the cells.

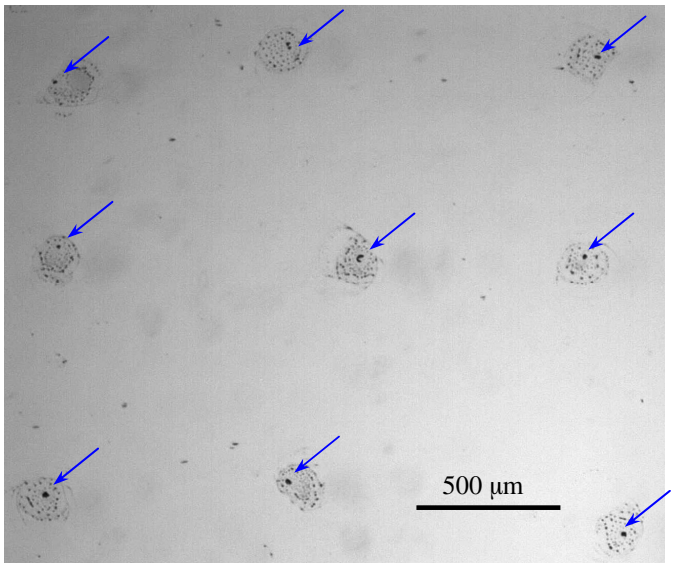

Figure 7. A 3 x 3 array of spots containing a single individual polystyrene bead each (blue arrows) deposited on a glass slide (the debris surrounding the beads is caused by salt crystals resulting from the drying of the PBS buffer).

CONCLUSIONS

The presented results can be considered as first step towards a fully automated technology for single cell printing. The combination of flow focusing and drop-on demand dispensing enables to combine methods of cell cytometry with inkjet printing of cells. Such technique can be of high relevance for single-cell analysis, tissue engineering or medical diagnostics.

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