A Non-Invasive Single Cell Dispensing Approach for 2-Dimensional Micro-Patterning

A. Yusof[1], L. Riegger[1], N. Paust[1], A. Ernst[1], R. Zengerle[1,2], P. Koltay[1,3]
[1]Laboratory for MEMS Applications, Department of Microsystems Engineering (IMTEK)
[2]Centre for Biological Signalling Studies (bioss)
University of Freiburg, Georges-Koehler-Allee 106, D-79110, Freiburg, Germany
[3] BioFluidix GmbH, Georges-Koehler-Allee 106, D-79110, Freiburg, Germany

Abstract:
We present a computer vision-based approach for detection of micro-beads and cells combined with a non-contact liquid dispensing system to pattern single polystyrene-beads and yeast cells in a 2-dimensional array. A so called NanoJet dispenser for dispensing liquid borne particles features the average droplet volume of 150pL to 950pL and a reproducibility of (CV) < 3%. The computer vision set-up consists of a CCD camera coupled with a magnifying lens, which was employed to detect the micro-beads close to the dispenser's orifice prior to dispensing. A real-time sequential image analysis was carried out by using a simple temporal differencing detection algorithm to identify single cells or particles in the proximity of the orifice that would be ejected with the subsequent dispense. Using this process arrays of polystyrene-beads and yeast cells were deposited onto glass slides attached to a programmable motorized stage. The overall deposition efficiency obtained was 70% with 58% of the spots contained single polystyrene-beads or cell respectively.

Keywords: single-cell, computer vision based detection, non-contact droplet dispensing, cell printing

Introduction

Significant progress in micro technology and optical detection in the past two decades has enabled the studies of living cells down to a molecular level. Although several protocols for single-cell analyses have been established however, the important component to initiate this rests on the availability of tools to prepare single-cells for subsequent analysis. In this work, we present a method to seed single-cells by using a non-contact droplet dispensing approach.

Single-cell dispensing system

The system to seed single-cells encapsulated in a micro droplet features three main elements as illustrated in figure 1: (1) a microfluidic based dispenser chip to deliver the cell suspension and to eject individual cells in a free-flying droplet, (2) an optical imaging system to detect the cells inside the dispenser chip and (3) a motorized linear stage for automated single-cell deposition on a substrate.

Dispenser chip

The ultimate role of the dispenser chip is to confine single-cells in a picoliter sized droplet that are ejected from the orifice of the dispenser. We used a NanoJet type dispenser chip (figure 3(a)) to deliver living cells and beads in buffer suspension. This dispenser chip features a drop-on-demand dispensing mechanism driven by piezo-actuator, and has an adjustable droplet volume range between 150pl to 950pl with excellent reproducibility [1]. Furthermore, an essential feature of the dispenser chip is that it is completely transparent from one side such that the cells or particles contained inside the chip prior to dispensing can be observed by the optical system from the outside.
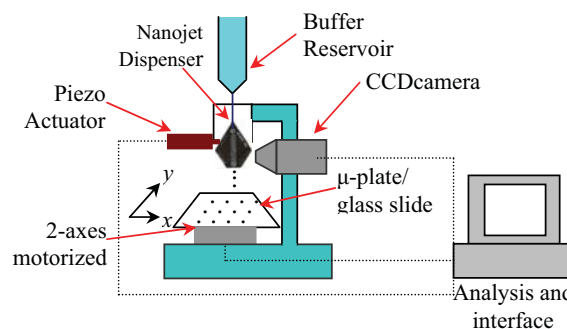

Fig. 1: *Schematic diagram of the single cell dispensing system*

Single-cell dispensing mechanism

The drop-on-demand dispensing nature of this dispenser chip requires a continuous dispensing activation to let cell suspension flow through the fluidic channel. Without any control by the optical imaging system this mechanism produces arbitrary number of cells passing across the nozzle and as a result, the number of cells confined in each droplet ejected from the nozzle is randomly distributed. The strategy to obtain a single-cell per droplet is the following:
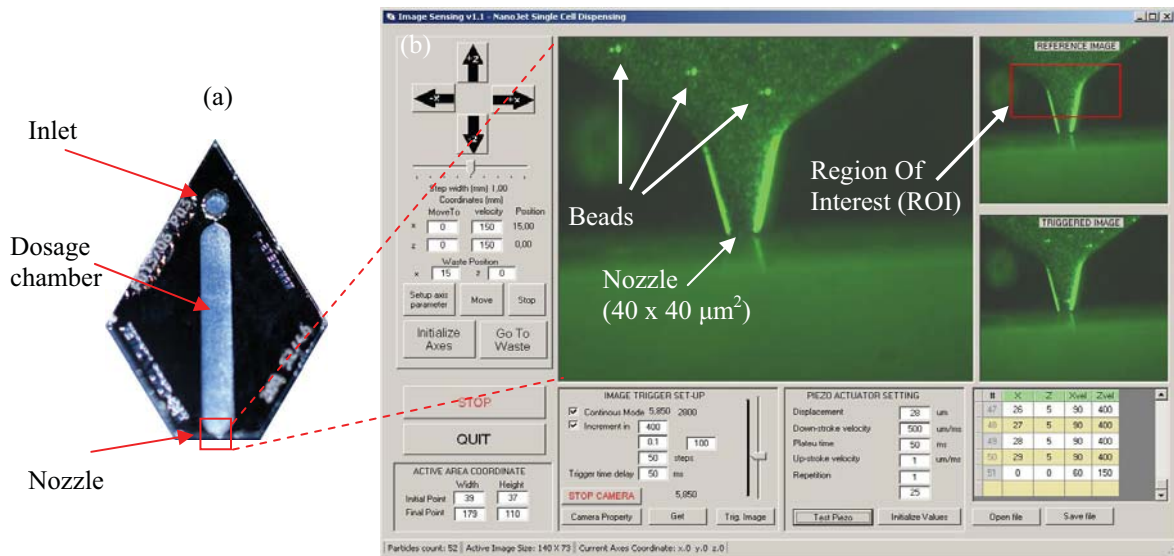
Fig. 3: *Nanojet dispenser (a) and GUI program (b) for controlling the complete system (dispenser, Optical system, positioning system). Live video frame shows dispenser nozzle at 7x objective magnification, while red rectangular box in reference image frame shows the region of interest (ROI).*
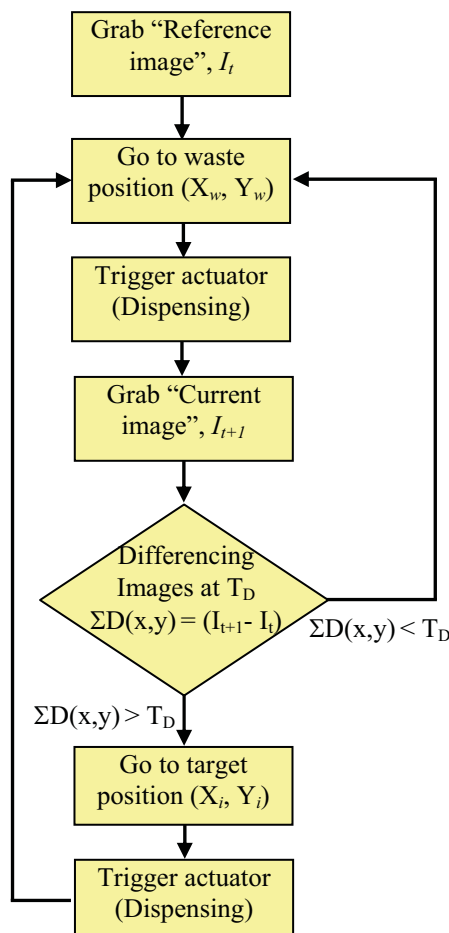

Fig. 2: *Control flow chart for 'sort-and-select' single cell*

While the dispenser is continuously producing droplets the optical imaging system is recording continuously the position and distribution of the cells inside the dispensers chip in close proximity to the nozzle. In some case no cells or several cells are in such close proximity to the nozzle that they would be expelled with the subsequent dispense. In such situations the generated droplets (containing none or many cells) are directed towards a waste position. However, in many cases exactly one single cell is such close to the nozzle that it will be ejected by the subsequent dispense. These droplets are directed towards the desired target position and do contain exactly one single cell.

In the presented experiments a computer vision mechanism was employed as optical sensor to detect the single-cells inside the dispenser chip. A CCD camera was mounted off-the-chip and focused at the nozzle section which is accessible optically. The sequential images streamed by the CCD camera were analysed using a simple motion detection algorithm [2] and enhanced with signal processing. A simplified sort-and-select dispensing mechanism was applied to discriminate between the images showing a single cell in region of interest (ROI) like shown in figure 2. The procedure starts by first defining a ROI by selecting a portion of the camera image close to the nozzle. It has to be noted that the selection of the position and size of the ROI depends on the fluid dynamics inside the dispenser chips and has a significant impact on the dispensing result, like explained in more detail later.

At the beginning of the algorithm a reference image without any particle flow is captured. Then the sequential image is captured while the dispenser is continuously delivering individual droplets to a

waste position (x_w, y_w), until the first particles/cells appear in the ROI. The two camera images (reference and current-sequential image) are set at grey scale and differentiate at threshold value (T_D) that unveils the background. As a result, new binary image will form that shows the differences on the foreground (image with bright spot if particle/cell exist in the current image frame). Principally, this algorithm analyses the images generated from the ROI to determine whether a single particle/cell is inside the ROI (i.e. close enough to the nozzle to be expelled by the next dispensing event). If a cell or particle is detected the dispenser is positioned at a predefined target position (x_i, y_i) and eventually dispenses a single droplet containing a single bead or cell. Afterwards the dispenser returns to the waste position and the procedure is repeated until the next single cell or bead is detected in the ROI.

Automated single-cell dispensing and patterning
We automate the dispensing mechanism, computer vision and translation motion of 2-axes motorized stage with in-house built software written in Visual Studio 2005. A graphical user interface (GUI-figure 3(b)) was designed that allows the user to define the coordinates for harvesting single-cells in 2-dimensional arrays position.

Sensing area (ROI) estimation
As mentioned before the size and position of the ROI is essential to achieve successful dispensing. Therefore, computational fluid dynamic (CFD) Simulations were performed to calculate the flow field inside the dispenser and to estimate the best position and size of the ROI as given in figure 4. Considering the dispensed droplet volume and the flow profile inside the nozzle one can approximate that all cells or particles residing at a distance of about 100µm from the orifice, will be ejected by the subsequent dispense. Of course this is a rough estimate only since the distance to the walls and the particle size play also a significant role for the drag forces exerted on the particles. In future work the exact position of the cell inside the ROI in relation to the flow field and cell size will be considered to account for these effects. Nevertheless, in the reported first experiments a simple rectangular ROI in appropriate distance to the nozzle turned out to be quite predictive. All results presented in this paper have been achieved applying this control strategy.
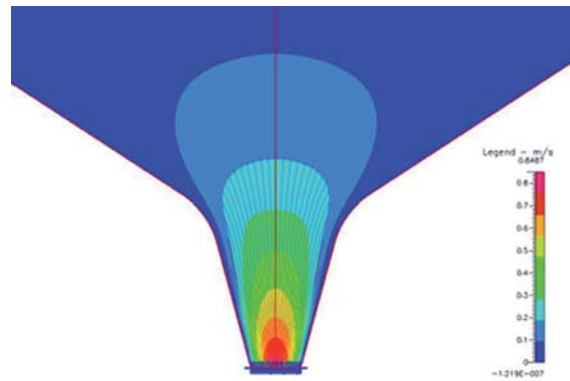

Fig.4: A 2-Dimensional CFD simulation shows the velocity flow profile at the nozzle to estimate the position and size of the ROI.

Results

Polystyrene beads micro-array
Polystyrene beads suspensions (average diameter 10µm) were prepared at different bead concentrations. The suspensions were supplied to the dispenser and automatically deposited on a glass slide according to the described algorithm. Figure 5 shows successful printing of polystyrene beads into 5x5 arrays (25 spots). The average dispensing efficiency (number of spots containing beads divided by total number of spots) to obtain polystyrene bead occupancy for deposited spot was 70% and 58% of the overall deposited spots comprising a single polystyrene bead. Figure 6 reveals that changes in the polystyrene beads concentration between 10^5 to 10^6 beads/ml, do not show a significant influence on single bead dispensing efficiency.
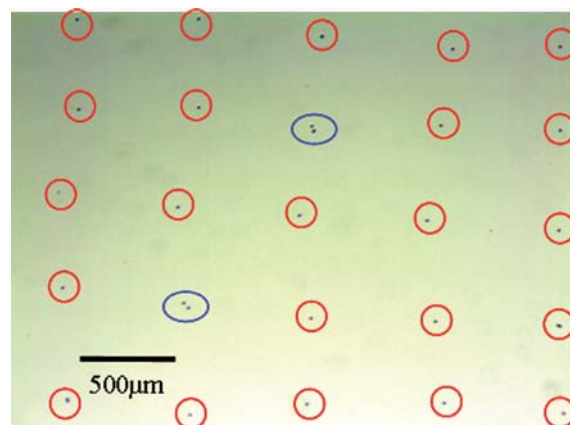

Fig.5: Micrograph of polystyrene beads deposited on a glass slide taken at 1.6x magnifications. Red circle represent spots containing single- polystyrene beads and blue circles mark spots containing more than a single- polystyrene bead.
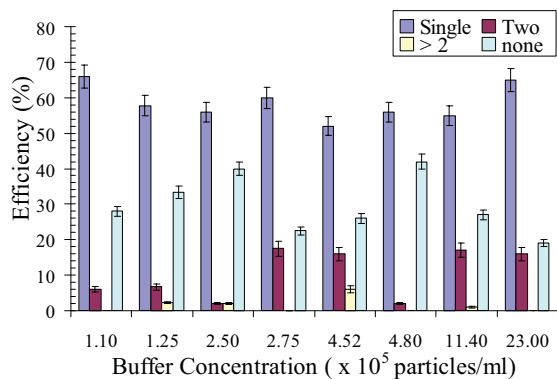
Fig.6: Result for polystyrene -beads deposition on glass slide. Average efficiency is 70% with 58% single bead occupancy

S.cerevisiae micro-array

To further assess the capability of the single cell / particle dispensing system, we used baker's yeast (*S.cerevisiae*) as a biological model and seeded the cells on culture media to observe its viability over a period of time. Fresh yeast cells were prepared in standard physiologic saline solution at concentration 2×10^5 cells/ml. Before supplying this suspension for seeding procedure, a viability test was performed. An aliquote of sample suspension was stained with *trypan blue* and the viable/non-viable cells were statistically counted using a hemocytometer (Neubauer). Only samples with at least 99.9% viability rate were used for printing experiments. Cell culture media was prepared in sterilized condition using premix yeast glucose (YGC) agar on a standard tissue culture dish.

Fig.7: Seeding single yeast cell array on culture media. Deposited spots containing yeast cell grow to form colonies after 72 hours incubation.

Result as shown in figure 7 reveals that 60% of the deposited droplets on culture media contain yeast cells and all remains viable after 72 hours incubated at 37°C.
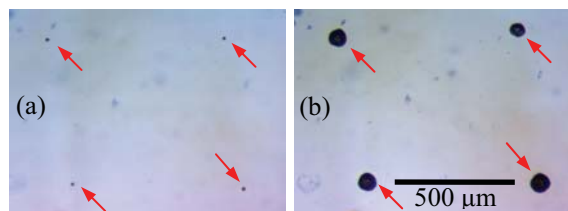

Fig.8: Fresh yeast cells has average diameter of 5 μm. (a) Four spots containing single yeast cell (red arrow). (b) Single seeded yeast cells grew up after 72 hours incubation. Picture was taken at 3.2x magnifications.

Figure 8(a) shows the enlarged view of 4 spots contain single yeast cells deposited on culture media. The seeded yeast cells were found proliferate during incubation (figure 8(b)) which indicates that the suitability of presented technique as a single-cell manipulation platform.

Conclusions

We have shown successful deposition of single polystyrene-beads as well as living cells (*S.cerevisiae*) encapsulated in free flying micro droplets to form 2-dimensional arrays with significant yield. Compared with other single cell array technique [3,4], the presented approach offers a minimally invasive single-cell manipulation and it can develop into a suite for single-cell applications in basic cell biology, drug discovery and stem cell research.

References

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