Label-free sorting and deposition of single bacterial cells using the Single-Cell Printer technology.

J. Riba, T. Gleichmann, R. Zengerle, P.Koltay

Laboratory for MEMS Applications, Department of Microsystems Engineering – IMTEK, University of Freiburg, Germany

Summary

We present a modified Single-Cell Printer (SCP) for sorting and deposition of individual bacterial cells by:

•Label-free optical cell detection

•Confinement of single cells in 35 picoliter droplets

•Non-contact drop-on-demand deposition onto a variety of substrates

We show that *Escherichia coli* cells can be deposited with a single cell printing efficiency of 78 %. Further, we deposited individual cells from a heterogeneous sample directly onto agar plates for subsequent clonal culturing.

Introduction

Increasing interest in single-cell analysis has aroused demand for technologies to sort and handle individual cells. We previously demonstrated that the Single-Cell Printer can be used to sort and deposit single mammalian cells onto various substrates for subsequent monoclonal culturing [1] and single cell genomics [2]. Compared to other technologies such as a FACS system the SCP is more flexible in terms of substrates and employs a disposable cartridge to prevent cross-contamination. Here, we present an advanced version of the instrument with high-resolution optical detection that has been developed with the aim to deposit bacterial cells. For the first time, we show label-free deposition of single bacterial cells using the SCP.

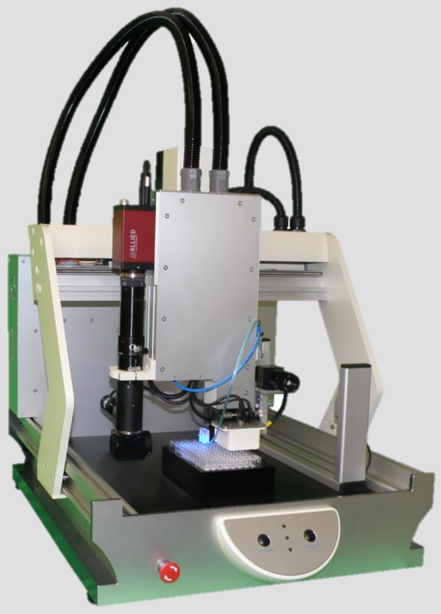
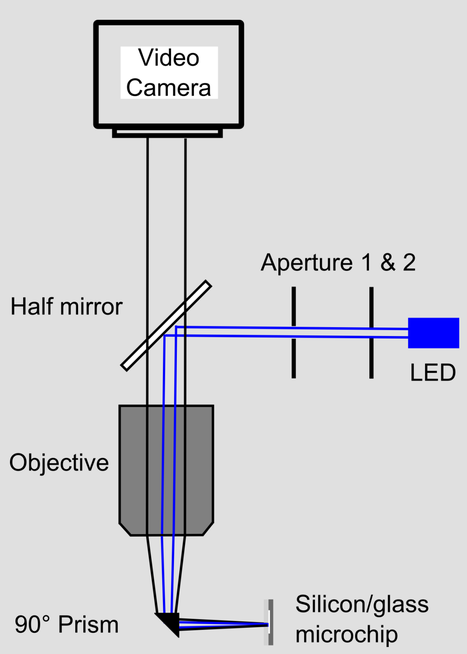


Figure 1: Single-Cell Printer

Design

For this work, the SCP described by Gross et al. [1] was equipped with a high-magnifying detection optic (Fig.2). Further, we fabricated dispenser chips with 20 µm nozzle size. The new chips dispense droplets 35 pl in volume and allow for detection of objects ~ 1 µm in size.



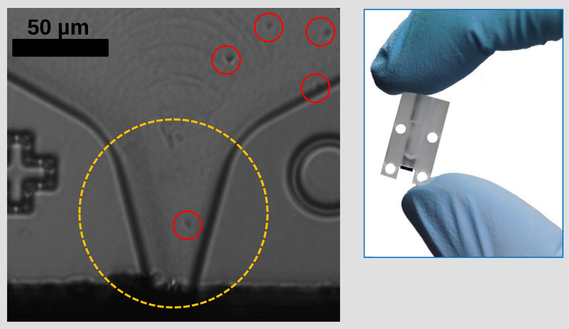


Figure 2: Brigth-field optical detection system (left) of the modified Single-Cell Printer. The Camera image (center) shows the region of interest (green) and *E.coli* cells (red circles) in the silicon/glass dispenser chip with a 20 µm sized nozzle. Assembled cartridge with dispenser chip (right).

Experimental Results

Single cell efficiency can be evaluated by printing fluorescently labeled bacteria cells. A GFP expressing *E.coli* strain was printed on glass slides in arrays of 100 droplets with 100 µm pitch. We classified whether a spot contained a single cell, no cell, or multiple cells via fluorescent microscopy (Fig.3). Out off 900 spots 699 contained a single cell (single-cell printing efficiency: 77.7 %).

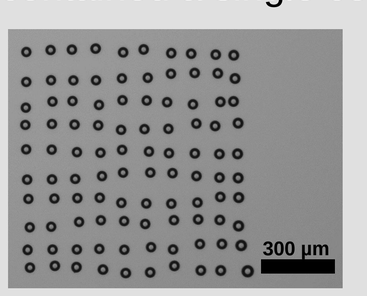
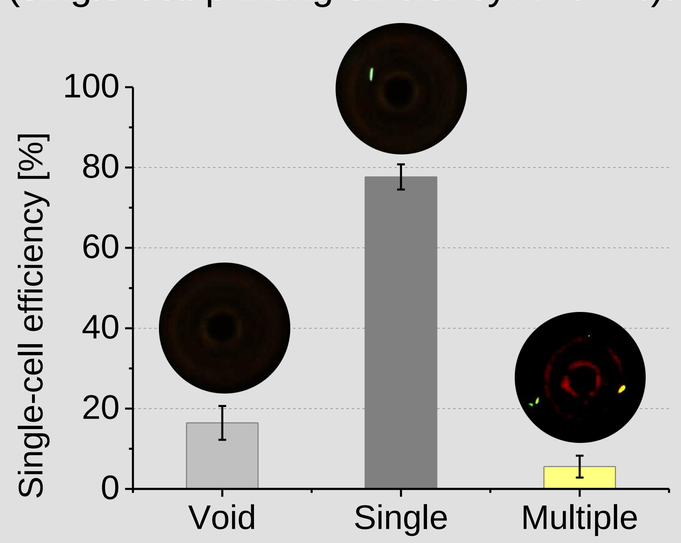


Figure 3: Droplet array (10x10) with printed *E.coli* cells (top). Single-cell efficiency was evaluated with fluorescent microscopy (right).



Single bacterial cells can be isolated from a heterogeneous sample resulting in clonal cultures. We deposited individual cells from a **binary mixture** of *E.coli* and *Enteroccocus faecalis* directly on **agar plates**. After cultivation, the colonies can be visually distinguished (Fig.4).

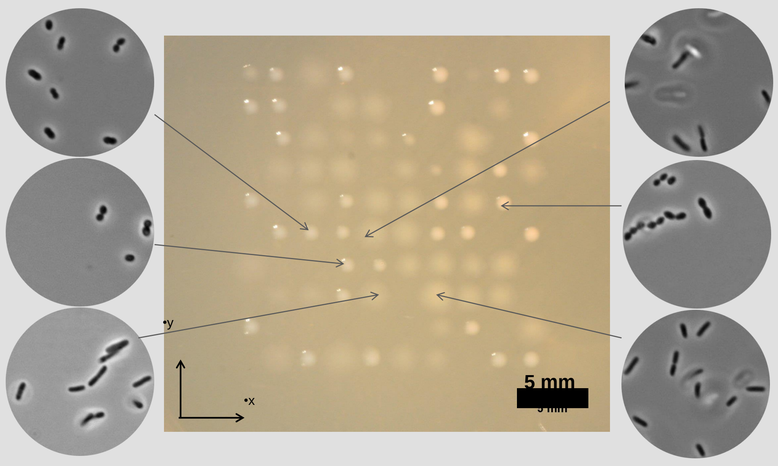


Figure 4: Clonal colonies of *E.coli* and *E.faecalis* after cultivation. Arrays of single-cells were printed directly on agar plates with 2 mm pitch.

Conclusions

We present a generic platform for label-free separation of bacteria. The Single-Cell Printer technology allows to isolate individual bacterial cells for subsequent clonal culturing on agar and micro-titer plates. Next, we aim to establish a highly automated single cell genomics workflow to apply the method for microbial community analysis.

Acknowledgements

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References

[1] Gross et al., *J. Lab. Autom.*, *18*(6), 504-518.

[2] Stumpf et al., *Biosens. Bioelectron.*, 69 (2015): 301-306.