A digital microfluidic platform for capture and selective retrieval of single bacteria

[1]*Phalguni Tewari Kumar*, [2]*Ioannis Passaris*, [1]*Deborah Decrop*, [1,4]*Tadej Kokalj*, [2]*Abram Aertsen*, [3]*Robert Puers*, [1]*Annemie Geeraerd and* [1]*Jeroen Lammertyn*
[1]*BIOSYST-MeBioS;* [2]*Laboratory of Food Microbiology;* [3]*MICAS-ESAT, KU Leuven, Belgium;* [4]*Institute of Metals and Technology, Ljubljana, Slovenia*

With the advent of powerful and automated fluorescence imaging techniques, in depth characterization of bacteria has become feasible. Although phenotypic screening is possible with the existing techniques[1], retrieval of interesting bacteria from a larger population for their subsequent analysis is still challenging. Moreover, the handling of such small cells suffers from low throughput and high manual work. Hence, novel technologies are required for capturing, isolating and subsequently analyzing single cells in a high throughput context.

Within the MeBioS Biosensor group, an innovative approach for conducting high throughput time-lapse studies on single non-adherent cells has been recently demonstrated, based on digital microfluidic platform (DMF)[2]. In the current work, we present an application of the adapted system, expanded with optical-tweezers[3], for capturing and releasing magnetic bead-coupled single bacterial cells from a microwell. The schematic of the protocol for capturing single bacteria cells (*Salmonella* Typhimurium) is illustrated in Fig.1. The microfluidic chip consists of (i) a grounding plate containing an array of 62,500 microwells (4 µm wide and 3 µm deep) and (ii) an actuation plate containing an array of electrodes. For obtaining suitable bacteria capturing efficiency, tosyl-activated superparamagnetic beads were conjugated with anti-*Salmonella* Typhimurium IgG1 at different antibody concentrations. By performing automated actuations, the microwells were seeded with antibody-functionalized-superparamagnetic beads, followed by an on-chip incubation of the beads with *Salmonella* cells. The array was then washed five times to remove unbound bacteria. As shown in Fig. 2a, a bacteria capturing efficiency of 48 % was obtained for an antibody concentration of 0.24 µg/µl (Fig.2b). To test the specificity of bacteria capturing, control samples with no-antibody-conjugated superparamagnetic beads were tested, which resulted in a capturing efficiency of less than 0.04% (Fig.2c). For retrieving bacteria bound magnetic beads, an IR optical-tweezers setup was built and the optimal buffer condition was determined for optically levitating the beads. As a proof of concept, bacteria bound to magnetic beads were optically retrieved from one microwell, transported and seeded in another empty microwell, as shown in Fig 3.

In conclusion, we demonstrate for the first time, a DMF platform enabled spatial isolation and selective retrieval of single non-adhering cells bound to magnetic beads using optical tweezers. Research focused on screening and selection of single cells within a large population, and identification of their genetic makeup is addressed with this novel digital microfluidic platform.

References

1. A. K. Wessel, L. Hmelo, M. R. Parsek, and M. Whiteley, *Nat. Rev. Microbiol.*, 2013, **11**, 337–48.

2. P. T. Kumar, K. Vriens, M. Cornaglia, M. Gijs, T. Kokalj, K. Thevissen, a. Geeraerd, B. P. a. Cammue, R. Puers, and J. Lammertyn, *Lab Chip*, 2015, 16–18.

3. D. Decrop, T. Brans, T. Kokalj, F. Beunis, and J. Lammertyn, 2014, pp. 1455–1457.

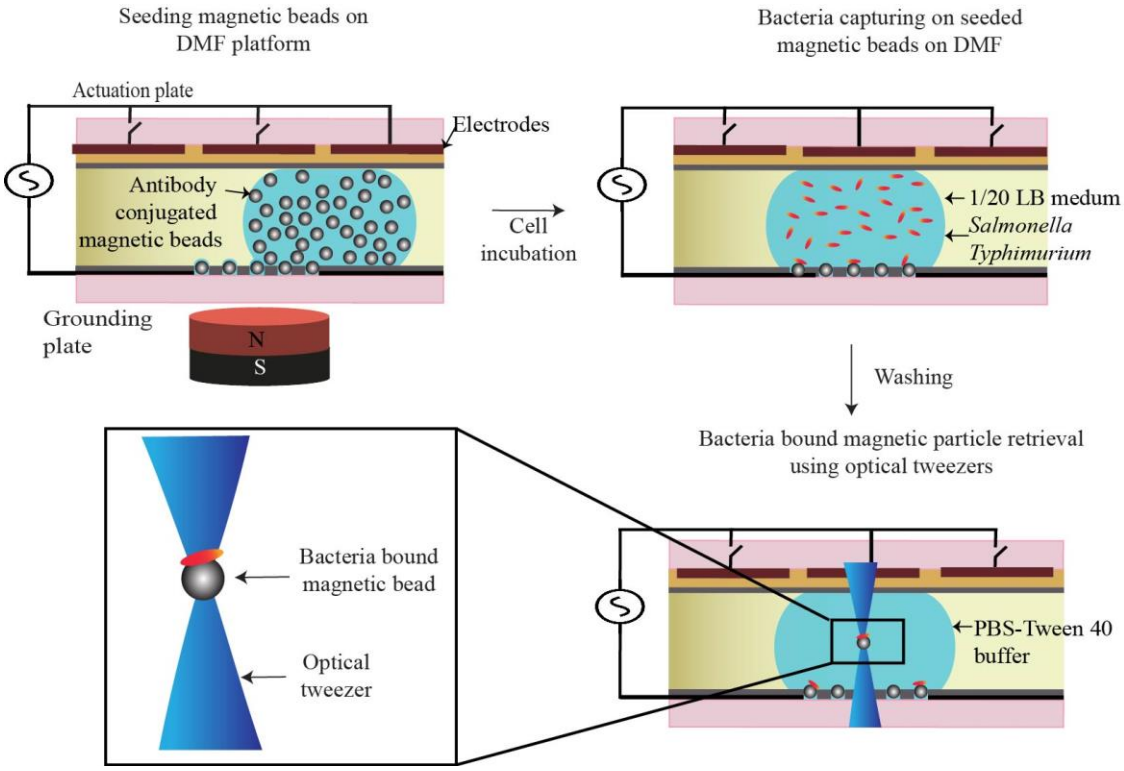

Figure 1: Schematic of optical tweezer-coupled-DMF chip for capturing single bacterial cells and single cell retrieval from microwells.

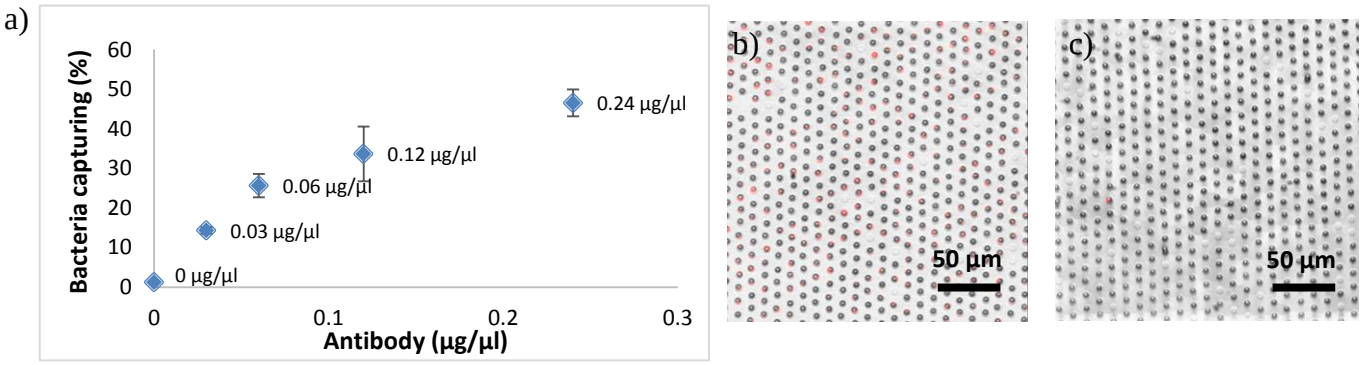

Figure 2: Bacteria capturing on superparamagnetic beads seeded in microwells. (a) The plot shows single bacteria capturing as a function of antibody concentration, error bars represent standard deviation of four replicate measurements; (b) an overlay of mCherry expressing bacteria cells (red) bound on magnetic beads (black circles) at 0.24 µg/µl antibody concentration; (c) control samples with an overlay of mCherry expressing bacterial cells bound on magnetic beads at 0 µg/µl antibody concentration.

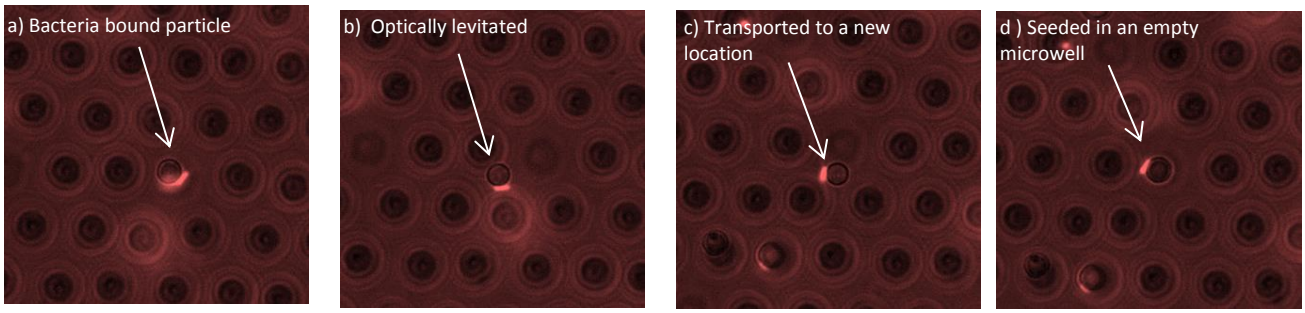

Figure 3: Time lapse images of bacteria bound magnetic bead retrieval. Images (c) and (d) are at different location on the microwell array in comparison with images (a) and (b).