

REVERSIBLE IMMUNOMAGNETIC CELL TRAPPING AND ANALYSIS ON AN ARRAY OF THIN-FILM PERMALLOY MICROFEATURES

Daniel Kirby, Éanna Bailey, Macdara Glynn, Charles Nwankire and Jens Ducreé*
*Biomedical Diagnostics Institute, National Centre for Sensor Research,
School of Physical Sciences, Dublin City University, Ireland*

ABSTRACT

Isolation and individual analysis of target cells from a bulk sample is vital in cell-based diagnostics. Previous work has established the alignment of single cells in a regular grid of 3-dimensional barriers [1]. We here present the magnetically controlled separation of specific target cells by a sputter-deposited thin-film of permalloy “teardrops”. Apart from simplified fabrication of our essentially 2-dimensional, thin-film architecture, our device also features high capture efficiencies, leaves flow lines unperturbed, assures homogeneous reagent exposure and suppresses particle clogging. Furthermore, the trapped cells can readily be released for further downstream analysis by removing the external magnetic field, thus fully regenerating the device for the next experiment.

KEYWORDS: Cell trapping, Cell alignment, Cell array, Magnetic trapping, Magnetic film

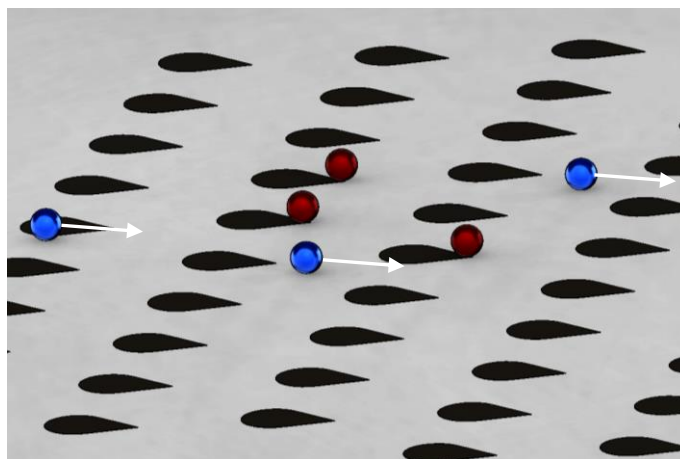


Figure 1: Magnetic bioparticles such as beads or tagged cells (red) are retained at the tip of the teardrop-shaped magnetic dots (black) where the local field is strongest while the unperturbed, non-magnetic particles (blue) continue in the downstream direction.

INTRODUCTION

Capture and analysis of individual cells from a mixed population is vital in cell-based diagnostics. Previous work has established the ability to array target cells using 3-dimensional geometrical obstacles such as micropillars and cups [1]. We here present a separation device that aligns immuno-magnetically tagged target cells into an ordered grid generated by a micropatterned, 160-nm thick permalloy film (80% Nickel, 20% Iron) deposited on the base of the chamber (Fig. 1). Maxima of the magnetic field arise in the vicinity of the sharp edges of these microfeatures, as outlined in the magnetic field simulations (Fig. 2). These potential wells serve to localize paramagnetic bioparticles (within a certain size range) such as tagged cells or beads during analysis or successive steps of washing and reagent exposure. Going

beyond the capabilities of geometrical barriers, our trapping mechanism is inherently reversible and can be easily adapted to suit specific particle sizes or flow conditions.

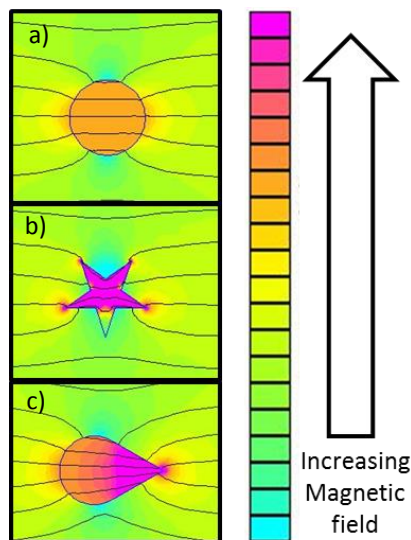


Figure 2: Simulation of local magnetic field for different shapes of a micropatterned permalloy film in a uniform external magnetic field. a) The round dot delivers a rather even distribution of the magnetic field. b) The star-shaped structure provides a larger field amplitude but it features several maxima. c) The teardrop shape focusses the magnetic field into a single maximum which turned out to be optimal for capture of individual bioparticles at high spatial definition.

PRINCIPLE OF OPERATION

Magnetic cell isolation has become widespread, with the majority of systems using bulk magnetic forces [2,3]. The novelty in our system is the arrayed trapping of the magnetically tagged cells (Fig. 3) where they can be studied individually and undergo media change, e.g. for fluorescent staining. The thin-film nature of these traps suppresses clogging and inevitable divergence of flow lines around 3D-obstacles, thus enhancing capture efficiency and assuring uniform exposure to reagents. Following on-array retention, the cells can be efficiently released into a downstream capture chamber by simply removing the magnetic field.

MATERIALS AND METHODS

The permalloy features are deposited on the floor of the chip by sputter-coating a UV-lithographically patterned (OAI, model 200) AZ-photos resist which was spin-coated on glass slides. A PMMA lid was cut by a CO₂ laser and bonded to the glass via a pressure-sensitive adhesive featuring an 86- μ m high microchannel for the passage of the cells. The MCF7 cancer cells were incubated with 4.5- μ m magnetic anti-EpCAM beads before being introduced to the system.

RESULTS AND DISCUSSION

This chip enables the separation of target bioparticles from an abundant background into an array of magnetic traps with single-occupancy distribution (Fig. 3a & b). We have measured a capture efficiency of over 99% for magnetic beads and a purity of 98.5%, with a non-magnetic particle purity of over 99.9% in the waste chamber.

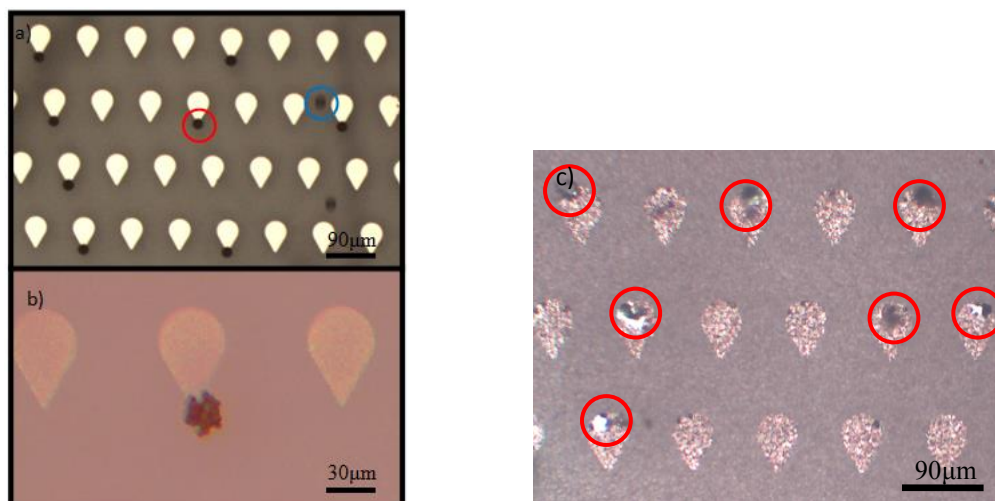


Figure 3: a) Microscope image of 20- μm magnetic beads (red circle) trapped by the local magnetic field maxima at the tips of the magnetic permalloy teardrops while the trajectory of non-magnetic beads (blue circle) remains unperturbed. The magnetic beads were captured with a sensitivity of 99.6% and a purity of 98.5%. Also, 99.95% of the non-magnetic beads were routed to the waste chamber. b) Magnetically tagged MCF7 cells were trapped on the magnetic permalloy teardrops, formed using an OAI 200 mask aligner. c) Capture of tagged MCF7 cells (circled in red) in a background of whole blood, which is flowing past. The MCF7 cells were captured with a sensitivity of 100%, while over 99% of the blood cells were routed to the waste chamber.

With a biomimetic sample of magnetically tagged MCF7 cells spiked into whole blood, we have furthermore shown a capture efficiency of 100% (Fig. 3c) while over 99% of the background blood cells advance to the waste chamber. We have also demonstrated media exchange without disturbing the retention of the cells and successfully released them for downstream analysis. We have also performed size separation by the variation of micro-dot geometry. Overall, this new and reversible magnetic alignment method of individual cells could easily be adapted to various formats of particle-based bioassays.

CONCLUSION

We have shown an immuno-magnetic method for high-efficiency, reversible trapping of magnetically tagged target cells suspended in a biological background into an array where they can be analysed by common bioanalytical protocols. The 2-dimensional nature of the system facilitates microfabrication, suppresses issues such as particle clogging and diverging flow fields that occur with common, 3D-geometrical obstacles and thus enhances capture efficiency.

ACKNOWLEDGEMENTS

This work was supported by the Science Foundation Ireland under grant No 10/CE/B1821. Fabrication was made possible by OAI, San Jose, CA.

REFERENCES

- [1] "Array-based capture, distribution, counting and multiplexed assaying of beads on a centrifugal microfluidic platform," R. Burger, P. Reith, G. Kijanka, V. Akujobi, P. Abgrall, J. Ducrée, Lab on a Chip, 12, 1289 (2012).
- [2] "Magnetic Separation of Malaria-Infected Red Blood Cells in Various Developmental Stages," J. Nam, H. Huang, H. Lim, C. Lim, S. Shin, Analytical Chemistry, 15, 7316 (2013).
- [3] <https://www.miltenyibiotec.com/en/products-and-services/macscell-separation/manual-cell-separation/separators.aspx>

CONTACT * Corresponding author: Jens Ducrée; +353 (1) 700 5377; jens.ducree@dcu.ie