LASER-BASED MANIPULATION AND FLUORESCENT DETECTION OF INDIVIDUAL, CENTRIFUGALLY ARRAYED BIOPARTICLES

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ABSTRACT

In this work we for the first time present a technology which pairs up fluorescence based detection / manipulation of individual particles using optical tweezers, with our highly efficient V-cup based trapping scheme. To the best of our knowledge, this is the first time that centrifugal microfluidics and optical tweezers have been combined to perform single particle manipulations.

KEYWORDS

Centrifugal Microfluidic, Cell Capturing, Optical Tweezers

INTRODUCTION

Centrifugal microfluidics for lab-on-a-chip applications is a field which has attracted considerable interest in recent years, both from academia as well as from industry [1]. The novel, here presented instrumental setup significantly expands state-of-art microfluidics to sophisticated particle manipulation and detection, thus for the first time enabling applications involving multiplexed, bead- and cell-based counting, ID, and "cherry-picking" of individual target particles. In particular, the integration of fluorescence detection comprises a seminal improvement of our preceding work, where the disc had to be cumbersomely moved to a separate microscope [2]. Furthermore we demonstrated in our previous work a magnetic actuator for the simultaneous retrieval of all captured particles [3]. In this paper we significantly improved the particle manipulation capabilities by adding an optical tweezers module to the setup, thus allowing manipulation of individual particles in the array.

WORKING PRINCIPLE

This work is based on our previously introduced, V-cup based particle capturing platform. In brief, particles (beads or biological cells) sediment under stagnant flow conditions into an array of scale matched V-cups, where they are mechanically trapped with a single-occupancy distribution. Earlier we demonstrated the very high (close to 100%) capture efficiency of this approach and the suitability of the system to perform bead-based immunoassays [2]. The major novelty of this work is the integration of an optical module which provides fluorescence detection as well as an optical tweezers function. The manipulation of particles using a laser has first been presented by Ashkin and co-workers in 1970 [4], followed by the demonstration of the trapping of living cells [5]. In brief, a laser beam is focused through a microscope objective with a high numerical aperture in order to create a highly focused spot with a steep field gradient. Dielectric particles such as polystyrene (PS) beads or cells are drawn towards this energy well where they are trapped. The captured particles can then be moved by displacing the laser focus, thus forcing the particle to follow in order to remain in the focus of the laser. For detailed description of the working principle in the ray optics regime (i.e. $d_{particle} >> \lambda_{laser}$) see [6].

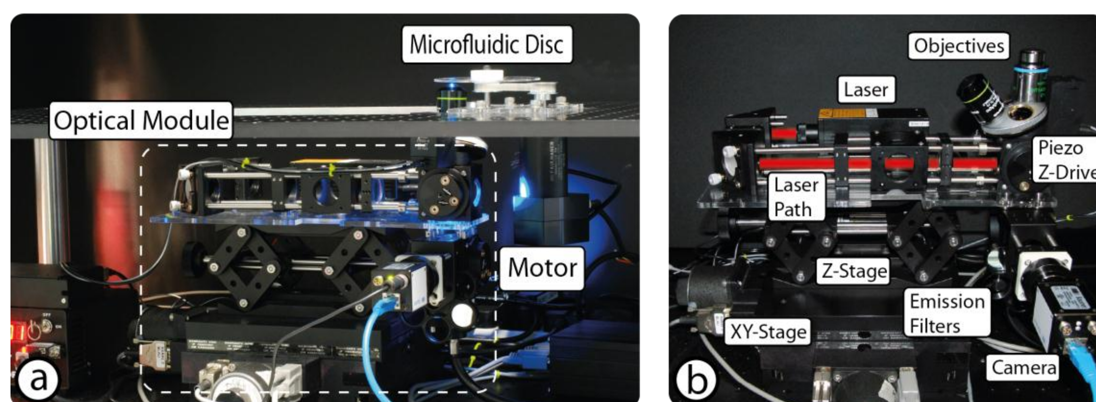

Figure 1: Centrifugal test stand comprising of motor and optical module (a). Optical module with optical tweezers setup, fluorescence detection and secondary camera for image acquisition during particle manipulation (b).

MATERIALS AND METHODS

The centrifugal test stand setup consists of standard components such as a computer controlled motor, camera and illumination. Furthermore it features an optical module (Fig. 1a). This module incorporates the optical tweezers to manipu-

late individual microparticles on disc using a 1-W, 1064-nm infrared laser (Roithner Lasertechnik, Austria). The laser is focused through a 40× oil immersion microscope objective with a numerical aperture of 1.3 (Zeiss, Germany). This setup allows a working distance of 200 µm. The module also features a fluorescence detection part at an excitation wavelength of 488 nm and up to six emission filters. Additionally, this module includes a secondary camera (TXG 14f, Baumer, Germany) which utilizes the same optical path as the laser to facilitate particle handling and acquisition of fluorescent images. The whole module is mounted on a computer controlled X-Y stage (Fig. 1b).

The microfluidic chips used in this work were manufactured in PDMS by casting on a lithographically structured SU-8 master. Following curing, the PDMS chips were cut to size, inlet holes were punched and the chips were irreversibly bonded to glass cover slides (thickness #1, VWR, Ireland) using O_2 plasma activation. The design of the chips and the holder to receive them on the test stand is outlined in Fig. 2.

For the experiments involving PS particles, we used FITC loaded beads with a diameter of 12.5 µm (PS FluoGreen) and non-fluorescent beads with a diameter of 20 µm (both from Microparticles, Germany). Cell experiments were performed using HL60 cells. The cells were fixed off-disc using 4% (v/v) formaldehyde (Sigma-Aldrich, Ireland), then captured and subsequently stained by incubation with Propidium Iodide (PI) (4 µl/ml, Invitrogen, Ireland) for 15 min. Subsequently the cells were washed twice with PBS containing 5% (v/v) FBS.

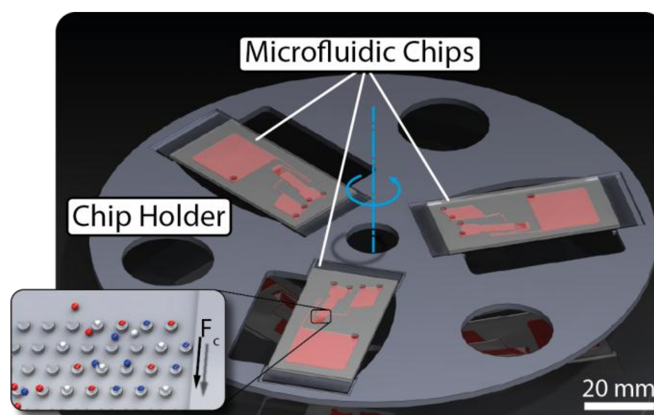

Figure 2: Microfluidic chips used in this work, attached to a holder to enable mounting on the centrifugal test stand. The insert shows the V-Cup based capturing principle.

RESULTS AND DISCUSSION

First, the manipulation of a captured PS particle using the optical tweezers is demonstrated. To this end, 20-µm beads were captured in the array and next one bead was selected and translated (Fig. 3 a-d). The optical tweezers then rearranged previously trapped, fluorescently-labeled beads to represent the letters “BDI” (Fig. 3 e-g). The main application of this platform is to capture cells from a suspension to identify and count subpopulations. This has been demonstrated by introducing a sample containing HL60 cells, capturing them in the V-Cups and staining them with PI. The stained cells were then imaged using the integrated optical module (Fig. 4 a and b). Furthermore, captured cells were then manipulated using the optical tweezers. The displacement of a trapped cell to a neighboring cup is shown in Fig. 4 c-f.

CONCLUSIONS AND OUTLOOK

In summary we present a decisively improved microfluidic platform which integrates centrifugal particle trapping, multi-color fluorescence detection and the capability to translocate single particles using optical tweezers. After demonstrating the successful on-disc capturing, staining, imaging and manipulation of PS beads and cells, work is currently underway to implement a complete cell screening assay using this platform.

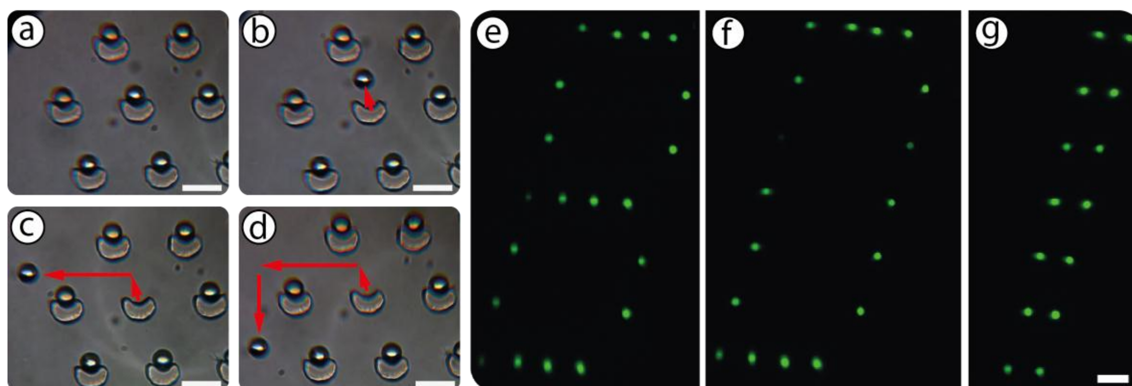

Figure 1: Single-bead manipulation using the optical tweezers module. After selecting a bead, the laser is turned on to trap the bead and "lift" it from the capturing element (a, b). The bead is then carried to the side and can be removed from the array for downstream analysis (c, d). Fluorescent PS beads with a diameter of 12.5 µm have been captured in the array and subsequently been re-arranged using the optical tweezers setup to display the letters "BDI" (e-g). Scale bars are 50 µm.

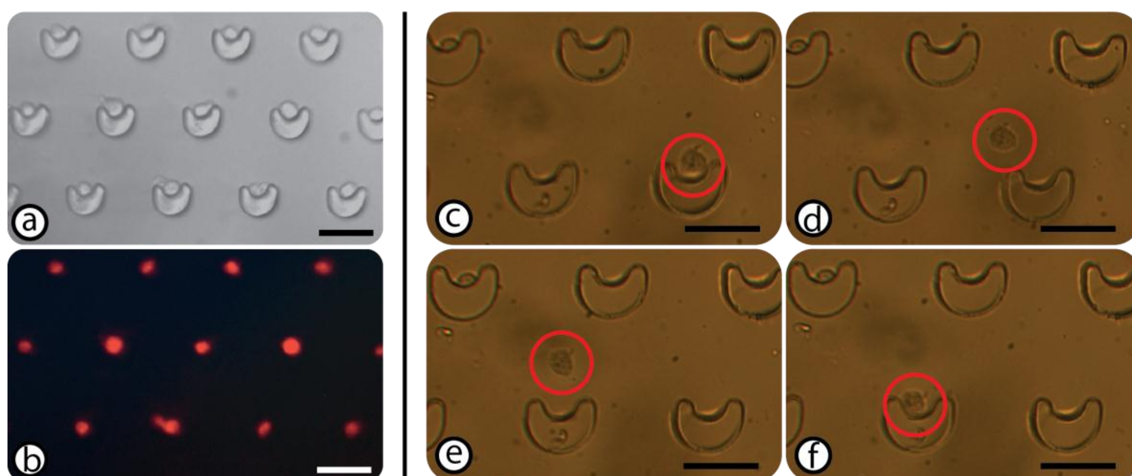

Figure 2: HL60 cells have been captured in the V-cup array, stained with PI and subsequently imaged using the optical module. Bright field image (a) and fluorescent image (b) of the same area. A captured HL60 cell is displaced from the initial capturing element to a neighboring V-cup using the optical tweezers module (c-f) Scale bars are 50 µm.

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