DISPOSABLE MICROFLUIDIC CHIP WITH INTEGRATED LIGHT SHEET ILLUMINATION ENABLES DIAGNOSTICS BASED ON MEMBRANE VESICLES

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ABSTRACT

Cell-derived membrane vesicles that are released in body fluids are emerging as potential non-invasive biomarkers for diseases like cancer. Techniques capable of measuring the size and concentration of such membrane vesicles directly in body fluids are urgently needed. Here we report on a microfluidic chip with integrated light sheet illumination, and demonstrate accurate fluorescence Single Particle Tracking measurements of the size and concentration of membrane vesicles in cell culture medium and in interstitial fluid collected from primary human breast tumours.

KEYWORDS: Light Sheet Microscopy, Lab-On-A-Chip, Diagnostics, Single Particle Tracking

INTRODUCTION

Nowadays, the relation between cell-derived membrane vesicles (MVs) in body fluids and the progression of diseases like cancer is receiving a lot of attention [1, 2]. MV properties that are of potential diagnostic and prognostic value are size and concentration, leading to many efforts to develop techniques that are suitable for MV characterization in terms of these properties [3]. Specifically, techniques capable of performing the characterization of MV size and concentration directly in body fluids are of major interest [4].

Fluorescence Single Particle Tracking (fSPT) is a technique that is capable of accurately measuring the size distribution and number concentration of fluorescently labelled nanoparticles directly in body fluids such as blood [5, 6]. However, because fSPT is based on epi-fluorescence microscopy, the contrast is limited due to fluorescence coming from out-of-focus particles or unbound fluorescent dye. Especially for fSPT characterization of MVs, the latter aspect can be problematic since the concentration of MVs is unknown a priori, so that a surplus of fluorescent label has to be added to ensure that all MVs will be stained. This, in turn, means that smaller MVs that contain less fluorescent dye may become invisible in the fluorescence background, resulting in an underestimation of the number concentration and a biased size distribution.

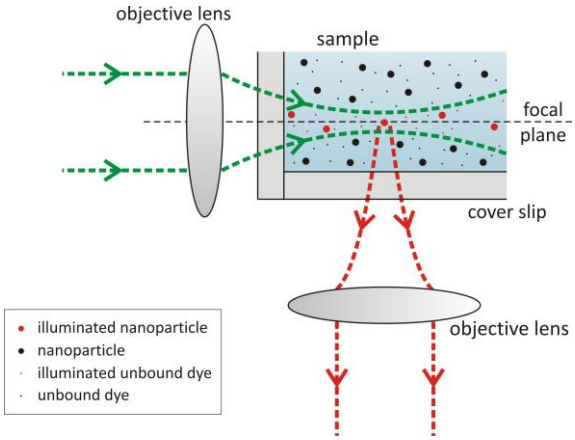

Figure 1: Illustration of the cross-sectional side view of the excitation (green) and fluorescence (red) light path in light sheet illumination.

An alternative to epi-fluorescence illumination that offers superior contrast is light sheet illumination [7]. As illustrated in Figure 1, in its classic form this requires a second objective lens for creating the light sheet, positioned

perpendicular to the imaging objective lens, and a sample holder that has two high-quality optical windows for illumination and imaging. However, as these sample holders are difficult and expensive to manufacture [8], they are not suitable for high-throughput diagnostic assays for which inexpensive disposable sample holders are preferred in order to avoid extensive cleaning procedures and sample contamination. We, therefore, created a mass producible microfluidic chip with integrated light sheet illumination for fSPT size and concentration measurements of fluorescently labeled MVs directly in body fluids.

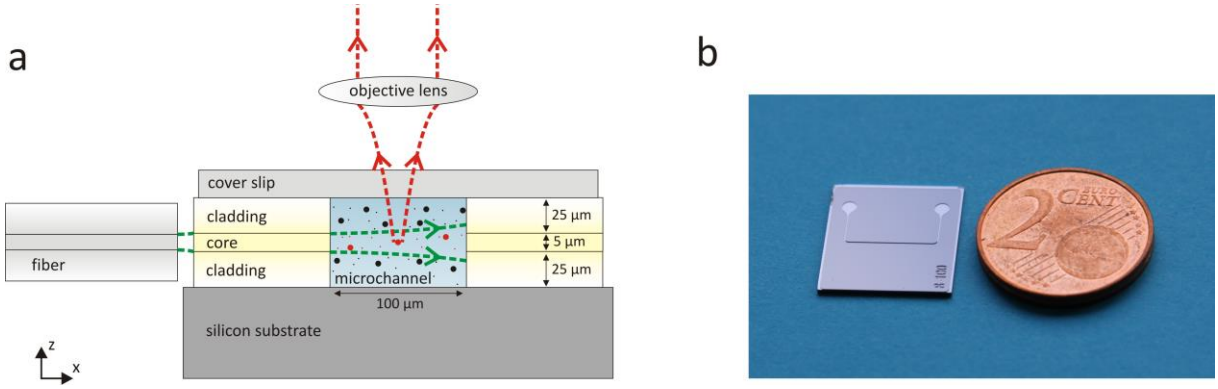

Figure 2: (a) Illustration of the cross-sectional side view of the microfluidic chip with silicon substrate and integrated waveguide for on-chip light sheet illumination. Laser light (green) enters the core of the planar waveguide by means of an optical fiber so that a sheet of light emerges in the microchannel. The fluorescence light (red) is collected by an objective lens. The drawing is not to scale. (b) A photograph of the microfluidic chip with silicon substrate (without a microscope cover slip).

EXPERIMENTAL

The chip is constructed on a glass or silicon substrate on top of which a planar waveguide structure is created consisting of one core layer of SU-8 between two cladding layers of SU-8 mixed with the epoxy resin D.E.R. 353 [9]. The three SU-8 layers are sequentially deposited by spin coating followed by a soft bake step. A microchannel of 100 µm width is created in the SU-8 waveguide using standard photolithography. The process is carried out on a 10 cm diameter glass or silicon wafer, so that multiple chips are fabricated simultaneously. Chips based on the glass wafer are covered with polydimethylsiloxane (PDMS) and imaging of the microchannel containing the body fluid with fluorescent MVs is performed through the glass substrate. Chips based on the silicon wafer are sealed with a microscopy cover slip, see Figure 2. The chips are mounted on a microscope and laser light is coupled into the waveguide using an optical fiber attached to a high precision alignment stage.

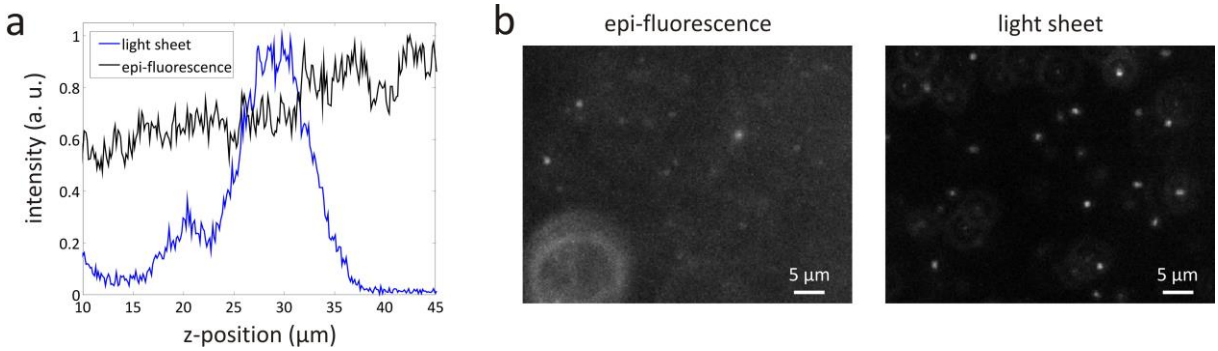

Figure 3: (a) The light sheet intensity profile along the z-axis. Contrary to epi-fluorescence illumination (black line), illumination through the planar waveguide results in excitation light that is restricted to a thin region along the z-axis at the centre of the channel with a full width at half maximum of ~9 µm (blue line). (b) Experimental characterization of the contrast using light sheet illumination versus epi-fluorescence illumination. Images are recorded with the microscope focused at the centre of the light sheet in the silicon chip.

RESULTS AND DISCUSSION

The light sheet generated by the planar waveguide was characterized by acquiring a z-stack through the microchannel containing a dispersion of fluorescent nanospheres. The intensity profile across the entire channel is found to have a full width at half maximum of ~9 µm, as shown in Figure 3a. The contrast was improved compared to epi-fluorescence illumination, as illustrated in Figure 3b. To demonstrate the potential of the chip as a diagnostic tool, fSPT measurements were performed on cell-derived MVs secreted in cell culture medium of breast cancer cells. As shown in figure 4, the majority of the MVs were situated in the 50 - 700 nm size range, and the concentration was ~$8 \cdot 10^8$ #/ml. Using epi-fluorescence illumina-

tion, a 4× lower concentration was found with a size distribution that is shifted towards larger values. On-chip fSPT measurements were also performed on cell-derived MVs secreted in interstitial fluid harvested from human breast cancer specimens. The MVs were found to be situated in the 90 - 900 nm size range, with a total number concentration of ~$4 \cdot 10^8$ #/ml. Using epi-fluorescence illumination, no meaningful size distribution or concentration could be determined. This demonstrates that improving contrast by light sheet illumination is essential for correct MV characterization.

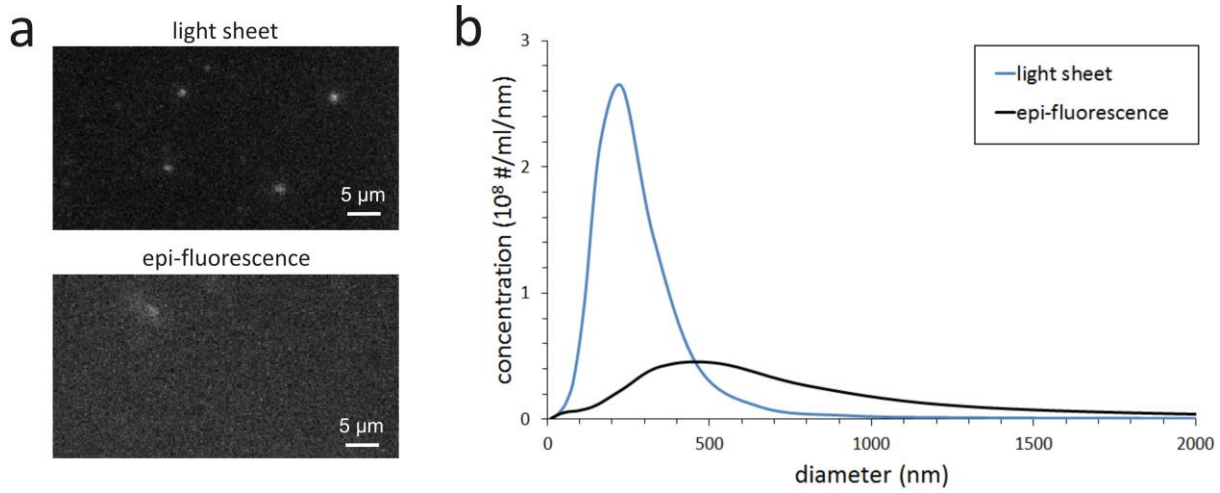

Figure 4: fSPT size and concentration measurements were performed on cell-derived MVs in cell culture medium. (a) The MVs are clearly visible when using light sheet illumination, while only a few MVs are visible for epi-fluorescence illumination due to the much higher background fluorescence. (b) The number concentration is underestimated and biased towards larger MVs for epi-fluorescence illumination versus sheet illumination.

CONCLUSION

The microfluidic chip presented here is simple in design, can be mass-fabricated at a low cost. It was successfully demonstrated that the chip enables accurate fSPT size and concentration measurements of MVs in cell culture medium and in interstitial fluid collected from primary human breast tumours. Thus, it opens the possibility to be used as a diagnostic tool that combines low cost, ease of use, and sensitivity.

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