AN INTEGRATED MICROPHOTONIC BIOSENSOR FOR SIMULTANEOUS REFRACTIVE INDEX AND DEFORMABILITY CELL DISCRIMINATION

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ABSTRACT

Effective refractive index and deformability of cells can be used to identify various cancers, infections and diseases. The proposed microphotonic biosensor measures the effective volume refractive index of deformed cells at a throughput as high as 6000 cells/s. These physical parameters yield dye-free discrimination for better and faster diagnostics. The biosensor discriminated three white blood cell populations: neutrophils, basophils and baseline myeloids. Notably, these cell populations are not distinguishable by flow cytometry. Thus, this device represents an important step towards discriminating different cell types and could be integrated in flow cytometry instruments and cell counter devices.

KEYWORDS: Microphotonic, Microfluidic, Fabry-Pérot, Optical microcavity, Biosensor

INTRODUCTION

Flow cytometers and Coulter counters are essential equipments in many hospitals and research centers. Owing to their high-throughput and specificity using Fluorescence-Activated Cell Sorting (FACS), they are tools of choice for clinical and research professionals. However, there is a high demand for improvements in these equipment amongst biomedical technologists. Indeed, these systems have a high cost, are space consuming and lack portability whereas the dyes used can be expensive, time consuming and increase cell death. Studies have shown that the refractive index can be used to identify various cancer cells and could help diagnose infections [1]. Furthermore, deformability can be used to identify different white blood cells [2]. We propose a microphotonic biosensor capable to measure the effective volume refractive index of mechanically deformed cells at a high-throughput. This device enables new dye-free discrimination parameters. To our knowledge, no dynamic or high-throughput measurement of refractive index and deformability for large cellular populations has been demonstrated simultaneously in a single integrated microsystem.

MATERIALS AND METHODS

Figure 1 shows a rendering of the assembled microphotonic biosensor with the highlighted highly sensitive Fabry-Pérot resonant cavity, $\Delta n = 1.6 \times 10^{-5}$, reported in previous work [3]. As a cell flows through the microcavity, the resonance peak shifts towards longer wavelengths since the refractive index of the cell is higher than its surrounding. A fast InGaAs infrared photodetector records optical power variations at a fixed wavelength and relays it to a high-speed digitizer. Myeloid cells derived from a promyelocytic leukemia cell line (HL60) were differentiated into basophils, neutrophils or maintained in a baseline differentiation state. We opted for these specific conditions since Forward Scatter (FSC) and Side Scatter (SSC) measurements used in flow cytometry cannot discriminate these cell types. Cells were injected in a microchannel, sheathlessly focused and ordered using inertial forces [4]. The straight microfluidic channel allows for shear-stress induced mechanical deformation on cells to be promoted or repressed simply by adjusting the flow rate. Considering imposed flow rates between 5 and 15 µl/min, microchannel dimensions of 35 µm in width by 15 µm in height and an optical mode around 45 µm in width, measurement rates at the cavity are between 2000 and 6000 cells/s.

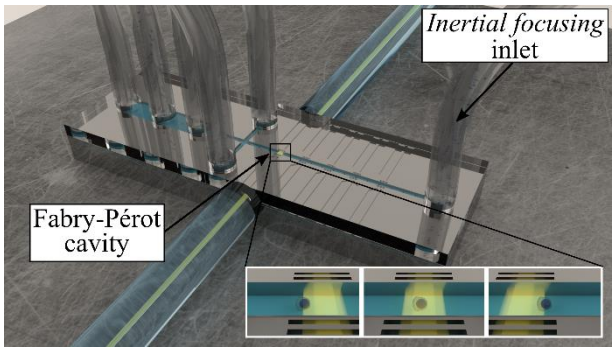

Figure 1: 3D rendering of the assembled microphotonic biosensor with insets showing a deformed cell flowing through the resonant cavity volume.

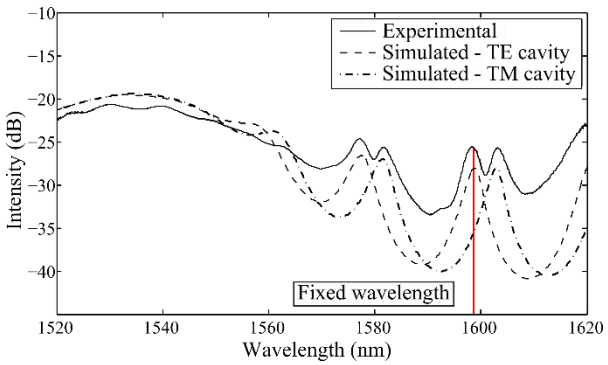

Figure 2: Fabry-Pérot spectrum showing separated TE and TM resonance. Red line represents the fixed wavelength used for cell sensing.

RESULTS AND DISCUSSION

Figure 2 shows the experimental spectrum of the Fabry-Pérot cavity with the fixed wavelength used for optical power variation measurements. TE and TM modes exhibit distinct peaks in the spectrum due to the combined effect of their difference in modal index, attenuation coefficients and mode size inside the very large cross-section rib waveguide. Figure 3 and Figure 4 show the typical signals in time for undeformed and deformed single cells measurement respectively. Parameters extracted from these curves are used to compare cell populations. Measurements on undeformed cells, represented as color-coded density dot plot, demonstrate discrimination only for neutrophils, as shown in Figure 5. Conversely, when shear stress is promoted, the device demonstrate discrimination for neutrophils, basophils and baseline condition. Figure 6 shows the color-coded density dot plot for deformed cells. Thus, simultaneous measurement of cells refractive index and deformation enhances discrimination.

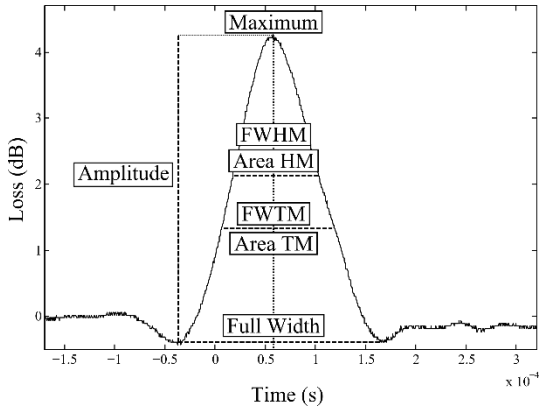

Figure 3: Typical signals for undeformed single cells with extracted parameters.

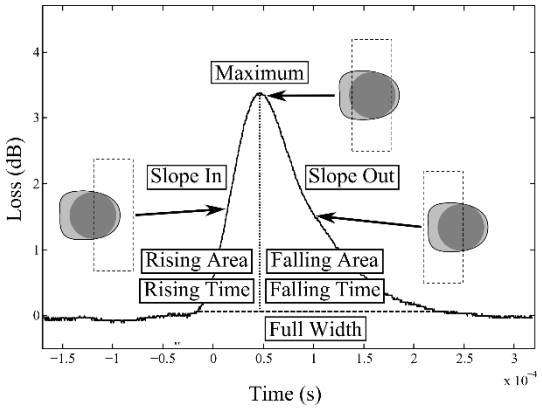

Figure 4: Typical signals for deformed single cells with extracted parameters and schematized cell flowing stages.

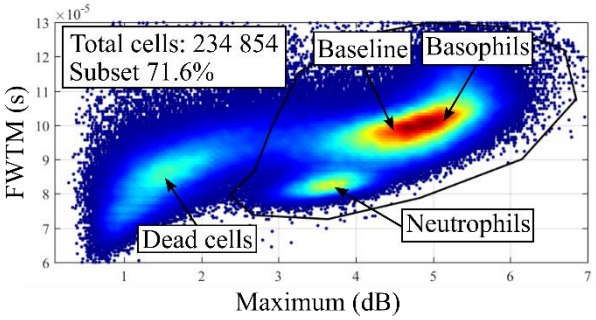

Figure 5: Color-coded density dot plot of undeformed cell populations showing only neutrophils discrimination. FWTM is the Full Width at one Third Maximum.

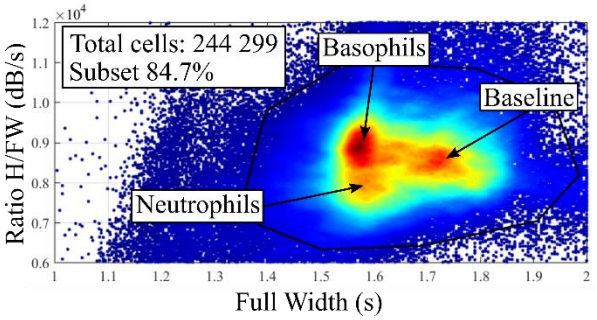

Figure 6: Color-coded density dot plot of deformed cell populations showing all populations discrimination. Ratio H/FW is the ratio of Maximum (H) over Full Width (FW).

CONCLUSION

Differentiated myeloid cells can be effectively separated by simultaneous measurement of refractive index and deformability as shown using our microphotonic biosensor. Thus, these physical characteristics can enhance cellular discrimination and improve flow cytometry capabilities. Integration of this device within a flow cytometry unit should improve cellular resolution, provide crucial information and thus meet the demand of biomedical technologists.

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