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INVESTIGATION OF FLOURESCENCE COHERENCE TOMOGRAPHY FOR OPTOFLUIDIC APPLICATIONS

Lukas-Karim Merhi[1], Bhuvaneshwari Karunakaran[2]*, Sae-Won Lee[1], Ash M. Parameswaran[1], Soumyo Mukherji[2], Debjani Paul[2], Mirza Faisal Beg[1], Marinko V. Sarunic[1]*

1) Engineering Science, Simon Fraser University, Burnaby, BC, Canada V5A 1S6
2) Biosciences and Bioengineering, Indian Institute of Technology Bombay, Mumbai, India 400076

* These authors contributed equally to this work

INTRODUCTION

Conventional flow cytometers (FCs) and fluorescence-activated-cell-sorters (FACS) are mechanically complex, bulky, and require specialized human operators, large sample volumes, and sample preparation procedures for proper diagnosis of diseases such as leukemia and malaria [1], [2]. For this reason, there has been an increasing demand for miniaturization, reduction of cost and portability of such devices. Lab-on-a-chip devices, which integrate microfluidics with other technologies, have been emerging as a potential solution to miniaturization of FC/FACS technology [3], [4]. One of the serious limitations of lab-on-a-chip devices is their inability to extract shape or morphological information which is very useful for cell differentiation and characterization [5], [6]. To meet this challenge, optical imaging techniques and microfluidics are combined to form a subset field in 'optofluidics' [7], [8].

In this report, as a proof of principle, we present the integration of a novel optical imaging technique called Fluorescence Coherence Tomography (FCT) with microfluidics in order to measure the cross-sectional position of a flowing particle in a microchannel. The preliminary results acquired through this work are important for future development of applications in the miniaturization of molecule specific flow cytometry. Example applications for this technology are motivated by recent work demonstrating that a cell's position in a microchannel is highly sensitive to its size and stiffness, which in turn are important biomarkers for cell classification [4]. By combining FCT with microfluidics, the long term goal is to provide researchers and scientists with new possibilities for biological investigations in optofluidic applications.

This report is organized as follows: the methods section introduces the theoretical concepts of FCT, the optical experimental layout, the preparation of stationary samples used in preliminary testing, and fabrication of optofluidic samples. The results section presents the optofluidic experimental outcomes. The report is concluded with a description of the system's limitations and a discussion on future work.

METHODS

FCT Concepts

FCT is a low coherence interferometric technique where signal is formed based on the amplitudes of the interferometric fringes, generated by a sample and reference reflection [9]. The key to FCT is the self-coherence. One can consider the fluorescence self-interference effect as a wave packet interfering with itself as illustrated by concentric circles in Figure 1 representing the emission wave packet of a fluorophore placed in front of a reflective surface.

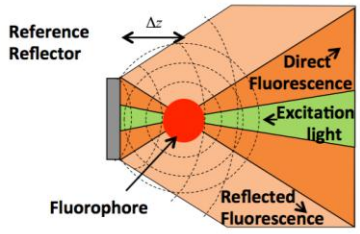

Figure 1: Schematic of the two optical paths of interest for the FCT signal

In Figure 1 the reflected fluorescence (with electric field E_R) and direct fluorescence (with electric field E_S) are shown to originate from a single fluorescent particle. They are combined to interfere and produce fringes as a function of the optical path length difference ($2\Delta z$) given by

$$I(k) = S(k) \cdot \left({E_R}^2 + {E_S}^2 + 2E_R E_S \cos(2\Delta z n k) \right), \quad (1)$$

where k is the wavenumber of the light, $S(k)$ is the spectral power density, and n is the index of refraction of the medium. To extract the axial location of fluorophores relative to the reference reflector, the Fourier transform of the interferometric signal, $I(k)$ in Eq. 1, is performed resulting in

$$\hat{I}(z) = \hat{S}(z) \otimes \left[2E_R E_S \cdot \left(\delta(z + \Delta z) + \delta(z - \Delta z) \right) \right], \quad (2)$$

where $\hat{S}(z)$ is the Fourier transform of the fluorescence spectrum. The delta functions, $\delta(z \pm \Delta z)$, describe the position of the fluorophore with respect to the reference reflector.

The coherence length of the fluorescent light, regarded as the source light in FCT dictates the system's axial resolution, which is dependent on the width of the fluorescence spectral range; the wider the spectral range is, the narrower the spread of $\hat{S}(z)$ becomes, increasing the precision of the axial measurement. In Fourier analysis, the Signal to Noise Ratio (SNR) can be expressed as the ratio of the peak value (μ) to the standard deviation of the noise floor (σ) in decibels (dB). Before performing the Fourier transform of the interferometric signal, $I(k)$ in Eq. 1, a few data processing steps are required such as: DC subtraction, linear sampling, Hann windowing, and sample padding.

FCT system Topology

The FCT system topology shown in Figure 2 is composed of a phase-stable common-path interferometer, and a custom-built spectrometer. In order to assist with sample alignment, a wide field imaging system (microscope) was incorporated with the setup. The FCT excitation light source used was a frequency doubled Nd:YAG laser (532 nm) with output up to 6.7 mW. The light source was directed to a 10x objective with a focal length of 16.6 mm, through a 593 nm single edge dichroic beam splitter. Instead of scanning the position of the beam, the sample assembly was equipped with linear actuators for x-y-z axis translation. In the common-path interferometer in Figure 2, the objective focused the laser beam down to a waist (w_0) of 5.6 µm at the sample with a depth of focus (*DOF*) of 370 µm. Fluorescent light excited at the sample was collected by the objective, and directed by a dichroic to the detector. Spectrally resolved detection of the fluorescent signal was performed using a custom lens based Czerny-Turner type spectrometer, shown in Figure 2. The first order of diffracted fluorescent light was focused onto the CCD detector (Basler Scout scA1390-17gm) with 1392 by 1040 pixels (4.65 µm square).

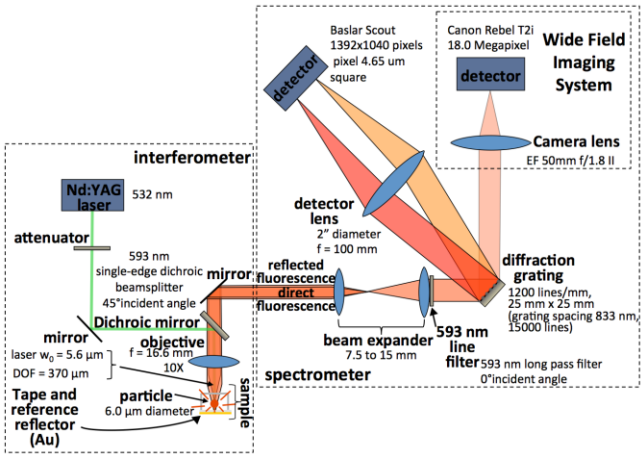

Figure 2 Schematic diagram of FCT setup.

Fluorescent Sample Preparation

In order to model cells in a microchannel, 6 µm in diameter fluorescent polystyrene microspheres (36-2FMT, Thermo Scientific, MA) were chosen to match the average diameter of human red blood cells (nominally 7-8 µm in diameter [10]). The 6 µm microspheres are dyed throughout with a proprietary "Firefli™" dye manufactured by Thermo Scientific. The "Firefli™" excitation and emission spectra have peaks at 542 nm and 612 nm, respectively, corresponding to a Stoke's shift of 70 nm.

To use the microspheres it was necessary to immerse them in an aqueous solution and serially dilute them to obtain a low enough concentration. This guaranteed that the detection of fluorescent emission was due to single particles in both stationary and optofluidic sample configurations. Visual inspection determined that the 0.14 mg/ml mixture contained sparse single particles.

Stationary Sample

The stationary sample consisted of a serially diluted deposition of 6 µm microspheres on a single strip of double sided tape (Scotch® Permanent Double Sided Tape) adhered to a gold-coated microscope slide (EMF Corporation, CA136 (Cr/Au)), illustrated in Figure 3 (a).

Optofluidic Sample

The master mold from which polymer replicas can be generated was made using standard photolithography techniques of patterning permanent epoxy negative photoresist SU-8 (2025 MicroChem, MA). The process involved spinning of SU-8 onto a silicon wafer, soft baking, defining the SU-8 structures by exposure to UV light using a photomask, post-exposure baking, and lastly SU-8 development. The microfluidic channel was 3 cm long with a rectangular cross section of 218 µm in width and 28 µm in height. The square reservoirs were 5 by 5 mm. The microfluidic device was fabricated with polydimethylsiloxane (PDMS) (Sylgard 184, Dow Corning, MI) using the standard soft lithography technique [11]. The thickness of the PDMS layer at the channel was maintained at ~3.7 mm. Holes were punched into the reservoirs for inlet and outlet ports for inserting polyethylene tubing (Intramedic, PE 190 ID 1.19 mm). The PDMS was tape bonded (Scotch® Permanent Double Sided Tape) to a gold coated microscope slide, and baked for 2 hours at 80 °C [12]. This fabrication technique allowed the production of device replicates shown in Figure 3 (b).

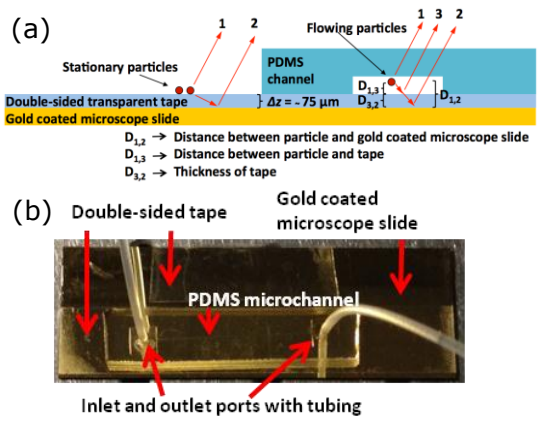

Figure 3: (a) Schematic diagram of the stationary and optofluidic sample. (b) Physical optofluidic sample

RESULTS

To perform FCT acquisition on the flowing microspheres, the optofluidic sample in Figure 3 (b). was placed in the interferometric configuration shown in Figure 2 and was connected to the syringe pump via the tubing. In order to detect faint fluorescence without introducing significant noise, the CCD detector was set with an analogue gain of 0 dB and an integration time of 55 ms. Figure 4 shows the results of the data processing steps for a single frame of the fluorescence signal from a flowing particle: (a) 33 rows of interferometric data (for this case), (b) vertically binned intensity profile of the rows from (a) shown in blue with a superimposed interpolated DC shown in red, and (c) intensity profiles of fringes after DC subtraction. Next, the Fourier transform of the interferometric fringe signal was computed as illustrated in Figure 4 (d). In Figure 4 (d), two peaks are visible, the first peak identified with blue markers corresponded to $D_{3,2}$, the thickness of double sided tape. The second peak identified with red markers corresponded to $D_{1,2}$, the distance between the microsphere and the mirror. $D_{3,2}$ and $D_{1,2}$ were measured as 76.9 ± 2.3 µm and 100.2 ± 2.3 µm, respectively, given by the center of the peak ± the coherence length. By subtracting $D_{3,2}$ from $D_{1,2}$, the position of the fluorescent particle in the channel away from the tape, $D_{1,3}$, was calculated to be 23.3 µm ± 4.6 µm. In this experiment, the laser was focused at the center of the channel therefore limiting the optical

detection system to fluorescent particles flowing right at the center and missing out on particles flowing off center. To overcome this limitation, a cross-sectional image of fluorescent particles flowing through the channel could be obtained by scanning the laser perpendicular to the direction of flow. Alternatively, a laser line focus with a non-confocal imaging spectrometer configuration can be used to obtain the cross-sectional image [9].

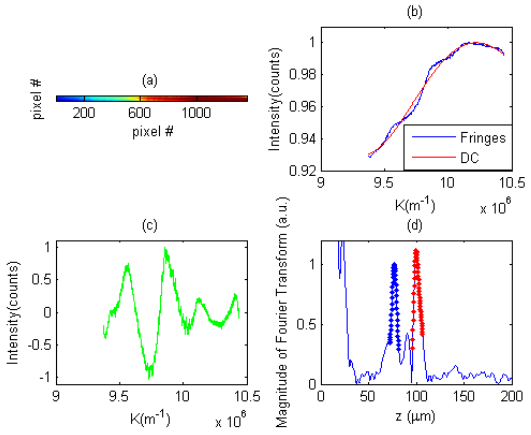

Figure 4: (a-d) processing steps of interferometric data

CONCLUSION

The concepts developed in this report established a technical foundation for the combination of Fluorescence Coherence Tomography with microfluidics to open up new possibilities for biological investigations in optofluidics. Fluorescent microspheres with a diameter of 6 μm were injected into a 28 μm tall PDMS microfluidic channel that was adhered to a gold coated microscope slide with double sided tape. The optical system was then used to perform FCT on these flowing fluorescent microspheres. The results showed that the system was able to resolve the axial position of the flowing particle in the microfluidic channel to within the coherence length.

Future Work

Beyond simple optics, detector changes, and new post processing techniques, the focus of future work should be to transfer the FCT technology from its current "bulky" state to a compact or on-chip implementation. However, for compact or on-chip imaging technique, self-interference fluorescence will be challenging. Nonetheless to benefit from the full potential of this technology as a portable hand-held device, the interferometer and spectrometer need to have a compact or on-chip implementation.

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