Optofluidic Microscopy using Femtosecond Micromachined Glass Microfluidics

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Abstract: Optofluidic microscopy is a versatile modality for imaging and analyzing particles and their properties in flow. In this paper, we demonstrate the applicability of microfluidic devices fabricated using femtosecond laser machining in fused silica for integration into optofluidic imaging systems. In addition, glass, being chemically inert, robust, and inexpensive, is ideal for field-deployable prototypes. By using glass for the sample handling component of our microscope, we are able to exploit its superior optical quality for imaging and biocompatibility. By integrating these glass microfluidic devices into a custom-built bright field microscope, we have been able to analyze red blood cells in flow with good fidelity.

1. Introduction

Of late, there has been a significant growth of development of microscopy systems based on microfluidic platforms. [1-5]. Microfluidics offers several degrees of design freedom and by developing custom designs for optofluidic components, researchers have been able to demonstrate imaging throughputs of up to 20,000 cells per second [1]. Specific modalities such as phase imaging [2], high speed fluorescence imaging [3], and 3D imaging of cells [4, 5] have been reported in literature. A vast majority of previously reported techniques employ PDMS (polydimethylsiloxane) microfluidic devices as the transport conduit(s) for the sample to be transported across the imaging field of view. The absorptive (chemical) nature of PDMS presents challenges for imaging systems. For example, PDMS is known to absorb small hydrophobic molecules (fluorescent labels like Nile red [6] and Rhodamine B [7]), both of which are commonly used for staining biological specimen for imaging. The absorption of these stains into the microfluidic device results in fluorescence emission from the PDMS device itself, leading to inaccuracies from deterioration of the imaging quality of the system.

Glass microfluidic devices, on the other hand, are more ideal for handling samples in microfluidics-based imaging systems. Glass is more robust and possesses superior optical quality as compared to PDMS. In addition, glass is bio-compatible and is not known to exhibit absorption of any organic compounds. Till recently, the fabrication of glass microfluidics was a significantly more complex process than soft-lithography (for PDMS). However, the advent of femtosecond micromachining has enabled rapid prototyping of glass microfluidic devices [8]. Using ultra-fast laser inscription (ULI) processes, we have fabricated glass microfluidic devices consisting of subsurface, channels. Multiple channels have been used to enable degeneracy as well as to obtain higher imaging throughputs. We have assembled a custom, bright-field transmission microscope to image red blood cells (RBCs) flowing through the microfluidic channels. In the following sections, details of the fabrication process of these devices and the experimental setup used to characterize these devices for imaging applications are described.

2. Fabrication of devices

The ULI technique uses short pulses from a laser to locally modify materials such as fused silica. The sample is transparent to the laser wavelength as such but absorbs through a multi-photon process when the light is focused onto it. Depending on parameters such as pulse energy, repetition rate and translation speed, several types of material modifications are possible. First is a refractive index modification in the material useful for creating waveguides. Second is the formation of nano-gratings which enables selective chemical etching. Third is the formation of voids in the material.

The sample is mounted on a high-precision translation stage and the femtosecond pulses are focused onto it using an objective. The sample can be moved relative to the laser to create 3D structures. The ability to easily create

3D structures is an advantage over other methods of fabricating microfluidic devices such as lithography using PDMS. Fused silica is also a more damage resistant material than PDMS and as previously noted, is compatible with many different biological samples and chemicals. ULI is also ideal for rapid prototyping as it does not require a mask to be created for each modification.

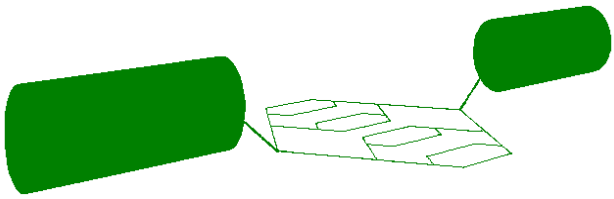

Figure 1: Schematic diagram of the microfluidic devices.

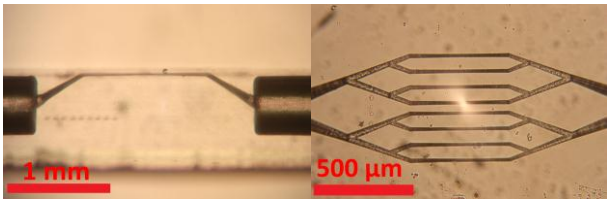

Figure 2: Device after etching. (a) Side view of the device showing the channel moving up to the surface for imaging. (b) Top view showing the individual channels.

The microfluidic devices used in these experiments consist of a series of parallel horizontal channels, as shown in figure 1, with widths varying from 5-20 µm and depths of ~20 µm. The design ensures that the cells in each channel are at the correct plane of focus for the microscope and prevents multiple cells from being present in the same channel position at once. The inlets of the device are offset vertically from the central channels and connected by angled channels to move the imaging region closer to the surface of the device.

The microfluidic devices used in these experiments were fabricated using ULI and hydrofluoric acid (HF) etching in fused silica. A Fianium HE-1060-1µJ femtosecond laser with a repetition rate of 500 kHz and pulse length of 330 fs and power of 250 mW was used to modify samples mounted on an Aerotech XYZ stage. After writing samples were etched for approximately 20 hours in 5% HF. Tubing from Upchurch Scientific (outer and inner diameter of 360 µm and 100 µm respectively) was bonded to the inlets using UV curing glue (Thorlabs MIL-A-3920). Figure 2 shows one of the devices after etching.

3. Design of custom bright field transmission microscope

Figure 3 depicts a schematic of the experimental microscopy setup. A high power blue LED (Holmarc HO-HBL-3B) was used to illuminate the iris. The illuminated iris was imaged onto the object plane using a plano-convex lens L2 (f=25 mm). A 40X (0.75NA) objective was used to magnify the cells inside the microfluidic devices. A low-frame rate digital camera (Imaging Source DMK 72BUC02) was used to acquire images of the cells.

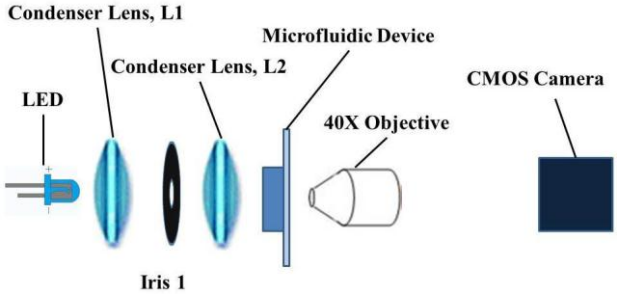

Figure 3: Experimental setup used for characterization of glass microfluidic channels

The glass microfluidic device was mounted on the optical setup (shown in figure 3) using a custom-designed sample mount and a three-axis translational stage (Melles Griot) for moving the flow channels into the field of view and focusing cells. Using a syringe pump (New Era Pump Systems NE-300), a suspension of red blood cells was flown through the microfluidic device at flow rate of 20 µl/hr. The exposure time of the CMOS camera was set to 100 µs and the frame rate was set to 52 fps. We developed an image processing algorithm was developed in MATLAB to analyze the videos of the flow stream and identify cells.

4. Results

By using simple background subtraction algorithms, we have successfully retrieved images of RBCs from the captured videos. A frame not containing any cells was defined as the background (shown in fig. 4(a)) and subtracted from all the other frames present in the video. The effect of background subtraction on a given frame is shown in figure 4.

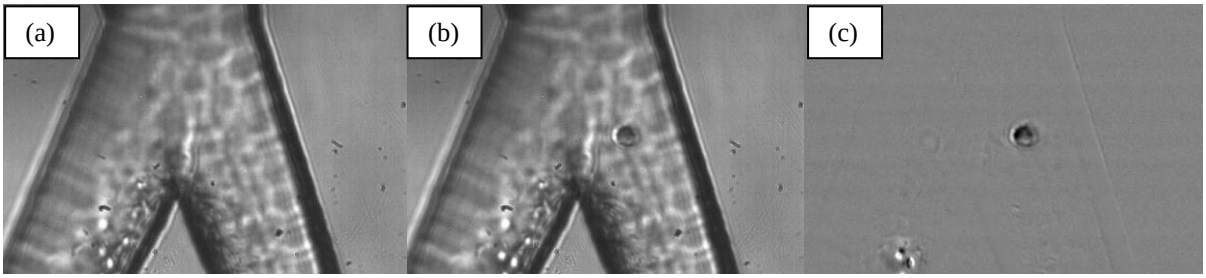

Figure 4 : (a) Background image not containing any cells. (b) Frame containing a cell before background subtraction. (c) Frame containing cell after background subtraction.

After this step, the frame was converted into a binary image with the use of appropriate thresholding. The threshold was chosen so that all the portions (pixels) of the image apart from the cell(s) were converted to zeros. On the thresholded image, connected component analysis was performed using the 'regionprops' function in MATLAB. The centroid of the cell was obtained and subsequently the portion of the frame containing the cell was cropped. Representative images of the cells obtained by processing few frames in the recorded video of the flow stream have been shown in the figure 5.

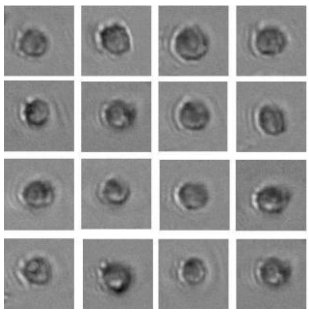

Figure 5: Representative images of red blood cells flown through the glass microfluidic device and acquired using the imaging cytometry system

5. Conclusion

In this paper, we have demonstrated the use of femtosecond-laser micromachined glass microfluidic devices for microscopy in flow. With the use of simple background subtraction algorithms, we have been able to obtain good fidelity images of cells flowing in these devices. We believe that the glass microfluidic platform provides a more robust and efficient alternative to PDMS microfluidic devices for use in optofluidic imaging applications.

4. References

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