

Flash imaging for microfluidics

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Abstract—Imaging of microparticles or biological entities inside microchannels provide informative data, given that the image quality is good and at par with the speed of the moving object. High-speed camera has been a boon for decades to capture fast moving objects, both in macro as well as micro world. This work reports a flash imaging system developed to image moving particles, thereby removing the need of conventional high-speed camera systems.

Keywords—flashing, LED pulsing, microfluidic imaging

I. INTRODUCTION

Microfluidics as a field is evolving in research for several applications ranging from lab-on-a-chip, inkjet technology, PCR applications to rapid diagnostic tests and many more. Cell imaging inside microfluidic channels provides the advantage of flow control, improved cell microenvironment, dynamic cell study and the provision of single cell analysis for studying cell-to-cell variability. As we go down the length scale, the time also scales down. This means that as the object under study reduces in size, the event becomes too fast to analyse. To examine the nature of such fast-moving particles inside a microfluidic channel, one of the conventional techniques is to image them with the help of a high-speed camera. However, in resource limiting settings, using such camera systems would be counterproductive due to the bulkiness and high cost associated with them. Also, huge amount of unwanted data is recorded and it is time consuming to process and filter out the important information. Other techniques involve fluorescence-based approaches to stain the cells and observe under fluorescence microscope or camera systems. However, such systems require elaborate pre-processing steps to label the cells before analysis. Hence, there is an urgent need to come up with a cost-effective and portable microfluidic imaging system to help researchers in this field, thereby reducing the number of pre-processing steps and providing relatively simple label-free imaging platform.

II. METHODOLOGY

The platform presented in this work comprises of a flashing circuit that is programmed to pulse high current in small time duration through an LED positioned on top of the microchannel with the help of a focusing illumination setup. The current pulsing circuit produces microsecond duration light of the order of $\sim 10\text{-}20\mu\text{s}$, by generating short current pulses of $\sim 3\text{-}4\text{A}$ amplitude whose duration and frequency are programmable. The circuit works in three modes which together constitute a charging discharging cycle and decides the frequency of operation of the circuit.

A. Setup

The flash circuit is pre-programmed with a specific current amplitude, pulse duration of flash and frequency of pulsing. Fig. 1 shows the schematic of the setup.

A high-power LED is positioned above the microfluidic device in line with a standard 21fps camera just below the device. The sample containing the blood cells is passed inside the microchannel with the help of a tubing through a syringe pump, connecting the sample filled syringe to the inlet of the device. The camera is turned on in video mode manually. As soon as the light flashes, the camera's shutter records the information of passing cell and captures an image during the short time when the LED flash is on. Since the light is flashed for a few microseconds, the cell appears to be virtually stationary and hence we obtain a blur-free image.

B. Working of the flashing circuit

Fig. 2 shows the fabricated pulser circuit board (Fig. 2(a)) along with its schematic (Fig. 2(b)) and the ideal waveforms (Fig. 2(c)) corresponding to the circuit. The design consists of multiple MOSFETs, diodes, inductor, and an LED. The idea is to charge the inductor in one cycle and in the next cycle discharge it through the high-power LED for a very short time and eventually fully discharge through the Zener diodes. The process is repeated for periodic flashing.

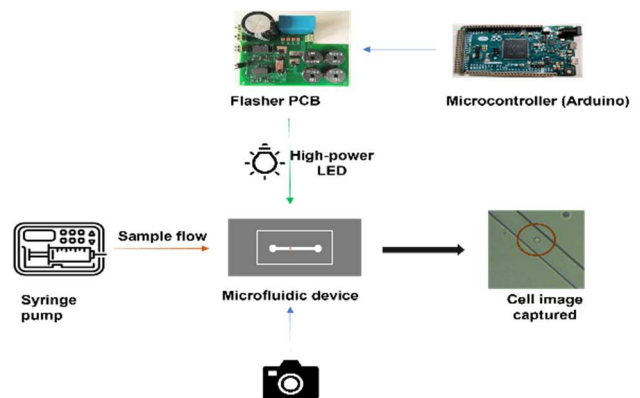


Fig. 1. Schematic of the experimental setup

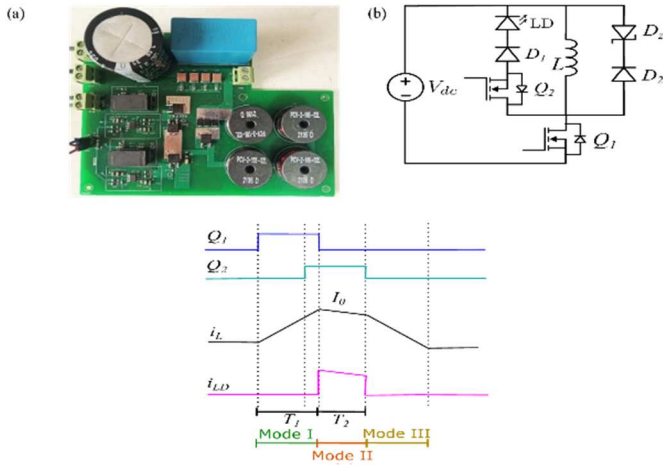


Fig. 2. (a) Fabricated flashing PCB (b) Schematic (c) Ideal waveforms

Mode I is responsible for charging the inductor up to the desired current amplitude. The first MOSFET, Q_1 is gated to turn ON by the microcontroller for a time duration T_1 . It charges the inductor up to amplitude I_0 . Just before turning Q_1 OFF, the second transistor, Q_2 is turned ON. As soon as Q_1 is turned OFF after time T_1 , Mode II commences and the inductor starts discharging through the high current LED, LD for a specified time duration, T_2 . This is the time when LD is ON and hence the light is flashed. The current through the LED remains almost constant ($=I_0$) for that time duration. After time T_1+T_2 , Q_2 is turned OFF. Mode III begins, and the inductor starts discharging through the Zener diode D_z for time T_3 . Once the inductor is fully discharged, first charging-discharging cycle is completed, and the next cycle can begin. The time $T = T_1+T_2+T_3$ decides the frequency of operation of the circuit and hence, flashing.

C. Fabrication of microfluidic device

The microfluidic device is fabricated from a silicon mould created using standard photolithography technique. Fig. 3 depicts the process steps involved in the fabrication of the device. A 4" Si wafer is cleaned using Piranha solution ($H_2SO_4:H_2O_2$) in the ratio of 3:1 for 10 minutes. After dehydration bake at $110^\circ C$ for 10 minutes, a positive photoresist is spin coated on the wafer. Once the wafer is relaxed, the desired patterns are created on the wafer by direct writing. Soon after writing, the wafer is developed in the photoresist developer solution for its specified time. This process creates the pattern in the photoresist.

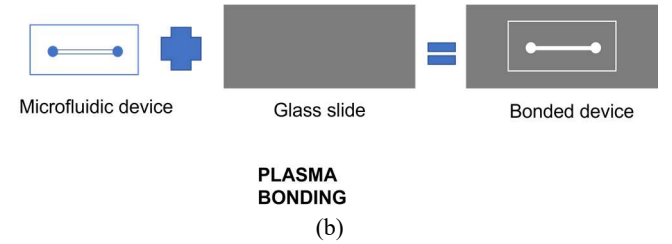
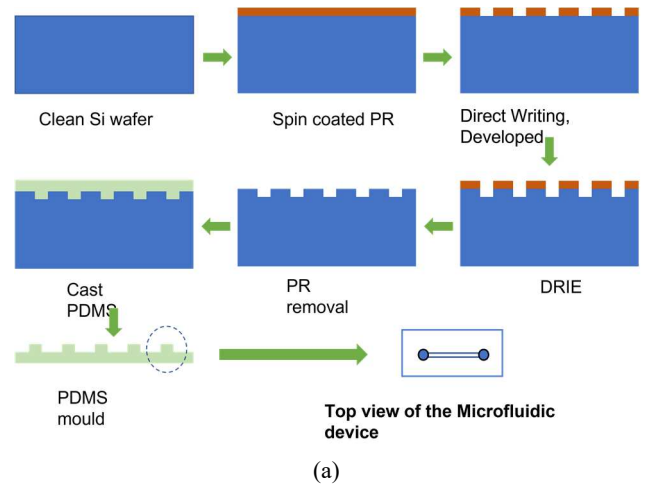


Fig. 3: Device fabrication process flow: (a) shows the creation of a device and (b) shows the bonding of the device to a glass slide using plasma.

To transfer the design on the wafer, the process of etching is carried out. Deep Reactive Ion Etching (DRIE) creates trenches in the wafer, thereby, transferring the pattern to the substrate. The photoresist is removed after etching to obtain the final mould. Channels of $20\mu m \times 20\mu m$ cross-section are created. The mould is casted over with Poly di-methyl siloxane (PDMS) polymer to create replica of the device in the polymer. The inlet and outlet holes are punched for insertion of the tubing. The device is bonded to a glass slide to cover the open channel from one side and is ready to use.

III. RESULTS

Fig. 4(a) shows the experimentally obtained waveforms of the current pulser. The green waveform represents the current through LD, $I(LD)$, which is pulsed for $12\mu s$. The other waveforms correspond to: voltage across Q_1 ($V(Q1)$ - blue), voltage across Q_2 ($V(Q2)$ - cyan) and voltage across LD ($V(LD)$ - pink).

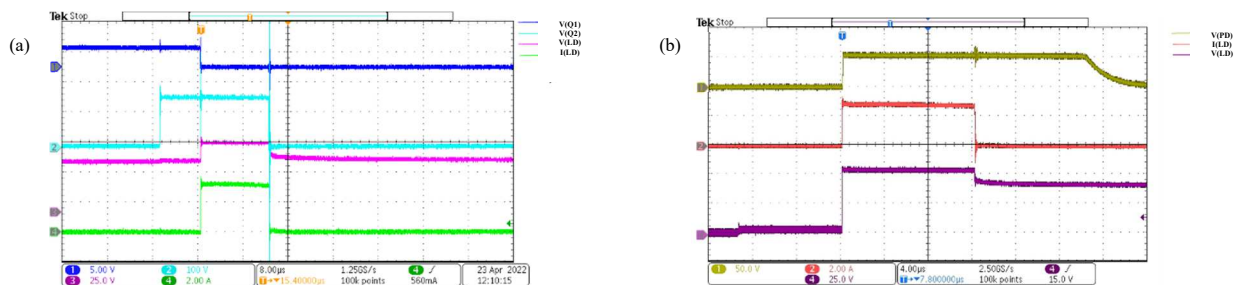


Fig. 4. (a) Experimental waveforms of the current pulser obtained in oscilloscope. (b) Photodiode response to LED flashing.

Fig 4(b) reports the response of a photodiode, $V(PD)$ denoted by the top waveform, corresponding to the flash of the LED. The other two waveforms correspond to the current through LD, $I(LD)$ and voltage through LD, $V(LD)$. It is thus demonstrated that the optical response of the LED is as fast as the electrical response.

Fig. 5 shows a series of images captured for the flowing red blood cells (RBCs) inside the fabricated microfluidic channel with programmed flashing. The flashing is carried out at 20Hz for a pulse duration of $10\mu s$ and the images are captured with the standard 21fps camera. The images are captured with a 10x objective lens. The red circle in each image encloses the RBC passing through the channel at the instant of image capture.

Fig. 6 shows a comparison of using continuous light to image the flowing cells vs the developed flashing light for imaging. It is evident that the cells are unclear and blurred in vision in the case of continuous light. This is because the cells traverse a significant distance throughout the time the shutter of the camera is on. However, in case of flash imaging, we can turn on the light for a short duration of time during the shutter on period, which virtually freezes the cell on the camera. Hence, we see clear cell boundaries with the help of integrated flashing system.

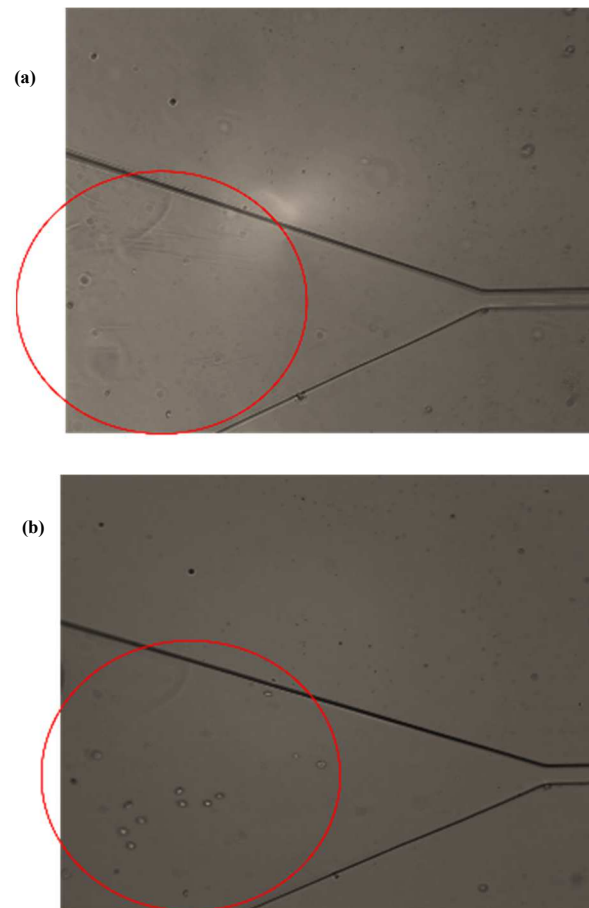


Fig. 6. Comparison of image capture with (a) normal light vs (b) flashing light.

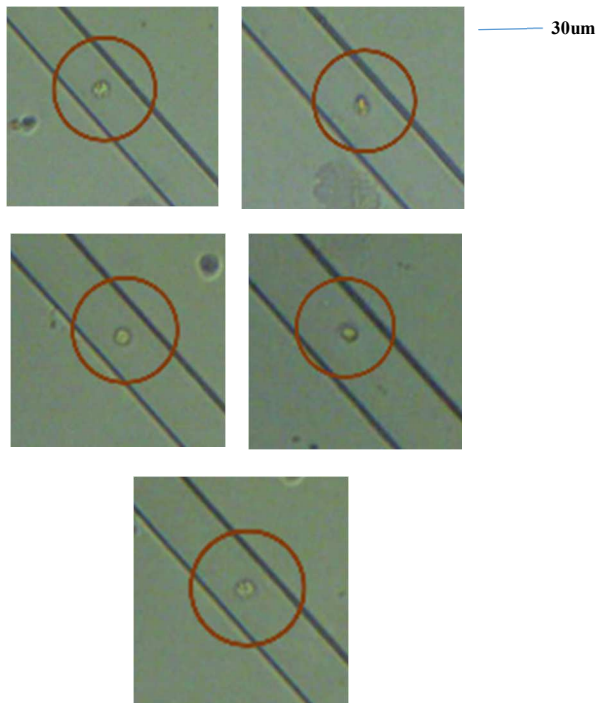


Fig. 5. Multiple cell images captured manually by flashing circuit.

IV. CONCLUSION

The flashing technique developed for microfluidic imaging has been tested and verified for the application as reported here. The proof of concept is supported by the images captured with the developed system inside microchannels. It could be extended for applications in other areas as well such as LED dimming, LED averaging, running other kinds of passive loads, etc.

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