Integration of Microfluidics in a Smartphone Microscopy Device for Particles Imaging

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Abstract: The necessity for decentralization of diagnostic technology, from a biomedical laboratory to near the patient sites, has driven the research community to engage the new generation smartphones with novel diagnostic and detection platforms. In this work, we will use state-of-the-art knowledge and state-of-the-art technology to construct an innovative platform on mobile devices with a future outlook of *in vivo* intervention. Specifically, we have built a low-cost smartphone-based microscopy technique by applying optics principles. With this technique we revealed the ability to identify PMMA beads circulating in a microfluidic channel, comparing its performance with the results obtained with an optical trapping device. The proposed platform could act as a detection and image analysis station for rapid and sensitive imaging of human cell collections from body fluids (biomedical diagnosis) or for screening of the water quality in remote areas (environmental pollution monitoring).

Keywords: Smartphone, optical tweezers, diagnosis, PMMA beads, microcopy.

1. INTRODUCTION

The need for developing of simple, light-weight, low-cost and rapid detection platforms for diagnosis, at real-time and patient's bedside conditions, is a significant challenge faced by humanity at the dawn of 21st century, for human health improvement. Especially the personal healthcare in the developing world and in remote areas, can be improved *via* patient-level health monitoring, sensing, and diagnostics technologies, in the framework of the metaphorically called "democratization" of science and technology achievements for those who would not normally have the opportunity or inclination due to their socio-economic status, local environment, or background [1]. According to Ozcan group [1], nanoscience and nanotechnology are the suitable areas for increased democratization, in both the development of label-based nanoscale imaging and sensing tools, and label-free nanoscale measurement tools. For the above mentioned it is logical that in the "combat" of diseases worldwide, one main strategy, complementary with improvement of treatments, aims at detecting the diseases at an early stage. Many of the approaches for that are based on the use of new communication technologies and in particular smartphones. The wide-spread use of a cell-phone all across the world created significant opportunities for healthcare applications using mobile devices. Nowadays, smartphone is the latest generation of cell-phones with excellent built-in equipment, such as touch-screen, multicore processor, digital camera, high image resolution, and open-source operation system. Smartphone is now becoming one of the most widely used mobile devices worldwide, while the number of users will be estimated to grow to 5.07 billion (64.5% of the world's population) by the end of 2019 [2]. The usage of smartphone in biosensor systems can take place as input button, data analyzer, screen display, and even as detector, which is designed in readout devices. The efficiently simplified electronic design, the minimized volume size and lowered overall cost of the systems allow portable and point-of-care (PoC) applications, outside biomedical laboratories. Therefore, the necessity for decentralization of diagnostic technology from a biomedical laboratory to near the patient sites has driven the research community to engage the new generation smartphones with novel diagnostic and detection platforms. Thus, several researchers have proposed a variety of designs on smartphones for portable healthcare diagnostics outside well-resourced laboratories [3, 4].

A custom-designed microscope attachment on a smartphone, integrating several optical elements, is configured to facilitate sample loading and to improve the diagnostic specificity. Most smartphone-based microscope systems are designed to be implemented on specific cell phone models, with small modifications required to adapt to a different type of cell-phones. Therefore, Breslauer *et al.* proposed a system in which an objective lens was adapted to the camera of a smartphone in order to capture bright field images of malaria parasites in blood smears [5]. Although the proposed system creates high-resolution images, the field of view is limited as only a part of the camera

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E-ISSN: 2311-8792/18

sensor is filled [5]. The smartphone microscope of Smith *et al.* utilizes a 1-mm ball lens for the magnification but creates images with aberrations that degrade the image quality over the bulk of the field of view [6]. Moreover, a low-cost smartphone microscope based on a 3-mm ball lens was deployed by Bogoch *et al.* to monitoring the helminth infections, but the effectiveness of this device was limited due to the low image quality [7]. Additionally, the lens-free holographic approach used by Ozcan *et al.* achieved high resolution over a large field but involves modification of the smartphone to remove the camera lens [8]. Furthermore, this approach cannot be used to image samples prepared on standard glass slides due to the requirement that the sample must be positioned in close proximity to the sensor. Recently, label-free detection, as the one-of the most promising fields in the biosensing research area, has also been integrated into a smartphone, utilizing the spectroscopy capability. Among the variety of label-free biosensing techniques, the surface plasmon polariton (SPR) optical biosensors are popular for quantitative analysis and characterization of biomolecular interactions [9]. The first smartphone label-free biosensor was described by Preechaburana *et al.* who reported a coupling of an angle-resolved SPR instrument to smartphone for detecting of β_2 microglobulin, which was one of the very important biomarkers for cancers, inflammatory disorders, and kidney diseases [10]. The platform consisted of a lab-on-a-chip device, an optical coupler instrument, and a smartphone. The phone screen illumination was configured to excite a plasmon on surface of the device through the optical coupler, while CMOS camera of the cell-phone captured color image for optical intensity at different angles. Later, Gallegos *et al.* in 2013 demonstrated the use of a smartphone-based photonic crystal (PC) to detect shifts in the resonant wavelength of a label-free biosensor [11]. The smartphone-based SPR sensor platform can also be used in transmission measurement for other nano-SPR devices [12]. Recently, Kwon *et al.* [13] published a comprehensive review of the research and development efforts for mobile detection instruments used to serve the need in global health, personalized medicine, and POC diagnostics. In this review, they consider the relative advantages of using the internal sensing capabilities of mobile communication devices compared to those of using a customized external sensor module, mentioning also some commercially available smartphone-based diagnostics devices [13].

In a complementary to modern biosensors research area, microfluidic devices can precisely manipulate microliter volumes of the body fluids in micrometer-sized channels [14,15]. Consequently, these devices can provide several advantages such as chemical and biological assays with reduced reagent or sample consumption, well-controlled microenvironments, high-throughput experimentation and precise control of the suspended sample [16]. They have been applied as powerful experimental tools in biomedical, physics, engineering and chemistry sciences [17-19]. Most of the microfluidic devices were developed on silicon, glass or polydimethylsiloxane (PDMS) by employing several fabrication techniques [20-23]. In our previous work, we studied the interplay between the geometrical characteristics, the flow velocity, and the optical trapping efficiency of microfluidic devices, fabricated using ultra fast laser inscription and chemical etching of fused silica glass [21]. Additionally, the evolution of fabrication materials technology enabled the research community to develop low-cost, smart diagnostic tools using microfluidic devices with built-in analysis capabilities [24, 25].

In this work, we report our effort on the development of a cost-effective microscope attachment on mobile phone camera, aiming to capture high resolution microscopy images and to perform image analysis to count the particles in a captured image. The main components of this system include holder assembly, lenses, objective and/or eyepiece lenses, filter, microfluidic device and a smartphone. The proposed scheme provides portability with low-cost and reduces the complexity. The observation of the processing area consists of a system of lenses and filters which embed on the mobile device camera. We evaluate the performance of this smartphone-based imaging by measuring the density of PMMA beads suspended in deionized water, which showed a good match to our measurement results obtained using the optical trapping technique. The proposed platform paves the way for a variety of applications with significant impact in healthcare.

2. MATERIALS AND METHODS

2.1. Fabrication of a Microfluidic Device

The microfluidic device was fabricated using two layers of PDMS by standard photolithography methods on a 3-cm thickness glass substrate with an area of 76x26 mm^2 as illustrated in Figure **1a** [21].

2.2. Operation and Detection System

In this work, we characterized the microfluidic device using a microscope by employing the optical

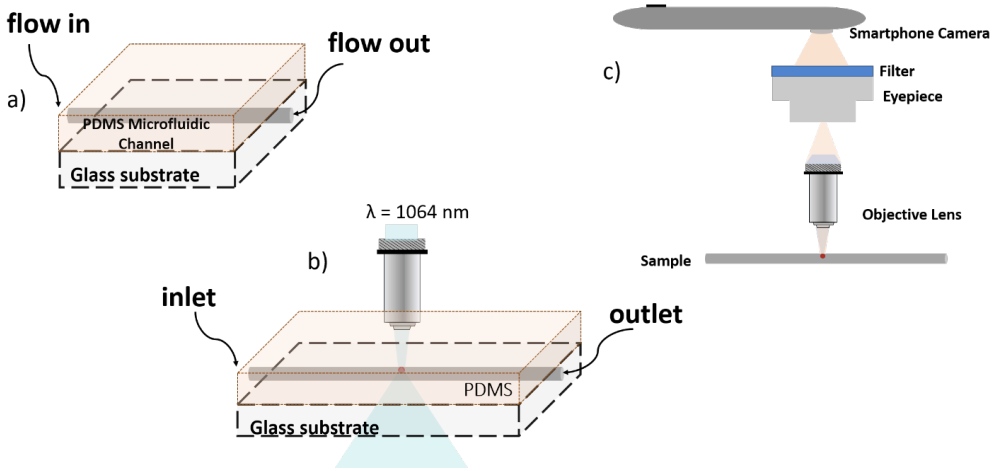

Figure 1: Various schematic diagrams of the experimental set ups are illustrated. (**a**) A schematic diagram of a microfluidic device. (**b**) The optical trapping layout for the evaluation of the microfluidic device. (**c**) Smartphone microscopy experimental set up.

trapping technique (Figure **1b**). The optical tweezers set-up, developed in our laboratory, is based on a continuous wave Nd:YAG laser (1064 nm, max:500 mW) [21, 26]. The objective lens for the optical tweezers experiments was a 100x oil-immersion with numerical aperture of 1.25. The microfluidic device was mounted on the x-y motorized translation stage. The imaging processing was monitored through a CCD camera connected to a PC. The samples were PMMA beads with 5 μm diameter which were suspended into deionized water and sonicated before their injection into the microfluidic channel though a syringe pump.

2.3. Smartphone Microscope

We used an iPhone 4S phone device for our microscope unit. This smartphone has an 8-megapixel (640x960 pixel) CMOS sensor, which is used to capture videos and images of the specimens. Moreover, the CMOS-BSI sensor of this smartphone has a built-in lens with a focal length of 4.28 mm. The imaging system consisted of an objective lens and microscopy eyepiece (Figure **1c**); the magnification and the image resolution could be adjusted by using various objective lenses. The smartphone and the optical components were mounted using a rail system.

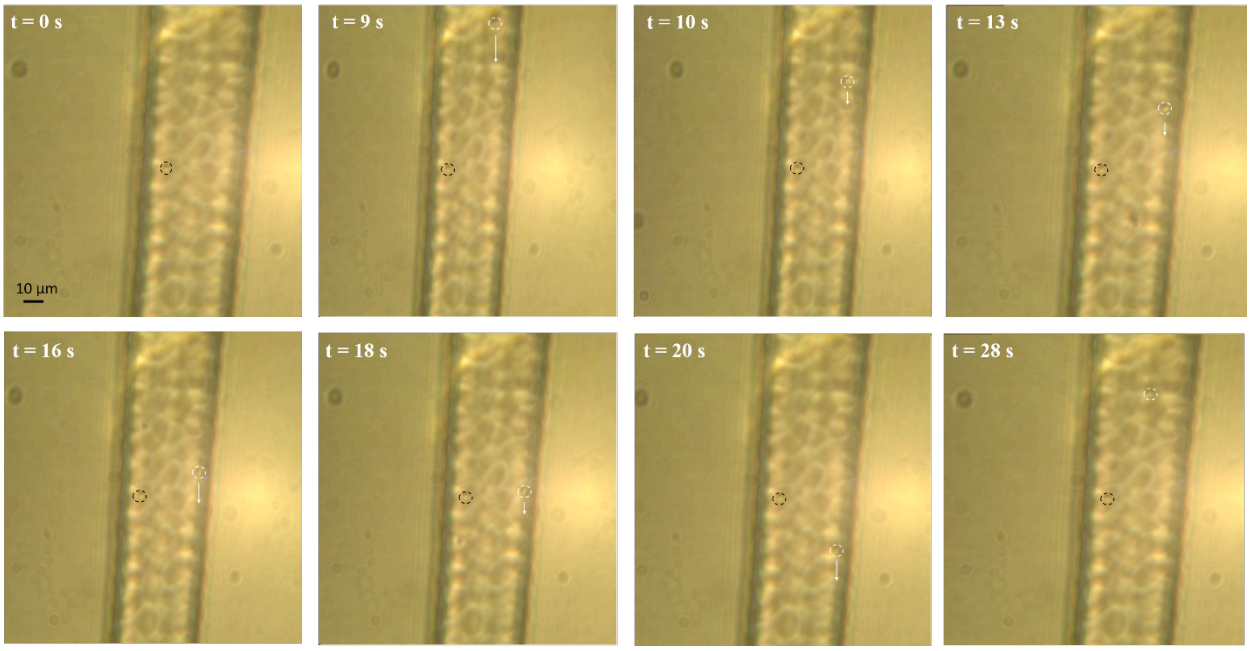

Figure 2: A series of single frames of trapped 5-μm PMMA bead using the optical tweezers set up. The black cycle represents the position of the trapped bead, while the white cycle and arrows represent the position of the beads as they flow though the microfluidic channel.

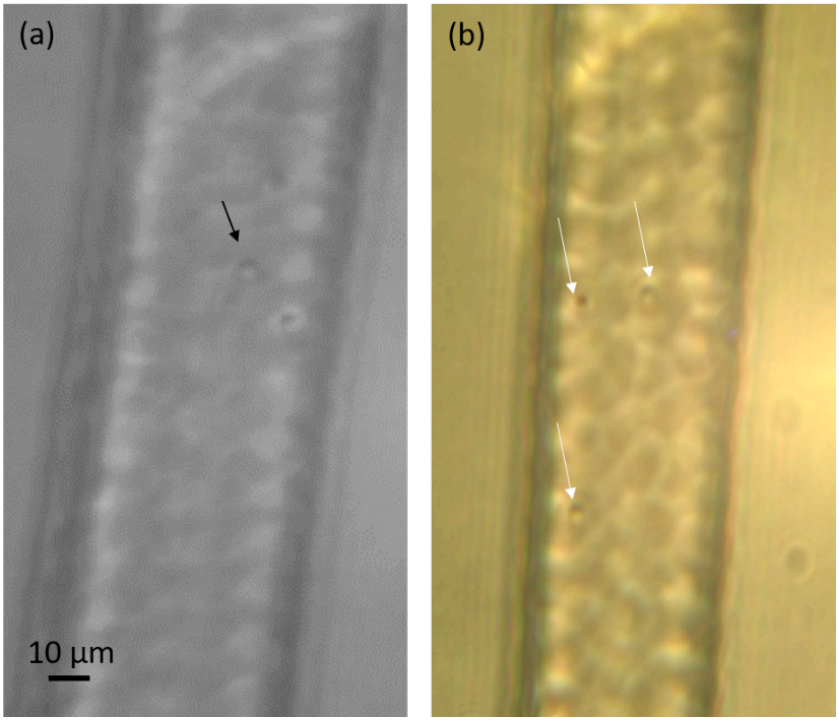

Figure 3: Images of PMMA bead (**a**) through smartphone microscope, (**b**) *via* a commercially available microscope. The arrows indicate the position of each bead.

The separation between the objective and eyepiece lens was kept at 160 mm in order to achieve the appropriate magnification according to the optics principles. Moreover, the eyepiece was separated from the smartphone camera by distance equal to the camera focal length. A filter was placed between the eyepiece and camera lens to both control the brightness of the imaging and to increase the performance.

3. RESULTS AND DISCUSSION

Firstly, we characterized the microfluidic device by employing the optical trapping technique. Figure **2** shows a series of single frames of the flow processing using the 100x oil-immersion objective lens. Specifically, a trapped PMMA bead was kept at stable position and we estimated the number of beads which appears as the fluid was flown with constant velocity. The black cycle in Figures **2** indicates the trapped PMMA bead. By applying constant fluid flow, several PMMA beads were moved through the microfluidic channel without disturbing the trapped particle. Using an image software, we calculated the amount of the particles in each captured image and we compared this value with the ones which were obtained following the same processing by employing the smartphone microscopy.

For the imaging experiments, with which we quantified the spatial resolution of the smartphone microscopy of the PMMA beads, initially we kept stationary conditions, *i.e.* without any fluid flow in order to compare them with the ones which were obtained by the optical trapping processing. The images captured by the smartphone microscopy are stored at the smartphone memory in jpg format and could be viewed through the smartphone screen directly or could be transferred to a computer for further digital processing or analysis. During the flow measurements, we used high definition mode of the smartphone to record videos of the flowing PMMA beads. Digital processing of the videos frames enabled us to automatically count the beads and then calculate their density for a given sample. For reasons of simplicity we implemented the beads count on a computer onto which we had transferred the images. Figure **3** illustrates the imaging performance of our smartphone microscopy using the 60x objective lens (Figure **3a**) comparing this with the one obtained with the conventional microscopy using the same objective lens (Figure **3b**). Specifically, all beads in images acquired by the microscope were clearly resolved as shown in Figure **3b**. For images taken by the smartphone microscopy, individual beads cannot be resolved due to strong background and possible due to lower light sensitivity. Moreover, imperfections and aberrations in the smartphone lens will also contribute to the non-diffraction limited performance. Despite the abovementioned limitations,

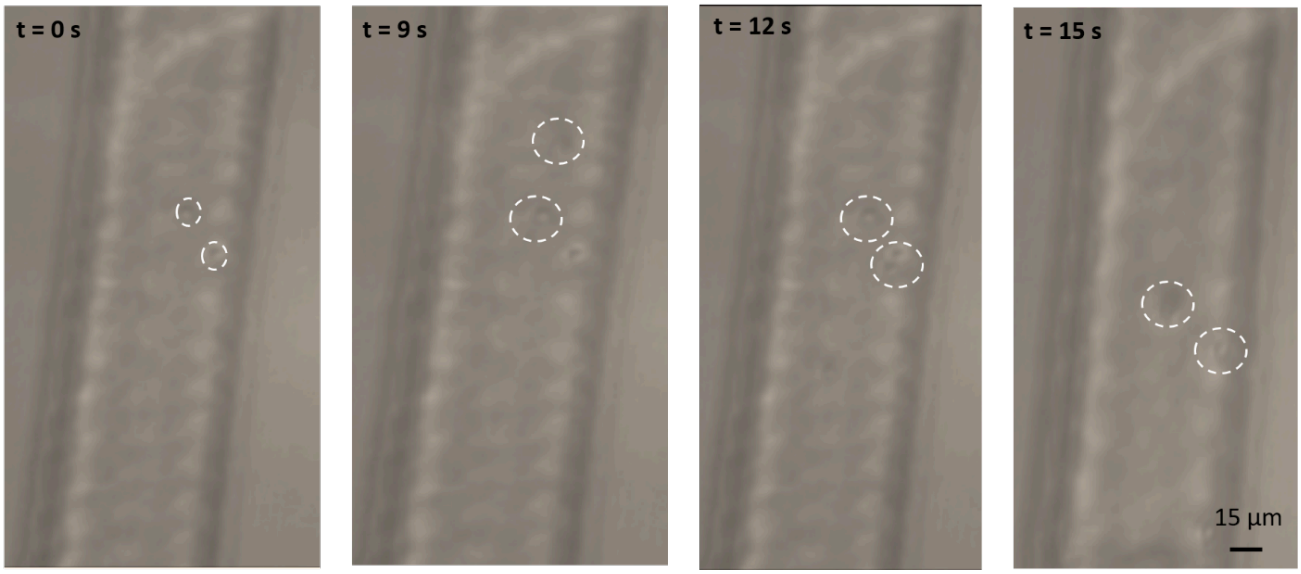

Figure 4: A series of single frames of flowing 5-µm PMMA bead using the smartphone microscopy set up. The white cycle represents the position of the bead, as they flow with constant flow velocity through the microfluidic channel.

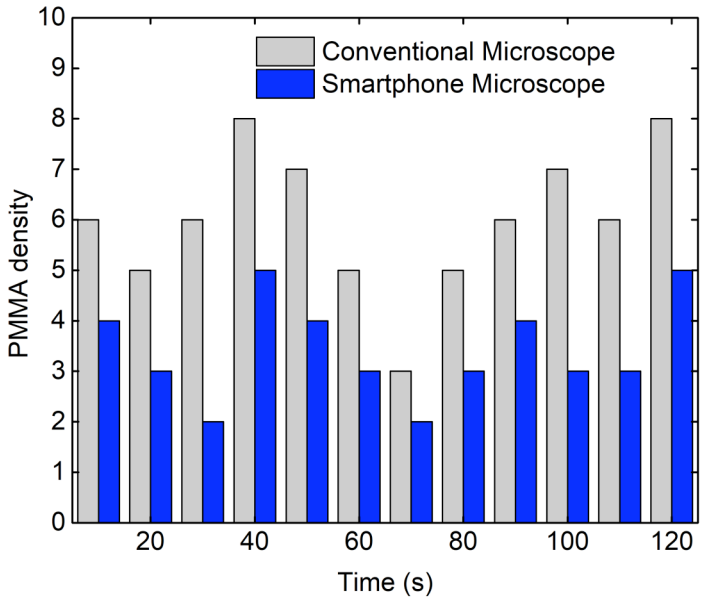

Figure 5: PMMA counting results obtained using a conventional microscope and our smartphone-based imaging platform.

the smartphone microscopy was able to capture images of PMMA samples with the required resolution for diagnosis. Figure **4** shows some representative snapshots, from a recorded video using the smartphone microscopy, illustrating beads in various positions as the fluid flows.

We used the software Image J in order to count the beads passing through the microfluidic channel. This involved a repeating sequence of bead detection and tracking. Starting with the first frame, every visible bead is detected. For every subsequent frame in each video, the bead detection process is repeated. By assuming that the flow rate between two frames is almost constant for each bead, the location of the bead from the one frame to the next can be predicted to be within a reasonable spatial range in image. Moreover, we averaged the number of the detected beads over multiple frames with longer time periods and over various distances in microfluidic channel in order to reduce the errors in detection. Figure **5** shows the PMMA beads density as a function of time for both the conventional and smartphone microscopy. As shown in Figure **5**, our beads density results obtained with the proposed smartphone platform matched well with that obtained by the conventional microscopy.

CONCLUSIONS

In this work, we demonstrated the integration of microfluidics on a smartphone using a compact and cost-effective platform which attach to the existing cell phone camera. The proposed device can reliably capture images of PMMA beads suspended in deionized water into a microfluidic channel as the fluid flows. We test the performance of our smartphone microscope by measuring the density of the beads in the microfluidic channel. We took further advantage of the digitized images to demonstrate automated the beads counting via image analysis software. We compare our experimental results with that obtained with a commercially available microscope which provides a good agreement. Due to the simple design of the proposed smartphone platform, it paves the way to increase the availability and the performance of the cell-phone microscopes and to accelerate their applications in resource-limited regions providing image-based diagnosis.

ACKNOWLEDMENTS

The authors gratefully acknowledge the funding by the “ELI-LASERLAB Europe Synergy, HiPER & IPERION-CH.gr (HELLAS-CH)” MIS 5002735 (Co-financed by Greece and the European Union - European Regional Development Fund). On of us, D.G. Kotsifaki gratefully acknowledges also the funding by the “IKY Fellowships of excellence for postgraduate studies in Greece-Siemens program”.

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Received on 27-03-2018 Accepted on 05-04-2018 Published on 01-06-2018

DOI: http://dx.doi.org/10.12974/2311-8792.2018.06.1