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Wide-field Fluorescent Microscopy on a Cell-phone

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Abstract— **We demonstrate wide-field fluorescent imaging on a cell-phone, using compact and cost-effective optical components that are mechanically attached to the existing camera unit of the cell-phone. Battery powered light-emitting diodes (LEDs) are used to side-pump the sample of interest using butt-coupling. The pump light is guided within the sample cuvette to excite the specimen uniformly. The fluorescent emission from the sample is then imaged with an additional lens that is put in front of the existing lens of the cell-phone camera. Because the excitation occurs through guided waves that propagate perpendicular to the detection path, an inexpensive plastic color filter is sufficient to create the dark-field background needed for fluorescent imaging. The imaging performance of this light-weight platform (~28 grams) is characterized with red and green fluorescent microbeads, achieving an imaging field-of-view of ~81 mm^2 and a spatial resolution of ~10 μm, which is enhanced through digital processing of the captured cell-phone images using compressive sampling based sparse signal recovery. We demonstrate the performance of this cell-phone fluorescent microscope by imaging labeled white-blood cells separated from whole blood samples as well as water-borne pathogenic protozoan parasites such as *Giardia Lamblia* cysts.**

I. INTRODUCTION

Cell-phones are widely used all over the world today. Late in 2010, cell-phone users have reached almost 5 billion worldwide and this number is further expected to grow up in the near future [1]. Most of these cell-phones are being used in the developing countries, which hold significant promise for a variety of telemedicine applications potentially impacting the fight against several global health problems [1]. So far various cell-phone based telemedicine technologies have been developed, including electrical impedance tomography, electrocardiography, fluorescent-microscopy, and lensfree on-chip microscopy [2-6].

Fluorescent microscopy is particularly important among these technologies and it has been widely used in biomedical research because of it specificity and sensitivity. In this work, we demonstrate wide-field fluorescent microscopy on a cell-phone (see Fig. 1) using compact, light-weight and cost-effective optical components that are directly attached to the existing camera-unit of the cell-phone. This platform can provide an ultra-wide imaging field-of-view (FOV) of ~ 81 mm^2 with a raw spatial resolution of ~ 20 μm, which can further be improved to ~10 μm using digital signal processing of the captured fluorescent images based on compressive sampling theory [7-8]. As shown in Fig.1, the fluorescent sample is pumped using battery-powered light-emitted-diodes (LEDs), which are butt-coupled to the sample from one side without the use of any lenses or micro-mechanical stages. The pump light is guided within the sample cuvette and it uniformly excites the labeled samples. The fluorescent emission from the sample is directly imaged on the cell-phone CMOS sensor through a simple lens that is placed in front of the existing cell-phone camera lens. A simple plastic absorption filter is sufficient to create the necessary dark-field background because the detection path is perpendicular to the excitation light path.

This cell-phone based fluorescent microscope has several advantages that make it a very promising tool for global health applications. First, major components of this fluorescent microscope attachment include a simple lens (cost: ~12 USD/piece), a plastic color filter (cost: ~1 USD/piece), 3 LEDs (cost: ~0.3 USD/piece), and a battery (cost: ~0.5 USD/piece), which make this fluorescent imaging platform on a cell-phone rather ***cost-effective***. In addition, this device has a large FOV (~81 mm^2) and a long depth-of-field (>1-2 mm), which allow imaging of large sample volumes. This is especially important for ***high-throughput imaging*** of microfluidic channels for quantification of e.g., rare cells or low concentration bacteria or pathogens. Moreover, the device is extremely ***compact and light-weight*** as illustrated in Fig. 1. The entire attachment unit, including optical components, battery, and mechanical components, only weighs ~28 gram (~1 ounce) and has dimensions of ~3.5 x 5.5 x 2.4 cm. This fluorescent microscope unit can be easily attached and detached to the cell-phone body without any fine alignment or tuning, which makes its interface very easy to use even in resource poor environments.

In the work described herein, the imaging performance of this cell-phone based fluorescent microscope was characterized with red and green fluorescent beads. The feasibility of using this platform to image fluorescently labeled cells and pathogens was also investigated. This compact and cost-effective cell-phone based fluorescent microscope could be a useful tool for biomedical research in general, and for resource limited settings, in specific. It can also be used for imaging and quantification of various lab-on-chip devices that have already been

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developed for global health needs.

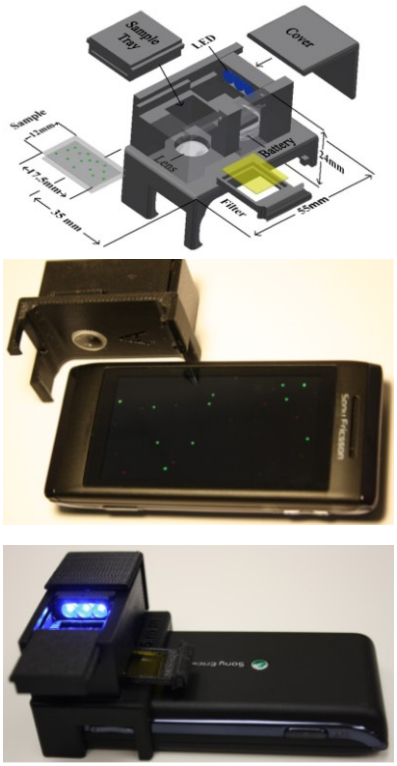

Fig. 1 (Top) Schematic diagram of the designed attachment for wide-field fluorescent imaging on a cell-phone. (Middle and Bottom) Different views of our cell-phone fluorescent microscope prototype. The whole cell-phone attachment weighs ~28 grams (~ 1 ounce) and has dimensions of ~3.5 x 5.5 x 2.4 cm. This mechanical unit can be attached and detached to the cell-phone body easily without the need for any fine alignment.

II. EXPERIMENTAL METHODS

A. Design of the Wide-field Fluorescent Microscope Installed on the Cell-phone

Sony-Erickson U10i Aino™ was chosen as the starting base for our fluorescent cell-phone microscope. However, we should note that the presented technique can be installed on any other cell-phone models independent of the operating system or the manufacturer. Sony-Erickson U10i Aino™ has an ~8 Mpixel color RGB sensor installed on it, which is used to capture the fluorescent images of the samples. The cell-phone digital camera unit has a lens with focal distance of $f \sim 4.65$ mm. We put another lens ($f_2 = 15$ mm) directly in front of the existing lens, which generates a system demagnification of $f_2/f = 3.2$ between the sample plane (located at the focal plane of f_2) and the camera CMOS sensor chip. The de-magnification factor can be easily changed by using another external lens with a different f_2 value and quite conveniently it is independent of the distance between these two lenses. Therefore, this system is very insensitive to vertical misalignments of the attached unit, making the system rather easy to use.

In our cell-phone based fluorescent microscope design, we have employed a different pumping scheme than a conventional fluorescent microscope. As shown in Fig. 1, the pump light generated by 3 LEDs is directly coupled to the sample holder from the side using lensfree butt-coupling. Since the LEDs have large cross-sections, this coupling is not sensitive to alignment. The sample holder can be treated as a multi-mode waveguide with three layers of refractive index (glass - liquid sample - glass) surrounded by air on both sides. Due to the strong refractive index contrast at the air-glass interface, the light is tightly confined inside this waveguide. In comparison to the air-glass interface, the glass-liquid sample interfaces have weak refractive index contrast; therefore a large portion of our pump photons can leak into the liquid sample to excite the fluorescently labeled specimens suspended inside the sample as shown in Figs. 2-5. Meanwhile, since the excitation light propagates perpendicular to the fluorescence detection path, a simple plastic absorption filter is sufficient to reject the scattered pump photons to create the necessary dark-field background.

B. Sample Preparation

White blood cell sample preparation: 1X red blood cell lysis buffer was purchased from eBioscience, Inc (San Diego, CA) and stored at 4°C. SYTO®16 green fluorescent nucleic acid stain (excitation/emission 488nm/518nm (+DNA) and 494nm/525nm (+RNA) was purchased from Invitrogen (Carlsbad, CA). To prepare labeled white-blood cell samples, 1mL of red blood cell lysis buffer was added to 200 µL of whole blood and incubated for 3 minutes. The lysed blood sample was then centrifuged and the white-blood cell pellet was re-suspended in 200 µL of PBS. Then 5 µL 1 mM STYO®16 solution was added to this 200 µL white blood cell sample and incubated in dark for ~30 minutes. After this incubation, the sample was centrifuged again. Supernatant was removed and the labeled white-blood cell pellet was re-suspended in PBS buffer. This labeled white blood cell solution was then placed between two glass slides (12.5 mm x 17 mm) and was imaged using our cell-phone fluorescent microscope, as illustrated in Fig. 4.

Giardia lamblia sample preparation: Giardia lamblia cysts were purchased from WaterBorne Inc. (New Orleans, LA, USA). The initial *Giardia lamblia* cysts concentration was ~$5x10^6$ parasites/mL. 100 µL of this sample was centrifuged and the pellet was re-suspended into 100 µL PBS buffer. 2.5 µL 1 mM SYTO®16 solution was added to this 100 µL Giardia lamblia sample and incubated in dark for ~30 minutes. After this incubation, the sample was centrifuged and the pellets were re-suspended in PBS buffer. The *Giardia lamblia* sample was then placed between two glass slides to be imaged using our cell-phone fluorescent microscope, as illustrated in Fig. 5.

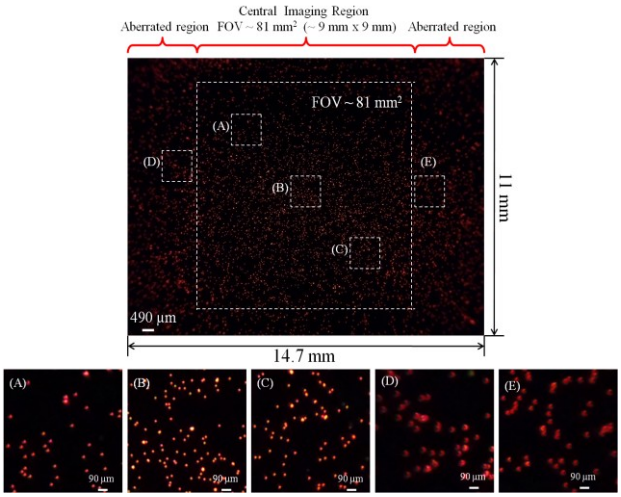

Fig. 2 Imaging performance of the cell-phone based fluorescent microscope is characterized with red fluorescent beads (10 µm diameter; excitation/emission: 580 nm/605 nm). The image has a central field of view of ~ 81mm^2 with good imaging quality. The region that lies outside this central region has aberrations and it is not included into the field of view.

III. RESULTS AND DISCUSSION

A. System Field of View

We used fluorescent micro-beads (10 µm in diameter) to test our cell-phone fluorescent microscope. As illustrated in Fig. 2, red fluorescent beads were imaged by our cell-phone fluorescent microscope over an area of 14.7 mm x 11 mm. This large imaging area has aberrations towards its edges (see e.g., Figs. 2(D-E)) and therefore only the central region with an area of ~ 9 mm x 9 mm shown good imaging quality (see e.g., Fig. 2(A-C)). This generates an effective FOV of ~81 mm^2 as indicated with the dashed square in Fig. 2. We should also noted that the aberrated regions can still be useful in certain applications, e.g., for counting of rare cells, even though the bead images in this region look distorted compared to the central FOV.

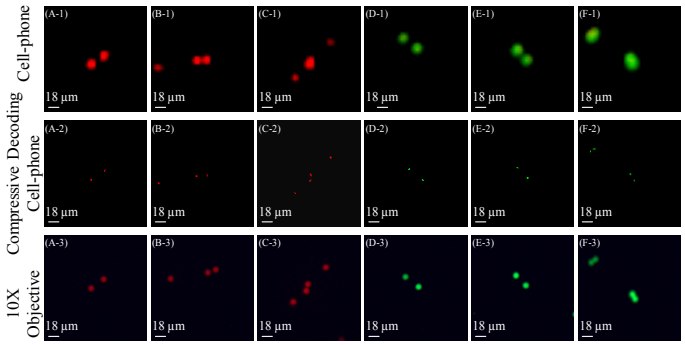

Fig. 3 Spatial resolution of the cell-phone based fluorescent microscope is characterized by red and green fluorescent beads (10 µm diameter). The top row is the raw cell-phone images of the beads which demonstrate ~ 20 µm resolution for both colors. The middle row shows the compressive decoding results of the top row cell-phone raw images, which improve the resolution to ~10 µm for both colors as shown in (C-2) and (F-2). The bottom row is the 10x microscope-objective (NA = 0.25) images of the same samples imaged by a conventional fluorescent microscope for comparison purposes. Note that these samples were suspended in solution, and therefore their relative orientation may be slightly shifted in microscope comparison images.

B. System Spatial Resolution

We characterized our system resolution using green and red fluorescent beads. Fig. 3 top row shows bead images obtained with our fluorescent cell-phone microscope. For comparison purposes, the same samples were also imaged using a conventional fluorescent microscope with a 10x objective lens (numerical aperture ~ 0.25) as illustrated in Fig. 3 bottom row. These results show that our cell-phone fluorescent microscope can resolve 2 beads that are separated ~ 20 µm (center to center). This resolution can be further improved by digital signal processing of the captured raw images through sparse signal recovery [7-8]. These processed images are shown in Fig. 3 middle row. After processing, 2 beads with a center-to-center distance of ~ 10 µm (Fig. 3(C-2) and Fig. 3 (F-2)) can be digitally resolved. Although the system has a modest resolving power (~10 µm), it could still be quite useful for various cell counting applications because of its ultra-wide FOV (~ 81 mm^2).

C. Cell-phone based Fluorescent Cell Imaging

After characterizing the performance of our cell-phone based fluorescent microscope, we investigated the feasibility of using it to image labeled cells. For this purpose we initially imaged labeled white blood cells. The sample was prepared as described in the experimental methods section. These labeled white blood cells were excited with blue LEDs (470 nm peak wavelength) and then imaged with our cell-phone fluorescent microscope. Figures 4(A-1) and (B-1) are digitally cropped from the central FOV of the full image (Fig. 4, top image), which show labeled white blood cells signatures. We also used a conventional fluorescent microscope (10x microscope objective-lens) to image the same regions of the sample to provide microscope comparisons (Figs. 4 (A-3 and B-3)). In addition, we have also compressively decoded Figs. 4(A-1 and B-1) to get better quality images as shown in Figs. 4(A-2 and B-2).

We also used water-borne parasites (*Giardia Lamblia* cysts) as the model system to demonstrate the feasibility of using this cell-phone based fluorescent microscope for water-quality monitoring. Fig. 5 (Top Row) illustrates the raw cell-phone fluorescent images of SYTO®16 labeled *Giardia Lamblia* cysts. These images were digitally cropped from a large FOV (~ 81 mm^2) and they match very well to the comparison images in Fig. 5 (Bottom Row), which were captured by a conventional fluorescent microscope (10x microscope-objective). As discussed earlier, this cell-phone based fluorescent microscope is able to rapidly image large sample volumes (e.g., > 0.1 mL), and this important feature makes our device quite powerful to image low concentration parasites in water samples even in resource poor settings.

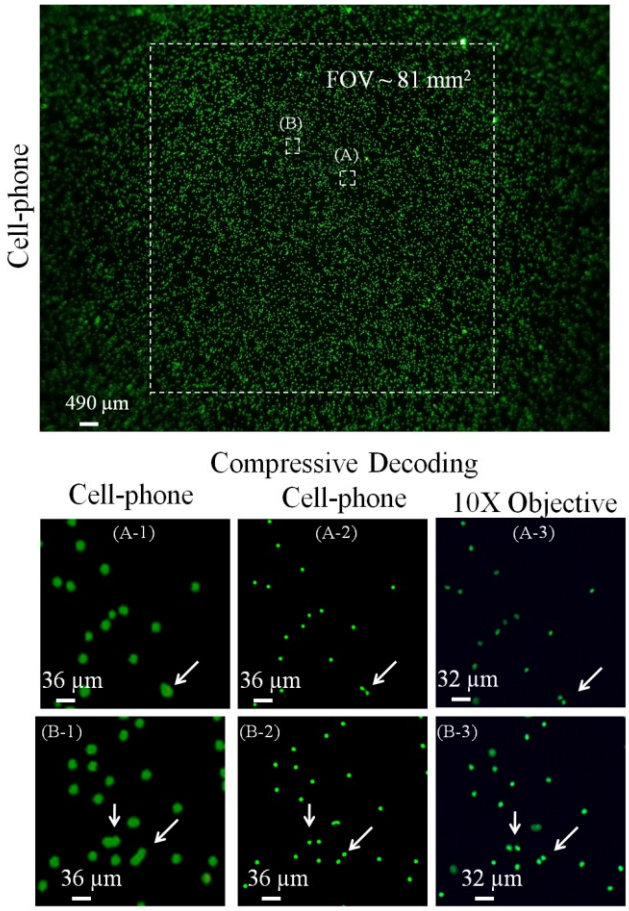

Fig. 4 Labeled white blood cell images are obtained with our cell-phone based fluorescent microscope. 10x microscope objective (NA = 0.25) images of the same samples, acquired with a conventional fluorescent microscope, are also provided for comparison purposes. White arrows point to cells that can be resolved using compressive decoding of our cell-phone images. Note that because the samples were suspended in a solution, their relative orientation might be slightly shifted in their corresponding microscope comparison images.

IV. Conclusion

In this work, we demonstrated wide-field fluorescent microscopy on a cell-phone. In this design, all the compact and cost-effective optical/mechanical components are directly attached to the cell-phone camera unit. This entire attachment only weighs ~ 28 grams (~1 ounce), which makes it quite portable. This microscope is able to achieve ~ 20 µm resolution without any digitally processing. With compressive decoding, the resolution can be further improved to ~10 µm over a field of view of ~ 81 mm^2. We also have explored the feasibility of using this cell-phone based fluorescent microscope to image labeled white blood cell and water-borne parasites, e.g., *Giardia Lamblia* cysts. This telemedicine device could a very powerful tool for wide-field imaging and quantification of various lab-on-a-chip devices developed for global health applications, such as remote monitoring of HIV+ patients for CD4+ T lymphocyte counts.

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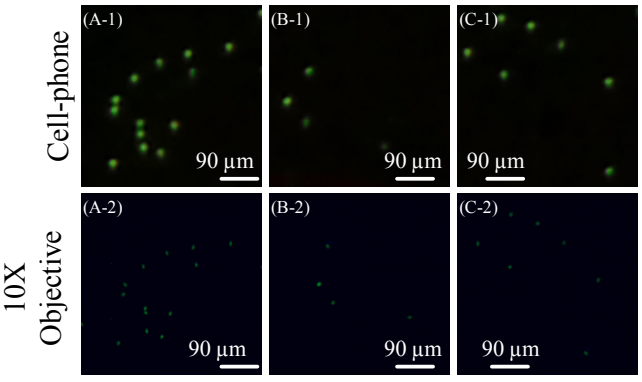

Fig. 5 (Top Row) Fluorescent cell-phone images of the *Giardia Lamblia* cysts. (Bottom row) 10x microscope objective (NA = 0.25) images of the same samples, for comparison purposes. Note that the samples were suspended in a liquid, and therefore their relative orientations might be slightly shifted in the microscope images. In (B-2) and (C-2) there are 2 dead-pixels at the microscope images that do not show up in our cell-phone images.