Video Article

Wide-field Fluorescent Microscopy and Fluorescent Imaging Flow Cytometry on a Cell-phone

Hongying Zhu[1], Aydogan Ozcan[1,2,3]

[1]Electrical Engineering Department, University of California, Los Angeles

[2]Bioengineering Department, University of California, Los Angeles

[3]California NanoSystems Institute (CNSI), University of California, Los Angeles

Correspondence to: Aydogan Ozcan at ozcan@ucla.edu

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Abstract

Fluorescent microscopy and flow cytometry are widely used tools in biomedical research and clinical diagnosis. However these devices are in general relatively bulky and costly, making them less effective in the resource limited settings. To potentially address these limitations, we have recently demonstrated the integration of wide-field fluorescent microscopy and imaging flow cytometry tools on cell-phones using compact, light-weight, and cost-effective opto-fluidic attachments. In our flow cytometry design, fluorescently labeled cells are flushed through a microfluidic channel that is positioned above the existing cell-phone camera unit. Battery powered light-emitting diodes (LEDs) are butt-coupled to the side of this microfluidic chip, which effectively acts as a multi-mode slab waveguide, where the excitation light is guided to uniformly excite the fluorescent targets. The cell-phone camera records a time lapse movie of the fluorescent cells flowing through the microfluidic channel, where the digital frames of this movie are processed to count the number of the labeled cells within the target solution of interest. Using a similar opto-fluidic design, we can also image these fluorescently labeled cells in static mode by *e.g.* sandwiching the fluorescent particles between two glass slides and capturing their fluorescent images using the cell-phone camera, which can achieve a spatial resolution of *e.g.* ~ 10 μm over a very large field-of-view of ~ 81 mm^2. This cell-phone based fluorescent imaging flow cytometry and microscopy platform might be useful especially in resource limited settings, for *e.g.* counting of CD4+ T cells toward monitoring of HIV+ patients or for detection of water-borne parasites in drinking water.

Video Link

The video component of this article can be found at https://www.jove.com/video/50451/

Introduction

Microscopy and flow-cytometry are widely used techniques[1-12] in biomedical and scientific research as well as clinical diagnosis for counting and characterization of various cell types. However, conventional microscopes and flow-cytometry instruments are relatively complex and expensive, which limits their use to mainly well-established central laboratories. Recently we have developed a compact and lightweight fluorescent imaging cytometry and microscopy device integrated on a cell-phone,[13,14] which shows promise to cost-effectively translate fluorescent microscopy, flow-cytometry and related micro-analysis techniques to resource-limited environments for various telemedicine applications impacting global health.

In the optofluidic flow-cytometry configuration (see *e.g.* **Figure 1C** and **1D**), a custom-designed polydimethysiloxane (PDMS) based microfluidic channel is positioned in front of the cell-phone camera-unit, where light-emitting-diodes (LEDs) are butt-coupled to the edges of the channel. This microfluidic chip, together with the liquid sample inside, forms an opto-fluidic planar waveguide (composed of for example PDMS-liquid-PDMS) such that the excitation light is guided to uniformly pump the fluorescent labeled specimens inside the micro-channel. The fluorescence emission from these labeled objects, *e.g.* cells, is further imaged through an additional lens placed right after the cell-phone camera-unit and is mapped onto the cell-phone Complementary Metal-Oxide-Semiconductor (CMOS) image sensor. Since the fluorescent emission is collected perpendicular to the excitation light path, an inexpensive plastic absorption filter is sufficient to remove the scattered excitation light and can provide a decent dark-field background required for fluorescent imaging. Using a similar opto-fluidic design, we can also image the fluorescent objects in static mode (see **Figure 1A** and **1B**), in which the fluorescent particles are sandwiched between two glass slides instead of flowing through a microfluidic channel and the fluorescent emission from these fluorescent particles are captured by the cell-phone CMOS image sensor for particle counting and characterization. Based on different application requirements, flow cytometry or wide-field fluorescent microscopy can be chosen. For example, cell-phone flow cytometry device could be especially useful for screening large volumes of liquid samples (*e.g.* a few ml) for the detection of rare cells or pathogens.

In this manuscript we review some of our recent results on the integration of fluorescent microscopy and imaging flow cytometry tools on a cell-phone using compact and cost-effective opto-fluidic attachments. These cell-phone-based micro-analysis, imaging cytometry and sensing

platforms could provide various opportunities for telemedicine and point-of-care diagnostics, especially impacting our fight against global health challenges in resource limited regions of the world.

Protocol

In this section, we introduce the experimental protocols for our cell-phone based wide-field fluorescence microscopy[13] and opto-fluidic imaging cytometry platform[14]. We will use fluorescent beads and fluorescently labeled white blood cells to test these imaging platforms.

A. Preparation of Cell-phone Based Wide-field Fluorescent Microscope and Opto-fluidic Imaging Flow Cytometer

The cell-phone based wide-field fluorescent microscope or flow cytometer consists of two major parts: a camera phone and a compact opto-fluidic add-on attachment.

1. The Camera Phone

While the presented techniques are applicable to almost any camera phone, we have chosen Sony Erickson Aino as the base for these devices. This cell-phone has an ~ 8 MegaPixel RGB CMOS sensor installed on it and a built-in lens that has a focal length (*f*1) of ~ 4.65 mm.

2. Opto-fluidic Attachment for Wide-field Fluorescent Microscopy

The optical attachment is designed by Autodesk and is printed by a Dimension Elite 3-D printer using ABSplus thermoplastic material. In this printing process, model and support materials are heated in an extrusion head within the printer and are deposited layer by layer on a modeling base. When this step is completed, the supporting material can be dissolved, leaving a robust 3D model of the desired prototype Our optical attachment design consists of LEDs (center wavelength at ~ 470 nm, Digikey), a plastic filter (#NT54-46, Edmund Optics), a sample tray, and a plano-convex lens *f*2=15 mm (# NT45-302, Edmund Optics). All the LEDs and plastic filters can be easily changed based on the fluorophores' spectra. The steps for assembling this opto-fluidic attachment include:

1. Place the lens into the attachment within its specific lens holder position.
2. Place the plastic filter onto the filter tray and slide it into the attachment; or tape the plastic filter in front of the cell-phone camera lens.
3. Insert the LED tray into the attachment.
4. Place the sample glass slides into the sample tray. Slide the sample tray into the attachment. Face the LEDs toward the sample.
5. Clip the attachment onto the cell-phone, such that the extra lens is directly in touch with the cell-phone camera lens.
6. Use the switch on the attachment to turn on the LEDs.
7. Image the sample of interest with the cell-phone camera unit using its "night mode".

3. Opto-fluidic Attachment for Fluorescent Imaging Cytometry

When there is a need to screen large volumes of liquid samples for the detection of rare events, optofluidic flow cytometry device could be preferred. We can modify our wide-field fluorescent microscope design and convert it into a flow cytometer, where a PDMS based microfluidic channel is used to continually deliver the liquid sample through the imaging volume. The optical attachment is also designed by Autodesk and printed by Dimension Elite 3-D printer. It also consists of LEDs (center wavelength at ~ 470 nm, Digikey), a plastic filter (#NT54-46, Edmund Optics), a sample tray, and an aspherical lens (*f* = 4.5 mm) (product # C230TME-A; Thorlab). The steps for assembling this opto-fluidic attachment include:

1. Place the aspherical lens into the attachment.
2. Place the plastic filter onto the filter tray and slide it into the attachment; or tape the plastic filter in front of the cellphone camera lens.
3. Slide the microfluidic channel into the same opto-fluidic attachment.
4. Clip the attachment onto the cell-phone such that the extra lens is directly in touch with the cell-phone camera lens.
5. Use the switch on the attachment to turn on the LEDs.
6. Connect the microfluidic channel to the syringe pump and deliver the liquid sample into the microfluidic device at a constant flow rate.
7. Capture a movie of the fluorescent cells/particles flowing through the microfluidic channel using the video mode of the cell-phone camera.

B. Sample Preparation

4. Preparation of Fluorescent Micro-particle Samples

1. Fluorescent beads with 10 µm diameter (red beads: product #F8834 excitation/emission 580nm/605nm; green beads: product #F8836: excitation/emission 505nm/515nm) are purchased from Invitrogen (Carlsbad, CA).
2. Mix 10 µl of green fluorescent beads, 10 µl of red fluorescent beads with 40 µl of DI water.
3. Place10 µl of this bead mixture on a glass slide using a micropipette and put another glass slide on the top of it to make a sandwich structure.
4. Insert this sandwich structure into the sample tray and slide it into the cell-phone attachment.

5. Preparation of Fluorescently Labeled White Blood Cells

1. Take SYTO16 nucleic acid fluorescent labeling kit (#S7578, Life Technology) and phosphate buffered saline (PBS) out from the refrigerator and bring them to room temperature.
2. Transfer 200 µl whole blood sample from EDTA blood collection tube to 1.5 ml polystyrene tube (# 05-408-129, Fisher Scientific).

3. Add 1 ml red blood cell lysing buffer (# R7757, Sigma-Aldrich) to the 200 µl whole blood sample and mix thoroughly.
4. After 5 min, centrifuge the lysed blood sample and remove the supernatant solution.
5. Resuspend the white blood cell pellet into 200 µl PBS buffer and gently mix them.
6. Add 5 µl 1 mM SYTO16 solution to the white blood cell sample. Wrap the sample with aluminum foil and incubate in dark environment for ~ 30 min.
7. Centrifuge the sample again. Supernatant is removed and the labeled white blood cell pellet is re-suspended into PBS buffer.
8. Place 5-10 µl labeled white blood cell liquid sample to a cover slip, and place a second cover slip on the top of the sample.
9. Insert the sandwiched sample slide into the sample tray and image it using the cell-phone fluorescent microscope.

Alternatively

10. Continually deliver the fluorescently labeled white blood cells through a microfluidic channel using an automated syringe pump, while also capturing a fluorescent microscopic movie of the flowing cells using the cell-phone camera in video mode. We should also emphasize that a portable battery powered syringe pump or even gravity force can be utilized to drive the flow through the microfluidic channel.

Representative Results

With our opto-fluidic pumping/excitation scheme (**Figures 1C** and **1D**), fluorescently labeled cells can be continuously delivered into the microfluidic channel using a syringe pump while the cell-phone camera records a time-lapse fluorescent microscopic movie of the flowing cells. These fluorescent movies can then be rapidly analyzed using contour-detection and tracking algorithms[14,15] to automatically determine the absolute number and the density of the cells flowing through the microfluidic channels, taking the function of a fluorescent imaging flow-cytometer. Based on the above described platform (**Figures 1C** and **1D**) and sample preparation protocols, we demonstrated accurate counting of total white-blood-cells (WBCs) in human blood samples, achieving comparable results against a standard hematology analyzer.[14] Alternatively, we can also image the fluorescent cells/particles in static mode,[13] such that without any fluidic flow, achieving a very large sample field-of-view of *e.g.* ~ 81 mm^2 with a spatial resolution of ~10 µm as illustrated in *e.g.* **Figures 2** and **3**. Sources of optical aberration can be reduced by designing a more complex lens system. Alternatively, it can also be partially corrected through digital image processing by characterizing the source of the aberration and their spatial patterns.

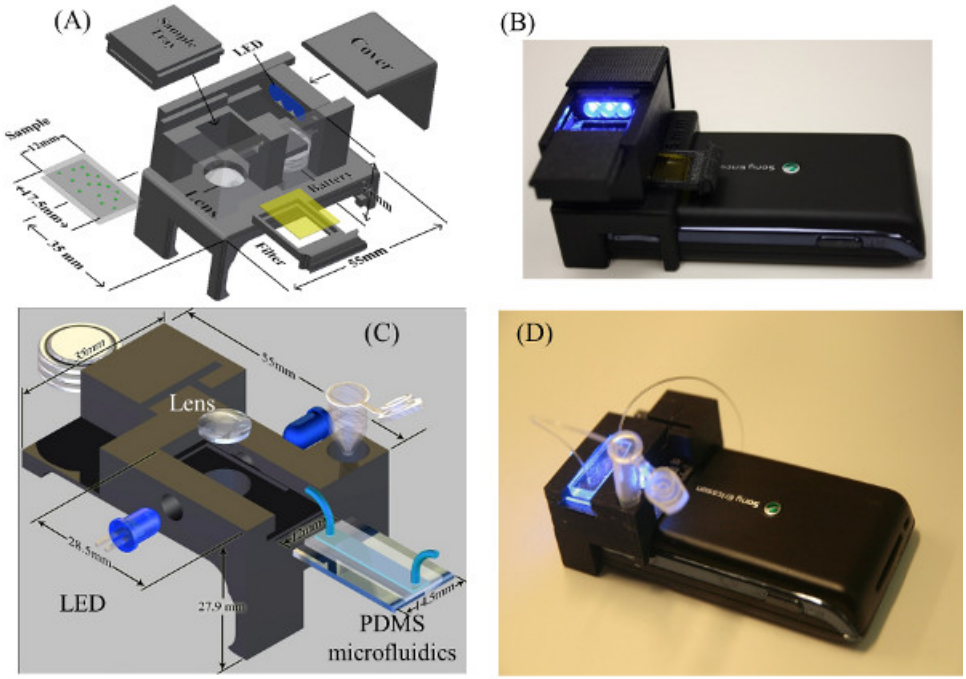

Figure 1. (A) Schematic illustration and **(B)** picture of a cell-phone-based wide-field fluorescent microscope.[13] **(C)** Schematic illustration and **(D)** picture of a cell-phone based imaging flow cytometer. [14] Click here to view larger figure.

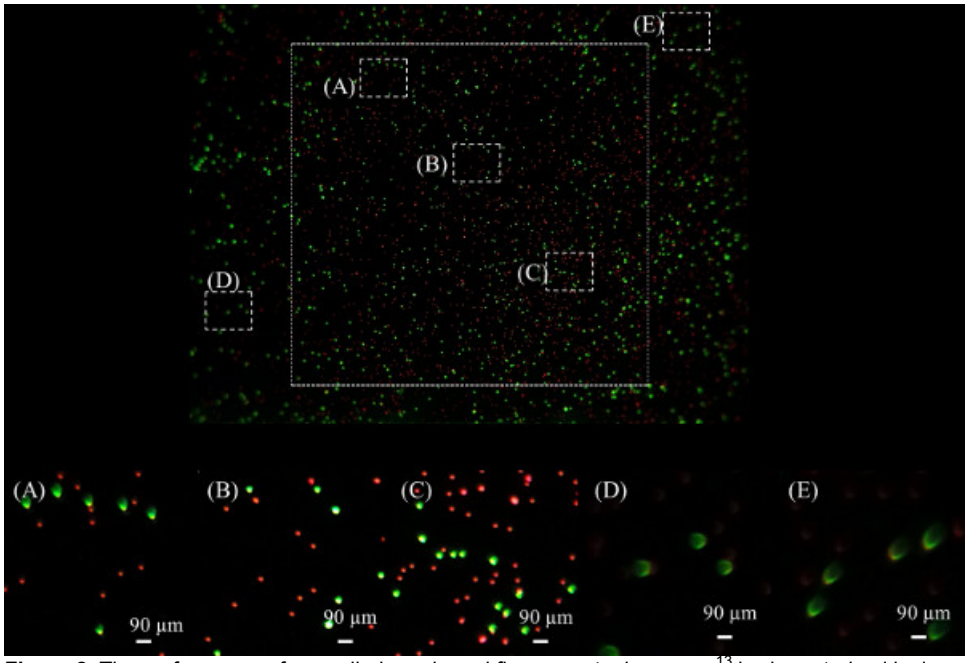

Figure 2. The performance of our cell-phone based fluorescent microscope[13] is characterized by imaging fluorescent beads (10 µm diameter green and red beads; red bead excitation/emission: 580 nm/605 nm, green bead excitation: 505 nm/515 nm). A decent image quality is achieved over a field-of-view of ~ 81 mm^2, see (**A,B,C**). Toward the edges of this central field-of-view, there are aberrated regions; see *e.g.* (**D-E**).

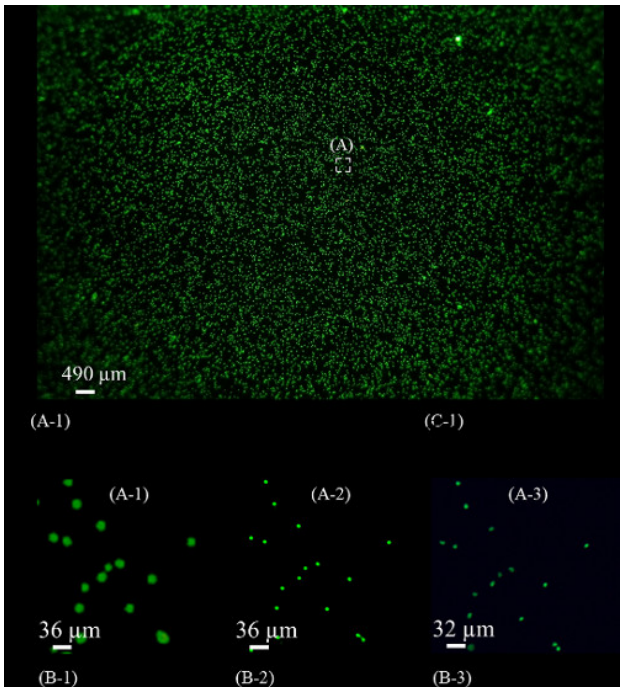

Figure 3. Top image: cell-phone images of fluorescently labeled white blood cells.[13] (**A-1**): Digitally zoomed cell-phone image of fluorescently labeled white blood cells cropped from the top image. (**A-2**): Compressive decoding results[18] for the cell-phone image shown in (**A-1**). (**A-3**): Conventional fluorescent microscope (10X objective, NA=0.25) comparison image of the same field of view.

Discussion

We have presented our recent results on cell-phone based wide-field fluorescent microscopy and opto-fluidic imaging flow cytometry using light-weight and compact opto-fluidic attachments to cell-phone cameras. Using this platform technology we imaged fluorescent objects including micro-particles and labeled white blood cells in whole blood samples. Therefore, this compact and cost-effective cell-phone based fluorescent imaging toolset might be useful for point-of-care diagnosis, especially in resource limited regions for combating various global health problems, such as monitoring of HIV+ patients for their CD4+ T lymphocytes counts.

Besides analysis of bodily fluids, the same cell-phone-based opto-fluidic cytometry platform can also be useful for other sensing needs such as quantification of fluorescent immunoassays for *e.g.* monitoring of water/food quality in resource-limited settings. Toward this end, we have recently demonstrated sensitive, specific and rapid detection of *Escherichia coli O157:H7* (*E. coli*) in water and milk samples using the same opto-fluidic cell-phone attachment[16]. We utilized surface functionalized glass capillaries to specifically and sensitively capture *E. coli* particles in liquid samples that were flown through each capillary. These captured *E. coli* particles were further labeled with quantum-dot (QD) conjugated secondary antibodies. The fluorescent emission from these functionalized capillaries was then detected using the opto-fluidic cell-phone imaging platform and the integrated fluorescence intensity along the capillary length was used to estimate the density of the captured *E. coli* particles in the target solution. With this opto-fluidic approach, we demonstrated a detection limit of ~5-10 CFU/ml in both water and milk samples[16].

Finally, we should note that depending on the application needs, the optical magnification and resolution of this platform can be tuned by changing f / f_2, where f is the focal length of the cell-phone camera lens and f_2 is the focal length of the external lens (**Figure 1A**). In addition to system magnification, we also should note that the LED tray and the associated filter can be easily changed to different colors to accommodate for various fluorophores or even immunochromatographic assays that might be used in specific applications.[17]

Disclosures

Dr. Ozcan is the founder of a start-up company that aims to commercialize computational imaging and microscopy tools.

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