3D-printed smartphone-based point of care tool for fluorescence- and magnetophoresis-based cytometry

Stephanie Knowlton,a Ashwini Joshi,a Philip Syrrist,b Ahmet F. Coskun,c and Savas Tasoglu,d,e,f

In developing countries, there are often limited resources available to provide important medical diagnostics, which severely limit our ability to diagnose conditions and administer proper treatment, leading to high mortality rates for treatable conditions. Here, we propose a multiplex tool capable of density-based cell sorting via magnetic focusing in parallel with fluorescence imaging to provide highly specific clinical assays. While many cell sorting techniques and fluorescence microscopes generally are costly and require extensive user training, limiting accessibility and usability in developing countries, this device is compact, low-cost, and portable. The device can separate cells on the basis of density, which can be used to identify cell type and cell activity, and image the cells in either brightfield, darkfield, or fluorescent imaging modes using the built-in smartphone camera. The combination of these two powerful and versatile techniques – magnetic focusing and fluorescence imaging – will make this platform broadly applicable to a range of biomedical assays. Clinical applications include cell cytometry and immunocytochemistry-based assays in limited-resource settings, which can ultimately help to improve worldwide accessibility to medical diagnostics.

Introduction

While there have been many recent advances in medical diagnostic tools, enabling physicians to make informed medical decisions around the world, access to such technologies in developing countries remains limited. In these areas, there are limited resources available to implement widespread disease testing programs, a lack of funds to pay for devices and consumables, challenges in training personnel to carry out complex tests, and, in some cases, little to no access to running water, light, or electricity.1 Infectious disease is responsible for many human deaths, but diagnosis of such conditions with limited resources remains a hurdle to improving public health. Therefore, there is a significant need to develop platform technologies with multiplex testing capabilities. Accessible cell sorting and microscopy technologies can be very useful in providing a tool with a broad range of medical diagnostic capabilities.

Magnetism has recently attracted attention for use in such technologies due to its label-free,2 highly sensitive,3 and highly specific3 advantages and its applications in microfluidics,4 microparticle detection,3 separation,3,5 and sorting.5,6 Magnetic focusing, which operates on the concept of negative magnetophoresis,2 involves suspending cells in a paramagnetic medium within a magnetic field, which causes a magnetic force to be exerted on the diamagnetic particles in the field. Further, if the magnetic field is aligned parallel to the gravitational force, the combination of magnetic and buoyant forces (governed by the density of the particles relative to that of the medium) causes particles in the magnetic field to levitate at a height determined by the particle’s density. It has been shown that less dense particles levitate higher in the magnetic field than denser particles, allowing rapid density-based separation of diamagnetic particles based on their densities.7–10 Because this technique requires only a paramagnetic medium and a magnetic field (formed by two permanent magnets with like poles facing each other), it shows promise for a variety of biomedical applications in low-resource settings. Recently, magnetic focusing has been shown to be useful for cell sorting and cytometry, including diagnosis of sickle cell disease, separation of lymphocytes,
leukocytes, and red blood cells, and monitoring leukocyte activation.11–13

Fluorescence microscopy is an advanced form of microscopy offering specificity by using fluorophore-conjugated antibodies or other fluorescent stains to identify certain cell markers. In fluorescence microscopic imaging, these fluorophores are excited when an electron absorbs energy from a specific wavelength of light; when the electron returns to its ground state, light is released (with a longer wavelength due to a small amount of energy loss) and is detected by a camera. A typical fluorescence microscope involves the use of an excitation filter, a dichroic mirror, and an emission filter to achieve optimal excitation of the fluorophore while reducing the noise from the excitation light as much as possible. This technology has broad applications in many fields of medical research and clinical disease diagnostics, including molecular and cell biology research,14–18 cancer research,18,19 bioluminescence imaging,20 and diagnosis of infectious diseases.21 However, the components of a typical fluorescence microscope (excitation lasers or light filters, dichroic mirrors, and emission light filters) introduce a high cost and low portability, preventing the use of fluorescence microscopy in resource-poor areas.

In the interest of providing portable and low-cost medical capabilities in low-resource settings, a significant amount of research is focused on fabricating point-of-care tools that are safe, cost-effective, and user-friendly.22 Specifically, mobile phone-based cell sorting and microscopy tools are particularly useful for point-of-care diagnostics. Once limited in their capabilities, smartphones have become complex processing machines. Not only has the computing power of these devices increased over the years, but so has the imaging capability. Today, the images obtained by mobile phones, particularly smartphones, can achieve resolution sufficient to image cells and even bacteria with sub-micrometer resolution. Smartphones thus provide a cost-effective alternative to traditional microscopic techniques due to their ubiquity. In 2020, it is estimated that 70% of the world’s population will be using smart devices and that 78% of these people will be using phones with the Android operating system.23

In order to adapt magnetic focusing for use in low-resource settings, a smartphone-compatible magnetic focusing platform was recently developed for density-based cell separation,24 which has also been applied to sickle cell disease diagnosis.25 This device performs density-based cell separation using two permanent magnets and leverages the onboard imaging and processing capabilities of the smartphone to analyze the spatial distribution of cells in the magnetic field. Thus, it can obtain quantitative measures, removing the need for additional processing steps by the user or for subjective interpretation. Here, we build on this technology by adding fluorescence microscopy, which will improve the multiplex test capabilities of this compact, low-cost, and versatile technology. Several studies have demonstrated the use of mobile phones for microscopic imaging, including several applications to medical diagnostics.26 Fluorescence microscopy has been incorporated into several mobile phone-based diagnostic tools,27–30 with broad applications for imaging and sizing DNA molecules,31 quantifying white blood cells via optofluidic analysis,32 detecting pathogens such as waterborne Giardia lamblia,33 and imaging viruses and bacteria.34

Here, the combination of these two highly versatile techniques – magnetic focusing and fluorescence microscopy – result in a powerful point-of-care tool. The proposed device is a low-cost, portable, smartphone-based tool which both separates cells via magnetic focusing and images them with either transmitted light or fluorescence imaging modes using the built-in camera of the Samsung Galaxy S6 smartphone. This technology may ultimately provide accessible research and diagnostic capabilities to low-resource settings.

**Methods**

**3D-printed design**

The 3D-printed fluorescence microscope was designed to be compatible with the Samsung Galaxy S6 (143.4 × 70.5 × 6.8 mm) because this smartphone offers manual focusing, allowing for detailed imaging. The entire fluorescence microscopy device consists of a phone case, a two-piece optical component, emission filter holders, and a shade (Fig. 1). The customized phone case (165 × 75 × 50 mm) attaches the optical component and shade to the smartphone. This phone case was designed in SolidWorks and was printed on a Stratasys Objet30 Prime polyjet printer using blue Rigid Opaque photopolymer (Stratasys, Eden Prairie, MN, USA). Combined, the case and other components weigh only 214 g (excluding the smartphone).

The case contains tracks for the smartphone and the optical component to slide into place, and the case’s base increases the stability of the setup. The optical component, emission filter holders, and shade were printed using a Formlabs Form 1+ stereolithography (SLA) 3D printer at a resolution of 0.1 mm with black resin (Formlabs Inc., Somerville, MA, USA). Each part of the optical component contains tracks that permit easy assembly and disassembly of the device as well as cost-effective modification. To allow for both brightfield and darkfield imaging, the device’s shade is easily attached and detached from the setup using tracks that align with the smartphone case.

**Optical components and imaging**

The optical component includes an electrical piece and a magnetic focusing piece. The electrical piece is designed to fit with a battery and switch and to exchange the microscope’s emission filters (Fig. 1a). The magnetic focusing piece includes spaces for two N52-grade nickel plated NdFeB 5 mm magnets (50.8 × 2 × 5 mm³) (custom design, K&J Magnetics, Inc., Pipersville, PA, USA) and a borosilicate glass micro-capillary tube (Square Boro Tubing #8270-050, VitroCom, Mountain Glass, NJ, USA) containing the sample (Fig. 1a); for
easily interchangeable LEDs to image the sample in the brightfield and darkfield configurations (Fig. 1); and for an aspheric lens with a diameter of 6.33 mm and numerical aperture of 0.64 mm (87–161, Edmund Optics, Barrington, NJ, USA) that magnifies the fluorescent image and focuses it on the smartphone camera. The camera application Camera FV-5 is used to image the sample.

In the optical setup for fluorescence imaging, the sample is excited using differently colored LEDs (Super Bright LEDs Inc., St. Louis, MO, USA). The LEDs are powered by two 3 V batteries (CR2032, Panasonic, Newark, NJ, USA) wired in series with a 220 Ω resistor, which provides sufficiently regulated power. To counter the high power drain of the LED, a slide switch (GF-1123-0025, CW Industries, Southampton, PA, USA) is attached between the battery and the diodes to prevent unnecessary current flow. Light is produced by the LEDs and directed towards the sample from the side to minimize the amount of background excitation light that is eventually captured by the camera. The emitted light from the sample travels through the external aspheric lens (87–161, Edmund Optics, Barrington, NJ, USA) and the magnified image is focused onto the smartphone camera (Sony IMX240, 1.34 μm pixel pitch). To maximize the clarity of the images, the lens and phone camera are aligned (Fig. 1a). The lens sits approximately 4 mm back from the top of the camera. Minor changes to focal length can be made using the manual zoom of the Samsung Galaxy S6. A similar setup has already been used to image microspheres and cells clearly using magnetic focusing and a brightfield configuration.22,27

Relying on relatively inexpensive commercially-available or 3D-printable components, the device presented here offers a low-cost alternative to traditional laboratory equipment designed for fluorescence imaging and/or cytometry. Table 1 displays a detailed cost breakdown for the device presented here. As evident in Table 1, the unit cost of the device is about $100 (assuming that the user provides the smartphone) as compared with tens of thousands of dollars for fluorescence microscopes or fluorescence-activated cell sorting.

<table>
<thead>
<tr>
<th>Item</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>3D-printed case, material cost</td>
<td>$18.23</td>
</tr>
<tr>
<td>Lens</td>
<td>$7.3</td>
</tr>
<tr>
<td>Permanent magnets (2)</td>
<td>$6.32</td>
</tr>
<tr>
<td>Battery</td>
<td>$0.34</td>
</tr>
<tr>
<td>Switch</td>
<td>$0.80</td>
</tr>
<tr>
<td>Resistor</td>
<td>$0.10</td>
</tr>
<tr>
<td>LEDs (3)</td>
<td>$2.09</td>
</tr>
<tr>
<td>Emission filter set (includes all filters used here)</td>
<td>$4.99</td>
</tr>
<tr>
<td>Total</td>
<td>$105.87</td>
</tr>
</tbody>
</table>

Per-use costs:

- Microcapillary tubes: $1 per test
- Gadavist (per 2 mL vial, sufficient for ~4000 assays): $17.43
- Staining: Varies by application
equipment. Each use of the device only requires replacement of the microcapillary tube and preparation of the sample in paramagnetic solution (~$1 per assay, excluding the cost of antibodies, which can vary greatly depending on the assay).

Characterization of setup

A comparison of the device's ability to image using both transmitted light for brightfield imaging and reflected light for darkfield imaging was first obtained using microspheres (Microspheres-Nanospheres, Corpuscular, Cold Spring, New York, USA 10516) of different sizes. Microspheres of 5.35 μm (C-PS-5.0), 10.4 μm (C-PS-10.0), 20.0 μm (C-PS-20.0), 40.0 μm (C-PS-40.0), and 79.0 μm (C-PS-80.0) were chosen for their clear difference in size and similarity to the size of various eukaryotic cells. A sample of the microspheres was mixed with a 100 mM solution of the gadolinium-based paramagnetic medium, Gadavist (Bayer, Whippany, NJ, USA), in Hank's balanced salt solution with a density of approximately 1 g cc$^{-1}$ and Optiprep medium with density 1.319–1.321 g cc$^{-1}$ (Sigma Aldrich, St. Louis, MO, USA) to create the final sample solution with 48–50 mM Gd concentration and final density of 1.096–1.156 g cc$^{-1}$. In these samples, the Gadavist functions as a paramagnetic agent while the Optiprep medium increases the levitation height of the microspheres, preventing them from reaching equilibrium at the bottom of the microcapillary tube where they would not be visible. This microsphere solution was then loaded by capillary action into the square VitroCom microcapillary tube, placed between the magnets, illuminated by a 3000 K warm white light LED (Digitkey, Thief River Falls, MN) and imaged by the smartphone camera using both the bright field and dark field configurations. Each size microsphere and light condition was imaged three times to analyze the size of 15 different beads in each test. The sizes of the beads for each condition were determined digitally using the ‘improfile’ function in MATLAB, which provides pixel intensity and location. Areas of low intensity corresponded to bead edges in bright field images while areas of high intensity indicated beads in dark field images. Bead sizes were then compared for the brightfield and darkfield states to determine image quality in the darkfield configuration.

Fluorescence was studied using three different colored fluorescent microspheres: red (UVPMS-BR-1.05, Cospheric LLC, Santa Barbara, CA, USA), green (UVPMS-BG-1.025, Cospheric LLC, Santa Barbara, CA), and blue (UVPMS-BB-1.13, Cospheric LLC, Santa Barbara, CA). These microspheres were excited by white (RL5-W6018, Super Bright LEDs Inc., St. Louis, MO, USA), blue (RL5-B5515, Super Bright LEDs Inc., St. Louis, MO), and UV light (RL5-UV0315-380, Super Bright LEDs Inc., St. Louis, MO), respectively, based on the emission spectra provided by Cospheric. To determine the effectiveness of low-cost plastic filters (Rosco Laboratories, Sun Valley, CA, USA), each type of microsphere was imaged with no emission filter, a red emission filter (Roscolux #19: fire, Rosco Laboratories, Sun Valley, CA), a green emission filter (Roscolux #389: gaslight green, Rosco Laboratories, Sun Valley, CA), and a blue emission filter (Roscolux #74: night blue, Rosco Laboratories, Sun Valley, CA). These filters were chosen based on the wavelengths that each transmits; the red, green, and blue filters were expected to allow the strongest signals to pass through for the red, green, and blue microspheres respectively. The microsphere samples were prepared using the Gadavist- and density-based protocol described above to bring them to the middle of the microcapillary tube. These samples were imaged in the darkfield imaging setup three times per test condition to analyze at least 15 beads for each combination of filter and digital channel. Images were analyzed using ImageJ by separating each picture into its constituent red, green, and blue channels and determining the signal strength, defined as the difference in mean signal pixel intensity and mean background pixel intensity, in each channel for each of the three different colored fluorescent beads with the four different filter conditions.

Optimization of device for cell imaging

To optimize our novel fluorescence microscope for biological applications, cells were stained with calcein (C3100MP, Thermo Fisher Scientific, Waltham, MA) and QTracker 625 (A10198, Thermo Fisher Scientific, Waltham, MA). Both of these substances stain the cytoplasm of cells and therefore were ideal candidates to test biological applications of the setup. Calcein, a green cytoplasmic stain, was used to optimize the setup for green fluorescence imaging. Breast cancer cells (MDA-MB-231, ATCC, Manassas, VA, USA) were cultured in RPMI medium, trypsinized, stained with calcein, and allowed to incubate for 30 minutes. Cells were then viewed under a laboratory fluorescence microscope to confirm incorporation of the fluorescent stain. The cells were then mixed in a 1:1 ratio with the 100 mM Gadavist solution in Hank's balanced salt solution for a final 50 mM Gadavist solution with the stained cancer cells. Based on the excitation and emission curves for calcein, the blue LED was used to excite the sample. The blue LED was chosen because it contained wavelengths required to excite the sample but did not contain wavelengths that may be mistaken for the green fluorescent light, unlike other possible LEDs such as an aqua or white light. To maintain the brightness of the excitation light and because the light did not contain a wide range of wavelengths, an excitation filter was not used. Three distinct, but similar, green emission filters were tested to determine the effect of the emission filter on a biological sample's image. The three different filters used were gaslight green (Roscolux #388: gaslight green, Rosco Laboratories, Sun Valley, CA), moss green (Roscolux #89: moss green, Rosco Laboratories, Sun Valley, CA), and pea green (Roscolux #86: pea green, Rosco Laboratories, Sun Valley, CA). All of these emission filters transmitted the most light around the 525 nm range emitted by calcein but showed lower transmittance for blue and red light. These samples were imaged in the darkfield...
imaging setup three times per test condition to analyze at least 30 cells for each emission filter, in each of the three digital channels. Images were analyzed using ImageJ by separating each picture into its constituent red, green, and blue channels and determining the signal strength for each combination of emission filter and channel. Because signal strength measures the contrast between signal and background for each test condition, this measurement indicates image quality, with high values corresponding to high quality. Additionally, the use of ImageJ for counting cells in each image digitally was investigated due to its relevance to future biological applications for this fluorescence microscope. Because of the high pixel intensity of the cells relative to the background, the “find maxima” function was tested at various noise tolerance levels.

A similar procedure was used to optimize the setup using red emission filters for QTracker 625, a red cytoplasmic stain. Prostate cancer cells (PC3, ATCC, Manassas, VA) were cultured in RPMI medium with QTracker 625 overnight. Based on the excitation and emission curves for QTracker 625, the blue LED was again used to excite the sample. A UV LED was also tested but did not provide as clear images as the blue LED. To maintain the brightness of the excitation light and because the blue light did not contain a wide range of wavelengths, an excitation filter was not used. Three distinct, but similar, red emission filters were tested to determine the effect of the emission filter on a biological sample’s image. The three different filters used were golden amber (Roscolux #21: golden amber, Rosco Laboratories, Sun Valley, CA), light red (Roscolux #26: light red, Rosco Laboratories, Sun Valley, CA), and fire (Roscolux #19: fire, Rosco Laboratories, Sun Valley, CA). All of these emission filters transmitted the most light around the 625 nm light emitted by QTracker 625 but showed lower transmittance for blue and green light. As in the calcine optimization, 30 cells were analyzed for each combination of emission filter and digital channel using ImageJ. Signal strength and cell counting potential were again used to determine image quality and usefulness in biological applications.

**Evaluation of clinical applications of device**

To evaluate effectiveness in a biological application, the device’s ability to count cells was evaluated. Breast cancer cells (MDA-MB-231) were again stained with calcine and allowed to incubate for at least 30 minutes. Cells in the stock solution of stained breast cancer cells were counted using a hemacytometer to determine the cell concentration. From this stock solution, five different dilutions were created consisting of 1%, 2%, 3%, 4%, and 5% of the stock solution with 99%, 98%, 97%, 96%, or 95% 100 mM Gadavist solution by volume, respectively. These five dilutions were tested in the fluorescence microscope presented here. The concentration of cells in each of the five samples was measured by visually counting the cells in a photo taken and using the “find maxima” function in the photo’s green channel in ImageJ. The volume of the capillary was used to convert these cell counts to cell concentrations. Concentrations measured visually and by ImageJ were compared to hemacytometer count to determine the accuracy of the device.

The limits of cell counting and cell detection were then investigated using ovarian cancer cells (HeyA8, ATCC, Manassas, VA) cultured using DMEM and stained with calcine. Again, the fluorescently stained cells were excited using a blue LED (RL3-B2030, Super Bright LEDs Inc., St. Louis, MO). The lower limit of cell counting was determined by capturing an image of a single cell in the field of view of the camera. The range of sizes of cells imaged with the smartphone camera was also assessed by staining cultured lung cancer cells (A549, ATCC, Manassas, VA) and measuring their diameters in ImageJ software. Measurements were taken in pixels and converted to μm using the 700 μm capillary interior for scale.

Lastly, the differences in two cell populations were analyzed in three ways. In the first test, a small population of QTracker 625-stained HeyA8 cells was mixed with a much larger population of calcine-stained HeyA8 cells. This setup mimics the detection of rare cells in a greater cell population. The paramagnetic agent, Gd, was added to the mixture to give a final concentration of 100 mM Gd. The sample was illuminated by a blue LED (RL3-B2030, Super Bright LEDs Inc., St. Louis, MO) and imaged using no emission filter, the gaslight green emission filter, and the fire emission filter. Images were also further filtered, digitally, in ImageJ to separate the red and green channels for each stain. The second test conducted between two cell populations mimics circulating tumor cells in the bloodstream. Cultured HeyA8 cells were again stained with calcine and mixed into diluted blood obtained via finger prick. Blood samples were obtained from humans with informed written consent; all experiments were performed in compliance with the relevant laws and institutional guidelines and all procedures were approved by the University of Connecticut Institutional Review Board Protocol #H15-048. The cells, which are expected to separate vertically in the magnetic field based on their different densities, are imaged in both brightfield and darkfield conditions. The third, and final, condition evaluates the potential use of the stain acridine orange for cell identification. Acridine orange stains both double-stranded DNA fluorescence green and single-stranded RNA fluorescence red. Due to their high replication rate, cancer cells tend to be stained more red than other cells. Given this trend, A549 and non-cancerous 3T3 cells (ATCC, Manassas, VA) were stained with acridine orange. Acridine orange was diluted to a concentration of 0.1 mg mL⁻¹ in phosphate buffered saline, and stained cells in suspension were incubated at 37 °C for at least four hours. After washing twice and resuspending in Hank’s balanced salt solution each solution containing one of the three types was made paramagnetic by the addition of 1 M Gd to reach a final concentration of 100 mM Gd. Each cell type was imaged when excited by a blue LED with no emission filter in order to capture all emission wavelengths. Images were split into
red, green, and blue channels in ImageJ, and the signal strength in the red and green channels was measured for both cell types in order to determine the red-to-green ratio in both acridine orange-stained populations.

Results and discussion
Effect of microsphere size and light conditions on image quality
Brightfield and darkfield imaging displays similar results for both conditions. The qualitative images in Fig. 2a shows comparable bead clarity, resolution, and detection for beads ranging from 5.35 μm to 79.0 μm. Although features of the 5.35 μm beads are not clear, their visibility indicates this microscope’s ability to detect small objects such as cells. Microspheres larger than 10.4 μm provide further detail in both types of images. Analysis of bead size in MATLAB for the five different sizes in both light conditions shows no significant difference between size seen in bright field and dark field images (Fig. 2b). Some limitations of the size measurement method used here include subjective interpretation of the bead edges based on the pixel intensity profiles, considering both shadows and scattering of light. However, the consistency of image quality and bead size across transmitted

---

**Fig. 2** Brightfield and darkfield imaging results. (a) Microspheres of various sizes (5.35 μm, 10.4 μm, 20.0 μm, 40.0 μm, and 79.0 μm) imaged under brightfield (BF)[left] and darkfield (DF)[right] conditions. (b) Representative image of how bead diameters were measured in brightfield (left) and darkfield (right) images based on pixel intensity using the improfile function in MATLAB. The black, double-headed arrow shows the horizontal distance considered to be the bead diameter for the beads marked in blue. (c) Diameter of the imaged beads for all sizes and both imaging conditions tested.
light and reflected light conditions indicate the suitability of our design for darkfield and fluorescence imaging.

**Effectiveness of fluorescence microscope**

Images of fluorescent microspheres taken with the proposed device display the effectiveness of the microscope in capturing emitted wavelengths. In Fig. 3, red, green, and blue fluorescent microspheres show the most contrast with the other colors using the appropriate physical channel and digital filter. For the red microspheres (Fig. 3a), the green and blue filters effectively reduce the signal strength in all three digital channels. With the green filter, the red signal strength is still comparable to the green signal strength due to the proximity of these wavelengths, the intensity of the red signal, and the imperfect boundaries of the digital channels (Fig. 3d). Using the red filter, the red channel's signal strength is significantly greater than the signal strength in the green or blue channels (Fig. 3d). Qualitatively, the image corresponding to both the red filter and the red digital channel is the brightest of the split images and contrasts most with other filter and channel combinations (Fig. 3a). The strength of the signal in the red channel with the red filters compared to that with other filters supports the hypothesis that the microsphere emits red light via fluorescence in response to white light excitation and that the filters used can effectively separate noise due to scattered white light. This allows the device to distinguish between fluorescent microobjects emitting different colors of light.

The green microspheres (Fig. 3b) also show the strongest filtered signal using the green emission filter and green digital channel. The red filter provides an attenuated signal in all three channels while the green filter maintained signal strength in both the green and blue channels (Fig. 3e). This comparable signal strength between the green and blue channels using the green emission filter can be explained by both the imperfect boundaries of the camera’s color sensitivity (Sony Corporation) and the fluorescent color of the microspheres. The peak emission for the green microspheres is actually approximately 500 nm (Cospheric LLC), which corresponds to a blue-green light. As a result, the signals in the blue and green channels with the blue emission filter are also relatively strong compared to the signal strength for the same conditions with the red microspheres. Qualitatively, the blue-green quality of the green microspheres can be seen in the brightness of the sample in the green channel with the green emission filter and in the blue channel for both the green and blue emission filters.

The blue microspheres show the strongest filtered signal using the blue emission filter and the blue digital channel (Fig. 3c). Although the blue channel shows a significantly stronger signal than the green or red channels in the composite image, the blue emission filter still attenuates the green and red channels (Fig. 3f). Furthermore, a red emission

---

**Fig. 3** Fluorescence imaging results. (a) Red fluorescent microspheres excited by white light under various physical filter (rows) and digital channel (columns) conditions. The physical filters are red (R), green (G), and blue (B) emission filters, with the leftmost column of the table showing the composite image in color. Each color image is also separated into its constituent red, (R), green (G), and blue (B) channels digitally in ImageJ, and these components are shown across the columns. (b) Green fluorescent microspheres excited by blue light. (c) Blue fluorescent microspheres excited by UV light. (d-f) Signal strength, defined as the difference between microsphere pixel intensity and background pixel intensity, for the red (d), green (e), and blue (f) fluorescent microspheres under different physical filter (represented by “No Filter”, “Red Filter,” “Green Filter”, and “Blue Filter”) and digital channel (represented by “R”, “G”, and “B”) conditions.
filter causes no emitted light to be captured by the camera due to the attenuation of blue light using a filter that allows only higher wavelengths (>500 nm) to pass through while greatly reducing the transmittance of light having a wavelength lower than 500 nm (Fig. 3c and f).

For all three differently colored microspheres, both physical and digital filters are needed to increase the contrast between the emitted fluorescent light and scattered or excitation light in the background. The filtered images are brightest and show the strongest signal relative to the background when the same-color emission filters and digital channels are used. This finding is significant for two reasons. First, the proposed fluorescence microscope picks up the light emitted by the fluorophore. Second, the dual filtering ensures that only the desired color of light is analyzed in the processed image. Like the traditional fluorescence microscope, an area of high pixel intensity corresponds to a fluorescent microobject emitting a desired wavelength of light. Also similar to more expensive designs, the proposed microscope can easily change the color of light picked up by switching out the physical filter and choosing a different digital channel in which the image is analyzed.

Filter and LED optimization for cell imaging
Given the effectiveness of the fluorescence microscope for microspheres, the device was optimized for fluorescent cell imaging using two different types of cytoplasmic stains: calcein and QTracker 625. Fig. 4a shows the excitation and emission spectra for the stains alongside the LED spectrum, emission filter light transmission, and smartphone camera’s green channel to indicate that the filtered images in the green channel and capture the calcein’s emitted light while

---

**Fig. 4** Live cell fluorescence imaging. (a) Wavelengths used to optimize filters for fluorescence imaging for calcein. From top to bottom: excitation and emission spectra for calcein (Thermo Fisher Scientific); blue LED emission (Super Bright LEDs, Inc.); transmittance for various green emission filters (Rosco Laboratories); relative absorbance of color channels in the Sony IMX240 camera (Sony Corporation). (b) Raw smartphone images of cells stained with calcein for no emission filter and gaslight green (GG), moss green (MG), and pea green (PG) emission filters. (c) Analysis of calcein fluorescence signal strength for four filter conditions and three digital channels. (d) Calcein-stained cells imaged with the gaslight green emission filter and split into red, green, and blue channels. (e) Representative image of cell counting using the “find maxima” function with a noise tolerance of 50 in ImageJ. (f) Wavelengths used to optimize filters for fluorescence imaging for QTracker 625. From top to bottom: excitation and emission spectra for QTracker 625 (Thermo Fisher Scientific); blue LED emission (Super Bright LEDs, Inc.); transmittance for various red emission filters (Rosco Laboratories); relative absorbance of color channels in Sony IMX240 camera (Sony Corporation). (g) Raw smartphone images of cells stained with QTracker 625 for no filter and golden amber (GA), light red (LR), and fire (F) emission filters. (h) Analysis of QTracker 625 fluorescence signal strength for four filter conditions and three digital channels. (i) QTracker 625-stained cells imaged with the golden amber emission filter and split into red, green, and blue channels. (j) Representative image of cell counting using the “find maxima” function with a noise tolerance of 30 in ImageJ.
reducing noise from the blue excitation wavelengths. The images in Fig. 4b visually display the effectiveness of all three emission filters in blocking the blue excitation light from being captured by the smartphone camera while allowing the green light from the cells to be imaged. All three green emission filters slightly attenuated the signal strength in the green and blue digital channels while significantly attenuating that in the red channel (Fig. 4c). Based on both the quantitative and qualitative analyses of the three green filters, the gaslight green filter was determined to be the most effective because it least attenuated the green signal. However, the similar effect of all three filters displays versatility in the design; in a low-resource setting, several types of green filters will suffice to image calcein-stained cells. With the gaslight green filter, individual cells were clear and bright in the green channel (Fig. 4d) and were able to be counted digitally in ImageJ (Fig. 4e). The ability to digitally count cells after simple filtering in this device increases its applications while reducing the manpower needed to analyze its results. However, if counting is done visually, the external magnetic field brings the cells into a line at equilibrium in the middle of the field of view, rendering them easier to count than if they were spread out on a glass slide under a traditional fluorescence microscope.

The results seen for the calcein are also seen for a cytoplasmic stain of a different color: QTracker 625 (Fig. 4f-j). Again, the emission filters transmit extremely low levels of the excitation light while allowing the emitted light to pass onto the lens and smartphone camera (Fig. 4f and g). Because of the great difference in wavelengths between the excitation and emission light for the QTracker 625, the images captured by the smartphone with only a plastic emission filter are primarily comprised of the fluorescence light. For this red stain, golden amber was chosen as the most effective emission filter due to its low attenuation of the red signal with high attenuation of the signals in the green and blue channels (Fig. 4h). As with the calcein, the red QTracker 625 shows clear individual cells (Fig. 4i) and an ability to digitally count cells (Fig. 4j). The effectiveness of the proposed device in both capturing fluorescence, bringing cells to equilibrium in the magnetic field, and digitally analyzing these signals shows promise for a variety of biological applications.

Characterization of cell imaging

Cell counting, one of the many possible biological applications for our device, was characterized using calcein-stained breast cancer cells in the proposed fluorescence microscope. Fig. 5a...
displays the images obtained for samples of five different concentrations. Because these images have been physically filtered using gaslight green plastic colored filter and digitally filtered by splitting channels in ImageJ, the areas of brightness in the photographs correspond to areas of high intensity green light, or to the stained cells. In Fig. 5a, the cells are aligned at equilibrium due to the external magnetic field; relative concentrations of the five dilutions are evident. A quantification of the cell concentrations shows that the proposed design accurately provides relative concentration measurements through either visual or digital methods (Fig. 5b). Both methods exhibit linearity \( R^2 > 0.95 \) with the theoretical percentage of the hemacytometer’s cell concentration determination. This reliable measurement of relative concentration in a small (10 \( \mu \)L) sample reduces the need to obtain, store, and analyze large volumes of biological samples and could be used to determine abnormal cell counts indicative of disease. For all five dilutions, the proposed fluorescence microscope’s images underestimate the theoretical cell concentration (Fig. 5b). This difference may be due to the error inherent in all three methods or due to cell death in the time elapsed between hemacytometer counting and sample imaging. While this slight underestimation presents an area for future optimization of the device, the strong correlation between measured cell concentration and true cell concentration shows promise for diagnostic capabilities of the proposed device.

The lowest cell concentration measured by this device is limited to one single cell in the smartphone camera’s field of view (Fig. 5c). At a zoom level typically used to capture images, this limit is 1 cell in 1.07 \( \mu \)L, or 935 cells per mL. However, it is possible to zoom out to a lower limit of detection of 1 cell in 1.47 \( \mu \)L or 680 cells per mL. The upper limit of detection for cell concentration is essentially unlimited; it is possible to dilute the sample for cells that are not resolvable due to high cell concentration. Another factor affecting cell detection is cell size. An analysis of the calcein-stained cells in a sample of A549 cells of various sizes showed that the device can detect cells with a minor ellipse axis as low as 8.17 \( \mu \)m (Fig. 5d).

Cell imaging applications

The device presented here has shown promise for a variety of applications involving the use of fluorescence
microscopy to distinguish two different cell populations. In Fig. 6a, cells stained with red QTracker 625 are much more rare than the cells stained with green calcein in the sample. The raw image with no emission filter shows the presence of both cell types, and splitting the image into green and red channels verifies that each color is indeed as it appears to the human eye. The image using the gaslight green filter again shows both cell types, which is likely due to the filter’s relatively high transmission of red wavelengths, above 600 nm (Fig. 4a). The fire filter, however, almost completely eliminates the visibility of the cells that are stained green. Due to the sensitivity of this filter, only the rare cell stained red is visible. This finding is significant because brightfield conditions would not be able to distinguish between the cells, but the fluorescence microscope with easily changeable and customizable filter sets can do so. Additionally, the magnetic field focuses these cells on a single plane such that the rare cell is in focus. Therefore, incorporation of appropriate filters and stains into this device can be utilized in identification and detection of rare cells in a sample with other, more populous cells.

The fluorescence imaging mode also has the capability to eliminate one population from the image. In Fig. 6b, a mixture of red blood cells and calcein-stained cancer cells is imaged in both brightfield and darkfield conditions. In brightfield conditions, both populations are seen. Both populations are spatially separated in the magnetic field due to their different densities. In fluorescence imaging mode, in contrast, only the cancer cells are seen because RBC staining with calcein is not observed. The RBCs do not interfere with the appearance of the cancer cells due to the aforementioned spatial separation. In this way, the combination of magnetophoresis and fluorescence imaging can both separate and differentially image the cell populations. This may be of interest in the context of blood pathologies, such as circulating tumor cells or circulating endothelial cells, which are currently difficult to detect.

Lastly, the use of another stain, acridine orange, may aid in cancer detection. In Fig. 6c, both cancer cells (A549) and non-cancerous mouse fibroblasts (3T3) are successfully stained with acridine orange and imaged with the smartphone camera. Because there is no emission filter in these images, all wavelengths of light are collected. The image can then be split into red, green, and blue channels digitally. In this application, red and green channels are of interest due to the high RNA content—and therefore more red emission—of rapidly dividing cells such as cancer cells. A comparison of the signal strength in the red and green channels for both images of similar quality shows that both types of cells have a much stronger signal in the red channel than in the green channel, and the cancer cells have a slightly higher red to green signal strength ratio than the 3T3 cells: 2.6 and 2.2, respectively. Again, the choice to image without an emission filter proves useful, and the capability to image and analyze the acridine orange stain shows promise for this device in a point-of-care setting.

**Conclusion**

A novel, low-cost fluorescence microscope incorporating magnetic focusing technology has been developed to increase accessibility to clinical and research tools in resource-limited settings. The use of the high resolution, manual-focusing camera of the phone prevents the need for a costly microscope while the darkfield configuration of the device eliminates expensive components such as a dichroic mirror. The different LEDs utilized, which cost under $1 each, excite the microspheres or stained cell samples while an emission filter minimizes the amount of light interference by reducing noise in the device. The unique approach of the modular design advances fluorescent technology by offering a cheap and portable, yet accurate, alternative to more traditional microscopic techniques. By successfully magnifying microscopic objects and providing similar conclusions to those offered by a more expensive and established fluorescence microscope, we have confirmed that the capability of the device is comparable to other, more expensive designs. This is coupled with magnetic focusing technology, which effectively focuses the microparticles into the camera’s focal plane and can separate cells of different densities to diagnose pathologies in fluids such as blood or urine.

This prototype has been shown to perform darkfield imaging with quality comparable the brightfield image quality in previously developed magnetic focusing devices. Additionally, the simple combination of a colored LEDs; a plastic emission filter; and digital separation of red, green, and blue channels effectively ensures that the intense pixels in a processed image correspond to the fluorescence emitted from microobjects in the sample. Thus, the need for expensive components such as an excitation filter and dichroic mirror is reduced. Finally, visualization and accurate counting of stained cells shows promise for use of the proposed design in biological applications in a research laboratory or in low-resource clinical settings.

The 3D printing fabrication method allowed for low-cost and facile rapid prototyping of this device. The design proposed presents many advantages due to its low cost, portability, versatility, and incorporation of multiple novel technologies. While a traditional fluorescence microscope may cost thousands of dollars, the 3D-printed components in the proposed design are easily fabricated with minimal resin in a desktop 3D printer, or may be mass produced from plastic; the filters, which represent the most expensive components of traditional microscopes, cost only a few dollars for a complete set. Because the device is relatively small and consists of a smartphone and plastic components, the design is lightweight and portable. Thus, the proposed device can be moved easily, shared among laboratories without the budget to purchase a fluorescence microscope, and used in mobile clinics in point-of-care settings. Finally, the modular components are easily exchanged such that if one part were to fail, the entire device would not need to be replaced. The interchangeable emission filters and LEDs allow users to select and optimize the correct imaging modality to their own needs.
microobjects of interest. Once in the device, microobjects such as cells are easy to view and count due to the reduction of noise by the filters and the bringing of cells to the middle of the vertical field of view by the external magnetic field. Furthermore, this external magnetic field separates cells based on density, and this device can be optimized to eventually diagnose diseases such as cancer and abnormal white blood cell counts in areas with limited access to healthcare resources. Thus, the proposed device distinguishes between cells based on multiple criteria: density, through magnetic focusing; size, through image analysis; and fluorescent stain color.

Future work will widen the range of the prototype's imaging modalities and further develop the biological applications of this device. In this study, only red and green fluorescent cytoplasmic stains were successfully tested and utilized to count cells. Future work should thus optimize the device in the blue channel and for nuclear and surface stains as well. We hypothesize that nuclear and surface stains are currently not seen due to their size relative to the cytoplasm of cells that has successfully been stained. Expanding the compatibility of this device to both nuclear and surface stains will greatly broaden its applications to live/dead assays, immunostaining, and more. Specifically, immunostaining may be used for cancer diagnostics, and live/dead assays using the proposed device will provide cost-effective insights into the results of cytotoxicity tests. Additionally, in live/dead testing, live cells will equilibrate to the middle of the capillary containing the sample while dead cells will settle to the bottom edge, and this spatial separation will help distinguish between the two types of cells. Other areas that will integrate fluorescence and magnetic focusing well include the aforementioned cancer detection and white blood cell abnormalities due to the differences in density between cancer cells, white blood cells, and red blood cells. In analyzing a blood sample, a calcinein-based assay has been shown to analyze the viability of red blood cells, which could prove useful in blood banks in limited-resource areas. Due to the range of research and clinical applications for which this prototype can be further optimized, the proposed design contributes a valuable tool to the field of point-of-care diagnostics.

Acknowledgements

ST acknowledges the American Heart Association Scientist Development Grant (15SDG25080056) and the University of Connecticut Research Excellence Program award for financial support of this research. SK acknowledges the support of the National Science Foundation Graduate Research Fellowship (DGE-1247393). AJ acknowledges the support of a University of Connecticut Summer Undergraduate Research Fellowship. We thank Ryan Lancaster, Peter McBride, Anton Razhov and the rest of the Fall 2015 3D Printing Class members for their enthusiastic and encouraging contribution to the final projects. We acknowledge Chu for his artful contribution to Fig. 1. ST and SK are founders of, and have an equity interest in mBiotics, LLC, a company that is developing microfluidic technologies for point-of-care diagnostic solutions. ST’s and SK’s interests were viewed and managed in accordance with the conflict of interest policies of the University of Connecticut. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

References