BioChip J. (2015) 9(4): 285-292
DOI 10.1007/s13206-015-9403-0

Original Article

A Smartphone-based Fluorescence Microscope Utilizing an External Phone Camera Lens Module

Jung-Hyun Kim[1], **Hong-Gu Joo**[2], **Tae-Hoon Kim**[3] **& Young-Gu Ju**[4,*]

Received: 14 May, 2015 / Accepted: 01 July, 2015 / Published online: 16 September, 2015

Abstract We constructed a smartphone-based fluorescence microscope by adding a phone camera lens module to the outside of a smartphone camera. The external lens module forms a relay system in conjunction with the built-in phone camera. The system benefits from the small pixel size of the smartphone and other advantages associated with the phone camera lens module such as its low cost, small volume, and low weight. To realize the fluorescence microscope function, the filters and illumination source were integrated into a simple mechanical structure with a focusing adjustment scheme. Based on an image analysis, the resolution of the microscope was approximately 2.5 μm and the field of view was as large as 1.2 mm × 1.2 mm. The fabricated microscope was used successfully to visualize the fluorescent paint and the low concentration quantum dots on a slide glass. The microscope obtained fluorescence images of cells stained with carboxyfluorescein succinimidyl ester (CFSE). This demonstration of a smartphone-based fluorescence microscope supports the feasibility of applications such as point-of-care diagnostic equipment based on fluorescence methods.

Keywords: Fluorescence, Microscopy, Smartphone

[1]Department of Physics, Kyungpook National University, 80 Daehakro, Bukgu, Daegu 702-701, Korea
[2]College of Veterinary Medicine, Jeju National University, 102 Jejudaehak-ro, Jeju-si, Jeju Special Self-Governing Province 690-756, Korea
[3]R&D Institute of Lumimicro Co. Ltd., Yongin, Gyeonggi-do 449-883, Korea
[4]Department of Physics Education, Kyungpook National University, 80 Daehakro, Bukgu, Daegu 702-701, Korea
*Correspondence and requests for materials should be addressed to Y.-G. Ju (✉ygju@knu.ac.kr)

Introduction

Smartphone technology is advancing very rapidly and has incorporated numerous existing electronic technologies such as the telephone, computer, internet, camera, and so on. Additional sensors and software are added to smartphones with growing computing power. In particular, recent progress with respect to camera performance in smartphones is staggering. It is not difficult to find a smartphone that has a camera with more than 10 million pixels and auto-focusing ability. Accordingly, it is natural to exploit these technological developments for a variety of applications. Biotechnology research has joined this trend and has examined applications of this device in tele-medicine or point-of-care (POC) testing, utilizing its telecommunication and imaging capability. The imaging capability of smartphones can easily extend to the function of a microscope by adding an external lens to the device's camera. In fact, this kind of microscope lens is available at a low cost in the market.

Some researchers have developed smartphone-based fluorescent imaging devices for use as portable diagnostic bio-equipment[1-4]. This type of imaging device is a miniaturized fluorescence microscope that utilizes a smartphone camera and telecommunication functions. The smartphone-based fluorescence microscope requires more expensive optical components such as an excitation light source, interference filter, and precision mechanical stage. Although smartphone-based fluorescence microscopes are not as widespread as non-fluorescent devices, they possess greater potential as portable bio-detection devices. In general, a fluorescence microscope can acquire both normal microscopic images and fluorescence images depending on the

filter and illumination source. Fluorescence is one of the most common techniques in the life sciences for the non-destructive tracking and analysis of biological molecules[5-8]. Its applications include fluorescence microscopy, DNA sequencing, DNA detection, protein detection, DNA micro-arrays, immunology, flow cytometry, and so on. Therefore, the study of smartphone-based fluorescence microscopes can facilitate the development of a portable diagnostic device that can access the established and rich toolbox of fluorescence methods.

In this paper, we present a simple fluorescence smartphone microscope that utilizes an extra phone camera lens module; this device has advantages in terms of resolution, volume, and cost. In addition, we fabricated various types of light sources covering both general microscope and fluorescence modes. After fabrication, we tested the smartphone-based microscope under a normal mode to determine its resolution and acquired fluorescence images of quantum dots and chromophore-stained biological samples.

Fabrication

The smartphone-based fluorescence microscope consisted of an external lens, filters, a sample stage, and an illumination source. The external lens had the greatest influence on the optical performance of the microscope, along with the lens inside the smartphone camera. The built-in lens in the rear camera of the smartphone is an infinite conjugate system, which assumes that an object is placed very far from the lens relative to the focal length. Thus, adding an external lens to the smartphone makes the whole system act as a relay lens. When an object is positioned at the focal point of the external lens, an image is formed at infinity. The built-in lens in the smartphone considers this image an object and forms a second image in its own sensor plane. The ray paths shown in Figure 1 illustrate this scenario. The ratio of the focal length of the built-in lens to that of the external lens is the magnification of the relay lens.

For the system fabricated in the experiment, we used an additional smartphone lens module as an external lens. The infinite conjugate side of the external lens module should face the built-in lens of the smartphone to form a relay lens. Although the lens module used for the external lens was not identical to the built-in lens of the smartphone used in the experiment, they were not appreciably different due to the lack of variation in focal length and entrance pupil diameter among systems. The specification of the external lens was as follows: the effective focal length, the back focal length, and the entrance pupil diameter were 3.97 mm, 0.64 mm, and 1.49 mm, respectively. When the focal length of the external module is similar to that of the smartphone, the magnification of the relay lens is approximately unity. Although the magnification is one, the resolution can be high as long as the numerical aperture of the built-in lens is high and the pixel size of the image sensor is very small. A recent advantage of smartphone cameras is their small pixel size. Many smartphone companies now produce image sensors with a pixel size as small as 1.0 μm. Accordingly, the camera can distinguish image points separated by 2.0 μm. However, the resolution also depends on the f/# (F-number) of the built-in lens, which is approximately 2. According to the Rayleigh criteria, the resolution limit is the wavelength multiplied by f/#. In our case, this limit was approximately 1.0 μm. Therefore, the theoretical resolution limit using the unit magnification relay system was approximately 2.0 μm.

Other benefits associated with the use of a phone camera module as an external lens are compact size, low weight, low cost, etc. Since it was developed for a smartphone, the module comprised multiple lenses for correcting higher order aberrations. In addition, they are produced in large volumes at low cost. We can take advantage of these outstanding features without extra investment in development.

Lens holders have screw threads on the side to adjust the focus while the center hole holds the lens module. Nowadays, many smartphones have auto-focusing functions. However, the range of lens movement is only a few hundred microns because the camera is an infinite conjugate system and the object is sufficiently far from the camera relative to the focal length. In a unit magnification relay system, the traveling distance of the lens module limits the focal adjustment. This auto-focusing ability is very useful for fine adjustment. However, some situations demand longer focal adjustment ranges. Therefore, the simple screw mechanism in the lens holder can extend the range of the focusing capability of the system without a loss of precision. Other smartphone-based fluorescence microscopes often have more precise mechanics, requiring more space and higher costs[1].

Filters are essential optical components to realize fluorescence microscope functions. The Kodak 47 blue filter used in the device removes red light from the light source. Even when a blue light-emitting diode (LED) or blue laser diode (LD) are used as illumination sources, they emit a small amount of red light owing to the long tail on the spontaneous emission spectrum. This small red light can reduce the contrast of the fluorescence image significantly owing to the weak signal from the

Figure 1. Schematic diagram of a smartphone-based fluorescence microscope.

fluorescent material. The blue filter is placed at the lower body of structure so that the light from the source passes through the filter before entering the sample on the slide glass, as shown in Figure 1. A Kodak 25[9] red filter and a red interference filter (Edmund Optics #52-528)[10] are located at the upper body of the structure after the point at which light passes the sample. The red filter allows light of wavelengths longer than 600 nm to pass. The blue light from the source excites the fluorescent material and converts it to red light. It passes the red filter and reaches the image sensor in the smartphone. The blue light that does not interact with the fluorescent material is removed by the red filters. Otherwise, it acts as noise. The combination of the two types of red filters enhances the extinction ratio, resulting in a higher signal-to-noise ratio.

The overall mechanical structure is very simple and practical. It comprises an upper and lower body. Each unit holds a lens module and an illumination module. A slide glass or a sample stage like a hemocytometer is placed between the two via a hole on the side. The two main bodies are fastened by screws. The smartphone can move over the upper body until its camera is aligned with the external lens module. If the phone has a rubber case, it is easy to maintain sufficient friction after the appropriate position is determined. In this manner, any type of smartphone can fit this mechanical structure. The exploded and assembly views are presented in Figure 2. The white plate between the upper body and the lower body, as shown in Figure 2, is the extra spacer used to accommodate a hemocytometer because it is much thicker than a general slide glass.

We fabricated three illumination sources, white LED, 405 nm blue LED and 445 nm blue LD (Figure 3). The white LED is used for the normal microscope mode and the blue LED and blue LD are for the fluorescence microscope mode. Since the white LED has a wide spectrum including red light, it can cause more noise in the fluorescence mode despite the use of a blue filter. Special care was paid to the fabrication of the blue LD illumination module. The blue LD used in the experiment has a maximum power of 1.0 W with an adjustment knob. The light from the LD enters the plastic fiber through the lens and the fiber delivers the blue light through a small hole at the end part. Since the end part has the same outer diameter as that of LED module, all three illumination sources are interchangeable.

Results and Discussion

Prior to analyzing the fluorescence mode, we tested the performance of the fabricated device as a normal microscope. Hemocytometer images taken by the smartphone microscope are presented with those taken by a conventional microscope. The hemocytometer contained a grid pattern in which the smallest square was 50 μm × 50 μm, as shown in Figure 4-(a). Further, 4 × 4 squares form a bigger square bordered by three lines. The picture in Figure 4-(b) shows both a square grid and its cells for a square of 200 μm × 200 μm. Each square contained approximately 10 cells, which have a round shape.

The initial pictures taken with the smartphone consisted of 3,264 × 2,448 pixels and could be enlarged to increase the viewing magnification. If the image in Figure 4-(a) is magnified using the software, the three lines appear clearer, similar to those shown in Figure 4-(c). Based on the size of the grid, the field of view (FOV) was estimated to be approximately 1.2 mm × 1.2 mm. In fact, the FOV of the system depends on the distance between the external lens and the built-in lens of the smartphone. The thickness of the red filters determines the spacing between the two lens modules. Therefore, the use of the thinner filters can increase the FOV.

For a better characterization of optical performance, we analyzed the resolution of the microscopic images taken using the smartphone. The profiles of the images were extracted using the image processing software

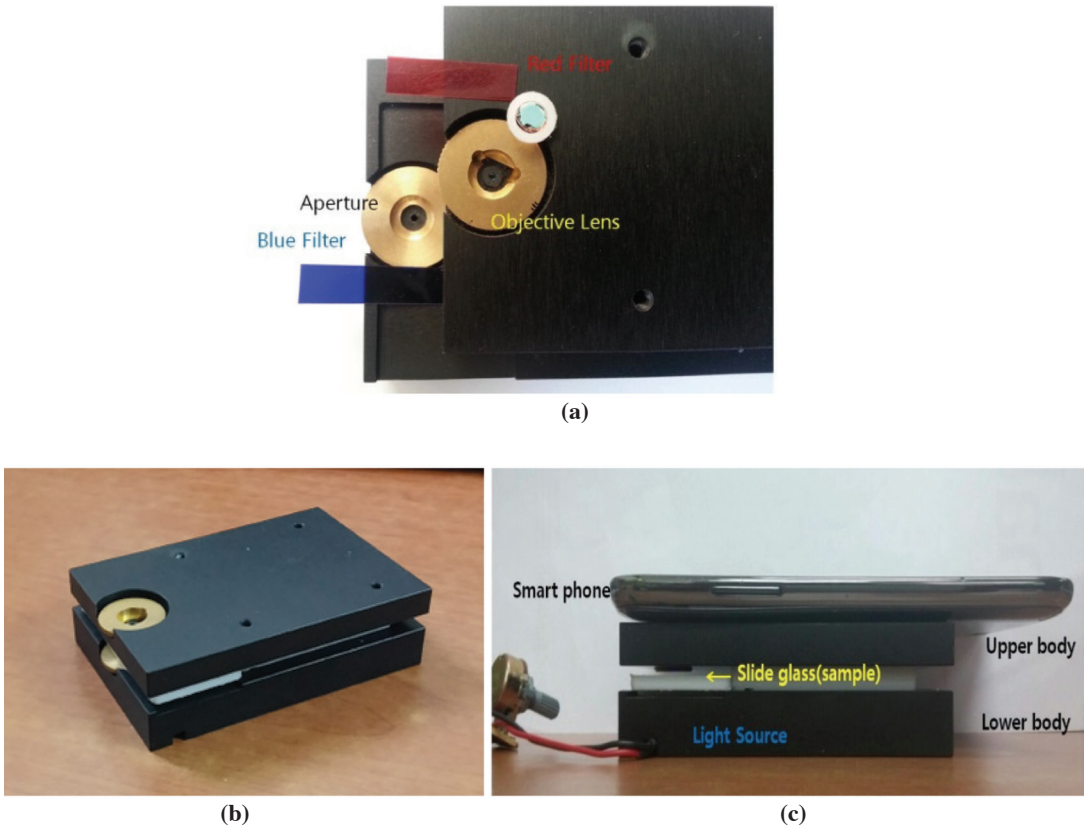


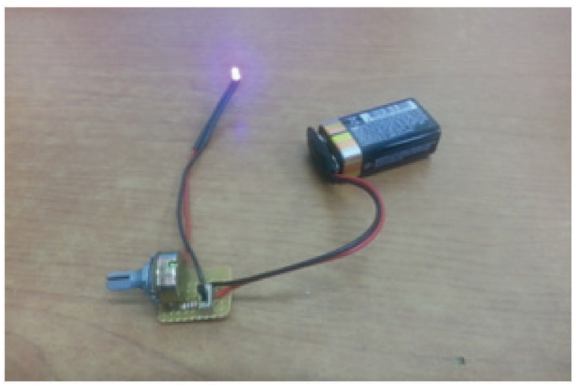
Figure 2. (a) The exploded view, (b) the assembly view without a smartphone, and (c) the assembly view of a smartphone-based fluorescence microscope.

ImageJ[11]. The intensity along the line shown in Figure 5-(a) is plotted as a function of pixel position. Each pixel was 0.38 μm wide because the distance between the two single peaks was 50 μm. The measured width of the peak shown in Figure 5-(b) was 5.5 μm. The original width of the line was 3.0 μm according to the calibration under the conventional microscope. Assuming that the magnification was approximately one, the geometrical image of the line should be approximately 3.0 μm wide. The point spread function of the optical system broadens the image of the line. A simulation based on convolution calculation revealed that the image of the line broadens to 5.5 μm when the resolution is approximately 2.5 μm. Consequently, the three lines were easily distinguishable, as seen in Figure 4 and Figure 5. The center-to-center distance between the three lines was 5 μm and the spacing between lines was 2 μm. Comparing to the reference[1], the resolution seems improved. The test target image taken by the reference system indicates that the contrast is about 0.11 for 1.8 μm line and spacing pattern. The resolution of the reference system can be estimated from the contrast. It is about 3.2 μm which is larger than that of our system.

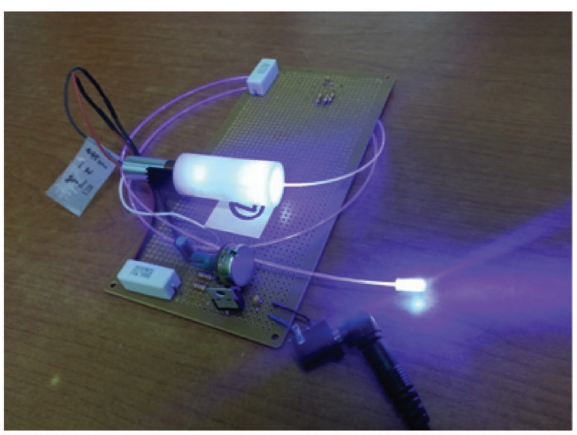
In our initial attempt at fluorescence imaging, we took a picture of fluorescent paint sprayed on a slide glass, as displayed in Figure 6. The fluorescence color was neon, which is suitable for the blue and red filter setup. The first picture, shown in Figure 6-(a), was taken under white LED illumination without a filter. This image taken in normal microscope mode shows the fluorescent particles clearly. Switching from the white LED source to the blue LED source changed the image, as seen in Figure 6-(b) and Figure 6-(c). The images differ with respect to whether the blue filter is absent or not. The overall color is blue since red filters are absent. The use of a red filter with the blue LED source displays the red fluorescence, as seen in Figure 6-(d). However, it suffered from low contrast because the blue LED contains a small amount of red light at the tail of the emission spectrum. This red light from the source acts as noise and degrades the contrast. The use of both blue and red filters increased the contrast of the fluorescent image greatly, as shown in Figure 6-(e).

It was relatively easy to obtain the fluorescence image from the fluorescent spray paint since the fluorescent particle was large and thus the signal was quite

(a)

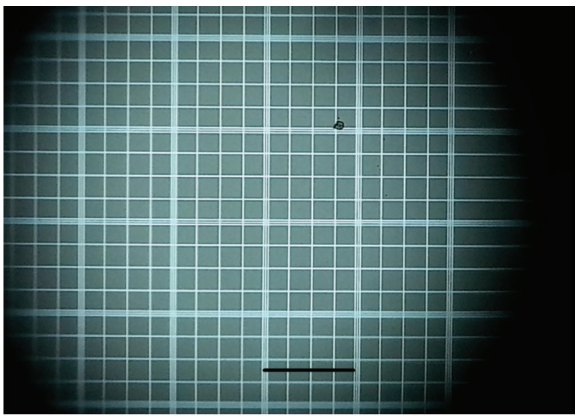


(b)

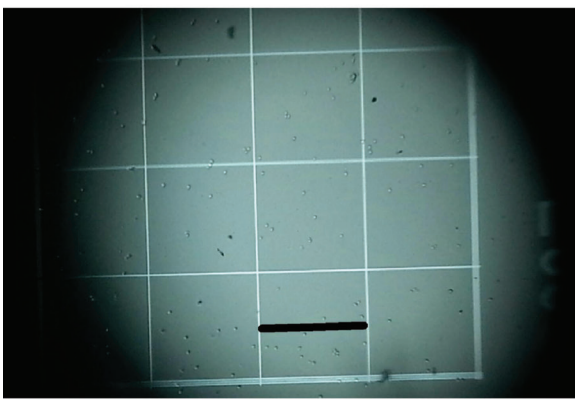


(c)

Figure 3. Three types of illumination light sources: (a) white LED, (b) 405 nm blue LED, and (c) 445 nm blue LD.



(a)



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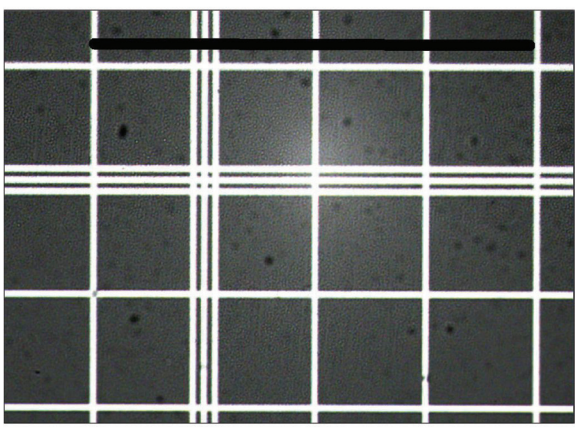


(c)

Figure 4. Pictures of (a) a hemocytometer using a smartphone microscope, (b) the cells on the hemocytometer using a smartphone microscope and (c) a hemocytometer using a conventional microscope. Scale bars indicate 200 μm.

large. We tried to reduce the amount of fluorescent material using a quantum dot solution with a concentration as small as 0.1 mg/mL. Figure 7 shows a clear fluorescence image of quantum dots at this concentration. The imaging was possible due to the increased power of LD illumination, which can increase the power up to 1 W. However, the actual output power of LD was much less than 1 W when the photo was taken. The quantum

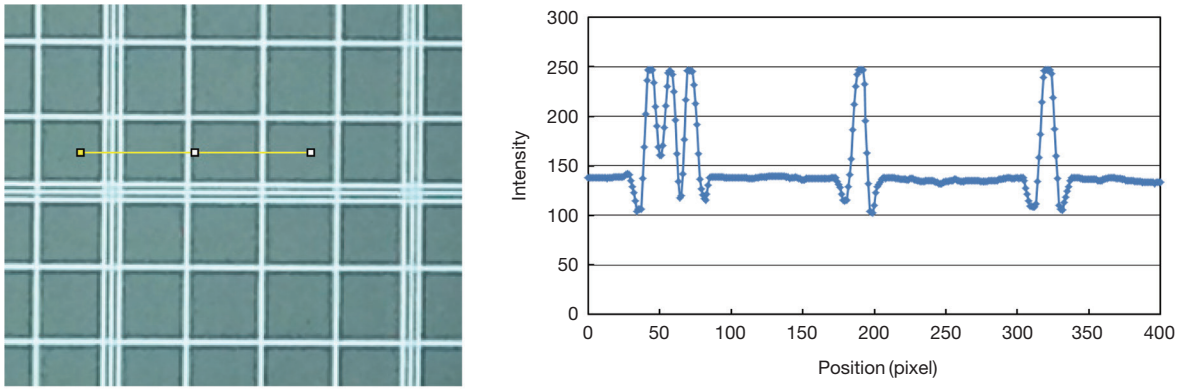


Figure 5. Measurement of resolution based on image analysis. (a) Photos of a hemocytometer under a smartphone microscope. Yellow line is a scanning line. (b) Profile of the image intensity as a function of position along the scanned line shown in (a).

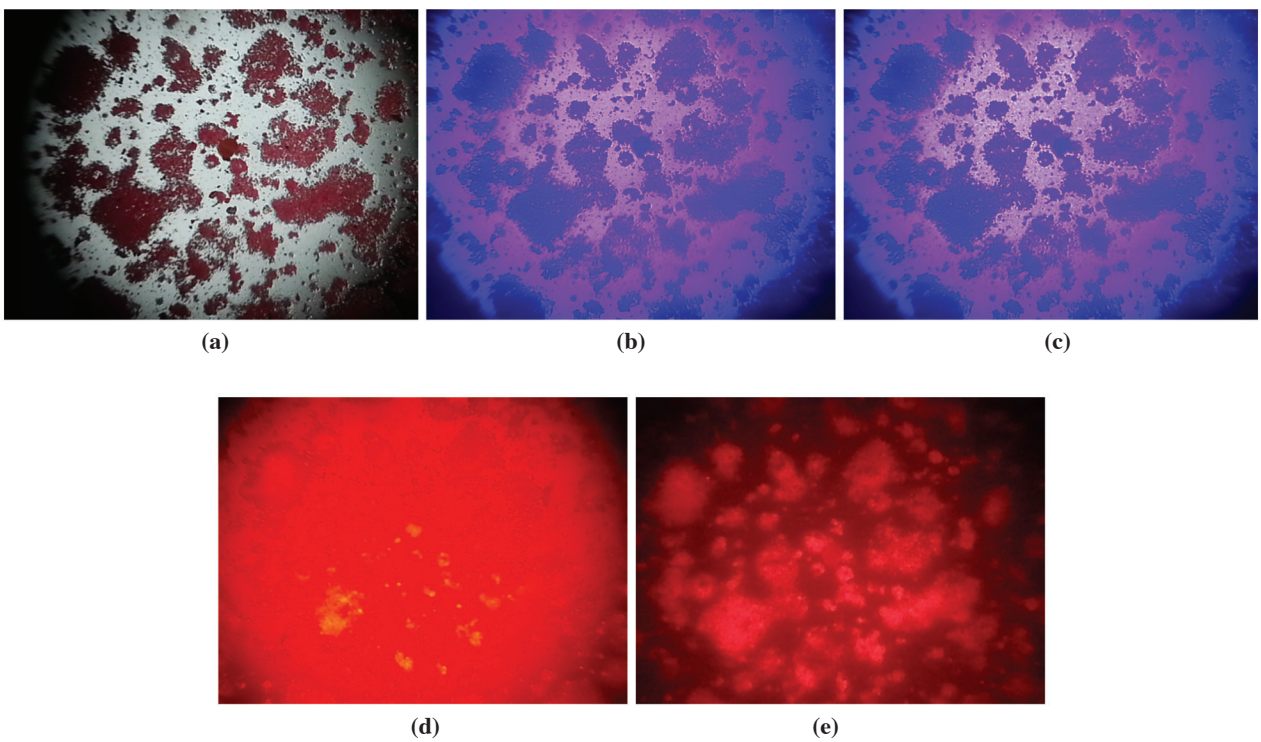


Figure 6. Images of fluorescent paint sprayed on a slide glass taken by a smartphone-based microscope under various illumination conditions: (a) white LED, (b) blue LED, (c) blue LED with blue filter only, (d) blue LED with red filter only, and (e) blue LED with both blue filter and red filter.

dots tend to aggregate and form a cluster, as seen in Figure 7, after the solvent evaporates. It was difficult to photograph a single quantum dot or quantum dots at a low concentration because it was difficult to separate a single quantum dot or disperse them at low concentration.

To test the fluorescence imaging of bio-samples, we prepared cells stained with carboxyfluorescein succinimidyl ester (CFSE). The cells used in this study were mouse spleen cells. Briefly, the cells were harvested from the spleens of C57BL/6 mice by mechanical forces and passed through a cell strainer (70-μm cutoff) to obtain single cells. For CFSE staining, the cells were resuspended in prewarmed phosphate-buffered saline

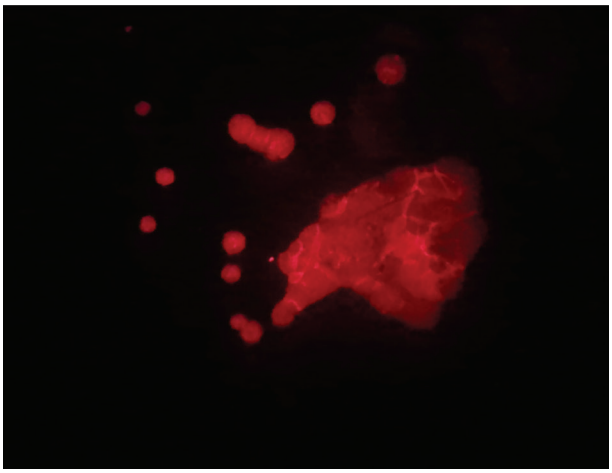
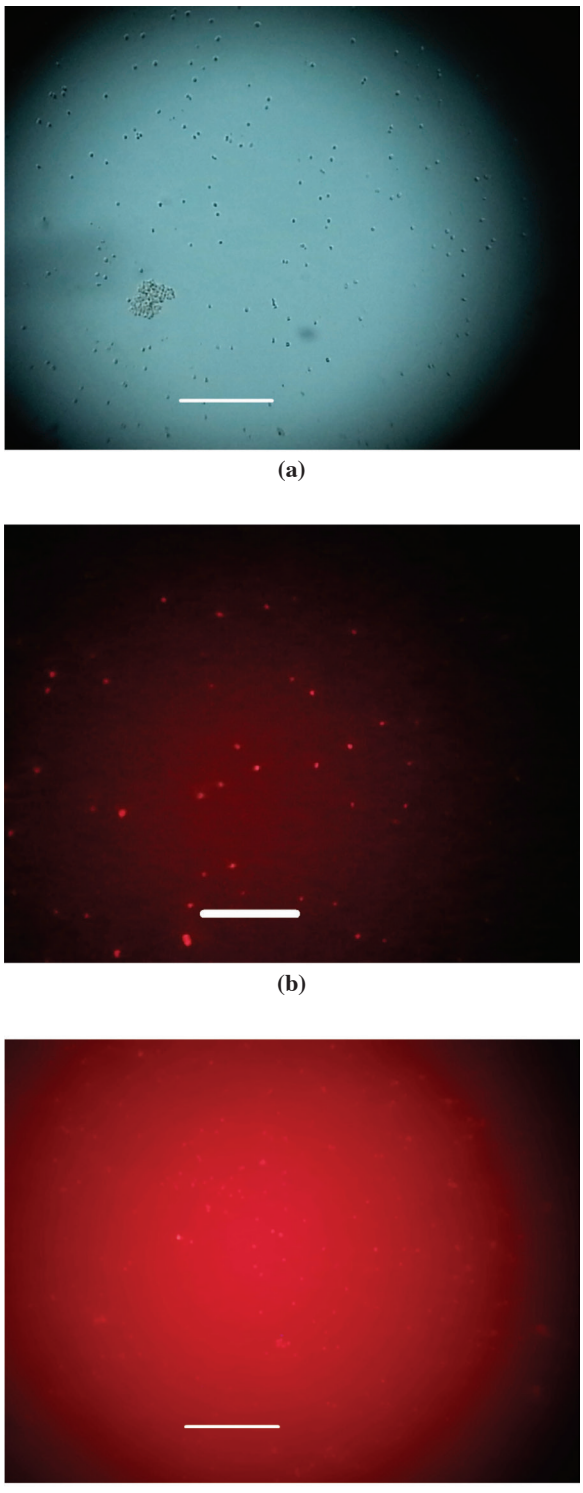


Figure 7. Fluorescence image of quantum dots for a concentration of 0.1 mg/mL. Blue LD excites quantum dots and the emission passes the blue and red filters.

containing 0.1% bovine serum albumin at a concentration of 10 million cells/mL and stained with 1 μL/mL of 5 mM CFSE solution.

Finally, we acquired fluorescence images of the cells stained with CFSE. The CFSE had excitation and emission peaks at 485 nm and 515 nm, respectively. Although these two peaks overlap at around 500 nm, fluorescence imaging was successful, as shown in Figure 8-(b). Since the red filter allowed light of wavelengths longer than 600 nm, the smartphone microscope captures fluorescence from the long wavelength tail of CFSE emissions. The small red dots represent cells stained with CFSE. The difference between the images in Figure 8-(b) and (c) is the spacing between the slide glass and cover glass. In Figure 8-(b), no intentional spacer was used and the spacing may reflect the height of the cell. In Figure 8-(c), a dam was built on the slide glass as high as 100 μm to provide space and prevent possible physical damage to the cells. However, the fluorescence image obtained using larger spacing suffered from lower contrast. This was mainly ascribed to the increased depth of solution, which resulted in increased background fluorescence. The solution may contain small amounts of fluorescence and can cause issues related to increased sample thickness.

For comparison purpose, we present the image of the cells stained with CFSE taken by a conventional fluorescence microscope as shown in Figure 9. The color of the cells seen in Figure 9 was green while the color of the cells seen in Figure 8-(b) is red. CFSE has the emission peak at 520 nm and the emission intensity at 520 nm is about 15 times higher than that at 600 nm. This is why Figure 9 shows much brighter image than



(a)

(b)

(c)

Figure 8. Microscopic images of cells stained with carboxyfluorescein succinimidyl ester (CFSE) fluorescence taken with the smartphone-based fluorescence microscope. The scale bars indicate 200 μm. (a) Conventional microscope mode with white light illumination (b) Fluorescence mode with blue LD illumination (c) Fluorescence mode with 100 μm spacing.

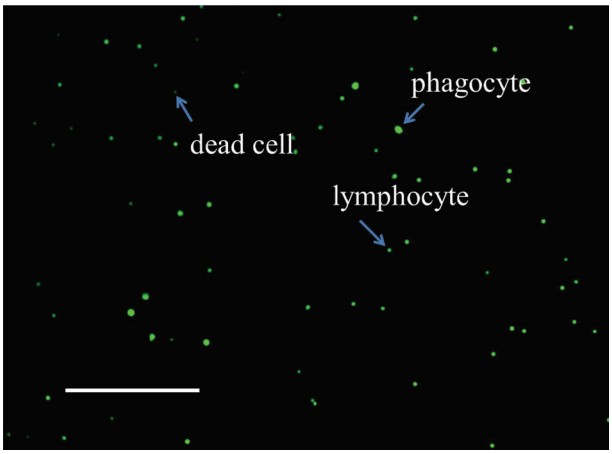


Figure 9. A microscopic image of cells stained with carboxyfluorescein succinimidyl ester (CFSE) fluorescence taken with a conventional fluorescence microscope. The scale bar indicates 200 μm.

Figure 8-(b). As matter of fact, our system was not intended for CFSE in the beginning. The suitable choice of a filter set may improve the intensity of image significantly. The measured sizes of the cells in Figure 9 are similar to those of Figure 8-(b). The size of lymphocyte is about 6 μm and the size of phagocyte is about 11 μm.

Conclusions

We constructed a smartphone-based fluorescence microscope by adding a phone camera lens module to the outside of a smartphone camera. The external lens module forms a unit magnification relay system along with the built-in phone camera. Despite a lack of magnification gain, the system benefits from the small pixel size of the smartphone and other advantages associated with the phone camera lens module such as its low cost, small volume, and low weight. To realize the fluorescence microscope function, the filters and illumination source are integrated into a simple mechanical structure with a focusing adjustment scheme. A combination of red and blue filters enhances the contrast of the fluorescence image when blue LED and blue LD illuminate the sample. The white light LED source also helps to obtain the normal microscope image. Based on an image analysis, the resolution of the microscope was approximately 2.5 μm and the FOV was as large as 1.2 mm × 1.2 mm. The fabricated microscope successfully showed fluorescence images of the fluorescent paint on a slide glass. The LD illumination source was powerful enough to delineate quantum dots formed from a low concentration solution. As a final stage, cells stained with CFSE fluorescence probes were examined. The fluorescence images clearly displayed red dots corresponding to the stained cells. Therefore, a smartphone-based fluorescence microscope can obtain fluorescence images of cells without relying on heavy and expensive equipment. Most importantly, the use of a smartphone ensures that the acquired image data can be transferred immediately to any storage system or any computer reachable by a network. The demonstration of this kind of device supports the feasibility of various applications such as POC diagnostic equipment or a rapid toolkit based on fluorescent methods.

Acknowledgements This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2011-0007648).

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