Time-Lapse Microscopy Using Smartphone With Augmented Reality Markers

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ABSTRACT A prototype system that replaces the conventional time-lapse imaging in microscopic inspection for use with smartphones is presented. Existing time-lapse imaging requires a video data feed between a microscope and a computer that varies depending on the type of image grabber. Even with proper hardware setups, a series of tedious and repetitive tasks is still required to relocate to the region-of-interest (ROI) of the specimens. In order to simplify the system and improve the efficiency of time-lapse imaging tasks, a smartphone-based platform utilizing microscopic augmented reality (μ-AR) markers is proposed. To evaluate the feasibility and efficiency of the proposed system, a user test was designed and performed, measuring the elapse time for a trial of the task starting from the execution of the application software to the completion of restoring and imaging of an ROI saved in advance. The results of the user test showed that the average elapse time was 65.3 ± 15.2 s with 6.86 ± 3.61 μm of position error and 0.08 ± 0.40 degrees of angle error. This indicates that the time-lapse imaging task was accomplished rapidly with a high level of accuracy. Thus, simplification of both the system and the task was achieved via our proposed system. *Microsc. Res. Tech. 77:243–249, 2014.*

INTRODUCTION

With the rapid growth of the smartphone market, smartphones have become widespread, and their cost has been significantly reduced. In spite of their consumer-level price, recent smartphones are equipped with state-of-the-art technologies, such as high-performance processors, sensors, touch-screen displays, and advanced camera systems. Accordingly, many researchers in various fields have noticed their advantages (e.g., cost-effectiveness, portability, and network functionality), and numerous approaches to utilizing smartphones have been studied. In particular, as the advanced imaging systems of smartphones provide high-performance and high-resolution imaging, they are highly suitable for biotechnology research involving microscopy, and various applications for microscopy and microanalysis have emerged. Recent research studies have successfully demonstrated the emerging use of smartphones and its feasibility for practical use. There are considerable examples, including lens-free holographic microscopes (Tseng et al., 2010), label-free photonic crystal biosensors (Gallegos et al., 2013), contact microscopy platforms (Navruz et al., 2013), fluorescent imaging platforms (Wei et al., 2013; Zhu et al., 2011a,b, 2013), dermatologic diagnostic tools (Lee et al., 2013), and tele-dermatopathology systems (Lehman and Gibson, 2013).

In this article, as another application using smartphones, we demonstrate a smartphone-based time-lapse imaging platform that simplifies conventional time-lapse imaging in terms of both the system and the task.

A time-lapse imaging method is generally required to monitor the long-term biological processes of live-cells in various biotechnology research projects (Hinchcliffe, 2005; Stephens and Allan, 2003). This commonly involves a set of consecutive tasks repeated for every specimen: removing the specimen from the incubator, finding and observing the region-of-interest (ROI), and putting the specimen back into the incubator. During these processes, rather than remaining in a strictly controlled incubator, the specimen is repetitively exposed to the external environment. In addition, a considerable amount of time is typically needed to restore the previous ROI of the specimen on a microscopic scale. Given that extended exposure to an uncontrolled environment can have a detrimental effect, minimizing the task time is indeed a crucial issue. In order to address these issues, two types of approaches have been studied. One is to integrate the incubator and the microscope, and the other is to minimize the ROI restoration time by tagging the position information on the microscopic stage.

While various types of integrated systems have been developed, they have all had weaknesses (e.g., large

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Fig. 1. The proposed smartphone-based platform for time-lapsed imaging. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

size, high price). Moreover, the microscope attached to the system could not be used for specimens other than those in the incubator (Burglin, 2000; LeSage and Kron, 2002). Instead of this approach, others have tried tagging the ROI information on the microscopic stage so that the movement of the stages could be recorded to restore a previous ROI and reduce searching time. Devices involved in these approaches included linear position encoders (Brugal et al., 1992), dial indicators (Kaplan et al., 2001), and optical mice (Ng, 2003; Ng and Cheong, 2004). However, for these solutions, additional equipment was required, and there was the danger of losing recorded position data if the device was dislocated. On the other hand, others have suggested engraving the roof of a microfluidic chamber so that the relative position of the stage to the chamber could be indexed (Hanson et al., 2011), which, unfortunately, had a rather low level of accuracy. Meanwhile, our previous research (Yun et al., 2013) was inspired by the effect of out-focusing, and it involved the design of a solution in which a film printed with a microscopic augmented reality (μ-AR) marker array was attached to the bottom of the specimen. By controlling the focal length at different levels, the specimen or an information indicator, the μ-AR marker, emerged accordingly. With this approach, which is both simple and inexpensive, an accurate and rapid ROI-restoring solution was realized.

Considering these previous studies, as mentioned above, we here suggest an interface utilizing

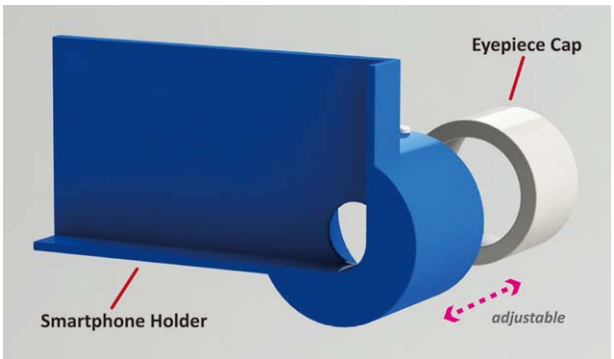

Fig. 2. A prototype of the mounting adaptor. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

smartphones to simplify the time-lapse imaging task and provide user convenience. As our previous work was based on computer vision and augmented reality technology, which are easy and appropriate to implement on smartphones, we again utilized μ-AR printed films. Hence, using a smartphone, we present an integrated application featuring the integration of ROI restoration, time-lapse imaging, and the managing of acquired images to maximize the level of user convenience. The important features of the proposed smartphone platform and application are summarized as follows:

System simplification: Replacing desktop computers and charged-coupled devices (CCDs), smartphone-based systems provide cost-saving and space-saving solutions with convenient installation. While desktop computer-CCD systems can usually only go around one microscope, smartphones with our simple mounting adaptor can go around multiple microscopes when needed. The mounting adaptor allows the smartphone to be placed on an eyepiece, where its camera captures images through one side of the eyepiece. Accordingly, it is unnecessary for the microscope and the smartphone to be modified to unite them.

Task simplification: The integrated camera functions of smartphones such as auto-focusing and auto-white balancing liberates users from the delicate manual controls of focus knob and the post-processing of acquired images. In particular, the auto-focusing function provides an effective and practical solution to the focus-drift problem commonly found in time-lapse imaging, even though, admittedly, it would not have the level of performance that is provided by the highest-priced systems. Imaging tasks are simplified into two steps: (1) Utilize μ-AR makers to find ROIs, and then (2) use the fully automated smartphone camera to save the observation result. The task is also simplified by a user interface created to manage several specimens and ROIs for the convenience of managing images.

High-resolution image acquisition: Most existing CCD components have a low resolution of 1–2 megapixels and come at a high price, whereas smartphones typically have a high resolution of 8 megapixels while also providing high-resolution imaging.

Network functionality: Utilizing online connectivity such as a 3G/LTE network, the application can be

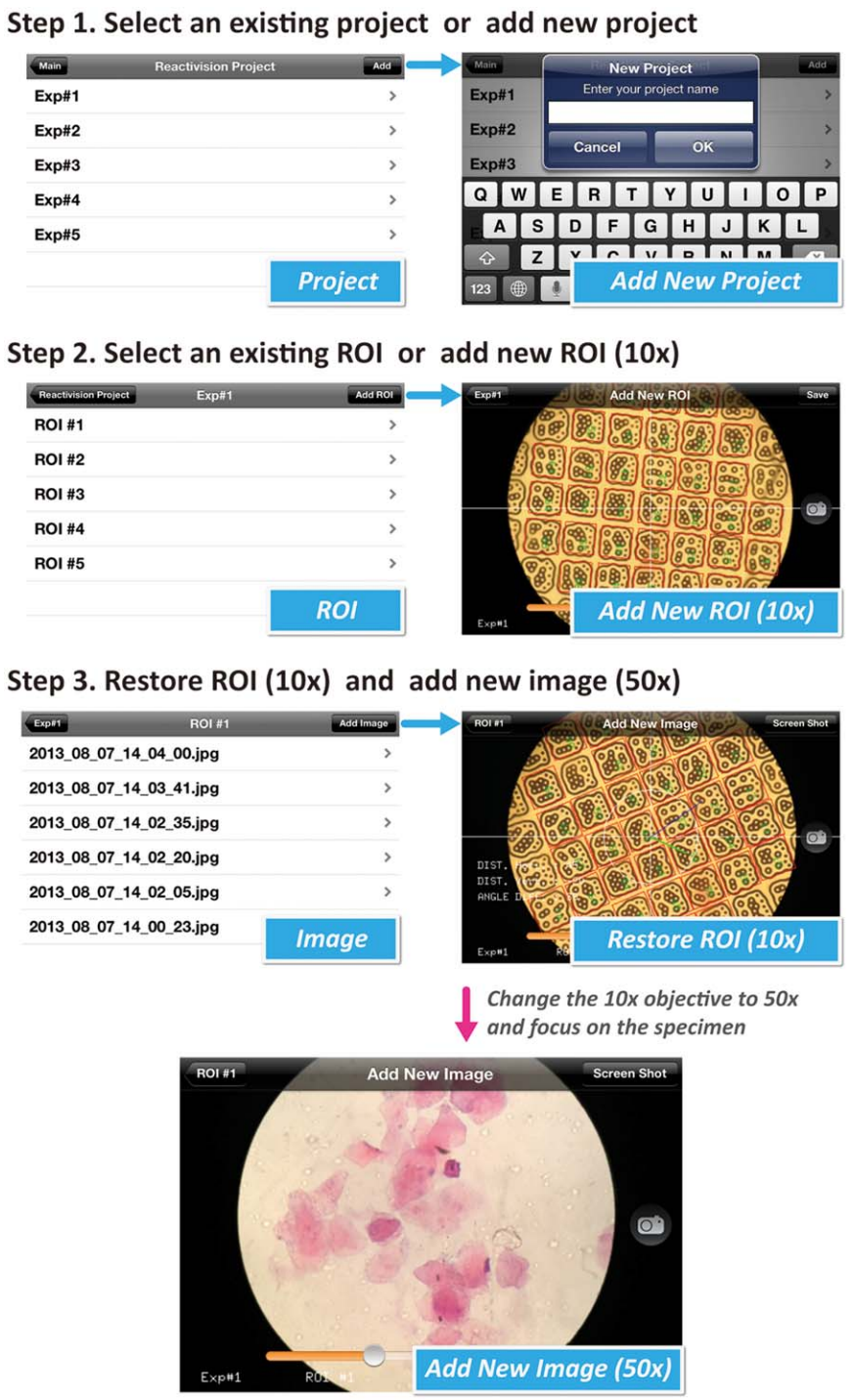

Fig. 3. The workflow of the application software. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

connected to a cloud media storage service. The function that uploads images of ROIs to the cloud image storage service allows the user to store, manage, and share images online. It can also gather and merge observation results from multiple or off-site microscopes, showing its feasibility for tele-diagnosis (Lehman and Gibson, 2013) or other applications such as crowd-sourced biomedical image analysis (Mavandadi et al., 2012).

Due to these features, a time-lapse imaging method using a smartphone makes microscopic experiments simpler and more flexible. In the following sections, the specific implementation of the smartphone platform for time-lapse imaging is described. In order to

evaluate the efficiency of the proposed system, a user test was designed and conducted, the details and results of which are presented below.

MATERIALS AND METHODS

The proposed smartphone-based platform is shown in Figure 1. It consists of a smartphone, an optical microscope, an adaptor for mounting the smartphone on a microscope, and a film printed with a μ-AR marker array providing the position coordinates of ROIs. As described above, the method developed in our previous work (Yun et al., 2013) to position the ROI was adopted in order to maintain its advantages. A prototype was implemented as follows.

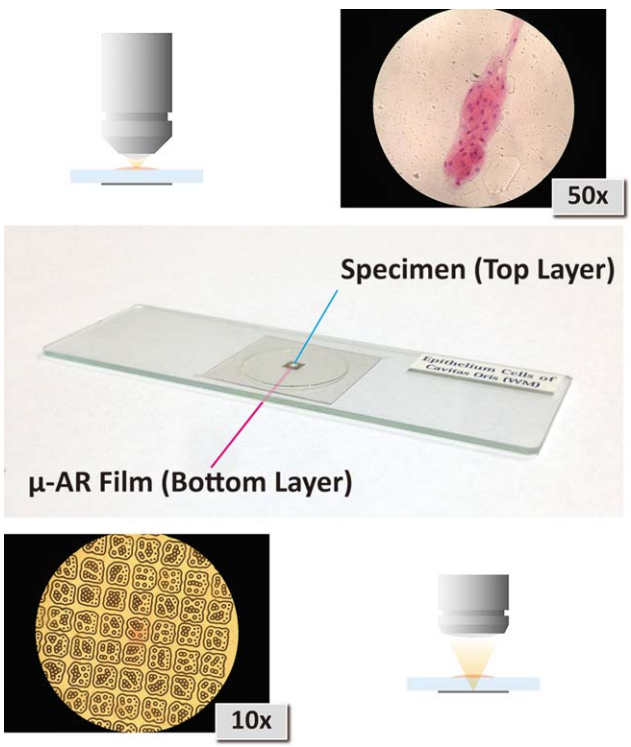

Fig. 4. Method of positioning ROI using film printed with μ-AR marker arrays (Yun et al., 2013). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Hardware

For the prototype platform, the iPhone 4S (Apple, USA) was used as the smartphone. Because its embedded camera has a resolution of 8 megapixels (3264×2448 pixels) and supports auto-focus and auto-white balance functions, it can acquire high-resolution images. For the optical microscope, an Axiolab A (Carl Zeiss, Germany) device was used equipped with objective lenses (Epiplan, ×5, ×10, ×20, ×50, and ×100; Carl Zeiss, Germany) and eyepiece lenses (E-PI ×10/20; Carl Zeiss, Germany).

To use the smartphone camera as a substitute for the existing CCD component, a means of mounting the smartphone onto the microscope was required. As a solution, a mounting adaptor that fit the eyepiece of the microscope was designed. Figure 2 shows a prototype design of the mounting adaptor. Considering the eye relief of the microscope, a proper distance from the final surface of the eyepiece lens to the smartphone camera sensor needed to be ensured to obtain a full field-of-view. Hence, the adaptor included an eyepiece cap to change the distance, as shown in Figure 2.

Application Software

The implemented software included functions for saving and restoring ROIs, imaging a restored ROI, and managing the captured images. The overall workflow of the software is shown in Figure 3.

For saving and restoring ROIs, the method developed in our previous work (Yun et al., 2013) was used. The specific method is illustrated in Figure 4. The center of Figure 4 shows that a film printed with a μ-AR marker array is located at the bottom of the specimen slide. The bottom and top of Figure 4 show the focal layer located on the film and the specimen layer, respectively. At the specimen layer, the initial ROI selection, observation, and imaging of the specimen are performed. At the film layer, the initially selected ROI is saved and restored.

Figure 5 shows a screenshot of the restoration of an ROI on the proposed smartphone platform. To increase the level of user convenience during the task of restoring

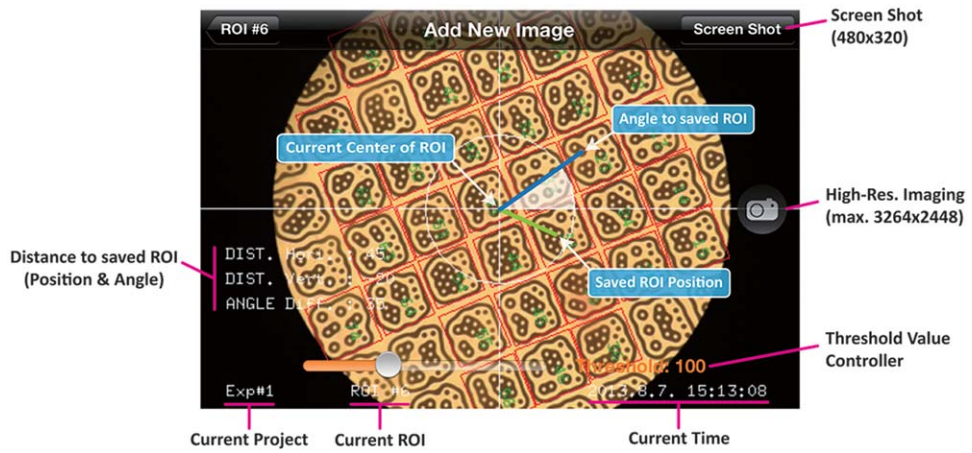

Fig. 5. Graphical user interface (GUI) for restoring and imaging ROI. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

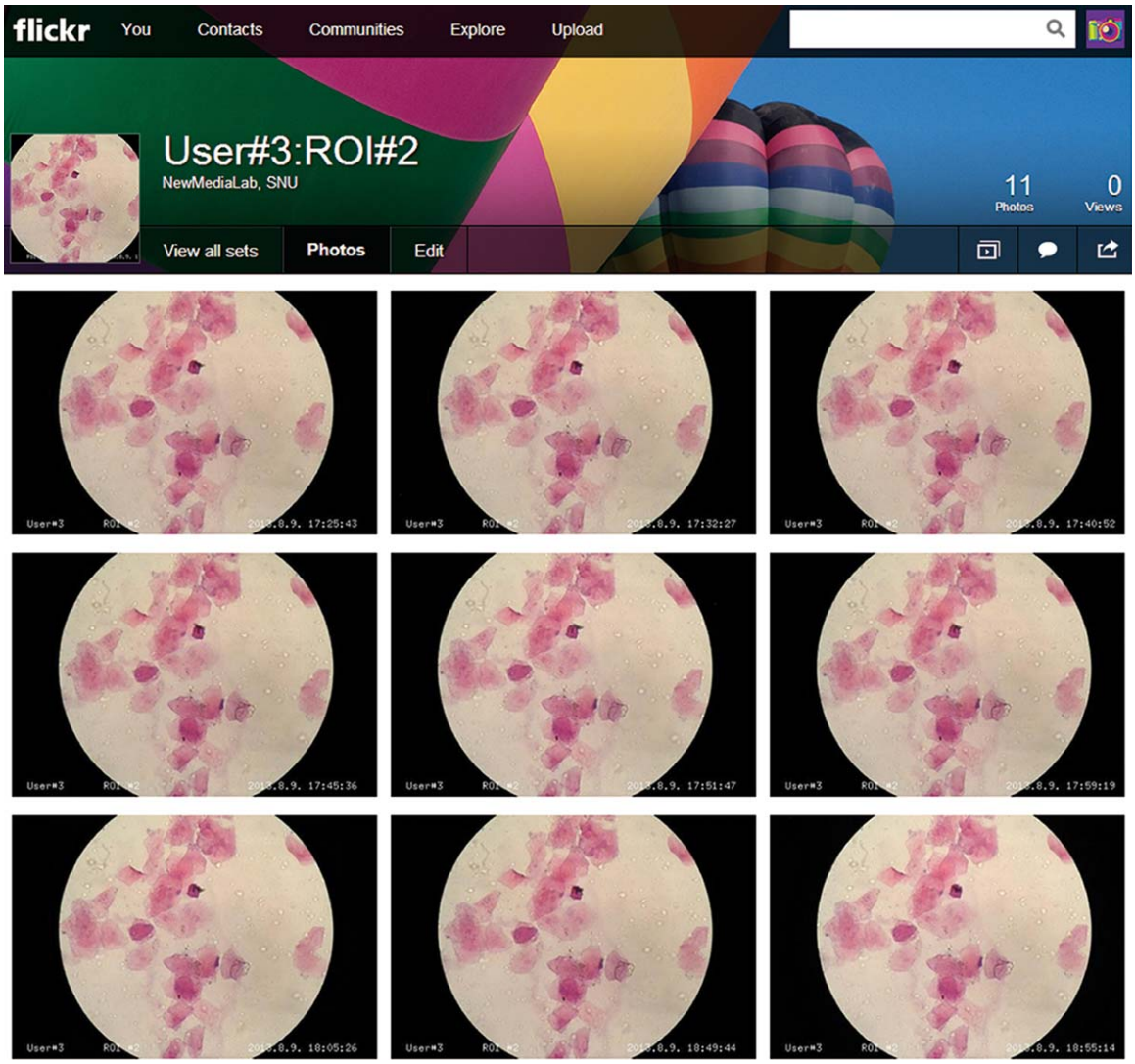

Fig. 6. An example of the images uploaded on *Flickr* cloud image storage service. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

an ROI, guidelines are displayed on the screen. The green line corresponds to the vector from the center of the current view to the saved position, and the blue line shows the amount of rotation from a saved angle in the counterclockwise direction. Extra information such as the name of the current project and ROI and a timestamp are also displayed. Occasionally, detection failures of the μ-AR markers may occur due to light or noise during the detection process. In most cases, controlling the threshold value using the slider at the bottom of the screen helps to resolve such detection problems. Imaging is done in two ways: by taking a low-resolution (480×320 pixels) image or taking a high-resolution (3264×2448 pixels) image. The first of these two methods is executed by touching the screen-capture button at the top right side of the screen and the second is done by touching the camera icon at the middle right side of the screen.

As time-lapse imaging usually involves numerous images in a time sequence, an efficient image-management scheme is important. The acquired images are saved in local memory with a filename that has the form of "*year_month_day_hour_minute_second.jpg*," and these files are automatically sorted in a time sequence. At the same time, they are also uploaded to the cloud media storage service. Recently, as various cloud media storage services have become available and easily accessible via the network functionality of smartphones, it has become highly convenient to store, manage, and share various types of media. In our prototype, the service offered by *Flickr*, a well-known media storage service, was used. An example of the images uploaded is shown in Figure 6.

RESULTS

As described above, minimizing the time of exposure to an uncontrolled environment is the most significant issue during the time-lapse imaging procedure. Thus, a user test was designed in order to evaluate the time efficiency of the proposed system. The test measured the elapse time for a trial of the task starting from the

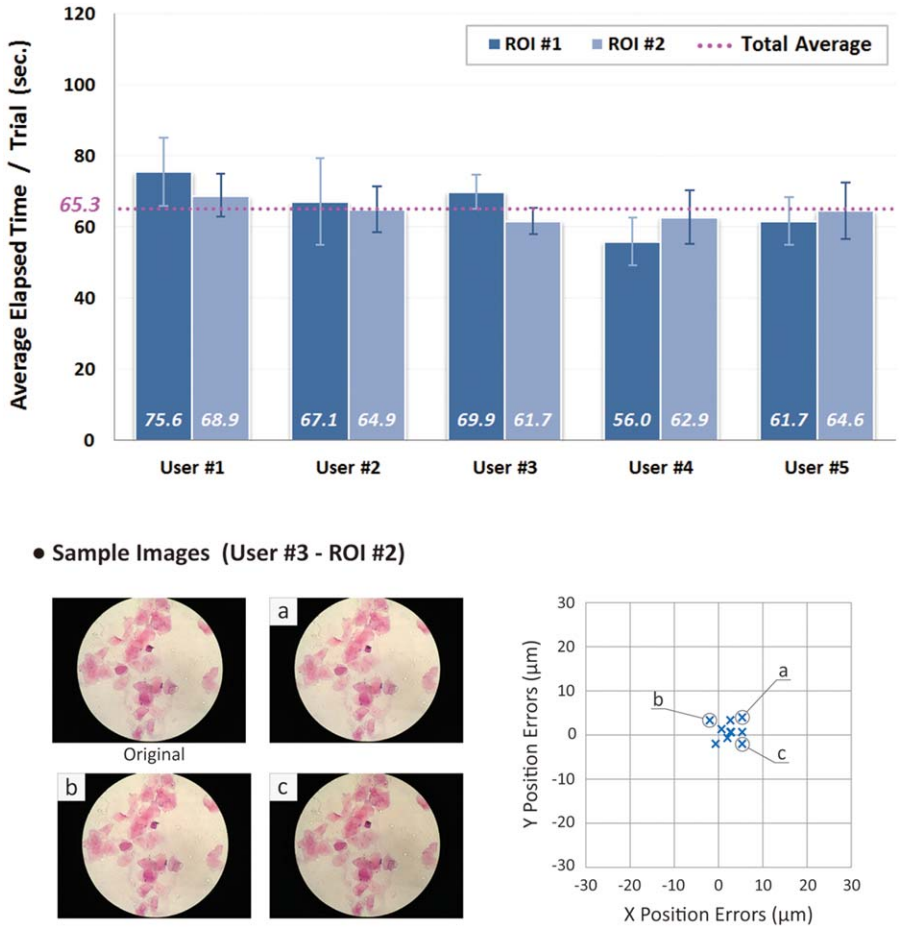

Fig. 7. The results of user test: average elapse time per a trial for each subject and ROIs (top) and sample images and its restoration accuracy from one subject (bottom: (**a**) 3rd trial, (**b**) 4th trial, (**c**) 8th trial). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

execution of the application software and finishing when imaging was complete. The specific procedure is described below.

Before testing, two different ROIs were saved in advance. At the beginning of a trial, a specimen was positioned at an arbitrary location on the microscopic stage. Subjects were requested to restore an ROI by manipulating the microscope with the assistance of our software and to capture an image of the specimen when the restoration step was complete. The restoring ROI step and imaging step were performed with the ×10 and the ×50 objective lenses, respectively. Instead of using live-cells, a permanent preparation slide of *epithelium cells of a human cavitas oris* was used as a specimen for convenience, because the aim of the test was not affected by the type of specimen. In addition, this made it possible to analyze the restoration accuracy from the resulting images more precisely, because there were no cell deformations or displacement, unlike in live-cells.

Five subjects—both of whom were skilled in operating an optical microscope and had knowledge of time-lapse imaging—were chosen to participate in the test. Specifically, they were requested to perform the tasks as rapidly as possible, as the accuracy of restoring an ROI using the μ-AR film had been verified in our previous work (Yun et al., 2013). For each of two different ROIs, 10 trials were performed, repeatedly. The captured image was automatically uploaded to the *Flickr* service immediately after the imaging process[1]. All of the images captured during the tests are available from our *Flickr* service account. Considering the uploading speed, we set the low-resolution images (about 100–200 kb) to be uploaded only for demonstration purposes, whereas the high-resolution images (about 3–4 MB) were saved only into local memory.

The results of the test are shown in Figure 7, which presents the average elapse time and the captured image sequence per subject. From the results, the average elapse time per trial was 65.3 ± 15.2 s with 6.86 ± 3.61 μm of position error and 0.08 ± 0.40 degrees of angle error. This indicates that the time-lapse imaging task was accomplished rapidly enough and with a high level of accuracy with our smartphone platform. Hence, it can replace the existing desktop platform in terms of both task efficiency and accuracy. Furthermore, as it includes all the advantages listed at the beginning of this article, it can be said that the

[1]All images during the user test are available on our Flickr account website: http://www.flickr.com/photos/nml_project/sets/

proposed method offers a practical and inexpensive solution.

CONCLUSIONS

This article presented a method that uses the widely popular smartphone as a microscopy platform for time-lapse imaging. In order to simplify the system and improve the efficiency of time-lapse imaging tasks, a mounting adaptor to hold a smartphone on a microscope was designed, and a smartphone application that integrates rapid ROI restoration utilizing μ-AR markers, time-lapse imaging, and convenient image management was created. To evaluate the feasibility and efficiency of the proposed system, a user test was designed and performed. The results of the user test showed that the time-lapse imaging task was accomplished rapidly with a high level of accuracy. Thus, both the system and the task were simplified via our proposed system.

REFERENCES

Brugal G, Dye R, Krief B, Chassery JM, Tanke H, Tucker JH. 1992. HOME: Highly optimized microscope environment. Cytometry 13: 109–116.

Burglin TR. 2000. A two-channel four-dimensional image recording and viewing system with automatic drift correction. J Microsc 200 (Part 1):75–80.

Gallegos D, Long KD, Yu H, Clark PP, Lin Y, George S, Nath P, Cunningham BT. 2013. Label-free biodetection using a smartphone. Lab Chip 13:2124–2132.

Hanson L, Cui L, Xie C, Cui B. 2011. A microfluidic positioning chamber for long term live cell imaging. Microsc Res Technol 74:496–501.

Hinchcliffe EH. 2005. Using long-term time-lapse imaging of mammalian cell cycle progression for laboratory instruction and analysis. Cell Biol Educ 4:284–290.

Kaplan S, Canan S, Aslan H, Unal B. 2001. A simple technique to measure the movements of the microscope stage along the *x* and *y* axes for stereological methods. J Microsc 203:321–325.

Lee KC, Cossman JP, Muglia J. 2013. Using smartphone cameras to photograph microscopic dermatologic images. Skin Res Technol 0: 1–2.

Lehman JS, Gibson LE. 2013. Smart teledermatopathology: A feasibility study of novel, high-value, portable, widely accessible and intuitive telepathology methods using handheld electronic devices. J Cutan Pathol 40:513–518.

LeSage AJ, Kron SJ. 2002. Design and implementation of algorithms for focus automation in digital imaging time-lapse microscopy. Cytometry 49:159–169.

Mavandadi S, Dimitrov S, Feng S, Yu F, Sikora U, Yaglidere O, Padmanabhan S, Nielsen K, Ozcan A. 2012. Distributed medical image analysis and diagnosis through crowd-sourced games: A Malaria case study. PLoS One 7:e37245.

Navruz I, Coskun AF, Wong J, Mohammad S, Tseng D, Nagi R, Phillips S, Ozcan A. 2013. Smart-phone based computational microscopy using multi-frame contact imaging on a fiber-optic array. Lab Chip 13:4015–4023.

Ng TW. 2003. The optical mouse as a two-dimensional displacement sensor. Sens Actuators A 107:21–25.

Ng TW, Cheong TL. 2004. The optical mouse as an inexpensive region-of-interest position recorder in optical microscopy. Microsc Res Technol 63:203–205.

Stephens DJ, Allan VJ. 2003. Light microscopy techniques for live cell imaging. Science 300:82–86.

Tseng D, Mudanyali O, Oztoprak C, Isikman SO, Sencan I, Yaglidere O, Ozcan A. 2010. Lensfree microscopy on a cell-phone. Lap Chip 10:1787–1792.

Wei Q, Qi H, Luo W, Tseng D, Ki SJ, Wan Z, Göröcs Z, Bentolila LA, Wu TT, Sun R, Ozcan A. 2013. Fluorescent imaging of single nanoparticles and viruses on a smart-phone. ACS Nano 7:9147–9155.

Yun K, Chung J, Park Y, Lee B, Lee WG, Bang H. 2013. Microscopic augmented-reality indicators for long-term live cell time-lapse imaging. Analyst 138:3196–3200.

Zhu H, Mavandadi S, Coskun AF, Yaglidere O, Ozcan A. 2011a. Optofluidic fluorescent imaging cytometry on a cell-phone. Anal Chem 83:6641–6647.

Zhu H, Yaglidere O, Su TW, Tseng D, Ozcan A. 2011b. Cost-effective and compact wide-field fluorescent imaging on a cell-phone. Lab Chip 11:315–322.

Zhu H, Sencan I, Wong J, Dimitrov S, Tseng D, Nagashima K, Ozcan A. 2013. Cost-effective and rapid blood analysis on a cell-phone. Lap Chip 13:1282–1288.