LOW-COST, NEAR-INFRARED, SCAN-LESS PORTABLE CONFOCAL MICROSCOPE

by

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ABSTRACT

Reflectance confocal microscope (RCM) is a diagnostic tool for various skin diseases, but the application of that is hampered by its high cost. In this thesis, we developed two versions of the low-cost, near-infrared scan-less portable confocal microscope. 1) High-speed RCM. We have used 840 nm central wavelength superluminescent LED as the light source. A confocal detection optics has been developed to maintain high lateral resolution even when a relatively large slit width was used. The material cost of the NIR RCM device was low as $5,200. The lateral resolution was 1.1 μm and 1.3 μm, along with the vertical and horizontal directions, respectively. Axial resolution was measured as 11.2 μm. In vivo confocal images of human forearm skin obtained at the imaging speed of 203 frames/sec clearly visualized characteristic epidermal and dermal cellular features of the human skin.

2) Speckle-free RCM. We have developed a portable confocal microscope (PCM) that uses a near-infrared (NIR) LED as the light source, and the speckle noise on the image was reduced due to the used of the spatially incoherent light source. The material cost of it is still low as ~$5,000 and weighed 1kg. The lateral resolution was measured as 1.6 μm, and axial resolution as 6.0 μm. The PCM device could visualize characteristic cellular features of human skin in vivo in a range from the stratum corneum to the superficial dermis. This capability is expected to facilitate the evaluation and clinical adoption of this low-cost diagnostic imaging tool. Dynamic imaging of blood flow in vivo was also demonstrated.
1. INTRODUCTION

The first confocal microscope was invented by Marvin Minsky early in 1955 [1]. Later, the confocal microscope was developed more and more robust; nowadays, the confocal microscope is a powerful tool for imaging different types of tissue[2,3].

A conventional confocal microscope typically uses a laser source as illumination, and it images a small voxel on the sample (tissue). The laser point scanning along two dimensions on the tissue, and an illuminated section of a plane is generated. A pinhole aperture is placed in front of the detector and focusing optics, where the out-of-focus light is filtered out. The position of the pinhole aperture is placed conjugate with the focal point on the tissue so that only the in-focus light can path through the pinhole. By changing the focal plane at different imaging depths, a 3D volume of image could be generated. The confocal microscope allows clinicians a view of the non-invasive way for human skin imaging with high resolution (Fig. 1.)

![Schematic of the RCM](image)

Figure 1. Schematic of the RCM
Considering the high cost of the confocal microscope, we had previously developed a smartphone-based confocal microscope. The material cost is only $4,200. It used a specially encoded method to generate a non-scan field of view, a detection slit aperture to generate a confocal situation[4]. This device was used in Uganda for skin cancer diagnosis.

In this thesis, we will present two versions of the low-cost portable confocal microscope to solve the challenges that the smartphone confocal microscope has. In chapter 2, the development of the high-speed (203 fps) near-infrared confocal microscope is presented. In chapter 3, the development of the speckle-free portable confocal microscope is demonstrated. Both of those two devices keep the advantage of low-cost, which is around $5,000.
2. LOW-COST, HIGH-SPEED NEAR INFRARED REFLECTANCE CONFOCAL MICROSCOPE

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2.1 Introduction

Skin biopsy histopathologic evaluation is the standard method for making a diagnostic assessment of most dermatological conditions. However, diagnosis often relies on clinical examination alone in remote and/or resource-scarce sites, which can lead to incorrect or delayed diagnosis [5,6] and inadequate treatment. Recently, smartphone-based microscopy devices have been developed with a goal of providing microscopy images at the point of care and subsequently improving the disease diagnosis in low-resource or distant settings. However, most of the smartphone-based microscopy devices are tailored for imaging excised and thinly-sectioned samples [7–11]. The sample acquisition and slide preparation remains challenging in these settings due to the lack of required equipment and trained personnel.

Reflectance confocal microscopy (RCM) is an in vivo microscopy technology that can examine cellular features of the skin without having to invasively sample the suspicious lesions [12]. RCM has been evaluated for the diagnosis of various skin diseases and shown to provide high diagnostic accuracy for major skin cancers in developed countries [13,14]. Recently, RCM has been also tested for imaging skin diseases prevalent in low-resource
settings such as Kaposi’s Sarcoma and Xeroderma Pigmentosum [15,16]. Clinical adaptation of RCM in low-resource or remote settings, however, has not been realized yet due mainly to the relatively high cost associated with the device.

We had previously developed a low-cost, smartphone-based confocal microscope and demonstrated human skin imaging in vivo [17]. The smartphone confocal microscope utilized slit confocal apertures, a broadband LED, and diffraction gratings to image multiple lines of the tissue simultaneously with each line associated with a distinctive wavelength [18]. As a result, two-dimensional confocal images were obtained without using any beam scanning devices and the confocal microscope was built at low cost (material cost = $4,200). While the smartphone confocal microscope successfully visualized cellular features of the skin, there were remaining technological challenges. Use of a relatively short wavelength (central wavelength = 590 nm) made it challenging to clearly image cellular features in the dermis. The color filter on the smartphone CMOS sensor inherently reduced the light collection efficiency, which in part limited the imaging speed to 4.3 frames/sec. The suboptimal imaging speed could lead to motion artifacts during in vivo skin imaging.

In this paper, we report the development of a low-cost, near-infrared (NIR) confocal microscope to address the aforementioned challenges. A new confocal detection optics is described and theoretical resolution simulation reported. Preliminary in vivo confocal images of the human forearm skin are presented.

### 2.2 Materials and Methods

#### 2.2.1 Low-cost near-infrared reflectance confocal microscope
The schematic of the low-cost confocal microscope is shown in Fig. 2.1. Light from a super-luminescent light emitting diode (sLED; EXS210040-01, Exalos; central wavelength = 840 nm; bandwidth = 50 nm; power = 10.5 mW; working current = 150 mA) was collimated by an aspheric singlet (f = 8 mm). The collimated light was then diffracted by a diffraction grating (grating 1; groove density = 1764.7 lpmm), focused by a cylindrical lens (focal length = 30 mm) and an objective lens (CFI60 Apochromat 40x, Nikon; NA = 0.8; water immersion) on the tissue. Use of the cylindrical lens generated a focused line on the tissue for each wavelength. A square aperture with the width of 2.8 mm and offset of 2.6 mm from the objective lens optical axis was used between the grating 1 and cylindrical lens, which resulted in the effective illumination NA of 0.28 and chief ray angle on the tissue of 21.4°.

Figure 2.1. Schematic of the low-cost NIR RCM device.
Reflected light from the tissue was collected by the objective lens and collimated. A circular aperture with a diameter of 3.5 mm and offset of 2.25 mm from the objective lens optical axis was used near the proximal end of the objective lens. The circular aperture made the effective detection aperture 0.35 and chief ray angle 18.7°. Light after the circular aperture was diffracted by another grating (grating 2; 1764.7 lp/mm) and focused by a focusing lens (f = 30 mm). A detection slit with the width of 50 µm and length of 3 mm was positioned at the focal plane of the focusing lens. After the detection slit, the light was collimated by the collimation lens (f = 30 mm), diffracted by the grating 3 (1800 lp/mm), and focused on the CMOS sensor (1280 × 1024 pixels; pixel size = 4.8 µm). A USB 3.0 cable was used to transfer the data from the CMOS sensor to a laptop (Surface Book Pro, Microsoft).

2.2.2 Confocal detection optics

We have developed a new confocal detection optics that provides better lateral resolution than the detection optics used in our previous smartphone confocal microscope. In RCM, a relatively large detection aperture is often used to reduce the speckle noise [19]. The sLED has a wide spectral bandwidth and therefore has a reduced speckle noise when the entire bandwidth is focused on the same point. In our confocal microscope, however, the effective spectral bandwidth that each pixel is detecting is small, which increases the coherence length and subsequent speckle noise. Therefore, a wide detection slit width is still needed even when the sLED with wide bandwidth is used as the source.
With the detection optics of the previous smartphone confocal microscope [17], an increase of the detection slit width directly degraded the lateral resolution. Comparison between the previous and present detection optics is shown in Fig. 2.2. The light beam color in Fig. 2.2. does not represent the actual light color of the light source but is indicative of the relative wavelength for the given beam path, red being a longer wavelength and blue being a shorter wavelength. The separation between the focused beams on the detection slit in Fig. 2.2. is exaggerated to better demonstrate the difference between the previous and present confocal detection optics. For an example detection slit width of 50 µm, which is also the separation between the blue and red beams, the corresponding spectral bandwidth between the red and blue beams was 0.6 nm.

Illumination optics is identical between the previous and present configurations. In the previous smartphone confocal microscope (Fig. 2.2a) [17], the grating in front of the camera lens (grating 3) was parallel to the grating in the detection path of the confocal optics (grating 2). When the detection light incident on a particular pixel on the CMOS sensor is backtraced to the tissue, the longer wavelength (red rays) passes through the top
edge of the detection slit. The longer wavelength is then incident on the grating 2 with a smaller angle than the central wavelength (green rays), which results in a larger field angle between the grating 2 and objective. This larger angle makes the detection point-spread function (PSF) left-shifted (red detection PSF). Likewise, the shorter wavelength (blue) can be back-traced to a right-shifted detection PSF (blue detection PSF). In summary, the detection pixel is not conjugate to a single point on the tissue but to multiple, laterally-shifted points. Confocal PSF for each pixel can be calculated as the sum of confocal PSF’s for all the wavelengths detected by that pixel. The confocal PSF for each wavelength is calculated by multiplying the illumination PSF with the detection PSF for the given wavelength. Therefore, in the previous detection optics configuration (Fig. 2.2a), the confocal PSF (dotted ellipse) becomes wider along the lateral direction as the detection slit width increases. Since confocal images are usually taken en face, resolution degradation along the lateral dimension deteriorates the image quality more severely than along the axial dimension.

In the present detection optics configuration (Fig. 2.2b), the grating 3 and grating 2 are mirror-symmetric relative to the detection slit. Due to this mirror symmetry, all the wavelengths detected by a particular pixel is back-traced to have the same field angle on the objective lens. Therefore, each pixel is conjugate to a single point on the tissue and produces a detection PSF without any lateral shift (white ellipse). The slit width determines the effective bandwidth each pixel is detecting. The confocal PSF for each pixel can then be calculated by summing the products between the detection PSF (white ellipse) and the illumination PSFs that correspond to the effective bandwidth (e.g., red, green, and blue illumination PSFs in Fig. 2.2b). As shown in Fig. 2.2b, the lateral extent of the confocal
PSF (dotted ellipse) is mainly determined by the lateral extent of the detection PSF and remains small even when the slit width is increased. The slit width increase, however, stretches the axial extent of the confocal PSF and degrades the axial resolution.

1.2.3 Resolution simulation

We have simulated the confocal PSFs for various slit widths for both previous and present detection optics (Fig. 2.3) using a custom Matlab code (Mathworks, Natick, MA). The simulation parameters were set according to the specifications of the components as described in section 2.2.1. The FWHM of the focused spot on the detection slit was 7.3 µm with the given detection beam diameter of 3.5 mm and focusing lens focal length of 30 mm. For the present detection optics, the resolution was calculated both along the lateral and axial directions of the tissue space (FWHMx and FWHMz) and along the minor and major axes of the confocal PSF (FHWMu and FWHMv). With the previous detection optics, the lateral resolution degraded significantly from 2.0 µm to 8.52 µm as the slit width increased from 10 µm to 50 µm, while the axial resolution did not change much. With the present detection optics, the lateral resolution (FWHMu) was maintained high, around 1 µm, while the axial resolution was increased significantly from 3.40 µm to 11.26 µm. While smaller axial FHWM is desirable for confocal microscopy, axial resolution of 10-15 µm was shown useable to visualize cellular features of human tissues [20,21] as long as the lateral FHWM is maintained small.
2.2.4 Imaging performance test

Lateral resolution of the low-cost NIR confocal microscope was measured by imaging a USAF resolution target. FWHM of the line spread function (LSF) was calculated along the spectrally-encoded and slit-length directions. Axial resolution was measured by translating a mirror along the objective lens optical axis with a motorized stage and calculating the FWHM of the axial response curve. The source power was attenuated during the resolution measurement to ensure that the pixel values were not saturated.

Tissue imaging performance was evaluated by imaging human forearm *in vivo* at different imaging depth levels. The forearm skin surface was placed parallel to the focal plane of the objective lens. Ultrasound gel with a similar refractive index to that of water was applied between the forearm and objective lens. The exposure time was set at 4.8 msec and the resulting frame rate was 203 fps. The microscope was translated relative to the forearm using a motorized stage. The motor speed was set to 1 mm/sec and the scan range 500 µm. The maximum acceleration of the motor was 4 mm/sec², which produced the

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Figure 2.3. Simulated PSFs of the previous and current confocal detection optics for a range of slit widths and lateral and axial FWHMs of PSFs. All FWHM values are shown in µm.
acceleration time of 0.25 sec and deceleration time 0.25 sec. At the center of the axial scanning, the uniform speed of 1 mm/sec was maintained over 250 µm range. Within the uniform speed region, the axial step size between frames was 5 µm. The skin surface was located at the beginning of the uniform velocity region. A bi-directional axial scan was conducted. The resulting 3D volume acquisition rate was 1.33 volumes/sec.

Images were saved as an AVI file using a custom LabVIEW code (National Instruments, Austin, TX). At the end of each axial scanning, the confocal FOV was manually moved to a new imaging location and the axial scanning was conducted at the new imaging location. After image acquisition, the AVI file was segmented into multiple image stacks with each stack representing one axial scan. The image stacks were analyzed in ImageJ [22]. The background intensity level was measured and subtracted. 3D rendering of the image stacks was conducted using 3D Slicer [23]. The speckle noise contrast was calculated by analyzing dermis images and dividing the standard deviation of the intensity values by the mean value at four 100×100-pixel regions that exhibited grossly uniform reflectivity without observable cellular features. The speckle noise contrast was measured at three different imaging depth levels.

2.3 Results

A photograph of the low-cost confocal microscope is shown in Fig. 2.4. The confocal microscope had a dimension of 15 cm (W) × 16 cm (H) × 4.5 cm (D), and the weight was 0.57 kg. The material cost for the confocal microscope was $5,188. The optical power on the specimen was 2.2 mW.
A confocal image of the USAF resolution target is shown in Fig. 2.5a. The smallest line pattern in group 9, element 3 (linewidth = 0.78 µm) was clearly distinguished along the slit length direction. The line pattern in group 9, element 2 (linewidth = 0.87 µm) was well resolved along the spectrally encoded direction. Lateral resolutions were measured as 1.05 ± 0.05 µm and 1.31 ± 0.06 µm along the slit length and spectrally encoded directions, respectively. The axial response curve is shown in Figure 2.3b. The axial FWHM was measured as 11.24 ± 0.13 µm. Both measured lateral and axial resolutions were in good agreement with the theoretically expected values shown in Fig. 2.3.

Figure 2.6 shows representative confocal images of human forearm skin in vivo. Each image had an image size of 678 µm × 543 µm. The image taken at the depth of 25 µm (Fig. 2.6a) shows high reflectivity of the stratum corneum. The image at the 60 µm depth (Fig. 2.6b) visualizes keratinocytes with dark cytoplasm and bright cell borders (arrows). At a deeper imaging depth of 80 µm (Fig. 2.6c), the keratinocytes have a smaller cell size.
Melanocytes or melanin-containing basal cells are visualized as bright dots (arrowheads) and dermal papillae (yellow asterisk) as dark openings at this depth. As the imaging depth is increased to 125 µm (Fig. 2.6d), the bright cells are distributed more towards the center of the FOV (dotted region), which is also observed in the video of the skin confocal images (Visualization 1). More dermal papillae are visualized at this depth. At a larger imaging depth of 195 µm (Fig. 2.6e), the fiber network in dermis generates bright signals while blood vessels are visualized as dark areas (white asterisks). Finally, at the imaging depth of 265 µm (Fig. 2.6f), the fiber network and blood vessels are visualized with reduced contrast. The speckle noise contrast was measured as 0.28, 0.26, and 0.24 for the imaging depth of 125 µm, 195 µm, and 265 µm, respectively.

Figure 2.5. Resolution measurement. A – Lateral resolution measurement with a USAF resolution target; and B – Axial response curve.

Visualization 1 shows a video of the sequential 2D en face confocal images from the first axial scan and a video of 3D rendering of 10 consecutive axial scans. The movie of the 2D confocal images is played at a 25 times slower speed than the image acquisition speed. The 3D rendering movie shows the possibility of rapidly examining multiple tissue lesions in 3D without having to identify the depth of the dermal-epidermal junction (DEJ).
Figure 2.6. In vivo confocal images of human forearm obtained at the imaging depth of 25 µm (A), 60 µm (B), 80µm (C), 125 µm (D), 195 µm (E), and 265 µm (F). Arrows – keratinocytes; arrowheads – melanocytes or melanin-containing basal cells; yellow asterisks - dermal papillae; white asterisks – blood vessels; and dotted line – a cluster of melanocytes of melanin-containing basal cells. Scale bar = 100 µm.

2.4 Discussion

In this paper, we have demonstrated that high-speed imaging of human skin in vivo with the low-cost NIR confocal microscope is feasible. The confocal images visualized characteristic cellular features of the skin. Our new confocal microscope achieved a fast image acquisition rate of 203 frames/sec, approximately 50 times faster than our previous smartphone confocal microscope and 20 times faster than commercial confocal microscope devices. The high imaging speed can be used to conduct real-time 3D confocal imaging of a region of interest or large-area imaging of the entire skin lesion within a short procedural time. We also expect that the low cost of the device will facilitate a wide adaption of the device in various clinical settings.
There were several remaining technological challenges found during the preliminary testing. Even though a relatively wide slit was used, the speckle noise was still prominent in confocal images, which hindered the image interpretation. Use of the wide slit degraded the axial resolution. In the future development, we will address these two issues by using a high-power LED, which has a significantly reduced spatial coherence and therefore allows for use of a narrow slit width. The volumetric imaging rate was limited to 1.33 volumes/sec mainly due to the acceleration and deceleration of the axial scanning stage. A piezoelectric transducer (PZT)-based scanner can be used to achieve higher volumetric imaging rate. In the new confocal detection optics, the CMOS sensor is located on the same side as the tissue, which will make it challenging to image certain anatomical locations such as back or face. A fold mirror can be used between the grating and camera lens to move the CMOS sensor away from the tissue and allow for imaging of a wider range of skin locations. In the future study of imaging suspicious skin lesions, we will evaluate the image quality of our microscope in comparison with the commercial confocal microscope and evaluate feasibilities of large-area scanning and real-time 3D imaging.

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**Disclosures**

DK and CG are inventors on a provisional patent application filed by the University of Arizona on the technology presented.
3. SPECKLE-FREE, NEAR-INFRARED PORTABLE CONFCOAL MICROSCOPE

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3.1 Introduction

Reflectance confocal microscopy (RCM) is a non-invasive optical imaging method that can examine cellular details of the skin with a quasi-histologic resolution[24–28]. Through numerous clinical studies, RCM has been shown to diagnose the vast majority of skin cancers with high sensitivity and specificity[14,29–33], which significantly surpass those of the clinical assessment. Recent studies show early evidence of how RCM can improve the skin cancer diagnosis and treatment: i) unnecessary biopsy of benign lesions can be reduced by 50-68%[34,35]; ii) treatment can be initiated during the initial visit without having to wait for the histologic diagnosis[36]; and iii) non-invasive treatment can be monitored at cellular resolution[37,38].

In 2016, current procedural terminology (CPT) reimbursement codes have been granted for RCM imaging of the skin[12]. Wide clinical uptake of RCM, however, has been hampered by the following factors: i) the device cost is high, ~$100,000; ii) training is required for reading confocal images; iii) the standard commercial RCM device (Vivascope 1500) is bulky[39]; and iv) the more portable commercial RCM device (Vivascope 3000) has a small field of view (FOV) without dermoscopic guidance resulting in sub-optimal diagnostic accuracy[40]. In response to the barrier associated with training requirement to interpret the RCM images, promising results have been recently reported from
development of automated RCM image analysis algorithms[41–43]. It is anticipated that either remote live interpretation or AI-based analysis will aid less experienced healthcare providers interpret confocal images. Major improvement of the RCM hardware to make the device portable and affordable, however, has been challenging.

Previously, we have reported on the development of a low-cost, smartphone-based confocal microscope[4]. In the smartphone confocal microscope, scan-free confocal optics was used, where each wavelength of the source is encoded with a transverse coordinate of the tissue. Since inexpensive optoelectrical components are incorporated in the smartphone confocal microscope, the overall material cost of the device was approximately $4,000. While the smartphone confocal microscope successfully visualized known RCM cellular features of human skin in vivo, there were several technological constraints: the imaging depth was limited due to the use of visible illumination light (central wavelength = 595 nm) and the imaging speed was relatively slow, 4.3 frames/sec (fps), which made the acquired confocal images prone to have motion artifacts.

More recently, we developed a near-infrared (NIR) portable confocal microscope (PCM) to address the aforementioned challenges found in the smartphone confocal microscope[24]. In the previous PCM device, a super-luminescent LED (sLED; central wavelength = 840nm; bandwidth = 50nm) was used with the goal of increasing the imaging depth and speed. While the sLED-based PCM device achieved a very high imaging speed of 203 fps, image quality was noticeably degraded due to the speckle noise. The speckle noise was generated by the use of a spatially-coherent light source (sLED) and detection of narrow spectral bandwidth (0.66 nm) by each pixel. The degraded image quality made it challenging to examine the RCM cellular features.
In this paper, we present a new PCM device that significantly reduces the speckle noise, making it possible to analyze the cellular structures from epidermis to superficial dermis. The new PCM device uses a spatially-incoherent light source, an NIR LED. Specific design of the LED-based PCM device is described. Process and results of simulating theoretical resolution are presented. Preliminary confocal images of human skin in vivo are presented and compared with the images taken from the previous sLED-based PCM device.

![Schematic of the NIR PCM device.](image)

**Figure 3.1.** Schematic of the NIR PCM device. black arrow – illumination; red arrow – detection.

### 3.2 Methods

#### 3.2.1 LED-based Portable confocal microscope

An LED (part number = SFH 4780S, Osram.; emission area = 0.75 × 0.75 mm²; viewing angle = 20º; maximum optical power = 660 mW) was used as the light source. The source central wavelength was 820 nm, and 40%-of-maximum bandwidth 40 nm. An aspherical collimator (f = 8 mm) and a cylindrical lens (f = 7.7 mm) were used to focus the light from the LED on an illumination slit (width = 25 µm; length = 3 mm). An achromatic doublet (f = 30 mm) was used to collimate the light from the illumination slit. The collimated beam...
was diffracted by a transmission grating (groove density = 1765 lpmm), which generated the full field angle of 5.9 ° for the spectral bandwidth of 40 nm. The diffracted light passed through a D-shaped aperture (inset, Fig. 3.1; width = 3.5 mm; height = 7.9 mm). A water-immersion objective lens (40x; NA = 0.8) was used to focus the illumination light on the tissue at the nominal incidence angle of 18.7º and effective NA of 0.35. The 5.9º field angle corresponded to the field size of 514 µm.

Light scattered back from the cellular structure of the tissue was collected by the same objective lens. Another D-shaped aperture was used for the detection beam path, which limited the effective detection NA to 0.35. The divided pupil approach, where the illumination and detection beam paths use separate regions of the pupil, was used to provide higher image contrast and reduce the contribution of the specular reflection from optical elements on the confocal image [44]. After the D-shaped aperture, the detection beam was diffracted by another transmission grating (1765 lpmm) and focused by an achromatic doublet (f = 30 mm) onto a detection slit (width = 25 µm; length = 3 mm). Light filtered by the detection slit was collimated by an achromatic doublet (f = 30 mm), diffracted by a transmission grating (1765 lpmm), and focused by a multi-element camera lens (f = 50 mm) onto a CMOS sensor (acA1300-200um, Basler; 1,280 × 1,024 pixels; pixel size = 4.8 µm). A fold mirror was added in the detection beam path to position the camera lens and CMOS sensor away from the tissue, which facilitated placement of the device on skin lesions. Most of the optical elements were assembled passively using custom, 3D-printed mechanical holders (printed by Form 3, Formlabs and uPrint SE Plus, Stratasys). The detection slit was mounted on a miniature, 3-axis translation stage to make the detection slit conjugate to the illumination slit and achieve proper resolution.

3.2.2 Resolution simulation

We have simulated the resolution of PCM by calculating the product of the illumination and detection point-spread functions (PSFs). Resolution simulation process is illustrated in Fig. 3.2. First, the 3D diffraction-limited PSF for the effective NA of 0.35 (water immersion) was calculated using the PSF Generator plug in in ImageJ[45]. The diffraction-limited 3D PSF was rotated by 18.7º (step 1) to represent the illumination beam that was introduced through the left half of the objective lens pupil (inset, Fig. 3.1) and incident on
the tissue at the nominal angle of 18.7°. The rotated PSF was convoluted with the image of the illumination slit on the tissue (step 2). With the magnification of 6 between the illumination slit and tissue, the size of the illumination slit image on the tissue was 4.2 (width) µm × 500 µm (length). The convoluted PSF showed the illumination PSF for a single wavelength when a 25-µm-wide illumination slit is used.

Next, we analyzed the effective spectrum each pixel was detecting. As was the case in the previous PCM device, each pixel on the CMOS sensor was conjugate to a single point on the tissue. The main role of the detection optics was to limit the spectrum that each pixel detected, which limited the width of the effective illumination beam. Given the groove density of the grating, 1765 lp/mm, focal length of the focusing lens, 30 mm, and detection slit width, 25 µm, the bandwidth of the spectrum that each pixel detects was 0.33 nm. The 0.33 nm bandwidth corresponded to a 4.2-µm lateral spread of the illumination beam. The effective illumination PSF was finally calculated by the convolution between the monochromatic illumination PSF for the 25 µm slit with the line width of 4.2 µm (step 3).

Detection PSF was calculated by rotating the diffraction-limited PSF for NA of 0.35 by -18.7° (step 1) to represent the detection beam that was collected through the right half of the objective lens pupil (inset, Fig. 3.1). The rotated PSF was convoluted with the image of each CMOS sensor pixel on the tissue (step 2). The pixel size of the CMOS sensor was 4.8 µm, and the magnification between the CMOS sensor and tissue was 10, which made the width of the pixel size on the tissue as 0.48 µm. Since the image of the individual pixel had a smaller width than the diffraction-limited PSF width, the final detection PSF was not widened noticeably. The confocal PSF was calculated as the product of the illumination
and detection PSFs. The cross sections of the confocal PSF were analyzed along the lateral (x) and axial (z) coordinates of the objective lens and minor (u) and major (v) axes of the PSF. FWHM values were calculated as 1.2 µm (x), 3.6 µm (z), 1.1 µm (u), and 7.2 µm (v).

### 3.2.3 Performance test

Lateral resolution was tested by imaging the USAF resolution target. The smallest pattern distinguished was analyzed. FWHM of the line-spread function was calculated along the horizontal and vertical directions of the confocal image. The axial resolution was calculated by imaging a mirror while translating the PCM device axially with a motorized stage. FWHM of the axial response curve was calculated.

Tissue imaging performance was tested by imaging human skin *in vivo*. During tissue imaging, the exposure time of the CMOS sensor was set as 0.05 second, and the corresponding imaging speed was 20 fps. The confocal images were transferred to a laptop (Surface Book Pro, Microsoft) via the USB 3.0 cable. Data were saved in real time using a custom LabVIEW code. The raw image data were compensated for the intensity non-uniformity using a custom Matlab code: A reference image was generated by i) acquiring a tissue image at the imaging depth larger than a typical confocal imaging depth limit, ~250 µm, so that the image does not exhibit any microscopic details but shows blurred overall reflectivity of the tissue, and ii) blurring the tissue image further using a Gaussian blurring filter with radius of 20 pixels. The reference image was normalized and used to divide each raw confocal image.

When imaging forearm and finger, the PCM device was mounted on a motorized stage to image the tissue for the imaging depth range of 0 to 300 µm. For the confocal images of forearm, we compared the images obtained by the current LED-based PCM device with the images of the same forearm acquired by the previous sLED-based device. Speckle noise contrast was measured for the image obtained from dermis. Four regions of interest (ROIs) with the area of approximately 4,000 pixels per each ROI that exhibit grossly uniform structure were chosen. Average and standard deviation of the intensity values within each ROI were calculated. The speckle contrast was determined as the ratio between the standard deviation and average. The PCM device was then unmounted from the motorized
stage and held by a single hand to image lower lip *in vivo*. The pressure on the tissue was gently varied to change the imaging depth.

### 3.3 Results

The NIR PCM device (Fig. 3.3a) was manufactured at the material cost of approximately $5,000. The PCM device had a dimension of 22 cm × 17.5 cm × 10 cm, and weighed about 1 kg. The illumination power on the tissue was 0.158 mW. The USAF resolution target image revealed that the pattern in the group 9, element 1 (linewidth = 0.98 µm) is distinguishable along the horizontal direction. The pattern in the group 9, element 3 (linewidth = 0.78 µm) was distinguished along the vertical direction, or the slit length direction. The FWHM of the line spread function (LSF) was measured as 1.68 ± 0.16 µm and 1.55 ± 0.13 µm along the horizontal and vertical directions, respectively. The average resolution is 1.62 µm. The measured FWHM of the axial response curve was 6.01 ± 0.11 µm.

![Figure 3.3. Photo of the PCM device (A) and confocal image of the USAF resolution target (B).](image-url)
Confocal images of a human forearm obtained at three imaging depths are shown in Figs. 3.4a-c. At the superficial region with the imaging depth of 26 µm (Fig. 3.4a), keratinocytes in epidermis including the stratum granulosum and spinosum are well visualized with dark cytoplasm and bright cell borders (arrows in Fig. 3.4a). At a larger imaging depth of 72 µm (Fig. 3.4b), dermal papillae (asterisk in Fig. 3.4b) are delineated by circular arrangement of melanocytes or melanin-containing basal cells (arrows in Fig. 3.4b). At the imaging depth of 232 µm (Fig. 3.4c), collagen fibers (arrowheads in Fig. 3.4c) in dermis are visualized. Speckle contrast in the dermis image (Fig. 3.4c) was measured as 0.08 ± 0.01, which was significantly lower than those values measured for the previous sLED-based device, 0.24-0.26.

Images taken with the previous sLED-based device are shown in Figs. 3.4d-f. While some of the cellular features shown in Figs. 3.4a-c are also observed in Figs. 3.4d-f such as keratinocytes (arrows in Fig. 3.4d) and dermal papillae (Fig. 3.4e), other morphologic features were difficult to appreciate. This comparison shows that the reduction of the
speckle noise and improvement of the axial resolution, from 11 µm to 6 µm, in the current LED-based device provides significantly better visualization of cellular features.

Images of the finger are shown in Fig. 3.5. In the superficial region (Fig. 3.5a), numerous keratinized cells (arrowheads in Fig. 3.5a) are shown. At the base of the epidermis (Fig. 3.5b), regular honeycomb pattern of cells (arrows in Fig. 3.5b) are observed, which might correspond to the single layer of basal generative cells. Under the basement membrane (Fig. 3.5c), dermal papillae are visualized as regularly-spaced dark openings (asterisk in Fig. 3.5c).

In the confocal images of the lower lip (Fig. 3.6), various structures are revealed. When the pressure on the tissue was light to make the imaging depth superficial, epithelial cell nuclei are visualized as bright dots (Fig. 3.6a). When the pressure on the tissue was increased to image the basal layer (Fig. 3.6b), blood cells inside capillaries are visualized (arrows in Fig. 3.6b, Visualization 1). At a deeper imaging depth inside the lamina propria (Fig. 3.6b), thin collagen fibers are clearly visualized (arrowheads in Fig. 3.6c).

Figure 3.5 Confocal image of human finger *in vivo* obtained with the LED-based PCM device. Different tissue layers are visualized: epidermis (A), basement membrane (B), and superficial dermis (C). arrowheads – hyper-keratinization; arrows – basal cells; and asterisk – dermal papillae.
3.4 Conclusions

In this paper, we have presented a NIR PCM and demonstrated cellular imaging of human skin in vivo. Compared to the previous sLED-based device, the current LED-based device significantly reduced the speckle noise, which facilitated the identification of cellular features in confocal images. Additionally, cellular features visualized in the present PCM device were similar to those presented in previous RCM literatures. This might suggest that the LED-based PCM device merit further evaluation for visualizing previously-validated RCM features associated with various skin diseases.

The material cost of our PCM device, ~$5,000, is higher than other smartphone-based portable microscopy devices. However, it is noted that the PCM device provides a unique capability of imaging cellular details of the skin in vivo, which might justify the use of the PCM device in certain resource-limited settings. In addition, we expect that the device cost will be significantly decreased in future commercialization by developing low-cost objective lenses and batch-producing optical elements. Compared to our previous smartphone-based PCM device, our current PCM device provided better image contrast and enhanced imaging depth. The imaging performance improvement was achieved by the
use of the NIR-based LED and the monochromatic CMOS sensor without the Bayer color filter. Use of a smartphone as part of the PCM device, however, provides some advantages: reducing the device cost and obviating the need for a laptop for data acquisition. In future development, we will explore a NIR-based, smartphone confocal microscope by removing filters from the smartphone camera module.

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**Disclosures**

CG and DK are the inventors of the provisional patent application related to the portable confocal microscopy technology presented in this paper. The University of Arizona has a technology-licensing agreement with a start-up company on the presented technology. CG and DK have rights to receive royalties as a result of this licensing agreement.
4. CONCLUSIONS

We demonstrated two versions of the low-cost near-infrared portable confocal microscope. Both of them can visualize cellular features in vivo for human skin.

The high-speed confocal microscope demonstrated the fastest imaging speed and can generate large areas of volume 3D images. However, because it used sLED as a light source, the speckle noise on the images is a challenge to be solved in the future. Potentially, an optical diffuser might be used to reduce the speckle noise level, and at the same time, the axial resolution can be benefited because of using narrower detection slit, where the speckle noise increases following the enlarger of the detection slit size.

The speckle free confocal microscope demonstrated high image quality and has been tested in clinics, UA Cancer Center (Tucson, AZ) (Fig. 4.) and Dermatology & Laser Center (Los Angeles, CA). However, the imaging speed was reduced due to the illumination efficiency. Potentially, a better light source format might be helpful in enhancing the SNR and imaging speed. Besides, customized collimation lenses might be able to help to improve the performance at the edge of the field of view.
We also hope to develop a new version of the low-cost confocal microscope, which has a dermatoscope function to guide the confocal image location—combining high speed and speckle free imaging function. A dry objective lens version of our confocal microscope can also have an application on cornea imaging. The final goal of the portable confocal microscope is making the device small as a smart phone and price of that is within $1,000.
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