Performance of full-pupil line-scanning reflectance confocal microscopy in human skin and oral mucosa in vivo

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Abstract: Point-scanning reflectance confocal microscopes continue to be successfully translated for detection of skin cancer. Line-scanning, with the use of a single scanner and a linear-array detector, offers a potentially smaller, simpler and lower cost alternative approach, to accelerate widespread dissemination into the clinic. However, translation will require an understanding of imaging performance deep within scattering and aberrating human tissues. We report the results of an investigation of the performance of a full-pupil line-scanning reflectance confocal microscope in human skin and oral mucosa, in terms of resolution, optical sectioning, contrast, signal-to-noise ratio, imaging and the effect of speckle noise.

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Introduction

Confocal point-scanning microscopes are being translated for clinical applications in dermatology and are showing promise for in vivo diagnosis of melanoma and basal cell carcinoma [1], pre-operative mapping of amelanotic and lentigo maligna melanomas to guide surgery [2] mosaicing of basal cell carcinomas in excised tissue from Mohs surgery [3,4] and detection of residual nonmelanoma skin cancer in shave biopsy wounds [5]. An early study in a generalized setting showed that basal cell carcinomas could be diagnosed in vivo with sensitivity of 92% and specificity of 97% [6]. More recent studies in generalized settings have reported that melanocytic lesions, both pigmented and nonpigmented (amelanotic), are detected with sensitivity of 92% and specificity of 69-84%, compared to specificity of 32-39% with dermoscopy [7,8]. Another study reported sensitivity of 97% and specificity of 98% for melanocytic lesions [9]. The latest study reports sensitivity of 93% and specificity of 82% for detecting lentigo maligna melanomas [10]. While the sensitivity is similar to that of the current clinical standard of visual examination by dermoscopy, the 2×-superior specificity with reflectance confocal microscopy represents a significant advance toward routine clinical utility [8,10]. However, the point-scanning system is relatively complex and expensive such that the technology remains confined to tertiary centers of care. To accelerate translation into the clinic, line-scanning may offer an alternative approach. With the use of a single galvanometric scanner, a linear-array detector and field-programmable gate array (FPGA)-based electronics, a low-cost approach to designing stand-alone confocal microscopes may be possible for diverse primary healthcare settings.

Line-scanning confocal microscopes [11,12], including a fiber-bundle based endoscope [13], were originally developed in the 1980-1990s. Applications have included reflectance imaging in ophthalmology [11] and fluorescence imaging of ovarian cancer [14]. More recently, the availability of inexpensive linear array detectors has enabled simpler designs and consequently led to a renaissance in line-scanning confocal microscopy [15–18]. While the technology has been rapidly developed and commercialized, the clinical applications to date have been confined to either relatively transparent tissues or relatively superficial layers of tissues. Of interest to us for cancer screening and diagnostic applications is imaging performance deep within the highly scattering and aberrating conditions of human tissues such as skin and oral mucosa. Studies of imaging performance are the necessary translational bridge from technology development to clinical implementation. To investigate performance under expected clinical conditions, the strongly scattering and aberrating conditions in human epidermis offers a realistic testing ground for both our immediate interest in skin cancer and for future application in other epithelial tissues.
Two pupil configurations are being investigated: divided pupil and full pupil [18,19]. Our initial studies with a divided-pupil line-scanning microscope were motivated by the original design of Koester [11]. The divided-pupil configuration demonstrated sharp optical sectioning, resolution and contrast for imaging nuclear and cellular detail in the epidermis of human skin and epithelium of oral mucosal in vivo [18]. However, the performance degrades in deeper dermis (in skin) and lamina propria (in oral mucosa). As an alternative, we implemented a full-pupil configuration to test performance in deeper tissues [19].

In this paper, we report the results of an investigation of the performance of a full-pupil line-scanning reflectance confocal microscope in human epidermis. A description of the FPGA-based electronics is included as well as an analysis of the noise performance of the linear-array detector. Imaging in reflectance of human epidermis down to the basal layer in vivo is shown. Imaging of oral mucosa in vivo is also shown. Varying slit widths and polarization configurations are investigated to reduce speckle noise. The present limitations of line-scanning in terms of background, contrast and speckle noise are discussed. The goal is to develop a clear understanding of line-scanning performance for potential translation to clinical applications.

1. Instrumentation and Methods

2.1. Optical design

Figure 1 shows the design of the line-scanning confocal microscope. The illumination is with a diode laser at wavelength 830 nm (L2 830S-120, Micro Laser Systems, Inc.). The 830 nm wavelength was chosen for its known ability to penetrate deeply into skin while maintaining sufficient optical sectioning. The beam is expanded using two convex spherical lenses (f1 = 17 mm, f2 = 65 mm) to a collimated beam of diameter D = 13 mm. A line is formed in the back focal plane of the objective lens using a cylindrical lens (f cyl = 150 mm). The line in the back focal plane is Fourier-transformed into a perpendicular line in the object plane. The line is scanned with a standard galvanometrically-driven mirror (MiniSax, GSI Lumonics) that is placed 35 mm from the pupil of the objective. The beam diameter of 13 mm is sufficient to overfill the 9 mm pupil to avoid vignetting, even as the deflection of ± 3 degrees results in a beam walk of ± 1.8 mm. The water-immersion objective lens (Lucid, Rochester, New York)

![Fig. 1. Optical layout. The blue line shows the path in the plane of the page and the red line shows the path in the perpendicular plane. The cylinder lens focuses the collimated beam into a line in the back focal plane of the objective, which is Fourier-transformed into a perpendicularly-oriented line in the object plane in the tissue. A mirror mounted on a galvanometric scanner sweeps the line across the object plane in the tissue. A polarizing beam splitter and quarter wave plate (QWP) remove back reflections from the optics.](image)
has a focal length \((f_{\text{obj}})\) of 4.58 mm, magnification 30 × and numerical aperture (NA) of 0.9. The length of the line in the object plane, which determines the field of view, is given by \(l = D(f_{\text{obj}}/f_{\text{cyl}}) = 0.40 \text{ mm}\). The measured field of view is 0.37 mm, due to the fact that the magnified line in the detector plane exceeds the detector length. Light that is scattered back from the object plane in the tissue is de-scanned and collected by a convex spherical lens \((f_{\text{det}} = 100 \text{ mm})\) which focuses onto a linear array detector (Panavision ELIS1024). The detector has 1024 pixels of dimensions 7 × 125 μm. A 50 μm chrome-on-glass slit (Applied Imaging, Inc., Rochester, NY) was glued to the frame of the chip. The magnification of the system is given by the ratio of the detector lens focal length to the objective lens focal length, \(\text{Mag} = f_{\text{det}}/f_{\text{obj}} = 22\). Additional details are available elsewhere [19].

A standard approach to eliminate back reflections from the optics is to use linearly polarized illumination along with a polarizing beam splitter (PBS) and a quarter-wave plate (QWP) placed behind the objective. However, in our line-scanner design, because there is no infinity space that is common to both the illumination and detection paths (see optical layout in Fig. 1), the QWP must be placed in the converging illumination beam. Nonetheless, considering that the beam is weakly convergent onto the back focal plane, we hypothesized that the resulting spherical aberrations from a thin QWP may be tolerable. Measurements of the effect of the resulting aberrations on the optical sectioning ability of the microscope are described in Section 2.3. For imaging, a 1 mm thick quartz QWP was placed on a mount at an angle of 4° with respect to the optical axis to prevent back reflections into the detector.

2.2. Electronics design

We are designing an FPGA-based approach to create an electronics platform that will be independent of conventional digital signal generators and synchronizers and peripheral devices such as frame grabbers and other acquisition cards. This is to eventually enable a low-cost, portable and robust standalone approach for line-scanning confocal microscopy. In the long term, such an approach that is independent of any particular computing platform may be necessary for successful implementation in diverse clinical settings. Figure 2 is a block diagram of the proposed electronics architecture.
diagram that shows the overall architecture of the electronics and the signal flow. We developed electronics using field programmable gate array (FPGA) logic to produce timing and synchronization signals for the linear detector array, frame grabber and galvanometric scanner. All three subsystems—detector board, frame grabber (IMAQ card) and galvanometric scanner—are synchronized by the FPGA (Altera) logic on the control board.

The ELIS-1024 linear array detector’s video output is analog. In our system it operates at a clock frequency of 10 MHz allowing us to acquire an image of 1024 pixels × 1024 lines at a maximum rate of approximately 10 frames per second.

The linear array detector receives the pixel clock (CLK), the line reset signal (RST), the shutter control signal (SHT) and the trigger to start video (DATA) signal from the control board. These signals are timed with the FPGA. The analog video output from the linear array is then transmitted through two operational amplifiers (OpAmp) on the detector board. These OpAmps set the black level (blk lvl) and the white level (wht lvl) of the video before being transmitted to the frame grabber (PCI-1410 from National Instruments). The frame grabber receives the pixel clock (PCLK) from the control board synchronous to the clock CLK, along with the synchronization signals horizontal-sync (HSYNC) and vertical-sync (VSYNC). A video frame is constructed in the frame grabber with these three signals: PCLK, HSYNC and VSYNC, in the form of a raster scan. The HSYNC defines the beginning of a line while the VSYNC defines the beginning of a frame.

The VSYNC is also synchronous to the beginning of the analog saw-tooth ramp which is provided to the Minisax Galvo Driver board to drive the galvanometric scanner. The amplitude and the center position of this saw-tooth ramp can be adjusted by two OpAmps. The amplitude corresponds to the scan angle of the galvanometric mirror while the offset corresponds to the center position of the scan.

2.3. Axial response measurements

Axial response measurements were made to determine the optical sectioning capability of the microscope. Axial response measurements were taken by translating a mirror through the focus of the objective lens using a motorized linear actuator (Nanomotion II, Melles Griot) while recording the signal as a function of axial defocus using a photodiode (Thorlabs SM05PD) behind a slit in the detector plane. The photodiode signal was collected using an oscilloscope (Agilent MSO6014A) controlled by Matlab via GPIB. The mirror was translated at a speed of 50 μm/second while the signal acquisition was at a rate of 50,000 points/second, resulting in a spatial sampling rate of 1000 points/μm. For these measurements, a water immersion objective lens with $f_{\text{obj}} = 3$ mm and 0.9 NA was used (Olympus LUMPlanFl/IR).

Hair gel with refractive index $n = 1.3457$ (Queen Helene Sculpturing Gel) was used as an immersion medium because it stays in place despite the 2.0 mm working distance of the lens, especially in the horizontal orientation of the setup.

The width of the slit determines the sectioning capability of the microscope. A sub-diffraction width will provide the best sectioning, but a wider slit may be needed when signal levels are low, such as imaging deep in tissue. A wider slit may also be used to reduce speckle noise, as described in Section 2.4. Four slit widths were used to illustrate the effect of slit width on sectioning. The slit width diameters used were 5, 10, 25 and 50 μm, which correspond to Airy units of 0.27, 0.54, 1.34 and 2.68, where the Airy unit (AU) is defined to be $0.61\lambda/\text{NA}$, and the magnification of this test system is 33. To determine how the sectioning ability worsens in tissue, the axial response was measured through full thickness specimens of human epidermis. The motivation for this experiment is to determine potential loss of optical sectioning when imaging through the epidermis and below the dermo-epidermal junction in the papillary dermis. Observation of cellular detail at the junction is important to clinicians and pathologists because disease and cancers originate at and spread from this location. The thickness of human epidermis is known to vary in the range of 50-100 μm [19]. Discarded human skin was obtained from Mohs surgeries under an IRB approved protocol (#08-006, “Procurement of Discarded Human Skin Specimens for Research Projects in Dermatology.”)

The epidermis was removed by soaking the tissue in warm water (50 °C) for two minutes and
then separating it from the underlying dermis with fine tweezers. The piece of epidermis was then placed on the mirror that was translated through the focus of the objective lens. The axial response measurements through epidermis were made with two slit widths corresponding to 0.27 and 1.34 AU.

In the optical layout in Fig. 1, the cylinder lens focuses light into a line in the back focal plane (BFP) of the objective lens. The location of the BFP in a commercially available objective lens is often not available and must be experimentally determined; therefore accurate placement of the line may not be easy and repeatable. To determine the sensitivity of optical sectioning to the placement of the cylinder lens and the corresponding line in the BFP, axial response measurements were made for four different positions of the cylinder lens. The cylindrical lens was placed at offset positions of +5 mm, 0 mm, −5 mm, −15 mm with respect to the nominal position, as measured along the optical axis, where the positive sign means toward the objective lens. Note that in practice the offset error will be within 1 mm in laboratory instruments and within 0.1 mm in manufactured products. Due to physical space constraints in our setup, the measurements could not be made for +15 mm.

2.4. Noise characterization of the linear array detector

The total system noise in the linear array detector (Panavision ELIS1024) was calculated by using the manufacturer’s specifications. Each source of noise was calculated in equivalent electrons and all sources were added in quadrature to determine total system noise. The sources of noise included shot noise, reset noise, quantization noise, amplifier noise and pattern noise. Figure 3 shows the results of our analysis.

Figure 3a is a plot of the total system noise and each of the component noise sources. For this detector, pattern noise is the dominant source of noise. Photoresponse nonuniformity (PRNU) pattern noise is a result of the difference in photoresponse from pixel to pixel, and does not change from frame to frame. PRNU is characterized as a fraction, $U_{PRNU}$, of the number of photoelectrons $n_{PE}$, such that the noise is $\langle n_{PRNU} \rangle = U_{PRNU} n_{PE}$. For the Panavision detector, the manufacturer specifies the $U_{PRNU}$ at 3%, which ultimately limits the signal-to-noise ratio to 33. Pattern noise can be measured by illuminating the detector uniformly to record the pattern and calculate the $U_{PRNU}$ at each detector pixel. The pattern can then be removed from every image based on the recorded signal level of each pixel. The pattern noise-corrected value of an image pixel is then $p = \text{signal}/(1 + U_{PRNU})$.

Reset noise represents the uncertainty that the capacitor in the read-out electronics dispels all the electrons upon resetting. Reset noise is $\langle n_{\text{reset}} \rangle = \sqrt{kTC}/q$, where $k$ is the Boltzmann constant, $T$ is temperature in degrees Kelvin, $C$ is the capacitance and $q$ is electron charge. The reset noise is 1569 electrons. Reset noise is a thermal noise, and may be limited by cooling the detector; however, this is inefficient, as the noise decreases only as the square root of the temperature. Reset noise is generally reduced by correlated double sampling, in which the difference between the reset voltage and the signal voltage is measured. However, this technique reduces the maximum frame rate by half.

Shot noise varies as $(n_{PE})^{1/2}$, where $n_{PE}$ is the number of photoelectrons. The signal to shot noise ratio is increased by increasing the signal, such that it is necessary to use the detector at high levels of illumination. The well depth of the detector is 800,000 electrons. Thus, for illumination levels varying from a quarter to full well depth, the shot noise varies from 447 to 894 electrons.

Quantization noise is the error introduced by binning the number of photoelectrons into 256 levels in the ADC. This noise level may be reduced by increasing the number of bits. For our image acquisition with eight bits, the quantization noise is 902 electrons.

Amplifier noise, also called the noise floor, is related to the frequency response of the electronics, and increases with increasing frame rate. The noise floor is 225 electrons.

Figure 3b shows a plot of the signal-to-noise ratio (SNR) versus number of photoelectrons incident on the detector. SNR peaks at 33 in this system.
Fig. 3. (A) Sources of noise in detection system. Pattern noise is the most significant source of noise. (B) Signal-to-noise ratio in the detection system. SNR levels off at one-quarter well, with a maximum SNR of 33, as limited by pattern noise.

2.5. Imaging methods

All imaging was performed with illumination wavelength $\lambda = 830$ nm and a water immersion lens with NA of 0.9 (Lucid, Rochester, NY). This lens is custom-designed to image through a 1 mm-thick glass slide which helps keep tissue flat and still. Ultrasound gel with refractive index $n = 1.3359$ (Sonigel, Mettler Electronics Corporation) was used as an immersion medium instead of water because it is viscous and stays in place while imaging. The Panavision detector with a 50 $\mu$m chrome-on-glass slit (Applied Imaging, Inc., Rochester, NY) was used for imaging. The 50 $\mu$m slit corresponds to 4.0 AU for the imaging configuration described in Section 2.1. Images were captured using a frame grabber (IMAQ PCI1410, National Instruments) and the Matlab Image Acquisition Toolbox (R2010a, Mathworks).

*In vivo* imaging was performed on the arm and lip of volunteers. To stabilize the tissue while imaging, the arm or lip was pressed against a metal plate. The plate has an aperture to allow the objective to access the tissue.

2.6. Investigation of speckle noise

The contrast due to speckle was quantified by calculating the speckle index [20] for each image as follows. For an $M \times M$ image of pixel values $p(m,n)$ for pixels $1 \leq m,n \leq M$, the local
mean $\mu(m,n)$ is defined as the mean of the pixel $(m,n)$ and its eight neighbors. The local standard deviation $\sigma(m,n)$ is defined as the standard deviation of that nine-pixel neighborhood. Because we expect the speckle size to be on the order of the resolution, which is approximately one pixel, a nine pixel kernel is appropriate.

The speckle index is then defined as

$$s = \frac{1}{M-2} \sum_{m=2}^{M-1} \frac{\sigma(m,n)}{\mu(m,n)}$$

The speckle index is the average of local contrast variations across the entire image. The range is limited from $(m,n) = 2$ to $M - 1$ to avoid edge effects.

The reduction of speckle in the system was investigated in three ways. First, speckle may be reduced by averaging successive image frames because tissue motion that may occur between frames will cause some spatial modulation of the noise pattern. Second, speckle was quantified in images taken with two different detector slit widths. Third, speckle noise was compared in images using the PBS/QWP scheme versus using a 50/50 beam splitter. Using the PBS/QWP results in circularly polarized illumination of the tissue and using the 50/50 BS results in linearly polarized light illuminating the tissue. Circularly polarized light depolarizes faster than linearly polarized due to the presence of relatively large scattering structures in skin [21].

To average frames, video was captured of human skin in vivo. In Matlab software, 52 pairs of adjacent images were selected and averaged. The speckle index of the first of each pair was calculated, and the speckle index of each averaged image was calculated.

To compare the effects of microscope configuration on speckle noise, three sets of 42 images were taken. Images in Set 1 and Set 2 were imaged using a PBS and QWP as described above. Set 1 was imaged using the Panavision detector with 50 µm chrome-on-glass slit. Set 2 was imaged with no slit on the detector, meaning the detector width of 125 µm (10 AU) serves as the slit width. For Set 3, a 50/50 beam splitter was used in place of the PBS and the QWP was removed. The detector with 50 µm slit was used. The speckle index was calculated for each image, and the mean and standard deviation of the speckle index was plotted for each set of images.

3. Results

3.1. Axial response measurements

Figures 4 and 5 show the results of axial response measurements for the line-scanning confocal microscope. In Fig. 5a, axial response measurements are shown for several detector-slit widths, which correspond to Airy units (AU) of 0.27, 0.54, 1.34 and 2.68. An Airy unit is defined as $0.61\lambda/NA$. The measured values for full-width at half-maximum (FWHM) of the axial response were found to be 1.6, 2.1, 2.5 and 3.6 µm, respectively. For comparison, these values are plotted with a theoretical model of a point scanning system [22] in Fig. 5 to demonstrate the sectioning capability of the line-scanner with respect to the standard confocal microscope. The semiconfocal nature of the line-scanning system accounts for about 20% increase in FWHM over the point-scanning system. Other misalignments in the system add to the discrepancy between the experimental and theoretical results. The objective lens is designed for water immersion, where the index of refraction of water $n = 1.33$. However, in the experimental setup we used gel with $n = 1.3457$. A difference of 0.01 in index of refraction results in a focal shift of 14 µm for a working distance of 2 mm. This produces spherical aberration of $-2.25$ waves. For a 3 AU pinhole, this results in a FWHM of 3.5 µm, as compared to the nominal 2.2 µm, as calculated by adding the spherical aberration to the sectioning integrals by Wilson [22]. This small change in index, in combination with other misalignments in the system, is responsible for the additional discrepancies between theory and experiment.

Axial response measurements through human epidermis are shown in Fig. 5b. Results varied from one specimen to another and also within a single specimen due to the natural
biological variability in the thickness of human epidermis. However, a factor-of-two increase in the FWHM of the axial response was typical. With a 5 μm slit (0.27 AU), the FWHM increased from the nominal 1.6 μm to 2.5 μm through epidermis. Using a 25 μm slit (0.54 AU), the FWHM increased from the nominal 2.7 μm to 3.7 μm through epidermis.

Figure 5c shows five axial response plots with plates of varying thickness placed behind the objective lens. The measured FWHM for each of these measurements was 2.2 μm, and no additional aberrations are apparent. It is clear that the addition of a thin QWP behind the objective lens does not significantly alter the sectioning capability of the microscope. The reasoning is that in one plane, the beam is collimated as it passes through the glass plate, and so suffers no spherical aberrations. It is in this plane that the objective pupil is filled and results in a tight focus in the focal plane. Thus, the line-scanner design allows for the use of a PBS and QWP, as in standard microscopes.

![Fig. 4. The FWHM of the axial response with increasing slit diameter in the line-scanning experiments (LS Exp) and increasing pinhole diameter in the point scanning theory (PS Theory). The experimental results in the line-scanning system are compared to the theoretical results for a point scanning system [22].](image)

Figure 5d shows four axial response plots for various displacements of the focused line with respect to the back focal plane of the objective. The measured FWHM for all four axial response measurements is 1.9 μm. For the offset position of z = −15 mm, some additional aberration is apparent. This is most likely due to an angular offset of the cylinder lens that may have occurred when realigning the lens position. The sectioning is therefore not sensitive to an axial offset of the focused line in the back focal plane of the objective lens.

Figure 5e shows the axial response plotted on a logarithmic scale for detector slit width of 25 μm with and without epidermis. The logarithmic scale emphasizes the tails of the axial response. It is important to note that while the FWHM of the axial response is generally used as the metric of optical sectioning, aberrations leading to significant side lobes result in a degradation of contrast and resolution in practice. The low spatial frequency background haze and loss of contrast seen in the images in Sections 3.2 and 3.3 can be partially attributed to the tails of the axial response.

3.2. Imaging of human epidermis in vivo

The imaging of human skin shows cellular and nuclear detail in the epidermis. Granular cells are typically 30 μm with nuclei of about 20 μm, spinous cells are smaller, typically 20 μm with 10 μm nuclei, and basal cells are smaller still, typically 10 μm with 5 μm nuclei. The optical sectioning measurements based on the FWHM of the axial response plots are visually confirmed nuclear detail on the order of 5 μm is observed with sufficient resolution and contrast.

Figure 6a shows the granular layer of the epidermis, with dark nuclei and bright cytoplasm in a regular pattern indicative of healthy tissue. Figure 6b shows the spinous layer with
Fig. 5. (A) Axial response (AR) measurements for varying detector-slit widths. Slit widths of 5, 10, 25 and 50 μm were used, corresponding to Airy units of 0.27, 0.54, 1.34, and 2.68. (B) AR measurements through full-thickness of human epidermis with detector-slit widths of 5 μm and 25 μm. These are compared to the nominal AR at the same slit width. (C) Five AR plots with plates of varying thickness behind the objective lens. Plate thicknesses of 40 μm, 170 μm, 1 mm and 3.5 mm were used. All measurements were taken with a 10 μm slit in place. (D) Four AR plots for various displacements of the focused line from the BFP of the objective lens. Displacements of +5, 0, −5 and −15 mm were used. (E) Axial response on a logarithmic scale for detector slit width of 25 μm with and without epidermis. Tails of axial response contribute to loss of contrast in imaging.
Fig. 6. Images of human epidermis *in vivo*. (A) Granular layer, (B) spinous layer, (C) basal layer.

Fig. 7. Frame from video clip (Media 1) of human epidermis *in vivo*.

Fig. 8. Images of human oral mucosa *in vivo*. (A) Superficial layer, (B) epithelial layer, (C) capillaries.

smaller cells in a regular pattern. Figures 6c shows the brightly scattering basal cells in the basal layer. The basal cells lining the dermal papillae are visible, which in cross section appear as bright rings. Figure 7 is a video of human epidermis *in vivo*. The video starts in the basal layer, with bright basal cells in ring formations. At the center of the ring near the left edge of the video, blood cells are seen as bright flickers against the dark lumen. The view then moves through the layers of the epidermis, starting from the deep basal layer, up through the spinous and granular layers. Dark nuclei appear within bright cytoplasm. The loss of contrast due to speckle is seen.
3.3. Imaging of human oral mucosa in vivo

Imaging of the lip (labial mucosa) shows bright nuclei and distinct cell membranes in the mucosa (Fig. 8). Deeper is the lamina propria. Brightly scattering blood cells are visible in capillary loops. Figure 8a shows the bright nuclei and cell membranes of the superficial layer of the mucosa. Figure 8b shows the bright nuclei slightly deeper in the mucosa. The lamina propria appears bright in Fig. 8c. Bright blood cells in dark capillary loops are visible, and are identified by viewing the live video, where the blood flow is visible. Figure 9 is a video of human oral mucosa. In the first half of the video, bright nuclei are seen in the epithelial layer. In the second half of the video, bright blood cells are seen flowing within the capillaries.

3.4. Speckle index

The mean and standard deviation of the speckle index is plotted in Fig. 10a for each case described in Section 2.6. Speckle was shown to be reduced most significantly by increasing the slit width from 50 μm to the full detector width of 125 μm. Two frame averaging also

![Fig. 9. Frame from a video clip (Media 2) of human oral mucosa in vivo.](image)

![Fig. 10. Comparison of speckle index.](image)
reduced speckle noise. The use of the 50/50 beam splitter does not significantly change the speckle noise in the system. The images in Fig. 10 are representative of those for each of the cases for which speckle indices were calculated. Figure 10b is a single frame image using a PBS and 50 μm slit. Figure 10c is a two-frame average that shows speckle reduction but also loss of resolution. Figure 10d is a single frame that was taken using a 125 μm slit. The speckle is significantly reduced. However, the increased background due to widening of the slit greatly reduces contrast in the image. Figure 10e is an image using the 50/50 beam splitter and 50 μm slit. This image shows similar speckle to that using the PBS.

4. Conclusions and Discussion

The full-pupil line-scanning confocal microscope demonstrates optical sectioning that compares to the expected axial response function calculations, and measurements through human epidermis shows degradation of sectioning by about a factor of two. Our results show that optical sectioning is sufficiently preserved through the epidermis and below the dermo-epidermal junction. The preservation of optical sectioning is consistent and sufficient when measured in several locations (5–10) across five epidermal specimens from different patients. These sectioning measurements are further confirmed by the imaging, which shows nuclear and cellular resolution down to the basal layer. These results suggest that line-scanning may allow observation of cellular-level detail at the dermo-epidermal junction, as required by clinicians and pathologists.

The main difficulty is the existence of speckle, which degrades contrast and the apparent resolution of the system. The speckle can be reduced by increasing the detector size and thus spatially averaging the noise. However, this approach leads to degradation in sectioning capability and an increase of background in the image. Speckle may be reduced with the use of broadband illumination sources such as a superluminescent diode or white light. Another possibility may be incoherent imaging such as fluorescence imaging. However, few dyes are available today for use in humans and detectability under nontoxic conditions in deep tissue remains to be investigated.

The line-scanner seems better-suited to imaging in less-scattering tissues, such as the oral mucosa, where the sectioning and resolution is maintained down to the lamina propria, and blood flow can be imaged. Future work will include comparisons of different detector and slit configurations for different tissue conditions.

The cost of materials to build a benchtop setup was about $15,000. The use of FPGA electronics may lead to standalone line-scanning microscopes for use in diverse settings.

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