MEMS-based handheld confocal microscope for in-vivo skin imaging

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Abstract: This paper describes a handheld laser scanning confocal microscope for skin microscopy. Beam scanning is accomplished with an electromagnetic MEMS bi-axial micromirror developed for pico projector applications, providing 800x600 (SVGA) resolution at 56 frames per second. The design uses commercial objective lenses with an optional hemisphere front lens, operating with a range of numerical aperture from NA=0.35 to NA=1.1 and corresponding diagonal field of view ranging from 653 µm to 216 µm. Using NA=1.1 and a laser wavelength of 830 nm we measured the axial response to be 1.14 µm full width at half maximum, with a corresponding 10%-90% lateral edge response of 0.39 µm. Image examples showing both epidermal and dermal features including capillary blood flow are provided. These images represent the highest resolution and frame rate yet achieved for tissue imaging with a MEMS bi-axial scan mirror.

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1. Introduction

The utility of confocal microscopy for skin imaging resides in its ability to provide cross-sectional images with cellular detail similar to that of histological techniques [1]. Recent work has largely been aimed at in vivo confocal imaging of skin, with a goal of providing a non-invasive sectional imaging method to replace existing biopsy and histology methods for diagnosis of suspected lesions. Though many studies show promising results with the use of bench-top confocal microscopes, clinical usage requires the use of a smaller handheld unit to allow for imaging of remote areas which are inaccessible by larger bench-top units [2]. In this paper we describe a portable handheld confocal microscope suited for in vivo skin imaging in a clinical setting. The compact design of this instrument is achieved by the use of a high-resolution, high-speed bi-axial microelectromechanical systems (MEMS) scanner developed for miniature projection, or pico projector, systems. A novel aspect of the instrument combines a fixed hemisphere front lens directly in contact with the tissue with commercially available air immersion objective lenses to achieve high numerical aperture (NA) and focus control ability without the need for fluid coupling between the objective lens and the sample.

Much prior research of in vivo imaging of skin has relied on the use of bench-top confocal microscopes, beginning with the use of a tandem scanning microscope and mercury lamp [3]. Since this initial investigation, the development of confocal laser scanning microscopes (CLSM) has allowed for increased optical power and specific wavelength selection. Because the primary application for confocal skin imaging is as a potential alternative to biopsy, many experiments have compared histological and confocal images, showing good agreement in cellular detail [1][4][5]. As an ex vivo tool, confocal microscopy has proven to be useful for immediate visualization of skin excisions during MOHS surgery, providing an alternative to frozen pathology [6][7][8]. The limitation of the instruments used in these experiments lies in their size and lack of portability. To date, there exists only one commercially available handheld confocal microscope designed for skin imaging (Lucid Vivascope 3000).

MEMS mirror technology has been used for confocal microscopy, most prominently in the development of confocal endoscopes [9][11][12][13][14], but also as a scanner for the Olympus OLS1100 bench-top confocal microscope [10]. Endoscopic confocal microscopes with MEMS actuators have been limited to low NA (0.25-0.4) imaging due to size constraints, making them unsuited for skin imaging where NA > 0.7 is desired [15]. Dual axis...
confocal configurations provide increased axial resolution over single axis counterparts, but suffer from decreased collection efficiency due to low NA collection optics [16][17]. High NA objective lenses for endoscopic confocal microscopes have been developed [18][19], but they are designed for use with coherent fiber bundles to relay scanned light to the sample. Without the size constraint of endoscopic variations, the use of larger commercial objective lenses for high NA skin imaging can be incorporated into a handheld design.

In this paper we describe a handheld MEMS based confocal microscope designed for in vivo reflectance skin imaging. Compared to previous demonstrations of confocal microscopy using a MEMS mirror, this paper illustrates that high fidelity imaging is possible using a mirror (manufactured by Microvision, Inc., Redmond, WA) that represents the current state of commercial development of bi-axial raster scanners [20]. Designed for SVGA resolution miniature projectors, this mirror uses position sensing and feedback control to achieve both high frame rate and high optical resolution imaging. Using this state of the art bi-axial MEMS scanner allows decreased instrument size relative to galvanometer based designs, and offers a path toward ultra-miniaturization of confocal microscopes. The small size of our first generation instrument makes it clinically viable, and we anticipate further miniaturization in future iterations. The size of the microscope presented here is not limited by the scanner, but by our desire to build a flexible prototype using interchangeable commercial objective lenses to allow investigation of skin imaging performance for various values of numerical aperture (0.35 < NA< 1). It also includes a CCD camera for wide-field imaging to register the confocal image relative to gross skin morphology. The result is a clinically useful instrument that can be used as a prototype for much smaller MEMS-based instruments in the future.

The first section of the paper describes the microscope layout and light delivery and collection. This is followed by a brief description of detection electronics and image formation. Optical performance is discussed in section three, followed by representative in vivo images and a discussion of our results.

2. Methods

A diagram of the handheld confocal microscope as well as the light launch and collection scheme is shown in Fig. 1. An 830 nm diode laser source (Sacher Lasertechnik) provides a collimated output with a maximum power of 180 mW. The output beam is incident on a polarizing beamsplitter in the reflection (s-polarization) mode, and then coupled into a two meter single mode polarization-maintaining (PM) fiber with 5.3 μm mode field diameter for delivery to the handheld microscope.

Linearly polarized light is collimated by a fiber port on the microscope to a diameter of about 1 mm. This beam is incident on a 1.5 mm diameter MEMS scan mirror which provides two dimensional raster scanning of the beam. The MEMS scanner is an electromagnetically actuated 2-axis scanner with a resonant, sinusoidal fast scan at a frequency of 22 kHz and sawtooth slow scan providing a 56 Hz frame rate. The resonant fast scan determines video timing, with HSYNC and VSYNC signals provided by the mirror controller for image formation. The mirror produces a rectangular scan pattern with a full optical angle of 22.4° for the fast scan direction and 17° for the slow scan direction.
After the MEMS scan mirror, a pair of achromatic lenses expands the input beam to fill the rear aperture of the objective lens and images the MEMS scanner into the back focal plane of the objective lens to ensure telecentric scanning. A quarter wave plate aligned at 45° to the polarization axis produces circularly polarized light at the sample. Because of our desire to provide variability in NA and field of view, the handheld microscope was designed to accommodate two objective lenses: a 50x 0.55 NA f=4 mm Nikon objective lens and a 100x 0.75 NA f=2 mm Leica objective lens. The field of view (FOV) is 260 µm by 198 µm for the 100x objective and 520 µm by 396 µm for the 50x objective. Both are extra long working distance objective lenses to provide the necessary space for an optional hemispherical front lens used to further increase NA. The constant beam expansion produced by the achromatic lens pair was optimized for the higher NA Leica objective, and does not fully fill the aperture of the 50x objective lens, resulting in an effective NA using this lens of 0.35.

The interface with the skin consists of a removable front piece with either a small aperture (1 mm diameter) or a BK7 hemispherical lens. The rigid front piece can be attached to the skin using adhesive tape, and mounts kinematically to the handheld microscope using magnets. The hemisphere front piece places the natural focus of the objective lens at the vertex of the hemisphere, so the wavefront of the converging beam matches the curvature of the hemisphere. This increases the NA and magnification by the index of refraction of the hemisphere (1.51 for BK7), giving further control over system NA.

Focus control is accomplished through translation of the stage on which the objective is mounted by a Tiny Picomotor actuator (New Focus). The Tiny Picomotor is a piezo driven, screw type linear actuator that allows for computer controlled movement or manual operation.
This mechanism allows for sub 30 nm incremental focus steps and constant velocity movement via computer control. The motor force and spring stiffness in the translation stage are sufficient to allow use of the microscope in any physical orientation without loss of focus control capability.

Upon reflection from the sample, circularly polarized light is collected by the objective lens and retraces the illumination path. A second pass through the quarter wave plate produces linearly polarized light that is orthogonal to the illumination light. The light is descanned by the MEMS scanner and coupled into the orthogonal polarization mode of the PM fiber. At the polarizing beamsplitter in the light launch and collection stage the light is aligned for transmission (p-mode) through the beamsplitter, and coupled into a second fiber for delivery to an avalanche photodiode (APD) detector. This polarization based detection ensures that reflections from any surfaces prior to the quarter wave plate on the illumination path do not get coupled to the APD (Hamamatsu C5460).

To provide a large area view of the skin surface and aid in alignment, a color CCD board camera (Videology) was integrated into the handheld microscope. Insufficient space between the objective lens and the skin required imaging through the objective lens to provide the large area CCD image. This is accomplished with a wedged beam sampler placed just behind the objective lens, and additional imaging lenses to relay the image to the CCD as seen in Fig. 1. White LEDs in front of the objective lens provide illumination for the large area image.

The measured optical power losses in the microscope are approximately as follows: 15% at the MEMS scanner, 5% in the telescope lenses and quarter wave plate, 30-55% in the beam sampler and objective, dependent on the objective being used (with the 100x objective being less transmissive at 830 nm). This corresponds to a one-way throughput of 56% for the 50x and 36% for the 100x objective. With up to 60mW of power entering the microscope, the intensity at the skin surface ranges from about 22 mW with the 100x objective to 34 mW with the 50x objective.

3. MEMS scanner and image formation

Fig. 2. Two-dimensional MEMS scanner, provided by Microvision, Inc. This mirror is electromagnetically actuated, with a resonant fast scan and sawtooth driven slow scan. The minor diameter is 1.5 mm.
3.1. Scanner resolution

The MEMS scanner, shown in Fig. 2, produces a rectangular scan pattern with a full optical scan angle of 22.4° in the fast scan (vertical) and 17° in the slow scan (horizontal) direction. The number of resolvable spots given the input beam diameter can be calculated using \( N = \beta B / \lambda \) where \( \beta \) is the full angle of the optical scan and \( B \) is the width of the scan aperture \([21]\). Because the illumination beam diameter of 1 mm under fills the MEMS mirror diameter of 1.5 mm, the beam diameter is used to calculate a resolution of 357 by 470 spots for the slow scan and fast scan directions respectively.

3.2. Imaging electronics

The APD used to measure the reflected light intensity has a bandwidth of 10 MHz. Image digitization and formation is accomplished using an image acquisition (IMAQ) card with an analog bandwidth of 30 MHz and maximum sampling rate of 40 MS/s (National Instruments PCI-1410). The amplification electronics in Fig. 3 interfaces between the APD and IMAQ card to allow the user to adjust gain and black level of the incoming signal. The electronics also allow the selection of either linear or logarithmic amplification, with the logarithmic amplification providing a compression ratio of 24 mV/dB. The signal from the APD is DC-coupled, so the black level adjustment is used to subtract any fixed background signal from the APD. As the IMAQ card expects an AC-coupled video signal with a 'back porch' black level reference, the HSYNC signal from the MEMS controller is used to control a CMOS switch which alternates between the video signal and ground, providing the necessary black level reference during this back porch time.

![Image of amplification and interface electronics for image formation. This scheme allows for the user to select between linear or logarithmic amplification and adjust black level and gain before the signal is digitized by the IMAQ card.](image)

Image formation is done onboard the IMAQ card with the HSYNC and VSYNC signals from the MEMS controller, using a non-standard image acquisition mode whereby the pixel clock is internally generated and triggered with the HSYNC signal. The number of pixels available in the slow scan direction (which is horizontal in our images) is set by the sawtooth slow scan to 320 pixels, while the number of pixels in the fast scan direction (vertical in our images) is set by the sampling rate of the IMAQ card. The resonant nature of the fast scan mode produces a sinusoidal scan pattern. Given the constant sampling rate, a stretching of the image occurs at the top and bottom of the image where the scanner is moving more slowly, as shown in Fig. 4(a). The pixel clock is set to achieve an aspect ratio of 1:1 at the center of the image where the fast scan is in the linear region of the scan. Doing so produces an image with 748 pixels, corresponding to a sampling rate of about 32.9 MS/s given the 22 kHz fast scan speed. Distortion due to the sinusoidal fast scan is corrected by a re-indexing of the image whereby rows at the top and bottom are discarded and rows in the center are reserved, as fit to...
an assumed sinusoidal position dependence. This technique has the benefit of computational quickness and removes most of the distortion from the MEMS scan pattern.

The APD bandwidth of 10 MHz and sampling rate of 32.9 MS/s ensures no aliasing in the fast scan direction of the image. However, the similarity of the optical resolution in the slow scan direction (357 resolvable spots) to the number of lines in the image (320) could lead to aliasing effects. Because the application of this microscope is skin imaging, periodic structures on this scale are not likely to be encountered, and this potential aliasing artifact does not appear to pose a problem.

Fig. 4. Image distortion due to sinusoidal fast scan of MEMS mirror in time (a) and the corrected image (b) accomplished by removing pixel rows on the edges of the image. This image of a sample MEMS device with a hole pattern on a 30 µm spacing; images were taken with the 50x objective and aperture front piece.

4. Optical performance

Optical performance of the handheld confocal microscope is readily described by the axial and edge response of the system. The afocal relay was designed to provide a 3x expansion to approximately fill the 3 mm diameter rear pupil of the 100x objective. This magnification
results in the image of the MEMS mirror having a diameter of 4.8 mm which exceeds the pupil diameter of the objective lenses used. Therefore, the limiting aperture in the system is the objective lens pupil. Using the analytical method of Gu [22] for Gaussian beam illumination the performance of a fiber confocal microscope can be modeled using a dimensionless “A-parameter” relating the squared ratio of the aperture radius $a_0$ to the illumination beam $1/e^2$ intensity radius $w$, where $A=2(a_0/w)^2$. When using the hemispherical lens front piece the NA is increased by 1.51 (the index of refraction for BK7), so the final NA using the hemispherical lens is 1.1 for the 100x objective and 0.83 for the 50x objective. The illumination beam radius was measured and A-parameter calculated for each of the following experiments.

4.1. Aperture front piece

The axial response of the system was measured in air by placing a polished silicon reflector in front of the objective lens, and translating the lens so the focus passed through the plane of the mirror. The reflected intensity on the APD was recorded as a function of translation to provide the measurement of axial response. Measured axial response full width at half maximum (FWHM) values were 2.24 µm and 5.73 µm for the 100x and 50x objectives, respectively, when using the aperture front piece (no hemisphere lens). With a value of $A=1.35$ for the 100x and $A=2.91$ for the 50x ($1/e^2$ beam radius of 0.86 mm), the method of Gu [22] produces theoretical values for the FWHM axial response of 2.25 µm and 4.64 µm for the 100x and 50x objectives, respectively.

The lateral resolution of the microscope, as represented by the edge response of the system, was measured with the MEMS scanner running by placing a cleaved silicon edge in the focal plane and recording the reflected intensity as a function of time on an oscilloscope. A 20 µm translation of the edge followed by a second oscilloscope trace allowed for the conversion from a time scale to distance. To ensure the measured performance was not limited by the low bandwidth of the imaging APD, a higher bandwidth APD (Hamamatsu C5331) was used for edge response measurements. Measured 10%-90% edge response distances were 0.61 µm and 0.94 µm for the 100x and 50x objectives, respectively. Theoretical edge response values were obtained by an integration of the point spread function derived by Gu et al. [22]. This method predicted 10-90% edge response of 0.45 µm for the 100x objective and 0.65 µm for the 50x objective.

4.2. Hemisphere Front Piece

A glass hemisphere centered on the beam focus introduces no aberration and increases the NA by the index of refraction of the glass. When the beam focus is not coincident with the hemisphere center, spherical aberration is introduced. As illustrated in Fig. 1, focus control is achieved by translation of the objective lens with respect to the sample plane. Therefore the system operates with the beam focus in a region beyond the center of the hemisphere lens, and the impact of the hemisphere on image formation was studied. OPD fan results from Zemax simulations of the hemisphere lens in the system are shown in Fig. 5. The effect of the hemisphere as a function of lateral displacement (within the image field of view) as well as depth into the sample was investigated. These models assume an illumination beam with NA in air of 0.75, corresponding to the 100x objective lens.

With the focus at the interface between the hemisphere and the sample (modeled as seawater in Zemax) the lateral displacement while maintaining a Strehl ratio of $> 0.8$, a goal for good performance, was found to be 60 µm (radius). Because the hemisphere provides an image magnification of 1.51, the diagonal FOV radius for our system is 109 µm. OPD plots in Fig. 5 are given for on axis, 60 µm and 100 µm lateral displacements for each depth.
When imaging at a depth of 50 µm into the sample, spherical aberration introduced by the hemisphere lens and the 50 µm layer of water degraded imaging performance so the on-axis Strehl ratio was below 0.8. To correct for this limiting spherical aberration, it was found that a flat piece of glass 135 µm thick introduced between the objective lens and the hemisphere lens introduced a correcting aberration. In effect, this correction glass optimizes the system for best performance at a depth of 50 µm. With the correction glass present, a lateral
displacement of 45 µm was achieved while maintaining Strehl ratio > 0.8. The thickness of the corrective glass is close to that of a #1 coverslip (150 µm), so we hypothesized that a coverslip could be used in practice to improve performance when imaging at this depth. When imaging at 100 µm depth, a slide thickness of 280 µm provides the best result, and can be achieved by stacking two #1 coverslips. With correction at this depth the Strehl ratio is 0.76 on axis and 0.69 at 45 µm off axis.

4.3. Optical performance with hemisphere

By taking into account the increase in NA due to the hemispherical lens, the analytical method of Gu [22] can be used to estimate the FWHM axial response as 0.71 and 1.47 µm for the 100x and 50x objective lenses respectively. For these measurements A-parameter values were A=1.35 using the 100x lens and A=2.29 for the 50x lens. Measurements were made using a polished silicon reflector in front of the hemisphere lens, with water coupling between the flat surface of the hemisphere and the silicon. The reflector was then translated axially away from the hemisphere surface to get information about the dependence of depth on axial FWHM. This data was gathered both with and without the corrective #1 coverslip, as seen in Table 1. The correction due to the coverslip was readily seen in axial response data using the 100x objective lens. As seen in Fig. 6, the best performance depth has moved from the surface of the hemisphere without the coverslip to a depth of 50 µm with the addition of the coverslip. This improvement is visible in both the axial FWHM and the collected intensity of reflected light.

**Table 1. Measured axial response in [µm] using the hemisphere front piece both with and without the corrective coverslip.**

| Depth into sample | 100x no correction | 100x with correction | 50x no correction | 50x with correction |
|-------------------|---------------------|----------------------|-------------------|---------------------|
| Tissue surface    | 1.89                | 2.07                 |                   |                     |
| 50 µm             | 2.45                | 1.14                 | 2.41              | 2.19                |

With the increase in NA due to the hemisphere, the theoretical 10-90% edge response is 0.30 µm for the 100x objective lens and 0.44 µm for the 50x objective lens. Measurements were again made with the MEMS scanner running while monitoring the signal from the APD on an oscilloscope. Water coupling was used between the flat hemisphere lens surface and silicon edge, which was oriented perpendicular to the fast scan direction. To help smooth noise visible in the edge response data, an error function was fit to each edge and the 10% and 90% locations found on the resulting fit line. Measurements were taken at the surface, 50 µm and 125 µm depths for the center of the image and 70% FOV lateral locations in the image. Measurement results are provided in Table 2. Comparing the edge response values at the center of the image, it can be seen that the addition of the corrective coverslip optimizes imaging performance at the 50 µm depth. Figure 7 shows the relationship between edge data, ERF fit and theoretical edge response.
Fig. 6. axial response for the 100x objective lens as a function of depth before (a) and after (b) coverslip correction. Notice the change in optimal location from the surface of the hemisphere in (a) to a depth of 50 µm in (b). Not only does the FWHM response improve, but the measured intensity increases. Also, the side lobes visible on the right side of the axial responses without correction (a) moves to the left side with correction (b) and can be seen to disappear around the 50 µm depth, reappearing on the right side in (b) at depth greater than 50 µm.

Table 2. Measured edge response using the hemispherical front piece (all units are µm). NA is 1.1 and 0.83 for the 100x and 50x objectives respectively. Values are given at the surface, 50 µm and 125 µm depths for the center of the image and 70% of the radial FOV. Values for 70% radial FOV were taken on both sides of the image center and averaged together. Shaded values correspond to the use of correction coverslip. For the 100x objective $A=1.35$ and for the 50x objective $A=2.91$. *Data for this location is not available.

| Objective | Axial Location | Theory | Center of Image | 70% FOV |
|-----------|----------------|--------|----------------|---------|
| 50x       | surface        | 0.53   | 0.64           | 0.69    |
|           | 50 µm          |        | 0.67           | 0.68    |
|           | 125 µm         |        | 0.73           | 0.72    |
|           | surface        |        | 0.67           | 0.67    |
|           | 50 µm          |        | 0.64           | 0.65    |
|           | 125 µm         |        | 0.65           | 0.67    |
| 100x      | surface        | 0.32   | 0.40           | 0.44    |
|           | 50 µm          |        | 0.49           | 0.53    |
|           | 125 µm         |        | 0.57           | *       |
|           | surface        |        | 0.50           | 0.52    |
|           | 50 µm          |        | 0.39           | 0.41    |
|           | 125 µm         |        | 0.49           | 0.51    |
5. In-vivo imaging results

Imaging in vivo was best achieved with the use of the hemisphere front piece. The lack of a solid interface between the aperture front piece and sample caused the sample to bulge into the aperture, creating a dependency between imaging depth and pressure against the sample. Because small changes in pressure caused the image plane to shift on the order of hundreds of microns, the hemisphere front piece was used exclusively for in vivo imaging of skin. Also, all images presented in this paper are shown as acquired by the data acquisition system, without post processing.

Fig. 8. In vivo confocal images of fingertip using the 100x objective lens left and 50x objective lens right in conjunction with the hemisphere front piece. The increased cellular detail with the 100x objective lens shows the improvement when using high NA when imaging skin. Image FOV height is 172 µm for the 100x image and 343 µm for the 50x image. The overlay CCD image shows the relative size of the CCD and confocal image areas, with the CCD image being approximately four times the width of the confocal image.
The flat bottom surface of the hemisphere front piece created an ideal interface for in vivo imaging. As seen in Fig. 8, images taken of a fingertip with the 100x objective show clear cellular detail while similar images taken with the 50x objective provide much less detail. This is not only due to a doubling of magnification with the 100x objective, but the increase in NA from 0.83 to 1.1 between the 50x and 100x objectives respectively. The large area CCD image of the skin surface in Fig. 8 shows the relation between the CCD image area and confocal imaging area. Field of view height is 343 µm and 172 µm for the 50x and 100x images respectively.

Visual confirmation of the cross sectioning ability of the handheld microscope can be seen in the video in Fig. 9(a). This video was taken using the 100x objective with hemisphere front piece, and shows a stepped axial scan of the fingertip of a Caucasian male. The depth increment with each step is 7 µm. Nuclei and cell membranes are clearly visible throughout the sample.

Comparison of the image areas and cellular detail visible with the two objective lenses is shown in Fig. 9(b) and Fig. 9(c). Fig. 9(b) was taken using the 50x objective with the hemisphere front lens, while Fig. 9(c) was taken with the 100x objective. Both are of the forearm region in a Caucasian male, and show capillary blood flow, confirming penetration to the epidermis-dermis boundary. An increase in dermal cellular structure detail is visible in the 100x video due to a doubling of magnification and NA (NA=1.1).

Fig. 9. Videos taken with handheld confocal microscope (Media 1, Media 2, Media 3). Cross sectioning capability of 100x objective is shown in (a) via stepped axial focusing, with focus increment of 7 µm. This video was taken on the fingertip of Caucasian male. Cellular detail in forearm region of Caucasian male is compared in (b) for the 50x objective and (c) for the 100x objective. In both (b) and (c) capillary blood flow is evident, confirming imaging penetration to the dermis. FOV height if 172 µm for 100x videos (a and c) and 343 µm for the 50x video (b). All videos were acquired using hemisphere front piece.
6. Discussion

Incorporating a MEMS scanner into a handheld instrument has the potential to dramatically decrease instrument size because of the small footprint of the scanner and also the capability for 2-axis scanning at a single reflection surface. Miniaturization of the confocal microscope is important for clinical use, facilitating handheld operation and enabling access to difficult skin locations. Because of the low mass of the MEMS mirror, high scan rates are possible. This will be important for vital confocal microscopy to minimize motion artifacts and to observe dynamic phenomena. The mirror that we used scans at 56 frames per second, adequate to observe blood flow in dermal capillary loops.

MEMS scanners have been demonstrated previously for confocal microscopy. The technology is maturing, however, and commercial development is accelerating. The mirror we used was engineered for laser-based video projection, which has performance criteria similar to scanned-beam microscopy. In particular, mirror flatness throughout the scan in the presence of large inertial forces is paramount, requiring optimized design of the mirror plate and flexures. Scan trajectory control must be accurate to much less than a pixel and scan timing must be similarly accurate at a sub-pixel level. The Microvision mirror incorporates integrated position sensing and closed loop control to achieve this level of performance. Next generation mirrors will add yet more resolution and will have smaller footprints, driven largely by the emerging market for so-called pico-projectors destined for cell-phone-sized packages. Vital microscopy will benefit from this continuing technology improvement, with highly compact scanners enabling handheld and even endoscopic platforms capable of high fidelity imaging.

Confocal reflectance imaging of skin benefits from use of high NA. Rajadhyaksha et al. showed that imaging performance is best with NA of 0.7 or greater [15]. In our setup with the 50x objective and hemisphere, the effective NA is about 0.5 due to the partial filling of the rear aperture, below the nominal minimum value for skin imaging. Lack of nuclear and fine cellular detail is evident when using the lower NA system (compare confocal images in Fig. 8 and Fig. 9). Using the lower NA 50x lens when imaging near the basal cell layer where capillary flow is visible, blood flow was sometimes evident as a blurring artifact which followed capillary paths. This artifact is likely due to inadequate cross sectioning ability and high side lobes of the axial PSF when imaging deep into the tissue, allowing highly scattering blood cells to contribute motional artifacts to the image even when the focal plane is above or below the capillary layer. By contrast, when imaging using the 100x objective, out of focus capillary flow is not visible in images taken above or below the capillary layer, while individual red blood cells are easily distinguished when the capillary layer is in the focal plane.

Our results indicate that when using higher NA, it will be important to correct for depth-dependent spherical aberration if one is to maintain cross sectioning ability and high contrast and sensitivity in the image. Figure 6 shows the clear improvement in the axial PSF with the 100x objective lens at a depth of 50 µm when a cover slip is inserted in the converging beam to partially compensate spherical aberration coming from the 50 µm water layer. In addition to a reduction of the FWHM of the PSF by more than a factor of two, the peak return signal increased by more than 60%. Managing aberration throughout the depth of interest will not only improve image quality but will maximize the depth at which useful imaging may be achieved. Variable compensation of depth-dependent spherical aberration is a topic of considerable interest for vital confocal microscopy, and our initial imaging results support the argument that spherical aberration will need to be managed for optimal performance of confocal microscopy of the skin or other tissues.

The polarization dependent detection employed in this instrument using a quarter wave plate near the objective lens is useful for suppressing reflections from the fiber end-face and from most of the lens surfaces. However, the optical return when imaging at increasing depth
into a scattering medium is reduced due to depolarization from increased scattering in the sample. An alternative configuration we investigated was use of a fiber circulator to separate the illumination and reflected light. While this proved useful when imaging strongly reflective objects, the return signal from skin was low enough that crosstalk in the circulator (specified to be less than 50 dB) caused interference artifacts and ultimately led us to prefer the polarization discrimination method as the best option for skin imaging using our instrument. With better cross-talk isolation the fiber circulator would provide an efficient and straightforward method of detection for fiber-based confocal imaging.

7. Conclusion

MEMS scan mirror technology has matured so that two-dimensional beam scanners are available with the speed, resolution and stability necessary to perform high-fidelity vital confocal microscopy. Because of their small size and ability to achieve two-axis scanning with a single reflection, these devices will enable the design of very compact scanning microscopes in handheld or endoscopic platforms. We have demonstrated this capability by constructing a MEMS-based confocal microscope that is handheld and that delivers nearly diffraction limited imaging with numerical aperture as high as NA=1.1. To our knowledge this is the first MEMS based confocal microscope to demonstrate in-vivo skin imaging at video rate with image quality comparable to galvanometer based microscopes. Skin images show the ability to penetrate through the epidermis and into the dermal skin layers in real time and with good image quality.

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