A multi-point scanner for high frame rate confocal microendoscopy

Andrew R. Rouse[a], Houssine Makhlouf[a,b], Anthony A. Tanbakuchi[a,b], Arthur F. Gmitro[a,b]

[a]Department of Radiology and [b]College of Optical Sciences, University of Arizona.

ABSTRACT

Slit-scanning geometries for confocal microendoscopy represent a compromise between acquisition rate and optical performance. Such systems provide high frame rates that freeze motion but recent Monte Carlo simulations show that scattered light severely limits the practical imaging depth for *in vivo* applications. A new multi-point scanning architecture for confocal microendoscopy has been developed. The new scanner is based on a relatively simple modification to the slit-scanning geometry that results in a parallelized point-scanning confocal microendoscope that maintains the high frame rate of a slit-scanning system while providing optical performance close to that of a single point scanning system. The multi-point scanner has been incorporated into an existing multi-spectral slit-scanning confocal microendoscope. The new confocal aperture consists of a slit and a rotating low duty cycle binary transmission grating, which effectively produces a set of continuously moving widely spaced illumination points along the slit. The design maintains the ability to rapidly switch between grayscale and multi-spectral imaging modes. The improved axial resolution of the multi-point scanning confocal microendoscope leads to significantly better confocal sectioning and deeper imaging, which greatly improves the diagnostic potential of the instrument.

Keywords: Microendoscopy, endomicroscopy, confocal, slit-scan, multi-point scan, optical biopsy, tissue scatter, axial resolution

1. CONFOCAL MICROENDOSCOPY

Confocal microscopy is an established method for imaging biological samples that has the ability to image selected depths within a sample while rejecting light from out-of-focus planes. In theory, this reduction in out-of-focus light allows one to image thick biological samples without the blurred background signal that degrades contrast. This "optical sectioning" also allows three-dimensional images to be reconstructed from a series of data obtained at varying depths.

The underlying technology of the benchtop confocal microscope has been extended to the application of in-vivo disease diagnosis through the use of endoscopic systems based on single mode fibers or fiber imaging bundles[1-8]. Such systems are typically called confocal microendoscopes or confocal endomicroscopes. The basic principle is to use a fiber to relay the focal plane of a confocal microscope to a remote location inside the body. Systems typically require a miniature objective at the distal tip to image the fiber into the tissue. As with benchtop systems, confocal microendoscopes are scanning optical systems. In some cases the scanning system is located in the proximal optical assembly and in other cases the scanning system is miniaturized and placed in the distal tip of the catheter.

The current state-of-the-art confocal systems at the University of Arizona's Biomedical Imaging Laboratory include a laboratory based multi-spectral confocal microendoscope and a clinical prototype grayscale confocal microlaparoscope[6-8]. The clinical system was specifically designed for in-vivo laparoscopic imaging of human ovary and it has successfully completed a 21 patient clinical feasibility study[6].

2. SCANNING GEOMETRIES AND OPTICAL SECTIONING

Confocal microendoscopes are scanning optical systems. In many cases, a point source is imaged to a point in the sample, and emitted fluorescence is re-imaged back to a pinhole aperture and a detector. The optical sectioning ability

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stems from the fact that light emanating from an out-of-focus point in the sample is largely rejected by the pinhole aperture. A two-dimensional image is built up by raster scanning the point illumination across the sample. This geometry can produce excellent axial resolution for high NA beams but typically requires relatively slow frame rates.

In an effort to increase throughput and frame rates, many confocal systems employ a line scanning geometry. Under this type of scanning mechanism, an anamorphic optical system shapes a laser source into a line of illumination that is scanned in one direction across the sample. Emitted fluorescence is re-imaged back to a slit aperture and a one- or two-dimensional CCD camera for signal detection.

The confocal microendoscope systems at the Biomedical Imaging Laboratory are based on a slit scanning geometry. The scanning system is simple and robust, and it provides the confocal microendoscope with high throughput and video rate imaging (30 frames-per-second). The tradeoff is some loss of performance in the axial resolution of the system. This was thought to be a fair tradeoff given that the theoretical axial performance of a slit scanning system is adequate for most imaging applications. Wilson[9], a pioneer in the characterization of confocal systems, stated that an ideal slit scanning confocal system should underperform an ideal point scanning system by approximately 40%. Given the high theoretical performance of a point scanning system, this 40% loss is often deemed acceptable. However, a recent Monte Carlo study[10] has concluded that the true resolution of a confocal system is dominated by tissue scatter and that theoretical non-scattering resolution predictions are not applicable to imaging in tissue. More importantly, the Monte Carlo simulations revealed that the slit scanning geometry is significantly more sensitive to the degradations caused by tissue scatter. These findings help to explain the discrepancy between the theoretical axial resolution of our system and the performance that we have observed in attempting to image below the surface of turbid media such as tissue.

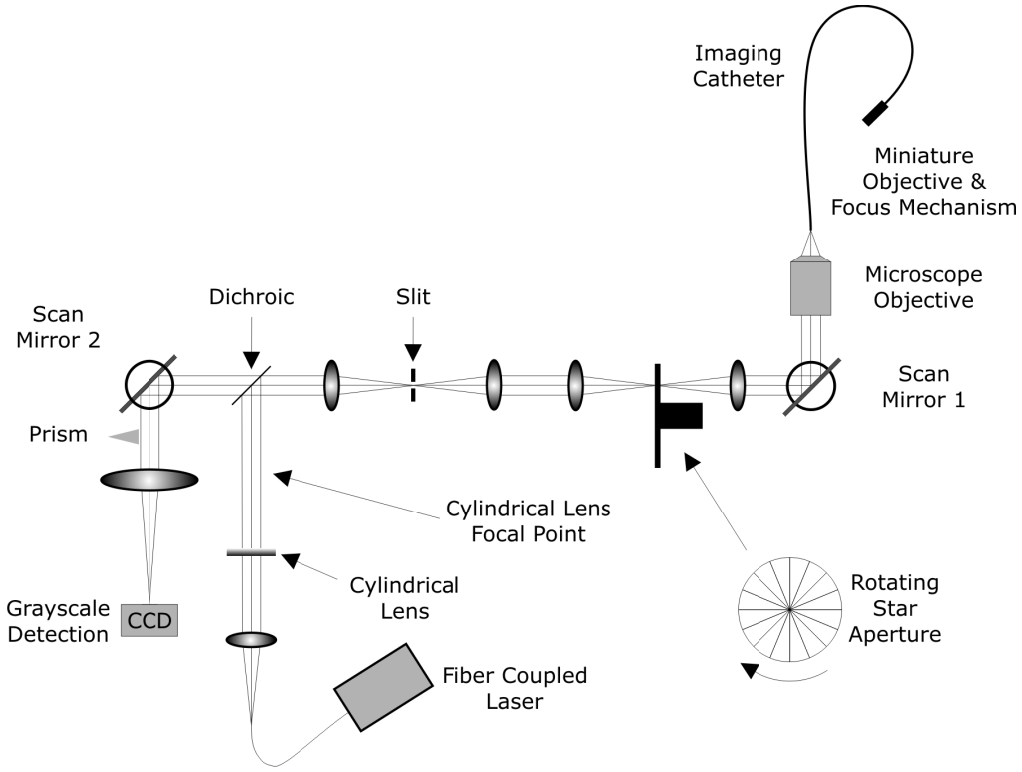

Figure 1. Layout of the parallelized point scanning confocal microendoscope. An anamorphic optical system forms a line of illumination at the slit plane. This line is relayed to the rotating star aperture. The combination of the slit and spinning aperture produces a line of pinholes that scan rapidly in one direction (in/out of page). The other axis of scanning is provided by the scan mirrors.

3. THE NEW PARALLELIZED POINT SCANNING GEOMETRY.

An ideal scanning system for in-vivo confocal microendoscopy would combine the best attributes of point and slit scanning geometries. In this paper, we introduce a new parallelized point scanning architecture that provides lateral and axial resolution comparable to that of a point scanning system while maintaining the high frame rate of a slit scanning geometry. The new technique involves a relatively minor change to the layout of the slit scanning confocal microendoscope but promises to yield significant improvements in axial resolution and better depth imaging in tissue.

Figure 1 shows the layout of the new multi-point scanning system. As with our previous slit-scan system, a 488nm solid state laser and an anamorphic optical system form a line of illumination at the slit plane. Two new lenses image the slit onto a rotating star aperture. The star aperture consists of a radial pattern of evenly spaced slits with a 10% duty cycle. By imaging the laser through the slit and star aperture we create a source profile made up of a line of uniformly spaced points. This profile is then imaged onto the proximal face of a 30,000 element fiber bundle (every 10^{th} pixel is illuminated) that relays the excitation points to the tissue. As the star aperture spins, these illumination points effectively provide the fast axis of a two dimensional raster pattern (in/out of the page in Figure 1). The slow axis is provided, as before, by the scan mirror located between the star aperture and the catheter (Scan Mirror 1 in the figure). A Full 2D image is generated at 30 frames-per-second by running the scan mirror back and forth at 15Hz while constantly spinning the star aperture. The spacing of the slit pattern on the rotating star aperture was chosen to provide point-scan like axial resolution to this system at a tissue depth of 100μm (based on Monte Carlo simulations). Very importantly, the new multi-point scanning geometry does not alter the fundamental slit-scanning layout. This allows us to maintain the multi-spectral and OCT aspects (see paper is 7558-19) of this multi-modality system.

4. CHARACTERIZATION OF THE MULTI-POINT SCANNER

The performance of the new scanning system was initially tested on a thin fluorescent planar target made from a mono-layer of 0.5μm diameter fluorescent beads adhered to a microscope slide. This phantom was imaged in 1μm steps through focus for both the normal slit scanning system and the new multi-point scanning system. The results of this experiment are shown in Figure 2. A small region of interest in the center of each image was averaged to plot the axial response of each system. The resulting axial resolution (FWHM) of the multi-point system was 7μm compared to 25μm for the original slit scanning geometry. This dramatic improvement in axial resolution was obtained while maintaining the 30 frames per second acquisition speed of the previous design.

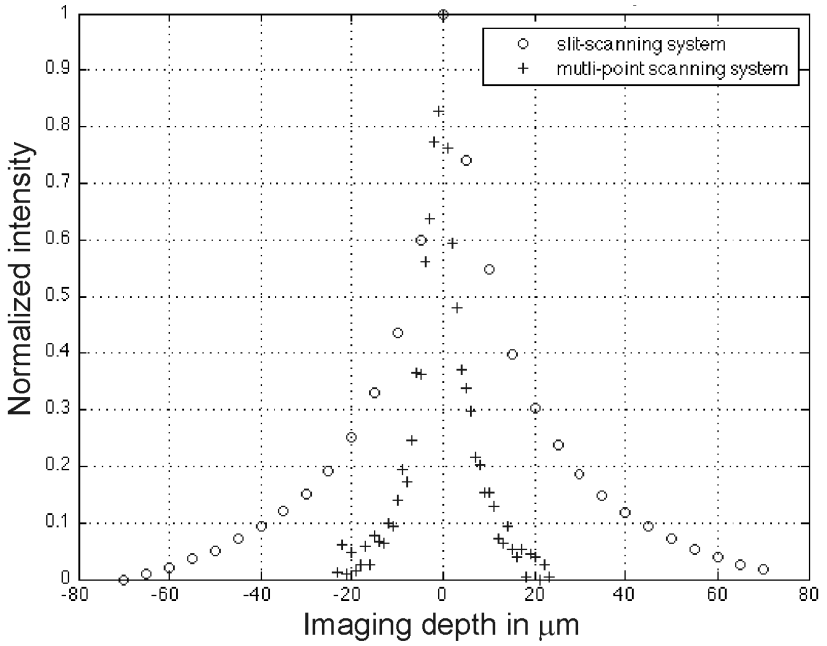

Figure 2. Comparison of the axial PSF of the slit scanning and new multi-point scanning geometries for the confocal microendoscope. Data collected from a single-layer fluorescent target scanned through focus. FWHM of the multi-point scanner is 7μm compared to 25μm for the conventional slit scanning system.

5. RESULTS

The prototype multi-point scanning confocal microendoscope was initially tested on a phantom made from cotton lightly soaked with acridine orange. The images in Figure 3 qualitatively compare the imaging performance of the new multi-point scanner to the original slit-scanning system. The figure shows images from the surface of the cotton and from a depth of roughly 40μm. The only change that was made when transitioning between the two scanning modalities was the addition or removal of the spinning aperture. The approximately 10-fold reduction in illumination power is evident in the images obtained with the multi-point scan system. However, even with this reduction in signal, the new multi-point scanning architecture shows a dramatic increase in contrast between in-plane features and the out-of-focus background. We attribute this improvement in imaging performance to the reduction of scattered light collection afforded by the multi-point scanner.

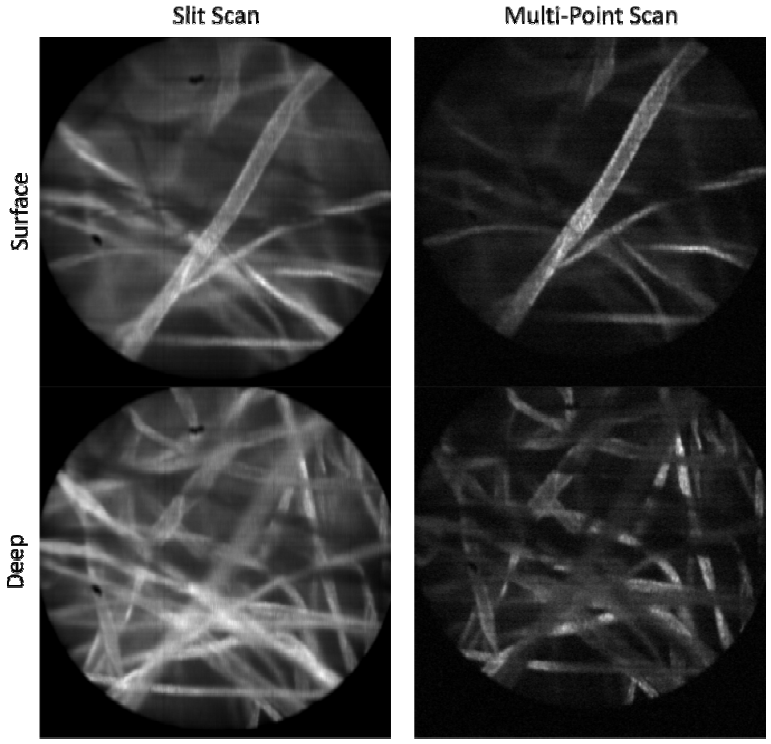

Figure 3. Imaging comparison between slit scan and multi-point scanning geometries. Data collected from a cotton phantom stained with acridine orange. The top and bottom rows represent two focus positions in the phantom. The approximately 10 fold decrease in illumination intensity is evident in the multi-point scan data. However, the multi-point scanning confocal microendoscope seems to provide noticeably improved contrast and out-of-plane rejection when compared to the slit scanning system.

Figure 4 shows data collected from a euthanized mouse using the new multi-point scanning system. All tissues were stained with acridine orange. Results show very high contrast images that clearly reveal differences in cell morphology between tissue types. Individual cell nuclei stand out in each of the examples. Figure 5 includes a pair of images acquired during the same experiment that provide a comparison between the performance characteristics of the slit scanning and multi-point scanning geometries. As with the cotton phantom, the image acquired with the multi-point scanner shows a decrease in signal power due to the 10% duty cycle of the spinning star aperture. However, even with this loss of illumination power, the multi-point scanning system reveals individual cells and cell nuclei with significantly higher contrast and higher resolution than its slit scanning counterpart.

6. CONCLUSION

There is an inherent tradeoff between the fast acquisition speeds of slit scan confocal microendoscopy and the improved axial resolution of point scanning systems. The multi-point scanning architecture introduced in this paper combines the technology of slit and point scanners and allows one to maintain the high frame rates of slit scanning systems while obtaining axial resolution similar to that of a point scanning system. The technology is still underdevelopment but early results clearly demonstrated the power of multi-point scanning confocal microendoscopy.

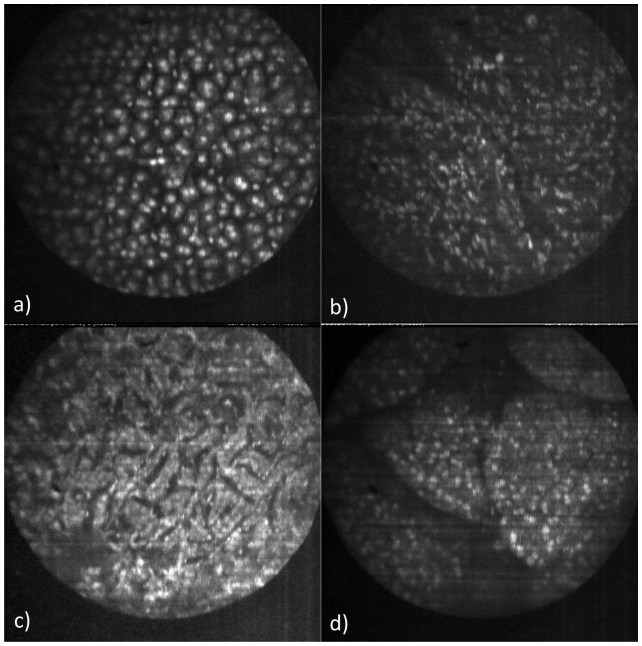

Figure 4. Euthanized mouse stained with acridine orange and imaged with the multi-point scanning confocal microendoscope. Images show a) liver, b) heart, c) kidney, and d) liver. The new system provides high contrast images that clearly reveal differences in cell morphology between tissue types.

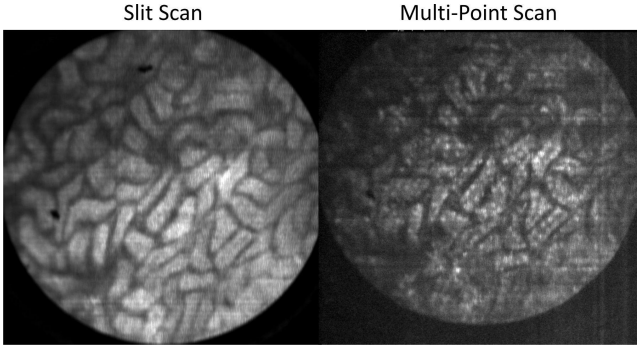

Figure 5. Euthanized mouse stained with acridine orange and imaged with the slit scanning and the multi-point scanning confocal microendoscope. The two images are from identical regions in the tissue. The image from the multi-point scanner shows lower signal but dramatically more cellular detail due to the improvement in axial performance.

7. ACKNOWLEDGEMENTS

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