Multispectral imaging with a confocal microendoscope

Andrew R. Rouse and Arthur F. Gmitro

Department of Radiology and the Optical Sciences Center, University of Arizona, Tucson, Arizona 85724

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The concept of a multispectral confocal microscope for in vivo imaging is introduced. To demonstrate the concept we modified a slit-scan fluorescence confocal microendoscope incorporating a fiber-optic catheter for in vivo imaging to record multispectral images. The system was designed to examine cellular structures during optical biopsy and to exploit the diagnostic information contained within the spectral domain. Preliminary experiments were carried out in phantoms and cell cultures to demonstrate the potential of the technique.

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Benchtop confocal microscopes are widely used to create high-quality optical images of biological samples. The fundamental characteristic of the confocal microscope is the ability to reject light from out-of-focus planes and provide a clear in-focus image of a thin section within the sample. This optical sectioning property is what makes the confocal microscope ideal for imaging thick biological samples.

Confocal microscopes have been adapted for in vivo imaging of skin, cornea, teeth, and cervix. For imaging deeper within the body, specialized endoscopic systems based on vertical-cavity surface-emitting laser arrays, micromachine scan mirrors, single fibers with dispersive elements, and coherent optical fiber bundles have been developed.

Confocal microscopes typically operate as epi-illumination systems in fluorescence or reflectance. However, fluorescence has emerged as the primary imaging technique because of the sensitivity and targeting specificity of fluorescent probes. The emission characteristics of many fluorescent probes used in microscopy are affected by the local environment in the sample. Therefore, multispectral fluorescence imaging provides the ability to determine properties of the local environment in a spatially resolved manner. Confocal microspectrofluorometers have been developed to measure spatially resolved fluorescence spectra and have been used to study drug–target interactions, ion concentrations, and pH levels in living cells. However, these devices are essentially benchtop systems with slow mechanical scanning and offer no solution for evaluating remote in vivo locations. In this Letter we present and demonstrate the concept of a multispectral confocal microendoscope (MCME) for remote in vivo imaging.

The MCME combines the features of a catheter-based confocal microscope with those of a microspectrofluorimeter. It has three main components: the illumination system, the fiber-optic catheter, and the detection system. The illumination consists of a light source, which is focused and scanned across the proximal end of a fiber-optic imaging bundle. The fiber-optic catheter transfers the scanned illumination profile to the distal, in vivo end of the catheter. A miniature objective lens images the distal end of the fiber bundle into the tissue, and a focusing mechanism controls the depth of the image plane. Induced sample fluorescence is imaged back through the catheter to the detection system and descanned onto the confocal aperture. Light transmitted by the aperture is dispersed (by a prism or a grating) and imaged onto an array detector.

To demonstrate the concept of the MCME we modified a slit-scan confocal system developed previously. The system was configured to collect spectral information across a 286-nm wavelength range centered at 600 nm with a spectral resolution of approximately 11 nm. However, the design is flexible and can be adapted to vary these spectral parameters. Figure 1 shows a layout of the MCME. An argon-ion laser operating at 488 nm and an anamorphic optical system produce a line of light that is scanned in one dimension across the proximal end of the fiber-optic catheter.

The catheter consists of a fiber-optic imaging bundle, a miniature objective, and a hydraulic focusing mechanism. The fiber bundle contains 30,000 optical elements with 3-μm center-to-center spacing. The overall diameter of the fiber is 1 mm, with an active image diameter of 720 μm. The miniature F/1 achromatic objective has a nominal magnification of 1.67 from tissue to fiber. A hydraulic focusing mechanism moves the distal face of the fiber with respect to the objective, which is in contact with the tissue. This mechanism allows focus control to 200 μm below the surface of the tissue.

![Fig. 1. Layout of the MCME.](image-url)
We modified the detection arm of the microendoscope to collect multispectral data by placing a collimating lens and a dispersing prism after the confocal slit. The dispersed light is imaged onto a cooled 512 × 512 CCD. At a fixed position of the scan mirror the two-dimensional light distribution on the CCD represents one (vertical) dimension of spatial information and one (horizontal) dimension of spectral information. A zoom lens mounted on the camera is adjusted such that the spatial extent of the image covers 256 pixels in the vertical dimension. At this magnification, the image of the slit aperture on the CCD is approximately 1 pixel wide. An 18° wedge prism is used to produce a smear in the spectral dimension of 26 pixels over a 286-nm range centered at 600 nm. The 256 × 26 pixel region of interest is read out of the CCD in 16 ms using a 1-MHz 12-bit digitizer. The scan rate of the mirror is adjusted so that the illumination moves one spatial resolution element during the 16-ms integration period. As the mirror is scanned, 256 frames of data are read out in 4.1 s to produce the full three-dimensional data set.

In terms of imaging performance, the spatial resolution of the MCME is essentially the same as that of the confocal microendoscope, since the components that determine the spatial resolution are unchanged. The lateral resolution of the MCME is fundamentally limited by the pixel spacing in the fiber bundle. The center-to-center spacing of the fiber pixels is 3 μm. The magnification of the distal objective is 1.67, yielding a theoretical lateral resolution limit of 1.8 μm in the tissue, which is the measured value within experimental error. The axial (or depth) resolution of the slit-scan confocal system, as measured by the response to a planar object scanned through focus, is 25 μm.

The spectral resolution in the MCME is determined by the dispersing power of the prism and the imaging optics in the detection arm. For the results presented here, a spectral range of 286 nm centered at 600 nm was spread over 26 pixels on the CCD. Figure 5 shows a plot of the spectrum obtained with light from a He–Ne laser fed back into the system. The FWHM of the distribution is 1.0 CCD pixel, showing that the spectral resolution of the system is indeed 11 nm.
We obtained the fine sampling of this distribution by tilting the CCD camera and collapsing the intensity distribution along the direction of the line image. The spectral resolution in the system can be adjusted by a change in the prism wedge angle or by variation of the tilt angle of the prism. Alternatively, the system can be configured with a diffraction grating if significantly higher spectral resolution is desired. The spectral range is also easily varied by altering the number of pixels read out of the CCD along the spectral dimension.

The frame rate of the system is currently limited by the readout speed of the CCD. A 256 × 256 × 26 multispectral image takes approximately 4 s to acquire. Higher frame rates can be achieved with a faster CCD. However, there is a trade-off among spectral sampling rate, data-acquisition speed, and signal-to-noise ratio. The data presented here represent a reasonable compromise for fluorescence imaging of cells in culture with AO as the fluorescent dye. However, specific applications may require coarse spectral sampling, which can be achieved with faster frame rates and (or) an improved signal-to-noise ratio. The optimum trade-off between these parameters will, of course, depend on the specific application.

Clearly, there is information stored in the spectral domain. Multispectral imaging systems, which usually employ switched spectral filters, have already shown promise for in situ diagnosis. The MCME discussed here provides high spatial resolution, the depth discrimination of a confocal system, and the potential for fine and optically efficient sampling of the spectral domain. Exactly how much spectral information is needed and how it is used are areas of continuing research. However, the exciting results being obtained with confocal microspectrofluorometers point the way toward applications that can now be extended to in vivo imaging of remote locations inside animals and humans. We believe that multispectral imaging can be used to improve the diagnostic utility of the in vivo confocal microendoscope. Color reconstructions appear to enhance image contrast and may be valuable for real-time in vivo visualization. Spectral data might be processed to yield functional information, such as pH maps, or to provide automated disease diagnosis.

In conclusion, we have introduced the concept of a multispectral confocal microendoscope for in vivo imaging. To demonstrate the principle we modified a slit-scan confocal microendoscope incorporating a fiber-optic catheter to collect multispectral data. Preliminary experiments demonstrated the ability of the system to provide high-quality multispectral images of live cells. Demonstration of the clinical utility of the instrument will require further work.

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References