

# Multispectral Endoscopic Imaging Enabled by Mapping Spectral Bands into the Time Domain

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**Abstract:** Rapid multispectral confocal imaging is performed with a single shot-limited detector. This approach uses fiber delay lines to map spectral bands into the time domain, and has been integrated with fiber bundles for endoscopic applications.

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## 1. Introduction

In-vivo, or fresh ex-vivo, biomedical imaging must be done rapidly. In many commercial products, long integration times allow pixel arrays and gratings to provide quality images in many color bands. Our imaging spectrometer uses serial arrays of reflecting fiber tips, delay lines between these elements, and a single element detector [1]. This approach merges fast spectroscopy with standard spatial scanning to create datacubes of weak signals in real time. Each spectrum is acquired in a few microseconds. These attributes are critical during real-time applications such as intra-surgical margin delineation and for ex-vivo studies before cell death. This paper describes an optical design for multispectral imaging of low light level fluorescence from endogenous fluorophores.

## 2. Optical Design

A schematic of our fiber based multispectral imaging system is shown in figure 1(L). Excitation is provided by a pulsed 488 nm or 405 nm laser beam, which propagates through the spatial scanners towards the tissue sample. As the two mirrors are scanned in an x-y raster pattern, three key planes are always conjugate - the distal end of the endoscope, the proximal end of the endoscope, and the entrance face of the multimode fiber that is connected to the beam splitter assembly. Since fluorescence from biological materials typically occurs within 1 to 5 nsec after excitation, both reflected and photoexcited signals propagate back through the x-y scanners to the fiber entrance face (the confocal pinhole) before the mirrors can move appreciably. This allows the mirrors to de-scan back propagating photons such that light from the focused spot on the sample indeed stays conjugate with the confocal aperture.

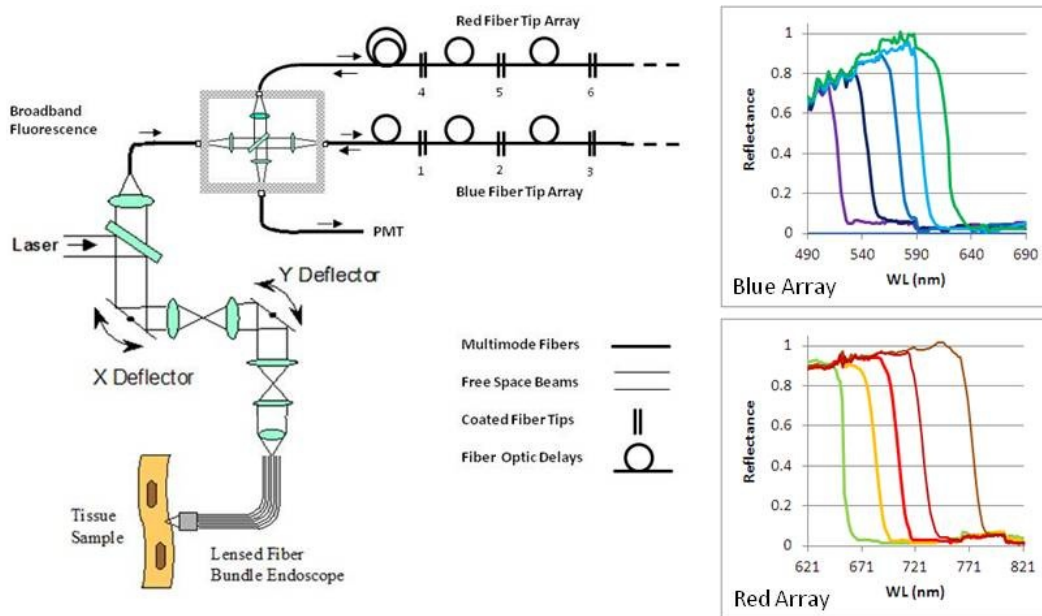


Fig. 1. Multispectral Endoscopic Imaging System (L), and Fiber Tip Reflectance (R)

After excitation by the laser pulse, broadband fluorescence from a biological tissue sample propagates into the detection array via the confocal aperture. After a color neutral 50/50 split into the red and blue arrays, the shortest wavelength light reflects from the first fiber tip, and the longest wavelength light reflects from the 10th fiber tip (figure 1(R)). The resulting signal is a series of ten time-indexed wavelength bands. The red fiber tip array contains an extra delay line equal in length to the entire blue fiber tip array, so the time indexing is seamlessly maintained. Propagation time through both fiber arrays matches the dwell time of one pixel in the spatial scans, while the propagation time between adjacent coated fiber tips matches the length of the laser pulse. The system depicted in figure 1(L) performs microscopy when a sample is placed at the focus of the objective lens, and microendoscopy when the proximal end of the endoscope is placed in the same location. For endoscopy, a coherent fiber bundle with 10,000 to 30,000 three or six micron diameter fibers is placed under the lens (see images at right of figure 2).

There are two key fiber optic issues in the above design. First, the serial arrays of coated tips are constructed with multimode fiber (typically 62.5 micron cores). This allows for acceptable collection efficiency. The coated tips perform as seen in figure 1(R) with the internal angles expected for multimode fiber [2]. Second, the fibers in the endoscopic bundle are composed of pure silica cores and fluorine doped clad glass. This avoids fluorescence from doped cores, which can mask the tissue fluorescence. The left side of figure 2 shows that this effect is significant across most of the visible spectrum. The effect is seen in doped-core fiber-bundles from multiple vendors [3].

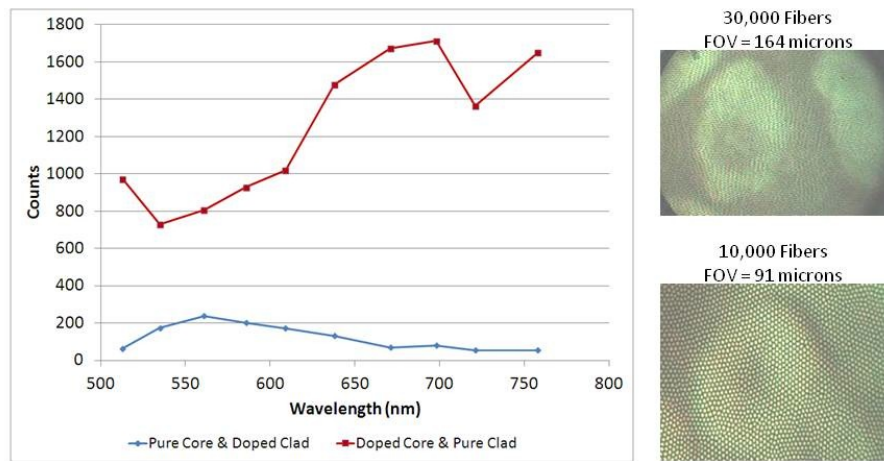


Fig. 2. Laser (488nm) Induced Fluorescence of Two Fiber Bundles with no sample (L), and with Mouse Kidney (R)

### 3. Applications

Useful biomedical images of low light endogenous fluorescence have been taken using these fiber enhancements. Reflections from each of the ten spectral tips depicted in figure 1 are corrected for cross-talk and detector response and used to generate raw datacubes. These datacubes are processed with a spectral angle mapping algorithm to create a single false color image displayed in real time. The false colors indicate a smooth variation from healthy to diseased tissue, and relate to chemical and physical aspects of the cellular environment. Experience has shown that these images must be acquired rapidly before spectral signatures change with cell death. These endoscopic bundles fit inside typical core biopsy needles – enabling future non-operative sampling of breast and lymph tissue.

### 4. References

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