Multiple-channel spectrally encoded imaging

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Abstract: Spectrally encoded endoscopy (SEE) uses miniature diffractive optics to encode space with wavelength, allowing video-rate three-dimensional imaging through sub-millimeter, flexible endoscopic probes. Here we present a new approach for SEE in which the illumination and the collection channels are separated in space, and spectral encoding is present only in the collection channel. Bench-top experiments using spatially incoherent white light illumination reveal significant improvement in image quality and considerable reduction of speckle noise compared to conventional techniques, and show that the new system is capable of high sensitivity fluorescence imaging of single cells. The presented new approach would allow improved functionality and usability of SEE.

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References and links

1. Introduction

Miniaturization of instrumentation for minimally invasive clinical intervention is a current trend in medicine, pressured forward by the constant advance in science and technology. The recent progress in solid state imaging technology allows current state-of-the-art endoscopes to perform various intervention and surgical procedures guided by high-resolution, real-time imaging deep inside the body. Since the mid-80’s, the remarkable progress in fiber optics and photonics technologies have opened new opportunities for imaging inside the body through more compact endoscopic instruments, owing to the small diameter and flexibility of silica optical fibers. Fiber bundle endoscopes [1–4] have made a considerable impact in clinical applications such as ductoscopy [5,6], embryoscopy [7], and angioscopy [8] which require imaging through small diameter probes. The use of a single optical fiber and a scanning mechanism at the distal end of an endoscope [9–11] eliminate pixelation artifacts and improve probe flexibility, at the expense, however, of the bulk of the mechanical scanning apparatus at the distal end of the endoscope. Spectrally encoded endoscopy (SEE), first presented in 1998 by Tearney et al. [12], uses wavelengths from a broadband source to encode a single lateral axis on the sample, while the second transverse dimension is scanned by slow probe rotation. SEE has been shown promising for high speed confocal microscopy [13–15] and for video-rate three dimensional endoscopic imaging through sub-millimeter, flexible probes [16–18].

In its current mode of implementation [17], SEE has several limiting factors which need to be addressed before its clinical promise could be realized. First, the use of wavelength to encode space imposes some difficulties on wavelength-sensitive imaging modalities. For example, fluorescence spectrally encoded imaging required a sophisticated optical setup for frequency-encoding [19]. Additionally, the use of spatially coherent illumination through a single mode fiber causes pronounced speckle noise, small depth of field, and poor signal collection efficiency which often requires the use of lasers, supercontinuum generation sources, or high power super-luminescent diode arrays. One possible solution for addressing these issues includes the use of a double-clad fiber [20] for spatially coherent sample illumination and incoherent signal collection. While double-clad SEE was demonstrated capable of speckle-free imaging with large depth of field, the endoscopic probe itself suffered from significant cross-talk between the illumination and the collection channels. Back reflections from the probe’s optics, which were efficiently collected by the large area and the high numerical aperture of the inner cladding, resulted with high image noise and required continuous background subtraction during image acquisition.

2. Multiple-channel SEE

Here we show a new approach for SEE, termed multiple-channel SEE (MC-SEE), which addresses many of the image quality concerns of SEE and further expands its functionality. While current forms of SEE involve spectral encoding in both the collection and illumination channels independently, effective encoded imaging is still feasible using only one encoded channel. The concept of imaging with a single encoded channel is schematically illustrated in Fig. 1, showing space-to-wavelength encoding of broadband light (e.g. fluorescence) emanating from a specimen.
At the sample, the spatial interval $\delta x$ along the $x$ axis near $x_0$, is related to the wavelength interval $\delta \lambda$ by [21]:

$$
\delta x = \frac{G \cdot f_1 \cdot \delta \lambda}{\cos \theta_0},
$$

where $\theta_0$ denotes the Littrow’s angle, $G$ denotes the grating groove density, and $f_1$ denotes the focal length of the imaging lens $L_1$. Neglecting optical aberrations, the lateral resolution $S_x$ at the center field of view is given by

$$
S_x = 1.029 \frac{\lambda f_1}{D},
$$

where $D$ denotes the diameter of the imaging lens $L_1$. Note that the resolution element is approximately 35% larger (resolution is lower) than that obtained with previous demonstrations of SEE ($S_{confocal} = 0.738 \lambda f_1 / D$ [21]) which were inherently confocal. The number of resolvable points $N_x$ along the $x$ axis would thus be smaller than in confocal SEE [21] by 35%:

$$
N_x \approx \frac{\sigma \lambda_0 GD}{\lambda_0 \cos \theta_0},
$$

where $\lambda_0$ denotes the center wavelength incident on the grating at Littrow’s angle $\theta_0$, and $\sigma_\lambda$ denotes the total spectrum emanating from the sample, which could originate from various sources such as reflectance of broadband illumination through a separate light guide, fluorescence excited by narrowband illumination, and other forms of broadband tissue luminescence.

Several bench-top experiments were conducted in order to study different sample illumination configurations (Fig. 2): wide-field (not encoded) front illumination, wide-field back illumination simulating diffuse light emanating from the tissue surface, and spectrally encoded illumination which utilized the imaging lens-grating pair but delivered through a separate multi-mode optical fiber. The spectrally encoded collection channel was comprised of a single-mode optical fiber (Nufern, S405-HP), an imaging lens (25 mm focal length, 25 mm diameter), a transmission diffraction grating (Wasatch photonics, 1200 lines/mm, maximum diffraction efficiency at 570 nm), and a spectrometer comprised of a collimation objective lens (Leica, Aacho 0.1 NA), a diffraction grating (Wasatch photonics, 1800 lines/mm, maximum diffraction efficiency at 532 nm), a multi-element lens (Nikon AFC, 50

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Fig. 1. Single channel spectral encoding. Schematic drawing of the optical paths in single channel space-to-wavelength encoding, illustrating the collection of a single wavelength from each lateral point on the sample.
mm focal length) and a high sensitivity back-illuminated electron multiplication charged coupled device (EMCCD) camera (Andor, DU970N-BV). Both grating-lens pairs at the two ends of the fiber were optimized to transmit wavelengths in the range of 450-650 nm, with 550 nm at Littrow’s angle.

Fig. 2. Schematic drawing of the experimental setups with front, back, and spectrally encoded illumination.

Front and back illumination of the sample were accomplished by directing a beam of white light (150W Halogen lamp) to the sample using a fiber bundle light guide (Schott ACE, A20500). The total power illuminating circular spot approximately 5 mm in diameter on the sample was 150 mW in the visible and the near infrared regions. We estimate that less than 1% of the illumination was overlapping with the imaged spectrally encoded line. Scanning in the direction perpendicular to the spectrally encoded line (x-axis) was achieved by moving the sample using a motorized translation stage. Exposure time was 100 ms per single line. MC-SEE images of a USAF-1951 resolution target printed in black ink on white paper (Newport, RES-2) using front and back sample illumination are shown in Figs. 3a and 3b, respectively. The images look nearly identical, with negligible speckle noise and resolution of approximately 64 line-pairs per millimeter (group 6, element 1) at the center field of view, which was limited primarily by the number of pixels (1600 horizontal pixels) in the spectrometer’s EMCCD. Some blurring, ghost pattern and a drop in resolution by a factor of 1.5 is noticeable in Figs. 3a and 3b, most likely caused by optical aberrations in the spectrometer’s grating. In order to demonstrate the advantages of spatially incoherent illumination over coherent illumination, the system was readjusted to employ titanium-sapphire laser illumination (Femtolasers Produktions GmbH, Rainbow) and the experimental setup was optimized for the near-infrared region of the spectrum (650-950 nm), including the replacement of the spectrometer’s grating (Wasatch photonics, 1800 lines/mm, maximum diffraction efficiency at 840 nm), the imaging grating (Wasatch photonics, 1200 lines/mm, maximum diffraction efficiency at 830 nm), and the single-mode optical fiber (Nufern, 780-HP). MC-SEE images of a portion of a 1 Euro cent coin using spectrally encoded coherent and incoherent front illumination are shown in Fig. 3c and Fig. 3d, respectively. Comparison between the two images reveal significant reduction in speckle noise using incoherent illumination (Fig. 3d), resulting in a more natural appearance with better discrimination of surface texture.
3. Fluorescence MC-SEE imaging

Fluorescent markers are widely used in biomedicine, and play invaluable roles in many clinical diagnostic applications. Yet, the low overlap between the excitation and emission spectra prohibits standard SEE from imaging fluorescence. In a previous work [19], an additional encoding interferometer was used to modulate the excitation wavelengths in SEE, so that the fluorescence signal could be spatially decoded based on the wavelength-dependent temporal modulation frequencies. In MC-SEE the additional interferometric frequency encoding is not required; instead, a conventional excitation light source, either narrowband or broadband, could be used to excite fluorescence which would then be collected and imaged using spectral encoding. In order to demonstrate fluorescence imaging using MC-SEE with a single spectrally-encoded channel, we replaced the imaging lens with a lens that allowed higher numerical aperture imaging (NA = 0.25, 11 mm focal length, 5.5 mm clear aperture) to improve resolution and signal collection efficiency.
An image of a USAF-1951 fluorescence resolution target (Edmund Optics, N57-792) was acquired using incoherent excitation light (Nikon Fiber Illuminator, 130 W mercury lamp) and a 360-440 nm bandpass excitation filter (Thorlabs, FB400-40), with exposure time of 100 ms per line (Fig. 4a). Along its wavelength (horizontal) axis, the fluorescence image revealed an intensity profile which resembles the emission spectrum of the fluorophore, resulting with a field of view of approximately 1.5 mm in the x axis. The fluorescence image shows resolution of approximately 144 line-pairs per millimeter (group 7, element 2), somewhat lower than the predicted value of 230 line-pairs per millimeter, most likely due to setup misalignment or some imperfections at the spectrometer’s diffraction grating. To demonstrate the potential of our approach to image weak fluorescence signals of biological specimen, we have imaged a culture of fixed human epithelial cells of breast adenocarcinoma origin (MDA-MB-231), whose membranes were labeled with a green fluorescent marker (DiOC$_{18}$), using a 465-495 nm bandpass excitation filter (Chroma Technology Corp.). The resulting MC-SEE fluorescence image of the cells, acquired with 500 ms line exposure time, is shown in Fig. 4b, next to an image of the same field of view using a conventional epi-fluorescence microscope (NA = 0.45, Fig. 4c). While the numerical aperture of the SEE lens (NA = 0.25) and the signal-to-noise ratio (6.3 dB) were too low to resolve sub cellular structures, single cells could be easily resolved and a good match was obtained between the two images. Non-uniformities in signal brightness in Fig. 4c are mainly attributed to photobleaching which occurred during the course of the experiment.

4. Discussion

The presented new approach for spectrally encoded imaging addresses several key challenges in this technology, including high back reflections from the probes, inefficient fluorescence encoding, and pronounced speckle noise. Using only a single encoded channel in the collection path, back-reflections from interfaces within the imaging probe could be minimized, as well as undesired coupling of the illumination light into the collection optical path. As a consequence of the low background signal, high sensitivity low noise cameras could be utilized. In our experiments, an electrically cooled EMCCD camera was used, capable of line rates of up to 1500 Hz, potentially allowing real-time, high resolution imaging. When illuminations is efficient and signal levels are sufficiently high, our
spectrometer allows capturing speeds of up to 2.5 frames per second, where each frame contains 800 x 600 pixels. Using white light incoherent illumination, MC-SEE images show significant improvement in quality with complete elimination of speckle noise and natural appearance. Similar image characteristics were previously obtained using SEE with a single double-clad fiber [20], however, the use of such fibers often required tedious alignment procedures of the optical setup, including the use of cross-polarizers to eliminate reflections from the fiber interfaces, spatial mode filtering to reduce coherent light collection through the fiber’s core, and real-time background subtraction due to varying back-reflections from the probe optics (which could not prevent the increase in shot noise).

However, low signal levels such as those emitted from fluorescence markers, would require longer exposure times and could reduce frame rates. A main cause for the relatively weak fluorescence signals is the fairly inefficient signal collection: using wavelength-encoding for imaging resulted in collection of only a small fraction $d\lambda/\sigma_\lambda = 1/N_s$ of the broadband fluorescence signal, where $d\lambda$ denotes the local bandwidth at each resolvable point. Several straightforward measures could be taken in order to improve signal efficiency, including the incorporation of a more intense spatially coherent supercontinuum source, and the use of dedicated line cameras specifically designed for the detection of low light levels at high line rates. When using less power-efficient broadband sources such as those used in this work, additional optics in the illumination channel such as cylindrical lenses, optical diffusers and diffraction gratings would improve spatial overlapping between the illumination and the imaged line. The physical characteristics of the fluorescence marker dictate not only its effective brightness, but also determine the field of view along the wavelength axis $\Delta X$, which is given by:

$$\Delta X = \frac{G_f \sigma^{in}_{\lambda}}{\cos \theta_e},$$

(4)

where $\sigma^{in}_{\lambda}$ denotes the emission spectrum of the fluorophore. Increasing the field of view would be possible by using fluorescent markers with broader emission spectra or, alternatively, by using multiple fluorophores with overlapping excitation spectra and complementary emission spectra, for labeling similar sites at the sample.

Finally, MC-SEE probe designs would have to include the illumination path either as an integral part of the imaging probe, or as separate endoscopic probes. While in previous implementations of SEE the illumination and collection paths were inherently aligned in space, MC-SEE would require careful alignment of the illumination and imaging channels to assure high signal efficiency. Using two fibers in a single probe could also be challenging in applications that demand full probe rotation, and would then require the use of a multi-channel rotary joint.

In summary, a new approach for spectrally encoded imaging is experimentally demonstrated, utilizing a single spectrally encoded imaging channel and a separate illumination channel. In addition to providing improved image quality, this technique is demonstrated useful for fluorescence imaging using a simple optical setup. Separate illumination and imaging channels would allow new endoscopic imaging capabilities using MC-SEE, including multiphoton fluorescence and high harmonics imaging, efficient scanningless endoscopic imaging [22], and other hybrid imaging modalities.

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