Near-infrared dyes as contrast-enhancing agents for spectroscopic optical coherence tomography

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Optical coherence tomography (OCT) images of biological tissues often have low contrast. Spectroscopic optical coherence tomography (SOCT) methods have been developed to enhance contrast but remain limited because most tissues are not spectrally active in the frequency bands of laser sources commonly used in OCT. Near-infrared (NIR) dyes with absorption spectra features within the OCT source spectrum can be used for enhancing contrast in this situation. We introduce and demonstrate the use of NIR dyes as contrast agents for SOCT. Contrast-enhanced images are compared with fluorescence microscopy, demonstrating a link between SOCT and fluorescence imaging. © 2004 Optical Society of America

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Spectroscopic optical coherence tomography (SOCT) not only provides a new way to obtain the functional and biochemical properties of tissues but also provides an important contrast mechanism in OCT images by providing spatially resolved spectroscopic information.[1] However, this contrast enhancement has limited use in imaging biological tissues because only a limited number of biological molecules are spectrally active in the near-infrared (NIR) spectrum commonly used for OCT imaging. Within the broadband laser spectra offered by state-of-the-art femtosecond titanium:sapphire lasers (centered at 800 nm), none of the major molecular components of tissue, namely, water, structural proteins without chromophores, most carbohydrates, lipids, and nucleic acids, are spectrally active. For this reason, anatomical and biochemical research using SOCT to date has been limited to a few types of naturally occurring NIR absorbers such as Hb/HbO_2 and melanin,[2] with studies done *in vitro*.[3,4] This situation is similar to conventional light microscopy imaging, where most tissue structures are colorless without the use of specific stains. It is therefore intuitive that spectroscopic staining with dyes that are spectrally active in the NIR region will enhance contrast in SOCT and potentially aid in differentiating tissue structures.

In the past, OCT contrast agents have been constructed to alter the intensity of backscattered light from specific locations. For example, air-filled microbubbles[5] and engineered microspheres[6] were used to increase backscattering from tissue, and molecular contrast was generated using a pump–probe technique to change the absorption.[7] The novel use of NIR dyes as contrast agents in SOCT imaging enables the regional spectral properties of a tissue to be altered, instead of altering only the intensity of the backscattered light. These agents may not only permit molecular OCT imaging of multiple species but also provide a link to a wide range of well-established fluorescent contrast agents since all fluorescent agents are also spectrally absorbing. Currently there are many commercially available NIR dyes, including fluorescent NIR dyes that are conjugated to different targeting molecules such as antibodies.[8] The NIR dye, Indocyanine Green, has also been approved for human use. These dyes can either be injected in free form or packaged within biocompatible microspheres to help target them to specific cells or tissue types.[6]

A fiber-based OCT setup was used for these studies. A diode-pumped mode-locked titanium:sapphire laser source with a center wavelength around 780 nm was used as the optical source. This laser pumped an ultrahigh numerical aperture (UHNA4, Nufern) fiber to spectrally broaden the output bandwidth to 120 nm.[9] Dispersion and polarization were matched in the interferometer arms. A precision linear optical scanner was used to scan the reference arm and the small (less than 0.5%) nonlinearity was corrected by calibration. The axial resolution of this system was measured to be 3 μm in air. A high-speed (5-Msamples/s, 12-bit) analog–digital converter (NI-PCI-6110, National Instruments) was used to acquire interferometric fringe data. Axial scans comprising the interferometric signals were sampled at 100,000 data points, and at 512 transverse positions to form two-dimensional images. The collected data were analyzed using Matlab for envelope detection and depth-resolved spectroscopic information. The short-time Fourier transform (STFT) was chosen as the time-frequency analysis method because it offered the best flexibility and controllability over other methods.[10] For experiments in which high depth resolution is not required, a STFT window size of 16,384 points (corresponding to a length of 327 μm in air) was chosen to allow for spectral resolution of 1 nm.[11] For experiments in which both spectral resolution and depth resolution were required, the STFT window size was chosen to optimize the time–frequency concentration,[12] typically

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using 1024 points (20 μm in air). To increase the signal-to-noise ratio when recovering the dye absorption spectrum, we averaged the absorption spectra calculated over 512 measurements in cases in which lateral resolution was not required. The NIR dye selected for this experiment (ADS7460, H. W. Sands, Inc.) has unique characteristics that make it attractive for SOCT. The absorption spectrum has a sharp peak at 740 nm. The dye, when used in appropriate concentrations, can absorb the shorter half of the laser spectrum wavelengths and transmit the longer half (Fig. 1), producing a predictable spectral signature that is useful for constructing SOCT images. The NIR dye has also been successfully encapsulated within protein microspheres made from bovine serum albumin that can act as delivery vehicles for OCT contrast agents.[6]

To establish the useful concentration of dye for detection with SOCT, we made various concentrations of dye solution. These solutions were placed in 1-mm-thick glass cuvettes (QS-459, Nova Biotech) and imaged with SOCT. The interference data from light scattered back from the top and bottom dye–glass interfaces were recorded and analyzed to extract the spectra. The absorption spectrum of the dye solution can be obtained using Beer's Law, as outlined by Faber *et al.*[2] Because the centroid of the backreflected light spectrum is typically calculated for displaying the spectroscopic data in a color image, this quantity was measured. Figure 1 plots the centroid of the backreflected light spectrum as a function of the dye concentration. As can be seen, the shift of the spectral centroid increases and then reaches a plateau with increasing dye concentration. The increase corresponds well to the theoretical calculation based on absorption data. Because OCT typically has penetration depths of 1–2 mm, we conclude that using a dye concentration of 50 μg/ml can produce the largest usable shift within this depth. At this concentration, most of the spectral center-of-mass shift occurs within 1 mm. A further increase in the concentration will limit the penetration depth of SOCT applications, whereas a decrease in the concentration will reduce the amount of spectral centroid shift. To test whether this NIR dye could be used for contrast enhancement at this concentration, we prepared an agar sample with two distinct vertical columns. One column contained a dye concentration of 50 μg/ml, and the second column contained no dye. The two columns were separated by a glass wall to prevent diffusion between agar columns. An equal concentration of 0.2% Intralipid solution was added to both columns for use as a scattering agent.[13] The resulting false-color hue-saturation SOCT image showed that the spectrum of the backreflected light from the column containing the dye had shifted toward longer wavelengths with increasing depth, whereas this effect was negligible for the column without the dye (data not shown). These results were consistent with previous studies using a color-glass wedge.[1]

Following these tissue phantom studies, we investigated the use of this NIR dye as a SOCT contrast agent in a biological environment and compared SOCT with fluorescence microscopy images. A stalk of green celery (*Apium graveolens* var. *dulce*) was used as a biological specimen. A celery stalk comprises two distinct tissue structures. The bulk of the stalk is composed of collenchyma tissue in which most of the cells are relatively large in size with thickened cell walls that mechanically support the stalk. Distributed around the center of the stalk are vascular bundles in which the cells are relatively smaller in size and form conducting vascular tubes to transport water and nutrients between the roots and leaves [Fig. 2(d)]. No significant differences between the spectral center of mass for these tissues were found with SOCT. We demonstrate that the contrast between these two tissues can be enhanced by use of a NIR dye. A celery stalk was cut near the root, leaving the upper leaves intact to facilitate transpiration. Control images were taken with SOCT before application of the dye. Subsequently, the root end of the stalk was submerged in a liquid mixture of 10-ml NIR dye (50 μg/ml) and 0.5-ml Rhodamine 5G (200 μg/ml) for 4 h to allow for capillary transportation. Rhodamine was added to permit acquisition of fluorescence microscopy images for comparison. The single-photon absorption spectrum for Rhodamine lies outside of the titanium:sapphire laser spectrum, and the two-photon absorption and emission efficiency is extremely low ($<10^{-10}$), resulting in no detectable contribution to the SOCT signal. Following capillary transportation of the dyes, SOCT data were collected from the same location as the control data. The celery stalk was cut in cross section at this imaging location for examination by fluorescence and light microscopy. Figures 2(a) and 2(b) show SOCT images with and without the NIR dye, respectively. For comparison, corresponding fluorescence and light microscopy images are also shown [Figs. 2(c) and 2(d)]. Enhancement of contrast is apparent in the vascular regions containing the NIR dye, where strong shifting of the spectral centroid is noted. The surrounding avascular collenchyma

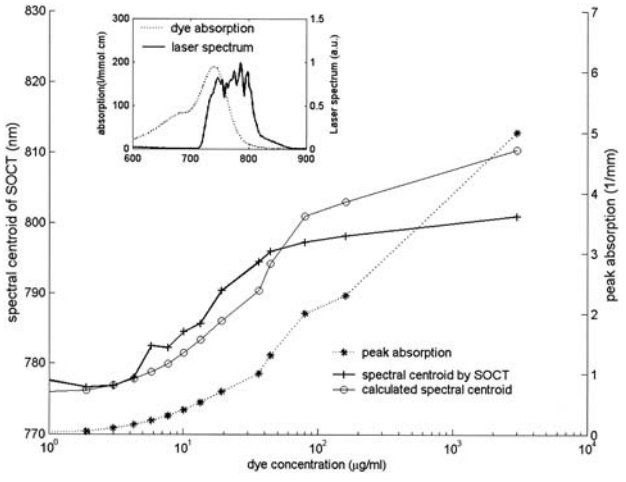

Fig. 1. SOCT detection of the spectral centroid shift with increasing dye concentration. The dotted curve shows the peak absorption ($\lambda = 740$ nm) measured by a spectrometer. Inset, spectra from the NIR dye and the titanium:sapphire laser used in this study.

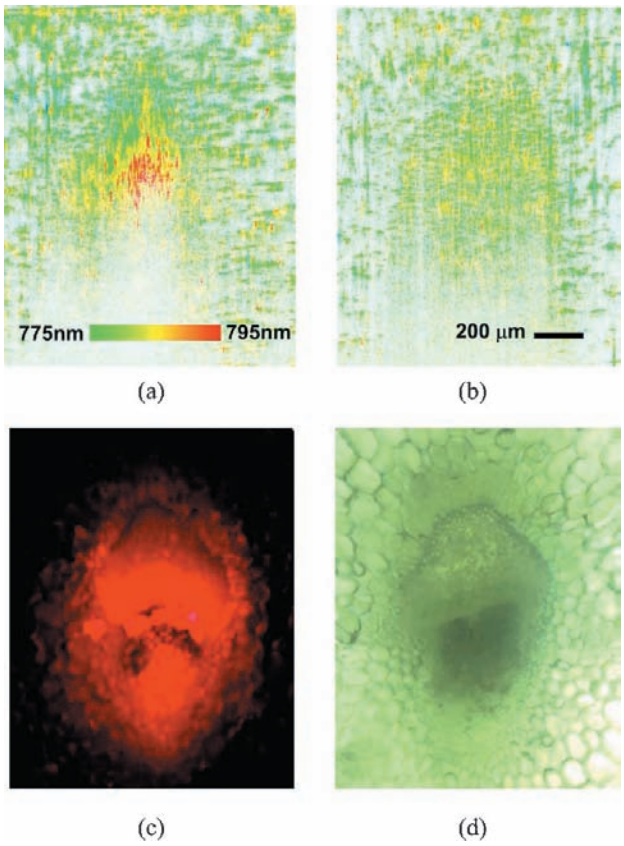

Fig. 2. NIR dye contrast enhancement in SOCT. (a) SOCT image of a celery stalk with dye present within the vascular bundle. The color bar shows the correspondence between pseudocolor labeling and the spectral centroid shift in the image. (b) SOCT image of the same area without dye. Note that the vascular bundle region is larger since the stalk was initially more hydrated. (c) Fluorescence microscopy and (d) light microscopy images showing the vascular bundle and the surrounding collenchyma tissue.

tissue shows minimal changes. The vascular bundle region showing strong SOCT contrast enhancement also correlates highly with the region showing strong fluorescence.

In summary, we have demonstrated a new category of contrast agent for OCT by use of NIR dyes in conjunction with SOCT image processing methods. Because NIR dyes can be easily administered and targeted to specific cell or tissue types, and since a few have already been approved for human use, there are multiple potential applications in humans, including contrast enhancement of early neoplasms and in OCT angiography. In contrast with OCT contrast agents designed to enhance scattering (engineered microspheres, gold nanoparticles), NIR dyes have the potential for multilabeling if the laser source spectrum is sufficiently broad. Several limitations are currently associated with the use of these dyes. Because the dye absorbs optical power, the imaging penetration depth may be reduced by approximately 20–30%. Since the contrast mechanism is based on absorption rather than increased scattering, high concentrations of dye may be required in small volumes (small collections of cells) because of current precision limitations associated with SOCT measurements. However, with the ongoing development of NIR dyes for fluorescence, confocal, and multiphoton microscopy, the detection of these OCT contrast agents targeted to specific cells and tissues appears feasible. In a similar manner, the SOCT methods presented here could be used for the detection of plasmon-resonant nanoparticle contrast agents with high extinction coefficients. Finally, the use of SOCT methods for the detection of NIR dyes links the coherence gating properties of OCT with the detection of a wide range of dyes that have traditionally been utilized as emitters of incoherent fluorescence.

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