Functional optical coherence tomography for detecting neural activity through scattering changes

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We have demonstrated functional optical coherence tomography (fOCT) for neural imaging by detecting scattering changes during the propagation of action potentials through neural tissue. OCT images of nerve fibers from the abdominal ganglion of the sea slug *Aplysia californica* were taken before, during, and after electrical stimulation. Images acquired during stimulation showed localized reversible increases in scattering compared with those acquired before stimulation. Motion-mode OCT images of nerve fibers showed transient scattering changes from spontaneous action potentials. These results demonstrate that OCT is sensitive to the optical changes in electrically active nerve fibers. © 2003 Optical Society of America

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Optical coherence tomography (OCT) is an emerging imaging technique that has been applied in a wide range of medical and biological fields.[1] OCT images are generated based on the variation of optical tissue properties from different structures. Typically the spatial variation of backscattered light produces contrast; however, tissue properties that change the amplitude, frequency, phase, or polarization of the incident light can also produce the desired contrast. In this Letter we demonstrate functional OCT (fOCT) for detecting optical scattering changes in individual nerve fibers during electrical activity. OCT is well suited for detecting neural activity in tissue because it provides noninvasive monitoring, high imaging resolution, fast acquisition rates, and high sensitivity to small optical changes.[2] Although standard OCT generates images based on architectural morphology, fOCT detects optical changes in cells and tissue during physiological events. A previous functional study demonstrated one-dimensional depth-resolved optical coherence domain reflectometry in a cat brain.[3] In this Letter we investigate electrical-activity-induced optical scattering changes in individual nerve fibers and visualize these changes with two-dimensional fOCT and motion- (M-) mode images.

Optical scattering changes in neural tissue occur through two physiological events: (1) changes in the local refractive indices of either the membrane or the cytoplasm and (2) changes in the geometry of the cell. The refractive index of a cell can be altered by a change in the ionic composition, such as during an action potential or following a reorientation of molecular dipoles when a potential difference exists across the membrane. The geometry of the cell can be altered by osmotically induced volume changes, as well as other physical forces. Detectable increases in light scattering during stimulation from crab and squid nerve fibers have been reported.[4] The intensity of scattered light has also been directly correlated with changes in membrane potential, suggesting that localized optical properties of neurons change as an action potential propagates because varying electric fields across the cell membrane cause ions in the cells to reorient.[5] Swelling and shrinking of axons have been attributed to the influx and efflux, respectively, of ions during action potential propagation.[6] Electrical and mechanical effects observed during electrical stimulation can produce optical changes detectable with fOCT.

For this study, we imaged the pleural–visceral connection at the abdominal ganglion of *Aplysia californica*. This animal model and ganglion were chosen because they have been studied extensively, and their electrical responses are well known. Neurons and nerve fibers are comparatively large (the latter are of the order of 250 μm in diameter), and their propagation velocities are relatively slow ($\sim$1 m/s). Nerve fibers consist of a bundle of axons surrounded by a muscle sheath (see the inset of Fig. 1 for a light microscope image of the fiber).

The OCT system used in this study consisted of a frequency-doubled Nd:YVO_4-pumped Ti:sapphire laser with a center wavelength of 800 nm, a bandwidth of 20 nm, and an average output power of 500 mW at an 80-MHz repetition rate. After fiber coupling into an ultrahigh-numerical-aperture fiber, nonlinear broadening of the optical spectrum to 130 nm improved the axial OCT resolution to 2 μm.[7] A fiber-based

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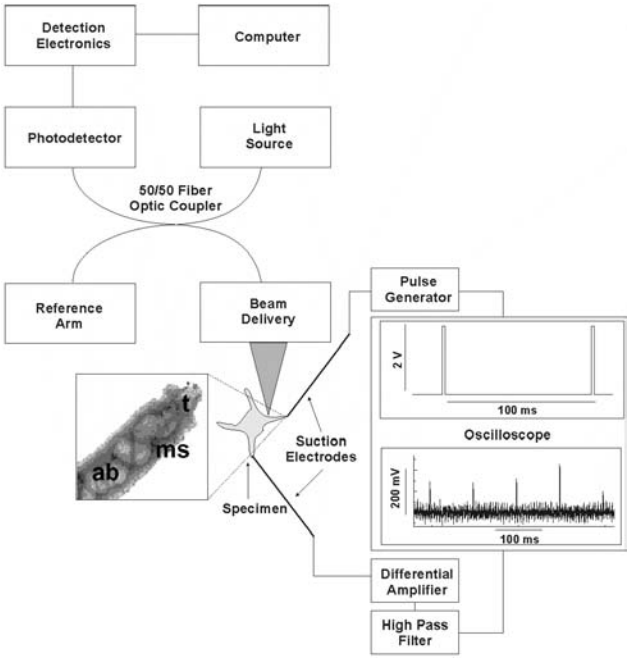

Fig. 1. Experimental setup. The inset shows a light-microscope image of a section of *Aplysia californica* nerve fiber at 4× magnification. ab, axon bundle; ms, muscle sheath; t, fiber tip.

Michelson-type interferometer was used. The reference arm contained a galvanometer-driven retroreflector operating at 40 Hz (one axial scan every 25 ms), and the sample arm consisted of a pair of orthogonal galvanometer-scanning mirrors. The 23-mW imaging beam was focused by a 20-mm focal-length lens to a 10-μm-diameter spot (transverse resolution). Two-dimensional fOCT images were acquired by lateral scanning of the incident beam and assembly of adjacent axial scans. We generated M-mode images by fixing the incident beam at one point on the tissue and acquiring axial scans over time.

The abdominal ganglion of *Aplysia* was dissected out after we anesthetized the slug with $MgCl_2$ (350 mM). The ganglion was pinned in a petri dish and maintained in physiological saline before OCT analysis to prevent dehydration and limit the amount of power deposited on the tissue. Stimulating and recording suction electrodes were attached to the ends of the fibers as shown in Fig. 1. The recording electrode was used to monitor the neural activity of the fiber.

The effect of external stimulation on optical scattering changes in the fiber was demonstrated by delivery of 2-V positive monophasic square pulses at 100 Hz for 6 min, followed by an 8-min recovery period. fOCT images were acquired before, during, and after stimulation at selected intervals. The experimental setup was not altered during image acquisition, and no movement was observed. No visual changes in the specimen were noted that would indicate injury, and no abnormalities in the electrical signal were observed.

Figure 2 shows a sequence of averaged fOCT images acquired before, during, and after stimulation. Averaging images over five scans reduced the appearance of speckle and improved the signal-to-noise ratio. Figures 2A–2C show localized regions of increased scattering during stimulation, which subsided after stimulation as the cells began to repolarize. The optical scattering changes observed with fOCT are consistent with earlier findings that light scattering from nerve fibers increases in response to electrical stimulation.

To quantitatively illustrate the changes in optical scattering that occurred during electrical excitation, in Fig. 2D we plot the average pixel intensity in a region of interest (shown in Fig. 2A) taken at 1-min intervals over a period of 24 min. Images were normalized and the same region of interest was considered in every image. This plot illustrates the direct relationship between electrical activity and scattering changes in fOCT images. Immediately after electrical stimulation began, scattering increased until stimulation was stopped, at which point scattering decreased but did not return to prestimulus levels. We believe that scattering did not decrease to prestimulus levels because most cells continued to exhibit a high level of spontaneous electrical activity as detected with the recording electrode. After an 8-min recovery period, stimulation resumed, and scattering again increased, as expected. We conclude that the scattering changes were not due to specimen heating based on temperature measurements, nor were they due to other imaging artifacts, because a decrease in scattering was observed after the end of stimulation, with a subsequent increase in scattering when stimulation resumed.

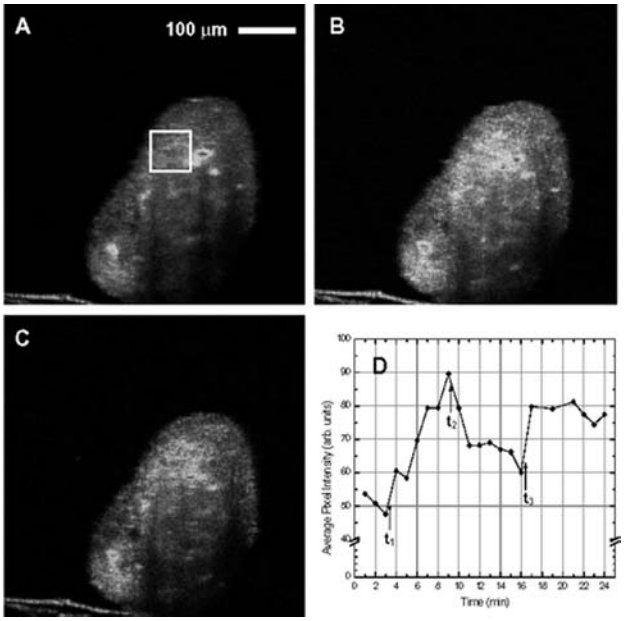

Fig. 2. Time sequence of fOCT images. Images represent an average of five scans acquired A, before; B, during; and C, 8 min after stimulation. D, plot of the average image pixel intensity over time in a region of interest denoted by the white box in A. Labeled arrows represent when electrical stimulation began (t_1), was stopped (t_2), and resumed (t_3). Brightness in OCT images corresponds to optical scattering.

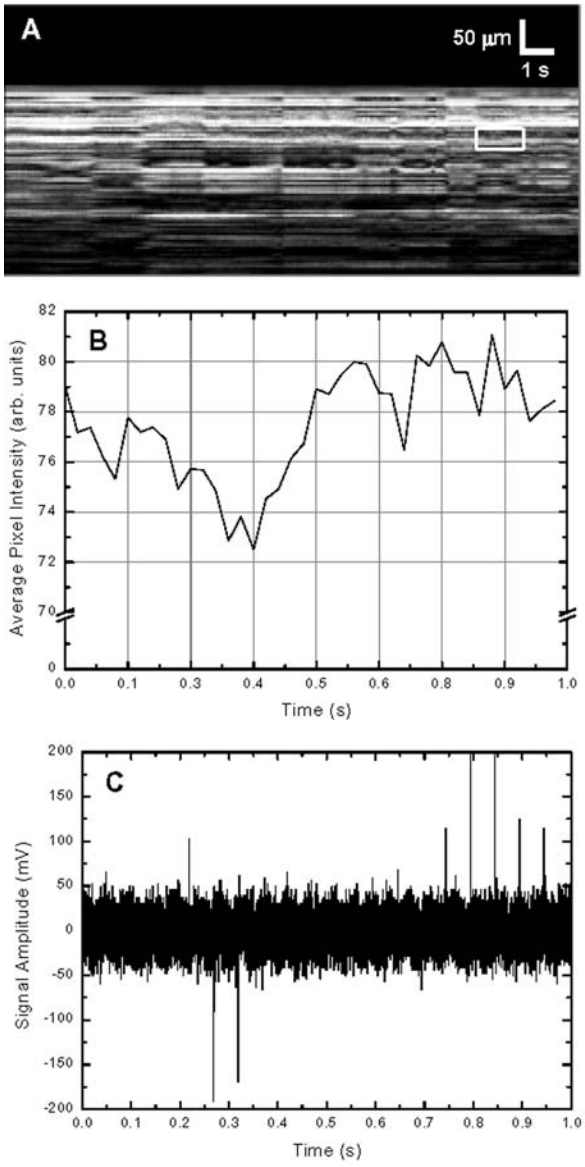

Fig. 3. Fast transient scattering changes. A, M-mode fOCT image. The horizontal axis represents time. B, plot of average image pixel intensity over time in the region of interest denoted by the white box in A. C, electrophysiological trace recorded from the nerve fiber imaged in A over the same time scale as the plot shown in B.

To demonstrate fast transient scattering changes as a result of spontaneous electrical activity, we acquired M-mode fOCT images over approximately 30 s, as shown in Fig. 3A. The average pixel intensity over time from a region of interest (box in Fig. 3A) was plotted in Fig. 3B. The recorded electrophysiological waveform is shown in Fig. 3C. It is important to note that we are not attempting to precisely correlate the plots in Figs. 3B and 3C. Since the fibers consist of a large number of individual axons, it is difficult to determine which axon produces each specific spike, and the M-mode and region-of-interest scattering changes may not have been acquired over that single axon. However, since the only variable in the experiment was the measured spontaneous electrical activity, we conclude that the transient changes observed in the M-mode fOCT images are due to this activity.

In this Letter we have demonstrated fOCT as a method of detecting optical scattering changes in neural tissue as a result of propagating action potentials. A motivation for this research is to replace complex parallel electronics used for multielectrode array recordings and the use of exogenous voltage-sensitive dyes in electrophysiology. Future work will therefore include imaging sparsely populated cultures of neurons to correlate individual action potentials with fOCT data as well as obtaining *en face* fOCT images to correlate spreading electrical activity with data from known neural networks. The use of fOCT to visualize neural activity could provide neurophysiologists with a new window into neural communication patterns and supplement current computed tomography and magnetic resonance imaging modalities with a real-time, cellular-resolution, optical technique.

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