Functional optical coherence tomography of neurophysiology

Stephen A. Boppart
Department of Electrical and Computer Engineering,
Beckman Institute for Advanced Science and Technology, Bioengineering Program, College of Medicine
University of Illinois at Urbana-Champaign, 405 N. Mathews Ave., Urbana, IL 61801
Email: boppart@uiuc.edu

Mariya Lazebnik, Claudio Vinegoni, Anupama Bowonder, Daniel L. Marks
Department of Electrical and Computer Engineering, Beckman Institute for Advanced Science and Technology
University of Illinois at Urbana-Champaign

Rhanor Gillette
Department of Molecular and Integrative Physiology, University of Illinois at Urbana-Champaign

Abstract: Functional optical coherence tomography (fOCT) is used to record optical changes that correspond to spontaneous and stimulated electrical activity in neural tissue. FOCT provides a high-resolution, real-time, non-invasive method for investigating neurophysiology.

OCIS codes: (170.4500) Optical coherence tomography; (170.3880) Medical and biological imaging; (170.5380) Physiology; (999.9999) Functional imaging.

1. Introduction

In contrast with standard optical coherence tomography (OCT) which can be used to generate images of neural tissue morphology [1], functional optical coherence tomography (fOCT) detects optical changes in cells and tissue during physiological events, such as electrical excitation [2]. Previous functional studies have used OCT to detect changes in mechanical cardiac parameters [3,4], and Doppler OCT techniques have been applied to measure blood flow [5,6]. Depth-resolved optical coherence domain reflectometry has been demonstrated in the living cat brain [7,8]. Limitations in recording techniques remain for studying complex neurophysiology and neural networks including single point recordings with micropipette electrodes, complex and sensitive banks of electronics for array recordings [9], and toxicity associated with voltage-sensitive optical dyes [10]. The development and use of fOCT for non-invasive, real-time, *en face* or depth-resolved detection of neurophysiological activity based on inherent changes in optical tissue properties has the potential to overcome many of these current limitations.

2. Methods

To demonstrate the use of fOCT, we imaged nerve fibers from the abdominal ganglion of the sea slug *Aplysia californica*, a well-characterized animal model with relatively simple neural networks [11]. The OCT system used in this study consisted of a titanium:sapphire source 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, the optical spectrum was nonlinearly broadened to 130 nm to improve the axial OCT resolution to 2 μm [12]. The 23 mW imaging beam was focused by a 20 mm focal length lens to a 10 μm diameter spot (transverse resolution). Axial scans were acquired at 30 Hz. Two-dimensional fOCT images were acquired by laterally scanning the incident beam. M-mode images were generated by fixing the incident beam at one point on the tissue and acquiring axial scans over time, enabling real-time acquisition of fast transients.

The dissected ganglion was pinned in a Petri dish and maintained in cooled physiological saline to prevent dehydration and to 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 Figure 1. Initially, optical changes in the tissue were elicited with biphasic stimulation since previous studies have noted that neural tissue, when stimulated with monophasic voltage or current pulses, may become injured due to vital cellular components (proteins, enzymes, and ions) being continuously displaced in one direction due to their electric charge. Presumably, these injurious displacements can be avoided by passing brief biphasic current pulses with a zero net flow of voltage or current through the cells, since this causes no net displacement of the cellular particles. The nerve fiber was stimulated with 4 $V_{p\text{-}p}$ biphasic pulses, at 100 Hz for 10 minutes, followed by 10 minutes of no stimulation. OCT images were taken before, during, and after stimulation. The nerve was then stimulated again for 10 minutes, followed by 10 minutes of no stimulation. To ascertain a controlled experiment, the setup was not altered during image acquisition, and no abnormalities in the recorded electrical signal were observed. Temperature measurements from the tissue and bath were acquired during imaging.

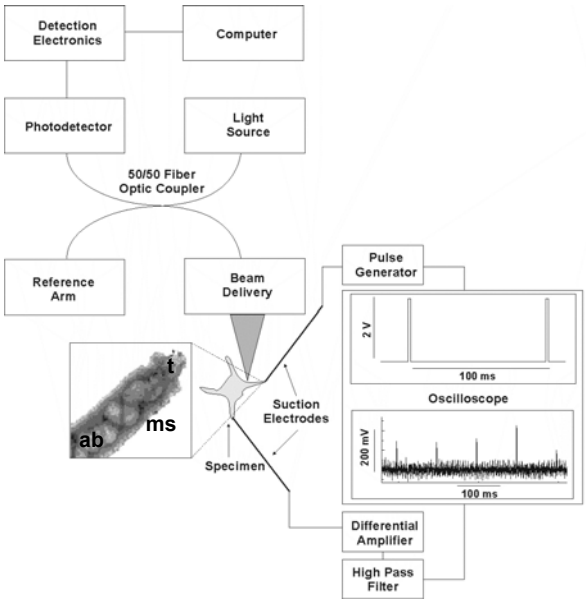

Fig. 1. Diagram of the experimental setup. Inset shows a light microscope image of a section of the *Aplysia californica* nerve fiber at 4x magnification. Abbreviations: ab, axon bundle; ms, muscle sheath; t, fiber tip.

3. Results

Figure 2 shows a sequence of averaged fOCT images obtained before, during, and after stimulation. Images were averaged to reduce the appearance of speckle and improve the signal-to-noise ratio. Figure 2B shows localized regions of increased scattering during stimulation, which subside after stimulation as the cells began to repolarize. As the nerve fiber was again stimulated, scattering again increased, and subsequently decreased upon the completion of stimulation. A plot of the total pixel intensity within the boxed region of Figure 2A over the time-course of this experiment is shown in Figure 2D. The optical scattering changes observed with fOCT are consistent with earlier findings that light scattering from nerve fibers increases in response to electrical stimulation [13]. We obtained similar results using monophasic stimulation as opposed to biphasic stimulation, which further support our conclusions. Based on temperature measurements, scattering changes were not due to specimen heating.

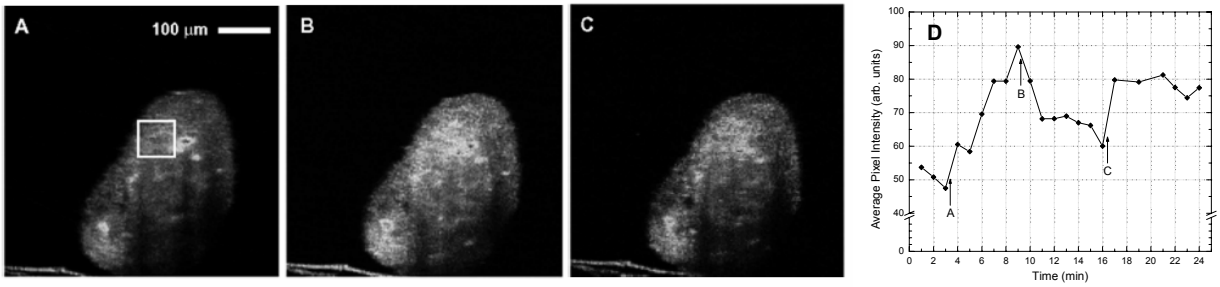

Fig. 2. Time-sequence of fOCT images. Images represent average of five scans, acquired (A) before biphasic stimulation, (B) during stimulation, and (C) 8 minutes after stimulation. (D) Plot of the average image pixel intensity over time in the region of interest denoted by the white box in (A). Labeled arrows represent when electrical stimulation began (A), was stopped (B), and resumed (C). Brightness in OCT images corresponds to optical scattering [2].

Finally, we acquired M-mode fOCT images to demonstrate fast transient scattering changes due to spontaneous electrical activity. A sample M-mode image is shown in Figure 3A over a 20-second time period. The M-mode image pixel intensity within the boxed region of interest in Figure 3A is plotted in Figure 3B. We simultaneously recorded with the recording electrode the spontaneous electrical activity of the nerve fiber. 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 were due only to this activity.

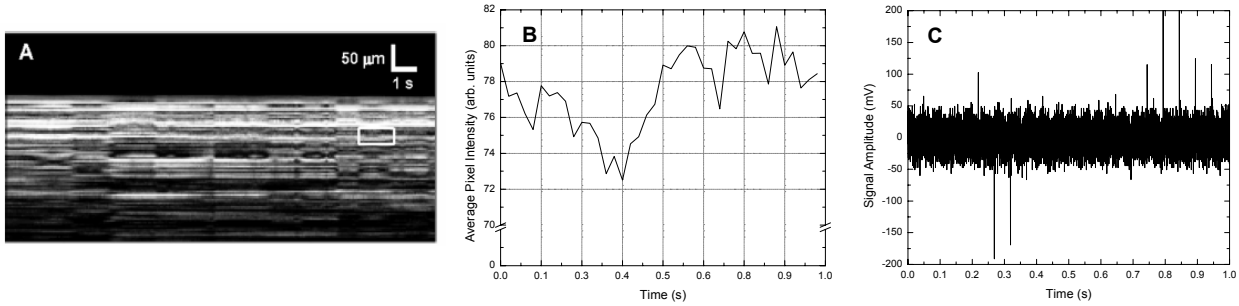

Fig. 3. Fast transient scattering changes. (A) M-mode fOCT image over a 20-second time period. The horizontal axis represents time. The backscatter intensity variations in the horizontal rows are due to spontaneous electrical activity in the nerve fiber. (B) Plot of average pixel intensity over time for 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) [2].

4. Conclusions

We have demonstrated fOCT as a method for detecting optical scattering changes in neural tissue resulting from propagating action potentials. Several investigations present themselves as the logical next steps in continuing this work. The first and most straight-forward is to directly correlate M-mode OCT data with electrophysiological data. By synchronizing fOCT data and image acquisition with electrical signal recordings, it will be possible to extract changes in optical properties from the tissue on time-scales consistent with individual action potentials. *En face* rather than depth-priority image acquisition will enable recordings of spreading electrical activity through the tissue. To extend this study, it will be possible to investigate the effect of inhibitors (such as tetrodotoxin) or pharmacotherapeutics on optical changes. Finally, since it has been proposed that a cell membrane illuminated with incident light beams of different polarizations can have different indices of refraction [13], subsequent studies will investigate whether polarization-sensitive OCT [14] can detect these different polarizations, and whether this will result in a more pronounced optical change.

The use of standard OCT and fOCT for visualizing neural anatomy and physiology has the potential to provide neurophysiologists with a new window into neural communication patterns at the cellular level, and supplement current computed tomography, magnetic resonance, and diffusion-based optical imaging modalities.

5. References

[1] S. A. Boppart, "Optical coherence tomography: Technology and applications for neuroimaging," Psychophysiology **40**, 529-541 (2003).
[2] M. Lazebnik, D. L. Marks, K. Potgieter, R. Gillette, S. A. Boppart, "Functional optical coherence tomography for detecting neural activity through scattering changes," Opt. Lett. **28**, 1218-1220 (2003).
[3] S. A. Boppart, G. J. Tearney, B. E. Bouma, J. F. Southern, M. E. Brezinski, and J. G. Fujimoto, "Noninvasive assessment of the developing *Xenopus* cardiovascular system using optical coherence tomography," Proc. Natl. Acad. Sci. USA **94**, 4256-4261 (1997).
[4] T. M. Yelbuz, M. A. Choma, L. Thrane, M. L. Kirby, J. A. Izatt, "Optical coherence tomography: A new high-resolution imaging technology to study cardiac development in chick embryos," Circulation **106**, 2771-2774 (2002).
[5] S. Yazdanfar, A. M. Rollins, J. A. Izatt, "Imaging and velocimetry of the human retinal circulation with color Doppler optical coherence tomography," Opt. Lett. **25**, 1448 – 1450 (2000).
[6] A. M. Rollins, S. Yazdanfar, J. K. Barton, J. A. Izatt, "Real-time *in vivo* color Doppler optical coherence tomography," J. Biomed. Opt. **7**, 123-129 (2002).
[7] R. Uma Maheswari, H. Takaoka, R. Homma, H. Kadono, M. Tanifuji, "Implementation of optical coherence tomography (OCT) in visualization of functional structures of cat visual cortex," Opt. Comm. **202**, 47-54 (2002).
[8] R. Uma Maheswari, H. Takaoka, H. Kadono, R. Homma, M. Tanifuji, "Novel functional imaging technique from brain surface with optical coherence tomography enabling visualization of depth resolved functional structure *in vivo*," J. Neurosci. Meth. **124**, 83-92 (2003).
[9] J. Csicsvari, D. A. Henze, B. Jamieson, K. D. Harris, A. Sirota, P. Bartho, K. D. Wise, G. Buzsaki, "Massively parallel recording of unit and local field potentials with silicon-based electrodes," J. Neurophysiol. **90**, 1314-1323 (2003).
[10] A. R. Cinelli, "High-definition mapping of neural activity using voltage-sensitive dyes," Methods **21**, 349-372 (2000).
[11] D. L. Glanzman, "Postsynaptic regulation of the development and long-term plasticity of *Aplysia* sensorimotor synapses in cell culture," J. Neurobiol. **25**, 666-693 (1994).
[12] D. L. Marks, A. L. Oldenburg, J. J. Reynolds, S. A. Boppart, "Study of an ultrahigh-numerical aperture fiber continuum generation source for optical coherence tomography," Opt. Lett. **27**, 2010-2012 (2002).
[13] R. A. Stepnoski, A. LaPorta, F. Raccuia-Behling, G. E. Blonder, R. E. Slusher, D. Kleinfeld, "Noninvasive detection of changes in membrane potential in cultured neurons by light scattering," Proc. Natl. Acad. Sci. USA **88**, 9382-9386 (1991).
[14] J. F. deBoer, T.E. Milner, "Review of polarization sensitive optical coherence tomography and Stokes vector discrimination," J. Biomed. Opt. **7**, 359-371 (2002).