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Detecting action-potential-correlated scattering changes in single neurons

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Optical coherence microscopy has potential as a tool for noninvasively measuring electrical activity in single neurons based on changes in their intrinsic optical properties.

A central goal of neuroscience is to understand how groups of neurons interact to give rise to the myriad functions of the nervous system. This task is extremely difficult in part because of the technical challenge of recording the electrical activity of many neurons simultaneously without disturbing their environment. Although several methods exist, new techniques are in strong demand. One promising approach is based on noninvasively detecting changes in the optical properties of neural tissue that accompany functional activity. Such changes are intrinsic and do not require the use of contrast-enhancing dyes. However, the effects are small and often accompanied by high levels of noise. Consequently, it takes optical-imaging techniques with high sensitivity to sense them.

Optical coherence tomography (OCT) is a biomedical-imaging modality that measures depth-resolved scattering of tissue using 'coherence-gated' detection. OCT can detect functional activity in neural tissue such as cortex[1] and retina[2] by measuring time-dependent changes in intrinsic scattering. Such studies have thus far focused on bulk neural tissue. But because neurons function as the fundamental unit of the nervous system, investigations would benefit from also being able to detect functional activity in individual neurons. Toward this goal, we have recently demonstrated the use of optical coherence microscopy (OCM) to measure functionally correlated scattering changes in single neurons.[3]

OCM combines the high spatial resolution of confocal microscopy with the interferometric detection of OCT.[4] OCM images are similar to those produced by confocal-reflectance microscopy, but the technique features improved sensitivity and penetration depth in highly scattering tissue. OCM is capable

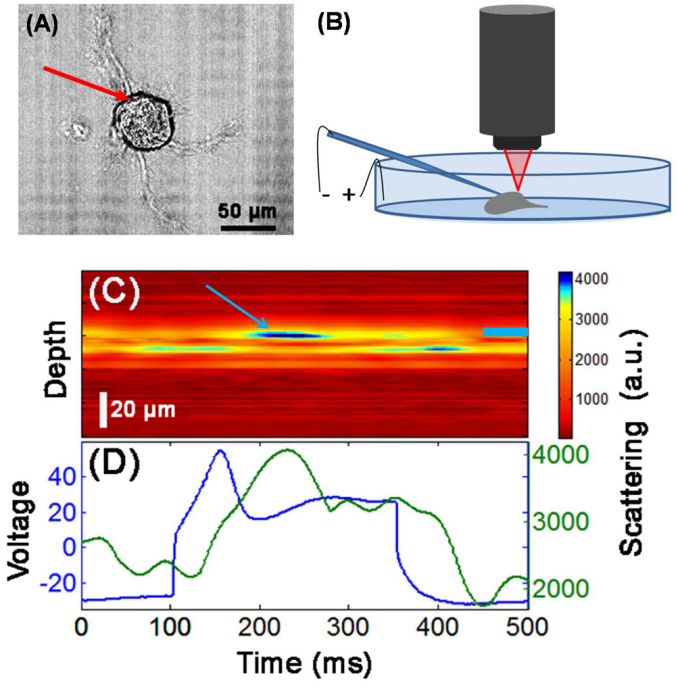

Figure 1. *Experimental setup and results for correlating scattering intensity and membrane voltage in a single neuron during an induced action potential. (a) OCM image of a 'bag-cell' neuron from Aplysia californica. (b) Schematic of experimental setup showing a neuron in culture impaled with a glass pipette microelectrode for stimulation and recording of the membrane voltage. Optical backscattering of the focused laser beam is measured by OCM. (c) Motion-mode OCM showing depth-resolved scattering intensity from the neuron as a function of time. The arrow points to an increase in scattering intensity from the neuron resulting from the action potential. (d) Membrane voltage (blue) and scattering intensity (green) from the neuron surface. Depth corresponding to the neuron surface is indicated by the blue bar in (c). a.u.: Arbitrary units. (Adapted from Reference 3.)*

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of achieving sensitivity greater than 100dB, spatial resolution on the order of 1μm, and temporal resolution of better than 1μs. This makes OCM ideal for detecting electrical activity from single neurons by measuring time-dependent scattering changes.

To demonstrate this potential, correlations between electrical activity and optical properties were made in individual cultured neurons—see Figure 1(a)—by measuring scattering changes during electrical stimulation. A schematic of the experimental setup is shown in Figure 1(b). A pulled-glass micropipette electrode was used to stimulate the neuron and simultaneously measure the membrane voltage while the OCM beam was focused on a fixed point on the neuron: see the red arrow in Figure 1(a). OCM can detect scattering from a range of depths in the sample, as in OCT. However, high spatial resolution is limited to a small range around the focal point due to the strong beam divergence. The motion-mode data in Figure 1(c) shows the scattering at different depths as a function of time during electrical stimulation. Figure 1(d) shows the membrane voltage along with the correlated scattering signal from the depth corresponding to the neuron's surface membrane. The increase in scattering from the neuron coinciding with the induced action potential is indicated by the blue arrow in Figure 1(c).

The observed changes were small and were accompanied by other fluctuations not related to electrical activity. For this reason, a small amount of temporal averaging was needed to produce the results shown. However, this is consistent with what is commonly done in many other electrophysiology measurements. Despite a delay between the membrane voltage and the scattering change, their time courses are closely related, indicating that the alterations may be caused by reorientation of charged molecules in the neuron's cell membrane.[5]

In conclusion, we have shown—for the first time—that OCM can detect intrinsic scattering changes in individual neurons that accompany electrical activity. These findings have implications for the further development of OCT and OCM as tools for noninvasive measurement of such activity in neural tissue. OCM also holds promise for studying the mechanisms of functionally correlated intrinsic scattering changes in neurons. Further experiments are needed to determine the conditions under which this technique could be used to independently detect electrical activity. As a next step, our laboratory will investigate the use of phase- or polarization-sensitive detection as a means of improving the signal-to-noise ratio.

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