

Optical measurement of neural activity using surface plasmon resonance

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We demonstrate that surface plasmon resonance (SPR) is applicable to the optical detection of neural signals. A low-noise SPR sensor was developed as a label- and artifact-free method for the extracellular recording of neural activity. The optical responses obtained from a rat sciatic nerve were highly correlated with simultaneously recorded electrical responses. Additional studies with stimulation intensity and lidocaine further confirmed that the optically measured signals originated from neural activities. © 2008 Optical Society of America

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Extracellular neural recording is a key technique used to measure neural activity. Conventionally, microelectrodes have been employed to record extracellular neural signals [1]. This electrical recording, however, involves stimulation artifacts when electrical stimulation is applied. Alternatively, voltage-sensitive fluorescence dyes can be used to optically record neural signals. Unlike electrical detection, optical recording has no stimulation artifacts. However, fluorescent dyes are expensive, toxic, involve a time-consuming labeling process, and can be affected by photobleaching [2].

Intrinsic optical properties in nonmammalian nerves and neurons have been examined for artifact- and label-free optical recording [3–6]. In particular, changes in scattering and birefringence of a nerve are correlated with neural activity in a complicated manner. When an action potential propagates through an axon in the nerve, reorientation of molecular dipoles across the membrane alters the refractive index of the axon membrane. In addition, the action-potential propagation produces the osmolality difference across the membrane, which in turn leads to cellular swelling, an increase in the cell volume through the influx of water molecules. These alterations in the refractive index and microanatomy of the nerve may result in optical scattering changes [3,4]. In contrast, the birefringence change associated with nerve activation presumably comes not only from conformational changes of a macromolecule but also from cellular swelling [4–6]. Although the biophysical mechanism underlying these fast intrinsic optical responses is not clearly understood at present, it is almost certain that the optical changes are very small in magnitude and are mostly localized near the membrane.

The surface-plasmon-resonance (SPR) sensor employs surface electromagnetic waves that propagate

through a conductor-dielectric interface. The SPR sensor has a highly sensitive resonance condition and a very small measurement volume at a thin layer on the conductor. These properties make the SPR sensor suitable for detecting neural activity, because action potentials are accompanied by small changes in the cellular volume and the membrane-localized refractive index [3–5]. In the current study, an SPR sensor is proposed as a new artifact- and label-free optical method for recording neural activity in mammalian nerves. An SPR system was developed to monitor the optical changes at the metal-nerve interface, and the optical responses were compared with simultaneously recorded electrical responses. This SPR-based recording system was sensitive enough to detect activity-induced intrinsic optical responses without signal averaging.

A schematic diagram of the SPR measurement system is presented in Fig. 1. We reported a preliminary version of the system in [7], but here we report the application of the improved system to neural recordings. The experimental setup was based on the attenuated total-reflection configuration, in which an incident beam was coupled through a BK7 prism on a glass slide. An SPR sensor chip with a 50-nm-thick gold-coated film on a microscopic BK7 glass slide was

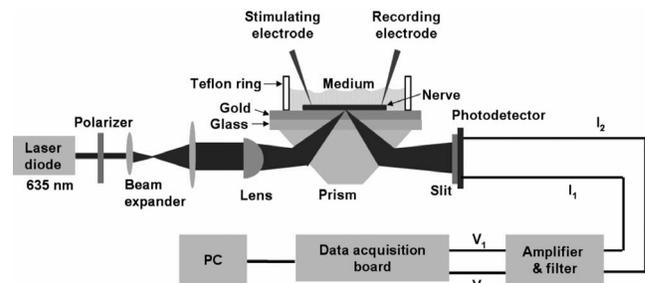


Fig. 1. Schematic of the presented SPR system.

modified for use as a recording chamber using a Teflon ring. A low-noise laser diode (Coherent, LabLaser C ULN) with a wavelength of 635 nm and an output power of 5 mW was used. The laser beam was focused onto a point with a beam diameter of 100 μm . Here we used an intensity-based SPR sensor, because its sensitivity is reported to be equal or superior to that of a phase-sensitive method [8]. The reflection intensity of the laser beam was detected through a multichannel photodetector array (Hamamatsu, S5981). The output currents (I_1 , I_2) from two vertical elements of the photodetector array were converted to voltage signals (V_1 , V_2), and amplified with a gain of 10,000 before low-pass filtering at the cutoff frequency (f_c) of 500 kHz (FEMTO, DLPCA-200). The SPR system was initially aligned such that those two signals were equal in magnitude, and then the difference between the two signals was monitored in order to remove common-mode noise. With additional noise reduction, the current system's ambient noise was less than 10 μV_{RMS} in magnitude.

Our system was built to record both optical and electrical signals simultaneously in response to electrical stimulation. A Teflon-insulated Pt-Ir wire (A-M Systems, Inc., 778000) electrode was placed on the proximal end of the nerve, while another wire was located on the distal end of it. The laser beam for the SPR detection was located between the two wires. Electrical responses were amplified a thousand times using a differential ac amplifier (A-M Systems, Inc., model 1700), filtered between 1 Hz and 10 kHz with a 60 Hz notch filter, and digitized at 20 kHz. The optical and electrical signals were simultaneously recorded and time labeled by a single DAQ board (National Instruments, NI 4462).

For neural recording experiments, sciatic nerves from knee (distal) to spinal cord (proximal) were dissected from male Sprague-Dawley (SD) rats. The rats weighed approximately 200 g. All tissues and blood vessels were removed to ensure that the sciatic nerve closely adhered to the gold surfaces in the recording chamber. The amputated sciatic nerves were immersed for 5 min in artificial cerebrospinal fluid (aCSF) at 37°C and then moved to the recording chamber. During each experiment, the viability of the nerve was continuously monitored for 30 min. All the animal experiments were performed under protocols approved by the Institute of Laboratory Animal Resources at Seoul National University.

Figure 2 shows the simultaneously recorded electrical (gray traces) and optical (black traces) responses evoked by biphasic pulses. The Y axis for the electrical signal is given in millivolts, while that for the optical signal is given in refractive index units (RIU). An ethanol test [9] was performed to calibrate the measured photodetector output voltage to the RIU. The electrical responses consisted of larger stimulus artifacts and smaller neural spikes. The smaller neural spikes had the waveform shapes typical of compound action potentials (CAPs). Reversing the stimulation polarity altered only the polarity of the stimulus artifacts. The electrical and optical responses, displayed in slower time scale [Fig. 2(a)],

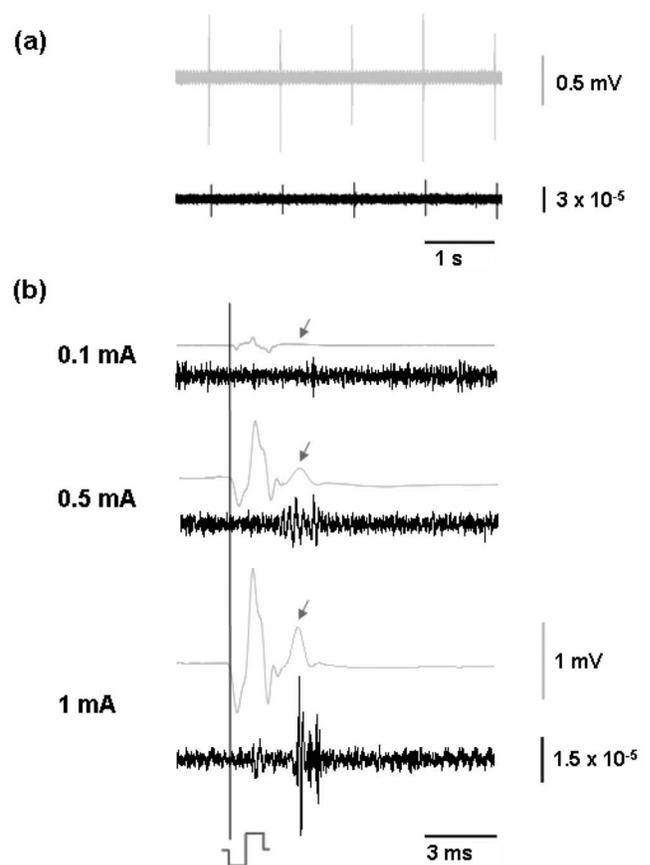


Fig. 2. Correspondence between electrical (gray trace) and optical responses (black trace) of a rat sciatic nerve with increasing stimulus intensity on the same time scale. A biphasic stimulation current (I_s) was either (a) fixed at 1 mA or (b) varied from 0.1 mA to 1 mA while the pulse duration (t_{pd}) and stimulation rate (f_s) were fixed at $t_{\text{pd}}=1.5$ ms and $f_s=1$ Hz, respectively. The vertical bar for the optical signal represents 10^{-5} changes of the refractive index unit (RIU). The arrows indicate peaks of neural responses.

showed strong correlation between the two. As shown when the responses are plotted with a faster time scale [Fig. 2(b)], both the electrical (gray traces) and optical responses (black traces) increased in magnitude when the stimulation intensity was increased.

Additionally, lidocaine (2%) was applied as a nerve blocker on the sciatic nerve segment. Lidocaine is known to be an effective nerve conduction blocker in rats, and 20 min was sufficient to develop maximal depression of CAP [10]. The electrical and SPR responses from the sample with the nerve blocker were shown to degrade within 20 min after the application (Fig. 3).

The dependency of the SPR response on stimulation amplitude and lidocaine further proved that the optical response originated from neural activities. However, we observed some differences in the characteristics of electrical and optical responses. The threshold for the evoked SPR response was slightly higher than that for the evoked electrical response. This may indicate that more fibers need to be excited for an SPR signal to be registered. Also, there was a time delay from the onset of the electrical response to that of the optical one, which varied between

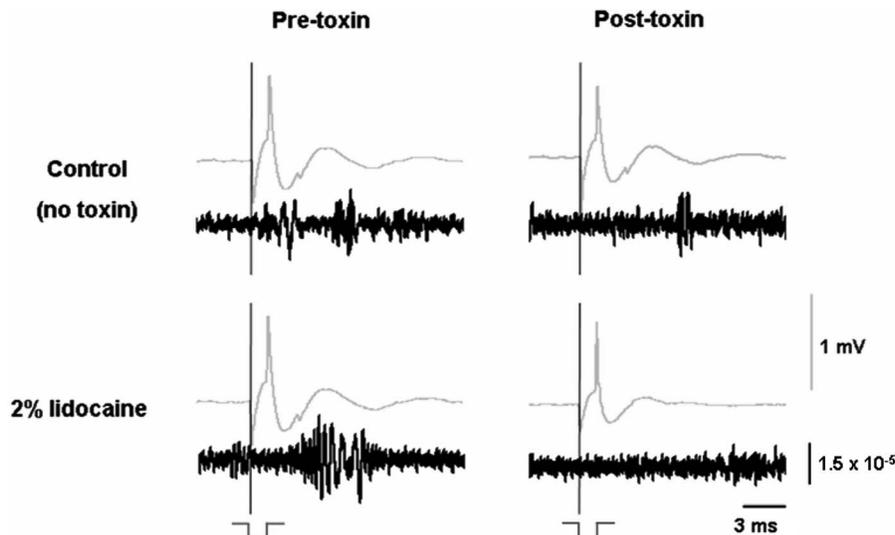


Fig. 3. Effects of a nerve-blocker application on the electrical (gray trace) and optical (black trace) responses. The post-toxin signals were recorded 20 min after nerve-blocker application. A monophasic current stimulation was used with $I_s=0.5$ mA, $t_{pd}=1.5$ ms, and $f_s=5$ Hz. The vertical bar for the optical signal represents 10^{-5} changes of the RIU.

0 and 5 ms, depending on the nerve preparation (length of the dissected segment, position of the nerve in the recording chamber, location of the electrodes and the probe beam, etc.). Although further study is needed to clarify these differences, they may be attributed to the different physiological origin of the optical response. The SPR signal may not represent the membrane potential itself but may vary with fluctuation in the refractive index of the measurement volume owing to cellular swelling. The delays may be attributable to the swelling caused by the slow diffusion of water molecules.

Lastly that the SPR signals had a good signal-to-noise ratio (about 4 to 1), so the recording did not require signal averaging. This can be compared with the well-defined responses of intrinsic optical neural recording obtained without signal averaging [6] or with other intrinsic optical neural recordings where signal averaging was required [4]. It is also noted that the SPR method may have a limitation of requiring the metal surface to be in close proximity to the nerve.

In summary, we introduced a new (to our knowledge) label- and artifact-free neural recording scheme based on SPR measurements. A low-noise SPR system was developed and used to measure neural activities in the sciatic nerves of rats. Through simultaneous recordings of electrical and SPR signals, the optical responses were proven to be associated with neural activity *in vitro*. Variable stimulation intensity was used, and a nerve blocker was applied to confirm the neural origin of the SPR responses. In the future, this study will be applied to neural recording *in vivo* using an optical fiber-based SPR sensor. Future work may also include imaging the propa-

gation of action potentials in cultured neural networks and further improving the signal quality by modifying the metal surface using, for example, nanoparticles and nanowires [9].

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