

Interferometric Detection of Action Potentials In Vitro

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Goal

Detection of individual action potentials in axons using intrinsic optical signals.

Area of Application:

The technique has been demonstrated using neuronal axons dissected from lobster. As implemented, it would further be applicable to the axons and active dendrites of cultured neurons with diameter somewhat larger than an optical wavelength. Using index matching techniques, it may be possible to extend the technique to smaller structures.

Materials

The essential optical components include a 5 mW polarized laser (HeNe); single mode, polarization preserving optical fiber with couplers; Wollaston prism, birefringent plate; water immersion 20x microscope objective; electro-optic modulator, and polarization analyzer. The interferometric microscope needs to be compact and stiff, a combination that can be achieved with Microbench components (LINOS Photonics; Milford, MA).

Protocol and Procedures

Early work has shown that light scattering from a network of small neuronal processes is modulated by the propagation of action potentials (Cohen et al. 1968, 1972a, 1972b). Studies of the scattering pattern obtained from a single axon were consistent with a change in the refractive index of the cell membrane which is proportional to the voltage across the membrane (Stepnoski et al. 1991), presumably arising from alignment of dipoles in the membrane. The magnitude of the change was determined for isolated axons from *Aplysia* neurons, for which the relative change in refractive index was $\Delta n/n = 50 \times 10^{-6}$ per mV. This corresponds to an equivalent optical phase shift of 0.1 to 0.2 mradian per spike. These relatively small changes could be detected, in single trials, by measuring changes in light scattering with darkfield illumination (Stepnoski et al. 1991).

The goal of this procedure is to make a direct confirmation of the index change by constructing a two-beam interferometer in which a beam that passes through


an axon interferes with a beam which bypasses the axon. A change in the relative optical phase of the two beams is a measure of the change in total optical density of the preparation. In order to avoid randomly fluctuating phase shifts we need the two beams to be transported by the same optical components. To accomplish this, we employ the apparatus shown in Fig 1(a), which uses polarization optics to create two distinct beams that propagate through the same optical system.

A polarization preserving single mode optical fiber that transports light from a HeNe laser acts as a point source for a microscope objective, producing a collimated Gaussian beam whose linear polarization angle is chosen to be at 45° with respect to the polarization optics that follow. The beam passes through an inclined quartz birefringent plate that causes a displacement of the S and P polarization components with respect to each other. The beams then pass through a Wollaston prism that introduces a relative angular deviation of the two polarization components, causing them to cross at a point beyond the rear face of the prism; the combined effect of the birefringent plate and Wollaston prism is similar to that of a Namarski prism with an unusually large divergence angle. The S and P polarized beams are incident on the rear aperture of a 20X water immersion microscope objective. The diameter of the beams are chosen to match the rear aperture of the objective and the crossing point is placed at the rear focal plane of the objective. The microscope objective brings the collimated beams to diffraction limited Gaussian focal spots in the image plane. The angular deviation of the two beams causes the focal spots to occur at distinct positions in the sample plane. The Wollaston angle was chosen to make this separation approximately $5 \mu\text{m}$; the choice of separation distance depends on the application. Since the two beams cross at the rear focal plane of the objective, their central rays are parallel. The specimen chamber is positioned so that the two beams are normally incident with their waist at the bottom reflective surface. As a result, the two beams are retro-reflected back into the microscope. The two beams are recombined by the Wollaston and birefringent plate and are coupled out of the apparatus by a 50% reflector.

The beam that emerges from the apparatus consists of a vertical polarization component that has passed through the axon and a horizontal polarization component that has bypassed the axon. The optical phase shift of the resting axon changes the polarization state of the output beam from linear to an arbitrary elliptical state. A change in the optical density or path length of the axon during an action potential would result in a perturbation to the elliptically polarized state.

To detect the small phase shifts expected in response to an action potential, the measurement must employ a differencing scheme to cancel technical fluctuations in the laser light intensity. Here, the output beam is passed through an electro-optic modulator (EOM), which is used to adjust the relative phase shift of S and P polarization components for the resting neuron. There are two ways the EOM and polarization analyzer may be used (Fig. 1d). The nominal phase shift between the two polarizations may be set to 90° . In this case the output beam has circular polarization (solid blue curve in Fig. 1b) and the analyzer, which is

oriented to measure diagonal components I_u and I_v sees a balanced signal. This corresponds to 90° in Fig. 1c. A deviation in the phase causes a deformation of the circular state (dashed blue curve in Fig. 1b) which imbalances I_u and I_v . As a result, the difference signal $I_u - I_v$ is a measure in the phase in which the laser intensity cancels.

A more effective detection method (called the dark fringe method) is to set the nominal phase shift between the two polarizations to 180° so that the output state is linearly polarized along v and the intensity I_u is zero (solid red curve in Fig. 1b). An additional phase shift results in a small deviation from linear polarization that causes I_u to become non-zero. This signal cannot be used directly, since the intensity  is second order in the phase Δ and would have a vanishing signal-to-noise ratio. However a first order signal can be obtained by superimposing a sinusoidal modulation of frequency f_s on the EOM phase shift. For the resting axon the modulation in the neighborhood of 180° produces no first order signal in I_u because the slope of the I_u vs phase curve is zero (see line marked b in Fig 1c). However, a positive phase shift in the axon would bias the net phase towards higher values (line marked c in Fig 1c) where the slope of the curve is positive. In this case the phase modulation produces a signal at frequency f_s in I_u with amplitude proportional to the axon phase shift. Similarly, a negative axon phase would bias the phase towards negative values (line marked a in Fig 1c) and produce a modulation with negative amplitude in I_u . The amplitude of the signal at frequency f_s (and therefore the axon phase retardation) can be measured using a lock-in amplifier referenced to the EOM modulation signal as shown in Fig 1e. The primary advantage of this technique is that the photodetectors have much less noise near the carrier frequency (chosen to be $>100\text{kHz}$) than near dc. In this configuration, both fluctuations in laser intensity and $1/f$ noise in the photodetectors are avoided and the principal sources of noise are shot noise and fluctuations in the preparation itself.

Short Example of Application

Figure 2 shows the preparation chamber for the walking leg nerve dissected from lobster and the optical phase shift recorded from a nerve. The nerve was micro-dissected so that only a small number of axonal fibers remained in the bundle. The second of the above detection schemes for phase-detection was used. Action potentials were elicited by stimulating the nerve with a voltage pulse set just above threshold; this minimized the number of axons excited. The extracellular signal from the action potential(s) were detected using electrodes placed beyond the imaging point. An optical phase shift of approximately 0.4 mradian was measured after averaging 100 pulses. This signal may contain contribution from one than one axon.

Advantages and Limits

The advantage of this technique is that a direct measure of change in optical density is made at a specific point. The measurement is performed in such a way that noise in the measurement process itself, e.g., from fluctuations in laser intensity, detector noise, etc., are minimized. The main limitations are that it requires an optically thin, in vitro sample, since any distortion in the beams degrades the sensitivity of the polarization analyzer. The final signal-to-noise ratio seems to be dominated by fluctuation in the specimen itself. Although these are hard to quantify, the main effects are probably Brownian motion of the physical structures and biochemical fluctuations in the axon cytoplasm.

References

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FIGURE LEGENDS

Figure 1 - Schematic of the interferometer and polarization-based detection system for changes in optical path length.

(a) Essential optics in the interferometer. The broad grey bar represents the total extent of the beam and the black line represents the central ray. After separation by polarization components, red and blue shading indicates S and P polarization components of the light. Laser light is introduced via a polarization preserving optical fiber at the top and propagates down to the preparation plane through a microscope objective, polarization components, and a second water immersion objective. The preparation plane is reflective at the excitation wavelength and the light is retroreflected and ultimately coupled out of the microscope using a 50% reflector. The output beam is passed through an electro-optic (EOM) modulator and is detected with a polarization analyzer shown in greater detail in part d. The principals of operation are described in the text.

(b) The electric field as a function of optical phase shift where the S, and P axes correspond to the polarization directions of the two rays (red and blue) in part a and the u, and v axes correspond to the two channels of the polarization analyzer. The projection of the curves on the u and v axes gives the amplitude of that component that is transmitted to the detectors.

(c) The intensity measured by the two channels of the analyzer as a function of optical phase shift. The short red lines are explained in the text.

(d) Detailed schematic of the phase-sensitive detection scheme. The EOM introduces a phase shift of the S polarized field with respect to the P polarized field. A DC voltage, denoted V_0 , is applied to correct for the phase shift of the resting axon. The $\pi/2$ plate rotates the polarization state so that the analyzer detects the intensity along two diagonal directions u and v. For balanced detection the intensities I_u and I_v are nominally equal (see text) and are subtracted to give a phase signal that is denoted Δ_b . For dark fringe detection, V_0 is set so that all light goes to the I_v detector. A phase modulation, denoted V_s with frequency f_s , is superimposed on the EOM drive signal. A lock-in amplifier reference to the modulation voltage V_s is used to perform a phase sensitive measurement of the amplitude of the f_s component in I_u . This amplitude is proportional to $(1/2)I_t(\Delta_d)$ where I_t is the total intensity and Δ_d is the deviation of the optical phase from the dark fringe configuration (in radians).

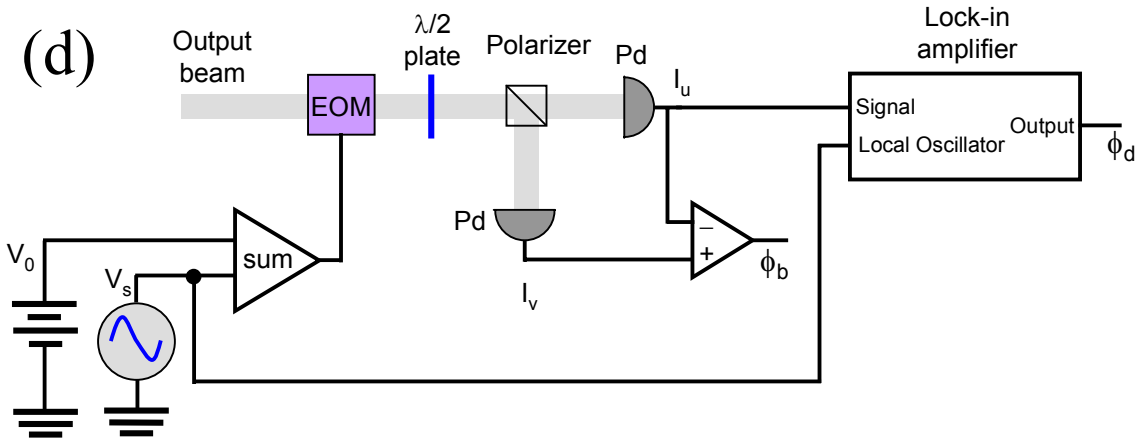
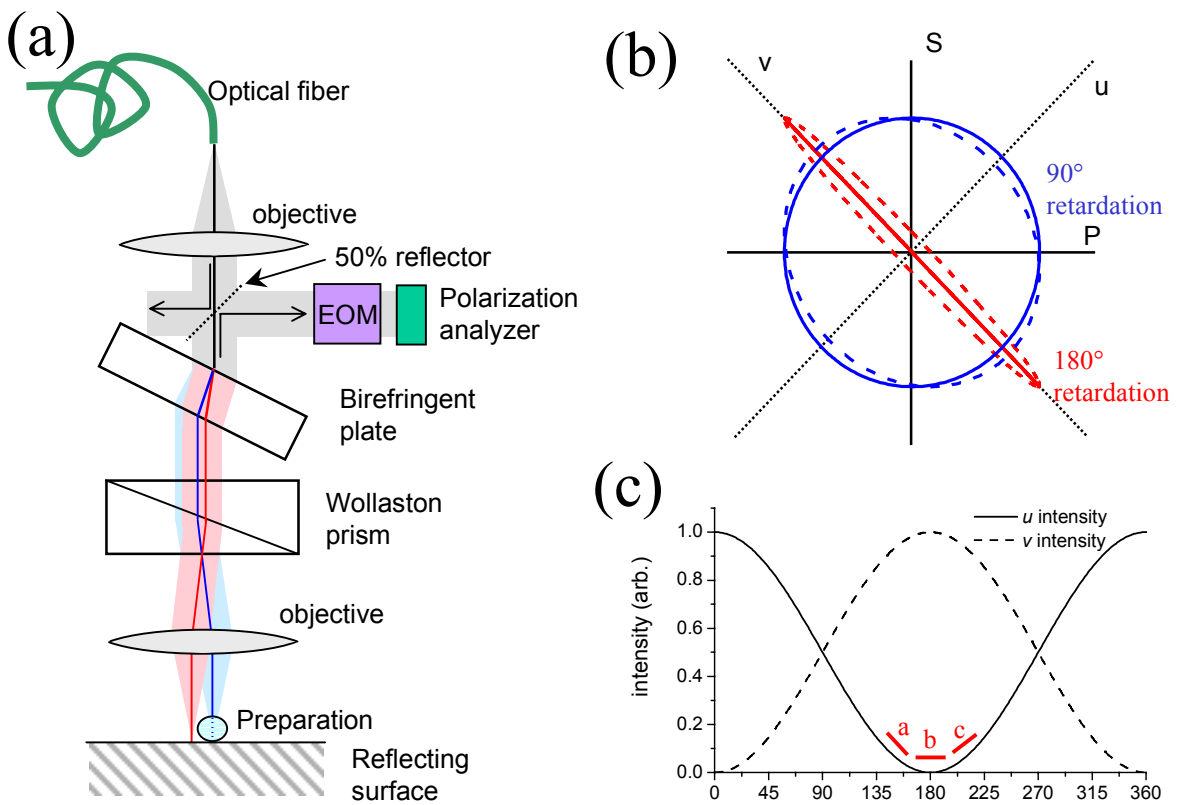
Figure 2 - Results for interferometric measurements through a walking leg nerve from lobster, *Homarus americanus*. The nerve is maintained in artificial sea water. The nerve was micro-dissected down to a few, but more than one, axonal fiber.

(a) The chamber that holds the preparation is a multicompartiment structure that positions the nerve across a dielectric mirror that has a maximal reflection at 630 nm, close to the HeNe emission line. One beam (P polarization, blue) passes through the nerve and the second (S polarization, red) passes about

5 μ m away from the nerve. The nerve further rests on 2 platinum wires at one end, which serve as the voltage stimulation source, and two at the other end, which serve as extracellular recording electrodes. Thin walls with slits that are filled with "Vaseline" provide electrical isolation between the chambers.

(b) The optical signal, denoted by the change in output phase $\Delta\phi$ between the two beams, in response to a just suprathreshold stimulus. Shown is the stimulus triggered average of 100 samples.

(c) The concomitantly measured and averaged electrical signal. It appears later than the optical signal since it is measured further along the axons.



LaPorta and Kleinfeld, CSHL Laboratory Manual - Figure 1

