

Detection of Action Potentials In Vitro by Changes in Refractive Index

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The detection of individual action potentials in nerve cells via intrinsic optical signals could open a new modality for the analysis of cellular properties in spatially extended cells and emergent properties in networks of neurons. Here we review the experimental evidence for the detection of changes in intrinsic optical properties, i.e., changes in optical refractive index, that covary with changes in the transmembrane voltage.

The pioneering experiments of Cohen, Keynes and Hille (1968) demonstrated that there is a change in optical properties, as revealed through light scattering and birefringence measurements on squid (*Loligo foresi*) giant axons, that faithfully tracks the change in transmembrane potential. The subsequent experiments of Stepnoski et al. (1991) made use of light scattering to characterize the optical properties of processes from identified invertebrate neurons (*Aplysia californica*) in culture and the change in these properties with a change in membrane potential. From the perspective of a laboratory tool, Stepnoski showed that standard darkfield microscopy could be used to record action potentials in real-time with signal-to-noise ratio of 10 or more (Fig. 1). Further, it was conjectured that the signal-to-noise ratio would be larger for the much finer processes that occur with cultured mammalian neurons.

Three essential results about the biophysics of neurons were concluded from the work of Stepnoski et al. (1991). We review these in the context of both recent and historical work.

1 - The average index of the cytoplasm relative to saline is

$$\langle n \rangle = 1.021 \pm 0.005 \text{ (mean } \pm \text{ SD).}$$

Further, the index varies across the diameter of the cell, such that is 1.6-times larger in the center than at the edges. This result confirms that the refractory properties of neuronal processes are dominated by the cytoplasm, although the membrane is the locus of voltage dependent changes.

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The cell membrane has an index that can be estimated to be 1.1-times that of saline. On the other hand, the 1 to 2 nm thickness of the membrane minimizes its contribution to the optical properties of the cell. The relative contribution could be enhanced by performing experiments in an index matching solution, such as by the addition of sugars to raise the index of the extracellular solution to 1.02 times the index of saline, i.e., to an absolute value of about 1.36.

2 - The change in index is linear in the membrane voltage, V_m , over a range that exceeded $-75 \text{ mV} < V_m < +75 \text{ mV}$ (Fig. 2A). This implies that the change in optical properties with membrane potential results from an interaction between the optical field and molecules with fixed dipole moments.

Analysis and physical modeling of the angular dependence of the voltage induced change in optical index strongly implied that the change in index is secondary to a net change orientation of dipoles in the membrane that is induced by a change in electric field across the membrane. This same model implies that the scattering should depend on the polarization of the light relative to the radial axis of the axon. Unfortunately, the polarization dependence was never reported, so the predictive aspect of the model remains untested.

The work of Cohen and colleagues (1972) addressed the polarization dependence of optical changes in squid giant axon as well as the extended voltage dependence of intrinsic optical changes. In particular, they showed that the birefringence of axons exhibits a quadratic voltage dependence, centered around $+130 \text{ mV}$, over the range $-200 \text{ mV} < V_m < +200 \text{ mV}$. This data provides support for the predicted polarization dependence for scattering from *Aplysia* neurons is likely to be true. On the other hand, the quadratic nature of the scattering with the squid axons implies that the change in optical properties results from neutral molecules in the membrane whose dipole moment is induced by a permanent transmembrane electric field. This field acts as a bias and could be caused by charges in proteins that span the membrane. As above, this induced dipole moment is then modified by a change in electric field across the membrane.

The linear voltage dependence observed in *Aplysia* over a limited range of voltages appears to be inconsistent with quadratic dependence detected over a large range (gray line in Fig. 2b). Thus, to the extent that a single mechanism for voltage dependent changes in the optical index applies across species and preparations, there is an inconsistency between the original squid data (Cohen et al., 1972) and the later *Aplysia* (Stepnoski et al., 1991) data.

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3- The change in index induced by changes in membrane voltage was $\Delta n = (1.2 \pm 0.4) \times 10^{-4}$ nm/mV. For a 1.8 nm thick membrane and a 50 mV action potential, this corresponds to a fractional change in index of

$$\Delta n / \langle n \rangle = (3.3 \pm 0.1) \times 10^{-3}.$$

Noting that the optical theorem related the forward scattering cross-section to the phase shift, this results corresponds to an equivalent optical phase shift of 1 to 2×10^{-4} radian per spike.

The phase shift can be directly measured by interferometer. Preliminary data was recently reported using a dual beam interferometer in conjunction with a walking leg nerve from lobster (*Homarus americanus*). The nerve was micro-dissected so that only a small number of axonal fibers remained in the bundle. Action potentials were elicited by stimulating the nerve with a voltage pulse set just above threshold (insert in Fig. 3a); 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 4×10^{-4} radians was measured after averaging 100 pulses (Fig. 3a). This signal may contain contributions from more than one axon yet was consistent in width with the concurrently measured electrical pulse (Fig. 3b). Nonetheless, there is semi-quantitative agreement between the inferred and directly measured values.

Future directions

Changes in intrinsic optical properties have never been fully exploited as a tool for biomedical research. The most pressing direction is to probe fine processes in cultured mammalian neurons. This should be most simple to accomplish with darkfield illumination or with total internal reflection microscopy (Axelrod et al. 1994). The latter method requires the use of glial-free cultures (Baker and Cowan 1977) or an equivalent mechanism to avoid distancing the processes from the glass surface (G.-Q. Bi, H. Davidowitz and D. Kleinfeld, unpublished). Basic biophysical issues, especially the polarization dependence of light scattering from axons, should be explored in the event that mammalian cultures can be probed in a useful manner.

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FIGURE LEGENDS

Figure 1 - Real-time measurement of spiking activity in a cultured left-upper-quadrant cell from *Aplysia* after 3 d in vitro. The cells were maintained in an artificial hemolymph. The train was induced by a current pulse, ΔI_m . The optical changes were recorded as the fractional change in scattered light, denoted $\Delta I/I$, from a field of fine processes illuminated with darkfield illumination. The amplitude of the spikes in the concurrently recorded intracellular membrane potential, denoted V_m , appears uneven as a result of undersampling. The top and bottom sets of traces are from two different cells. Previously unpublished data from the study of Stepnoski et al. (1991).

Figure 2 - Dependence of intrinsic optical signals on transmembrane voltage as measured in two preparations using voltage clamp.

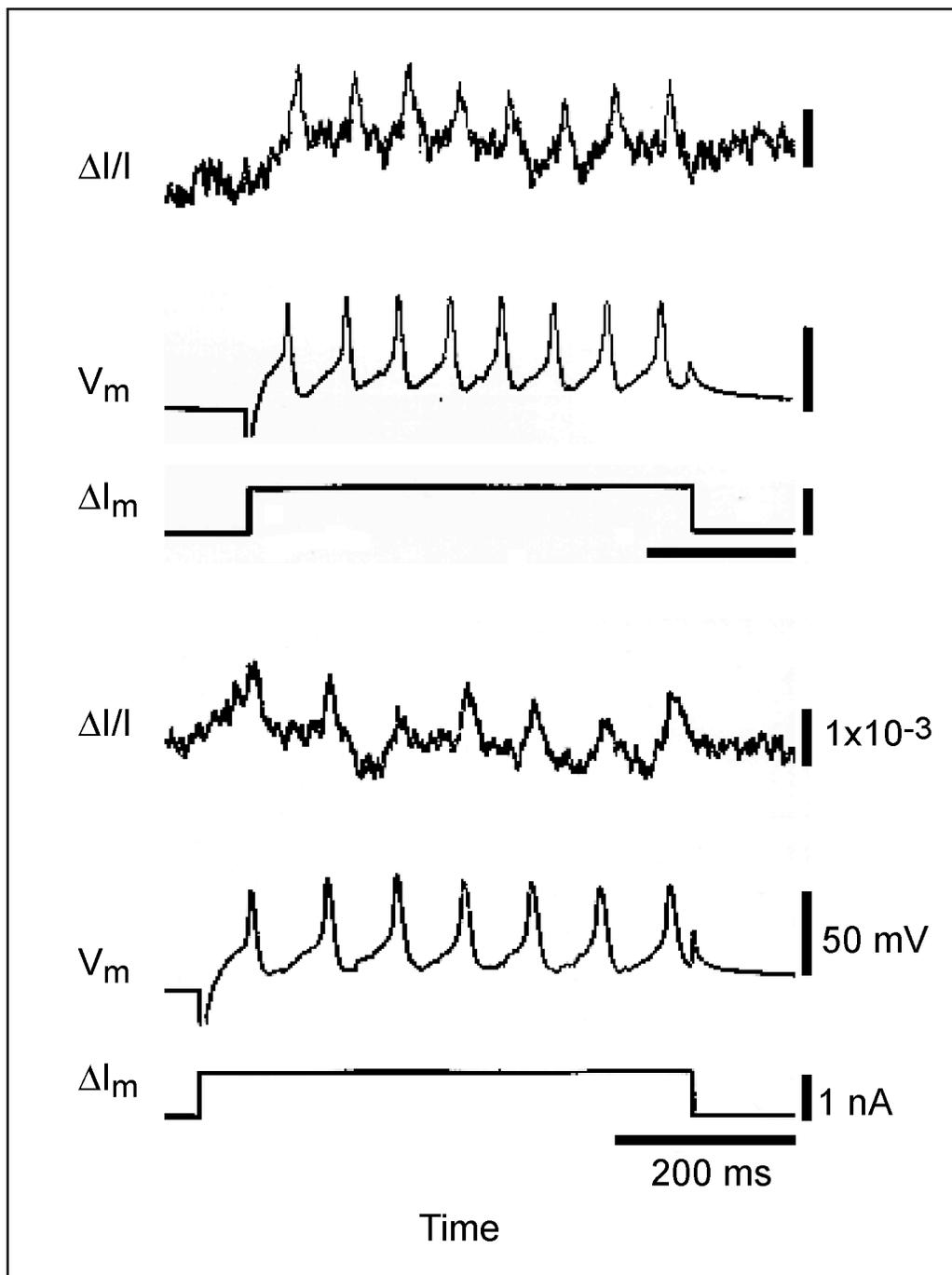
(a) Results for the intensity of the change in scattered light, measured under darkfield conditions, as a function of intracellular voltage (2 electrode voltage clamp) for *Aplysia*. A 2-electrode voltage clamp was used, monitoring the intracellular potential at the soma and injecting current, I_{feedback} , in the proximal process. Panel adapted from Stepnoski et al. (1991).

(b) Results for the intensity of the fast, initial change in birefringence measured for the squid giant axon. The gray straight line was drawn to illustrate the best fit of a linear relation between -85 mV and +75 mV that is constrained to pass through the origin. Panel adapted from Cohen et al. (1972).

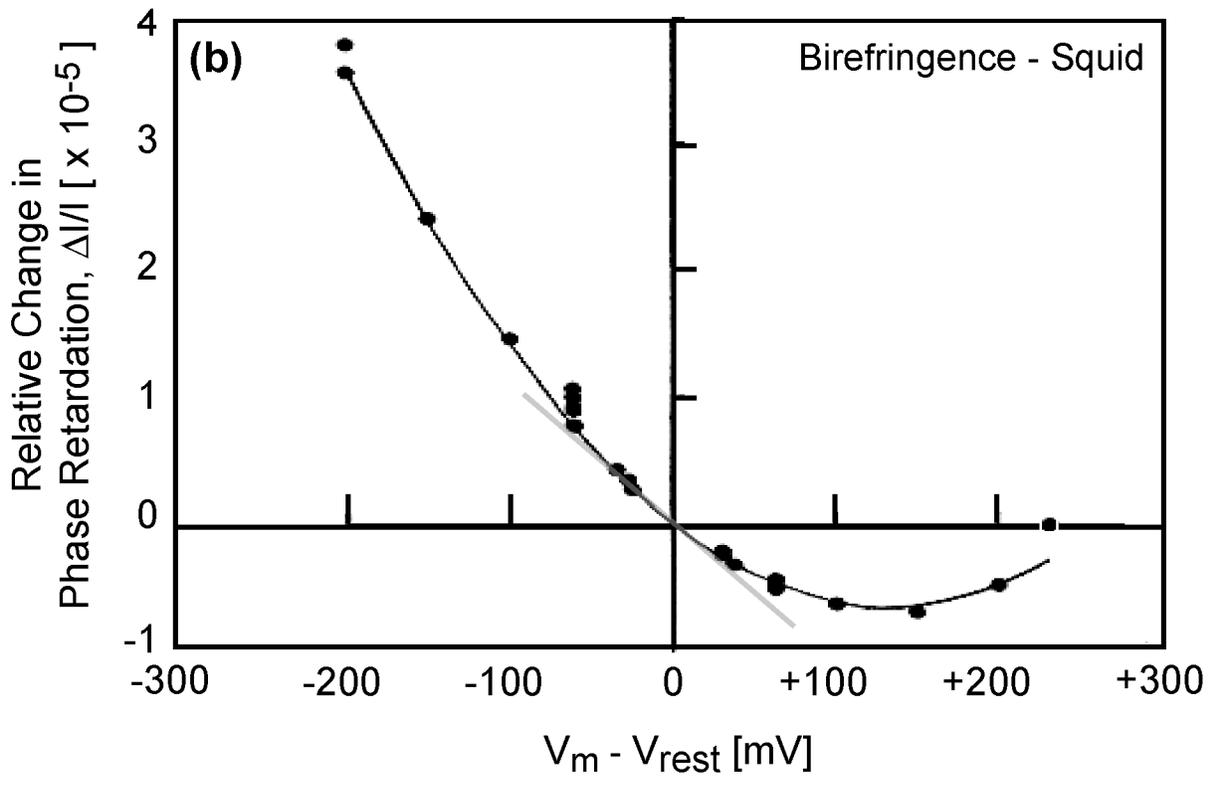
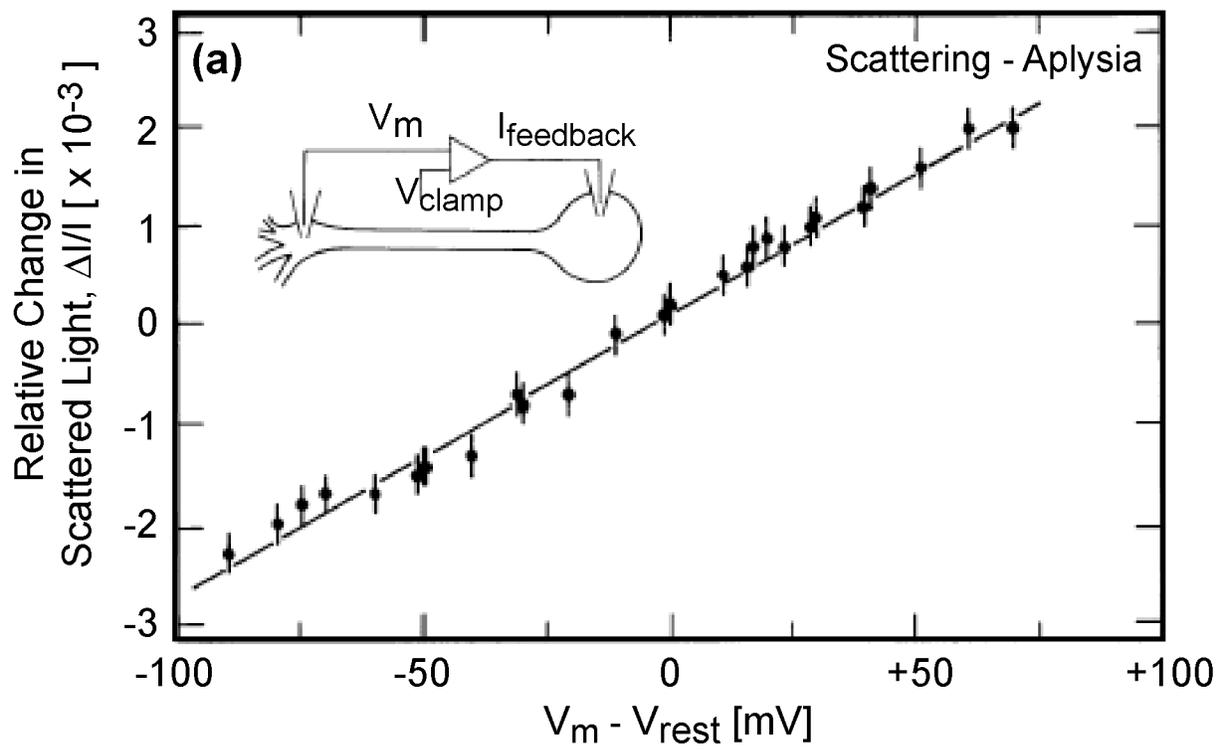
Figure 3 - Interferometric measurements through a walking leg nerve from lobster. The nerve was maintained in artificial sea water. The nerve was micro-dissected down to a few, but more than one, axonal fiber.

(a) 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.

(b) The concomitantly measured and averaged electrical signal. It appears later than the optical signal since it is measured further along the axons. Figure adapted from LaPorta and Kleinfeld (2003).



Kleinfeld and La Porta - Figure 1



Kleinfeld and La Porta - Figure 2

