Noninvasive functional imaging of the retina at cellular resolution

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Intrinsic optical signal imaging allows noninvasive identification of localized retinal neural dysfunction, promising early diagnosis of eye diseases.

It is well established that many eye diseases, such as age-related macular degeneration, retinitis pigmentosa, glaucoma and diabetic retinopathy are associated with pathological changes in the retina in multiple cell classes. Different eye diseases affect different types of retinal cells, some of which occur in localized areas of the retina in the early stage of the disease. Accurate identification of retinal dysfunction at the local, cellular level is essential for early disease detection and reliable treatment evaluation. However, given the delicate structure of the retina, functional examination of individual cell types is challenging.

Electroretinogram (ERG) and perimetry measurements can provide valuable assessment of retinal function. However, spatial resolution and signal selectivity of these established methods may not be high enough to allow precise separation of outer and inner retinal cell dysfunction. While it is possible to combine morphological imaging, such as high-resolution optical coherence tomography, with functional assessment to improve retinal study and diagnosis, conducting these separate measurements is time-consuming and cost-inefficient. In order to achieve high-resolution imaging of retinal function, we are exploring high-resolution intrinsic optical signal (IOS) imaging of retinal activation.

Without the requirement of exogenous biomarkers, IOS imaging is label-free and totally noninvasive. In principle, both stimulus-evoked retinal neural activity and corresponding hemodynamic (changes in blood supply) and metabolic changes can produce transient IOSs associated with retinal stimulation. While hemodynamic and metabolic change-correlated IOSs can provide important information in healthy assessment of the visual system, they are relatively slow and cannot directly track fast neural activities in the retina. Fast IOSs, which have time courses comparable to electrophysiological kinetics, can provide direct evaluation of physiological health of retinal photoreceptors and inner neurons.

It was generally believed previously that fast IOSs correlated with stimulus-evoked neural activity were inherently tiny signals that were only obtainable at high background light intensity and difficult for practical applications. However, using optimized near-IR light illumination, improved imaging resolution and localized retinal stimulation, we have recently demonstrated fast IOS imaging of stimulus-evoked activities in animal retinas. High-spatial (μm) and high-temporal (ms) resolution imaging have revealed robust IOSs which had time courses comparable to ERG kinetics.1

We used freshly isolated animal retinas for preliminary validation of fast IOS imaging. While we used the flat-mounted retina to achieve spatiotemporal IOS mapping of individual functional layers,1 we employed retinal slices comprising a cross-section of the retina to demonstrate simultaneous IOS monitoring of visual signal propagation among multiple functional layers.3 High-resolution imaging revealed both negative (decreasing)
and positive (increasing) signals in adjacent retinal areas (see Figure 1), suggesting that high resolution is critical to minimize spatial pooling for robust detection of fast IOSs.

In vivo IOS imaging of intact animals (the *Rana Pipiens* frog) was also recently validated. In order to achieve high spatio-temporal resolution imaging, we constructed a rapid line-scan confocal ophthalmoscope. Using precise image registration, the confocal imager provided enough resolution to differentiate individual photoreceptors in vivo (see Figure 2, left panel). A movie clip is available online to illustrate the stability of in vivo retinal imaging of intact animals (frogs). With visible light stimulation, rapid imaging disclosed fast IOSs that were tightly correlated with retinal stimulation (see Figure 2, right panel).

Fast IOS imaging promises to be a noninvasive method to provide cellular resolution tests of retinal function. So far, in vivo IOS imaging of normal animals has been demonstrated. Our next step is to employ diseased animal models to test the relationship between IOS abnormalities and retinal dysfunctions and to further instrument developments to pursue functional IOS imaging of human retinas.

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**References**


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