Aberration in 3-Photon Transcranial Calcium Imaging of Cortical Activity

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Abstract: 3-photon excitation permits fluorescence microscopy through the intact skull, but is highly sensitive to aberration. We estimate aberration encountered in focusing 1250 nm light through a glass-on-acrylic window for chronic transcranial *in vivo* imaging.
OCIS codes: (180.2520) Fluorescence microscopy; (180.4315) Nonlinear microscopy; (090.1000) Aberration compensation

Dissection of complex neural computations, such as those underlying visually guided behavior, requires analysis of patterns of activity of single neurons wired in local circuits, as well as of meso-scale connections between distant cortical areas involved in sensory processing, decision making, and motor output. Widefield transcranial optogenetic mapping and fluorescence imaging of genetically encoded calcium indicators, such as variants of the widely used GCaMP-family of fluorescent proteins, have enabled analysis of large-scale activity patterns and connectivity between cortical areas on the millimeter scale [1,2]. On the level of single neurons, however, multiple scattering of incoming excitation light and outgoing fluorescence in thick bone and in neuropil has largely restricted direct imaging of single neuron activity to 2-photon imaging in preparations which involve surgically penetrating [3,4] or thinning [5] the skull.

Progress in leveraging 3-photon excitation in fluorescence microscopy has extended *in vivo* multiphoton imaging into novel territories [6], primarily through reducing out-of-focus and scattered excitation, and improving signal-to-background contrast. In particular, fluorescence imaging through the intact skull has been shown to be possible without optical or computational scattering compensation [7]. Exciting with ~40 fs (transform-limit) pulses at 1250 nm, we have imaged fine neuronal structure and calcium activity *in vivo* with single cell resolution through the skull with only the skin and periosteum removed, conditions in which conventional 2-photon microscopy performs poorly (Fig. 1).

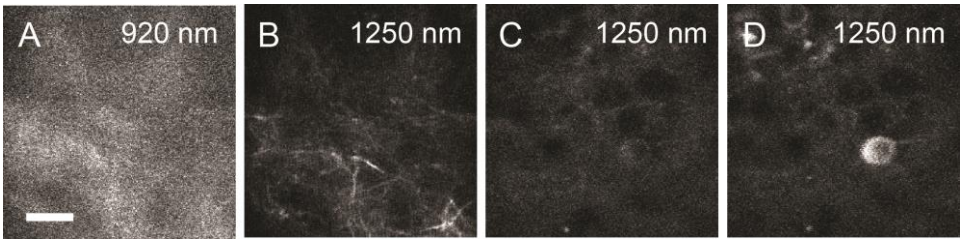

Figure 1: Transcranial fluorescence imaging of neuronal structure and activity through intact skull. A, 2-photon (920 nm excitation) transcranial fluorescence image of dendritic YFP at a depth of 150 μm below the pial surface of cortex. B, 3-photon (1250 nm excitation) transcranial fluorescence image of dendritic YFP in the same mouse and field-of-view as in (A). C, 3-photon transcranial fluorescence image of resting GCaMP6s fluorescence. D, 3-photon transcranial fluorescence image of a spontaneously active neuron in the same field-of-view as in (C). Scale bar: 20 μm.

To extend these capabilities to transcranial preparations suitable for widefield imaging and meso-scale access to multiple cortical areas, aberrations introduced by the sample preparation must be addressed. While the higher-order

nonlinearity of 3-photon over 2- and 1-photon excitation improves image quality in the presence of scattering and volume fluorescence, the nonlinear dependence of signal on intensity also increases sensitivity to aberration-induced spreading of the microscope focus [8]. Compensation of focus-degrading aberrations is thus expected to be of even greater benefit than in 2-photon microscopy, particularly when penetrating through turbid layers due to the background contribution from scattered excitation light. As an initial step toward performing widefield and 3-photon transcranial imaging in the same preparation, we have characterized aberrations arising from imaging through a chronic glass-on-acrylic window of a through-skull imaging preparation used in widefield fluorescence imaging of cortical activity (Fig. 2).

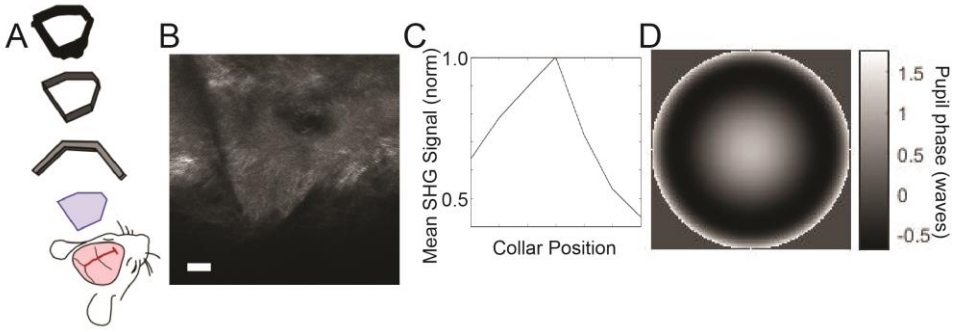

Figure 2: Estimation of spherical aberration from focusing through a chronic, transcranial window. A, schematic of transcranial window preparation. A no.1 glass coverslip adhered to the bare skull with transparent dental acrylic permits widefield fluorescence imaging in a headfixed, awake, behaving mouse. B, second harmonic image of skull surface. Scale bar: 20 μm. C, dependence of second harmonic signal on compensating spherical aberration applied by rotating the objective correction collar. D, aberrated pupil phase estimated from primary, second-order, and third-order Zernike spherical modes [9] corresponding to optimal collar correction in (C). The estimated pupil phase is in good agreement with the pupil phase calculated from a model of focusing through layers of varying refractive indices [10].

These results identify significant spherical aberration of several orders resulting from propagation through the layers of glass and acrylic, and provide an estimate of the aberrated wavefront and its consequences for imaging. These results will guide the development of corrective measures toward the goal of bridging micro- and meso-scale imaging of cortical activity and analysis of neural computation.

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