CORTEX-WIDE, CELLULAR-RESOLUTION TWO-PHOTON MICROSCOPY

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Functional imaging of the mouse brain in its extreme complexity involves substantial trade-offs. An optical intrinsic spectroscopy system can image the entire cortex but at the expense of spatial and temporal resolution [1]. A two-photon microscope (TPM) can image single neurons with high temporal resolution, but the field of view (FOV) is generally restricted. Advanced techniques like random-access scanning allow for imaging single neurons that are millimeters apart but only by ignoring the neurons and tissue in between [2]. By carefully considering the properties of the optical components as well as the imaging requirements, we present a TPM capable of imaging nearly the entire mouse cortex with 15 Hz frame rates and single neuron resolution.

Designing an effective calcium imaging TPM requires paying careful attention to the optical components, which determine the FOV and resolution, and the properties of the fluorophore, which determine the requisite signal-to-noise ratio (SNR) and frame rate. As in any laser scanning microscope, the galvanometers and relay lenses must be chosen to ensure that the optical quality of the laser beam is not degraded before it gets to the objective. To simplify design choices, we first choose an objective and then focus on the optical invariant of each component. By ensuring each component's optical invariant matches (or exceeds) that of the objective, even a basic objective can yield a large FOV and a good laser spot size, resulting in a large space-bandwidth product [3]. Furthermore, sacrificing some resolution, such as by limiting the excitation numerical aperture, can lead to large FOV gains while still being able to resolve single cells. Next, each GCaMP6 mouse line has characteristic time constants and fluorescence contrast ratio for calcium binding. With these properties in hand, we design the TPM to have a high enough SNR and frame rate to image the transients effectively. Unfortunately, a large space bandwidth product requires scanning the laser quickly, limiting the pixel dwell time and therefore the SNR. Fortunately, the nonlinear nature of two-photon fluorescence means that the signal increases quadratically with laser power. Of course, increasing the power will, in turn, adversely affect fluorophore bleaching, photodamage, and thermal damage. We show that these damage mechanisms, however, all scale beneficially with increasing spot size and FOV, so the laser power can be increased safely and substantially. With these design considerations, calcium transients at the whole cortex level can be imaged with cellular resolution, see Figure 1.

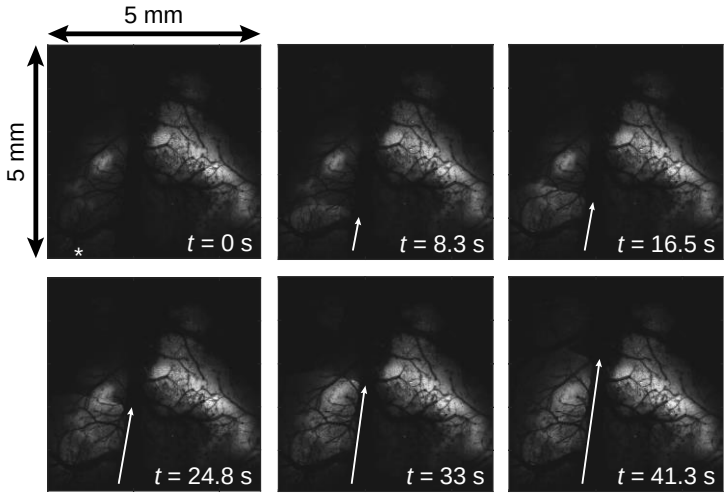

Figure 1 – Six frames of a cortical spreading depression (CSD) measured on a mouse with a full craniotomy. The field of view is 5 mm by 5 mm, and the frame rate is 15 Hz averaged down to 3 Hz for signal clarity. In the first frame, upper left, the CSD begins at t = 0 s and its location is marked with an asterisk. The CSD front propagates from posterior to anterior in the left cortex, marked by an arrow in each frame. The entire CSD lasts approximately one minute.

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2. N. J. Sofroniew, et al., "A large field of view two-photon mesoscope with subcellular resolution for in vivo imaging," eLife 5 (2016).
3. J. R. Bumstead, et al., "Designing a large field-of-view two-photon microscope using optical invariant analysis," Neurophoton. 5(2), 025001 (2018)