

**OPTICAL METHODS AND INTEGRATED SYSTEMS FOR BRAIN  
IMAGING IN AWAKE, UNTETHERED ANIMALS**

by

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# Abstract

Imaging is a powerful tool for biomedical research offering non-contact and minimally or non-invasive means of investigating at multiple scales - from single molecules to large populations of cells. Imaging in awake, behaving animals is an emerging field that offers the additional advantage of being able to study physiological processes and structures in a more natural state than what is possible in tissue slices or even in anesthetized animals. To date, most imaging in awake animals has used optical fiber bundles or electrical cables to transfer signals to traditional imaging-system components. However, the fibers or cables tether the animal and greatly limit the kind and duration of animal behavior that can be studied using imaging methods. This work involves three distinct yet related approaches to fulfill the goal of imaging in unanesthetized, unrestrained animals - optical techniques for functional and structural imaging, development of novel photodetectors and the design of miniaturized imaging systems.

I hypothesized that the flow within vessels might act as a contrast-enhancing agent and improve the visualization of vascular architecture using laser speckle imaging. When imaging rodent cerebral vasculature I saw a two to four fold increase in the contrast-to-noise ratios and was able to visualize 10-30% more vascular features over reflectance techniques.

I designed a complementary metal oxide semiconductor (CMOS) photodetector array that was comparable in sensitivity and noise performance to cooled CCD sensors, able to image fluorescence from a single cell, while running at faster frame rates.

Next, I designed an imaging system weighing under 6 grams and occupying less than 4 cm<sup>3</sup>. The system incorporated multispectral illumination, adjustable focusing optics and the high-sensitivity CMOS imager. I was able to implement a variety of optical modalities with the system and performed reflectance, fluorescence, spectroscopic and laser speckle imaging with my device, which had a footprint small enough to mount on a rat skull. I imaged cortical vessels down to 10-20  $\mu\text{m}$  in rodents and functionally imaged hemoglobin / oxy-hemoglobin concentration changes under hypoxic conditions. Finally, I designed a protocol for the attachment of the head-mounted imaging system to rodent skulls and demonstrated imaging in freely-moving rodents without any tethers.