

Special Section Guest Editorial: Imaging Neuroimmune, Neuroglial, and Neurovascular Interfaces

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In this [Special Section of *Neurophotonics*](#), we focus on optical imaging techniques and their application to studies of neuroimmune, neuroglial, and neurovascular interfaces. Lacking its own energy reserve, the brain's metabolic demands rely heavily upon a constant supply of blood. A dense network of microscopic blood vessels (arterioles, capillaries, and venules) ensures proper delivery of blood to the brain. However, the vasculature is not just static plumbing. It is highly reactive to surrounding neurons and its response can shape brain activity. In particular, the microvasculature can respond to vasoactive signals released by neighboring neurons and astroglia, and promote blood flow during periods of increased metabolic demand (i.e., neurovascular coupling).¹ Many additional vascular functions are also crucial to brain health including regulation of molecular transport into and out of the brain via the blood-brain barrier,² clearance of brain waste along cerebrospinal fluid (CSF)-filled perivascular routes,^{3,4} and regulation of immune cell entry into the brain.⁵ Our understanding of these vital functions has been accelerated by optical imaging techniques that facilitate both *in vivo* and *ex vivo* studies of the neurovasculature. The articles published here cover diverse topics and highlight a variety of innovative techniques and concepts from preclinical to clinical applications. They also showcase the exciting work being performed by young scientists that will usher in a new era of discovery.

The brain is composed of many functional regions with distinct circuitries. It is not surprising that the blood vessels serving these distinct tissues also differ in structure, cellular composition, cellular morphology, and genetic profiles.^{6,7} In addition to defining principles of blood supply to the brain, cross-regional comparisons will clarify why some brain areas are more vulnerable to pathology or why they generate different signals during fMRI. However, the scale of these cross-regional studies needs to be increased. Large-scale mapping of brain vasculature in fixed tissues is becoming more routine with simpler tissue clearing techniques and availability of light-sheet microscopy. Bennett and Kim discuss the various modalities to image whole brain vasculature, and weigh strengths and weaknesses of these approaches.⁸ They further discuss strategies to label different neurovascular cell types, which is crucial to understanding diversity in the neurovascular unit – the collection of cell types that comprise the blood vessel wall in different blood vessel types. In these endeavors, approaches are needed to label vascular cell types specifically and brightly.⁸ Frietas-Andrade et al. present an optimized approach to label the endothelium in fixed tissues and introduce software for analysis of vascular structure.⁹

When considering aging and age-related diseases, the cerebral white matter and deeper brain regions appear to be more vulnerable than cortex and the basis for this vulnerability has remained poorly understood.^{10,11} *In vivo* optical imaging has historically been limited to more superficial aspects of the cortex. Recently, three-photon imaging has become the tool of choice to image deeper into brain tissues at cellular resolution, allowing access to cerebral white matter, hippocampus and other subcortical structures without the need to remove overlying tissue.^{12,13} Thornton et al. describe how three-photon microscopy can be used for multi-color fluorescence imaging of white matter in mice using red fluorophores to image oligodendrocytes and

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neurons.¹⁴ This approach will be important for understanding the vascular basis of myelin degeneration in models of vascular cognitive impairment and Alzheimer's disease.

While whole brain imaging is possible for fixed specimens, *in vivo* imaging remains more restrictive with small fields of view. There is a need to understand how behaviors of individual small arterioles and capillaries are coordinated to regulate blood flow broadly across vascular networks. To address this challenge, some imaging techniques strike a balance between imaging resolution, field of view, and acquisition speed. Optical coherence tomography angiography (OCTA) resolves microvascular structure and flow more broadly than multiphoton microscopy. Li and Tang describe the principles of OCTA and how to overcome challenges of tailing artifacts caused by blood vessels that limit 3D imaging capacity.¹⁵ Tournissac et al. describe a bihemispheric cranial window surgery to improve optical access for longitudinal imaging of brain vasculature *in vivo* at multiple scales and across brain hemispheres.¹⁶ Imaging broad fields of view is also critical to clinical applications. Miller et al. show how laser speckle contrast imaging can provide vascular flow information across a surgical field to guide neurosurgical procedures.¹⁷

How neurons communicate with blood vessels during neurovascular coupling is a topic of great interest and debate. Several signaling pathways may be involved. Astrocytes serve as an intermediary to convey signals from neurons to arterioles by enwrapping both synapses and the vascular wall.¹⁸ Cam H. Tran synthesizes current knowledge on astrocyte roles in neurovascular coupling and new imaging approaches.¹⁹ Gorza et al. provide a comprehensive review of opportunities and challenges to imaging subcellular astrocyte calcium activity using multiphoton microscopy.²⁰ Recent work also suggests that neurons and astroglia provide signals to capillaries, which then rapidly conduct signals upstream to arterioles leading to vasodilation.²¹ Jeffrey et al. describe an isolated preparation with parenchymal arteriole and attached capillary networks ideal to probe the underpinnings of capillary-to-arteriole conductive signaling.²² Some experiments may require *ex vivo* brain slices approaches for rigorous pharmacology, and Bojovic et al. describe the use of acute brain slices as a model in neurovascular research.²³ Studies are also increasingly in need of fast imaging of calcium and voltage sensitive dyes across tissue volumes; Har-Gil et al. provide new tools to address this need.²⁴ This collection of approaches provides a strong platform for mechanistic studies.

Beyond neurons and astrocytes, other cells of the neurovascular unit are also being actively investigated. Vast capillary networks lie beyond the arterioles and blood flow regulation within capillaries is still not well understood. Recent studies have shown that capillary pericytes serve a role in local blood flow regulation but may contribute to slower aspects of this regulation.²⁵ This may be due to distinctions in the contractile machinery of pericytes, compared to more dynamic tone changes controlled by smooth muscle cells. Erdener et al. provide a deep dive into the cytoskeletal elements that may control pericyte contraction.²⁶ Occupying a perivascular niche next door to smooth muscle and pericytes of the arteriole-capillary transition zone, perivascular fibroblasts have recently come into the spotlight as potential regulators of vascular structure and function.^{27,28} They may regulate the structure of the vascular basement membrane, an acellular layer of matrix proteins in the vascular wall, or communicate with smooth muscle cells or pericytes. However, their physiological roles remain largely unknown. Jones et al. demonstrate how fibroblast localization can be mapped in cleared brains of fluorescent transgenic mice.²⁹ Vascular cell types also communicate through release of extracellular vesicles, which carry signaling molecules and microRNAs with broad effects on brain function. Bağcı et al. describe approaches to define the molecular heterogeneity of extracellular vesicles.³⁰

In vivo imagers are acutely aware of the observer effect, as one can easily disrupt the delicate physiological processes they intend to study. It is therefore paramount to use approaches that minimize the disruption of the brain and physiological state of the animal. Anesthetics commonly used during *in vivo* imaging shift our observations away from normal physiology. Zhang et al. provide a detailed guide on best practices to image and measure neurovascular responses in awake mice.³¹ Studying vascular development *in vivo* is also challenging because it is a highly orchestrated process³² easily derailed by neuroinflammation associated with skull removal during cranial window generation. Coelho-Santos et al. demonstrate how to minimize perturbations by creating thinned-skull windows in mouse pups for longitudinal imaging.³³ Similarly, flow of cerebrospinal fluid (CSF) through perivascular routes during clearance of brain waste is a delicate process that can be disrupted by invasive cranial window implantation.

Kiel et al. demonstrate how infrared imaging of CSF tracers can be used to track CSF tracer flux broadly in the brain through an intact skull.³⁴

Immune cells within the blood, CSF, and marrow of the skull, can interact with and affect cerebrovascular function. This interplay becomes particularly important during brain disease, as recruited immune cells can release vasoactive substances, penetrate the brain tissue, obstruct blood flow, and damage vascular walls. Barkaway et al. provide a review that beautifully illustrates concepts and directions at this intersection between neuroimmunology and cerebrovascular research.³⁵ Faulhaber et al. introduce easy-to-use reagents that label circulating immune cells for *in vivo* imaging that may facilitate new discoveries in this area.³⁶ Further, Fukada et al. provide an innovative photothrombotic approach to occlude single brain vessels at depth that could be applied to precision studies of immune cell-vascular responses to brain injury.³⁷

In summary, cerebrovascular research is clearly alive and well. There is no shortage of questions, powerful techniques, or innovative young researchers in our quest to uncover mechanisms of cerebrovascular function in health and disease. We are honored to have served as guest editors for this exciting collection of papers.

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