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A Systemic Triple Label Strategy for Fluorescent Microscopy of Inflammation in CNS and Non-CNS Tissue.

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A variety of histology and microscopy procedures have been developed to investigate trauma and inflammation of CNS and non-CNS tissue in rodent animal models. However, typical methods require thinly slicing tissue section (<60 μm) and post-processing the individual sections in expensive histological labels. Finely slicing and manipulating tissue slices during processing can morphologically distort the tissue [1]. Instead, imaging thick sections of tissue can 'capture' the injury site and surrounding tissue intact for imaging [2]. Here we describe pairing multi-fluorescent labeling strategy to systemically label blood vessels and blood-brain barrier leakiness along with a commercially available transgenic mouse to produce a systemic triple labeled mouse for histological analysis of trauma and inflammation.

To induce a replicable, small brain injury trauma, a microelectrode array shank was surgically implanted into the cortex of eGFP-CX3CR1 mice, which express the fluorescent protein eGFP primarily in macrophages and microglia immune cells. Mice were later given an injection of Evans blue dye solution 6 hours before perfusion. Mice were perfused under deep anesthesia with PBS, followed by DiI lipophilic dye, and finally 4% formaldehyde. Brains were collected and sliced in thick (0.5 mm) sections to 'capture' the implanted device and surrounding brain tissue. These slices and other, non-CNS tissue of interest were placed in optical clearing solution and imaged using confocal microscopy.

We determined that collecting Evans blue, DiI, and eGFP fluorescent signal was achievable simply through sequential collection of each fluorescent signal. Although Evans blue dye has a long history of use as a blood-brain barrier leakage marker, it is seldom if even imaged under laser confocal microscopy; we report that Evans blue in CNS tissue appears to collect within neuron cell bodies over time, and not microglial cells (Figure 1). Evans blue decreased in intensity with distance from injury, corresponding with activated microglia morphology.

Imaging non-CNS tissue revealed Evans blue concentrated in areas of high vascular perfusion, such as DiI-labeled capillary beds at the tips of fingers and the salivary glands (Figure 2a, 2c). Macrophages and some lymphatic vessels of the immune system were imaged expressing eGFP (Figure 2b).

The features of this systemic labeling combination, described here in brief, are very well suited for studying the impact of trauma and inflammation in both the mouse CNS and non-CNS tissue. Through utilizing optical clearing solutions and laser-based microscopy tools, the outlined histological strategy can be used to analyze largely intact tissue specimens for assessment of trauma and inflammation, such as around implanted devices [3]. [4]

References:

[1] AJ Woolley, *et al*., Journal of Neuroscience (2011)

[2] AJ Woolley, *et al*., Journal of Visualized Experiments (2013)
[3] AJ Woolley, *et al*., Journal of Neural Engineering (2013)
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Figure 1. Maximum intensity projection images through 60 μm of tissue are shown collect with confocal microscopy at the split between right and left hemispheres in the cortex (longitudinal fissure). Evans blue (a), delivered 4 hr before perfusion, is visible in the injured (right) hemisphere both within neuronal cell bodies and dispersed throughout the parenchyma. DiI (b), injected in the vasculature, and eGFP (c), expressed in microglia, cleanly separate by imaging sequentially (d). Scale bar: 100 μm.

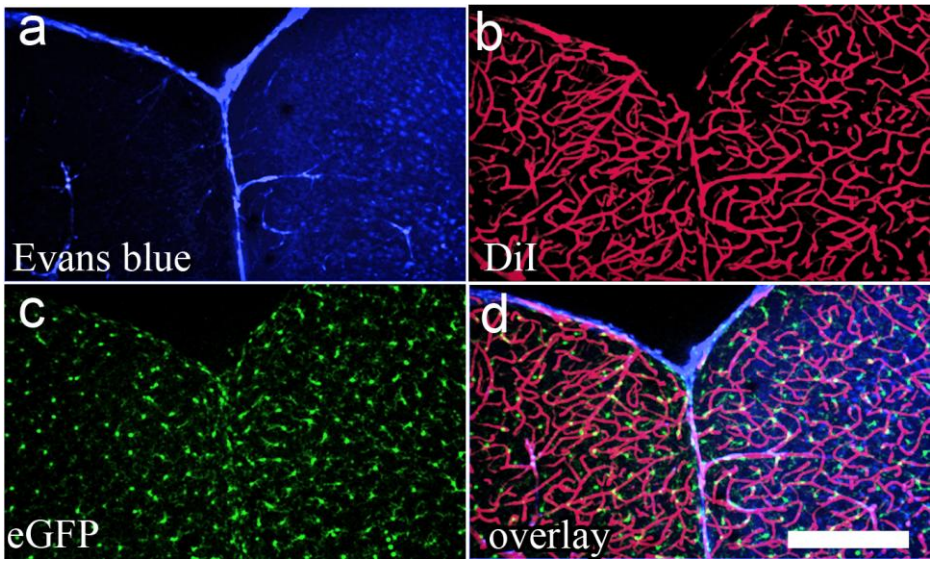
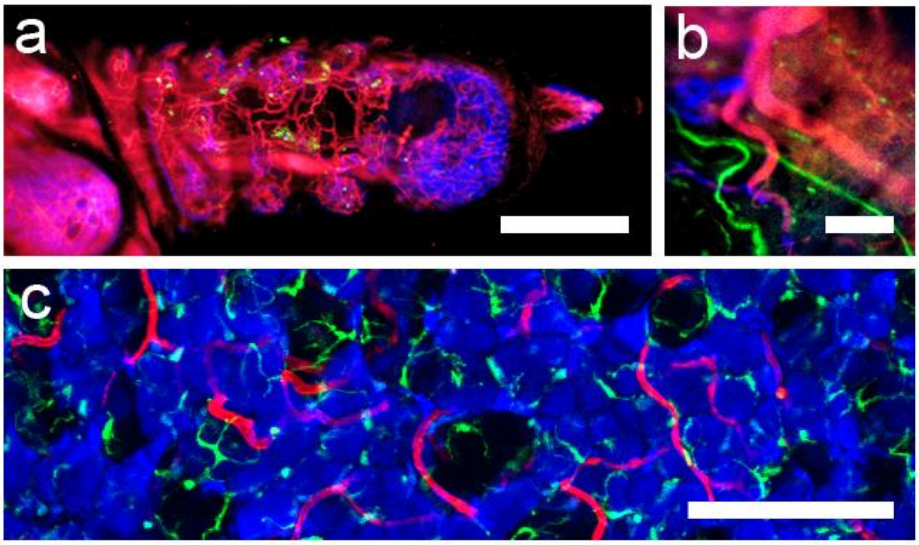


Figure 2. Adult mouse expressing eGFP (green) in macrophages was injected with Evans blue (blue) and perfused with DiI (magenta); details views of the fingers (a), palms of the feet (b), and salivary gland (c) are shown. Evans blue was widely seen in areas of fine capillary beds. Macrophages and some lymphatic vessels (b) were visible in the eGFP channel. Scale bars: 400 μm (a), and 60 μm (b, c).



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