

## **In vivo quantification of microglia dynamics with a scanning laser ophthalmoscope in a mouse model of focal laser injury**

Clemens Alt, Charles P. Lin

*Advanced Microscopy Program, Wellman Center for Photomedicine and Center for Systems Biology, Massachusetts General Hospital, Harvard Medical School*

Microglia are the resident immune cells of the central nervous system and play a crucial role in maintaining neuronal health and function. Their dynamic behavior, that is, the constant extension and retraction of microglia processes, is thought to be critical for communication between microglia and their cellular neighbors, such as neurons, astrocytes and endothelial cells of the vasculature. Here, we investigate the dynamics of retinal microglia in vivo under normal conditions and in response to focal laser injury of blood vessel endothelial wall.

We have developed a scanning laser ophthalmoscope specifically for mouse retinal imaging that allows retinal microstructure, such as the processes of microglia and retinal vasculature, to be visualized. In order to generate focal laser injury, a laser photocoagulator was adapted to the SLO. An acousto-optic modulator chopped pulses from a continuous wave laser. A tip-tilt-scanner was used to direct the laser beam into a blood vessel of interest under SLO image guidance. Mild coagulation was produced using millisecond-long pulses.

Microglia react dynamically to focal laser injury of blood vessel endothelial walls. Under normal conditions, microglia soma remain stationary and the processes probe a territory of their immediate environment. In response to local injury, process movement velocity approximately doubles and amplitude increases several fold within minutes after injury. Moreover, the previously unpolarized process movement assumes a distinct directionality towards the injury site, indicating signaling between the injured endothelial cells and the microglia. In vivo retinal imaging is a crucial tool for understanding the dynamic behavior of retinal cells.