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High-speed cellular level brain imaging in freely moving mice using fluorescence microendoscopy

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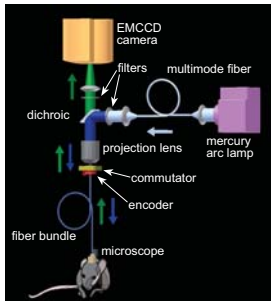
Introduction

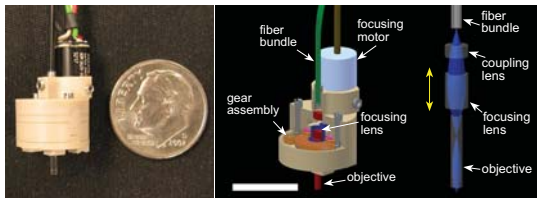
A longstanding research goal has been to develop flexible fiber-optic fluorescence brain imaging techniques with micron-scale resolution, for the purpose of studying animal behavior and underlying cellular properties concurrently. To achieve this goal, we have created a portable one-photon fluorescence microendoscope based on fiber-optics, micro-optics, and a custom gearbox for focal adjustments. We concentrated our instrumentation design efforts on the use of mice, because of the wide availability of transgenic mouse lines with genetically targeted alterations to cellular properties and resulting behavioral deficits. Our device is about 1 cm in lateral extent and about 2 g in mass, sufficiently small to be borne on the head of an adult mouse that is freely behaving but tethered via flexible fiber optics. The use of minimally invasive gradient refractive index (GRIN) micro-lenses permits imaging of cells with cellular level resolution in either surface or deep brain areas. To test the capabilities for fluorescence imaging using this approach, we acquired high-speed videos of microcirculation within neocortical and hippocampal area CA1 capillaries at frame rates of up to 100 Hz in freely moving mice. Individual red blood cell movements were readily apparent, allowing capillary blood flow speeds to be computed. We expect that fluorescence imaging in freely moving mice will allow detailed comparisons of animal behavior, physiological dynamics, and cellular properties between normal and transgenic mouse subjects.

System for imaging in freely moving mice



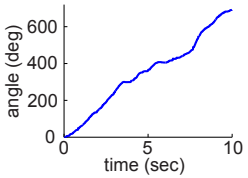
The mouse is tethered via a flexible optical fiber bundle. A commutator relieves torsional strain by allowing the bundle to rotate as the mouse turns. An encoder measures this rotation to enable software re-alignment of the images. A high-speed CCD camera acquires fluorescence images at up to 100 Hz frate rates.

Miniature head-mounted microscope

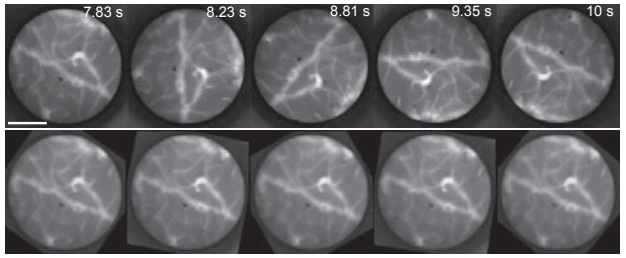


Left: Photograph of miniature microscope next to a dime.
Middle: Computer Aided Design (CAD) schematic of microscope.
Right: CAD schematic of the triplet lens assembly that relays fluorescence emissions from the mouse brain to the fiber bundle.

Computation methods and image alignment

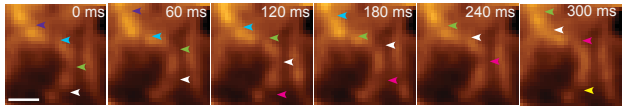


Left: encoder trace for image sequence shown below.
Right: flow chart describing computational methods for aligning raw image sequences and analyzing them.



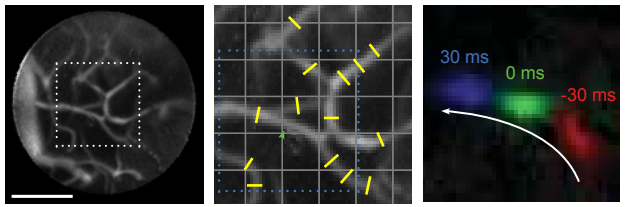
Top: Raw image sequence of neocortical vasculature. Scale bar is 100 µm.
Bottom: Aligned image sequence after utilizing encoder measurement and image registration algorithm

Tracking red blood cells



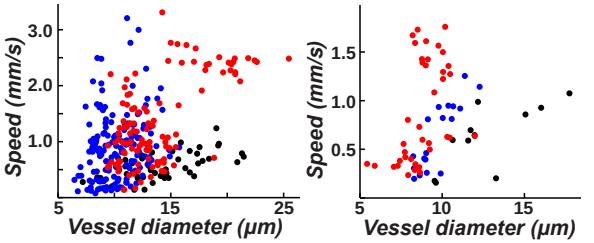
Sequence of aligned panels from the neocortex of a freely moving mouse. Image acquisition frame rate was 100 Hz, but only every 6th frame is shown. Colored arrowheads indicate the location of individual red blood cells within the vessel. Scale bar is 20 µm.

Computing speeds and diameters



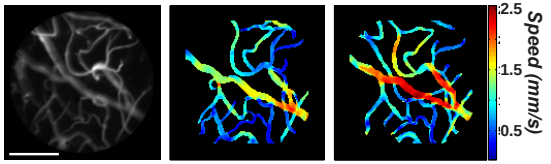
Left: Map of motor cortical vasculature in mouse. Scale bar is 100 µm.
Right: Zoom-in of dotted region in left panel. To ensure homogenous sampling of the field of view, the image is divided into a grid. Within each grid box, a single point is chosen at which the vessel diameter is measured (yellow lines) and the average red blood cell speed through that point is computed using two-point cross-correlations.
Middle: Overlayed correlograms of a pixel near the green arrowhead in the middle panel with its surrounding pixels at various time delays (shown in red, blue, and green). The speed computed equals the distance between the centroids of the correlograms divided by their relative time delay. The white arrow depicts flow direction.

Flow speeds and vessel diameters



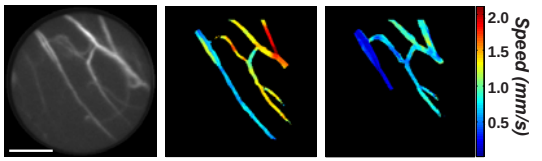
Left: Plot of red blood cell speed vs. vessel diameter in the motor cortex of three freely moving mice (red, blue, black).
Right: Plot from the hippocampus of three freely moving mice (red, blue, black).

Red blood cell speed map in motor cortex



Left: Map of motor cortical vasculature in an adult mouse. Scale bar is 100 µm.
Middle: *Anesthetized.* Color map of red blood cell speeds (averaged over 10 seconds).
Right: *Freely moving.* Color map of red blood cell speeds (averaged over 10 seconds).

Red blood cell speed map in hippocampus



Left: Map of hippocampal vasculature in an adult mouse. Scale bar is 100 µm.
Middle: *Anesthetized.* Color map of red blood cell speeds (averaged over 10 seconds).
Right: *Freely moving.* Color map of red blood cell speeds (averaged over 10 seconds).

Conclusions

1. **Miniature fiber bundle epifluorescence microscope**
   - GRIN micro-lenses for both surface and deep tissue imaging
2. **Commutator relieves torsional strain on mouse**
   - Encoder measures fiber bundle rotation, used for rough alignment
   - Image registration algorithm for fine alignment of image sequences
3. **Cellular level fluorescence imaging**
4. **High-speed (100 Hz) image acquisition**
5. **Brain imaging in freely moving mice**
   - Cortical and hippocampal blood vessels
   - Measurements of average red blood cell speeds and vessel diameters

Acknowledgments

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2. We thank the Varian Machine Shop for fabricating the microscope housing.