# New views into the brain of mice on the move

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A portable fiber-optic epifluorescence microscope allows real-time imaging of brain function with cellular spatial resolution in freely moving mice.



Remarkable correlations have been found between behavior and brain activity, mainly measured through electrophysiological recordings and functional magnetic resonance imaging. Nevertheless, neuroscientists are still far from understanding behavior in terms of the underlying dynamic patterns of activity in neuronal networks. Such an understanding is dependent on observing neural activity at the cellular level in animals as they perform normal behaviors requiring freedom of movement. A report in this issue of *Nature Methods* now demonstrates fiber-optic imaging with high temporal and spatial resolution, suitable for measuring single-cell activity in freely moving mice[1].

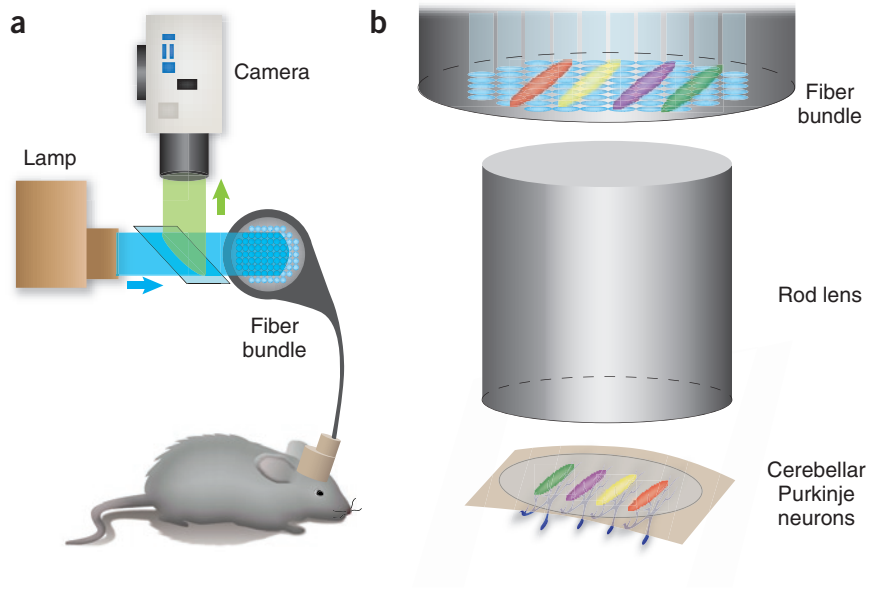


**Figure 1** | High-resolution fiber-optic imaging in the brain of mobile mice. (**a**) Excitation light propagates in one direction through an optical fiber bundle while emitted fluorescence returns via the same path, is reflected by a dichroic mirror and is imaged by a camera. (**b**) Microlenses project and enlarge small fluorescent brain structures onto a fiber bundle containing well-ordered arrays of glass cores for flexible image transfer from the mouse to the camera. Viewed from the brain surface, the co-planar dendritic arborizations of cerebellar Purkinje cells labeled with calcium-sensitive fluorescent dye appear as parallel stripes.

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With the advent of new fluorescent markers and new microscopy techniques, optical imaging has evolved as a powerful alternative to electrophysiological approaches for revealing both temporal and spatial aspects of brain function. In particular, high-resolution *in vivo* imaging using two-photon microscopy has contributed enormously to our understanding of cellular and subcellular aspects of brain function[2]. Most of our knowledge, however, has been gained from imaging anesthetized and head-restrained animals. Optical recording in freely moving animals has been far more challenging.

Researchers have followed two principle routes in their attempts to image awake, mobile animals. In one approach, high-resolution imaging was recently demonstrated in head-restrained mice while they were running on an air-supported styrofoam ball[3]. Alternatively, the microscope can be miniaturized so that it can be carried by the animal in a piggyback fashion. Flexible optical fibers are key to this approach as they enable researchers to transmit light back and forth between a small head-mounted front end and the remote light source and photodetector. Following a first proof-of-principle demonstration that high-resolution imaging of blood capillaries can be achieved in moving animals using a portable two-photon microscope[4], various types of compact imaging instruments have been devised based on either single-photon or two-photon excitation and comprising laser-scanning as well as wide-field illumination systems[5]. So far none of these devices have achieved measurements of single-cell activity in a freely running animal.

Flusberg *et al.*[1] now present a miniaturized epifluorescence microscope using single-photon excitation that achieves this goal (**Fig. 1a**). The design principle is simple and relies on image transfer through a well-ordered fiber bundle (**Fig. 1b**). These fiber bundles typically contain thousands of fiber cores, whose centers are spaced about 10 micrometers apart. In the simplest approach, their remote end can be placed directly on the brain surface, while the other end is imaged by a camera. This method has previously been used for fast voltage-sensitive dye

imaging of sensory processing in freely moving mice[6], albeit at a spatial resolution far from identifying individual cells. To achieve near-micrometer resolution capable of discriminating individual cells Flusberg *et al.*[1] added an extra post-fiber imaging stage with appropriate magnification using thin rod-like lenses (**Fig. 1b**). The core of the mini-microscope they present is about the size of a cherry, weighs only 1.1 grams and is easily carried by a mouse. Optionally they included a motorized focusing lens, which enables remote adjustment of the focal plane. Using a fast charge-coupled device (CCD) camera they achieved image acquisition rates of up to 100 Hz over imaging fields of a quarter to a third of a millimeter.

Flusberg *et al.* demonstrate the potential of their imaging approach by applying it to two questions of fundamental importance to neuroscientists[1]. First, they filled blood vessels with a fluorescent marker and measured blood flow by tracking unstained red blood cells[7]. By imaging blood flow in the microvasculature of both neocortex and hippocampus (inserting the front lens through an implanted guide tube in the latter case) they found a positive correlation of speed with vessel diameter and constructed maps of flow speed in behaving animals. As blood-flow changes contribute to signal contrast in functional magnetic resonance imaging experiments, high-resolution imaging of microvasculature might be extremely helpful in furthering our understanding of hemodynamic changes during behavior in different brain regions.

The second major achievement of this new study is the first imaging of activity in the cerebellum with single-cell resolution in freely moving mice. Neuronal activity in cerebellar cortex can be directly visualized by loading calcium-sensitive fluorescent dye into cerebellar neurons[8]. The highly ordered arrangement of the co-planar Purkinje cell dendrites can be viewed from the cerebellar surface as thin stripes and thus is readily amenable to epifluorescence imaging[9,10]. By resolving the stripe-like activation of individual neighboring Purkinje neurons, Flusberg *et al.*[1] report that Purkinje neurons are more active during locomotion and that co-active Purkinje neurons appear to be spatially clustered into microzones[9]. Fiber-optic imaging of Purkinje cell activity in moving mice will be of great value because the cerebellar cortex is critically implicated in motor coordination as well as motor learning, but the relevance of particular activation patterns to behavior has remained elusive.

These first high-speed cellular level movies of blood flow and neuronal activity are remarkably stable during movement of the mouse. This stability may be attributed to the compactness, lack of optical sectioning and relative simplicity of the imaging device, which should also make it readily applicable in other laboratories. Different from imaging using miniature scanning devices, fiber-bundle imaging does not require any moving parts, which presumably renders it less sensitive to head accelerations. Another advantage is that the temporal resolution is only limited by camera speed and signal-to-noise ratio. Nevertheless, several problems remain to be solved, most prominently the limited depth penetration of single-photon excitation and the lack of optical sectioning for applications that require it. In this respect two-photon fiberscopes, which are now available in comparable size[10,11], may still be advantageous for imaging activity in deeper neuronal populations or in fine dendrites.

With the emerging technique of imaging in head-restrained animals[3] and the repertoire of fiber-optic imaging systems now at hand, ranging from low-resolution systems for bulk measurements from brain regions[12] or subregions[13] to high-resolution systems for cellular imaging as shown in the new report, neuroscientists have gained powerful tools to obtain direct views of neural activity patterns during specific behaviors. These advances promise exciting times ahead!


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