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350-μm side-view optical probe for imaging the murine brain *in vivo* from the cortex to the hypothalamus

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Abstract. Miniature endoscopic probes offer a solution for deep brain imaging by overcoming the limited depth of intravital microscopy. We describe a small-diameter (350 μm) graded-index optical probe with a side-view design for *in vivo* cellular imaging of the mammalian brain. The side-view probe provides a unique view of the vertical network of neurons and penetrating blood vessels. At a given insertion site, the translational and rotational scanning of the probe provides access to a large tissue area (>1 mm^2) across the cortex, hippocampus, thalamus, and hypothalamus. © *2013 Society of Photo-Optical Instrumentation Engineers (SPIE)* [DOI: 10.1117/1.JBO.18.5.050502]

Keywords: miniature endoscope; side-view probe; deep brain imaging; neuronal imaging.

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Radiological brain imagings based on magnetic resonance imaging and computed tomography are highly useful in clinical medicine, but mechanistic studies of diseases often require cellular-level details that are beyond the resolution of whole-body imaging techniques. High-resolution optical imaging, championed by intravital fluorescence microscopy,[1] has proven a useful tool in brain research by visualizing the murine cerebral cortex at the cellular level through a transparent cranial window[2] or thinned skull.[3] While this approach allows for longitudinal observation over several weeks, the access is limited to depths of 500 to 750 μm from the surface within the cerebral cortex. Recent developments of new light sources in the near-infrared range at 1300 to 1800 nm and three-photon nonlinear processes were shown to extend the penetration depths up to 1.6 mm in a mouse.[4] However, light penetration is fundamentally limited by scattering in the tissue. To overcome this problem, there has been considerable interest in optical endoscopes.[5–8] Miniature optical probes are typically based on commercially available graded-index (GRIN) rod lenses. The smallest probes demonstrated to date have a diameter of 0.6 mm,[5] with a straight view design like conventional objective lenses. A probe, once inserted into the brain, cannot be moved laterally without causing significant damage to the surrounding tissue. The field of view (FOV) of a straight-view probe is limited to 50 to 200 μm, depending on the diameters of the lens and the numerical aperture.

Here, we present an optical probe with a side-view design [as shown in Fig. 1(a)], which is distinctly different from the previous front-view probes and solves the problem of the limited view area. Side-view brain imaging has been demonstrated by implanting a prism with a relatively large size of 1 mm into the cortex.[9,10] To minimize tissue damage, we fabricated our probe with the smallest commercially available GRIN rod lenses with a diameter of only 350 μm; despite the small diameter, the side-viewing probe[11] allows a large volume in the brain to be visualized by scanning and rotating the probe with minimal invasion, as shown in Fig. 1(b).

The optical probe was constructed with three GRIN rod lenses (NSG America, Somerset, New Jersey) with diameters of 350 μm. A coupling lens (ILW025) was glued to the proximal end of a relay lens (SRL200) using transparent UV curable epoxy (NOA81, Norland). An imaging lens, prepared by shortening an ILW025 lens to a pitch of 0.16, was attached to the distal end of the relay lens, and an aluminum-coated right-angle prism (250 μm base length; BK7 glass; Precision Optics, Inc., Gardner, Massachusetts) was attached to the imaging lens. The combined pitch of the imaging lens and the prism is 0.25, such that the overall pitch of the probe is 2.5. Light focused at the proximal surface is relayed to a focus at the distal prism surface. The total length of the probe is 32 mm, approximately three-quarters of which was inserted into a stainless metal sleeve (I.D. = 370 μm, O.D. = 610 μm) for mechanical reinforcement. The remaining distal part (~5 mm in length; O.D. = 350 μm) was inserted into the brain. The optical probe was mounted on a three-axis translational and rotational stage [shown in Fig. 1(c)] and coupled into a custom-built confocal microscope system, which we previously described.[12] The focal plane in the depth could be adjusted by translating the coupling objective lens (40×, NA = 0.6) in the microscope without moving the probe. The intrinsic FOV of the 350-μm probe was about 90 μm. The transverse and axial resolutions (in air) were about 1 μm and 10 μm, respectively.

For imaging, a mouse was anesthetized by intraperitoneal injection of ketamine (90 mg/kg) and xylazine (9 mg/kg). The head was shaved using a rodent trimmer and depilatory cream. Before inserting the probe through the frontal or parietal areas of the skull, we performed a craniotomy by using a microdrill to expose the cortex. The bone fragment was carefully lifted away from the skull with 20-μm tipped forceps. Then the anesthetized mouse was laid down on a temperature-regulated animal stage. The stage temperature was maintained at 37°C. The mouse head was fixed on a stereotaxic device mounted on the animal stage. The stage was controlled to align the probe to the target site in the exposed cortex. A drop of saline was applied

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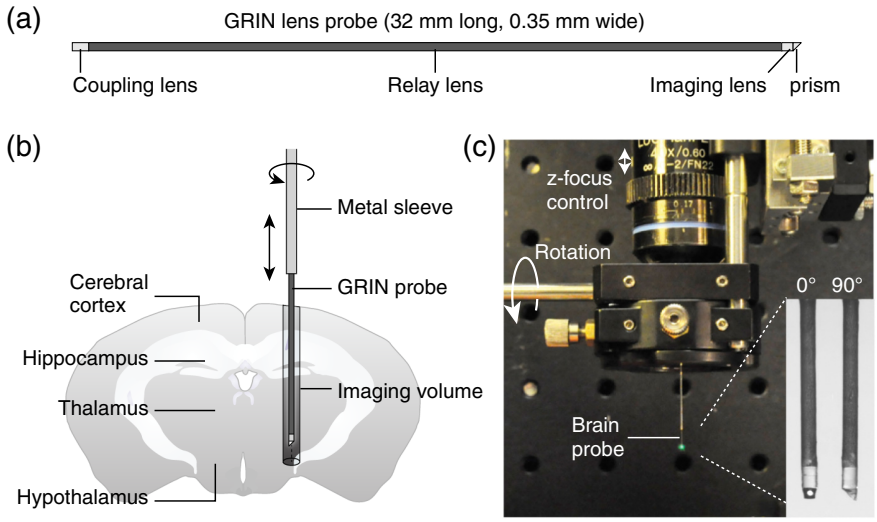

Fig. 1 Thin side-view probes for deep brain imaging. (a) Schematic of a side-view probe. (b) Illustration of wide-area brain imaging. (c) Picture of the imaging setup. Z-focus control is achieved by moving the objective lens. Insets show the pictures of the side-view probe taken from two different angles.

on the tissue, and the probe was gently inserted into the brain by moving up the animal stage slowly. The 350-μm probe was stiff enough to penetrate into the soft brain tissue.[13] Besides, the sharp edge of the prism and the narrow diameter fostered the smooth insertion of the probe without significant collateral damage to the surrounding tissue. After the probe was fully inserted into the brain, we scanned the tissue to change the imaging site by slowly moving the mouse down (and up again) and spinning the probe with the rotational stage.

To visualize neurons, we used Thy1-YFP mice (C57B6 background, Jackson Laboratory, Bar Harbor, Maine), where yellow fluorescent protein (YFP) is expressed in neuronal cells, such as motor and sensory neurons in the cerebrum and granule cells and mossy fiber in the cerebellum.[14] With a single insertion, the side-view probe delivered high-resolution images of the axon of neurons and a subset of the neuronal cells across all layers in the cerebral cortex *in vivo*, as shown in Fig. 2(a). Scanning the probe along the insertion site yielded a wide-area view of the neural circuitry in the intact brain tissue surrounding the inserted probe, as shown in Fig. 2(b). Individual neuronal cells were visualized clearly within the hippocampus [as shown in Fig. 2(c)–2(e)], thalamus, hypothalamus [as shown in Fig. 2(f)], olfactory bulb, midbrain, and medullar oblongata [as shown in Fig. 2(g)].

The side-view probe is also suited for assessing blood flow in deep-penetrating arteries and arterioles, which is important for function analysis of specialized vascular architecture. To visualize blood vessels, we used Tie2-EGFP mice (FVB/N background, Jackson Laboratory), where Tie2+ vascular endothelial cells express enhanced green fluorescent protein (EGFP). We injected fluorescent microspheres intravenously into a Tie2-EGFP transgenic mouse and acquired a high-frame-rate movie of the microspheres flowing in the blood vessels in a region about 1 to 1.5 mm below the cortical surface, as shown in Fig. 3(a). The measured flow speed was about 200 and 400 μm/s in the arterioles with diameters of 5 μm and 30 μm, respectively, as shown in Fig. 3(b). Both rates are slightly slower than the previous measurements for superficial vessels.[9,15]

Although physical damage to tissue by the probe is inevitable, the optical probe offers access to the deep regions of the brain inaccessible by current noninvasive microscopies based on objective lenses. To check the tissue damage by the probe, we examined the brain tissue after the careful insertion and removal of probes. We compared two probes with different diameters: 350 μm, as described above, and 1250 μm, as used by Kim et al.[11] The histology showed that the 350-μm probe left much less of an insertion mark and produced little hemorrhaging [as

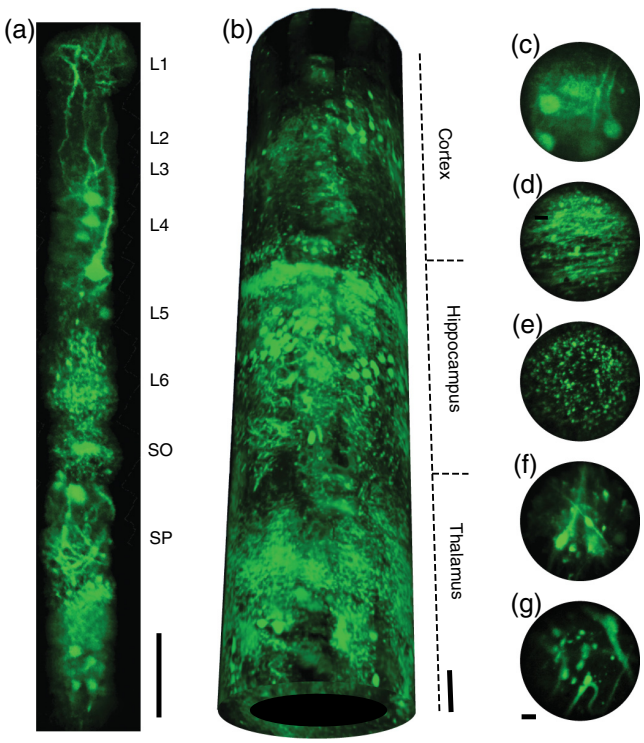

Fig. 2 *In vivo* images of the brain obtained with a 350-μm probe in a Thy1-GFP mouse. (a) A mosaic image of neurons and a subset of neural cells in the cerebral cortex and hippocampus. L1 to L6: Cortical layers 1 to 6, SO: Stratum oriens, SP: Stratum pyramidale. (b) A rendering of wide-area neuronal images obtained with the side-view probe. (c) Pyramidal cells in the hippocampus. (d) Basal dendrites in the stratum oriens. (e) Neuropils in the hippocampus. (f) Neuronal cells in medulla oblongata. (g) Neuronal cells in the hypothalamus. Scale bars: 100 μm in (a, b), 10 μm in (c)–(g).

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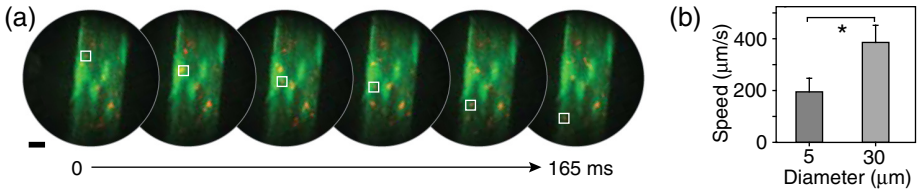

Fig. 3 *In vivo* image of nano-particles (red) flowing in a penetrating blood vessel in the brain. (a) Image of fluorescent beads (red) flowing into a penetrated blood vessel in a Tie2-EGFP mouse. Individual particles can be tracked using the side-view probe shown in the box. (b) Flow speeds of the penetrating blood vessels. $*P < 0.05$. Scale bar: 10 μm.

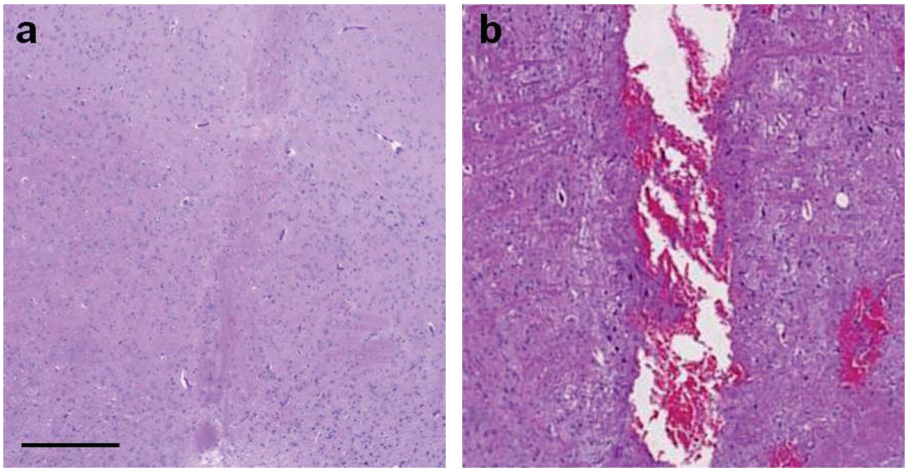

Fig. 4 H&E histology of brain tissue after probe insertion at the injection sites of (a) the 350-μm-diameter probe and (b) the 1250-μm probe. Scale bar: 500 μm.

shown in Fig. 4(a)] in comparison to the larger probe [as shown in Fig. 4(b)]. The narrow wound made by the 350-μm probe was fully closed by interstitial tissue pressure.

In conclusion, we have presented a new and promising ultra-thin side-view probe for imaging the brain *in vivo* with a cellular resolution at penetration depths up to several millimeters. In many applications, the probe can be inserted without perturbing the brain site to be interrogated. The imaging depth of the probe can be made several hundred microns or beyond by using near-infrared multiphoton excitation, allowing the majority of the mouse brain to be visualized from the insertion site. We anticipate that this technique based on a side-view probe will facilitate various studies in neurosciences, neurobiology, and mouse models of brain disease.

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