Extended Field-of-View Deep Brain Imaging using Aberration Correction in GRIN Microendoscopes through 3D Printed Polymer Microlenses

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Abstract: We report the development and application of a new approach to correct aberrations in GRIN microendoscopes using 3D printed polymer microlenses. Corrected microendoscopes are validated performing functional imaging experiments in the mouse brain *in vivo*. **OCIS codes:** (180.2520) Fluorescence microscopy; (190.4180) Multiphoton processes; (100.2960) Image analysis.

Two-photon fluorescence imaging allows high-resolution anatomical and functional visualization of neuronal circuits hundreds of micrometers deep in the brain [1]. Light scattering, however, affects the propagation of excitation and emission photons, making imaging increasingly difficult with tissue depth [2,3]. Despite various non-invasive strategies were developed to improve imaging depth in multi-photon fluorescence microscopy [4–8] and to reach areas 1-1.5 mm below the brain surface, the use of implantable microendoscopic probes [9,10] is required to access deeper regions.

A critical barrier to progress is the lack of availability of microendoscopic devices that combine cellular resolution across a large field-of-view (FOV) and small cross-sections, to allow high-resolution population imaging while minimizing tissue damage. Microendoscopes currently applied for deep imaging use GRIN lenses (diameter: 0.35-1.5 mm), which exhibit intrinsic optical aberrations [9]. These aberrations decrease the spatial resolution and lower the excitation efficiency, degrading image quality and restricting the FOV [11]. Importantly, GRIN endoscopes have non-uniform spatial resolution across the FOV (especially in the axial direction), with spatial resolution worsening with the radial distance from the optical axis.

Here we report the development and application of a new method to correct aberrations and extend the FOV in ultrathin (diameter ≤ 0.5 mm) microendoscopes. Extended FOV (eFOV) microendoscopes are composed of a GRIN rod lens, a glass coverslip and a small diameter aspheric lens (Fig. 1a, b), which was fabricated using two-photon polymerization [12].

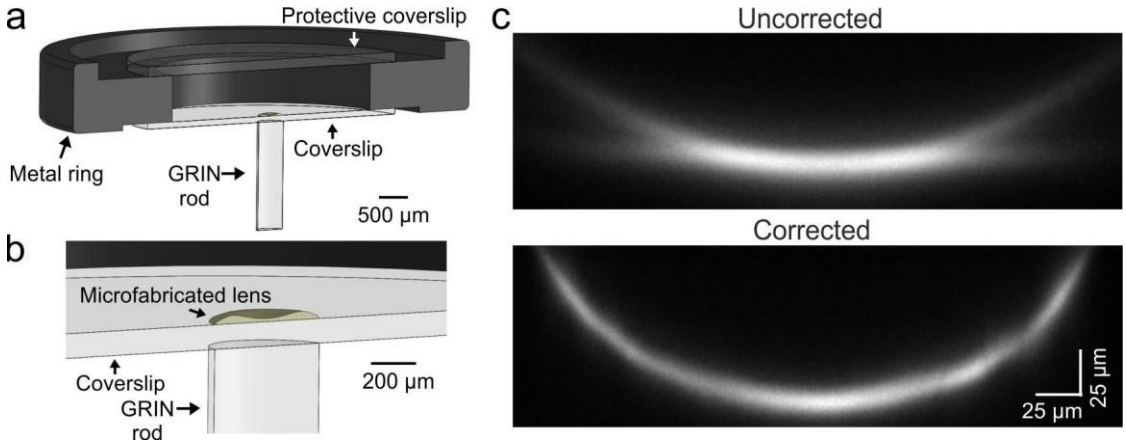

Fig. 1. Aberration correction using microfabricated lenses in ultrathin microendoscopes. a-b) schematic of the eFOV-microendoscope mount for head implant. c) x,z projections of a z-stack of two-photon laser scanning images of a subresolved fluorescent layer (thickness: 300 nm) obtained using microendoscopes without (uncorrected, top) and with (corrected, bottom) the microfabricated corrective lens.

We characterized eFOV-microendoscopes coupling them with a two-photon laser scanning imaging system and acquiring z-stacks of a subresolved thin fluorescent layer [13]. eFOV-microendoscopes showed a wider area with higher axial resolution compared to uncorrected microendoscopes. (Fig. 1c), a result which was confirmed imaging neurons expressing the green fluorescence protein (GFP) in fixed brain slices.

We then used eFOV-microendoscopes to image neurons expressing the genetically encoded calcium indicator GCaMP6s in the CA1 hippocampal region. Active regions of interest were homogeneously distributed across the extended ^{2020}FOV, including the peripheral regions (Fig. 2a-c).

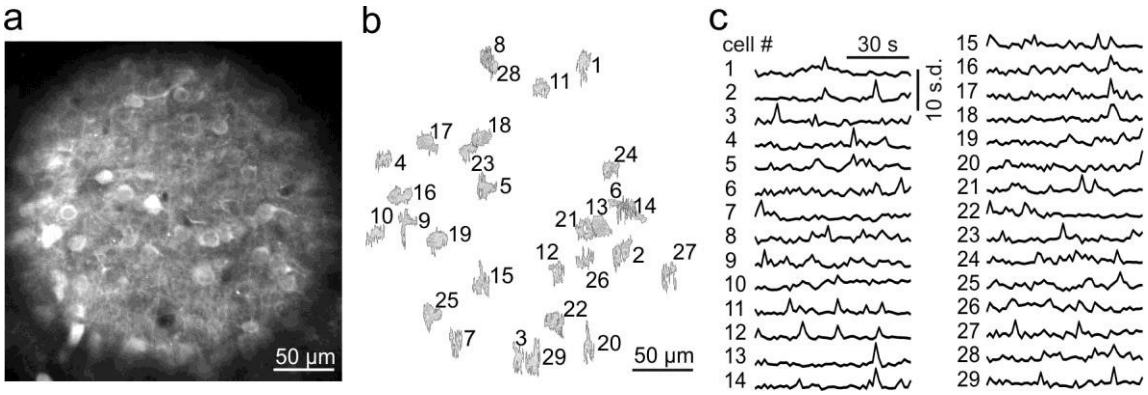

Fig. 2. a) GCaMP6s expressing hippocampal neurons imaged *in vivo* with an eFOV-microendoscope of diameter 0.35 mm. b) Active ROIs are identified and numbered. c) Fluorescence signals over time for the ROIs displayed in b.

In conclusion, we designed, developed, and validated a method to correct for optical aberrations in ultrathin (diameter ≤ 0.5 mm) microendoscopes using small corrective lenses microfabricated through direct laser lithography. These novel endoscopic probes will allow extended FOV imaging of neuronal populations in deep structures with minimal invasiveness and no change in the optical path of the microscope.

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