Quad-SPIM: a novel light-sheet microscope for high-speed imaging of human brain tissue

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Abstract: Light-sheet fluorescence microscopy is a powerful imaging technique for neuroscience, enabling high-resolution, fast volumetric imaging of large brain tissues. We present a novel microscope using four wavelengths to simultaneously reveal structural details with micrometre precision.

1. Introduction

Light-sheet fluorescence microscopy (LSFM), also known as selective plane illumination microscopy (SPIM), is one of the most significant advancements in optical imaging in the past two decades. Unlike traditional microscopy techniques, LSFM illuminates specimens with a thin sheet of light, capturing images plane by plane. This approach drastically reduces photodamage and photobleaching while providing high-resolution, high signal-to-background images. These characteristics enable researchers to study living organisms over extended periods of time with minimal disruptions. The evolution and increasing customization of LSFM have revolutionized fields such as developmental biology, neurobiology, and other disciplines focused on cellular and subcellular dynamics. In neuroscience, LSFM has been pivotal in mapping neural circuits and brain activity in both live and fixed samples [1], [2], as well as in studying brain vasculature [3]. Additionally, it enables sub-micron resolution imaging of cleared brain tissues, yielding new insights into brain function and diseases affecting the nervous system [4], [5], [6], [7], [8], [9]. As LSFM technology evolves, efforts are focused on enhancing imaging depth, resolution, and the range of detectable fluorophores so as to broaden its possible applications. A key challenge lies in imaging large or densely packed specimens, where light scattering and absorption may severely degrade image quality. Indeed, LSFM requires biological samples to be optically transparent to light to enable their volumetric reconstruction [6], [10], [11]. Advances in multiphoton excitation and non-linear optics hold promise for overcoming these limitations [12]; furthermore, integrating LSFM with other modalities, such as magnetic resonance imaging (MRI), positron emission tomography (PET), Polarized Light Imaging (PLI), or Optical Coherence Tomography (OCT) can offer a multimodal approach to studying biological structures and functions [13][14], [15], [16].
Here, we present an innovative light-sheet microscope capable of imaging large cleared human brain tissues with multiple wavelengths simultaneously. This advancement enables the detailed investigation of various structural features with micrometer resolution and exceptionally high imaging speed.

2. Materials and Methods

2.1. Setup construction

The newly-designed LSFM setup enables rapid, multi-channel imaging of human brain tissues with micrometre resolution. It integrates four diode-pumped solid-state lasers with the following excitation wavelengths: 488 nm, 561 nm, 594 nm, and 647 nm. The lasers are combined into a single collimated beam via dichroic mirrors and directed through an Acousto-Optic Tunable Filter (AOTF) for precise wavelength selection. A diffraction prism separates again the beam into four slightly separated beams, which are scanned by a galvanometric mirror and focused into digitally scanned light sheets using an achromatic doublet and an illumination objective (2X/0.10 NA). The detection system uses a 5X/0.15 NA objective, which is tilted by 20° with respect to the illumination one, and an achromatic doublet acting as a tube lens. The emitted fluorescence is split into four channels via dichroic filters (cut-off wavelengths: 520 nm, 581 nm, and 604 nm). Each channel is captured by a dedicated sCMOS camera (Orca Flash 4 v.3, Hamamatsu), with band-pass filters to prevent cross-talk. Built ad hoc from a PVC block, the specimen holder accommodates up to eight 60 mm × 60 mm slides, and is provided with a drainage system for the imaging medium.

The entire chamber is mounted on motorized XY and Z stages, allowing precise 3D scanning of samples for high-throughput volumetric imaging.

2.2. Sample preparation

Human brain tissue slices were optically cleared and stained with the SHORT protocol [7], [17], [18], [19] and then mounted between a 60 mm × 60 mm × 1 mm optical glass slide and a 60 mm × 60 mm × 1 mm quartz slide (RI = 1.46) filled with TDE [20] before imaging.

3. Results

3.1. System characterization

The newly built LSFM microscope was fully characterized in terms of resolution, signal-to-noise and signal-to-background ratios, and imaging speed. The spatial resolution of the setup has been determined through the evaluation of the point spread function (PSF) of the optical system. For a correct measurement, sub-diffraction sized fluorescent beads were used as point light sources (500 nm spheres, TetraSpeck, Invitrogen). Beads were diluted 1:10000 v/v in the imaging medium (91% glycerol v/v, RI=1.46). The samples were excited with each laser line and imaged in the four channels for a thorough assessment of the PSF. After imaging, the intensity profiles (N=100 for each channel) were projected on three lines drawn along the three axes of the beads. A gaussian fitting was performed for each bead axis and the corresponding full width at half maximum (FWHM), which can be considered equal to the size of the PSF, was evaluated. The measurements from each channel and each axis were then averaged to obtain the final values reported in Table I, which define the resolution of the microscopy system.

Table 1. PSF of 500 nm-diameter fluorescent spheres (µm)

488 nm			561 nm			594 nm			647 nm		
X	Y	Z	X	Y	Z	X	Y	Z	X	Y	Z
2.8±0.1	2.5±0.2	5.9±0.3	2.9±0.2	2.9±0.3	5.6±0.27	3.6±0.3	2.9±0.3	7.6±0.4	3.5±0.2	3.2±0.2	7.7±0.4

SBR was computed from a human brain tissue slab from the Broca's area stained with NeuN primary antibody and Alexa Fluor 647 secondary antibody. The mean intensity signal (μ_s) was estimated from 100 regions of interest (ROIs), whereas 10 ROIs were taken into consideration for evaluating the background mean intensity (μ_b). The SNR was calculated from the same ROIs adopted for the SBR. The obtained SBR and SNR are:

$$SBR = \frac{\mu_s}{\mu_b} = 1.7 \pm 0.1 \quad [1] \quad \text{and} \quad SNR = \frac{\mu_s - \mu_b}{\sigma_b} = 11.5 \pm 0.2 \quad [2]$$

3.2. Evaluation of the imaging speed

The imaging speed of the apparatus was evaluated and compared to the one of an inverted dual-view SPIM setup already in use in our labs [7]. This was done by imaging a 0.5 mm-thick human Broca's area slab, measuring 40 mm x 40 mm laterally, that was previously processed with the SHORT tissue transformation method and stained with anti-NeuN, -Calretinin and -Somatostatin antibodies for visualizing different neuronal subpopulation. The three primary antibodies were respectively conjugated with Alexa Fluor 647, 561, and 488 secondary antibodies (Figure 1).

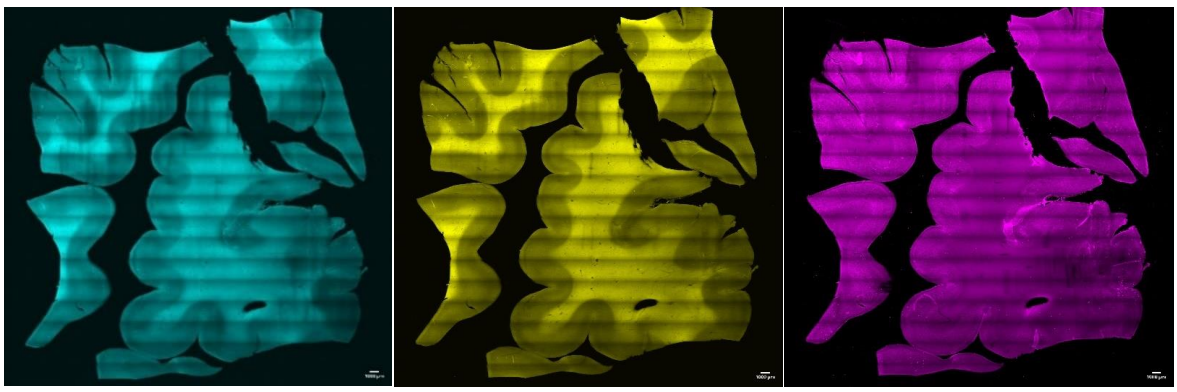

Fig.1. Maximum intensity projections (MIPs) of human brain Broca's area stained with (from left to right) Calretinin, Somatostatin, and NeuN primary antibodies.

The dual SPIM system performed 3-colour imaging in 4 hours and 35 minutes in two successive 2-channel acquisitions, whereas the quad-SPIM acquisition took 23 minutes to image the three channels simultaneously, resulting in an overall 12-fold improvement in the imaging speed of the same human brain sample.
This speed boost enables volumetric imaging at a rate of 2.1 cm^3/h in four colors, vastly outperforming the previous rate of 0.17 cm^3/h available in our labs. Rapid tissue acquisition is critical for imaging large and complex brain samples, reducing the time needed for data collection while maintaining an adequate spatial resolution enabling, for instance, the fine-grained visualization of neuronal cell bodies and the delineation of nerve fiber bundles[21], [22].

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