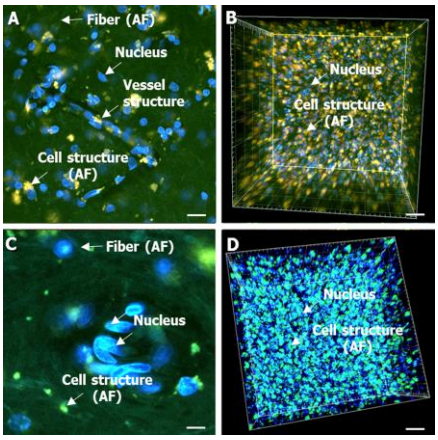
A platform for deep two-photon microscopic imaging of optically cleared and stained human brain tissue

A Schueth, S Hildebrand, S Foulquier, M van Zandvoort, A Roebroeck
Faculty of Psychology & Neuroscience, Maastricht University
Oxfordlaan 55, 6229 EV, the Netherlands
E-mail : anna.schueth@maastrichtuniversity.nl

KEY WORDS: two-photon laser scanning microscopy, optical clearing, mammalian brain tissue, human brain, DAPI, SYTO

We set out to establish an imaging platform for two-photon microscopic imaging of optical cleared mammalian brain samples to investigate gray matter cytoarchitecture. To date, we have performed two-photon laser scanning microscopy (TPLSM) [1] using cleared, fixed, 3-5 mm thick, adult human brain samples (age range between 50 and 65 years) as well as Thy-1-YFP mice brains. The chosen clearing protocols were iDISCO+ [2] and a modified version of the FRUIT [3] protocol. For nuclear labeling of the human brain samples, DAPI (Carl Roth GmbH, Germany) and SYTO dyes (Thermo Fisher Scientific Inc., the Netherlands) were used. Our TPLSM is a Leica TCS SP5 MP (Leica Mikrosysteme Vertrieb GmbH, Germany) with a HXC APO L 20x / 1.00 W 2 mm working distance water immersion objective. For microscopic data acquisition we have used excitation wavelengths ranging from 750 to 810 nm and a laser power between 6 and 45% from a Coherent Inc. Chameleon Ultra II Ti:Sa laser; field-of-view (FOV) was 738 x 738 µm and the frame rate was 0.8 frames/sec.



TPLSM imaging allowed the investigation of fixed, optical cleared mammalian brain of a stack thickness of up to 2 mm. Furthermore, TPLSM enabled the visualization of DAPI/SYTO up to an imaging depth of approx. 1 mm and the YFP signal up to a depth of 2 mm. We observed better clearing results for iDISCO+ cleared human brain samples for the FRUIT variant, likely due to better delipidation. Moreover, we could show the advantage of TPLSM for neuroanatomical examinations of cleared brain samples for fluorescent labels with short excitation wavelengths. This platform will be extended to light-sheet imaging to provide good imaging at high depth and with a larger FOV.

Fig. 1. TPLSM imaging of human visual cortex samples, DAPI (blue) to detect cell nuclei. Autofluorescence (AF) of tissue compartments, such as fibers (green) (A-D). A, C: Vessels containing DAPI stained nuclei (blue) (C showing a magnification). B, D: High-resolution TPLSM images and stacks allowed for the production of 3D volume units. Scale bars: 15 µm.

References:

1. Denk W, et al. Two-photon laser scanning fluorescence microscopy. Science. 1990, 248(4951):73-6.
1. Renier N, et al. Mapping of Brain Activity by Automated Volume Analysis of Immediate Early Genes. Cell. 2016, 165(7):1789-802.
2. Hou B, et al. Scalable and DiI-compatible optical clearance of the mammalian brain. Front Neuroanat. 2015; 9:19.