Three-dimensional cytoarchitectonic analysis of the human brain

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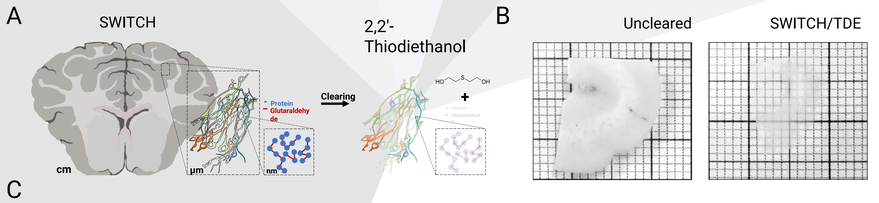
Abstract

Studying the three-dimensional architecture of the human neuronal networks in large tissue at subcellular resolution is one of the biggest challenges of our days. Commonly, sampled slices of the tissue of interest are individually stained and imaged. This approach in addition to being time-consuming does not consider space cell organization and sampling errors, leading, in the best case, to loss of information, and in the worst case to wrong analysis. To overcome 2D imaging's limits, in this study we developed a methodology that allows analyzing the cytoarchitecture of mm^3 of the human brain in three dimensions at high resolution. We successfully integrate the SWITCH immunohistochemistry technique (Murray et al. 2015) with the TDE clearing method (Costantini et al. 2015) to image tissues from different subjects with two-photon fluorescence microscopy. Quantitative analysis of brain cytoarchitecture of the different samples was performed using a machine learning approach that allows an automatic segmentation of neurons in three-dimension with high specificity and sensitivity. The identification and localization of the neurons obtained with this new approach enable to characterize and classify large human brain specimens with a high-resolution optical technique, giving the possibility to expand the histological studies to the third dimension.

SWITCH/TDE clearing enables multiple staining of human brain

In order to obtain a reliable methodology applicable to human brain samples to reach high transparency while preserving the tissue structure, the SWITCH permeabilization protocol (Murray et al. 2015) was modified and combined with the 2'2 thiodiethanol (TDE) clearing method (Costantini et al. 2015). The SWITCH/TDE clearing protocol can be applied to both paediatric, adult, and elderly human brain slices. The combination of the two techniques not only achieves high transparency while preserving the tissue structure, but also allows deep tissue labelling: small molecules can be imaged up to 1 mm while antibody can homogeneously label 500 μm think slices.



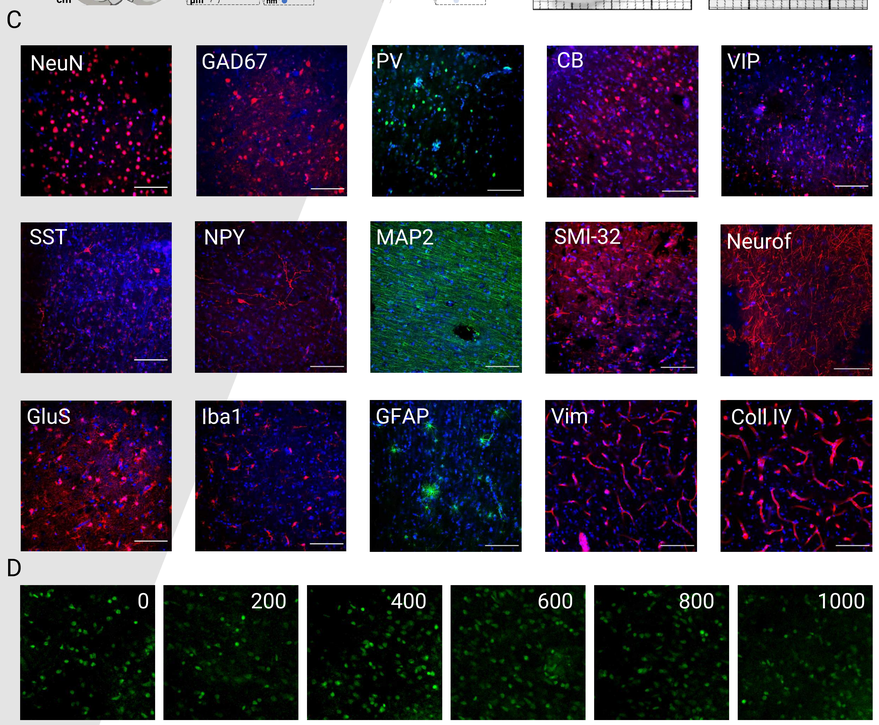


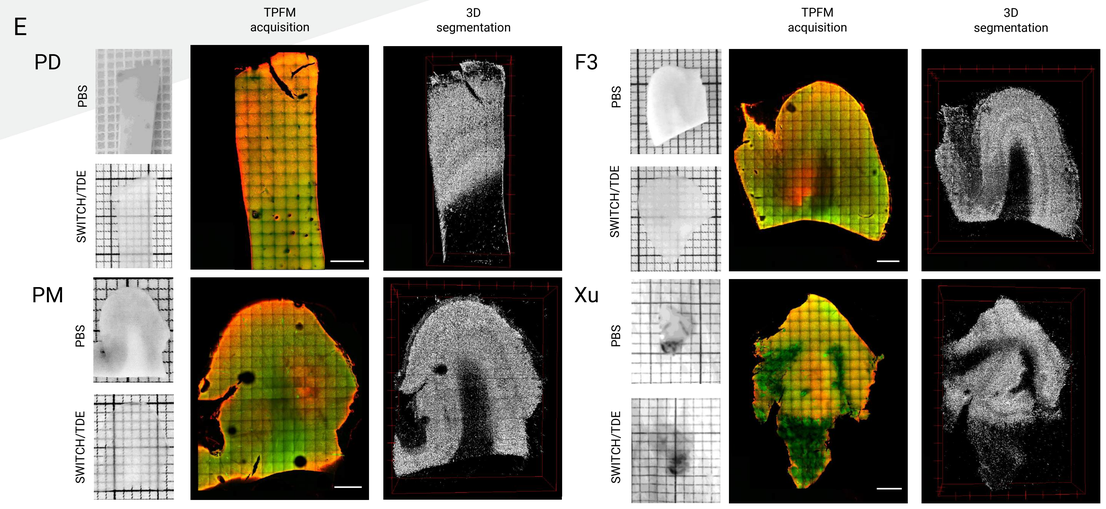
Figure A: schematic illustration of the SWITCH/TDE clearing. Figure B: 1mm slice of an adult human brain cortex before and after the treatment. Figure C: representative images of cleared tissues immunostained with various antibodies: NeuN (all neurons), glutamic acid decarboxylase (GAD67; all GABAergic interneurons), Parvalbumin (PV; GABAergic interneurons subtype), Calbindin (CB; GABAergic interneurons subtype), Vasointestinal peptide (VIP; GABAergic interneurons subtype), Somatostatin (SST; GABAergic interneuron subtype), Neuropeptide Y (NPY; GABAergic interneuron subtype), Microtubule-Associated Protein 2 (MAP2; pyramidal cells), Nonphosphorylated neurofilament protein (SMI32; pyramidal cells), Neurofilament (Neurof), Glutamine synthetase (GluS), Ionized calcium Binding Adaptor molecule 1 (Iba1; glial cells), Glial Fibrillary Acidic Protein (GFAP; glial cells) Vimentin (Vim; Microvasculature), Collagen IV (Coll IV; microvasculature). Figure D: images of Sytox Green label tissue at various depth. Imaging was performed with a custom made two-photon fluorescence microscope (TPFM) with a 25x tunable immersion objective from Zeiss (LD LCI Plan-apochromat). Scale bar = 50 µm.

Mesoscopic reconstruction and segmentation

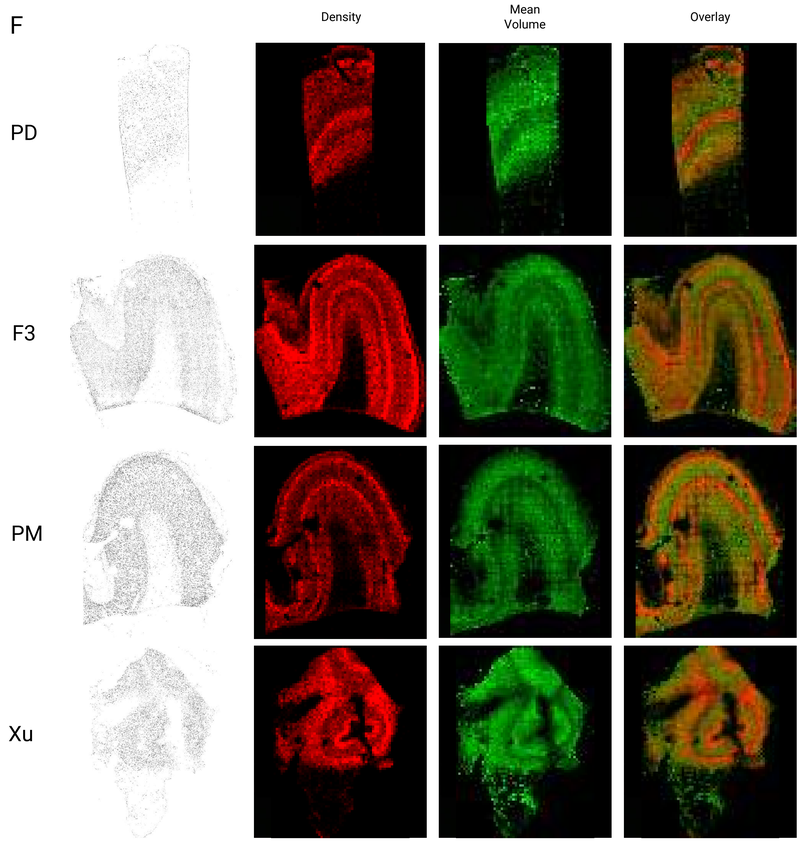
Samples from healthy and affected patients were analyzed for quantitative evaluation. The samples were treated with the SWITCH/TDE clearing method, stained with NeuN (in red) and DAPI (in green), then mesoscopic reconstructions (mm-size volume) were acquired with a custom-made two-photon fluorescence microscope (resolution: 0,44 x 0,44 x 2 µm). The datasets were analyzed with an automatic segmentation tool able to produce masks of every single neuron through the whole volume.

Samples PD and F3 are two different portions of the left prefrontal cortex from an adult and elderly subject, respectively. Instead, samples PM and Xu are surgically removed pieces of pediatric patients affected by Focal Cortical Dysplasia Type 2a (FCDIIa) and Hemimegalencephaly (HME), respectively.

The image E shows for each sample the picture of the tissue before and after the clearing. A representative plane of the mesoscopic reconstruction obtained with the TPFM (stitching of the single stacks are performed with a custom-made software: Zetastitcher), and, finally, the 3D rendering of the neuronal masks obtained after the segmentation. Scale bar = 1 mm

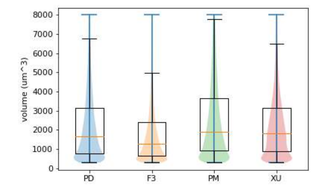


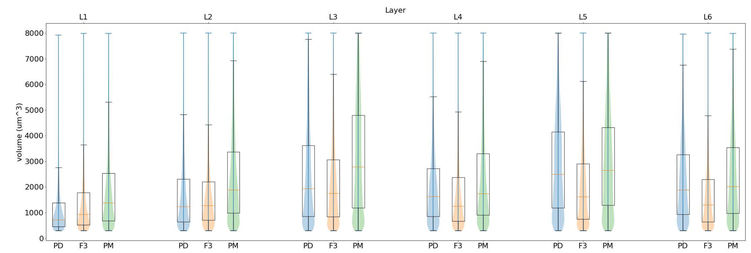
3D neuronal distribution: size and density

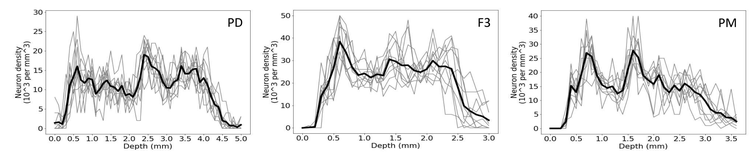


The 3D mask clouds were analyzed to obtain information about neuronal size distribution and density. In order to remove border artifacts, a volume thresholds were applied to count the neurons: volumes lower than 300 and higher than 8000 µm3 were eliminated. The total numbers of neurons analyzed are: 45'132 (PD), 160'129 (F3), 117'217 (PM), 108'176 (Xu). For each sample, 3D maps of volume distribution and neuronal density were produced (1px represent the mean of a 100 x 100 x 100 μm^3 volume).

Figure F: The combination of the two maps highlights the presence of inhomogeneity between layers in terms of neuronal density and cell volume, allowing the identification of the six expected layers of the cortex. The HME biopsy (Xu) shows a disruption of the structural organization of the cortex making layer classification impossible.







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