Combining Brain-Clearing and Light-Sheet Fluorescece Microscopy with Tape-Transfer Assisted Sectioning and Histochemistry for Whole Mouse Brain and Spinal Cord Imaging

Mouse Brain Architecture

G. Giese[1]
A. Scherbarth[1]
V. Pinskiy [2]
A. Tolpygo [2]
F. Mechler [2]
P. Mitra [2]

[1] Max Planck Institute for Medical Research Heidelberg

[2] Cold Spring Harbor Laboratory

Overview

Objective & Approach

Recently it has become feasible to routinely image entire brains, particularly for Mouse, advancing neuroscience research by permitting the unbiased study of the whole brain.

There are currently three basic approaches (see table below), with trade-offs. Our aim is to combine approach (c) and (a), in that order and in the same brain, to gain the advantages of both approaches.

method	(a) cryo-sectioning + on-slide HC + scanning light microscopy	(b) block-face sectioning + scanning two-photon microscopy	(c) brain clearing + light-sheet fluorescence microscopy
registration	requires computation	**Automatic**	**Automatic**
histochemistry (HC)	**thin section: fast/routine**	Possible with diffusion into block (slow); usually not done	Possible with diffusion into block (slow); usually not done
label persistence with sample age	**sections preserved HC permanent** F decays	sections not preserved F decays	F very stable (EGFP, mRFP1)
Imaging speed	intermediate	slow	fast/intermediate
resolution in-plane	~ 0.5 µm	< 0.5 µm	~ 1.0 µm
resolution b/w-plane for similar scan times	~ 10 µm	Typically ~ 50 µm	~ **1-4 µm**

Approach (c). Brain Clearing & Light Sheet Fluorescence Microscopy (LSFM)

(A) Tissue Clearing Method (modified "BABB") as implemented in Giese Lab (manuscript submitted)

Prior to LSM scanning, whole mouse brains were fixed, extracted, dehydrated, and cleared.

Fixation Protocol:
- transcardiac perfusion with 4% PFA in PBS
- fixed extracted brains were incubated in cold PBS
- perfusion-to-dehydration time was minimized

Dehydration protocol (Dodt et al., 2007) modified in the Giese Lab:
- Series of increasing concentrations of alcohols in dH_2O (with modifications)

Clearing Protocol (Dodt et al., 2007) modified in the Giese Lab:
- incubation in BABB = Benzyl Alcohol + Benzyl Benzoate 1:2 vol;
- ~ 25% (anisometric) linear shrinking
- refr. index ~ 1.558 (BABB, as well as the cleared specimen);

(B) Light Sheet Fluorescence Microscopy (LSFM) implemented in Giese Lab
- 3D imaging large samples (~ cm) with µm-resolution
- 2 scanners (scanned laser light sheet with shadow suppression)
- long working distance excitation and emission objectives (~ 5 mm focus depth)
- brains scanned in horizontal plane (mosaic of 3D image plane series)
- 1.6 µm x 1.6 µm pixel size in specimen plane; 3.2 µm z-step
- hemispheres scanned separately with overlap
- image cube size ~ $2*10^{10}$ voxels (2500 x 7000 x 1500)
- Stitching of mosaic image plane series
- correction for attenuation of excitation / emission signal by tissue

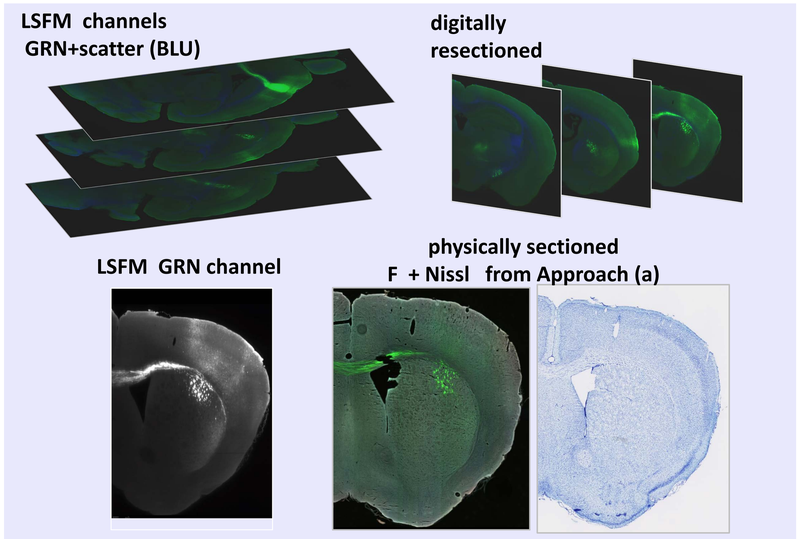
channel	Excitation [nm]	Emission [nm]
GRN	488	503-537
RED	561	570-640
side scatter	488	454-496

Main Results

Feasibility and benefits of the combo Approach (c)+(a) demonstrated

(1) near isotropic scanning resolution at the cellular level (~1 µm)
(2) preservation of high S/N fluorescent signal after rehydration
(3) high-resolution brain-wide cytoarchitecture & histochemistry

Example:
Whole brain mapping of sensorymotor cortex projections



Mouse injected in somatomotor cortex with anterograde fluorescent tracer

(adeno-associated virus; cytoplasmic expression of EGFP; PTM562 Left-Hemi)

Approach (a). Histological Tissue Processing Pipeline

(A) Tissue Rehydration Method implemented in both the Giese and Mitra Labs

Cleared brains were rehydrated for histological processing.
Rehydration protocol:
- Incubation steps with modified alcohol / water mixtures, as in dehydration protocol but in reversed order
- Final step: PBS

(B) Histological Processing Methods (MBA pipeline) implemented in Mitra Lab

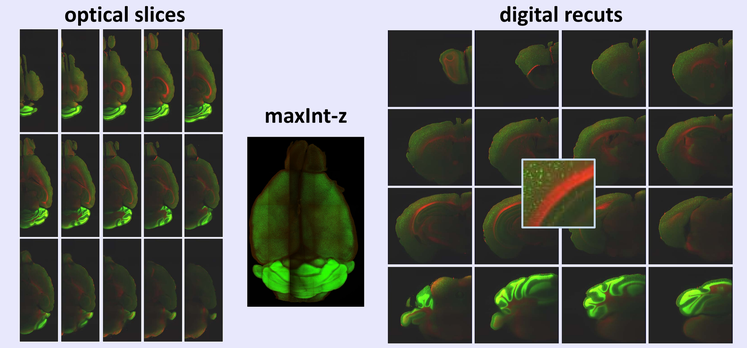
After rehydration, brains were processed in the semi-automated Mouse Brain Architecture (MBA) Project tissue processing pipeline.

- brain coronally cryo-sectioned to 20 µm thickness
- sections transferred to glass slides using the tape-transfer system (this prevents tissue distortions or displacement)
- alternate sections developed, on slide, for label (histochemistry or fluorescence) and Nissl stain
- whole slides imaged using the NanoZoomer 2.0HT (0.46 µm/pixel in plane)

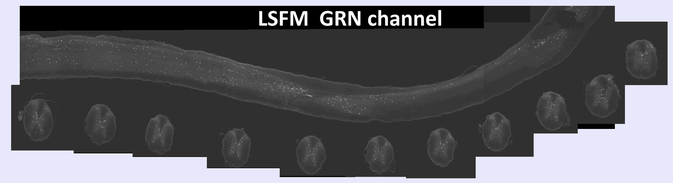
Pilot Results

These pilot studies were completed only for Approach (c). They highlight the potential application of the combined approach to studies that demand preserving high spatial resolution in the z-dimension that serves rapid, unbiased comprehensive brain-wide cell & process detection together with high-resolution anatomical localization.

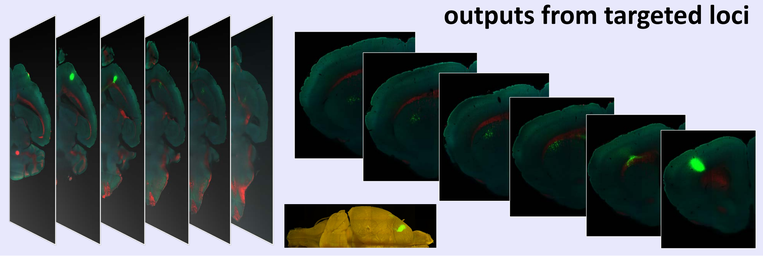
(A) Whole-brain mapping of genetically targeted neuron class



PV-Cre (nuclear + cytoplasmic label) mouse brain & spinal cord - Approach (c)



(B) Whole-brain mapping of outputs from targeted loci



Mouse MO/SS cortex injected in with anterograde AAV EGFP - Approach (c)

Future Work

- Improve rehydration method to better preserve tissue integrity
- Integrate clearing method into the MBA histological pipeline prior to the sectioning stage
- Digitally align the (i) image stacks obtained from the cleared brain with light sheet scan with (ii) the Nissl and fluorescent stacks obtained from the MBA pipeline.

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