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A Methodological Pipeline for Serial-Section Imaging and Tissue Realignment for Whole-Brain Functional Connectomics

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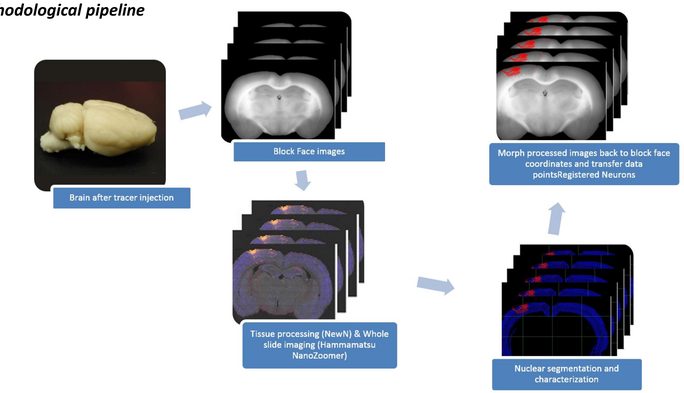
Introduction

Understanding the neurobiological basis of cognition and behavior, and disruptions to these processes following brain injury and disease, requires a large scale assessment of information exchange amongst populations of neurons, as well as knowledge of their patterns of connectivity. We present an approach that allows for a large-scale investigation of brain connectivity.

This open-source toolkit allows an investigator to visualize and quantify whole brain data in 3-D and additionally provides a framework that can be rapidly integrated with user-specific analyses and methodologies.

Methods

Methodological pipeline



Pipeline for whole-brain functional connectomics. Serial section (block-face) images provide an un-distorted whole brain reference coordinate system.

Histological processing

An image was taken of each section just prior to sectioning (Block-face image).

Then each section was processed for either the neuronal marker (NeuN) for neuronal segmentation and automatic detection of tracer filled cells, or with a stain to aid in identification of regional boundaries (Parvalbumin or Cresyl Violet).

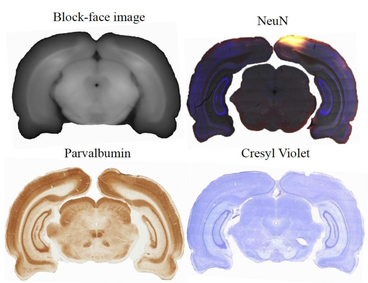


Image acquisition

Rapid image acquisition of entire coronal or sagittal sections was conducted using NanoZoomer whole-slide scanning microscopy (NanoZoomer Digital Pathology RS, Hamamatsu Photonics), which is capable of automatically capturing wide-field multispectral fluorescent images over entire brain sections at high resolution.

The objective was focused on the middle of the section in the z-plane and image acquisition was conducted with 40x magnification with a multi band pass filter cube (DAPI/FITC/Texas Red).

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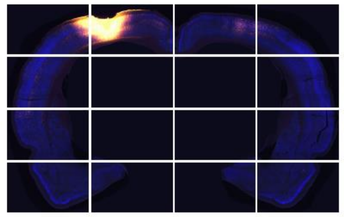
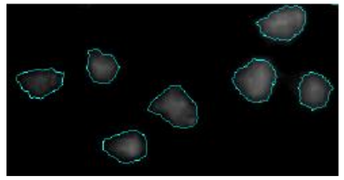


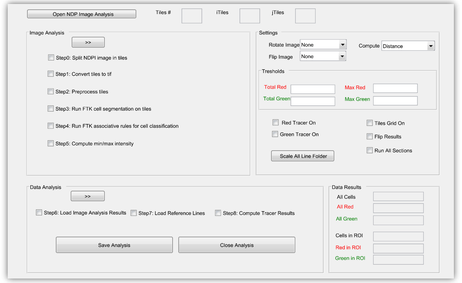
High resolution (40x) tile images are created



Neuronal segmentation using Farsight toolkit

Automated detection of retrograde tracer

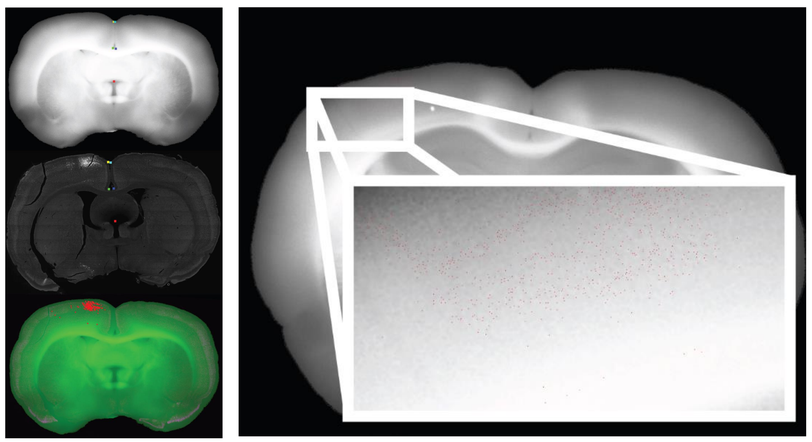
A custom software platform was developed in **Matlab**. It is using the open source image processing software **Fiji** and **Farsight** to accommodate automated neuronal segmentation and realignment of identified tracer filled cells with serial-section block-face imaging.



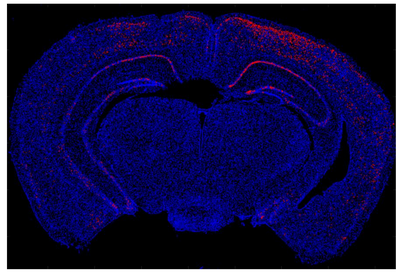
The automated segmentation of neurons allows for measurement of the precise x- y- position of each neuron, as well as the intrinsic neuronal features including the intensity values. The user has the option to specify their own parameters in order to classify cells according to a specific neuronal marker.

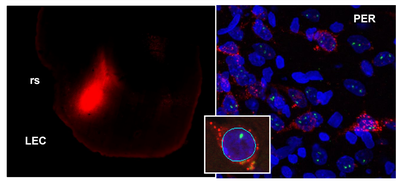
Registration to Block Face Image

We implemented methods that allow re-alignment of detected tracer positive cells to the corresponding block-face image by using a vector field based registration toolkit **ANTs**. Common points on processed tissue and pre-cut image of the same section are marked to improve registration. Reference points which could clearly be identified on both the pre-cut image of the section (left-top) and the same section following histological processing (left-middle) were marked (color coded squares) using **ITK-SNAP Tool** and used to facilitate registration (left-bottom).



Results





Whole Brain Immediate Early Gene Detection

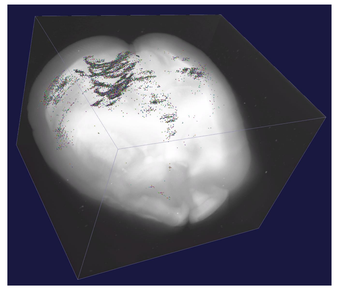
As a proof of principal for functional connectomics, we performed additional analyses. We conducted an experiment for the detection of the IEG Homer1a on a data set of serial images from a mouse brain. Homer1a and other IEGs (e.g., Arc, Narp, c-Fos) are transcribed rapidly following neuronal firing (Montes-Rodriguez et al., 2013), and can therefore be used as markers of behaviorally driven ensemble activity following a specific event.

The FARSIGHT toolbox can also accommodate detection of both immediate-early gene expression and tracer within tissue sections. For instance, individual nuclei (DAPI stain) or neuronal cell bodies (NeuN stain) can be segmented and the integrated intensity for other fluorophores can be measured within or surrounding the segmented objects

3D Rendering

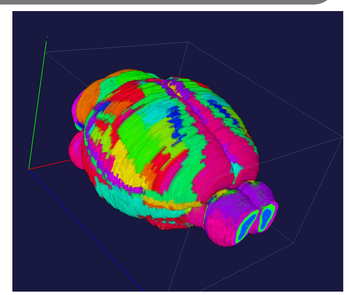
Finally, individual sections were extruded in the z-plane to match the section thickness (50um) and allow rendering of data in 3D using **Vaa3D Tool**.

For the present analyses anatomical data labeled sections with quantified counts (1:6; Movie 1) were interspersed with Cresyl violet stained, parvalbumin immunostained and non-quantified tracer labeled sections. Any configuration of stained and/or quantified material could be reconstructed using this method.



Conclusions and Discussions

- We describe a custom software platform that accommodates an automated analysis pipeline to enable 3D visualization and analysis of whole brain connectivity data.
- Our software platform allows for the integration of additional functionality, for example, work with the Scalable Brain Atlas database to allow region based 3D analysis.
- In future refinements of our software platform, additional work could be integrated improving the quality of segmentation of NeuN tagged cell bodies, or possibly by testing using other antibody and fluorophores combinations.



Acknowledgements
This research was supported by the Alberta Innovates Health Solutions Polaris Award to BLM, MH46823-16 to BLM, Alberta Innovates Health Solutions Fellowships to AAW and BJC, MH099682 and AG049090 to AAW, and a Natural Sciences and Engineering Council of Canada Fellowship to BJC. The block face imaging system was developed with support from Neuroelectronic Research Flanders (NERF).