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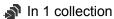
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## Micro-CT scanning and fiber localization



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#### **ABSTRACT**

We have developed a new micro-fiber array approach capable of chronically measuring and optogenetically manipulating local dynamics across over 100 targeted locations simultaneously in head-fixed and freely moving mice, enabling investigation of cell-type and neurotransmitter-specific signals over arbitrary 3-D volumes . This protocol includes the micro-CT scanning and fiber localization steps. Please contact us (mwhowe@bu.edu) if you are interested in using this technique.



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### Perfusion and dissection

- Mice were injected intraperitoneally with 400-500 mg/kg Euthasol (Covetrus Euthanasia III), and then perfused transcardially with 20mL 1% phosphate buffered saline (PBS, Fisher), followed by 20mL 4% paraformaldehyde in 1% PBS.
- After perfusion and decapitation, the lower jaw and front of the skull were removed in order to allow diffusion of solution into the brain while still keeping the implant intact.

## **Preparation for CT scanning**

- The brain was soaked in the 4% paraformaldehyde solution for 24h, rinsed three times with 1% PBS, and then transferred to a diluted Lugol's solution, to provide tissue contrast for computerized tomography (CT) scanning (Metscher, 2009).
  - 3.1 The Lugol solution was prepared by diluting 10mL 100% Lugol's Solution (Carolina, 10% potassium iodide, 5% iodine) with 30mL deionized water, a dilution chosen to be approximately isotonic to biological tissues61.
  - 3.2 Note on soak time: Initially, samples were soaked in this diluted Lugol's solution in a foil-wrapped 50mL conical centrifuge tube on an orbital shaker plate for 10-14 days. We have more recently found that using 4 oz specimen cups instead of the 50mL conical centrifuge tubes enables better diffusion of the Lugol's solution, and adequate contrast can be achieved in three to four days.
- 4 After soaking, the skulls were rinsed three times with 1% PBS, and secured in a modified centrifuge tube.



## Micro-CT scanning

5 The implanted skulls were imaged in a micro-CT scanner (Zeiss Xradia Versa 520, a core instrument of the Boston University Micro-CT and X-ray Microscopy Imaging Facility) with the following parameters: 140kV, 10W, HE1 filter, 0.4X objective, 2s exposure time, 1001 projections, 12-micron voxel size.

## CT registration and fiber localization

6 The CT was then registered to the Allen Mouse Brain Common Coordinate Framework 3D 10-micron reference atlas (Wang et al., 2020) to bring individual mice into a common coordinate system, and then fibers were identified and mapped from the recording tip up to the grid. This process was carried out using a combination of FIJI (https://imagej.net/software/fiji/) and MATLAB (Mathworks, version 2020b) using a combination of existing MATLAB functions and custom lab-written functions and GUIs. This pipeline is now publicly available at https://github.com/HoweLab/MultifiberLocalization.