**Based on codes from Kim et al. 2023, Neuron, "Whole-brain mapping of effective connectivity by fMRI with cortex-wide patterned optogenetics".**

Note: The procedure described here was run on Ubuntu 22.04 and requires installation of AFNI (https://afni.nimh.nih.gov/), FSL (<https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/>), ANTs (<http://stnava.github.io/ANTs/>) and MATLAB (R2023a).

**File descriptions in pattern\_generation directory**

**Allen\_annotation\_modified.nii**: Allen mouse brain reference atlas (<https://atlas.brain-map.org/>) downsampled and modified from the original file (P56\_Annotation\_downsample2.nii.gz).

**Brain\_template.nii**: A customized T2-weighted mouse brain template on the same space with *Allen\_annotation\_modified.nii.*

**OI\_bead.jpg**: A fluorescence image of the mouse brain acquired through a bundle fiber. This image shows fluorescence expression of the dorsal cortex and fluorescence reference tubes for coregistration.

**star\_800x500.png**: A star-shaped reference pattern input to DMD, created by analysisImaging/DMD/showStar.m (previously DMD\_ref.jpg).

**star\_800x500.tif**: The star-shaped pattern reflected at the bundle fiber end and imaged by an sCMOS camera (previously OI\_ref.jpg).

**Allen\_pills\_mask.nii**: Masks to detect reference tubes in Allen space, created by create\_Allen\_pills\_mask.m

**File descriptions in matlab\_functions directory**

**MR2DMD.m**: The wrapper to do coregistration of a specified subject, using Allen\_annotation\_modified.nii, Brain\_template.nii and Allen\_pills.mask.nii, which runs

1. **Atlas\_T2\_coreg\_DS.sh**: Pipeline for coregistration of the atlas (*Allen\_annotation\_modified.nii*) and the structural image (*xxx.nii*). Then it detect reference tubes (FindPillsExp\_Allen.py)

Output: T2w\_resample.nii (T2\* MR image), Atlas\_anno\_to\_T2.nii (Allen annotation data, registered to individual brain)

1. **DMD\_pattern\_prep.m**: Define ROI for optical imaging, save as *ROI\_info* Coregister MRI and optical imaging using detected reference tubes, and save as *tform*. Then coregister optical imaging and DMD using star\_800x500.png/tif, and save as *tform2*.

Output: Atlas\_reg\_info.mat, containing

ROI\_info: CCF 60 regions info containing name and #pixels

Proj\_brain: CCF mapped to DMD input

TotalBrainImage: superposition of 60 regions of Proj\_brain

tform: transform from MR to optical images

tform2: transform from optical image to DMD input

mapconfwarpedtoDMD: brain boundary mapped to DMD input

**DMD\_pattern\_generation.m:** run DMD\_pattern\_generation(ROI\_index) on the MATLAB command window. For example, DMD\_pattern\_generation(31) makes the binary image of the left MOp pattern, and saves it as a png file (ROI\_31.png). ROI\_index can be a row vector to generate a stimulation pattern of multiple ROIs. For example, DMD\_pattern\_generation([28 29]) makes the pattern of the right VISa+VISrl.

**Imupdatepair.m**: (not yet tested) compare two widefield images, one reference and the other latest within a specified directory. Used to align macroscope positions across sessions.