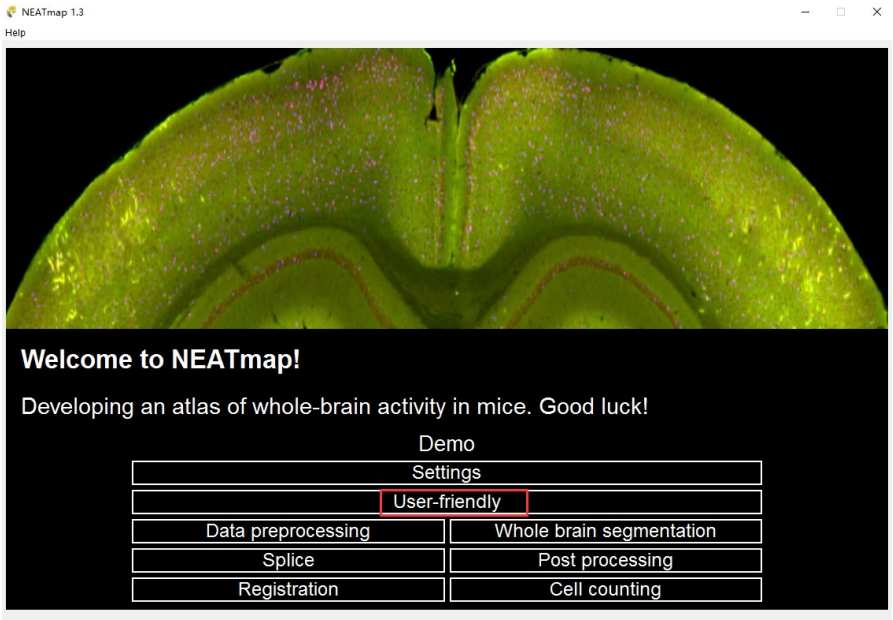
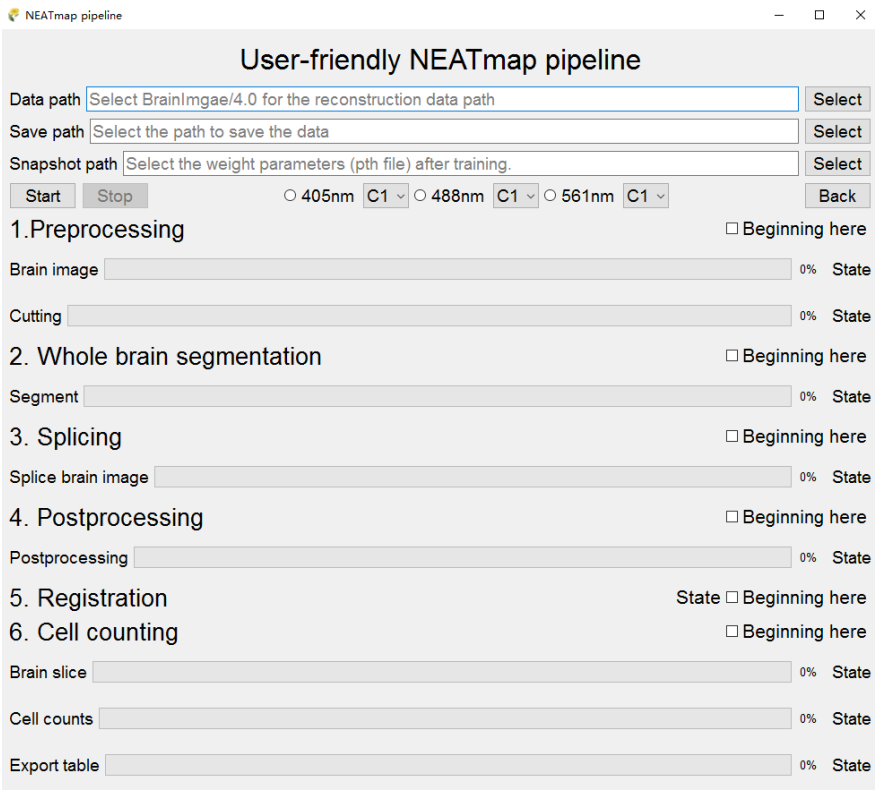
NEATmap Software User Guide

User-friendly



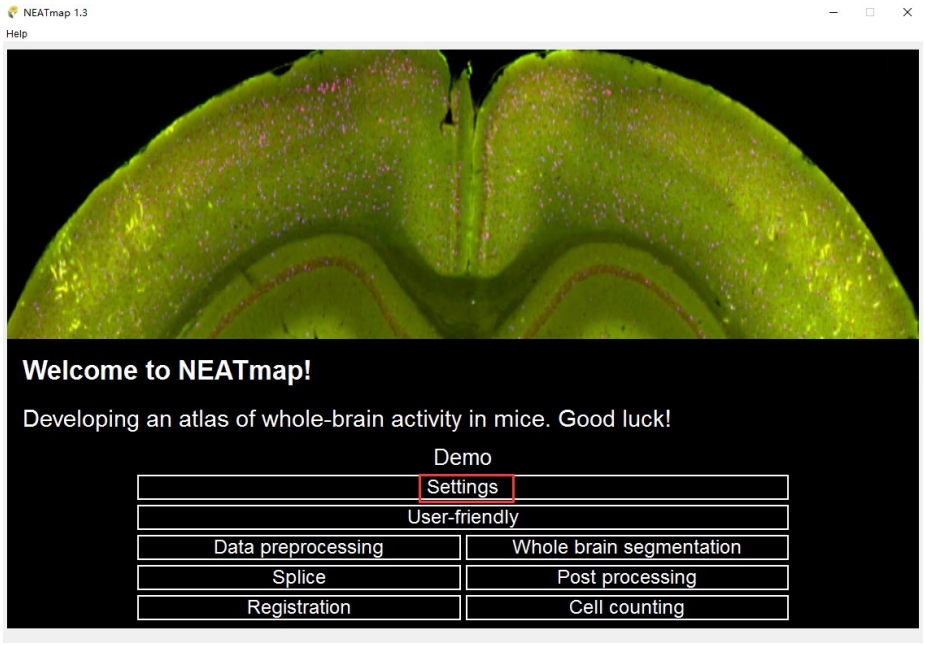


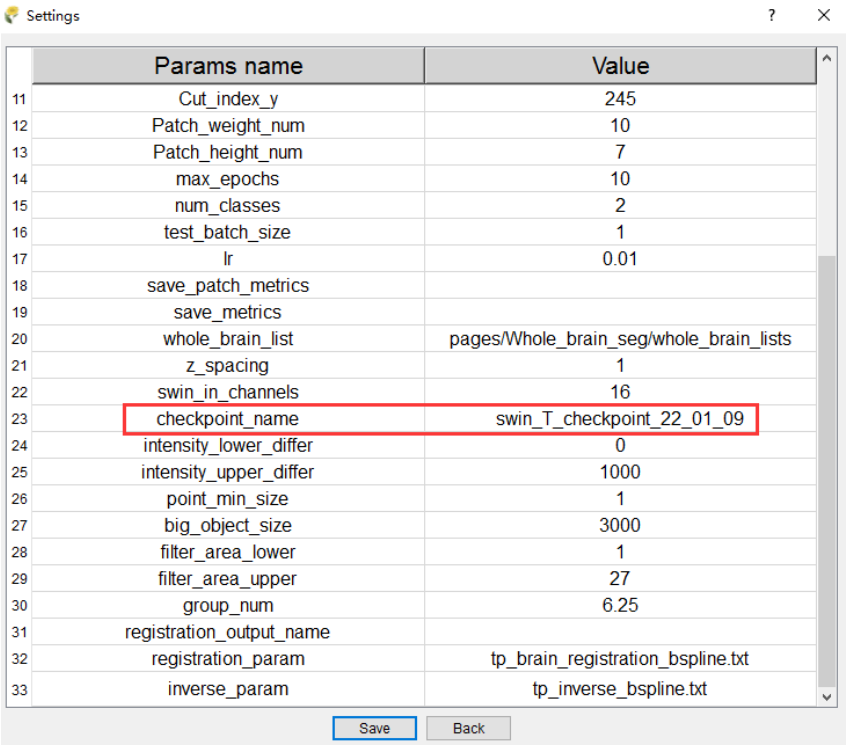
The **Data path** is BrainImage/4.0 after reconstruction. **Snapshot path** is the path for loading the trained parameters of the

neural network. The default path for the trained parameters is:

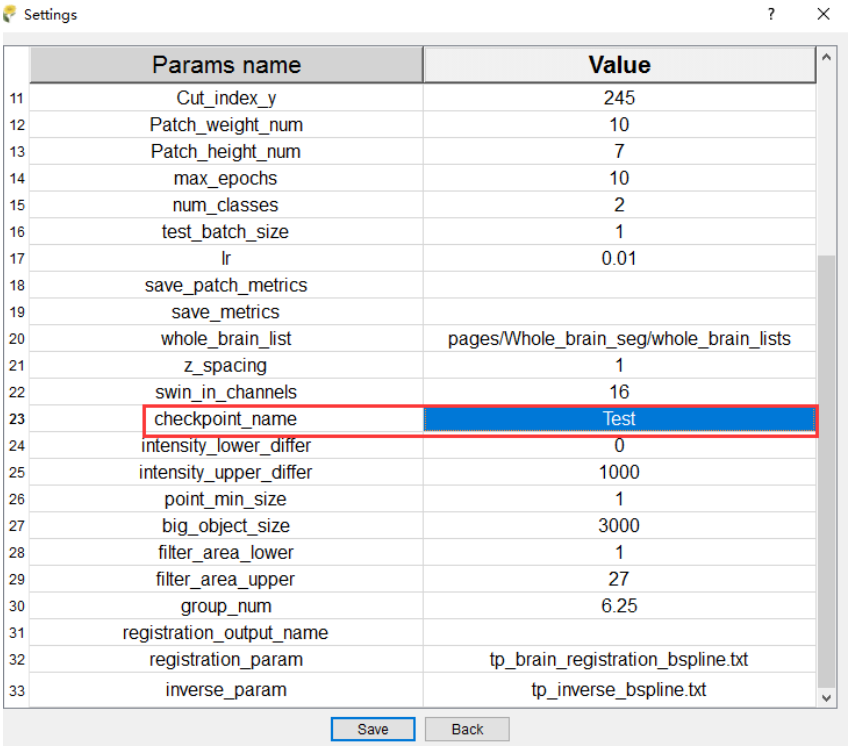
pages\Whole_brain_seg\swin_T_checkpoint_22_01_09.

If you want to change the network parameters, you need to modify the checkpoint name on the **Settings** page to the name of the folder where the network parameters are located.





For example, if the network parameters you want to use are in the folder name **Test**, modify them as shown below:

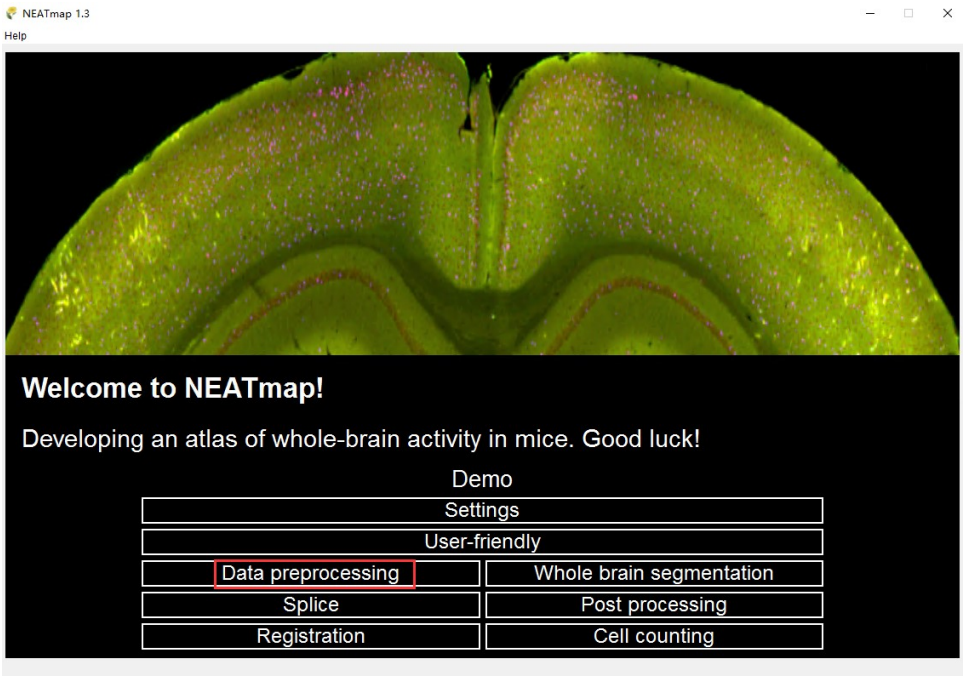


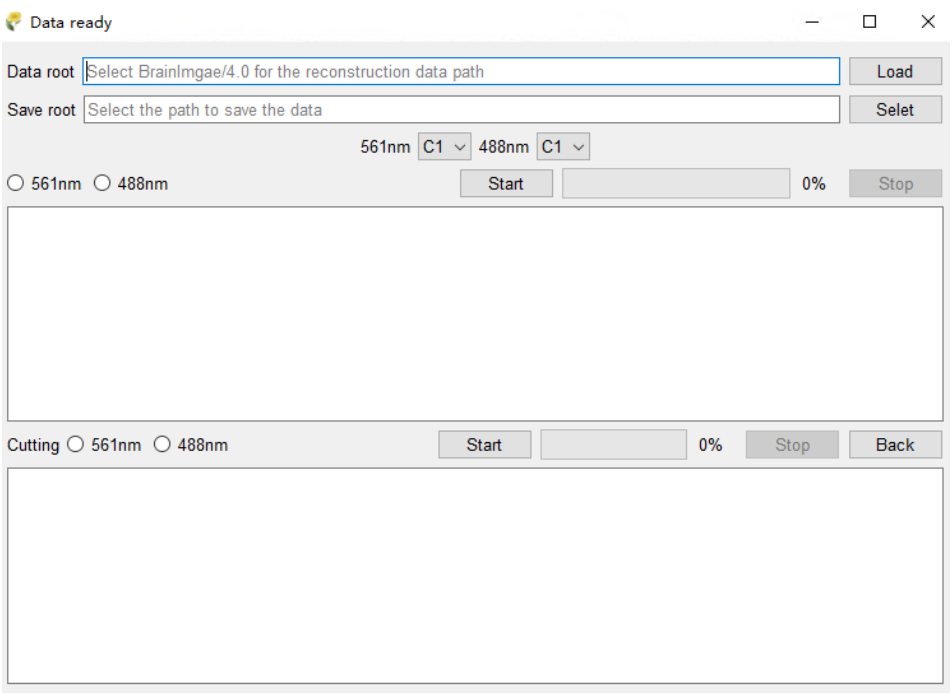
Then, click **Save** to complete the modification of network parameters.

Finally, the number information of each channel must be selected correctly, and click **Start** to start running. On this page, Data preprocessing to export of cell counting results will be completed.

If you want to skip **Preprocessing**, you can check **Beginning here** to the right of **Whole brain segmentation**. Other operate similarly.

1. Data preparation





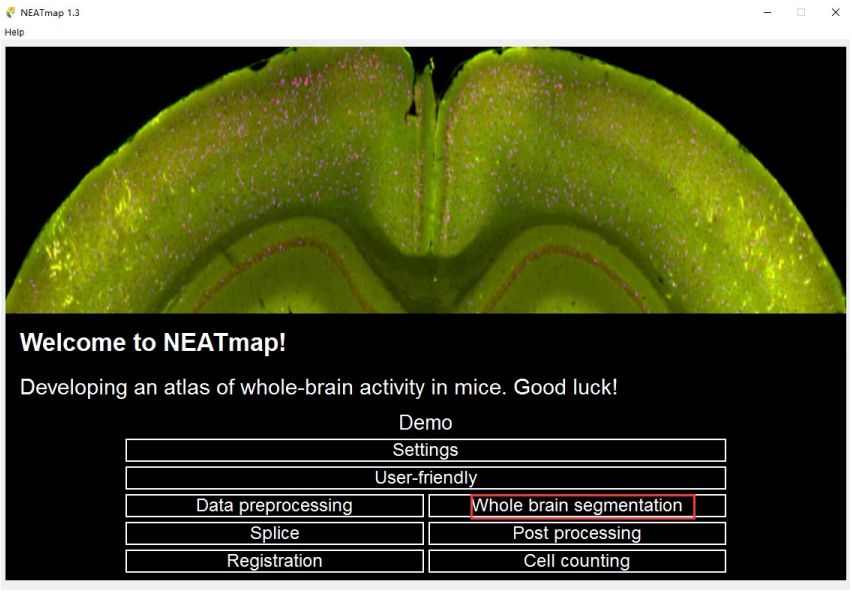
(1). Data preparation for 3D brain slices: The data root is BrainImage/4.0 after reconstruction. Select channels corresponding to 561nm and 488nm. By default, the 3D data size is (3500, 2500, 64) for (x, y, z) dimensions. Click **Start** to begin the preparation

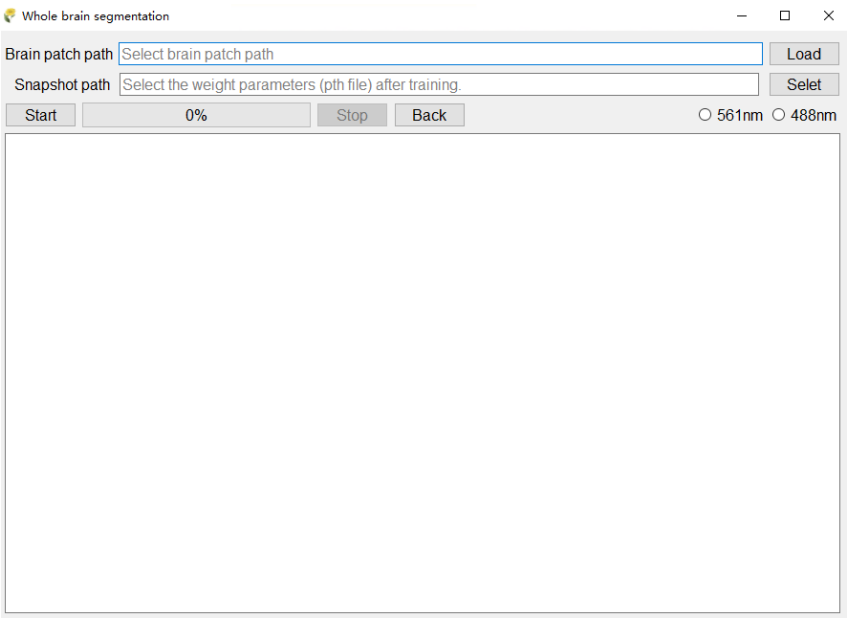
process for either 561nm or 488nm channels. **Stop** will change to **Finished** when a single data preparation is complete.

(2). After the 3D data preparation is complete, start cropping the data. Crop one data into 70 subvolume brain images with dimensions (256, 256, 64) for (x, y, z). Perform the operation as before, and click **Back** to return to the main page after completion.

2. Whole-brain immunolabeled signal segmentation

After cropping, start the whole-brain automated segmentation of c-Fos+ and spontaneous fluorescence signals in the whole brain.



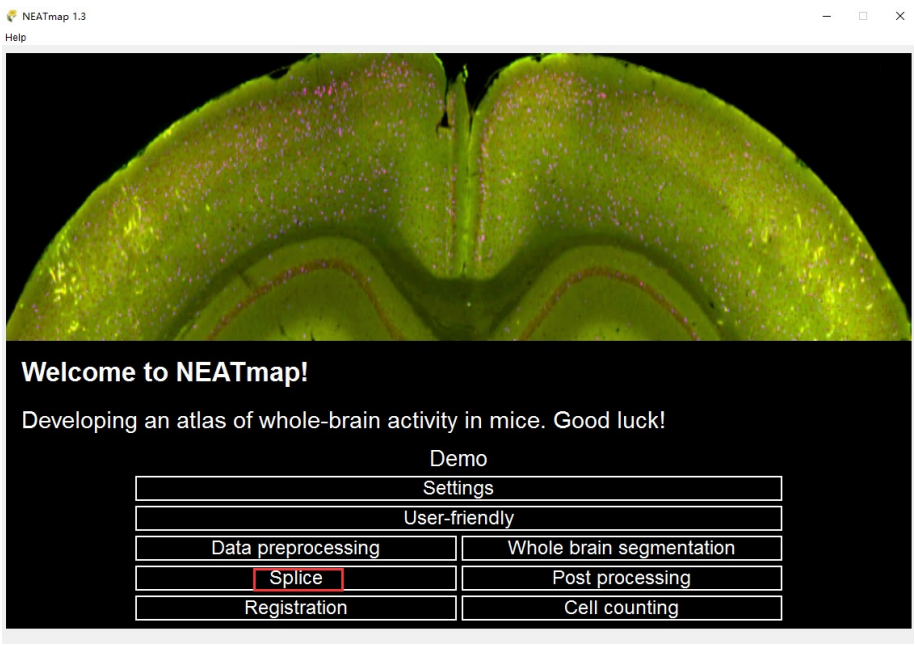


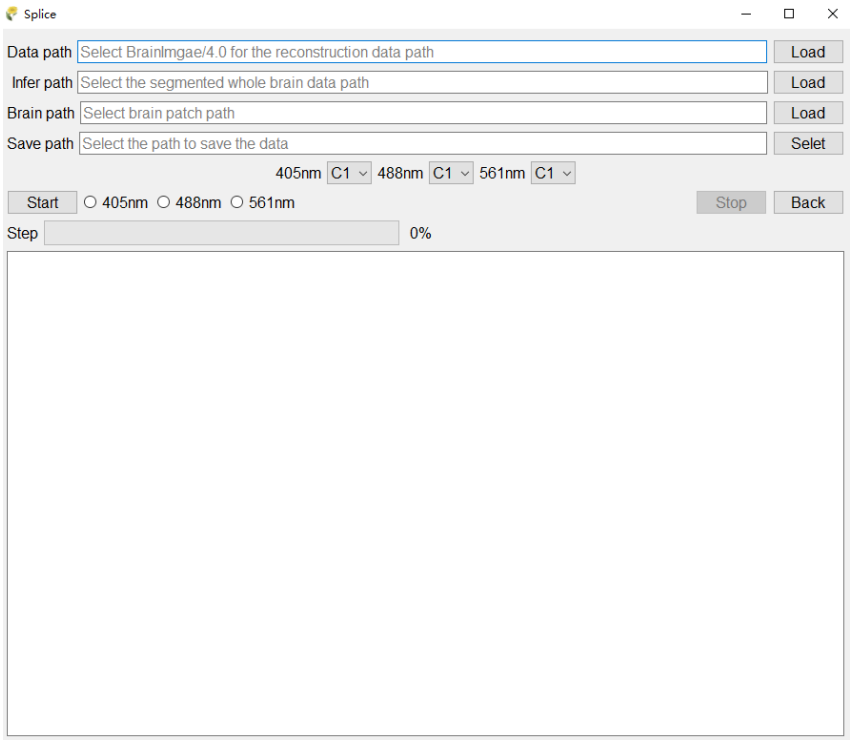
Brain patch path refers to the parent path of the Subvolume brain images after cropping. Snapshot path is the path for loading the trained parameters of the neural network. The default path for the trained parameters is:

pages\Whole_brain_seg\swin_T_checkpoint_22_01_09.

3. Whole brain splicing of segmentation results

After completing the whole-brain signal segmentation for the dual channels, it is necessary to restore the segmentation results of the Subvolume brain images to the original size of the brain slices.





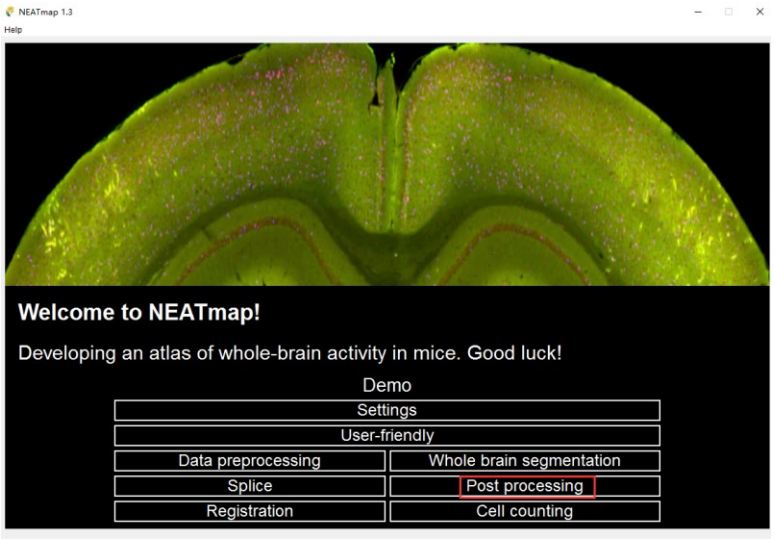
The data root path is as described above. The Infer root refers to the path of the whole-brain dual-channel segmentation results, and the Brain root refers to the parent path of the Subvolume brain images.

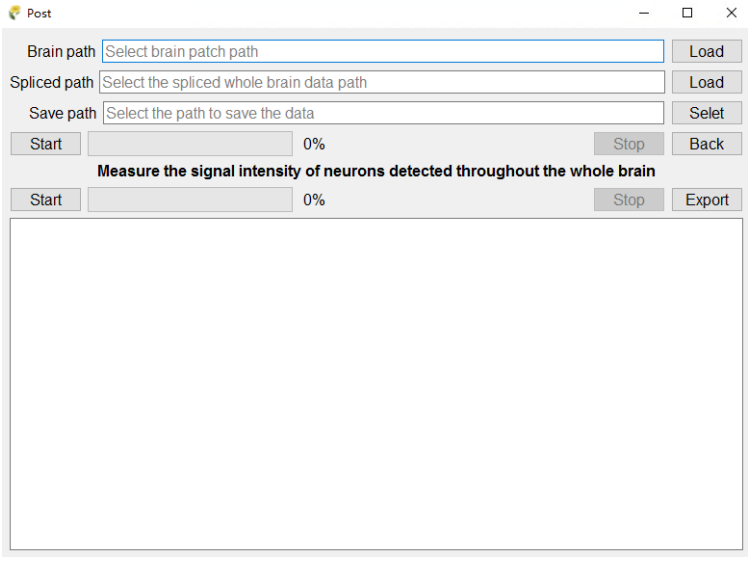
The stitching process consists of a total of 2 steps. The first step

is to convert the 3D segmentation results to 2D and stitch them in a 2D layer-wise manner for the whole brain (3500, 2500). The second step is to combine all stitched 2D segmentation results to generate a 3D volume (3500, 2500, 64).

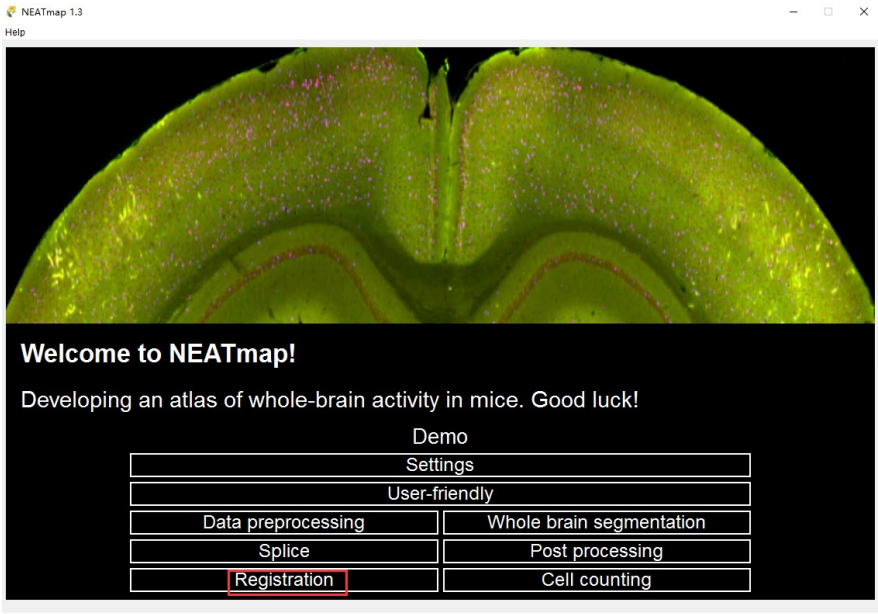
4. Post processing

After completing the whole-brain signal segmentation stitching, post-processing operations are performed. The splice path refers to the parent path of the stitched results.



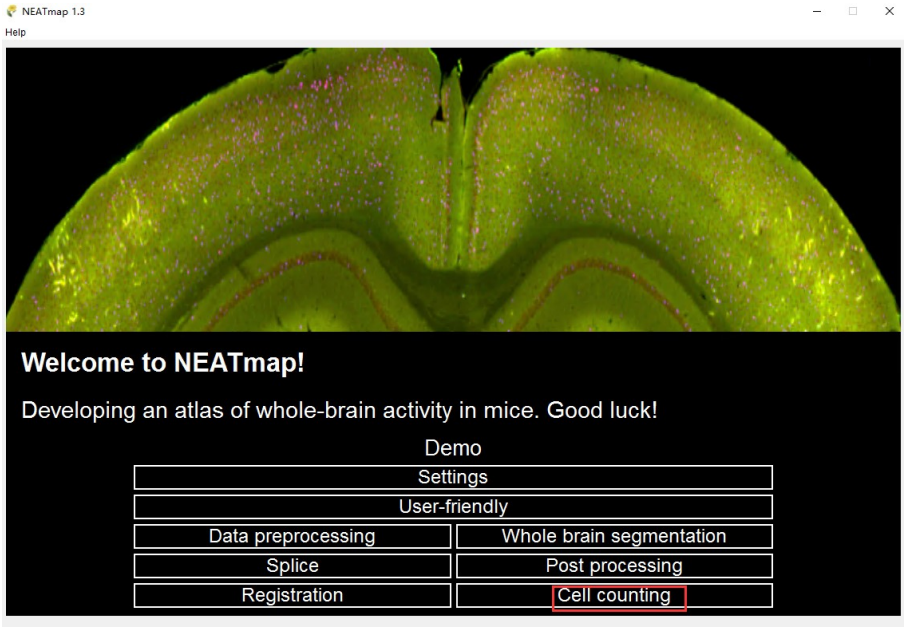


5. Registration

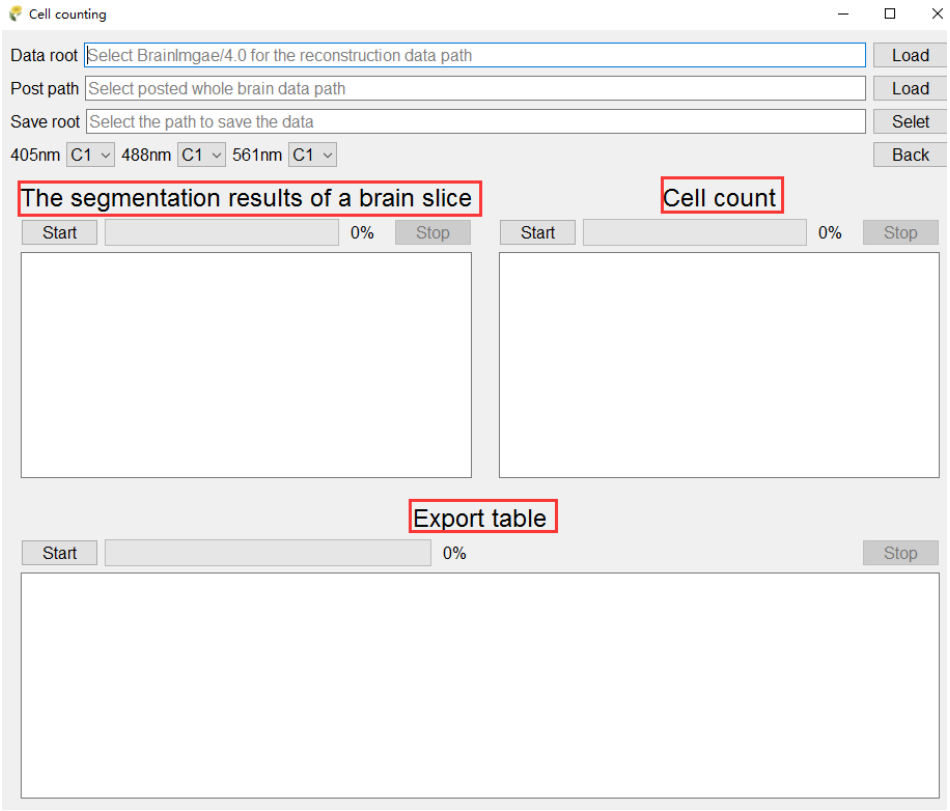


This module can register the whole brain to the Allen Common Coordinate Framework atlas. The operation is the same as above, and when finished, the **Stop** button displays **Finished**.

6. Cell counts



After registration and post-processing are completed, the segmentation results need to be mapped to the Allen CCFv3. The Post path refers to the parent path of the post-processing results.



(1) The module is used to restore the size of the segmentation results from (3500, 2500, 64) to (3500, 2500, 75), which corresponds to a thickness of 300 μm (75 x 4 μm).

(2) Cell count. This module is used to count cells in the whole brain signal segmentation results.

(3) Export table. This module is used to generate a csv file that can be imported into Freesia, which records the centroid coordinates of each segmented c-Fos signal.

Exported file