TRACER-3D User manual

Toolkit for Reconstructing Anatomical Coordinates in Rats in 3 Dimensions

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Introduction

TRACER-3D is a useful toolbox for visualizing the trajectories of recording electrodes (e.g Neuropixels) through different sub-regions of the rat brain. Anatomical delineations are referenced from the Waxholm Space atlas of the adult Sprague Dawley rat brain (https://www.nitrc.org/projects/whs-sd-atlas). There are three packages in the TRACER-3D toolbox. One is for locating electrode tracks in the brain post-hoc using histological images, one is for generating coordinates prior to surgery in order to target specific brain regions, and one for visualizing the virus expression. NOTE: For Neuropixels, we included the 175um tip + 9600um electrode surface length (20um * 960 electrodes *1/2 row/electrodes). So happy tracing!

Contents

1 Requirements

In order to start working with TRACER-3D you need to have:

- Individually sliced images of rat brain slices (coronal, sagittal, or horizontal) in .jpg format.
- Python 3.8.5 (Spyder IDE 4.1.5).
- A computer mouse with scroll wheel and a functional keyboard.
- The atlas files. They can be downloaded from the NITRC website (https://www.nitrc.org/projects/whs-sd-atlas). The files that you should have are:
  - `WHS_SD_rat_T2star_v1.01.nii.gz`
  - `WHS_SD_rat_brainmask_v1.01.nii.gz`
  - `WHS_SD_rat_atlas_v3.nii.gz`
  - `WHS_SD_rat_atlas_v3.label`

2 Setup of the environment

It is recommended to use Anaconda for non experts in Python, this will help with the downloading of the necessary packages and for the updating of the existing ones. Anaconda can be downloaded for free from their website (https://www.anaconda.com). Once installed Anaconda will already include Spyder. To create a new environment in anaconda:

- go to `Environments` on the left panel,
- click `create` in the bottom left,
- name your environment and in `Packages` select both Python (3.8) and R.

Once a new environment is created you should install the packages necessary to run TRACER-3D, this is done with the following steps:

- open the terminal by clicking the arrow near the name of you environment,
- navigate to the folder where requirements.txt is
- run `pip install -r requirements.txt`

The final steps to have everything ready to run are:

- create a folder named Files where to save the file created with `Useful_File_Creation.py`;
- change the paths to the folder Files, the one where the Atlas files are, and the other paths in the script that should be changed in each script of TRACER-3D;
- run file `Useful_File_Creation.py` that is in the repository to generate files that are too large to be added to the GitHub repository.

3 Post-surgery/recording process

TRACER-3D main task is to help the user with the registration and study of the brain regions traversed by the probe. This is achieved by running the following scripts:

- `Preprocess_Histology.py`
- `Register_probes.py`
- `3D_Visuals_Probe.py`
- `Analize_Clicked_Points.py`

3.1 Preprocessing histological Images

This functionality helps you obtain processed, clear, nice looking histology images with desired sizes and contrast. You should run this file to properly register the histology images. Make sure your brain slices are all saved in the same folder. It is recommended to have the images with consecutive names (e.g. histology01, histology02, etc.).

Open `Preprocess_Histology.py` and first only run this `%matplotlib inline` line of code in your console before running the entire script.

NOTE: If you are a Mac user, make sure that Python as the security authorization to access input from keyboard. If you get the error in Figure 1 use the sudo command in your terminal to open your desired IDE (Spyder is recommended). Use the command `sudo spyder`. Follow the steps below to properly run the script.

```
Exception in thread Thread-12:
Traceback (most recent call last):
  File "/opt/anaconda3/lib/python3.8/threading.py", line 932, in _bootstrap_inner
    self.run()
  File "/opt/anaconda3/lib/python3.8/threading.py", line 870, in run
    self._target(*self._args, **self._kwargs)
  File "/opt/anaconda3/lib/python3.8/site-packages/keyboard/__init__.py", line 294, in listen
    _os_keyboard.listen(self.direct_callback)
  File "/opt/anaconda3/lib/python3.8/site-packages/keyboard/_darwinkeyboard.py", line 430, in listen
    raise OSError("Error 13 - Must be run as administrator")
OSError: Error 13 - Must be run as administrator
```

Figure 1: Error for Mac users when running `Preprocess_Histology.py`.

- Add the correct path for your folder:
  `histology_folder = Path('/Users/....../Histology/')`
- Choose the plane of view with the keyboard letters (c for coronal, s for sagittal, h for horizontal).
- Once you have adjusted the histology image using the controls exhibited in the console press 's' on the keyboard to finalize your editing and then save.
- You will find the processed histology image in the folder "Processed", that will be crated as subfolder of the Histology.

3.2 Registering the probe track and navigating the atlas

This file runs based on the images saved from the pre processing step. You can then select the corresponding brain slice with the Waxholm atlas, overlay the histology to the atlas, and register the probe. Make sure you have the right path to the preprocessed images obtained from the previous script, and to the other relevant folders. Open the script named: `Register_probes.py` and follow the steps below.

- Close the console where `Preprocess_Histology.py` was running and open a new one.
- Add the path here:

  `processed_histology_folder =`
  `Path('/Users/..../Histology/processed').`
- There are two figures popping up in the screen and to perfectly overlay your histology image with the atlas you need to click 't' to activate the overlay points.
- Choose at-least 4 points in the same order for both the histology slice and the atlas image that is on your computer screen. Accuracy in selecting the points at this stage will determine a good fit of the histology image to the atlas. The numbering will help you remember the ordering of the clicked points (Figure 2). Figure 3 shows the Nissel stained version of a histology slice.

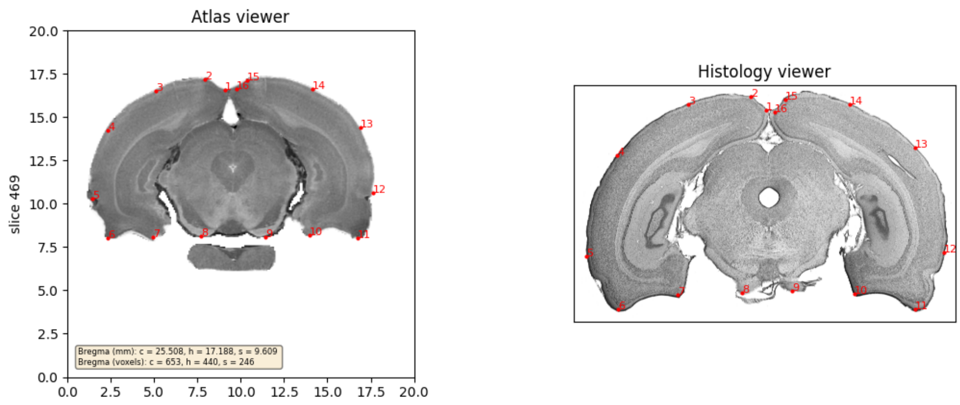

Figure 2: Left: clicked points on the atlas. Right: clicked points on the histology.

- Press 'h' to match the histology with the atlas.
- Press 'a' to visualize the regions boundaries of the Waxholm atlas (Figure 4).
- After having pressed 'h' and 'a' it is possible to either visualize the regions colors by pressing 'v' or start registering the probes by pressing 'r'.
- Click the points on the probe tracks.
- When registering probes, it is important that the first and the last point clicked correspond to the entry point and to the last visible point of the probe. This applies for all the histology images you want to click the probe points.

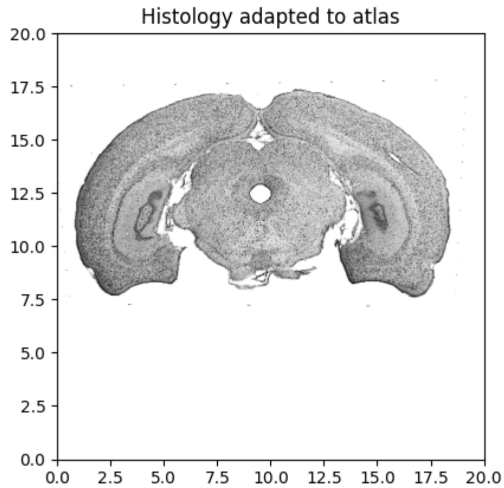

Figure 3: Histology overlayed with the selected slice of the atlas.

- Press 'c' to delete the probe points.
- Press 'w' to visualize probes in '2D' (Figure 5).
- Press 'e' to save the histology and proceed with the registration of the next slice. The slices will appear according with the ordering given by their names.
- If you have multiple images, once you finish registering the electrode track of one histology image, you'll find the next one lined up on your desktop screen. Follow the same procedure as you did for the first histology image.
- For more options follow the instructions on the console.

NOTE: In case you want to change the background colour of the image or the atlas, you can do it by changing the following: Go to line 21 (Figure 6) in the `Tracker.py` file and change the 'cmap' colour to either white or black depending on your choice of image. To change the colour of the gridines of the atlas, go to code line 367 in the `Register_probes.py` file and change to 0 for black, 1 for white or 0.5 for gray.

NOTE: when interacting with keyboard make sure that one figure between Atlas viewer, Histology viewer or Histology adapted to atlas is selected otherwise the the software will not interact with the keyboard.

3.3 Visualizing probes in the 3D atlas

Now you have saved and registered points of the probe track(s). You can further visualize your probe tracks in 3D by running this script `3D_Visuals_Probe.py`.

This script gives you the information about the probe insertion angle, regions transverses by the probes, the location and the number of channels in the probe (e.g. neuropixel) that has been recording (Figure 7 left)

In the processed folder on your PATH you will then find a `.txt file` in which you will find relevant information about the probe you have currently visualized (Figure 7 right). Close and open a new console every time you run this script.

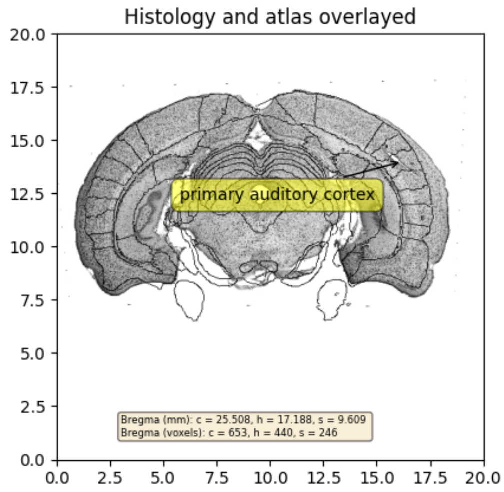

Figure 4: Activated grid lines showing the regions of the rat brain in the coronal section.

3.4 Visualizing clicked points in the 3D atlas

Running `Analize_Clicked_Points.py` the user can obtain information about the coordinates an the regions of the points clicked to register the probe. Additionally, the clicked point are displayed in the 3D image of the Waxholm atlas (Figure 8).

4 Pre-surgery preparation

The other important feature of TRACER-3D is to help the user to plan the electrode insertion before surgery, showing the inclination and insertion distance necessary to target specific brain's regions. For the insertion planning the scripts to run are:

- `Plan_probe_Insertion.py`
- `3D_Visuals_Probe_Insertion.py`

4.1 Planning probe insertion

The script `Plan_probe_Insertion.py` is essentially useful to plan electrode insertion before any surgeries. Once you run the script you will be displayed with the Waxholm atlas. You can then scroll through the slices until you reach the slice of your satisfaction. Then use the commands displayed on the console to view boundaries and determine where in the brain region you want to insert the electrode. Remember you can add more than one probe.

4.2 Visualizing probes in the 3D atlas for insertion

Now you have saved and registered points of the probe track(s). You can further visualize your probe tracks in 3D by running this script `3D_Visuals_Probe_Insertion.py`

This script gives you the information about the probe insertion angle, regions transverses by the electrode, the location and the number of channels that can be used for recording neural signals. Close and open a new console every time you run this script.

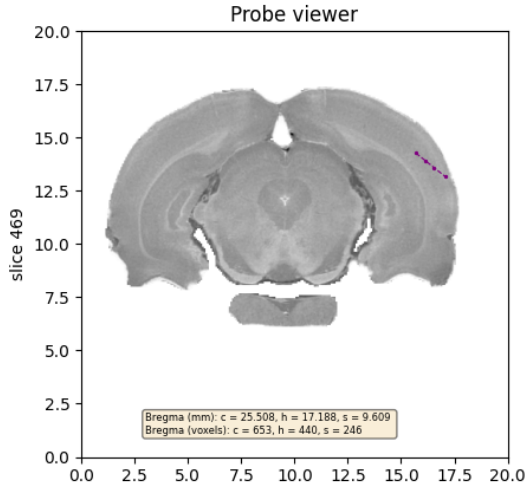

Figure 5: 2D representation of the rat brain with he reconstructed probe.

```
        self.im = ax.imshow(self.X[:, self.ind, :].T, origin="lower", extent=[0, 512*pixdim, 0, 512*pixdim], cmap='gist_yarg')
        #cmap='gist_yarg' if white background wanted
        #cmap='gray' for a gray or black background
```

Figure 6: Code to change background colours.

5 Virus expression visualization

Similarly to what is done to register and visualize probe tracks, the software can help the user to visualize the virus in the brain. The scripts to run for this purpose are:

- `Register_virus.py`
- `3D_Visuals_Virus.py`

5.1 Registering the virus and navigating the atlas

This file runs based on the images saved from the pre processing step. You can then select the corresponding brain slice with the Waxholm atlas that is available. ake sure you have the right path to the preprocessed images obtained from the previous script, and to the other relevant folders. Open the script named: `Register_virus.py` and follow the steps below.

- Close the console where `Preprocess_Histology.py` was running and open a new one.
- Add the path here:

  `processed_histology_folder =`
  `Path('/Users/.../histology/processed')`
- There are two figures popping up in the screen and to perfectly overlay your histology image with the atlas you need to click 't' to activate the overlay points.
- Choose at-least 4 points in the same order for both the histology slice and the atlas image that is on your computer screen. The numbering will help you remember the clicked points.

Figure 7: Number of recording channels in the electrode.

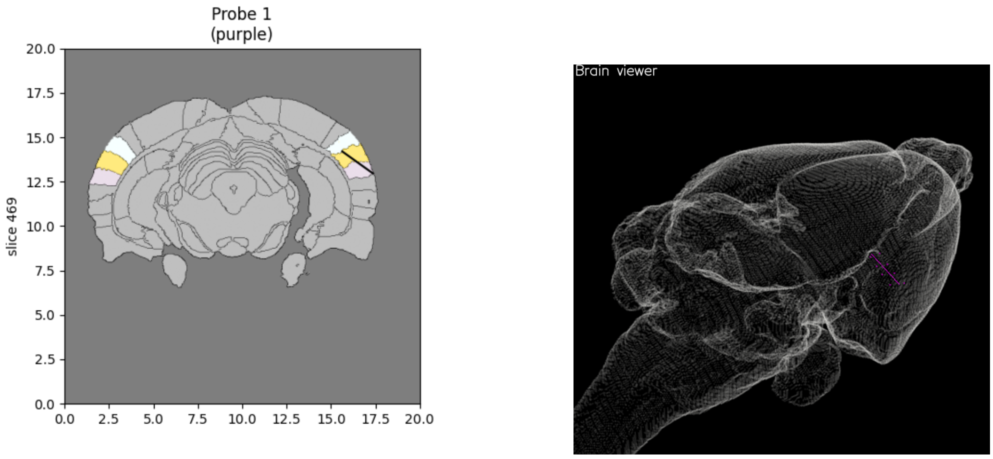

Figure 8: Left: Regions of the rat brain that the electrode has traversed. Right: 3D constructed electrode passing through different regions of the rat brain.

- Press 'h' to match the histology with the atlas.
- Press 'a' to visualize the regions boundaries of the Waxholm atlas.
- After having pressed 'h' and 'a' it is possible to either visualize the regions colors by pressing 'v' or start registering the probes by pressing 'r'.
- click the points where you can see the virus expression.
- Press 'c' to delete the virus points.
- Press 'e' to save the registered slice of histology with the virus. Add a name for the file.
- If you have multiple images, once you finish registering the electrode track of one histology image, you'll find the next one lined up on your desktop screen. Follow the same procedure as you did for the first histology image.
- For more options follow the instructions on the console.

NOTE: when interacting with keyboard make sure that one figure between Atlas viewer, Histology viewer or Histology adapted to atlas is selected otherwise the the software will not interact with the keyboard.

5.2 Visualizing virus in the 3D atlas

Now you have saved and registered points of the virus. You can further visualize the virus in 3D by running this script `3D_Visuals_Virus.py` This script gives you the information about the region and location coordinates of the virus points. Additionally, it creates `info.txt` file where the information are saved. Close the console every time you run this script. Figure 9 shows the 3D visualization of the virus expression in the Waxholm atlas.

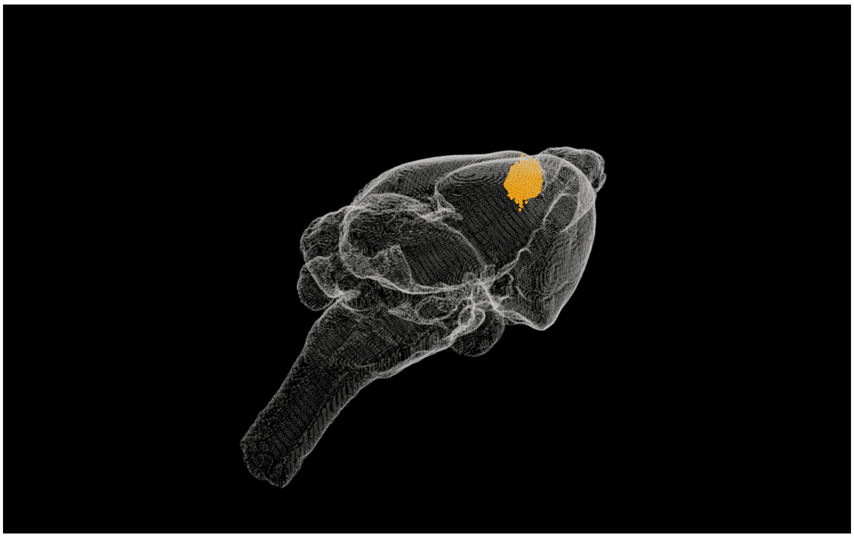

Figure 9: 3D representation of the virus.