

Example: Atlas-based imaging data analysis
tool for quantitative mouse brain histology
AIDAhisto

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This is a step-by-step procedure to count all cells visible in the red channel of a whole brain slice as shown in Figure 1.

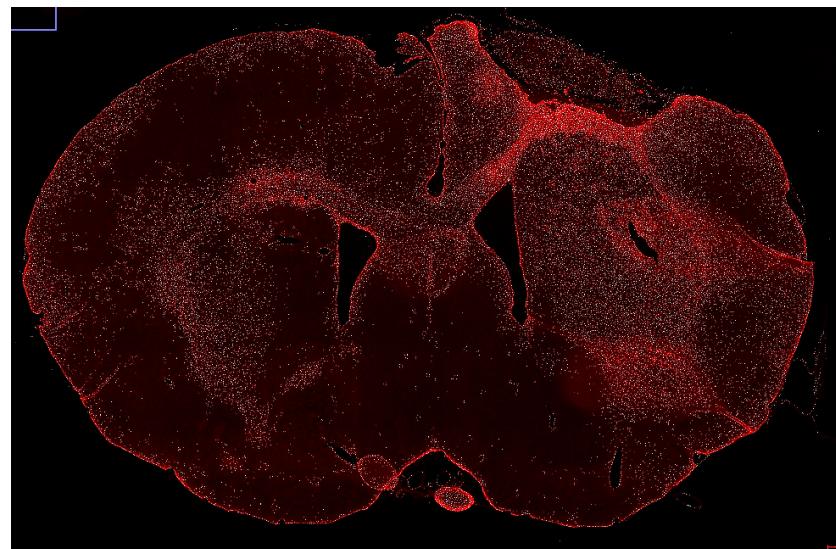


Figure 1: Example microscopy image (red channel): white spots indicate results of cell counting.

1 Download & Install

1. Download the zip-File `AIDAhisto-master` using the following [Link](#).
After opening the web page, the zip-file can be downloaded (Figure 2).

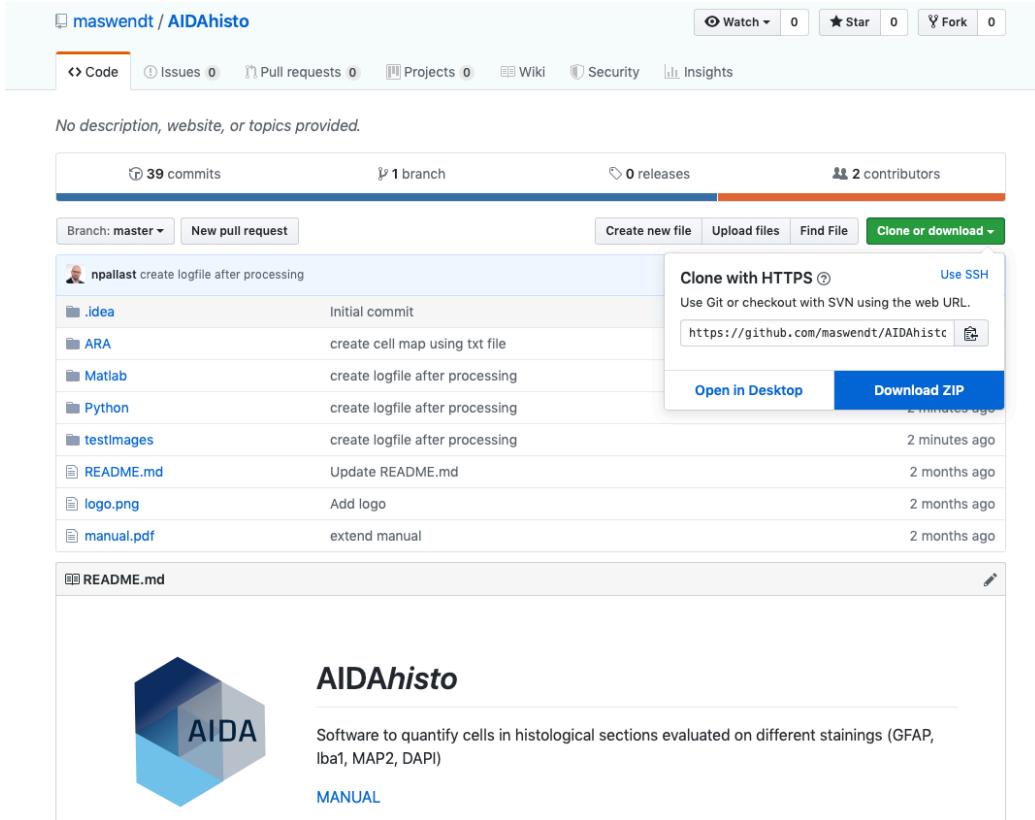


Figure 2: Click on `Clone or Download` → `Download ZIP` to download the zip-File.

2. Download & Install Python 3.6 or higher using [Anaconda](#) and enter the following command to install all necessary Python packages
`pip install numpy=1.14.3 argparse=1.4.0 scipy=1.2.1 matplotlib=3.0.3`
3. The code was also implemented in Matlab (tested with version R2018a).
Note: Matlab processing is faster and visualization better compared to Python; otherwise there are no differences.

2 Atlas Transformation

In the provided example files in the folder `.../AIDAhist-master/testImages/wholebrain_atlas.` the transformed atlas slice is already included. In order to perform the atlas transformation, conduct the following steps:

1. Load ARA with highest resolution ($10\mu m$) from the folder
`.../AIDAhisto-master/ARA/annotation_10.nii.gz`
2. Load microscopy image in ImageJ from the folder
`.../AIDAhisto-master/testImages/wholebrain_slice.tif`
3. Select the matching slice in the ARA and make a substack with ImageJ:
Image → Stacks → Tools → Make Substack
4. Choose the corresponding slice number of the ARA and save the substack.
5. Choose "Multi-point" in ImageJ and place 20-40 landmarks in the microscopy image and the corresponding positions in the ARA (Figure 3). Note: that step requires some experience in mouse brain anatomy to match in atlas landmarks with the microscopy - especially if like in this example the mouse brain tissue is strongly deformed due to a local brain lesion.

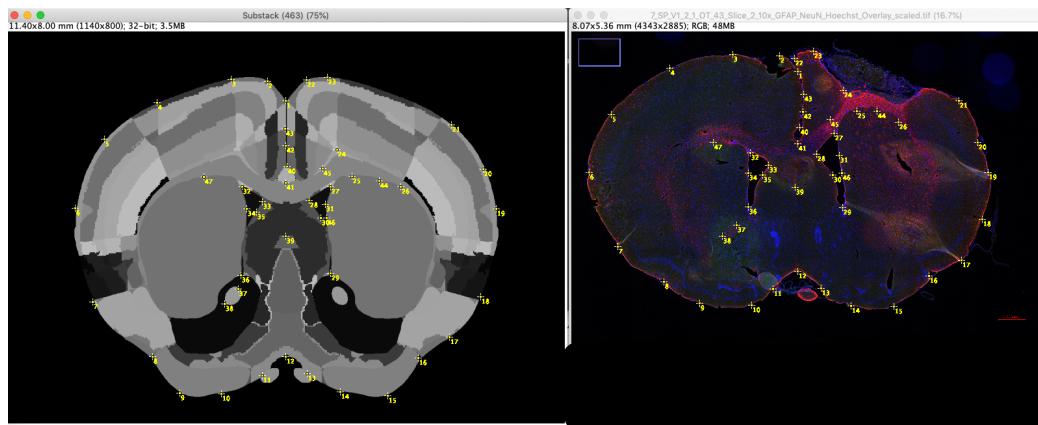


Figure 3: 30-50 landmarks in the microscopy image and the corresponding positions in the ARA

6. Landmark registration with ImageJ: Plugins → Transform → Landmark Correspondences
7. Choose the substack of the ARA as "source image" and the microscopy image as template image in the transform menu
8. Choose: Transformation method: Moving Least Squares (non-linear), Transformation class: Affine, No interpolation (Figure 4).

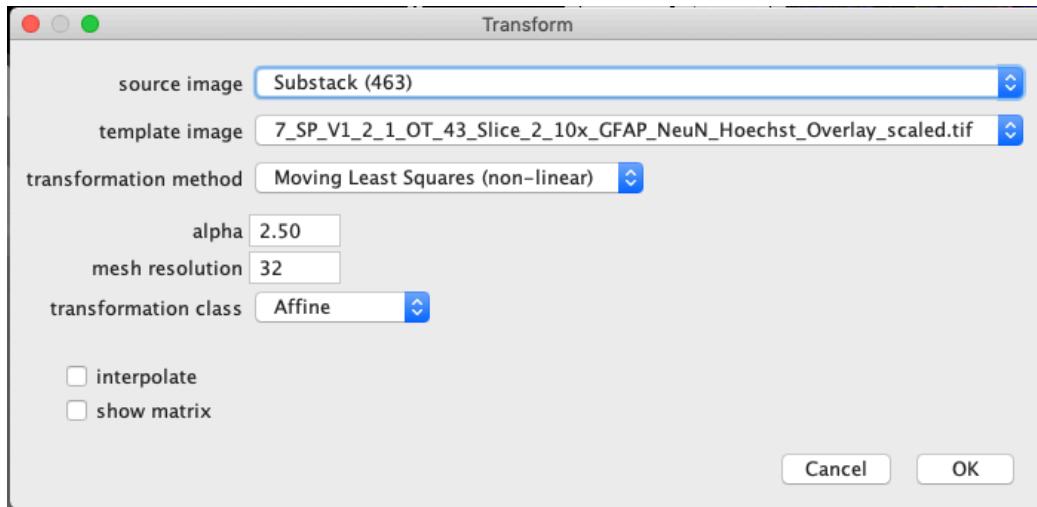


Figure 4: Choose the shown tranformation parameters to register the atlas with the microscopy image

3 Count Cells

1. Count all cells in the given image with Matlab: Here, you have to open Matlab and set `.../AIDAhisto-master/Matlab` as "Current Folder" (Figure 5A). Type the same command in the "Command Window" (see Figure 5B) like shown in figure 6.

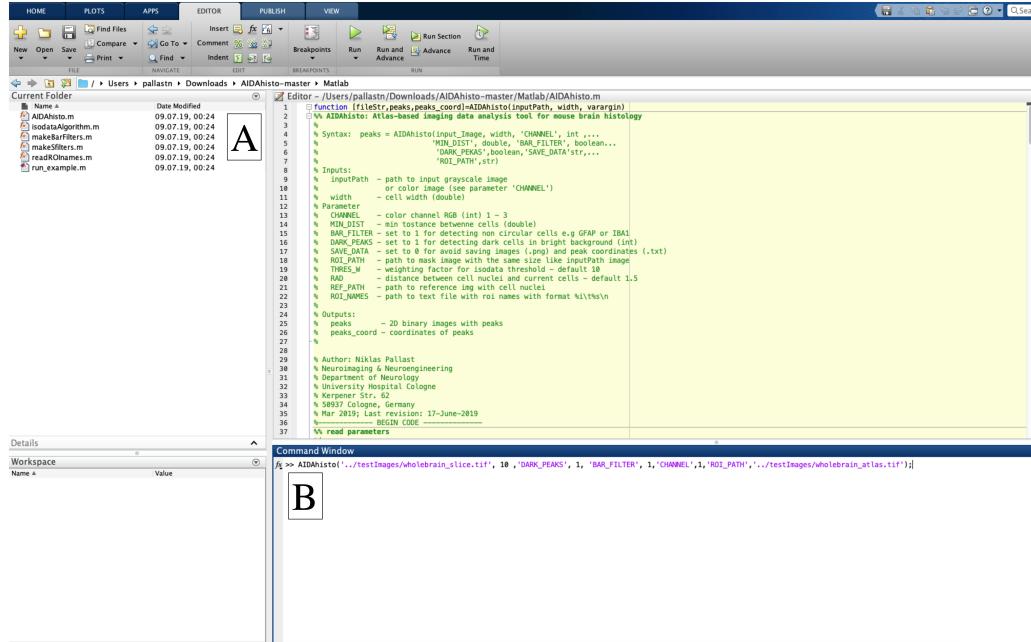


Figure 5: Set the `.../AIDAhisto-master/Matlab` as "Current Folder" (A) and type the command of figure 6 in "Command Window" (B)

```
f1 >> AIDAhisto(''..//testImages/wholebrain_slice.tif'', 10 , 'DARK_PEAKS' , 1, 'BAR_FILTER' , 1,'CHANNEL',1,'ROI_PATH','..//testImages/wholebrain_atlas.tif');
```

Figure 6: Type the shown command in the "Command Window"

2. Count all cells in the given image with Python: Open the Command Prompt if you use a Windows System or Terminal if you use a Macintosh System. Set `AIDAhisto-master/Python` as your current folder using the command `cd` (see figure 7)

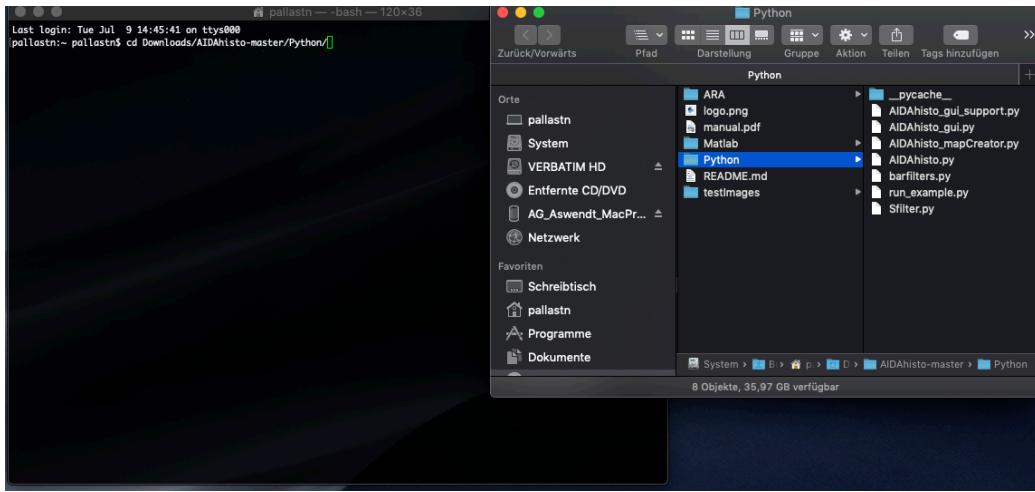


Figure 7: Set the Python folder as your current folder using the command `cd`

3. Type (not copy) the following command in the command window.

```
python AIDAhisto.py ../testImages/wholebrain_slice.tif
-f -w 9 -d -c 0 -a ../testImages/wholebrain_atlas.tif
-l ../ARA/acronyms_ARA.txt
```

4. The results are stored in the folder `AIDAhisto-master/testImages` and can be easily overlaid in ImageJ like described [here](#). Note: the counted cells will be represented by a single white pixel, which might be difficult to see in the overlay. Try to use the dilate function in ImageJ on a binarized version of the counting result.

4 Results

After successful completion of the previous steps you will end up with two text files and an image file:

- **wholebrain_slice_ch1_cC.tif**: A binary image which can be overlaid with the related red channel of the original image to visualise the result.
- **wholebrain_slice_ch1_cC.txt**: A text file which provides the number of identified cells and the x-y-coordinates of each cell position.

- **wholebrain_slice_ch1_cCROIs.txt** A text file with three columns. In the first column contains the pixel value of each region in the given atlas. The second column contains the acronyms of ARA. The third column contains the number of identified cells for each region.