

Example: Atlas-based imaging data analysis  
tool at histological resolutions  
AIDAhisto

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Conduct the following steps to count all cells for the red channel of a whole brain slice like shown in figure 1.

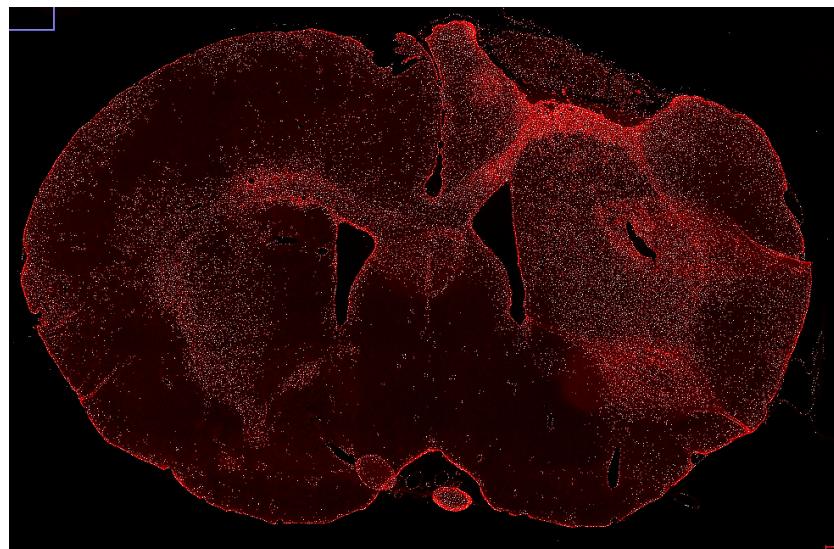


Figure 1: Example image overlaid with white cell count

# 1 Downaload & Install

1. Download the zip-File `AIDAhisto-master` using the following [Link](#).

After opening the web page, the zip-file can be downloaded as shown in the figure 2.

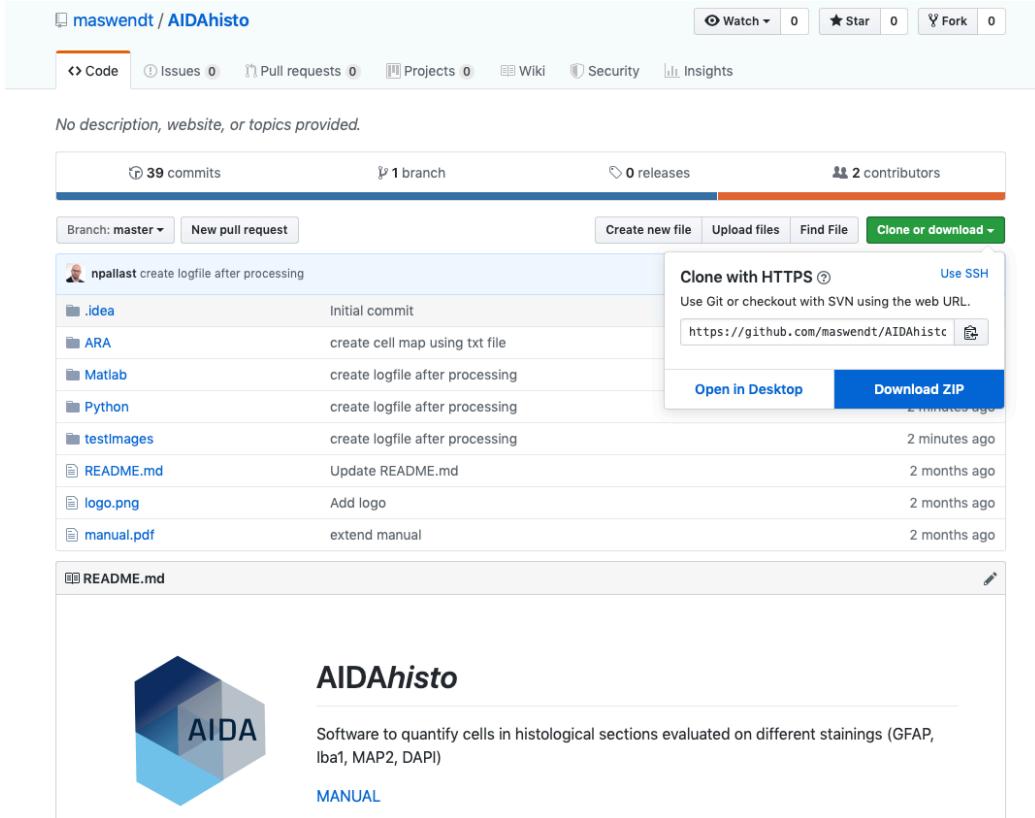


Figure 2: Click on **Clone or Download** → **Download ZIP** to download the zip-File.

2. Download & Install Python 3.6 or higher using [Anaconda](#) and enter the command to install necessary packages

```
pip install numpy=1.14.3 argparse=1.4.0 scipy=1.2.1 matplotlib=3.0.3
```

3. Otherwise a current version of Matlab is required.

## 2 Atlas Transformation

For the present example the transformed atlas slide is already available in the folder `.../AIDAhist-master/testImages/wholebrain_atlas.tif`. Nevertheless, to transform the atlas conduct 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 30-50 landmarks in the microscopy image and the corresponding positions in the ARA (see figure 3)

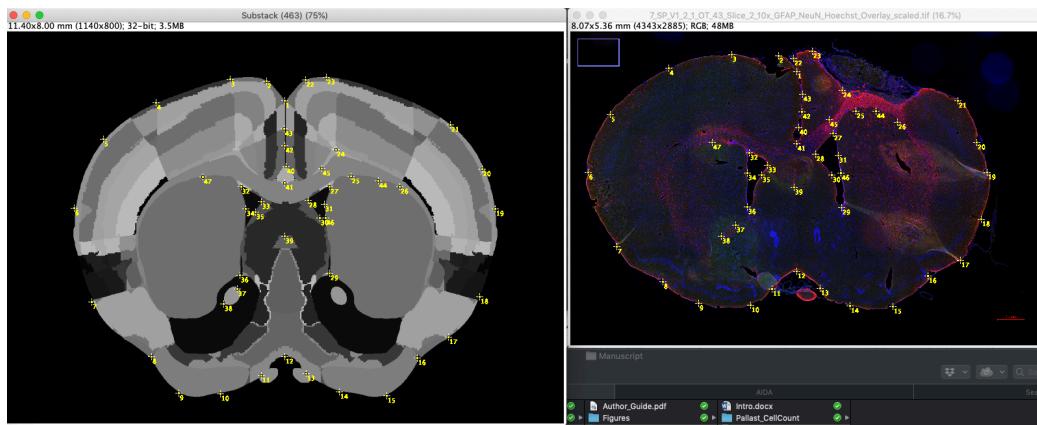


Figure 3: 30-50 landmarks in the microscopy image and the corresponding positions in the ARA

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 (see figure 4)

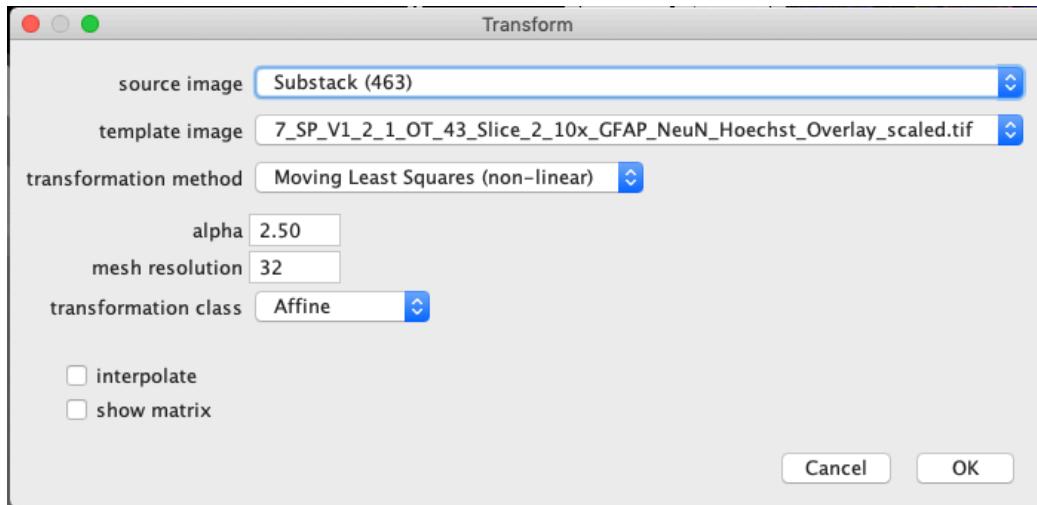


Figure 4: Choose the shown transformation parameter to register the atlas with the microscopy image

### 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" (see figure 5A). Type the same command in the "Command Window" (see figure 5B) like shown in figure 6.

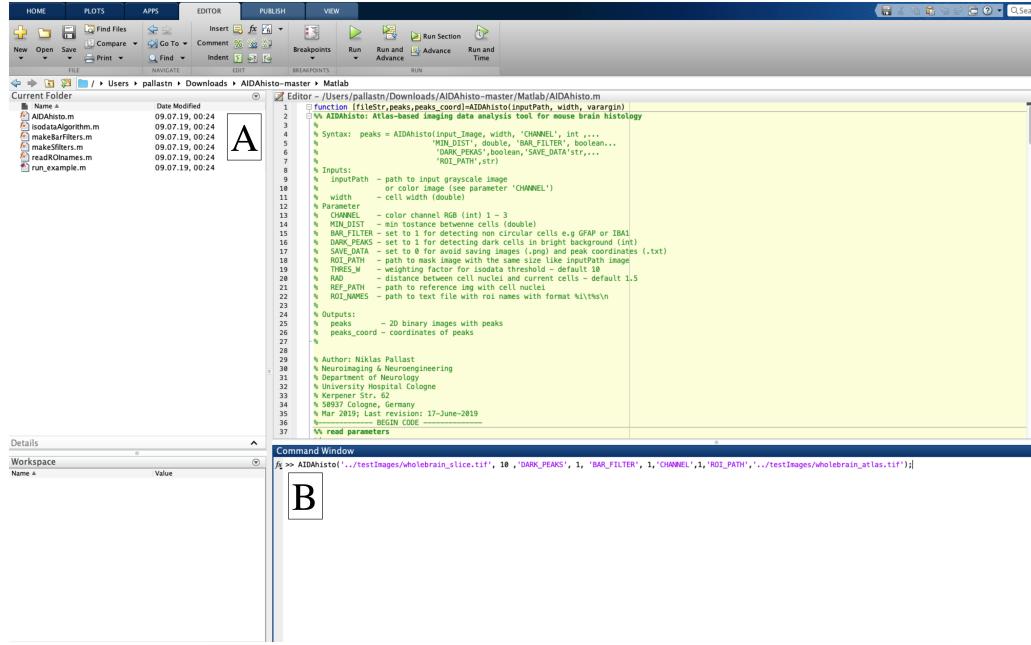


Figure 5: Set the `.../AIDAhisto-master/Matlab` as "Current Folder" (A) and type the command of figure 6 in "Command Window" (B)

```
fct =>> 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 Open 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)

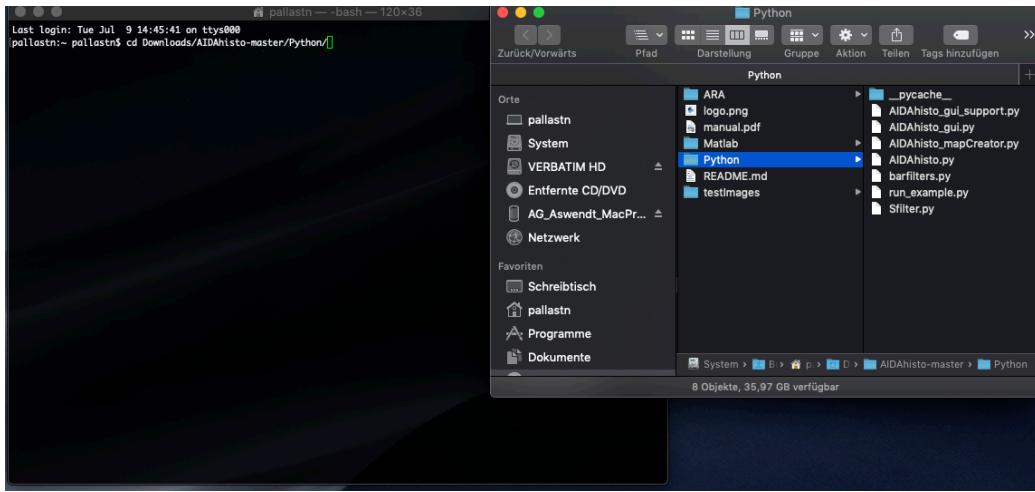


Figure 7: Set the Python folder as your current folder using the command `cd`

3. Type 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 easily overlaid in ImageJ like described [here](#).