

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

AIDAhisto

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1 Introduction

AIDAhisto provides accurate and fast results for cell nuclei as well as immunohistochemical stainings of neurons, astrocytes and immune cells in the mouse brain with respect to the associated regions of the Allen Brain Reference Atlas (ARA). The transformation between the atlas as a source and the brain slice as a target image was conducted by a landmark based registration.

2 Download & Install

1. 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==1.1.0 matplotlib==3.0.3`
2. The code is also implemented in **Matlab** (tested with version R2018a).
Note: Matlab processing is faster and visualization better compared to Python; otherwise there are no differences.
3. Download the zip-File **AIDAhisto-master** using the following [Link](#). After opening the web page, the zip-file can be downloaded (Figure 1).

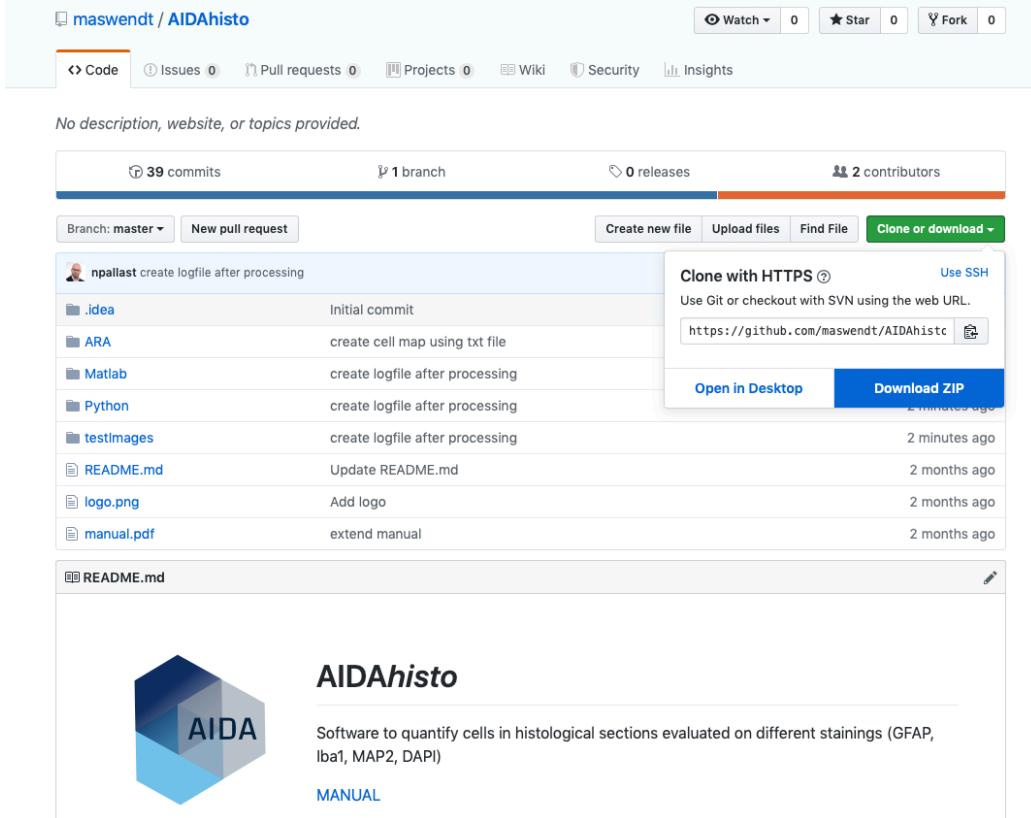


Figure 1: Click on **Clone or Download** → **Download ZIP** to download the zip-File.

In the following, the content of each folder is explained exactly:

ARA Contains the atlas in 10um and 50um with parental and original regions. Each atlas is also in a split version to distinguish the cell count between the left and right hemisphere. Furthermore, the acronyms are included that correspond to those of the Allen Brain Institute

Matlab Contains the Matlab code with the appropriate program call `AIDAhisto.m` and `run_example.m`

Python Contains the Matlab code with the appropriate program call `AIDAhisto.m` and `run_example.m`

testImages Contains sample images of different staining (GFAP, IBA1) with different resolutions. There is also an already transformed atlas in original version `wholebrain_atlas.tif` and split version `wholebrain_atlas_splitted.tif` which is overlaid with the brain slice `wholebrain_slice.tif`. The results after the processing are described in detail in the manual.pdf. Those who process these images are listed in the logfile `wholebrain_slice.tif_process.log`

3 Atlas Transformation

In this example we conduct the registration process using a simple landmark-based registration with imageJ as described below. For more information follow the link: [ImageJ Landmark Correspondences](#). Other tools allow even stronger transformations and thus facilitate the choice of the correct layer in Atlas. These tools are listed below, but are not described in detail

- [BigStitcher](#)
- [QuickNII Tool](#)

The transformed atlas slice (`wholebrain_atlas.tif`) is already included in the folder `.../AIDAhist-master/testImages/`. 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` and open the image with ImageJ
2. At first a black picture appears because the color space is not scaled correctly. To adjust the color space follow these steps:
 - Select slide 855
 - Open the B&C Tool with Image → Adjust → Brightness/Color
 - Click Auto
3. Load microscopy image in ImageJ from the folder `.../AIDAhisto-master/testImages/wholebrain_slice.tif`
4. Select the matching slice in the ARA and make a substack with ImageJ: Image → Stacks → Tools → Make Substack

5. Chose the corresponding slice number of the ARA and save the substack.
6. Choose "Multi-point" in ImageJ and place 20-40 landmarks in the microscopy image and the corresponding positions in the ARA (Figure 2). 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.

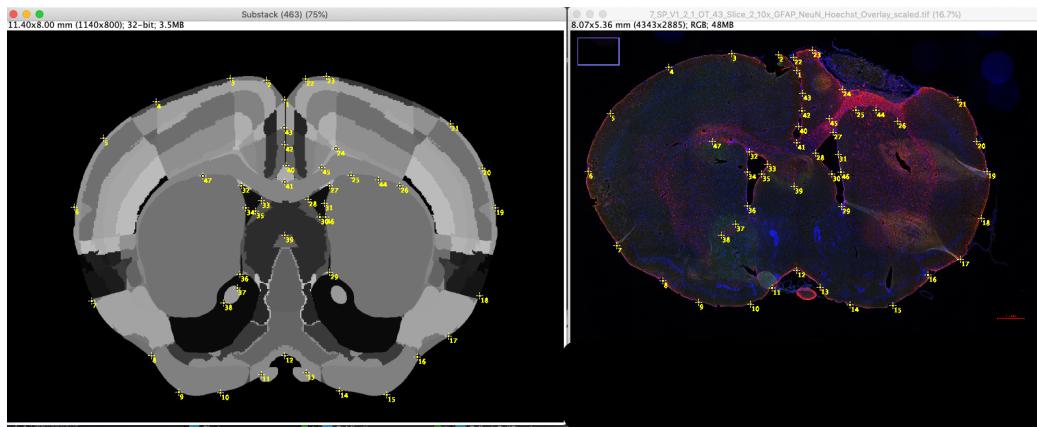


Figure 2: 30-50 landmarks in the microscopy image and the corresponding positions in the ARA

7. Landmark registration with ImageJ: Plugins → Transform → Landmark Correspondences
8. Choose the substack of the ARA as "source image" and the microscopy image as template image in the transform menu
9. Choose: Transformation method: Moving Least Squares (non-linear), Transformation class: Affine, No interpolation (Figure 3).

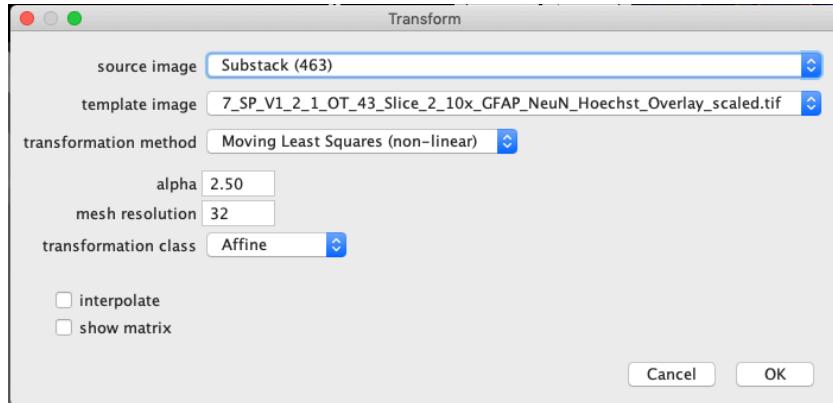


Figure 3: Choose the shown transformation parameters to register the atlas with the microscopy image

4 Count Cells

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.

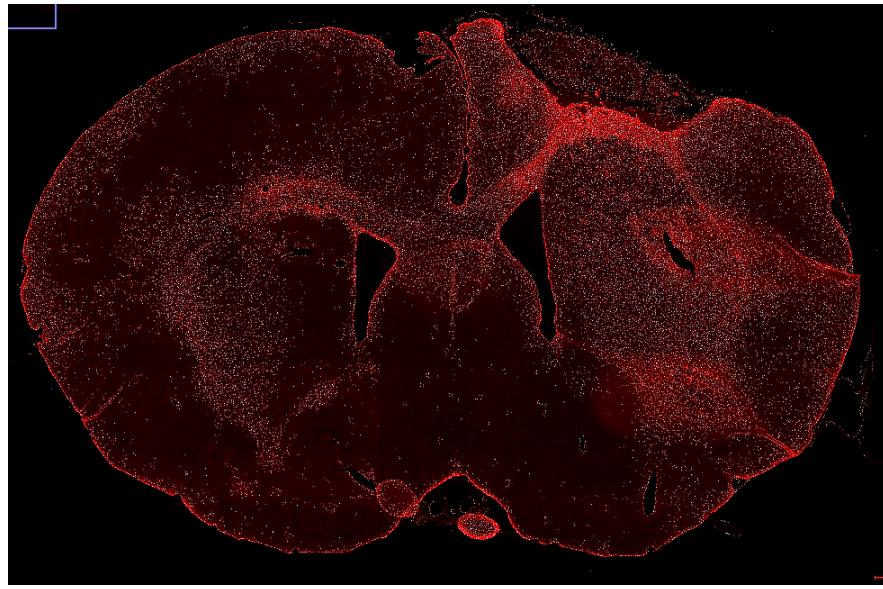


Figure 4: Example microscopy image (red channel): white spots indicate results of cell counting.

- 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.

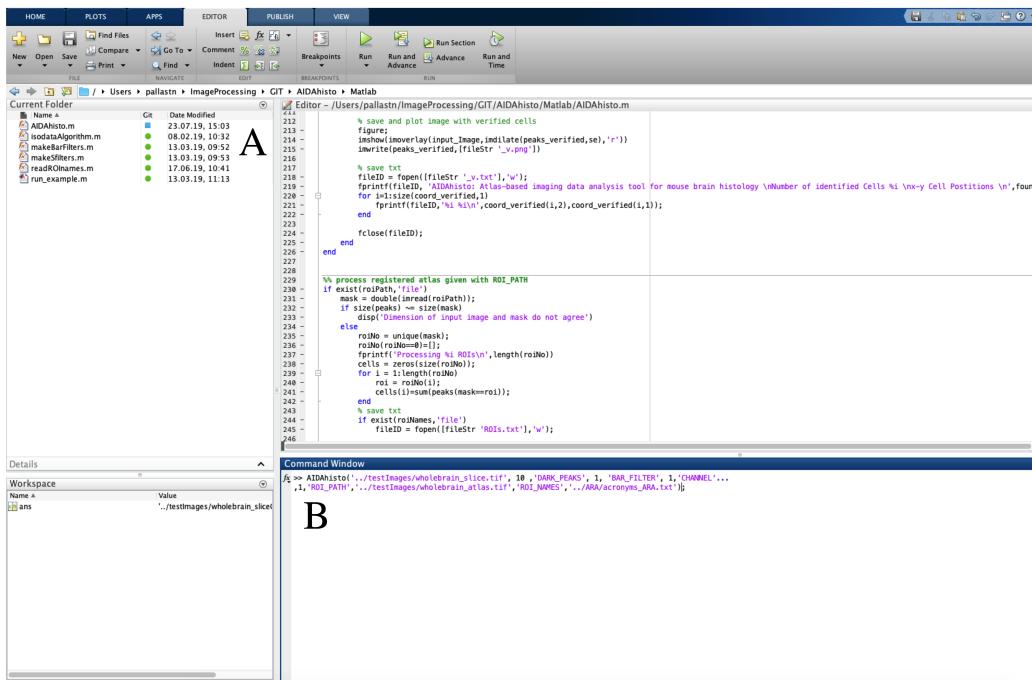


Figure 5: Set the `.../AIDAhisto-master/Matlab` as "Current Folder" (A) and type the command of figure 6 in "Command Window" (B)

Command Window

```

f>> AIDAhisto('..\\testImages\\wholebrain_slice.tif', 10 , 'DARK_PEAKS', 1, 'BAR_FILTER', 1,'CHANNEL',...
    ,1, 'ROI_PATH','..\\testImages\\wholebrain_atlas.tif','ROI_NAMES','..\\ARA\\acronyms_ARA.txt');

```

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

- 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)

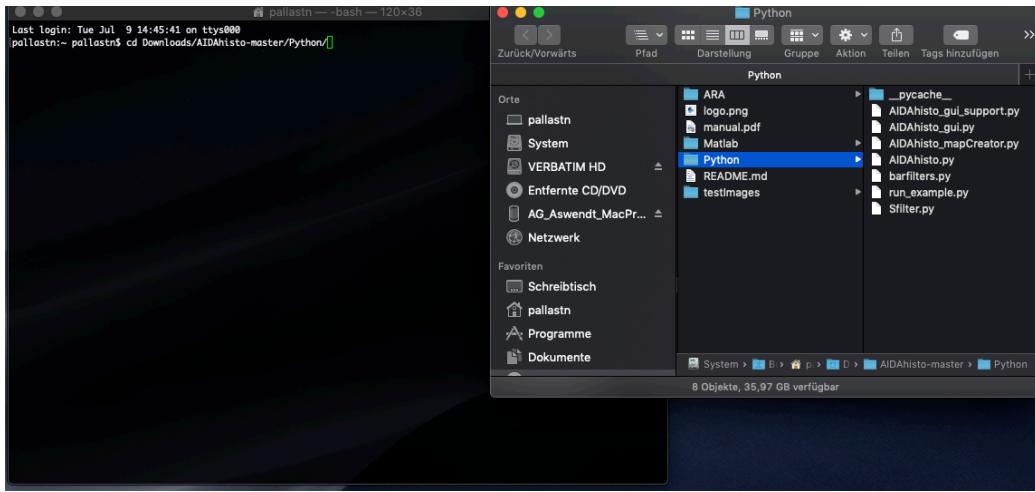


Figure 7: Set the Python folder as your current folder using the command `cd`

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

```
python AIDAhistro.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 `AIDAhistro-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.

5 Results

After successful completion of the previous steps you will end up with two text files (see Fig. 8) 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.

```

Number of detected cells: 31053
cell positions (xy):
68 2441
73 2436
106 2419
129 2421
131 2189
135 2292
136 2195
138 2211
139 2163
140 2339
144 2225
144 2258
145 2219
146 2259
147 2459
148 2240
148 2423
149 2266
150 2145
150 2153
151 2444
152 2431
153 2442
156 2243
157 2459
158 2511
160 2236

```

(a) wholebrain_slice_ch1_cC.txt

Region	Pixel Value	Count
SSp-m6b	74	1
119	14	1
36	GU1	152
72	ADP	4
81	VL	111
117	och	129
120	ADP1	152
129	V3	8
148	GU4	49
163	ATp2/3	98
180	OTp2/3	191
187	GU5	34
211	ACAd2/3	213
226	LPO	16
232	VL	71
258	LSr	871
263	AVP	8
272	AVPV	3
296	ACAv2/3	229
297	NW	43
314	ATp6a	17
320	MOp1	394
342	SI	74
344	ATp5	27
351	BS7	172
450	SSp-u1	236
452	MEPO	6
477	STR	473

(b) wholebrain_slice_ch1_cCROIs.txt

Figure 8: a) The first text file contains the number of all identified cells with x-y-positions in the input image. b) The second text file contains acronyms and pixel values of the given ARA with related cell number.

- **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.

6 Usage of AIDAhistro GUI with Python

With the implementation in Python, a generic user interface has been implemented to support the arrival of AIDAhistro. To start the GUI set /Python as your current folder in the command window of your System and open the GUI (see 9) by typing `python AIDAhistro.gui.py`.

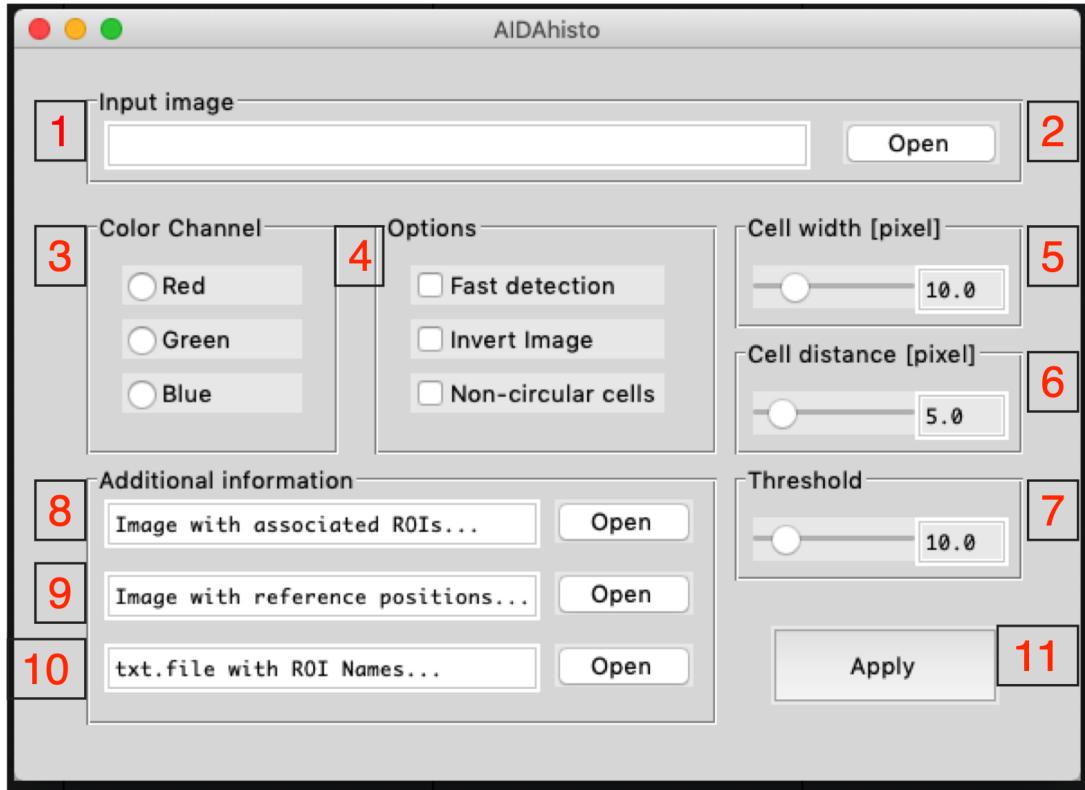


Figure 9: Description of the user interface to process with all provided input parameters.

The user only can choose an image file and adapt the cell width (5) to run AIDAhisto, but we also provide some parameters to optimize the output and to meet all individual requirements of manifold investigations. Therefore, the following is a detailed explanation of the numbering in Figure 9:

1. Path the the file of the input image with the postfix .jpeg, png, tiff
2. Press button to open file dialog and select a image file
3. Choose the color channel that should be evaluated. If only one channel is present, the first channel will always be examined.
4. • For very large images, the process can be accelerated by choosing *Fast detection*

- If the cells are dark and the background is bright, the image should be inverted by choosing *Invert image*.
 - If the cells are not round and have a different shape choose *Non-circular cells*.
5. Choose the cell size in pixels.
 6. The minimum cell distance is pre-setted but can also be adapted.
 7. The automatically calculated threshold value can be adjusted and weighted here.
 8. If regions are superimposed with the image, the region image can be selected here.
 9. You can enter the image of a previous investigation here and take it as a reference.
 10. If certain names should be noted in the output file instead of the pixel value, the name of the respective pixel value can be entered here as a text file.

7 Allen Mouse Brain Atlas Database

In order to simplify the search for specific region numbers (e.g. 582 Caudoputamen) and list the related parental and child regions, we have summarized the **ARA data available** from (©2017 Allen Institute for Brain Science. Allen Mouse Brain Atlas (ccf3)) in an online database software, which we are using also for managing research data <https://doi.org/10.1093/database/bay12>. To use the database, create an account here <https://ninoxdb.de/de/templates/research> and import via the "Import archive" function this file.