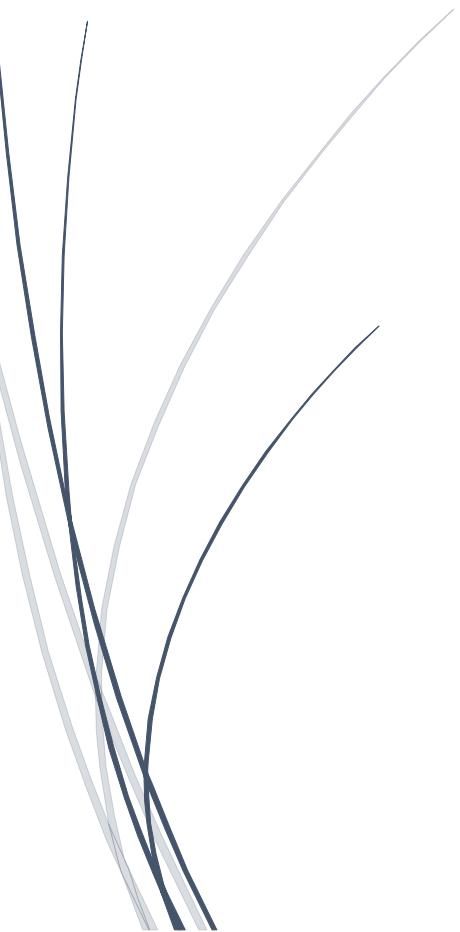




# DMC-BrainMap

User guide



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## Introduction

This is a guide on how to install and use the DMC-BrainMap plugin. DMC-BrainMap makes it possible to analyze your data from processing raw images to visualization without the need for coding or editing scripts. This user guide will guide you through all possible tools of the plugin and give examples for cell/projection segmentation, estimation of optic fiber/neuropixels probes and analysis of injection sites as well as how to incorporate spatial transcriptomics data into the workflow. Often, the commands are very similar for different kinds of analysis and the tools in the plugin are self-explanatory when you hover over the text for the input information. There are different points where you can import pre-analyzed data into the plugin, or export data to use it outside of the plugin, which are described as well.

For installing and opening the plugin, commands should be given through command/anaconda prompt. These commands are highlighted in gray. Command/anaconda prompt can easily be opened through the search bar on your computer.

The values and parameters used in this user guide are based on images of coronal sections in mice from a fluorescent microscope. This could not be ideal for your data, so we advise first to find appropriate parameters before doing any large batch processing.

Step 1 and 4 explain how to install napari and the plugin, this should be only performed once and can be skipped afterwards.

## STEP 1: Installing napari

The plugin runs in napari, which is a Python library for n-dimensional image visualization, annotation, and analysis. The first thing you need to do is to install napari (<https://napari.org/stable/>). We recommend using conda for it, but using venv should also be fine. If you don't have anaconda/miniconda pre-installed, this needs to be installed as well (read <https://www.anaconda.com/> or <https://docs.anaconda.com/> for more information).

Using command prompt/anaconda prompt:

```
>> conda create -y -n napari-env -c conda-forge python=3.9  
>> conda activate napari-env  
>> pip install "napari[all]"
```

You can also name the 'napari-env' differently if this would be more convenient and to avoid dependency issues with other napari-plugins. This step should only be performed once to install napari.

## STEP 2: Starting napari

1. Open command prompt and enter:

```
>> conda activate napari-env
```

2. You now work inside of a napari-env and your command prompt should look like this: (depending on users and folder you want to work in this can look a bit different)

```
Microsoft Windows [Version 10.0.22621.1702]
(c) Microsoft Corporation. All rights reserved.

C:\Users\sabin>conda activate napari-env

(napari-env) C:\Users\sabin>
```

3. Enter:

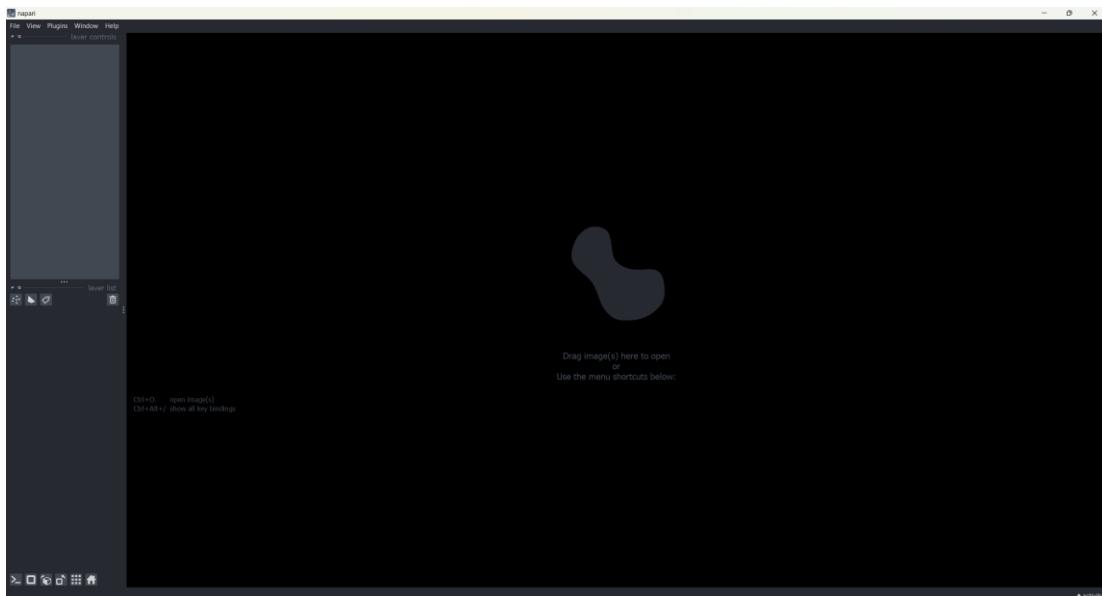
```
>> napari
```

4. Napari should now open

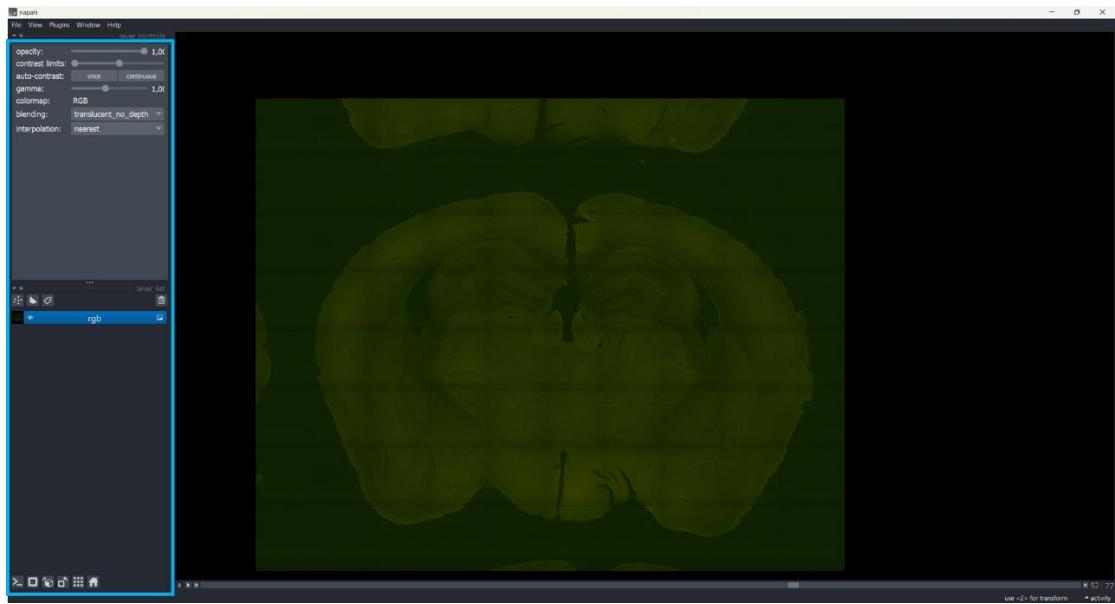
### STEP 3: Short introduction to napari

Here, we shortly describe the main tools in napari. We only describe the tools that are useful in combination with our plugin. Please visit the napari website (<https://napari.org/stable/>) for more information on and other possibilities in napari.

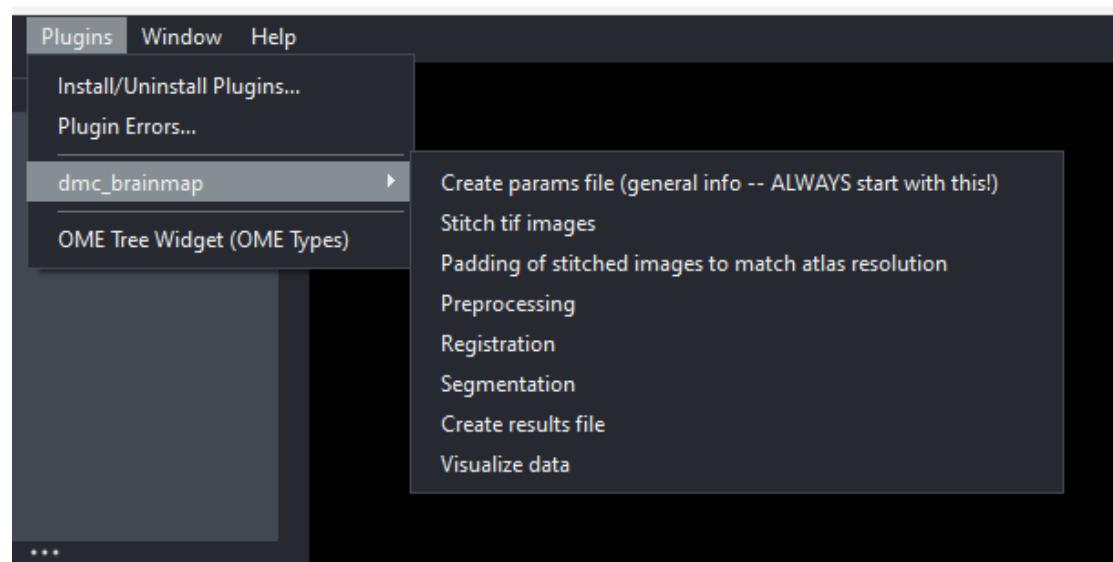
1. This is how the home screen of napari looks like when you execute step 2:



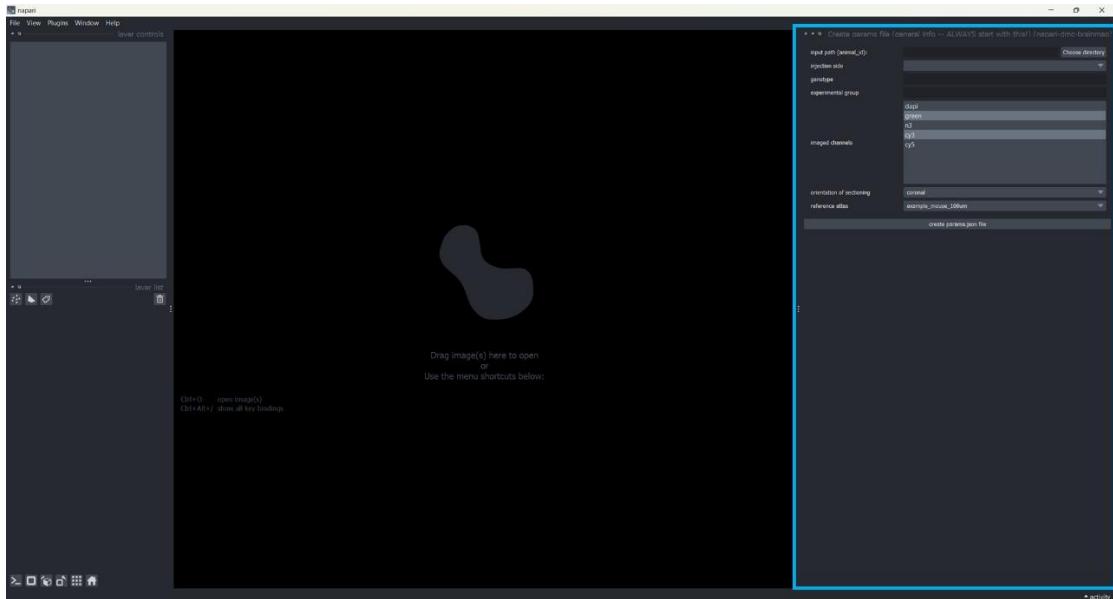
2. If you want to inspect images, you can drag an image (folder) into the middle screen with the napari logo.
3. The panel on the left gives you different options for your images.
  - a. You can select the layer and change opacity/contrasts...
  - b. By right clicking on the displayed layer, you can split RGB into different layers, duplicate layers...



4. In the left upper corner, you find different buttons. Most important is the “Plugins” button: if you click on Plugins, there will be a drop-down menu with all installed plugins. Via this way you will navigate through all the tools of the DMC-BrainMap plugin (after installation step 4).



- When opening a plugin it will appear on the right side of the screen.



- Often, when hovering over the text of the different tools, you get more information about the input you need to provide.

## STEP 4A: Installing the DMC-BrainMap plugin through github

- Download the plugin codes from github (<https://github.com/hejDMC/napari-dmc-brainmap>) by opening a terminal (and navigating to the installation destination) and entering:

```
>> git clone https://github.com/hejDMC/napari-dmc-brainmap
```

- Install the plugin to your napari version. Activate your napari environment (as in step 1) and change your directory into the napari-dmc-brainmap folder and run the installation command

```
>> conda activate napari-env
>> cd >> INSERT napari-dmc-brainmap DIRECTORY HERE <<
>> pip install -e .
```

- This is an example on how it looks on a windows computer:

```
Microsoft Windows [Version 10.0.22631.3447]
(c) Microsoft Corporation. All rights reserved.

C:\Users\sabin>conda activate napari-env

(napari-env) C:\Users\sabin>cd desktop\napari-dmc-brainmap

(napari-env) C:\Users\sabin\Desktop\napari-dmc-brainmap>pip install -e
```

In the first line, we activate the napari environment. You see the name of the environment in the brackets in the beginning of the second line, indicating that the environment is now active.

The current directory (where you want to install the plugin) is shown before the ">" sign and afterwards follows the command)

## STEP 4B: Installing the DMC-BrainMap plugin through napari (to be implemented)

1. NOTE: this option is currently not available
2. Open command prompt and activate the napari-env:

```
>> conda activate napari-env
```

3. You now work inside of a napari-env and your command prompt should look like this: (depending on users and folder you want to work in this can look a bit different)

```
Microsoft Windows [Version 10.0.22621.1702]
(c) Microsoft Corporation. All rights reserved.

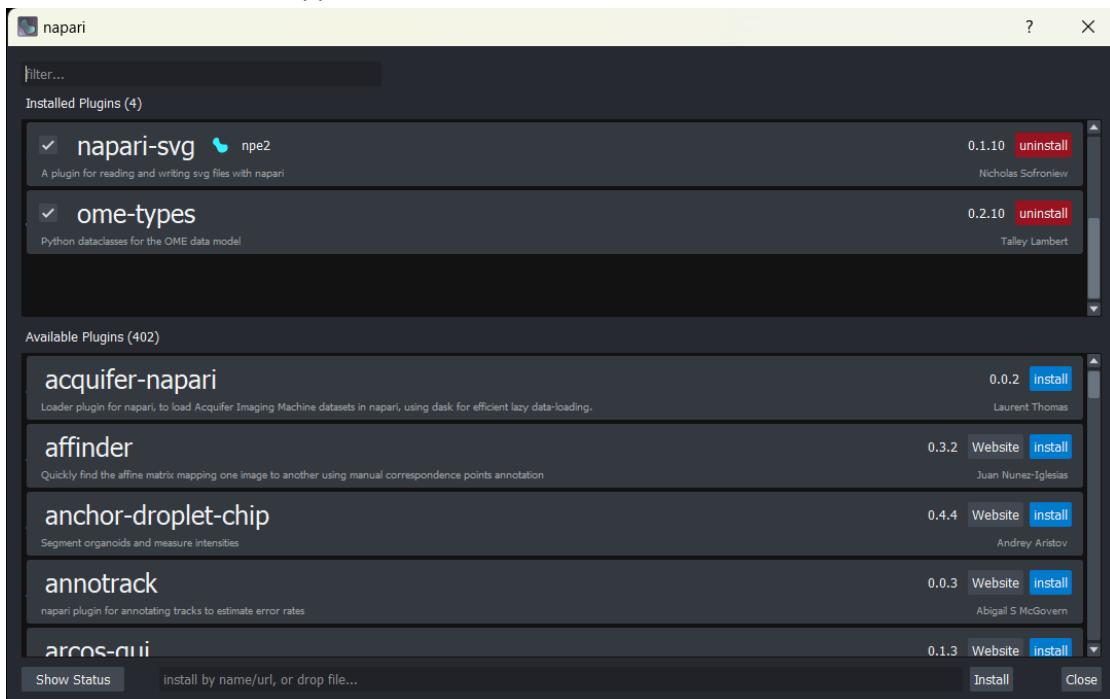
C:\Users\sabin>conda activate napari-env

(napari-env) C:\Users\sabin>
```

4. Launch napari by entering:

```
>> napari
```

5. Go to Plugins and click on install\uninstall plugin
6. The next screen should appear:

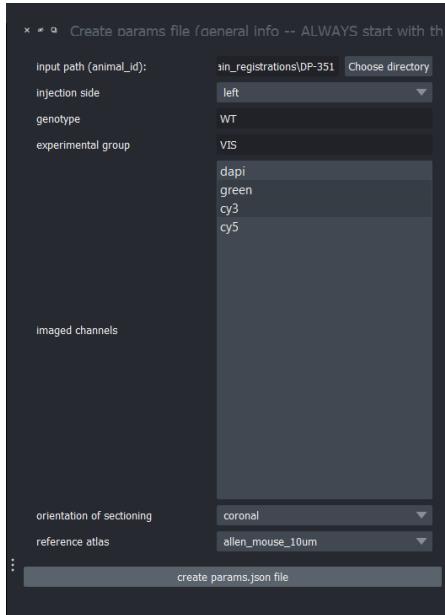


7. Search for the DMC-BrainMap plugin and press install.
8. After installation is completed, close and reopen napari.
9. The plugin is now ready to be used.
10. This step should only be performed once to install DMC-BrainMap.

## STEP 5: Making a params.json file – always the first step

Before being able to process or analyze your images you need to make a params file which holds all important information about the histology data of this animal. In the first step, you add all basic information and when performing different functions throughout the plugin, the information will be added automatically (for example the parameters used for making RGB images).

1. Launch the params tool by pressing **Plugins>dmc\_brainmap>Create params file**.
2. The panel on the right should look like this:



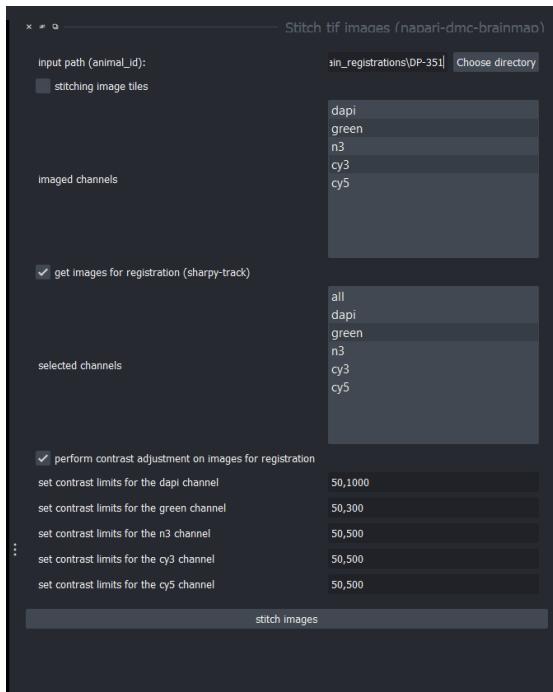
For genotype, injection side and experimental group you can fill in the info you want. Important! You need to provide the channel, orientation, and atlas info to continue. Even if you don't want to register, still give the atlas info.

3. Press 'create params.json file'
4. The params.json file should now be created in the animal folder.
5. The params.json file will automatically be updated after every processing step you complete in napari.

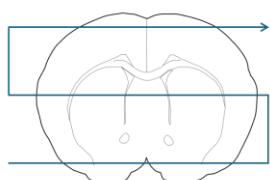
## STEP 6: Stitching .tif images (optional)

This tool stitches images acquired with DMC-FluoImager (<https://github.com/hejDMC/napari-dmc-fluoimager>). In case you acquired images with another microscope, please go to step 7: padding of stitched images.

1. The raw data should have the format described in Appendix 1.
2. Launch the stitching tool by pressing **Plugins>dmc\_brainmap>Stitch tif images**
3. The panel on the right should look like this:



- 4.
5. Choose the right data folder, channel for registration, parameters...
6. Leave box for stitching image tiles unchecked when automatic scanning with DMC-FluoImager.
7. If you haven't used the DMC-FluoImager, but acquired individual tiles, check the box for stitching imaging tiles.
  - a. The data should be stored in a logical way (see Appendix 1). Be aware the stitching



will happen in the following way:

- b. Make sure you have also imaged your slices in the same orientation and the individual image tiles have a dimension of [2048,2048].
8. If you want to register the brain in the plugin, check the box for sharp\_y\_track images and choose the channel(s) for registration. You only need to register one channel and this info can be used for segmentation of all channels.
9. In command prompt you can follow the progress of the stitching (and downsampling).
10. When finished you should have the following inside the animal folder (Appendix 2):
  - a. Stitched folder
  - b. (Sharp\_y track folder with inside separate folders for the channels you selected.)
  - c. image\_names.csv file with the image names connected to the number of the images for the registration later (Appendix 3).
11. You can skip step 7: padding of stitched images since these images are already padded for atlas dimension aspect ratio.

## STEP 7: Padding of stitched images to match atlas resolution

This step can be skipped for images stitched in the DMC-BrainMap plugin.

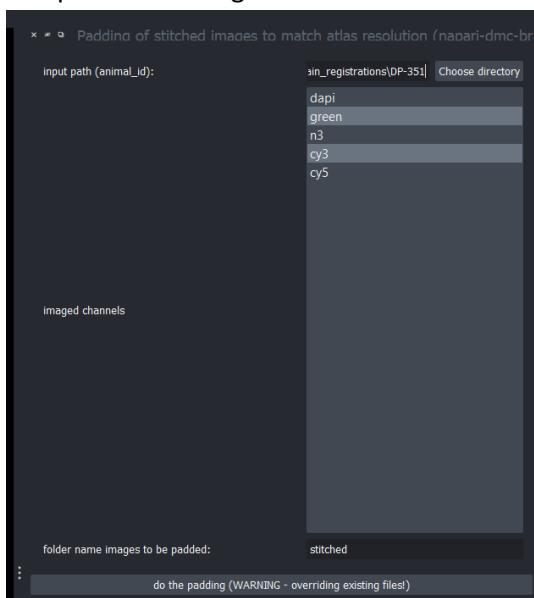
In general the DMC-BrainMap requires:

- 16-bit
- single channel
- grayscale
- .tif format

Furthermore, they must be in a folder with format: animal\_id\stitched\channel\_name

The channel names should be: dapi, green, n3, cy3 or cy5.

1. Launch the padding tool by pressing **Plugins>dmc\_brainmap>Padding** of stitched images to match atlas resolution.
2. The panel on the right should look like this:



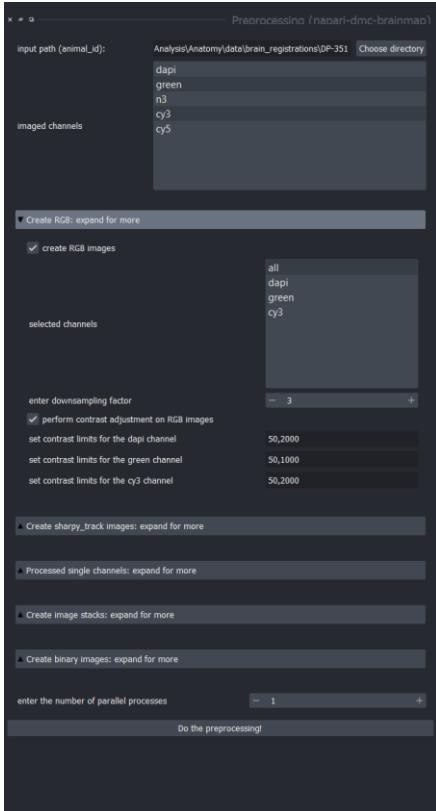
3. Press: 'do the padding'
4. In command prompt you can follow the progress.
5. Warning, this will overwrite your images. If you want to keep the original images, make a copy.
6. You will find the stitched images in the original folder and can use them for all following steps.
7. An image\_names file will be created (Appendix 3).

## STEP 8: Preprocessing

Here you can do different types of preprocessing: making RGB images, sharp-track images (needed for registration!), single channel downsampled images, image stacks, binary images... all functions work the same as the RGB example described below.

1. Launch the preprocessing tool by pressing **Plugins>dmc\_brainmap>preprocessing**

2. The panel on the right should look like this:

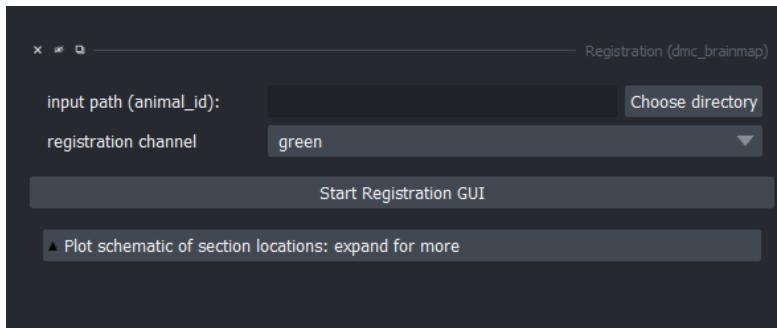


3. For every function you want to execute **you need to check the box**.
4. Select the imaged channels.
5. Select the channels you want for the RGB images (in case you imaged less channels, you can still select 'all' and RGB images will be created with only these channels).
6. Enter the downsampling factor (default 3)
7. Press: 'Do the preprocessing!'
8. In Command prompt you can follow the progress of the preprocessing.
9. You should now have a `rgb` folder in the animal folder (Appendix 2).

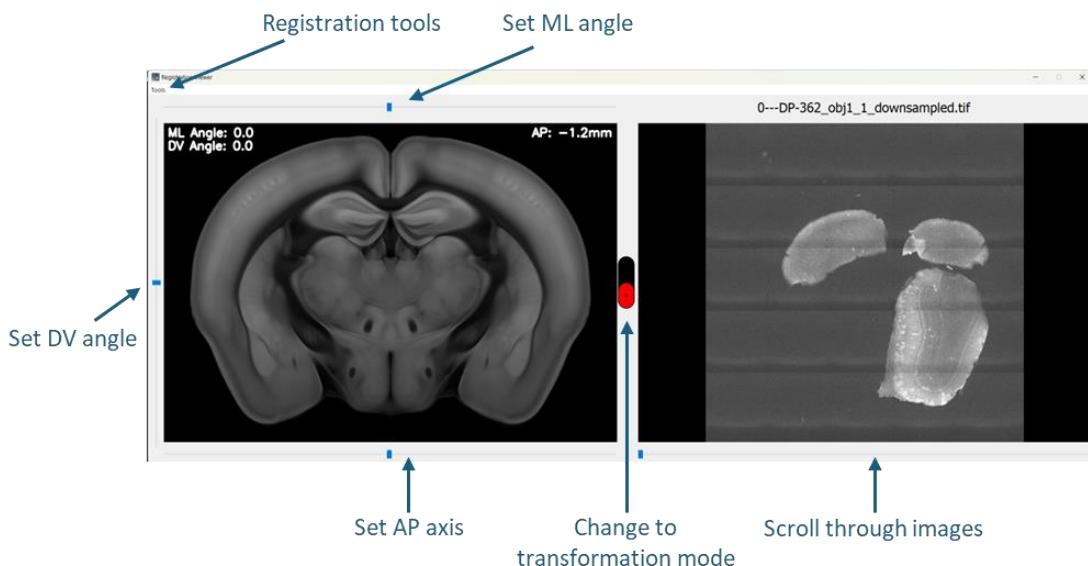
## STEP 9: Registration using SHARPy-track

For registrations, DMC-BrainMap uses its own tool called ***SHARPy-track***. Below, you find a short overview of how to use SHARPy-track. The atlas and orientation of sectioning you want to use is defined in the params file, here we will use the coronal allen\_mouse\_10 mm as an example. You can find examples of other atlases/atlas orientations in Appendix 4/5. In case you want to use other orientations, make sure to have the same image orientation as the atlas. If you need to rotate your images, make sure to do the padding on the stitched images after rotating them.

1. Launch the registration tool by pressing `Plugins>dmc_brainmap>registration`
2. The panel on the right should look like this:

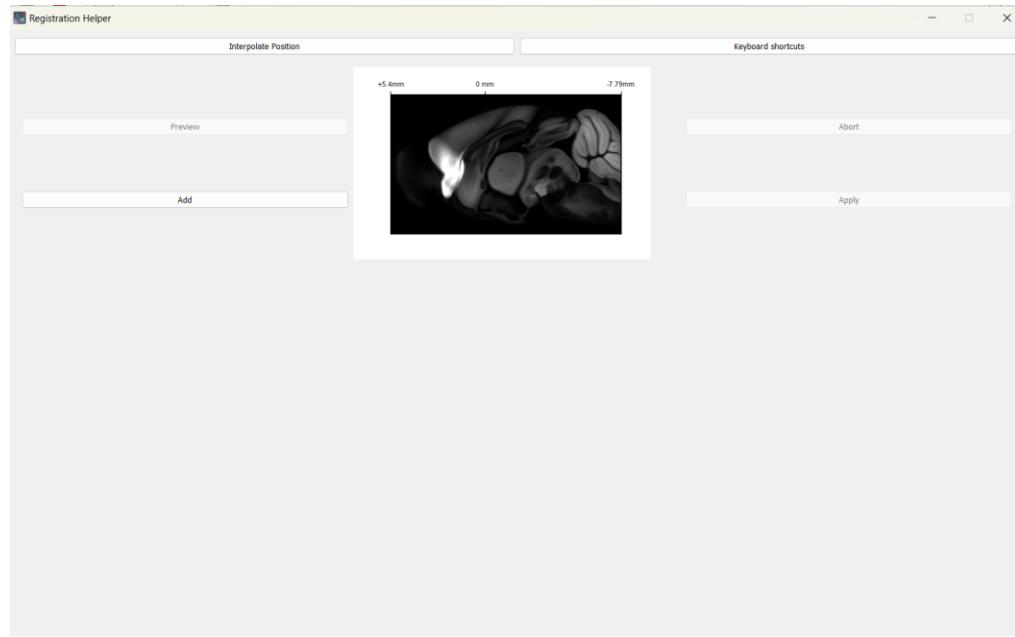


3. Enter the correct data folder and the channel you want to use for registration. Important: images need to be sharp\_y\_track format and in a folder sharp\_y\_track\channel\_name format to continue. This should be automatically correct when created in the plugin.
4. Press: 'start the registration GUI'.
5. The next screen should appear:

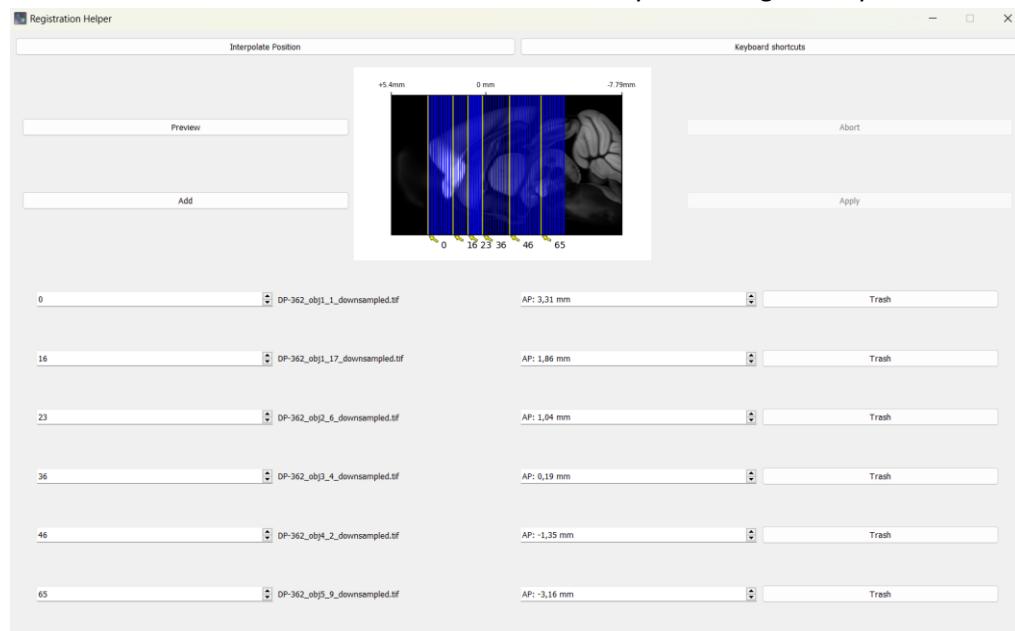


6. If you already have registered images, answer yes if asked if you want to load it. Otherwise, the previous data will get overwritten!
7. Registration
  - a. Set DV and ML angles.
  - b. Angles can be changed by using the sliding bars on top and side of the atlas view.
  - c. Find the correct AP coordinates of the atlas slice for the microscope slice you want to register.
  - d. Switch the transformation button in the middle to green (transformation mode)
  - e. Add dots by clicking on the atlas screen. Accordingly, dots will appear on the microscopy slice. Adapt the dots on the microscopy slice by dragging them to the wanted location.
  - f. After adding 5 dots the transformation will appear on the atlas screen.
  - g. You can make the registration better by adding more dots or dragging the transformation.
  - h. Press 'Z', 'X', 'C' to switch view between '100% Atlas', '50% Blend', '100% Sample' modes. Press 'A' to show or hide brain region outline.
  - i. When happy with the transformation, go to the next slide.

- j. When done, you can just close the registration gui, the data is saved automatically.
8. Tools:
- a. If you go to the tools button, you have two tabs: ‘Interpolate Position’ and ‘Keyboard shortcuts’.
  - b. If you are on ‘Interpolate Position’, the screen looks like this:



- c. By using this tool, you can automatically calculate the AP coordinates for your images.
- d. Put the right angles and AP axis for an image and press ‘Add’.
- e. Info will be added for every image you add.



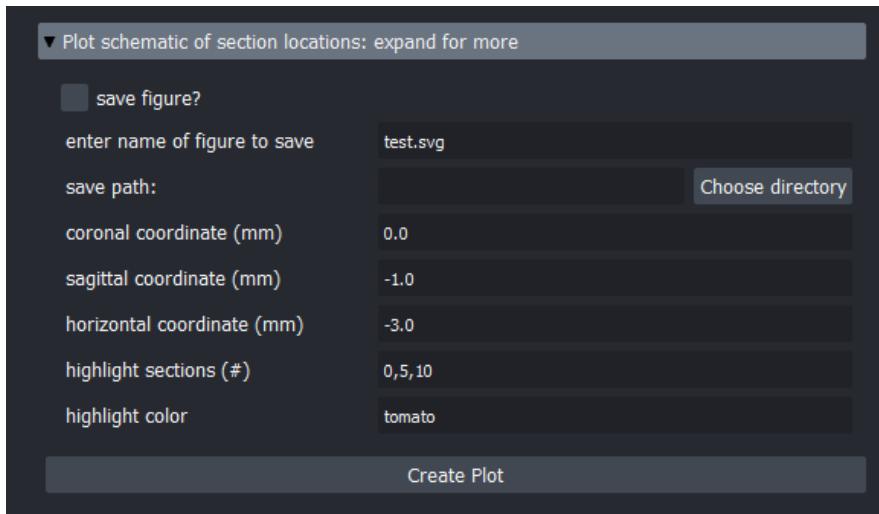
- f. Press preview to scroll through your images and the atlas.
- g. Press Apply if you are happy with the result, Abort if you want to make changes.

- h. If you press apply you get following screen:

Confirm or cancel change(s)					
	slice_id	pre_AP	post_AP	type_of_change	registered
0	0	none	3.54	added	NO
1	1	none	3.44	added	NO
2	2	none	3.33	added	NO
3	3	none	3.22	added	NO
4	4	none	3.12	added	NO
5	5	none	3.02	added	NO
6	6	none	2.91	added	NO
7	7	none	2.8	added	NO
8	8	none	2.7	added	NO
9	9	none	2.6	added	NO
10	10	none	2.49	added	NO
11	11	none	2.38	added	NO
12	12	none	2.28	added	NO
13	13	none	2.17	added	NO
14	14	none	2.07	added	NO

- i. Select the images for which you want to use the calculated AP.  
j. The 'Keyboard shortcuts' tab gives you a quick overview of all shortcuts in Sharpy.

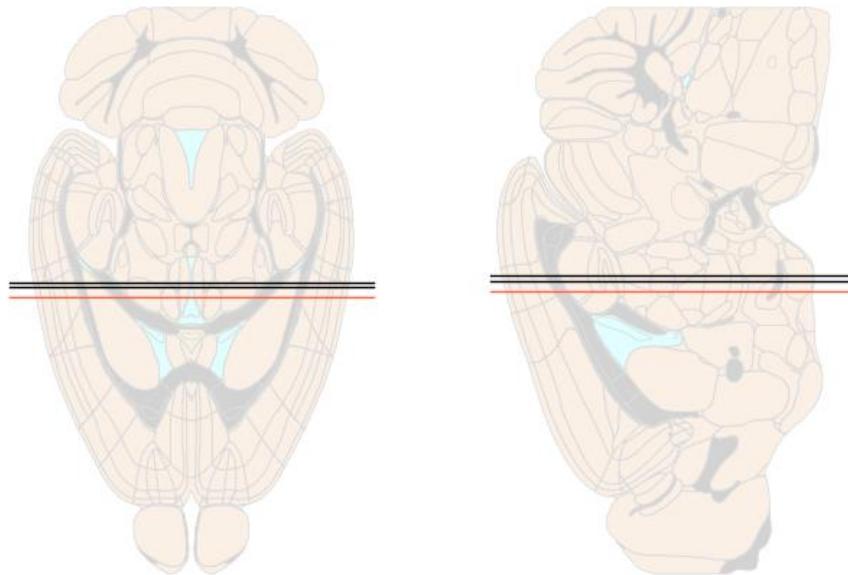
In addition, DMC-BrainMap allows you to visualize the location of your sections on schematized brain section. For this open the drop-down window “Plot schematic of section locations: expand for more”:



1. The input path and registration channel need to be set as for opening the Registration GUI.
2. Enter the coordinates of the sections you want to plot the sections on (ignore the coordinate for the orientation of your actual brain sections, i.e. if you have coronal sections, enter coordinates for the sagittal and horizontal orientation).

3. If you want to highlight a subset of sections (lines on the plots) enter the indices of this sections (starting with 0) and enter the color name. For selecting colors see Appendix 21.
4. If you want to save the figure, tick the “save figure?” box, set a name and enter the path you want to save the figure in.

An example output looks like this (animal with three coronal sections, first section highlighted in ‘tomato’ color):



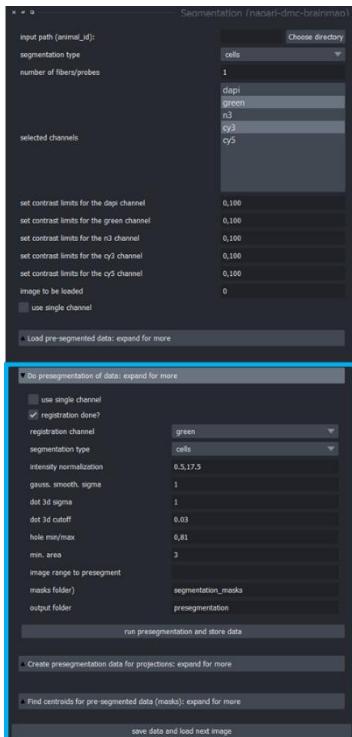
## STEP 10A: Segmentation of cells axonal projection densities

For cell segmentation, both automated and manual segmentation can be used. Presegmentation performed in other tools (like napari-allencell-segmenter, Imaris, Wholebrain...) can be loaded into the plugin to manually edit if the format of segmentation data is according to the one of DMC-BrainMap (e.g. names of headers/folder compositions, see Appendix 7 and description below).

### Automated presegmentation soma/nuclei

We have a presegmentation workflow based on the Rab5a workflow from napari-allencell-segmenter included in the DMC-BrainMap plugin (<https://github.com/AllenInstitute/aics-segmentation>). We use this workflow for nuclear localized signals. You can find more information on using the napari-allencell-segmenter workflows and importing them into the DMC-BrainMap plugin under Appendix 6.

1. Launch the segmentation tool by pressing **Plugins> dmc\_brainmap>segmentation**
2. The panel should look like this:



3. Fill in the animal/image information and select for type of segmentation “cells”.
4. If you don't use RGB images, thick the box for ‘use single channel’.
5. If you have previously registered these brain slices, tick the box for ‘registration done?’ This way, all fluorescent signal segmented outside of the brain slice will be deleted automatically.
6. Fill in the parameters for the Rab5a workflow, please find more information on: <https://github.com/AllenInstitute/aics-segmentation> .
7. To find the right parameters you will have to try and see what is best for your images.
8. Enter the preferred name for the output folders.
9. Press ‘run segmentation and store data’.

- This should give you a folder (with chosen name) with subfolder cells which has subfolders for different channels (example: 'presegmentation\cells\cy3') that contain a .csv file for every image with xy coordinates for all the cells (format as Appendix 7).

#### Create presegmentation data for projections

This tool makes it possible to provide presegmentation data for projections. The presegmentation is based on binary images that you need to create beforehand (read step 8: preprocessing). It is necessary to find an appropriate threshold value, to make the segmentation as good as possible.

- Launch the segmentation by pressing **Plugins>dmc\_brainmap>segmentation**.
- The panel should look like this:



- Fill in the animal/image information.
- Open the tab for presegmentation of projections.
- Fill in the input (binary, which has subfolders for each channel) and output folder (choose name).
- Press 'create presegmentation of projections data'
- This should give you a folder (with chosen name) with subfolder cells which has subfolders for different channels (example: 'presegmentation\projections\cy3') that contain a .csv file for every image with xy coordinates for all the segmented pixels (Appendix 8).
- You can load this presegmentation data into the manual segmentation tool by selecting 'projections' as segmentation type and following the instructions of the manual segmentation cells below.

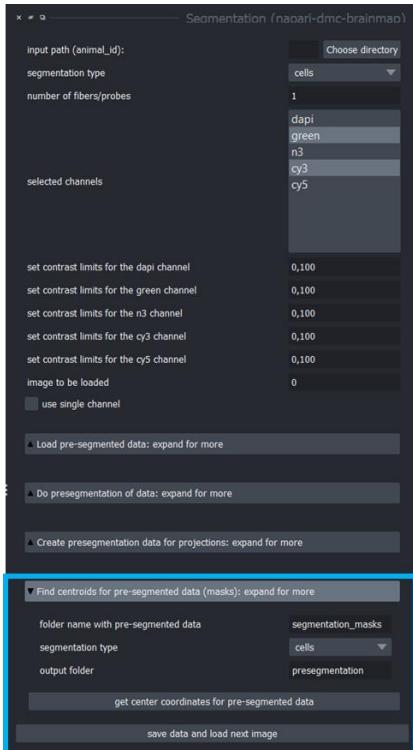
#### Find centroids for presegmented data (masks)

In case you use a presegmentation tool (like the separate napari-allencell-segmenter) creating binary images with 'masks', you need to find the centroids of the masks.

If you presegmented in the DMC-BrainMap plugin, you can skip this step.

- Launch the segmentation by pressing **Plugins>dmc\_brainmap>segmentation**.

10. The panel should look like this:



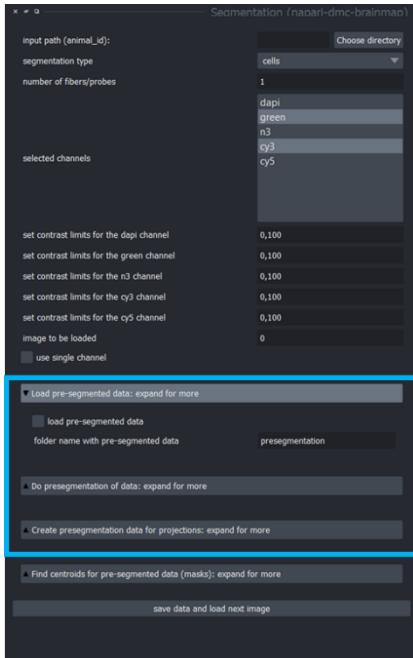
11. Fill in the animal/image information.
12. Open the tab for centroids detection.
13. Fill in the input (previous output folder for automatic segmentation) and output folder (choose name)
14. Press 'get center coordinates for pre-segmented data'
15. This should give you a folder (with chosen name) with subfolder cells which has subfolders for different channels that contain a .csv file for every image with xy coordinates for all the cells.

This data can be loaded into the manual segmentation tool.

#### Manual segmentation/editing automatic segmentation

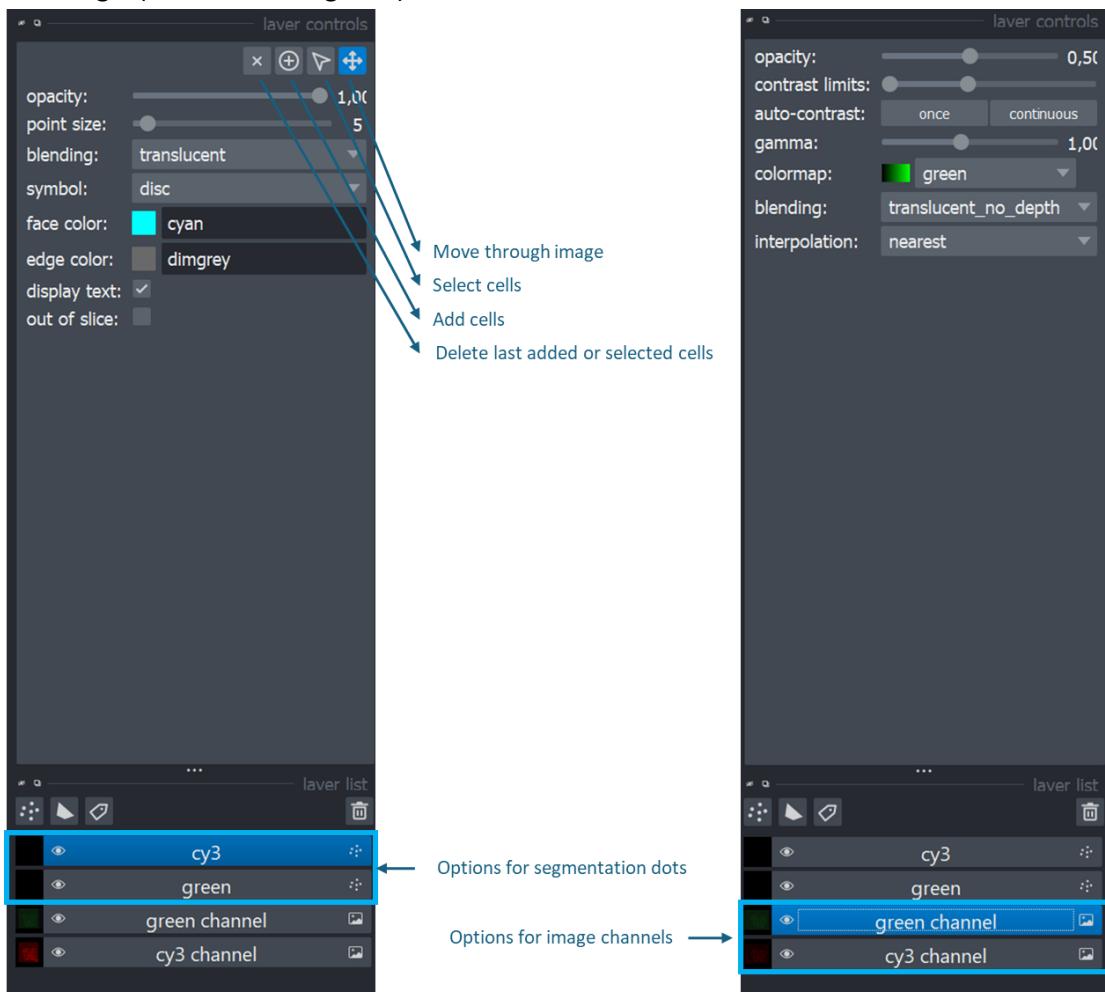
1. Launch the segmentation by pressing Plugins> dmc\_brainmap>segmentation

2. The panel should look like this:



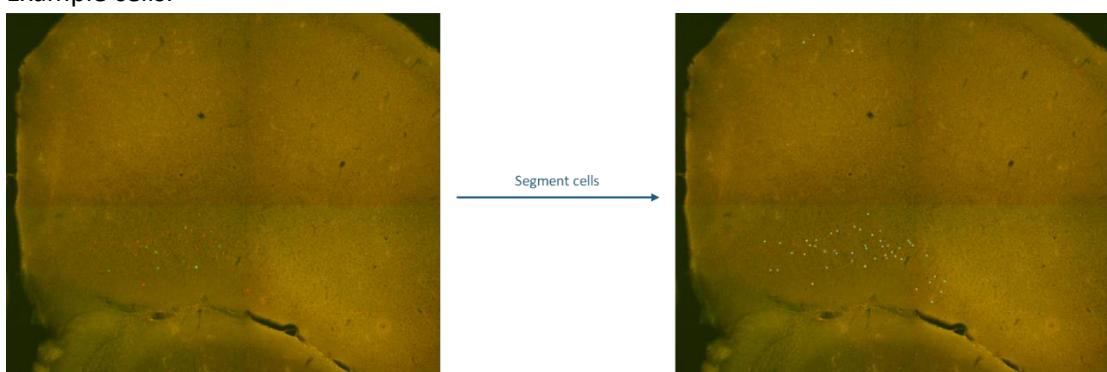
3. Fill in the animal/image information and select for type of segmentation 'cells' or 'projections'.
4. If you want to segment dapi, green and/or cy3 channel, you can use the RGB images. If you want to segment n3 or cy5 (or you rather segment on single channel images), you need to tick the box 'use single channel'.
5. You can set the contrast limits for the channels you use (without changing the actual images), but you can also use the sliding bars when your images are loaded (see image below).
6. Optionally, presegmented data can be loaded (tick box). This data should have the following format: see Appendix 8 and should be in a folder 'chosen name\cells\' with subfolders for every channel (for example 'presegmentation\cells\cy3'). As input, you give the main folder (here 'presegmentation').
7. Fill in the number of the image you want to load (numerical order of the images can be found in the image\_names file).
8. Leave the 'number of fibers/probes' on 1.
9. Press save and load next image → this will load the image you asked for and fills in the number of the next image automatically.
10. With the panel on the left, you can both change the image channels (opacity, brightness, colour...) and use the segmentation tools by clicking on the image channels or segmentation channels respectively at the bottom.
11. Some useful keyboard shortcuts:
  - Holding space while dragging screen → moving along image while in adding cells mode
  - v → make channel that is selected visible or not
  - a → select all segmentation dots in the selected channel
  - 1/del → delete selected segmentation dots

12. Now you can easily add/remove dots on the cell bodies by selecting the correct channel and selecting + (or X for deleting dots):



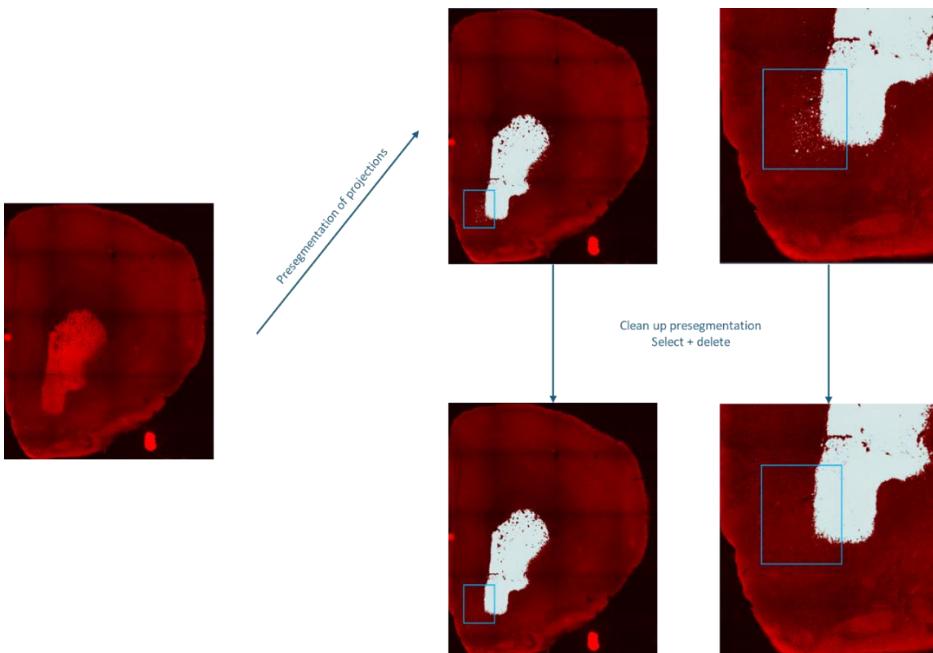
13. You can segment cell bodies/nuclei in different channels. Every channel will be marked with a different coloured dot.

Example cells:



14. You can delete wrongly segmented projections from the presegmentation data for projections (see above) by selecting and deleting dots.

Example projections:



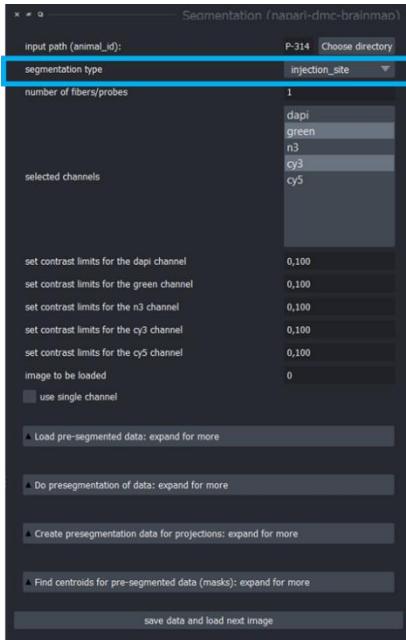
15. When happy with the segmentation, press 'save and load next image'.
16. When you segmented all images you wanted, press once more 'save and load next image' and simply close the tool.
17. Now a folder 'segmentation' will be created, with subfolders for every type of segmentation and in these folders are subfolders for every channel (example: animal\segmentation\cells\cy3).
18. The data is saved as a .csv file for every image, for every channel, with xy coordinates for every dot (cells) or pixels (projections) (Appendix 7-8).

## STEP 10B: Segmentation injection site

This tool was created to assess the brain region(s) covered by the viral injection. As an output, you get an overview of all pixels in the segmented areas on the image.

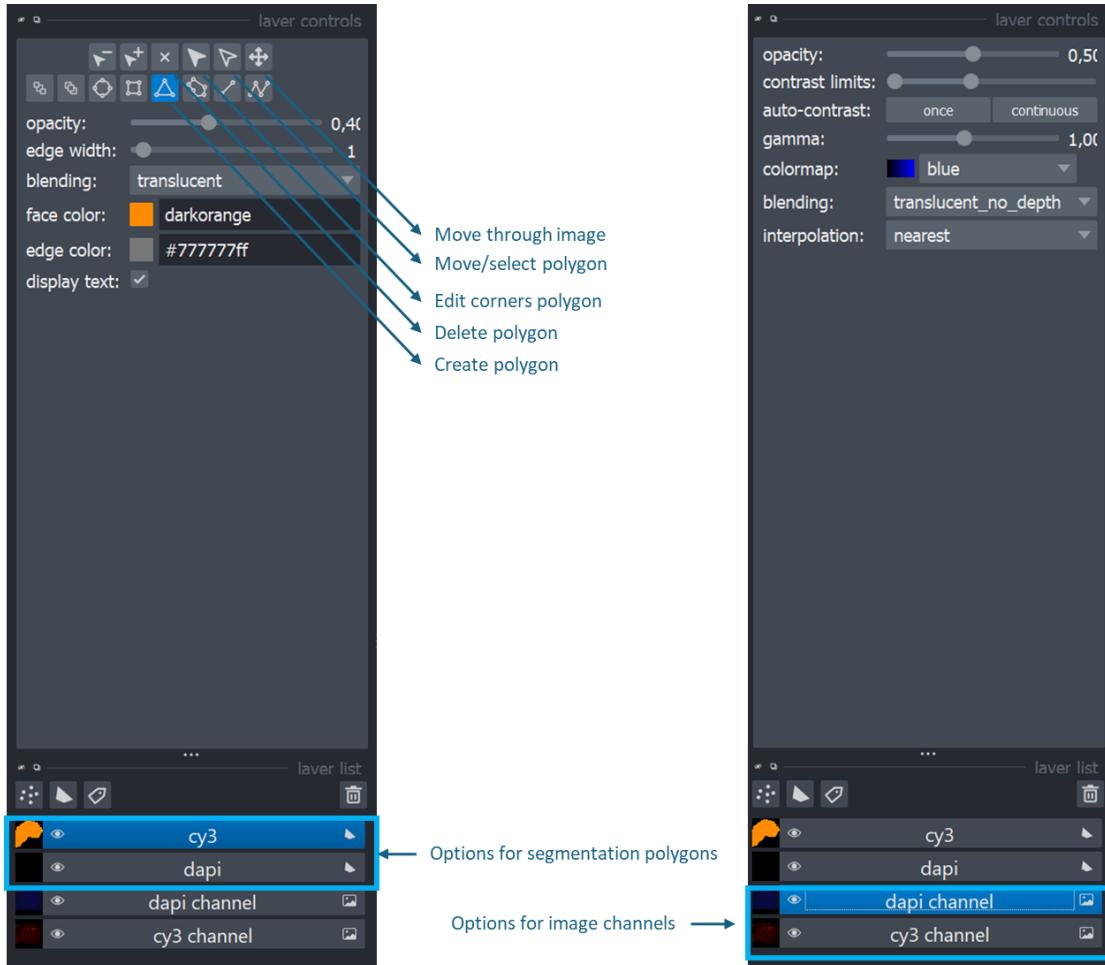
1. Launch the segmentation by pressing `Plugins>dmc_brainmap>segmentation`

2. The panel should look like this:



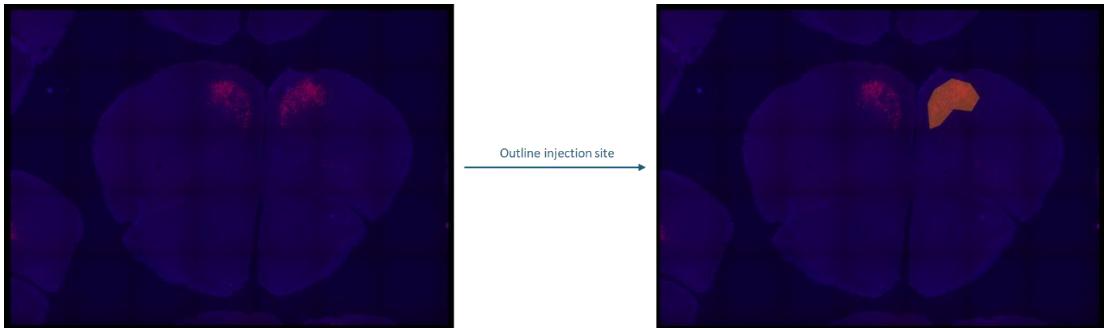
3. Fill in the animal/image information and select for type of segmentation “injection\_site”
4. If you want to segment dapi, green and/or cy3 channel, you can use the RGB images. If you want to segment n3 or cy5 (or you rather segment on single channel images), you need to tick the box ‘use single channel’.
5. You can set the contrast limits for the channels you use (without changing the actual images), but you can also use the sliding bars when your images are loaded (see image below).
6. Optionally, presegmented data can be loaded (tick box). This data should have the following format: see Appendix 9 and should be in a folder ‘chosen name\optic\_fiber\’ with subfolders for every channel (for example ‘presegmentation\optic\_fiber\cy3’). As input, you give the main folder (here ‘presegmentation’).
7. Fill in the number of the image you want to load (numerical order of the images can be found in the image\_names file).
8. Leave the ‘number of fibers/probes’ on 1.
9. Press save and load next image → this will load the image you asked for and fills in the number of the next image automatically.
10. With the panel on the left, you can both change the image channels (opacity, brightness, colour...) and use the segmentation tools by clicking on the image channels or segmentation channels respectively at the bottom.
11. Some useful keyboard shortcuts:
  - Holding space while dragging screen → moving along image while in adding polygon mode
  - v → make channel that is selected visible or not
  - a → select all segmentation dots in the selected channel
  - 1/del → delete selected segmentation polygons

12. Select the channel you want to segment and press the 'create polygon' or button.



13. Make an outline of the injection site by clicking on the screen to make the corners of the polygon.

14. Example:



15. When finished, press escape to finish the polygon.

16. Depending on how your criteria are, you can also add multiple polygons for the same channel in one image (everything will be summed up in the results file).

17. You can also segment in multiple channels on the same image (will be saved as separate results).

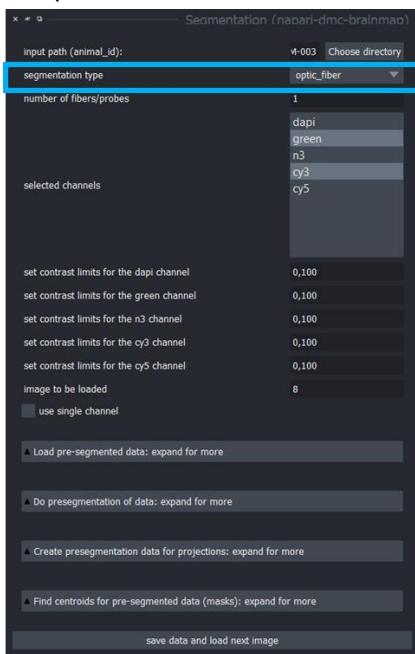
18. When happy with the segmentation, press save and load next image.

19. When you segmented all images you wanted, press once more save and load next image and simply close the tool.

20. Now a folder segmentation will be created, with subfolders for every type of segmentation and in these folders are subfolders for every channel (example: animal\segmentation\injection\_site\cy3).
21. The data is saved as a .csv file for every image, with xy coordinates for every corner of the polygon (Appendix 9).

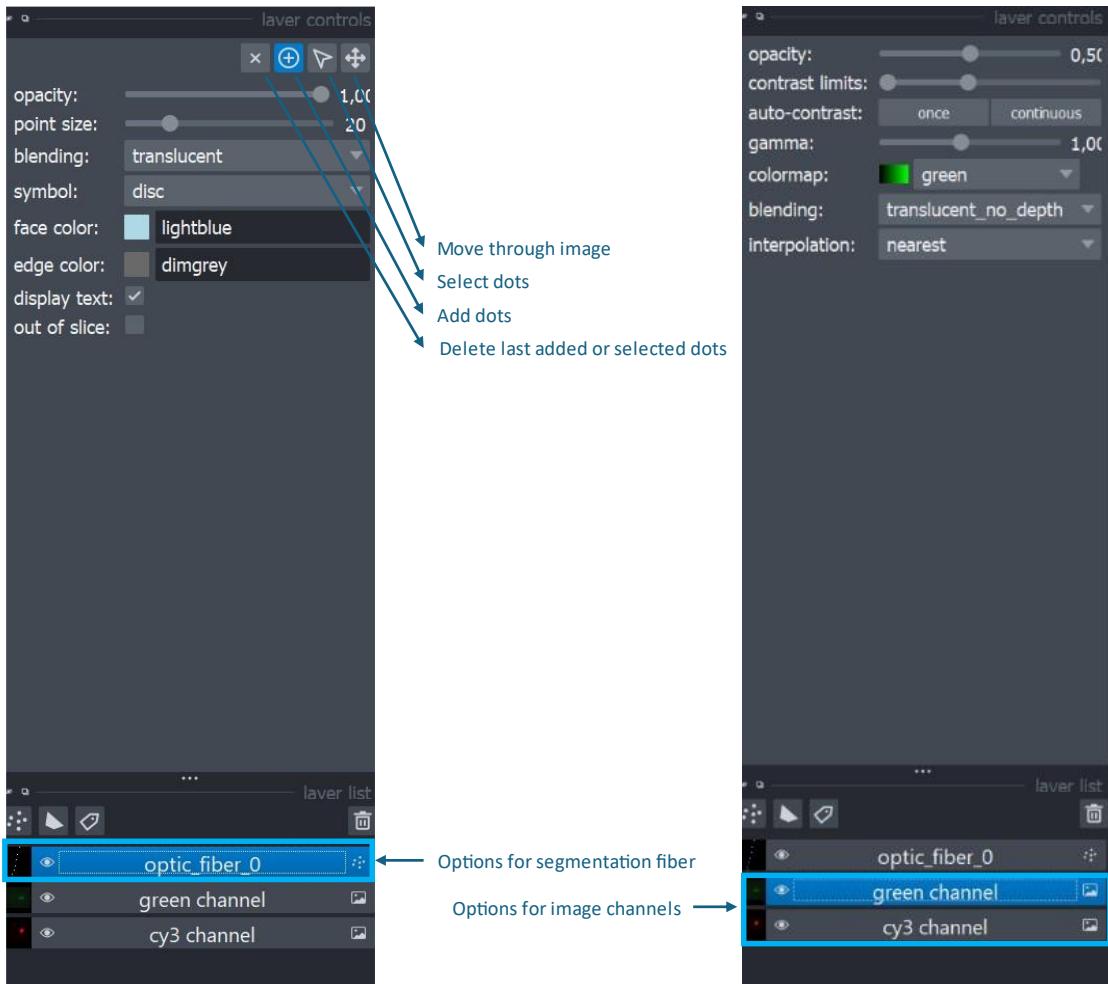
## STEP 10C: Segmentation optic fiber

1. Launch the segmentation by pressing **Plugins> dmc\_brainmap>segmentation**
2. The panel should look like this:



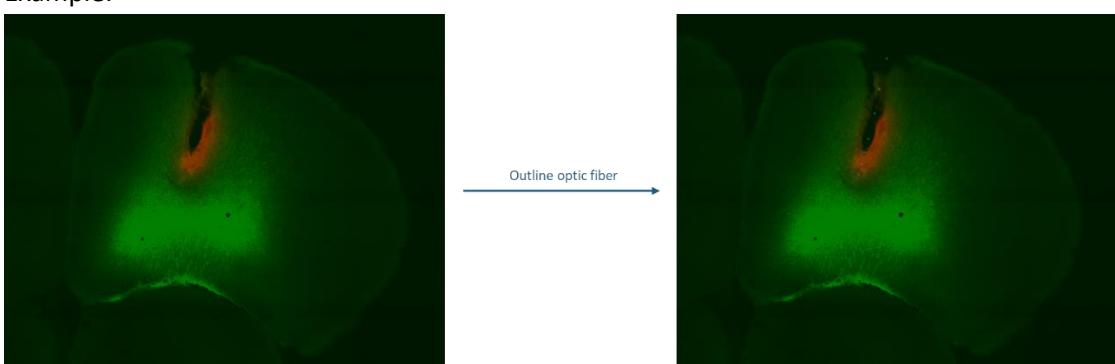
3. Fill in the animal/image information and select for type of segmentation ‘optic\_fiber’.
4. If you want to segment dapi, green and/or cy3 channel, you can use the RGB images. If you want to segment n3 or cy5 (or you rather segment on single channel images), you need to tick the box ‘use single channel’.
5. You can set the contrast limits for the channels you use (without changing the actual images), but you can also use the sliding bars when your images are loaded (see image below).
6. Optionally, presegmented data can be loaded (tick box). This data should have the following format: see Appendix 10 and should be in a folder ‘chosen name\optic\_fiber\’ with subfolders for every channel (for example ‘presegmentation\optic\_fiber\cy3’). As input, you give the main folder (here ‘presegmentation’).
7. Fill in the number of the image you want to load (numerical order of the images can be found in the image\_names file).
8. Select the ‘number of fibers/probes’.
9. Press save and load next image → this will load the image you asked for and fills in the number of the next image automatically.
10. With the panel on the left, you can both change the image channels (opacity, brightness, colour...) and use the segmentation tools by clicking on the image channels or segmentation channels respectively at the bottom.
11. Some useful keyboard shortcuts:

- a. Holding space while dragging screen → moving along image while in adding cells mode
  - b. v → make channel that is selected visible or not
  - c. a → select all segmentation dots in the selected channel
  - d. 1/del → delete selected segmentation dots
  - e.
12. Now you can easily add/remove dots on the optic fiber tract by selecting the correct fiber number and selecting + (or X for deleting dots).



13. You can segment different fiber tracts by placing dots along the tract. Every fiber will be marked with different coloured dots.

Example:

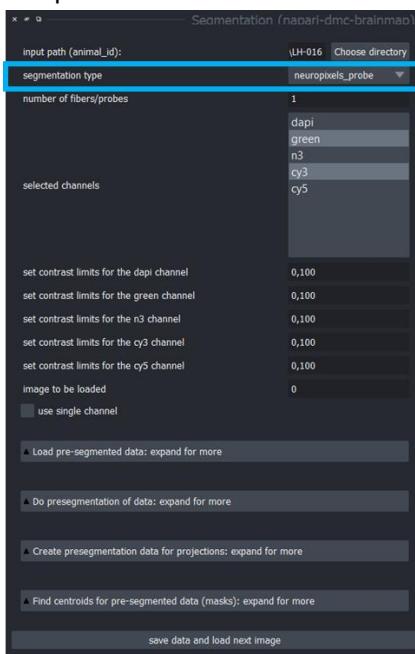


14. When happy with the segmentation, press 'save and load next image'.

15. When you segmented all images you wanted, press once more ‘save and load next image’ and simply close the tool.
16. Now a folder ‘segmentation’ will be created, with subfolders for every type of segmentation (here optic\_fiber) and in these folders are subfolders for every optic fiber (example: animal\segmentation\optic\_fiber\optic\_fiber\_0).
17. The data is saved as a .csv file for every image, for every channel, with xy coordinates for every dot (Appendix 10).

## STEP 10D: Segmentation Neuropixels probe

1. Launch the segmentation by pressing **Plugins> dmc\_brainmap>segmentation**
2. The panel should look like this:



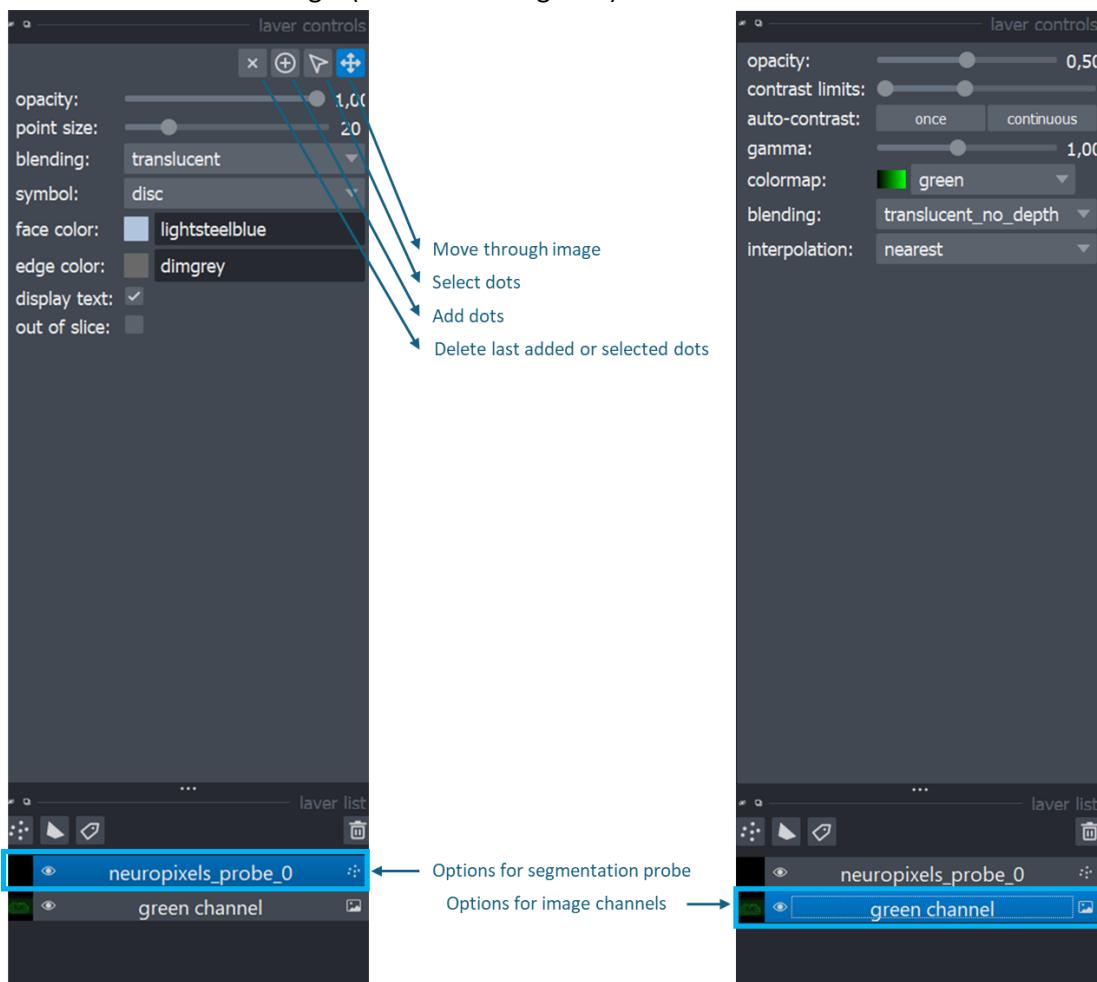
3. Fill in the animal/image information and select for type of segmentation ‘Neuropixels\_probe’.
4. If you want to segment dapi, green and/or cy3 channel, you can use the RGB images. If you want to segment n3 or cy5 (or you rather segment on single channel images), you need to tick the box ‘use single channel’.
5. You can set the contrast limits for the channels you use (without changing the actual images), but you can also use the sliding bars when your images are loaded (see image below).
6. Optionally, presegmented data can be loaded (tick box). This data should have the following format: see Appendix 11 and should be in a folder ‘chosen name\neuropixels\_probe\’ with subfolders for every channel (for example ‘presegmentation\neuropixels\_probe\cy3’). As input, you give the main folder (here ‘presegmentation’).
7. Fill in the number of the image you want to load (numerical order of the images can be found in the image\_names file).
8. Select the ‘number of fibers/probes’.
9. Press save and load next image → this will load the image you asked for and fills in the number of the next image automatically.

10. With the panel on the left, you can both change the image channels (opacity, brightness, colour...) and use the segmentation tools by clicking on the image channels or segmentation channels respectively at the bottom.

11. Some useful keyboard shortcuts:

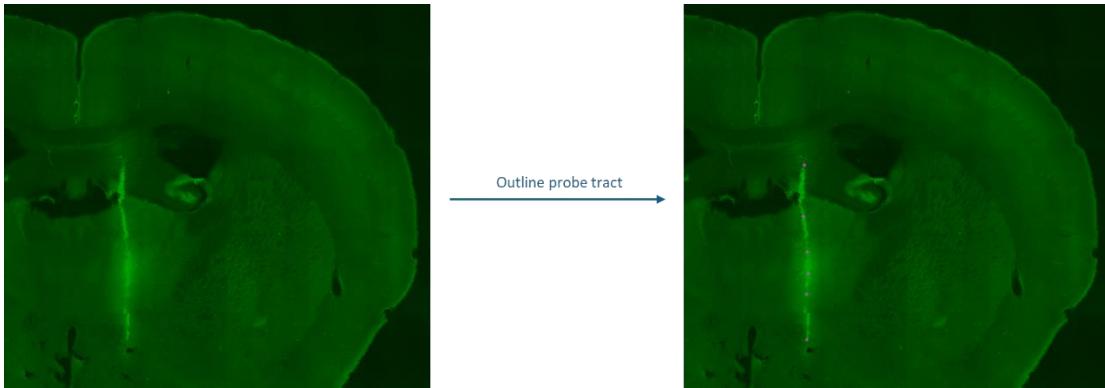
- Holding space while dragging screen → moving along image while in adding cells mode
- v → make channel that is selected visible or not
- a → select all segmentation dots in the selected channel
- 1/del → delete selected segmentation dots

12. Now you can easily add/remove dots on the Neuropixels probe tract by selecting the correct fiber number and selecting + (or X for deleting dots):



13. You can segment different probe tracts by placing dots along the tract. Every probe will be marked with different colored dots.

Example:



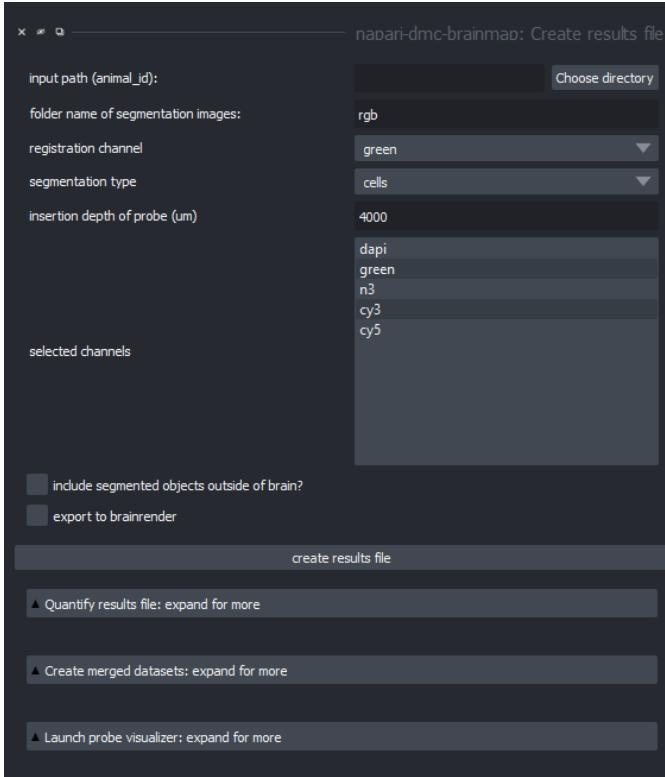
14. When happy with the segmentation, press 'save and load next image'.
15. When you segmented all images you wanted, press once more 'save and load next image' and simply close the tool.
16. Now a folder 'segmentation' will be created, with subfolders for every type of segmentation (here neuropixels\_probe) and in these folders are subfolders for every neuropixels probe (example: animal\segmentation\neuropixels\_probe\neuropixels\_probe\_0).
17. The data is saved as a .csv file for every image, for every channel, with xy coordinates for every dot (Appendix 11).

## STEP 11A: Create result files

If you have both registered and segmented the images, you can combine them into a result file. This file will contain every dot, area... (depending on the type of segmentation) and its location in the reference atlas.

1. To make the results file launch the results tool by pressing `Plugins>dmc_brainmap>Create results file`

2. The panel should look like this:

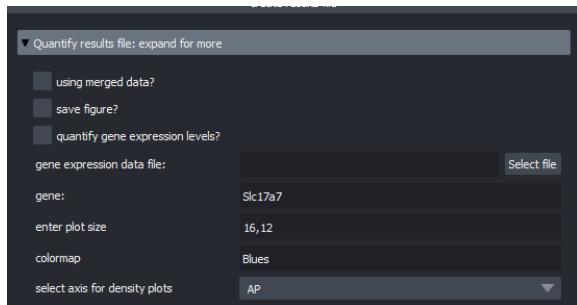


3. Give animal and image info and fill in the type of segmentation you want the results from.
4. By default segmented objects outside of the brain (based on registration data) will be excluded, if you want to keep them, tick the 'include segmented objects outside of brain' button
5. If you want to create a results file for optic\_fibers or neuropixels\_probes, you can enter the insertion depth of the fiber/probe (comma-separated list for multiple probes/fibers). If you leave this box empty, the insertion depth will be estimated based on your segmentation.
6. If you want to export your data formatted for visualization using brainrender (<https://brainglobe.info/documentation/brainrender>), tick the respective button
7. Press 'create results file'.
8. A folder 'results' will be created with subfolders for the different segmentation types, which have subfolders for every channel (example: animal\results\cells\cy3).
9. You can use these files for further data analysis (both with the visualize data option in the plugin or external analysis)
10. Data results file format:
  - Cells:
    - Coordinates and ABA regions for all segmented cells (ex: Appendix 12)
    - For every channel separately
    - Folder example: results\cells\cy3
  - Projections
    - Coordinates and ABA regions for all segmented pixels on projections (ex: Appendix 13)
    - For every channel separately
    - Folder example: results\projections\cy3

- Injection site:
  - Coordinates and ABA regions for all pixels in the segmented injection site (ex: Appendix 14+ see below for more options).
  - For every channel separately
  - Folder example: results\injection\_site\cy3
- Optic fiber:
  - Coordinates and ABA regions for all dots on the optic fiber tract (ex: Appendix 15).
  - For every optic fiber separately
  - Folder example: results\optic\_fiber\optic\_fiber\_0
- Neuropixels probe:
  - Coordinates and ABA regions for all dots on the probe tract (ex: Appendix 16).
  - For every neuropixels probe separately
  - Folder example: results\neuropixels\_probe\neuropixels\_probe\_0
  - .json files for the ProbeViewer (see below)
  - .svg files with a visualization of the probe, the channels and corresponding brain regions based on anatomy. A confidence metric is for each electrode location is calculated the following: from the 3D center of a recording site (channel), a  $21 \times 21 \times 21 = 9261$  voxels cube (with recording site center in the center of cube) is calculated, from which a spherical shape is estimated by selecting voxels that fulfil:  
 $\text{euclidean\_distance}(\text{cubecenter\_xyz} - \text{voxel\_xyz}) \leq 10 \text{ voxels}$   
 This selects a spherical shaped volume of 4169 voxels.  
 We then find allen brain structure id for all these 4169 voxels. If there is any structure\_id different than recording site structure\_id, we will use the closest to center one to calculate the confidence value:  $\text{euclidean\_distance}(\text{spherecenter\_xyz} - \text{different\_id\_xyz}).\text{min}() * \text{voxel\_size}$   
 For example, channel center has coordinate [0,0,0] with structure\_id=123, it's closest different structure is at [0,0,5] with structure\_id=234. Then confidence is calculated as: 5 voxels \* 10um/voxel = 50um.  
 If all structure id within sphere are the same as center, then the confidence value is 10 voxels \* 10um/voxel = 100um

## STEP 11B: Quantify results file

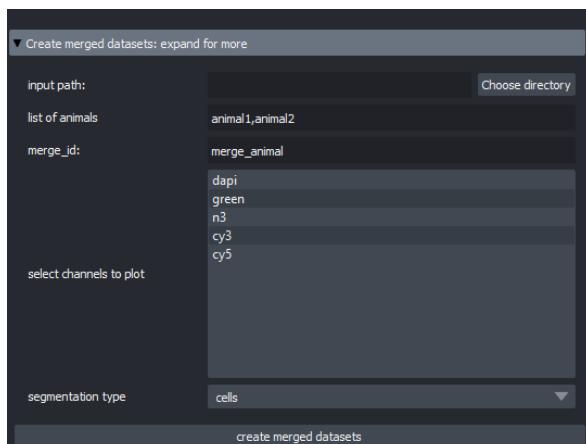
After creating a results file, DMC-BrainMap allows you to quantify the data. Open the 'Quantify results file' tab and enter the correct parameters:



The output will create a .csv file containing information on the distribution of your segmented object with regard to brain regions (in %). The quantified results will be also visualized as a pie chart and a density plot (<https://seaborn.pydata.org/generated/seaborn.kdeplot.html>). You can save this figure by ticking the ‘save figure?’ option. If you use merged data from several animals (see STEP 11C below), tick the respective option. If you want to visualize gene expression data, locate the gene expression data file.

## STEP 11C: Create merged datasets

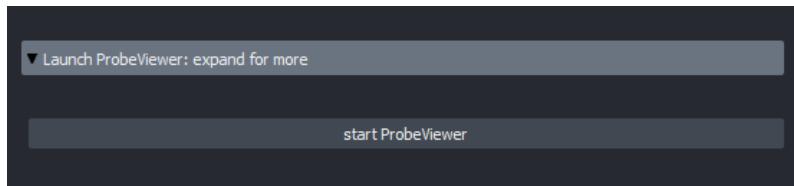
In case you are planning to use custom scripts for further data analysis and visualization, DMC-BrainMap can merge datasets from individual animals. Open the ‘Create merged datasets’ tab:



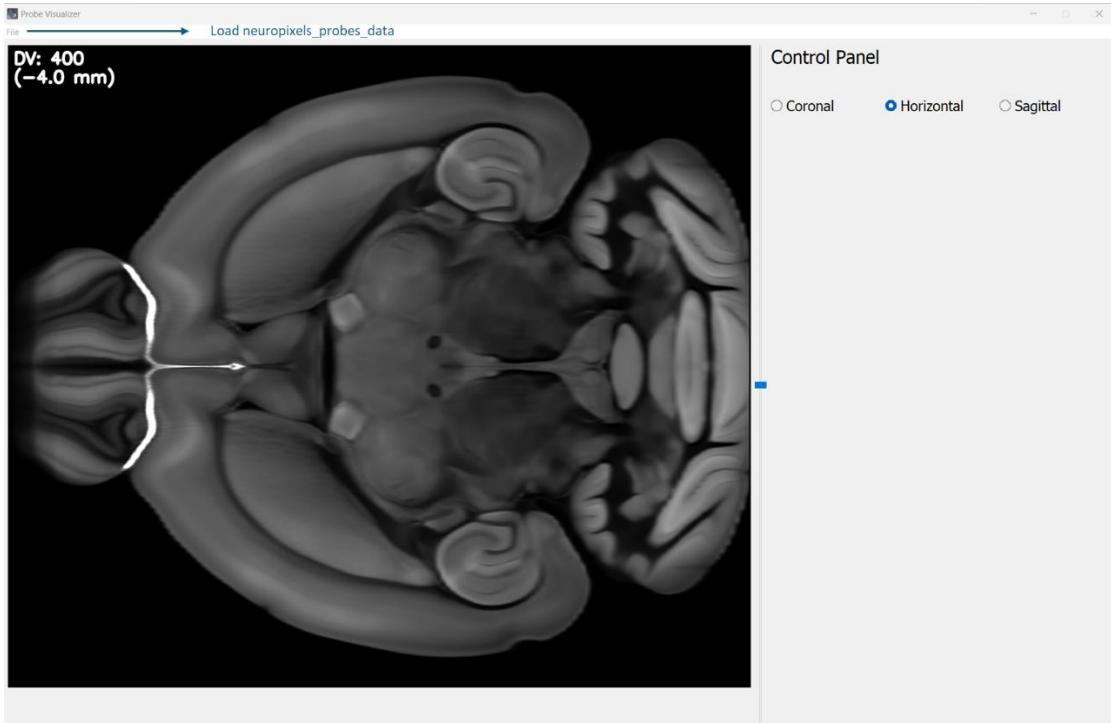
1. Specify the ‘input path’: the directory containing the folders with the analyzed data of multiple animals
2. Specify the animal\_ids of the animals to be merged
3. Specify a ‘merge\_id’: the name of the folder containing the merged data
4. And select the channels and segmentation type
5. If you want to quantify the results file from the merged dataset (see above), change the directory in the ‘Create results file’ widget above (‘input path (animal\_id)’) accordingly

## STEP 11D: Launch the ProbeViewer

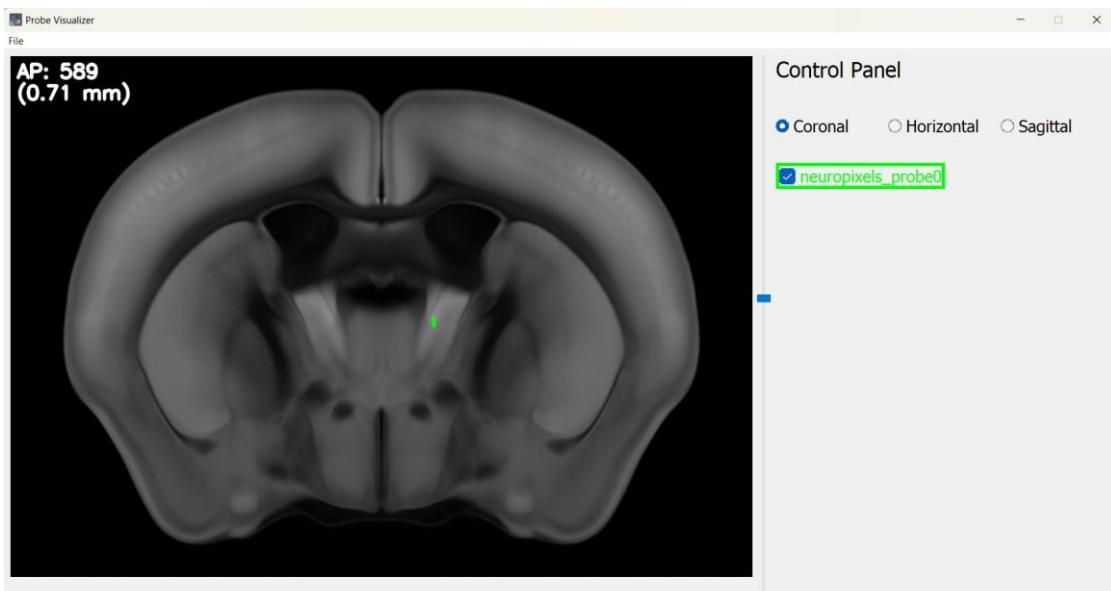
DMC-BrainMap comes with an integrated tool to visualize Neuropixels probe trajectories called **ProbeViewer**. After having created the results file for your probes (see above), you can launch the viewer by opening the ‘Launch ProbeViewer’ tab and pressing the ‘start ProbeViewer’ button:



1. The next screen should appear:



- 2.
3. Load the neuropixels\_probes\_data by pressing file>Load probes json and selecting the correct neuropixels\_probes\_data.json.
4. The screen should look like this:

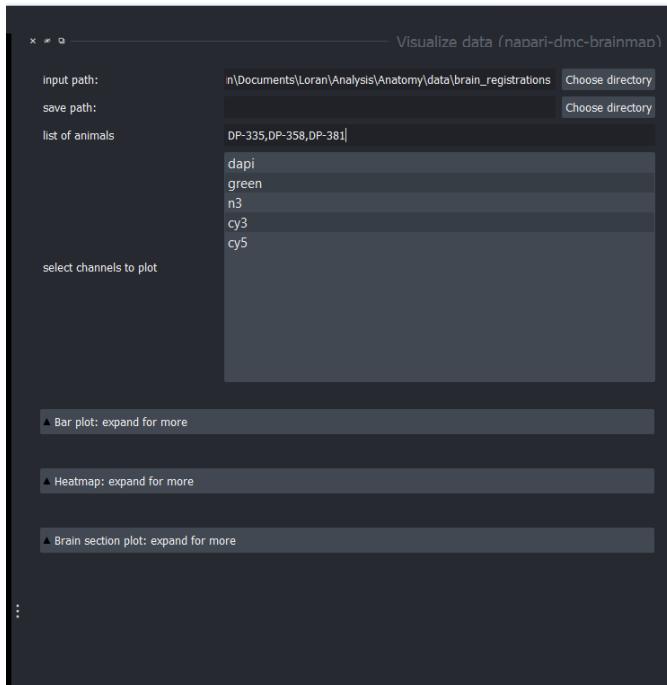


5. You can switch between coronal, horizontal, and sagittal view and (de)select probes to visualize them in the 3D brain volume.

## STEP 12: Visualize data

In addition to the option to quantify data as pie charts and density estimates (see Step 11), DMC-BrainMap allows users to visualize their data in 3 ways: as barplots, heatmaps and plotted on schematized brainsections. It supports to visualize data from multiple animals of different groups. **All animal\_id folders need to be placed in the same directory, i.e. not placed in subdirectories for different experimental groups.** For selecting colors see Appendix 21.

1. Launch the visualize data tool by pressing **Plugins>dmc\_brainmap>Visualize data**
2. The panel should look like this:



3. As input path enter the folder that contains the animal id folders. As list of animals, include the name of the animal id folders that you want to include in the results.
4. Give channel information.
5. You can create 3 types of plots with the plugin:
  - Bar plots
    - For segmented cells, injection sites, projections and gene expression data
    - Possibility to make groups based on channel, experimental group or genotype
    - Make sure to add the list of brain areas in correct acronyms of the reference atlas used
    - Example Appendix 17
  - Heatmaps
    - For segmented cells, injection sites, projections and gene expression data
    - Heatmaps in bins along the AP axis and brainregions (optional: with subregions)
    - Example Appendix 18
  - Brain section plot
    - For all segmented items

- Representation of segmented objects on reference atlas brain sections in coronal, sagittal or horizontal orientation
- Example Appendix 19

## STEP 12A: Barplot visualization

1. Select the base parameters (see above) and set a save path if you wish to save the data.
2. Open the drop-down menu ‘Bar plot: expand for more’
3. Select the item you want to plot and set the hemisphere from which you like to collect data
4. If you intend to plot channels/groups/genotypes or animals separately, select the appropriate options. Groups and genotypes need to be specified in the params file (see STEP 5) and be spelled the same (not ‘Cre’ in one animal and ‘cre’ in the other etc)
5. Select the areas you which to plot (acronyms according to reference space used)
6. Set the other parameters on e.g. axis labels, figure size etc.
7. If you wish to visualize individual data points, tick the ‘plot individual data points’ options and select a colormap (default ‘c:Greys’)
8. You can quantify data as absolute numbers (e.g. numbers of cells) or as a percentage of all cells in the dataset (‘percentage\_dataset’) or a percentage relative to the selected brain areas (‘percentage\_selection’) in the ‘plotting absolute numbers or percentage’ option
9. Click ‘Create bar plot’ the generate the plot.
10. If you saved data, a folder with the save name is created in the specified location, containing an .svg file with the plot, a .csv file containing the data used to create the plot and a .json file containing the plotting parameters
11. Example plots are in Appendix 17

## STEP 12B: Heatmap visualization

1. Select the base parameters (see above) and set a save path if you wish to save the data.
2. Open the drop-down menu ‘Heatmap: expand for more’
3. Select the item you want to plot and set the hemisphere from which you like to collect data
4. If you intend to create a difference plot between groups (e.g. differences between experimental groups), select the option otherwise leave it unset (default). Groups and genotypes need to be specified in the params file (see STEP 5) and be spelled the same (not ‘Cre’ in one animal and ‘cre’ in the other etc)
  - a. If you choose to plot a difference plot. Select the two items that are going to be subtracted from each other. E.g. when intending to plot the difference between ‘experimental’ and ‘control’ groups enter: “experimental-control”. This will subtract the values for the control group from the experimental group. Group names need to be specified in the params file of all mice (see above).
5. Select the areas you which to plot (acronyms according to reference space used) and specify the edges of the bins along the AP axis.
6. Set the other parameters on e.g. axis labels, figure size etc.
7. If you wish to include subregions, select the ‘include descendants’ options. As an example, ACA1, ACA2/3 etc. are descendants from ACA (allen reference atlas).
8. You can quantify data as absolute numbers (e.g. numbers of cells) or as a percentage of all cells in the dataset (‘percentage\_dataset’) or a percentage relative to the selected brain areas (‘percentage\_selection’) in the ‘plotting absolute numbers or percentage’ option
9. Click ‘Create heatmap’ the generate the plot.

10. If you saved data, a folder with the save name is created in the specified location, containing an .svg file with the plot, a .csv file containing the data used to create the plot and a .json file containing the plotting parameters
11. Example plots are in Appendix 18

## STEP 12C: Brain section visualization

12. Select the base parameters (see above) and set a save path if you wish to save the data.
13. Open the drop-down menu ‘Brain section plot: expand for more’
14. Select the item(s) you want to plot and set the hemisphere from which you like to collect data, the hemisphere you like to plot and the orientation of the schematized brain section. You can plot data in all orientations, irrespective of the orientation of the actual brain sections.
15. If you wish to highlight some brain areas, enter the acronyms as a comma separated list (e.g. “ACAd,ACAv,PL,ILA” and specify the corresponding colors as a comma separated list (e.g. “green,red,blue,yellow”).
  - a. If you want to color all brain areas in a brain section type “ALL”
  - b. If you want to use the default atlas colors, type “ATLAS”
16. When visualizing cells or projections, can also color brain areas (as heatmaps) according to the number of cells/projection density. Tick the ‘color brain areas according to cell/projection density’ option.
17. Select the sections (in mm) you wish to plot and the range (in mm) around each section you like to collect data from.
  - a. E.g. when you select the sections “-0.5, 1.0” and the range “0.05”, you will plot the sections at the atlas locations -0.5 mm and 1.0 mm relative to bregma and collect data +/- 0.05 mm around each section
18. If you intend to plot channels/groups/genotypes or animals separately, select the appropriate options. Groups and genotypes need to be specified in the params file (see STEP 5) and be spelled the same (not ‘Cre’ in one animal and ‘cre’ in the other etc)
19. If you intend to create a difference plot (cells and projections only) between groups (e.g. differences between experimental groups), select the option otherwise leave it unset (default). Groups and genotypes need to be specified in the params file (see STEP 5) and be spelled the same (not ‘Cre’ in one animal and ‘cre’ in the other etc)
  - a. If you choose to plot a difference plot. Select the two items that are going to be subtracted from each other. E.g. when intending to plot the difference between ‘experimental’ and ‘control’ groups enter: “experimental-control”. This will subtract the values for the control group from the experimental group. Group names need to be specified in the params file of all mice (see above).
20. Set all parameters for the items you selected to plot.
21. For spatial transcriptomics data:
  - a. If you intend to visualize the results of a clustering of the gene expression data, the cluster\_ids need to be specified in the results file for each ‘spot’
  - b. Clustering results, can be either visualized by color-coding the spots of the data according to cluster ID, or you can color code brain areas according to the majority of clusters in that particular brain regions (‘winner-takes-all’) or you can create a Voronoi tessellation
  - c. Similarly, when visualizing gene expression data (enter gene name and gene\_expression file in the pop-up menu), you can color code the spots of the data

according to gene expression, or you can color code brain areas according to the average gene expression values, or you can perform a Voronoi tessellation (rounding the expression data might improve your results, enter the value to round to the number of digits after point in the pop-up menu)

22. Click 'Create brain section plot' to generate the plot.
23. If you saved data, a folder with the save name is created in the specified location, containing an .svg file with the plot, multiple .csv files (one for each section) containing the data used to create the plot(s) and a .json file containing the plotting parameters
24. Example plots are in Appendix 19

## Appendix

This appendix contains examples of how the data should look. This is only as example and the data shown is not analyzed in detail.

### Appendix 1: Example of raw data format needed for stitching.

(animal ID and obj naming can be chosen, but other names should be accordingly)

#### Option a: DMC-FluoImager

Different object slides of 1 animal

In channel folder

In meta data folder

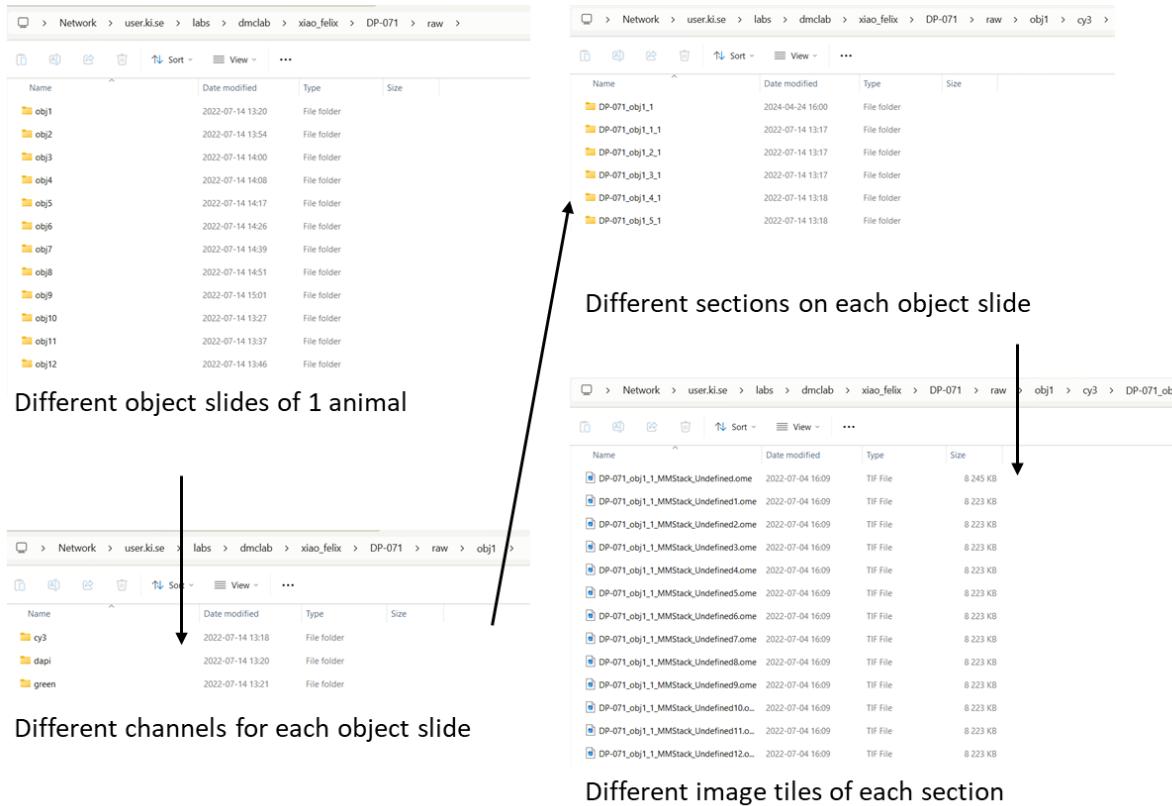
Different channels and meta data for each object slide

Name	Date modified	Type	Size
obj1	2023-09-12 17:24	File folder	
obj2	2023-09-12 17:48	File folder	
obj3	2023-09-12 18:16	File folder	
obj4	2023-09-12 18:42	File folder	
obj5	2023-09-12 19:08	File folder	
obj6	2023-09-12 19:23	File folder	

Name	Date modified	Type	Size
display_settings	2023-09-08 10:10	Text Document	1 KB
NDTiff.index	2023-09-08 10:10	INDEX File	68 KB
obj1_cy3_NDTiffStack	2023-09-08 10:07	TIF File	4 181 823 ...
obj1_cy2_NDTiffStack_1	2023-09-08 10:10	TIF File	1 131 553 ...

Name	Date modified	Type	Size
display_settings	2023-09-08 09:30	Text Document	1 KB
NDTiff.index	2023-09-08 09:30	INDEX File	21 KB
obj1_meta_NDTiffStack	2023-09-08 09:30	TIF File	2 254 889 ...
region_focus_plane	2023-09-08 09:56	Microsoft Excel Co...	3 KB
regions_pos	2023-09-08 09:48	JSON File	24 KB
regions_pos_withz	2023-09-08 09:56	JSON File	37 KB
stitched	2023-09-08 09:30	TIF File	1 851 020 ...
stitched_boundary_xystage	2023-09-08 09:48	Microsoft Excel Co...	3 KB
stitched_regions	2023-09-08 09:48	Microsoft Excel Co...	1 KB
stitched_regions	2023-09-08 09:48	TIF File	10 837 KB

## Option b: manual images



## Appendix 2: Folders created after stitching and preprocessing

Name	Date modified	Type	Size
rgb	2023-08-09 16:58	File folder	
sharpie_track	2023-08-09 16:58	File folder	
stitched	2023-08-09 17:04	File folder	
image_names	2023-08-09 16:26	Microsoft Excel Co...	2 KB
params	2023-08-09 16:29	JSON File	2 KB

### Appendix 3: Format image\_names file

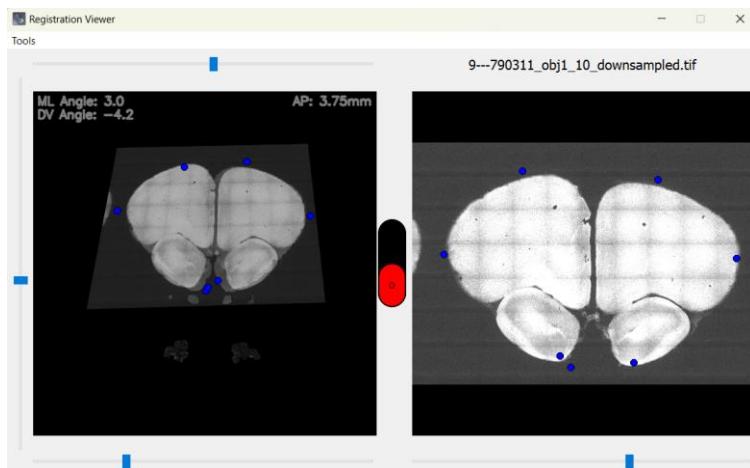
A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB	AC	AD	AE
1																														
2	0 DP-353_oh1_1																													
3	1 DP-353_oh1_2																													
4	2 DP-353_oh1_3																													
5	3 DP-353_oh1_4																													
6	4 DP-353_oh1_5																													
7	5 DP-353_oh1_6																													
8	6 DP-353_oh1_7																													
9	7 DP-353_oh1_8																													
10	8 DP-353_oh1_9																													
11	9 DP-353_oh1_10																													
12	10 DP-353_oh1_11																													
13	11 DP-353_oh1_12																													
14	12 DP-353_oh1_13																													
15	13 DP-353_oh1_14																													
16	14 DP-353_oh1_15																													
17	15 DP-353_oh1_16																													
18	16 DP-353_oh1_17																													
19	17 DP-353_oh1_18																													
20	18 DP-353_oh2_1																													
21	19 DP-353_oh2_2																													
22	20 DP-353_oh2_3																													
23	21 DP-353_oh2_4																													
24	22 DP-353_oh2_5																													
25	23 DP-353_oh2_6																													
26	24 DP-353_oh2_7																													
27	25 DP-353_oh2_8																													
28	26 DP-353_oh2_9																													
29	27 DP-353_oh2_10																													
30	28 DP-353_oh2_11																													
31	29 DP-353_oh2_12																													
32	30 DP-353_oh2_13																													
33	31 DP-353_oh2_14																													
34	32 DP-353_oh2_15																													
35	33 DP-353_oh2_16																													
36	34 DP-353_oh2_17																													
37	35 DP-353_oh2_18																													
38	36 DP-353_oh2_19																													
39	37 DP-353_oh2_20																													
40	38 DP-353_oh2_21																													

#### Appendix 4: Example sagittal and horizontal registration



#### Appendix 5: Example other species registration (rat atlas: whs\_sd\_rat\_39um)

For the rat atlas it is best to lower the resolution of your screen to get a better view on the registrations (example 1280x960)

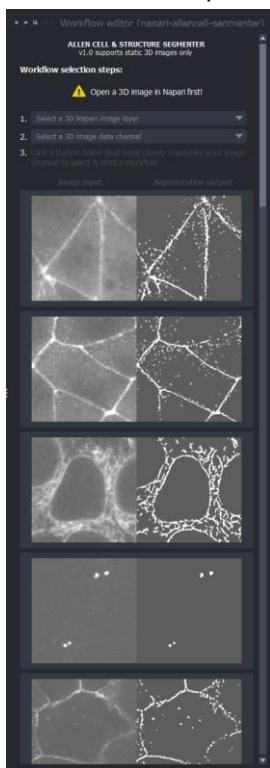


## Appendix 6: Additional information napari-allencell-segmenter

Here we will use the napari-allencell-segmenter tool. More info on the tool please see  
<https://github.com/AllenCell/napari-allencell-segmenter>  
<https://www.youtube.com/watch?v=Se612Vj4ges&t=1522s>  
<https://www.allencell.org/segmenter.html>

To install the plugin, simply open napari, go to plugins>install/uninstall plugins and look for the napari-allencell-segmenter and install. You may have to restart napari after this. The idea of this segmenter tool is that you first find the correct parameters for segmentation of your images and later use this to run the tool over a batch of images.

16. Launch the workflow editor by pressing Plugins>napari-allencell-segmenter>workflow editor
17. The panel should look like this:



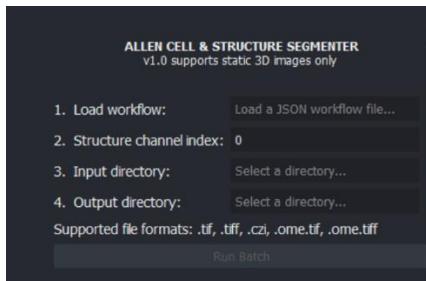
18. Choose an image with good example of what you want to segment (the better your example image, the better your results in the end)
19. Simply drag the image into the middle field of napari.
20. Sometimes the image is not recognized as a 3d image (3 layers, here RGB). Then the error message that you must load a 3d image and the “frozen” buttons remain. You can simply solve this by pressing right mouse click on the image name, duplicating your image and then selecting both images and stack them again (see image). If your image is immediately recognized as 3 layers, you can skip this step. It does not matter if your rgb image is only made from green and red image for example, if it has the rgb format (see preprocessing)
21. Choose the image layer (usually just the name of your image) Choose the channel (R-G-B → 0-1-2), be aware which channel you choose, since the workflow you make for this should also only be used for the cells in the color of this channel! Choose a segmentation type that resembles most to your images. depending on which one you choose, you will have more parameters/processing steps. For

cell bodies I prefer to use: (option 8)



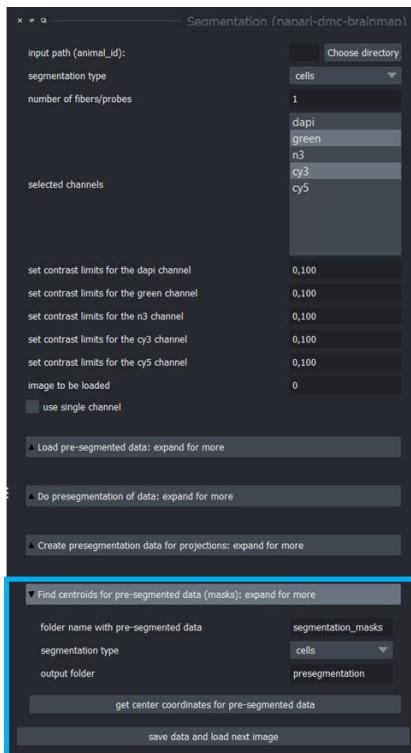
22. Play around with the parameters to find a workflow that is best for your images (and the specific channel). Once you are happy with the parameters, it is best to try it on 1-2 more images before pressing “save workflow”.
23. Then a workflow.json file is made. It is best to rename this file to for example workflow.json so you can see the difference.
24. Repeat this for the other channels you want to perform segmentation on.
25. Launch the batch processing by pressing Plugins>napari-allencell-segmenter>batch processing

26. The panel should look like this:



27. Fill in the information (make sure the workflow file and the channel are accordingly!)
28. Input directory = RGB images
29. Output directory = channel specific so it should have a name like preseg/cells/cy3 (the cells and channel need to be there!)
30. Press run batch
31. This should create segmented images (black and white) and an extra file in the folder you chose.
32. Repeat for the other channels
33. After these steps you have presegmented images with “masks”. Now we need to find the center of these masks. From here we use again the DMC-BrainMap plugin
34. Launch the segmentation by pressing Plugins>dmc\_brainmap>segmentation

35. The panel should look like this:



36. Fill in the animal information.

37. Open the tab for centroids detection.

38. Fill in the input (previous output folder for automatic segmentation) and output folder (choose name)

39. Press 'get center coordinates for pre-segmented data'.

40. This should give you a folder (with chosen name) with subfolder cells which has subfolders for different channels that contain a .csv file for every image with xy coordinates for all the cells.

41. This data can be loaded into the manual segmentation tool.

## Appendix 7: Data format (pre)segmentation cells

A screenshot of a Microsoft Excel spreadsheet titled "DP-311\_obj1\_12\_RGB\_cells". The ribbon is visible at the top with tabs for File, Home, Insert, Page Layout, Formulas, Data, Review, View, Automate, and Help. The "Home" tab is selected. The toolbar below the ribbon includes icons for Cut, Copy, Paste, Format Painter, Clipboard, Font, Alignment, and Number. The active cell is A1. The data starts at row 1 with columns A, B, and C labeled "Position Y" and "Position X". Rows 2 through 12 contain numerical values.

	A	B	C	D	E	F	G	H	I	J	K	L
1		Position Y	Position X									
2	0	1454.578	2941.155									
3	1	1419.398	2947.691									
4	2	1484.952	2987.869									
5	3	1577.804	2984.793									
6	4	1504.176	3038.621									
7	5	1528.59	3051.693									
8	6	1553.966	3064.189									
9	7	1540.125	3122.245									
10	8	1531.282	3137.817									
11	9	1493.603	3148.39									
12	10	1486.49	3143.968									

## Appendix 8: Data format (pre)segmentation projections

A screenshot of a Microsoft Excel spreadsheet titled "DP-117\_obj4\_3\_RGB\_projections". The ribbon and toolbar are identical to Appendix 7. The active cell is A1. The data starts at row 1 with columns A, B, and C labeled "Position Y" and "Position X". Rows 2 through 12 contain numerical values.

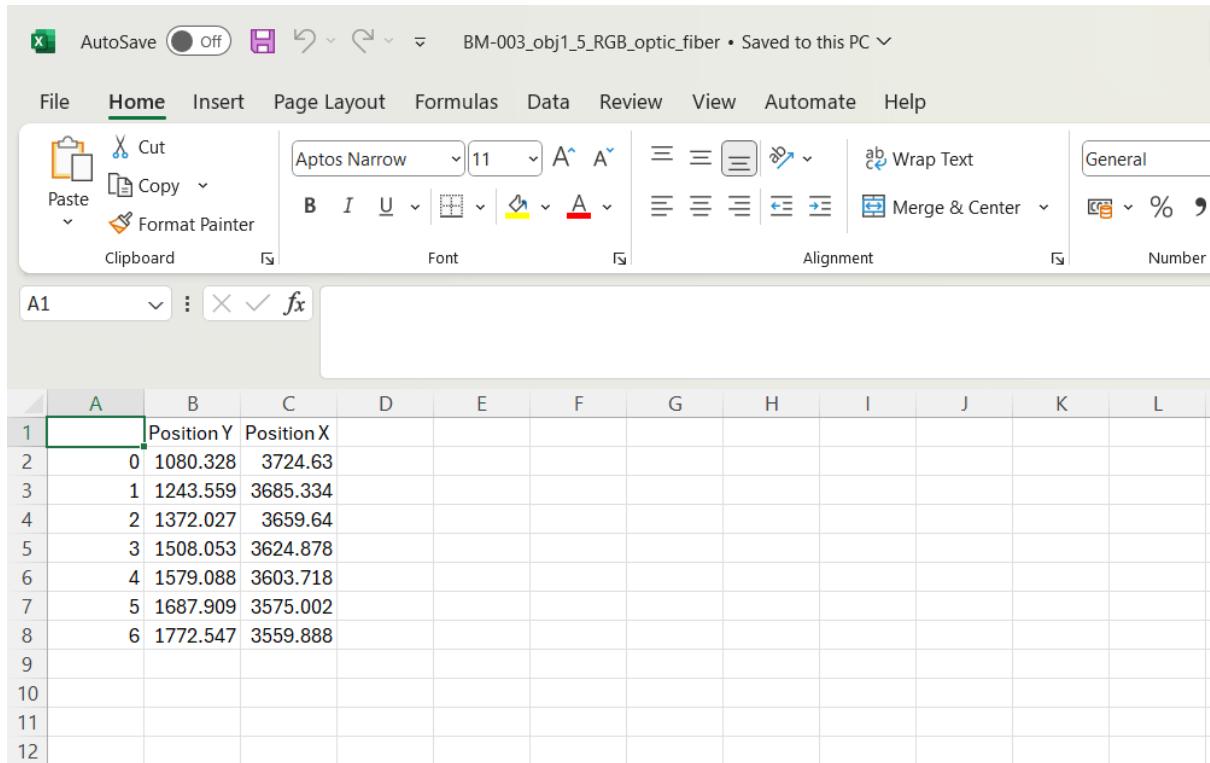
	A	B	C	D	E	F	G	H	I	J	K	L
1		Position Y	Position X									
2	0	0	555									
3	1	0	559									
4	2	0	563									
5	3	0	565									
6	4	0	566									
7	5	0	567									
8	6	0	568									
9	7	0	569									
10	8	0	571									
11	9	0	572									
12	10	0	574									

## Appendix 9: Data format (pre)segmentation injection site

The screenshot shows a Microsoft Excel spreadsheet titled "DP-452\_obj5\_3\_RGB\_injection\_site • Saved to this PC". The ribbon menu is visible at the top, with "Home" selected. The toolbar below includes options for Paste, Cut, Copy, Format Painter, and various font and alignment tools. The active cell is A1. The data is organized into 12 columns labeled A through L. Column A contains row numbers from 1 to 12. Columns B and C contain numerical values for "Position Y" and "Position X" respectively. Column D contains values for "idx\_shape". The data starts from row 2 and ends at row 10.

	A	B	C	D	E	F	G	H	I	J	K	L
1		Position Y	Position X	idx_shape								
2	0	1214.878	5347.934	0								
3	1	1364.439	5586.012	0								
4	2	1507.896	5662.318	0								
5	3	1582.677	5578.381	0								
6	4	1691.033	5354.039	0								
7	5	1645.249	5248.735	0								
8	6	1588.782	5173.954	0								
9	7	1401.067	5070.177	0								
10	8	1282.028	5129.696	0								
11												
12												

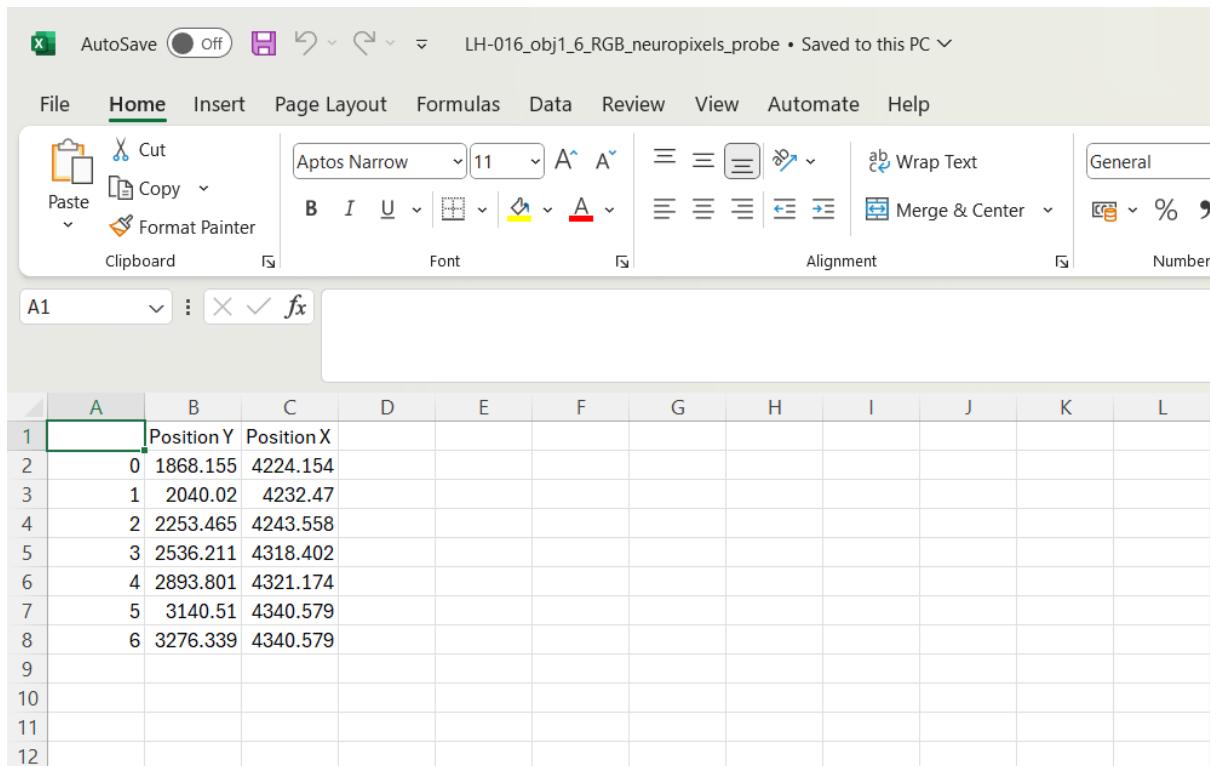
#### Appendix 10: Data format (pre)segmentation optic fiber



A screenshot of Microsoft Excel showing a data table for optic fiber segmentation. The table has columns labeled A, B, and C, with headers "Position Y" and "Position X". The data consists of 8 rows of coordinates:

	A	B	C
1	Position Y	Position X	
2	0	1080.328	3724.63
3	1	1243.559	3685.334
4	2	1372.027	3659.64
5	3	1508.053	3624.878
6	4	1579.088	3603.718
7	5	1687.909	3575.002
8	6	1772.547	3559.888

#### Appendix 11: Data format (pre)segmentation Neuropixel probe



A screenshot of Microsoft Excel showing a data table for neuropixel probe segmentation. The table has columns labeled A, B, and C, with headers "Position Y" and "Position X". The data consists of 8 rows of coordinates:

	A	B	C
1	Position Y	Position X	
2	0	1868.155	4224.154
3	1	2040.02	4232.47
4	2	2253.465	4243.558
5	3	2536.211	4318.402
6	4	2893.801	4321.174
7	5	3140.51	4340.579
8	6	3276.339	4340.579

#### Appendix 12: Data results file cells

AutoSave (Off) DP-347\_cells • Saved to this PC

File Home Insert Page Layout Formulas Data Review View Automate Help

Cut Copy Format Painter Paste

Font Alignment Number

A1

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1		name	acronym	structure_iap_mm	dv_mm	ml_mm	ap_coords	dv_coords	ml_coords	section_name				
2	0	Orbital are	ORBI5	630	3.16	-2.95	-1.24	224	295	694	DP-347_obj1_4_downsampled.tif			
3	0	Orbital are	ORBI2/3	412	3.07	-3.42	-1.36	233	342	706	DP-347_obj1_5_downsampled.tif			
4	0	Secondary MOs5		767	2.95	-2.56	-1.18	245	256	688	DP-347_obj1_6_downsampled.tif			
5	1	Frontal pol	FRP5	5.26E+08	2.95	-2.64	-1.26	245	264	696	DP-347_obj1_6_downsampled.tif			
6	2	Frontal pol	FRP5	5.26E+08	2.97	-2.94	-1.5	243	294	720	DP-347_obj1_6_downsampled.tif			
7	3	Frontal pol	FRP5	5.26E+08	2.96	-2.98	-1.42	244	298	712	DP-347_obj1_6_downsampled.tif			
8	4	Secondary MOs5		767	2.97	-3.13	-1.67	243	313	737	DP-347_obj1_6_downsampled.tif			
9	5	Orbital are	ORBI5	630	2.97	-3.17	-1.59	243	317	729	DP-347_obj1_6_downsampled.tif			
10	6	Orbital are	ORBI5	630	2.98	-3.29	-1.62	242	329	732	DP-347_obj1_6_downsampled.tif			
11	7	Orbital are	ORBI5	630	2.97	-3.08	-1.51	243	308	721	DP-347_obj1_6_downsampled.tif			
12	8	Orbital are	ORBI5	630	2.97	-3.12	-1.46	243	312	716	DP-347_obj1_6_downsampled.tif			
13	0	Orbital are	ORBI5	630	2.78	-3.21	1.68	262	321	402	DP-347_obj1_7_downsampled.tif			
14	1	Secondary MOs5		767	2.77	-2.78	1.69	263	278	401	DP-347_obj1_7_downsampled.tif			
15	2	Secondary MOs5		767	2.79	-2.54	0.92	261	254	478	DP-347_obj1_7_downsampled.tif			
16	3	Secondary MOs5		767	2.78	-2.43	0.86	262	243	484	DP-347_obj1_7_downsampled.tif			
17	4	Orbital are	ORBI2/3	412	2.88	-3.93	-1.6	252	393	730	DP-347_obj1_7_downsampled.tif			

### Appendix 13: Data results file projections

AutoSave (Off) DP-117\_projections • Saved to this PC

File Home Insert Page Layout Formulas Data Review View Automate Help

Cut Copy Format Painter Paste

Font Alignment Number

A1

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1		name	acronym	ap_mm	dv_mm	ml_mm	sphinx_id	zpixel	ypixel	xpixel				
2	0	Nucleus α\ACB		1.82	-5.74	-1	576	358	574	670	DP-117_obj1_17_downsampled.tif			
3	1	Nucleus α\ACB		1.82	-5.74	-1	576	358	574	670	DP-117_obj1_17_downsampled.tif			
4	2	Nucleus α\ACB		1.82	-5.74	-1	576	358	574	670	DP-117_obj1_17_downsampled.tif			
5	3	Nucleus α\ACB		1.82	-5.74	-1	576	358	574	670	DP-117_obj1_17_downsampled.tif			
6	4	Nucleus α\ACB		1.82	-5.72	-1	576	358	572	670	DP-117_obj1_17_downsampled.tif			
7	5	Nucleus α\ACB		1.82	-5.71	-0.99	576	358	571	669	DP-117_obj1_17_downsampled.tif			
8	6	Nucleus α\ACB		1.82	-5.71	-1.02	576	358	571	672	DP-117_obj1_17_downsampled.tif			
9	7	Nucleus α\ACB		1.82	-5.71	-1	576	358	571	670	DP-117_obj1_17_downsampled.tif			
10	8	Nucleus α\ACB		1.82	-5.71	-1.01	576	358	571	671	DP-117_obj1_17_downsampled.tif			
11	9	Nucleus α\ACB		1.82	-5.71	-1.01	576	358	571	671	DP-117_obj1_17_downsampled.tif			
12	10	Nucleus α\ACB		1.82	-5.71	-0.99	576	358	571	669	DP-117_obj1_17_downsampled.tif			
13	11	Nucleus α\ACB		1.82	-5.71	-1.01	576	358	571	671	DP-117_obj1_17_downsampled.tif			
14	12	Nucleus α\ACB		1.82	-5.71	-0.99	576	358	571	669	DP-117_obj1_17_downsampled.tif			
15	13	Nucleus α\ACB		1.82	-5.71	-1	576	358	571	670	DP-117_obj1_17_downsampled.tif			
16	14	Nucleus α\ACB		1.82	-5.71	-1.01	576	358	571	671	DP-117_obj1_17_downsampled.tif			
17	15	Nucleus α\ACB		1.82	-5.71	-1	576	358	571	670	DP-117_obj1_17_downsampled.tif			

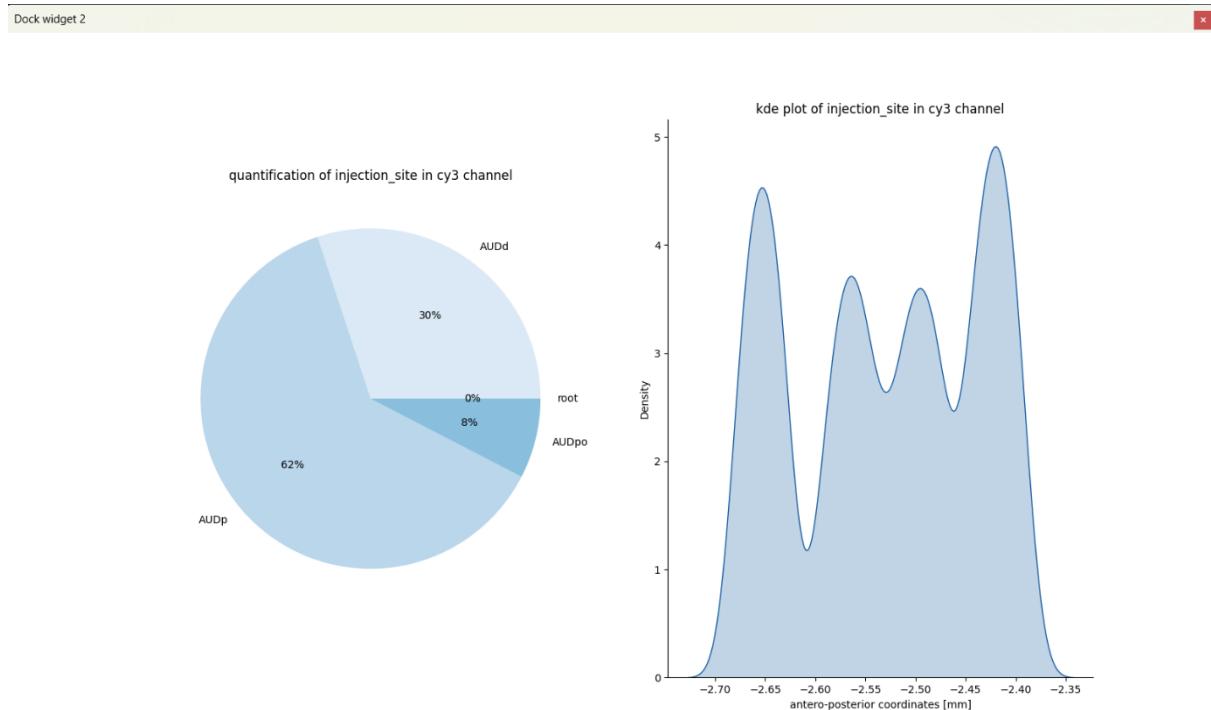
#### Appendix 14: Data results file injection site and quantification\_injection\_site

DP-452\_injection\_site • Saved to this PC

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1		name	acronym	structure_id	ap_mm	dv_mm	ml_mm	ap_coords	dv_coords	ml_coords	section_name			
2	0	Dorsal aud	AUDd2/3	600	-2.46	-1.95	-4.26	786	195	996	DP-452_obj5_3_downsampled.tif			
3	1	Dorsal aud	AUDd2/3	600	-2.46	-1.95	-4.27	786	195	997	DP-452_obj5_3_downsampled.tif			
4	2	Dorsal aud	AUDd2/3	600	-2.46	-1.95	-4.28	786	195	998	DP-452_obj5_3_downsampled.tif			
5	3	Dorsal aud	AUDd2/3	600	-2.46	-1.95	-4.29	786	195	999	DP-452_obj5_3_downsampled.tif			
6	4	Dorsal aud	AUDd2/3	600	-2.46	-1.95	-4.3	786	195	1000	DP-452_obj5_3_downsampled.tif			
7	5	Dorsal aud	AUDd2/3	600	-2.46	-1.96	-4.23	786	196	993	DP-452_obj5_3_downsampled.tif			
8	6	Dorsal aud	AUDd2/3	600	-2.46	-1.96	-4.24	786	196	994	DP-452_obj5_3_downsampled.tif			
9	7	Dorsal aud	AUDd2/3	600	-2.46	-1.96	-4.25	786	196	995	DP-452_obj5_3_downsampled.tif			
10	8	Dorsal aud	AUDd2/3	600	-2.46	-1.96	-4.26	786	196	996	DP-452_obj5_3_downsampled.tif			
11	9	Dorsal aud	AUDd2/3	600	-2.46	-1.96	-4.27	786	196	997	DP-452_obj5_3_downsampled.tif			
12	10	Dorsal aud	AUDd2/3	600	-2.46	-1.96	-4.28	786	196	998	DP-452_obj5_3_downsampled.tif			
13	11	Dorsal aud	AUDd2/3	600	-2.46	-1.96	-4.29	786	196	999	DP-452_obj5_3_downsampled.tif			
14	12	Dorsal aud	AUDd2/3	600	-2.46	-1.96	-4.3	786	196	1000	DP-452_obj5_3_downsampled.tif			
15	13	Dorsal aud	AUDd2/3	600	-2.46	-1.96	-4.31	786	196	1001	DP-452_obj5_3_downsampled.tif			
16	14	Dorsal aud	AUDd2/3	600	-2.46	-1.96	-4.32	786	196	1002	DP-452_obj5_3_downsampled.tif			
17	15	Dorsal aud	AUDd2/3	600	-2.46	-1.98	-4.18	786	198	988	DP-452_obj5_3_downsampled.tif			

quantification\_injection\_site • Saved to this PC

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	animal_id	AUDd	AUDp	AUDpo	root									
2	DP-452	0.30053	0.623589	0.075832	4.91E-05									
3														
4														
5														



## Appendix 15: Data results file optic fiber

File Home Insert Page Layout Formulas Data Review View Automate Help

AutoSave (off) Search

Cut Copy Paste Format Painter

Font Alignment Number

General Conditional Formatting

A1

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1		name	acronym	structure_i	ap_mm	dv_mm	ml_mm	ap_coords	dv_coords	ml_coords	section_name			
2	0	Secondary MOs5		767	2.48	-2.19	-1.1	292	219	680	BM-003_obj1_4_downsampled.tif			
3	1	Orbital are	ORBvl6a	608	2.48	-3.04	-1.07	292	304	677	BM-003_obj1_4_downsampled.tif			
4	2	Secondary MOs6a		1021	2.48	-2.61	-1.07	292	261	677	BM-003_obj1_4_downsampled.tif			
5	0	Secondary MOs2/3		962	2.37	-1.73	-1.22	303	173	692	BM-003_obj1_5_downsampled.tif			
6	1	Secondary MOs5		767	2.37	-2.07	-1.17	303	207	687	BM-003_obj1_5_downsampled.tif			
7	2	Secondary MOs5		767	2.37	-2.34	-1.15	303	234	685	BM-003_obj1_5_downsampled.tif			
8	3	Secondary MOs6a		1021	2.37	-2.63	-1.11	303	263	681	BM-003_obj1_5_downsampled.tif			
9	4	Secondary MOs6a		1021	2.37	-2.78	-1.08	303	278	678	BM-003_obj1_5_downsampled.tif			
10	5	Orbital are	ORBvl6a	608	2.37	-3.01	-1.04	303	301	674	BM-003_obj1_5_downsampled.tif			
11	6	Orbital are	ORBvl6a	608	2.37	-3.18	-1.03	303	318	673	BM-003_obj1_5_downsampled.tif			
12	0	Secondary MOs1		656	2.31	-1.56	-1.19	309	156	689	BM-003_obj1_6_downsampled.tif			
13	1	Secondary MOs5		767	2.31	-1.98	-1.12	309	198	682	BM-003_obj1_6_downsampled.tif			
14	2	Secondary MOs6a		1021	2.31	-2.53	-1.08	309	253	678	BM-003_obj1_6_downsampled.tif			
15	3	Orbital are	ORBvl6a	608	2.31	-3.04	-1.03	309	304	673	BM-003_obj1_6_downsampled.tif			
16	4	Orbital are	ORBvl6a	608	2.31	-3.28	-1.03	309	328	673	BM-003_obj1_6_downsampled.tif			
17	5	Orbital are	ORBvl5	1125	2.31	-3.58	-0.99	309	358	669	BM-003_obj1_6_downsampled.tif			

## Appendix 16: Data results file Neuropixels probe

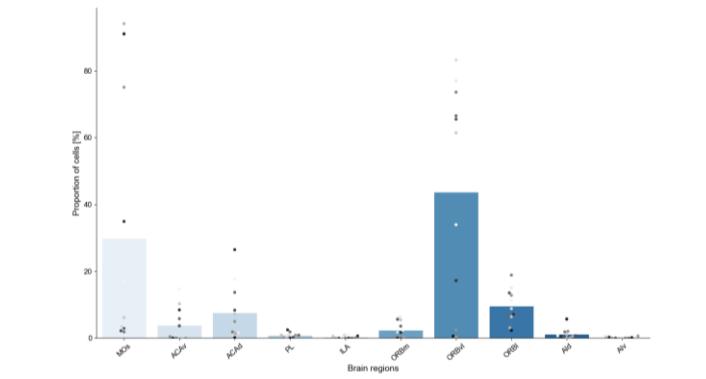
Screenshot of Microsoft Excel showing the "LH-016\_neuropixels\_probe" spreadsheet. The ribbon tabs are Home, Insert, Page Layout, Formulas, Data, Review, View, Automate, Help. The Home tab is selected.

The table contains 17 rows of data, starting from row 1. The columns are labeled A through N. The data includes names, acronyms, coordinates, and section names.

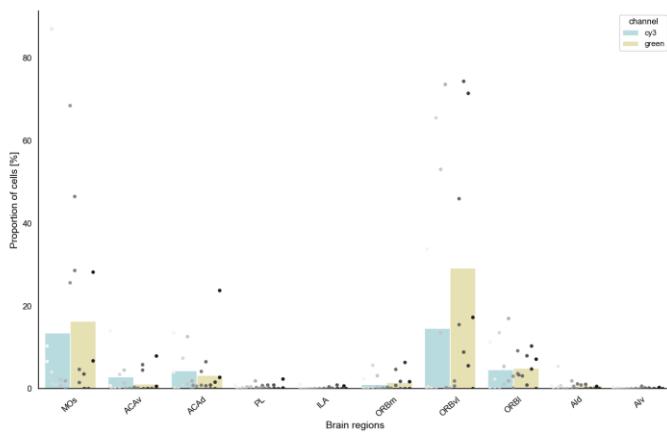
	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1		name	acronym	structure_i	ap_mm	dv_mm	ml_mm	ap_coords	dv_coords	ml_coords	section_name			
2	0	Primary mx	MOp2/3		943	-0.12	-0.81	-0.86	552	81	656	LH-016_obj1_2_downsampled.tif		
3	1	Secondary	MOs5		767	-0.13	-1.2	0.83	553	120	653	LH-016_obj1_2_downsampled.tif		
4	2	Secondary	MOs6a		1021	-0.13	-1.59	-0.8	553	159	650	LH-016_obj1_2_downsampled.tif		
5	3	Anterior ci	ACAd6b		927	-0.14	-1.99	-0.77	554	199	647	LH-016_obj1_2_downsampled.tif		
6	0	root	root		0	-0.2	-0.65	-0.41	560	65	611	LH-016_obj1_3_downsampled.tif		
7	1	Secondary	MOs5		767	-0.2	-1.04	-0.43	560	104	613	LH-016_obj1_3_downsampled.tif		
8	2	Anterior ci	ACAd5		1015	-0.21	-1.63	-0.49	561	163	619	LH-016_obj1_3_downsampled.tif		
9	3	Anterior ci	ACAv6a		810	-0.22	-2.16	-0.53	562	216	623	LH-016_obj1_3_downsampled.tif		
10	0	Secondary	MOs6a		1021	-0.3	-1.74	-0.85	570	174	655	LH-016_obj1_4_downsampled.tif		
11	1	cingulum	c ing		940	-0.3	-1.95	0.84	570	195	654	LH-016_obj1_4_downsampled.tif		
12	2	corpus cal	ccb		4.85E+08	-0.31	-2.3	-0.82	571	230	652	LH-016_obj1_4_downsampled.tif		
13	3	third ventri	V3		129	-0.33	-3.8	-0.83	573	380	653	LH-016_obj1_4_downsampled.tif		
14	0	Anterior ci	ACAv6a		810	-0.35	-2	-0.61	575	200	631	LH-016_obj1_5_downsampled.tif		
15	1	corpus cal	ccb		4.85E+08	-0.36	-2.36	-0.61	576	236	631	LH-016_obj1_5_downsampled.tif		
16	2	Lateral sepi	LSc		250	-0.36	-2.67	-0.6	576	267	630	LH-016_obj1_5_downsampled.tif		
17	3	third ventri	V3		129	-0.38	-3.56	-0.67	578	356	637	LH-016_obj1_5_downsampled.tif		

## Appendix 17: Barplots

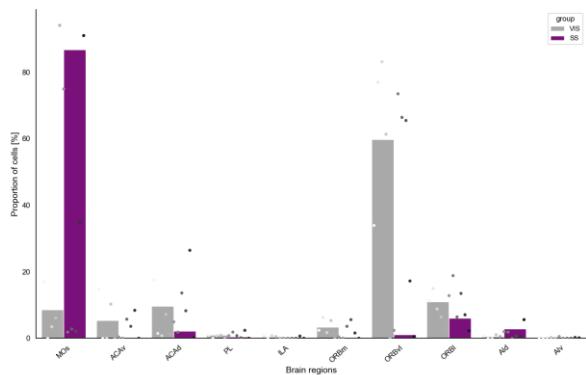
*Barplot: all cells*



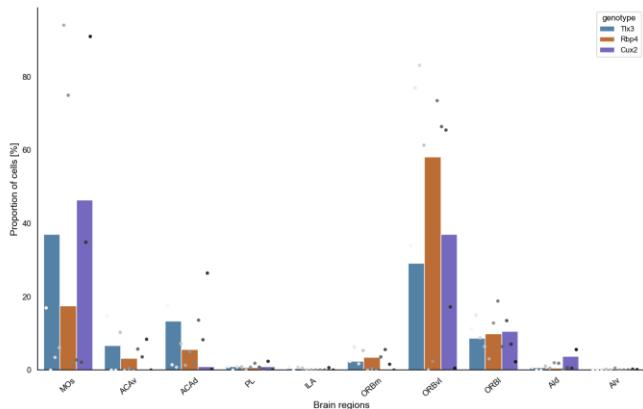
*Barplot: split by channel*



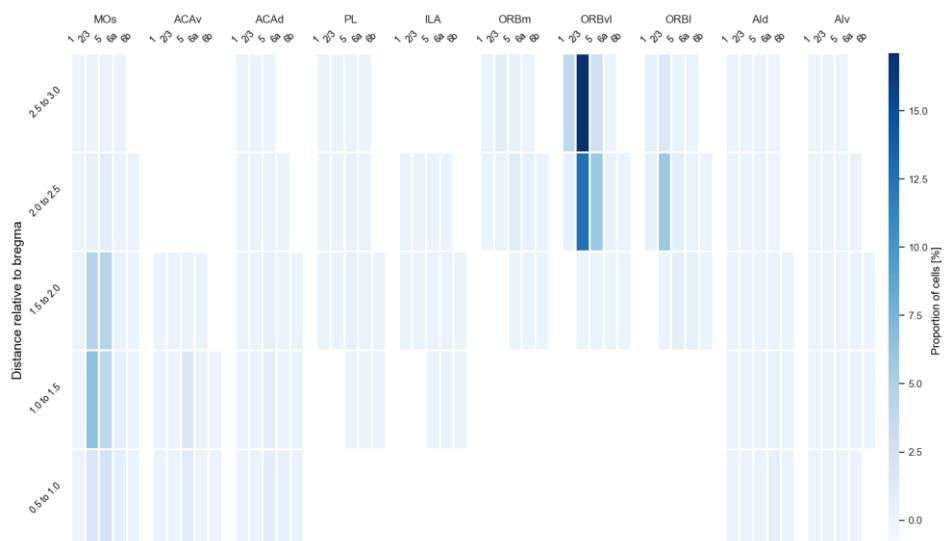
*Barplot: split by experimental group*



*Barplot: split by genotype*

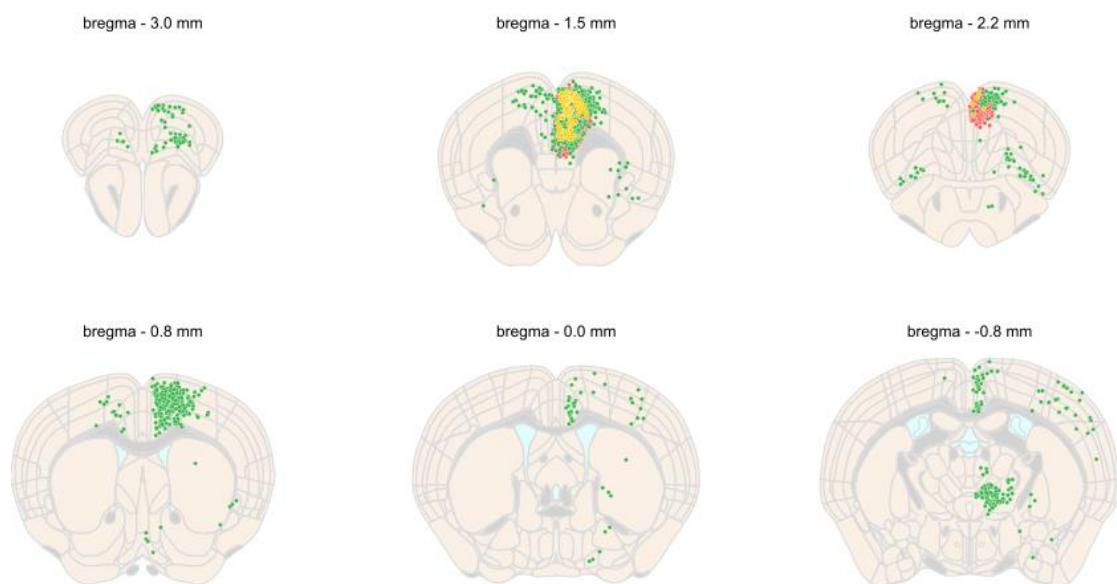


### Appendix 18: Heatmap

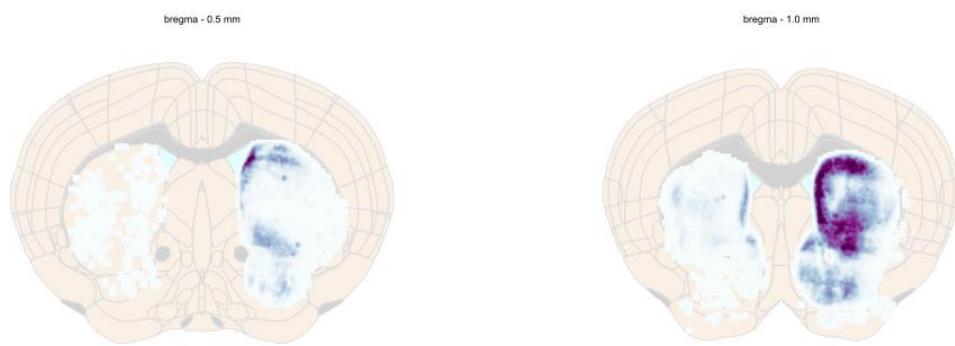


### Appendix 19: Brain sections

#### *Brain sections plot: cells*



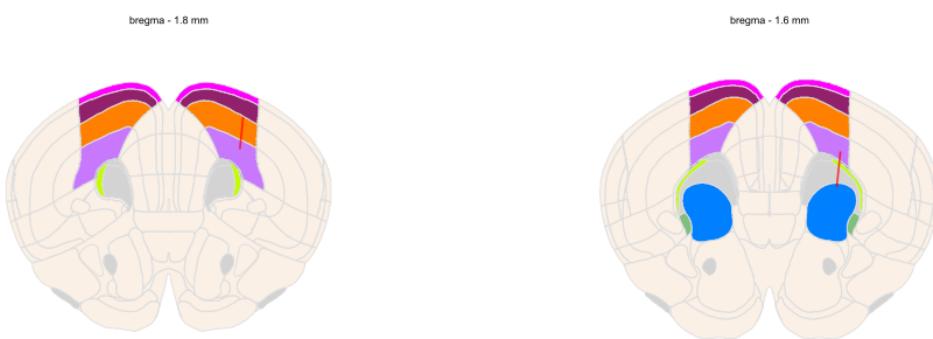
*Brain sections plot: projection density*



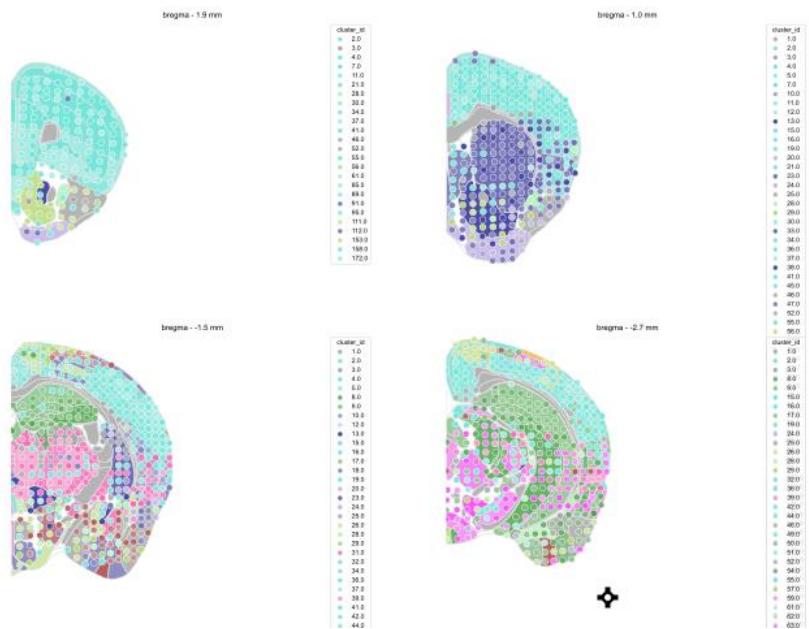
*Brain sections plot: injection site and optic fiber*



*Brain sections plot: neuropixels probe and selected brain areas colored*

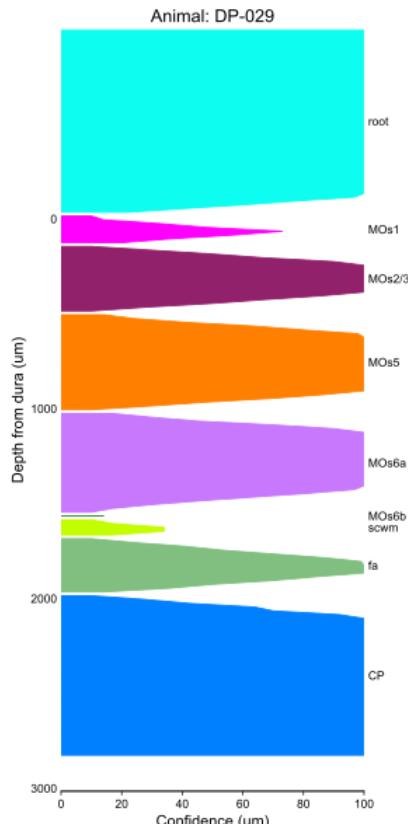


*Brain sections plot: spatial transcriptomics data (brain areas colored according to max. cluster identity; spots according to cluster identity)*



## Appendix 20: Estimate and visualize neuropixels probe

### A: Neuropixels\_probe\_0.svg



### B: Neuropixels\_probe\_0\_data.csv

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A1

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1	Channel_L	Channel_R	Distance_I	Depth(um)	Inside_Bra	Voxel_AP	Voxel_DV	Voxel_ML	structure_i	Acronym	Name		Distance_To_Nearest_Structure(um)			
2	0	1	2	175	5233	TRUE	606	582	660	1097	HY	Hypothalai	30			
3	1	3	4	195	5213	TRUE	606	580	660	1097	HY	Hypothalai	45			
4	2	5	6	215	5193	TRUE	606	578	659	1097	HY	Hypothalai	40			
5	3	7	8	235	5173	TRUE	605	576	659	1097	HY	Hypothalai	41			
6	4	9	10	255	5153	TRUE	605	574	659	1097	HY	Hypothalai	42			
7	5	11	12	275	5133	TRUE	605	572	659	1097	HY	Hypothalai	48			
8	6	13	14	295	5113	TRUE	605	570	659	1097	HY	Hypothalai	51			
9	7	15	16	315	5093	TRUE	605	568	659	1097	HY	Hypothalai	59			
10	8	17	18	335	5073	TRUE	605	566	659	1097	HY	Hypothalai	60			
11	9	19	20	355	5053	TRUE	604	564	659	1097	HY	Hypothalai	60			
12	10	21	22	375	5033	TRUE	604	562	659	1097	HY	Hypothalai	61			
13	11	23	24	395	5013	TRUE	604	560	658	1097	HY	Hypothalai	59			
14	12	25	26	415	4993	TRUE	604	558	658	1097	HY	Hypothalai	71			
15	13	27	28	435	4973	TRUE	604	556	658	1097	HY	Hypothalai	83			
16	14	29	30	455	4953	TRUE	603	554	658	1097	HY	Hypothalai	91			
17	15	31	32	475	4933	TRUE	603	552	658	1097	HY	Hypothalai	100			
18	16	33	34	495	4913	TRUE	603	550	658	1097	HY	Hypothalai	89			
19	17	35	36	515	4893	TRUE	603	548	658	1097	HY	Hypothalai	72			
20	18	37	38	535	4873	TRUE	603	546	658	1097	HY	Hypothalai	56			
21	19	39	40	555	4853	TRUE	602	544	658	1097	HY	Hypothalai	44			
22	20	41	42	575	4833	TRUE	602	542	657	1097	HY	Hypothalai	28			
23	21	43	44	595	4813	TRUE	602	540	657	1097	HY	Hypothalai	14			
24	22	45	46	615	4793	TRUE	602	538	657	797	ZI	Zona incer	10			
25	23	47	48	635	4773	TRUE	602	536	657	797	ZI	Zona incer	22			
26	24	49	50	655	4753	TRUE	601	534	657	797	ZI	Zona incer	22			
27	25	51	52	675	4733	TRUE	601	532	657	797	ZI	Zona incer	33			
28	26	53	54	695	4713	TRUE	601	530	657	797	ZI	Zona incer	43			
29	27	55	56	715	4693	TRUE	601	528	657	797	ZI	Zona incer	53			
30	28	57	58	735	4673	TRUE	601	526	657	797	ZI	Zona incer	61			
31	29	59	60	755	4653	TRUE	600	524	656	797	ZI	Zona incer	65			
32	30	61	62	775	4633	TRUE	600	522	656	797	ZI	Zona incer	73			
33	31	63	64	795	4613	TRUE	600	520	656	797	ZI	Zona incer	78			
34	32	65	66	815	4593	TRUE	600	518	656	797	ZI	Zona incer	64			
35	33	67	68	835	4573	TRUE	600	516	656	797	ZI	Zona incer	53			
36	34	69	70	855	4553	TRUE	599	514	656	797	ZI	Zona incer	53			
37	35	71	72	875	4533	TRUE	599	512	656	797	ZI	Zona incer	41			
38	36	73	74	895	4513	TRUE	599	510	656	797	ZI	Zona incer	22			
39	37	75	76	915	4493	TRUE	599	508	656	797	ZI	Zona incer	10			
40	38	77	78	935	4473	TRUE	599	506	655	262	RT	Reticular n	14			

## C: Neuropixels\_probes\_data.json

## Appendix 21: Choosing colors in DMC-BrainMap

Unless otherwise specified in the respective tooltip of the entry, colors in DMC-BrainMap are specified in the following way:

1. Provide a comma separated list of colors, e.g. “green,red,blue”
  - a. This will explicitly name the colors for each subgroup, if less items than colors are given, only the first matching colors are used, if less colors than items are given, random colors are added
2. Provide a colormap by adding a “c:” before the name of the name of the colormap, e.g. “c:Reds”
  - a. This will use the specified cmap
3. Create a custom cmap by entering a color name or a combination of colors (in case cmaps are required, e.g. for heatmaps):
  - a. Case 1: create a colormap for a specified color by entering the name of the color, e.g. “lightgreen”. DMC-BrainMap will create a custom, linear colormap from white to the given color (in this case lightgreen)
  - b. Case 2: create a custom, linear colormap for two colors by entering the names of the colors separated by “-”, e.g. “blue-darkorange”. This will create a linear colormap from blue to darkorange
  - c. Case 3: Create a custom, diverging colormap for two colors by entering the names of the colors separated by “:”, e.g. “green-red”. This will create a diverging colormap from green to red centered on white
  - d. Case 4: Create a custom, diverging colormap for three colors by entering the names of the colors separated by “:”, e.g. “green:blue:red”. This will create a diverging colormap from green to red centered on blue.