

HERBS Cookbook

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HERBS (Histological E-data Registration in rodent Brain Spaces), is an open-source graphical user interface which allows users to perform four core functionalities: (i) plan electrode insertions and target viral or other injections before surgery, (ii) process and edit histological images, (iii) register objects such as probes, virus, cells and slice boundaries from histological images, (iv) visualize the brain in 3D with defined functions.

HERBS was developed initially to reconstruct the trajectories of Neuropixels probes, and to identify which electrode sites traversed which brain regions during *in vivo* recordings. Accordingly, HERBS can reconstruct multiple electrodes within one animal, and place electrode reconstructions across animals in common reference coordinates. Similar to previous mouse brain tool kits ([shamash2018tool](#)), it can calculate anatomical coordinates to target desired brain regions when planning a surgery. In addition to electrophysiological functionalities, HERBS can register and visualize 3D volumes of virus expression, anatomical tracers, lesions or neurodegeneration extending through multiple sections, and it works equally well in coronal, horizontal and sagittal planes of sectioning. It also allows users to rotate the atlas brain template up to 30 degrees along X, Y and Z axes to compensate for out-of-plane tissue sectioning. The current version of HERBS can load light-sheet microscopy images for basic visualization, but the full suite of functionalities for light-sheet data will come in a later release.

HERBS provides manual and automatic cell counting features, as well as contouring brain tissue in 3D space. It is compatible with potentially any method of tissue staining, a range of common image formats (e.g. .jpeg or .tiff), and any microscopy data so long as the regions of interest are visible and can be delineated by the user.

To maximize general accessibility, HERBS was written as a downloadable Python package that does not require coding knowledge beyond running a single command line for installation. HERBS is available openly on GitHub, PyPI and is compatible with Windows, macOS and Linux operating systems. HERBS currently runs both rat (Waxholm Space Atlas of the Sprague Dawley Rat Brain ([papp2014waxholm](#))) and mouse (Allen mouse brain atlas([wang2020allen](#))) atlases as plugins. Several of the core functionalities of HERBS can be also accessed in a related, previously released Python-based software, TRACER (Tool kit for Reconstructing Anatomical Coordinates in Rats) which is also available through the Whitlock lab GitHub repository.

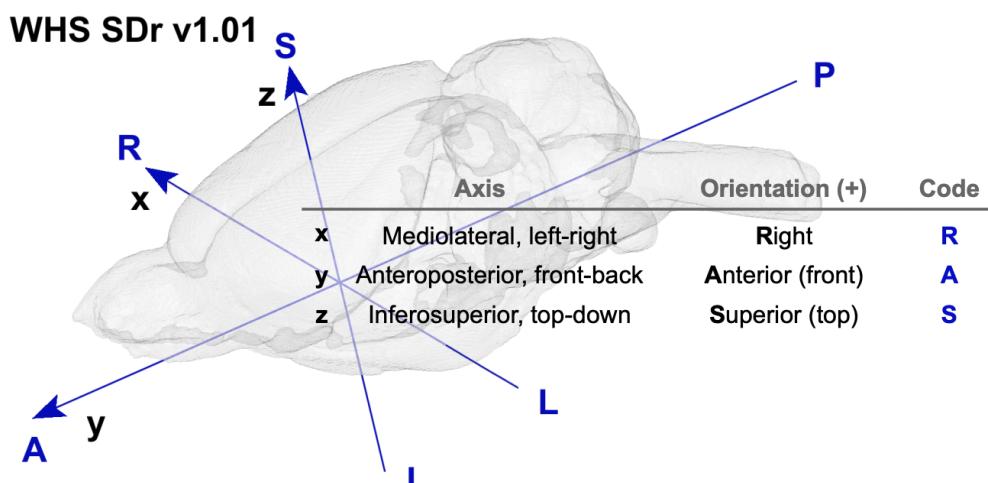
VOLUME ATLAS COORDINATES

A volume (or volumetric) atlas is based on high-resolution volumetric images typically obtained by magnetic resolution imaging and are in three dimensional space.

In the HERBS world, users can upload any volume atlas they like as long as the atlas has the same coordinate system as specified in HERBS. The coordinate system in HERBS is the same as the Waxholm space (WHS) atlas v1.01 (illustrated below). The Waxholm space atlas of the Sprague Dawley rat brain is an open access volumetric atlas. The digital atlas contains more than 200 detailed regional delineations, acquired from high-resolution MRI and DTI scans of an 80 day old male rat. HERBS also provides downloading and processing functions for the Allen mouse brain atlas with sections in three different thicknesses (10, 25 or 50 microns). The Allen Mouse Brain atlas exists in a different coordinate system than the WHS atlas. To make it compatible, HERBS modifies the original Allen atlas coordinate system to make it suitable for the HERBS environment.

For more information on the Waxholm Space atlas visit:

<https://www.nitrc.org/projects/whs-sd-atlas> Coordinates for navigating the brain volume in HERBS are defined by the Waxholm Space coordinate system shown here:



Chapter 3

TESTED OPERATING SYSTEMS AND PACKAGE DEPENDENCIES

HERBS requires 64bit Operating Systems and 64bit Python, and was built with a MacBook Pro (Big Sur). For wider usability with other machines, we have tested HERBS on other operating systems and different versions of Python with their different respective dependencies. Testing is ongoing and we list here only a portion of the tested combinations.

HERBS uses **OpenGL** for 3D visualisations. For linux and Windows users, OpenGL must be installed and, for macOS users, **Xcode** must be installed before using HERBS.

3.1 MacOS

- Monterey 12.4; 3.8 GHz 8-Core Intel Core i7; 64 GB RAM
- Big Sur 11.6; 2.3 GHz Quad-Core Intel Core i7; 32 GB RAM

Both machines work outstanding with Python 3.8.10 and Python 3.9.9 specifications in PyCharm and Terminal virtual environment.

3.2 Windows

- Windows 10; 3.20 GHz Intel core i7; 32 GB RAM
- Windows 10; 3.10 GHz Intel core i5; 32 GB RAM

Note: HERBS installation with Windows is currently only compatible with Python 3.8. We recommend Windows user to install Python 3.8. Making HERBS compatible with Python 3.9 is under testing.

3.3 Linux

- Kubuntu 18.04; 2.20 GHz Intel core i5; 16 GB RAM
- Ubuntu 18.04; 1.90 GHz Intel 8core i7; 32 GB RAM

3.4 Tested Python and Dependencies

3.4.1 Python 3.8.10

Click on hyperlink to download Python 3.8.10

aicspylibczi 3.0.3	nibabel 3.2.1	Tifffile 2021.11.2	csv
PyQt5 5.14.2	Opencv-Python 4.5.4.60	PyOpenGL 3.1.5	QtRangeSlider 0.1.5
Numpy 1.20.3	Scipy 1.7.3	Numba 0.54.1	Pandas 1.3.5
Pathlib 1.0.1	Pyqtgraph 0.12.3	Requestes 2.26.0	Natsort 8.0.2
queue	pickle	colorsys	warnings

3.4.2 Python 3.9.9

Click on hyperlink to download Python 3.9.9

aicspylibczi 3.0.3	nibabel 3.2.1	Tifffile 2021.11.2	csv
PyQt5 5.14.2/5.15.6	Opencv-Python 4.5.4.60	PyOpenGL 3.1.5/3.1.6	QtRangeSlider 0.1.5
Numpy 1.20.3	Scipy 1.7.3	Numba 0.54.1/0.55.1	Pandas 1.3.5
Pathlib 1.0.1	Pyqtgraph 0.12.3	Requestes 2.26.0	Natsort 8.0.2
queue	pickle	colorsys	warnings

To install HERBS you need Python installed on your computer (version 3.8.10 is recommended).

4.1 Install through PyCharm

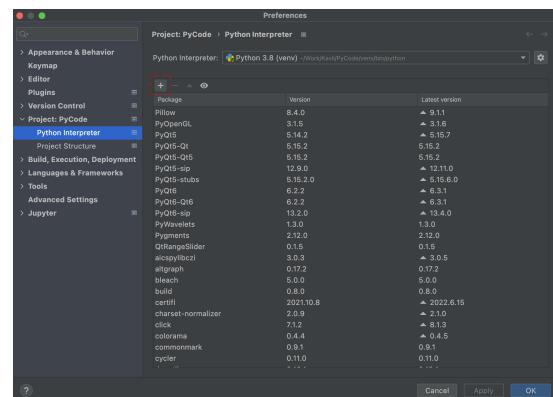
In this section, we will illustrate how to install herbs using PyCharm assuming that Python requirements are satisfied.

If PyCharm is not installed, please install PyCharm.

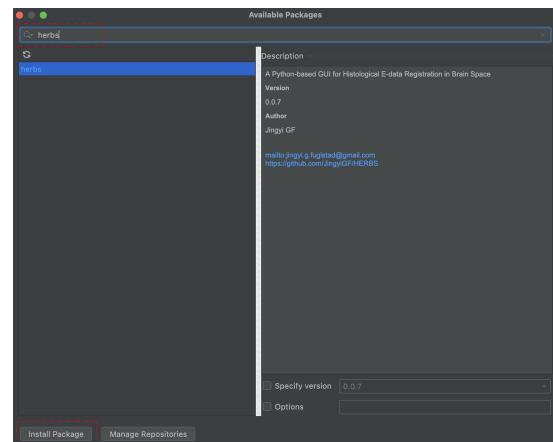
Follow link for more details about on how to install and set up PyCharm to create a Python project

Install, uninstall, and upgrade packages (<https://www.jetbrains.com/help/pycharm/installing-uninstalling-and-upgrading-packages.html>)

- Go to Python Interpreter
- Windows and Linux: **File Settings –> Project –> Python Interpreter**
- macOS: **PyCharm –> Preferences –> Project –> Python Interpreter**
- And press + button.



- Type **herbs**
- Specify the latest version
- Click on **Install Package** button

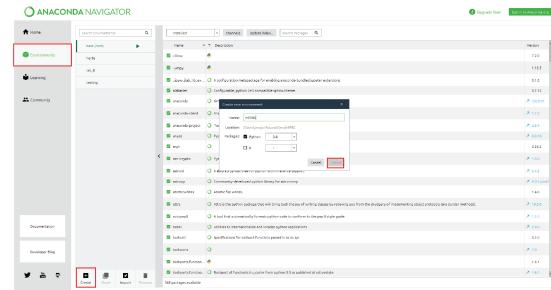


4.2 Install from Anaconda

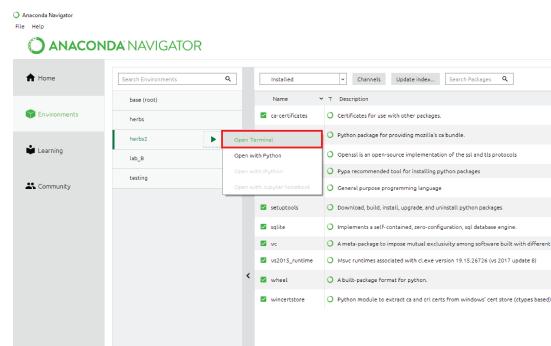
In this section installation of HERBS through Anaconda will be illustrated.

If Anaconda is not installed, please install Anaconda. To create a conda environment:

- Open Anaconda Navigator:
Click Environment -> Create -> name of the Environment -> Select Python version -> Click on Create



- Click on the environment name to activate it
- Left click on the mouse
- Click on Open Terminal



Using your conda environment terminal, you can open the HERBS window with:

```
1 import herbs
2 herbs.run_herbs()
```

If opening a conda terminal directly from the start menu provided the suitable Python version has been already installed, type:

```
1 conda activate herbs
```

and then -

```
1 import herbs
2 herbs.run_herbs()
```

More information on installing can be found in the project description: <https://pypi.org/project/herbs/>

4.3 Install from GitHub (advanced installation)

If you, as a user is skilled at using terminal and/or command prompt; Navigate to the path directory where you will store the software using a terminal or command prompt and enter the command:

```
1 git clone https://github.com/Whitlock-Group/HERBS.git
```

5.1 Graphic User Interface

HERBS has five different interactive working areas: the **Menu Bar**, **Side Bar**, **Tool Bar**, **Plot Windows** and **Status Bar** (Figure 5.1). In this chapter, we will introduce each area separately and illustrate every widget listed in HERBS.

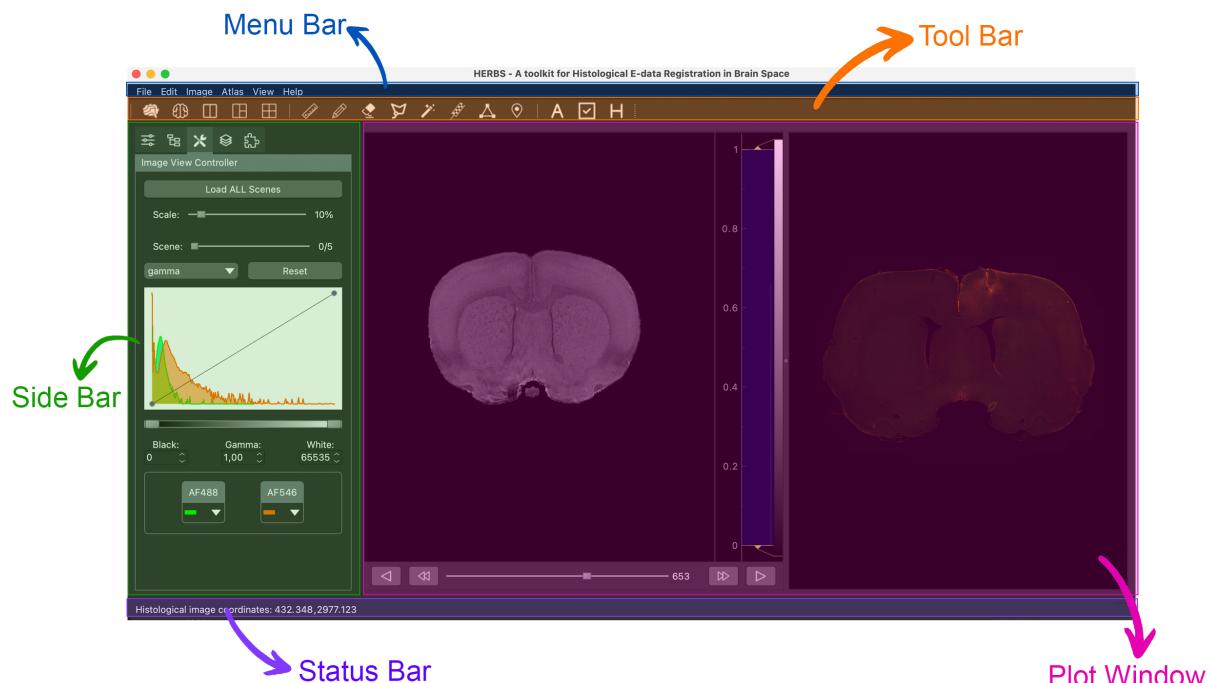
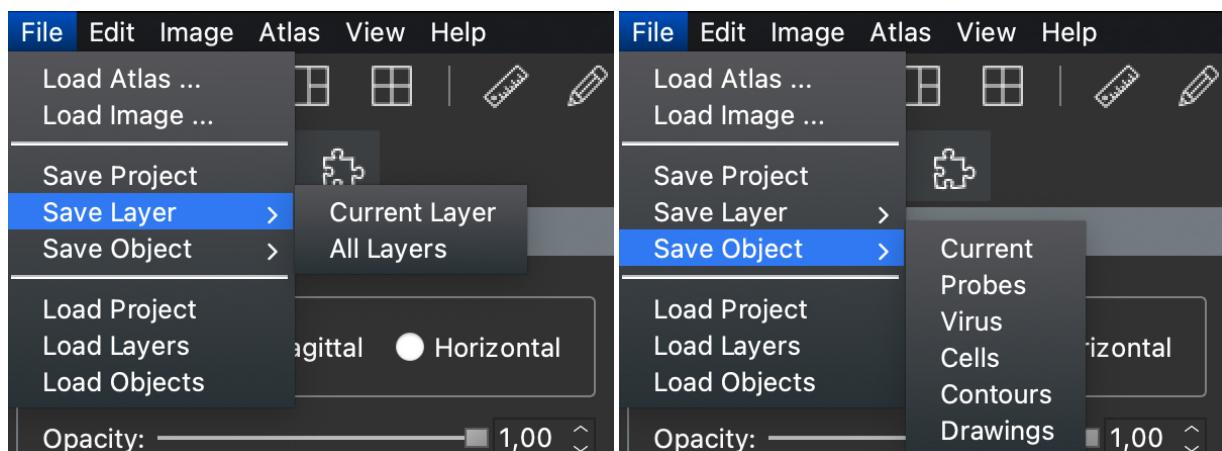


Figure 5.1: Illustration of working areas in the HERBS GUI.

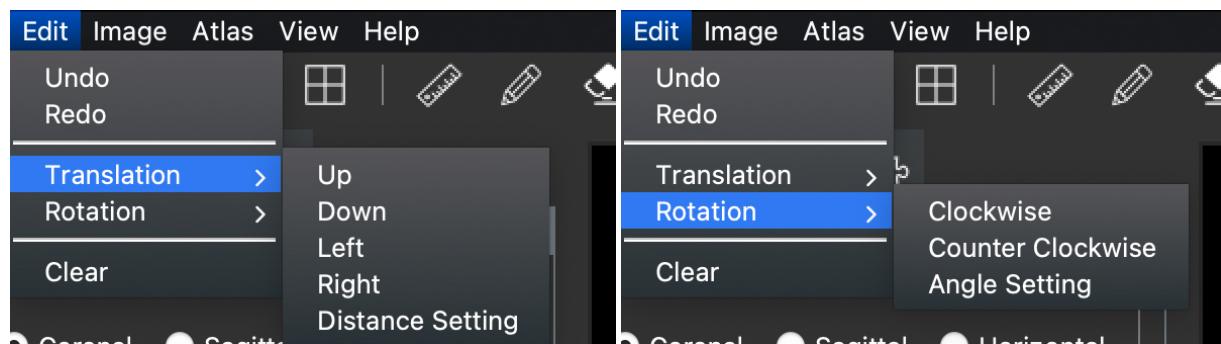
5.2 Menu Bar

5.2.1 File Menu



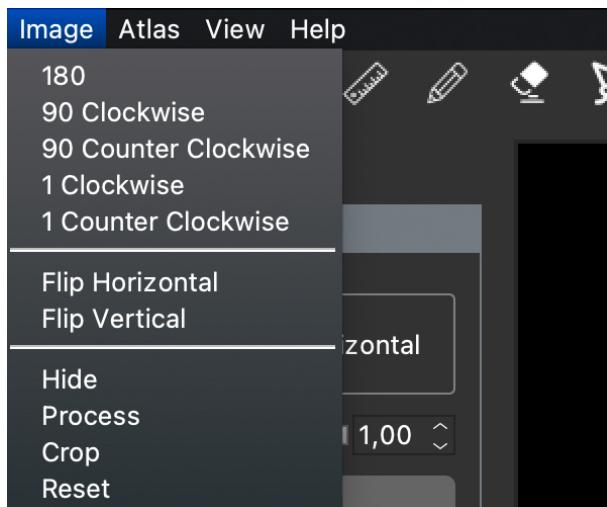
Menu	Description
Load Atlas...	Click to open a file dialog to select the folder where a volume atlas is saved and load the atlas to the Volume Atlas Window . (Volume atlas here and subsequently refers to the WHS rat atlas or the Allen mouse atlas .)
Load Image...	Click to open a file dialog to select a histological image and load the image to the Image Window .
Save Project	Click to open a file dialog to select the folder in which the current working project will be saved.
Save Layer	Save the data associated with the current active layer or save all data for every single layer.
Save Object	Save data for current active object or all data in a specific group.
Load Project	Load a previously saved/ongoing project.
Load Layers	Load layer related data.
Load Objects	Load object related data.

5.2.2 Edit Menu



Menu	Description
Undo	Undo an action associated with a layer.
Redo	Re-do a performed action on a layer.
Translation	Move a layer on a flat X-Y plane.
Rotation	Rotate a layer around a central pivot point.
Clear	Clear all existing layers and related data.

5.2.3 Image Menu

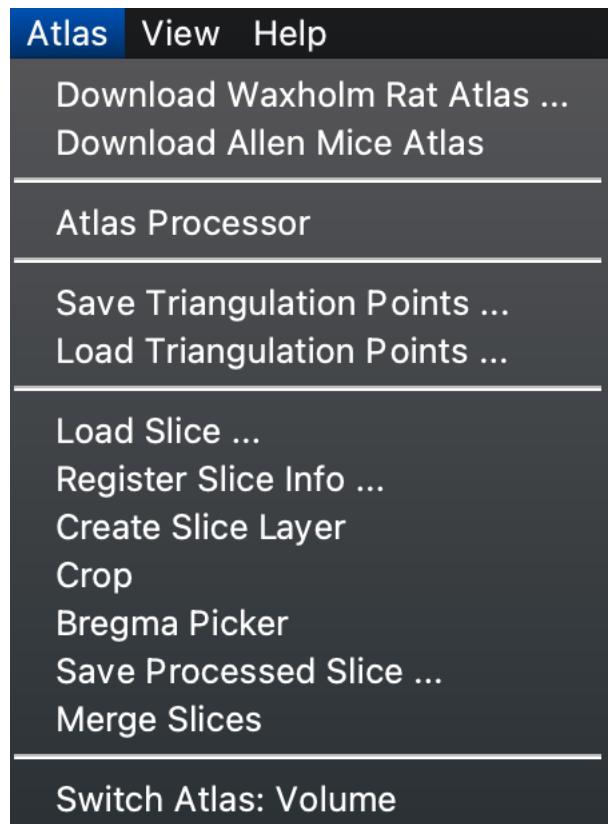


The [Image Menu](#) has three separated blocks. The top block contains menus for rotating the current image in the [Image View](#) window. The middle block is for flipping the current image, and the bottom block contains basic image editing functions.

Menu	Description
180	Rotate the image 180 degrees.
90 Clockwise	Rotate the current image 90 degrees clockwise.
90 Counter Clockwise	Rotate the current image 90 degrees counter-clockwise.
1 Clockwise	Rotate the current image 1 degree clockwise.
1 Counter Clockwise	Rotate the current image 1 degree counter-clockwise.
Flip Horizontal	Flip the current image horizontally.
Flip Vertical	Flip the current image vertically.
Hide	Checkable, when checked, the current image is set to be invisible.
Process	Generate a copy of the current image for further processing, such that further processing will not affect the current image.
Crop	Crop the current image.
Reset	Erase the processing and rewind back to the original image.

5.2.4

Atlas Menu

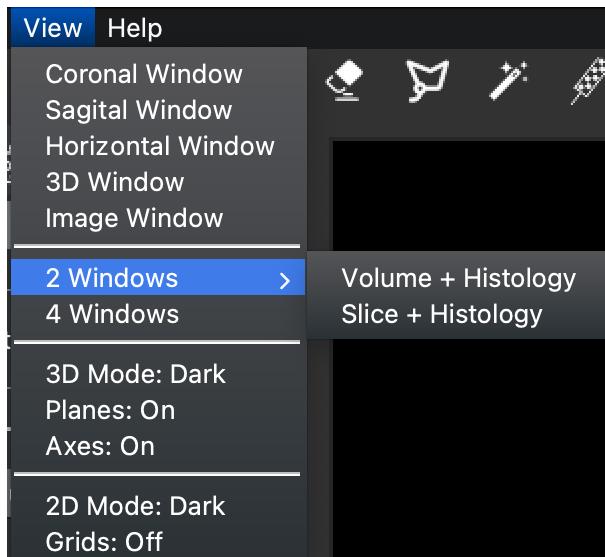


The [Atlas Menu](#) has five separated blocks. The menu serves for downloading atlases, processing custom atlases, triangulation points, Slice Atlases and switching atlases from top to bottom respectively.

Menu	Description
Download Waxholm Rat Atlas	Automatically downloads the required files of the WHS rat brain atlas to run in HERBS.
Download Allen mouse Atlas	Automatically downloads the required files of the Allen Mouse Brain atlas to run in HERBS.
Atlas Processor	To process custom defined atlases (in development).
Save Triangulation Points	Saves the points clicked from the warping process.
Load Triangulation Points	Loads the saved triangulation points.
Load Slice	To load one page of Slice Atlas(Book Atlas) of choice.
Register Slice Info	To add the information of an uploaded image from the book atlas
Create Slice Layer	To turn the Slice Atlas into a processing status.
Crop	Image crop function.
Bregma Picker	Selects the Bregma point for the uploaded slice.

Save Processed Slice	Saves a processed atlas slice.
Merge Slices	To merge all pages of processed Slice Atlas into a 3D mesh (in development).
Switch Atlas: Volume	To switch between Volume Atlas and Slice Atlas when both types of Atlases are uploaded.

5.2.5 View Menu



The **View Menu** has four separated blocks. The menu serves for showing single window, showing multiple windows, controlling 3D window and controlling 2D Volume Atlases slice windows from top to bottom respectively.

Menu	Description
Coronal Window	Shows the coronal section of the atlas in the PlotWindow.
Sagittal Window	Shows the sagittal section of the atlas in the PlotWindow.
Horizontal Window	Shows the horizontal section of the atlas in the PlotWindow.
3D window	Shows the 3D volume of the atlas in the PlotWindow.
Image Window	Shows the uploaded image in the PlotWindow.
2 Windows	Opens 2 windows at a time for viewing.
4 Windows	Opens all 4 windows for viewing.
3D Mode:Dark/White	Selects dark or white mode to view the 3D volume of the brain.
Planes:On/Off	Selects whether to visualize the 3 planes of sectioning in the 3D volumes.

2D Mode:Dark/White	Changes the background color (dark or white) for the atlas viewer window.
Grids:On/Off	Select to view or turn off grids.

5.3 Tool Bar



Note: All working tools are checkable. When a working tool is checked, a gray line will appear underneath it, and a group of settings and control widgets will pop up (See Figure 5.2 for example).



Figure 5.2: Illustration of how the ToolBar looks when the Probe Tool is checked.

5.3.1 Ruler Pop Up Widgets



- Color Button: click to change the color of ruler path.
- Size Slider: move the slider to change the width of the ruler.
- Length: displays the length measured by the ruler.

5.3.2 Pencil Pop Up Widgets



- Color Button: click to change the color of the drawing path.
- Size Slider: move slider to change the width of drawing path.
- Path Type Button : checkable. The drawing path will become closed when the button is checked, otherwise the drawing path appears as a line by default.

5.3.3 Magic Wand Pop Up Widgets



- Color Button: click to change the color of the selected area in the mask layer.
- Tolerance: determines how different a pixel must be in tone and color from surrounding pixels to be included in the selection.

Tool Icon	Checkable	Description
	No	Atlas Loader Tool: click to open file dialog to select the folder where a volume atlas is saved and load the atlas to Volume Atlas View windows.
	No	Image Loader Tool: click to open file dialog to select the histological image file and load it to Image View window.
	No	Two Plot Window Tool: click to show one section window on the left and histological image window on the right. The displayed section window will depend on the atlas currently selected.
	No	Three Plot Window Tool: click to show one section window on left, histological image window on top right, and 3D window on bottom right.
	No	Four Plot Window Tool: click to show all four Volume Atlas View windows.
	Yes	Ruler Tool: used to measure the length under the ruler path.
	Yes	Pencil Tool: used for drawing lines or areas as desired on histological images.
	Yes	Eraser Tool: used to delete the area covered by the eraser symbol.
	Yes	Lasso Tool: used to create a region of interest.
	Yes	Magic Wand Tool: used to select a group of pixels which have the same or similar colors.
	Yes	Probe Tool: used to draw points along the trajectories of probes and to plan probe coordinates.
	Yes	Triangulation Tool: used for drawing triangulation points to match histological and atlas images.
	Yes	Cell Selector Tool: used to specify the location of cell bodies.
	Yes	Transform to Atlas Tool: used to match the histological image to the atlas slice.
	No	Accept Transformation Tool: accepts the transformation after matching histological image to atlas.
	Yes	Transform to Histological Image Tool: used to match the atlas slice to the histological image.

- Kernel Selector: select to change the shape of the kernel. Kernels are used in morphological transformations to filter the selected area.
- Size (Kernel size): sets the size of the selected kernel.
- Virus Button  : click to turn the current mask layer into a virus expression layer.
- Contour Button : click to turn the current mask layer into a contour layer.

5.3.4 Triangulation Pop Up Widgets



- visualize Tri-Line Button  : checkable. When checked, the icon changes to  and the triangles generated by the triangulation points are visible, otherwise the triangles are hidden (default).
- Bounding rectangles matching Button  : click to create matching bounding boxes for the atlas or histology image.
- Color Button : click to change the color of triangulation points.
- Points Number Input: sets the number of triangulation points on the boundary of histological and atlas slices.

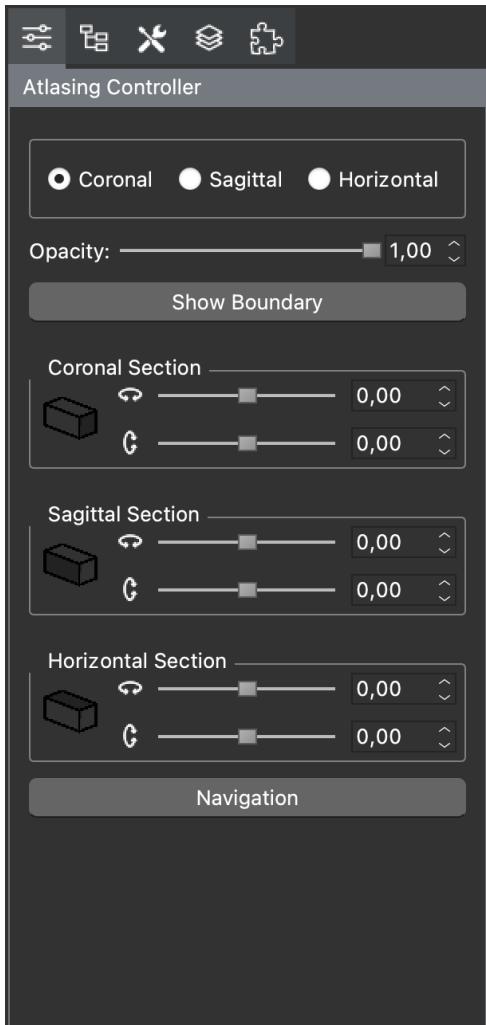
5.3.5 Cell-Body Pop Up Widgets



- Color Button : click to change the color of selected cell bodies.
- Manual Cell Body Selector  : checkable. When checked , users can select a cell body manually by clicking on the image. Each scatter marker indicates one cell body.
- Example Cell Body Selector  : checkable. When checked , users can select all the pixels of a single cell body as an example cell body for automatic cell body detection.
- Auto Cell Body Detector  : (under development) click to detect cell bodies with a similar color, size and shape as the example cell body.
- Cell Count Area: displays the number of cell bodies on each color channel after a cell body is selected or detected when the loaded image is not an RGB image.

5.4 Side Bar

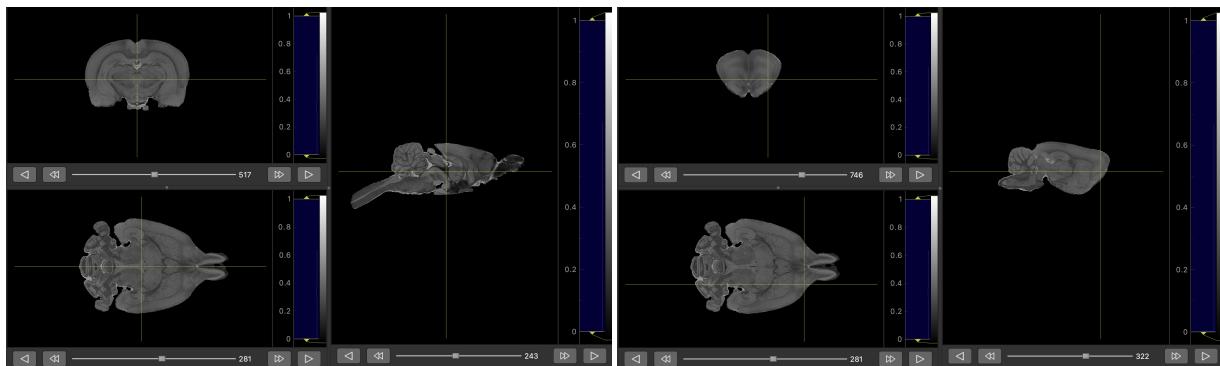
5.4.1 Atlasing Control Panel (Ctrl + 1)



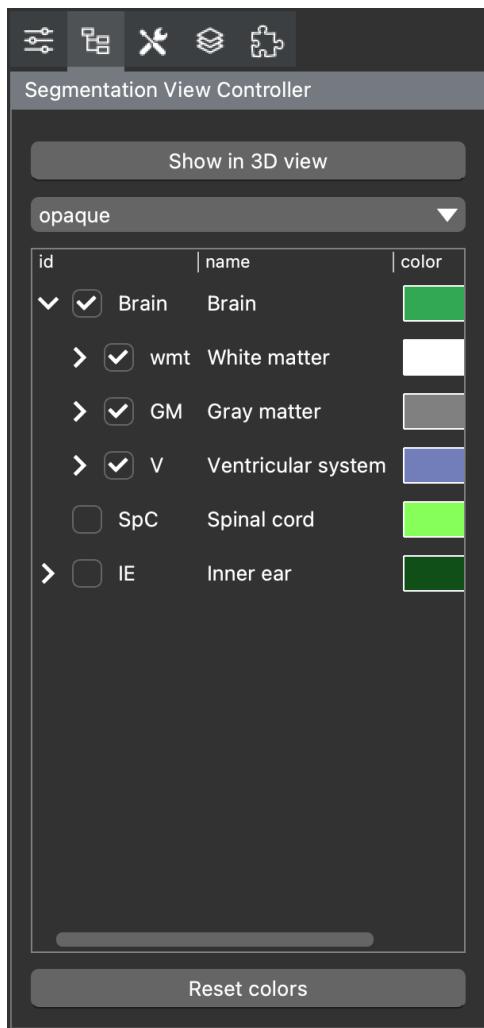
Atlasing Control panel is used to control the visualization of a volume atlas. The panel will be enabled when a volume atlas is loaded, but not enabled when an image from a book atlas (e.g Paxinos & Watson) is loaded. The panel has five different widget groups:

- **'Atlas Display'** button group is used to choose which plane of sectioning to display from the atlas.
- **'Label Opacity'** slider is used to change the opacity of label colors for the different brain regions.
- **'Show Boundary'** button is checkable; it shows the boundaries between brain areas in the atlas.
- **'Atlas Section Rotation'** slider group is used to adjust the plane of sectioning of a volume atlas within -30 and +30 degrees.
- **'Navigation'** button is checkable and works only when all 4 **Volume Atlas View** windows are all displayed. When the button is checked, a crosshair indicator will appear in each of the **Section View** windows. The sagittal slice and horizontal slice, for example, are related to the crosshair in the **Coronal View** window. When moving the crosshair, the sagittal slice and horizontal slice will change accordingly. See example below.

For more examples, see section 6.2. Scrolling through the atlas.



5.4.2 Segmentation View Controller Panel (Ctrl + 2)



Segmentation View Controller is used to control the visualization of different brain regions in a volume atlas. Similar to the Atlasing Controller, this panel is enabled when a volume atlas is loaded, but not enabled when an image slice atlas(an atlas slice from a book) is loaded. The panel contains four widgets:

- 'Show in 3D view' button is checkable. When the button is checked, the selected brain region will show up in the **3D View** window.
- 'Blending Mode' selector is used to control the opacity of the selected brain region which is shown in **3D View** window ("opaque" is selected in the example to the left).
- 'Brain Regions/Labels Information Container' contains the information of all delineations in a volume atlas.
- 'Reset colors' button: click to erase changes in label colors and revert to the default colors.

For more examples, see section 6.2. Scrolling through the atlas.

Brain Regions/Labels Information Container

- **Arrow indicator:** a tree structure indicator, the left arrow of a label indicates that more sub-regions are included in the parent region.
- **Check Box:** check to visualize the colored brain region.
- **Acronym:** abbreviated name of the brain regions. The abbreviations are used in the **Status Bar** when the cursor hovers on the **Sectioning View** windows.
- **Name :** the full name of each brain region.
- **Color button:** click to open a color dialog to change the color of the brain region.

5.4.3 Image View Controller Panel (Ctrl + 3)

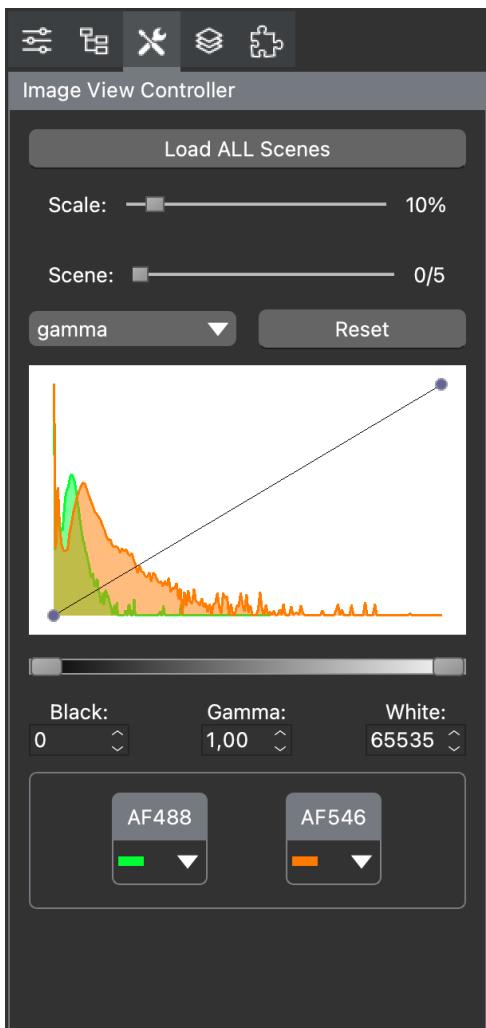


Image View Controller is used to control the visualization of the histological image. The panel is enabled when an image is loaded; it contains five widgets:

- 'Load ALL Scenes' button is checkable, and can be used only when a .czi format file is to be loaded. When the button is checked, all scenes contained in the file will be loaded. Otherwise only the first scene will be loaded.
- 'Scale' slider is used to resize a .czi formatted image.
- 'Scene' slider is used to slide between multiple scenes when available. If a file has only one scene the slider will be hidden.
- 'Curve' widget is used to control the brightness and contrast of an image (controls are "Black", "Gamma" and "White").
- 'Channel Color Selector' group is for changing the visibility and color of each individual channel. Click the name of the channel to change its color and visibility.

For more examples, see subsection 6.3.3. Change Brightness and Contrast of Images.

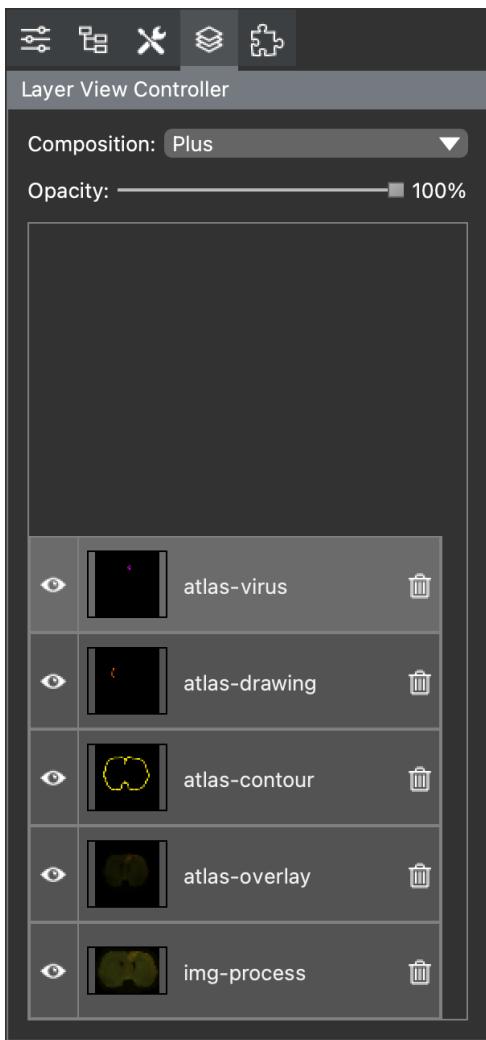
Curve Widget

- **Curve Line Type** selector: use to define the curve type (e.g. linear, gamma).
- **Reset** button: click to erase changed curve lines and reset to baseline.
- **Curve**: by default it's a straight line from bottom left to top right – this is the baseline. The colored histogram(s) in the background correspond to the colors of each channel in the histological image.
- **Black/White** slider: adjusts the endpoints of the baseline to darker tones (left) or lighter tones (right).
- **Value boxes**: use to set the input levels directly without moving the slider.

To change the Curve, users can change the **Gamma Value** box when the line type is gamma, or click on the baseline to create a control point when line type is linear or a spline. By dragging the point up, you will lighten the image at those tones and surrounding tones. Drag down, and you'll see the image darken at tones around that point.

Users can also change the endpoints on the baseline by moving the handles on **Black/White** slider, or by changing the value in the **Black Value** or **White Value** boxes.

5.4.4 Layer View Controller Panel (Ctrl + 4)



The **Layer View Controller** is used to control the working objects generated by actions in both **Atlas View** and **Image View** windows. The panel contains three widgets:

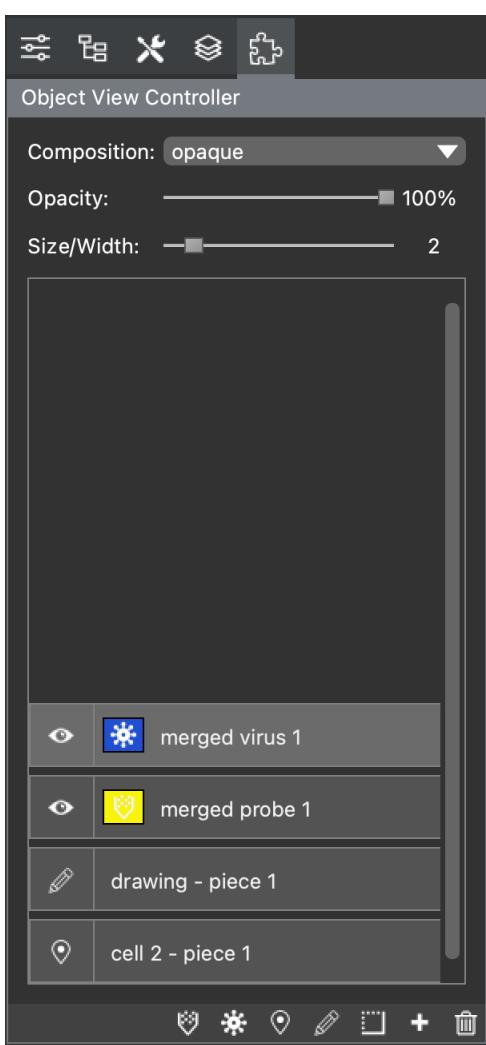
- **'Composition'** mode selector: use when overlaying layers.
 - SourceOver: Image replaces the background if it is opaque. Otherwise, it uses the alpha channel to blend the image with the background.
 - Overlay: Image color is mixed with the background color to reflect the lightness or darkness of the background.
 - Plus: Both the alpha and color of the image and background pixels are added together.
 - Multiple: The output is the image color multiplied by the background.
- **'Opacity'** slider: use to change the transparency of the selected layer.
- **'Layer Container'** contains the created layers. The active layer is in lighter gray. Click the layer to activate/inactivate. Multiple layers can be selected by holding down the Command key or Ctrl key while clicking on them.

Layer Container

- **Eye button:** use to change the visibility of the corresponding layer.
- **Thumbnail:** generated automatically when a change happens to a layer.
- **Layer Name:** generated automatically when an action is called.
- **Trash button:** click to delete all data related to the layer.

Currently, the available layer names are 'img-process', 'img-mask', 'img-probe', 'img-virus', 'img-cells', 'img-contour', 'img-drawing' and 'img-overlay' when an action happens in the **Image View** window. 'atlas-slice', 'atlas-mask', 'atlas-probe', 'atlas-virus', 'atlas-cells', 'atlas-contour', 'atlas-drawing' and 'atlas-overlay' appear when an action happens in the **Atlas View** window. Refer to section 7.7 for examples.

5.4.5 Object View Controller Panel (Ctrl + 5)



The **Object View Controller** is used to control the registered objects. The panel contains five widgets:

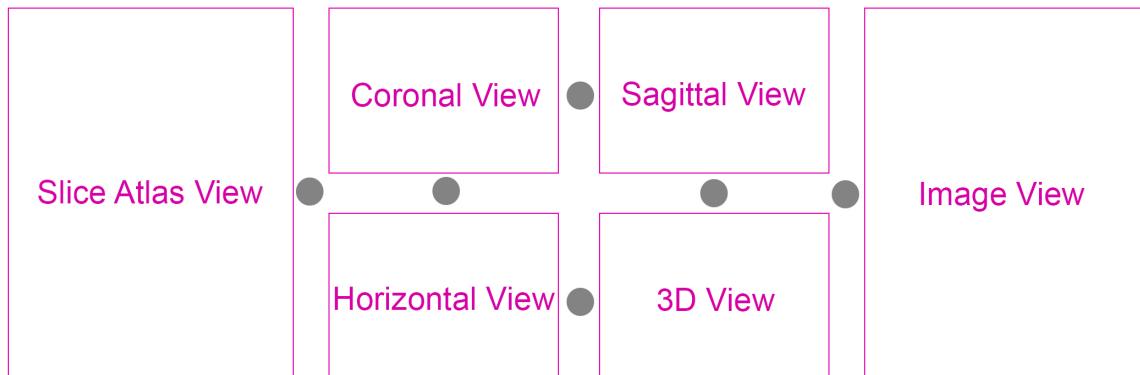
- **'Composition'** mode selector: use to control the blend mode of the selected objects in the **3D View** window.
- **'Opacity'** slider: use to control the transparency of the selected objects in the **3D View** window. Works when **Composition Mode** is 'translucent'.
- **'Size/Width'** slider: use to control the size of the selected objects in the **3D View** window.
- **'Object Container'** contains all registered object layers. The active object layer is in lighter gray.
- **'Object Action'** buttons (icons at the very bottom) are used to register or remove objects. From left to right these are:
 - **Merge Probe Piece** button
 - **Merge Virus Piece** button
 - **Merge Cells Piece** button
 - **Merge Drawing Piece** button
 - **Merge Contour Piece** button
 - **Add Object Piece** button
 - **Delete Object (Piece)** button

Objects Container

- **Object Piece**
 - **Object Icon**: generated automatically when **Add Object Piece** button is clicked.
 - **Object Piece Name**: also generated automatically when **Add Object Piece** button is clicked. The name of the piece can be changed but the format of **Object Piece Name**(E.g. probe object piece, virus object piece) is conserved. For HERBS to recognise the pieces, the name of the pieces must have the format 'object name i - piece j', where 'i' is the index of objects after merging, and 'j' is the index of pieces to be merged.
- **Merged Object**
 - **Eye** button: use to change the visibility of the object in the **3D View** window.
 - **Object Icon**: click to change the color of the object in the **3D View** window.
 - **Object Name**: generated automatically when the **Merge Object Piece** button is clicked. Object names cannot be changed.

Examples in which Object View Controller is used are shown from Section 7.4 onwards.

5.5 Plot Window



HERBS has six plot windows, and each window can be resized by dragging any of the small gray dots between windows. The **Coronal View**, **Sagittal View** and **Horizontal View** windows are **Section View** windows. These three windows, together with **3D View**, are **Volume Atlas View** windows and work with the WHS or Allen atlases (or other volumetric reference atlases loaded by the user). The **Slice Atlas View** window is only for when the atlas is a pdf or digital book (e.g. a Paxinos & Watson atlas). The **Volume Atlas View** windows and **Slice Atlas View** windows cannot be displayed at the same time. If both types of atlases are loaded, the default displayed view will depend on which type of atlas was loaded later.

5.6 Status Bar

The **Status Bar** lives at the bottom of HERBS. It shows all the information that HERBS provides. After HERBS is launched, the message 'Ready' is printed.

Ready

When moving cursor on the **Image View** window, the coordinates (in pixels) of the image appear in the status bar.

Histological image coordinates: 2322.118,1428.331

When moving the cursor on the **Volume Atlas View** window, AP coordinates relative to Bregma are shown, as well as DV coordinates with respect to the brain surface, as are regional delineations (per the loaded Atlas Volume).

Atlas voxel:(314,653,283),ML:2689.25um,AP:0.0um,DV:-6105.48um w.r.t Bregma,DV:-5128.92um w.r.t Surface: [197]GM > Tel > SubPAL > Str > CPU : Caudate putamen

Error messages are printed in red in the **Status Bar** while working in HERBS.

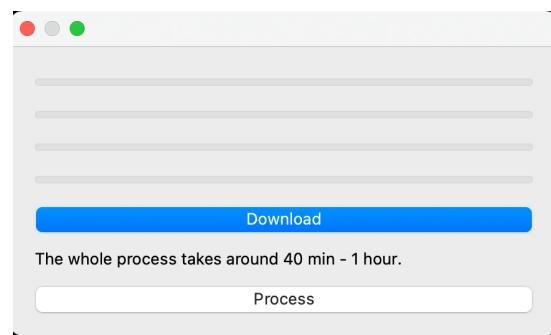
No required Processed image is created.

6.1 Download and Process Atlases

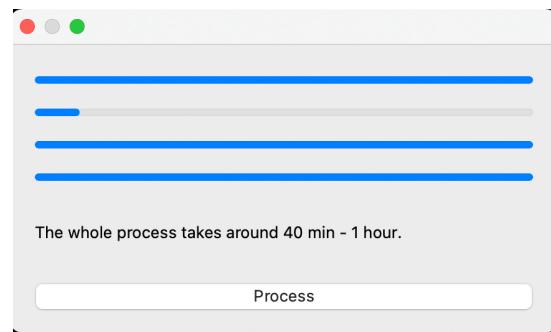
6.1.1 Download Waxholm Space Rat Brain Atlas, v4

In this section, we show how to download the Waxholm Space Rat Brain atlas raw data through HERBS, and how to process the raw data so it can be used in HERBS.

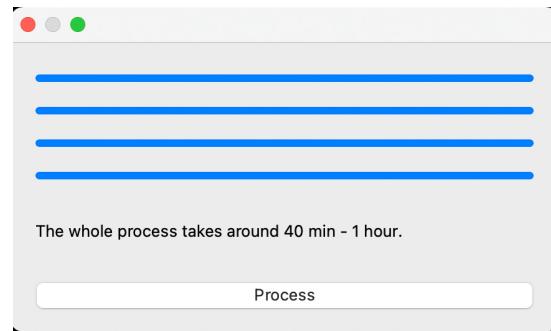
- Click the 'Atlas Menu' in the MenuBar, and click 'Download Waxholm Rat Atlas' in the drop-down menu. A popup window will appear afterwards.



- Click the 'Download' button, and either choose a folder or create a new one to save the downloaded atlas files. After downloading starts, the 'Download' button will be invisible. Downloading can take up to 1 hour due to the number and size of the atlas files.



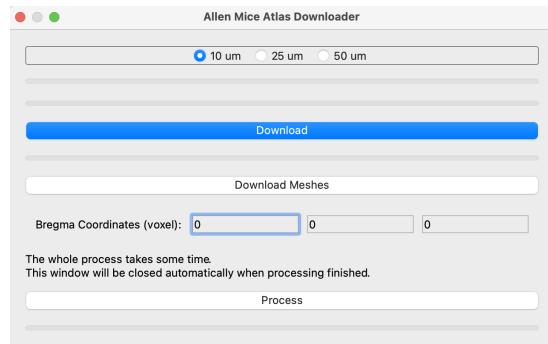
- After all four raw data files are downloaded, the 'Process' button will be enabled and must be clicked to process the raw data for HERBS. Processing will take at least half an hour. Once processing is completed, a window will pop up asking if you want to leave. Click 'yes' to close the download process.



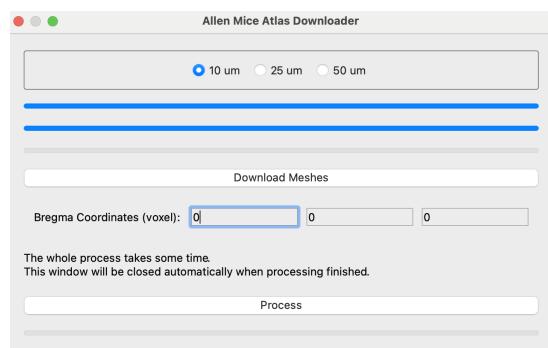
6.1.2 Download Allen Mouse Brain Atlas

In this section, we will introduce how to download the Allen Mouse Brain Atlas raw data through HERBS, and how to process the raw data so it can be used in HERBS.

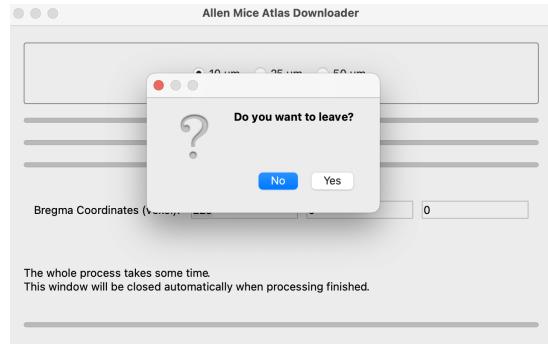
- Click the 'Atlas Menu' in the Menu Bar and click 'Download Allen Mice Atlas' in the drop-down menu. A pop window will appear. Select the desired atlas slice thickness (will vary for different applications, but we suggest starting with 10 microns).



- Click the 'Download' button and wait until the 2 blue lines are complete. Then start the download of the meshes by clicking 'Download Meshes'. The process will take some time due to large file sizes.



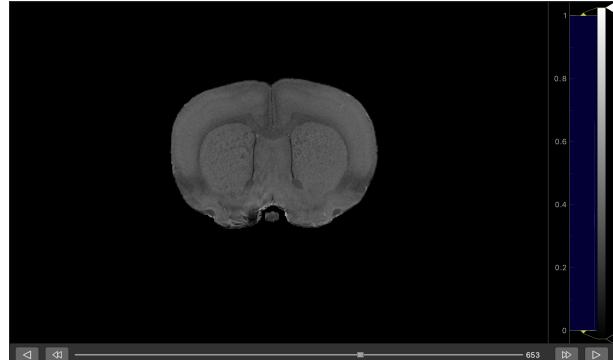
- Once all the process has been completed, a window will pop up asking if you want to leave. Click 'yes' to close the download process.



6.2 Scrolling through the atlas

After a volume Atlas is downloaded and processed, users can load the atlas to HERBS and scroll through it. Click the 'Atlas Loader'  tool or click 'File' in the Menu Bar and 'Load Atlas' in the drop-down menu to load the atlas. Note that the 'Load Atlas...' menu will always open a file dialog to ask the user to choose the folder where the atlas will be saved. The 'Atlas Loader' tool will automatically load the atlas from the folder where the atlas has been saved before.

- After an Atlas is loaded, the Coronal section window is displayed by default.



- Users can use the page slider at the bottom of the section window to scroll between atlas slices, and use the vertical slider to the right of the section window to change the brightness and contrast of the atlas slice.



- To display another section window, select the 'Atlas Display' button accordingly. For instance, after the 'Sagittal' button is selected,

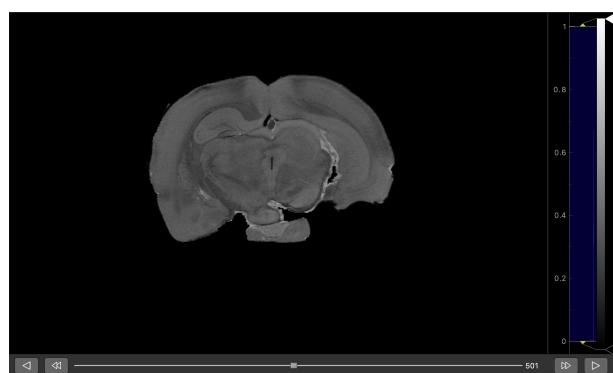
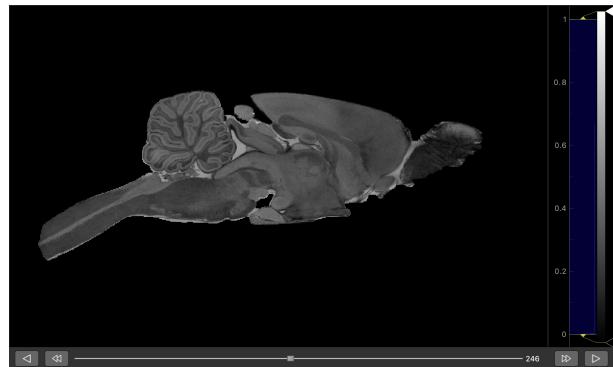


a sagittal section window will be displayed.

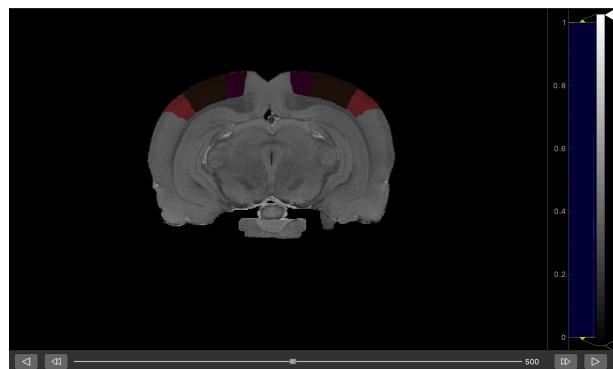
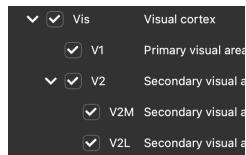
- Users can rotate the volume atlas arbitrarily up to 30 degrees using the 'Section Rotation' sliders. When the 'Coronal Section' slider is set to -17.87 degrees horizontally:



the atlas slice will rotate accordingly. This facilitates matching between the plane of sectioning of the histological image and the atlas template.



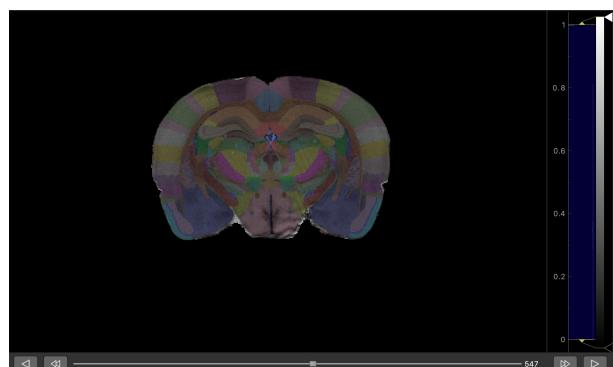
- To see different brain regions, go to the 'Segmentation View Controller' panel in the SideBar and check the desired brain regions. The example here shows when visual cortex is selected.



- The boundaries of all brain regions can be visualized by checking the 'Show Boundary' button in the 'Atlassing Controller' panel in the SideBar.



- Using the 'Opacity Slider' in the 'Atlassing Controller' panel, users can control the opacity of the colors overlaid on the regions. An example with opacity set to 0.38 is shown here.



6.3

Process Histological Images

Processing histological images is a functionality HERBS provides for users' convenience. To go through the processing steps, an image must first be loaded by clicking the 'Image Loader'  tool button in ToolBar or selecting 'Load Image...' from the drop-down menu under 'File'.

Three widgets are used to control loading settings when loading a .czi image file: the 'Load All Scenes' button, the 'Scale Slider' and 'Scene Slider' (Figure 6.1). The 'Load ALL Scenes' button can only be used before loading images, whereas 'Scale Slider' can be used both before and after an image is loaded. Other compatible formats are .jpg, .tiff, .png, .bmp, but the 'Scale Slider' and 'Scene Slider' do not appear for them.

When loading an image with the 'Load All Scenes' button checked, all scenes (if the file contains more than one scene) will be loaded at once, and scaled as shown in the 'Scale Slider'. Otherwise, the first scene will be loaded with the scaling set per the slider. To load images in other scenes, users can move the 'Scene Slider' to a desired scene index. If the file contains only one scene, the 'Scene Slider' will be hidden after the image in the first scene is loaded.

The 'Scale Slider' controls the resolution of the loaded image. As histological images are usually high resolution and large in size, loading the entire image with the original size can be time consuming. If full resolution is not needed for a given job, the 'Scale Slider' can be used to shrink the size of image as desired.

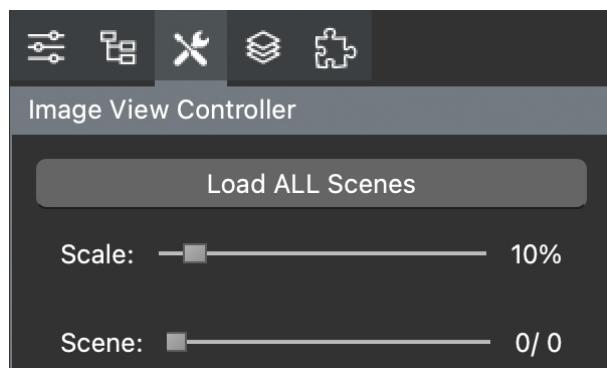


Figure 6.1: The 'Load All Scenes' button, 'Scale Slider' and 'Scene Slider' are found in the 'Image View Controller' panel in the SideBar. NOTE: 'Scene' and 'Scale' sliders only appear with .czi formatted images.

6.3.1 Crop Image

After an image is loaded, users can crop the image to remove unnecessary parts and keep the desired portion for further processing.

- Load an image.



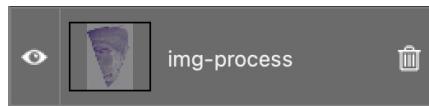
- Select the 'Lasso' tool in ToolBar, and click on the image to define a region of interest (ROI).



Note: The lasso path closes when the clicked path arrives back to the first clicked point. Once the outline is closed the dashed line will turn into a solid line.



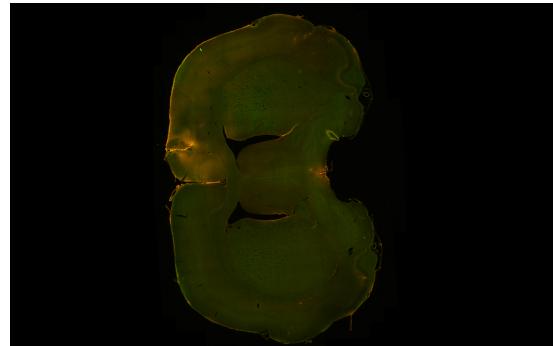
- Next, go to the 'Image' menu and click 'Crop' in the drop-down menu. HERBS will form the ROI as a rectangular bounding box and keep the area inside of it. An 'img-process' layer will be generated in 'Layer Container' in the panel to the left.



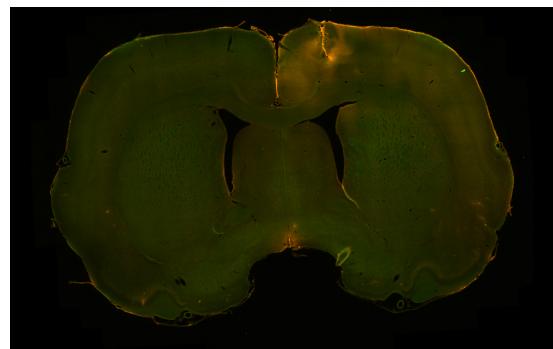
6.3.2 Rotate Image

If an image is not in the desired orientation, users can flip and rotate it in HERBS.

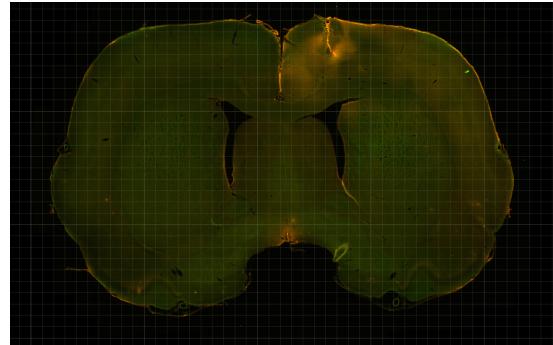
- Load an image.



- Go to the 'Image' menu and click to rotate the image by larger increments (90 or 180 degrees). In this example, we selected '90 Clockwise'.



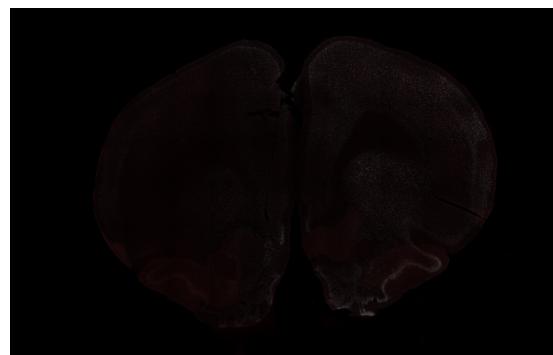
- For fine tuning image orientation, we recommend first turning on grid lines, which is done by clicking 'Show Grids' in the drop-down menu under 'View'. Then, back in the 'Image' menu, rotate the image one degree at a time by clicking '1 Clockwise' or '1 Counter Clockwise' to attain the desired final orientation.



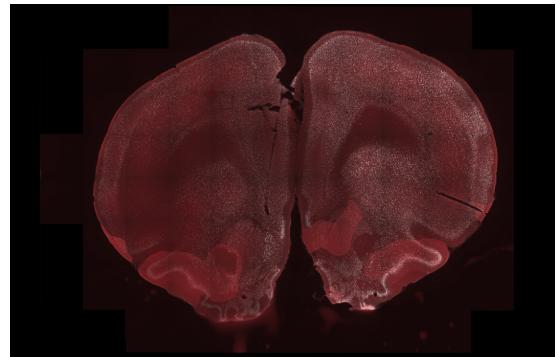
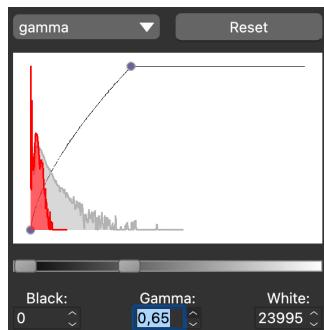
6.3.3 Change Brightness and Contrast of Images

Users can change the brightness and contrast of images as well as the colors of individual channels using the widgets listed in the 'Image View Controller' panel in the SideBar.

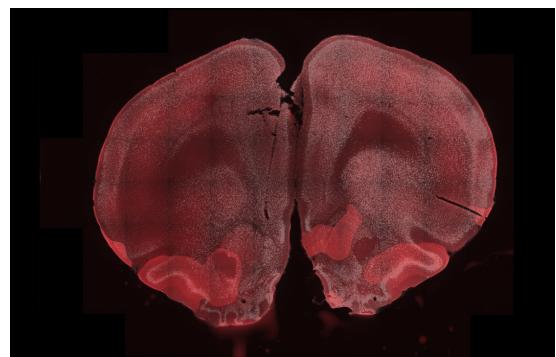
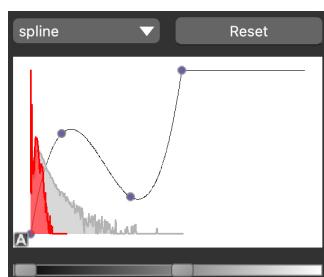
- After loading a dark image, the user can enhance it for better visualization.



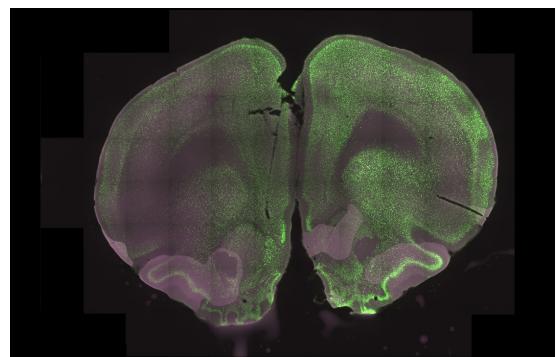
- This can be done by changing the 'White Slider' and 'Gamma Box'.



- Adjust specific aspects of the image by changing the shape of the curve, e.g. adding two points on spline curve.



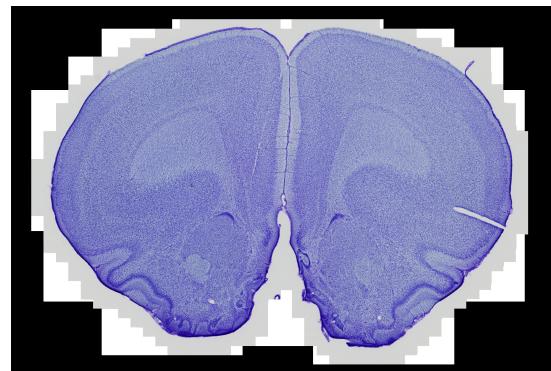
- To change the color of a two-channel image, simply select the desired color in 'Channel Color Selector' (which is independent of the color of the original fluorophores).



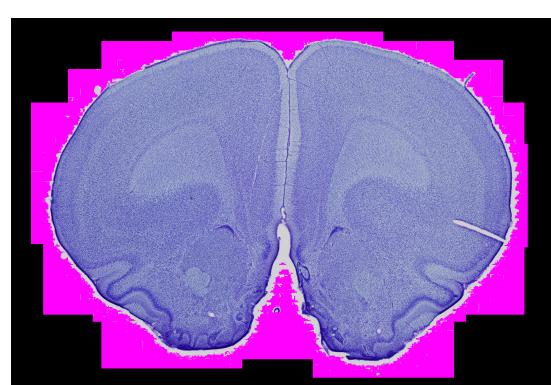
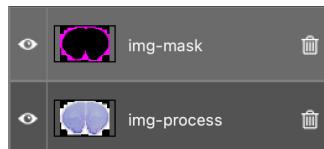
6.3.4 Remove Unnecessary Background

When scanning brain tissue, the scanner or microscope may generate a background around the tissue sections. If desired, the background can be removed following the steps in this section.

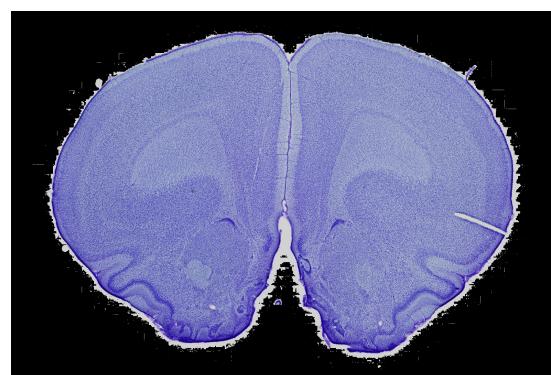
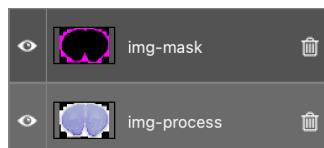
- First, load an image by clicking the 'Image' menu, then select 'Process' in the drop-down menu to turn the current image into a layer which can be processed. An 'img-process' layer will be generated in **Layer Container** to the left.



- Next, select the 'Magic Wand' tool and adjust the value of 'Tolerance' as preferred. Click anywhere on the image to activate the 'img-mask' layer. An area will be selected and an 'img-mask' layer will be generated.

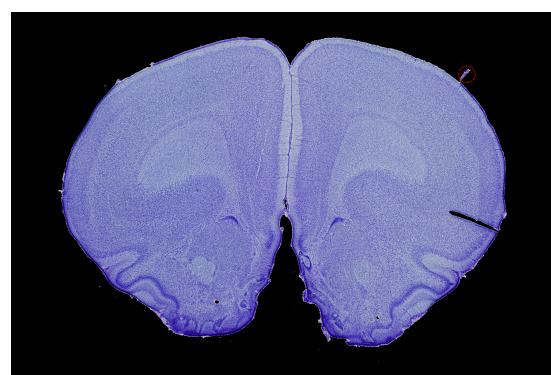


- Click on the 'img-process' layer to change the focus from the 'img-mask' to 'img-process', and move the cursor to the image window and click on the image at the boundary area.



Press the 'Delete' or 'Backspace' key on the keyboard and the selected area will be removed.

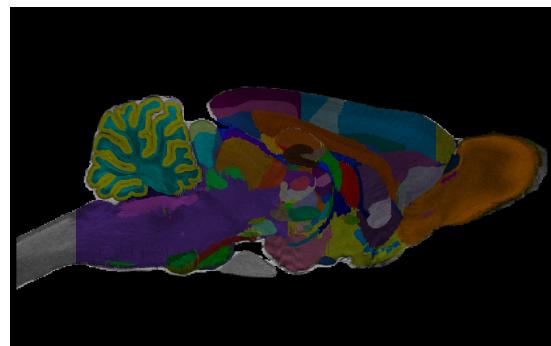
- Repeat these steps to further remove background until until the desired image is obtained. The 'Eraser' tool can also be used to remove small details.



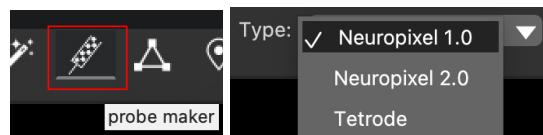
6.4 Generating pre-surgical coordinates

One of the essential steps in planning a surgery is defining the stereotaxic coordinates to reach specific brain areas of interest. HERBS significantly facilitates this process by giving the user a means to calculate the insertion parameters to target potentially several regions along a linear trajectory. In this section, we illustrate how to obtain coordinates for inserting a NP2 recording probe or targeting an injection using HERBS.

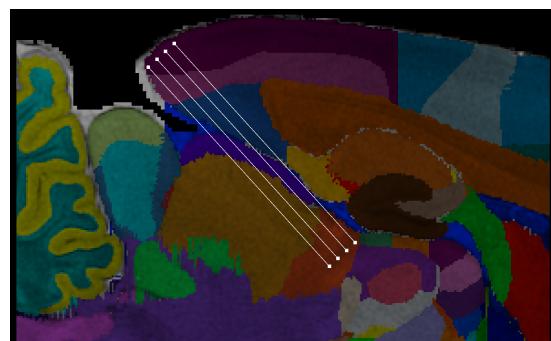
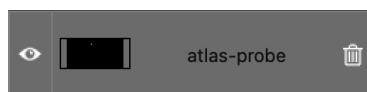
- After loading the appropriate atlas, choose the desired plane of sectioning, scroll to the desired location using the page scroller with/without colored brain regions and/or regional boundaries on (See Section 6.2).



- Select the ‘Probe marker’ button in the Toolbar and select the type of electrode - e.g. Neuropixel 1.0/2.0/Tetrodes. In this example we will use a Neuropixels 2.0, which has 4 shanks.



- Single-click on the atlas slice to set the desired start point of the probe, then zoom in and move the mouse and single-click at the desired end point. Note that an atlas-probe layer in the ‘Layer View Controller’ panel is generated automatically once the end point has been selected.



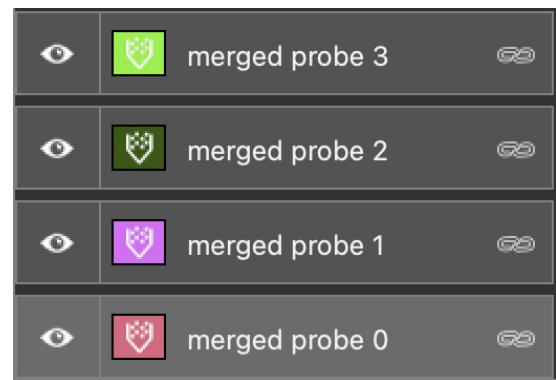
- Go to the ‘Object View Controller’ Panel and click the ‘Add Object Piece’ button listed on the bottom of the panel to create all four pieces for four probes respectively.



	probe 3 - piece 3
	probe 2 - piece 2
	probe 1 - piece 1
	probe 0 - piece 0

6.4 Generating pre-surgical coordinates

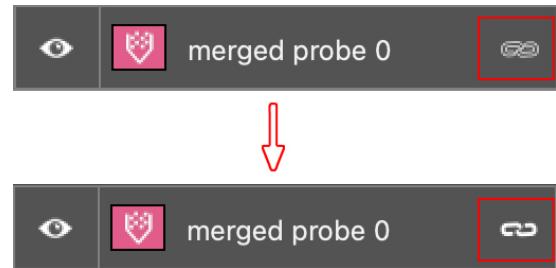
- Click the 'Merge probe' button to create all available probes. Note that the individual pieces will disappear and probe objects will be created in the panel.



- To visualize the readout of a single probe, click on the desired probe in the Object View Controller and a corresponding information window will pop up.

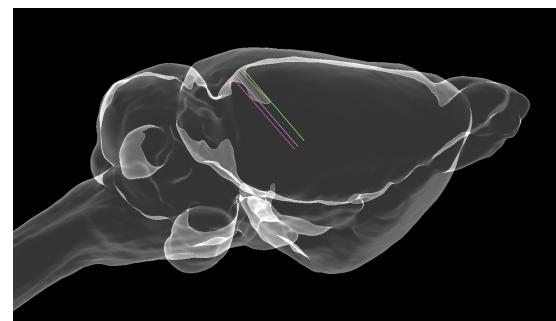
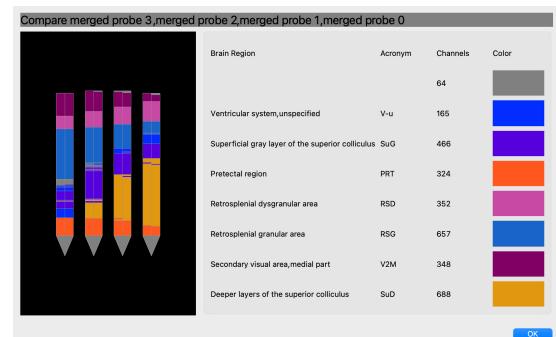
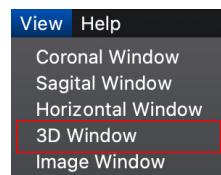


- To compare two or more probes, click the link button on the right side of each probe of interest. Note that the status of the link button will turn from *empty* to *linked*.



- Click the 'Compare' button to visualize the information of the linked probes.

- To visualize the probe in a 3D view, click the 3D Window menu in the popup menu of View menu. The 3D atlas mesh with the probe object will appear after rendering.



6.5 Matching Histological Images with the Atlas

6.5.1 Selecting landmark points

Once the appropriate atlas has been downloaded and processed, the next step is to match histological images with a template slice from the atlas. This places the histological slice in the coordinates of the reference atlas volume. It is a critical step for subsequent quantitative comparisons of brain regions and object locations within and between brains. In this section, we will show the steps to match the histological images and the atlas.

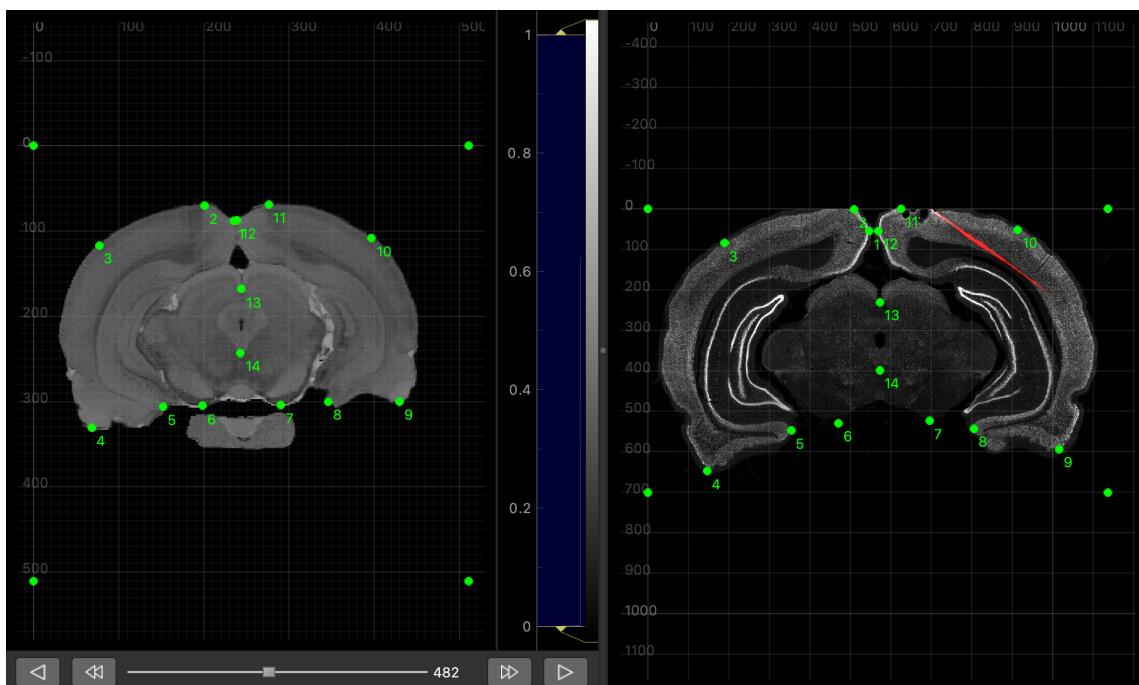
- Load the atlas and histological image. Slide the volume atlas scroller to the desired slice in the desired plane and at the desired angle (see section 6.2). Pre-process the histological image as needed (e.g. remove background, rotate, etc.; see section 6.3.2).



- Check the 'Triangulation'  button in the Toolbar, and select a bright color which has high contrast against both the atlas slice and histological image. Next, type in the desired number of boundary points (the default value is 2 but we typically use between 10 to 20).

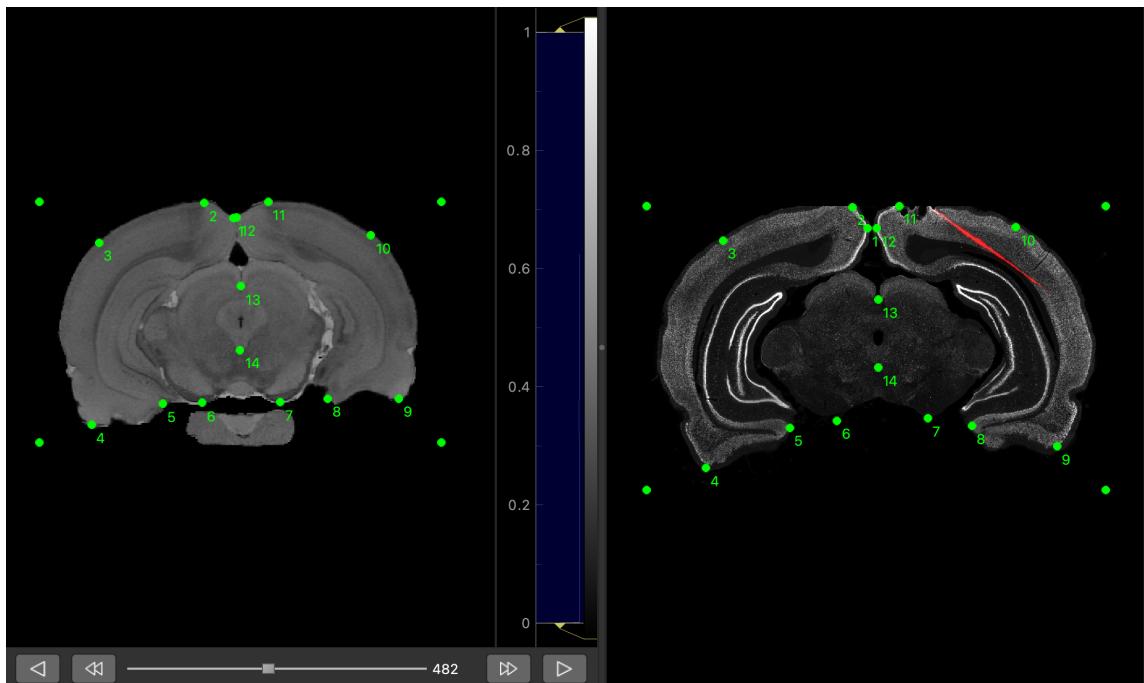


- Click on the atlas slice window and histological image window to select landmark points. The number of selected landmarks and the order of selection **must be the same** in both windows. The selected landmark points are movable and the corresponding order numbers are shown next to the points, whereas the bounding points (forming a large outer rectangle in either window) are not movable and have no order number.



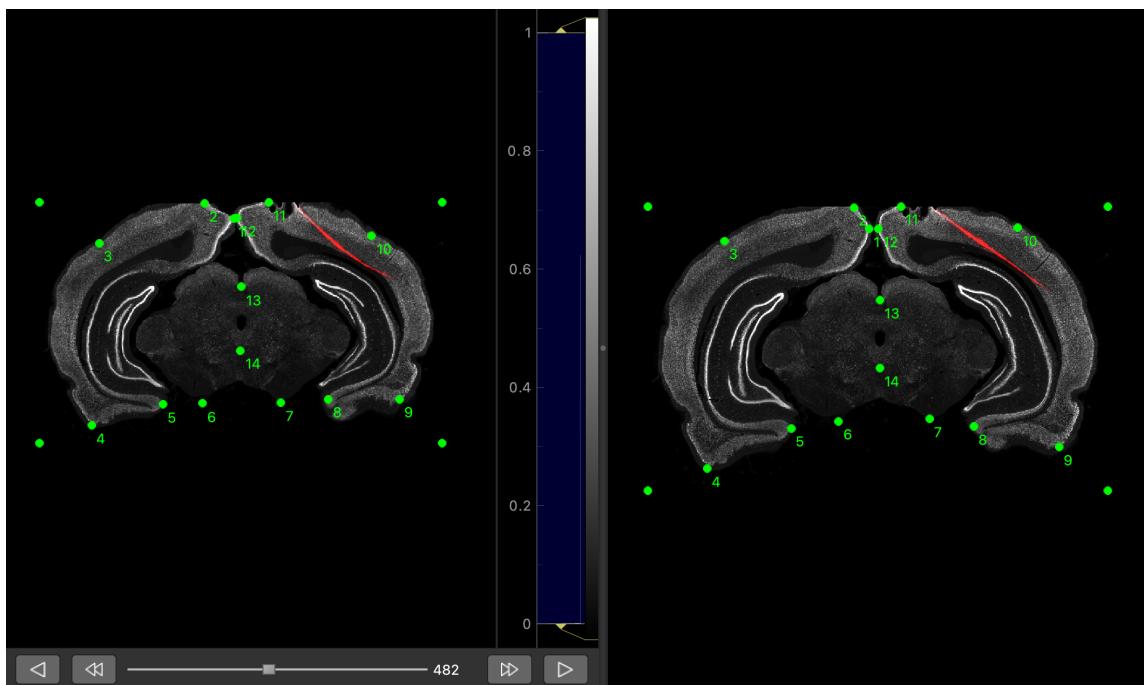
- In this example, we can see that the bounding points are farther away from the atlas slice compared to the histological image. To match them, click the 'Bounding Rectangles'

Matching' button  in the pop up tool when the 'Triangulation' button is checked, and HERBS will correct them.

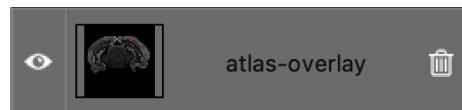


6.5.2 Overlay histological image onto Atlas

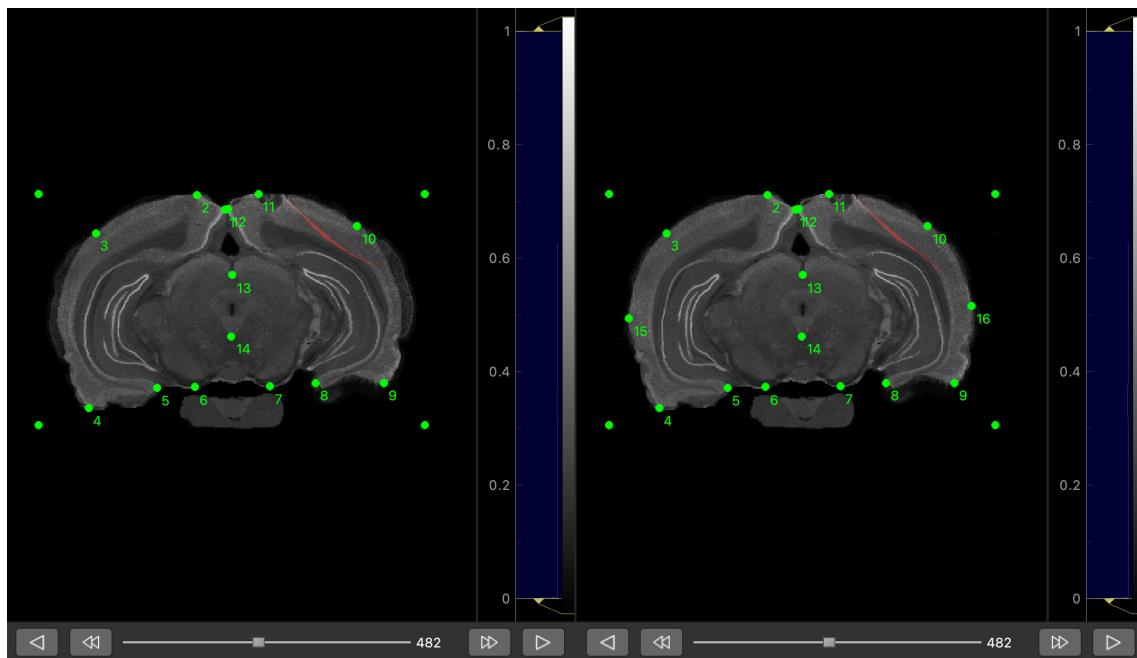
- After selecting all landmarks, click the 'Transform to atlas tool' button  to overlay the histological image onto the atlas slice.



Note: The status of the 'Transform to atlas tool' button has now changed from *need overlay*  to *delete overlay* , and an atlas-overlay layer is generated in the Layer View Controller panel.



- If the desired transformation is not achieved click the 'Transform to atlas tool' button with *delete overlay* status, or click the trash button in the atlas-overlay layer. Add more landmark points by clicking on both windows and/or drag the points to the desired location and rematch until a satisfactory overlay is achieved. The left figure shows the overlay matching using 14 selected points whereas the right figure shows when 16 points are used, and both figures are visualized with layer opacity around 50%.
- Pro tip: (i) Place the anchor points at key architectural locations like the highest, widest and/or lowest points around each slice. Anchor points can be placed inside the slices as well. (ii) Once your section has been matched to the atlas you can view the regional boundaries from the WHS or Allen Atlas overlaid on your histological section. Do this by selecting the "Plus" style of Composition in the Layer View Controller, and sliding the opacity to 50

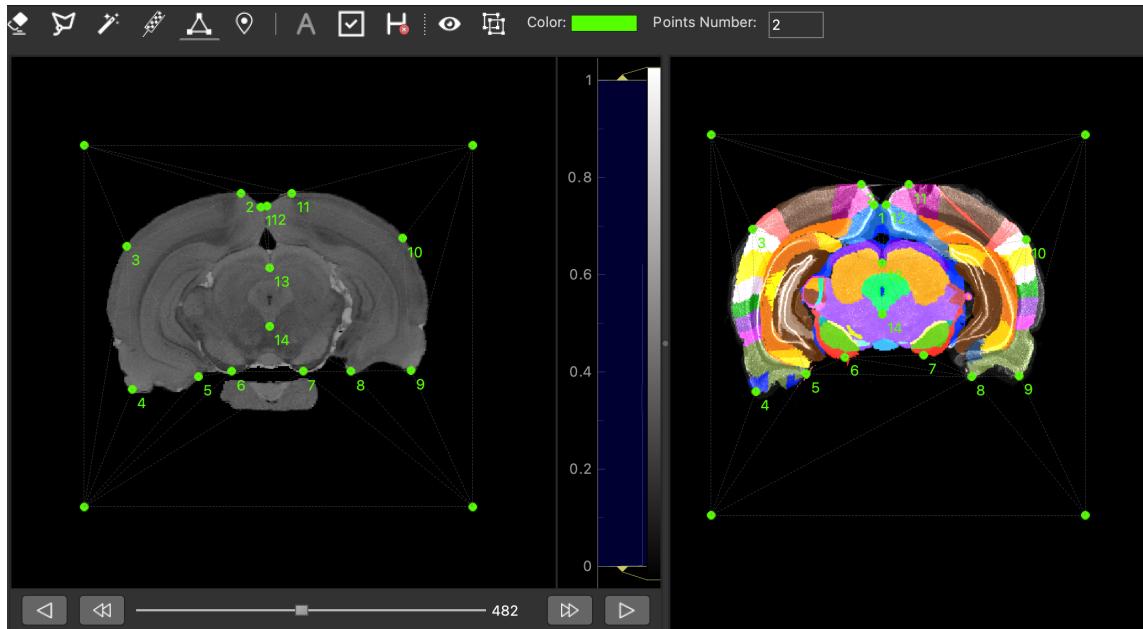


6.5.3 Overlay atlas slice onto histological image

This part of HERBS is still under development. The goal is to help anatomist visualize how atlas brain regions fit onto individual histological slices from different brains, with the understanding that every brain is unique.

- The procedure is the same as that described above, only instead click the 'Transform to histological image tool' **H** with the same points clicked on both images. The color-coded atlas image is then warped onto the histology image, and is also an effective method for visualizing the anatomical boundaries around a recording or injection site.

6.5 Matching Histological Images with the Atlas



6.6 Probe Trajectory Registration

To reconstruct probe trajectories after surgeries or *in vivo* recordings, histological images must first be overlaid on their corresponding atlas images (see section 6.2 and 6.3). Before constructing a probe trajectory, the 'Probe Tool' button needs to be checked and the correct probe type must be selected in the pop up tool menu. For standard electrodes, linear probes, or electrode arrays, choose "Tetrode" for each electrode to be reconstructed.

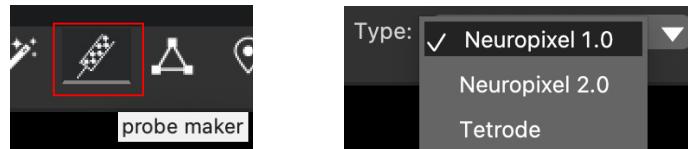
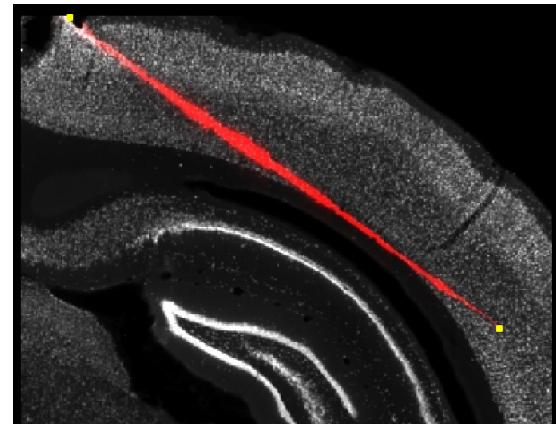


Figure 6.2: Left: The 'Probe Maker' button is shown in the red box. Right: 'Probe Type' selector.

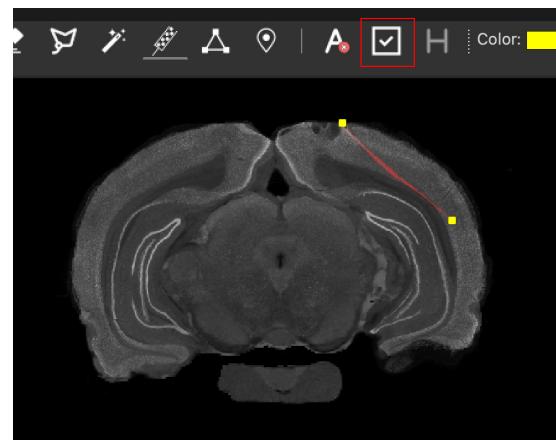
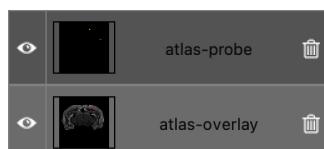
6.6.1 Probe(s) in a single slice

In this recipe we illustrate how to register a probe trajectory when the reconstruction can be made using a single histological image. In the example image, the probe (a version 1.0 Neuropixel, IMEC, Belgium) was coated with DiI (Vybrant247 DiI, catalog no.V22888, Thermo Fisher Scientific, USA).

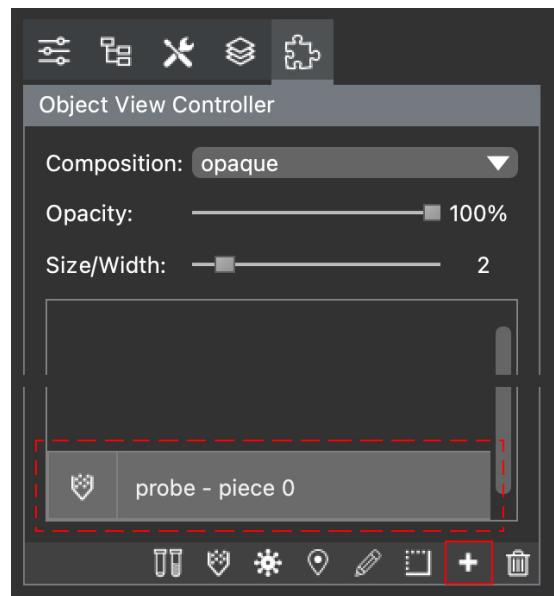
- Once the histology images has been warped to fit the atlas, click points along the probe trajectory on the histological image in histological image window. After a point is selected, an 'img-probe' layer will be generated in Layer View panel.



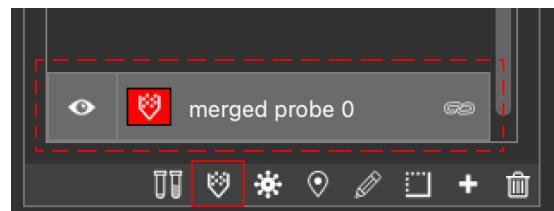
- Click the 'Accept and Transfer' button in Toolbar to transfer the selected points from the histological image window to the atlas slice window. Note. an 'atlas-probe' layer will be generated and the 'img-probe' layer will disappear.



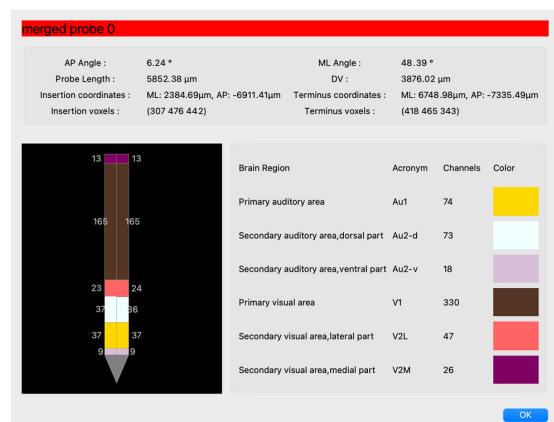
- After transferring, go to the 'Object View Controller' panel and click the 'Add object piece' button, located at the bottom of the panel, to generate all available object pieces. All available pieces will then be shown in the panel. In this example, only one piece is generated.



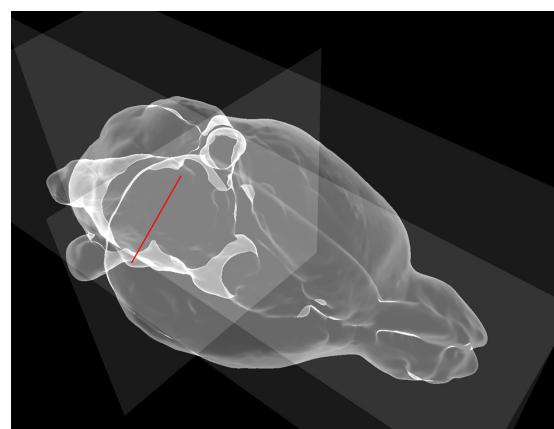
- Click 'Merge Probe' to merge the probe object pieces into a single probe object.



- To obtain probe and electrode information, click on the probe object. A pop up window contain the information will appear.



- To visualize the probe in a 3D view, click the Show 3 Windows button in the Tool bar and drag the window connector (grey dot) which shows up at the bottom of histological image window. The 3D atlas mesh with the probe object will appear after rendering.

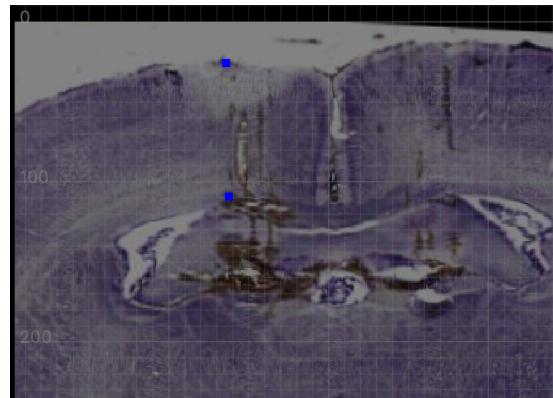


6.6.2 Probe registration across multiple slices

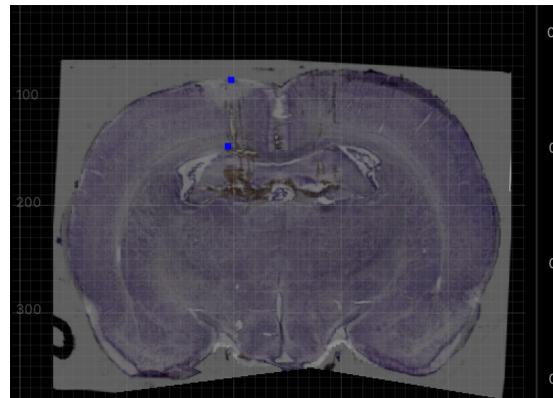
In this recipe we describe how to register a probe when its trajectory traverses multiple histological images. This example uses a case where two Neuropixel 2.0 probes (with 4 shanks) are inserted in both the hemispheres of a rat brain. For simplicity we only use the probe tracks in the left hemisphere.

NOTE: Registering a multi-shank probe is different from generating pre-surgical coordinates for a multi-shank probe (as shown in section 6.4). This is because generation of pre-surgical coordinates uses a template in which all 4 shanks of the probe are included. However, when registering a multi-shank probe, the 'probe piece' elements must be added individually as separate 'object piece's before merging all the shanks together.

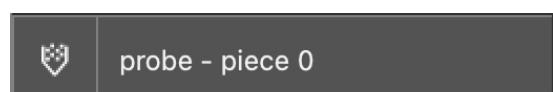
- After overlaying one of the histological images onto an atlas slice, click points along one of the probe trajectories on the histological image.



- Click the 'Accept and Transfer' button in the Tool bar to transfer the selected points from the histological image window to the atlas slice window.



- Go to the 'Object View Controller' panel and click the 'Add object piece' button to generate all available object pieces.



- Repeat the above steps on the same image for each shank until all probe pieces are created.

	probe - piece 3
	probe - piece 2
	probe - piece 1
	probe - piece 0

- Rename all probes by double-clicking the piece name and type in the correct index. Note that **probe** and **piece** are reserved for a probe object piece, and an empty space is required between the reserved keywords and the indexes.

	probe 3 - piece 0
	probe 2 - piece 0
	probe 1 - piece 0
	probe 0 - piece 0

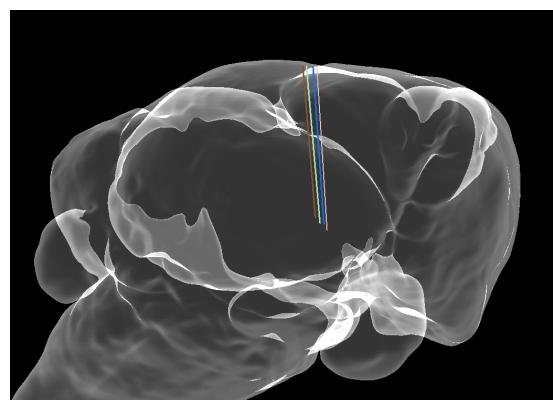
- Repeat the steps above for each of the relevant histology images and rename the probe pieces accordingly, corresponding to probes added for each histological slice. In this example we used two histological slices which contain 4 shanks. Each shank is considered as a single probe. Since we used two images to trace the probe tracks, we named the probe pieces 0 and 1, and the index for the single shanks range from 0 to 3 (e.g: 0, 1, 2, 3).

	probe 3 - piece 1
	probe 2 - piece 1
	probe 1 - piece 1
	probe 0 - piece 1
	probe 3 - piece 0
	probe 2 - piece 0
	probe 1 - piece 0
	probe 0 - piece 0

- Click 'Merge Probe' to merge the probe object pieces into probe objects. In this case, 4 probes will be generated. Click on any of them to see the associated readout, and compare them by linking the objects and clicking the 'Compare' button (see last step in section 6.4).

		merged probe 3	
		merged probe 2	
		merged probe 1	
		merged probe 0	

- Click the Show 3 Windows button in the Tool bar and drag the window connector (grey dot) which appears at the bottom of histological image window.



6.7

Virus Expression Registration

The purpose of this functionality is to visualize the regions that an injected virus is expressed (or which contain an anatomical tracer) rather than the exact coordinates individual cells that show expression. Therefore, the selected points are highlighted based on pixels rather than vectors.

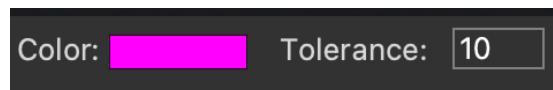
NOTE: The process described here is equally applicable for registering & visualizing anatomical tracers, lesions or neurodegeneration.

6.7.1 Single type of virus expression

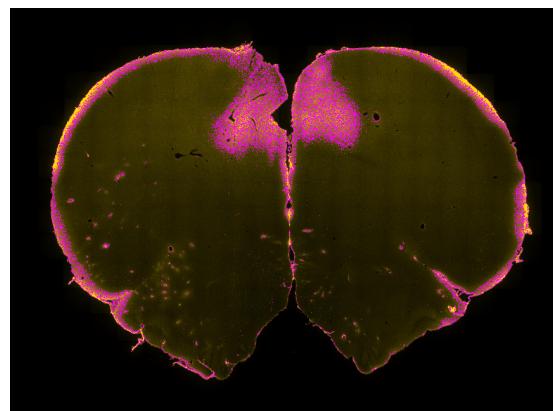
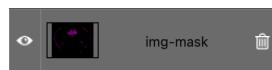
In this recipe, an AAV5-mDIx-Chr2-mCherry-Fishell-3 virus has been injected bilaterally in the cingulate cortex, and viral expression appears in multiple histological images. However for the sake of simplicity and illustration we will take 3 images.

NOTE: In procedure, each of the images is loaded, processed, and the data are saved one at a time. Once the virus expression is defined and saved for a section, the next section needs to be loaded, and the processing steps are repeated.

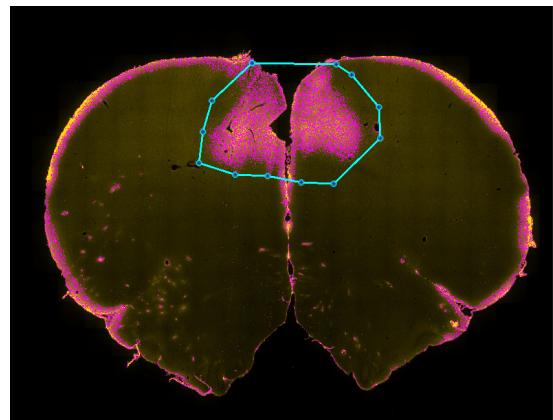
- After matching the first histological image to the atlas slice, check the 'Magic Wand' button. Select a suitable color that contrasts with the virus expression, and select the desired tolerance.



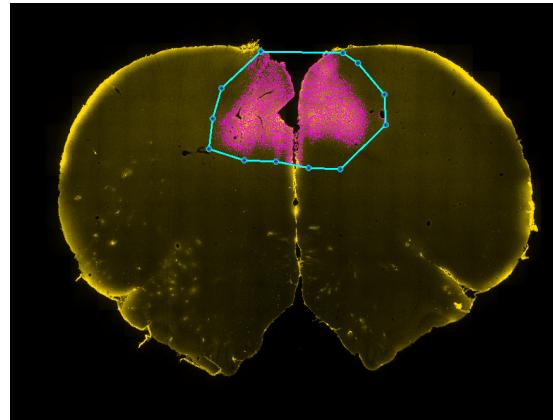
- Click on the histological image in the window on the right where viral expression can be seen. Hold the 'Shift' key and click on the image until the virus is covered completely. It may be necessary to try higher or lower Tolerances before finding the ideal for a given image. Highlighting the virus creates an img-mask layer in the 'Layer View Controller' panel.



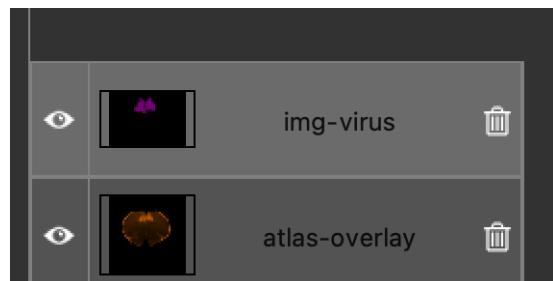
- Check the 'Polygon Lasso' tool and select the area where the virus actually spread by clicking points around the area. Note that when the start point and end point meet, the dashed lasso line becomes solid.



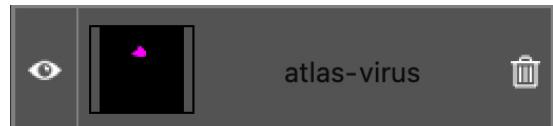
- Press 'delete' key or 'backspace' key (depending on your computer) to delete the irrelevant parts labeled of the selected area. The region is deleted both on the histology image and in the img-mask in the Layer View Controller.



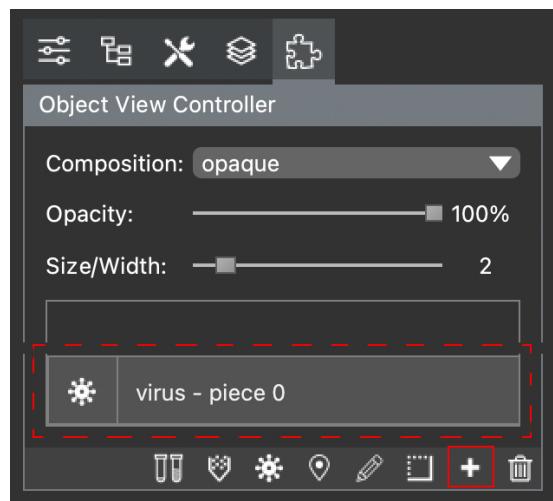
- Click the 'Magic Wand' button again, then click the 'Virus button' to transform the 'img-mask' layer into an 'img-virus' layer. The virus selected will be transferred to the atlas and can be seen as a layer in the Layer View Controller.



- Click on the 'Accept Transformation' button in the Toolbar to transfer selected virus from the histological image window to the atlas slice window. Note that an 'atlas-virus' layer is created in the Layer View Controller. If the glowing viral region disappears from the histology image, click on the Eyeball icon next to the 'atlas-virus' layer and it will appear over the atlas slice.



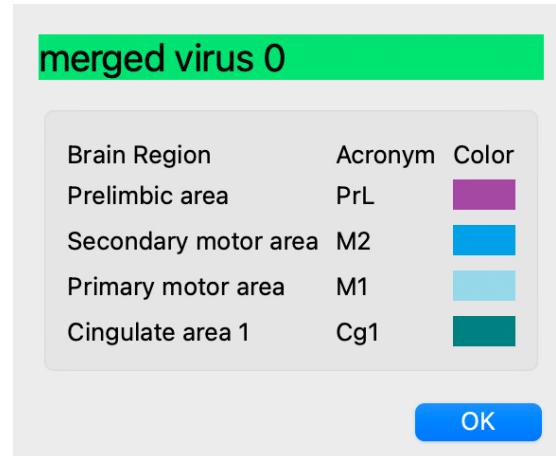
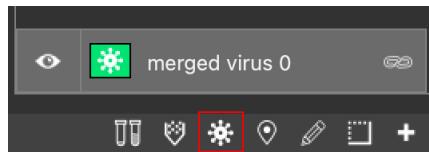
- Go to the 'Object View Controller' panel and click on the 'Add Object Piece' button listed at the bottom of the panel to make a virus piece.



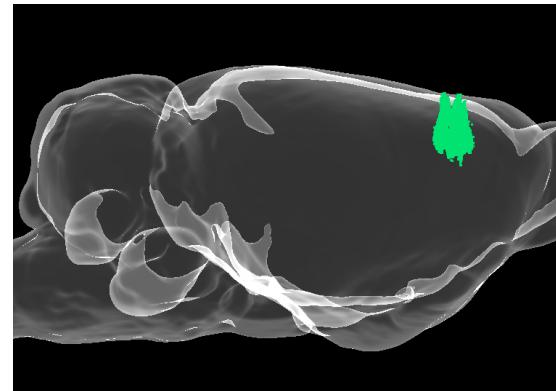
- Repeat the above steps for the next histological images. In this recipe, we use 3 histological images that show expression for the same virus so that 3 virus pieces will be generated.

	virus - piece 2
	virus - piece 1
	virus - piece 0

- To obtain a list of regions containing viral expression, click the 'Merge Virus Piece' button at the bottom of the panel. A merged virus object will be generated and the individual virus pieces will disappear. To view the regional viral read out, click on the merged virus object so that the information window will pop up.



- To view the 3D reconstruction, click the 'Show 3 windows' button in the Toolbar or go to the 3D Window in the popup submenu of the View Menu. Remember to drag the window connector (small grey dot) up to enlarge the 3D window (see section 6.6.1).



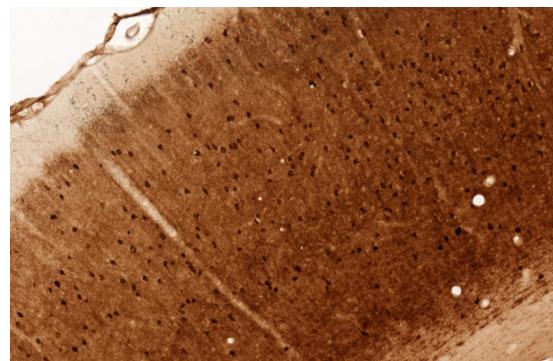
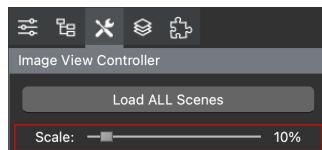
6.8 Cell Body registration

The long-term goal of this functionality is to provide automatic cell counting, but at the moment it provides an interactive way to perform cell body counting. It is worth noting that this functionality works differently when the histological image is an RGB image or immunostained tissue. For RGB images, the cell count only reflects the total count of selected cells. That means if two or more types of cellular staining appear in the histological image, there is no way to distinguish them in the cell count in HERBS. Whereas for immunostained or fluorescent labeled tissue, the cell count considers different colors of labeling of the cells (shown in different layers with different colors). They can be visualized and counted separately since they appear in different color layers when they are selected.

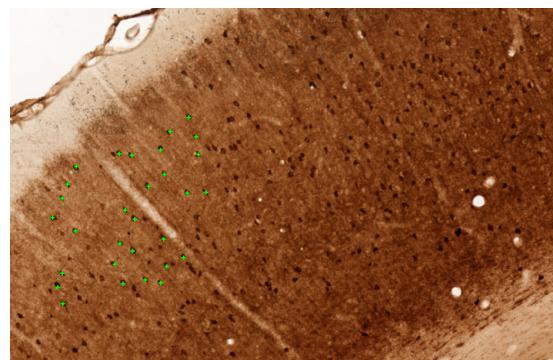
6.8.1 RGB Histological Image

In this recipe, we will illustrate how to do interactive cell counting when the histological image is RGB. For the sake of illustration we choose only 30 cells.

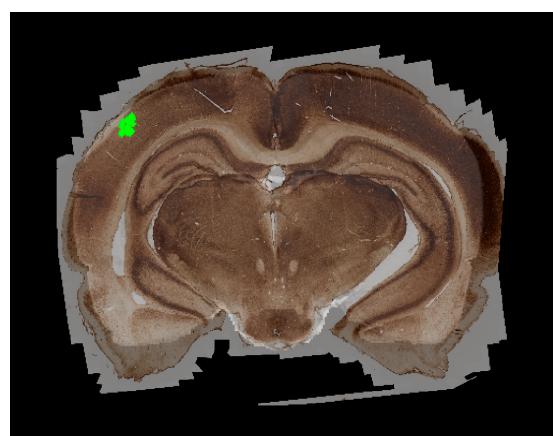
- After overlaying the low resolution histological image to the atlas slice, users can change the histological image to a higher resolution by sliding the 'Scale' slider in the 'Image View Controller' panel, and zoom in the histological image to where cells are located.



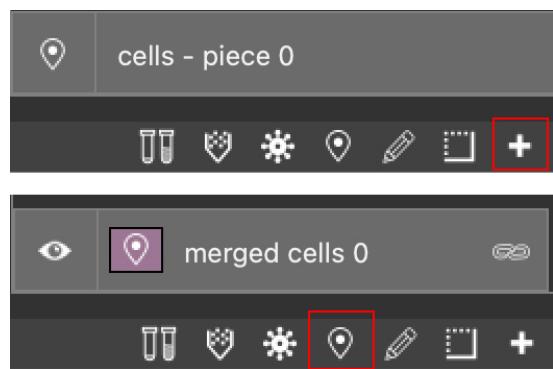
- Check the 'Cell Selector' button in the Tool bar and check the 'select cells manually' button in the popup tool area. Click on the histological image to select the cells of interest. While cells are being selected, the cell count number will increase as well. Note, an 'img-cells' layer will be generated after a cell is selected.



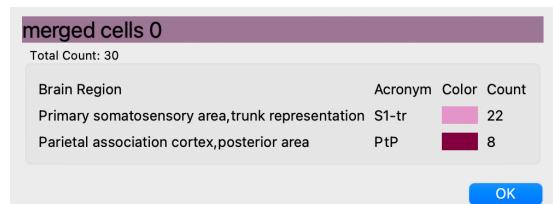
- Click on the 'Accept Transformation' button in the Toolbar to transfer selected cells from the histological image window to the atlas slice window. Note that an 'atlas-cells' layer is created and the 'img-cells' layer will disappear.



- Go to the 'Object View Controller' panel and click on the 'Add Object Piece' button at the bottom of the panel to make a 'cell piece', and turn this piece to a 'cell object' by clicking the 'Merge Cells' button.



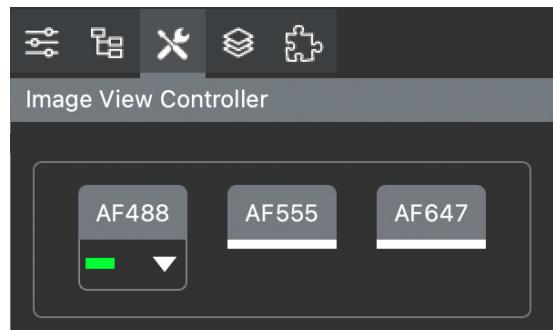
- Click the cell object to see a read out of the cell count in each brain region.



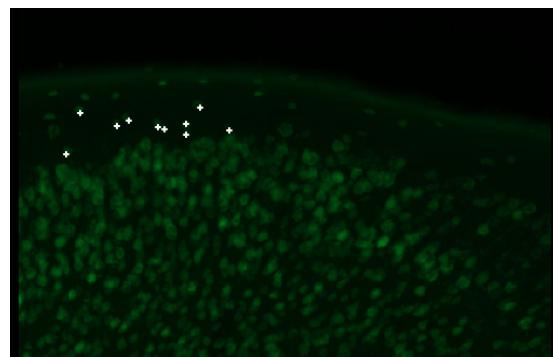
6.8.2 Immunostained tissue

In this recipe, we use a histological section from a mouse brain injected with the retrograde tracers Ctb-555 and Ctb-647, and immunostained with NeuN antibodies to detect cells in the tissue sections. Since different cells are labeled different colors by the tracers or antibody, they occupy different color layers in the histological slice image. In this example, we show how cells in different color layers can be counted by selecting different colors from the layer view controller.

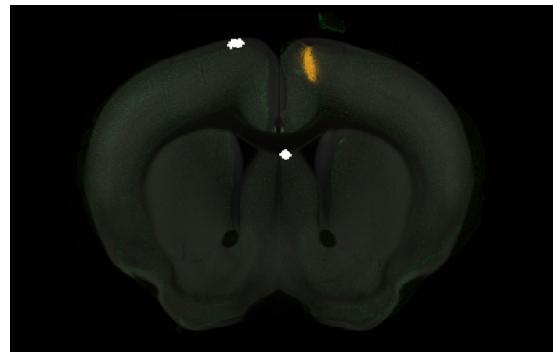
- After overlaying the low resolution histological image to the atlas slice, users can change the histological image to a higher resolution (see section 6.8.1). The color channels can each be toggled on or off by clicking the channel names in the 'Image View Controller' panel.



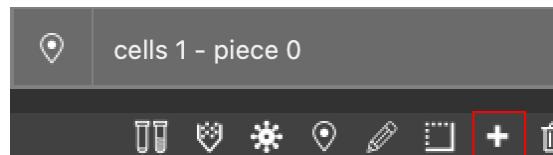
- Check the 'select cells manually' button and select cells on the visible layer of the histological image.



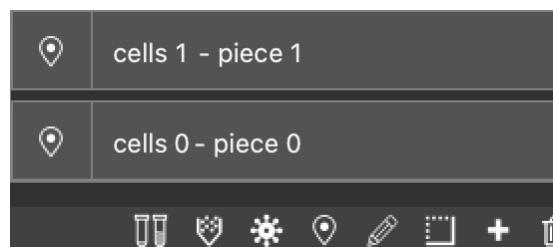
- Click on the 'Accept and Transform' button in the Toolbar to transfer selected cells from the histological image window to the atlas slice window.



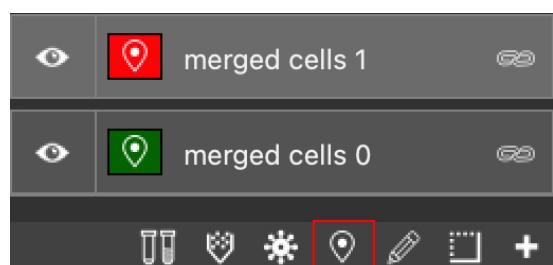
- Go to the 'Object View Controller' panel and click on the 'Add Object Piece' button at the bottom of the panel to make a 'cells piece'.



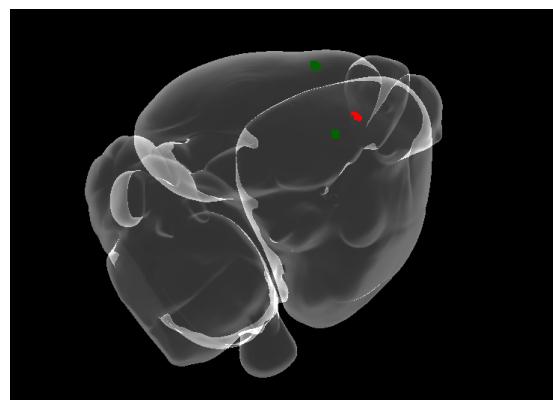
- Repeat the above steps for each of the individual channels until all pieces for all different types of cells are created. Rename the cell pieces to the desired index.



- Click the 'Merge Cells' button to make all 'cell pieces' into 'cell objects'.



- Click the 'Show 3 windows' button in the Toolbar or go to the 3D Window in the popup sub-menu of the View Menu to visualize the labeled cells in 3D. Remember to drag the window connector (small grey dot) up to turn on and enlarge the 3D window (see section 6.6.1).



6.9 Contour of Brain Tissue

This functionality generates boundaries around individual tissue slices in order to construct a 3D mesh for a single brain (still in development). Following the steps below, users can match the histological image to the atlas slice and transfer the boundary to the atlas window.

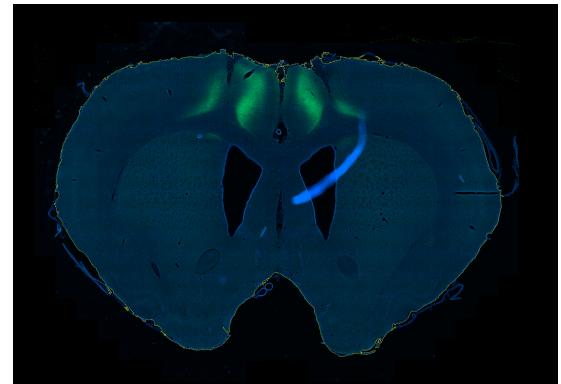
- After loading a histological image, click the 'Magic wand' button in the Toolbar and select a suitable color and tolerance (users should try higher tolerance values here, on the order of 100).



- Click on the histological image where the slice is covered with the selected color. The area of the slice should be covered completely.



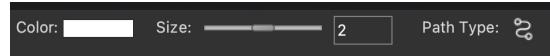
- With the 'Magic Wand' button still selected, click the 'Contour' button in the popup tool.



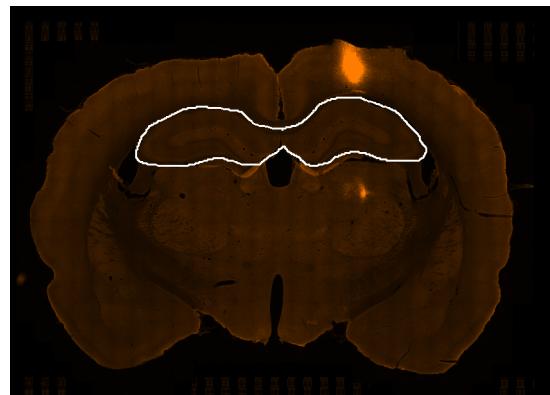
6.10 Draw area/locations of interest

The purpose of this functionality is to record a user-defined line or area of interest which is not a probe trajectory, virus expression or cells in the brain.

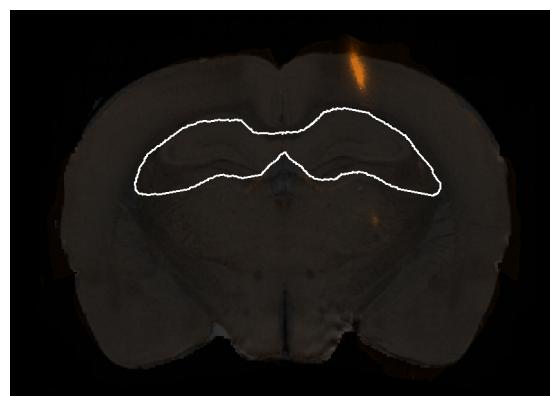
- After matching the histological image to the atlas slice, check the 'Pencil' button in the Toolbar and select a suitable color and preferred path type.



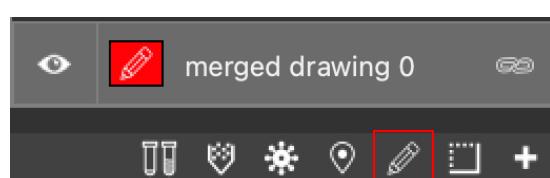
- Click on the histological image once where you would like to begin drawing, and a line will follow the path of the mouse. Click again to stop drawing. Once the line or region of interest is drawn, an 'img-drawing' layer is created in the 'Layer View Controller'.



- Click the 'Accept and Transfer' button to transfer the drawing from the image window to atlas window. When the 'atlas-drawing' layer is created, the 'img-drawing' layer will disappear.



- Click the 'Add Object Piece' button at the bottom of the 'Object View Controller' to make a 'drawing piece', and click the 'Merge Drawing' button to merge individual 'drawing piece's to a 'drawing object'.



- Click the 'Show 3 windows' button in the Toolbar or go to the 3D Window in the popup sub-menu of the View Menu to visualize the hand drawing in 3D. Remember to drag the window connector (small grey dot) up to turn on and enlarge the 3D window (see section 6.6.1).

