ABBA

Aligning **B**ig **B**rains & **A**tlases

User's Guide

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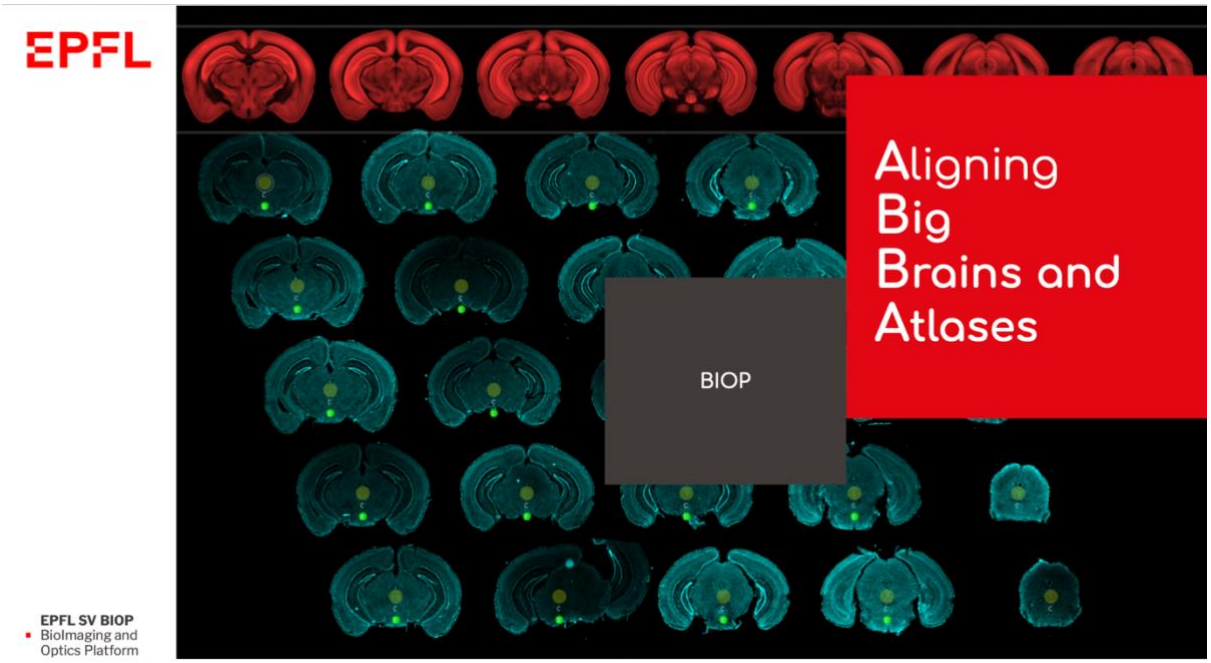
INRTRODUCTION

Overview of ABBA

ABBA (Aligning Big Brains & Atlases) is a Fiji plugin developed by the BioImaging & Optics Platform at EPFL for registering serial biological tissue section images to reference brain atlases. In this workflow, QuPath is used to import and organize experimental brain section images into a project structure compatible with ABBA. The images are then exported from QuPath and processed in Fiji using ABBA for slice registration.

This approach enables accurate alignment of experimental datasets to the 3D Allen Mouse Brain Atlas, facilitating consistent region mapping and quantitative analysis. Registration results are saved in formats suitable for downstream processing without requiring further steps in QuPath.



Purpose of this protocol

This protocol provides step-by-step instructions for installing ABBA, importing and exporting images via QuPath, performing registration in Fiji, and saving the aligned results. It aims to ensure reproducibility and efficiency in brain atlas mapping workflows.

INSTALLATION INSTRUCTINONS

System Requirements

- Operating systems supported（Windows, Linux, MacOS）
- Fiji Installed
- Elastix 5.2.0 installed
- QuPath installed

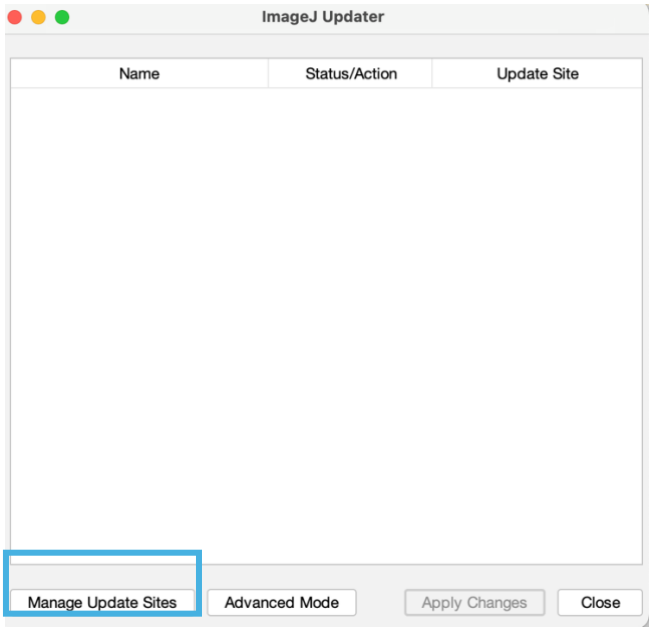
Installing ABBA in Fiji

1. **Download and install Fiji**

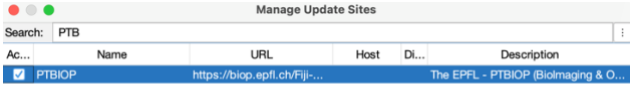
   If Fiji is not already installed, download it from fiji.sc/fiji.latest and install it.

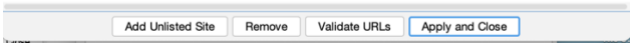
2. **Add necessary update sit**

   - Click Help > Update... > Manage update sites;



   - Tick the checkbox PTBIOP;





   - Click Apply and Close, then Apply Changes;
   - Restart Fiji.

3. **Install elatix/transformix**

   elastix, a toolbox for 2D in-plane registration, is an indipendent program that is executed by ABBA under the hood in order to apply affine and spline transformations to the images.

   - download elastix version 5.2.0 for your operating system;
   - extract it to a convenient location (e.g., C:\ on Windows, /opt/ on Linux, Applications on MacOS).

   Windows

   Download Visual C++ redistributable (`vc_redist.x64.exe` for 64-bit systems) and install it.

   Linux

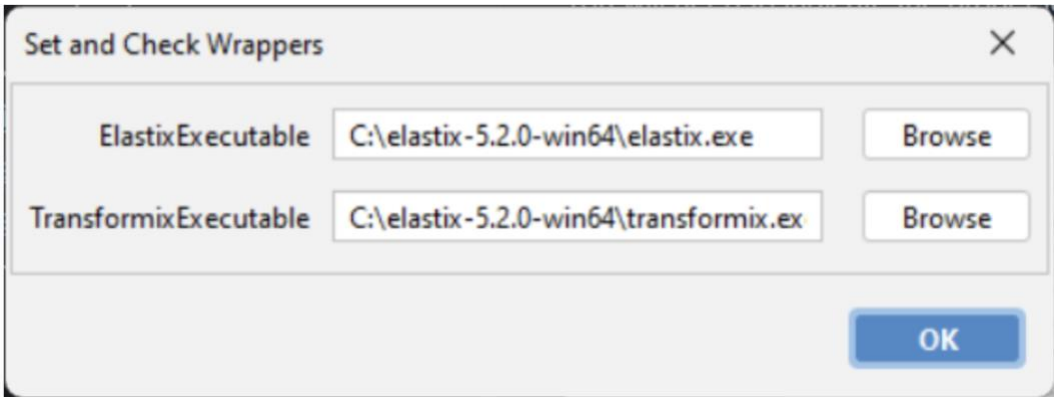
   No special steps are required.

   MacOS

   Since macOS treats elastix and transformix as software from "unknown developers," you need to create security exceptions for both executables to bypass repeated warnings.

4. **Set elastic paths in Fiji**

   Now, you have to tell Fiji where to find your installation of elastix and transformix by clicking on Plugins > BIOP > Elastix > Test elastix. When asked, specify the paths of elastix and transformix executables. For instance:



   In Fiji's console (Window > Console, if closed), you should see a confirmation messages like:

```
[INFO] Elastix  ->      set :-)
Transformix     ->      set :-)
```

5. **Install QuPath extension for ABBA**

   if QuPath is not already installed, download it and install it;

Lastly, you will be asked to start a registration test. Run it and check its result.

PREPARING YOUR DATA

Setting up a dataset of brain sections in QuPath

As recommended, you should begin by creating a QuPath project that contains all the brain slices you wish to register, typically from one animal.

1. **Choose a folder**

The first step of creating a project is to create an empty folder somewhere on your computer. You can then set this to be the project directory in one of two ways:

- Through File > Project… > Create new project
- Drag the folder on top of QuPath

2. **Add images**

The easiest way to add images to a project is usually to drag them on top of QuPath.

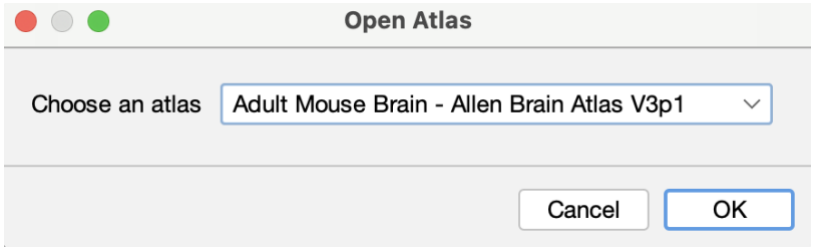
This opens a dialog box, which shows a list of images to import and provides some options to customize how it will happen.

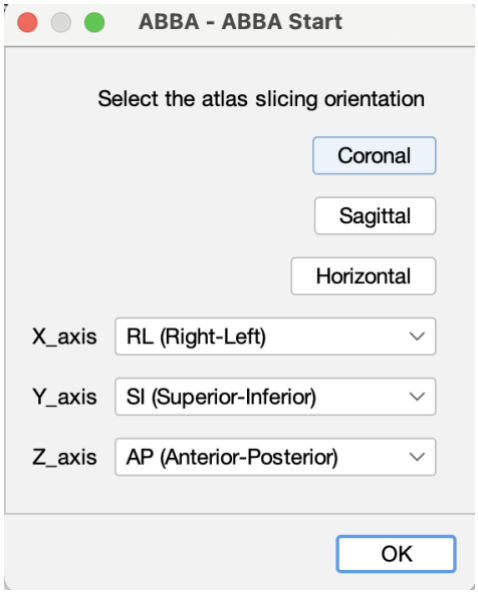
3. **Click Import**

At this point, your work in QuPath is complete. You can leave QuPath open as you proceed with the rest of the workflow.

NAVIGATING & SELECTING DATA

Start Fiji or launch the ABBA application if you used the installer, then start the ABBA plugin (type ABBA in Fiji's search bar or go to Plugins > BIOP > Atlas > ABBA - ABBA Start). You will then need to choose an atlas (this documentation uses the Allen adult mouse brain atlas) and select between three slicing orientations: coronal, sagittal, or horizontal. The examples provided use the coronal orientation, as it is the most common, but ABBA functions similarly in all orientations.





Navigating ABBA's BigDataViewer

ABBA utilizes Fiji's BigDataViewer to display multiresolution images in a highly responsive way. However, the BigDataViewer interface is quite different from the standard ImageJ display, so it's essential to get familiar with the basic navigation controls:

- hold and drag right-click pan across the image
- mouse wheel zoom in / out
- up / down keys zoom in / out
- shift + up / down key fast zoom in / out

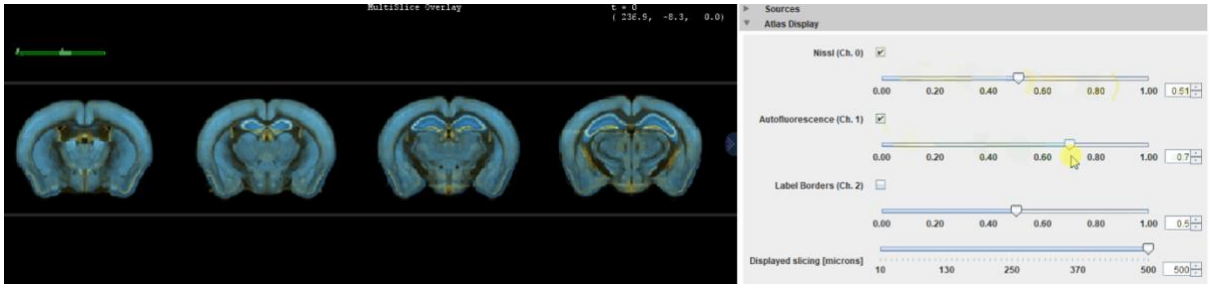
Take some time to practice these commands so you can efficiently navigate across the atlas.

Atlas display options

When ABBA starts, you will see the atlas dataset, sliced regularly along the Z-axis. The dataset consists of a 3-channel image, which in the case of the Allen Brain Atlas CCFv3, includes:

- Channel 0 - Nissl (Ch. 0) – staining nuclei
- Channel 1 - Allen Reference Atlas(ARA) (Ch. 1) – Auto Fluorescence
- Channel 2 - Label Borders (Ch. 2)

You can toggle these channels on or off using checkboxes and adjust their visibility using sliders to emphasize or de-emphasize each one.

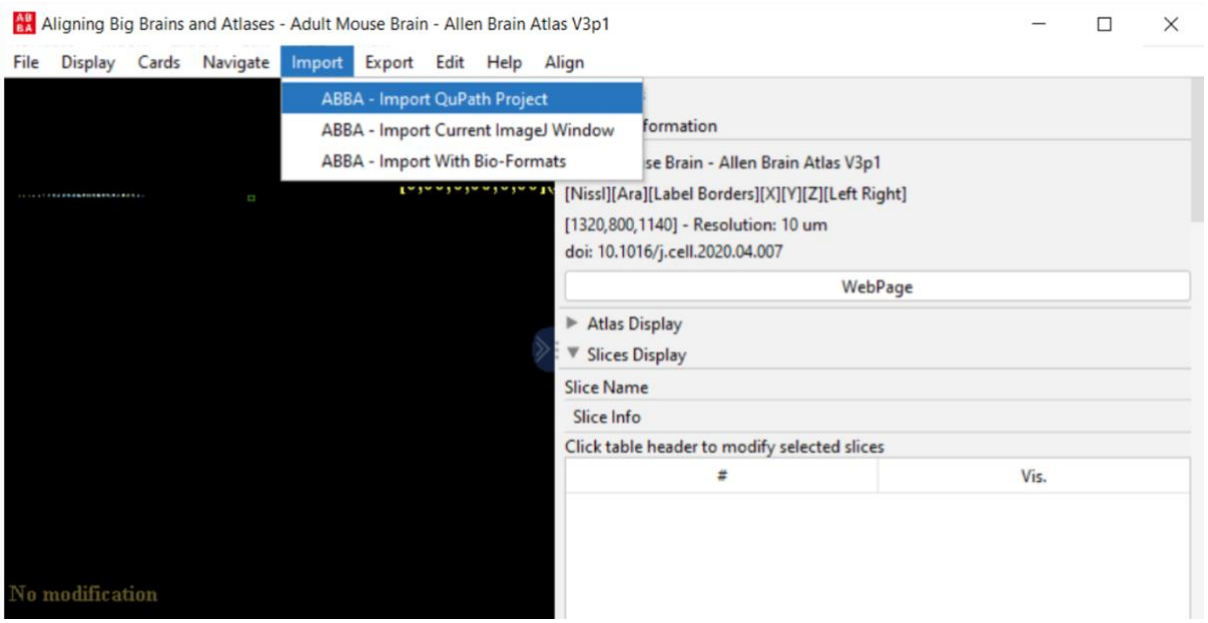


Control the displayed spacing between slices with the displayed slicing [microns] slider:

- 10 steps = 100 microns between slices
- 50 steps = 500 microns between slices

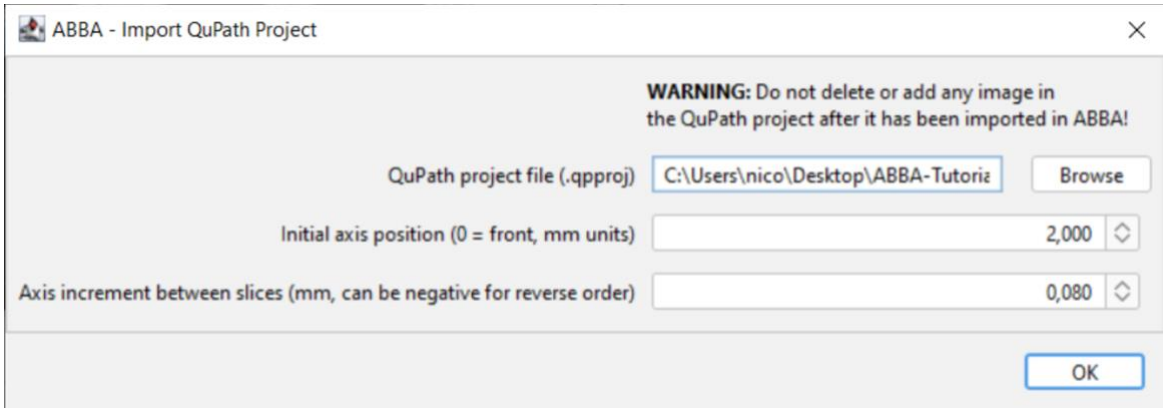
Importing a QuPath Project in ABBA

Once you're comfortable with the navigation, you can proceed to import a QuPath project. In the ABBA window menu, click Import > ABBA - Import QuPath Project:

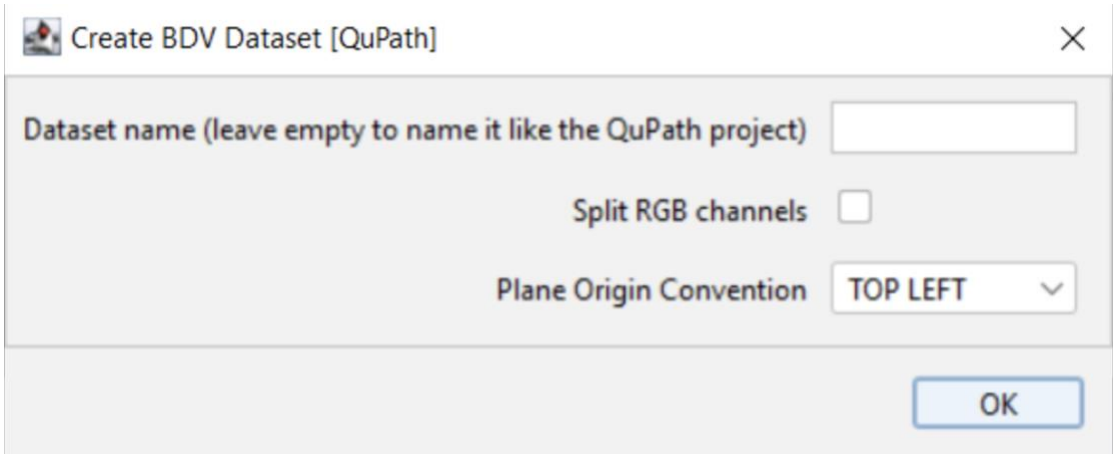


Note: *You can also import the image currently displayed in the active ImageJ window directly. Make sure that the pixel size is set correctly, ideally in millimeters.*

Next, select your project file, specify the initial position of the first slice, and enter the approximate slice spacing in millimetres (the demo dataset uses 80-micron spacing). These values are just starting estimates and will be fine-tuned later.



After this, a second window will appear with advanced import options. The default settings (as shown below) should work for most cases.



The plane origin convention doesn't matter for most users.

The initial import process may take up to a minute. Once the project is loaded, you should see an image similar to this:

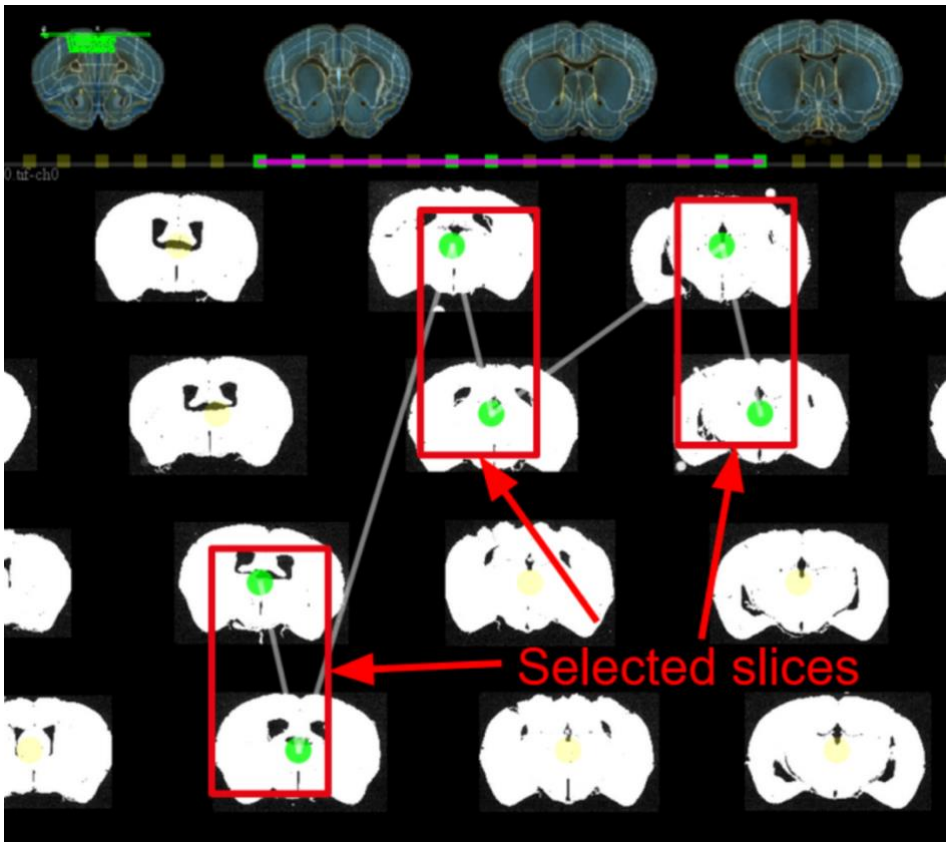
SLICES SELECTION AND DISPLAY

Once your dataset is opened in ABBA, you'll be able to position slices along the slicing axis ("Z") and adjust each slice in 2D (including tilt and roll corrections, 2D affine, and spline in-plane registrations).

When ABBA starts, you'll begin in Positioning mode, where the Allen brain atlas is displayed with regularly spaced slices overlaid on your dataset.

Selecting Slices

ABBA allows you to select and perform actions on specific slices. Each slice is represented by a round handle, which indicates whether it's selected (green) or not (yellow).



There are two ways to select slices:

Rectangle Selection with the Mouse

You can select slices by drawing rectangles around them. There are also modifier keys for adding or removing slices from the current selection:

- Hold and left-click: Draw a rectangle to select slices.

- Ctrl + hold and left-click: Remove slices from the current selection.
- Shift + hold and left-click: Add slices to the current selection.

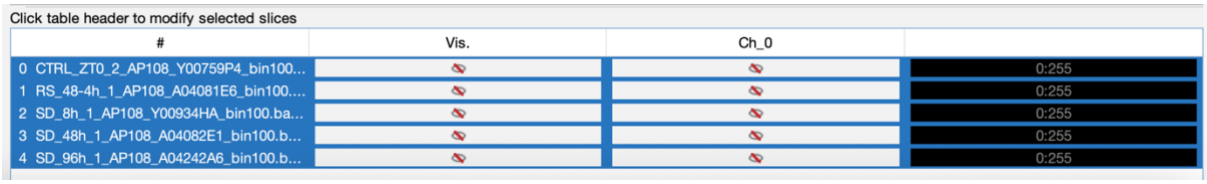
Slice display table

You can also select slices in the Slices Display card table:

The selection behaves as a standard table (in windows: shift to select a range, ctrl to toggle the selection of a single slice). And there are additional shortcuts:

- Ctrl + a: Select all slices.
- Ctrl + shift + a: Deselect all slices.

For Mac users, use cmd instead of ctrl.



Removing Unwanted Slices

In some cases, especially with multi-series files like VSI files, you might encounter unwanted images, such as labels or macro images. These unwanted slices, typically RGB images, will often appear black in the slice display table, making them easy to identify.

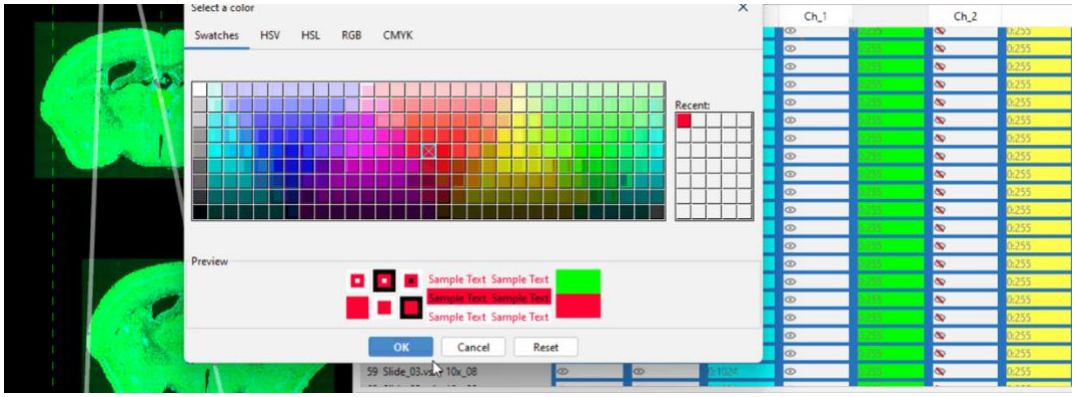
To remove unwanted slices:

- Select the slices that appear black in the table.
- Right-click in the ABBA viewer window and select Remove Selected Slices (or go to Edit >ABBA - Remove Selected Slices in the menu bar).

Displaying Specific Channels

Often, slices have multiple channels, but not all of them are useful for registration or analysis. To focus on the relevant channels, you can control their display by toggling selected channels through the header of the slice display table.

Additionally, you can adjust the color, and the minimum and maximum display values for better contrast (pay attention to where you need to click in the column headers).



If necessary, the display for each slice can be further customized by modifying its corresponding row in the table.

REGISTRATION WOTRKFLOW

The registration process starts with a manual step, which serves two purposes:

- Estimate the position of each slice along the “Z” axis of the atlas.
- Adjust the slicing angles of the atlas.

To help position each slice along the slicing axis, ABBA offers an interface designed for easy manipulation of a series of slices.

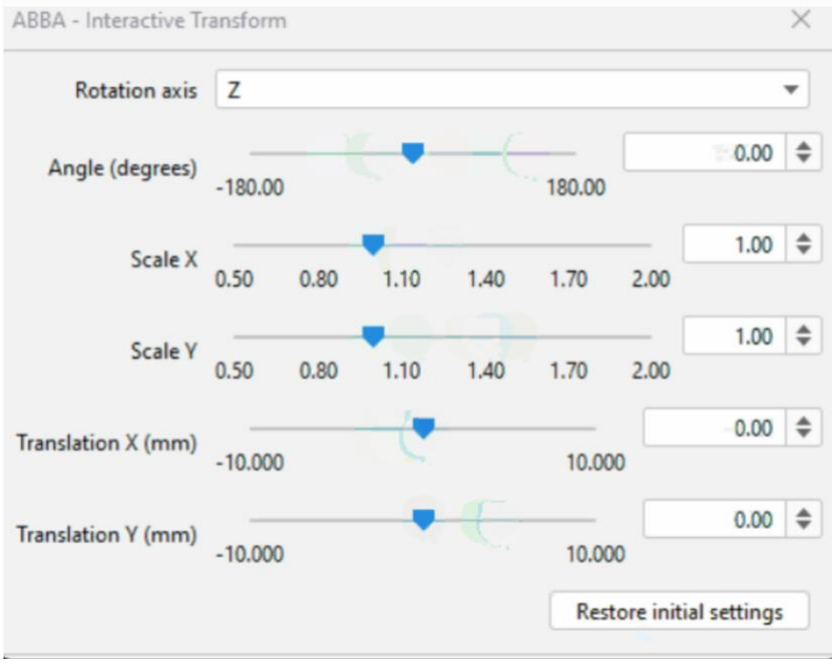
Flip and/or rotate slices

In some cases, the acquired slices might be flipped or rotated relative to the atlas. You can correct this by using the Edit Selected Slices tab, which provides four options:

The first two buttons rotate the selected slices 90° clockwise (CW) or counterclockwise (CCW). The next two buttons flip the slices either vertically or horizontally.

Manual interactive transformation of slices (Scale, Translate, Rotate)

To apply manual transformations (such as rotation, translation, and scaling) to the selected slices, go to the top menu bar and select Register > Affine > Interactive Transform. This tool allows you to rotate, translate, and scale the slices anisotropically (for example, compensating for the common 20% shrinkage in the Y direction due to slicing).



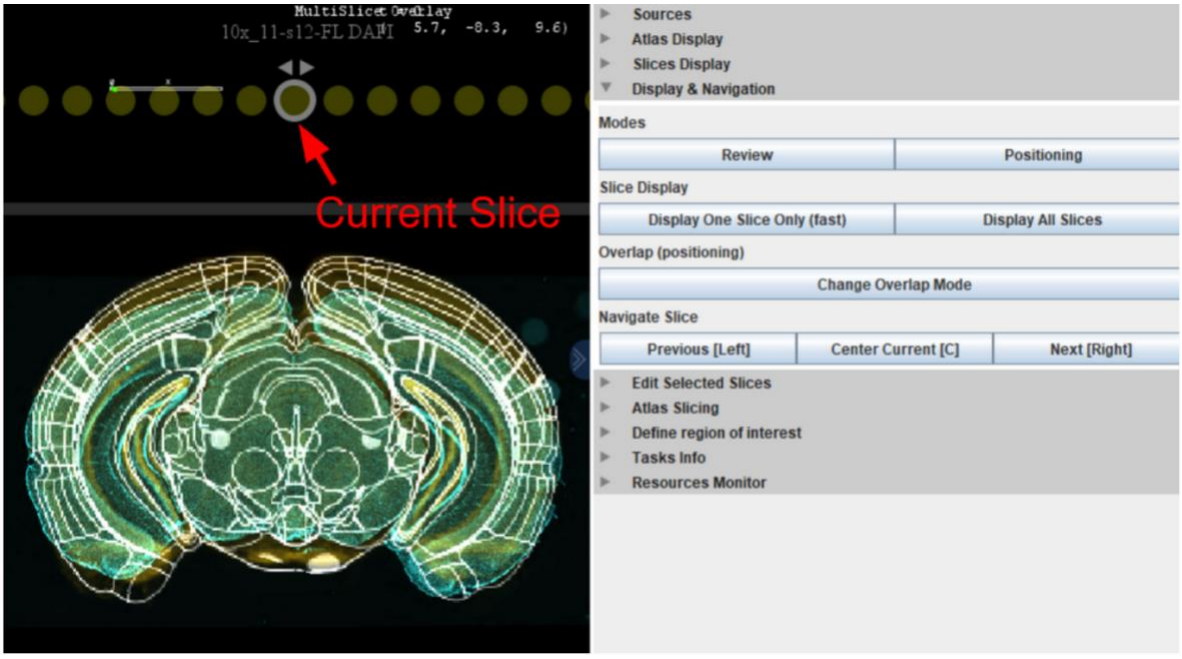
This feature provides fast visual feedback as you apply transformations. Once you're satisfied with the adjustments, simply close the Interactive Transform window.

Using the review mode to investigate the position of slices along the atlas

Positioning mode allows easy movement of slices but is not optimal for overlaying sections onto the atlas. You can switch to review mode to display a single slice at a time, overlaying it with the atlas for inspection.

To switch to review mode:

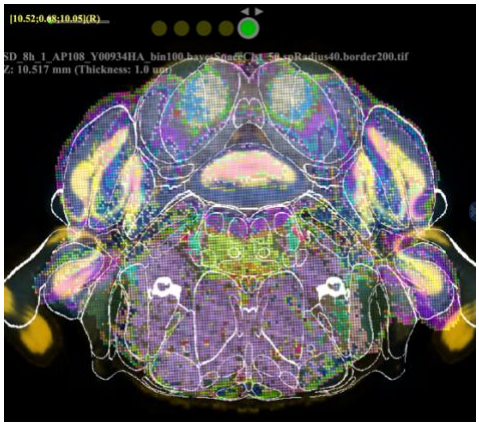
- Press the shortcut key r.
- Or click Review in the Display & Navigation > Modes card.
- Or Select Display > Review Mode from the menu bar.



In review mode, the current slice (indicated by a white circle around the handle) is displayed. Navigate between slices with the left and right arrow keys or use the Previous and Next buttons in the Display & Navigation card.

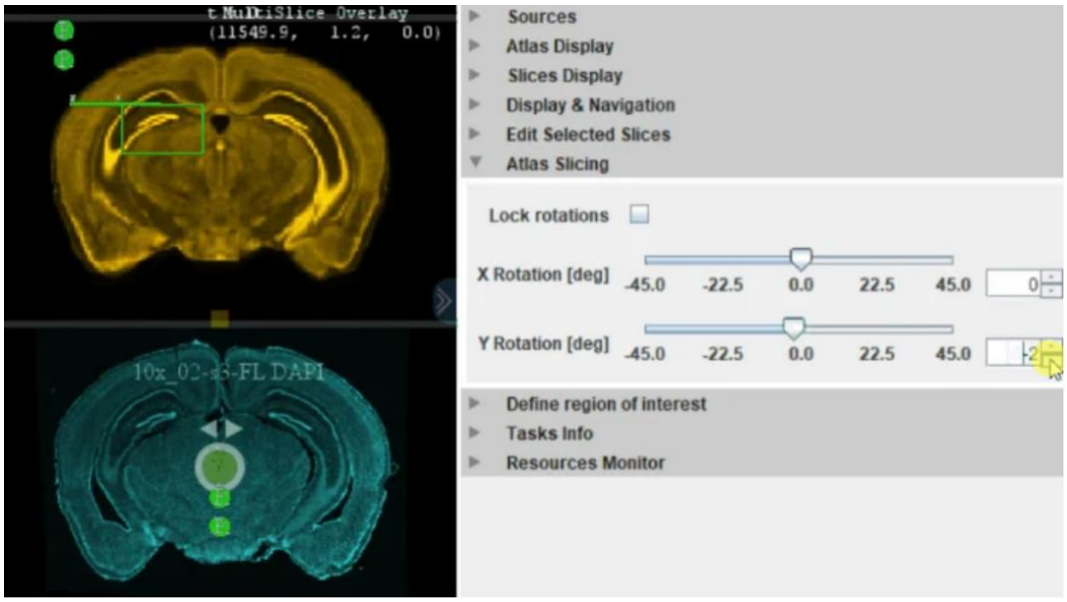
If adjustments are needed, you can return to positioning mode at any time.

Note: *You can also use the Interactive Transform tool in review mode to improve the registration results.*



Correcting atlas slicing orientation

The Atlas Slicing card contains two sliders to adjust the slicing angles of the atlas:



Use slices with easily identifiable features to set the slicing orientation.

EXPORTING & ANALYZING RESULTS

Exporting Results

To export the current results: Go to the top menu bar and select

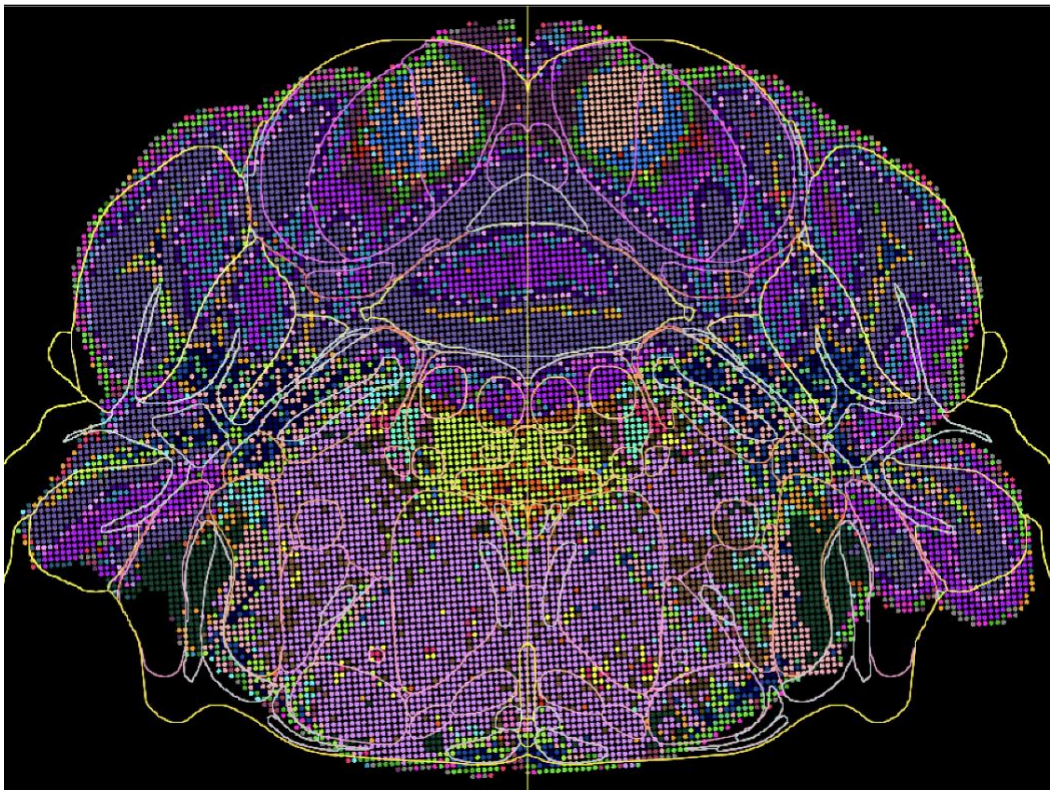
Export > ImageJ > Export Regions To ROI Manager or Export Regions To File.

The latter will save the results as an ROI .zip file, which can then be edited in ImageJ.

Note: *You can also choose to export ROIs using acronyms, in which case the region names will be displayed.*

Analyzing Results

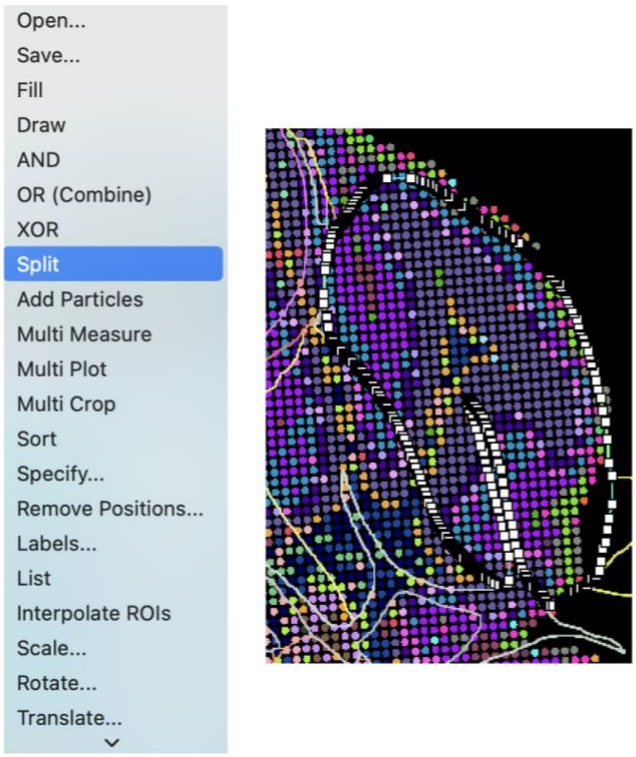
Open the exported ROIs in Fiji, an example result may look like this:



Editing regions

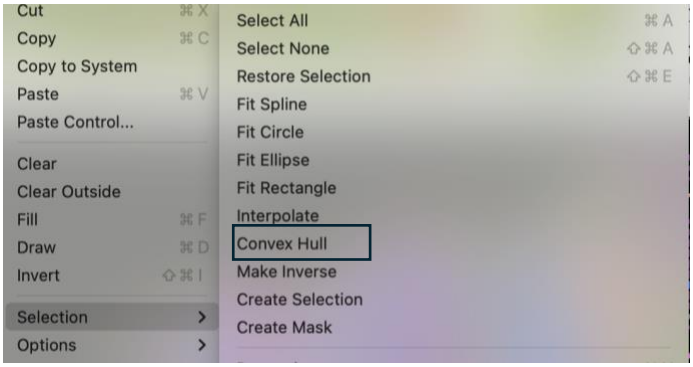
- By default, each region is stored as a *composite ROI*.
- To edit an individual region:
    a) Right-click the ROI and select Split.

    This will convert the composite ROI into individual ROIs with editable points (polygon ROIs).



    b) In some cases, after splitting, only one of the regions will contain points.

    For regions without points, go to Edit > Selection > Convex Hull to convert them into polygon ROIs.



Once converted, you can move points to adjust the region’s shape as needed.

Saving Adjusted Results

After making all necessary adjustments to the ROI, Save the ROIs as a .zip file in your desired location.

TUTORIAL & RESOURCES

For more detailed guidance and examples on using ABBA, refer to the following resources:

1. **Video Tutorial** (YouTube, 2022)
   https://www.youtube.com/watch?v=sERGONVw4zE
2. **Written Documentation**
   https://abba-documentation.readthedocs.io/
3. **Slide Tutorial** (Google Slides)
   https://docs.google.com/presentation/d/1c5yG-5Rhz5WlR4Hf9TNVkjqb6yD6oukza8P6vHGVZMw/edit?slide=id.g216719c3381_0_385#slide=id.g216719c3381_0_385

References

If you use ABBA in your work, please cite the following reference:

Chiaruttini, N., Castoldi, C., Requie, L., et al. (2025). ABBA+BraiAn, an integrated suite for whole-brain mapping, reveals brain-wide differences in immediate-early genes induction upon learning. *Cell Reports*. https://doi.org/10.1016/j.celrep.2025.115876