

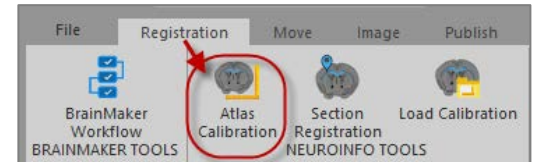
There are three main steps involved in the brain region identification process: atlas calibration, section registration, and anatomical identification.

CALIBRATE THE ATLAS

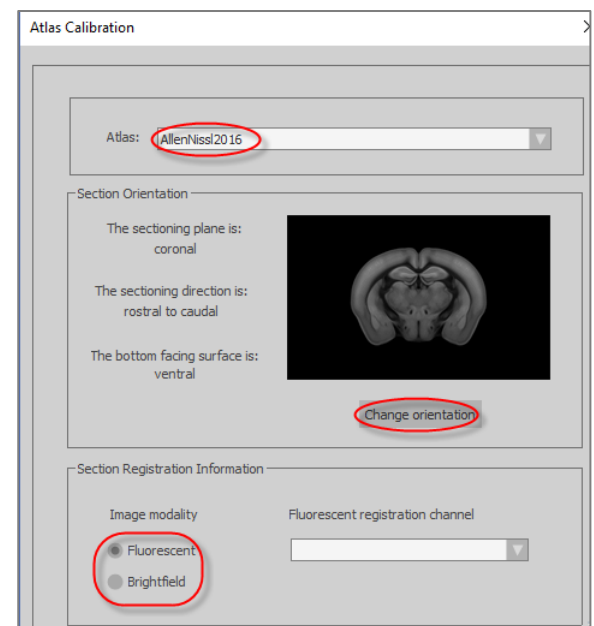
1. Click the *Open an image file* button in the Quick Access bar to open your image.



2. Click **Registration>Atlas Calibration** to open the *Calibration* window.

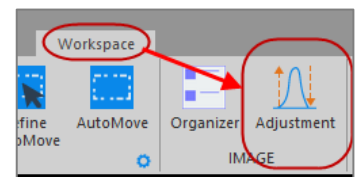


3. In the *Atlas Calibration* window:
 - a. Select an **atlas** (we provide Allen atlases by default but you can add your own).
 - b. Optional - Define the orientation of your experimental section: Click **Change Orientation**.
 - c. Select the experimental **image modality**.



For fluorescence:

- i. Select the channel to be used for registration from the drop-down menu.
- ii. Go to **Workspace>Adjustment** to open the *Image adjustment* panel and select the registration channel to display that channel only in the experimental section.



- d. Click **Next**. The *Atlas Calibration* window changes to display more settings, and the *Atlas* window opens, showing a 2D reference section on the left and the 3D reference brain volume on the right.
4. Match reference and experimental sections as closely as possible using the *Atlas* window and the *Atlas calibration* settings.
 - a. To help with visualizing the two sections, try **Show Tissue Overlay**, **Use Color [...]** or adjust transparency with the slider: the closer to **Section**, the more visible the experimental section; the closer to **Atlas**, the more transparent the experimental section.



- b. Navigate in Z to find the closest matching reference section: use the Z slider (drag the slider or scroll the mouse wheel to move along the slider).
- c. Adjust the scaling (scaling sliders) and orientation (shift and rotation sliders) of the reference section so that it matches the experimental section as closely as possible.

Select a plane at a location approximate to the cut plane.

Z :

Adjust scaling until the sections are the same size.

X :

Y :

Adjust shift until sections are centered.

X :

Y :

Adjust rotation until section overlap as closely as possible.

X :

Y :

Z :

Automatically refine the manual adjustments

- d. Click **Automatically Refine** [...] to finish the calibration.

7. Save your calibration: Click **Next**, enter a calibration name, check **Set as default** [...] and click the **Save calibration** button.

Atlas Calibration

Save the atlas calibration for use with similar experimental sections.

Lab3-Calibration

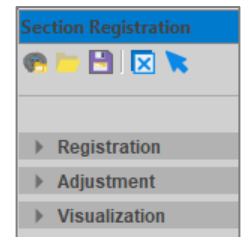
Save Calibration

☒ Set as default atlas calibration

REGISTER SECTIONS

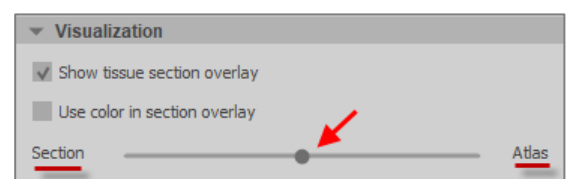
Here, we'll assume that the sections we want to register are complete sections (see User guide for instructions for partial sections) and that we want to use the calibration we just performed.

1. From the *Registration* ribbon bar, click **Section Registration** to open the *Section Registration* window. The panel provides three types of registration controls: Registration, Adjustment, and Visualization. The calibration settings you saved as the default settings in the previous step will be applied.



2. In the *Section registration* window, use the *Visualization* options to help you visualize the overlapping sections.

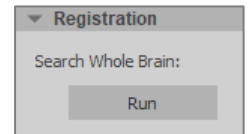
Adjust transparency with the slider: the closer to **Section**, the more visible the experimental section; the closer to **Atlas**, the more transparent the experimental section.



- Under *Registration*, click **Search Whole Brain>Run** to identify the position of the section within the atlas by scanning along the sectioning axis.

If you're not satisfied with the results:

- Use the *Adjustment* controls to align the section manually.
- Under *Registration*, click **Search Locally>Linear**. Local search only considers section positions that are similar to the current section position.



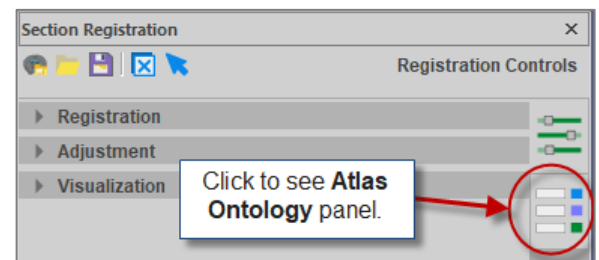
- Save the registration: Click the button in the top bar of the *Section Registration* window.



IDENTIFY ANATOMICAL STRUCTURES

Use the Atlas ontology to automatically identify structures once you've registered a section. The ontology provides a comprehensive list of anatomical structures.

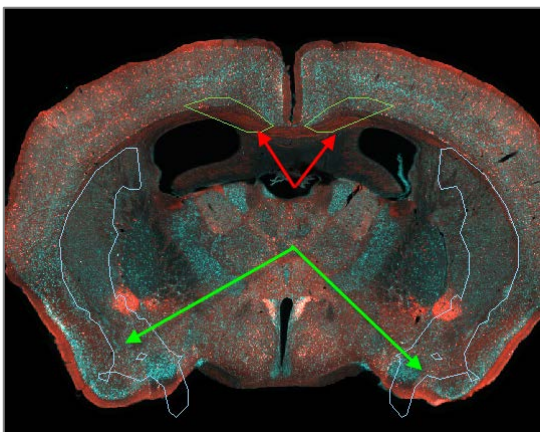
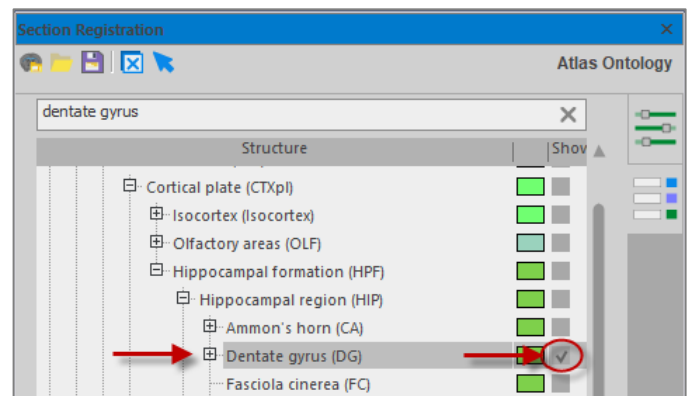
- In the *Section Registration* window, click the **Ontology** button on the right hand side.



- Use one of these two methods to delineate and identify a region:
 - Click the **Select Region** button in the top bar then click the region in the section.

OR

- Find the desired structure in the ontology list and check the corresponding checkbox.



Whether you click in the section or select a structure from the list, the structure is automatically delineated.