# Pathology Identification and 2D Image Registration w/ arivis Pro

*Carl Zeiss AG*

## Introduction

The goal of this tutorial is to instruct a user on performing image registration of the Allen Brain Atlas onto whole mice brain slices and identify cells/pathology within certain brain regions using arivis Pro with a Python script.

## Roles and Responsibilities

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Technical Contact: Jason Fung (jason.fung@zeiss.com)

## Folder Package

This project is contained in the folder titled “Wellington Lab Histology Images”. The following **folders/subfolders** and *files* are listed here:

1. **Data/**
   - **757317_DAB**
   - **769746_GFAP_Iba1_costain-001**
     - *769746_GFAP_Iba1_costain-001.**czi***
     - *769746_GFAP_Iba1_costain-001.**sis***
       - Demo dataset
2. **Documentation/**
   - **"**DATA_to_Atlas_brain_registration_Zeiss_arivis_Pro**.pdf"**
   - **"**summary_Atlas_Brain_registration_Zeiss_arivis_Pro**.pdf"**
   - **"**Pathology Identification and Registration Manual**.docx"**
   - **"**Atlas_to_DATA_brain_registration_Zeiss_arivis_Pro**.pdf"**
3. **Pipelines/**
   - *"step_1_pre_registration_processing**.pipeline**"*
   - *"step_2_label_registered_atlas**.pipeline**"*
   - *"step_3_tau_identification**.pipeline**"*
   - *"step_4_compartmentalize_segments**.pipeline**"*
4. **Registration Files/**
   - **ABA_Tags_Import/ –** *folder containing Allen Brain Atlas tags for areas of the brain*
   - **Atlas_files/ –** *Folder containing the .**sis** file of the annotated volume of the Allen Brain Atlas*
   - *ants_reg_env**.yml** – yaml file for setting up the Anaconda python environment*
   - *Atlas_to_DATA_registration_2D_only_v3**.py**- python file for running registration*
5. **Scripts and codes/**

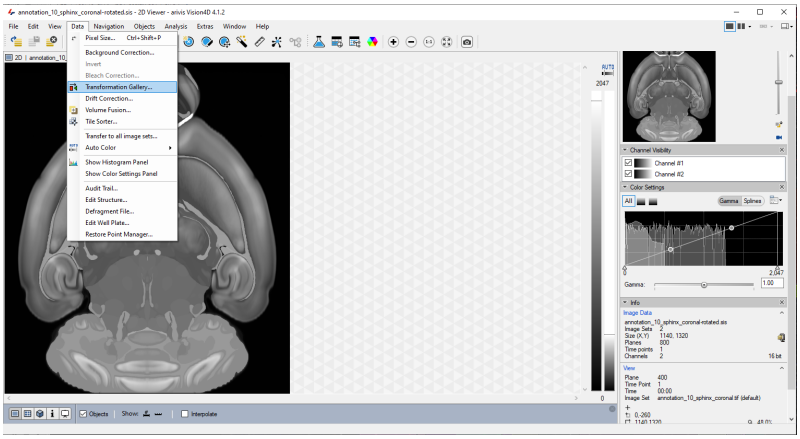
- *ABA_annotation_changing**.ipynb** – jupyter notebook for editing and simplifying the annotation atlas.*
- *ABA_get_anatomical_name_list**.ipynb** – jupyter notebook for getting the original list of subregion names along with its annotation IDs*
- *Mouse_connectivity_manifest**.json - .json** file to extract configurations and metadata from the brain atlas API*

6. **Templates/**
   - **Annotation/**
     - *Annotation**.nrrd** – unmodified annotation volume from the allen brain atlas API*
     - *Full_atlas_template**.tiff** – **.tiff** file of the annotation.nrrd file.*
   - **Volume/**
     - *Autofl_volume**.nrrd** – unmodified autofluorescence volume from the allen brain atlas API*
     - *Autofl_volume**.tiff** - **.tiff** file of the autofl_volume.nrrd file*

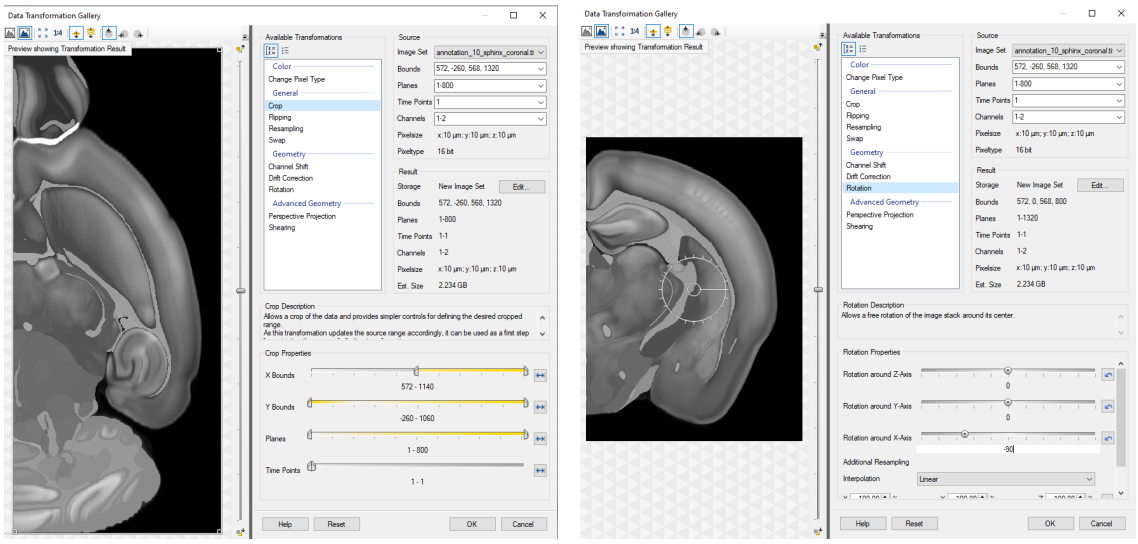
## Procedure

### Creating atlas (Option 1) Cropping and editing the original .sis file

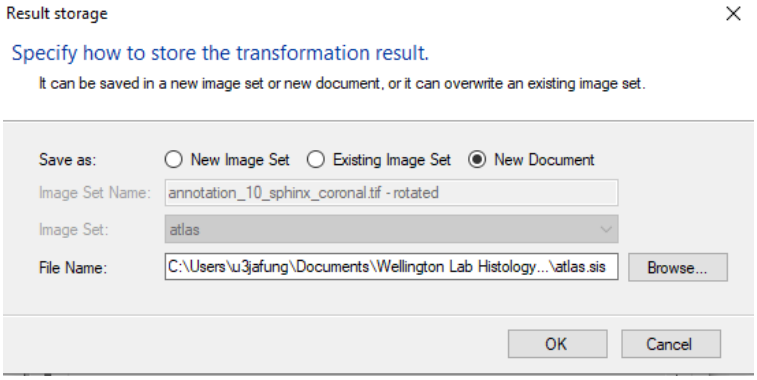
1. In "**Registration Files/Atlas_files",** and open "*annotation_10_sphinx_coronal-rotated.**sis**".*
2. Under the Data panel at the top bar in arivis, select Transformation Gallery.




3. In the Data Transformation Gallery window, perform a Crop operation first and then a Rotation operation.
   a. For halved coronal section: crop half the image and rotate -90 degrees about the x-axis.




4. Under the Result section, click the Edit button next and change the storage to New Document. Type in the desired File Name and make sure it is saved in a easily accessible folder. Click OK.




5. Click OK to start transformation.

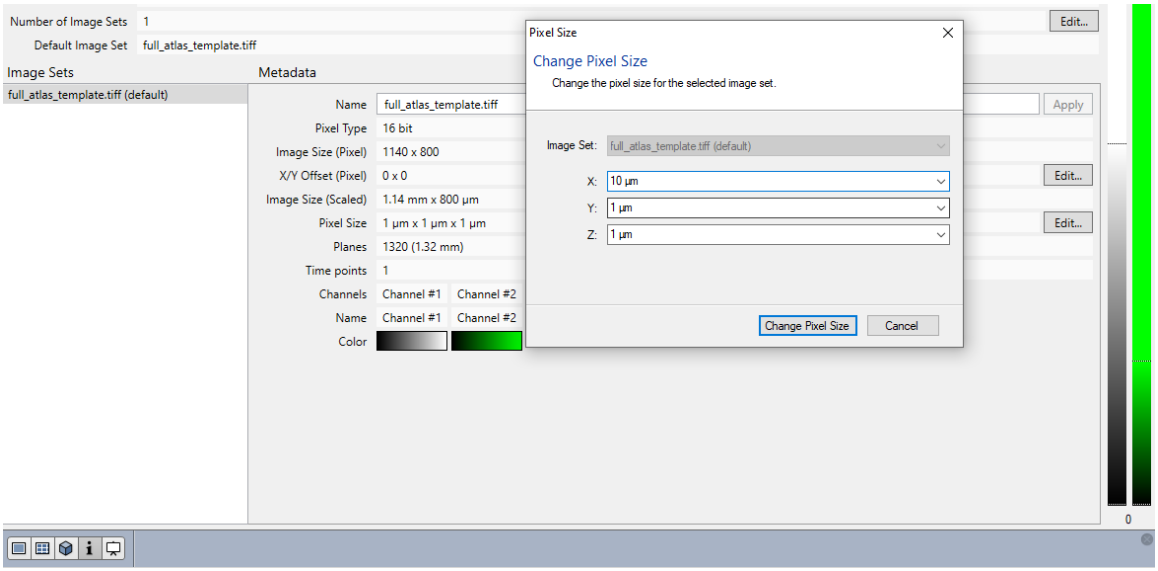
## Creating atlas (Option 2) from scratch

There may be a case where the annotation volume has subregions pruned and edited to simplify analysis. folder "**scripts and codes/**" In this case a new **.sis** file needs to be recreated. To simplify the structure, refer to file *ABA_annotation_changing.**ipynb.***

After simplifying your structure using the python notebook file, follow the instructions below:

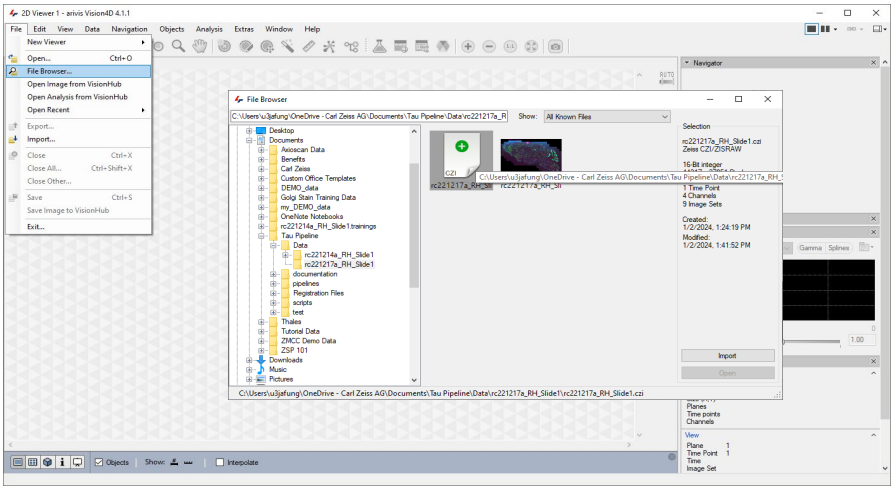
1. Import the annotation volume first by dragging the file *full_atlas_template.**tiff*** into arivis and save as a New File.
2. Then drag the file *autofl_volume.**tiff*** into the same viewer.
3. The pixel info will not be correct. To change the pixel size for the imageset, at the bottom of the arivis window, click on the information tab with the icon  and edit the

Pixel Size from 1 um x 1 um x 1 um -> 10 um x 10 um x 10 um. Make sure to also change the Name to “atlas”.

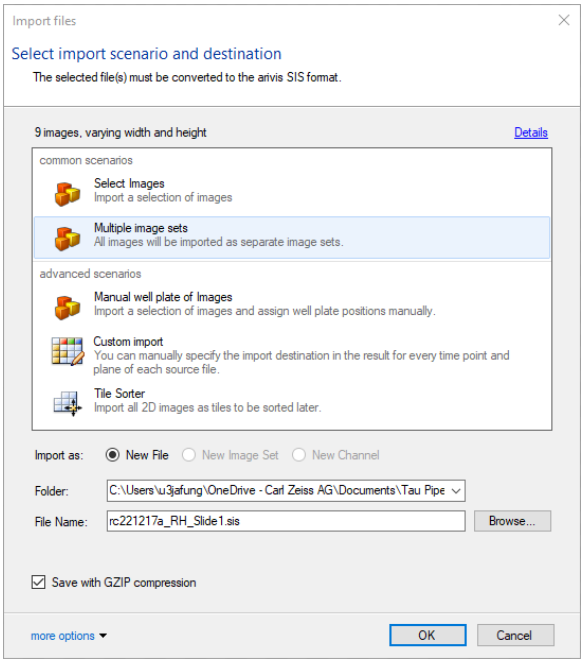



## Importing .czi Data to .sis

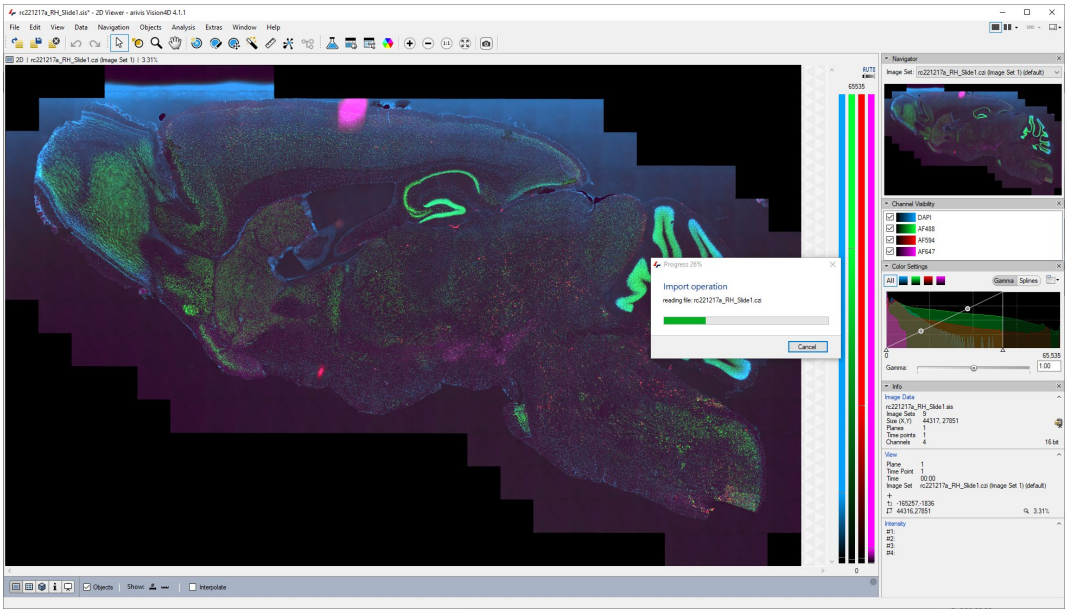
1. In arivis Pro, select the “File Browser” and navigate to the folder containing .czi file



2. Select the .czi file you want to convert. An “Import Files” tab will pop up with import configurations. If there are multiple images in the .czi file, choose “Multiple image sets”. Otherwise, select the image sets in “Select Images” to choose individual images to be imported.



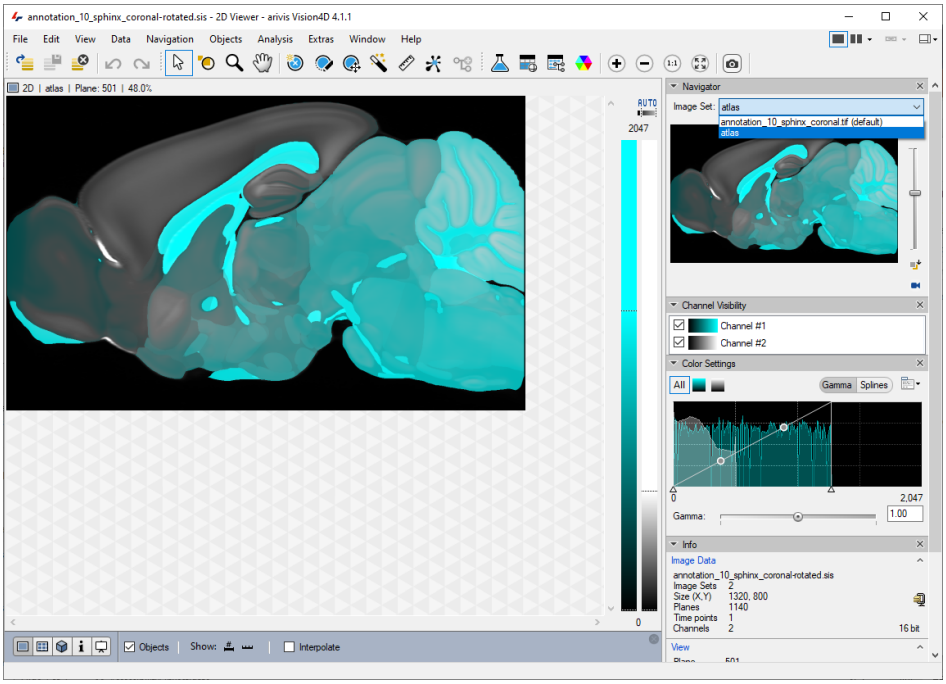

3. Allow the program to load the image. If multiple large images are selected, this will take around a few minutes to load.



## 2D Registration

### Image Downsampling

1. In the **Registration Files/Atlas_file** folder, open the arivis file *atlas*.**sis**. Make sure the **.sis** file has the 'atlas' Image Set shown in the navigator tab. You should now have two arivis windows open, one for the sample data, and one for the brain atlas.

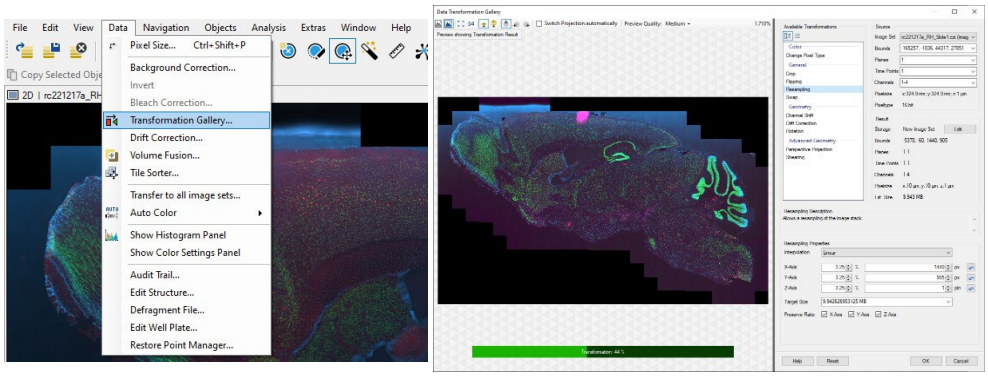



2. To register the Allen Brain Atlas to the sample image slice, the image needs to be downsampled to the same resolution as the slices in the Allen Brain Atlas.

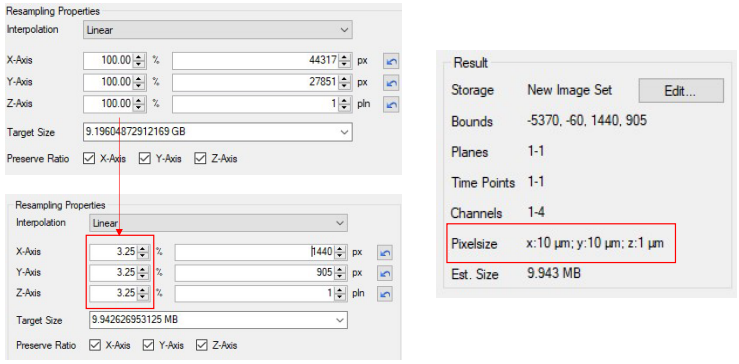
Metadata

| | |
|---|---|
| Name | atlas |
| Pixel Type | 16 bit |
| Image Size (Pixel) | 1320 x 800 |
| X/Y Offset (Pixel) | -90 x 0 |
| Image Size (Scaled) | 1.32 cm x 8 mm |
| Pixel Size | 10 µm x 10 µm x 10 µm |
| Planes | 1140 (1.14 cm) |
| Time points | 1 |

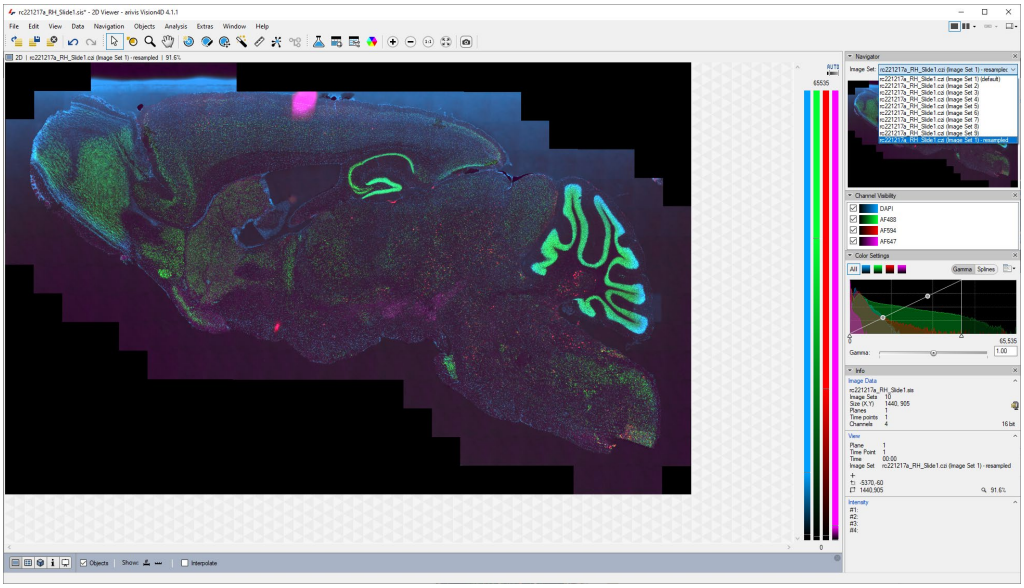
   a. In the sample image Arivis window, navigate to the "Data" tab at the top and select "Transformation Gallery".




b. In the transformation galley window, in the resampling tab, change the resampling properties: X,Y,Z-axis to 3.25%. This will result in the X,Y pixel size in the resampled image slice to be equivalent to the Allen Brain Atlas pixel size.



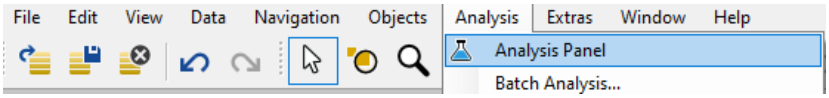

c. Now in “Image Set” drop down in the Navigator tab should show a resampled image.



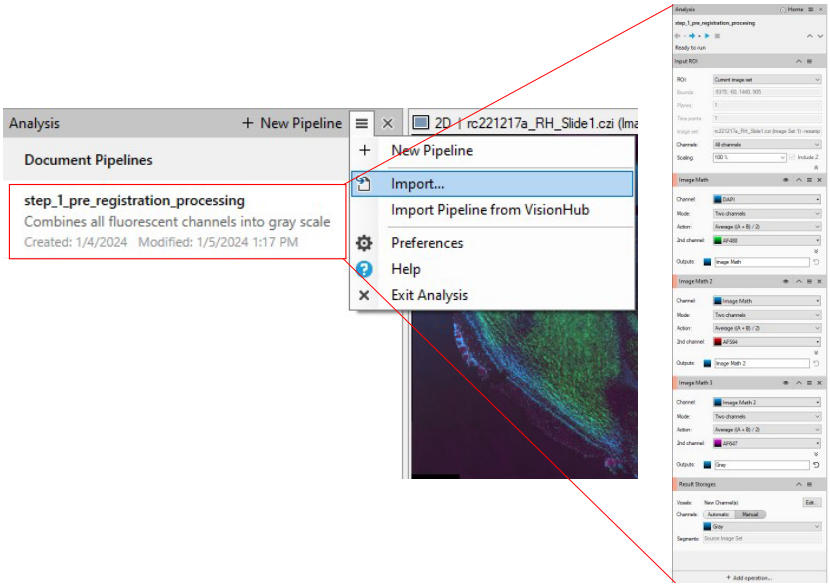
## Image Preprocessing – Combining Channels

Ideally, the image should reflect closely to the allen brain atlas, where the contrast in finer detail regions should be structurally similar. Therefore, the following image processing section shows you how to combine the channels. **If** a channel already shows similarity you can ignore this step.

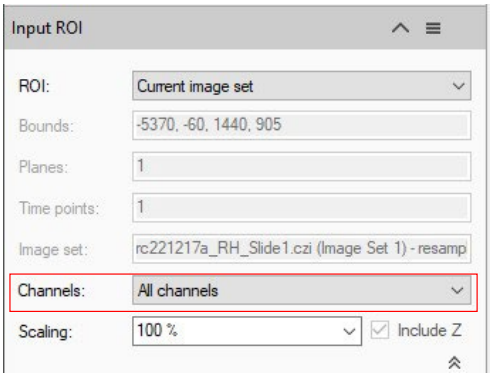
1. Select the resampled image set, in the navigator panel.
2. In the “Analysis” tab at the top, select “analysis panel” to open the analysis pipeline window. This will open up an analysis pipeline panel.



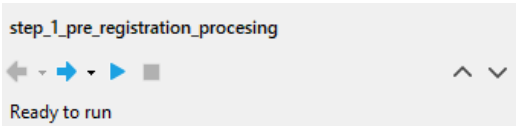

3. In the Analysis panel, click on the  button to import a pipeline. Select the file *step_1_pre_registration_processing**.pipeline*** file in **pipelines/** folder.



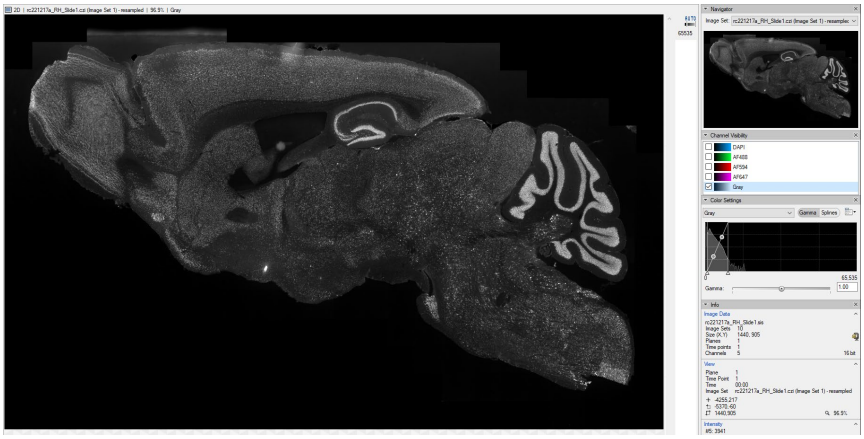

4. After importing *step_1_pre_registration_processing**.pipeline*** file, make sure the correct image set is selected and all 4 channels are selected in the Input ROI section of the pipeline.




5. The pipeline loaded should not need to have any parameters changed. To run the pipeline, click the button at the top of the 'Analysis' tab.




6. The result should be a gray image channel that gets produced for the resampled image set.



7. Ctrl+S to save your result and exit the pipeline by clicking 'x' on the top right corner of the analysis document tab.

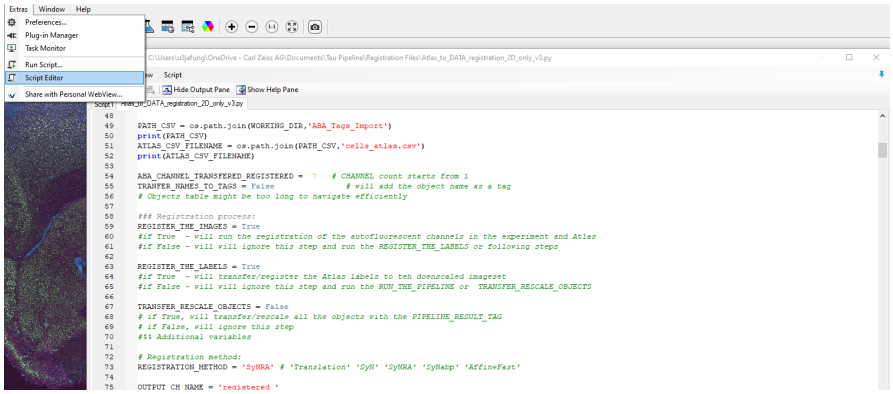
## Running 2D Registration

1. 2D registration uses a python script loaded with Anaconda. If haven't already, refer to sections 5-7 (pg. 15-17) in pdf located in the documentation folder, to download/install Anaconda:

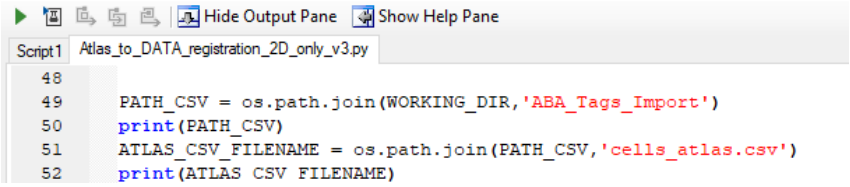
Tau Pipeline/documentation/Atlas_to_DATA_brain_registration_Zeiss_arivis_pro.pdf

2. In **Registration Files/**, a python script called *Atlas_to_DATA_registration_2D_only_v3**.py** should be in the folder

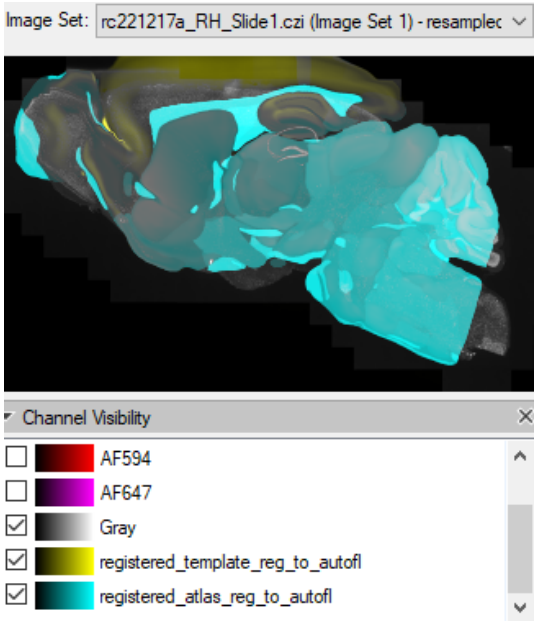
3. In the Arivis window that is showing the image dataset, click on the *Extras* tab at the top to open the python file *Atlas_to_DATA_registration_2D_only_v3**.py***



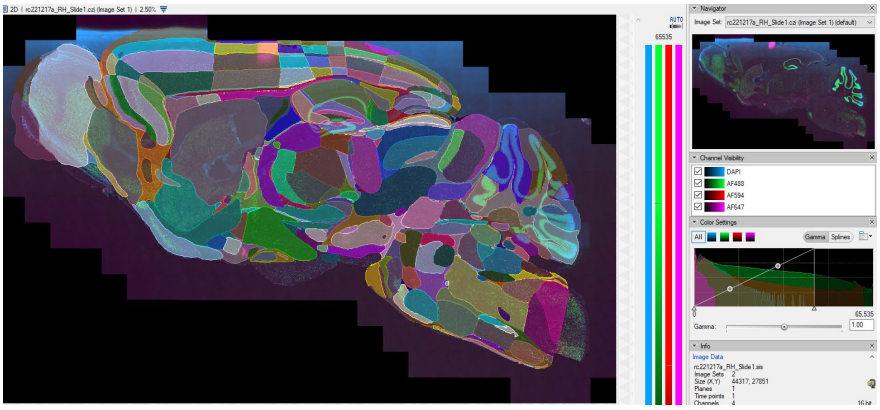

4. Another parameter to change in the script is Line 40, which is the atlas plane chosen from the Allen Brain Atlas
5. **IF** channels were not combined into one channel and there is a specific channel that wants to be used, change Line 35 to the number representing the position of the channel in the Channel Visibility of the experimental image dataset in the navigator.
6. The process is separated into two steps, the first is by running the script with Line 59 and 63 as True and Line 67 as False. This is to perform the registration of the Atlas onto the image slice.
7. In the script editor, run the script by clicking the green icon at the top:




8. The program will run and generate two channels that represents the registered atlas onto the resampled image



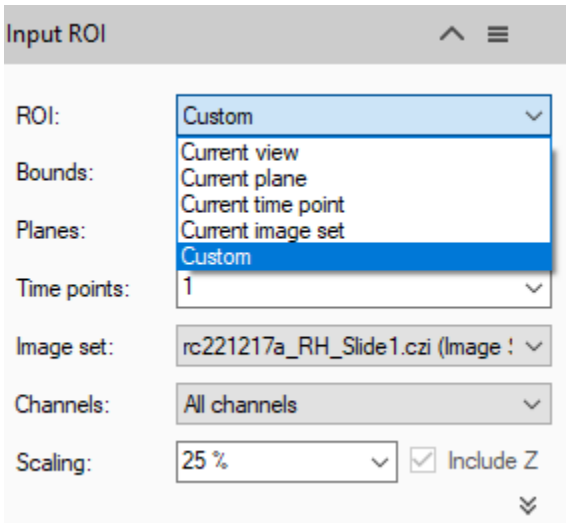

9. Back in the analysis tab, import *step_2_label_registered_atlas**.pipeline*** pipeline file from the **pipelines/** folder. Run the pipeline, as this will use the last channel to segment the areas in the sample data with the atlas.
10. Once this is done, change Line 59 and 63 to ***False*** and Line 67 as ***True***. This is to rescale the registered atlas into the original image.
11. Switch over to the original image set in the navigator tab, and the image should be populated with multi colored regions on the image.



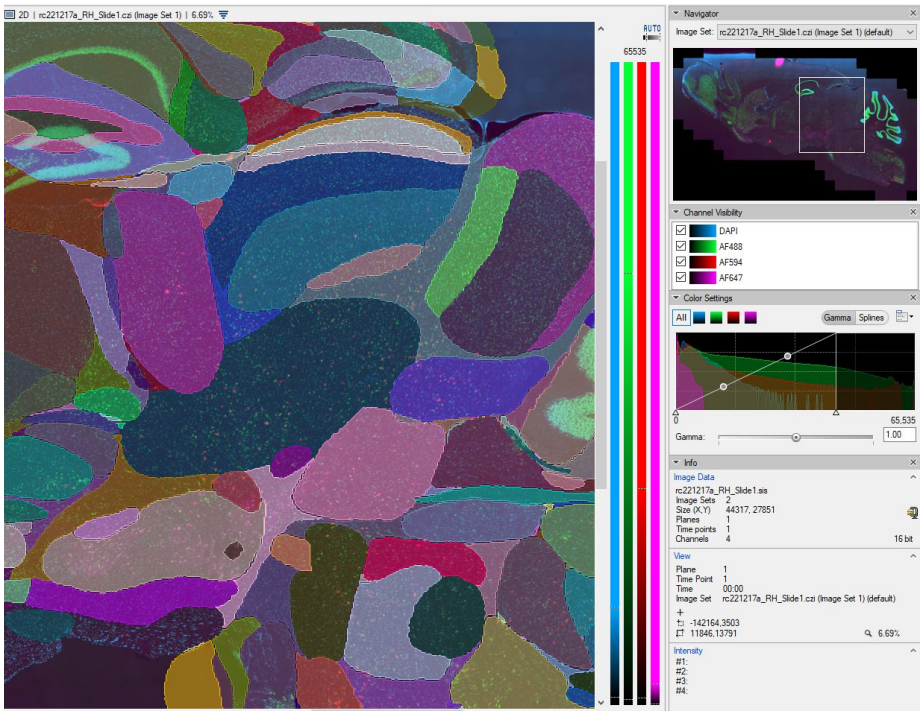
12. This concludes the registration process.

## Pathology/Cell Identification

1. In the Analysis panel, click on the  button to import a pipeline. Select the file '*step_3_tau_identification**.pipeline*** in **pipelines/** folder.
2. Once the pipeline is loaded, in the Input ROI tab, set the ROI drop down to the desired setting.



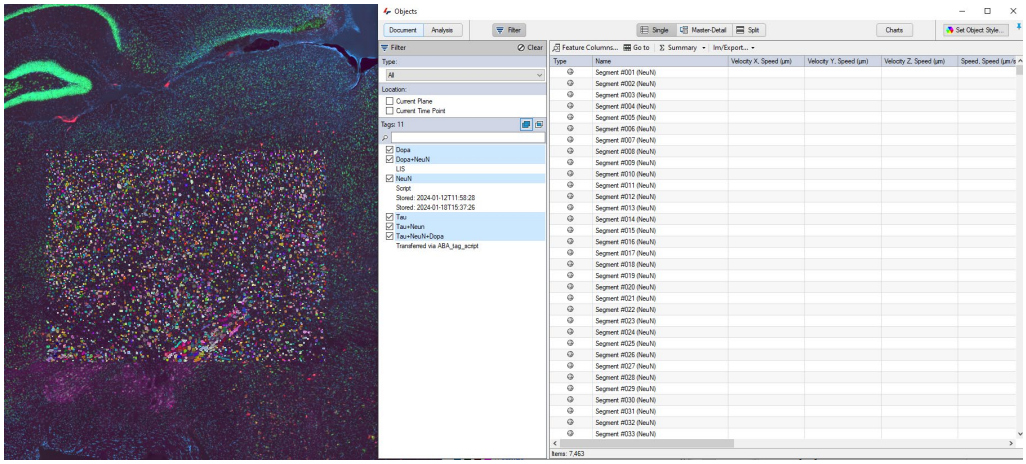

a. If only want to run the identification pipeline on one part of the image, zoom into the desired area of interest, and choose “Current view”. Otherwise, choose current Image set or Current plane.




b. Make sure the channels dropdown is selected as “All Channels”, and the scaling dropdown is selected as “25%”. This is to improve computational processing speed, while preserving the structural information of the imaged cells.

3. To run the entire pipeline, click the  button at the top of the analysis tab.

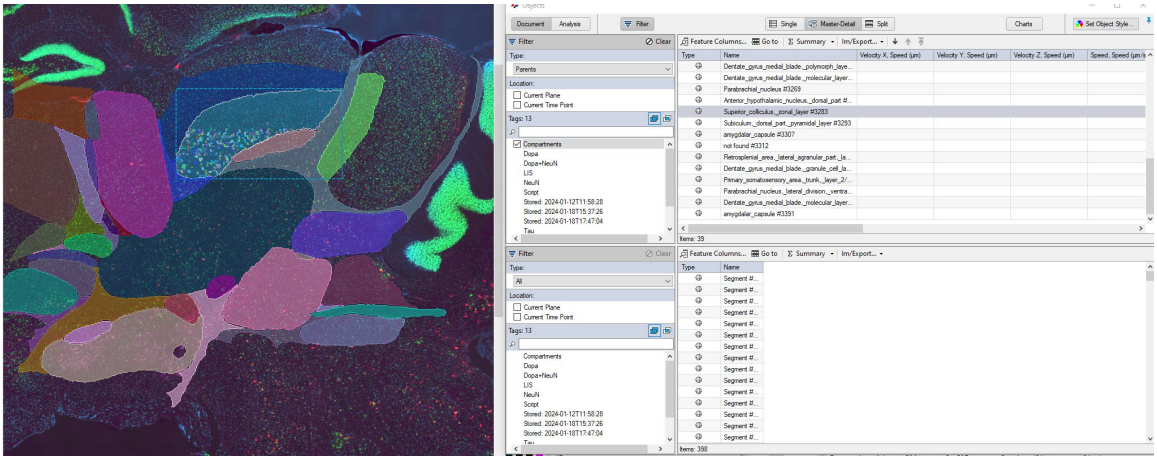
4. The result is a table of objects from the identification pipeline



5. For more information on the different parameters to change, see supplementary materials (WIP)

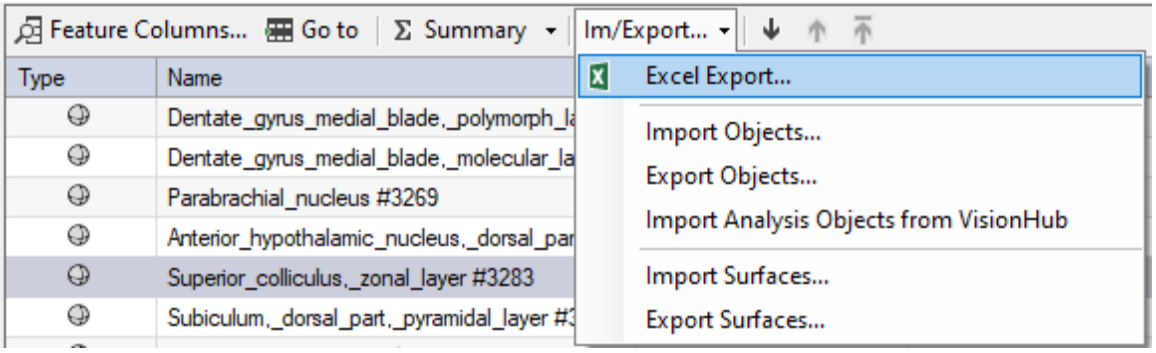
## Atlas-Identification Compartmentalization

1. In the Analysis panel, click on the  button to import a pipeline. Select the file '*step_4_compartmentalize_segments**.pipeline*** in ***pipelines/*** folder.
2. To run the entire pipeline, click the  button at the top of the analysis tab.
3. The result is a tag in the objects table that shows "compartment". This procedure created parent-child objects, where segments are compartmentalized and grouped together. In the objects table, click on Master-Details to show a high level overview of the resulting objects in "compartment".



4. Clicking and selecting an object in the viewer can now show what pathology segments are in specific regions in the registered atlas. From

here an excel spreadsheet can be exported by selecting the 'import/export' tab




5. In the excel exporter, click on Master-Detail report to organize regions-segmentation.