Tutorial for registering Histology to MRI

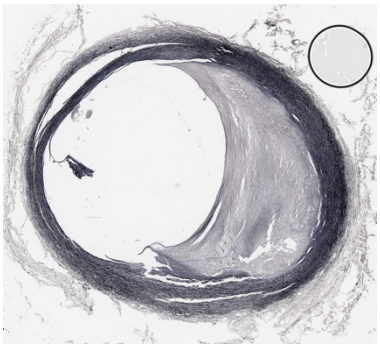
This tutorial describes how to register semi-quantitative histology performed in quPath to MRI images

Histology

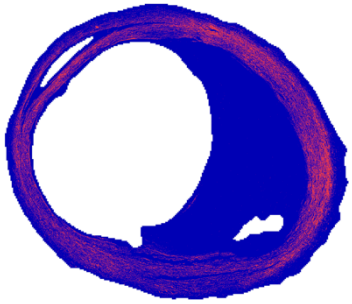
To begin you will need the *Brightfield* and *Stained Area Fraction* images exported by quPath (you can find a tutorial on how to do that here). The images output from this process have been included here (*input/histology_BF.png* and *input/histology_SAF.png*). These examples are for Verhoeff's elastin stain

Step 1

The first step is to mask the Brightfield and Stained Area Fraction images. This is done in MATLAB using format_histology.m and takes *images/input/histology_BF.png* and *images/input/histology_SAF.png* as input and gives *images/output/histology_BF_masked.png* and *images/output/histology_SAF_masked.png* as output.

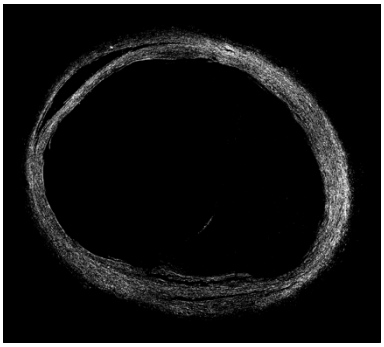


images/input/histology_BF.png



images/input/histology_SAF.png

images/output/histology_BF_masked.png



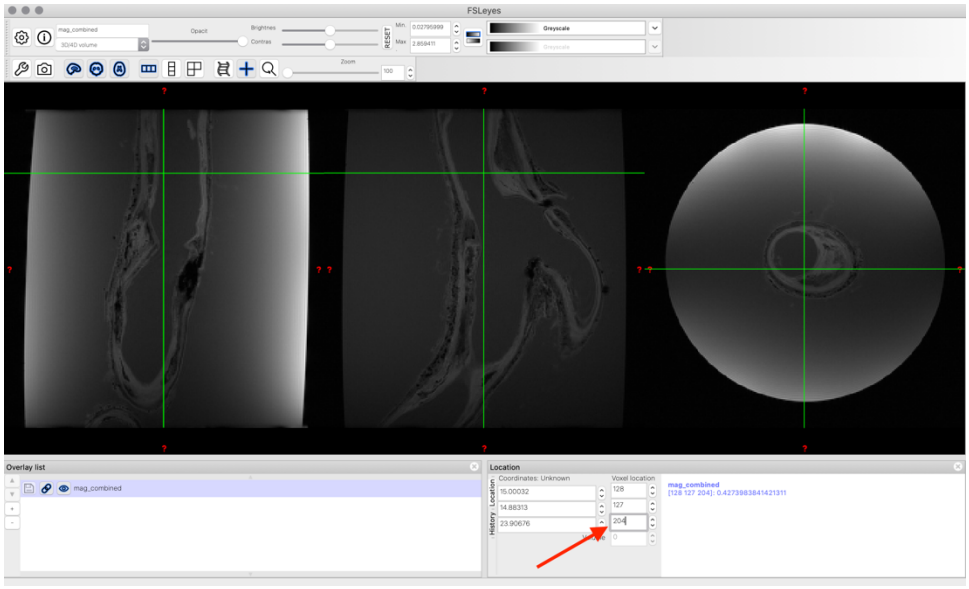
images/output/histology_SAF_masked.png

Manual alignment of histology and MRI

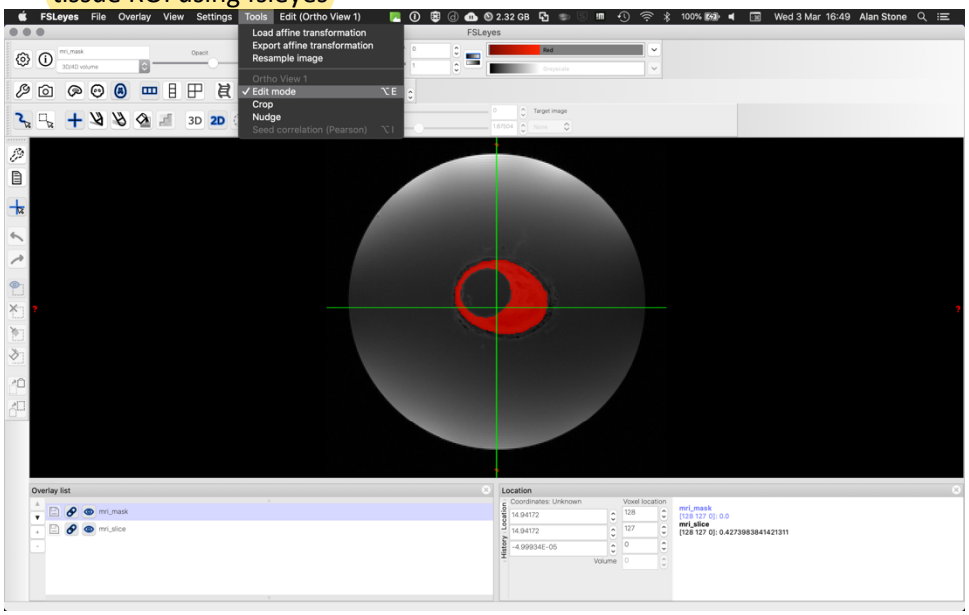
The next step is to manually align the MRI and histology images. For this part you will need to use FSL (here)

Formatting MRI

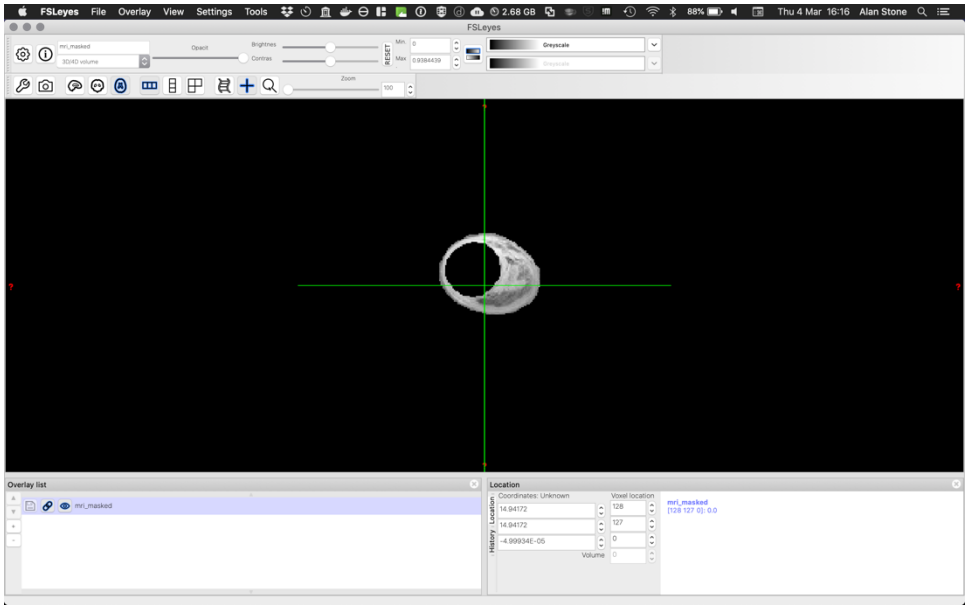
- Using the FSL viewer (fsleyes) identify the MRI slice number that aligns with the histology slice. In this case it is 204



- In the script **extract_mri_slice.sh** manually change the variable *slice_id* to this number (204) and run the script in terminal. This will produce *images/output/mri_slice.nii.gz* and *images/output/mri_mask.nii.gz.* The mask image is empty and we will use it to manually draw a tissue ROI using fsleyes

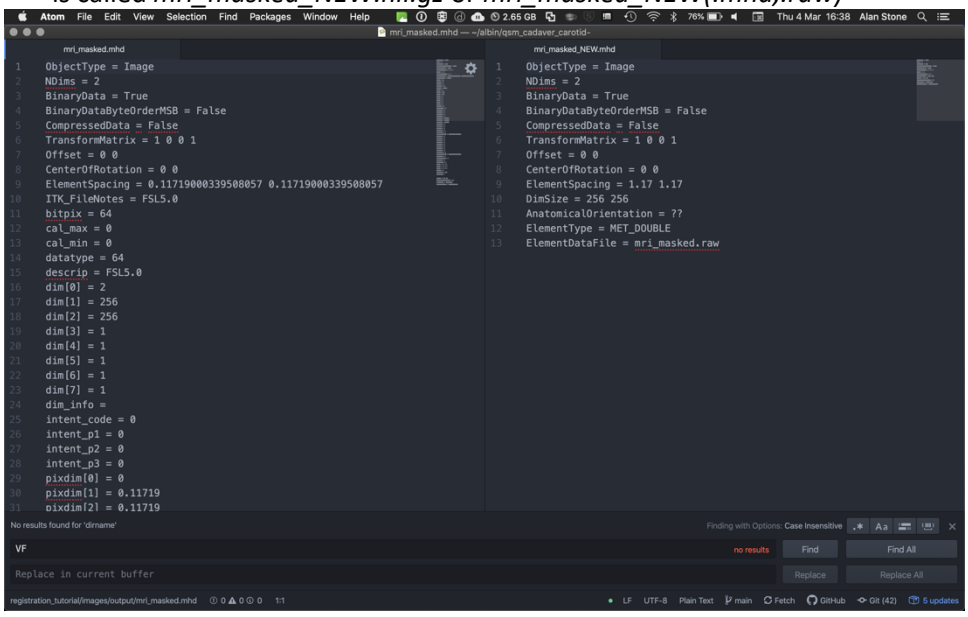


- When you have drawn and saved the mask run **mask_mri_slice.sh** to produce the tissue masked MRI slice (*images/output/mri_masked.nii.gz*)



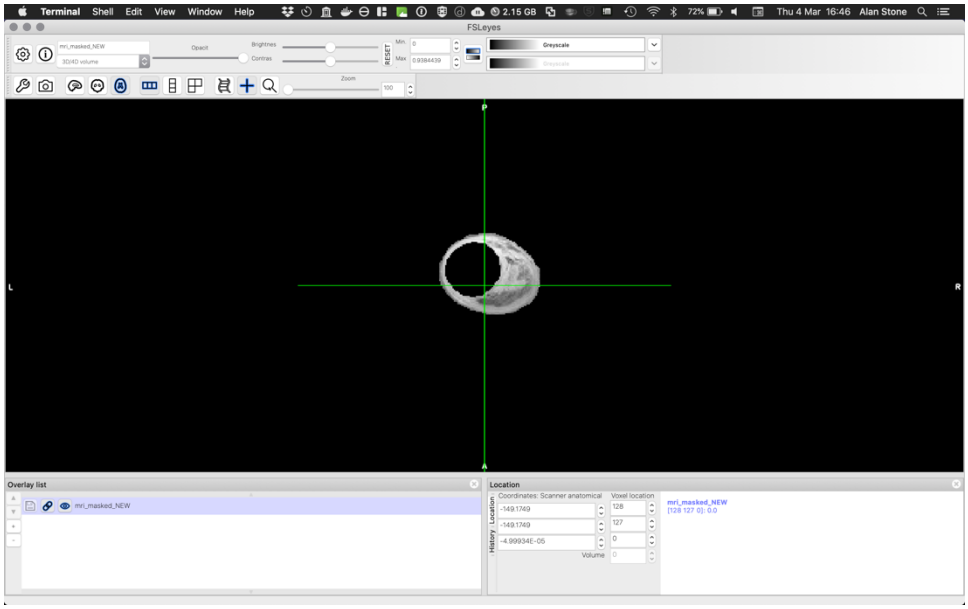
- The next step is to format the masked MRI image for registration. This is done in Python using the SimpleITK library. If you are new to python you will need to install Anaconda (https://docs.anaconda.com/anaconda/install/) and simpleITK (conda install -c simpleitk simpleitk)

- Run **format_mri_for_reg.py** in ipython. The "formatting" involves manually scaling the MRI so that image sizes are comparable with histology and the registration routine. We do this by writing a new header file (.mhd) with the relevant changes and saving a new image. To facilitate this there is an intermediate step where .nii files are converted to .raw format and back again. You can see the difference between the old and new header files below. The newly scaled image is called *mri_masked_NEW.nii.gz* or *mri_masked_NEW(.mhd,.raw)*



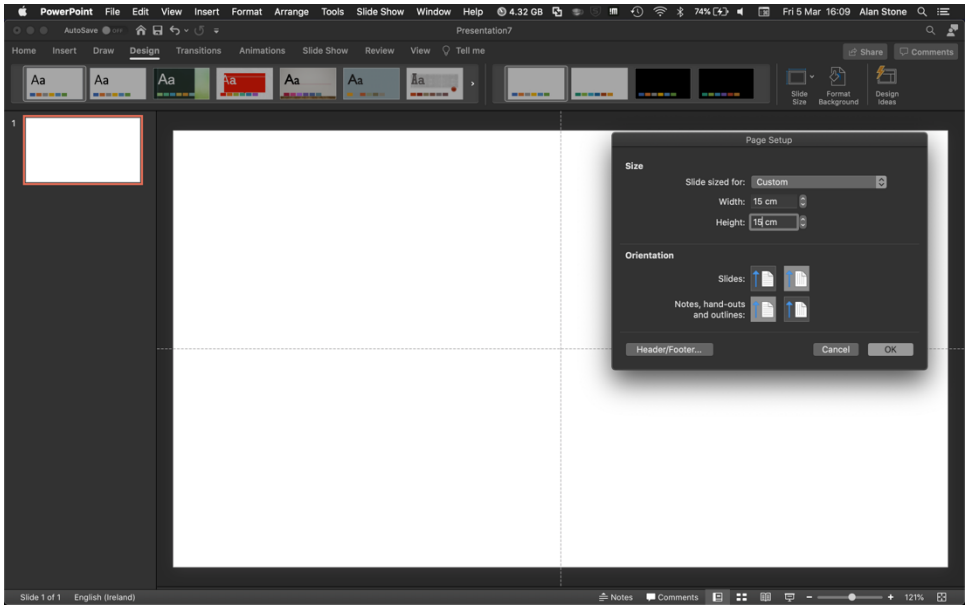
Formatting histology

- The formatting of the histology images is more manual. To begin, we need a representative screenshot of the MRI image. It is important that this image represents the scale and ratio of the MRI image. To achieve this it is best to view the image in fsleyes, using the green cross-hairs to ensure that you are only selecting the field-of-view in your screen shot

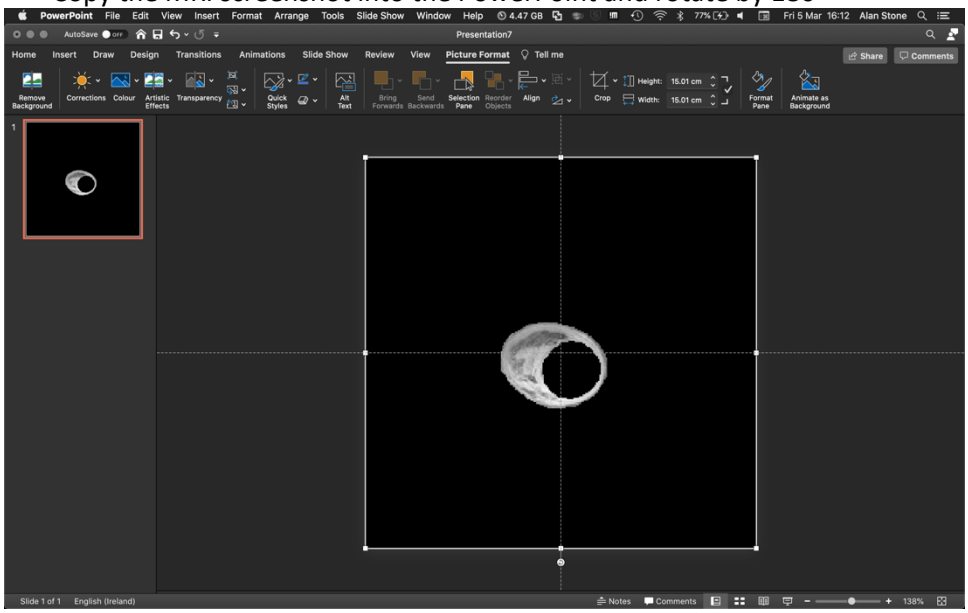


- An example of the image is attached below.

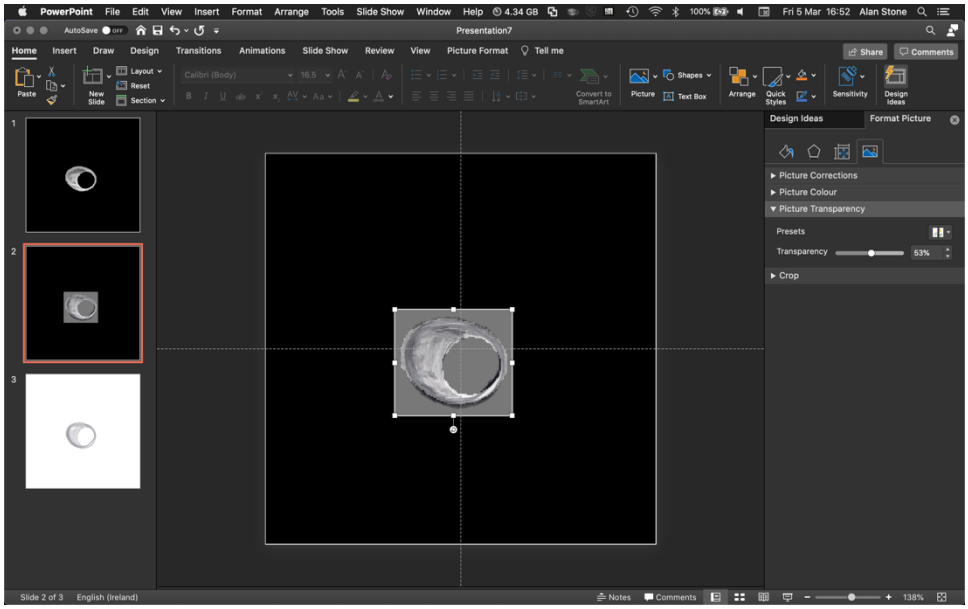
- The next step is to copy the file into PowerPoint. Most of the MRI field-of-views we have used are square so change the page setup in PowerPoint to square



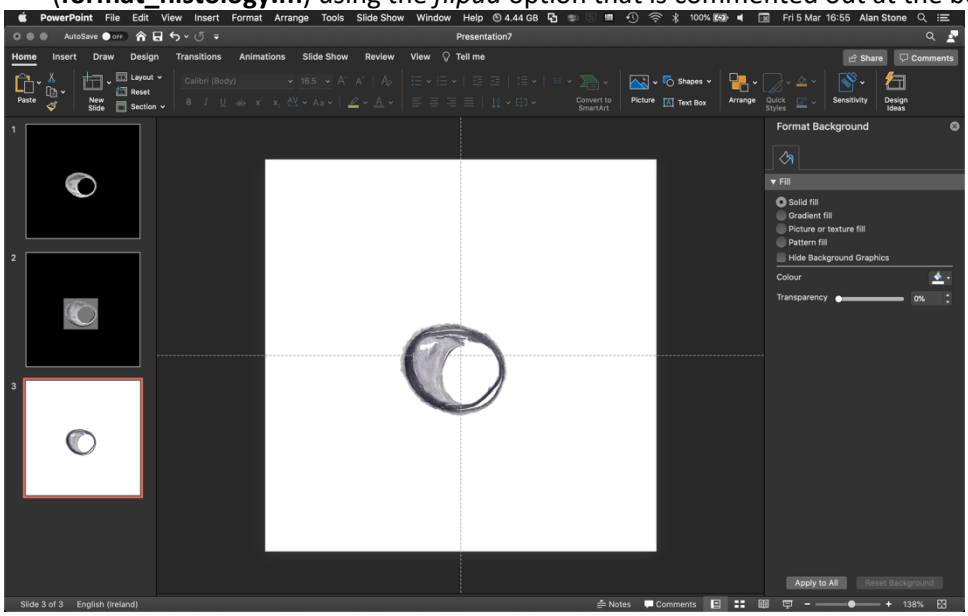
- Copy the MRI screenshot into the PowerPoint and rotate by 180°



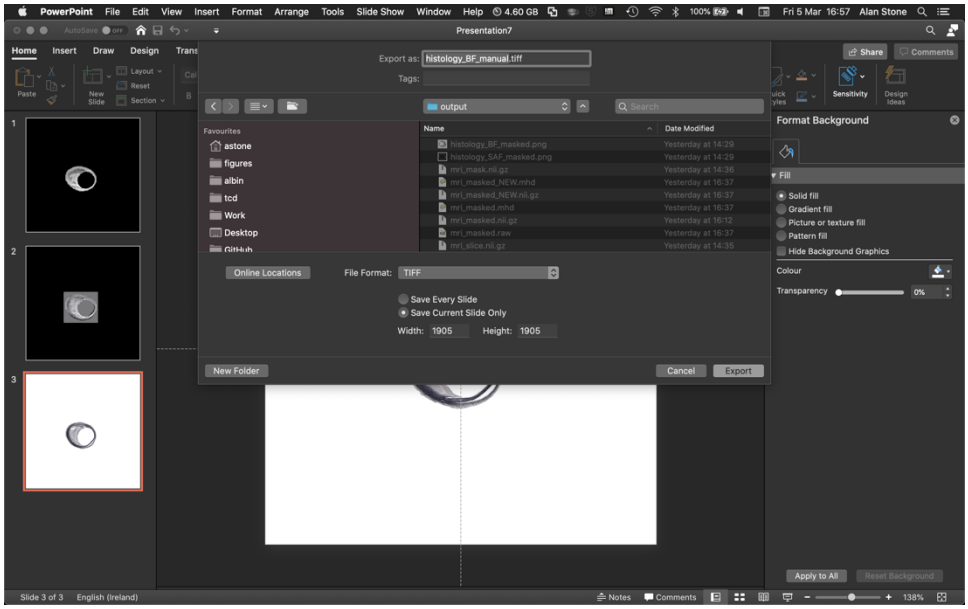
- Copy the *images/output/histology_BF_masked.png* and *images/output/histology_SAF_masked.png* into the PowerPoint file. Change the transparency and manually align the masked brightfield histology to the MRI.



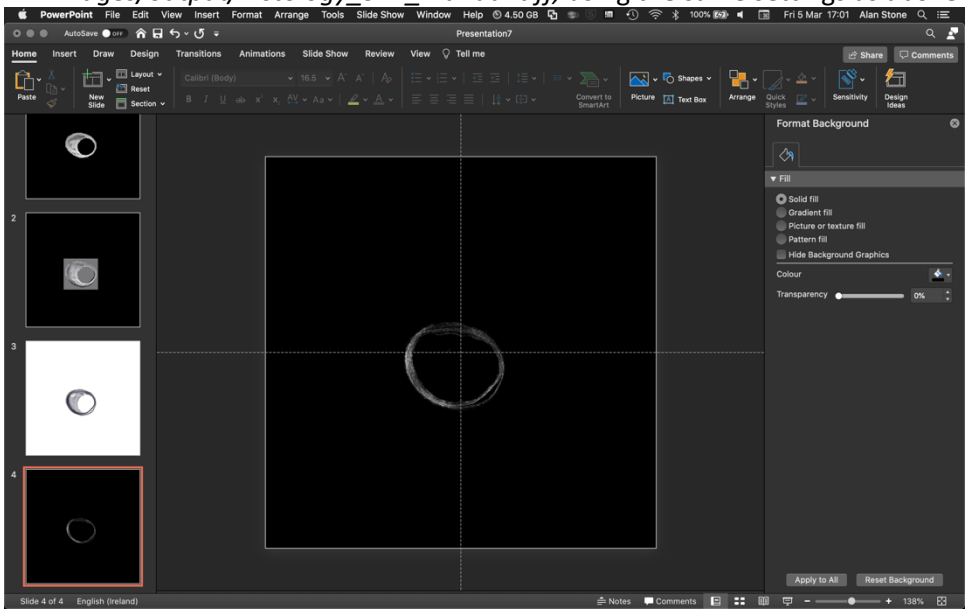
- Copy to its own slide and turn transparency off (i.e. 0%). Note that manual alignment should only be done by rotating and scale, try to avoid anything that will distort the original image e.g shearing. Also if the image needs to be reflected, try outputting the masked histology (**format_histology.m**) using the *flipud* option that is commented out at the bottom of the script



- Export as a tiff - *images/ouptut/histology_BF_manual.tiff*. I found the resolution settings in the screenshot below work best for registration later



- In PowerPoint, manually align the masked SAF image with the manually aligned Brightfield image. Copy to its own slide and turn transparency off. Export as a tiff, *images/output/histology_SAF_manual.tiff,* using the same settings as above



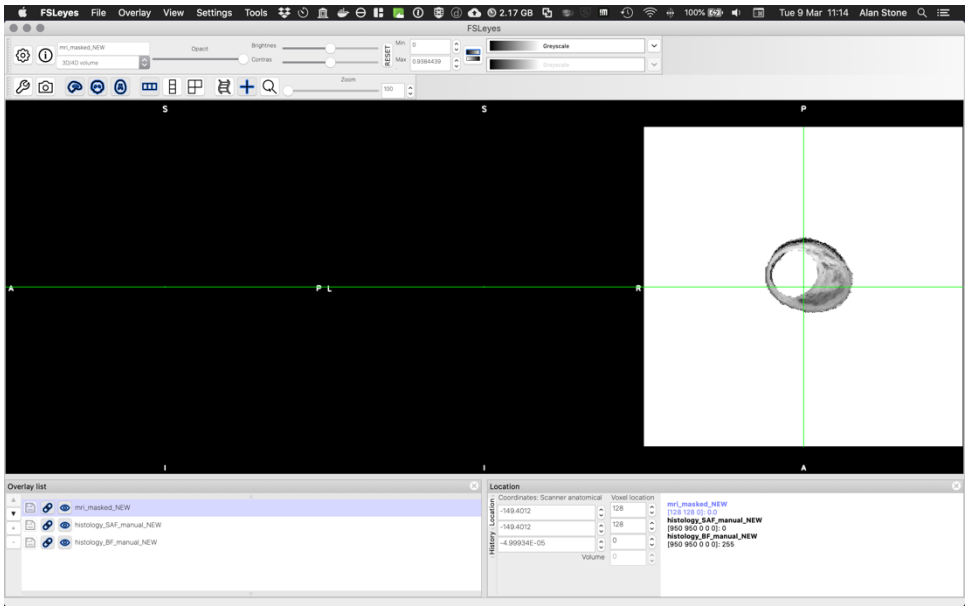
- The final step for preprocessing the histology is to format it for registration. This uses the same process as the MRI formatting above (Python / SimpleITK library) with a few tweaks.

- Run **format_histology_for_reg.py** in ipython. The "formatting" involves manually scaling the histology so that image sizes are comparable with MRI ("*_NEW.nii.gz") and the registration routine. We do this by writing a new header file (.mhd) with the relevant changes and saving a new image. To facilitate this there is an intermediate step where nifti files are converted to .raw format and back again. You can see the difference between the old and new header files below. The newly scaled image are called *images/output/histology_BF_manual_NEW.nii.gz* and *images/output/histology_SAF_manual_NEW.nii.gz*

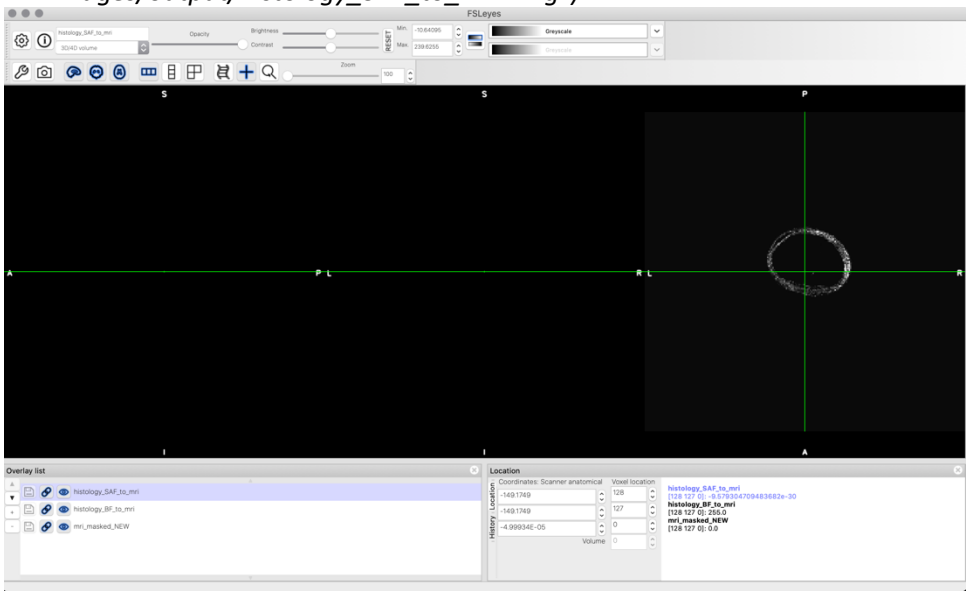
Registration

- You can now visually check the manual alignment of the MRI and histology images using the FSL viewer:

```
fsleyes *_NEW.nii.gz
```



- We can now run the registration using elastix. You will need to install elastix (see here). Then run **run_registration.sh** from the terminal. The last line of the script calls FSL to view the registered histology that are now in MRI space (*images/output/histology_BF_to_mri.nii.gz* and *images/output/histology_SAF_to_mri.nii.gz).*



- Now that the histology is registered to the MRI you can perform regional analysis using k-means clustering as described here