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Imaging- Bright field

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Protocol status: Working
We use this protocol and it's working

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Abstract

Protocol for imaging using Hamamatsu NanoZoomer slide scanner. Sections for analysis should be mounted on slides, stained for appropriate markers, and coverslipped. This protocol is using a Carl Zeiss LSM 880 confocal microcope.

1 Image slides at 40x magnification.

2 Extract individual sections using the NDP.view software (https://www.hamamatsu.com/eu/en/product/life-science-and-medical-systems/digital-slide-scanner/U12388-01.html).

3 Save images as tiff files.

4 Open images from same structures as one single stack using the merge function.

5 Using the crop, rotate, and move functions align all images from the same stack as best as possible. Note: Using 2-3 specific anatomy points to align the structures make the process easier.

6 Crop the entire stack at the same dimensions.

7 Save each image as a new tiff file (it is recommended to save it in a new folder).

8 Open the imageJ macro provided on Zenodo: https://doi.org/10.5281/zenodo.10822458.
Note: A README is provided at the beginning of the macro in order to convert all images in a 0-255 bit image.

9 Save the images as a new tiff file (it is recommended to save it in a new folder).

10 Open matlab and run for each structure the protocol provided on the Zenodo: https://doi.org/10.5281/zenodo.10822458.
Note: Follow the information provided on the code to align images.