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Autofluorescence Reduction and Imaging Marmoset NHP Tissue with a TissueFAXS Imaging System

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Autofluorescence Reduction 10m

1 1) Wash samples in 1X PBS for **00:02:00** .

2 2) Stain samples free floating with **.05 % volume** TrueBlack in **70 % volume** Ethanol for **00:03:30**

3 3) Rinse samples in 1X PBS for **00:10:00** .

4 4) Mount samples on a slide with ProLong™ Gold Antifade Mountan.

Imaging Samples 1w

5 Image samples with a 5x overview scan to determine the tissue regions and boarders.

6 Switch to 20x and image both slices with DAPI and 488 to determine the location of GFP+ cells.

7 Create "Defined Regions" around GFP+ cells.

8 Once these defined regions are created, switch to the 40x water immersion objective and "Force Focus" on multiple points across the tissue to find upper and lower bounds for focusing.

9 Compute focus points for each defined region -- Options, Focus (1x1) -- and set your upper and lower bounds for imaging.

10 Image each defined region with **0.5 µm** steps so that cells can be reconstructed for morphology.

11 Export the FOVs for morphology reconstruction. Be sure to uncheck “Use Stich” as the stitching is done later in the reconstruction pipline. Save the Z-stack with "Original" and "16-bit" format.