ESRF	**Experiment title:** Revealing brain nanostructure for better MRI biophysical model	**Experiment number**: LS-2702 Ref. No 76330
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Shifts: 15	**Local contact(s)**: Alexandra Joita Pacureanu	*Received at ESRF:*

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Report:

The project aimed at collecting large-field-of-view high-resolution 3D synchrotron data of axons from monkey brains, and uses phase contrast x-ray tomography to validate axon diameter estimations obtained from low resolution MRI.

MRI scanning: First, we collected MRI scans of the fixed whole brain monkey.

Tissue preparation and embedding for synchrotron imaging: Tissue samples (diameter: 1 mm and 2-3 mm long) were stained in osmium (OsO_4) to enhance the contrast to the myelin that is wrapped around axons. Two types of tissue samples were prepared: Paraffin or EPON (plastic) embedding.

ESRF – ID16A beam line: EPON- and paraffin-embedded samples were imaged at ID16A, but the EPON samples were judged to be more mechanically stable with regard to the high photon density at the beamline and the comparatively low melting point of paraffin.

The acquired 3D images, at isotropic voxel resolutions between 75-100 nm and 2048 voxels in each dimension, were astounding and revealed new insight into axon densities, geometry of the myelin sheaths and axon trajectories in the different regions of the monkey brain. The acquired data contains a multitude of novel information and we are currently in the process of setting up segmentation methods to extract vessel and axon information for anmalysis.

The first, preliminaty 3D segmentation results from the monkey brain are shown in Figure 1 and depict axonal twisting, non-straight trajectories and a complex axonal environment never before described.

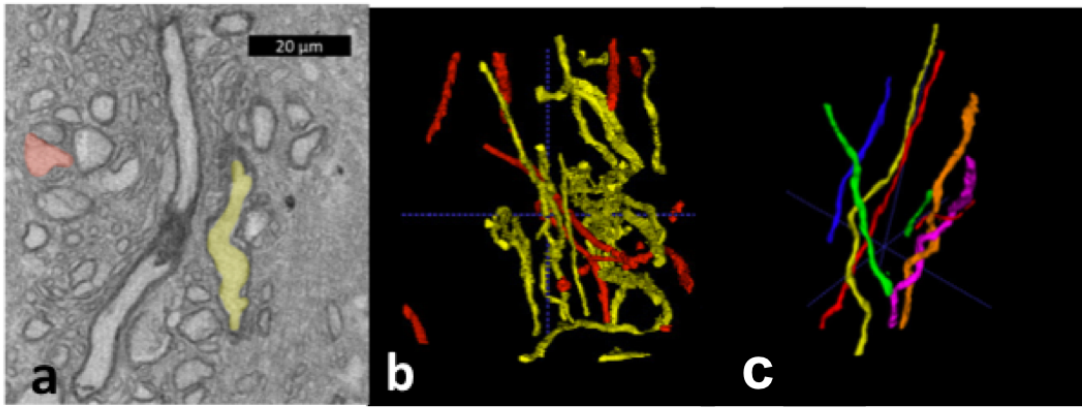

Figure 1. Data obtained from ID16A, ESRF showing axons (yellow) and blood vessels in (red) a) Slice showing crossing fibre region of monkey brain. b) Crossing fibre region 3D view c) (Assorted colours) large axons in crossing fibre-region, 3D view.

Encountered Problems and Improvements: The staining did not penetrate the whole tissue volume as we had expected from pilot samples. Instead, the OsO4 only penetrated the rim of the cylindrical biopsies. Therefore, we could only image the axons within this stained rim. The obtained results, however, are unique to our research field and are of very high quality. We have now resolved the staining issue and plan to prepare samples that are 0.3 mm in diameter if future beamtime is granted for further experiments. The homogeneous staining and smaller samples will ensure as high reconstruction quality as possible (the centre of the sample will be imaged to reduce reconstruction artifacts related to a varying sample volume in the field of view during sample rotation), and improve the already impressive signal-to-noise ratio to make even smaller axons distinguishable.

Perspective: We have gained an understanding of not only the technical aspects of synchrotron imaging, but also of its great potential within our field. We are convinced that the collected data will form the basis of a scientific publication in cooperation with Alexandra Pacureanu of ESRF when the segmentation and data analysis algorithms are up and running. The beamtime was a great success and we are applying for more time at ID16A in the March-round. The new proposal builds on the data we obtained from our first beamtime, described in this report, and aims to study de- and remyelination processes using a rat brain disease model of Multiple Sclerosis.