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MicroCT Scanning of Pig Vagus Nerves

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We use this protocol and it's working

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Abstract

This protocol describes the scanner setup, the parameters required and the reconstruction of microCT scans for imaging pig vagus nerves and the fascicles within them.

Materials

- Nikon XT H 225 microCT scanner
- Nerve sample - previously stained and prepared for scanning
- Sample mount

Troubleshooting

Safety warnings

Please ensure proper training and permissions before using X-ray equipment.

Nerve sample preparation

1 See protocol **Nerve Sample Preparation for MicroCT Scanning Protocol** for nerve sample preparation.
Ensure the cranial ends are down and the caudal ends are up (upside down) in the holder.

MicroCT scanner setup and parameters

2 A Nikon XT H 225 microCT scanner (Nikon Metrology, Tring, UK) was used in this protocol.

3 Home the scanning stage.

4 Condition the X-ray filament at 200 kVp for 10 minutes.

5 Change and ensure the target is set to molybdenum.

6 Place the nerve sample within the machine and secure it to the stage.

7 Move the stage to visualise the bottom of the nerve and ensure, by rotating the sample, that the sample is completely in the field of view at every angle.

8 Take note of the Y-axis co-ordinates (this will allow for calculation of the overlap and shift between the multiple scans).

9 Start a program scan setup (When scanning a long nerve sample, despite it being cut into 4 cm lengths, at a desired resolution of 7 µm with a smaller field of view, multiple scans are required to cover the length of the nerve samples that have been wrapped around a sponge. Four (overlapping) scans are usually required.)

10 Set the scanning parameters of the first scan to the following:
35 kVp energy, 120 µA current, 7 W power, an exposure of 0.25 fps and 4 seconds, and a resolution with isotropic voxel size of 7 µm.

11 Adjust the focus accordingly for the sharpest image, using the display screen to visualise.

12 Create a reference with the following parameters:
2 images, 64 frames. This should require 8 mins 55 seconds time.

13 In the reconstruction options, do not crop the image bounding box. This can be done afterwards and is better for the stitching process down the line if all scans are not cropped.

14 Choose optimised projections (around 3186) and save the scanning setup.

15 Add another scan to the program - it should replicate the previously setup parameters.

16 Shift the Y-axis by 12.74mm (or calculate your level of overlap desired and shift accordinigly) by moving the nerve sample down.

17 Use the same reference that was just created and save the scan setup.

18 Do this again for another two scans (or however many are required to visualise the full nerve).

19 Save the entire program and start the scan. This will be ~14 hours so it is advised to start it for an overnight scan.

Reconstruction

20 Open the xteckt file in CT Pro 3D software (Nikon's software for reconstructing CT data generated by Nikon Metrology, Tring, UK).

21 Calculate the centre of rotation manually with dual slice selection.

22 Perform a beam hardening correction with a preset of 2 and coefficient of 0.0.

23 Crop the image if desired but ensure the nerve is within the cropped region throughout the length by toggling between the visualisation windows and plane directions.

24 Reconstruct as 16-bit volumes and triple TIFF 16-bit image stack files allowing for subsequent image analysis and segmentation in various software.