Beamtime at ID-16-NIA
-experiment MD1311:
Misdirection of axonal outgrowth of nerve fibers after nerve injury and repair/reconstruction

Team and staff present at beamtime:

Lars B. Dahlin, Martin Bech, *Lena Stenberg, Nathaly de la Rosa*; Department of Translational Medicine – Hand Surgery, Lund University and Department of Hand Surgery, Skane University Hospital, Malmö, SWEDEN; Medical Radiation Physics Department, Lund University, LUND SWEDEN

Local contact: *Dmitry (Dima) Karpov*

Aim

The general aim – as stated in the proposal - is to image outgrowing nerve fibers in different environments with the focus how it looks like in three dimensions.

Particularly, our aim was to analyze the role of tissue niche relevant for the extent of misdirected growth occurring in regeneration at a specific time point after nerve injury and repair/reconstruction in healthy and diabetic rats with the following models: 1) **nerve repair** (direct repair by sutures), 2) nerve **reconstruction** = a) **autologous nerve, b) nerve conduits** (i.e. hollow tubes), and c) **nerve allograft** (extracted nerve from cadavers) to bridge **nerve defects**. 3) The **nerve conduit** was modified by a **matrix - silk threads** (spider fibres; a recombinant silk protein functionalized with a motif from fibronectin, FN-silk, as ECM-like support) - to **support** outgrowing axons. The models were applied in healthy and diabetic (Goto-Kakizaki) rats. The plan was also to create images from normal, uninjured, nerves from the two different rat models.

Samples measured

We have measured a total of 8 samples, where we had problems to navigate in the specimens even if we tried to define the distance from the proximal repair border (defined as 4 mm; marked at the plastic embedding; se figure below).
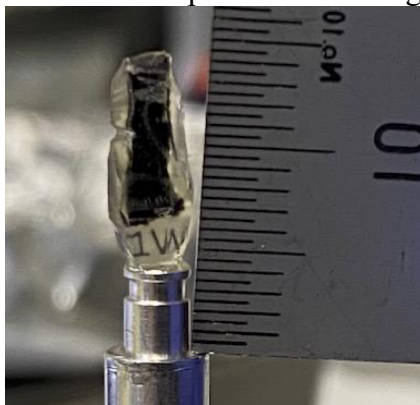
Some samples were measured several times in order to find an optimal location in the peripheral nerve. We were able to measure **a)** one control from a healthy rat (nerve fibers observed and defined), **b)** conventional nerve sutures from a healthy rat (nerve fibers identified) and from a diabetic rat (no nerve fibers identified), **c)** autologous nerve graft from a healthy rat (nerve fibers identified) and from a diabetic rat (no nerve fibers identified), **d)** nerve allograft from a healthy rat (nerve fibers identified) and from a diabetic rat (no nerve fibers identified), **e)** nerve conduit with silk fibers from a healthy rat (silk fibers observed, but no nerve fibers identified).

The samples were all imaged with a voxel size of 120 nm (13 scans). Further, samples were re-measured with 60 nm voxel size (15 scans).
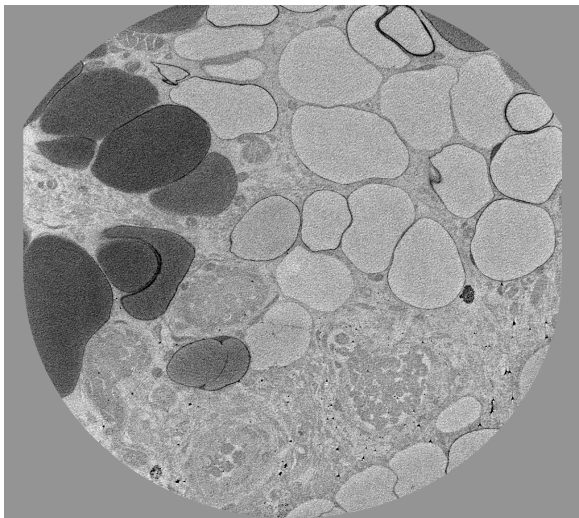

Figure showing a blood vessel filled with erythrocytes in the lower part of the image (from nerve suture specimen – healthy rat).
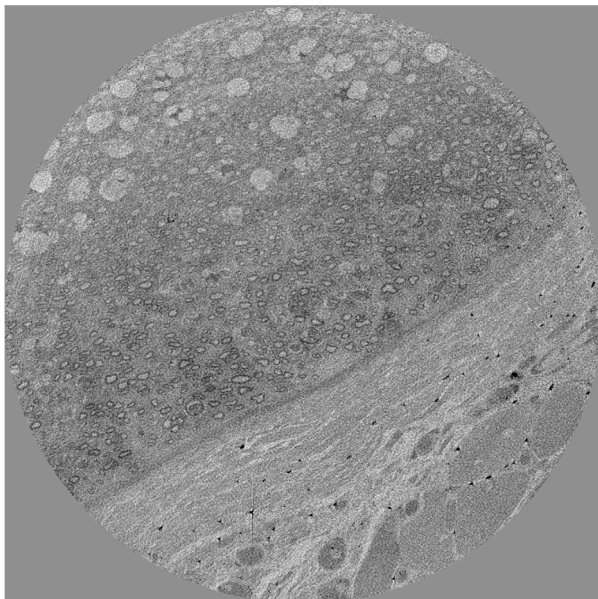

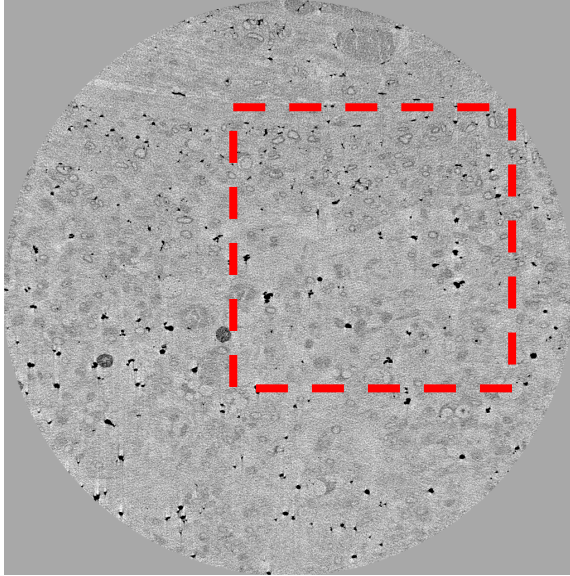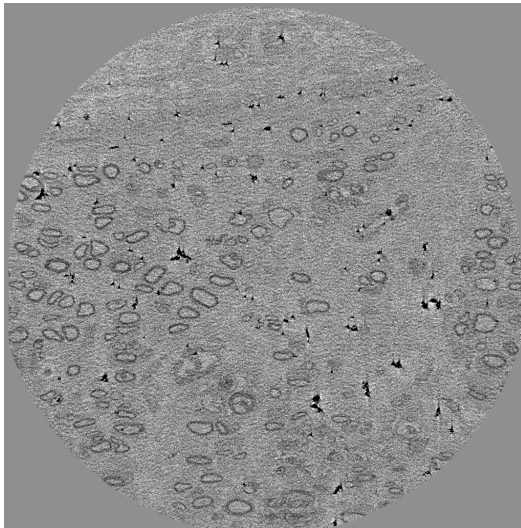Figure showing nerve fibers from the same specimen as above (nerve suture healthy rats)
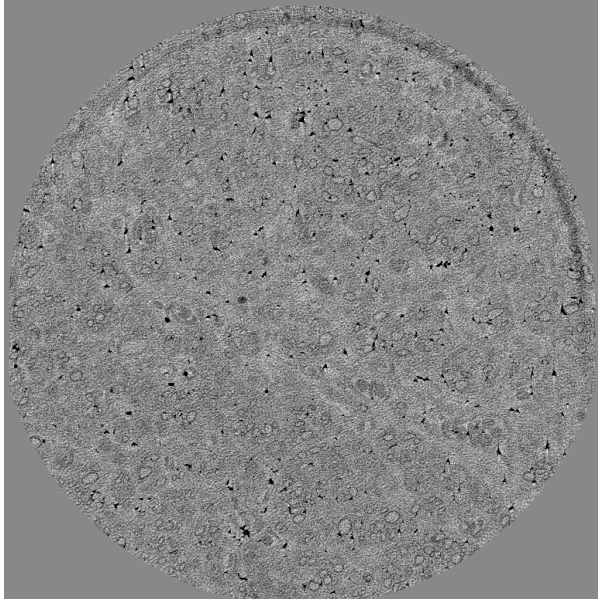
Figure showing nerve fibers in a specimen from a diabetic rat (nerve suture).

Detail of nerve fibers after a nerve reconstruction with an autologous nerve graft in a healthy rat.

Nerve fibers in a nerve allograft from a healthy rat.

Data analysis

The data from the repaired/reconstructed and control samples are currently being analyzed and re-scanned with nano-CT in order to get a fully view for facilitating orientation in the specimen. Initial visual inspection of the data, with scrolling through the specimen, gives us hope for quantification of misdirection of the outgrowing nerve fibers.

Impact

Though the data is not fully analyzed, the preliminary results have already been used in a scientific presentation. The images inspire also colleagues and we have applied for further beamtime to scan the rest of the specimens. Meantime we scan the specimen with nano-CT as well as perform conventional light microscopic images to facilitate orientation and identification of the nerve fibers.