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Ultra-high resolution imaging of the cat spinal cord

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Simulation of spinal cord...

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We use this protocol and it's working

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Abstract

Detailed description of steps to extract and prepared tissue samples to acquire ultra-high resolution images of the cat spinal cord.

Materials

Tissue Sample
- Formalin fixed cat (Ward's Science, Catalog # 470154-114/470154-122)

Chemicals & Solutions
- 1x Phosphate Buffered Saline
- Sodium Azide (S2002, Sigma Adrich, St. Louis, MO)
- Gadavist^{TM} Gadobutrol 1 mmol / mL Injection (Bayer Healthcare, Berlin, Germany)
- Fomblin Y LVAC 16/6

Imaging Equipment
- Vertical-bore 11.7T/89mm Actively Shielded Bruker AVANCE AV3 HD MicroImaging System
- Magnetic field gradient sets: Micro 2.5 Gradient set for 30 mm RF coil and Mini0.75 for 40 mm RF coil
- 1H quadrature 30 or 40 mm birdcage
- 1H/19F birdcage resonator for fluorine imaging
- Small animal CT scanner (Si78; Bruker BioSpin GmbH, Ettlingen, Germany)

Other
- 3 cm glass tubes
- vacuum pump
- ruler
- dissection instruments
- Parafilm

Troubleshooting

Sample Preparation

1 Place the cat specimen in a prone position and identify the desired spinal segments by counting the spinous processes.

2 Measure that the length of the desired segment does not exceed 10 cm in total.

3 Start the dissection with a dorsal incision along the side of the spinous process, and carefully dissect laterally to expose the vertebral column.

4 Confirm the vertebral segments of interest, then carefully transect the spinal column at least one vertebra above and below the desired segments with a bone rongeur and sharp blade. Make sure not to pull on the spinal cord during this process or the spinal roots may detach.

5 Cut the lateral muscles and connective tissue to detach the spinal segment (with bone intact). Keep the sample more than 3 cm wide.

6 Once the spinal segment is detached from the rest of the cat, trim the muscles (and spinal processes if necessary) until the sample is about 3 cm in diameter, and check that it fits snugly inside the 3 cm glass tube.

7 Put a suture on select spine levels, usually placed on the vertebrae at the center of the desired field of view (4 cm).

8 Put the sample next to the ruler on a piece of paper or chuck pad, label the corresponding levels of each vertebra of the sample, and take a picture of the sample to keep as reference.

9 Dab the sample dry on paper towels, then transfer it to the 3 cm glass tube with caudal side down, and cover with Parafilm to prevent the tissue from drying out. The sample is now ready for the high-resolution CT scan (see Imaging Protocol).
Note: the sample should be kept in the glass tube from here on, so make sure it is centered in a good position for the MR scanner. This will ensure good registration between CT and MRI later on.

10 After the CT, submerge the sample in 1x PBS + 0.2% Gadavist solution for around 1-2 weeks at 4 ^{O}C (keep sample inside glass tube). The PBS rehydrates the tissue, which is critical for MRI acquisition, and Gadavist increases tissue contrast to reduce imaging time. You can also add 0.3% NaN_3 (sodium azide) which will help prevent bacterial growth in the process.

11 Two days before the MR scans, immerse the sample in dH2O + 0.2% Gadavist solution to increase tissue water contents to help restore some T2 relaxation time.

12 Remove the solution from the sample glass tube, dry the sample as much as possible without removing it from the tube. Then, fill the tube with Fomblin, making sure the sample is fully submerged. Place the tube in a vacuum pump for 1-2 hours. The sample is now ready for high-resolution MR imaging.

Imaging Protocol

13 Perform **high-resolution CT imaging** (50-100 um resolution) on the sample to obtain good images of the vertebral column.

13.1 Place the glass tube in the microCT scanner horizontally. Check with a pilot scan that the sample is centered in the field of view.

13.2 Use a low dose 1 mm aluminum filter, acquire a CT scan with the "step and shoot" method (0.6-degree gantry step) at 100×100×100 µm resolution, with a field of view of approximately 33.6×31.9×100 mm, but this should be based on length of your sample. Use the filtered back projection algorithm to reconstruct the image.

14 Perform a series of **high-resolution MR imaging** (50-200 um resolution) on the sample to obtain good soft tissue and neural fiber distributions.

14.1 Center the sample in the field of view (FOV). Then, load the sample vertically into the scanner. The 30mm or 40mm gradient coils have approximately a 4cm long FOV.

14.2 MRI sequences and parameters:

Sequence	RF coil	TE/TR range (ms)	Average	Echo	Flip angle	Resolution	FOV(mm)
T2 TurboRARE_3D	1H 30mm or 40mm	17-22/300-500	4	4	90 deg	50um iso	19.2×40x25.6
Fat enhanced	1H 30mm or 40mm	14-38/300-500	1-4	4-8	90 deg	100×100x50um	19.2×40x25.6
DWI (b1000/2000, >= 19 dir)	1H 30mm or 40mm	30/300-500	1	1	90 deg	100-200um iso	19.2×40x25.6
19F	1H/19F 30mm	16-28/500-600	2-4	4-16	90 deg	100×100x50um	32×40×32

	Sequence	RF coil	TE/TR range (ms)	Average	Echo	Flip angle	Resolution	FOV(mm)
		or 40mm						

Note:

- selective fat excitation and refocusing was achieved with RF frequency at 1.2 ppm
- selective fluorine excitation was achieved with RF frequency at -82ppm for Fomblin

14.3 If your spinal segment of interest is longer than 4cm, you will need to move the sample and acquire another set of images, then merge the segments together. Make sure there is at least 5mm overlap between the segments to ensure good registration.