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High-resolution imaging of the human cadaver 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 human cadaver spinal cord, following a SpIC3D pipeline.

The cadaver sample was obtained from the University of Pittsburgh School of Medicine, protocol approved by University of Pittsburgh CORID, ID: 1070.

Materials

Tissue Sample

- Formalin fixed cadaveric human

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 9.4T/72mm Bruker AVANCE AV3 HD MicroImaging System
- Siemens, Prisma 3T
- Flexible coil
- CT Scanner, Epica VIMAGO HU system

Other

- Polycarbonate cylindrical rigid container
- Vacuum pump
- Dissection instruments

Troubleshooting

Sample Preparation

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

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

3 Confirm the vertebral segments of interest, based on the insertion of the twelfth rib at the T12 spinal segment, then carefully transect the spinal column at least one vertebra above and below the desired segments with a bone rongeur, sharp blade, and other appropriate cutting tools. Make sure not to pull on the spinal cord during this process or the spinal roots may detach.

4 Cut the lateral muscles and connective tissue to detach the spinal segment (with bone intact).

5 Once the spinal segment is detached from the rest of the cadaveric human, trim the muscles (and spinal processes if necessary) until the sample is about 5 cm in diameter.

6 Put a suture on select spine levels, usually placed on the vertebrae at the center of the desired field of view.

7 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.

Imaging Protocol

8 First, perform high-resolution CT imaging (250 µm resolution) on the sample to obtain good images of the vertebral column.

9 Submerge the sample in a sealed bag with 1x PBS and 0.3% Azide for at least one week at 4º C. For Cadaver 2, switch to 1x PBS with 0.2% Gadavist for around 1-2 weeks at 4º C (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). 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.

10 Before acquiring MRI imaging, remove the sample from PBS and drain thoroughly. Submerge the sample in a bag with Fomblin and place upright in a vacuum chamber under vacuum for at least 12 hours to remove air bubbles. Wrap rubber bands around the bagged specimen to reduce the volume of the bag and the required amount of Fomblin. Seal the open end of the bag.

11 If using a 3T scanner, place the specimen in the MRI scanner with the ventral side down, wrapping a flexible coil around it and making sure it cannot move during the scan time.

11.1 If using a 9.4T scanner, place the plastic bag with Fomblin in a polycarbonate cylindrical rigid container, with caudal side down. Make sure to minimize any wrinkles in the bag along the walls of the cylinder as best as possible. This will help to minimize any air/tissue interfaces along the long axis of the sample, helping with shimming the magnetic field and making it as homogeneous as possible for high-resolution imaging.

12 Center the sample in the field of view (FOV). Find the optimal sequences and parameters, in order to achieve the best images quality possible.

Images sequences and parameters

13 Specimen 1, 3T: T2 SPACE, sagittal orientation, resolution 0.306×0.306×0.306 mm^3, Repetition Time 900 ms, Echo Time 134 ms, Number of Averages 4.
Specimen 2, 3T: T2* FLASH, axial orientation, resolution 0.293×0.293×1 mm^3, Repetition Time 20 ms, Echo Time 3.36 ms, Number of Averages 2.
Specimen 2, 9.4T: T2 Turbo RARE, sagittal orientation, resolution 0.13×0.13×0.13 mm^3, Repetition Time 800 ms, Echo Time 26 ms, Number of Averages 1.
Specimen 2, 9.4T: Fluorine RARE, sagittal orientation, resolution 0.260×0.260×0.260 mm^3, Repetition Time 1500 ms, Echo Time 26 ms, Number of Averages 1.
Specimen 2, 9.4T: Fat enhanced RARE, sagittal orientation, resolution 0.13×0.13×0.13 mm^3, Repetition Time 500 ms, Echo Time 40 ms, Number of Averages 1.
Specimen 3, 3T: T2* FLASH, axial orientation, resolution 0.293×0.293×1 mm^3, Repetition Time 20 ms, Echo Time 10 ms, Number of Averages 1.
Specimen 3, 3T: T1 vibe, sagittal orientation, resolution 0.293×0.293×0.5 mm^3, Repetition Time 8 ms, Echo Time 2.72 ms, Number of Averages 6.
Specimen 4, 3T: T2* FLASH, axial orientation, resolution 0.293×0.293×1 mm^3, Repetition Time 20 ms, Echo Time 13 ms, Number of Averages 2.
Specimen 4, 3T: T2* FLASH, axial orientation, resolution 0.293×0.293×1 mm^3, Repetition Time 10 ms, Echo Time 3.27 ms, Number of Averages 2.
Specimen 6, 3T: T2* FLASH, axial orientation, resolution 0.293×0.293×1 mm^3, Repetition Time 20 ms, Echo Time 3.36 ms, Number of Averages 1.

Note:

- Selective fat excitation and refocusing was achieved with RF frequency at -3.5 ppm.
- Selective fluorine excitation was achieved with RF frequency at -85ppm for Fomblin.