Image Registration Framework for Pixelwise Comparison of Ex Vivo MRI and Histology in Rat Model of Contusion Spinal Cord Injury

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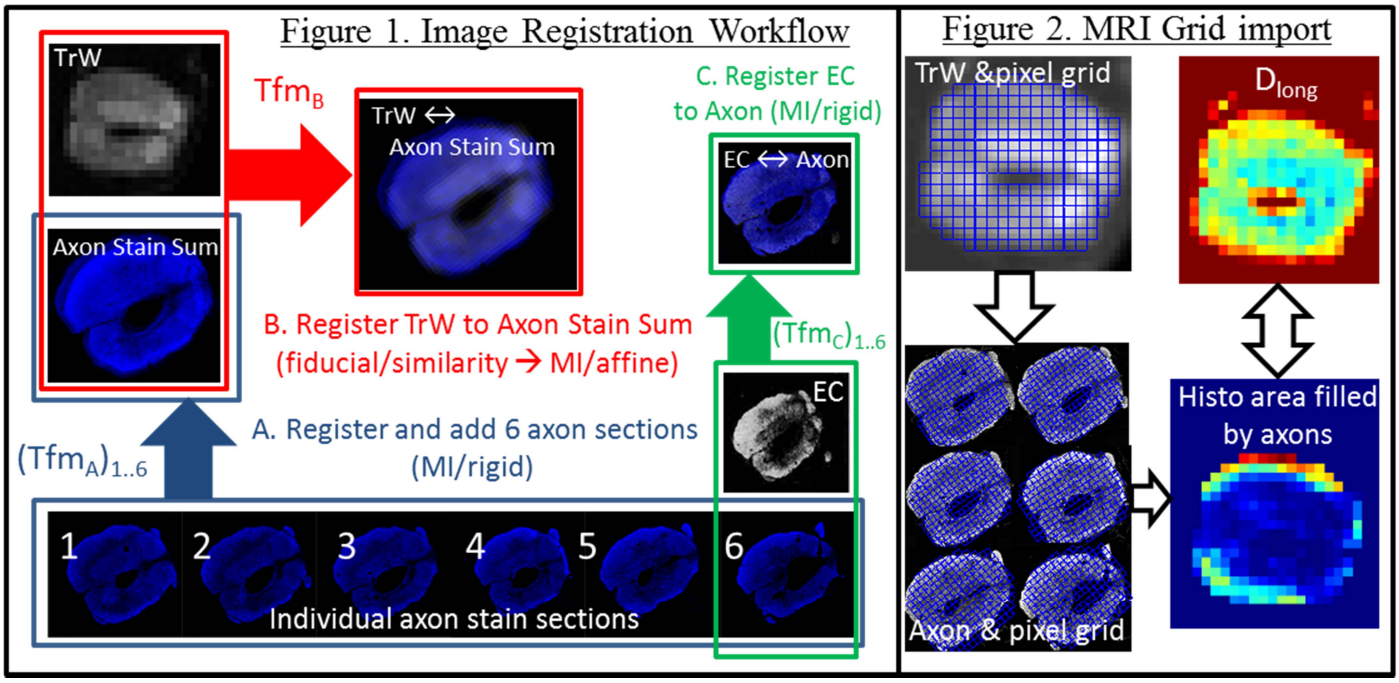
Target audience: Researchers seeking to improve comparison between MRI and histology in spinal cord injury and disease.

Purpose: Comparison with histology is necessary in validating MRI measurements of white matter microstructure in experimental spinal cord injury (SCI), with the literature mostly reporting ROI analysis of easily definable anatomical regions[1]. However, this ROI approach may introduce manual observer bias, and becomes difficult in clinically relevant injury models such as contusion, where the highly variable injury pattern may obfuscate the identification of particular tracts. We present here an image registration procedure which ameliorates this issue by allowing pixelwise comparison or automated ROI import between ex vivo MRI (DTI) and histology (axon and myelin stains) for rat SCI.

Methods: An image registration workflow was tested on contused thoracic T9/T10 rat spinal cords, perfused and extracted at 8 weeks post-injury (N=4) and 27 weeks post-injury (N=5). Spin-echo DTI was acquired with a Bruker 7T preclinical scanner from 5 axial slices spread evenly across the lesion extent (caudal edge, mid-caudal, epicentre, mid-cranial, cranial edge) at 100 µm in-plane resolution and 1mm slice thickness (TE/TR = 21.3/1500ms, 6 directions, b=750 s/mm^2, NA=4, FOV=1.28 cm). Spinal cord samples were cut into 20 µm axial cross-sections and subdivided into 10 interleaved sets with member sections 200 µm apart. One set was immunostained for axons (neurofilament/βIII-tubulin) and myelin content (Myelin Basic Protein) while an adjacent set was stained for eriochrome cyanine alone (alternative marker for myelin), with both sets imaged at 10x magnification on a Zeiss Axioplan 2 microscope. **Figure 1** illustrates the image registration workflow (3D Slicer was used). The MRI epicentre slice was aligned with the histology epicenter as determined at the midpoint between the manually selected caudal and cranial lesion edges. The 6 axon stain sections corresponding to one MR slice were first rigidly registered by mutual information and added to make an "axon stain sum" image, which was registered to the DTI-derived trace weighted (TrW) image. The registration was initialized by similarity transform to minimize the average distance between sets of manually-defined fiducials in the two modalities around the boundary of the cord. Mutual information with affine transformation was used to warp the TrW image to the axon stain sum image space. EC sections were aligned with their corresponding axon/MBP stain section by rigid registration and mutual information. The target registration error (TRE) was assessed by measuring the distance between corresponding sets of internal landmarks in the axon stain sum and TrW image, typically including the location of the ventral fissure, tips of the gray matter butterfly and the centre/edge of internal cavities (~4 landmarks per MRI slice).

Results & Discussion: The mean TRE was 110 ± 260 µm over the 180 defined landmarks (approximate dorsoventral length of normal thoracic cord ~ 2 mm). ROIs of arbitrary shape can be transferred from one modality to the other. **Figure 2** shows one application of the linear transformations produced by the registration, where the boundaries of the MRI pixels have been converted into warped squares on the histology sections. In this way, a histological measure (e.g. number of axon stain pixels above a threshold) can be calculated for histology regions encompassed by each MR pixel without distorting the original histological image, thus allowing generation of histology parameter maps for 1-to-1 comparison with MR parameter maps (e.g. longitudinal diffusivity). The TrW image was chosen for registration purposes due to its clear contrast between cavity, gray matter and white matter. Other MR maps (e.g. myelin water fraction) acquired with known in-plane offset from the TrW image can then be compared to histology. Experience showed that rigid/affine warping was more stable than nonlinear b-spline warping, except where extensive cavitation made the tissue too floppy (these regions can be excluded from further analysis). A previous report of pixelwise MR-histology comparison in rodent spinal cord[2] used fiducial-based registration for an experimental model of multiple sclerosis; our technique has proven successful in the "messier" contusion injury model with nominally more objectivity due to the use of the mutual information metric in the final step of the registration.



Conclusions: We present a robust registration scheme which allows pixelwise correlation between ex vivo MR and histology parameter maps, which will allow more powerful validation of MR measures of white matter integrity. **References**: [1] Kozlowski et al, J Neurotrama 2008:653-76. [2] Budde et al, J Neurosci 2009:2805-13.