Registration of In-vivo MR Images to Triphenyltetrazolium Chloride Stained Sections in Small Animal Models

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Introduction: To establish in-vivo biomarkers, signal changes associated with newly developed high-resolution in-vivo imaging techniques must be directly correlated with histological markers of disease. In this study, an effective method was developed to register in-vivo MR images and digitized histological brain sections to compensate the distortions that occur in the brain during the extraction, fixation, and staining process. The purpose of this study was to determine the registration accuracy of the method using T_1-weighted MR images and 2,3,5-triphenyltetrazolium chloride (TTC) stained sections, and to establish the minimum number of landmark points required for accurate registration.

Methods: Ten male New Zealand white rabbits were studied using a 4 Tesla whole body MR scanner, consisting of a Varian Unity INOVA console, and Siemens Sonata gradient system. T_1-weighted anatomical images were acquired with FOV=8 x 8 x 4 cm^3, TR/TE/TI = 15/7.6 /500 ms, 256 x 256 x 8 acquisition matrix. After imaging, rabbits were sacrificed and the brains cut into 5 mm coronal slices. Tissue sections were incubated with 2% TTC for 30 minutes to identify tissue with viable mitochondria. The stained sections were scanned (~500 x 400 pixel resolution) with an Epson Perfection 636U scanner. An image warping paradigm was implemented using VTK/Python (version 4.2/version 2.1). Registration validation and determination of accuracy was performed using 10 simulated histological sections created by warping the T_1-weighted images to resemble their corresponding TTC stained sections. The warping of TTC sections using an optimized protocol was evaluated by visual inspection. For registration, images were first brought into rough alignment by manually rotating, translating, and magnifying the MR image. Target points in the MR image and source points in the histological section were chosen for the non-linear registration using a newly developed guideline assisted method that calculates the relative angle of the target point based on the angle of the source point in relation to a fixed arc defined by three easily identifiable anatomical landmarks. Landmark points were defined along all visible brain layers. Root mean squared (RMS) registration errors were calculated and registration methods compared by t-test ($p<0.05$ considered significant) using a grid of evenly distributed test points. The minimum number of landmark points required for optimal registration was also defined.

Results: In the simulated data, linear registration produced an average RMS registration error of 974 ± 336 μm, and the subsequent use of non-linear registration with guideline assistance significantly decreased ($p<0.01$, paired, two-tailed t-test) the error to 411 ± 105 μm. Figure 1 demonstrates the decrease in RMS error as the number of landmark points increases, and shows significantly smaller RMS error ($p<0.03$, paired, two-tailed t-test) associated with guideline assistance (diamonds) compared to no assistance (squares) when ≥ 50 landmark points were used. A typical T_1-weighted anatomical image (Fig. 2A) and corresponding TTC stained section following warping (Fig. 2B) demonstrate the successful application of the method. Gridlines superimposed on the TTC section (Fig. 2B) provide an indication of the applied non-linear warping.

Fig.1

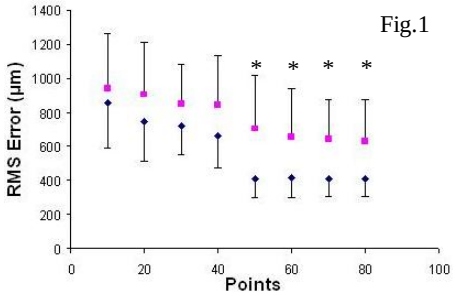


Fig.2A

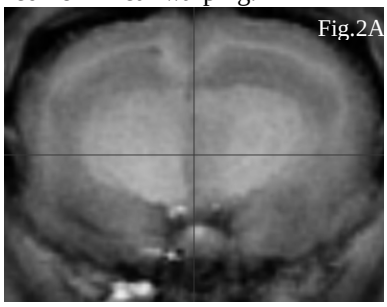
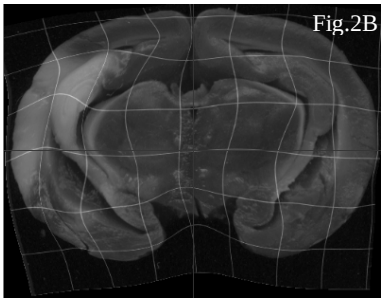


Fig.2B



Discussion: An effective image registration method was developed to register histological sections to in-vivo MR images in small animal models. The method combines the selection of source and target landmark points based on anatomy with radial guideline assistance based on the position of the source point relative to an arc defined by easily resolved anatomical features. The method provides accurate registration to within a RMS error of 411μm, equivalent to approximately 2.5 pixels in the MR image. Visual inspection of the TTC section following warping demonstrates satisfactory results and allows the correlation of infarct location with in-vivo MR signal changes. Future work must include a 3D software slicer to correct minor differences in image orientation by re-slicing the 3D MR images to match the histological section.

Acknowledgement and References:

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