Registration of In-Vivo MRI to Histology using 3D Block Face Imaging as common reference: Application to Cell Tracking in a Murine Model of HIV-1 Encephalitis

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Introduction: Registration of MRI to histopatological sections is useful for characterization and validation of the in-vivo results. Commonly used methods for registration fall short of this goal secondary to shrinkage and tearing during tissue preparation. To address this problem, we developed a technique using 3D block face histology[1] as the common space for the registration process. Technique utility is demonstrated through immunocyte tracking, from blood to brain, in a mouse model of HIV-1 encephalitis (HIVE).

Methods and Applications: Superparamagnetic Iron Oxide (SPIO, Feridex, Bayer Health Care) labeled bone-marrow derived macrophages (BMM)[2] were injected intravenously and tracked across the blood-brain barrier (BBB) using T_2*-weighted MRI in four mice using a Bruker 7T/21cm Biospec MRI system. MRI scans were conducted before (Pre MRI) and 7 days after BMM tail vein injection (Post MRI). Brains were removed, fixed and then embedded in paraffin. Brains were sequentially photographed during tissue slicing in the cryostat to obtain images for creating the blockface volume (BF Histo). Histological sections (HS) were stained with Prussian blue to identify cells containing iron (SPIO). MRI volumes were registered to the blockface volume.

1-Volume Reconstruction - MRI scans were subimaged, removing tissue outside of the brain (Fig.1A). *BF Histo* is subimaged from digital photographs using a region growing algorithm. Brain volumes were then reconstructed from the stack of coronal digital BF Histo images after sequential 2D linear alignment in the longitudinal direction (Fig.1B). Similarly, *HS volume* is reconstructed from stained slides, subimaged, aligned to blockface sections and warped back to the original shape (Fig.1C).

2-Registration and Warping – MRI volumes were registered to BF Histo using affine transformation. Matched MRI and HS slices are warped to corresponding BF slices individually using thin-plate splines with optimized landmarks and minimum bending energy (Fig. 2).

3-Cell Detection –Signal corresponding to cells detected by MRI (MRI cells) are processed by registration, histogram based signal normalization and subtraction of Post MRI from the Pre MRI scan. The resulting signals are leveled to just above the noise and edge artifacts are removed. Resulting signals (MRI cells) are overlaid on Pre MRI for visualization (Fig. 2A). Cells in HS were detected by segmentation of the Prussian blue stain from digitized histological sections (Fig. 2C).

4-Comparison – Volumes were created from MRI cells and HS cells for visualization and comparison purposes (Fig. 3). The area containing cells in each slice were expressed as a percent of slice area in both MRI and histology for each coregistered slice (Fig. 4).

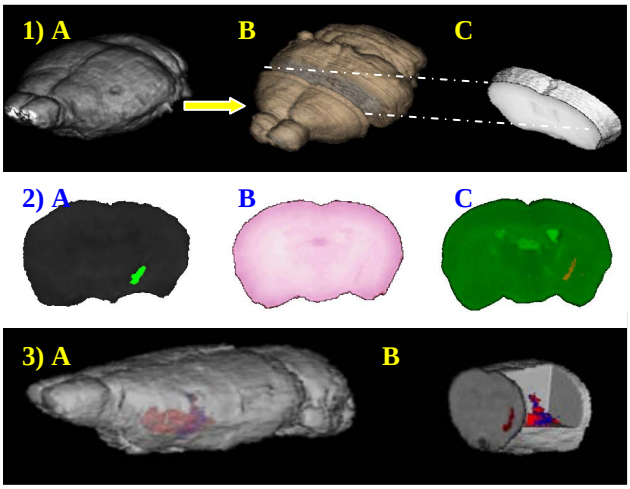

Figure 1–**A**: *MRI mouse brain (anterior-posterior view) before registration to BF Histo.* **B***: BF Histo (reference).* **C***: HS Histo collected from BF Histo for histological analysis.*

Figure 2–**A**: *MRI Cells (green) overlapped on Pre MRI slice.* **B***: BF Histo slice (common reference).* **C***: Matching HS sections (HS Cells in orange).*

Figure 3–**A**: *3D rendering of Pre MRI (50% transparent) containing MRI Cells object (red) and HS Cells object (blue).* **B***: Pre MRI volume portion corresponding to the HS volume with MRI Cells and HS Cells objects. Note that since the MRI sensitivity to cells is higher than the red object (MRI Cells) contains the blue object (HS Cells). The red region beyond the blue volume is beyond the range of the slices selected for histological staining.*

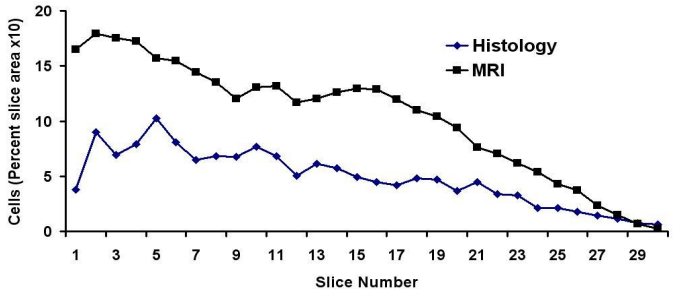

Figure 4: *Cells detected using in vivo MRI and histopatological*

Results: Registration and Warping of MRI volumes to HS were conducted in four mice with HIV-1 encephalitis. Registration of MRI and HS was accurate within the histological slices as can be seen in Figure 2. Spatial distribution and cell areas detected with in vivo MRI show a good correlation with histopatology (Figure 4), although the regions with few cells on histology were undetected by MRI (Figure 3B). In addition, we found that the cell areas were overestimated by 30-40% comparing the MRI detection of SPIO labeled BMM to coregistered histology (Fig. 4).

Discussion: MRI can be used in vivo to track cells migrating across the blood-brain barrier in a mouse model of HIV-1 encephalitis. However, accuracy of the method requires further study to more accurately determine sensitivity and specificity of the method. The 2D warping algorithm provides good results, but requires extensive user interaction. A more automated 3D warping algorithm is being explored. In-vivo MRI shows high sensitivity to clusters of cells, however isolated or few cells are not detected at a resolution of 150 microns isotropic with Feridex labeled cells. This study has demonstrated that the dynamics of the migration of BBM into the nervous system can be monitored by MRI and validated by histological coregistration. Such observations, to our knowledge, have not been reported elsewhere and provide a new biomarker to track disease onset and progression relevant to the neuropathogenesis of HIV-1 infection. Of interest, coregistration of histology to imaging can also be modified to track other cell types as well as correlating a wide range of neurohistological measures with MRI and/or MRSI measures, making these observations broadly applicable for neuroscience research.

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References: 1: Toga AW, Ambach KL, Schluender S. Neuroimage. 1:334-344.**(**1994) **2**: Zelivyanskaya M.L., Nelson J.A., Poluektova L., Uberti M., Mellon M., Gendelman H.E., Boska M.D. J. Neurosci Res. 73(3):284-295 (2003).