# Co-registration of DTI and MTR MRI Data to Quantitative Immunohistochemistry in a Novel Mouse Model of Inflammatory Cerebral Demyelination

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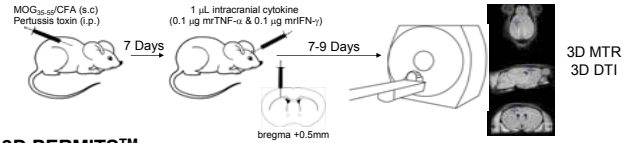
## Introduction

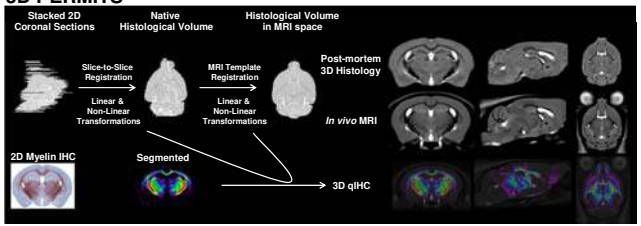
The objective of this study was to determine the neuropathological correlates of *in vivo* magnetization transfer imaging (MTI) and diffusion tensor imaging (DTI) MRI measures using a novel mouse model of inflammatory cerebral demyelination. Conventional MRI is routinely utilized to evaluate the efficacy of therapeutic agents in multiple sclerosis (MS) clinical studies. However, the relationship between non-conventional, *in vivo* imaging measures and the underlying pathophysiological processes remains poorly understood. In order to maximize the information gleaned from MRI data and appropriately steer clinical development of novel therapeutics, we have performed a rigorous correlation analysis between *in vivo* 3D MRI measures and gold-standard, post-mortem, quantitative immunohistochemistry (qIHC) measures in mice with focal inflammatory/demyelinating cerebral lesions using 3D PERMITS™.

## Methods

### Focal Brain Lesion in C57Bl/6 MOG/EAE and MRI Acquisition




### 3D PERMITS™

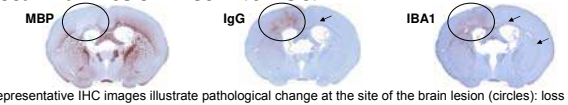



PERMITS™ uses multi-step, image registration to generate 3D qIHC volumes (Grand'Maison, 2013):

- Mouse brains tissue were fixed and embedded in paraffin, then sectioned into 5 µm sections with ~120 levels covering the entire brain
- Tissue sections were stained for Myelin Basic Protein (MBP), Immunoglobulin-G (IgG) or the microglial/macrophage marker IBA-1 and counterstained with Acid Blue 129 (Zehntner, 2008)
- IHC sections were digitized using Zeiss MIRAX Scan150 whole slide scanner
- Image registration employed between-section alignment in a coarse-to-fine fashion, proceeding from an initial center-of-mass alignment, through affine alignment, and then several passes of non-linear between-section alignment
- Registration of the resulting 3D volume to an anatomical MRI template using a coarse-to-fine, multi-resolution, nonlinear registration process
- Two-dimensional (2D) qIHC maps were generated for each section using an artificial neural network (ANN) classifier (Zijdenbos, 2002),in a fully-automated manner, to generate unbiased, binarized (chromogen vs. non-chromogen) images
- The concatenated transformations derived from the 3D reconstruction process were then applied to the 2D qIHC maps to generate 3D qIHC volumes of MBP and IgG

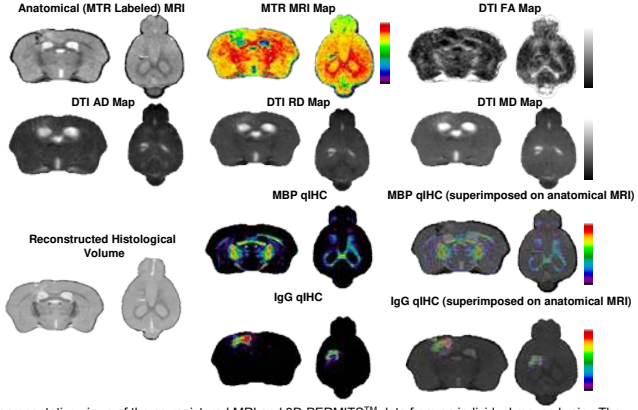
## Results

### Focal Brain Lesion in C57Bl/6 MOG/EAE




Representative IHC images illustrate pathological change at the site of the brain lesion (circles): loss of myelin integrity (MBP), blood-brain barrier permeability covering both white and grey matter (IgG), and microglia-activation/macrophage-infiltration (IBA-1). Arrows indicate areas of inflammation in the right hemisphere.

### Co-Registered 3D MRI and 3D PERMITS™




Representative views of the co-registered MRI and 3D PERMITS™ data from an individual mouse brain. The anatomical volume was derived from the MT-labeled images. The MTR MRI parametric data is illustrated using a spectral color scale (low MTR signal in the colder colors and high MTR signal in the warmer colors). The DTI MRI maps (FA, MD, AD, and RD) are illustrated using grayscale (low signal in black and high signal in white). The cytokine-induced lesion is readily apparent in the left hemisphere in all MR images. The reconstructed histological volume utilized in 3D PERMITS™ was derived from the IHC tissue stain. The MBP and IgG qIHC maps represent the quantitative MBP and IgG load, respectively.

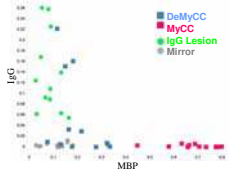
### Lesion-Based Analysis

The corpus callosum ROI was sub-parcellated into demyelinated (DeMyCC = blue) and myelinated (MyCC = pink) regions by thresholding the 3D MBP IHC data. The IgG ROI was defined by the IgG staining (IgG ROI = turquoise), while the contralateral ROI was defined as the mirror of the IgG staining region (contralateral mirror = gray).

| qIHC | Demyelinated CC | % Difference | Myelinated CC |
|---|---|---|---|
| MBP | 0.205 ± 0.089 | 30 | 0.686 ± 0.101 |
| IgG | 0.053 ± 0.079 | 2,650 | 0.002 ± 0.003 |

| qIHC | IgG Lesion | % Difference | Mirror |
|---|---|---|---|
| MBP | 0.083 ± 0.043 | 76 | 0.109 ± 0.051 |
| IgG | 0.138 ± 0.076 | 3,450 | 0.004 ± 0.004 |

Summary qIHC measured as staining density (mean ± s.d. & % difference)



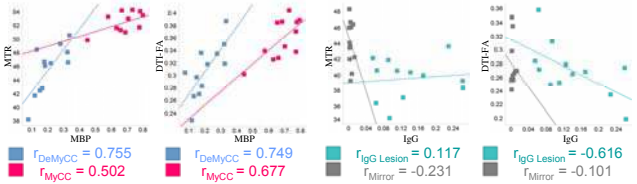

Individual qIHC measures within the corpus callosum and IgG ROIs

| MRI | Demyelinated CC | % Difference | Myelinated CC |
|---|---|---|---|
| MTR | 45.762 ± 3.59 | 87 | 52.598 ± 1.443 |
| DTI-FA | 0.31 ± 0.043 | 86 | 0.36 ± 0.033 |
| DTI-MD | 0.834 ± 0.097 | 109 | 0.763 ± 0.046 |
| DTI-AD | 1.099 ± 0.105 | 104 | 1.053 ± 0.051 |
| DTI-RD | 0.701 ± 0.097 | 113 | 0.617 ± 0.048 |

| IgG Lesion | % Difference | Mirror |
|---|---|---|
| 39.692 ± 2.574 | 88 | 44.140 ± 2.776 |
| 0.272 ± 0.04 | 97 | 0.279 ± 0.033 |
| 0.850 ± 0.092 | 112 | 0.759 ± 0.082 |
| 1.078 ± 0.096 | 111 | 0.973 ± 0.092 |
| 0.737 ± 0.092 | 113 | 0.652 ± 0.079 |

Summary MRI measures within the corpus callosum and IgG ROIs (mean ± s.d.; % difference)

### Lesion Based Correlation Analysis




Correlation analysis of the MTR and DTI-FA MRI measures within the corpus callosum and IgG ROIs

## References

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## Acknowledgements & Disclosure

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## Conclusions

- The focal lesions were clearly visible on the MR images, and co-registration between MRI and qIHC data was achieved using Biospective's PERMITS™ technology.
- The lesions demonstrated variable degrees of demyelination and IgG staining across animals.
- Strong correlations were observed between demyelination and both MTR & DTI-FA in the demyelinated corpus callosum ROI.
- It will be important to examine these correlations in the context of remyelination.
- A strong correlation was also observed between IgG and DTI-FA in the cortical ROI
- The combination of *in vivo* MRI and post-mortem 3D qIHC studies is a valuable strategy for interrogating the relationship between quantitative neuroimaging and gold-standard neuropathology measures.
- This unique approach is expected to provide improved interpretation of MRI data from pre-clinical and clinical therapeutic efficacy studies.