3D Relaxomic Analysis of a Tumor Laden Mouse Brain

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Introduction. Contrast in MRI is usually derived from the heterogeneous distribution of intrinsic relaxation and other properties. Quantitative images of NMR parameters provide a basis for tissue characterization, but the manner in which these correspond to underlying tissue composition is often poorly understood. A variety of dynamic interactions between tissue macromolecules and water such as magnetization transfer may influence NMR relaxation rates. We hypothesize that correlations between the molecular weight distribution of tissue proteins and quantitative MR parameters, on a voxel-by-voxel basis, may provide insights into explaining tissue MR properties. We term the integration of tissue relaxation and proteomics as relaxomic analysis.

We employ matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI-IMS) and coregister the spatial proteomic information to NMR imaging for our analysis. The MALDI process uses the interactions between laser energy and an acidic matrix solution to liberate ionized macromolecules from tissue samples for mass spectrometry analysis. This technique has been used to characterize the distributions by molecular weights of tissue proteins as large as 100 kDa. More recently, MALDI-IMS techniques have been developed to study spatial distributions of tissue proteins in situ by rasterizing the conventional MALDI process across a plated tissue section.

In previous reports we demonstrated a technique to register single MALDI-IMS images to volumetric NMR data [1]. We have extended the MALDI-IMS acquisition to three dimensions and register the MALDI-IMS volume to parametric NMR volumes. To demonstrate the correlation between the two imaging modalities in a disease state we coregistered the MRI and MALDI-IMS data for a tumor laden mouse brain.

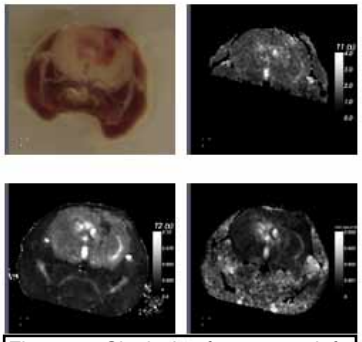

Figure 1. Clockwise from upper left: blockface, T_1, T_2, and ADC images from a tumor laden mouse brain.

Methods. A six to eight week old athymic nude (nu/nu) mouse was obtained (Charles River Laboratories, Wilmington, MA). Following intraperitoneal anesthesia using ketamine (30 mg/Kg) and xylazine (30 mg/Kg), the right striatum was stereotactically injected with 10^5 GL26-glioma cells in 2.5 µL of PBS. Three weeks after tumor implantation, quantitative volumetric NMR images of the mouse's brain were acquired at 7T (Varian, Inc.). T_1 maps were obtained using multiple flip-angle gradient-echo multi-slice images (96x96x9, TR=200 msec, TE=4.5 msec, θ={15,30,45,60,75,90} degrees). T_2 maps were obtained using multi-echo spin-echo multi-slice images (96x96x15, TR=4 sec, TE={12,24,36,48}). ADC maps were made using multiple diffusion gradient spin-echo multi-slice images (96x96x15, TR=2 sec, TE=30 msec, b-values = {0, 100, 200, 400, 800} s^2/mm). All measurements were made with a voxel size of 200x200x200 µm.

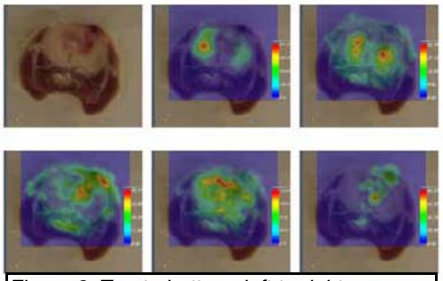

Figure 2. Top to bottom, left to right, corresponding slices from: blockface volume, striatum, nucleus accumbens, tumor, myelin, and injection insult ion volumes overlaid onto the blockface volume. Each ion volume was generated by integrating (m/z) ranges for each ion type from the mass spectra data.

After NMR imaging, the mouse was ex-sanguinated and perfused with saline. The head was frozen and sectioned using a cryo-microtome (Leica, Inc.) at 40 µm slice thickness. Blockface images were acquired of each section using a digital camera (Canon, Inc.). Sections from approximately bregma +1.0 to -8.0 mm were collected every 160 µm for MALDI-IMS acquisition using an Ultraflex II MALDI/TOF system (Bruker, Inc.). Approximately 4000 mass spectra were acquired at 150x150 µm resolution for each MALDI-IMS section collected.

The MALDI-IMS data was coregistered to the NMR data using previously reported methods [1]. After registration, the individual MALDI-IMS sections were concatenated to generate a MALDI-IMS volume. The NMR data was also registered to the blockface volume using voxel based registration techniques.

The result of the registration placed the MALDI-IMS and NMR data in the same reference frame and allowed for relaxomic analysis. Six mass-to-charge (*m/z*) peaks were manually selected from the MALDI-IMS data that spatially highlighted distinct features in the brain: striatum (6741 *m/z*), nucleus accumbens (7346 *m/z*), tumor (13820 *m/z*), myelin (14128 *m/z*), and injection insult (14966 *m/z*). Mass spectra in each section were integrated over these peaks to generate scalar ion images where the intensity in the image represented the relative amount of that ion. Each of the 3D ion images were classified into 5 intensity classes using a K-means algorithm (MATLAB, Inc.) and average T_1, T_2, and ADC values were calculated for pixels which were classified as having the highest ion intensity.

Table 1. Average NMR parameters as a function of MALDI-IMS ion type.

	T_1 (s)	T_2 (ms)	ADC (10^{-3} mm^2/s)
Tumor	0.85±0.82	33.5±18.2	0.92±0.62
Insult	1.27±0.46	38.4±11.8	0.97±0.44
Nucleus Accumbens	0.88±0.70	46.6±7.7	0.92±0.24
Striatum	1.21±0.51	43.6±6.0	0.99±0.30
Myelin	1.63±0.62	56.8±22.7	1.34±0.61

Results. The acquired NMR and MALDI data encompassed approximately 1.2 cm^3 of the brain. Representative slices of each modality registered to each other are shown in Figure 1.

Figure 2 shows scalar ion images of the previously identified peak ranges and the corresponding blockface slice. Each ion image in Figure 2 shows clear contrast in the spatial distribution of its corresponding ion. The registered ion images correlate well with corresponding features in the blockface volume.

Table 1 contains the results of the clustering analysis for each ion volume. An interesting result is the distribution of myelin basic protein, as observed by the myelin ion volume, and its overlap with edematous tissues in the brain as observed by examining structural cues in the NMR data. This leads to the higher than normal T_1, T_2 and ADC parameters for myelinated structures (see Table 1). This result may have been caused by the insult rupturing the corpus callosum upon injection and the ensuing edema promoting distribution of the myelin basic protein into the ventricles. Other results, such as the striatum and nucleus accumbens are in agreement with expected values of T_1, T_2, and ADC in mouse brains at 7T.

Conclusion. This abstract demonstrates preliminary results in using spatially resolved 3D MALDI-IMS to elaborate on observed NMR relaxation phenomena in diseased tissue. The results in the brain tumor model provide insight into the contrast seen in the parametric NMR images. Future work using relaxomic analysis will focus on validation and expansion of these results to other small animal models of disease and to explaining the variations of relaxation within tissue in terms of variations in protein contents.

References.

[1] Sinha, T.K., Luci, J.J., Shahidi, S.K., Cornett, D.S., Gore, J.C., Dawant, B.M. Registration of MALDI mass spectrometry images and MR parametric maps for relaxomic analysis. 14th Annual meeting of the ISMRM, Seattle, WA, 2006.