Correlation of tissue proteomic profiles with MR measures in a rat brain

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Introduction. The heterogeneous distribution of contrast in MR images can be attributed to spatial variations of relaxation and other NMR properties of tissue. Quantitative MR images can provide a basis for tissue characterization, but the relationship between these measures and the underlying tissue composition is not well established. We are using proteomic profiling provided by matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI IMS) as a means to characterize tissue composition and to correlate these observations with *in vivo* MR measures.

Here we investigate the quantitative relationship between MR measures with proteomic profiles in a normal rat brain. The goal is to demonstrate the covariance of varying protein profiles on the observed MR signatures measured *in vivo*. MALDI IMS is capable of generating spectral images of tissue sections, where each pixel in the image represents a spectrum of constituent proteins within the tissue at a given spatial location. In earlier work, we examined single peaks (or molecules) within these proteomic images and looked to establish relationships with corresponding MR information. More recently we have refined and extended our approach to examine the entire proteomic spectrum at each pixel and its relationship with quantitative measures from MR. We hypothesize that this spectral correlation will yield insights into the heterogeneous distribution of MR measures observed *in vivo* and their relationships with the underlying tissue composition.

Methods. Rat brain was imaged *in vivo* under gaseous anesthesia (98% oxygen/2% isoflurane) at 9.4T (Varian, Inc.). A coronal gradient-echo 3D scan was acquired for co-registration purposes (TR=25msec, TE=4msec, flip angle = 20, matrix=192x192x192, FOV=38.4x25.6x25.6mm). A saturation recovery protocol was used to derive quantitative T_1, T_2, and M_0 measures. Three coronal multi-slice image sets were collected using a spin-echo sequence (matrix=128x128, FOV=25.6x30.0, NEX=4, # of slices=27, slice thickness=300um) with the following T_R/T_E combinations: 2000/10, 2000/45, and 8000/10 msec. These weighted images were non-linearly fit to the equation:

$$S = M_0 * (1-e^{(-T_R/T_1)}) * e^{(-T_E/T_2)},$$

to generate quantitative maps of T_1, T_2, and M_0.

After imaging, the rat was exsanguinated and sectioned to generate MALDI IMS data. The whole head (with skull intact) was sectioned coronally and optically imaged using a digital camera every 30 microns. The resulting blockface images were reconstructed and co-registered rigidly with the *in vivo* 3D gradient-echo image mentioned previously. Sections approximately 5 mm deep in the brain (relative to the dorsal side of the animal) were plated for MALDI IMS analysis. Each section was imaged at 200um in-plane resolution, resulting in an image with 121x217 points across a region encompassing the rat brain, and with a spectral dimension of 25203, resulting in an average spectral resolution of approximately 1.5 Da.

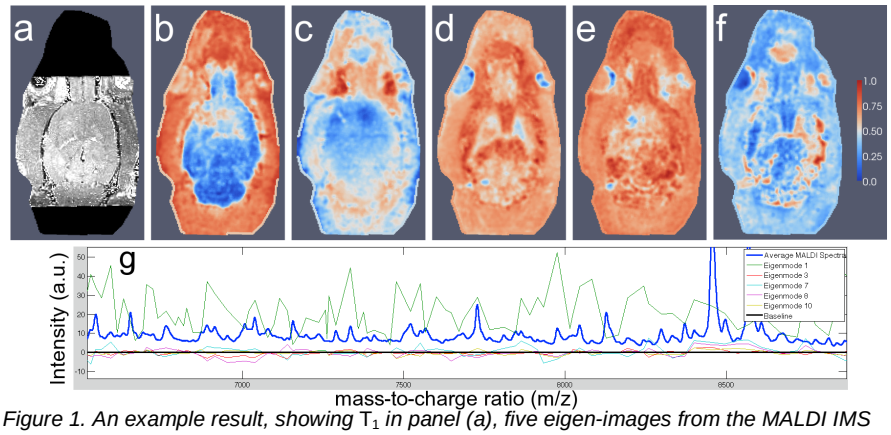

Figure 1. An example result, showing T_1 in panel (a), five eigen-images from the MALDI IMS data (b-f) for a single section in a rat head. Using ANOVA analysis with 5-way interactions, the data in (b-f) were found to significantly correlate with the voxel-by-voxel T_1 measures seen in (a). The data in (b-f) provide a direct measure of tissue proteomic profiles, and thus can be used to relate protein changes in this tissue to changes in T_1. Panel (g) demonstrates this principal by showing a portion of the spectral dimension acquired in this section. The blue line represents the average MALDI signature across all spatial points, while the black line provides a baseline reference at 0. The other colored lines show the variations of each eigen-mode found to correlate with the spatial distribution of T_1. These results demonstrate how one can investigate signal contrast changes in MR in light of changes in the underlying macromolecular profile of tissue.

A single MALDI IMS section was coregistered and correlated to the corresponding quantitative MR measures. The co-registration process is described thoroughly in [1]. In brief, each MALDI IMS dataset is co-registered to its blockface image, which is localized in the 3D blockface volume. The 3D blockface volume is then coregistered with the 3D *in vivo* MR image. This ultimately allows one to navigate from the 2D MALDI IMS data to corresponding locations in the 3D MRI data. The scale of the MALDI data (26257 pixels by 25203 spectral points) necessitated reduction of dimensions via principal component analysis to allow for computationally tractable correlation analysis. Each mass spectra was further condensed by thresholding for peaks above an arbitrary threshold prior to PCA. Combining these two techniques resulted in reduced dataset of 10 principal component spectra, each with a spectral dimension of 367 peaks, which accounted for approximately 96% of the variation in the original spectral data. The principal spectra were then used to generate eigen-images for each eigen-mode (i.e. images which demonstrated spatial distribution of each principal component).

N-way analysis of variance (ANOVA) tests were used to determine the covariance of T_1 with respect to each eigen-image on a pixel-by-pixel basis. The T_1 estimate was corrected for variations due to M_0, using a model for water relaxation in fast-exchange, resulting a map of T_1 where the influence of M_0 was mitigated.

Results. Five-way interactions in our ANOVA tests showed significant correlations between T_1 and a number of eigen-images. Out of 637 possible correlations, 17 demonstrate significance in their *p*-value ($p<0.05$; uncorrected). An example significant correlation is shown in Figure 1. In this example, eigen-images 1, 3, 7, 8, and 10 all correlate significantly with T_1 (Fig 1a-f). Examining the corresponding eigen-modes (1,3,7, 8, and 10) can provide quantitative estimates of which *m/z* peaks varied relative to the mean spectral content of the tissue (Fig 1g). That is, given a significant correlation between certain eigen-images and T_1, one can ascertain which *m/z* peaks were involved in generating that correlation by investigating the corresponding eigen-modes.

Conclusion. We have demonstrated a method to quantitatively correlate MR metrics with proteomic information from MALDI IMS. Building on previous work that demonstrated the quantitative co-registration of these two modalities, this abstract demonstrates our techniques to digest the feature-rich MALDI IMS data and proposes a method to correlate these the spectral changes in a tissue's proteomic profile with changes in MR parameters. We are planning to use techniques such as *in situ* trypsin digest to provide accurate protein identification of the interesting *m/z* changes we observe using the techniques described here. Finally, we are currently using these techniques to investigate MR signal and correlated proteomic changes in pathophysiological states such as brain tumors.

References. [1] Sinha, T.K., et al. 3D MALDI imaging mass spectrometry integration with *in vivo* MRI. Nature Methods: 5(1). 2008