Brain tumor imaging based, histology trained maps (IBHTMs) of cellularity predict tumor presence in pathologically confirmed regions sampled ex-vivo

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Target Audience: Scientists and clinicians interested in brain cancer imaging methods.

Purpose Recent advances in the voxel-wise co-registration of histology obtained from ex-vivo whole brain samples and in-vivo imaging have been used to determine the level of diffusion restriction necessary for defining regions of brain tumor hypercellularity and diffusion restricted necrosis[1]. The methodology presented in this recent study has opened up the possibility of training algorithms to predict histological features based on the MR voxel values and the co-registered histological features of interest.

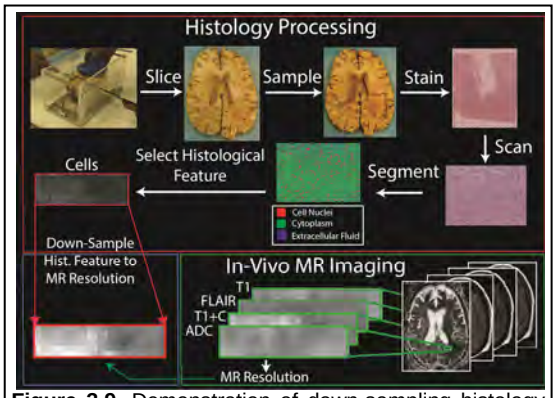

Figure 3.9. Demonstration of down-sampling histology to clinical MRI resolution for a voxel-wise comparison[1].

Methods *Patient Population* Seven patients with high-grade gliomas were included in this IRB approved study. Patients donated their brains following death. *Ex-vivo Histology Processing* Large tissue samples (approximately 4cm^2) were taken from regions suspicious of tumor and free from MR acquisition artifacts in each of the 7 patients. Histological samples were hematoxylin and eosin (H&E) stained. Each slide was then photographed at 10x using a motorized microscope stage and Nikon Instruments software (Melville, NY). Each photo was individually segmented to locate cells. The process began with a white background correction, where the average of 20-40 photos taken from regions of empty slide (i.e. no tissue in view) was calculated and subtracted from each image. The RBG photos were reduced to the red component alone giving the best black and white contrast among the tissue types for the H&E stained slides. A contrast optimization was applied to best segment the images. A k-means clustering algorithm implemented in Matlab was then used to segment each photo. A representative segmentation is shown in the center of Figure 1.

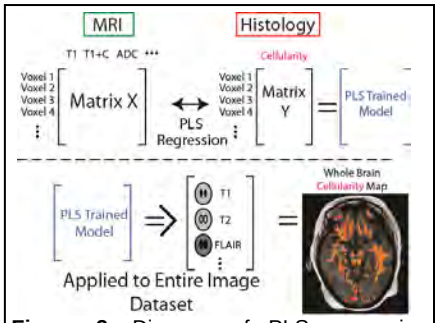

Figure 2. Diagram of PLS regression trained model applied to entire image dataset for generation of an IBHT map of cell count (i.e. cellularity).

Precise Histology to MRI Correlation Co-registration of histology to MRI was performed using a manually defined linear rotational and translational transformation applied to align each histology slide to the MRI. The location of each sample was matched visually to the MRI slice that best represented the sample's location[1].

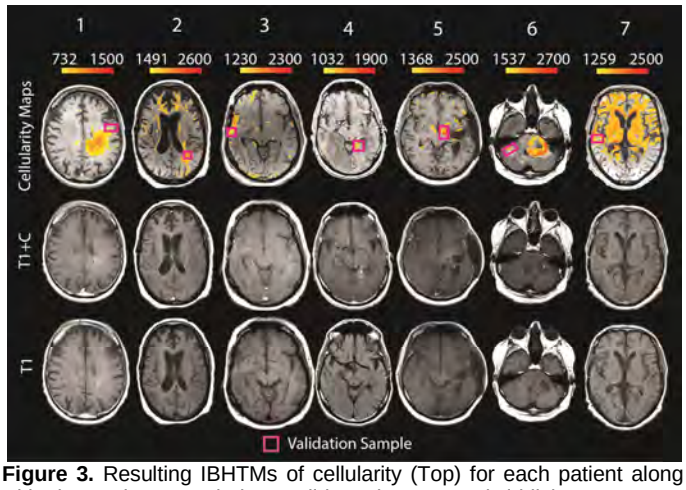

Figure 3. Resulting IBHTMs of cellularity (Top) for each patient along with the region sampled to validate the maps. (Middle) T1+contrast (Bottom) T1 images in the same slice.

Histology from within each region of interest drawn using the HISTtoMRI toolbox was then down-sampled to the MRI resolution for a direct 1 to 1 comparison (method shown in Figure 1). Histological segmentation values, along with the MRI values within each voxel were then extracted and combined across all samples.

Partial least squares (PLS) regression was applied to the MRI values using cellularity as the independent variable to train a model. The PLS trained model was then applied to the patient's entire stack of whole brain MR images to generate imaging based histology trained maps (IBHTMs) of cellularity (Figure 2). Voxels outside the brain were excluded. The resulting maps were thresholded based on a 95% confidence interval determined from cellularity calculated within a normal histology sample for each patient (Figure 3, Top).

To validate the algorithm's accuracy, additional histology samples were gathered from regions indicated as hypercellular by the IBHTM. These samples were scanned and the predicted values were compared to the actual cellularity values using a Pearson correlation.

Results Figure 3 shows the IBHTMs of cellularity for each of the seven patients. Samples gathered in regions implicated, all demonstrated viable tumor. Pearson correlation coefficients ranged from 0.31 to 0.69 and all were significantly positively correlated, $p<0.00001$. We found viable tumor in regions that appeared normal on conventional imaging in 5 of 7 patients.

Discussion We present a novel method for mapping brain cancer cellularity with imaging based histological trained maps. This new method will potentially improve surgical planning, radiation guidance, and tumor progression detection.

References 1. LaViolette, P.S., *et al. Neuro-Oncology.* **In Press**(2014).

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