Fiducial Markers for Correlation of Whole-Specimen Histopathology with MR Imaging at 7T

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INTRODUCTION

Histology provides the "gold standard" for tumor delineation and assessment of tumor response to cancer treatment. There is a growing role for image registration with histology in radiotherapy in order to estimate tumor anatomical changes during and following treatment. However, correlating cross-sectional images to histopathology is difficult because the fixation procedure and subsequent processing techniques change tissue properties non-uniformly (1). Also, often between histological and in vivo images there are differences in sectioning angle and slice thickness. Anatomical landmarks (e.g. the urethra in prostate) can be used to correlate histology with MR images. However, anatomical landmarks are not always readily visible in all sections in MR and histology images. The use of injectable markers (2) that are visible in MR imaging and histology is an interesting option since such markers can be used to define points or planes (groups of points) in each MR image and histology section. These reference points can serve either of two purposes: to register MR images with histopathology images, or to align adjacent histology images for 3D histopathology volume reconstruction. This abstract presents the preliminary results of testing two injectable fiducial markers for whole-specimen histopathology correlation with high-field (7 T) MR imaging. These markers were previously found suitable for histology and for pre-clinical in vivo and ex vivo MR imaging at 1.5 T (2). The future goal of this work is to use this technique for whole-specimen histopathology registration with MR images using human prostate organs resulting from salvage prostatectomy.

METHODS

Sample preparation: Two iridescent acrylic paints (2) were tested undiluted and at various dilutions as potential fiducial markers: Iridescent Bronze (IB) (iron oxide coated mica particles and 'PhthaloGreen') undiluted and at dilutions of 1:2, 1:5 and 1:10 by mixing with 'Polymer Medium (Gloss)' (2); Iridescent Stainless Steel (ISS) (iron, chromium, nickel), undiluted and diluted at 1:10 separately with 'Hansa yellow light' paint and 'PhthaloBlue' paint. (All paints and the polymer medium and were supplied by Golden Artist Colors, New Berlin, NY).

Experiment 1: For each paint mixture (7 combinations), 5 injections were made in each specimen (~ 27 cm^3 volume) of fresh porcine kidney. The volume of paint per each injection varied as a function of specimen size with at a ratio of ~ 0.1ml paint/cm delivered using a 27-G syringe needle. The syringe needle was pressed fully into the sample prior to release of the paint, and then slowly drawn backwards while the paint was deposited in an even track through the tissue. The samples were MR imaged twice: fresh and after 24 hrs fixation in 10% neutral buffered Formalin solution. The specimens were processed for histology after the last MR imaging session. To correlate the visibility on MR images with histology, samples for histology were taken at different depths of the tissue sample perpendicular to the direction of the needle track.

Experiment 2: The 4 best paint combinations from the first experiment (i.e. the 4 IB dilutions) were injected twice by the same method into separate fresh porcine liver samples (~ 8 cm^3 volume, 2 sections of tissue per paint dilution, 8 samples in total). Four liver samples (1 for each dilution) were fixed for 24 hrs, and the fresh and fixed samples were MR imaged.

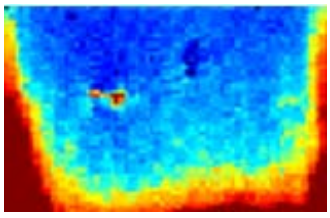
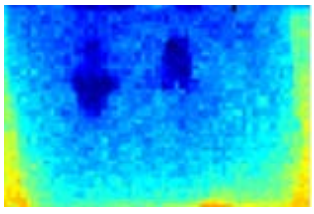
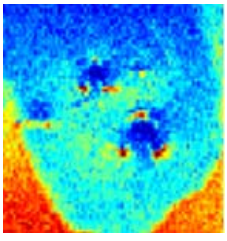
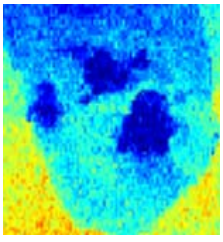
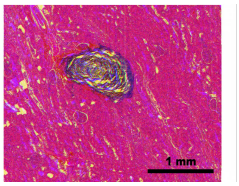
MRI imaging: The samples were embedded in a block of a mixture of gelatin (4%) and agarose (1.5%) in groups of 3 or 4 prior to imaging, to reduce susceptibility artefacts at the sample edges. All images were acquired on a 7T pre-clinical MRI scanner (70/30 BioSpec, Bruker, Ettlingen, Germany), where a quadrature volume resonator (15.5 cm inner diameter) was used for transmission and reception. Each set of samples was imaged using two different imaging sequences: a spin-echo (SE) sequence, RARE (Rapid Acquisition with Relaxation Enhancement) with effective TE = 20 ms, TR = 200 ms, signal averages = 2, echo images = 1; a gradient echo (GE) sequence, FLASH (Fast Low Angle Shot), TE = 7 ms, TR = 380 ms, flip angle = 30°. For both sequences volume acquisition was made of the tissue-gel block with approximately 15 slices of 2 mm thickness, with an acquisition matrix of 256 × 256 matrix (~ 0.5 × 0.5 mm^2 pixel dimension). The readout bandwidth was 50 kHz for all acquisitions.

RESULTS

Experiment 1: The ISS acrylic paint in both undiluted and diluted concentrations created excessive susceptibility artefacts resulting in distortion of the entire image. Therefore ISS was not considered for further examination in histology. Where not occluded by the ISS artefacts, the IB paint was visible on MRI slices. IB was also visible on Hematoxylin and Eosin (H&E) staining, exhibiting a small ellipsoidal shape (~1 mm largest diameter) with a stable dimension through different histological sections (Figure 1).

Experiment 2: All dilutions of IB were visible in both SE and GE MR images, with the GE images displaying susceptibility artefacts several times the size of the SE artefacts in each instance. It was not apparent from this study if artefact dimension was a function of paint dilution, however the distribution of the paint in the needle track was not strictly controlled. Also it was noted that in some samples the paint leaked through cavities in the liver tissue, to create the appearance of more than two markers in certain slices.

Figure 1. MR images (fresh and fixed ex vivo porcine liver) and histology (ex vivo porcine kidney) showing the appearance of IB markers. (a)-(d) SE and GE MR images for non-diluted IB. In (c) and (d) a third apparent marker is visible due to paint leakage. (e) The IB presented a well-defined shape in H&E images with no diffusion through the tissue structures.

(a) MRI fresh SE	(b) MRI fresh GE	(c) MRI fixed SE	(d) MRI fixed GE	(e) H&E staining
				

CONCLUSION

In conclusion the IB acrylic paint could be used as a fiducial marker for registration of histology sections with high-field MR images, whereas the ISS marker was found to produce excessive susceptibility artefacts. No significant changes in the size of the susceptibility artefacts or the anatomical size in the H&E image with different dilution were observed. Further studies will test this technique in other types of tissue with the goal of using IB acrylic paint for registration of whole mount ex vivo human prostate histology with MR images. This work will provide input to developing a deformable registration strategy for the assessment of prostate geometrical change during and after radiotherapy (3).

REFERENCES

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