**Purpose:**

Within the last few years, 19F MRI was successfully applied to numerous research questions including those focusing on pulmonary imaging using fluorinated gases, measurement of the partial oxygen pressure and 1H-based cell tracking. Regarding 19F-based cell tracking, ex-vivo MRI in combination with immunohistochemistry is often performed to validate in-vivo findings. Unfortunately, due to different sample geometries and sizes, the correlation of the different imaging modalities is limited. For 1H MRI, coils optimized for imaging of histological samples have been successfully applied. Using these coils, 1H MRI images of histological samples could be obtained, allowing exact correlation of histological findings with MRI data. The current work expands the concept of 1H MRI on histological tissue samples to 1H/19F MRI, thus allowing correlation between the 1H/19F MR data and histology.

**Methods:**

**Tumor Model and In-vivo Labeling Procedure:**

An attenuated oncolytic Vaccinia virus (strain GLV-1h68) was injected i.v. into a mouse bearing a subcutaneous 1936-MEL melanoma of 150 mm³. Four days after GLV-1h68 injection, 100 μl of perfluoropolyether (PFC) emulsion was injected i.v. As mentioned in1, PFC emulsion nanoparticles are internalized by phagocytic cells (monocytes, macrophages). The tumor was excised 11 days post infection.

**Experiment Procedure:**

Fig.1A shows a sketch illustrating the preparation and measurement procedure. After freezing, fixation, rinsing, and embedding, the tumor was sectioned into 150 μm thick slides using a Vibratome. The sections were labeled with primary CD68 antibodies (monocytes, macrophages) and secondary Cy3-conjugated anti-rat antibodies and mounted on glass slides (Fig. 1A). More details of this preparation procedure can be found in8. In a second step, (fluorescence) microscopic images were obtained from an antibody-labeled tumor section (Fig. 1B). Finally, 1H/19F MR projection images were obtained from the same tumor section as used for fluorescence microscopy (Fig. 1C).

**MRI:**

For MRI, a customized double-tunable 1H/19F surface coil was built (2 cm side length). Similar coils have been shown to deliver a high sensitivity and sufficient in-plane B1 homogeneity to perform MRI on histological slices. Imaging of the tumor slice was performed using a 7T small animal scanner. 1H imaging parameters: Sequence = Multi spin echo, TIE/TR = 9/1000ms, Res = 0.1x0.1mm², NA = 16. 19F imaging parameters: Sequence (low-resolved) = Turbo spin echo (TSE), TE/TR = 6.2/1000ms, Res = 0.4x0.4 mm², TF = 48, NA = 3600; Sequence (high-resolved) = TSE, TE/TR = 6.3/1500ms, Res = 0.12x0.1mm², TF = 64, NA = 14400.

**Results:**

Fig.2 shows the MRI results. High-resolution 1H MRI data as well as low- and high-resolution 19F data were obtained. The 1H MRI data and the low-resolved 19F MRI data were both obtained in approximately 1h measurement time (Fig. 2A and B). The high-resolved 19F MRI data were obtained in 24 h (Fig.2C). The 19F/1H overlay in Fig.2D shows that the 19F signal was restricted to the tumor margin.

Fig.3 presents the histology results. In Fig.3A, a top-light image of the tumor section is shown. The green GFP signal in Fig.3B and the CD68 signal (Fig.3C and 3D) from CD68-positive cells was located at the tumor margin. Correlation of the histological and the 19F signal and the CD68 signal (Fig.3C and 3D).

**Discussion and Conclusion:**

Using an optimized 1H/19F surface coil, the 19F signal distribution in a thin tumor section was obtained in acceptable measurement times. Since the same section was measured using both fluorescence-microscopy and MRI, a correlation of the MRI findings with histology was enabled. Thus, in the future this technique can improve the validation of in-vivo MR findings. Furthermore, markers visible in both microscopy and MRI could further enhance data correlation. In this proof-of-principle study, a single, small 1H/19F surface coil was used. Since only one tumor section could be investigated per measurement session using this setup, investigating the entire tumor would be a time consuming process. In the future, double-resonant coil arrays might overcome the limitation of single coils, thus allowing simultaneous measurement of multiple tissue sections.

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**References:**

[7] Temme et al. (2012), WIREs Nanobiotechnol. 4
[8] Weibel et al. (2008), Cellular Microbiology. 10

**Target Audience:** 19F MRI, cell tracking, tissue engineering and regenerative medicine communities