In vivo Multicolor Imaging of Perfluorocarbon Emulsions using ultrafast Spectroscopic Imaging (F-uTSI)

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For Molecular Imaging by MRI, several approaches have been suggested. Most methods employ the use of T_1 or T_2^* based contrast agents. In general, these agents are functionalized with ligands that bind specific biomarkers or cellular uptake [1,2,3]. The presence of the biomarker is associated with local contrast changes in the MR images. One prominent limitation of this approach is that if a mix of agents is used, each binding a different marker, they cannot be distinguished in the MR images, solely based on the contrast change. Alternatively, the use of ^{19}F agents, such as perfluorocarbon (PFC), emulsions has been suggested [4]. Efficient images of PFC emulsion is possible with the Fluorine ultra-fast Turbo Spectroscopic Imaging (F-uTSI) sequence [5,6]. This technique has been further optimized for efficient spectral coverage of large 3D volumes. Here we show that *in vivo* F-uTSI, without additional increase in scan time, can distinguish various ^{19}F compounds based on chemical shift differences allowing for 'multicolor' imaging. Multicolor imaging has proven its versatility with optical imaging [7]. Additional advantages of ^{19}F include absolute quantification, high intrinsic specificity, and no need for pre-contrast imaging.

Methods

The study was performed on a 3T clinical whole-body scanner (Achieva, Philips Healthcare, The Netherlands) using a dual tuned transmit/receive surface coil (7×12 cm) and a dual ^{19}F/^{1}H spectrometer system. All *in vivo* experiments were conducted on black mice (C57BL/6) and were approved by the animal experimental committee of the Maastricht University. All animal care and protocols were in accordance with the rules and regulations as stipulated in Dutch law. For anesthesia, an intra-peritoneal (IP) injection of KMA mix (Ketamine 75 mg/kg; Medetomidine 1.0 mg/kg; Atropine 0.04 mg/kg) was used. During scanning the anesthesia was maintained by continuous IP infusion of the KMA mix. The body temperature of the animal was supported by a heated animal bed. The contrast agents were introduced into the colon via the rectum. The contrast agents are: 25% (v/v) emulsion of perfluoro-octyl bromide (PFOB) and a 16% (v/v) emulsion of perfluoro-15-crown-5-ether (PFCE).

The ^{19}F data were collected with the F-uTSI technique [5,6] which is based on acquiring long spin-echo trains, *e.g.* 16-32 echoes per excitation, where each echo corresponds to one point in k-space. A 3D data with a resolution of 48x48x14 mm has been recorded with 24 echoes per excitation. The duration of the echo-spacing is 5 ms, during this time the 19F spectral data were acquired with a BW 28000 and the resolution is 437.5 Hz/point. The voxel size is 2x2x3 mm, TR/TE/ES: 285/5/5 ms, NSA: 1, and a scan-time of 6:23m.

The center frequency was set on the ^{19}F resonance line of perfluoro-15-crown-5-ether. Spatial reconstruction was done using the standard software available on the scanner. These data were exported and ^{19}F images were created by integrating the signal intensity of the single -CF_2 resonance of the PFCE and the –CF_2 resonances of the PFOB, using the 3DiCSI software package developed at the Hatch MR Research Center, Columbia University, New York, USA. High-resolution T1-weighted GRE images were recorded for anatomical co-registration (resolution 0.55×0.55×4.0 mm^3, TR/TE=24/6.5 ms, α=35).

Results and Discussion

The F-uTSI sequence can perform 3D spectroscopic imaging with excellent spatial resolution at clinical relevant scan-times. To achieve this, the actual data sampling is performed at low spectral resolution, e.g. 437.5 Hz/point. The data shown in fig.1 demonstrate that, despite this low spectral resolution, both PFOB and PFCE can be distinguished clearly in the spectra. Also corresponding images can be reconstructed from these spectra, demonstrating PFOB and PFCE in the mouse colon. An additional advantage of the F-uTSI approach is that all images are free of any chemical shift artifact. Simultaneous acquisition of the different PFC emulsions is not only time efficient, it also the offers additional advantages that more complex biological processes can be imaged by MR, such as experiments with co-injection of emulsion functionalized for different markers. Again, F-uTSI allows imaging with optimal resolution. It should be noted that spectral width of the various PFCs is large compared to the bandwidth of RF pulses that are currently used. The limit in the RF pulses is set by SAR considerations as well as hardware limitation. We are investigating the use of broader excitation profiles using optimized pulse shapes.

Conclusion

3D F-uTSI is a time efficient spectroscopic imaging technique which allows for *in vivo* multicolor ^{19}F imaging. The multicolor images are reconstructed from a single 3D F-uTSI data set resulting in a time efficient experiment. Furthermore, complex biological process can be imaged using co-injection of differently functionalized PFC emulsions, e.g. the multi-color imaging using F-uTSI will offer the possibility to visualize and quantify biomarker ratios.

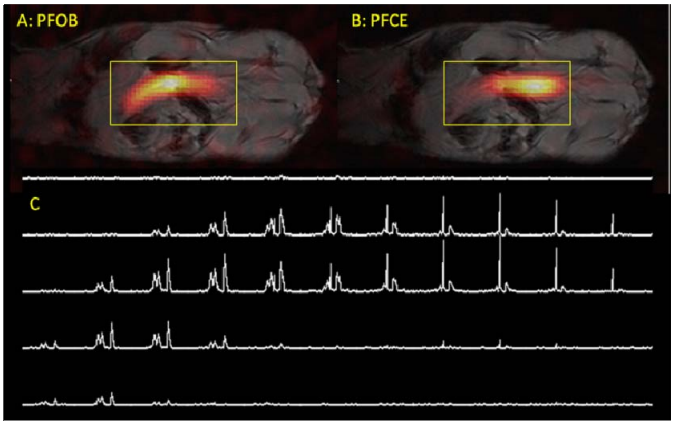

Fig 1. Multicolor 3D F-uTSI. Shown is slice 5. Panel A shows the PFOB image obtained by integrating the –CF_2 resonances, while the PFCE image (B) is obtained from the single –CF_2 peak. The yellow box indicates the area from which the spectra are shown in panel C. The single -CF_2 resonance line (-7 ppm) of PFCE is clearly visible, so are the resonance lines of PFOB, i.e. from left to right: -CF_2Br (18 ppm), -CF_3 (3 ppm) and the CF_2 resonances (from -35 to -44 ppm). Note: the ppm values are given with respect to the resonance frequency at which the data were recorded.

References

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