Ex-vivo Diffusion Imaging at Ultra-High Fields

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HIGHLIGHTS

- Ex-vivo diffusion MRI is challenging due decreased T2's and ADCs compared
- Long acquisition times, shorter T1's, tissue preparation, high-end hardware and specialized pulse sequences can compensate
- Ex-vivo dMRI at 7T and above is becoming an important technique in cortical architecture studies with high resolution MRI

TARGET AUDIENCE

Neuroscientists, Physicists and clinicians wanting to learn about the challenges, methods and possibilities of diffusion imaging on ex-vivo tissue samples

OUTCOME/OBJECTIVES

1. An understanding of the challenges of ex-vivo diffusion imaging at 7T and above
2. Knowledge of the array of techniques (deviating from in-vivo methods) available
3. Be informed of seminal ex vivo diffusion studies reporting validation and basic neuroscience results

PURPOSE & METHODS

A number of investigations over at least a decade have examined brain tissue samples post-mortem at ultra-high fields (UHF; >=7T) mostly using animal scanners or spectroscopy systems. Some of these investigations have recently started to use diffusion MRI (dMRI) as their main MRI modality. The purposes of these investigations are varied, but mostly consist of at least one of the following three objectives. First, performing both MRI and histological analysis on sections of the same tissue allows exploring the physiological underpinnings of basic contrast mechanisms [1, 2] in both healthy and diseased tissue, such as the basic physiological compartments in which diffusion influences the MRI signal. Second, somewhat related, it allows validation of model fitting and inference techniques that try to go beyond the basic information in the data, such as validation of diffusion MRI tractography [3, 4] or axon diameter estimation [5, 6]. Third, the very high spatial resolution that can be achieved can be used for basic neuroscience investigations into the architecture and microstructure of neural tissue, at mesoscopic scales [7-10].

A number of challenges have to be overcome to acquire high quality UHF ex-vivo diffusion MRI, some of them specific to changing relaxation parameters at high B_0 fields, other specific to fixed ex-vivo tissue. Smaller T2's at higher main field strengths are an innate disadvantage to diffusion imaging that mostly uses relatively long-TE pulsed gradient spin-echo (PGSE) sequences. Moreover, fixed ex-vivo tissue has further reduced T2's and reduced water diffusion compared to in-vivo tissue. Water diffusion is reduced partly due to the lower temperature and partly due to effects of tissue fixation. Apparent diffusion coefficients (ADCs) measured with dMRI have been reported to be reduced by as much as 50% in unfixed ex-vivo white matter and 80% in fixed white matter with respect to in-vivo values [1]. Although diffusion anisotropy, a crucial marker for neurite orientation and microstructure estimation, remains intact [11, 12], reduction of absolute diffusivity entails a reduction of contrast-to-noise ratio to detect these important markers. Techniques available to counter this loss in water diffusivity are [1]: increasing sample temperature during scanning (raising white matter ADC by approximately 95% for a 20^{o}C increase) and soaking in Phosphate Buffer Solution (PBS; raising white matter ADC by approximately 30%). The adverse effects of lower T2 and ADC are partly compensated by reduced T1's for ex-vivo tissue, allowing more SNR efficiency at short TRs, which can be made even more beneficial by soaking specimens in gadolinium contrast agent [1]. Combining several strategies, Dyrby et al. [13] established an ex vivo tissue imaging pipeline including tissue fixation, storage, preparation, scanning and data processing stages. They recommend allowing temperature and unstable tissue mechanics to stabilize prior to actual data acquisition and choosing a b-value of about 4000 mm^2/s with standard PGSE sequences. The main additional tools in the hands of the investigator for ex-vivo high field diffusion investigations are: high gradient strength (mostly in the range 140mT/m – 1500mT/m for preclinical systems), long scanning times (ex-vivo examinations often run continuously over days), small close-fitting RF-coils and cryo-cooled RF-coils, and optimized MR pulse sequences. Although STEAM diffusion-weighting preparation has some advantages, particularly for long diffusion-time experiments, the bulk of UHF ex-vivo diffusion experiments are performed with PGSE sequences often with 3D gradient-echo [1] or GRASE readout [14], although 2D acquisitions are also often used, despite their SNR inefficiency per unit time [15]. A high degree of k-space segmentation, with 1 to 15 line read out per excitation and diffusion preparation module is obligatory given low T2 and T2*. As an interesting alternative a diffusion weighted balanced steady state free precession (bSSFP) sequence was recently proposed for efficient whole ex-vivo human brain dMRI on clinical MRI systems [16, 17].

RESULTS & DISCUSSION

Using the techniques described above, a number of studies have reported important basic neuroscience results concerning dMRI contrast mechanisms, histological validation and cortical architecture. Leergaard et al. [18] quantitatively compared fiber orientation distributions (FODs), obtained from spherical deconvolution analysis [19] in an ex vivo rat brain against histological measurements of rat brain myeloarchitecture. They show high FOD accuracy to an orientation error of approximately 5–6 degrees. This quantitative validation of FODs in rat brains was recently revisited with 2D structure tensor analysis of histological sections [20]. Moving the validation from local orientations to entire tractography streamlines, Roebroeck et al. [3] validated streamline tractography of known human optic chiasm pathways at 9.4T and investigated the effects of spatial

resolution of the diffusion data. Seehaus et al. [4] combined DTI at 9.4T with carbocyanine die tracing in the same human temporal lobe tissue block. Since axons are selectively labelled along their length, robust definitions of sensitivity and specificity for DTI tractography could be given and its accuracy could be assessed. DTI streamline tractography was shown to have sensitivity and specificity of greater than 80% over distances of several centimeters with voxel sizes in the 100s of microns. Finally, moving from orientation and tractography validation to multi-compartment microstructural model estimation, Assaf et al. [5] and Dyrby et al. [6] were able to validate axon diameter estimates against histology gold standards and report good accuracy, especially for larger axons with large gradient system strengths.

Most recently, ex-vivo diffusion imaging has gained popularity as a method for basic neuroscience results and reference atlases. Aggarwal et al. [21] created a high resolution 3D atlas (125-255 µm isotropic) of the human brain stem with DTI at 11.7T. They reconstructed fiber pathways with high fidelity using DTI streamline tractography including the decussation of the pyramidal tract fibers. They also used ADC maps to delineate gray matter nuclei to similar precision as that accessible from myelin-stained sections of the same specimen. Dell'Aqua et al. [8] combined dMRI of the human cerebellum down to 100um resolution at 7T with immunohistochemistry labelling and light microscopy to distinguish cerebellar cortical layers and model the trajectory of most of its small-scale circuit fibers. The immunohistochemistry was used to show that diffusion characteristics for each cerebellar cortical layer reflects known architectural patterns. Leuze et al. [10] could model layer-specific intracortical connectivity in human V1 by tracking intracortical fiber pathways running tangentially and radially within specific cortical layers. They validated these dMRI-based findings (acquired at 242um at 9.4T) with cell and myelin stains and polarized light imaging [PLI; 22, 23] for the same tissue blocks. Kleinnijenhuis et al. [9] could similarly distinguish cortical layers and the V1/V2 border based on dMRI signal properties acquired at 300um at 11.7T. They showed fractional anisotropy (FA) derived from DTI analysis varies with cortical depth and reduces in the stria of Gennari, the inner band of Baillarger and the deepest layer of the cortex. In [24] they elaborated this result further with neurite orientation dispersion and density imaging [NODDI; 25], a more sophisticated multi-compartment dMRI signal model. Moving ex vivo dMRI even further into the realm of MRI-based architectonic classification, Bastiani et al. [7] showed that features derived from the voxelwise orientational ADC profile (at 340um at 9.4T) can be used to automatically classify cortical layers in human motor cortex.

CONCLUSION

The sophistication of specific tissue preparation techniques and pulse sequences has increased to a level where ex-vivo dMRI has quickly become an important tool in cortical architecture studies with mesoscopic spatial resolution. In addition, ex-vivo dMRI has had an important role in validating the accuracy and precision of both fiber microstructure and orientation estimates; and tractography results for about a decade. Both uses are likely to continue and be combined with more sophisticated histology techniques and be expand to larger tissue samples in larger bore-size systems such as human UHF systems.

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