High resolution diffusion MRI of the unfixed post mortem brain

Christian Langkammer[1], Nikolaus Krebs[2], Christoph Birkl[1], Lukas Pirpamer[1], Florian Borsodi[1], Michaela Haindl[1], Gernot Reishofer[3], David Andrew Porter[4], Eva Scheurer[2], Franz Fazekas[1], and Stefan Ropele[1]

[1]Department of Neurology, Medical University of Graz, Graz, Austria, [2]Ludwig Boltzmann Institute for Clinical-Forensic Imaging, Graz, Austria, [3]Department of Radiology, Medical University of Graz, Graz, Austria, [4]Siemens Healthcare, Erlangen, Germany

Purpose: The ability to determine the direction of the neuronal fibers in-vivo rendered diffusion MRI to one of the most widely utilized imaging modalities in current neuroscience research. *Connectomics* of the human brain has recently gained increased interest but the underlying mechanism for restricted diffusivity is only partly understood. So far, effects of tissue compartmentalization, composition and geometric factors of myelinated fibers were mainly investigated using formalin-fixed tissue with relatively long MRI acquisition times [1]. However, formalin fixation dehumidifies brain tissue and consequently reduces the freedom of motion of water protons, yielding low SNR and severely impacting the quality of tractography [2]. *Thus, the aim of this study was to investigate the feasibility of high-resolution diffusion MRI in the unfixed post mortem human brain as basis for validating diffusion-derived measures with histology.*

Methods: Twenty-one deceased subjects (age at death: 42-92 years) underwent in situ MRI at 3T (Magnetom Trio, A Tim system, Siemens Healthcare, 12 channel phased-array head coil) directly after death (post mortem interval < 72 hours). MRI included a single-shot DTI sequence (TR/TE=7.3s/106ms, 12 diffusion directions, resolution 2x2x3mm³, 50 slices, 8 averages, TA=13min). In four cases, high resolution diffusion MRI [3] was additionally obtained by a readout-segmented EPI sequence (TR/TE=10s/94ms, 12 diffusion sensitizing directions, resolution=1x1x3mm³, 50 slices, 2 averages, TA=57min). According to the lower tissue temperature the b-value was adjusted between 1500 and 2000 s/mm². Diffusion sequences covered the entire brain – exceptional sequences with resolutions of 0.5x0.5x3mm³ and 0.5x0.5x1mm³. Acquired DTI data were processed with FSL and by automated tractography using TRACULA [4]. After MRI, brains were extracted and fixed in 4% neutral buffered formalin for at least 3 weeks. Following fixation, tissue specimens with a slice thickness of 1 to 5 mm were taken from pre-specified regions of two subjects and optically cleared using benzyl alcohol-benzyl benzoate (BABB) to investigate possible application with higher-harmonic generation imaging [5].

Results: Figure 1A shows a conventional diffusivity image (trace-weighted), while 1B and 1C show higher-resolution images obtained with the readout-segmented EPI sequence. Higher resolution allowed a more detailed delineation of anatomical structures as shown in Figure 1D. We observed a significantly reduced mean diffusivity when compared to in vivo measurements – depending on the investigated region at least by a factor of 2. Hypo-intense basal ganglia proved to be a shine-through effect of iron induced T2 shortening. Concerning tissue preparation, we found that optically-cleared tissue samples up to a thickness of 3 mm demonstrated sufficient transparency for subsequent microscopy or optical tomography (Figure 1E and 1F).

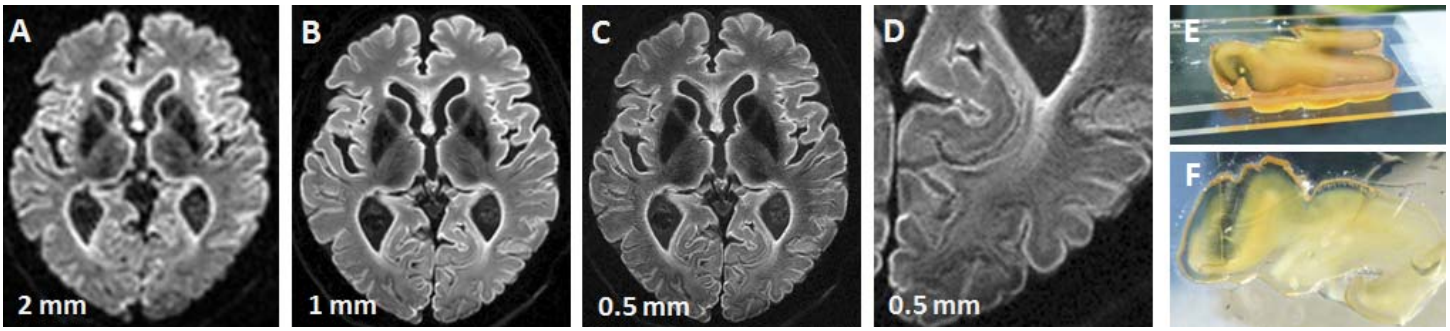

Figure 1: Post mortem trace-weighted diffusivity maps with different image resolutions (A-C). Higher imaging resolution enables investigations of small anatomical structures (D). Optically-cleared 3 mm-thick tissue slice for microscopy (E-F).

Discussion and Conclusion: This study investigated the feasibility of post mortem diffusion MRI in situ. However, those measurements have reduced diffusivity and SNR in respect to in vivo, but the improvement in SNR and image resolution compared to DTI of the formalin fixed brain is remarkable [1,2]. Without clinical scan time restrictions, images can be acquired at spatial resolutions beyond conventional DTI, which is a prerequisite for resolving small structures and tissue compartments such as the cortex. Ongoing work focusses on systematically compensating for temperature effects on restricted diffusivity as well as on evaluating appropriate histological techniques. Complementary to microscopy of micrometer-thin sections, higher-harmonic generation imaging can assess several millimeters thick tissues. The ability to determine also the orientation of structures such as myelinated fibers three dimensionally renders higher-harmonic generation imaging a promising tool for the validation of diffusion MRI [6]. In concert with optical imaging, high-resolution post mortem diffusion MRI in situ allows further correlations of diffusion-derived measures, which is especially of interest in small white matter structures and regions with fiber crossings.

References: [1] McNab JA, 2009, Neuroimage, 46(3):775, [2] D'Arceuil H, 2007, Neuroimage, 36(1):64, [3] Porter DA, 2009, MRM, 62(2):468, [4] Yendiki A, 2011, Front Neuroinform,5:23, [5] Schriefl AJ, 2012, J R Soc Interface; 10(80):20120760, [6] Farrar MJ, 2011, Biophys J;100(5):1362.