Ultra-high field MRI and spatially resolved spectroscopy of organoids at 28.2 T

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Introduction: Organoids, 3D cell cultures mimicking organs, are an interesting platform to study disease mechanisms and have the potential to partially mitigate the need for animal testing. Currently, light microscopy methods and electron microscopy are predominantly used to study the structure of organoids [1]. Magnetic resonance imaging and spatially resolved spectroscopy can complement microscopy techniques and provide translatable insights for clinical imaging and diagnosis. Due to the small size of organoids <3mm, high signal-to-noise ratios (SNR) are needed to obtain high-resolution images or localized spectra from small volumes of interest. High SNR values can be obtained by using ultra-high field strengths, such as a 28.2 T NMR spectrometer equipped with a microimaging probe (Fig.1a). Previously, we have demonstrated the feasibility of high-resolution organoid MRI towards structural imaging, diffusion imaging and localized spectroscopy [2-3]. Here, we show that very high-resolution diffusion MRI experiments with isotropic voxel sizes below 20 µm are feasible and compare the metabolite profile using single-voxel spectroscopy with and without adiabatic pulses.

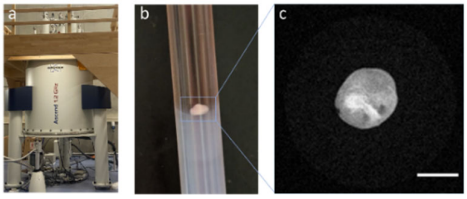

Fig. 1a. 28.2 T system. b. Organoid specimen atop a layer of agarose gel. c. 2D diffusion-weighted image (PSGE, slice thickness 0.1 mm) of a cross-section across the organoid. The scale bar represents 1 mm.

Methods: Organoid specimens: Cortical organoids (DIV55-110 [4]) were positioned in 3 and 5 mm tubes atop a layer of agar and suspended in medium (Fig 1b). MRI system: Experiments were performed on a 28.2 T magnet equipped with an Avance Neo console, a microimaging probe (Iprobe: coil diameter 5mm, 3T/m gradient set), ParaVision 360 V3.3, all Bruker, Ettlingen, Germany). The temperature was controlled at 310K. High-resolution diffusion MRI experiments: PGSE EPI 3D, voxel size 19.5x19.5x19.5µm^3, Δ 2.7ms, δ 0.9ms, 9 directions, repetition time 3.8s, echo time 9.4ms, 8 segments, 2 repetitions, b 0.1 and 1.0ms/µm^2, experiment time 21h 37min. Localized spectroscopy: sLASER/PRESS, TR 2s, TE 11.8ms(sLASER)/8.5ms(PRESS), voxel size (600µm)^3, water suppression - VAPOR, experiment time 34 min, working chemical shift 3 ppm.

Results and discussion: Diffusion-weighted images (Fig.1c) show variability in the internal structure of organoids. Using 3D PGSE EPI, an isotropic image resolution of (19.5 µm)^3 was obtained (Fig.2a). Mean diffusivity (MD) analysis confirmed spatial heterogeneity within the organoid (Fig.2a). Single-voxel spectroscopy was performed using sLASER to minimize the chemical shift displacement effect and was compared to PRESS (Fig.2b).

Conclusion: High-resolution imaging and localized spectroscopy of organoids are feasible. Further correlation with immunohistochemistry to determine structural heterogeneity and HR-MAS or MS to validate the metabolite composition is needed.

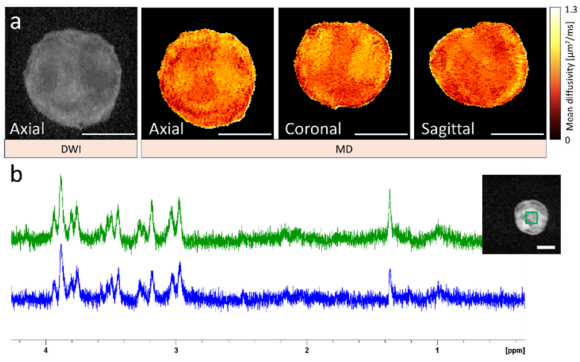

Fig. 2a. Diffusion-weighted image (DWI) and mean diffusivity (MD) maps of 3D PGSE EPI at a spatial resolution of (19.5µm)^3. For the MD analysis, a denoising protocol was used. b. sLASER (green) and PRESS (blue) spectra are shown. The voxel size and location are shown on a reference image. Scale bars represent 1 mm.

References: [1] Brémond Martin et al., Front Neurosci.(2021).[2] Krug et al., ESMRMB Proceedings (2024) [3] Nikolaeva et al. ICMRM (2025) [4] Yoon et al., Nat Methods (2019).