

Phase contrast MR microscopy of neuronal architecture of the living rat and mouse brain at 7T and 9.4T

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Introduction: The signal phase information recently brought significant advantages in revealing anatomical details of the human brain yielding a higher contrast to noise ratio between white matter (WM) and gray matter (GM) together with an excellent depiction of cortical morphology and substructure. While cortical cytoarchitecture has been extensively studied via histology [1], ex-vivo MRI [2] or MEMRI [3], the detection of cytoarchitectural boundaries non-invasively was demonstrated in this study by exploiting the MR signal phase. We demonstrate for the first time the potential of phase images in solving the challenge of visualizing *in vivo* the cortical lamination of the rat isocortex and mouse cerebellum. Phase contrast MR microscopy of the living rat and mouse brain was performed to quantitatively analyze the frequency shifts occurring within and between WM/GM and cerebellar cortex at two field strengths.

Material and Methods: Experiments were performed at 9.4 T and 7 T on a Bruker Biospec System. High-resolution 2D gradient echo flow compensated images were acquired with the parameters: *Mouse* ($n=5$), *7T*: $TR = 1937$ ms, $TE = 16$ ms, flip angle = 72 degrees, resolution $35 \times 35 \times 250 \mu\text{m}^3$, acquisition time 17 min 18 s; *cerebellum*: resolution $30 \times 30 \times 200 \mu\text{m}^3$, acquisition time 12 min 50 s. MR microscopy measurements were performed using a cryogenically-cooled quadrature-resonator (Bruker, Ettlingen, Germany). *Mouse* ($n=5$), *9.4T*: $TR = 400$ ms, $TE = 14$ ms, flip angle = 35 degrees, resolution $78 \times 78 \times 500 \mu\text{m}^3$, acquisition time 13 min 39 s; *Rat* ($n=5$), *9.4T*: $TR = 960$ ms, $TE = 20$ ms, flip angle = 51 degrees, resolution $97 \times 97 \times 500 \mu\text{m}^3$, acquisition time 13 min 3 s. Images were processed offline with custom-made software developed in Matlab. In order to reduce the noise, the Fourier reconstruction was replaced by the following regularization technique: $\arg \min \left(\|F \cdot x - y\|_2^2 - \lambda \|TV(x)\|_1 \right)$, where x represent the reconstructed image, y the acquired k -space data, F the Fourier transform, TV the total variation and λ the regularization parameter representing a data consistency tuning constant. This reconstruction was performed for each individual acquisition channel. In order to preserve the phase information, the complex images acquired with each individual channel were combined using the adaptive reconstruction method [4]. The background field inhomogeneity resulting from imperfect shimming was removed by: 1) low pass filter of the complex image (Gaussian filter equal to the image size); 2) complex division of the complex image by the low pass filtered image. Minimum intensity projection was performed over 2 slices for better visualizing the venous rat and mouse cerebral architecture. Frequency shift variations were computed as follows: *i*) 20 projections perpendicular to the surface of the cortical gray matter, running along the external capsule and from the corpus callosum to the hippocampus and *ii*) 20 projections along the WM, granular and molecular layer of the mouse cerebellar cortex. Statistical analysis was performed to quantify frequency shifts occurring between GM/body of the corpus callosum (bcc), GM/external capsule (ec), between the neighboring layers of the rat isocortex and mouse cerebellar cortex at 7T and 9.4T respectively. An alpha level of 0.05 was used for all statistical tests.

Results/Discussion: Excellent contrast between WM and GM was obtained in all cases proving that signal phase brings significant advantages over the corresponding signal magnitude. The contrast enhancement evidenced the brain microanatomy, identifying the neuronal cell layers in the rat isocortex and mouse cerebellum as illustrated in figure 2b and 1a. So far, *in vivo* delineation of mouse cortex cerebellar layers was obtained only after extensively long scan times [5]. In this study, a laminar pattern was clearly observed on the phase images that directly correspond to histology (Fig 1a). The WM appears as dark contrast (-6 ± 1.3 Hz) surrounded by the brighter granular layer (2.5 ± 0.2 Hz), followed by a dark rim (-1.65 ± 0.3 Hz) representing the Purkinje cell layer and the molecular layer having frequency shift of -0.3 ± 0.2 Hz. All along the rat isocortex, two stripes of negative phase values (-0.34 ± 0.11 , -0.23 ± 0.17 Hz respectively) surrounded by two inner brighter laminae (0.37 ± 0.2 , 0.33 ± 0.19 Hz respectively) are observed as indicated in figure 2b. The WM/GM frequency shift measured at 7T and 9.4T are illustrated in figure 2d. Minimum intensity projections performed on the high-pass filtered phase images improved the rat cerebral venous contrast without any administration of exogenous contrast agents as shown in Fig. 2c. As expected, the contrast on the phase images is significantly higher at 9.4 T compared to 7T (Fig 1b, 2d). All results displayed here were compared visually with the Fourier reconstructed data. The regularized technique showed effective noise reduction in the resulting phase images. This technique proved to be efficient to overcome the reduced contrast on the phase images at 7T enabling the delineation of structures located close to the interface between air/bone and sinuses that are generally canceled out in the mouse brain at 9.4 T.

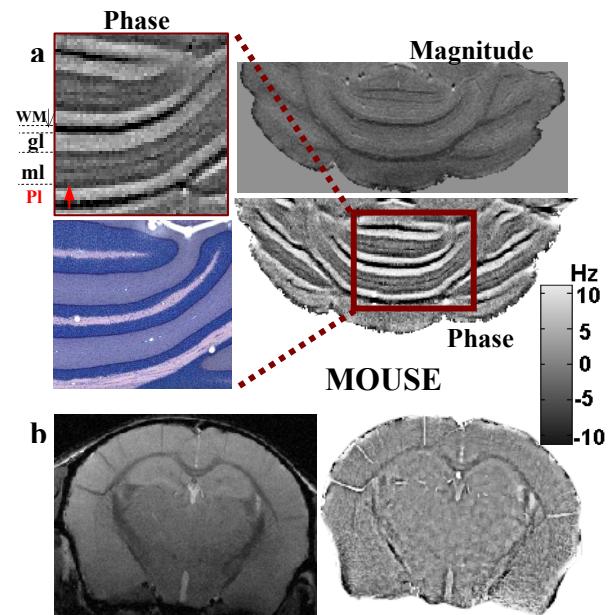


Figure 1: *a*) right, coronal view - Magnitude and high-pass filtered phase images depicting the cortical layers of the living mouse cerebellum (*ml* - molecular layer, *gl* - granular layer, *WM* - white matter, *Pl* - Purkinje cells). Left - Magnified view of the high-pass phase image in comparison to a corresponding Giemsa stain (www.brainmaps.org). *b*): Axial view of the mouse brain-Magnitude and phase image, 7T.

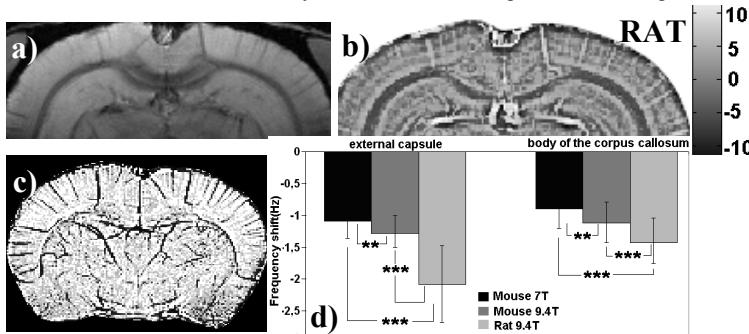


Figure 2 : Magnitude (*a*) and high-pass phase image (*b*) revealing five cortical layers of alternating brightness; 9.4T, axial slice. *c*) MR venography of the rat brain. *d*) Statistical analysis of the frequency shifts between WM/GM measured in the rat and mouse brain at two field strengths (** $p < 0.01$, *** $p < 0.001$).

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Conclusion: The present findings further enhance the potential of *in vivo* phase contrast MRI for yielding remarkable anatomical contrast of the living rodent brain at high field. The presented results make this method very suitable for application on animal models of demyelination to detect subtle changes induced by myelin loss, gliosis or axonal damage. This method is also adapted for checking the localization of demyelinated lesions in areas with high vascular density as observed in multiple sclerosis patients [5] and to differentiate between different types of plaques.

References: [1] Polleux P et al, J. Comp Neurol. 1997; 385:95-116. [2] Johnson AG et al, NeuroImage. 2007; 37(1):82-89. [3] Silva AC et al, J