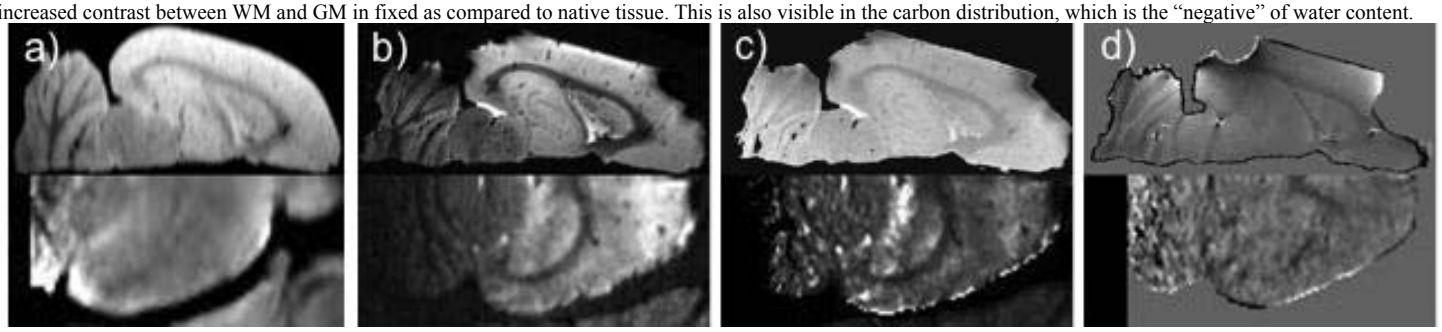
Changes in MR contrast after fixation: insight from M0, R2*, phase and element imaging by LA-ICP-MS

Ana-Maria Oros-Peusquens[1], Andreas Matusch[1], Johannes Lindemeyer[1], Johanna Sabine Becker[2], and N. Jon Shah[1]
[1]*INM-4, Research Centre Juelich, Juelich, Germany,* [2]*Central Division of Analytical Chemistry, Research Centre Juelich, Juelich, Germany*

Introduction Recently, several aspects of the MRI contrast have been compared for the first time with images showing the quantitative distribution of several elements (Fe, C and P) [1]. A poor correlation was found in the fixed brain of a young rat between susceptibility contrast and either the iron distribution or the carbon and phosphorus distributions, considered to describe the myelin concentration as represented by phospholipids. The conclusion of the study [1] was that the interpretation of phase contrast as being representative of either iron or myelin concentration needs to be made on a case-by case basis. Much effort is currently being invested in the study of iron, microstructure and myelin content *in vivo* using phase information and quantitative susceptibility mapping [2-5]. Clarifying the correlation between MR contrast and the presence of different elements seems more important than ever. The aim of this study was manifold: to investigate quantitative changes in the element distribution related to fixation; to correlate element distributions with MR contrast in a more mature rat brain with shorter fixation time than reported before [1]; to investigate whether specific contrast changes in MRI can be related to changes in the concentration/distribution of a few elements only.

Materials and methods Measurements were performed on a home-built 9.4T animal scanner comprising a 21cm horizontal bore Magnex magnet equipped with a 12cm ID, 600mT/m, 100μs rise time Agilent gradient coil and interfaced to a Siemens Tim Trio console running on the syngo™ platform. An RF birdcage coil was used for transmit and a quadrature surface coil (Rapid) was used for signal receive. All procedures were in accordance with the German animal protection legislation. A male Wistar rat (489g, 4 months old) was first scanned *in vivo* under isofluorane anesthesia. The brain was subsequently excised and divided: one half was shock-frozen in isopentane at -40C and kept for 16 days at -80C; the other was fixed in a 4% formalin solution for 8 days and scanned afterwards in Fomblin by high-resolution MRI. Sixteen days after excision, the two brain halves were cryocut in orientations as similar as possible, the slices were mounted on glass supports and analysed with Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) [6]. The MRI examinations *in vivo* as well as post mortem included a high-resolution 3D GRE scan (TR=50ms, 50deg, 4 echoes) for T2* and phase contrast and a multiple-echo long-TR (TR=10/8s, 32/20 echoes, 90deg) scan for M_0 mapping. The phase images corresponding to each echo were unwrapped and local phase contrast obtained by applying a composite filtering method [7]. This includes a low-order 3D polynomial fit, spherical harmonics decomposition, thresholding and fitting with a superposition of external dipole fields. A single-exponential fit to the signal intensity delivered T2* and M0 (signal intensity at TE=0) maps. LA-ICP-MS measurements for two-dimensional imaging of biological tissues were performed by line scanning ablation of thin cryocut tissue sections as described in [8]. The diameter of the focused laser beam during ablation was 100um and the gap between lines 60um. Quantification of analytical data was performed using matrix-matched home-made brain laboratory standards with known element concentration, measured together with the tissue samples under the same experimental conditions. Due to the multi-element capability of the novel LA-ICP-MS technique nearly all trace elements and minor elements were analyzed directly without any additional sample preparation in one measurement of the tissue section.

Results and Discussion Given the very high complexity of the problem we will limit ourselves in the following to a qualitative discussion. Fig. 1 shows M0 (a), long-TE magnitude (b), T2* (c) and phase (d) contrast obtained *in vivo* (lower part), to be compared with the same quantities obtained on the other brain half after fixation (upper part). In Fig. 2, some of the conspicuous changes (K, Mg; also Na, Cl, Mn, Zn, not shown) and constants (C, P, Fe, Cu) in the element distribution are shown. Since the two brain slices were measured by LA-ICP-MS with identical settings and nearly simultaneously, counting rates can be compared directly for all elements. This is beneficial for addressing changes in the concentration of elements which are not easily calibrated, such as carbon. Given the different constraints on measurement time, the resolution and SNR achieved in vivo were substantially lower than those on fixed tissue. It was therefore not possible to compare similar levels of anatomy visibility *in vivo* and post mortem at high resolution. However, even at low resolution, glimpses of cortical layers in some slices were possible for the in vivo data. In contrast, and despite the much higher resolution, SNR and overall contrast, the layer structure was not convincingly visible post mortem. A possible mechanism responsible for the visualisation of layers in phase contrast at 14.1T in vivo was assigned to K [9]. A factor 10 increase in the potassium level and a change in its distribution in tissue was noticed after fixation, most probably due to diffusion of the element from the fixative into the tissue. Perhaps these changes are responsible in a convoluted way for the lack of visibility of cortical layers in fixed tissue. However, the change in visibility of cortical layers is not to be assigned to a correlation between synaptic density shown by K and iron concentration [9], since the latter remains unchanged. The cerebellar T2* and phase contrast observed in fixed as well as fresh tissue are very high. We can infer that the measured changes in K concentration do not affect the cerebellar WM-GM and intracortical contrast. We notice here the visibility of three layers in cerebellar grey matter by combining the MR phase and magnitude information. The proton density (water content) shown in Fig. 1, has increased contrast between WM and GM in fixed as compared to native tissue. This is also visible in the carbon distribution, which is the "negative" of water content.



The mean carbon levels increase by 15%, likely reflecting an increase in density in the WM; the C content in cortical GM is nearly unchanged whereas it is increasing by nearly 30% regionally in the cerebral and cerebellar WM. The same effect was observed for phosphorus with slightly higher amplitude, probably due to the buffer solution. We stress that these numbers are only a rough estimate of the effect, since the two slices do not exactly reflect identical anatomy and some fluctuation in the counting rate cannot be excluded. Qualitatively these results agree with results of water mapping on fixed human brains, where higher WM-GM contrast and water content (magnetisation density) were observed than in vivo [10]. Last, very similar iron levels were observed in fixed and native tissue; a strikingly high concentration was observed in the cerebellum and the granular layer of the hippocampus, which have high phase contrast. A low concentration was seen in WM. The correlation of iron concentration with MRI T2* and phase contrast was found to be qualitatively better than reported before [1] and unchanged between native and fixed tissue. A plausible cause for this correlation is the increase in Fe concentration with age (4 months vs 1 month). In conclusion, the combination of MR contrast and element analysis allows one to investigate the tissue microsctructure on its most fundamental level.

References: ; [1] A.M. Oros-Peusquens, Proc. ISMRM 2011, 122; [2] Duyn J et al. PNAS 2006; [3] JH Lee PNAS 2009; [4] He and Yablonskyi PNAS 2009; [5] Li et al., NeuroImage, in press; [6] Becker JS, textbook.; [7] Lindemeyer et al., submitted to present ISMRM; [8] JS Becker et al. 2005, Anal At Spectrom 20, 912.; [9] J Marques et al., NeuroImage 46, 2009.; [10] A.M. Oros-Peusquens et al., Proc. ISMRM 2009, 1549.