Optimization of sample preparation for MRI of formaldehyde-fixed brains
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### Context: MRI of post-mortem samples

Post-mortem MR imaging is useful for several applications:
- acquiring high quality anatomical reference images thanks to long scanning time;
- comparing MR images with gold standard histological imaging;
- studying autopsies samples;
- testing sequences on an anatomically realistic phantom.

However, post-mortem tissue needs to be fixed in order to prevent its degradation. **Fixation modifies the properties of the tissue (relaxivity, diffusion)** by inducing chemical changes [Tovi and Ericsson, 1992].

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### Purpose and objective

The change of tissue properties affects MR imaging: in particular, $T_2$ is decreased by fixation, which is detrimental to SNR.

Understanding the kinetics of the fixation and associated tissue changes is required to improve the preparation of tissue samples for MR imaging. Therefore, this study measures the evolution of several parameters relevant to MR imaging during post-fixation in formaldehyde over a period of months.

Moreover, it has been shown that soaking a fixed tissue sample in saline solution prior to imaging can partially restore the $T_2$ of the tissue and be beneficial to the SNR of acquired MR data. [D’Arceuil et al, 2007][Shepherd et al, 2009]

Therefore, this study also measures the evolution of the same parameters during subsequent soaking in saline solution.

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### Overall design of the study

Two healthy eves (adult two-year-old female sheep, cow's aries) were used, in accordance with local animal regulation (authorisation ATJ981 of the French Ministry of Agriculture).

The following procedure was used for each animal:
- euthanasia using massive injection of barbiturate;
- immediate perfusion of the head with 4 L PFA at 4°C to prevent early tissue degradation;
- brain extraction;
- immersion of the brain in PFA.

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### Imaging and analysis methods

**High-resolution anatomical image**

During each session, an anatomical image was acquired for registration using a 3D turbo spin echo sequence with variable flip angle, at 0.3 mm isotropic resolution (SPACE, TR = 6000 ms, TE = 275 ms, GRAPPA 3, turbo factor 164, echo train length 654 ms, bandwidth 250 Hz/Px, acquisition time ≈ 150 s).

In addition, one session was dedicated to acquiring an image at 0.3 mm isotropic resolution for anatomical reference, using a 3D turbo spin echo sequence with variable flip angle (SPACE, TR = 4000 ms, TE = 275 ms, turbo factor 164, echo train length 951 ms, bandwidth 183 Hz/Px, acquisition time 1 h 24 min).

The longitudinal relaxation time $T_1$ was mapped using variable flip angle–actual flip angle imaging (VAPF) [Harutyunyan et al, 2012]. The variable flip angle acquisition used a prepared-steady-state free precession sequence (FSPGR, with 1 mm isotropic resolution, TR = 3 s, TE = 14 ms, FA = 7°, PA = 45°, GRAPPA 1, 3rd June 2015, ISMRM, Toronto).

The effective transverse relaxation time $T_2$ was measured using the M0-Andersen method [Hurley et al, 2012].

**Diffusion imaging**

Diffusion-weighted images were acquired using echo planar imaging (EPI) with 1.7 mm isotropic resolution, 55 linearly spaced TE values between 10 ms and 90 ms, 50 flip angles, 1560 Hz/Px, acquisition time 11 min 45 s.

The signal decay across echoes was fitted with a least-squares regression of a single exponential decay using a Levenberg-Marquardt algorithm implemented in PTK, an in-house software suite.

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### Results

Both brains were imaged repeatedly on a clinical 7 T MRI system over a period of 3 months during immersion in PFA.

After that, one brain was immersed in isotonic saline solution for washing, and imaged repeatedly for 3 months.

Initially two acquisitions per day were performed, then the frequency was decreased. For the duration of each acquisition session the brains were transferred in Fluorinert™, a fluorocarbon-based fluid that creates no signal and has a similar susceptibility to cerebrospinal fluid.

Maps of $T_1$, $T_2$, and diffusion were acquired during each session. Average values over white matter and grey matter were extracted in hand-delimited regions of interest (see details below).

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### Discussion and conclusion

- **Fixation in PFA is stable after 8 weeks**;
- **soaking in saline solution achieves maximum recovery after 3 weeks**;
- these values are expected to be longer for larger specimen such as human brains, which require longer penetration time.

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### References


400–404 (2007).

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**Table: T1, T2, and Diffusion Values**

<table>
<thead>
<tr>
<th>Time (not to scale)</th>
<th>T1 (ms)</th>
<th>T2 (ms)</th>
<th>Diffusion (mm²/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1200</td>
<td>1400</td>
<td>0.20</td>
</tr>
<tr>
<td>10 hours</td>
<td>1200</td>
<td>1400</td>
<td>0.20</td>
</tr>
<tr>
<td>12 weeks</td>
<td>1200</td>
<td>1400</td>
<td>0.20</td>
</tr>
</tbody>
</table>

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**Figure: Example axial view of relaxometry maps, 4 weeks into PFA**

Each brain is represented by a different curve. Error bars represent average standard error of the mean.