Routine Development for Artefact Correction and Information Extraction from Diffusion Weighted Echo Planar Images of Rats

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Rutinutveckling för Artefaktkorrigering och Informationsextrahering från Diffusionsviktade Eko-Plana bilder av Råtta

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Biologists and physicians study complex biologic phenomena in which they use advanced imaging methods. They acquire images containing a lot of information which must be extracted in a correct way. This requires computer skills and knowledge in image processing methods which they seldom have. To overcome the problem, this master thesis aimed to develop a routine for artefact correction and information extraction from images acquired in a research project at the Karolinska Institutet in Stockholm. By developing the routine, the thesis showed how software developed for images of human can be applied to images of rats. The routine handles formatting issues and artefact corrections, calculates diffusion metrics, and performs statistical tests on spatially aligned magnetic resonance images of rats acquired with diffusion weighted echo planar imaging. The routine was verified by analysing the images that it had processed and was considered to create reliable images. Future studies within the field should focus on developing atlases of rats and continue the work with identifying how software developed for images of human can be applied to images of rats.

**Keywords:** Image Processing, Rats, Routine, Artefact correction, Diffusion Weighted Imaging, Echo Planar Imaging
Sammanfattning


Nyckelord: Bildprocessning, Rätta, Rutin, Artefaktkorrigering, Diffusionsviktning, Eko-planartechnik
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List of Abbreviations

ADC - Apparent Diffusion Coefficient
DTI - Diffusion Tensor Imaging
DW - Diffusion Weighting
DWI - Diffusion Weighted Imaging
EC - Eddy Currents
EPI - Echo Planar Imaging
FA - Fractional Anisotropy
MD - Mean Diffusivity
MRI - Magnetic Resonance Imaging
NMR - Nuclear Magnetic Resonance
PE - Phase Encoding
RA - Relative Anisotropy
SE - Spin Echo
TE - Time to Echo
TR - Time to Repeat
Chapter 1

Introduction

The increasing interest to use images when studying complex biological phenomena creates a need of user friendly software to allow biologists to extract information from their images. The need has been pointed out and arise from biologists poor computer skills, which therefore need easily manoeuvrable tools [1]. Software developed for analysis of images of humans exist, but applying these on images of animals, on which a lot of research is performed, sometimes require modifications and hence more user knowledge. One example when problems may arise is in brain imaging, as the brains from different species have large anatomical differences. One method to image the brain is to use Magnetic Resonance Imaging (MRI), a non-invasive method that allows to image different anatomic phenomena [2]. One application of MRI is Diffusion Weighted Imaging (DWI) which gives information about the diffusion of water molecules in the imaged object. To extract information about the diffusion in acquired images, open software distributed free for research can be used. These does though require user knowledge, which is not always the case for biologists and physicians performing research.

One group of physicians with this need work in a research project at the Karolinska Institutet, Stockholm, Sweden. They aim at investigating the brain development in humans born with a single sided hearing loss due to atresia in one ear canal, which means that the ear canal is missing [3]. The disability hinders sound to reach the inner ear, and thereby transportation to the brain nuclei, and has a prevalence of 0.5 to 1 per 10,000 born children every year [4]. The children usually experience difficulties in their daily life, have poor school results and miss the possibility of stereo hearing and sound localization [5]. To enable a longitudinal investigation of the brain development in individuals with the disorder, a rat model has been established since longitudinal investigations in humans are time consuming and sometimes unethical. The rats underwent surgery during which one ear canal was closed and were thereafter imaged with Diffusion Weighted (DW) MRI [5]. Images of the rats with one closed ear canal, further on referred to as obstructed, are to be statistically compared with images of animals with two ear canals, further on called controls, with the aim to detect eventual differences in the groups’ brain development. The main goal for the research group is to compare brain tracts and nuclei belonging to the auditory system between the obstructed and control animals. To complete their project, they need an easily manoeuvrable tool to process and extract information from their acquired images.

Having an interest in connections between the sound processing nuclei in the brain, the group analyses the diffusion of water in the brain. When water molecules diffuse without restrictions, it is referred to as isotropic diffusion. Hindered by for example blood vessel walls and myelinated
Chapter 1 Introduction

Anisotropic diffusion occurs. Diffusion causes a signal loss which only can be measured along the axis of the applied DW gradient and hence DW gradients are always applied in several directions. The three dimensional diffusion can be expressed by a tensor using Diffusion Tensor Imaging (DTI). The tensors give information about the diffusion and by using their eigenvalues and eigenvectors, metrics can be calculated to extract information about the diffusion in imaged tissues. Anisotropic diffusion can be used to extract information about nerve tracts. As demyelination of nerve tracts decrease the appearance of anisotropic diffusion while isotropic diffusion increase, the degree of myelination can be assumed to affect the diffusion type in brain matter.

To image the diffusion of water in the body, DW images can be acquired with DW spin echo gradient schemes and a Echo Planar Imaging (EPI) read out. EPI uses long phase-encoding gradient trains after each 90°-excitation, during which spins affected by different magnetic fields have a long time to accumulate large phase differences. Local variations in the magnetic field occur due to the different magnetic properties of the imaged tissues. These properties arise from the proportionality constant describing how much a material is magnetized when put in an external magnetic field, called susceptibility. The local susceptibility variations induce a magnetic off-resonance field which causes artefacts that have large impact on images in areas including boundaries of tissue and air.

One method to correct for the artefacts caused by the variations in susceptibility is to use two images acquired with opposite Phase-Encoding (PE) directions and thereby model the off-resonance field by comparing the artefacts’ behaviour in the two images. Other methods acquire the off-resonance field during image acquisition and use it to remove the induced artefacts. Corrections with acquired off-resonance fields have been judged to not give satisfying results for high magnetic field strengths. A third method to correct susceptibility induced artefacts is by registering one EPI image without DW to a T2-weighted image. The registration gives a distortion field which thereafter is used to correct the artefacts in the DW EPI images.

DW EPI also suffers from eddy current induced artefacts which should be corrected for before further analysis. Recently, it was showed that estimation of the off-resonance field from two EPI images with different PE direction followed by a combined correction of eddy current and susceptibility induced artefacts resulted in better artefact reduction compared to a separately correction of the artefacts. The method used the off-resonance field when the eddy current induced artefacts were estimated and hence only required one re-sampling of the images which reduced number of potential errors.

One method to statistically test differences between groups of images is to perform voxel-wise statistical tests. Doing so requires spatial aligned images which can be performed manually or by using mathematical transformation algorithms.

The goal of this master thesis was to investigate how software developed for processing of images of human could be applied to images of rats. Furthermore, it aimed at developing a routine to be used for artefact correction and information extraction from DW EPI images of rats. The routine had to be easy to use for physicians, handle data formatting issues, correct for eddy current and susceptibility induced artefacts, calculate diffusion metrics to describe diffusion and statistically test eventual differences between two groups of data; controls and obstructed animals. A reader unfamiliar with the field of study is recommended to read the literature study in Appendix A before continue the reading of the main chapters of the thesis.
Chapter 2

Materials and Method

This chapter lists the software and material used in the developed routine and describes the routine development. Complementary details can be found in Appendix B and in the end of this chapter, tasks implemented in the routine are described by figure 2.3. The subtitles describing steps in the chapter are reused in the Result chapter.

2.1 Materials

The existing software and toolboxes used for the routine development are presented below.

- FSL 5.0.9 (Analysis Group, FMRIB, Oxford, UK)
- FSL view 3.2.0 (Analysis Group, FMRIB, Oxford, UK)
- ITK-SNAP 3.4.0 (Paul A. Yushekevich and Guido Gerig, University of Pennsylvania and University of Utah, USA)
- Matlab R2016a (The MathWorks Inc., Natick, MA, USA)
- SPM8 v6313 (Wellcome Trust Centre for Neuroimaging, London, UK), Matlab toolbox
- "Tools for NIfTI and ANALYZE image" 1.27 (Jimmy Shen, 2014), Matlab toolbox

The work was performed on a MacBook Pro with 2.8 GHZ Intel Core i7 processor, 16 GB RAM-memory and 1 TB flash memory.

DW images of rat brains acquired with spin echo EPI in a 9.4 Tesla Varian animal scanner were used when the routine was developed and verified. The images consisted of two data files acquired identically, but with opposite PE direction by a 180°-rotation of the scanner’s coordinate system around the slice-selective z-axis. DW gradients had been applied in 48 directions, each sixth without DW, b-value equal to zero. The images consisted of 128x128x25 pixels with voxel size 0.25x0.188x0.5 mm. Additionally, T2-weighted images of the animals with 256x256x40 pixels and voxel size 0.125x0.125x0.315 mm were available.
2.2 Artefact Correction

Images were initially displayed as five-dimensional as described in Appendix B. They were changed to be four-dimensional with DW in the fourth dimension, as the software used required that format. Changing the image dimensions was performed by editing the image header with the Nifti-toolbox. Images with different PE directions were displayed in different rotations, why one image from each animal was rotated 180° in the xy-plane by inverting the x- and y-axis using FSL.

Images were corrected for susceptibility and eddy current induced artefacts using the FSL tools topup and eddy. topup investigated the differences in artefacts in the images acquired without DW from the two acquisitions, 6 from each acquisition and 12 in total. Doing so, the off-resonance field was modelled by using cubic splines. Additionally, information about the PE directions and the assumption that the susceptibility induced artefacts were identical but with reversed behaviour were used in the modelling [13]. An even number of voxels in all image directions was required by topup and hence the bottom slice in the z-direction was duplicated. Except modelling the off-resonance field, topup removed eventual movement artefacts from the image acquisitions. After running topup, eddy used the directions of the DW gradients, the b-values and the off-resonance field to predict the image distortions induced by eddy currents [17]. Distortions caused by eddy currents and susceptibility variations were removed simultaneously by eddy. More detailed descriptions of the algorithms implemented in topup and eddy are given in Appendix B.

topup was run in accordance with the FSL recommendations, but length of splines, warpres, and degree of smoothing, fwhm, were reduced by a factor 10. Brain masks were created in ITK-SNAP using the half-automated Snake- and the manual Paintbrush modes. Masks were created from the first of the 12 motion corrected b0-images and the T2-weighted image of each animal.

After artefact corrections, the results were verified using the Pencil function in FSL view shown in figure 2.1. The brain edges before and after artefact correction were drawn and compared using the skull and the knowledge that bone have low intensity in MR images.

![Figure 2.1: Brain edge computation by drawing the pixels at the inner border of the skull.](image)
2.3 Tensor Reconstruction and Diffusion Metrics

The brain mask from the $b_0$-image, artefact corrected images, $b$-values and DW gradient vectors were used for diffusion tensor reconstruction in the FSL-tool DTIFIT. Using the eigenvalues belonging to the tensors’ eigenvectors, the diffusion metric fractional anisotropy was calculated which is detailed described in Appendix A. Additionally, the first eigenvalue was used as a measure of the axial diffusivity to describe the main direction of the diffusion.

2.4 Spatial Alignment

After artefact correction and tensor reconstruction, images from all animals were spatially aligned to enable voxel-wise statistical tests. The alignment was performed using a linear affine transformation implemented in the FSL-tool FLIRT. Before the alignment, the $T_2$-weighted images assumed to be free from geometrical artefacts, were multiplied with their corresponding brain masks. By thereafter registering the brains from the $T_2$-weighted images to the animal best positioned during the image acquisition, a 4x4 matrix describing the transformation was calculated for the five registrations. The matrices were reused to align the DW images describing fractional anisotropy. The similarity measure mutual information was used when determining the transformation matrix.

2.5 Statistical tests

The aligned images describing fractional anisotropy enabled voxel-wise statistical tests to be performed. The tests consisted of unpaired two-sample T-tests in SPM8, in which voxel-wise mean values of fractional anisotropy in the two groups were compared. Testing was performed using equation 2.1 and the tested hypothesis investigated if there were higher values of fractional anisotropy in the control group. The $p$-value under which the null hypothesis $H_0$ was to be rejected or not was 0.05.

\[
T = \frac{X - Y}{\sqrt{\frac{s_x^2}{n_x} + \frac{s_y^2}{n_y}}}
\]  

(2.1)

With:

- $T$: t-value of tested data, to compare with table value
- $X$: mean value obstructed
- $Y$: mean value controls
- $s_x$: variance obstructed
- $s_y$: variance controls
- $n_x$: number of obstructed animals
- $n_y$: number of control animals

Degree of myelination was also compared between the right and left side of the nuclei in the auditory system in images of obstructed animal 1. Regions of interest, the nuclei, were drawn on the FA image using ITK-SNAP and a paper brain atlas [18]. Mean values of FA in each nuclei including the standard deviation were computed and compared between nuclei in the right and left side of the brain.
Chapter 2  Materials and Method

(a) Intensity corrected image of obstructed animal 1. Target image for spatial alignment.
(b) Obstructed animal 2.
(c) Obstructed animal 3.
(d) Control animal 1.
(e) Control animal 2.
(f) Control animal 3.

Figure 2.2: $T_2$-weighted images from the six animals used for computation of transformation matrices, which were reused for spatial alignment of FA images. The top of the brain is missing in (c) and the image in (a) had been corrected for intensity variations arisen during the image acquisition. No image except (a) underwent intensity corrections.

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2.6 Routine development

The tasks presented above were implemented in a routine described in figure 2.3. The work flow was initially solved for one animal and thereafter were scripts and guidelines created. Verification of the routine was performed by processing and analysing images of the other five animals. The scripts included artefact corrections, diffusion tensor reconstructions, calculation of fractional anisotropy and spatial alignment of images. The guidelines described how to use graphical user interfaces for the statistical tests in SPM8.

Figure 2.3: Workflow for the developed routine. Brain masks and statistical tests were performed manually. The automated parts were implemented in scripts in Matlab.
Chapter 3

Results

This chapter presents the results achieved during the thesis work. The logic follows the Method chapter and verification of the routine is performed by presenting the images that it processed.

3.1 Artefact correction

Running \texttt{topup} resulted in an off-resonance field of the underlying variational magnetic field, caused by the susceptibility variations, shown in figure 3.1. A brain mask from a DW image of the same animal is shown in figure 3.2, which was used by \texttt{eddy}. Comparisons of image 3.3 and 3.4 show that brain edges were modified and how initially compressed areas with high signal got stretched, with the total intensity in the right-left direction preserved. Some geometrical deformations were though visible also after the corrections. The result from one animal is presented in figure 3.4, which shows how intensity was more evenly distributed over the brain after correction. Figure 3.3c and 3.4c show that the area between the brain edges of images from opposing PE directions was decreased by the artefact corrections. The symmetry of tissue within the brain was also increased by the corrections.

![Figure 3.1: Off-resonance field modelled by \texttt{topup} for obstructed animal 1. Bright areas correspond to a local increase in the magnetic field while dark areas correspond to a local decrease. Gray areas imply an unchanged magnetic field. Areas with a change in the magnetic field were most affected by artefacts.](image)
Chapter 3  Results

Figure 3.2: Brain mask created for an image from the first phase-encoding direction for obstructed animal 1, before running eddy.

Figure 3.3: Comparison of brain edges before artefact correction in the transversal plane, images from obstructed animal 1. The mask from (a) is red in (c) and the mask from (b) is blue in (c). The area between the edges in (c) indicates the mismatch caused by susceptibility variations.
3.2 Tensor Reconstruction and Diffusion Metrics

The diffusion metric fractional anisotropy and the directions of the first eigenvector for one obstructed and one control animal are presented in figure 3.5 and 3.6. No explicit difference was achieved by visually comparing the images from the obstructed and the control animal. By analysing brain edges and brain structures it is seen that the animals were differently positioned during the image acquisition and that the brains may be of different sizes.

3.3 Spatial Alignment

The results from the spatial alignment are presented in figure 3.7 and 3.8. The misalignment of obstructed animal 3 and control animal 1 seen in figure 3.7 was increased when the transformation matrices were reused on the images showing FA, which is seen in figure 3.8. Because of the misalignment, the two animals were excluded from further analysis.
Figure 3.5: Fractional Anisotropy (FA) and the first eigenvector of the diffusion tensors in obstructed animal 1. High FA values correspond to high intensities, which are present in areas of white brain matter. The red-green-blue color coding used in (d), (e) and (f) describes the main direction of diffusion referring to the coordinate system used during image acquisition and explained in Appendix A. Red corresponds to diffusion in the cranial-caudal direction, green to right-left and blue to ventral-dorsal. Anatomical areas of interest are the ones belonging to the auditory system, which are shown in figure 2 in Appendix A.
3.3 Spatial Alignment

Figure 3.6: Fractional anisotropy and main direction of the diffusion in control animal 3. High FA values correspond to high intensities, which are present in areas of white brain matter. The red-green-blue color coding used in (d), (e) and (f) describes the main direction of diffusion referring to the coordinate system used during image acquisition and explained in Appendix A. Red corresponds to diffusion in the cranial-caudal direction, green to right-left and blue to ventral-dorsal. Anatomical areas of interest are the ones belonging to the auditory system, which are shown in figure 2 in Appendix A.
Figure 3.7: Result of spatial alignment of $T_2$-weighted images presented in figure 2.2 using affine transformation. The images in (c) and (d) are tilted and in (c) the top of the brain is missing. Random intensity variations are visible in the five animals aligned to the target in (a).
3.3 Spatial Alignment

Figure 3.8: Result of spatial alignment of FA images by reusing the 4x4 affine matrices created by aligning the $T_2$-weighted images in figure 2.2. Areas of high FA are displayed in bright and low FA correspond to dark areas. The bright structures in (c) were stretched by the alignment. The image in (d) remained tilted, identified by the different bright patterns in the brain. The overall intensity in (a) is lower compared to the other images.
Chapter 3  
Results

3.4 Statistical tests

Results from the T-test are presented in figure 3.9. Red voxels does according to the test have a significant higher FA value in the control animals, for a p-value of 0.05. The brain edges and some areas in the brain were presented to have higher values of FA in the control group.

![Figure 3.9: Result from T-test.](image)

The nuclei used as regions of interest when comparing mean value of FA are shown in figure 3.10 and the colour coding is presented in figure 3.11. Analysing the mean values and the standard deviations in table 3.1, it was concluded that no difference in myelination appeared between the nuclei on the right and left side in obstructed animal 1. Additionally, no difference was found when all nuclei from the right side were compared with the nuclei from the left side as clusters. Lowest mean value was found in the auditory cortex and medial geniculate body 2, with FA average 0.15 and standard deviation 0.05 respectively 0.07. Highest mean value was found in the superior olivary complex 2 with average 0.28 and standard deviation 0.09.
Figure 3.10: The figure contains nuclei in the auditory system in rats. (a), (b), (c) show the same view as presented in figure 3.9a. No difference in mean value of FA was found between corresponding nuclei in the right and left side of the brain. Blue dashed lines in the images show the slices positions in the brain.
Chapter 3  Results

<table>
<thead>
<tr>
<th>Region</th>
<th>Mean value [FA]</th>
<th>Standard deviation [FA]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Auditory Cortex 1</td>
<td>0.17</td>
<td>0.07</td>
</tr>
<tr>
<td>Auditory Cortex 2</td>
<td>0.15</td>
<td>0.05</td>
</tr>
<tr>
<td>Cochlear Nucleus 1</td>
<td>0.19</td>
<td>0.08</td>
</tr>
<tr>
<td>Cochlear Nucleus 2</td>
<td>0.18</td>
<td>0.08</td>
</tr>
<tr>
<td>Inferior Colliculus 1</td>
<td>0.13</td>
<td>0.05</td>
</tr>
<tr>
<td>Inferior Colliculus 2</td>
<td>0.13</td>
<td>0.05</td>
</tr>
<tr>
<td>Lateral Lemniscous 1</td>
<td>0.24</td>
<td>0.11</td>
</tr>
<tr>
<td>Lateral Lemniscous 2</td>
<td>0.23</td>
<td>0.14</td>
</tr>
<tr>
<td>Medial Geniculate Body 1</td>
<td>0.16</td>
<td>0.07</td>
</tr>
<tr>
<td>Medial Geniculate Body 2</td>
<td>0.15</td>
<td>0.07</td>
</tr>
<tr>
<td>Superior Olivary Complex 1</td>
<td>0.27</td>
<td>0.09</td>
</tr>
<tr>
<td>Superior Olivary Complex 2</td>
<td>0.28</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Table 3.1: Table of mean values with standard deviation for the region of interest analysis of FA values. By comparing auditory cortex 1 and 2, it was concluded that no detectable differences in FA existed.

Figure 3.11: Colour coding used when identifying the nuclei in the auditory system shown in figure 3.10.
3.5 Routine development

The developed routine handles formatting issues, corrects for artefacts, reconstructs diffusion tensors, calculates diffusion metrics and performs statistical tests on two groups of data. Its workflow was presented in figure 2.3 and details can be found in Appendix C. Formatting, artefact corrections, tensor reconstruction, calculations of diffusion metrics and spatial alignment were implemented as scripts in Matlab. Brain mask creation in ITK-SNAP and statistical tests in SPM8 were performed manually.

Modelling the off-resonance field required 45 minutes per animal. Manual creation of brain masks performed after that required 40 minutes for each animal. Following, estimation of eddy current induced artefacts including correction of susceptibility and eddy current induced artefacts, plus tensor reconstruction and calculation of diffusion metrics took 1 hour and 20 minutes for each individual. Spatial alignment required 5 minutes per animal by the script, in which the transformation matrix was computed and reused to align FA images. Finally, set up and execution of the statistical test needed 20 minutes for the group of four images included in the test.
Chapter 4

Discussion

This chapter motivates the decisions for the developed routine and thereafter discuss how it was verified. Limitations in the verifications and the routine’s contributions to future research are presented. Proposals for further improvements of the routine are given as well as recommendations on future studies within the field and relevant investigations based on the statistical results achieved during the thesis. Finally the thesis work is evaluated.

4.1 Routine Development and Verification

The local behaviour of the artefacts caused by varying magnetic properties in tissues motivated spline functions for modelling the off-resonance field. Spline functions were also used by eddy as the corrections of the off-resonance field varied with the position. The similarity measure sum-of-squared-difference was used when susceptibility and eddy current induced artefacts were corrected, as its sensitivity for intensity variations gave valuable information about the correctness in the artefact correction.

When modelling the off-resonance field, lengths of the spline functions, warpres, was recommended to correspond to the images’ voxel dimensions in mm [19]. To avoid too expensive execution times and inspired by [20], the spline lengths were shortened by a factor 10 to achieve a transformation adapted to images of rats. The smoothing implemented in the algorithm used a Gaussian deconvolution kernel [19], defined by its bandwidth in mm at full-width-half-max, fwhm. This was also reduced by a factor 10, with the same motivation as for the spline lengths. warpres and fwhm were the only parameters expressed in mm by topup and hence the only parameters that must be changed when applying topup to images of rats. To improve the model of the off-resonance field, a total of 12 b0-images, 6 from each phase-encoding direction, were used in the modelling. That also enabled correction of eventual movements of the animal during the acquisition and increased the signal to noise ratio.

Drawings of brain edges was performed manually and hence user dependent. They were though created by only one user, which decreases the variability of the drawings. As the user was not blinded, the chance of subjective created drawings cannot be neglected.

Statistical T-tests were chosen because of their simplicity and the possibility to give meaningful results on small amounts of data. The results must be interpretable by physicians and the developed routine was supposed to be verified using available data. The p-value 0.05 was used as it is common practice and hence the results can be compared with other research projects.
Chapter 4  Discussion

T-tests require normal distributed data, which, despite the small data samples, was assumed to be true as biologic data as a rule is normal distributed. Additionally, T-tests have shown good performance on small samples when comparing mean values from two groups and are common for hypothesis testing of non-discrete data, which here was the case.

Brain masks were created manually during the thesis work, which is common practice in animal research. This is though time consuming and quality of the masks cannot be guaranteed because of the user dependence. Development of brain atlases of rats would streamline the brain extraction even if manual adjustments would remain necessary.

4.2 Contributions to field of study

The result of this thesis plays an important role in the research project at the Karolinska Institutet that it was a part of. The project studies brain development in individuals with one respectively two ear canals. The developed routine has an objective position to the results of the research project and contributes with reliable images. If the difference between the obstructed and control group can be strengthen and statistically proved, children may in the future receive individualized aids that they today do not. In the long perspective this contributes to better school results and reduced number of costly interventions later in life.

Except the research project for which the routine has been developed, other researchers are free to use it. It may need some modifications for images of other formats and dimensions, but the believe is that it at least will serve as a guidance in how artefacts can be reduced and information extracted from DW EPI images. With the knowledge that topup requires an even number of voxels in the x-, y- and z-directions, future research projects acquiring images that must be corrected for susceptibility induced artefacts will be able to exclude one step in the routine. By from the beginning acquiring images with even number of voxels in the 3 directions, duplication of edge slices can be avoided through good planning.

The statistical T-test indicated a decrease in FA values in the obstructed animals. One of the identified areas, positioned at the blue cross in figure 3.9a, is positioned close to the auditory system which is seen when comparing with figure 3.10. The area did according to the test have a higher value of FA in the control group, which implies a higher degree of myelination in the rats with two ear canals. No such change was detected when the right and left side of the brain was compared in an obstructed animal. The voxels expressed as having a higher FA in the control group should therefore be carefully handled. Unexpected red voxels along the brain edges and in the top of the brain, and the absence of right-left differences in the region of interest based analysis, suggest the result to be a possible coincidence.

Additional reasons for handling the statistical results with carefulness is the small number of images used in the computations. Small erroneous differences may have affected the result in two ways; actual biological changes may have been suppressed and random errors may have been amplified. Another issue with the image analysis is the limited voxel resolution. The areas of interest are small and eventual differences caused by obstructing one ear canal would probably not be large enough to affect one voxel. As a result, undetected anatomical changes may exist. Additionally, the spatial alignment may not have succeeded completely and hence may have affected the result. The intensity variations shown in figure 3.7, could have affected the intensity when aligning the FA images. The unexpected results from the test does though probably depend on a geometrical misalignment and not errors from intensity variations.

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4.3 Limitations

The major limitation in verifying the routine was the small number of available images. Visual inspection of images before and after artefact correction proves the reduction of artefacts by using the routine. The DTI calculations gave reliable diffusion maps which indicates the correctness of the developed routine, which also was verified by the overall agreement in the statistical test. Conclusions about biological changes could anyhow not be made because of the small amount of images and the randomness in the statistical test.

4.4 Evaluation

The routine corrects for artefacts and extracts information from images acquired with DW EPI. An improvement of the routine would be to enable spatial alignment of images that do not have the same content, as the linear transformation could not align images where parts of the brain were missing. Additionally, the routine should include mapping of brain tracts using tractography. By using the mask in figure 3.10, the nerve tracts transporting signals between the nuclei could be mapped using deterministic or probabilistic tractography methods in DSI Studio (Fang-Cheng Yeh, Department of Psychology Carnegie Mellon University, Pittsburgh, PA, USA). As 42 DW gradients were used during the image acquisition, a proposal for future studies is to use HARDI based tractography. It handles the problem of having several nerve tracts within one voxel and utilize more information than DTI.

The major goal of this thesis, to develop a routine that creates images free from artefacts, has been reached. It also reconstructs diffusion tensors in artefact free DW images and enables statistical tests of two groups of images. By applying the routine on DW images of obstructed and control animals, it has been indicated that obstructed animals may have a lower degree of myelination in areas close to the auditory system compared to the control group.
Chapter 5

Conclusion

This thesis has showed that software developed for images of humans can be applied to images of rats, but that it requires modifications and verifications by the user. By developing a routine it was showed that image processing and information extraction from images of rats can be streamlined, but that the lack of atlases is a reason for the limited degree of automation. Future studies should therefore focus on the creation of atlases of rats. They should also continue the work of identifying how software developed for processing of images of human must be changed before applied to images of rats. By applying the routine on the images it was developed for, it was indicated that rats with only one ear canal may have a lower degree of myelination around the nuclei in the auditory system compared to rats having two ear canals. No difference in myelination between the right and left side was detected when the nuclei in the auditory system in one obstructed rat were compared. The degree of myelination must though be further investigated before any conclusions can be made.
Chapter 6

References


Appendix A

Introduction

Literature Study

This chapter aims to give the reader the technical and anatomical background information necessary to understand the thesis. The main project which this thesis was a part of, searches for hypothetical anatomical changes in the brain as a result of single-sided hearing loss. The thesis evaluated the possibility of adopting existing software tools developed for human images to images of rats. By doing so, the rat auditory system was analysed using diffusion tensor reconstruction of acquired Diffusion Weighted (DW) images. In this appendix, an introduction of the rat auditory system is followed by a brief introduction to Nuclear Magnetic Resonance (NMR) and Magnetic Resonance Imaging (MRI). Thereafter, the specific imaging process Diffusion Weighted Imaging (DWI) followed by the analysis method Diffusion Tensor Imaging (DTI) is described. Finally, the most recent research within the subject is presented.
Appendix A

A.1 The Rat Auditory System

The outer and middle ear are responsible for collecting and transporting mechanical sound waves to the inner ear where it is translated to electrical signals [1]. From the inner ear, the cochlea, signals are transported via the auditory nerve to the cochlear nucleus [2]. Described in figure 1, the sound signals travel from the cochlear nucleus through the auditory pathways and the intermediate nuclei in subcortex where it is further processed [1]. From the cochlear nucleus, signals are transmitted to the superior olivary complex where the source of the sound is localized. Thereafter, signals are transmitted to the lateral lemniscus and inferior colliculus [3]. Seen in figure 1, the inferior colliculus also receives signals from cochlear nucleus and integrates sound information from the two previous nuclei [4]. The medial geniculate body is a relay centre positioned in the thalamus which receives signals from inferior colliculus and projects them to the auditory cortex, where the final sound processing occurs. Some signals are directly projected to the auditory cortex without passing through the medial geniculate body. An anatomical visualization of the nuclei involved in the auditory system and the auditory cortex is given in figure 2.

Figure 1: Schematic figure of the rat auditory system. Own work with inspiration from [5], [2] and [3].

Nerve cells, neurons, transfer electrical stimuli from the cell body, the soma, to the dendrites through myelin shielded axons [5]. Tractography is a method to map the axons, which in this thesis also are referred to as nerve tracts. Interneurons, cell bodies and dendrites constitute the nuclei described above. Interneurons are nerve cells with short tracts which improves or inhibits a signal when transferred between two tracts [3], [5].
Figure 2: Anatomical positions of the five nuclei included in the auditory system and the auditory cortex. Three-dimensional volumes are shown in b) and the coronal slices in c) are from marked areas in a). The red-yellow scale in c) visualizes degree of activation in nuclei when the animal was exposed by sound stimulations. Abbreviations: Cochlear Nucleus (CN), Superior Olivary Complex (SOC), Lateral Lemniscous (LL), Inferior Colliculus (IC), Medial Geniculate Body (MGB), Auditory Cortex (AC). Reprinted from [2] with permission from Elsevier.
Appendix A

A.2 Physics

This chapter aims to give an overview of underlying principles in MRI and DWI. The basics in MRI are followed by a more detailed presentation of DWI, which function as basis knowledge in the next chapter where tasks for processing of DW images are presented.

A.2.1 Magnetic Resonance Imaging

A traditional MRI pulse sequence consists of a slice selective $G_z$-gradient simultaneous with a $90^\circ$-pulse flipping spins to the transversal xy-plane [6]. In the xy-plane, spins dephase which yields a signal decrease from the plane [7]. In the meantime, spins slowly realign along the longitudinal $z$-direction to reach minimal energy state. The images used in this thesis were acquired with a Cartesian coordinate system as shown in figure 3. Examples of gradient schemes are Spin-Echo (SE) and Gradient-Echo (GE), which are described below. SE combined with Echo-Planar-Imaging (EPI) read out was used when the images used in this thesis were acquired, which is presented in figure 4.

![Figure 3: Cartesian Coordinate system. Images used in the thesis were acquired with read-out in the cranial-caudal direction along the x-axis, right-left PE along the y-axis and slice-selection as ventral-dorsal along the z-axis.](image)

SE sequences consist of one $90^\circ$-pulse flipping the spins within a certain slice to the xy-plane, followed by a $180^\circ$-pulse. The spins will defocus in the xy-plane and the measured signal therefore decreases after the $90^\circ$-pulse [8]. By applying a $180^\circ$-pulse for the chosen slice, the spins refocus and an echo is generated, which is measured after a certain time, Time-to-Echo (TE) [9]. After some additional waiting, Time-to-Repeat (TR), the experiment can be repeated by a new initializing $90^\circ$-pulse. The main difference between SE and GE sequences is that GE imaging does not use any $180^\circ$ refocusing pulses [8].

The acquired signals correspond to entries in the k-space, which is the Fourier transform of the image. Thus, for Cartesian acquisition of the k-space, the final image can be reconstructed by taking the inverse Fourier transform of the acquired k-space [7]. One method to acquire the k-space is EPI, which covers the whole k-space within one single $90^\circ$-pulse excitation using a long TR [9]. This is based on a combination of PE gradients and read-out gradients, the latter with varying signs as seen in figure 4.

A4
A.2.2 Diffusion Weighted Imaging

DWI is a method to image motion of water in the brain and is used for studying the motion of water molecules in the body. Diffusion of water molecules is random [10], and when diffusion is free and not restricted, it is referred to as isotropic diffusion. If the diffusion is hindered, anisotropic diffusion occurs. Membranes such as blood vessel walls and myelinated axons restrict the probability of molecules to pass through the walls, yielding anisotropic diffusion.

One of the methods for acquiring DW images is based on SE-EPI as illustrated in figure 4 [9]. It consists of a dephasing diffusion gradient after the slice selective 90°-pulse, followed by a 180°-pulse and a rephasing diffusion gradient. Doing so, spins that moved along the gradient direction during the pulse sequence gain measurable phase differences. The resulting decrease in signal intensity is caused by spins in water molecules that underwent diffusion, which in turn gives a signal attenuation.

Figure 4: A pulse sequence diagram demonstrating how images used in this thesis were acquired. Grey diffusion gradients are applied along each axis before and after the 180° refocusing pulse. LaTex figure created by modifying code from a macro-file created by M. J. White as a part of his PhD thesis, Copyright (c) 2004 Mark J White [11]. Pulse sequence design inspired by [12].
Expressing the three dimensional diffusion of water with a tensor allows for further calculations and information about observed tissues [13]. Mapping the anisotropic diffusion gives information about nerve tracts [14], which is described as tractography in the next chapter. Demyelination of nerve tracts in the brain in patients with Multiple Sclerosis decreases the appearance of anisotropic diffusion and increases the isotropic diffusion [15]. Degree of myelination may thus affect the diffusion type in brain matter.

**A.2.2.1 Measuring Diffusion**

Diffusion of water molecules attenuates the measured signal in NMR [16], where high degree of diffusion implies a large signal decrease and low diffusion gives less reduction of the signal [17]. Diffusion is detected by assigning spins with different frequencies using DW gradients, as described in figure 4. These are applied in many directions and their strengths are determined by their $b$-values, expressed in equation 1. It uses $\gamma$ as the gyromagnetic ratio, $\delta$ length of the diffusion gradient, $g$ strength of diffusion gradient and $\Delta$ the time between two gradients.

$$b = \gamma^2 \delta^2 g^2 \Delta$$

Because of the movement of some spins between two applied gradients, these will not be refocused by the second gradient and a signal loss arise. The signal loss gives a measure of the degree of diffusion, which is expressed by the fraction between $S$ and $S_0$ in equation 2. The equation uses $S_0$ as the initially measured MR-signal without DW gradients and $S$ as the signal measured with DW gradients. In addition to the $b$-values describing the strength of the gradients, the signal decrease depends on the diffusion coefficient $D$ in the tissue [18].

$$\frac{S}{S_0} = e^{-bD}$$

Equation 1 describes how large $b$-values, high diffusion weighting, require DW gradients with high amplitude $g$ and long duration $\delta$. Alternatively, long time $\Delta$ between the gradients can be used to enable long diffusion distances and thereby a large signal attenuation. For $b$-values equal to zero, no DW is used and pure anatomical images are acquired. As diffusion only can be measured along the axis of the DW gradient, DW gradients are applied in several directions to detect the three-dimensional phenomenon.

**A.2.2.2 Artefacts**

A fast readout and k-space filling in EPI makes the method less sensitive to patient movements [12], while variations in susceptibility have a major impact on the acquired data [19]. Artefacts arising from different susceptibility properties in tissues have large impact on the images in areas including boundaries of tissue and air, while they are smaller in boundaries of tissue and bone [20].

Susceptibility is a proportionality constant describing how much a material is magnetized when put in a magnetic field [21]. Different properties of magnetic susceptibility of tissues in the imaged object generate local differences in the magnetic field which result in locally varying spin frequencies, also called off-resonance fields. With long trains of PE gradients after the $90^\circ$-excitation in EPI, spins affected by the different magnetic fields will during this time accumulate large phase differences [16]. The impact of the off-resonance field depend on the relation to the PE-gradient [22] and the artefacts induced by it are common along the PE direction in EPI, due to the low bandwidht along the PE-axis [19]. Furthermore the PE-gradients in EPI must have a
small bandwidth to avoid coverage of more than one voxel at each sampling step. The artefacts caused by the off-resonance field are proportional to the main magnetic field $B_0$ and acquisition time, whilst inverse proportional to the gradient size [23]. The off-resonance field cause areas in the image to be stretched and compressed along the PE direction. Stretched areas are displayed with decreased signal intensity while compressed areas get improved signal intensity [24].

High amplitude of the diffusion gradients and short read-out times imply a fast switching of strong gradients in DWI [25]. As a result, eddy currents are induced in magnetic materials in the scanner yielding geometrical image artefacts. Eddy currents yield several artefacts in the image, such as compression, dilation, shifting and shearing. A global uniform field change yields an image shift in the PE direction [26]. Furthermore, scaling occurs in the PE direction when a erroneous gradient occurs along the PE axis. A change of the magnetic field in the slice-selective direction results in a reorientation of slices and image shifts. When a change in the gradient strength occurs along the read-out axis, a shearing effect may occur.
Appendix A

A.3 Image Processing

Processing of DW images can be performed in accordance with the model presented by Hasan et al., which includes correction of artefacts, DTI reconstruction, tractography and analysis with statistical tests [27]. This chapter presents the processing steps for DW images acquired with SE-EPI sequences.

A.3.1 Artefact corrections

Before tensor reconstruction and image analysis, the appearance of artefacts must be reduced. Some artefacts common in MRI are patient movements, chemical shifts, magnetic susceptibility and eddy currents [7]. As the rats were asleep while being imaged, the appearance of movement artefacts were minor. Chemical shifts cause artefacts around boundaries between water and fat. These can be prevented by fat saturation, which was used when the images used in the thesis were acquired. Susceptibility and eddy current induced artefacts had though to be corrected for during the thesis work and are presented below.

Eddy current induced artefacts can be corrected for by using a reference image free from eddy currents, showing how the non-distorted image should look like [28]. The reference image can be acquired during the scanning [26] or estimated [29]. Calculating phase shifts in all directions (x, y, z) yield an estimate of phase-shifts in the distorted image, which thereafter can be corrected.

One method to correct for susceptibility induced artefacts is the one implemented by Emberton et al., where two images with opposite PE directions were acquired [30]. By calculating the integral over the signal along the PE axis in both images, they could compare the total signal and find corresponding voxels in the two images. Repeating the integration followed by voxel matching for each line in the PE direction, the off-resonance field was estimated. A similar approach was used when Andersson et al. presented and implemented their method [31]. By modelling the displacement field between two acquired images having opposite PE directions, they estimated the off-resonance field using splines.

One task not directly related to artefacts, but is usually performed together with artefact corrections, is extraction of brain matter from other tissues in the image. Brain extraction is performed as brain analysis such as tractography only is interested in the brain matter. When available, a mask or atlas defining the brain edges can be used. Using an atlas or mask requires good correspondence to the studied object regarding age, gender, species, etc. [32]. Additionally, atlases should preferably be built on a large population which requires large amounts of data [33].

A.3.2 Diffusion Tensors

To describe the three dimensional diffusion phenomenon, DTI reconstructs the raw DWI images through second-order tensors, which can be visualized as ellipsoids seen in figure 5. These tensors consist of six unique values and are expressed with 3x3 matrices [34], as described in equation 3.

\[
D = \begin{pmatrix}
D_{xx} & D_{xy} & D_{xz} \\
D_{yx} & D_{yy} & D_{yz} \\
D_{zx} & D_{zy} & D_{zz}
\end{pmatrix}
\]  

The expression of a tensor as a matrix implies the possibility of applying matrix operations to the tensor [35]. As the tensor matrix in equation 3 is symmetric, it can be diagonalized to get
its eigenvalues $\lambda_1$, $\lambda_2$, $\lambda_3$ and corresponding eigenvectors $\vec{e}_1$, $\vec{e}_2$, $\vec{e}_3$ [36]. Shown in figure 5, the eigenvalues written in decreasing order determine the tensor’s direction, while the orthogonal eigenvectors define its orientation [37]. The tensor described in equation 3 and figure 5, has 6 unique values. DTI therefore require at least 6 different DW gradients during the image acquisition. A seventh acquisition without diffusion weighting gives $S_0$ and is always performed to have a reference image available. The diagonal elements in $D$ are obtained from pure x-, y- and z- gradient acquisitions $D_{xx}$, $D_{yy}$, $D_{zz}$, while the off-diagonal elements are obtained when gradients are combined in two orthogonal directions [37].

The difference between $S$ and $S_0$ in equation 2 determines the signal attenuation caused by diffusion. This does in turn determine the Apparent Diffusion Coefficient (ADC), which is calculated by extracting $D$ from the equation [27]. Diffusion is only sensed along the axis of the diffusion gradient [34] and applying a diffusion gradient along the x-axis, $G_x$, enables calculation of the ADC-constant in that direction.

Figure 5: A three dimensional tensor with eigenvalues labelled in decreasing order and corresponding orthogonal eigenvectors. The tensor takes the form of an sphere when isotropic diffusion occur and as an ellipsoid in anisotropic diffusion.

Using the eigenvalues and eigenvectors from $D$, measures can be determined to extract information about the diffusion in imaged tissues. Mean Diffusivity (MD) is a synonym to ADC and is the measure of diffusivity in tissue without regards to tensor orientations [14]. It describes the strength of the diffusion and is determined using equation 4, which uses the diagonal elements from the diffusion tensor $D$. Using the eigenvalues $\lambda_1$, $\lambda_2$, $\lambda_3$ to $D$ would be equivalent [27].

$$MD = \frac{D_{xx} + D_{yy} + D_{zz}}{3}$$ \hspace{1cm} (4)

To describe the diffusion with dimensionless indices, Basser et al. introduced the terms Fractional Anisotropy (FA) and Relative Anisotropy (RA). FA is calculated using equation 5 and measures the overall anisotropy of a tensor which can be used in tractography. Tissues with pure isotropic diffusion have FA values close to zero, while those with high degree of anisotropic diffusion have FA values close to one [38].

$$FA = \sqrt{\frac{3}{2}} \sqrt{\frac{(\lambda_1 - MD)^2 + (\lambda_2 - MD)^2 + (\lambda_3 - MD)^2}{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}}$$ \hspace{1cm} (5)
Appendix A

RA measures the relation between a tensors anisotropic and isotropic diffusion indices expressed in equation 6 [38]. The fraction determines a relation between the eigenvalues’ standard deviation and mean diffusivity. Tissues with anisotropic diffusion have RA values close to one while isotropic tissues have RA values close to zero.

\[
RA = \frac{1}{\sqrt{3}} \sqrt{\frac{(\lambda_1 - MD)^2 + (\lambda_1 - MD)^2 + (\lambda_2 - MD)^2}{MD}}
\]  

(6)

A.3.3 Tractography

Fibre tracking, tractography, can be performed with deterministic or probabilistic methods [39]. The main difference between the approaches is that probabilistic tractography takes uncertainty into account, which deterministic tractography does not.

Deterministic tractography uses a starting seed and determines the path for tracts projected from that seed [40]. Paths are determined by following the first eigenvector in the tensors leaving the seed point. At the end of a tensor, thresholds for angular changes and FA values decide if a path shall continue to a connecting eigenvector or terminate [41]. Smaller acceptance for angle in the connection and requirement of high FA values decrease the amount of identified tracts. Tractography does in general give more reliable results when larger amount of diffusion gradients are used during acquisition, but deterministic tractography has shown to yield satisfying results with only six gradient directions [42]. Other constraints for the reliability of deterministic tractography results are patient movement, artefacts and noise in the images. This implicates the importance of proper artefact correction and data treatment before tractography can be performed.

To decrease the impact from the limitations with artefacts in deterministic tractography, one can use probabilistic tractography which instead of calculating the most probable tract paths, also investigates the uncertainty in the paths [41]. In doing so, it repeats the calculations multiple times and constructs a probability map of the tracts based on degree of repetitions in which certain tracts were found [43]. Probabilistic tractography may loose some details, but has proved to be more robust than deterministic tractography [39]. Probabilistic tractography has more expensive computations compared to deterministic methods.

Examples of existing tractography methods are

- Fiber Assignment by Continuous Tracking, FACT, a DTI based method [44]
- Tensor Deflection, TEND, a DTI based method using the three eigenvectors instead of only using the first vector [45]
- Protrackx implemented in FSL, a probabilistic approach handling crossing fibres [46]
- High Angular Resolution Imaging, HARDI, commented below

A limitation in both deterministic and probabilistic tractography methods is the image resolution in MRI. DTI expresses each voxel as a diffusion tensor, but may in fact consist of several nerve tracts. This implicates that each tensor represents tracts with different directions and sometimes tracts with crossing paths. As a result, tracts that by defined thresholds should have terminated at a certain point may continue, and tracts that should have continued may terminate earlier [47]. The problem is common when imaging rats, because of the small brain and limited resolution.
Additionally, the auditory system consist of crossing fibres, and hence even a good resolution does not avoid the problem. One solution is to use High Angular Resolution Imaging (HARDI) based tractography instead of DTI, which tackles crossing fibres better. HARDI requires images acquired with preferably 50 DW gradients or more [48], [49]. The images used in this thesis were acquired with 42 DW gradients, and hence HARDI based tractography would not undoubtedly handle the crossing fibres in the images.

A.3.4 Statistics and Data Analysis

Data Analysis of DW images mainly consist of comparisons of images on group level where previous processing outcomes are compared between individuals [27]. This may be achieved by performing statistical tests on the constructed tracts or voxel-wise on the calculated diffusion tensors and metrics.

Voxel-wise statistical computations can be performed using a T-test, which compares the mean value between two dependent or independent groups of data [50]. A requirement for T-tests is the assumption of having normal distributed data and they offer three different tests of the null hypothesis $H_0$: Group 1 = Group 2, Group 1 $\geq$ Group 2 and Group 1 $<$ Group 2.

Smith et al. developed a method called tract based spatial statistics, in which they combined voxel-wise and tract based information. This was performed by calculating FA values for all individuals in their group and choosing one of the images that was most typical for their group [51]. They thereafter used a non-linear registration algorithm to align all individuals’ FA images with the typical image. With the images aligned, a mean image of the data was created. In the mean image, thresholds for FA values were used to amplify the brain tracts’ occurrence. After an additional projection of each data set to the amplified FA mean image, voxel-wise statistical tests were performed and gave a measure of similarities and dissimilarities in the population. They implemented the method as a part of FSL.
Appendix A

A.4 Previous Research

Several software packages useful in analysis of DW images exist and are available for free download. Some of these are the Free Software Library (FSL, http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/) [52], Camino (http://camino.cs.ucl.ac.uk/index.php) [53], DSI-studio (http://dsi-studio.labsolver.org) and ExploreDTI (http://www.exploredti.com) [54]. FSL includes a package called FDT which contains tools for processing and analysis of DW images [52]. FSL also includes tools to correct for artefacts caused by eddy currents [28] and susceptibility variations [31]. Camino is written in Java and was developed as an analysis tool for DTI images offering, among others, tools for deterministic and probabilistic tractography [53]. DSI-studio enables image analysis including tools such as deterministic tractography [55] and comparisons within populations [56]. Developed in Matlab, ExploreDTI includes modules for eddy current correction, processing and post-processing of data [54].

Budin et al. developed a processing routine for rodent data which handles image registration, skull-stripping and several computations including analysing statistics [57]. They concluded that their routine can be used for tractography, but before that it, would need modifications.

Gyengesi et al. evaluated different tractography methods and pointed out the importance of correctly calculated eigenvalues when mapping neural tracts [58]. Errors in the Diffusion tensor and its eigenvalues could lead to erroneous tractography results.

Previous studies of DWI processing in rats have mostly focused on tractography or skull-stripping using atlases. Detailed studies concerning artefact corrections of rat DW images have not been found. One solution proposed by Wu et al. used distortion free $T_2$ images as target and registered EPI acquired images without diffusion weighting to them [19]. They used mutual information as similarity measure and deformable transformation. The deformation field was expressed with splines of degree three and they evaluated their method as successful.
A. References


A. References


A. References


A. References


Appendix B

Complementary description of the materials used in the thesis.

Figure 1: Example of one four dimensional file containing one acquisition from one rat. The 42 DW images were acquired in combination with 6 images without DW, using the same SE-EPI gradient scheme. The 6 images from each acquisition, in total 12 per rat, were mainly used for artefact correction.

The scanner, a Varian 9.4 Tesla animal scanner, saved the DW images as five dimensional with the order $[x \ y \ z \ time \ DW]$ expressed as $[128 \ 128 \ 25 \ 1 \ 48]$. The software used in the developed routine read DW images as four-dimensional with $[x \ y \ z \ DW]$. Based on the software requirements, the dimensions and header of the acquired images were changed using the Nifti-toolbox in Matlab, in which the fourth dimension was removed and replaced by the fifth dimension $[128 \ 128 \ 25 \ 48]$. Images were thereafter read as four-dimensional by the software in the routine.

The susceptibility variations within an object depend on the tissue and hence are constant during the acquisitions. The susceptibility induced local variations in the magnetic field can therefore be assumed to remain the same when two images of one object are acquired identically. Acquiring two images with opposite phase-encoding directions yield images with identical but reversed susceptibility artefacts. By using these differences, the module topup in FSL models the local variations in the magnetic field caused by susceptibility variations [?]. The modelled off-resonance field can thereafter be used by other tools to correct the susceptibility induced artefacts, which in the thesis was performed with eddy.
Appendix B

Using the estimated off-resonance field in combination with phase encoding directions, time between centre of first and last echo, diffusion gradient vectors and strength of diffusion gradients, eddy implemented in FSL can be used to correct for susceptibility and eddy current induced artefacts [?]. Its algorithm models the eddy current field and predicts an image free from artefacts. The modelled eddy current field is added to the artefact free image to create an estimated distorted image. This image is compared to the acquired image using sum-of-squared-difference and in a iterative process is the estimated eddy current field refined a pre-defined number of times to reach an estimated image free from artefacts.
Details of the developed routine

The script below takes two DW images acquired with opposite phase-encoding directions saved as `image001.nii` and `image002.nii` as input. The script has to be run in the same directory as the images are saved. Before the script can be run, reset both images’ headers using the Reset-function in SPM8. To do so, first type the following lines in Matlab.

```matlab
addpath /Applications/MATLAB_R2016a.app/spm8
spm fmri

SPM8 will open, press on Display in the Menu window. Chose one image to display. The chosen image will be shown in the Graphics window. Press Reset... in the Graphics window, and chose the image files for which the headers are to be reset. Notice the change of the parameters in the matrix Dir Cos, which displays how the images are oriented according to the header. Now run the script below.

%% Load Nifti-toolbox and FSL. Manually chose the directory where images are stored.
addpath /Applications/MATLAB_R2016a.app/Nifti
setenv('FSLDIR','/usr/local/fsl');
setenv('FSLOUTPUTTYPE','NIFTI'); % Save images as .nii
fprintf('Enviroments loaded 
')

%% Change header format on both images
a1=load_nii('image001.nii');
b1=reshape(a1.img,[128 128 25 48]);
a1.hdr.dime.dim=[4 128 128 25 48 1 1 1];
a1.original.hdr.dime.dim=[4 128 128 25 48 1 1 1];
a1.img=b1;
save_nii(a1,'im001.nii'); % Save the new image as im001.nii
fprintf('Header 1 changed 
')

a2=load_nii('image002.nii');
b2=reshape(a2.img,[128 128 25 48]);
a2.hdr.dime.dim=[4 128 128 25 48 1 1 1];
a2.original.hdr.dime.dim=[4 128 128 25 48 1 1 1];
a2.img=b2;
save_nii(a2,'im002.nii'); % Save the new image as im002.nii
fprintf('Header 2 changed 
')

%% Extract b0-images
```

C1
Appendix C

% Rotate first image to fit the second
rot = '/usr/local/fsl/bin/fslswapdim im001.nii -x -y z im001rot.nii';
system(rot);
fprintf('Image 1 rotated 
')

% Create images with even number of slices (z-direction)
slices1 = '/usr/local/fsl/bin/fslroi im001rot im001rotbottom 0 128 0 128 0 1';
% Create copy of the bottom slice
system(slices1);
merge1 = '/usr/local/fsl/bin/fslmerge -z im001rot26 im001rotbottom im001rot';
% Add the copied slice to the image
system(merge1);
slices2 = '/usr/local/fsl/bin/fslroi im002 im002bottom 0 128 0 128 0 1';
% Create copy of the bottom slice
system(slices2);
merge2 = '/usr/local/fsl/bin/fslmerge -z im00226 im002bottom im002';
% Add the copied slice to the image
system(merge2);
fprintf('Even number of slices created 
')

% Create b0-images from both scan directions, also create folders and
% move time slices to them
split1 = '/usr/local/fsl/bin/fslsplit im001rot26 im001rot_ -t'; % Split the 4D
series to 48 3D images
system(split1);
b01 = '/usr/local/fsl/bin/fslmerge -t im001rotb0 im001rot_0000 im001rot_0008
im001rot_0016 im001rot_0024 im001rot_0032 im001rot_0040'; % Merge the b0-images and save as im001rotb0.nii
system(b01);

split2 = '/usr/local/fsl/bin/fslsplit im00226 im002_ -t';
system(split2);
b02 = '/usr/local/fsl/bin/fslmerge -t im002b0 im002_0000 im002_0008 im002_0016
im002_0024 im002_0032 im002_0040'; % Merge the b0-images and save as im0020.nii
system(b02);

b0 = '/usr/local/fsl/bin/fslmerge -t b0 im001rotb0 im002b0'; % Create a file
with 12 b0-images
system(b0);
fprintf('B0 images extracted and pure b0 images created 
')

mkdir('TimeSlicesIm001rot26'); % Move 3D slices to created directories
movefile('im001rot_*','TimeSlicesIm001rot26');
mkdir('TimeSlicesIm00226');
movefile('im002_*','TimeSlicesIm00226');

%% Run topup
topup = '/usr/local/fsl/bin/topup --imain=b0.nii --datain=acqparams.txt
--out=splinefield --fout=offresfield --iout=unwarped --config=b02b0.cnf --fwhm=0.8
Appendix C

--warpres=1 -v'; % Save modelled off-resonance field as offresfield.nii and splinefield.nii, the latter
system(topup)
fprintf('Topup finished \n')

Create a brain mask of unw0000 in ITK-SNAP and thereafter perform artefact correction using the script below. Save the brain mask as unw000Mask.nii

%% Run eddy to perform artefact correction
imain = ’/usr/local/fsl/bin/fslmerge -t imainEddy im001rot26 im00226’; % Merge the two 4D uncorrected DW images to one image file and use as input to eddy
system(imain);
fprintf(’Image created\n’);
eddy = ’/usr/local/fsl/bin/eddy --imain=imainEddy --mask=unw00Mask --index=indices.txt --acqp=acqparams12.txt --bvecs=all_bvecs.txt --bvals=all_bvals.txt --topup=splinefield --out=eddy -v’; % Use the off-resonance field from topup saved as splines
system(eddy)
fprintf(’Finished\n’);

% Extract one b0-image from eddy
im = ’/usr/local/fsl/bin/fslroi eddy eddy00 0 1’;
system(im);
fprintf(’b0-image from eddy extracted.\n’);

Verify the artefact correction by using the Create Mask and Pencil functions in FSL view. Choose one slice showing the phase-encoding direction and draw a mask along the brains’ edges. Draw brain edges for im001rot26, im00226, eddy00 and eddy18. Open the masks from im001rot26 and im00226 simultaneously, followed by eddy00 and eddy18. Compare the area between the masks before and after artefact correction and analyse geometrical changes inside the brain.

Calculate metrics describing the diffusion by using DTIFIT in the script below.

%% DTIFIT
dti = ’/usr/local/fsl/bin/dtifit --data=eddy.nii --out=FDT_male_60_2_control --mask=unw00Mask.nii --bvecs=all_bvecs.txt --bvals=all_bvals.txt -V’;
system(dti);
fprintf(’DTIFIT finished \n’);

%% Radial Diffusivity can be calculated if it is of interest
add = ’/usr/local/fsl/bin/fslmaths FDT_4plus40_L2 -add FDT_4plus40_L3 FDT_4plus40_L2L3’;
system(add);
div = ’/usr/local/fsl/bin/fslmaths FDT_4plus40_L2L3 -div 2 FDT_4plus40_RA’;
system(div);
fprintf(’RA image created \n’);

Spatially align images from all animals to enable the statistical test described in the following. Create a brain mask for the $T_2$-weighted images from each animal, and use the the brains to calculate transformation matrices. Reuse the matrices to align FA images to one chosen target image.

C3
Appendix C

%% Align T2

t2 = '/usr/local/fsl/bin/flirt -cost mutualinfo -in T2image -ref T2targetImage -out alignedT2 -omat transfMatrix -v';
system(t2);

%% Align FA

% Uses the matrix transfMatrix from the alignment above
fa = '/usr/local/fsl/bin/flirt -cost mutualinfo -applyxfm -init transfMatrix -in FAimage -ref FAtargetImage -out alignedFA -v';
system(fa);

% Repeat for all individuals to be aligned to the target image.

Open SPM8. In the Menu window, press Specify 2nd-level. A Batch Editor is opened. Decide a Directory in which results from the tests shall be saved. Chose Two-sample T-test as test design and assign images from the control and obstructed animals to group one and two. Save the Batch and run it. The Graphics window will now show the design of the test. Go to the Menu window and press estimate. Select the created Batch file as design matrix and press run. The program now loads data from the image files and prepares for upcoming tests. Go to the Menu window and press Results. Load the Batch file and the window SPM Contrast Manager will open. Chose t-contrast and thereafter Define new contrast.... Now set up the hypothesis to be tested voxel-wise and thereafter press Done. Two hypotheses can be tested: 1 -1 implies testing if Group1 > Group2 ? -1 1 implies testing if Group1 < Group2 ?

The window Stats: Results will now open. Chose none for apply masking, set a title for the comparison, chose p-value by pressing none and typing in the text box. Decide a threshold for how many voxels with a statistically significant result must be clustered to be presented in the resulting figure. Press Display sections and chose one image on which the statistical results will be presented. Coloured voxels correspond to clusters larger than the threshold value containing statistically significant results of the tested hypothesis.
Appendix D

Additional images created in the thesis work.

Figure 2: Comparison of brain edges before artefact correction. The mask from (a) is red in (c) and the mask from (b) is blue in (c). Control animal 2.
**Appendix D**

Figure 3: Masks after artefact corrections. The mask in (a) is red in (c) and the mask from (b) is blue in (c). Control animal 2.

Figure 4: Comparison of brain edges before artefact correction. The mask from (a) is red in (c) and the mask from (b) is blue in (c). Control animal 3.
Appendix D

Figure 5: Masks after artefact corrections. The mask in (a) is red in (c) and and the mask from (b) is blue in (c). Control animal 3.

Figure 6: Comparison of brain edges before artefact correction. The mask from (a) is red in (c) and the mask from (b) is blue in (c). Control animal 3.
Appendix D

Figure 7: Masks after artefact corrections. The mask in (a) is red in (c) and the mask from (b) is blue in (c). Control animal 3.