**Overview of the pipeline of the image processing tasks used in our study**

**Step 1. Manual masking of brain tissues on the first image frame of the DTI scan.** We used Slicer 3D software to manually draw the outlines of the brain on the first non-B0 image frame.

**Step 2. Normalization of intensity profile along the Z-axis.** Motion causes spin history artefacts which may affect each imaging slice differently, due to the echo-planar imaging technique, causing large jumps in image intensities along the off-plane axis (in our case, the Z axis profile). It was therefore necessary to correct for inter-slice image intensity differences. Using the mask from Step 1, we extracted the brain tissue from the images, and performed a “rough” realignment of image frames to the first non-B0 volume with the MCFLIRT algorithm from the FSL tool. Although this motion correction step is suboptimal compared to that of the Step 4, the purpose was to generate a temporal average of the diffusion images for the intensity normalization (=mean DWI). Each slice along the Z axis of each image frame was then separately normalized so that the mean image intensity value equals that of the mean DWI. For the following processing steps, this normalized image was used.

**Step 3. Automatic propagation of mask for all the DTI frames.** In order to ensure that the brain remains within the mask in all DTI frames, it is necessary to refine the mask on every frame. Instead of drawing the mask manually, we used a random-forest based image classifier, implemented in the Convert3D software package, as part of ITKSNap (http://www.itksnap.org/) to achieve this goal. The first image frame from the DTI was used to train the classifier with the hand-drawn brain mask for each case individually, to most efficiently predict the borders of the mask. Once trained, the same classifier was used on unlabeled successive image frames. By thresholding the probability map to 50%, a binary mask was generated which accounted for rotations and displacements in the DTI.
Step 4. **Motion correction.** We co-registered each of the corrected DWI frames with the first non-B0 frame using the FLIRT algorithm from the FSL software package. A weight image was generated for each image frame, which was used to weight the cost function towards the inner regions of the brain, to reduce the confounding effects of tissues that surround the brain and to account for the possible remaining imprecision of the brain mask. Due to the relatively large field inhomogeneity and the presence of confounding factors such as susceptibility artifacts from maternal tissues, a 12 degrees of freedom linear transformation was used with the squared difference as cost metric. The mean frame-wise displacement for each subject was calculated from the transformation matrices and was used as an indicator of the degree of head movement.

Step 5. **Correction of the b-vectors.** Due to the fact that the fetal head moves and rotates under the influence of the diffusion-weighting gradient, the b-vector orientations have to be re-oriented after the correction of head movement based on the rotation component of the transformation matrix (rotate_bvecs algorithm in FSL).

Step 6. **Censoring image frames of excessive motion or severe artifacts.** Next, we estimated the similarity of each image frame to the first non-B0 image using the mutual information metric. The frames that fall under the lower 5th percentile of the image similarity were censored (typically image frame 0 or 1 in the case of a 15 diffusion weighting orientation scheme).

Step 7. **Diffusion tensor estimation.** Based on the re-oriented b-vectors and the motion corrected, masked image, diffusion tensors were estimated in the FSL software using the diffit command. A weighted least squares estimation algorithm was used, which also took into account the censoring and rejected the image frames which were marked during step 6.

Step 8. **Scalar map calculation.** The parallel diffusivity was estimated from the first eigenvalue of the diffusion tensor. The fractional anisotropy and mean diffusivity were calculated using the following equations:

\[ FA = \sqrt{\frac{2}{3} + \frac{(\lambda_1 - \bar{\lambda})^2 + (\lambda_2 - \bar{\lambda})^2 + (\lambda_3 - \bar{\lambda})^2}{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}} \]

\[ MD = \frac{\lambda_1 + \lambda_2 + \lambda_3}{3} \]
where $\lambda_1$, $\lambda_2$, $\lambda_3$ are the eigenvalues of the diffusion tensor and $\bar{\lambda}$ is the mean of the eigenvalues.

References

