# Covalidation of Anisotropy using Polarized Light Microscopy and Diffusion Tensor Imaging in White Matter of a Mouse

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**INTRODUCTION:** Polarized light microscopy (PLM) can be used to measure anisotropic structures in thin histological sections. This relies on the ability of tissue with directional architecture to rotate the angle of polarized light that is shined through them [1,2]. This is similar to the information provided by Diffusion Tensor Imaging (DTI), which provides directional information of nerve fiber bundles in white matter (WM). DTI data has been used to reconstruct and visualize large fiber bundles in the brain and has been successful in reproducing known fiber anatomy from pathological dissection and anatomy textbooks, but remains incompletely validated on smaller structures. The purpose of this project was to compare directional anatomy of WM from PLM with DTI data.

**DTI METHODS:** Two male Swiss Webster mice (7 weeks of age) were used. All procedures were in compliance with the National Institute of Health *Guide for the Care and Use of Laboratory Animals*. The mouse was anesthetized and placed in a custom made stereotaxic device. This was positioned in a second cradle that fits into an Oxford instruments 200/330 (4.7 T) magnet equipped with a 15-cm inner diameter gradient coil. A multislice diffusion weighted spin-echo imaging sequence was acquired with a slice thickness of 0.5 mm, field of view was $1.5 \times 1.5$ cm$^2$, and data matrix was $128 \times 128$ (zero-filled to $256 \times 256$). Diffusion-sensitizing gradients were applied along six directions with two diffusion-sensitizing factors, $b$ values = 0 and 0.785 ms/um$^2$.

**PLM METHODS:** The animals were killed with intraperitoneal injection of pentobarbital. The brain of the mouse was removed from the skull and fixed in 4% formalin solution and was embedded in gelatine. The brain was sectioned with a cryostat microtome CM3050 S (Leica Microsystems, Bensheim, Germany) at a thickness of 100µm in the sagittal plane. The sections were serially collected, mounted with Aquatex© (Merck, Darmstadt, Germany), and coverslipped without staining. Ninety serial, sagittal sections of the mouse brain were produced. PLM was used to quantitatively estimate the fiber orientation in each point of the sections as described before [1,2]. In short, an optical system was used consisting of a pair of horizontally-mounted crossed-polars and a removable quarter-wave plate. Light is passed through the system from below and the transmitted light is imaged by a downwards pointing CCD camera with a basic resolution of 1300×1030 pixel (Axiocam HR, Carl Zeiss, Göttingen, Germany). The filters can be rotated while keeping the sample fixed so that their relative orientation remains constant. The images were acquired using the AxioVision software (Carl Zeiss, Göttingen, Germany). The magnification of the camera was adjusted such that one pixel represents $64 \times 64$ µm$^2$ in-plane of the section (over a section thickness of 100 µm). For each section, nine images separated by 10° rotations of the filters were acquired using the polarization filters only. The maximum intensity of this sequence was used to calculate the angle of inclination (out-of-plane orientation) in each pixel of the section (0 to 90°). Additional nine images separated by 20° rotations of the filters were acquired using the additional quarter wave plate. Sinusoids were fitted to the nine intensity values at each pixel to recover the angle of direction (in-plane orientation), which is represented by the angle of the filter combination at the minimum light intensity. These steps of image processing were realized using Matlab scripts (MathWorks Inc., Natick, MA, USA).

**COREGISTRATION METHODS:** Coregistration between the DTI and PLM data was performed. The maximum signal intensity images (MSI), obtained from the PLM in the sagittal plane and the DTI data were each combined to form a 3D data set. Using a cross modal measure a volumetric alignment was performed between the MSI and the anisotrpy data sets, which is close to it in contrast characteristics. This was done to compensate for distortions that occur in the reconstruction of the volumetric PLM data from serial sections. The alignment was performed using a general, 12 parameter, affine warp and an affine transform matrix was computed. In some cases the registration was done with a restricted field of view to compensate for non-linear distortions in one or the other data set. The 3D DTI data set was resampled in register with the PLM volume. Using the previously calculated transform matrix the DTI data was transformed into the PLM coordinate space and the DTI directional information was corrected using a modification of the method of Alexander et al. [3]. A goodness of directional fit image was computed in each case by calculating the square of the dot product between the normalized direction vectors in both data sets (good correspondence = high signal intensity).

**RESULTS:** Figure (a) demonstrates a DTI anisotropy image in the sagittal plane off midline. Figure (b) is a MSI image from the corresponding slice in the PLM data set. Figure (c) is a goodness of directional fit image ($cos^2\theta$), which demonstrates strong correlation between the two methods in regions of high anisotropy in the corpus callosum and cerebellum.

**CONCLUSION:** PLM can provide similar and validating data to that provided by DTI imaging in anistorpic WM.

**REFERENCES:** [1] Axer H, et al., Microscopy and Research Technique 51:48--492. [2] Axer H, et al., Journal of Neuroscience Methods 105:121-131. [3] Alexander DC, et al., IEEE Transactions of Medical Imaging 20:1131-1139.