Applying COMMIT on Histological Data

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ABSTRACT

As diffusion tensor imaging is used widely as a tool to explore the microstructure and connectivity of the nervous system, research regarding histological validation of diffusion tensor tractography is of increasingly importance. Currently, structure tensor analysis is one primary method to analyze histological data and validate dMRI findings. However, there is no guarantee that it recovers the exact ground truth fiber connectivity, since the signal-to-noise ratio is limited and that the tracking algorithms are not completely quantitative. To approach this problem, a Volume Fraction Model within COMMIT framework was applied to the structure tensor tractography here. It was shown that this model could improve the accuracy of the structure tensor tractography and get the tractograms closer to the ground truth quantitatively.

INTRODUCTION

Diffusion tensor imaging (DTI) offers a unique method to noninvasively characterize the brain tissue on the microscopic level, which makes it a widely used tool in clinical and neuroscience applications to reconstruct the neuron connectivity. However, DTI is an indirect measure since it derives the fiber orientations via the mobility of water molecules; besides, the complex in vivo situations, such as crossing fibers, make it difficult for DTI to recover accurately enough connectivity. To approach these problems and to better understand how well DTI can model the anatomical ground truth, it is important to conduct validation experiments. Currently, the comparison of DTI findings with histological situation in post mortem tissue is the primary validation method [1,2], as it allows direct comparison of DTI findings with histological ground truth.

Based on histological data, one primary method to estimate fiber orientation and reconstruct neuron connectivity is through structure tensor (ST) analysis. Compared to other fiber orientation estimation methods, such as manually tracing fibers, ST analysis is more

quantitative, automated and highly efficient. Structure tensor analysis was first proposed by Buddle et al as a method to validate dMRI tractography [3]. They calculated structure tensor for a given image pixel by determining direction-dependent spatial derivative in a local area and averaging the products of spatial derivatives over a certain neighborhood of the pixel. This method was later extended to 3D by Admad R.K. to analyze serial image stacks [4]. Analogous to diffusion tensor, local fiber orientations are determined by the eigenvector of the structure tensor. However, there is no guarantee that this method recovers the exact ground truth fibers, since the signal to noise ratio of the images is limited, and that the current tracking algorithms are not completely quantitative [5].

To approach this problem, COMMIT (Convex Optimization Modeling for Microstructure Informed Tractography) framework was implemented in the project. The COMMIT framework essentially works on diffusion tensor tractography. It evaluates and improves the plausibility of generated tractograms by integrating tissue microstructure estimation and tractography [6]. By minimizing the error between the original diffusion MRI data and the simulated diffusion signal, it guarantees to recover a global optimum solution.

Here, Volume Fraction Model within COMMIT framework was applied to structure tensor tractography based on CLARITY image. In Volume Fraction Model, CLARITY signals (voxel values) are modeled by the number of fibers going through that voxel. By minimizing the error between the original signal and the simulated CLARITY signal, each fiber is assigned a weight: fibers with certain weights are eliminated. Through removing globally unfit fibers, it is expected that this model can improve structure tensor tractography and get the corresponding tractograms closer to the ground truth fibers quantitatively.

MATERIALS AND METHODS

The images (*figure 1*) were obtained through two photon microscopes with a resolution of 1.5μm x 1.5μm x 1.5μm. The tissues were from part of a monkey's visual cortex, which became transparent to light after being processed with CLARITY [7]. The images were preprocessed to reduce artifacts and to enhance the contrast.
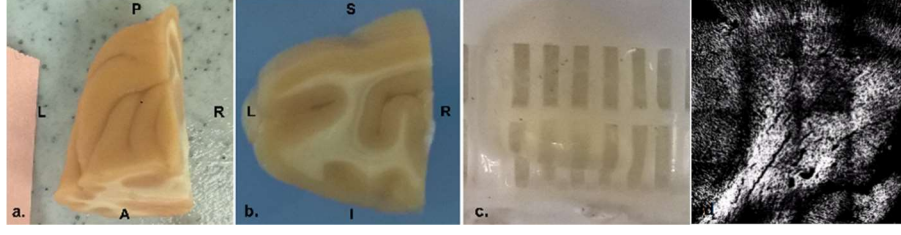
Figure 1. a) the monkey's visual cortex b) the slice of tissues used c) the tissue after being processed with CLARITY d) the two-photon image after being preprocessed

Structure Tensor Imaging

After preprocessing the image stack, 3D structure tensor was calculated according to the published formulas [4]. The voxels in the image stack were grouped into different patches, which were the units for structure tensor calculation. The image stack was first convolved with a Gaussian window function *w (x, y, z)*, then at each patch *(x, y, z)*, the spatial derivatives with respect to *x, y, z* were calculated as (1).

$$I_x(x,y,z) = -(1/\sigma^2)[x' G(x',y',z') * I(x',y',z')](x,y,z) \qquad (1)$$

The structure tensor $[S_{ij}]$ corresponding to each patch was taken as the local mean of the inner product of $[I_x\ I_y\ I_z]^T$ and $[I_x\ I_y\ I_z]$ (2).

$$S(x,y,z) = \begin{pmatrix} \langle I_x I_x \rangle_N & \langle I_x I_y \rangle_N & \langle I_x I_z \rangle_N \\ \langle I_y I_x \rangle_N & \langle I_y I_y \rangle_N & \langle I_y I_z \rangle_N \\ \langle I_z I_x \rangle_N & \langle I_z I_y \rangle_N & \langle I_z I_z \rangle_N \end{pmatrix}. \qquad (2)$$

By varying the number of voxels contained in each patch, structure tensor maps with different resolutions can be obtained. Here, structure tensors with resolutions of 12µm x 12µm x 12µm, 25µm x 25µm x 25µm, 50µm x 50µm x 50µm, 100µm x 100µm x 100µm, were calculated. To characterize the structure tensor anisotropy, eigenvalues and eigenvectors of each structure tensor were calculated, with the eigenvector (*V3*) corresponding to the minimum eigenvalue being the direction parallel to the local fiber orientations. Fractional anisotropy (*FA*) maps were derived and used as the mask for tractography seeding. The decomposition of structure tensor map was done using *fslmaths* from *FSL*.

Tractography

Tractography was generated through Fiber Assigned by Continuous Tracking (FACT) algorithm. FACT algorithm was first introduced by Mori S. [8], and is now one of the most frequently used fiber tracing algorithm. It generates tractograms by connecting voxels to the adjacent one to which the fiber direction is pointing.

Here, *V3* maps was taken as the fiber tracking direction, and *FA* maps was used as the mask for seeding. Based on the four structure tensor maps calculated in the previous step, tractograms of different resolutions were generated through *MRTrix* and visualized through *Trackvis*.

COMMIT Model

To improve the tractography, the Volume Fraction Model was applied. The model takes the CLARITY signal and the corresponding tractogram as inputs, and gives a weight vector corresponding to the streamlines in the tractogram as the output. In brief, each fiber is assigned a weight; the weights of all fibers going through a voxel sum up to the voxels' intensity value. By solving the multivariate system of equations and setting a threshold to the weights, a subset of fibers is selected as valid, while other fibers are eliminated as being false positives.

Model Testing

To evaluate the model, ground truth data is required. To obtain the ground truth data, a CLARITY image was synthesized based on a tractogram generated from the raw CLARITY data (*ground truth tractogram*). It was desired that the synthetic CLARITY image has the same resolution as the original one, however, due to computational limitation, the synthesized CLARITY image had a resolution of 15μm x15μm x 15μm (*tckmap*). A new tractogram was generated from the synthetic CLARITY data as described before (*testing tractography*). COMMIT was applied to the testing tractogram; fibers with certain weights were selected, which formed a new tractogram (*COMMIT tractogram*). Subsequently, the same number of fibers as *COMMIT tractogram* were selected randomly from *testing tractogram*

(*random_tractogram*). For *random_tractogram*, fibers were selected randomly for 20 times and the average performance was taken as the final performance.

To evaluate whether Volume Fraction Model could improve tractography quantitatively, three metrics were implemented.

Mean fiber distance matrix This matrix measures how single fiber in two tractograms resembles each other. In brief, every fiber in the *COMMIT tractogram/ random tractogram* and the *ground truth tractogram* are downsampled to have the same number of nodes (7 nodes here). The distance between fiber i in one tractogram and fiber j in another tractogram was calculated as the mean distance between each pair of nodes (*figure 2*) (3).
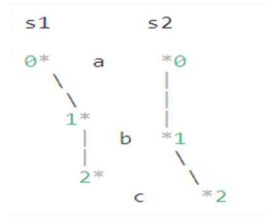
$$d_{ij} = (d_{aa} + d_{bb} + d_{cc})/3 \qquad (3)$$

Figure 2. Illustration of the calculation between two fibers (downloaded from *DIPY* documentation)

The distance between a fiber and a tractogram was estimated as the minimum distance between the fiber and fibers in that tractogram. The distance between two tractograms was calculated as the mean distance between fibers in one tractogram and the other tractogram.

Binary Map This matrix explores whether there is a fiber connecting two points. The binary map of a tractogram is generated by putting the voxel value as 1 if there are fibers going through that voxel, and as 0 if not. Two binary maps are compared by summing up the absolute differences between corresponding voxel values (4).

$$y = \sum \left| map^1_{groundtruth}[i, j] - map^1_{COMMIT/random}[i, j] \right| \qquad (4)$$

Non-binary Map The non-binary map of a tractogram was generated by putting the voxel value as N if there were N fibers going through that voxel (for *COMMIT tractography*, the weight of each fiber is considered in by using *tckmap: -tck_weights_in*). After obtaining all

voxel values, the map was normalized by dividing the map by its sum, after which the voxels in the map summed up to 1. Two non-binary maps are compared by summing up the absolute differences between corresponding voxel values as (4).

The first two metrics are simpler and more intuitive to compare tractography. The third metric is stricter since its penalizing scalar difference in each voxel.

RESULTS AND DISCUSSION

Structure Tensor and Tractography

Figure 3 shows the fiber orientation distribution (top) and tractogram (bottom) generated with different resolutions.
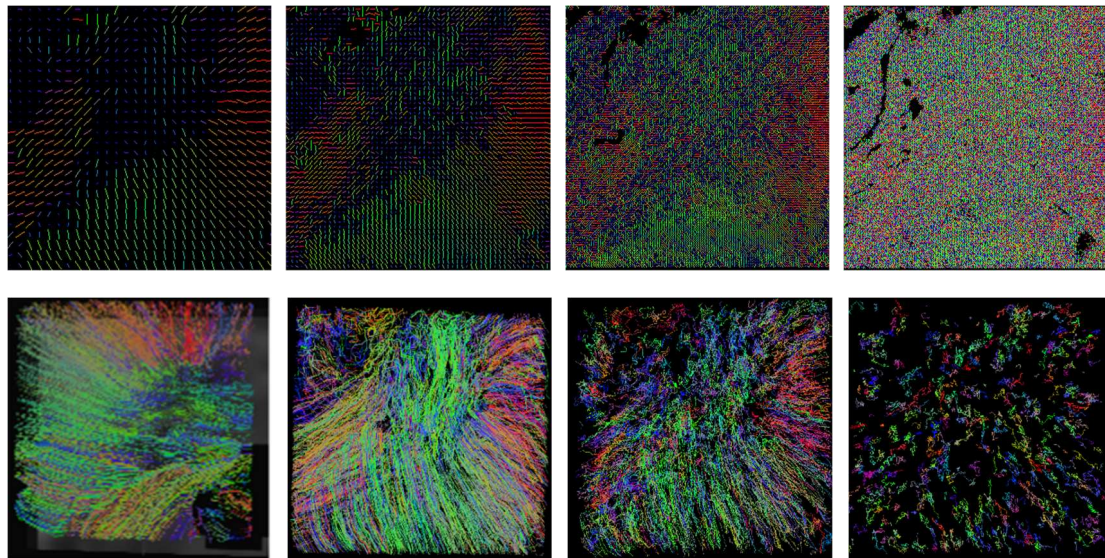
Figure3. a-d) fiber orientations (V3) with resolution of 12 x 12 x 12, 25 x 25 x 25, 50 x 50 x 50, 100 x 100 x 100 (μm^3) e-f) tractography with resolution of 12 x 12 x 12, 25 x 25 x 25, 50 x 50 x 50, 100 x 100 x 100 (μm^3)

Generally, structure tensors with higher resolutions should give tractograms which better describes the real fiber pathways in the brain. However, it was shown that tractograms with resolution of 12μm x 12μm x 12μm and 24μm x 24μm x 24μm revealed quite few about the fiber pathways, while tractograms with lower resolution showed some patterns. This might be due to that structure tensors calculated based on less patch voxels were more likely to be

influenced by artifacts, thus resulted in a discontinuous fiber orientation distribution. The tractogram with resolution of 50μm x 50μm x 50μm and 100μm x 100μm x 100μm could cover the whole area, while the latter one seemed smoother than the former one, thus tractography with resolution of 100μm x 100μm x 100μm were used in the next steps. It was noteworthy that compared to diffusion tensor tractography with resolution of *500μm*, the resolution obtained here was much higher.

COMMIT Model

COMMIT tractogram and random tractogram were shown in *figure 4*. It was noteworthy that due to the reduced resolution and subsequent parameter changes in structure tensor calculation, tractograms generated from synthetic CLARITY data were not as smooth as the ground truth tractogram.
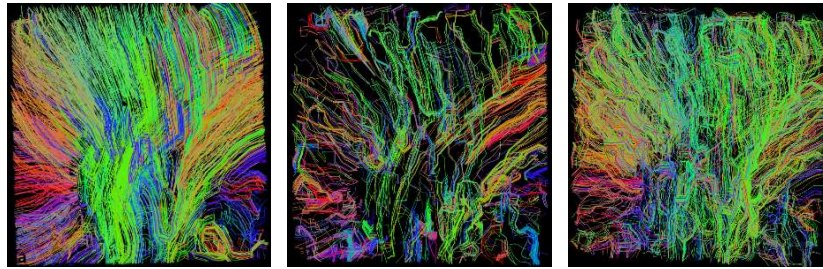

Figure4 a) *Ground Truth Tractogram* b) *COMMIT Tractogram* c) *Random Tractogram*

Figure 5 showed the results of the Mean Fiber Distance Matrix. The distance given by COMMIT was significantly lower than that of random compared to the ground truth tractogram. *Figure 6* showed the results of the Binary Map Matrix. Similarly, the error given by COMMIT tractography was significantly lower than that of random. These indicated that the Volume Fraction Model could improve the accuracy of tractography to better encode the CLARITY signal.
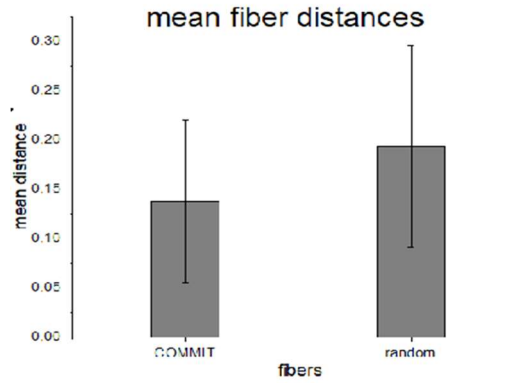
Figure 5. Mean fiber distance between *COMMIT tractogram/ random tractogram* to *ground truth tractogram*
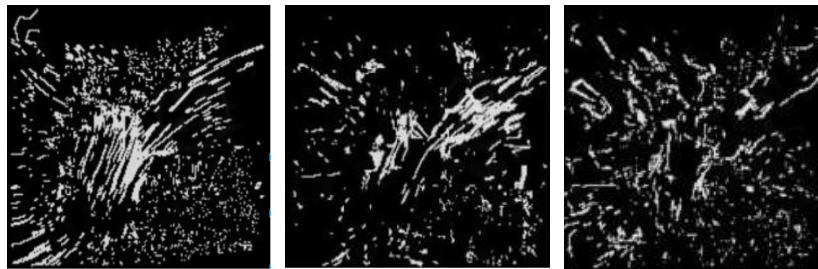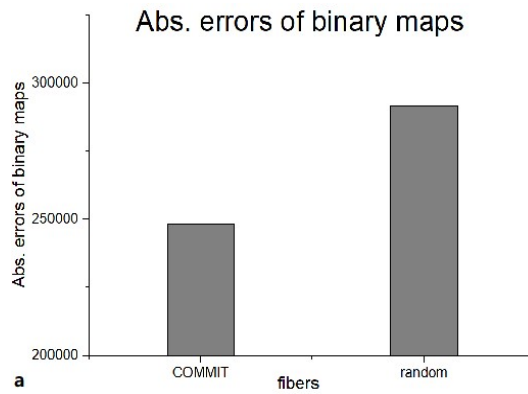

Figure 6. (a) absolute errors between *COMMIT tractography/ random tractography* and *ground truth tractography* b) c) d) binary map of ground truth, COMMIT and random tractography

Figure 7 presented the results of the Non-Binary Map Matrix. It showed that COMMIT tractography tend to give a higher error than random tractography compared with the ground truth, which was unexpected. The reasons behind this were complex. One factor that resulted in this results might be that shorter fibers were more likely to be selected (figure 8, figure S2)

by the Volume Fraction Model; shorter fibers tend to go through less voxels, thus there would be less constraints on them, which made them less likely to be given a zero weight.

The voxel value of Non-Binary Map Matrix is highly sensitive to fiber coordinate and the number of fibers: it is possible that a small change, such as a shift of fibers, can cause a big deviation in the results. Besides, the way of normalizing the map and simulating the intensity value (i.e, taking the number of fibers or the total length of fibers going through a voxel as the voxel value) also played an important role in terms of the results. Since the Non-Binary Map Matrix was very complex and not mature now, it was not taken as a main measurement here..
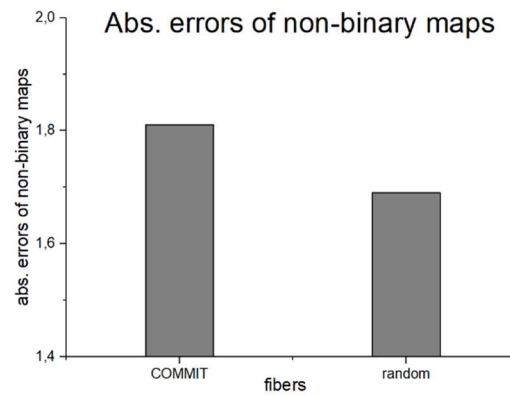

Figure 7. Absolute errors between *COMMIT tractography*/ *random tractography* and *ground truth tractography*
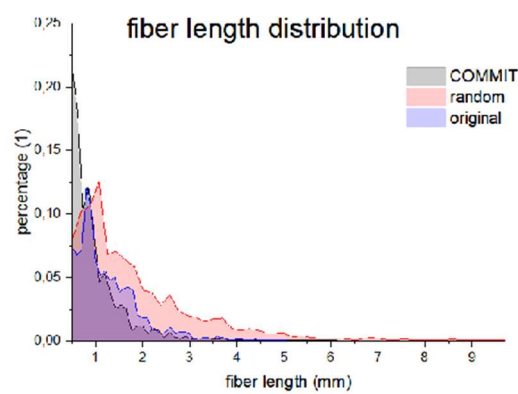

Figure 8. Fiber length distribution in *ground truth, COMMIT* and *random tractography*

CONCLUSIONS

Here, I generated a tractogram based on histological data (CLARITY image) through FACT algorithm, and demonstrated that COMMIT/Volume Fraction Model could improve the accuracy of this tractography and thus get the tractograms closer to ground truth. This was achieved by assigning fiber weights and eliminating zero-weighted fibers; here, shorter fibers are less likely to be removed. However, this model assumes that the intensity of the neurofilaments should be constant along the tract, which is not necessarily true. Further development of this model can allow some degree of freedom to the streamlines, which goes towards a model which is more complex but closer to the real situations.

It is envisioned that CLARITY can offer a method of histological validation to diffusion tensor tractography, as CLARITY has a higher resolution and maintains microstructures well. Future work can focus on further development of the Volume Fraction Model and the comparison between diffusion tensor tractography and structure tensor tractography to better understand how diffusion MRI can encode information of the geometry of the neurons.

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Supplementary Information

Image Preprocessing

To reduce the artifacts in the CLARITY data and to find the best contrast, the images were preprocessed via Fiji. First, bleach correction and a high-pass filter (through Gaussian filter) were applied to compensate for the intensity decay along z-axis and to flat-field correct the image stacks. To enhance the outline of the fibers, a median filter was applied, and the contrast was adjusted (*https://imagej.net/Preprocessing(MTrack)*).

Threshold Choosing

Considering that it was difficult to set an absolute value regarding fiber weights as the threshold, thresholding by different percentage of top-weighted fibers was adopted (*figure S1*). 0.5%, 1%, 1.5%, 2% and all fibers with non-zero weights were tried (usually, Volume Fraction Model gave about 2.5% of fibers with weights larger than 0). For each percentage, the experiment was conducted 10 times. It was shown that there was no significant difference made by varying the threshold in terms of mean fiber distances; regarding the errors of binary maps, the higher the percentage was, the larger the difference of errors between COMMIT and random was. Besides, to main more fibers, all fibers with non-zero weights were selected.
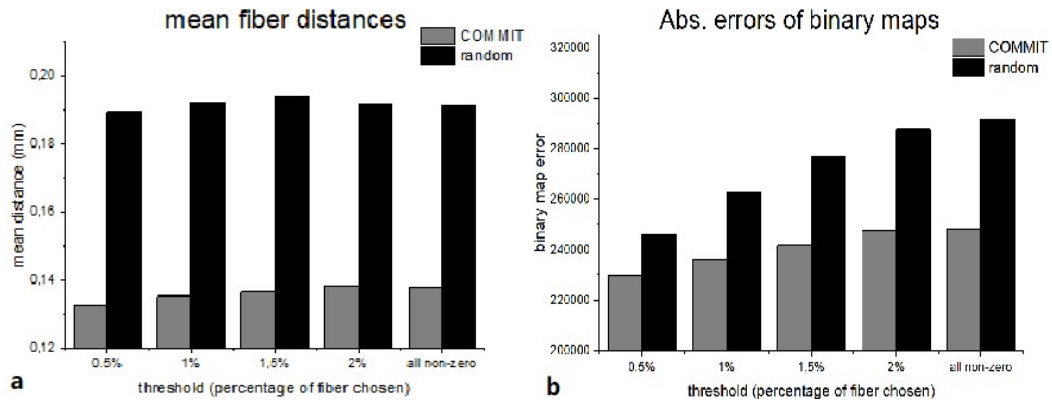
Figure S1. Relationships between the results of Mean Fiber Distance Matrix/ Binary Maps measurements and the percentage of fibers selected by COMMIT

Mean Fiber Length Matrix

In *figure 6*, it was shown that ground truth fibers were in general shorter than COMMIT/random tractography. This was due to the change of resolution while synthesizing CLARITY data and subsequent parameter change in structure tensor calculation. The tractography based on the raw CLARITY data tended to give shorter fibers than that of synthetic data (*figure S2*).

To see if these changes had influence on the results, the experiment was done completely on the synthetic data once (*i.e,* the *ground truth tractogram* was also generated based on CLARITY image with resolution of 15μm). The results showed the same pattern (*figure S3*).
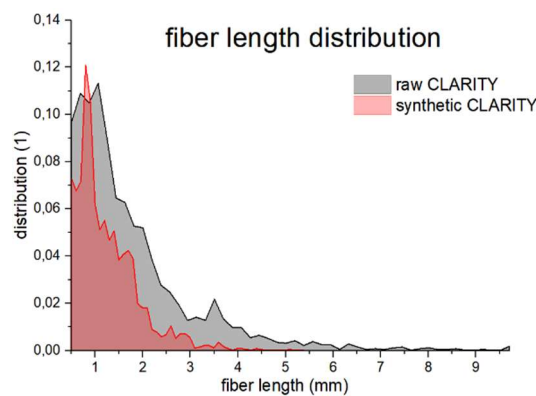

Figure S2. fiber length distribution in tractography generated based on raw CLARITY data and synthetic CLARITY data
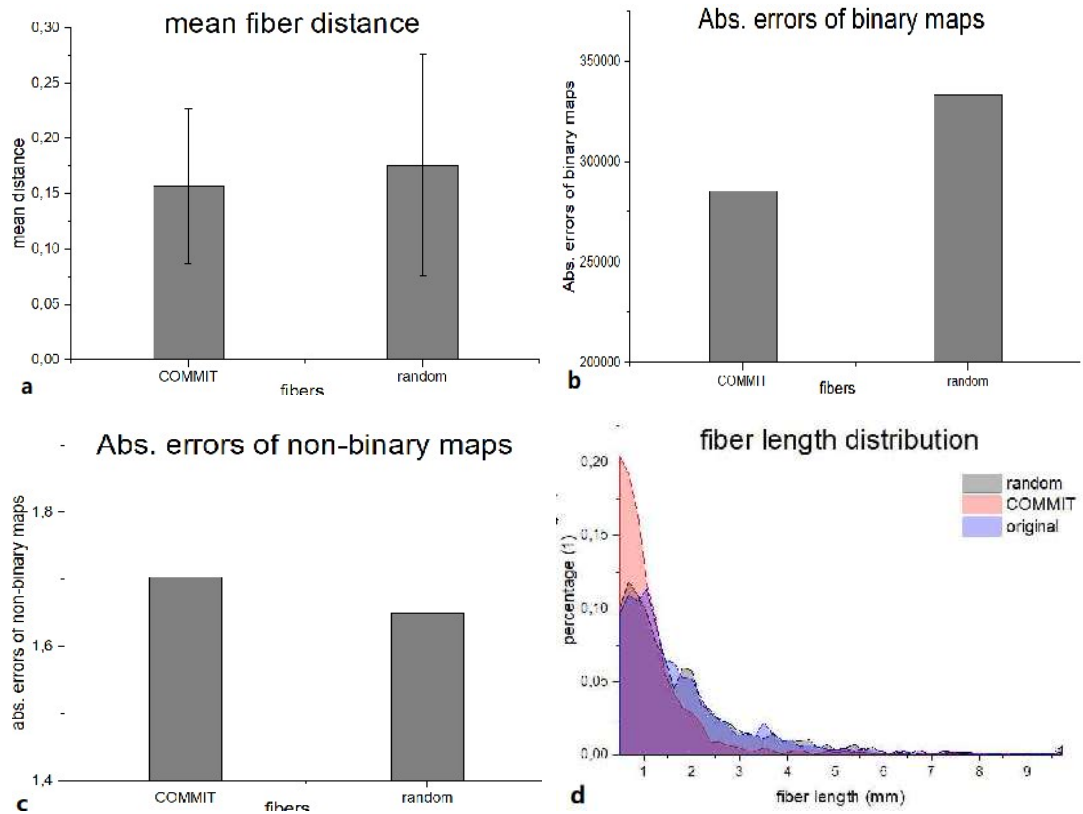
Figure S3. Results of parallel experiments where the *ground truth tractogram* was generated on CLARITY images with the same resolution as the *testing tractogram* a) mean fiber distance b,c) absolute errors of the non-binary/binary maps d)fiber length distribution of the *COMMIT tractogram/ random tractogram* and the *ground truth tractogram*