Methods for validating the anatomical trajectory of reconstructed fibre tracts in diffusion magnetic resonance fibre tractography

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Abstract
The introduction of diffusion tensor imaging fifteen years ago revolutionised the way of studying white matter disorders through the means of diffusion magnetic resonance fibre tractography. It remains the only method available to date to non-invasively study white matter architecture in-vivo. However, there are a number of limitations and pitfalls associated with this technique that calls to question the validity of fibre tractography. In particular the lack of a genuine gold standard prohibits quantitative analysis on the performance of fibre tractography algorithms. It is therefore of great importance for proper validation of fibre tractography to establish robust methods that may serve as a gold standard. In this review, several classical and contemporary methods used to study white matter architecture are discussed that can be used to validate the anatomical trajectory of white matter bundles in the brain derived by fibre tractography with high precision. It emphasises the need for true three-dimensional acquisition of ground truth data at high resolution from realistic specimen in order to accurately resolve the correct trajectory of white matter tracts in the presence of crossing fibres.

Keywords
diffusion magnetic resonance imaging, fibre tractography, validation methods, ground truth, gold standard

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Contents

Introduction

Diffusion MRI is a powerful method for measuring white matter architecture non-invasively and in-vivo (Johansen-Berg & Behrens, 2009; Jones, 2011). Based on the assumption that diffusion of protons is restricted by macromolecular structures such as myelin sheets wrapped around axons (Le Bihan & Johansen-Berg, 2012), details about white matter architecture can be derived from the principle direction of diffusion (Basser, Mattiello, & Le Bihan, 1994). With the introduction of the first diffusion tensor fibre tractography algorithm by Mori, Crain, Chacko, and van Zijl (1999) more than a decade ago a whole near era of brain research began. The unique ability diffusion tensor imaging to visualise the complex white matter architecture of the living brain caused many research groups targeting neurological disorders to quickly adopt the technique (Ciccarelli, Catani, Johansen-Berg, Clark, & Thompson, 2008). Moreover, fibre tractography is extensively used in a new field entitled connectomics that studies the structural and functional connectivity of the brain (Hagmann et al., 2010). The strong resemblance of fibre tractography with known anatomy of white matter structures detailed in anatomical atlases (Figure 1) ensured the technique was readily accepted by the research community.

However, although fibre tractography produces qualitatively good results, its reliability and robustness remain largely unproven. This is a serious concern, as the entire processing pipeline of fibre tractography suffers from a number of lim-

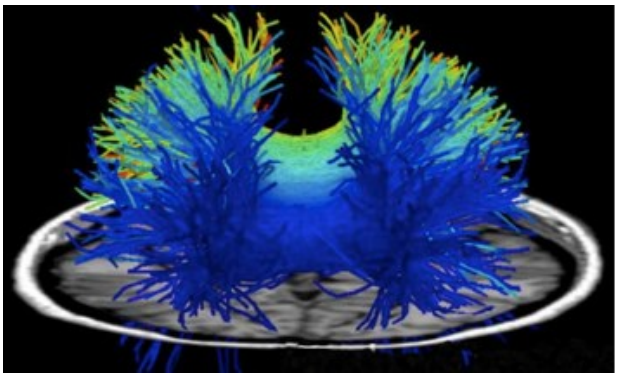

Figure 1. Diffusion tensor imaging of the corpus callosum using the streamline fibre tractography algorithm shows a strong correspondence with known anatomy of the commissural tracts and cortical projection tracts. Adapted from (Jones, 2011).

itations that could produce errors in the results. Diffusion MR fibre tractography is challenged with three major limitations (Jbabdi & Johansen-Berg, 2011). First of all, it is an indirect measure of white matter architecture. Secondly, the accuracy of reconstructed fibre tracts is considerably low in the presence of crossing fibres. And thirdly, the results are difficult to quantify. The errors introduced by these limitations can have detrimental effects on the validity of the results. Caution should therefore be applied when interpreting fibre tractography results without proper control experiments (Jones, Knösche, & Turner, 2013).

To test the reliability of a novel techniques, the results are often compared to well-established methods that proof the integrity of the new technique. Although the similarities are strong in the comparison of fibre tractography with known white matter anatomy (Figure 1), a more quantitative approach to prove the reliability and robustness of fibre tractography is preferred. This is particularly true if the technique is to be used in clinic settings. A quantitative analysis can be achieved by comparing the results with a known ground truth, or so called gold standard. Unfortunately, diffusion MR is currently to only method to visualise white matter architecture non-invasively and in-vivo. It is therefore extremely difficult to acquire solid ground truth in live specimen using any other well-established method. As a consequence, there exists no genuine gold standard for fibre tractography.

Nevertheless, several attempts have been made to validate fibre tractography, although mostly on postmortem ex-vivo specimen. The methods that have been used for this purpose are covered in the two overviews by Johansen-Berg and Behrens (2009) and Jones (2011). In this master thesis, a review of recent advances in validation methods for diffusion MR fibre tractography will be provided. In particular, the focus will be on validating the precise anatomical trajectory of the reconstructed fibre tracts. Good and reliable localisation of white matter tracts plays an important role in surgery where sub-millimetre precision is critical during resection of brain tumours near white matter tracts or during disconnection of disrupting white matter pathways in patients with epilepsy.

This master thesis is structured as following. The next chapter briefly covers the fundamental steps of fibre tractography, starting from the acquisition of diffusion MR images up to tracing white matter bundles with fibre tractography algorithms. The third chapter takes a critical look at limitations to each step and indicates possible sources of errors in localisation of reconstructed tracts. Once the need for validation of fibre tractography is made clear, the fourth chapter presents an overview of methods that can be used for verify of the precise anatomical trajectory of white matter tracts. Each validation method is introduces with a brief description on how it works and how it compares to diffusion MRI before it is closed with a discussion on the advantages and disadvantage of the method. The fifth chapter tries to bring all validation methods together by presenting the methods in a comparison chart for evaluation. In this chapter, the common components of image registration and evaluation metric shared by all validation methods are discussed. As well as some prospects for future research. Finally, the master thesis ends with a conclusion on what makes a good validation method.

Fundamentals

The processing pipeline of fibre tractography consists of many consecutive steps. From high abstraction point, the entire process can be reduced to three important phases (Figure 2). The first phase is the acquisition of diffusion MR images. During this phase, diffusion of protons is measured with an MR scanner and diffusion-weighted images are reconstructed. The second phase involves modelling of diffusion profiles from the acquired data. This phase is critical if fibre tracts are to be traced correctly in the presence of crossing fibres. The final phase is the actual reconstruction of tracts from the modelled data. During this phase, fibre tractography algorithms are applied to modelled data in order to extract continuous fibres that can be visualised.

This chapter is only briefly covers the important steps in diffusion MR fibre tractography. For a more extensive overview on the techniques described in this chapter, the reader is referred to (Johansen-Berg & Behrens, 2009; Jones, 2011) and references cited in this chapter.

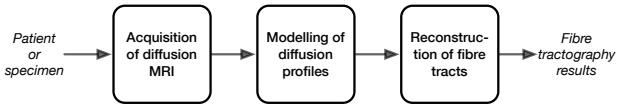

Figure 2. The three consecutive phases in the fibre tractography pipeline, from acquisition of diffusion MR data from patients or specimen to the fibre tractography results of fibre tractography algorithms.

Diffusion MR

Acquisition of diffusion MR images relies on the random diffusion of molecules, a process also known as Brownian motion. Thermal energy causes the molecule to diffuse a certain distance D, depending on the mass of the molecule, the temperature, and the viscosity of the medium (Einstein, 1905). In diffusion MR imaging, the molecule under investigation is typically the unbound water proton H^+.

In a homogenous medium, protons can diffuse freely in all three directions. But in a heterogenous medium the diffusion of the proton is hindered or restricted by macromolecular structures and the actual diffusion distance is reduced. There is still some dispute about the right model for what cellular components and compartmentalisation are responsible for the limited diffusion (Le Bihan & Johansen-Berg, 2012). In one model, four types of limited diffusion are described by restriction, tortuosity, molecular crowing, and permeable barriers (Figure 3). Regardless of the right model, the principle idea behind diffusion MRI is that the restriction to the displacement of proton between measures can be quantified. The reduction in diffusion distance can then be exploited to uncover information about the surrounding of the proton.

The first successful acquisition of a diffusion-weighted MR image was achieved by Le Bihan et al. (1986). In the same publication, the well-known b-factor was introduced. This b-factor plays an essential role in quantifying the average diffusion distance D of molecules based on the signal attenuation A from the MR acquisition, as shown in equation 1.

$$A = exp(-bD) \tag{1}$$

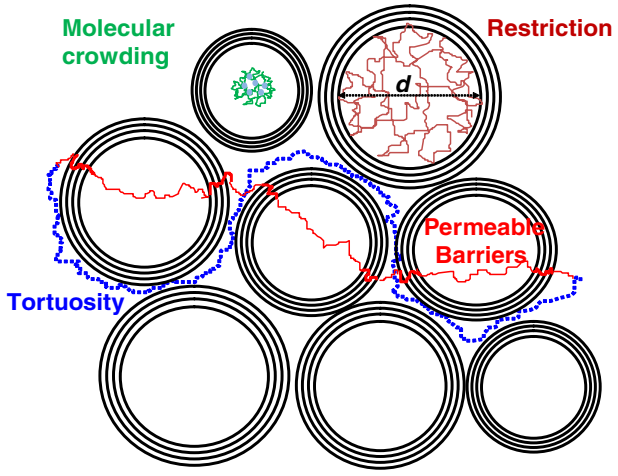

Figure 3. In the model buy (Le Bihan & Johansen-Berg, 2012), four sources of restricted diffusion are explained as restriction of protons in compartments, molecular crowding due to high affinity of the proton to a stationary component in the cell, tortuosity from moving freely around cells, and diffusion of the proton across permeable barriers. All four types restrict the diffusion distance of a proton compared to one in a homogeneous medium. Adapted from (Le Bihan & Johansen-Berg, 2012).

Long diffusion times results in an exponential decay of the measured MR signal. However, signal attenuation A does not accurately measure the amount of diffusion at a voxel, as it is confounded by other parameters like T_1 and T_2, especially in pathological tissue (Le Bihan & Johansen-Berg, 2012). It is therefore important to acquire pure quantitative diffusion maps, usually done by acquiring at least two diffusion MR images at different b-factor values b and b_0 to cancel out these confounding factors using equation 2.

$$ADC = ln\left[A(b_0)/A(b)\right]/(b-b_0) \tag{2}$$

This provides a map of the amount of diffusion at every voxel in the image. This image is not a diffusion-weighted MR image anymore, but rather a true diffusion MR image. ADC maps have become an accepted measure for diagnosis in the clinic, for example in the diagnosis of the brain region affected by stroke (Figure 4). However, the ADC maps do not provide information about the direction of diffusion that is required in fibre tractography.

Diffusion models

To resolve the direction of diffusion, more information needs to be retrieved from the data than just the apparent diffusion coefficient. Mathematical models are applied to the diffusion data in order to achieve this goal. The first of such mathematical models is the diffusion tensor model by Basser et al. (1994). The idea begin the diffusion tensor model is based on the Stejskal-Tanner equation. The diffusion tensor itself is a three by three symmetrical matrix that represents the diffusion direction in three dimensions (Figure 5). In order to reconstruct the diffusion tensor model, at least six diffusion MR

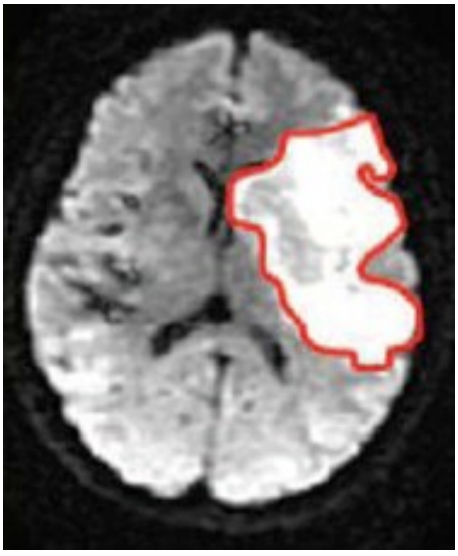

Figure 4. An ADC map of a patient who suffered from a stroke. The red boundary highlights the region affected by the stroke. This region can easily be identified on the maps because of slow diffusion rates in the affected tissue. Adapted from (Le Bihan & Johansen-Berg, 2012).

$$\underline{\mathbf{D}} = \begin{matrix} D_{xx} & D_{xy} & D_{xz} \\ D_{xy} & D_{yy} & D_{yz} \\ D_{xz} & D_{yz} & D_{zz} \end{matrix}$$

Figure 5. Diffusion tensor model. Since the matrix is symmetrical, it suffices to take at least six measurements in orthogonal directions to fill the matrix.

measurements are required in orthogonal directions. The principle direction of diffusion can be derived by decomposition of the diffusion tensor matrix into its eigenvectors. The principle direction of diffusion is assumed to be the eigenvector with the largest eigenvalue. The principle direction of diffusion is sufficient information to reconstruct fibre tracts with streamline fibre tractography algorithms; for a final results, see Figure 1.

However, the diffusion tensor model is a relatively simple model. Indeed, only taking into account the principle diffusion direction is not always sufficient to describe the fibre configuration in the presence of crossing fibres. The diffusion tensor model is accurate when fibres run parallel to each other, for example in the corpus callosum (Behrens, Johansen-Berg, Jbabdi, Rushworth, & Woolrich, 2006). But when fibres start to fan out, cross over, kiss each other or bend together, the diffusion tensor models produces the exact same tensor (Figure 6). To solve this problem, more complex model such a Q-ball imaging, High-Angular Resolution diffusion Imaging (HARDI) or diffusion spectrum imaging (DSI), have been proposed that take into account these common fibre configurations (Assemlal, Tschumperlé, Brun, & Siddiqi, 2011). The variety of models sample the specimen at many different

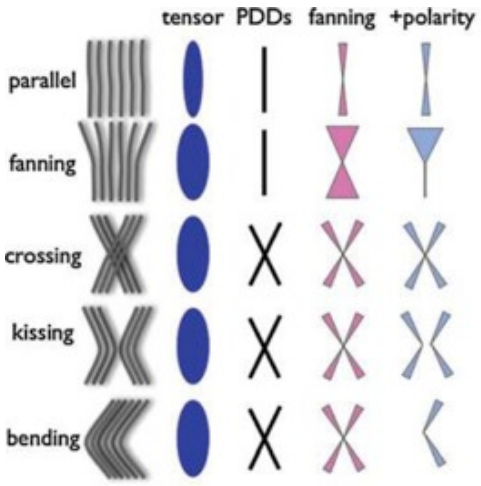

Figure 6. Different configurations of fibre orientations results in the same diffusion tensor. This makes it difficult to distinguish between the different configurations is such a simple model is applied. More advanced model that incorporate more information are able to resolve this situation more precisely. Adapted from (Jbabdi & Johansen-Berg, 2011).

angles, different b-values, different spatial distributions, or combination thereof. This leads to additional information about the white matter architecture, just enough to resolve the complex fibre configurations. The trade-off to acquire this information is either an increase in scan time or a decrease in resolution. Consequently, the relatively simple but fast diffusion tensor model remains the preferred protocol for use in the clinic.

Fibre tractography

Regardless of what model is used to derive diffusion profiles of the protons, this information is input to the wide variety of fibre tractography algorithms available to date. They all have the same goal, but try to achieve that goal through different means. Some of the algorithms work only on local regions, others try to incorporate global criteria. In general, one important distinction can be made between fibre tractography algorithms; those that reconstruct a single fibre tract orientation from the model data and those that consider the distribution of possible fibre orientations for a particular voxel.

Streamline fibre tractography

The first fibre tractography algorithm by Mori et al. (1999) belongs to this first class of so called deterministic, or streamline, tractography algorithms. Starting from a seed point in the data, the next voxel on the tract is determined by the principle direction of the tensor at the current voxel (see Figure 7). Two stopping criteria are used to determine when the tracing procedure should terminate. The first is based on the fractional anisotropy (FA) of a voxel. If the fractional anisotropy drops below a certain threshold (e.g., the orange track on the bottom right in Figure 7d hit a grey voxel with low fraction anisotropy), it becomes difficult to determine in which direction to go based on the principle diffusion direction. The second criteria is based on the maximum bending energy of tracts. If the track makes a sharp bend (e.g., the orange track on the bottom left in Figure 7d), it is unlikely the track follows this path.

Several improvements have been proposed since the introduction of the streamline algorithm. For example, region of interests can be selected for reconstructed fibres to pass through in order to filter out non-relevant or erroneous tracts. Other have tried to improve on the integration procedure used to determine the direction of the tracts. Whereas the original streamline algorithm by Mori et al. (1999) would use discreet temporal steps during integration of the partial derivative equation used in streamline fibre tractography algorithms, the Euler's method uses a fixed spatial step-size and the Runge-Katta method uses an adaptive spatial step-size (Jones, 2011). All the former approach reconstruct similar tract, only the smoothness of the reconstructed curve is affected and at voxels with complex fibre configurations slightly different pathways may be chosen.

Another major improvement to the streamline fibre tractography algorithm is using all the available information from the diffusion tensor data, rather than just the principle diffu-

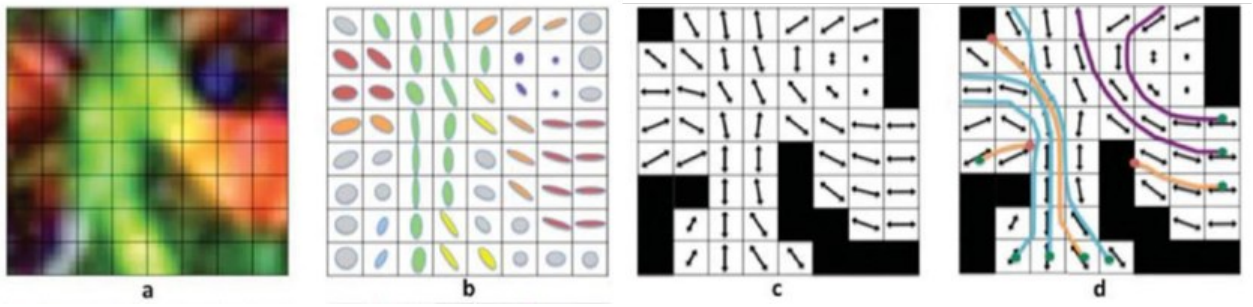

Figure 7. Example application of a streamline fibre tractography algorithm at work. The diffusion tensors of (b) can be visualised in a colour-coded scheme (a) where red indicated fibres running left-right, green indicated fibres running top-bottom, and blue indicated fibre running in-out-plane. Grey indicated isotropic tensors. The principle diffusion direction (c) can be extracted from the diffusion tensor (b) as the eigenvector corresponding to the largest eigenvalue. Starting from the seeding points in (d) indicated by green dots, the fibre tractography algorithm traces lines through the vector field. The traced curve remains parallel to the principle diffusion direction. The tracing procedure is terminated if the track hits a voxel with low fractional anisotropy, e.g., the orange track on the bottom right, or when the track makes a sharp turn, e.g., the orange track on the bottom left. Adapted from (Jones, 2011)

sion direction. The tensor deflection (TEND) algorithm by (Lazar et al., 2003) determines the direction in which to the reconstructed fibre tract should bend based on the full tensor of a voxel (see Figure 8). This approach can partially solve the crossing fibre problem as it can continue to trace curves through voxels with low fractional anisotropy due to crossing fibres. It also makes the algorithm less sensitive to noise or deviations from propagating localisation errors.

An alternative reconstruction approach is the G-TRACT algorithm by Cheng et al. (2006). Two termination points are selected in the brain and the algorithm tries to find a connection between the two. The final tract does not necessarily have to be the one with the lowest diffusion hindrance (i.e. conform to the principle diffusion direction). This approach enables more tracts to be found, but requires additional heuristics or constraints to filter out erroneous tracts.

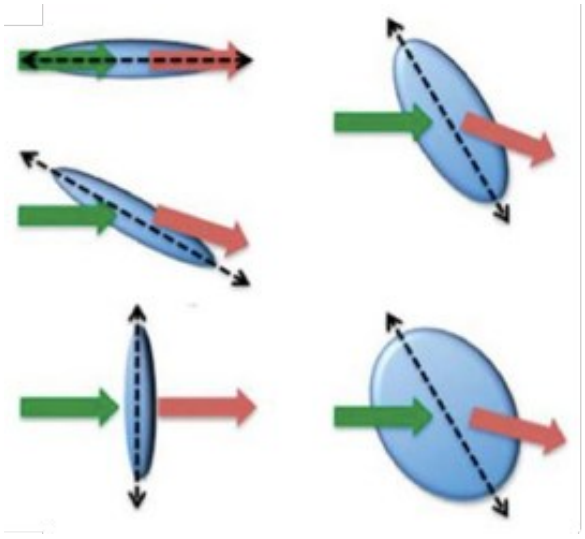

Figure 8. The diffusion tensor deflection model by (Lazar et al., 2003) can continue the tracing procedure even in the presence of high uncertainty. For example, when the fractional anisotropy is low (i.e., the lower right example), or when the track hits an orthogonal tensor (i.e., the lower left example). In all examples the fibre tractography algorithm uses the full information of the tensor to determine in which direction the track should continue. Adapted from (Jones, 2011).

Probabilistic fibre tractography

Streamline fibre tractography algorithms all face a major challenge in the presence of crossing fibres. Since crossing fibres are extremely common in every brain, a different approach to tracing fibre tracts is required. The second class of fibre tractography algorithms tries to solve this problem to some extent by determining the likelihood two end points are connected through a particular voxel, rather than tracing a single fibre tract.

Probabilistic fibre tractography algorithms often apply simulated diffusion random walk or front-wave propagation techniques to determine to what degree voxels are connected to each other. Since they do not try to find the most optimal track based on the principle diffusion direction, these algorithms can deal with uncertainties from low fractional anisotropy in the presence of crossing fibres (Figure 9). At each intersection, the likelihood a path exists is decreased until it approaches zero or some other constraint. The result is a map of probabilities of voxels which are likely connected to the seeding point (Figure 10).

Limitations

Thus far the three steps in the processing pipeline for fibre tractography has been presented. However, there has been little discussion about the limitations and pitfalls of each step that contribute to errors in fibre tractography. The possible sources of errors will be addressed in this chapter. Errors in the fibre tractography pipeline can be classified into three categories (Johansen-Berg & Behrens, 2009; Jbabdi & Johansen-Berg,

2011). First, there is the acquisition error that involves mostly signal noise and motion artefacts during acquisition of the diffusion-weighted scans. Next, there is the modelling error that results from the assumptions we make about the underlying model for proton diffusion in white matter architecture. This type of error becomes particularly important when dealing with the crossing fibre problem. Finally, there is the reconstruction error that plays a critical role during the actual tracing of the white matter tracts in the diffusion-weighted images.

The errors from all three stages accumulate throughout the process of tracing white matter bundles in the brain. This accumulation of errors may result in large deviations of the final reconstructed tracts. Deviations of the reconstructed tracts from the true anatomy can have major implications for the application of fibre tractography in both research and clinic applications where precision is key.

Acquisition

Diffusion MRI suffers from a number of limitations that can have a dire influence on the efficacy of fibre tractography in brain connectivity analysis. The main concern is with what we actually measure with fibre tractography. What we would like to measure is the white matter bundles coursing through the brain. However, we should keep in mind that what we actually measure in diffusion MRI is the dephasing of spins when a spatially-varying gradient is applied to the system (Jones et al., 2013). This is not the same as white matter bundles. In fact, the diffusion of molecules is restricted by all types of micro-structural components in the tissue, like cell membranes, myelin sheaths and microtubules (Beaulieu, 2002). One of the fundamental limitations of fibre tractography is that diffusion MR is an indirect probe for white matter architecture (Jbabdi & Johansen-Berg, 2011). Other acquisition schemes such as magnetised transfer may hold more merit in localising myelinated white matter bundles (Jones et al., 2013), but it remains to be seen if this approach can also be used in fibre tractography as it lacks any directional data similar to diffusion-weighted MR. In addition, myelin is not required for diffusion anisotropy, although has been proven to modulate the degree of anisotropy (Le Bihan & Johansen-Berg, 2012).

Although diffusion MRI is the only technique available today to measure diffusion in vivo non-invasively, a major disadvantage of the technique is the trade-off between resolution, SNR and scanning time. Diffusion MRI is an inherently noisy acquisition scheme, resulting in low SNR. Repeated measurements can be used to improve the acquired signal strength. However, for typical clinical applications, we would like to keep the scanning time to a minimum. On top of that, the models used to resolve crossing fibres require acquisition of the same voxel at multiple angles. This does not help to improve the SNR, but does increase the scanning time, leaving little time for repeated measurements. Sacrificing spatial resolution for improved signal strength is not a good option either. The partial volume effect, resulting from low spatial resolution, is a major obstacle in resolving crossing fibres. Regarding the acquisition of diffusion-weighted MR images, Jones et al (Jones et al., 2013) recommend pushing for the highest possible spatial resolution, as long as the voxels remain isotropic and the SNR does not drop below 3:1 unless appropriate models for the noise distribution are used to improve the SNR of the acquired images.

Other limitations that should be taken in to account during acquisition of diffusion MR images is that the use of live subjects is likely to introduce motion artefacts into the images. Since several acquisitions at different gradient directions are necessary to measure diffusion in enough directions for the model to derive a diffusion profile, registration of subsequent images is essential. The inherently low SNR make this a tricky procedure. For postmortem specimens, change in morphology

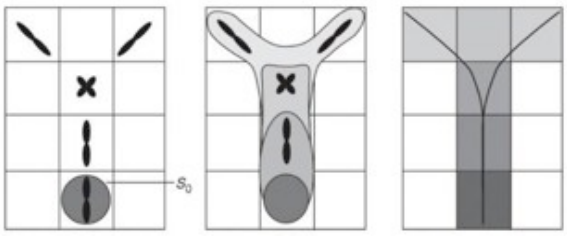

Figure 9. The front wave approach in probabilistic fibre tractography algorithms is capable of dealing with uncertainties at voxels where fibre tracts diverge. Adapted from (Jones, 2011).

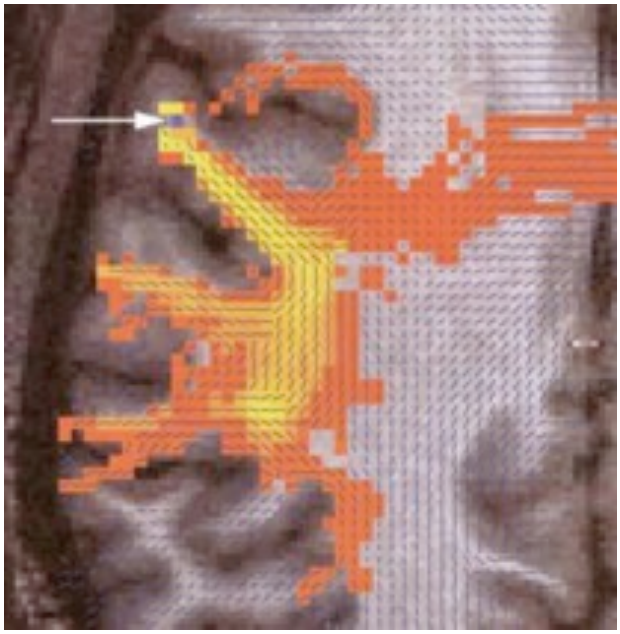

Figure 10. Results of probabilistic fibre tractography algorithm with simulated diffusion random walk. The fibre tractography algorithm was initiated at the voxel indicated by the arrow. Yellow voxels represent high probability that it is connected to the seeding point whereas dark orange voxels represents low probability. Adapted from (Jones, 2011).

and diffusion of protons need to be taken into account (Miller et al., 2011).

Modelling

Extracting more parameters from the acquired data is crucial to model the diffusion profiles correctly in the presence of crossing fibres. Of course, the information that can be extracted from the data is limited by the SNR, spatial resolution, partial volume effects and b-values of the acquired images. Consequently, there is an interaction between the acquisition parameters and the quality of the model. The more information that is needed for extraction of the diffusion profiles, the more images need to be acquired. For clinical settings this means a trade-off between scan time and resolution.

The white matter achitecture is vastly more complex (Behrens et al., 2006) than can be represented by current models. Consequently, modelling errors are extremely difficult to quantify. Since the physiological properties of white matter architecture are large unknown, we do not have to right statistical tools to evaluate our models regarding their accuracy (Jbabdi & Johansen-Berg, 2011). As is often the case when dealing with pathology, the diseased tissue may exhibit other properties than healthy brain tissue. For this reason, diffusion MRI is in patients suffering from stroke since the contrast between diseased tissue and healthy tissue is high. In the same way, the diffusion constant for the developing brain in children is higher than those of the adult brain (Hüppi & Dubois, 2006). Consequently, the b-values selected for imaging should be set slightly lower. Models need to take these variations into account in order to provide accurate descriptions of the diffusion profile. Although more biophysicial model have been proposed that describe fibre orientation distributions rather than diffusion profiles (Johansen-Berg & Behrens, 2009).

This dependency on models to generate diffusion profiles is a major disadvantage. For this reason acquisition schemes have been proposed that are free of modelling, i.e., they do not rely on prior biological assumption about white matter architecture or diffusion of protons. Examples of such acquisition scheme include Q-ball imaging and diffusion kurtosis imaging (Assemlal et al., 2011). The disadvantage of these acquisition schemes is the vast number of images required to construct diffusion profiles that can resolve complex fibre configurations accurately.

Reconstruction

Most streamline tractography algorithm depend on this one assumption; that the tangent of the curve traced by the algorithm is parallel to the peak in the data estimated from the data by the chosen model. The principle diffusion direction may not always correspond with the orientation of the fibre (Jones, 2011; Johansen-Berg & Behrens, 2009). This may be particularly true when fibres intersect/cross and the diffusion tensor is modified. Other criteria may be applied, such as the maximum curvature of the tracts or the minimum fractional anisotropy (FA) threshold level. But as Jones et al (Jones et al., 2013) righteously point out, the values chosen for these parameters are hardly ever justified in literature. In fact, selecting the right parameters depends on the regions of the brain that is being processed. Structures with largely parallel white matter bundles, such as the corpus callosum, are much easier to extract than twisting and crossing white matter bundles in Meyer's loop.

Figure 11. Variations in noise can cause streamline fibre tractography algorithms to deviate from the actual white matter tracts. The dispersion has a linear relationship to the distance of the reconstructed tract. Adapted from (Lazar et al., 2003).

Another major problem in reconstructing fibre tracts is that reconstruction takes several steps. In addition, the integration steps are evaluating in an imaging grid while reconstruction is done in continuous space (Johansen-Berg & Behrens, 2009). This makes fibre tractography extremely sensitive to noise from for example distortion, motion artefacts, or ghosting (Jones, 2011). Errors during the integration procedure propagate to the next step. In particular long continuous tracts are subject to propagation of this errors. The dispersion of the reconstructed tract from the actual white matter bundle is linear to the distance from the seed point (Figure 11). This errors can easily be up to 1 mm for longer pathways (40mm or more) and also depends on the SNR (Lazar et al., 2003). Probabilistic fibre tractography algorithms also suffers from an artificial reduction in probability for longer tracks (Jones, 2011). Some solutions are offered in term of diffusion tensor regularisation to make the procedure less sensitive to noise (Lazar et al., 2003). Robust artefact correction for imaging artefacts and image registration for motion artefacts is needed.

Another major limitation to diffusion MR fibre tractography is that orientation in the term of polarity, i.e., whether the pathway is afferent, efferent or bidirectional, cannot be determined with diffusion MRI (Jbabdi & Johansen-Berg, 2011). This information can be used to determine flow of information in connectivity studies, but also help determine the underlying fibre configuration when fibres cross. Other imaging modalities such as electroencephalography (EEG) or magnetoencephalography (MEG) can measure polarity. In the section of validation methods a techique for MR-visible tracers is described that allows polarity of fibre tracts to be determined with MRI in vivo.

Nevertheless, the major issue remains the inability to resolve crossing fibres due to limited spatial resolution and partial volume effects of MRI (Jones et al., 2013; Johansen-Berg & Behrens, 2009). Streamline algorithm cannot properly deal with crossing fibres. Moreover, due to partial voluming effect a single voxel is compromised of a heterogenous mixture of tissue (Beaulieu, 2002). Tissue other than white matter may have an influence on the diffusion of the proton as well, thereby confounding the diffusion MR measurements.

Probabilistic fibre tractography tries to deal with this issue by taking into account possible errors made in the acquisition or modelling because of noise and artefacts. However, the computational overload of reconstructing tracts from many different seeding points is quite high for probabilistic fibre tractography algorithms as compared to streamline algorithms (Johansen-Berg & Behrens, 2009).

On a final note on the interpretation of fibre tractography results is that it can be difficult to interpret. The algorithms produce wonderful images of major pathways in the brain.But given the pitfalls mentioned in this chapter, without proper validation of fibre tractography cautious should be used about drawing conclusion from these images. Interpreting the data derived from probabilistic fibre tractography algorithms is even more tricky, as it is tempting to interpret the probabilities derived from the algorithm as a measure of connectivity. However, the probabilities in probabilistic fibre tractography represent likelihood that a tract originating from the seeding point end up at that particular voxel, rather than providing a probability on the connection strength (Johansen-Berg & Behrens, 2009; Jones et al., 2013). A final word of caution on interpreting the results from fibre tractography is that the fibre count of white matter bundles cannot be accurately determined (Jones et al., 2013).

Need for validation

In conclusion on the limitations of diffusion MR fibre tractography, it is an indirect measure to track white matter bundles through the brain. The plenty pitfalls throughout the entire process of fibre tractography should call for caution when interpreting the results. Since there is no intrinsic measure for tract localisation errors in diffusion MRI, the reliability of the fibre tractography results are difficult to quantify (Jones, 2011). Nevertheless, the ability of diffusion MR fibre tractography to non-invasively and in-vivo image white matter structures and its strong colocalisation of fibre tractography results with known major white matter tracts has made it a widely adopted technique in both research and clinic. Because of its wide use and uncertainty about its reliability, proper validation of fibre tractography algorithms is required before relying too heavily on its results in research studies and clinical applications.

Validation methods

Long before diffusion MR became available, people have been studying white matter structures in the brain using classical dissection methods. More recently, studies using histological sectioning and studies into white matter pathology are at the root of today's white matter atlases. The use of immunological staining methods and tracers have boosted research into cell-specific studies, resulting in improved accuracy of white matter atlases. With MR scanners, physical phantoms and software simulations are common provide new opportunities for repeated measures.

The ability to measure white matter pathways in the human brain in vivo spawned a new era of connectome studies (Hagmann et al., 2010; Sporns, 2013). These studies concern themselves with the structural connectivity between regions of the brain, i.e. to what extent brain regions are interconnected. For these kind of studies, validating the existence of a connection between two brain regions is necessary and sufficient. At least, for as long as we cannot accurately determine the strength of the connection (Hagmann et al., 2010; Sporns, 2013; Jones et al., 2013).

However, clinical applications of diffusion MR fibre tractography tend to be more concerned with the anatomical trajectory of the fibres rather than structural or functional connectivity, i.e. determine the exact spatial location of white matter pathways in the human brain. After all, precision is key when it comes to dissecting a brain tumour or transection of the cerebral cortex in the presence of epilepsy. For clinical application it is therefore not only important to validate what white matter pathways connect to which brain region, but also validate their exact course through the brain.

In this chapter, we will discuss the wide range of methods available for validation of the precise anatomical trajectory of diffusion MR fibre tractography and address their benefits and drawbacks.

Physical phantoms

One common approach to validating the accuracy and performance of clinical scanners of any kind is the use of physical phantoms. At present, two distinct classes of physical phantoms are available. The first class of phantoms are the phantoms constructed from artificial fibrous materials such a polymer or yarn. The second class of phantoms are the biological tissue samples.

Procedure

Phantoms are constructed from materials with known properties and modelled in such a way it represents the part of the body of interest. Finding the right materials and constructing a descent model are the major challenges for physical phantoms. Obviously, materials that consists of strands are most appropriate for use in diffusion MR phantoms. However, not every fibrous material is suitable. Glass capillaries are too rigid to bend in curves and strands of Teflon® capillaries are too thick to properly represent white matter bundles (Pullens, Roebroeck, & Goebel, 2010). In recent year, polymer fibres, such as polyethylene, polyamide, or Dyneema®, have become increasingly popular as material to work with in physical phantoms not only for their flexibility and small diameter, but also for similar values of anisotropy and T_2 to human white matter (Pullens et al., 2010).

Individual fibres are usually constructed by twisting many fibres of polymer together into a single large strand that represents fascicles of individual neurones. These strands are then bundled together to form a larger single fibre that represents a white matter bundle. Strands can also be interwoven to represent crossing fibre bundles in the brain (Figure 12). Fibre

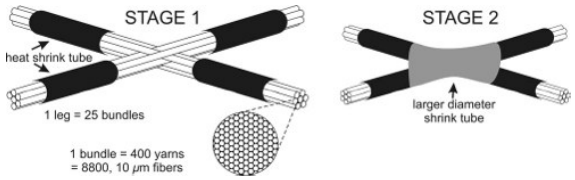

Figure 12. Construction of a simple physical phantom with crossing fibres from strands of Dyneema®. Crossing of the fibres is achieved by weaving the strands at the intersection. Additional shrink-wrapping is performed to tighten the strands together and keep the fibres from falling apart. Adapted from (Pullens et al., 2010).

bundles can be shrink-wrapped to tighten the strands together and increase the anisotropy. The fibre bundles can than be positioned inside the container of the physical phantom.

For biological phantoms, tissue needs to be excised from a subject. The best tissue is one that has a known white matter structure, or at least a white matter structure that is easy to predict. For example, Campbell, Savadjiev, Siddiqi, and Pike (2006) spinal cord tissue from a rodent was used because spinal cord are long parallel fibres with relatively simple and well-known surrounding tissue. The excised tissue can then be arranged into any configuration preferred before it is scanned (Figure 13).

Advantages and limitations

Physical phantoms have some severe limitations with application to validation of fibre tractography. First of all, the phantoms tend to be overly simplistic compared to the complex structure of the brain, although an attempt has been made to create a semi-realistic phantom for use in the FiberCup competition (Figure 14). This semi-realistic phantom contains all forms of white matter tracts that are common to the human brain; i.e., commissural tracts, lateral tracts and projection tracts. Moreover, model contains several sites of crossing fibres.

Another limitation to physical phantoms is that it can be difficult to select the right material to match properties of white matter. Although Dyneema® comes close to matching the T_2 and anisotropy of human white matter, real white matter is not a homogeneous material. Therefore any polymer material would face the same limitations. This limitation has been overcome by using biological tissue, such as spinal cords from rodents (Campbell et al., 2006) or asparagus (Lätt et al., 2007). The tissue is arranged in a known position before imaging (Figure 13. Biological tissue may have the advantage of having the natural complexity of fibres, their ground truth is much harder to establish accurately. This would require dissection of the specimen to reveal the internal structure of the fibres. For this reason, biological phantoms are limited to tissue for which it is known in what orientation fibres run from anatomical studies.

Finally, the physical phantoms are often used in studies that focus on the crossing fibre problem. These studies are mainly interesting in resolving the crossing fibre problem to ensure fibre tractography algorithm select the correct branch for continuous tracking. That is, well are crossing fibres resolved, rather than the localisation of tracts. The precise location cannot accurately be controlled in the construction of the phantom and therefore are less suited for this application.

As mentioned before, one of the major limitations to hardware phantoms are the very simplistic structure that can be made using the polymer fibres. One solution is to use biological tissue excised from postmortem animals or plants, such as spinal cord from rodents (Campbell et al., 2006) or asparagus (Lätt et al., 2007). The use of biological tissue has the advantage that it is the most natural product resembling white matter tissue with regard to its properties. However, there are some limitations to using ex-vivo tissue samples, since the fibre composition inside the sample remains largely unknown, unless the tissue sample is further analysed by one of the subsequent techniques described in this article. Moreover, the effects of postmortem delays on the diffusion property of

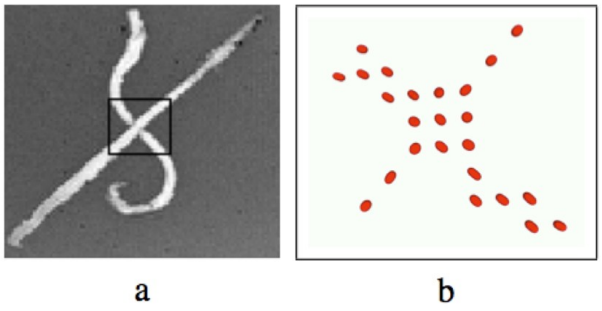

Figure 13. Two strands of spinal cord tissue excised from a rodent are positioned in such a way the strands overlap in a crossing fibre configuration (a). The results of diffusion tensor imaging of the bounding box is shown in (b). The effect of overlap as a decrease in fractional anisotropy is small but present at the intersection. Adapted from (Campbell et al., 2006).

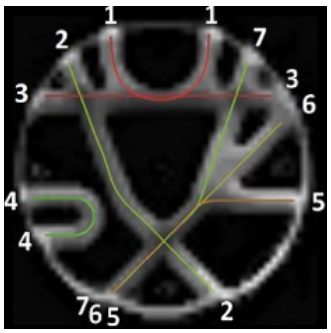

Figure 14. A semi-realistic hardware phantom used in the FiberCup competition. The model contains commissural tracts, lateral tracts and projection tracts resembling structures common in the human brain. The ground truth of the seven pathways and their corresponding termination sites are shown on top of the image. Adapted from (Côté et al., 2013).

the tissue need to be determined prior to its use. This makes the use of biological tissue less ideal for the validation of the precise course of fibres.

Software models

Another validation technique that belongs to the phantom class is software models. Based on the models on white matter structures from neuroanatomy that are available in large-scale brain maps and atlases, artificial diffusion-weighted MR images can be synthesised. Modelling an entire human brain at the cellular level is still beyond the reach of current day knowledge and computational power. Fortunately, such overly complex models are not needed for validation of fibre tractography algorithms. These algorithm usually run on a subset of measures derived from white matter architecture. For this reason it suffices to use synthetic diffusion tensor fields, unless the algorithm incorporates additional measures.

Procedure

The first step in creating synthetic tensor fields involves creating tubular structures that represents the white matter tracts to be traced. This structure can vary from simple in plane piecewise linear line segments (Figure 15) up to rather complex three dimensional curvilinear structures (Figure 16). From these tracts, the eigenvalues for each voxel are determined to construct the final tensor model for the artificial image. At this stage of modelling, noise can be added to the diffusion direction and orientation. Finally, additional imaging noise and artefacts can be added when necessary.

Right after the initial explosion of deterministic fibre tractography algorithms developed, Tournier et al. (2002) performed an initial analysis of these algorithms using computer-simulated data. The purpose of the study was to determine how different parameters including noise affects the performance of the fibre tractography algorithms, assuming different algorithms are susceptible to parameters in varying degrees. Very simplistic models were used (Figure 15), but this level of complexity was sufficient for a first error analyses.

As indicated by Leemans, Sijbers, Verhoye, Van der Linden, and Van Dyck (2005), simple models may suffice for analysing the effect of different properties of white matter tracts on accuracy of the fibre tractography algorithms, such as curvature, noise or fractional anisotropy. More complex models are required to models important properties of white matter tracts, such a the effect of partial volume effect in crossing fibres or at termination sites near the grey matter cortex. Most importantly, white matter fibres do not have constant fibre density as they mix and mingle with other fibres. In addition, the transition between the fibre tract and surrounding tissue is rarely sharply defined as is often the case in simple models. For this reason, additional filtering steps were introducing to the process of generating synthetic diffusion tensor fields to make the models more realistic.

Over time, even more complexity has been added to the process of synthesising diffusion tensor fields. The complex models focus in particular on partial volume effect due to the crossing fibre problem as this has currently the most detrimental effect on the performance of fibre tractography algorithms. Fibre bundles are now models as set of individual fibre strands and are interwoven into complex patterns similar to the patterns found in the human brain (Figure 16). Even semi-realistic models based on diffusion tensor brain atlases have been proposed (Leemans et al., 2005; Barbieri, Bauer, Klein, Nimsky, & Hahn, 2011). Here, actual diffusion MRI scans are used as a basis on which to build synthetic white matter tracts through the brain.

Advantages and limitations

The main advantage of software simulations is that any shape imaginable can be modelled using synthetic tensor fields. Moreover, the imaging parameters can be set to any preferred value and the exact position of the tracts in known with high

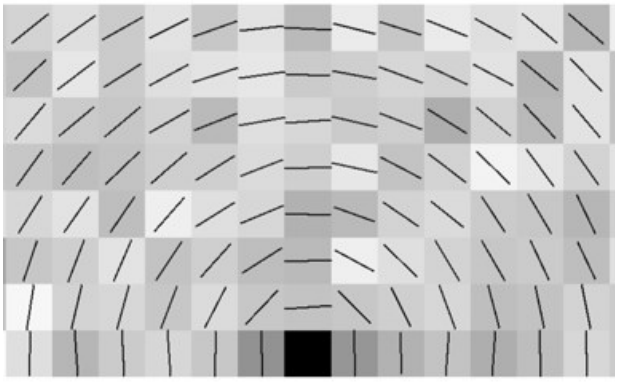

Figure 15. Example of a simple software model for the analyses on the performance of fibre tractography algorithms. Semicircular paths are simulated in a two-dimensional plane for testing fibre tracking at different curvatures with noise added to the tensor image. Adapted from (Tournier et al., 2002).

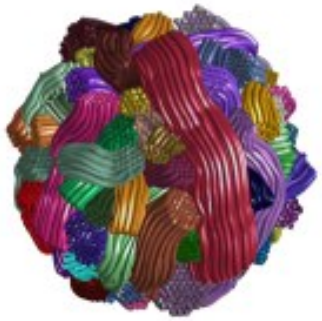

Figure 16. Example of a complex artificial white matter structure with crossing fibres synthesised by a software model. The complexity of the model is in having a three-dimensional structure with strands of fibres that twist and bend much like natural white matter tracts. Packaging them densely together creates crossing fibre configurations for which the ground truth of the fibres is known with high precision. Adapted from (Close et al., 2009).

precision. No other class of phantoms or validation technique allows this much control over the parameters. Additional benefits are achieved when comparing the derived results with the ground truth as no registration of the two images is requires since they operate within the same coordinate space.

A slight disadvantage of this technique is that generation of a realistic model is computationally intensive. Computing a single instance of the model from Close et al. (2009) requires about one day. This is mainly due to iterative process of compacting the bundles together without causing them to run through each other. Fortunately, this disadvantage can be downplayed by the fact a model needs only be generated once and can then be used many times. However, selecting the right parameters in a complex model is a challenging task. Most models used to date use relatively simple mathematical descriptors of shapes that are fast to compute but no where close to true biological complexity. Nevertheless, the high precision of ground truth and extensive control over the parameters make software models an interesting options for validation of fibre tractography.

Dissection

Long before radiography was available to view inside the human body, physicians have been studying the human anatomy through dissection studies. The first dissection studies date back to the Greek physician Galen, around 130-200 AD (Johansen-Berg & Behrens, 2009). But it wasn't until 1543, when the Belgian physician Andreas Vesalius published his anatomical masterpiece De Humani Corporis Fabrica (On the Structure of the Human Body), that a detailed map of the human brain was available that clearly differentiated between white matter structures and grey matter cortex (Figure 17). In the early twentieth century, a renewed interest into the workings of the human brain revitalised interest in dissection studies and promoted the advancement of techniques for dissection studies.

Procedure

A major breakthrough in studying white matter structures came from Klingler (1935). In his article, he describes a preparation method that involves repeatedly freezing the formalin-fixed postmortem brain. The ice crystals that form during freezing loosen up the tight white matter bundles in the brain. This allows for detailed dissection and visualisation of separate white matter fibres.

Klingler's dissection technique is still used to date to study gross anatomical tracts in prepared specimen of both human and animal postmortem brains. The technique can expose white matter bundles in exquisite detail. It even allows fibre tracts to be traced in 3D space, offering a greater potential than 2D histological sectioning since the fibre tracts are not cut to thin pieces (Jones, 2011; de Castro, de Holanda Dde H Christoph, dos Santos, & Landeiro, 2005; Türe, Yaşargil, Friedman, & Al-Mefty, 2000). The dissection technique particularly excels in the presence of complex fibre orientations, such as in Meyer's Loop where the complex fibre orientations can be carefully dissected (Figure 18) (Agrawal et al., 2011). Although histology has taken away some of the attention from dissection studies, the ability for the dissection technique to study small fibre tracts makes it still as valid as any histology technique available (Agrawal et al., 2011).

Advantages and limitations

However, anatomical dissection is a labour-intensive task that requires fine motor skills, plenty of patience, and extensive prior anatomical knowledge for good quality dissections (Johansen-Berg & Behrens, 2009; Jones, 2011; Agrawal et al., 2011). Although mapping of the 3D course of the fibre tracts is feasible with dissection, it is a rather complex process to accurately keep track of the spatial position of the fibres (Jones, 2011). Usually only one major fibre tracts per postmortem brain can be studied, since the process of dissection tends to cut away surrounding fibre tracts in a destructive manner that makes studying the smaller fibres located particularly near terminations sites in the cortex difficult (Jones, 2011; Türe et al., 2000; Fernandez-Miranda et al., 2008). In addition, it can still be challenging to distinguish between white matter

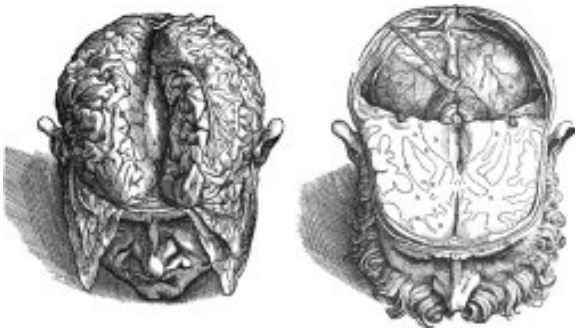

Figure 17. Anatomical dissection of the human brain as visualised in Humani Corporis Fabrica by the Belgian physician Andreas Vesalius. Adapted from Johansen-Berg and Behrens (2009)

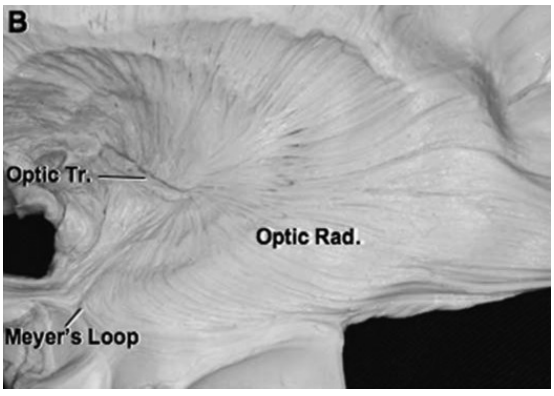

Figure 18. Klingler's dissection technique applied to a prepared specimen of a postmortem human brain exposing the white matter fibres of the optical tracts, including the complex configurations in Meyer's loop. Aapted from (Agrawal et al., 2011).

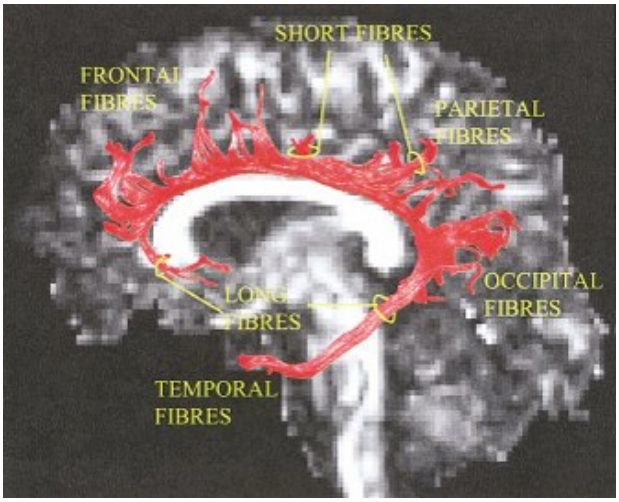

Figure 19. Strong correspondence of the reconstructed tracts in the corpus callosum with the known anatomy of the fibres tracts in the corpus callosum. Adapted from (Catani et al., 2002).

and grey matter without staining, even for trained experts (de Castro et al., 2005).

Anatomical dissection and diffusion MR tractography have a reciprocal relationship from which both techniques can benefit. The prior knowledge required for robust dissection means new tracts are hard to discover in exploratory dissection. However, fibre tractography prior to dissection can aid the process of dissecting by visualising major fibre tracts in the specimen before dissection starts to improve the quality of the dissection (Skadorwa, Kunicki, Nauman, & Ciszek, 2009). Because of the non-destructive nature of diffusion MR tractography, this technique has found its way into neuroanatomical education as a virtual dissection training tool (Agrawal et al., 2011; Skadorwa et al., 2009; Catani, Howard, Pajevic, & Jones, 2002; Hagmann et al., 2003).

Diffusion MR tractography can benefit from dissection studies as a validation measure. Diffusion MR tractography shows close resemblance to known major white matter tracts. In a study by Catani et al. (2002), a virtual dissection tool was created using diffusion tensor images. White matter tracts were selected using a set of region of interests (ROIs) the fibre pathway had to pass through. These ROIs were selected using white matter atlases constructed by Ludwig and Klingler (1956) based on dissection. Upon qualitative inspection, the reconstructed tracts showed a strong correspondence with the known white matter anatomy of the brain for all the major fibre tracts (Figure 19). Hagmann et al. (2003) used a similar approach to develop a virtual dissection tool and came to the same conclusion that diffusion MR tractography offered a good matching with known anatomical data.

Because most brain atlases, including the ones on white matter structures, tend to be based on a single brain specimen, there might be a difference in overlap between reconstructed pathways and actual white matter tracts due to difference in brain morphology. To properly validate the reconstructed pathways using classical dissection, Lawes et al. (2007) first constructed a white matter atlas from 15 young healthy volunteers to capture intersubject variability in the white matter tracts. These results were then compared to tracts dissected from several postmortem formalin-fixed brains. The results showed quite promising overlaps (Figure 20). However, particularly near the termination sites of a white matter tracts where intersubject variability is high, the results showed a diminished overlap with the dissected tracts.

In addition, diffusion MR tractography can assist careful dissection of brain specimen, as shown by Kier, Staib, Davis, and Bronen (2004). Dissection requires extensive prior knowledge about white matter structure. Using diffusion MR tractography, the dissection can be assisted for precise localisation of brain white matter tracts. Coregistration of the intermediate MR images with the pictures of the specimen as layers of the brain are peeled back plays an essential role for precise localisation of the tracts. Using this method, successful dissection is permitted without extensive prior knowledge and experience, and reduces that chance of accidentally cutting tracts.

Pathology

Closely related to anatomical dissection is the use a pathology to trace white matter tracts in the brain. Assuming diffusion MR tractography traces white matter structures, any changes to these structures should be visible on the scans. Several neurodegenerative diseases are assumed to affect the integrity of the white matter. Such diseases include Alzheimer's disease, multiple sclerosis, and amyotrophic lateral sclerosis. Other processes may introduce changes to white matter structures as well, such as myelination of nerve fibres during childhood and neuroplasticity after stroke or the presence of brain tumours.

Procedure

Nerve fibres tend to degenerate progressively, either starting distal to the site of injury, a processes known as anterograde degeneration, or proximal to the site of injury, called retrograde degeneration. Progressive degeneration have originally been verified by staining methods in histology (Johansen-Berg & Behrens, 2009) which will be covered in the next section. Degeneration can be induced artificially by cutting or damaging axons, but this approach is limited to animal studies. Despite that, this approach has also been applied to postmortem human brains after brain injury (Johansen-Berg & Behrens, 2009)].

Studying patients with progressive neurodegeneration has great potential to identify both the origin and termination site of nerve pathways and possibly deduce polarity of pathways (Johansen-Berg & Behrens, 2009). Where original staining methods were invasive, much like the classical dissection approach, diffusion MR tractography allows in vivo visualisation of neurodegeneration (Ciccarelli et al., 2008). As myelin is progressively degenerated, either anterograde or retrograde, the changes it causes to white matter structures can be visualised with diffusion MR imaging. Pierpaoli et al.

Figure 20. The right temporo-parieto-occipital pathway. (i) reconstructed pathway form diffusion tensor imaging. (ii) the pathway exposed in the partially dissected brain specimen (iii) the white matter tracts extracted from the brain specimen. Adapted from (Lawes et al., 2007)

(2001) showed that Wallerian degeneration can be detected using DTI. Other diseases and processes affecting nerve fibres can be visualised similarly using diffusion MR tractography, e.g. in patients suffering from stroke (Xue et al., 2001) or myelination of nerve fibres during maturation of the brain in childhood (Maas et al., 2004; Knickmeyer et al., 2008). Longitudinal studies of patients with neurodegenerative diseases in combination with a functional evaluation study can reveal the location of entire pathways as they are progressively degenerated or myelinated. Degeneration of white matter can be classified by a decrease in fractional anisotropy and a slight increase in mean diffusivity of voxels affected by the degeneration (Ciccarelli et al., 2008; Jones, 2011). Disease monitoring is usually done by quantifying these measures along a selected reconstructed pathway (Ciccarelli et al., 2008).

Advantages and limitations

Based on the correlation between functional deficiencies and affected white matter pathways we can determine the location of white matter pathways. However, there are limitations to how much we know about the cause, process and effect of neurodegenerative diseases on white matter integrity. This makes studying changes to white matter structures in neurodegenerative diseases difficult. Studying pathology may not be the most accurate measure to validate the precise course of fibre tracts if the exact location of the lesions cannot be determined. It may however prove useful in validation of global functional connectivity studies.

Moreover, there are ethical consideration of putting patients with a debilitating neurodegenerative disease in longitudinal studies. Given the time course and degeneration of quality of life for patients with neurodegenerative diseases, the demands put on by the trials may be strenuous for the patients. An alternative can be found in artificially induced degeneration of neurones. However, these kind of studies are limited to animals only for obvious reasons and a one-on-one mapping of white matter tracts cannot not always be made in translational studies between animals and humans. Regardless of the ethical considerations for animal studies, artificially induced lesions do offer extensive control over the location of neurodegeneration. Additionally, planned termination of the animal's life can be carried out in order to freeze the brain at its current state of degeneration for further post-mortem studies using any of the techniques mentioned in this article.

Histology

Another well-established classical method for studying white matter architecture is the use of histology with additional staining techniques. Histology is the study of structure of tissues using microscopy techniques. For this purpose, a specimen needs to be fixated before it is sliced into sections. A device called a microtome cuts very thin sections with a thickness down to several micrometre from the specimen. These sections are then transferred to a coverslip or other medium for storage and handling prior to inspection. Before analysing the sections, additional staining techniques can be applied to increase to contrast between structures of interest and surrounding structures. Finally, the sections are analysed using light microscopy techniques.

Procedure

Histology operates on ex-vivo specimen that need to be fixated before they are slices into sections. The fixation procedure is similar to that of Klingler's dissection method, except that repeated freezing and thawing of the brain specimen in order to loosen up white matter fibres is not necessary and best avoid to preserve morphology. The brain specimen is either emerged in paraformaldehyde (PFA) or the specimen is perfused with PFA through the cardiovascular system for several weeks until it stiffens. Once the specimen has been set, it can be sliced into sections. The thickness of the sections can vary from a centimetre when slices are cut by hand down to several micrometre when slices with a microtome. Typical histology produces a single two-dimensional image of the surface of a section. Consequently, the thickness and spacing between sections determines the axial resolution of the reconstructed 3D images.

Sections derived from fixated specimen can be studies directly with light imaging techniques such as light cameras or light microscopy. However, the contrast between tissue types may be poor. For improved contrast, additional staining techniques may be applied to the sections prior to inspection.

Many dyes are available to stain specific structures of tissue. For studying white matter architecture in the brain, dyes that target the lipid layers of myelin wrapped around axons can be used. The first myelin staining was developed by Carl Weigert in 1862 (Johansen-Berg & Behrens, 2009). Sections are first treated with chrome and copper solutions which binds primarily to the lipid structure of myelin. After treatment with hematoxylin, the myelin is stained with a deep blue to black colour, making it easy to distinguish myelinated white matter structures from surrounding tissue. Nowadays, Luxol fast blue is a commercially available dye that has replaced Weigert's method for staining myelin. Other neuronal markers, such as Nissl staining or Black-Gold II staining for neuronal cell bodies have also been used (Flint et al., 2010; Hansen et al., 2011).

Another class of staining techniques that is of particular interest to fibre tractography are tracers (Johansen-Berg & Behrens, 2009; Jones, 2011). Tracers are substances that are either actively or passively transported by the neurones or it can be a virus that infects neuronal cells. This property makes them particularly interesting for studying connectivity, but they can also be used to selectively stain a small set of fibres in a region of the brain e.g., in a study by Seehaus et al. (2013). Tracers have to be injected into the specimen prior to fixation. After injection, the substance or virus is taken up by surrounding axons. Depending on the substance use, retrograde or anterograde transportation of the substance labels the entire neurone. Most substance tracers are limited to single neuronal transmission, although most viruses and some substances can be transferred between neurones at synaptic terminals (Ciccarelli et al., 2008). This allows longer tracts to be visualised. Viral tracers require an additional processing step in which the infected neurones are labeled using immunochemistry.

After sections have been stained with dyes or tracers, light imaging techniques can be used to visualise the white matter structure. The human eyes can be used to study sections in which a dye was used to label white matter structures, but a microscope is preferred to study the microscopic structure. A fluorescent microscope is required for analysing sections stained with tracers since the substances and immunochemical labels used in the process are not visible without. The result of the analysis are two-dimensional images of the face of the section in which white matter or select white matter fibres are clearly visible. Another approach is to use laser scanning confocal fluorescence microscopy that allows additional optical sectioning of the section to reconstruct small three-dimensional volumes rather than only surface images.

Advantages and limitations

Histology is a labour-intensive procedure for much the same reasons as dissection. The process is limited to ex-vivo specimen of brains that need to be fixated and processed before sections of the brain can be analysed. The advantage of histology over MRI is the superior spatial resolution that can be achieved. Resolutions higher than MRI can already be achieved using light imaging with a high-quality camera. With the additional use of a light microscope, the resolution can be increased to the nanometre level. Moreover, modern immunochemical staining are highly selective with high affinity for their target. This offers much more certainty about white matter structures than with the indirect probe used in diffusion MRI to measure white matter structures. However, histology requires a well-equipped laboratory, and the use of viral tracers requires the right permissions to use the technique. Some tracers rely on active transportation of the substance. It is uncertain for how long this process continues after death. Consequently, the distance tracers can travel to label neurones is limited to this process.

Figure 21. A tracer has been injected into the cortex of the brain at the upper left corner of the image. The substance is distributed in the vicinity of the injection site, before it is taken up by a fibre pathway. This fibre pathway is clearly visible from the data in the encircled region. Adapted from (Seehaus et al., 2013).

Polarised light imaging

Histological staining methods requires a biological laboratory that is allowed to work with genetically modified materials and that may not always be available at every research institute. Rather than staining histological brain section with antibodies, the same histological sections can be studies using polarised light imaging (H. Axer, Axer, Krings, & Keyserlingk, 2001; M. Axer, Amunts, et al., 2011; M. Axer, Gräßel, et al., 2011; Choe, Stepniewska, Colvin, Ding, & Anderson, 2012).

Procedure

Polarised light imaging relies on the anisotropic structure of the lipid layers in myelinated axons, nerve fibres that exhibit a birefringent property. The birefringent properties of tissue bends the polarisation of linearly polarised light that is transmitted through the section. Using polarisation filters and an additional quarter wave retardation plate, the out-plane inclination and in-plane direction of nerve fibres can be visualised.

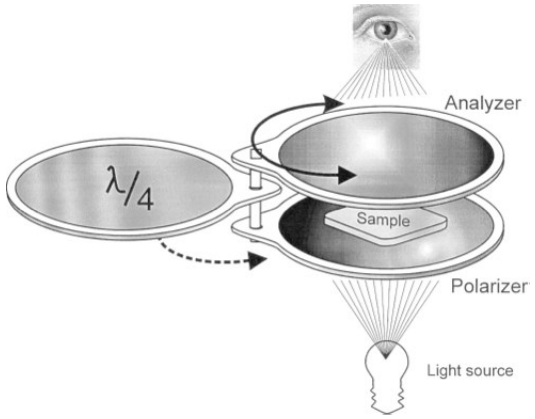

Figure 22. The setup for polarised light imaging. The sample is placed between to opposing polarised that can be rotated while the sample remains fixed. Light is linearly polarised by the polariser beneath the sample, birefringent tissue retards the polarised light, the second polarised filters out only the retarded component of the light, and finally the light is measured with a camera. An additional quarter-wave retardation plate can be inserted after the sample. Adapted from (H. Axer et al., 2001).

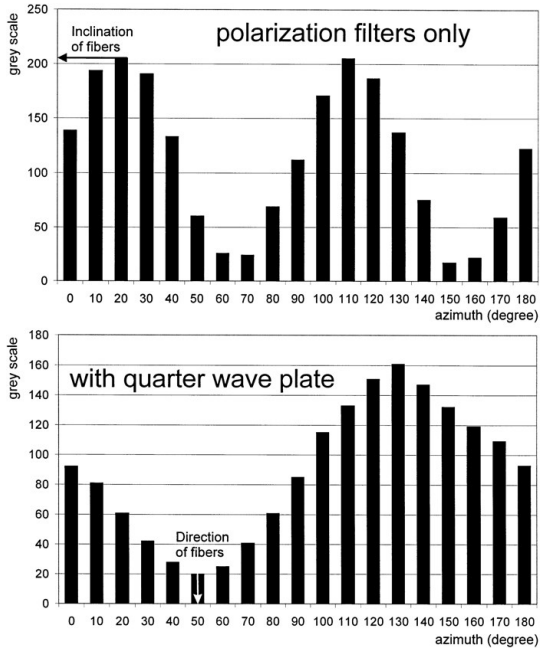

Figure 23. Example of light intensity of a voxel in the specimen measured at discrete angles. In the upper graph, the inclination of the fibre is determined by two global maximum. Hence, ambiguity between the sign of the inclination needs to be solved using a tilting stage (M. Axer, Gräßel, et al., 2011). In the In the lower graph, the direction of the fibre at a given voxel can be determined by the global minimum in measured light intensity. Adapted from (H. Axer et al., 2001).

The result after registration of the separate 2D sections in a depth stack produced a high-resolution 3D vector field that constitute the fibre tracts in the specimen.

The preparation step of obtaining histological section is the same as with staining procedures. The brain specimen needs to be fixated in formaldehyde over a prolonged period. Often, the specimen is encased in gelatine to create a solid block for better alignment during sectioning (M. Axer, Gräßel, et al., 2011). Imaging of the histological section is done by placing the sample between two opposing polarisers, a method known as cross polarisation (Figure 22). Light rays emitted from a light source is linearly polarised by the first polariser. As the polarised light rays pass through the sample, the light rays are retarded by the birefringent properties of the sample. Depending on the angle of the nerve fibre and the density of the nerve fibres, light rays are affected to different proportions. The second polariser is perpendicular to the first polarisers. As light rays hit the second polariser, only the retarded component is allowed to pass through. Finally, the transmitted light is captured by a camera. And additional quart-wavelength plate can be placed between the specimen and the second polariser to add an additional quarter wavelength retardation to the transmitted light.

Light intensity measures are acquired with the cross polarisers at several angles for each histological section. The number of acquisition angles determine the angular resolution of the acquired data. Without the additional quarter-wavelength retardation plate, the global maximum light intensity measured by the camera reflects the out-plane inclination of the nerve fibre for each pixel in the section (upper graph in Figure 23). When using the addition quarter-wavelength retardation plate, the in-plane direction of the nerve fibre for each pixel in the section can be determined by the global minimum in light intensity (lower graph in Figure 23). In standard polarised light imaging, there is an ambiguity in the inclination of the detected fibres; i.e., an inclination of $\alpha \in [0,90] = -\alpha$ (upper graph in Figure 23). To resolve this ambiguity, M. Axer, Gräßel, et al. (2011) used a tilting stage to image the section at different angles to create a stereoscopic view. From this data, the sign of the inclination can be determined.

Two-dimensional maps of fibre inclination and fibre direction can be derived from polarised light imaging at a resolution of up to 100μm (M. Axer, Gräßel, et al., 2011). The results show a striking resemblance to diffusion MR fibre tractography when colour-coded with the same orientation scheme (Figure 24). In fact, using the data acquired by polarised light imaging, slightly modified diffusion tensor imaging fibre tractography algorithm can be applied to the images to trace tracts (Figure 25 (M. Axer, Gräßel, et al., 2011). For quantitative analysis, a robust comparison method needs to be developed to analyse the differences between the two images of different spatial resolution. As described in the previous section on histology, downsampling the polarised light image to the spatial resolution of the diffusion MR image would be a simple and straightforward solution, but one that includes the loss of information from the detailed polarised light images. More advanced comparison techniques are needed to take advantage

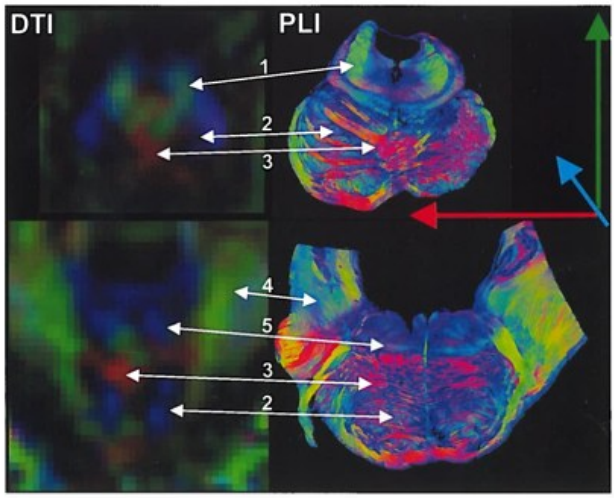

Figure 24. Qualitative comparison between fibre tractography results using diffusion tensor imaging and the results of PLI on the same section of a specimen shows a strong correspondence between fibre orientations. Adapted from (Jones, 2011).

of the increased spatial and angular orientation of polarised light imaging.

The polarised light imaging method has been validated using confocal microscopy by staining the histological sections for myelin with DiI (H. Axer et al., 2001). In the same study synthetic images were used to validate polarised light imaging. It was shown that the inclination and direction of the nerve fibres in the section derived by polarised light imaging conforms with the results of the well-established staining method and the synthetic images. This confirmation with the true anatomy could help to establish polarised light imaging as a solid and robust validation method for fibre tractography results.

Advantages and limitations

The slice thickness of the sections is an important parameters in polarised light imaging. If the slices are too thin, the retardation of the polarised light is too small to be detected by the camera. If the slices are too thick, attenuation and scattering of the photons become an issue. Optimal slice thickness was determined to be 100 μm by H. Axer et al. (2001). This puts a limitation on the axial resolution of this method. Since the total retardation of the light depends on the thickness of the slice, the slice thickness needs to remain constant throughout the experiment and the camera needs to be calibrated for the selected thickness.

Other tissue in the brain may also exhibit the birefringent property. For example, collagen, a substance found in blood vessels, exhibits the birefringent property. Fortunately, the amount of collagen present in cerebral blood vessel in rather small (H. Axer et al., 2001). Still, tissue surrounding nerve fibres can distort the image. Even at higher resolution, the partial volume effect continues to play a role. The orientation of the measured fibres is the result of a summation of fibres passing through the thin section of the specimen. In addition, from stained sections placed under a confocal laser scanning microscope, it is shown that even in white matter structure where the fibres are assumed to run mainly parallel, the architecture of the structure is not homogeneous (M. Axer, Amunts, et al., 2011). Consequently, the measured data are averaged over this heterogenous distribution of nerve fibres.

The resolution of polarised light imaging can be even further improved by using polarised light microscopy. Using this technique, the resolution can be improved up to 1.6 μm for modern day microscopes (M. Axer, Gräßel, et al., 2011). Single nerve axons can be imaged at this resolution that allows clear distinction between crossing fibres (Figure 25). However, a major limitation remain the thickness of the histological section. Even with current microscopy techniques, the slice thickness cannot drop below 20 μm, resulting in anisotropic voxels (M. Axer, Gräßel, et al., 2011). Another disadvantage of polarised microscopy is that only small sections can be imaged at a time, with each dimension of the section up to several mm. Finally, however small the voxel are, partial voluming effect still plays a role. Especially at the boundary between white matter and grey matter, where the partial volume effect may result in steep inclinations or non-existing fibre tracts.

The time it takes to prepare a brain specimen and to image an entire human brain with polarised light imaging remains a major disadvantage of the technique. With polarised light microscopy, the amount of time it takes to image an entire human brain is even worse, as only small sections of the specimen can be imaged at a time. However, a multi-resolution approach can be applied to save time, where only troubling sections with many crossing fibres are analysed using polarised light microscopy while other sections are analysed with polarised light imaging.

Manganese tracer

Finally, there is one last validation methods that deserve mention and that is the manganese tracers. This tracers works similar to substance and viral tracers mentioned in the section on histology, but the added benefit of manganese is that it is visible on MR images. This enables a whole new class of validation studies as it can be performed in vivo.

Procedure

A manganese (Mn^{2+}) solution is injected at a site where it can be taken up by the fibre pathway. In the study by Pautler (2004), the eye of the rodent was chosen as site. This makes injecting the tracer at the correct site more accessible, but limits imaging to the right optical pathway (Figure 26). The manganese tracer is allowed to diffusion over a period of one day before the brain is imaged using T_1-weighted MR imaging. The manganese tracer shortens the relaxation time of the tissue, thereby enhancing its contrast on the T_1-weighted MR image. Subtraction from non-enhanced T_1-weighted images reveals the position of the pathway. Imaging at time intervals can even reveal in which direction the tracer diffuses.

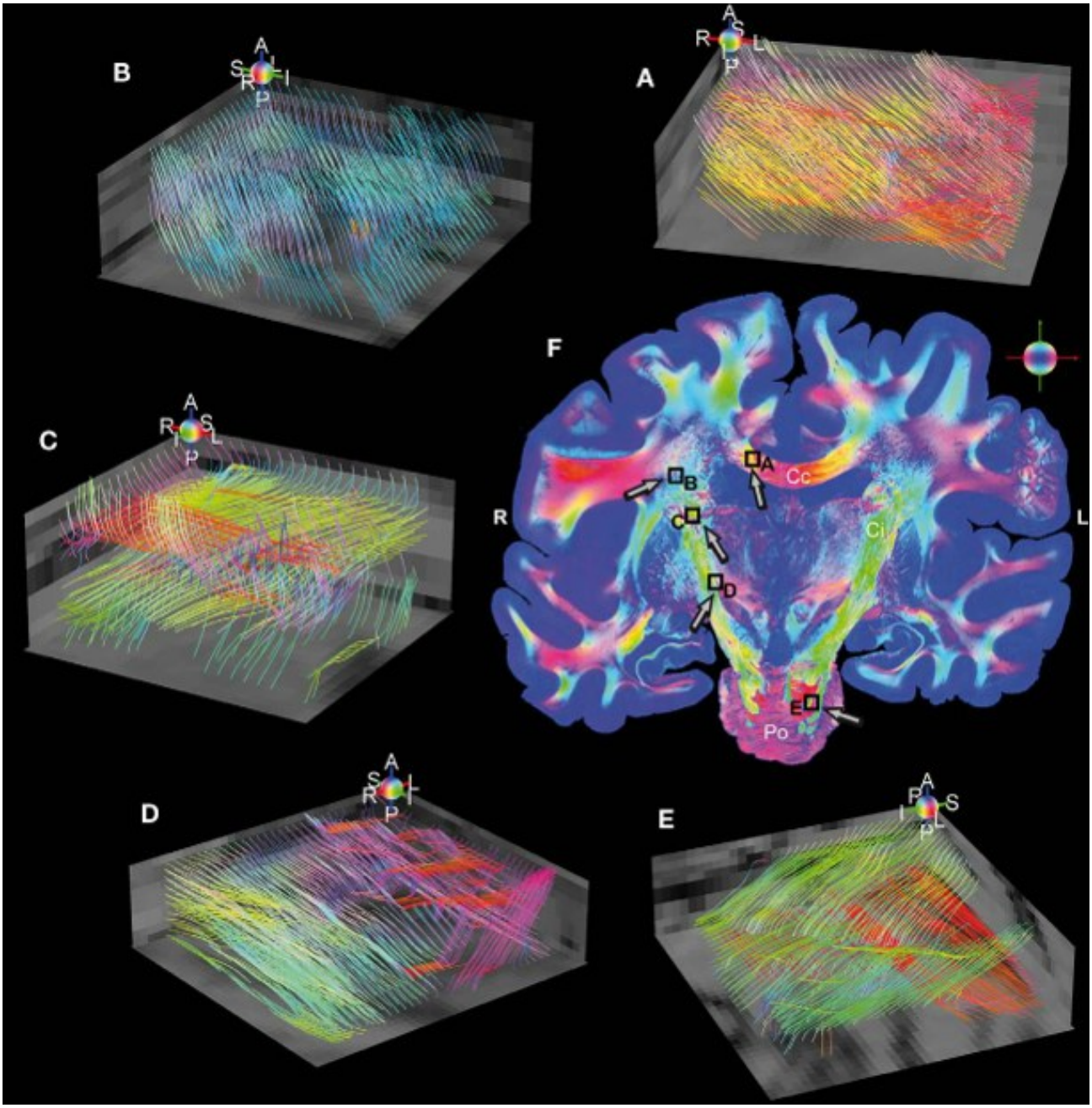

Figure 25. Several sections (A-E) are analysed from a single slice (F) using polarised light microscopy. With a spatial sampling size below the size of a single axon, minor deflections in the fibre tract can be visualised. In particular, appreciate the resolution in the presence of crossing fibres in subfigure C through E. The tracts are reconstructed using slightly modified diffusion tensor imaging tractography algorithms. Adapted from (M. Axer, Gräßel, et al., 2011).

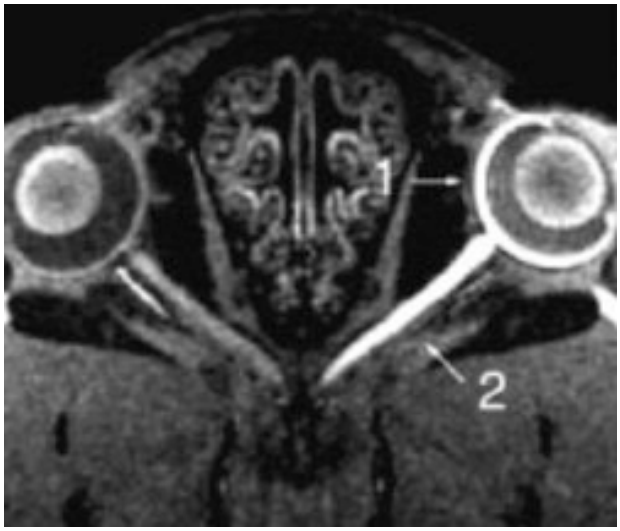

Figure 26. A manganese solution was injected into the right eye. After 24 hours, the retina and optical pathway are enhanced by the manganese tracer. Adapted from (Pautler, 2004).

Advantages and limitations

As already mentioned, the main advantage of the manganese tracer is that it is visible on MR images. Consequently, single pathways can be visualised in-vivo with the use of this tracer at the same time as diffusion MR images are acquired. Where most substance tracers are limited to single neuronal transmission (Ciccarelli et al., 2008), thereby restricting its use to studies that focus on a single axons, manganese can be transferred across synapses (Pautler, 2004). In this study, the entire optical pathway of the rat was studies (Figure 26). The disadvantage is that the entire pathway is enhanced and no distinction can be made between merged fibre pathways. Additional injection sites in the same subject could be used, but may confound the results if the tracers merge onto the same tract. Although manganese tracer is eventually washed out of the system in about three to four days, depending on the concentration and size of the subject. Since the manganese tracer is toxic, its application is limited to animal studies only. This requires translational studies to map the results to human anatomy.

Discussion

In the previous chapter several method for validation of the trajectory of reconstructed fibre tracts in diffusion MR fibre tractography and their individual advantages and limitations have been discussed. In this chapter, the different techniques are compared with each other based on the following criteria: what is measured, at what spatial resolution, specimen preparation, and the complexity of the method. After the discussion on validation methods, the process of image registration of multimodal images and the metric common to all validation methods are also discussed in this chapter. Finally, some speculative ideas for future research are presented.

Validation methods

One of the major problems with diffusion MR fibre tractography is that the probe used to measure white matter architecture is indirect. Diffusion of protons in not only regulated by white matter, but also by surrounding tissues, temperature and viscosity of the medium. Therefore, a quality of a gold standard should be that is accurately measures the desired object of interest. Achieving highly selective probes with high affinity can be a challenging quest in the natural world because of the complexity of nature. Many types of tissue share similar properties. Even though modern staining methods are highly selective and have high affinity, even here false positive may occur. The promising technique of polarised light imaging relies on the birefringent property of myelin sheet, but the birefringent property is also shared by other cellular components such as microtubule. The best approach to deal with this limitation is to use software models, since they only model the object of interest. However, software model tend to be an oversimplification of the real world and actual performance of fibre tractography algorithms should therefore always be tested on realistic data as well.

A second major issue with diffusion MR fibre tractography is the limited spatial resolution that can be achieved even with ultra-high field MR scanners. Partial volume effects are introduced into the measurement as a result of the limited spatial resolution. In turn, the partial volume effect is at the root of the crossing fibre problem. Diffusion tensor microscopy can solves this problem, but this technique is limited to small samples rather than the whole brain. However, it can help in providing a solid ground truth of white matter architecture using the same imaging probes as traditional diffusion tensor imaging. To effectively solve the problem of crossing fibres and establish a ground truth in which the true orientation and direction of fibres is known even in the presence of crossing fibres, an imaging modality at the level of several micrometers is required. Physical phantoms and maganese tracers are both limited to the same spatial resolution since they use MRI as well. The resolution of dissection is tricky to define, but dissecting small fibres bundles continues to remain problematic. Only techniques using microscopy, such as histology and polarised light imaging over the superior resolution that can solve this problem.

Diffusion MRI does offer the advantage of in-vivo three-dimensional imaging. No other technique can match that. This means that most validation studies are performed on post-mortem ex-vivo brain specimens. Postmortem human brains are scarce and using animal brain requires careful ethical considerations. In addition, preparing the brain specimens for analysis takes quite some time and requires special equipment and expertise. More importantly, the diffusion parameters of tissue may alter depending on the postmortem delay of the specimen. If the postmortem brain specimen is used in histology or polarised light imaging, additional sectioning of the brain is required. The major complaint about histology of brain specimens is that sectioning of the specimen introduces

additional artefacts. Although this issue can be regulated with images registration, as is discussed in the next section. Still, brain volumes are reconstructed from two-dimensional slices. Polarised light imaging offers something in between 2D and 3D imaging, as it is also able to measure out-of-plane inclination of fibre orientations. However, this technique still requires sectioning of the specimen, even when the block-face technique is applied.

There is often a tradeoff between the amount of effort required to obtain the ground truth and the precision of tract localisation. Or rather, solving the crossing fibre problem by applying high-resolution whole-brain imaging is a tremendous tasks that produces enormous amounts of data. Although this approach is used today by Amunts et al. (2013) in the BigBrain project, as will be discussed in the section on future research. These high-resolution approaches often also require laboratory equipment and permissions to work with the materials used. In addition, working with these methods often require high levels of expertise, such as is the case in dissection or histology. Actually, high resolution for the entire brain may not be necessary after all. In white matter structures with mostly thick parallel fibres, techniques offering lower spatial resolution but larger volume coverage per unit time could be used. While for complex white matter structures, high resolution images can be used to establish ground truth for these parts. A multi-scale approach may therefore be the best option. Such an approach was used by (M. Axer, Gräßel, et al., 2011) where whole brian slices were images using polarised light imaging and brain region with complex fibre configurations were further analysed using polarised light microscopy (Figure 25).

Finally, an inherent limitation of diffusion MR fibre tractography is the inability to measure polarity of pathways. Although this property becomes only of interest in connectivity studies, it is worthwhile to mention that tracer studies, whether they use histology or manganese, can reveal this kind of information about pathways and may be a valuable addition to such studies but could also be of benefit to reconstructing fibre pathways.

Image registration

Nearly all validation methods, with the exception of software models, work with imaging modality other than MRI to acquire ground truth. In addition, sectioning of the specimen with the microtome may introduce shearing and tearing artefacts that need to be corrected. Consequently, registration of the two types of images is required before they can be used for validation. This holds also true for physical phantoms and maganese tracers where diffusion MR and structural MR images need to be registered.

With physical phantoms, fiducial markers can be added to the frame that holds the artificial fibres. This relatively easy registration of the images with the established ground truth. Validation studies using manganese tracers requires image registration as well. For software models there is often no need for image registration as the simulation can be run in the same spatial coordinate system as the ground truth; i.e., the images are already aligned.

For the other validation methods, in particular histology and polarised light imaging, image registration can be complicated due to orders of magnitude differences in resolution and difference in contrast between tissue types. In addition, three-dimensional volumes need to be reconstructed from the two-dimensional images obtains from the sections in histology and polarised light imaging. For histology, the registration of stained sections with anatomical MRI scans is detailed in a recent manuscript by Stille, Smith, Crum, and Modo (2013). Manually selecting landmarks is a laborious task. However, automated image registration is complicated by the fact that the relationship between contrast of tissue in the different imaging modalities can be complicated to describe. The best alternative would be to use automatically detected landmarks in histological and MR images (Stille et al., 2013). Using this technique, a registration error of approximately 0.3mm can be achieved.

An alternative approach to avoid the need for complex registration procedures is to prepare the ex-vivo specimen as a block face construct, as is done by M. Axer, Gräßel, et al. (2011) and Choe et al. (2012) for polarised light imaging. With block-face imaging, the brain specimen is encased in a transparent solution that will hold the specimen in position during imaging and provide the specimen with additional integrity during sectioning with the microtome. Fiducial markers can be added to the solution to aid the registration procedure. Although this procedure prevents most of the shearing and tearing during microtome sectioning, other deformations from misaligned sections or sections lost in the process can still occur.

When light microscopy is used to image histological slices with either staining methods or polarised light imaging, the process of image registration is even more complicated because only small volumes can be used with light microscopy, with volume sizes of only a couple of cubic millimetres. With the limited resolution of MRI with voxel sizes about the same order of magnitude, automatic registration is likely to fail. The best approach for image registration is still open for discussion, but manual registration remains an option.

Evaluation metric

One more challenges still remaining in validating tractography is defining a proper evaluation metric for comparing the tractography results with the established ground truth. Fibre tractography in its current state is inherently limited by the spatial resolution of MRI and noise. Tractography results are therefore unlikely to exactly coincide with the true anatomy. If we want to compare the performance of fibre tractography algorithms, we not only need to establish a ground truth, but also require a metric to compare the performance.

A common metric in image analysis with respect to segmentation is the use of volume overlap to determine how well

two segmentations coincide, e.g. using the DICE index. However, using volume overlap to compare the results of fibre tractography would be a poor choice since overlap may occur irrespective of the origin, destination and curvature of the tracts. A proper metric should include this information along the full length of the tract.

Several metrics have already been developed for global connectivity assays. For example, in (Côté et al., 2013) several metrics have been introduced for connectivity analysis that makes use of expected ROIs to classify fibres bundles into valid connections, invalid connections, or non connections. Expected ROIs are also used to determine valid and invalid bundles. Although volume overlap may not be the best metric by itself, it can still be used to determine the average bundle coverage for the entire brain. A similar approach of using ROIs for global connectivity has been used in Close et al. (2009), where strands are classified as valid connections if they terminate within respectable distance from the target ROI.

The number of incoming and outgoing connections between nodes and finding the right target may suffice for validation of global connectivity assays. For precise tract localisation, the full length of the fibres need to be validated. In (Barbieri et al., 2011), one such approach is used to measure the fibre-tracking error along the full length of a fibre bundle. A minimum safety radius is determined such that circle with this radius aligned at fibres inside the bundle cover the cross section of the modelled fibre bundle. This safety radius is calculated at regular intervals along the fibre bundle.

However, the approach by Barbieri et al. (2011) is rather computational intensify, as it requires computation of the Voronoi diagram at each cross section. Depending on the output of the tractography algorithm, the mean squared difference between the reconstructed tracts and the ground truth may be sufficient for tracts represented by piecewise curvilinear functions or b-splines (J. Li & Wunsche, 2005). An addition step of curve fitting might be needed for voxel-based representations to convert the data into piecewise curvilinear functions or b-splines.

This relatively simple but robust evaluation metric has also been used in (Tournier et al., 2002). Four metric are defined as (i) the absolute difference in distance between the end-points of the reconstructed tract and the ground truth, (ii) the root-mean-square difference in distance between points along the tracts, (iii) the maximum difference in distance from points along the tracts, and (iv) a success rate classification based on the maximum deviation from the tract. Other properties of tracts, such as the total length of the tract (Leemans et al., 2005), can also be used as an evaluation metric.

Future research

Advances in ultra-high field MRI enables visualisation of the brain at increasing resolution in limited amount of time. Future progress in this field can aid advances in fibre tractography by improving the resolution of voxels with crossing fibres. However, for clinical applications, the resolution of MRI is inherently limited by the available scan time and signal-to-noise ratio of diffusion MR. In the previous section on histological methods we have already seen what ultra-high field MRI can reveal about white matter structures when applied to small volumes of brain specimen in diffusion tensor microscopy (Figure 25). For whole-brain diffusion MRI of the human brain, the level of resolution from diffusion tensor microscopy may be beyond the reach in clinical application, with current spatial resolution at 7 tesla limited to about 0.5mm and in experimental setups at 11.7 tesla limited to 125-255μm (Aggarwal et al., 2013). This expresses the continuing need to establish tools for validating diffusion MR fibre tractography in the presence of crossing fibres.

In the previous section we have seen that for validation of tractography we either have to move to simplistic models constructed at macroscopic scale, or we have to move a microscopic analysis of anatomical samples. The latter has preference if the tractography algorithms are to be used in clinical settings. Dealing with microscopic analysis has its downsides. For one, only small volumes can be processed at a time. Moreover, most techniques described in the article use 2D sections for the microscopic analysis. Since fibres tend to run in all three dimensions, these approached severely limit the accuracy of the ground truth, even if a 3D volume is reconstructed from the 2D sections. Only polarised light imaging, and polarised light microscopy for improved resolution, offer a certain degree of three dimensional imaging of a 2D slices of the specimen. That is, the technique can measure the in-plane direction of fibres as well as the out-of-plane inclination of fibres.

There is one promising combination of techniques that, as far as the author knows is only a speculative application to validation of diffusion MR fibre tractography, holds merit to move whole-brain microscopic analysis to true three-dimensional analysis. A recently developed technique called CLARITY (Chung et al., 2013; Chung & Deisseroth, 2013) that removes lipid structures from organs. This morphological modifica-

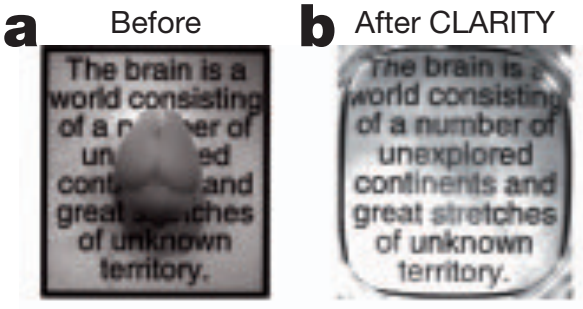

Figure 27. Example of a rodent brain before CLARITY is applied (a), and the rodent brain after CLARITY has been applied (b). The removal of lipids from the organ makes the structure transparent to light, allowing deeper penetration of photons in light microscopy imaging. Adapted from (Chung et al., 2013).

tion turn the entire organ, for example the brain, transparent to the eye (Figure 27). Using multi-photon confocal light microscopy, the entire organ can be imaged in all three dimensions without having to section the specimen. This results in true 3D imaging at microscopy level. At the current stage of the technique, it is restricted to small volumes like the rodent brain due to limitations on the lipid removal from structure deep inside the organ and limitations on the depth of penetration of photons for light microscopy. The disadvantage to using CLARITY is that it requires extensive preparation of the brain specimen prior to imaging. During the preparation, lipids are removed from the organ that may alter the morphology of the organ. In particular, there are some concerns about the lipid myelin sheets wrapped around axons. This problem can be avoid by a surprising simple alternative of soaking the organ in sucrose solution that turn the organ transparent without altering its morphology using a technique called SeeDB (Ke, Fujimoto, & Imai, 2013). Together with the BrainBow technique (Livet et al., 2007; Lichtman, Livet, & Sanes, 2008), that uses a transgenic lineage of a species that expresses a whole range of fluorophores with different colour for individual neurones, it enables tracing of neuronal circuits at microscopic level (Figure 28). Although this approach is incredibly time consuming, it would allow the creation of brain maps of both neuronal localisation and connectivity in exquisite detail. Combined with pre-preparation and post-preparation high resolution diffusion MR images of the specimen, a near-golden standard could be established for validation of diffusion MR fibre tractography.

An alternative approach is high-resolution micro-optical sectioning tomography with additional staining for neuronal structures. It has previously been applied to the rodent brain (A. Li et al., 2010) and more recently to the human brain as well (Amunts et al., 2013). This automatic approach to sectioning large volumes enables the creation of highly detailed maps of the brain at unprecedented resolution in the range of 1μm for the rodent brain and 10μm for the human brain. However, regardless of the resolution, this technique is still very much time consuming, the brain is still sectioned into 2D slices and the large volume of data is complicated to analyse. Although polarised light imaging could be used in addition to standard optical microscopy to extract out-of-plane fibre inclination to reconstruct a proper 3D volume of the 2D sections.

One of the methods that may seem to be missing from the list is cryogenic transmission electron microscopy (Glaeser, 2008). Although this technique is known for its exquisite spatial resolution beyond the range of light microscopy. It is most often used only for imaging of subcellular components. This level of resolution is probably not needed for fibre tractography. In addition, the disadvantage of using electron microscopy is the destructive properties of the electron beam on the specimen and again it requires 2D sectioning of the specimen. Thereby deeming it unnecessary and unsuitable as a validation method for diffusion MR fibre tractography.

Finally, there is an post-processing step to diffusion MR fibre tractography that holds promise for super-resolution imaging of white matter structures. This technique, called track-density imaging or track-weighted imaging, allows tracks to be visualised up to a resolution of 125μm using clinical high-field MR scanners at 3 tesla (Figure 29) (Calamante et al., 2010). However, since the technique relies on the same method we want to validate the accuracy for, it would be contradictory to use this technique as a validation method. Still, the techniques faces the same problems as diffusion MR fibre tractography, and they could both benefit from their progress in established a proper ground truth for validation. Several

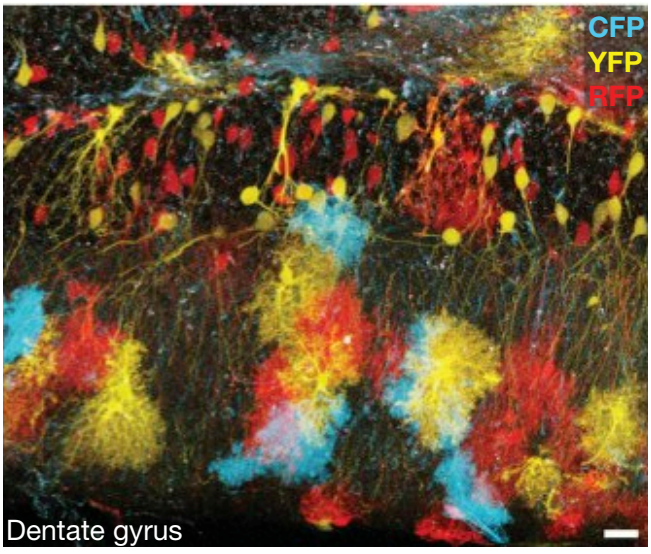

Figure 28. Example of a neuronal network stained with the BrainBow colouring technique in the dental gyros of a rodent brain. Adapted from (Livet et al., 2007).

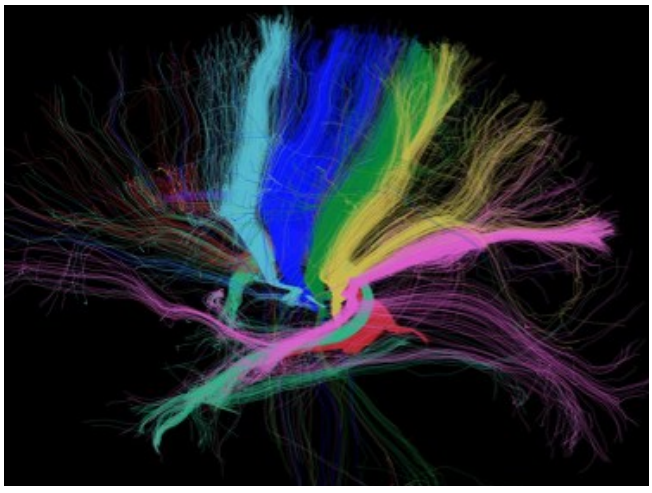

Figure 29. Example of the spatial resolution that can be achieved in super-resolution track-density imaging of tracts seeded from the human thalamus with seven manually selected ROIs. Adapted from (Calamante et al., 2010)

attempts have been made to validate the correctness of the super-resolution property for this technique (Calamante et al., 2011, 2012). One way that this technique could be used is to use the post-processed super-resolution track-density images and compare them to polarised light images of the same resolution, rather than downsampling high-resolution microscopy images to the scale of diffusion MR images.

Conclusion

Diffusion MR fibre tractography is the only method available to date to non-invasively measure white matter structures in the living human brain. For this reason, it is an indispensable tool for analysing white matter pathology in diseases and disorders. However, due to its intrinsic limitations on resolution and signal-to-noise ratio, proper validation of tractography results is essential before it can be used reliably in the clinic.

In this review, a concise overview of various methods for validation of the precise anatomical course of white matter tracts measured with diffusion MR fibre tractography is provided. No one method excels above all others. The choice of the right method often depends on the resources available to the researchers and to what extend validation is necessary. Higher precision in establishing ground truth requires methods that have an intrinsic higher resolution, such as microscopy or histology compared to physical phantoms, dissection or MR-visible tracers, but these methods are often more labour intensive. Software phantoms may provide a practical solution since it can generate large datasets of ground truth with only few resources. But like physical phantoms, the major drawback is the lack of realism, since the data is only as good as the model used to generate the data.

There is one particular method that stands out between all others. Polarised light imaging, including polarised light microscopy, shows a lot of promise. Although this method can also only be applied to postmortem brains, its relatively easy setup to scan whole sections of the brain and the amount of information that can be derived from the high-resolution realistic data is encouraging. In particular, the option to use additional staining of viral tracers on the same sections and the ability to use light microscopy on sections with complex fibre configurations is a valuable combination.

Acknowledgments

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Note: references with the title typeset in **boldface** are used as primary literature for this master thesis. All other references are used as secondary literature.

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