Strategies and Challenges in DTI Analysis

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Learning Points

- DTI analysis forms only one part of a DTI study and is mutually dependent on other stages in the DTI pipeline, such as data acquisition.
- There are many different approaches for analyzing DTI and the most optimal method depends on the goal(s) of the DTI investigation.
- DTI analysis methods can be categorized into three main classes: whole-brain, regional, and voxel-based approaches.
- There are pros and cons in all DTI analysis approaches, and there is no single best or worst analysis method, but a range of techniques that are more or less suited to any given application.

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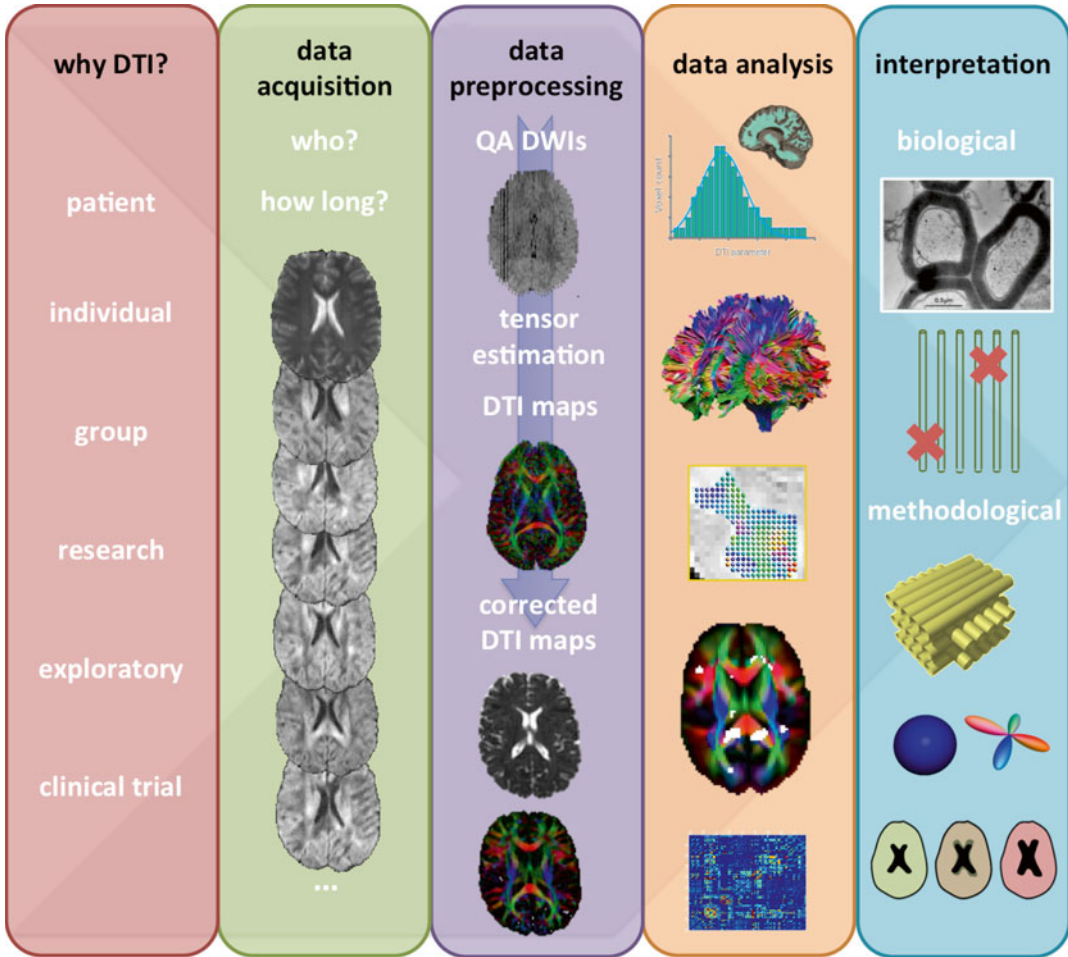Universitair Psychiatrisch Centrum (UPC) - KU
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- Many software packages and tools are available to process and analyse DTI data, which vary considerably in functionality.
- The broad range of analysis approaches and heterogeneous functionality in software packages contributes to a lack of standardization that complicates the analysis of DTI data and the interpretation of results.

Introduction to DTI Analysis

Since its introduction, DTI has been used to study microstructural tissue changes in a wide range of neurologic and psychiatric disorders, as well as in normal development and ageing [1]. Many approaches have been proposed to extract DTI measures from the data and compare them across subjects. As each of these methods have some advantages and limitations, the most optimal analysis approach will depend on the clinical and research questions that need answering. Furthermore, the limitations of the selected method should be considered during the interpretation of the results. This chapter provides a brief overview of the different options that are available for the analysis of DTI data. In the following chapters, more detailed information is provided about three main analysis techniques, i.e., region of interest analysis (Chap. 9), voxel-based analysis (Chap. 10), and tractography and connectivity analysis (Chap. 11).

Fig. 8.1 Prototypal DTI study pipeline. Whole-brain tractogram and connectivity matrix. [Reprinted from Caeyenberghs K, Leemans A, Leunissen I, Gooijers J, Michiels K, Sunaert S, et al. Altered structural networks and executive deficits in traumatic brain injury patients. Brain Struct Funct. 2014 Jan;219(1):193–209. With permission from Springer Verlag]. Voxel-based analysis figure. [Adapted from Emsell L, Langan C, Van Hecke W,Barker GJ, Leemans A, Sunaert S, et al. White matter differences in euthymic bipolar I disorder: a combined magnetic resonance imaging and diffusion tensor imaging voxel-based study. Bipolar Disord. 2013 Jun;15(4):365–376. With permission from John Wiley & Sons.]Axon micrograph. [Reprinted from Beaulieu C. The basis of anisotropic water diffusion in the nervous system—a technical review. NMR Biomed. 2002 Nov–Dec;15 (7–8):435–455. With permission from John Wiley & Sons, Inc.] Corrected DTI maps. [Courtesy of A. Leemans]

Analyzing DTI data is only one part of the whole DTI processing pipeline. Figure 8.1 summarizes a prototypal DTI pipeline, from the goal of the DTI study, through to data acquisition, data analysis, and interpretation of the results. As highlighted in Chap. 2 and discussed in more detail in the chapters of Section 2, many choices have to be made at each step of this pipeline. Note that these different steps are not independent of each other; for example, the most optimal analysis technique will depend on the quality of the data and how it is acquired.

Why Do We Need to Analyze DTI Data?

Diffusion-weighted imaging (DWI) is widely used in clinical practice as it provides unique, rapidly accessible information that can be used in the assessment of ischaemic stroke, to differentiate vasogenic versus cytotoxic oedema and to characterize intracranial lesions such as pyogenic abscess, infections, tumors, and trauma [2]. However, whilst the processing of DWI data is relatively easy, the analysis of DTI data is significantly more complex. For example, the need for more diffusion-weighted images makes the acquisition longer and more challenging. In addition, motion correction becomes more important, and the tensor estimation is more complex compared to ADC calculations. There are also more techniques available for analyzing DTI data compared to DWI. In clinical practice, DWI information, typically the DWI and ADC maps, is interpreted visually by a radiologist. It has been demonstrated that DTI can be useful in evaluating changes in the normal appearing white matter. However, qualitative assessment of DTI information, such as FA maps, may be more difficult there.

To illustrate the challenge of qualitatively assessing scalar DTI maps, consider the axial color-encoded FA maps in Fig. 8.2. This random assortment of images comprises seven pairs of axial slices generated from patients with pathology that has been associated with changes in white matter microstructure, and two healthy subjects. There are two patients with tinnitus, two with cerebral palsy, two with multiple sclerosis,

Fig. 8.2 A matching puzzle with DTI. Match the axial colour-encoded FA maps with the correct pathology. In addition to two healthy subjects, there are two images of patients with tinnitus, cerebral palsy, multiple sclerosis, schizophrenia, Alzheimer's disease, spinocerebellar ataxia, and amyotrophic lateral sclerosis
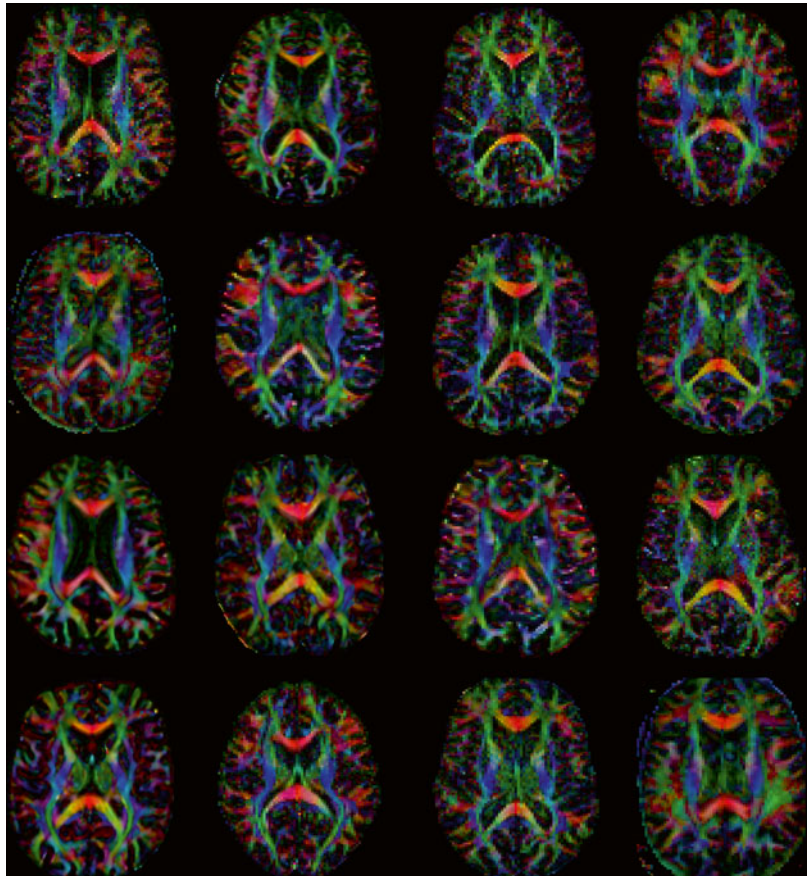
two with schizophrenia, two with Alzheimer's disease, two with spinocerebellar ataxia and two with amyotrophic lateral sclerosis. Is it possible to match the FA maps with the correct pathology and identify the healthy controls?

For all subjects, a similar axial slice was selected. Data from the subjects with the same pathology were acquired using the same protocol in the same study, whereas data from subjects with different pathologies were acquired in different studies (and therefore mostly with different acquisition protocols). Hence, it may be possible to match some subjects based on image quality or based on prior knowledge about the presence of neurodegeneration and ventriculomegaly in some of these disorders. However, when these factors are excluded from the visual assessment of the data, it becomes very difficult to match the pathology to the DTI data. This demonstrates firstly, that changes in FA that occur due to pathology are not always readily visualised on colour FA maps, and secondly, that such FA changes are not specific to one particular disorder. Although visual assessment of colour FA maps can be useful, in general, there is a need for reliable quantitative analysis methods that allow meaningful conclusions to be drawn from the DTI data.

DTI Analysis Techniques

Many different DTI analysis techniques and approaches have been applied to study a range of pathologies and include region of interest analysis, tractography, histogram analysis, atlas-based segmentation, quantification of graph-based connectivity networks, and voxel-based analysis to name but a few. Each of these techniques has its own strengths and limitations and there is no single technique that can be regarded as superior to all the others. The most optimal analysis approach depends on many factors, including:

- The purpose of the analysis (e.g., to delineate a known fibre bundle, to explore the data)
- Whether it is for a single subject or group comparison
- If there is a hypothesis about the location and extent of change or difference in DTI measures
- The data acquisition protocol (e.g., # of directions, b-value, voxel size)
- The data quality
- …

For simplicity, the different techniques that are available to analyze DTI data sets can be classified into three categories:

- Whole-brain analyses
- Region-specific analyses
- Voxel-based analyses

This subdivision of analysis techniques is based on the scale that is used to evaluate the DTI measures in the brain. As shown in Fig. 8.3, DTI analysis can be performed at the level of the whole brain (Fig. 8.3a), at a regional level (Fig. 8.3b), or at the smallest scale, i.e., the voxel (Fig. 8.3c).
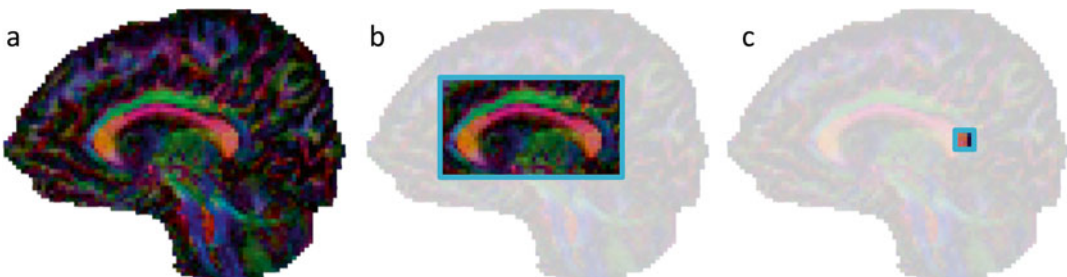

Fig. 8.3 Subdividing DTI analysis methods into three parts: whole-brain analysis approaches (**a**), region-specific analysis methods (**b**), and voxel-based analysis methods (**c**)

In most voxel-based analysis approaches, DTI measures are evaluated at the voxel level, but at the same time in every voxel of the brain. As such, this method can also be regarded as a whole-brain analysis technique. This next section provides a brief overview of each of these major classes of DTI analysis methods.

Whole-Brain Analysis Techniques

The general concept of whole brain DTI analysis techniques is to obtain quantitative DTI measures from all the voxels that include brain white matter, and can thus be subdivided into two parts (see Fig. 8.4):

- An approach to define which voxels are part of the brain white matter
- An approach to extract relevant DTI information from these voxels

The selection of the voxels to be included in the analysis can be done using brain segmentations from anatomical MRI data sets (located in the same image space as the DTI data) or by performing whole-brain tractography. In whole-brain tractography, all brain voxels are used as seed regions to start the tractography process. Using specific parameter constraints such as an FA and curvature threshold, the tracts will mainly traverse white matter voxels, as the FA is lower in grey matter and cerebrospinal fluid.

Once the voxels are selected, the DTI information can be extracted. If anatomical MR based segmentations are used, it is important to ensure that the anatomical image and the DTI data set are located in the same space. It is therefore necessary to register both images to each other (image registration is introduced in Chap. 10). Usually, the anatomical image is transformed to the non diffusion-weighted image using a rigid-body or affine transformation. As all the diffusion-weighted images should already be in the same space as the non-diffusion-weighted image (done during the motion correc-
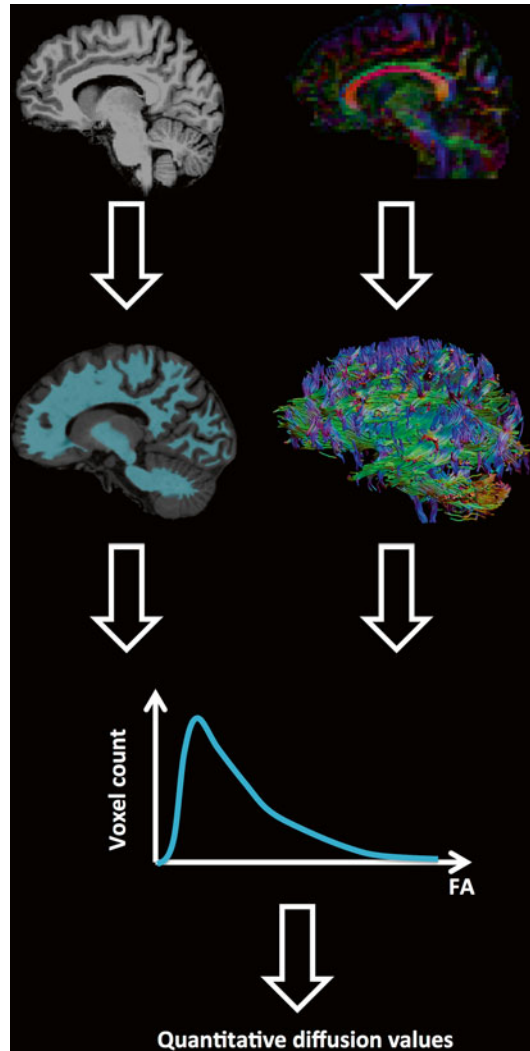

Fig. 8.4 An example of whole-brain analysis of DTI measures. Brain or white matter voxels are defined by a mask created from either an anatomical MRI segmentation or by performing whole brain tractography. A histogram of the diffusion values in these voxels is obtained and relevant information can be extracted and compared

tion, see Chap. 7), the calculated tensors and diffusion metrics will also be aligned with the anatomical MRI. Extracting the diffusion information after whole-brain tractography doesn't involve image registration with an anatomical MR image. DTI measures from voxels that contain a streamline from the whole-brain tractography result will be selected.

Histogram Analysis

Once the DTI measures have been extracted from the selected voxels of interest, they can be summarized using a histogram (see Fig. 8.4). This histogram is a frequency distribution that displays the number of voxels with a specific value of the diffusion measure (e.g., FA). From this histogram, the following parameters can be extracted:

- Mean or median of the diffusion measure values
- The peak height of the histogram: voxel count of the value that is present the most
- The peak location of the histogram: the diffusion measure value that is present the most in the data set

Usually, studies will only obtain the mean or median value of the diffusion measure. The resulting values can then be statistically compared across groups of subjects or correlated with other variables, such as clinical, neuropsychological or other test scores.

Whole-brain analysis of DTI data has the following strengths and limitations:

Strengths
- Does not require prior knowledge of where hypothesized differences could be found
- Less reliant on user intervention than other approaches
- Results obtained quickly, without labor-intensive interventions
- Fewer statistical tests (i.e., multiple comparisons), compared to other techniques as only one set of diffusion measures is obtained for the whole brain

Limitations
- Regional information is lost as DTI measures are averaged over the whole-brain white matter
- Results are sensitive to partial volume effects due to atrophy
- Results can depend on segmentation/registration accuracy or whole-brain tractography parameters

Region-Specific Analysis Techniques

In region specific analysis techniques, diffusion measures are obtained in one or more predefined areas of the brain. DTI measures, such as the FA and MD are thus statistically evaluated in an anatomical region or white matter tract reconstruction. There are two main approaches:

- Region of interest analysis
- Tractography analysis

Region of Interest Analysis

In *region of interest (ROI) analysis*, diffusion measures are obtained from a specific brain region, which is defined by manual delineation or by automated segmentation or parcellation. As automated segmentations are less observer dependent and thus more reproducible, they have some clear advantages over manual delineations. However, automated segmentations are not always appropriate, for example due to ill-defined boundaries in regions of pathology.

Manual delineation of ROIs is typically performed by freehand drawing of the region or by placing basic shapes such as circles or squares on 2D slices. Due to the manual interaction that is needed, the results are observer dependent. In addition, manually delineating specific regions in a group of subjects is time consuming. This is especially the case when white matter fibre bundles need to be delineated, as they run through several slices, and thus many 2D ROIs need to be drawn in order to delineate as much of the bundle as possible. Ideally, ROIs should be drawn on maps that are independent of the diffusion measures of interest. For example if FA maps are used to delineate regions, and the FA is a measure of interest, a bias can be introduced in the results because ROIs are typically drawn around regions with a higher FA. However, FA might be lower in areas of pathology, which could then be excluded from the analysis, thereby artificially decreasing differences with the control group. In contrast, regions delineated on an anatomical MR (T1/T2) image are drawn independently of the diffusion measures that will be analyzed. However, this approach also has some potential
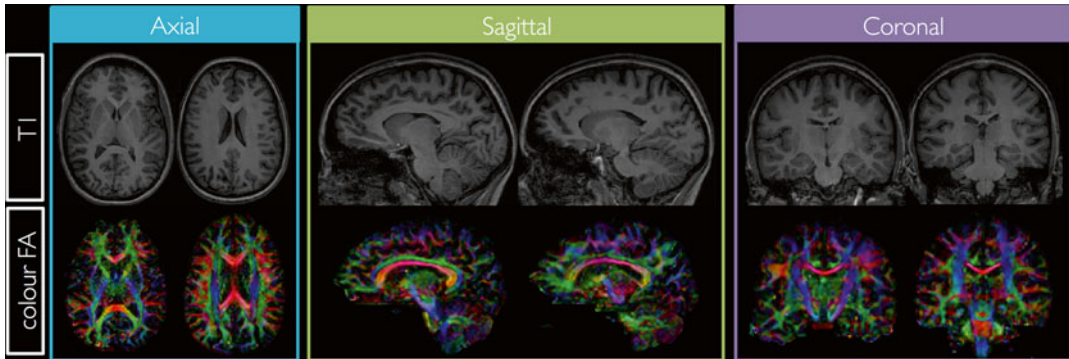
Fig. 8.5 Axial, sagittal, and coronal slices of a T1 weighted image and the color-encoded FA maps of a healthy subject

limitations, as the anatomical MRI data set needs to be registered accurately to the DTI data set, which is not always straightforward due to different distortions in both images [3]. An alternative approach is to delineate the regions on the non-diffusion-weighted image, which should be in the same space as the quantitative diffusion maps after motion correction. However, the delineation of white matter bundles on either the anatomical scans or non-diffusion-weighted images is confounded by the lack of orientational contrast (which is provided by the color FA map). This is illustrated in Fig. 8.5, which shows axial, sagittal, and coronal slices of a T1-weighted image and corresponding color-coded FA slices of a healthy subject

In the presence of lesions, ROI analysis (actually all DTI analyses) can become challenging. This is demonstrated in Fig. 8.6, which illustrates axial non-diffusion-weighted and color-encoded FA slices from five patients with cerebral palsy. The lesions in the left hemisphere clearly affect the visualization of the corticospinal tract (CST). Reliably comparing diffusion values from the left CST with the contralateral CST in this population or of a healthy population would be difficult. For example, delineating the ROI based on the color-encoded FA maps can be biased by the lower FA values in the lesion. However, drawing the ROI on the non-diffusion-weighted image, which is independent from the diffusion measures, is also challenging.

Instead of delineating regions and structures manually, automatic segmentation methods can be used. Such automated methods are especially useful when structures or lesions can be accurately segmented on the anatomical MRI. As an example, T2 lesions could be segmented in a patient with multiple sclerosis. After registering the T1/T2 MR image to the DTI data set and applying the deformation field to the segmented lesion masks, DTI measures can be derived from these lesions. Bear in mind that these results will strongly depend on the segmentation and registration accuracy, especially when some of the lesions are small. In addition, the resolution of the DTI image is typically lower than the resolution of the anatomical MRI that is used for the segmentation, leading to partial volume effects. Finally, note that it is not easy to obtain automatic segmentations of white matter tracts based on anatomical MR images.

Region-specific analysis of DTI data by using the ROI approach has the following advantages and limitations:

Strengths
- In comparison to whole-brain analyses, more regionally specific information is obtained
- Manual delineation is closer to the original data than other techniques which require more complex modeling and image processing
- ROI analysis is less dependent on parameter settings than tractography or voxel-based analysis
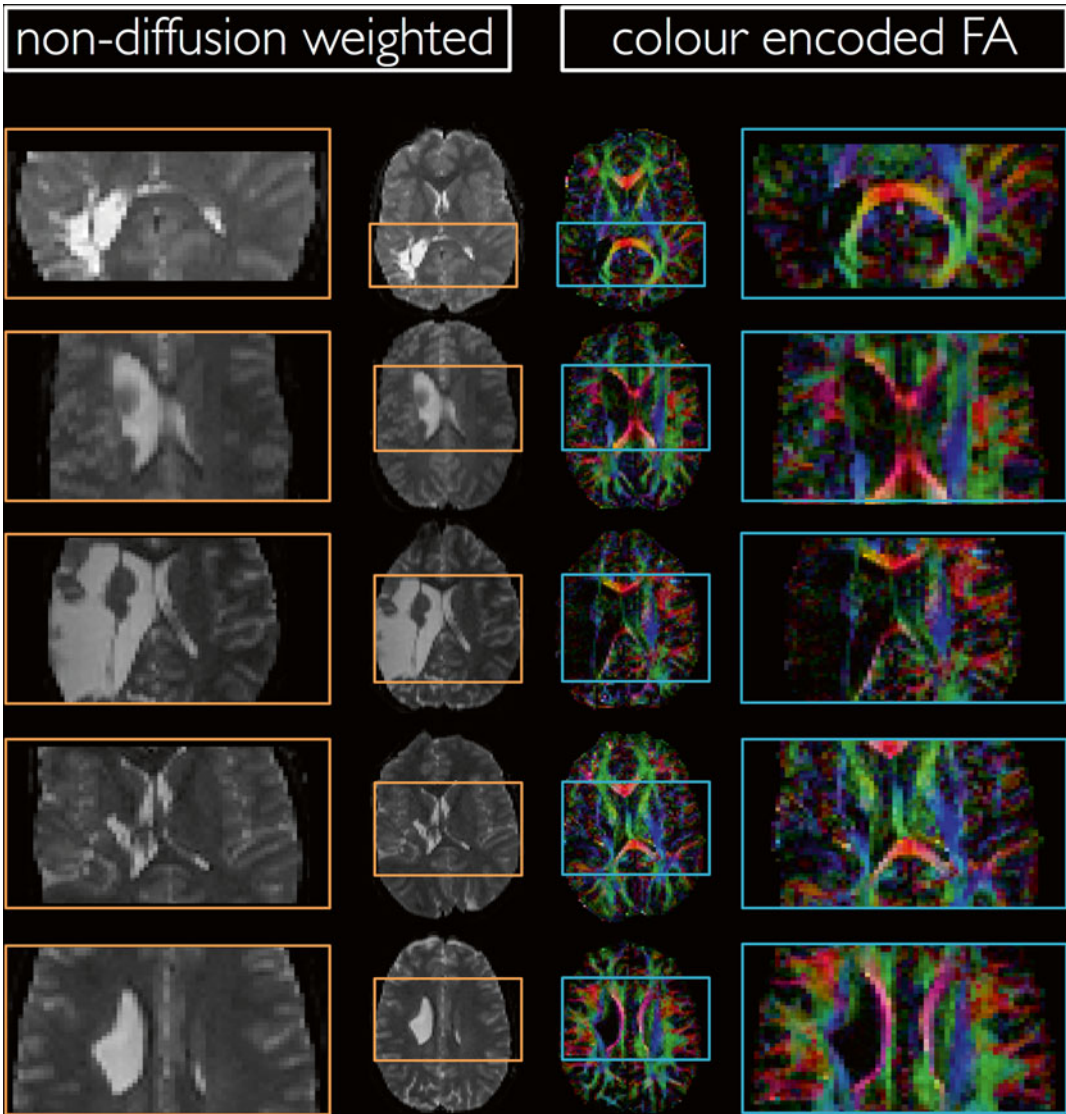
Fig. 8.6 Axial non-diffusion-weighted and color-encoded FA slices in five patients with cerebral palsy. The presence of lesions make ROI delineation challenging

Limitations
- Requires a prior hypothesis about where differences could be found, as that is where the ROI will be placed.
- Intra- and inter-observer reproducibility of results should be assessed, as manual delineation is subjective.
- Requires clear guidelines that describe how the ROI should be defined (e.g., size, anatomical location, boundaries).
- The selection of many ROIs increases the number of statistical tests that are performed and therefore correction for multiple comparisons is required.

- Results can be biased if ROIs are drawn on the parameter map of the measure of interest, e.g., drawing an ROI on a color FA map when investigating FA.
- Results can depend on segmentation/registration accuracy when ROIs are delineated on anatomical MR images.
- Delineating regions manually is very time consuming and laborious.
- Excludes (potentially valuable) information from regions that are not selected/studied.
- Drawing an ROI or segmenting a structure can be challenging in the presence of pathology.

Tractography Analysis

The delineation of white matter tracts using only 2D manually drawn ROIs or anatomical MR images is not optimal for the reasons outlined previously. However, by using the inherent directional diffusion information in the DTI data set, virtual representations of white matter fibre bundles can be reconstructed, using *tractography* (or "fibre tracking"). Tractography refers to the mathematical reconstruction of white matter fibre bundle representations by integrating the local diffusion tensor information from every voxel. In its simplest form, tractography can be compared with a puzzle "connecting the dots." As shown in Fig. 8.7, by following the letters alphabetically, and drawing lines between subsequent letters, one can complete the drawing and the global picture.

How diffusion tractography relates to "connecting the dots," is shown in Fig. 8.8. Instead of the alphabet and the natural sequence of letters, the orientational diffusion information can be followed and connected to create a more global picture of the white matter bundle. Consider two voxels in the brain, i.e., the green and blue voxels that are shown in Fig. 8.8a. The DTI data can be used to estimate tensors in every voxel. Recall that these tensors can be represented by an ellipsoid whose longest axis represents the direction of maximal diffusion. For visualization purposes, only the relevant tensors between the green and blue voxels are displayed, as shown in Fig. 8.8b. As explained in Section 2 of this book
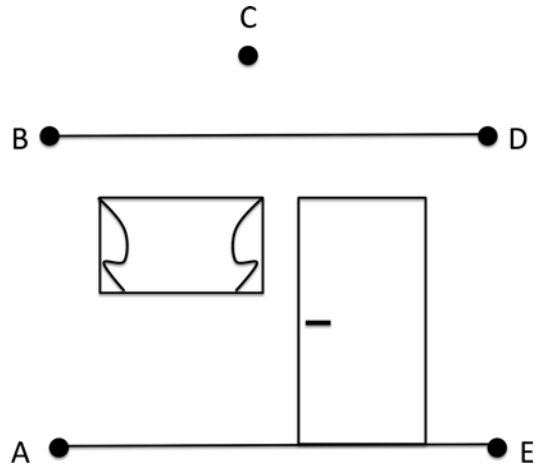

Fig. 8.7 Connecting the dots: by following the alphabet and drawing a line between subsequent letters, the global picture (i.e., a house) becomes clear

(Chaps. 3–5), the orientation of the estimated tensor is assumed to relate to the underlying white matter architecture, as the amount of diffusion along the axonal bundles will be greater compared to the amount of diffusion perpendicular to them. DTI tractography is based on the assumption that by following the maximal amount of diffusion in a given direction (i.e., the longitudinal axis of the ellipsoid) in each voxel, the orientation of axon bundles can be followed, and hence the tensors provide an indirect, simplistic, discrete representation of white matter fibre pathways, as shown in Fig. 8.8c. In practice, these assumptions suffer major flaws, which are discussed in detail in several other chapters (see especially Chaps. 5, 11 and 21).

If the tractography process is started in the green voxel A (referred to as the seed voxel), the main direction of diffusion is followed, until a new voxel is reached (voxel B in Fig. 8.8d). This process is then repeated until a certain stop criterion is reached. Typical tracking initiation and termination criteria are based on selection and exclusion ROIs, and FA, fibre length and curvature thresholds. For example, tracking may be stopped when the FA in a voxel is below 0.2, to prevent streamlines going into low anisotropy grey matter or CSF. These ROIs and thresholds determine the number of streamlines and how

Fig. 8.8 A simplified
example or diffusion tensor
tractography. Two voxels
are selected in the brain
(**a**) and the relevant tensors
in between the voxels are
visualized (**b**). As these
tensors are representations
of the underlying white
matter axonal bundles (**c**),
they can be used to
mathematically reconstruct
virtual representations of
these bundles (**d** and **e**)
[Courtesy of A. Leemans]
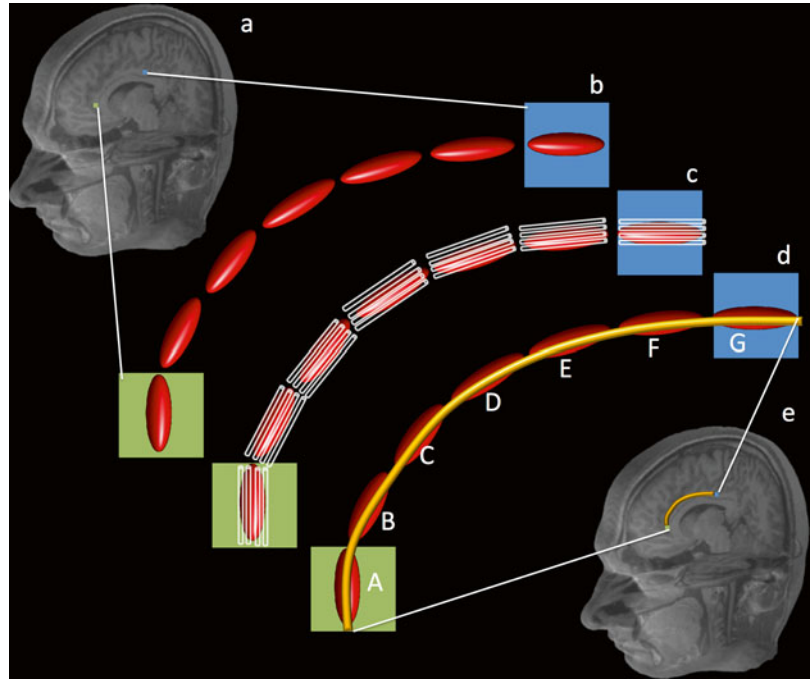
they travel through the data, and hence the final tract reconstruction. It is therefore important to realize that tractography is both operator and parameter dependent, and there is no 'ground-truth' solution to validate tracking results.

In the example of Fig. 8.8, tracking ends in the blue voxel, as shown in Fig. 8.8d. The pathway from seed voxel to end point can be represented by a streamline. In this simplified case, a single the streamline represents a fibre tract (see the orange line in Fig. 8.8e, representing part of the cingulum); however in practice, many stream-lines make up a fibre tract.

It is worth noting that some of the terminology used in tractography can be confusing. In tractography, a fibre, streamline or track is *not* synony-mous with an actual nerve fibre in the biological sense, and a fibre tract is not synonymous with an anatomical fibre bundle (even in the case of fibre bundles that include "tract" in their anatomical name, such as the corticospinal tract!). These concepts are explained in more detail in Chap. 11. In this context, it is very important to under-stand that the resulting fibre tracts are virtual

mathematical reconstructions that bear some resemblance to parts of axonal bundles. Therefore the thickness, length or number of these recon-structed tracts cannot be *directly* related to the underlying microstructure or anatomy.

As tractography uses directional diffusion information to reconstruct connections in the brain, it is an elegant technique for obtaining dif-fusion measures from specific white matter bun-dles. One of the most useful and common applications of tractography is the noninvasive, virtual dissection of fibre bundles in 3D, i.e., seg-mentation. The segmented tract is equivalent to a 3D ROI from which diffusion measures can be calculated. This obviates the need to delineate the bundle manually by using 2D ROIs on different slices or to apply segmentation methods to ana-tomical MR images, which contain less specific white matter tract information. Typically, the average of the DTI measure, e.g., FA, is calcu-lated from all voxels that are part of the delin-eated tract. A less commonly used, but useful strategy is to also measure the value at predefined points or along the length of the bundle. Such

Fig. 8.9 An example of a tractography analysis. A sagittal slice is selected (**a**) to draw a seed region for tractography (**b**). Diffusion measures can then be calculated from the resulting tracts (**c**)
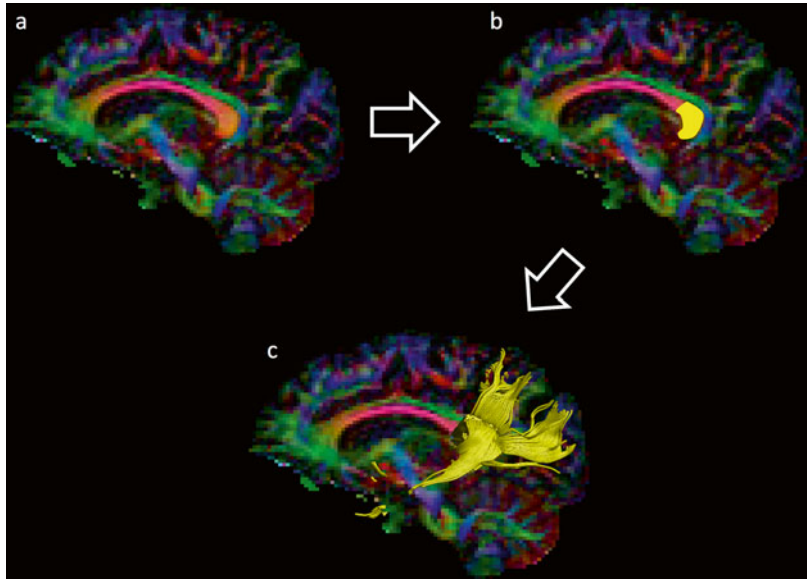
tract profiles or distributions may reveal more localized differences that are lost when averaging over the length of the tract. Some people refer to this as "tractometry" [4].

An example of a tractography analysis is shown in Fig. 8.9. Starting from a sagittal color-encoded FA slice (Fig. 8.9a), a region of interest is drawn as a seed region for tractography (Fig. 8.9b). The resulting tracts, in this case a representation of the splenium of the corpus callosum, are shown in Fig. 8.9c. Diffusion measures can then be extracted from these tracts and compared across subject groups or correlated with clinical or neuropsychological scores.

Region specific analysis of DTI data using tractography has the following strengths and limitations:

Strengths
- In comparison to whole brain tractography or histogram analyses, more regionally specific information is obtained.
- Tractography provides an intuitive way of reconstructing 3D virtual representations of white matter bundles in vivo using diffusion information.

- As typically only very few ROIs are necessary to calculate the tracts, it is in general more reproducible compared to ROI-based methods.

Limitations
- Requires a prior hypothesis about where differences could be found as DTI measures will only be analyzed in the tracts that are reconstructed.
- Tract reconstructions depend on many parameters.
- Tractography results are often affected by the "crossing-fibre" problem.
- In non-automated methods, the use of manually defined ROIs for tract selection means that tractography results are observer dependent. Ideally, clear guidelines should be followed regarding ROI placement.
- Noise and other artifacts affect tract reconstruction, and therefore the selection of voxels that will be used in the analysis.
- The selection of many tracts increases the number of statistical tests that are performed and therefore correction for multiple comparisons is required.

- Pathology can affect the tractography result, again potentially creating a bias.
- There is no ground truth to validate tractography results.

Note that region-specific analyses can also be performed using automated approaches and templates/atlases. This will be discussed in the next section on voxel-based analysis.

Voxel-Based Analysis

One of the advantages of region-specific analyses compared to a whole-brain analysis is that information can be obtained from specific brain areas of interest. As such, the obtained DTI measures have the potential of being more sensitive (not averaged out over the whole brain) as well as specific (localized changes might be related to a certain pathology). Voxel-based analysis techniques take this idea further by evaluating and comparing DTI measures at the smallest imaging scale possible, i.e., the individual voxel. At the same time, DTI measures are compared in all voxels, so this analysis method could also be regarded as a whole-brain analysis technique.

One of the main challenges of any group analysis, and particularly voxel-based analysis, is selecting spatially corresponding voxels across subjects to compare the DTI values. If this condition is not satisfied, it does not make sense to compare the voxel measures. The process of aligning corresponding voxels in different data sets is referred to as image registration, and is an important step in the voxel-based analysis pipeline. Between-subject image registration is especially challenging because the brains of different subjects can vary in size and shape at the global as well as local level. However, when correspondence between images can be achieved at the voxel level, voxel-based analysis is a powerful tool to analyze DTI data. Since it is highly automated, there is no need for an a priori hypothesis about the location of anticipated changes, and the observer dependence of the results is minimized.

Typically, a voxel-based analysis pipeline consists of the following steps:

1. Selection of the atlas/template space to which all data will be aligned
2. Alignment of all data to this atlas using global and local registration methods
3. Smoothing of the aligned data sets
4. Statistical analysis in every voxel

For each of these steps, there are a number of choices to be made, both in terms of selecting the appropriate approach as well as choosing the specific parameters that will be used. As it has been shown that voxel-based analysis results depend on these choices, every step of the pipeline should be considered with care and parameter selections should be justified.

An overview of VBA is provided in Fig. 8.10. Voxel-based comparison of FA values is performed for two groups of subjects, each consisting of five subjects. In Fig. 8.10c, d. Although these images are warped during spatial alignment, the registration process ensures that voxels in the spatially aligned images retain the same quantitative diffusion values as in the original data, thereby allowing statistical comparisons to be made. Depending on the type of VBA implementation used, the warped images may be smoothed, for example to increase signal-to-noise in the parameter maps (smoothing is discussed in detail in Chap. 10). A specific voxel with the same x, y, and z coordinates in the atlas space is then selected across subjects and subject groups (as shown by the blue and green lines in Fig. 8.10c, d). The FA values from the different subjects in that voxel can be visualized by a histogram (as shown in Fig. 8.10e). FA values in that specific voxel can then be compared statistically between the groups. When statistical significance is reached, the voxel can be given a colour, as shown in white in Fig. 8.10f). This process of statistical testing of FA values between groups is repeated for every voxel, resulting in a VBA map that displays the voxels and regions in which a statistical difference is found between the groups.
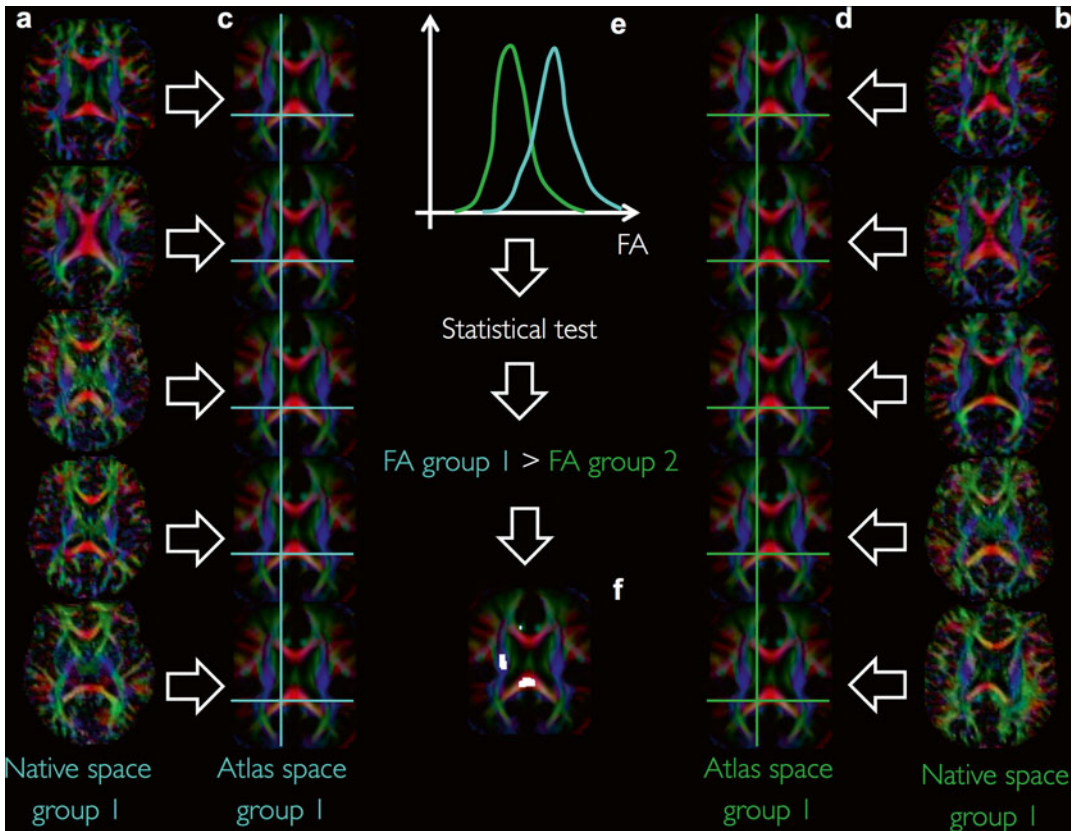
Fig. 8.10 An example of a voxel-based analysis of two groups of five subjects. The original data sets (**a** and **b**) are transformed from their native space to the atlas space (**c** and **d**). Within each voxel of the registered data sets the diffusion measures, such as the FA value, can be evaluated statistically (**e**). Statistically significant voxels are then highlighted, for example by labeling with a specific color (here, *white*) or by coloring according to a test statistic. This provides a visual map of group differences (**f**)

As there are many thousands of voxels in a typical DTI parameter image, many thousands of statistical tests need to be performed in VBA, making it necessary to perform some sort of correction for multiple comparisons, to reduce the number of false positive findings. The number of statistical tests can be reduced by limiting the analysis to, for example:

- White matter
- Manually drawn regions in atlas space
- Specific regions, as derived from atlas parcellations
- Specific white matter tracts, by performing tractography in atlas space (tensor information should then be available in the atlas)

These "hybrid" analysis methods combine the strengths of the different analysis techniques and try to avoid specific limitations of them.

Voxel-based analysis of DTI data has the following advantages and limitations:

Advantages
- The data is analyzed at the smallest scale, i.e., at the voxel level.
- The whole brain is evaluated as all voxels are included in the analysis.
- No a priori hypothesis about the location of the expected differences is needed.
- The manual observer interaction and therefore the observer dependence of the results is minimized.

Fig. 8.11 Capabilities and limitations of different analysis approaches

Limitations

- Results depend on the parameters that are chosen in the voxel-based analysis pipeline.
- As statistical analysis is performed in every voxel, there is a chance of false positive findings and multiple comparison correction should be applied.
- Diffusion measures are compared in every voxel, not in specific tracts.
- Results are only meaningful when accurate image registration can be achieved.
- Pathology and lesions can affect the results, especially when the location of the lesions is variable across subjects.

Choosing an Optimal Analysis Approach

Unfortunately, there is no single DTI analysis approach that is optimal for evaluating diffusion MRI measures for all studies and purposes. As different analysis techniques each have their own strengths and weaknesses, and rely on various assumptions, choosing the most optimal analysis approach for a given purpose is an important step in the DTI pipeline. For example, Fig. 8.11 provides a summary of what can and cannot be done using different analysis approaches.

In this section, a short and non-exhaustive overview of factors and guidelines is provided to help select the best analysis technique(s) for different applications. Ideally, these considerations should be made *before* acquiring the data. A much more detailed overview of factors that need to be considered when using DTI in clinical populations can be found in Chap. 13. It is important to stress that these guidelines are not prescriptive and the choice of which methodology to choose ultimately rests with the DTI user. The important point is that each choice should be appropriately reasoned and justified.

Things to Consider before Starting DTI Data Analysis

Goal and Hypothesis

The choice of which DTI analysis technique to apply will depend on the *general goal* of using DTI, i.e. whether the data will be used for a group study in a research setting or for individual patient analysis in clinical practice. For example:

- In research studies, typically, a longer DTI acquisition can be performed compared to the clinical routine, which can impact the selection of a DTI analysis approach. For example, some of the more advanced tractography techniques (see Chaps. 11 and 20) require the acquisition of a large number of diffusion-weighted images acquired along different gradient directions.
- Not all DTI analysis techniques can be easily applied in individual patients, e.g., voxel-based analyses.
- The use of DTI for an individual patient in clinical practice requires the use of CE/FDA approved software, thereby limiting the possible DTI analysis options.

The presence or absence of a *specific hypothesis* about the nature and/or location of the expected diffusion changes can also influence the selection of an appropriate analysis technique.

- *Region-specific DTI analysis* methods can be used to evaluate the diffusion measures in areas where changes are expected. When differences are hypothesized to be present in specific white matter bundles, fibre tractography can be used to reconstruct virtual approximations of these pathways. To evaluate the diffusion measures in lesions or specific parts of a white matter bundle, region of interest analysis can be applied.
- *Voxel-based analysis* can be used for exploratory studies or if no clear hypothesis can be made about the location of the expected differences in diffusion parameters. Recall that in a voxel-based analysis, it is assumed that the changes in the diffusion measures occur in similar regions of the brain in different patients. This is unlikely to be the case in many clinical populations, e.g., traumatic brain injury.
- *Whole-brain analysis* methods can be applied if more global diffusion changes are expected or if the location of diffusion changes is heterogeneous between patients.

The Study Population

With regard to the study population, the following factors should be considered:

- *Population composition*: Can the patient group be regarded as one homogeneous group, or does it need to be subdivided into different subgroups? Is there a need for a matched healthy control group?
- *Population size*: How many subjects should be included in each group in order to be able to draw meaningful conclusions? This will depend on the magnitude of expected differences or changes in diffusion parameters. For example is the amount of change likely to be statistically or visually detectable given the unavoidable presence of noise or artifacts in the data?
- *Population characteristics*: Different factors, such as age, gender, IQ, handedness, etc. may affect the diffusion measures. Different subject

groups should therefore be carefully matched with respect to these factors. For example, if children or elderly subjects are scanned, the choice of which DTI analysis technique to apply can be affected, because:

– The DTI acquisition time may be shorter and data quality may be affected by increased subject motion.
– Of differential rates of brain structural change due to development or neurodegeneration.
– Image registration of DTI data from children or elderly to an adult atlas can introduce errors, which will therefore impact analysis techniques that don't make use of appropriate population atlases [5].

• *Population pathology*: The presence and nature of brain lesions can complicate DTI analysis by distorting normal anatomy (see Fig. 8.6), and hindering image registration and tractography. The degree to which analysis will be affected or the affect on method selection will depend on:

– If the lesions are focal or diffuse
– The size and location of the lesion/s
– The number of lesions
– Variability of location across patients

– In addition to the presence of lesions, neurodegeneration can affect DTI analysis and interpretation, because of:

– The increased presence of partial volume effects
– The challenges associated with image registration to a healthy adult atlas

The Data Acquisition

Data quality and data analysis are affected by the choice of *DTI acquisition* parameters such as the:

• Number of diffusion directions
• Image resolution
• *b*-Value
• Number of *b*-values
• Number of averages

For example, the tensor estimation, tractography result and image registration result depend on the data quality, which depends on which DTI acquisition parameters are chosen. Some types of analysis techniques, such as tractography are indeed more suited to acquisition schemes with more gradient directions. In longitudinal or multicenter studies, the scanner performance and acquisition parameters should be monitored. A DTI hardware phantom is useful for quantifying data quality over time and across centers.

The Resources

The following resources should be considered with regard to DTI analysis:

• *People*: Which clinical and technical/software expertise is present or needed to perform the analysis and interpret the results?
• *Time*: How much scan time is available to obtain the DTI data? Is there time to evaluate different analysis approaches and compare the results, or perform labour-intensive analysis methods such as a region of interest analysis or non-automated fibre-tracking? Is there time to run complex computational processes that may take hours or several days to complete, or are results required immediately?
• *Software and hardware for analysis*: Will the data be processed on the scanner or off-line on a separate computer or server? Which software will be used?
• *Money*: Is there money available to buy specific software packages or licenses, to acquire enough data sets, or to outsource part of the analysis?

Selecting an Optimal DTI Analysis Approach

When faced with so many factors to consider, it is easy to become overwhelmed with choices and lose sight of the reason for acquiring DTI data in the first place. The decision scheme in Fig. 8.12 therefore aims to guide the DTI user in choosing which type of analysis technique to
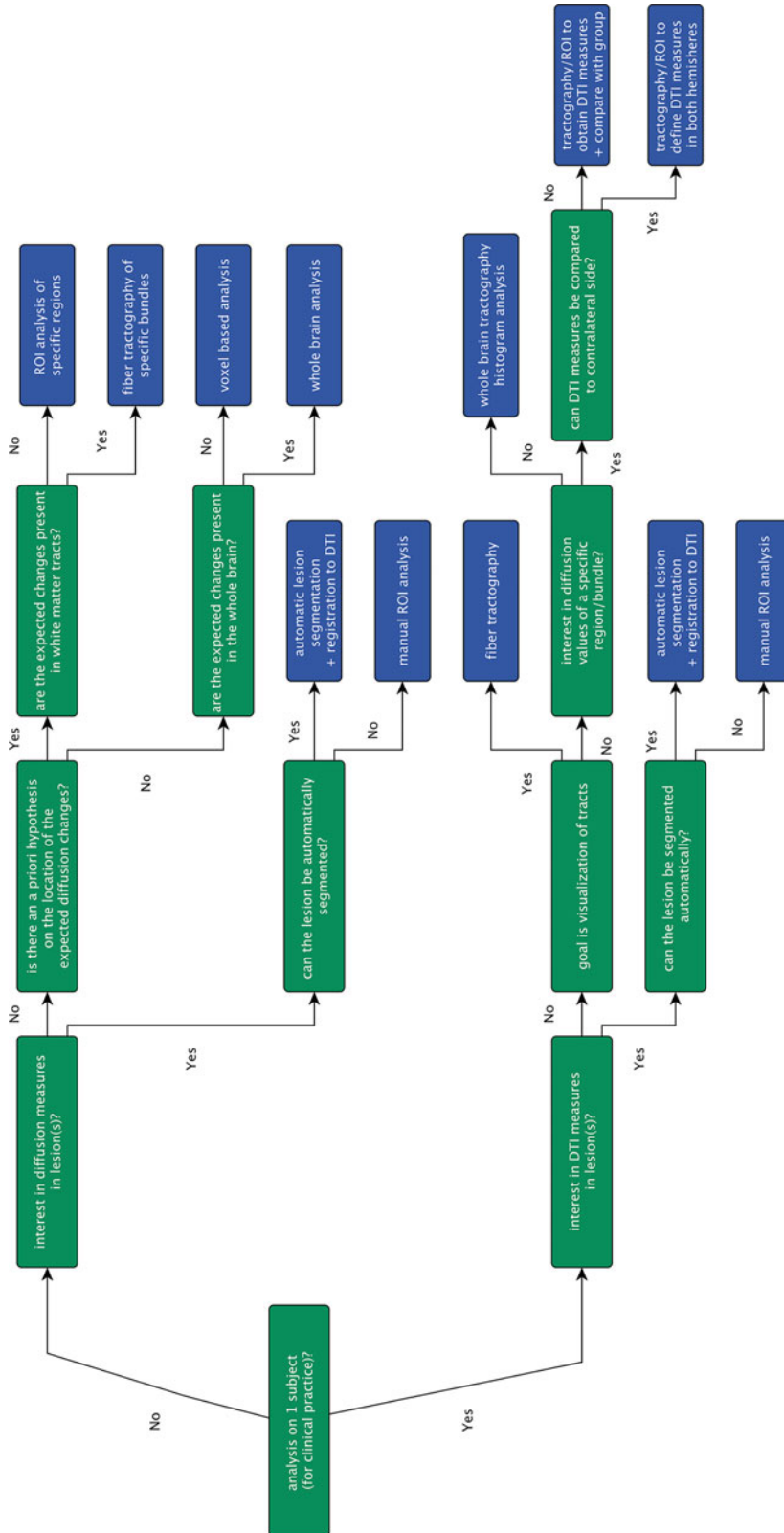
Fig. 8.12 A decision scheme to assist with DTI analysis method selection based on the aim of the DTI study

use according to the initial goal of the DTI investigation.

It is important to stress that this decision scheme does not provide a complete and comprehensive overview of all the relevant questions that could be asked when choosing a DTI analysis method, nor does it provide formal solutions or strict answers. Figure 8.12 should be interpreted as an example of how knowledge about the different analysis options and their pitfalls can be incorporated into an informed decision process, which can assist in the selection of a specific analysis approach.

Selecting a Software Package to Analyze the Data

Many DTI software packages are available, all with different functionalities, ranging from data import, basic image viewing and processing, image quality correction, registration, automatic segmentation, and DTI tractography to higher order diffusion modeling and advanced tractography. Most of the packages can perform the fundamental pre-processing needed for DTI analysis, such as tensor estimation, and visualization of scalar diffusion maps and glyphs. However, the specific approaches for preprocessing, e.g., the mathematical model for tensor estimation, and motion and artifact correction methods, can differ. In addition, there are a wide range of different options and approaches for tractography, which vary according to the algorithms used in the software package and the parameters that can be chosen to control them.

This inconsistency between different DTI analysis tools is further complicated by the use of different terminology for both the same and different operations across packages. It is (unfortunately) possible to perform (apparently) the same analysis using (apparently) the same parameters on the same dataset and obtain different results when using different packages [6], or even using different software versions of the same package. This is because of (sometimes subtle) differences in the way the software

developers have integrated the continuously evolving theoretical methods that underlie DTI data processing into their applications, as well as the way their code interacts with different software programs and operating systems. Not only does this add to the challenges of interpreting findings, but means that it is extremely important to use the same package and software version for the analysis of all the datasets in the same study. This is particularly important in longitudinal investigations and may require analyzing new data with older software, or preferably, all the data with the most up-to-date software version.

In this context, it is also important to become familiar with the different parameter settings and how changing them affects the final results. Although most packages provide reasonable default settings, the most optimal results may require some empirical parameter adjustment. This is particularly relevant in VBA and tractography-based analysis, and indeed in any analysis employing image registration (including data correction strategies). Chapter 11 provides some compelling visual examples of how changing just a single parameter can drastically alter tractography results.

New DTI software packages and tools are continuously being released whilst older ones are being developed to incorporate new features, bug fixes, and enhancements. For this reason, specific DTI software tools and their functionality are not listed in this chapter. Instead, we recommend consulting The Neuroimaging Informatics Tools and Resources Clearinghouse (www.nitrc.org) and 'I do Imaging' (www.idoimaging.com) websites which list many of the latest noncommercially developed DTI software. Details about proprietary DTI vendor software can be obtained from the respective MRI scanner manufacturer applications specialists. Further details about DTI analysis software can be found in Chap. 13, including topics such as data storage, export/import and file formats, version control, and licensing. Figure 8.13 provides a summary checklist of considerations related to choosing DTI analysis software.

Fig. 8.13 Checklist of considerations and features to DTI analysis software

- Is it approved for clinical use? (e.g. FDA approved)
- Has its use been validated scientifically? (e.g. in peer-reviewed publications)
- Is it easy to install and run?
- What system requirements does it need? (e.g. operating system, memory, processer etc.)
- Does it require additional software (including specific libraries/scripts etc.) to run?
- How much does it cost?
- Is there any technical support available? (e.g. instruction manual, mailing list, contact person or helpdesk)
- How much technical support is available? (e.g. level of detail in manual, activity on mailing list, responsiveness of development team)
- Are change-logs provided that document changes to the source code and software functionality between versions or following software upgrades?
- Is it compatible with or does it generate files that are compatible with other commonly used software packages?
- Is it command-line based or use a graphical user interface?
- Is the source code available to view or modify? (e.g. for trouble-shooting, development and to customize for in-house use or public distribution)
- Is it possible to check the data at each stage of the analysis pipeline?
- Does it include any of the following features?
    - Image viewer (2D / 3D)
    - DICOM or Vendor format file import
    - Data quality assessment and / or correction tools
    - A choice of tensor estimation strategies
    - Histogram analysis tools
    - Region-of-interest analysis tools
    - Tractography analysis tools
    - Group analysis tools (e.g. VBA, atlas-based tractography)
    - Other image processing tools (e.g. registration, editing etc.)

Conclusion

In conclusion, the analysis of DTI data sets forms only one part of a DTI study. In each phase of a DTI investigation, choices and decisions have to be made. The most optimal analysis approach will therefore depend on the decisions made in earlier stages of the DTI pipeline.

There are many options available for analyzing DTI data sets, ranging from whole brain to regional and voxel-based analysis. Knowing the

advantages and pitfalls of each analysis technique can help with selecting the best strategy for a given application.

The following chapters in this section provide a more detailed overview of the most commonly used DTI analysis techniques.

Appendix: Memory Game Solution

In Fig. 8.14, the solution to the memory game in Fig. 8.2 is provided. Was it possible to match the pathologies with the FA maps?
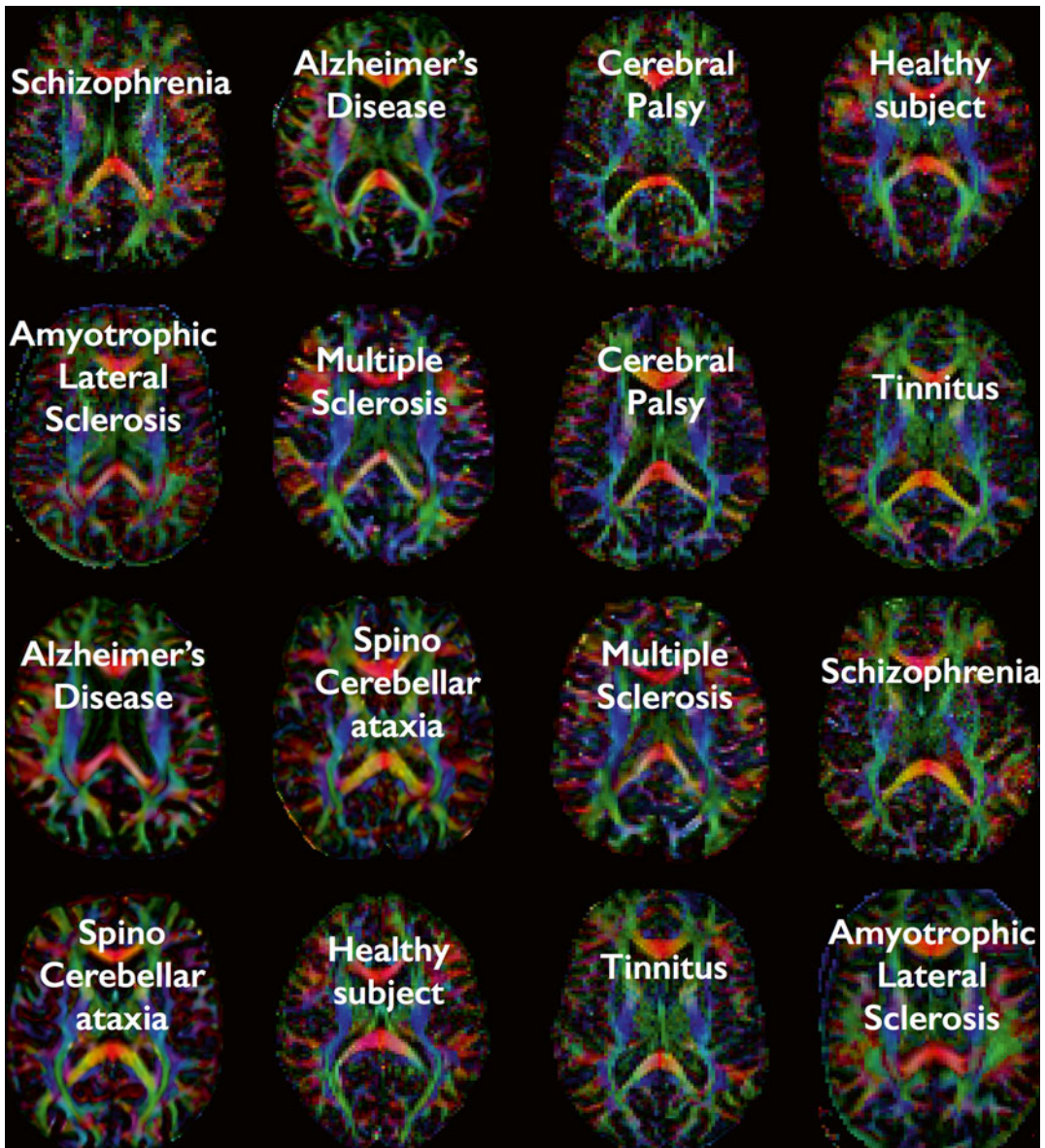

Fig. 8.14 Solution to the DTI memory game

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Suggested Reading

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