TAMPERE UNIVERSITY OF TECHNOLOGY
Department of Biomedical Engineering

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THE EFFECT OF MAGNETIC FIELD STRENGTH ON THE DETECTION OF MULTIPLE SCLEROSIS USING TRACT-BASED SPATIAL STATISTICS

Master of Science Thesis

Supervisors: Professor Hannu Eskola and Ullamari Hakulinen M.Sc.

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ABSTRACT

TAMPERE UNIVERSITY OF TECHNOLOGY
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In recent years, diffusion MRI has been instrumental in detecting microstructural changes early enough inside the brain for diagnosis of neurological diseases. The change in diffusion metrics is used as a measure for identification of pathological abnormalities. Diffusion MRI has been used for detection of multiple sclerosis (MS), a neurological problem occurring due to environmental factors and immune system attack against the central nervous system (CNS) which comprises of brain, spinal cord, and optic nerves.

In this research, the effect of magnetic field strength was tested by comparing control subjects of 1.5T and 3T diffusion MRIs (DMRIs) with the patients who had 1.5T diffusion MRIs for clinically isolated syndromes (CIS) indicative of multiple sclerosis (MS).The experiment was divided into three groups and all comparisons were done by Tract-Based Spatial Statistics (TBSS) method with relation to age.

Tract based spatial statistics (TBSS) is a method which is used to compare two diffusion MRI image groups. It is a method which uses the advantage of voxel based morphometry (VBM) and tractography that is by being fully automated and cover full area without specifying tracts and solving alignment and smoothing issues. In TBSS, the first process is preprocessing the images for possible artifacts like eddy current. Next is aligning the images nonlinearly using affine transformation using medium degree of freedom. This stage is important to create mean FA skeleton which is used as a frame work for comparison between image groups. Skeletonisation is done by applying 'thinning' (non-maximum-suppression perpendicular to the local tract structure). Then Project each FA data onto the skeleton by filling the skeleton with FA values from the nearest track center. Finally carry out voxelwise statistics across subjects for possible difference between the two groups.

The experiment done for the effect of magnetic field strength showed there is high significant difference between control groups (1.5T and 3T) because of magnetic field strength difference and the same result occurred between same magnetic field strengths groups (1.5T control and 1.5T MS patients) due to the effect of multiple sclerosis. While comparison between 3T control groups and 1.5T MS patients show the highest difference due to effects of both MS and magnetic field strength.

PREFACE

This Master of Science thesis work was carried out at the Department of Radiology, Medical Imaging Center, Tampere University Hospital, Tampere, Finland, in cooperation with the Department of Biomedical Engineering, Tampere University of Technology, Tampere, Finland.

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Dibaba, Freazer Dandena

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Freazer Dandena Dibaba

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LIST OF SYMBOLS AND ABBREVIATIONS

AD	Axial Diffusion
ADC	Apparent diffusion coefficient
APP	Amyloid beta precursor protein
BET	Brain Extraction Tool
CC	Corpus callosum
CIS	Clinically isolated syndrome
CNS	Central Nervous System
CSF	Cerebrospinal fluid
CoFG	Center of gravity
DOF	Degree of freedom
DTI	Diffusion tensor imaging
DICOM	Digital Imaging and Communication in Medicine
EDSS	Expanded Disability Status Scale
FA	Fractional Anisotropy.
FMRIB	Functional MRI of Brain
FSL	FMRIB Software Library
FID	Free Induction Decay
FWHM	Full width half maximum
GM	Gray Matter
GUI	Graphical user interface.
GLM	General Linear Model
MNI	Montreal Neurological Institute
MS	Multiple Sclerosis

MRI	Magnetic Resonance Imaging
MD	Mean Diffusivity.
NAWM	Normal Appearing white matter
NAGM	Normal appearing gray matter
NIFTI	Neuro imaging Informatics Technology Initiative
PPMS	Primary progress Multiple Sclerosis.
PD	Parkinson Disease
ROI	Region of Interest
RD	Radial diffusivity
RF	Radio frequency
RRMS	Relapsing Remitting Multiple Sclerosis
SN	Substantia Nigra
SNR	Signal to noise ratio
SA	Signal Abnormalities
TBI	Traumatic brain injury
TAI	Traumatic axonal injury
TBSS	Tract based spatial statistics
VBM	Voxel based Morphometry
VEP	Visual evoked potential

1. INTRODUCTION

In diagnosis of diseases, magnetic resonance imaging (MRI) has become a major tool for detection and progress of pathological problems. MRI's enhanced capacity to identify contrast in soft tissues and being free from hazards associated with ionizing radiation have been vital for its success. Diseases need early detection for proper medication and treatment and one of MRI methods used to facilitate early detection is diffusion MRI.

Diffusion MRI measures the changes in intensity due to motion of hydrogen molecules. The signal measures the mean distance of hydrogen molecules per unit time based on random translation motion. Diffusion abnormalities have been instrumental in detecting pathological problems in a given area as change in diffusion measurements is related to microstructural changes in a certain region of body [1].

Diffusion tensor imaging (DTI) is an increasingly used method to investigate brain white matter in research and clinical application. The principle of DTI is that tissues are represented in rotationally invariant ellipsoids in which the three principal directions of diffusion are shown in three dimensional axis of plane. Higher value of diffusion represents diffusion along parallel direction, while the other two represent in transverse direction. Three tensor values are used to fit into 3D ellipsoid. There are different parameters used in DTI, for example, frictional anisotropy (FA) and apparent diffusion coefficient (ADC). FA value is used for comparison between two subjects in this research [2-4].

Tract Based Spatial Statistics (TBSS) is a method which is used to identify any significant difference between two groups of diffusion MRI images for possible abnormalities. This method uses advantage of both voxel based morphometry (VBM) and tractography methods, that is, by solving alignment and smoothing issues, and by being fully automated and covering the whole brain without prespecifying tracts [5].

Multiple sclerosis (MS) is a neurological problem which is caused by environmental factors and an immune system attack on the central nervous system (CNS), which comprises of brain, spinal cord, and optic nerves. Symptoms range from mild numbness in the limbs and muscular weakness to severe paralysis or loss of vision. The immune system attack on CNS causes damage to myelin sheath, a fatty substance that surrounds and protects nervous fibers. This causes interruption and disturbance of nerve impulse which travels to and fro between brain and spinal cord. The damaged myelin forms scars (sclerosis) and thus the disease is given its name [6].

Clinically isolated syndrome (CIS) is the first symptom to appear which lasts for at least 24 hours and is caused by inflammation or demyelination of the central nervous

system (CNS). These symptoms can be monofocal or multifocal, that is, symptoms can be experienced by single lesion or multiple lesions respectively. The likelihood of a person with CIS to develop MS depends on MRI-detected brain lesions. If the lesions resemble that of MS, then the subject has higher chance of developing MS and diagnosis is inevitable [7].

Despite technological improvement in imaging, multiple sclerosis (MS) remains a clinical diagnosis that is supported but not replaced by laboratory or image findings. However, imaging is essential for current diagnostic criteria of MS, predicting likelihood of MS for clinically isolated syndrome (CIS), correlation with lesion pathology and assessment of treatment outcome [8].

In this research, CIS MS data is analyzed using diffusion MRI for improved detection using 3T- and 1.5T-magnetic field strengths. The comparison between these two data groups is done using TBSS by FSL (FMRIB software library). This software is mainly written by the members of Oxford Center for Functional MRI of the Brain (FMRIB).

2. THEORETICAL BACKGROUND

2.1. MRI TECHNIQUES

Magnetic resonance imaging (MRI) has become one of the most promising instruments in medical imaging in research and diagnosis because of its enhanced capacity to identify the contrast in soft tissues and being free from hazards associated with ionizing radiation. It shows the significance of physical science to play a role in supporting clinical application and developing new techniques. As a result, its use has increased significantly in recent years as the development of new techniques has emerged on a regular basis. Here the basics of MRI principles and, specifically, the diffusion weighted imaging will be discussed in detail [9].

The main concept behind MRI imaging is the interaction between an external magnetic field B_0 and spin magnetic moment of protons μ, which results in precession of proton spins around B_0 axis. The rate at which proton rotate around the axis of an external magnetic field is given by the equation called Larmor equation:

$$\omega_0 \equiv \gamma \times B_0 \tag{1}$$

Where γ is the gyromagnetic ratio (measured in megahertz per tesla), ω_0 is the angular frequency of proton, and B_0 is the external magnetic field strength (measured in teslas). MRI active nuclei are odd-numbered so that their spins would not overlap. Usually, hydrogen nuclei are used as they are abundant in the human body [10-11]. When B_0 magnetic field is applied, majority of nuclei align with the direction of external magnetic field while some of them in the opposite direction. When radio frequency (RF) signal is applied, the net magnetization flips to a certain degree which causes two magnetization vector components, longitudinal magnetization, and transverse magnetization. The latter precess around the receiver coil and induce current in that coil according to Faraday law of induction. This current is considered as MRI signal [12].

Conventional MRI is a commonly used MRI in which when the radio frequency (RF) pulse is turned off, the induced signal or current in the receiver coil gradually dies off because the net magnetization vector in transverse plane decreases as precessing protons begin to dephase. This is known as free induction decay (FID). The turning off of RF signal leads to two most important processes: T_1 recovery and T_2 decay. T_1 indicates how quickly protons can emit or exchange their absorbed energy to their neighboring atoms. T_2 indicates the dephasing of precessed protons as these protons become dephased. The net magnetization vector in transverse direction decays because

each proton will experience an external self-generated magnetic field of its neighboring protons. This is termed T_2 decay or spin-spin relaxation [13-14].

Functional based MRI is another type of MRI, which is used for processing of sensory data from eyes and ears or motor tasks as moving fingers. The basic principle for identification is the oxygen level in the part of brain to be studied. Active region has higher concentration of blood, and it will affect the magnetic property of the blood. [15].

2.2. Diffusion based MRI

Diffusion weighted imaging measures the change in intensity due to the motion of hydrogen molecules. The signal is measured by the mean distance hydrogen molecules moves per unit time based on random translational motion. The signal detected will depend on the speed of molecules movement across magnetic field gradient [1].

The movements of water molecules can be uniform in all directions, that is, isotropic with respect to magnetic field or it can also be anisotropic in which the movement of molecules is directional as tubular or layered environment as shown in Figure 1 below. Nearly, isotropic environment is experienced inside the cerebrospinal fluid (CSF) in the ventricles or cystic fluids.

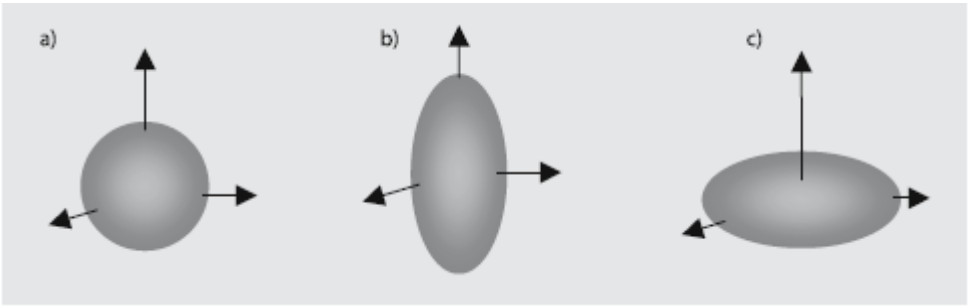

*Figure 1. Diffusion tensor ellipsoids for **(a)** isotropic **(b)**, tubular and **(c)** layered environments [1]*

In heterogeneous environments, method of diffusion depends on the direction of gradient field. Water molecules inside the brain diffuse faster in the direction of axon with intact myelin sheath than in perpendicular direction. Their movement in anisotropic diffusion is observed when axons are in a bundle and arranged in parallel [1].

Diffusion weighted imaging is acquired with the use of echo planar imaging sequence as shown in Figure 2 in which a pair of same polarized signals are excited between 180^0 RF pulse. Afterwards, the signal will be read for any divergence in phase. A change in phase is transmitted to those spins that move along the gradient field while the pulses are being applied. The spins in a voxel which have experienced different shifts are no longer coherent, and they will produce weaker MRI signal. The magnitude

of this signal will depend on the strength and duration of the gradient pulses, their spacing, and the diffusion constant along their direction of gradient field [16].

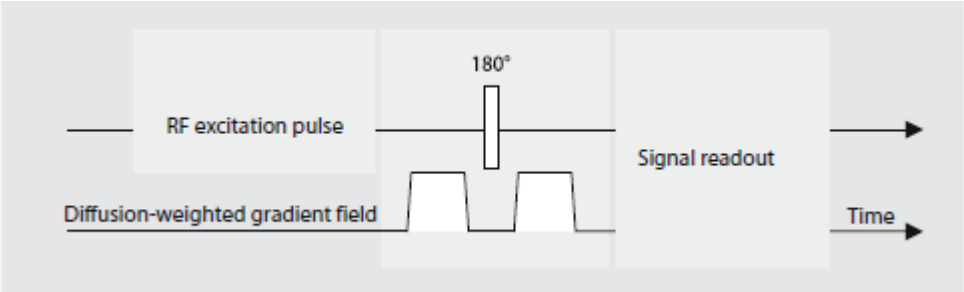

Figure 2. Diagram of a diffusion-weighted sequence [14].

For a given gradient pulse pair and inversion pulse, the amount of diffusion weighted is denoted by ***b value***. This value expresses the signal loss which is expected from a given pulse sequence. By measuring with different b values, a diffusion constant of a given biological tissue can be calculated and the measured diffusion constants are represented by apparent diffusion coefficient (ADC). The color of ADC image is affected by the amount of diffusion, that is, dark ADC image shows there is less variation in diffusion while bright image shows high variation. One application of this is the significant change in color of the diffusion weighted image to detect early regions of tissues which are affected by stroke just within six hours. This is one advantage of diffusion weighted imaging over the conventional T_2 weighted imaging. After a given time, the movement of water molecules will resume so that both areas will have the same color [17].

Although the determinants of water diffusion have not been fully understood, there is a general agreement in which physiochemical properties of tissue like viscosity and temperature as well as structural components (macromolecules, membranes, and intercellular organelles) can have greater effect on water diffusivity. For example, in tissues with random microstructure (isotropic diffusion), the measured ADC have the same value in all directions. While those with ordered organelles, like in white matter, in skeletal, cardiac and uterine muscle, and some portion of kidney have varied measures in ADC. From this, it can be concluded that pathologic conditions will not only affect the bulk diffusivity but also anisotropic diffusion character of water or metabolites [2][17].

Intentions in making ADC into two or three directions have been made by assuming higher value in ADC represents diffusion flow in longitudinal axis while smaller values represent diffusion flow in transverse directions. This method has shortcoming in the heterogeneously oriented tissues, such as brain white matter as it is difficult to align diffusion gradients in the axis fibers in all voxels. The assumption of cylindrical symmetry of tissues and two directions of diffusion is too simplistic for many tissues.

This could lead to erroneous conclusions about tissue structure and diagnostic error. In order to overcome this, diffusion tensor imaging is proposed [17].

2.3. Diffusion tensor imaging

Diffusion tensor imaging (DTI) is an increasingly used method for investigation of brain white matter integrity in both research and clinical applications. The principle of DTI is that tissues are represented in rotationally invariant ellipsoids in which the three principal directions of diffusion are shown in three dimensional axis of plane. The highest value of diffusion is indicated by parallel direction while other two transverse directions show diffusion of lesser magnitude. One of the crucial advantages of using DTI in clinical investigation is as *surrogate marker*. The requirements for surrogate markers are: (1) the marker should predict future clinical disability and (2) any intervention must alter both surrogate marker and the clinical outcome by the same mechanism. Another requirement is, the change in the marker should correlate with clinical change and there should be standardization in DTI measurements, if there are different scanners in use. Familiarity with normal variation of fractional anisotropy (FA), apparent diffusion coefficient (ADC) values, and measurement reproducibility are essential when DTI measurements are interpreted in clinical patients [2-3].

2.3.1. DTI parameters

The estimation of diffusion tensor D has been proposed in which six independent scalar elements of the diffusion tensor are used to characterize diffusion in all directions. By using them, we can describe intrinsic properties of the tissue that are rotationally invariant. These are the three principal diffusivities which are perpendicular to each other and define the 'fiber' frame of reference. As shown in Figure 4B, they can be sorted in decreasing magnitude (higher, intermediate, and lower) diffusivity. The first represents diffusion coefficient in parallel direction while the other two represent in the transverse direction. The measured diffusion along different direction can fit into a 3D ellipsoid. The length and orientation of the 3D ellipsoid are defined by six parameters. To convert measurements to these six parameters, a 3 x 3 matrix called tensor is used, and hence the name diffusion tensor imaging. Several parameters can be found, for example, degree of anisotropy by using the difference of the three Eigen values $(\lambda_1 - \lambda_2)^2 + (\lambda_1 - \lambda_3)^2 + (\lambda_2 - \lambda_3)^2$. Here the Eigen value λ represents diffusion in the major axis direction. If the value of this measurement is 0, then the diffusion is isotropic, and if the value is higher, it shows higher diffusion anisotropy. The other derivative is fractional anisotropy (FA) [4].

$$\mathrm{FA}=\frac{1}{\sqrt{2}}\left(\sqrt{\left((\lambda_1-\lambda_2)^2+(\lambda_1-\lambda_3)^2+(\lambda_2-\lambda_3)^2\right)}\right)\times\frac{1}{\sqrt{\left(\lambda_1{}^2+\lambda_2{}^2+\lambda_3{}^2\right)}} \qquad (2)$$

FA value will be used to compare control and patient groups throughout this study. The principle of DTI and color contrast is shown in Figure 3 below.

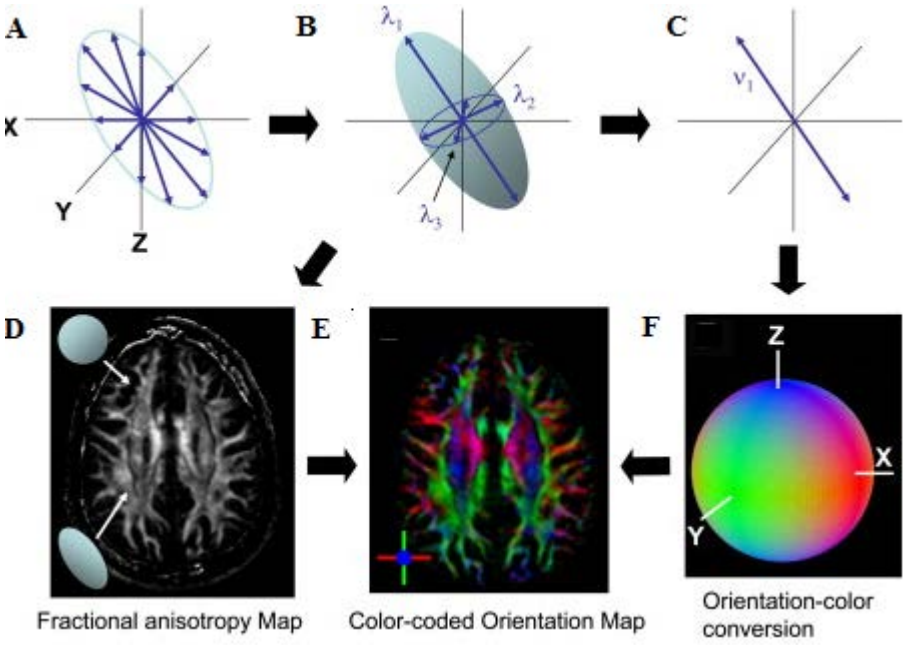

Figure 3. The Principle of DTI and Contrast Generation From diffusion measurements along multiple axes (A), the shape and the orientation of a "diffusion ellipsoid" is estimated (B). This ellipsoid represents what an ink stain would be if ink were dropped within the pixel. An anisotropy map (D) can be created from the shape, in which dark regions are isotropic (spherical) and bright regions are anisotropic (elongated). From the estimated ellipsoid (B), the orientation of the longest axis can be found (C), which is assumed to represent the local fiber orientation. This orientation information is converted to a color (F) at each pixel. By combining the intensity of the anisotropy map (D) and color (F), a color-coded orientation map is created (E) [4].

FA and ADC values are compared by the use of either voxel based morphometric (VBM) or region of interest (ROI) methods. Voxelwise analysis is less operator dependent, easily automated, and it requires intersubject registration and image smoothing. This can cause error when computing for FA. For clinical purpose, ROI based method is used.

FA values are usually used as an indicator for white matter tissue integrity. Although it does not reflect any distinctive specific tissue property, it is influenced by tissue hydration, myelination, cell-packing density, fiber diameter as well as directional coherence [2]. High FA values are found in those white matter areas where there is uniform orientation and tightly packed fibers. Higher values of FA are reported from corpus callosum which represents thick bundle of mediolaterally oriented fibers

connecting the cerebral hemispheres. The FA values for the posterior part, that is, splenium, are higher than the anterior parts. In some regions, anisotropy in white matter is considerably low because of complexity and crossing fiber orientation. FA values are generally lower for gray matter than white matter [2]. Recent findings show that FA values vary regionally from 0.121 for the deep gray matter of putamen to 0.806 for the genu of the corpus callosum in 31 healthy subjects with 3T MRI [18].

ADC values show relatively less regional variation in healthy adult brain parenchyma. In surveys, it was found out that ADC values for most regions average 0.7-0.8*10^{-3} mm^2/s. Compared with genu and splenium of the body, corpus callosum shows higher FA values and lower ADC value due to its smaller size and vulnerability to partial volume effect [2].

During measurement with ROI, care must be given to inter- and intraobserver differences, because they will exist even if the experiment is done in a standard procedure. [19]. One factor affecting the repeatability of the experiment is the size and placement of circular ROI used. It is suggested that small circular region of interest (ROI) should be placed in a region of high anisotropy as a slight difference in the ROI placement and section shift in ROI location can lead to differences in FA and ADC values [20][21]. Good repeatability can also be found by using the polygonal free hand ROI [22].

2.3.2. Image alignment

In order to compare diffusion parameters across subjects, it is necessary to solve image misalignment. Correction is done by aligning different subjects on the same platform or space and by comparing their corresponding parts. The process of alignment is called image registration. It is the spatial adjustment of one image to match another one. The contents of input image are moved around within an image matrix until they are well aligned with the contents of the reference image. The input is a single subject's brain while the reference image can be a different image of the same subject, different subject, or 'template' image which is formed by averaging of subject's image in a common space such as MNI152 (Montreal Neurological Institute) [5]. There are two methods of registrations, and they are linear registration and non-linear registration.

2.3.2.1. Linear registration

This type of registration consist of low degree of freedom (DoF) transformations, like global translation, rotation, scaling and shears (square become parallelogram). It is mainly used in intrasubject registration, for example, between one subject's FA images to the same subject's T_1-weigted structural image. It is not accurate enough to compare different subjects as warping is required in these cases due to detailed difference in the shapes of brain [5].

2.3.2.2. Nonlinear registration

It is another type of registration which is used to align different subjects or a single subject to standard template image. This registration can be applied to local warps which are simple with low degree of freedom (DoF) or to very finely detailed, complex warps (high DoF) for aligning input image to reference image as much as possible. Nonlinear registration is normally initiated by linear registration to get both general orientation and size matched globally.

In diffusion MRI, a common step in aligning multiple images of the subjects to each other is first linearly, then nonlinear registrations driven by FA images. It is essential to keep the general structure intact to achieve maximum registration possible. At low DoF, there is insufficient accuracy of alignment even within major tracts, while at high DoF extreme (very high dimensional warping), it is possible to align two images perfectly so that they look exactly like each other, but doing so results in warping the images many times and it could possibly distort the overall structural homology. Therefore, it is necessary to use intermediate DoF [5].

Templates used for aligning FA images of the subjects are MNI152 (Montreal Neurological Institute) T_1-weigted average image or FMRIB_58 (functional MRI of brain), average of FA image from 58 adults in MNI152 standard space. Working in a common standard space is convenient to report results as it could be understood by other researchers. However, it is not always the case when considering very young infants or subjects who have severe pathology. In these cases, by aligning all subjects and averaging them, case specific template should be developed [5].

2.4. Voxel Based Morphometry (VBM)

This method is used to compare various structural imaging studies by putting them in one common place and study the differences among different structures. The first step for the voxel based morphometry (VBM) is to align subject's structural images into a chosen template using affine and low degree of freedom (DoF) nonlinear registration; then segmenting of each subject's structure into different gray matter (GM) output and smoothing it. Smoothing is essential as it represent local 'gray matter density,' that is, the local balance between the count of GM and non-GM voxels. It also helps to reduce the effect of misalignment as the registration is imperfect and help the data to be more Gaussian oriented so that it will be more valid for the Gaussian random field thresholding. Usually between 4- and 16-mm full-width half maximum (FWHM) smoothing is applied (with Gaussian Linear Filter) [23].

The next step in VBM is to operate voxelwise statistics and compare the patient group with control one and then thresholding. Voxelwise statistics is carried out using any relevant covariates for the design matrix. A simple example would model group membership (patient and control) with appropriate contrasts comparing the group

means. The standard approach is to use simple univariate statistics, meaning that each voxel is processed separately, the data for each voxel constitute a 1D vector of values, where that one dimension is subject number, and the model is fit separately to each voxel's 1D data vector. Lastly, threshold the resulting T statistic (F or Z) while correcting for multiple comparison across voxels. VBM is usually carried out by SPM software package [23].

When using VBM things that should be considered are alignment issue and smoothing. The alignment issue describes whether the same part of white matter (anatomical) is included from all the subjects in the corresponding voxel. This registration problem is not solved even by using high degree of freedom, that is, by making images look similar. The other issue is smoothing and it is evident that smoothing helps to reduce residual misalignment and enhance sensitivity of detecting changes. It also renders the data to be more Gaussian distributed by improving the validity to use the commonly used Gaussian random field (GRF) theory thresholding approach. The problem with smoothing is in the extent of using it as too much smoothing may remove important information. Typically between 4- and 16-mm full-width half maximum (FWHM) smoothing (with a Gaussian linear filter) is applied [24].

One of the draw backs of VBM is alignment problem. Care must be given not to misinterpret residual misalignment. It is not certain whether a given voxel (the final voxel where the comparisons for two subjects is made) contain anatomically corresponding regions that is same part of white matter tract from the subjects involved. Even by using from low to medium degree of freedom, there is ambiguity whether two images are aligned completely or not. It is possible to align them to look completely alike by using high degree of freedom, but there is a possibility that a given tract can be broken into two or two tracts can merge into one.

One example for residual alignment issue is given by comparing a patient group with higher ventricular size to a control group as both groups have the same white matter integrity. Because of the ventricular size, conventional registration (low to medium) DOF causes the anterior section of the corpus callosum (CC) more anterior in patients' group than control's group. This has caused change in FA when VBM is carried out. At the front of CC, FA value of patients is greater than that of controls. The reverse is true when implied at the back [25].

The second problem with VBM is the extent of smoothing. It is known that smoothing helps to reduce misalignment issues though not in a well-controlled way. It also helps to improve sensitivity of detecting changes if the smoothing matches with spatial resolution of the image. Since the extent of smoothing cannot be decided early, it can cause distortion and affect interpretability of the images. It also increases the partial volume effect so that it will be difficult whether the changes occur because of the increment in FA value or due to different tissues. If it is possible, spatial smoothing is a good remedy for this ambiguity.

This smoothing problem is seen in the VBM analysis of schizophrenia (neurological problem) data in which different smoothing extents (from 0 to 16 mm FWHM) are used; as a result, different group differences appear and disappear across the range [26]. In another study on the asymmetry of schizophrenia that was studied using 3.6- and 9-mm FWHM smoothing, the resulting asymmetries were different for each case [27].

In order to solve the above listed problems, that is, alignment and smoothening, region of interest (ROI) method has been proposed. This method involves drawing extent of smoothing region of interest (ROI) usually by hand on white matter tracts for control and patient images separately and comparing their FA values in their corresponding regions [28-29]. This method is helpful for comparing major tracts, but it is difficult to place ROI in smaller and thinner tracts due to partial volume effect. The comparison using ROI method also shows the changes are restricted to ROI but not to the whole brain [30].

2.5. MR Diffusion Tractography

MR diffusion tractography is a method that is used to identify white matter pathways in human brain. These pathways form substrates for information between different brain regions; therefore, their study is vital to identify normal brain from the diseased one. This method is the only noninvasive and in vivo method to identify and measure these pathways. By comparing with invasive measurements, tractography measurements are indirect, difficult to interpret quantitatively and error prone. However, their ease of measurement and noninvasive behavior give extra advantage to answer clinical and scientific questions which cannot be answered by any other method [5].

The basic assumption for all diffusion tractography method is when a number of axons align in a common space; the diffusion of water molecules is preferred along a longitudinal direction than transverse direction. In constructing fiber bundles or drawing axonal connectivity, all tractography algorithms aim to find path where diffusion is least hindered.

Tractography is a method which integrates voxelwise fiber orientations into a path way which connects different brain regions. The most common tractography method used is the streamline method. As it is shown in Figure 4, streamline through a vector field is any line in which all the tangents are in parallel direction with the field. It is constructed by starting at seed point and by following the local vector information on step by step method joining the arrows of the tangent vectors.

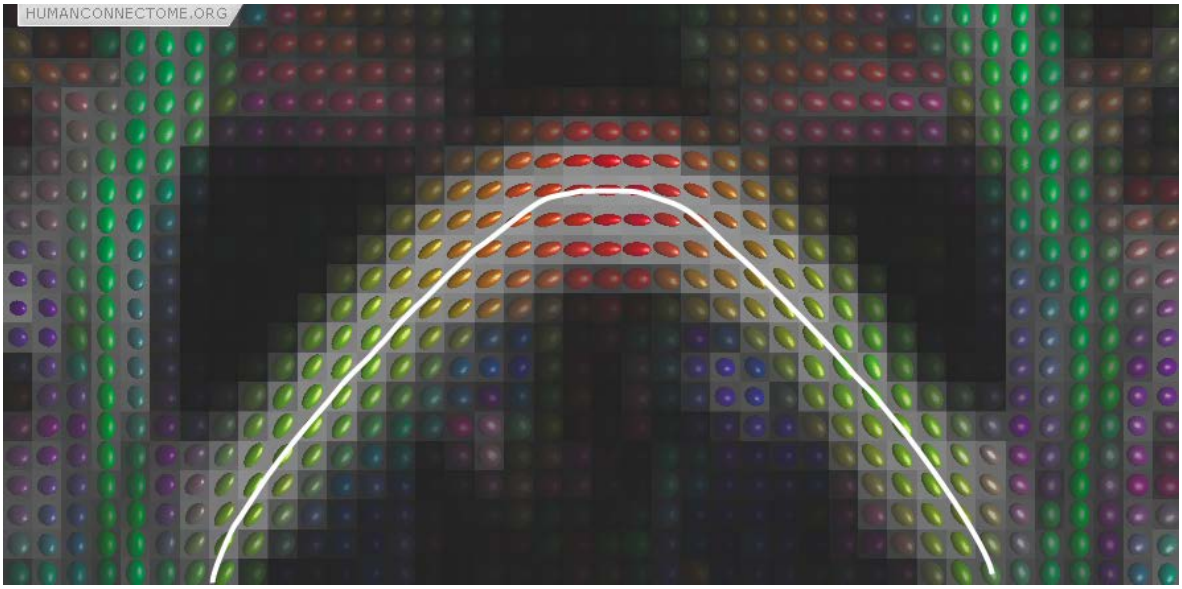

Figure 4. streamline tractography from conceptual perspective.The white stream line follows the orientation of least hindrance to diffussion (here the principal axis of diffusion tensors) *[5].*

A stream line is represented in 3D space curve in which the streamline **r** is expressed with function of arc length **s**, the distance along streamline from the start. The tangent is assumed to be the first eigenvector of the diffusion tensor, that is, t(s) =e(r(s)) as r(s) is the [x, y, z] location along distance **s**. The equation describing the formation of streamline is given by

$$d\mathbf{r}(s)/d(s)=e(\mathbf{r}(s)) \qquad (3)$$

The above equation is a differential one so it calculates the changes that streamline makes along the arc **r(s)**, not the actual location of the streamline. It also implies that there are errors along the path and will be compounded as streamline propagates. In order to create a continuous streamline from the discrete measurements in each voxel, interpolation is used. Streamline tractography is used for in vivo dissection, that is, to isolate and delineate major white matter tracts in human brain. This is done by combining anatomical knowledge of trajectories of major fiber bundles in the brain [5].

In the above-mentioned tractography method, the problem of aligning still persists and it can be overcome by taking the mean FA tractography result for each major tract before comparing control and patient data [26]. Another method to overcome the alignment problem is to parameterize the tract obtained by tractography using, for example, the distance along the tract. This will help cross subject comparisons of FA values between control and patient without accurate aligning. This method is the basics for the tract based spatial statistics (TBSS) method which combines the advantage of VBM and tractography [1].

There are mainly three drawbacks of tractography. First, FA parameterization can be obtained for only tracts that can reliably be traced as there is no robust, fully automated method that can classify all the tracts available. The second one would be it is not straightforward to identify or localize the different tracts from start to end as it can differ from subject to subject. The third drawback concerning tractography is partial volume effect as it is difficult to separate the tract voxel from nontract voxel which could affect the FA value.

2.6. Tract based spatial statistics (TBSS)

Tract based spatial statistics (TBSS) is a method which is used by combing advantages of both VBM and tractography methods. That is by solving alignment and smoothing issues and by being fully automated and covering the whole brain without prespecifying tracts.

TBSS is achieved first by taking the average of all the FA values of major white matter tracts as FA skeleton and then by projecting all the images of subjects to this FA skeleton. The last step is to carry out voxelwise statistics across all subjects on skeleton space FA data. This method is executed by the software FSL (FMRIB software Library). The summary of TBSS is presented as follows [5].

- Assign common registration target and align all the FA images to this target place. Registration is done by nonlinear method as perfect alignment is not required
- Create mean FA skeleton image from all aligned images by applying ‘thinning’ (non-maximum-suppression perpendicular to the local tract structure). Threshold this to remove areas of low FA values and areas with high intersubject variability.
- Project each FA data onto the skeleton by filling the skeleton with FA values from the nearest track center.
- Carry out voxelwise statistics across subjects on the skeleton-space FA data [5].

The detail of each step is described in the section below.

2.6.1. Pre processing

The first step in TBSS is preprocessing FA image. It includes removing eddy current which is created by gradient coils of MRI and head motion during scanning. Head motion creates rigid body image motion while eddy current forms first order linear image transformation [31]. Eddy current is removed by using built-in software *eddy current correction* in FSL. After preprocessing, diffusion tensors can be calculated. The three principal directions of diffusion, that is, primary, secondary and tertiary can be

extracted from diffusion tensors. Then it is straight forward to calculate FA [32-33]. Finally, BET *brain extraction tool* is used to exclude nondiffusion brain voxels from further considerations [34].

2.6.2. Nonlinear alignment

The next step for aligning multiple FA images to each other is using nonlinear alignment with intermediate DOF. Here, the basics of the images will not change and keeping the general tract structure intact is important to prepare for the next stage (projection of data on tract skeleton). The two other extremes of DOF (low and high) are avoided as low DOF do not guarantee sufficient alignment even for the major tracts as voxelwise statistics is not accurate. While at high degree of freedom (DOF), images can be aligned to look similar but doing so could result in distortion of original image as it may have been warped so much and some parts could split into two or some merge into one.

There are three different ways to complete nonlinear registration:

1. Registering FA images to FMRIB58_FA standard-space image, an averaged FA map included with the software package.

2. Registering FA images to a user defined target image or

3. By identifying the "most representative" target from the FA images and using it as a target image.

The first option is the optimum and recommended one as it involves only one registration for each subject. The second method also needs one registration for each subject to a user selected target, but it is not suitable for subjects who are different from the ‘generic’ ones, for example, children as the adult oriented FMRIB_58 model is not suitable for alignment. The third option is to register each image to every other image and choose the one which is most ‘typical’ for the group with least warping needed (minimum mean distance from the rest). However, this method takes higher computation and longer time [5][23].

2.6.3. Creating Mean FA Image and Skeleton

After choosing the most typical subject for the target, all FA images are aligned to this and the entire data set is affine transformed into $1mm^3$ MNI152 space. All subsequent processing is carried out using this space and resolution. The space is chosen for its interpretation, display and analysis result. Working in higher resolution prevents from further interpolation blurring (increase in partial volume) with slightest increase in computation time. Affine and nonlinear registrations are combined in order to avoid resampling the images twice. The mean FA image is created by averaging the transformed images. This image is smooth due to averaging and resolution upsampling.

It is then used to create the skeleton image which represents all the ‘common’ tracts of the aligned images. It is shown in the left side of Figure 5 below that most contiguous set of tracts appear to be curved sheets with certain thickness. The resulting skeleton is set to be a thin curved surface in middle. While some other tracts look cylindrical tube (right side in Figure 5), their skeleton are made to be a thin line across the center of the tube. At a distance away from the middle surface or center line, FA values decrease gradually as moving away from white matter [5][23].

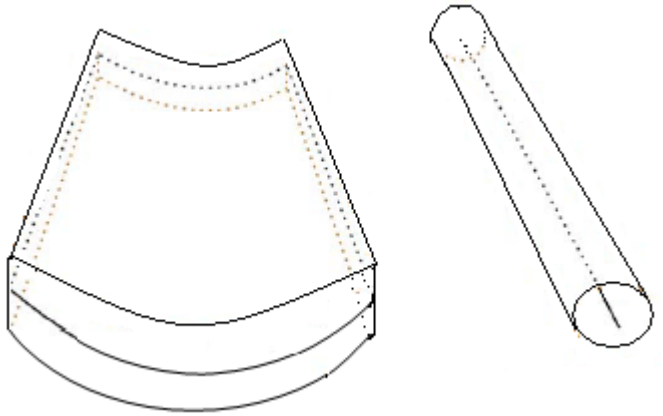

Figure 5. Examples of fiber bundles; a ‘‘thick sheet’ with a thin surface as its skeleton, and a ‘‘tube’’, with a line as its skeleton [23].

The next step involves making skeleton image from the mean FA image. The first step is to estimate local surface perpendicular direction (at all voxel in the image) and compute nonmaximum suppression in this direction. By searching all voxels in this direction (tract perpendicular direction), the one with the highest FA value is taken as the center of tract.

The tract surface orientation can be found in two ways. If the voxel of interest is far from tract center, FA value will be higher in the neighboring voxels on one side of the voxel than on the other side and the direction in which it is highest shows toward the center of the tracts. This can be explained briefly by using 3 x 3 voxel neighborhoods as in Figure 6 below. First we take the center of gravity of the neighborhood by the first derivative of FA image. The vector which points from the current voxel center to current local center of gravity (CofG) should point to tract center which is perpendicular to local tract structure. As seen in Figure 6(1), it is possible if the center of voxel is not near to CofG (0.1mm range). On the other case, if center of voxel is near CofG as in (2) by taking the second derivative of the FA image, the remaining perpendiculars can be found.

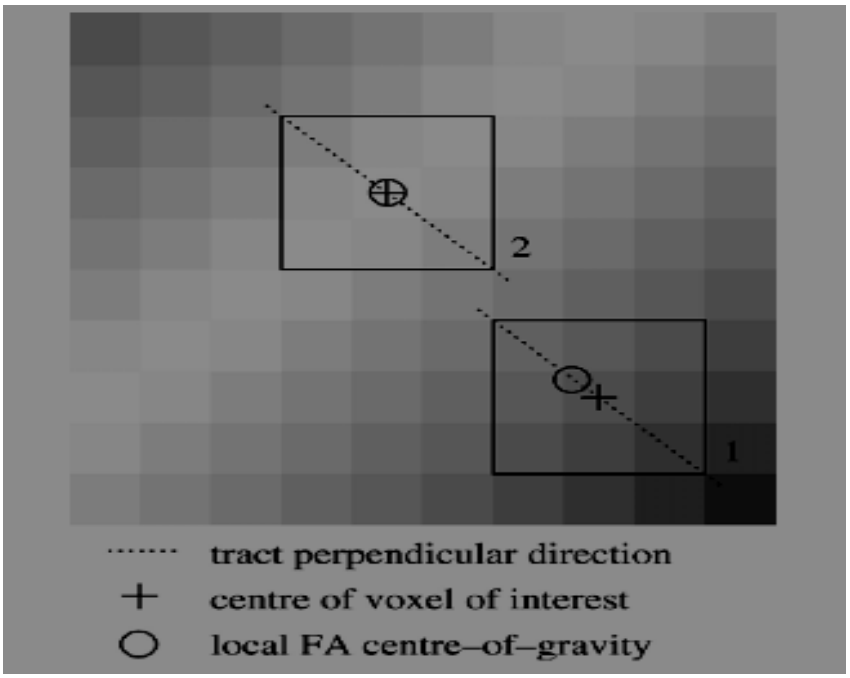

Figure.6. Example of (1) a voxel where the local centre-of-gravity (CofG) points in the local tract perpendicular direction, and (2) a voxel lying on the tract centre [23].

After the perpendiculars are defined, the skeleton can be prepared. For each voxel, its FA value is compared with its two neighboring voxels; if the voxel in question has higher FA values than its neighboring voxels, then it is considered to be on the skeleton tract. By doing the same thing for all voxels, the skeleton tract can be constructed.

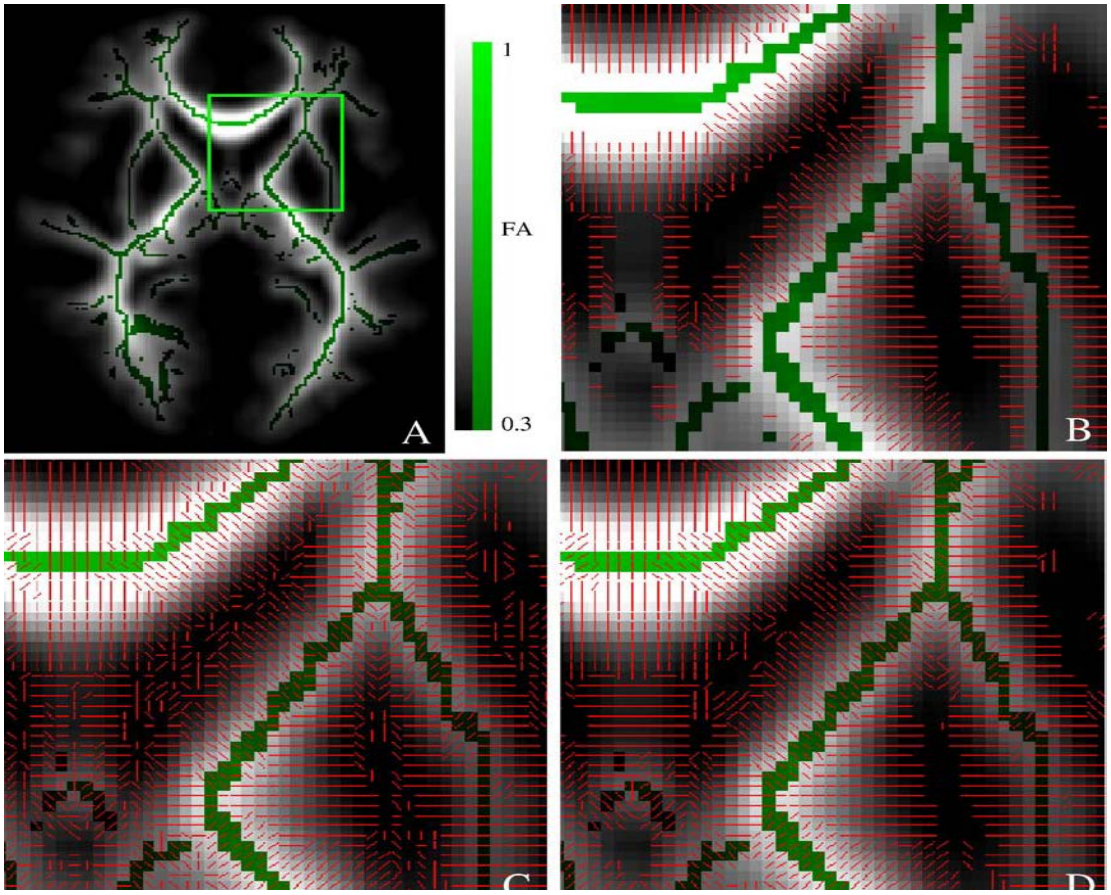

Figure 7. Different skeletonisation stages. (A) Original mean FA image with final skeleton and the ROI used for the remaining sub-images. (B) Skeletonisation stage 1, using local FA centre-of-gravity to find tract perpendiculars. (C) Skeletonisation after stage 2, using FA image second-derivative to find remaining perpendiculars. (D) Result of smoothing the perpendicular direction vector image. Note that the tract appears more than a single voxel thick in some places, because of its 3D nature; where the fibre bundle surface lies partially parallel to the plane being viewed, it will not appear thin, though would do if viewed with a different 3D slicing [23].

The steps involved to change mean FA image into skeleton is shown in Figure 7 above. The top left image shows an example of axial slice through mean FA image with the final skeleton on top with the region of interest (ROI) for the remaining sub-images is shown. On top right, it shows the first estimate in perpendicular direction to the local tract structure. The lines show direction estimated on the basis of local center of gravity. The bottom left shows the second derivative for the CofG that has not estimated the perpendicular tract. In the bottom right, the direction estimate have been smoothed by taking 3 x 3 neighborhood direction [5][23].

The constructed skeleton FA value should be tresholded in order to remove areas with large intersubject variations. The optimum value for threshold is between 0.2 and 0.3. The skeleton can contain disconnections in its tract structure due to crossing fibers as the nonmaximum suppression tracts are not well defined at junctions [5].

2.6.4. Projecting FA to skeleton

After the Skelton tract is ready, we now ‘project’ each subject FA image into mean FA skeleton. This projection from the tract center to the mean skeleton is accurate even if there are some misalignments between subjects.

For each voxel in the skeleton tract, a maximum FA value in a perpendicular direction is searched from a subject’s FA image. The perpendicular directions of the skeleton are already prepared from the previous skeleton formation. Then the maximum value of FA is assigned to the skeleton voxel in question. This assignment is valid only in perpendicular direction as the change in FA value is greatly pronounced in this direction than parallel direction.

There are two limitations for the searching of maximum FA value to skeleton tract. The first limitation is search is limited to voxels closer to starting section of the skeleton than that of the farther section of the skeleton. Space between two separate sections of skeleton are divided into two and each skeleton section can search voxels from its space. This is achieved by using distance map which shows the distance of each voxel from the nearest skeleton voxel. This constraint also ensures each voxel in the image is mapped only to a single point in the skeleton. The distance map used in this experiment is shown in Figure 8 below [23].

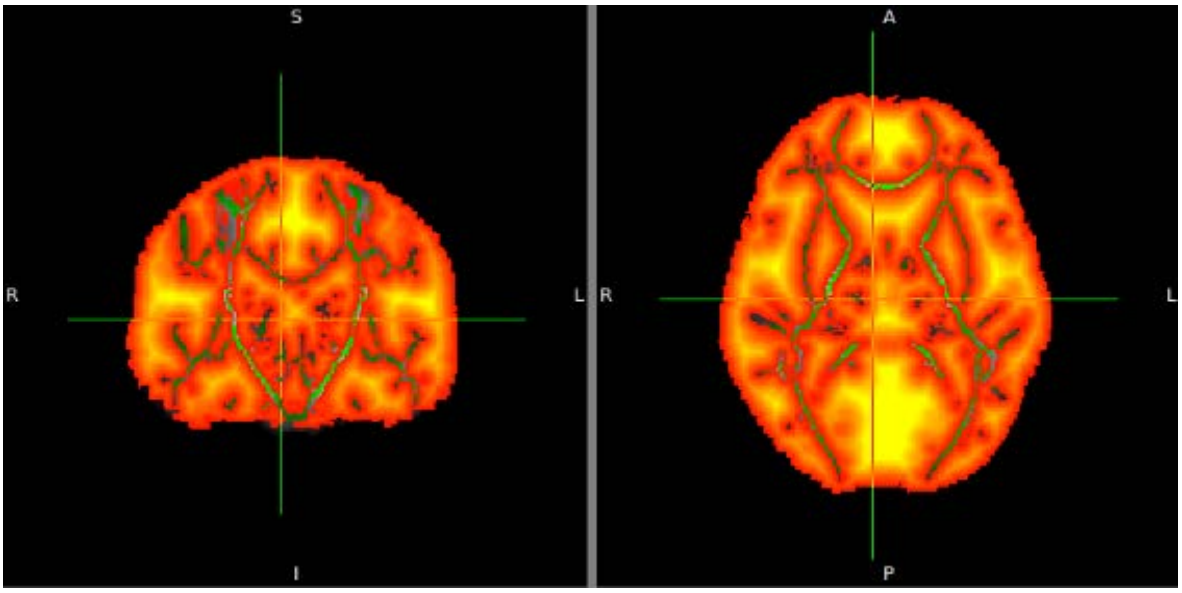

Figure 8. Distance map with green skeleton and the red regions show near the skeleton, yellow represent distance from the skeleton.

The other constraint for the search of maximum FA is via soft distance limit. A wide Gaussian function (FWHM 20mm) is applied to weighting function of FA values when searching for maximum FA. The function deweights the most distance voxels in smooth

pattern and emphasizes the nearby FA values effectively. After finding the voxel with the maximum value, its original value is placed to the skeleton voxel.

2.6.5. Statistics and Thresholding

At this stage the data is ready to be fed into voxelwise cross subject statistical analysis. Since each FA image of the subjects is aligned into a common space using constrained nonlinear registration, common tract skeleton has been formed, and each subject FA has been projected into the skeleton via perpendicular search for local tract center.

The simplest approach is to use univariate linear modeling, which is to process each skeleton voxel independently, applying general linear model (GLM) and multiple regressions across subjects. In order to find group differences between two groups, an unpaired t-test is used. The FA value data distributions in the skeletons are of Gaussian form and simple parametric regression and inference can be used.

Multisubject (group) comparison is done by using multiple comparison permutation test approach by testing voxel t-value or cluster size against null distribution of maximum values of test statistics [35]. The null distribution in this type of problems is unknown and thus generated by random permutation of subject ID. This approach gives good control over probability of falsely rejecting voxel hypothesis.

2.7. Medical application

Diffusion MRI is used for the study of neurological diseases because it provides in vivo measures that reflect the underlying tissue abnormalities. It includes dementia, multiple sclerosis, traumatic brain injury, stroke, epilepsy, brain tumors, and movement disorders. Diffusion MRI has been used to obtain information about the mechanism of diseases, to support diagnosis and predict prognosis. Its clinical research application using DTI includes detecting brain positions for new therapeutic interventions and to develop outcome measures that can be used for clinical trial by increasing the efficiency for new treatment. It has an advantage of providing insight into pathological changes that affect the tissue structure, improve our understanding of pathogenesis of neurological disease and lead to development of appropriate treatment. Its use in routine clinical trials has varied significantly as it provides good diagnosis and formulating prognosis for stroke while it remains limited for other diseases, like multiple sclerosis and dementia [36].

Parkinson's disease (PD) is a progressive neurodegenerative dysfunction with motor-like resting tremor, bradykinesia, rigidity, resting tremor, and postural abnormality symptoms. It is primarily associated with progressive neuronal loss in substantia nigra (SN) and other brain structures. It has been revealed that the FA value in substantia nigra of the DTI measurement is lower for Parkinson’s disease patients than healthy controls (0.403 vs. 0.415; p=0.001). Multiple regression analysis revealed that clinical

severity of PD is inversely correlated to FA value in the SN of the patient with PD (regression coefficient of -0.019). No other significant change for FA and ADC values are obtained in the other parts of brain [37].

In another study, it has been shown that the mean diffusivity (MD) has increased and the FA decreased in the genu of corpus callosum and in the superior longitudinal fasciculus, while in cingulum, only the MD was altered. From this, it can be concluded that there is a progressive degeneration of axons in the major fiber bundles [38].

Many factors influence the ability of DTI to differentiate patients with PD from the healthy ones. These are the field strength of magnet, spatial resolution, signal-to-noise ratio, contrast-to-noise ratio, image artifacts, and zones within the SN where the ROI are drawn. Comparing studies conducted on 1.5T MRI with one large 40 mm^3 region of interest (ROI), using 3T and three 30.6 mm^3 ROI shows the latter has higher resolution and can detect PD effectively in the selected areas 100% [38].

Traumatic brain injury (TBI) is one of the neurological conditions that occur in the brain due to sudden injury or external force. It includes motor vehicle accidents, falls, assaults, or blast injury. The effects occur immediately or after a certain period of time. It includes cognitive and motor defects. Traumatic axonal injury (TAI) also known as diffusive axonal injury (DAI) is a major contributor to cognitive dysfunction following TBI. It is characterized by silver stains, horseradish peroxide uptake, and, recently, neurofilament or amyloid beta precursor protein (APP) immunohistochemistry. APP normally traverses along the length of axon and will accumulate on the axonal retraction bulbs and varicosities at the time of injuries. The structural integrity is distorted during injury; thus it leads to increment in neurofilament immune reactivity [39].

DTI has been instrumental in detecting TAI as it measures the change in white matter orientation and provides information about brain microstructure using isotopic and anisotropic water diffusion. Diffusion is greater along axonal direction than perpendicular direction as most of the axons are aligned in parallel direction while the transverse or perpendicular is restricted to myelin sheath. Therefore, when an injury occurs, the axonal diffusion will be impaired and water may be forced to flow in perpendicular directions if the myelin sheath is compromised. It has been revealed that DTI is able to detect regions which are histological-verified traumatic axonal injury. Two DTI parameters, relative anisotropy and axial diffusivity, have shown statistically significant decrease in the areas of white matter during trauma [2][39].

There is also a decrease in cognitive capabilities in those regions of white matter with a decreased FA values and disrupted white matter structures. These include executive, memory, and attention. The axonal damage occurs for both mild and moderate trauma while irreversible radical damage, which impairs the myelin sheath, occurs for the severe case. The decrease in capabilities is correlated with the severity of the damage as it is comparatively lower for the mild one while it increases for the severe case [39].

2.7.1. Detection of Multiple sclerosis

The word sclerosis means hardening or stiffening of a structure. Multiple sclerosis (MS) is an autoimmune disease which is characterized by the dissemination in space and time in the central nervous system (CNS) of demyelinating lesions or plaques. It mainly affects the white matter of the brain but symptoms are also seen in the gray matter and outside visible lesions. The symptoms include demylenating and axonal loss which ultimately lead to disability [6].

DTI is mainly used to provide understanding of the mechanism of damage in the brain, spinal cord and optical nerve by MS. In particular, DTI is better in detecting pathological changes which are not visible in conventional MRI. This is mainly used for finding pathology for normal-appearing white matter (NAWM) and gray matter (GM) which can lead to disability. DTI is not used routinely in the diagnosis of MS because of lack of standardization of measurement for multicenter studies. Its ability to detect beyond lesions and its sensitivity to structural damage and improvements in high resolution scanners and hard ware are encouraging to be used in multiple clinical sites. The description of DTI findings in MS are discussed below [40].

MS Lesions

DTI studies in MS has consistently demonstrated that there is a decrease in FA value in MS lesions compared to NAWM and normal brain. There is high diffusion abnormalities found in T_1–hypointense lesions as they are affected by severe tissue disruption. But there are also some reports which suggest a decrease in ADC value. This creates ambiguity in differentiating tumor-like MS lesions and tumors [5][41][42].

Table1. Summary of the main findings of DTI studies in MS with their interpretation. [5].

Tissue type	*Diffusion findings*	*Conclusion*
MS lesion	1 Increased MD and reduced FA 2 Heterogeneity of values 3 Highest degree of abnormalities in non –enhanced lesion	1 Different abnormalities are present in lesions(demyelination, edema, inflammation) 2 Axonal loss and gliosis are dominant in chronic lesions
Normal-appearing white matter (NAWM)	1 Enhanced MD and reduced FA 2 Significant correlation between disability and FA or MD in ´´clinically eloquent `` regions 3 limited correlation with T2 LL	1 DTI is sensitive to NAWM diffuse changes 2 Regional NAWM measures correlate with disability (axonal loss and gliosis) 3 NAWM processes independent from T2 lesions
Normal-appearing gray matter (NAGM)	1 Increased or unchanged MD and reduced FA 2 Poor correlation with T2 LL	1 DTI sensitive to NAGM diffuse changes 2 NAGM measures correlate with disability

NAWM Abnormalities and Their Clinical Relevance

Reduced FA and increased MD have been detected in the NAWM of patients with MS compared to white matter of healthy subjects. Diffusion measures in the NAWM have been related to clinical disability. Both FA and MD in the cerebral peduncle are inversely related in expanded disability status scale (EDSS) which reflect neurological impairment, functional score and motor dysfunction. In other study, MD of corpus callosum segmented with tractography was found to be higher in patients with relapsing remitting (RR) MS compared with healthy controls. Generally, clinical correlation reports suggest that pathological damage detected by DTI in NAWM regions is a significant factor which leads to disability and progression in MS [42-43].

Recent study using tract based spatial statistics (TBSS) over the whole brain has shown that there is a relation between FA and dysfunction [44]. However, there is no

significant relation between primary progressive MS (PPMS), diffusion indices and disability. This uncertainty results has been seen with histogram method on PP MS [45]. This lack of correlation is due to limitation in clinical scales, such as EDSS and the role of spinal cord damage in determining disability in this form of MS.

GM abnormalities

DTI results using histogram method has shown an increased MD in the normal appearing gray matter (NAGM), including cortical and deep regions, in patients with MS [46-47]. There is evidence that diffuse image in the NAGM contribute to clinical disorders, like severity of language, attention and memory deficits in patients with RR MS [42]. Longitudinal changes in NAGM diffusivity were shown in patients with RR MS and progressive MS [48-49]. On the contrary, diffusion abnormalities have been found in the basal ganglia analyzed using ROI method [50]. The tissues NAGM in these studies include lesions as they are derived from segmentation of conventional images on which GM lesions are not visible [5].

Spinal cord and optic nerve

Pilot studies have been made using DTI for cervical spinal cord, and it shows patients with MS have greater correlation with diffusion indices. These studies were made using ROI and histogram method [51-52]. There is difficulty in measuring in these regions due to their small size, motion artifacts and cerebrospinal fluid (CSF) pulsation. However, pilot measures of optical neuritis indicate a diffusion change compared to control groups but fails to correlate any relation with visual function [53-54].

2.8. The effect of magnetic field strength

In using 1.5T and 3T MRI separately, it shows that there is a small change in the mean FA and ADC values. The difference is small when compared to the mean values. DTI at 3T has 40% increase in signal to noise ratio (SNR), offers better image resolution or shorter imaging time than 1.5T. There is no significant difference in FA or mean diffusivity values [2]. MR systems operating at higher field strength, such as 3.0 T, has recently become available not only for research purposes but also for clinical use in patients. Since SNR increases linearly with increasing magnetic field, high-field-strength systems are promising for diffusion weighted MRI as well as DTI. Susceptibility artifacts, however, increase exponentially with field strength. This has shown to cause significant geometric image distortions, stretching, and blurring on diffusion weighted MRI. Susceptibility artifacts are caused by materials of high magnetic susceptibility which can cause changes in magnetic field orientation. Examples include medical devices in side or near magnetic field or implants inside the human body. These artifacts cause bright areas (unregistered areas) and dark areas with no signal near the magnetic material. Parallel imaging has been shown to be an efficient

tool for speeding up image acquisition through a reduction of the number of phase-encoding steps that are necessary for image generation. The resulting gain can be used not only to cut down on MR image acquisition time but also to prevent susceptibility artifacts. In particular, in single-shot echo-planar MR imaging, a reduction of phase-encoding steps translates directly into a reduced echo train length that in turn prevents or reduces phase errors [55-56].

3. MATERIAL AND METHODS

3.1. Healthy Controls

There are two groups which are used as a control group in this research. The first one consists of 10 subjects with 1.5T scan while the second group consists of another 10 subjects with 3T scans. Both groups are free from neurological diseases and there is no age difference between the groups. The mean age for the control groups is 40. In this research, the control groups were compared with each other and with patient data to detect the effect of magnetic field strength and multiple sclerosis. The schematic diagram in Figure 9 below shows the comparisons in this research.

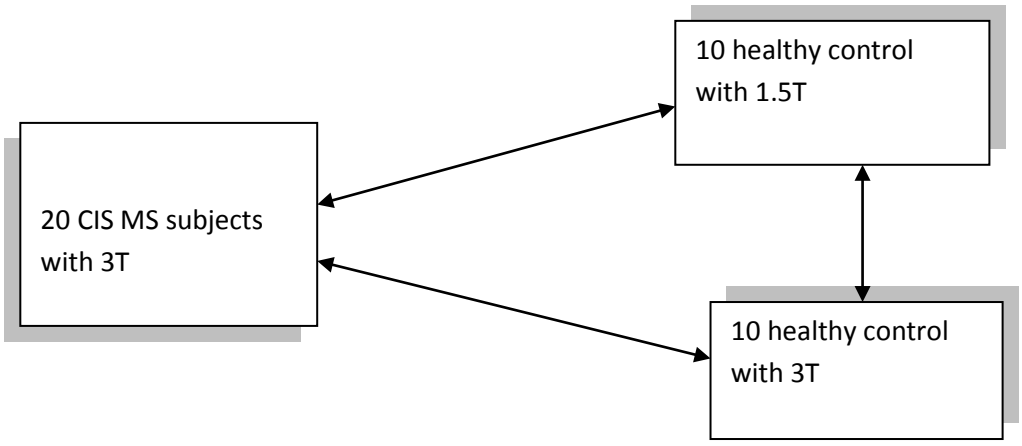

Figure 9. The control and patient data with their specification and pattern for the experiment.

3.2. Multiple sclerosis patients

The patient data used in this research were 20 centrally isolated syndrome (CIS) cases indicative of multiple sclerosis (MS) scanned with 1.5T. CIS MS is the beginning stage of the multiple sclerosis in which a subject has high probability of developing MS.

In this case, the subjects taken were from longitudinal study so there in no ambiguity over the presence of MS. Here also there is no age difference compared with control groups as it can be shown in the Figure above. The MS subjects were compared with

1.5T control subjects for the effect of MS on the subjects while comparisons with 3T subjects were used to detect the effect of magnetic field strength and multiple sclerosis.

The images of the control and patient groups with their FA value distribution in their comparisons are shown below. For the first comparison between two control groups, that is, between subjects with 1.5T control data and 3T control data, the original image for their respective groups and their FA value pattern is shown below in the following images.

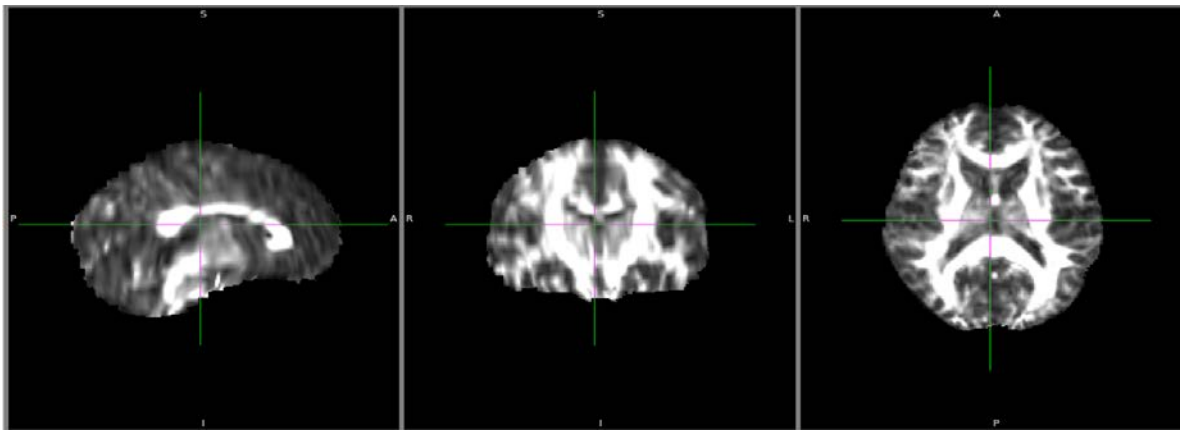

Figure 10. Original image of healthy control data with 1.5T in three directions.

The other control group is subjects with scan of 3T. Ten subjects are used in this experiment and their original image is shown in the figure below.

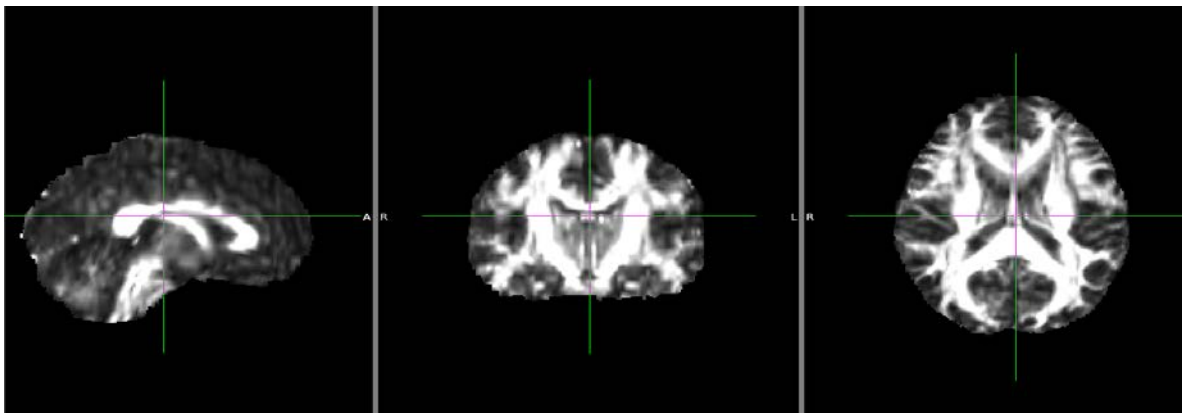

Figure 11. Original image for control subjects with scan of 3T in three directions

As it can be seen from the above two images, scans with 3T images are brighter and each tract can be seen more easily than 1.5T scans due to high magnetic field strength. Subjects with CIS MS are scanned with 1.5T and are shown in the figure below.

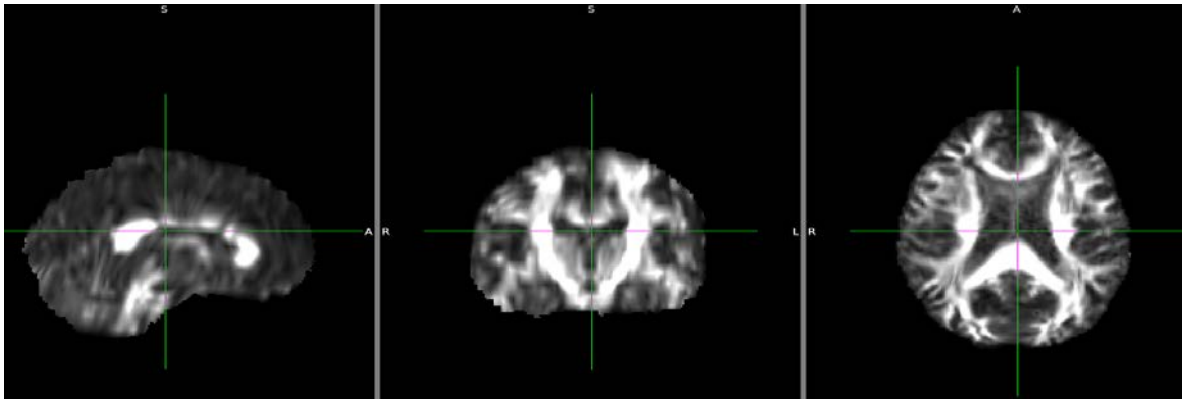

Figure 12. Original image of centrally isolated syndrome (CIS) MS scanned with 1.5T.

In the above images (Figure 12), it can be seen that CIS MS images show blurred and some irregularities around the major tract compared to scans of control subjects with 1.5T and 3T seen in Figures 10 and 11 respectively.

Before comparison of MS data to control groups and comparison between control groups, it is essential to observe the FA values of each respective group in the comparison to understand the characteristics of each group and predict the outcome. This can be extracted from ***time series*** application of FSL.

For comparison of the two healthy control groups, that is, subjects with 1.5T scan and 3T scans, the FA values for each subject in the group, that is, 10 for each group is shown in Figure 13 below.

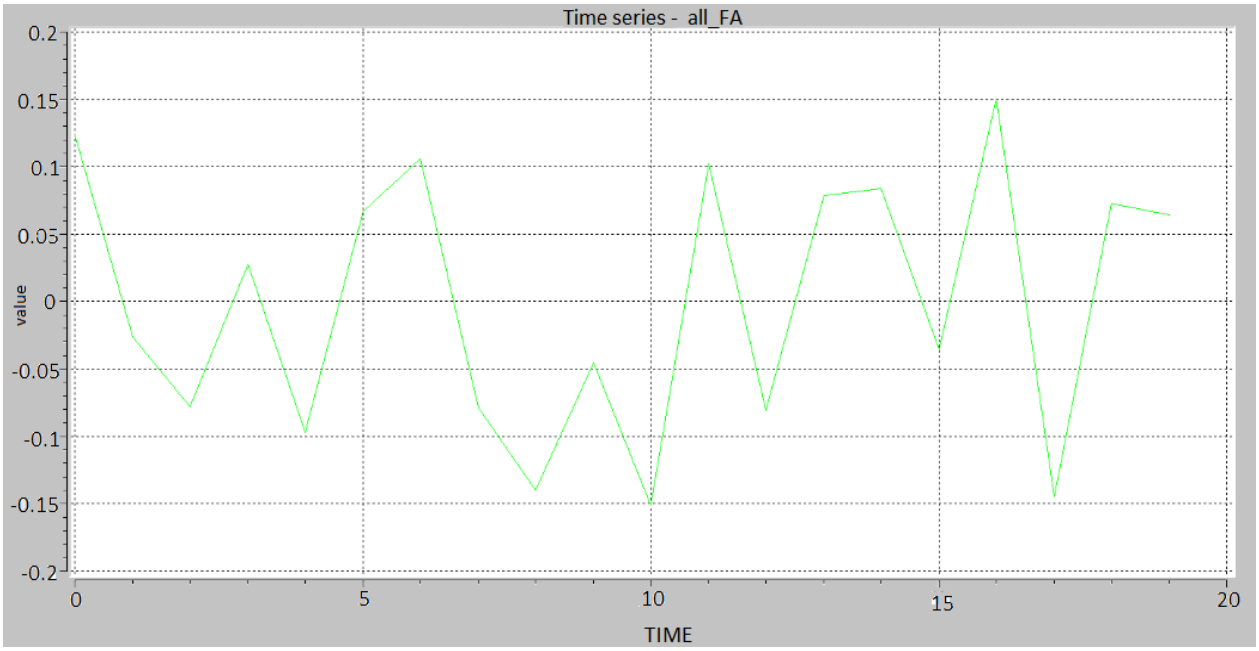

Figure 13. The original FA values of the control groups in which the first ten represent scans for 1.5T while the rest ten represent for 3T scans.

As seen in the above figure, FA values for 1.5T is from 0 to 9 while 3T is from 10 to 19. By comparing the two groups, FA values for 3T is relatively higher than that of 1.5T.

When comparing between 1.5T control group and 1.5 T CIS MS group, it is shown in Figure 14 below that the first 10 FA values, that is, from slice number 0 to 9 is for control group while the rest 20 subjects from 10 to 29 slice numbers are for MS scans. It can be observed that subjects with MS are relatively higher and have variable FA value.

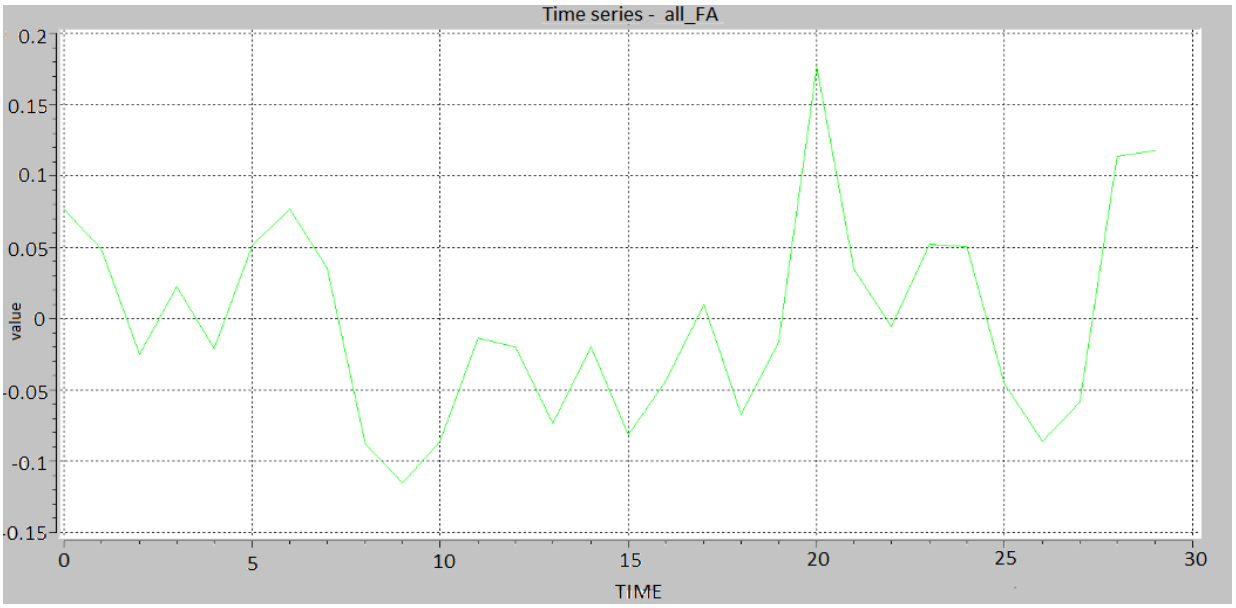

Figure 14. Comparison between the original FA values of 1.5T control scans with 1.5T MS data. The first ten (0-9) show control data while the rest twenty (10-29) represent MS data.

The last comparison is between 3T control data and 1.5T MS data. The original FA value for the control and MS scan is shown in the Figure 15 below. It can be observed that the control group has higher FA value because of its higher magnetic field strength while MS scans have irregular pattern of FA values due to the effect of MS.

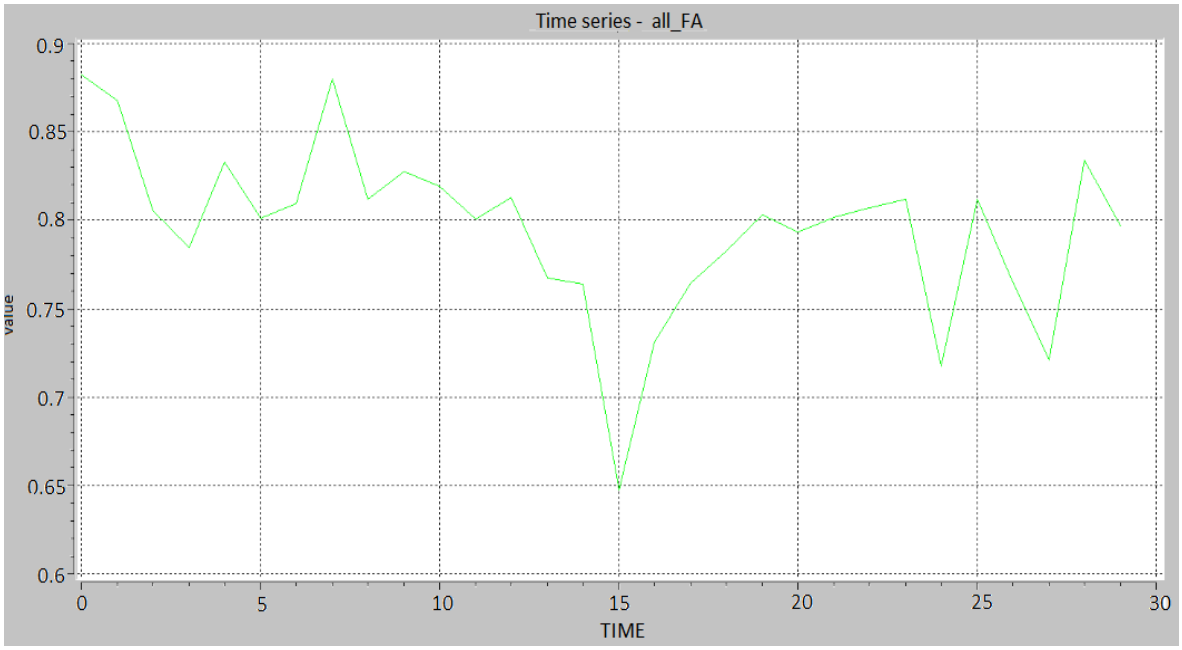

Figure 15. The original FA values of 3T Control group (0 -9) and 1.5T MS data (10-29).it is shown that the control scans have higher FA while that of MS data are irregular.

3.3. Magnetic resonance imaging acquisition

The MRI acquisitions for the control and patient groups are given in the following section below.

Controls

The examinations for 3T control group were taken by Siemens Trio (Siemens Healthcare, Erlangen, Germany), while for 1.5T control group, it is Siemens Avanto Scanners (Siemens AG Medical Solutions, Erlangen, Germany), and for both cases, a 12-channel head matrix coil was used.

The DTI protocol consisted of single-shot diffusion-weighted echo planar imaging sequence. The parameters of DTI for the 3T scanner were matrix 128 x 128, 3 averages, slice/gap 3.0/0.9 mm, voxel dimension, b-factor 0 and 1000 s/mm^2, repetition time (TR) 5144 ms, echo time (TE) 92 ms, FOV 230 mm, and 20 diffusion gradient orientations with total scan time of 50 min.

The 1.5 T scanner parameters were: matrix 128 x 128, 3 averages, slice/gap 5.0/1.5 mm, b-factor 0 and 1000 s/mm^2, TR 3600 ms, TE 96 ms, FOV 230 mm, and 12 diffusion gradient orientations with total scan time was 30 min.

In addition to DTI, the MRI protocol included sagittal T_1-weighted 3D is prepared gradient echo, axial T_2 turbo spin echo, conventional axial and high resolution sagittal FLAIR, axial T_2, and axial SWI (susceptibility weighted imaging) series.

MS patients

The patient images were acquired with a Siemens 1.5T MR scanner (Siemens, Avanto SQ, Siemens Medical Solutions, Erlangen, Germany) with the following magnetization prepared rapid gradient echo T_1-weighted MR parameters: TR 1160 ms, TE 4.24 ms, inversion time (TI) 600 ms, flip angle 15 degrees, slice thickness 0.9 mm, (axial) in-plane resolution 0.9 mm x 0.9 mm and acquisition matrix size of 256 x 224 pixels.

The DTI images were acquired in the same imaging session as the T_1-weighted images. Typical parameters for the DTI single-shot diffusion-weighted echo-planar imaging (EPI) sequence were TR 3500 ms, TE 96 ms, FOV 230 mm x 230 mm, matrix 128 x 128, 3 averages, slice/gap 5.0/1.5 mm, voxel dimension 1.8 mm x 1.8 mm x 5.0 mm, b-factor 0 and 1000 s/mm^2 and 12 gradient orientations. This study was approved by the Ethics Committee of Tampere University Hospital. Informed consent was given by the patients.

Data analysis

The analysis for the effect of magnetic field in DMRI and detection of MS is carried out by the FSL (FMRIB software library) Release 4.1.9. This software is mainly written by the members of Oxford Center for Functional MRI of the Brain (FMRIB). It is mainly used for noncommercial purpose [57].

FSL is not a single program but a collection of different tools and subprograms for different MRI data. It is Linux based operating system which can be installed in Window PC and Mac OSX with emulation software.

The steps used for the comparison of different groups of control and patient groups are described in the following sections.

3.4. Steps for group data analysis

The procedure used to compare the two groups of DMRI data is addressed in the following subsections. It includes the change in data format and FSL preprocessing before TBSS is carried out. Finally, statistical comparison is carried out between the created skeletons. The schematic flow for this process is shown in Figure 16 .

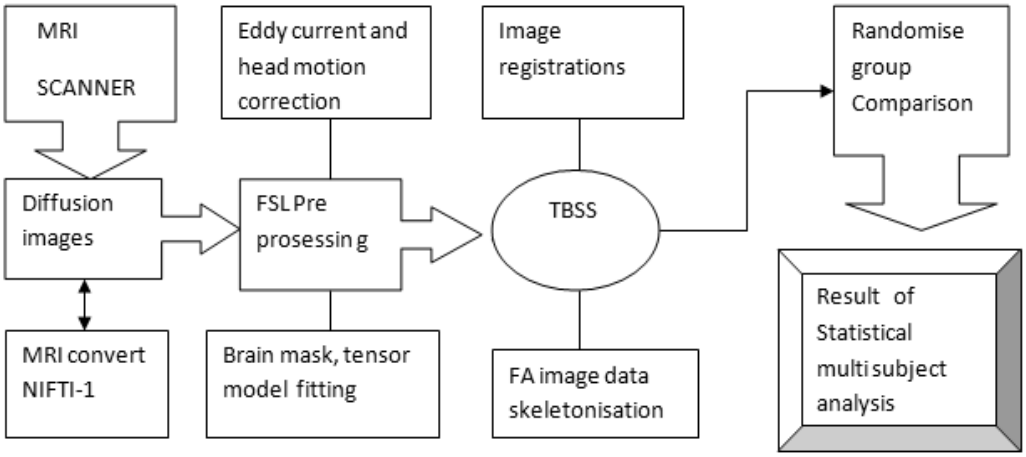

Figure 16. Steps used for white matter neural pathway analysis including conversion of data format, preprocessing, TBSS and statistical analysis.

3.4.1. DMRI brain format

The first step in the analysis of DMRI data is to change the format from DICOM (Digital Imaging and Communication in Medicine) to NIFTI-1 (Neuroimaging Informatics Technology Initiative) data format as FSL use NIFTI-1 as default. The conversion is done by using free online software called *MRI- Convert*.

3.4.2. Artifact reduction

Artifacts arising from eddy current and head movement are corrected by built-in software in FSL called *eddy current correction.* It corrects eddy current which arises from gradient coils and head movement. This software can be implemented from GUI (graphical user interface) or by writing the command line on the UNIX shell.

```
eddy_correct <input file> <output file> <reference No.>,
```

Where <input file> is a 4D image in NIFTI-1 format while <output file> is the file name for the output. The <reference No.> is the chosen register volume in which all other volumes in 4D input are registered. In this case, it is 0 as there is no registration to input file. The additional dimension is ID for the data.

3.4.3. Brain mask creation

After the input image is corrected for the artifacts, the next step is to create brain mask image. This is done by using the program *brain extraction tool* (BET) in FSL. It can be operated from GUI or by writing the *bet* command in the UNIX shell.

```
bet <input file> <output file> <options>,
```

Here the options are additional parameters to customize the output of brain extraction; in this case, -m is used to create binary mask creation.

3.4.4. Diffusion tensor data compiling

This stage is the last preprocessing stage before the start of real TBSS work. It calculates diffusion tensors from raw diffusion data. This is computed by using simple least square fit of the tensor model to the diffusion data. The tool DTIFIT is used for this computation and it takes input 4D input images, BET binary mask, diffusion gradient direction (b-vectors) and diffusion weighting factors (b-values). It can be run from GUI or writing the command below.

```
dtifit -k <dti data file> -o <output basename> -m <binary mask file> -r <b vectors file> -b <b values file> <options>,
```

The <dti data file> refers to eddy current corrected 4D diffusion image, including volumes with and without diffusion weighting; <output basename> is a user defined output name for the DTIFIT output; <binary mask file> is used for BET created binary mask file; <b vectors file> includes the direction for the NIFTI-1 file; <b values file> containing b-value for the NIFTI-1 file. Here care must be given to the order of b-value and b-direction. The <options> provide additional parameters, but it is not mandatory. DTIFIT creates many output files. The basic output files are listed below.

• Three eigenvector image files; 1st (<basename>_V1), 2nd (<basename>_V2), and 3rd eigenvectors (<basename>_V3).

• Three eigenvalue images, suffixes _L1, _L2, _L3.

• Mean diffusivity image, better known as apparent diffusion coefficient (ADC) map (_MD).

• Fractional anisotropy, suffix _FA.

• Mode of anisotropy (suffix _MO) with scale: oblate=-1, isotropic=0, prolate= 1.

• Raw T_2 image with no diffusion weighting, suffix _S0.

For TBSS computation, the important value is FA value. Before the actual TBSS starts, the FA values computed in the DTIFIT stage should be copied to a new folder (directory) and renamed to prefix PAT- and CON- to indicate the patient and control data. This name is also essential for the statistics comparison for the two groups in the later stage.

After renaming and copying the FA value in specified folder, the first TBSS command is used to preprocess the NIFTI files and put them into new subdirectory FA and original FA file into directory origdata.

```
tbss_1_preproc *nii.gz
```

This command will remove some part of FA data and set all voxel values at the end to 0 to remove possible errors during the previous stage, tensor fitting. This script also creates another program slicesdir which create another webpage that show static view of the FA values.

The next stage in TBSS is image alignment in this case to FMRIB58_FA template.

```
tbss_2_reg <options>
```

The option in this case is -T for FMRIB58_FA and -t <imagefile> for a given image file from list and -n for the most representative image by aligning one image to the other rest.

Creating mean FA and mean FA skeleton is done by using the command below.

```
tbss_3_postreg <options>
```

Here -T is used in our case as the mean FA and skeleton is computed from FMRIB58_FA template. While -S is used to compute directly from the original data. The generated mean FA and skeleton can be viewed in stats folder.

In order to view all the FA images under the FA skeleton the following command is used.

```
fslview all_FA -b 0,0.8 mean_FA_skeleton -b 0.2,0.8 -l Green
```

Here 0.2 and 0.8 are threshold values while green is the color for skeleton.

The last stage of TBSS is to threshold the mean FA skeleton. Values below the threshold are cut off from the skeleton.

```
tbss_4_prestats <threshold>
```
threshold assigned in this case is 0.3.

3.4.5. Inference

Statistical comparison is conducted between the two created FA skeletons for possible differences. This is done by creating GLM (General Linear Model) for unpaired t-test.

```
cd stats

design_ttest2 design `cat ../contamount` `cat ../dataamount`
```

`Contamount` refers to control data amount while `data amount` is patient data.

3.4.6. Running permutation test

After the creation of GLM and t-contrast files, then follows the permutation process by program *Randomise* to run voxelwise statistics to determine the difference between control and patient groups.

`randomise -i all_FA_skeletonised -o tbss -m mean_FA_skeleton_mask –d <design.mat> -t <design.con> -n 5000 <options>.`

`<input>` is a 4D image showing all FA skeleton, `all_FA_skeletonised,<output>` is a user defined file, in this case `tbss_FA` is used, `-m mean_FA_skeleton_mask` feeds the threshold skeleton area to the program, `-d <design.mat>` is the filename for created design matrix file and `-t<design.con>` is the filename for the created t contrasts file. The number of iteration is 5000 and selection for TBSS in the option is -T2.

4. RESULTS

In this chapter, the results of the experiments will be discussed. The results are subdivided into three parts. Firstly, the effect of magnetic field strengths will be discussed by comparing two groups which have different magnetic fields, and in this case, 3T and 1.5T with the same age range. Here the subjects on the study are free from any neurological disorders. In this TBSS analysis, subjects with 3T diffusion MRI have larger area of FA values compared to 1.5T diffusion MRI subjects. This can be demonstrated by their histogram and the skeletonized FA image. As it can be shown in the Figure 17 below, FA images of 3T subjects cover more areas of variation from the skeleton threshold.

4.1. Comparison between 1.5T and 3T of control FA data

In the first section of the result, the comparison between 1.5T and 3T control groups is presented with images of areas of high difference in significance with red-yellow color and histogram which shows the number of voxels vs 1-p values of significantly different regions.

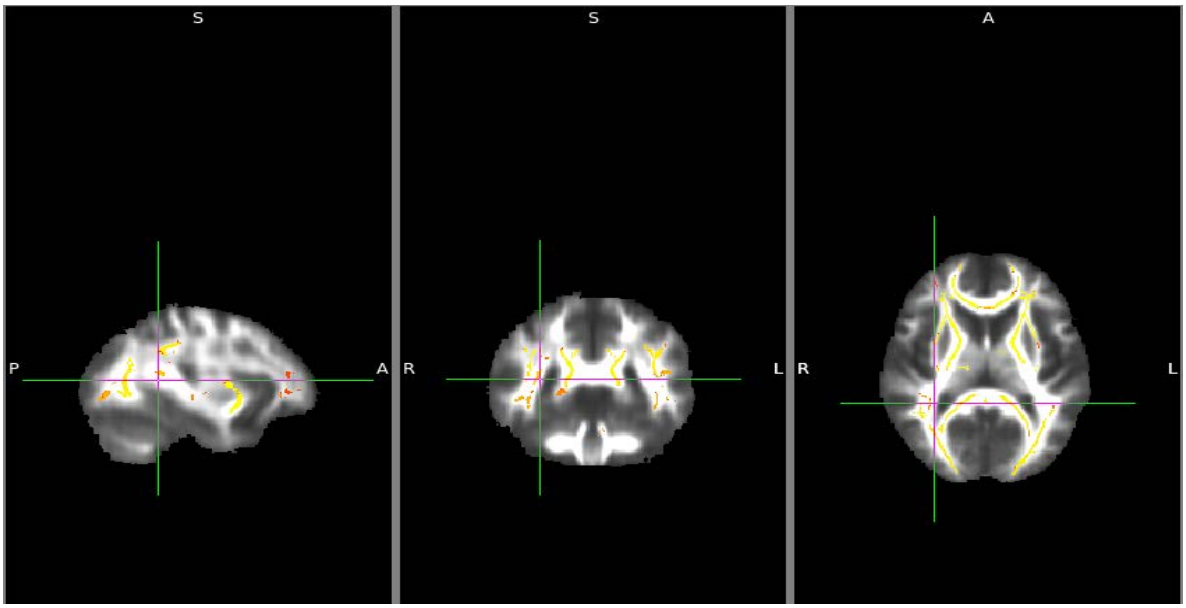

Figure 17. Regions with red-yellow color show areas with significant difference. Here both groups are free from neurological differences.

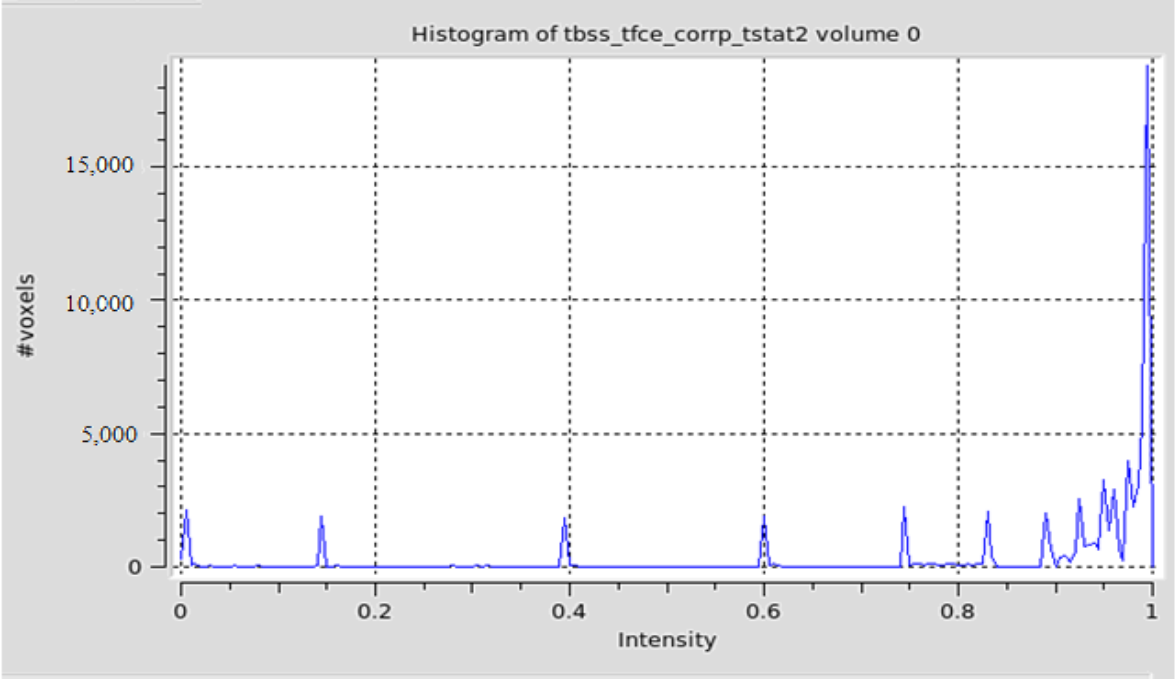

Figure 18. The histogram for the comparison between control groups (1.5T&3T) in which 1-p values vs voxel amount show high significance between them.

4.2. Comparison between 1.5T control data and 1.5T MS data.

The second section is comparison of 1.5T control data with 1.5T multiple sclerosis. In this study, 20 subjects from patient data are compared with 10 control data. Regions with significant difference and histogram of these regions are shown in the two figures below.

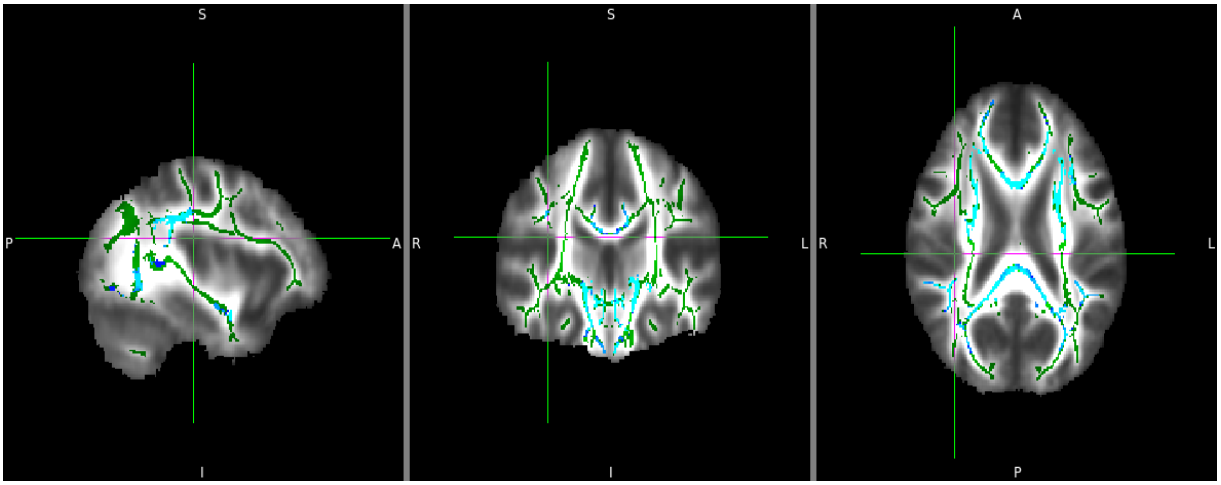

Figure 19.Regions with light blue show higher significant difference with green skeleton

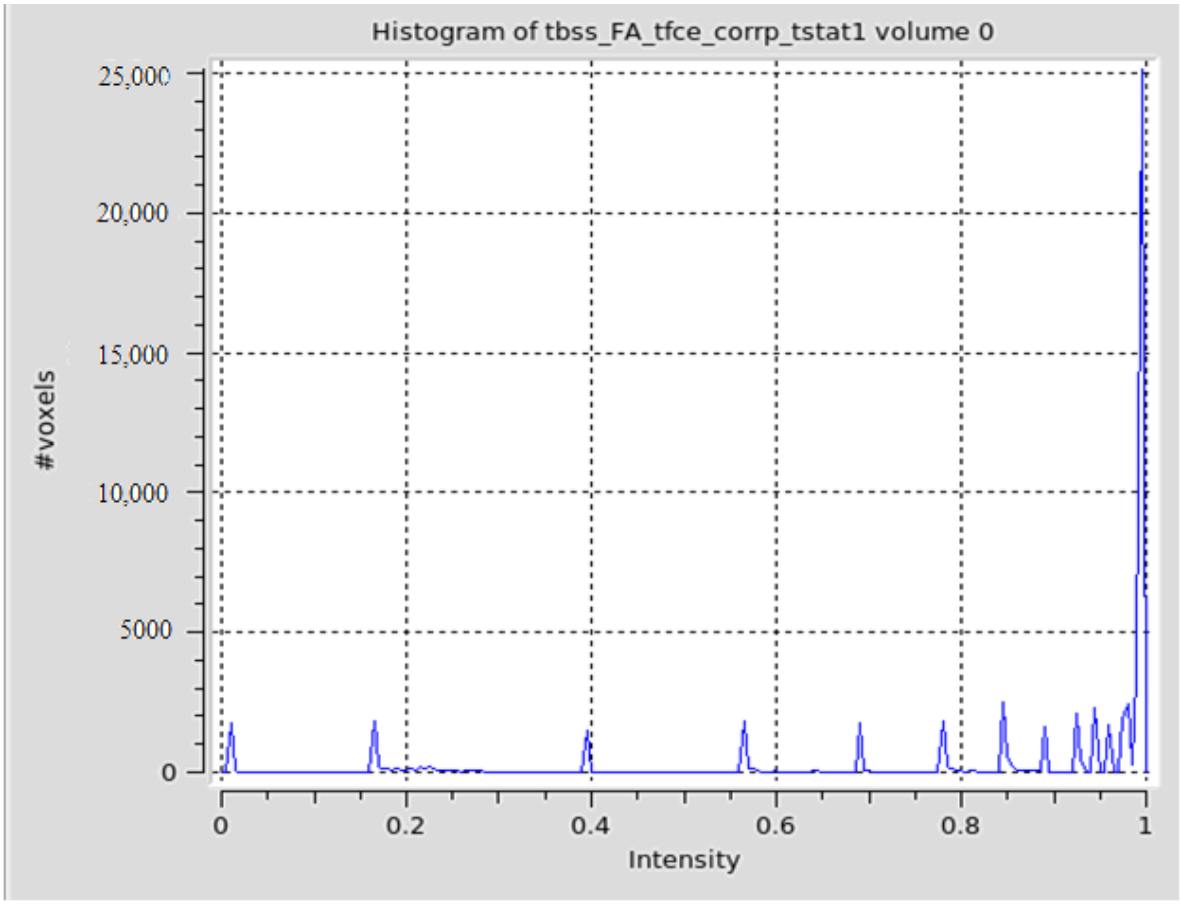

Figure 20. The histogram for the comparison between1.5T control group & 1.5T MS data in which 1-p values vs voxel amount show high significance between them.

4.3. The effect of magnetic field strength and multiple sclerosis (MS)

When comparing the effect of magnetic field strength with that of multiple sclerosis by comparing 1.5T MS patient with 3T control data, it can be seen that some of the changes occur due to magnetic field strength difference while the rest due to multiple sclerosis (MS). This is shown in Figure 21& 22 by adding both effects on the image data and by subtracting the effect of magnetic field. This method is performed by adding the effect of magnetic field strength (change between 1.5T and 3T control data) and multiple sclerosis (change between 1.5T control data and 1.5T MS data) by clicking them on the original image. Subtracting can be done by double clicking the respective effect from the menu bar.

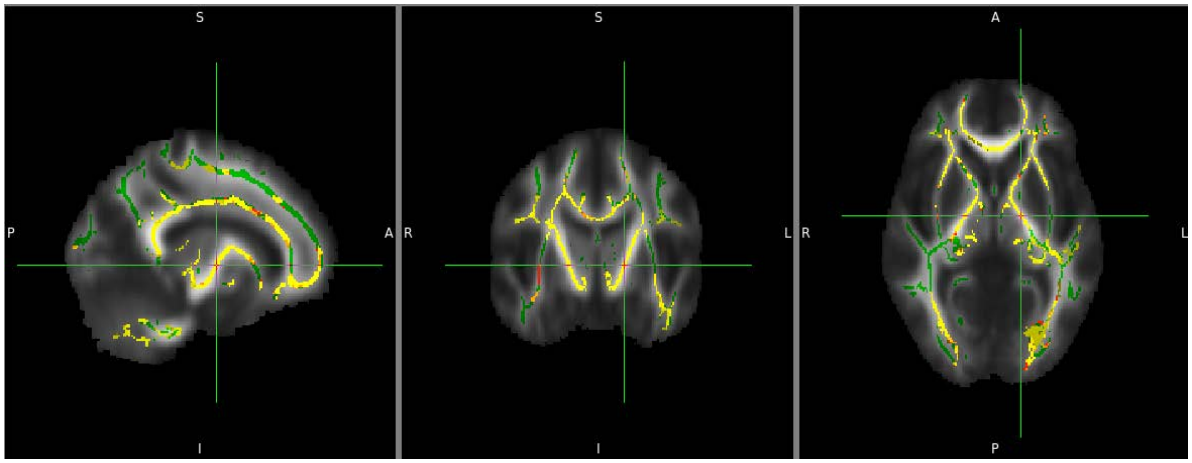

Figure 21. The effect of both magnetic field and multiple sclerosis (MS) by putting one effect on another.

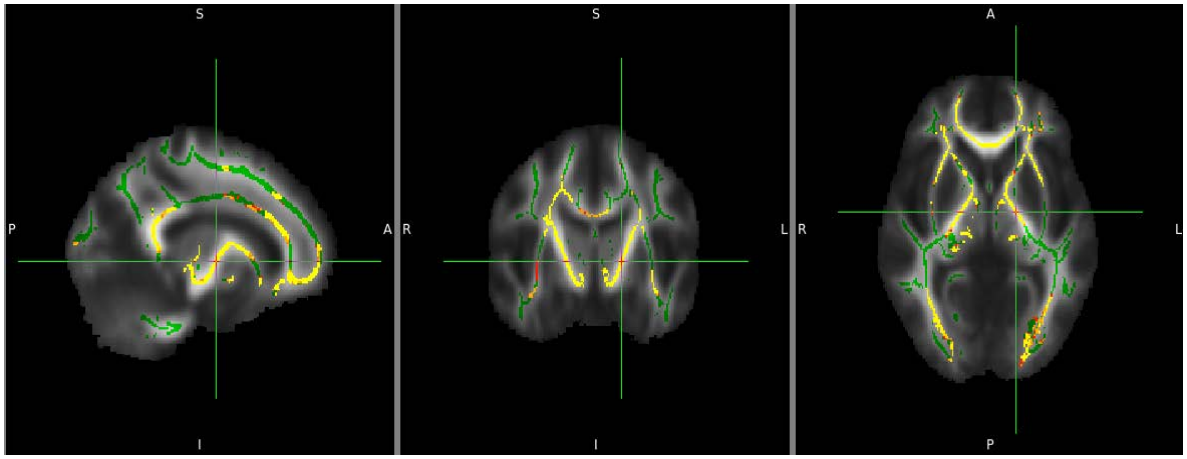

Figure 22. Brain images when the effect of magnetic field is subtracted

In both the Figures 21 & 22, the green area shows the skeleton image while the red-yellow regions show areas with significant difference between their respective images. As seen in Figure 22, some parts of the yellow-red region are lost when the effect of magnetic field strength is subtracted.

In comparison between 3T control data and 1.5T image with multiple sclerosis (MS), it is seen in Figure 23 below that significantly larger areas of brain have differences due to higher magnetic field from 3T.

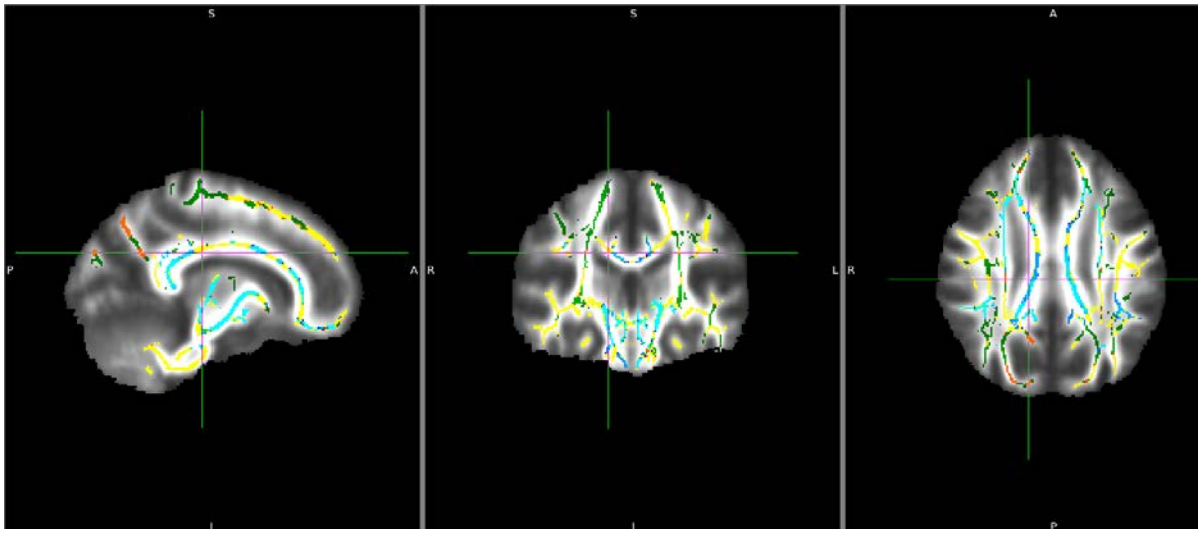

Figure 23. Comparison between 1.5 T MS data and 3T control data

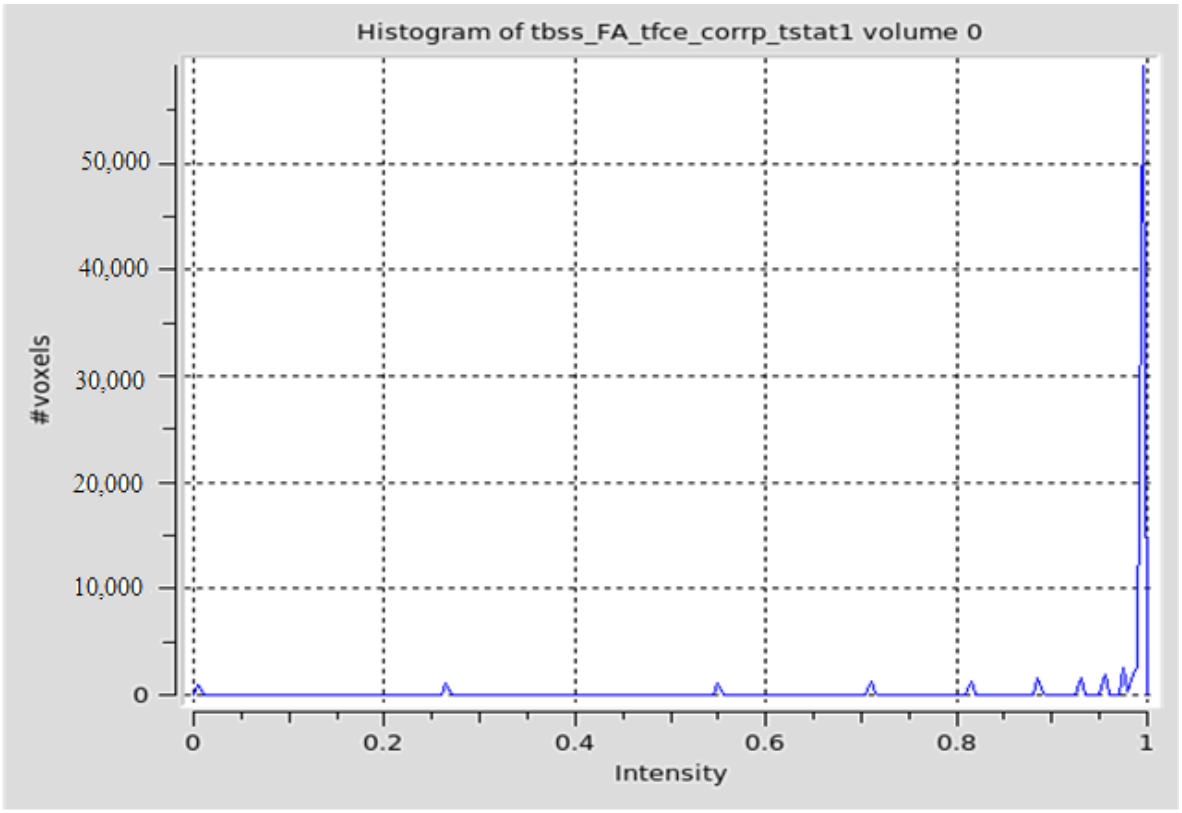

Figure 24. The histogram for the comparison between 3T control group & 1.5T MS data in which 1-p values vs voxel number show high significance difference between them

5. DISCUSSION

This aim of this thesis work is to identify the effect of magnetic field strength in the detection of pathological damage by multiple sclerosis (MS) using diffusion MRI images. Diffusion MRI, specifically, diffusion tensor imaging (DTI) is a unique and most promising technique with regard to MS as it quantifies the amount of nonrandom water diffusion within tissues and provide unique *in vivo* information about pathological process that affect water diffusion as a result of brain microstructural damage [58]. It has a distinct advantage over other MRI methods, like conventional MRI and functional MRI, as it can detect changes in short time and help to detect microstructural damage of brain.

In this research, 1.5T MS images have been used to compare with 1.5T and 3T control groups. Their respective comparisons will be discussed in the subsections below.

Comparison of 1.5 T and 3T control data

As seen in the Figure 17, the red-yellow regions show significant differences between the two groups due to the effect of magnetic field strength. The histogram in Figure 18 shows the numbers of voxels which are significantly different are greater than 15,000. Here, in this case, both control groups are free from neurological problems.

Comparison between 1.5 T MS and 1.5T control data

When we are comparing 1.5T MS with 1.5T control data as seen in Figure 19, light blue color shows a significant difference between the two groups. It is also seen in Figure 20 that the histogram shows around 25,000 significantly different voxels. This value is caused only by the effect of MS, and it exceeds the effect of magnetic field shown in Figures 17 and 18. There is an abnormal diffusion change in widespread white matter regions of MS patients. Specifically, low FA value, increased mean diffusivity (MD ($\lambda_1+ \lambda_2+ \lambda_3$)/3), axial diffusivity AD (λ_1), and radial diffusivity (RD= ($\lambda_2+ \lambda_3$)/2). The latter plays predominant role in detecting subtle pathological changes in MS [33]. The reason behind the increment of RD, MD, and AD is because disruption of white matter tract causes increment of diffusion in tract directions other than major tracts (λ_2 and λ_3 in Figure 3). This direction includes axial (perpendicular) and radial (horizontal) directions which cause increments of RD and AD. The increment of diffusion in other tracts causes the difference among the Eigen values in three directions to decrease as seen in equation 2 and hence the reduction of FA values. In other study of comparing MS patients with normal control of same age range, there is increment in RD value and slight increment of AD in reduced FA regions. These regions include the fornices, the

left corona radiata, the inferior longitudinal fasciculus in both hemispheres, both optic radiations, and parts of the corpus callosum [59].

Comparison between 1.5T MS and 3T control data

When comparing 1.5T MS data and 3T control data, the difference observed is due to the effect of both magnetic field strength and pathological disturbances caused by MS. As it can be seen in Figure 23, those regions with blue, red, and yellow color show significant differences with control data. The number of voxels with significant difference highly exceeds as shown in Figure 24 as it comprises both effect of magnetic field strength and MS. In other study for the effect of magnetic field strength, specifically, high-field MR imaging, it was observed that it has a substantial influence on the classification of patients with CIS according to imaging and a mild influence on the classification according to diagnostic criteria for MS, leading to consequences for prognostic classification, imaging guidelines, and clinical trials [60]. Comparison between 1.5T and 3T MS images shows that the number, volume, and spatial distribution of signal abnormalities (SA) for a pairwise T_2 and T_1 analyses are higher for 3T compared to 1.5T. Logical regression analysis also shows that the probability of missing SA is higher for 1.5T than 3T, and, significantly, more regionally distinct spatial SA difference is observed on 3T than 1.5T [61].

The advancement of new diagnostic method, like diffusion tensor imaging (DTI), has improved the detection of MS. On the contrary, similar studies conducted using T_2-weighted MRI imaging show mild results for CIS with McDonald criteria. These criteria state that in order to detect MS from visual evoked potential (VEP) and cerebrospinal fluid, at least three out of four of the following requirements should be fulfilled: (i) at least one gadolinium-enhancing lesion or at least nine lesions on T_2-weighted MRI; (ii) at least one juxtacortical lesion; (iii) at least three periventricular lesions; and (iv) at least one infratentorial lesion [62-64]. Hence diffusion tensor imaging (DTI) is more simplistic and less time consuming in comparison to T_2-weighted MRI imaging method.

Reliability of the results

The data that is used for this study are carefully taken and full information about each data is available. The MS data used in this research is a part of longitudinal study for the progress of the disease so that no ambiguity could be raised. Since the control data is free from any neurological impairment, the comparison between control and patient is reliable.

Diffusion MRI is one of the new advanced methods used to study detection of neurological problems, and in this case, MS, as it can detect changes in microstructures of human brain by using changes in diffusion metrics for example FA and ADC. This enables detection in faster and reliable way compared to other MRI methods. The comparison method TBSS used for comparing control and patient FA data uses

advantages of both VBM and ROI methods, and significantly different regions can easily be seen on color-coded areas of the image and histogram figures. This quantitative data (histogram) makes the conclusion more powerful than the qualitative data (color-coded areas).

The limitations in this research are first age indifference; since both controls and patient data are age related, effects caused due to age differences cannot be addressed. The number of data is relatively low and the result would be more reliable and complete if the number of participants is large. Usage of one type of MS type, that is, CIS, limits the extent of the research, and inclusion of other stages or types of MS would have made the research more complete. Lastly but not the least, since the MS data was taken only by 1.5T, the analysis on the detection of higher magnetic field strength could not be used for the analysis of magnetic field strength

Criticism of TBSS

In this section, some of the drawbacks and limitations of TBSS and, in general, diffusion MRI are discussed. The first limitation of TBBS involves around skeletonisation. In TBSS, skeletonisation depends on finding maximum FA value across perpendicular direction of local tracts. If there are some abnormalities, FA value of their corresponding voxel will decrease and other voxel with lesser FA value will be selected for skeleton image; thus this would create ambiguity. But this abnormality can also indicate microstructural change, which is the intended aim of this method. The other case regarding skeletonisation and coregistration is that images should be registered correctly before they are skeletonised as it would be difficult to spot registration errors since TBSS is completely an automatic process. If this is not done correctly, it would give biased results [65].

The second pitfall regarding TBSS and skeletonisation is partial volume effect that is occurrence of many fibers in a single voxel; this will ultimately lower the FA value in the skeleton and give biased results and wrong directions [65].

The third limitation goes in general to diffusion MRI and DTI as larger acquisition time is the main source of flaws as patient’s motion during scan time is inevitable, which causes distortion in the acquired images. Image preprocessing is the remedy for this distortion. Affine image registration is commonly used to efficiently remove patient movement. The other point to note is, when comparing control data and patient data, the parameters should be similar, that is, the equipment should be similar with their corresponding parameters [65].

6. CONCLUSIONS

Diffusion tensor imaging is a new emerging method to detect pathological lesions by using the change in diffusion parameters, mainly FA values. It has advantage over other methods in which it give faster results for early diagnosis and helps to understand the progression of MS.

From the results, it can be concluded that higher magnetic field strength detect MS better than lower magnetic field strength due to improved signal to noise ratio and chemical shift. The results obtained by comparing 1.5T MS data with 3T MS data shows it is difficult to get clear distinction between the effect of magnetic field and that of MS. Further clinical studies with larger number of subjects and different types or stages of MS should be done in order to improve the sensitivity and specificity of this method.

REFERENCE.

1 Gong, G., Jiang, T., Zhu, C., Zang, Y., Wang, F., Xie, S., Xiao, J., Guo, X., 2005. Asymmetry analysis of cingulum based on scale-invariant parameterization by diffusion tensor imaging. Hum. Brain Mapp. 24, 92– 98

2. A. Brander, A. Kataja, A. Saastamoinen, P. Ryymin, H. Huhtala, J. Öhman *et al.* Diffusion tensor imaging of the brain in a healthy adult population: normative values and measurement reproducibility at 3 T and 1.5 T Acta Radiologica, 51 (2010), pp. 800–807

3 Prentice RL Surrogate endpoints in clinical trials: definition and operational criteria. Stat Med. 1989 Apr; 8(4):431-40.

4 Mori S, Zhang J. Principles of diffusion tensor imaging and its applications to basic neuroscience research. Neuron. 2006; 51:527–539.

5 Heidi Johansen-Berg, Timothy E.J Behrens Diffusion MRI from quantitative measurement to in vivo neuroanatomy 2009, pages 180-183.

6 Bjartmar C, Trapp BD Axonal and neuronal degeneration in multiple sclerosis: mechanisms and functional consequences. Current opinion Neurol. 2001 Jun; 14(3):271-8. Review

7 http://www.nationalmssociety.org/about-multiple-sclerosis/what-we-know-about-ms/diagnosing-ms/cis/index.aspx

8 Anthony Kam Commentary**:** The past, present and future of imaging in multiple sclerosis *Journal of Clinical Neuroscience, Volume 17, Issue 11, November 2010, Page 1482*

9 Bloch F. Nuclear induction. Phys Rev 1946;70: 460–474.

10 Purcell EM, Torrey HC, Pound RV. Resonance absorption by nuclear magnetic moments in a solid. Phys Rev 1946;69:37–38.

11 Bloembergen N, Purcell EM, Pound RV. Relaxation effects in nuclear magnetic resonance absorption. Phys Rev 1948;73:679–712.

12. Hoult DI, Richards RE.The signal-to-noise ratio of the nuclear magnetic resonance experiment. J Magn Reson 1976;24:71–85.

13. Westbrook Catherine.MRI at a glnace. Oxford, England 2002, Blackwell Science, 128pp.

14. Bitar R., Leung G., Perng R., Tadros S., Moody A. R., Sarrazin J., McGregor C., Christakis M., Symons S., Nelson A., Roberts T. P. MR Pulse Sequences: what every radiologist wants to know but is afraid to ask. Radiographics 26(2006)2, pp. 513-37.

15 Keevil, S F 2001 Magnetic resonance imaging in medicine Physics Education 36 476-485

16 Dominic Weishaupt, Victor D. Köchli, Borut Marincek. How Does MRI Work: An Introduction to the Physics and Function of Magnetic Resonance Imaging

17 Pierpaoli C, Jezzard P, Basser PJ, et al. Diffusion tensor MR imaging of human brain. Radiology 1996; 201: 637–648

18. Lee CEC, Danelian LE, Thomasson D, Baker EH. Normal regional fractional anisotropy and apparent diffusion coefficient of the brain measured on a 3T MR scanner. Neuroradiology 2009; 51:3 –

19 Marenco S, Rawlings R, Rohde GK, Barnett AS, Honea RA, Pierpaoli C, et al. Regional distribution of measurement error in DTI. Psychiatry Res 2006; 147:69 – 78.

20 Ozturk A, Sasson AD, Farrell JAD, Landman BA, da Motta ACBS, Aralasmak A, et al. Regional differences in diffusion tensor imaging measurements: assessment of intrarater and interrater variability. Am J Neuroradiol 2008; 29:1124 – 7.

21 Bisdas S, Bohning DE, Besenski N, Nicholas JS, Rumboldt Z. Reproducibility, interrater agreement and age-related changes of fractional anisotropy measures at 3 T in healthy subjects: effect of the applied b-value. Am J Neuroradiol 2008; 29:1128 – 33.

22 B onekamp D, Nagae LM, Degaonkar M, Matson M, Abdalla WMA, Parker PB, et al. Diffusion tensor imaging in children and adolescents: reproducibility, hemispheric and age-related differences. Neuroimage 2007; 34:733 – 42.

23 Smith MS, Jenkinson M, Johansen-Berg H, Rueckert D, Nichols TE, MackayCE, et al. Tract-Based Spatial Statistics: Voxelwise Analysis of Multi-Subject Diffusion Data. FMRIB Technical Report TR05SS1 (a related paper has been accepted for publication in NeuroImage).2005;p26

24 K. J. Worsley, A. C. Evans, S. Marrett, P. Neelin A three-dimensional statistical analysis for CBF activation studies in human brain. J Cereb Blood Flow Metab. 1992 November; 12(6): 900–918. doi: 10.1038/jcbfm.1992.127

25 simon T ,Ding L, Bish J, McDonald –McGinn D,Zackai E,Gee J (2005) Volumetric, Connective, and Morphologic changes in the brains of children with chromosome 22q11.2 deletion syndrome: an integrative study. NeuroImage 25:169-180

26 Jones D, Catani M, Pierpaoli C,Reeves S ,Shergill S, O'Sullivan M ,Golesworthy P, McGuire P,Horsefield M,Simmons A,Williams S, Howard R (2006) age effect on diffusion tensor magnetic resonance imaging tractography measures of frontal cortex connections in schizophrenia. *Hum Brain Mapp* 27(3):230-238

27 Park H-J,Westin C-F,Kubiccki M,Maier S,Niznikiewicz M,Baer A,Frumin M,kikinis R, Jolesz F, McCarley R, Shenton M (2004) white matter hemisphere asymmetrics in healthy subjects and in schizophrenia: a diffusion tensor MRI study .*NeuroImage* 23:213-223

28 Ellis C, Simmons A, Jones D, Bland J, Dawson J , Horsfield M, Williams S, Leigh P(1999) Diffusion Tensor MRI assesses corti- cospinal tract damage in ALS .Neurology 53:1051-1058

29 Kubicki M,Westin C-F,Nestor P,Wible C, Frumin M,Maier S,Kikins R,Jolesz F,McCarley R, Shenton M(2003) Cingulate fasciculus integrity disruption in schizophrenia : a magnetic resonance diffusion Tensor imaging study. *Biological psychiatry* 54:1171-1180

30. Park H-J Kubicki M Maier S,Niznikiewicz M,Baer A,Frumin M,Kikinis R, Jolesz F,Westin C-F(2003) Spatial normalization of diffusion tensor MRI using multiple channels *NeuroImage* 20:1995-2009

31 Horsfield, M., Mapping eddy current induced field for the correction of diffusion weighted echo planar images. Magnetic Resonance Imaging 17, 1335–1345 1999.

32 Basser, P., Matiello, J., Le Bihan, D., 1994. Estimation of the effective selfdiffusion tensor from the NMR spin echo. J. Magn. Reson., B 103, 247– 254

33 Pierpaoli, P., Basser, P., 1996. Toward a quantitive assessment of diffusion anisotropy. Magn. Reson. Med. 36, 893–906.

34 Smith,S., 2002. Fast robust automated brain extraction. Hum. Brain Mapp. 17 (3),143– 155.

35 Nichols, T.E., Holmes, A.P., 2001. Nonparametric permutation tests for functional neuroimaging: a primer with examples. Hum. Brain Mapp.15,1– 25.

36 Benedetta Bodini, Olga Ciccarelli *Diffusion MRI, 2009, Pages 175-203*

37 Chan LL, Rumpel H, Yap K, et al. Case control study of diffusion tensor imaging in Parkinson's disease**.** J Neurol Neurosurg Psychiatry 2007;78:1383–86

38 Vaillancourt DE, Spraker MB, Prodoehl J, Abraham I, Corcos DM, Zhou XJ, Comella CL, Little DM: High-resolution diffusion tensor imaging in the substantia nigra of de novo Parkinson disease**.** *Neurology* 2009, **72**(16):1378-1384.

39 Kraus MF, Susmaras T, Caughlin BP, et al. White matter integrity and cognition in chronic traumatic brain injury: a diffusion tensor imaging study. Brain. 2007;130:2508–19. [PubMed]

40. Werring DJ, Clark CA, Barker GJ, Thompson AJ, Miller DH. Diffusion tensor imaging of lesions and normal-appearing white matter in multiple sclerosis. Neurology.1999 May 12; 52(8):1626-32.

41 Armstrong MA, McDonnell GV, Graham CA, Kirk CW, Droogan AG, Hawkins SA. Mult Scler. Relationship between tumour necrosis factor-alpha (TNFalpha) production and a specific multiple sclerosis (MS) associated TNF gene haplotype1999 Jun;5(3):165-70.

42 Rovaris M, Gallo A, Valsasina P et al (2005) Short term accrual of gray matter pathology in patients with progressive multiple sclerosis: An in vivo study using diffusion tensor MRI Neuroimage 24:1139-1146

43 Griffin CM, chard DT,Ciccarelli o ET AL (2001) Diffusion tensor imaging in early relapsing – remitting multiple sclerosis, Mult Scler 7: 290-297

44 Bodini B, Mandarelli G, Tomassini V, Tarsitani L, Pestalozza I, Gasperini C, Lenzi GL, Pancheri P, Pozzilli C. Alexithymia in multiple sclerosis : relationship with fatigue and depression. Acta Neurol Scand. 2008 Jul; 118(1):18-23.

45 Rocca MA, Matthews PM, Caouto D, Ghezzi A, Falini A, Scotti G, Comi G, Filippi M(2002).Evidence for wide spread movement –associated functional MRI changes in patients with PPMS . *Neurology* .58 866-872

46 Filippi M, Cercignani M, Inglese M, Horsfield MA, Comi G. Diffusion tensor magnetic resonance imaging in multiple sclerosis. Neurology. 2001 Feb 13;56(3):304-11.

47 Bozzali M, Cercignani M, Sormani MP, Comi G, Filippi M. Quantification of brain gray matter damage in different MS phenotype by use of diffusion tensor MR imaging . AJNR Am J Neuroradiol. 2002 Jun-Jul;23(6):985-8.

48 Oreja-Guevara C, Rovaris M, Iannucci G, Valsasina P, Caputo D, Cavarretta R, Sormani MP, Ferrante P, Comi G, Filippi M. Progressive gray matter damage in patients with relapsing-remitting multiple sclerosis: a longitudinal diffusion tensor magnetic resonance imaging study .Arch Neurol. 2005 Apr; 62(4):578-84.

49 Montalban X, Sastre-Garriga J, Filippi M, Khaleeli Z, Téllez N, Vellinga MM, Tur C, Brochet B, Barkhof F, Rovaris M, Miller DH, Polman CH, Rovira A, Thompson AJ. Primary progressive multiple sclerosis diagnostic criteria: a reappraisal. Mult Scler. 2009 Dec; 15(12):1459-65. doi: 10.1177/1352458509348422. Epub 2009 Dec 7

50 Cercignani M, Inglese M, Siger-Zajdel M, Filippi M. segmenting brain white matter ,gray matter and cerebro –spinal fluid using diffusion tensor –MRI driven indices .Magn Reson Imaging. 2001 Nov; 19(9):1167-72

51 Agosta F, Benedetti B, Rocca MA, Valsasina P, Rovaris M, Comi G, Filippi M.Quantification of cervical cord pathology in primary progressive MS using diffusion tensor MRI. Neurology. 2005 Feb 22; 64(4):631-5.

52 Hesseltine SM, Law M, Babb J, Rad M, Lopez S, Ge Y, Johnson G, Grossman RI Diffusion tensor imaging in multiple sclerosis: assessment of regional differences in the axial plane within normal-appearing cervical spinal cord. AJNR Am J Neuroradiol. 2006 Jun-Jul;27(6):1189-93

53 Rocca MA, Hickman SJ, Bö L, Agosta F, Miller DH, Comi G, Filippi M Imaging the optic nerve in multiple sclerosis. Review PMID Mult Scler. 2005 Oct; 11(5):537-41

54 Trip SA, Wheeler-Kingshott C, Jones SJ, Li WY, Barker GJ, Thompson AJ, Plant GT, Miller DH.Optic nerve diffusion tensor imaging in optic neuritis. Neuroimage. 2006 Apr 1;30(2):498-505. Epub 2005 Oct 20.

55. Kuhl CK, Gieseke J, von Falkenhausen M, et al. Sensitivity encoding for diffusion-weighted MR imaging at 3.0 T: intraindividual comparative study.Radiology 2005;234:517–26

56 Gillard JH, Papadakis NG, Martin K, et al: MR diffusion tensor imaging of white matter tract disruption in stroke at 3 T. Br J Radiol 2001; 74: 642 – 647.

57 http://www.fmrib.ox.ac.uk/fsl/

58 Y. Liu, Y. Duan,Y.He et al., “Whole brain white matter changes revealed by multiple diffusion metrics in multiple sclerosis: a TBSS study,” *European Journal of Radiology*, vol. 81, no. 10, pp. 2826–2832, 2012.

59 Roosendaal S.D., Geurts J.J.G., Vrenken H., Hulst H.E., Cover K.S., Castelijns J.A., PouwelsP.J.W., BarkhofF. Regional DTI differences in multiple sclerosis patients (2009) *NeuroImage*, 44 (4) , pp. 1397-1403.

60 M.P. Wattjes, M. Harzheim, C.K. Kuhl, J. Gieseke, S. Schmidt, L. Klotz *et al*. Does high-field MR imaging have an influence on the classification of patients with clinically isolated syndromes according to current diagnostic MR imaging criteria for multiple sclerosis? AJNR Am. J. Neuroradiol., 27 (2006), pp. 1794–1798

61 Di Perri C., Dwyer M.G., Wack D.S., Cox J.L., Hashmi K., Saluste E., Hussein S., (...), Zivadinov R. Signal abnormalities on 1.5 and 3 Tesla brain MRI in multiple sclerosis patients and healthy controls. A morphological and spatial quantitative comparison study (2009) NeuroImage, 47 (4), pp. 1352-1362.

62 W.I. McDonald, A. Compston, G. Edan *et al*. Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis Ann Neurol, 50 (2001), pp. 121–127

63 F. Barkhof, M. Filippi, D.H. Miller *et al.* Comparison of MRI criteria at first presentation to predict conversion to clinically definite multiple sclerosis Brain, 120 (1997), pp. 2059–2069

64 M. Tintore, A. Rovira, M.J. Martinez *et al.* Isolated demyelinating syndromes: comparison of different MR imaging criteria to predict conversion to clinically definite multiple sclerosis Am J Neuroradiol, 21 (2000), pp. 702–706

65. Jones DK, Cercignani M. Twenty-five pitfalls in the analysis of diffusion MRI data. NMR in Biomedicine. 2010;23(7):803–820.