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The CONNECT project: Combining macro- and micro-structure

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ABSTRACT

In recent years, diffusion MRI has become an extremely important tool for studying the morphology of living brain tissue, as it provides unique insights into both its macrostructure and microstructure. Recent applications of diffusion MRI aimed to characterize the structural connectome using tractography to infer connectivity between brain regions. In parallel to the development of tractography, additional diffusion MRI based frameworks (CHARMED, AxCaliber, ActiveAx) were developed enabling the extraction of a multitude of micro-structural parameters (axon diameter distribution, mean axonal diameter and axonal density). This unique insight into both tissue microstructure and connectivity has enormous potential value in understanding the structure and organization of the brain as well as providing unique insights to abnormalities that underpin disease states.

The CONNECT (Consortium Of Neuroimagers for the Non-invasive Exploration of brain Connectivity and Tracts) project aimed to combine tractography and micro-structural measures of the living human brain in order to obtain a better estimate of the connectome, while also striving to extend validation of these measurements. This paper summarizes the project and describes the perspective of using micro-structural measures to study the connectome.

Introduction

Studying the connectome of the brain has become a popular research topic that is rapidly gaining in importance. Non-invasive assessment of the structural connectome (large scale connections) can only be achieved by magnetic resonance imaging techniques, specifically — tractography applied to diffusion imaging data (structural connectivity) (Axer et al., 2011; Bastiani et al., 2012; Cammoun et al., 2012; Johansen-Berg and Behrens, 2009; Le Bihan and Johansen-Berg, 2012; Owen et al., 2013; Sporns, 2012; Sporns et al., 2005; Toga et al., 2012; Van Essen and Ugurbil, 2012). Although tractography provides a powerful measure of large scale brain connections, it is not a direct or comprehensive measure of the connectome. Moreover, diffusion tensor imaging and tractography also suffer from well described methodological and technical limitations that often lead to artifacts and potential errors in the assessment

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of connectivity (Assaf and Pasternak, 2008; Jones and Basser, 2004; Jones and Cercignani, 2010).

A range of definitions of the connectome have been proposed. One general definition of this brain property refers to a combination of a multitude of neural characteristics that describes the ability of the brain to transfer information between different areas at different length and time scales. While the neuronal pathways and synchronized activity are the main observable features of the connectome, they are built from smaller components that assemble, piece by piece, the complex network between neurons (and glia). This micro-structural characteristic of neural tissue may refer to architecture of cell bodies and neurite arrangement within the gray matter or axonal properties (size distribution, density, myelin content) in the white matter.

The EU CONNECT consortium (Consortium Of Neuroimagers for the Non-invasive Exploration of brain Connectivity and Tracts) (www.brain-connect.eu) set out to explore if micro-structural measures of the white matter can be used, to better understand and define the structural connectome. The CONNECT project envisioned that a combination of diffusion MRI based tractography and various micro-structural measures of the white matter can provide better characterization of neural tissue that can constrain and refine the assessment of the connectome.

The project was built in a multi-level design consisting of four parts: methods development, enhanced tractography and connectivity, validation and applications. In methods development, the CONNECT project aimed to further develop and optimize micro-structural measurement techniques to provide robust and comprehensive assessment of neural tissue micro-structure (see methods description below). In the enhanced tractography and connectivity part the aim was to combine the micro-structural measures with current tractography algorithms to constrain and enhance the validity of connectivity measures from diffusion MRI. In the validation part of the project several approaches were implemented to verify that the advanced micro-structural techniques do indeed measure real micro-structural properties of the tissue. In the application phase of the project we aimed to explore the variability of the advanced measures over a large cohort of subjects and to construct the first in-vivo atlas of tissue micro-structure. In addition we wished to demonstrate the utility of these methods in a practically realizable clinical setup: in development and pathological conditions.

In this perspective report we provide our view of measuring brain connectivity using novel micro-structural measures (and their relation to the connectome) with diffusion MRI, and how these are likely to take center stage in the burgeoning field of brain connectomics. We will describe here the unique approaches that were developed to validate the advanced methods used in this project. Lastly, we also provide here a first view of the micro-structural atlas and the new opportunities it opens in neuroimaging and neuroscience.

Diffusion methods for studying tissue micro-structure

Diffusion tensor imaging (DTI) is the state-of-the-art methodology for white mater imaging (Assaf and Pasternak, 2008; Basser, 1995; Basser and Jones, 2002; Basser et al., 1994, 2000; Catani et al., 2002; Johansen-Berg and Behrens, 2009; Le Bihan et al., 2001; Pierpaoli et al., 1996). However, this methodology suffers from inherent limitations and its relation to tissue micro-structure is not straightforward (Alexander et al., 2002; Assaf and Basser, 2005; Assaf and Pasternak, 2008; Basser et al., 1994; Beaulieu, 2002; Le Bihan and Johansen-Berg, 2012; Oouchi et al., 2007; Tuch et al., 2002). In recent years, several acquisition and analysis frameworks were developed to provide a more direct and specific estimation of micro-structural features of the white matter (Alexander, 2008; Alexander et al., 2010; Assaf and Basser, 2005; Assaf et al., 2004, 2006). Within the work of the CONNECT project, we aimed to further develop, generalize and extend the applicability of these methods and acquire a database to investigate the variance of these features over the healthy population. Among the methods that were used are: CHARMED, AxCaliber, ActiveAx and double PFG.

1. The CHARMED (composite hindered and restricted model of diffusion) framework (Assaf and Basser, 2005; Assaf et al., 2004) models white matter by combining two diffusion components: hindered and restricted. Within CHARMED it is assumed that diffusion in the axonal space is restricted while elsewhere it is only hindered. The analytical diffusion signal descriptions of each of these diffusion processes are different. The hindered diffusion component is characterized by a regular diffusion tensor as it follows the theory of Stejskal and Tanner (Assaf et al., 2004). The restricted diffusion component is modeled by previously developed analytical expressions for diffusion within cylinders (Callaghan, 1995; Neuman, 1974; van Gelderen et al., 1994). By assuming a total independency of these two diffusion processes, the CHARMED model fits the raw data simultaneously to both, estimating their volume fractions (fr and fh) as well as the diffusivity parameters (Dh, Dr) and axonal orientation(s) (Assaf et al., 2004).
2. The AxCaliber framework, in common with CHARMED, is also a two-compartment white matter model[2] that provides estimates of the axonal diameter distribution (ADD) within each voxel (Assaf et al., 2008; Barazany et al., 2009). AxCaliber requires acquisition of the data perpendicular to the fiber orientation necessitating careful planning of the experiment by controlling the relative placement of the specimen and with respect to the applied gradient direction scheme. The idea behind AxCaliber is that axons of different diameters will experience restricted diffusion at a different diffusion time. By simultaneous analysis of multi-diffusion time diffusion data, the estimation of the ADD, modeled as a gamma function, is feasible (Assaf et al., 2008; Barazany et al., 2009).
3. ActiveAx (Alexander et al., 2010) is a further method used to study axonal properties. Unlike AxCaliber, it models the mean axon diameter rather than the full axon diameter distribution; however it has the advantage of a less demanding acquisition protocol and it also allows estimation of the mean axonal diameter for any orientation of the fiber system. ActiveAx uses the four compartment minimal model of white matter diffusion (MMWMD), which is an extension to the CHARMED model. The four compartments in ActiveAx are the intra-axonal water, adjacent extra-axonal water, CSF water and glial cell water (Alexander et al., 2010). While each of these compartments has different diffusion properties, the intra-axonal water in particular, is influenced by the axonal diameter. A more recent development of this technique (Zhang et al., 2011b) also incorporates fiber orientation dispersion, which provides an additional useful parameter (the fiber dispersion index) and improves the estimate of the axon diameter and density.
4. DTI, CHARMED, AxCaliber and ActiveAx are Single-Pulsed-Field-Gradient (s-PFG) methods. Despite their widespread use for characterizing WM tracts, these methods can have difficulties in areas of complex fiber configurations, for example when diffusion appears macroscopically isotropic, and conveys little useful micro-structure information as occurs for example in the gray matter (GM). The double pulsed field gradient (dPFG) (Callaghan and Komlosh, 2002; Komlosh et al., 2007; Shemesh et al., 2009, 2010, 2011) sequence uses two pairs of diffusion gradients and thus can provide a link between two non-parallel motions that the same spin experiences. This approach has significance for measuring anisotropic motion of spin within neuronal fibers on the microscopic level while the macroscopic organization of the fibers is isotropic — as happens frequently in the gray matter or areas of the complex white matter.

[2] Which becomes a three-compartment model once cerebro-spinal fluid free diffusion is also included.

These methods, as well as high angular resolution diffusion imaging (HARDI) and various relaxometry measures were the methodological bases for the CONNECT project. Fig. 1 demonstrates the micro-structural features that can be extracted from the above mentioned methods.

With the development of axon diameter estimation methods (AxCaliber, Assaf et al., 2008 and ActiveAx, Zhang et al., 2011b), the applicability of these methods in clinical setup was questioned due to the limited strength of the gradients. However, traditional q-space analysis gives some insight into sensitivity to the axon diameter distribution even with clinical gradient strengths. The length-scale of particle displacements to which PGSE measurements are sensitive is roughly $1/q$ (where q is defined as $q = \gamma\delta g$). The maximum q-value in, for example, the human in-vivo ActiveAx protocol (Alexander et al., 2010) is around $2.1 \times 10^5\ m^{-1}$ so that $1/q = 4.8\ \mu m$. Indeed, the numerical simulations and analysis (Alexander, 2008) show that at clinical gradient strengths, we get most sensitivity to diameters around 5 μm. However, the range of sensitivity has a window around that value and extends down to around 2 or 3 μm. Recent work (Dyrby et al., 2010, 2012) provides a detailed numerical analysis to study the window of sensitivity as a function of increasing gradient strength. The results show the significant benefits of increased gradient strength in separating the sizes of smaller axons, but also that useful sensitivity remains even at low gradient strength. Whatever the gradient strength, the axon diameter estimation methods can still detect small axons as smaller than the range of sensitivity, yet simply cannot quantify their size precisely. Axon diameters are in the range of 0.1–10 μm, so the measurements from human protocols can only distinguish diameters towards the higher end of the natural distribution (2–10 μm). However, smaller diameters still contribute to estimates of the mean of the distribution, as in ActiveAx, or the gamma model of the distribution, as in AxCaliber.

A recent additional class of technique, including NODDI (Neurite Orientation Dispersion and Density Imaging) and the ball-and-rackets model, that emerged from the work of the consortium focuses on estimating fiber density and dispersion (Sotiropoulos et al., 2012; Zhang et al., 2012). These features are simpler to estimate than the axon diameter so they require less demanding imaging protocols. The imaging techniques thus provide the first examples of microstructure imaging techniques that are economical enough to be incorporated routinely into clinical studies; as such their uptake has been rapid in a range of applications.

Validating micro-structural measurements

With the inability to obtain the ground truth in the living human brain, alternative validation of diffusion MRI connectivity and micro-structure measures is essential. In the CONNECT project, this issue was explored on several levels.

The most obvious form of validation for structural MRI measures is through comparison with histology. However, one should bear in mind that histology suffers from various limitations that reduce the strength of this comparison: (a) Histology procedures often cause tissue distortions (especially shrinkage), which not only change the dimensions of the tissue (a problem that can be partially resolved by image processing) but also change the micro-structural features (e.g. fiber density); (b) Histology can be performed on several levels

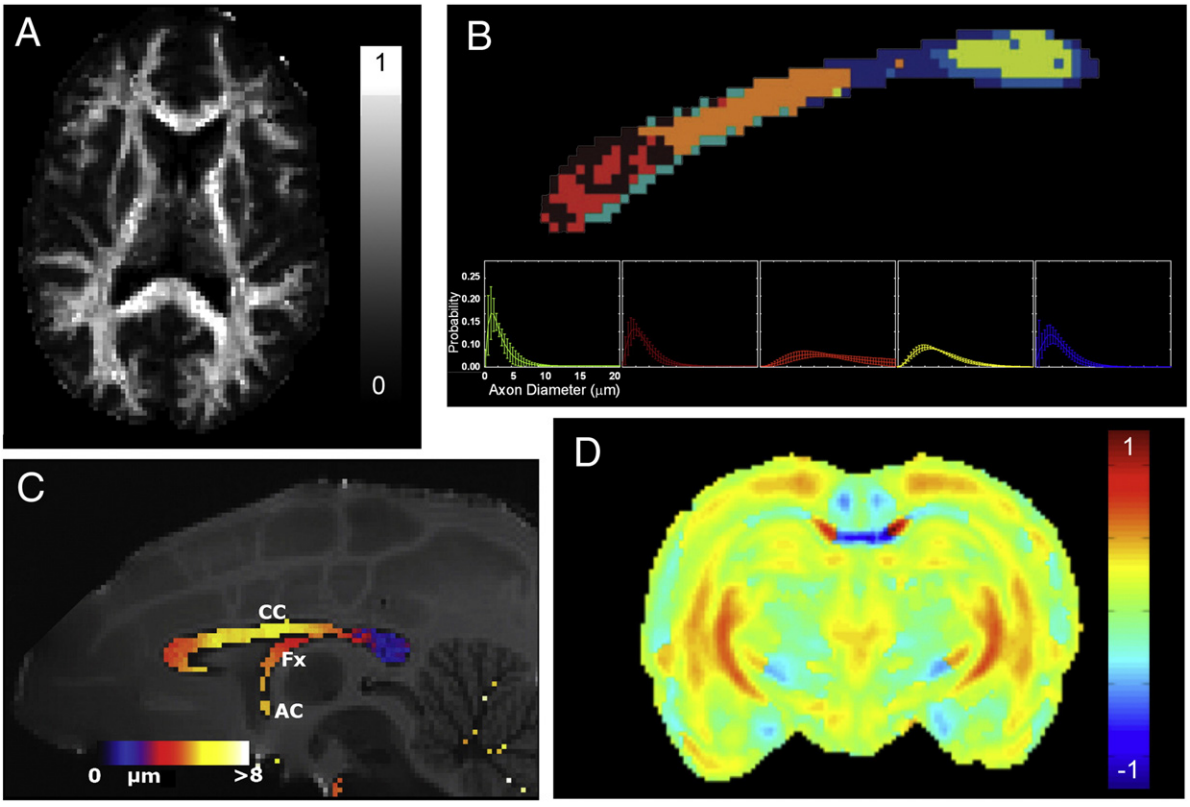

Fig. 1. Micro-structural features extracted from diffusion MRI. (A) Axonal density maps computed from CHARMED for a healthy subject. As expected the axonal density is high in areas of the white matter and reduces gradually in the gray matter; (B) Axon diameter distribution along the corpus callosum of the rat extracted from AxCaliber (Barazany et al., 2009). The ADD is sharp and narrow at the genu and splenium of the CC and broader at the body of the CC; (C) ActiveAx of the fixed Vervet monkey brain. In the mid sagittal slice axon diameters are found to vary across the corpus callosum (CC), fornix (Fx) and anterior commissure (AC). Axons in CC and AC project into the plan (right–left) whereas in Fx axons project within the plan (superior–inferior) (Dyrby et al., 2012); (D) Mean eccentricity map of the dPFG pulse sequence in the rat brain showing high contrast towards cortical fiber orientation (Shemesh et al., 2012).
Panel B was reproduced by permission from Barazany et al. (2009); panel C was modified and reproduced by permission from Dyrby et al. (2012); panel D was reproduced by permission from Shemesh et al. (2012).

revealing different magnitudes of detail of the tissue. However, the information provided by histological analysis is very specific for the chosen marker deployed, thus its relevance to MRI measurements can be limited; (c) Direct measurement of micro-structural features (such as axon diameter by electron microscopy) can only be performed on extremely small portions of the tissue (~tens of microns) which are hardly representative of one MRI voxel (~mm^3). On the other hand histological analysis of tractography by tract tracing does provides invaluable information on the existence or otherwise of a connection, and large amounts of knowledge of anatomical connections in a range of species have been accumulated by numerous groups over many years. However this approach is time-consuming and painstaking, revealing only one pathway per experiment, making overall validation of whole-brain tractography results, for example, unrealistic.

Despite the limitations of histology it appears that it does have a significant added value and can help in increasing the overall confidence in the extracted MRI parameters. Thus, in CONNECT, we have performed histological validation of our diffusion MRI based observations at several levels:

1. Comparison of axon diameter measures with electron microscopy.
2. Comparison of axonal density and orientation measures with immuno-histochemistry of specific cellular markers for myelin and neural fibers (Dyrby et al., 2012).
3. Comparison of tractography results with Mn^{+2} based tract tracing estimating false negative and false positive imaging observations (Dyrby et al., 2007, 2011).

Overall these comparisons have demonstrated the robustness of micro-structural measures (summarized in Fig. 2). Briefly, the consortium was able to demonstrate that:

(a) Diffusion MRI based axonal diameter measurements correlate well with histological measures of ADD although there is a scaling factor in the quantitative values between the two methods (Fig. 2A). While histology typically underestimates the axonal properties due to shrinkage of the tissue, diffusion MRI tends to overestimate the same feature. This overestimation arises in part from limitations in the data, for example not only due to insufficient gradient strength (Dyrby et al., 2012) on clinical MRI scanners limiting sensitivity to smaller diameters (Dyrby et al., 2012), but also due to limitations in the model, which account for fiber dispersion, membrane permeability and other cellular compartments simplistically at best (Sotiropoulos et al., 2012; Zhang et al., 2011b, 2012). Thus, although the correlation between the two methods is significant, current diffusion MRI technology falls short in estimating the small diameter axons (e.g. <1 μm). With the advent of new gradient technologies for clinical scanners, it may be that more accurate estimation of even smaller axons will be feasible in the future (McNab et al., 2012a,b).

(b) Axonal density measures, as extracted from CHARMED, are linearly correlated with myelin basic protein immuno-reactivity. This result, although providing a very important validation, is expected since myelin content is correlated with axonal diameter which, in turn, influences the density of the fibers. However, such correlation demonstrates that although diffusion measures cannot extract true density (by means of fibers/mm^2) the extracted restricted diffusion volume fraction does represent fiber density in a quantitative and meaningful manner.

(c) In recent years, examination and interpretation of diffusion measures in the gray matter have been more deeply investigated (Dyrby et al., 2011; Heidemann et al., 2010; Leuze et al., 2012; McNab et al., 2009, 2013). It appears that with the development of technical advancements in diffusion imaging (parallel imaging, higher gradient strength, better sampling protocols), the noise levels of extracted parameters in the gray matter reduce, revealing interesting features of neural fiber architecture within the cortex. Using DTI, CHARMED and dPFG, as well as the more recent NODDI technique (Zhang et al., 2012) that emerged from work in the CONNECT project, we were able to demonstrate that diffusion imaging is sensitive to differences in neurite density and architecture within the gray matter opening new realms of opportunities for diffusion imaging that had traditionally been optimized to study the white matter architecture opening the way to cytoarchitectonic studies in the brain cortex.

(d) Direct comparison of tractography with manganese based tract tracing was able to demonstrate the weaknesses and strengths of tractography (Dyrby et al., 2007; Knösche et al., 2011). Specifically it should be noted that tractography is prone to false positive observations — i.e. demonstrating fiber pathways that do not exist in reality (Fig. 2B) as well as false negative results (i.e. not depicting real fibers). This issue should be considered

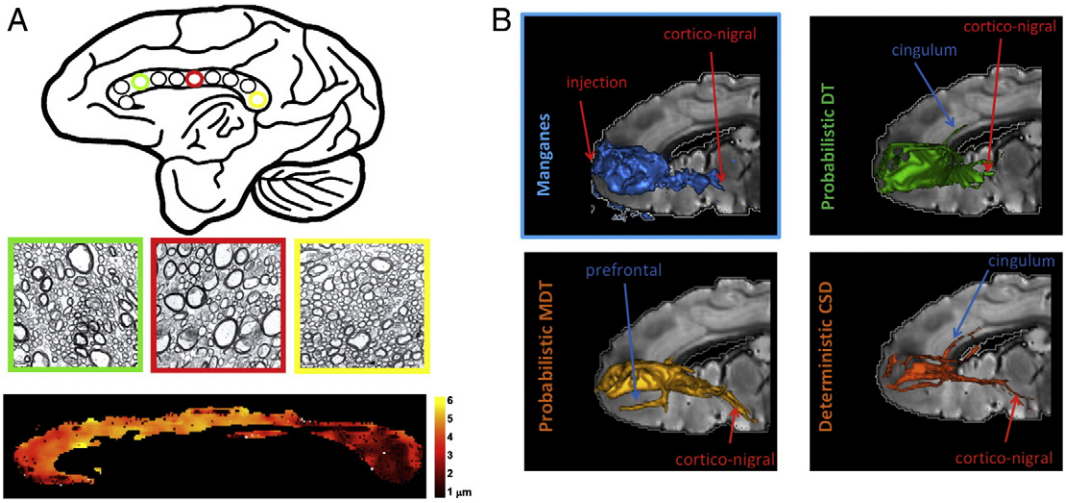

Fig. 2. Validation of diffusion MRI micro-structural measures. (A) For validation electron microscopy (EM) was used for validation of axon diameter methods. The corpus callosum was punctured in ten regions and forty EM images were manually analyzed for diameter, fiber density, etc. EM results support the high–low–high gradient of axon diameters along the CC found with ActiveAx. Three EM example images and ex vivo ActiveAx are shown for a 3 year old Vervet monkey brain. An ActiveAx protocol optimized for Gmax of 300 mT/m was used. (B) Comparison between tractography and tract tracing (by manganese) underscoring the false negative and false positive artifacts of tractography.

when one tries to perform connectivity analysis solely based on tractography. However, within the CONNECT project we demonstrate (see later on in 'combining tractography in micro-structure') that synergizing micro-structure measures and tractography may increase dramatically the confidence of tractography based observations.

Although the histological comparison yielded important validatory evidence for the diffusion MRI measures, in CONNECT, an optimized phantom setup was also developed to explore the methodology and robustness of the different measures. Phantoms that mimic the white matter macro-structure have already been demonstrated in previous studies highlighting the problems of DTI based tractography and enabling refinement of diffusion imaging analysis procedures. In CONNECT, a unique phantom based on biomimetic principles was developed that allows investigation of tissue micro-structure as well. The phantom development was performed under the hypothesis that validation of tissue microstructure measurements and of tractography results requires ground truth information derived from samples with known properties that are close to those observed in human neuronal tissue (Hubbard and Parker, 2009).

Methods were developed for coaxial electrospinning (Co-ES) of materials with microscopic dimensions comparable to axons (Fig. 3) (Zhou et al., 2011). The microscopic features of the axon-mimetic structures are determined by the choice of polymer materials and Co-ES process parameters such as voltage, translation stage velocity, polymer flow rate and needle-surface working distance. The orientation of these structures is controlled via the use of translation stages or mandrels in combination with optimized choices of Co-ES process parameters. Using this setup, several stages were used to generate a range of geometries including crossing, bending and fanning structures. This flexible approach to fiber deposition allowed the production of structures of similar dimensions (of the order of few microns) to those found in the white matter tracts of the brain. The diffusion MRI measurements on the phantom at 3 T, 4.7 T and 7 T MRI scanners demonstrate that ADC and fractional anisotropy measurements are consistent along each phantom, indicating uniform internal structure (Fig. 3), and are within the range of biological tissues. It is anticipated that such a phantom will become the optimal setup for exploring and validating existing and future MRI methods for studying brain microstructure.

Combining micro- and macro-structural measures of white matter pathways

One of the main aims of the CONNECT project was to demonstrate the added value in combining micro- and macro-structural measures of the white matter. The main motivation for this was that although DTI opened an opportunity to investigate brain connectivity via tractography, the use of this method to extract structural connectivity is limited due to the inability of this model to cope with partial volume effects and to provide information on tissue micro-structure.

The combination of micro- and macro-structural measurements introduced several challenges including: the need to correct motion artifacts in high b-value diffusion weighted images, co-registering DTI and other micro-structural measures and optimizing the micro-structural protocols to fits within the experimental time frame typically required for clinical imaging. These issues were addressed and mostly resolved in the CONNECT project (Alexander et al., 2010; Ben-Amitay et al., 2012; De Santis et al., 2013b; Drobnjak et al., 2010; Dyrby et al., 2011, 2012; Siow et al., 2011).

Following successful optimization of the micro-structural measurements, we first wished to demonstrate that acquiring the micro-structural information provides an added value over conventional methods. In a database of ~20 healthy subjects (aged 20–30 years) that were scanned with both DTI and CHARMED protocols we set out to explore the benefits and potential of combining CHARMED information into tractography (detailed description of the experimental protocols and analysis procedures is given in Appendix A). Following transformation of the quantitative measures to MNI space further analysis was performed on both the single-subject and at the group level. The group level MNI normalized data of each subject were combined to produce an average brain DTI data set with the corresponding restricted diffusion volume fraction map. Fig. 4 shows reconstruction of the corona radiata passing through the cerebral peduncles. When the reconstructed fibers are colored according to their FA values, the crossing fiber artifact of this fiber system with the superior longitudinal fasciculus is very apparent (see arrow on the left side of Fig. 4). When looking at the CHARMED data (right side of Fig. 4), this artifact is significantly reduced demonstrating the added value of incorporating micro-structural measures into tractography providing a more reliable and accurate micro-structural characterization of specific fiber systems.

At the following step of combining tractography and micro-structural measures, we aimed to combine ADD measures with tractography to explore the micro-structural properties of neuronal fibers. To demonstrate the usefulness of this approach a phantom consisting of two spinal cord segments was constructed (Barazany et al., 2011): one sectioned out from the fasciculus gracilis and the second from the anterior cortico-spinal tract. These two segments were chosen as they vary significantly in their axon diameter properties. The two sections were placed in a plate perpendicularly one above the other.

To allow the synergism of ADD measures with tractography, the AxCaliber framework was extended to 3 dimensions. The original version of AxCaliber was limited to measuring the ADD for fibers with

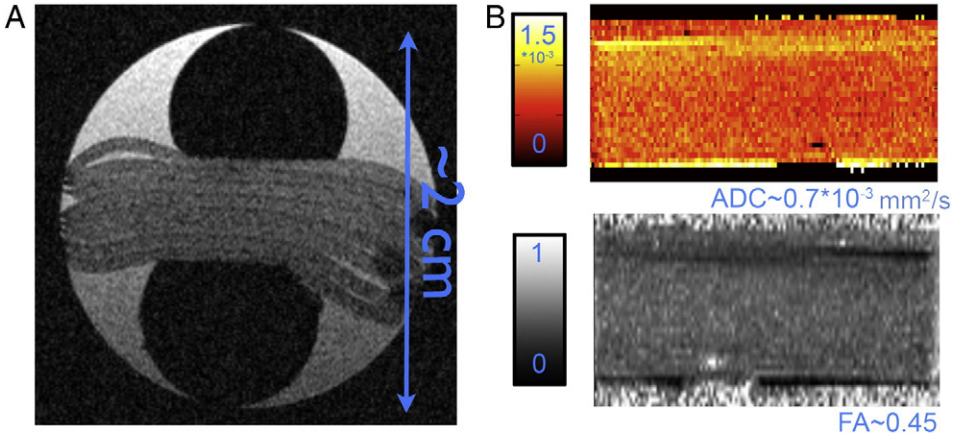

Fig. 3. White matter biomimetic phantom. (A) A tissue mimetic phantom created by coaxial electrospinning material. (B) Diffusion MRI measures of the phantom depicting the FA and ADC maps at 7 T.

a-priori known orientation and was previously applied mainly to the corpus callosum. To extend AxCaliber to 3D a multi diffusion-time CHARMED acquisition was used that provides, for each voxel, the volume fraction of hindered and restricted diffusion, the fiber orientations (two in each voxel), and the hindered and restricted diffusivities as well as the noise floor. From this, a full AxCaliber data set can be re-sampled exactly perpendicular to the fitted fiber systems (independent of its original orientation). As a final step of the development of 3D AxCaliber, the axon diameter distribution of each voxel was then calculated from the re-sampled data (Barazany et al., 2011). Fig. 5 shows an example of the 3D AxCaliber analysis pipeline where the CHARMED analysis recognizes the two fiber systems at the crossing region (Fig. 5A). Fig. 5B shows spherical de-convolution analysis of the DTI data set. This analysis allows characterization of the diffusion properties of each of the fiber segments separately using the 3D framework described above. Following re-sampling of the data to create a full AxCaliber data set it was possible to calculate, for each image voxel, and for each fiber within it, the axon diameter distribution as shown in Fig. 5C. These results obtained from AxCaliber and ActiveAx (Zhang et al., 2011a) techniques indicate that the two intersecting fiber systems can be separated based on their axon diameter properties underscoring the potential in combining micro-structural features into tractography.

Additional developmental work within the project demonstrates the longer-term potential benefits of combining both microstructure estimation and connectivity mapping. Preliminary experiments with the MicroTrack algorithm (Sherbondy et al., 2010) show in a simulation that estimating microstructural features of tracts at the same time as estimating the connecting pathways themselves increases confidence in the microstructural parameter estimates and reduces false positives in tractography. In particular, it offers a way to circumvent some classical confounds of traditional tractography, such as distinguishing kissing and crossing fibers. Preliminary results in post-mortem monkey brains (Sherbondy et al., 2011) give further supporting evidence for this.

Towards the micro-connectome: atlas of brain micro-structure

Once reliable indicators of brain microstructure and optimal approaches to incorporating these to provide robust estimates of brain connectivity were established, our next and final step of the project was to produce initial reference databases of brain connectivity and microstructure. There have been atlases produced in the past – derived from a single subject (19) – and just using DTI data. Although useful, such atlases were built from the data of a single subject. By pooling data from multiple subjects, we were able to place confidence bounds, in each pixel in the image, on the quantitative metrics of microstructure and connectivity. The next paragraphs will summarize the different atlases the CONNECT project aims to create. Full description and demonstration of the atlas are beyond the scope of this report thus only sample examples will be provided. As the CONNECT project embraces a handful of methodological advances, the atlases were produced in several centers, each with a different aim:

(a) Atlas of brain connectivity (NeuroSpin, France)
(b) Atlas of brain micro-structure (axonal density and myelin) (Tel Aviv, Israel and Cardiff, UK)
(c) Atlas of axonal diameter (London and Manchester, UK and NeuroSpin, France).

(a) Atlas of brain connectivity
This atlas is based on the ≪ARCHI≫ database acquired in NeuroSpin. The ARCHI database includes MRI acquisitions performed for 79 young healthy subjects between 18 and 40 years old. The analysis routine used to construct the atlas included the following steps: (a) labeling of the white matter fiber bundles at the individual scale; (b) reconstruction of quantitative maps at the individual subject level; and (c) matching of all the subjects to a common space to create the micro-ATLAS. Fig. 6 depicts the WM bundles obtained for the

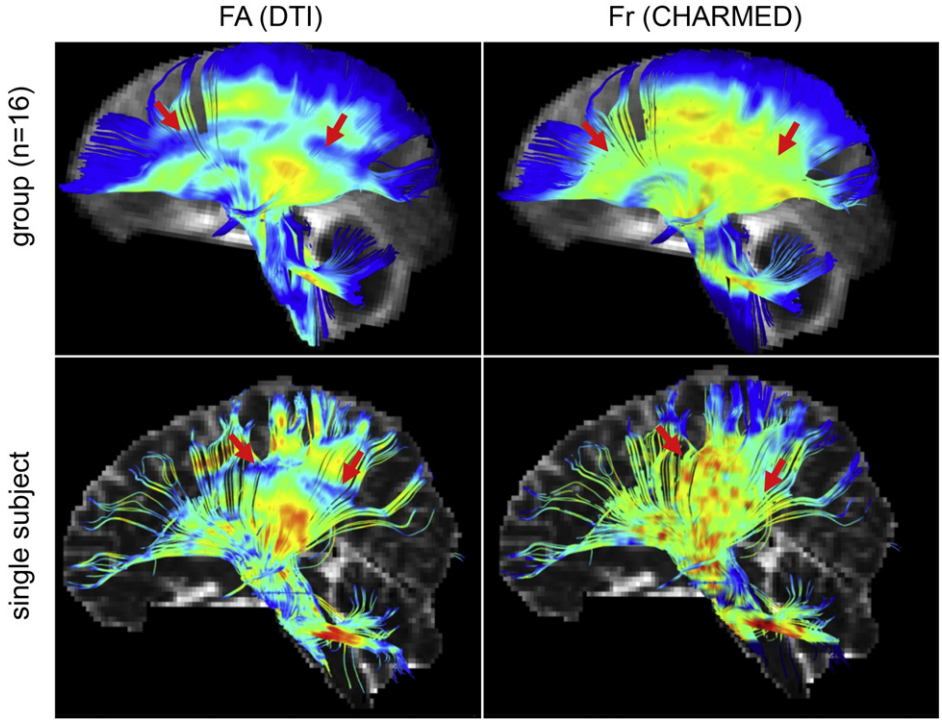

Fig. 4. Combining CHARMED and tractography. Reconstruction of the corona radiata on an averaged DTI data set (n = 16) (top row) and representative single subject (bottom row) colored according to FA (left column) and Fr (restricted volume of CHARMED) (right column). Note that the area of reduced FA (marked by arrows) in the FA maps is less apparent in the Fr maps.

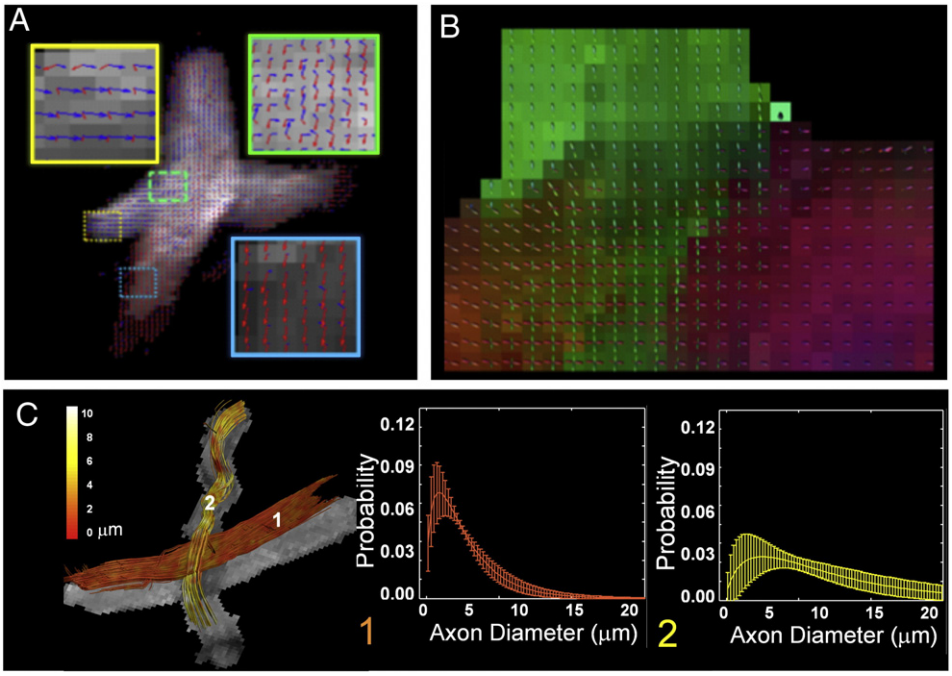

Fig. 5. AxCaliber 3D. (A) CHARMED analysis of crossing fiber phantom depicting the fiber orientations at each pixel resolving the crossing fibers at the cross-section. (B) Spherical harmonics de-convolution analysis of the DTI data set. (C) ADD extracted from the re-sampled CHARMED data set projected into the fiber-tracts of the two specimens.

79 subjects of the ARCHI database including 38 long WM bundles and 94 short WM bundles that could be identified and matched in all the subjects. A specific color is attributed to each fiber bundle (Schmitt et al., 2013). This atlas was implemented in the CONNECTOMIST software following the method described in Guevara et al. (2011, 2012).

(b) Atlas of brain micro-structure (axonal density and myelin)
This part of the atlas incorporates micro-structural information (axonal density, axonal diffusivity, myelin water fraction) provided in standardized space as well as for specific fiber systems (De Santis et al., 2013a). In general, tractography was executed using the spherical deconvolution approach for 4 major pathways: the arcuate fasciculi, the cingulum, the uncinate fasciculi, and the superior longitudinal fasciculi.
For each subject, all the parametric maps (FA, MD and axonal density) were non-linearly registered to the anatomical scan. The latter was used to normalize the brain in MNI space using non-linear warping. The same transformation was then applied to the parametric maps and to the binarized tracts. Mean and standard deviation were calculated for the parametric maps. Visitation count was calculated for the tracts and thresholded at 70%. The whole brain tractography of one subject was warped to MNI space and the thresholded visitation count was used to define the ROI to reconstruct the fiber pathways.
Fig. 7 shows an example for atlas reconstruction for one specific fiber system — the arcuate (superior longitudinal) fasciculus. For this tract (as well as for the others studied although not shown in this paper), mean values, standard deviations and correlations between the parametric maps (FA, MD and Fr) are shown. Moreover, the distribution of the mean and the standard deviation of the parameters are projected along the tract.

(c) Atlas of mean axon diameter
10 volunteers from the ARCHI database were also scanned with the ActiveAx-optimized protocol (Based on protocol in Appendix A) in order to provide mean axon properties for the entire brain and specific fiber systems. Fig. 8 shows the resultant estimates of axon diameter for an entire hemisphere of one of the volunteers. This is the first demonstration of the orientation-independent mapping of microstructure in the majority of the white matter in vivo.

Additional and future aspects of the CONNECT project

The functionality of brain tissue is strongly related to its morphological and microstructural features. Quantification of many of these features is currently only achieved through invasive histological techniques. The consortium developed diffusion MRI into a true microstructural probe that enables virtual biopsy and virtual dissection of the human brain in-vivo reliably and non-invasively. As such the main impact of the consortium is the development of diffusion-based MRI to such an extent that it becomes a robust and reliable tool with which to investigate the white matter and its connections both in routine clinical and neuroscience research settings. It is expected that the applicability of the methods presented in this overview will increase following the introduction of fast diffusion imaging acquisition techniques (Feinberg et al., 2010). This unprecedented insight into the white matter opens up new realms of possibilities in terms of both diagnostic and therapeutic strategies, as well as providing fundamental new insights to the connectivity and workings of the brain. By being able to probe the white matter to this new level of detail, and combined with detailed assessment of brain function, an unparalleled holistic view of the brain is within reach.

Conflict of interest

A.A.G. receives royalties for books and essays focused on patient safety and medicine. C.L. receives royalties for a book focused on healthcare management. The other authors have no conflicts of interest.

Appendix A. Experimental protocol

Optimized micro-structural protocols for the human brain were implemented in the clinical scanners at Tel Aviv Medical Center, Cardiff University, Manchester University, Geneva University and

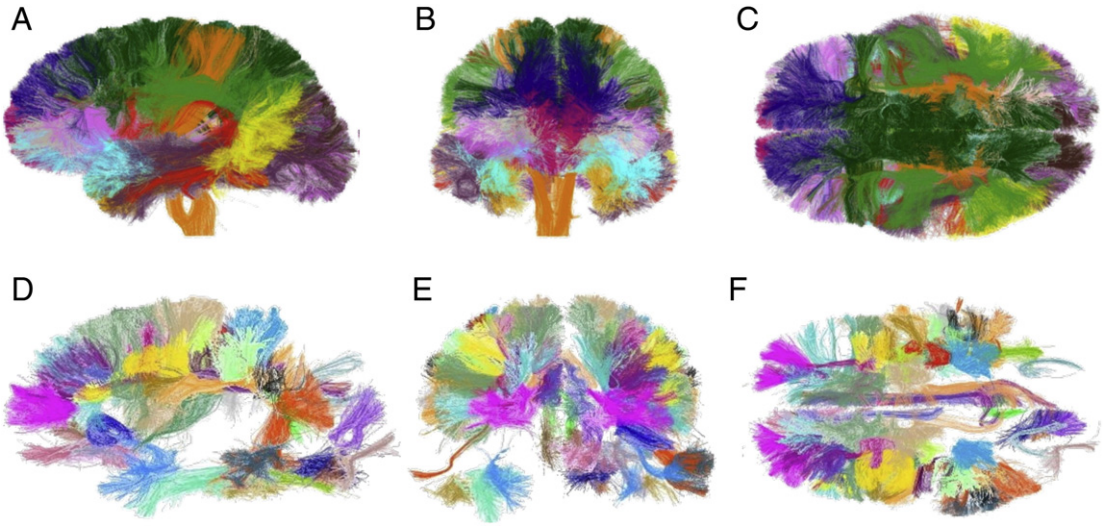

Fig. 6. Connectivity atlas. Rendering of the long WM fiber bundles (A,B,C) and of the short WM fiber bundles (D,E,F) for subject 1 of the ARCHI database. Fibers were constructed using the following steps: (1) quality check and q-space sampling to detect and correct various artifacts; (2) modeling of the orientation distribution function (ODF) fields; (3) producing tractography mask; (4) tractography over the entire brain using a regularized streamline probabilistic approach; (5) bundle maps filtered according to curvature, tortuosity and density; (6) creation of the connectivity matrix; (7) intra- and inter-subject fiber clustering; (8) automatic labeling of the bundles.

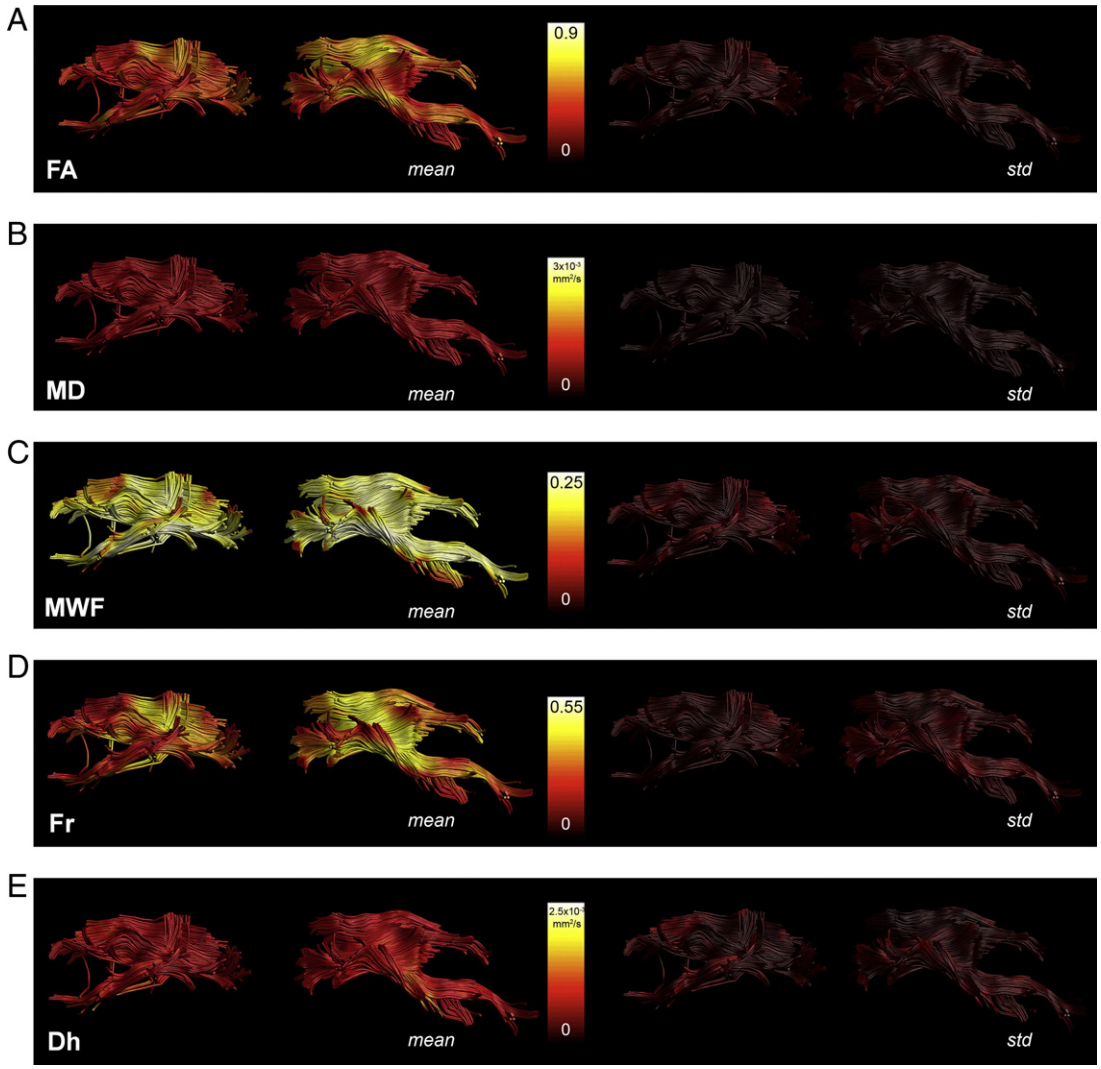

Fig. 7. Micro-structural atlas: relaxometry and diffusivity. In this part of the atlas, different micro-structural parameters (axonal density, myelin water fraction, etc.) were projected onto specific fiber systems. In this figure the superior longitudinal fasciculus fiber system is depicted and colored according to the: (A) fractional anisotropy; (B) mean diffusivity; (C) myelin water fraction; (D) axonal density (Fr); and (E) extra-axonal diffusivity (Dh).

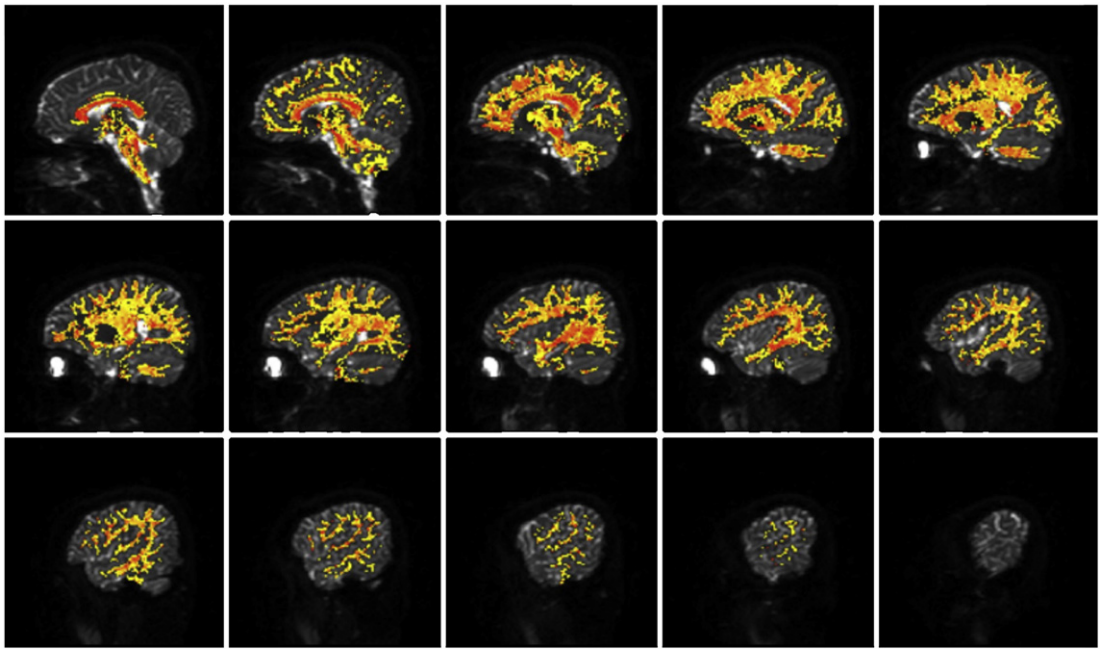

Fig. 8. Micro-structural atlas: mean axonal diameter. In this part of the atlas we created maps of mean axon diameter (extracted from ActiveAx analysis) for 10 subjects. The figure shows the orientation-independent white matter axon diameter measurement of the right hemisphere in a single individual (range 0–8 μm).

Neurological Institute of Milano for: 1. CHARMED; 2. AxCaliber; and 3. ActiveAx. The general experimental conditions are summarized in the following table:

In addition to micro-structural protocol, a HARDI data set was also acquired consisting of 60 gradient directions measured at b = 1000 s/mm^2 and whole brain coverage at a cubic resolution of 2.1 mm^3.

The analysis procedure included the following frameworks:

Connectivity analysis:

1. Open Walnut: www.openwalnut.org
2. BrainVisa: http://brainvisa.info/
3. ExploreDTI: http://www.exploredti.com/.

Micro-structure analysis:

1. CHARMED/AxCaliber: http://neuroimaging.tau.ac.il/yawiki/index.php/CHARMED_code
2. ActiveAx: http://cmic.cs.ucl.ac.uk/camino/.

Data sets for Connectivity and Micro-structure analysis: http://www.brain-connect.eu.

ActiveAx and Tractography: http://dig.drcmr.dk/downloads/.

	CHARMED	AxCaliber	ActiveAx
Matrix size	80 × 80	128 × 128	128 × 128
Resolution	2.4 × 2.4 × 3.0	1.5 × 1.5 × 3	1.8 × 1.8 × 3.9
Gradient strength (G/cm)	~4	~3.5–4	~4
Δ/δ (ms)	~38/32	(88, 68, 48, 28)/21	(70, 15, 70)/15
TR/TE (ms)	17,000/~90	6000/137	6000/122
Maximal b value (s/mm^2)	4000	4000	500, 700, 2770, 2880
Brain coverage	Full	Single slice	Full
Gradient scheme	3 at b = 0 6 at b = 1000 15 at b = 2500 16 at b = 4000	Single direction Perpendicular to the CC. 28 b value incremented linearly from 0 to 4000	90 directions per each b value
Acquisition time	14 min	12 min	36 min

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