Unraveling Grey Matter with Diffusion MRI: Challenges and Possibilities

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Plain language summary — The cerebral cortex, the outer layer of the brain, contains the vast majority of neurons. It plays an important role in several disorders, like dementia or autism spectrum disorder and is comprised of so-called grey matter (GM). GM is characterized by a high percentage of cells, which makes it different from white matter (WM), which comprises most of the rest of the brain and mainly contains connections between the cells in the cortex.

These structures can be studied with diffusion MRI. This method can both unravel the microstructure of and the connections in the brain. Diffusion MRI measures the movement of water molecules over very small distances throughout the brain. This movement can be hindered or constrained by cellular structures in the brain, which can be reconstructed by interpreting the diffusion MRI signal. Most diffusion MRI methods have been developed for studying WM, and not GM despite being an important brain structure. Because of the differences between the two structures, methods developed for studying WM cannot be used for studying GM without adapting them. This literature review presents an overview of diffusion MRI methods which have been used to study GM in the cerebral cortex. The presented methods belong to two types of diffusion MRI methods: i) biophysical models and ii) tractography.

Biophysical models try to translate the MRI signal to measures which would normally only be obtainable with microscopy, like the size of cell bodies. Microscopy is only possible *ex vivo* (in studies of dead brains), while diffusion MRI is possible *in vivo* (in studies of living brains) which enables researchers to get microscopic information from living patients. Biophysical models divide the brain microstructure into different compartments (cell bodies, neurites & extracellular space) and conceptualize these with certain parameters describing the (interactions between the) compartments. Computer techniques are used to obtain the parameter values from the MRI signal. The presented methods in this review are promising, but are not ready for use in the clinic yet. This is because the models have not been used with data from scanners used in the clinic. This data has several limitations compared to data from scanners used in research. Moreover, biophysical models are still mainly useful for healthy brains, but not yet for studying disorders with changes in the GM.

Tractography, on the other hand, is a method to follow brain fibers which connect two different regions of the cortex. To do this, directional information about the water displacement is used. The assumption is that the main displacement observed with diffusion MRI comes from displacements within a fiber. These directions are then pieced together throughout the brain, so fibers can be followed. Fibers often begin in the GM, after which they travel through the WM to a different GM region. Currently, it is hardly possible to obtain the exact beginning and end points of these fibers in the cortex. Tractography methods for GM try to obtain this information, and to follow fibers in the GM itself. This is still challenging, however, for example because it is challenging to reconstruct where fibers enter the GM. More issues exist for GM tractography and are discussed in this review, alongside the methods being developed to overcome them.

Abstract — The cerebral cortex is a grey matter (GM) structure in the brain that contains the vast majority of neurons. As such, it plays an important role in the functioning of the brain. GM changes are also associated with several neurodegenerative and neurodevelopmental disorders. Despite the importance of GM, there is currently a lack of methods able to provide non-invasive insights into its structure. Two emerging approaches might bridge this gap of knowledge by providing a complementary structural characterization of the cortex: biophysical modelling might allow to capture the microstructure of the cortex, whereas fiber tractography could reconstruct the spatial organization of fibers in cortical circuits. This literature review investigates the possibilities and challenges of using biophysical modeling and tractography to study the GM.

Biophysical models try to resemble a non-invasive *in vivo* microscope. They describe the GM as a sum of microscopic compartments, and try to fit model parameters to the diffusion signal to obtain microstructural information. Preclinical results have been promising, but the development of clinically feasible acquisition protocols is challenging. Furthermore, current models are specific to healthy tissue, which makes the extension to pathologies challenging.

The application of tractography to GM suffers from multiple unresolved challenges, e.g., limited spatial resolution, the gyral bias and the reconstruction of reliable fiber orientations. Multiple promising methods have been developed to overcome these challenges and are discussed in this review, but it will remain a challenge to combine these different methods to simultaneously overcome the different challenges.

Due to a lack of a ground truth except histology, validation is often complicated for these two methods.

Index Terms — biophysical models, cerebral cortex, diffusion MRI, grey matter, tractography

I. Introduction

Diffusion magnetic resonance imaging (diffusion MRI) is a non-invasive method which can be used to obtain *in vivo* information about the brain microstructure. The main feature of diffusion MRI is its ability to measure the displacement of water molecules at a microscopic scale, because the signal is sensitized to this motion (Jones, 2010). Cellular structures can hinder water molecules from moving in specific directions, which affects the diffusion MRI signal, and can be studied by fitting appropriate models. Applications of diffusion MRI are diverse and cover a wide range of areas (Tournier, 2019). Traditionally, diffusion tensor imaging has been the most known method and has been applied to study a wide range of brain conditions (Pierpaoli et al., 1996; Mori & Zhang, 2006; Jones & Leemans, 2011; Lerner et al., 2014; Atkinson-Clement et al., 2017).

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Diffusion MRI is often used for brain imaging. An important brain structure is the human cerebral cortex, which is made up of grey matter (GM). Dynamic changes in the cortex have been associated with brain development, learning, aging and major neurodevelopmental and neurodegenerative disorders (Olesen et al., 2022). For example, Alzheimer's disease is in the early stages primarily a cortical disease where microstructural changes and degeneration take place (Weston et al., 2015). Moreover, relatively consistent abnormalities in GM microstructure in several cortical regions have been reported for autism spectrum disorder (Nazeri et al., 2020). Neurology might therefore benefit from unraveling the cortical GM.

Nevertheless, most diffusion MRI work has been focused on white matter (WM) and not on (cortical) GM. Whereas the WM structure is found to be relatively homogeneous, the GM structure is more heterogeneous (Lee et al., 2020). This makes that techniques used for WM imaging often cannot be used for GM imaging without adapting them. Two promising diffusion MRI methods to cope with the GM heterogeneity are tractography and microstructural imaging with biophysical models. Tractography methods based on diffusion MRI are used to reconstruct fiber pathways throughout the brain, which are useful to study brain function or to plan neurosurgeries. On the other hand, microstructural imaging using diffusion MRI focuses on measures of key microstructural features. It attempts to obtain *in vivo* information that has long been considered purely the domain of histological studies (Tournier, 2019).

The aim of this review is to present an overview of GM applications of i) biophysical models for microstructural imaging and ii) tractography. The focus mainly lies on the current possibilities and existing challenges.

II. Methods

A. Literature search

Since the use of biophysical models and tractography are two separate subjects within the field of diffusion MRI, a different search was conducted for the two of them. PubMed was used for both literature searches.

For the search of biophysical models, the following PubMed search was used:

((biophysical model [Title/Abstract]) OR (model [Title/Abstract])) AND (diffusion MRI [Title/Abstract]) AND ((grey matter [Title/Abstract]) OR (gray matter [Title/Abstract]) OR (cortex [Title/Abstract]) OR (cortical [Title/Abstract]) OR (laminar [Title/Abstract]))

This gave a total of 249 hits. For the search of tractography methods within or near the GM, the following PubMed search was used:

((tractography [Title/Abstract]) OR (connectivity [Title/Abstract]) OR (circuitry [Title/Abstract])) AND ((grey matter [Title/Abstract]) OR (gray matter [Title/Abstract]) OR (cortical [Title/Abstract]) OR (cortex [Title/Abstract])) AND ((diffusion MRI [Title/Abstract]) OR (diffusion-weighted [Title/Abstract]))

This resulted in a total of 1,188 hits. The number of hits for both searches was reduced by limiting the publication year to 2020 or later. The underlying assumption for this was that the most promising methods which have been proposed before 2020 would be cited in the publications of 2020 and later. This led to 109 hits for the biophysical models and 455 hits for tractography methods.

Moreover, several selection criteria were used for title screening and afterwards abstract screening. First, the method should have a clear focus on GM or the WM-GM boundary. Especially many tractography methods were focused on deep WM. Second, the publication should have a global focus on the cerebral cortex, and not a regional focus on one specific cortical region. Third, the publication should be focused on imaging healthy subjects, since different pathologies might affect the requirements of the methods. Fourth, the primary focus was on human brain imaging, but especially for the biophysical models it was not possible to only use methods applied to human brain imaging, since the state of the field is not this far yet. Apart from these literature searches, secondary searches were done by screening the publications in which certain studies cited in this work, were cited.

B. Review structure

After providing an introduction to the cortical structure, we will first present a review of biophysical models developed for GM imaging. Subsequently, we will present a review of tractography methods that have been used in and near the cerebral cortex. For each of the two categories, we also review the challenges that are still open.

III. Introduction to the Cortical Structure

The cortex is histologically divided into 6 laminar layers, from outer to inner layer: i) molecular, ii) external granular, iii) external pyramidal, iv) internal granular, v) internal pyramidal and vi) multiform. This division in 6 layers is done based on the characterization of the cell bodies or somas in these layers (i.e., its cytoarchitecture, see *Figure 1*). Each of these layers has its own characteristics and has certain connections to other layers or other parts of the brain like the thalamus. Being composed of neurons, the cortex also has a great amount of neurites with a certain direction. The direction is most often either radial (perpendicular to the cortical surface) or tangential (parallel to the cortical surface). Not all GM regions exhibit exactly the same structure, there are at least five different types of cortical tissue structures, as shown in *Figure 2*. These tend to differ in for example the density of neurites (Nazeri et al., 2020). Some of the cortical regions exhibit more myelination (the existence of a myelin shaft around the neurite, as is seen in WM) than other cortical regions, which may complicate segmentation in structural images (Kim et al., 2015). The cortical thickness varies between brain regions between 1 and 4.5 mm, with an

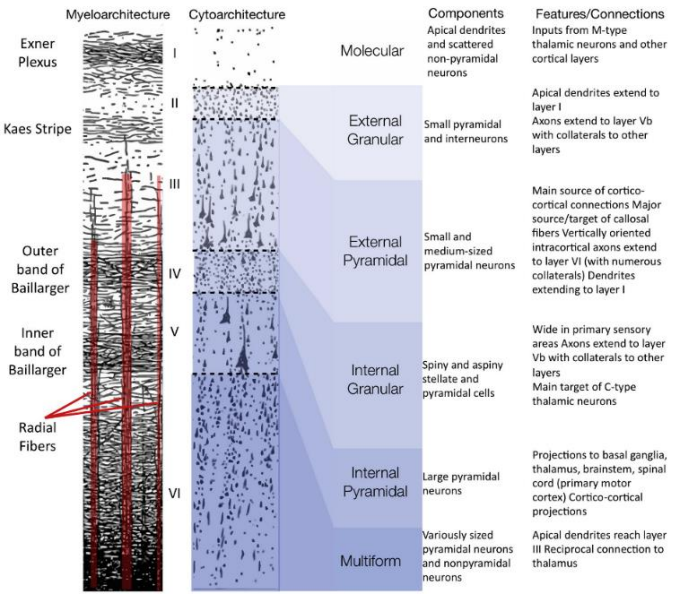

Figure 1: The microstructure of the cerebral cortex. Based on its cytoarchitecture, the cortex can be divided into six layers. Two types of fibers can be distinguished with different orientations: radial and tangential. This image is adapted from (Nazeri et al., 2020).

average thickness of 2.5 mm (Fischl & Dale, 2000). Another distinctive feature of the cortex is its folding, resulting in gyri (convexities) and sulci (concavities) in the brain. Due to this gyrification of the cortex, the cortex has been able to expand compared to when no gyrification would have occurred (Kleinnijenhuis et al., 2015). For a zoomed-in schematic overview of the GM microstructure, see *Figure 4* in the next section.

In the next sections, biophysical models and tractography for GM purposes will be discussed. The goal of biophysical models is to estimate tissue properties which would otherwise only be accessible by histological studies, i.e., "to bring MRI to the level of a non-invasive in vivo microscope" (Jelescu et al., 2020).

Tractography methods, on the other hand, are used to track fibers throughout the brain, in order to obtain information about brain connectivity. Most fibers throughout the brain end and/or begin in the cortical GM, as can be seen for the radial fibers in *Figure 1* which enter the WM at the boundary with WM. These fibers can end/begin in different layers of the cortex (Barbas & Rempel-Clower, 1997). Tractography is possible because diffusion MRI can obtain the direction in which the water molecules move. This direction is then assumed to be correlated with neurite orientations and by piecing these orientations together, long-range fiber pathways can be obtained (Jeurissen et al., 2019).

IV. Biophysical models: the Standard Model and beyond

A. Key biophysical models of WM

The past years multiple authors have tried to develop biophysical models which can be used for structural imaging of

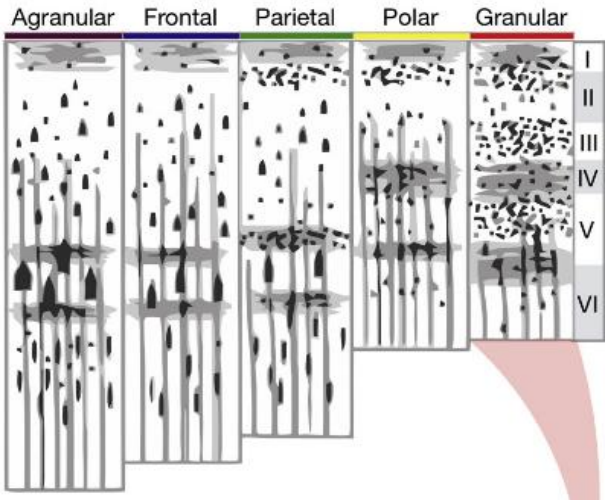

Figure 2: five different types of cortical microstructure. Different cortical regions have different structures. They might differ in thickness, neurite density, cyto-architecture and more. This figure is adapted from (Nazeri et al., 2020)

the brain using diffusion MRI. In order to obtain microstructural information via biophysical modeling, it is first needed to conceptualize what tissue features most strongly contribute to and affect the signal that is obtained during a (clinical) scan. Second, it is needed to optimize the model by estimating its parameters. Biophysical models are distinct from so-called signal representations like diffusion tensor imaging (DTI) or diffusion kurtosis imaging (DKI), methods that are often used in diffusion MRI. Whereas models have an underlying theory to make sense of the measured data, signal representations lack this theoretical foundation and can therefore be seen as a mathematical expression or formula. Signal representations might be sensitive to specific pathologies or underlying processes, but cannot be validated against histology like biophysical models can (Novikov et al., 2018). For WM imaging, various similar models have been presented, which are summarized in the Standard Model (SM) (Novikov et al., 2019).

The SM conceptualizes brain tissue as a collection of two or three compartments in which anisotropic, Gaussian diffusion can be observed. The first compartment is modeled as sticks, which represent neurites in the brain oriented in a specific direction. The diffusion direction is captured by the fiber orientation distribution (FOD) within a voxel. The second compartment is the extra-neurite space, which represents the space surrounding the bundles of neurites in the brain. Extra-cellular diffusion is modelled as anisotropic and Gaussian with a so-called Zeppelin, which is a cylindrically symmetric tensor. Sometimes, a third compartment is added to the model, representing the cerebrospinal fluid, in which free diffusion can be observed. Exchange of water between these compartments is neglected (Novikov et al., 2019; Jelescu et al., 2022).

Compared to signal representations, biophysical models such as the SM offer more specific and more meaningful explanations of signal changes. For example, multiple microstructural changes could explain an increase in diffusion

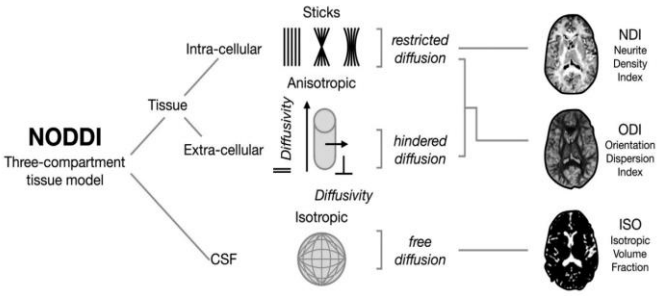

Figure 3: the NODDI model. This model is an example of what is summarized in the SM. The microstructure is divided in three compartments with each a different kind of diffusion. Neurites are modeled as sticks and the extra-neurite space as a zeppelin-like compartment. NDI-, ODI- and ISO-measures can be obtained with NODDI. This image is adapted from (Nazeri et al., 2020)

perpendicular to axons in WM (radial diffusivity). DTI could be used to measure this increase, but it cannot differentiate between demyelination, axonal loss or edema as a cause for this increase. Biophysical models, however, can be used to find out the cause of this increase in radial diffusivity (Jelescu et al., 2020). This provides additional physiological insights for the end user.

One of the models resembling the SM is Neurite Orientation and Dispersion Distribution Imaging (NODDI), first introduced by Zhang et al. (2012). This is a three compartment model and can be used to obtain three parameters: neurite density index (NDI), orientation dispersion index (ODI) and free-water isotropic volume fraction (ISO). The NDI describes the density of neurites within a voxel and the ODI describes the configuration of neurite orientations within a voxel. The ISO is considered to be a measure of CSF (Nazeri et al., 2020). NODDI has been a popular model in clinical research because of its shorter acquisition requirements, which are achieved by fixing the number of sticks to 1.

Albeit being developed for the brain WM, NODDI has also been applied to investigate GM in several neurological and neuropsychiatric disorders, although not without limitations. These limitations include constraints on model parameters, neglect of inter-compartmental exchange for unmyelinated neurites, neglect of cell bodies (somas) and low image resolution on clinical scanners resulting in partial volume effects (Nazeri et al., 2020).

B. Biophysical models of GM

Whereas the SM is thought to be a good minimal model for conceptualizing the WM, it has found to be insufficient for the conceptualization of GM, especially at higher b-values (Palombo et al., 2020). This is due to the microstructural differences between WM and GM and has led several authors to extend the SM to better be able to conceptualize the GM as well. Jelescu et al. (2022) have stated that the SM might have to be supplemented with at least three signal contributions in order to represent GM. First, an important difference between GM and WM is the exchange rate of water between different parts of the microstructure. Since neurites in WM are mostly myelinated, water exchange between the neurite and the extra-neurite space is very slow. GM neurites can also be unmyelinated, which makes water exchange possible at a higher rate. Exchange might thus have to be accounted for in GM models. Second, structural disorder within compartments in the GM might lead to non-Gaussian diffusion within compartments. Thirdly, whereas cell bodies or soma constitute 5-10% of the WM and are therefore considered neglectable, soma constitute 10-20% of the GM. Therefore, the signal contribution of soma might not be neglectable in GM imaging.

Multiple contributions have been made on these three issues in biophysical modeling of GM. Jelescu et al. (2022) have presented Neurite Exchange Imaging (NEXI) which incorporates the exchange across neurite cell membranes and they compared this to the work of Palombo et al. (2020) who presented Soma And Neurite Density Imaging (SANDI). SANDI is an extension of the SM in which the contribution of soma is accounted for. Combining the two models results in SANDI with exchange (SANDIX) as proposed by Olesen et al. (2022), who also proposed a SANDIX model with a subpopulation of impermeable neurites – which would

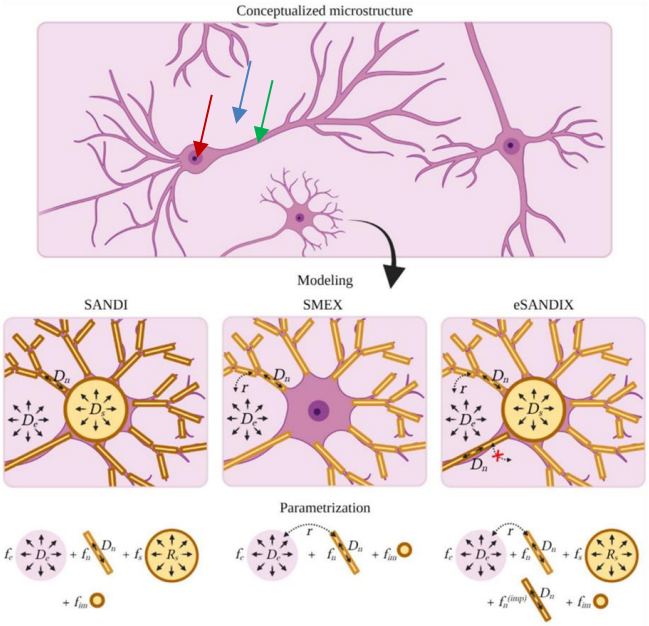

Figure 4: schematic presentation of the GM microstructure and how this is modeled by the different models. CSF is neglected in these models and this microstructure. The red arrow points to a soma, the green arrow to a neurite and the blue arrow to extra-neurite space. Exchange of water between these compartments is possible. The rate at which this happens depends among others on the membranes of the compartments.

All models represent the neurites as a collection of consecutive sticks. SANDI and eSANDIX model the soma as an individual compartment, whereas NEXI (SMEX in this figure) adds the soma to the extra-neurite space. NEXI and eSANDIX account for water exchange between the neurite and extra-neurite space. eSANDIX also models myelinated axons where no exchange is possible, as depicted by the red cross through the exchange arrow in the eSANDIX model representation.

This image is adapted from (Olesen et al., 2022).

Table 1: Overview of the presented models used for GM imaging.

Model	Compartments	Measures
NODDI (Zhang et al., 2012)	- Neurites - Extra-neurite space (ENS) - CSF - *no neurite-ENS compartment exchange*	- Neurite density index (NDI) - Orientation dispersion index (ODI) - Free-water isotropic volume fraction (ISO)
NEXI (Jelescu et al., 2022)	- Neurites - Extra-neurite space (ENS) - *with neurite-ENS compartment exchange*	- Compartment fractions - Compartment diffusivities - Exchange rate
SANDI (Palombo et al., 2020)	- Neurites - Extra-neurite space (ENS) - Somas - *no neurite-ENS compartment exchange*	- Compartment fractions - Compartment diffusivities - Soma radius
SANDIX (Olesen et al., 2022)	- Neurites - Extra-neurite space (ENS) - Somas - *with neurite-ENS compartment exchange*	- Compartment fractions - Compartment diffusivities - Soma radius - Exchange rate
eSANDIX (Olesen et al., 2022)	- Myelinated neurites - Unmyelinated neurites - Extra-neurite space (ENS) - Somas - *with neurite-ENS exchange*	- Compartment fractions - Compartment diffusivities - Soma radius - Exchange rate

represent myelinated axons in the brain – called eSANDIX. These models are represented in *Figure 4*. An overview of the compartments and measures used in each model can be found in *Table 1*.

Olesen et al. (2022) compared these models on both Monte Carlo diffusion simulations of GM neurons and animal data to study the characteristics of GM tissue. Mainly, Olesen et al. were interested in finding an explanation why neurites in the GM do not exhibit the same signal behavior as neurites in the WM. It has been shown that the diffusion-weighted signal $S(b)$ in WM is approximately proportional to $b^{-1/2}$, where b is the diffusion-weighting gradient strength (b-value). Veraart et al. (2019) have shown that this relation is apparent for strong diffusion-weighting, and they use this finding to suggest that neurites in WM can indeed be modeled as sticks in the SM. This power-law relationship between the signal and the b-value is not observed in GM, which is another indication that the SM does not suffice for GM.

By comparing NEXI, SANDI, SANDIX and eSANDIX, Olesen et al. (2022) could study the contributions of soma and exchange to the diffusion-weighted signal. It was also studied whether the curvature of neurites in GM could be responsible for the difference in signal. It was found that the stick power-law is in GM affected by both the contribution somas have on the signal and the exchange which can happen with GM neurites because most are not myelinated. In regions where GM neurites are myelinated, the stick power-law becomes apparent again. Due to the effects both exchange and somas have on the diffusion-weighted signal, Olesen et al. conclude that a potential GM model should include both contributions, like SANDIX.

It should be noted that SANDIX only models exchange between neurites and extra-neurite space, and somas are modeled to be impermeable. In NEXI, however, somas are not considered as an additional compartment. They are modeled to be one with the extra-neurite space, making it that they are modeled to be fully permeable. It is therefore expected that NEXI and SANDIX respectively over- and underestimate exchange between somas and the extra-neurite space (Olesen et al., 2022). Others have focused on exchange between cells and the extra-cellular or extra-neurite space. The model called Cellular Exchange Imaging (CEXI) consisted of a spherical cell and an extra-cellular space, which was used to investigate the impact of permeability. This model is not made to represent GM microstructure, but the simulations with this model might still provide interesting insights for GM modeling. It was shown that diffusion is dominated by exchange mechanisms with longer diffusion times in permeable tissues. Time-dependencies should therefore be taken into account with permeable tissues like somas. Moreover, for highly permeable tissues, non-Gaussian diffusion might have to be accounted for (Gardier et al., 2023). Accounting for exchange between somas and extra-neurite space might thus be a next extension of the GM models. This could for example be useful for studying tumors in the brain (Reynaud, 2017).

C. Current challenges for microstructural diffusion MRI with biophysical models

Models like SANDIX can thus approximate GM anatomy, but they also need to be compatible with available acquisition protocols so they can be used during image processing. As explained by Jelescu et al. (2020), being able to use specific models requires the use of dedicated acquisition protocols. This is why new models cannot always be tested on existing dMRI datasets. So far, Palombo et al. (2020) have proven that SANDI

is applicable to data from the human connectome project (HCP) (Van Essen et al., 2013), but the used scanner in the HCP is not representative for clinical scanners. The other promising GM models mentioned above (eSANDIX and NEXI) have only been used in *ex vivo* animal studies (Olesen et al., 2022; Jelescu et al., 2022), which requires different protocols than *in vivo* human brain dMRI on (pre)clinical scanners. Therefore, research not only has to be done on the validity of GM models for the human brain, but also on compatible acquisition protocols which would in the end be feasible on clinical scanners.

Another challenge to the development of biophysical models is validation. For validation purposes in microstructural dMRI, histological data is often seen as the gold standard to compare the results of biophysical modelling to. This has for example been used to check the performance of biophysical models in measuring the neurite density, orientation and dispersion, axon diameters, cell shapes and heterogeneity, and myelination of neurites. However, other validation methods have been used as well in previous studies, and some features like intra-compartment diffusivities or inter-compartment exchange times cannot be studied with histology (Jelescu et al., 2020). There are for example possibilities to use numerical simulations, like Olesen et al. (2022) did, but most of these tend to be oversimplifications of real microstructures in the human brain, especially for GM (Jelescu et al., 2020). A full overview of validation practices is outside the scope of this paper and can be found in (Jelescu et al., 2020). Between models, inconsistencies have been reported in exchange times (Chakwizira et al., 2023). Moreover, all of the above models are relatively insensitive to variations in compartment diffusivities, and soma sizes tend to be overestimated (Gardier et al., 2023). This shows the need for proper validations of and comparisons between models.

Another challenge lies in the data fitting that is needed to translate the signal into the biophysical measures. The method used most often for this is the non-linear fitting, but this is not without its challenges. First, it is possible that the signal is equally well explained by multiple sets of model parameters, or in other words that model degeneracies exist. This can be overcome by using model constraints, but these are often arbitrary and might therefore obscure the results. Other questions are how to deal with noise in the obtained signal during the data fitting and how to determine the initial values of the model parameters in the optimization algorithm. Especially for complex models parameter initialization is an important aspect of data fitting. Lastly, computational times can be high for biophysical models, but this can (partly) be overcome by parallel computation, dictionary matching/learning or machine learning (Jelescu et al., 2020).

Pathologies might also pose challenges to biophysical models, since these might alter anatomical features. Depending on the pathology, different features might be expected to change, e.g. compartment fractions, exchange rates, compartment shapes and sizes, etc. The main promise of biophysical models is their specificity. This means that parameters estimated with a biophysical model should be strongly associated to a biophysical characteristic of tissues, but these characteristics might change due to a pathology. It is therefore questionable whether one single model could be used to incorporate the variety of possible pathologies, especially when using model constraints to overcome model degeneracies (Jelescu et al., 2020; Gardier et al., 2023).

A last challenge lies in the clinical translation. So far, clinical use of biophysical models has been limited for several reasons. Biophysical models require advanced acquisition protocols which often take too long for clinical practice and are not feasible on clinical scanners due to differences in hardware between clinical scanners and animal and human research scanners. This results in a low signal-to-noise ratio (SNR) and a low spatial resolution when using these acquisition protocols on clinical scanners, which affects the performance of the biophysical models (Jelescu et al., 2020).

V. Fiber reconstructions in the cortical GM and near the WM-GM boundary

Whereas biophysical models can capture the GM microstructure, one might also want to reconstruct the structural organization of the fibers in the GM circuits. This is what tractography methods can be used for. Tractography methods use diffusion MRI data to reconstruct the fiber pathways of the brain non-invasively, and are mainly used to track WM pathways that connect two (sub)cortical regions (Zhang et al., 2022). Since the start of the Human Connectome Project, the field of tractography based on diffusion MRI has seen a boost in innovation, with even a dedicated Connectome scanner made for diffusion MRI purposes in this project resulting in high quality data (Setsompop et al., 2013). While this has resulted in many breakthroughs in WM tractography, the field still faces multiple open challenges. Particularly, tractography in the cortex or at the WM-GM boundary is challenging, creating uncertainty on where tracts terminate within the cortex, both laterally and radially (Shamir & Assaf, 2023). In order to be able to image full fibers starting and ending in the cortex, De Luca et al. (2020) identified four requirements:

i. being able to estimate reliable WM FODs,
ii. being able to cross superficial WM at the WM-GM boundary,
iii. being able to overcome the gyral bias,
iv. being able to estimate reliable GM FODs.

In this section, the most promising methods for requirement iii and iv will be discussed.

A. *Intra-cortical tractography*

One of the first attempts for intra-cortical tractography was done *ex vivo* at high spatial resolution (242 µm) using spherical deconvolution. The cortex was divided into four different layers, because the spatial resolution did not allow for a distinction of six cortical layers. It was found that these layers all had different diffusion properties, and both radial and tangential fibers were reconstructed in this study. The

reconstructed orientation of tracts were in alignment with histological results. In the superficial layer, mostly tangential fibers were observed parallel to the cortical surface. Further down towards the WM-GM boundary, radial fibers were observed as well, just like in *Figure 1*. In these layers closer to the WM-GM boundary, crossing fibers had to be accounted for in the tractography algorithm (Leuze et al., 2014).

Recently, De Luca et al. (2020) proposed the multiple FOD (mFOD) framework to improve tractography in cortical GM. This was a revisitation of the spherical deconvolution approach towards FODs, where the FODs for WM and GM are in this method represented by different models: DKI for WM and NODDI for GM. The framework was both tested on simulations and on *in vivo* data from the HCP. Tractography using this mFOD framework led to an increase in fiber pathways ending in the cortical GM compared to state-of-the-art tractography. This is mainly due to the fact that the mFOD framework reconstructs more plausible and accurate GM FODs, which is essential for GM tractography.

Another work focusing on reconstructing FODs in the cortex is (Avram et al., 2022). They used high-resolution *ex vivo* mean apparent propagator MRI (MAP-MRI) to obtain diffusion properties of the cortex in the rhesus macaque brain. MAP-MRI has already been shown to be clinically feasible, and is used to measure probability density functions of the motion of water molecules in brain tissue. This study specifically focused on diffusion measures in and close to the cortex, and the high resolution (200 µm) made it possible to differentiate between cortical layers. The extracted FODs in the different layers were in line with the histological results of the same brain and with previous studies on fiber orientations in the cortex. The FODs were not used for tractography in this study, but they hold the potential to be used for intra-cortical tractography. Since this was an *ex vivo* study, a long scan time could be used. The clinical translation of high-resolution MAP-MRI still poses technical challenges regarding gradient heating and scan efficiency.

Another method to improve cortical tractography is the use of a knowledge-based model, in which macrostructural information about connectivity is combined with microstructural information of the laminar structure of the cortex. This has been shown to be both feasible for the macaque brain and the human brain (Shamir & Assaf, 2023). For the brain, a model was built based on multiple histological studies of laminar connectivity in both human and nonhuman brains. In the model, a distinction was made between horizontal and radial connections. Horizontal connections are interregional, either within or between the two hemispheres, whereas radial connections are intraregional connections in the cortex or connections between the cortex and subcortex. Rules for connectivity were identified for both types of connections, which define the start- and endpoints of tracts based on the regions that are connected by the tract, the granularity index of these regions and their laminar composition. It should be noted that the modeled intracortical connections are not based on tractography with this method, but on assumed connections from the laminar composition. Using this model requires a multimodal MRI acquisition to get both microstructural information about the laminar composition and connectivity information from diffusion MRI metrics (Shamir & Assaf, 2021). The feasibility of this method for healthy human brain mapping was shown in (Shamir et al., 2022), but there are still limitations to this method. The model reduces the cortical composition to three layers instead of the six that have been identified in histological studies, and the model is not able to estimate the probability of the connections (Shamir & Assaf, 2023).

B. Short association fibers along the WM-GM surface

Short association fibers are WM fibers that make a connection between two adjacent cortical regions. These short association fibers include U-fibers which run through the superficial WM, the WM that is closest to the cortex. It has been estimated that U-fibers compose around 60% of all WM pathways, but until recent they have not gained much attraction in neuroimaging. Being so close to the cortex, tractography of U-fibers in specific, but also short association fibers more generally, results in partial volume effects which make tractography difficult. This makes the U-fibers especially prone to the gyral bias, and tractography of U-fibers is additionally complicated by crossing, bending, kissing and fanning fibers. Filtering of fibers is complicated as well because there is no universally accepted definition of what should be considered a U-fiber or short association fiber. In a method specifically designed for U-fiber tracking, a cortical mesh is obtained from the T1-weighted image and used for seeding. Then, probabilistic tractography was performed using the FODs from the diffusion MRI data with three filters. The first filter is that both the startpoint and endpoint should be in GM. Secondly, fibers can only connect two GM regions within the same hemisphere and third, the full tract should travel through WM. This method led to a great cortical coverage and a majority of fibers connecting gyri, which should be expected. However, some limitations were presented as well. Histological validation was not possible because of a lack of a whole-brain ground truth. Moreover, using a cortical mesh based on the T1-weighted image makes the method dependent on registration quality, which would be deteriorated in distorted data sets or subjects with unusual anatomy like tumors (Shastin et al., 2022).

C. Overcoming the gyral bias: knowing where tracts enter the cortical GM

One of the reasons for the unknown lateral terminations along the WM-GM surface is the so-called gyral bias. This bias describes the observation that tracts tend to primarily terminate on gyral crowns rather than on sulcal walls or fundi. This bias was proven by intra-subject comparisons to histological findings. The main source for this bias is the seeding strategy and connectivity quantification used in the tracking algorithm. This bias could not be resolved by more advanced diffusion models or tracking algorithms, or by higher spatial resolutions (Schilling et al., 2018). *Figure 5* shows the gyral bias.

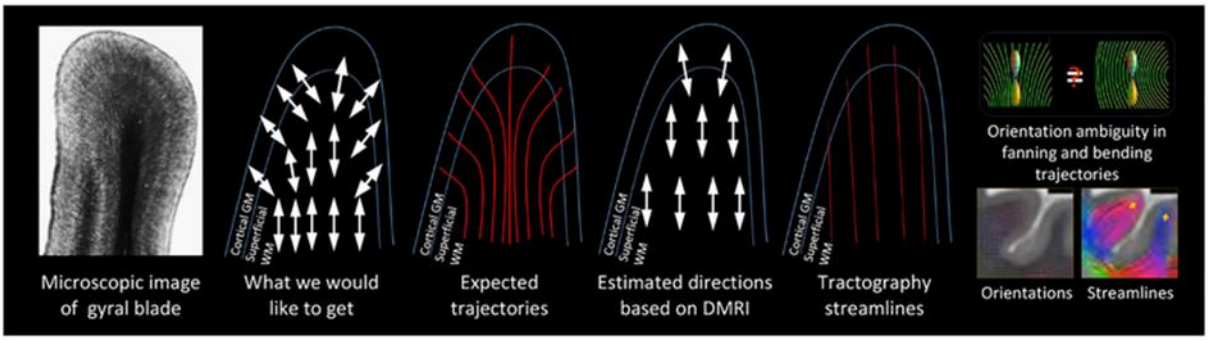

Figure 5: a representation of what is meant with the gyral bias. At the far left, a microscopic image of a gyral blade is shown. Next to it, the fiber orientations are shown which one would expect to obtain, and further to the right the resulting fiber tracts one would expect. This is however not what is obtained with diffusion MRI. What is obtained is shown in the next two images, this is what we call the gyral bias. This image is adapted from (Wu et al., 2020).

Overcoming the gyral bias has been one of the goals of Frank et al. (2020) in proposing Joint Estimation Diffusion Imaging (JEDI). This is a method in which single pulsed field gradient (sPFG) imaging is combined with double pulsed field gradient (dPFG) imaging, to combine, respectively, macroscopic and microscopic anisotropy information. The method reduces two problems resulting in the gyral bias, partial volume effects of voxels containing both WM and GM, and curvature constraints in tractography algorithms. By combining sPFG data with dPFG data, microscopic anisotropy in GM or voxels with both WM and GM can be detected, which makes it possible to follow tracts into the GM. The curvature constraints are not an issue in JEDI because the authors make use of a tracking method (GO-ESP, see (Galinsky & Frank, 2014)) which sets no constraints on curvatures. The results show JEDI's potential to overcome the gyral bias, since it is able to tract more complete fibers within both WM and GM which appear to be in line with expected fibers. However, the acquisition protocol should be enhanced to be in line with clinical scan times and further validations against histological data should be executed.

Another method to reduce or overcome the gyral bias is the use of asymmetric fiber orientation distributions (AFODs). Here, information from neighboring voxels is used to adapt the FOD of a voxel to an asymmetrical FOD, allowing for better reconstructions of complex tract configurations. This method does not require advanced acquisition protocols or constraints and assumptions based on additional structural images like T1-weighted images, like other methods for mitigating the gyral bias. The use of AFODs results in a more complete coverage of both gyri and sulci and higher reconstructed connectivity between cortical regions. However, there are limitations. The GM diffusion was assumed to be isotropic, which it is not, and the method presumably only works with multi-shell diffusion MRI data (Wu et al., 2020). Following up on this study, active cortex tractography was proposed by Wu et al., which is an adaptive tractography method based on the AFODs. Apart from considering AFODs, they also use anatomical information of the WM-GM boundary to improve cortical tractography and further mitigate the gyral bias. The preliminary results were promising and have been presented in (Wu et al., 2021).

Cottaar et al. (2021) showed that it is also possible to mitigate the gyral bias by using a different tractography method within the gyral blade than for the rest of the brain. After segmenting the white matter in gyral blades, a continuous vector field is modeled and (iteratively) improved taking into account the cortical fold geometry and fiber densities and orientations. Crossing fibers are not possible in this gyral blade WM model and tracts are only allowed to terminate in the cortex, not in WM. The modeled vector field is then used for tractography within the gyral blade, from the WM-GM boundary to the deep WM (the WM outside the gyral blade). Although successful in mitigating the gyral bias, a major limitation is the loss of information about short-distance connections like U-fibers or connections within the gyrus. Reconstructing long-distance connections seems to be more accurate with this vector field method.

Focusing on the superficial WM, St-Onge et al. (2018) proposed surface-enhanced tractography (SET) to mitigate poor resolution at diffusion MRI images, the partial volume effect and the gyral bias. They use the T1-weighted, image which is typically acquired as well during diffusion MRI exams, to retrieve the cortical surface geometry which is used to enhance tractography. By including this structural information, the following properties were meant to be considered in the proposed model with constraints along the cortical surface: i) parallel orientation of fibers to the gyral wall, ii) fibers end orthogonal to the WM-GM boundary, iii) fiber terminations are present along the full WM-GM boundary and iv) smooth fiber trajectories. This resulted in a reduced gyral bias, as well as a reduction of the length bias and a reduction of the amount of false positive reconstructed fibers (St-Onge et al., 2018). The gyral bias was still present however, with a great amount of sulcal banks and fundi uncovered (Rheault et al., 2020). Combining this with adaptive and dynamic cortical seeding methods results in a further reduction of gyral bias and a fuller coverage of the cortex (St-Onge et al., 2021).

D. Persisting challenges

In their review of diffusion MRI possibilities for imaging cortical structures, Assaf (2019) posited that one of the major limitations is the relatively low spatial resolution compared to the structures one might want to image in the cortex. The resolution of diffusion MRI is typically 1.5 mm isotropic at *in vivo* scans with 3T scanners, while the cortex itself has an

average thickness of 2.5 mm. It is therefore difficult to bring tractography to the level of cortical layers, but innovations in hardware, such as the development of the 2nd generation Connectome scanner (Huang et al., 2021), promise the possibility of higher spatial resolutions.

Moreover, the diffusion FODs, which are used for fiber tractography, are not only correlated with the neurite orientations. The processes in other structures like glial cells have also been found to contribute to the FOD. This might complicate fiber tracking (Assaf, 2019).

The curvature of the cortex, especially the curvature arising from gyri and sulci, also poses challenges for tractography. This is mainly problematic because of the use of a Cartesian coordinate system, which is well-suited for problems with planar symmetry or where no symmetry exists. For the cerebral cortex, using a Cartesian coordinate system may not be optimal and could be replaced with using curvilinear coordinates. Hussain et al. (2021) used a curvilinear coordinate system for tractography within the hippocampus at HCP data. Using this coordinate system served as an anatomical prior for them and hereby informs the tractography. Since the cerebral cortex has regions with high curvature, the authors expect that tractography near and in the cortex will also benefit from using a curvilinear coordinate system. Until then, curvature of the cortex remains a challenge for tractography methods.

Two challenges in WM tractography which might also be apparent in tractography in and near the cortex, are the crossing fibers problem and the bottleneck problem. The crossing fibers problem occurs when multiple fibers cross the same voxel, which complicates the interpretation of the signal and therefore also tractography (Figley et al., 2022). The appearance of crossing fibers in cortical GM was for example shown in (Leuze et al., 2014). The bottleneck problem has been identified as one of the biggest challenges in WM tractography. This problem occurs when multiple fibers converge in one voxel, and later on diverge again. It is in such cases unknown which fiber propagates which way after diverging from the common voxel, resulting in both false positives and false negatives (Schilling et al., 2022a). To what extent this also occurs in cortical GM is unknown, but should be kept in mind.

VI. Discussion

The goal of this literature review was to investigate the possibilities and challenges of charting the cerebral cortex using diffusion MRI, specifically the use of biophysical models and tractography for this matter. In this section, we will first consider overlapping points of discussion for both methods, after which points of discussion specific to either one of the methods are discussed.

Both methods have previously mainly been developed for WM imaging, and advances in the field of GM are all relatively recent. As a result, research on both methods is still in the preclinical phase, either *ex vivo* on animals or *in vivo* on the human brain with preclinical scanners. Results should therefore be handled with care, since data from clinical scanners will suffer from several limitations like lower spatial resolution and SNR. Moreover, diffusion characteristics can differ between *in vivo* and *ex vivo* subjects. Nevertheless, this preclinical phase is invaluable to advance the field (Schilling et al., 2022b). First of all, comparisons can be made to histological results of the same subject, making it possible to investigate what microstructural features affect diffusion MRI measures. Second, more advanced acquisition protocols can be used for *ex vivo* imaging, making it possible to seek the boundaries of what diffusion MRI is capable of and to get more information about the studied structure. However, translation to the clinic of the methods discussed in this review remains a challenge for now.

Validation is an essential but difficult aspect for both methods as well. *In vivo* studies to show the feasibility of new methods, although very valuable in the developing process, have hardly no possibilities for validation against a ground truth. Options for validation against a ground truth include comparing *ex vivo* imaging to histological results of the same subject, or using realistic phantoms (Schilling et al., 2019). However, both methods have their difficulties. Histological studies require a lot of resources which far from every institute has available, and representing the full complexity of the brain has proven to be very complicated when manufacturing phantoms. Atlases or models based on multiple histological studies, like the one built in Shamir & Assaf (2021) for fiber tracts, offer opportunities to compare results to what can be expected. But variations between subjects and model complicate validation of methods. Especially the cited tractography methods in this review have not yet been validated against a ground truth.

A. Biophysical models

The literature on biophysical models is focused on augmenting the SM with GM-specific characteristics. There seems to be agreement between authors that a biophysical model should at least incorporate somas and intra-compartmental exchange. For now, the diffusion processes in the proposed models have been described as Gaussian, while structural disorder within compartments might lead to non-Gaussian diffusion. In WM, accounting for non-Gaussian diffusion by using DKI has led to better characterization of the WM structure (Steven et al., 2014). GM models might become even more accurate when accounting for non-Gaussian diffusion, so this could be a subject for future studies.

Moreover, the current methods mainly focused on structures in the cerebrum, but the brain has other GM regions as well which might have other characteristics. It is therefore still a question to what extent these methods can be used for a global brain analysis. As explained by Jelescu et al. (2020), the models are also specific to healthy tissue and incorporating pathologies might be complex, resulting in the expected need of specific models for different pathologies. Diagnostics might therefore be more complex using biophysical models, but the study of the progression of a pathology could be possible with the use of biophysical models.

A more fundamental question for the use of biophysical models lies in the complexity of the proposed models. The more complex the model is made, the more accurate the model might be in representing the tissue type which is supposed to be modeled. However, the more complex a model is, the more difficult it could get for it to be useable in the clinic. More complex models will tend to have more parameters to be tweaked, which could lead to more degeneracies of the model and longer computation times. It is an open question what level of complexity is needed for the models to be sufficiently useful in the clinic.

B. Tractography within cortical GM and near the WM-GM boundary

As denoted by De Luca et al. (2020), improvements on several fronts have to be made before the field of diffusion MRI is able to track full fiber pathways throughout the brain. This includes being able to reconstruct reliable FODs in GM, overcoming the gyral bias and crossing the superficial WM. The literature reviewed in this paper tend to focus on either one of these issues. Although the advancements on the several fronts seem to be promising, no efforts are made yet to combine them into a unified approach for fiber tractography. It is also still an open question to what extent each of the proposed methods can be combined with other methods proposed for different issues.

In this review, multiple different tractography methods have been presented. Ideally, we would be able to critically compare the results of these methods with each other, concluding on what would be the best method to continue with. However, due to variations in acquisition protocols and evaluation metrics of the methods' performance, this is quite complicated. Take for example the methods focused on mitigating the gyral bias. Even though active cortex tractography (Wu et al., 2021), SET (St-Onge et al., 2021) and the vector field tractography (Cottaar et al., 2021) were all tested on HCP data, the methods are hard to compare. Active cortex tractography was for example mainly evaluated qualitatively, while the other two were evaluated quantitatively. SET was evaluated by the percentage of cortical coverage and end-point distributions along the cortical surface, but these measures are less informative for the vector field tractography because of its constraints. Moreover, none of these methods was validated against histological data, so it could only be compared with expected fibers. A comparative study might therefore be needed to compare the methods on the same subject with the same acquisition.

Although methods have been proposed on reconstructing reliable FODs within the GM, it has mainly been used to reconstruct the propagation of WM fibers entering the GM, which are mainly radial fibers. Reconstructions of tangential fibers through the cortex are still relatively unstudied in the field of diffusion MRI tractography. More work might therefore be needed on this aspect.

C. Combining biophysical models and tractography

As we have seen in this literature review, biophysical models and tractography are used for different purposes. Biophysical models are used to get microstructural information about the tissue that is studied, while tractography aims to reconstruct the connections throughout the brain to obtain connectivity information. Both types of information might be simultaneously of interest in clinical exams. Therefore, it would be an advantage if acquisition protocols could be developed which would obtain signals that could be used for both the use of biophysical models as well as for obtaining FODs.

The studies cited in this literature review either focus on biophysical modeling or on tractography. The two of them have not been combined. However, we have seen that the NODDI model has been used for microstructural imaging, although with limitations for GM (Nazeri et al., 2020), and for representing the GM FOD (De Luca et al., 2020). It might thus be possible to combine the both, but it is unknown to what extent the acquisition protocols of the other, more advanced GM models can be used to obtain reliable FODs which can be used for fiber tractography in both GM and WM.

VII. Conclusions

This literature review investigated the possibilities and challenges of using diffusion MRI methods for unraveling GM. Specifically, the use of biophysical modeling for microstructural imaging and tractography for fiber tracking in the cortex were studied. The earlier identified challenge of limited spatial resolution for both methods still persists. Moreover, the lack of a gold standard except histology complicates validation. Validation, however, is an essential part for the development of both methods.

The field of GM biophysical modeling seems to converge to advancing the SM, which is used in WM, by complementing it with GM characteristics, and the first preclinical results seem promising. The field of GM tractography, on the other hand, seems to be more diverse. WM tractography is already widely used, but also suffers from challenges which also extend to GM tractography. Contributions are made on several fronts with promising methods to overcome specific issues (gyral bias, reliable FODs, crossing fibers and bottleneck problem, etc.), but no efforts are made yet to combine the advancements on the different issues in tractography. Until these methods can be combined, GM tractography will remain challenging.

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