

Studying neuroanatomy using MRI

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The study of neuroanatomy using imaging enables key insights into how our brains function, are shaped by genes and environment, and change with development, aging and disease. Developments in MRI acquisition, image processing and data modeling have been key to these advances. However, MRI provides an indirect measurement of the biological signals we aim to investigate. Thus, artifacts and key questions of correct interpretation can confound the readouts provided by anatomical MRI. In this review we provide an overview of the methods for measuring macro- and mesoscopic structure and for inferring microstructural properties; we also describe key artifacts and confounds that can lead to incorrect conclusions. Ultimately, we believe that, although methods need to improve and caution is required in interpretation, structural MRI continues to have great promise in furthering our understanding of how the brain works.

The study of neuroanatomy can be traced back to ancient Egypt and was brought into the modern era by the seminal tracings of the brain and nerves by Thomas Willis. The brain was long viewed as the seat of consciousness and thought. A direct linkage between cognition and individual brain areas arose in the 19th century, as epitomized by the work of Broca, Wernicke and Lichtheim¹. Detailed studies of the composition of cell types across regions of the brain by Ramon y Cajal, von Economo, Brodmann and others then laid the foundation of our current understanding of the anatomy of the central nervous system (reviewed in ref. 1). As we became more and more certain that functions were localized to specific brain regions, questions arose about the evolution of these brain regions. This in turn led to questions about the possible relationship between their size or shape and interindividual variations in cognitive abilities or progression of a disease.

The advent of MRI in the late 1970s and early 80s revolutionized the study of neuroanatomy, as for the first time it could be investigated *in vivo* with sufficient contrast to differentiate brain compartments. The 90s introduced computational approaches to MRI-based studies of neuroanatomy. At the same time, novel quantitative or semiquantitative MRI techniques emerged, allowing researchers to estimate

microstructure within a voxel. The advent of automated processing pipelines further enabled large scale analyses of population imaging data in ways that were previously time-prohibitive.

The ability to obtain high-quality, detailed information from *in vivo* imaging has clearly revolutionized our understanding of neuroanatomy and structure–function relations and shed insights into multiple disease processes. Yet there are myriads of issues surrounding acquisition, analysis, study design and interpretation that need to be considered. This review provides readers with an overview of these issues to equip them to select the most appropriate toolkit for their needs.

Overview of the methods

Key to understanding MRI based measures of neuroanatomy is that, with MRI, we are not directly measuring the cellular compartments we would like to make inferences on. Limited resolution, combined with indirect measurements, cautions against simplistic extrapolation from MRI findings to neurobiological conclusions. In contrast to the common metaphor of ‘taking a picture of the brain’, MRI instead measures radio-frequency signals emitted from hydrogen atoms after the application of electromagnetic (radio-frequency) waves, localizing the signal using spatially varying magnetic gradients. Contrast from each voxel (a three-dimensional pixel) depends on the density of protons within the voxel and properties of the local tissue microenvironment that are either directly related to the magnetic properties of hydrogen or that can be detected through manipulation of magnetic fields. The contrast produced is dependent on the precise timing of the magnetic field manipulations (pulse sequences), as shown in **Figure 1**.

To provide more detail, tissue contrast is dependent on T_1 and T_2 relaxation, two independent processes that describe how spins (i.e. magnetic properties) behave after the application of radio-frequency pulses. T_1 represents the time constant for a system of hydrogen protons to return to equilibrium after radio-frequency radiation². T_1 values are determined by macromolecule concentration, water binding and water content³, with myelin shortening T_1 and edema lengthening T_1 . T_2 , or transverse, relaxation describes the process by which spins are taken out of alignment with each other due to variations in the local

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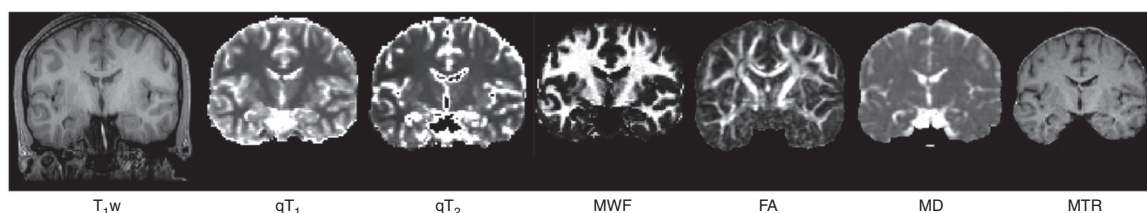


Figure 1 Coronal slices of multimodal images of brain structure acquired in members of a birth cohort when they reached 20 years of age. Leftmost: the T_1 -weighted (T_1w) image most commonly used for analyzing brain volumes, voxel based morphometry, cortical thickness, etc. Next, from left to right, are quantitative T_1 and T_2 (qT_1 and qT_2 , respectively) and myelin water fraction (MWF) maps, estimated using the multicomponent driven equilibrium single pulse observation of T_1 and T_2 (mcDESPOT) sequence⁴⁵. Right three images: FA and mean diffusivity (MD), both from diffusion imaging, and (rightmost) a magnetization transfer ratio (MTR) map. These data indicate the types of rich information about brain structure that can be obtained from MRI in a single session. Sample images acquired from the ALSPAC MRI study, which was approved by the North Somerset and South Bristol Research Ethics Committee and the Baycrest Research Ethics Board and conducted in accordance with its guidelines. Informed written consent was obtained from all participants.

tissue environment at the micro- or nanoscopic scale⁴. T_2 effects are observed as signal decay, with gray matter having a longer T_2 relaxation time than white matter. Structural MRI studies almost universally use ‘weighted’ imaging, in which the signal intensity is related to T_1 and T_2 , rather than quantifying these or other properties (because these are generally more efficient, giving greater signal to noise for a given acquisition duration).

Once images have been acquired, measuring brain structure involves two complementary approaches: (i) the macro- or mesoscopic, considering sizes and shapes across multiple voxels, and (ii) the microscopic, obtaining information from within voxels.

Macroscopic and mesoscopic neuroanatomy. There are five domains to studying size and shape at the macro- or mesoscopic scale:

- (1) Manual volumetry involves trained anatomists manually segmenting regions of interest from brain scans. While in many ways manual volumetry is still the gold standard, it is hard to scale to multiple scans (especially for modern studies running into the thousands of participants)^{5,6} and multiple brain regions. Thus, the primary use of manual volumetry is in smaller studies with a single, focused hypothesis, as well as for the creation of data sets to be incorporated into automatic segmentation algorithms.
- (2) Automatic segmentation algorithms aim to replace manual volumetry for most applications. In some cases manually segmented data sets are used to parcellate new databases on some combination of linear and nonlinear image registration, tissue classification and related image features, while for other algorithms no manual training is necessary. The time of trained anatomists is traded for computer time, with computationally intensive multiatlas approaches and/or additional surface-based constraints adding extra accuracy⁷. Agreement between manual volumetry and automatic segmentation indicates ever-improving correspondence between the two.
- (3) Two classes of morphometry algorithms are commonly used to analyze neuroanatomy without the prior constraint of defined regions of interest: voxel-based morphometry (VBM) and deformation-based morphometry (DBM). In VBM, illustrated in **Figure 2**, the brain is classified into white matter, gray matter and cerebrospinal fluid; a single tissue class is selected, then blurred with a Gaussian kernel to give an estimate of the local amount of that tissue type at every voxel, then compared across subjects after linear or coarse nonlinear alignment⁸. In DBM, brains are nonlinearly deformed toward a common space or

each other, and deformation fields are either directly analyzed⁹ or reduced to a scalar measure of volume change, the Jacobian determinant, before comparisons across scans¹⁰. These two methods are joined in optimized VBM, wherein the tissue density measure is modulated by the Jacobian determinant¹¹.

- (4) The complex folding pattern of the cerebral cortex challenges computational neuroanatomy, though this has significantly improved with the development of surface-based algorithms, as shown in **Figure 3**. Here the inside and outside surfaces of the cortex are segmented using deformable models, and measures such as cortical thickness and surface area are extracted^{12–14}. The diverse anatomical metrics that can be extracted from three-dimensional models of the cortical surface capture different developmental processes and show dissociable correlations with demographic^{15,16}, genetic^{17,18}, environmental¹⁹ and clinical^{20,21} variables, highlighting the value in moving classical volumetric approaches to anatomical analysis. Surface-based methods also provide an improved coordinate system for the cerebral cortex, allowing for smoothing of signal on the cortical sheet and surface-based alignment to bring individuals into closer correspondence for statistical comparisons^{13,22}.
- (5) White-matter tract locations and sizes can be estimated using diffusion tractography. Here measures of water diffusion, described in greater detail below, are used to trace the pathways of the brain, followed by inferences on properties of each tract. The benefits and caveats of tracing the macroscopic connections of the brain were described in greater detail in a recent review²³, to which we refer the reader for a more in-depth description.

Since MRI is not a direct picture of the underlying anatomy, the outcomes of derived measures, such as cortical thickness or VBM, depend on sequence and hardware choices^{24,25}. Measures of cortical thickness at 7 tesla (T) are, for example, lower than when measured at 3T²⁶. Varying tissue properties across the cerebral cortex present different degrees of challenge in separating gray from white matter; the motor cortex is heavily myelinated in its lower layers, and thus magnetic resonance (MR)-based thickness estimates (especially in early studies) tend to underestimate its thickness compared with histological measurements²⁷. Constant improvements in hardware along with MR-sequence development are bringing imaging measures ever closer to post-mortem ground truth, but the nature of the MR signal needs always to be kept in mind when offering interpretations of imaging data; what we measure is not always identical to what the anatomists of old (and today) would have estimated. This becomes

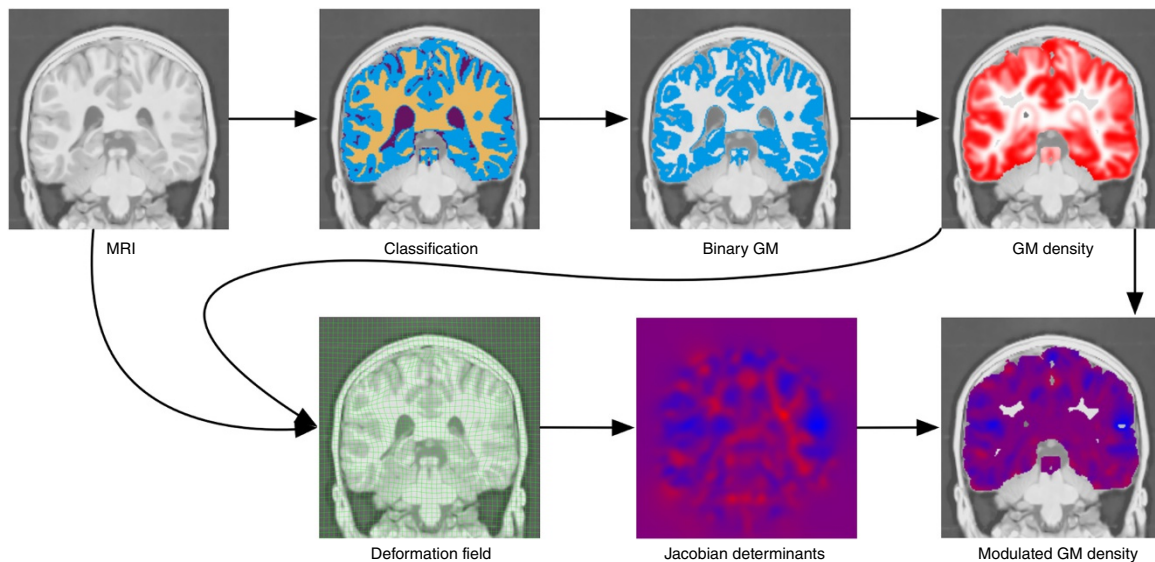


Figure 2 Voxel-based morphometry (VBM). VBM was the first widely adopted technique for determining alterations in neuroanatomy across sets of subjects. VBM entails classifying the brain (MRI) into white matter, gray matter (GM), cerebrospinal fluid and background (classification), extracting one of the classified tissues types (binary GM), then smoothing the extracted tissue type with a Gaussian kernel. The final product is thus an image (GM density), in linearly registered stereotaxic space, with values ranging from 0 to 1 representing the amount of gray matter within a local neighborhood as determined by the blurring kernel⁸. A modification to the basic VBM protocol was proposed in 2001 (ref. 11), wherein nonlinear registration, based on either aligning the T₁-weighted MRIs or the GM density maps, is incorporated to provide better spatial alignment. This optimized VBM procedure also combines the nonlinear registration (deformation field) with the tissue density map obtained from classic VBM by multiplying (or modulating) the tissue density map by the Jacobian determinant of the nonlinear deformation field to produce the modulated GM density map. Sample images were obtained from the POND study, which was approved by The Hospital for Sick Children Research Ethics Board and conducted in accordance with its guidelines. Informed written consent was obtained from all participants and/or their parents.

an even greater issue when potential artifacts segregate with the study population, as discussed later in this review.

Microstructure: MRI at the microscopic level. The study of microstructure, which in MRI refers to the distribution of the contents of a voxel, has been primarily the domain of diffusion MRI. Here the thermally-driven, random motion of water molecules is a probe of the local microenvironment, and the restriction of that motion is used to infer the organization of the tissue inside the imaging voxel.

Initial studies modeled the scatter pattern of diffusing water molecules with an ellipsoidal shape, represented by a tensor²⁸. Various metrics can be derived from the diffusion tensor, such as the fractional anisotropy (FA, representing how elongated the shape is), as well as the mean, axial and radial diffusivity (i.e., the radius of the ellipse along different axes)²⁹. Variations in these metrics reflect variations in the profile of water diffusion and have been therefore associated with alterations in the underlying tissue microstructural boundaries. They can be compared across individuals at every voxel after alignment (for example³⁰), or averaged across tracts after tractography-based tract segmentation (for example³¹). To overcome registration issues, skeleton-based approaches were introduced, whereby values are projected to an alignment-invariant mean-FA skeleton representing the center of major tracts, which also eliminates partial-volume effects at the edge of tracts^{32,33}.

Tensor-based modeling of diffusion MRI data, while sensitive to alterations in microstructure, is not specific to a given types of variation in tissue properties²⁹. Furthermore, in regions with complex fiber patterns or significant restriction (for example, entrapment of water in a particular cellular compartment), the tensor model does not capture the underlying structure and only provides an average unimodal approximation.

Acquiring multiple 'shells', or diffusion MRI acquisition with varying sensitivity to diffusion process (typically quantified by the experimental parameter *b*), opens up further potential for analyses. Multishell measurements enable deviations from the tensor model (and therefore complex microstructure) using higher-order approximations, such as the diffusion kurtosis model³⁴. Even kurtosis estimates of diffusion measures still lack direct biological interpretation, similarly to parameters in the tensor model; however, model-based mappings³⁵ offer potential work-arounds (see also **Box 1**). As both modeling and acquisition methods evolve, estimates with greater specificity can be obtained. Possible inferences range, for instance, from mapping crossing-fibers in white matter to estimating neurite densities in gray matter. These methods are summarized in **Box 1**.

While diffusion based approaches have become the dominant method for inferring microstructure, other quantitative imaging techniques still provide unique information. Magnetization transfer (MT) imaging reflects the interaction between free-water protons and protons bound to large macromolecules³⁶, such as proteins. MT is often employed in studies of the structural properties of white matter, where the macromolecules of myelin are the dominant source of the signal³⁷. MT is also used to probe damaged tissue and inflammation due to its sensitivity to protein content³⁸. The fastest way to acquire MT data is to use two acquisitions, with and without a MT saturation pulse, and thereafter calculate the MT ratio as the percent signal change between the two acquisitions³⁹. A variant of MT is chemical exchange saturation transfer (CEST)⁴⁰, which has applications in detecting pH changes in stroke⁴¹ and in tumor delineation⁴².

Instead of using weighted images for subsequent image processing, one can also quantify T₁ and T₂ directly. The aim is to allow greater inference about local tissue composition: for example, by using the very short T₂ associated with myelin compared to other water within

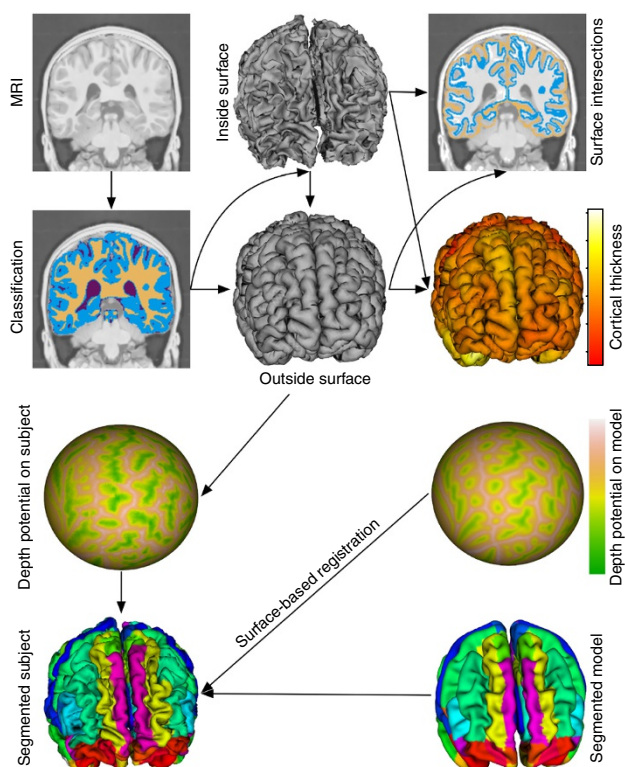


Figure 3 Surface-based analyses. The inside and outside surfaces of the cortex are extracted based on a mix of tissue classification and deformable model segmentation. Cortical thickness can then be computed based on the distance between the inside surface and outside surface, and surface area computed on either surface (not shown). For the sake of intersubject statistics as well as to aid in segmenting the cortex into constituent lobes, sulci and gyri, the curvature (or, alternately, some measure of sulcal depth or depth potential) is computed on the surface (depth potential on subject) as well as for a model (depth potential on model). Surface-based registration then takes the segmented model and uses it to parcellate the input surface (segmented subject). Sample images obtained from the POND study.

a voxel⁴³. The main downside of quantitative T_1 and T_2 mapping is the significantly greater amount of time required for equivalent signal-to-noise ratio (SNR) and resolution, as the gold-standard methods require acquiring data at multiple echoes or inversion times^{43,44}. This is compounded by the general need to estimate multiple T_2 values within each voxel if one wants to map specific compartments like myelin. Faster approximations exist^{45–47}, yet these methods have significant variation in reported metrics, such that calibration to the gold standard is still required⁴⁸.

The sequences described above all rely on the magnitude information and discard phase information. Phase information can, however, be used to identify susceptibility changes between tissues. For example, phase images can be used to quantify the mean magnetic susceptibility of the tissue in a voxel, which is arguably a more direct measure of the tissue magnetism than many of the relaxation time-based methods described above. This method, known as quantitative susceptibility mapping, has been demonstrated to change with tissue iron, myelin and calcium⁴⁹.

Applications of structural MRI

The spatial organization of the brain and its microstructural properties are the physical manifestations of the information encoded in the genome, developmental history and experienced environment, and thus touch on just about every facet of neuroscience. As described above,

MRI enables the derivation of multiple (semi-)quantitative features of brain structure in a reliable manner (test–retest correlations > 0.75), over short timescales (~ 1 h) and on a large scale ($> 1,000$ participants). It is clearly impossible, within this review, to provide an overview of all aspects of neuroscience touched on by MRI studies of brain structure. Instead we provide two illustrative examples. The first relates to combining genetics and epidemiology with brain imaging to study populations (**Box 2**). Second, interindividual differences in adult cognition, behavior and psychiatric disease can often be linked to earlier developmental variations that emerge across infancy, childhood and adolescence. Structural neuroimaging plays a special role in unraveling these developmental associations since changes in brain structure work across both short and long time-scales (**Box 3**).

Finally, brain structure need not be only analyzed as a series of independent segmented regions or even voxels. Instead neuroanatomy can also be studied in terms of networks of covariance. Studying brain networks using brain imaging first came out of activation and resting state functional MRI or positron-emission tomography (PET) studies. Similar networks emerged when correlating seed regions with the rest of the brain using either VBM⁵⁰ or cortical thickness⁵¹ across a population of subjects. Variations in such structural covariance networks have been linked to both normal development and aging, as well as multiple disease processes^{52,53}. The link to other measures of networks, whether from functional imaging or tract tracing is, however, ambiguous⁵⁴, and clearly more work is needed to understand the origins and significance of structural covariance networks.

Forward problem, inverse problem: what are the signal sources, and what kind of inferences can we make?

Once imaging results have identified a finding of interest, establishing their molecular and cellular bases is often elusive. The study of brain plasticity with MRI provides an illustrative example. The early identification of altered ‘concentration’ of hippocampal gray matter (as measured by VBM) in London taxi drivers suggested that the processes of learning and memory, so long studied in animal models, might be detectable by MRI in humans⁵⁵. Additional evidence came from studies showing that teaching adults how to juggle altered gray-matter concentrations in the visual and parietal cortex⁵⁶, a change that occurred in as quickly as 7 d (ref. 57) and was accompanied by changes in water diffusion in underlying white matter⁵⁸. Microstructural changes have even been detected using diffusion MRI within 2 h of playing a video game⁵⁹. Further work by multiple groups has shown that MRI can detect brain plasticity in motor coordination tasks⁶⁰ and musical training⁶¹, amongst others. Intriguing as they are, these studies are not without some refutation and debate. For example, one danger of misinterpretation can be seen in Bengtsson *et al.*⁶², where reported areas of change after piano practice are mostly on the very edge of the white matter tracts and therefore likely represent changes in tract thickness rather than microstructural properties of the tracts. Juggling, the most-studied training task for inducing structural brain plasticity, often shows changes in the visual and parietal cortices, but the precise localization of those effects is poorly reproducible⁶³. Detecting brain plasticity in a study of adults trained on a joystick task was found to be extremely dependent on methodological choices made during image processing and appeared to be artifactual⁶⁴.

Studies of human brain plasticity were then back-translated into animal models, in particular mice and rats. The advantage of rodent models is, of course, that invasive histology and immunohistochemistry experiments can be carried out after MRI assessments. Initially, it was established that learning causes changes in regional brain volumes⁶⁵ and diffusion properties⁶⁶. Such alterations could be

Box 1 Advanced diffusion modeling

Tensor-based modeling of diffusion MRI data assumes that water molecules disperse randomly according to a Gaussian distribution that is in general anisotropic (i.e., described by an ellipse). The tensor model can provide markers that are sensitive to alterations in tissue microstructure, but tensor-based metrics are not specific to biologically interpretable tissue properties. Furthermore, tensor-derived measures like FA (**Fig. 4a**) reflect geometrical properties (fiber complexity; **Fig. 4b**), biophysical properties (size of fibers and/or fiber density) and aspects of the measurement (diffusion time and/or b -value).

A number of biophysical models have been proposed as an alternative to the tensor to aid interpretation by parameterizing the observed signal as a function of intrinsic tissue properties (see¹²⁸ for a review and¹¹⁹ for a taxonomy of these models). Early on, the observation of multiexponential decay of the diffusion signal (particularly at high b -values)^{135,136} motivated multicompartment models (**Fig. 4c**; modified from ref. 119, Elsevier), where the signal is assumed to arise from nonexchanging cellular environments. For example, early models treated the extracellular space as giving rise to hindered diffusion (Gaussian movement but with a reduced diffusion coefficient) and intracellular spaces as having restricted diffusion (which is inherently non-Gaussian)¹³⁷. Several approaches have expanded on these earlier studies, in particular for modeling white matter. The ball-and-stick model¹³⁸ captured restricted, perfectly anisotropic intra-axonal diffusion (stick) and isotropic extra-axonal diffusion (ball). The composite hindered and restricted water diffusion (CHARMED) model¹³⁹ replaced the sticks with cylindrical fibers of fixed diameter and modeled the extra-axonal space using a tensor. AxCaliber¹⁴⁰ further added a distribution of axon diameters and included a compartment to represent stationary or glial cell water¹⁴¹. Alexander *et al.*¹⁴² used modified versions of the above models were to provide a model for white matter diffusion that was relatively simple and yet complex enough to probe axonal density and diameter. Fieremans *et al.*³⁵ also proposed a mapping from nonspecific diffusion kurtosis metrics to white matter microstructure features (including axonal water fraction, intra-axonal diffusivity and extra-axonal diffusivities).

The previous models assume homogeneity in fiber orientation. A plethora of methods exist for the estimation of the fiber orientation density function (fODF), which describes the distribution of fiber orientations within each imaging voxel (see ref. 143 for a review). fODF estimation methods are mostly designed and aimed for tractography, although some microstructure information can be extracted (for instance, refs. 118,144). Nevertheless, fiber dispersion is represented explicitly in more recent multicompartment white matter models (**Fig. 4d**; modified from ref. 129, Elsevier) that relax the assumption of orientation homogeneity (for example, refs. 145, 146; see also the “Structural Imaging: current possibilities” section of this paper). Dispersion models are also increasingly being used to make inferences about gray matter compartments that collectively represent neurites, i.e., projections from cell bodies, either dendrites or axons⁷³. Recently, the NODDI (neurite orientation dispersion and density imaging) model has enabled similar measures to be estimated from images acquired on commonly available clinical scanners using scan times that are feasible for *in vivo* imaging (**Fig. 4e,f**)¹⁴⁷.

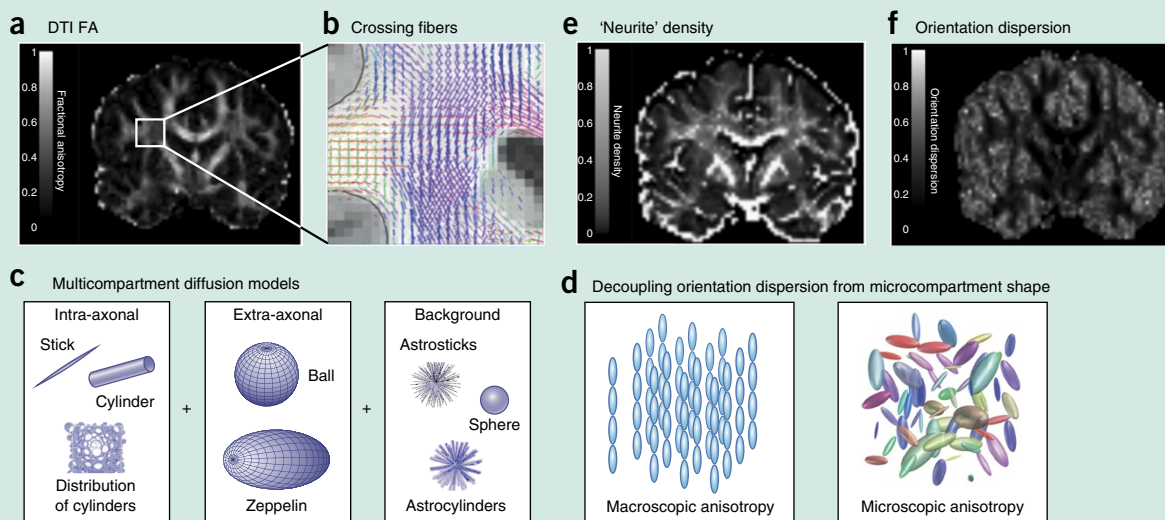


Figure 4 Overview of diffusion modeling advances.

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detected within 1 d and yet seemed to last for weeks or months⁶⁷. At the level of correlations, diffusion properties changes in white matter were correlated with myelin markers⁶⁸; diffusion in gray matter correlated with astrocytic and synaptic markers⁶⁶. Volumetric changes similarly correlated with synaptic markers, in particular GAP43 (an axonal growth cone indicator^{65,69}), dendritic spine counts⁷⁰ and glutamate concentration⁷¹. Establishing more causal connections between cellular and mesoscopic changes is just beginning; irradiation, for example, has been shown to stop both neurogenesis and exercise-related increases hippocampal volume⁷². Genetic mouse models in particular promise to play an important role in linking molecular mechanisms to MRI outcomes.

Even with using animal models to provide direct mechanistic explanations of MRI signal, interpretation will remain contextual.

An increase in gray matter in the hippocampus might be conclusively linked to synaptic changes in the future but only within the context of healthy individuals engaging in a learning task. In pathological states, the same change in hippocampal volume could be caused by entirely unrelated cellular mechanisms. Advanced MR sequences, particularly microstructure modeling approaches, offer the promise of greater specificity than volumetric measures. Working with a rodent model, Jespersen *et al.*⁷³ compared *ex vivo* multishell diffusion data with a combination of myelin and cell body staining. They identified a high degree of correlation between neurite density (estimated from advanced diffusion MRI models) and myelin maps; moreover, the biophysical models based on assumptions regarding cellular structure clearly outperformed the simpler tensor model⁷³. Similarly, related work identified a strong relationship between myelin density in the

Box 2 Population neuroscience

Population neuroscience integrates epidemiology, genetics and neuroscience to identify influences shaping the human brain from conception onwards^{5,148,149}. As we discussed elsewhere¹⁵⁰, such efforts face three key challenges: (i) an infinite combination of factors influencing the brain from within (genes and their regulation) and from without (social and physical environment); (ii) the presence of developmental cascades that carry such influences from one timepoint to the next (for example, prenatal to postnatal), from one organ to another (for example, cardiometabolic organs to brain) and from one level of organization to a different one (for example, behavior to gene regulation and vice versa); and (iii) the structural and functional complexity of the human brain.

These challenges can be met by conducting studies in large samples of participants drawn from the general population and evaluated with state-of-the-art tools for assessing genes and their regulation, external and internal environments, and brain properties, all done in an integrative fashion and across lifespan. Unlike clinical (case-control) studies, population neuroscience does not focus on patients. An ideal (i.e., representative) sample includes a mix of healthy individuals, individuals in preclinical stages of a disease and those with a fully blown disorder, with numbers corresponding to the population prevalence of different conditions (and their antecedents) at a given age. Broad sampling of environments and genomes is essential for identifying the key influences shaping the brain capacities (and health) under different circumstances.

The usefulness of various data sets for answering certain questions is determined not only by what was acquired but also by who participated in the study. Ascertainment (to 'ascertain' is to discover or to determine) refers to the way we identify individuals for the purpose of recruiting them into a given study. Ascertainment bias refers to a sampling (or selection) bias. Such a bias is more likely to occur in case-control studies (for example, patients with a certain disease versus healthy controls) but may be present also in population-based studies recruiting participants in the community. In all cases, it is crucial to document (and report) recruitment strategies, target populations and, if possible, response rates and characteristics of those who declined to participate (or who might have been excluded by investigators).

To date, most of the population-neuroscience work has been done in high-income countries, which of course carries limitations in generalizing across cultural contexts, and has been carried out through observational studies. This is the case for the great majority of sites participating in the Enhancing Neuroimaging Genetics through Meta-Analysis (ENIGMA) consortium¹⁵¹ and the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium¹⁵². These two consortia brought together various studies employing brain imaging and genetics in disease-oriented research carried out mostly in North America, Europe, Australia and Japan.

Many of the population-based studies have combined MR imaging with detailed assessments of various environments, including internal milieu (for example, plasma levels of hormones, lipids, micronutrients), physical (for example, air pollution) and social environment (for example, social structure of the family and neighborhood), as well as previous experiences (for example, stressful life events). Assessing 'external' environments is challenging. Asking a series of questions using a standard survey is the most common way for collecting information about the individual's physical and social environment (for example, <https://www.phenxtoolkit.org/index.php>). One can also use aggregate data (collected by local, state or federal governments) to characterize physical and social environments in which an individual spends most of his/her time (for example, home or workplace). This can be done using geographic information systems that allow researchers to map data in space and time, providing multiple layers of information about the individual's physical and social environment at a given time point (for example, <http://www.esri.com/>). Furthermore, the emergence of tools such as Google Street View and social media, including Twitter and Facebook (see Odgers *et al.*¹⁵³ for an example), provides new opportunities for characterizing physical and social environment at an aggregate level.

In addition to being scanned and assessed in a number of domains (for example, cognition, mental and cardiometabolic health), cohort participants provide a blood sample that can be used subsequently in genome-wide genomic, transcriptomic and epigenomic analyses. It is notable that proper acquisition, processing and storage of blood and its derivatives (for example, plasma, serum, blood cells) provide a rich source of biological material to be used with technologies often unavailable when a study started (for example, whole-genome sequencing, derivation of inducible pluripotent stem cells). To some extent, this is also true for imaging data: new image-analysis algorithms may be used to derive new phenotypes from old images.

Let us finish this section with one example of integrating system-level and molecular phenotypes derived, respectively, from *in vivo* (MRI) and *ex vivo* (gene expression) studies. For many years, multimodal integration of various structural and functional features of the human brain has been enabled by the use of a common reference system¹⁵⁴. This mapping principle has been used to bring gene-expression data contained in the Allen Brain Atlas into a FreeSurfer-based anatomical parcellation of the cerebral cortex¹⁵⁵. This allowed us, for example, to show that regional differences in cortical thickness associated with early cannabis use follow a regional gradient in the expression of *CNR1* (ref. 156).

corpus callosum as measured using a modified variant of the NODDI model (Box 1 and Fig. 4) and electron microscopy⁷⁴. Combining quantitative magnetization transfer with multishell diffusion imaging, Stikov and colleagues showed that the g-ratio, the ratio of the inner to outer diameter of the myelin sheath, could be estimated accurately as compared against electron microscopy⁷⁵. These examples illustrate that the field is moving ever closer to accurate estimates of direct measures of neural morphometry, though clearly much more work is needed to both establish the levels of accuracy and robustness of existing measures and to extend into new biology-based indices. We need to keep in mind that an indirect mapping or inference is necessary to go from what we measure to tissue biophysical properties, to avoid over-interpretation of results⁷⁶.

An expensive motion detector? MRI artifacts and caveats

MRI is not a simple picture of the brain, which has advantages—namely, the multiple contrasts that can be obtained by different pulse sequences—but also leads to a series of possible artifacts that can

confound easy interpretation of results and, at worst, even lead to entirely incorrect conclusions. The ubiquitous and varied nature of artifacts in MRI stems from the fundamental physical difficulty of manipulating the magnetic field (B_0) to be either uniform in space or form linear intensity ramps and manipulating radio-frequency (RF) fields (B_1) to be uniform in space. Furthermore, these fields are required to be rapidly, accurately and reproducibly modulated in time to encode the image. These requirements are compounded by the fact that data encoding occurs in the spatial frequency domain, resulting in MR artifacts having quite different characteristics than one might encounter in a camera or other imaging system. Additionally, even subtle intersubject differences, such as hydration levels⁷⁷ or time of day⁷⁸ influence outcomes. Lastly, the image processing, particularly the spatial normalization, used to derive the metrics that go into the ultimate statistical analyses also come with potential confounds. Thus, when performing a morphometry study it is critical to identify the potential sources of artifacts and explicitly investigate whether they may be the underlying source of a perceived biological effect when confounds segregate with predictors

Box 3 Developmental structural neuroimaging

Our first major longitudinal insight into the dynamics of *in vivo* human brain development came from a structural neuroimaging study¹⁵⁷, which revealed that the maturational trajectory of human gray matter volume follows a curvilinear ‘inverted U’ rather than showing a linear progression to adult values. Since this seminal study there has been a steep climb in the number of large-scale longitudinal structural neuroimaging data sets¹⁵⁸ and associated research reports detailing the spatiotemporal heterogeneity of neuroanatomical maturation within the brain^{16,159} and charting how the dynamics of structural brain development can vary as a function of genetic¹⁶⁰, environmental¹⁹, demographic (for example, biological sex¹⁵⁹), cognitive¹⁶¹ and clinical¹⁶² factors. Several special opportunities and challenges of structural neuroimaging are brought to the fore by developmental applications and deserve special mention.

First, advances in automated image analysis mean that a vast number of morphological estimates can now be extracted from any given structural neuroimaging scan. For example, modern methods for cortical morphometry measure cortical thickness, surface area, volume, curvature and sulcal depth relative to the brain hull at tens of thousands of points across the cortical mantle in a single scan. It has become clear that these diverse metrics follow distinct developmental trajectories in health¹⁶³, which reflect non-overlapping sets of genetic and environmental influences¹⁸ but can be interrelated in a spatiotemporally specific manner¹⁶⁴. These normative observations carry major consequences for the optimal design of structural neuroimaging analysis in clinical populations, because conclusions regarding the presence and regional distribution of cortical abnormalities in a given genetic disorder can vary greatly across different morphometric features¹⁵⁹.

Second, any effort to localize anatomical changes that segregate with a demographic or clinical variable of interest needs to be balanced against the recognition that regional variations in brain size and shape are not independent from each other or from variations in overall brain size. From a statistical perspective, this observation raises the question about how to best ‘account for’ total brain size when analyzing a region of interest (ROI); this has traditionally been addressed by either proportionalizing ROI measures or including total brain volume as a linear covariate when modeling variation in ROI anatomy. However, a number of recent studies^{165,166} have underlined that relationships between ROI size and overall brain size are often profoundly nonlinear and have shown how the inability of traditional methods to control for these nonlinearities can lead to false inferences regarding the presence and location of regional brain changes. The importance of using an allometric framework in analysis of morphometric data is especially pronounced when studying groups that differ markedly in overall brain size due to demographic (e.g., males versus females) or clinical (for example, neurogenetic syndromes versus controls) variables¹⁶⁶.

Third, in addition to raising important analytic challenges, population-level patterns of neuroanatomical covariation represent a special window into brain organization that is uniquely provided by structural neuroimaging data and offers new ways of detecting brain changes secondary to developmental or disease-related processes. Thus, patterns of structural covariance are known to vary over childhood and adolescence⁵¹, track interindividual differences in cognitive ability¹⁶⁷, be altered by disease processes¹⁶⁸ and recapitulate patterns of maturational coupling¹⁶³, interregional connectivity and coordinated functional activation within the human brain^{169,170}. Crucially, normative patterns of anatomical covariation in the human brain appear to constrain the spatial distribution of disease effects^{168,171}, which advances our ability to assay and interpret structural neuroimaging phenotypes in clinical populations.

Fourth, advances in image acquisition and image processing allow us to probe very early brain development. 3D *in utero* imaging has to cope with large rotations of the fetus during the scans. By taking multiple stacks of 2D slices, where each slice is acquired fast enough to negate most motion, and then accounting for motion between slices, a 3D image can be reconstructed¹⁷². Furthermore, rapid myelination and similar maturational changes in the developing brain result in MR contrast changes (i.e., T₁ and T₂) throughout the first 24 months of life. Image processing algorithms thus have to adapt to these changing contrasts¹⁷³. Lastly, premature and very prematurely born subjects are also providing new insights into early brain development without the constraints of *in utero* acquisitions¹⁷⁴. These populations can furthermore capture effects of altered environments on early brain development¹⁷⁵.

(i.e., patients moving more than controls), so as to not end up using MRI as an overpriced motion detector.

Subject motion. The most common potential confound in morphometry studies is subject motion. While the fact that subject motion can contaminate or even induce MRI findings has been known for decades, the subtle and pernicious nature of motion confounds has only recently been quantified. The heightened concern regarding motion related biases in neuroimaging first emerged in the context of functional neuroimaging data⁷⁹ and then rapidly extended into the structural neuroimaging community^{80,81}. Accounting for subject motion is fundamentally difficult because (i) acquisition is performed in the spatial frequency domain, so the effects of motion on the reconstructed image will vary depending on the timing, direction and amount of motion, and (ii) in the vast majority of group studies the amount of motion will be correlated with the effect we are trying to study (for example, elderly subjects move more than middle aged; subjects with more advanced diseases move more than those earlier in the course; subjects may move differently with medication). Furthermore, it has been shown⁸⁰ that motion changes the information content of the images in the direction we would typically expect in an atrophy study: more motion induces an apparent reduction in gray matter that is difficult to distinguish from true atrophy. For diffusion MRI-derived microstructure estimates,

motion can also induce spurious differences between groups, even in cases of comparing groups with control subjects only, when no differences are expected⁸². These phenomena pose special challenges for the collection, analysis and interpretation of developmental neuroimaging data given that (i) motion is nonrandomly distributed with respect to age (children move more than adults), sex (males move more than females) and clinical status⁸³, and (ii) motion-induced biases are prominent in brain regions (for example, prefrontal cortices) that are notable for displaying anatomical differences as a function of age, sex and disease status⁸¹. Indeed, the adoption of approaches to excluding scans based on motion artifacts impacts conclusions regarding trajectories of neuroanatomical change in brain development¹⁵.

Approaches for addressing motion-related confounds in neuroimaging can be divided into *post hoc* strategies that attempt to minimize the impact of motion on already gathered images and prospective strategies that seek to reduce motion contamination during image acquisition. The most basic *post hoc* strategy is to estimate the amount of motion in each scan and then exclude any scans that exceed a set motion cut-off and/or match motion magnitude in individuals across groups. This of course can be inefficient as it sometimes requires discarding one's best data and can rule out certain designs. Estimates of in-scanner motion could potentially also be used as regressors in statistical analysis in an attempt to ‘control’ for motion effects⁸².

For diffusion MRI, in which multiple measurements are obtained for each voxel, motion can blur the estimated maps due to misalignment of the measured volumes. Furthermore, subject motion can be difficult to estimate, as it interacts with hardware-related artifacts (eddy currents) and field-inhomogeneity distortions (see next section). However, recent frameworks offer improved and robust performance in detecting and correcting these artifacts (for example⁸⁴). After having estimated motion and artifacts, one can detect outlier data⁸⁵ and either downweight them or replace them with average predictions obtained from nonoutlier measurements to minimize bias and effects on subsequent analysis⁸⁶.

In contrast to these *post hoc* approaches, prospective strategies seek to detect and account for subject motion during the acquisition itself. Approaches to motion detection and correction take two basic forms: (i) the detection is provided by an external tracking device or (ii) the MRI signal itself is used to track motion. The latter can be implemented directly⁸⁷ or through the use of a short-duration 'navigator' sequence embedded within the structural or diffusion scan in order to detect and account for motion. Examples of the former include cameras with reflective markers, RF markers, magnetic markers and inertial navigation systems^{88–90}. The downside of marker- and sensor-based systems is that the marker or sensor is typically attached to the skin, which may not move rigidly with the brain. Attachment to the upper teeth is more reliable but requires special expertise to set up and assumes that the patient has teeth. An alternative system uses facial geometry itself as the tracked pattern⁹¹. Camera-based systems require a clear line-of-sight to the subject's head.

An appealing alternative to the hardware and subject preparation involved with external tracking systems is the use of the MR scanner itself to track and remove motion effects^{92–94}. If additional navigators are used in the sequence, they may increase scan time but are sometimes inserted in the 'dead time' present in many MR sequences during which the scanner is idle while magnetization evolves (either decays or recovers). These methods typically track motion on a slower time-scale than external tracking systems but require no special setup.

Field inhomogeneities. B_0 (main magnetic field) inhomogeneities and susceptibility regions. Ideally, the main magnetic field (B_0 field) should be spatially uniform. Spatial information is encoded by applying linear ramps in the magnitude of the field that vary with position. In practice, the initially uniform field is also modified by the presence of the human subject. Magnetic susceptibility is the material property that describes the amount of magnetization of a material relative to the applied magnetic field. Brain tissue is weakly diamagnetic, dispersing the magnetic field, whereas air, due to the oxygen content, is slightly paramagnetic, concentrating the magnetic field. Some regions of the brain are close to air spaces, such as the inferior frontal region (near the sinuses) and the lateral temporal regions (near the air canals). Shimming is the process of applying additional spatially tailored magnetic fields to compensate for the variation in the magnetic field in these regions. Since the fields generated by the shim coils are fairly smooth, they cannot compensate fully for field inhomogeneity in regions of sharply changing susceptibility. **Figure 5** shows how the remaining inhomogeneity results in geometric distortion in the image. In non-EPI (echo planar imaging) acquisitions (such as T_1 -weighted and T_2 -weighted), the distortion is in the readout direction, whereas in EPI acquisitions (such as in diffusion MRI) it is along the phase encode direction. As a result of these geometric distortions, the cortex is shown to be compressed or stretched depending on the polarity of the readout (phase-encode for EPI) gradient or, equivalently, the sign of the field offset (**Fig. 5c,d**). The magnitude of the distortion scales inversely with the readout bandwidth (echo spacing in EPI).

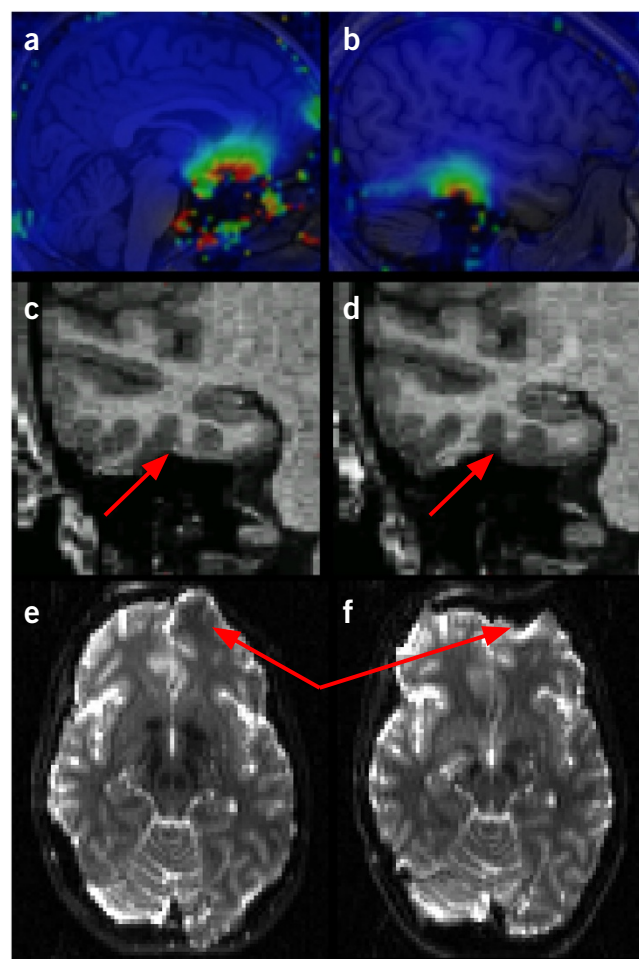


Figure 5 Acquisition parameter effects on anatomical readouts. From left to right, B_0 field maps in (a) orbitofrontal and (b) lateral temporal regions showing large inhomogeneities in the magnetic field. (c,d) FLASH scans in which the polarity of the readout gradient is changed from (c) positive to (d) negative, changing the direction of the distortion. Red arrow: changing the polarity of the readout gradient results in no apparent cortex in c but quite thick cortex in d. (e,f) The effect is even more dramatic when changing the polarity of the phase encode direction in diffusion images; red arrows point to dramatic alterations in frontal cortex topology. Data acquired with informed written consent; all studies were approved by the Partners Human Research Committee.

Gradient nonlinearities. Designing a gradient system with high gradient strength and high slew rate (peak gradient strength divided by time to reach that strength), while maximizing spatial linearity and minimizing eddy (i.e., swirling) currents, is an engineering challenge. Linearity is always compromised to some extent. Gradient nonlinearity results in geometric distortion of the images and is the same for all imaging sequences. Since nonlinearity is part of the gradient system design, it does not vary with the patient and, given detailed information about the gradient design, can be corrected on the scanner or offline⁹⁵.

Chemical and/or fat shift. Magnetic spins precess (change orientation of the rotational axis) at a rate proportional to the magnetic field that they experience, and the precession frequency encodes the position of the tissue containing the spins. It is most commonly the hydrogen nuclei (protons) in water that are imaged in biological tissue. Water protons precess at a higher frequency than protons in lipids. This difference in precession frequency is called the chemical shift.

Since the scanner is tuned to hydrogen nuclei in water, hydrogen nuclei in lipids resonate at the ‘wrong’ frequency, i.e., the frequency of the received signal does not correctly reflect the spatial origin of the source. The result is that the fat signal is shifted in the image relative to the water signal. Just as in regions of B_0 field inhomogeneity, the fat shift is inversely proportional to the readout bandwidth. With lower bandwidths, orbital fat or scalp fat may shift sufficiently to overlap with cortex, confounding morphometry studies. The fat signal may be ameliorated through the use of narrowband or composite ‘water excitation’ RF pulses that excite only the water spins and not the fat spins, or through the use of saturation pulses that suppress the fat signal.

RF (B_1) inhomogeneities. RF pulses provide the energy that perturbs the spins, which then emit this energy during the relaxation process. The energy experienced by an ensemble of spins is expressed as the ‘flip angle’, because it describes the resulting magnetization in terms of a vector with transverse and longitudinal components (perpendicular and coaxial with the B_0 field, respectively). Ideally all the spins within the receive coil should experience the same transmit field (B_1+) during an RF pulse. In practice, the B_1+ field is not perfectly homogeneous, and the inhomogeneity is exacerbated at higher field strengths where the RF wavelength is closer to the scale of the body region being imaged⁹⁶. In the head, this effect leads to brightening of the center of the image. This corresponds to varying flip angle across the head. Note that the result is not simply a scaling of the image intensity but rather a variation of contrast across the image if multiple tissue types are present. Therefore a simple multiplicative intensity normalization cannot completely correct for this effect. One approach is to map relaxation parameters, taking into account the B_1+ map⁹⁷. Achieving a uniform B_1+ field in the first place is ideal, and B_1+ uniformity can be improved with parallel transmit methods, in which the contributions of multiple transmit coils are combined to achieve a uniform resultant field⁹⁸. Since the combination is phase sensitive and interference can be constructive, great care is necessary to avoid creating RF energy ‘hot spots’.

Once transmitted, RF energy is subsequently emitted from the imaged object during the spin relaxation process. This energy is captured by the receive coils as measured signal and is used to form an image. Multiple receive elements enhance the SNR of the received signal and enable acceleration of image acquisition. However, multichannel receive coils result in an inhomogeneous RF receive field (B_1-). This inhomogeneity results in a scaling of the image intensity and can be corrected with a B_1 receive map. Optimally the correction should be done by weighting the contributions from each coil element with the coil sensitivity profile before forming the combined image⁹⁹.

Dura. The human brain and spinal cord are enveloped by three membranes known collectively as the meninges. Of these three—the dura mater, the arachnoid mater and the pia mater—it is the thick dura that can corrupt estimates of cortical volume and thickness. While the dura has MR characteristics that are quite different than gray matter, by unfortunate coincidence the T_1 , T_2 and proton density (PD) values of the dura results in it being close to isointense with cortical gray matter in many typical T_1 -weighted structural imaging sequences. This fact, combined with the close proximity or even physical adjacency of the dura to the cortex, makes it difficult or impossible to avoid having the dura contaminate estimates of cortical thickness and volume from only a T_1 -weighted MRI. In **Figure 6** one can see many locations where dura is close enough to cortical gray matter that it can be difficult or impossible to resolve the tissue between them and avoid labeling the dura as gray matter. This can lead to substantial overestimation of cortical thickness volume and, more importantly, to significant overestimation of atrophy rates in neurodegenerative diseases when the

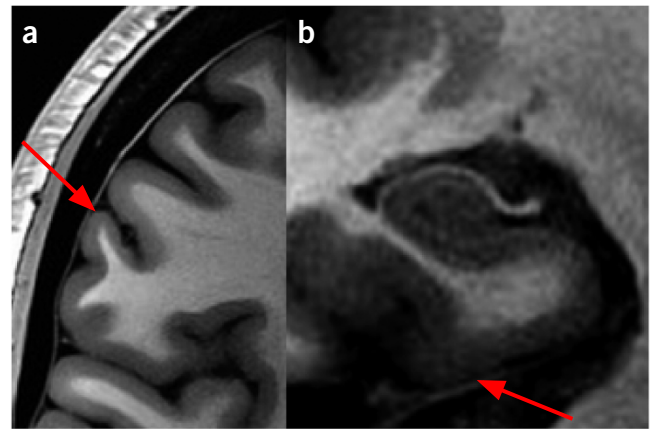


Figure 6 The dura and the cortex. $380\ \mu\text{m} \times 380\ \mu\text{m} \times 1\ \text{mm}$ image (average of 7 scans of 9 min each, motion corrected $T_1 = 900\ \text{ms}$; $\text{TR} = 2,250$, $\text{TE} = 4.35$, flip = 9° ; 3T; 32-channel array) highlighting the dura and its proximity to (a) superior lateral cortex and to (b) entorhinal cortex.

retraction of the gray matter allows the dura to be differentiated from cortex in patients while it is not differentiated in healthy controls.

Fortuitously, dura has short magnetic decay properties T_2 and T_2^* , and thus even a modest increase in echo time can allow it to be visually distinguished from adjacent gray matter. Unfortunately simply lengthening echo time (TE) is rarely an optimal strategy for a T_1 -weighted scan as gray–white contrast reduces monotonically with increasing TE. A better approach is to either use multiecho acquisitions¹⁰⁰ where T_2^* information is encoded in the multiple echoes, or to acquire a bandwidth-, readout- and/or geometry-matched T_2 -weighted scan in which the dura will be significantly darker than the cortex.

Spatial normalization and image processing. After acquisition is complete, image processing pipelines both extract key metrics and identify corresponding spatial mappings between subjects, as described above and illustrated in **Figures 2** and **3**. While accumulation of computational errors adds noise, it is in the identification of spatial homology that problems can in particular arise. At coarse spatial scales, as well as usually subcortical regions, correspondence between subjects is readily identified and computed. The complex folding patterns of the cerebral cortex and (with sufficient resolution) the cerebellar cortex present a more fundamental problem, in that there is no 1:1 homology between any two cortices. The sulcal patterns vary from cortex to cortex. To give just one example, some subjects have a single-branched collateral sulcus in the medial temporal lobes, about one-third have two branches to the sulcus and about one-fifth of subjects have an interrupted sulcus¹⁰¹. Furthermore, the branching pattern is related to volumes of surrounding regions¹⁰¹. When such diverging branching patterns exist between subjects—and they exist across many cortical sulci—then it is usually not clear how to the correct point-to-point matching between subjects is, never mind how to create a correct computational algorithm. This is particularly a problem with volumetric registrations and therefore with VBM¹⁰².

There are multiple approaches to addressing misregistration problems. The first is to acknowledge that registration issues present one form of signal¹⁰³ (i.e., a group difference in gray matter density might be due to differential cortical folding rather than true gray matter amounts), and thus a finding in VBM provides an indication of where to look in more detail at anatomical alterations. Second, explicitly aligning cortical folding patterns significantly improves localization.

The best illustration for this improvement is in the cross-subject correspondence of Broca's areas (cortical subdivisions based on neuron types and distributions invisible on MRI) after either volumetric or surface-based registration, with the surface-based algorithm considerably improving colocalization¹⁰⁴. Even surface-based registrations cannot completely account for differential folding patterns (where the true solution is not even known), and thus the third approach is to explicitly map sulcal shapes. The best of known suite of algorithms for sulcal identification and study is in BrainVISA¹⁰⁵. Here sizes, location, branching patterns, etc., can be studied and compared across groups or other metrics of interest. Ultimately, these methods are complementary, and as with acquisition artifacts, understanding the role of possible confounds on final outcomes is key in best advancing science using structural brain imaging.

Structural imaging: current possibilities

Advances in ultrahigh field strengths (for example, 7T) offer a route for increased baseline signal (and therefore SNR). This comes at other costs, particular for acquisitions that are affected by shorter T_2 relaxation times, worse RF field homogeneity, greater RF power deposition and increased magnetic susceptibility and potential for distortions⁹⁶. In particular, methods such as diffusion and fast acquisition are challenging to implement robustly at higher field. Nevertheless, establishing the right balance between all these factors allows data of very high quality and high spatial resolution^{106,107}. Ultrahigh fields can also have transformative impacts for certain modalities that carry limited information at lower fields. Quantitative susceptibility mapping is an example, with increased contrast at 7T enabling whole-brain quantitative susceptibility mapping⁴⁹ and higher spatial resolution providing iron deposition imaging even in small subcortical nuclei¹⁰⁸.

At the same time, multichannel receive coil arrays allow for higher SNR and simultaneous acquisition of more than one slice. The volume extent to be imaged can be separated into a set of equally spaced slices, each driving a high signal in a unique subset of coil elements. The slices can then be acquired simultaneously and the spatial sensitivity of each coil element can be used to separate the signal from different slices. Such simultaneous multislice (or multiband) acquisitions^{109,110} reduce the time required to scan brain volumes and currently permit 2- to 5-fold acceleration of diffusion MRI scan time¹¹¹, translating to higher spatial and/or angular resolution and/or SNR per unit time. Multiband acquisitions have been key in improving data quality in different projects (and contexts), such as the adult Human Connectome Project⁶, UK Biobank⁵ and the developing Human Connectome Project¹¹².

Modern clinical scanners are equipped with gradient systems that can deliver greater spatial magnetic fields gradients G_{\max} (~80 mT/m versus 40 mT/m). In diffusion imaging, higher gradient strength leads to greater signal contrast in terms of the signal change for a given displacement of water due to diffusion. In addition, experiments with precise control over the gradient strength and duration (collectively pooled in the parameter q) can permit measurement of small differences in diffusion displacement that may enable estimation of specific microstructural features of tissue. Furthermore, tight head-gradient sets allow fast switching of the gradients (i.e., faster 'slew rates') compared to whole-body gradient systems. These features collectively allow shorter and stronger gradient pulses for diffusion preparation, which are beneficial in a number of ways, including higher SNR, spatial resolution and angular contrast, and therefore more accurate q -space measurements that technically require infinitely short gradients. Human MR systems have been recently developed with ultrahigh G_{\max} , such as the Connectome Skyras, which uses 100 and 300 mT/m^{96,113},

allowing very high data quality^{114,115}. Such hardware capabilities have been very beneficial in mapping microstructure in animals using small-bore systems, and the potential to translate these advantages into human studies has recently been demonstrated^{116,117}.

The future. Advances in acquisition, image processing and modeling will assuredly keep structural imaging at the forefront of understanding brain-behavior relations and how they are altered in development, aging and disease.

A primary focus for diffusion MRI research is in microstructural models that go beyond tensor-derived metrics, such as FA, which are inherently nonspecific. For instance, a reduction in FA has been associated with loss of structure and/or 'integrity', while an increase in FA can be indicative of degeneration¹¹⁸. In regions with complex fiber patterns, selective degeneration of one fiber population can lead to an apparent increase in structural coherence; what is left looks macroscopically more 'organized'.

More complex biophysical models along with sophisticated measurements can more precisely explain the measured signal¹¹⁹ and aid interpretation by providing specific markers of microstructure changes^{120–122}. For example, the diffusion signal measured at a range of q -values (gradient areas) exhibits a characteristic diffraction pattern in which zero-crossings indicate the size of restricted compartments¹²³. Estimation of microscopic features, such as compartment shape and size, using these diffusion measurements have been demonstrated on nonbiological model systems using high-performance scanners¹²⁴. This approach might ultimately enable estimation of mean axon diameters from measurements made perpendicular to a perfectly coherent white matter fiber bundle. In practice, however, such a diffraction pattern cannot be measured with the standard (single-pulse) diffusion sequences or using current hardware available on human scanners. Moreover, these models typically neglect the heterogeneity of fibers within a voxel, which can drive similar signal properties to those of compartmental shape. A recent method¹²⁵ aims to estimate per-axon diffusivities and anisotropy (microscopic anisotropy), which are inherently free from orientation homogeneity and heterogeneity assumptions and focus solely on microscopic features of interest. Changing the gradients used for diffusion encoding, primarily involving double-diffusion-encoding approaches such as double-pulse diffusion sensitization or oscillating gradients, offer alternative approaches for estimating compartment shape and size, as diffraction measurements of zero-crossings are robust to heterogeneities of the imaged system^{124,126,127}. Such sequences are also promising to better characterize restricted diffusion and to probe anisotropy in gray matter voxels or complex crossing white matter bundles (for example^{128–130}; and see **Box 1** and **Fig. 4**). They can even capture diffusional water exchange, a cell membrane permeability-dependent parameter¹³¹. While these various methods have demonstrated remarkable potential in model systems, translation into *in vivo* human imaging remains limited by the hardware and measurement techniques available on human MRI scanners, though this is rapidly improving (for example^{125,131}) and will likely provide exciting breakthroughs in the future. These improvements in diffusion imaging techniques will furthermore assist with better tractography, thereby enabling improved localization of the signals origins.

We face an exciting future for the study of neuroanatomy as methods advance, artifacts are understood and their impacts reduced. Our understanding of how the brain changes, be it across development or aging, in response to novel environments or cognitive challenges, or in disease, is still rudimentary. Combining estimates of volumes, thickness, etc. from high-resolution anatomical images with multiple

microstructure modalities, including double-diffusion-encoding approaches, magnetization transfer and susceptibility-weighted imaging, and quantitative T_1 and T_2 mapping, is especially promising. Combining *in vivo* human imaging with experimental models, with all their genetic and molecular tools, will also provide novel insights. Ultimately, we will come closer and closer to not just mapping what is happening where but to understanding the underlying molecular and cellular bases of our signals.

Also, brain structure provides a common framework to unify multiple neuroscience investigations. Recent years have seen a proliferation of spatially comprehensive and publicly available maps of human brain organization, which are all anatomically grounded but together encompass a vast array of phenotypic dimensions. Salient examples include spatial maps of gene expression (for example, the Allen Brain Atlas, at <http://www.brain-map.org/>), cytoarchitectonics (http://www.fz-juelich.de/JuBrain/EN/_node.html) and cognitive associations (<http://neurosynth.org>). The diverse modalities represented by these maps emphasize the fact that structural neuroimaging provides just one of many ways of modeling the brain and also reinforces the fundamental importance of anatomy as the common spatial framework within which all other phenotypic properties of the brain are embedded¹³². This broad point is well illustrated by several relatively recent studies that use structural neuroimaging data to calculate physical distances between different brain regions and then demonstrate that these distance-based representations of the brain can predict spatial patterns of functional connectivity, metabolism, gene expression and cognitive specialization within the human brain^{133,134}. Thus, the *in vivo* measures of brain organization provided by structural neuroimaging are not only highly informative and discriminative phenotypes in their own right but also describe the basic anatomical scaffold within which our brains evolve, develop and operate.

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AUTHOR CONTRIBUTIONS

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