NeuroImage 129 (2016) 185–197

Contents lists available at ScienceDirect

NeuroImage

journal homepage: www.elsevier.com/locate/ynimg

Comparison of 3D orientation distribution functions measured with confocal microscopy and diffusion MRI

Kurt Schilling [a,b,*], Vaibhav Janve [a,b], Yurui Gao [a,b], Iwona Stepniewska [c], Bennett A. Landman [a,b,d], Adam W. Anderson [a,b]

[a] Vanderbilt University Institute of Imaging Science, Vanderbilt University, Nashville, TN, USA
[b] Department of Biomedical Engineering, Vanderbilt University, Nashville, TN, USA
[c] Department of Psychology, Vanderbilt University, Nashville, TN, USA
[d] Department of Electrical Engineering, Vanderbilt University, Nashville, TN, USA

ARTICLE INFO

Article history:
Received 9 October 2015
Accepted 11 January 2016
Available online 21 January 2016

Keywords:
Diffusion MRI
Validation
Fiber orientation distribution
DTI
Crossing fibers

ABSTRACT

The ability of diffusion MRI (dMRI) fiber tractography to non-invasively map three-dimensional (3D) anatomical networks in the human brain has made it a valuable tool in both clinical and research settings. However, there are many assumptions inherent to any tractography algorithm that can limit the accuracy of the reconstructed fiber tracts. Among them is the assumption that the diffusion-weighted images accurately reflect the underlying fiber orientation distribution (FOD) in the MRI voxel. Consequently, validating dMRI's ability to assess the underlying fiber orientation in each voxel is critical for its use as a biomedical tool. Here, using post-mortem histology and confocal microscopy, we present a method to perform histological validation of orientation functions in 3D, which has previously been limited to two-dimensional analysis of tissue sections. We demonstrate the ability to extract the 3D FOD from confocal z-stacks, and quantify the agreement between the MRI estimates of orientation information obtained using constrained spherical deconvolution (CSD) and the true geometry of the fibers. We find an orientation error of approximately 6° in voxels containing nearly parallel fibers, and 10–11° in crossing fiber regions, and note that CSD was unable to resolve fibers crossing at angles below 60° in our dataset. This is the first time that the 3D white matter orientation distribution is calculated from histology and compared to dMRI. Thus, this technique serves as a gold standard for dMRI validation studies — providing the ability to determine the extent to which the dMRI signal is consistent with the histological FOD, and to establish how well different dMRI models can predict the ground truth FOD.

Introduction

Diffusion magnetic resonance imaging (dMRI) has become a mainstay in neuroimaging studies due to its ability to provide, noninvasively, unique biological and clinical information about tissue composition, microstructure, and architectural organization (Basser and Pierpaoli, 1996; Beaulieu, 2002). Of particular interest is the ability to estimate the distribution of neuronal fiber orientations in each voxel from a set of diffusion measurements, an object often referred to as the fiber orientation distribution (FOD). By following these fiber orientation estimates from voxel to voxel throughout the brain, intricate maps of brain connectivity can be created. This process of mapping brain connectivity using dMRI data has been termed "fiber tractography" (Mori et al., 1999; Mori and van Zijl, 2002), and has been used in applications ranging from delineating brain networks (Hagmann et al., 2007), to studying the changes associated with disease (Kitamura et al., 2013; Koenig et al., 2015), psychiatric disorders (White et al., 2008), and traumatic brain injury (Shenton et al., 2012).

Diffusion tensor imaging (DTI) was the first MRI method to allow mapping of fiber orientations throughout the brain (Basser et al., 1994), and remains the most common. However, this uni-modal Gaussian diffusion model is known to be inadequate for characterizing diffusion in voxels with complex fiber structure (Wiegell et al., 2000) and has been shown to lead to erroneous tractography results. A number of methods have been introduced to address this "crossing fiber" problem (Anderson, 2005; Assaf and Basser, 2005; Behrens et al., 2007; Behrens et al., 2003; Descoteaux et al., 2007; Jansons and Alexander, 2003; Ozarslan et al., 2006; Tournier et al., 2004; Tuch, 2004; Tuch et al., 2002; Wedeen et al., 2005). Typically, these

Abbreviations: dMRI, diffusion magnetic resonance imaging; FOD, fiber orientation distribution; DTI, diffusion tensor imaging; ODF, orientation density function; WM, white matter; GM, gray matter; CSD, constrained spherical deconvolution; SH, spherical harmonics; PSF, point spread function; ABA, adaptive bases algorithm; PPD, preservation of principal directions; SNR, signal to noise ratio.

* Corresponding author at: Vanderbilt University Institute of Imaging Science, 1161 21st Avenue South, Medical Center North, AA-1105, Nashville, TN 37232-2310, USA.
E-mail address: kurt.g.schilling@vanderbilt.edu (K. Schilling).

http://dx.doi.org/10.1016/j.neuroimage.2016.01.022
1053-8119/

approaches solve for the fiber orientation by estimating the FOD or the orientation density function (ODF) — another spherical function, which reflects the relative number of spins that have diffused in any given direction. Tractography algorithms then exploit local peaks in the FOD or ODF to propagate tract streamlines.

While these techniques have produced improvements in white matter tractography, there has been no clear consensus on a "gold standard" for validating the underlying orientation distributions. The most common method to date has been validation using synthetic data (Alexander, 2005; Sakaie and Lowe, 2007). However, these simulations rely on assumptions and approximations to generate the modeled MR signal, and are likely to be inadequate for validation in the living brain. Physical phantoms can be used to provide more realistic experiment conditions (including artifacts inherent to dMRI) and allow control of the ground truth orientation distribution. Yet, these capillary-based (Lin et al., 2003; Yanasak and Allison, 2006) or synthetic fiber-based (Farrher et al., 2012; Fieremans et al., 2008; Perrin et al., 2005) phantoms can still fail to replicate the structural characteristics typical of neuronal tissue, including axon diameter, membrane permeability, and most importantly, the enormous geometric complexity seen in the central nervous system.

To overcome these limitations, several studies have validated orientation measures using post-mortem histology. From stained tissue sections, techniques such as manual tracing (Leergaard et al., 2010), structure tensor analysis (Seehaus et al., 2015), and Fourier analysis (Choe et al., 2012) have been used to quantify the histological FOD. However, two potential disadvantages have plagued histological validation studies to date. First, many have been limited to two-dimensional (2D), in-plane analysis of tissue sections. Thus, they rely on tissue sectioning in a plane parallel to the direction of fibers, and analysis is restricted to only those fibers oriented in that plane. Recently, this limitation of validation studies has been circumvented through the use of confocal microscopy (Jespersen et al., 2012; Khan et al., 2015) and optical coherence tomography (Wang et al., 2015). However, no method has been presented which characterizes the full fiber orientation distribution in white matter voxels, but rather recent studies estimate a single dominant orientation in areas equivalent in size to an MRI voxel (Khan et al., 2015; Wang et al., 2015), or determine the orientation distribution in axons and dendrites of the cerebral cortex (Jespersen et al., 2012). Second, comparing MRI and histology is often done through manual alignment (Khan et al., 2015; Leergaard et al., 2010; Seehaus et al., 2013) of the data, which is prone to error and can lead to geometric mismatch and a bias in the validation results. Consequently, there is a need for a method to compare dMRI estimates of white matter pathways to direct measurements of axonal orientations on a voxel-by-voxel basis — one which allows three-dimensional (3D) analysis and addresses accurate, reproducible registration.

In this study, using post-mortem histology and confocal microscopy, we develop an approach to extend histological validation of orientation functions to 3D. We also describe appropriate alignment and orientation of the histological data to MRI data. The intention of this work is not a comprehensive validation of the strengths and weaknesses of the various dMRI algorithms, nor determination of optimal acquisition parameters. Rather, the focus is on the technique itself, which represents an advance in the development of a "gold standard" for the purposes of validating fiber orientation information. We begin with an in-depth description of the method, which employs a common image processing technique – structure tensor analysis – in order to extract the 3D FOD in areas equivalent in size to an MRI voxel. Next, we describe the sensitivity of this approach to confocal acquisition and image processing parameters. Finally, we apply this technique to both single fiber and crossing fiber white matter (WM) regions, and, as a methodological benchmark, make quantitative comparisons of the histological-FOD to the corresponding MRI-FOD derived using constrained spherical deconvolution (CSD) with the damped Richardson–Lucy algorithm (Dell'acqua et al., 2010).

Materials and methods

MRI acquisition

All animal procedures were approved by the Vanderbilt University Animal Care and Use Committee. Diffusion MRI experiments were performed on an adult squirrel monkey brain that had been perfusion fixed with physiological saline followed by 4% paraformaldehyde. Prior to fixation, the brain had undergone micro-electrode array recording experiments for an unrelated study. Due to this, there was slight atrophy in one hemisphere near the motor and pre-motor areas, resulting in asymmetric hemispheres in both histology and MRI. However, this does not impact the results of our study, which is focused on characterizing white matter structure by determining the underlying fiber orientation distribution. The brain was then immersed in 4% paraformaldehyde for 3 weeks. The brain was transferred into a phosphate-buffered saline medium for 24 h and scanned on a Varian 9.4 T, 21 cm bore magnet using a multi-shot multi-slice spin echo EPI sequence (TR = 6.7 s; TE = 42 ms; δ = 8 ms; Δ = 27 ms; max gradient strength = 30 g/cm; voxel size = 400 μm isotropic; partial Fourier = .75; NEX = 5).

A 30-direction diffusion-sampling scheme based on an electrostatic repulsion algorithm (Jones et al., 1999) was used to acquire 30 diffusion-weighted images at a b-value of 3200 s/mm^2, and 2 additional images were collected with b = 0. This set of data was used for calculating diffusion tensors using a weighted linear least squares fit. Next, a 90-direction scheme was used to acquire diffusion weighted-images at a b-value of 6400 s/mm^2, and 6 additional images at b = 0. From this dataset, the MRI-FOD was estimated using constrained spherical deconvolution with the damped Richardson–Lucy algorithm (Dell'acqua et al., 2010) and fit to 8th order spherical harmonic (SH) coefficients. MRI data processing was done using the high angular resolution diffusion imaging (HARDI) toolbox for MATLAB, available at http://neuroimagen.es/webs/hardi_tools/.

Histological procedures

After imaging, the brain was sectioned on a cryomicrotome at a thickness of 80 μm in the coronal plane and mounted on glass slides. Using a Canon EOS20D (Lake Success, NY, USA) digital camera with a zoom lens of 70–300 mm, the tissue block was digitally photographed prior to cutting every other section, resulting in a 3D "block-face" volume with a through-plane resolution of 160 μm.

The tissue sections were mounted on glass slides and stained following the procedures outlined in (Budde and Frank, 2012). Briefly, tissue sections were rinsed in PBS and dehydrated through graded ethanol solutions. The fluorescent lipophilic dye, "DiI", (1,1′-dioctadecyl-3,3,3′3′-tetramethylindocarbocyanine percholarate) in 100% ethanol (.25 mg/mL) was rinsed over sections for 1 min. The stained sections were then rehydrated through graded ethanol solutions, and coverslipped with Fluoromount-G mounting medium.

Confocal acquisition

All histological data were collected using an LSM 710 inverted confocal microscope (Carl Zeiss, Inc. Thornwood, NY, USA). For all selected tissue slices, confocal acquisition consists of two protocols: [1] creating a 2D montage of the entire tissue and [2] constructing a 3D high-resolution image in a selected region of interest. The 2D montage (Fig. 1A) consists of approximately 600–900 individual tiles acquired using a 10× air objective at a resolution of 0.80 μm^2, which are stitched together using Zeiss software, ZEN 2010. Acquisition for a single slice takes approximately 30 min. To correct for image inhomogeneity and tiling effects in the image, we found it useful to increase the zoom feature to 1.5× or higher at the expense of collecting more tiles. This 2D montage is used for image registration, and for localizing the 3D high-resolution region of interest.