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A 3D fiber model of the human brainstem

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Abstract

A new neuroanatomic approach to evaluate the fiber orientation in gross histological sections of the human brain was developed. Serial sections of a human brainstem were used to derive fiber orientation maps by analysis of polarized light sequences of these sections. Fiber inclination maps visualize angles of inclination, and fiber direction maps show angles of direction. These angles define vectors which can be visualized as RGB-colors. The serial sections were aligned to each other using the minimized Euclidian distance as fit criterion. In the 3D data set of the human brainstem the major fiber tracts were segmented, and three-dimensional models of these fiber tracts were generated. The presented results demonstrate that two kinds of fiber atlases are feasible: a fiber orientation atlas representing a vector in each voxel, which shows the nerve fiber orientation, and a volume-based atlas representing the major fiber tracts. These models can be used for the evaluation of diffusion tensor data as well as for neurosurgical planning.

Keywords: Central nervous fiber architecture; Atlas; Fiber orientation maps; Three-dimensional reconstruction; Polarized light

1. Introduction

Diffusion tensor mapping in neuroradiology allows to derive information about the three-dimensional orientation of fiber tracts in the living human brain [1]. The method is based on the preferential diffusion of molecules along the major fiber tracts, while perpendicular to the fibers diffusion is limited [2–5]. Thus, diffusion weighted magnetic resonance imaging measures the anisotropy of diffusion in the brain, which resembles the orientations of nerve fibers. Based on diffusion weighted MRI data, diffusion ellipsoids can be calculated, which represent the orientation of the major fiber tracts. Inspired by this magnificent new technique we developed a new neuroanatomic approach to evaluate the fiber orientation in gross histological sections of the human brain [6,7].

Our method is able to obtain similar information about the orientation of fiber tracts in anatomic serial brain sections as diffusion tensor mapping does but with higher magnification. This paper gives an overview of the methods used to explore these anatomical data.

2. Material and methods

The lower human brainstem (pons and medulla oblongata) taken from a 70 year old female who donated her body for anatomical study, was fixed in 4% aqueous formalin solution for at least 3 weeks and dissected carefully.

At first the brainstem was embedded in gelatine and hardened in formalin. Afterwards the specimen was sliced into four 1 cm thick slabs perpendicular to the axis of Meinert. After cryoprotecion the gelatine embedded slabs were sectioned serially at 100 μm using a cryomicrotome (HM 500 OM, Microm, Waldorf, Germany). A thickness of 100 μm was found to be optimal for estimating the 3D fiber course [7]. The serial sections were coverslipped without staining and used to derive fiber orientation maps (FOMs) as described earlier [6,7]. These serial FOMs were aligned to each other for three-dimensional reconstruction and for segmentation of the major fiber tracts of the brainstem.

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Table 1
Parameters applied for serial image alignment

Consistent matrix transformation	Cross correlation coefficient	Euclidian distance
$D = \sum_{i}^{n} \left(\frac{(I_{A_i} - I_{B_i})}{C_i} \right)$ with $C_i = \max\left(\frac{I_A}{I_B}, \frac{I_B}{I_A} \right)$	$C = \frac{\sum_x \sum_y A(x,y) * B(x,y)}{\left[\sum_x \sum_y A(x,y)^2 \right]^{1/2} * \left[\sum_x \sum_y B(x,y)^2 \right]^{1/2}}$	$\mathrm{ED} = \sqrt{\sum_{i}^{n} (I_{A_i} - I_{B_i})^2}$
Minimize	Maximize	Minimize

2.1. Acquisition

In short, polarized light is used to estimate the three-dimensional course (angles of direction and inclination) of nerve fibers in brain sections [7]. The myelin sheaths of the nerve fibers are birefringent. Light becomes plane polarized by transmission through a polarizing filter (the polarizer). The radially oriented lipids of the myelin sheaths of the nerve fibers are able to twist the light [8], so that it can pass through a second polarizing filter (the analyzer) with a polarizing plane perpendicular to the first polarizing filter.

The 258 serial sections of the brainstem sections were digitized under azimuths from 0 to 80° using two polars only (in steps of 10°). These sequences were used to estimate the angle of inclination of fibers (in the width of the sample). The same sections were digitized under azimuths from 0 to 160° in steps of 20° using a quarter wave plate additionally. The quarter wave plate is a compensator capable to impose a phase shift of 1/4 cycle on the light wave, so that all directions of the fibers from 0 to 180° can be distinguished unambiguously from each other according to that azimuth where the smallest intensity is found. Otherwise, those two directions of fibers, which are perpendicular to each other cannot be distinguished [7]. These sequences were used to estimate the angle of direction of the fibers (in the cutting plane of the sample).

The sequences were digitized using the 3CCD video camera Sony DXC-930P, which was connected to a Pentium personal computer using Windows NT (Microsoft). In this study, the magnification of the camera was adjusted that one pixel of the digital image represents a volume of $100 \times 100 \times 100\ \mu m^3$ in the sample. The settings of the imaging system (magnification, contrast, brightness) were constant throughout the study. Image processing was performed using algorithms written for MATLAB 6.0 (MathWorks Inc., Natick, MA, USA) with the Image Processing Toolbox.

2.2. Visualization of the data

The angles of inclination and direction can be visualized as two distinct gray scale images. Fiber inclination maps visualize angles of inclination from 0 to 90° and fiber direction maps show angles of direction from 0 to 180° in each pixel of the image.

To allow visualization of fiber orientation in one image those angles were transformed into unit vectors with *x*-, *y*-, and *z*-coordinates which in turn can be visualized as R-, G-, B-colors in one color image. Fibers running from left to right are shown in red, from up to down in green and from anterior to posterior in blue. This method is inspired by the visualization of diffusion tensors [2].

2.3. Automatic image alignment

Rigid (isomorphic) transformations were computed on the serial sections of the brainstem. Each image is translated and rotated in respect to its predecessor. Different methods of automatic image alignment are described in the literature: Fiducial markers [9], principal axis alignment [10], consistent matrix transformation [11,12], cross correlation coefficient [10], and the maximal area of overlap [12].

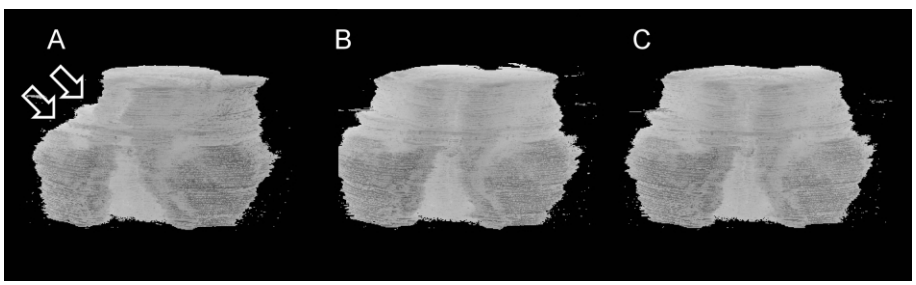

Fig. 1. Reconstructed volumes of the pons. (A) Cross correlation method. This method tends to rotate the sections (arrows) and thus is not suited for this purpose. (B) Consistent matrix transformation. (C) Euclidian distance.

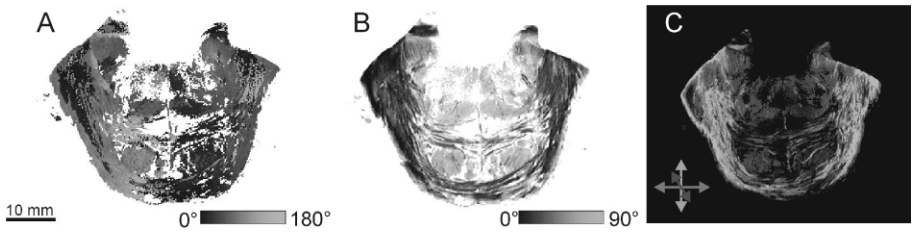

Fig. 2. Fiber orientation maps of a section of the pons. (A) Fiber direction map. Angles of direction (*xy*-plane of the section, 0–180°) are visualized as grayscale values. (B) Fiber inclination map. Angles of inclination (*z*-direction of the sample, 0–180°) are visualized as grayscale values. (C) RGB-coded orientation map.

Two of these parameters (consistent matrix transformation and cross correlation coefficient) were applied to the fiber inclination maps of our data set in order to define the optimal fit of the images (Table 1). In addition, the Euclidian distance was used as a fit criterion. The optimal fit is the situation where the fit criterion becomes minimal (or maximal using the correlation coefficient as fit criterion). The Euclidian distance method and the consistent matrix transformation yielded the best results (Fig. 1), while the cross correlation method caused rotational errors of the reconstructed volume. Thus, we used the Euclidian distance method as standard procedure for image alignment. The rigid transformations were applied to all images (fiber inclination maps, fiber direction maps, and RGB-images).

2.4. Three-dimensional reconstruction of the brainstem and segmentation of major fiber tracts

The aligned RGB-sections were imported into the software 3D Slicer (Massachusetts Institute of Technology, USA) [13]. Since the 3D Slicer reads raw data files with 256 × 256 pixels, the sequential images were translated into this format at first. The software allows slicing the volume data set and has different tools for segmentation and three-dimensional reconstruction of anatomical structures in the volume. Thus, major fiber tracts in the brainstem were segmented manually and then reconstructed three-dimensionally. This way a 3D fiber tract model of the brainstem was developed.

3. Results

Two hundred fifty-eight serial axial sections of the human brainstem were imaged, each yielding fiber inclination maps, fiber direction maps, and RGB images (Fig. 2). Automatic image alignment was performed using the minimized Euclidian distance as fit criterion. The three-dimensional data sets of all sections were imported into the 3D Slicer.

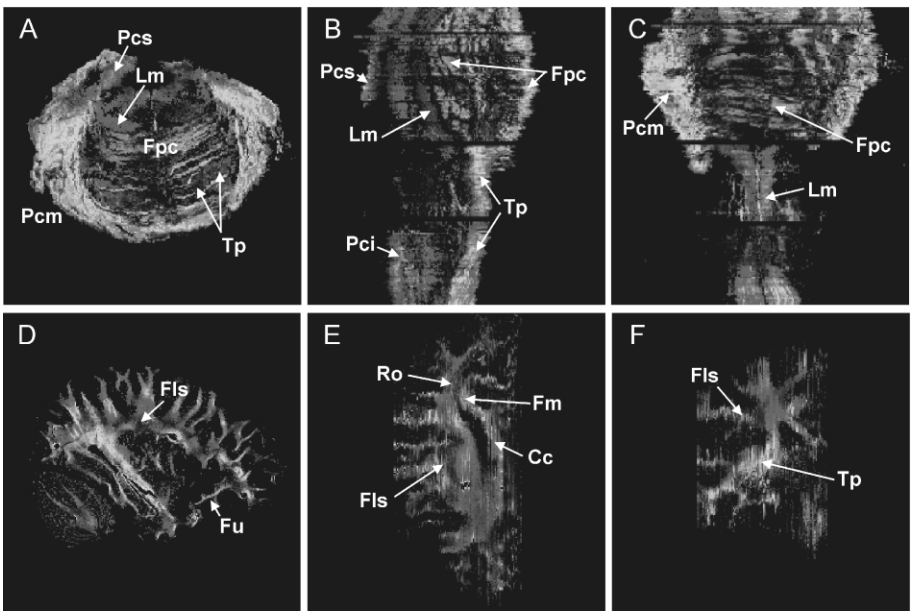

Fig. 3. Three-dimensional data set of the brainstem (A–C), which was reconstructed from serial axial sections, and the human brain (D–F), which was reconstructed from serial sagittal sections. The colors visualize different major fiber tracts. (A) Axial slice of the pons. (B) Sagittal slice of pons and medulla oblongata. The gaps show the borders between the four slabs of the brainstem. (C) Coronal slice of pons and medulla oblongata. (D) Sagittal slice of the human brain. (E) Horizontal slice of the human brain. (F) Frontal slice of the human brain. Abbreviations are: Pcs, pedunculus cerebellaris superior; Pcm, pedunculus cerebellaris medius; Pci, pedunculus cerebellaris inferior; Fpc, fibrae pontocerebellares; Lm, lemniscus medialis; Tp, tractus pyramidalis; Fls, fasciculus longitudinalis superior; Fu, fasciculus uncinatus; Ro, radiatio optica; Fm, forceps major; Cc, corpus callosum.

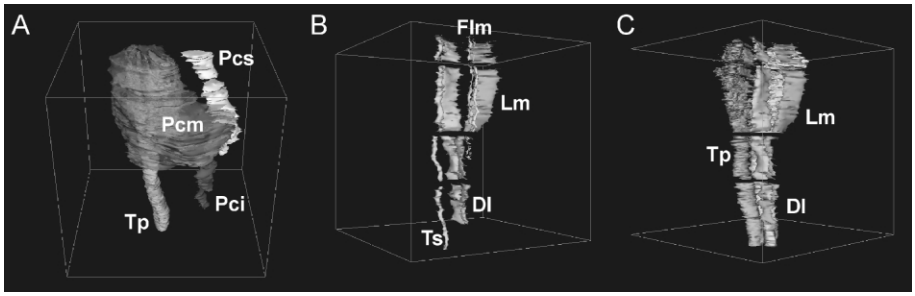

Fig. 4. 3D reconstructed models of the major fiber tracts of the brainstem. (A) Cerebellar peduncles. (B) Medial lemnisci, right spinothalamic tract, and medial longitudinal fascicles. (C) Medial lemnisci and pyramidal tracts. Abbreviations are: Pcs, pedunculus cerebellaris superior; Pcm, pedunculus cerebellaris medius; Pci, pedunculus cerebellaris inferior; Lm, lemniscus medialis; Dl, decussatio lemniscorum; Ts, tractus spinothalamicus; Tp: tractus pyramidalis.

Fig. 3(A)–(C) show sections through the brainstem volume. The red color visualizes fibers running from left to right, the green color shows fibers running from up to down, and blue shows fibers running in the axis of the brainstem. Directions in between these major axes are visualized as mixed colors in the RGB-color space. This way the major fiber tracts can be visually distinguished. Thus, the data set allows of manual segmentation of the major fiber tracts of the brainstem, e.g. the cerebellar peduncles, the medial lemniscus, the spinothalamic tract, the pyramidal tract, and the medial longitudinal fasciculus. In a similar data set of a human hemisphere the major fiber tracts of the telencephalon (Fig. 3(D)–(F)) can also be seen.

After the segmentation of the fiber tracts of the brainstem these tracts were reconstructed three-dimensionally (Fig. 4). This way, the 3D course of these fiber tracts was visualized, and the spatial relationship of the tracts shown. Most of these tracts are compact fiber bundles, but e.g. in the case of the pyramidal tracts the pyramidal bundles are intermingled with the pontocerebellar fibers at the level of the pons (Fig. 4(A)). The decussatio lemniscorum is shown in Fig. 4(B) in the middle level of the medulla oblongata, while the spinothalamic tract fibers cross at spinal segment level.

4. Discussion

Since the described anatomic method has a much higher resolution than diffusion tensor mapping [7], it allows the generation of a digital fiber model of the human brain. This model could be used as a fiber atlas for neurosurgical planning since major fiber tracts such as the pyramidal tract have to be carefully avoided in a neurosurgical procedure [14,15].

In this study the human brainstem was chosen as a model for developing this method. It was possible to visualize the major fiber tracts of the brainstem. The intention of our future work is to develop a fiber atlas of the entire human brain. A first data set of a human hemisphere is shown in Fig. 3(C)–(F). Most of the neuroanatomic atlases [16,17] show mainly the gray matter of the brain since these atlases are based preferentially on cytoarchitectonic staining procedures. Thus, a reliable model of the fiber architecture of the human brain is still lacking. The described methodology is the basis for realizing such a digital human fiber model.

While the large fiber tracts of the brainstem are single compact fiber bundles, the fiber tracts in the telencephalon are highly intermingled [18]. This poses a problem for the segmentation of these fiber tracts. Sufficient algorithms are needed to perform an automatic segmentation. In addition, the magnification of such a data set should be as high as possible in order to visualize small bundles of fibers also. The 3D Slicer uses a resolution of 256 × 256 pixels, but the resolution of the original images is much higher (760 × 574 pixels). Thus, the visual information can be improved by using maximum resolution of the data sets.

In addition, the presented method could be used for evaluation of the diffusion tensor maps known from magnetic resonance imaging. Diffusion weighted MRI

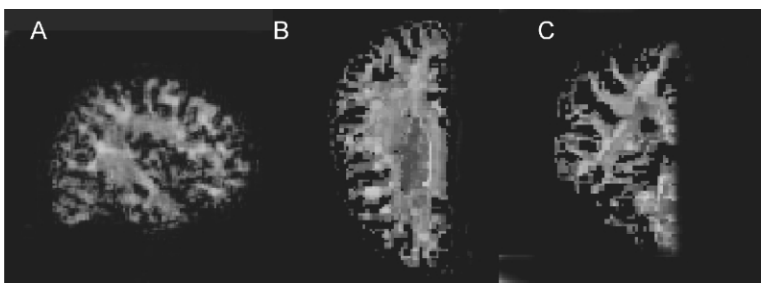

Fig. 5. Diffusion tensor imaging. The slices were manually selected from MR-DTI volume (different brain) to correspond with the polarized light sections. The same color coding scheme is employed, however the direction is set by the principal eigenvector of the diffusivity tensor. (A) Sagittal section of the human brain. (B) Horizontal section. (C) Coronal section.

gives information about the three-dimensional orientation of the major fiber tracts [19–23].

The visualization of diffusion tensors produces images representing the orientation of the fiber tracts. Interpretation of these images, however, was performed up to now by referring to gross anatomical atlases of the major fiber tracts [20]. Fig. 5 shows an example of diffusion tensor imaging slices through the human brain [24,25]. These images contain similar information about the fiber tracts as the images (Fig. 3(D)–(F)) produced by the method presented here. However, the colors look different, which may be due to the different physical principles the two methods are based upon. The analysis of these differences remains an interesting focus of our future research.

We demonstrated that the use of digital processing of polarized light images obtained directly from brain sections yields FOM, which additionally display smaller bundles of fibers [7].

In addition, diffusion tensor data can be used to trace single fiber bundles by following their orientation in the 3D data set, i.e. to perform a 3D fiber tracking [1,26–29]. A similar procedure is also conceivable with the presented FOM, which consist of vectors representing the orientation of the nerve fibers.

The presented method is able to provide two kinds of atlases: a fiber orientation atlas representing a vector in each voxel (Fig. 3), and a volume-based atlas representing the major fiber tracts in the brain (Fig. 4). These models can be used for evaluation of diffusion tensor data as well as for neurosurgical planning.

5. Summary

A new neuroanatomic approach to evaluate the fiber orientation in gross histological sections of the human brain was developed. Sequences of polarized light images of serial sections of a human brainstem were used to derive FOM using image processing tools. The calculated angles of nerve fiber orientation are represented in FOMs. Fiber inclination maps visualize angles of inclination, and fiber direction maps show angles of direction. These angles define vectors which also can be visualized as RGB-colors. The FOMs have a higher magnification than diffusion tensor mapping has. Thus, the method can be used for developing fiber models for evaluation of diffusion tensor mapping. The serial sections were aligned to each other using the minimized Euclidian distance as fit criterion. In the 3D data set of the human brainstem the major fiber tracts were segmented, and three-dimensional models of these fiber tracts were generated. The presented results demonstrate that two kinds of fiber atlases are feasible: a fiber orientation atlas representing a vector in each voxel, which shows the nerve fiber orientation at each point, and a volume-based atlas representing the major fiber tracts. These models can be used for the evaluation of diffusion tensor data as well as for neurosurgical planning.

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