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How to asses, visualize and compare the anisotropy of linear structures reconstructed from optical sections—A study based on histopathological quantification of human brain microvessels

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ABSTRACT

Three-dimensional analyses of the spatial arrangement, spatial orientation and preferential directions of systems of fibers are frequent tasks in many scientific fields, including the textile industry, plant biology and tissue modeling. In biology, systems of oriented and branching lines are often used to represent the three-dimensional directionality and topology of microscopic blood vessels supplying various organs. In our study, we present a novel $p(\chi^2)$ (chi-square) method for evaluating the anisotropy of line systems that involves comparing the observed length densities of lines with the discrete uniform distribution of an isotropic line system with the χ^2-test. Using this method in our open source software, we determined the rose of directions, preferential directions and level of anisotropy of linear systems representing the microscopic blood vessels in samples of various regions from human brains (cortex, subcortical gray matter and white matter). The novel method was compared with two other methods used for anisotropy quantification (ellipsoidal and fractional anisotropy). All three methods detected different levels of anisotropy of blood microvessels in human brain. The microvascular bed in the cortex was closer to an isotropic network, while the microvessels supplying the white matter appeared to be an anisotropic and direction-sensitive system. All three methods were able to determine the differences between various brain regions. The advantage of our $p(\chi^2)$ method is its high correlation with the number of preferential directions of the line system. However, the software, named esofspy, is able to calculate all three of the measures of anisotropy compared and documented in this paper, thus making the methods freely available to the scientific community.

1. Introduction

Cerebral circulation is fundamental to health and the maintenance of brain tissue (Monson et al., 2008; Schlimper et al., 2010). Recently, modern imaging methods, such as different magnetic resonance imaging (MRI) techniques, have been used to directly visualize blood flow through the brain, as demonstrated by nearly 5000 entries for [(MRI) AND (blood flow) AND (brain)] in PubMed (National Library of Medicine, Bethesda, MD, Pubmed (2010)). Although computed tomography (CT) or MRI are routinely used for diagnosis or experiments (reviewed by Giachetti and Zanetti (2006)), the possibilities of using MRI and CT for quantitative description and quantification of vessel orientation are less obvious (Inoue, 2010; Schlageter et al., 1999). The quantification of vascular or blood flow parameters in various visualization techniques (imaging, histology, etc.) allows for statistical comparisons between different conditions, such as healthy and diseased brains, untreated and treated tissue or gray and white matter from different brain regions. However, if the goal is to link changes in circulation to certain pathological changes in the nervous tissue or if the resolution of the chosen imaging method is not sufficient for the topic of interest, histological examination of brain samples is still necessary (Grinberg et al., 2009; Kiselev et al., 2005). In addition, the orientation of vessels within the brain determines their visibility in different imaging techniques (Park et al., 2008). Quantitative data on vessel

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orientation might provide important insight into changes in brain perfusion and thus neuronal function (Gardner, 2010) and represent a prerequisite for modeling cerebral blood flow.

1.1. Geometrical representation of blood vessels as oriented branching fibers

In biology, systems of oriented and branching lines or fibers are often used to represent the three-dimensional (3D) directionality and topology of microscopic blood vessels supplying various organs. The blood microvessels comprise arterioles, metarterioles, capillaries and venules. These objects can be quantified by their volume, surface area, length, cross-sectional area, vessel diameter, vessel number/microvessel density, tortuosity, intervessel distance and their spatial arrangement can be visualized (Schlageter et al., 1999; Park et al., 2008; Bullitt et al., 2009; Ding et al., 2008; Janácek et al., 2009; Jirkovská et al., 2002; Jirkovská et al., 2008; Konerding et al., 1995; Kubínová et al., 2001; Kubínová and Janáček, 2001; Lametschwandtner et al., 2004; Mathieu et al., 2006; Salu et al., 2002; Tonar et al., 2011).

References to the orientation of cerebral blood microvessels are usually made with respect to conventional anatomical planes and directions, e.g., anterio-posterior, latero-lateral, radial, tangential, etc. However, if a statistical description of hundreds or thousands of microvessel orientations in each sample is required, tools for analyzing and summarizing these systems are necessary. For maximal data mining, such tools should also identify any preferential directions, if possible.

The two of the most frequently used characteristics that can be used to conceptually describe the fiber orientation are the rose of directions and the rose of intersections (Mecke, 1981; Rataj and Saxl, 1989). The rose of intersections (Fig. 1A) is a polar plot of the mean density of intersections of the fiber system with a line of given orientation (Spodarev, 2001; Spodarev, 2003; Stoyan et al., 1996; Sundararaghavan and Zabaras, 2004). Briefly, an image of the fiber system is intersected by lines in various directions and the number of intersects or the variation of gray level (in the case of gray scale digital images) along the lines is observed. The orientation distribution is related to the intensity of the intersections in relevant directions (Stoyan et al., 1996; Kärkkäinen and Jensen, 2001).

The rose of directions (Fig. 1B) is defined as the length-weighted orientation of the segments, typically piecewise continuous curves (Stoyan et al., 1996; Tunak and Linka, 2007). The relationship between the rose of intersections and the rose of directions has been described by several groups (Beneš and Gokhale, 2000; Kiderlen and Pfrang, 2005; Prokešová, 2003).

Both the rose of intersections and rose of directions allow for the numerical description of the orientation of each line segment (e.g., each vessel) in a system and their assignment to classes for further statistical analysis (Stoyan et al., 1996; Howard and Reed, 1998). Furthermore, these measures provide a first impression of the uniformity or non-uniformity of fiber (e.g., blood vessel) orientation, known as isotropy or anisotropy (see below). The degree of anisotropy of a cerebral vascular tree might represent an additional important measure that characterizes nervous tissue nutrition and function (cf. Inoue, 2010; Schlageter et al., 1999; Gardner, 2010).

1.2. Exploring of blood vessel orientation in the form of numerical data

In the two-dimensional (2D) evaluation of line systems, a plane can be divided into as many equiangular parts as the accuracy required with respect to the length density. Individual parts are characterized by angle intervals covering $[0, 2\pi[$. For example, the plane can be divided in 4 quadrants. In this case, one quadrant is given by angle interval $[\langle k*(\pi/2), (k+1)*(\pi/2)[$, where $k=0, 1, 2, 3$. The lengths of lines in individual regions are divided by the total length of all lines in the system to get the length density. These length densities are graphically represented by a polar plot (see Fig. 1B), giving information about the 2D anisotropy of system.

For numerical analysis of 3D line systems, such as cerebral blood vessels, the evaluation can essentially follow the 2D model. The space can be divided in regions given by angles of elevation (or latitude) and azimuth (or longitude) (Fig. 2E) in such a way that all tetragons on the surface of the sphere bounded by subsistent azimuths and elevations have equal area. Azimuths correspond to equiangular parts of the 2D case from the interval $[0, 2\pi[$. Elevations are from the interval $[0, \pi/2]$ taking the symmetry with respect to the origin into account. The length densities of lines in given directions (regions) are counted and plotted into the rose of directions. If the spatial points of the rose of directions form a sphere, we can say that the system is isotropic.

1.3. Anisotropy and isotropy of line systems

Anisotropy is defined as a property of a system having a different value when measured in different directions. The an/isotropy of

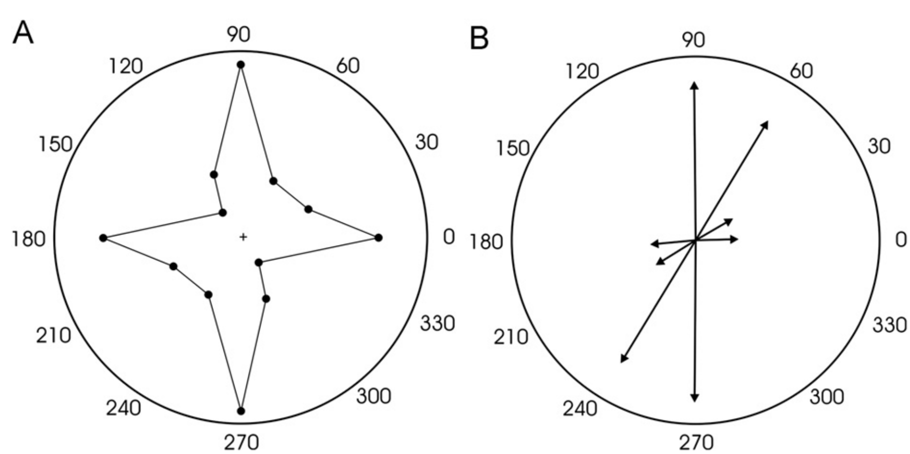

Fig. 1. (A) An illustration of the rose of intersection. The shape gives information about the an/isotropy of the structure. A circular shape means that the structure is isotropic, while the star-like spikes show the anisotropic arrangement of a system. (B) An illustration of the rose of directions. The arrows in individual directions show the length (or angular) density of the lines of a system in the associated direction. If there is a uniform distribution, the arrows have the same size. In the case of anisotropic system, the lengths of arrows are higher in the preferential directions. Angles are in degrees in both cases.