Biomaterials 116 (2017) 34–47

Contents lists available at ScienceDirect

Biomaterials

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

Automated quantification of three-dimensional organization of fiber-like structures in biological tissues

Zhiyi Liu [a], Dimitra Pouli [a], Disha Sood [a], Aswin Sundarakrishnan [a], Carrie K. Hui Mingalone [b], Lisa M. Arendt [b], Carlo Alonzo [a], Kyle P. Quinn [a, d], Charlotte Kuperwasser [b], Li Zeng [b], Thomas Schnelldorfer [c], David L. Kaplan [a], Irene Georgakoudi [a, *]

[a] Department of Biomedical Engineering, Tufts University, Medford, MA, 02155, USA
[b] Department of Developmental, Molecular and Chemical Biology, Tufts University School of Medicine, Boston, MA, 02111, USA
[c] Lahey Hospital & Medical Center, Burlington, MA, 01805, USA
[d] Department of Biomedical Engineering, University of Arkansas, Fayetteville, AR, 72701, USA

ARTICLE INFO

Article history:
Received 24 July 2016
Received in revised form
12 November 2016
Accepted 24 November 2016
Available online 25 November 2016

Keywords:
Three-dimensional organization
Collagen fiber
Neuronal axon
Cancer
Traumatic brain injury
Multi-photon microscopy

ABSTRACT

Fiber-like structures are prevalent in biological tissues, yet quantitative approaches to assess their three-dimensional (3D) organization are lacking. We develop 3D directional variance, as a quantitative biomarker of truly 3D fibrillar organization by extending the directional statistics formalism developed for describing circular data distributions (i.e. when 0° and 360° are equivalent) to axial ones (i.e. when 0° and 180° are equivalent). Significant advantages of this analysis include its time efficiency, sensitivity and ability to provide quantitative readouts of organization over different size scales of a given data set. We establish a broad range of applications for this method by characterizing collagen fibers, neuronal axons and fibroblasts in the context of cancer diagnostics, traumatic brain injury and cell-matrix interactions in developing engineered tissues. This method opens possibilities for unraveling in a sensitive, and quantitative manner the organization of essential fiber-like structures in tissues and ultimately its impact on tissue function.

1. Introduction

Advances in high resolution optical imaging have enabled assessments of the structure of essential tissue components with micron scale resolution and are expected to contribute significantly towards our understanding of structure-function relationships that ultimately define cell-matrix interactions [1–4]. Disruption of cell-matrix interactions is associated with a wide range of diseases such as cancer, fibrosis, and neurodegenerative diseases [5–7]. Modified interactions have major effects upon key functional cellular features including gene regulation, cytoskeletal structure, differentiation, and cell growth control [8–10], as well as upon the matrix biophysical and biochemical properties, including collagen fiber organization and mechanical properties [11]. Thus, improved characterization of these structure-function connections may have important implications for our understanding of tissue growth, development, wound healing and disease progression.

Confocal and multi-photon microscopy, especially second harmonic generation (SHG) and two-photon excited fluorescence (TPEF) [12,13], provide the necessary axial resolution to capture fine structures within cells and the ECM in a 3D context. However, even as high-resolution 3D images of fiber-like tissue structures become more readily accessible, quantitative analysis algorithms for their orientation and organization have largely remained limited to analysis of 2D images [14–21], with only a few notable exceptions [22–25]. Schriefl et al. used polarized microscopy to determine the fiber orientation relying on the birefringence of collagen fibers, by sequentially rotating the sample slide in the azimuthal and the elevation plane to find the two Euler angles to depict an orientation in 3D space. This method eliminates the need for 3D imaging, which can be time consuming; however, it is limited to thin specimens (less than 10 μm), it requires picrosirius staining for enhancement of birefringence, and it doesn't work well for wavy fibers [22]. Lau et al. acquired 3D SHG image stacks and used

* Corresponding author.
E-mail address: Irene.Georgakoudi@tufts.edu (I. Georgakoudi).

http://dx.doi.org/10.1016/j.biomaterials.2016.11.041
0142-9612/

Fourier transform based approaches to quantify 3D orientation of collagen fibers at discrete regions of interest (ROIs). The overall orientation of collagen fibers within each ROI is determined by the filter bank method. The filter bank consists of various 3D orientation filters constructed in the Fourier space, and the filter orientation that corresponds to the maximum likelihood to the Fourier transform of each ROI is designated as the fiber orientation. However, the ROI size is a tradeoff between determination accuracy and computational time [23]. Further approaches, including inertia moments [24] and diffusion tensor imaging (DTI) in combination with two-photon microscopy [25], may be restricted by the characteristics of fibers (e.g., shape, cross section or thickness) or tissues (e.g., heterogeneity). Generally, these methods have been often limited by their computational expense or their inability to provide organization readouts at the microenvironment scale. Recently, we developed a 3D weighted vector summation algorithm, which provides voxel-wise orientation information with high accuracy that is not limited by the waviness or thickness of the fibers, and offers a number of computational time and sensitivity advantages over previous methods [26].

Here, we develop a metric, 3D directional variance, as a quantitative biomarker of truly 3D organization of fiber-like structures, based on this voxel-wise orientation algorithm. Analogous to variance in linear data which measures how far a set of numbers is spread out, directional variance extends this measure to a set of orientations — in this case, in a 3D context. A lower directional variance value reveals more highly aligned fibers, while a higher one corresponds to more random fiber alignment. In this study, we develop the formalism for extracting the 3D directional variance of fiber-like structures within 3D image stacks, and then apply this metric to the organization characterization of several such structures, including collagen fibers, neuronal axons, and fibroblasts, present within 3D multi-photon image data sets of different tissue systems. By using these tissue systems, we illustrate the capability of this approach to characterize 3D fiber-like structure organization in heterogeneous samples (cartilage) or as a detailed function of distance from a certain point of interest (traumatic brain injury model). In addition, we show that the choice of window over which 3D organization is assessed may be critical in some cases for identifying changes (human peritoneal metastasis model) and not in others (mouse breast cancer model) depending on the size and the tissue-level organization of the fibers. Finally, this approach is well-suited to assess alignment of multiple types of fiber-like structures within the same specimen, as exemplified by the lung engineered tissue model. Together these applications reveal a broad potential of this newly-developed metric for biomedical uses.

2. Materials and methods

2.1. 3D directional variance formalism to quantify fibrillar organization

The development of 3D directional variance relies on concepts from directional statistics [27]. Directional variance for circular data (for which orientations of 0° and 360° are equivalent), V_{3D}, is defined as [27]:

$$V_{3D} = 1 - \overline{R}_{3D} \tag{1}$$

where $\overline{R}_{3D} = (\overline{C}^c_{3D}2 + \overline{S}^c_{3D}2 + \overline{Z}^c_{3D}2)^{1/2}$, and $\overline{C}^c_{3D} = (1/n)\sum_{j=1}^{n}\sin\varphi_j \cos\theta_j$, $\overline{S}^c_{3D} = (1/n)\sum_{j=1}^{n}\sin\varphi_j \sin\theta_j$, $\overline{Z}^c_{3D} = (1/n)\sum_{j=1}^{n}\cos\varphi_j$. The superscript c refers to the circular data, and n is the total number of voxels that contribute to the determination of the directional variance. θ and φ are the azimuthal and polar angles used to depict an orientation in 3D space, respectively (Fig. 1a). The azimuthal angle θ is determined by projecting fibers to a fixed 2D plane, i.e., the xy plane (Fig. 1a). However, according to the definition of the polar angle, φ, there is no fixed 2D plane onto which fibers could be projected for the determination of all possible φ orientations. To address this problem, we consider additional angles β and γ (Fig. 1a), which are azimuthal angles, like θ, and related to φ by Ref. [26]:

$$\tan^2\varphi = 1\Big/\tan^2\beta + 1\Big/\tan^2\gamma \tag{2}$$

The determination of β and γ can be achieved using the same approach as that for θ. After projecting fibers to a fixed 2D plane, the orientation is determined from the weighted vector summation algorithm we described recently [18,26]. Briefly, to acquire the θ orientation of the center voxel of a $n \times n \times n$ voxel cube window, we first average the cube along the z direction to project the cube window to a square window ($n \times n$ pixels), and then calculate the orientation of the center pixel of the square, based on the basic premise shown in Fig. 1b and c. First, we define the vectors passing through the center pixel (marked in purple) of the window size of choice (11 × 11 pixels in this case). Then these vectors are weighted by their length and intensity fluctuations along their direction [26], as shown in Fig. 1b. The orientation of the center pixel is defined as the summation of all these weighted vectors and shown in Fig. 1c, which corresponds well to the direction of fiber alignment. The determination of angles β and γ is achieved by averaging the cube along the other two directions, followed by the identical weighted vector summation algorithm.

Once we acquire the values of θ and φ for each voxel of a 3D stack containing fiber-like structures, whose orientations are equivalent for 0° and 180°, we need to modify the definition of 3D directional variance. A general approach to transforming axial (fiber like) data to circular data is to multiply angular values by 2. This strategy is sufficient for transforming the azimuthal angle θ, but not for the polar angle φ. Again, we use azimuthal angles β and γ to represent the polar angle φ, and define $b_j = \sqrt{1/\tan^2(2\beta_j) + 1/\tan^2(2\gamma_j)}$ to derive the modified components as:

$$\overline{C}^a_{3D} = (1/n)\sum_{j=1}^{n}(b_j\Big/\sqrt{1+b_j^2})\cos(2\theta_j) \tag{3}$$

$$\overline{S}^a_{3D} = (1/n)\sum_{j=1}^{n}(b_j\Big/\sqrt{1+b_j^2})\sin(2\theta_j) \tag{4}$$

$$\overline{Z}^a_{3D} = (1/n)\sum_{j=1}^{n} SI\Big/\sqrt{1+b_j^2} \tag{5}$$

Where $SI = (-1)\cdot(\varphi - 90)/|\varphi - 90|$ when $\varphi \neq 90^o$, and $SI = 1$ when $\varphi = 90^o$. The superscript a refers to the axial data. Using these modified components, we acquire the modified $\overline{R}_{3D}$, and finally the V_{3D} that is suitable to quantify the organization of 3D fiber-like structures. The custom code for the assessment of the 3D directional variance is written in MATLAB.

Orientation maps of two typical planes, as marked by green and blue (Fig. 1a), are plotted in Fig. 1d and e respectively, which serve as a reference for assessing the orientation values. Throughout this study, optical sections are all along the xy plane, and 3D image stacks are then reconstructed based on these sections at different z depths. Note that a φ of 90° corresponds to fibers that are parallel to the optical sections.

In this study, the 3D directional variance is assessed typically