Horny L, Hulan M, Zitny R, Chlup H, Konvickova S, Adamek T (2009) *Computer-aided analysis of arterial wall architecture*. IFMBE Proceedings 25(4):1494-1497. MANUSCRIPT VERSION.

Computer-aided analysis of arterial wall architecture

L. Horny[1], M. Hulan[1], R. Zitny[2], H. Chlup[1], S. Konvickova[1] and T. Adamek[3]

[1] Department of Mechanics, Biomechanics and Mechatronics, Faculty of Mechanical Engineering Czech Technical University in Prague, Prague, Czech Republic
[2] Department of Process Engineering, Faculty of Mechanical Engineering Czech Technical University in Prague, Prague, Czech Republic
[3] Department of Forensic Medicine, Third Faculty of Medicine, Charles University in Prague, Prague, Czech Republic

Abstract— **Realistic simulations and engineering innovations help to improve therapy of blood vessel diseases by involving detailed information about a blood vessel wall architecture. This information is especially needed in a constitutive modeling of tissues. This study presents a computational method for evaluating of arterial internal structure based on analysis of histological sections. Presented algorithm searches dominant directions in binary pixel maps of histological sections filtered according to a constituent, which was previously stained. Particular constituent is represented by pixels with the value one in a black and white image. Neighborhood of each non-zero pixel is analyzed by a rotating line segment. Dependence between the number of non-zero image pixels shared by the line segment and the segment angle is found. The rotation with maximum number of non-zero pixels determines local dominant direction for the pixel in the given neighborhood. By applying this procedure to all non-zero pixels of the binary image an empirical distribution function of preferred directions in the whole image is provided. This method was tested in four histological sections of human abdominal aorta. A distribution of collagen fibrils' directions was evaluated for medial and adventitial layer. Two sections for every layer were analyzed – one with a long axis aligned to the axial direction of the aorta and one aligned circumferentially. Graphs of empirical probability density functions are presented. Results proved that collagen fibrils are not aligned in finite number of directions. However, the distribution in adventitial layer had two significant peaks almost symmetrically disposed to circumferential and axial direction of the artery wall. Peaks correspond to ≈67° and ≈112° (angle is measured from axial to circumferential direction). Sections of the medial layer did not provide unambiguous results.**

Keywords— **aorta, collagen, constitutive model, distribution function, image analysis.**

I. Introduction

Realistic simulations should improve therapy of blood vessel diseases by involving detailed information about material composition and tissue structure. Especially constitutive models of arteries should consider an internal structure and mechanical behavior of their constituents. However, biological tissues comprise large number of different cells, matrix proteins and bonding elements. Moreover, there are many complex interrelations between these constituents. Thus a description of mechanical response of a tissue based on microstructure observations is a complicated problem. Nowadays models based on nonlinear solid mechanics consider only a limited number of constituents and special assumptions about their arrangement are employed.

Constitutive models are suggested within framework of mixture theory. The most frequent models incorporate information about collagenous fibrils and their arrangement [1], [2]. Wall is assumed to be a basement matrix in which fibers are embedded and finite number of preferred directions is supposed. Collagenous component is typically arranged in two families of helical fibers. Slightly different considerations about the matrix are made in [3]. Recently Rezakhaniha and Stergiopulos published a modified mixture model which incorporates anisotropic response of elastin [4] and applied this idea for modeling of a venous wall. Threefold mixture was considered by Zulliger et al. in [5]. They suggested a constitutive model which incorporates elastin, collagen and smooth muscle cells. Models with more than three constituents are rare if ever occur.

Models with continuous distribution of preferred directions also exist. One such model was recently suggested by Gasser et al. in [6]. Driessen et al. utilized another continuous model in [7]. This way seems to be in a better agreement with reality than the previously described models. However, obtaining empirical distribution functions which describe arrangement of fibrils requires fine microstructure analysis. There are plenty of papers which report histological observations of arterial walls. But detailed analyses providing experimentally determined distribution functions of wall constituents are rare. Billiar and Sacks published data for bovine pericard and porcine aortic leaflet [8]. Analysis of cellular nuclei orientation in human aortic media was performed in [2]. Up to date overview of collagenous fibrils organization in arterial wall can be found in [9].

The purpose of this study is to extent knowledge of collagen organization in aortic wall. An automatic method evaluating directional arrangement of fibers in a histological

section is presented and applied to collagen fibrils in human abdominal aorta.

II. Image analysis algorithm

Obtained histological sections were digitalized and tresholded with respect to particular color of histological staining. The example of such an image is in Fig. 1.

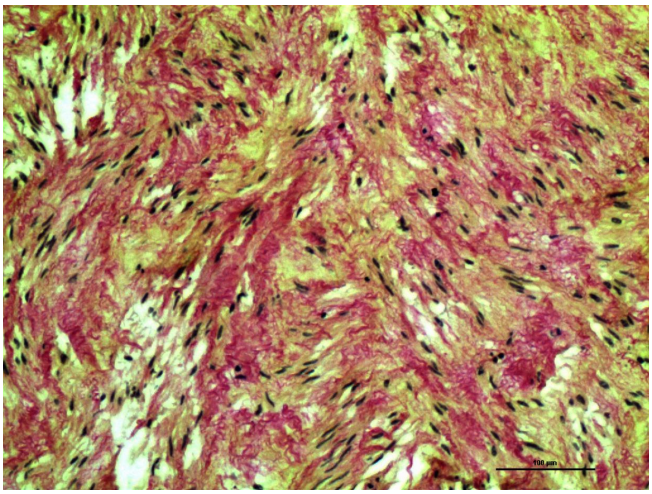

Fig. 1 Transversal section of abdominal aorta stained with van Gieson. Collagen fibrils are stained red. Black smooth muscle nuclei are also apparent. Scale corresponds to 100μm.

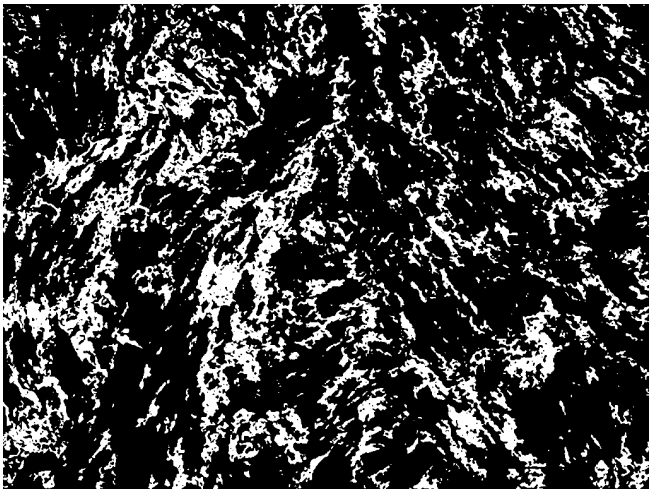

Fig. 2 Binary map of transversal section of abdominal aorta stained with van Gieson (from Fig. 1). White color corresponds to collagenous fibrils.

Filtered image is then transformed to a binary pixel map with logical one in pixels associated with the selected color, see Fig.2. In such a way prepared binary pixel map is the input of the *rotating line segment algorithm* (RLSA) providing directions distribution of the sparse structure represented by non-zero pixels. In each non-zero pixel of the input binary image the algorithm looks for a best line segment (and its angle) sharing maximum *non-zero* pixels in the current neighborhood. This angle of maximum non-zero pixels incidence gives a local dominant direction.

Let us consider non-zero pixel of the binary pixel map and its square neighborhood of size k x k. k must be odd and higher than one. This neighborhood is represented by square logical matrix M $(k \times k)$. A set of pixels L in square grid which satisfies (1) or (3) is called a discrete native line (the set L includes all points having the distance less than $\omega/2$ from the line $ax+by+c=0$)

$$L(a,b,c,\omega)=\left\{[x,y]\in\mathbb{Z}^2;0\le ax+by+c+\frac{\omega}{2}<\omega\right\} \qquad (1)$$

Parameters in (1) have to satisfy constraints (2).

$$\omega=\max(|a|,|b|) \qquad a,b,c\in\mathbb{Z} \qquad (2)$$

Numerical range of the line angle is $\langle 0;\pi)$ to take into account the symmetry of the rotating line. The rotated line expressed by one parameter α (rotation angle), is defined by equation (3).

$$L^{\alpha}=\left\{[x,y]\in\mathbb{Z}^2;0\le x+y\tan\alpha+\frac{\max(1,|\tan\alpha|)}{2}<\max(1,|\tan\alpha|)\right\} \qquad (3)$$

Details can be found in [12]. Each L^{α} can be represented by a square matrix k x k of logical values. Pixels which satisfy the condition (3) have values of one, other pixels have zero values. Generated discrete lines having inclination within a prescribed interval of angles $\langle\alpha, \alpha+\Delta\alpha)$.

A level of the discrete line fitting to the neighborhood area M is measured by matching coefficient $C(\alpha)$.

$$C(\alpha)=\frac{\sum_{i=1}^{k}\sum_{j=1}^{k}M(i,j)\cdot L^{\alpha}(i,j)}{k};\quad C(\alpha)\in\langle 0;1\rangle \qquad (4)$$

$C(\alpha)$ is obtained as the sum of element by element multiplication of matrices M and L^{α}, normalized with respect to size of neighborhood k. $C(\alpha)$ is never equal to zero because both matrices includes at least one non-zero pixel. Maximum value of $C(\alpha)$ corresponds to the angle of local dominant direction in a given pixel.

Two ways from the local (pixel) to the global (image) information transfer exist. First, one may create a local em-

pirical distribution function in each non-zero pixel of the image and averages over the whole image (method I.). The second way is to represent each non-zero pixel in the image only by the maximum value of $c(\alpha)$, and to create a histogram of local dominant directions in the image (method II.). In an ideal case of image with homogenously distributed structure both alternatives converge to each other. Methods were programmed within MATLAB (Mathworks, USA) platform. Alternatives to the above described algorithm exist. Holzapfel et al. [2] used an automatic technique based on second-order moment evaluation. A ridge and valley method is applied in [11]. Detailed information about presented algorithm and its testing with different images can be found in [12].

III. Collagenous fibrils distribution

Four histological images obtained form 36-years-old male abdominal aorta were analyzed by the algorithm described above. They were stained with van Gieson, digitized and filtered to extract the collagenous component. One pair of images was obtained from adventitial part of a wall and one from a medial layer. Optical magnification was $\times 20$. The distribution of collagen fibrils in histological images was determined using the angle resolution $\Delta\alpha=\pi/16$. Orientation of analyzed angle α is shown in Fig. 3.

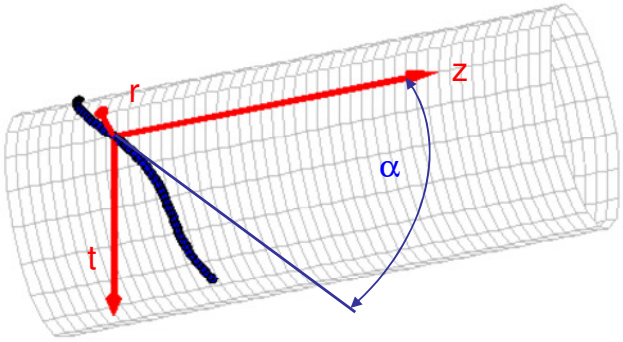

Fig. 3 Sketch of resulted angle. The angle is measured between axial and circumferential direction.

IV. Results and conclusion

Obtained results may be affected by a choice of the parameter k (length of the rotating line segment). After several tests we suggest that the best choice for k is to be proportional to a characteristic dimension of the analyzed structure. In the present analysis the k was set to k=51 pixels, because this value refers to the typical size of collagen fibers in the analyzed image.

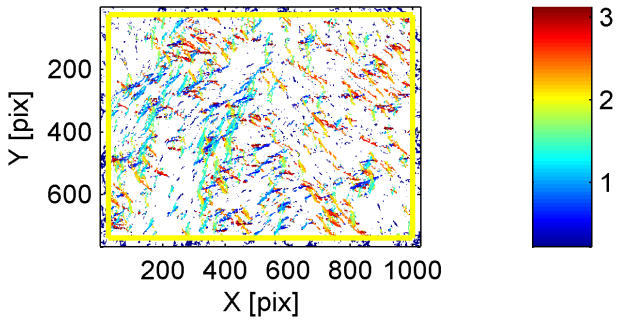

Fig. 4 The map of local dominant directions for adventitial circumferential section (same section as in Fig. 1 and 2). Horizontal and vertical axis in pixels (compare with neighborhood size k=51). Color map in radians. White color corresponds to zero-pixels.

Fig. 4 shows the map of results for circumferential section form adventitial layer. It proves that neighborhood of pixel is proportional to colored areas which represents non-zero pixels. Local distribution of dominant angles is shown in Fig 5 and 6. A length of arrow is normalized and corresponds to the value of matching coefficient. It is seems to be obvious that a continuous distribution of collagen fibers rather than a finite number of preferred directions exist. However, only two significant peaks are presented in Fig. 5 (adventitial layer). It confirms that models of collagenous architecture with two families of helical fibers are close to a real structure. These peaks represent values of α ≈67° and ≈112° (with respect to angular step 11.25°).

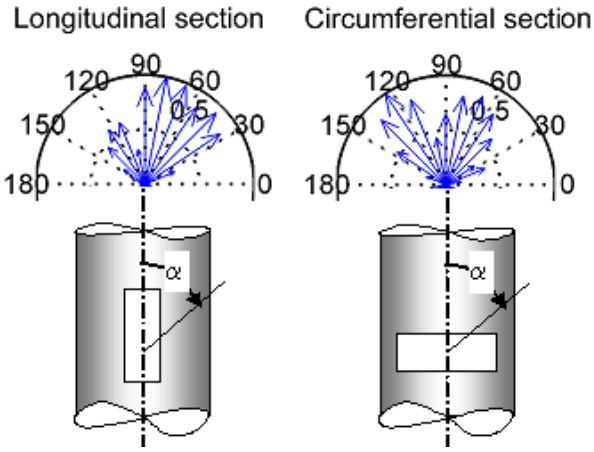

Fig. 5 Local directions histogram for adventitial layer (polar projection). Two almost symmetrically disposed directions are confirmed (method II.)

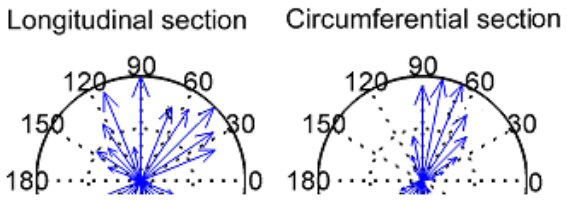

Fig. 6 Local directions histogram for medial layer. The result does not provide unambiguous conclusion. Method II.

This result is in accordance with [1]. Medial distribution is in Fig. 6. On contrary to the adventitia we may not confirm existence of two dominant directions. Longitudinal sections suggest similar result but circumferential section is obviously with a one peak only. For completeness graphs of average probability density evaluated by method I in adventitia are presented in Fig. 7. Results obtained by method I and II qualitatively agree, however method II predicts sharper distributions and therefore the method II is a better guide for modeling based upon the finite number of directions.

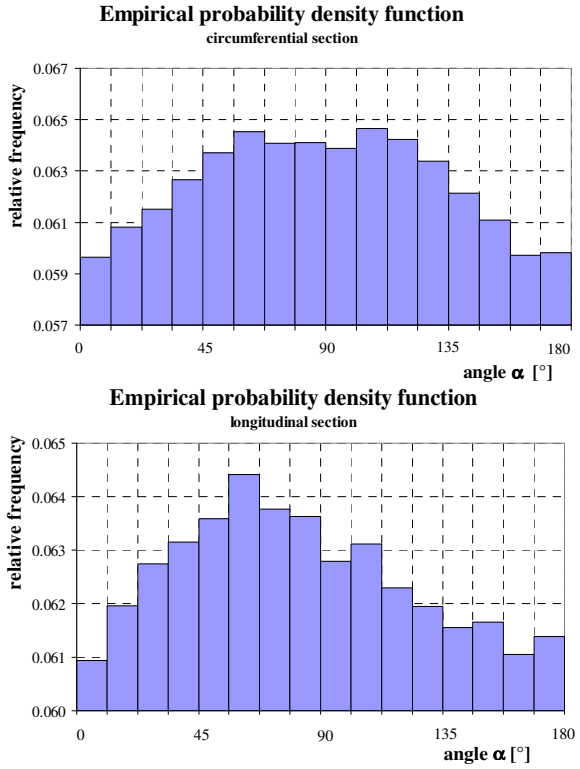

Fig. 7 Average empirical probability density in adventitia based on $C(\alpha)$. Circumferential section is on top and longitudinal section is at bottom. Method I.

Finally, we may conclude that the RLSA successfully identifies dominant direction in histological images. Moreover, comparable results were obtained from different histological sections. Obtained results for adventitia are in accordance with literature, and all presented data are available on request.

Acknowledgment

This research has been supported by Czech Ministry of Education project MSM 68 40 77 00 12 and Czech Science Foundation GACR 106/08/0557.

References

1. Holzapfel GA, Gasser TC, Ogden RW (2000) A new constitutive framework for arterial wall mechanics and a comparative study of material models. J Elast 61:1–48
2. Holzapfel GA, Gasser TC, Stadler M (2002) A structural model for viscoelastic behavior of arterial walls: Continuum formulation and finite element analysis. E J Mech A/Solids 21: 441–463
3. Zulliger MA, Fridez P, Hayashi K, Stergiopulos N (2004) A strain energy function for arteries accounting for wall composition and structure. J Biomech 37:989–1000
4. Rezakhaniha R, Stergiopulos N (2008) A structural model of the venous wall considering elastin anisotropy. J Biomech Eng 130, DOI 10.1115/1.2907749
5. Zulliger MA, Rachev A, Stergiopulos N (2004) A constitutive formulation of arterial mechanics including vascular smooth muscle tone. AJP Heart 287:1335–1343
6. Gasser TC, Ogden RW, Holzapfel GA (2006) Hyperelastic modelling of arterial layers with distributed collagen fiber orientations. J R Soc Interface 3:15–35
7. Driessen NJB, Bouten CVC, Baaijens FPT (2005) A structural constitutive model for collagenous cardiovascular tissue incorporating the angular fiber distribution. J Biomech Eng 127:494–503
8. Billiar KM, Sacks MS (1997) method to quantify the fiber kinematics of planar tissue under biaxial stretch. J Biomech 30:753–756
9. Holzapfel GA (2008) Collagen in Arterial Walls: Biomechanical Aspects in: Collagen: Structure and Mechanics. Ed.: Fratzl P, Springer 2008 DOI 10.1007/978-0-387-73906-9
10. Brimkov VE, Barneva R (2005) Analytical honeycomb geometry for raster and volume graphics. Comput J 48:180–199
11. Elbischger PJ, Bischof H, Regitnig P, Holzapfel GA (2004) Automatic analysis of collagen fiber orientation in the outermost layer of human arteries. Pattern Anal Applic 7:269–284
12. Hulan M (2008) Evaluation of Dominate Directions in Binary Pixel Maps, 9th Workshop on Applied Mechanics. Prague: CTU, Faculty of Mechanical Engineering, 2008, vol. 1, pp 19-21

Author: R. Zitny
Institute: Faculty of Mechanical Engineering CTU in Prague
Street: Technicka 4
City: Prague
Country: Czech Republic
Email: Rudolf.Zitny@fs.cvut.cz