Horny L, Kronek J, Chlup H, Zitny R, Hulan M. (2010) *A distribution of collagen fiber orientations in aortic histological section.* IFMBE Proceedings 29:772-775. DOI: 10.1007/978-3-642-13039-7_195 MANUSCRIPT VERSION
Publisher link http://dx.doi.org/10.1007/978-3-642-13039-7_195

A distribution of collagen fiber orientations in aortic histological section

L. Horny[1], J. Kronek[1], H. Chlup[1,2], R. Zitny[3] and M. Hulan[1]

[1] Laboratory of Biomechanics, Faculty of Mechanical Engineering, Czech Technical University in Prague, Prague, Czech Republic
[2] Institute of Thermomechanics, Academy of Sciences of the Czech Republic, Prague, Czech Republic
[3] Department of Process Engineering, Faculty of Mechanical Engineering Czech Technical University in Prague, Prague, Czech Republic

Abstract–**Distributions of collagen fibrils and smooth muscle cells nuclei (SMC) orientations were investigated in histological sections obtained from medial layer of human thoracic aorta. The sections were stained with van Gieson. Digital image of the sections was converted to binary pixel map with target component represented in white (logical unity). Selected image was indicated to elucidate the sensitivity to threshold conditions and three different binary conversions were performed. Consecutively images were processed by in house developed software BinaryDirections which uses an algorithm of the rotation line segment to determine significant directions in digital images. The algorithm operates in the way that in each target pixel a line segment is rotated step by step to explore neighborhood of the pixel. Exploring the neighborhood the number of unity pixels in each rotating step is determined. The distribution of orientations in the entire image is gained after normalization either as averaged density distribution from all pixels or as an histogram of the most abundant directions in the image. It was found that both collagen fibrils and SMC nuclei analyses give significant peak in distributions. Its value ranges between 45° - 65° (defined as declination from longitudinal axis of an artery in a tubular configuration) depending on the method. It implies that preferred direction in aortic medial layer was oriented circumferentially rather than longitudinally. This conclusion was almost independent of the threshold setup. Results suggest that the orientations of SMC nuclei and collagen fibrils are mutually correlated and determination of collagen fibril orientations, which may be stained in insignificant manner, could be supported with SMC nuclei orientations to obtain more realistic models.**

Keywords– **aorta, anisotropy, collagen, fiber distribution, histology, probability density.**

I. Introduction

Innovative biomedical engineering moves towards patient-specific (bio-)artificial implants and computational simulations. It involves a development of sophisticated constitutive models for biological tissues. These models have to account for complex material structure. Biological tissues comprise large number of different cells, matrix proteins and bonding elements. Moreover, there are many complex interrelations between these constituents.

If an arterial wall is considered, there are three main layers usually distinguished, Holzapfel et al. [1]. Each layer has different function and a composition. Briefly, innermost layer called *tunica intima* consists of endothelial cells which rest on basal lamina. There is also a subendothelial layer dominated with collagen and smooth muscle cells (SMC). Tunica intima and subendothelial layer are very thin and compliant in physiological state; however they may become mechanically considerable in pathological situation, see Holzapfel et al. [3]. *Tunica media*, middle layer of an artery, has distinctive three-dimensional network structure. It is arranged into so-called musculoelastic fascicles which consist of SMC, bundles of collagen fibrils and elastin. They are separated with fenestrated elastin laminae and repeat through medial layer [2]. Directional arrangement of fibrillar components in media is helical with small pitch (almost circumferential orientation) [1,2]. Tunica adventitia is the outermost layer where especially fibroblasts, fibrocytes and collagen fibers are presented. Collagens form two helically arranged families of fibers with significantly dispersed individual fibers. Above mentioned layers are separated by elastic laminae. However, described structure is valid only in the case of elastic artery. Histological structure of muscular arteries may differ significantly. For more details see [1] and [2] and references therein.

Models of internal structure are being implemented in constitutive theories. Nowadays constitutive theories based on continuum mechanics can incorporate information about fibrillar structure to predict appropriate anisotropic behavior of a material. The anisotropy may rise from either finite number of preferred directions or their continuous distribution (for finite number of directions see Holzapfel et al. [1,6] or Lanir [4]; and for continuous distribution Gasser et al. [2], Lanir [4] or Driessen et al. [5]). It is also possible to build up a model with composite structure (mixture theory) which will be intrinsically multiphase. Well known is the fact that fibrillar components of biological tissues are usually crimped. Models accounting for an uncrimping and successive straining within a loading process have also been proposed; see Lanir [4], Freed and Doehring [7] or Cacho et al. [8].

This paper deals with internal structure of arterial wall based on observations made within an inspection of histological sections. There are different ways to obtain such information. Analysis of histological sections and employing transmitted light microscopy (TLM) is probably

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the oldest and simplest one. Nevertheless any drawbacks exist. First of all a section is mechanically loaded during sectioning and chemical fixation (in formalin) may also change internal structure. Moreover this method does not seem to be suitable for an investigation of rearrangements in internal structure under a loading, what is of cardinal importance, because of complicated load fixation. However, such papers exist; see Sokolis et al. [9], they reported straightening of elastic lamellae under uniaxial tension. The polarized light microscopy (PLM) may be used in the same way because of the collagen and SMC birefringence, Canham et al. [10], [11]. Both studies revealed approximately circumferential orientations of collagen fibrils and SMC in coronary arteries and internal mammary artery.

Small-angle scattering methods may be used as an alternative to the optical microscopy. Small-angle X-ray scattering (SAXS) seems to be capable to reveal a relation between fibers orientation and external load in a nano-scale, Schmid et al. [12]. Small-angle light scattering (SALS) method may also be employed rather than for nano-scale in micro-scale investigations of collagen organization in a tissue, Sacks et al. [13].

The main goal of this paper is to present findings obtained within investigation of histological sections of human abdominal aorta using digitalized images. In house software BinaryDirections with implemented algorithm of the *rotating line segment* (RLS), see Horny et al. [14], was used to quantify an organization of collagen fibrils and nuclei of SMC. As first RLS algorithm is described and obtained results follow.

II. Methods

Histological sections were obtained from abdominal aorta of 36-years-old male donor. They were stained with van Gieson and digitalized under 10 x magnifications. Four images were the same as in [14] and were used to confirm previous results. It was due to the fact that the algorithm in BinaryDirections (RLS) has been improved in 2009. Additional image from medial layer was indicated to examine if the correlation between collagen fibers orientation and SMC nuclei exists. This image was also used in the analysis of threshold sensitivity to elucidate an influence of a binary conversion on results; threshold of RGB filter which transforms stained collagen to white (logical unity) pixels and non-collagen components to black (logical zero) pixels.

III. RLS alghorithm

Exact mathematical formulation of the rotating line segment (RLS) was described in details in [14]. Here only key ideas will be repeated with emphasis on improvements made in 2009. The RLS processes binary pixel maps as follows. In non-zero pixel (target pixel) of the processed image the line segment is step by step rotated in order to evaluate the number of non-zero pixels in the target pixel neighborhood. The evaluation is based on so-called matching coefficient, $C(\alpha)$, which is normalized number of non-zero pixels shared with the line segment and neighborhood of target pixel at given rotating step α. Thus RLS is two-parametric algorithm which depends on given α and positive integer N. N is the linear dimension of the neighborhood (square with side length equal to N). The neighborhood and the line segment are represented as matrices with elements equal to zero or one depending on filtered pixels' values. The rotation of the line segment is performed as a creation of new matrix with different non-zero elements. The number of non-zero pixels (collagen) in given direction (rotating step) is obtained as a product between elements in neighborhood matrix and line segment matrix, where corresponding positions in matrices are multiplied and the products are summed. This number is then normalized with respect to dimensions of selected neighborhood and the line segment, thus matching coefficient $C(\alpha)$ is obtained.

There are two ways to reveal relevant information about directional frequency of non-zero (collagen) pixels in the neighborhood of target pixel. First, one can reduce information to the most abundant angle (rotation step) only, and create an histogram over entire image. A dominant direction distribution is then obtained. If full information is stored then average distribution of collagen pixels frequency over entire image can be computed after normalizing to unit surface. If multiple maxima in the matching coefficient are obtained, they are added to their corresponding orientations weighted with multiplicative inverse of their multiplicity (newly added). The second improvement implemented in BinaryDirections software is a penalty function which suppresses contribution of almost uniform distributions in averaging procedure.

IV. Results

Selected results for processed images are shown in Fig. 4–6. Fig. 1 shows original digital image of used histological section (abdominal aortic media). Filtering this image to collagen bundles is in Fig. 2; smooth muscle cell nuclei, easy distinguishable in Fig. 1, are filtered in Fig. 3. Fig. 2 and Fig. 3 are overlaid with orange grid spaced every 100 pixels to highlight length scales. A distribution of

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orientations is build up using information from a pixel neighborhood, thus its dimension (N) should be chosen with respect to dimensions of image texture.

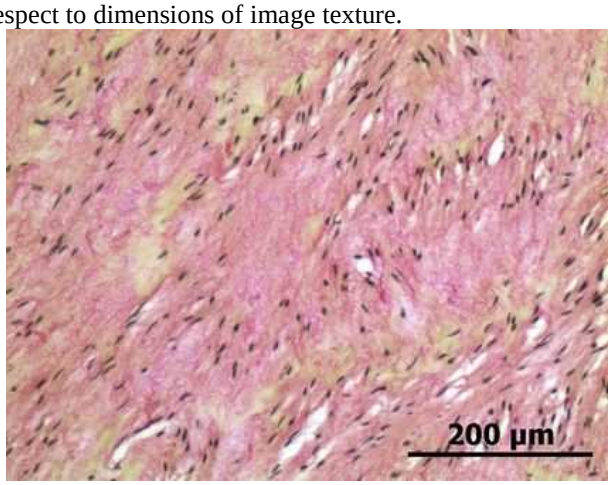

Fig 1 Additional histological section obtained from abdominal aortic media; stained with van Gieson. Bundles of collagen fibrils are stained in red. Black nuclei of smooth muscle cells are also apparent.

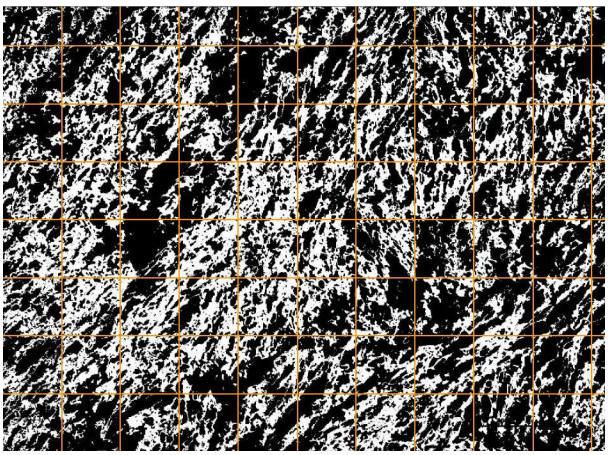

Fig. 2 Binary pixel map of the section from Fig. 1 filtered with respect to collagen (white). Orange grid spacing is 100 pixels; 0.64μm/pix.

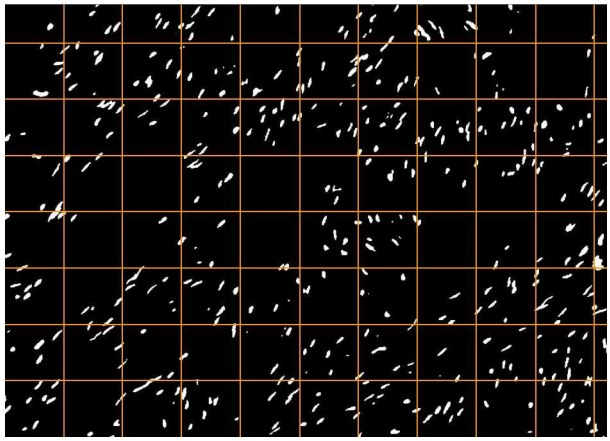

Fig. 3 Binary pixel map of the section from Fig. 1 filtered with respect to SMC nuclei (white). Orange grid spacing is 100 pixels; 0.64μm/pix.

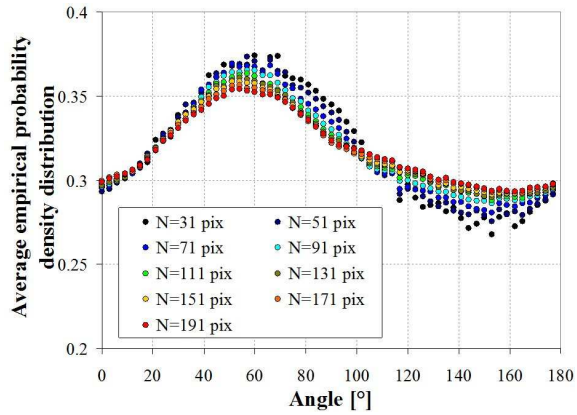

Fig. 4 Empirical probability density distribution of collagen fiber orientations obtained from the binary map in Fig. 2. Sensitivity to neighborhood dimension is shown.

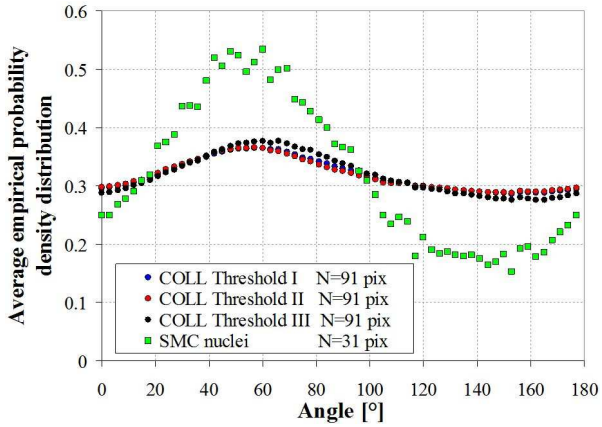

Fig. 5 Comparison between empirical probability density distributions of collagen fibers orientations obtained form Fig. 2 with different filter thresholds and empirical probability density distribution of smooth muscle cell nuclei in Fig. 3.

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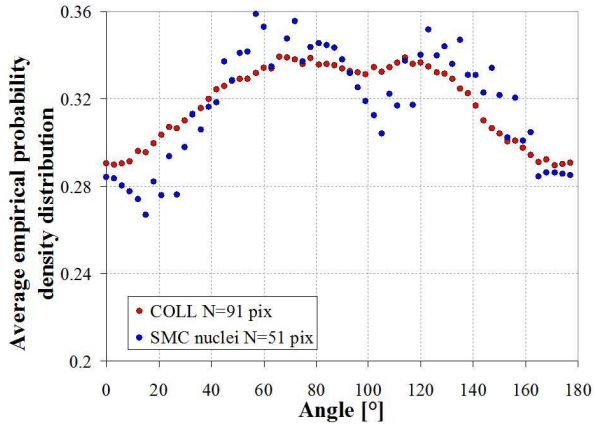

Fig. 6 Comparison between empirical probability density distribution for collagen orientation and smooth muscle nuclei orientation for medial section published in [14].

Fig. 4 shows small sensitivity of the texture (Fig. 2) on N. Density peak is located approximately at 55°.

Filtering to target component is not unambiguous process and may depend on individual skills of an operator. To find out this influence the section (Fig. 1) was binary converted three times with small perturbations in collagen threshold. Fig. 5 shows that all images gave similar results, the peak at ≈55°.

Due to existence of so-called musculoelastic fascicles in aortic media it was hypothesized that the correlation between collagen fibers and SMC orientations may exist. In the case of histological section in Fig. 1 (and its binary conversions above mentioned) this hypothesis was confirmed (Fig. 5). Both methods revealed density maximum between 45°-65°. Fig. 6 shows that the section previously analyzed in [14] suggests correlation between SMC and collagen orientations. However, additional analyses of further sections did not provide this result in every case.

V. Conclusions

The results suggest that the rotation line segment may be powerful instrument in analyses of arterial architecture. It was shown that analyzed histological image from human aortic media (Fig. 1) gives continuous density distribution with maximum at app. 55° for the collagen (the angle is defined with respect to longitudinal axis in cylindrical configuration). Similar result was obtained within the analysis of smooth muscle cell nuclei orientations (continuous distribution with one peak located between 45°-65°). This may not be a rule, but may serve as confirmation for purely collagen-based analyses. The correlation between SMC nuclei and collagen fibrils orientations may be expected especially within medial layer where so-called musculoelastic fascicles are presented. Prospective mismatches between SMC and collagen distributions may be attributed to finite thickness of histological section (5μm in this study) which may contain more that one fascicle. Results show that preferred direction is oriented circumferentially rather than longitudinally, however the orientations density maximum does not meet exactly circumferential direction as in [6]. Within analysis of histological sections published in [14] with improved algorithm, obtained results were similar in character with reported in [14].

Acknowledgment

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

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Author: Lukas Horny
Institute: Fac. of Mech. Eng. Czech Technical University in Prague
Street: Technicka 4
City: Prague
Country: Czech Republic
Email: Lukas.Horny@fs.cvut.cz