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AUTOMATIC IDENTIFICATION OF THE COLLAGEN ORGANIZATION IN THE VASCULAR WALL FROM HISTOLOGICAL STAINS

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SUMMARY

In this study a new automatic method for quantitative capturing of fiber distribution in the arterial wall is presented. Method is based on Fast Fourier Transform (FFT). Powering and re-normalizing of the obtained histogram is the key step in the proposed method. We demonstrated that this simple operation allows us to improve the original qualitative results so that they represent now the true fiber distribution even quantitatively. The proposed method is validated against real images analyzed via classical FFT approach. All results confirm practical applicability of the proposed method for quantitative capturing of the distribution of collagen fibers in the arterial wall.

INTRODUCTION

Detailed micro-histological knowledge of arterial wall is crucial to develop vascular constitutive models, i.e. mathematical descriptions of biomechanical wall properties[1]. Most important, histo-mechanical constitutive models allocate macroscopic stress to the different micro-structural components and, hence, are able to link the macroscopic loading state with potential cellular responses involved in wall remodeling, for example.

FFT –based methods are commonly used for fiber analyses [2,3] and so far they are able to provide reliable information about dominant directions in the image but not to capture the dispersion.

The objective of the present study is to develop a novel FFT-based method that is able to extract quantitative collagen fiber distribution from histological images, i.e. to compute the orientation density of collagen fibers.

METHODS

FFT is applied to each image so it gives the amplitude spectrum which is divided into 180 sectors, each 1° wide, and pixel values in each sector are summed.

It is noted that the center of the FFT spectrum represents the lowest frequencies, whereas higher frequencies are represented by points more distant from the center. Low frequencies refer to the average brightness of the image and its roughest components, while high frequencies represent the finest details (and noise) in the image. This allows deciding whether to investigate the fiber waviness or the principal fiber orientation.

Powering and re-normalizing of the obtained histogram is the key step in the image analysis.

$$s(\alpha) = h^w(\alpha) \quad for\ 0 \leq \alpha < 180° \tag{1}$$

Here w is the power applied to the original histogram $h(\alpha)$. Then the modified histogram $s(\alpha)$ was normalized, which in turn defined the orientation density function of the fiber distribution. Clearly, powering with $w>1$ enhances high values and suppresses low values of $h(\alpha)$. The higher power was applied, i.e. the higher the parameter w was set, the more noise (low values) was suppressed while the peaks were simultaneously enhanced. Unfortunately, setting the w value depends on the quality of the image, nature of the investigated structure and also on the analysis objective to be achieved.

The above concept was applied to histological stains in order to quantify the collagen organization in the wall of abdominal aortic aneurysms (AAA). To this end the coefficient of determination R^2 value was used as a quality measure.

In the first step, a single wide-field image (magnification: 20; pixel size: 0.392μm; image size: 2592x1944; see **Chyba! Nenalezen zdroj odkazů.**top) was taken. In the second step, the same area of the histological slice that was pictured during step 1, was taken through some 600 sub-images (magnification: 500; pixel size: 0.062 μm; image size: 1280x960). Each of these sub-images was analyzed separately by a classical FFT [2], i.e. the w value was set to 1. It was assumed that all fibers in the sub-image have the same orientation, and the angle corresponding to the highest probability was stored in a histogram with a class width of 1°. Finally, the obtained histogram was smoothed (floating average with the base of 5) and normalized. Solid line in Figure 1bottom illustrates the resulting distribution, which was then considered as ground truth information, i.e. used to

identify the *w* value using eq.(1) that led to the best fit with the modified FFT method described above.

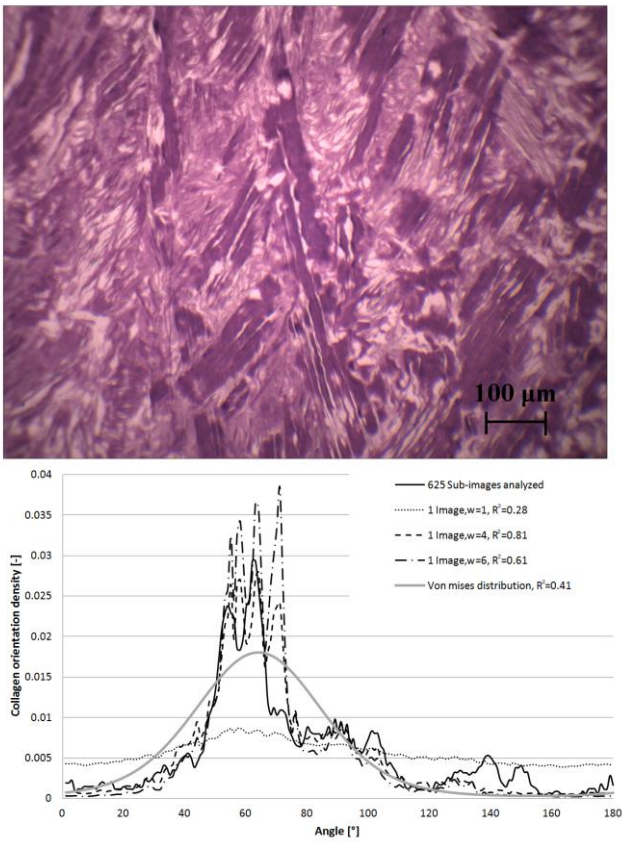

Figure 1. Top - one of the analyzed images of aneurysmal wall stained by Elastica van Gieson. Bottom - effect of the *w* value introduced in eq.(1) on the computed collagen orientation density (dashed lines) of the presented image. Ground-truth data from a sub-image analysis. (solid line). Best correlation is given for *w=4*.

The above outlined method was applied to 8 images in total and the best *w* value was then confirmed by a set of paired t-tests.

RESULTS AND DISCUSSION

Figure 1 shows clearly that the histogram without powering (*w =1*, dotted line) cannot capture the collagen distribution while a proper *w* value can increase the correlation up to R^2=0.8. For comparison we show also the best fit of von Mises distribution (grey line) which is commonly applied in material models to describe the anisotropy of fibers. For the other investigated images the results were similar with the proper *w* value *w=3 or 4.* It was confirmed by a statistical analysis that the results obtained with values *w=3 or 4* give a statistically equal accuracy, while the results achieved with the lower or higher *w* values are always significantly worse.

CONCLUSIONS

The classical FFT was modified, such that it facilitates a quantitative analysis of fiber organizations, i.e. allows not only measuring the dominant fiber orientations as in the previous studies [2,3] but provides also correct information about dispersion. The accuracy reached here is higher than currently needed by most of material models [1]. Finally, the proposed method is fast and operator independent.

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