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# AUTOMATIC EVALUATION OF COLLAGEN FIBRE DIRECTIONS FROM POLARIZED LIGHT MICROSCOPY IMAGES

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## Introduction

Polarized light microscopy (PLM) is generally considered as a powerful method for detecting and describing anisotropy of any specimens which turn the plane of polarized light. This method is also widely used for detecting directions of collagen fibres in the arterial wall [1], [2]. The PLM exploits birefringent feature of collagen enhanced by its staining with Picrosirius red, see Figure 1. However, manual measurement of the fibre direction takes a lot of time; its detection in one point of the image took us approximately 45s. Therefore most studies present a very limited number of measured points per sample, typically less than 50 [1], [[2]. Therefore we **proposed an automated procedure** based on phase correlation [3] to evaluate in-plane collagen fibre orientations from PLM images. Moreover, this procedure eliminates the operator dependence of the manual measurements.

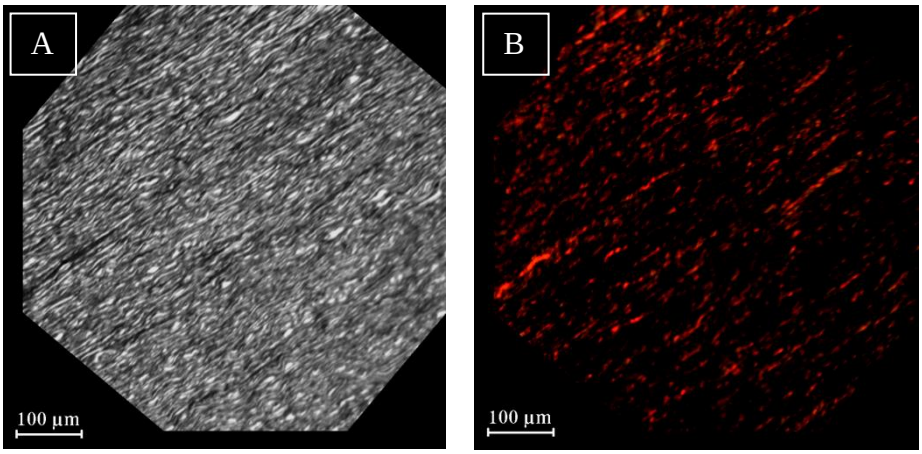


**Figure 1. A) Investigated image stained with Picrosirius red captured using standart light, B) The same image as A, captured using polarized light.**

## Methods

Six porcine aortas were harvested, and an axial (5µm thick) cut from each was stained using Picrosirius red. We captured three images of the chosen area rotated clockwise by an arbitrary angle using both standard and polarized light (magnification 100x, resolution 960x960px).

Standard light images (SLI) were used to determine rotation angle and shift between individual images using phase correlation. Then we rotated and shifted also polarized light images (PLI) in the same manner so all PLIs displayed with varying pixel intensities (due to rotation). In the next step, each PLI was divided into small areas of interest (10x10px) which were further considered as points. In each of these points we obtained 3 intensities for 3 angles. Next, we fitted these three values using sin function and determined the minimum of this function which corresponds to the angle of collagen fibres (principal material direction) in the investigated point. This approach was validated against manual measurements of 2 operators. Each of them measured manually 100 points for 3 different sets of PLIs.

**Results and discussion**

The average angle deviation between operators and the algorithm was $-9.3° \pm 8.6°$ and $-3.8° \pm 8.6°$, respectively. It is evident that the operator related error is comparable with the deviation between operator and the proposed algorithm. Standard deviation was the same for both operators so they differ only in the mean value. Remarkably both operators achieve negative mean difference which means that they measured lower angles than determined by the proposed approach. This can be explained by limited human eye sensitivity to the light intensity. In other words, point appearing already black for the human eye had generally some residual intensity therefore our algorithm continued to rotate and obtained a larger angle value.

**Conclusion**

We proposed a new procedure for evaluating directions of collagen fibres on the basis of phase correlation between images [3]. This **approach is several orders faster** than manual measurement. Although some 3000 points were measured in each set of PLI, our evaluation took only 20 minutes with a standard PC, including the time for capturing the images. Manual measuring of the same number of points would take several days of work. It also means that the proposed approach is able to provide not only qualitative but also quantitative insight on the collagen fibre distribution.

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