

# USE OF CONFOCAL MICROSCOPE IMAGES FOR IMPROVING AORTIC VALVES MECHANICAL PROPERTIES CHARACTERIZATION

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## Introduction

The aortic valves (AV) have an oriented network of collagen fibers embedded in an elastin matrix. This architecture is responsible for their hyperelastic and anisotropic behaviors. Non-linear transverse isotropic constitutive equations are often used to model them, assuming a macroscopically-identifiable preferred fiber direction. In previous studies [1], a new method was proposed to estimate relevant material and structural properties of AV while reducing the number of complex and time-consuming experiments. An inverse analysis procedure based on a series of finite element simulations of planar biaxial tensile tests was first used to set up a reduced protocol. Using real samples, we highlighted the necessity to include local information, such as local strain fields, in order to get relevant parameters of such anisotropic soft tissues. At that time, the same fiber orientation was assigned to each element. The aim of the current work was to use confocal microscopy to obtain local planar angular collagen fibers distribution in the fibrosa layer in order to assess or not the previous assumption and to improve AV mechanical properties characterization.

## Methods

**Biaxial testing experiments:** AV leaflets were excised from fresh porcine hearts. For each specimen, one square sample of about 10 mm width was isolated from the central area of the leaflet. A homemade rake-based planar biaxial tensile testing device was used to apply seven loading conditions (difference force threshold ratios were applied on each axis). In order to capture local strain using full-field measurement, a speckle pattern was made on the surface of the sample as shown on figure 1a.

**Fibers orientation measurement:** Confocal laser scanning microscopy enables the non-destructive high-resolution imaging of AV. This was performed on the same sample as the one previously used for the biaxial experiment. The sample was glued to the bottom of a Petri dish filled with a 0.9% isotonic saline solution, in its undeformed state. Cyanoacrylate glue was applied on the ventricularis side so that the fibrosa layer can be easily observed. Sample was manually moved under the objective. The red spots on figure 2a show approximately the locations where fibers orientation measurements were performed.

**Parameters identification:** An inverse analysis approach was used to identify the rheological parameters, first without the fibers orientation (only thanks to the biaxial tensile test force-displacement curves and 3D-Digital Image Correlation strain field

measurements) and secondly by taking them into account.

## Results

Highly heterogeneous strain fields were obtained during the biaxial tensile tests, for all loading conditions (Figure 1b). Local strain concentration near the hooks does not seem to strongly affect the central area of the sample.

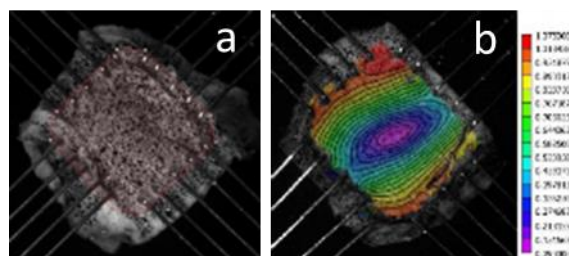


Figure 1: (a) Speckle pattern and analyzed area (in red); (b) norm of the displacement field (mm)

Confocal microscopy showed that once the fibrosa is reached, the collagen fibers orientation becomes quite homogeneous between 110 and 140  $\mu\text{m}$  depth (figure 2b). Fibers are mainly oriented in the focal plane.

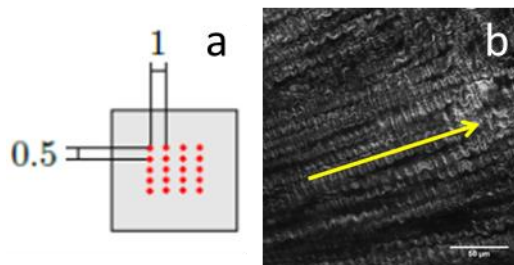


Figure 2: (a) areas of analysis with multiphoton microscopy; (b) yellow arrow shows the main collagen fibers orientation for the selected picture

## Results and discussion

Taking account the fibers orientation during the inverse analysis step clearly speeded up and improved the identification of the mechanical parameters, even if the areas where measurements have been performed were insufficient, which forced us to interpolate data between the different measurement locations.

## References

1. Laville et al., Comput Methods Biomech Biomed Engin, 18sup1:1976-1977, 2015

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