

## Heart valve heterogeneity

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# Heart valve heterogeneity

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## Introduction

Current research on heart valve tissue engineering focusses on the macro- (overall structure and performance) and micro-level (local tissue remodeling and underlying mechanisms) (figure 1). To couple these properties, more information on the meso-level (layer related structural and mechanical properties) or heterogeneity of the valves is needed. In view of this, the focuss of this study is to investigate the possibility to separate the distinct valve leaflet layers, and to visualize leaflet structures in 3D.

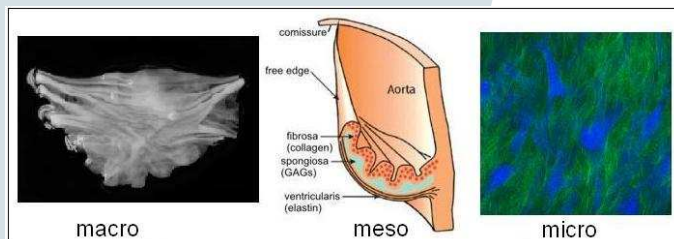


Figure 1 The macro-, meso-, and micro level of heart valves.

## Methods

### layer separation

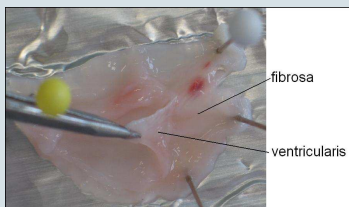


Figure 2 Separation of valve leaflet layers.

Porcine aortic valve leaflets were separated by dissecting the connections between the fibrosa and ventricularis (= spongiosa) (figure 2) [1]. Intact leaflets and individual layers were histologically evaluated using Masson Trichrome staining to confirm separation.

### 3D leaflet visualization

To visualize the 3D collagen structure, porcine aortic valve leaflets were fluorescently stained with CNA35-OGG488, optically cleared, embedded in agarose and scanned using optical projection tomography (OPT) (figure 3) [2].

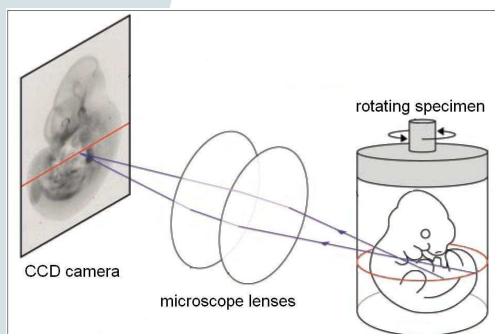


Figure 3 Schematic representation of the OPT system. Light transmitted from the rotating specimen is focused by lenses onto a CCD camera.

## Results

### layer separation

Histological evaluation indicated that the fibrosa and ventricularis were separated successfully (figure 4). Separation of the layers resulted in unwrinkling of the fibrosa, and swelling of the layers, probably due to exposure of the GAG-rich spongiosa to a moisturized environment.

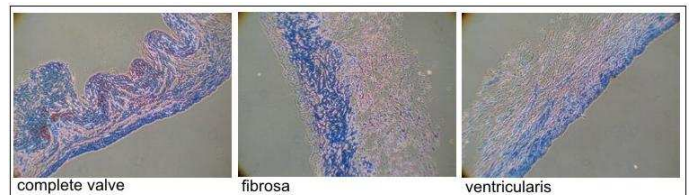


Figure 4 Histology sections, stained with Masson Trichrome, of a complete leaflet and separated fibrosa and ventricularis (blue = collagen, red = muscle; magnification 10x).

### 3D leaflet visualization

OPT images showed only staining of collagen at the edges of the leaflet (figure 5), probably due to poor probe diffusion, or over-staining of the outer regions of the specimen. Within the stained regions, no specific structures could be distinguished.

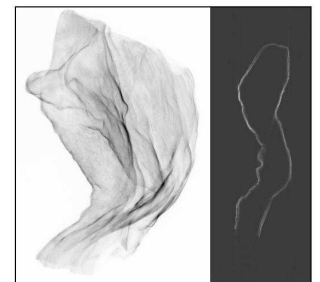


Figure 5 3D image (left) and reconstructed cross-section (right) of collagen in a valve leaflet, visualized by OPT.

## Conclusion and future plans

The dissection method to separate distinct valve layers has shown to be feasible. For imaging 3D leaflet structures, OPT may be a promising technique, but requires protocol optimization. Applicability of other imaging modalities (OCT, MRI, NIR) will be explored.

To couple local valve properties to overall performance, future research will furthermore focuss on biochemical (collagen types, elastin) and mechanical (mechanical properties, anisotropy) layer characterization.

Results will be coupled in a prediction model for valve functionality, which can be used for the development of pre-implantation criteria for tissue engineered heart valves.

### References:

- [1] I. VESELY AND R. NOSEWORTHY *ASAIO J.* 1996; 42(5): 739-746
- [2] J. SHARPE ET AL. *Science* 2002; 19(296): 541-545