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The heart in 4D: novel tools to quantify ECM dynamics and tissue morphology in cardiac development

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Abstract Text

Heart morphogenesis is a complex asymmetric process that requires the timely coordination of distinct events to form a mature heart. One of the most important stages in heart development occurs when the linear heart tube undergoes a series of morphological rearrangements known as cardiac looping and ballooning. Disruptions in these processes can cause cardiac malformations characterised by defects in heart morphology and chamber misalignment. These defects are challenging to characterise using traditional two-dimensional approaches, consequently, new studies have started to develop diverse three-dimensional (3D) quantification methods to better define cardiac development[1].

During development, the vertebrate heart tube is formed by an outer layer of myocardial cells and an inner layer of specialised endothelial cells (endocardium), between which lies a layer of extracellular matrix (ECM) known as the cardiac jelly. Recent studies have demonstrated that during development the ECM is a dynamic structure, and regionalised processes such as ECM deposition, remodelling and degradation are crucial to spatially fine-tune organ morphology[2].

To understand the morphogenetic events and underlying mechanisms that drive cardiac morphogenesis, the zebrafish has been shown to be a valuable vertebrate model. Using live in vivo light-sheet imaging of zebrafish embryos, we observe an asymmetric expansion of the cardiac jelly on the left side of the heart tube at the onset of cardiac looping. We hypothesise that early differences in the

composition and regionalisation of the ECM may promote cardiac morphogenesis. To understand the spatiotemporal dynamics of the ECM throughout heart development and its role in cardiac morphogenesis, an image analysis pipeline was developed that allows the segmentation of the ECM from **negative space** by simultaneously imaging the myocardium and endocardium.

High-resolution live images of arrested zebrafish hearts that express actin-tagged GFP in the myocardium and actin-localised RFP in the endocardium were imaged in the light-sheet microscope (A-A'). To remove noise artefacts and enhance layer borders, channels were pre-processed and filtered using arivis Vision4D and Fiji (B-B''). Individual slices making up each filtered channel go through a process of contour detection to extract all the contours that delineate each tissue completely (C-C''). To segment the cardiac jelly, the contours that outline the lumen of the myocardium and the external face of the endocardium are used to create a mask containing the ECM (D). Resulting contour-masks are finally used to render volumetric meshes of each tissue layer (E-E''), where tissue distribution and morphology can be characterised (F-F''). 3D renders of the ECM at different developmental stages confirm the cardiac jelly undergoes dynamic regionalised remodelling during morphogenesis.

This pipeline provides the **first 3D reconstruction of the ECM** in the developing heart. In addition, it allows not only an in-depth description of the heart and its tissue layers as it develops but also of cardiac abnormalities in ways not previously possible. Moreover, this detailed 3D description of cardiac jelly distribution and regionalisation through time, combined with functional analysis of its various components, will provide invaluable insights into ECM dynamics, and deepen our understanding of its role in the context of cardiac morphogenesis.

A Image Acquisition

B Pre-processing and Filtering

C Segmentation

Myocardium & **Endocardium**

Close tissue contours

Select tissue contours

Cardiac jelly

Myocardial filled internal contours

XOR

Endocardial filled external contours

result

Cardiac jelly contours

mask   mask

E 3-Dimensional Reconstruction

Tissue volume reconstruction

F 3-Dimensional Analysis of the Heart and its Comprising Layers

A′ Imaging of arrested heart

Light-sheet chamber

B′ *Tg*(*myl7:lifeActGFP*)
Tg(*fli1a:AC-TagRFP*)

B″

C′ **Myocardial Channel**

Slice number | [i] Selected tissue contours | [ii] Filled internal contours | [iii] Filled external contours | [iv] Tissue layer contours

090

145

C″ **Endocardial Channel**

[i] Selected tissue contours | [ii] Filled internal contours | [iii] Filled external contours | [iv] Tissue layer contours

E′ Myocardial volume   Endocardial volume

Anterior
Left
Ventral

D **Cardiac Jelly**
Slice number
090   145

E″ Cardiac jelly volume

F′ **Morphological Analysis**

Ventral view   Left view

Chamber orientation, size and shape

F″ **Cardiac Jelly Validation** → **ECM Distribution Analysis**

ECM: layer in between the myocardial and endocardial tissue layers

Thickness heat map [um]

0.0   18.0