Measuring the diameter of embryonic arteries: the method & first biometric data

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This presentation introduces a method for conducting highly significant measurements of the diameters of multiple blood vessels along the complex blood vessel tress of embryos of biomedical model organisms. Beside describing and discussing the method, it also presents results from applying the method for measuring the diameter of the great intrathoracal arteries of 30 mouse embryos (OF1 strain) of developmental stage (Theiler stage, TS [1]) 22. It also presents evaluations of the accuracy and reproducibility of these measurements.

Background: For researching the genetic factors underlying cardiovascular malformations, mouse lines lacking functional genes for driving essential steps in cardiovascular morphogenesis are designed. Often the severity of the cardiovascular defects in homozygous offsprings causes prenatal death and enforces morphological analysis of embryos [2, 3]. Especially the diagnosis of stenosis or dilation of embryonic blood vessels is a major challenge. Significant measurements of the diameter of embryonic blood vessels can only be created from high resolution, high quality digital volume data. Only such data permit cutting virtual resections perpendicular (in respect to all spatial directions) to the longitudinal axis of multiple embryonic blood vessels at comparable positions.

Goal: We aim at presenting a method, which fits for measuring the diameter of the blood vessels of mouse embryos and fetus. We further aim to present first biometric reference data of the lumen of the great intra-thoracal arteries of early mouse fetus by using this method and to evaluate the significance and the accuracy of the obtained measurements.

Material & Method: We harvested mouse fetus of the Him:OF1 strain at 14.5 days post conceptionem (dpc) and staged them according to Theiler [1]. The great arteries of 30 fetus of early to late Theiler stage 22 were measured. The thoraces of the embryos were separated and subjected to high resolution episcopic microscopy (HREM). HREM [3] is an episcopic data generation method, which generates volume data of a near histological quality and a voxel size of 2µm x 2µm x 2µm. 3D models of the blood vessels were created with the volume and surface rendering tools of the software package Amira (Mercury systems, Figure1). These models were used for defining comparable positions along the pulmonary trunk, the ascending aorta and the descending aorta immediately distal to the connection with the ductus arteriosus. Then the models were toggled to define a virtual resection plane through the original volume data, which, at the measurement position cut through the blood vessel perpendicularily to its longitudinal axis (Figure1). The perimeter of the blood vessel lumen was measured and used for calculating the diameter. Statistics were preformed with the software packages Excel (Microsoft) and SAS (www.sas.com).

Results: We present: Firstly, biometric data describing the lumen diameters of the measured blood vessels (pulmonary trunk: mean = 148.1µm, range = 102µm – 207.8µm; ascending aorta: mean = 139.6µm, range = 96.5µm – 216.4µm, descending aorta: mean = 160.8µm, range = 104.6µm – 218.3µm) and secondly, evaluations of the significance and

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accuracy of the proposed method. We evaluated three aspects: 1) We tested the accuracy of tracing blood vessel lumina and asked one researcher to trace and calculate the diameter of the ascending aorta and pulmonary trunk in virtual resections of 20 mouse embryos for 20 times. A break of at least 1 hour was held between each measurement cycle. The values obtained differed less than 3 pixels. 2) We tested the bias introduced by the subjective definition of blood vessel borders and handed ten researchers identical virtual reslice images of the pulmonary trunk. They were asked to trace the perimeter of the ascending aorta and pulmonary trunk and to calculate the diameter. Their results differed less than 4 pixels. 3) For testing the interindividual differences in defining the correct resection plane, 10 researchers received the volume data and 3D models of the pulmonary trunk of 20 mouse embryos. They defined comparable resection planes and reoriented the 3D model to allow cutting perpendicular to the longitudinal axis of the pulmonary trunk. The measurements differed less than 11 pixels. 75% of the measurements differed less then 6 pixels.

Conclusions: We present biometric reference data of the lumina of the great intrathoracal arteries of early mouse fetus. These data can be used for identifying stenosis or dilation in genetically engineered mouse lines bred on the OF1 genetic background. We also present evaluations of our measuring method. These evaluations reveal that high-resolution of the volume data is essential for obtaining significant measurements. An approximate measurement error of 7 pixels must be taken into account. Therefore the diameter of the pulmonary trunk of TS22 mouse fetus (mean diameter of 140µm to 150µm) can be measured with an accuracy of approximately +5% in volume data with 2µm x 2µm x 2µm voxel size (HREM data). However, the same diameter can be only measured with an accuracy of +15% in optical projection tomography (OPT) data of 6µm x 6µm x 6µm voxel size and +25% in magnetic resonance imaging (MRI) data of 10µm x 10µm x 10µm voxel size.

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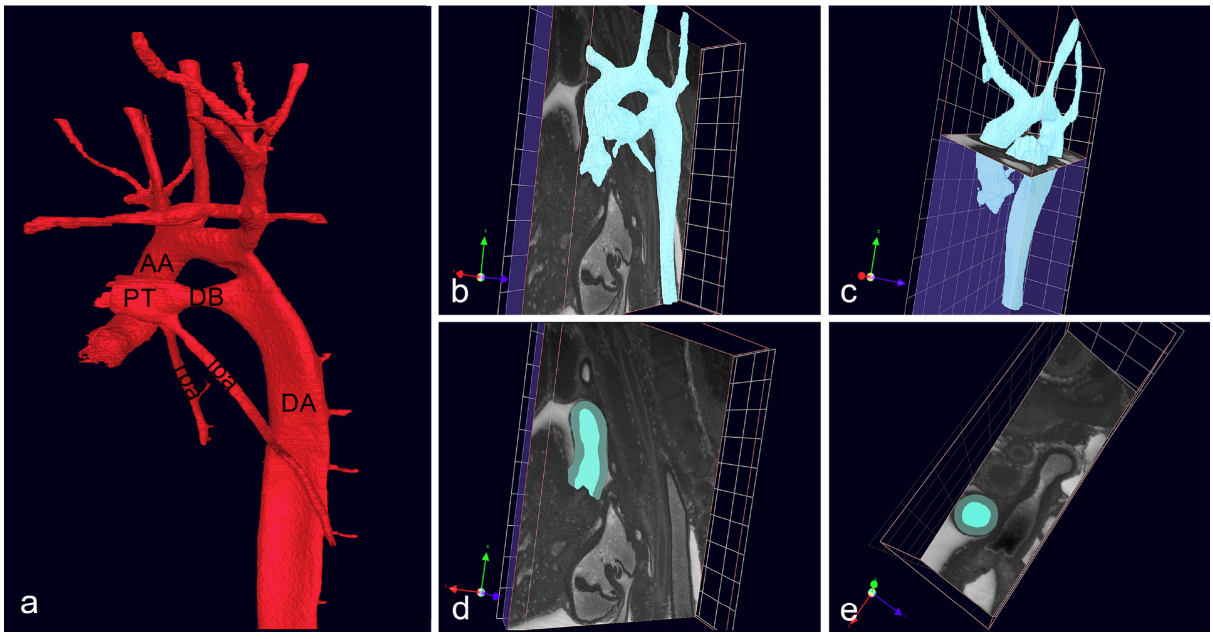

Figure 1. HREM volume data and 3 computer models of the great intra-thoracal arteries of TS22 mouse embryos. a. 3D model b-e. Steps in generating the virtual resection plane and in measuring the perimeter of the ascending aorta.