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# Exploring damaged elastic arteries by Synchrotron X-ray Micro-CT

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## 1. Introduction

Diabetes is a chronic pathology affecting about 10% of the world population. It accelerates arterial stiffness and increases the probability of cardiovascular diseases. The effect of diabetes on the stiffness of the vascular system has been broadly estimated to correspond to 6–15 years of chronological aging (Vatner et al. 2021). This pathology is mainly characterized by an important level of blood glucose leading to the formation of cross-links within elastin fibers and collagens, resulting in a decrease of arterial wall distensibility. Large arteries such as the aorta contain, in their extracellular matrix, an amorphous elastic hydrophobic polymer named elastin, which is the major component of elastic fibers. Its entropic elasticity and its concentric lamellar organization within the aortic wall allow the aorta to remain in a reversible deformation domain during the cardiac cycle, which alternate phases of overpressure and contraction. Alterations to elastin due to a pathological situation or aging permanently affect the arterial mechanics. Synchrotron X-ray microtomography ( $\mu$ -CT) enables imaging of large sample volumes with sub-micrometric resolution associated to millimeter size field (Weitkamp et al. 2020; Ben Zemzem et al. 2021). We have developed a protocol to harvest mice aorta, with a limited collapse, for  $\mu$ -CT imaging.

## 2. Methods

### 2.1 Animals and Sample Preparation

Mouse procedures were authorized and realized in accordance with local laws (agreement D-51-454-2). The mice, 6-month-old C57BL6J (control,  $n = 4$ ) and 6-month-old *db/db* (diabetic,  $n = 6$ ), were purchased from C. River and share the same genetic background.

Aortas were collected after euthanasia and heparin injection, the heart and aorta were then flushed with phosphate buffered saline to remove residual blood. The aorta was further prefixed by injecting 4% formalin then filled of 1% low melting agarose to keep the aorta opened and to prevent collapse during dehydration and inclusion steps. Gel solidification in the lumen exerts a counterpressure to avoid collapse. The heart and aorta were collected with surrounding tissues. Finally, the samples were fixed in 4% formalin, dehydrated, and embedded in paraffin. The final samples were about 4-cm-long paraffin rods containing the heart and aorta.

### 2.3 Imaging Procedures

Synchrotron X-ray  $\mu$ -CT was performed on the ANATOMIX beamline (SOLEIL synchrotron, Gif-sur-Yvette, France) with an effective pixel size of 0.65  $\mu\text{m}$  at the sample level. Tomographic reconstruction was performed using the standard processing pipeline at the beamline. The reconstructed volume stacks for each scan contained 2048  $\times$  2048  $\times$  2048 voxels of size (0.65  $\mu\text{m}$ )<sup>3</sup>, giving a 32 GB file per stack.

### 2.4 Arterial Structure Segmentation

Arterial structures were segmented automatically from reconstructed stacks with bespoke Matlab scripts as previously described (Ben Zemzem et al. 2021). The result of this segmentation for each tomography volume scan is a 2048-image 3D stack containing 8-bit voxels of the arterial wall.

### 2.5 Reserve Length of Elastic Lamellae

Elastic lamellae are first “fragmented” using thresholding. Typically, about 15–30 fragments are kept per image, leading to about 2,000–30,000 fragments per stack. 10 images stacks were retained for control mice, 11 for diabetic mice.

## 3. Results and Discussion

Figure 1a shows a typical reconstructed raw 2D image plane extracted from a synchrotron X-ray  $\mu$ -CT acquisition ( $2048^3$  voxels) at  $0.65 \mu\text{m}/\text{voxel}$  for an aortic segment of a 6-month control mouse. The sample was not sectioned and not stained.

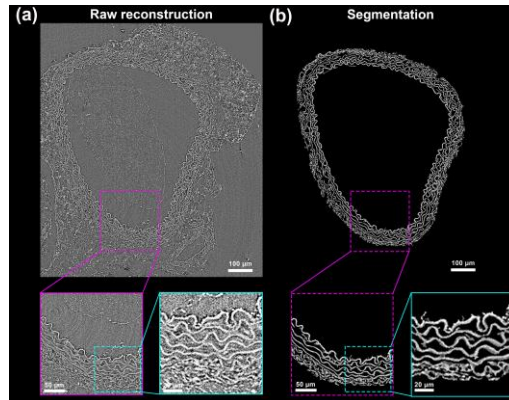


Figure 1. 2D-plane reconstruction from synchrotron acquisition and its automatic segmentation.

The phase-contrast image exhibits the different structures of the aortic environment from the thoracic region. Figure 1b shows an automatic segmentation of elastic structures by our program.

Elastic lamellae are altered in diabetic mice (Fig. 2 upper row versus lower row). The control presents a wavy aspect indicating a reserve length. This reserve length allows a spring to be stretched above its apparent length, i.e., its end-to-end distance. If a spring is stretched too much or altered, it loses its reserve and its ability to be reversibly deformed when overstretched. To assess the reserve length of these lamellae, we compute, for a given segment, the ratio ( $r$ ) between its Euclidean (i.e., end-to-end) and geodesic lengths (i.e., its real length). 127,180 measurements were made on control mice ( $r=0.823$ ,  $s=0.047$ ), 159,485 on diabetic mice ( $r=0.889$ ,  $s=0.026$ ) to have an exhaustive view over the length, for each elastic lamella and in each orientation. Our analysis shows that diabetic mice have lost a significant part ( $p<0.004$ ) of their reserve length (59.5 % less) as compared to the control group (Ben Zemzem et al. 2022).

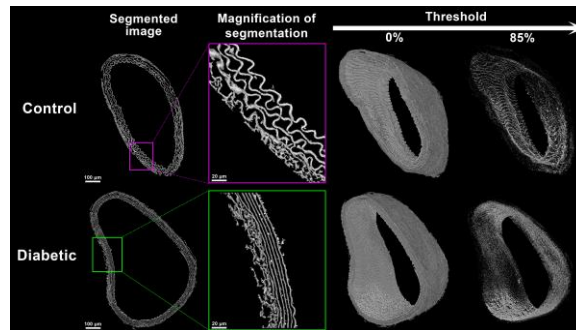


Figure 2. Comparison of control (upper row) and diabetic (lower row) elastic structures in one 2D plane (left) versus 3D perspective of 800 planes (right).

We have previously shown (Ben Zemzem et al. 2021) that an inner lattice-like network (Fig 2., upper right image) is present inside the elastic structure, to which it seems to confer flexibility and resistance. 6-month-old diabetic mice exhibit a drastic destruction of this network (Fig 2., lower right image) in comparison of normal mice of the same age, suggesting a structural role for this lattice-like network.

To go further in our study, we have developed an approach combining standard image analysis and deep learning approaches to segment and separate finely the elastic lamellae to study them one by one, associated with the capabilities of the ROMEO supercomputer (Reims), allows us to extract parameters such as local geometry (thickness, density, local curvature) and the geometry of arteriola branching.

## 4. Conclusions

In-line phase-contrast synchrotron X-ray microtomography of the arterial wall with label-free tissues grants access to fine details of elastic structures. The strength of this method lies in its large field, its excellent resolution, and a good contrast for intramural elastic structures. Coupled to an automatic image analysis program, this technique provides information that cannot be obtained using other imaging methods. Moreover, for each sample, it allows the computation of thousands of measurements for a given feature, such as the ratio between Euclidean and geodesic distances of a segment of an elastic lamella.

Our results show that the reserve length of elastic lamellae is reduced in diabetic mice together with the loss of filaments that support the wall. This suggests that elasticity is correlated to the existence of this lattice-like network that has disappeared at 6 months of diabetes.

The challenge is to extract measurements that allow us to accurately estimate the physiological age of the artery. We plan to develop a pressurization system to image and measure the deformation of arterial wall structures under X-ray beam, so as to decipher the link between small scale alterations and their biomechanical incidence. Further, as the methods we use allow us to make contiguous tomographic scans, we can merged them into a global view of the murine arterial network. This aorta envelope will be used to model the blood flow inside this deformable network.

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