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# X-ray micro computed tomography for the visualization of an atherosclerotic human coronary artery

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**Abstract.** Atherosclerosis refers to narrowing or blocking of blood vessels that can lead to a heart attack, chest pain or stroke. Constricted segments of diseased arteries exhibit considerably increased wall shear stress, compared to the healthy ones. One of the possibilities to improve patient's treatment is the application of nano-therapeutic approaches, based on shear stress sensitive nano-containers. In order to tailor the chemical composition and subsequent physical properties of such liposomes, one has to know precisely the morphology of critically stenosed arteries at micrometre resolution. It is often obtained by means of histology, which has the drawback of offering only two-dimensional information. Additionally, it requires the artery to be decalcified before sectioning, which might lead to deformations within the tissue. Micro computed tomography ( $\mu$ CT) enables the three-dimensional (3D) visualization of soft and hard tissues at micrometre level.  $\mu$ CT allows lumen segmentation that is crucial for subsequent flow simulation analysis. In this communication, tomographic images of a human coronary artery before and after decalcification are qualitatively and quantitatively compared. We analyse the cross section of the diseased human coronary artery before and after decalcification, and calculate the lumen area of both samples.

## 1. Introduction

The development of atherosclerosis is a complex process. It causes thickening of the vessel wall and a constriction of the vessel lumen that can block the blood flow for example known as heart attack or stroke. To counter the constriction in case of an infarct, vasodilator drugs are often employed. The first aid ambulance medication given to the patient includes intravenous injection of nitroglycerine to treat clotted blood vessels and to restore the blood flow in critically constricted parts of the arteries. Systemic administration of such vasodilator, however, leads to undesired hypotension and decreased blood perfusion of the heart. Up to 15 or even 20 % of people struck by heart attack die before arriving to hospital. Critically stenosed segments of arteries (e.g. 80 % stenosis) reveal an at least 10 times higher wall shear stress, compared to the healthy parts [1]. One can take advantage of such blood flow-specific changes and employ them as purely mechanical trigger for the delivery of vasodilators. The therapeutic approach focused on the application of shear stress sensitive nano-containers, which become mechanically activated by shear forces, has been proposed [2, 3]. Such nanometre-sized lipid-based liposomes can offer local, rapid and non-invasive treatment, before the patient reaches the hospital.

To optimize the mechano-sensitive liposomes, the morphology of atherosclerotic vessels including its lumen is required. These data can be used to simulate the blood flow [4]. The standard procedure of characterization is histology, which provides sub-micrometre resolution in the lateral dimensions.



The tissue morphology, however, can be altered owing to preparation artefacts, which can originate from decalcification, cutting and embedding, leading to tissue shrinkage or deformation. In particular, the histological evaluation provides only limited information in third dimension. This drawback significantly limits the application in blood flow simulations. X-ray tomography can master this challenge, providing non-destructive, quantitative 3D visualization of both, soft and hard tissues containing specimens [4-9]. A variety of operation modes of  $\mu$ CT can be applied for specimen characterization, depending on the defined task and type of information required. One of them is absorption contrast, which is mostly used to discriminate anatomical structures with reasonable differences in X-ray absorption, including the determination of plaque morphology within artery and to determine the extent of stenosis resulting in lumen segmentation. X-ray absorption is described by the imaginary part of the refractive index  $\beta$ , which is related to the attenuation coefficient [6]. Since the difference in absorption contrast within soft tissues is weak, differentiation among tissue types is challenging. Therefore, phase contrast (PC) modalities are preferred for such purpose. Here, the decrement of the real part of the refractive index is measured, which is two to three orders of magnitude larger than  $\beta$  at the X-ray energies considered. Thus, PC modalities provide significantly improved contrast for soft tissue differentiation [5, 7, 8] compared to the absorption contrast mode.

In this study, we present the tomography data of a diseased human coronary artery, acquired using conventional laboratory  $\mu$ CT in absorption contrast mode. A human artery containing a calcified atherosclerotic plaque was measured before and after the decalcification process to evaluate the cross section of the lumen, to calculate the non-constricted area of the artery, and to determine the cross-section changes due to decalcification procedure.

## 2. Materials and methods

### 2.1. Human coronary artery preparation

The artery was obtained from a donated body (Institute of Anatomy, University of Basel). The responsible Ethical Committee approved the request and written consent of the donor has been obtained. A 2.2 cm-long section was cut from the atherosclerotic human coronary artery. The artery was cleaned from surrounding tissues using a scalpel and fixed in 4 % paraformaldehyde (PFA). Afterwards, the sample was dehydrated by soaking, firstly, in ethanol, then in xylene overnight at reduced pressure (Tissue-Tek VIP E300 Histokinetic automated dehydrator, Sakura). Subsequently, the artery was immersed in molten paraffin at a temperature of 60 °C. After the paraffin solidification, the shape and size of the block were adjusted with a scalpel to the size of artery-containing region.

To decalcify the sample, the paraffin embedded artery was heated to a temperature of 60 °C and immersed in decalcifier solution (distilled water: formic acid: PFA, 87: 8: 5 v/v) at a temperature of 37 °C for a period of two days. The dehydration steps were repeated as described above to re-embed.

### 2.2. Advanced laboratory X-ray microtomography system

The laboratory  $\mu$ CT system nanotom<sup>®</sup> m (Phoenixlx-ray, GE Sensing & Inspection Technologies GmbH, Wunstorf, Germany) operating in absorption contrast mode was used to visualize the calcified and decalcified parts of the human artery. The instrument is equipped with a 180 kV/15 W nanofocus X-ray source with a W target. An accelerating voltage of 60 kV, which results in a mean photon energy of 40 to 50 keV, and a beam current of 310  $\mu$ A were used. The sample was mounted on a high-precision manipulator and rotated through the incoming X-ray beam. During the rotation over an angular range of 360° 1,000 equiangular projections were recorded. For each projection three images were acquired with a total exposure time of 3.0 s (calcified sample) and 3.5 s (decalcified sample), respectively. A pixel size of 17.78  $\mu$ m (calcified sample) and 13.73  $\mu$ m (decalcified sample) as well as a field of view of 1,300  $\times$  2,400 pixels (calcified sample) and 2,000  $\times$  2,400 pixels (decalcified sample) were used, respectively. The acquired projections were reconstructed using the cone-beam, filtered back-projection algorithm of the phoenix datoslx 2.0.1-RTM software. Subsequently, the lumen of the artery was visualized. For the segmentation purpose of the artery lumen, we have selected dedicated slices to start the region-growing algorithm tool in VG Studio Max 2.1 (Volume Graphics, Heidelberg, Germany).

### 3. Results and discussions

#### 3.1. Visualization of the plaque containing coronary artery

The preparation steps during fixation and embedding as well as decalcification can significantly modify the vessel morphology. Therefore, each step has to be carefully investigated. In this work, we visualized the human coronary artery using  $\mu$ CT. It is beneficial, because one can decrease X-ray energy or accelerating voltage, which provides increased contrast between soft tissue components. It allows us to visualize the precise geometry of the vessel wall and observe changes in lumen morphology in comparison to the originally calcified artery.

Figure 1 compares slices of plaque-containing artery (A – top view, C – lateral view) and the same artery after decalcification process (B – top view, D – lateral view). In Figure 1A, the artery walls and plaque are clearly visible, however, the contrast in the surrounding fatty tissue is low. On the contrary, in Figure 1B, better contrast in the soft tissue is present, and the artery wall is clearly visible. However, the hard tissue residuals from the plaque decalcification are still present in the surrounding tissue (shown by yellow arrows). One can also notice the presence of preparation artefacts including air bubble in the left lumen of calcified artery (Figure 1A), and small paraffin cracks inside the lumens of the decalcified artery (Figure 1B).

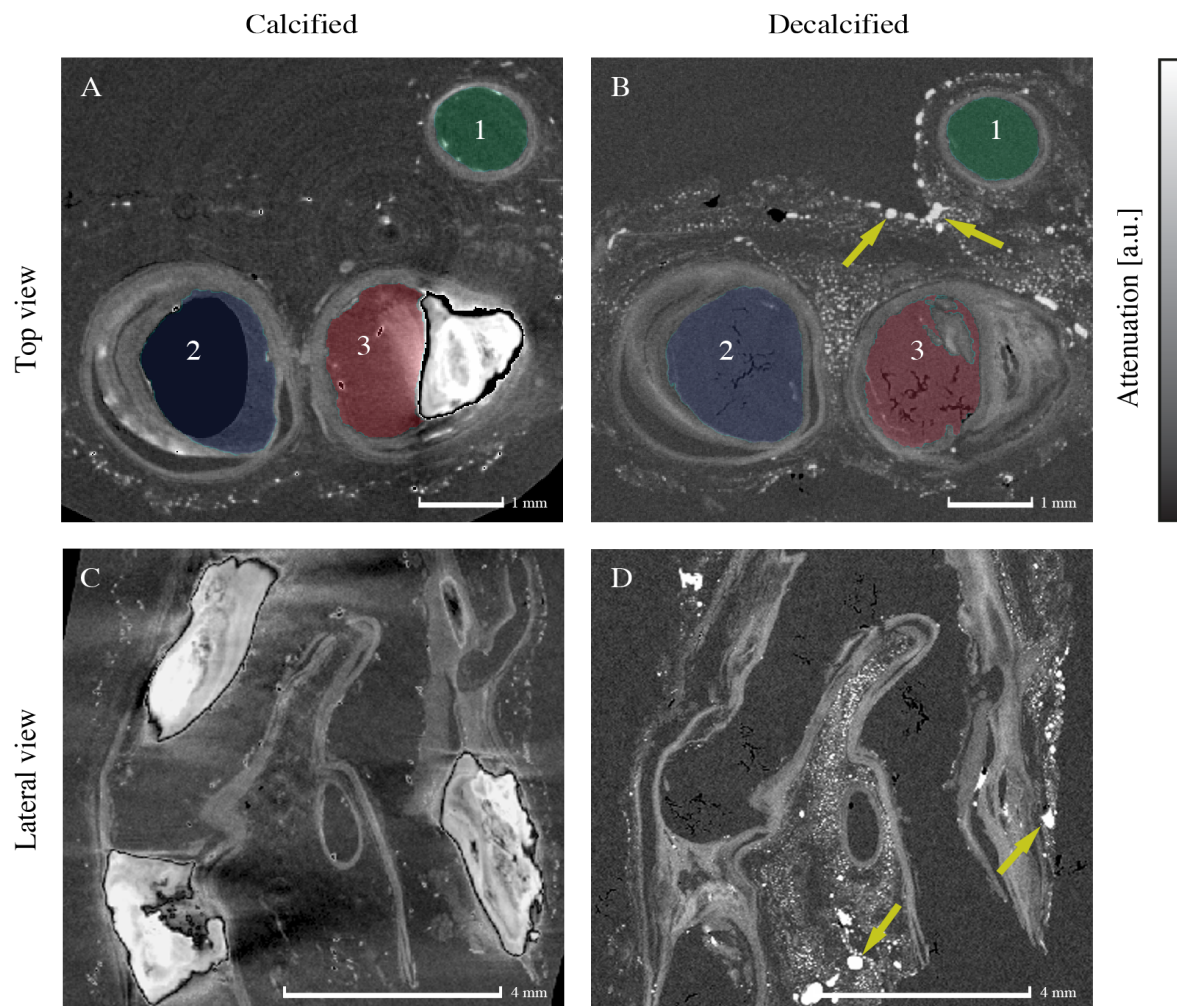


Figure 1. Axial slices of a calcified (A – top view, C – lateral view) and decalcified (B – top view, D – lateral view) human coronary artery embedded in paraffin, measured in absorption contrast  $\mu$ CT. Region 1 of images A and B corresponds to the cross-sectional area of lumen coloured in green, Region 2 coloured in blue and region 3 coloured in red, respectively. Region 3 of image A reveals presence of calcified plaque, depicted in bright white colour.

Streak artefacts (whitish colour on red background) around the highly X-ray absorbing calcified plaque were observed in the calcified sample (Figure 1A). These artefacts overlap with the morphology of the lumen and make segmentation more challenging.

The cross-sectional area of both calcified and decalcified samples was extracted using the region-growing segmentation tool of VG Studio Max 2.1, which is a fast and convenient method to obtain the morphology of the lumen. The lumen area is used for flow simulations. The cross-sectional area was calculated and the results are presented in Table 1.

**Table 1.** The cross-sectional area of the artery lumen.

	Calcified artery area, mm <sup>2</sup>	Decalcified artery area, mm <sup>2</sup>
Region 1 (green)	0.84 ± 0.02	0.80 ± 0.02
Region 2 (blue)	2.42 ± 0.02	2.40 ± 0.02
Region 3 (red)	1.86 ± 0.02	1.82 ± 0.02

Numbers and related colour indicate each region of the artery. The cross-sectional area of the segmented slice was calculated and found to be within the error bars between the two samples before and after decalcification process: from 0.84 to 0.80 mm<sup>2</sup> (Region 1), from 2.42 to 2.40 mm<sup>2</sup> (Region 2), and from 1.86 to 1.82 mm<sup>2</sup> (Region 3). The shrinkage of the artery, hence decrease in all lumen areas, could be caused by the chemical treatment within the decalcification process. The area of Region 3, which contained an atherosclerotic plaque, was not notably decreased at the location of the removed plaque, because the soft tissue was still present. The area of the calcified plaque was calculated to be 1.07 mm<sup>2</sup>, whereas the area of the soft tissue residuals after decalcification was equal to 1.02 mm<sup>2</sup>. Comparing these two slices from calcified and decalcified datasets, the decalcification process does not significantly change the cross section of the lumen.

The authors have realized the surprisingly high contrast for unstained soft tissue imaged. One can reasonably assume that the dehydration is the key factor, as it leads to increased tissue density. This increase gives a rise to the observed attenuation differences. Recently, it has been shown that paraffin-embedded human brain tissue yields significant contrast even in laboratory-based  $\mu$ CT [8].

#### 4. Conclusion

Atherosclerosis is a complex inflammatory disease, related to the damage of endothelium and its inflammation as well as to the accumulation of the lipids and white blood cells, proliferation of intimal-smooth-muscle cells, followed by tissue calcification that lead to a fibro-fatty plaque formation. Here, we show the difference in lumen morphology between calcified and decalcified human coronary artery. We present the quantified cross-sectional area of the lumen region for both samples. In future, it will be beneficial to compare artery lumen areas between samples fixed in water, formalin, paraffin, or after a decalcification process, and histology in order to better understand how each processing affects the artery wall. Micro computed tomography is a valuable tool to study the morphology of diseased atherosclerotic artery, containing soft and hard tissues, as previously demonstrated for formalin fixation of the human brain [10].

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