Non-enhanced micro-CT of paraffin embedded coronary vessels: a tool for experimental atherosclerosis

Poster No.:	C-2353
Congress:	ECR 2013
Type:	Scientific Exhibit
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Keywords:	Vascular, Computer applications, CT-High Resolution, Experimental, Computer Applications-3D, Laboratory tests, Calcifications / Calculi, Arteriosclerosis, Hemodynamics / Flow dynamics
DOI:	10.1594/ecr2013/C-2353

Purpose

Atherosclerosis (ATS) is a chronic proliferative and inflammatory disease characterized by accumulation of lipids, migration-proliferation of synthetic smooth muscle cells (SMCs) and inflammatory cells infiltration in arterial vessel walls, which results in the formation and growth of plaques. The mechanisms which determine local formation of atherosclerotic plaques and their progression in a specific vascular district are only partly known [1].

Endothelial wall shear stress (WSS) is currently accepted as the major predictive factor of plaque location and growth on the basis of strong experimental and clinical evidence that arterial sites with the lowest WSS within an individual artery coincide with the most atherosusceptible. WSS modeling requires precise information about the three-dimensional shape and size of arterial vessels under real physiological conditions; however, physical limitations of current in vivo imaging modalities pose a severe constraint on the actual ability to correlate WSS to the precise plaque localization, shape and extension.

This work aims to assess and quantify morphometric parameters of full-length coronary arteries in three dimensions at high resolution by ex vivo micro-CT (µCT), and to evaluate the possible integration with in vivo information by invasive coronary angiography (ICA) and and intravascular ultrasound (IVUS).

Methods and Materials

Excised left coronary artery (LMCA-LAD) and underlying myocardium from pigs fed with a high-cholesterol diet were fixed in formalin, dehydrated and embedded in paraffin inside a Falcon-type test tube (3 cm of diameter). In vivo information obtained by ICA and IVUS were also available for the same animals.

A micro-CT scanner with variable magnification, built by our group, was used for the experiments [2]. The voltage and current of the x-ray tube were set up to 20 kVp and 0.7 mA, respectively. All the tomographic acquisition were made with 720 projections over 360 degrees, for a total scan time of 54 min and a total exposure of 2268 mAs. Two scans per sample have been done in sequence, resulting in a total axial Field of View (FoV) of 8 cm that was sufficient to cover the entire length of the coronary artery. For each sample, we have obtained a reconstructed volume of 512x512x1400 isotropic voxels, with a voxel size of 57.4^3 μm^3. After the micro-CT scan, the samples were further processed in order to perform the histological examination with hematoxilyn and eosin stain. To this end, the

samples were first removed from the Falcon test tube and disembedded from paraffin. Then, the samples were cut in sections of 5 mm each and embedded again in small blocks of paraffin to allow the sectioning of slices on a microtome.

ImageJ (NIH, Bathesda, USA, ver. 1.47b, http://rsbweb.nih.gov/ij/) was used to crop the image to the minimum volume of interest, thus reducing the space on disk and the amount of memory required for the subsequent analysis. OsiriX (ver. 5.0.1 32 bit, http://www.osirix-viewer.com) was used to perform 3D curved multi-planar reformation (MPR) of the coronary artery, image segmentation and 3D volume renderings (VR).

Images for this section:

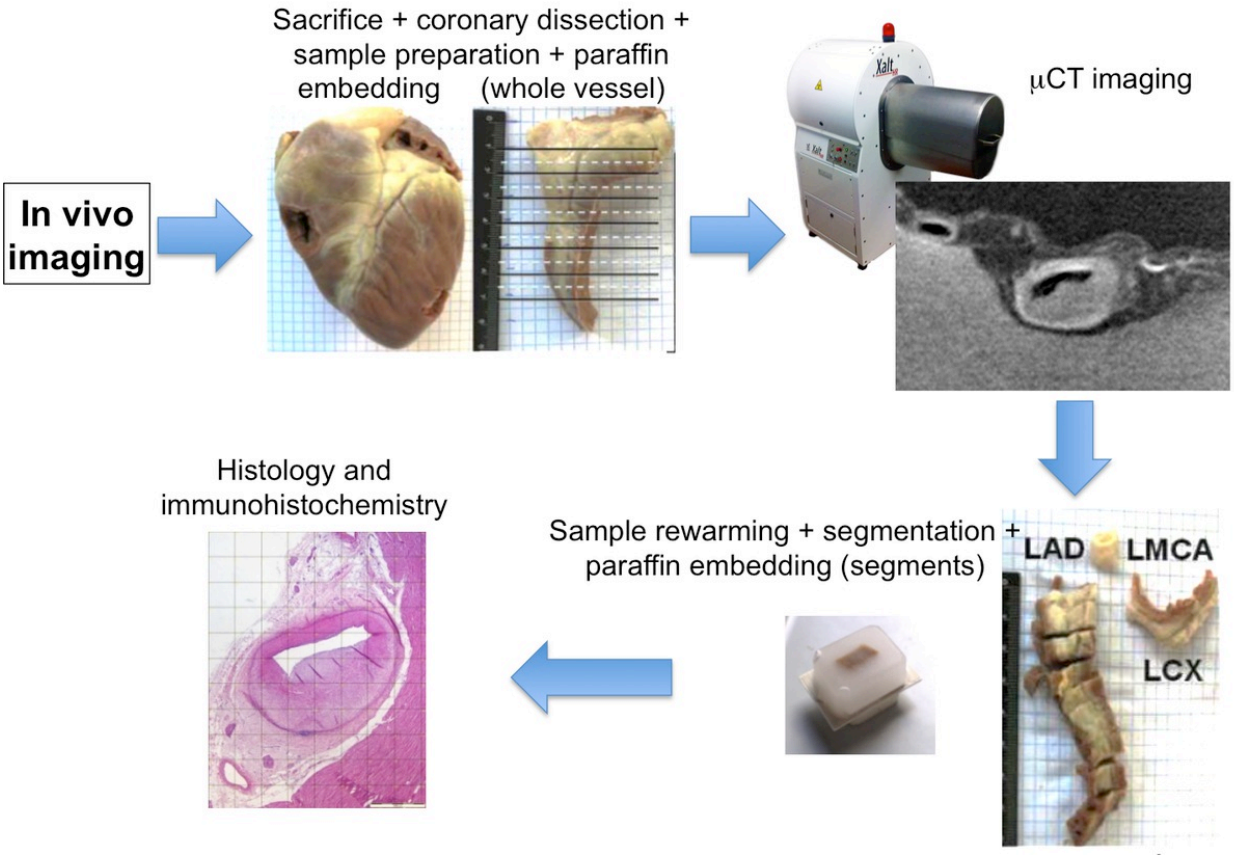

Fig. 1: After in vivo imaging, the animal is sacrified, heart excised and fixed in formalin. LAD and part of the underlying myocardium is dissected, dehydrated and embedded in paraffin for micro-CT imaging. The sample is further processed for histology and immunohistochemistry.

Results

Formalin fixed vs. paraffin embedded sample preparation

Some samples underwent micro-CT imaging before and after paraffin embedding to compare the image quality in the two conditions. The difference is shown in Fig. 2 on page 5. After paraffin embedding (Fig 2B) the µCT images show good discrimination between fat, muscle and calcium thus allowing a reliable segmentation of walls and lesions. This is less apparent on the same sample after formalin fixation only (Fig 2A).

Another difference between the two conditions is shown in Fig. 3 on page 6. The dehydration prior to paraffin embedding led to a significant shrinking of the tissues, and hence to a shortening of the coronary vessel [3]. This distorsion shall be taken into account in order to reliably correlating the ex vivo information of micro-CT with the corresponding images obtained in vivo.

Quantitative profiling of plaque area and vessel structure

The quantitative measurement of wall area and calcium was done automatically by threshold-based segmentation. The 3D Curved MPR analysis implemented in OsiriX allowed the visual inspection of the entire coronary artery in "one-shot", as series of 2D longitudinal slices. Fig. 4 on page 7 shows how micro-CT images of the paraffin embedded coronary vessels can be imported on OsiriX for centerline reformation prior to image segmentation. The profiles of total area, wall area and calcium area along the coronary artery allowed a comprehensive profiling of intimal lesions and calcification (Fig. 5 on page 7 and Fig. 6 on page 8). Microcalcifications down to 0.2 nl (nanoliters) were localized.

The difference in both visual appearance and quantitative results for mild (Fig. 5 on page 7) and severe (Fig. 6 on page 8) high-fat diet induced lesions is evident. In some cases, the semiautomated quantification algorithm was not carried on because sample decalcification was required for subsequent immunohistochemistry (Fig. 7 on page 9). This type of sample processing is currently being used for studying the correlation of plaque radiodensity with cellular composition.

Integration with in vivo imaging and with histology

A major challenge in the exploitation of high resolution information from ex vivo micro-CT is in the integration with the information obtained in vivo, i.e., under real physiological conditions.

ICA allowed to reconstruct the three-dimensional shape of the coronary tree but with limited information on the plaque localization and extent. IVUS partially overcome this limitation and provided information about the vessel lumen and plaque extension in vivo, but with a very low spatial and contrast resolution. IVUS and ICA can be coregistered by tracking the guide catheter under fluoroscopy. The spatial matching of micro-CT with IVUS is more difficult because of the tissue shrinkage due to the sample preparation, as shown in Fig. 3 on page 6. This drawback can be overcome by spatial matching of reference points such as calcium deposits, vessel branches and curves.

The integration of micro-CT with histology is easier because the samples are imaged at the same processing stage in the two modalities. This has been already shown in a previous work of the same group [4] (Fig. 9 on page 10). Histological cross-sections can be reliably coregistered to the corresponding micro-CT cross-sections thus allowing the correlation between volumetric plaque morphology and cellular composition.

Images for this section:

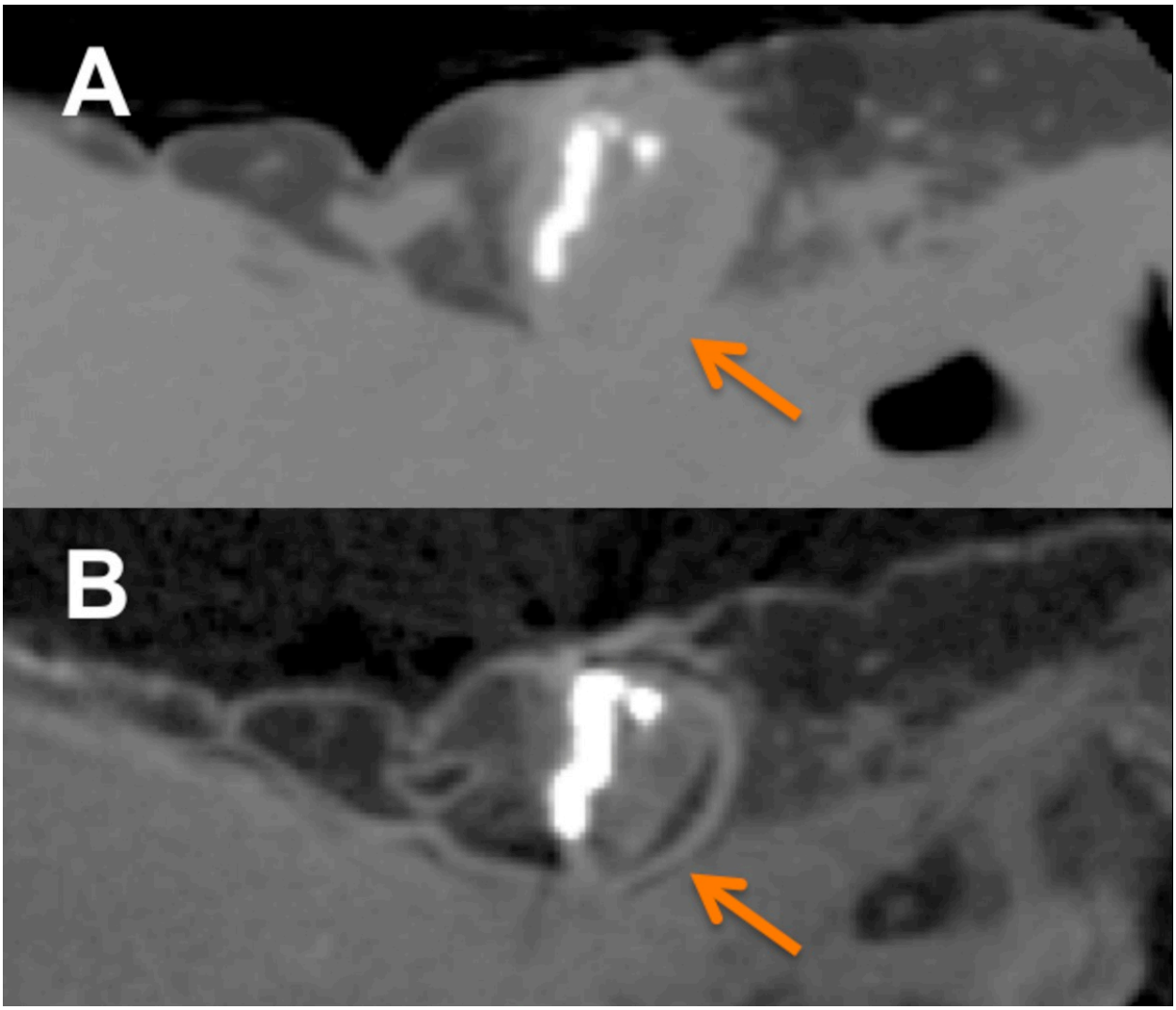

Fig. 2: Cross sectional images of a coronary artery with calcified plaque. (A) Image obtained after sample fixation (formalin). (B) Same sample of (A) imaged after dehydration and paraffin embedding.

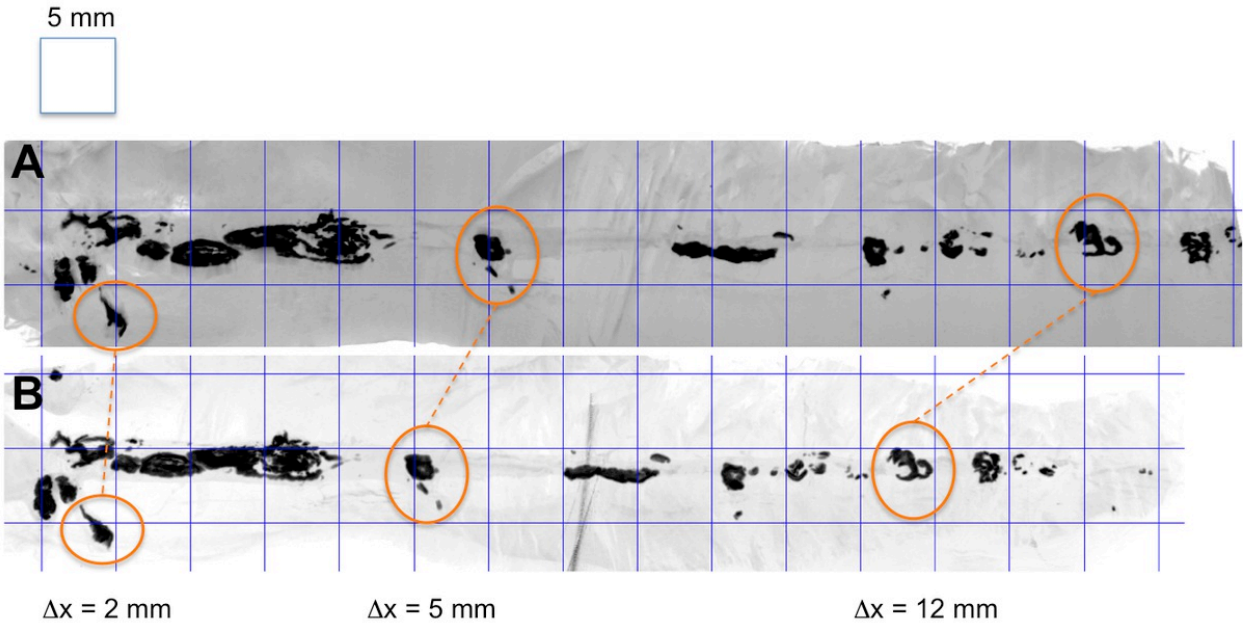

Fig. 3: Maximum intensity projection (MIP) of the longitudinal reformation of the micro-CT images shown in Fig.2 (inverted gray look-up-table). The dark spots are calcified plaques. (A) Image obtained after fixation in formalin. (B) Same as (A) after dehydration and paraffin embedding. The shortening of the vessel after paraffin embedding is evident.

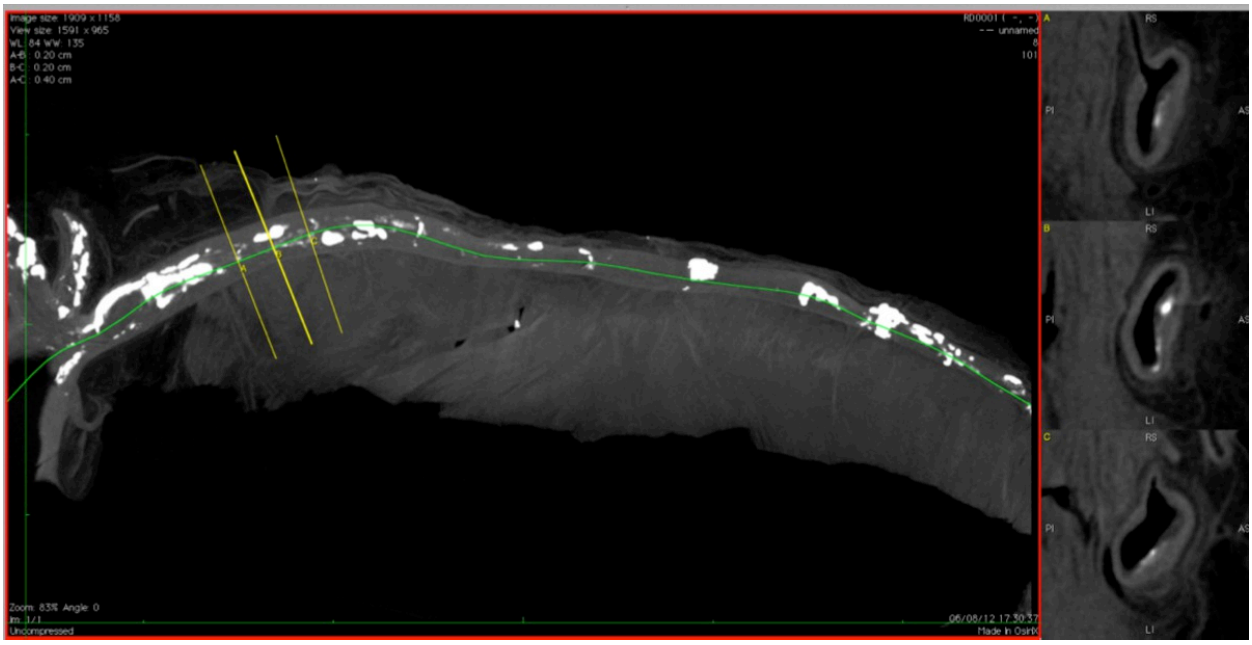

Fig. 4: Example of use of the 3D Curved-MPR plugin of Osirix for the curved multiplanar reformation of a micro-CT image of a coronary artery .

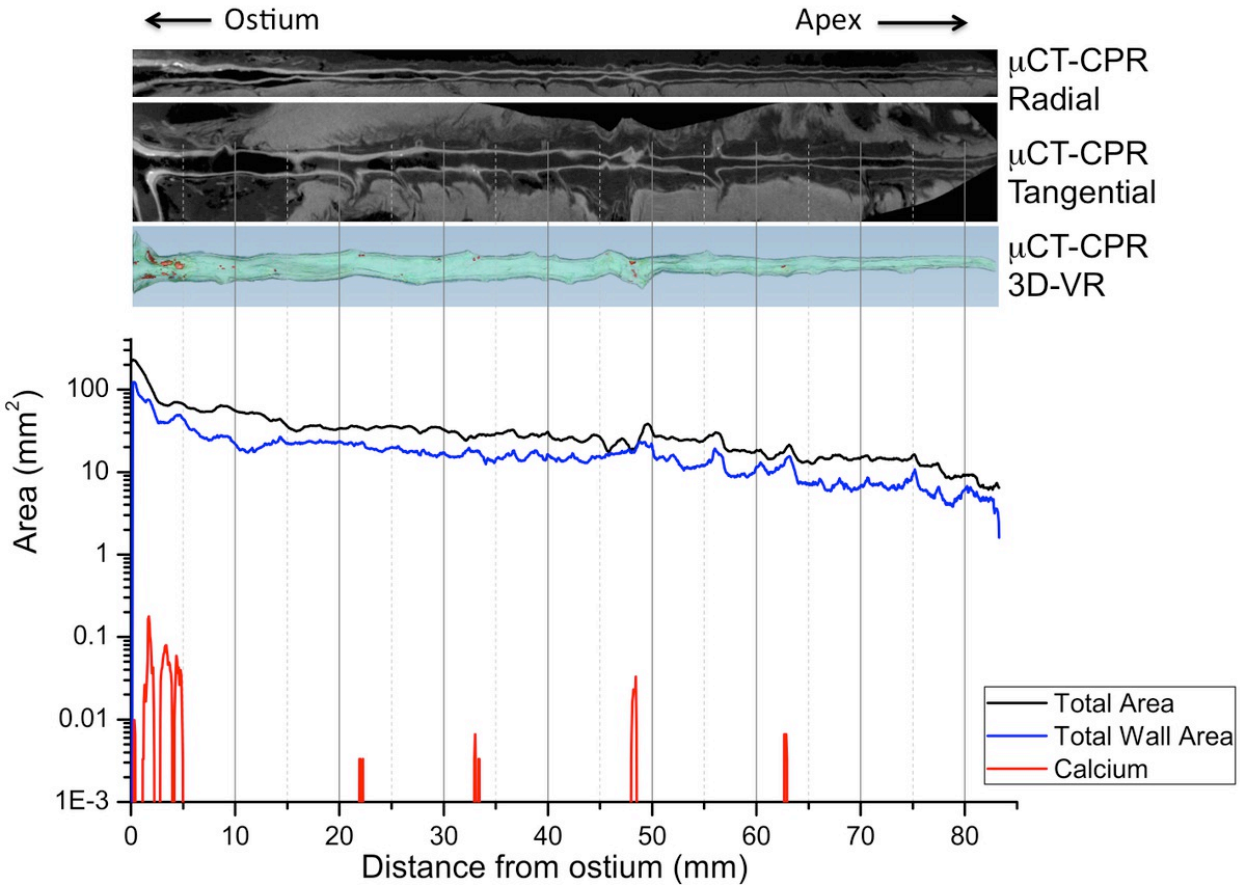

Fig. 5: Longitudinal profile of a left coronary artery (LAD) of a pig with mild intimal changes. The profiles have been obtained via a semi-automatic segmentation of the micro-CT image obtained after paraffin embedding. At each point from the ostium, the curves represent the total area (black line), the total wall area, i.e., the total area minus the lumen area (blue line) and the calcium area (red line).

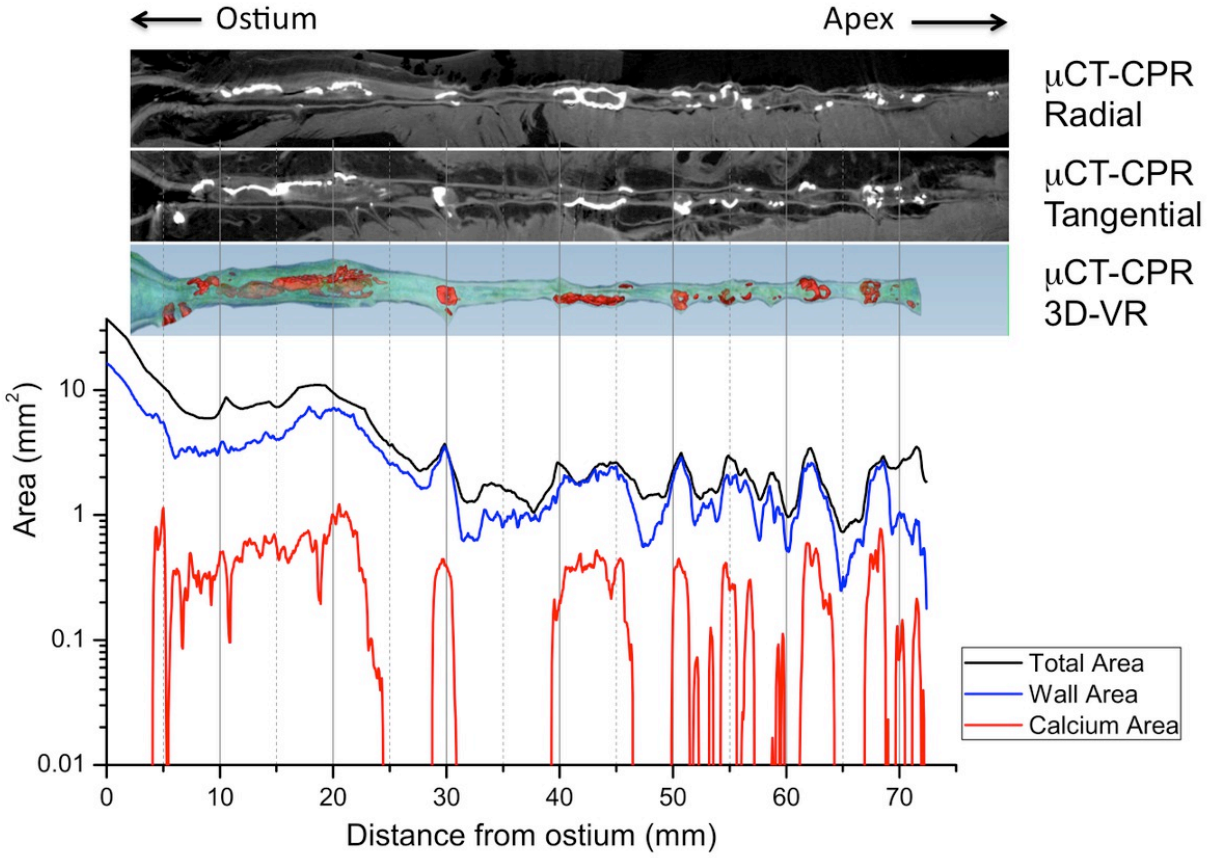

Fig. 6: Same as Fig. 5, for the LAD of a pig with high-fat induced severe coronary lesions.

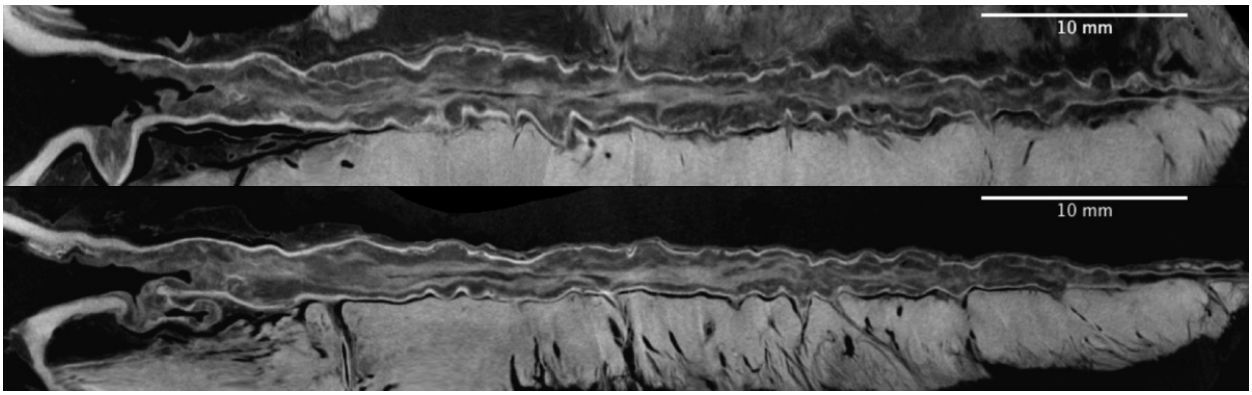

Fig. 7: In some cases, sample was decalcified before micro-CT imaging to allow subsequent immunohistochemistry. Apparent lumen collapse is due to tissue shrinkage. Micro-CT imaging with samples which underwent this type of processing are being used to better study the correlation between radiodensity and cellular composition inside plaques.

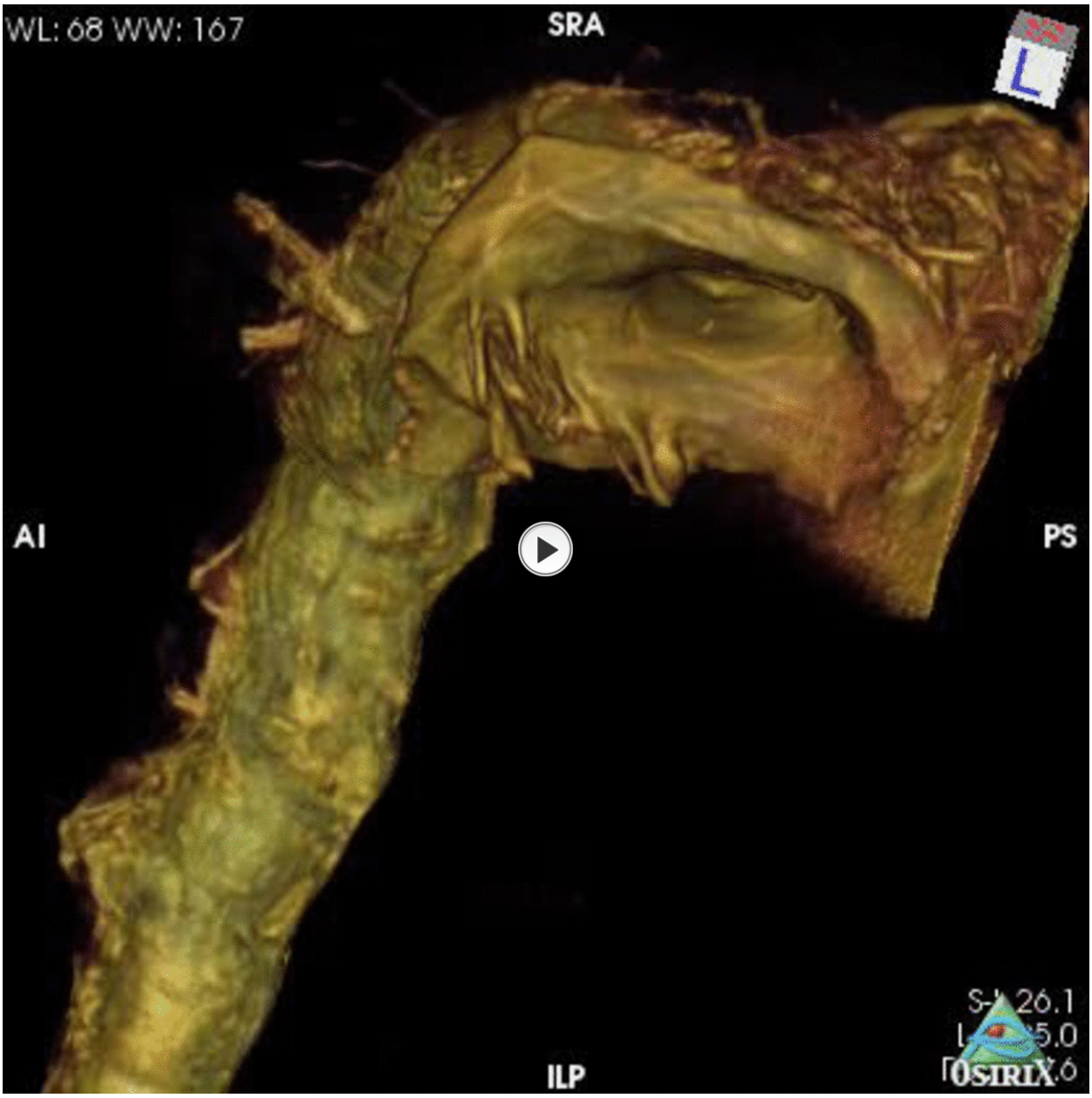

Fig. 8: Fly-through of the 3D volume rendered micro-CT of the LAD shown in Fig. 7.

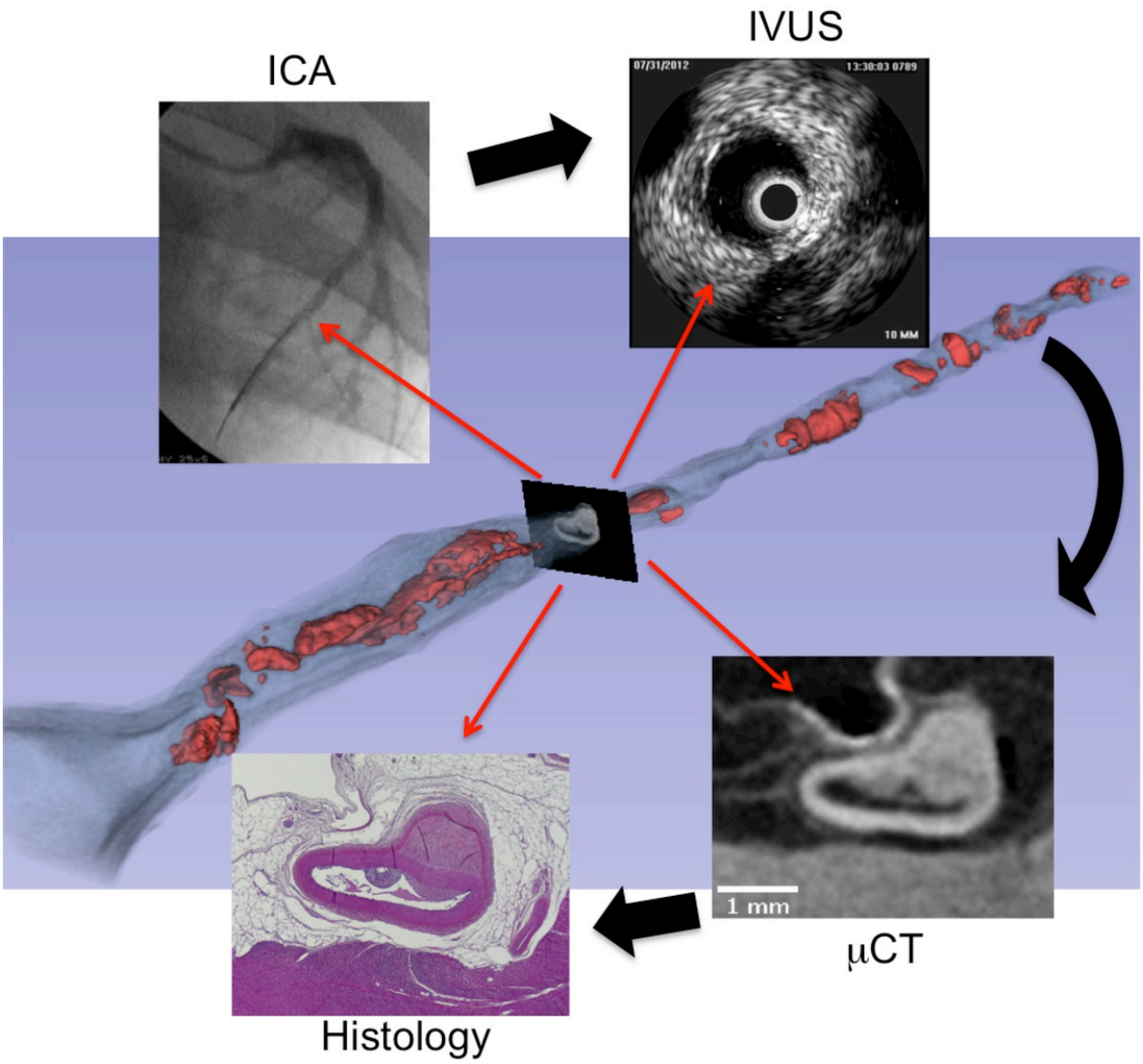

Fig. 9: The integration of different imaging modalities in vivo and ex vivo can lead to a better understanding of the correlation between coronary anatomy, WSS and plaque extension, location and composition.

Conclusion

As compared to the current state of the art, the use of micro-CT allows us to raise the level of spatial resolution in a comprehensive profiling of coronary artery lesions; this could be a more suitable approach for the study of hemodynamic-cellular correlation in plaque development and growth. Combined micro-CT, histology and immunohistochemistry in each individual coronary plaque is a prerequisite for evaluating the role of plaque morphology in three-dimensions on cellular composition, activation, and remodelling.

Acknowledgements

The study presented in this poster is partly financed by the EU-FP7 Project ICT n. 224297 "ARTreat".

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