

# Morphology of urethral tissue: a combined synchrotron radiation-based micro-tomography and optical microscopy study

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## Aims

The 3D anatomy of urethral tissue is only partially known. Available histological data do not show for instance the two states (open/close), which are characteristic for the function of the sphincter. Developments on artificial urinary sphincters for the treatment of severe incontinence require morphological information on the 3D structure of the urethral tissue. Micro computed tomography ( $\mu$ CT) provides the necessary spatial resolution, but investigating soft tissue the contrast is often too weak for meaningful images. Contrast enhancement is achieved by means of staining and embedding procedures [1]. Alternatively, hard X-ray phase-contrast imaging such as grating interferometry can be applied to the explanted tissue without applying extended preparation procedures [2]. In this paper, the power of synchrotron radiation-based  $\mu$ CT in absorption and phase contrast modes is demonstrated and compared with conventional histology. The study is based on sheep and porcine urethral tissues. Sheep and especially pig are used for in-vivo tests of artificial urinary sphincters.

## Method

Specimens were derived by cutting parts of approximately 10 mm length from freshly explanted urethras of domestic pigs and sheep.

After extraction, the specimens for investigation by absorption contrast  $\mu$ CT and histological analysis were fixated in a 5% formaldehyde solution. After fixation, specimens were dehydrated in an ascending alcohol series (50, 70, 80, 90, 96 and 100%). Each processing step so far was maintained for 24 h. To remove grease, the specimens were immersed in xylol for 5 min. Subsequently, the specimens were embedded in polymethylmetacrylate. The embedding solution consisted of 40 ml methylmetacrylate, 10 ml dibuthylphtalate (softener) and 1 g perkadox (activation agent). The polymerization took approximately 8 h at room temperature. Specimens for optical microscopy were further cut by a rotary microtome in 10 – 20  $\mu$ m thick slices. These slices were colored with toluidin blue or according to giemsa staining. Images were taken with an optical microscope type TMRM of the company Leica®.

The specimen for investigation by phase-contrast  $\mu$ CT was measured freshly, directly after extraction. During the measurement, the specimen was kept in pure water.

The SR $\mu$ CT measurements were carried out at HASYLAB/DESY in Hamburg, Germany. The beamlines used are BW 2 for absorption contrast and W 2 for phase contrast, both operated by GKSS Research Center.

For absorption contrast measurements the photon energy was set to 15 keV. The pixel size of the acquired projections corresponded to 5.1  $\mu$ m. The spatial resolution of the entire setup was determined to 8.42  $\mu$ m using the 10% value of the modulation

transfer function [3]. The 3D data were reconstructed by means of the standard filtered back-projection reconstruction algorithm out of 1441 projections (360° scan) [4]. For easier handling and to increase the contrast, the amount of data was reduced by binning with a factor 4, resulting in a pixel size of 20.4  $\mu\text{m}$  [5].

For the grating-based phase contrast measurements a monochromatic x-ray beam of 25 keV at the 6<sup>th</sup> fractional Talbot order was applied. The effective pixel size corresponded to 9.9  $\mu\text{m}$ . Projection radiographs were taken in 721 steps over a range of 180°. At each projection angle, eight phase-stepping images were taken over two periods of the interferometer fringe pattern. The phase contrast projection data set was reconstructed using a modified filter kernel in combination with standard filtered-back projection [6]. The data were binned twofold, which leads to a pixel size of 19.8  $\mu\text{m}$ .

## **Results and discussion**

High-resolution tomographic imaging in absorption contrast mode allows visualizing two layers of sheep urethral tissue (see figure 1). The brighter areas in the image correspond to less absorbing tissue. The inner layer is the tunica mucosa, consisting of an epithelium and the lamina propria. The epithelium is the interface between lumen and tissue. In between epithelium and muscles, a layer of connective tissue (lamina propria) is located. Typical for mammalian domestic animals are the caverns, which can be found in the connective tissue [7]. The outer layer is the tunica muscularis, which is built of skeletal muscles. The border between these two layers is associated with smooth muscles. Histological data as represented in figure 2 confirm these general findings, even if the epithelium cannot be seen because preparation artifacts are dominant, here.

The images of a porcine urethra obtained by phase contrast  $\mu\text{CT}$  also show the layered composition (see figure 3). Compared with histological data, the layers might be classified in the following way [7]. The lumen, which has an irregular branched shape, is surrounded by a darker feature in circular manner. This feature more or less homogeneous refers to the tunica mucosa. The epithelium, which is a narrow cell line bounding the lumen, cannot be seen in the tomographic images. Around the tunica mucosa a darker ring-like structure appears. This structure could be related to the tela submucosa, which is a connective layer between tunica mucosa and tunica muscularis. The layer lying beneath the tela submucosa is the tunica muscularis, which appears in a brighter colour and is also wider than the layers described above. It consists mainly of skeletal muscles. In the tunica muscularis different structures can be identified showing the different orientations of the skeletal muscles. The patterned structure close to the tela submucosa could be the result of cells, which are arranged around the skeletal muscle fibres. These muscle fibres are axially orientated and therefore transversally cut. The non-patterned area thus should refer to circular orientated skeletal muscles. Around the tunica muscularis, a third layer can be seen. This layer is displayed by a more evenly and slightly darker colour and is associated with the tunica adventitia. The tunica adventitia is the outermost layer of the urethra and consists basically of low-dense connective tissue.

## **Conclusion**

Synchrotron radiation-based  $\mu\text{CT}$  is an appropriate method to visualize soft tissues such as urethras. Both modes, absorption and phase contrast, allow for reasonable discrimination between circularly arranged tissue layers. Conventional histological images of selected slices confirm the morphological microstructure derived from SR $\mu\text{CT}$  investigations. With optical microscopy, the lateral spatial resolution is certainly higher, but the third dimension is lost as the result of sectioning. For the development and the testing of artificial urinary sphincters structural information in axial and transversal directions are necessary; the SR $\mu\text{CT}$  has proven to provide useful details in all three direction with reasonable spatial resolution and contrast.

The information can be corroborated using conventional histology of pre-selected slices. The deformations and the shrinkage of the high-resolution histological slices can be corrected using the less detailed tomography data [8]. In this way the two approaches are complementary.

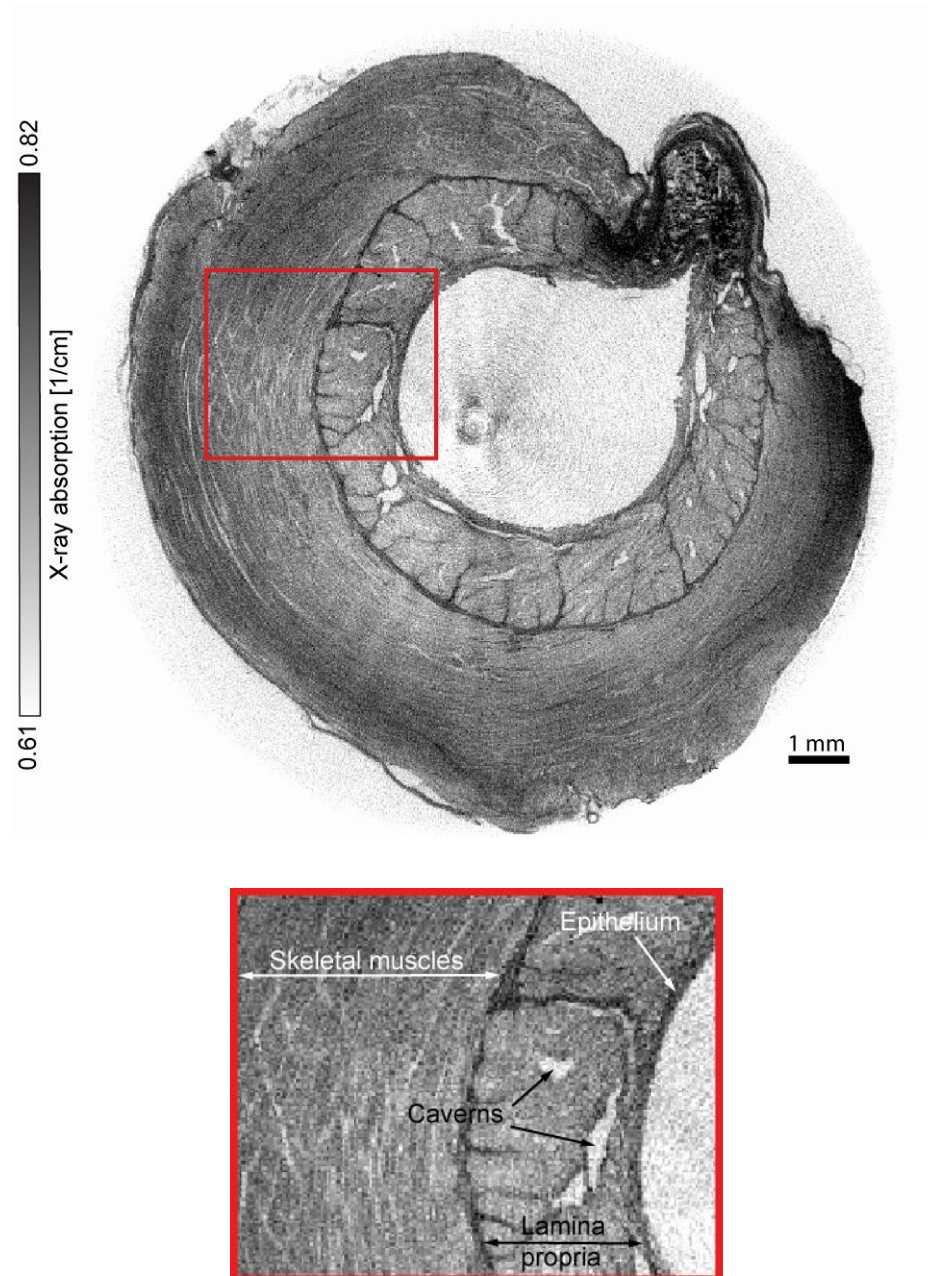


Figure 1: Virtual slice of SR $\mu$ CT (absorption contrast); transversal cut through a sheep urethra.

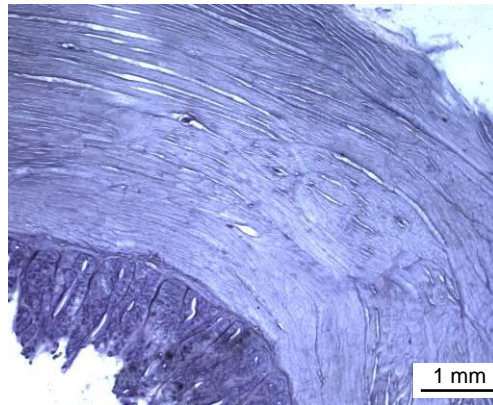


Figure 2: Part of a histological slice from a transversal cut through a sheep urethra.

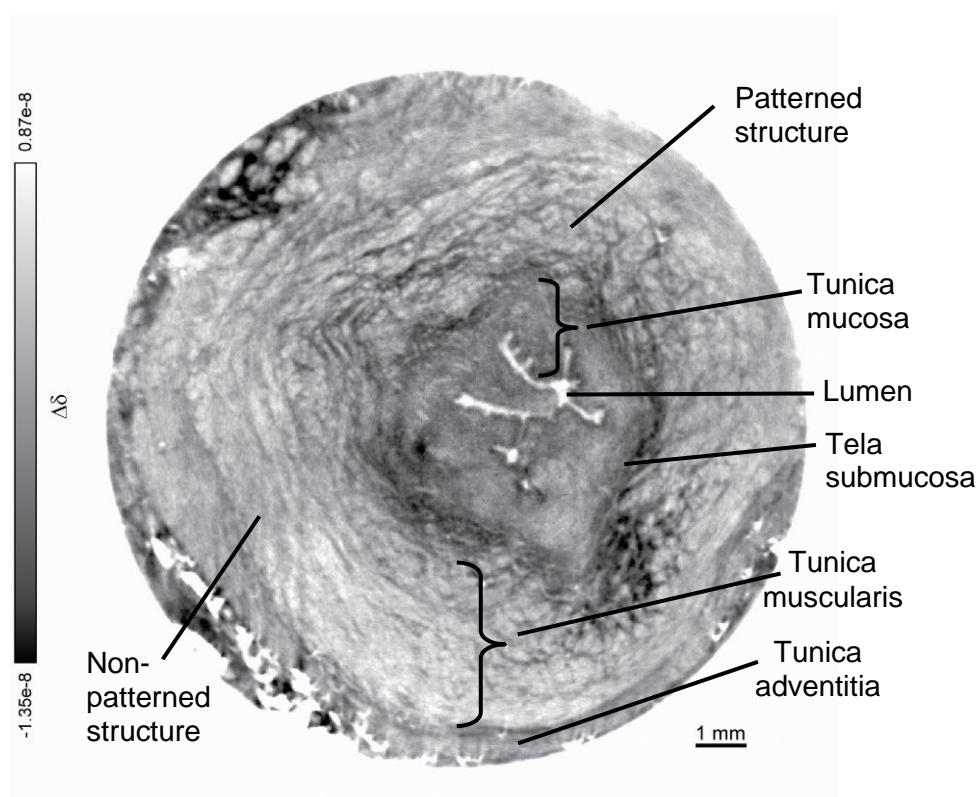


Figure 3: Virtual slice of phase-contrast SR $\mu$ CT (grating interferometry), transversal cut through a porcine urethra.

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