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The nanostructure of biological tissues: a scanning X-ray scattering study

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INTRODUCTION: The human body is composed of functional micro- and nanostructures, which are usually organized in three-dimensional fashion and exhibit preferential orientations. While the microstructures are accessible for example by micro computed tomography, the nanometer-scale features are generally only uncovered on surfaces by means of optical methods or electron microscopy. The present communication demonstrates the power of synchrotron radiation-based scattering by means of a focused X-ray (5 μ m \times 20 μ m) that rasterizes tissue slices. The acquired scattering data yield the abundance and orientation of all components with extension from atomic to sub-micrometer periodicities. This means, all kinds of nanostructures such as hydroxyapatite crystallites or collagen fibrils can be quantitatively gathered. As prominent examples, we show teeth¹, brain, and urethra specimens.²

METHODS: The data were recorded at the cSAXS beamline of the Swiss Light Source (Paul Scherrer Institut, Villigen, Switzerland) as described previously.³ The wet tissue slices some hundred micrometer thin were put into polyimide sachets to prevent dehydration. Some specimens were resin-embedded for conventional histology.

RESULTS: Fig. 1 shows the scattering intensities of a 380 μ m-thin section of an embedded male porcine urethra parallel to the cavity. The images were generated from about 40'000 scattering patterns obtained with an 11.2 keV X-ray beam, scanned along the specimen in 75 and 50 μ m steps in x- and y-directions, respectively. The specimen-detector distance was about 7.1 m.

The images of Fig. 1 show the presence of highly oriented nanostructures with extensions between 5 and 300 nm. The colours relate to the preferential orientations according to the wheel on the right, whereas the brightness corresponds to the nanostructure's abundance.

The liquid-like soft urethral tissue consists of epithelium, connective tissue (*lamina propria*) and muscles (*tunica muscularis*). This layered composition is represented on the nanometer scale by the elongated parallel turquoise-coloured features with oriented nanostructures.



Fig. 1: The series of scanning SAXS images shows the characteristic anatomy of the urethra. The sagittal images from top-left to bottom-right represents the nanostructures ranging from 5 to 8 nm, 11 to 20 nm, 38 to 57 nm, 76 to 102 nm, 114 to 131 nm, 152 to 176 nm, 200 to 229 nm, and 269 to 305 nm, respectively. The colours are chosen according to the nanostructure's orientations, see colour wheel, their brightness relates to their abundance. The bar corresponds to 2 mm.

CONCLUSIONS: Scanning X-ray scattering provides the abundance and orientation of all nanostructures with micrometer resolution in real space along sections of hard and soft tissues.

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