

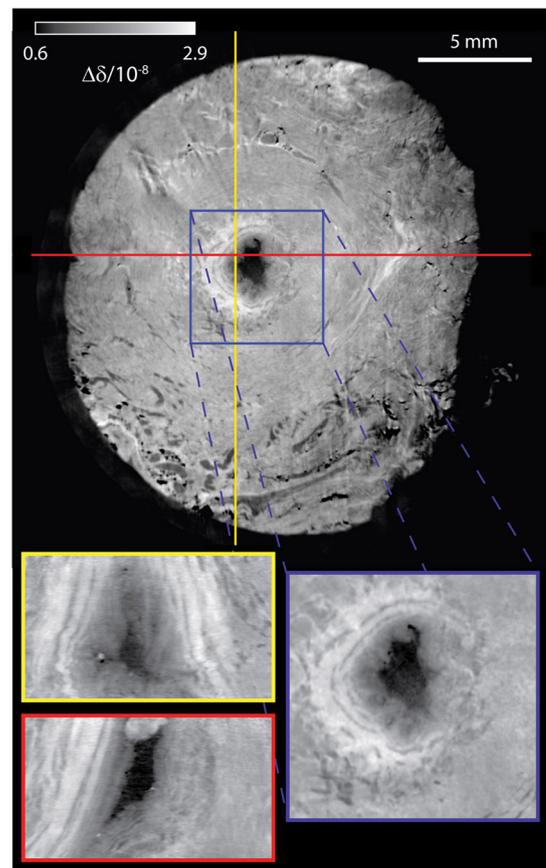
# Phase-contrast x-ray tomography reveals the micro-anatomy of soft tissues

**Bert Müller**

*Employing the sensitivity of grating interferometry constitutes a major advance in building artificial muscles to treat severe urinary incontinence.*

Try closing a hose pipe by putting your hands around it, and you will find it is impossible. The urinary sphincter, however, uses just such a mechanism to close the urethra. The trick is in the characteristic liquid-like properties of urethral tissue:<sup>1</sup> no artificial engineering material has yet been able to duplicate it. The detailed anatomy of the urethra on micrometer scales has thus far attracted little attention. By the same token, because incontinence is becoming an increasingly important problem in our aging society, demand for artificial muscles is picking up. Only a few implant systems are commercially available, and their results are unsatisfactory. Up to 50% of American Medical Systems 800™ devices require revision in the first five years after implantation.<sup>2</sup> Understanding the micro-architecture of urethral tissue may help speed development of more sophisticated, artificial analogs of natural muscles.

Today, optical microscopy of histological slices is the only technique available for studying the urethra in detail. Histology is extremely helpful in understanding structure-function relationships, but preparative procedures introduce many artifacts, such as irregular local deformations and shrinkage of the anisotropic soft tissue (i.e., it exhibits different structural and mechanical properties depending on direction). Even for serial sectioning, true micrometer resolution in 3D is lost. Unfortunately, nondestructive 3D approaches using x-ray computed tomography in absorption-contrast mode do not provide enough contrast. The application of staining protocols and other preparation techniques such as embedding and erosion casting are obviously much more demanding for urethral tissue than for blood vessels<sup>3</sup> and are unlikely to be successful.



**Figure 1.** Virtual cuts through a grating-based tomography data set of a human urethra.  $\Delta\delta$ : Angular resolution (in rad).

Compared to the widely used attenuation (or absorption)-contrast mode, the phase-contrast mode is known to be much more effective for soft tissue.<sup>4</sup> Several approaches have been introduced since the pioneering work of Ulrich Bonse,<sup>5</sup> but so

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far the urethra has not been the specimen of interest. Recently, Timm Weitkamp and colleagues<sup>6</sup> invented grating-based microtomography, which shows extremely high sensitivity combined with a large field of view. In fairly short order, grating-based microtomography became available at a number of synchrotron-radiation sources. Very recently, for example, such a system was brought into operation at the HASYLAB synchrotron laboratory in Hamburg (Germany).<sup>7</sup> The method enables in vitro visualization of explanted urethras in a liquid-filled container.

Using images taken at HASYLAB, we carried out some of the first successful measurements done on an explanted human male urethra, with virtual cuts in three orthogonal directions (see Figure 1).<sup>8</sup> The projections are recorded using beam-splitter and analyzer gratings, both placed between specimen and conventional detector. The specimen deflects the x-rays and, therefore, disturbs the interference pattern. These slight distortions are detected by displacing the analyzer grating ('phase stepping'). In the center of the cut perpendicular to the axis of symmetry, we were able to clearly identify the lumen and get a general idea of how the urethra closes and opens for a restricted period of time to pass water. The irregularly branched lumen is circularly surrounded by the more or less homogeneous tunica mucosa. The epithelium—a thin layer of cells that is the interface to the lumen—is only vaguely resolved. Inhomogeneous tissue surrounds the rather structured layers near the urethra's center.

The resolution power of the grating-based microtomography setup (characterized using the measurement sensitivity for the angular resolution) is huge and corresponds to the order of  $10^{-9}$  rad.<sup>9</sup> This value is tantamount to being able to see a car on the moon from Earth. The size of the cubic voxels ( $10\mu\text{m}$  on a side), however, must be improved to allow visualization of single biological cells within the tissue. We recently imaged unstained cells in the human cerebellum for the first time using grating-based microtomography at the ID19 beamline at the European Synchrotron Radiation Facility in Grenoble (France).<sup>10</sup>

Together with industry (Nanopowers SA, Lausanne, Switzerland), we are developing artificial urinary sphincters and are testing them in vitro in our laboratory. The 3D micro-anatomy of human and porcine urethras is helping to optimize the experimental setup and is advancing fundamental understanding of the closing and opening mechanism. We intend to image the morphology of the urethra, and especially of the lumen, at pre-selected stages during closing and opening. This 3D data should allow building a model similar to the urethra-compression model<sup>11</sup> that will support realization of high-performance artificial sphincters for both sexes. The focus of our studies is ultimately the good of patients suffering from severe urinary incontinence.

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