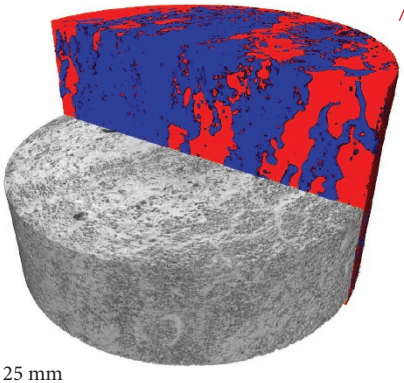
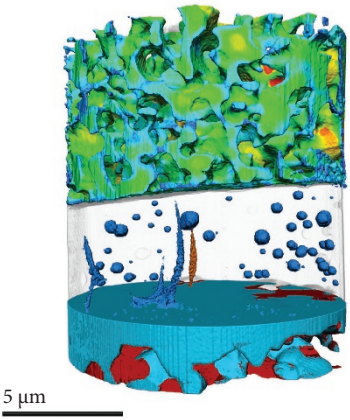
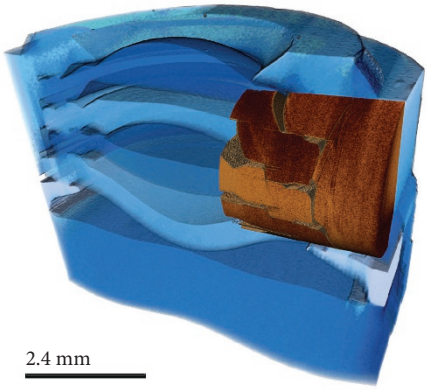
MICROSCOPY AND ANALYSIS







An Overview of 3D X-ray Microscopy

Essential Knowledge Briefings

Second Edition, 2020

ZEISS

Cover: Selection of images produced by X-ray microscopes of different materials, including geological, electrical and advanced materials (clockwise from top).

Segmentation showing the lithological classification of a 100 mm carbonate rock core. Imaging was performed using the FPX detector on a ZEISS Xradia 520 Versa X-ray microscope. This rendering was created with ORS Visual SI Advanced.

Mobile phone camera lens assembly imaged by ZEISS Xradia 520 Versa. Brown section is overlay of an interior tomography scan.

A portion of a solid oxide fuel cell (SOFC) was imaged using ZEISS Xradia 810 Ultra. There are three layers of the SOFC visible. The porous top section is the cathode, which is a lanthanum-strontium-manganite (LSM) composition. The LSM network has been color labeled according to its local thickness. Blue is thin and red is thick. The center of the sample is the electrolyte, which is made of yttria-stabilized zirconia (YSZ). In this portion of the sample, the image does not show the solid YSZ, but actually the voids that exist within the YSZ. One void is labeled orange because it also connects to the pore network in the lower portion of the cell. The bottom layer is the anode, which is a porous composite of nickel and YSZ. YSZ is blue, nickel is red.

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Essential Knowledge Briefings, published by John Wiley & Sons, comprise a series of short guides to the latest techniques, applications and equipment used in analytical science. Revised and updated annually, EKBs are an essential resource for scientists working in both academia and industry looking to update their understanding of key developments within each specialty. Free to download in a range of electronic formats, the EKB range is available at www.essentialknowledgebriefings.com

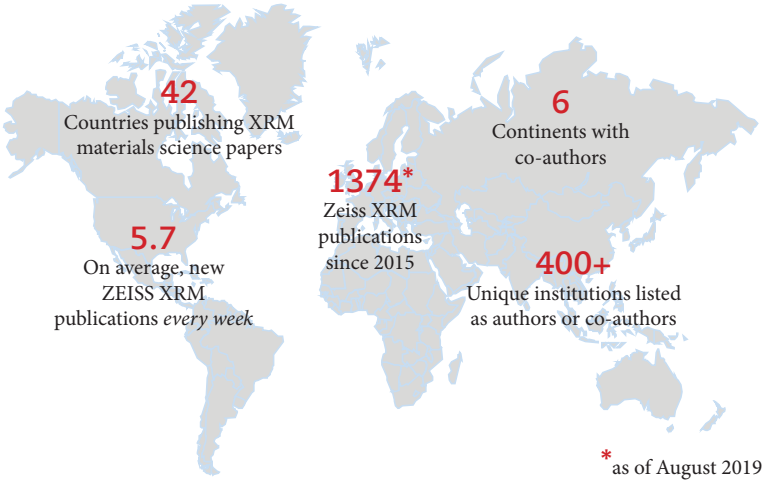
INTRODUCTION

Scientists have long used X-rays to peer within solid objects, but the recent rise of X-ray microscopy (XRM) means they are now able to probe the secrets of matter in ever greater detail. Unlike most forms of microscopy, XRM can deliver high resolution and contrast in three dimensions, and can do so without destroying samples.

Recent advances in XRM technology have come from pioneering work in synchrotron facilities across the world. In these facilities, researchers use brilliant beams of X-rays produced by particle accelerators to achieve unprecedented levels of resolution and contrast. While synchrotron scientists continue to look ever deeper into materials, commercially available, lab-based XRM systems are now benefiting from their developments in detector and focusing optic technologies.

These commercial lab-based XRM systems have a key role to play in three-dimensional (3D) imaging and tomography, offering resolutions well beyond that of classical X-ray tomography or micro-CT. They can provide non-destructive 3D imaging of samples across a wide range of length scales, revealing features from nanometers to millimeters. They also offer a unique opportunity to study samples *in situ* to examine how the microstructure changes over time, known as four-dimensional (4D) imaging. With these strengths, it should come as no surprise that XRM is being used to study an ever-increasing range of materials, from biological samples to batteries to advanced alloys to geological material, for both research and industrial applications.

The amount of data being produced by the systems around the world is impressive, both in its breadth and scope.

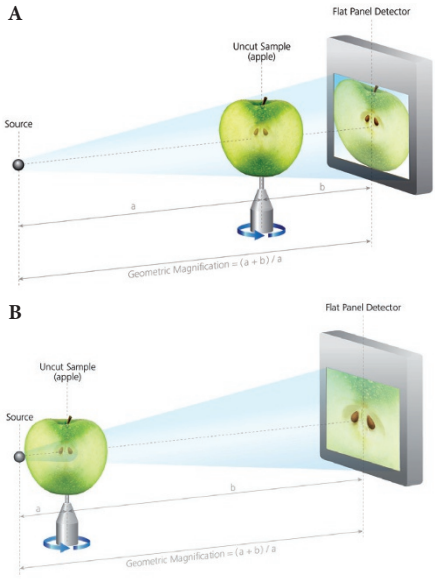


INTRODUCING X-RAY MICROSCOPY

With micro-CT architecture, you can image the whole object (e.g. an apple), but if you want to see small things inside (e.g. a seed), you need to cut it open. Cutting an apple might be OK, but what if:

- It is a precious sample you can't destroy?
- It is an intact device (battery, electronics component)?
- Cutting your sample risks damaging the structure?
- You need to preserve your sample for future studies?
- You have sparse features and don't know where to cut?
- You are working inside an *in situ* chamber or rig?

There are frequent cases where working with larger or intact samples is beneficial. Only an X-ray microscope can scan an apple seed at high resolution *without cutting* the apple open (Figure 1).



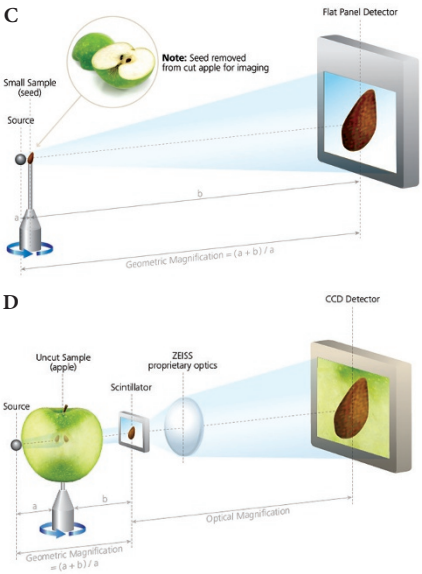


Figure 1. With micro-CT architecture you can image the whole apple, but if you want to see the seed, you need to cut it open (A–C). Only an X-ray microscope can scan an apple seed at high resolution without cutting the apple open (D)

X-ray microscopes use a powerful X-ray source and advanced optics to create compelling 3D images of samples. Because X-rays can penetrate through solid objects, there is no need for sectioning thick or opaque samples. Instead, by taking multiple X-ray projections from different angles as the sample is rotated, X-ray microscopes can build up a detailed 3D representation of a sample's internal structures.

At their simplest, X-ray microscopes comprise an X-ray source, usually an X-ray tube, and a detector, usually a combination of a scintillator to convert the X-rays into visible light and a charge-coupled device (CCD) to detect this light. The sample is placed between the source and the detector and illuminated with X-rays. Although X-rays can

pass through solid objects, they are absorbed to different extents by different materials based on the local density, and it is this contrast that produces the image on the detector.

With the combination of the latest scintillators carefully tuned to enhance sensitivity, glass lenses to induce varying degrees of optical magnification, and the latest CCDs containing small detector pixel sizes, it becomes possible to achieve imaging resolutions of 500 nm and voxel sizes as small as 40 nm, as seen within the ZEISS Xradia Versa family of X-ray microscopes (Figures 2 and 3). But even higher resolutions, defined as the smallest individual features that can be resolved in the magnified image, can be achieved by taking advantage of the latest advanced X-ray optics (Figure 4).

In the ZEISS Xradia Ultra family of X-ray microscopes, X-rays emitted from a source initially pass through a capillary

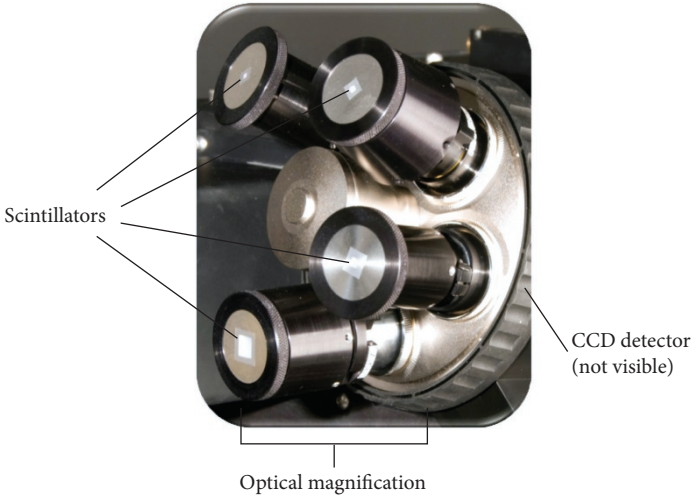


Figure 2. ZEISS Xradia Versa: multiple scintillator-coupled optics for different magnifications

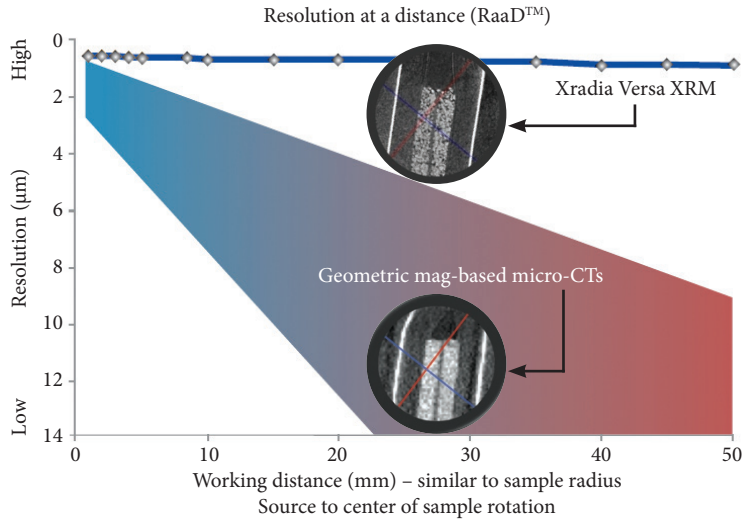


Figure 3. A comparison of X-ray microscopy and traditional micro-CT architectures

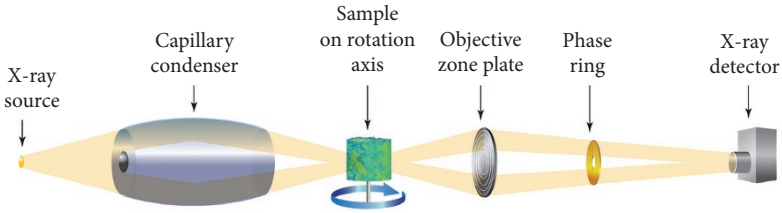


Figure 4. Schematic diagram of the optics for ZEISS Xradia Ultra

condenser (Figure 4) to produce even illumination upon the sample. After passing through the object, the transmitted X-rays are then focused by a Fresnel zone plate objective lens to produce a magnified image. Optionally, the X-rays can also pass through a Zernike phase ring to produce an edge-enhancing phase contrast effect before hitting the detector. With the addition of these unique X-ray optical elements, this set-up can achieve resolutions as low as 50 nm.

BOX 1. A century of X-rays

X-rays are a form of electromagnetic radiation, with wavelengths much shorter than visible light, between 0.01 and 10nm. Their short wavelengths mean that, unlike visible light, X-rays can penetrate visually opaque objects, making them ideal for medical and security scanning, and various research applications.

German physicist Wilhelm Röntgen was the first to discover X-rays, in 1895, and to create an X-ray image of the human body – his wife's hand. His discovery was quickly leapt upon: within six months, his mysterious 'X' rays were being used by battlefield medics to locate bullets inside injured soldiers.

The development of XRM as an advanced microscopy technique took a century longer. On the one hand the short wavelengths of X-rays mean they penetrate opaque objects and interrogate internal structures, offering new information beyond what can be obtained from light microscopy. But conversely, this

Figure 5. SEM image of Fresnel zone plate

characteristic also gives rise to the challenge that X-rays can't be focused using the familiar glass lenses that produce magnification in light microscopes.

So producing magnified X-ray images at high resolutions has required the development, over the past few decades, of appropriate scintillators, advanced optics, and detectors with tiny pixel sizes. These optics include capillary condensers for evenly illuminating samples and Fresnel zone plates, which comprise concentric rings of opaque and transparent zones that can focus X-rays via diffraction.

Originally developed for use in synchrotrons, which produce powerful X-ray beams by accelerating electrons around a circular track at high speeds, these technologies have since found their way into commercially available laboratory-scale X-ray microscopes, such as the Versa and Ultra families of X-ray microscopes.

BOX 2. Focus on magnification

There are two ways to produce a magnified image: geometric magnification and optical magnification. Geometric magnification is generated purely by the relative distance between the light source, the object and the detector. A huge shadow of a small object projected by a light source onto a wall behind it is produced by geometric magnification – like a shadow puppet.

To make the shadow larger, and thus increase the magnification, either the light source needs to be moved closer to the object or the object needs to be moved further away from the wall, or both. As the magnification increases, the sharpness of the image necessarily decreases, a phenomenon referred to as penumbral blur. This blur, and the practical distances between light source and detector, set boundaries on the magnification that can be realized in a system relying solely on this geometric effect.

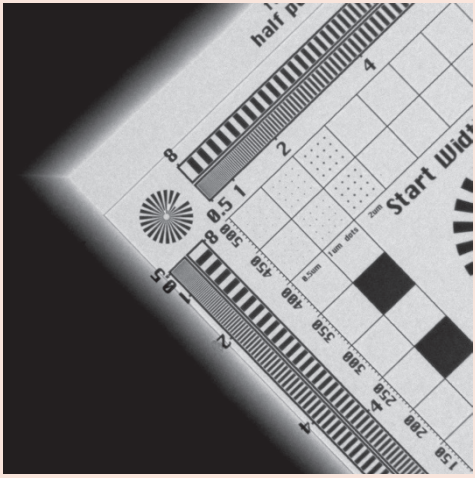


Figure 6. Image of resolution measure

Optical magnification is conventionally produced by glass lenses. It allows for a much greater degree of magnification than geometric magnification, while retaining high levels of resolution. By focusing and refracting the light interacting with an object, the lenses in a modern light microscope can magnify the object by a factor of hundreds.

But because the glass lenses that focus and refract visible light don't work with X-rays, magnification of X-ray images has traditionally been limited by the geometric effect alone. This is fine for generating X-ray images of broken limbs or cancerous tumours, which don't require much magnification, but it has prevented X-rays from being used to study materials at microscopic or even sub-micrometer scales.

This all changed with the development of dual-stage magnification systems, combining moderate amounts of geometric magnification with user-selected optical magnification. This is enabled by means of scintillator-coupled optics, whereby an X-ray image is first formed on a scintillator and then further magnified by an optical lens before reaching the CCD detector plane. Combining these magnification schemes produces a level of resolution and flexibility not previously accessible in laboratory tomography.

Furthermore, additional technological advances have produced focusing optics that act upon X-rays directly, namely capillary condensers and Fresnel zone plates, further pushing the limits of the total magnification achievable by XRM.

FLEXIBLE X-RAY MICROSCOPES WITH SUB-MICRON RESOLUTION

A powerful range of X-ray microscopes that uses advanced optics and detector technology to resolve features as small as 500 nm, comprising four different models – 410, 510, 610 and 620 – that differ in their precise mix of features, the ZEISS Xradia Versa family employs standard geometric magnification as well as novel optical magnification implemented between the scintillator and the CCD. With this two-stage magnification, the level of detail that can be resolved is nearly independent of the relative positions of the X-ray source, sample and detector. We call this 'resolution at a distance' (RaaD) – capable of maintaining 1 µm spatial resolution even at 50 mm source-sample distance – ideal for larger samples or samples contained within *in situ* chambers.

The Xradia Versa family benefits from many other advanced features. These include several methods for improving image contrast in instances where X-ray absorption by different materials in a sample is very similar

Figure 7. ZEISS Xradia 620 Versa

(see Box 3) and the ability to zoom in on interesting features, just like with a light microscope. It also boasts proprietary stabilization mechanisms, a sample stage with multiple degrees of freedom and automated workflows for high volume, repetitive scanning.

For ZEISS Xradia 510/610/620 Versa models, there is an optional flat panel detector, which extends the application space to larger volumes, allowing entire large samples – such as an intact smartphone – to be imaged rapidly in 3D. The technically most advanced model of the family, ZEISS Xradia 620 Versa also offers optional modes for studying the crystal structure of samples (see Box 4) and for manipulating X-ray images taken at two different wavelengths to discern subtle details, such as mineral phases in a geological sample.

With these advanced features, the Xradia Versa instruments allow scientists to conduct studies that would be impossible with conventional X-ray imaging systems. Take, for example, an investigation of how tiny silicon particles are distributed in a chunk of aluminium-silicon alloy treated with heat and friction to make it less brittle. Using propagation phase contrast, scientists can detect the silicon particles in the processed part of the sample and then use optional grain structure analysis to examine their orientation. Other applications include mapping the location of individual neurons in brain tissue in 3D, or high-resolution inspection of defects and failures within large electronics packages.

BOX 3. Enhancing contrast

Absorption contrast imaging is the standard imaging mode used in hospital X-rays. It produces a shadow picture where materials that absorb the most X-rays – such as bone – produce the most contrast. Utilizing optimal scintillator and detector designs, the Versa and Ultra X-ray microscopes maximize the sensitivity to these small absorption variations. In the case of polychromatic X-rays as used in Versa, this demands enhanced detection of low-energy photons and decreased detection of contrast-reducing high-energy photons. However, for samples containing materials that absorb X-rays to very similar extents, absorption contrast imaging on its own may not be sufficient for producing a detailed image. Examples of such low-contrast samples include soft tissue, polymers and fossilized organisms encased in amber.

So the Xradia Versa and Ultra families of X-ray microscopes employ alternative methodologies to enhance the contrast. These

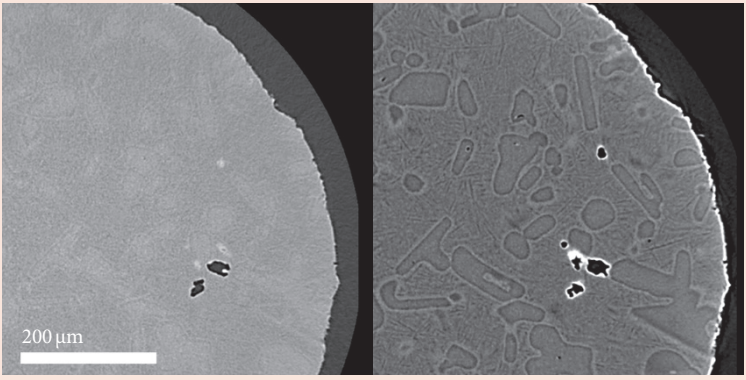


Figure 8. Propagation phase contrast image of aluminium silicon (right) alloy discerns whisker interfaces not present in absorption image (left)

include phase contrast imaging, which takes advantage of the fact that all light, including X-rays, will pass through materials at different speeds, altering the phase of the light wave. Using appropriate detectors and acquisition geometry, this phase difference can be used to enhance contrast, especially at the interfaces between materials. In ZEISS Xradia Ultra, a Zernike phase ring is inserted into the beam path and used to amplify this effect, leveraging a variation of a technology originally developed for light microscopy.

BOX 4. Transforming crystallography

X-ray crystallography is a technique for studying the regular arrangement of atoms in crystalline materials, such as ceramics and metals. When a crystal is illuminated by an X-ray source, the atoms cause the X-rays to spread out – or diffract – and then interfere with each other to create a pattern specific to that material.

Traditionally, the X-ray diffraction (XRD) technique has been used as an analytical approach for identifying and describing crystalline phases, but without the power to produce spatially resolved images. Concurrently, electron backscatter diffraction (EBSD) in electron microscopes has developed to provide 2D maps of the crystal structure of surfaces, but lacks the power to generate 3D information. But, since the early 2000s, scientists have been developing a technique known as diffraction contrast tomography (DCT), using the powerful X-ray beams

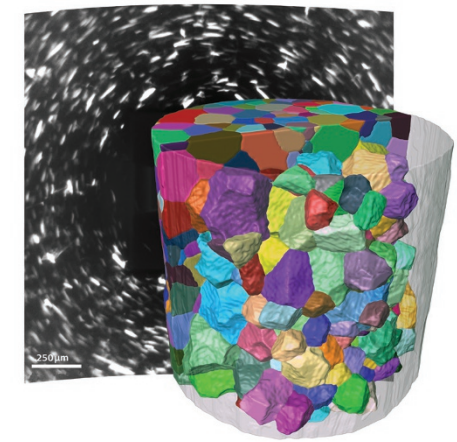


Figure 9. Non-destructive LabDCT 3D grain structure of iron. Internal crystallography (colour) revealed by diffraction information (black and white)

produced by synchrotrons to visualize cracks, impurities, locations, shape, and orientation of grains in 3D. Unfortunately, only a handful of facilities worldwide offer this technology, despite a growing demand from material scientists.

ZEISS Xradia 620 Versa offers DCT in a commercially available X-ray microscope, bringing 3D non-destructive grain mapping into the lab. With the LabDCT module, grains in the sample act as tiny lenses to focus diffracted X-rays onto a detector. The GrainMapper3D software then reconstructs the positions, shapes and orientations of the grains in 3D from a combination of these diffraction patterns and ordinary X-ray absorption images.

ADVANCED X-RAY MICROSCOPES WITH NANOSCALE IMAGING

The ZEISS Xradia Ultra family are the first X-ray microscopes to bring non-destructive 3D imaging at nanometer-scale resolutions into the laboratory. Comprising two different models – 800 and 810 – that differ in their X-ray energies and precise mix of features, the Xradia Ultra family uses advanced optics and detector technologies adopted from synchrotron-based designs to achieve resolutions down to 50 nm, intermediate between light microscopy and electron microscopy.

Models with two different X-ray energies to enhance contrast are available. ZEISS Xradia 800 Ultra uses a higher-energy X-ray source (8.0 keV) for imaging thicker, denser samples, because more powerful X-rays are required to penetrate them, while Xradia 810 Ultra uses a lower-energy X-ray source (5.4 keV) for thinner, less dense samples. The advantage of using lower energy X-rays where possible is that they are absorbed more strongly by the sample and therefore provide a higher contrast. So whereas Xradia 810 Ultra is more suitable for analyzing polymers, energy storage materials and soft tissue, Xradia 800 Ultra is more suitable for analyzing electronic components and metals.

With the Xradia Ultra family, the option of adding a phase ring behind the Fresnel zone plate is also offered to further improve contrast, which can be useful for studying low density samples as well as tiny features like cracks and defects. In addition, an optional Load Stage can be integrated to perform *in situ* 3D nanomechanical testing (see Box 5). The

Xradia Ultra family also benefits from a low vibration platform and precise positioning stages for holding the microscope steady during several hours of unattended imaging.

With its unprecedented resolution and advanced features, the Xradia Ultra microscopes can take XRM to places where it's never been before. For example, scientists are using Xradia Ultra to determine the internal porosity of shale at nanoscale resolutions and to explore dendritic structures in advanced metal alloys. With the optional Load Stage, they are able to study crack formation in dentin, a major component of teeth that protects them from fracture, by observing how cracks initiate, propagate and ultimately lead to failure.

BOX 5. Imaging under pressure

Scientists have long used microscopes to study materials under strain, but these techniques have generally required very thin samples (e.g. TEM) or were limited to surface deformation (e.g. SEM or most light microscopy).

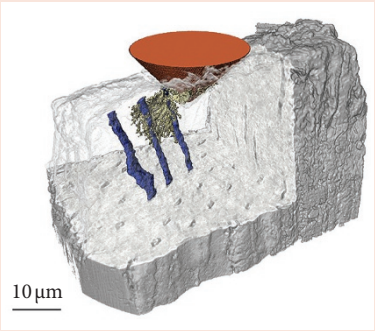


Figure 10. A rendering showing the 3D morphology of a selected crack (gold) in dentin (grey) in relation to neighboring tubules (blue) and the indenter tip (orange)

The Ultra Load Stage is an optional accessory for ZEISS Xradia Ultra that overcomes these limitations. It allows, for the first time, the effects of compression, tension and indentation on whole samples to be imaged in 4D at nanoscale resolutions.

The stage consists of a motor, a force sensor, and two anvils at the top and bottom that exert force on the sample while being rotated and imaged by XRM. In indentation mode, for example, the top anvil presses down on the surface of a sample to reveal how micro-cracks appear and propagate through the sample.

Applications of this innovative new technology include studying how building materials fail when compressed or stretched, and how this relates to their microstructural features. For example, scientists can observe whether damage initiation is localized or widespread, what are the related structural features, and how does it propagate through the sample with increasing load.

HIGH-RESOLUTION *IN SITU* EXPERIMENTS

Science is increasingly moving towards looking at samples *in situ* in order to fully understand how different materials perform under real-life working conditions. As previously stated, one of the advantages of RaaD is that it allows you to perform high-resolution scans at source-to-sample distances that a traditional micro-CT architecture cannot match. While a sample may be able to be reduced in size, this is not the case if you want to perform an *in situ* experiment: your effective sample size is no longer the sample, but the size of the *in situ* chamber.

Taking advantage of this unique feature of XRM, you can look at your samples in a wide variety of *in situ* experiments, using either commercially available *in situ* chambers or building your own. These can range from micromechanical studies on tension, compression or indentation, to temperature or atmosphere control.

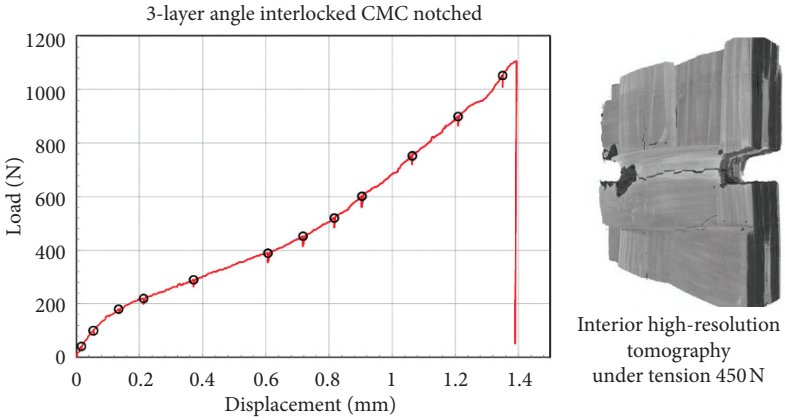


Figure 11. An *in situ* experiment looking at how a woven ceramic matrix composite performs under tension

BOX 6. Correlative microscopy

Correlative microscopy, in which several different microscopy techniques are combined into a common workflow, is becoming an increasingly popular way to study a range of different samples, including biological specimens, geological samples and advanced functional materials such as battery electrodes. The advantage of using several different microscopy techniques is that it allows scientists to cover a broad range of length scales and dimensions consistent with the often-hierarchical nature of their samples, therefore providing an improved level of contextual understanding for their data as well as a more complete representation of its complex structure.

With its ability to non-destructively produce 3D and 4D images of the interior of samples at sub-micrometer resolutions, intermediate between light microscopy and electron microscopy,

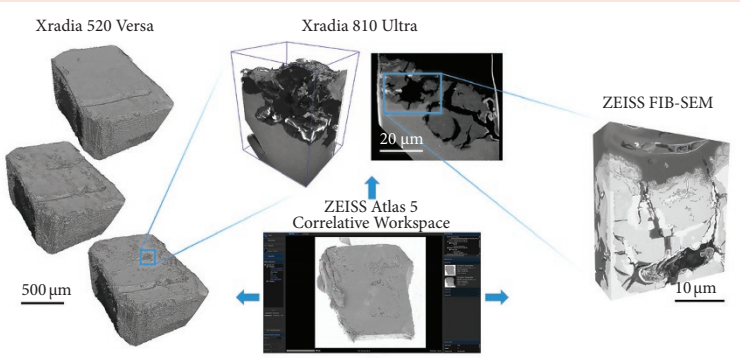


Figure 12. Experimental overview. This multiscale correlation study was designed to better understand corrosion damage in a Mg AZ31B alloy sample. The graphical user interface of ZEISS Atlas 5, designed as a correlative workspace enabling the user to combine data from different modalities, was used to link and navigate between imaging performed on ZEISS Xradia 620 Versa, ZEISS Xradia 810 Ultra and ZEISS FIB-SEM microscopes

XRM is becoming an integral part of such studies. In one recent example, a group of scientists used ZEISS Xradia Versa to monitor corrosion in a magnesium alloy over the course of a few hours, and subsequently identify a particularly corroded region within the sample for further study at higher resolution. They then extracted this region using focused ion beam milling (on a ZEISS FIB-SEM), and performed more detailed 3D analysis first with ZEISS Xradia Ultra and then FIB-SEM tomography (which destroyed the sample in the final step). Such a 'correlative tomography' workflow has the power to provide 3D information at targeted regions of interest spanning from the mm to nm scale.

ZEISS Atlas 5 is a solution designed to aid such correlative microscopy studies, by automatically integrating images produced by different microscopy techniques (even 3D) so that users can seamlessly identify and navigate to specific features for further interrogation.

THE MANY APPLICATIONS OF XRM

XRM uses X-rays to penetrate deep inside solid objects, revealing their internal structures, down to the nanoscale. It is of invaluable use across a wide range of scientific disciplines, from life sciences to geology to advanced materials.

The microscopic structures of almost all materials can change over their lifetime, as a result of physical stresses or exposure to environmental conditions such as moisture and heat. XRM offers a unique opportunity to understand the mechanisms and dynamics of these changes inside a sample in 3D. Examples include improving our understanding of corrosion inside lightweight magnesium alloys, and studying the formation of micro-fractures within human bones.

The electronic devices essential to modern life demand powerful rechargeable batteries, with long battery life and fast charging times. XRM can image whole batteries in 3D,

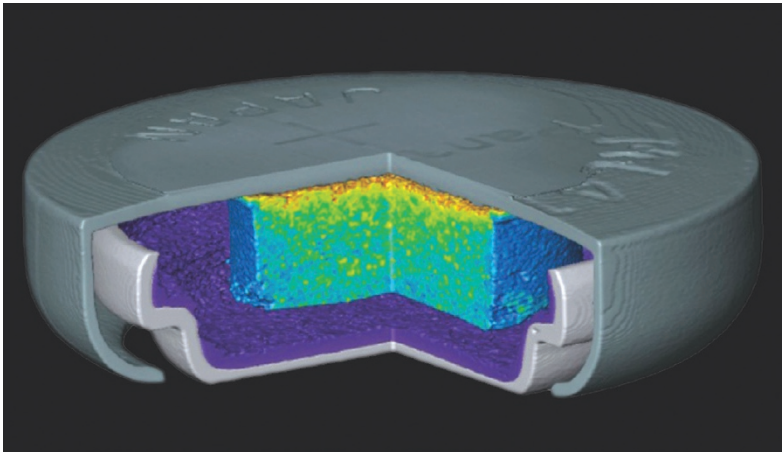


Figure 13. 4D non-destructive XRM study tracking the change in microstructure of lithium ion battery materials as a result of charge cycles. Field of view: 2 cm

without disturbing the packaging, to reveal the micro-cracks and structural defects that can cause them to fail.

XRM is also at the forefront of cutting-edge geological investigations. In industrial oil and gas extraction, XRM is regularly used to provide estimates of yield from potential drilling sites, especially from today's less conventional oil sources, such as shale. XRM can also produce 3D images of the pores and fractures in the rocks that dictate the likely flow and accessibility of its oil or gas.

Visible light microscopy including laser scanning confocal microscopy has transformed the life sciences, but is limited to thin or translucent samples, with relatively small volumes. XRM can complement these techniques by creating 3D images of large specimens such as entire zebrafish or other organisms without damaging them, providing a tantalizing glimpse of their tissues and organs at the resolution of individual cells.

EXAMPLE: ADDITIVE MANUFACTURING

As the development of additive manufacturing (AM) for mainstream production continues to grow, so too will the need for linking the impact of process conditions to material performance, from raw stock material to final part. Defect detection and characterization within additive manufactured parts is a key to efficient and effective process development. However, the process parameters for fabrication can vary dramatically for different materials and shapes, resulting in unwanted defects in the final part.

The unique architecture of ZEISS Xradia Versa, most notably the variable resolution detector coupled with a flexible sample stage, allows easy collection of high-resolution tomography information for detailed analysis of complex AM parts. In the case that higher resolution is required, ZEISS Xradia Ultra offers a 50 nm resolution.

For AM using feedstock powder, the powder needs to be uniform in morphology, shape and size. Satellite particles

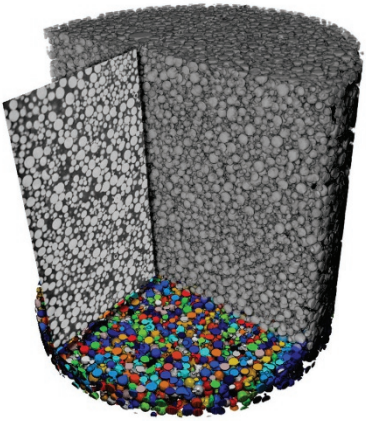


Figure 14. Ti-6Al-4V powder for PBF, DED. Defects in the raw powder material for additive manufacturing can be analyzed using XRM

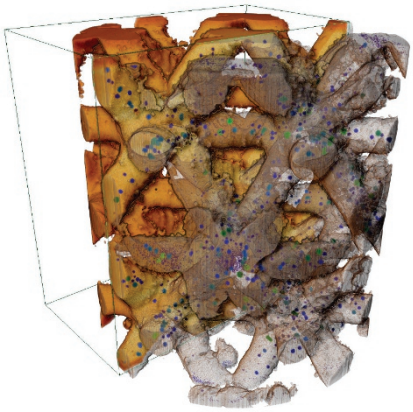


Figure 15. An Inconel 3D-printed lattice structure with the porosity shown by the blue dots. Sample courtesy of Kavan Hazeli, Mechanical and Aerospace Engineering, The University of Alabama, Huntsville, USA

and internal porosity also must be avoided in order to avoid the appearance of defects in the final part. In Figures 14 and 15, we are able to see a number of defects in the raw material, which may lead to an inferior final product, depending on their quantity and distribution.

We are also able to get statistical analysis of final parts, such as porosity. Ideally, metal AM parts, such as the Inconel sample in Figure 15, have a density greater than 99.5% to match physical properties and the reliability of traditionally machined/formed parts. An unwanted concentration of porosity can cause failures in your final product.

EXAMPLE: BATTERIES

Analysis of batteries is challenging – many critical efficiency and safety effects only become apparent with aging, and thus studies of microstructure over time are critical to understand lifetime dynamics. This makes non-destructive measurement techniques particularly appealing. However, this is complicated by the fact that we would prefer to measure intact devices to see their microstructure. Many X-ray CT systems claim sub-micron resolution, but this is only possible

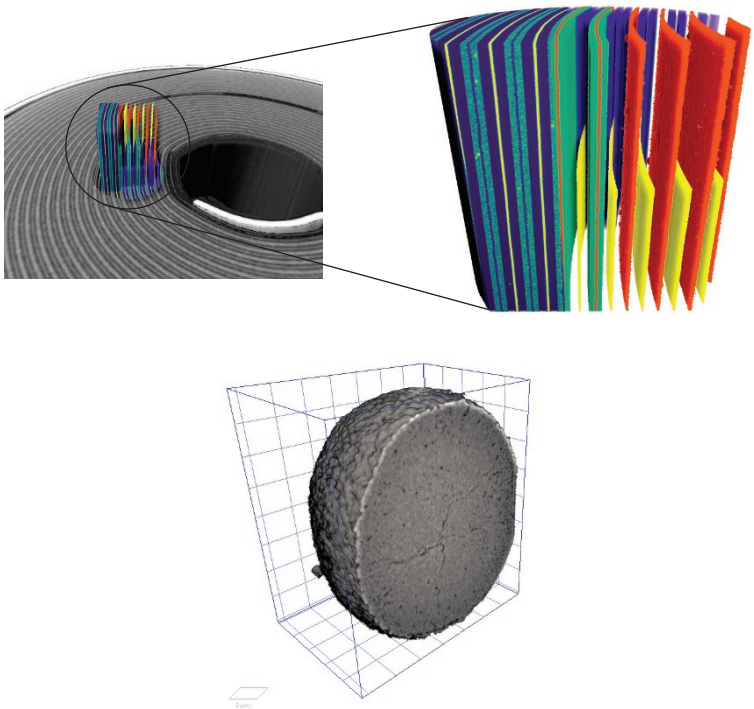


Figure 16. Top: An 18650 Li-ion battery measured with ZEISS Xradia 620 Versa. Bottom: A single cathode particle of NMC 111 imaged in High Resolution Zernike Phase Contrast mode on ZEISS Xradia 810 Ultra, voxel size 32 nm

when the sample is prepared very carefully – usually cut down to a small piece, and often requires a special filament exchange and adjustment of the X-ray source. Luckily, with RaaD we are able to scan the entire device to identify areas of interest and then zoom in for high-resolution imaging.

In Figure 16, we see an example of XRM's ability to measure intact devices with a measurement of a commercial 18650 Li-ion battery measured with ZEISS Xradia 620 Versa. We are clearly able to distinguish between the different layers in the battery and can see how charge cycling, for example, will affect each of them.

If nanometer-scale resolution is required, we can correlatively turn to ZEISS Xradia Ultra to measure battery materials. In the example shown in Figure 16, we see a single cathode particle of NMC 111. In this case, the study was motivated by understanding how morphological changes in the particle will affect device performance, such as any changes in the internal crack visible in the scan. Due to the non-destructive qualities of XRM, this high-resolution imaging can fit seamlessly and correlatively into your workflow with other imaging modalities.

EXAMPLE: CRYSTALLOGRAPHY

With LabDCT, ZEISS brings you the first-ever laboratory-based diffraction contrast tomography imaging module. This unique grain imaging analytical technology enables non-destructive mapping of orientation and microstructure in 3D. No longer confined to conventional 2D metallography investigations, direct visualization of 3D crystallographic grain orientation opens up a new dimension in the characterization of metal alloys and polycrystalline materials.

You can combine 3D grain orientation with 3D microstructural features such as defects or precipitates that you have observed in tomography, thus seeing new possibilities for characterizing damage, deformation and growth mechanisms – or even to couple with modeling.

LabDCT extends metals research to 3D – and on to 4D with routine tool access for longitudinal studies such as

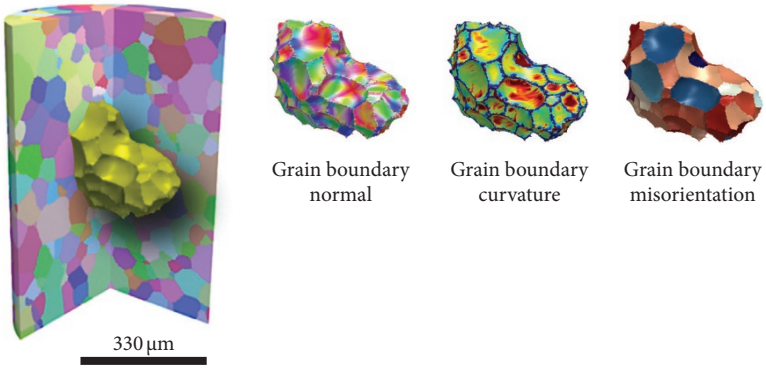


Figure 17. Abnormal grain growth in Armco iron using LabDCT. Laboratory diffraction contrast tomography is a contrast method that is uniquely available on the ZEISS Xradia Versa XRM instruments. Sample courtesy of Prof. Burton R. Patterson, University of Florida, USA

corrosion. Compared to measurements done in a synchrotron, being able to expose your samples to environments in the microscope across days, weeks or even months is a unique strength of laboratory-based XRM experiments.

The possibilities cover a wide range, from metals and alloys, such as in the example shown in Figure 17 of abnormal grain growth in Armco iron, to abrasives, energetic materials, or semiconductors. By being able to routinely acquire grain statistics on larger volumes at faster acquisition times, the crystallographic information provided by LabDCT lets you supplement other analyses like EBSD or synchrotron methods.

EXAMPLE: BIOMATERIALS

From the smallest features measured by ZEISS Xradia Ultra microscopes to larger samples, XRM has a lot to offer for biomaterial samples. With the Scout and Zoom approach, you can observe interior details of larger samples without destroying them, which is particularly advantageous for precious samples such as fossils.

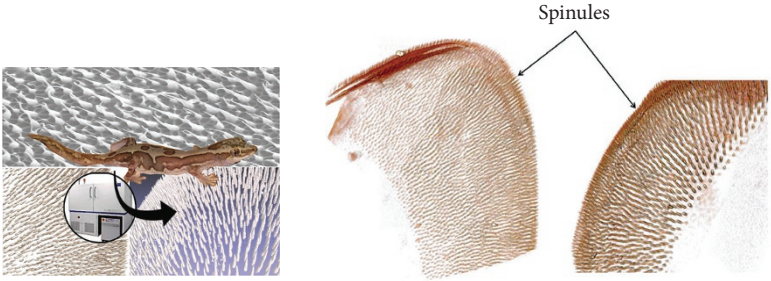


Figure 18. Using ZEISS Xradia Ultra XRM, digital copies of natural and copied Gecko-lizard nanotipped microspinules are generated with unprecedented levels of accuracy to provide design instructions for soft lithography replication. Moreover, the X-ray data are easily transcribed *in silico* for fine editing and refinement before eventual real-life projection into 3D printouts. https://doi.org/10.1002/admi.201800201

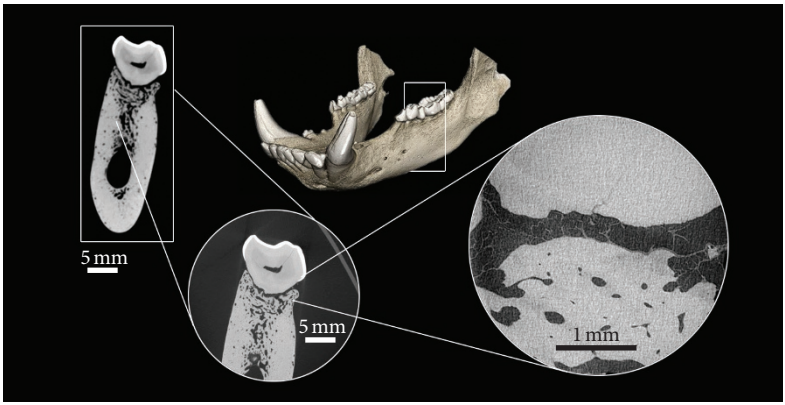


Figure 19. A bear jaw imaged with ZEISS Xradia Versa, using the Scout and Zoom software that enables the creation of efficient workflows by using a simple control system to scout a region of interest and specify scanning parameters

EXAMPLE: SEMICONDUCTORS

Over the last decade, ZEISS Xradia Versa 3D X-ray microscopes have become the standard for non-destructive failure analysis of semiconductor packages. Xradia 620 Versa RepScan, a sub-micron resolution, 3D non-destructive imaging solution for inspection and measurement, adds a new dimension of capabilities to XRM by enabling linear and volumetric inspection and measurement of critical buried structures in advanced semiconductor packages.

RepScan enables automatic loading, scanning and unloading of identical samples without the need for operator intervention. Scan results may be automatically transferred to a separate workstation where a variety of measurements can be executed semi-automatically. This establishes a new benchmark for non-destructive off-line measurements that support process optimization, product development and quality analysis and control of complex fine-pitch 3D

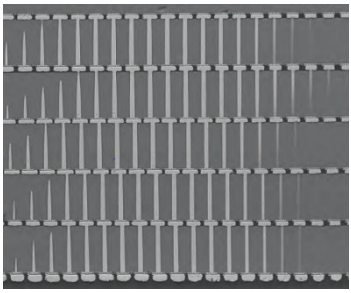


Figure 20. Image taken by manual cross-section
- **Mechanically destructive to sample**
- **Linear measurements in only one plane**
- **Difficult to position/capture fine features**

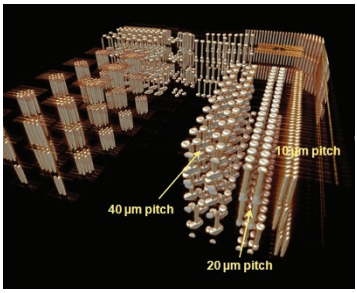


Figure 21. Image taken by 3D X-ray microscope
- **Not mechanically destructive**
- **Linear and volumetric measurements**
- **Infinite flexibility for all planes/ features**

architectures, including 2.5D interposers, high bandwidth memory stacks with TSVs and microbumps, wafer-level packages with package-on-package interconnects, and ultra-thin memory with multiple chips in a stack.

By being able to image and measure buried features non-destructively with sub-micron resolution, you can base development decisions on richer, higher-accuracy engineering data and use acquired data for designs of experiments, process skews, corner lot or other statistical process analysis.

CASE 1: From advanced materials to insect pests

Since 2017, Ralf Wehrspohn, director of the Fraunhofer Institute for Microstructure of Materials and Systems IMWS in Halle and professor of Microstructured Material Design at the Martin Luther University Halle-Wittenberg, Germany, has been using ZEISS Xradia 810 Ultra to study advanced materials developed by his group. But now, he is also beginning to apply the expertise his group has built up in 3D XRM to other, completely different scientific fields.

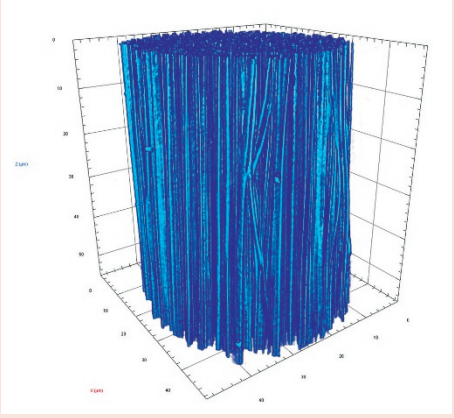
'In our group, we use XRM to produce 3D virtual representations of the samples non-destructively,' explains Wehrspohn. 'These representations enable the morphological characterization of the samples and also the estimation of structural parameters using software analysis. From those 3D images, we have already characterized polymer fibers, protein fleeces, porous materials of diverse compositions, catalysts and drugs.'

For example, Wehrspohn and his group used ZEISS Xradia 810 Ultra to study novel electrodes they had developed for splitting water by electrolysis, which offers an efficient way to produce hydrogen for energy applications. The electrodes consist of stainless steel coated in oxidized carbon nanotubes and then covered in catalytic particles of platinum or ruthenium dioxide, with the carbon nanotubes providing an effective way to link the catalytic particles to the stainless-steel support. With ZEISS Xradia 810 Ultra, the scientists were able to confirm that the stainless-steel support

had become fully coated with carbon nanotubes, increasing its surface area by almost seven times.[1]

They also used ZEISS Xradia 810 Ultra to study polymer yarns that are simultaneously strong and tough, properties that are usually mutually exclusive in synthetic materials. These polymer yarns consist of nanofibrils of polyacrylonitrile aligned in the same direction and connected together via a polymer called poly(ethylene glycol) bisazide. The yarns are then heated while being stretched to enhance their strength and toughness. With ZEISS Xradia 810 Ultra, the scientists were able to confirm that the preparation process had aligned all the nanofibrils in the same direction.[2]

For Wehrspohn, the main advantage of 3D XRM is that it is non-destructive. 'It means that we can visualize in detail the internal parts of the sample without the need to cut or destroy it, which is usually necessary for other microscopy techniques,' he says.



Three-dimensional X-ray image of the polymer yarn, showing the nanofibrils aligned in the same direction

He and his group are now bringing this advantage to biological samples. Recently, in conjunction with colleagues from the Institute of Biology at the Martin Luther University Halle-Wittenberg, they used ZEISS Xradia 810 Ultra to study structures known as setae at the tip of the abdomen of a species of thrip.[3] The thrip uses these setae to hold a drop of fluid that it can launch at predators as a defense mechanism.

'In the future, we plan to explore more consistently the use of XRM to develop new methods for analyzing biological samples,' Wehrspohn says.

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MICROSCOPY AND ANALYSIS

Essential Knowledge Briefings