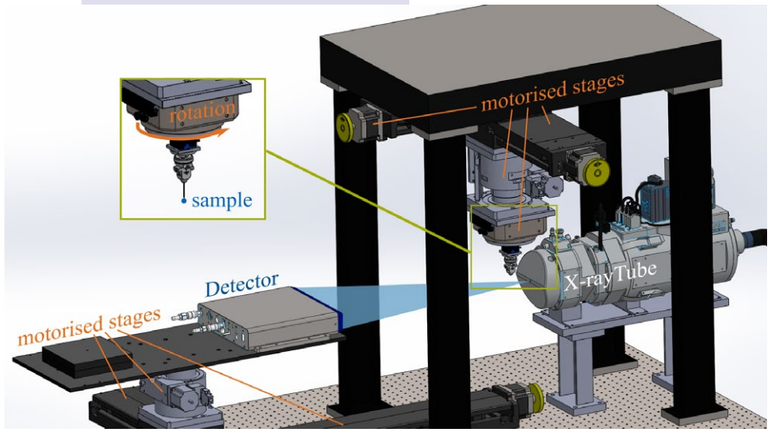
Capturing three-dimensional cell structure with X-ray tomography

Seeing cells is no easy task. Most cells are smaller than a tenth of the size of a human hair, making them impossible to see by eye. Optical microscopes, with the help of cell-staining to colour cells, can help us peer into the invisible world of cells. However, they only show us a 2D image of a very thin slice of tissue. But how can we see what the cells and tissue actually look like in 3D? To do just that, Dr Madleen Busse from the Technical University of Munich has been developing X-ray stains that can be used to visualise cells and tissues in 3D using cutting-edge X-ray imaging techniques developed by her colleagues Prof Franz Pfeiffer and MSc Simone Ferstl.

To see into the invisible world of objects that are less than a tenth of a millimetre in size, we need to find ways of magnifying the object. One way of magnifying an object may be to use something like a magnifying glass or, where greater magnification is needed, an optical microscope.

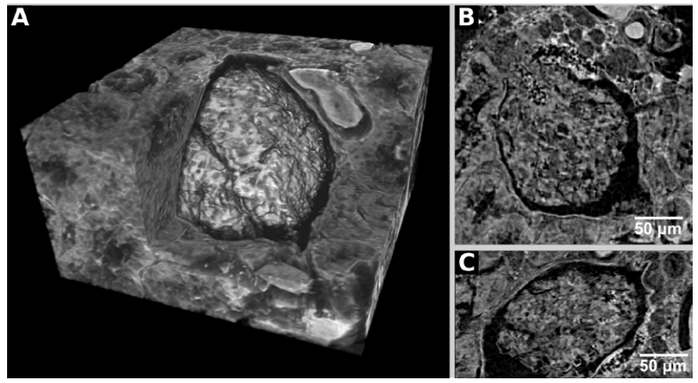
With sufficient magnification on a microscope, it is possible to produce a clear and detailed image of even tiny objects, like cells, that are on the order of tens to hundreds of micrometres in size, depending on the type of cell. The smallest object that can be seen by the human eye is about one tenth of a millimetre, meaning cells are still a ten to a hundred times too small to be seen by the naked eye without any magnification. Fortunately, achieving magnification levels on this order and preserving the image quality is relatively straightforward. Therefore, microscopy and visual inspection of cells has become a routine tool in medical diagnosis.



What makes microscopy so useful for diagnosing different illnesses is that many diseases, such as cancer, cause changes at the cellular level. Cancerous cells have many visual differences to normal, healthy cells, including changes to the cell structure and even changing how the cells form tissue clusters and clump together. This is why taking a biopsy, or a small tissue sample, is a standard part of cancer diagnosis and the process of looking at the anatomy of cells in this way is known as histology.

To help identify cell types and differentiate between healthy and infected cells, just using a good quality microscope is not enough. The cells and structures to investigate need to be coloured first to see them under the light microscope. A commonly used technique for this is cell staining, where dye-like molecules are used to preferentially colour certain parts or certain types of cells. This can help to increase the contrast between different areas of the cell. We often see pictures of bacteria or cells that are beautifully coloured due to this reason. Some cell stains can even be used on living cells to see how they function and how their metabolic processes work.

However, light microscopy is not the only method to examine cells. Therefore, there are also cell stains which are designed to work with other imaging methods than with optical microscopy. Dr Madleen Busse has been developing cell stains which are specifically designed for X-ray imaging. Under the supervision of Prof Franz Pfeiffer, together with Simone Ferstl and their team at the Technical University of Munich, she has been working on combining these cell stains with an in-house-developed 3D X-ray microscope to look at the smallest details of cells.



NanoCT and histology tissue images from a human kidney cortex neighbouring cancerous tissue: (A) Representative NanoCT volume with an effective voxel size of ~ 730 nm (250 µm x 250 µm x 135 µm) highlighting a renal corpuscle with glomerulus and Bowman's capsule. Glomeruli are actively involved in the filtration of the blood to form urine. (B) and (C) Individual NanoCT slices derived from orthogonal planes through the volume seen in (A).

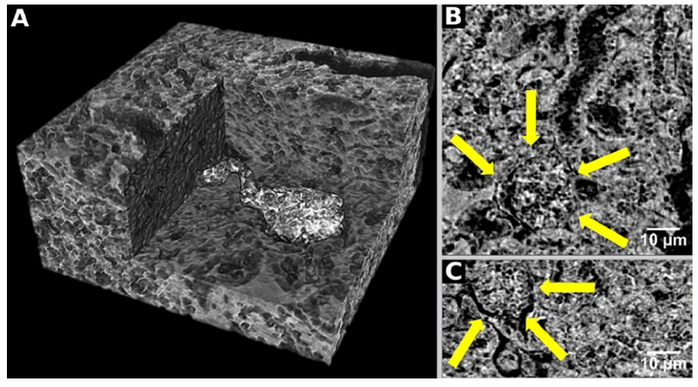
Dr Busse has developed a staining method that makes it possible for the first time to look at the cytoplasm of cells.

3D X-RAY TOMOGRAPHY

Usually optical microscopy gives a flat, two-dimensional image of an object that shows the surface layers of what is in the tissue. For medical diagnosis, it is often helpful to be able to look at an object in full 3D space. This is why computed tomography (CT) was developed – rather than just the traditional flat X-ray radiograph. In a CT device, the camera is installed on a large circular mount. During the CT scan the camera moves in a circle around a patient and takes snapshots at all different angles. Computer algorithms are then used to reconstruct a full 3D visualisation from all these different images.

3D X-ray tomography cannot only be used on patients, but also to look at tissue biopsies for histological studies, in a similar way optical microscopy does. The advantage of using X-ray light rather than visible light is that X-rays can penetrate through the cell and the tissue, meaning a full 3D reconstruction can be done, whereas optical microscopy can only look through a thin tissue slice (of a couple of hundreds of micrometers).

While X-ray tomography makes it possible to gain a whole new perspective on tissue samples, there are a few challenges when it comes to using it. For soft tissues, the contrast between different areas of the tissue is very poor as the X-rays are only weakly absorbed. This makes it hard to recognise the cellular structures needed for histology studies.



NanoCT slices and 3D rendering of a mouse kidney cortex with an effective voxel size of 400 nm: (A) Perspective view of a volume rendering with glomerulus and the proximal tubule modeled in 3D. (B) and (C) Two orthogonal NanoCT slices through the cuboid volume shown in (A) with the glomerulus (yellow arrows) embedded into the tubular network.

CELL STAINING

This is where the work of Dr Busse comes in. She has developed a staining method which can be combined with advanced X-ray tomography technologies with micrometre or nanometre resolution, which have been developed by Prof Pfeiffer and his team. This makes it possible to look at the cytoplasm of cells. The team designed their staining method to make use of eosin – a dye that has been extensively used in histological studies as it binds very efficiently to proteins and peptides in the cell cytoplasm.

This novel staining method is non-destructive and completely compatible with standard histological techniques. It allows for the visualisation of the internal and external structural details of the cytoplasm. As the staining methods developed can be automated, the technique has a great deal of potential for clinical diagnosis as well and there is future potential for customisability of the staining approaches.

Once the team had successfully shown this technique could work for visualising structures in mouse kidneys, they then revealed the first 3D images of human renal cell carcinoma tissue – cancerous tissue in kidneys. Kidney cancer has a 40% mortality rate. The X-ray tomography could identify not just the cancerous cells with the same accuracy as visible light microscopy but also see deeper into tumours and distinguish kidney cells that, while they

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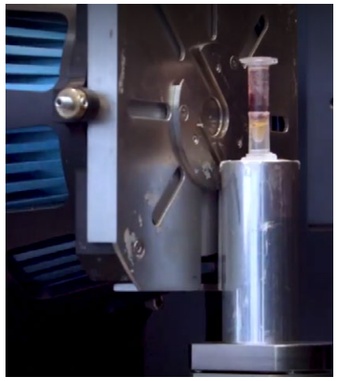

Mounted soft-tissue sample in front of an X-ray source of a microCT scanner.

Simone Ferstl is mounting a stained soft-tissue sample on the presicion stage in the NanoCT. The mounting of the sample is very crucial to the tomography – a slight move of the sample may make a 3D reconstruction of the acquired data impossible.

were not cancerous, had undergone degenerative changes.

THE INFORMATION NUCLEUS

The team have continued developing new cell staining protocols, including one that can be used to look at the cell nucleus. The nucleus of a cell is where all the genetic material of the cell is stored, which in turn provide all the information and coding for the actions and behaviour of the cell. It is also one of the cellular sub-structures that is dramatically changed when cells become cancerous. Apart from that, the distribution of cell nuclei in the tissue gives a lot of information about a disease. A high number of cell nuclei in a tissue region, for example, can be a sign for an inflammation. In contrast to that, the absence of cell nuclei may stand for dead tissue, or tissue necrosis. The cell nuclei and their distribution therefore are of interest for disease diagnosis and progression monitoring.

In collaboration with the Radiology and Pathology Department of the TUM Klinikum rechts der Isar in Munich, the team of Prof Franz Pfeiffer has been using nanometre resolution 3D X-ray microscopy to visualise the cell nuclei in 3D. Currently, they are working on ways to progress this development into clinical settings. Here, the penetrating power of X-rays makes it possible to look at how the cell nuclei are distributed in 3D inside the tissue.

Dr Busse sees these developments in making 3D X-ray tomography a feasible tool for histology studies as an important breakthrough due to the technique's ability to reveal additional layers of information that cannot be seen with the current gold standard histology techniques. More information on multiple layers of tissue will make it possible to monitor disease progression with more detail and see how far through the tissue diseases have spread. This will offer a greater number of options for personalised medicine and better treatment outcomes.

The X-ray tomography could identify not just the cancerous cells but see deeper into tumours and distinguish kidney cells that had undergone degenerative changes.

Dr Busse is setting the parameters for a microCT measurement. Parameters such as voltage, power, filter, exposure time, number of projections, voxel size and binning will determine the image quality.

Behind the Research

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Research Objectives

Prof Pfeiffer, Dr Busse and MSc Ferstl have developed a methodology using a cytoplasm-specific X-ray stain in order to visualise sub-cellular structures.

Detail

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Bio

Madleen Busse holds a PhD in chemistry from Monash University in Melbourne, Australia. In 2015, she was awarded the TU Munich Foundation Fellowship and one year later, received a Marie Skłodowska-Curie Individual Fellowship to build her profile in targeted contrast agent design, staining protocol development and X-ray computed tomography.

Simone Ferstl is a PhD candidate in physics working in Prof Pfeiffer's group at the Technical University of Munich (TUM). Her research focuses on an in-house-built laboratory 3D X-ray microscope (NanoCT). The majority of her work is optimising the imaging system and exploring new fields of application with the NanoCT.

After overseas stays in France (ESRF & ILL Grenoble), the USA (APS Argonne) and Switzerland (PSI Villigen), **Franz Pfeiffer** was appointed professor at the TUM in 2009. Here, he was awarded the DFG Leibniz Prize in 2011. He is leading the chair of Biomedical Physics and is director of the TU Munich School of BioEngineering since 2017.

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Collaborators

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- Radiology Department at TUM Klinikum rechts der Isar: Melanie A. Kimm, Daniela Pfeiffer, Alexandra Gersing and Ernst J. Rummeny

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Personal Response

What cellular sub-structures will you be targeting next?

" My vision is to non-destructively observe specific sub-cellular organelles in soft tissue in 3D providing structural and functional information at multiple scales. The motivation for such an endeavour is the aspiration to link X-ray attenuation-based CT with targeted soft tissue analysis. Additionally, I strive to understand disease development and progression to provide the tools needed for more personalised medicine. Future projects will focus on the visualisation of other cellular sub-structures such as the mitochondrial network architecture. This will allow me to transform 3D virtual X-ray histology into a valuable and reliable tool for tissue analysis in medical research and medicine. "

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