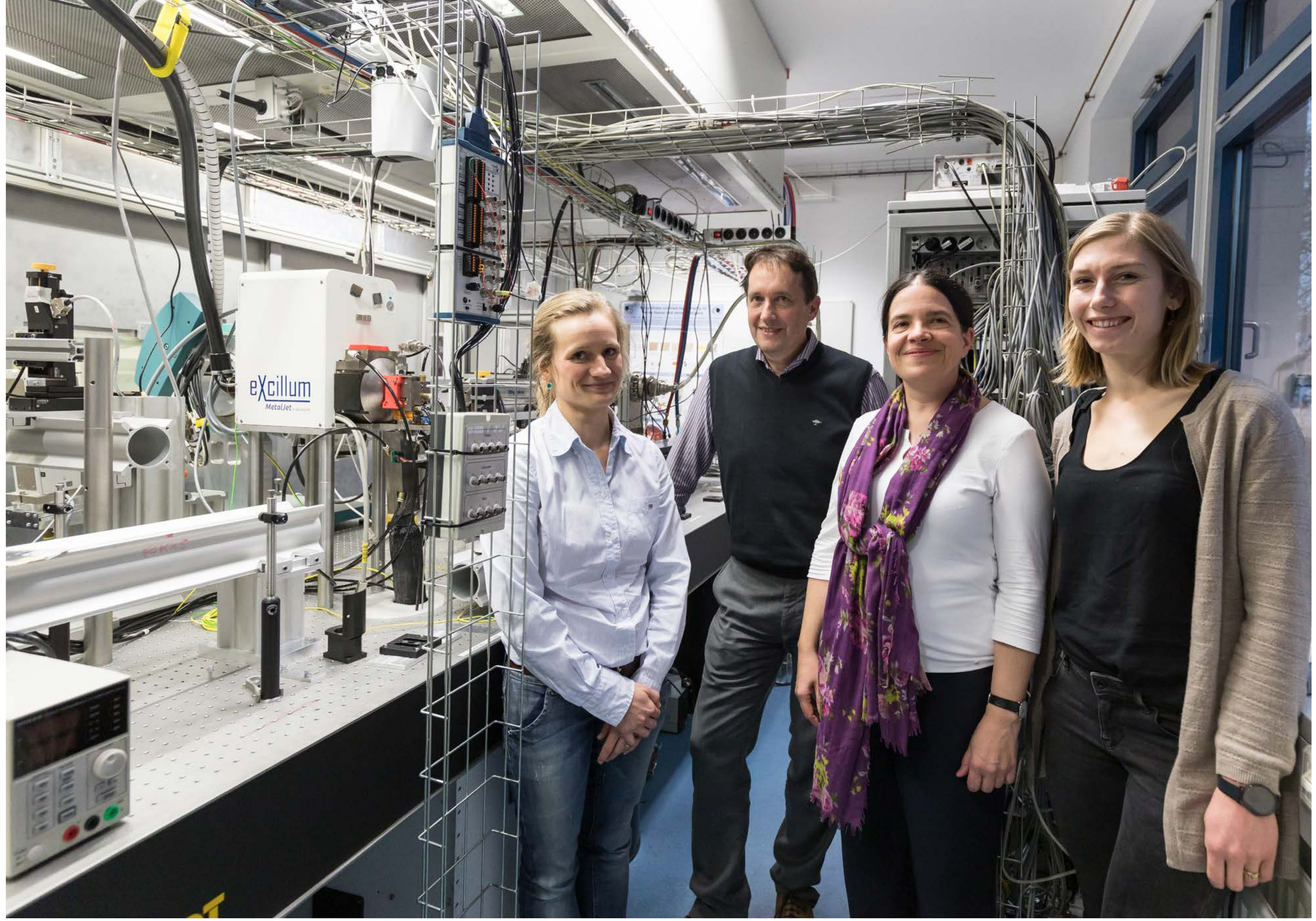
Scientists at the University of Göttingen have developed a special type of X-ray imaging that offers unprecedented insights into the fine structure of nerve cells. In an interview on location, the researchers explain how the virtual study of brain tissue could lead to a better understanding of neurodegenerative diseases, such as multiple sclerosis (MS) and Alzheimer's, and to new diagnostic applications.



Collaborating on phase-contrast tomography: Tim Salditt, Professor at the Institute for X-Ray Physics at the University of Göttingen and (from left) Franziska van der Meer, PhD, Professor Christine Stadelmann-Nessler, Director of the Institute for Neuropathology, and doctoral student Marina Eckermann.

Zooming in on the Brain's Microarchitecture to Better Understand Diseases

Text: Matthias Manych | Photos: Heiko Meyer

The tissue sample, which is x-rayed by photons, is only about one by three millimeters in size. It comes from the cerebellar cortex, a region of the brain that is very densely packed with nerve cells. At the end of the experiment, when all the X-ray signals recorded by the detector behind the sample have been converted into image data, an impressively detailed picture emerges: The dendrites of Purkinje neurons protrude like branches from the cell-packed granular layer. Around 1.8 million cerebellar cells are visible in this three-dimensional reconstruction.

Phase contrast measures the difference

This high-resolution zoom into the neural microarchitecture is made possible by a special X-ray imaging called phase-contrast tomography (see sidebar on page 4). A particularly powerful implementation of this technique for high resolution was developed by Professor Tim Salditt from the Institute for X-Ray Physics at the University of Göttingen and his team.

Unlike conventional radiography, which relies on the absorption of X-ray beams, the phase-contrast technology is about the refraction of the beams in the tissue sample, as Salditt explains: "When the beams pass through the object, this is reflected in their propagation speed. These small time delays are exploited in phase contrast techniques. We can make them visible by a propagation of the beam through free space between the object and the detector, and from the detected image then calculate and display information about biological structures."

Virtual histology – adding the third dimension

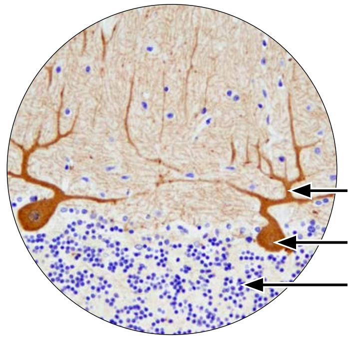
The development of phase-contrast tomography for use in virtual histology is the result of a joint project between the Salditt group and a team led by Professor Christine Stadelmann-Nessler, Director of the Institute of Neuropathology at the Göttingen University Medical Center. The neuropathologist says she required more than the two-dimensional information provided by classical light microscopy, fluorescence and electron microscopy. For her research focus on MS, Stadelmann-Nessler wanted to determine precisely which cells are destroyed by the onset of MS. Through her contact with Professor Salditt, she developed the idea of switching from 2D to the spatial dimension of 3D.

The cerebellum was ideal for their joint work on phase-contrast tomography. It is already well defined histologically and is particularly affected by MS. But until now it was practically impossible to quantify the cells of the granular layer, for instance. "We still have to answer questions about the exact number and location of cells, how they are connected to neighboring cells, which cell types are found at a specific location and how they are interconnected," says Stadelmann-Nessler. And virtual histology by phase-contrast tomography should provide the answers.

Validating a groundbreaking method

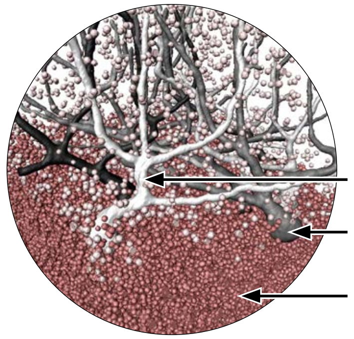
Franziska van der Meer, PhD, scientific assistant at the Institute of Neuropathology, is clearly excited about the possibilities. "If you now zoom into the 3D image, you can see what is happening in the cell below, how the fine cell structures are connected to each other," explains the neuroscientist. The findings of the phase-contrast tomography instrument were validated at the DESY German Electron Synchrotron in Hamburg where the Göttingen scientists had the opportunity to install a comparable instrument to serve as a reference standard.

Conventional histology



Two-dimensional conventional microscopic image of an immunohistochemically prepared section of a cerebellum tissue sample

Virtual histology



The same section of cerebellum tissue recorded with phase-contrast tomography and reconstructed as a 3D image.[1]

By using Synchrotron radiation, the team generated much higher resolution image data than they were previously able to. As a result of this validation, the 3D reconstructions created in the Göttingen laboratory were proven to be very good. In particular, the specially developed algorithm proved impressively effective in displaying separately identifiable cells. Salditt believes that – through close cooperation and mutual inspiration – the Göttingen biophysicists and neuropathologists currently enjoy a technological advantage at a national as well as international level.

Optimizing the method for future goals

Following the proof of concept, the scientists are now working on refining the method, improving the resolution and processing the massive amounts of data faster by using machine learning. And they are already working on the next set of goals. Apart from MS, they aim to achieve a better understanding of other neurodegenerative diseases such as Parkinson's and Alzheimer's. In Göttingen, virtual histology studies on the relationship between Alzheimer's plaques and the surrounding vessels have already been initiated.

Another important goal is the translation of the diagnostic method to routine clinical practice. Before that can happen, larger studies are needed to prove the correlation between virtual histology and classical pathological findings. Then the technology and algorithms need to be integrated into a simple and robust workflow. That could be achieved in eight to ten years, Salditt reckons. He adds: "Our long-term goal is to have a phase-contrast tomograph in every pathology lab." ●

[1] M. Töpperwien, F. v.d. Meer, C. Stadelmann, T. Salditt, Three-dimensional virtual histology of human cerebellum by X-ray phase-contrast tomography, PNAS vol. 115 | no. 27, 6940ff. https://www.pnas.org/content/115/27/6940. Last accessed Feb. 21, 2020

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In eight to ten years, phase-contrast tomography could be routine clinical practice, reckons Professor Tim Salditt.

Details of method

The phase-contrast tomograph in the Göttingen X-ray physics laboratory consists of a liquid-metal X-ray source, the sample holder, the detector, and a tube with optical lenses. In the X-ray tube, electrons hit a fine beam of liquid metal. The resulting photons hit the sample and are then recorded by the detector. The optics serve as a microscope. Measurement times of 30 minutes and less are possible with high-resolution detectors.

For the DESY method, a certain amount of energy has to be filtered out of the synchrotron radiation spectrum and monochromatized. The beam defined in this way is then focused using a mirror system (Kirkpatrick-Baez optics) and finally reduced to only 15 nanometers in diameter using a waveguide. The rays then hit the sample and finally the detector.