UNIVERSITÄTSMEDIZIN GÖTTINGEN UMG

MBExC Multiscale Bioimaging Cluster of Excellence

## Press Release

### ONE microscopy: from molecule to 3D structure with conventional microscopy

**Researchers at the University Medical Center Göttingen (UMG) have developed a new method that makes it possible for the first time to image the three-dimensional shape of proteins with a conventional microscope. Combined with artificial intelligence, One-step Nanoscale Expansion (ONE) microscopy enables the detection of structural changes in damaged or toxic proteins in human samples. Diseases such as Parkinson's disease, which are based on protein misfolding, could thus be detected and treated at an early stage. ONE microscopy was named one of the "seven technologies to watch in 2024" by the journal Nature and was recently published in the renowned journal Nature Biotechnology.**

Göttingen, October 9, 2024 – Fluorescence imaging is one of the most versatile and widely used tools in biology to observe biological processes in living cells. Despite advances in technology and improvements in resolution, the visualization of single molecules and the organization of molecular complexes using fluorescence microscopy remains a challenge. Until now, this was only possible using expensive structural biology methods such as electron microscopy (EM) and, in particular, cryo-EM, in which the samples are imaged with a very strong electron beam at an extremely low temperature.

A research team led by Professor Dr. Silvio O. Rizzoli, director of the Department of Neuro- and Sensory Physiology at the University Medical Center Göttingen (UMG), spokesperson of the Center for Biostructural Imaging of Neurodegeneration (BIN) and member of the Cluster of Excellence "Multiscale Bioimaging: from molecular machines to networks of excitable cells" (MBExC), and Dr. Ali Shaib, group leader at the Department of Neuro- and Sensory Physiology at the UMG, have now developed a method using a few simple but effective tricks to visualize individual molecules in detail using conventional light microscopy. Instead of using expensive, high-resolution microscopes to improve the resolution, they developed One-step Nanoscale Expansion (ONE) microscopy. In this method, the volume of the sample is increased by binding the cells and the structures therein to a water-absorbing gel that penetrates the cells. By absorbing water, the gel increases up to 15 times its volume. This causes the molecules in the sample to move apart evenly and also become larger, so that they can be imaged with a light microscope after being specifically labeled with fluorescent molecules. Combined with a method based on artificial intelligence to evaluate the fluorescence changes, the scientists have succeeded for the first time in doing what was previously only possible with high-resolution cryo-electron microscopy and X-ray technology: "We are now able to reconstruct 3D protein structures from two-dimensional fluorescence images," says Professor Rizzoli. This offers an unprecedented opportunity to directly visualize fine structural details of individual proteins as well as multiprotein complexes in cells or in isolation. Changes in the spatial structure of proteins can also be easily detected with ONE microscopy. In a collaboration with colleagues from Göttingen and Kassel, molecular protein aggregates, which are typical of Parkinson's disease, were imaged and classified in cerebrospinal fluid samples from patients. This is

promising for improved early detection of Parkinson's disease, which affects millions of people worldwide.

ONE microscopy is a simple and cost-effective method that can be carried out in any laboratory with a conventional microscope and achieves a resolution level of around one nanometer. This is about 100,000 times smaller than the diameter of a human hair. The authors provide the necessary software as a free open source package. The newly developed method has now been published in the renowned journal Nature Biotechnology. Due to its high potential, Nature has included ONE microscopy in its list of “seven technologies to keep an eye on in 2024”.

**Original publication:**

*Shaib AH, Chouaib AA, Chowdhury R, Altendorf J, Mihaylov D, Zhang C, Krah D, Imani V, Spencer RKW, Georgiev SV, Mougios N, Monga M, Reshetniak S, Mimoso T, Chen H, Fatehbasharzad P, Crzan D, Saal KA, Alawieh MM, Alawar N, Eilts J, Kang J, Soleimani A, Müller M, Pape C, Alvarez L, Trenkwalder C, Mollenhauer B, Outeiro TF, Köster S, Preobraschenski J, Becherer U, Moser T, Boyden ES, Aricescu AR, Sauer M, Opazo F, Rizzoli SO (2024) One-step nanoscale expansion microscopy reveals protein shapes using conventional microscopes. Nature Biotechnology, 2024. DOI: 10.1038/s41587-024-02431-9.*

**About the ONE microscopy method**

The resolution of conventional light microscopes is limited by the laws of optics: Objects smaller than 200 nanometers, such as antibodies with a size of around 15 nanometers, appear blurred and cannot be made visible separately if they are less than 200 nanometers apart. Super-resolution microscopy circumvents this diffraction limit with optical tricks, so that resolutions of up to ten nanometers and less can be achieved. However, this requires very expensive microscopes. ONE microscopy relies on a magnification of the sample volume in order to circumvent this diffraction limit. Cells and the structures they contain are first chemically bound to a water-absorbing gel, as found in baby diapers. By absorbing water, the gel expands together with the sample, causing the individual molecules to move away from each other. The additional effect of heat or enzymes leads to the splitting of the protein molecules. Individual fragments are formed, which are moved evenly in different directions during the large-scale expansion by up to 15 times, while their spatial arrangement is retained. Targeted labeling with fluorescent molecules then allows the individual protein fragments, which are now located at a distance above the diffraction limit, to be imaged using a conventional light microscope. “We were surprised to see that we can actually visualize the Y-shape of antibodies with fluorescence microscopy,” says Professor Rizzoli. “Combined with artificial intelligence (encoder-decoder model), we have succeeded for the first time in reconstructing the three-dimensional structure of individual protein molecules from two-dimensional fluorescence images, based on conventional light microscopy.”

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A comparison was made between ONE microscopy and high-resolution cryo-EM microscopy. The determined protein structure of the $GABA_A$ receptor, which controls the activity of nerve cells in the brain and spinal cord, showed how well the new method worked. “ONE microscopy makes it possible to visualize the entire structure of the $GABA_A$ receptor. This consists of 15 to 20 percent disordered loops, which have to be averaged from up to one hundred thousand image data using cryo-EM. As these loops are flexible components, i.e. they can vary from receptor to receptor, these image data cannot be averaged adequately. The loops are therefore not recognizable. With ONE microscopy, the first individual images of the entire molecule are available less than 72 hours after the start of expansion,” says Dr. Shaib. “This technique makes it possible to achieve resolutions of more than ten nanometers even with an older light microscope. For comparison, our genetic material, DNA, has a diameter of around 2.5 nanometers and could be imaged using this technique. Any laboratory, regardless of its financial resources, can produce very high-resolution images using the ONE microscopy technique. This is a revolution in microscopy with long-term implications for both science and technology.

**Various application potential for diagnostics**

ONE microscopy makes it possible to detect changes in the shape of damaged or toxic proteins in human samples and therefore offers a wide range of potential applications. In order for proteins to perform their correct function within the cell, they must adopt a three-dimensional structure. This is achieved by folding the protein, a process in which errors can occur. Misfolded proteins are either degraded or lead to toxic deposits in the cells. In addition, there is a deficiency of the corresponding protein and an associated loss of function in the cell and in the organism as a whole. “ONE microscopy could enable a visual diagnosis of protein misfolding diseases such as Parkinson's disease based on blood samples,” says Professor Rizzoli. Using ONE microscopy, it has already been possible to image and classify the aggregates of the alpha-synuclein protein in cerebrospinal fluid samples from Parkinson's patients. A misfolding of alpha-synuclein leads to the formation of these aggregates, which are deposited in the brain and are responsible for the death of nerve cells. “Since we can easily recognize the shape of these aggregates, there is the possibility of an early diagnosis of this neurodegenerative disease,” says Professor Rizzoli. “This would give patients access to early, effective and personalized treatment before the brain is too severely damaged.”

*The Göttingen **Cluster of Excellence 2067 Multiscale Bioimaging: From Molecular Machines to Networks of Excitable Cells (MBExC)** has been funded since January 2019 as part of the Excellence Strategy of the German federal and state governments. In a unique interdisciplinary research approach, the development of new imaging technologies is being driven forward in order to map the disease-relevant functional units of electrically active heart and nerve cells, from the molecular to the organ level. To this end, MBExC brings together numerous university and non-university partners at the Göttingen Campus. The overarching goal is to understand the connection between heart and brain diseases, to*

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*link basic and clinical research and thus to develop new therapeutic and diagnostic approaches with social implications.*

**Further information:**
Department of Neuro- and Sensory Physiology at the UMG: https://www.rizzoli-lab.de
The Cluster of Excellence „Multiscale Bioimaging: from molecular machines to networks of excitable cells“ (MBExC): https://mbexc.de

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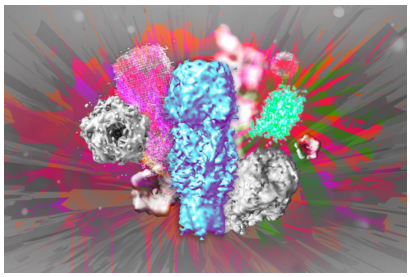
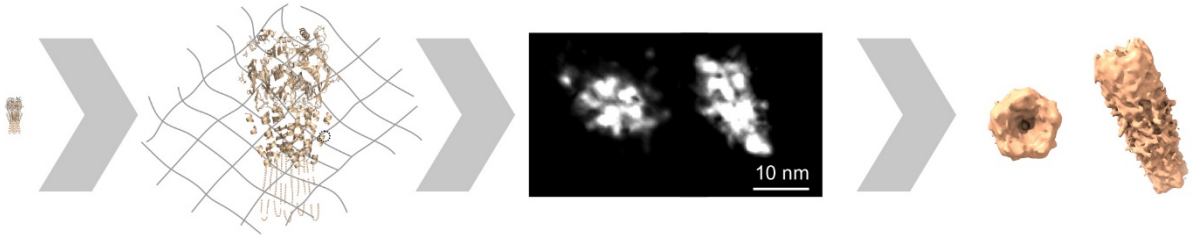
## Images and captions

The inventors (from left), group leader Dr. Ali H. Shaib and Professor Dr. Silvio O. Rizzoli, director of the Department for Neuro- and Sensory Physiology at the University Medical Center Göttingen (UMG). Photo: A. Shaib.



Artistic impression of the first protein structure of the $GABA_A$ receptor solved by ONE microscopy. Picture: Shaib/Rizzoli, umg/mbexc




The ONE microscopy, from molecule to 3D-structure. Figure: Shaib/Rizzoli, umg/mbexc