

Shapes, not Colors

Prof. Gil Westmeyer visualizes molecular information that imaging techniques have not yet been able to access. One prime example is the molecular processes involved in signal transmission between nerve cells, such as when the connections between neurons strengthen during learning. Together with his team, Westmeyer is developing new markers for electron microscopy.

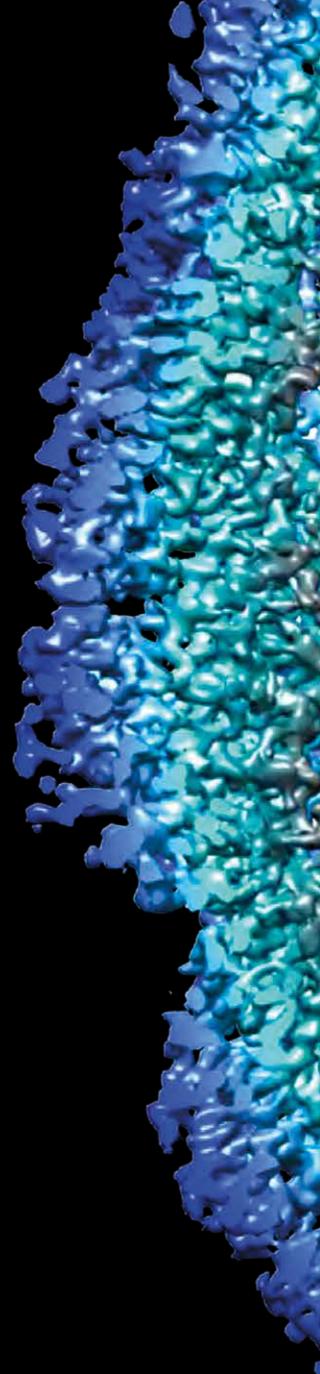
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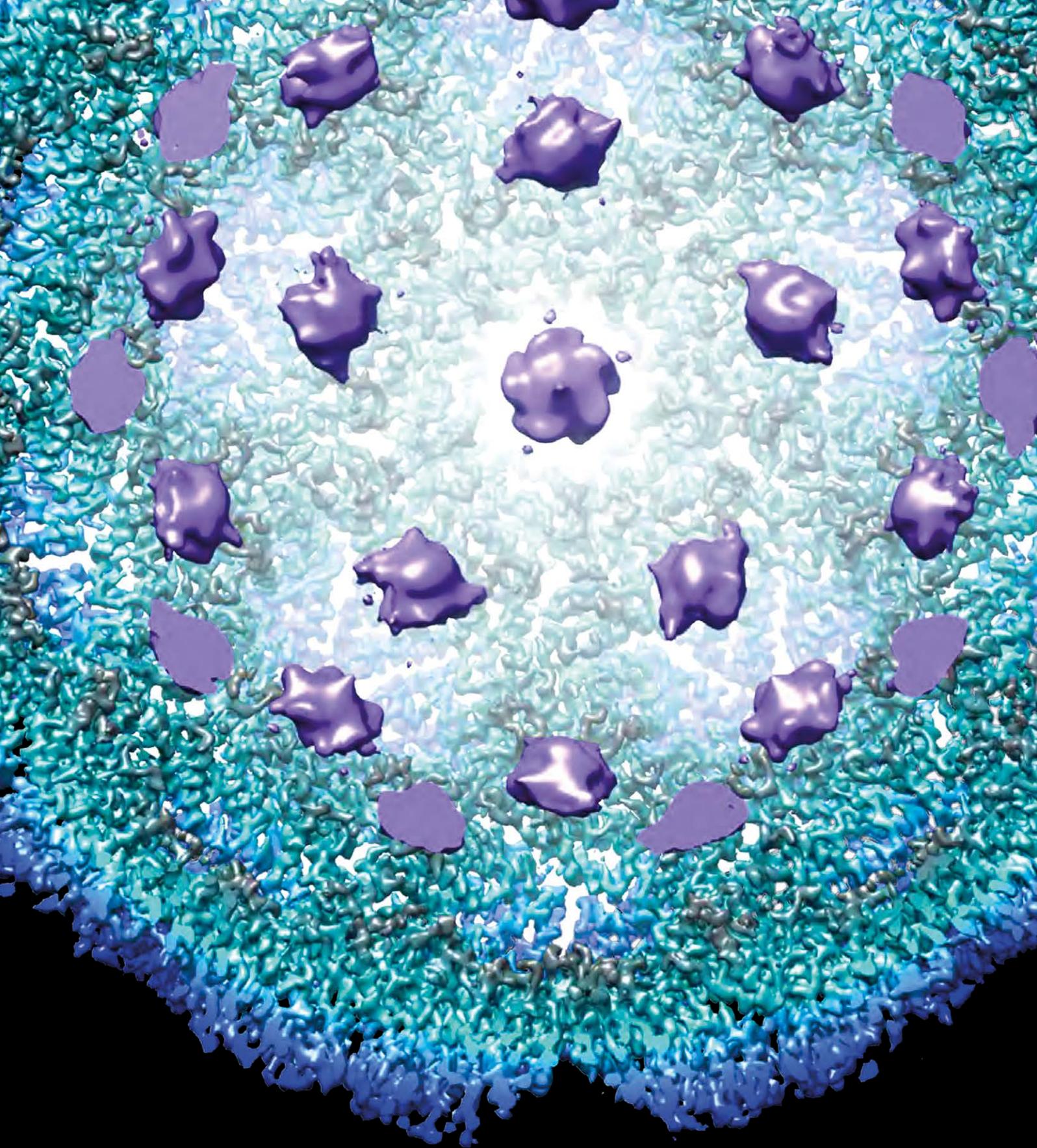
Wenn Zellen aktiviert werden, verändert sich ihre Genexpression und damit ihre Proteinproduktion. Das ist auch bei Nervenzellen der Fall, wenn im Rahmen von Lernprozessen die Signalübertragung verstärkt wird. Außerdem produzieren die Zellen dann unterschiedliche Proteine, etwa für die Veränderung von Synapsen. Bisher ist es nicht möglich, diese molekularen Veränderungen mittels Hochdurchsatz-Mikroskopie zu visualisieren, da die Fluoreszenzmikroskopie zwar Methoden zur Markierung von Molekülen bietet, aber nicht die notwendige Auflösung liefert.

Prof. Gil Westmeyer hat mit seinem Team deshalb neuartige Marker für die Elektronenmikroskopie entwickelt. Sie ermöglichen, eine Vielzahl „molekularer Spieler“ parallel in sehr hoher Auflösung zu visualisieren. Dafür nutzt der Forscher Designer-Proteine, die von Nervenzellen in Zellkulturen oder von gentechnisch veränderten Modellorganismen produziert werden, etwa wenn die Zelle aktiviert wird. Die Proteine bauen sich in der jeweiligen Zelle dann selber zu Nanostrukturen zusammen. Das Aussehen dieser Strukturen lässt sich durch geschicktes Design der Proteinbausteine variieren. Im Elektronenmikroskop können auf diesem Weg unterschiedlichste molekulare Informationen parallel ausgelesen werden. Die Methode lässt sich vielseitig nutzen, etwa um molekulare Vorgänge von Lernprozessen zu untersuchen oder um das Verständnis für neuropsychiatrische Erkrankungen zu verbessern. Eine wichtige Rolle könnte die Technologie außerdem bei der Entwicklung zukünftiger Zelltherapien und der Entwicklung von Schnittstellen zwischen Nervenzellen und Computern spielen. □



1 nm

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Asked about the applications for the latest methods he has developed, Gil Westmeyer first outlines the bigger picture. The connections between nerve cells, known as synapses, have a hugely important role to play in memory formation. New synapses develop, and existing synapses become more receptive to signals. The next time the same cell is stimulated, the response from the receiving cell is significantly stronger.

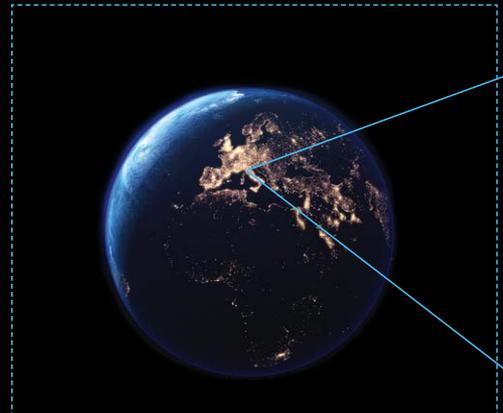
Researchers worldwide hope that by revealing the connections between nerve cells, they can infer their functions. This impetus has led to the emergence of a new branch of neuroscience called connectomics – a reference to genomics, which considers a complete set of genes. Scientists in this field are working to create the connectome – akin to a wiring diagram for the brain – by eventually mapping all synapses of a brain. Some researchers even believe that the connectome is at the core of what we are – in other words, our memories, our cognition, and our thoughts.

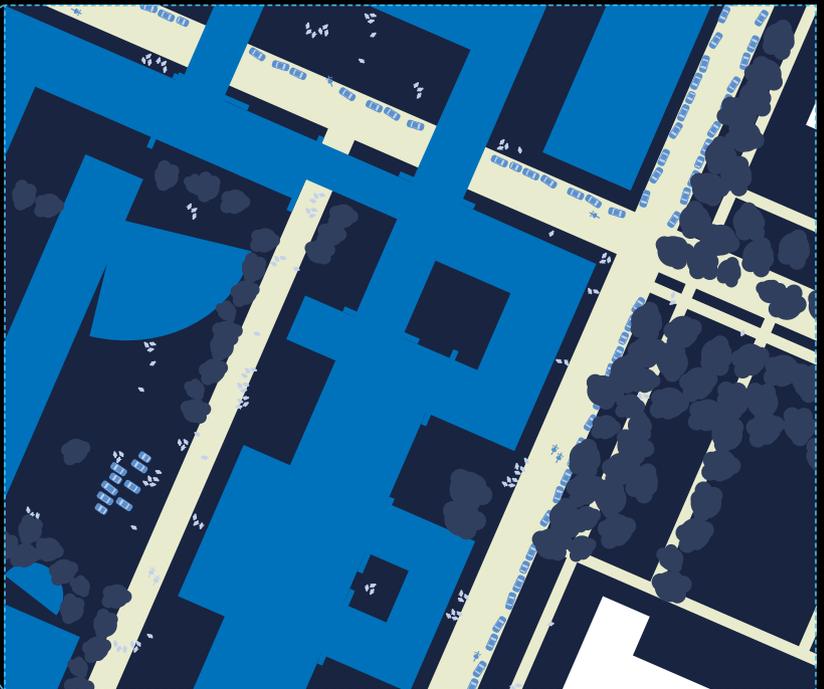
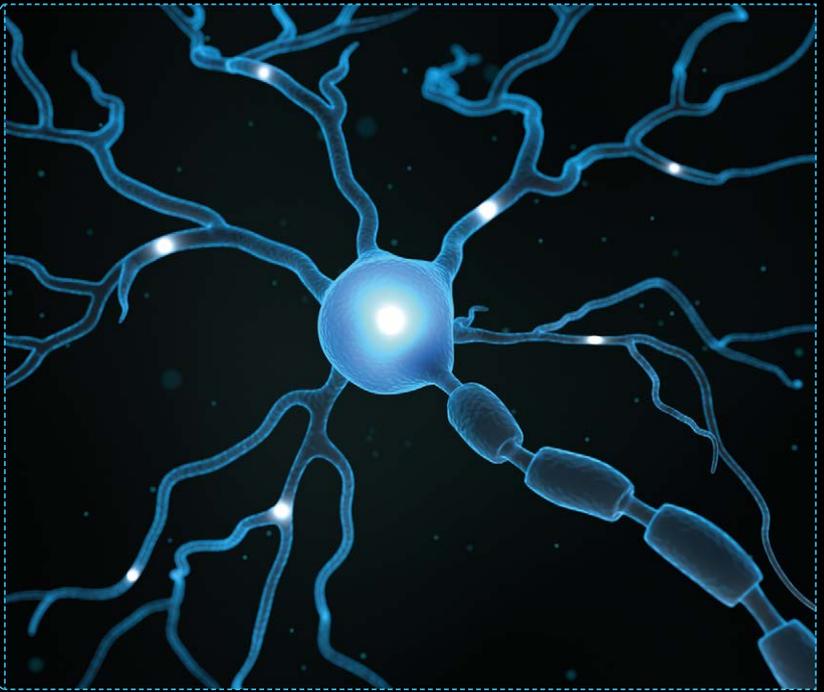
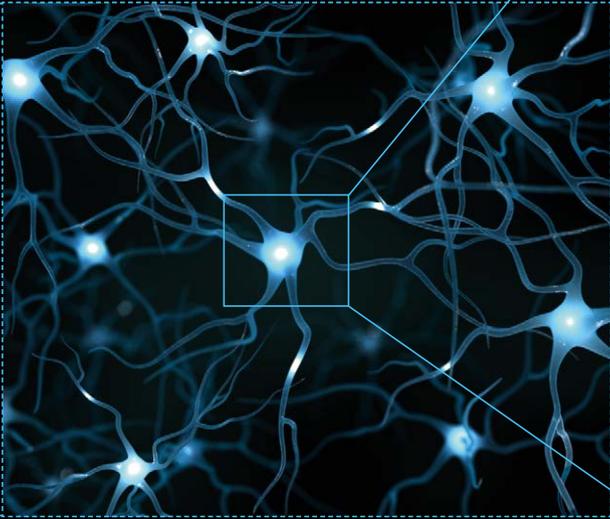
The connectome – a wiring diagram for the brain

Scientists have already successfully mapped the connectomes of certain model organisms, such as the roundworm *Caenorhabditis elegans* and the fruit fly *Drosophila melanogaster*. With around 100,000 neurons, a fruit fly's brain is compact enough for researchers to examine in its entirety. By comparison, a human brain has around 100 billion nerve cells. Westmeyer compares the overview provided by the connectome to a street map of a large, dynamic city. "While we can see the major anatomical connections, we want to zoom in even further and see what traffic volumes are located where, how the traffic light intervals are organized, and where road construction zones crop up and disappear," he states.

Gil Westmeyer heads up the Institute for Synthetic Biomedicine at Helmholtz Zentrum München and is Professor of Neurobiological Engineering at TUM. His research program focuses on developing biomolecular and genetic methods for capturing and controlling fundamental cell processes. Such techniques make it possible to visualize the underlying patterns in molecular mechanisms and refine current models of information processing. These insights will also help us understand the mechanisms involved in a biological learning process. ▶

In analogy to a detailed road map, a brain's connectome details the connections between all of its neurons.





Fluorescence microscopy

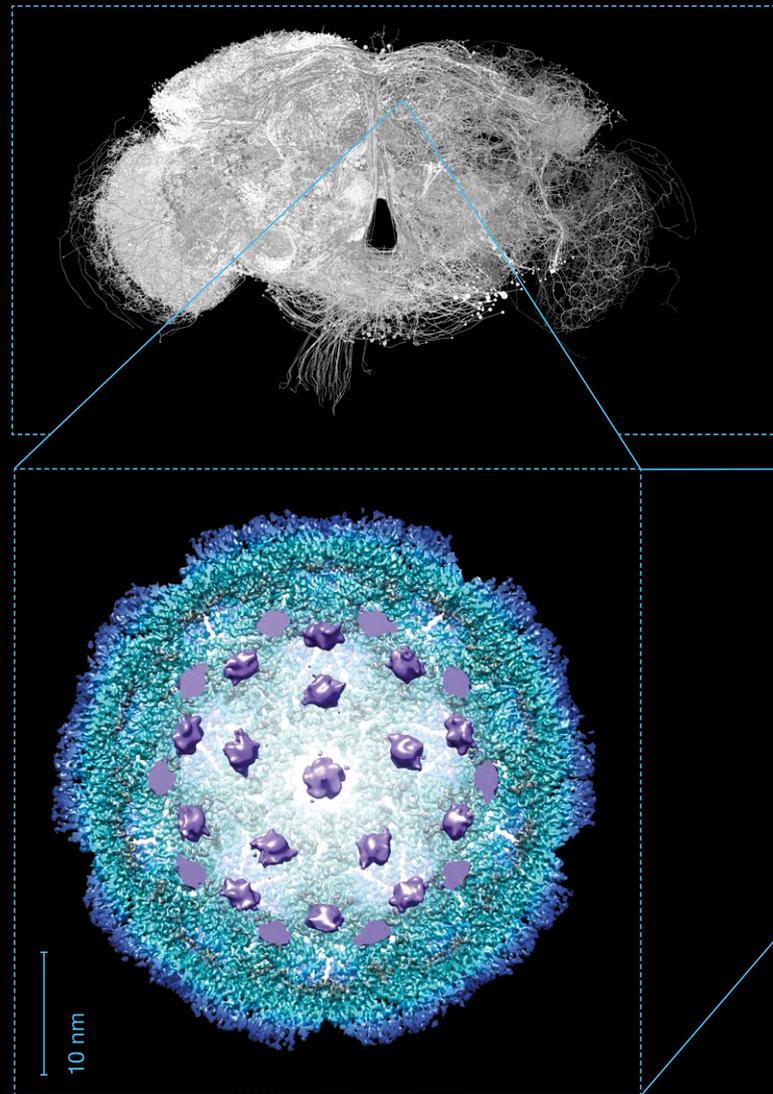
Fluorescence microscopy is a specific form of optical microscopy based on the physical effect of fluorescence. When fluorescent compounds are excited with light of a certain wavelength, they emit light of a different, longer wavelength. This form of microscopy uses fluorescent dyes that attach themselves to certain structures with the help of, say, antibodies or fluorescent proteins produced by genetically modified cells. Light has wavelengths of well under one micrometer. Due to the diffraction barrier, an optical microscope cannot distinguish between objects smaller than several hundred nanometers in size. By spatially restricting possible fluorescence emissions or isolating the fluorescence signals, super-resolution fluorescence microscopy can overcome the resolution barrier and localize fluorophores measuring just a few nanometers across.

Volume electron microscopy

For visualizing cell-cell contacts and intracellular organelles in a block of tissue with nanometer resolution, the block is ablated (with either diamond knives or ion beams) and analyzed sequentially using scanning electron microscopy on either the individual sections or the block remaining after each section is removed. The tissue is then virtually assembled from the 2D sections into a 3D digital model. Computer algorithms help to identify the individual cell structures.

Source: *Drosophila* Brainbow: a recombinase-based fluorescence labeling technique to subdivide neural expression patterns Reprinted (adapted) from 2011 Springer Nature; EM reconstruction of *Drosophila* neurons by the Full Adult Fly tracing community. Visualisation by Philipp Schlegel (Drosophila Connectomics Group, University of Cambridge) 2018 Springer Nature, Reprinted Zheng, Lauritzen et al., Cell Resource (2018); Reprinted (adapted) with permission from "Iron-Sequestering Nanocompartments as Multiplexed Electron Microscopy Gene Reporters". Copyright 2019 American Chemical Society

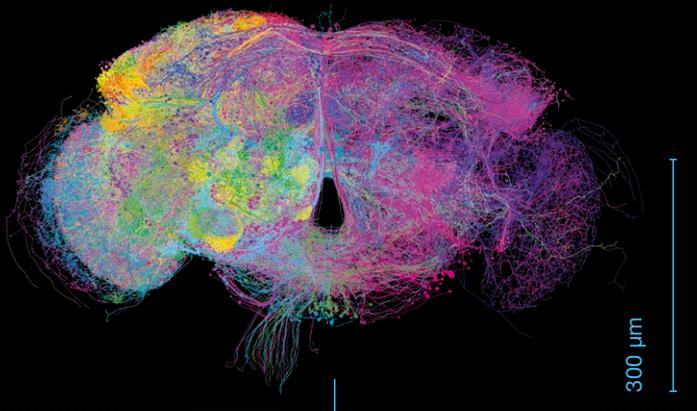
High-throughput volume electron microscopy reveals the "connectome" of a fly's brain, i.e., all connections between its neurons.



Nanosized gene reporters are genetically expressed by defined nerve cells under specific conditions.



Fluorescence microscopy can reveal cell function but lacks the resolution to resolve the exact contacts between nerve cells.



Gene reporters for electron microscopy augment the brain's wiring diagram with "multicolor" information on cell function.

Learning processes at the molecular level

Any change of state in a cell is closely associated with changes in gene expression and, consequently, protein production. The fact that signal transmission intensifies after a learning process is partly based on the synthesis of new proteins, both in the cell body and in the processes of nerve cells, which branch out considerably.

Electron microscopy (EM) is the imaging technique of choice for mapping the trajectories of nerve cells. "Volume electron microscopy provides detailed information about the connections within neural networks. However, the images produced in high-throughput microscopy are imprecise depictions of the molecular players on the cell's playing field, such as mRNA or proteins," explains Westmeyer.

To capture these molecular cell processes, fluorescence microscopy (FM) is a suitable technique. FM uses special fluorescent proteins to provide multicolored markings and has established itself as an indispensable technique in the field of biomedicine. Unfortunately, FM does not offer the resolution required to examine the nerve cells' delicate processes. Westmeyer and his team hope to resolve this issue with a new method they have developed for electron microscopy. It involves markers that can visualize information in a similar way to fluorescent proteins, just with an electron microscope instead – therefore offering considerably higher resolution.

In order to achieve this, the scientists rely on protein complexes that self-assemble within the nerve cells. They use these complexes in neurons, in cell cultures, or in model organisms such as fruit flies, in which nerve cells are genetically modified to produce corresponding

markers, for instance, when they are activated or produce certain proteins for new synapse formation. One class of protein complexes is known as encapsulins, which self-assemble into hollow nanocompartments with defined sizes of 20, 30, or 40 nanometers. Nerve cells, with diameters of several micrometers, are around 1000 times larger than these nanocages.

Proteins or enzymes, such as the enzyme ferroxidase, can be encapsulated inside the nanocages. The enzyme catalyzes the oxidation of iron ions that enter the nanocages through their pores, creating iron-oxide species of low solubility that are trapped in the nanocages. Metals have a higher density than proteins and therefore improve the contrast so that the nanocages are clearly visible in EM images. What is even more ingenious is that, by skillfully designing the proteins' building blocks, scientists can create nanostructures with different contours that are distinguishable in EM imaging. "This means we can generate a whole range of structures, which allows us to examine several parameters of a cell's state at the same time. Given the complexity of the nervous system, this is a significant advantage," says Westmeyer. It is all made possible by the fact that the nanocages are non-toxic and so small and inert that they do not hinder or disturb cells. The protein complexes serve as markers for genetic activities (gene reporters), which means that different molecular states of cells can effectively be visualized with different "colors" in an electron microscope. The project has received a prestigious Consolidator Grant from the European Research Council.

Westmeyer thinks one step further. An electron microscope cannot visualize activities in living cells. To bridge

this gap and make it possible to examine dynamic processes in the future, fluorescent proteins will also be added to the nanocages inside the nerve cells. "This will allow us to make the markings so bright that we could even conduct measurements on living cells using high-performance, super-resolution fluorescence microscopy – with a potential resolution of several nanometers," says Westmeyer. Theoretically, a subsequent step could see cells examined under a fluorescence microscope being additionally examined under an electron microscope so that the cell structures can be analyzed in even greater detail. "It might sound simple, but the validation required means it is still a long way off," notes Westmeyer.

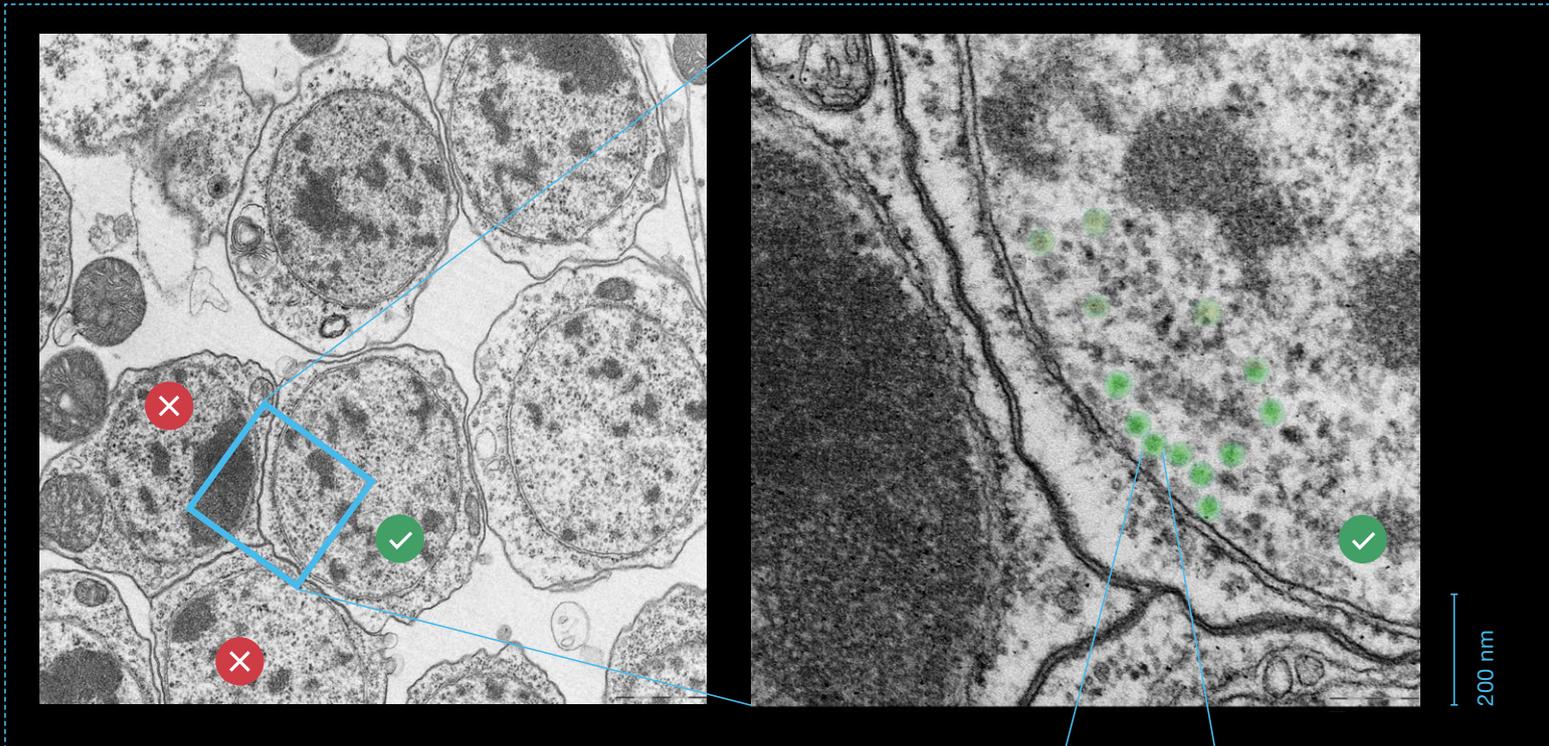
Monitoring human-computer interfaces

Westmeyer is fizzing with ideas of other use cases for his methods. For instance, insights gained into the dynamic interactions between patterns of brain activity – i.e., the cell states identified by markers – and connections between nerve cells, in other words, the paths of nerve cells identified from EM images, could improve our understanding of neuropsychiatric disorders such as autism and Alzheimer's. The novel marker methods could also contribute to the development of future cell-based therapies. The aim is to use a combination of different molecular methods to visualize as many aspects of cellular processes as possible, thus enabling researchers to refine and support these innovative treatment approaches. Westmeyer's method could also help uncover the architectural principles of neuronal circuitry, which could then provide inspiration for the design and development of neuromorphic computer chips. This chip architecture ▶

“Our method allows us to augment high-resolution anatomical brain maps with ‘multicolor’ functional information.”

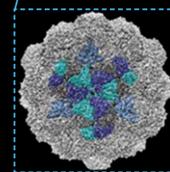
Gil Westmeyer





Visualizing molecular information in neurons.

Left: Electron microscopy of a section through a fruit fly's brain. EM gene reporters are expressed in the nerve cell labeled with the green checkmark to report on the cell's genetic state.



Link
www.westmeyerlab.org

seeks to recreate the circuitry optimized over the course of biological evolution and would then, for example, be able to perform pattern recognition algorithms very efficiently at the hardware level.

Furthermore, the EM markers could prove helpful in creating new interfaces between nerve cells and computer chips. In a new project at the Munich Institute for Biomedical Engineering (MIBE) at TUM, conducted in collaboration with the Technical University of Dresden and Bernhard Wolfrum of TUM, Westmeyer hopes to grow nerve cells directly onto the circuitry of computer chips. The scientists want to measure the electrical and electrochemical properties of the nerve cells and deliver electrical stimulation in return. They then plan to use electron microscopy to examine and improve cell-chip contacts. The marker systems Westmeyer's team has developed for electron microscopy could provide crucial additional information about the functional state of the nerve cells used in the experiment, thus allowing scientists to monitor and optimize the interfaces. This iterative approach could make future interfaces between neurons and a connected device, such as a computer, safer and more precise. One potential application for this technology would be to control a paralyzed patient's bionic arms or legs.



Karoline Stürmer



Prof. Gil Gregor Westmeyer

studied medicine and philosophy in Munich. He conducted his doctoral work on the molecular basis of Alzheimer's disease in Prof. Christian Haass' laboratory before receiving a part of his clinical education at Harvard Medical School in Boston, Massachusetts (USA). He then worked in Prof. Alan Jasanoff's laboratory at MIT before being appointed to TUM in 2012. Gil Westmeyer is currently Professor of Neurobiological Engineering at TUM. He is also Director of the Institute for Synthetic Biomedicine at Helmholtz Zentrum München.
