Molecular bright spots

STED microscopes can produce extremely detailed images of everything from the transport of individual proteins or tiny membrane vesicles in living cells to the synapses of neurons or the skeletons of tumor cells. The technique was invented by **Stefan Hell**, Director at the **Max Planck Institutes for Biophysical Chemistry** in Goettingen **and Medical Research** in Heidelberg. Now, the spin-off company Abberior Instruments sells the highest-resolution fluorescence microscope on the market – and researchers at both the Institutes and the company continue to push the resolution to its ultimate limit: the single nanometer size scale of a molecule.

TEXT **JANOSCH DEEG**

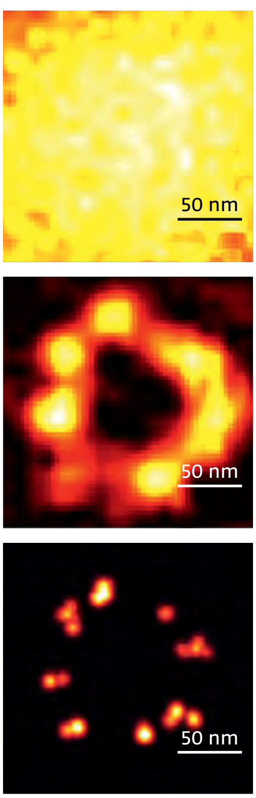
The black box measures almost one and a half meters square and has a height of perhaps 40 centimeters. Usually, black boxes are associated with disasters – but not so in this case. Here, it stands for a real success story from physical research. The box contains an assortment of lasers, lenses, mirrors, and numerous other components, which together make up the latest microscope from the company Abberior Instruments. Going by the name of MINFLUX, it will soon be available worldwide and will once again take fluorescence microscopy to a new level. In fact, the company's existing models have already set new standards, producing images around ten times sharper than experts thought possible just 20 years ago.

This ultrasharp form of optical fluorescence microscopy was invented by one of the company's seven founders, and he was even awarded a Nobel Prize for it. With his work, he proved all of the experts wrong in their belief that optical microscopy had already reached the limits set by the laws of physics in the 20th century. It's no wonder, therefore, that the company has seen impressive rates of growth. Its latest innovation is MINFLUX, a fluorescence microscope that will peer down into even smaller dimensions, reaching as far as the size of a molecule. But first things first. You could say that the story of Abberior Instruments actually began with a setback if not with a series of small disasters.

In the 1980s, a physicist in his mid-twenties by the name of Stefan Hell was working on his doctoral thesis

A sprouting neuron: the axon of a nerve cell uses a growth cone such as this to search for its target. Captured using a STED microscope, this image offers a detailed view of the fine projections (green), with which the rounded end of the axon explores its environment. The red and blue staining reveals proteins of the cytoskeleton, which provide the growth cone with its structure and mobility.

Breakthroughs in the resolution of fluorescence microscopy: developed by Stefan Hell and colleagues, STED microscopy (center) achieved a resolution about ten times higher than the widely used confocal microscopy (top) over a decade ago. Now, with MINFLUX (bottom), the researchers have once again increased the definition by a factor of ten – making this technique 100 times more powerful overall and allowing it to image the molecular scale.



in Heidelberg. Using laser scanning microscopes, he was examining semiconductor chips at Heidelberg Instruments, a company his doctoral supervisor had recently founded with a group of other professors. Things were looking good, and wealthy financial backers were coming on board. Everything revolved around the new technology, however, and too little attention was paid to customer requirements. The company was ultimately broken up, and its workforce of almost 100 employees were either laid off or split up among successor companies by the investors. But this no longer affected Stefan Hell's plan. With his doctorate almost under his belt, he was ready to dive into the world of science – although he took one valuable piece of experience away with him: "I saw what not to do when founding a hi-tech spin-off," the researcher smiles. "Ideally, you design products for which customers already exist. And you don't rely on financial backers who don't really understand the subject matter," he says, lounging casually in his chair in a conference room at Abberior Instruments. He knows he chose a better approach.

By the early 1990s, Hell was working as a young scientist in Finland. Up there in the cold north, he was chasing a hot lead: what if the resolution limit of optical microscopes could be overcome with the help of a quantum optical effect? This was a radical idea at the time, because the resolution limit of optical microscopes was carved in stone. In fact, this had been the case since 1873, when the physicist Ernst Abbe formulated the relevant law: an optical microscope cannot visualize structures smaller than half the wavelength of visible light. This diffraction limit meant that optical microscopes could only resolve structures down to a size of 200 nanometers (nm) – fine enough to discern individual cells and their larger components, such as the nucleus or other organelles, but not the cell's molecular machinery. Modern electron microscopy can penetrate down to this scale, but only if the cells are first killed and prepared in a laborious process. This wouldn't be necessary with the device that Hell had in mind – which would therefore be especially useful in biomedical research.

When Hell became a Junior Research Group Leader at the Max Planck Institute for Biophysical Chemistry in Goettingen in 1997, he was able to build a microscope based on his ideas. Around the turn of the millennium, the device was ready – and it could ultimately resolve details with dimensions of just 20 to 30 nanometers. Since then, the technology has been known as STED microscopy (see box on page 27).

STED WINS THE RESOLUTION RACE

In 2004, another young physicist was looking for a topic for his doctoral thesis and was impressed by Hell's invention: "I was fascinated to see how you can bypass a law of physics," says Gerald Donnert, who is now Managing Director of Abberior Instruments. The same year, he persuaded Stefan Hell, who was by then Director of the Department of NanoBiophotonics at the Max Planck Institute for Biophysical Chemistry, to take him on as a doctoral researcher.

The young scientist found Hell to be not only highly focused but also a very exacting boss – a great match for the ambitious and highly motivated Donnert, who was tasked with building the highest-resolution STED microscope ever built. "It was an extremely exciting time," he recalls – after all, the year 2006 saw the emergence of another high-resolution form of fluorescence microscopy in the U.S. It was a bit like a race between top athletes, he says: "Who had the highest resolution? >

Photos: MPI for Biophysical Chemistry

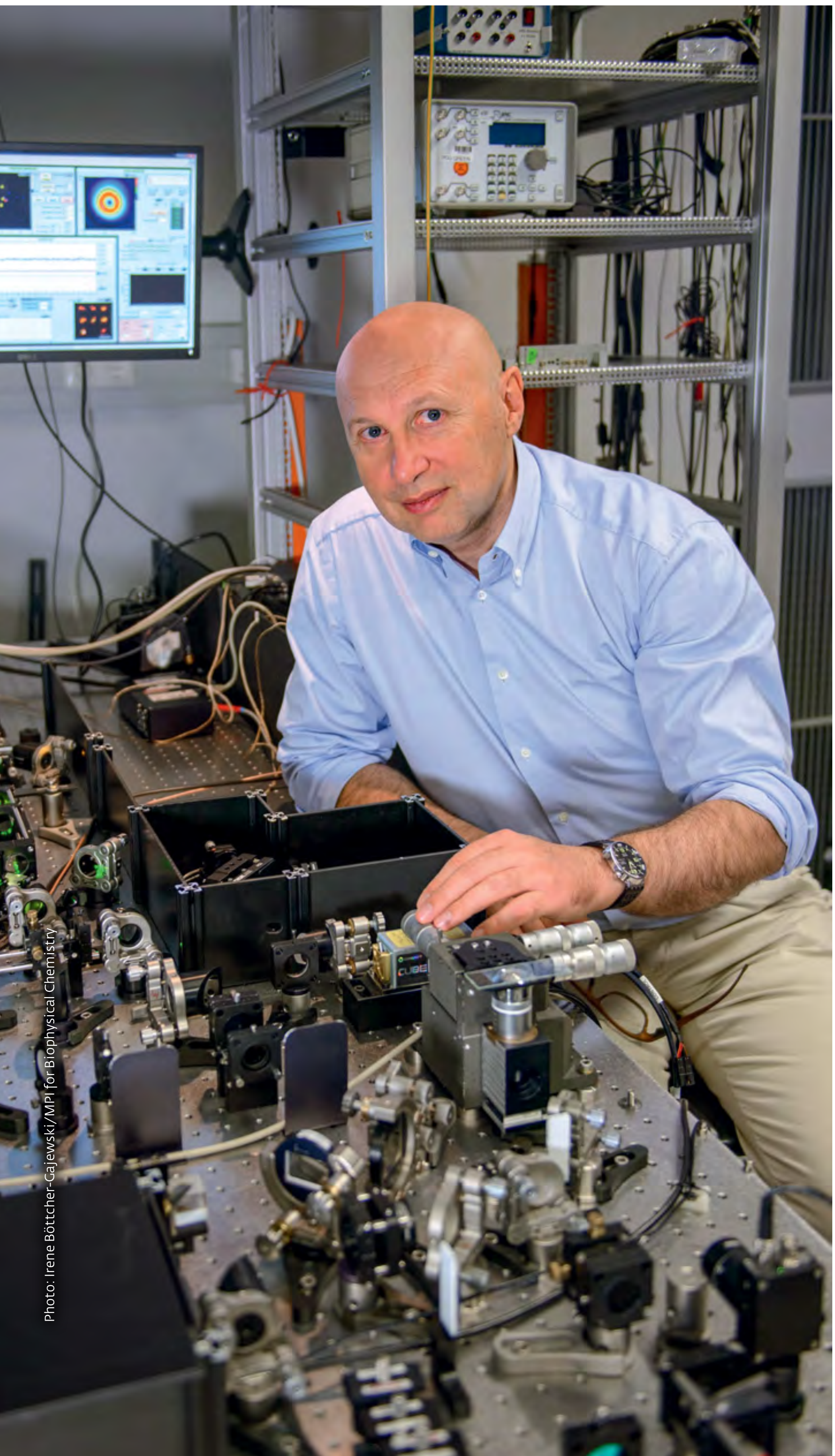


Photo: Irene Böttcher-Gajewski/MPI for Biophysical Chemistry

STED

STED stands for "stimulated emission depletion" and is a high-resolution form of fluorescence microscopy that involves the specific, temporary deactivation of tiny fluorescent dye molecules. In fluorescence microscopy, the interesting areas of the sample – such as specific structures of a cell – are generally marked with fluorescent dyes prior to examination. After the sample is briefly illuminated with light, these areas begin to glow. But two fluorescent molecules that are very close together can only be distinguished in this way if the light waves produced during fluorescence do not overlap too strongly. For this reason, the resolution is limited to around 200 nanometers (nm) at best.

The special thing about STED is that it ensures that fluorescent molecules in close proximity to one another do not emit light at the same time. To achieve this, a donut-shaped laser beam switches some of the molecules off so that light is only emitted by those in the hole of the donut beam. Nearby molecules that have been switched off can no longer interfere with those that are glowing. By scanning across the sample, this method produces a fluorescence image with a resolution of 20 to 100 nanometers, depending on the configuration.

A result of basic research: the first STED microscope was built by Stefan Hell at the Max Planck Institute for Biophysical Chemistry.

Track of a molecule diffusing within the cell membrane. Its position was determined approximately 10,000 times a second to a resolution of 20 nanometers (nm).

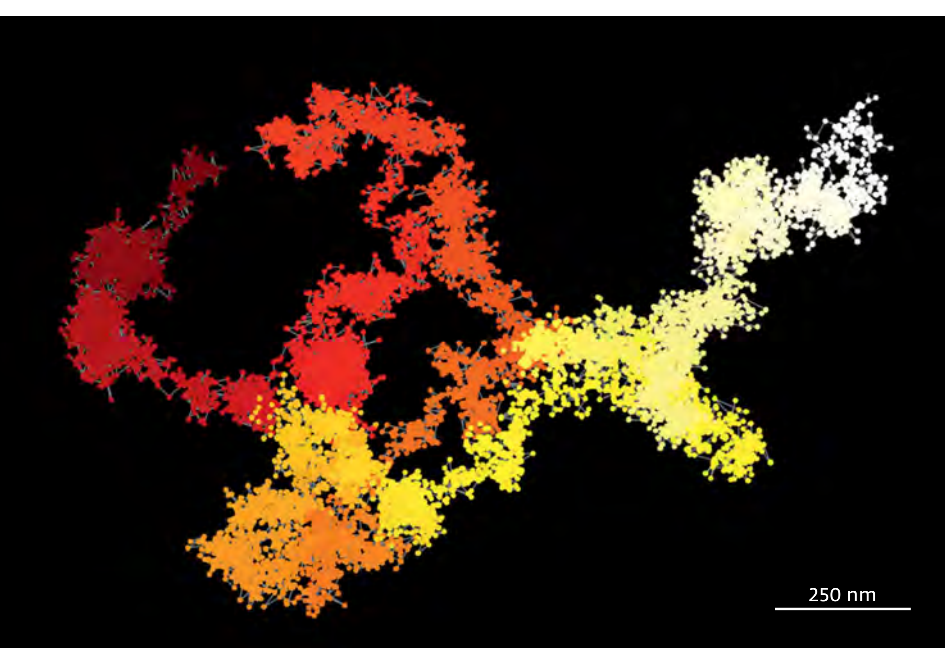


Photo: Stefan Hell/MPI for Biophysical Chemistry

Whose method would be the most useful one?" Donnert completed his doctoral thesis in under three years – an exceptionally short time in a subject like physics – and his microscope ultimately won the race.

The U.S. National Academy of Sciences invited him to Washington and presented him with a prize for his work. But despite this success, he initially felt an urge to get away from science: "I wanted to experience something different," he says. When he was offered a job at McKinsey & Company, a top address in the world of management consulting, it was an opportunity he could not refuse. During his time at the company, he learned how business works and realized that you have to be willing to take risks in order to achieve success. In the meantime, he stayed in touch with Hell. The two researchers had a hunch that the potential market for ultrasharp microscopes was much bigger than the established microscope manufacturers believed.

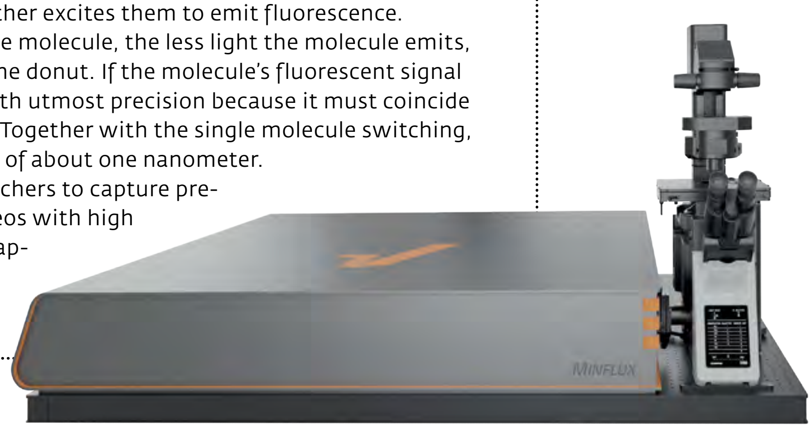
Indeed, in the hope of quickly popularizing the technique, Hell had initially teamed up with a well-known company that had acquired the patent license for STED microscopy. But there wasn't as much demand for the devices as he'd hoped. Nor did the idea of marketing STED microscopy through a smaller and more versatile joint venture come to fruition, an approach suggested by Dieter Treichel, Senior Start-up & Portfolio Manager at Max Planck Innovation. "Large companies are more interested in selling their well-established products for as long as possible," says Treichel. "STED was seen as the icing on the cake, rather than the new standard in confocal fluorescence microscopy. In this situation, it made sense to found a start-up, even though we lacked sales channels of our own at first."

With that in mind, Hell wasted no time in teaming up with his former doctoral student Gerald Donnert to improve the availability of the most powerful fluorescence microscopes in the world. Donnert's considerable knowledge of business strategies, which he had acquired during his time at McKinsey, would now be a priceless asset. But as well as a watertight business plan, the two researchers took another key factor into consideration: "The success of a company depends on its people." They brought a further five colleagues on board as co-founders of Abberior Instruments GmbH, bringing together a range of different skill sets from the worlds of physics, chemistry, and biology. All of them were former doctoral students with Hell and knew they could work well as a team. All of them were familiar with STED technology from the ground up and believed in its potential. Stefan Hell, their former mentor, would continue to support them as an advisor.

The team received assistance from Max Planck Innovation to found the company, especially from Dieter Treichel,

MINFLUX

MINFLUX (from “minimal fluorescence flux”) is a combination, so to speak, of the fundamental strength of STED and another high-resolution strategy – PALM or STORM. These techniques use a burst of light over a wide area of the sample to switch on a few molecules at random – just enough so that the probability of two dye molecules in close proximity fluorescing at the same time is small. By bringing together a large number of individual images, computer algorithms can then reconstruct the positions of the fluorophores – provided that they glow very brightly. Since this is rarely the case, however, PALM and STORM seldom deliver a higher resolution than 20 to 40 nanometers. MINFLUX also switches the fluorescent molecules on individually, but the molecules are scanned using a donut-shaped laser beam in order to determine their definitive position. Unlike in STED, the donut laser beam doesn’t switch the molecules off but rather excites them to emit fluorescence.
The closer the center of the beam comes to the molecule, the less light the molecule emits, as no excitation occurs in the dark center of the donut. If the molecule’s fluorescent signal disappears, its position can be determined with utmost precision because it must coincide with the known position of the donut center. Together with the single molecule switching, this localization results in a spatial resolution of about one nanometer.
For the first time, MINFLUX also allows researchers to capture precise images of molecular paths, such that videos with high spatial and temporal resolution can now be captured of proteins diffusing rapidly, for example, within the interior of a living cell.



The MINFLUX microscope, which combines the conceptual strengths of STED with that of another microscopy technique, is the latest commercial product from Abberior Instruments. It is the first commercially available microscope to achieve a resolution of one nanometer, i.e. the size of a molecule.

Photo: Abberior Instruments GmbH

both in the early days and when they came to establish a U.S. subsidiary a few years later. The young entrepreneurs deliberately made do without external financial support and instead opted to pull together the seed capital from their own savings: EUR 200,000 in total. In other words, there were no external backers, because Hell was keen to avoid a repeat of his experience at Heidelberg Instruments.

ALMOST TWICE AS MANY ORDERS FROM YEAR TO YEAR SINCE 2014

Abberior Instruments was recorded in the register of companies in 2012. Two orders had already been received verbally – from “family and friends,” as Hell jokingly refers to the company’s first customers: overseas research colleagues who knew him personally and needed high-resolution microscopes for their research. In the early days, the team met in what they called the “garage” – an approximately 25-square-meter room that they had rented on the ground floor of a rather unassuming three-story building on the University of Goettingen campus. It was here that the researchers built the first microscopes. Although they offered customers from the world of science more capabilities than established devices on the market, they were not yet STED microscopes, since the patent licenses were still held exclusively by the big company. Even so, everything was going to plan, although one risk remained: “If a customer hadn’t paid for a microscope that they’d ordered, we’d have run into serious difficulties. At that time, the capital was all tied up in the shipped devices,” Donnert recalls. But the customers were satisfied and paid their invoices, allowing the young company to build more devices and grow.

Word of their success got around: in January 2014, Abberior Instruments was awarded the Innovationspreis der deutschen Wirtschaft (German Business Prize for Innovation) in the “Startup” category. And in spring of the same year, the exclusive patent license of the major company expired, clearing the way for Abberior Instruments to offer STED microscopes as well. And then came the news that no one was

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really expecting: in fall 2014, Stefan Hell was awarded a Nobel Prize for the invention of STED microscopy. According to Donnert, interest in the devices rose significantly as a result: "From that point onwards, our rate of growth has been remarkable." Hell disagrees, though: "The Nobel Prize affected our growth less than you'd imagine." He believes it had more to do with the power of the microscopes, as well as their fair price and the company's expert customer service. "In 2014, the price of STED microscopes almost halved thanks to the presence of Abberior Instruments as a second supplier. That was not only good for the researchers and the grant funding agencies, but also boosted demand. And it had nothing to do with the Nobel Prize." In any case, the number of orders has almost doubled from year to year since 2014.

The company's rapid growth also called for bigger premises, and the "garage" became an entire floor of the same building, with several offices, laboratories, workshops, a showroom, a meeting room, and a break room equipped with a coffee machine and a football table – in other words, everything you need to provide a relaxed, creative working environment for 40 people. In addition, there were now a further 20 staff members working at three smaller locations – Heidelberg, Basel, and Jupiter in Florida. Incidentally, the Heidelberg branch just happens to occupy the rooms where Hell worked on his doctorate some 30 years ago.

The staff members of the research and development Department, who are responsible for developing new methods and devices, make up an unusually large proportion of the workforce – and they're good: "Abberior Instruments has the world's best development team in the field of laser scanning microscopy," says Stefan Hell. As Donnert has also found, this is something that customers value: "We're perceived as being the most innovative company in superresolution fluorescence microscopy."

ABBERIOR INSTRUMENTS LEAVES THE START-UP PHASE

The Abberior Instruments range offers something for every requirement: "For those who want to obtain good, high-resolution images as quickly as possible as well as for experts who want to derive the maximum benefit," explains Hell. The products therefore range from very easy to operate, compact STED microscopes to more powerful models and even tailor-made solutions. In addition, an independent sister firm by the name of Abberior markets the fluorescent dyes that go with the microscopes. That's also part of the strategy – everything comes from a single source in order to ensure the best possible results for researchers.

There was just one problem: many potential customers were still unaware of Abberior Instruments. The team therefore stepped up their efforts to establish contact with interested parties. Here too, rather than simply attending scientific conferences, they explored new approaches. In 2015, for example, a number of employees embarked on a two-week tour of Germany. They installed an STED microscope in a container and did the rounds of universities across the country. Every day, at a new location, they followed the same routine: set up, demonstrate, dismantle. "People were quite impressed that our systems produced top-notch superresolution in a movable container – reliably," says Donnert.

To improve their positioning in the major markets of the U.S. and Asia, the company has also teamed up with two industry giants. Since 2019, Abberior Instruments' compact STED model has been marketed by Nikon in the U.S. and Zeiss in China. Donnert is proud to report that the companies examined the devices thoroughly and were very impressed. At least now that Abberior Instruments has begun collaborating with these major players, the company evidently has left the start-up phase. Hell is amazed at how fast the company has grown in the space of seven or eight years. "Most people overestimate what they can do in one year and underestimate what they can achieve in ten years," he says, quoting Bill Gates. "The same thing happened with Abberior and Abberior Instruments."

That said, it hasn't even been ten years since the company was founded, and everything suggests that it will continue on a steep upward trajectory. The market for STED microscopes continues to expand, and the

Part of the Goettingen team with Managing Director Gerald Donnert (front row, fourth from left). In total, Abberior Instruments employs around 60 members of staff, many of whom work on ongoing research and product development.

next high-end product is already waiting in the wings: MINFLUX will be able to resolve tightly packed individual molecules in three dimensions (see box on page 29). This seemed unthinkable not all that long ago and will take biomedical microscopy in particular to a whole new level. Here, too, it was Hell who came up with the idea that underpins the method. The Max Planck Society secured the patent rights back in 2011, and the first scientific publication on MINFLUX came at the end of 2016. Abberior Instruments then acquired the licenses from the Max Planck Society – with the promise that it would develop a product from the physical concept as quickly as possible. And that's precisely what it did. Just over three years later, the prototype in the black box is so advanced that it is now a microscope that biologists can use. "That's only possible because Abberior Instruments is a slender, streamlined company in which everyone making decisions about a product also has a detailed understanding of it. And that goes for everything from the technology to its application," says Gerald Donnert. "And because all of the decision-makers are hand-picked and don't depend on financial backers," adds Stefan Hell. Once again, this goes to show that it was the right decision to found a start-up in which no one has a say but the expert scientists. ◂

GLOSSARY

Fluorescence microscopy: In this special variant of optical microscopy, fluorescent molecules are used as tags to highlight the molecules of interest, such as specific proteins. To this end, the sample is illuminated with light, causing them to glow. As the emitted fluorescence light has a longer wavelength than the illumination light, the latter can be filtered out so that the image shows the fluorescent structures only. Individual organelles – such as the Golgi apparatus, for example – can also be marked with fluorescent molecules.

Confocal laser scanning microscopy: In this form of microscopy, the sample is scanned with a focused laser beam rather than being illuminated all at once. The laser beam excites suitable markers point by point in the sample, causing them to fluoresce.