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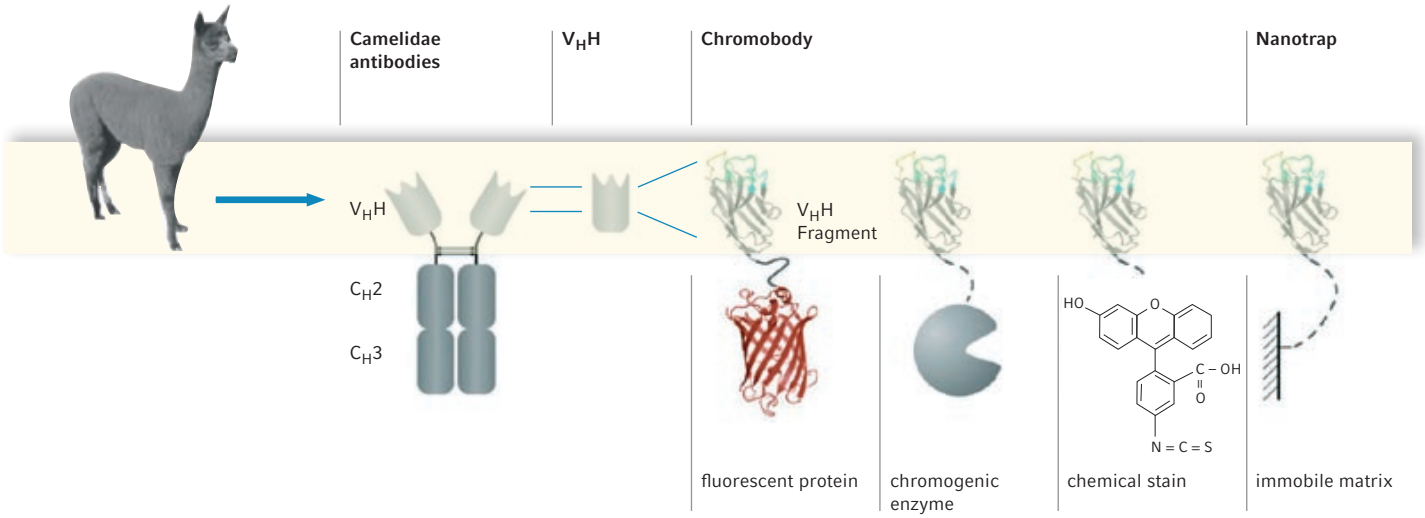
LIFE IN THE DWARF DIMENSION

The intricacy of the cell has now been resolved to greater detail than ever – thanks to a groundbreaking technique in microscopy. Biologist Heinrich Leonhardt and his fellow researchers have used this new technology to reveal the structures of a mammalian cell for the first time in 3D, in multiple colors and at unprecedented resolution. Now details such as DNA, the enshrouding nuclear envelope and even individual nuclear pores can be seen. These researchers plan to record cellular processes as a 3D movie.

We have come a long way since the Dutchman Antoni van Leeuwenhoek built the first microscopes in the 17th Century. His devices were actually little more than extremely powerful magnifying glasses, but they were nevertheless the tools with which he discovered bacteria. Present generation optical microscopes let us take a much closer look at the minute structures inside a cell. They work by bundling light through several lenses after it has reflected off or passed through the tiny object of interest. A widely used advancement on this technology is fluorescence microscopy. This is where fluorescent stains light up specific structures, making them visible so that we can locate and track them in the cell. However, there has always been one limit to optical microscopy in all its variants: the resolution, that is the ability to distinguish the tiniest of structures, is dictated by the wavelength of visible light, and is around 200 nanometers in the best case. One nanometer is one billionth of a meter. The prefix "nano", incidentally, is derived from the Greek word for "dwarf".

"It was the German physicist Ernst Abbe from Eisenach in the 19th Century who discovered this limit to optical microscopy, which is named the Abbe limit after him," reports Professor Heinrich Leonhardt. "But the limit is not absolute: there have been different approaches to getting around it, yet none of these techniques has made it into widespread use so far." That is why scientists have long been trying to combine the methods normally used for imaging molecular structures with the advantages of optical microscopy – particularly its ease of use – to break the Abbe barrier. "Working together with the research group of Professor John Sedat of the University of California in San Francisco, we have now succeeded

in doing just that," says Heinrich Leonhardt. "We achieved double the resolution: down to about 100 nanometers. The basis of our 3D SIM method is still conventional optical microscopy, but we use a special lighting technique (from which it gets its name: structured illumination microscopy, SIM). What we do is actually something you ought to avoid at all costs in optical microscopy: we use structured lighting. That is where we shine a very fine, known pattern of light onto our sample, a cell for instance. That leads to interference with the structures of the sample, which show up as patterns of shadows – which you would normally want to avoid." A similar type of waveform interference, known in a graphical context as the Moiré effect, can appear when you scan a printed picture, for example. "These interference patterns, which are bothersome at first sight, actually turn out to contain valuable information," Heinrich Leonhardt tells us. "With a lot of mathematics and a good computer, we can calculate from this raw data the tiniest of structures that cannot be resolved using a simple optical microscope." This method is repeated stepwise through different planes in the cells using different colors. Out of this data, a multicolored, three-dimensional image of the cell is ultimately reconstructed in the computer at exceptionally high resolution. "Using this technique, you can reveal cellular structures that can't be captured using conventional optical microscopes," says Heinrich Leonhardt. "This allows an entirely new kind of analysis in the life sciences: anywhere from pure research to applied problem solving."



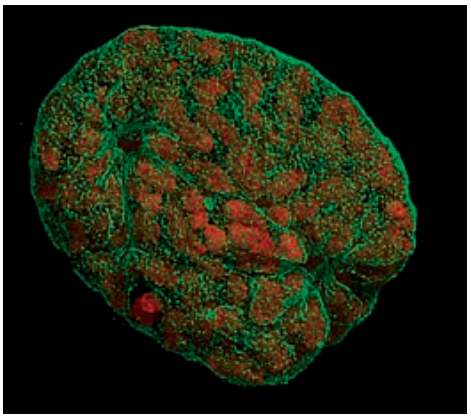
▲ In their approach, Heinrich Leonhardt and his fellow researchers use the antigen-binding domains of a single-chain antibody obtained from camels or alpacas. This is ten times smaller overall than a conventional antibody, and is appropriately called a nanobody. By fusion with fluorescent proteins, the researchers create fluorescent nanoprobes, which they call chromobodies. Thanks to their small size and stability, chromobodies can even be used inside living cells.

INSIGHTS INTO THE CRITICAL PHASE OF CELL DIVISION

Our researchers were also in for some fascinating new insights even while they were still developing the device. They were able to observe the nucleus of a mammalian cell shortly before division – a critical moment in its lifecycle. During this phase, the two copies of genetic material must be shared to utmost precision between the two daughter cells. Errors

in this sensitive process can be fatal: cell death is the comparably harmless consequence, where the much more dangerous outcome would be cancer. Using this new technology, the scientists have also taken a good look at the nuclear envelope in detail, where they saw the membrane surrounding the partially visible DNA as well as individual nuclear pores, which are openings in the nuclear envelope and important transport paths. "What we also found especially interesting were two large invaginations in the nuclear envelope," explains Heinrich Leonhardt, "namely where so-called centrosomes localize. From this point, long threads then form, along which the genetic material migrates in the form of chromosomes into the daughter cells." This fundamental biological process has never before been seen in such richness of detail. There are even new details discernable on the surface of the chromosomes, that is on the DNA itself. This is only possible because the different structures are stained with different colors – still only using conventional stains.

There is in fact another device, the STED microscope, that can already "see" below the Abbe limit, but it is limited by its technical intricacy and the need to use special stains. Also, compared to any optical microscope, electron microscopes can achieve much, much higher resolutions still, but are extremely complex and cannot produce multicolored images. "So, producing 3D images in three or even four colors is still only possible using 3D SIM technology," says the LMU researcher. "What is more, we can use established, easy methods for preparing and staining the samples." We should see the transition from current 3D SIM prototypes to commercially available equipment very soon. "Of course, such large-scale projects as the development and first ever use of this technology can only ever be achieved as a collective team effort," stresses Heinrich Leonhardt. "In our case, for instance, we had hardware and software engineers working very closely with physicists and cellular biologists. Our long-term goal now is to improve the method even further so that we can actually make 3D movies of the processes in living cells."



▲ This picture shows a cell in its prophase, that is at the beginning of cell division. One can clearly see the condensation of chromosomes (red/magenta) and the surrounding nuclear envelope (green), on which invaginations and the onset of tearing can be seen.

UNPARALLELED DIMENSIONS IN MICROSCOPY

The biologist has advanced into unparalleled dimensions in living cell microscopy in yet another project. "Essentially, what we want is to understand cellular processes better, and above all pathogenic processes at a molecular level," he tells us. "But that's only possible if you have reliable information on the quantity, localization and changes in the cellular components concerned. In recent years, antibodies have become our most valuable tool in this quest, because they can be used to detect biological molecules and structures for research, diagnostics and therapy." Antibodies, or immunoglobulins, are part of the defense arsenal of higher vertebrate immune systems, and are genuine quick-change artists. Each one of

these immunomolecules recognizes another structure, called an antigen, highly specifically. They patrol the blood and other body fluids on the lookout for pathogens and other foreign objects. The general structure of all antibodies is the same, and is much like the letter "Y", except that the very ends of the two short arms are extremely variable. It is with these domains that each antibody recognizes its antigen and latches onto it. The many millions of antibodies in an organism differ only by these "hypervariable regions", by which they can recognize just as many different antigens.

The highly specific binding ability of immunoglobulins, which can recognize just about every antigen and every cellular structure there is, is used to enormous success in biomedicine. Antibodies are produced specifically to seek out wanted biomolecular suspects, and are coupled with a marker. If the suspect in question is present in a cell or tissue, then the antibody will grab hold of it and in turn be detected from its marker. This marker is typically a fluorescent stain that can be rendered visible under a microscope. In short: where the marker lights up, that's where the antibody and, with it, the targeted molecule is. "Antibodies are undoubtedly the most important tool in biomedical research and diagnostics," says Heinrich Leonhardt. "But they have a definite downside: for many studies, antibodies are simply too large, even if we have already developed smaller molecules. Also, they only allow us to take isolated snapshots, and so far it has been impossible to investigate dynamic processes within living cells." These dynamic processes, however, are precisely what scientists are eager to learn about. Weary of simply studying where and in what quantity a protein is present in a cell at any given instant, they now want to investigate how proteins move within cells, and how they interact and change as they do. The trouble is, all of these things can only be studied inside living cells, while the natural antibodies in the body only occur outside cells. If you try to produce antibodies inside a cell, they will be nonfunctional. This is because, being proteins, they must fold up into a specific three-dimensional shape in order to fulfill their function. As it is, inside cells they tend to fold up wrongly, making them useless.

HOW TO MAKE CHROMOBODIES

The conventional antibodies we have been talking about so far consist of four chains in total. Two heavy chains, which bind to one another in various places, form the basic frame that makes the "stem" of the Y-shaped structure. Two light chains each bind to the free ends of these two molecules, becoming the two short arms of the Y, the tips of which are solely responsible for recognizing the corresponding antigen. "Camels and their relatives the alpacas, however, also have significantly smaller, single-chained antibodies," reports Heinrich Leonhardt. "They lack the light chains. Their antibodies bind at a single variable region. It is the smallest antigen-binding fragment we have encountered in nature." Therefore, the researchers logically decided to use only the antigen-binding domain of such a single-chained antibody in their approach. Overall, it is ten times smaller than a conventional antibody, and has been accordingly named a nanobody. By fusion with fluorescent proteins, Leonhardt and his co-worker Dr. Ulrich Rothbauer then created fluorescent

nanoprobes, which they called chromobodies. Thanks to their small size and stability, chromobodies can even be used inside living cells. "Our fluorescent designer molecules can be produced by the cells themselves. Then they latch onto the antigens of interest, and track their path and destiny within the cells." This method is not only limited to tracking proteins, either. Protein chemical modifications and other cell components can now be studied as well, which has been impossible until now. Given their small size, chromobodies are very easy to produce and extremely stable. There are even more reasons why these camel antibodies are a welcome alternative: until now, conventional antibodies have always been produced in animals, especially in rabbits, mice, rats, chickens, goats and sheep. This is no longer necessary with the advent of nanobody technology. Thanks to the simple structure of nanobodies, the desired antibody diversity can be created in artificial molecular libraries. The group of Ulrich Rothbauer and Heinrich Leonhardt has already generated large libraries that contain billions of chromobodies, although the present libraries were still obtained from immunized camels and alpacas. In future, however, this technology should phase out the use of animals. Select nanobodies can be produced in almost any desired quantity from bacteria. These libraries have already proven valuable: "We have obtained quite a few specific probes for various biological cell structures," reports Heinrich Leonhardt. "With the help of these chromobodies, we can follow, influence and even block processes in cells. That allows entirely novel functional studies. For example, we have developed a ‚nano trap', which we can use to detect, catch and isolate interacting proteins in the cell. The possibilities are almost limitless."

Prof. Dr. Heinrich Leonhardt has been Professor for molecular human biology at the BioCenter of LMU Munich since 2002. He is a member of the clusters of excellence „Nanosystems Initiative Munich" (NIM) and „Center for Integrated Protein Science Munich" (CIPSM).
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