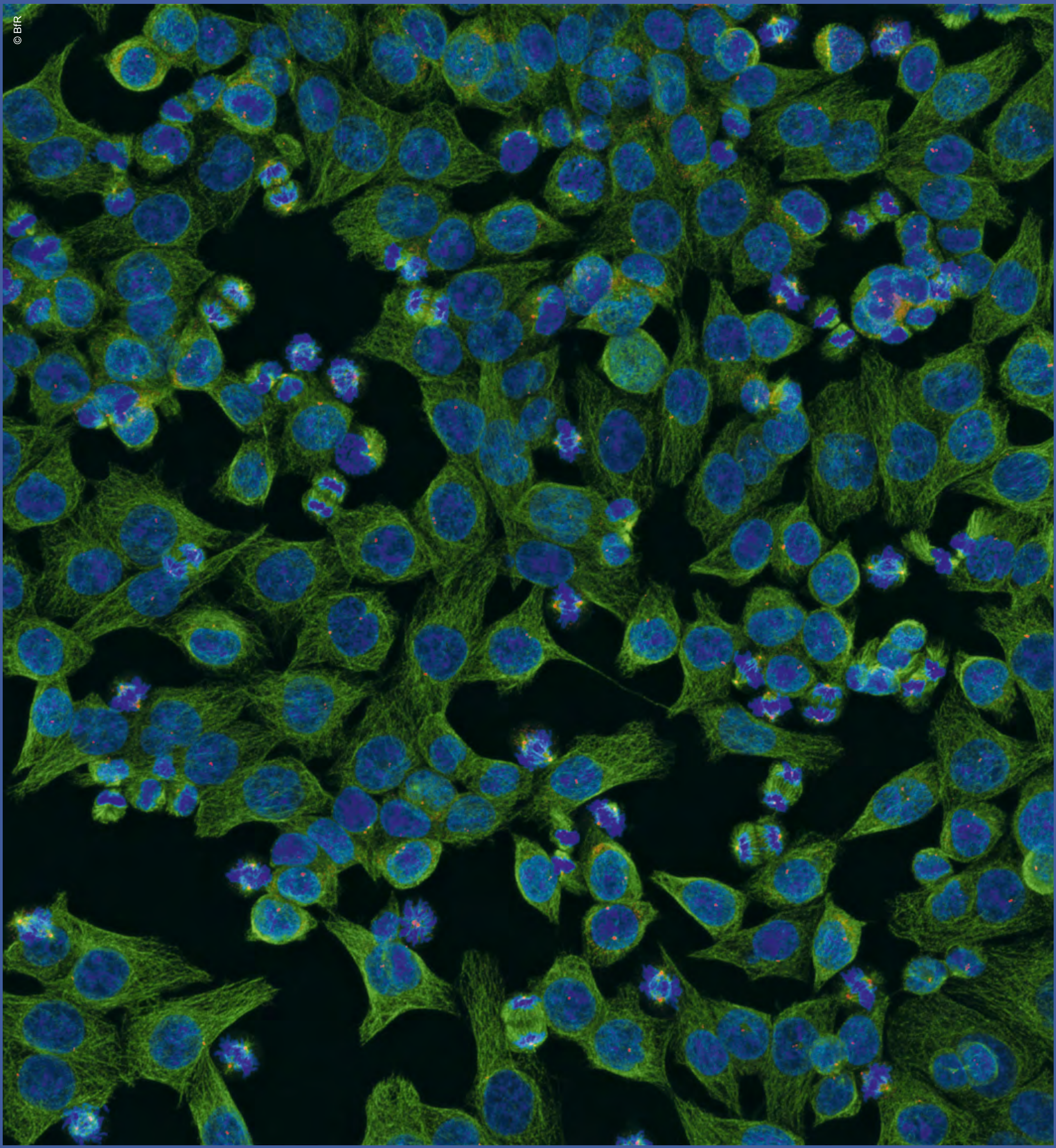


Divided risk: colon cancer cells under the microscope, many of them recorded during cell division.

Cell nuclei are coloured blue; the microtubules (“spindle fibres”) involved in cell division as a “spindle apparatus” are coloured green.

Hormone traces in the nano-world

Scientists at the BfR are using high-resolution microscopes to examine how oestrogen-like substances alter the body's cells. This research also serves to replace animal experiments.

Gilbert Schönfelder has a cold and therefore has several packs of paper tissues at the ready. This is quite appropriate, as he can use the tissues to illustrate how breast cancer cells proliferate. The physician and toxicologist at the BfR puts down three packs of tissues close to one another. „The mammary gland cells are in a row like a picket fence", explains Schönfelder. If a cell breaks free from its anchorage, then it has to break off the close contacts with its neighbours." The scientists demonstrates this by pulling the middle pack out of the row. This separation from a group of cells is a fateful step. It can signal the start of a metastasis. "After all, 90 percent of cancer sufferers die from the metastases and not from the original carcinoma itself", says Schönfelder.

People understand by observing

Observing promotes understanding. Humans rely on what they can see. When we see something, we can understand it better. This also applies to processes that take place in the microcosm of the cell and its surroundings. Even in the age of laboratory analysis, bioinformatics and genetic data, therefore, observation is still of great benefit in medical research. Indeed, it is even making a comeback. Together with computer-assisted evaluations, observation in the form of microscopic images constitutes an important source of information for scientists like Schönfelder – when, for example, the job is to determine which chemical substances from the environment can separate the mammary gland cell from its surroundings, resulting in gaps in the picket fence.

"To make the small world large" is the goal of Schönfelder and his team. He set up a microscopy centre at the BfR together with biotechnologist Konrad Gulich. The "BioImagingCenter" is located in a darkened, vibration-protected room with a constant temperature on the ground floor of the BfR complex in Berlin-Marienfelde. The microscopes are high-resolution microscopes that are capable of capturing images and processing them instantly – the computer has been given "eyes".

The research at the BioImagingCenter provides important support for the German Centre for the Protection of Laboratory Animals (Bf3R) at the BfR in the development of novel methods that can replace and supplement animal experiments. This means that researchers who can observe through the microscope how chemicals alter the properties of cells (keyword: cancer) can refrain from conducting one or more animal experiments (or design the experiments in a more meaningful and targeted manner).

A nanoscope for the inside of the cell

The centrepiece of the BioImagingCenter is the PALM/STORM microscope. It has a room all to itself and is currently the "highest-resolution light microscope in the world", explains Gulich, not without pride. "Its magnification factor is around 20 times higher than that of conventional microscopes". A green-coloured something can be seen on the screen of the "PALM/STORM" with two closely located blue circles at the centre. It is a cell that is in the process of dividing, and one cell becomes two. The cell is highly sensitive during this division phase: the chromosomes – the carriers of the genetic material – dock on to fine protein fibres, known as the "spindle apparatus". The fibres "pull" the chromosomes in the direction of the newly developing daughter cells.

"Some substances can hinder this process, as can be observed under the microscope", says biologist Ailine Stolz. "We are researching the potential of these substances." Stolz uses the PALM/STORM microscope to study the way in which hormone-like substances act during cell division and can possibly therefore cause or at least promote cancer. The huge level of accuracy of the microscope is based on an invention that won the Nobel Prize for Chemistry in 2014. The new technology turned the microscope into a nanoscope, as the Noble Prize Committee wrote in its reasons for choosing this technology.

The limit of resolution

It was physicist Ernst Abbe from Jena who defined the seemingly unsurmountable limit of resolution way back in 1873. According to Abbe, the microscope can no longer distinguish between two dot-shaped objects if they are less than 0.2 micrometres apart. Generations of researchers who succeeded Abbe were unable to overcome this constraint.

In 2005, American physicist Eric Betzig succeeded in outmanoeuvring nature. His "PALM" method (PALM stands for "photoactivated localisation microscopy") is based on the realisation that molecules that have been previously marked with fluorescence are caused to light up with a small light impulse. This impulse is so weak that only a few molecules flicker in each case. Moreover, they are more than 0.2 micrometres apart – in other words, they obey Abbe's Law and are visible independently of one another. The trick is that an overall image with an extremely high resolution down to 10 nanometres (0.01 micrometres) is only achieved after thousands of individual images are combined in the computer. "It's like a night sky in which more and more stars gradually begin to appear until, finally, they're all shining", is how Gulich explains the PALM principle.

The high-throughput microscope in the next room is based on a different approach. The focus is not on small details in the cell but rather on changes in many cells that are magnified by the microscope. The computer registers the images and evaluates them statistically. "We are developing a test method that will help us to better understand the effects of hormonally active substances", explains biologist Sebastian Dunst. On the screen showing the microscopic image, it is possible to recognise tightly packed and blue-coloured nuclei of breast cancer cells with green-sprinkled boundaries – the cell membranes.

More metastases due to hormones?

The substances that colour green on the cell membrane are adhesion proteins. They connect cells, they glue the cells together, so to speak. Dunst and his colleagues noticed that adhesion proteins can change under the influence of certain substances, strengthening or weakening the bond between the cells as a result. This may mean that the risk of metastases is lower when the connection is stronger – because the cell remains in its "picket fence" structure. The theory behind this is that substances that have a similar effect to that of the female sex hormone estrogen loosen the cell contacts, thereby increasing the risk of metastases. In contrast, substances that block estrogens reduce the risk. This is probably also due to the effect on the adhesion proteins.

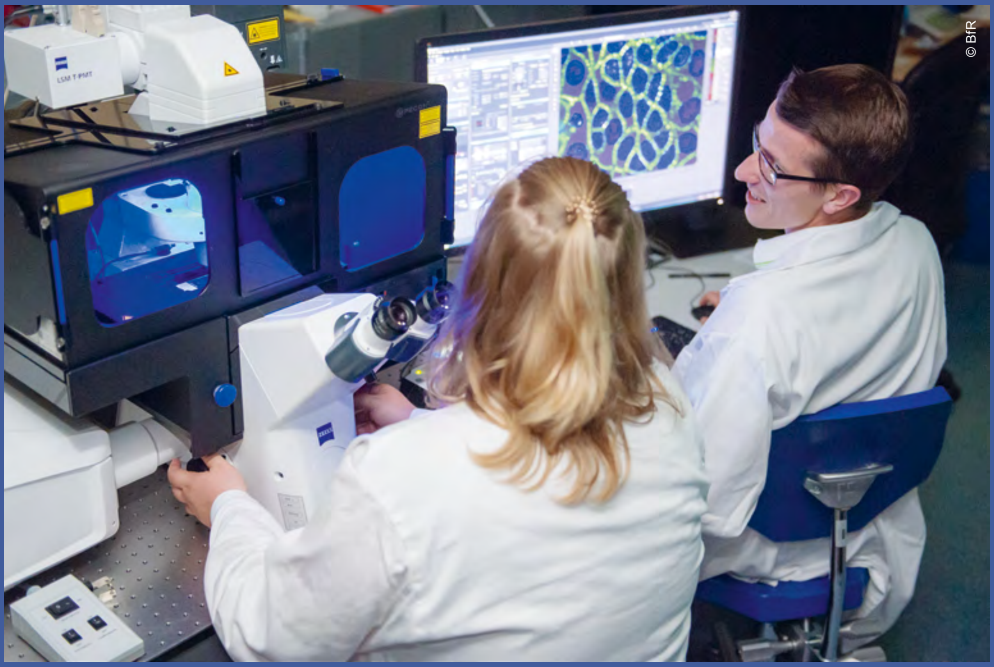
When the high-throughput microscope is used to examine cell cultures, it is possible to study exactly what happens when the dose of a substance is increased. If a chemical substance is used to block the hormone estrogen, the cells adhere better to each other and the cell is less mobile. If, on the other hand, estrogen-like substances are dripped onto the cells, the reverse happens. "We have tested more than 20 active substances with estrogen-like effect and succeeded in observing clear effects", Dunst reports.

High-penetration laser for deep insights

Multi-photon microscopy provides deep insights into living organisms and is also used at the "BioImagingCenter". The researchers use long-wave laser light that can penetrate tissue up to a depth of one millimetre. This may not sound a lot but it represents a major step in the microcosm of life –1,000 micrometres no less.

This technique makes it possible to study life processes in complete organisms, such as zebrafish embryos in organ-like microstructures, known as organoids – and to do so cell by cell, so to speak. Organoids are to be used increasingly in future for testing medications or chemicals." With this approach, we are working to reduce the number of animal experiments in basic research", says Schönfelder, as he looks at a hugely magnified organoid. If only microscope pioneer Abbe could look over his shoulder, he would be astounded. ◘

Cell in focus: impressions from the BioImagingCenter



We want to make the small world large!

Professor Dr. Gilbert Schönfelder, toxicologist