Altered blood profile: in leukemia, greater numbers of white blood cells are formed, here colored brown and turquoise in this image from a scanning electron microscope. Conversely, the number of other blood cells, red blood cells for instance, decreases. The disease can also be diagnosed based on the mechanical properties of the patient's white blood cells.

IMAGE: SCIENCE PHOTO LIBRARY/GSCHMEISSNER, STEVE

CELLS UNDER PRESSURE

TEXT: ROLAND WENGENMAYR

To date, medical science has shown little interest in how easily cells deform. As Jochen Guck, Director at the Max Planck Institute for the Science of Light in Erlangen, and his team have discovered, this attitude is unjustified. As it turns out, the mechanical properties of cells can be used to diagnose cancer and possibly also inflammation. The scientists are currently testing the method together with University Hospital Erlangen – and have already gathered useful insights into COVID-19.

It could lead to a whole new way of testing blood that can be monitored directly on a screen. Small, dark shadows stream through a funnel-shaped narrowing into a slender channel. Within this, they accelerate, become deformed and are then swept back out again. They're blood cells, and the images were taken by a high-speed camera fitted to a microscope. "The channel is just 20 micrometers in diameter and 300 micrometers long," explains Martin Kräter – in other words, its diameter is about the same as that of a fine human hair, and on the lab-on-a-chip that the biologist is showing, the channels can only be discerned by the indistinct way they reflect light.

With a PhD in hematology, Kräter is an expert on blood, and the laboratory is located at the Max Planck Institute for the Science of Light in Erlangen, Germany. It is an unusual place for biology-based research. That can be explained by Jochen Guck's career, which has transcended the boundaries between specialist disciplines and was one of the reasons he joined the Institute as its Director in October 2018. Guck is a physicist who, among other things, uses laser light as a tool. And that's why he fits the profile of the Institute, in particular because his field of research is physics and, more specifically, the mechanics of living cells. This is precisely what his work on blood cells is all about. As a young researcher, Guck performed experiments on cancer cells in which he observed that they are mechanically softer than their healthy counterparts. When a force is applied to them, they deform to a greater degree than healthy cells do, and this can be seen under the microscope as they pass through the channel. Scanning cells in this way could become a new method of medical diagnostics, for example in cancer therapy and screening, as well as to identify inflammation. Looking at the mechanical properties of the cells should also help in understanding certain diseases, such as COVID-19.

This technique has opened up a new dimension in medicine, and Jochen Guck's team has now advanced it to the point where it has even given rise to an award-winning start-up company, Zellmechanik Dresden. The first generation of such blood testing devices has now been launched on the market. However, its use is still limited to research purposes and the market is therefore very small, but Guck expects this to change. Indeed, some of the devices are already running in test mode in a laboratory at the children's hospital at Erlangen University Hospital. Guck's team is working on site with the oncologist and physician Markus Metzler and his research group. They are testing whether the technique can aid in diagnosing leukemia, the most common childhood cancer. This cooperation also exemplifies the research of the Max Planck Zentrum für Physik und Medizin in Erlangen, in which

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the Max Planck Institute for the Science of Light, the University of Erlangen-Nuremberg and University Hospital Erlangen are collaborating. The center's new building is currently under construction, and Guck's team will move into it in 2024, as one of its five major working groups.

As a young physicist, Jochen Guck never imagined that one day he would become involved in biomedical science. "I opted out of biology at school," he says with a grin. Biology, he felt, was too unstructured, requiring too much rote memorization of facts. He was drawn to math and physics. And so Guck went on to become a physicist specializing in lasers and, in a roundabout way, this led to his meeting Josef Käs in Austin, Texas, who was interested in capturing and investigating the biomechanical properties of cells with the aid of lasers (a technique referred to as "optical tweezers").

Laser tweezers deform cells

In encountering Käs, Guck also came upon the scientific field that drives him to this day. "I was into laser technology at the time," he says. "The fact that it involved cells was something I tended to put up with." He seized the opportunity to set up a laboratory tailored to his needs and wishes. Being accessible to disabled people was a must; Guck uses a wheelchair. "I made sure the optical tables were high enough to park a wheelchair under them," he stresses. At the time, he discovered experimentally that cells react to laser light quite differently than hard glass or plastic particles of a similar size. The device Guck built consisted of two opposing laser beams. A channel transporting the cells ran between them. If a cell entered the light field, it was captured. But instead of being compressed by the opposing laser beams, as the physicists had expected, to Guck's surprise the opposite happened: the cells reacted to the pressure of the laser light by elongating along the beams. The optical tweezers unexpectedly turned into an optical stretcher. Guck managed to solve the riddle. "That was my first scientific eureka experience," he recalls. And it fired up his fascination for cells. Back then, scientific evidence was already emerging that the mechanical behavior of cells could reveal something about their internal state: cancer cells had been found to be softer than their healthy counterparts. His discovery led Guck to systematically investigate this observed behavior and to show that it could be used as a marker for cancer.

By the time he arrived in Erlangen in 2018, Guck was already envisioning the development of a routine testing procedure based on the biomechanical analysis of cells that could be employed in day-to-day clinical practice. His biggest challenge was to develop a method that could reliably screen a large number of cells in an amount of time that was also feasible for use in the real world. For although the optical stretcher developed by Guck in Austin was the fastest method around at the time and could analyze approximately one hundred cells per hour, it was still far too slow. Martin Kräter sums up the challenge they faced: "When you're examining a blood sample, only about one in every thousand bloods cells is white; the rest are red!" However, the diagnostic technique must capture a sufficient number of these rare white blood cells within a reasonable period of time. It's the white cells that are pathologically transformed in leukemia, and they also provide crucial information about the state of the immune system and about possible sites of inflammation.

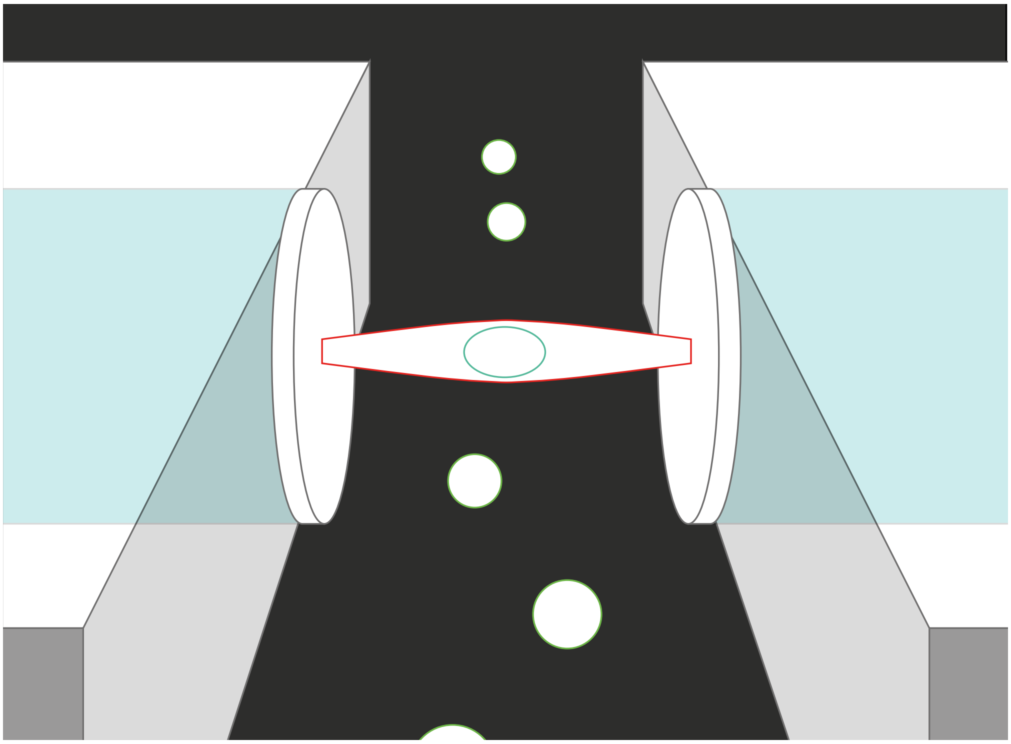
So they needed to discover a much faster process. Guck and his team came up with the idea of using "microfluidics" to capture blood cells in a rapid flow through a microscopic narrowing. This formed the basis of the method demonstrated by Kräter in the laboratory. Passing a fluid containing blood cells through a tiny channel sounds simple. However, its success as a diagnostic tool depends on precise control of the flow, allowing them to compare the deformation of similar cells under precisely reproducible conditions. A trick was required. The scientists developed an enveloping flow that surrounds the actual flow of the solution containing the cells, and both are pushed through the micro-funnel into the channel. This prevents cells from sticking to the walls of the channel. In addition, the flow of the fluid in the channel needs to be completely free of turbulence. A "laminar" (smooth) flow has a flow profile that is physically precisely calculable. The flow velocity is lowest along the wall, while it increases towards the center of the channel. This precisely predictable flow profile provides the key to reliable analysis of cell deformation. It causes the blood cells in the channel to be pushed in the direction of the flow at their center and slowed down at their lateral edges, deforming them into their projectile-like shape.

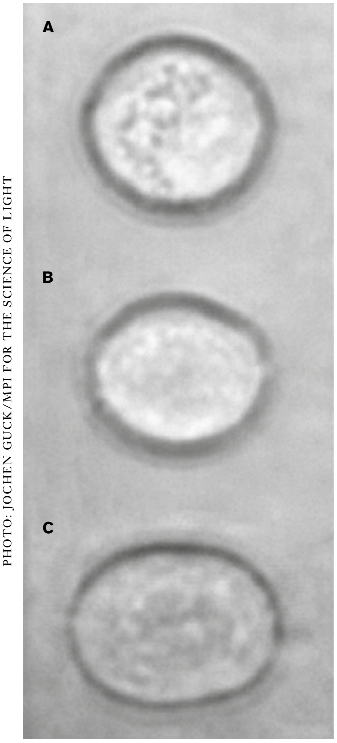
SUMMARY

Cancer cells are softer than healthy cells. The mechanical properties of cells can help to diagnose some cancers even earlier than techniques based on biochemical signals.

A team from the Max Planck Institute for the Science of Light is currently testing deformability cytometry with Erlangen University Hospital, primarily to diagnose leukemia.

The researchers are also investigating how useful the technique might be in diagnosing and studying inflammation. They have, for instance, discovered that red blood cells from COVID-19 patients deform less readily than those from healthy individuals, even after recovering from an infection. This could explain the symptoms and long-term effects of long COVID-19.





Optical stretching device: intense laser beams can grip cells just like a pair of tweezers (left). Right: healthy cells (A) deform significantly less in the process than tumor cells (B). Metastatic cells are the most readily elongated (C).

A high-speed camera synchronized with a bright light-emitting diode flash can record the deformation of a thousand cells per second using this technique. "That's 36,000 times faster than the optical stretcher," summarizes Guck. "Using the stretcher, it would have taken us about a hundred years to record the same number of cells we can now analyze in a day!" It was this huge increase in throughput that was the breakthrough. "Real-time deformability cytometry" of this kind has made it feasible to detect pathological changes in cells in clinical settings.

"The term 'cytometry' simply means 'measuring cells'," explains Guck. "Real-time", however, involves processing hundreds of thousands of images in a short period of time with no delay. No human being can analyze such an enormous amount of data. The team, therefore, utilizes artificial intelligence and machine learning. The technology of artificial neural networks is now well established and is ideally suited to detecting patterns. It automatically sorts the passing cells by shape, as Kräter demonstrates on the screen. However, the system also needs to reliably recognize cell forms. To accomplish this, the AI system needs to be trained with blood samples from as many patients as possible. That's precisely what happens in Markus Metzler's laboratory at the Erlangen children's hospital.

Biomechanics as an early warning system

To appreciate the benefits of using diagnostics based on touch (tactile sensing) in the field of oncology, we need to take a look at the cell and its mechanics. The fact that a cell reveals something about its inner workings by means of its mechanical properties alone has to do with its "cytoskeleton," a term Guck is not a fan of. This skeleton does indeed play a role in structural support, similar to our bones. If we were single cells, however, we would be able to change our shape, just like Harry Potter, by rapidly restructuring our skeleton. And this is exactly what a cell does continuously in order to react to its environment or to changes in its interior – or to divide. So what's the best way to visualize such a skeleton? "It's best to think of it like a gel," says Guck. If you imagine a cell as a tube of moisturizer, it wouldn't just passively sit around in your bathroom cabinet. The gel in it would take on a life of its own, constantly deforming the tube and even causing it to roam around inside the cabinet. But such continuous activity comes at a price. "A considerable amount of a cell's energy is devoted to its cytoskeleton," explains Guck, "as much as 30 to 40 percent!"

What is decisive for diagnostics is that a cancer cell, for instance, has already remodeled its cytoskeleton and softened even before standard clinical diagnostics discover its familiar characteristics: surface-bound proteins that can be identified with color-tagged antibodies. "In a manner of speaking, we can now analyze samples blindfolded," explains Guck: "If its mechanics have altered, the function of the cell must have already changed as well." As a result, biomechanics may become an early warning system for malignant changes in cells. In addition, real-time deformability cytometry instantly provides meaningful images, without extra procedures in the lab, such as staining.

This early warning function and the ability to directly identify pathological changes are advantages that have also convinced Markus Metzler of the merits of the joint research project. "We're a really good match for each other," he enthuses, referring to the fact that his team brings clinical practice research to the table, while Guck's group contributes cutting-edge technology from basic research: "If anyone can advance this new technology, we can." Metzler is confronted with the suffering and fears of his young cancer patients and their parents on a daily basis at the Erlangen children's hospital, and this provides him with additional motivation to drive the development of new diagnostic methods forward. It is the reason why the researchers are operating their own laboratory directly integrated into the health care system. There, they perform routine examinations that are already well-established in medical practice. Alongside this, real-time deformability cytometers from Guck's group are already up and running. They're utilizing the large number of blood samples drawn by the clinic to train their AI system to detect abnormally transformed cells.

Markus Metzler even expects that the AI training phase with data from various diseases will progress reasonably quickly. And he's got his sights set on more than cancer cells. "It also works reasonably well for certain forms of inflammation," he says, referring to the fact that white blood cells can also be exploited as messengers for inflammation concealed in the body. In and of itself, this is nothing new; standard blood testing involves counting leukocytes. What is novel, however, is being able to assess their biomechanical state, which can provide additional information. How this can be used to

Automated analysis: a liquid containing cells passes through the tubing over a microfluidic chip, while a high-speed camera installed on an optical microscope records the cells. Software then identifies diseased cells that deform more in the flow than healthy ones.

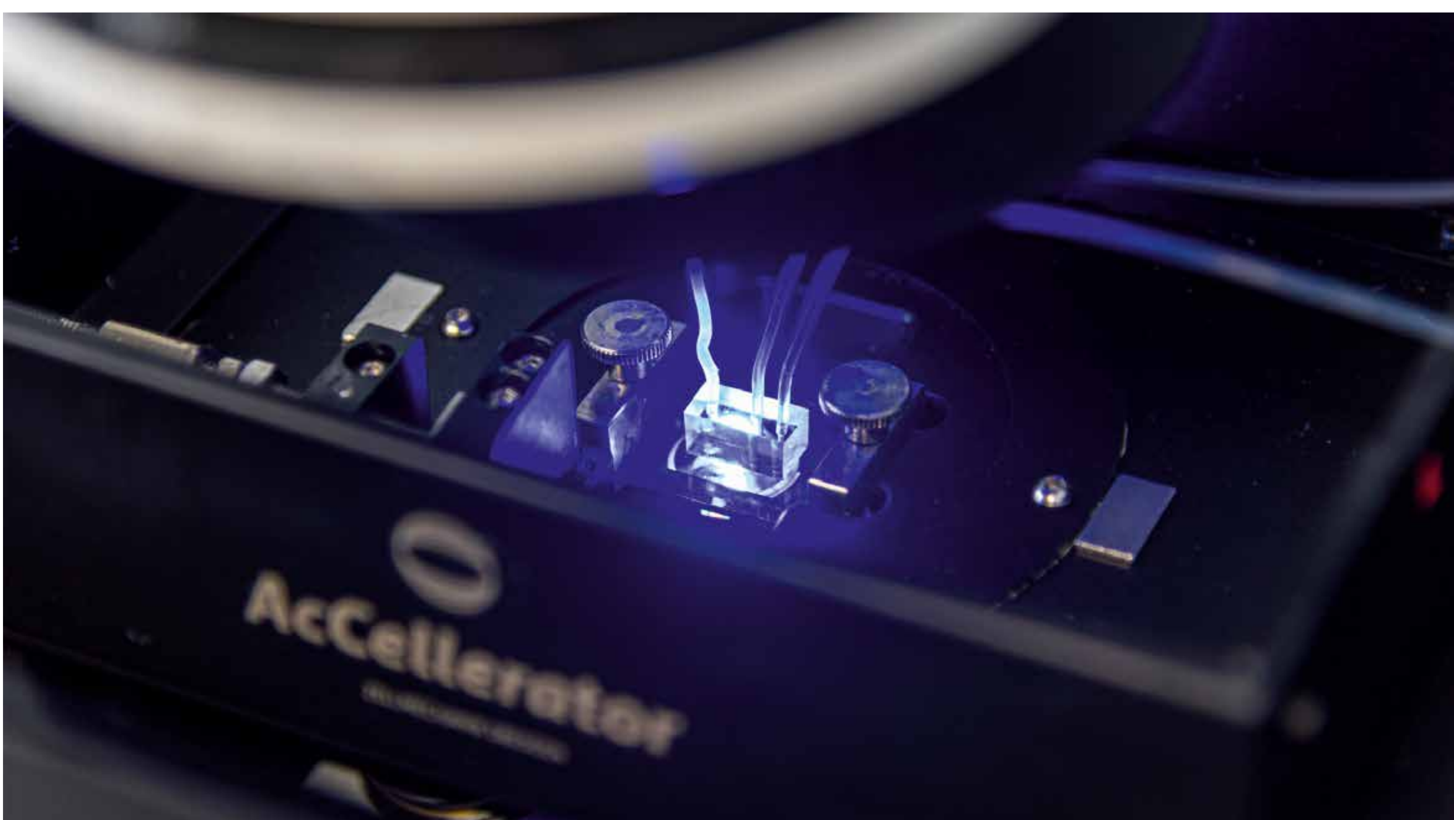
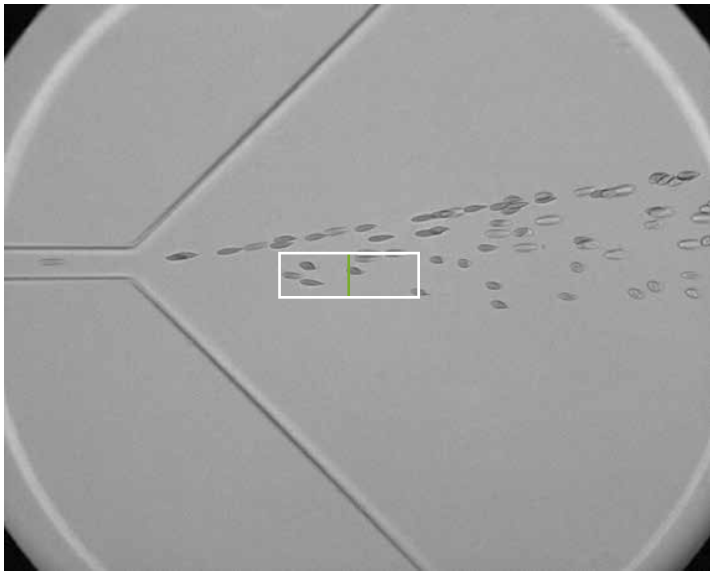


PHOTO: JOCHEN GUCK/MPI FOR THE SCIENCE OF LIGHT



Biomechanical rapid test: the extent to which cells are distorted when they flow through a fine channel on a chip reveals whether they are healthy or diseased. The actual measurement occurs in the marked rectangle at the center of the image once this area is positioned over the channel.

PHOTO: MARTIN KRÄTER/MPI FOR THE SCIENCE OF LIGHT

improve diagnostics and therapy is a question that Jochen Guck's team are hoping to clarify with other medical research partners.

One mechanism behind COVID-19 damage

"Our method can detect a property of the cells that, to date, has remained untested," emphasizes Markus Metzler. It is a little like examining a patient with suspected appendicitis. "If we only recorded the size of the patient's abdomen and skin color, but didn't palpate the abdomen at all," says the professor of medicine, "we would be missing crucial diagnostic information." On the other hand, if the patient merely had a hematoma on her abdomen, the physician could make a correct diagnosis just by visually examining her. In precisely the same way, real-time deformability cytometry can be useful in some diseases, but in others, well-established diagnostic techniques are all that is required.

As a physician, however, Metzler is also taking the rigors of everyday clinical practice into account. Both he and chemist Manfred Rauh, head of the lab at the children's hospital, have seen promising medical innovations fail in practice. In a hospital, things need to be quick and uncomplicated: load up your blood sample, press a button and let the device do the rest – a requirement that a real-time deformability cytometer also needs to meet. Therefore, the next generation of the device is going to include an autosampler function for automated testing. Guck is already collaborating with a team at the Fraunhofer Institute for Process Optimization and Automation in Mannheim to develop the system. He envisages a time when deformability cytometry becomes a standard procedure for blood testing in all laboratories. It could potentially be used to diagnose inflammation and, even more importantly, for the early detection of leukemia and other types of cancer, such as lung cancer. Beyond this, the technique could eventually become an element of regular monitoring during and after tumor therapy. Metzler believes that medicine first needs to discover the specialized ways biomechanical diagnostics can establish itself in routine practice.

In the current situation, the technique can apparently help to explain some of the symptoms and long-term effects of COVID-19. Examining the blood of COVID-19 patients in the Erlangen intensive care unit has revealed, among other things, that the erythrocytes (red blood cells) of patients had become less elastic compared to those of healthy individuals. "However, the erythrocytes need to be able to deform in order to pass through the fine blood capillaries," explains Martin Kräter: "If they can't, they can block the finest blood vessels, such as those in the lungs – and this, significantly, is one of the leading causes of death from COVID-19."

Especially interesting is the finding that such changes in the blood are still observable six months after patients have recovered from the disease. This could provide the physiological explanation for why some people who have seemingly recovered often still experience respiratory problems, reduced physical fitness, and even neurological deficits. "Some of the patients we studied experienced, among other things, reduced concentration capabilities," says Kräter. In such cases, the mechanical properties of blood cells could, for the first time, conclusively clarify health problems that were previously inexplicable. "Our pre-publication results are creating quite a stir," says Guck. This highlights how helpful biomechanics might be in medical research and diagnostics. And Jochen Guck is convinced that this will also hold true for a variety of other diseases.

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GLOSSARY

DEFORMABILITY CYTOMETRY
A technique based on studying the mechanical properties of cells to better understand various diseases and to improve our ability to diagnose them.