By Kurt Kleiner
Photographs by Hadas Parush

GIVING TUMOURS AN IMAGE MAKEOVER

USING ADVANCED IMAGING TECHNOLOGY, SYSTEMS BIOLOGIST LEEAT KEREN OPENS A WINDOW ON LIFE IN THE CELLULAR WORLD

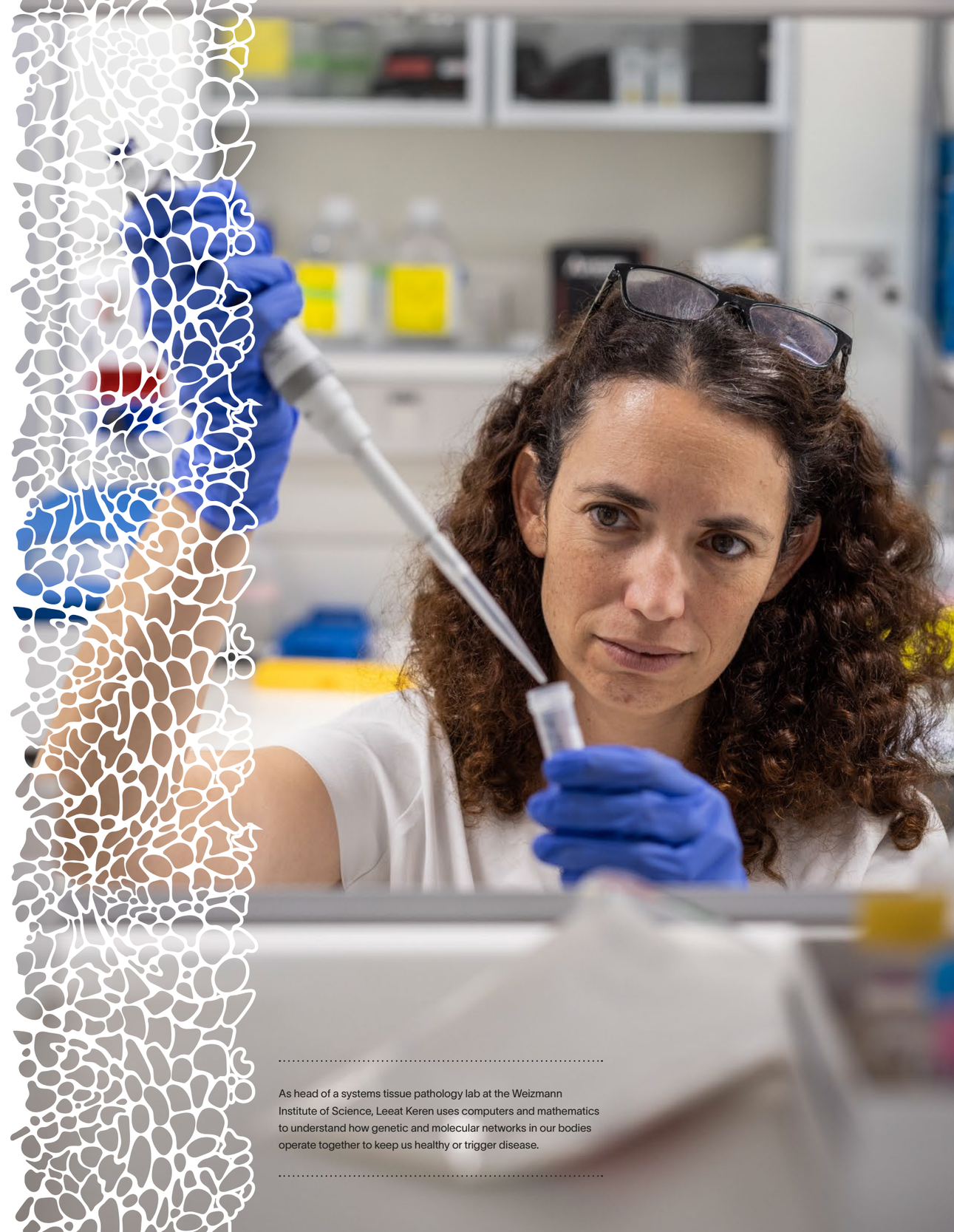
Leeat Keren was fresh from completing her PhD in computational biology at the Weizmann Institute of Science in Israel. As a systems biologist, she was interested in putting pieces together. She had been trained to use computers and mathematics to figure out how the genetic and molecular networks in our bodies operate together to keep us healthy or break down and cause disease.

She thought the field of immunology was primed for disruption by the systems biology approach and was emboldened while attending a presentation by Michael Angelo, a Stanford University pathologist, at an immunology conference.

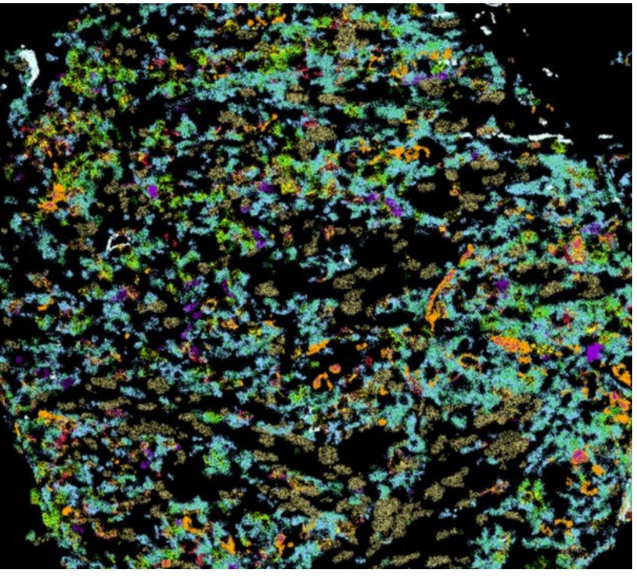
"He presented this idea of a new instrument he was building to be able to visualize many proteins inside the tissue at once without disrupting it," Keren says. "And I remember I came back to my office and told my roommates, 'This is what everybody is going to be doing a few years from now.' I fell in love with this technology and the prospects of what it could do."

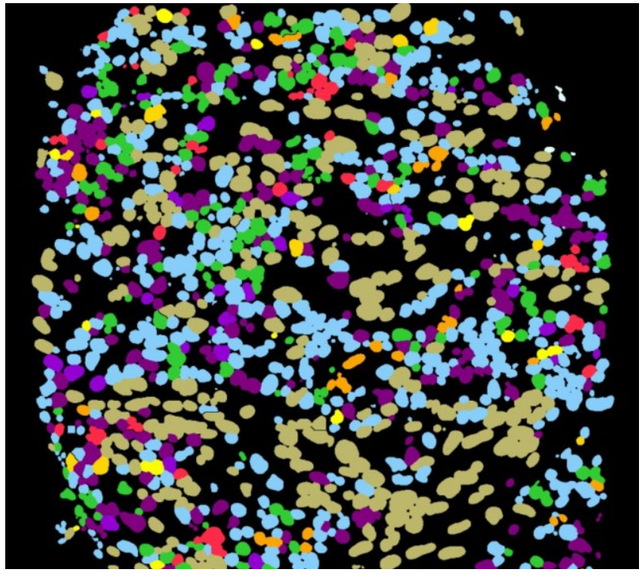
The technology is called multiplexed imaging. For more than a century, pathologists have been examining tumours by placing them under microscopes and studying the shapes of the cells and how they were organized. More recently, they have been able to do tests that reveal the genetic activity in the tumour cells and which proteins are being produced by those genes.

The trouble is that these tests require the cells to be pulled apart. Scientists see how the cells are behaving overall but lose information about how these cells communicate with each other, relate to one another, cooperate or compete.



As head of a systems tissue pathology lab at the Weizmann Institute of Science, Leeat Keren uses computers and mathematics to understand how genetic and molecular networks in our bodies operate together to keep us healthy or trigger disease.





COURTESY OF THE KEREN LAB, WEIZMANN INSTITUTE OF SCIENCE (BOTTOM LEFT AND RIGHT)

Multiplex imaging, a technology Keren helped develop, allows pathologists to visualize the number and location of proteins inside a tissue, identify tumour and immune cells and observe how cells communicate. The resulting raw data (far left) is fed into a mathematical model that maps the tissue's cells (left). Tumour cells appear in brown.

Multiplexed imaging offers scientists the best of both worlds. They can look for dozens of proteins at once and use the information to tell which cells are present, how they are functioning, and how they are communicating with one another (see related story).

After seeing Angelo's presentation, Keren worked with him as a postdoctoral fellow to develop the instrument and, most crucially, to create the computational pipelines to analyze the massive data sets generated by the new technology. Fortunately for Keren, the advancements in multiplexed imaging coincided with the revolution in artificial intelligence. She was able to borrow sophisticated tools from the realms of computer vision and apply them to make sense of the thousands of images that she generated.

Today, Keren runs her own lab at Weizmann. An Azrieli Early Career Faculty Fellow (2020 to 2023), she is interested in the smallest details of tumours: how they organize themselves at a cellular level, how they subvert the body's natural defenses and how understanding them can lead to better cancer treatments.

Gerard Socie, head of hematology-transplantation at Hôpital Saint-Louis in Paris, says Keren is "a worldwide leader in the field in developing these new technologies." He works with her lab now to try to understand why some patients who have hematopoietic stem cell transplants suffer serious side effects in the gastrointestinal tract. "She's really one of the smartest people I've ever worked with," he says.

Keren takes a systems biology approach, which involves studying the complex interactions within biological systems (such as cells, organs or entire organisms) in a comprehensive and integrated manner. It often leverages computational tools and mathematical modelling to understand the behaviour and function of a system rather than focusing on individual components separately. It is an especially useful approach to study tumours and how they interact with the immune system.

Keren is interested in the nitty-gritty of tumours: how they're organized at a cellular level, how they subvert the body's natural defenses and how understanding them can lead to better cancer treatments

Our body has a sophisticated regulatory system that controls how cells grow and are replaced. When cells start to grow out of control, they are usually detected by the immune system and destroyed before we are even aware of them.

A tumour is a group of cells that have somehow escaped these regulatory systems and grown abnormally. Although we tend to think of tumours as an undifferentiated mass, they have their own structures and internal functions. "A tumour that ultimately thrives and succeeds and becomes a cancer — this tumour had to do a lot of things," Keren says. "It had to make space for itself. It had to draw in blood vessels so that it would have nutrients. And one of the things that it needed to do is escape from the immune system."

Tumours can evade the immune system in several ways, including by producing proteins that signal immune cells to turn off. In recent years, medical researchers have developed a class of treatments called "immunotherapy" that tries to overcome these tumour defenses and help the immune system fight cancer.

"We're trying to understand how this process functions as a system," Keren says. "And we're trying to then correlate that with larger-scale outcomes. So, whether this patient will respond well to a particular immunotherapy, whether this tumour will progress or stay static, whether this tumour will recur."

Keren and colleagues, for example, have been studying a type of cancer called triple-negative breast cancer, an aggressive form of cancer that can spread quickly and is difficult to treat. Like other cancers, it manipulates the immune system to avoid being attacked. One of the ways it does this is using a signalling protein called PD-L1, which turns off a kind of immune cell called a T-cell. This prevents the T-cells from attacking cancer cells.

Doctors often treat triple-negative cancer with a drug that blocks the effect of PD-L1, hoping to turn the T-cells back on and get them to attack the cancer. But the treatment does not always work.

In research that was published in 2019, Keren and her Stanford colleagues used multiplex imaging to try to understand which cells were generating the PD-L1, and whether there were differences between tumours that progressed and those that did not. They looked at tissue from tumours that had been removed from 41 patients with triple-negative breast cancer and used multiplex imaging to find the amounts and locations of 36 different proteins and immune cell types.

They found that tumours containing many immune cells could be organized in one of two ways. Some were "compartmentalized" — the immune cells were organized in large clumps well separated from the tumour cells. Others were "mixed," where immune cells were scrambled up with tumour cells. Moreover, different cells were responsible for making PD-L1 in these different types of tumours. In the mixed group,

PD-L1 was made by tumour cells, but in the compartmentalized group it was made by immune cells called myeloid cells. If scientists measured the number of immune cells, the two kinds of tumours were virtually the same. But how the cells were organized made a difference: people with compartmentalized tumours had better outcomes than those with mixed tumours.

In subsequent work, Keren and colleagues went further, examining the complicated interactions among cells. They were able to detect specific interactions in some tumours that predicted the likelihood of a cancer recurring or spreading.

"We can now tease apart the cell types that are actually residing in this particular tumour of this particular patient," says Keren. "And we can also see how they're communicating with each other because the way that immune cells communicate with each other is through proteins."

Keren senses that systems biology is on the verge of a revolution similar to what happened 20 years ago in genetics, when researchers moved from studying one gene at a time to understanding how genes worked together in networks. In the same way, researchers are moving from understanding the workings of individual cells to understanding the importance of interactions between and among cells.

Our knowledge of these microenvironments is still in its early days, she says. The interactions are complex. And researchers are still developing the tools they need to probe the environments, manipulate them and understand how they work.

Researchers working with microenvironments need analogous tools that let them manipulate tissues to understand the interactions. There is progress, Keren says, such as research into using 3D printers to produce tissues with different cell types in different structures to see how functions are affected. "The field is really moving at a super, super fast pace," Keren says.

In large part, it is Keren who is setting the pace. ▲●■

An Azrieli Early Career Faculty Fellow (2020 to 2023), Keren senses that systems biology is on the verge of a revolution, with the rise of computational and mathematical modelling of complex biological systems. Scientists are moving from a focus on the workings of individual cells to the importance of interactions among cells.

One day, 3D printers could produce tissues with different cell types in different structures to see how functions are affected. This is part of the revolution in systems biology

GETTING PROTEINS READY FOR THEIR CLOSE-UP

An important item in Leeat Keren's toolkit is a technology she helped develop while at Stanford University known as multiplexed ion beam imaging by time of flight (MIBI-TOF).

The technology lets her understand what is going on both inside and between cells: what types of cells are in the tissue sample, how the cells signal to one another, whether the immune cells are actively fighting the cancer cells and other information.

At its simplest, MIBI-TOF uses a beam of ions to knock tiny pieces of labelled proteins off a tissue sample. The labels are detected by a mass spectrometer, and computer programs use the information to generate images that show the structure of the tissue and where the proteins are found.

Here's how it works.

Proteins are important for the structure, function and regulation of cells. By looking for certain proteins, scientists can figure out what kind of cell they're looking at and how it is functioning. Cells also signal each other by passing proteins back and forth, so scientists can understand how the cells are communicating as well — for instance, how a cancer cell tells an immune cell to turn off.

Scientists start by deciding which proteins they want to study. Then they take antibodies that have been designed to attach to those proteins. Each antibody only attaches to one kind of protein, and each also has a tiny piece of a different kind of metal attached to it.

When they stain a tissue sample with the labelled antibodies, each protein being studied ends up with a specific antibody attached to it, and attached to that is the metal label that allows the protein to be identified.

Once the proteins are labelled, the researchers run the sample under a machine that shoots a tiny stream of charged particles called ions, one small section of the sample at a time. These ions knock loose the proteins and their labels that are then detected by a mass spectrometer.

Because the metal labels each weigh a slightly different amount, each takes a different amount of time to reach the detectors in the spectrometer. By computing these different times of flight, the spectrometer can tell which label it is detecting and which protein is in that part of the sample.

All the information is used to create a series of pictures, each one showing the location of one protein. An image can be made up of as many as four million pixels, and each pixel contains information on up to 42 different proteins.

With so much information to process, scientists use computational methods, including advanced machine learning and artificial intelligence techniques, to get a complete picture of what they're looking at.

Keren and her colleagues developed algorithms for identifying individual cells in the tissue and understanding the type of cell being studied. In the same way that Google Lens can "see" an image of a cat and identify it as a cat, she says, "we have algorithms that "look" at a cell and can say, for example, that this is a lymphocyte." ▲●■