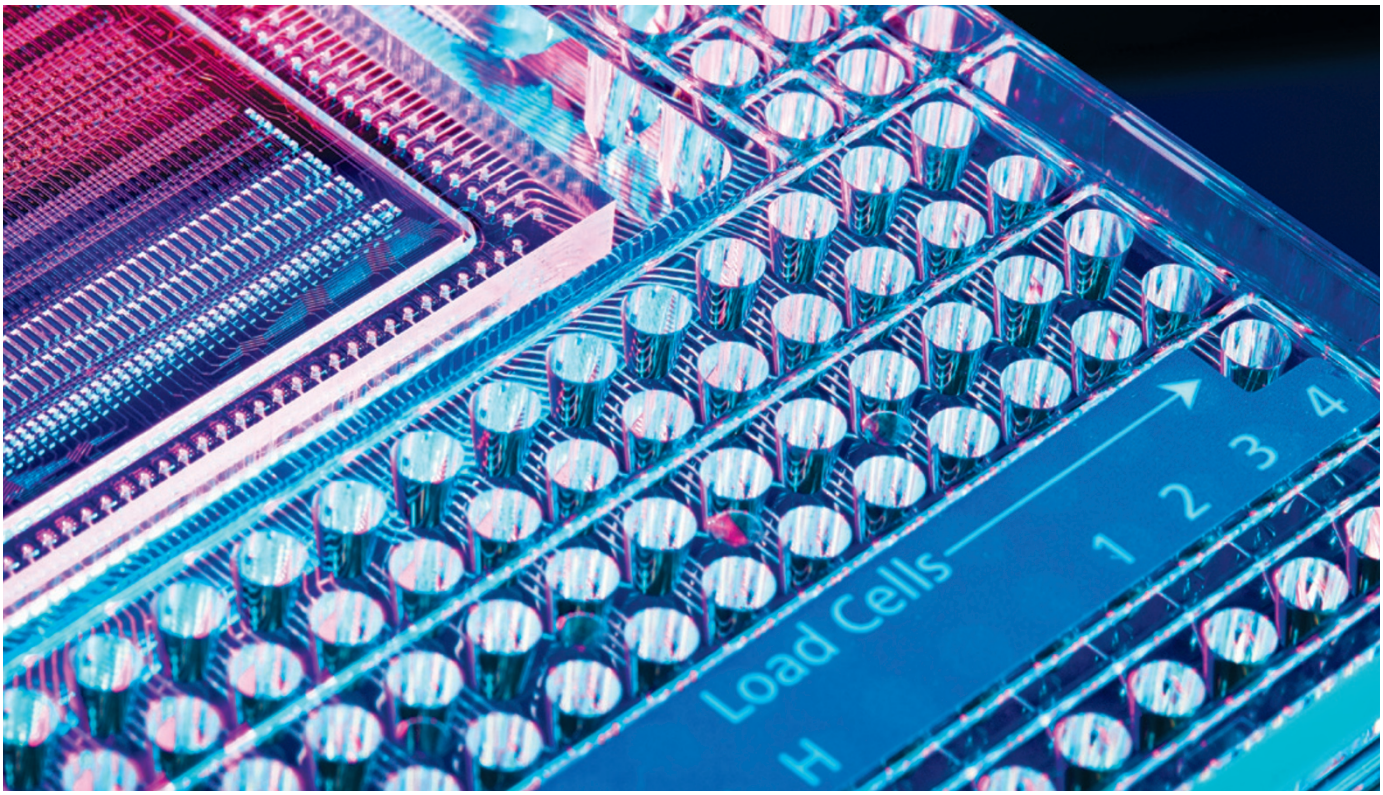
TECHNOLOGY FEATURE

CANCER SHOWS STRENGTH THROUGH DIVERSITY

Tumours are made up of disparate cell populations that often resist treatment — but understanding this heterogeneity could provide ways to improve chemotherapy.

FLUIDIGM



Tiny channels in microfluidics chips such as this one from Fluidigm can isolate individual cancer cells for study.

BY CAITLIN SMITH

Cells come in all shapes and sizes — boxy epithelial cells, discoid red blood cells, delicate, threadlike neurons and the behemoth human egg that is just visible to the naked eye. Even among cells of the same basic type, no two are identical. And the same is true of cells within a cancerous tumour, where differences in size and shape can have profound implications for the progression of a patient's disease. As a result, researchers are keen to get to grips with cell heterogeneity. Developing the tools and techniques to rationalize this cellular chaos has been a slow process, but the latest methods for imaging, modelling and sorting cells are at last coaxing them to relinquish their secrets.

For cancer, this may help to explain why a tumour that has been shrunk by chemotherapy suddenly kicks back into life and starts growing again. The plasticity of individual tumour cells lets them modify their behaviour in response to external cues, says Nicholas Saunders, a cancer biologist at the University of Queensland in Brisbane, Australia. One such cue is chemotherapy, and although the heterogeneity of tumour cells makes it harder to predict how each will respond to treatment, "we now have tools that allow us to interrogate this issue in a relatively definitive way," he says. Recent techniques for sequencing the DNA of single cells from tumours, for instance, has fired up this area as scientists explore ways to use the technology, says Saunders.

SINGLE LIFE

To investigate how cancer cells survive chemotherapy, researchers are moving into the challenging realm of single-cell analysis. At this small scale, it becomes hard to separate true variations between cells from technical errors in measurement, says Nicholas Navin, a molecular geneticist from the University of Texas MD Anderson Cancer Center in Houston. When differences between cells are detected, scientists can question whether the observed variations are important.

Researchers are particularly interested in ▶

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the individual cells shed by tumours into a patient's bloodstream. Carried around the body, these 'metastatic' cells can initiate fresh tumours, allowing the disease to progress. But capturing these roaming cells for study is tricky, because they are mixed in with multiple cell types in the bloodstream.

One system Navin is using to isolate single tumour cells from blood is DEPArray, an instrument made by Silicon Biosystems of Bologna, Italy. This can isolate, move and image one tumour cell from a mixture of 100,000 cells.

Metastatic cells in the blood sample are first tagged with a chemical marker that emits light under a fluorescence microscope. In the DEPArray system, the individual cancer cells are then imprisoned in 'cages' created using an electric field. Viewed on a monitor, these cages can be manipulated to move a single cell into a collection vessel, ready for further study. The lack of physical contact helps the cells to stay alive during the manipulation.

"We think of these cells as extreme shape-shifters that can do anything."

Nevertheless, sorting takes time, says Navin. Initially, the DEPArray system took around an hour to isolate one tumour cell, but improvements to the technology and software mean that it can now move multiple tumour cells simultaneously from the mixture to the collection vessel. "The current system can route 13 cells in about 4 hours," says Navin.

Navin is also working with a system made by Fluidigm in South San Francisco, California. This captures 96 cells in one run, says Ken Livak, a researcher at the company. However, unlike DEPArray, it does not image cells to help with visual sorting, so it is best for isolating previously sorted cells, he says.

Fluidigm's system features a device about the size of a postage stamp that contains tiny channels, valves and chambers. Minute amounts of fluid, along with cells, are driven through channels across the chip by opening and closing the valves. The channels contain a series of alcove-like capture sites. An unoccupied site will trap and hold an individual cell, but if the site is already occupied, the cells bypass it and move to the next one, until all 96 sites hold cells.

SHAPE-SHIFTERS

Chris Bakal, a cancer biologist at the Institute of Cancer Research in London, is hunting for patterns in the diversity of cancer-cell shapes. He and his team study metastatic melanoma cells, which are notorious for making drastic changes to their shape so that they can infiltrate far-flung reaches of the body.

The team's work centres on analysing images derived from spinning-disk confocal microscopy. In this technique, a laser illuminates the cells, and the microscope scans the light bouncing off them at many points simultaneously, gathered through pinholes in a spinning disk. The method is more sensitive than conventional confocal microscopy, which detects only one point of light at a time.

Bakal and his group have seen the diverse shapes of some cancer cells[1], and are now using statistical and computational analysis to try to identify which shapes are important.

"We think of these cells as extreme shape-shifters that can do anything," says Bakal. But generating and maintaining more diversity in cell shape than is needed may simply squander energy and drain the population of the cell shapes that are most useful, he says. Bakal and his team have found that, in fact, metastatic melanoma cells generally assume one of two shapes: rounded or spindle-shaped, each with its own advantages. "If you're a metastatic cell, you want those two shapes because a rounded shape migrates through soft tissues like the brain or the circulatory system," he says, "whereas the spindle shape is good for bone and hard tissues."

Bakal thinks that looking at various aspects of heterogeneity in single cells will prove useful. "You might see genetic heterogeneity in this experiment, and you might see shape heterogeneity in another experiment", he says, but notes that it may not always be clear whether the two observations are connected. To determine if there is a link, he plans to sequence the DNA from individual cells after imaging them to see if he can find mutations that correlate with one shape or the other.

ORDER OUT OF CHAOS

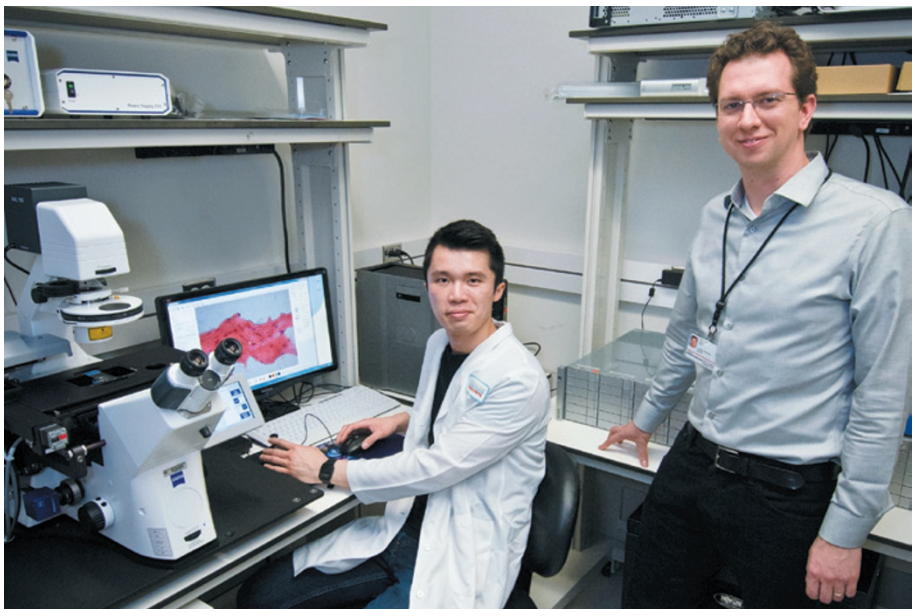
Despite their heterogeneity, tumours cannot be totally chaotic, says Garry Nolan, a cell-signalling researcher at Stanford University in California. He thinks that there must be organization somewhere within the diversity, so his approach to studying individual cells focuses on differences in the patterns of the myriad proteins that cells express. He believes that the complement of proteins alters as a normal cell becomes cancerous. As a result, the different protein complements seen in a sample of cancer cells could be related to the past history of those cells.

So far, Nolan's group has tracked more than 100 proteins simultaneously in individual cells, using a technique called mass cytometry. This is similar to flow cytometry, which separates cells according to fluorescently labelled proteins of interest. However, Nolan and his team wanted to look at many more proteins than is possible with flow cytometry, which is limited to the analysis of only a handful of proteins by the number of fluorescent tags that can be used. To solve this problem, the researchers developed mass cytometry so that they could identify tens or hundreds of proteins at the same time[2].

In mass cytometry, instead of proteins of interest being labelled with fluorescent markers, they are tagged with small metal particles that differ in mass. Once tagged, each cell is ionized and sent to a mass spectrometer, which separates the metal-tagged labels by mass. Unlike the fluorescent signals of flow cytometry, the mass measurements are relatively easy to distinguish from one another. Another benefit of this method is that it can measure proteins within the cell, because the cell is essentially vaporized during the process.

Nolan and his team are now developing their mass-cytometry technique to measure hundreds of proteins per cell, enabling them to piece together the puzzle of how cells become cancerous. The team has discovered a group of heterogeneous cancer cells that each have "their own little time-stamp signature on them", Nolan says. The varying complement of proteins on each cell indicates how far it has passed along the path to becoming cancerous, he adds.

By arranging the cancer cells according to these time-stamp proteins, the researchers



UNIV. TEXAS MD ANDERSON CANCER CENTER

Marco Leung and Nicholas Navin (right) study genetic differences in cancer cells to gauge tumour activity.

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created a timeline for a cell's physiology. Nolan believes that what seems to be a heterogeneous group of cancer cells is actually a snapshot of cells that represent different stages on a pathway leading to fully fledged cancer cells[3]. Viewed one by one, the mix may look wildly variable. But when viewed as a time-stamped group, "there is order there, waiting to be understood", Nolan says.

PERSONAL SPACE

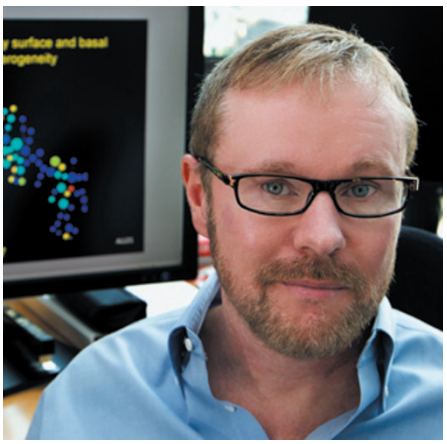
As well as presenting deviant shapes, cancer cells have a tendency to disregard the normal rules that other cells use for spacing themselves in three dimensions. "Basically they don't sit in these nice structures within the tissue like most cell types do," says Navin of breast-cancer cells. "They don't respect their neighbours."

The three-dimensional position of cancer cells within the tissue, as well as the areas immediately surrounding them, influence tumour formation and growth. Researchers hope that studying cellular heterogeneity in three dimensions — which more closely resembles real tissues — will deliver insights that could help to fight cancer.

Lucas Pelkmans, a researcher at the Institute of Molecular Life Sciences at the University of Zurich in Switzerland, studies how cells are affected by their surroundings. He and his team use automated, high-resolution imaging of millions of cells to monitor hundreds of parameters, including a cell's shape, distance from neighbouring cells and position within a tissue.

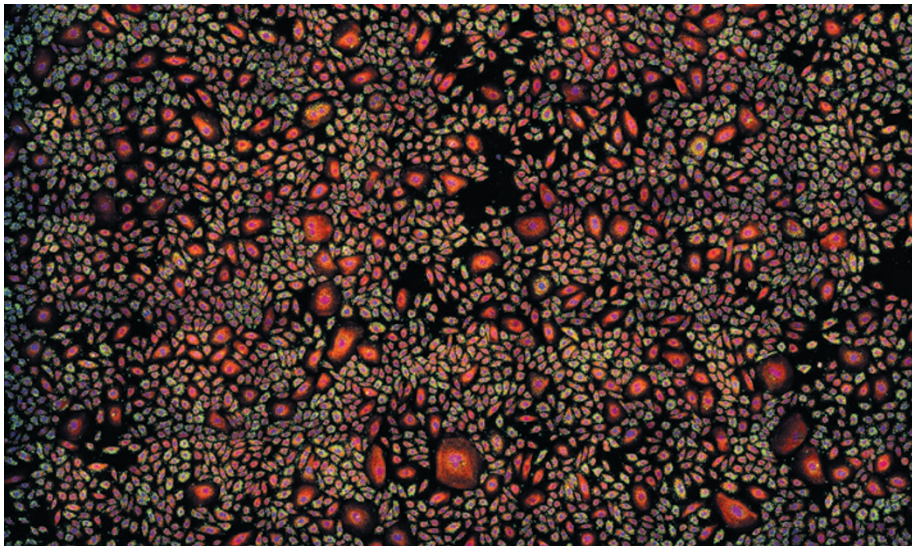
Pelkmans and his team then correlate these parameters with other measures of cellular activity, such as the molecular composition of cell membranes and the abundance of messenger RNA (mRNA) molecules, which are transcribed from DNA to serve as templates for the production of proteins in cells.

The team developed a technique that attaches fluorescent labels to single mRNA molecules of interest within individual cells, and then massively amplifies the fluorescent signal. "With that, you get a bright spot inside single cells," says Pelkmans. "By counting the number of spots, you basically get a read-out of the number of mRNAs in one cell." This can reveal whether a particular cell has different levels of gene expression compared to another; if it does, this might suggest that the two cells will go on to have different roles.



Garry Nolan uses mass cytometry to track proteins that reveal a timeline of cancer-cell physiology.

GARRY NOLAN/STANFORD UNIV.



Using high-throughput fluorescence imaging of cells, a team at the University of Zurich is studying messenger RNA levels to understand the importance of a cell's spatial positioning within tissues.

LUCAS PELKMANS/UNIV. OF ZURICH

The varying types of correlation between the measured parameters create a tell-tale cell signature. "These signatures clearly can be different for different genes, but there are strong signatures," says Pelkmans. The signatures indicate a kind of tumour geography and hint at the functional role of a cancer cell at a given position. Cells can grow together as a community, but those on the periphery can show different signatures to those in the interior. Interpreting these signatures can help researchers to understand how signals exchanged between cells influence tumour growth.

The movement of cells within tumours has piqued the interest of Kornelia Polyak at the Dana-Farber Cancer Institute and Harvard Medical School in Boston, Massachusetts. She is studying the spatial changes that occur during cancer treatments. Using measurements from real tumour cells obtained from cancer patients, she and her team have built a computer model that simulates tumour growth.

The model allows researchers to take virtual samples of the simulated tumour at different times and places, Polyak says. They can even subject the simulated tumour to a course of cancer treatment. Although it is not yet ready for clinical use, Polyak hopes that the model will ultimately act as a surrogate patient, allowing clinicians to try out different simulated therapies and assess predicted outcomes before they treat patients.

"We could actually use this for designing the best treatment strategy," she says. "But the treatment itself changes the tumour, so you have to know how the tumour changes." Models might help physicians to get one step ahead of the tumour, Polyak suggests, allowing them to anticipate the survival of a small population of drug-resistant cells and so quickly fight back against cancer recurrence[4].

Key to the issue of resistance is knowing how many tumour cells already have the genetic mutations that make them resistant to drugs, and to what extent chemotherapy itself induces such mutations. Studying individual cells may provide the answer. "The more genetically diverse a tumour is, the more likely it is to be resistant to certain therapies, so that's one potentially useful parameter that you could get for patients," says Navin.

Understanding the degree of heterogeneity within a tumour is important in assessing the severity of the cancer. Individuals with diverse tumours might be more likely to harbour metastatic cells or be more resistant to therapy, compared with patients whose tumours are more homogeneous, says Navin. "If we can measure the extent of heterogeneity of a tumour-cell population, then we may be able to use this index to predict which patients will have invasive or metastatic tumours, and which will respond to chemotherapy or show poor survival," he says.

Cell heterogeneity gives normal cells the power to react to the environment, but it also underlies the ability of some tumour cells to emerge unscathed from even the strongest chemotherapy. If researchers can uncover how cancer cells adapt to cancer treatments, cell heterogeneity might ultimately be turned to the patients' advantage. ■

Caitlin Smith *is a freelance writer in Portland, Oregon.*

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