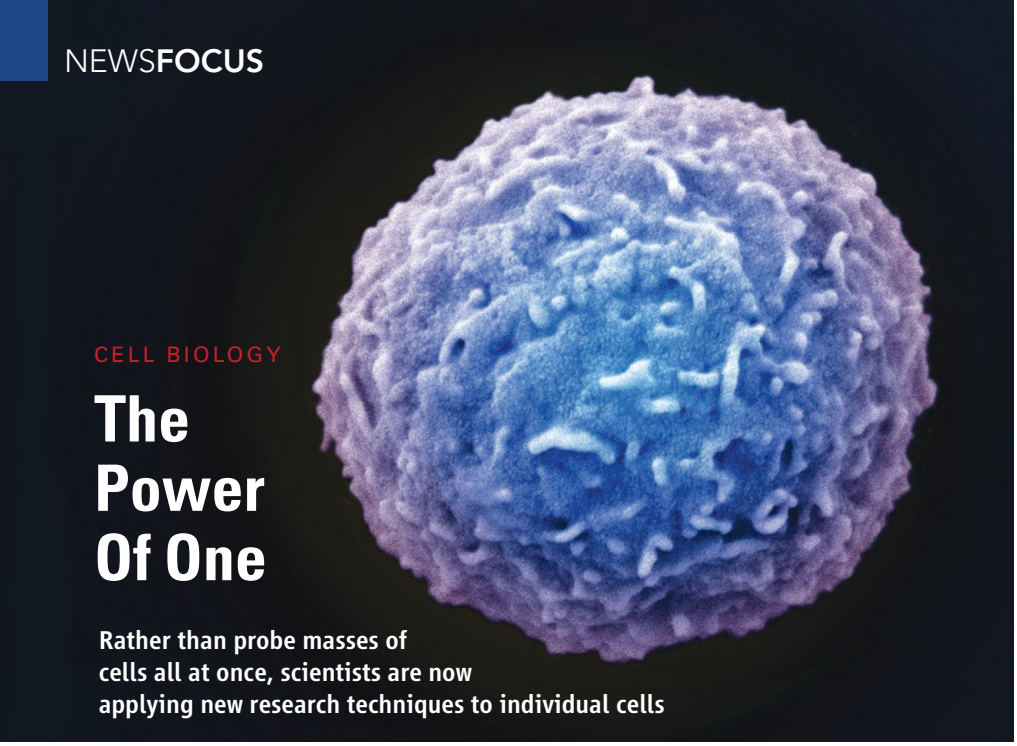

CELL BIOLOGY

The Power Of One

Rather than probe masses of cells all at once, scientists are now applying new research techniques to individual cells

Small sample size. Researchers are exploring new ways of investigating individual cells such as this human white blood cell.

1) Measure out a heaping portion of cells.
2) Grind until thoroughly mixed.
3) Analyze.

Cellular studies still pretty much stick to this traditional recipe, whether the goal is probing bacterial metabolism, following differentiation of stem cells, or tabulating gene activity in tumors. But mashing up a multitude of cells—one common method of studying gene expression typically requires more than 10,000—obliterates key differences between cells, researchers have come to realize. "If you take an average of a large number of cells, you get an average answer," says analytical chemist Renato Zenobi of the Swiss Federal Institute of Technology in Zurich.

That's why more and more scientists are opting for the alternative approach of taking the measure of individual cells. Although much of this work is in its early stages, "there is an increasingly diverse set of examples where single-cell studies have provided qualitative insights that couldn't be obtained from population-level studies," says biophysicist Michael Elowitz of the California Institute of Technology in Pasadena.

Scientists have already recorded the most accurate measurements of how much an individual cell weighs and gauged how much oxygen one requires. They've flagged specific cancer cells resistant to chemotherapy and developed ways to pinpoint rare, disease-causing bacteria among swarms of harmless microbes. Developmental biologists have tallied gene activity as a fertilized egg starts its course of division and specialization, work that might help clarify the factors that spur a cell in the embryo to become one tissue and its seemingly identical next-door neighbor to become something else. And Elowitz and other researchers have spelled out how individual cells not only cope with but actually benefit from "noise," random fluctuations in their internal and external conditions.

Of course, scientists have paid attention to single cells ever since the first microscopes were invented. What's changed is that researchers are now applying to individual cells the powerful techniques, including genome sequencing, mass spectrometry, and gene expression analysis, that formerly required batches of cells. "Real biological tissues are complex, and if you want to dissect that complexity and heterogeneity, you have to have tools to do it at the single-cell level," says biophysicist Stephen Quake of Stanford University in Palo Alto, California.

Good technique

Single-cell research tools range from old standbys to cutting-edge inventions (see sidebar for a sample of methods). Many of them allow researchers to get into what Quake calls "production mode," analyzing large numbers of individual cells in parallel or over a short period of time. The technology he calls "absolutely central" to the surge in single-cell research is microfluidics, which uses miniaturized networks of channels, valves, pumps, and chambers to control microscopic quantities of liquid. So-called lab-on-a-chip devices combine microfluidic circuits and can perform several analytical steps.

An example of how microfluidics can elucidate single-cell behavior comes from Quake, his Stanford colleague Markus Covert, and their colleagues. Last July in *Nature*, the

Single-Cell Tech Primer

Microfluidics is the hot technique in the single-cell field (see main text). However, it's just one of the methods that are enabling researchers to delve into individual cells.

• Gene Expression

Many modern gene-expression studies apply the mingled contents of thousands of cells to devices called microarrays that look like glass slides or microchips. But with microfluidic chips, researchers can make the same measurements on one cell. Take the gene-expression chips from California-based Fluidigm, a company co-founded by Stephen Quake of Stanford University in Palo Alto, California. The devices isolate samples from individual cells and mix them with the chemicals necessary for quantitative polymerase chain reaction, a technique that determines gene activity by measuring how much messenger RNA a gene makes. The company's most powerful version allows researchers to simultaneously gauge the activity of 96 genes in 96 individual cells, churning through a batch in about 4 hours.

Another new technology, known as RNA-seq, provides an alternative to microfluidic chips for measuring gene activity in one cell. An offshoot of next-generation genome sequencing, the procedure involves converting a cell's mRNA molecules back into short strands of DNA, sequencing those DNA fragments, and then matching them up with the gene that originally spawned the mRNAs. Last May in *Cell Stem Cell*, molecular geneticist M. Azim Surani of the University of Cambridge in the United Kingdom and colleagues reported one of the first studies to apply the RNA-seq technique to single cells, revealing the expression of 385 genes in individual embryonic stem cells. Fans of RNA-seq emphasize that it can measure more genes at once than can microfluidic chips. Meanwhile, chip devotees tout their method's superior speed.

• Flow Cytometry

A classic technique, flow cytometry sifts and counts cells based

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team reported using a microfluidic chip to dose individual cells with different concentrations of tumor necrosis factor α (TNF-α), a protein that can trigger inflammation and cell suicide. Previous work on cells en masse suggested that they respond to a range of TNF-α concentrations. But the researchers found that sensitivity to TNF-α varied from cell to cell—a result that might help clarify how the body fine-tunes TNF-α's effects.

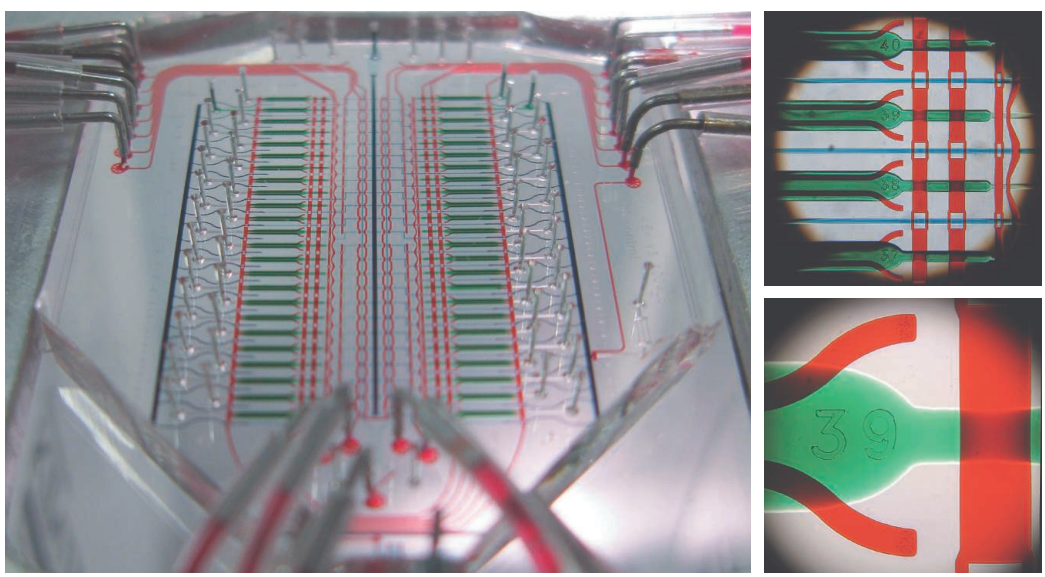
Single-cell techniques also allow researchers to ferret out rare troublemakers such as *Escherichia coli* bacteria of the O157:H7 strain. Just a handful of the malignant microbes in undercooked hamburger could be enough to cause fatal food poisoning, so detection methods need high sensitivity. Last year, biophysical chemist Richard Mathies of the University of California (UC), Berkeley, and colleagues revealed a strategy that can finger a single cell of the lethal strain from among as many as 100,000 normal *E. coli*. The key step in their method, which the researchers described in *Analytical Chemistry*, involves a microfluidic circuit that creates tiny, uniform oil droplets in which DNA sequences from normal and lethal bacteria are copied.

Predicting which patients will benefit from cancer treatment could be another use of single-cell methods. Immunologist Garry Nolan of Stanford and colleagues have profiled individual cancer cells with phosphospecific flow cytometry, a technique for determining whether proteins have undergone certain chemical modifications that can change cell behavior. For cancers such as acute myeloid leukemia and lymphoma, Nolan and colleagues showed that some abnormal cells contain patterns of protein modifications indicating resistance to chemotherapy. Based on these patterns, "you could pick out patients who would respond to chemo and [those] who wouldn't," he says.

Gone genome fishing

Microbial ecologists and microbiologists are looking to single-cell techniques for help with their culture problem. More than 99% of microbial species remain enigmatic because they refuse to grow under lab conditions and scientists can't obtain enough of the microbes' DNA to sequence their genomes. To fill in the gaps, researchers have turned to metagenomics (*Science*, 30 March 2007, p. 1781), trawling environments such as the open ocean and the human colon for any microbial DNA and then sequencing it to identify genes. Such an analysis can reveal the metabolic capabilities of an entire microbial community, and in a few cases researchers have been able to piece together an unculturable bacterium's genome from the jumble of DNA fragments. But it's usually impossible to pin down which genes belong to a specific microbe. That means researchers can't determine which species perform which ecological roles or reconstruct their evolution, notes microbial ecologist Ramunas Stepanauskas of the Bigelow Laboratory for Ocean Sciences in West Boothbay Harbor, Maine.

In 2001, however, genome biologist Roger Lasken, now at the J. Craig Venter Institute in Rockville, Maryland, and colleagues unveiled a method called multiple displacement amplification (MDA) that can expand the minute amount of DNA in a single bacterial cell by



Dynamic fluids. With microfluidic chips like this one, researchers can sort single cells, copy their DNA, measure their gene activity, and perform other kinds of analysis.

CREDITS: PAUL C. BLAINEY/STEPHEN R. QUAKE GROUP/STANFORD MICROFLUIDICS FOUNDRY

on characteristics such as the presence of certain proteins on their surface. Researchers use it today for single-cell work because it can handle large numbers of cells quickly. Garry Nolan of Stanford and colleagues have turned to phosphospecific flow cytometry, a version that detects whether some of a cell's signaling proteins have been tagged with phosphate groups, which are chemical switches that can turn the proteins on or off. Measuring the phosphorylation status of the proteins in a circuit that controls a particular cellular function provides an indication of a cell's status: how other cells have influenced it and how it might respond in the future.

Along with more accurate cancer prognoses, potential uses of phosphospecific flow cytometry include drug screening, Nolan says. The technique is even more powerful when combined with mass spectrometry, he notes. In phosphospecific flow cytometry, the small number of fluorescent tags limits researchers to measuring about 15 of a cell's phosphorylated proteins. But a new instrument developed by DVS Sciences in Canada uses different isotopes of elements such as lanthanum as labels and mass spectrometry to detect the tags, hiking the number of proteins researchers can monitor to approximately 100. "This is not your grandmother's flow cytometry," Nolan says.

• Mass Spectrometry

Mass spectrometry alone might also provide insights into individual cells. Renato Zenobi's lab at the Swiss Federal Institute of Technology in Zurich, for example, is one of several taking advantage of the method to tally the products of biochemical reactions one cell at a time. The cellular concentrations of metabolic compounds such as ATP, the molecule cells use to store energy, are well within the sensitivity of mass spectrometry, he says. The trick now, adds Zenobi, is figuring out what questions biologists can answer by performing mass spectrometry on an individual cell. "We need to show a really hot application of the data, and that will convince" more researchers that adopting it is a good idea.

–M.L.

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