

Cell by Cell: Moving Biology toward a More Predictive Future

FOR years, physicists and chemists at the Laboratory have reaped the benefits of the versatility and power of computer codes designed to study the physical and chemical processes underlying high-explosives detonations. Now, biologists are also looking to computer codes for understanding the intricate processes underlying cellular reactions.

A growing interest exists within Livermore's Biology and Biotechnology Research Program (BBRP), and the biology community, to pursue "quantitative biology." This emerging discipline, targeted at the understanding of biological processes, will integrate experimental data into predictive models that harness the same computational power used by physicists and chemists. Computational results can, in turn, be used to design and evaluate new experiments.

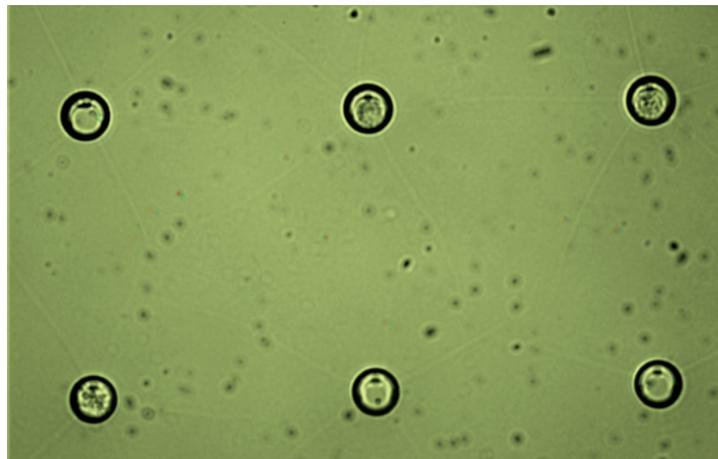
BBRP is taking on a new initiative to develop a system for gathering massive amounts of individual cellular data. These data will be used to help build computational models of cells, which, in turn, will help scientists achieve a quantitative understanding of the cellular life cycle. "Computational modeling offers many benefits that biologists hope to apply in guiding experiments and understanding the results of research efforts," says Livermore biologist Allen Christian. "The thrust of this effort is the gathering of quantitative cellular measurements for input into computer codes. The codes can then be used to model how cells react to various environments and to different stimuli or disturbances."

Piecing together cellular processes helps scientists gain an understanding of stepwise cellular pathways, enabling them to predict cellular reactions. This knowledge could lead to the discovery of new technologies ranging from drug therapies to protection against bioterror attacks.

Gathering the Data to Feed the Codes

Sophisticated computer modeling provides the framework to help design experiments. With computer modeling, hypotheses can be developed and testable predictions made. Currently, computer codes are being used at Livermore to study cell signaling through the calcium ion channels between epithelial cells. (See *S&TR*, January/February 2003, pp. 15–18.) Codes such as these represent a first step toward the more comprehensive goal of quantifying a library of myriad cellular processes.

One of the greatest challenges biologists face in developing biological computer models is the need for precise data on the



In the instrumented cell system, a microchip is designed to capture, sustain, and experiment on a single cell. Each circular capture well is approximately 10 micrometers across and contains a single human cancer cell.

concentration and distribution of cellular components, such as nuclei and mitochondria. However, gathering the massive data necessary to feed a useful code is much more difficult for biologists than for their physicist and chemist counterparts. For the most part, first principles can be used in physics and chemistry to supply consistent and reliable data. Biology is different. Although the cell—the basic unit of life—can be generally characterized, each individual cell reacts differently than its neighbor when picked and prodded with an experimental disturbance—be it a pathogen, enzyme, electrical current, optical interference, or some other disruption. And the information collected in conventional experiments that aggregate in one sample the data from many cells simply isn't sufficient for computational modeling. To be useful in a computer code, data must reflect the diverse conditions found in individual cells.

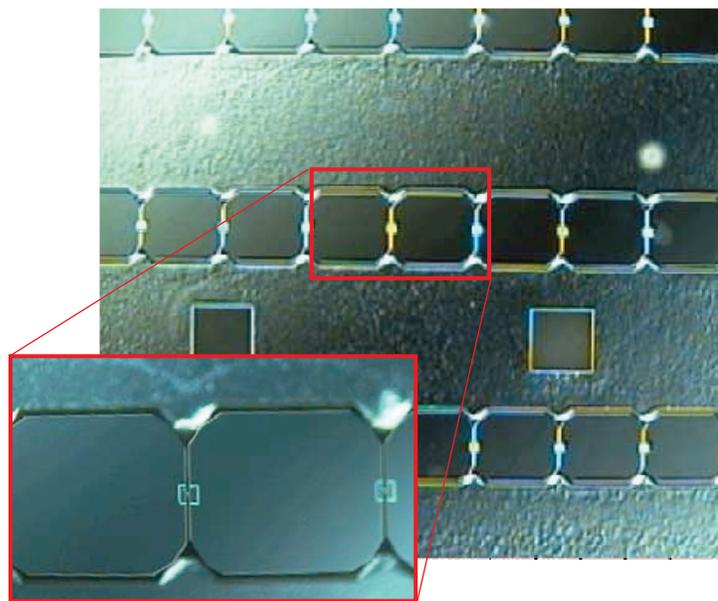
Mike Colvin, a computational biologist whose work has advanced the idea of a system capable of taking individual cell measurements, explains that a number of phenomena prevent the accurate representation of cellular processes when using pooled or aggregate data. "We lose information about what individual cells are doing," says Colvin. For example, conventional experimental techniques, such as pooling dissolved cells, destroy data about a

cell's location. Colvin also points out that many biologic processes create asymmetries at the cellular level. While these variable properties may not be accurately reflected in pooled data, single-cell measurements can provide averages and reflect variance in cellular processes. Thus, quantitative data from individual cells are essential to create accurate and reliable computational models that can lead researchers to a predictive understanding of cellular pathways.

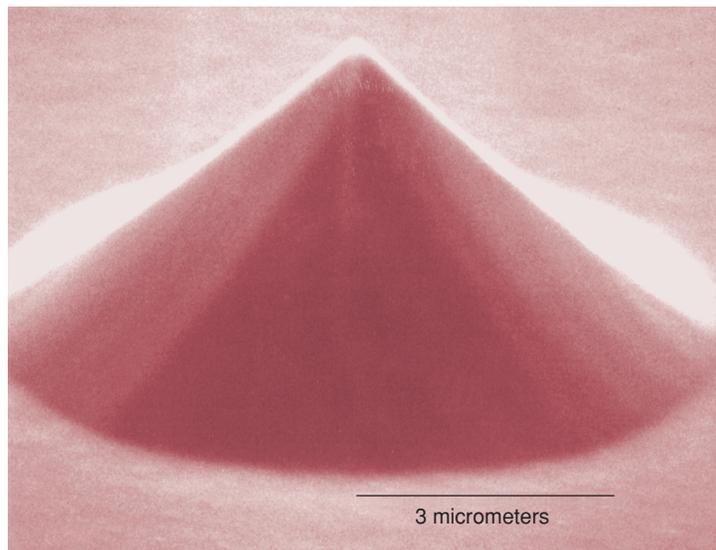
New System Speeds Accurate Cellular Measurement

For the Department of Energy's Genomics:GTL (formerly Genomes to Life) Program, the development of an "instrumented cell" system is expected to be the enabling technology that kick-starts the data gathering. With funding from the Laboratory Directed Research and Development (LDRD) Program, Christian's group is creating an instrumented cell system that allows high-throughput measurement of individual cells. Studies of specific cellular responses require the ability to index cells in a way that preserves their identity during an experiment. Christian's group envisioned the configuration of an array of individual cells, each fixed in a specific location. This idea led to the development of a microchip that Christian and his team have fabricated to capture, sustain, and experiment on individual cells.

The microchip consists of wells that are just the right size to hold only one cell. Because cells vary considerably in size, the



This image shows a microchip with arrays of capture wells that are small enough to trap individual bacteria. The inset is a magnified view of a single well, which is located in the vertical space between two adjacent rectangular bases. In the space is a narrow gap where a bacterium is trapped as the flow is drawn through.



A Livermore-designed glass microneedle will be manufactured in arrays and used to inject and sample large numbers of captured cells at once. This manipulation technology, combined with the technology for capturing and maintaining cells, will ensure the high throughput necessary to produce large amounts of data quickly.

microchip will eventually be made with interchangeable wells. Single cells are captured in the wells by first flowing a liquid containing the cells onto the chip. A syringe pump then pulls the liquid through an opening in the bottoms of the wells, which are small enough to trap the cells inside.

Keeping the cells alive after capture is one of the project's greatest challenges—and what makes this capability unique in the world. The liquid environment in which the cells are maintained amounts to an artificial blood stream that must deliver nutrients and maintain the proper level of carbon dioxide and a temperature of 37°C, among other variables. The slightest deviation in any of these variables from what the cell is accustomed to can cause the cell to behave differently—which would defeat the purpose of studying the response of cells under normal conditions.

The team is also fabricating glass microneedles for injecting substances into the captured cells and sampling their interior. The needle will be fabricated in arrays corresponding to the position of each cell-capture well in order to inject multiple cells at once. The ability to simultaneously capture and manipulate large numbers of cells will ensure the high throughput necessary to produce large amounts of data quickly. The team is also writing software to automate the entire process, further enhancing the accuracy of analysis and reducing costs.

The instrumented cell system can be fitted with standard measurement and imaging instruments, such as infrared

spectroscopes, fluorescence microscopes, and bright-field microscopes. The system is currently capable of arraying large numbers of human cells, but plans are in place to expand its use to bacterial cells.

A Systems Approach to Biology

“The whole field of biology is moving toward systems biology, whether that system be one cell or an entire organism or environment,” says Colvin. With the completion of the Human Genome Project, scientists have a rich understanding of the genome and many of the proteins constructed by genes. Much is known about biochemical pathways and intracellular transport. The workings and functions of organs and organelles are well understood. However, despite the enormous amount of knowledge gleaned about myriad pathways, biochemicals, enzymatic reactions, cellular receptors, ion channels, protein–protein interactions, and so forth, little is known about how all these units work together as a system.

By breaking down living organisms into manageable units, researchers have been able to study individual parts—indeed parts of parts. This effort has led to the mapping of many cellular pathways, but few quantitative data have come from it. New techniques such as computational modeling are now helping biologists to develop a systems approach to understanding and predicting cellular processes. The challenge is to gather the

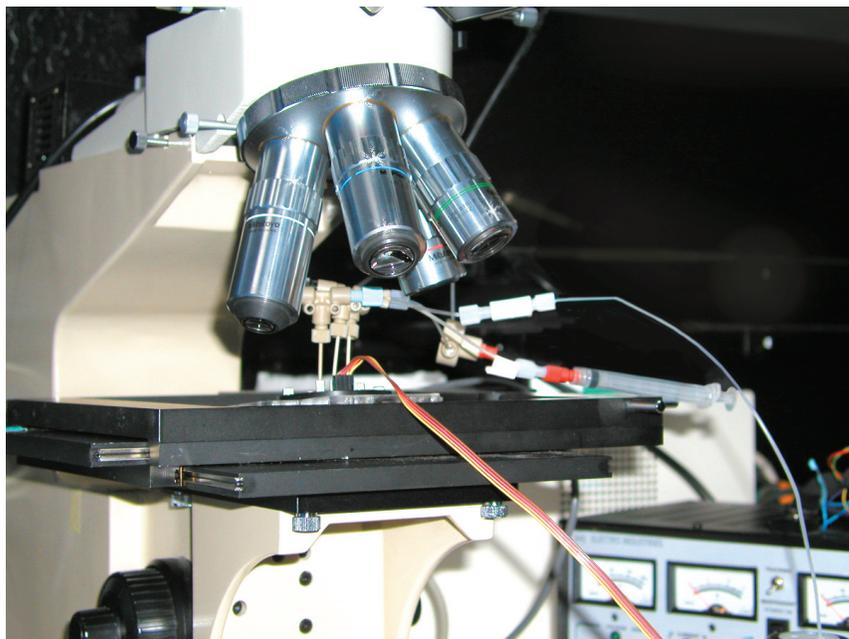
massive amounts of data necessary to feed computational codes that are so extraordinarily complex, they simulate life.

Integrating the System with Gene-Silencing Technology

Christian plans to combine the new instrumented cell system with his group’s award-winning siHybrid gene-silencing technique, which thwarts gene expression by inserting short interfering RNA–DNA hybrid molecules into cells. (See *S&TR*, October 2004, pp. 10–11.) The recently developed siHybrids, funded by LDRD, enable the robust study of how genes create proteins and how those proteins function. The siHybrids will help generate some of the key data to be quantified using the instrumented cell system.

The instrumented cell effort is expected to spawn breakthroughs in many Laboratory- and DOE-relevant biological applications, including improved explication of the DNA-repair system, the elucidation of host–pathogen interactions, and the understanding of microbial communities. Colvin points to the Laboratory’s analytic and computational capabilities as strengths that can establish Livermore as a leader in this new era of biology. Core technologies that have been developed for other applications, such as microfabrication, advanced imaging, and mass spectrometry, will help the Laboratory play a leading role in moving biology from what many consider a descriptive science to a quantitative and, ultimately, predictive science.

—Maurina S. Sherman



The instrumented cell system can interface with standard imaging and measurement instruments, such as microscopes and spectroscopes.

Key Words: Biology and Biotechnology Research Program (BBRP), cellular processes, computational biology, Genomes to Life, genomics, GTL:Genomics, Human Genome Project, instrumented cell, Laboratory Directed Research and Development (LDRD), microfluidics, quantitative biology, systems biology.

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