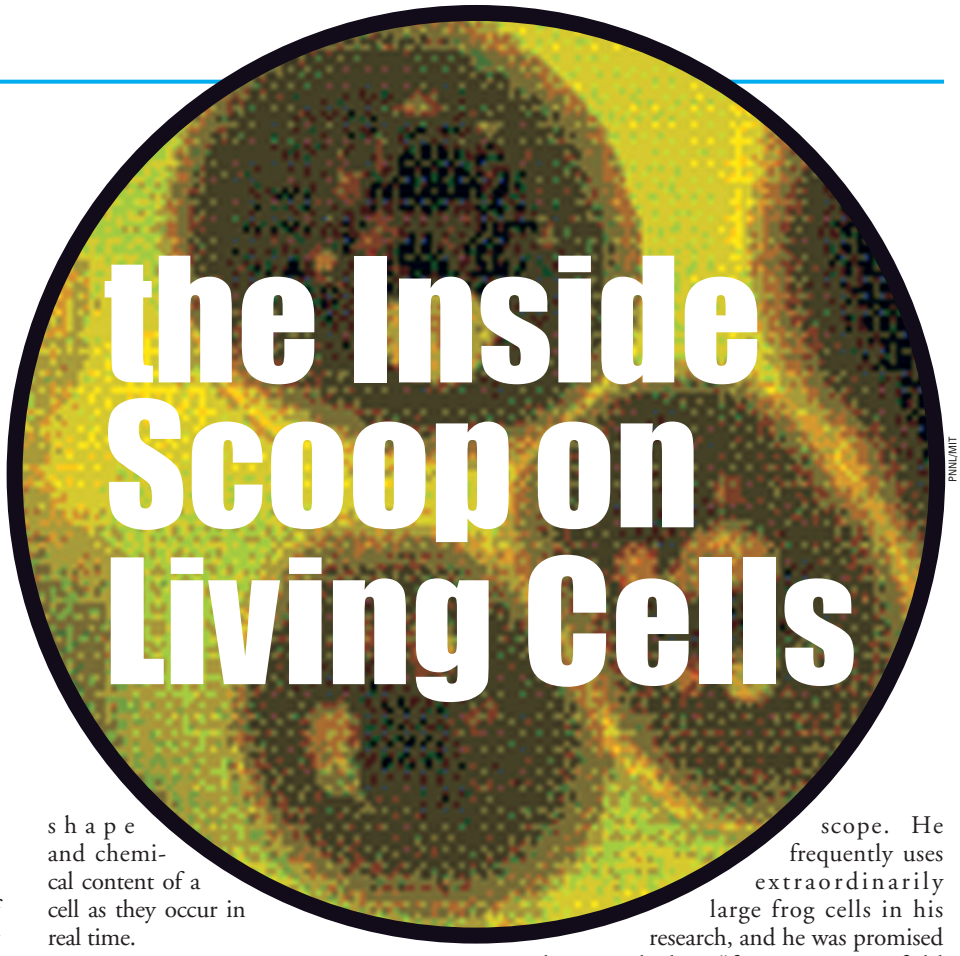
New Microscope Gives Scientists the Inside Scoop on Living Cells



PNNL/MIT

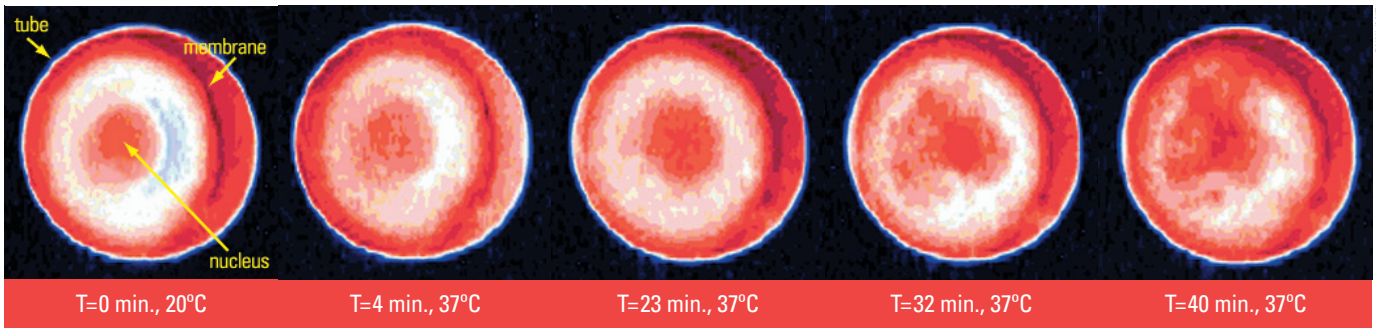
Scientists at Pacific Northwest National Laboratory (PNNL) in Richland, Washington, have teamed together to develop a unique imaging system capable of focusing on a single living cell. This new technology will be used in what the multidisciplinary team has termed a "cellular observatory" to study the effects of environmental insults on live cells. The new technology combines nuclear magnetic resonance (NMR) technology with optical microscopy to deliver information about a cell's physical and chemical composition without invading or destroying the cell. This high-tech hybrid system can zero in on the very nucleus of a living cell, offering scientists fresh insights into the way cells work. And because the cells remain alive during the imaging and analysis procedures, researchers can track changes in both the shape and chemical content of a cell as they occur in real time.

Zoom with a view. Water-selective NMR microscopic imaging and spectroscopy are combined to produce images of a *Xenopus laevis* oocyte reacting to stress as it is heated from 20°C to 37°C. The images show irregularities in water distribution in the cell as the nucleus empties water into the cytoplasm and is later partially emitted from the cell.

A Team Approach

A physicist by training, Robert Wind's professional life thus far has been focused on magnetic resonance and spectroscopy. He came to the PNNL six years ago, drawn to the lab's extensive store of magnetic resonance equipment and yearning for an opportunity to apply existing NMR technology in a novel fashion. Wind eventually persuaded cell biologist Eric Ackerman to join the PNNL's growing multidisciplinary team. Ackerman, who had studied the molecular biology of DNA repair and replication at the National Institutes of Health in Bethesda, Maryland, saw promise in the PNNL's unique capabilities and resources. "One of the attractions is that there are instruments here that are unique in the world," he says. Ackerman was swayed, in part, by the prospect of gathering research data quickly with the integrated microscope. He frequently uses extraordinarily large frog cells in his research, and he was promised by Wind that "for every two-fold increase in cell size, we could increase data collection by a factor of four."

Traditional analytical methods require the destruction or significant modification of live cells to examine chemical changes within them. Thus, valuable information is lost, resulting in an inaccurate description of the cell. In many techniques, scientists start by killing the sample cells and staining them with dye, which Ackerman says "automatically perturbs [the cells'] response." In his opinion, the PNNL's new noninvasive imaging system is significant because "it leads to new kinds of biological data" and thus will open up new roads for biologists to explore. "With this technique, we can observe changes in metabolites throughout a single cell, [as opposed to] an average change over many cells," Ackerman explains. "And for the first time, we will be able to do this at the



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same time with two of the most powerful microscopy technologies available."

Technology Times Two

Here's how the two pieces of equipment work together. The confocal microscope obtains a three-dimensional image of the cell in just a few minutes. This optical image is used as a guide to help researchers zoom in on a particular section of interest within the cell, which they then analyze using NMR. The magnetic resonance imaging takes longer—eight hours to complete a three-dimensional image of an oocyte with a 10-micrometer resolution (or only eight minutes for a two-dimensional image). However, with the integrated microscope, these time-consuming NMR images are no longer necessary. The magnetic resonance component of the microscope "helps us find the compartments of a cell and get chemical information," Wind explains. "The greater the resolution, the more information we can get," Ackerman adds. In future work, the researchers will focus more closely on individual organelles, or parts of the cell. "If particular organelles—or organelles located in a specific part of the cell—respond differently to environmental stress, this would provide important insights into stress responses," he says. "For example, if the mitochondria near the membranes behaved differently from the mitochondria near the nucleus, this might illustrate a new kind of intracellular communication."

The cell the PNNL team is using now is a frog cell, a *Xenopus* oocyte, that is 100,000 times bigger than a somatic cell and contains nearly the largest of all nuclei known to biology (the oocyte's nucleus is itself larger than most entire somatic cells). Using the integrated optical magnetic resonance microscope, the researchers will be able to look at changes that occur inside the nucleus and in other cellular compartments. "We have been able to obtain chemical information from a living cell without destroying the cell or invading it in the process," Wind says. "And, for the first time, we have been able to do this on the cell nucleus, the internal control center of the cell." Wind says the team is combining two techniques to give new insights into the study of cellular changes. He estimates that 10 or so labs worldwide possess equipment and capabilities similar to that of the PNNL team, but admits that "our image quality may be better."

Twice the Potential

Wind and Ackerman expect a final version of both components of the combined imaging system to be complete within the next two to three months. While awaiting completion of construction of the integrated microscope, the group began studies of heat shock on the frog oocyte and recently obtained initial results. Says Wind, "Expression of heat shock is a well-established cellular response to multiple kinds of stress. The heat-shock proteins are pretty well conserved from [the fly species] *Drosophila* to humans, and one of the roles of heat shock–like proteins is to aid in protein folding. Any increased understanding or new insights [into] how frog cells respond to stress is likely to also be relevant to human cells."

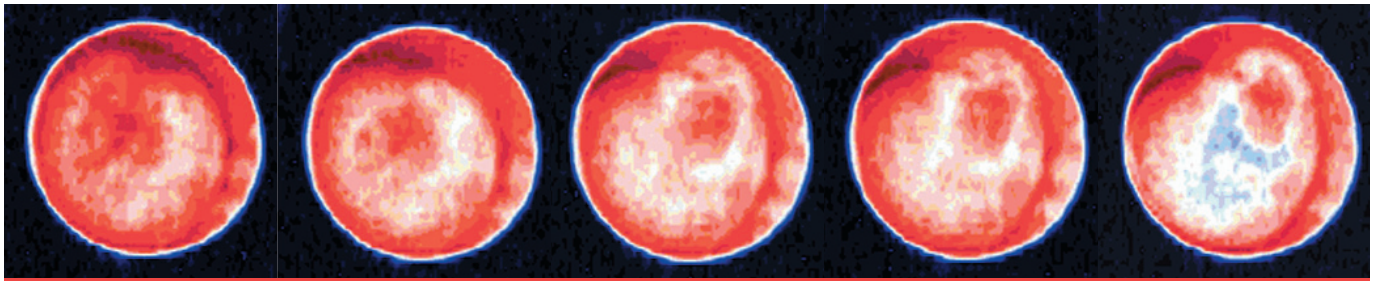
During these experiments, the cell released six or more proteins in response to heat stress. Within a temperature range of 20–37°C, the group observed profound changes in the water distribution inside the cell cytoplasm and around the cell nucleus. In similar experiments the group also observed differential effects on the intensity and location of several NMR resonances following the oocyte's exposure to elevated temperatures. Ackerman and Wind believe these effects stem from the stress response, not simply the temperature increase, "because different resonances in different-sized oocytes change differently," Wind explains. Ackerman says these results represent an important step in improving understanding of the links between the molecular and cellular events in response to environmental stress.

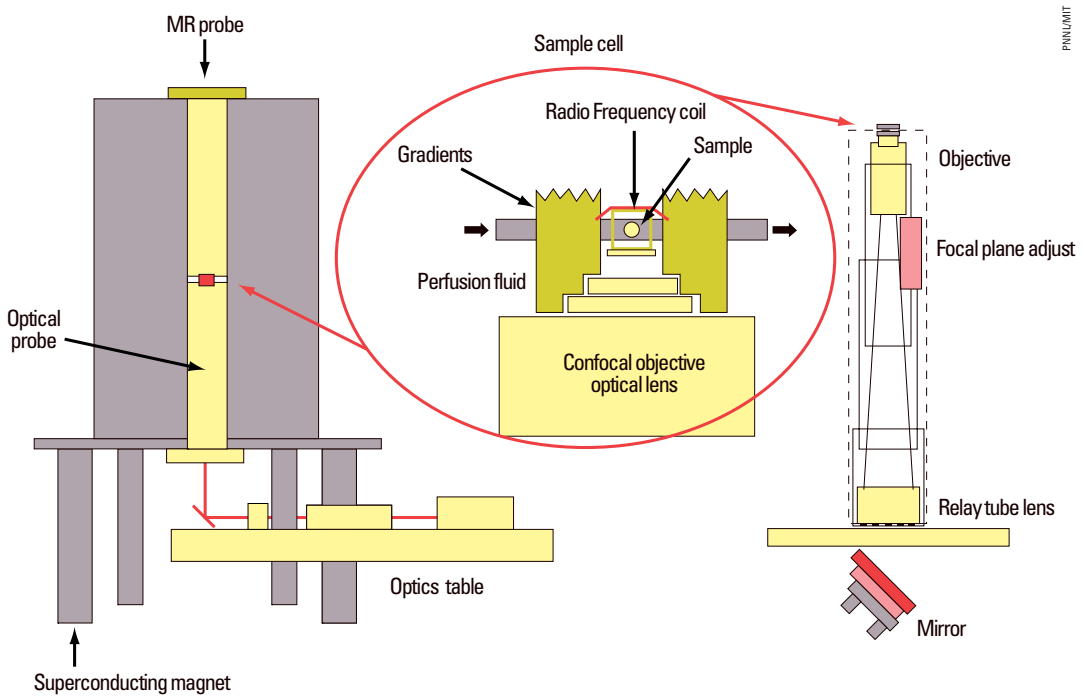
Maurice Montal, a professor of biology at the University of California at San Diego, says that the new capability offered by the PNNL imaging system is of paramount importance to environmental laboratories interested in studying environmental toxicants. Montal, whose specialty is studying nerve cells, has toured the PNNL facilities and seen the new equipment firsthand. "I don't know of another like it in the world," he says of the integrated optical magnetic resonance microscope. "It was particularly interesting to see that you could identify the state of water within a cell," he says. "You could determine the movement of lipid molecules in the membrane of the egg and measure the molecular motion in two dimensions. That's quite impressive."

Montal was also interested by the ability to detect the activity of single molecules. Montal, who has been invited to collaborate with the PNNL team on future research, says that eventually "this combination of two techniques would allow us to measure simultaneously the dynamics of proteins undergoing conformational change in the membranes of cells. That would allow new capabilities to understand basic processes in the cell"—a prospect Montal says he finds very exciting.

Because the PNNL imaging system is extremely sensitive, it is well-suited to looking at the effects of drugs or toxicants on cells, which are extremely sensitive to a host of environmental threats including organic solvents, lead, free radicals, gases, and alcohol. The PNNL team hopes to use the imaging system to learn more about these effects. "The connection between environmental exposure and human health problems has long been known, but we know precious little about what really happens when a chemical substance enters the body," says Gerald Stokes, associate laboratory director of the Environmental and Health Sciences Division at the PNNL. "This event puts us on the path toward our goal of being able to study living cells."

The PNNL team hopes that studying live cells in real time will eventually help scientists learn how and why some cells fight off diseases and others don't, how to predict and prevent diseases, and how to





The first generation. In the first combined optical/NMR microscope, a cell sample holder containing a cell perfusion system, a radio frequency coil (for NMR experiments), and a magnetic gradient system (for MRI experiments) is situated at the interface of an optical and NMR probe. The combined microscope will be used to analyze 3-D systems such as large single cells, cell clusters, and small tissue samples.

follow disease treatment. Also, they say, it will be possible to study the effects on cells exposed to multiple contaminants at the same time and, ultimately, to relate these cellular responses to the effects of environmental exposures on human health. This capability would vastly improve upon traditional animal studies, which typically expose mice or rats to chemicals in concentrations far higher than humans would usually encounter in the environment. In such studies, researchers are unable to screen for multiple chemical effects at the same time.

Ackerman believes the PNNL cellular imaging system could be used to study the combined effects of pesticides and other toxic chemicals, which would aid in making regulatory decisions as well. "This technique gives new and better information," he says. "That could lead to better regulations—help us find out if some regulations are too stringent, for example, or not stringent enough."

Yet another potential application is cancer research. The PNNL cellular observatory could be used to study cell apoptosis (programmed cell death that affects the formation of cancerous tumors) and perhaps lead to new diagnostic tools or treatments that suppress tumor formation. A better grasp of what's happening on the cellular level might put researchers a step closer to the development of agents to interfere with the process of cell death. The National Cancer Institute has already funded research—slated to begin within the next couple of months—in which PNNL researcher Brian Thrall will join the team to look at tumor formation in mammalian cells.

The Cost of New Capabilities

The chief drawback of the integrated optical magnetic resonance microscope is its cost of $300,000–400,000. That expense may limit access to the technology, because only a few labs in the United States have the financial resources necessary to buy, run, and maintain such equipment. "I don't think that it could be replicated in, say, a university laboratory," says Montal.

Montal adds that the complicated combination of computers, lasers, magnets, and sophisticated imaging equipment "requires a collection of specialized human expertise all converging together. That's why a national laboratory is ideal for this work." And unless the technology could somehow be commercialized, he doesn't consider it practical to expect such an imaging system to be replicated elsewhere anytime within the next three to five years.

While their work thus far shows extraordinary promise, Wind and Ackerman are both quick to point out that the new imaging and analysis capabilities aren't a quick fix that will lead to easy answers to questions of environmental health. Instead, Ackerman sees the work as a significant starting point from which future research will stem. "One of the reasonable hopes is that this will illuminate whole new areas of research to be looked at using other methods," he says. With a statement that hints at promising research still to come, Wind agrees: "These are initial steps on a very long path."

Jennifer Medlin

Suggested Reading

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