

With a new technique called intravital imaging, pioneered at Albert Einstein College of Medicine, scientists can for the first time observe the behavior of individual cells deep within living animals.

These unprecedented views are yielding novel insights into how cancer cells spread throughout the body—and how they can be stopped.

# FANTASTIC VOYAGES

BY GARY GOLDENBERG

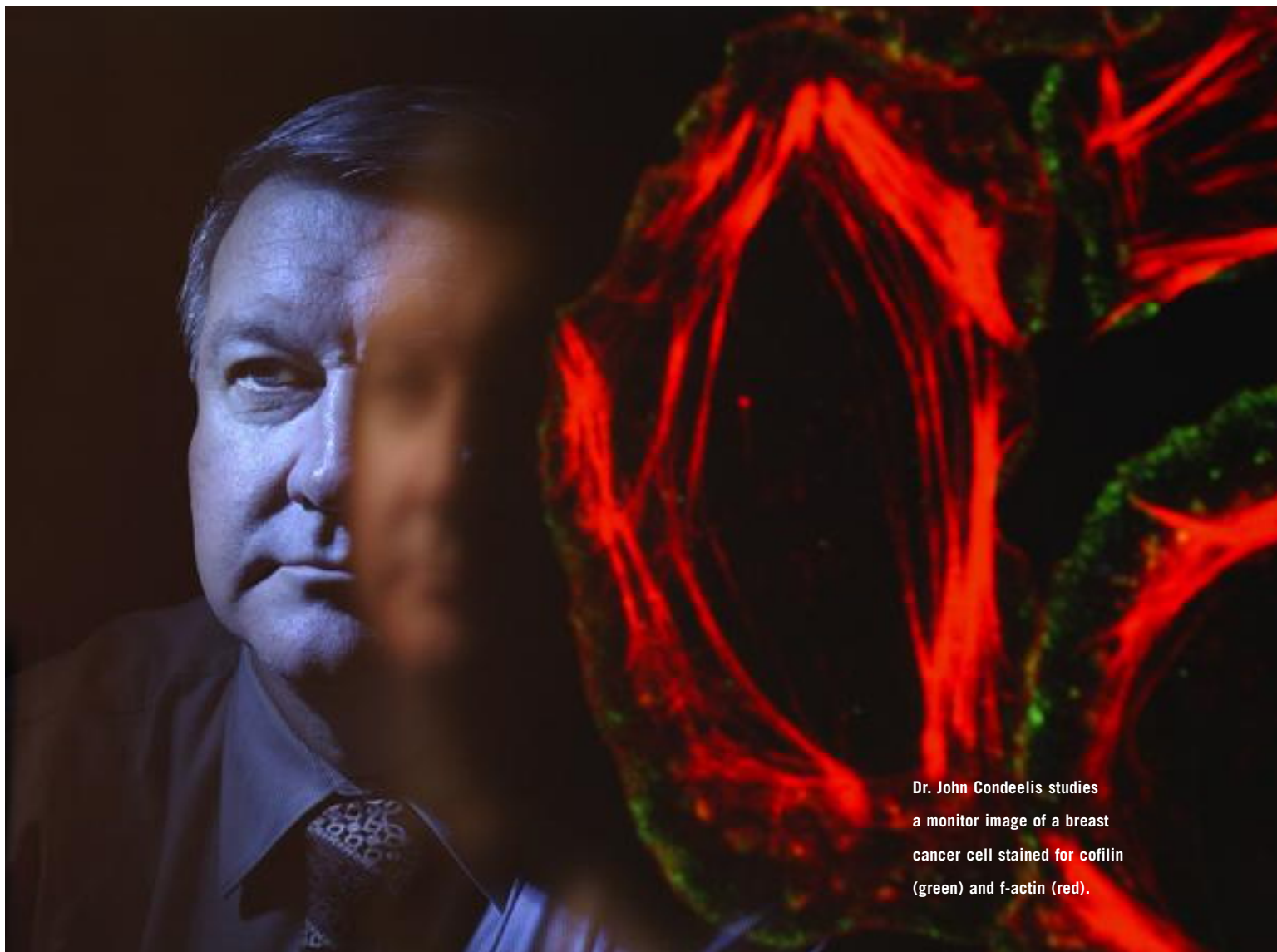
*“We’re going to see things no one ever saw before. The actual physical process of life itself—not something under a microscope. Just think of it.”*

**S**ci-fi fans may recall these words of Cora Peterson, a medical assistant in the kitschy cinematic classic “Fantastic Voyage.” It’s 1966, the height of the Cold War. A leading scientist defects to the West and is shot before he can divulge his secrets. The only way to save his life is to inject a miniaturized submarine into his bloodstream to seek out and destroy a clot lodged deep within his brain. The surgery takes place at a top-secret military hospital, where the submarine *Proteus* and its crew are reduced to the size of a microbe and injected into the scientist’s carotid artery. It’s not

long before they are attacked by voracious antibodies and tentacled macrophages, but nothing diminishes their awe of the complexity and beauty of life at the cellular level.

John Condeelis, PhD, and Jeff Segall, PhD, professors of anatomy and structural biology at Albert Einstein College of Medicine, know the feeling well. For several years, they have been voyaging among the cells of live animals, not in a tiny sub, but with a tool called “intravital imaging” (IVI). An amalgam of advanced light microscopy, genetic engineering, and computer processing, IVI is fundamentally changing the way scientists view “inner space.”

Using this technique, Drs. Condeelis and Segall and their colleagues have achieved the first high-resolution images of individual tumor cells in a living animal, unearthing invaluable clues as to how these cells metastasize, the leading cause of death in cancer patients. The scientists are now searching for substances that might be used to inhibit the wanderlust of cancer cells.



Dr. John Condeelis studies a monitor image of a breast cancer cell stained for cofilin (green) and f-actin (red).

## The voyage begins

Their fantastic voyages began about a decade ago when they were studying slime molds in order to decipher the molecular mechanisms that govern cell movement. Using genetic engineering, they would knock out or overexpress individual genes in these single-celled protozoans and then examine the consequences under the microscope. The setup was far from ideal because changes in cell motility and behavior are difficult to observe with conventional microscopy. Furthermore, the cells were being observed in an artificial environment. “By watching cells in vitro you get a skewed, if not artificial, view of how cells move in a body,” Dr. Condeelis explains.

With colleagues at Einstein, Dr. Condeelis began searching for new ways to study cell movement. Electron microscopy (EM) was no help; EM yields highly detailed images, but only of inanimate specimens. It’s just the opposite with CT and MR scanning, which can produce images of living things, though not at the cellular and molecular levels.

Instead, the solution would come from a convergence of technologies, including genetic engineering, fluorescence microscopy, and computers.

Genetic engineering played a major role, as it does in most of the current biological research, allowing the team to render cancer cells highly visible. The key ingredient was a jellyfish protein called GFP (green fluorescent protein). In 1994, scientists at Columbia University discovered how to attach the gene for GFP to any gene in a foreign organism. When the target gene is expressed, the protein it produces shines like a Day-Glo stick at a rock concert, providing a highly visible and specific marker of gene activity in individual cells.

In search of suitable animal models, Drs. Condeelis and Segall turned once again to genetic engineering. “You can take cell lines derived from a human cancer, inject them into animals and cause a tumor, but the tumor doesn’t look anything like what you would see clinically,” says Dr. Condeelis,

## RESEARCH AT EINSTEIN

who is scientific director of Einstein's Analytical Imaging Facility, which offers advanced light and electron microscope imaging services to the entire medical center. "We solved this problem by implanting human oncogenes, like HER2neu, the gene for breast cancer, into mice. Then we crossed those mice with a strain of mice whose cells express GFP and they become cancerous."

Next, the team needed a way to visualize GFP, which is where fluorescence microscopy came into play. But the standard technique would not suffice. "To excite molecules deep inside a living animal, you have to push an intense amount of laser light through tissue," says Dr. Condeelis. "This causes tremendous phototoxic damage." The solution was found

tion, you can see these cells crawl along on these collagen fibers," he says. "This had never been seen before. You could not have anticipated this in culture."

He clicks the computer mouse again, launching another movie of tumor cells mingling with macrophages (immune system scavenger cells), which cluster along blood vessels that feed the tumor. It turns out that these clusters are where tumor cells enter the blood vessels, which they use as expressways to distant parts of the body, in a process called intravasation.

Dr. Condeelis' studies also show that only tumor cells and macrophages are able to enter the tumor's blood vessels, and they do so in tandem. Clearly, the two cell types are commu-

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in a quantum mechanical effect called multiphoton excitation, which holds that a single full-strength laser beam can be replaced with two synchronized half-strength laser beams. Each half-strength beam is too weak to cause phototoxicity, yet together they are strong enough to excite the target molecules.

Finally, computers were employed to control the microscope and take digital snapshots of slices of the target tissue, which were then combined to make short movies. The latest innovation, unique to Einstein, was to take multiple image slices at each position, allowing the researchers to follow the cells in three dimensions.

### Seeing is believing

For the generation brought up on the eye-catching graphics of GameBoy or PlayStation, IVI's relatively murky images may be a disappointment. But for biomedical researchers, the technique is nothing less than revolutionary.

"IVI has led to astounding new insights that we couldn't have imagined by looking at these cells in culture," beams Dr. Condeelis, sounding much like the mesmerized scientists aboard the *Proteus*. To demonstrate, he clicks on an icon on his computer monitor, launching a brief movie of a tumor cell as it inches toward a blood vessel, thrusting out one pseudopod (foot-like projection) after another. "At high magnifica-

nicating, with tumor cells getting the upper hand. But how? Clues would come from a laboratory technique called "gene expression analysis," which can detect the presence and expression levels of thousands of genes at a time. From this data, researchers were able to explain the signaling pathways that govern tumor cell-macrophage interaction.

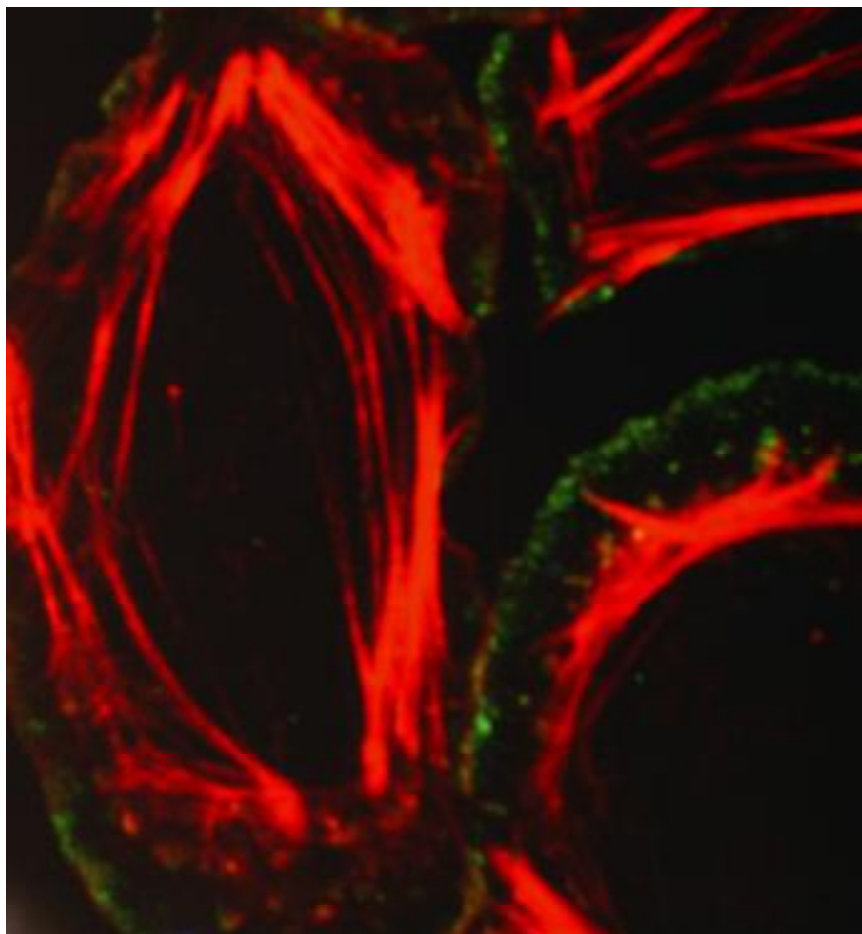
"A lot of papers in the past 10 years said that intravasation didn't play any role in metastasis. People started to look elsewhere for potential therapeutic interventions. Our work has completely turned this around," reports the researcher.

One possible target for intervention is the pseudopod, the cell's steering wheel, which is controlled by five different biochemical pathways. "We've learned that in the most invasive tumors cells, the genes that control pseudopods are overexpressed. As a result, these cells are hyper-pumped, like little Arnold Schwarzeneggers. With IVI, you can see them move more than 10 times faster than the cells from which they were derived just a few generations ago. It's an amazing transformation," says Dr. Condeelis.

### What are they thinking?

The next goal is to conduct gene expression analysis directly inside a living cell, which would allow the researchers to watch the cell "think."

Gene expression analysis is currently done by extracting



and processing genetic material (RNA) from cells, which is then placed on DNA microarrays (glass chips on which thousands of complementary DNA samples have been deposited in a defined configuration by high-speed robotic printing). By analyzing the resulting pattern of RNA-DNA binding, it is possible to discern exactly which genes are expressed in the sample cells.

It's a powerful technique, though it shows only averages of gene activity across a group of cells. Dr. Condeelis would like to see what is transpiring genetically in a single living cell as it moves and communicates. To do this, Dr. Condeelis and Robert Singer, PhD, inventor of gene expression analysis technique for living cells, are working to tag individual genes with differently colored oligonucleotides (small strands of DNA), which will fluoresce when the genes are expressed. Eleven genes have been tagged thus far.

## A group effort

As Dr. Condeelis is quick to note, IVI has been a group effort from the start, involving specialists in imaging, optics, biophysics, computers, DNA microarrays and transgenic animals, as well as structural, molecular and developmental biology, organic chemistry, and molecular pharmacology. Key Einstein contributors include: Jeffrey Pollard, PhD, Betty and Sheldon Feinberg Senior Faculty Scholar who is professor of developmental and molecular biology and of obstetrics and gynecology and women's health, and director of the transgenic animal facility; Dr. Segall, the researcher behind the animal models; Dr. Singer, professor of anatomy and structural biology and of cell biology; and Richard Stanley, PhD, the Reneé and Robert A. Belfer Professor and Chairman of Developmental Biology, the discoverer of a critical

motility-related receptor on macrophages (CSF-1). Several years ago, the researchers joined to create the Signaling, Tumor Cell Motility and Invasion Group within the Albert Einstein Comprehensive Cancer Center, with major funding from the National Cancer Institute.

IVI is not limited to cancer research. Other researchers at Einstein are using IVI to study the function of kidney cells and the growth of new blood vessels (angiogenesis) in heart muscle. Says Dr. Condeelis, "With micro-lenses and light pipes—technologies now under development at a number of places—it will eventually be possible to observe cells deep inside the brain or inside the chambers of the heart."

At one point during the voyage of the *Proteus*, crewmember Cora Peterson exclaimed, "I never ... never imagined it could be anything ... like this." Neither did Dr. Condeelis and his crew of researchers. ■

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