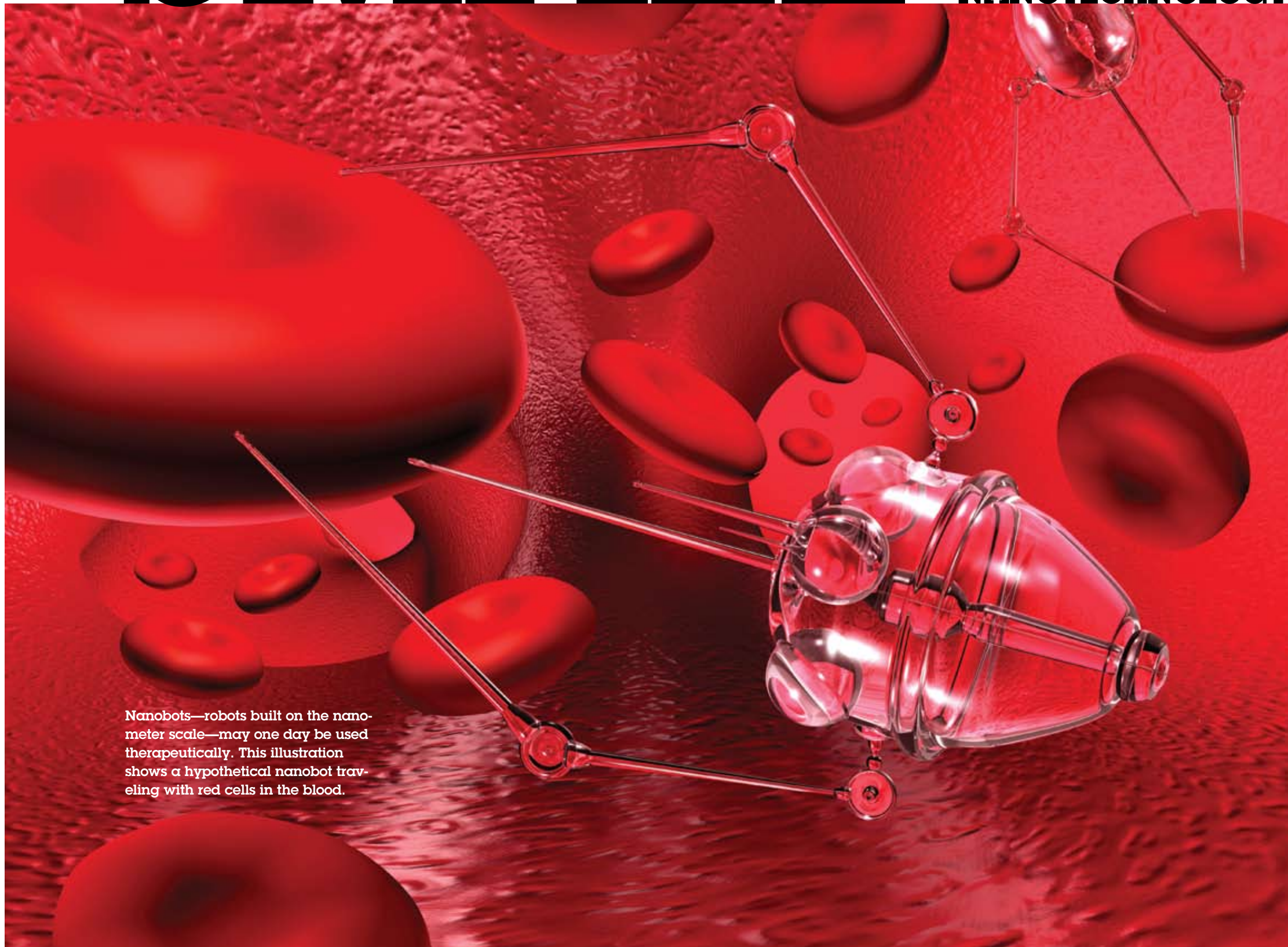


LET'S GET SMALL

NANOTECHNOLOGY: the next BIG thing in medical research



Nanobots—robots built on the nanometer scale—may one day be used therapeutically. This illustration shows a hypothetical nanobot traveling with red cells in the blood.

Comedian Steve Martin once entertained audiences with an absurdist routine about giving up hard drugs in favor of a substance whose only effect was to make users small. It was all fun and games, he joked, until some tall people came over or you were reckless enough to get small while driving.

Three decades later, researchers at Einstein are doing Mr. Martin's "Let's Get Small" shtick one better. They're getting really small. It's not for kicks, of course, but to observe and manipulate biological processes at the nanometer level — the scale of molecules and structures inside cells.

Thanks to a convergence of technologies—genetic and biochemical engineering, supercomputing, advanced microscopy, and microchip manufacturing—researchers are gaining unprecedented access to the cellular universe, with far-reaching consequences for biomedical science and, ultimately, for patient care.

It is now possible, for example, to build cancer detection devices so small that a dozen could fit on the head of a pin, or to turn on a single gene in a single cell and watch that snippet of DNA do its work. Both of these nanotechnologies are now under development at Einstein.

Nanotechnology may well be the platform that launches the era of molecular therapy, in which treatments are based on an understanding of what is happening at the cellular level and applied directly to individual cells rather than administered in broad strokes to tissues,

organs, or whole bodies with a host of unwanted side effects.

"In the future, you are not going to pump drugs into people's veins from a bottle hanging on an IV rack—that's a century-old technology," says one of Einstein's nanotech pioneers, John Condeelis, Ph.D., professor and co-chair of Anatomy & Structural Biology and co-director, with Robert Singer, Ph.D., of the Gruss Lipper Biophotonics Center. "Ultimately, we are going to develop some kind of way of handling drugs at the cellular and subcellular level, where they have to do their business."

HOW SMALL IS SMALL?

Nanotechnology is usually defined as the manipulation of matter from 1 to 100 nanometers (nm) in size—a scale that is meaningless in everyday life. (A nanometer is one billionth of a meter.) For perspective, a grain of salt is a 300,000 nm in length while a human hair is about 100,000 nm wide. Most

HOW SMALL IS SMALL? IT'S ALL RELATIVE.

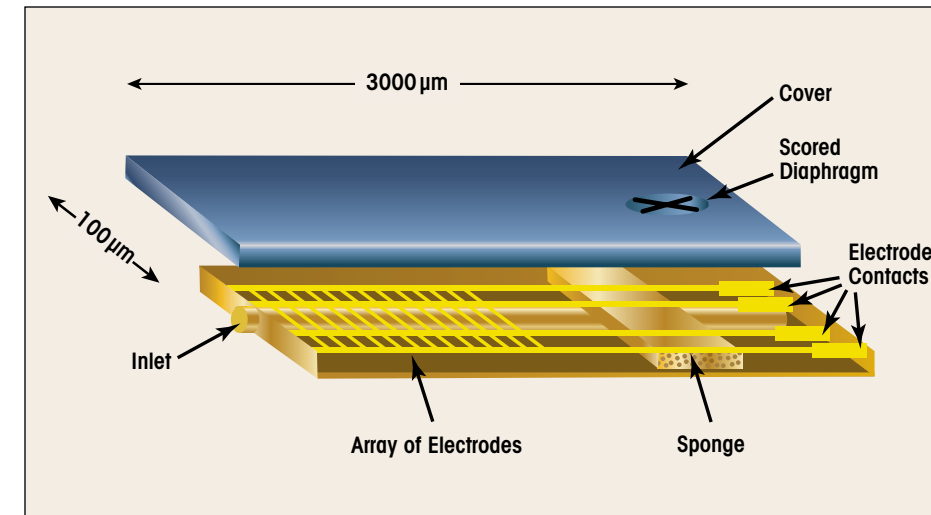
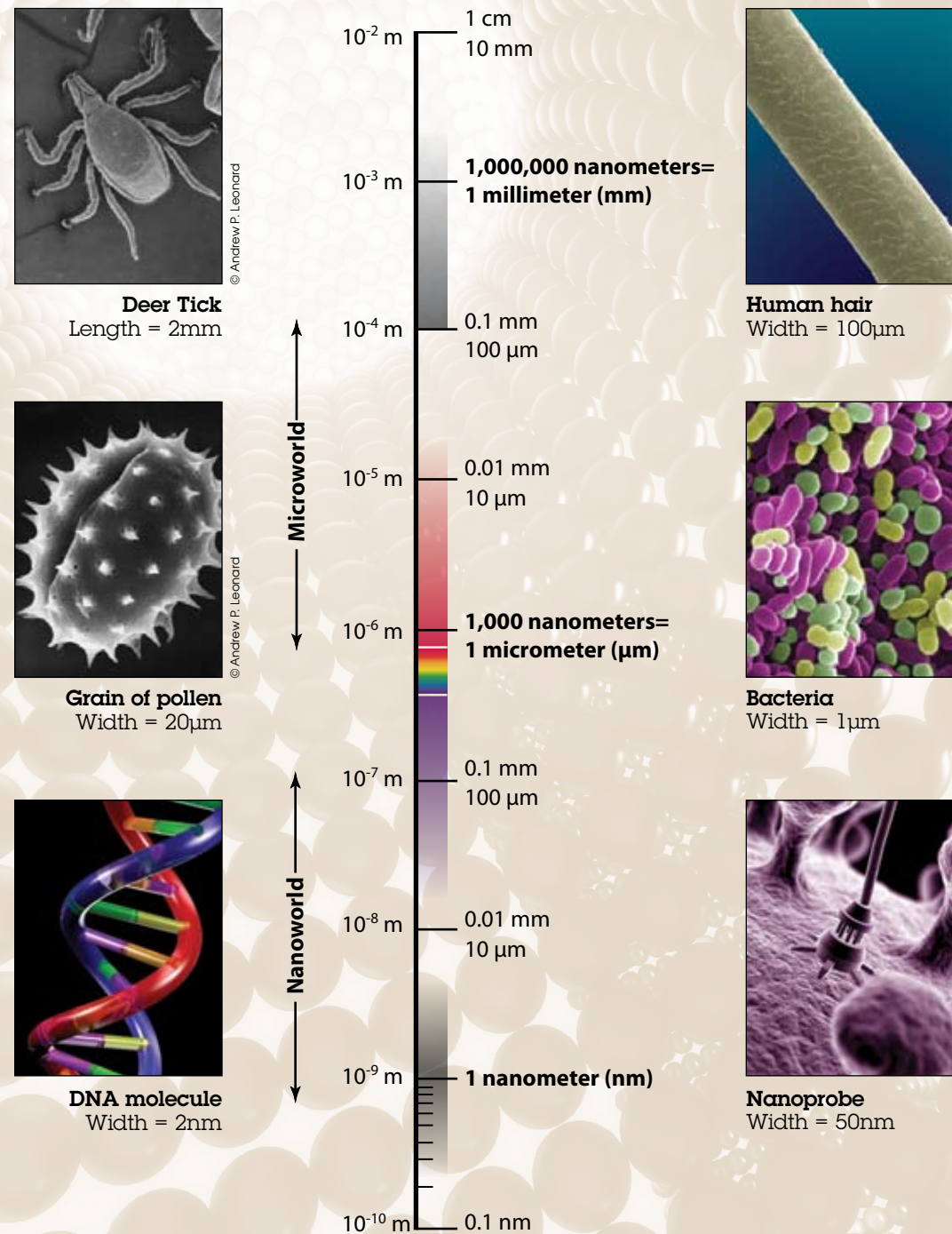


Diagram of a first-generation NANIVID (nano intravital device) that Dr. Condeelis and his colleagues are designing for use in human tissue. The NANIVID's individual components are nanoscale structures.

everything less than a hair's breadth is invisible to the naked eye. Below that lies the microscopic world, but even the best optical microscopes cannot discern objects less than 200 nm (a limitation tied to the wavelength of visible light). For Dr. Condeelis, that's where things start to get interesting.

A few years ago, Dr. Condeelis and his colleagues in Einstein's Analytical Imaging Facility devised a way to capture the first high-resolution, three-dimensional images of individual tumor cells inside a living animal (see *YU Review*, Summer 2003). Their novel technique is known as intravital imaging—an amalgam of genetic engineering, advanced microscopy, and computer-controlled image processing. It allowed the team to open a new window on how breast tumor cells metastasize.

"We found that the cells move from the primary tumor mass across vast expanses of normal tissue—hundreds of cell diameters in length—traveling along a superhighway of collagen fibers," he explains. A determined bunch, these tumor cells make a headlong dash for blood vessels, which they locate by sensing a gradient of growth factors, insidiously exploiting the infrastructure laid for the normal development and maintenance of breast tissue.

To test whether they had identified the critical ingredients needed for the tumor cells to spread, the team constructed an artificial blood vessel (a small catheter filled with growth factors and other substances), which was then placed inside the breast

tissue of mice with genetically engineered tumors. Some 90 minutes later, a line of tumor cells could be seen wending its way toward the catheter. With this rudimentary device, the team could predict whether breast cancer cells had the potential to metastasize.

Dr. Condeelis realized that he had the blueprint for a potentially powerful research and diagnostic tool. At the very least, such a tool could be useful for learning more about the micro-environment of tumors. In addition, it might also prove valuable for early detection of breast cancer well before clinical signs such as lumps arise, or for monitoring the progression of cancer in patients with breast tumors, alerting doctors to the need for more aggressive therapy.

But first, the tool would have to be miniaturized to the point where it could be easily inserted into a mammary gland. It would also have to be outfitted with reservoirs for holding the growth factors and releasing them in a controlled fashion, sensors for detecting and identifying cells, a transmitter for reporting results, and a port for retrieving cells for further study—quite a small order, as it were.

"That's where nanotechnology comes in," says Dr. Condeelis.

THE ALBANY CONNECTION

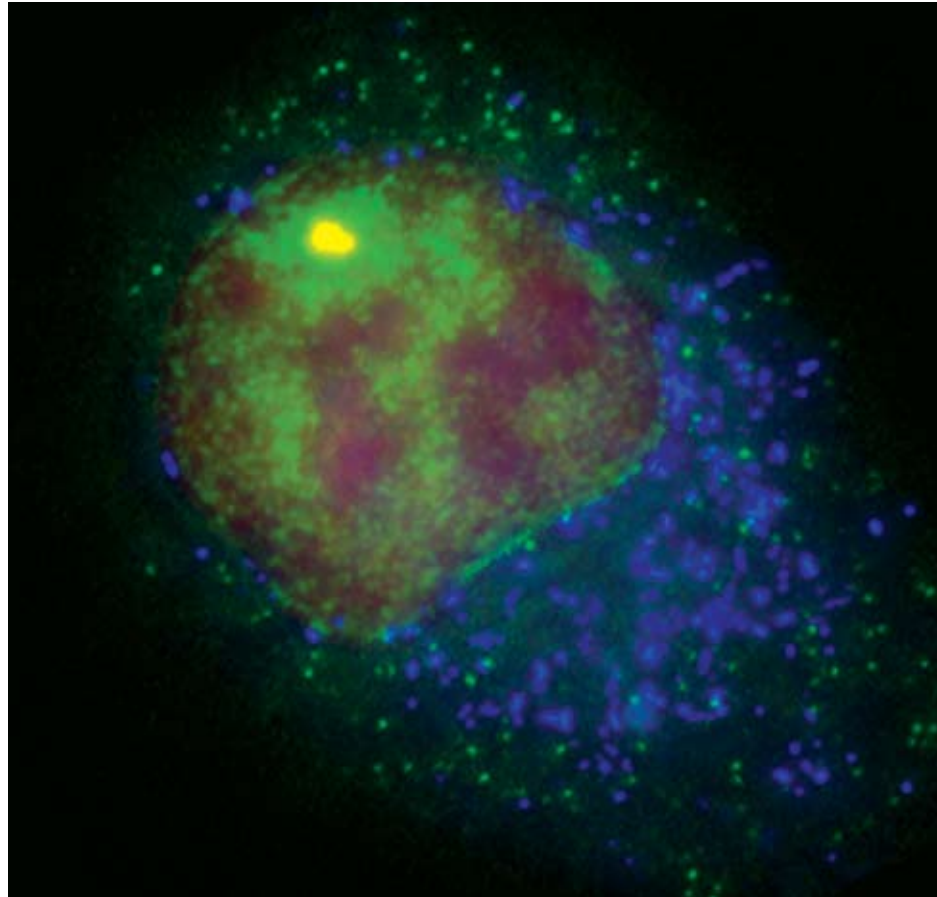
For help in getting really small, Dr. Condeelis turned to James Castracane, Ph.D., professor and head of the Nanobiosciences Constellation at the College of Nanoscale Science and Engineering (CNSE), University at Albany-SUNY, the first college of its kind. Funded by a five-year, \$2 million grant from

the National Cancer Institute, the two scientists have begun building a microchip version of the artificial blood vessel, called a NANIVID, which is short for nano intravital device.

An expert in micro-electro-mechanical systems—which combine mechanical elements, sensors, actuators, and electronics on a silicon wafer—Dr. Castracane was well versed in cramming a lot of stuff into tiny packages. But this particular microchip presented unusual challenges. First, the basic materials had to be biocompatible. "You have to prepare these chips so that the cells are happy to be around them," Dr. Castracane explains. The CNSE team also had to create nanosponges that could control release of the "biomolecular bait" (for attracting tumor cells and their associated helper cells) and to design hardware and software for tiny sensors that could detect and discriminate among different tumor cell types by virtue of their unique electrical signatures.

Devising a practical method of retrieving data from the device posed yet another challenge. In the first-generation NANIVID, tiny wires will be used to get information from the chip. "But eventually, we are going to develop a way to access the device remotely," perhaps using a tiny radio-frequency transmitter, like that used in an EZ Pass, says Dr. Castracane.

Einstein's researchers hope to begin evaluating the chip in laboratory animals this year. If all goes well, the NANIVID may help answer a host of questions about breast cancer, for example: What types of cells are involved in each stage of the disease? Why do certain patients respond to chemotherapy while others do not? How do tumor cells



Observing nanoscale phenomena: By attaching fluorescent tags to RNA polymerase II (the enzyme responsible for transcription), Dr. Singer observed the stages of transcription in vivo and in real time. At left is a fluorescently labeled cell with the locus of transcription in yellow. Messenger RNA is green and the translated protein product is blue.

for patient care. The inability to see beyond averages is perhaps the greatest barrier to understanding the inner lives of cells and, by extension, to designing therapies that work at the molecular and cellular level.

It appears that this barrier has been surmounted. Dr. Singer and his colleagues at Einstein, in a remarkable feat of nano-engineering, have crafted a way to trigger the expression of a single gene and observe its function in a living cell, a longtime dream of molecular biologists. The technique, known as single-cell gene-expression profiling, begins with the transcription factor for the gene under study. (A transcription factor is a protein that attaches to and activates a gene.) Using a trick of biochemistry, the transcription factor is put under lock and key by binding it to a so-called caging group. The bond is engineered to be photo-cleavable, so that it can be broken by a nano-sized sliver of light. In this way, the researcher can activate a single gene in a single cell with the flip of a switch.

The next step is to make the gene visible, which is accomplished by tagging it with a fluorescent protein that lights up when the gene becomes active. The corresponding mRNA and the protein that it produces can also be made to glow, in different colors, allowing their movements to be followed throughout the cell.

All of this happens at the nano level and thus is invisible to the naked eye. To view these colorful molecules, the researchers must employ ultra-sensitive cameras, high-powered computers and a special microscope known as the intravital imaging microscope—the same one that Dr. Condeelis uses in his research.

become resistant to drug therapy? The research may also point the way to new strategies for drug design and help clinicians assess whether a particular therapy is working.

As configured, the NANIVID would be applicable only to breast cancer. “The basic principle might also work in other cancers that spread through the bloodstream,” says Dr. Condeelis. “But first, we would have to gain more knowledge of the cell types and growth factors involved in those cancers.”

The Condeelis-Castracane collaboration marks the beginning of a formal alliance between Einstein and CNSE to advance education and research in nanobiotechnology and its application to health care (nanomedicine). The programs will focus on six areas: developing the nanoscale knowledge base for disease identification, therapy design and evaluation, clinical implementation, drug discovery and delivery, toxicology detection and cure, and medical devices and components demonstration and deployment.

THE INNER LIVES OF CELLS

“Every cell is doing something unique—expressing a combination of genes that is different from other cells,” says Robert Singer, Ph.D., professor of Cell Biology and co-chair of Anatomy & Structural Biology. The possible number of gene-expression combinations runs into the millions, making for a lot of cellular diversity, even within a highly specific type of tissue.

But the subtleties of gene expression—the conversion of DNA code into messenger RNA (mRNA) and then into a protein—are lost on researchers. Because of technological limitations, researchers know only the state of an average cell, gleaned from analyses of the large masses of cells that are needed to obtain measurable thresholds of biologic molecules.

“Basically, you grind up millions of cells and get an ensemble measurement of a huge series of events all homogenized together—all the things that are going on in all the cells, seen as an average. But you don’t know what an individual cell is doing,” says Dr. Singer.

While this may seem like an esoteric point, it has enormous implications for biomedical research and

It’s not exactly riveting viewing—until you realize you’re actually watching a gene, life’s fundamental biological unit, do its magic.

“Capturing these images becomes a bioinformatics problem,” says Dr. Singer, whose studies are funded, in part, by the National Nanotechnology Initiative, a program of the National Institutes of Health. “Each image is 1,000 by 1,000 pixels—that’s a million points of data for every image that you take. You can take 1,000 images a second, so imagine the data buildup that occurs.” This trove of data must then be run through special computer algorithms in order to sort out the subtle, glacially slow movements of the tagged molecules from the background noise.

The resultant video, pieced together from the thousands of still images, is a murky soup of moving smudges dappled with small dots of bright color. It’s not exactly riveting viewing—until you realize that you’re actually watching a gene, life’s fundamental biological unit, do its magic.

APPLICATIONS TO CANCER

Still in its infancy, single-cell gene-expression profiling is already affecting biomedical research. Dr. Singer is currently adapting the technique to devise a tool for diagnosing the severity of prostate cancer.

Presently, it is hard to tell whether prostate cancer that is confined to the gland is relatively harmless (as is usually the case) or highly aggressive. As a consequence, physicians and patients are hard pressed to choose between conservative treatment, (“watchful waiting” which carries with it the risk that the cancer will spread) and invasive therapies such as surgery (which can involve side effects such as incontinence and impotence).

Single-cell gene-expression profiling may provide some clarity. With Jeffrey Levisky, an M.D.-Ph.D. student at Einstein, Dr. Singer developed a way to view the expression of as many as 11 different genes in a cell at once, allowing for an unprecedented glimpse at a cell’s true nature.

This process was subsequently applied to prostate cancer cells, focusing on five genes that have been implicated in the disease. Studies revealed that prostate cancers of different aggressiveness have distinct gene-expression signatures, forming the basis for a diagnostic test now under development at Aureon Laboratories of Yonkers, N.Y., a company Dr. Singer helped found. In practice, such a test could be used to characterize individual cells in the prostate and determine whether enough cells have clicked into a pattern of expression that is cause for worry. This level of specificity cannot be attained with other gene-expression technologies, such as DNA microarrays, which measure the average expression of genes across a large number of cells.

His lab is also applying the technique to colon cancer. “There is only one drug, 5-fluorouracil, that is effective against colon cancer,” says Dr. Singer. “The problem is that only 30 percent of patients respond to this chemotherapy, and we have no idea who they will be.” As a result, countless patients are needlessly subjected to chemotherapy, dramatically affecting their quality of life and wasting valuable time for trying second- and third-line treatments.

Using single-cell gene-expression profiling, Rossanna Pezo, an M.D.-Ph.D. student in Dr. Singer’s lab, was able to identify a gene-expression pattern unique to colon cancer cells that respond to 5-fluorouracil. A diag-

nostic test based on this discovery is now being developed for a clinical trial at Montefiore Medical Center in collaboration with another M.D.-Ph.D. student, Saumil Ghandi.

STAY TUNED

It’s all too easy to overstate the promise of new biomedical technologies. Gene therapy, which has thus far failed to live up to expectations, is a case in point. Nonetheless, Dr. Condeelis is convinced that nanotechnology will make a huge difference in biomedical research and health care. “It’s like saying, 150 years ago, that chemistry is going to be important in drug development,” he says. “At the time, everybody knew it, though they may not have been able to give any examples. That is where nanotechnology is today. We know it’s going to be important, and I can already give you a thousand examples of where it’s heading. So, stay tuned.”

Dr. Singer is similarly enthusiastic: “The next generation is going to look back at our current treatments, like chemotherapy, the way like we look at 19th-century practices like studying bumps on the head and bleeding people. Medicine is going to be completely different when we understand what is going on at the molecular level.”

Evidently, Steve Martin was onto something. But getting small is much more than fun and games—it may well be the future of medicine. **E**