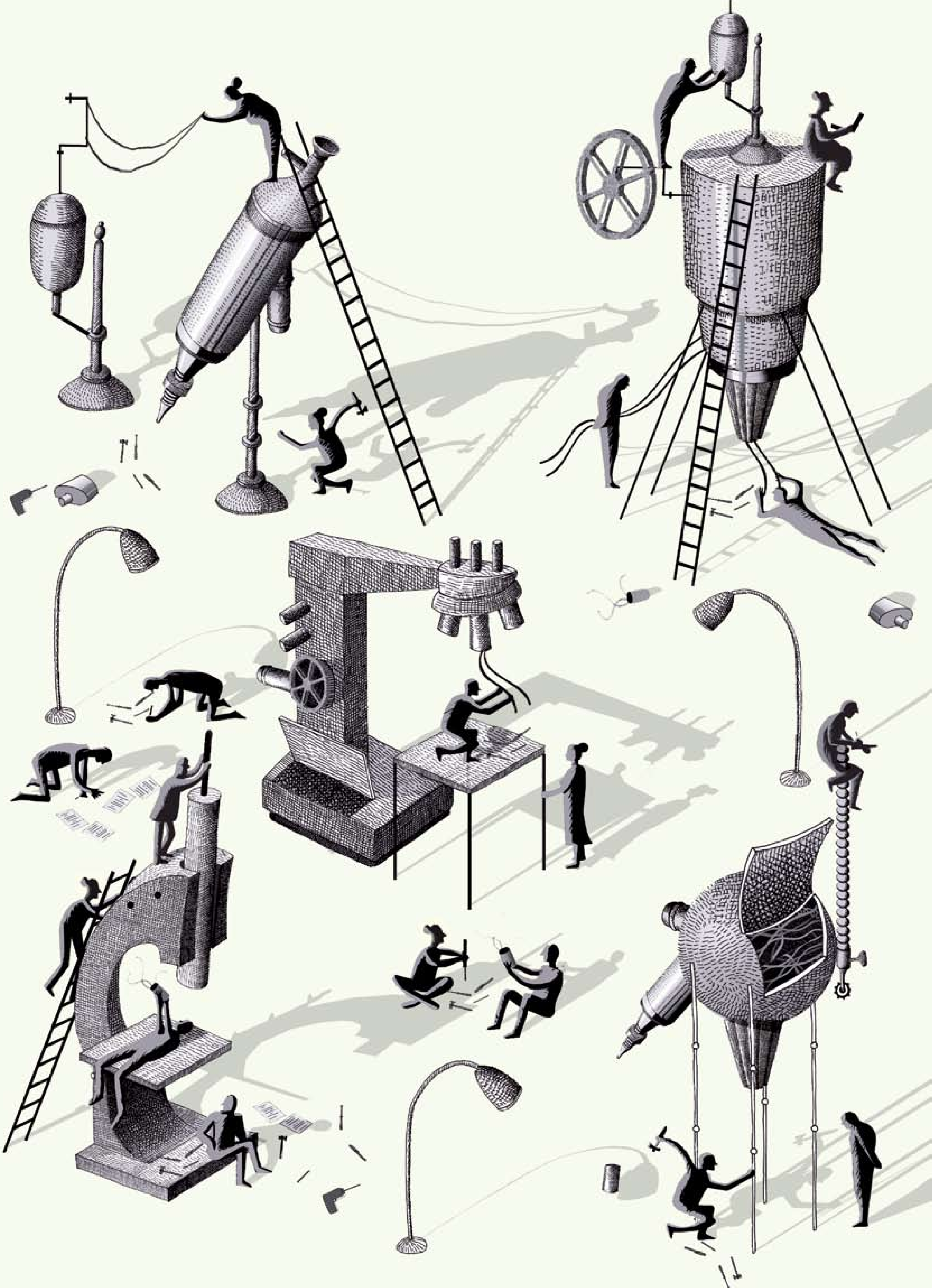


A DIFFERENT MINDSET

TO TRUE TINKERERS, THE LIMITS OF THE PRESENT ARE NEVER PERMANENT BARRIERS, MERELY OFFERS THEY CAN'T REFUSE.

by Tim Friend and Jennifer Michalowski
illustration by Adam Simpson

To most people, a microscope is a microscope—a device for revealing structures too small to see with the naked eye. But to some, a microscope or any other tool is a collection of parts that might be reconfigured to do its designated task better, or perhaps accomplish something else entirely. Their world is a collage of potentially useful bits and pieces. By shuffling the pieces and fitting them together in inventive ways, they come up with new tools—many of which are redefining the limits of biological research. Tinkerers fiddle and adapt, look across disciplines, test and redesign, and ultimately devise methods to explore areas of research that once seemed unapproachable. For example, the semiconductor industry, which continues to find ways to cram more information onto a computer chip, has some lessons to teach the alert biologist interested in observing life at its smallest scale but stymied by the capabilities of modern microscopes. Similarly, bulky needles used to inject labeling molecules can damage cells, but scientists who dabble in nanotechnology are producing thinner and stronger materials that are less likely to disturb the sample. HHMI investigators Carlos Bustamante, Taekjip Ha, and Carolyn Bertozzi, and Janelia Farm scientist Herschel Marchman, are making such innovations. They craft tools to help explore the scientific mysteries that compel them personally, but their gadgets are enabling a far broader community of researchers to address diverse and intriguing biological questions.

Start with a Need

CARLOS BUSTAMANTE GREW UP IN PERU, WHERE HIS KNACK for tinkering became apparent at a very young age. Playing with his toy cars meant taking them apart and putting them back together. As the space age dawned, his interests turned skyward. He built rockets and experimented with different types of fuels. But his world changed at about age 12 when his father, a physician, brought him a microscope from the United States.

"I thought it was the most marvelous thing. You could peer into this microscopic world, where all these exciting things were happening," says Bustamante, now an HHMI investigator at the University of California, Berkeley, and head of the Advanced Microscopies Department at Lawrence Berkeley National Laboratory.

In the late 1980s at the University of New Mexico, he and his colleagues used fluorescence microscopy and wire electrodes to coax DNA to "inch along like a caterpillar," enabling real-time study of DNA movement through a gel during electrophoresis. Then he added magnetic tweezers, figuring out how to anchor one end of a DNA molecule to a glass slide and attach magnetic beads to the other end to characterize the elastic response of the molecule—a first.

Bustamante is probably best known for tinkering with optical tweezers—a laser-based technique physicists invented in 1970 that uses the minute forces exerted by light waves to manipulate molecules. He adapted optical tweezers in the 1990s so that a molecule, trapped in a kind of Star Trek tractor beam, can be poked, prodded, and analyzed. This method has become a standard for measuring single molecules.

Because good can always be better, the upgrading in Bustamante's lab continues—at smaller and smaller scales. He says his most exciting invention so far is an ultra-high-resolution optical tweezers machine. "Measuring changes down to the angstrom, it opens up a way of following the dynamics of biological systems," says Bustamante. He is currently searching for nanoscale handles he can attach to molecules to obtain even more precise information. Those handles will likely be carbon nanotubes, he says—cylindrical carbon molecules 1–3 nanometers in diameter that are unusually strong and have unique electrical and heat-conducting properties.

Bustamante says he was deeply inspired in his youth, and adulthood, by two of humanity's greatest scientists—and tinkerers *extraordinaire*. "Galileo, my all-time hero, was a professor of mathematics, but he was always building things. It is breathtaking the way he put reasoning into instruments that allowed him to arrive at such profound conclusions as the law of inertia."

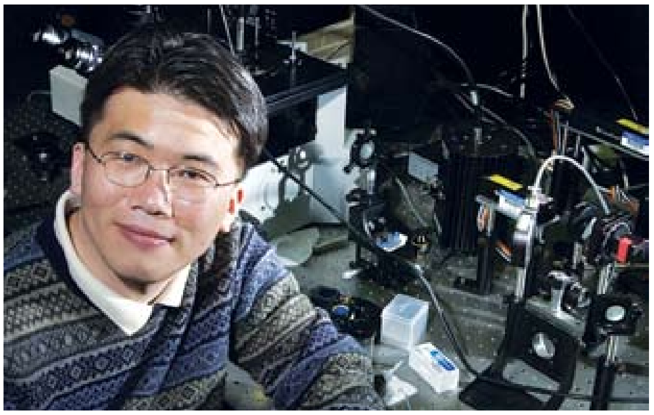
Louis Pasteur's elegant logic similarly impressed Bustamante. Pasteur discovered stereochemistry when he precipitated tartaric acid crystals from a solution and, to his surprise, found two mirror-image shapes. Curiosity piqued, Pasteur used a small stick to separate the crystals into two bunches, using his microscope, and proceeded to dissolve them and study further. He found the solutions identical in all aspects, except that one rotated the plane of polarization of light clockwise and the other counterclockwise. "Pasteur arrived at the remarkable conclusion that molecules can exist in two different forms that are mirror images of one another. That's tinkering for you, at its best."

A scientist can accomplish remarkable results just by reflecting that same curiosity-driven spirit, according to Bustamante. "There is nothing written—no rule—that says scientists should work only with instruments that exist. You start with a need and develop the instrument to fit that need," he says.

"Oftentimes, tinkering doesn't involve sophisticated thinking," he insists. "It involves simple logic, and the simpler a system is the better it will be."

Andrew Nagata

Extreme Techniques



AS A YOUNG STUDENT IN KOREA, TAEKJIP HA WAS A SELF-described "test taker." He didn't discover his inventive side until he became a researcher with goals that were unattainable with existing technologies.

"I was just a very good student who did what I was told to do," he recalls. "But once I began to work on my own projects, it was a whole new world. I began to think 'How can I do measurements?' That is when I became a tinkerer," says Ha, a biophysicist and HHMI investigator at the University of Illinois at Urbana-Champaign. Since then, he has immersed himself in the role, even ordering off-the-shelf parts to build a microscope from scratch.

Ha's overriding goal has been to observe single molecules in action. For example, his group recently devised the "nanocontainer"—a nanoscale test tube with a diameter one-thousandth that of a human hair—to enable scientists to observe the behavior of single molecules of DNA, RNA, or proteins. In the June 11, 2007, online issue of the *Proceedings of the National Academy of Sciences*, the team described how they intentionally made the nanocontainers porous; the large subject molecules cannot escape, but smaller molecules such as ions and the cellular energy source ATP can be added to start reactions. Ha calls the technique "another cool addition to the tool set that is being made available by our research."

That tool set got its start when Ha was challenged to improve fluorescence resonance energy transfer (FRET) as an optical measurement technique. At the time, FRET could measure the distance between only two points. The "donor" and "acceptor," two different colors, are attached to two points on a molecule, or on two molecules. If the distance between them remains larger than 10 nanometers, their colors do not change. But if the dyes come into closer proximity, indicating the molecules are interacting, the ratio of the two colors—and thus the intensity—changes.

Unsatisfied, Ha continued to experiment, building an apparatus that combines single-molecule FRET with optical tweezers. Today he is performing FRET in three colors, allowing measurements of three different molecular distances at a time. "With complex molecules there is more than one moving part," he explains.

"My ultimate goal is to reconstitute the entire DNA replication system," says Ha. "When all these proteins are labeled with different molecules and we have nanoscale handles to manipulate and study their coordination in great detail, we will see how molecules really work."

Ha's graduate advisor, Shimon Weiss, now at the University of California, Los Angeles, considers Ha exceptional. "Taekjip has the rare ability to identify the bottlenecks in any problem he attacks, and then come up with the most correct, simple way to overcome those bottlenecks. Never overdesigning to solve a problem, he optimizes his work investment and stops where the solution is just adequate."

Ha says he has developed an instinct for choosing people with a similar bent to work in his lab—and he pushes them to use it. "I tell my students to find ways to reduce the setup time for their experiments by a factor of two to four. I encourage them to change the whole process of doing measurements to make things more reliable and efficient so they can do better science." There's no need for him to push them very hard, Ha adds. "We always have pressure to do more tinkering."

> "I was just a very good student who did what I was told to do. But once I began to work on my own projects, it was a whole new world." — TAEKJIP HA

John Dixon / AP, ©HHMI

Fantastic Vision

CAROLYN BERTOZZI'S PENCHANT FOR TECHNICAL INGENUITY showed itself when she was a youngster playing dolls with her sisters. "We had great fun taking dolls apart and reassembling them as alien creatures," she recalls. The gadgets that her father, a physicist at the Massachusetts Institute of Technology, brought home were also an outlet. "A great favorite was a strong magnet. My sisters and I used it to build strange sculptures from nails, staples, nuts, and bolts."

Now a chemist and an HHMI investigator at the University of California, Berkeley, Bertozzi exercises her creativity in the field of glycosylation—the cellular process by which sugars are added to proteins or other molecules. The resulting "glycans" govern a variety of cell-to-cell interactions. Scientists had known for decades that changes in glycosylation were associated with cancer, bacterial infections, and other illnesses, but they had no way of studying how molecules at the cell surface use sugars. Bertozzi's lab devised a method of imaging glycosylation—something not thought possible.

Her group developed a chemical reporter, or label, that can be installed in the glycans of cells in culture or in living animals, such as worms, zebrafish, and mice. The reporter, linked to a simple sugar and introduced into the media of worms or fish, or injected into mice, is metabolized and incorporated into cell-surface glycans. After chemical reaction with a probe molecule, the reporter can be visualized by using an imaging technique such as fluorescence microscopy or positron emission tomography.

Bertozzi says her inspiration for building gadgets comes from "the demands of research at the interface of disciplines," whereby members of one community express a need that can be met by another. As director of the Molecular Foundry at the Lawrence Berkeley National Laboratory—a user facility that supports research in nanoscience worldwide—she orchestrates that kind of interaction, and participates herself. "Of the many tools my group has worked on," she says, "the coolest is probably the carbon nanotube-based 'nanoinjector,' which we developed with Alex Zettl's lab in the Physics Department here at Berkeley."

Barbara Ries

In this case, carbon nanotubes served as new building blocks for injecting imaging agents, called quantum dots, into cells, and conducting surgery on single cells. Carbon nanotubes, being harder than steel and manipulable with angstrom-resolution precision, make ideal injectors for delicate cell membranes.

In collaboration with Zettl, Bertozzi's lab mounted a single carbon nanotube to an atomic force microscope (AFM)—a widely used instrument that scans surfaces by manipulating a probe at subnanometer scales. The tip of this "nanomanipulator" can be moved in three dimensions—X, Y, and Z. After loading the carbon nanotube with quantum dots and other molecules, the device can be positioned just above a cell. The AFM can then push the carbon nanotube through the cell membrane and deliver the load of quantum dots, bright enough to track single particles, into the cell's interior. The nanoinjector is described in detail in the May 15, 2007, issue of the *Proceedings of the National Academy of Sciences*.

For a devoted tinkerer like Bertozzi, life can imitate art: "In one of my favorite movies, *Fantastic Voyage* [1966], a group of scientists and physicians miniaturize a ship, and themselves, to navigate inside the human body in order to diagnose and treat disease. This fantasy may now essentially come true as nanoscale probes and manipulators revolutionize medicine. What was fiction a few decades ago may ultimately be realized in the hands of scientists, engineers, and physicians working together."

HOW SMALL ARE WE TALKING?

1 millimeter =	1 nanometer =	1 angstrom =
0.03937 INCH	0.000001 MILLIMETER	0.1 NANOMETER OR 1/250 MILLIONTH OF AN INCH

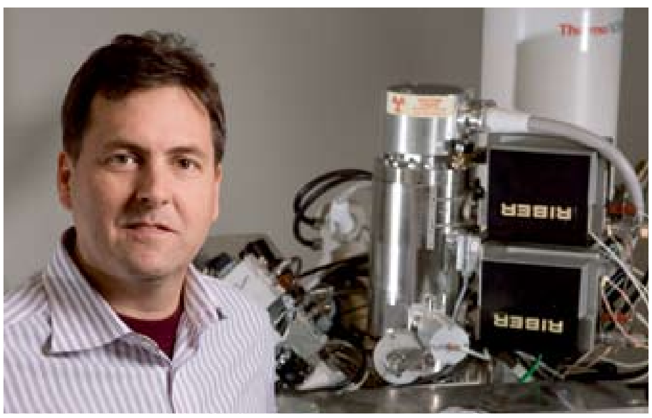
Inner Engineer

WHEN HERSCHEL MARCHMAN SAYS "WE NEED TO LOOK AT things in different ways," he's not just talking about the view through a microscope. Marchman is intent on expanding researchers' options through creative variations on existing technology, and his unique perspective provides a wellspring of ideas for vexed scientists who think they have hit a wall in their research because of technological limitations.

Marchman, a senior scientist working in Harald Hess's applied physics and instrumentation group at HHMI's Janelia Farm Research Campus, approaches such issues as an engineer, often seeing parallels to problems the semiconductor industry—in which he spent some 15 years—has already solved. He readily borrows tools from that field, adapting them in ways he anticipates will dramatically affect biological discovery.

High on his priority list is reducing the need to stain biological samples, which enhances their contrast but also imposes constraints. In the case of scanning electron microscopy, for example, users are limited to imaging only those cellular structures that can be labeled with metal stains during sample preparation. "I'm sure there's more information there that we're missing," Marchman says.

To begin with, he'd like to harness the power of deep-ultraviolet illumination (DUV), a favorite of computer-chip lithographers because its ultra-short wavelength allows them to print exceedingly tiny patterns. Though DUV has not been applied to biological samples for high-resolution imaging, Marchman thinks that, with some experimenting, it might allow researchers to detect contrast patterns in cells more naturally, without using stains.



Paul Fetters

"When we tinker we don't want to build something completely from scratch. We want to add value to what you can buy by modifying it." —HERSCHEL MARCHMAN

Meanwhile, he's working with Hess to design a detector for a scanning electron microscope that will break from the current paradigm, in which electrons bounce off a sample's surface. The modification should bring the instrument closer to the far superior resolution of transmission electron microscopy. It's an example of Marchman's way of improving on the status quo.

"When we tinker," he says, "we don't want to build something completely from scratch. We want to add value to what you can buy by modifying it."

Marchman has some high-tech tools to enable his innovations. Many of his projects depend on a focused ion beam, which allows him to etch and deposit materials at the nanoscale level. Marchman sees an opportunity to make the standard focused ion beam he has purchased even more useful: he plans to add a photocell and a gas chamber to the instrument so that he can perform chemical reactions directly on the small-scale parts he produces.

This setup, Marchman believes, will help meet the needs of Janelia Farm researchers in a variety of ways. For example, he imagines using the gas chamber to fill fruit fly brains with a resin so they can be sliced thin for microscopy, eliminating the need for microsurgery, which is time-consuming and tends to destroy delicate brain tissue. Again borrowing from the semiconductor industry, he will take advantage of hydrocarbon gases mixed with metals. With a vacuum, the metals can be deposited onto a highly localized region of a computer chip. Marchman will use the technique to deposit a spot of gold onto a single cell, making the gold a simple, inert marker for navigating back to that cell under the microscope.

"If you really understand the technology," he says, "you can modify it to your own problem." ■

FOR MORE INFORMATION: To read about other HHMI tinkerers, visit the *Bulletin* online at www.hhmi.org/bulletin/aug2007.