Seeing Is Knowing

Caltech scientists and engineers create new lines of sight with innovative imaging techniques

BY KATIE NEITH

Photographs add emotional context to current events, bring loved ones to mind with a single glance, and keep the past alive when our memories begin to fade. In science and engineering, still and moving images provide a similarly essential service, offering insight into—and sometimes even answers for—the biggest questions researchers are asking about how the world works.

At Caltech, a number of scientists and engineers are opening up new views into our environment and are exploring how we can best visualize the structures and processes that make up the chemical compounds and tiniest biological elements of the physical world.

"There is so much about cells that we don't understand," says structural biologist Grant Jensen. "But we're learning that many biological processes can be understood just by imaging the machinery working in a cell, doing its job. Just a picture of it reveals basically how it works."

Up Close and Biological

Jensen says that, growing up near Los Alamos National Lab in New Mexico, "I imagined I would become a physicist like everybody else in town." But soon after he started his undergraduate studies in physics at Brigham Young University, a research project led to his being captivated by the wonders of and potential advances to be made in structural biology. He never looked back.

"I learned that some of the highest-impact work waiting to be done in biology involved image processing, math, and three-dimensional reconstructions, which is all stuff I love," Jensen recalls. "As a postdoc, I saw that it was possible to take multiple images of a single object from different points of view and merge them into a 3-D reconstruction, and I realized that we could do this to cells. Immediately, I knew that this was what I would be doing for many years."

Jensen says his imaging research is guided by the words of an unlikely scientific muse, baseball's Yogi Berra, who once famously said: "Sometimes you can see a lot just by looking." To do that looking, his lab has become one of just a handful in the world to own and operate an electron cryomicroscope—a unique type of microscope that enables a novel imaging technique called electron cryotomography (ECT), which allows Jensen and his team to observe biological samples in a near-native state. Developed in Europe in the 1980s, the electron cryomicroscope produces a magnified image by illuminating samples—kept at cryogenic temperatures of below –150 degrees Celsius—with an electron beam. Unlike in traditional microscopy, for which samples must be fixed, embedded in plastic, sectioned, and stained, samples for ECT are frozen so quickly that they become almost immediately fixed within a layer of transparent, glass-like ice.

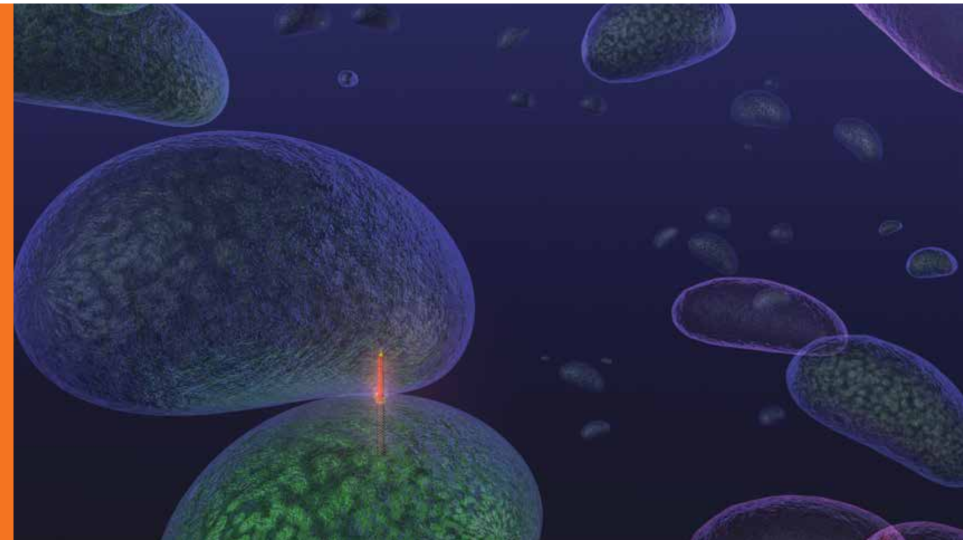
Once the sample is frozen, it is rotated around an axis while a specialized digital camera takes a series of high-resolution images; the information gathered by the camera allows the team to reconstruct the object in three dimensions and then analyze it in detail.

"Caltech is positioned to do this kind of work because a generous gift from the Gordon and Betty Moore Foundation allowed us to buy the world's very best electron cryomicroscope in 2002," Jensen says. "Only one or two other labs are doing similar work today; we really have a unique niche in the world."

ECT recently helped his group identify the way in which the cholera-causing bacterium *Vibrio cholerae* kills its intestinal competition, the common bacterium *Escherichia coli*, by delivering a toxin. By imaging the cholera cells, the researchers discovered tubes inside the bacteria: sometimes they were long, skinny, and filled with toxin; at other times, they were short, wide, and empty.

"Using fluorescence light microscopy and ECT, we were able to discover that these tubes are outer sheaths assembled around an inner javelin-like rod. When a cholera cell bumps into an *E. coli* cell, the outer sheath contracts and propels the inner rod of toxin through a port in the cholera cell's own membrane," explains Jensen. "The rod then punctures the *E. coli* cell and delivers the toxin. We call that a spring-loaded molecular dagger." Jensen says this discovery may lead to using this structure for medical purposes, such as designing entirely new cells to treat infection or disease.

Jensen's lab was also the first to obtain 3-D images of a complete bacterial flagellar motor. These rotary nanomachines power the miniscule whip-like flagella that are responsible for propelling bacteria through the body; their exact structure, however, had been a biological mystery until



Above: An artist's illustration shows a cholera cell injecting toxin into an E. coli *cell via a spring-loaded molecular dagger (in orange.) Grant Jensen's lab at Caltech was the first to discover this phenomenon using sophisticated imaging techniques.*

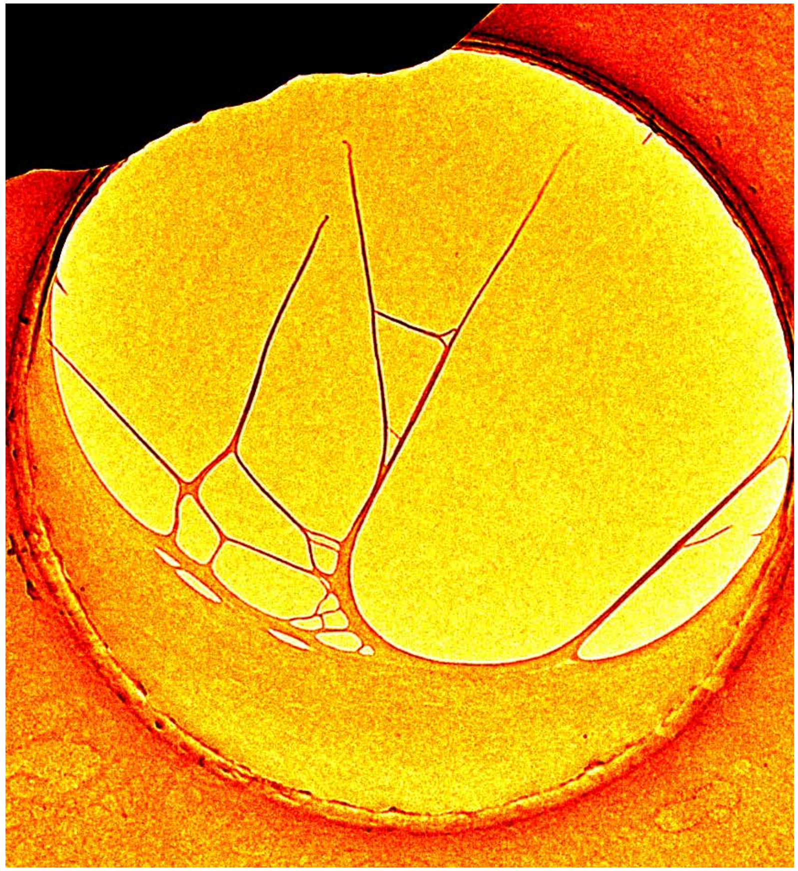
Jensen used ECT to unveil the details of how they are assembled. Thanks to that technological peephole, he and his colleagues were able to visualize how—like an outboard motor anchored to a boat—the flagellar motor in a bacterium is held in place on the outside of the cell membrane by a structure that remains fixed, allowing the flagellum itself to spin and move the cell through liquid.

One of Jensen's other significant ECT-powered advances in structural biology is the development of a better picture of how some key HIV structures form; determining the 3-D arrangement of layers present at different stages of the virus's development has helped us understand how certain antiviral drugs block HIV's growth and proliferation, he says. His lab has also helped quiet the debate over the existence of cytoskeletons in bacteria—an idea that had previously been dismissed by researchers who simply did not have powerful enough imaging technologies to see the microbes' intracellular filament skeletons.

"We've made a lot of progress in understanding why bacteria and viruses have the shapes that they do, which is key to learning about how they work," Jensen says.

Jensen compares ECT to taking apart a car; you may know nothing about cars, but if you pull an engine apart to look at all of its pieces and how they fit together, you can at least begin to intuit how it works.

"What satisfies me most is understanding how biological processes work at a mechanical level," says Jensen. "Taking pictures of the cells can, at times, advance our understanding faster than any other approach."



Visualizing Space and Time

For chemist Ahmed Zewail, however, "taking pictures" isn't quite enough. Zewail's goal is not only to visualize biological specimens—or, in his case, any kind of molecule or nanostructure—at their most basic level, but also to see the details of the fundamental reactions they undergo…in real time.

He tackled the time element first, pioneering the field of femtochemistry, the study of chemical reactions occurring at the timescale of the femtosecond, which is one-millionth-of-a-billionth of a second. For these innovative efforts, he was awarded the 1999 Nobel Prize in Chemistry. Less than 10 years later, Zewail revolutionized the field yet again by building on his early femtochemistry work to create the four-dimensional (4D) electron microscope, which reveals objects not only in the usual three dimensions, but incorporates time into the image as well.

Most electron microscopes use a steady stream of electrons for illumination; Zewail's new technology, on the other hand, employs the precision-timed release of individual electrons—doled out one by one—to produce images of objects at the atomic scale. Each electron contributes to a picture representing a still at a given point in time. Like the frames in a film, the sequential images generated by many millions of such electrons can be assembled into a digital movie of motion at the atomic scale.

"We used to use laser light to probe and interrogate chemical systems, but that has been supplanted by the electron because the electron has properties that make it very convenient for looking at extremely small things," says Spencer Baskin, a senior scientist who has worked in Zewail's lab for 24 years.

Resolving the details of such "extremely small things" is impossible to do with laser light, since its wavelengths are much larger than any given nanostructure. To get a "picture" of something, you need a wavelength that's at least on the same scale or, preferably, smaller than the object you are trying to resolve. Electrons are perfect for this task because their wavelengths shrink as their velocity increases; thus, they can be accelerated so that their wavelengths are a picometer, or a trillionth of a meter, in length, making it possible for researchers to capture the details of nanostructures at very high resolutions. By sending individual electrons out at known intervals of time, the 4D microscope takes that process to the next level, not only capturing tiny objects but also tracking their precise motions in real time.

"Electron microscopes have been around for a long time, but the 4D microscope extended the technology to the time domain, so we can resolve very fast processes that couldn't be seen before," says Ulrich Lorenz, a postdoctoral scholar in Zewail's lab. "This is where the power of this technique comes in: combining time and spatial resolution to get completely new information about how processes evolve."

The 4D microscope was invented at Caltech's Physical Biology Center for Ultrafast Science and Technology (UST). The center is directed by Zewail, who created it in 2005 to investigate the fundamental physics of chemical and biological phenomena. Thus far, UST researchers have been able to use the 4D microscope to study a wide range of processes in different materials, including thermal expansion and phase transitions, chemical bond dynamics, and nanomechanical motions. The latter studies yield information about mechanical properties such as stiffness, which is of particular importance for maintaining strength and integrity in everything from colossal edifices to the tiniest of nanoscale structures.

"What satisfies me most is understanding how biological processes work at a mechanical level. Taking pictures of the cells can, at times, advance our understanding faster than any other approach."

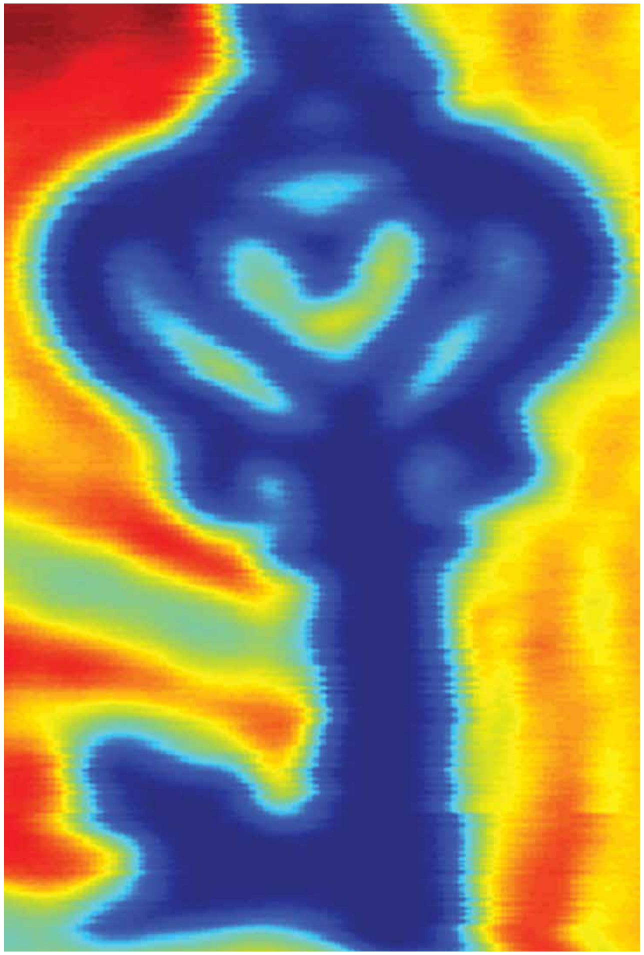
Such insights are similarly important when talking about biological structures; knowing the mechanical properties of fabrications made of DNA, for instance, is crucial to building sturdy biotech tools, such as DNA nanotubes for drug delivery in the body. And, indeed, earlier this year, Zewail and his group reported on a breakthrough 4D experiment that began with DNA stretched over a hole embedded in a thin carbon film.

They cut away several DNA filaments from the carbon film to create a three-dimensional, free-standing arrangement under the 4D microscope. Next, the scientists set the DNA strands vibrating using heat generated by a laser and imaged those motions using electron pulses. By determining the frequency of the DNA's oscillations, they were able to directly measure their construction's stiffness, a measurement that had never before been possible.

Recently, using a similar approach, the group also studied the material properties of protein assemblies called amyloids, which are believed to play a role in many neurodegenerative diseases.

"Because we're using an instrument that had never been built

Above: A DNA nanostructure as seen through the 4D electron microscope invented by chemist Ahmed Zewail at Caltech's Physical Biology Center for Ultrafast Science and Technology.



Above: A terahertz image shows a key inside an envelope; without the use of this technique, the object would be invisible to the naked eye.

before, everything we look at is something new that no one has ever clearly seen in motion before," Baskin says. "The difficulty is in finding the things that are going to be extremely important."

The end goal, he notes, is to be able to see individual molecules and atoms in the process of rearranging, taking a series of snapshots of where the atoms in a molecule are at each point in time as it goes through a reaction.

"People have frequently called our end goal a molecular movie," says Lorenz. "If you could watch chemistry—this rearrangement of atoms to make new things and transform compounds—in a movie, then you would really have it all. That would be incredible."

Transparent Technology

Engineer Ali Hajimiri isn't as interested in looking at things, per se, as in looking *through* them. It's a sideline of the work his lab has done for the past 15 years, exploring solutions for a host of different problems in electrical engineering. Five years ago, he began to work on silicon microchips able to generate and radiate high-frequency electromagnetic waves, called terahertz (THz) waves. These waves fall into a largely untapped region of the electromagnetic spectrum and were being used to penetrate numerous materials and render image details in high resolution—but only in systems that were generally too bulky and expensive for widespread use.

"There were clearly potential applications for these terahertz waves—like advanced security operations and medical diagnostic purposes," says Hajimiri. "We saw it as a big challenge worthy of our efforts, so we decided to see how far we could get with a terahertz system—how small we could make it."

In December 2012, Hajimiri's team published a paper announcing that they had developed THz imaging chips that used the high-frequency waves to see through—and image items cloaked under—materials such as fabric, plastic, paper, and wood. While the THz waves work much like X-rays, they do not carry enough energy to remove electrons from atoms or molecules and so don't create the same ionizing damage. In addition to providing images of things that are normally hidden, the system can also use spectroscopic data to detect the chemical signatures of things like chemical weapons, illegal drugs, and explosives.

"What we did was find new ways of making complete THz systems using an integrated-circuit chip-manufacturing process similar to those used to make image sensors for cell phones," says Hajimiri. "Done in large volumes, it's extremely low cost—approximately a dollar per chip set. Therefore, THz chips could be used extensively across multiple platforms ranging from cell phones to computers to other handheld devices. I think they could be ubiquitous in the long run."

In addition to being cheap, the new chip set—in which one chip acts as a light source and the other as a detector, or camera—is no bigger than a fingertip and sends out signals that are more than a thousand times stronger than those possible using existing approaches, Hajimiri says.

He and his lab members were able to use the THz chip set to detect objects hidden inside all kinds of items; for instance, they've been able to recognize a bullet stashed inside a teddy bear and reveal a razor blade in a piece of plastic.

"The first time we saw such an image—a snapshot literally representing years of effort—we were jumping up and down with excitement," says Hajimiri. "It's one thing to create a device or component that you think could be used to do certain things in the future. It's a completely different thing to actually see it working."

The beauty of the technology, Hajimiri says, is that the system can be adjusted and dynamically controlled. If, for instance, you want to image the inside of something that's soft and that, therefore, the waves can see through more easily, you can operate at a lower power. If you need to see through a much more complex or dense object, you can just crank up the power.

The technology has seemingly limitless potential applications, Hajimiri says. A THz scanner could look into large packages, crates, or even machinery. Various industries could use the scanner to check equipment for defects without having to take the object apart. For gaming or human-machine interfaces in general, THz systems could have even more impressive implications. Since the technology can be used to track movement rather than as an imaging technique, people can use it to communicate with their computers from across the room through certain gestures or even eye movements, says Hajimiri.

"Current human-machine interactive gaming systems like Kinect for Xbox are really responding to big movements of the limbs," he notes. "With terahertz waves, a gaming system would be able to detect the slightest movements of the eye, track where a user is looking, monitor their breathing, or even detect a heartbeat—it can detect even such very small displacements."

Hajimiri thinks this terahertz technology could even be used for medical applications, such as searching for tumors inside the body noninvasively, with just the wave of a handheld scanner.

"There are always new applications that we haven't thought about yet," he says. "Imagine having that sensor on your phone, and just sliding your phone across something to see what's inside it. I find all the possibilities very exciting and challenging."

"Everything we look at is something new that no one has ever clearly seen in motion before."

Indeed, Hajimiri says, the only limits in imaging technology—as in most of science and engineering—are researchers' imaginations, not their abilities.

"To use the old cliché, we need to be able to really think outside of the box and come up with new ways of doing things instead of succumbing to preconceived notions about what can or cannot be done," he says.

And when it comes to getting the first glimpse at something nobody has ever seen before, Caltech's imaging researchers are not only thinking outside the box, they're building there, too.

After all, as Lorenz points out, "Seeing something that you normally wouldn't be able to see is just so cool. It's why people do science!" E&S

John S. (Spencer) Baskin is a senior scientist in chemistry and chemical engineering.

Ali Hajimiri is the Thomas G. Myers Professor of Electrical Engineering.

Grant Jensen is professor of biology and an investigator with the Howard Hughes Medical Institute.

HHMI, the National Institutes of Health, the Beckman Institute, and gifts from the Gordon and Betty Moore Foundation help support his ECT work.

Ulrich Lorenz is a postdoctoral scholar in chemistry.

Ahmed Zewail is the Linus Pauling Professor of Chemistry and professor of physics.

His lab's 4D electron microscopy studies are sponsored through grants from the Gordon and Betty Moore Foundation, the National Science Foundation, and the Air Force Office of Scientific Research.