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In The Niche Of Time

Unique ultrafast electron imaging tools use time to help elucidate function

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Robert Paz/Caltech

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Time Bandits Zewail (center) and colleagues pose with the second-generation ultrafast electron microscope.

Imagine being able to see every bit of a chemical reaction as it happens. To observe reactants form fleeting intermediates that seamlessly transform into products. Or to watch a movie of a protein as it folds in a nanosecond. Not a representation, not a model or simulation, but actual pictures showing what molecules, cells, and proteins look like and how they move.

Researchers at California Institute of Technology are capturing such images with the world's only ultrafast electron microscope. For example, they have spied channels open and close in semiconductor crystals, a phenomenon never seen before.

Time is of the essence for this microscope, which can track three-dimensional structural changes on the atomic timescale. [Ahmed H. Zewail](#), a professor of chemical physics and of physics at Caltech, refers to it as four-dimensional imaging.

Bond-making and bond-breaking between atoms occurs on the femtosecond (10^{-15}) timescale and at picometer spatial resolution. This makes those transient structures of chemical reactions hard to catch, but this microscope can see them, Zewail says. To get photos at these space and time resolutions, analytical techniques that rely solely on radiation or light (such as spectroscopy and optical microscopy) won't work. Those techniques are physically limited to nanometer resolution, and conventional electron

microscopes do not have the temporal component, he adds.

Zewail and his colleagues can see the structure and dynamics of a transient intermediate with the microscope because it probes samples with packets of single electrons at femtosecond time intervals.

Experts in microscopy consider the Caltech microscope revolutionary. This kind of imaging allows unprecedented ability to simultaneously determine molecular structure and the dynamics of how molecules move, says Sir John M. Thomas, a former head of physical chemistry at Cambridge University who has closely followed the development of this ultrafast technique. "In a sense, I wish I were 20 years younger so that I would be around to follow the future progress of this spectacular work," he says.

Thus far, Zewail and his colleagues have used ultrafast electron microscopy to directly image ordered systems, such as crystalline molecular materials. But they're eyeing applications far beyond. The Caltech researchers suggest that ultrafast electron imaging of biological particles, such as ribosomes, will provide more general information about complex energy landscapes. Ultimately, they want to unravel the functioning of more complicated biological systems such as whole cells.

"The idea that you could acquire real images with subpicosecond resolution was a change in the way chemists, physicists, and biologists could think about dynamics in the molecular systems," says [David A. Tirrell](#), chair of the division of chemistry and chemical engineering at Caltech.

Tirrell notes that many areas of science will benefit from the ultrafast electron microscope. He suggests that breakthroughs in chemistry, materials science, and surface science could stem from the ability to, for example, watch charges get transferred across interfaces from one material to another in energy conversion systems.

For Zewail and other Caltech researchers interested in physical biology, which is generally defined as using physical concepts to explore and understand biology, the interest in ultrafast techniques highlights a growing appreciation in biology for quantitative approaches, Tirrell adds.

Zewail says he recognized the scientific need for ultrafast electron diffraction and microscopy several years before he received the [1999 Nobel Prize in Chemistry](#) for the development of femtosecond chemistry. "I realized that as we get more and more involved in complex systems such as in biology and materials science, it is not enough to either determine the static structure or measure the time alone," he says. "To get at the function—let's call it the behavior—of either materials or biological systems, we have to integrate tools of structure and dynamics."

The only way to get an image is by magnifying a sample through microscopy, Zewail says. Direct imaging via ultrafast electron microscopy became reality in 2005, thanks to a \$17.5 million grant from the [Gordon & Betty Moore Foundation](#).

The ultrafast electron microscope at Caltech is a towering, modified transmission electron microscope interfaced to an expansive tabletop femtosecond-timed laser system, which is why researchers and visitors must wear safety glasses with dark lenses. Part of the laser beam is used to excite the sample, and the remainder is converted to femtosecond pulses, which result in single-electron packets suitable for probing molecular structures. Because electrons repel each other, single electrons are critical to achieving atomic-scale femtosecond time resolution, Zewail explains. Instead of bringing in all of the electrons at once and having them repel each other, as in some conventional electron microscopes, electrons come in one at a time in the ultrafast electron microscope.

When a single electron bounces off the excited sample in the microscope and hits the instrument's detector, the lenses inside the microscope magnify the object into a single frame that represents the femtosecond resolution and appears as an image on a computer screen. The process of acquiring frames is sequential, and the researchers can put all of the frames together to make a digital movie of what is happening on the atomic level.

The first generation of the microscope, which accelerated electrons to 120 keV, provided images of both

inorganic materials and stained cell slices at better spatial resolution than a conventional transmission electron microscope did.

The higher resolution second-generation microscope runs at 200 keV and is currently in use at Caltech (*Nano Lett.* **2007**, 7, 2545). It has helped researchers discover, for example, a previously unknown mechanical phenomenon in copper tetracyanoquinodimethane (CuTCNQ), a well-researched nanomaterial.

CuTCNQ, a quasi-one-dimensional semiconductor, has been studied for about 40 years, but the microscope's unique capabilities of spatial and temporal resolutions elucidated unexpected behavior, says David J. Flannigan, a postdoc in Zewail's group. Flannigan explains that initial images of the material from the ultrafast electron microscope showed a row of crystalline rods ranging from several micrometers to tens of nanometers. These images revealed a channel that forms and disappears in the row as a near-infrared laser pulse is turned on and off. The researchers captured the expansion and contraction of the CuTCNQ crystal frame by frame and combined them into a short movie (*Angew. Chem. Int. Ed.* **2007**, 46, 9206).

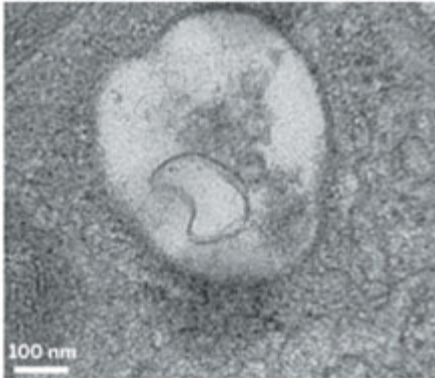
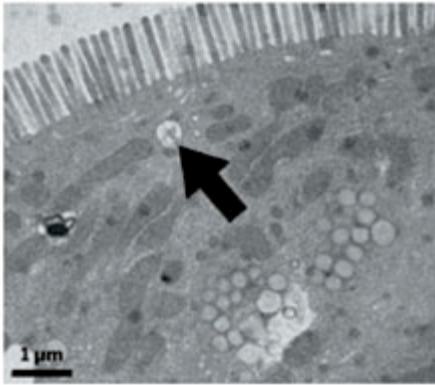
The researchers think that the opening and closing of the channel is due to a modulation of the π -electron interactions of the stacked TCNQ molecules. The pulse created a mixed valence structure that led to charge transfer and the formation of a new lattice along the stacking direction. In effect, the rods swelled and closed the channel. The results may be valuable for developing molecular nanoswitches, the researchers suggest.

Despite the advance in the second-generation microscope, it is not optimal. Samples must be kept under vacuum to prevent them from reacting with ambient gases. Zewail says the highest resolution third-generation microscope, which is slated to run at 300 keV and be operational next year, will be able to run samples under more natural conditions, such as atmospheric pressure or in a liquid.

Still, the microscope setup cannot study solid surfaces or handle gas samples. For this reason, Zewail's group continues to conduct research with ultrafast electron diffraction and crystallography, the precursors of ultrafast electron microscopy. Though these two earlier techniques do not provide direct images, they are still useful because they can provide temporal and dynamic information about samples that the microscope can't handle.

To handle gas samples, Zewail's group developed ultrafast electron diffraction. The technique produces diffraction patterns, which, after mathematical manipulation, yield structural information. And for more complex crystal samples, they developed ultrafast electron crystallography, which also produces diffraction scattering patterns that must be transformed mathematically to yield structural information.

As they do with ultrafast electron microscopy, the Caltech researchers use ultrafast electron crystallography to make movies of structural dynamics of, for example, interfacial macromolecular assemblies, nanostructures, and crystalline fatty acid bilayers. These movies have subnanometer spatial and subpicosecond temporal resolutions.



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Seeing Biology Details of a rat cell as imaged by ultrafast electron microscopy.

All three ultrafast electron techniques are based on the "pump and probe" concept. A sample is stimulated with light and then probed with electrons. The resulting data appear as patterns in the diffraction and crystallography techniques but as direct images in microscopy.

The earlier ultrafast techniques are operationally similar to conventional X-ray diffraction and crystallography. They involve probing the sample; obtaining information about the locations of atoms in space as a pattern of spots, lines, or rings; and using mathematical transformations to convert the patterns into molecular structures. But in ultrafast electron diffraction and crystallography, the delivery of electrons can be timed so that researchers can also determine dynamics in samples of gases and nanomaterials.

The ultrafast diffraction and crystallography instruments require a high-vacuum scattering chamber about the size of a kitchen oven. (At Caltech, it happens to be covered in aluminum foil and referred to as "the baked potato.") The chamber is integrated with a laser system. The laser generates femtosecond light pulses that are split between initiating a reaction in the sample and generating femtosecond-timed electron probes. After passing through a series of apertures and deflectors, the electron hits the excited sample.

Crystallography samples are kept under vacuum to prevent interaction with surrounding gases. Diffraction patterns are recorded by a camera equipped with single-electron detection capability. By comparing the changes among different pattern features and by using mathematical transformations, the researchers can parse out structure and dynamics.

For example, Zewail's group has used ultrafast crystallography to study the structure and dynamics of interfacial water, a thin layer of water in close proximity to a surface. It behaves differently than bulk water and is difficult to investigate experimentally. Hydrophobic and hydrophilic interactions of macromolecules with the thin water layer around them, however, play an important role in deciphering research questions such as how proteins fold. The group's ultrafast electron crystallographic study of a monolayer of ice on different substrates yielded long-sought information on bond distances (*Science* **2004**, *304*, 80).

[Nuh Gedik](#), a postdoctoral fellow in Zewail's group who will start as an assistant professor of physics at Massachusetts Institute of Technology next month, says one of the most rewarding parts of his work at Caltech was to be able to use ultrafast electron crystallography. He used the method to investigate structural dynamics of a cuprate superconductor that has fleeting phase transitions (*Science* **2007**, 316, 425). Because the team was able to directly watch the evolution of a lattice structure, he says, they discovered a photoinduced structural phase transition that cannot be seen with conventional techniques.

Zewail's group has helped answer other questions about phase transformations. For example, a study demonstrated that crystals undergo sequential atomic motions as they pass through phase transitions, which is similar to what happens with individual molecules during chemical reactions (*Science* **2007**, 318, 788).

In that study, "we looked at vanadium dioxide, a material that forms a crystal that is electrically insulating at room temperature but undergoes a phase transition to a metallic structure at 68 °C," explains Peter Baum, formerly a postdoctoral fellow in Zewail's group. Baum is currently setting up a physics research lab at Ludwig Maximilians University, in Munich, Germany. By using ultrafast electron crystallography with near-infrared sample excitation, the researchers could record the atomic motions in all three spatial dimensions and in time as they happen during the phase transition. "Vanadium atoms first separate from each other for a very short time before they move sideways toward their final positions," he says.

Baum and Zewail have looked deeper into the ultrafast realm and hope to generate attosecond (10^{-18}) electron pulses. These pulses would help extend the domain of ultrafast electron techniques to the subfemtosecond and attosecond timescales.

Getting to the attosecond timescale would allow researchers to probe the dynamics of electron motion. "What has not been observed so far is how the electrons react in space and time and how they move to the final configuration after an impulsive disturbance such as ultrafast ionization," Baum says.

Other researchers have reported creating light pulses as short as 130 attoseconds. And recent work by the Caltech researchers suggests that subattosecond electron pulses are conceptually feasible (*Proc. Natl. Acad. Sci. USA* **2007**, 104, 18409.)

Zewail has teamed up with other Caltech scientists to use the ultrafast techniques to answer otherwise intractable scientific questions. For example, [Grant J. Jensen](#), an assistant professor of biology, points out that much more is known about the biochemistry of a cell than about the cell's biomechanics. He predicts that for many samples, ultrafast electron methods will prove a more useful probe of biological structure and dynamics than techniques such as traditional crystallography and nuclear magnetic resonance.

In particular, Zewail and Jensen think they know how to get an image of a whole cell with the ultrafast electron microscope. They plan to image whole cells, which can contain more than 10,000 proteins, to further understand how structures with many components and possible conformations operate. "We are going to be able to image cells even though those proteins are randomly oriented everywhere," Zewail says. What those images will look like has yet to be determined. They have secured a grant from the National Institutes of Health but declined C&EN's request to elaborate on the proposed experiments.

Soon Caltech may not be the only research facility boasting an ultrafast electron microscope. Caltech, which owns a patent on the design, is negotiating an agreement to commercialize the technology with FEI, an electron microscope company based in Hillsboro, Ore., which Caltech contracted to build the researchers' original design. So from now on, peeking into the mysteries of chemical reactions or cells will no longer be left only to the imagination.

Channel Gating

With the ultrafast electron microscope, Caltech researchers gathered frames to create this movie of a channel that opens and closes in crystals of CuTCNQ as electron pulses are turned on and off.

Courtesy of David Flannigan

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