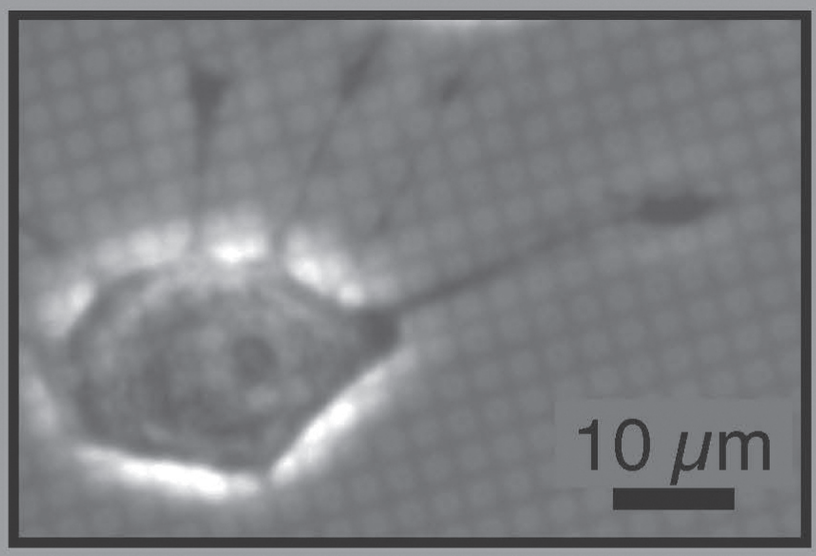
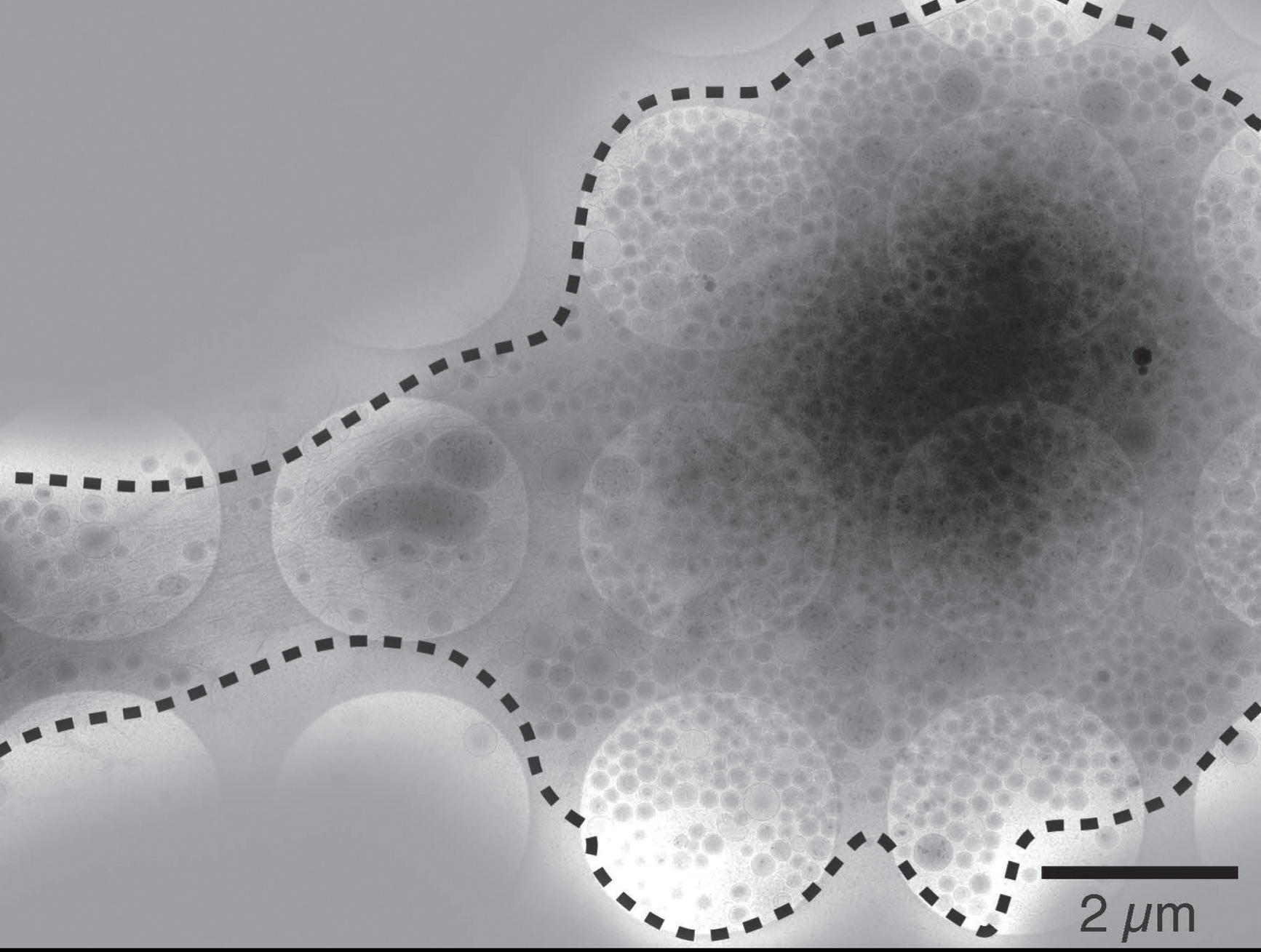


ANOTHER LOOK

NEW TECHNIQUES REVEAL ONCE-HIDDEN CLUES TO GUIDE DRUG DESIGN

BY SHARON TREGASKIS

IMAGES REPRINTED FROM "RIBOSOME-ASSOCIATED VESICLES: A DYNAMIC SUBCOMPARTMENT OF THE ENDOPLASMIC RETICULUM IN SECRETORY CELLS," S. D. CARTER ET AL., SCIENCE ADVANCES. LICENSED BY CC BY 4.0.



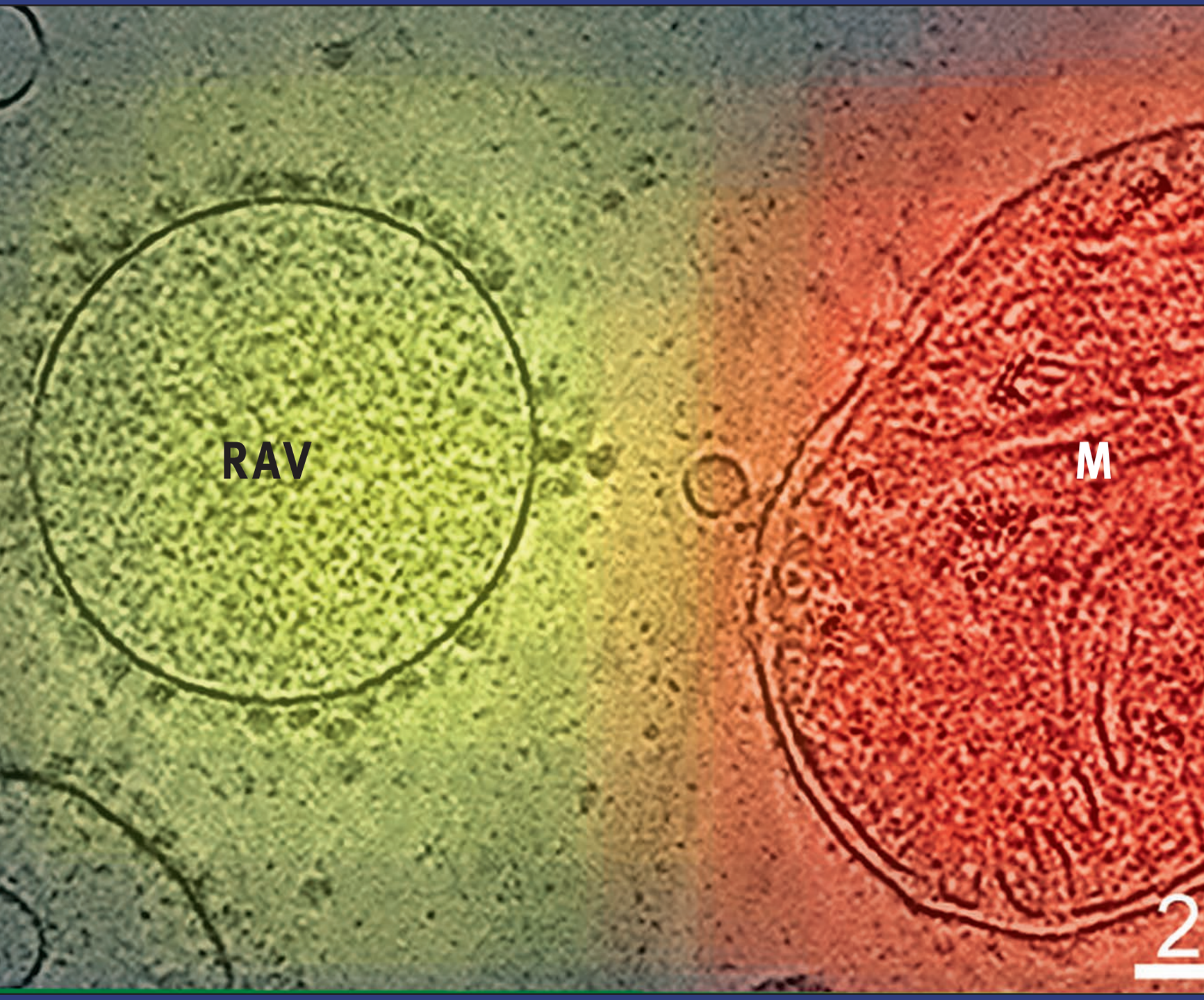
Margaret Bourke-White was 8 years old in 1912 when she accompanied her father to a foundry. The fiery spectacle of molten metal gleaming through smoke and grime captivated the girl. Fifteen years later, as a budding industrial photographer, she would talk her way into Cleveland's Otis Steel Co., intent on recreating the images burned in her mind's eye.

The camera, however, failed to capture what Bourke-White had seen. Her black-and-white film was sensitive only to blue light, and in her early prints, the luminous metal visible to the naked eye was as black as the soot that coated the factory.



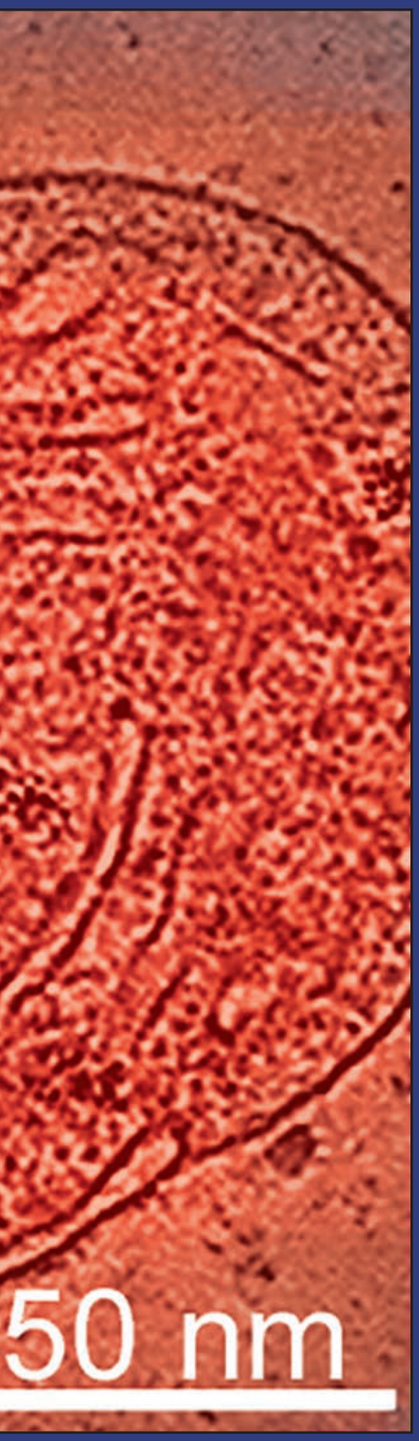
Like a photographer modifying aperture, exposure time and lighting to capture a fleeting moment, Zach Freyberg tweaks the tools of cryo-electron microscopy and tomography to visualize the subcellular machines that produce and transport proteins within intact cells. His findings suggest new ways for understanding chemical signaling throughout the human body and rational strategies for drug design. Here, a thin protrusion extends from the periphery of a secretory cell grown directly on a grid to produce images that preserve vital contextual details.

PHOTO: POURED MOLTEN STEEL IN THE OTIS STEEL MILL, CLEVELAND, OHIO, 1928. (BY MARGARET BOURKE-WHITE/ THE LIFE IMAGES COLLECTION VIA GETTY IMAGES)



Consider a tale of two brick towers—one a sturdy, 10-story building and the other featuring the same number of bricks, but at a 20° angle to the ground (an exaggerated Leaning Tower of Pisa). That second tower obviously wouldn't be habitable. But depending on the scale and style of analysis, says Freyberg, that fact might not be clear. "If you just ground up the bricks, ran them on a gel and compared them, those two structures would look the same." So when he started investigating how secretory cells produce and deliver chemical signals, Freyberg was committed to imaging whole cells to preserve context; that choice would unleash a flood of data. To focus its attention, the team, including collaborators in the laboratory of Grant Jensen at Caltech, used a combination imaging technology (dubbed CLEM) to color code: Red shows mitochondria, and yellow is the endoplasmic reticulum (ER). The fluorescence drew their eyes to a previously unknown, mobile form of the ER—the ribosome-associated vesicle (RAV).

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Throughout the winter of 1927–28, the photographer tweaked exposure, vantage point, printing process. Her skin burned and her camera blistered. At last, using a lighting technique developed in Hollywood, Bourke-White got her shot. "Saturate yourself with your subject," she would later write, "and the camera will all but take you by the hand and point the way."

Zach Freyberg has spent more than a decade tweaking techniques for another kind of imaging—cryo-electron microscopy and tomography inside cells. He is intent on seeing the engines of subcellular protein manufacture.

In April, Science Advances published a series of images captured by Freyberg that may add a chapter to basic biology textbooks. Freyberg is both a psychiatrist and a cell biologist—and a University of Pittsburgh assistant professor with appointments in both of those departments. His work reveals a previously unknown form of the endoplasmic reticulum. Freyberg collaborated with Nobel laureate Joachim Frank, Caltech's Grant Jensen and others on the study. The newly recognized form is dubbed RAV—for ribosome-associated vesicle. The discovery suggests a mechanism by which secretory cells throughout the body provide instantaneous, localized response to activity among cells. The work also suggests fresh treatment strategies for diseases like schizophrenia, Parkinson's and diabetes that trace their roots to glitches in chemical signaling.



Like Bourke-White's Otis Steel photos, the Freyberg images deploy novel imaging techniques to investigate the synergy of form and function. "If you can see the machinery that's responsible for local [protein] translation, especially in response to different levels of activity in the cell, the next step is learning to control it," says Freyberg, who sees the work as a step toward rational design of medications that coax plasticity in the aging or diseased brain.

Freyberg's work is inspired by a clinical conundrum that has plagued his own psychiatry practice since residency. It's an issue that stretches back to the 1950s, when antipsychotic meds were first introduced.

For every prescription penned for antipsychotics like chlorpromazine or risperidone, psychiatrists hazard as much harm as good. On the upside, after only a few doses, people with schizophrenia often report that their hallucinations have diminished in intensity.

Frequently, however, the drugs also precipitate rapid weight gain, insulin resistance, type 2 diabetes—life-shortening metabolic issues. Some patients are plagued by spasms and other neuromuscular side effects; a few suffer severe cardiovascular symptoms. For some 40 percent of people with schizophrenia, risk outweighs reward, and they quit their meds.

Those stats should surprise exactly no one in medicine. "While we still have many questions and poorly understand the therapeutic benefits of these medications in schizophrenia," says Freyberg, "we have even less understanding of why these medications have such profound metabolic side effects."

By the time Freyberg started his postdoc at Columbia, a growing body of literature suggested that like jostling a radio to boost its audio reception and eventually snapping a part, antipsychotics were rattling dopamine signaling throughout the human body. In one study, Freyberg and collaborators found that the pancreas produces its own dopamine, has its own dopamine receptors and uses dopamine to regulate insulin secretions.

Freyberg set out to figure out how antipsychotics altered that process. He decided to complement more conventional analyses with high-resolution 3D images of the structures within certain cells while the cells were intact. He looked in the pancreas, where dopamine impedes insulin production; in the kidneys, where it helps regulate blood pressure; and in the brain, where it influences the biochemistry of motivation and perception.

As an undergraduate in the mid-90s, Freyberg had become sensitized to the profound ways in which the processes of imaging itself can transform a subject. At the time, he was using various microscopy methods to study living sea urchin eggs. Delicate stuff.

"For the last 70 years, we've been looking at samples that were fixed, sectioned, exposed to heavy metals like lead and uranium," he says. "All of these treatments cumulatively affect the cell, so a lot of what's in the textbooks and what we think we know is a product of these processes."

X-ray crystallography, for example, generates extremely detailed 3D images of proteins, but the crystallization process destroys context. Transmission electron microscopy (TEM) produces detailed 2D images, but only works if electrons can move through a sample. Scientists fix a specimen with chemicals like formaldehyde; mount it in resin; and shave off slices measured by the nanometer. Then they bombard those slices with electrons.

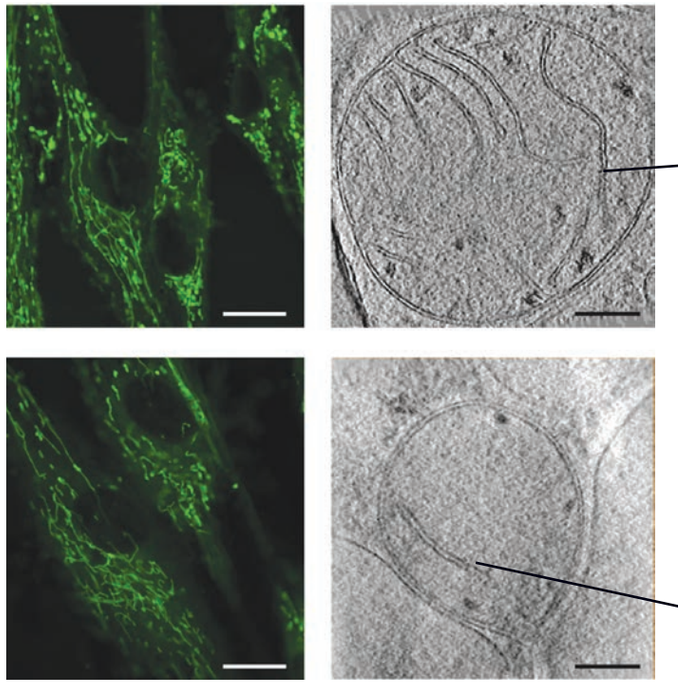
To study dopamine-secreting cells, Freyberg chose a technique that wouldn't be so rough on his samples: cryo-electron microscopy. CryoEM, as it's called, uses a deep freeze instead of chemicals to fix samples and lower-dose electron beams than conventional TEM, allowing the study of single proteins, nucleic acids and other relatively fragile biomolecules. And Joachim Frank was already using cryoEM at Columbia to study protein biosynthesis in the ribosome. So when Freyberg joined Columbia's faculty in 2011, he introduced himself.

Frank was imaging purified organelles in two dimensions. Freyberg opted to sacrifice resolution for context and expose whole cells to the electron beam. First, he grew native cells from the rat pancreas and rat cortical neurons as well as connective tissue from mouse embryos on cryo-electron microscopy grids, the inert scaffolds used to hold samples in place. Then, using a technique known as a "tomographic tilt series," the team captured dozens, and sometimes hundreds, of views of the same intact cell at 1° increments spanning 120° and digitally reassembled them into 3D representations.

"Tomography is definitely an area where we'll see the biggest payoff in the future," says Angela Gronenborn, chair of Pitt's Department of Structural Biology. "We'll do cellular biology in atomic detail, and up until now, that was impossible. It's no longer divide and conquer, but look at your targets or molecules in the normal cellular setting."

The Science Advances paper isn't the first time Freyberg has used such techniques to uncover insights obscured by other methods. In a paper for iScience, he and another team at Columbia, including Frank, identify structural anomalies associated with Leigh syndrome, a fatal neurological disorder caused by mutations that affect mitochondrial energy production.

By growing whole cells from a patient and a healthy control directly on the cryoEM grid, the team could analyze tomograms of the mitochondrial cristae. The cristae are the protruding folds within the organelle's inner membrane where a cell's energy source is synthesized; more cristae mean more energy. They documented profound differences in the cristae volume, shape and orientation from the patient and control.

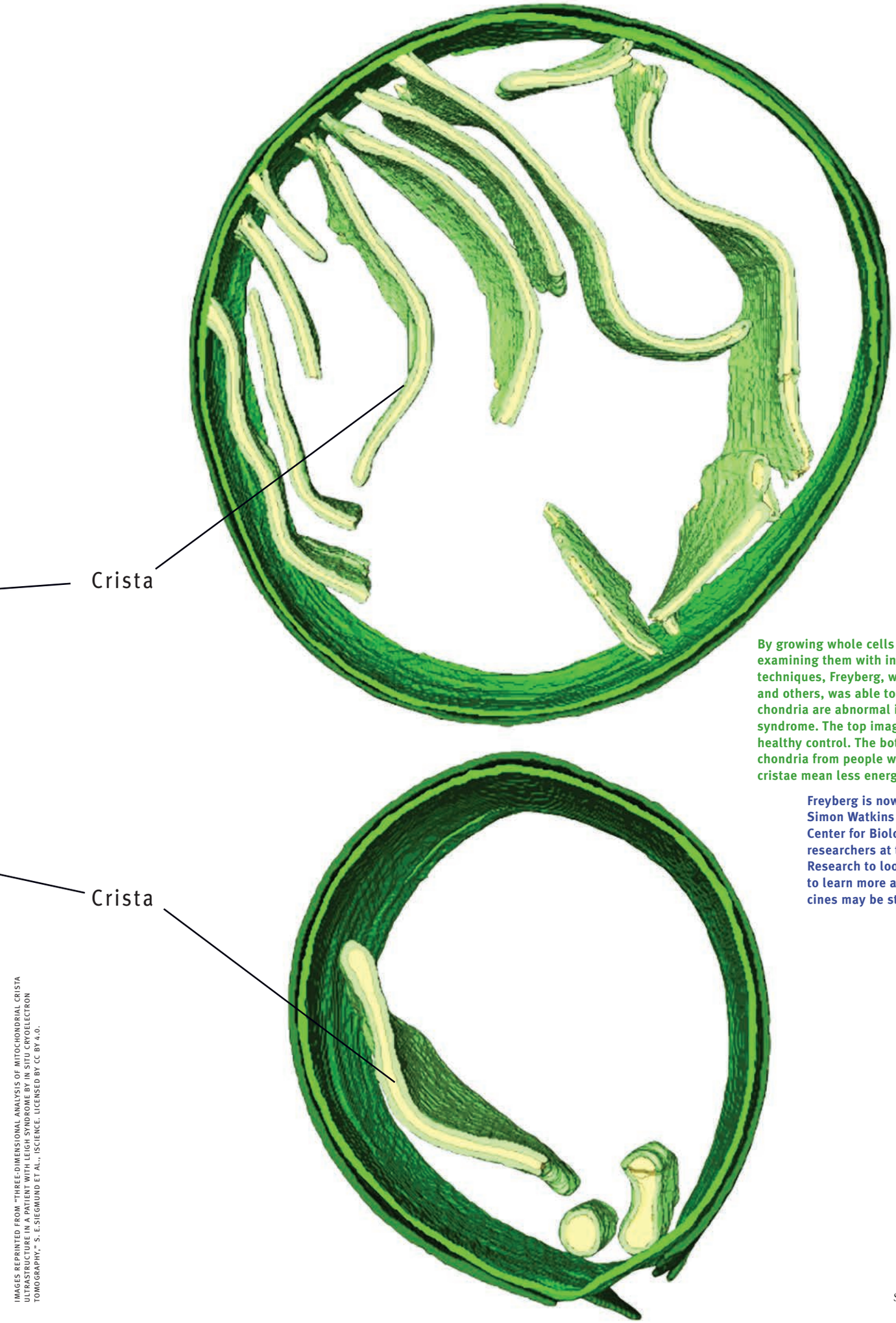


Scale bars represent 5 µm.

Last summer, Pitt acquired a new cryoEM instrument known as the Krios, capable of greater resolution and faster image collection than its predecessor. In partnership with the manufacturer, Freyberg has been refining tomographic imaging approaches to enhance his capacity to capture RAVs at greater resolution within cells. He speculates that the newly discovered vesicle plays a role in neuroplasticity, which isn't always desired. For instance, people with schizophrenia seem to have fewer dendrites. (Pitt's psychiatry chair David Lewis discovered this.) And Parkinson's seems to involve less dopamine production. Maybe figuring out how to alter RAV function will lead to tailored treatments that boost neurological function without compromising signaling elsewhere in the body.

Margaret Bourke-White was not yet 50, covering the Korean War, when she began experiencing the symptoms of Parkinson's disease that would end her career. She had followed her camera for more than three decades as a staff photographer first at Fortune and later LIFE, from the Great Depression to the American civil rights movement. She perched atop the gargoyles of the Chrysler Building, rode in a bomber over Tunisia, learned from Mahatma Gandhi how to spin.

"If you want to photograph a man spinning," she would later write, "give some thought to why he spins. Understanding for a photographer is as important as the equipment he uses."

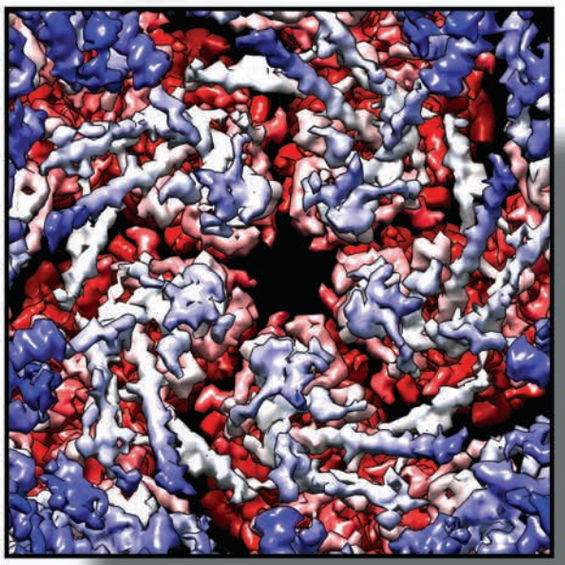


By growing whole cells on a grid, then examining them with inventive imaging techniques, Freyberg, with Joachim Frank and others, was able to see how mitochondria are abnormal in Leigh's syndrome. The top images show the healthy control. The bottom shows mitochondria from people with Leigh's. Fewer cristae mean less energy.

Freyberg is now partnering with Pitt's Simon Watkins and Alan Watson at the Center for Biological Imaging along with researchers at the Center for Vaccine Research to look at COVID-19. They hope to learn more about how the new vaccines may be stopping infection.

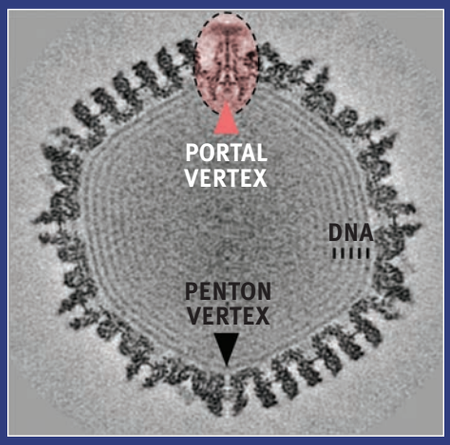
IMAGES REPRINTED FROM "THREE-DIMENSIONAL ANALYSIS OF MITOCHONDRIAL CRISTA ULTRASTRUCTURE IN A PATIENT WITH LEIGH SYNDROME BY IN SITU CRYOELECTRON TOMOGRAPHY," S. E.SIEGMUND ET AL., ISCIENCE. LICENSED BY CC BY 4.0.

James Conway's collaboration with Pitt professor Fred Homa focuses on the herpes simplex virus and the "portal vertex," where DNA moves in and out of the protein shell (or capsid). Shown here, a cryo-electron micrograph of herpesvirions superimposed with a model of the capsid and a close-up of the interior surface revealing numerous corkscrew-like structures (alpha-helices). To understand the roles of the shell proteins, Conway and group member Alexis Huet use genetically engineered variants of the capsid with slightly modified DNA when imaging. This and other approaches are giving them clues about how form affects function. Conway's knack for coaxing forth high-resolution data has led to a wealth of collaborations. He's coauthored structural analyses on hepatitis B virus, papillomavirus, enteroviruses, canine parvovirus as well as bacterial viruses (phages) HK97, lambda and T5, among others. "It's hard to say no," he concedes.



BUT WAIT, THERE'S MORE

BY SHARON TREGASKIS



What makes viruses so formidable? Their protective shells, called capsids, play a large part. Like a fireproof safe, the capsid contains and protects the genetic strands that give HIV, Zika and the flu their destructive powers. Identify each protein within the shell of a particular virus, in its relative position, and a vaccine or antidote designed to target the weakest link can crack the viral defense system without affecting the health of its host. Game over.

That's the hope. Pitt professor of structural biology James Conway has spent more than 25 years using cryo-electron microscopy to produce 3D maps of capsids by knitting together thousands of 2D images. He's shed light on the herpes capsid in particular. Vaccines and medications designed to target the weak links Conway uncovers could furnish more effective protection from Epstein-Barr, shingles and other herpes simplex viruses, with fewer side effects.

When Angela Gronenborn founded Pitt's Department of Structural Biology in 2004, in addition to recruiting talented researchers like Conway, she outfitted the basement of BST3 with the best imaging tools on the market—including a Polara cryo-electron microscope, which Conway has gotten to know quite well. So when the manufacturer announced a few years ago that the Polara would be end-lifed in 2018, Gronenborn wasted no time arranging to replace it with the latest model.

Pitt's brand new Titan Krios was installed last July.

"In structural biology this is *the* equipment," says Gronenborn. At a cost of $6 million, the unit represents a big financial commitment from the University and the National Institutes of Health. "It was very easy for the NIH to give us the money," says Gronenborn, "because we had a major commitment from the chancellor and Dr. Levine."

Conway served as principal investigator for the National Institutes of Health grant that brought the Krios to Pitt, and he managed the installation process to get it online.

Like the slicing, dicing Veg-O-Matic of late-night infomercial fame, the Krios operates in multiple modes. That flexibility makes it an attractive tool for scientists across Pitt's campus and elsewhere who are intent on imaging tiny structures while they are intact, including viruses, enzymatic machines and even suborganelles within whole cells.

In basic imaging mode, the machine generates thousands of single 2D images at extremely high resolution—so high it's measured by the Ångstrom. (That's one hundred-millionth of a centimeter, a unit typically used to characterize wavelengths and arrangements of atoms in space.) Using the Polara, Conway coaxed the resolution of his work from 10 Ångstroms down to 4. With the Krios, he's already reached a target of 2.1 Ångstroms. "[That] is very high," says Conway, "the kind of thing you expect from X-ray crystallography." (That's the technique Rosalind Franklin used that gave us the first view of the double helix.)

Conway expects to use the Krios to analyze particles not suitable for X-ray crystallography.

The machine is also able to bring larger (though still tiny), asymmetric objects into focus. In a tomography mode, it can knit together hundreds of 2D images into 3D representations. The approach has Pitt's Zach Freyberg making waves in cellular biology. (See p. 22.)

Conway is exploring a joint project with Pitt vaccine developers to visualize the spike protein that gives coronavirus its name (corona is Latin for crown). The researchers want to look at how the spike is organized and how antibodies or drugs bind to neutralize it.

His work on solving other viruses will help him hit the ground running. ■