People & Ideas

John Heuser: Capture the moment

Heuser uses quick-freeze, deep-etch electron microscopy to explore cellular ultrastructure.

As a postdoc working on synaptic transmission, electron microscopist John Heuser faced a seemingly intractable problem: the techniques available to him at the time were unable to capture the lightning-quick cellular events he wanted to study (1, 2). Rather than choose a different biological problem to study, Heuser began working on a technological solution to the issue: a new electron microscopy technique called quick-freeze, deep-etch electron microscopy (EM) (3).

Throughout his career, Heuser has made a close study of cell physiology. Having perfected his new EM technique, he has used it (together with other approaches) to generate striking images and new discoveries about the inner workings of cells (4, 5).

To get a closer look at his work and career, we called him at his EM facility at Missouri's Washington University. He'd just returned from a trip to his new lab in Kyoto, Japan, where he's teaching a new generation of students the art of quick-freeze, deep-etch EM and using the technique to probe the mysteries of stem cell biology.

BEST EXPOSURE

When were you first exposed to electron microscopy?

When I was an undergraduate at Harvard. I was planning to major in biochemistry, but they assigned me a tutor who was a pure electron microscopist, a fellow named J. David Robertson. He had trained with the first generation of electron microscopists like Frank Schmitt and Keith Porter, and, by the time I got to know him at Harvard, Dave had started up a crackerjack EM lab that would make major technical advances that would lead to the "unit membrane" concept [the idea that all cellular membranes share the same basic organization].

I was plopped into his lab as a junior in college, and I liked it so much I stayed there all the way through medical school. Photography was already a passion of mine—mainly 35mm film—and it was just incredible to be able to carry my hobby into the electron microscope and photograph cells at such high magnifications.

What sorts of things do you like to photograph, besides cells?

Well, I've spent a lot of time photographing my children, probably over-documenting the process of their growing up. I have so many photos that, looking back now, I sometimes think I should have stepped out from behind the camera more often. Even back in medical school, whenever we went on camping or canoeing trips, my classmates were always yelling at me, "Stop pointing the camera at us—stop making your 'thin sections of life'—and start paddling!"

But besides family, friends, and molecules, the thing I best like to photograph is people, in all their guises. I particularly like to photograph circuses, dancing, and things that are lively—I try to capture the moment that's the epitome of the event, when people are at their very best during their performance. I guess I've been after something similar with the electron microscope all this time, too.

> "I try to capture the moment that's the epitome of the event."

You mentioned that you trained as an MD…

Yes, I got my MD from Harvard also, but that was because I couldn't get into the graduate school there. They took four people a year into Steve Kuffler's neurobiology program, and I wasn't one of the four, so I went to medical school instead. I actually enjoyed medicine a lot but didn't stick with it. I graduated in a class with amazing people like Tom Pollard and Freddie Goldberg, and afterwards I went to London for a postdoc with Bernard Katz.

Katz is famous for his theory that synaptic vesicles discharge neurotransmitters and that this is the fundamental mechanism for communication in the nervous system. He won the Nobel Prize for this. When I arrived in his lab he said to me,

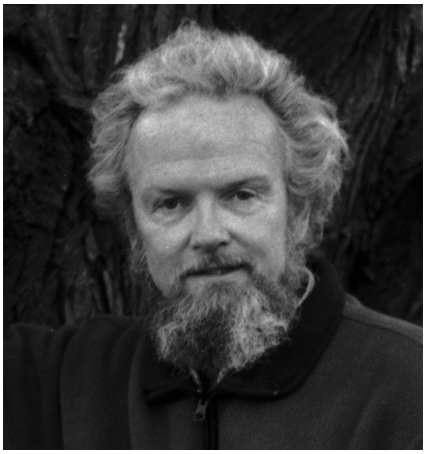

PHOTO COURTESY OF RON VALE

John Heuser

"Okay, we've got this electrophysiological evidence that some small compartment is doing this. You're an electron microscopist; go to the microscope and show it." And I did, although it took 15 years and some great collaborations to pull it off.

TIMING THE SHOT

Why was that?

Because chemical fixatives are so much slower than the process I was trying to capture! I could stimulate nerves and then throw them in fixative right away, but all that gave me was the aftermath of the event, which people at the time thought looked like ultrastructural damage. But I'd trained at Harvard with Robertson and Porter, so, the second I saw this aftermath, I guessed I'd caught the way the nerve recovers after a bout of discharge and that this was happening through endocytosis.

Soon after that, I managed to get a position at NIH working with Thomas Reese, who was a master of the horseradish peroxidase (HRP) technique. Together we showed that HRP added to the outsides of nerves during electrical stimulation ended up inside their synaptic vesicles if the nerves were stimulated. This proved that those vesicles had once been in continuity with the cell surface and had been endocytosed back in from the surface during their recycling. So we'd proven vesicle recycling, but

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I still hadn't gotten what I had started out for, which was to capture the discharge event.

So you worked out another approach…

The discharge event happens on a millisecond timescale, so right away Tom and I realized we couldn't wait for fixative to work; we had to immediately freeze our samples. Our Holy Grail was to get our samples into the microscope in an unfixed, frozen state, without ice crystals.

I'd always wanted to be an inventor, like my mother's father, who had patented some of the very early designs for lightbulbs, radio tubes, and other things. I thought maybe this would be my chance to follow in my grandpa's footsteps!

What Tom and I did was to basically improve on a design by Van Herreveld at Caltech, who had the brilliant idea of "slamming" a piece of tissue into a very, very cold block of metal to quick-freeze it. We perfected a more robust and workable version of his "slam-freezer," and it worked beautifully for imaging nerve terminals, so we got what we were shooting for in the end. The other payoff was that, gee whiz, we'd frozen our sample so quickly that there wasn't enough time for very large ice crystals to form in them, so we could use this technique to look at darn near anything in an unfixed state!

PHOTO COURTESY OF JOHN HEUSER

A ca. 1990 photo by John Heuser: Acrobats Sacha Pavlata and Lisa Giobbi, at the peak of a performance.

Later, while teaching in the neurobiology course at the Marine Biological Laboratory in Woods Hole, we and our students discovered that we could observe more detail in the samples if we let some of the water evaporate from our sample after freeze-fracturing—that's the "deep-etch" part of our "quick-freeze, deep-etch" technique.

THE BIGGER PICTURE

And you have used this technique to look at a lot of different things…

Yes. This approach is particularly good for looking at structures at or near the plasma membrane, so we've had a lot of collaborations that focused on things like endocytic structures or the assembly of pathogen proteins at the plasma membrane. One drawback of this technique, though, is that it freezes only a very thin film of material—10 µm at most. Things look great at 5 µm, though, so it works well for looking at tissue cultures, where we can readily expose membrane surfaces, either inside or outside of the cell.

Another criticism many people have of our approach is that, in order to visualize structures, we have to add a contrast agent. As is pretty standard in all of freeze-fracture EM, our contrast agent has always been a 2-nm–thick film of platinum, evaporated onto the sample's surface in a vacuum. People say that this metal film obscures too much detail; the metal film is like the frosting on a cake, so you don't quite see the real cake. But nothing has come along yet that will let us image our deep-etched samples any better. Maybe future scanning EMs will help.

By now, everyone agrees that freezing a sample is preferable to chemical fixation, but most people are using a different freezing technique that uses high pressures to avoid ice crystal formation. This lets you freeze much bigger samples, but they can't be freeze-fractured or deep-etched, and they don't yield enough contrast in the EM to see membrane surfaces "en face." In comparison, that's where quick-freeze, deep-etch EM excels.

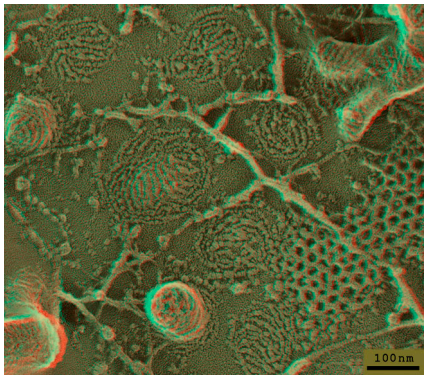

IMAGE COURTESY OF JOHN HEUSER

A quick-freeze, deep-etch EM anaglyph stereo image showing caveolae at the plasma membrane (circular, striped areas).

You've also used this to look at individual molecules…

Yes, our technique is good for looking at freeze-dried molecules, so probably 50% of my effort has involved helping people get to know their pet molecule. Of course, down at the molecular level, 2 nm of platinum frosting on the surface makes a big difference, because your molecule's only 10–30 nm in size to start with. Still, we've been pretty successful at providing folks with helpful "first looks" at their molecules.

> "Our Holy Grail was to get our samples into the microscope in an unfixed, frozen state."

We're actually doing a lot of this kind of work in my new lab, which I recently started at the Institute for Integrated Cell-Material Sciences in Japan. This is where induced pluripotent stem cells (iPSCs) were discovered, and people there are developing new nanomaterials that affect iPSCs as well as embryonic stem cells. These materials are right at the scale where our EM technique works best, so we're using it to help evaluate them. It's fun work, and, even though quick-freeze, deep-etch EM does not appear to be in vogue in America right now, a lot of people in Japan are interested in learning our techniques, so I'm enjoying it.

1. Heuser, J.E., and T.S. Reese. 1973. *J. Cell Biol.* 57:315–344.
2. Heuser, J.E., et al. 1979. *J. Cell Biol.* 81:275–300.
3. Heuser, J.E. 2011. *J. Electron Microsc. (Tokyo).* 60:S3–S29.
4. Heuser, J.E., and S.R. Salpeter. 1979. *J. Cell Biol.* 82:150–173.
5. Heuser, J.E. 1983. *J. Mol. Biol.* 169:155–195.