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The Guts of a Cell, Frozen in Time

A novel 3-D imaging technique provides a first look at the internal structure of human skin cells.

By Jocelyn Rice

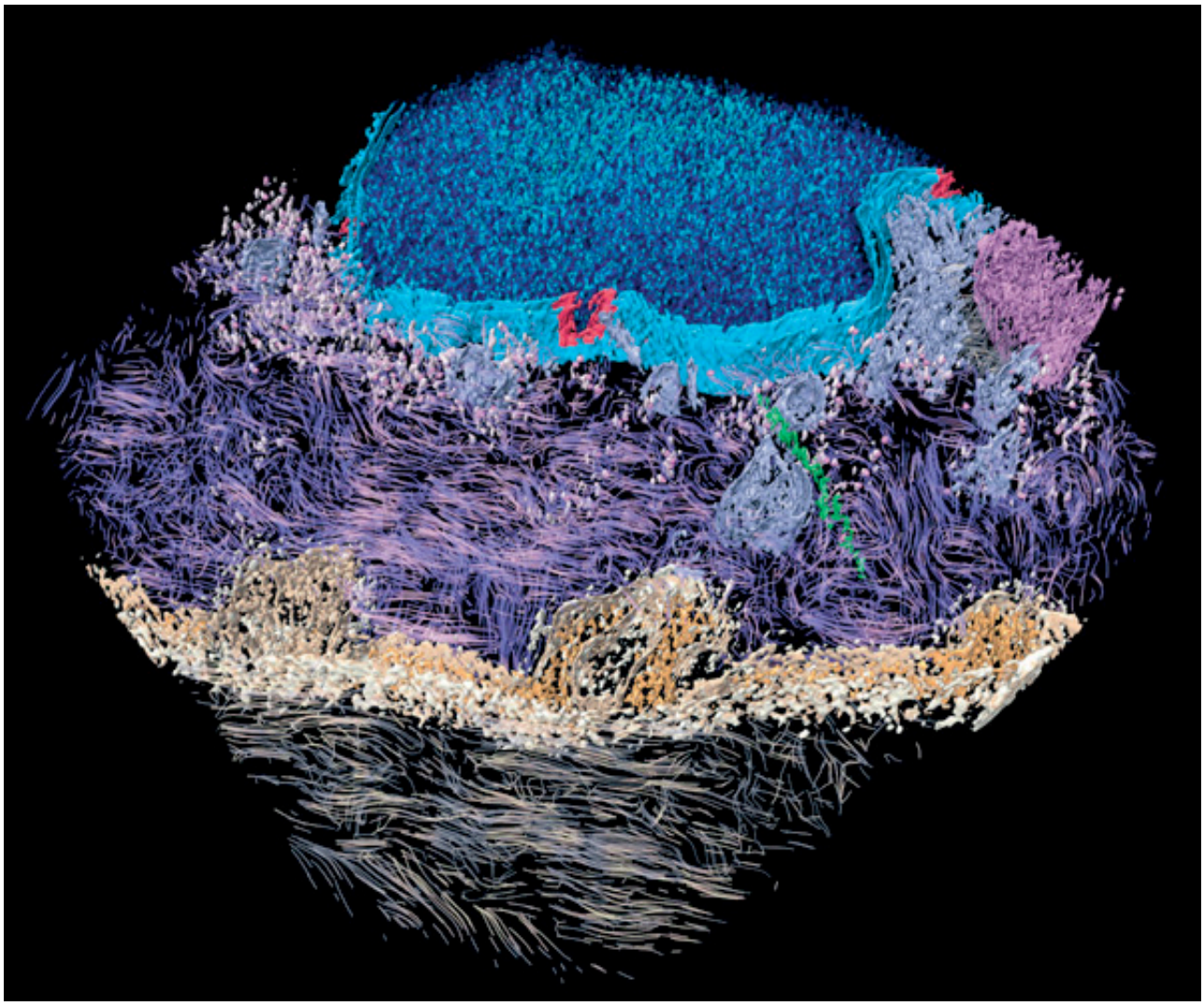
A new twist on a technique called cryo-electron tomography offers a closer-than-ever look inside a human skin cell: it generates a 3-D image with resolution fine enough to distinguish the structures of proteins. The new method, which involves freezing a cell and slicing it into thin sections, will allow scientists to probe how proteins organize and interact deep within a cell without disturbing them from their native states.

"At this resolution, the cell is essentially an uncharted territory," says Achilleas Frangakis (http://www-db.embl.de/jss/EmblGroupsOrg/per_3219.html) , a biologist at the European Molecular Biology Laboratory, in Heidelberg, Germany, who led the work. The images have a resolution of three to four nanometers, allowing scientists to discern the structures of individual proteins. Because the proteins have not been disrupted from their native positions, the scientists can glean clues about how they function and interact with one another in a living cell. "When you see the proteins, you immediately also see their interaction partners--how they interact in an undisturbed environment," says Frangakis.

Traditional electron tomography can generate 3-D extreme close-ups of cells, but the procedure comes at a cost. Samples to be studied typically undergo elaborate chemical treatment that allows them to withstand the vacuum within the microscope and the powerful beam of electrons used to generate the image. However, that chemical processing also disturbs proteins and organelles from their natural configurations, destroying valuable information about how they function.

Scientists can circumvent this problem by freezing a sample so quickly that ice crystals--which would ravage the cell's delicate internal structures--don't have time to form. But since samples must be extremely thin for cryo-electron tomography to work, most cell types were ineligible. Only tiny bacterial cells and the thin fringes of eukaryotic cells made the cut.

Now Frangakis and his team have developed a way to cut frozen cells into miniscule slices, revealing the previously unavailable innards of much thicker cells. This includes eukaryotic cells--cells with nuclei--like those that make up human tissues. The scientists then use a lower-power electron beam to image the sample, so that it holds up longer in the microscope. They have also refined the software needed to build a 3-D representation of the slice.



Frozen cells: *A thin, frozen slice of a human skin cell was bombarded with electrons and then reconstructed with specialized software to create this 3-D color-coded image. Each cellular structure has its own color: blue for the nucleus and its envelope, red for nuclear pores, purple for mitochondria, and brown for the cadherin proteins that allow the cell to adhere to its neighbors.*

Frangakis's group tried out its new procedure on human skin cells, which are thick enough that their inner workings are invisible using conventional cryo-electron tomography. The team first froze cells from the arm of a healthy donor to a frigid -200 ºC by plunging them into liquid nitrogen. Keeping the cells' temperature below -140 ºC, the scientists sliced them into 50-nanometer-thick sections with a diamond-bladed knife and then bombarded them with a relatively low-power beam of electrons in the microscope. Special software pared down the resulting image into even thinner virtual slices--as tiny as half a nanometer thick--to construct a 3-D representation called a tomogram. The results were published last week in the journal *Nature (http://www.nature.com/nature/)* .

These tomograms allowed the research team to resolve, with unprecedented detail, the Velcro-like mechanism that skin cells use to attach to one another. Proteins called cadherins protrude from the cells' surfaces and hook together in elaborate structures that had previously remained obscure. Thanks to the new imaging technique, Frangakis was able to determine precisely how the cadherin molecules interact. Each protein linked up with other cadherins in two different ways--one for proteins from the same cell, and one for proteins from a neighboring cell--to form an interlocking lattice.

Frangakis's accomplishment is "truly a first," says Grant Jensen (http://www.jensenlab.caltech.edu/) , a biologist at the California Institute of Technology who specializes in cryo-electron tomography. While using electron tomography on thinly sliced frozen cells had been attempted before, he says, "it never worked well enough to make any significant

conclusions from it."

Visualizing cells in as close to their natural state as possible is the key benefit of cryo-electron tomography, says Jensen. Frangakis's slicing technique, known as cryo-sectioning, opens the door to reaping that benefit on virtually any cell--not just on skinny ones. "Cryo-sectioning is going to allow us to look at any cell we want," Jensen says.

For his next project, Frangakis plans to use his new technique to plumb the innermost realms of rat kidney cells. He hopes to tease apart the structure of nuclear pore complexes--sophisticated protein assemblies that act as gatekeepers to the cell's nucleus, allowing only certain molecules to pass through.
