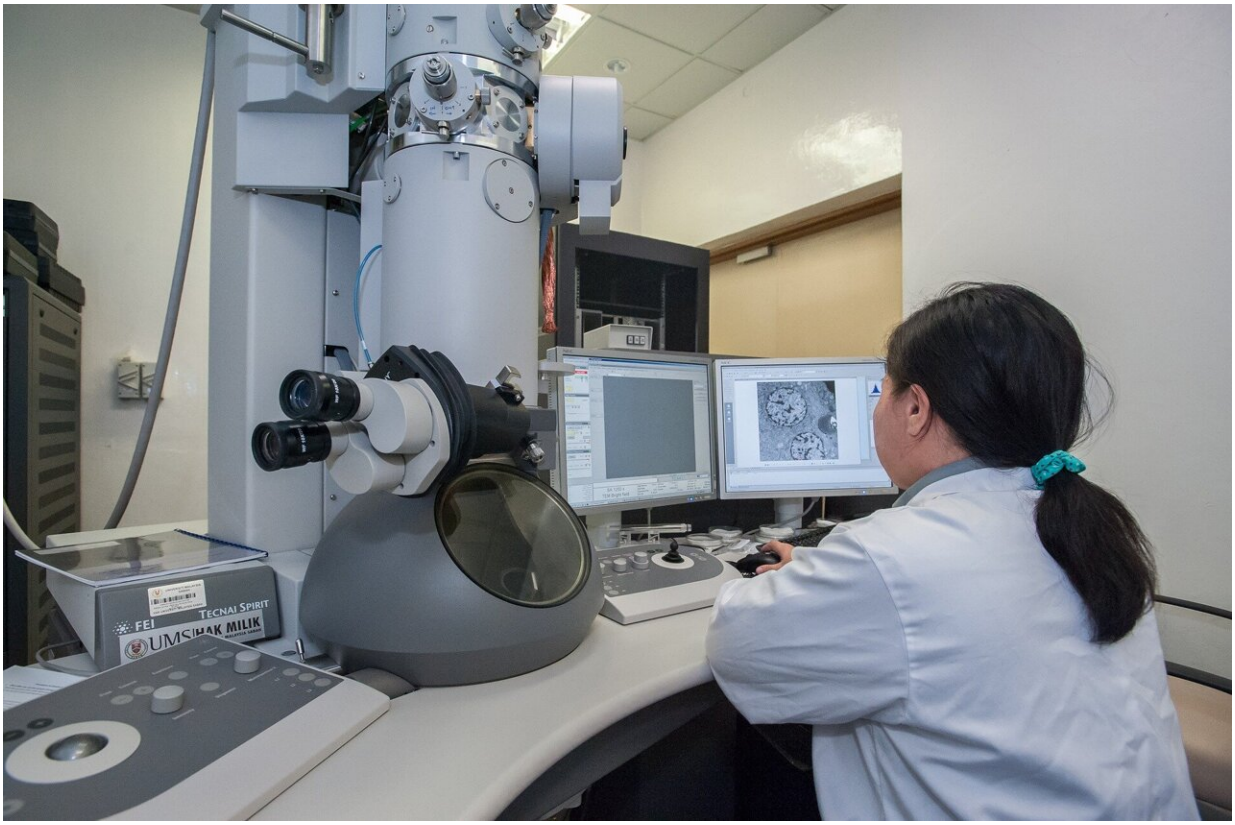
New technique boosts cryo-electron microscopy clarity, safety

September 21 2021, by Joshua A. Krisch



Credit: Pixabay/CC0 Public Domain

When scientists needed to visualize the structure of the spike protein, which coronaviruses use to infiltrate human cells, they turned to cryo-electron microscopy. One of the most powerful imaging tools in a

researcher's arsenal, cryo-electron microscopy (cryo-EM) can visualize proteins, pathogens and sundry cell components almost down to their individual atoms.

But preparing samples for cryo-EM is cumbersome process that relies on ethane—a powerful coolant in liquid form, and a flammable gas at room temperature prone to explosions.

A new study published Sept. 7 in the *International Union of Crystallography Journal* demonstrates that cryo-EM samples can be prepared with a safer and less expensive coolant—liquid nitrogen—and these samples can produce even sharper images than those prepared with ethane. The findings upend conventional wisdom dating back to the 1980s, and may improve the safety and quality of cryo-EM.

"Ethane is not a standard lab chemical. It is hazardous, and using it adds additional complications," said senior author Robert Thorne, professor of physics in the College of Arts and Sciences and a Weiss Presidential Fellow. "Liquid nitrogen is the coolant of choice."

Cryo-EM works by firing electrons through molecules that are flash frozen in a glassy sheet of water, capturing multiple fuzzy images of the molecules within the ice. Sophisticated software can often average those blurry clips into one crisp 3D image, but not consistently.

Some of the blurriness comes from the sample itself. When the water encasing the molecules is cooled too slowly, it forms ice crystals that degrade the image. Scientists circumvent this problem by using ethane to cool the water so quickly that it snaps into a glassy, crystal-free sheet. But such rapid freezing puts stress on the sheet, which rests on a thin film of gold. When the electron beam hits the sheet, stress causes the molecules to move, blurring the final image in a phenomenon known as beam-induced motion.

"We have two opposing factors," Thorne said. "We want to cool the samples fast, to prevent ice crystal formation and to capture the biological structure of the molecules. But we also want to cool the samples as slowly as possible to minimize their motion during imaging."

Ethane cools samples very quickly. But researchers must use liquid nitrogen to convert ethane gas into a liquid, and then more liquid nitrogen to store samples after they are frozen. "Ethane is cumbersome, it's dangerous, and, ultimately, the samples end up in liquid nitrogen anyway," Thorne said.

Liquid nitrogen cools at rates roughly 50 times slower than those of ethane, according to reports over the last 40 years, and that's not fast enough to convert water into a glassy sheet. But in 2006, Thorne's research group discovered the main factor slowing down nitrogen was cold gas hovering above the surface of the liquid, which cooled small samples before they ever got to the liquid.

Thorne's company, MiTeGen, eventually developed an automated cooling instrument for X-ray crystallography—another method used to image protein molecules—that removes the cold gas just before a sample is plunged into nitrogen, and found that cooling rates increased to a mere six times slower than ethane. MiTeGen staff then adapted their cooling instrument for cryo-EM samples and collaborated with staff at the Cornell Center for Materials Research and postdoctoral associate Jonathan Clinger to collect and analyze cryo-EM data.

As the new study reports, nitrogen cools at the perfect speed for cryo-EM sample preparation—fast enough to avoid significant ice crystal formation, but slow enough to reduce beam-induced motion later on.

"Ethane is overkill," Thorne said. "For speed you don't need, you're getting blurry images with beam-induced motion, and that is more

problematic than any ice crystals that form from slightly slower cooling."

And all-liquid nitrogen cooling, Thorne said, will simplify cryo-EM workflows, removing the extra steps demanded by ethane and making it easier to design automated cooling instruments that meet current lab safety standards.

"This is a nice illustration of how basic academic science—looking into how small objects cool and how ice forms within them—can lead to practical solutions and commercial products."

More information: Tyler Engstrom et al, High-resolution single-particle cryo-EM of samples vitrified in boiling nitrogen, *IUCrJ* (2021). DOI: 10.1107/S2052252521008095

Provided by Cornell University