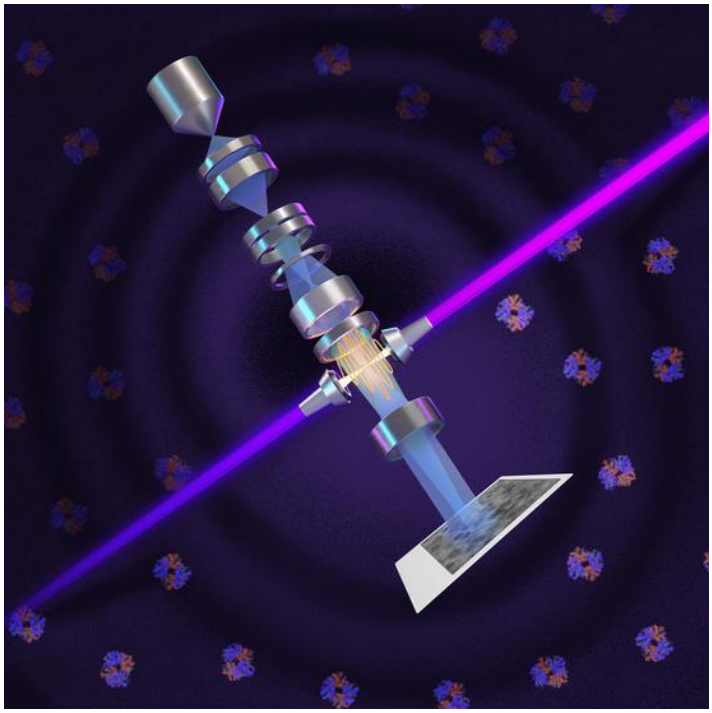
Laser Beam Brings Contrast to Electron Microscopy

Physicists have demonstrated a laser-based method that enables a cryogenic electron microscope to image small protein structures with greater detail than previously possible.

By **Susan Curtis**

Cryogenic electron microscopy, more commonly known as cryo-EM, has become a vital tool for elucidating the structures of complex biomolecules. But even the best instruments struggle to produce clear images of small proteins, which constitute around 90% of those found in the human body. Now Holger Müller and colleagues at the University of California, Berkeley, have developed a laser-based method for controlling the phase of an electron beam that leads to higher contrast in cryo-EM images [**1**]. In demonstrations, the method enhanced the resolution of small-protein images, paving the way for biologists to uncover a new domain of protein structures that are beyond the reach of current techniques.



The laser phase plate makes high-contrast cryo-EM images possible by exploiting an intense laser beam to alter the phase of the electron beam.
Credit: Sayo Studio

Cryo-EM reconstructs 3D images from frozen samples containing thousands or even millions of identical molecules. Freezing the samples preserves them in a near-natural state, while also reducing the damaging effects of the electron beam. However, electrons interact only weakly with biological specimens, which limits the image contrast. Combined with high noise levels, the weak image contrast places a lower limit on the size of the molecules that can be studied.

An obvious way to improve the contrast would be to insert a so-called phase plate, a component that shifts the phase of a wave by a set amount (usually denoted by an angle). Phase plates that change the phase by 90°, also called quarter-wave plates, are widely used in optical microscopy to visualize specimens that are almost transparent. Light passing through the sample is not attenuated, but some of the light acquires small phase changes when it is diffracted by the specimen. The phase plate is used to shift the phase of the undiffracted reference signal. With this additional phase shift, interference between the diffracted and undiffracted beams produces contrast when the two beams hit the camera.

Previous studies have attempted to replicate this scheme in a cryo-EM, but adding a physical phase plate has been shown to

cause signal loss and even compromise the resolution of the images. To overcome this problem, Müller and colleagues have been developing a laser-based phase plate, a solution that the group first proposed in 2010 [**2**]. The idea is to use an intense laser that interacts with the electron beam through stimulated Compton scattering. This scattering alters the phase of the electron waves that make up the beam.

However, achieving a phase shift of 90° requires a continuous laser intensity of around 400 GW/cm^2, which had previously only been produced by pulsed lasers. In 2021, the team achieved this record-breaking performance for the first time by trapping a laser beam inside an optical cavity with concave mirrored surfaces [**3**]. Resonance effects inside the cavity amplify the power of the laser beam to 80 kW while also focusing it to a spot size of 6.7 µm. At such high intensities, sustaining the resonant conditions inside the cavity becomes a major challenge. "The radiation pressure pushes the mirrors apart, so we need to optimize multiple feedback loops to counteract the length changes and keep the cavity precisely in resonance," Müller says.

Initial experiments with an older electron microscope proved the concept of the laser phase plate, but the resulting images did not show improved resolution. In this new work, the researchers fitted the laser phase plate to a state-of-the-art cryo-EM and tested its performance using two specimens. One was aldolase, a protein found in muscle that is large enough to be imaged with a conventional cryo-EM. The other was hemoglobin, a blood-based protein that is small enough to test the lower limit of current machines.

The laser phase plate was shown to improve the image resolution for both proteins, but the benefit was more notable for the smaller hemoglobin molecule. In one experiment, the resolution of the hemoglobin structure increased from a relatively poor 4.46 angstroms to 3.09 angstroms when the laser was switched on. For both samples, the laser phase plate yielded other improvements in imaging performance that could be particularly important for a newer technique, called cryo-electron tomography. This "cryo-ET" can be used to create 3D images of individual objects inside cells or other complex samples.

Müller believes that using the laser phase plate could lead indirectly to further gains in resolution. In conventional cryo-EM, the electron beam is intentionally defocused to provide partial phase contrast, but the laser phase plate makes this strategy unnecessary. "Moving to in-focus imaging would boost the resolution by at least as much as we have shown in the current work," Müller says.

Richard Henderson, who shared the 2017 Nobel Prize in Chemistry for his work on cryo-EM, is excited by the development, as it could extend the reach of cryo-EM to smaller proteins. "Cryo-EM desperately needs a good quarter-wave plate, and the laser phase plate being developed at Berkeley shows great promise for fulfilling this need."

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REFERENCES

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