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Laser Phase Plate: Advancing Beyond Proof-of-Concept

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In cryogenic single-particle electron microscopy (cryo-EM) and tomography (cryo-ET), a phase plate can provide optimum image contrast for weak-phase objects. It does so by selectively phase-shifting the undiffracted part of the electron wave function by 90° relative to the diffracted part in the back focal plane (BFP) of the objective lens. We have demonstrated a laser phase plate (LPP), based on coherently manipulating the electron wave function with a continuous laser beam, which is built up to an intensity of ~ 400 GW/cm^2 by resonance in a Fabry-Perot cavity. To overcome space limitations in the BFP, and to obtain a low cut-on spatial frequency (where phase contrast becomes effective) of ~ 0.004 nm^{-1}, we use a relay lens to generate a 5.7-fold magnified conjugate BFP, corresponding to an effective focal length of f_{eff} = 20 mm of the electron optics, compared to 3.5 mm for the objective lens alone. This comes at the cost of increased spherical and chromatic aberration: Cs = 4.7 mm compared to 2.7 mm and Cc = 7.6 mm compared to 2.7 mm.

Initial work obtained a high-resolution map of 20S proteasome particles (Fig. 1, left) and demonstrated both the long-term stability of the LPP during data collection and the high contrast of images [1]. Processing of the images required no special expertise beyond that needed to process standard cryo-EM data. The phase shift can be tuned from 0° to at least 135°. We then attempted to reconstruct other particles and to improve the resolution or the number of particles needed for a reconstruction beyond what can be achieved without phase contrast. This, however, revealed a systematic limit on the resolution, in addition to the expected falloff of the temporal coherence envelope.

Surprisingly, our experiments have shown thermal magnetic-field fluctuations [2] to be the cause (Fig. 1, right). Thermal currents (Johnson noise) in the beam liner tube (of length L and diameter D) at a temperature T generate fluctuating magnetic fields, and thus random deflections of the electron beam, with a variance of $\langle\theta^2\rangle \sim 0.67\,(e/p)^2\,\mu_0\,k_B\,T\,L/D^2$ when $L \gg D$, where p and e are the electron momentum and charge, μ_0 the vacuum permittivity, and k_B the Boltzmann constant [3]. Such fluctuations are usually negligible. In the current prototype of the LPP, however, the electrons pass through a long and narrow hole, L = 20 mm and D = 2 mm. Along with the long effective focal length of the relay optics, this increases the effect on the resolution.

To verify this, we characterized the contrast transfer function (CTF) by comparing the amplitude of Fourier transforms of images of thin carbon films in phase plate mode and in standard mode, where the relay optics are deactivated. This method factors out the structure factor of the carbon films and the detective quantum efficiency of the camera. The CTFs shown in Fig 1 (right) have been obtained by multiplying the measured ratio by the CTF in standard mode, obtained from manufacturer's specifications. We then replaced the LPP with mechanical dummies with progressively larger hole diameter and even removed the dummies completely, which is expected to improve the resolution if magnetic field fluctuations are the cause. Our data shows good agreement with a model combining thermal magnetic field fluctuations with the temporal-coherence envelope. With a D = 4-mm and especially 8-mm hole size, the CTF is as good as without LPP, and consistent with temporal coherence being the only limitation. We are currently manufacturing a functioning LPP with an 8-mm hole and expect to test it in May 2022.

Soon, we hope to also overcome the current limit from chromatic aberration by installing a gun monochromator to reduce the electron-beam energy spread. This is currently scheduled for June 2022. In addition, to compensate for the increase in Cs due to the presence of the relay optics, we will begin working with a microscope equipped with a Cs corrector, in addition to a gun monochromator. This microscope will also feature a post-column energy filter as well as an improved camera and make use of a newly designed dual LPP with crossed laser beams, which we expect to suppress the weak ghost images observed with a single LPP. This instrument is projected for delivery in Q1, 2023. Looking even further ahead, we are currently exploring the fabrication of a miniature LPP, which could be placed directly into the back focal plane of the objective lens. This would avoid the need for relay optics, at a cost in performance at the lowest spatial frequencies [4].

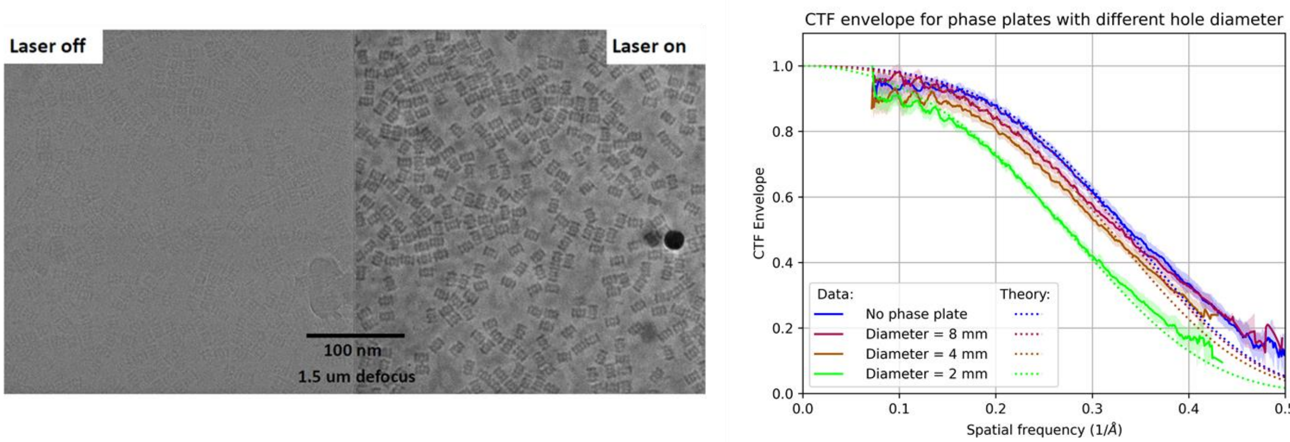

Figure 1. Left: Images of 20S proteasome with and without the LPP, using the same gray scales. **Right:** Contrast transfer function (CTF) envelope in phase plate mode measured without any LPP and with LPP dummies having different hole diameters. Theory based on Johnson noise and the increased Cc of the relay optics (dotted lines) and experiment (solid lines) agree well and indicate that the performance of an LPP with 8 mm hole diameter is indistinguishable from that expected from temporal coherence alone.

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