Phase Contrast Electron Microscopy Using Lasers

Jeremy J. Axelrod¹* , Sara L. Campbell¹, Osip Schwartz¹, Carter Turnbaugh¹, Robert M. Glaeser²,³, and Holger Müller¹,³

¹. Department of Physics, University of California—Berkeley, Berkeley, USA.
². Department of Molecular and Cell Biology, University of California—Berkeley, Berkeley, USA.
³. Lawrence Berkeley National Laboratory, Berkeley, USA.
* Corresponding author: jaxelrod@berkeley.edu

Transmission electron microscopy (TEM) of thin phase objects provides only low image contrast due to incomplete conversion of phase modulation to amplitude modulation in the transmitted electron’s wave function. Defocusing the imaging system is the most common technique by which this incomplete conversion is accomplished. However, defocusing cannot generate contrast for low spatial frequency components in the image. In fields like cryo-electron microscopy, these low spatial frequencies are needed to align particle images for averaging to increase the signal-to-noise ratio. The transmitted electron wave function consists of two components: the scattered wave, which has interacted with the sample, and the unscattered wave, which has not. If a 90° phase shift is applied between these two components of the wave, they interfere at the image plane providing maximal amplitude contrast. Such a phase shift can be applied in the diffraction plane where the unscattered wave is focused to a single point. (see figure 1). This technique is known in optical microscopy as Zernike phase contrast [1], and the device used to generate the phase shift is referred to as a phase plate. Application of this technique to TEM has long been considered, with initial proposals coming as early as 1947 [2]. However, realizing the requisite phase shift for an electron wave function is non-trivial. Most phase plate designs require matter to be placed in or near the electron beam, which causes varying degrees of electron loss due to scattering, and instability of the imaging transfer function due to electrostatic charging. The most successful phase plate to date is the Volta phase plate, which has been used to provide impressive contrast enhancement, but still suffers from the afore-mentioned issues [3].

To avoid such issues, we have designed and implemented a phase plate which uses a tightly-focused, high-intensity laser beam to phase shift the unscattered wave [4]. Since no material is placed in or near the electron beam, the phase plate does not charge or scatter electrons. The phase shift arises from the ponderomotive potential of the laser beam’s electromagnetic field. A Fabry-Perot optical cavity is used to both focus and resonantly enhance the amplitude of the light. This allows sufficient light intensity to be achieved with a continuous-wave laser, making the device compatible with the continuously-operating (non-pulsed) electron guns found in most TEMs. The focusing provided by the cavity allows for the unscattered wave to be selectively phase shifted relative to the scattered wave, since the scattered wave spreads over a much larger area than the width of the laser beam focus. Initial tests of our prototype device have shown that it can provide up to 45° of phase shift for an 80 keV electron beam (half of this for a 300 keV beam) [5]. So far, the maximum phase shift has been limited by the quality of mirrors used in the Fabry-Perot cavity and the Pound-Drever-Hall laser frequency feedback system used to keep the laser light resonant with the cavity. Figure 2 shows a comparison between images of a thin carbon film taken with and without the laser phase plate, displaying the increase in image contrast at low spatial frequencies. We expect that further improvements to the optics in our setup will soon allow for a full 90° phase shift to be achieved for 300 keV electrons. Our efforts will then turn to using the device as a stable phase plate for image contrast enhancement in cryo-electron microscopy.

Figure 1. Zernike phase contrast in a TEM using a laser beam. The laser beam (red) is focused to a small spot in the diffraction plane where it intersects the unscattered electron wave. By applying a 90° phase shift to the unscattered beam, phase modulation in the electron wave is converted to amplitude modulation (equations on the right).

Figure 2. Left: an image of a thin amorphous carbon film, close to focus, with the laser phase plate deactivated. The inset displays the Fourier transform of the image, showing little contrast at low spatial frequencies (center of inset). Right: an image of the same sample, close to focus, with the laser phase plate activated, providing approximately 20° of phase shift. The Fourier transform (inset) shows a substantial increase in image contrast at low spatial frequencies. The dark stripe in the Fourier transform is due to the presence of the laser beam.