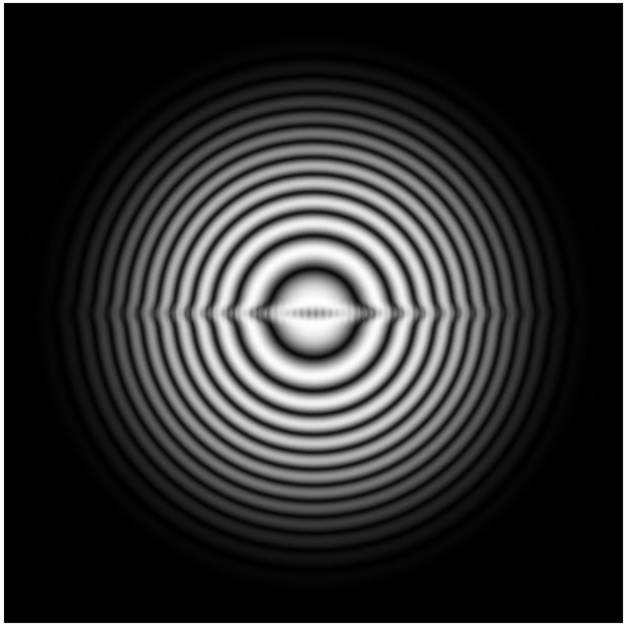
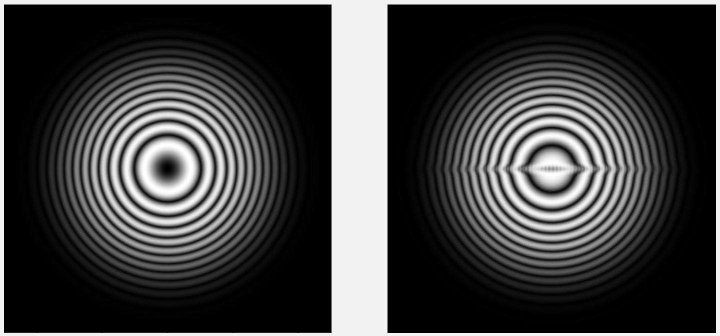
Laser phase-contrast transmission electron microscopy and associated computational opportunities

Petar Petrov
UC Berkeley

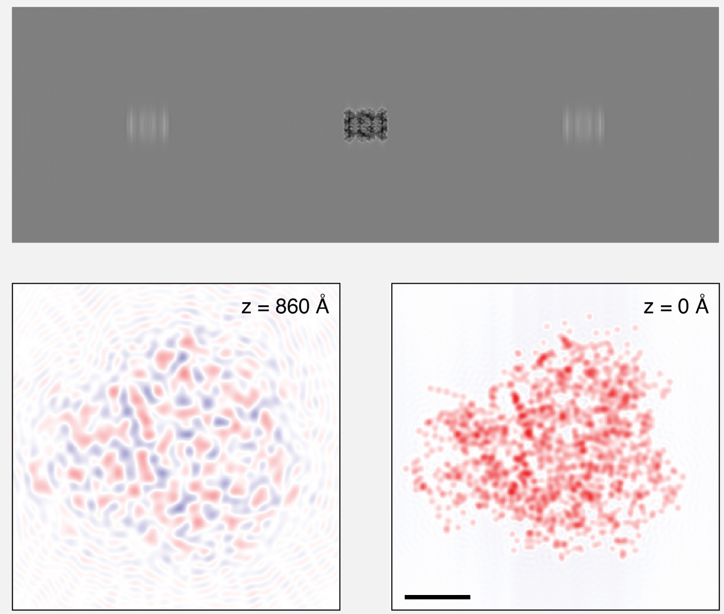


Los Angeles, CA
November 15, 2022

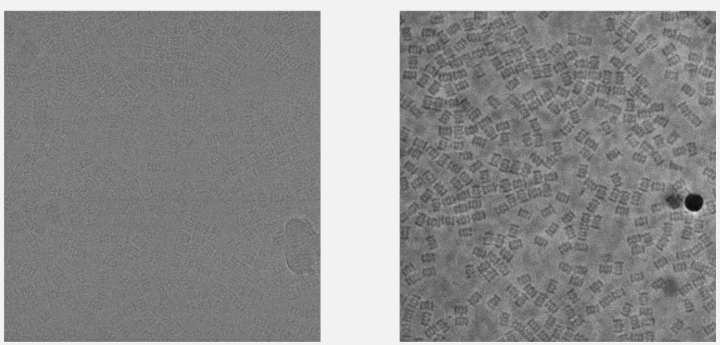
Part I:
Laser-Based Phase Contrast



Part II:
Assorted Computational Opportunities



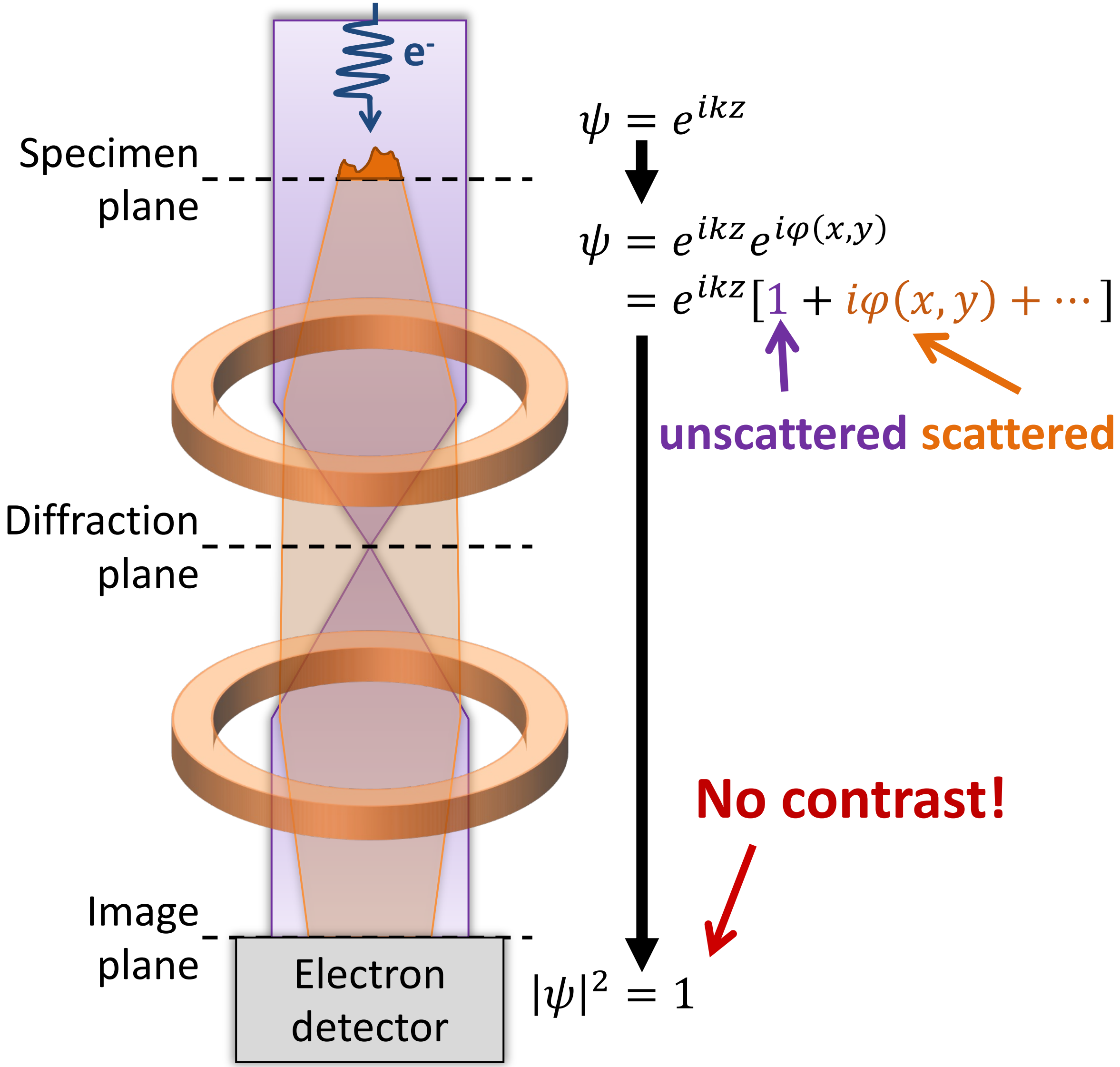
Part III:
Recent Experimental Progress



Part I
Laser-Based Phase Contrast

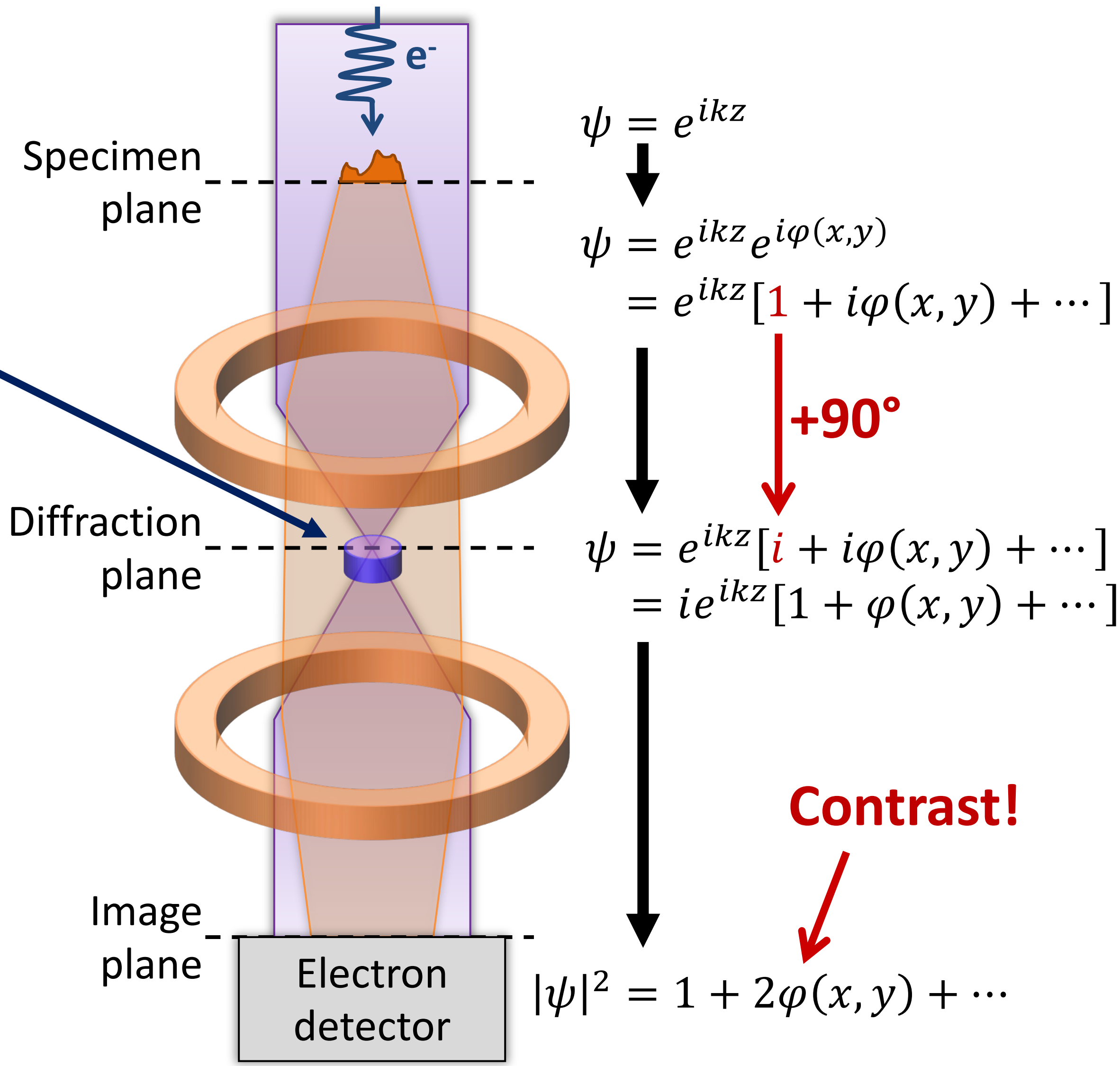
Biological specimens are weak-phase objects

- Biomolecules interact weakly with the illuminating beam, imparting a small phase shift $\varphi \ll 1$
- The image has **no contrast**
- Defocus f introduces phase $\exp(-i\pi\lambda_e f s^2)$ in the diffraction plane
    - Varies with spatial frequency s.



A 90º phase shift enables phase contrast imaging

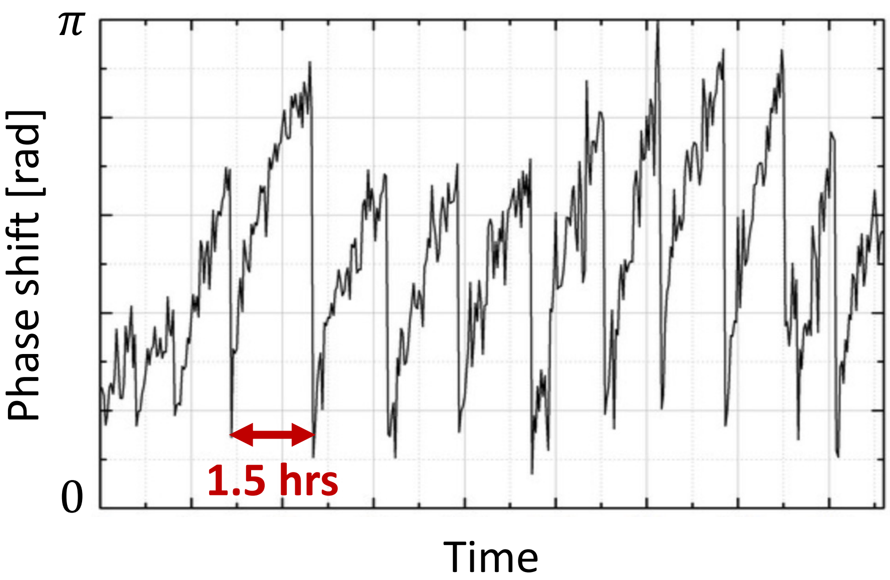
- Scattered and "unscattered" beams are spatially separated in the diffraction plane
    - Tune their phases separately with a phase plate
    - First implemented by Zernike for optical microscopy (1942)
- Experimentally challenging in electron microscopy
    - Proposal: Boersch (1947)
    - Volta phase plate: Danev (2014)

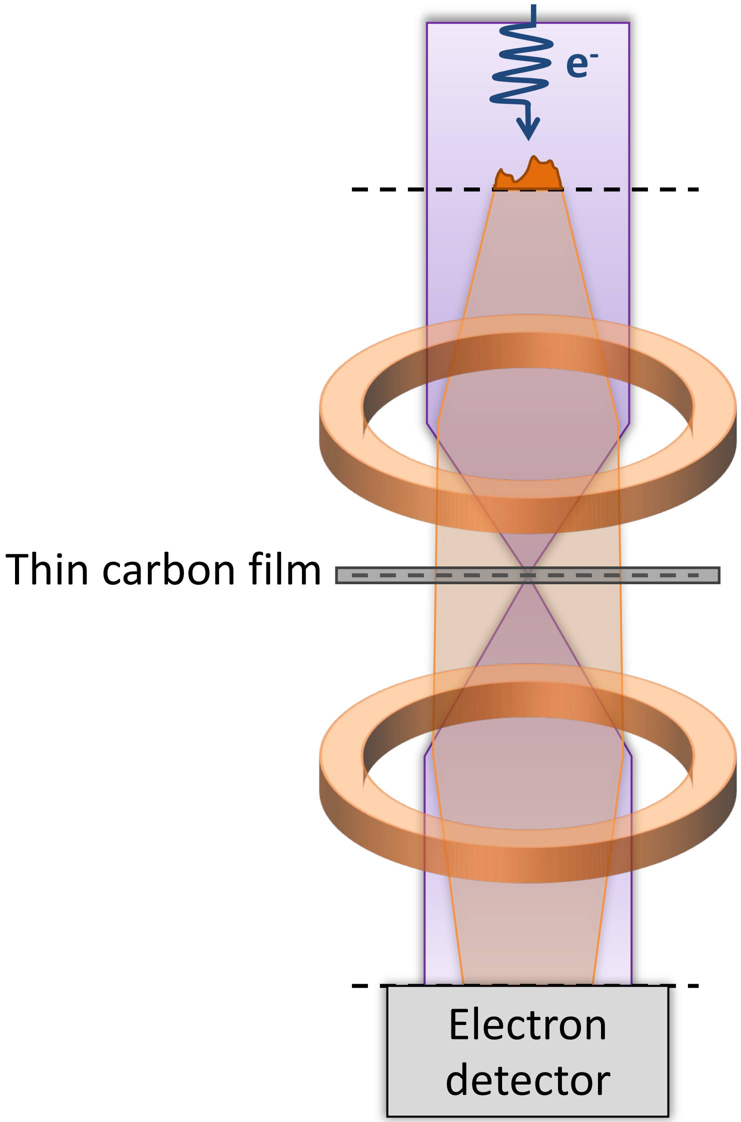


Boersch & Naturforsch, *Phys. Sci.* **2** (1947)
Glaeser, *Rev. Sci. Instrum.* **84** (2013)
Danev et al., *PNAS* **111** (2014)

"Volta" phase contrast is elegant, but…

- Volta phase plate has been the most successful to date





- Poorly-understood mechanism
- Phase shift is not stable over time
- Film scatters away ~20% of electrons
  - Loss is worst at high spatial frequencies

Danev et al., *eLife* **6** (2017)
Buijsse et al., *Ultramicroscopy* **218** (2020)
Danev et al., *Nat. Commun.* **12** (2021)

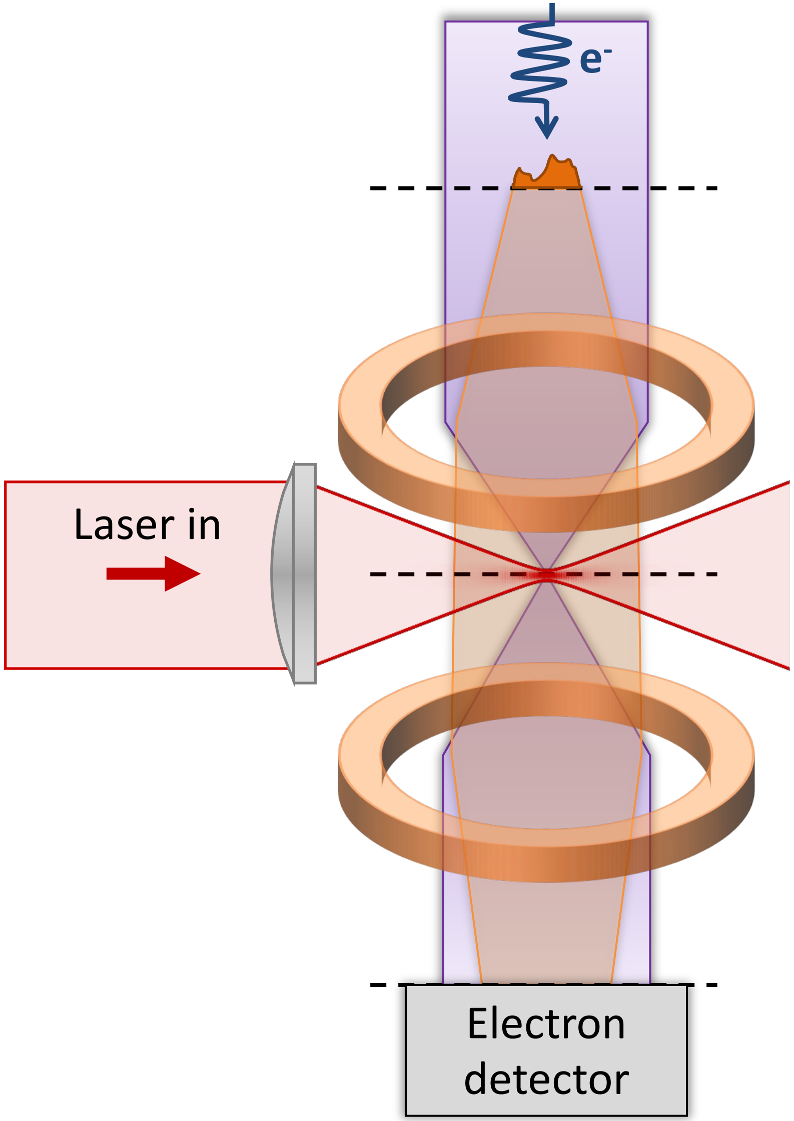
The laser phase plate (LPP)

- Electrons are phase-shifted by light via stimulated Compton scattering
  - Electron feels a ponderomotive potential $\propto E^2$

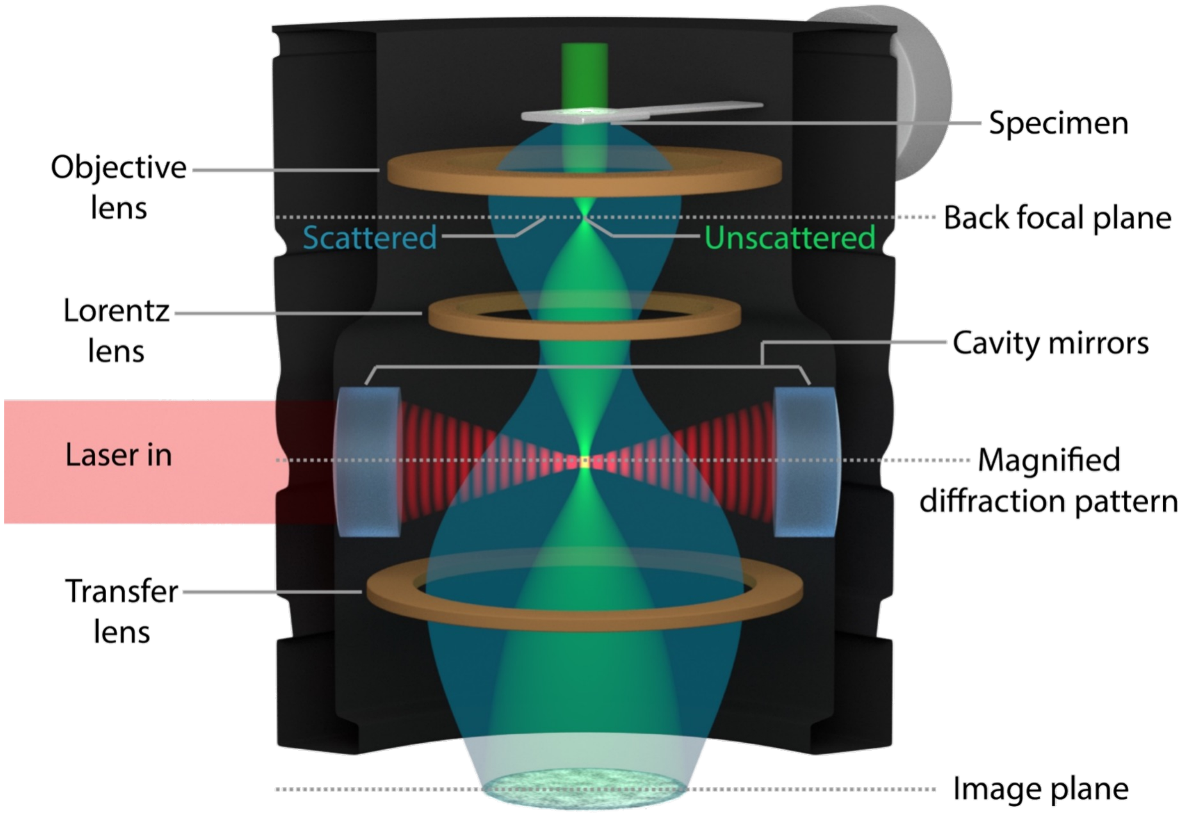
Advantages:

- **Stable, controllable phase shift**
- **No material in the electron beam**

- 90º phase shift of 300 kV electrons requires **extreme light intensity** ~400 GW/cm^2



The laser phase plate (LPP)



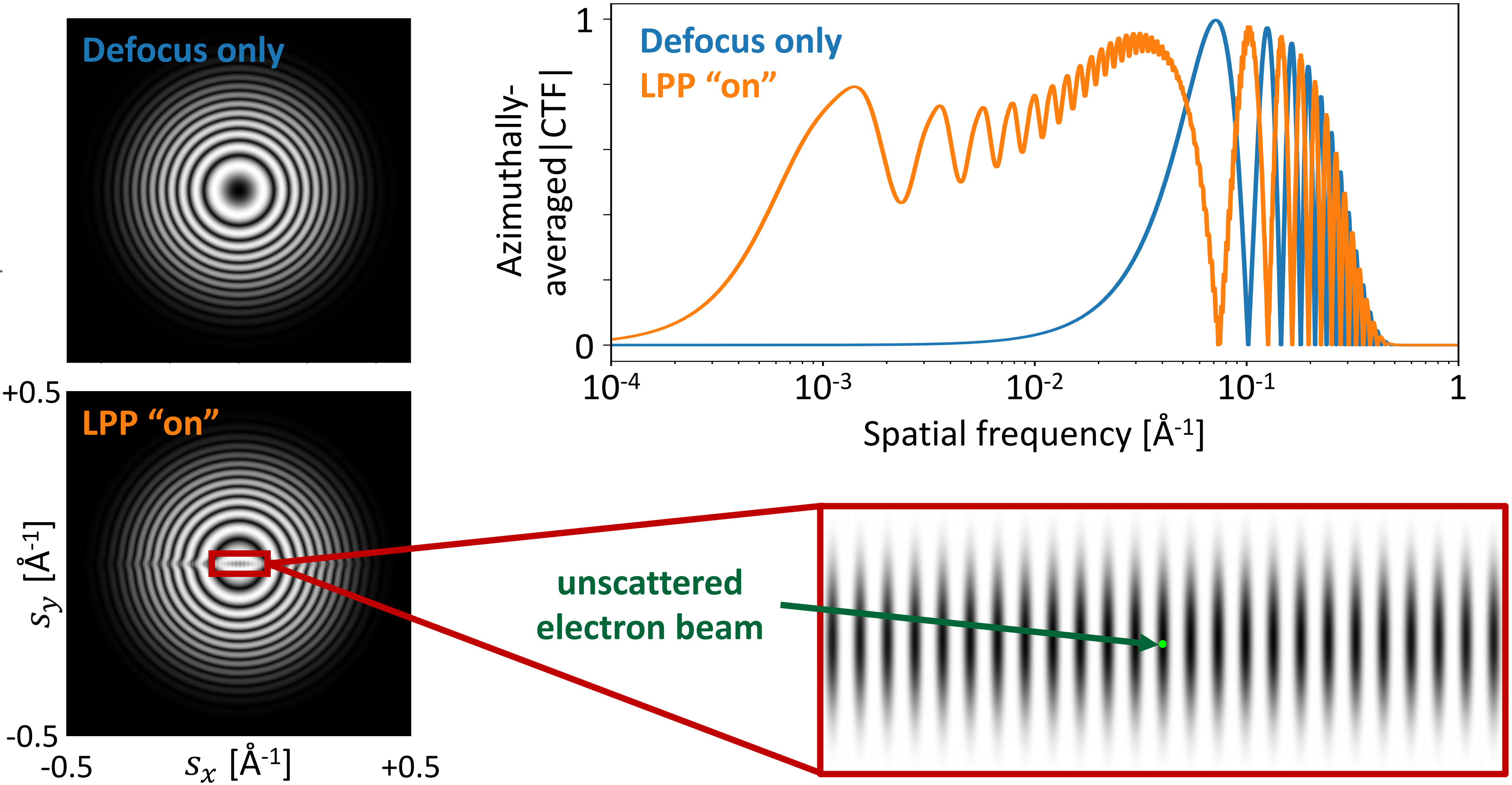
Light is **amplified** and **tightly focused** by a high-NA cavity

- Input: ~15 W continuous-wave 1064 nm laser
- Power amplification: ~7000×
- Beam waist: 8.5 μm (NA ~0.05)

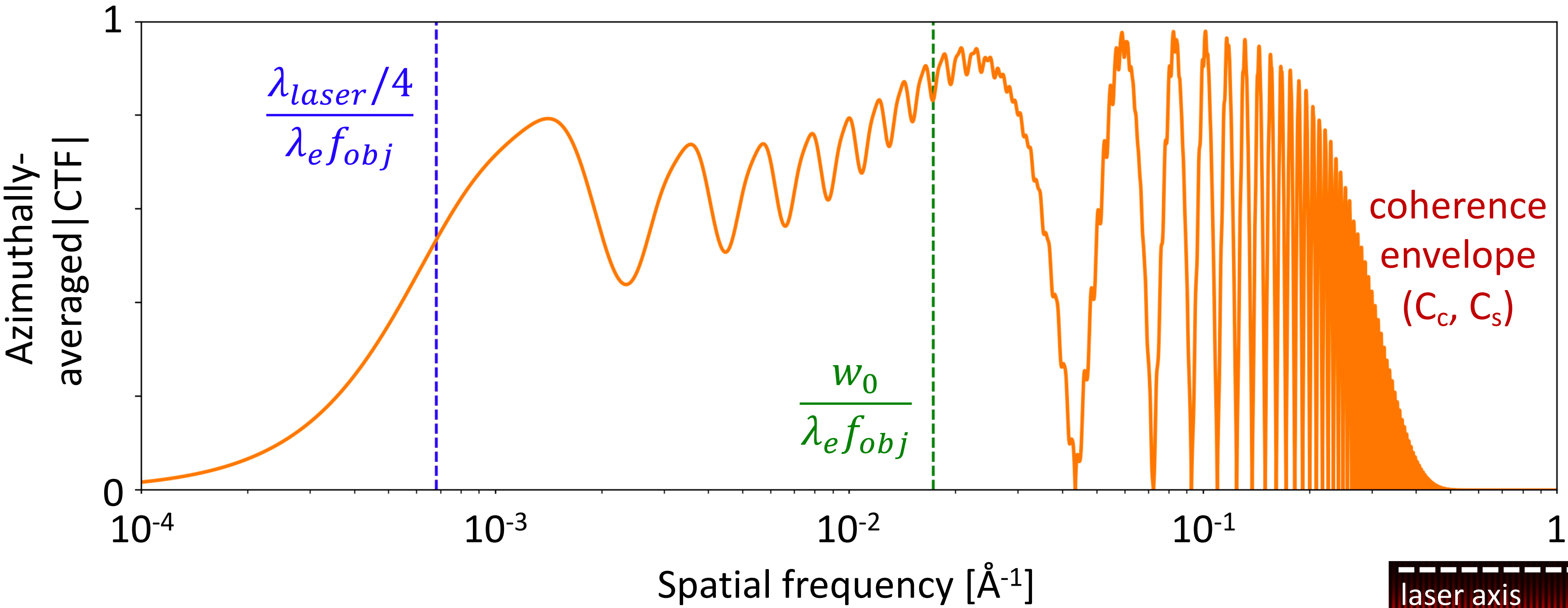
Schwartz et al., *Opt. Express* **25** (2017)
Schwartz et al., *Nat. Methods* **16** (2019)

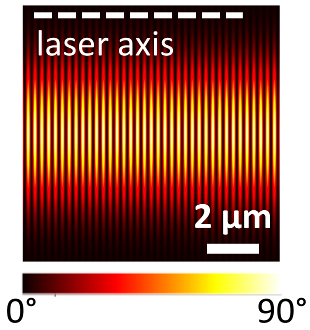
Contrast transfer function (CTF) of the LPP

- CTF describes information transfer at each spatial frequency
- LPP significantly enhances **low-frequency contrast**



Contrast transfer function (CTF) of the LPP





- **First cut-on frequency** determined by laser wavelength λ_{laser}
- **Second cut-on frequency** determined by laser waist w_0
- Not unique to the LPP:
  - **High-frequency oscillations** due to defocus, spherical aberration
  - **High-frequency attenuation** due to standard coherence envelope

Part II
Assorted Computational Opportunities

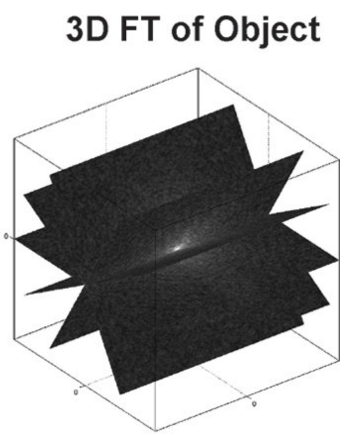
1. Tomography
2. Spherical aberration correction
3. Focal series
4. High-resolution template matching
5. Exit wave reconstruction
6. Image de-ghosting

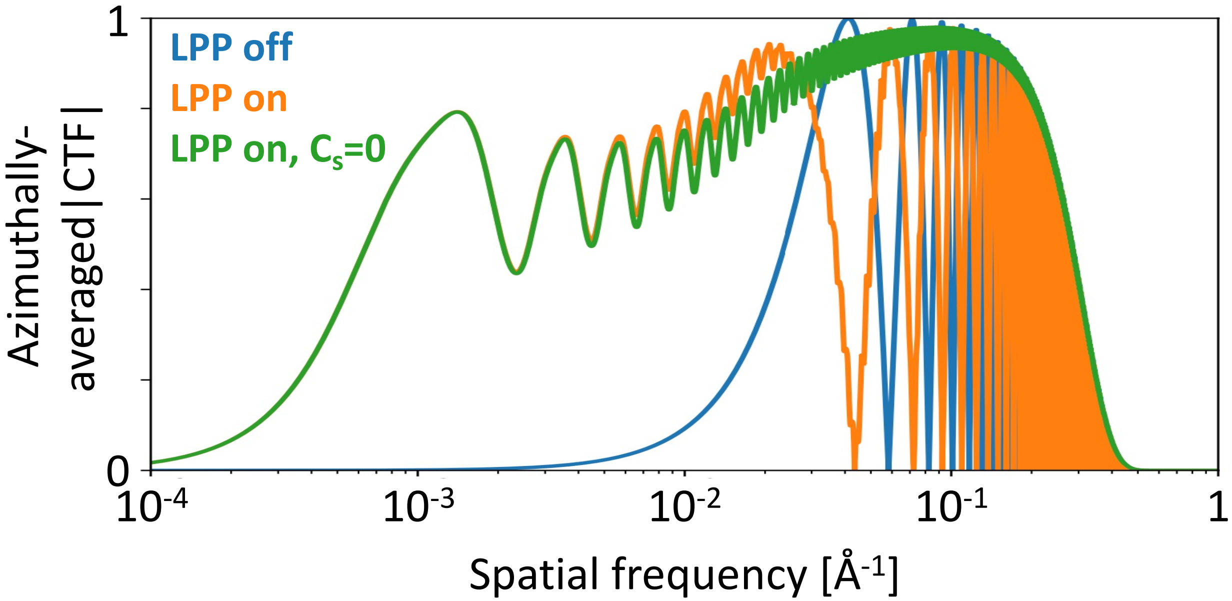
1. The LPP can improve electron tomography

Cryo-electron tomography (cryo-ET) enables volumetric reconstruction

Challenges:

- **Electron "budget" is spread thin** across N images: $N = \pi \frac{D}{d}$
- **Defocus-based contrast causes a tradeoff** between high and low (spatial) frequency information



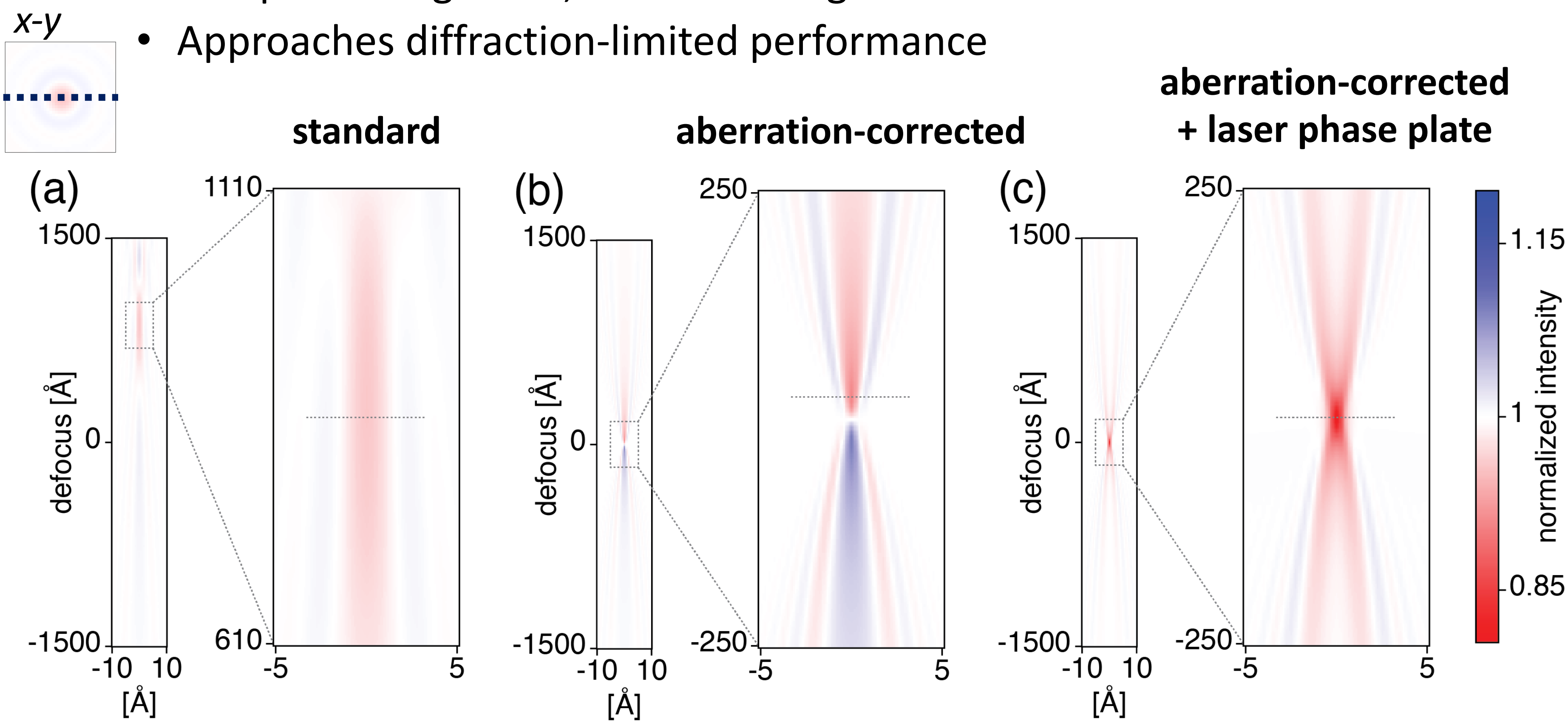


- Enhanced contrast reduces minimum dose per image
- **LPP enhances low frequencies without sacrificing high ones**

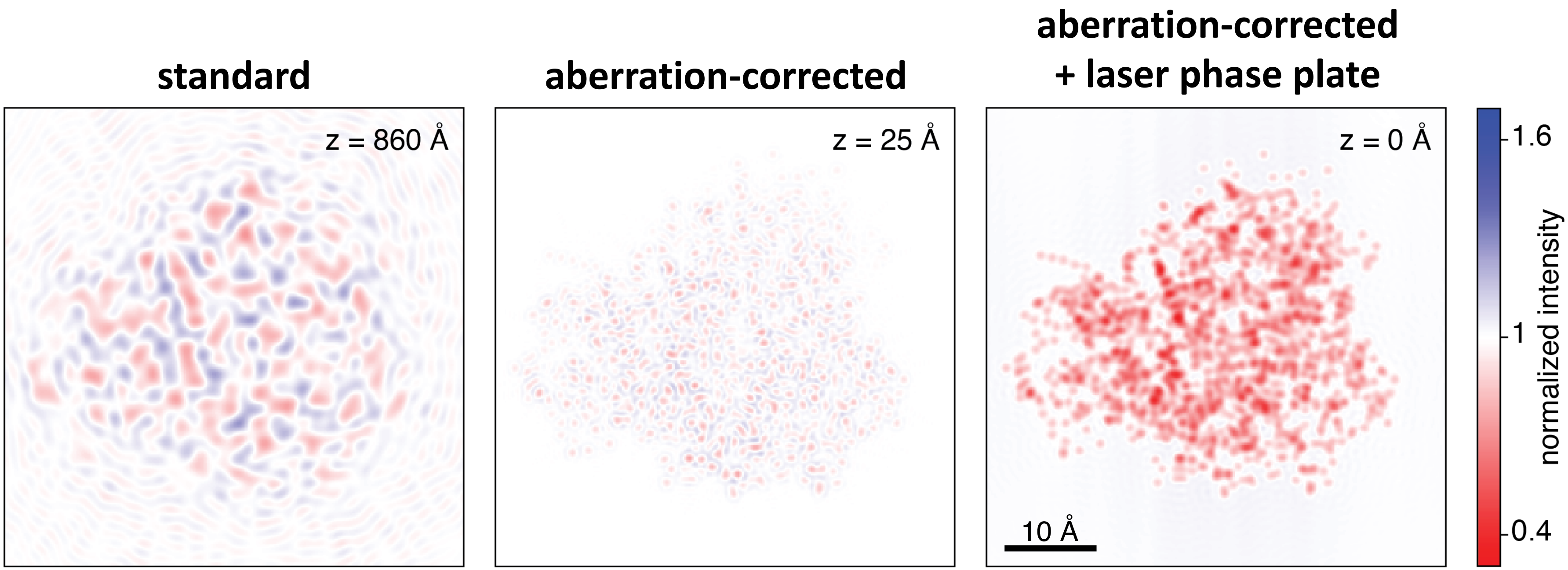
Bracewell, *Aust. J. Phys.* **9** (1956)
Crowther et al., *Nature* **226** (1970)
Petrov et al., *J. Struct. Biol.* **214** (2022)

2. Aberration-corrected phase contrast TEM has a very localized point spread function

- Spherical aberration correction ($C_s = 0$) increases *z*-dependence of the point spread function ($\mathcal{F}^{-1}[\mathrm{CTF}]$) but causes contrast inversion at *z* = 0
- Phase plate removes this contrast inversion, tightly localizes signal in 3D
  - Sharpens foreground, reduces background
  - Approaches diffraction-limited performance



2. Simulations demonstrate improved contrast and point resolution

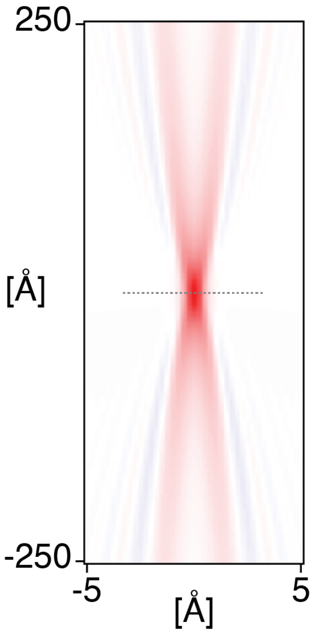


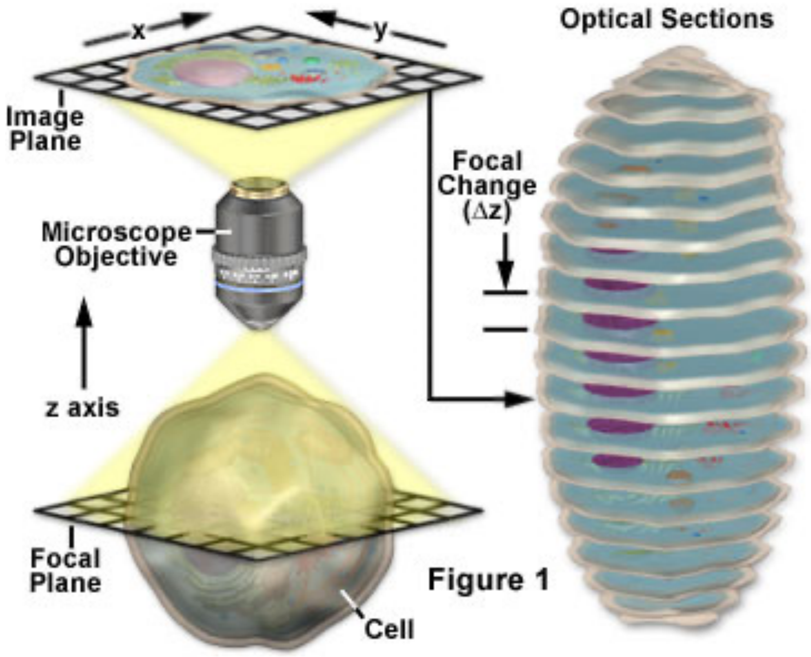
- Simulations of myoglobin at highest-contrast *z* position reveal the benefits of aberration-corrected laser phase plate imaging
  - All atoms have negative contrast
  - **Note:** continuum model of solvent simulated here

Petrov et al., *J. Struct. Biol.* **214** (2022)
Solvent: Shang & Sigworth, *J. Struct. Biol.* **180** (2012)

3. Focal series may be a useful paradigm

- Focal series are well established in fluorescence microscopy
  - Utilize strong *z*-dependence of the point spread function to reject background
  - Scan only defocus value, not stage tilt
  - Borrow deconvolution tools from fluorescence microscopy
  - Replace Crowther limit with Shannon limit (~63% of Crowther)





$$N = 2\frac{D}{d}$$

Hernandez Candia et al., *PLoS ONE* **9** (2014)
Jenkins & Gaylord, *Appl. Opt.* **54** (2015)
Waugh et al., *PNAS* **117** (2020)
Croxford et al., *PNAS* **118** (2021)
Petrov et al., *J. Struct. Biol.* **214** (2022)

3. Focal series versus tilt series

	Tilt series	Focal series
Missing wedge	Yes	No
Sampling limit	Crowther	~0.63× Crowther
Scanning	Stage tilt	Objective defocus
Background	High (z projection)	Low (optical sectioning)
Radiation damage	Varies with spatial frequency	Varies with axial position

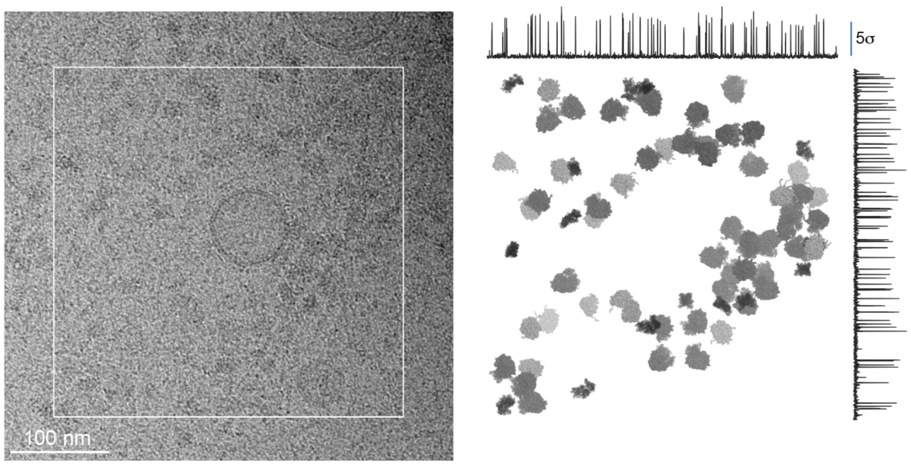
- Anticipate low background and high stability
- Obtain high-resolution information from thin axial section
- May reduce *z*-dependence of radiation damage with an interleaved approach
  - Analogous to dose-symmetric tilt series
- Combine focal series with low-resolution tilt series as in hybrid scheme

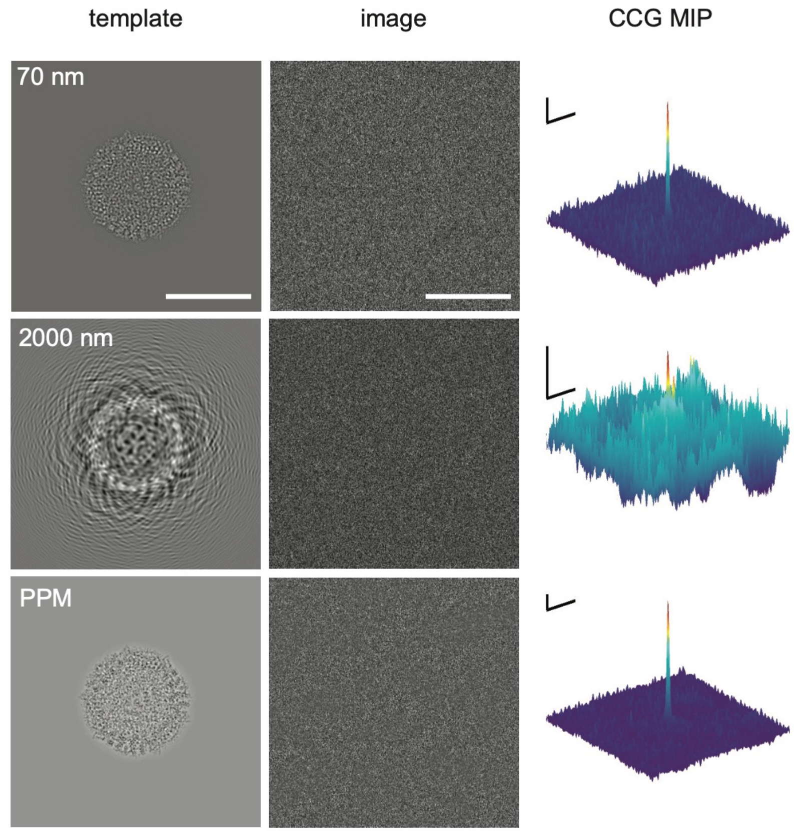
DeRosier, *Ultramicroscopy* **81** (2000).
Yalisove et al., *Phys. Rev. Applied* **15** (2021)
Ren et al., *Ultramicroscopy* **208** (2021)
Gureyev et al., *Ultramicroscopy* **218** (2021)
Petrov et al., *J. Struct. Biol.* **214** (2022)

4. The LPP can improve high-resolution template matching

- Cross-correlograms C_j obtained by correlation of a micrograph I with templates T_j

$$C_j = \mathcal{F}^{-1}\left[\mathcal{F}[I] \cdot \mathcal{F}\left[T_j\right]\right]$$





- **Phase plate improves detection SNR** ~3× relative to defocus
- Removal of low-frequency structural noise due to other particles improves detection
  - Low-resolution tomograms enable background subtraction

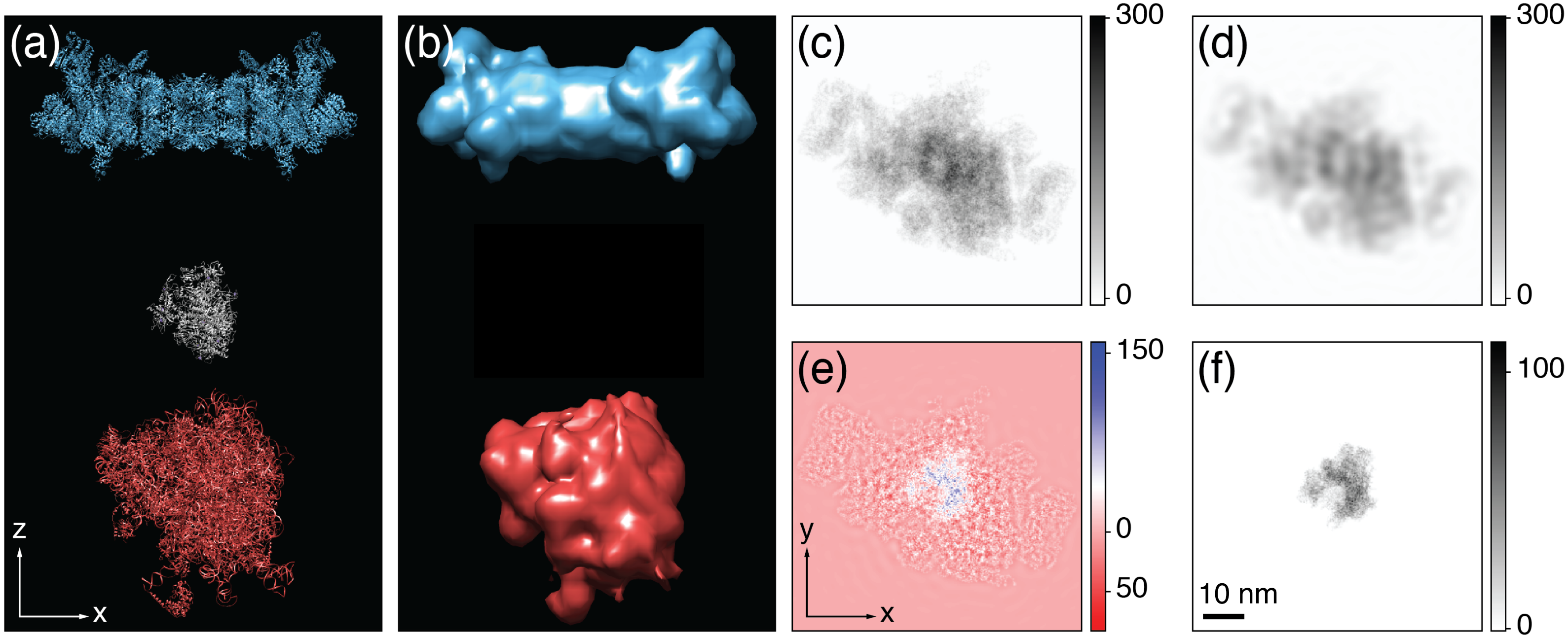
Rickgauer et al., *eLife* **6** (2017)
Rickgauer et al., *bioRxiv* (2020)
Petrov et al., *J. Struct. Biol.* **214** (2022)

4. Low-resolution tomograms enable background subtraction

Untilted image	Tilt series
High-resolution information	
	Axial (*z*) information
Low-resolution information	*Low-resolution information*

Background subtraction

1. Project tomogram onto *x*-*y* plane
2. Subtract projection from untilted image



5. The LPP enables exit wave reconstruction

- Zernike phase plates enable full reconstruction of the wave function from two images (in the weak scattering limit: μ, ϕ both small)

phase plate "off"

$$\psi(\boldsymbol{x}) \sim e^{\mu(\boldsymbol{x})} e^{i\varphi(\boldsymbol{x})}$$

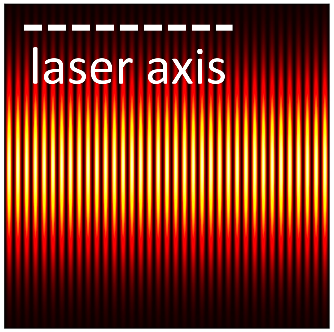
phase plate "on"

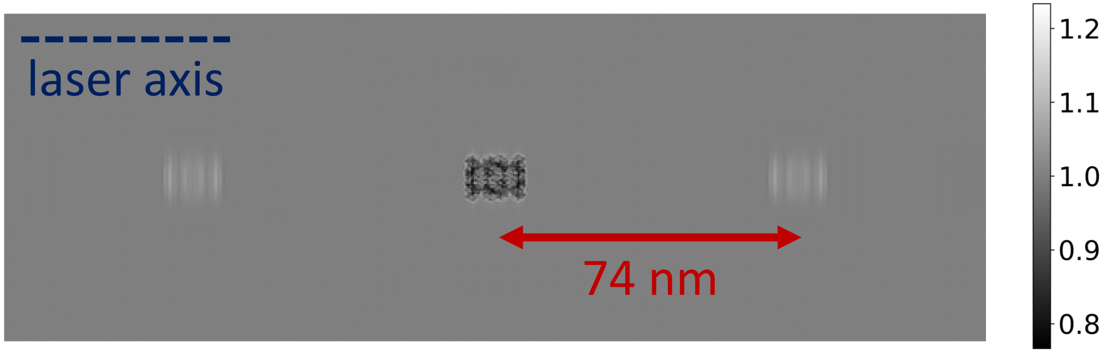
- The LPP can approximate this:

- These methods work best at **zero defocus** and with **C_s correction**
  - Accurate low-defocus imaging is a research endeavor

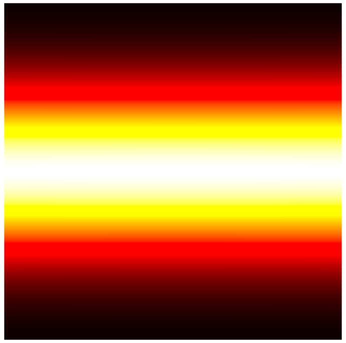
6. The LPP diffracts electrons into ghost images

- Laser standing wave acts as a phase grating
- Diffraction of electrons generates weak “ghost” images



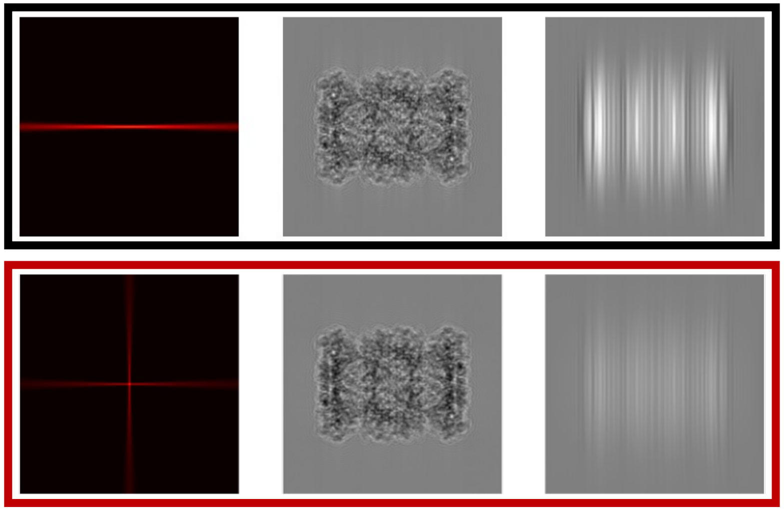


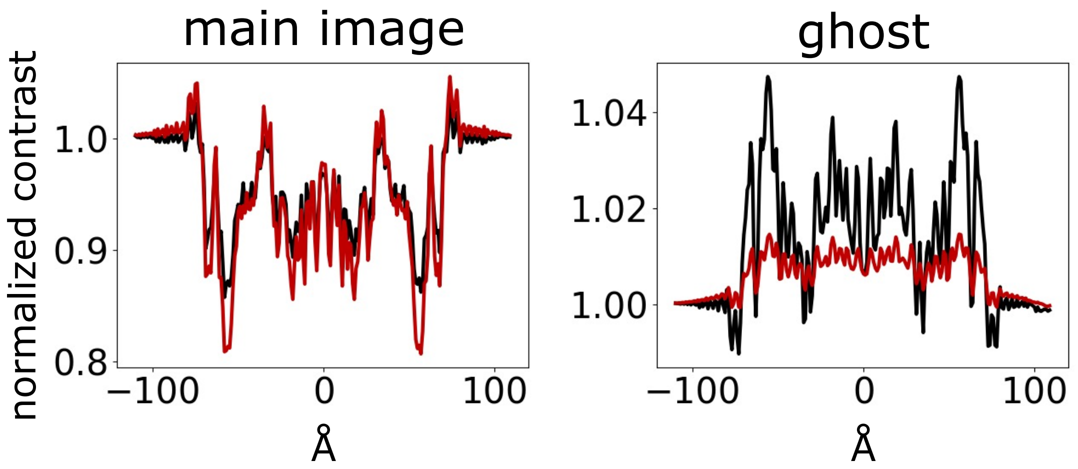
- Ghost spacing is $\frac{2\lambda_e f_{\mathrm{obj}}}{\lambda_{\mathrm{laser}}}$, or 74 nm in our instrument
- Relativistic reversal of the ponderomotive potential can eliminate standing wave structure
  - Ghosts perfectly eliminated
  - Cut-on frequency of phase plate is ~20× higher



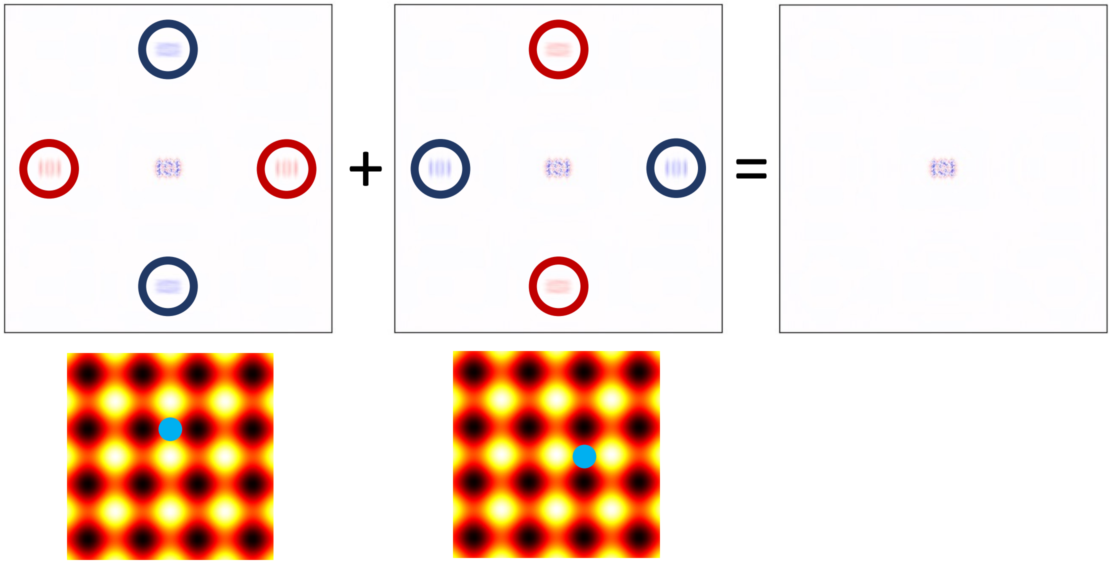
Axelrod et al., *Phys. Rev. Lett.* **124** (2020)
Turnbaugh et al., *Rev. Sci. Instrum.* **92** (2021)

6. Ghost images are reduced by crossed phase plates





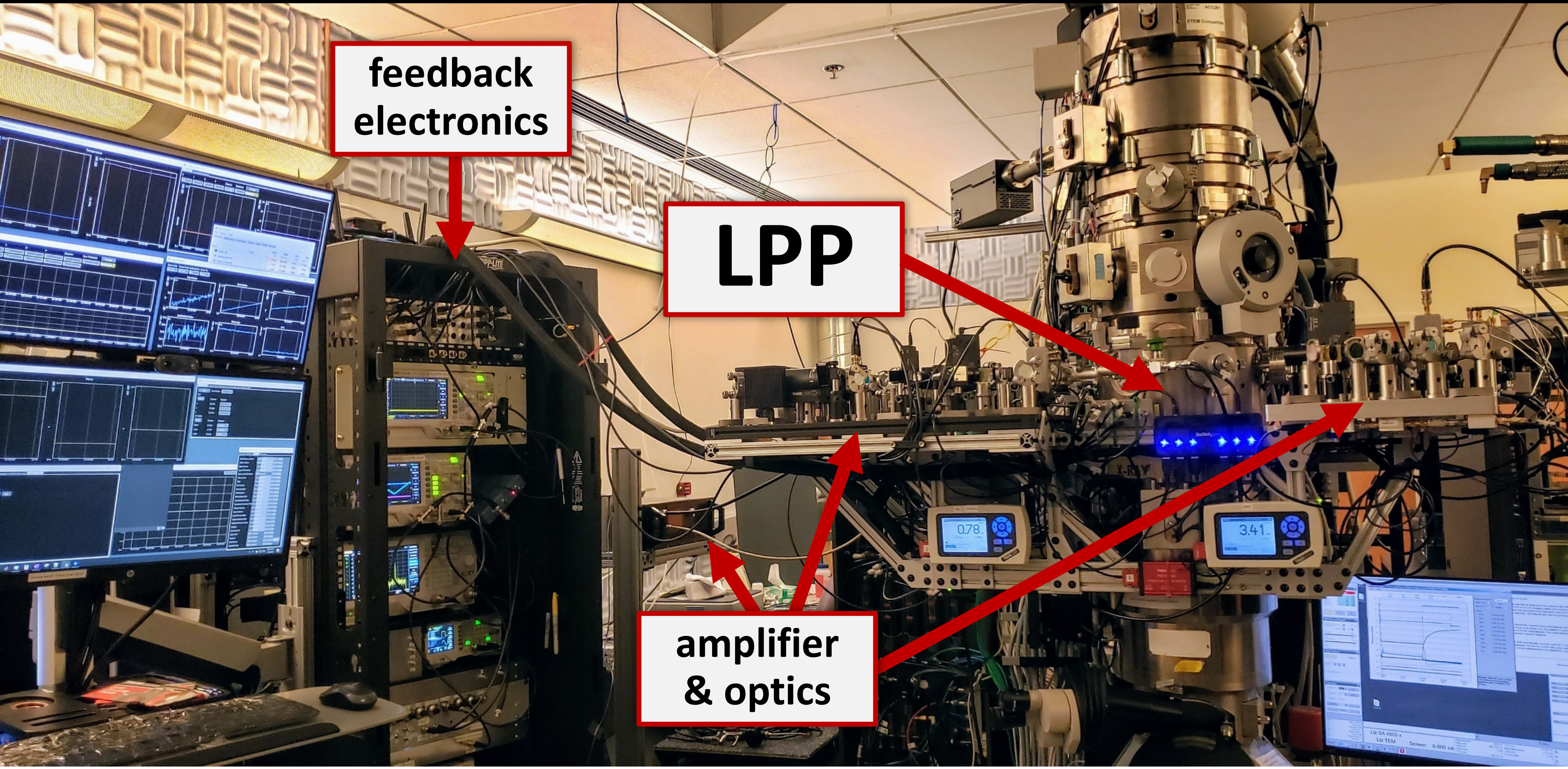
- Planned “crossed LPPs” reduce ghost intensity 3-4×
- Cavity power requirements reduced by 50%
- Two-image sequence can reduce ghosts >250× just by summing intensity



Axelrod et al., *Phys. Rev. Lett.* **124** (2020)
Turnbaugh et al., *Rev. Sci. Instrum.* **92** (2021)

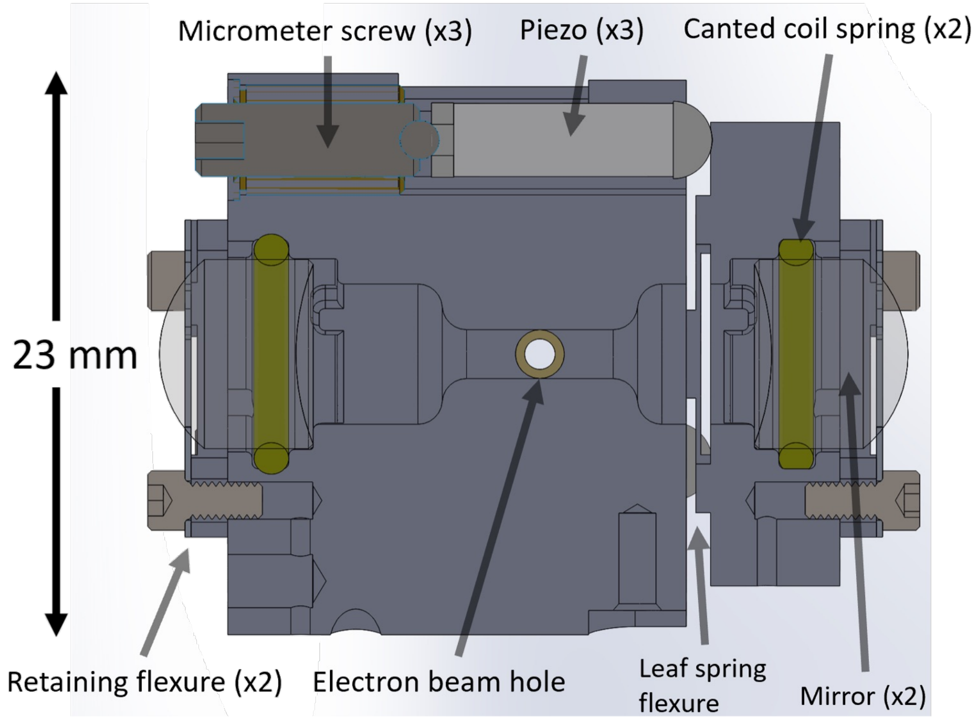
Part III
Recent Experimental Progress

Laser phase plate in the lab

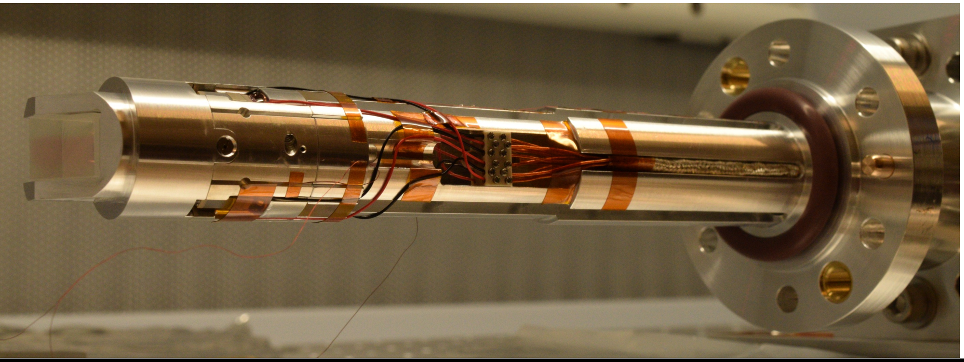


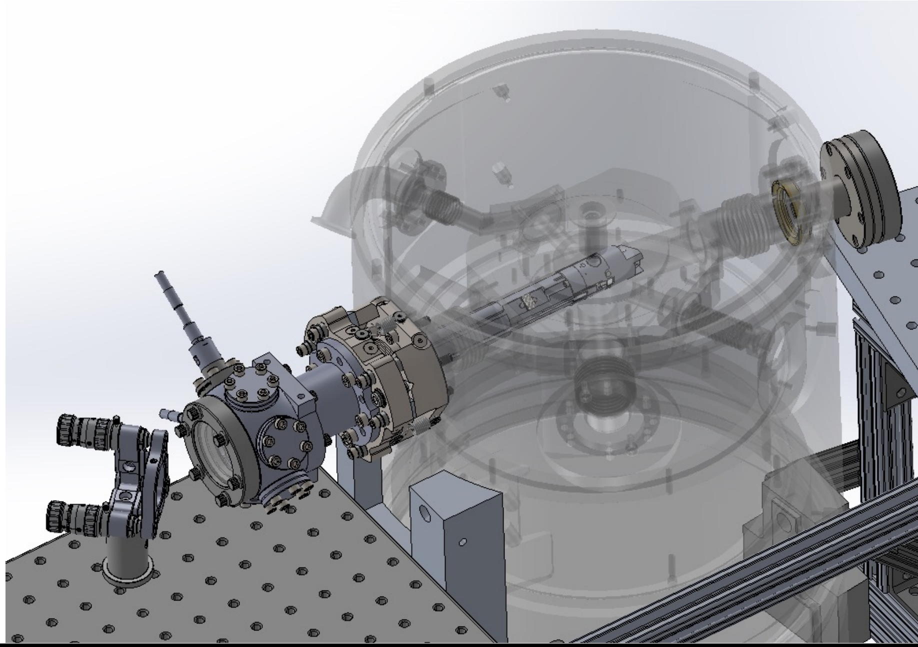
- **FEI Titan 80-300** equipped with transfer optics module
- **50 W fiber amplifier** and breadboards for mode-matching, beam monitoring
  - LPP **circulating power >100 kW** after amplification
- **Feedback electronics** stabilize laser frequency, mode position, cavity length

Design of the laser phase plate



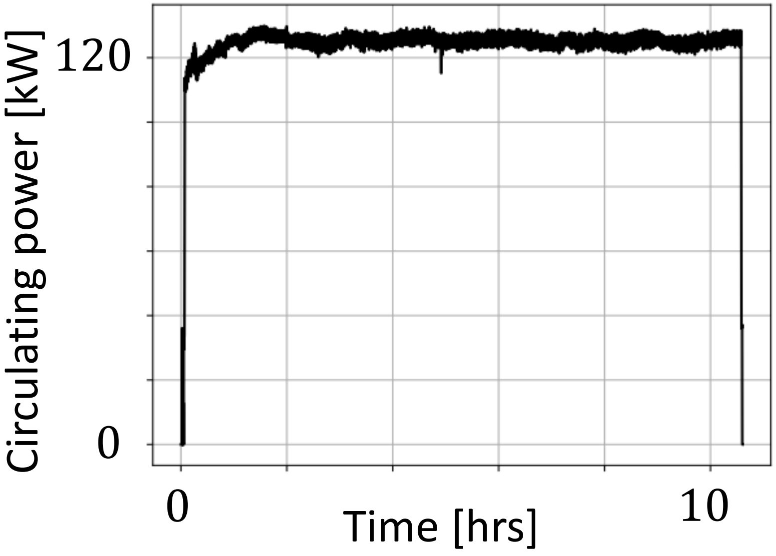
- **Vacuum-compatible** (10^{-7} mbar)
- **Non-magnetic** (Al, Ti, …)
- **Super-polished** mirrors (~1 Å rms roughness), surface figure <λ/100

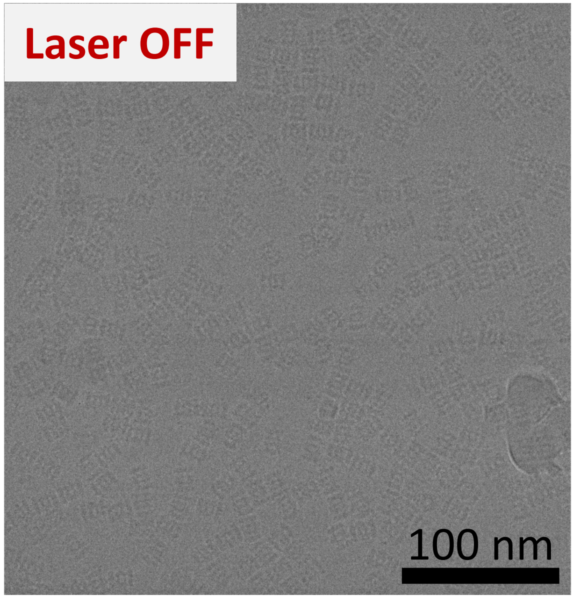


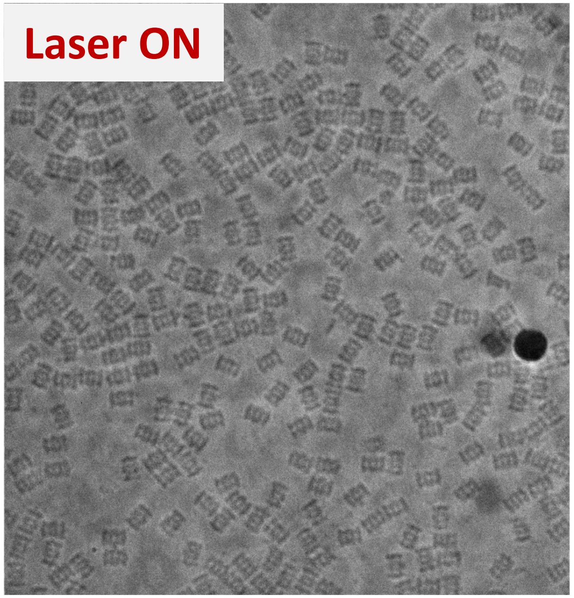


The LPP enables phase-contrast TEM

- Stable cavity power for hours at a time
  - Stable power → stable phase shift
- Constant performance over >400 total hours of high-power operation
- Have achieved up to ~120° phase shift





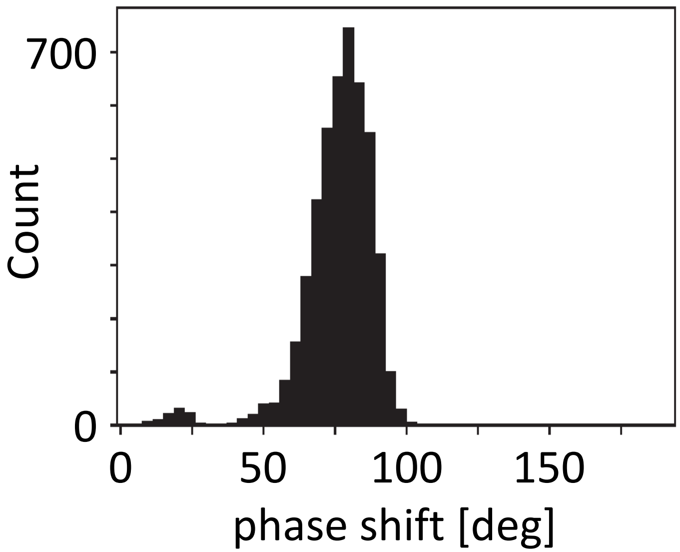


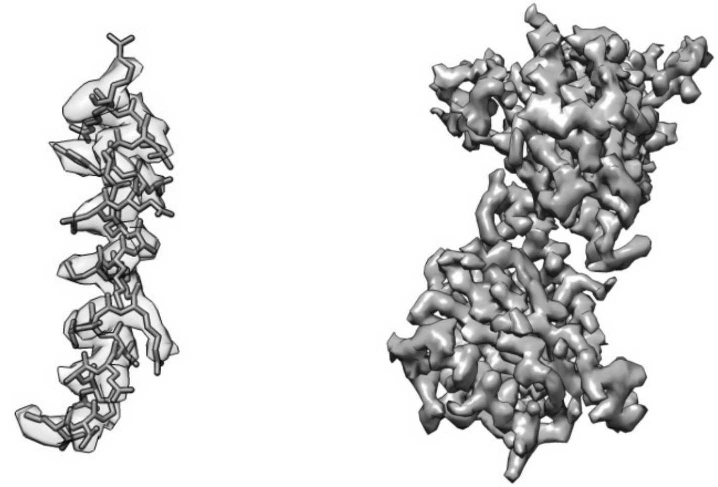
defocus is 1.5 µm in both images

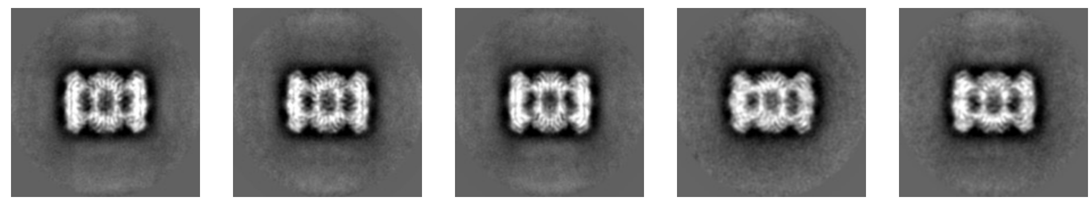
Turnbaugh et al., *Rev. Sci. Instrum.* **92** (2021)

Proteasome reconstruction with laser phase contrast

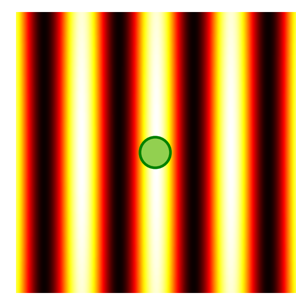
- Reconstructed 20S proteasome core particle (~680 kDa)
  - RELION 3.1 workflow
  - Resolution (FSC): 3.8 Å
  - Total particles: 4789
  - B-factor: 131 Å^2







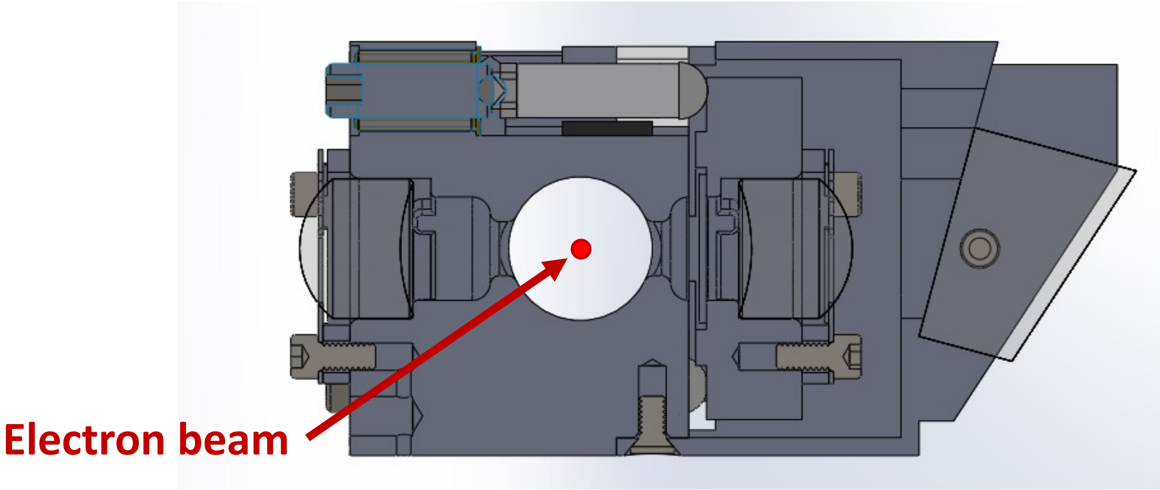
- Electron beam periodically aligned to laser using deflectors:



Recent experimental challenges

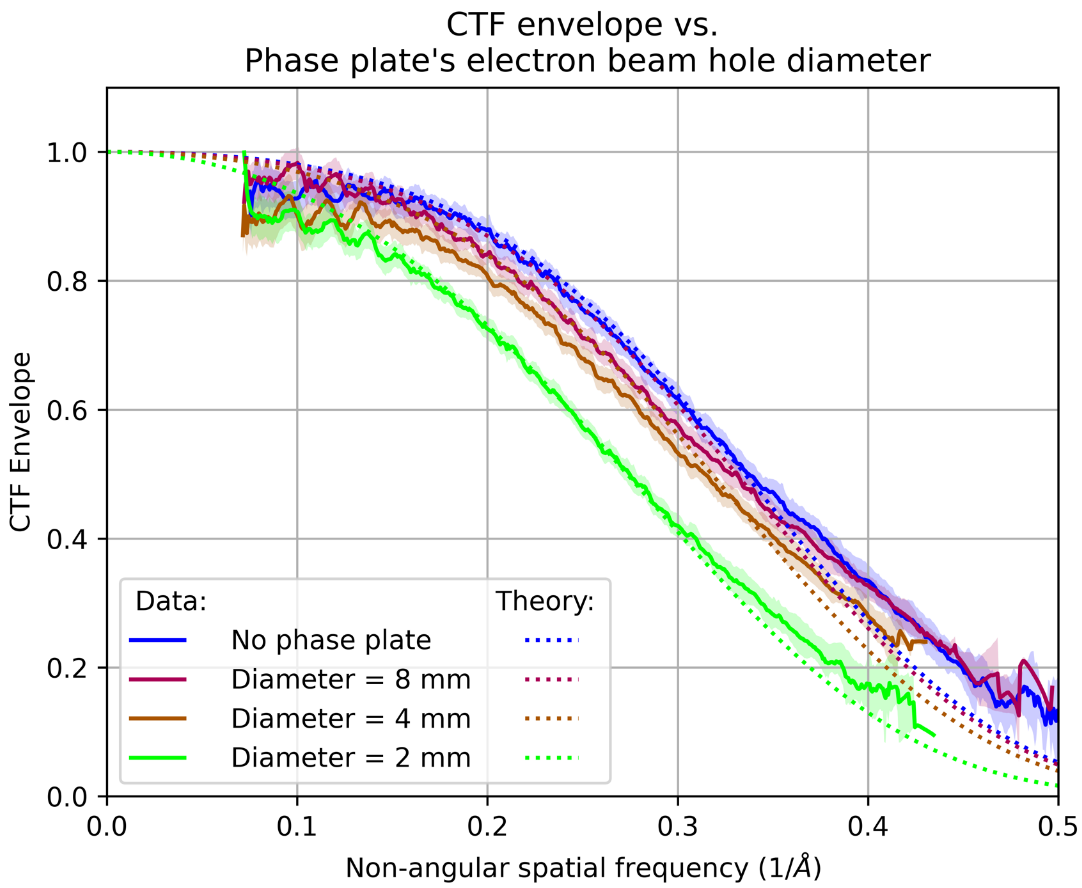
A few hurdles…

1. **Transfer optics** increased aberrations (spherical, chromatic)
   → Installed gun monochromator
2. **Ice contamination** rate limited imaging time (~30 min)
   → Added extra vacuum pump on the column
   → Inserted objective aperture in the back focal plane
3. **Thermal magnetic field noise** blurred images
   → Enlarged the LPP electron beam hole



Thermal magnetic field noise decoheres electron beam

- Thermal fluctuations in aluminum cavity mount (and titanium column liner tube…) induce magnetic field noise, **reduce coherence envelope**
  - Systems with transfer optics are particularly sensitive ☹



$$\langle x^2 \rangle = \int dz \, \frac{C\lambda^2 e^2 \mu_0 k_B T}{h^2} \frac{X^2(z)}{D^2(z)}$$

$$E(s) = \exp(-2\pi^2 \langle x^2 \rangle s^2)$$

D: hole diameter
X: marginal ray distance
T: temperature
C: material-dependent constant
s: spatial frequency

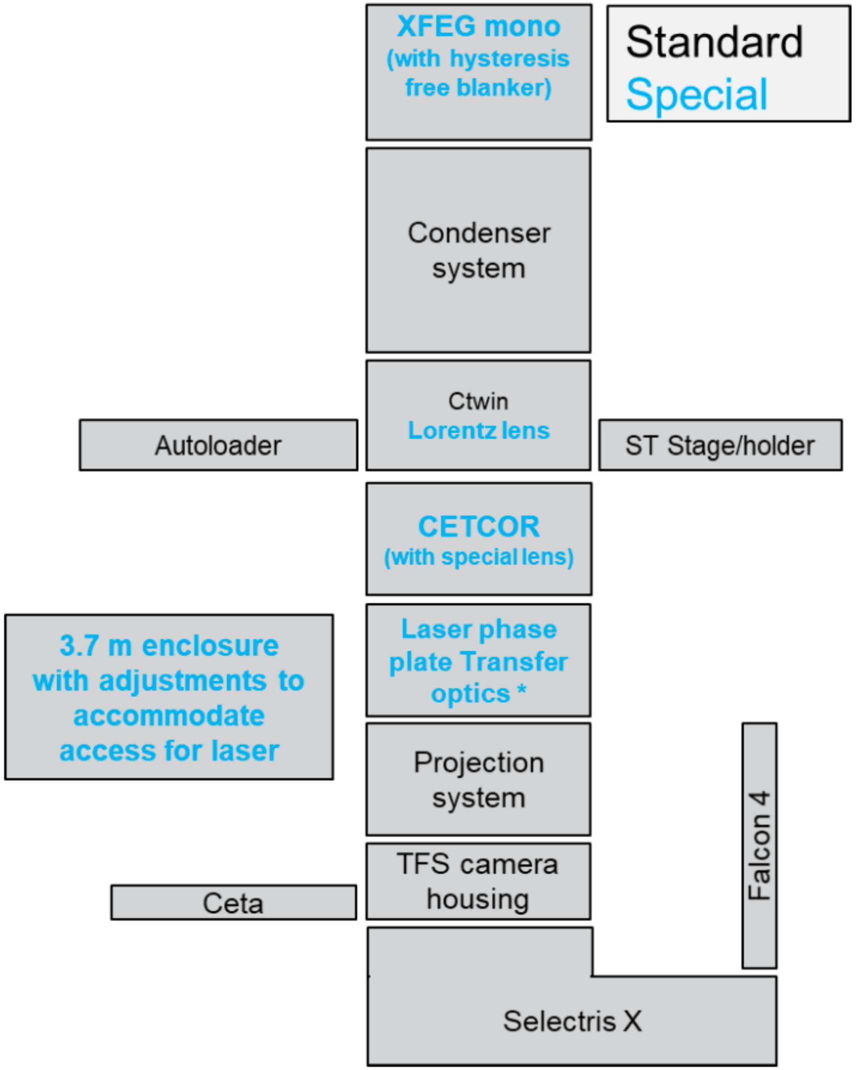
Fixed by larger electron beam hole

Uhlemann et al., *Phys. Rev. Lett.* **111** (2013)
Uhlemann et al., *Ultramicroscopy* **151** (2014)
Lee & Romalis, *J. Appl. Phys.* **103** (2008)

New instrument coming soon

Custom Titan Krios G4 arriving in Berkeley soon (~Q2 2023):

- *Better* transfer optics
- Autoloader
- Gun monochromator
- Post-column energy filter
- Latest-generation camera
- **Spherical aberration corrector**
- **Crossed laser phase plate**



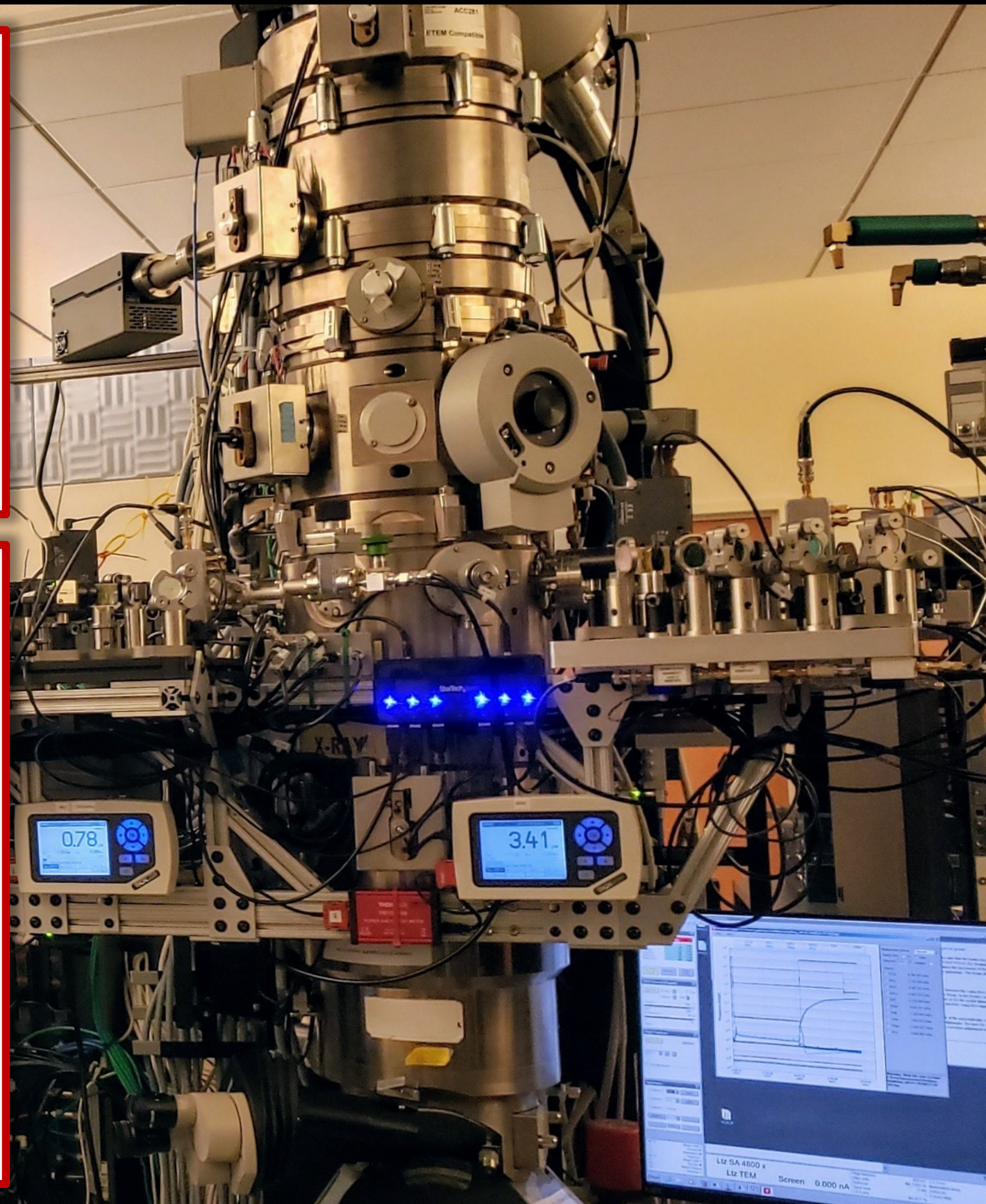
The future of laser phase-contrast cryo-EM

LPP development:

- Crossed phase plates
- Aberration correction
- In-focus imaging
- LPP-specific processing
- Cavity miniaturization

Cryo-EM experimental design:

- Reduced exposure time
- Tomography
  - Fewer tilts?
  - Focal series?
- Template matching
  - Background subtraction
- Exit wave reconstruction



Acknowledgements

LPP Team

- **Holger Müller**
- **Bob Glaeser**
- Jeremy Axelrod
- Jonathan Remis
- Shahar Sandhaus

Alumni

- Osip Schwartz
- Sara Campbell
- Carter Turnbaugh
- Jeske Dioquino

Collaborators

- Eva Nogales (Berkeley)
- Yifan Cheng (UCSF)
- Zanlin Yu (UCSF)
- Thermo Fisher Scientific/FEI

NIH

CZ

GORDON AND BETTY MOORE FOUNDATION