High-intensity cavities for electron microscopy and molecular traps

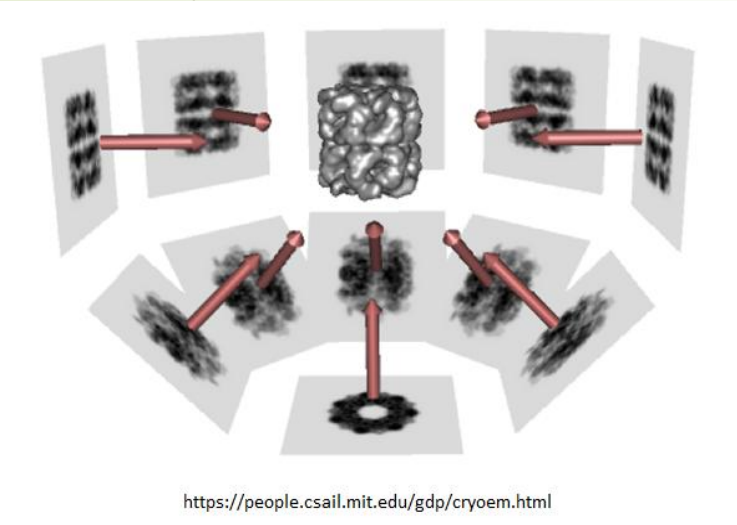
Holger Müller

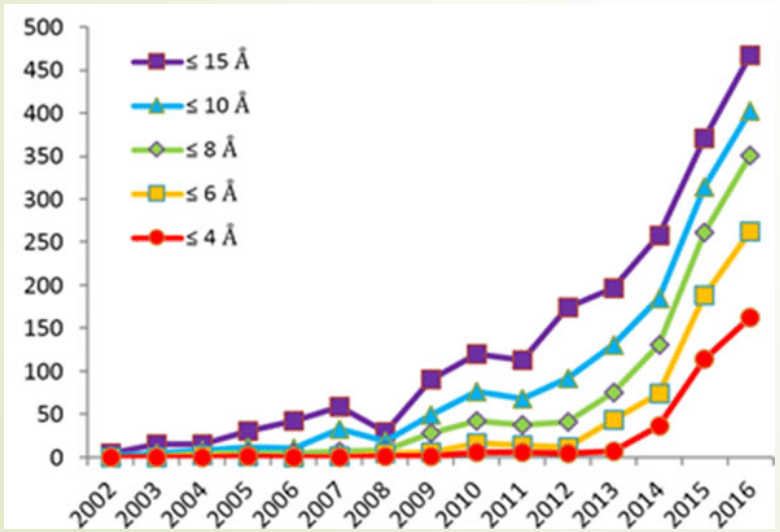
https://what-if.xkcd.com/13/

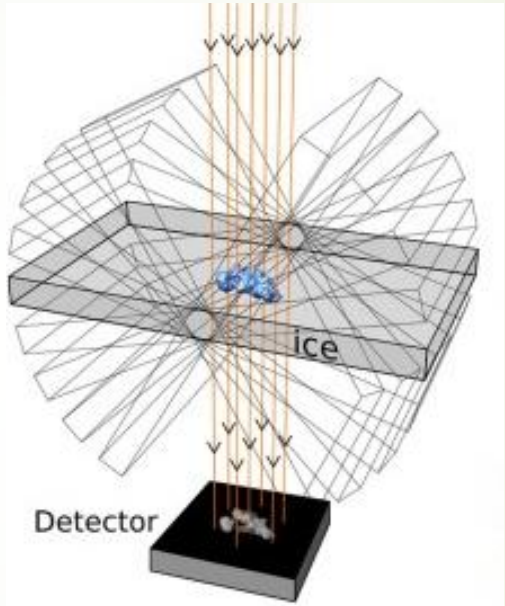
“Make the electron microscope 100 times better.”

R. P. Feynman, December 29, 1959

Single-particle cryo-EM is growing quickly…





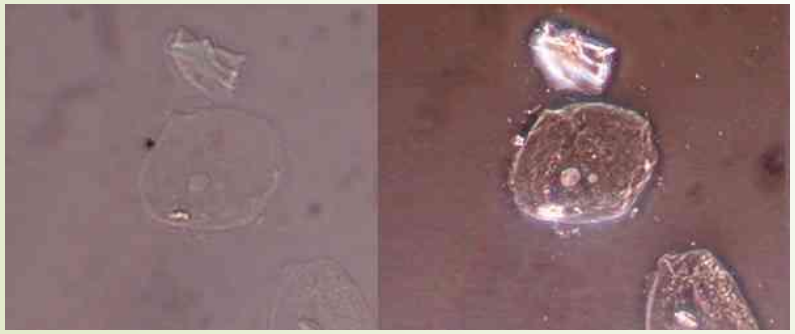


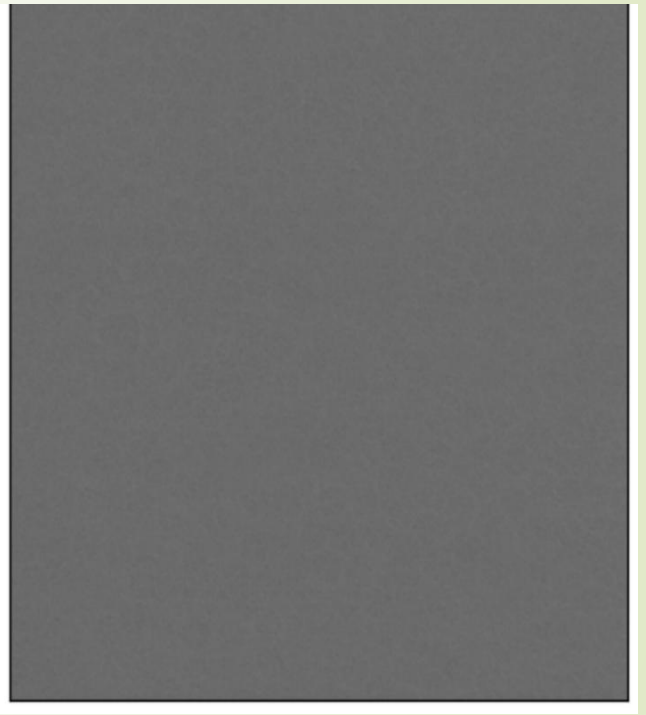
Koning et al., Ann. Anatom. (2018); ThermoFisher

…and we want cryo-ET to grow equally well very soon!

What's so hard about that?

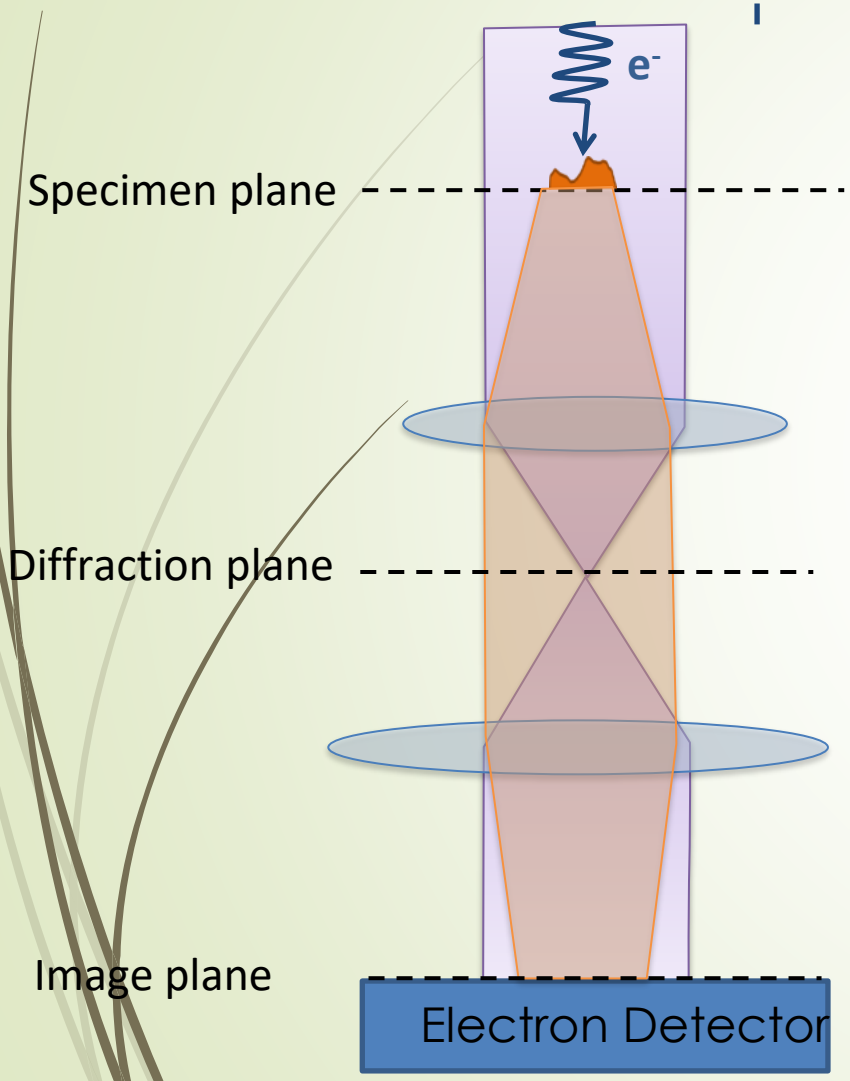
- Specimens are weak-phase objects
- Low amplitude contrast
- Radiation damage: 10 e-/Å2 alter structure
- Tomography extremely difficult
- Phase contrast!





Raw data example

Weak phase objects

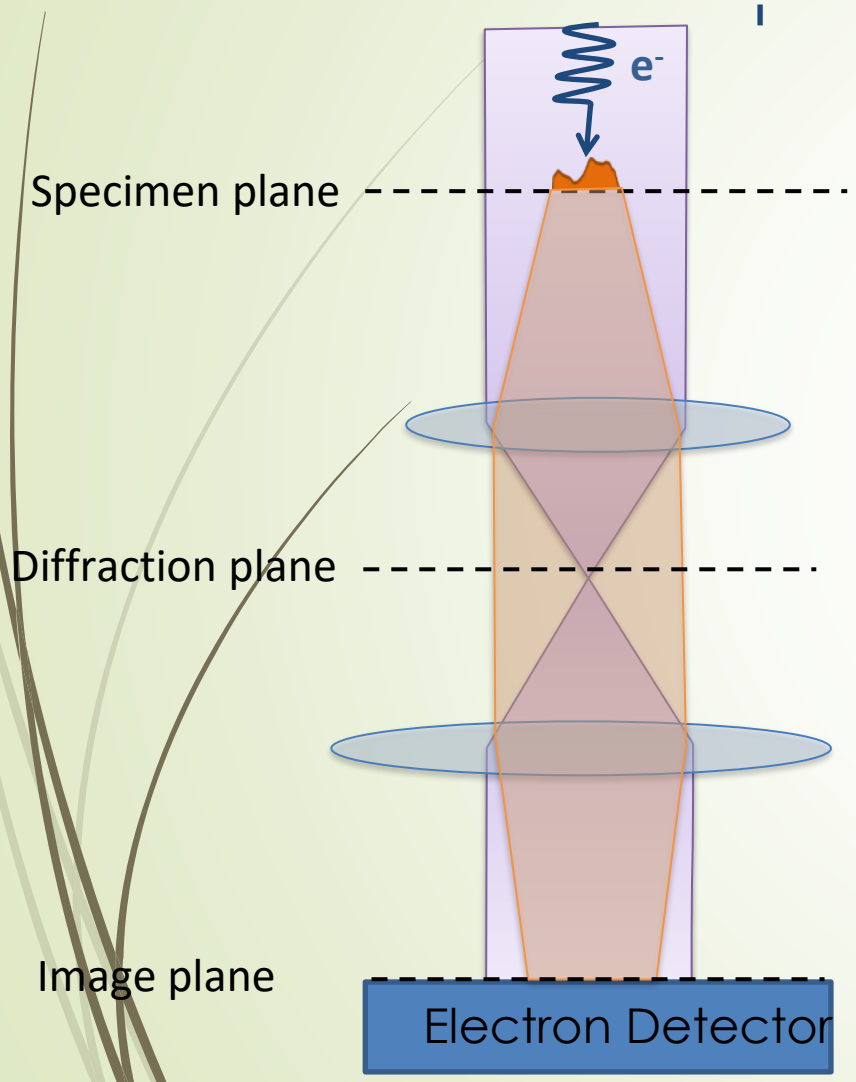


$$\psi = e^{ikz}$$

$$\psi = e^{ikz} e^{i\phi(x,y)} = e^{ikz}\left(1 + i\phi(x,y) + \ldots\right)$$

$$\text{Image}: \quad |\psi|^2 = 1$$ No contrast!

90° phase shift enables phase contrast



$$\psi = e^{ikz}$$

$$\psi = e^{ikz} e^{i\phi(x,y)} = e^{ikz}\left(1 + i\phi(x,y) + \ldots\right)$$

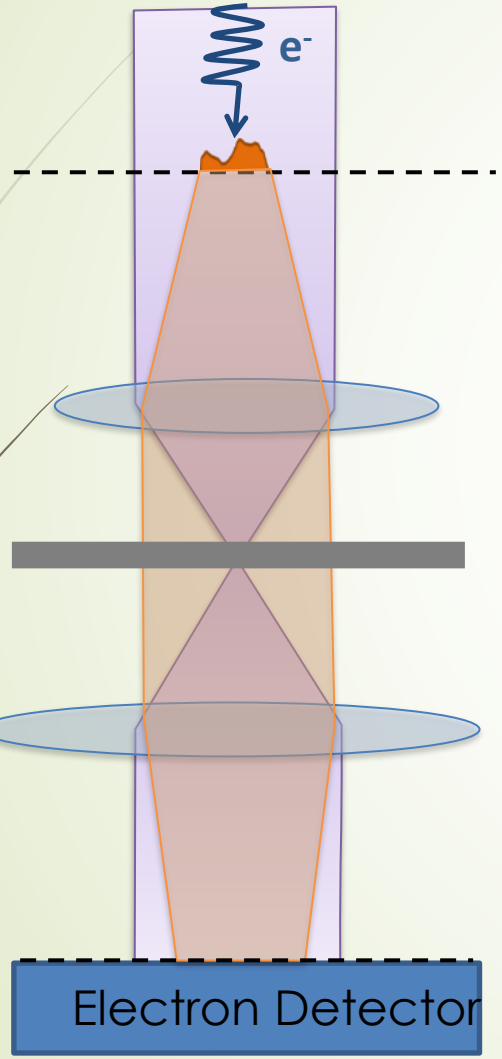
+90°

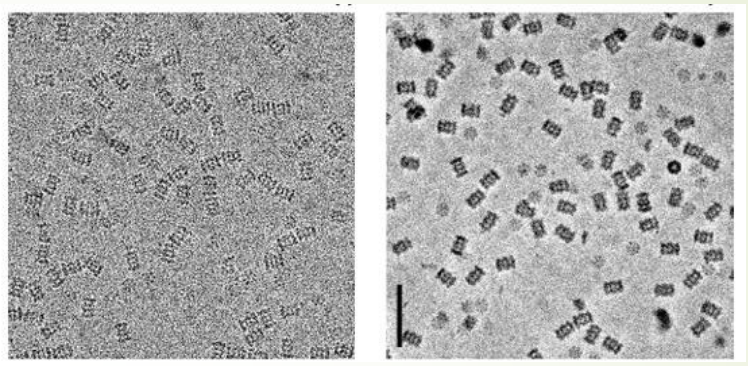
$$\psi = e^{ikz}\left(i + i\phi(x,y) + \ldots\right)$$

Contrast

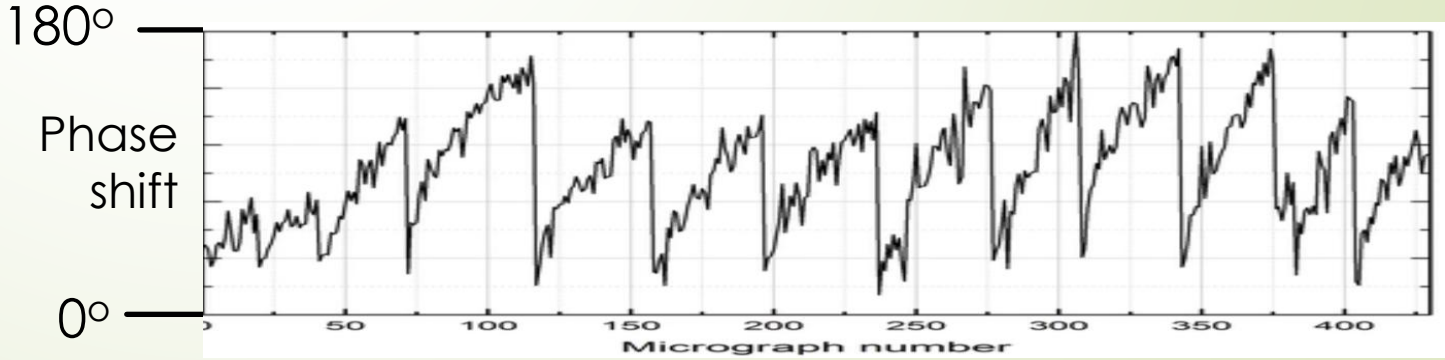
$$\text{Image}: \quad |\psi|^2 = 1 + 2\phi(x,y) + \ldots$$

Volta phase contrast is elegant, but…

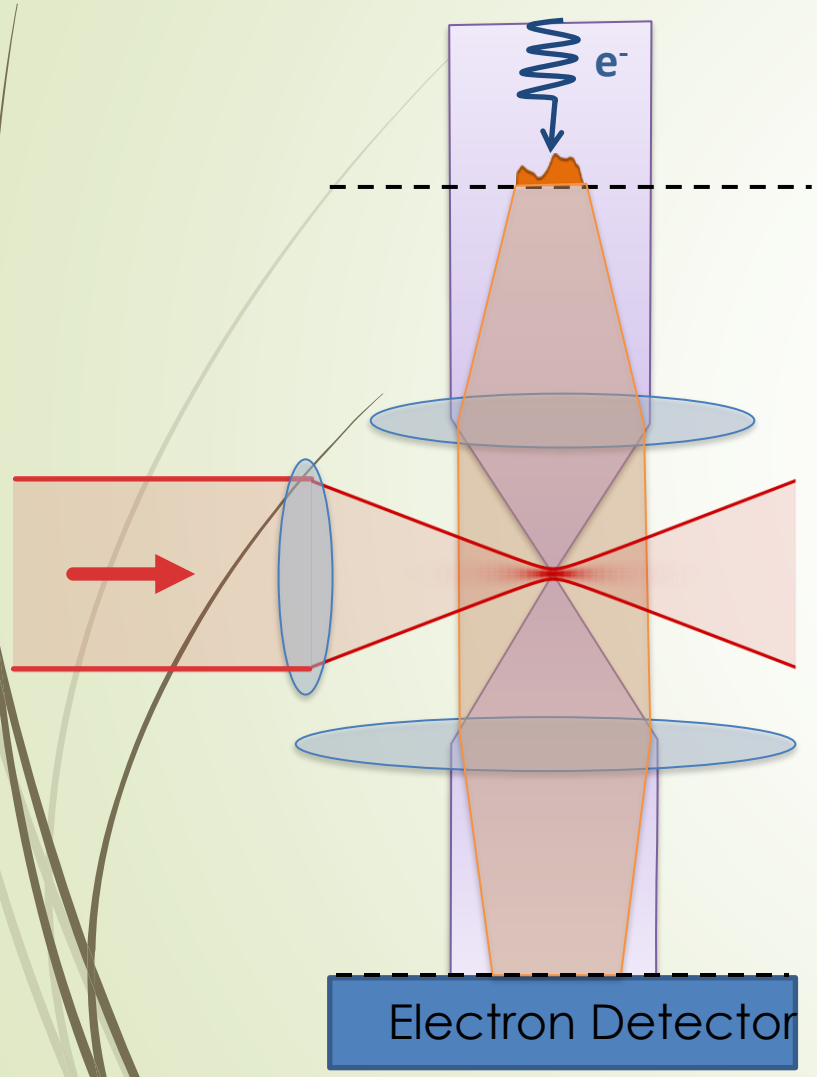




- …effect is not well-controlled
- Few labs have successfully tried it



Laser Phase Contrast



- Laser creates phase shift through "ponderomotive potential"

- No charging
- No radiation damage
- Well-controlled

- Need 75 kW focused to 8 μm, or 300 GW/cm^2, for 300 keV beam

New J. of Physics 12, 073011 (2010)

Need a big laser?...

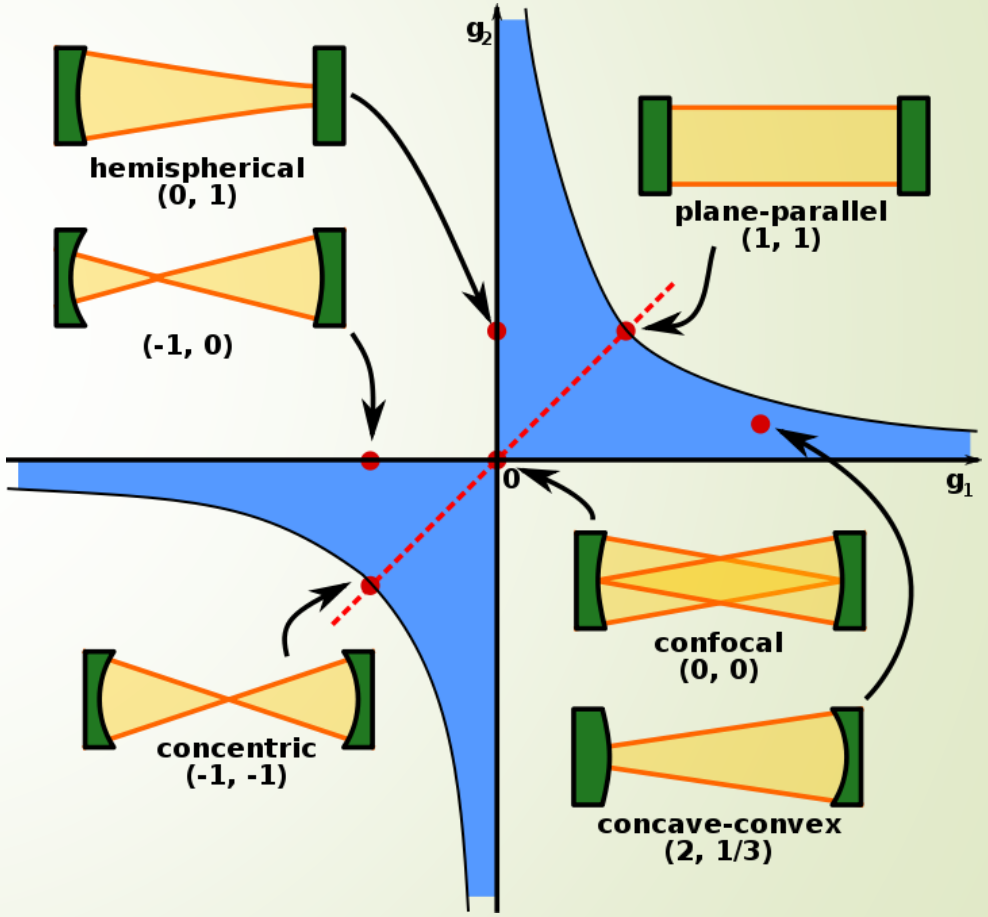
- 10 kW industrial laser (IPG)
- Needs a lot of room
- Prohibitively expensive
- We'd need 7 or 8 of those

- 30 kW military laser, Lockheed Martin
- Potentially unsafe
- Insufficient power

Use a cavity!

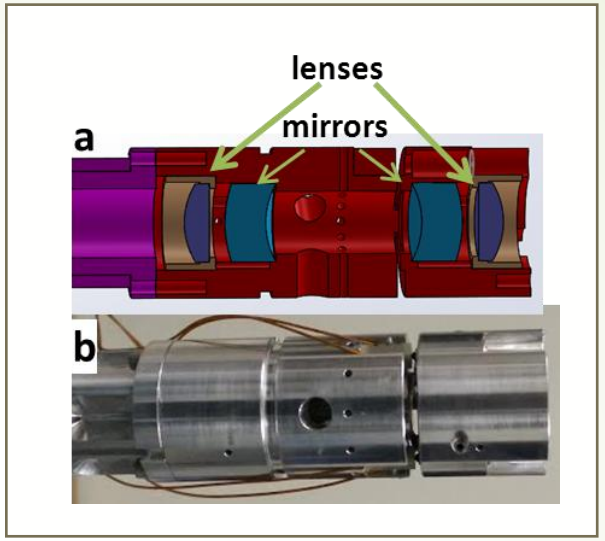
Design principles

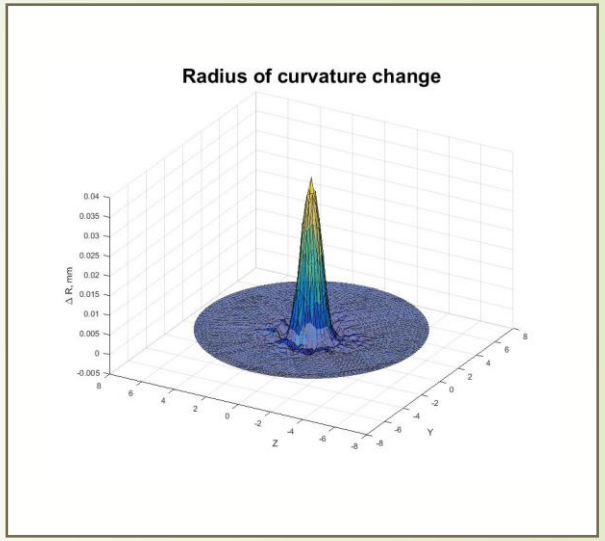
- Unknowns about contamination, thermal lensing, damage threshold
- The best part is no part
- Two mirrors
- Near concentric for focusing

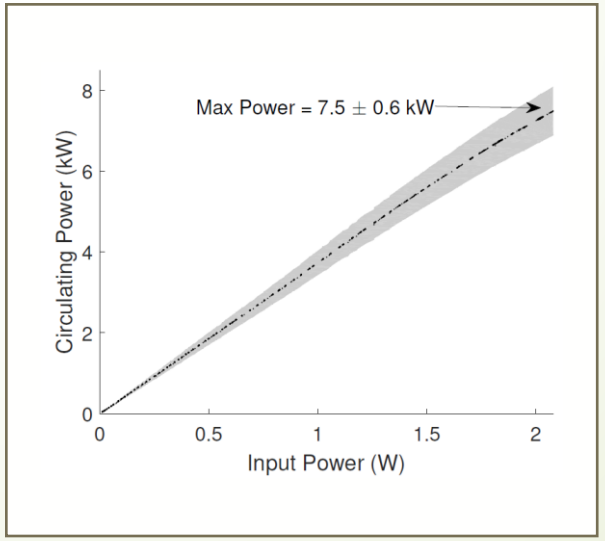


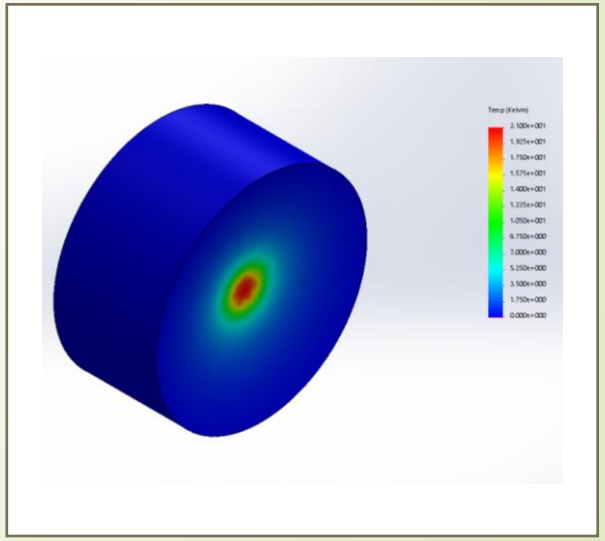
2016: 7.5 kW, limited by thermal expansion

Schwartz et al., Opt Express **25,** 14453 (2017)





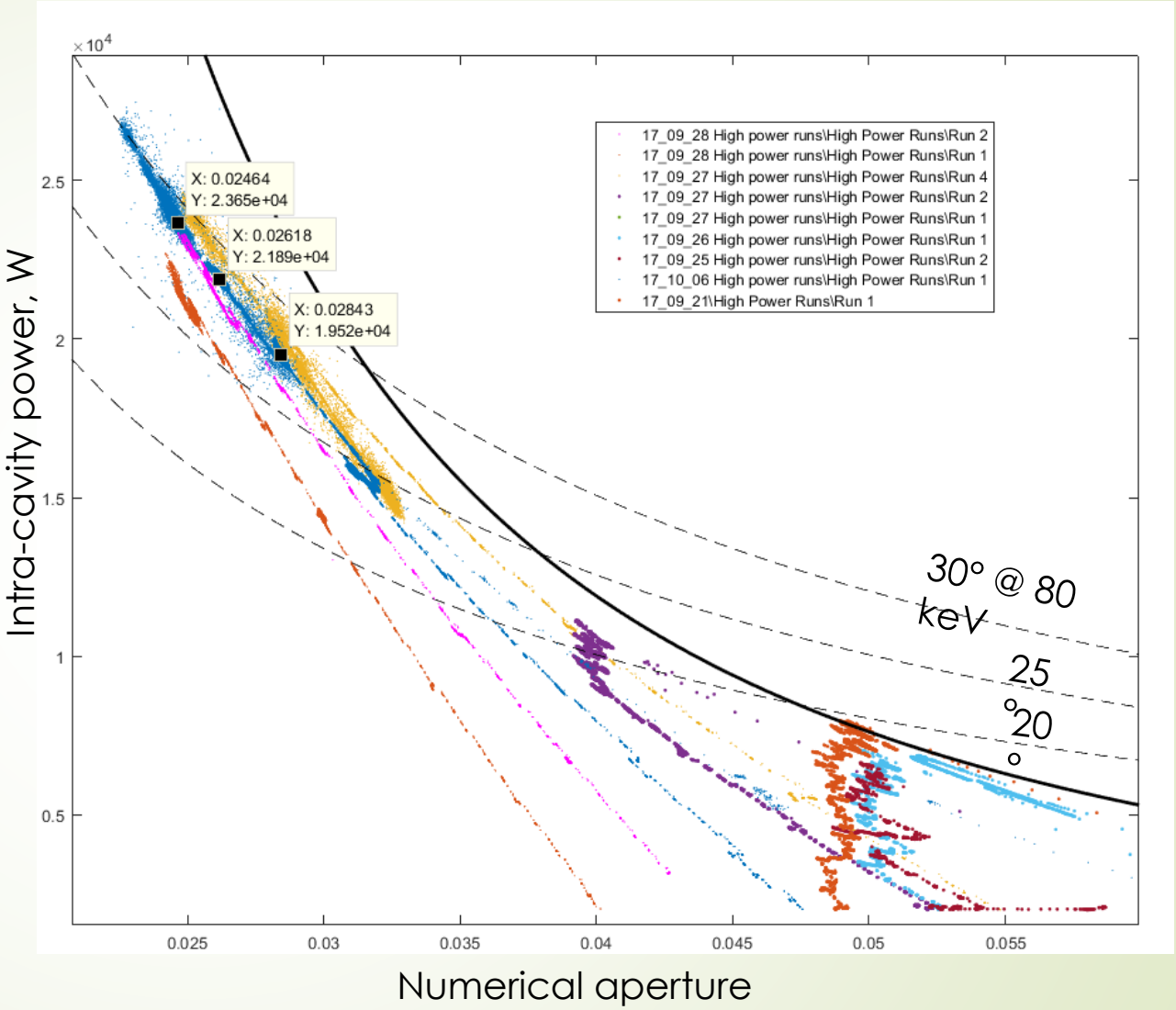


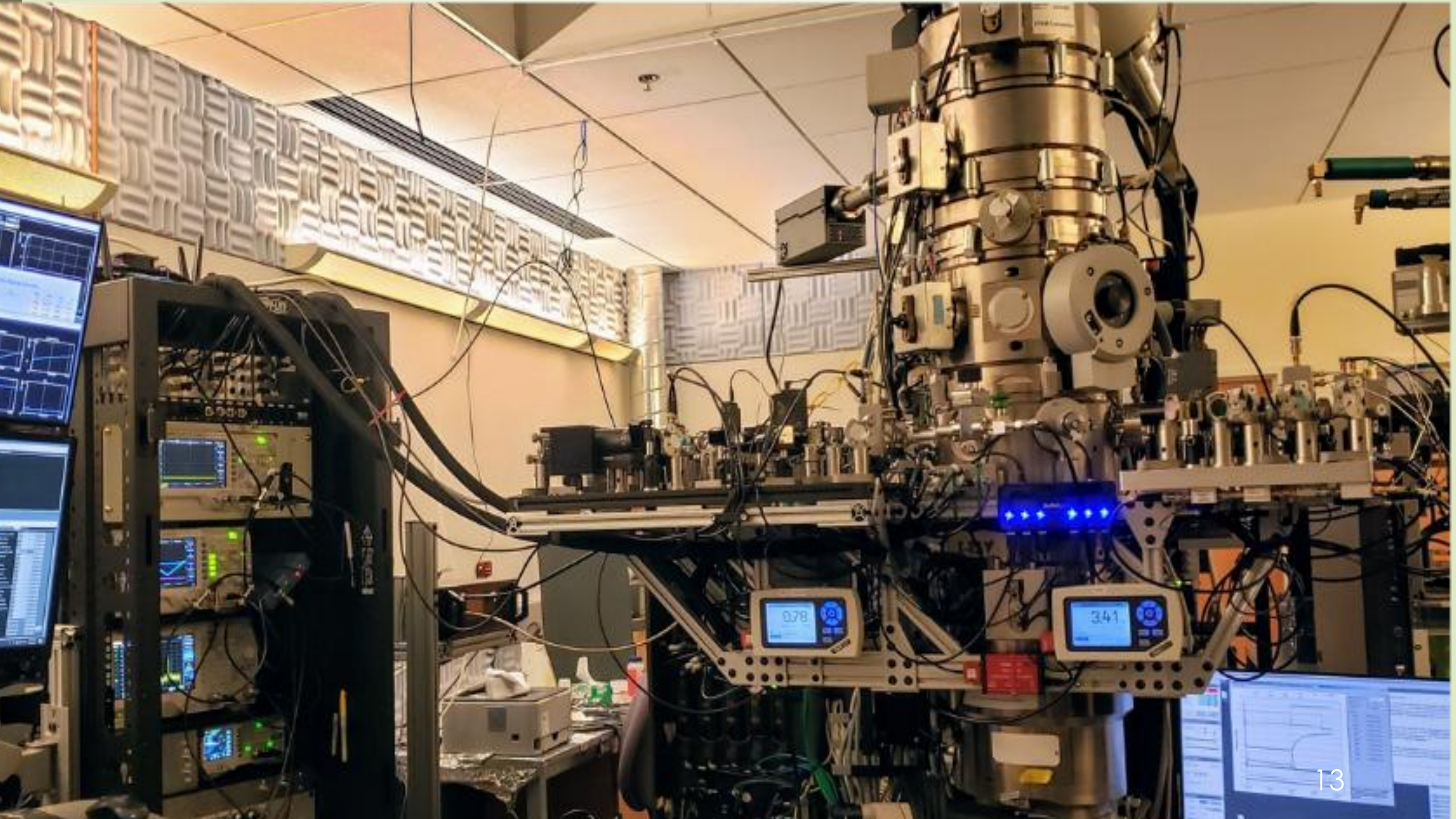


2017: More power, at the cost of NA…

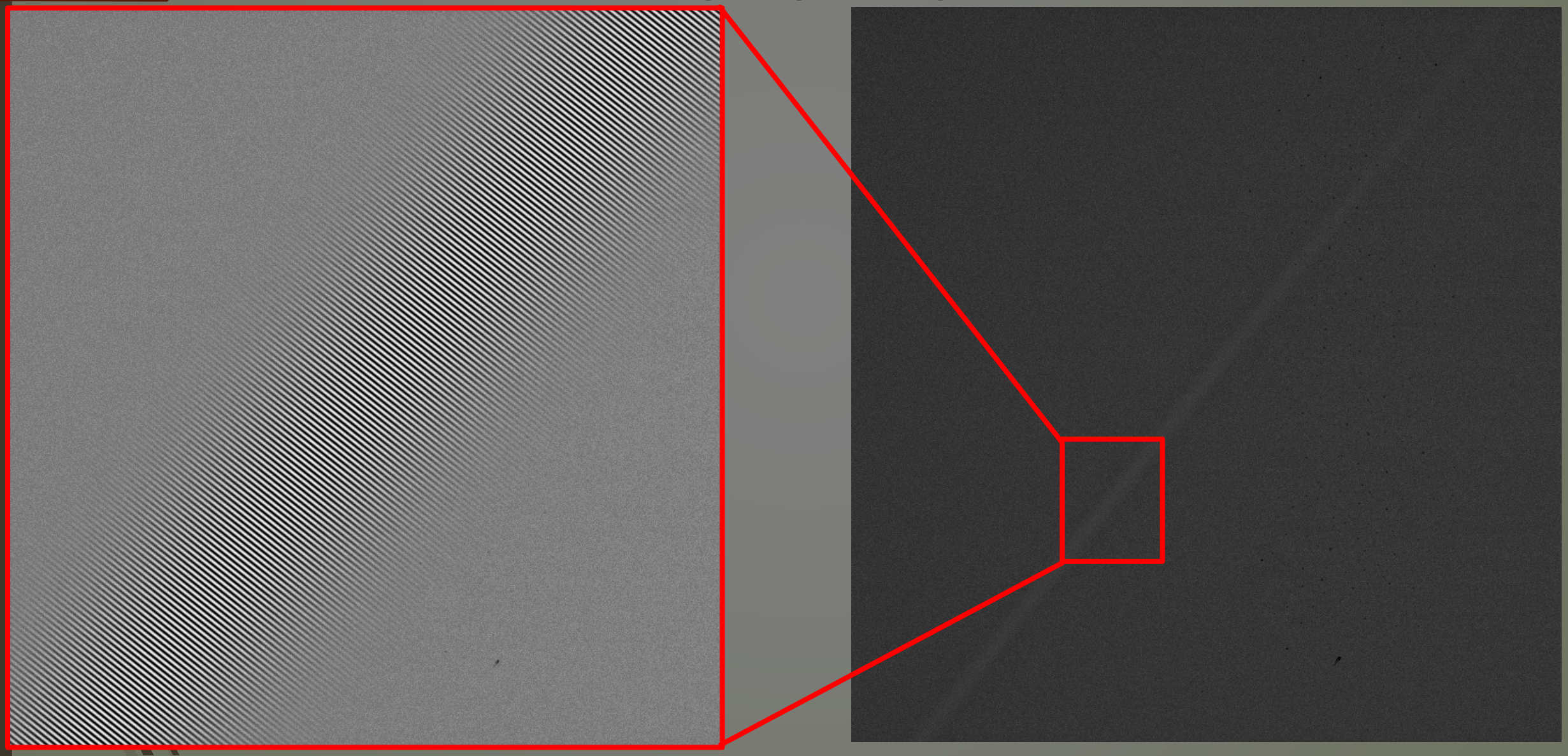
What's the way out?

1. Better coating: LIGO has C_{abs} =1 ppm, we have C_{abs} = 10 ppm
2. Substrate material:
   a. Corning ULE would allow 10x-100x improvement depending on temperature
   b. Sapphire would allow for 2x improvement at room temperature but 3000x at liquid nitrogen temperature
   c. Diamond: 1000x higher already at room temperature




0.78
3.41

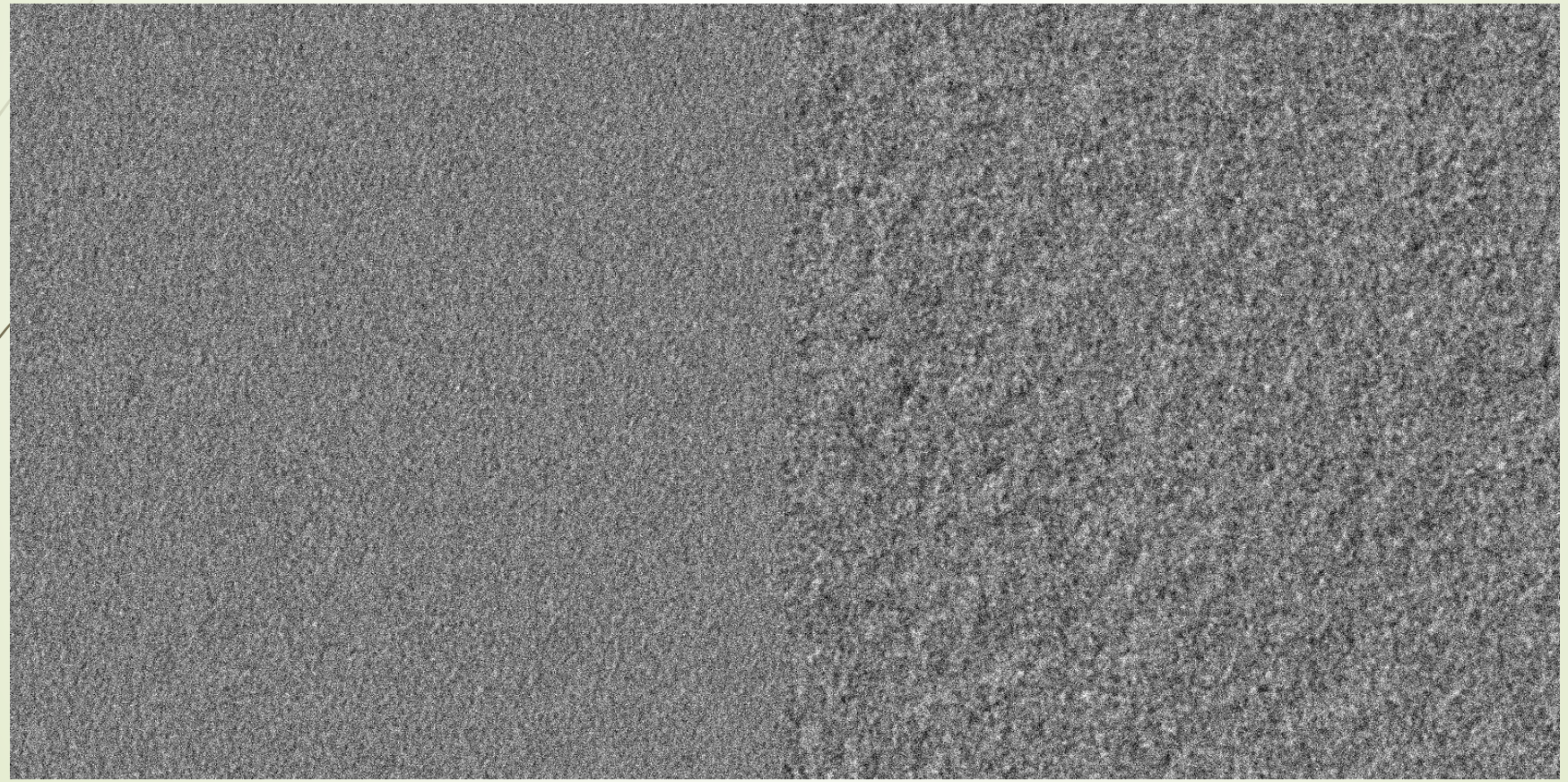
Results: Imaging a light wave!



… and initial phase-contrast images!

Laser off

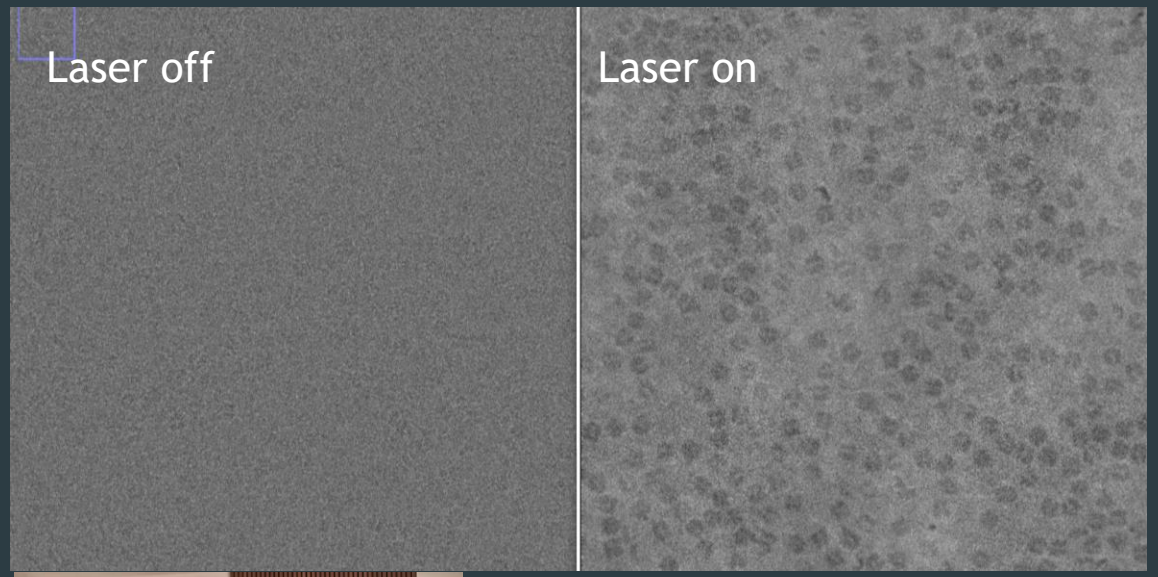
Laser on



Thin carbon film (~3 nm), 20 s exposure, 80 keV

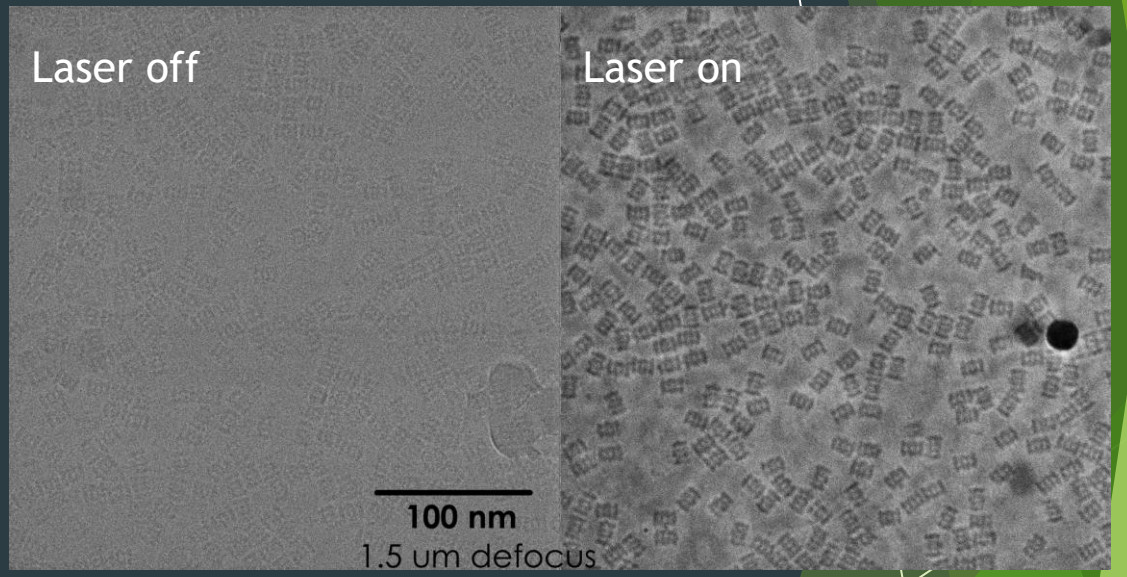
2019: Finally 90 degrees
(and beyond… 97 kW, 450 GW/cm^2)

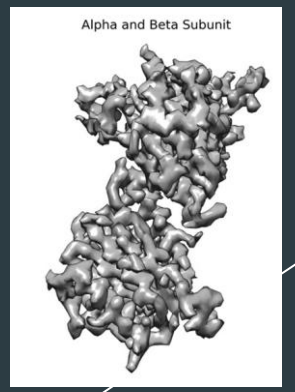
Rubisco



Carter Turnbaugh, et al., Rev. Scientific Instrum. 92, 053005 (2021)

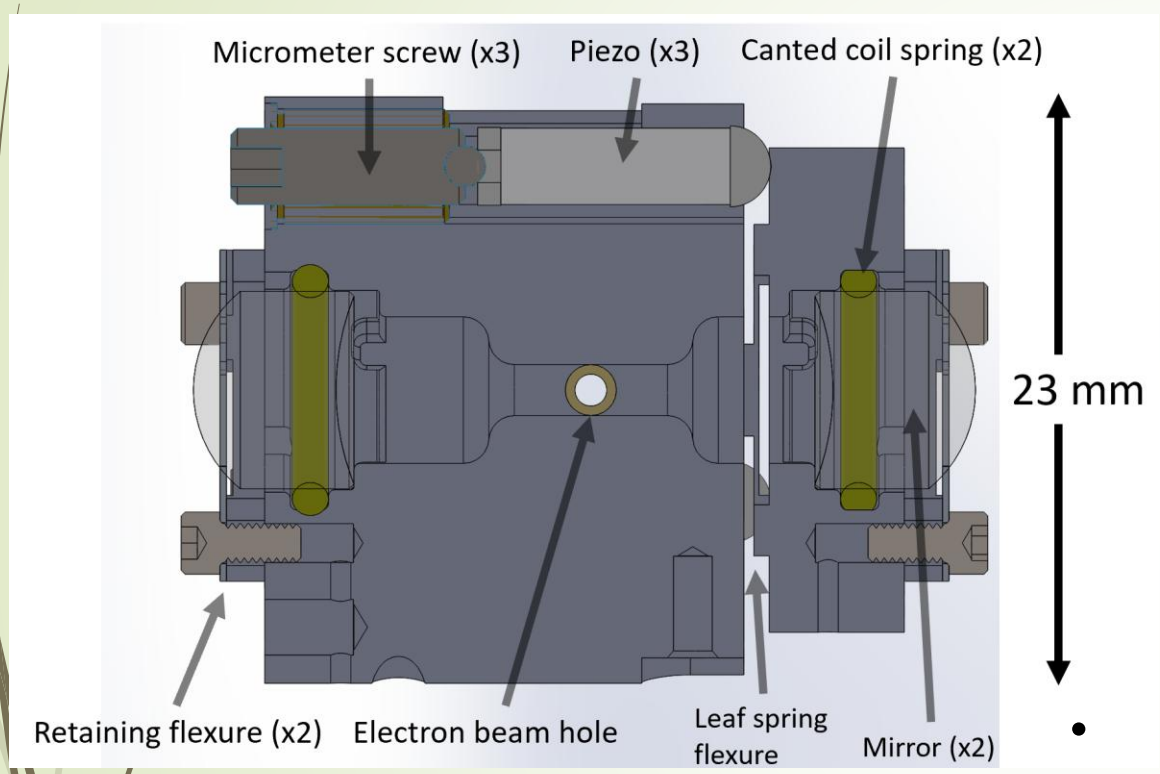
20S proteasome

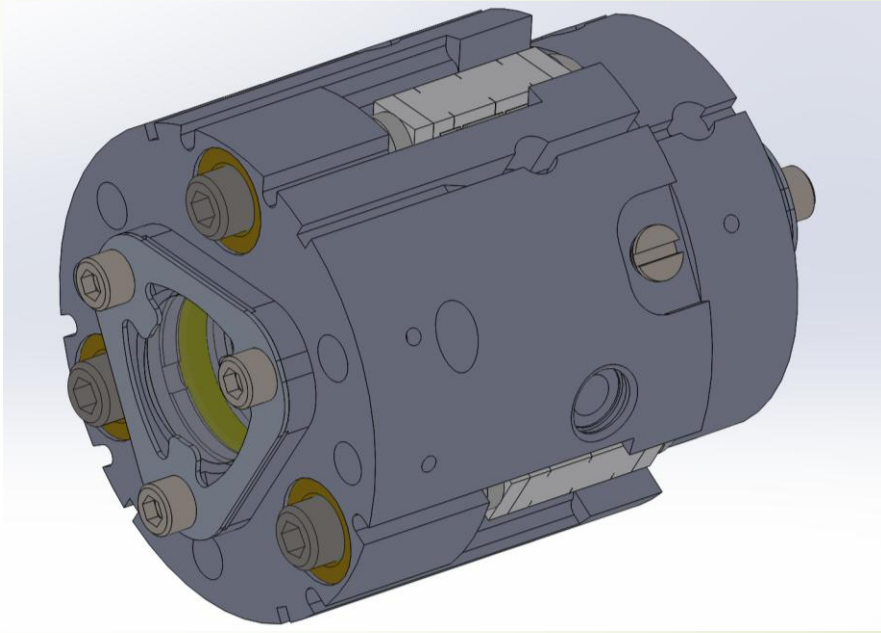




Thanks to Yifan Cheng!

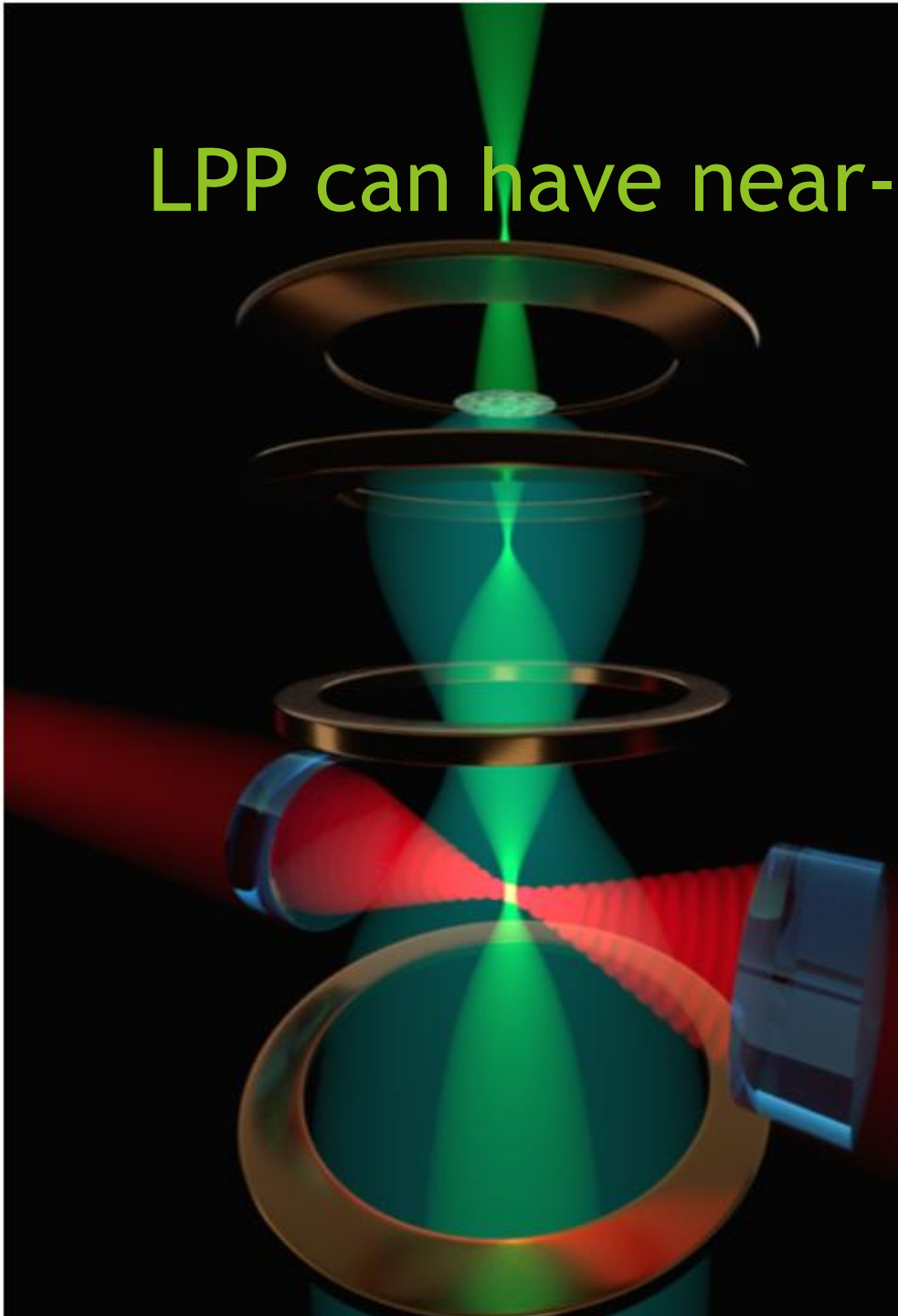
Cavity Design



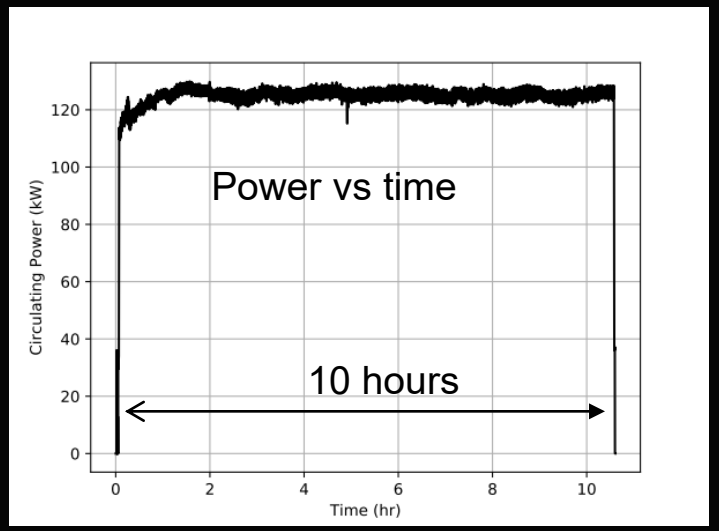


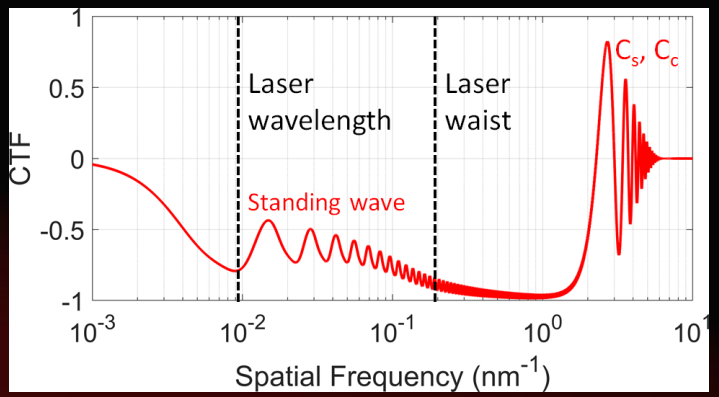
- 10 mm curvature radius ULE mirrors, < 20 ppm loss
- Monolithic aluminum mirror mount
- Leaf spring flexure for near-concentric alignment
- Tripod actuator configuration for tip/tilt/length control

LPP can have near-ideal properties



300 GW/cm^2 cw!!

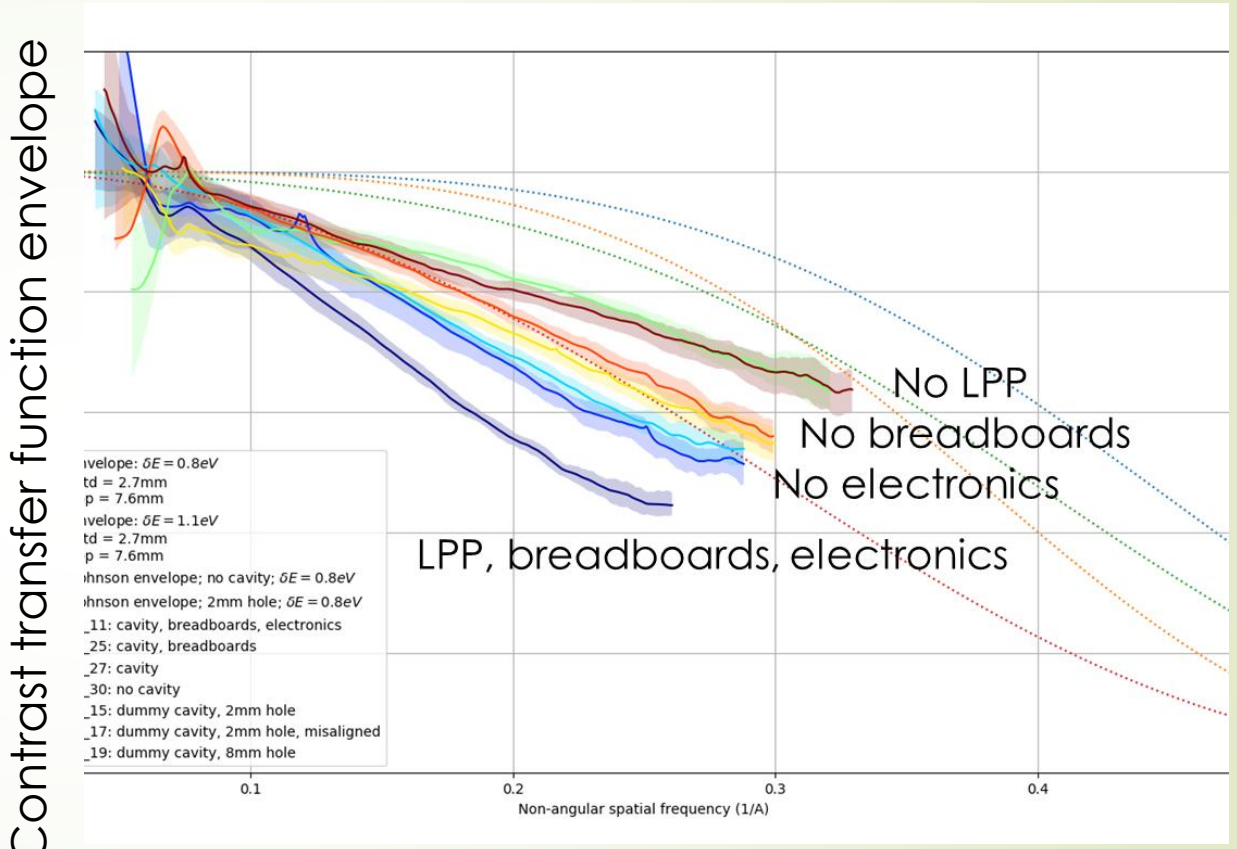




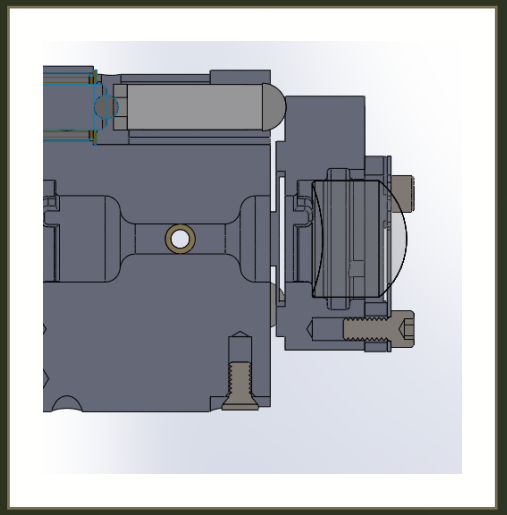
…but needed relay optics triples Cc currently

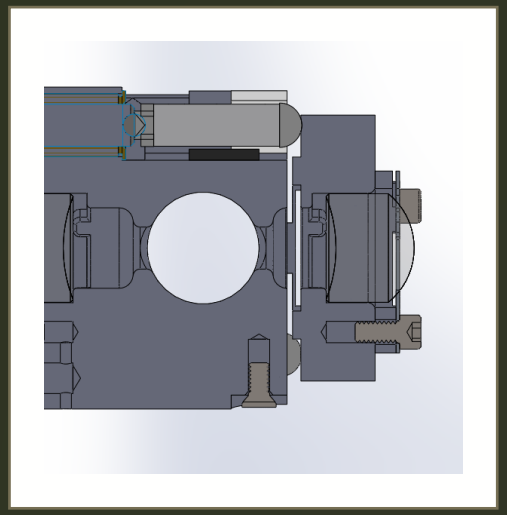
But limitations still apparent…

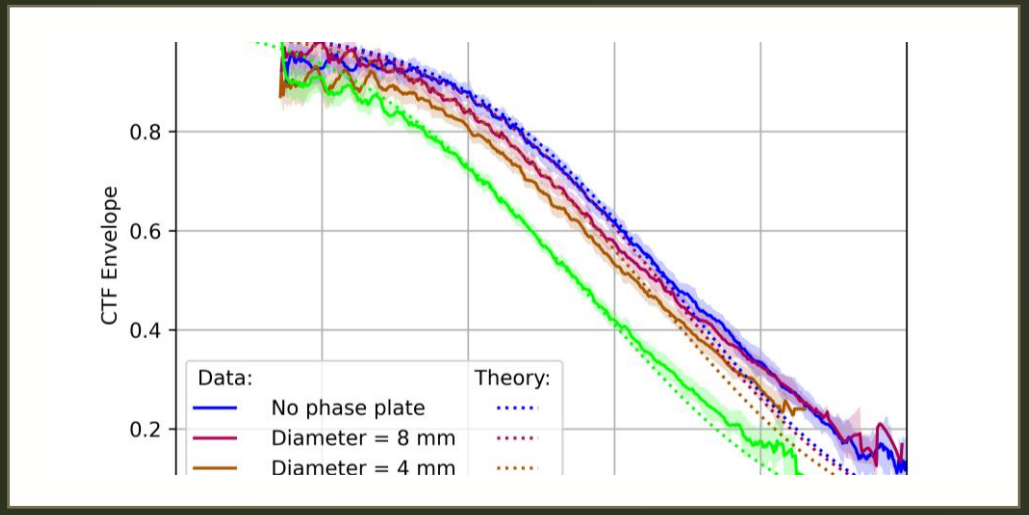
- 64 micrographs, 5789 particles, 3.8 Å resolution
  - Cc (as expected…)
  - Ice contamination
  - Johnson noise (surprise!)



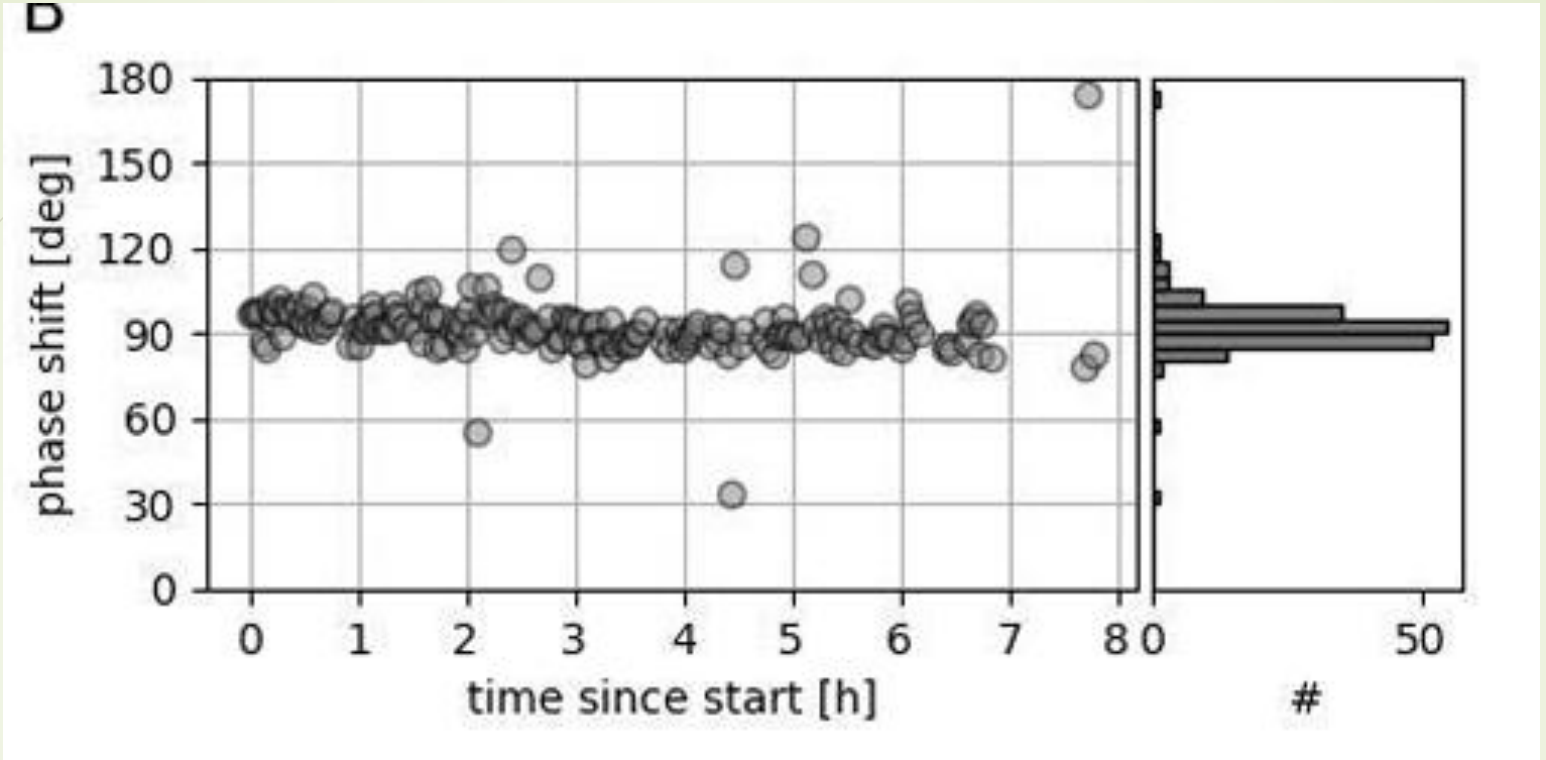
Spatial frequency





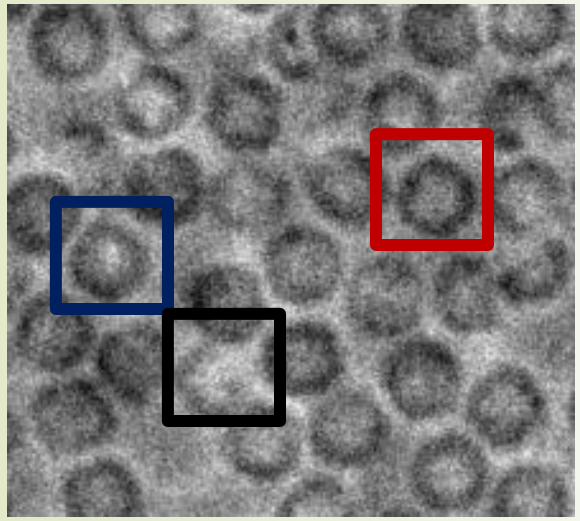


Overcoming resolution loss from Johnson noise

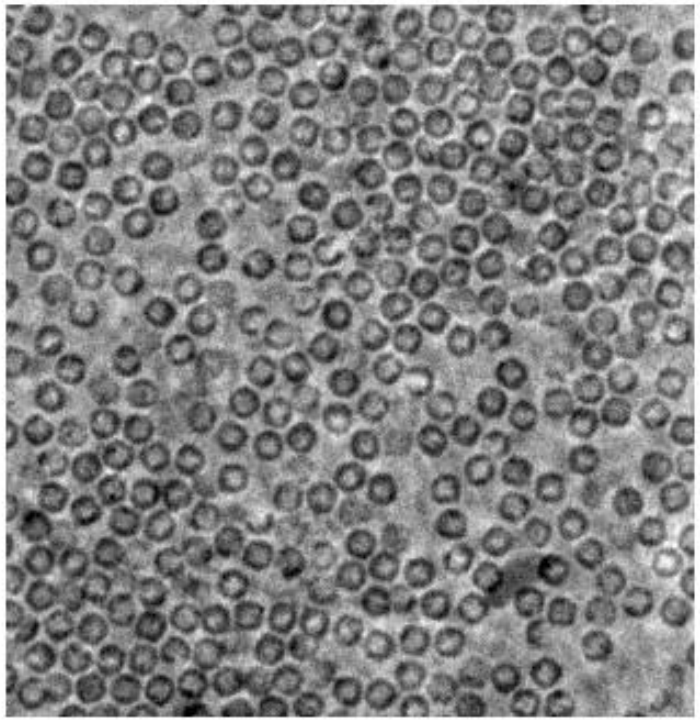


Phase shift is stable

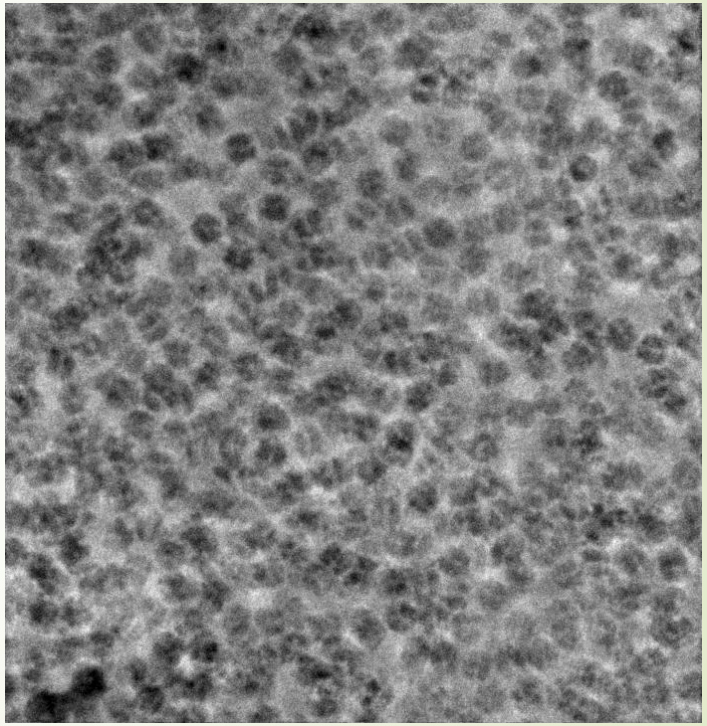
LPP reveals an astonishing degree of particle heterogeneity…



Light center (“ring”)
Dark center (“disc”)
Incomplete rings (“Gs”)



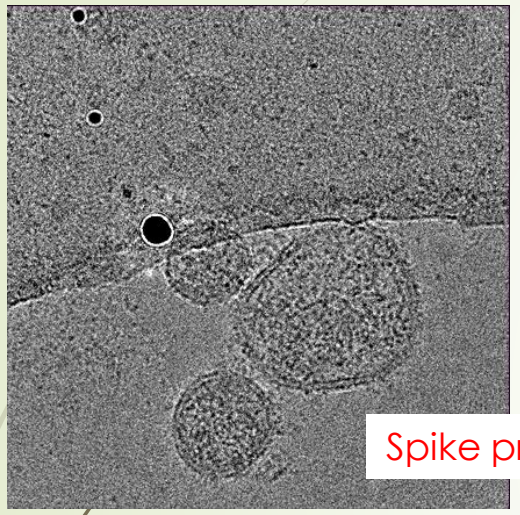
ApoF

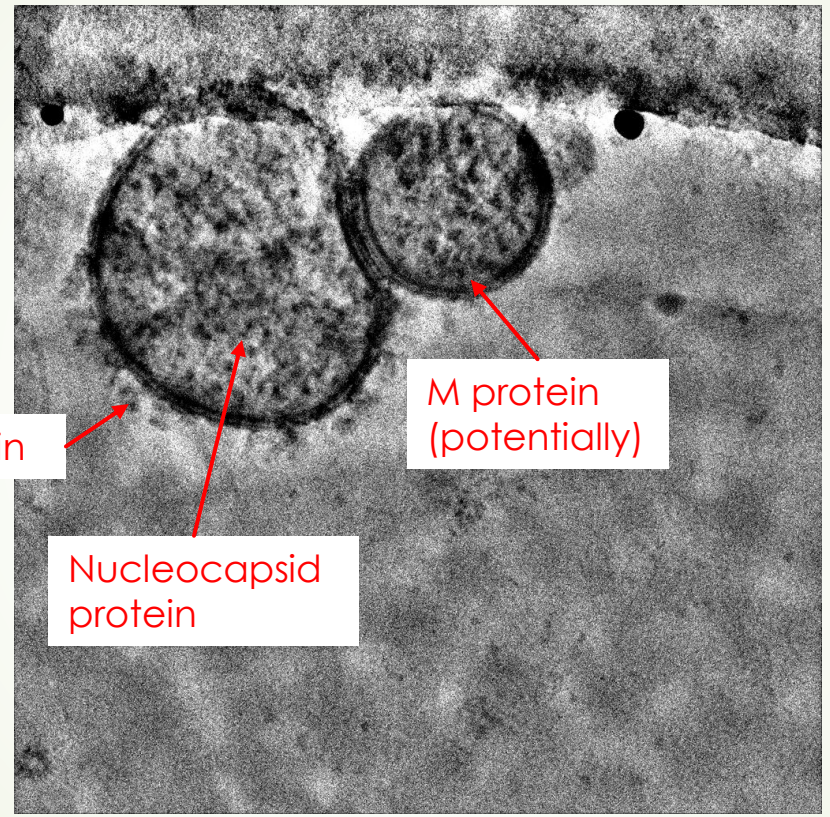


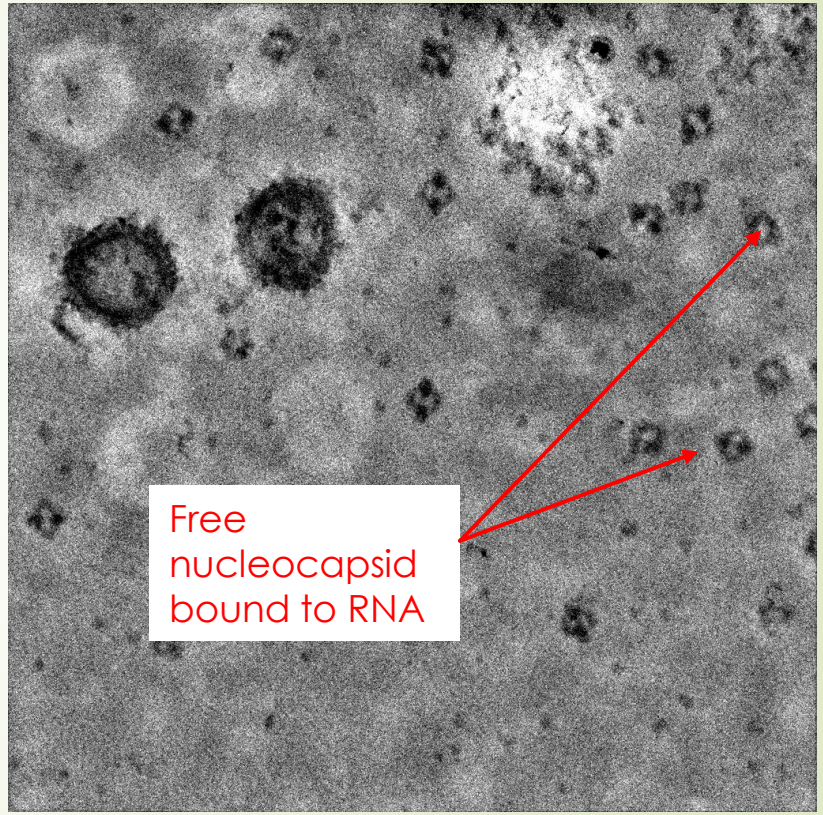
Rubisco

…enabling HI and AI to see variations between individual particels, not just class averages

Imaging Covid Virus-Like Particles





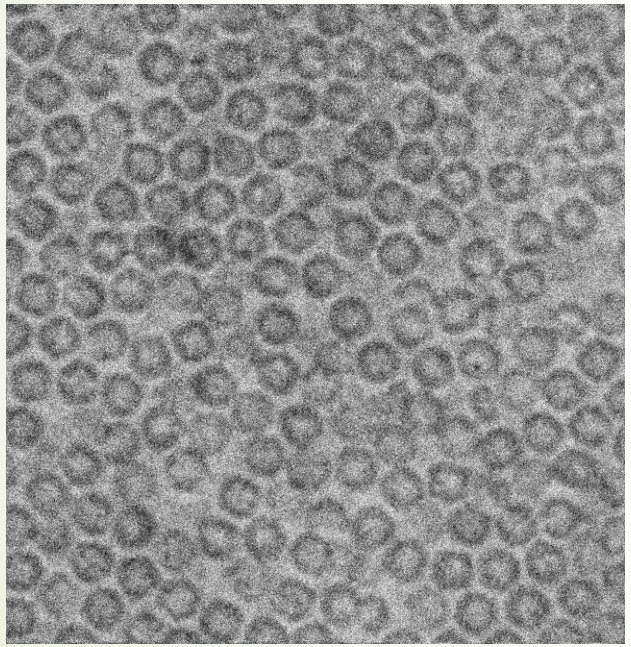


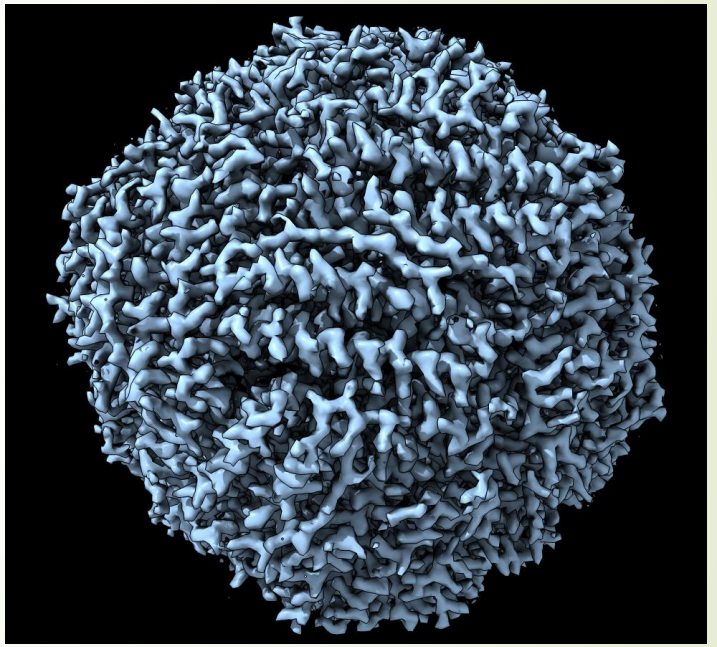
Still limited by Cc (as expected…)

Resolution now limited by Cc…

Laser on, 2.8 A with 15,000 particles

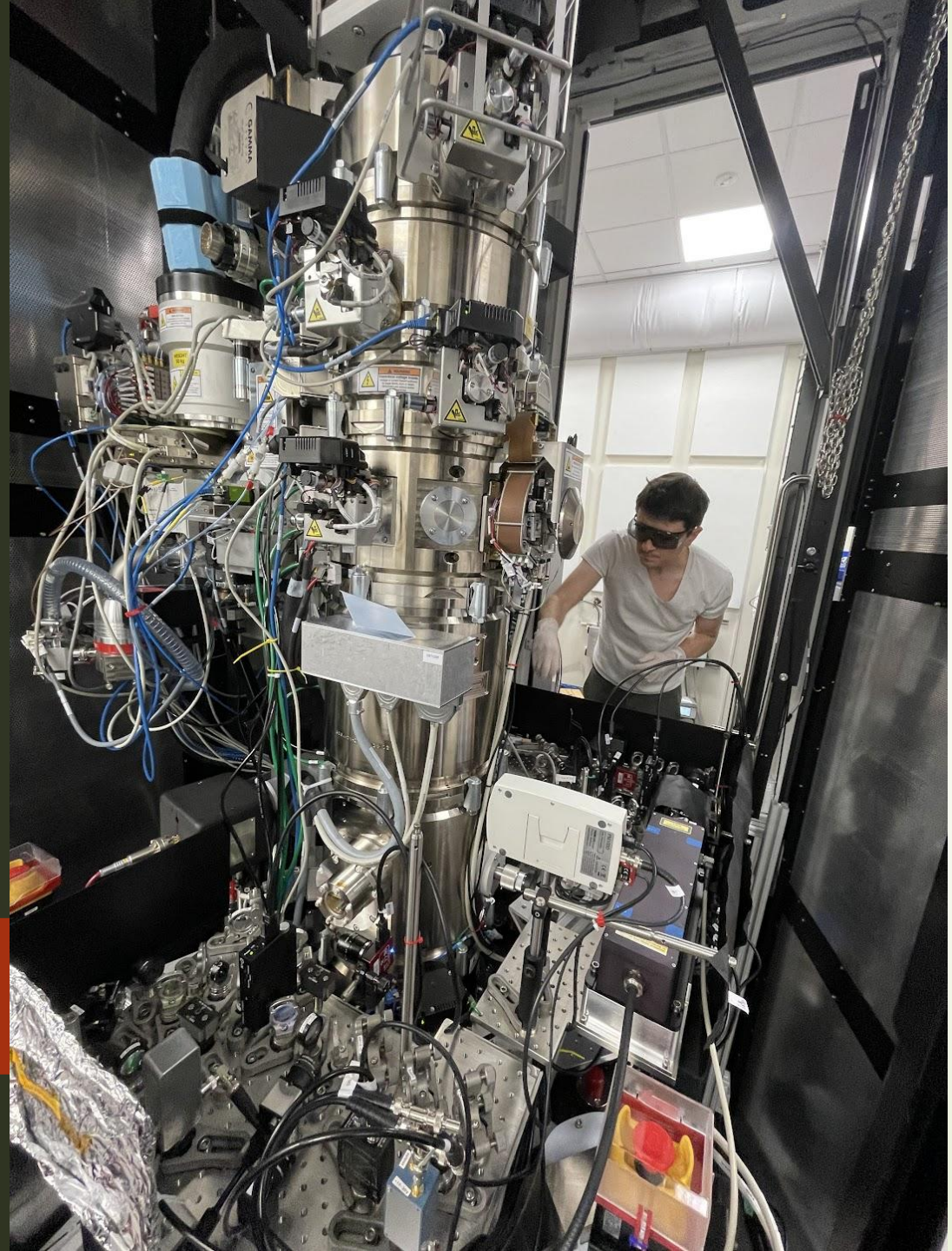
800 nm defocus
50e-/Å² tota; dose





…and we expect much better results with the new microscope soon!

New microscope!!



C-FEG to overcome Cc: 1 Å resolution instead of 3 Å

Automation for high throughput

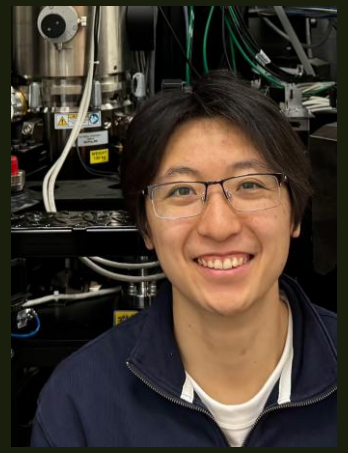
Cs- correction

Long effective focal length

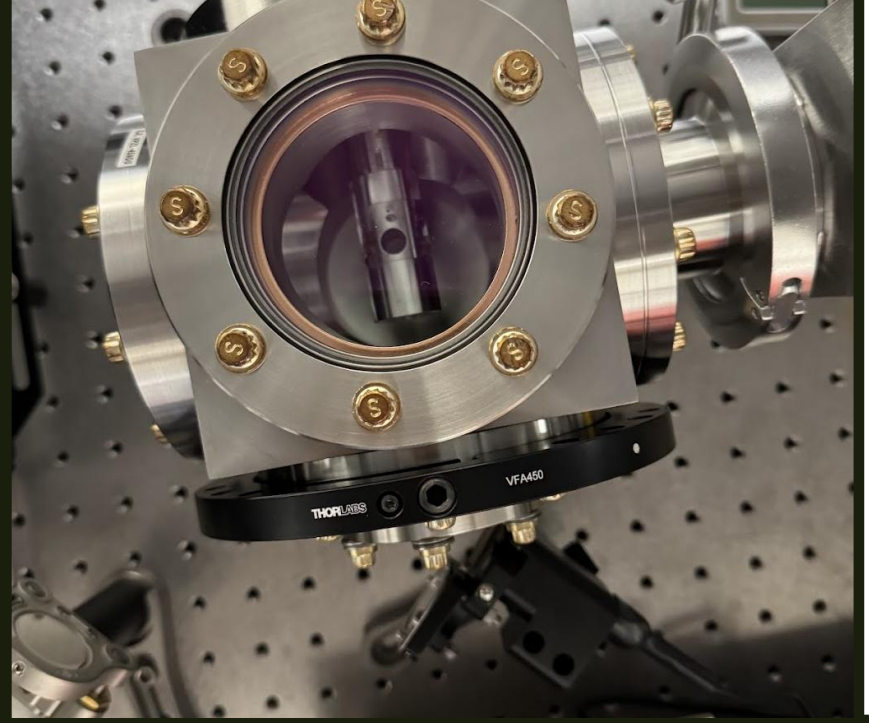
Selectris & Falcon 4

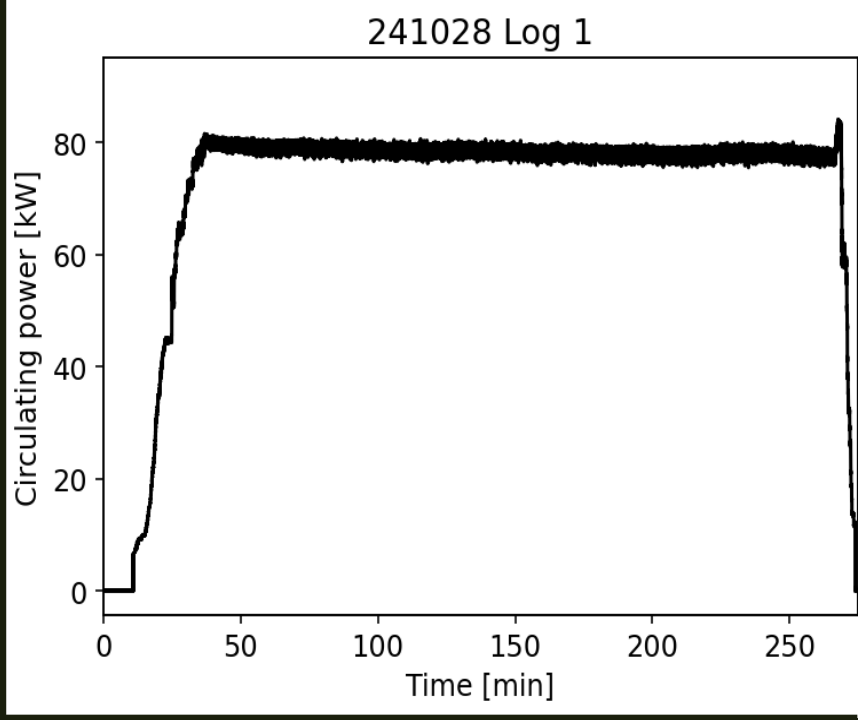
Chan Zuckerberg Initiative

Petar Petrov

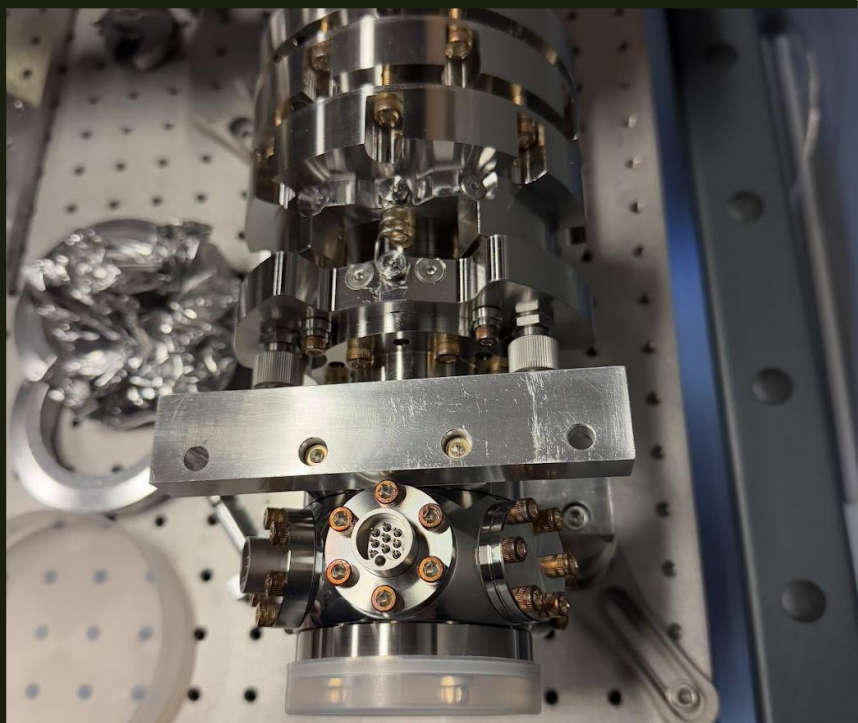


Jessie Zhang

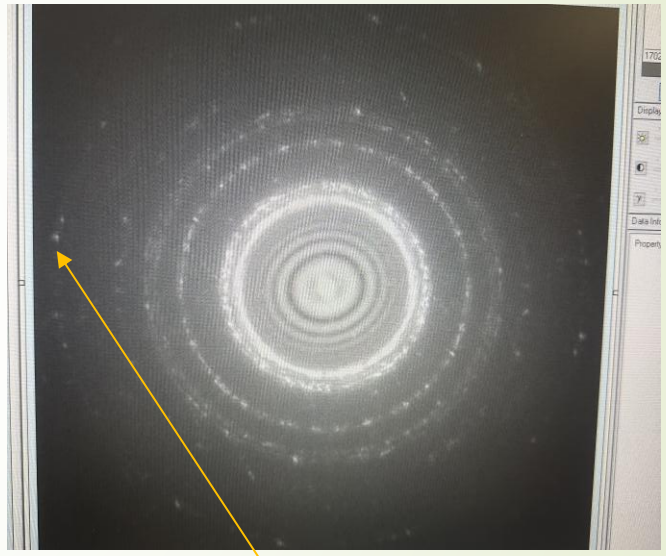




T-1 cavity built and tested



Installing the phase plate…

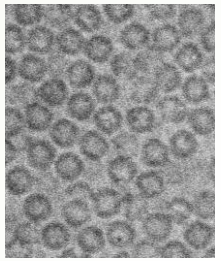


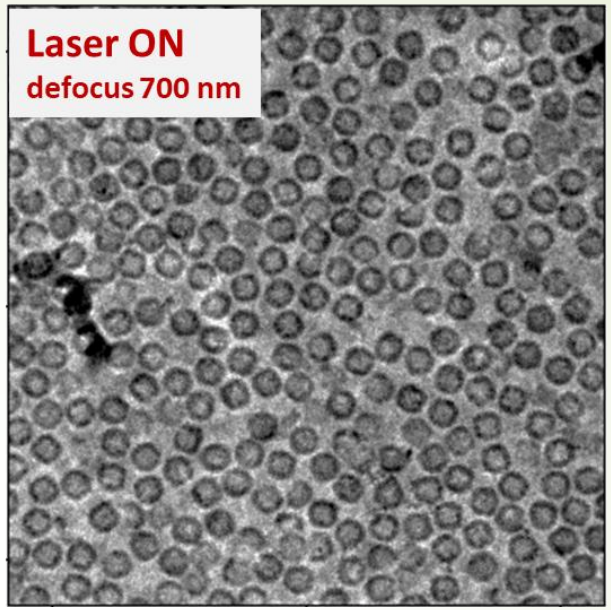
0.7 A resolution!

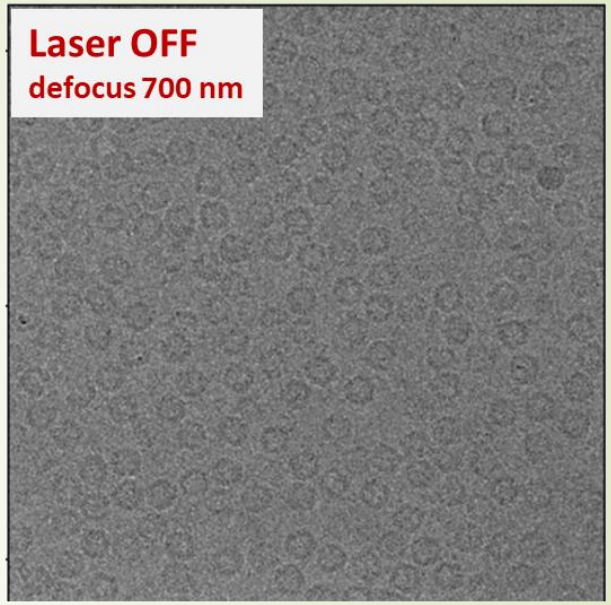
Petar? Jessie?

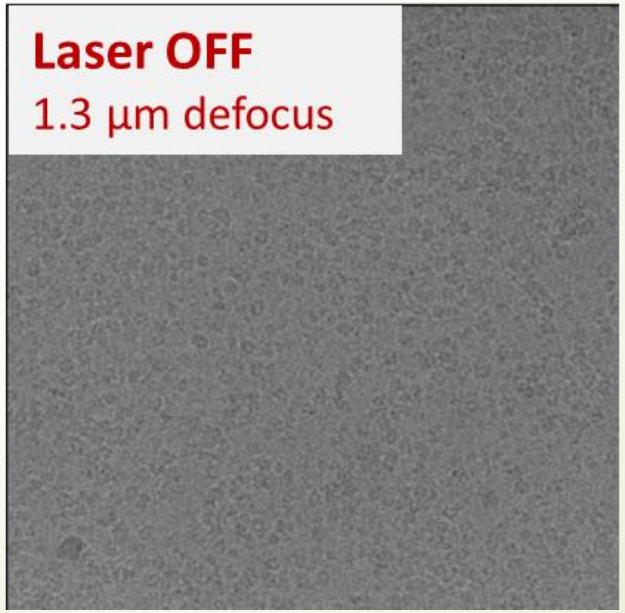
SPA: Apoferritin & Hemoglobin

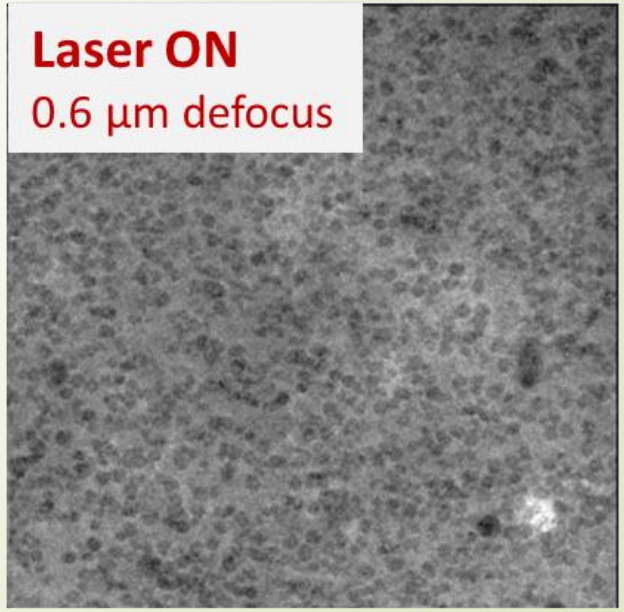
- Apo:
  - First thing we looked at.
  - Got a reconstruction out to 2.8 Å from about 50 images.
- Hemo:
  - Some class averages from ~ 50 images we took.
  - There are 10 classes and a total of 10800 particles here.
  - Likely to yield full reconstruction once we have more data



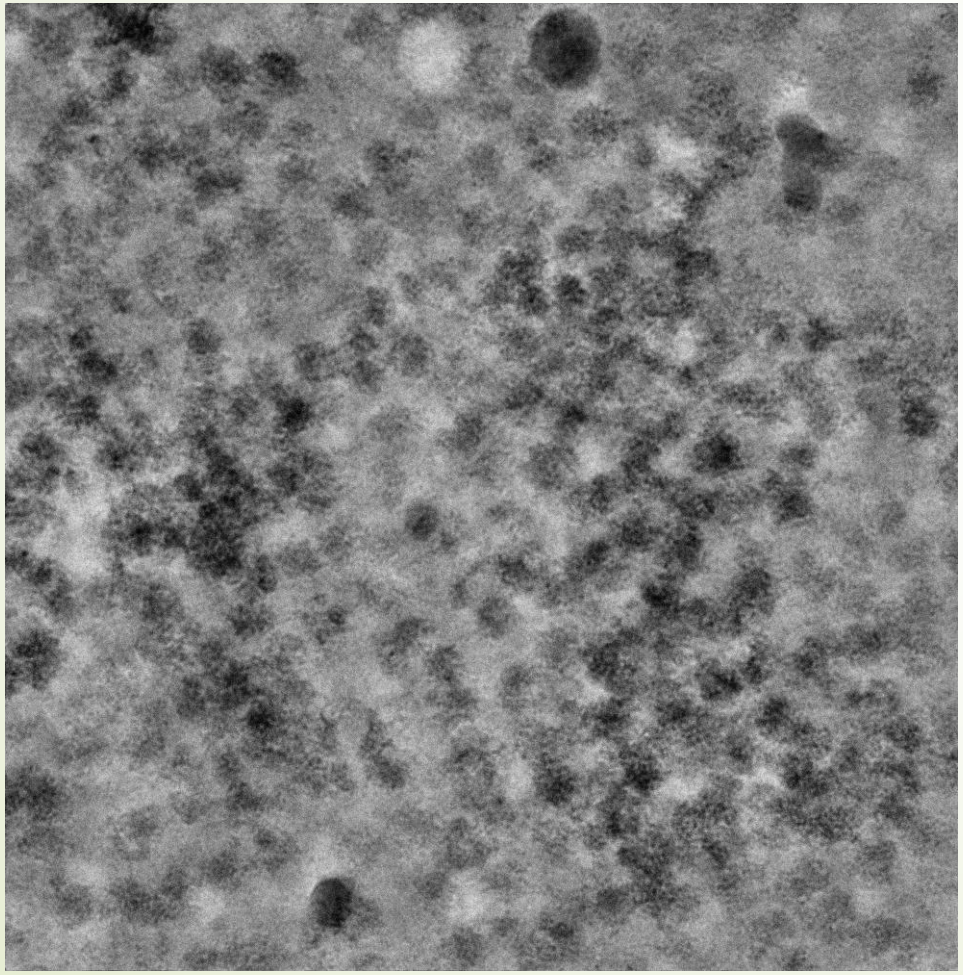


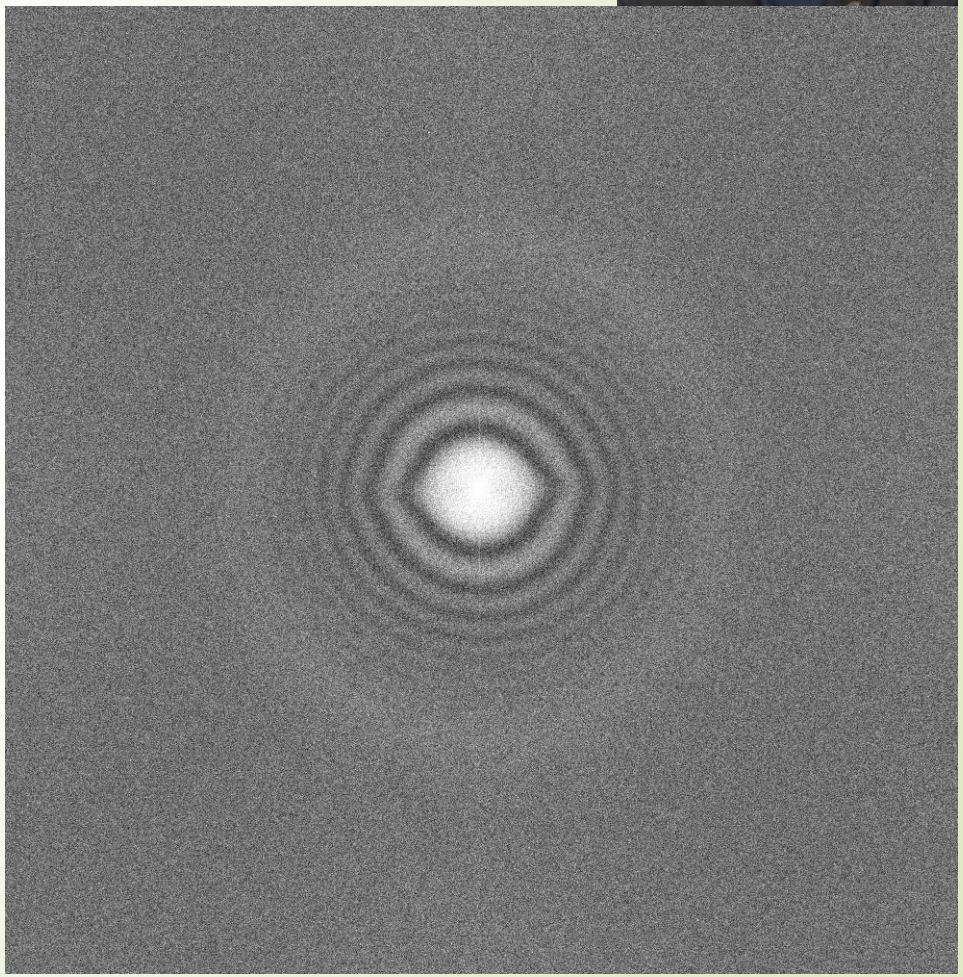




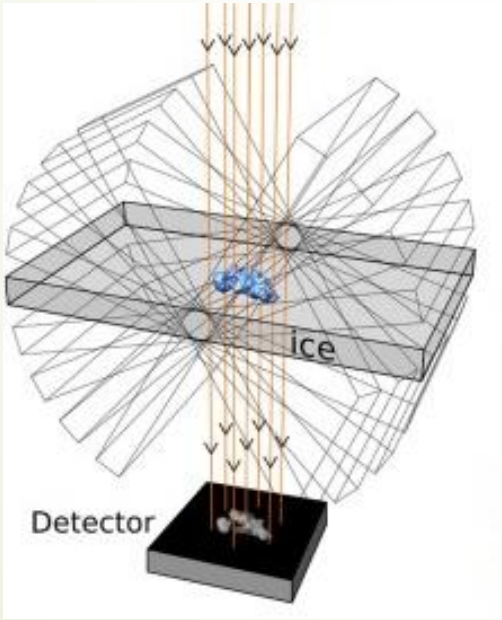


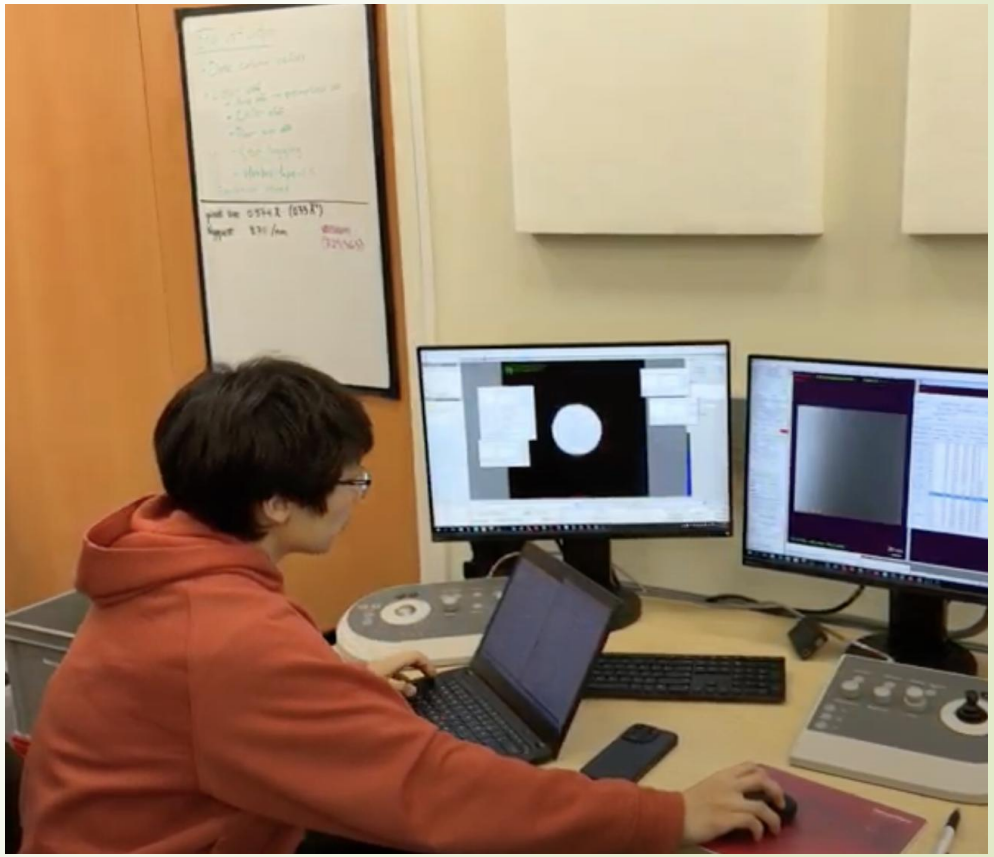
Ribosomes - thanks to Chandrima Majumder, Jamie Cate Lab





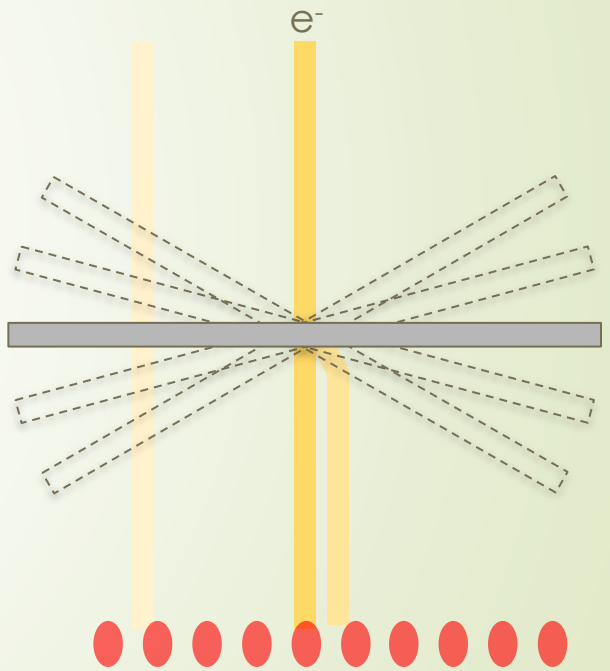
Tomography is the next frontier





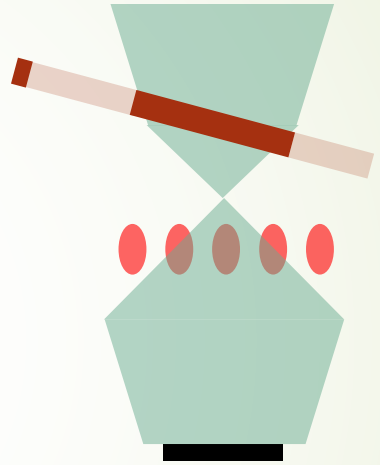
…but the electron beam needs to be aligned at each tilt angle

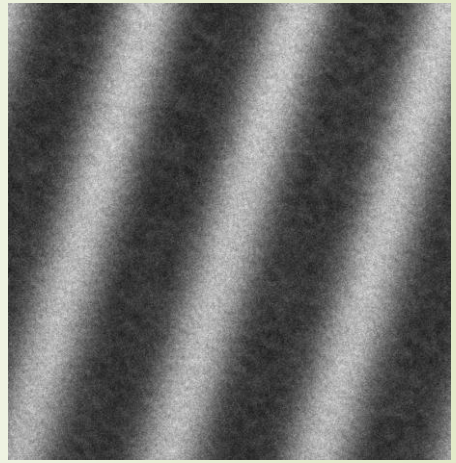
- Centered on sample stage tilt axis
- Aligned to the laser beam
- Sample-charging deflects the e- beam and may ruin alignment

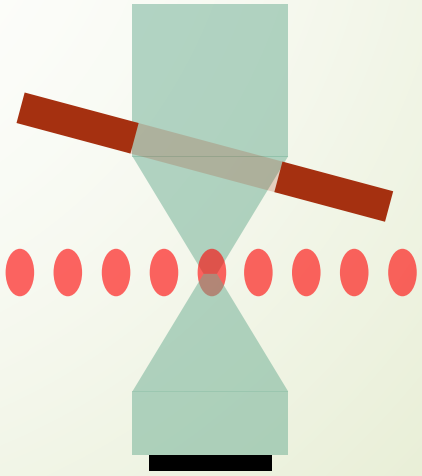


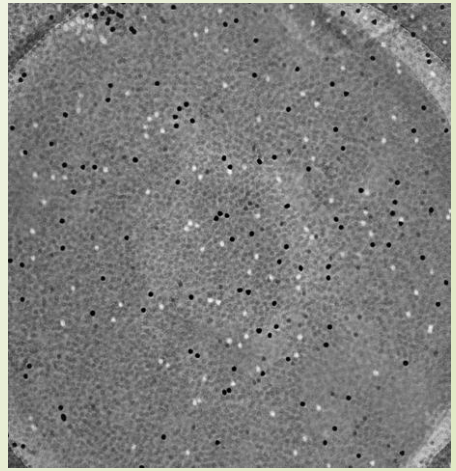
Automated laser-electron alignment

1. Set tilt angle
2. Shift beam out of hole
3. Change illumination to record Ronchigram
4. Determine alignment and apply corrections
5. Shift beam back to hole center
6. Record movie



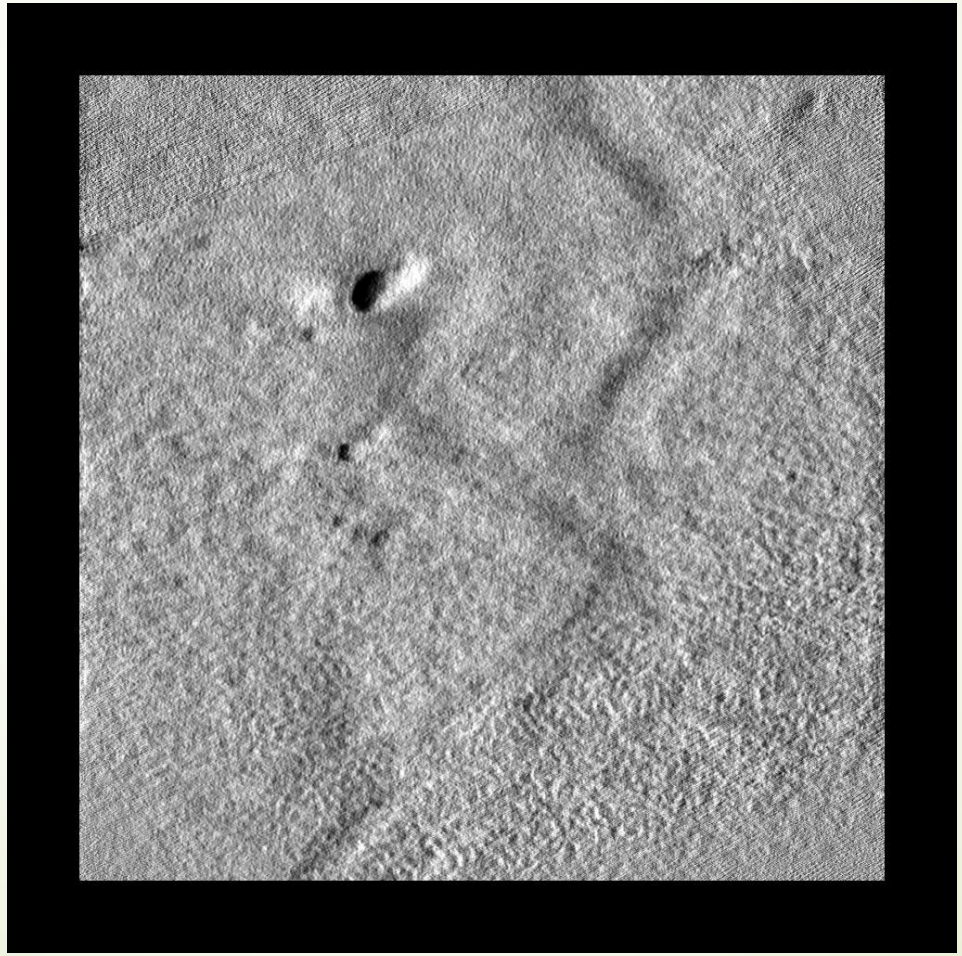






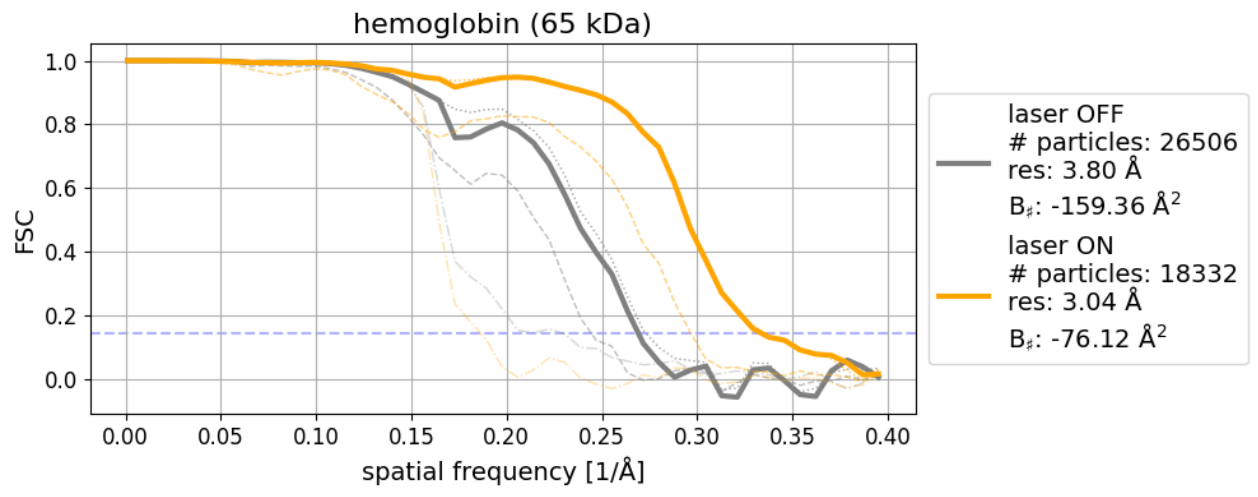
Tomogram of mammalian HSC-3 cells

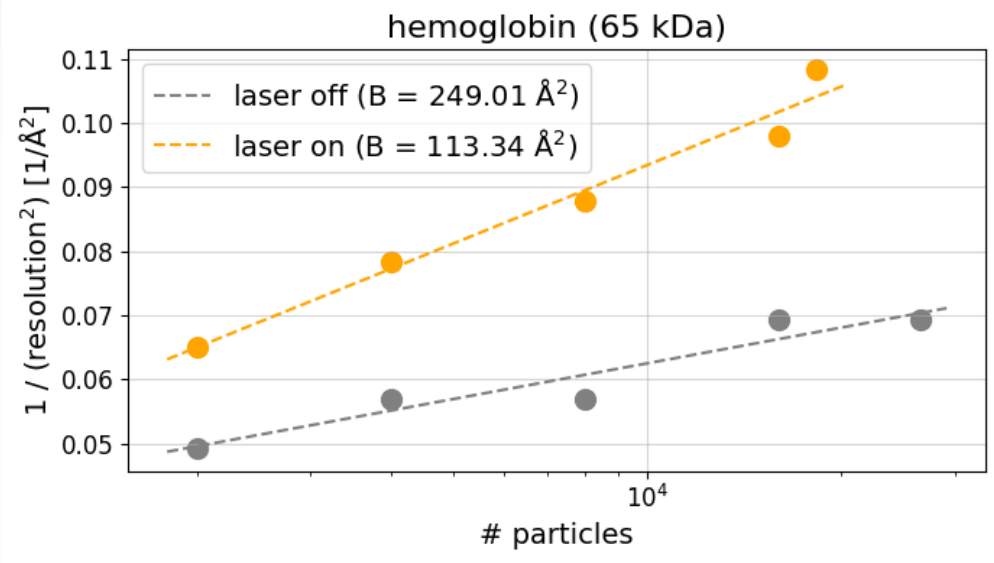
- -39 to +39 degrees
- 3-degree steps,
- dose-symmetric out to 27 degrees.
- 2.6 e/A^2 /tilt step

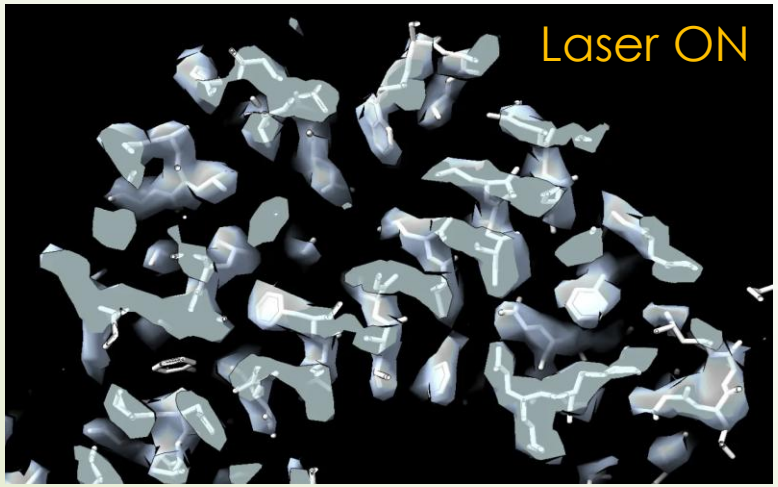


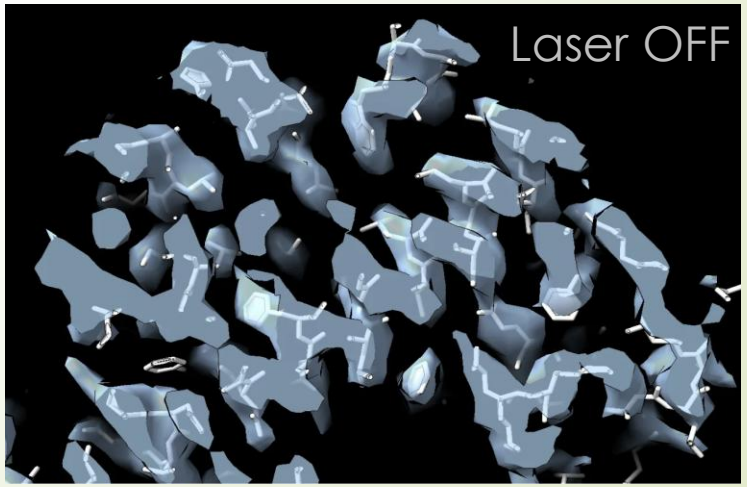
Hang Cheng

FSC improvement, B factor boost

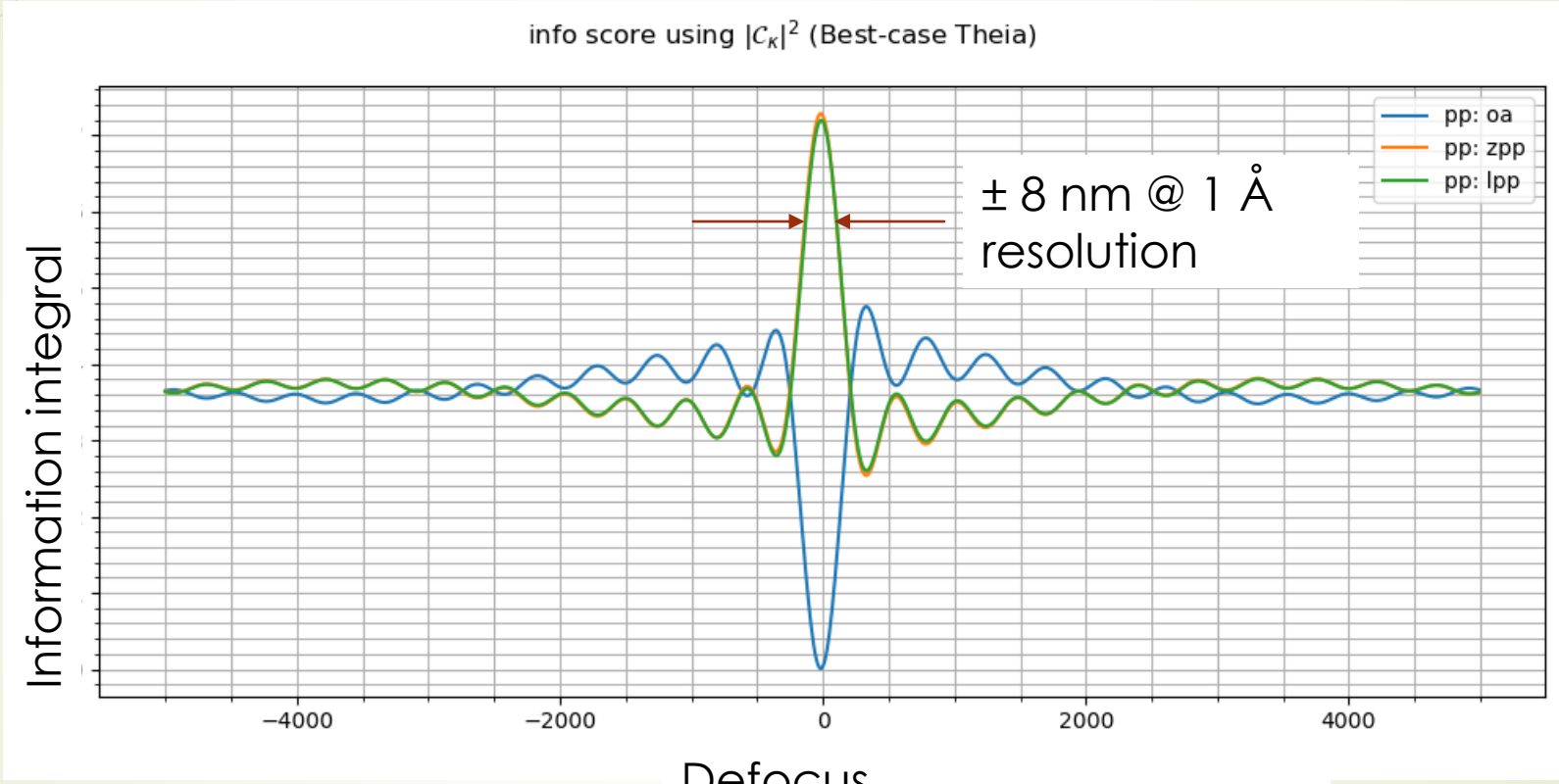








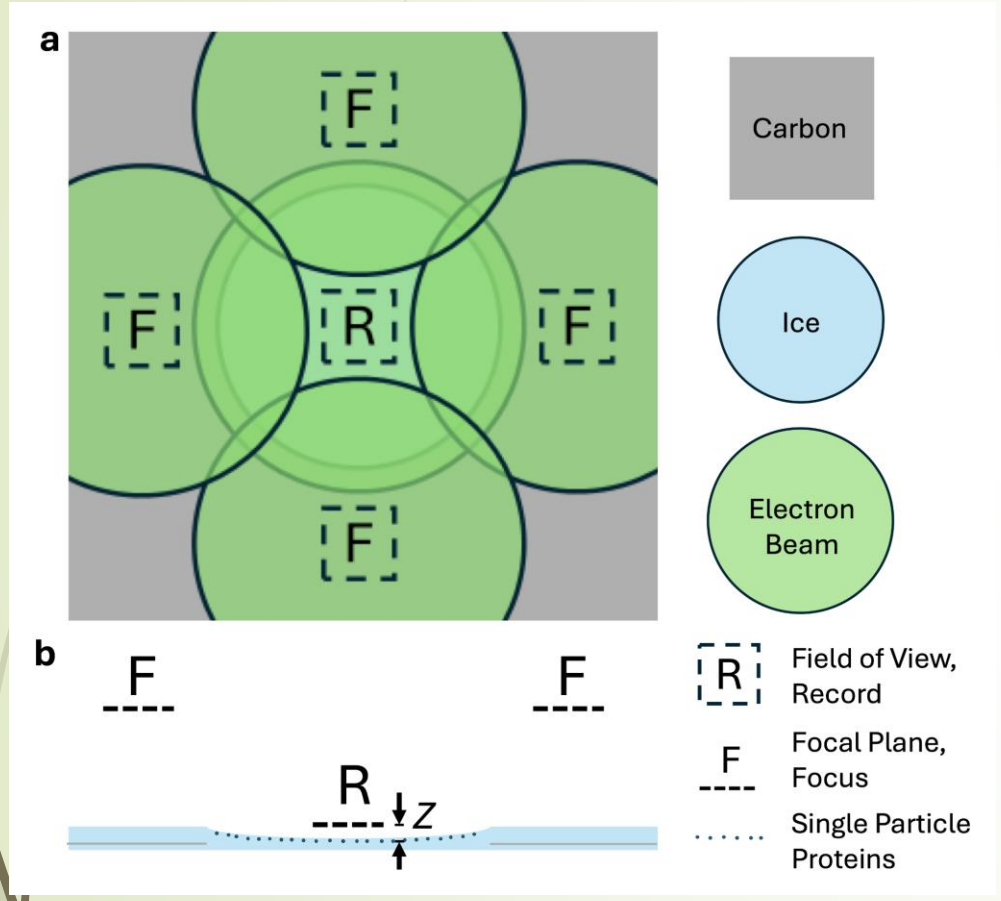
In-focus imaging: a long-sought goal



LPP + Cs correction eliminates oscillations in the CTF

Nearly 100% of the information that is carried by the electron beam can be accessible

Clover-leaf strategy (on the Titan)



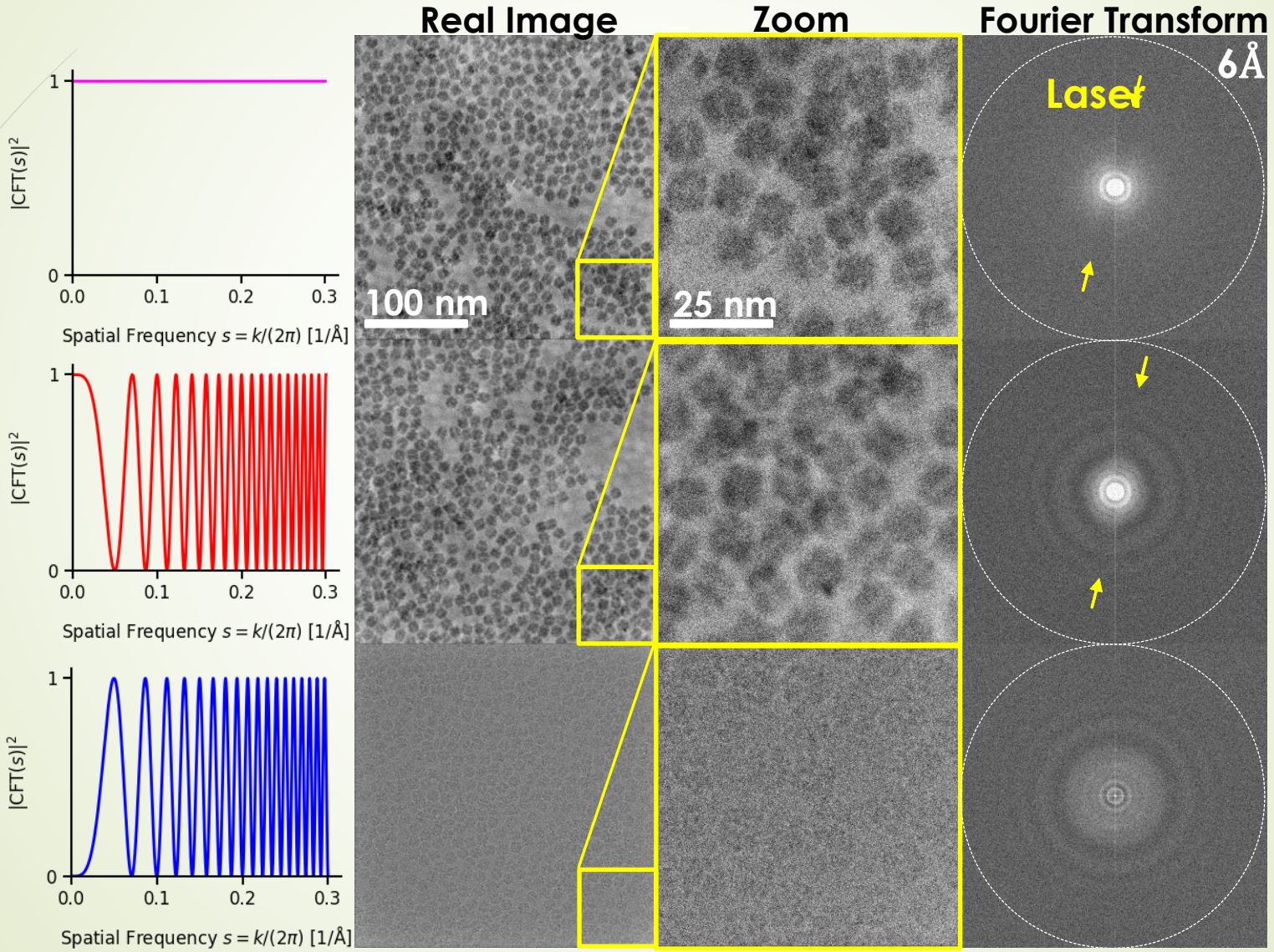
	σ_z [nm]	Phase shift	*N*
Room temp, laser ON	5.2 ± 0.6	82°	36
Cryo, laser OFF	9.8 ± 1.3	-	30
Cryo, laser ON	13.5 ± 3.2	68°	10

Quantifoil grids, Target defocus 1 µm

Next step: verify defocus with template matching

Radostin Danev

Ian Hicklin, Eric Cooper



Laser on, in-focus (Scherzer, 60 ± 14 nm)

Laser on, defocused

Laser off, 1 µm defocus

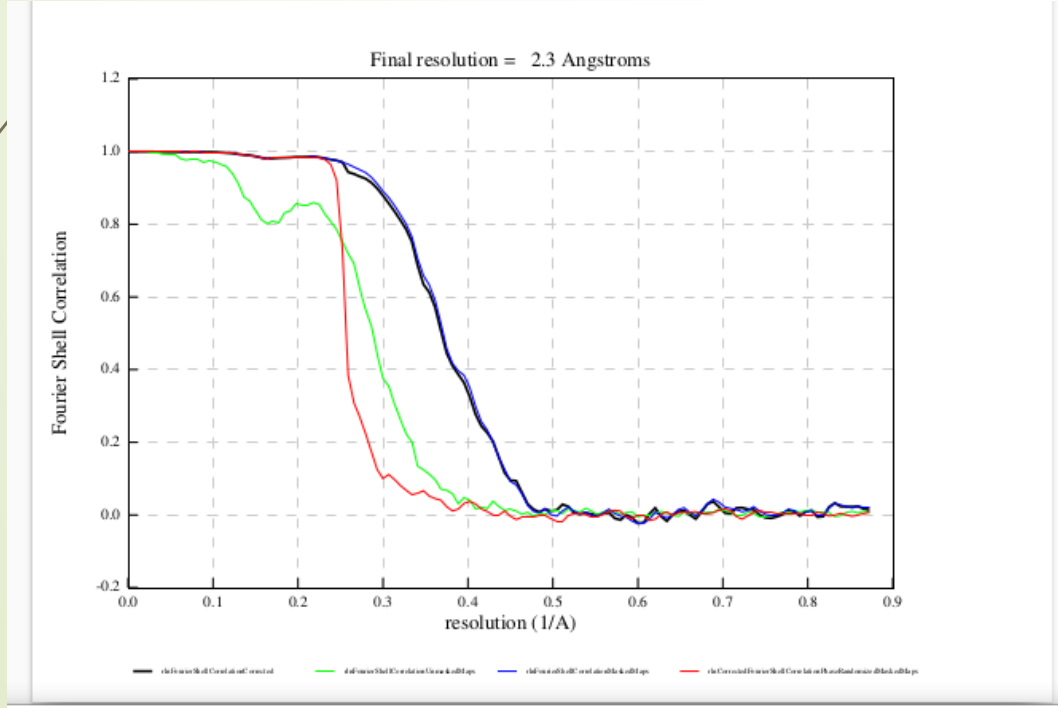
State-of-the art: ~2.3 A from ~ 10,000 images

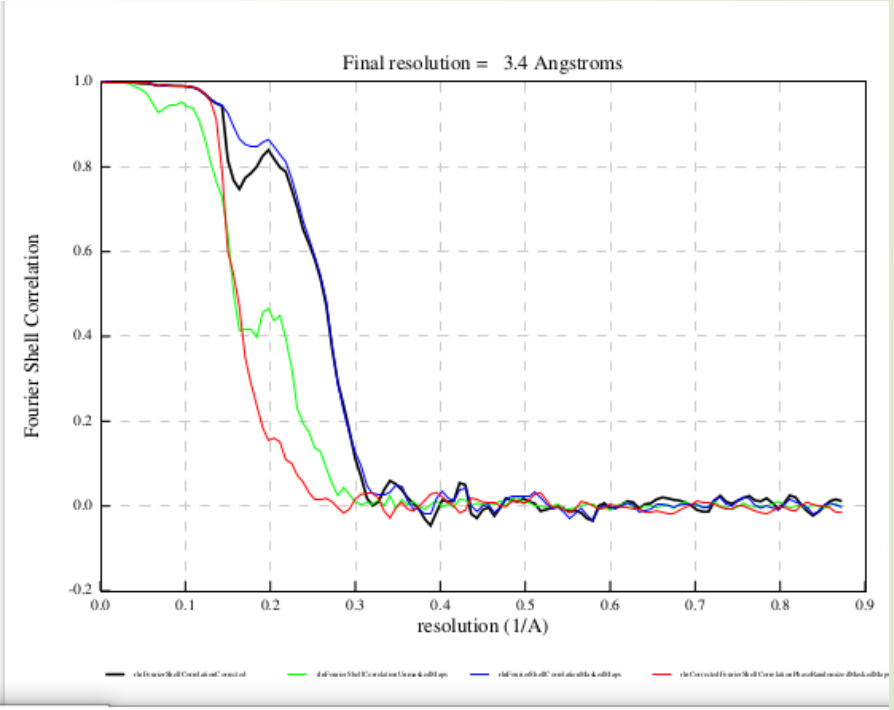
Table 1 | Data collection and refinement statistics for all models

PDB EMDB	CO-HbAM 8WIX 37571	O_2-HbAM 8WIY 37572	Deoxy HbAM 8WIZ 37573	CO-HbA 8WJ0 37574	O_2-HbA 8WJ1 37575	Deoxy HbA 8WJ2 37576
Data collection/processing						
Magnification	165,000	165,000	165,000	165,000	165,000	165,000
Voltage (kV)	300	300	300	300	300	300
Electron exposure ($e^-/Å^2$)	60.0	60.0/58.4	58.4	58.4	58.4	58.4
Defocus range (μm)	−0.4 to −1.2	−0.4 to −1.2	−0.4 to −1.2	−0.4 to −1.2	−0.4 to −1.2	−0.4 to −1.2
No. of frames	54	54	54	55	55	54/56
Pixel size (Å)	0.51	0.51	0.51	0.51	0.51	0.51
Micrographs	5775	16,056	12,001	8994	9002	16,579
Initial particles	7,290,950	16,003,848	6,875,397	4,939,556	4,542,816	8,382,020
Final particles	751,964	372,582	316,544	504,650	550,843	460,786
Symmetry imposed	C2	C2	C2	C2	C2	C2
Map resolution (Å)	2.29	2.31	2.20	2.24	2.27	2.35
FSC threshold	0.143	0.143	0.143	0.143	0.143	0.143
Map sharpening B factor ($Å^2$)	−85.5	−78.1	−85.7	−82.7	−88.2	−70.3
Refinement						
Initial model used (PDB code)	-	-	-	2dn1	2dn1	2dn2
Model resolution (Å)	2.3	2.3	2.2	2.2	2.3	2.3
FSC threshold	0.5	0.5	0.5	0.5	0.5	0.5
Model composition						
Non-hydrogen atoms	4980	5162	5102	4790	4732	4948
Protein residues	574	574	574	566	566	574
Ligands	8	8	6	8	8	4
B factors ($Å^2$)						
Protein	59.2	48.0	47.8	55.3	59.3	52.9
Ligand	47.5	34.1	35.2	41.9	46.0	39.0

In-focus using 113 images

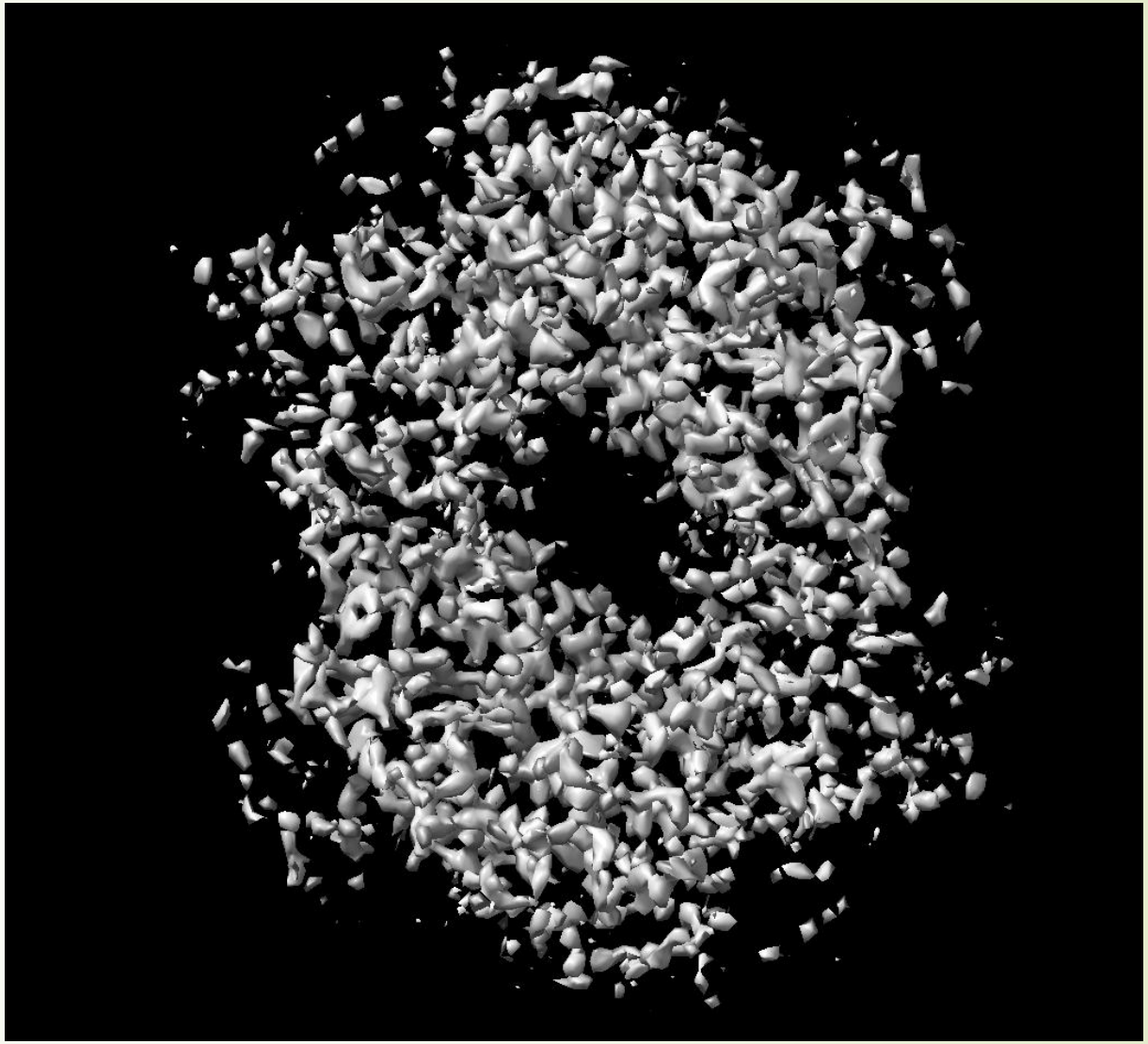
- 113 movies both in focus and out of focus
- No CTF correction needed, super easy processing



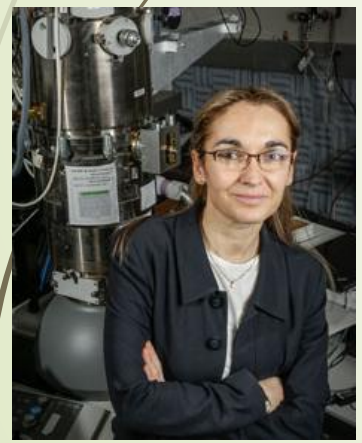


2.1 A resolution with 113 images: making the electron microscope 100 x better

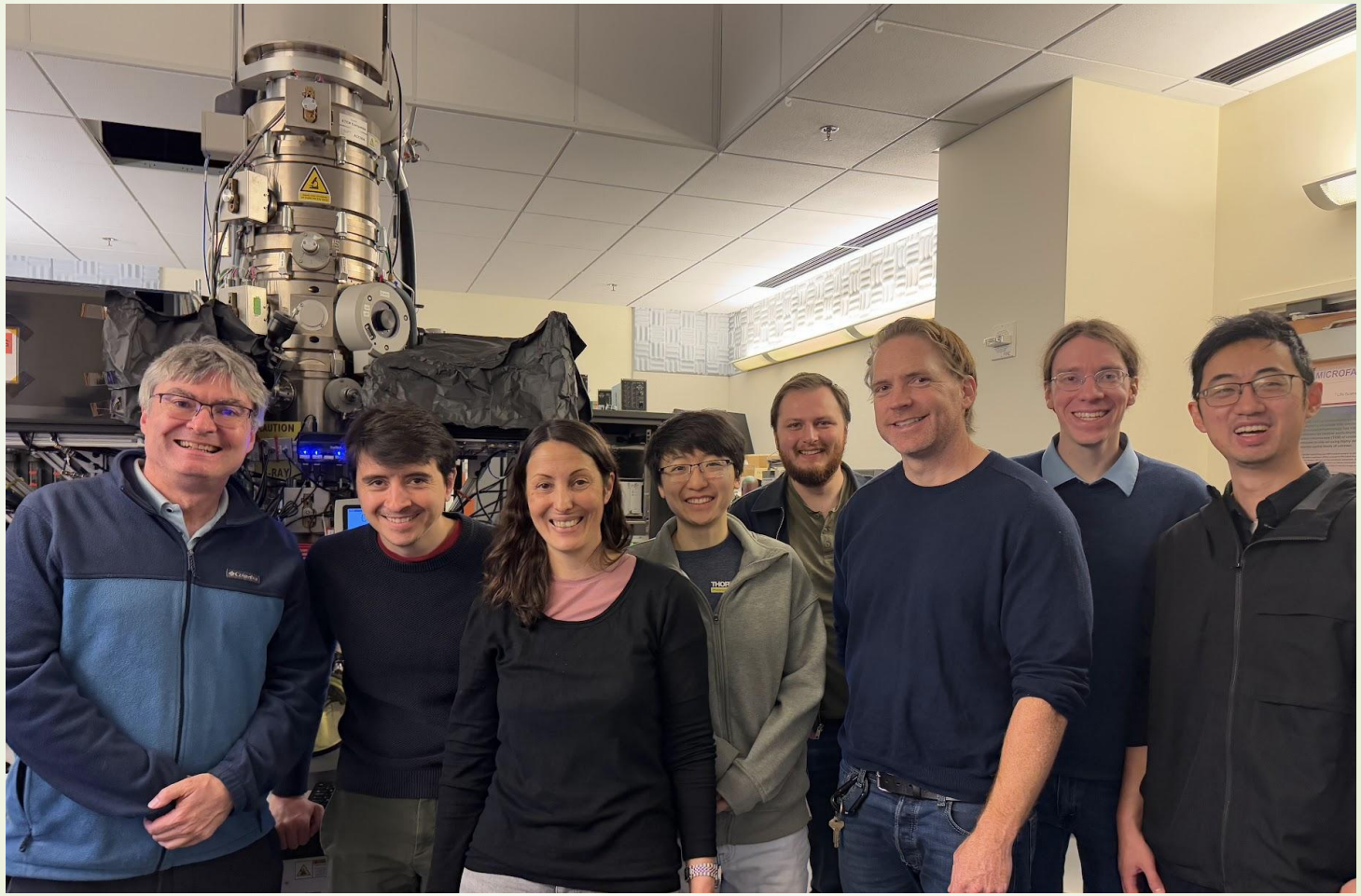
- Since the PSF is localized now, can lower to box size, reject more noise



Robert Glaeser



Eva Nogales



HM, Petar Petrov, Jessie Zhang, Ian Hicklin, Jonathan Remis, Eric Cooper, Hang Cheng. Former members: Jeremy Axelrod, Osip Schwartz, Sara Campbell, Jeske Dioquino, Carter Turnbaugh

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