Phase-contrast Presentation
Nov. 13, 2012

HIGH-END INSTRUMENTATION:
STATUS REPORT ON IN-FOCUS PHASE CONTRAST

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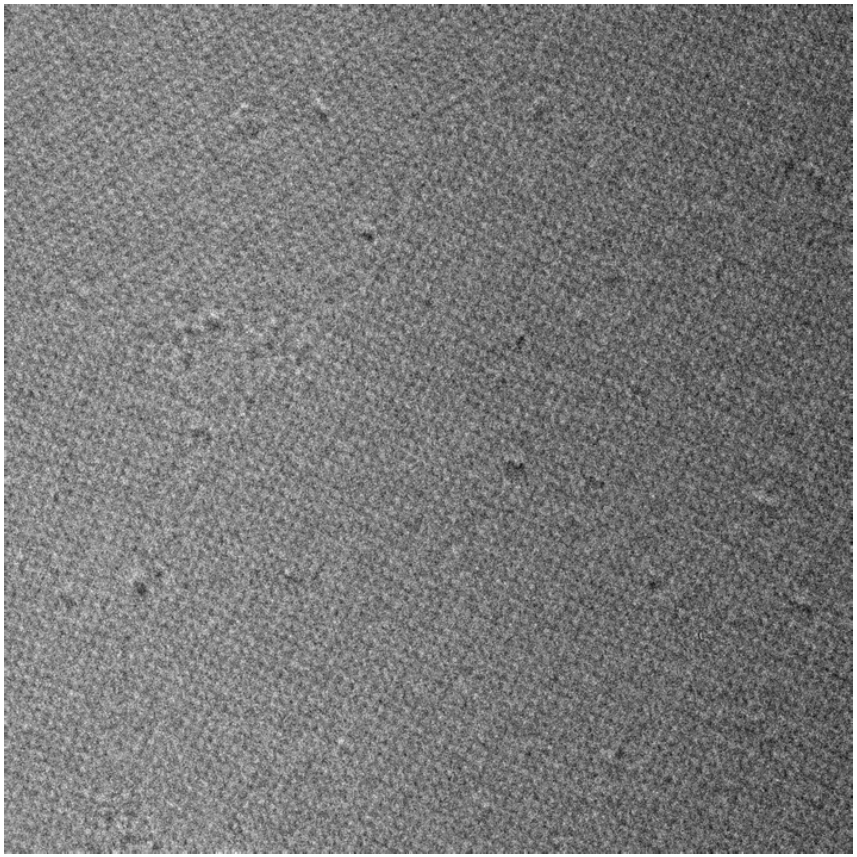
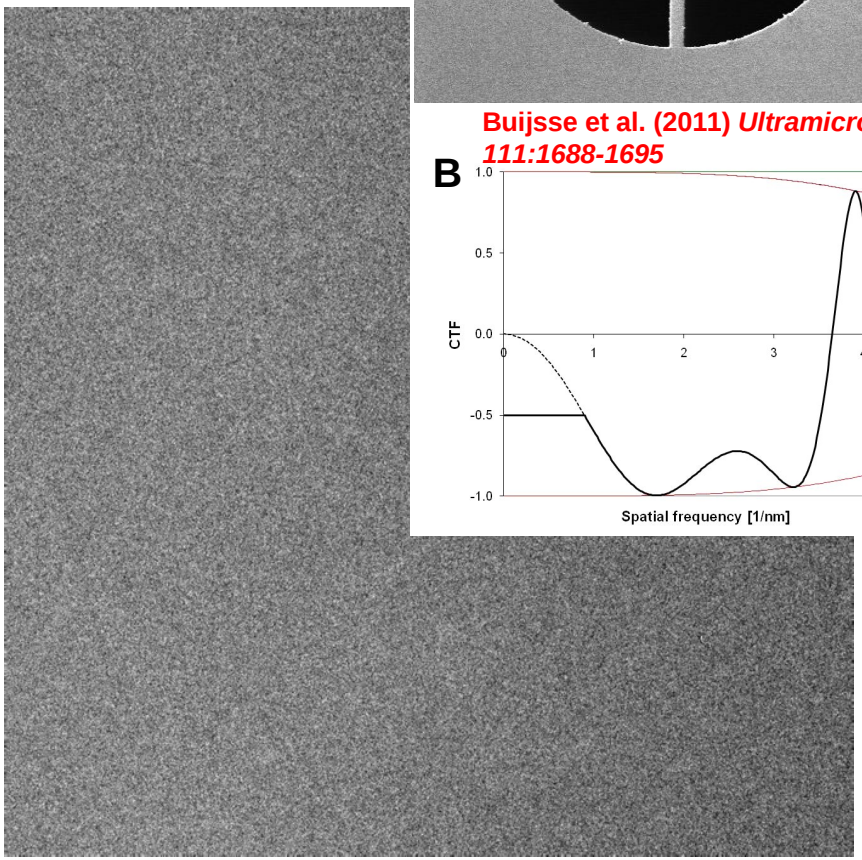
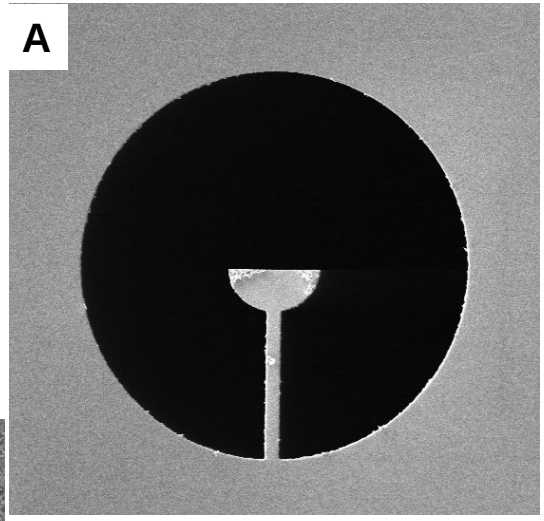
THE “TULIP” APERTURE IS A “PASSIVE” PHASE-CONTRAST APERTURE, NOT A “PHASE PLATE” APERTURE

CONTRAST = 0.5 IN SSB DOMAIN AND γ(s) SHOWS UP AS A PHASE ERROR







Buijsse et al. (2011) *Ultramicroscopy 111:1688-1695*

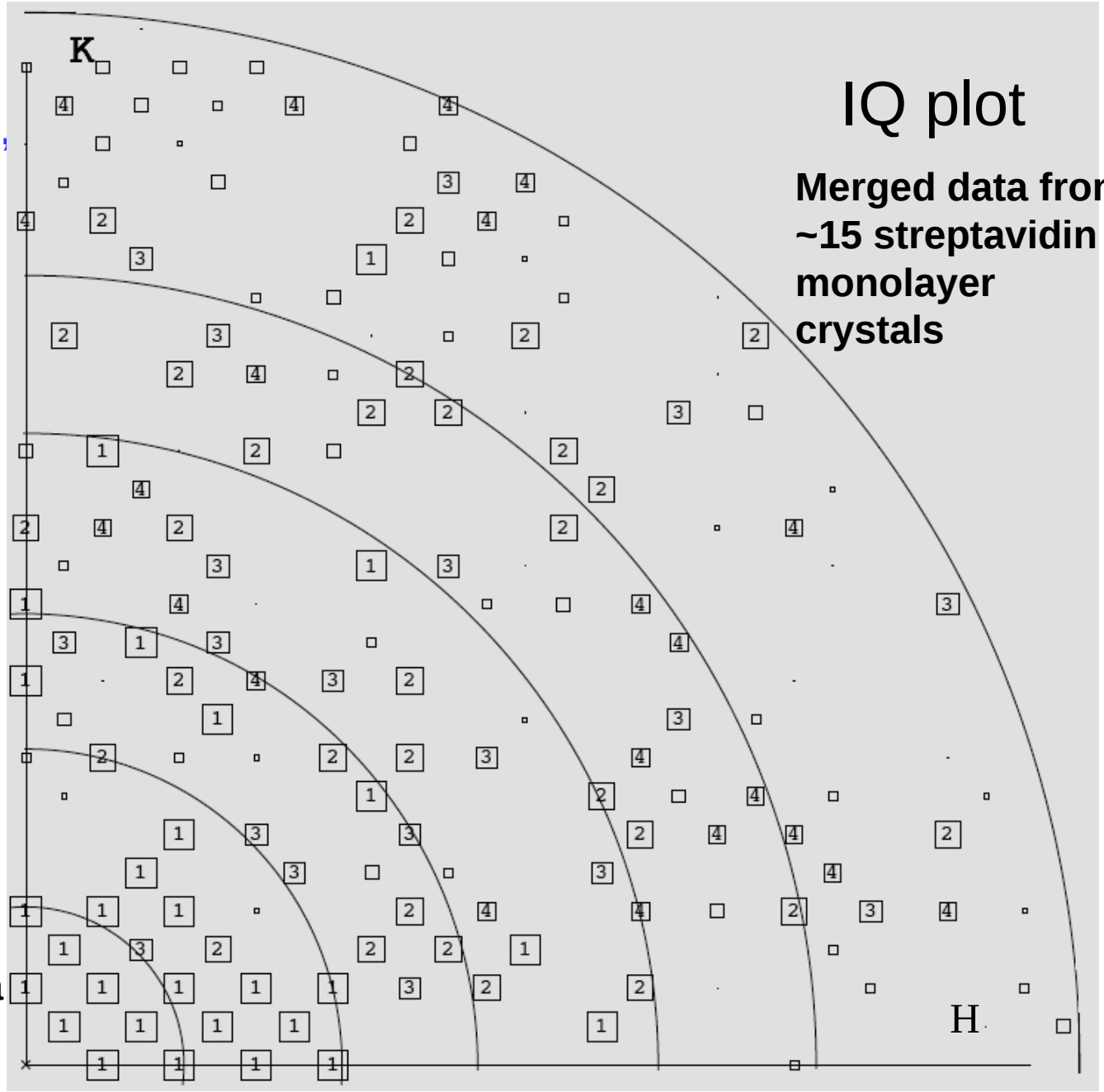
Cryo-EM images of the same area of a streptavidin monolayer crystal
Both images taken with the same defocus value, close to Scherzer

STRUCTURE FACTORS FOR "CTF-CORRECTED, UNBENT" IMAGES

Spots with IQ 4 or less, shown with numbers, have expected phase errors ~ 22° or less

Outer circle is drawn at 3.0 Å: aperture is compatible with high resolution

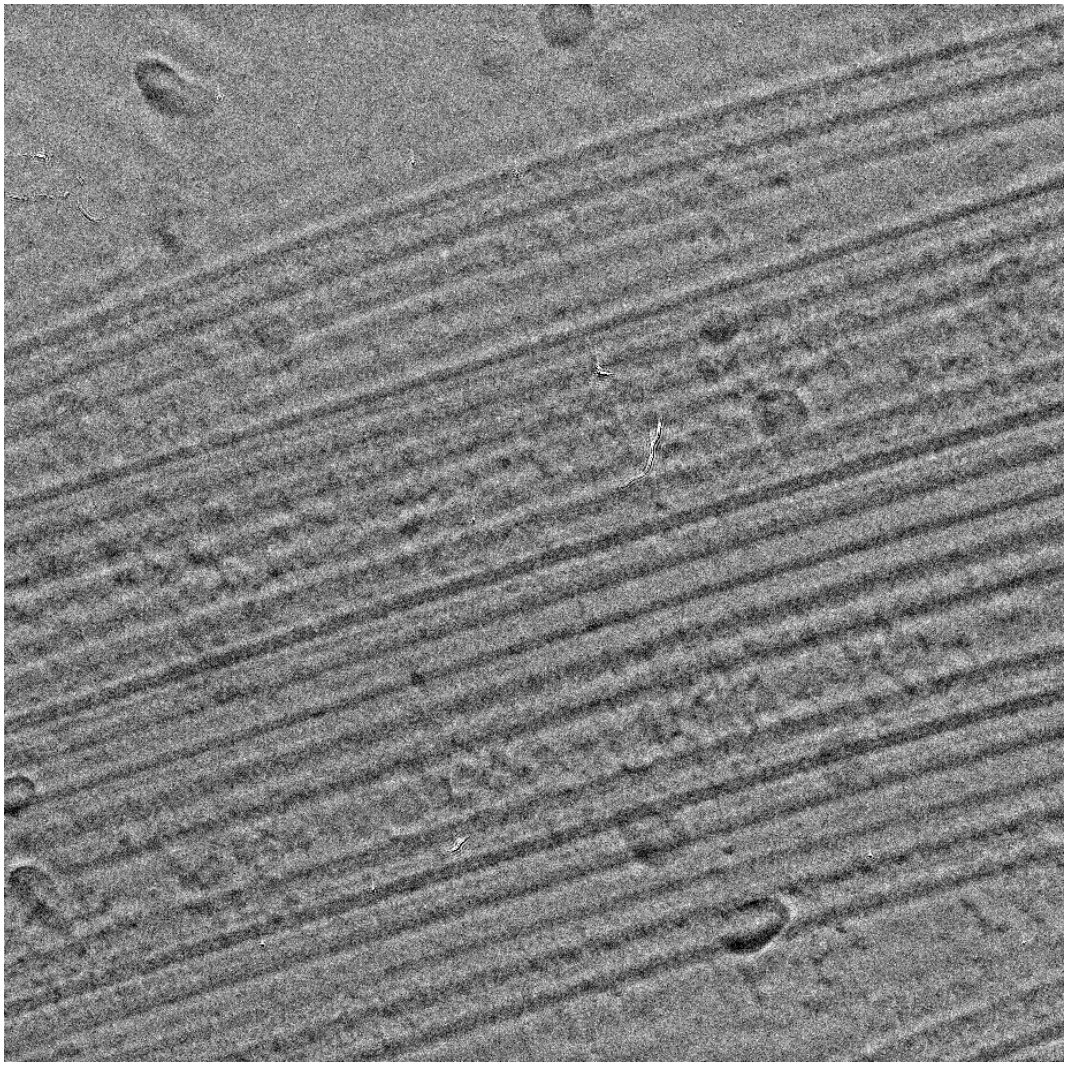
~25,000 unit cells in this merged data set of images recorded with the K2 camera

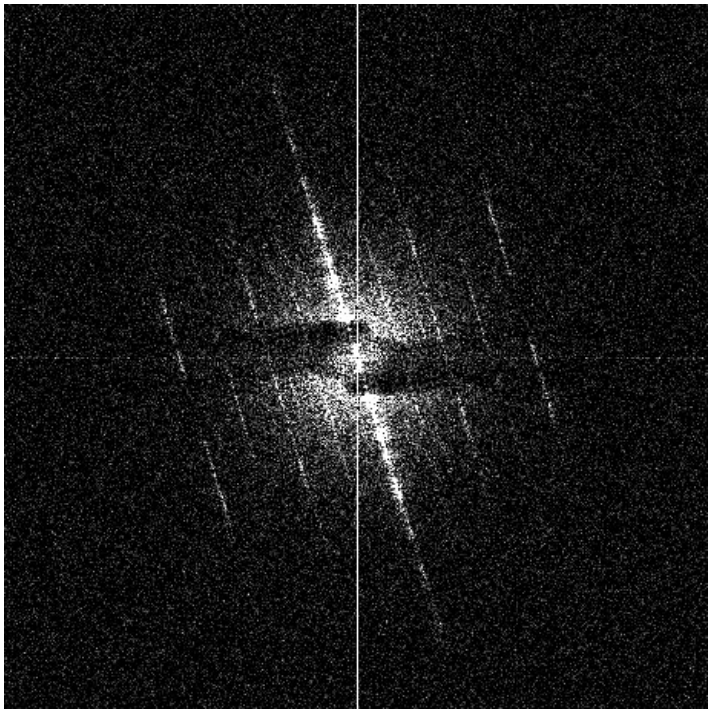


IQ plot

Merged data from ~15 streptavidin monolayer crystals

MICROTUBULE DOUBLETS
(Puey Onjai and Ken Downing)

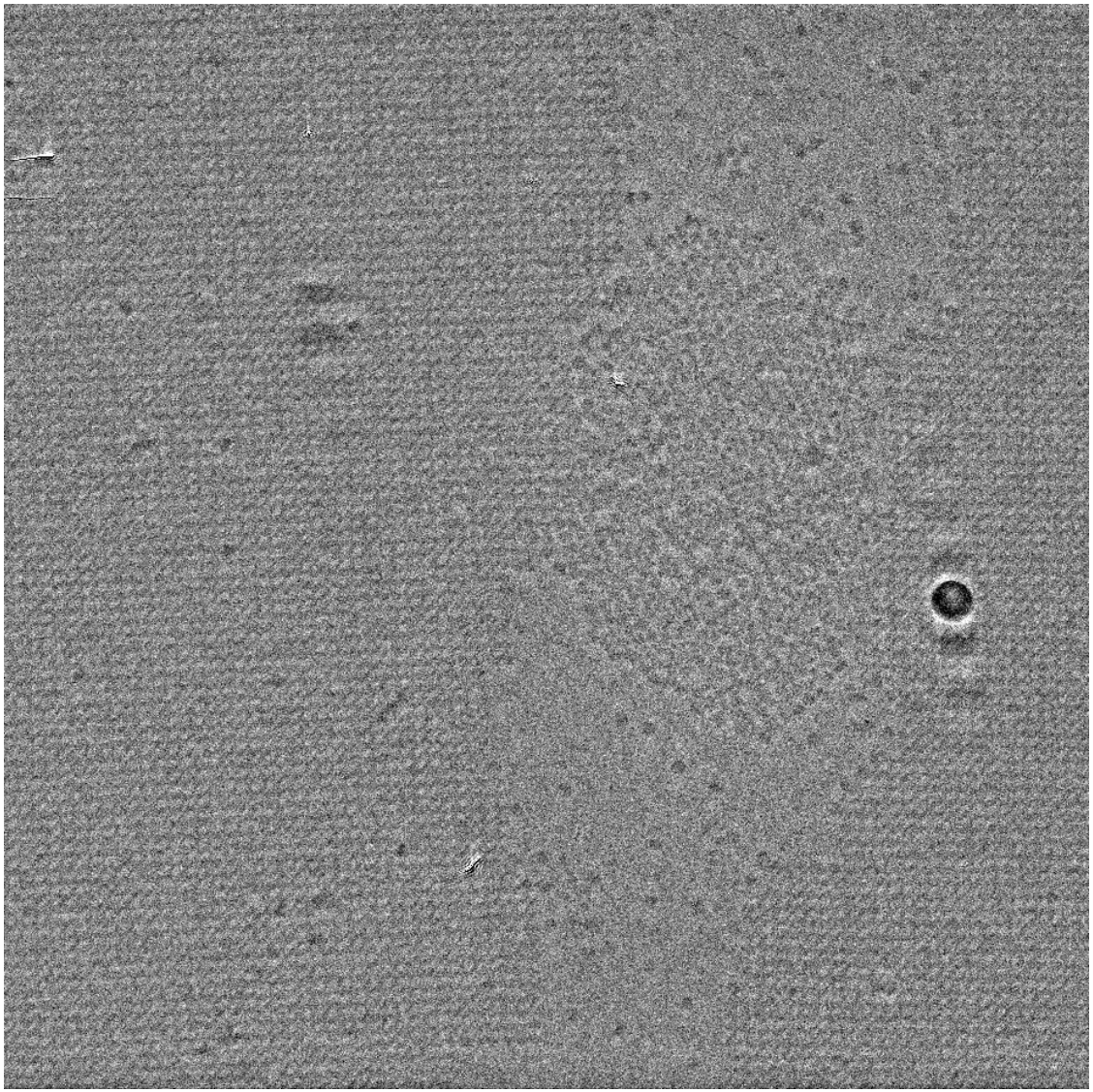




Note: much better contrast transfer for the low-resolution sections of layer lines that cross the DSB “gap” (didactic example of the effectivenesss of the SSB aperture)

NON-CRYSTALLINE REGION BETWEEN TWO SEPARATE CRYSTALS

- You can spot individual streptavidn tetramers, Mr ~ 55k!
- This capability should increase the coverage of proteome that is possible by a large factor- guestimate as much as 100X?
- Results are similar to the best obtained with a thin carbon-film (“Zernicke”) phase plate
- Similar problems are also encountered with
  - Unreliable manufacture
  - Short lifetime when there is a good one



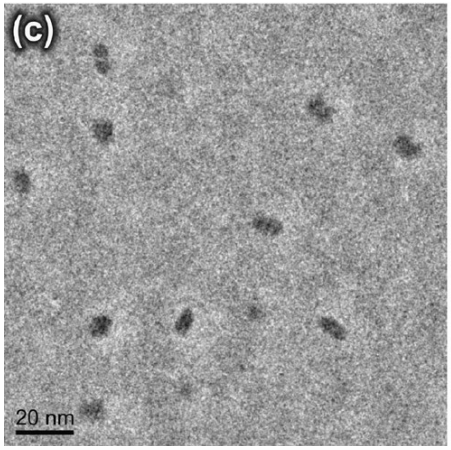
In-focus image, tulip aperture

MOTIVATION TO DEVELOP IN-FOCUS PHASE CONTRAST

- In-focus phase contrast of low-resolution features should be ~0.028 D, where D = particle diameter in nm

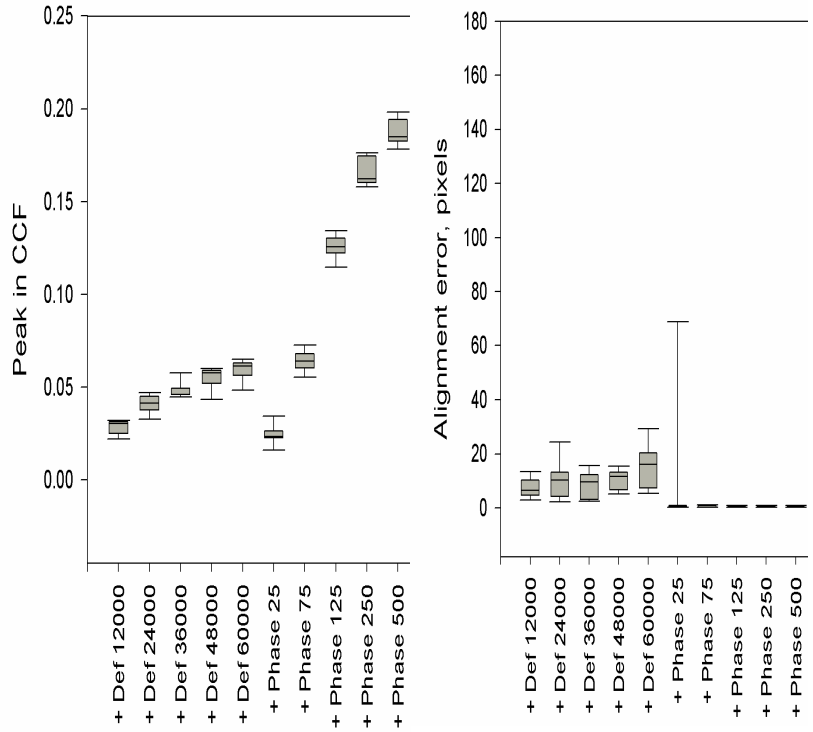
  Glaeser & Hall (2011) Biophys J. 100:2331-7

  - Defocus contrast is only a few percent of this, due to the fact that its CTF falls to zero at low spatial frequencies
- The increased SNR at low spatial frequencies should
  - Improve particle alignment and assignment of Euler angles, even for quite small particles
  - Improve assignment of structurally distinct particles into separate conformational or compositional classes



In-focus cryo-EM image of a 200 kDa homodimer

Danev et al. (2009) Ultramicroscopy 109:312-325



Simulation of potential alignment accuracy: phase-contrast images of a 100 kDa protein

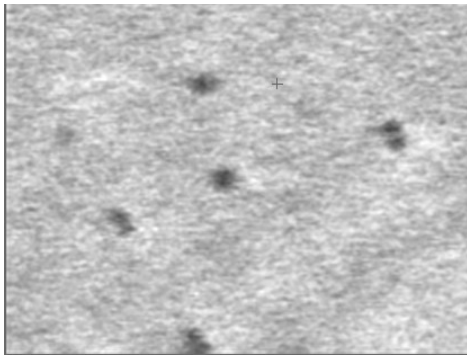
Hall et al. (2011) J. Struct. Biol. 174:468-475

2-nm FEATURES MAY BE THE "DETECTABLE LIMIT" IN CRYO-EM TOMOGRAPHY

$(\rho_{particle} - \rho_{ice})$ values required for S/N > 3σ, for different voxel sizes and electron exposures

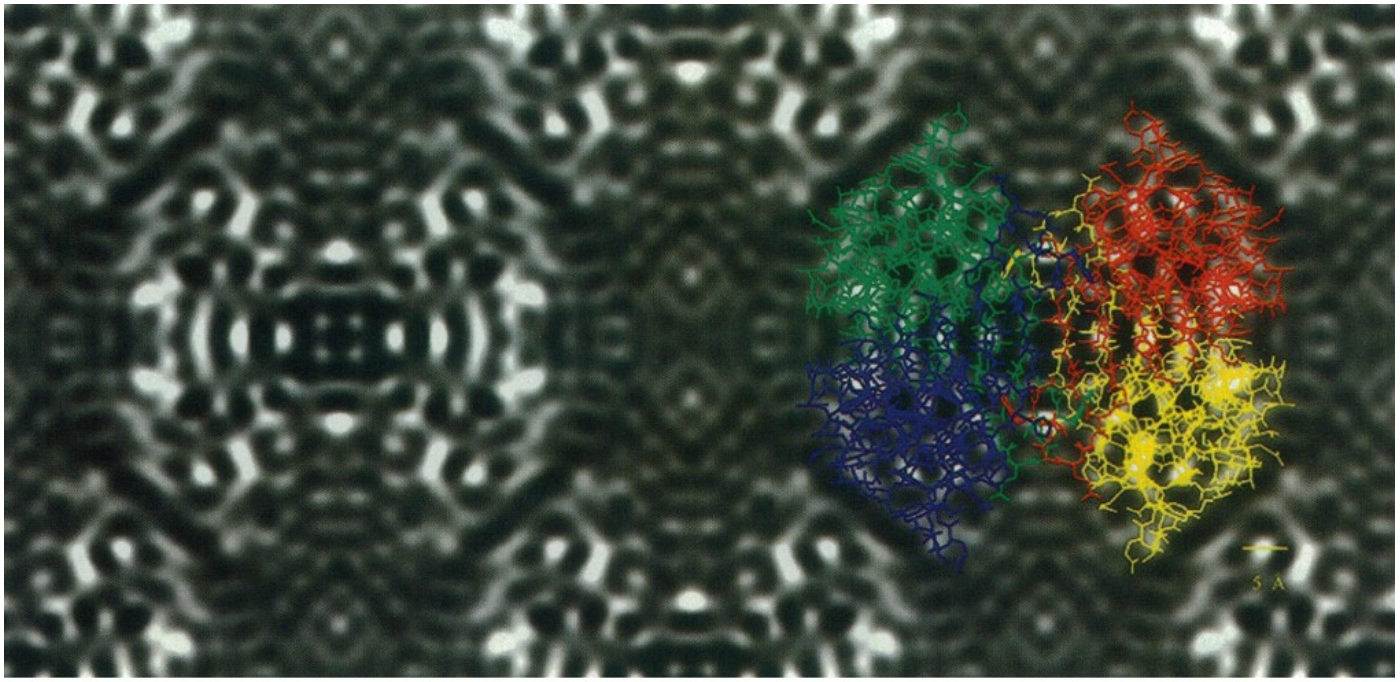
p (e nm^{-2})	0.5 nm	1 nm	2 nm	3 nm	5 nm
30	31	7.7	1.9	0.86	**0.31**
100	17	4.3	1.1	0.48	**0.17**
300	10	2.5	0.62	**0.27**	**0.10**
10^3	5.4	1.4	**0.34**	**0.15**	**0.06**
$3x10^3$	3.1	0.79	**0.19**	**0.09**	**0.03**
10^4	1.7	0.43	0.11	0.05	0.02
$3x10^4$	~~1.0~~	~~0.25~~	~~0.06~~	~~0.03~~	~~0.01~~
10^5	~~0.54~~	~~0.14~~	~~0.04~~	~~0.02~~	~~0.006~~

Table from: Glaeser & Hall (2011) Biophys J. 100:2331-2337



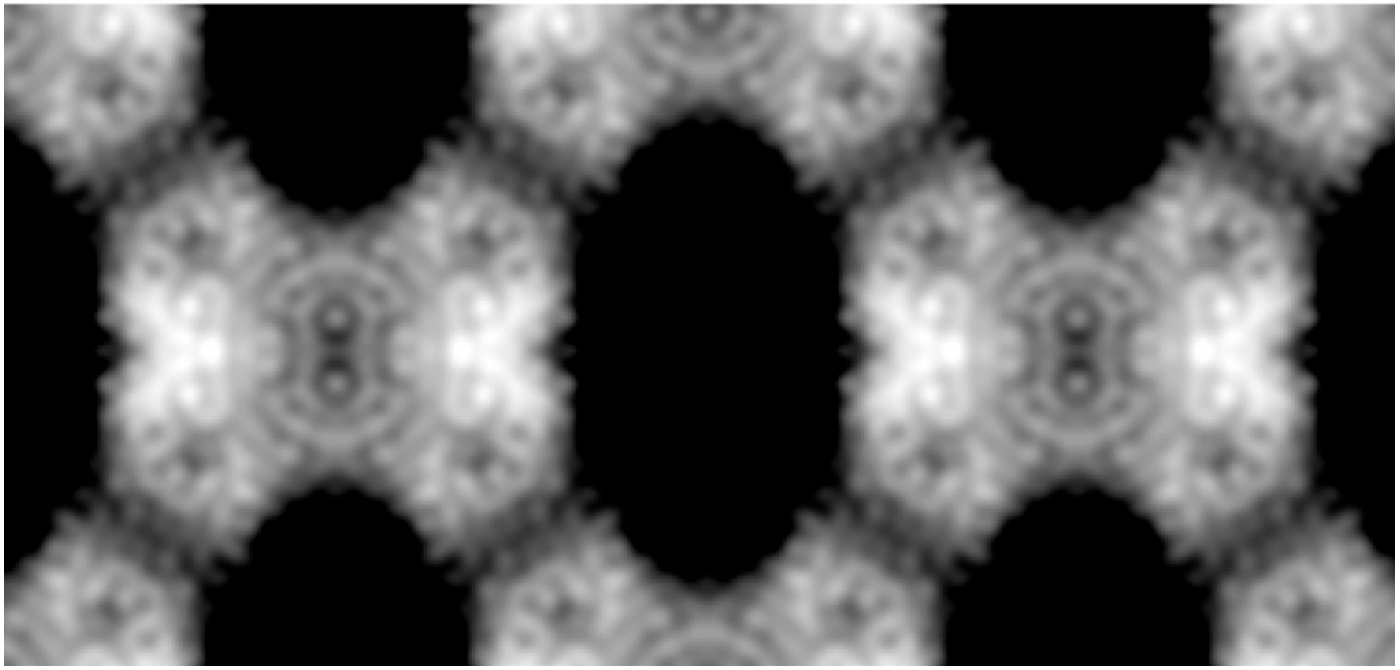
Tomogram of a dimeric, 200 kDa particle suspended In vitreous ice
Danev & Glaeser, unpublished

- **In-focus phase-contrast EM tomography of suitably thin (weak-phase) objects should just barely reach a feature-detection limit of ~2 nm (Fourier resolution of 4 nm)**
- **Density values must be multiplied by a factor of 14 for amplitude-contrast images** Saxberg & Saxton (1981) Ultramicroscopy 6:85-90
  - **This would be the case for (incoherent) annular dark-field STEM images**
  - **It is also likely to be the case for CTEM images of thick specimens, even when a C_c corrector is used**



Avila-Sakar, A.J. and Chiu, W. (1996) Biophys J. 70:57-68

400 keV, film
Close-to-focus image phases merged with diffraction amplitudes
3.0 Å map



Han, B-G., Sassolini, S. and Glaeser, R.M., Unpublished

300 KV, K2 camera
In-focus, “tulip” Phase-contrast aperture
3.0 Å map