

Advantages of Exploiting the Coherence in X-ray Diffraction Studies of Biological Materials

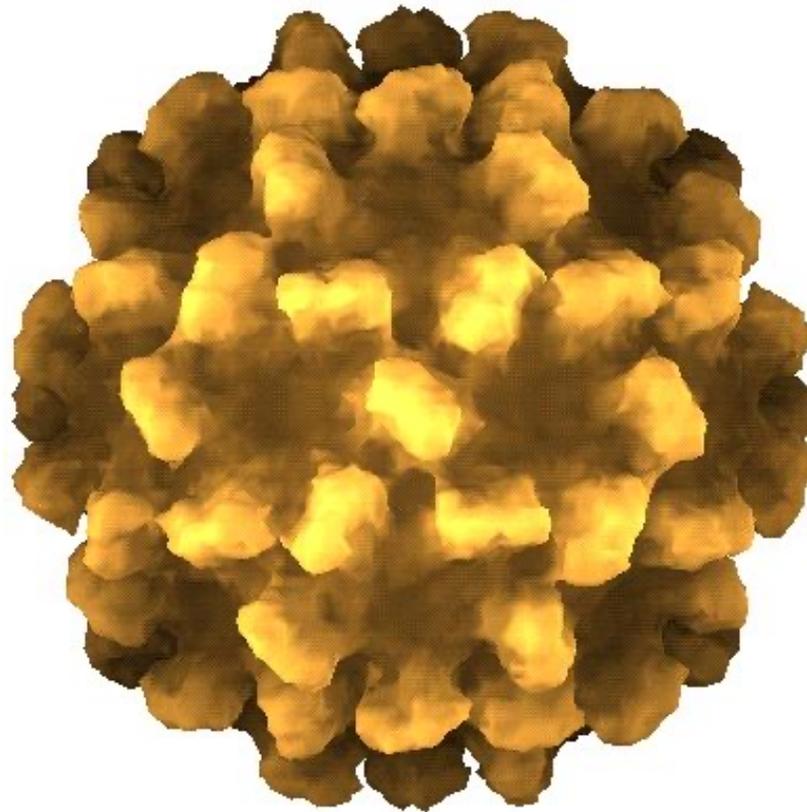
- Ian Robinson
- Sébastien Boutet
- Franz Pfeiffer
- Ivan Vartanians
- Ross Harder
- Meng Liang
- Garth Williams
- Mark Pfeifer
- Jing Tao
- Jim Zuo

Departments of Physics
and Materials Science
University of Illinois

Danish Cryst. Meeting,
Sandbjerg Estate,
May 2005

Tomato Bushy Stunt Virus

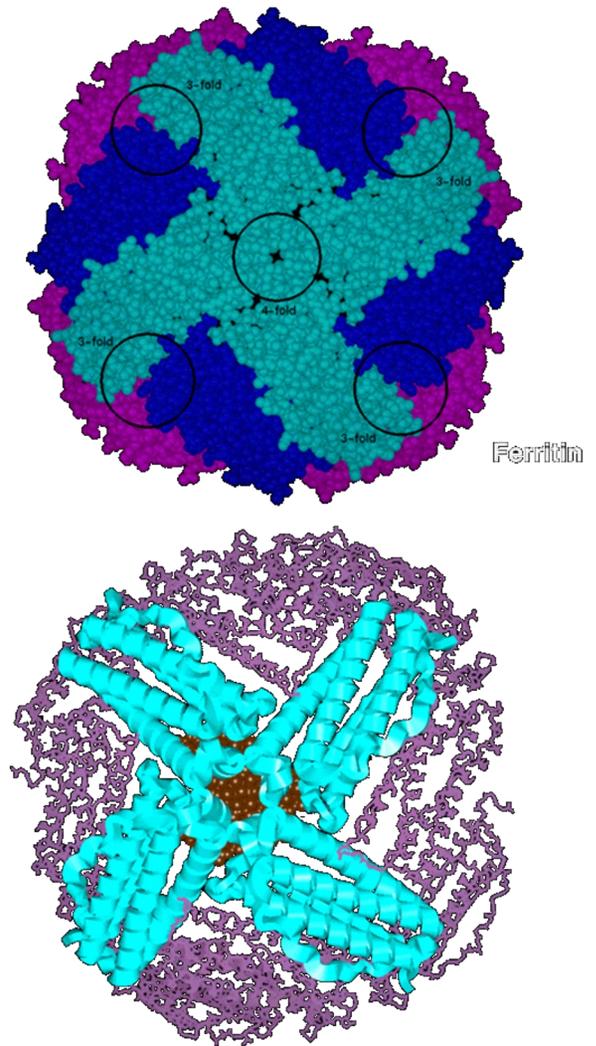
1980



I. K. Robinson DANSYNC Sandbjerg

Horse Spleen Ferritin

- Ferritin used in almost all living things for Iron Storage
- Made of 24 identical protein subunits arranged in 432 symmetry
- Crystallizes as FCC (I23)
- Inner shell diameter 80Å
- Outer shell diameter 130Å
- Iron core: ferrihydrite form
- With Iron : **Holo**ferritin
- Empty Shell : **Apo**ferritin



Critical Nucleation

Yau S.-T. and Vekilov P.G., *J. Am. Chem. Soc.* (2001), 123, 1080-1089

- a) Nucleation via planar 2D clustering
- b) Nucleation via compact close-packed 3D clustering
- Possible also via non-crystalline (eg icosahedral) nuclei.

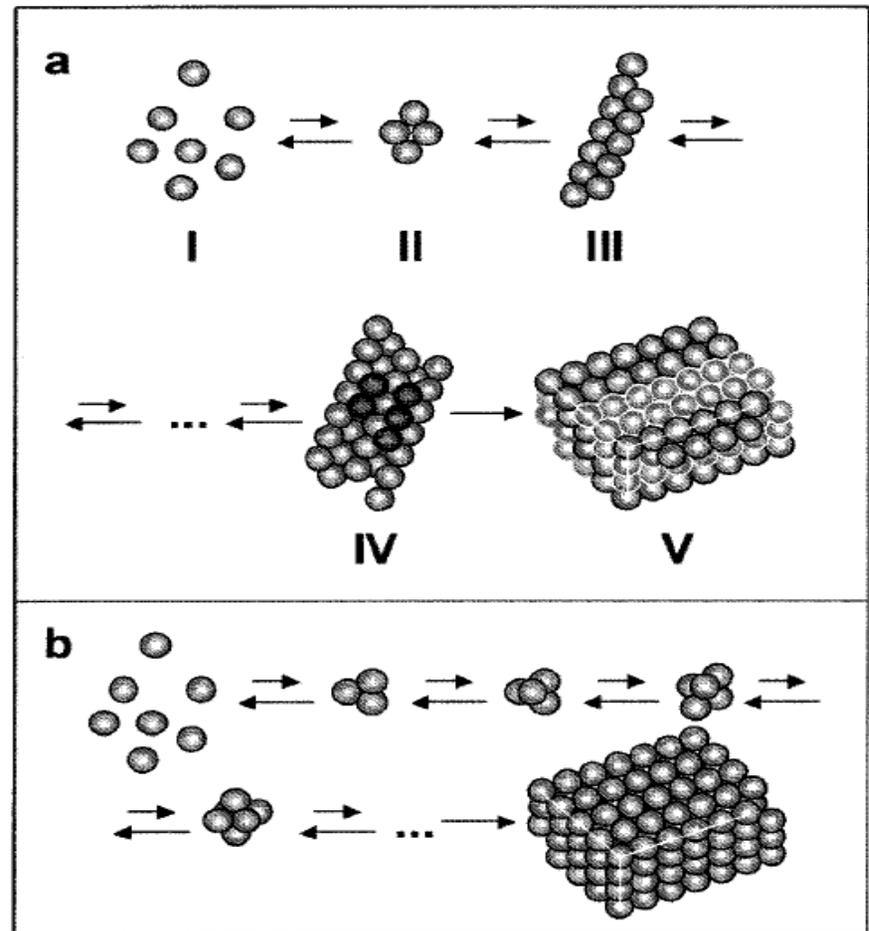
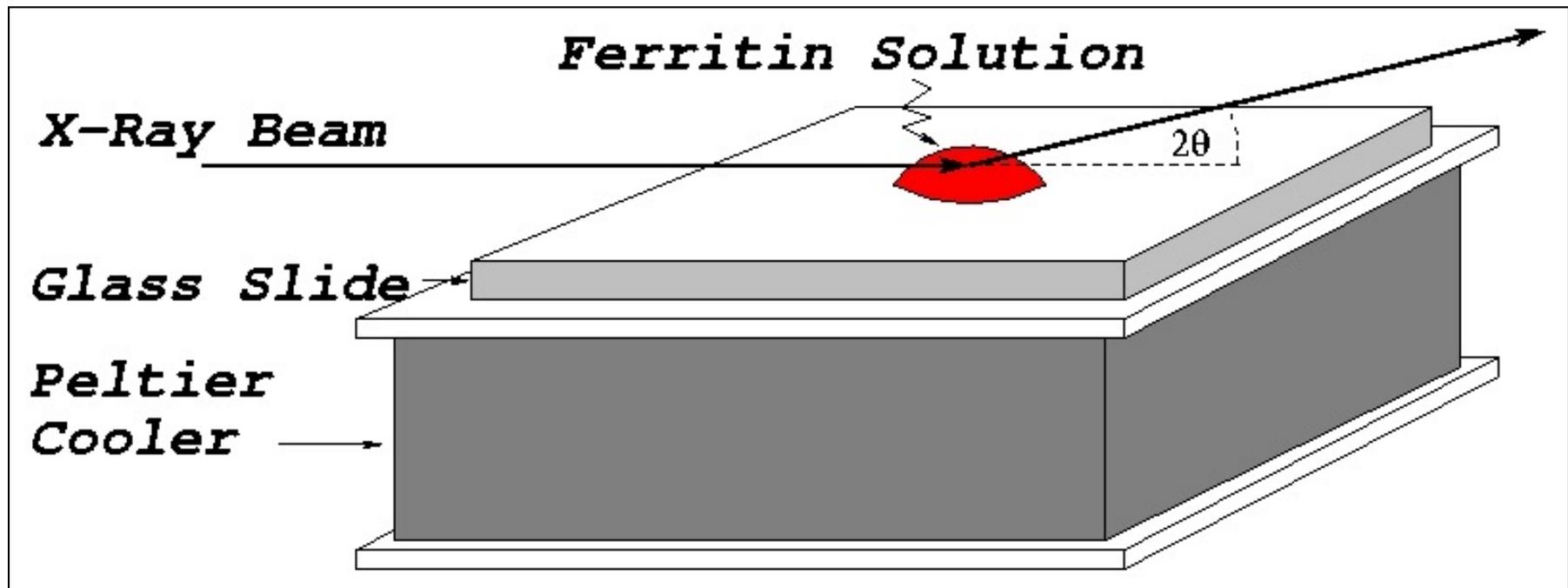
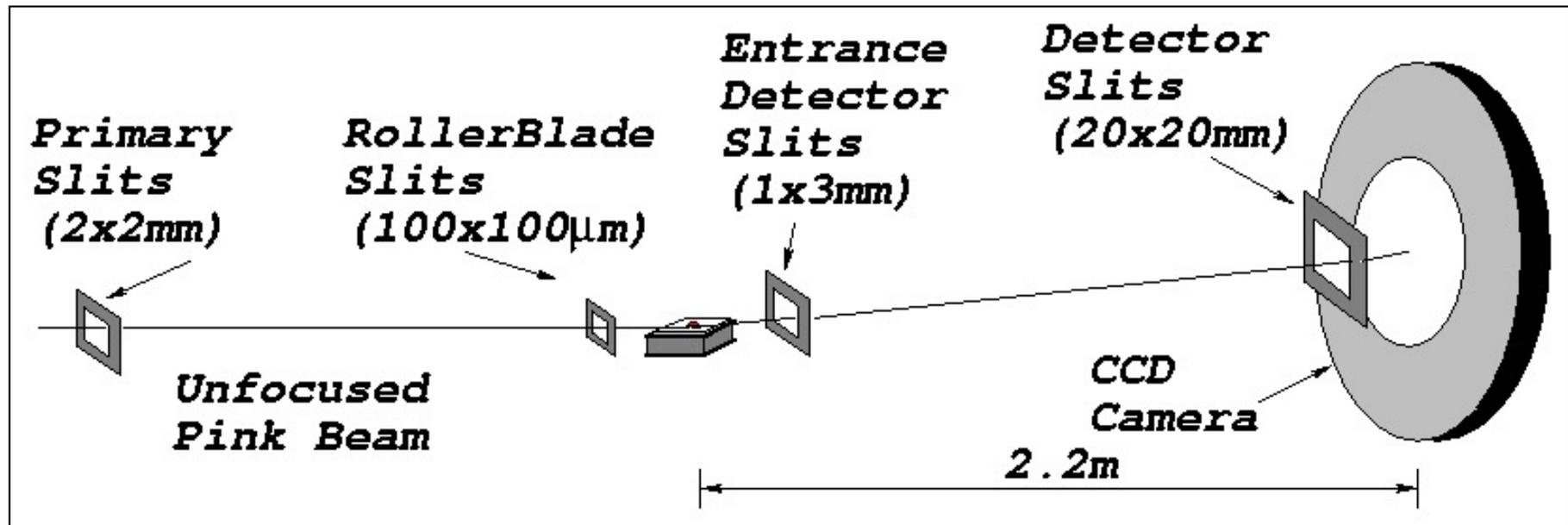


Figure 10. Schematic illustration of two nucleation pathways: (a) via a planar critical cluster (in IV molecules belonging to the second layer are shown in a lighter shade; in V the (110) layers that stack up to form this crystal are delineated by lighter and darker contours) and (b) via compact critical cluster.^{5,6}

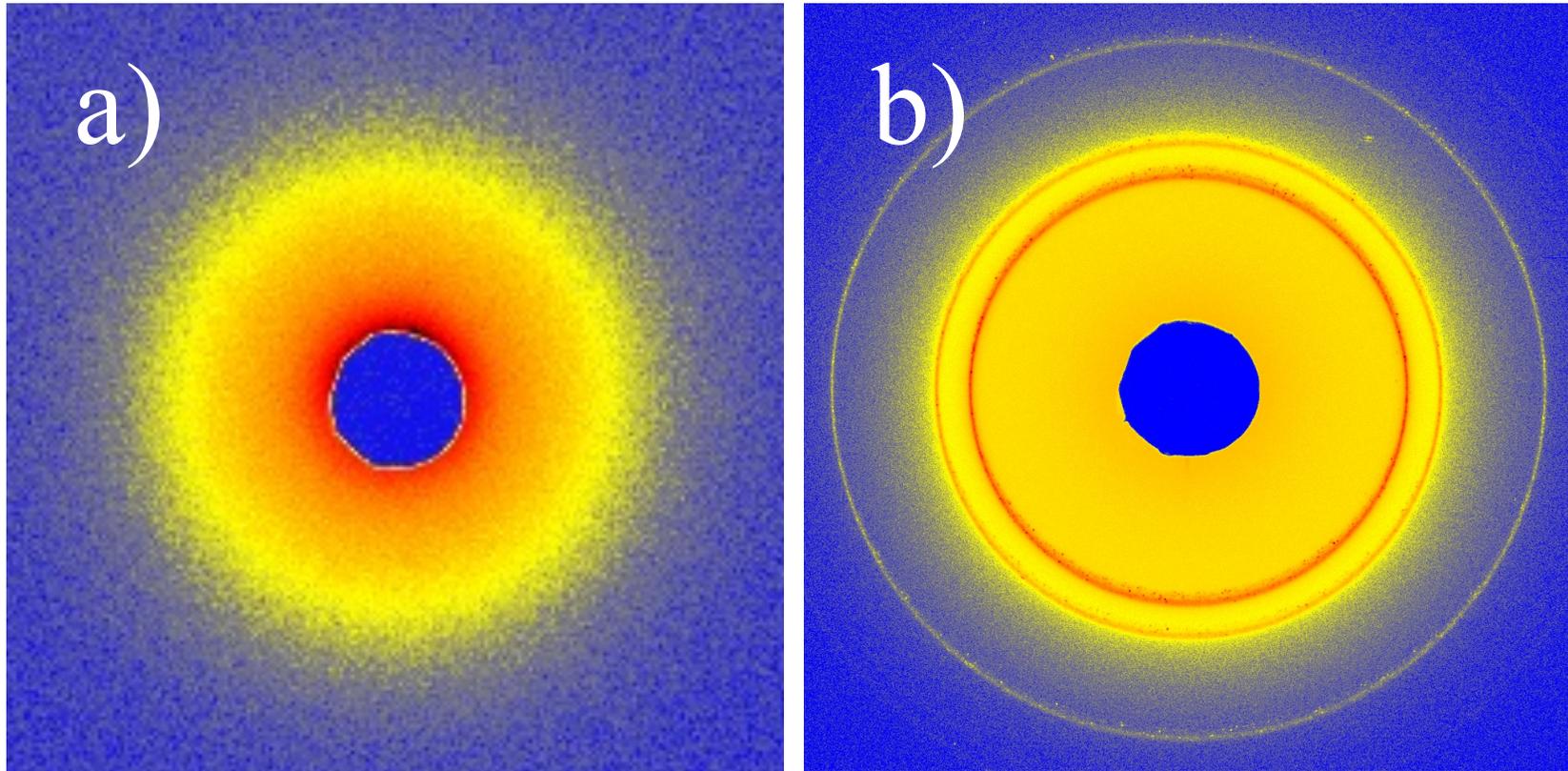
In-situ Study of Crystallization



Experiment at APS Sector 34



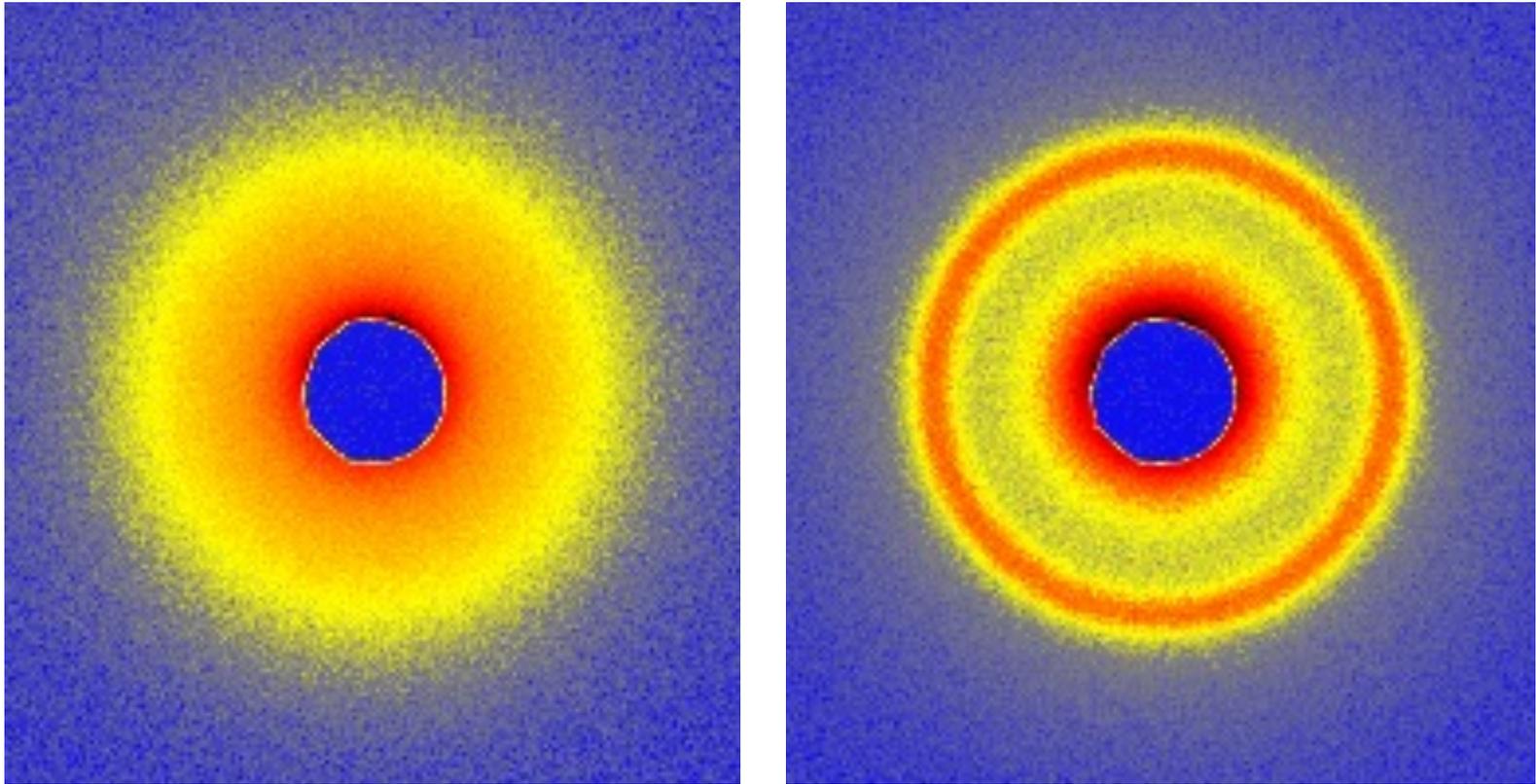
Crystallization of Holoferitin: SAXS



a) SAXS pattern of holoferitin at 10°C.

b) SAXS pattern after adding Cadmium salt

Effect of Temperature

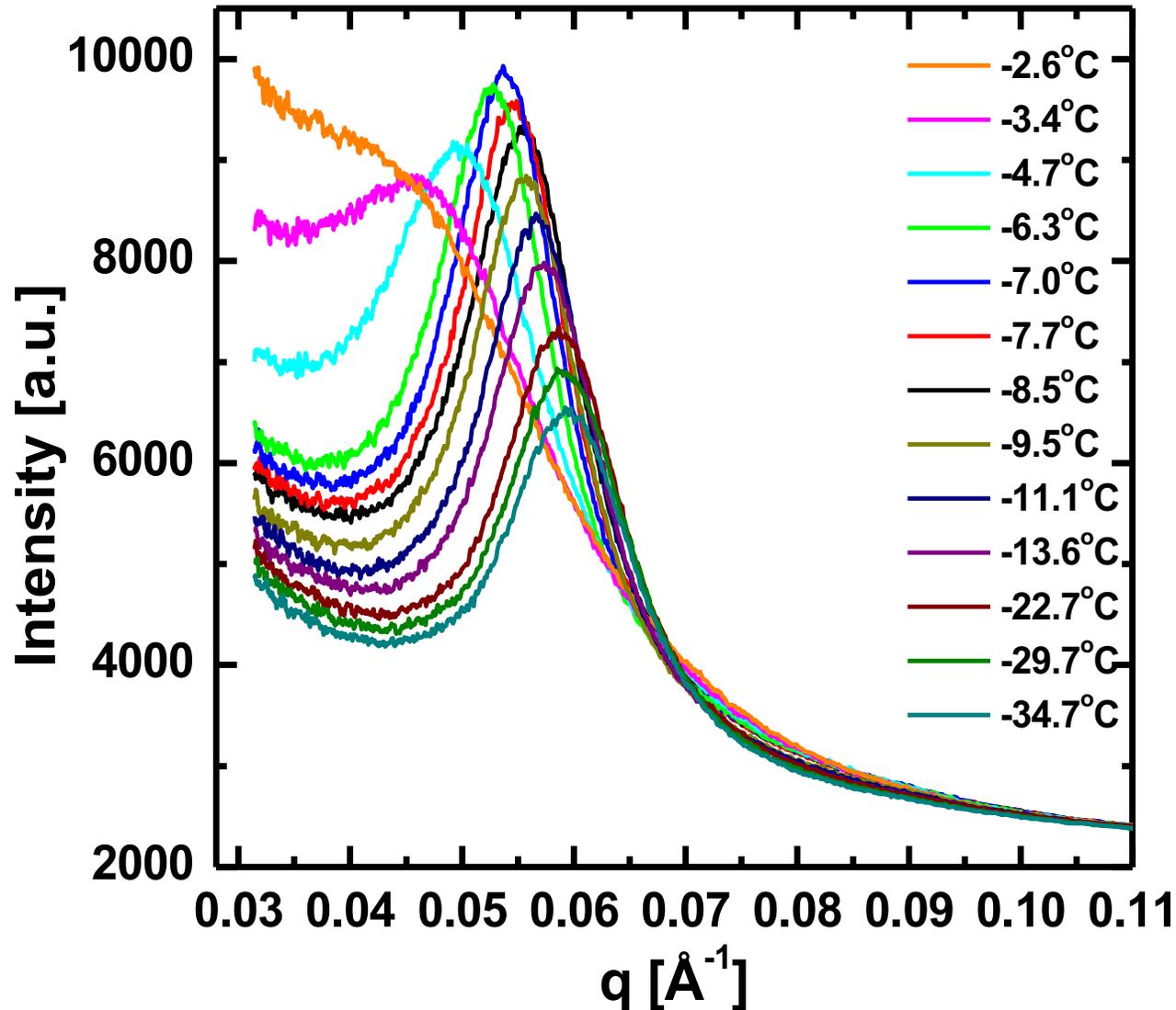


SAXS pattern at $T=+10^{\circ}\text{C}$ and -10°C .

Holoferritin in dilute (10mg/ml) NaCl only.

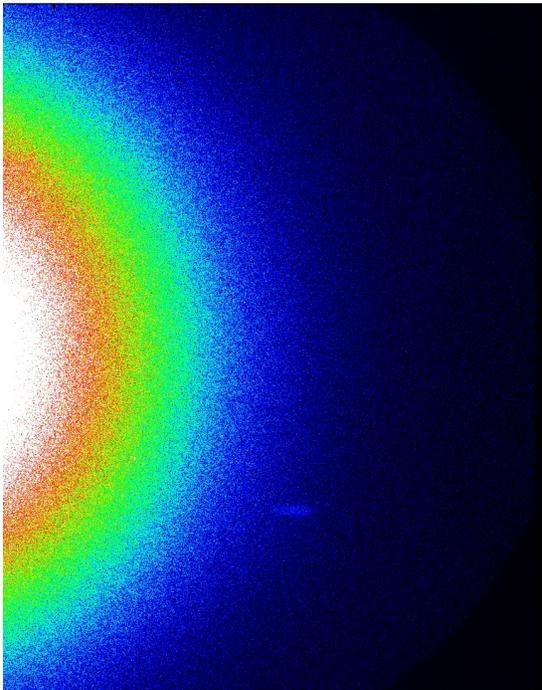
I. K. Robinson DANSYNC Sandbjerg

Integrated SAXS Data

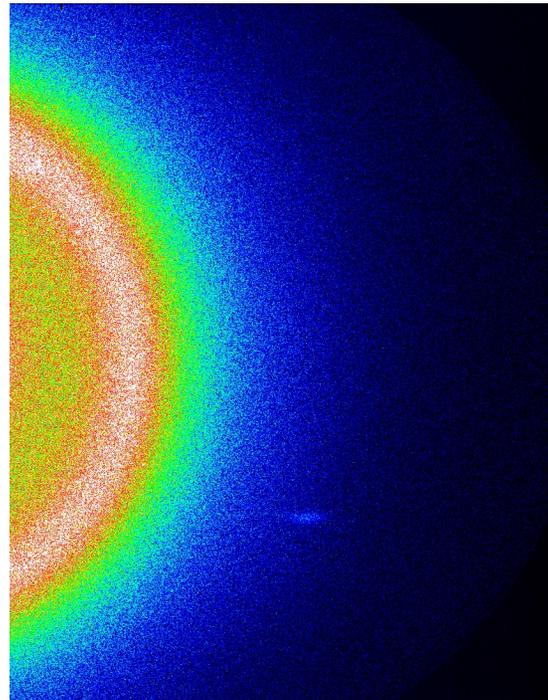


- The SAXS pattern shows a broad peak which shifts in position and changes in width as a function of temperature

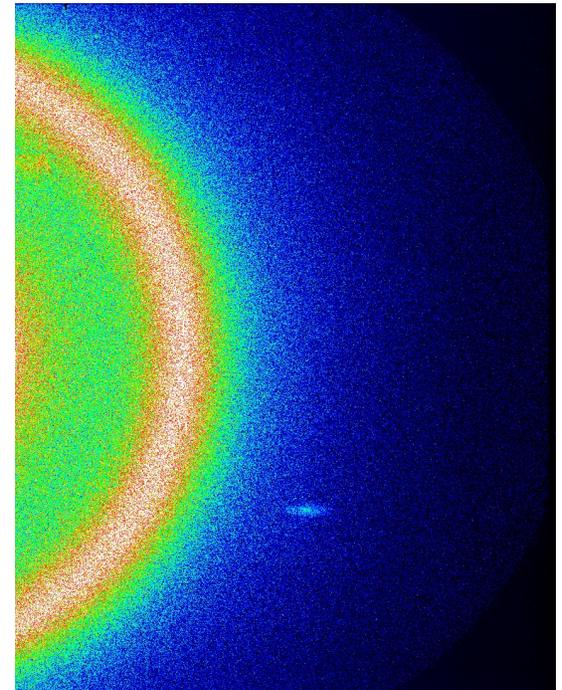
Ferritin Solution upon Freezing



T=13.3

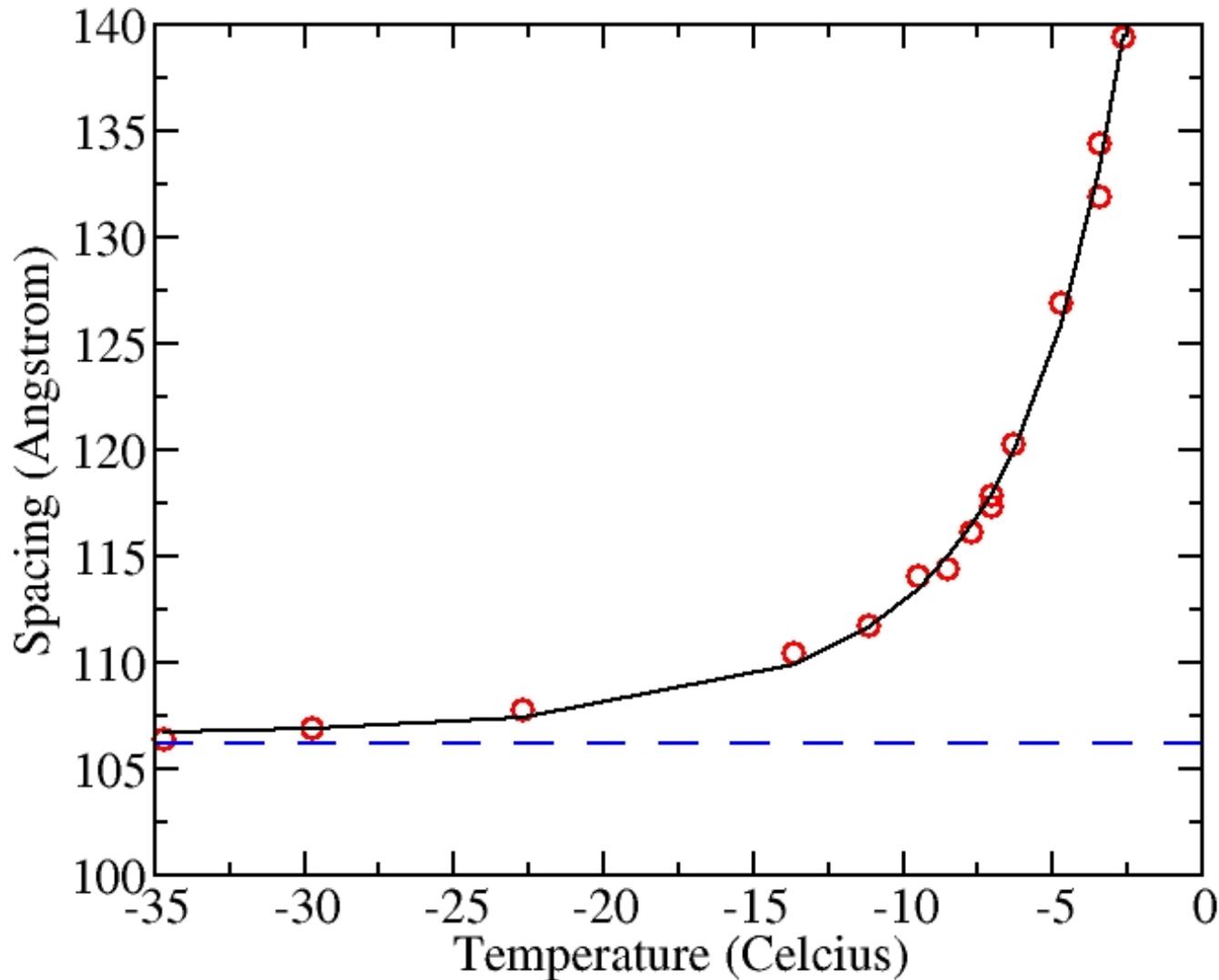


T=-4.7

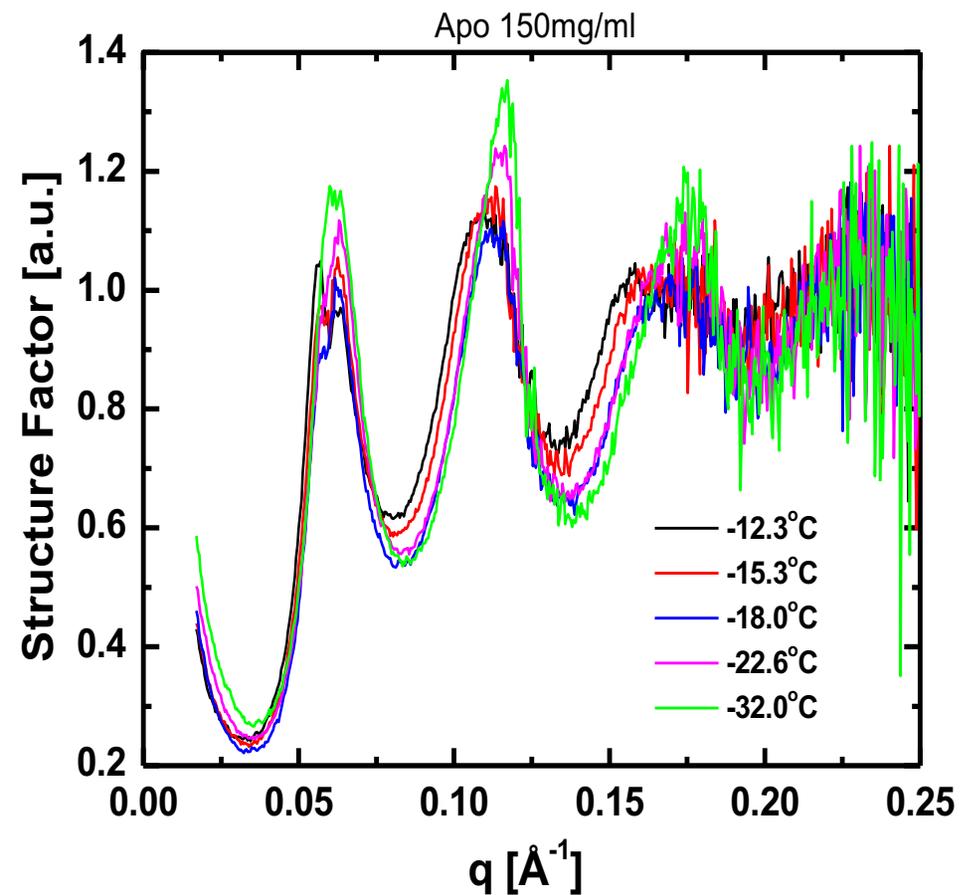
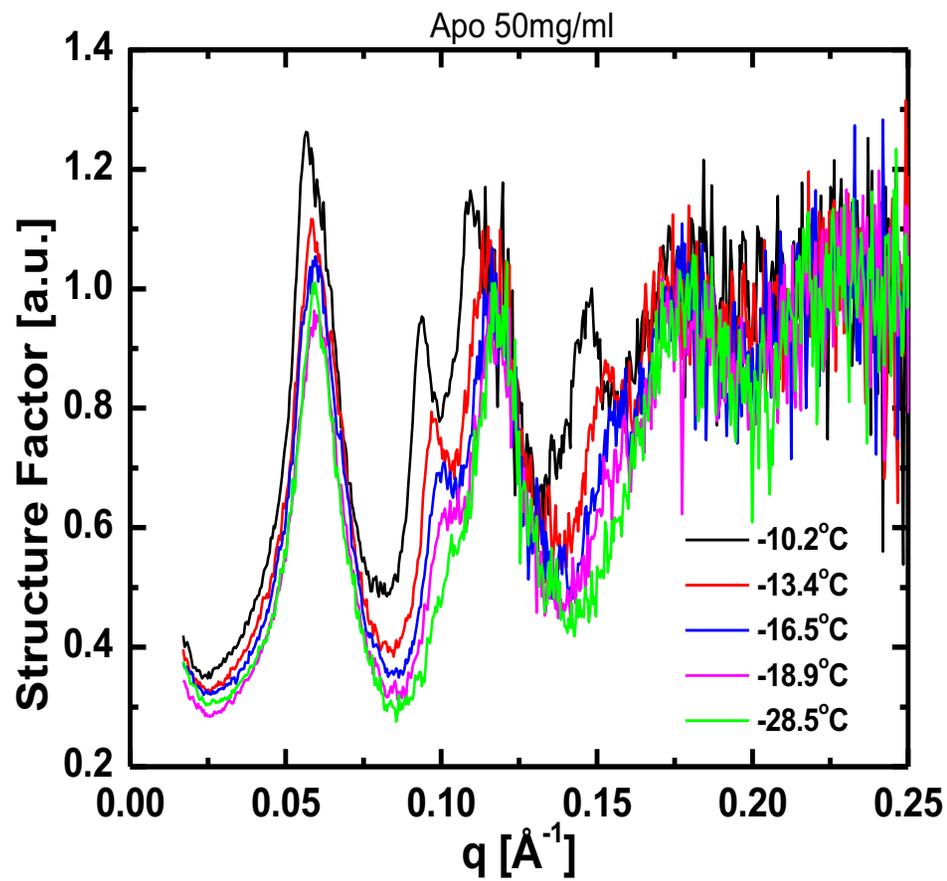


T=-34.7

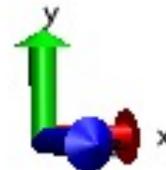
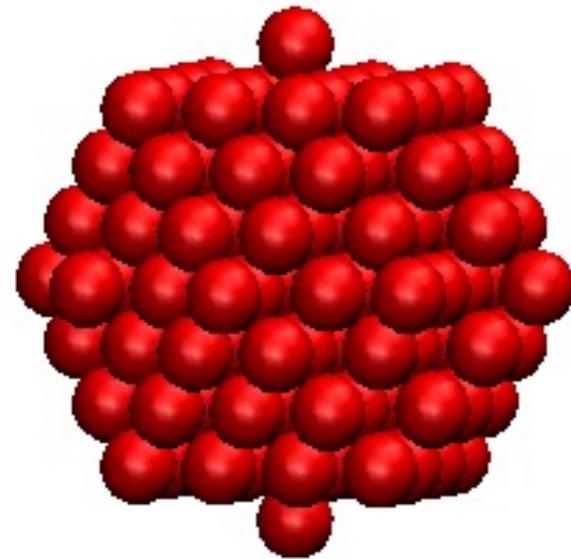
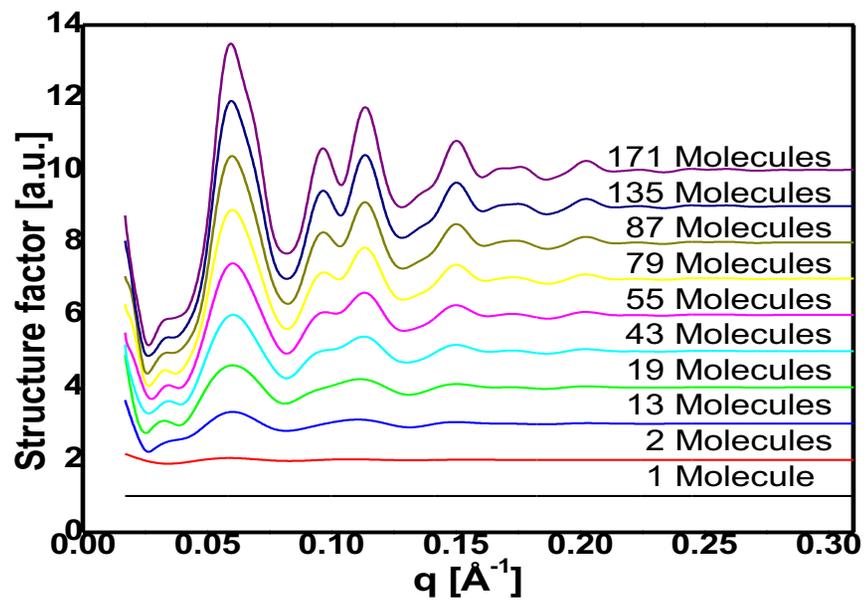
Temperature Variation of Peak Position



Structure Factors of Apoferritin

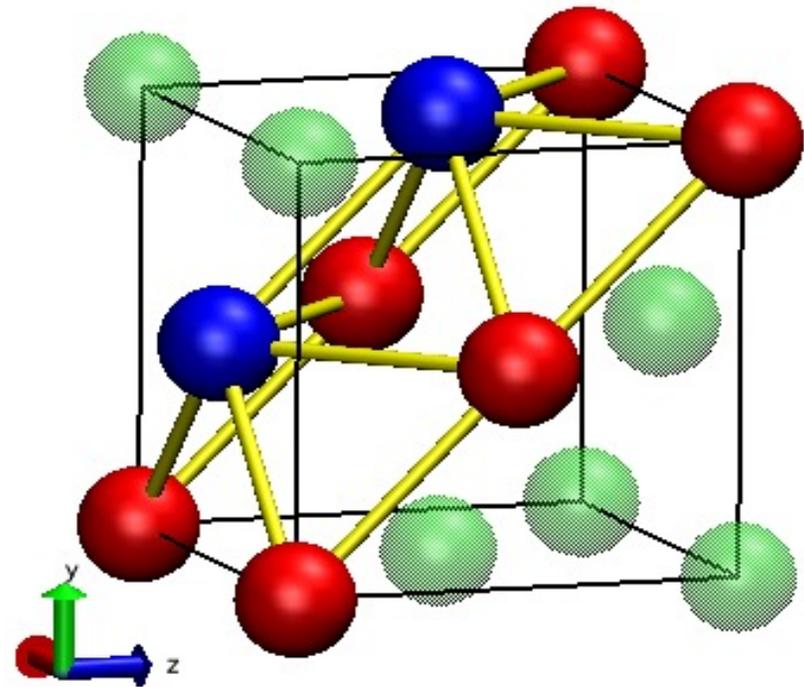
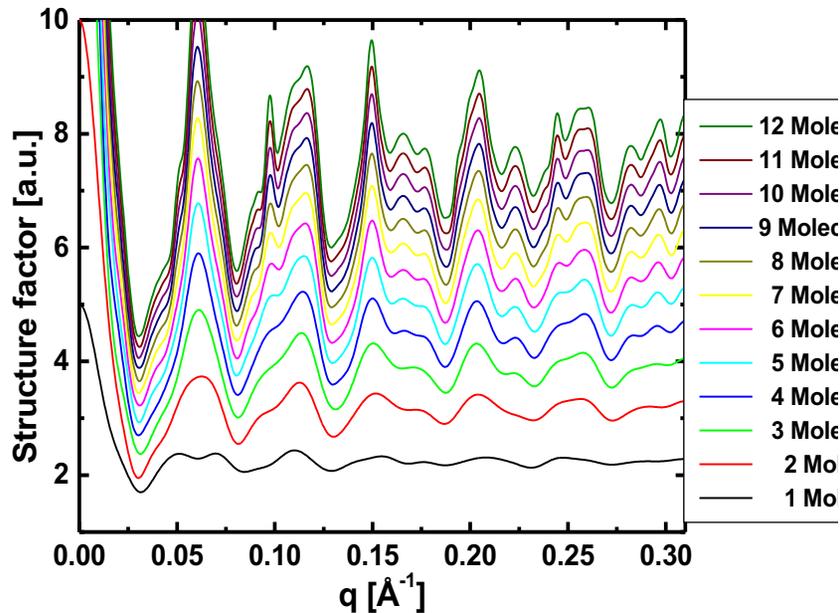


FCC Spherical Clusters



Planar Clusters of 110 Rods

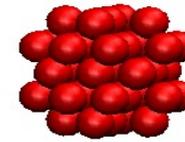
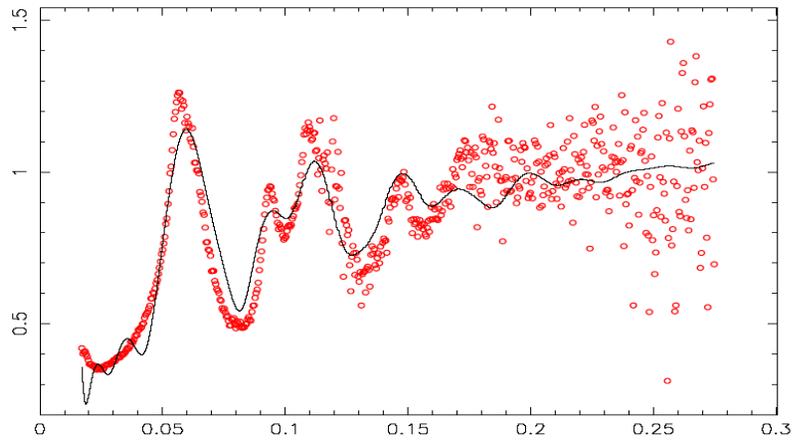
Structure factor simulation as 5 $\langle 110 \rangle$ rods with
1 to 12 molecules per rod



Best Fits

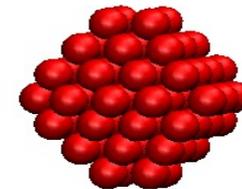
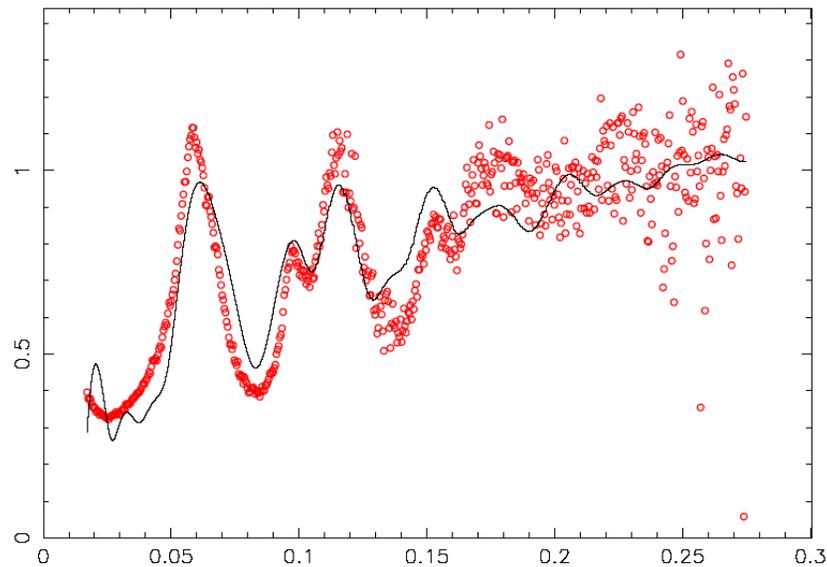
$T = -10^{\circ}\text{C}$

Cluster Size = 36



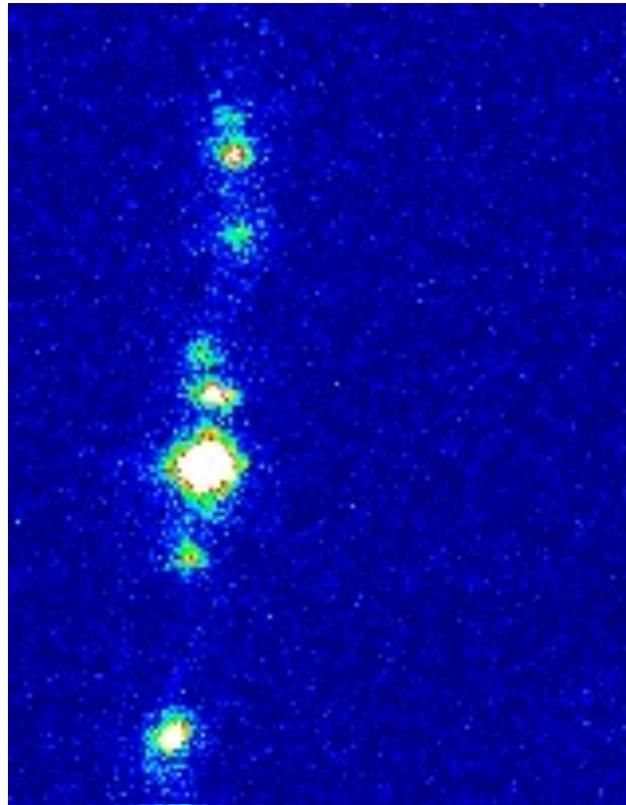
$T = -13^{\circ}\text{C}$

Cluster Size = 63

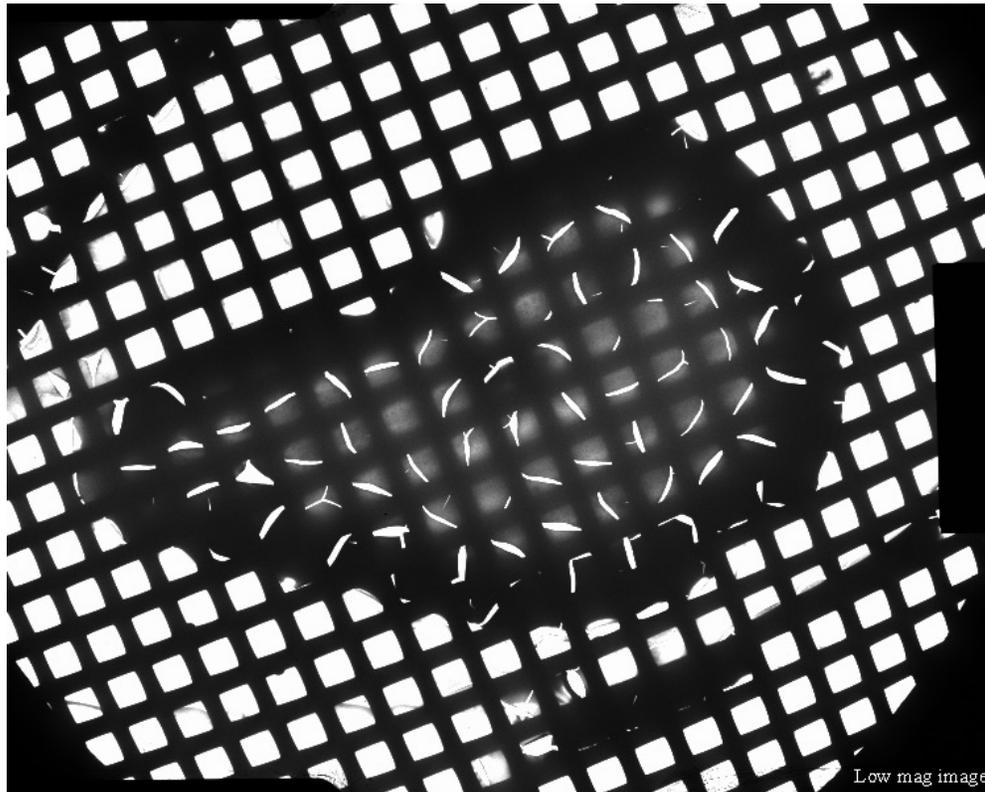


Ferritin (111) Powder Ring

- 50 frames
- 30sec exposure
- 0.3sec playback
- 150x200 pixels of 22.5 μm

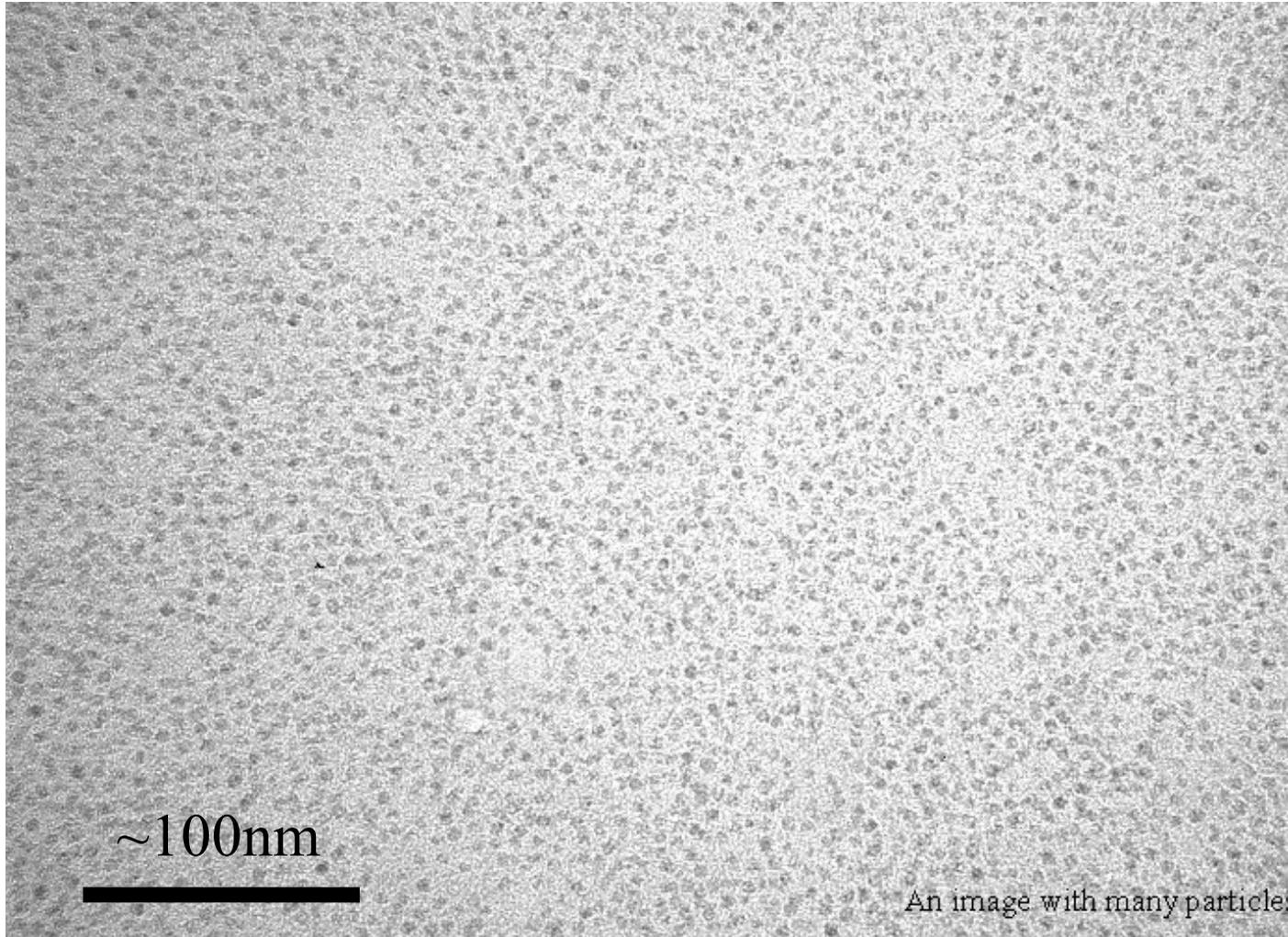


Overview of EM grid



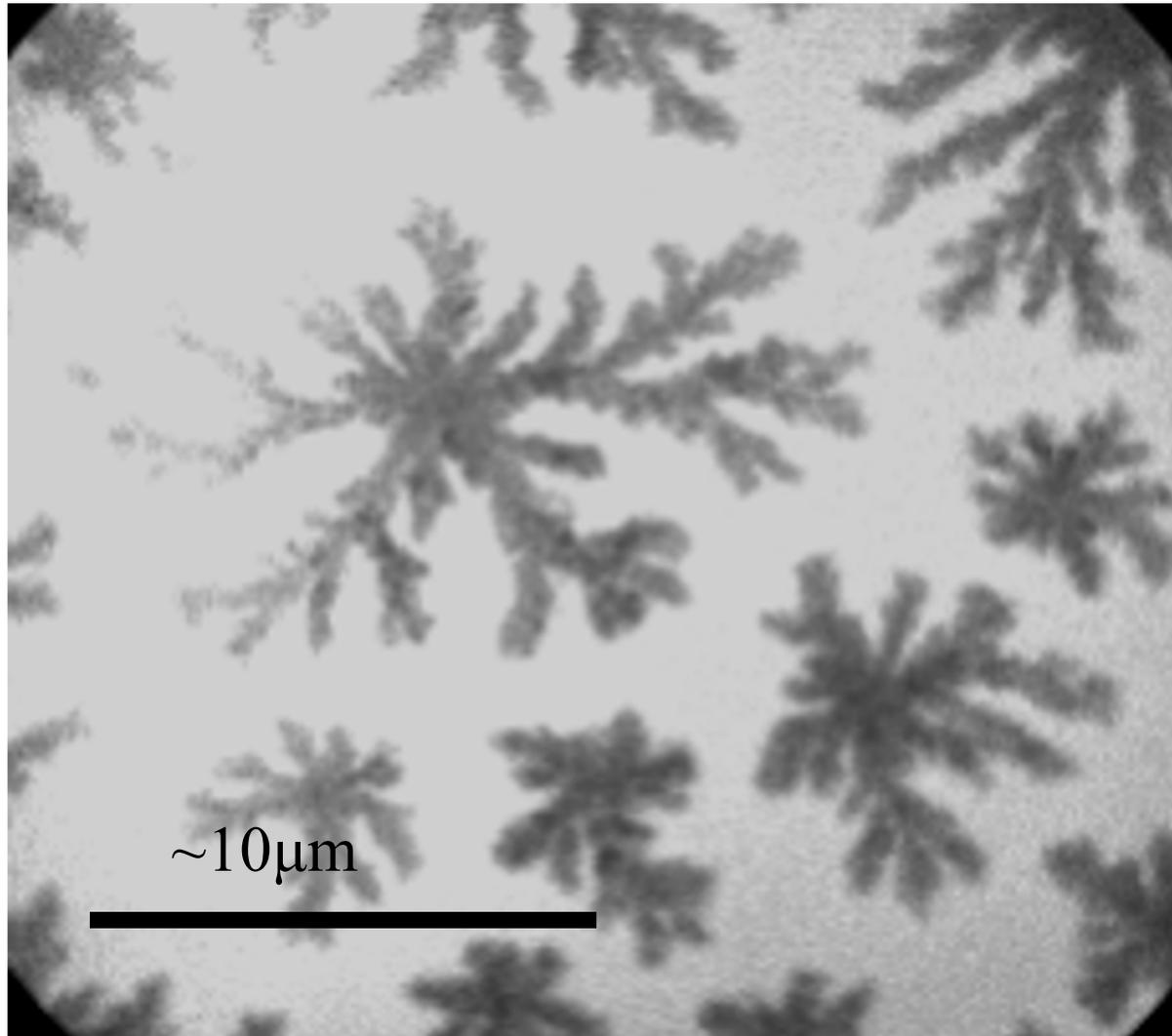
- 3mm diam grid
- a-carbon + “Formvar”
- 10mg/ml Ferritin
- 10mg/ml NaCl
- dipped grid
- dried in air
- no cryo-cooling
 - ▷ three regions of different density

Border Region (low density)



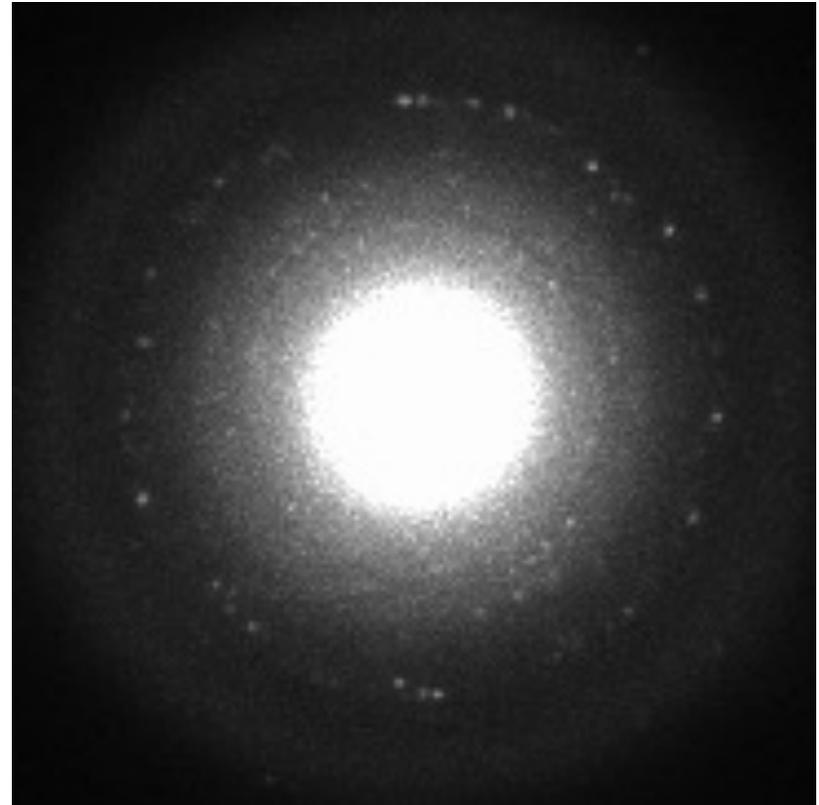
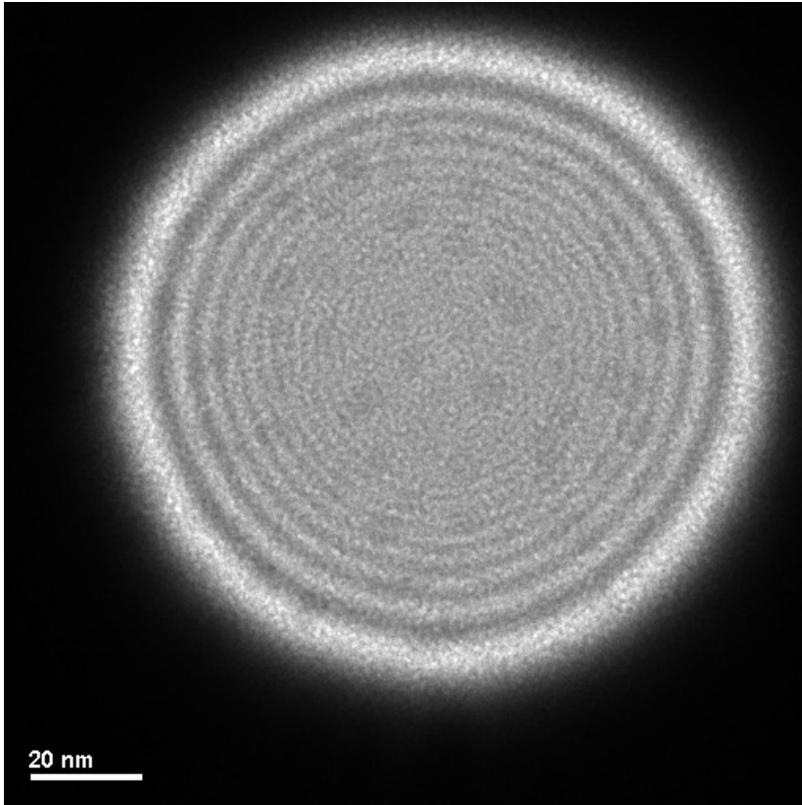
I. K. Robinson DANSYNC Sandbjerg

High Density Ring

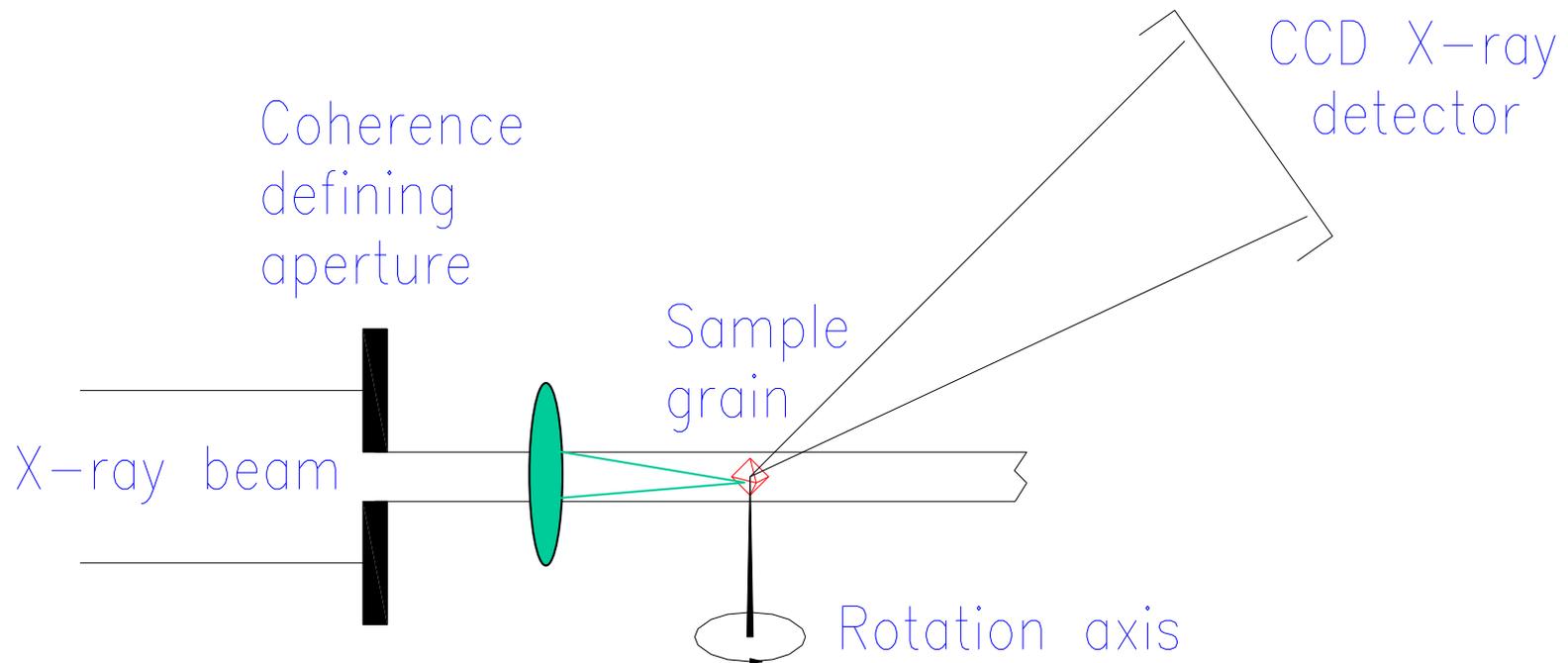


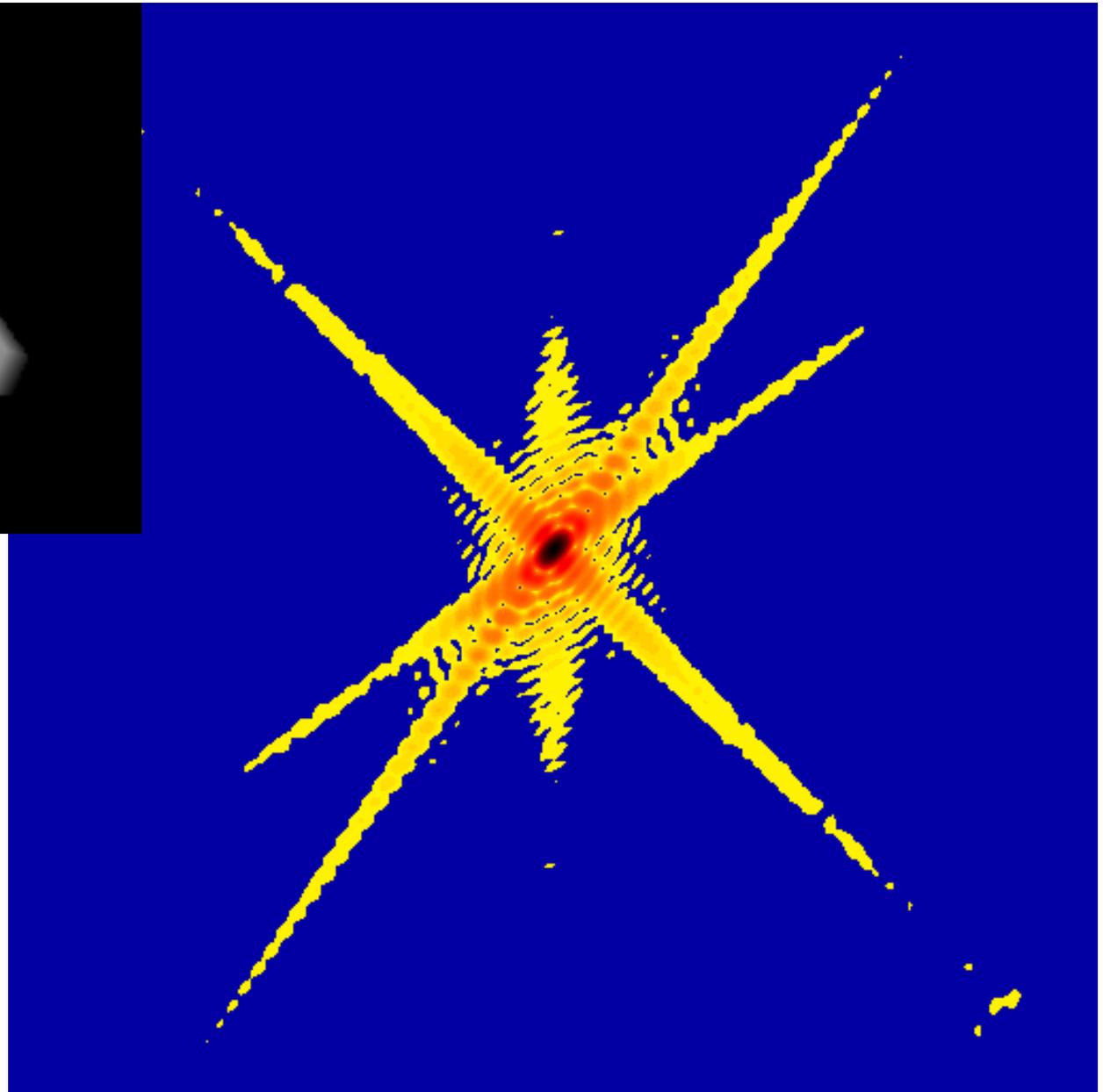
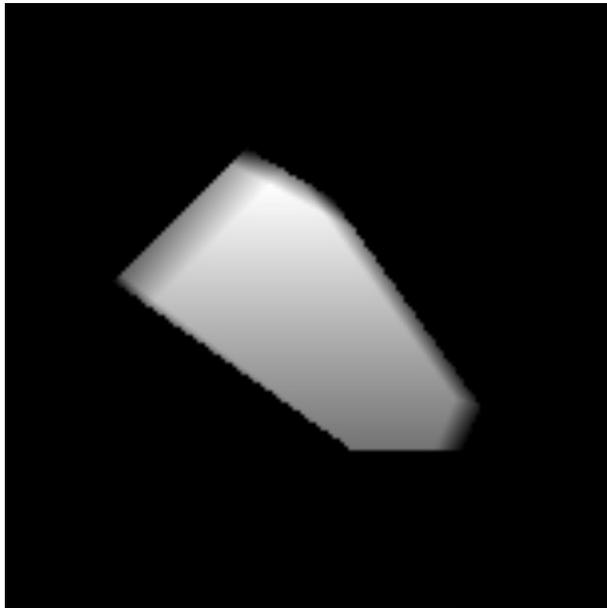
I. K. Robinson DANSYNC Sandbjerg

Diffraction from Border Region



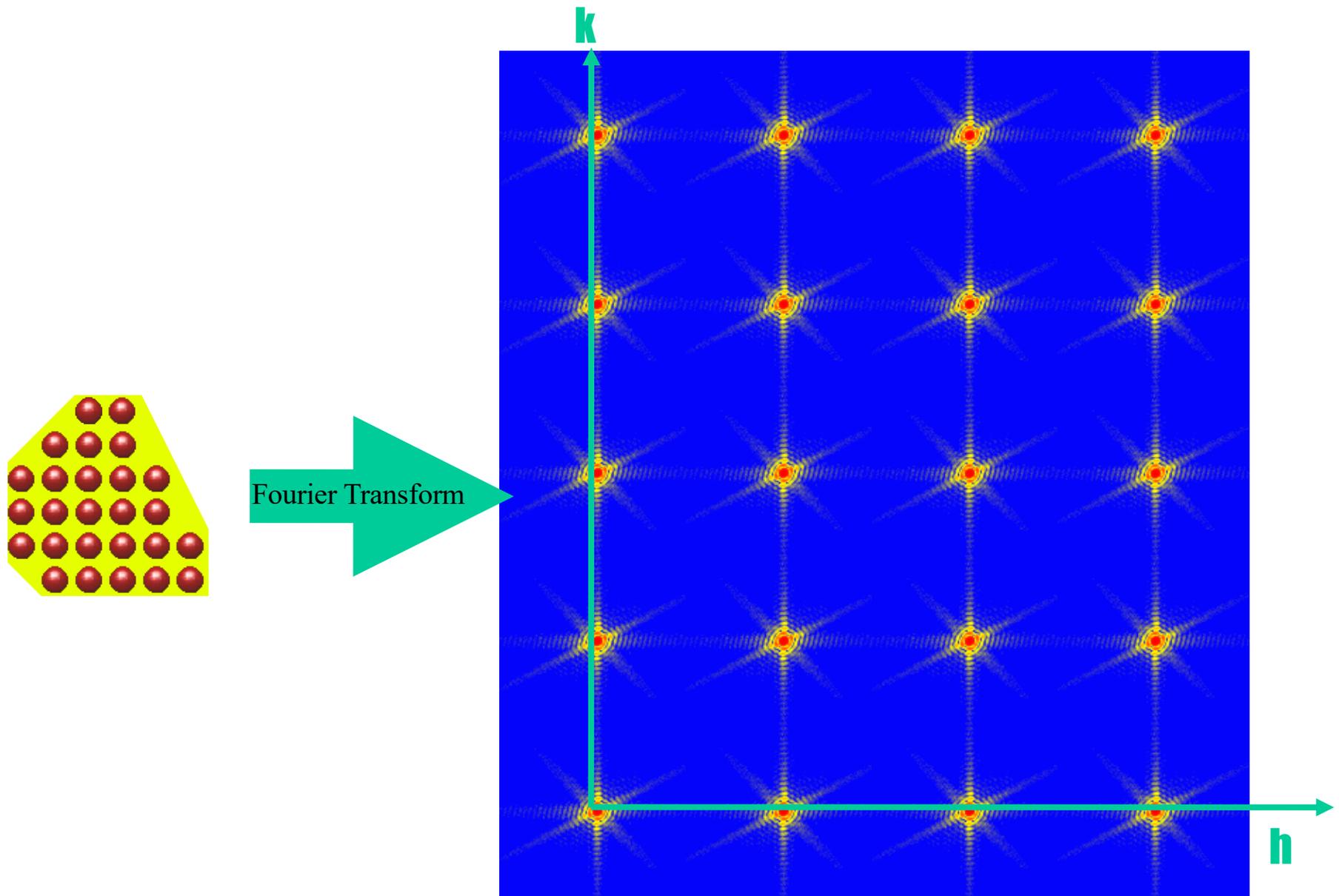
Lensless X-ray Microscope



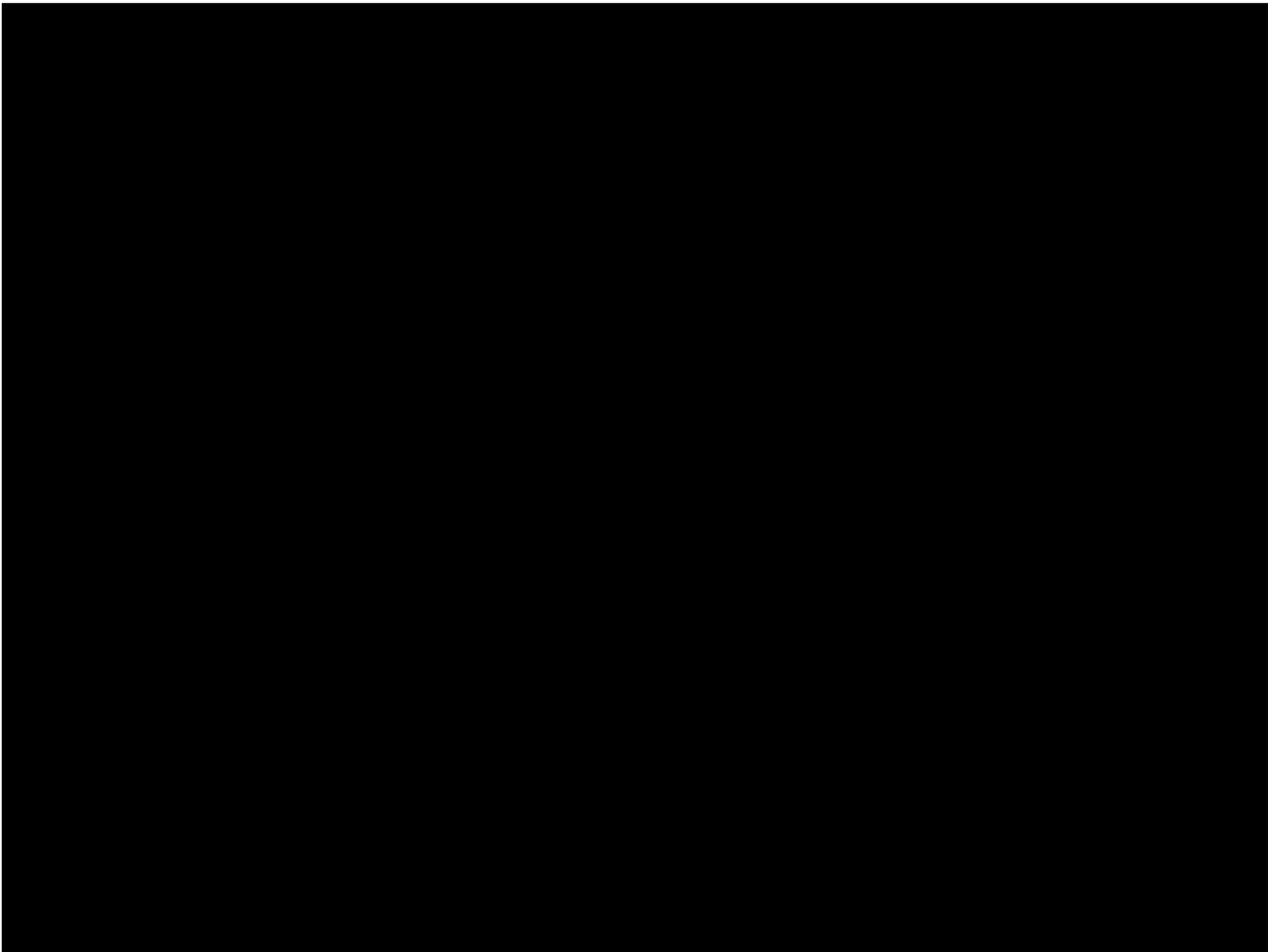


I. K. Robinson DANSYNC Sandbjerg

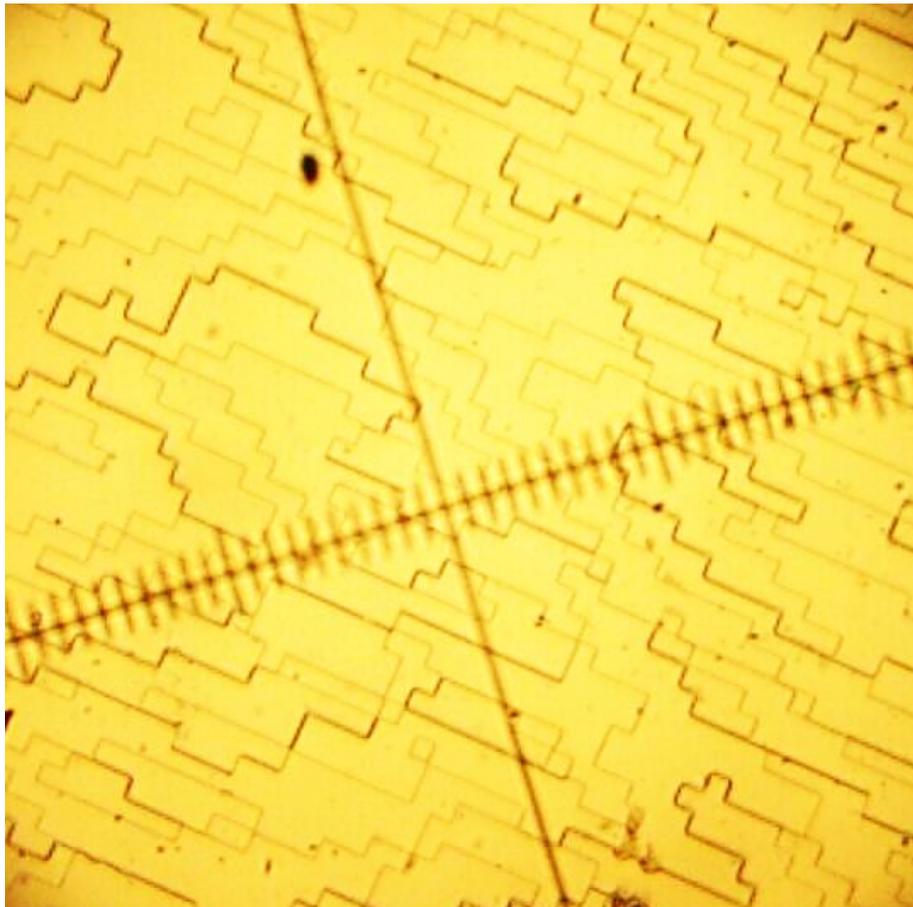
Coherent Diffraction from Crystals



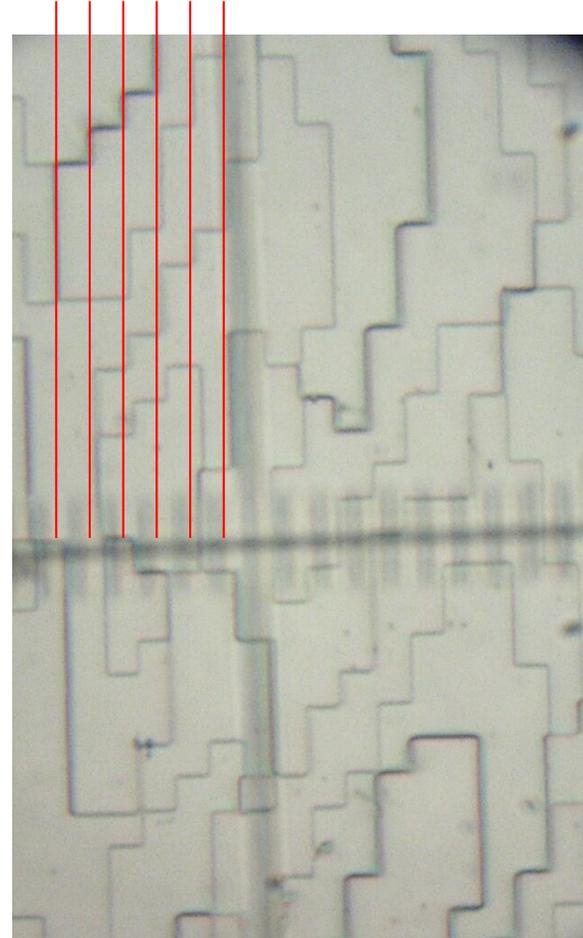
I. K. Robinson DANSYNC Sandbjerg



Microscope Images of Gratings

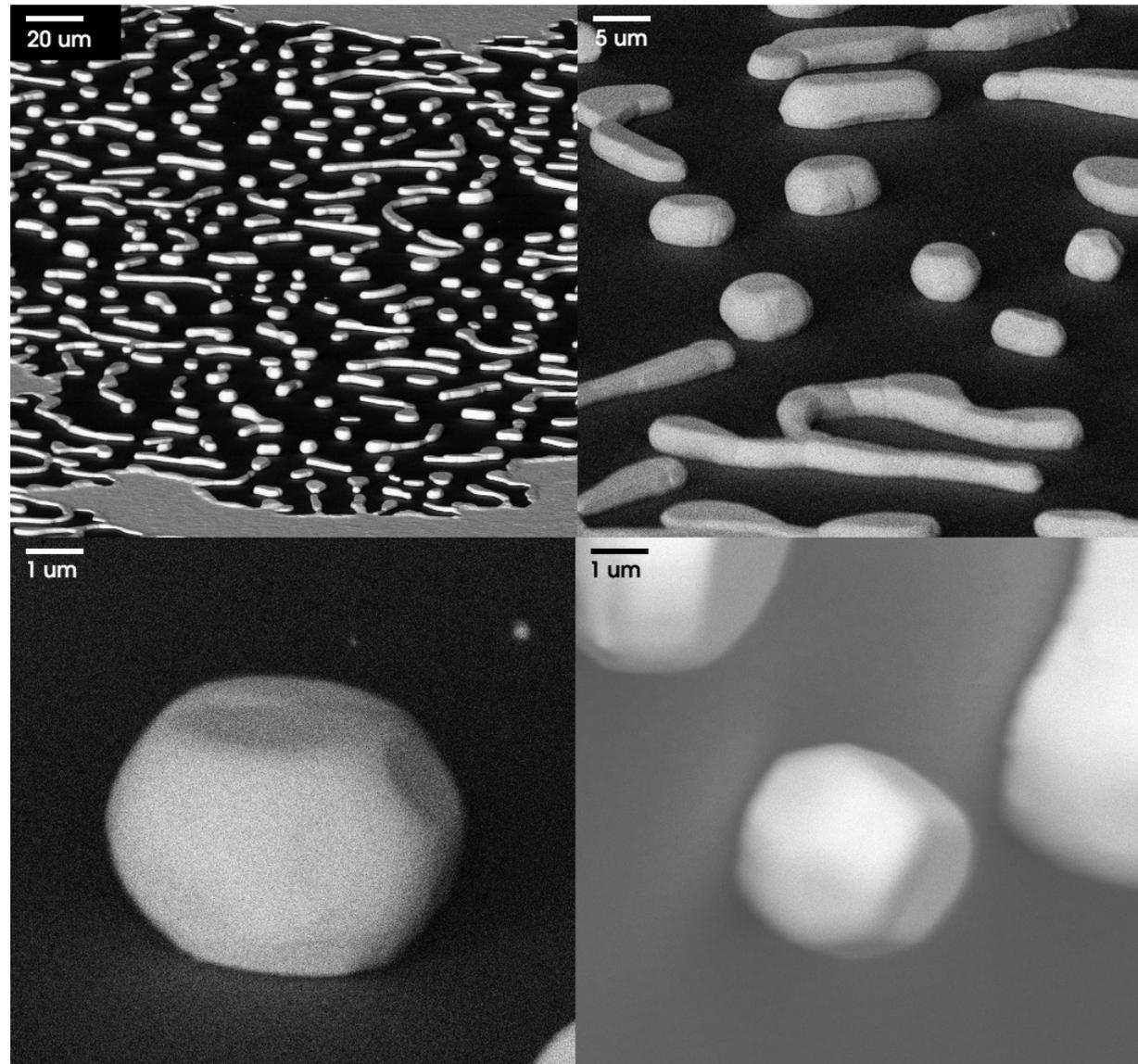


5 μ m grid lines



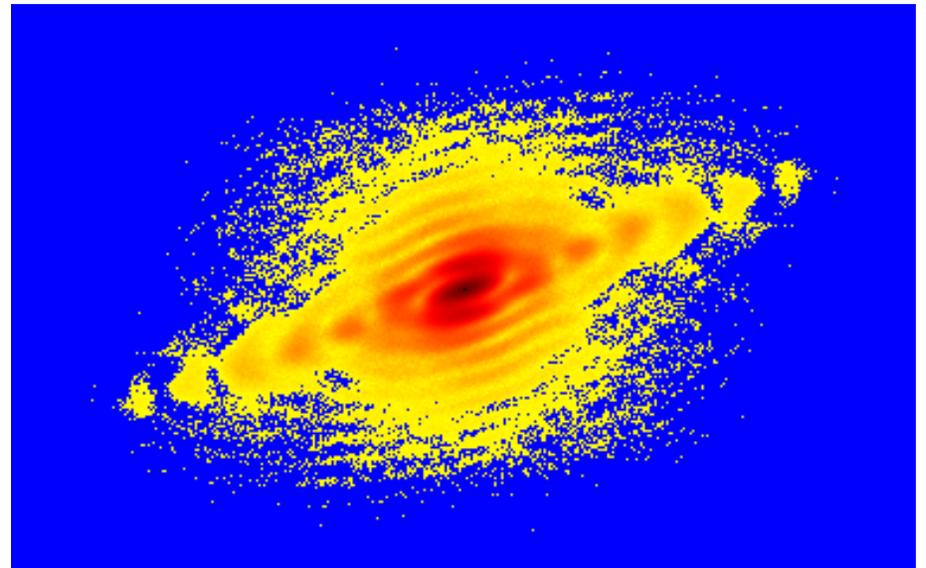
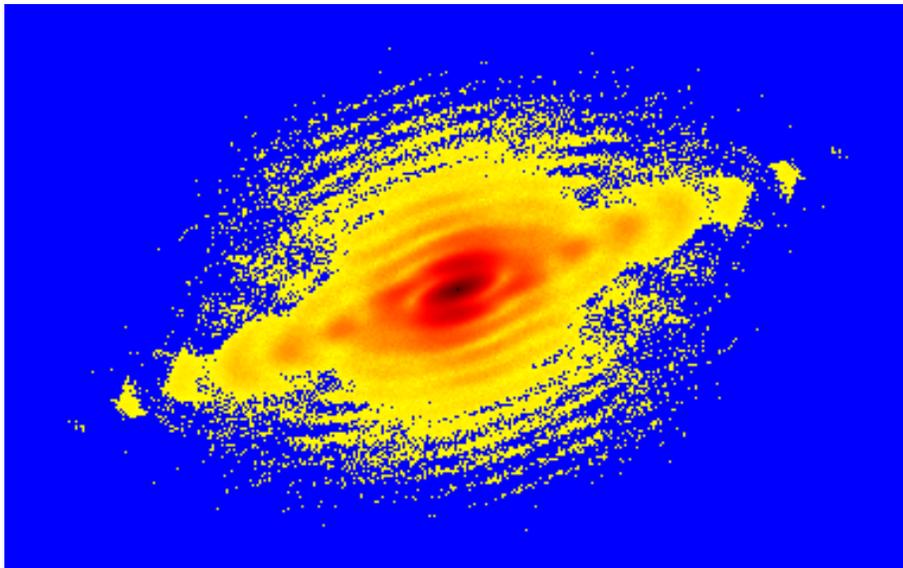
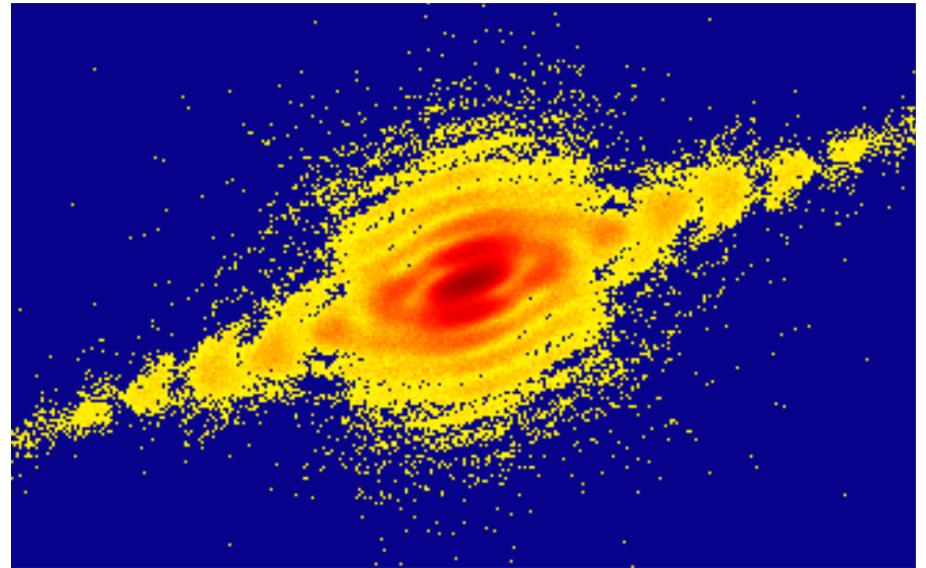
SEMS

- Au blanket film
- Quartz substrate
- Annealed at 950°C for 70 hrs.

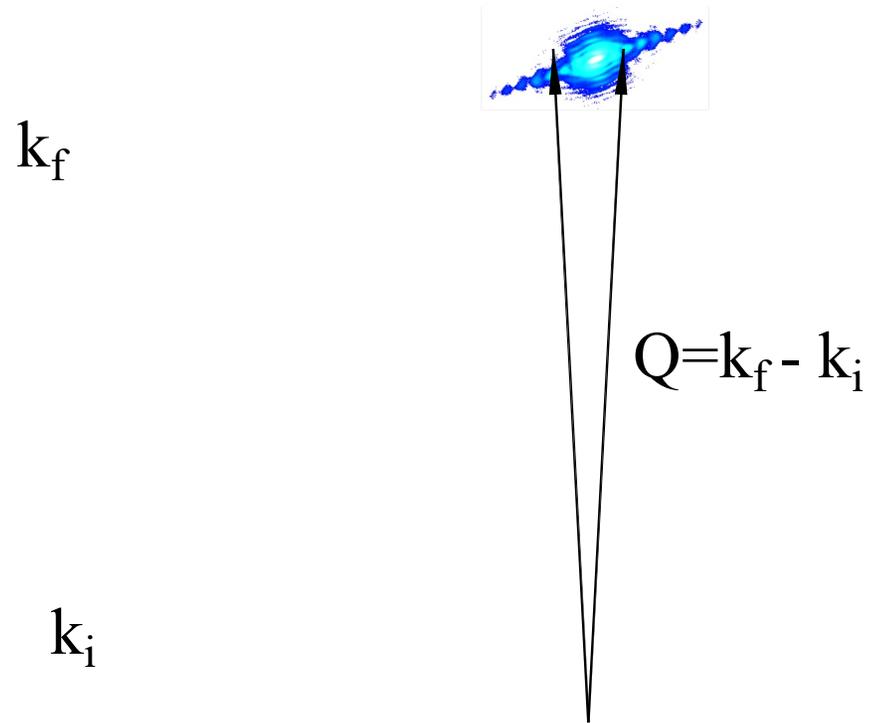


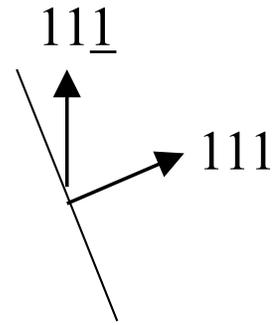
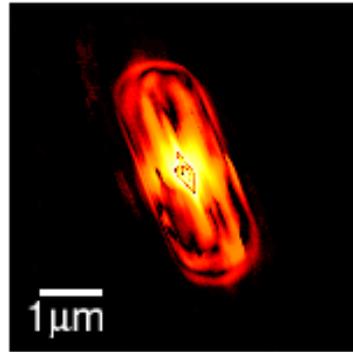
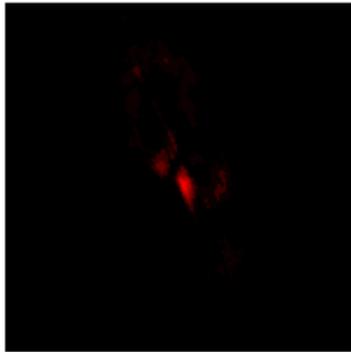
Symmetrized Data and two best fits

Chisq=0.0005

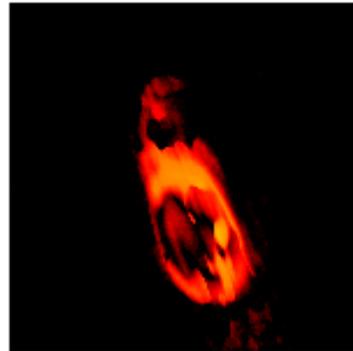
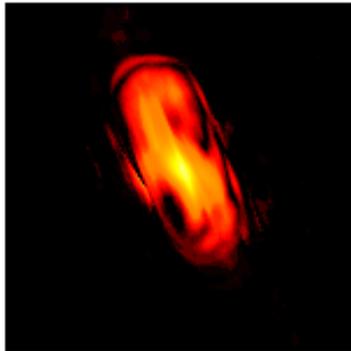
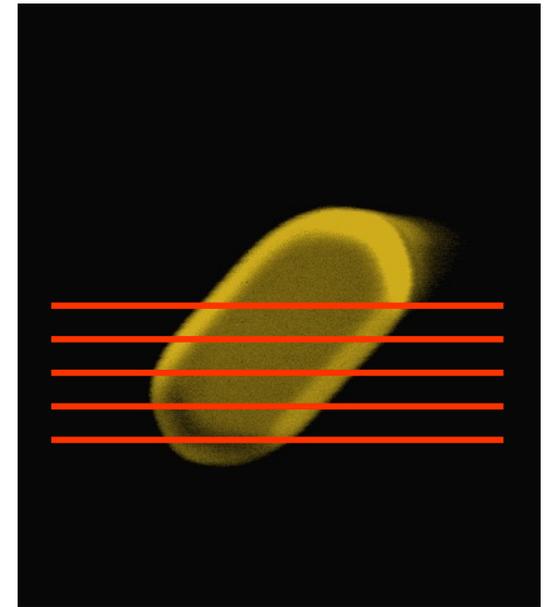
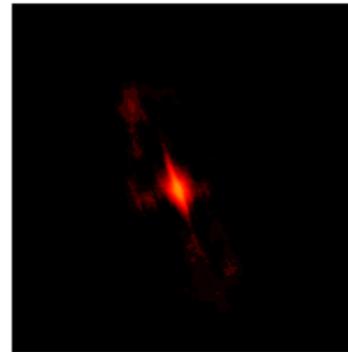
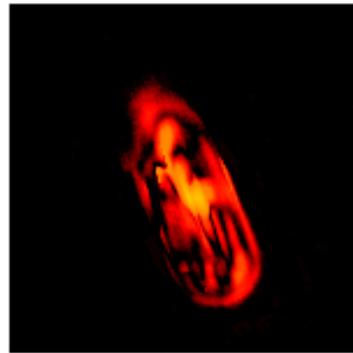
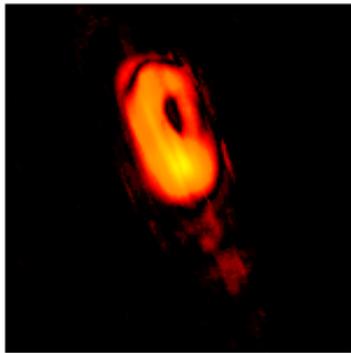
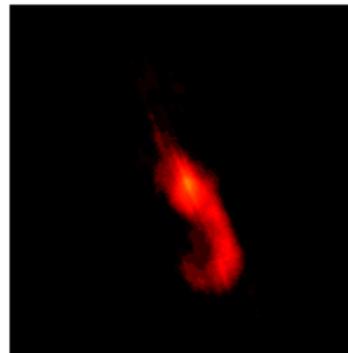
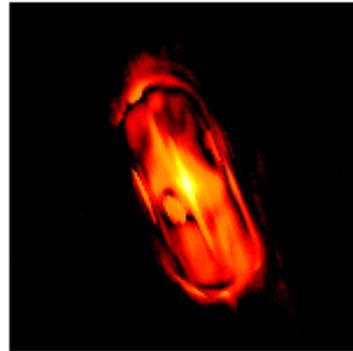
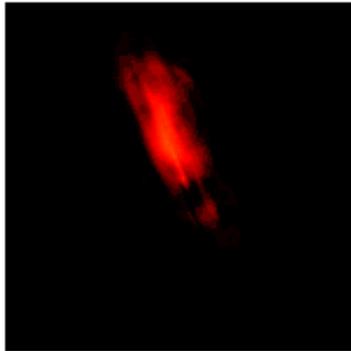


3D Diffraction Method

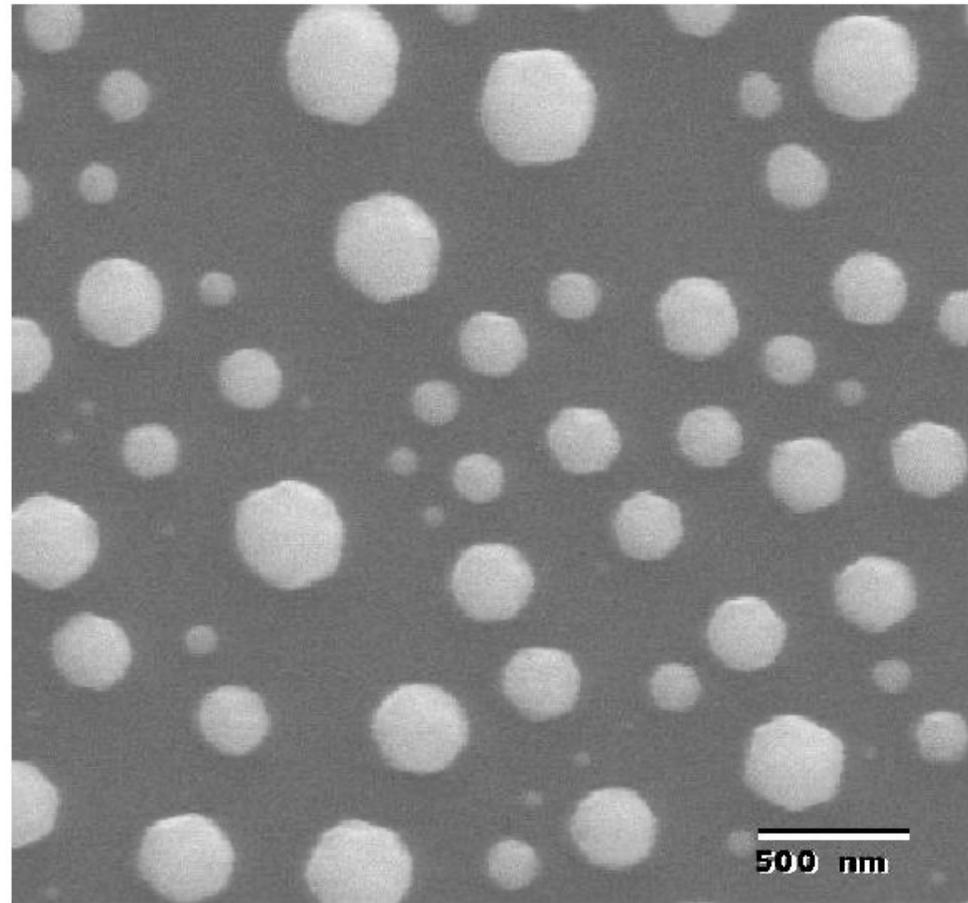




Slices through
plan view SEM:



In situ growth of Pb crystals



I. K. Robinson DANSYNC Sandbjerg

Good statistics, 3D diffraction data

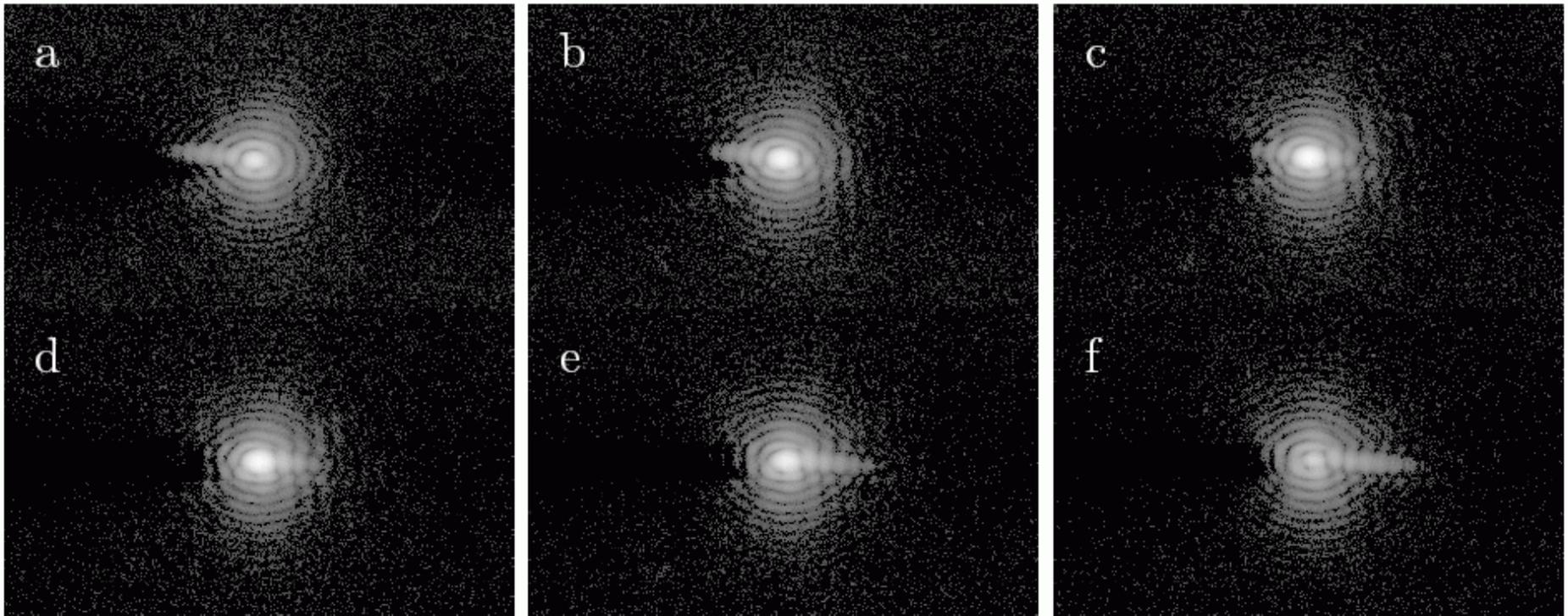
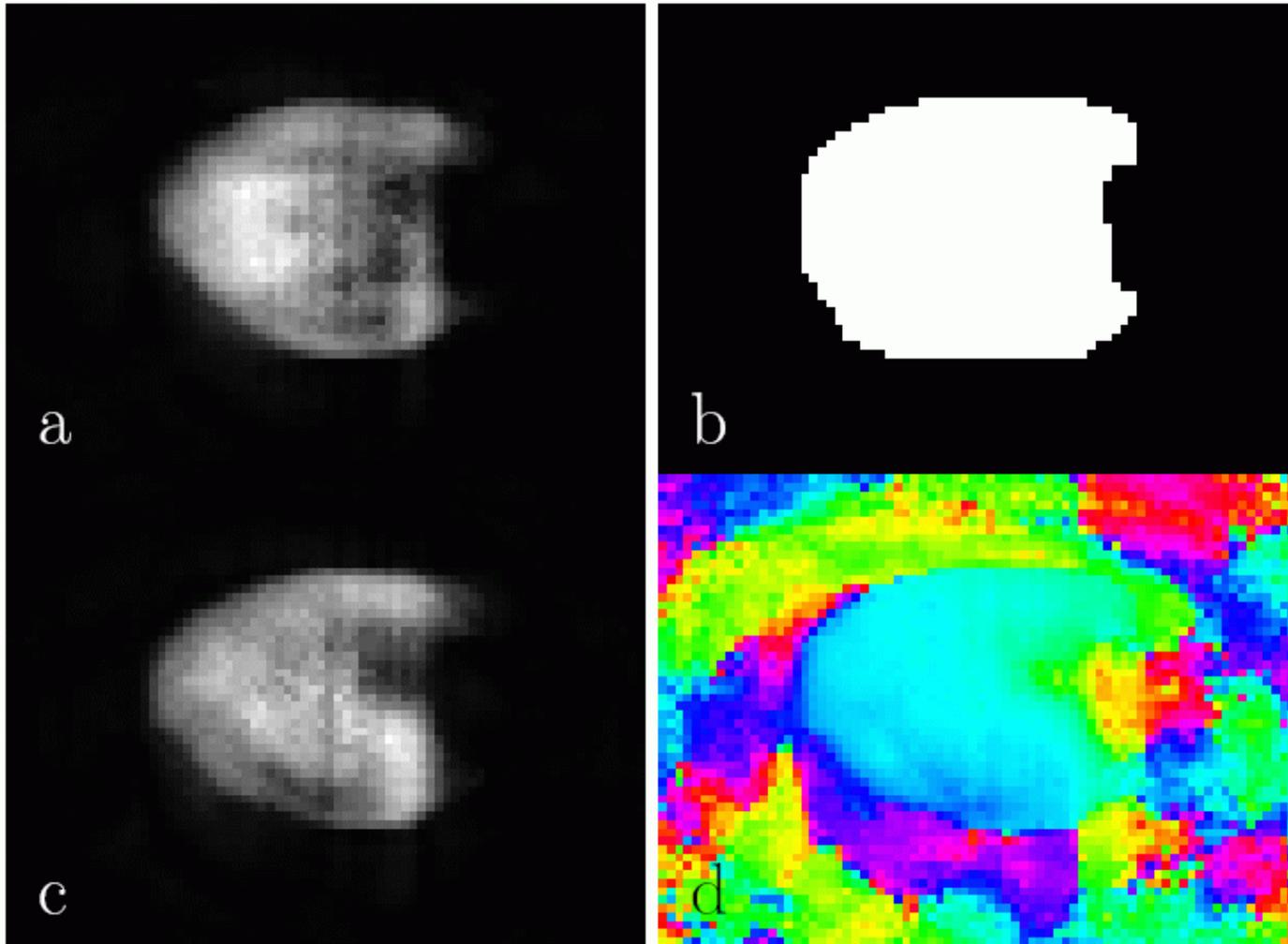
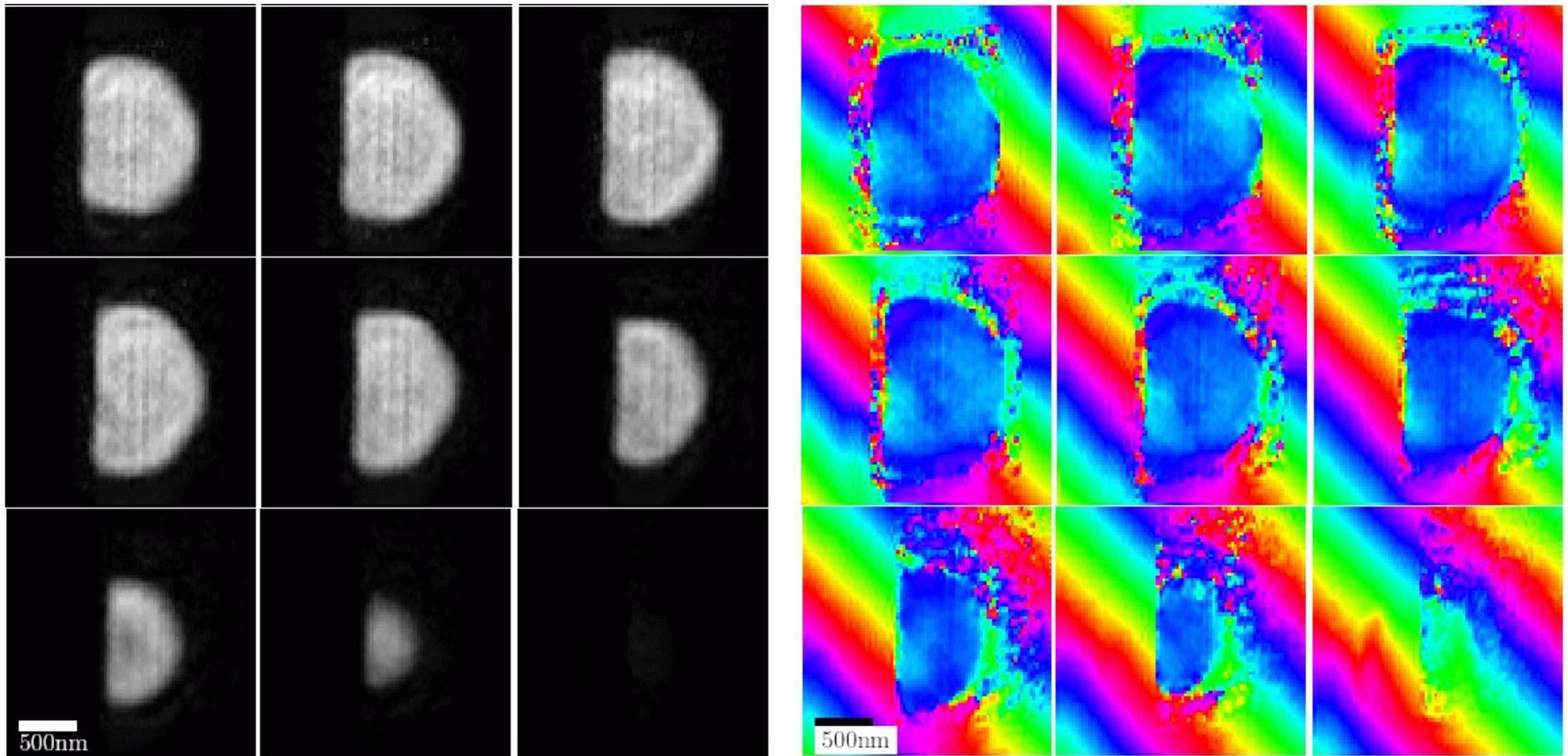


Figure 4.12: Center slices from 3D CXD pattern from Pb sample, on a log scale. Data file 296 from 10/03.

Learn shape of “tight” support

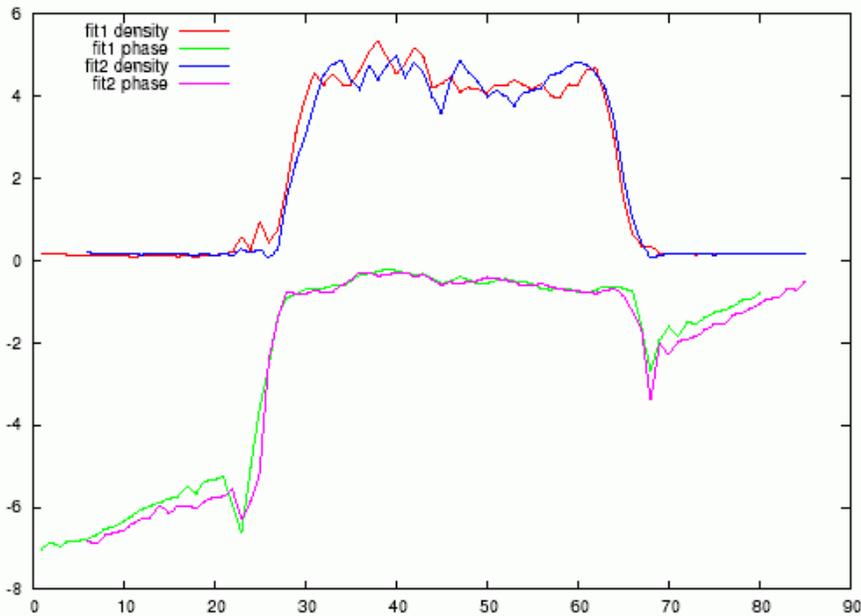


Then refine amplitude *and* phase

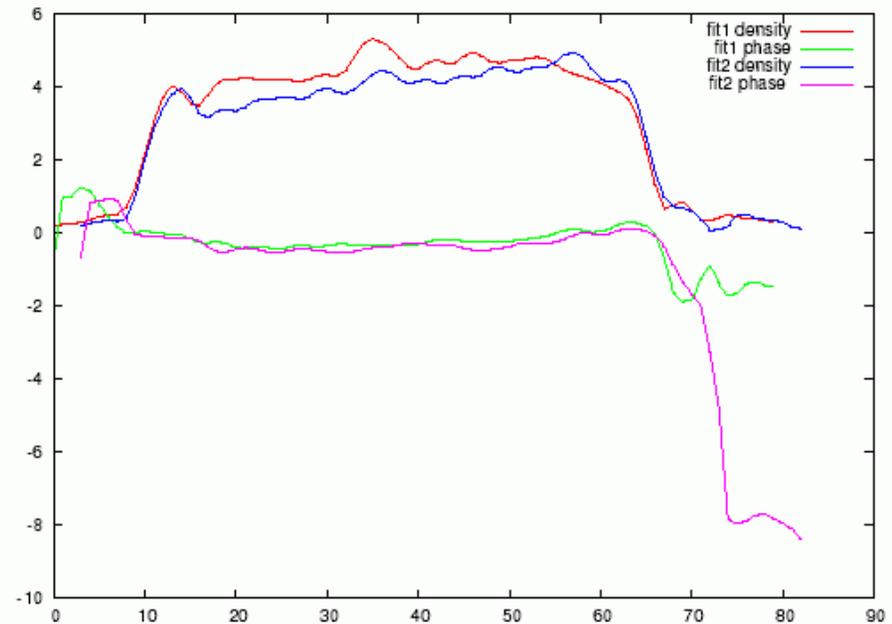


Amplitude/phase cross sections

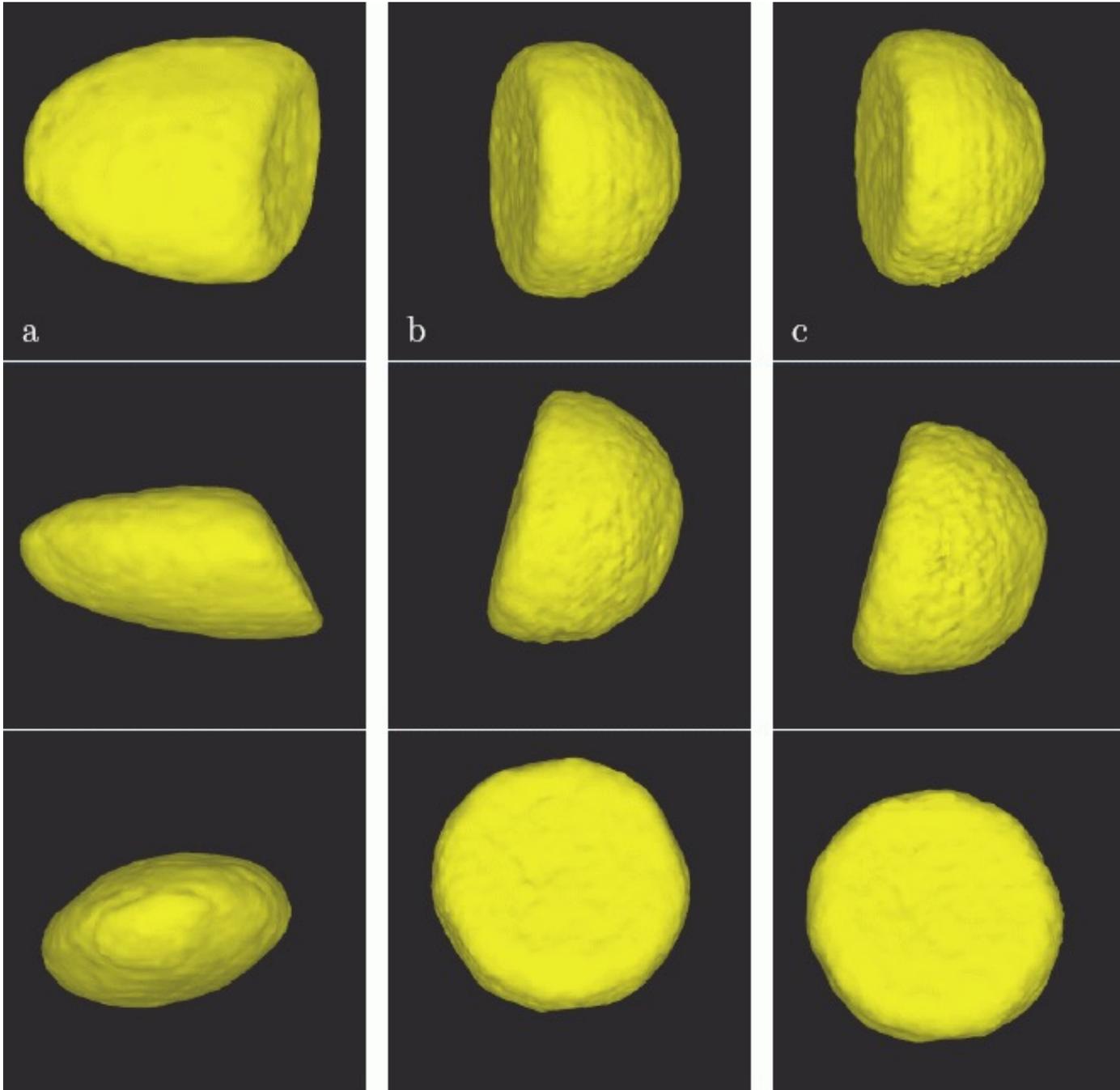
x-striations do not reproduce



x-coordinate

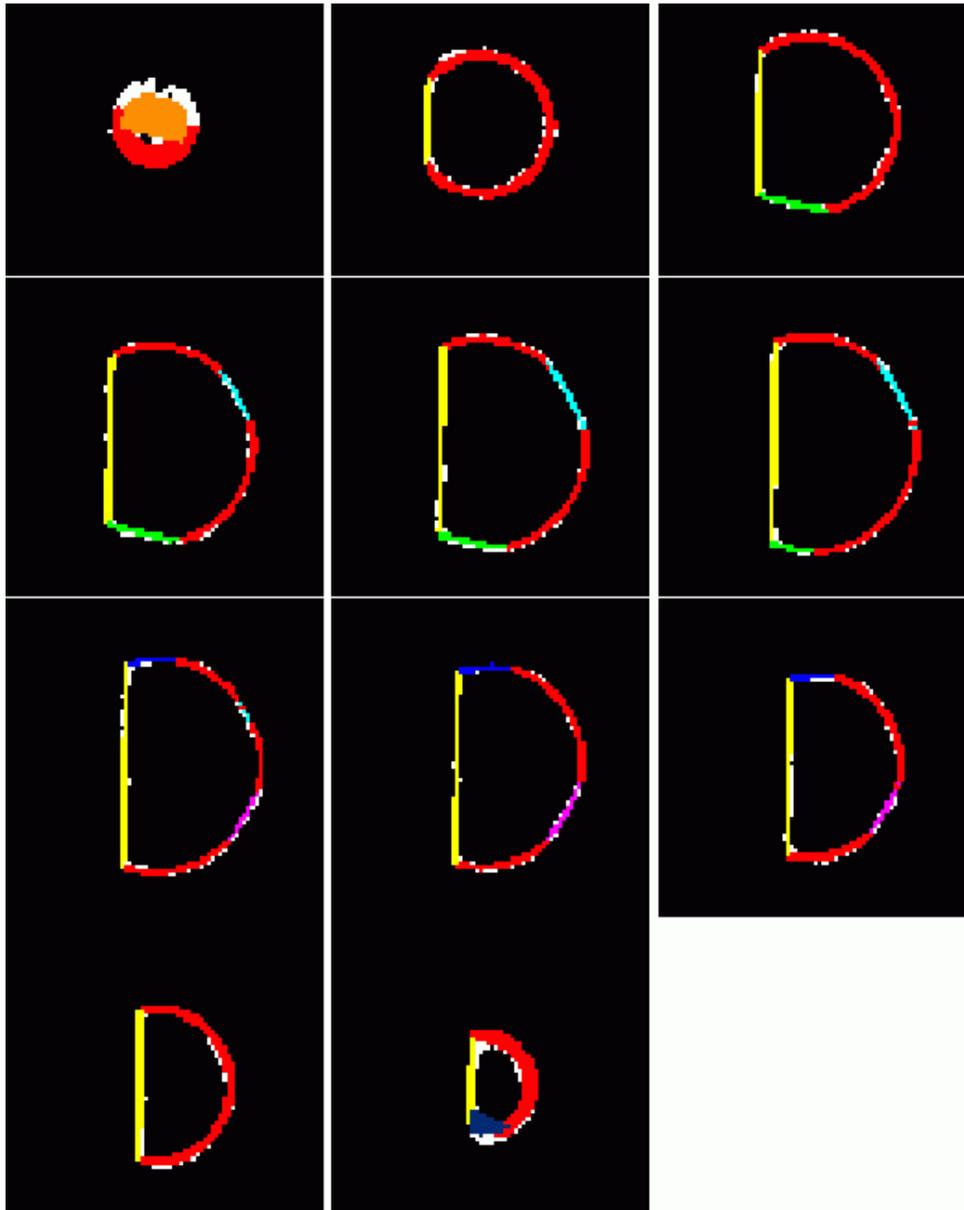


y-coordinate



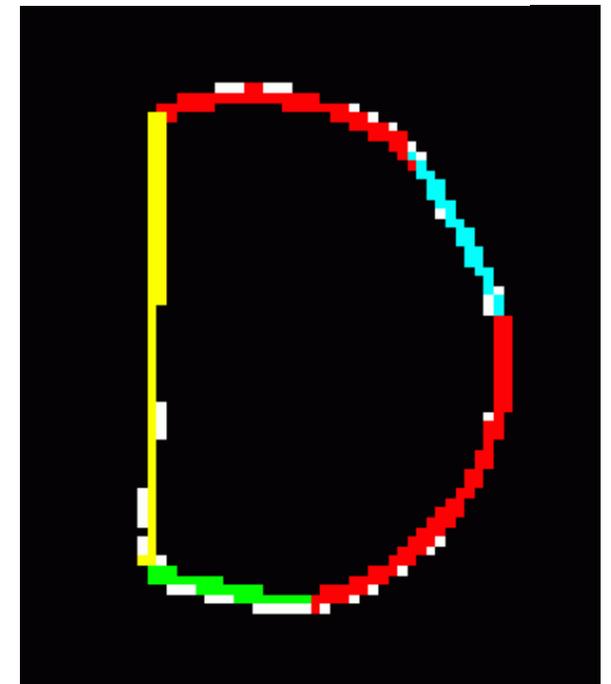
I. K. Robinson DANSYNC Sandbjerg

Fitting to faceted shape



| | $ R $ | | P0 | P1 | P2 | P3 | P4 | P5 | P6 |
|---|-------|----|----|----|-----|-----|-----|-----|-----|
|  | 9.4 | P0 | 0 | 85 | 149 | 79 | 134 | 106 | 71 |
|  | 25.7 | P1 | | 0 | 123 | 164 | 83 | 76 | 102 |
|  | 25.1 | P2 | | | 0 | 72 | 67 | 74 | 110 |
|  | 25.9 | P3 | | | | 0 | 111 | 106 | 76 |
|  | 25.4 | P4 | | | | | 0 | 113 | 68 |
|  | 25.4 | P5 | | | | | | 0 | 176 |
|  | 26.0 | P6 | | | | | | | 0 0 |

Angles between facets

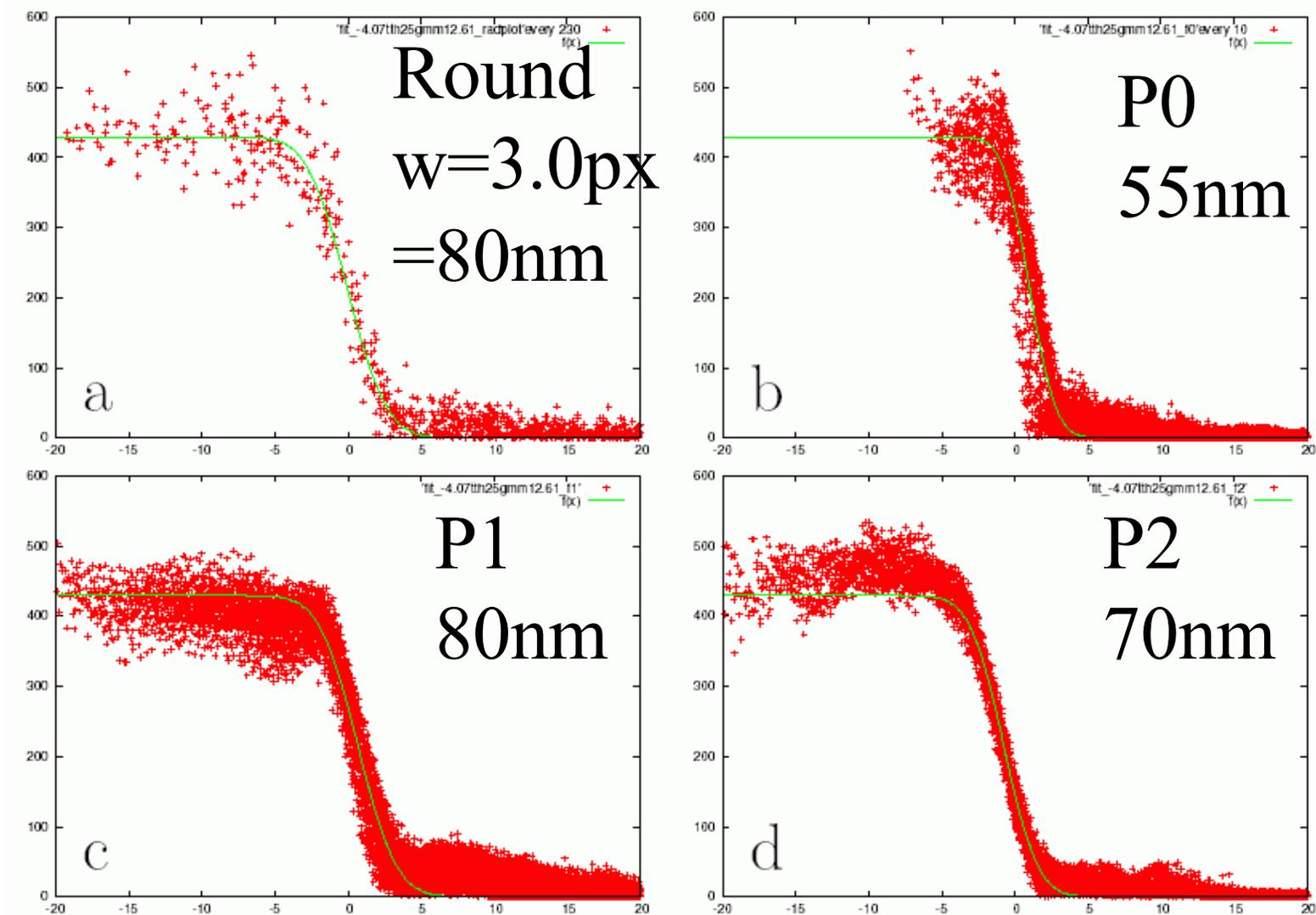


Facets of Equilibrium Crystal Shape

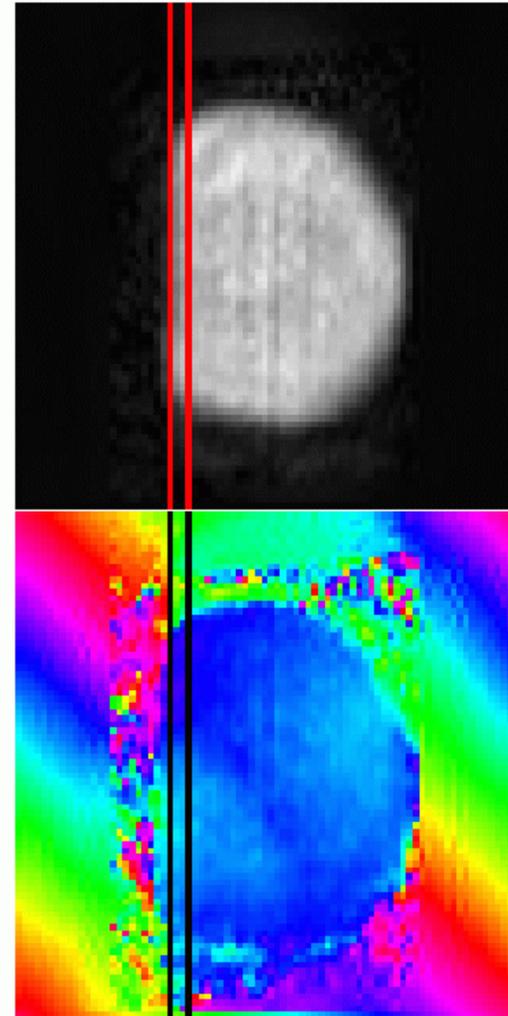
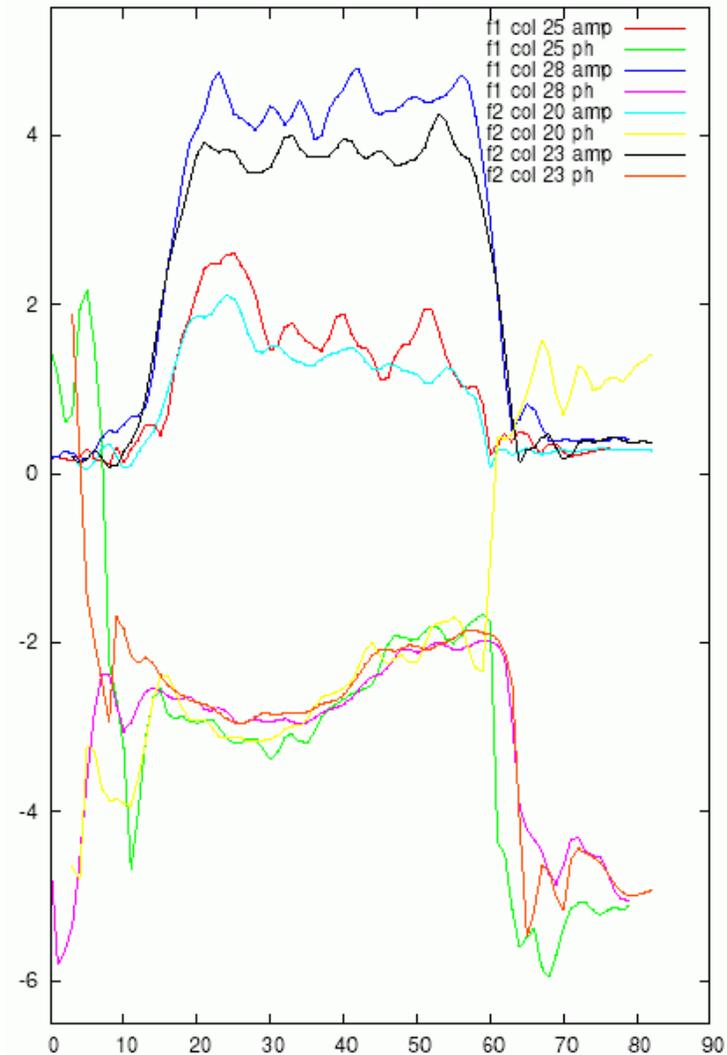


I. K. Robinson DANSYNC Sandbjerg

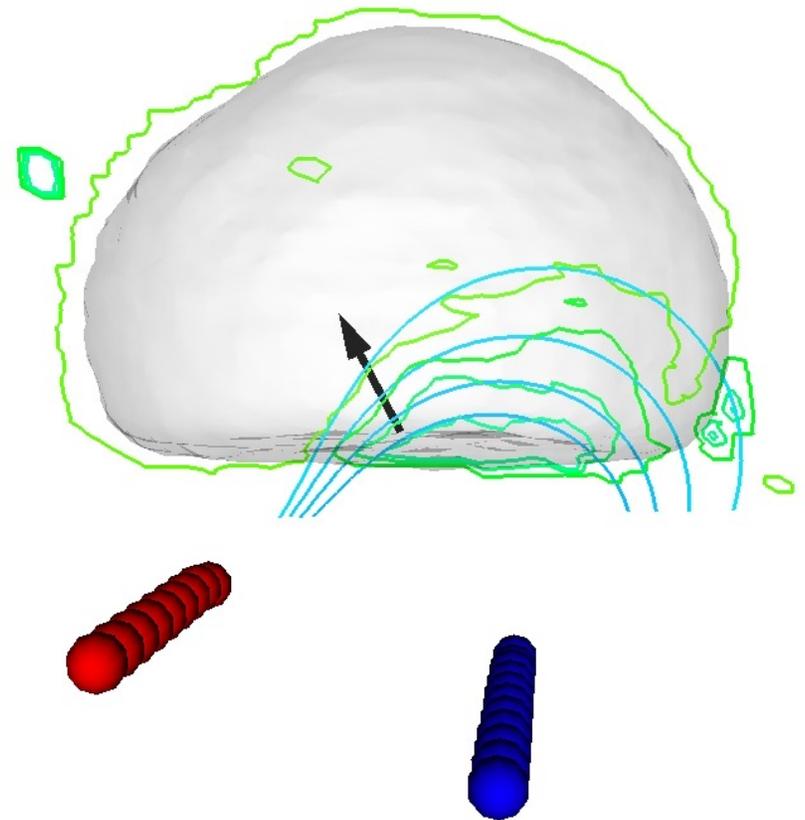
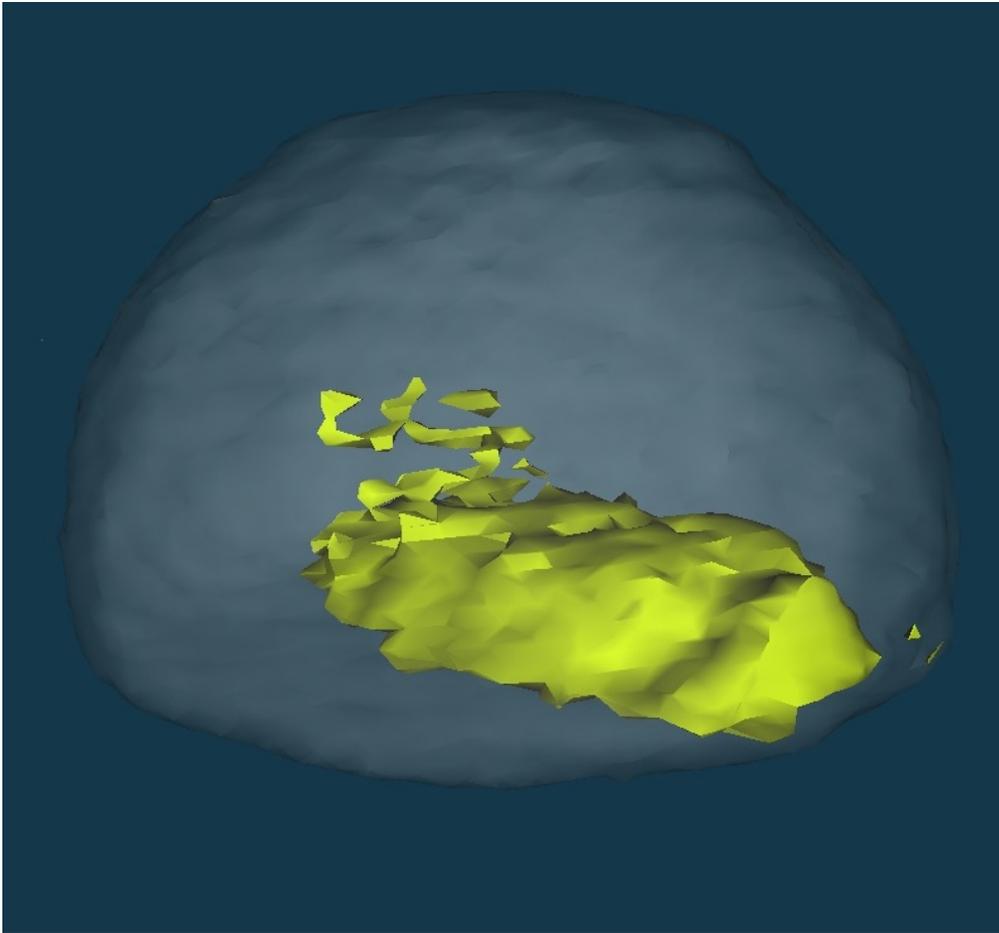
Density distribution across surface



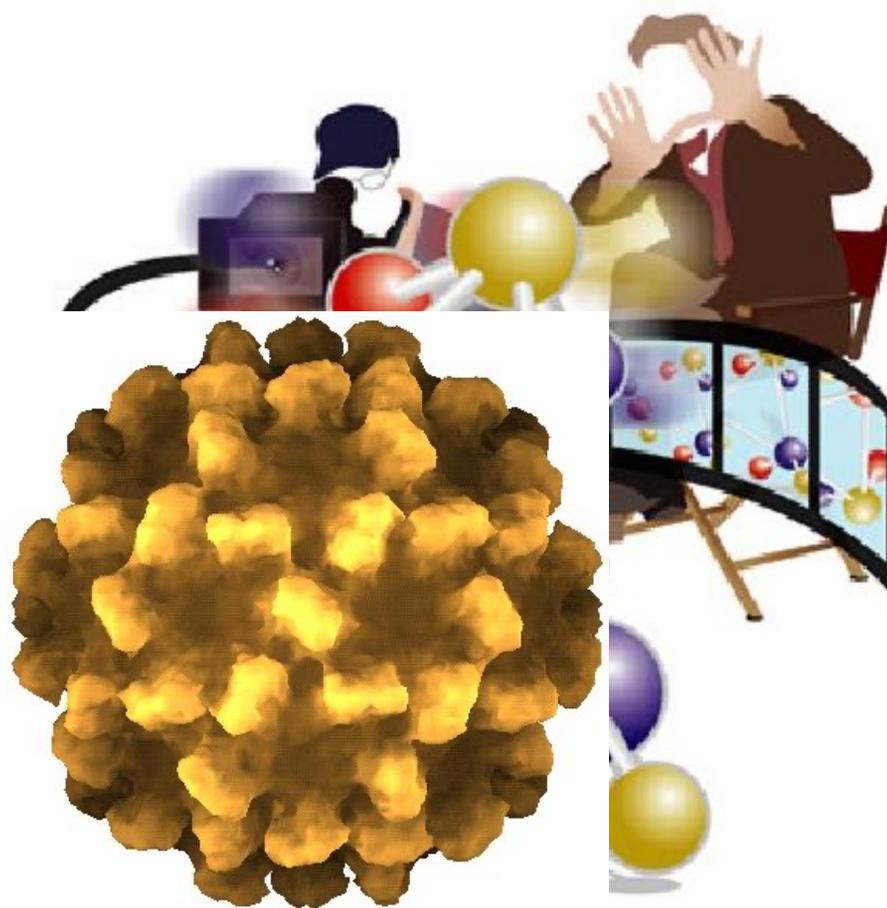
Phase structure near substrate interface



Modeling of 3D Phase Bump



Molecular Movies using XFEL



I. K. Robinson DANSYNC Sandbjerg

Conclusions

- Smallest Ferritin crystals about 3 microns
- Implosion due to radiation damage
- Expanded aggregate state upon freezing
- Model SAXS using clusters
- Single molecule diffraction ideas can be tested using nanocrystals
- Phase objects 'equally' accessible