Future of CryoEM

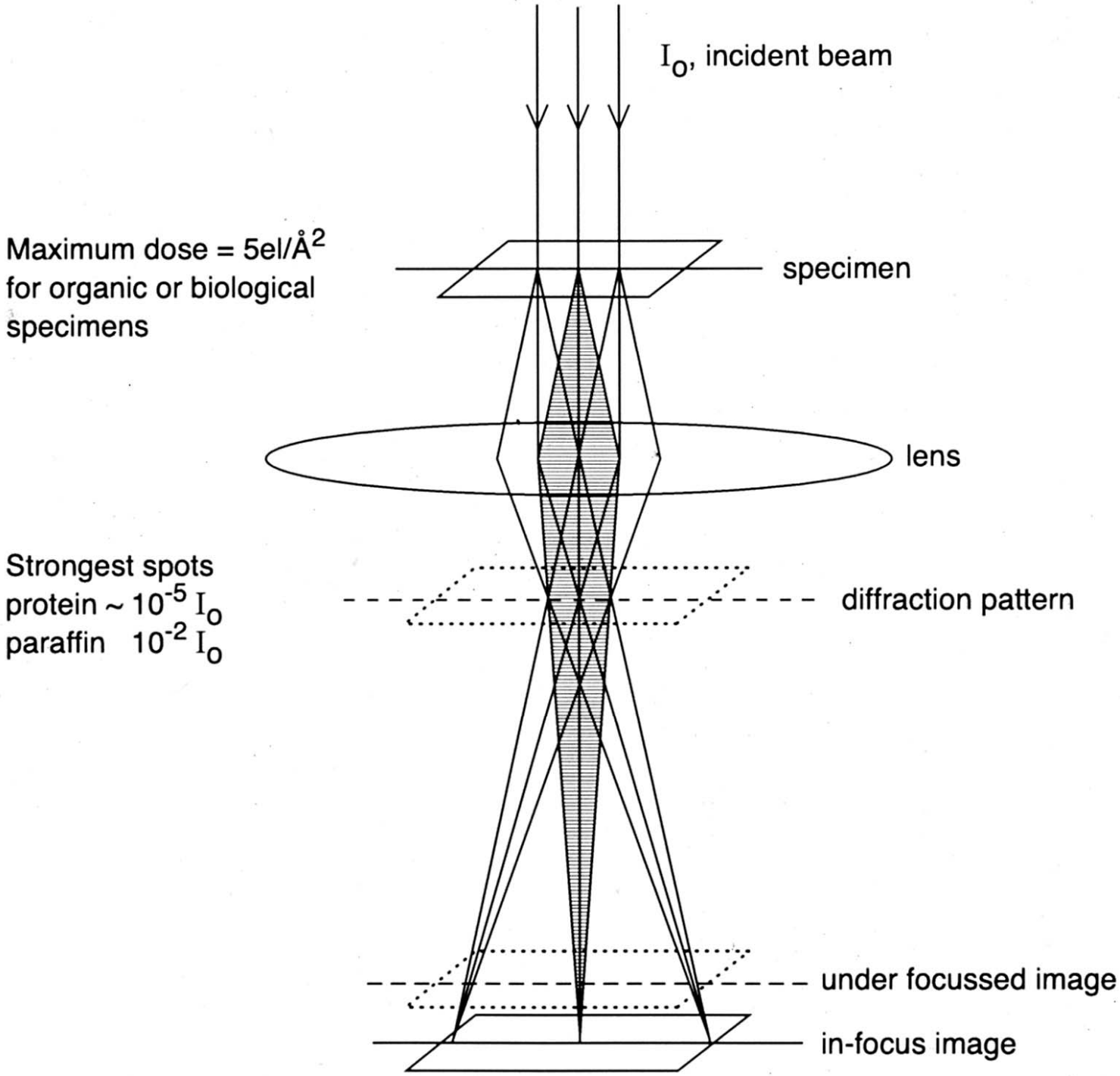
Optimistic

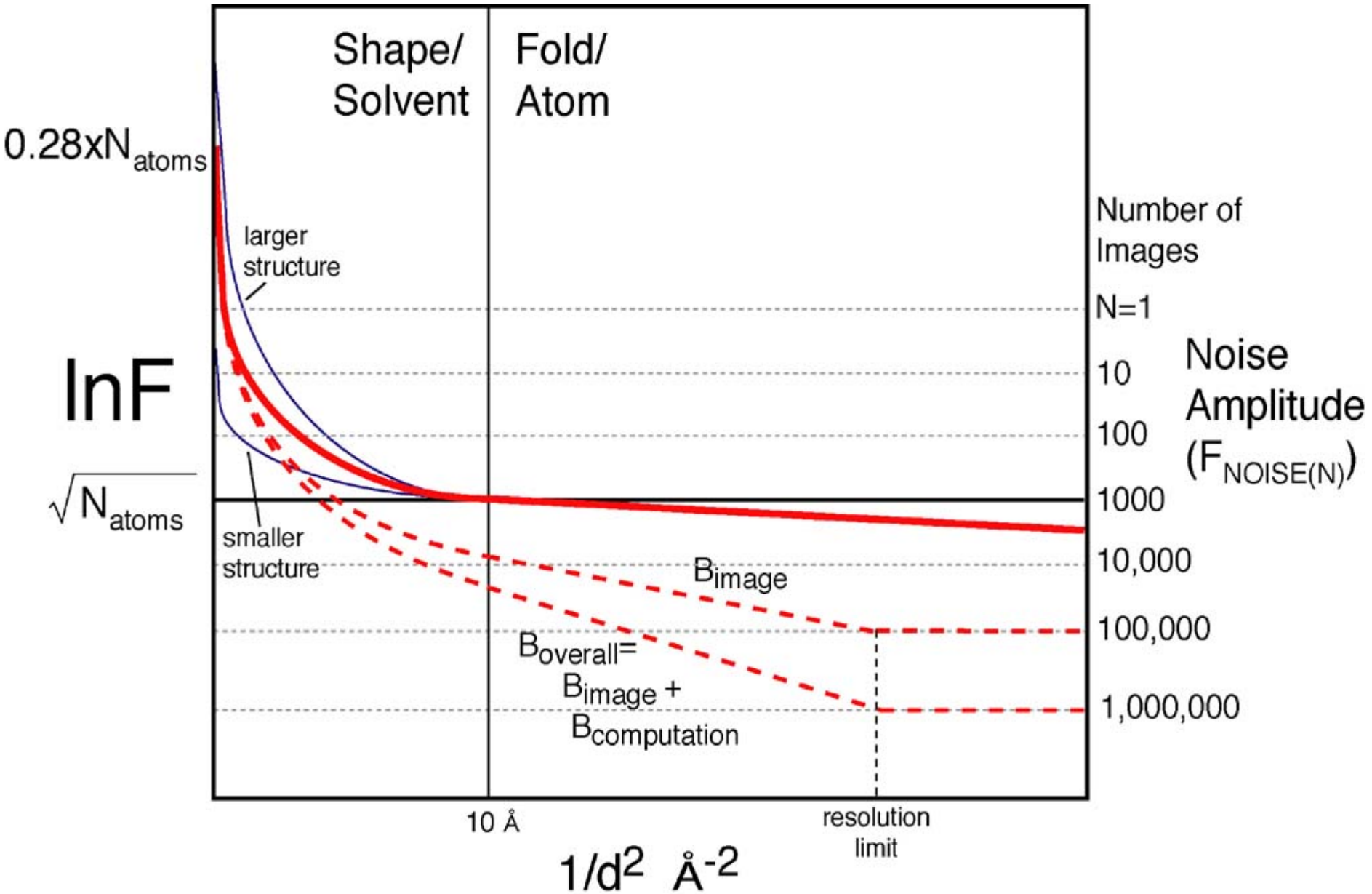
- Instrumental problems will all be solved
- Specimen preparation will become straightforward
- Beam-induced charging and movement will be minimised
- Structures will be obtained routinely at resolutions beyond 3Å
- Reliable indices will be developed which differentiate good structures from bad structures

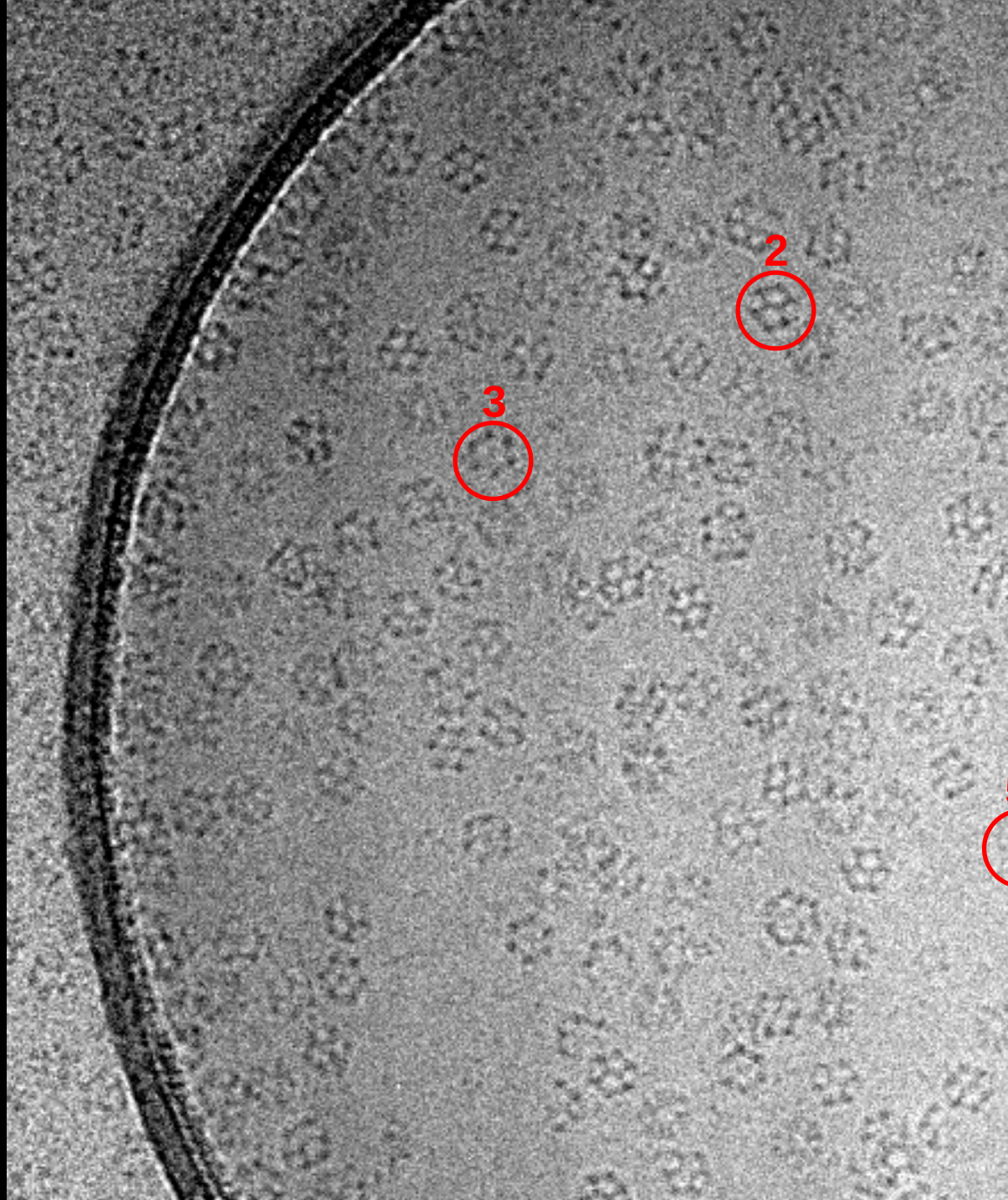
Pessimistic

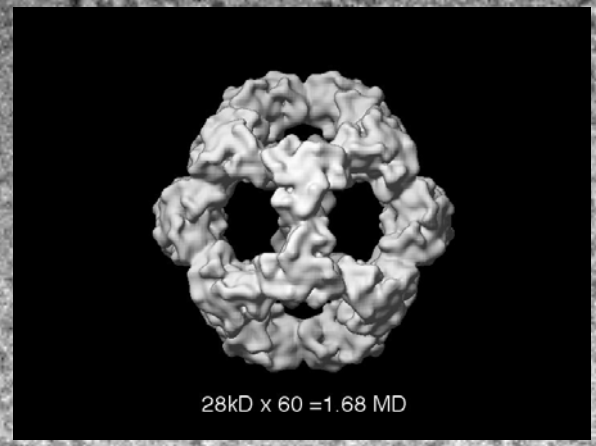
- Progress with equipment will be slow and increasingly expensive, requiring regional centres (c.f. synchrotrons for X-ray diffraction)
- Beam-induced charging and movement will be intractable
- A few atypical structures will be determined at high resolution but resolution will be routinely stuck at 6-10 Å
- There will be some high profile scandals with serious errors in published structures

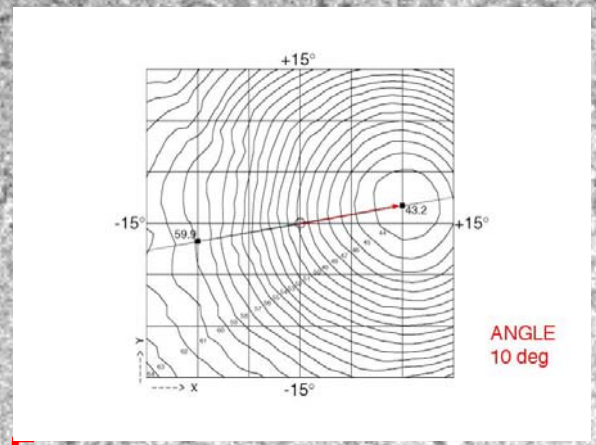
Overview of electron cryomicroscopy


I_o, incident beam
Maximum dose = 5el/Å^2
for organic or biological
specimens
specimen
lens
Strongest spots
protein ~ 10^{-5} I_o
paraffin 10^{-2} I_o
diffraction pattern
under focussed image
in-focus image

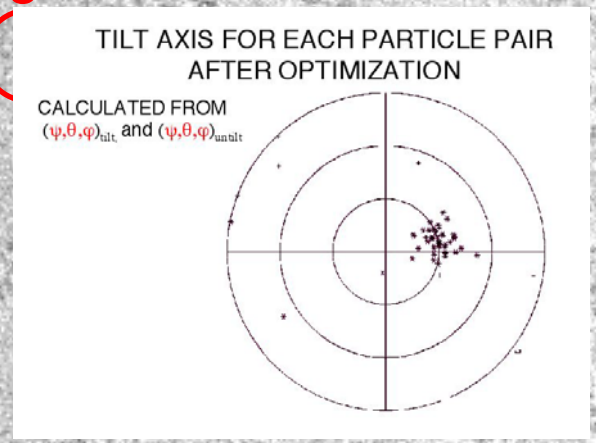

Shape/
Solvent
Fold/
Atom
0.28xN atoms
larger
structure
lnF
√N atoms
smaller
structure
Number of
Images
N=1
10
100
1000
10,000
100,000
1,000,000
Noise
Amplitude
(F NOISE(N))
B image
B overall=
B image +
B computation
10 Å
resolution
limit
1/d2 Å-2

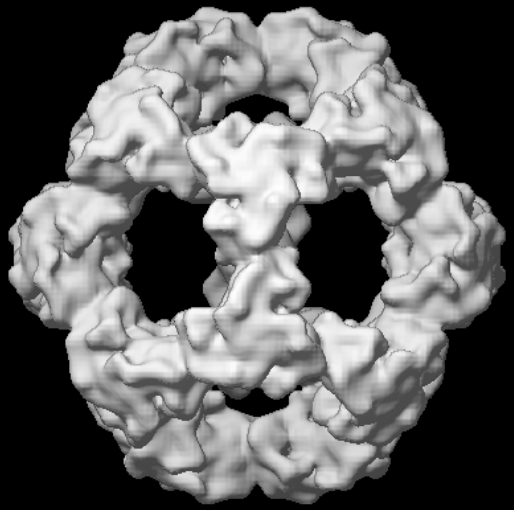

2
3

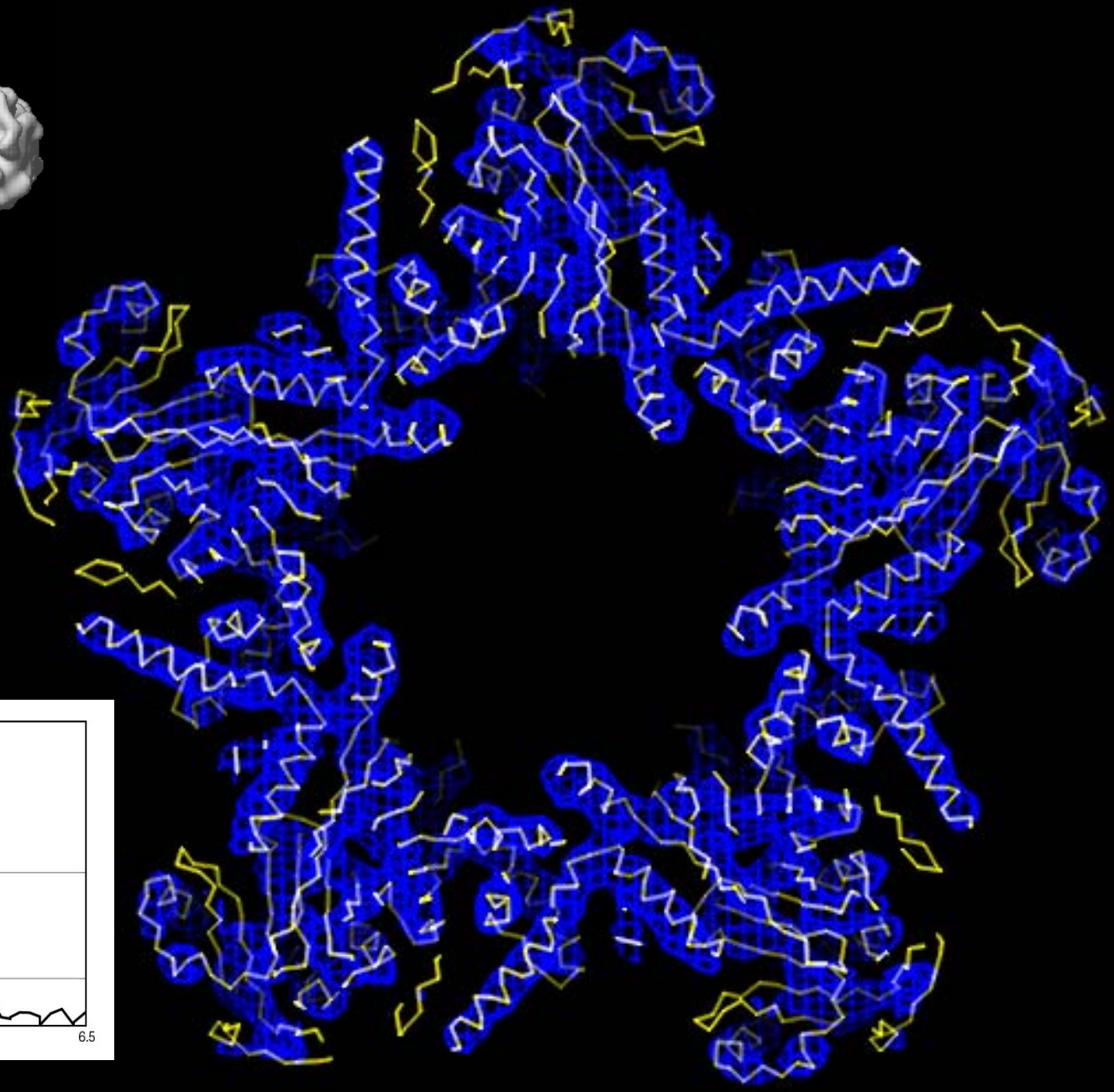

28kD x 60 =1.68 MD

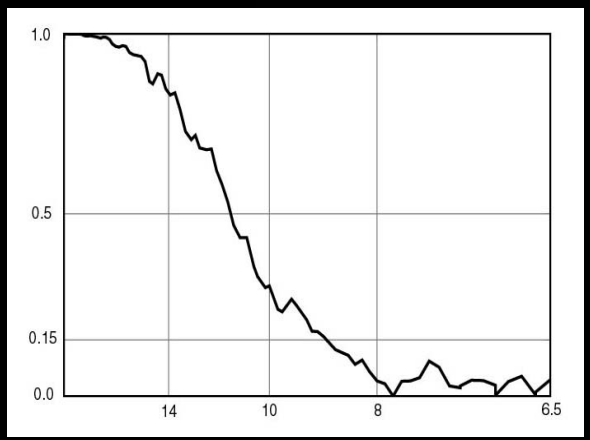

+15°
-15°
+15°
-15°
59.9
43.2
ANGLE
10 deg

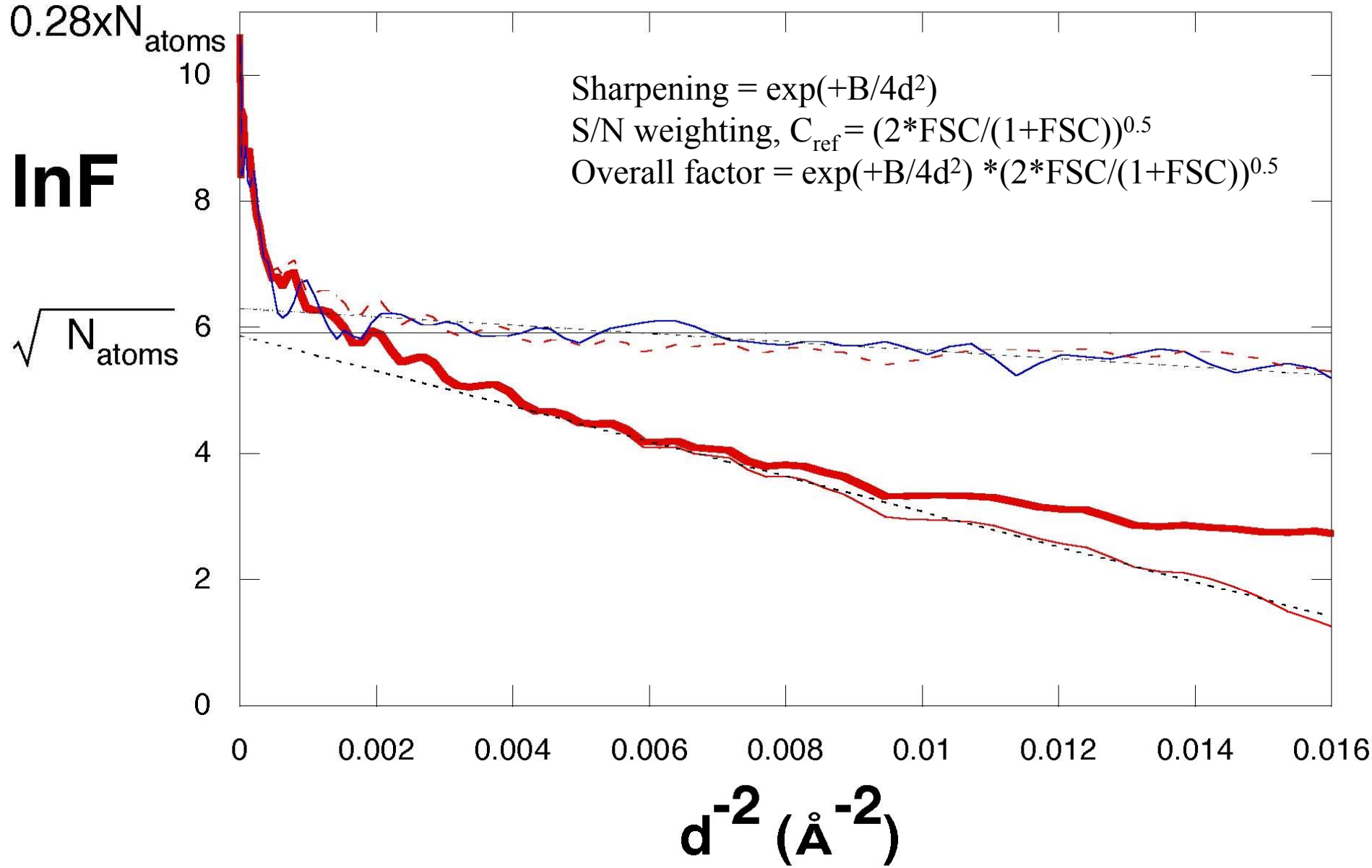
5


TILT AXIS FOR EACH PARTICLE PAIR
AFTER OPTIMIZATION
CALCULATED FROM
(ψ,θ,φ)tilt and (ψ,θ,φ)untilt






1.0
0.5
0.15
0.0
14
10
8
6.5


0.28xN atoms
lnF
√ N atoms
Sharpening = exp(+B/4d²)
S/N weighting, C ref = (2*FSC/(1+FSC))^0.5
Overall factor = exp(+B/4d²) *(2*FSC/(1+FSC))^0.5
10
8
6
4
2
0
0
0.002
0.004
0.006
0.008
0.01
0.012
0.014
0.016
d-2 (Å-2)

For two independent half sets of data

Cross-correlation = Ctest

$Ctest = \Sigma(S + N1)(S + N2) / \Sigma(S^2 + 2SN + N^2)$

$= S^2 / (S^2 + N^2)$

where S = signal and N = N1 = N2 = noise in half dataset

Comparing the full set of data to a perfect reference set

Cross-correlation = Cref

$Cref = S^2 / (\sqrt{S^2} \cdot \sqrt{(S^2 + N^2/2)})$

$= \sqrt{(S^2/(S^2 + N^2/2))} = (2 \cdot Ctest / (1 + Ctest))^{1/2}$

Therefore

When $S^2 = N^2$ Ctest = 0.500 and Cref = 0.816 = fom

When $6S^2 = N^2$ Ctest = 0.143 and Cref = 0.500 = fom

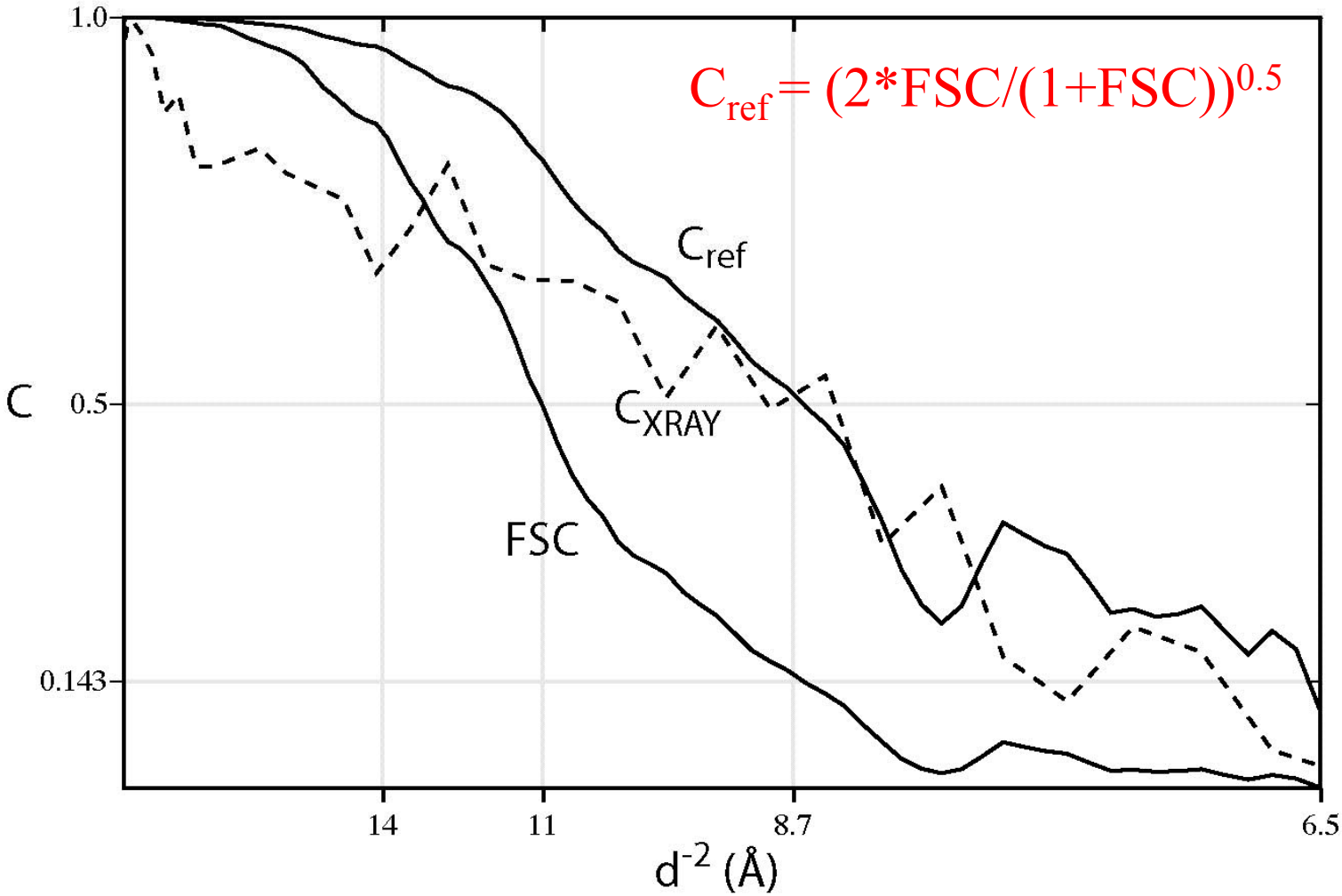
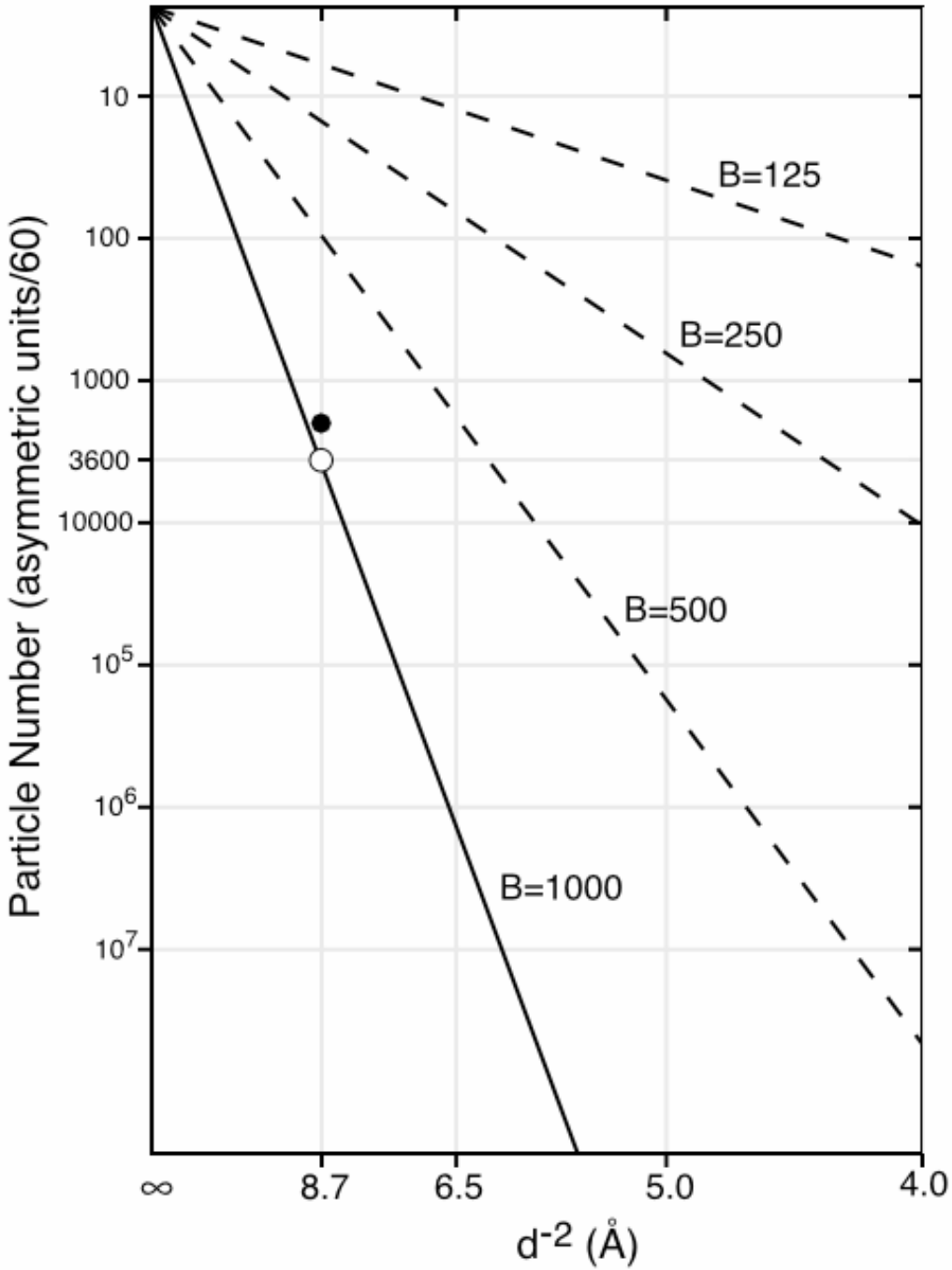

$C_{ref} = (2*FSC/(1+FSC))^{0.5}$
1.0
0.5
0.143
C
C_{ref}
C_{XRAY}
FSC
14
11
8.7
6.5
d^{-2} (Å)

TABLE 2

Type of molecule	Approx. M.W. (Daltons)	D (Å)	N_c, number of carbon atom equivalents	N_s, number of unique diffraction spots to resolution of d = 3Å in projection	f, fraction of electrons elastically scattered out to 3Å resolution	$\frac{\langle I_{OBS} \rangle}{Io}$	$\frac{\langle F_{OBS} \rangle}{Fo}$	Phase contrast = total image fractional contrast = signal	Fractional noise level in pixel of dimension $\left(\frac{d}{2}\right)^2$ = 1.5Å x 1.5Å	Can single molecule be detected? How many times > noise	Multiple of sigma expected within unit cell at random	Multiple of sigma expected within entire volume of 5 parameter space at random	Can single molecule alignment be carried out in practice?	Minimum number of images needed for structure with average Fourier component to be >3 σ in projection	Total number of images in 3D x $[\frac{\pi R}{d}]$ De Rosier & Klug (1967)
large virus	300M	900	25,000,000	141,371	0.0520	0.184×10^{-6}	0.429×10^{-3}	0.322	0.30	644	5.2	8.5	yes	13	12600
small virus	11M	300	936,000	15,707	0.0173	0.552×10^{-6}	0.743×10^{-3}	0.186	0.30	124	4.8	7.7	yes	40	12600
ribosome	3.3M	200	277,000	6,981	0.0115	0.827×10^{-6}	0.910×10^{-3}	0.152	0.30	68	4.7	7.5	yes	60	12600
	1.4M	150	117,000	3,926	0.0087	1.103×10^{-6}	1.050×10^{-3}	0.132	0.30	44	4.6	7.3	yes	80	12600
multimeric enzyme	420K	100	35,000	1,745	0.0058	1.654×10^{-6}	1.286×10^{-3}	0.107	0.30	24	4.4	7.1	possibly	120	12600
	180K	75	14,600	981	0.0043	2.206×10^{-6}	1.485×10^{-3}	0.093	0.30	16	4.2	6.8	possibly	160	12600
	52K	50	4,330	436	0.0029	3.309×10^{-6}	1.819×10^{-3}	0.076	0.30	8.4	4.1	6.7	possibly	240	12600
small protein	18K	35	1,500	213	0.0020	4.727×10^{-6}	2.174×10^{-3}	0.064	0.30	4.9	3.9	6.3	no	345	12600
very small protein	7K	25	540	109	0.00144	6.618×10^{-6}	2.572×10^{-3}	0.054	0.30	3.0	3.5	5.9	no	480	12600
equation	(1)	-	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(13)	(16)	(17)	(10)	(11)
relation to D	$0.418 \times D^3$	D	$0.0346 \times D^3$	$0.01745 \times D^2$	$5.7 \times 10^{-5} \times D$	$1.654 \times 10^{-4} \times D^{-1}$	$0.0128 \times D^{-\frac{1}{2}}$	$0.0107 \times D^{\frac{1}{2}}$	-	$0.02388 \times D^{\frac{1}{2}}$				$12087 \times D^{-1}$	-
dependence on resolution d	-	-	-	$\alpha \frac{1}{d^2}$	-	-	-	$\alpha \frac{1}{d}$	$\alpha \frac{1}{d}$	$\alpha \frac{1}{d}$				-	$\frac{38,000}{d}$

Parameters in electron microscopy of single protein molecules or molecular assemblies. To simplify the presentation, it is assumed that the molecules are arranged in a closely-packed 2-dimensional crystal with a square unit cell as shown in Fig. 3. The formulae used to derive Table 2 are given in the Appendix.

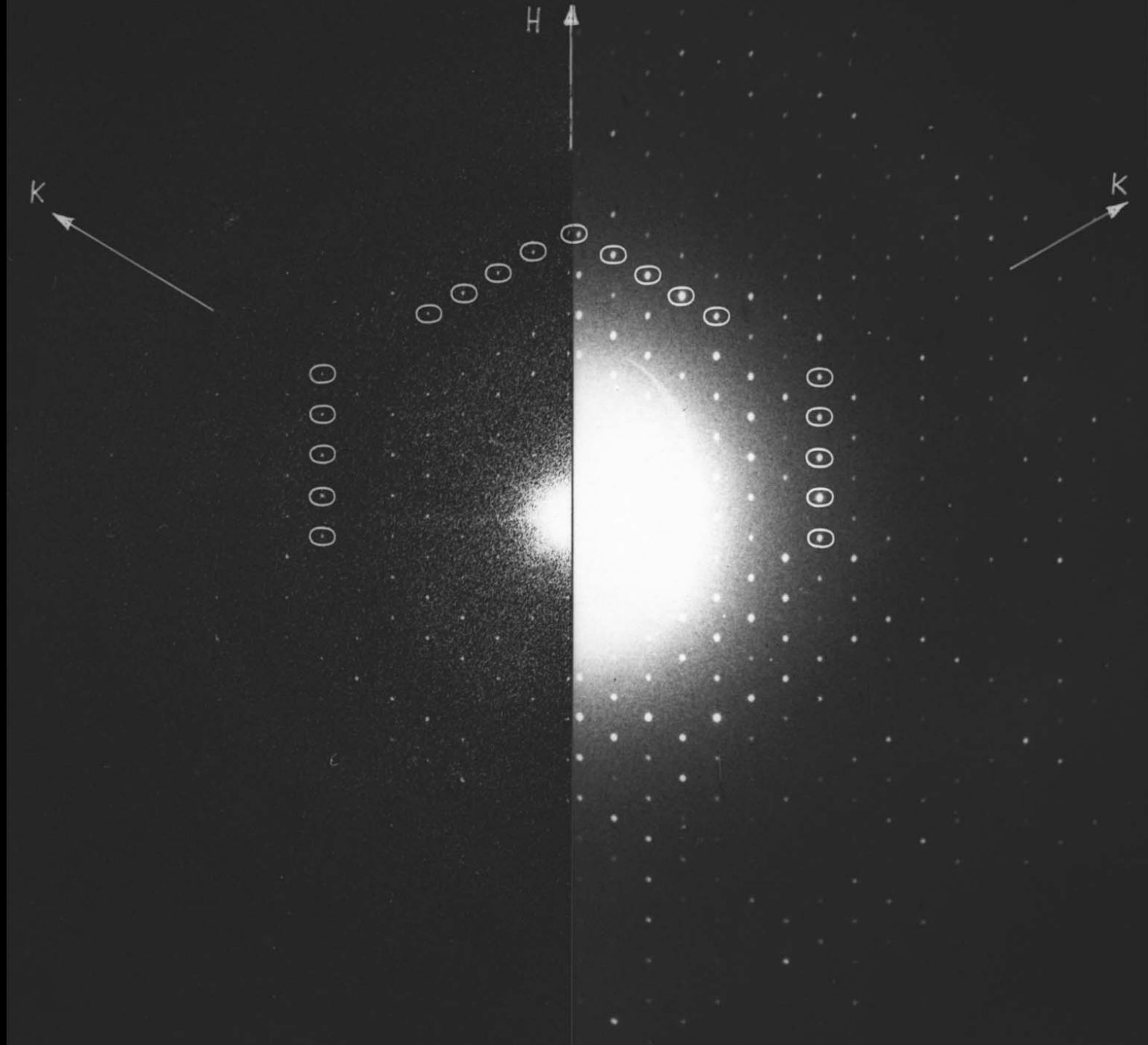

Particle Number (asymmetric units/60)
10
100
1000
3600
10000
10^5
10^6
10^7
B=125
B=250
B=500
B=1000
∞
8.7
6.5
5.0
4.0
d^{-2} (Å)

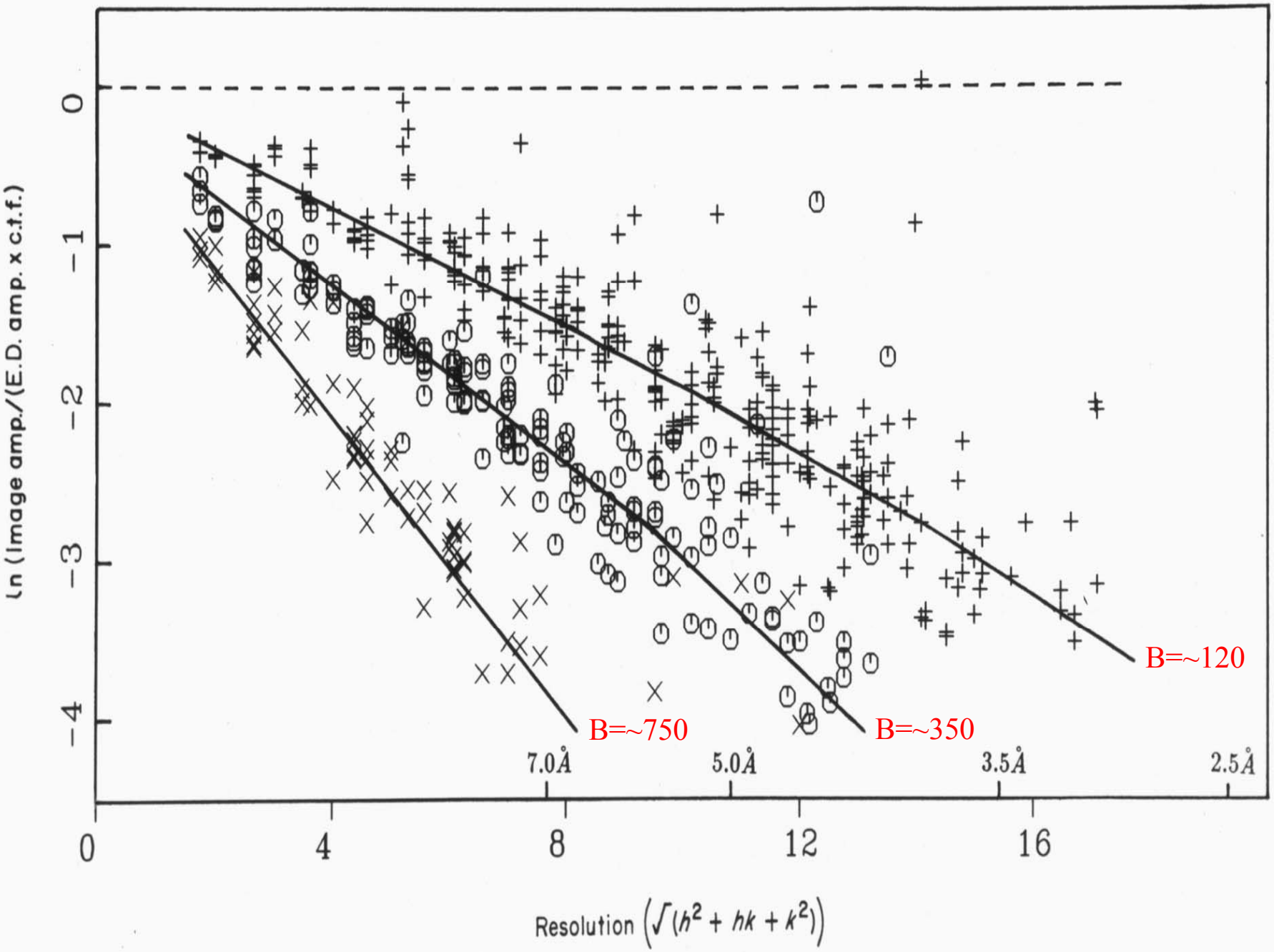
Specimen types - 2D crystals

+ can average many unit cells on good crystals
+ lattice defines position and orientation accurately

- resolution only as good as crystal order (c.f. X-ray)
- lattice can block structural changes or induce disorder

Potential for better images which would allow more powerful processing (treat as single molecules, with individual (restricted) position and orientation), hence overcome limitations.


H
K
K


ln (Image amp./(E.D. amp. x c.t.f.))
0
−1
−2
−3
−4
0
4
8
12
16
7.0Å
5.0Å
3.5Å
2.5Å
Resolution $\left(\sqrt{(h^2 + hk + k^2)}\right)$
B=~120
B=~350
B=~750

DOC Purple Membrane

Table 2. Average signal-to-background ratio of all spot intensities in the resolution range 3.0 Å to 4.0 Å, obtained for one image using different reference areas to find the position of unit cells

Area in pixel	Unit cells	% Fourier averaging	Reflections found with IQ⩽7	Signal/Background
300 × 300	73	99	79	10/7
200 × 200	33	98	88	25/7
130 × 130	14	91	107	41/7
70 × 70	4	80	100	37/7
34 × 34	1	0	70	4/7
Unprocessed image			40	0/7

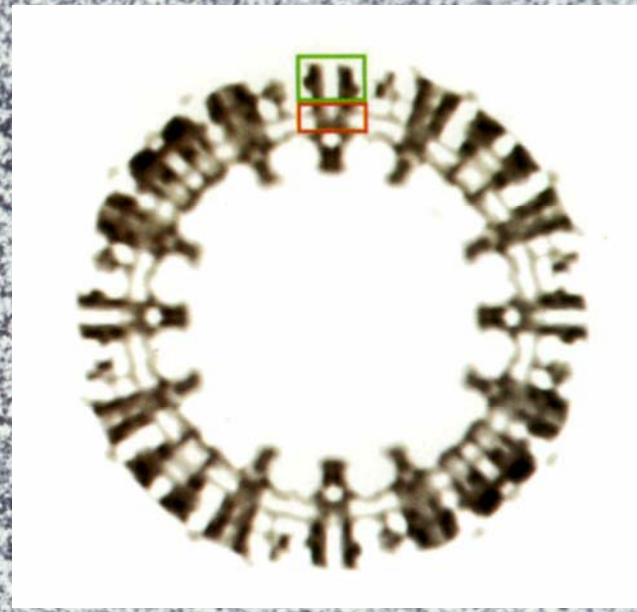
In each case, Fourier averaging was applied to the image before unbending except in the last two rows. The value (in %) in the third column refers to the area masked out by the Fourier mask applied to the image transform. The signal-to-background ratio was calculated after subtraction of the background from each integrated spot intensity. The total number of reflections in the chosen range was 222. The signal refers to the average intensity above background at the position expected for the diffraction peaks from the crystal. The background is the average intensity in the immediately surrounding area; it is normalized to 7. The standard deviation of the background is the background fluctuation after the intensity of all reflections has been averaged. It was determined to be 1.0 for the single molecule cross-correlation in the second last row. Thus, a peak of 4 is above a background of 7 by 4 × the standard deviation. The signal-to-background ratio for the unprocessed image was included for comparison in the last row.

Specimen types - helical arrays - 1.5D crystals

+ single picture provides many views, no missing cone
+ filaments often more strongly bonded than 2D crystals

- often the helix bends and twists a lot (small diameter helices)
- large diameter tubular arrays may be deformed (squashed)

Potential to correct for substantial distortions if images were more perfect. Potential to image thicker specimens with Cc corrector.



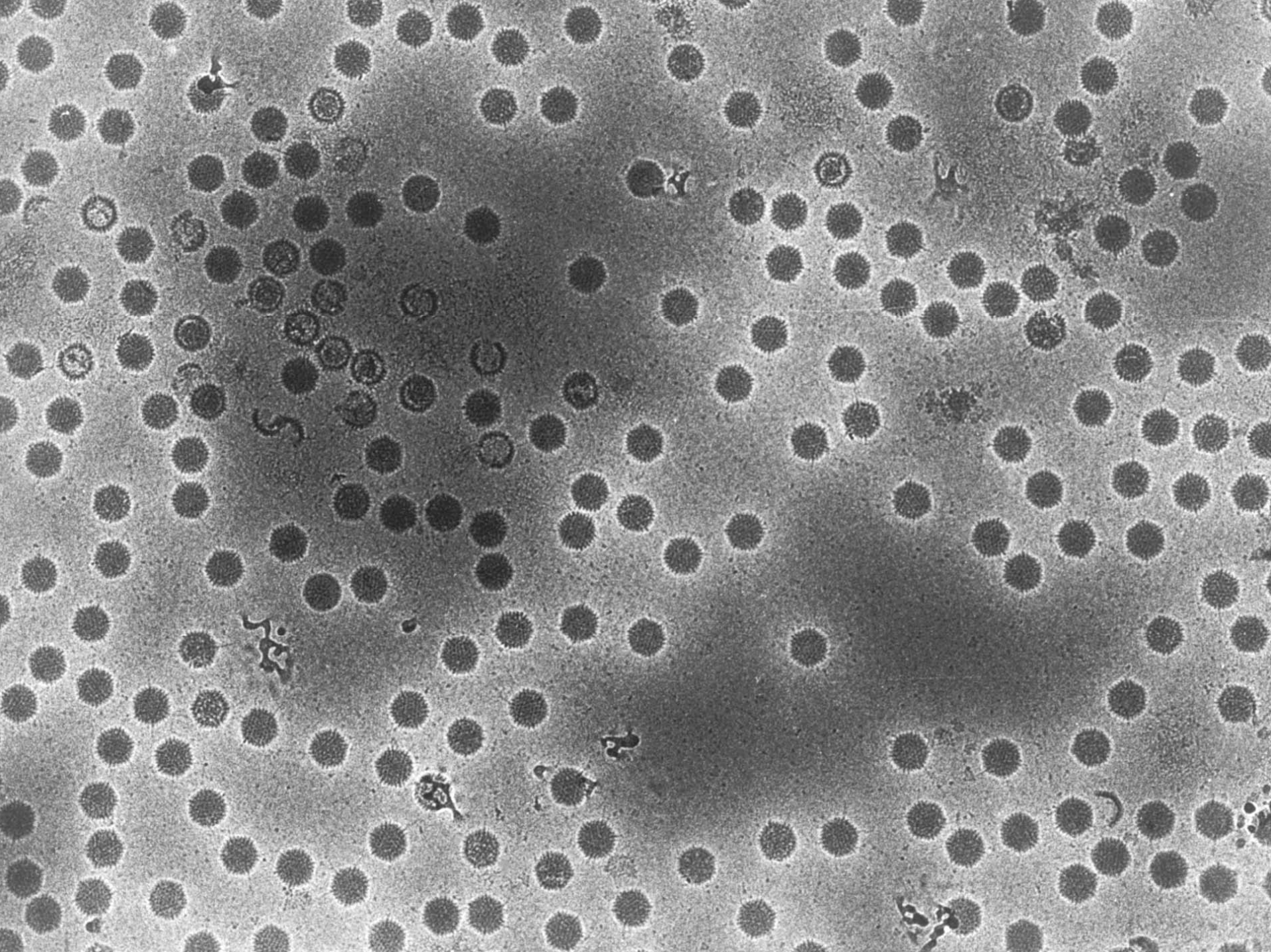
Nigel Unwin

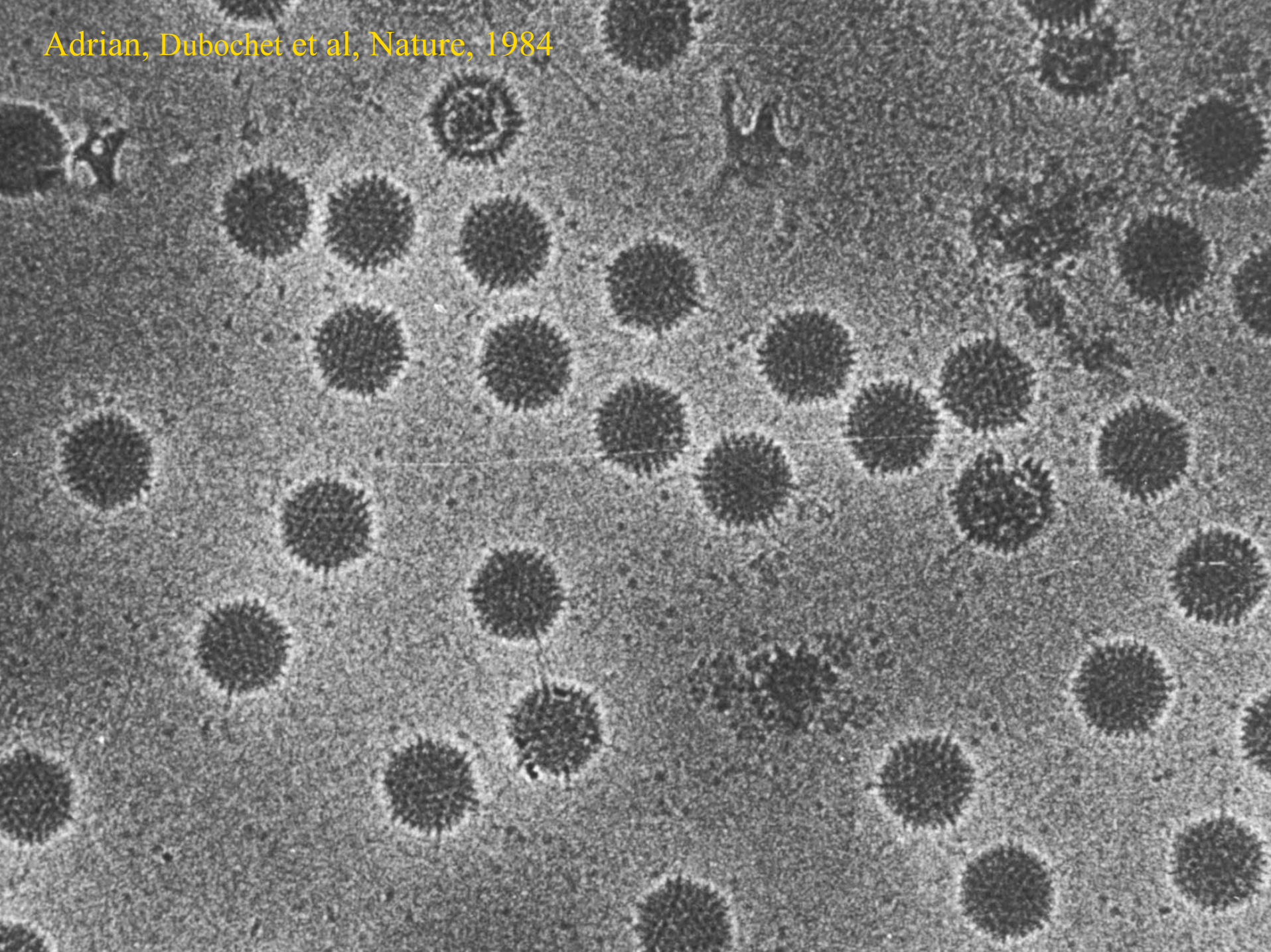
Specimen types - single particles – 0D crystal

+ no crystallisation required
+ extensive purification not required (purify in the computer)
+ most generally applicable specimen preparation method

- lack of neighbours may allow increased flexibility & disorder
- lack of intermolecular contact may allow more beam-induced image blurring

No reason in principle why it should not be possible to reach 3-4 Ångstrom resolution using single particle approaches. Better images will allow finer classification.




Adrian, Dubochet et al, Nature, 1984

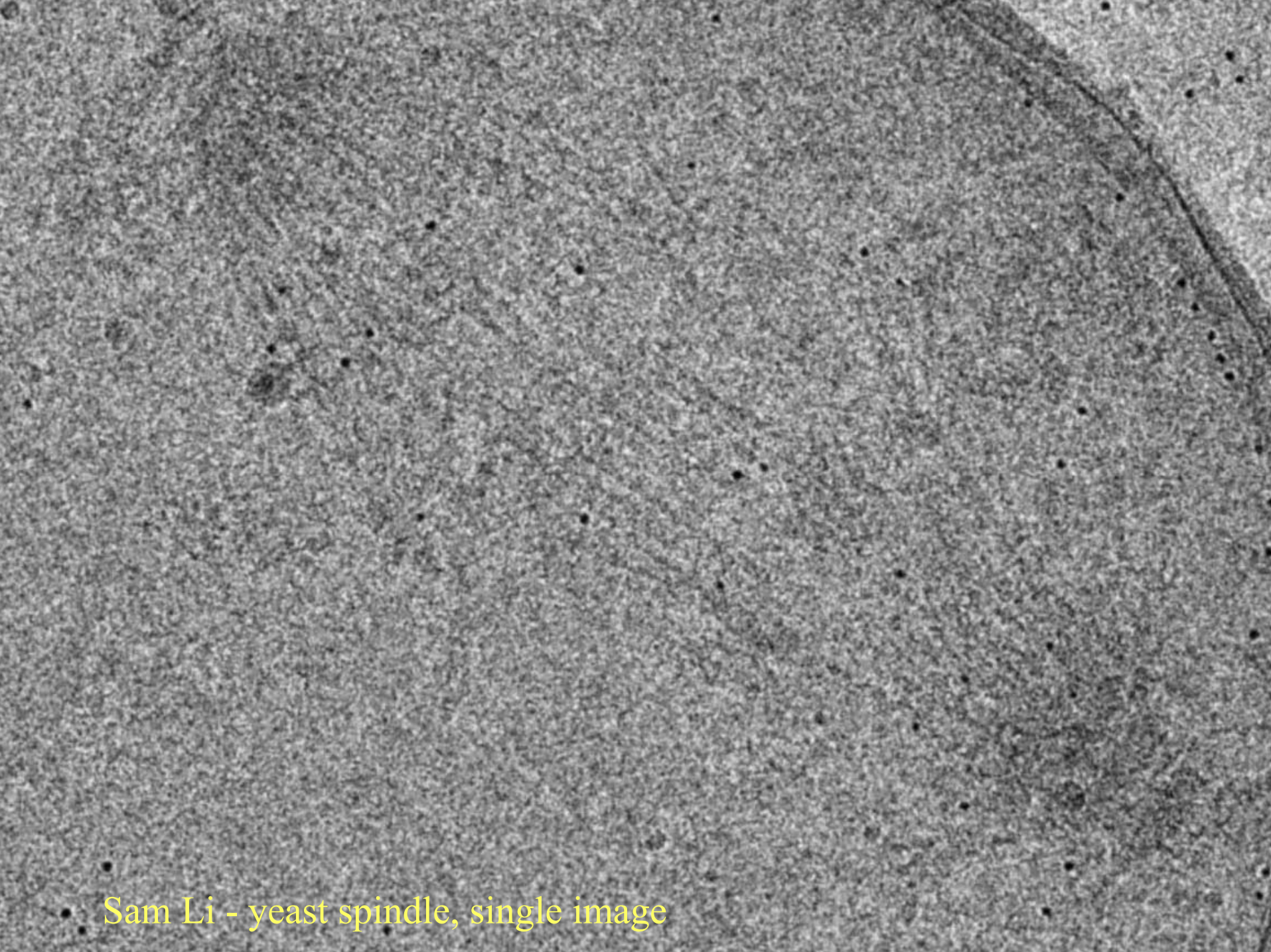
Specimen types - tomography of sections or spreads

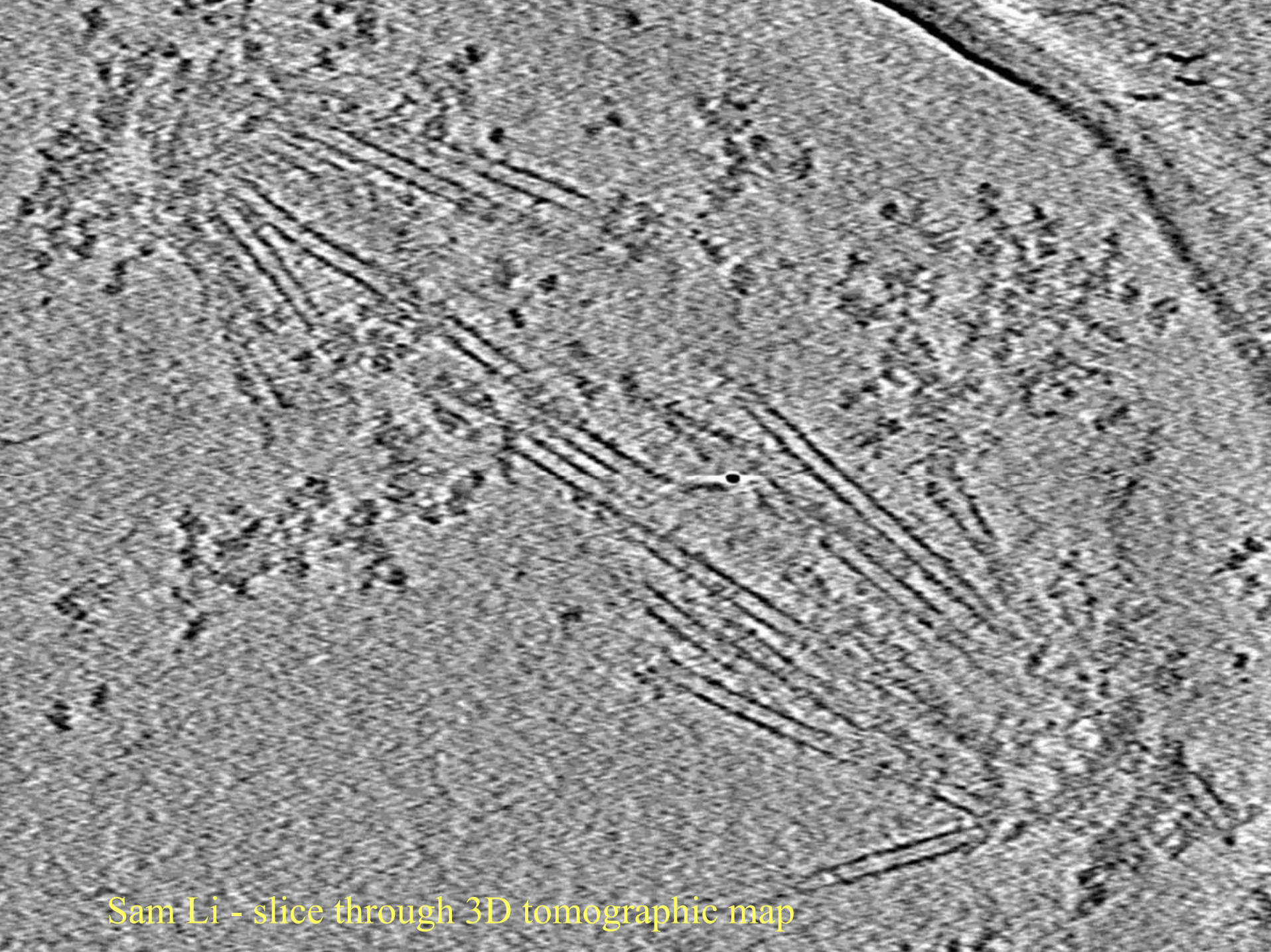
+ simultaneous alignment of entire field
+ colloidal gold can improve alignment
+ no need for homogeneity or symmetry, very general

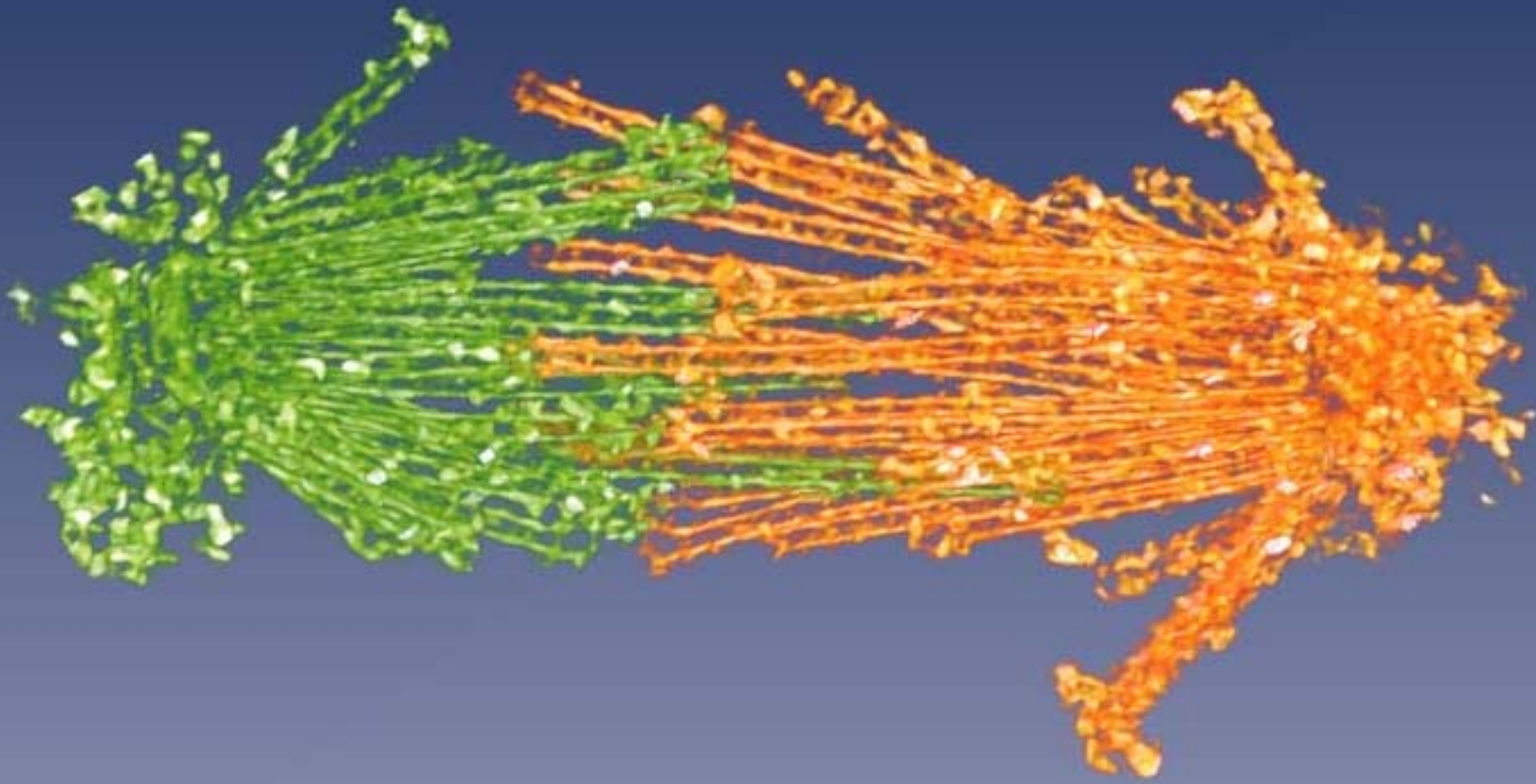
- structure may change during acquisition of tilt views
- without later real-space averaging, resolution always < 1/20 $Å^{-1}$

Critical to make use of every electron and get maximum MTF/DQE. In principle, all cryoEM could be done by tomography.

For example, crystallographic, helical or single particle analyses can be carried out with subsequent real-space averaging of the tomographic 3D density – What is optimal strategy? – collection of 1, 2, 3, or 50 tilt views of a field of homogeneous particles?


Sam Li - yeast spindle, single image


Sam Li - slice through 3D tomographic map

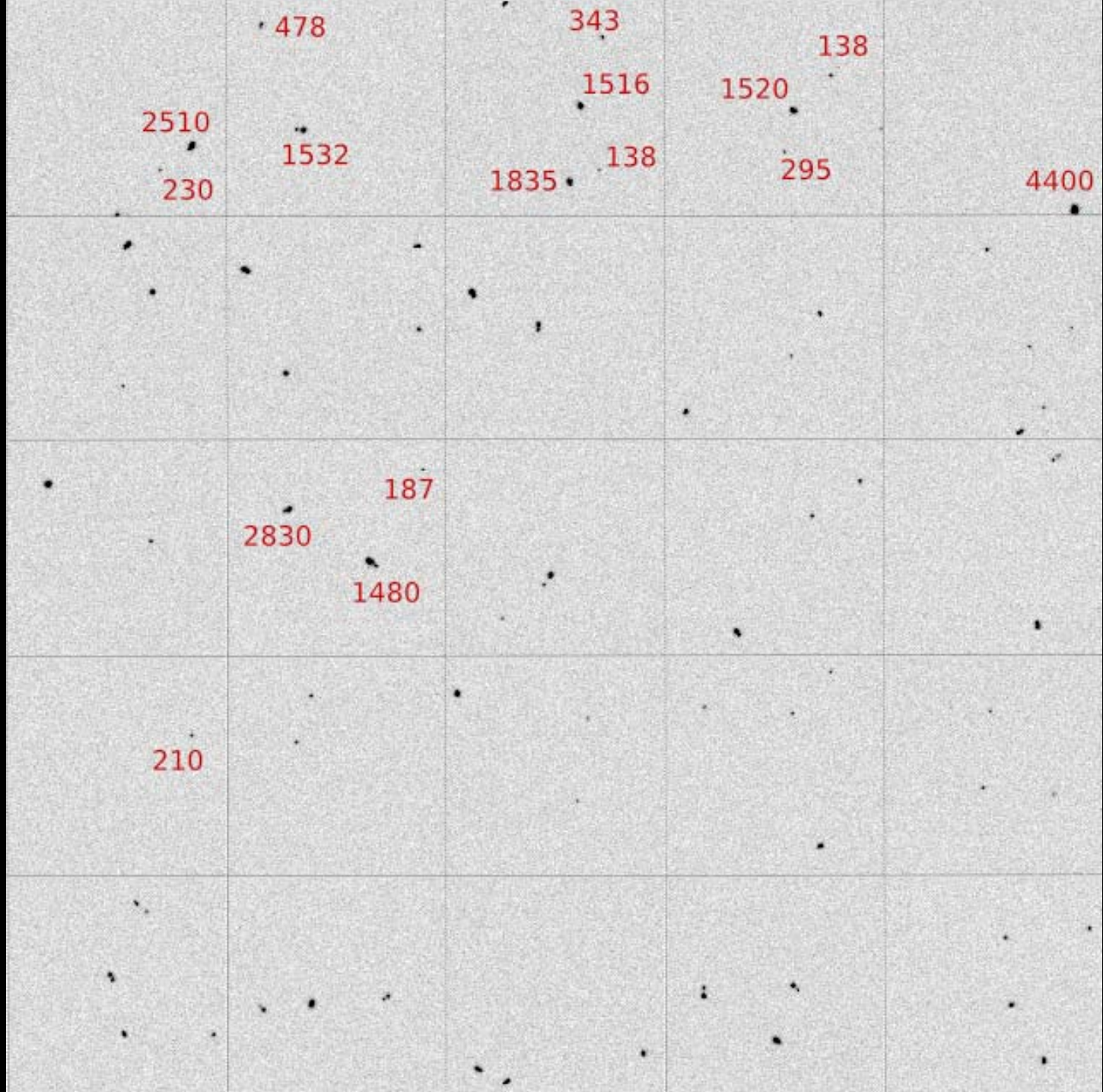


Sam Li

- **Better microscopes (Cc corrected, efficient phase plates)**
  Cc = 0 especially useful in tomography
  phase plate most useful for single particle alignment

- **Better detectors (What do we want)**
  high DQE(0) and DQE(Ny)
  large field of view
  low noise (< 0.1 el/pix)

- **Understand why the images we get are so bad**
  ice contamination, charging, beam-induced movement


478
343
138
1516
1520
2510
1532
138
295
230
1835
4400
187
2830
1480
210