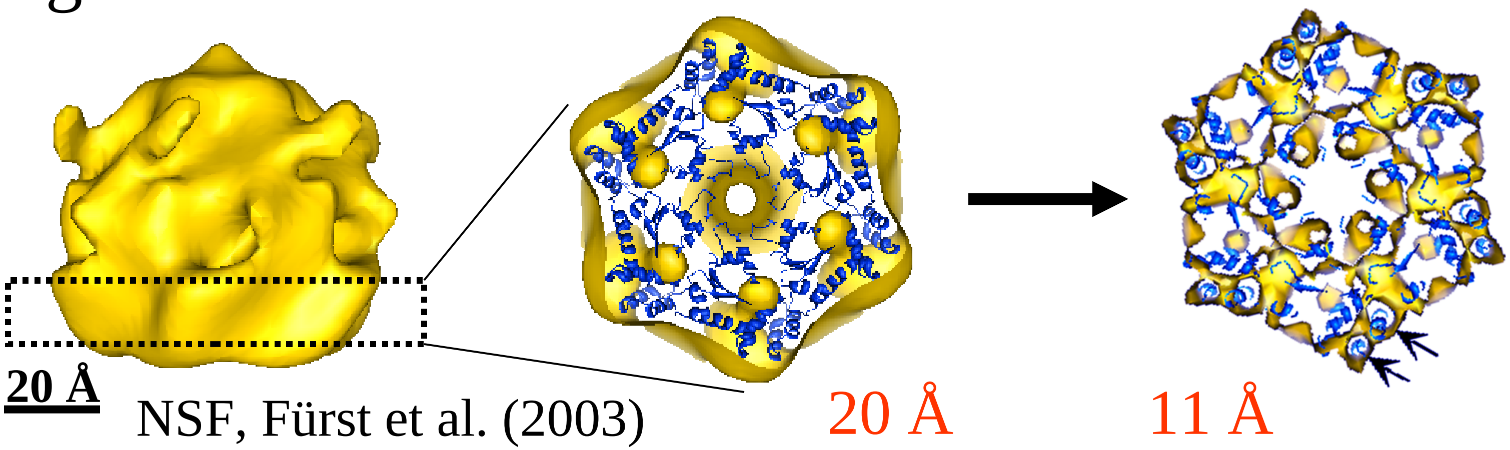
Refinement Strategies for Single Particle Structure Determination

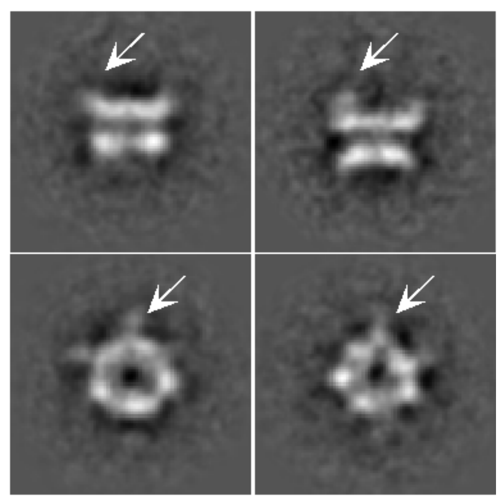
N. Grigorieff

Goals

- Higher resolution



- Sorting of structural heterogeneity



The Prophecy

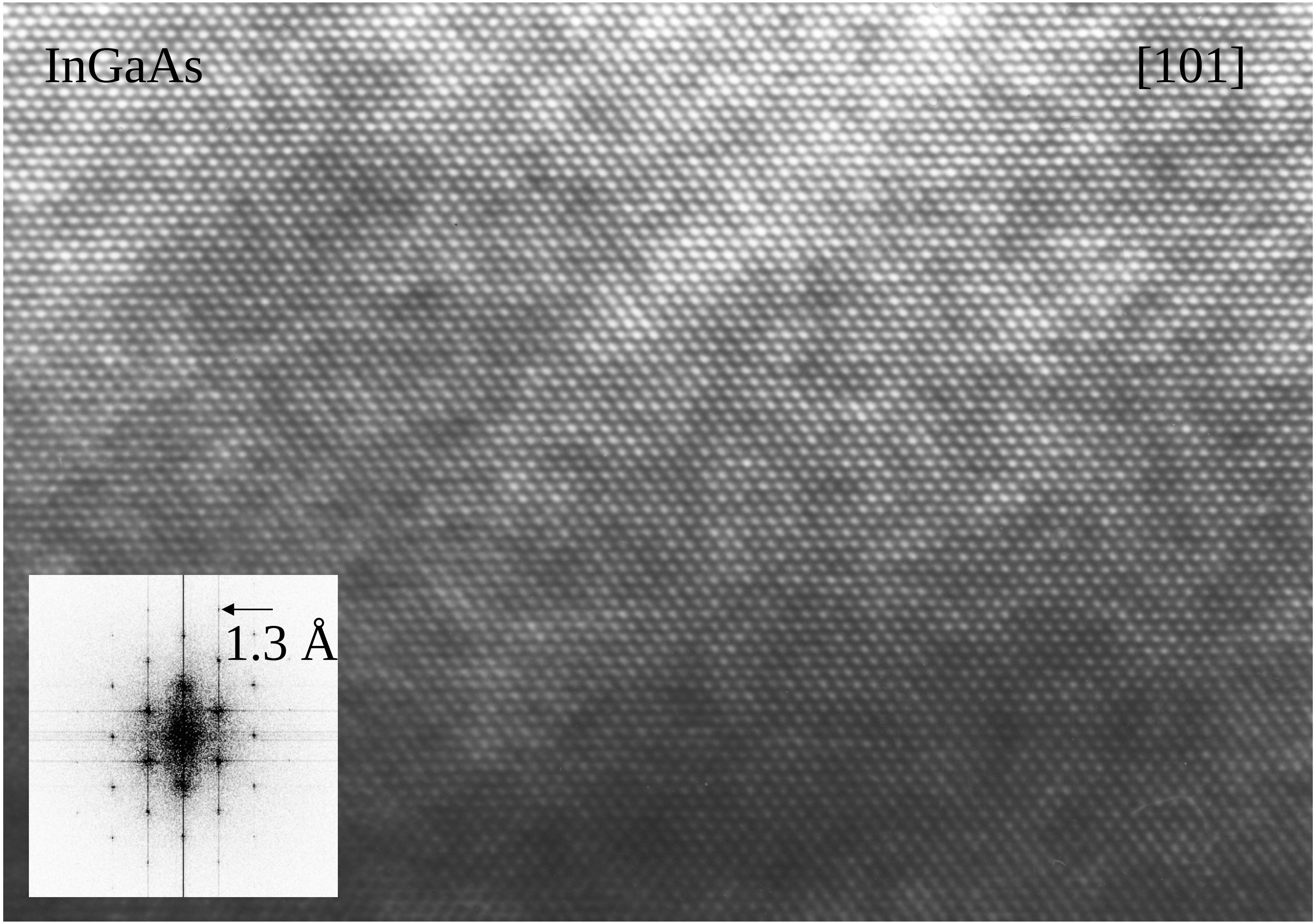
King Richard hath decreed… (QRB, 1995)

- Use 5 e^- per $Å^2$
- Demand a signal-to-noise ratio of 9 or better
- Aim for 3 Å resolution

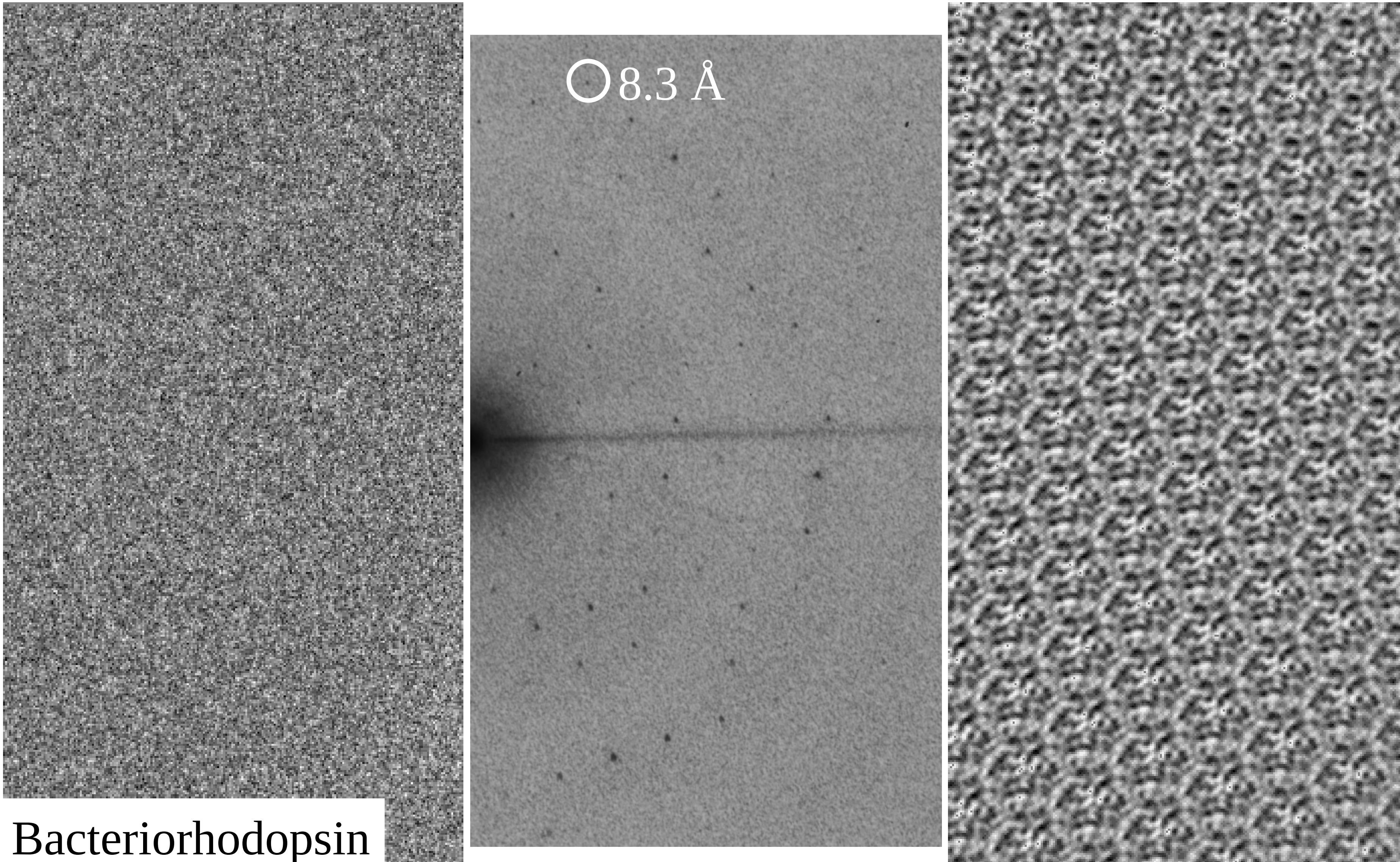
② Thou shall need to image 13,000 molecules

② For 6 Å, thou shall need only 7,000 images

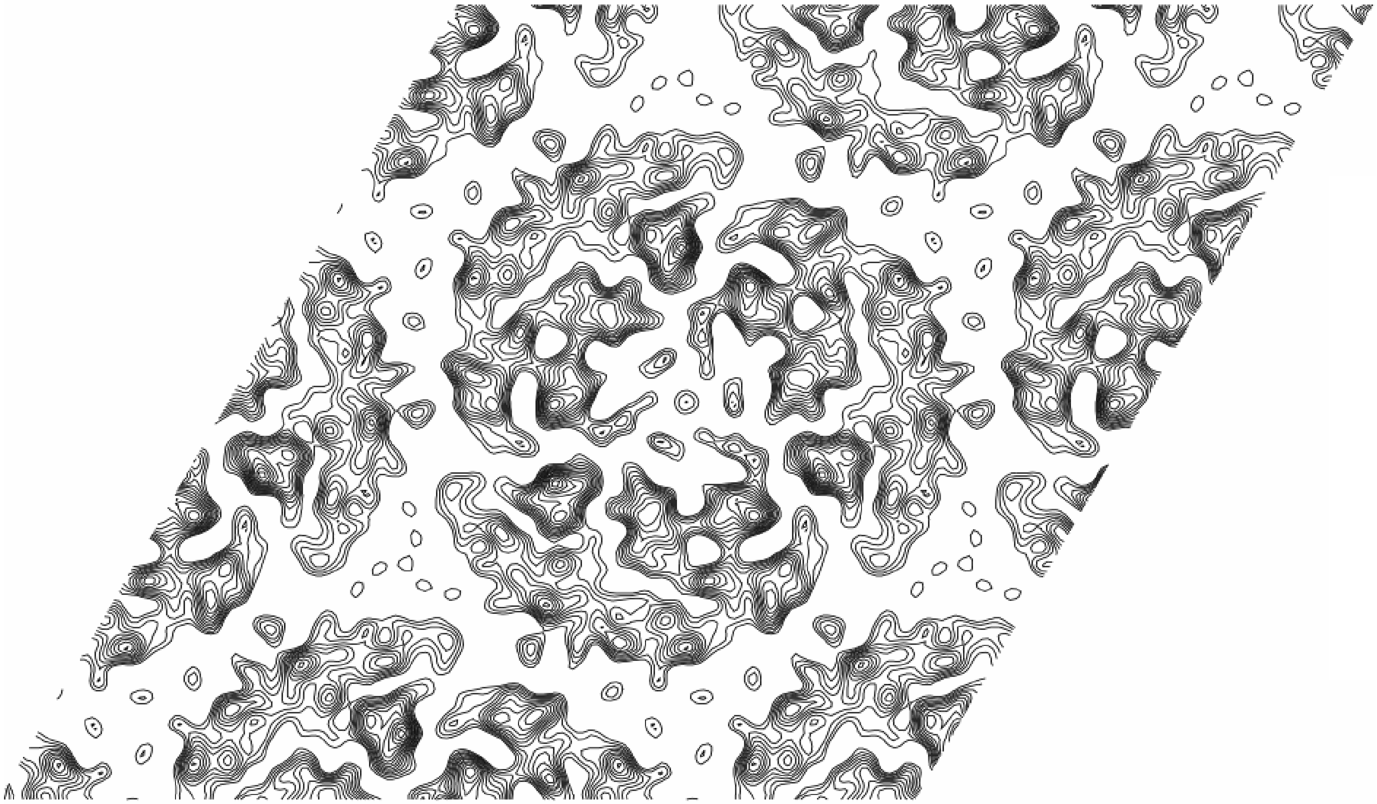
Resolving Power



Protein Crystals

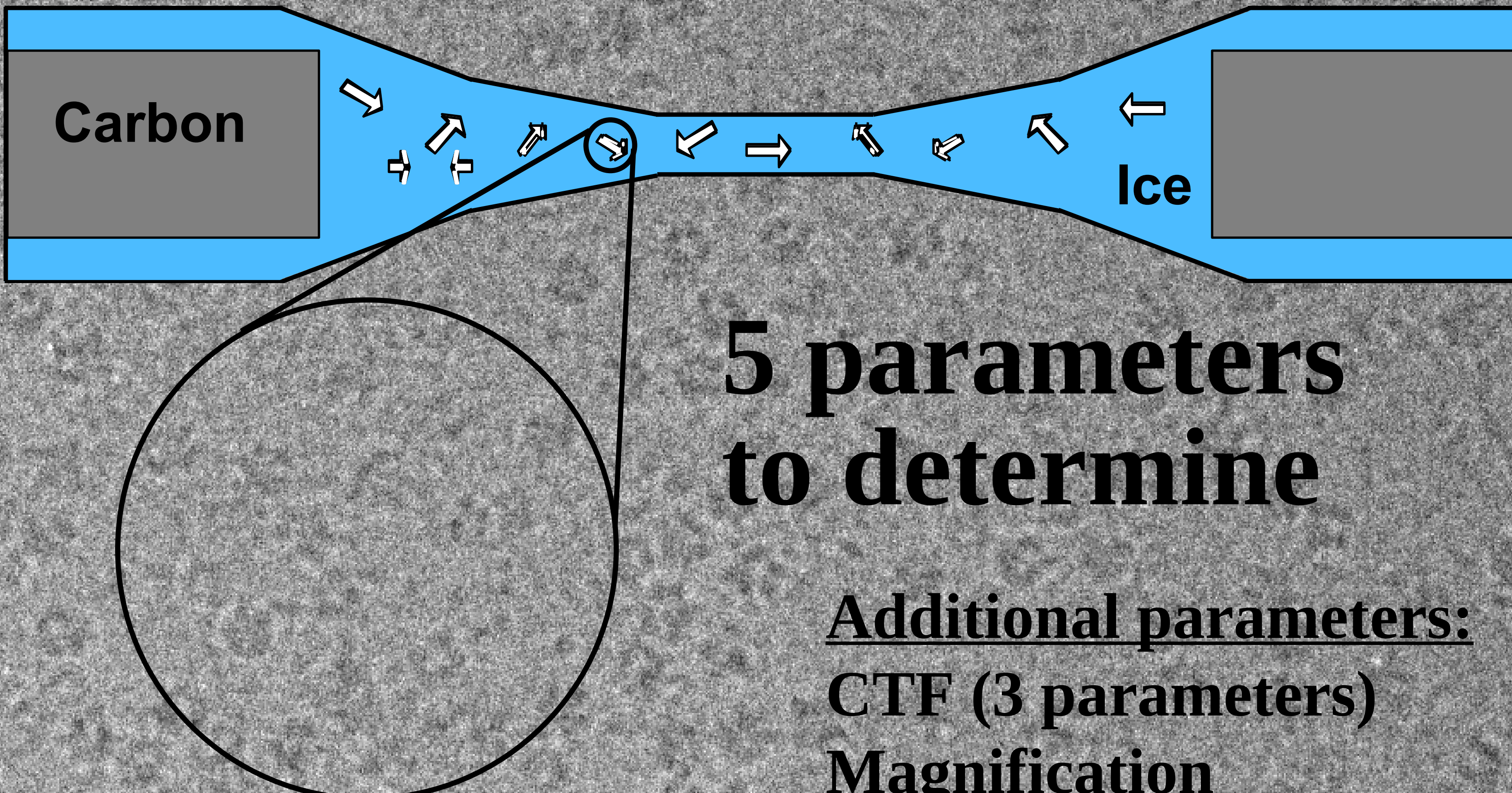


Purple Membrane



2.6 Å resolution

The Puzzle



5 parameters to determine

<u>Additional parameters:</u>

CTF (3 parameters)

Magnification

Beam Tilt (2 parameters)

200Å

A Crazy Idea

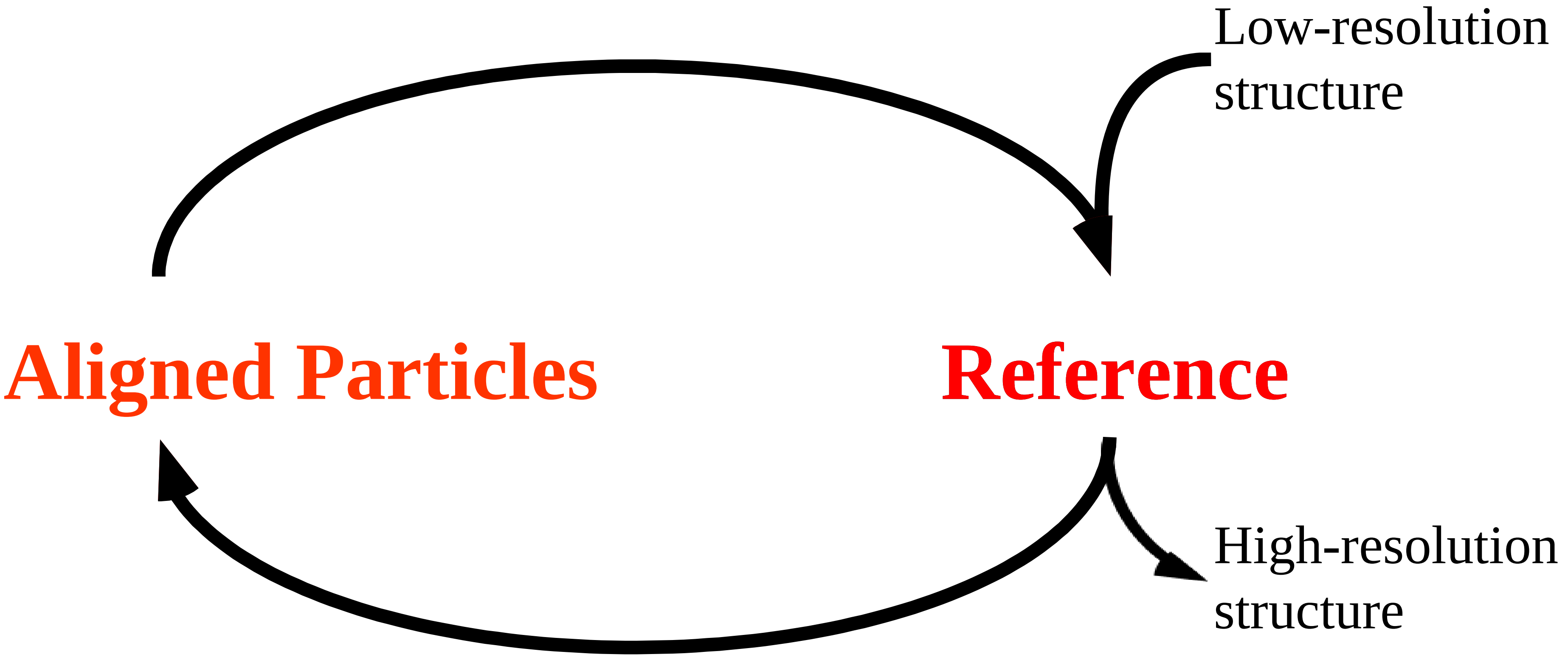
- Assume reliable resolution measure
- Search entire parameter space for highest resolution
- Given enough images, atomic resolution is reached
- Example:

  3 angles, 1 deg step; two coordinates, 1 pixel step:

  $360 \times 360 \times 360 \times 100 \times 100 = 5 \times 10^{11}$

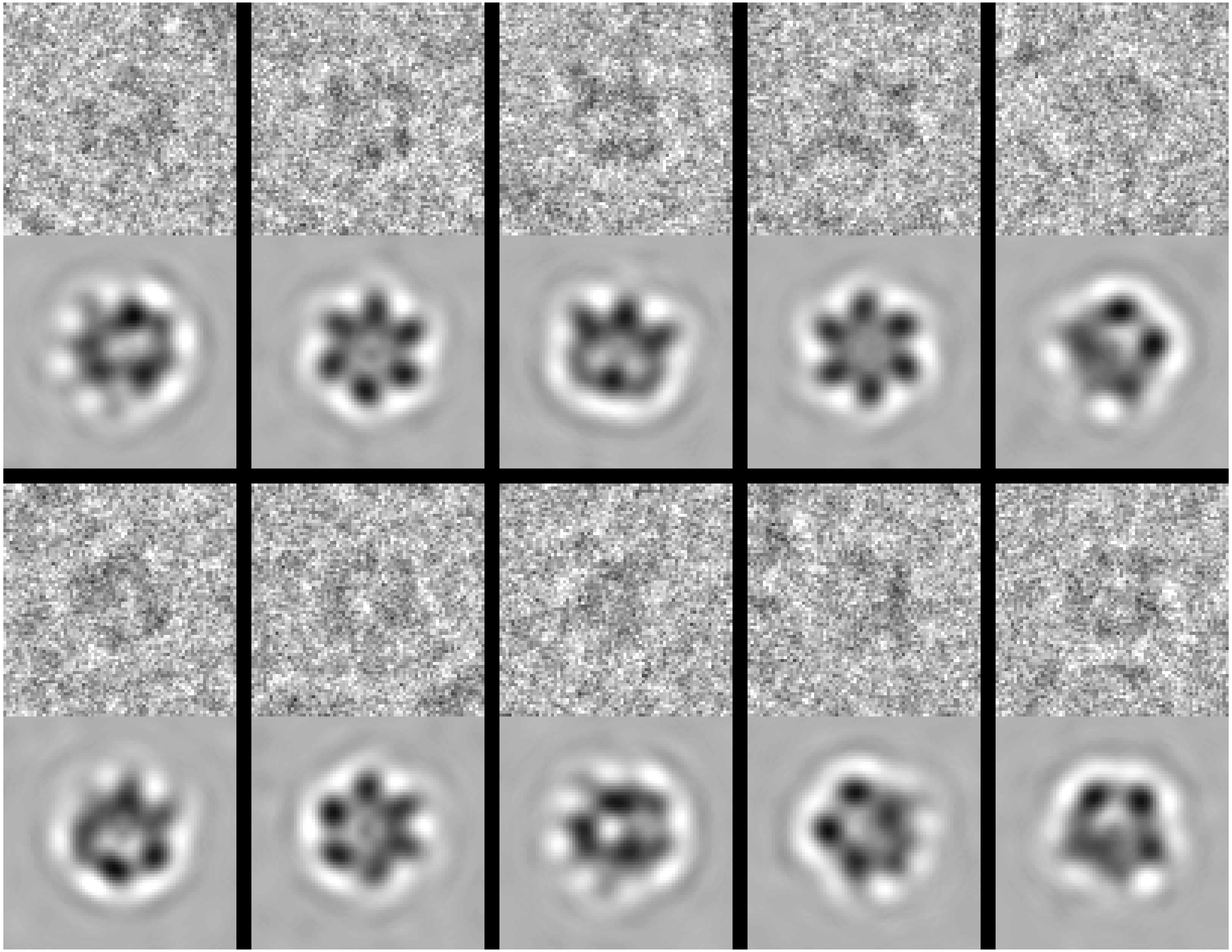
  13000 particles: $(5 \times 10^{11})^{13000}$ structures to search
- This is a big number!

Refinement



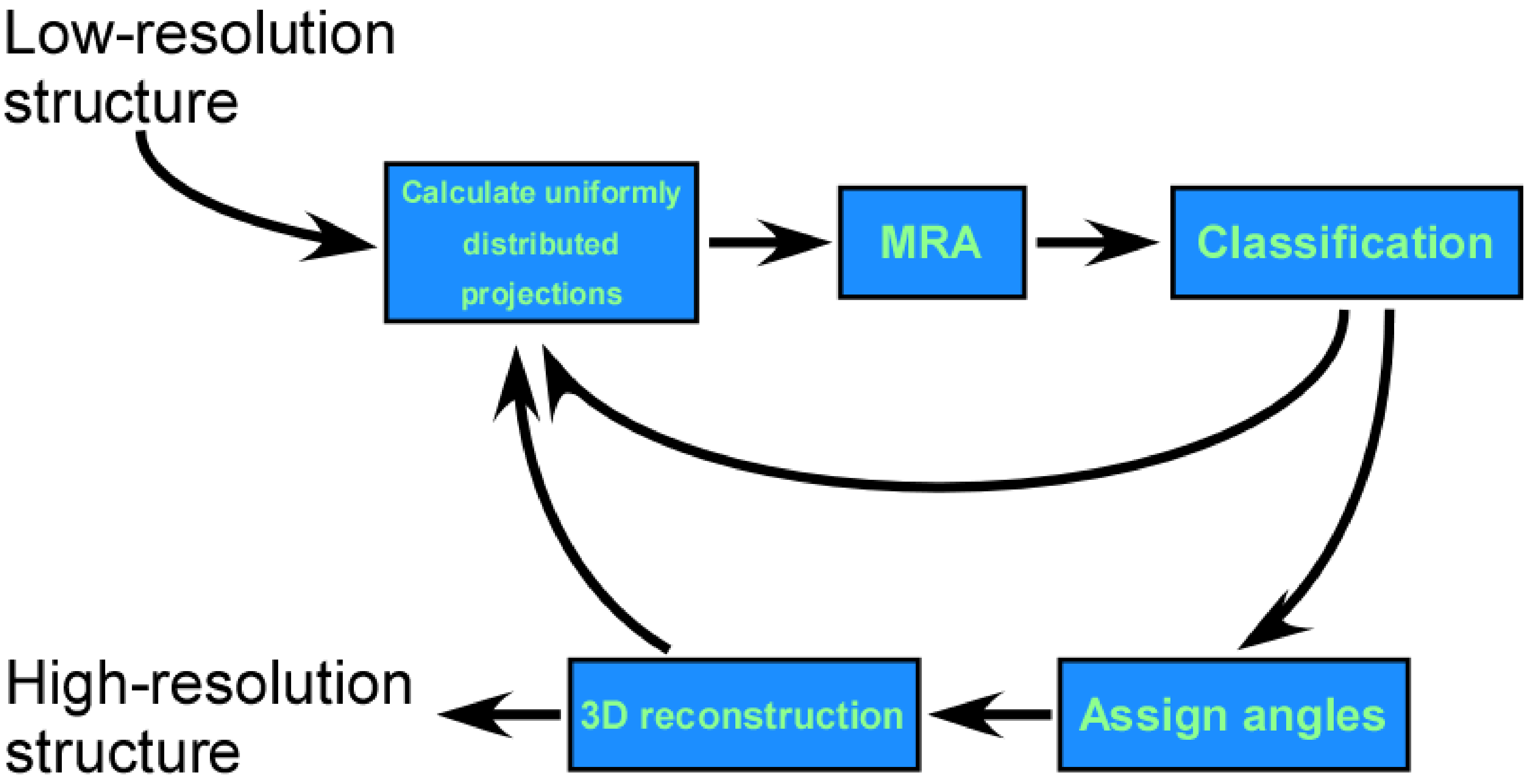
(Expectation maximization)

Strategy 1: Projection Matching



Strategy 2: Alignment in Reciprocal Space

Strategy 3: MRA and Classification



Strategy 4: Maximum Likelihood

Structure for *n*+1 iteration

$$A^{(n+1)} = \frac{1}{N}\sum_{i=1}^{N}\frac{\int X_i(\phi)\,p_i\left(\phi,\Theta^{(n)}\right)\mathrm{d}\phi}{\int p_i\left(\phi,\Theta^{(n)}\right)\mathrm{d}\phi}$$

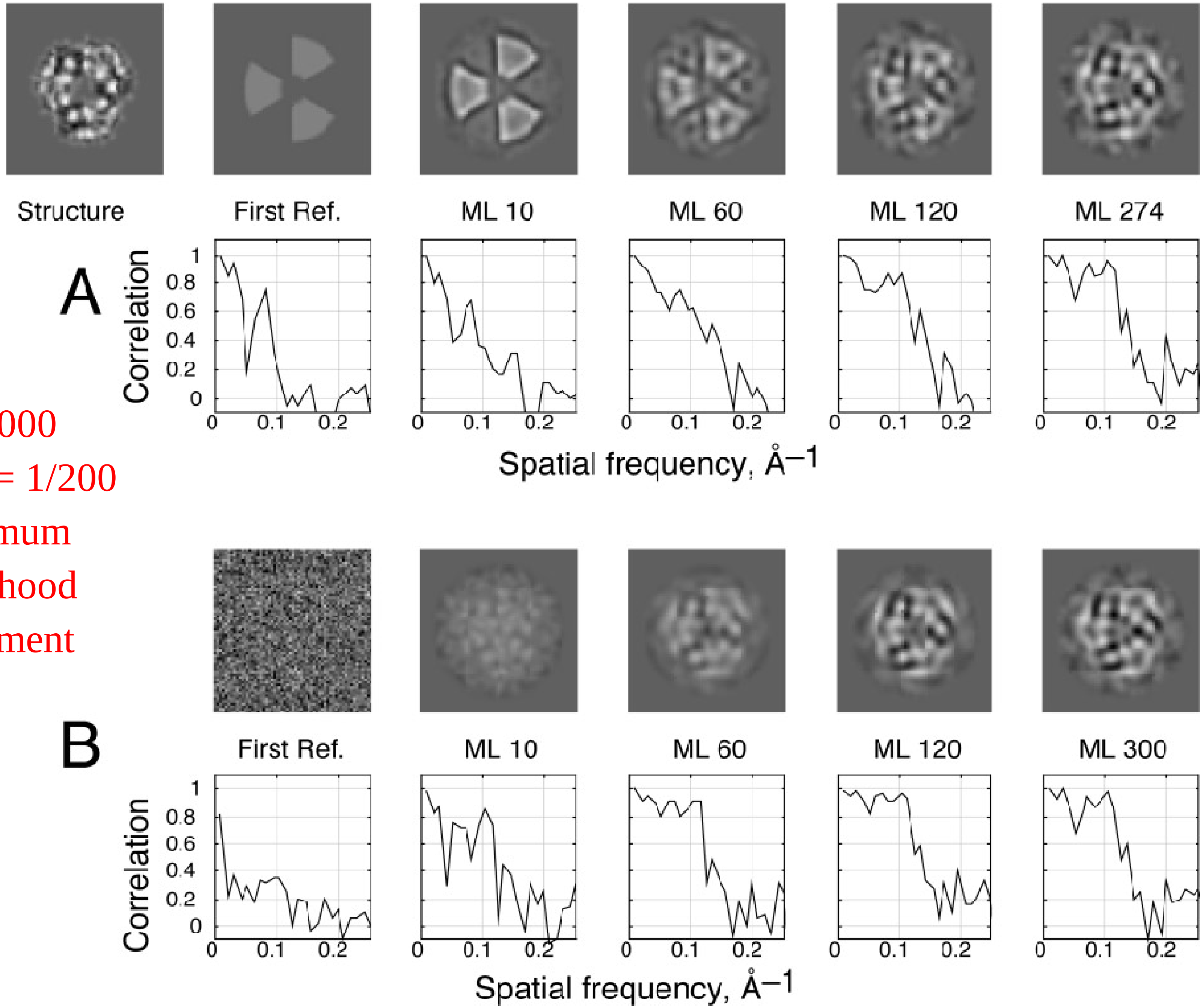
Probability function

$$p_i(\phi,\Theta)=\left(\frac{1}{\sqrt{2\pi}\sigma}\right)^{M}\exp\left[-\frac{\left|X_i(\phi)-A\right|^2}{2\sigma^2}\right]f(\phi\,|\,\Theta)$$

X_i: ***i*th image** | N: **# of images** | ϕ: **alignment parameters**

Θ: **model parameters** | σ: **noise in images** | f : **positional probab.**

Sigworth (1998), J. Struct. Biol. 122, 328-339



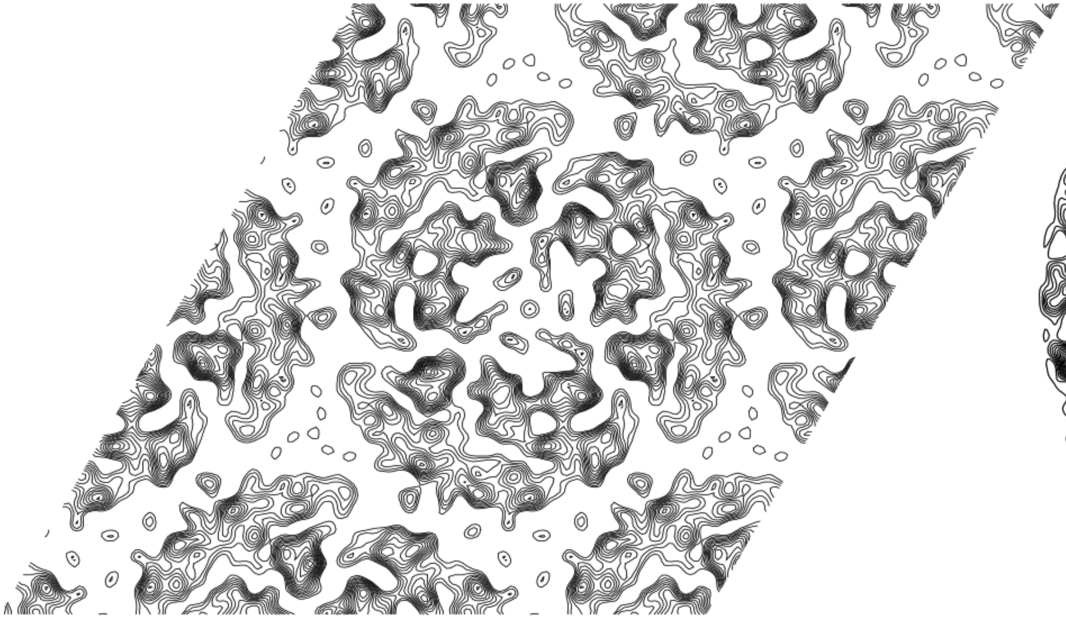
N = 4000

SNR = 1/200

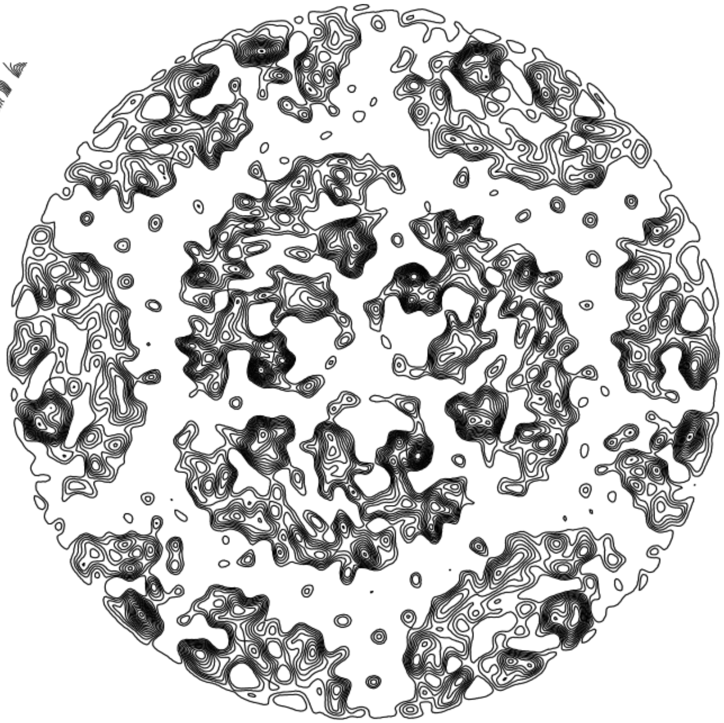
Maximum likelihood alignment

Sigworth (1998), J. Struct. Biol. 122, 328-339

ML processing of 2D crystals

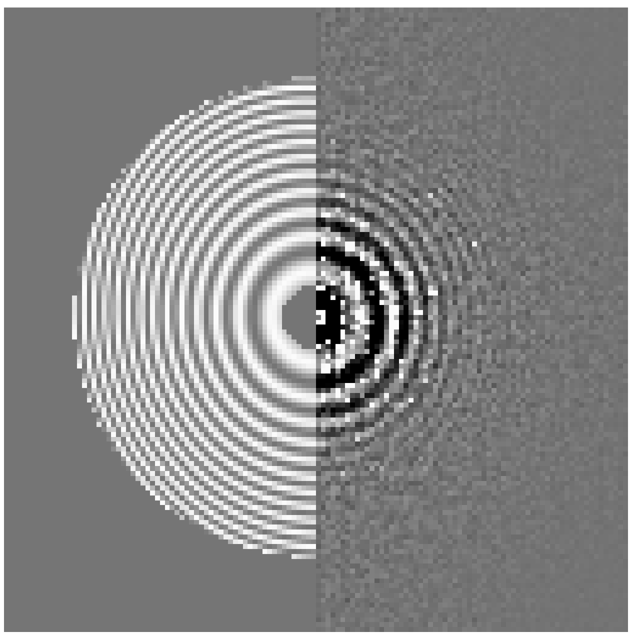


Crystallography

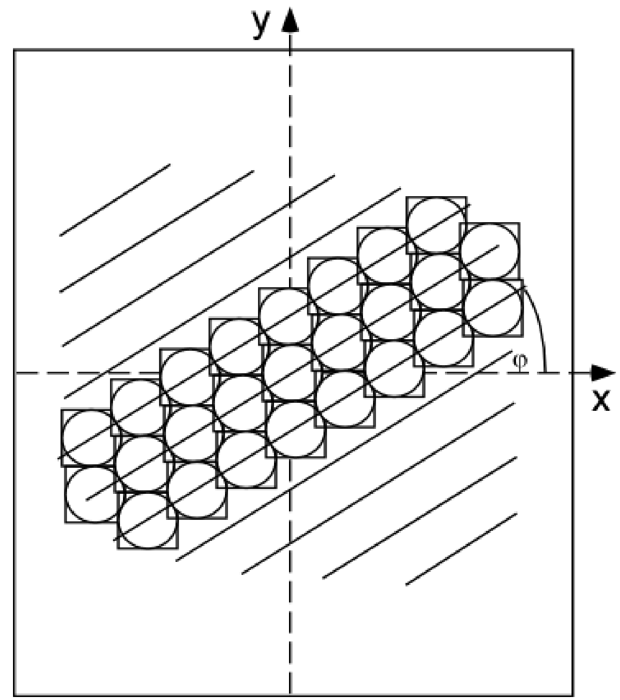


Alignment of
individual unit cells
using ML approach

Defocus/Astigmatism and Magnification

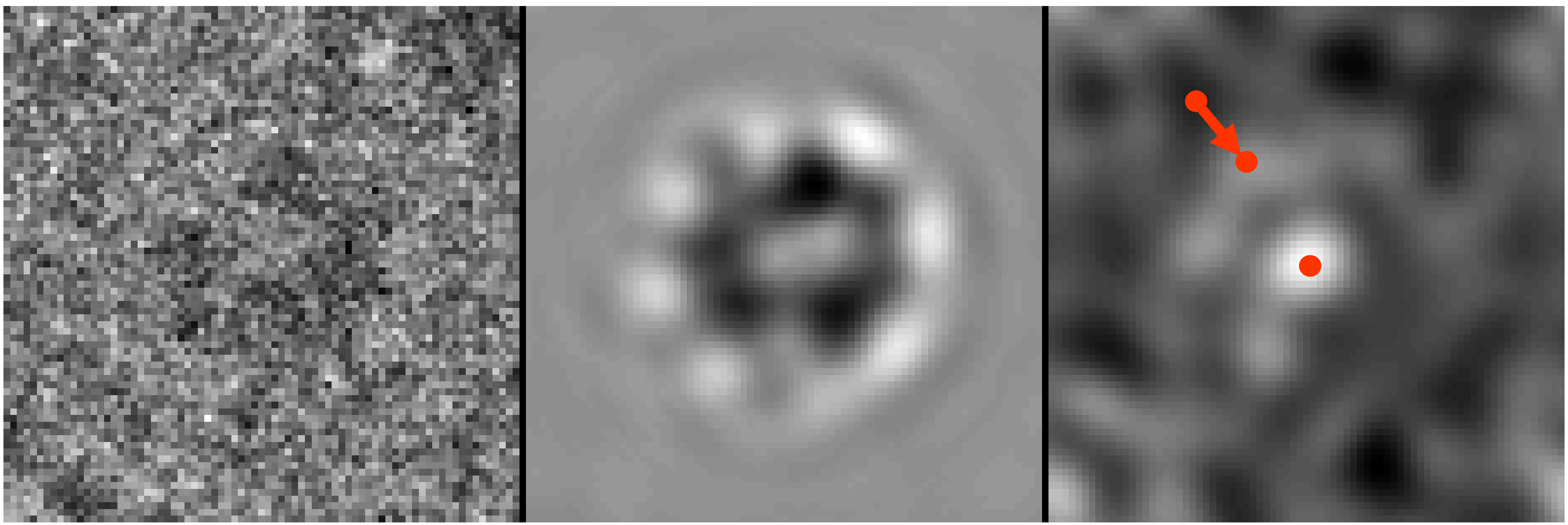


CTFFIND3



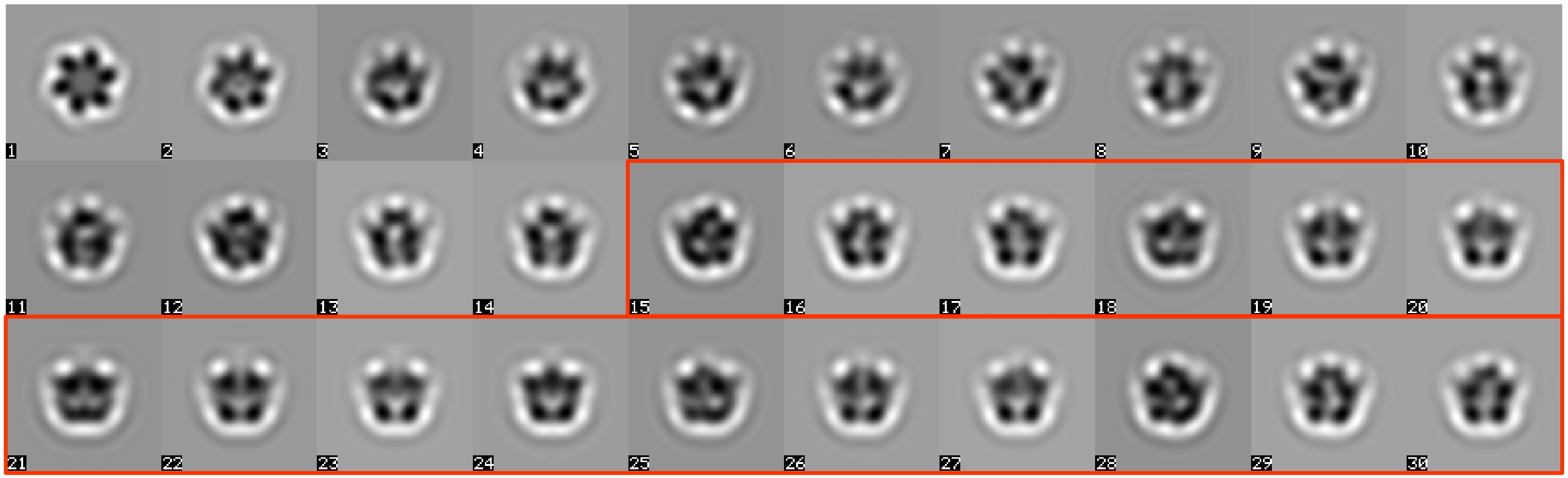
CTFTILT

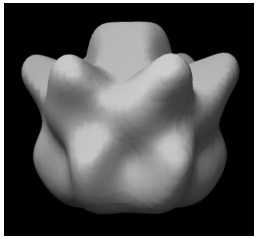
Problem 1: Local Optima



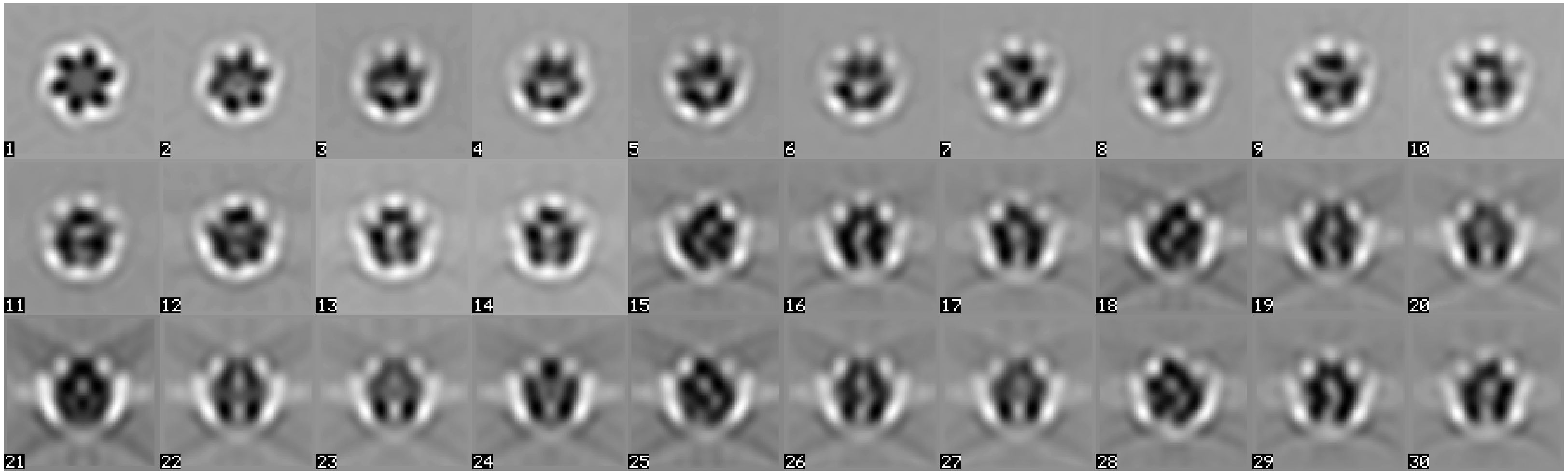
Particle Reference

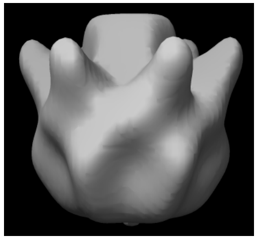
Problem 2: Missing Views



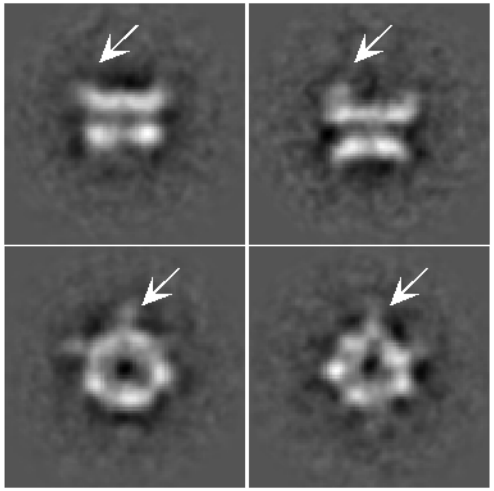


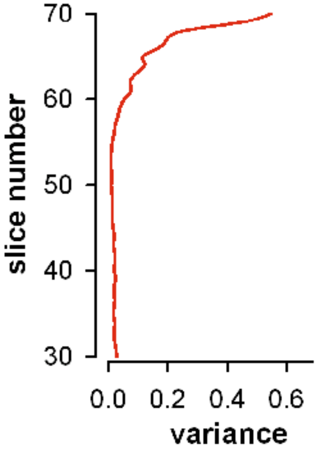
> 60º

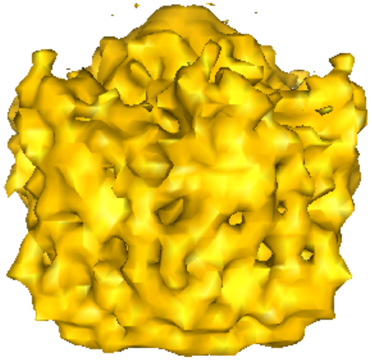




Problem 3: Heterogeneity







- Misalignment of particles
- Lower resolution in disordered regions
- Loss of features

Classification Using ML

Structure for *n*+1 iteration

$$A_k^{(n+1)} = \frac{1}{\sum_i q_i^k(\Theta)} \sum_{i=1}^{N} \frac{\int X_i(\phi) p_i^k\left(\phi, \Theta^{(n)}\right) d\phi}{\sum_k \int p_i^k\left(\phi, \Theta^{(n)}\right) d\phi}$$

Probability function

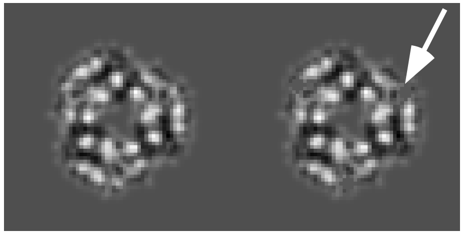
$$p_i^k(\phi, \Theta) = \left(\frac{1}{\sqrt{2\pi}\sigma}\right)^M \exp\left[-\frac{\left|X_i(\phi) - A_k\right|^2}{2\sigma^2}\right] f(\phi \mid \Theta)$$

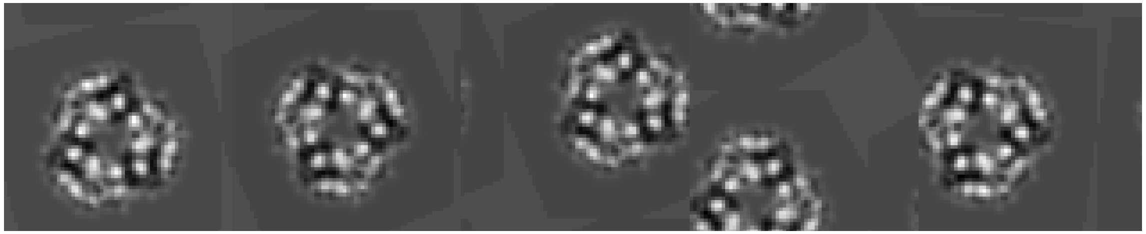
Probability for class *k*

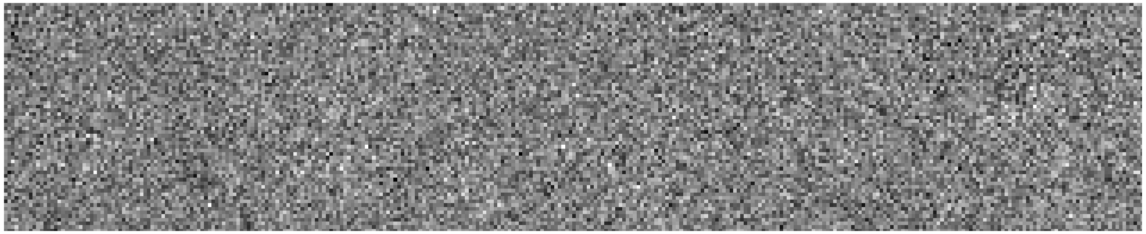
$$q_i^k(\Theta) = \int p_i^k(\phi, \Theta) d\phi$$

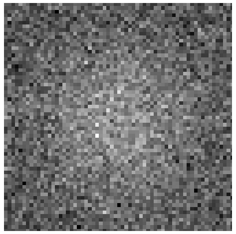
X_i: ***i*th image**
N: **# of images**
ϕ: **alignment parameters**
Θ: **model parameters**
σ: **noise in images**
f : **positional probab.**

Classification Using ML

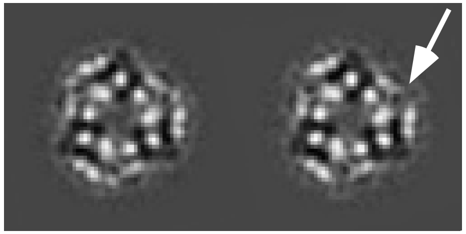


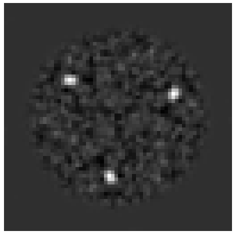






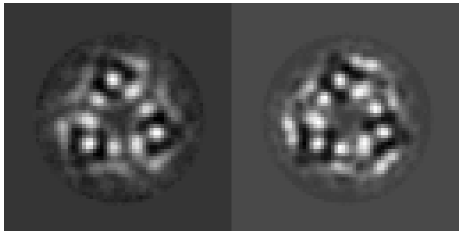
SNR = 1/50
N = 2000

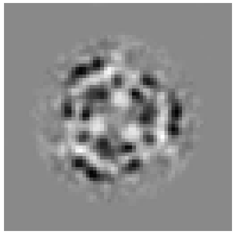




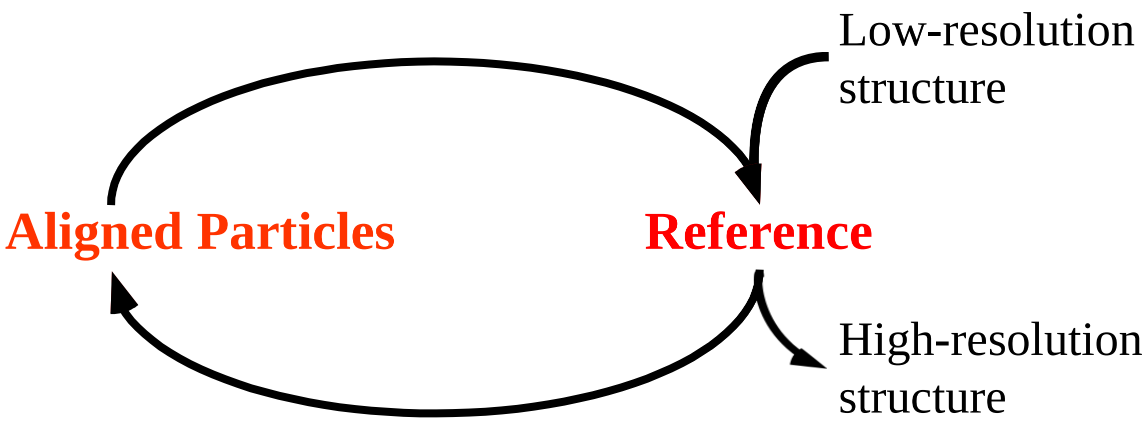
Difference map

Correlation alignment

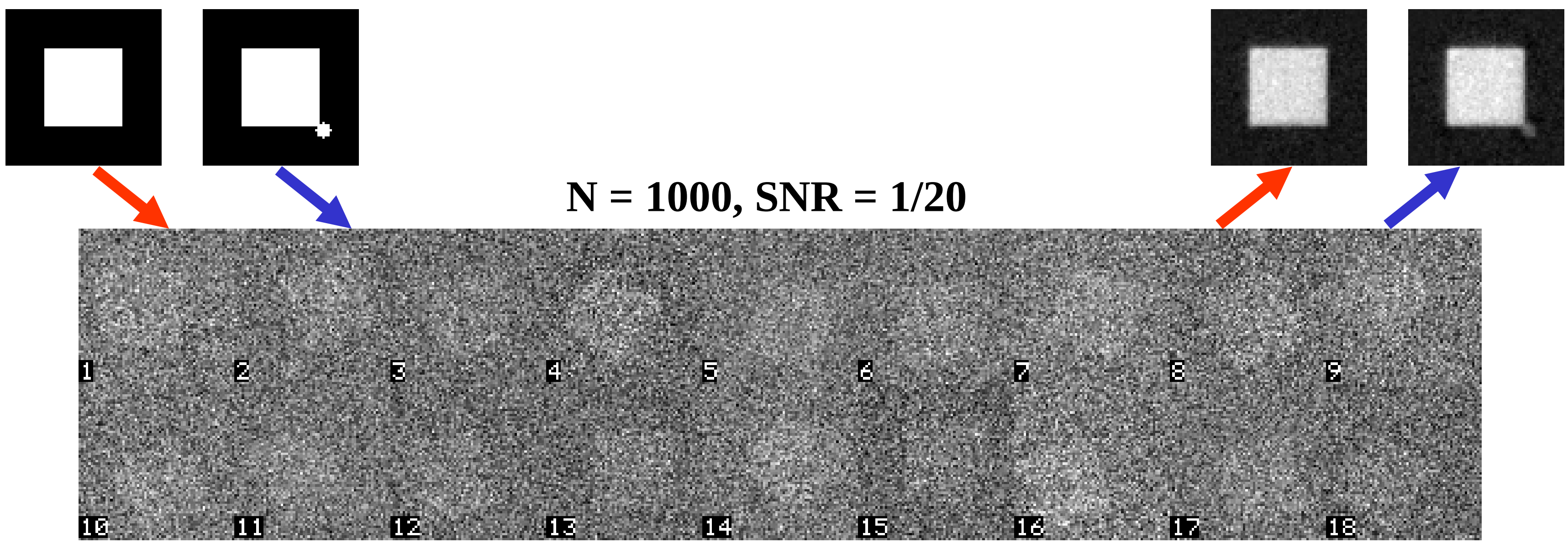




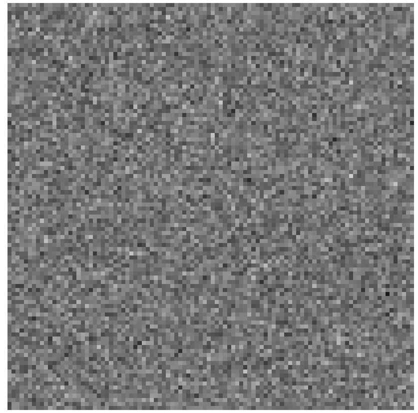
Problem 4: Processing Artifacts



- Interpolation errors
- Masking
- Negative B-factor
- …



Problem 5: Noise Bias

 ⊗  **= 0 on average**

 ⊗ 

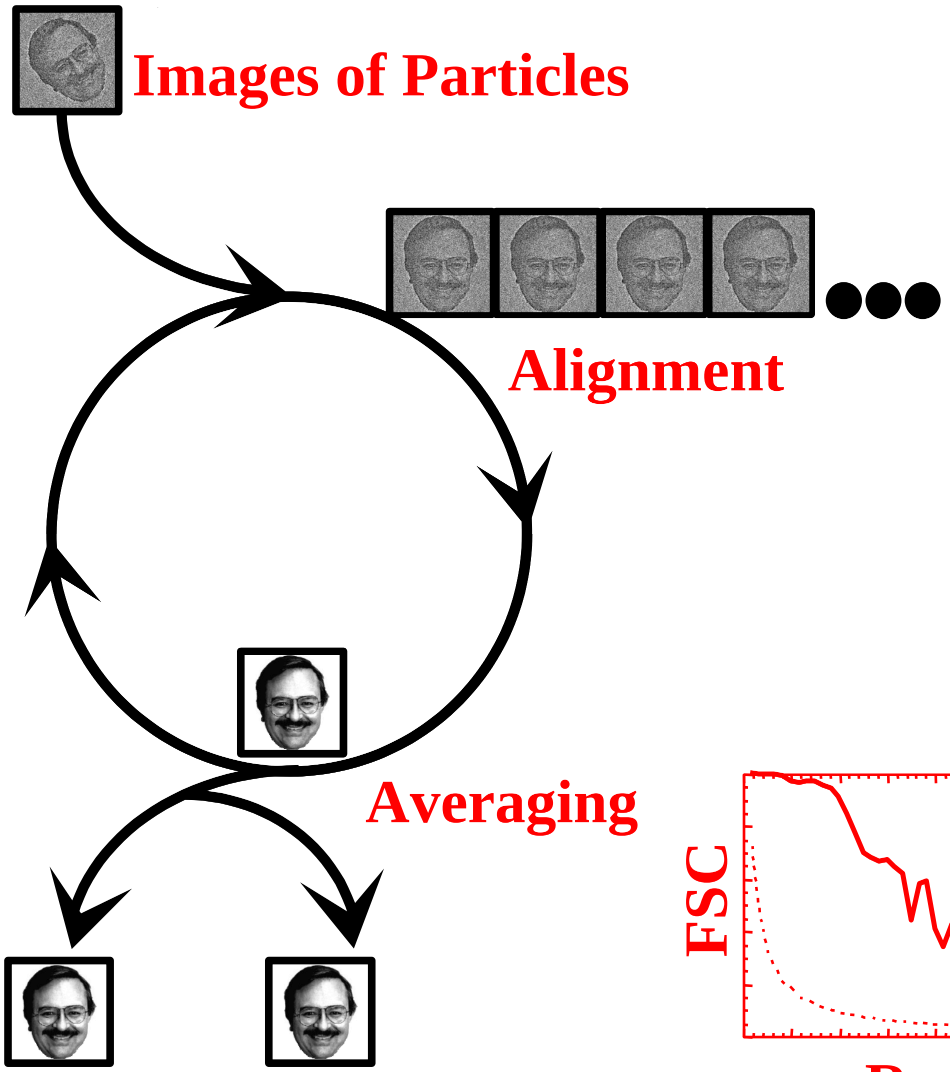
> 0

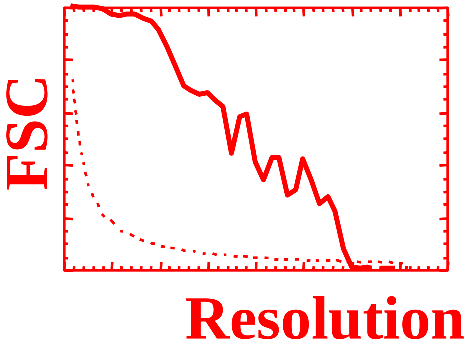
for 64x64 image: average correlation = 0.064

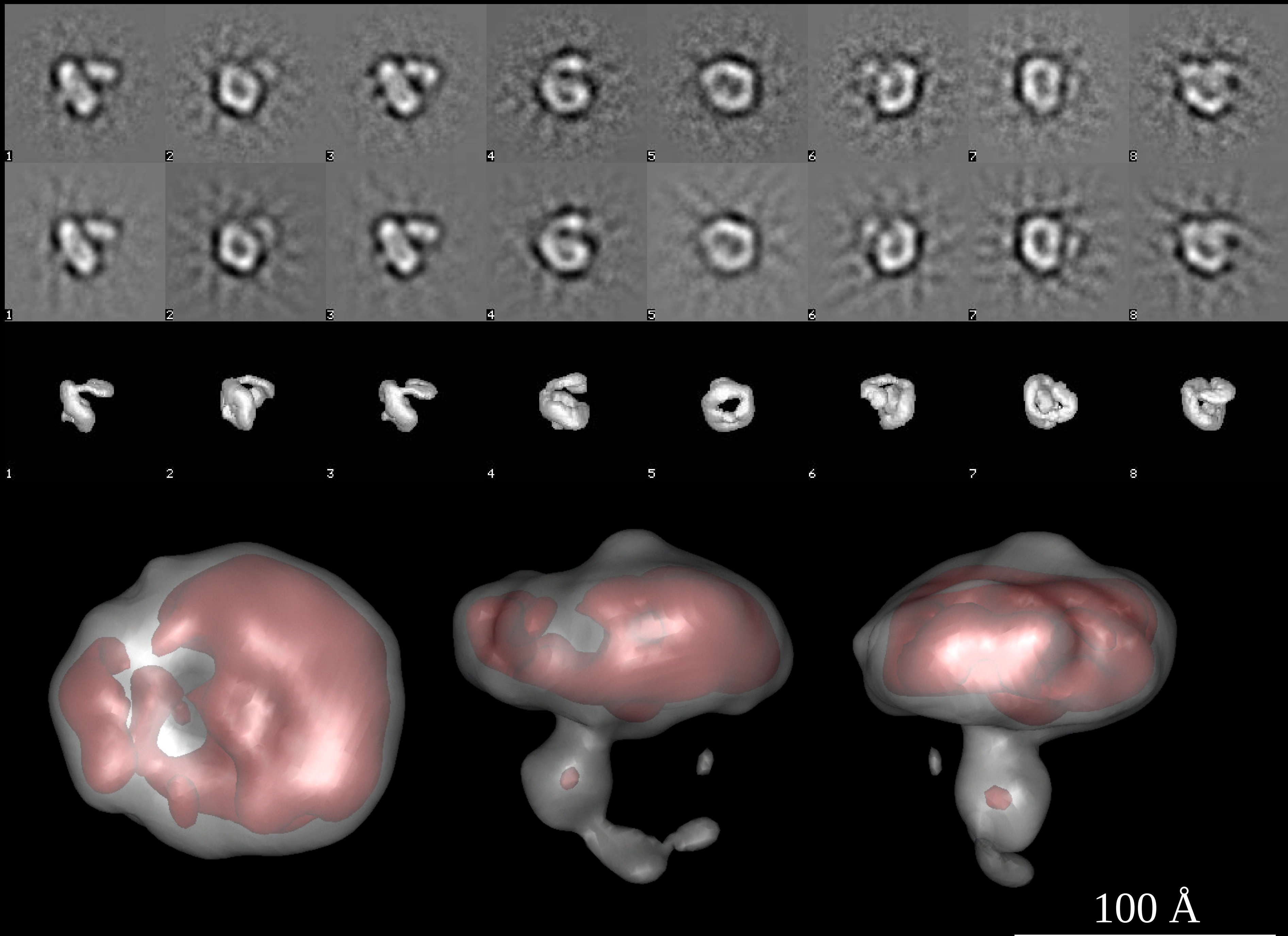
Seeing is NOT Always Believing

100 Images   1000 Images   Reference

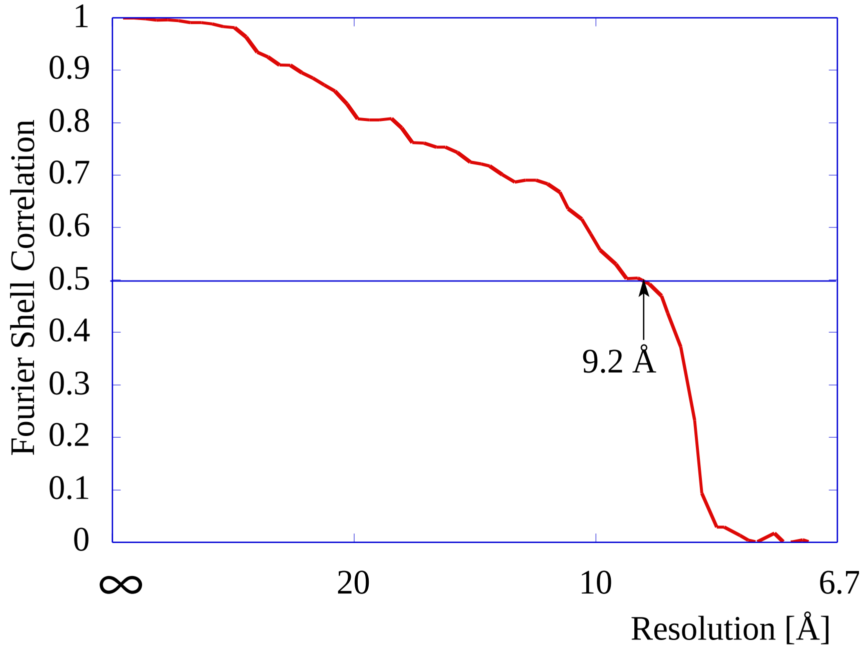
Resolution Measurement

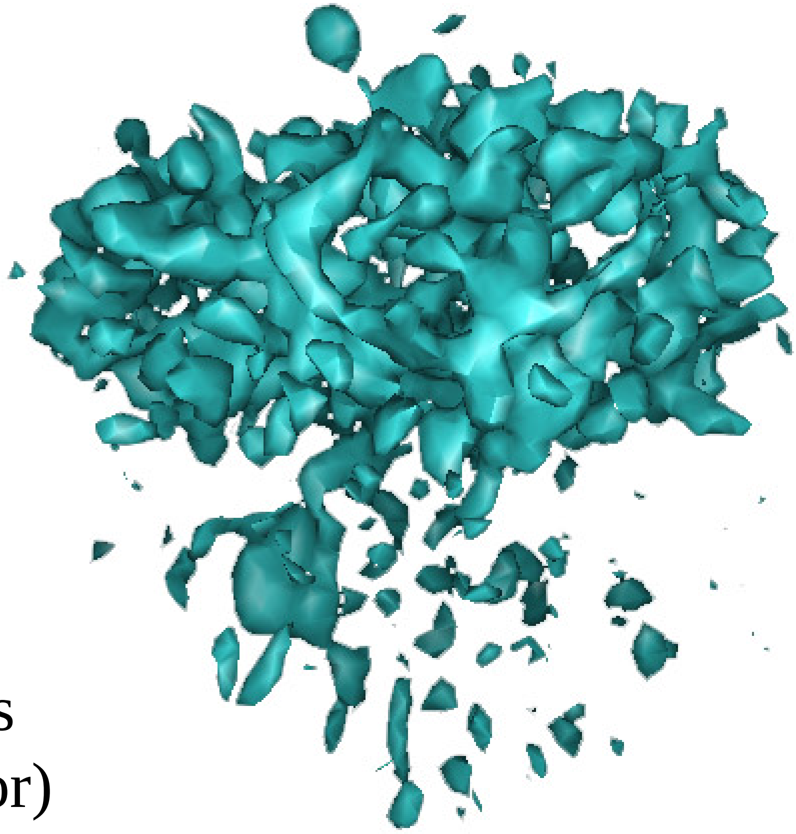





1
2
3
4
5
6
7
8
1
2
3
4
5
6
7
8
1
2
3
4
5
6
7
8
100 Å

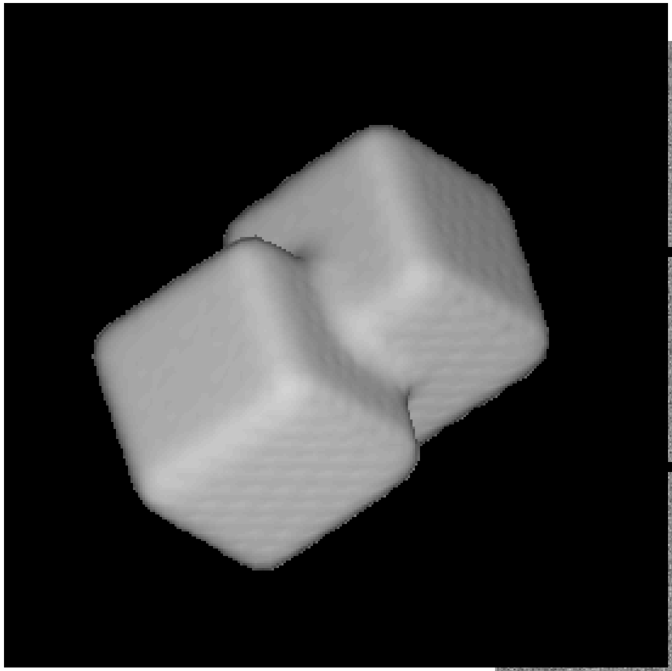
Swiss Cheese

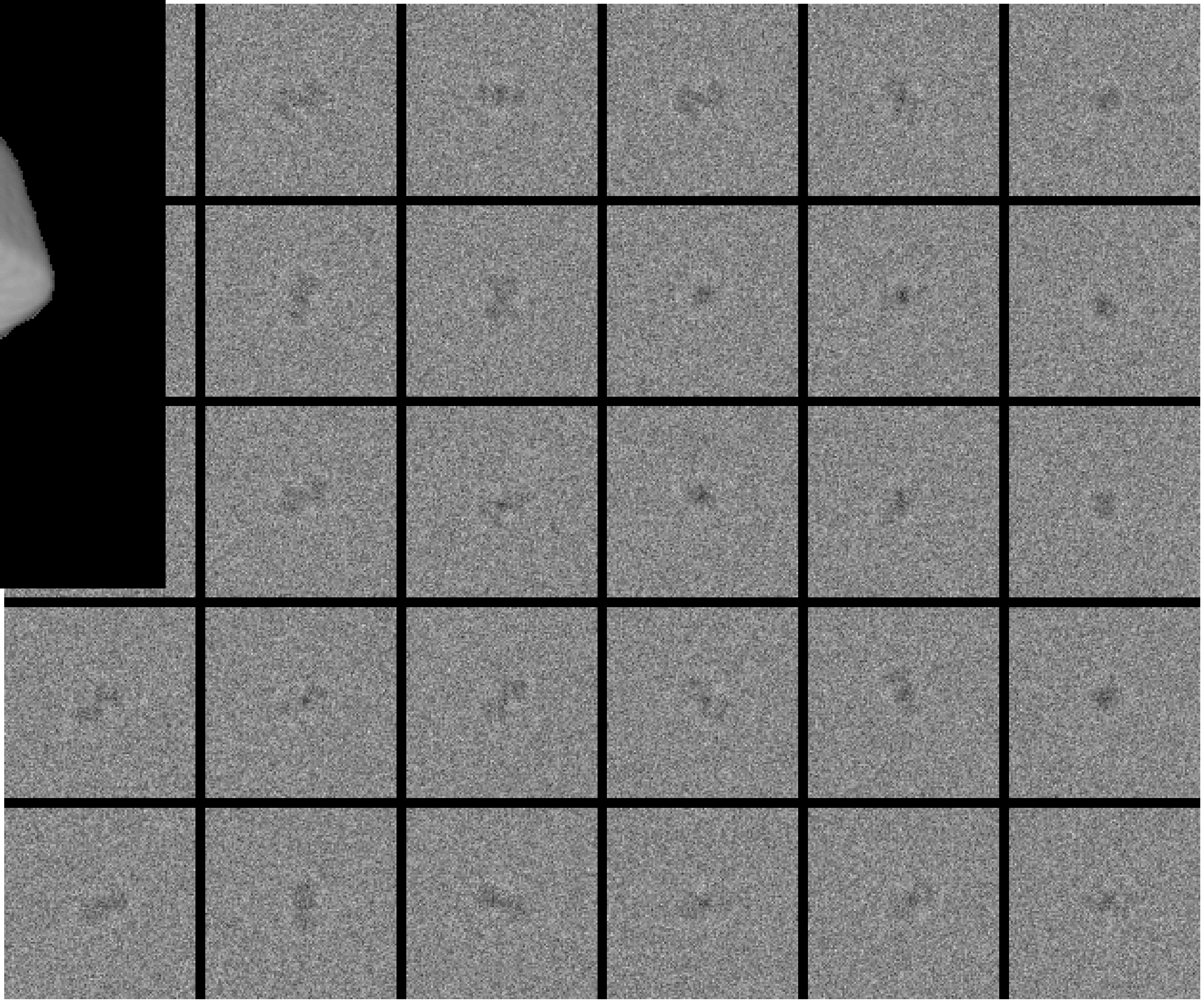




Dangerous:
Boosting of high-resolution terms
(application of a negative B-factor)

Gedanken Experiments

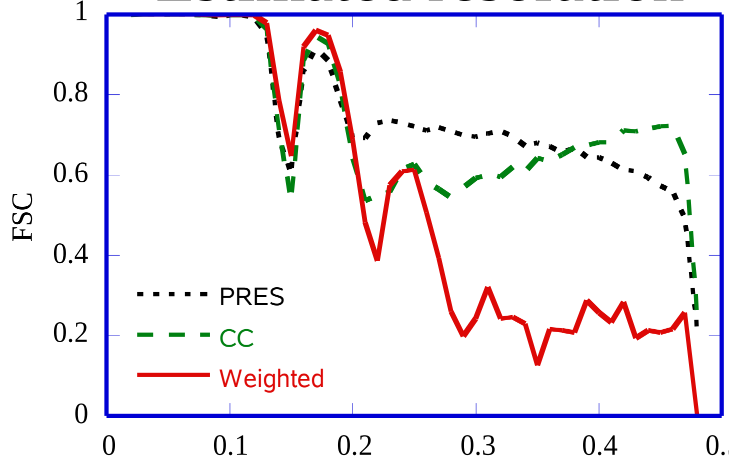




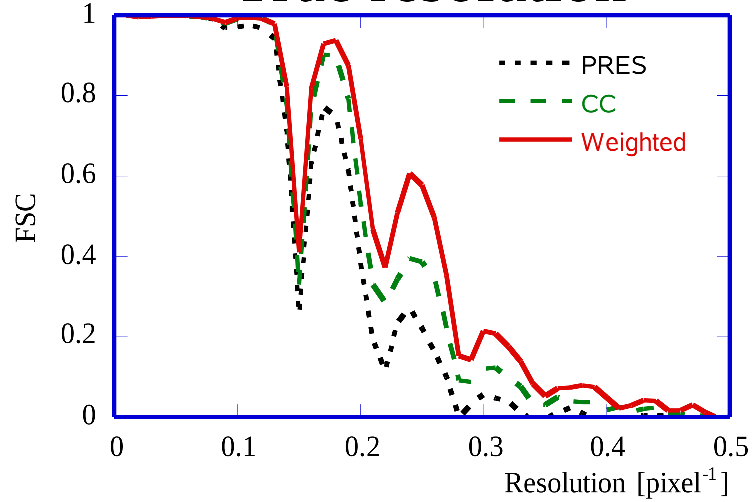
N = 30000
SNR = 1/50

Weighted Correlation

Estimated resolution



True resolution

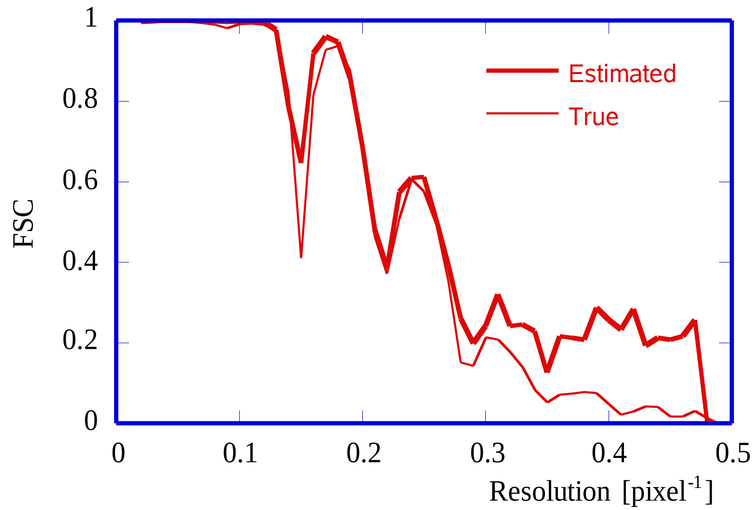


$$\mathrm{PRES}(X,Y) = \frac{\sum_{k\in[0,0.5]} \Delta\Phi_{X,Y}(\mathbf{k})\left|F_X(\mathbf{k})\right|}{\sum_{k\in[0,0.5]} \left|F_X(\mathbf{k})\right|}$$

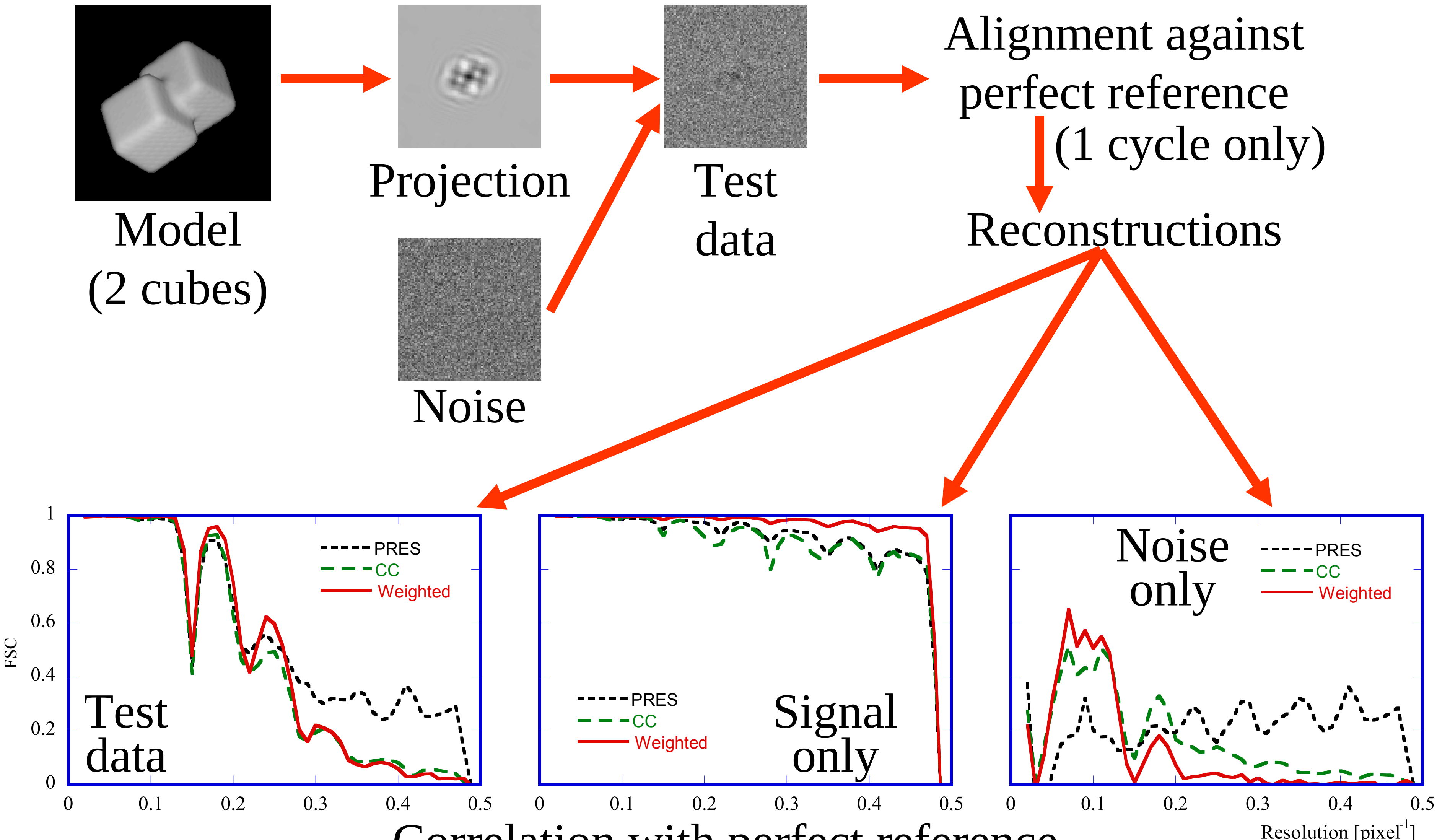
$$\mathrm{CC}(X,Y) = \frac{\sum_{k\in[0,0.5]} F_X(\mathbf{k}) F_Y^{\dagger}(\mathbf{k})}{\sqrt{\sum_{k\in[0,0.5]} \left|F_X(\mathbf{k})\right|^2 \sum_{k\in[0,0.5]} \left|F_Y(\mathbf{k})\right|^2}}$$

$$\mathrm{CC}_W(X,Y) = \sum_{k\in[0,0.5]} W(\mathbf{k}) F_X(\mathbf{k}) F_Y^{\dagger}(\mathbf{k})$$

$$C_W\left(\sum_i W_i\right) \approx k^3$$



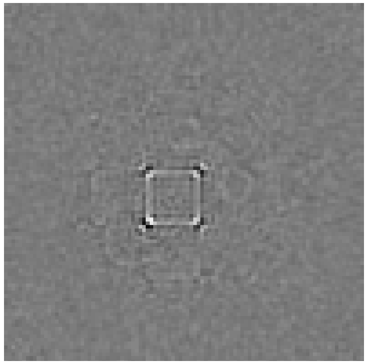
Noise Bias



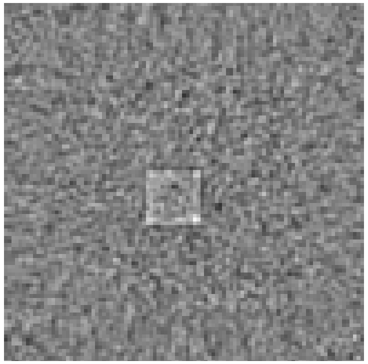
Correlation with perfect reference

Noise Reconstruction

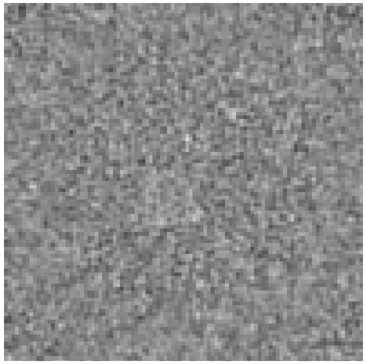
Phase residual



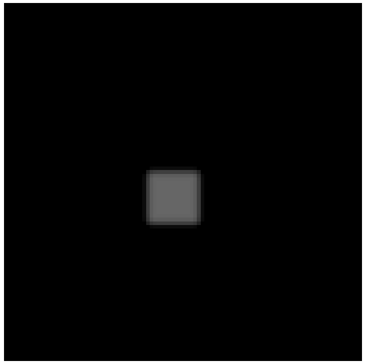
Linear correlation coefficient



Weighted correlation coefficient

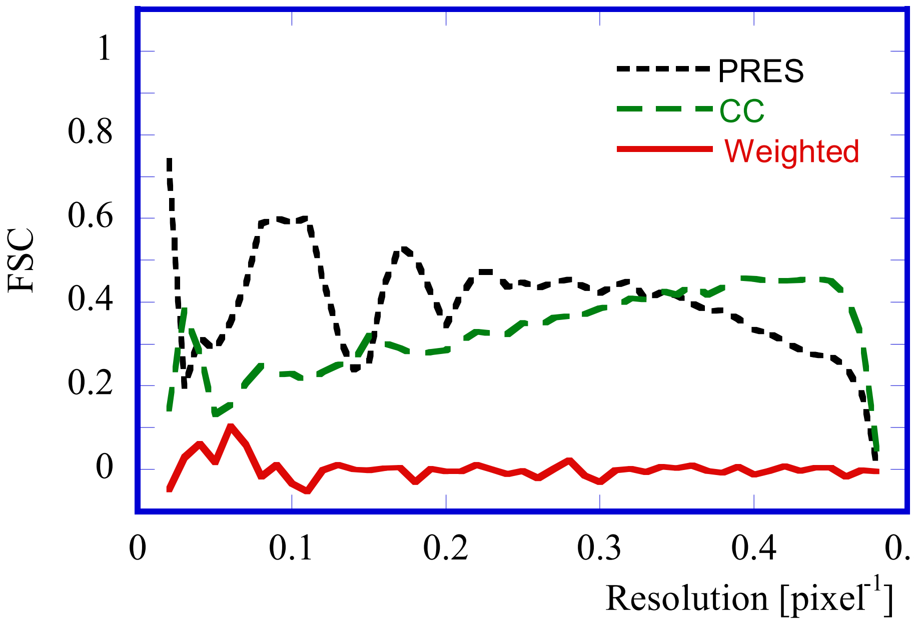


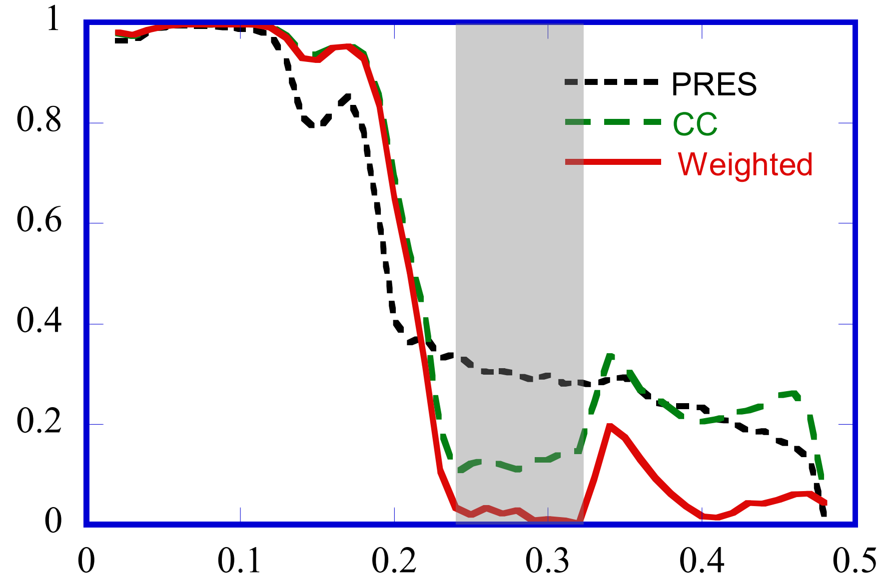
Reference



Coherence Constraint

$$\mathrm{CC}_W\left(X,Y\right)=\sum_i\left|\mathrm{CC}\right|_i^3$$





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- NSF/20S — Johannes Fürst (Axel Brünger)
- Noisy Face — David DeRosier
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"The shores of Valinor" by Ted Nasmith.

Scanned by Eriol.