Reconstruction from projections

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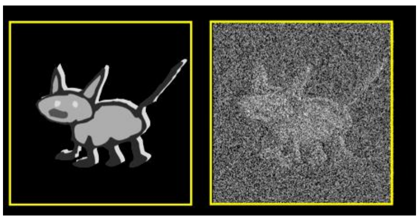
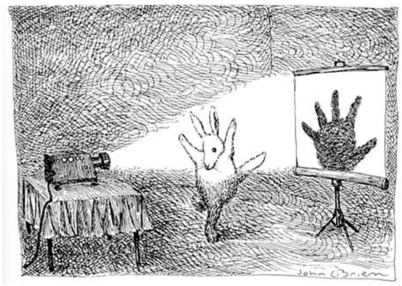
EMBO course 2015

What is a cryoEM image?

- It's a projection image…

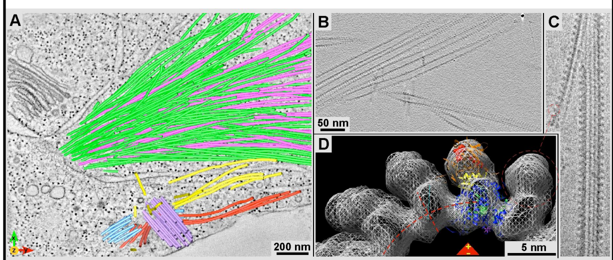


How to go from the images to a 3D reconstruction?



How, Why, What?

- What to take into account when choosing the reconstruction algorithm?



Courtesy of Andy Hoenger, Boulder

With your neighbour

- Think of 4 factors in your dataset that you think might be important to consider…
- You have 5 minutes to write down your answers

- Target: 11 factors.. or more

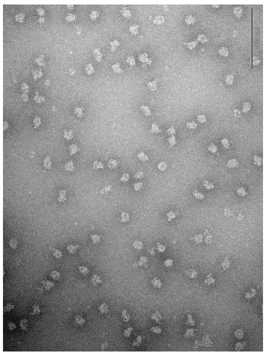
Image processing

- 2 key concepts:
- We need different views of the object
- We need to average many views
- We need to combine these views together to generate the 3D volume.
- The data set is incomplete.

Obtaining different views

- Single particle

(ideally) the particles are randomly oriented, and are all the same. These correspond to different views of the same object.



Obtaining different views - tomography

Computer tomography of a human being:

Rotate an X-ray source around the body, collect multiple projection pictures, unique object

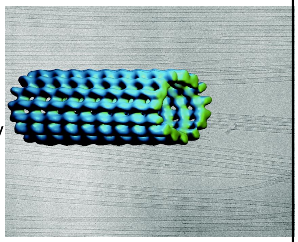
Electron tomography:

Rotate a sample in an electron microscope, collect multiple projections from the same area with known angular separation

Obtaining different views

- Helical

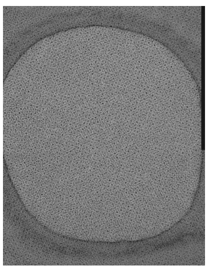
Within one helix, we see the basic building block, (the unit cell) from many directions. The image of one helix contains many different views of the unit cell.



Obtaining different views

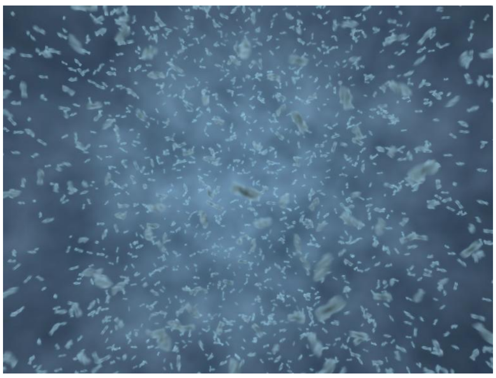
- 2D crystals

All of the unit cells are in the same orientation – we collect images of multiple different crystals, each tilted to different angles within the microscope.



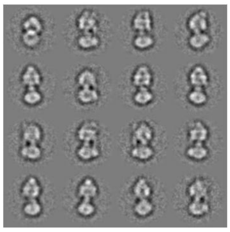
Computational work flow to produce a 3D structure from projections

- CTF correction
- Filtering
- Alignment
- Classification
- Averaging
- Angle assignment
- 3D reconstruction
- Resolution assessment
- Refinement
- Sharpening/weighting

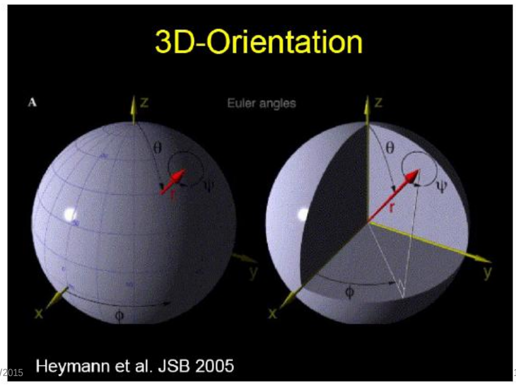


Angle assignment

- How do these views all relate to each other?



Angle assignment

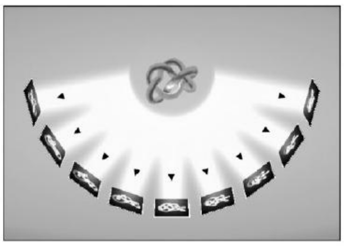


3 approaches to angle assignment

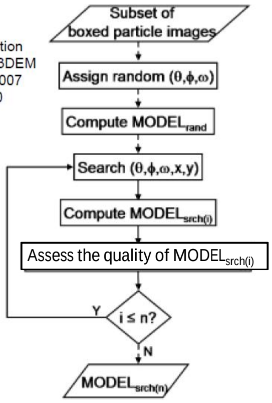
1. based on starting model (projection matching)
2. ab initio (common lines, angular reconstitution)
3. known angles (random conical tilt, tomography)

If you have a starting model

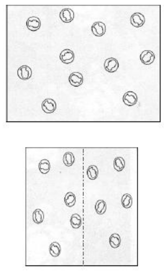
Projecting a model and compare images to the projections



Ab initio model generation implemented in AUTO3DEM
Yan et al. JSB 2006, 2007
Sanz-Garcia JSB 2010



Random conical tilt c.f. tomography

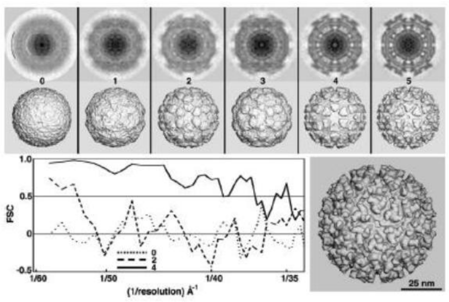


Untilted image:
a) Find particles with the same orientation/view
b) Find rotation angles

Tilted image:
a) Find tilt angle
b) Find particle's positions
>> all spatial relation-ships known !!
c) Use tilted views for 3-D reconstruction

Figures: M.Radermacher, J.Elect.Microsc.Techn. 9 (1988) 359-394

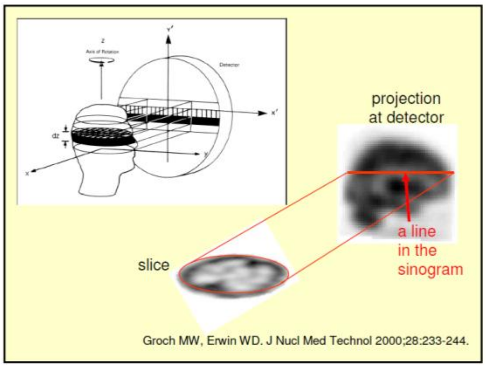
One example iteration of a starting model using 150 of furthest from focus particles, converges very rapidly to 35 Å



Yan et al. JSB 2006, 2007 Fuller et al. JSB 1996 "the uncommon line"

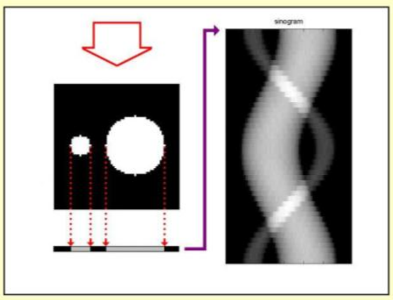
Next generate a 3D representation of all your data

- In real space or in Fourier space
- Analytical reconstruction algorithms (e.g. filtered back projection, Fourier transform interpolation in cartesian coordinates). Fast, elegant.
- Iterative reconstruction algorithms. Slower, less efficient, more versatile? Algebraic reconstruction technique (ART), simultaneous iterative reconstruction technique (SIRT),
- Newer applications including maximum likelihood estimates (Relion)



Groch MW, Erwin WD. J Nucl Med Technol 2000;28:233-244.

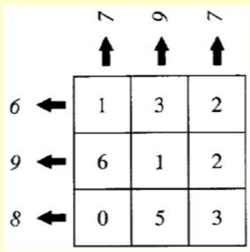
Sinogram = collection of projections of a single slice



Exercise: Given this slice, obtain the sinogram

1	3	2
6	1	2
0	5	3

Solution: Given this slice, obtain the sinogram



The sinogram contains 2 rows:
(7,9,7) and (8,9,6).

Analytic reconstruction methods

(projection - backprojection algorithms)

filtered back-projection

back-projection filtering

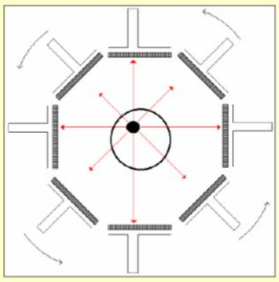
Radon J.

On the determination of functions from their integrals along certain manifolds [in German].

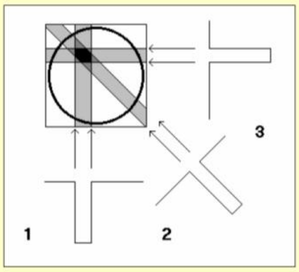
Math Phys Klass 1917;69:262-277.

Included in handout: ANGULAR RECONSTITUTION IN THREE-DIMENSIONAL ELECTRON MICROSCOPY: HISTORICAL AND THEORETICAL ASPECTS, van Heel et al. 1997

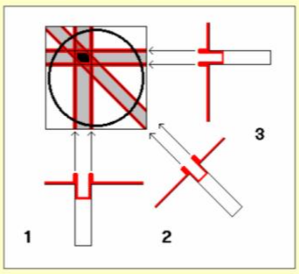
Recording projections of a slice

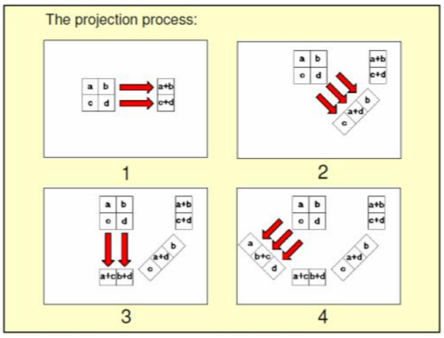


Back projection (BP)



Filtered back-projection (FBP)





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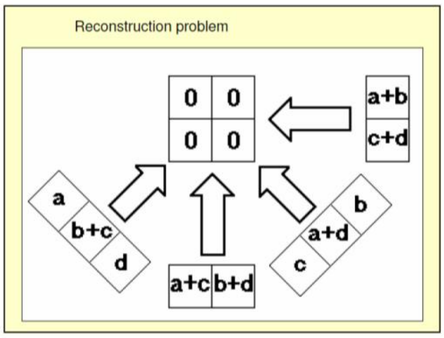

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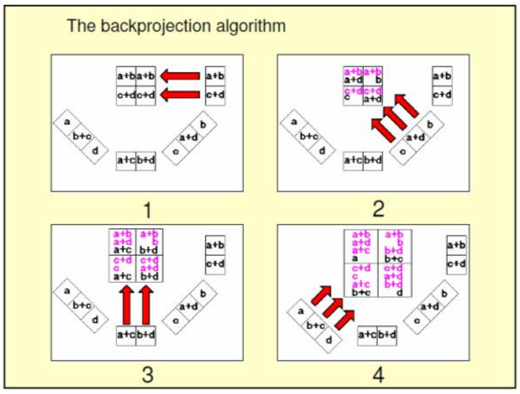

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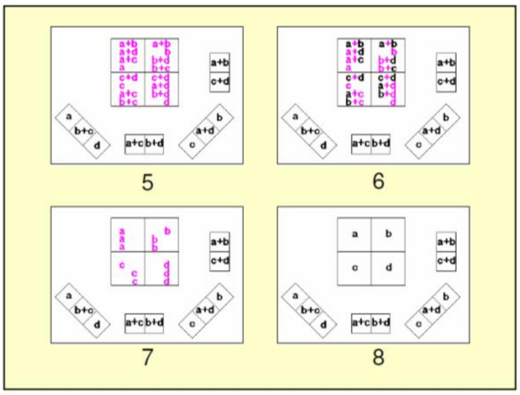

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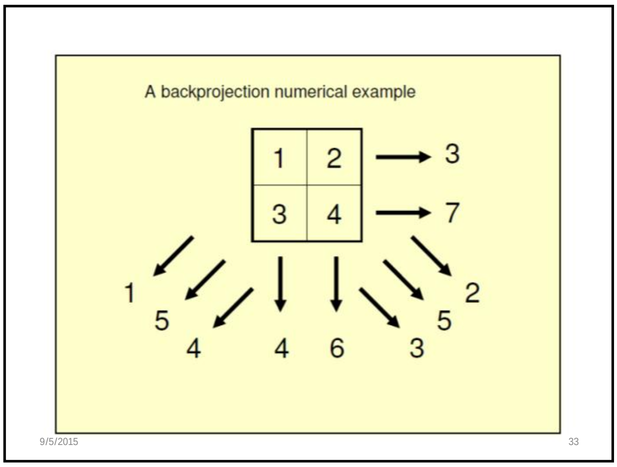
A backprojection numerical example
1 2
3 4
3
7
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5
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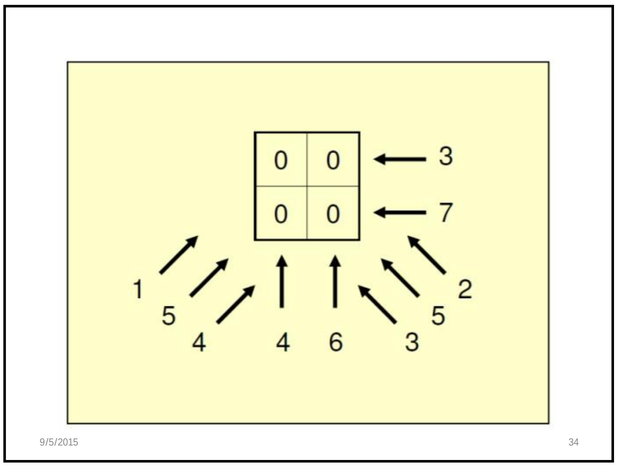
33


0 0
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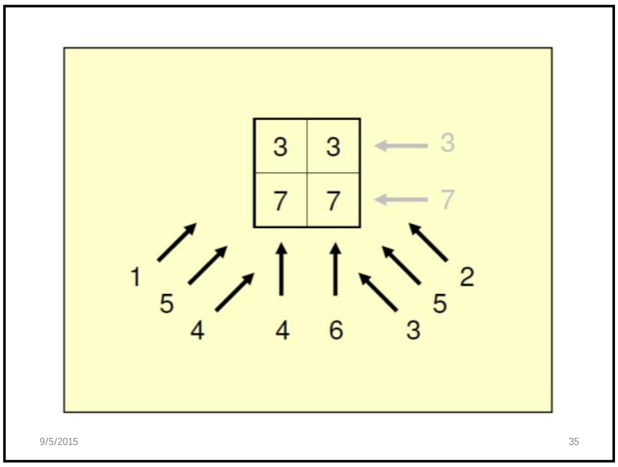
34


3 3
7 7
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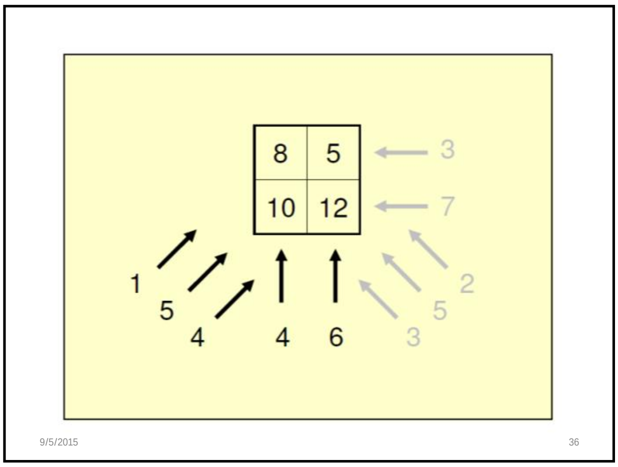
35


8 5
10 12
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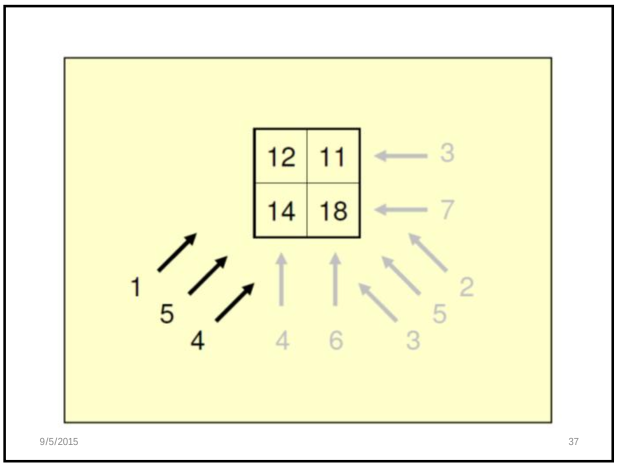
36


12 11
14 18
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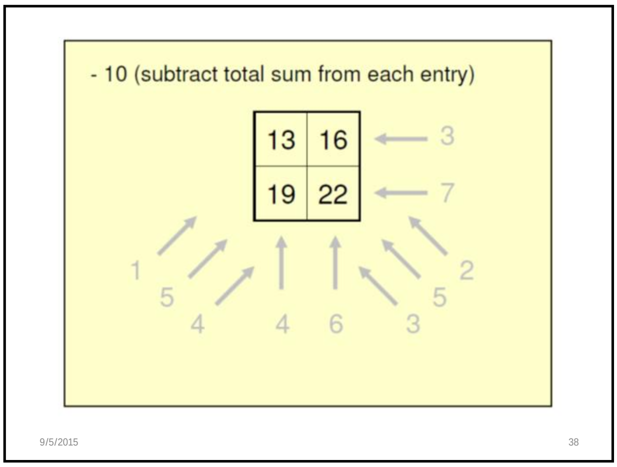
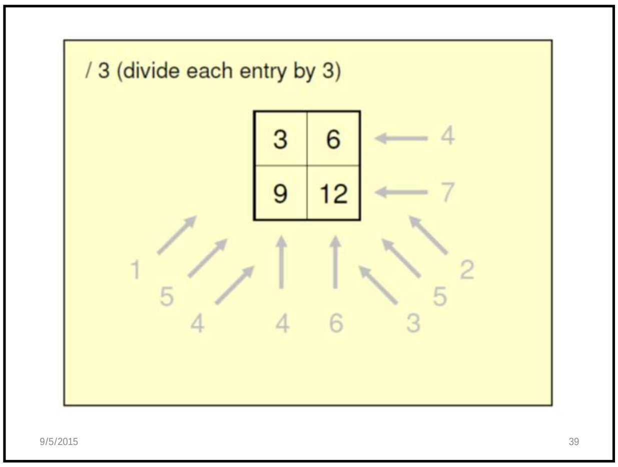
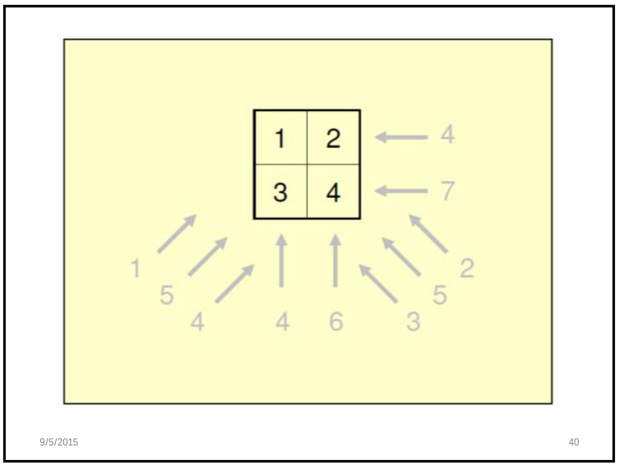
37


- 10 (subtract total sum from each entry)
13 16
19 22
3
7
1
5
4
4 6 3
5
2
9/5/2015
38


/ 3 (divide each entry by 3)
3 6
9 12
4
7
1
5
4
4 6 3
5
2
9/5/2015
39


1 2
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4 6 3
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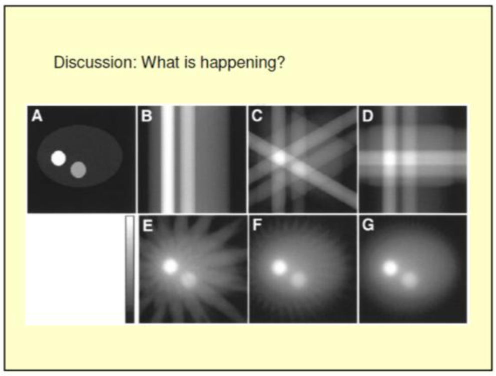
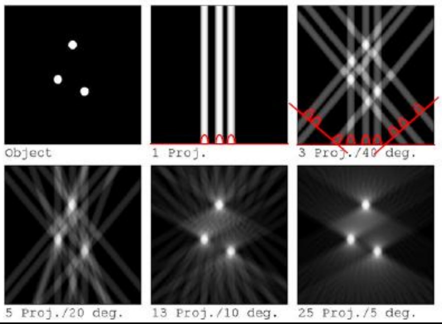
40

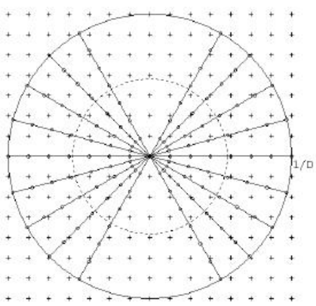


 Consider what would happen if the views are not evenly spread?

Back projection, anisotropic resolution due to uneven distribution (typical in tomography and 2D crystals)



How many images do I need??

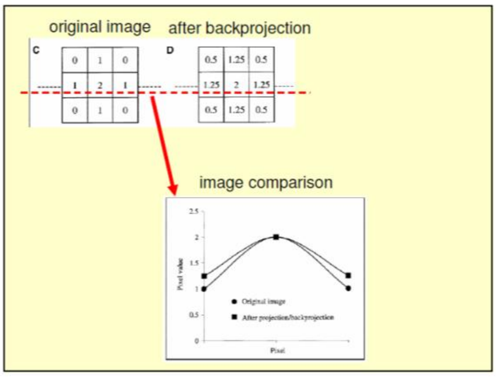


Crowther Criterion

$$R=K/\pi D$$

R: Resolution limit
K: Number of projections
D: Object size

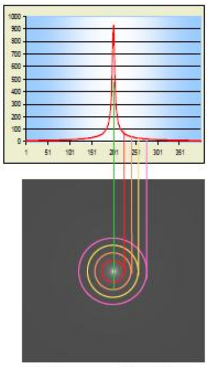
Crowther et al. 1970



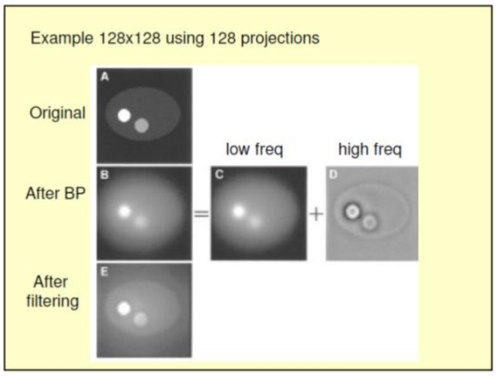
Back projection

The simplest algorithm has one serious limitation.

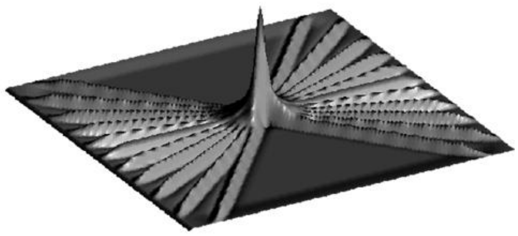
If we are reconstructing only one point, it will be surrounded by background that is proportional to 1/r, where r is the distance from the point. That background is proportional to intensity of the local point. It makes the whole reconstruction smeared.



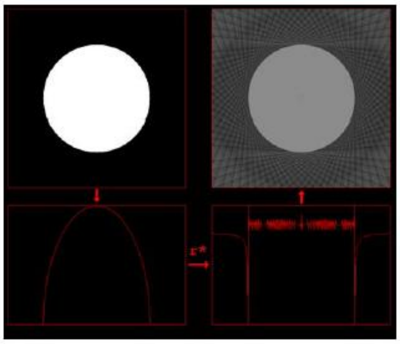
Point spread function



Weighted Backprojection

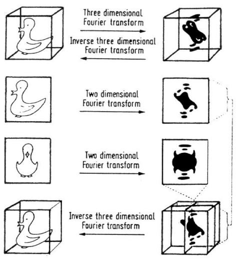


Weighted Backprojection



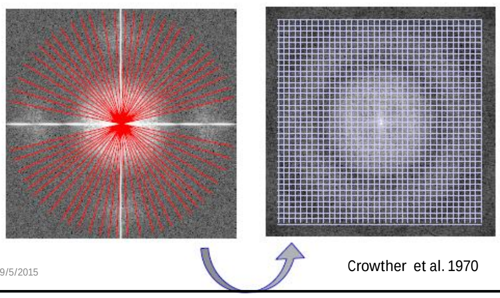
Fourier Methods
Central Projection theorem

- 2D Fourier transform (FT) is calculated for every projection
- These correspond to central sections in 3D FT of the object
- Reconstruction can be done by "filling" the 3D Fourier space with these sections



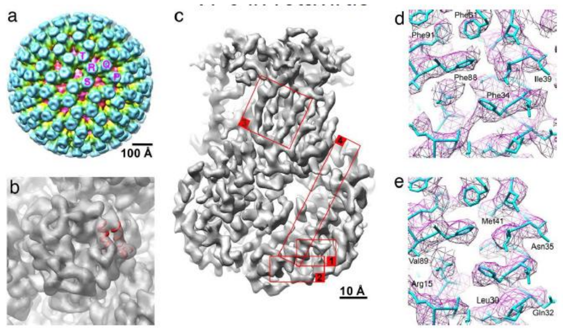
Interpolate on Cartesian coordinates to restore the discrete Fourier transform on each pixel of the grid. apply filters, symmetry, ctf correction. (Freealign, auto3dem)

Fourier methods



Crowther et al. 1970

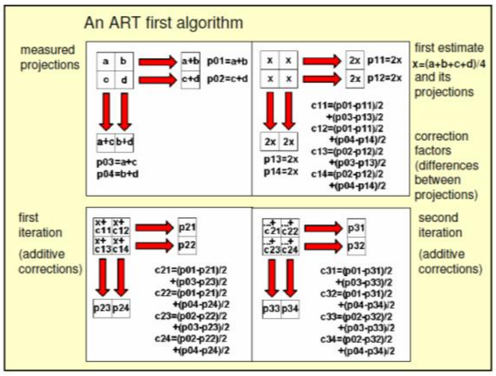
Even though one set of rules were applied during the reconstruction, additional averaging of repetitive elements can also be carried out later.



Zhang X. et al. PNAS 2008;105:1867-1872

Iterative reconstruction methods

- Principle is to find a solution by successive estimates. The projections corresponding to the current estimate are compared with the measured projections. The result of the comparison is used to modify the current estimate, thus creating a new estimate.
- The algorithms differ in the way the measured and the estimated projections are compared and the kind of correction compared to the current estimate. Start the process by arbitarily creating a first estimate e.g. all pixels =0



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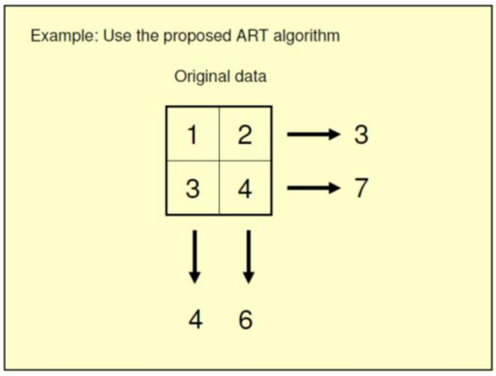

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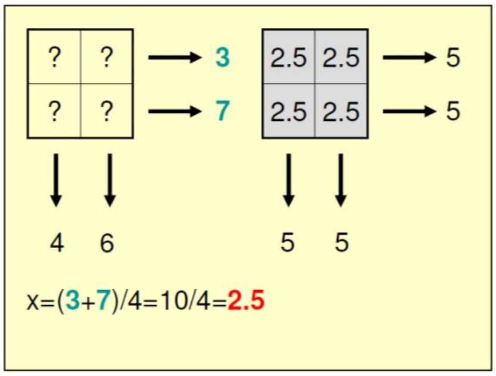

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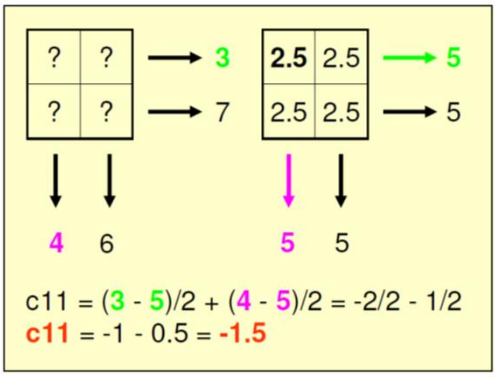

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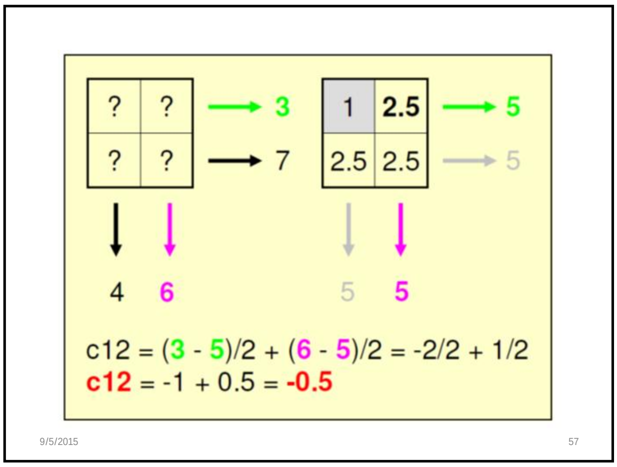
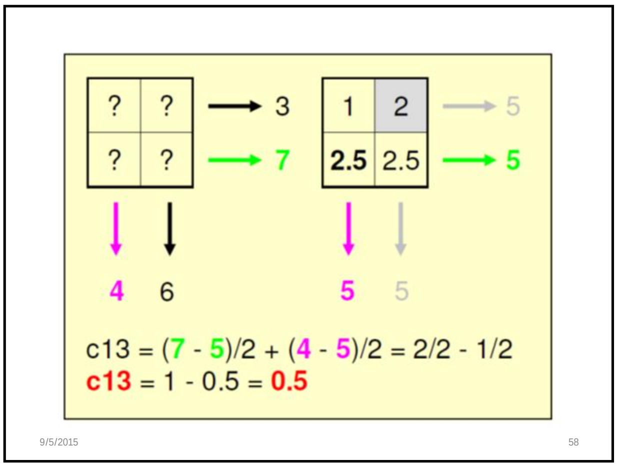
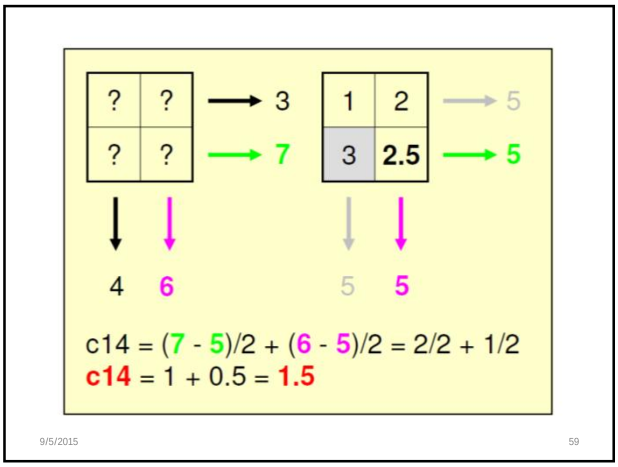
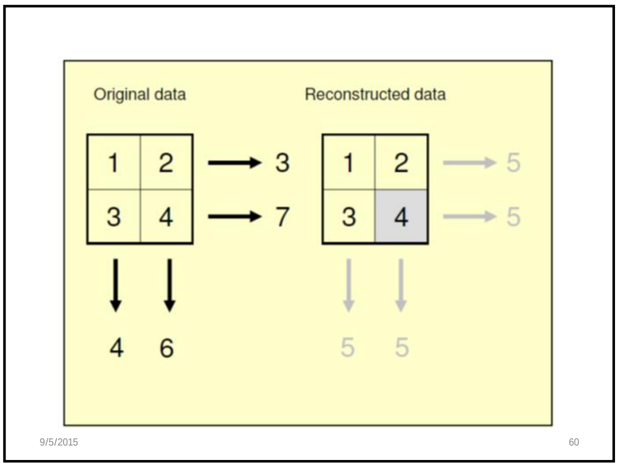
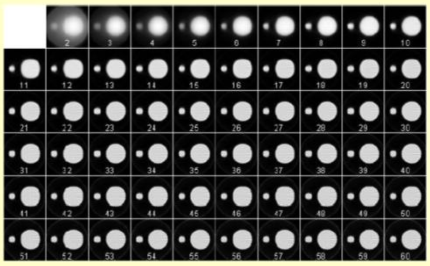

? ? → 3
? ? → 7
4 6
1 2.5 → 5
2.5 2.5 → 5
5 5
c12 = (3 - 5)/2 + (6 - 5)/2 = -2/2 + 1/2
c12 = -1 + 0.5 = -0.5
9/5/2015
57

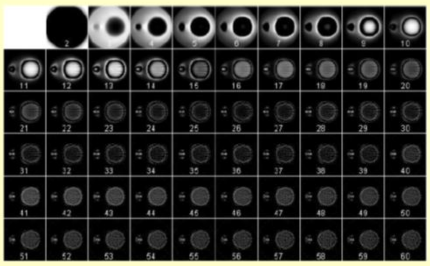

? ? → 3
? ? → 7
4 6
1 2 → 5
2.5 2.5 → 5
5 5
c13 = (7 - 5)/2 + (4 - 5)/2 = 2/2 - 1/2
c13 = 1 - 0.5 = 0.5
9/5/2015
58


? ? → 3
? ? → 7
4 6
1 2 → 5
3 2.5 → 5
5 5
c14 = (7 - 5)/2 + (6 - 5)/2 = 2/2 + 1/2
c14 = 1 + 0.5 = 1.5
9/5/2015
59


Original data
Reconstructed data
1 2 → 3
3 4 → 7
4 6
1 2 → 5
3 4 → 5
5 5
9/5/2015
60

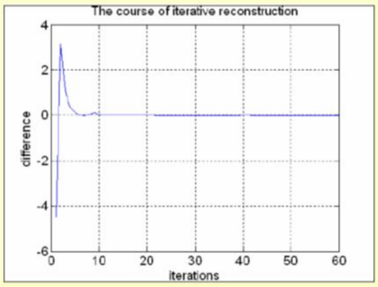


Iterative reconstruction - multiplicative corrections



Iterative reconstruction - differences between individual iterations

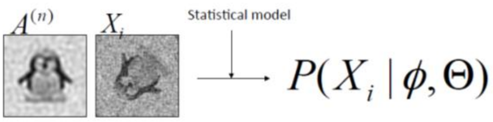
Iterative reconstruction - multiplicative corrections



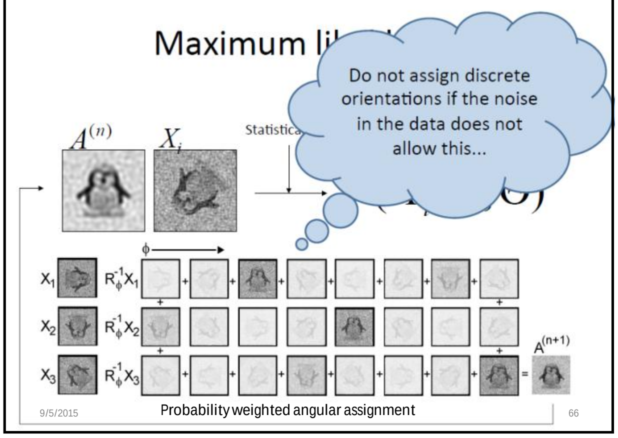
Important developments

- Most frequently used methods are all iterative (FT, WBP, SIRT).
- Maximum likelihood methods include the possibility that even with 2D classification, there is heterogeneity within the dataset, and several models may infact be represented by the data.
- 3D classification can enable even better sorting of the data
- ML deals well with electron dose and fine drift refinement from DED, as more heavily irradiated data, or heavily drifted frames have less weight in the model

Maximum likelihood



Maximum likelihood



Probability weighted angular assignment

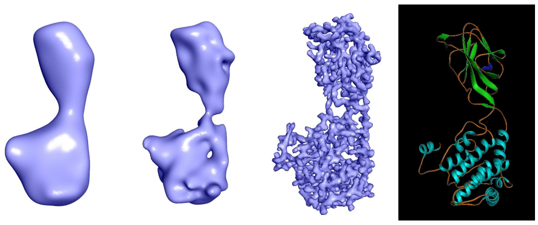
In Relion, Bayesian approach is

- Used to approximate noise
- Used to approximate orientations
- Seed refinement with multiple reconstructions
- Used to pull out heterogeneous structures (up to 3-4?)
- Much of this is automatic, and computational slow.
- Biggest advantage with DED data, large datasets, known starting model

I have a 3D reconstruction, what does it mean?

- How do I know which of the features are reliable?
- I could compare two independent reconstructions.
- The smallest features are most likely to be wrong.
- I need a measure of the resolution.
- Is the resolution the same across the whole structure, or is it better in some areas than others?

Structures at different resolutions



Low 15 Å Shape, size

intermediate 9Å domains, helices

high resolution 3Å backbone trace

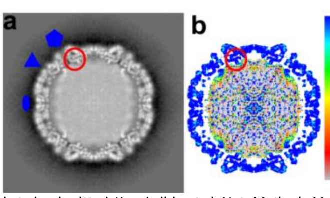
When it all goes wrong…

Check for the following:

- The number of projections is too small
- Distribution of defocii does not fill all the zeroes in the CTF
- Angular range is not uniformly filled
- Signal to noise ratio is low
- Projections are not centered
- Angles are not accurately defined
- CTF correction is incorrect
- Data are over-refined
- Averaging method is inappropriate

Variation within the reconstruction?

- Although the whole volume is reconstructed, there is variation as to how well each feature is represented. (Resmap)



Shakeel et al. submitted; Kucukelbir, et al. *Nat. Methods* 11, 63-65,

- Thanks to Bettina Böttcher, Andy Hoenger, John Briggs, Nicolas Boisset, Elena Orlova, Juergen Plitzko, Achilleas Frangakis Villa Uriol, Sjors Scheres and others for images and slides.