

This material is provided for educational use only. The information in these slides including all data, images and related materials are the property of :

Michael Moody

School of Pharmacy

University of London

29/39 Brunswick Square

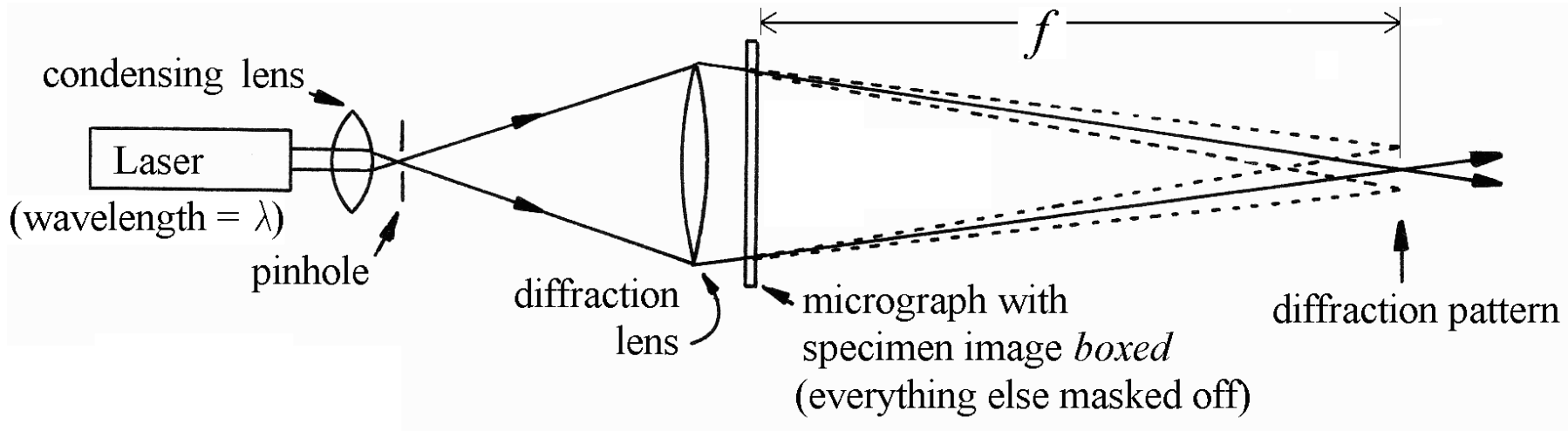
London WC1N 1AX, U.K.

No part of this material may be reproduced without explicit written permission.

FUNDAMENTALS OF IMAGE ANALYSIS & AVERAGING. (M.F. Moody)

- Getting F.Ts. (analogue & digital).
- Using F.Ts. in image processing: **averaging** and **image matching**.
- Using F.Ts. in **3D reconstruction**.

Getting analogue (continuous) F.Ts.: the optical diffractometer.



Scaling optical diffraction patterns.

$$dd^* = f\lambda, \text{ where}$$

d (cm.) = repeat distance in micrograph ;

d^* (cm.) = corresponding repeat in pattern ;

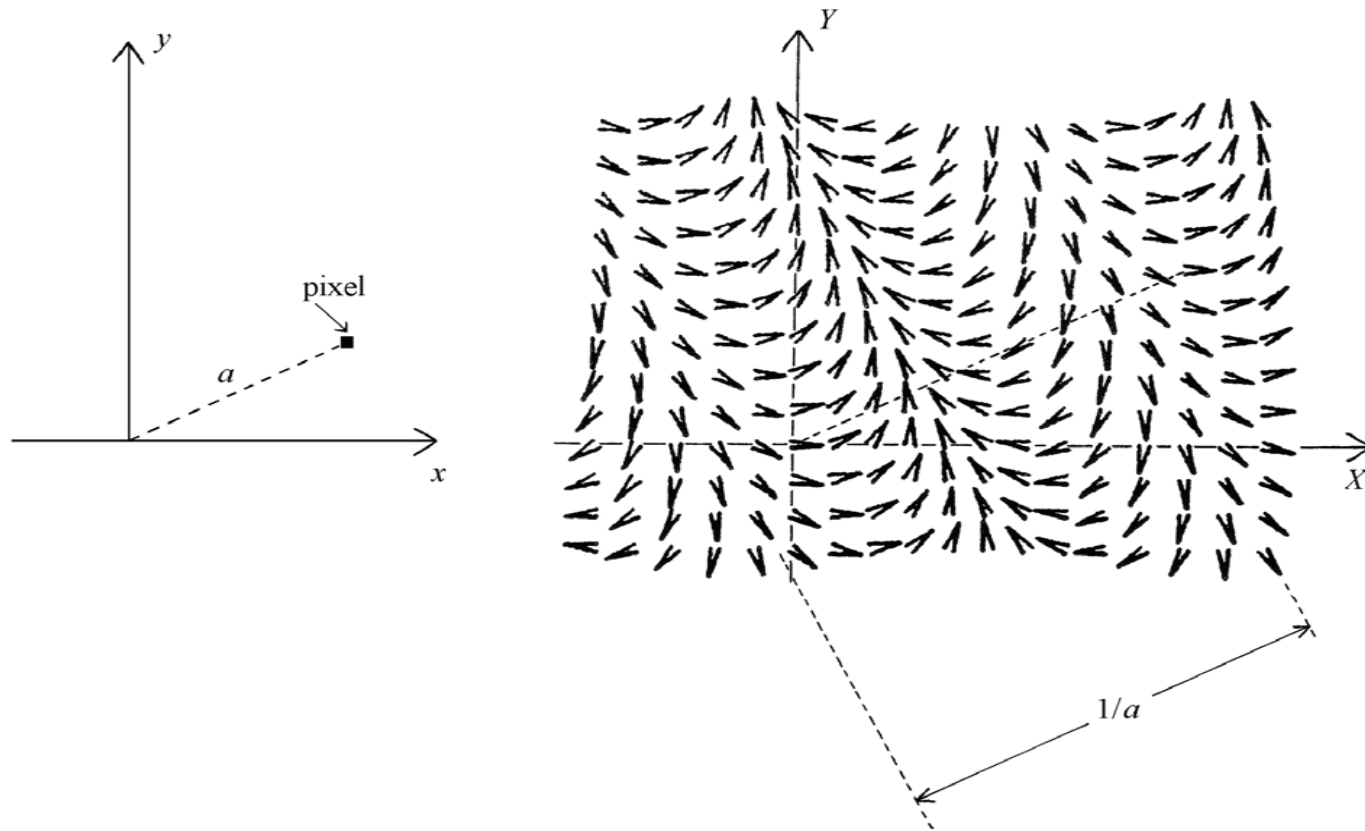
f (cm.) = distance from micrograph to pattern ;

λ (cm.) = wavelength of light.

Analogue & digital F.Ts.

- Optical diffractometers give **continuous** F.Ts., but only their amplitudes, neither their phases nor numbers.
- Computers, which provide these, input and output **discrete** numbers.
- So they calculate the Discrete (or Digital) Fourier Transform (**DFT**), which has **sampled** input and output.

Getting a DFT: basic principle.



- Divide image into pixels.
- Each pixel \rightarrow phase-wave.
- Calculate its vectors at each point of F.T.

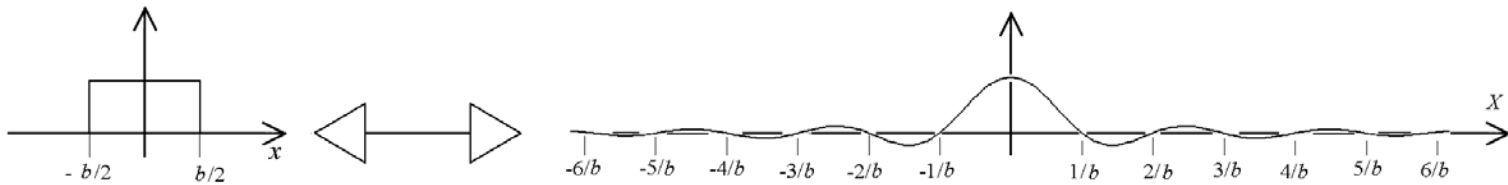
Size of DFT calculation.

- Image has N pixels, and F.T. has N sampling points, each with a vector.
- For each image pixel, find every F.T. vector and add it to F.T. at that sampling point.
- So no. of calculations is proportional to (no. of image pixels)(no. of F.T. sampling points).
- Each no. = N , so no. of calculations is proportional to N^2 .

Fast Fourier Transform (FFT).

- Simple FT: no. of calculations is proportional to N^2 .
- But the simplicity of sines/cosines allows a more efficient arrangement: the **FFT**.
- FFT: no. of calculations is proportional only to $N \cdot \log(N)$ [using logarithms to base 2].
- Therefore the calculations take only $\log(N)/N$ times as long.
- Thus, for a 1D image with $N = 1024$ [where $\log(N) = 10$] the FFT is a hundred times faster.
- For a 2D image, with N^2 pixels, the speed gain would be 10000.

Preparing for the DFT: image selection.

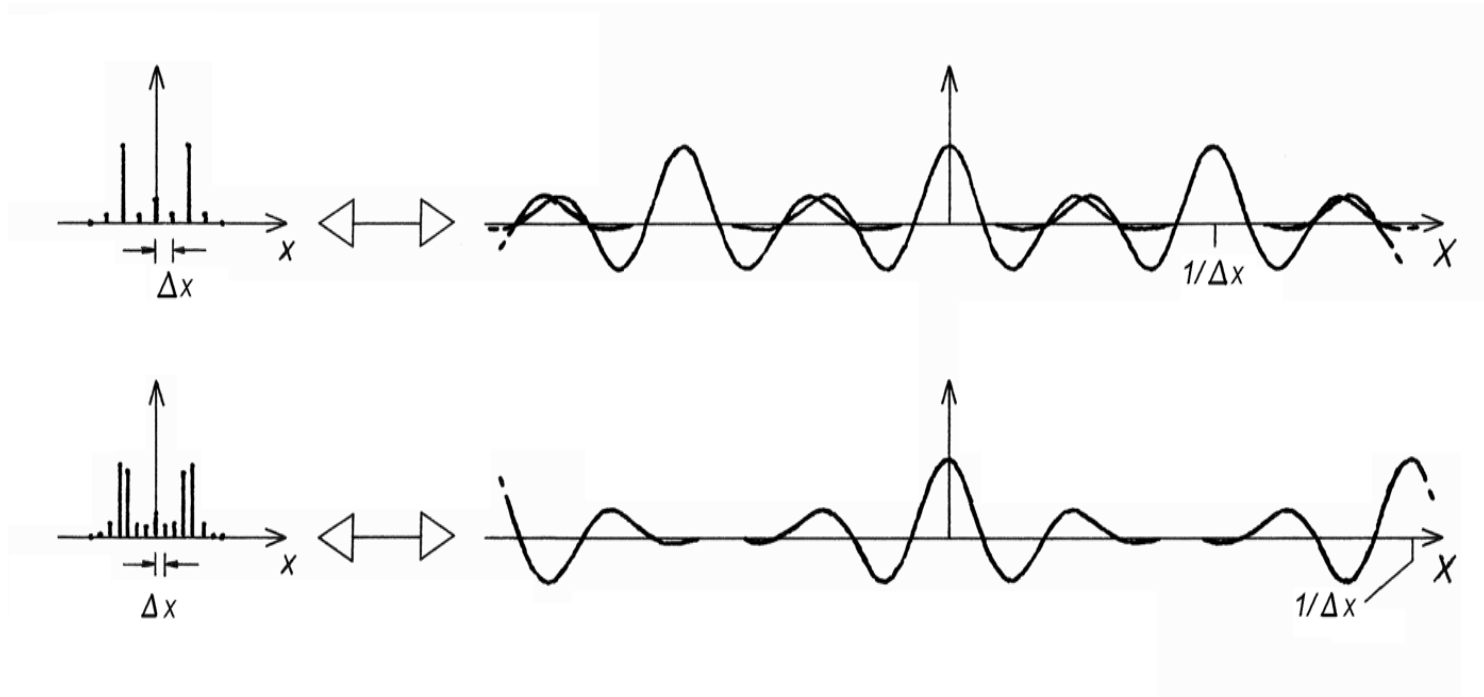


- Isolating particle with a mask = “*boxing*”.
- But the sharp edges of the box interfere with the F.T.
- So we remove these by minimising the density change at the edges (“*floating*”) and blurring them (“*apodization*”).

Preparing for the DFT: image digitisation.

- Chosen particle image is now isolated and surrounded by a blank (zeros).
- The image is densitometered, but we must choose **how finely to sample it**.
- We need to surround the image data with an area of zeros, and must choose how wide to make this “**zero-padding**”.
- Zero-padding is important since the DFT implicitly makes both image and F.T. **periodic**.

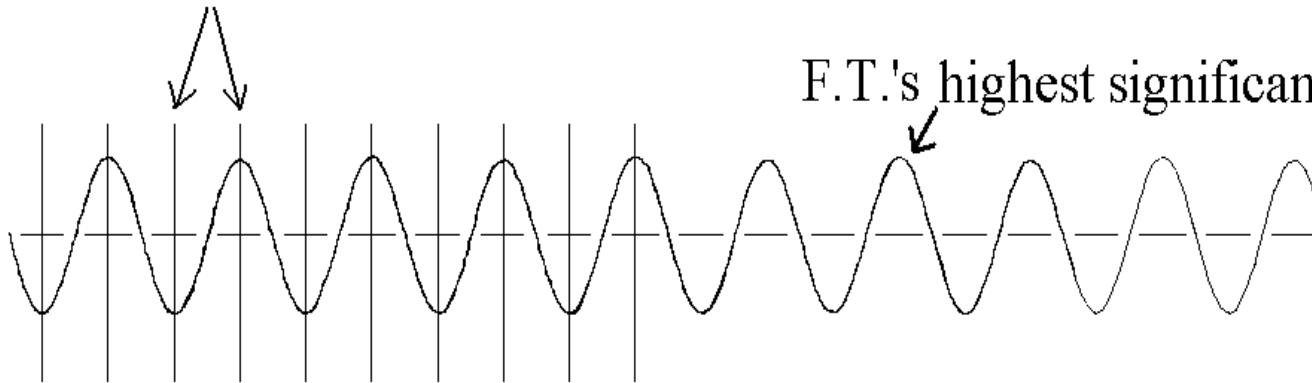
Need for fine image sampling: **aliasing**.



- DFT has copies of image's FT separated by $1/(\text{sampling distance in image})$.
- This separation distance must be big enough to avoid overlap of neighbouring copies of image's F.T.
- Overlap affects outer parts of F.T., i.e. higher spatial frequencies of image.

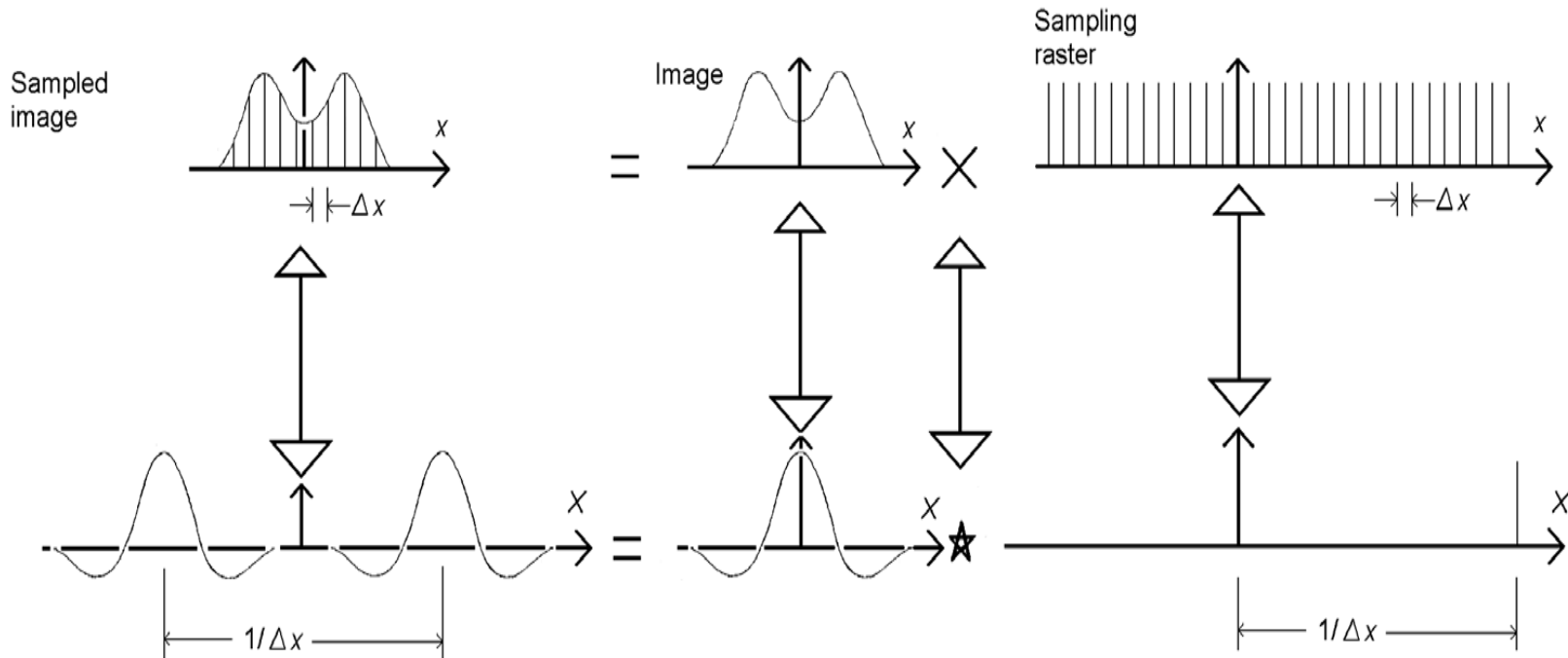
Nyquist critical sampling frequency.

Nyquist sampling frequency



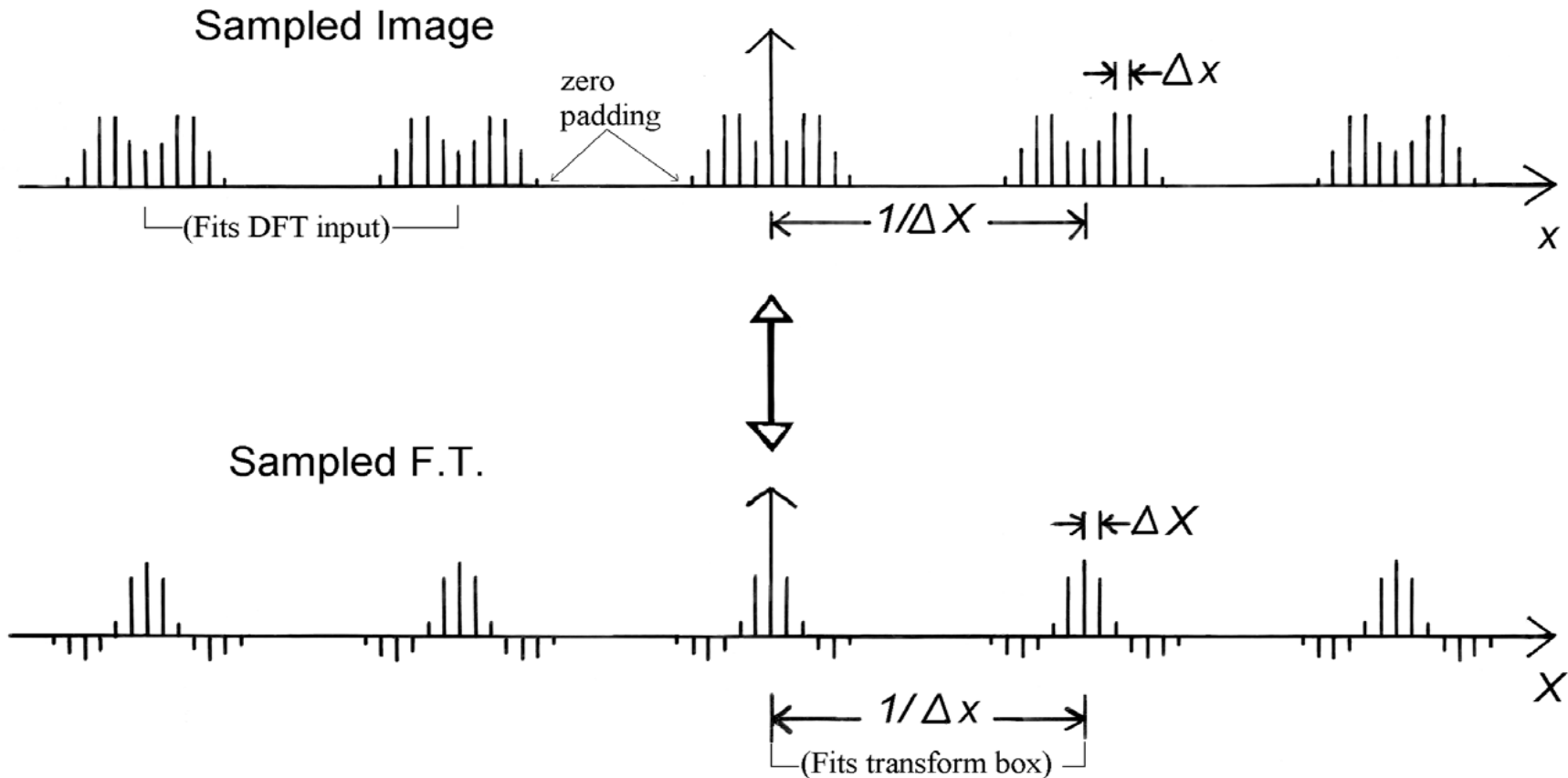
F.T.'s highest significant spatial frequency

DFT = copies of sampled image's F.T.



- Sampled image = continuous image \times sampling raster.
- So $FT(\text{sampled image}) = FT(\text{image}) * \text{reciprocal lattice of sampling raster}$.
- Also, back-transformed image is convoluted with $FT(\text{sampling raster of FT})$.

Zero-padding \leftrightarrow fine sampling.



- Finer image sampling \rightarrow "zero-padded" transform box, avoiding aliasing.
- "Zero-padding" of image \rightarrow finer transform sampling, allowing interpolation.

Scaling DFTs.

$dd^* = \Delta x(N/M)$, where

d (nm) = repeat distance in specimen;

d^* (transform units) = length in DFT;

Δx (nm) = pixel-pixel distance in micrograph;

M = magnification of micrograph;

N = no. of transform units along box side.

Example.

A micrograph taken with a magnification of 40000 was scanned using a raster of 25 microns, and the transform was calculated at 256 points (transform units) along the box edge.

The specimen contains a repeat of 40 Å; at how many transform units will lie the corresponding peak?

$$d = 40 \text{ \AA} = 4 \text{ nm}; \quad \Delta x = 25 \mu\text{m} = 25000 \text{ nm}; \quad N = 256; \quad M = 40000.$$

$$\text{So } 4d^* = 25000 \times 256/40000 = 6625/40.$$

$$\therefore \underline{d^*} = 6625/40 \times 4 = \underline{41.4 \text{ transform units.}}$$

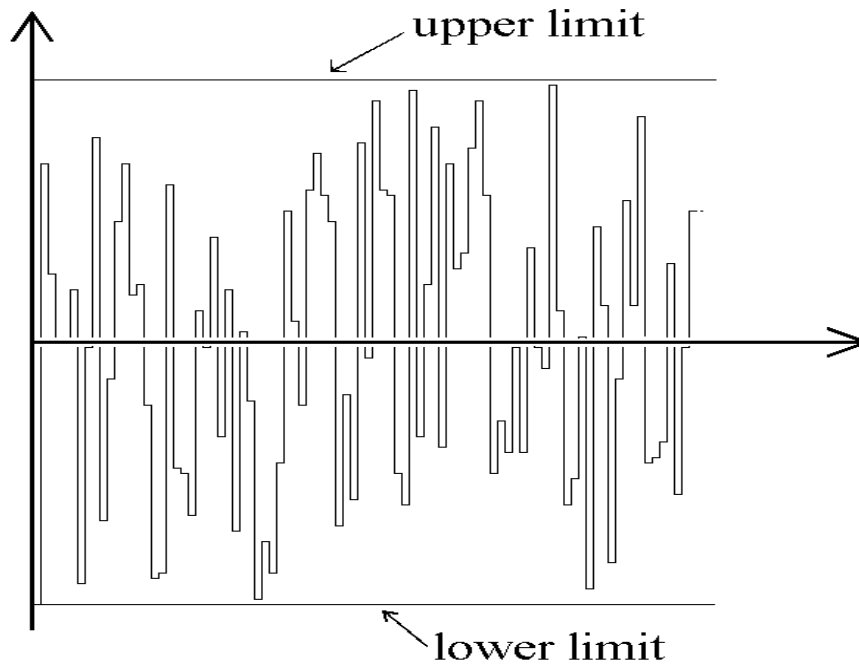
IMAGE PROCESSING: IMAGE AVERAGING.

- Reducing micrograph noise needs many different images to be **averaged**.
- This can be done by **Fourier filtering**, when the particle has translational symmetry (e.g. 2D crystals or helices).
- Alternatively, different particles can be averaged, but this requires us to know their **relative positions**.
- These are found by **matching** the particles' images.

Fourier filtering.

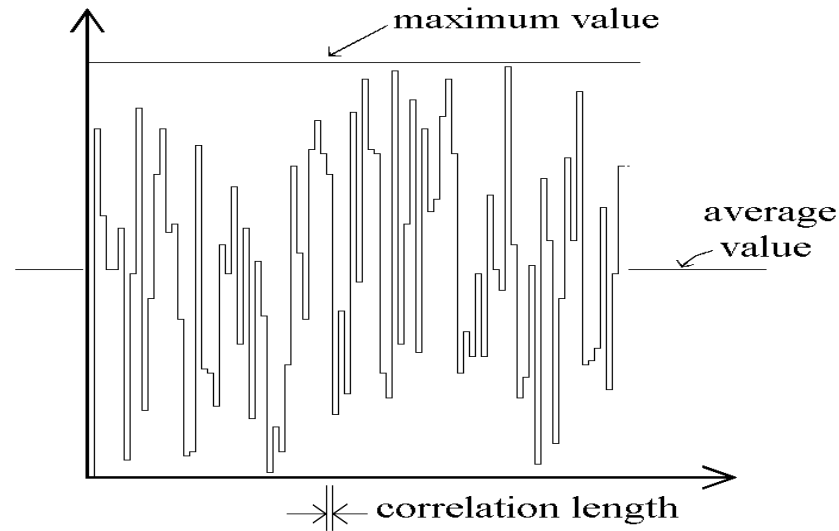
- This is a 3-stage process:
- (1) take F.T. of noisy image of specimen with subunits in a lattice.
- (2) In F.T., select peaks at reciprocal lattice points.
- (3) Back-transform just these selected peaks.
- This removes much of the image **noise**.
- To understand it, we need FT(noise).

F.T.(+/-noise).



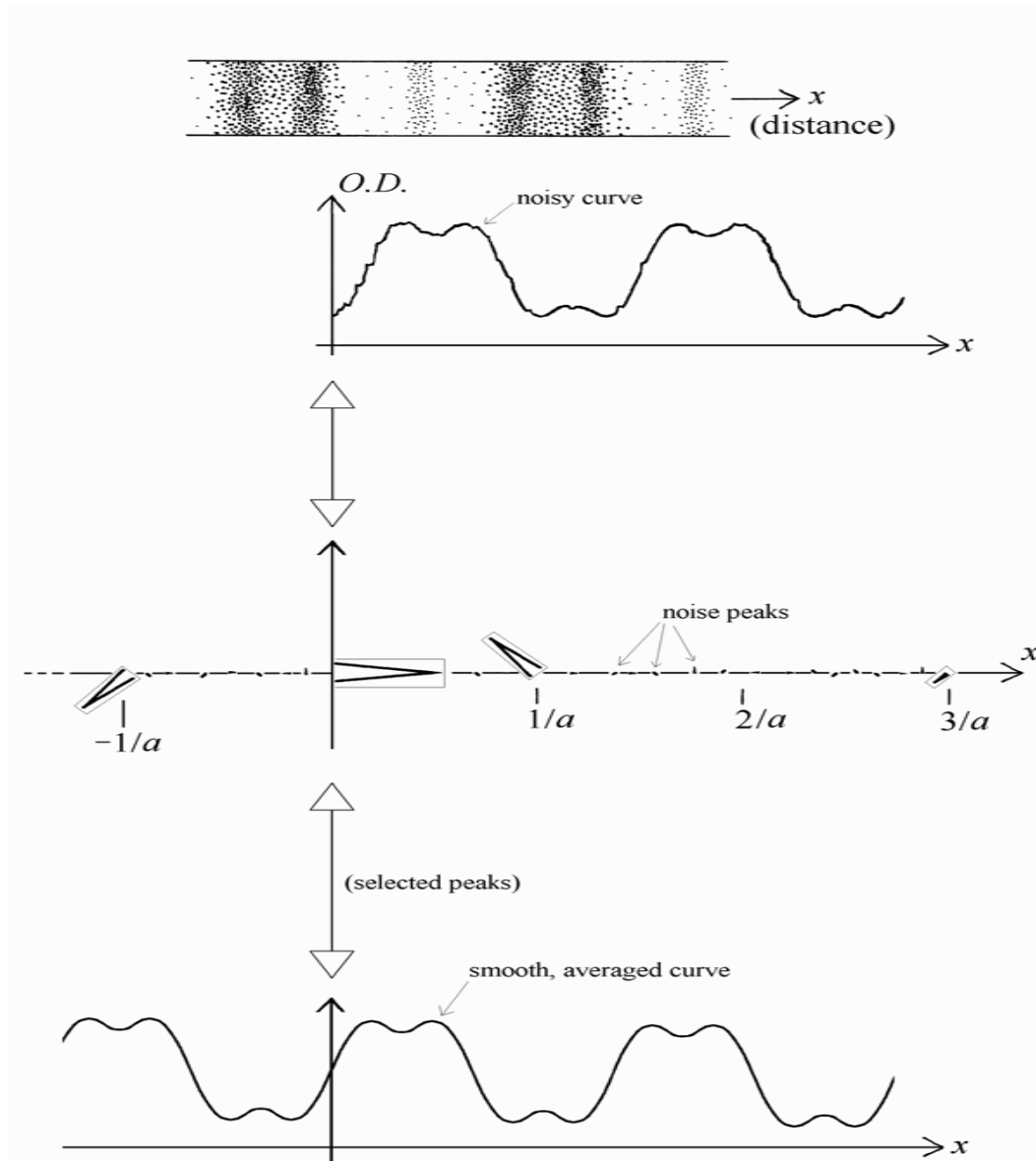
- Noise lacks any pattern, so it has no predominant spatial frequency.
- So there is no frequency peak in its F.T.
- Also, noise is the most complex data (given max. value and correlation length).
- So its F.T. must also be the most complex, i.e. noise; but not the same noise (there is more variety in noise than in any pattern).
- Thus **F.T.(+/-noise) = [different] +/-noise.**

F.T.(+noise)

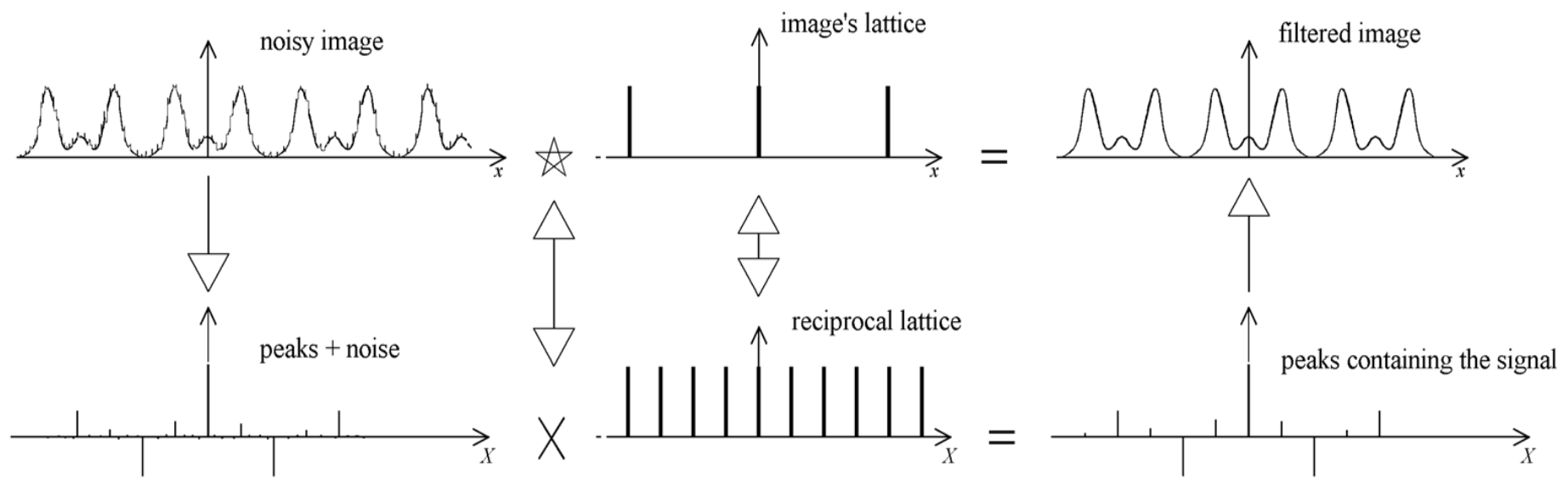


- This (like most) noise is always positive.
- So it equals +/-noise minus the [negative] lowest value.
- So its F.T. = F.T.(+/-noise) + F.T.(constant)
- This is: noise + peak at origin.

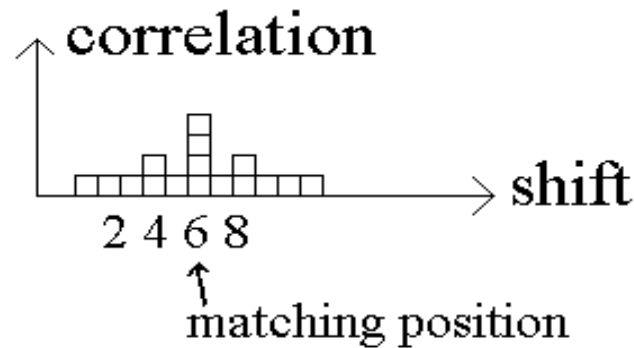
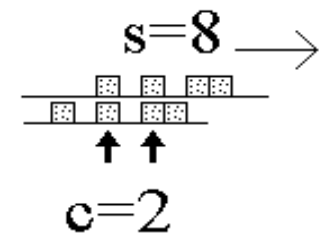
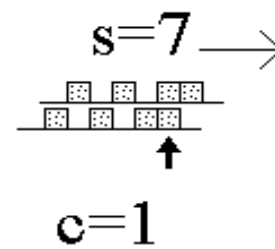
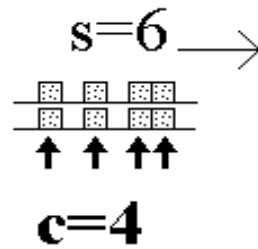
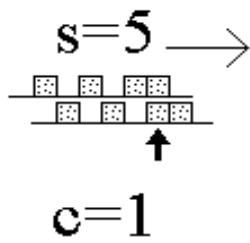
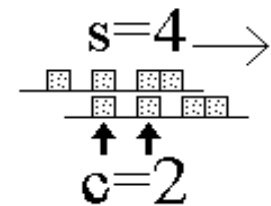
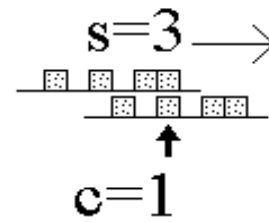
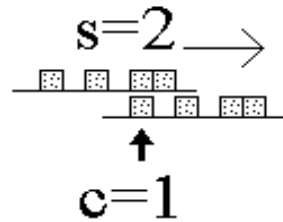
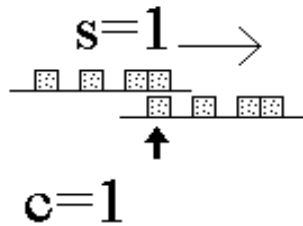
Filtering = noise removal.



Filtering = averaging repeats.



Matching images: the correlation function.



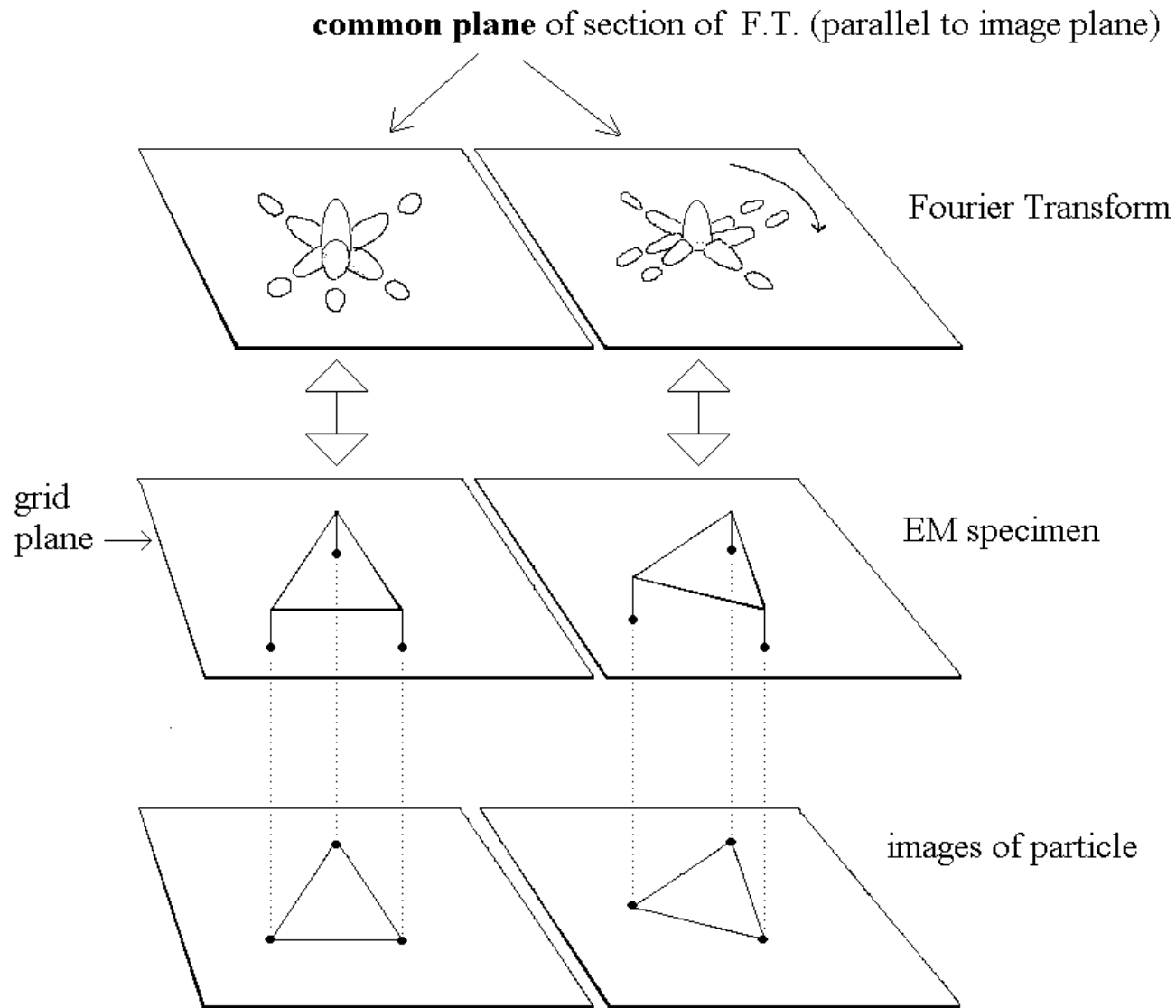
Correlation functions: ACF & CCF.

- Correlation functions can be used to find shifts &/or rotations needed to match images.
- Autocorrelation function (ACF) matches the image with itself.
- It has a peak when 2 copies are in register.
- Cross correlation function (CCF) of 2 different images.
- This has a peak where 2 images match best.

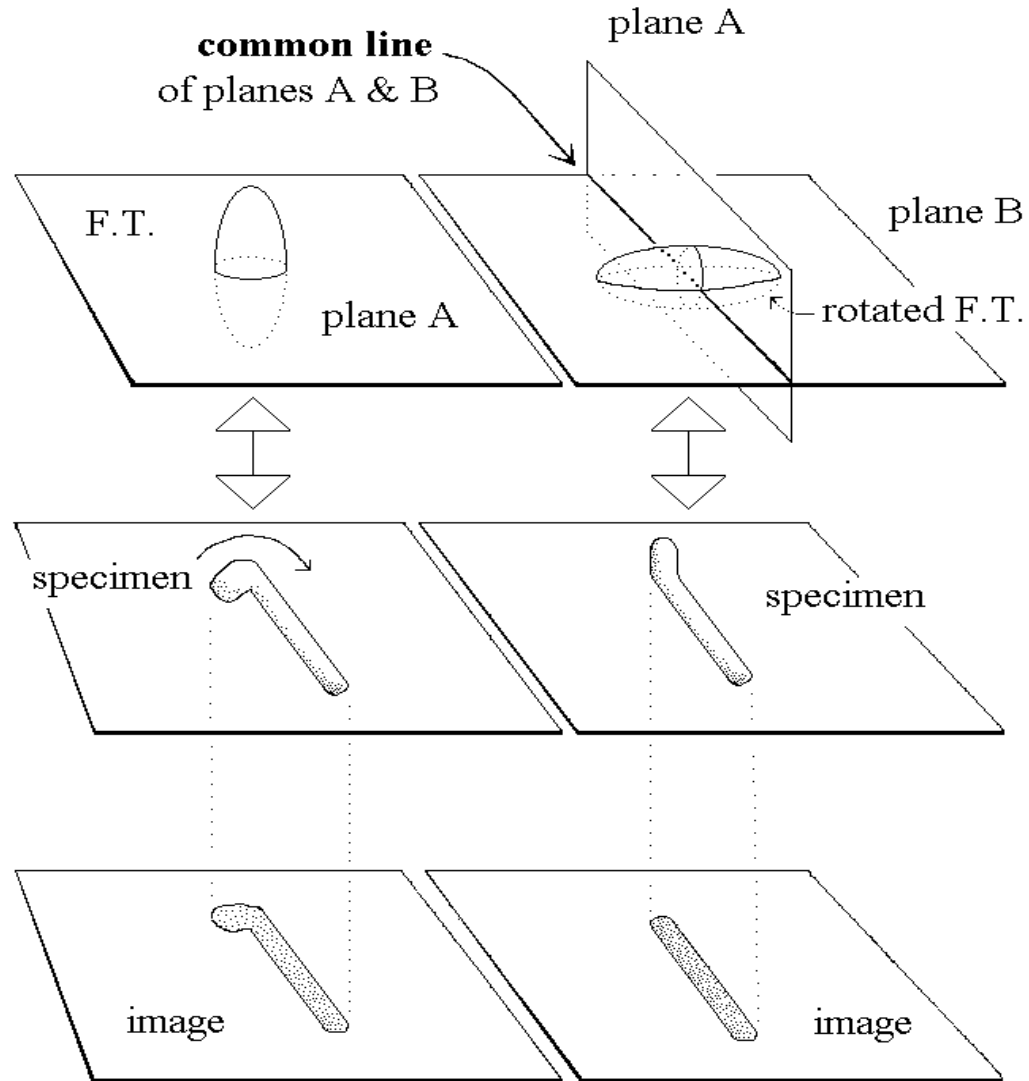
Matching images & F.Ts.

- Correlation functions relating 2 particles are usually calculated from the F.T. sections relating to their images.
- But which F.T. sections are used?
- This depends on the movement that related the 2 particles.
- **Pure shifts** show the same particle view or projection, so they give the **same F.T. section plane**.
- But **rotations** can give **different** F.T. planes.

Rotation parallel to microscope axis.



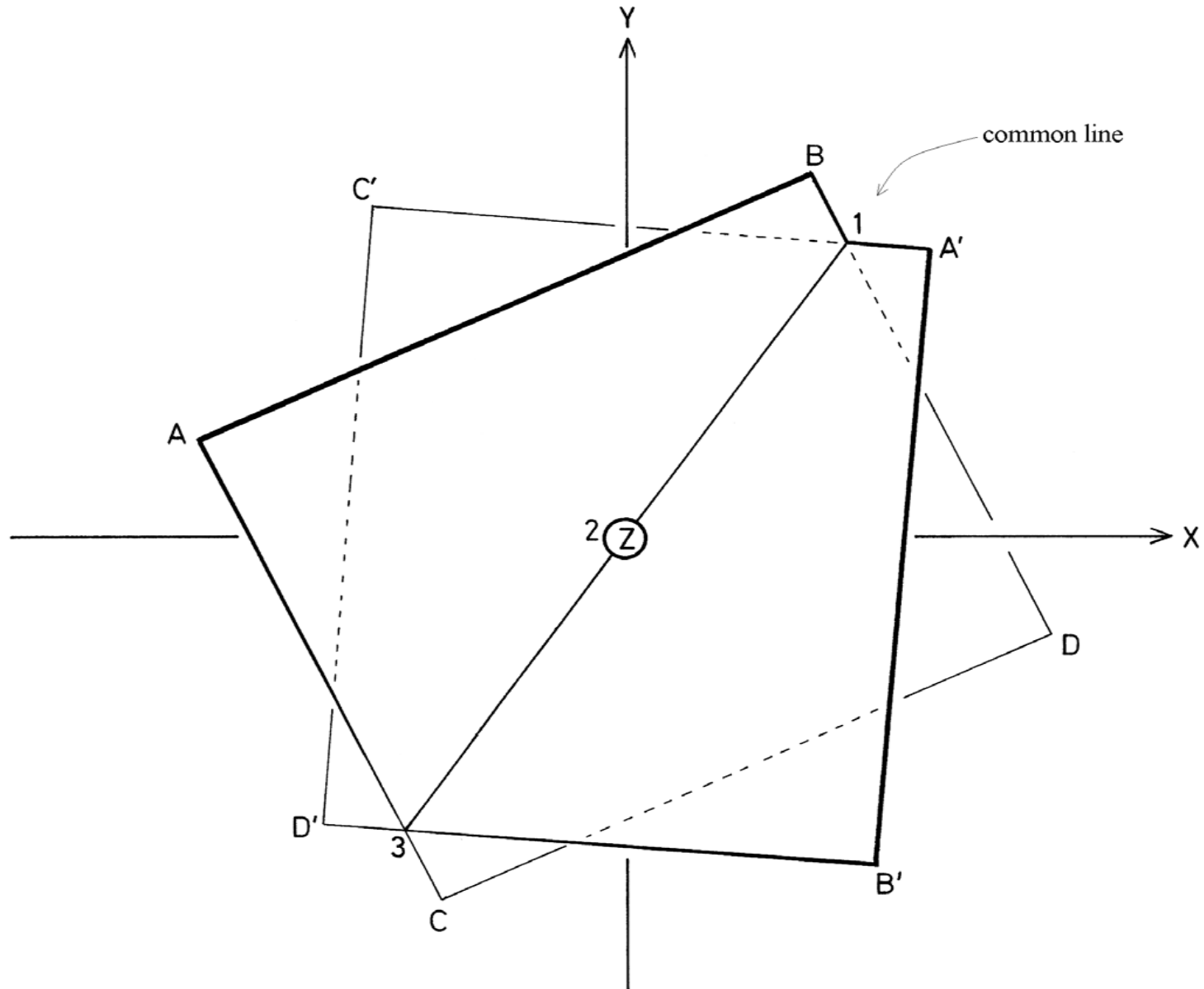
Rotation perpendicular to microscope axis.



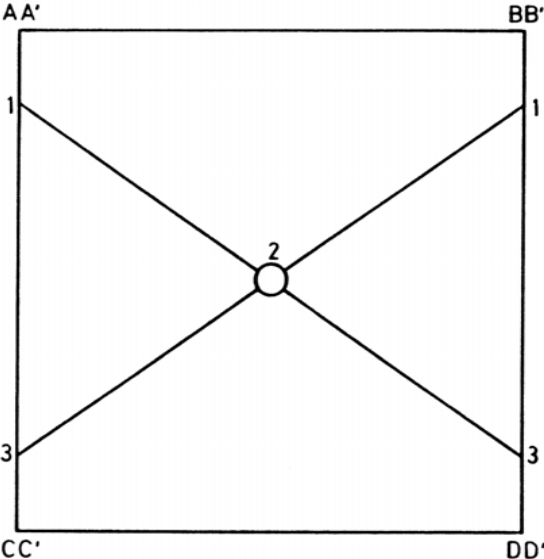
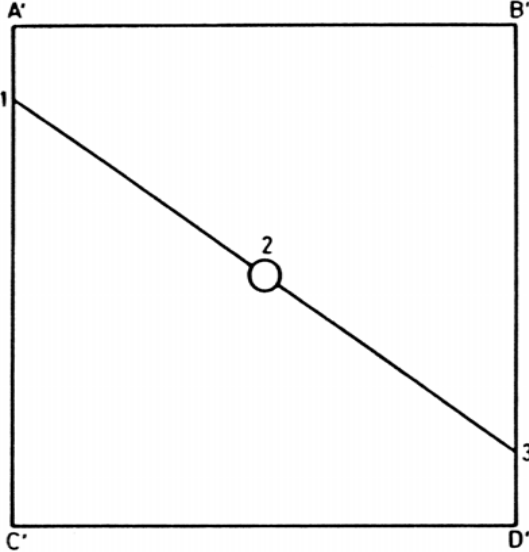
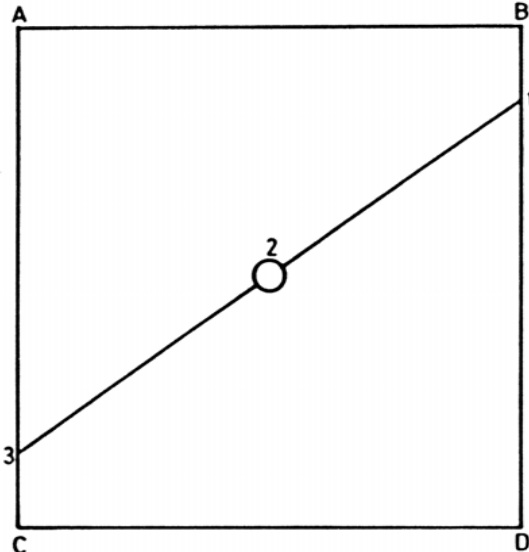
Comparing particles' F.Ts.

- If all particles show the *same* view (*i.e.* only shifts or rotations *parallel* to the microscope axis are possible), then we compare **common planes** of their F.Ts.
- If particles show *different* views (*i.e.* they can rotate about axes *not* parallel to the microscope axis), then we can only compare **common lines** of their F.Ts.

Symmetry produces common lines within the particle F.T. (1).



Symmetry produces common lines within the particle F.T. (2).



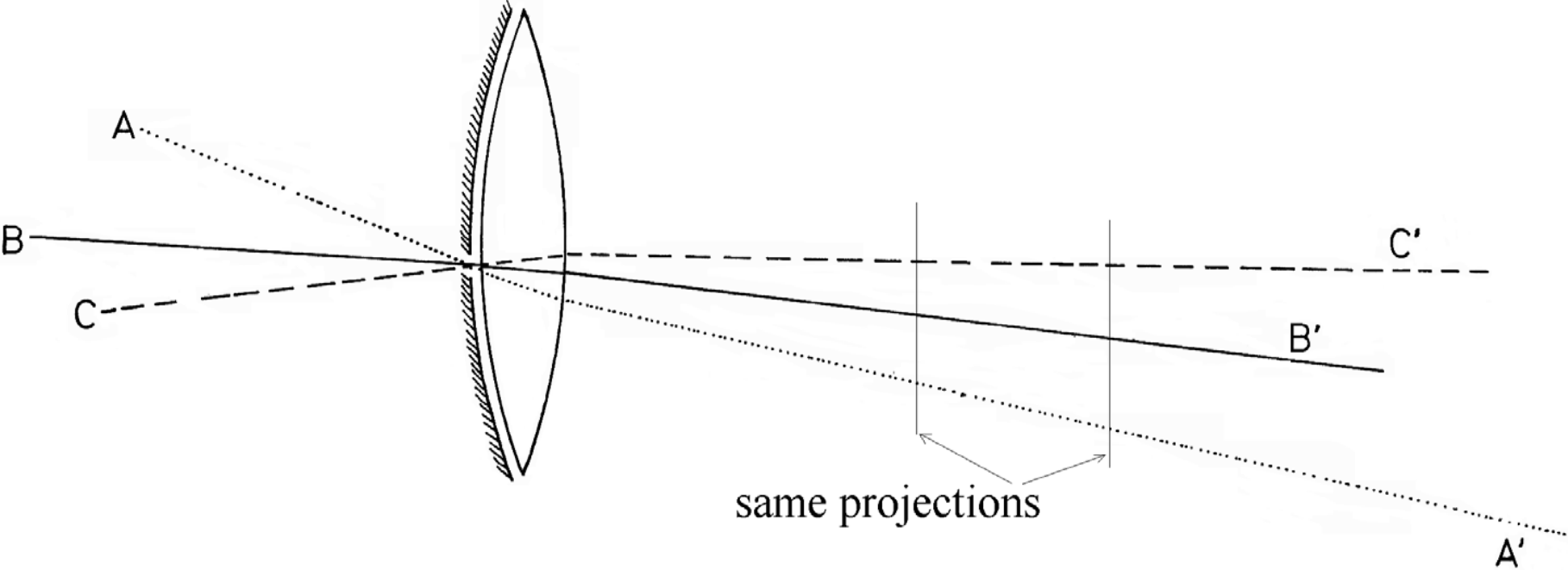
Common line pattern yields orientation information.

- **Inter-transform common lines (cross-common lines)** tell us about the relative orientations of the different particles.
- **Intra-transform common lines** tell us about the orientation of a particle's symmetry axes, i.e. about the particle's orientation.
- Particles with high symmetry (e.g. icosahedral) have complicated patterns of common lines that can determine their orientation accurately enough for 3D reconstruction.

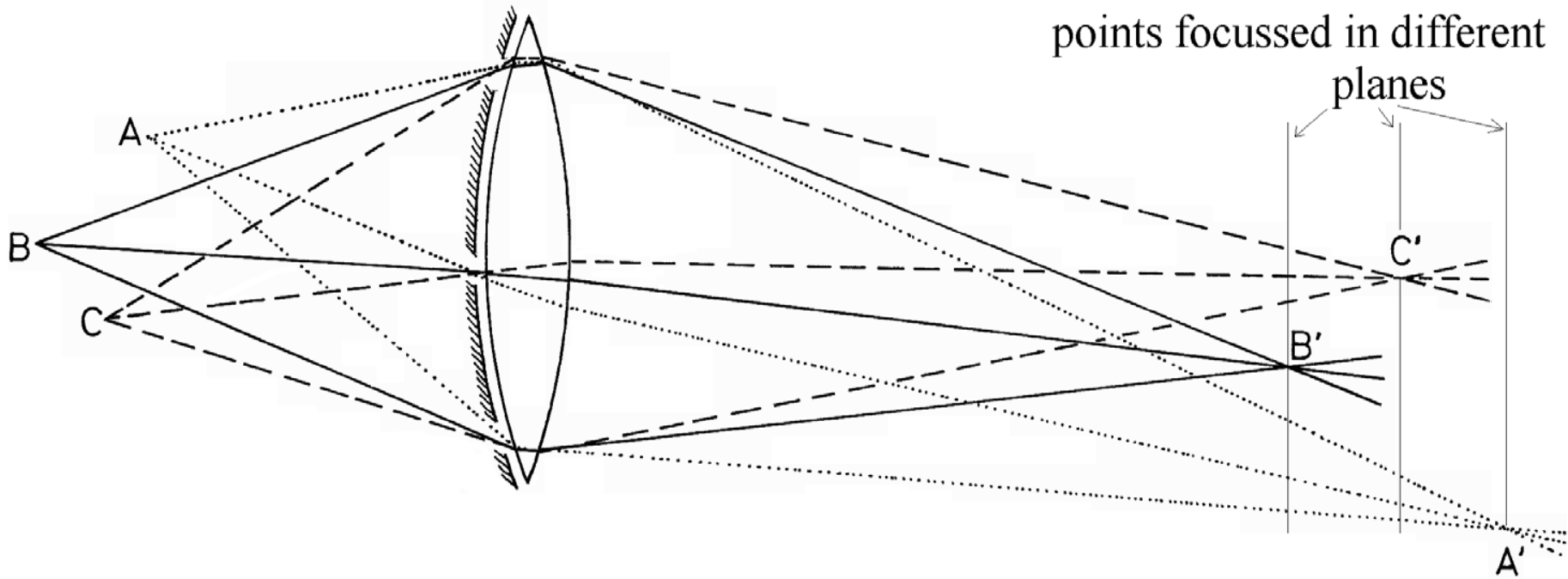
3D RECONSTRUCTION.

- Why is it so necessary in electron microscopy?
- Reconstructions classified by **space**: do they work in *real* or *reciprocal* space?
- Reconstructions classified by **data type**: do they use particle *symmetry*, or combine *different views*?

Narrow-aperture imaging yields projections



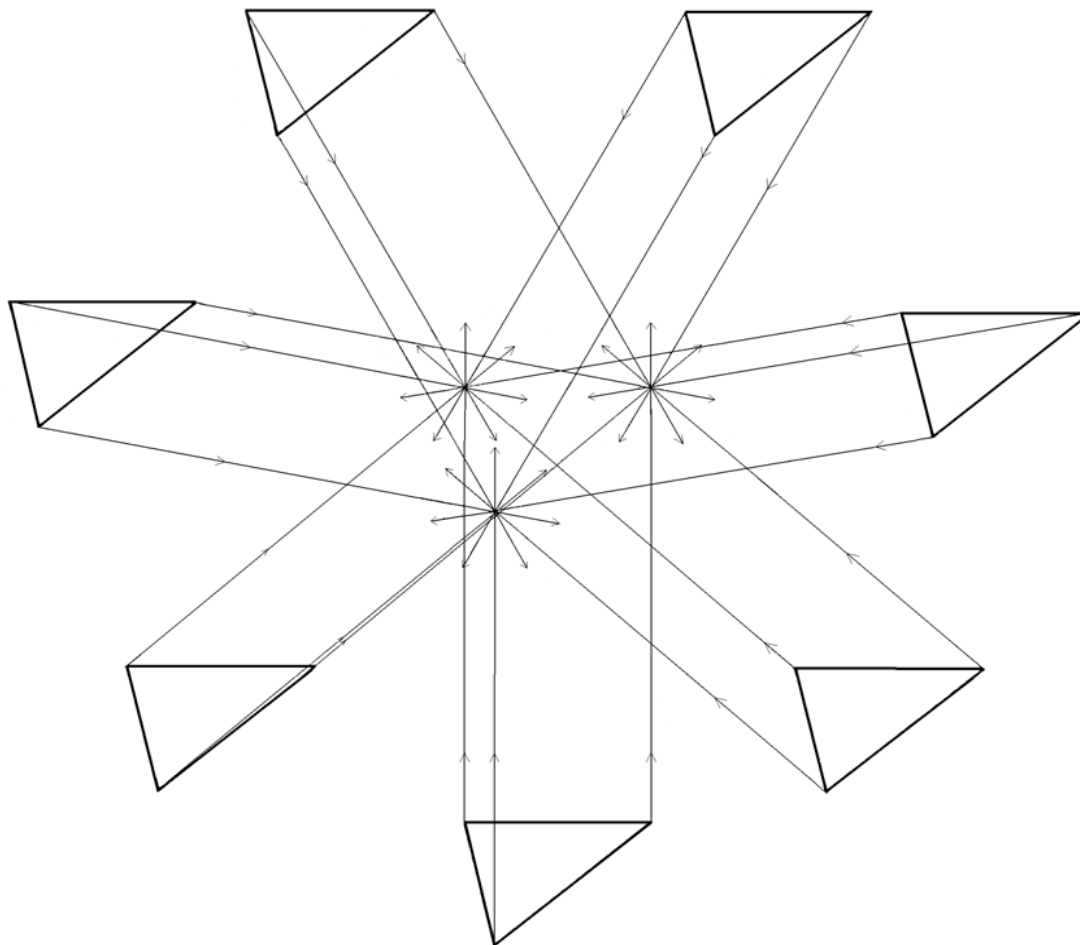
Wide-aperture imaging preserves some depth information.



Real-space 3D reconstruction: **back-projection.**

- Each electron micrograph corresponds to one pinhole image (single viewpoint).
- A wide-aperture lens *combines* pinhole images from *different* viewpoints to give depth information.
- So try combining, like a wide-aperture lens, micrographs taken from different viewpoints .

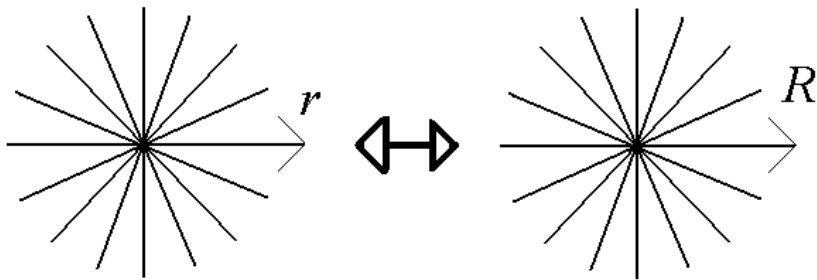
Back-projection.



Clarifying back-projection images.

- Simple back-projection yields images with every point surrounded by a fan of rays.
- Thus the image is the true image convoluted (*) with a fan of lines, so
B.P. image = (true image)*(fan of rays).
- To get the true image, we need to *deconvolute* the simple back-projection using FTs.
- $FT\{\text{B.P. image}\} = FT\{\text{true image}\} \cdot FT\{\text{fan of rays}\}$
so
 $FT\{\text{true image}\} = FT\{\text{B.P. image}\} / FT\{\text{fan of rays}\}$
- What is $FT\{\text{fan of rays}\}$?

F.T. {fan of rays}.



- The F.T. of each line is the perpendicular line, so
 $FT\{\text{fan of rays in } r\} = \text{fan of rays in } R$.
- With enough rays, a fan of rays in R is like a revolving thin propellor, *i.e.* is just a peak of density $1/R$.

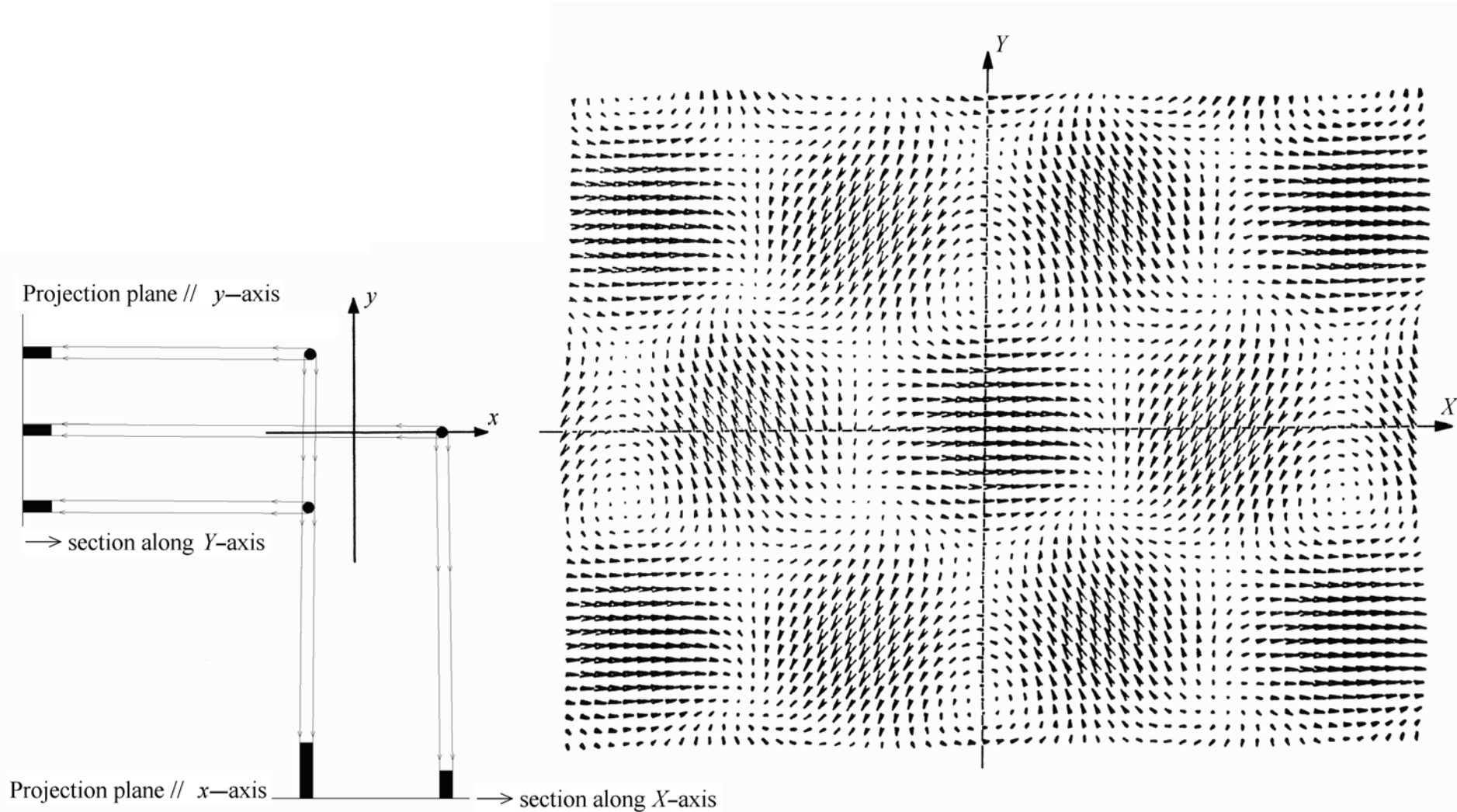
R-weighted back-projection images.

- $FT\{\text{true image}\} = FT\{\text{B.P. image}\}/FT\{\text{fan of rays}\}$, and $FT\{\text{fan of rays}\}$ is just a broad peak of density $1/R$.
- But dividing by a function of density $1/R$ is the same as multiplying by R , so:
- $FT\{\text{true image}\} = R \cdot FT\{\text{B.P. image}\}$.
- Thus the back-projection FT is **R-weighted**.
- Then the clarified image is obtained by back-transformation.

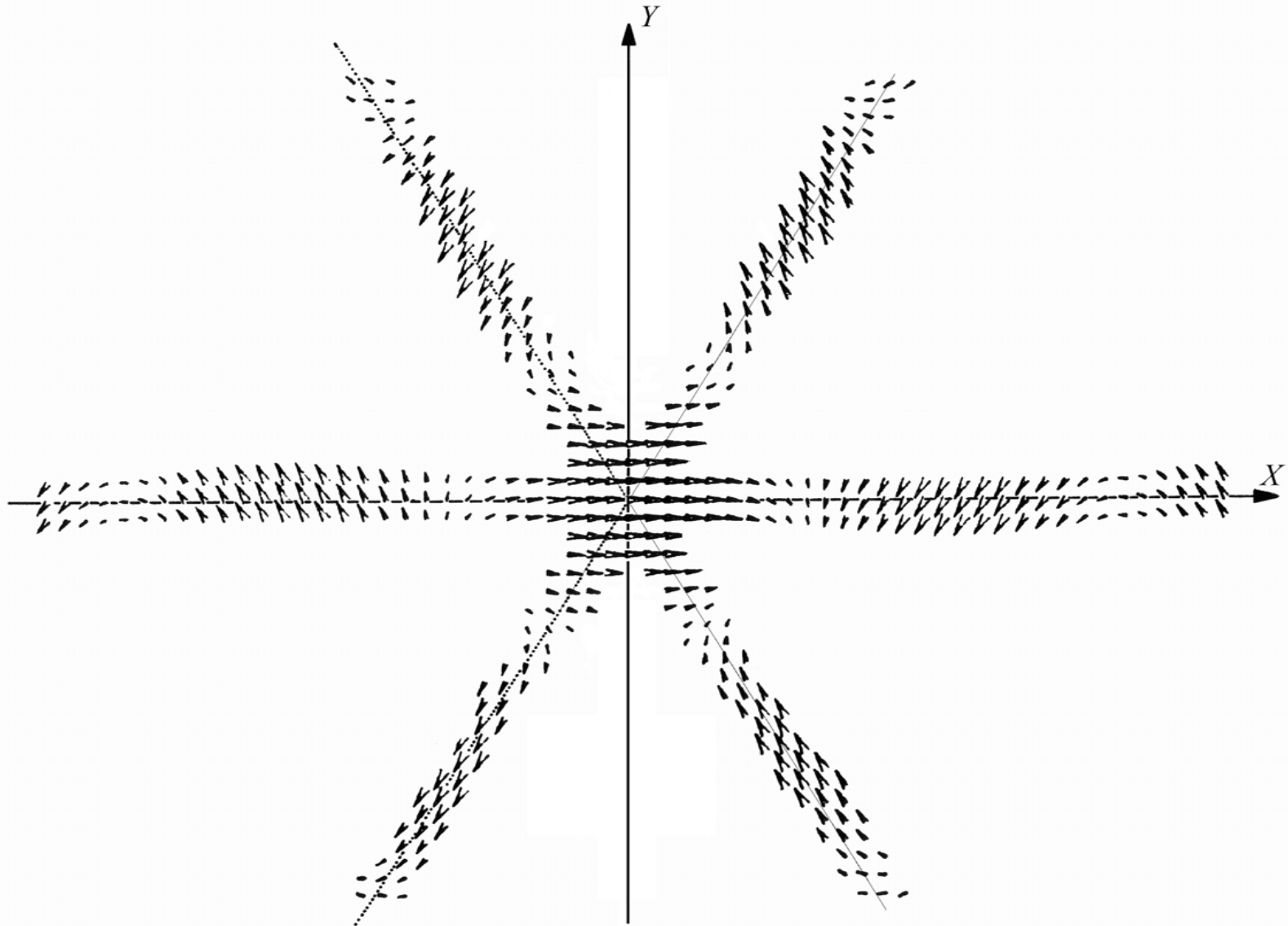
Reciprocal-space (Fourier) 3D reconstruction.

- This starts from the F.T. inversion theorem: the 3D F.T. of a structure can be inverted to give the structure.
- Therefore our task is to find the 3D F.T. of the structure of interest.
- Each image gives us one projection; by the projection theorem, its F.T. is a parallel central section of the 3D F.T.
- Enough central sections can be built up to give us the 3D F.T. and hence the 3D structure.
- But how can 2D sections give us a 3D F.T.?

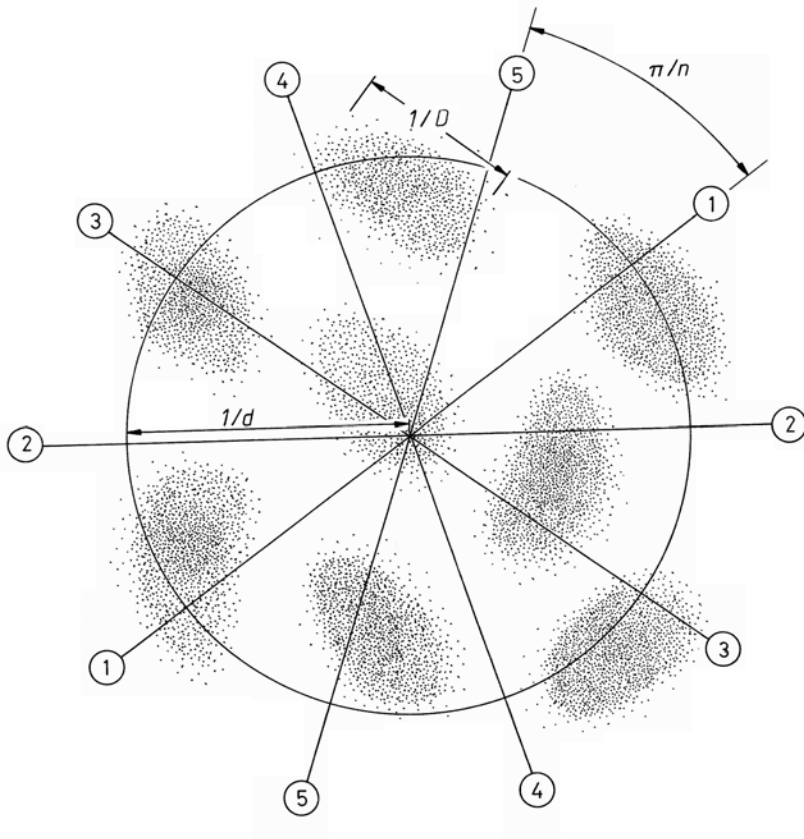
Fourier 3D reconstruction: different views.



Fourier 3D reconstruction: symmetry.



What resolution shall we get?



- A particle of width D has an F.T. made of “lumps” roughly $1/D$ across.
- We can find the whole F.T. provided our sections don’t miss any “lump”.
- For n sections, this will be true until the F.T. radius is π/n .
- That F.T. radius sets the reconstruction resolution:
- $(\pi/n)(1/d) = 1/D$ or
- $d = \pi.D/n$.

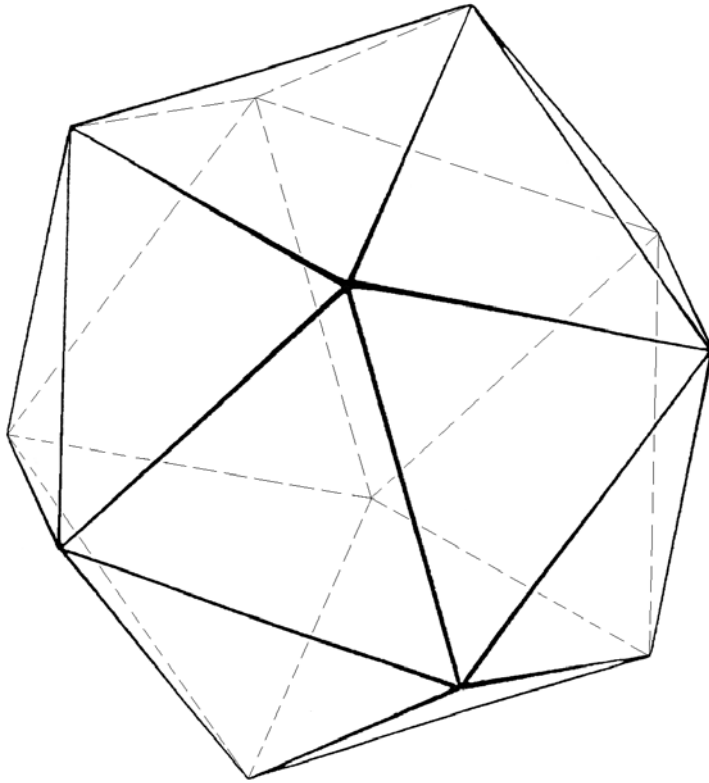
Reconstruction by combining different views.

- Each view gives a different F.T. section.
- Resolution is defined by the F.T. radius within which all transform “lumps” are defined by an experimental a F.T. section.
- Main problem is to get adequate resolution in all directions (e.g. “missing cone”).

Reconstruction using particle symmetry.

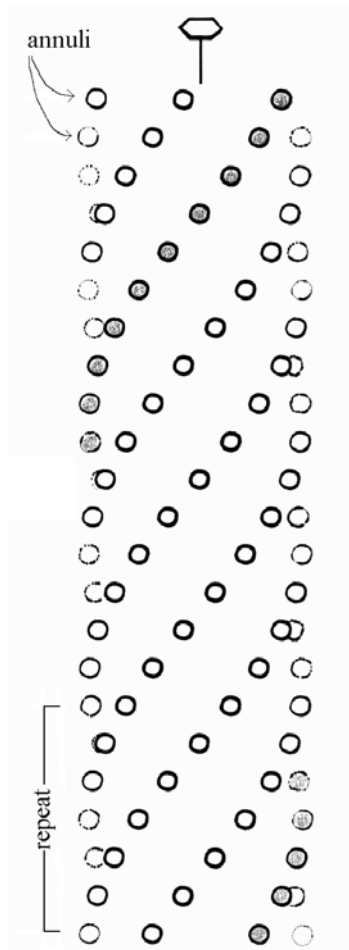
- Symmetrical particles have subunits in different orientations.
- Each orientation corresponds to a different view, potentially a contribution towards a 3D reconstruction.
- Two symmetry-types (*icosahedral* & *helical*) have enough orientations to get a useful reconstruction from **one** particle.
- Reconstructions from such particles use reciprocal (Fourier) space methods.

Icosahedral particles provide 60 views.



- The icosahedron has 12 faces.
- 3 subunits can be accommodated symmetrically per face.
- So $3 \times 12 = 60$ subunits can be accommodated symmetrically in a particle with icosahedral symmetry.

Helical particle shows new views within its repeat.



- Particle consists of annuli of 6 subunits each.
- Repeat = 7 annuli, so the particle provides 7 different views of the subunit.
- The particle contains several repeats; these contribute to averaging of the structure.