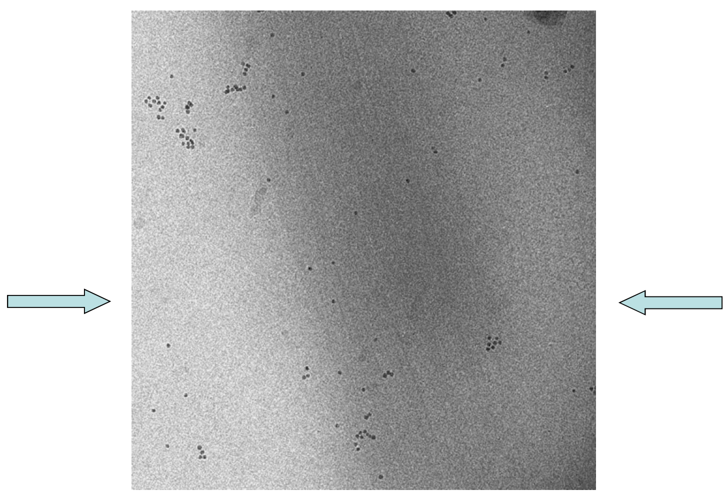
Principles and General Aspects of Electron Tomography

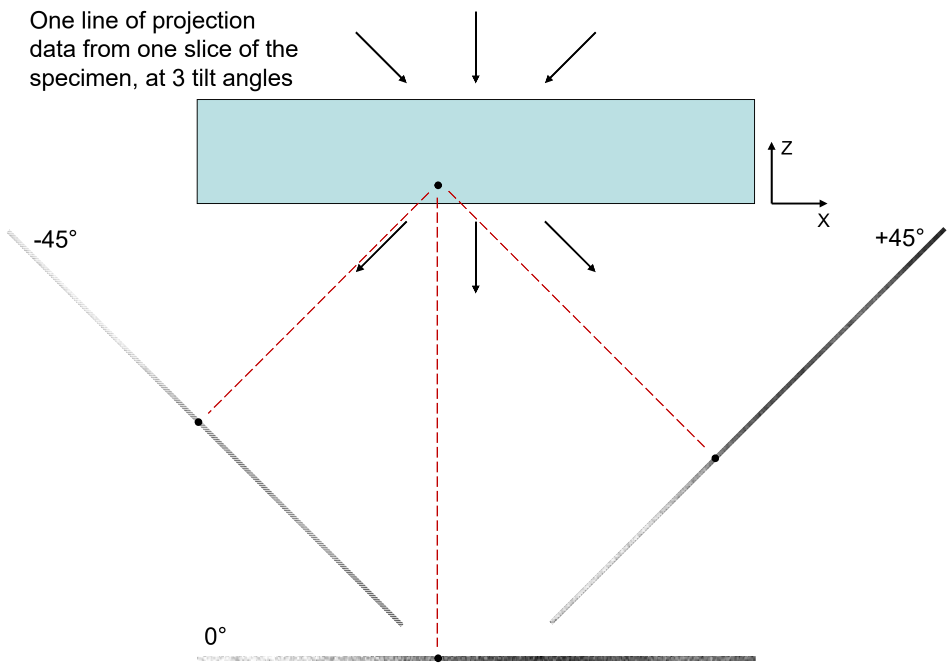
Aligned Cryo-Tilt Series of Flagella of a Giardia Cell

±60º at 2º increments



Horizontal line of projection data

One line of projection data from one slice of the specimen, at 3 tilt angles

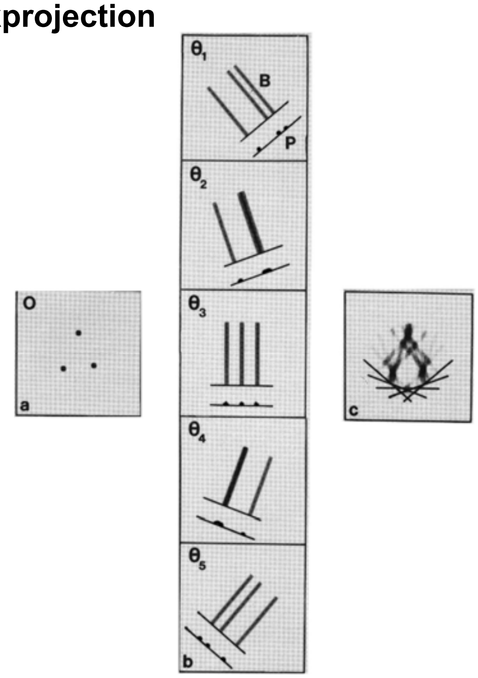


Backprojection

P - Projection of 3 points onto a line at various tilt angles

B – Backprojection of projection data into reconstruction volume

(From Radermacher, 1992)

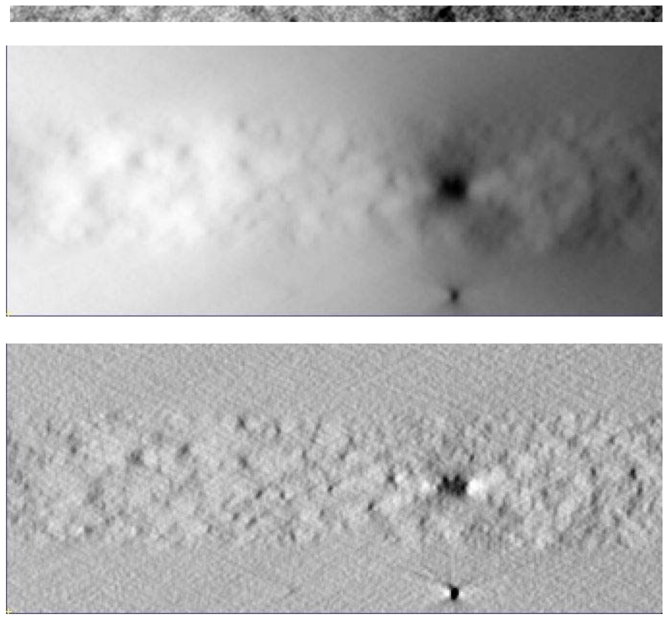


Backprojection without and with R-weighting

Nearby lines of tilt series image at 0°

X/Z slice of backprojection without R-weighting

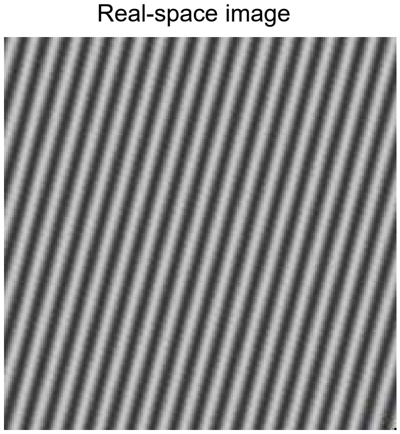
X/Z slice of backprojection with R-weighting



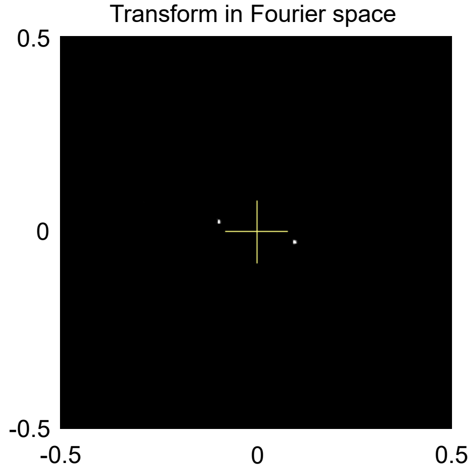
Fourier Transform of Sine-wave Image

- Every image can be represented as a sum of sine waves at various frequencies and orientations

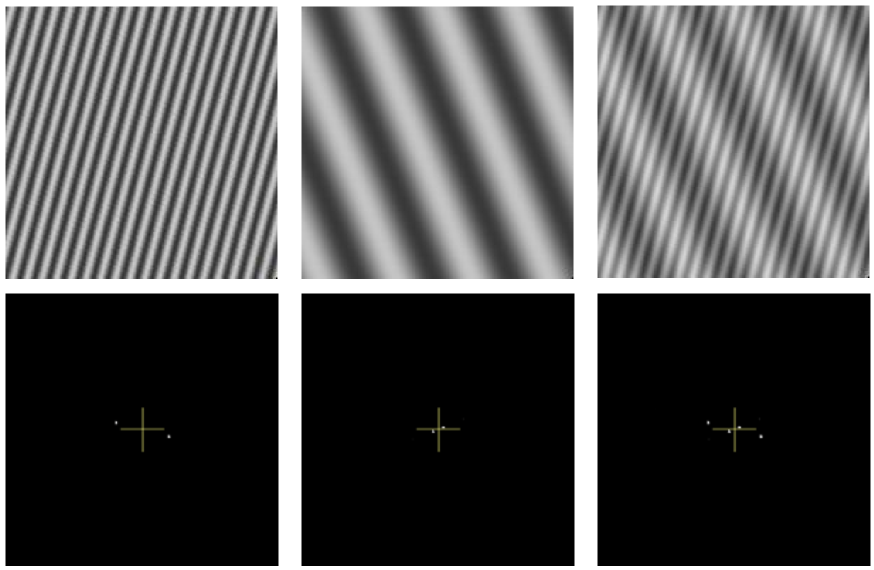
Real-space image



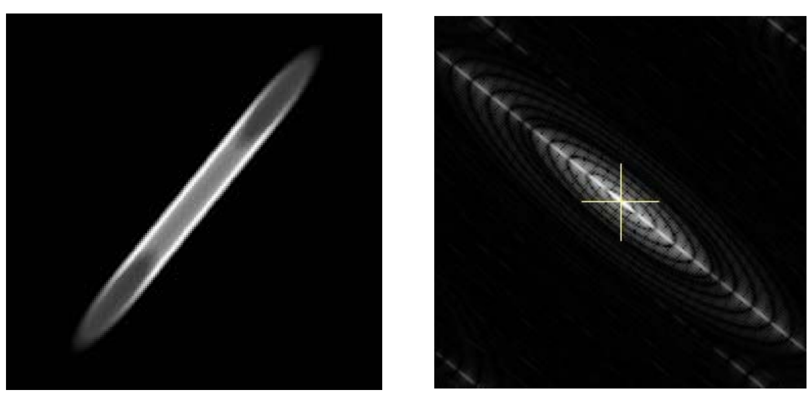
Transform in Fourier space



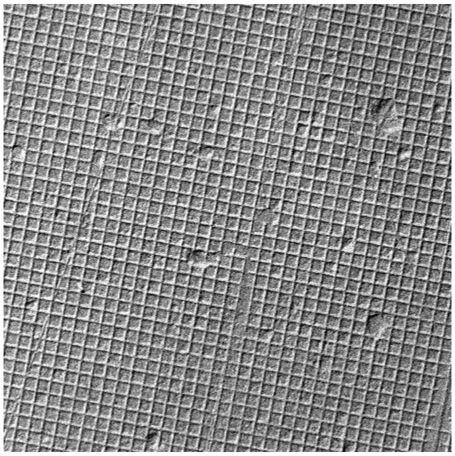
Sine-wave Images and Fourier Transforms

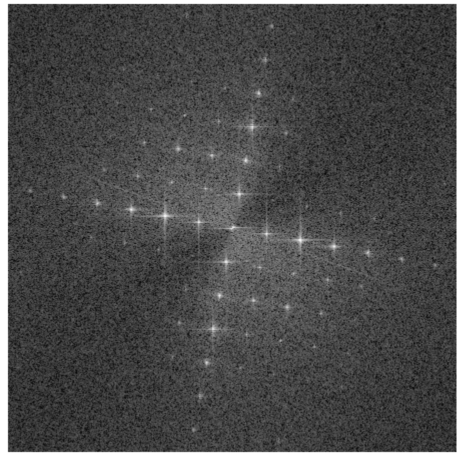


Fourier Transform of Isolated Ideal Structure

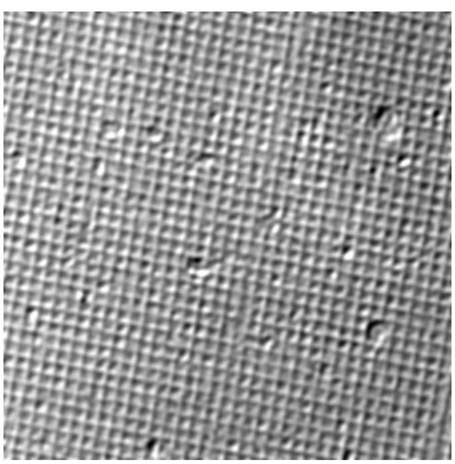


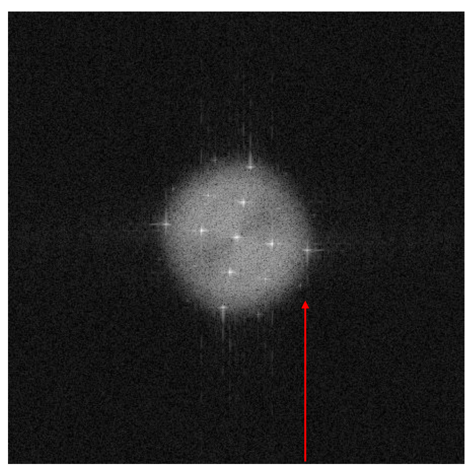
Fourier Transform of Periodic Structure





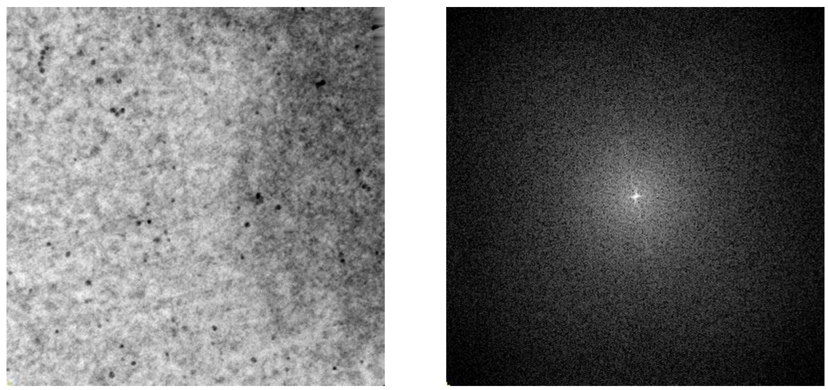
“Low-pass” Filtering Removes High Spatial Frequencies (Fine Details)



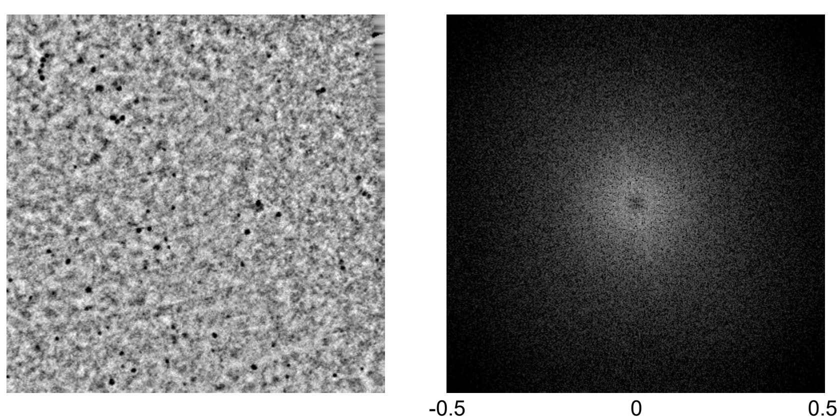


Frequencies above ~0.15 cycles/pixel filtered out

Fourier Transform of Image without Periodic Structure



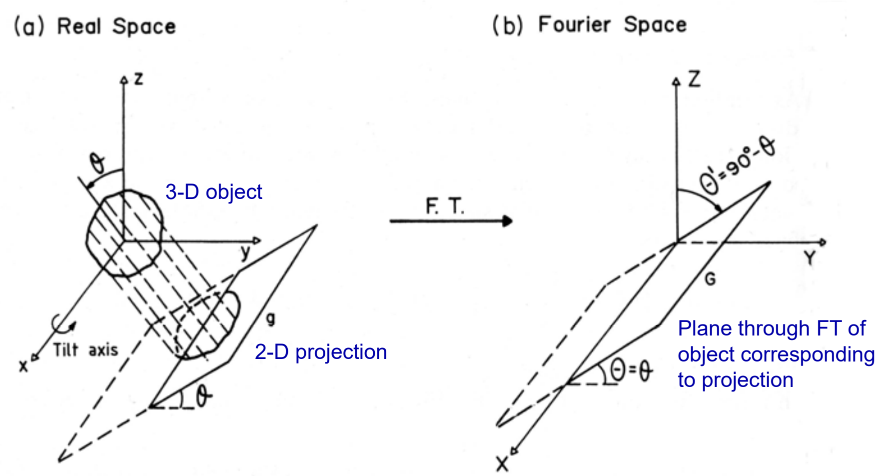
“High-pass” Filtering Removes Low Spatial Frequencies (Large-scale Intensity Changes)



Frequencies below ~0.03 cycles/pixel filtered out

The Central Section Theorem

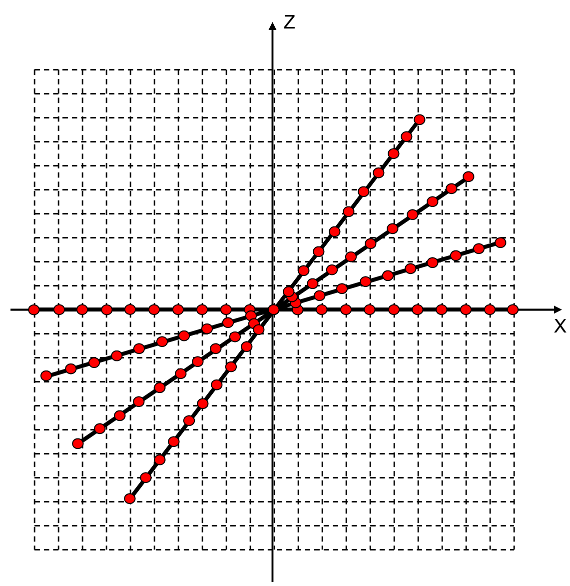
The 2-D Fourier transform of a projection image is a central plane through the 3-D Fourier transform of the object



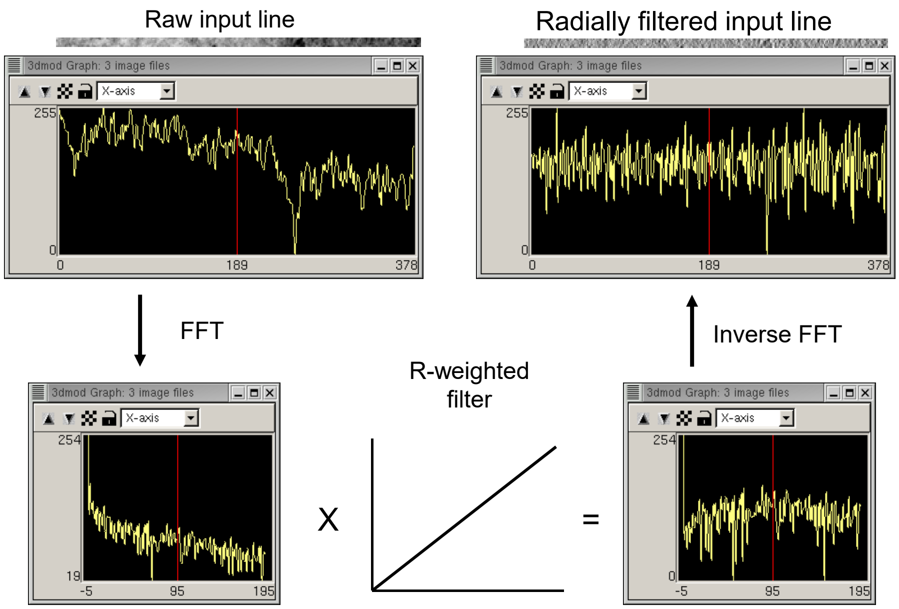
(From Carazo, 1992)

Problems Revealed by Projection Data in Fourier Space

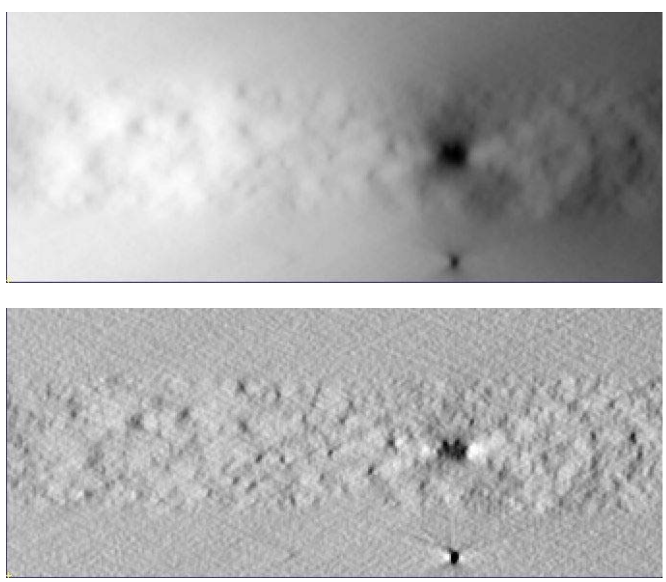
- Sample points from projection lines do not fall on rectangular lattice -> interpolation needed for inverse Fourier method
- Fourier space is oversampled near the origin -> R-weighting needed in backprojection
- Samples are too sparse at high frequencies -> resolution is limited by number of projections
- Samples are nonexistent above highest angle -> missing wedge of data



Radial Filtering of Projection Line before Backprojection



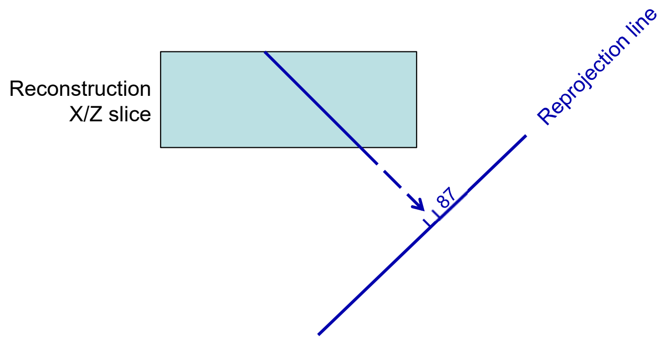
Backprojection Without and With R-weighting



R-weighting keeps low frequencies from dominating

Iterative Reconstruction

- In an iterative method, each iteration involves:
  - Reprojecting from the current estimate of the tomogram (i.e., adding up the densities along each ray line at an given angle)



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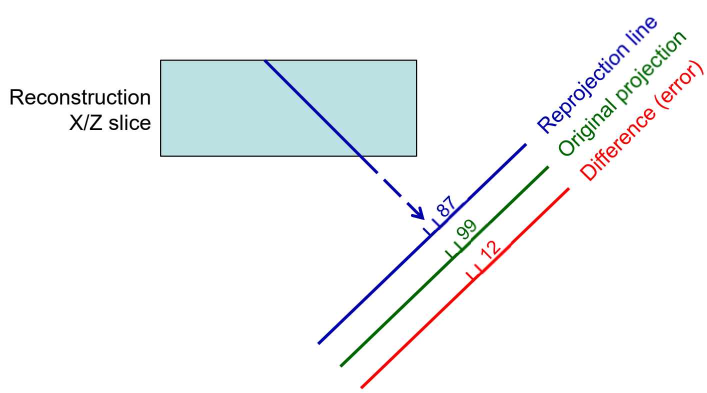

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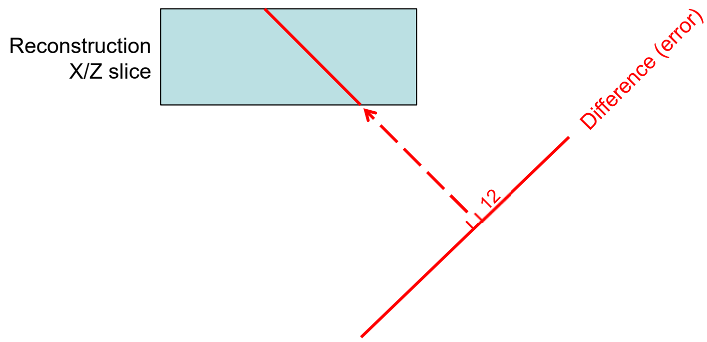
  - Adjusting tomogram by distributing difference for each projection pixel into the pixels along the ray through reconstruction



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- In ART (Algebraic Reconstruction Technique) this operation is done one angle at a time
- In SIRT (Simultaneous Iterative Reconstruction Technique) the reprojections are computed for all angles first, then the tomogram is adjusted by all the differences
  - Requires more iterations than ART but is more resistant to noise

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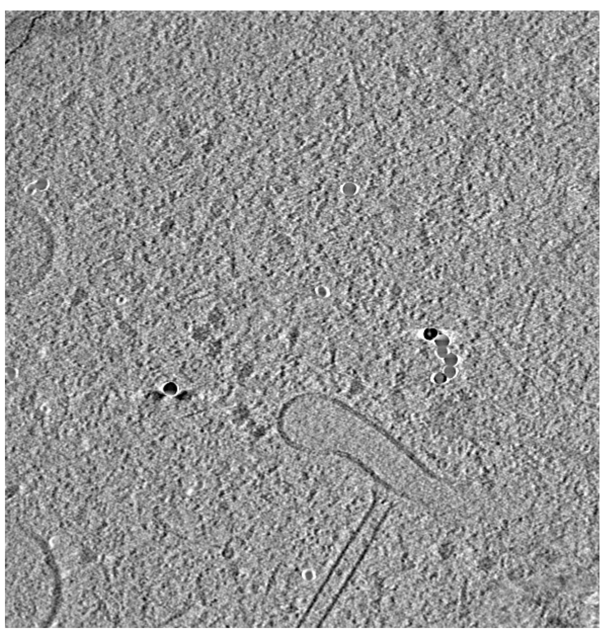
- In SIRT (Simultaneous Iterative Reconstruction Technique) the reprojections are computed for all angles first, then the tomogram is adjusted by all the differences
  - Requires more iterations than ART but is more resistant to noise
- The starting reconstruction is generally an unweighted backprojection, so it starts dominated by low frequencies, and higher frequencies are added in through the iterations

Cryotomogram Computed with Backprojection

Cryotomogram Computed with SIRT (16 iterations)

Cryotomogram Computed with SIRT (8 iterations)



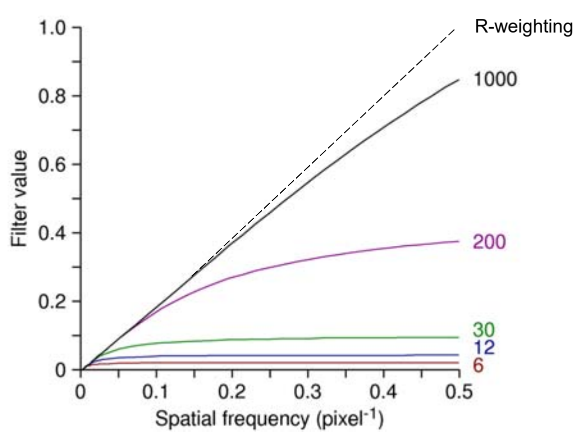
Cryotomogram Computed with Backprojection

Cryotomogram Computed with SIRT (16 iterations)

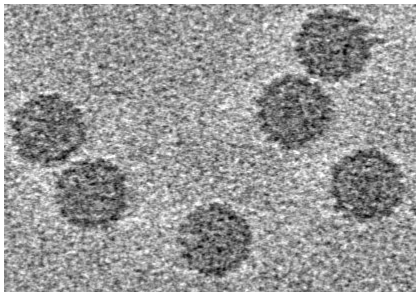
Cryotomogram Computed with SIRT (8 iterations)

SIRT Is Just an Expensive Filter

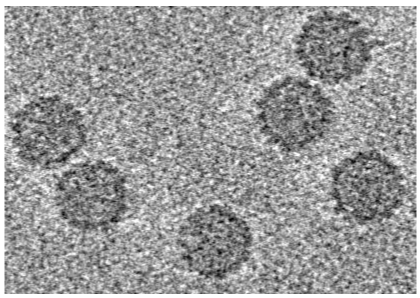
- N iterations of SIRT is mathematically equivalent to doing back-projection with a radial weighting function proportional to
  $f * (1 – (1 – a/f)^N)$ instead of f
  - Zeng, G.L., 2012, A filtered backprojection algorithm with characteristics of the iterative landweber algorithm, Med. Phys. 39: 603-607
  - The constant "a" and an adjusted "N" value are determined by matching output to that from SIRT



Comparison of SIRT and SIRT-Like Radial Filter

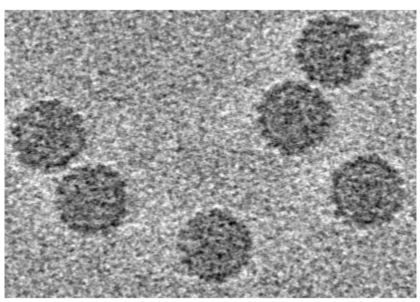


SIRT
6 iterations

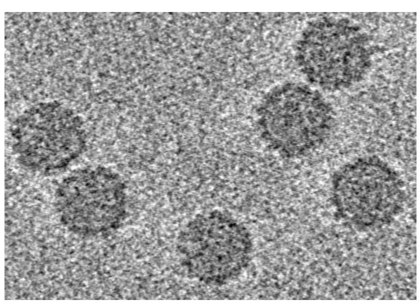


SIRT
12 iterations

Comparison of SIRT and SIRT-Like Radial Filter



SIRT-like filter
equivalent to
6 iterations



SIRT-like filter
equivalent to
12 iterations

Factors Limiting Tomogram Resolution

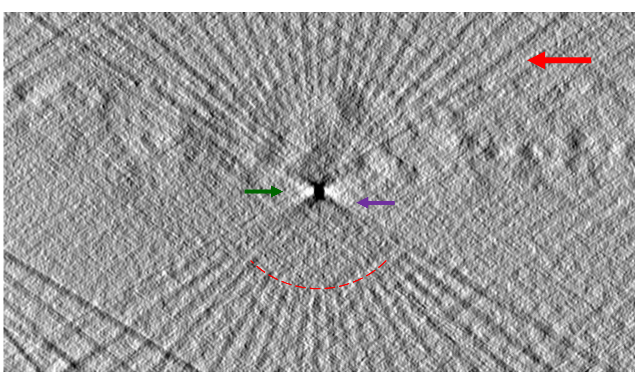
Resolution here refers to the ability to resolve features within the tomogram reliably; this is independent of the resolution that can be achieved by averaging information above the tomogram resolution.

Factors are:

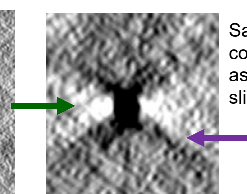
- Number of projections relative to thickness of material
- Density of material within the volume
- Signal-to-noise ratio of input images, determined by electron dose and efficiency of camera
- Resolution of imaging system (microscope and camera)
- Quality of alignment of data entering into backprojection

The Point Spread Function from Single-Axis Tilting

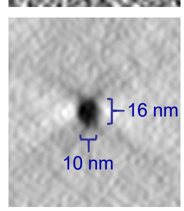
- Elongation in Z from limited tilt range (1.6 x for ±60º range)
- Fringes (overshoots) in X from limited tilt range
- Artefactual rays at each tilt angle starting at a distance that depends on tilt increment and size of the density
  - These start closer and are more prominent at higher increment
  - Stronger rays start right at the density for the terminal angles



X/Z slice from BBb (tutorial data set) with 6º increment

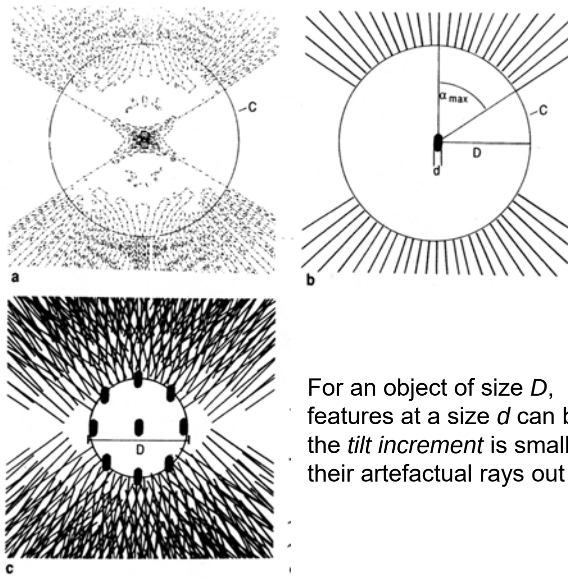


Same contrast as full slice



Less contrast – no saturation

The Point Spread Function and its Effects on Resolution



For an object of size *D*,
features at a size *d* can be well-resolved if the *tilt increment* is small enough to keep their artefactual rays out of the object

(From Radermacher, 1992)

The Crowther Resolution Formula

(Crowther, DeRosier, and Klug 1970)

1. For D = diameter of reconstructed volume
   $\Delta\theta$ = tilt increment (radians)
   resolution $d = D\,\Delta\theta$

2. For $\Delta\theta$ = tilt increment (degrees)
   f = resolution in frequency (reciprocal space) units
   $f = 57.3 / (D\,\Delta\theta)$

3. For n = number of views
   θ_{max} = maximum tilt angle
   $f = 28.5\,n / (D\,\theta_{max})$

4. For $\theta_{max} = \pm 60^{o}$
   $f = 0.48\,n / D$

Tomograms Often Beat the Formulas

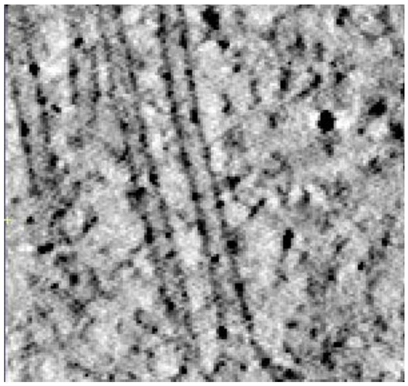
- Stained and especially frozen-hydrated material may be sparse – the “object” of diameter D is not the whole specimen but structural components in it
- The artefactual rays are not of paramount importance at relatively fine tilt intervals – they are just another source of noise

Factors Limiting Tomogram Resolution

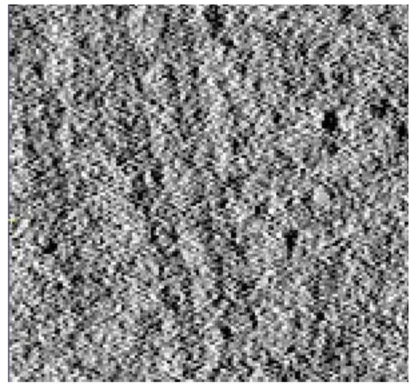
- Number of projections relative to thickness of material
- Density of material within the volume
- Signal-to-noise ratio of input images, determined by electron dose and efficiency of camera

Effect of Noise on Resolution

There is no averaging of high frequency data in the reconstruction process: the SNR of the input data determines the SNR of the reconstruction



Reconstruction from original data



Noise added equivalent to 100 electrons/pixel

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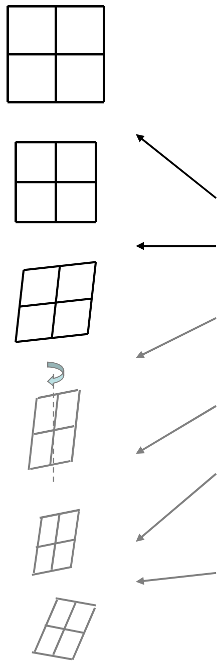
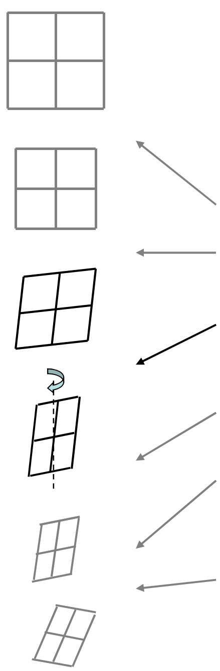
Tiltalign’s Model for Fiducial Positions



Fiducial positions in every projection view are derived from a single set of positions in 3D by the following changes or operations:

1. The specimen shrinks uniformly in 3D
2. The specimen shrinks along one axis in the plane of the specimen
3. The specimen is tilted around the Y axis, which need not be perpendicular to the beam axis, and the tilt axis could change with tilt direction
4. An image is projected with a magnification that may vary among the views
5. The image is rotated during projection (the tilt axis is not vertical on the camera) and may also stretch along an axis during projection
6. The image is shifted due to variations in specimen position



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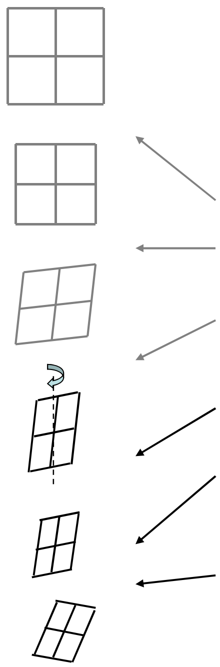

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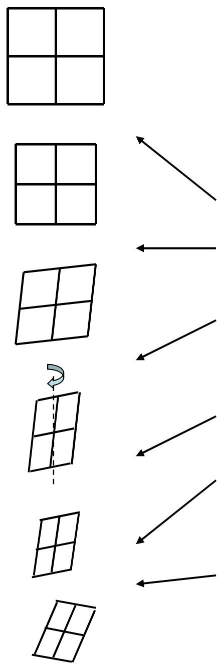
The Tiltalign Variables

- The specimen changes and imaging operations are expressed in terms of 5 variables that Tiltalign can solve for at each tilt:
  1. **Mag**: a uniform change in specimen size or microscope magnification
  2. **Tilt**: the tilt angle
  3. **Rotation**: the rotation of the tilt axis from the vertical
  4. **X-stretch (Dmag)**: a shrinkage/stretch along the X-axis in the plane of the specimen
  5. **Skew**: a change in the angle between X and Y axes
- X-stretch and Skew together represent a linear shrinkage along an arbitrary axis (distortion)
- Tiltalign can also solve for three variables that are the same for all views:
  1. **Beam tilt**: the angle between the tilt axis and the perpendicular to the beam axis
  2. **Projection skew**: a change in the angle between X and Y axes resulting from stretch during projection
  3. **X-axis tilt**: a tilt around the X axis between two halves of a bidirectional tilt series

Tiltalign's Model for Fiducial Positions

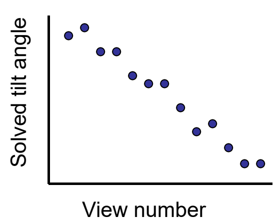


Fiducial positions in every projection view are derived from a single set of positions in 3D by the following changes or operations:

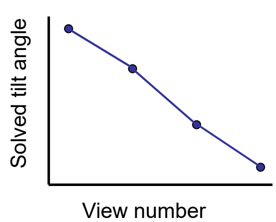
1. The specimen shrinks uniformly in 3D (**Mag**)
2. The specimen shrinks along one axis in the plane of the specimen (**X-stretch** and **Skew**)
3. The specimen is tilted around the Y axis, which need not be perpendicular to the beam axis (**Tilt**, **Beam tilt**), and the tilt axis could change with tilt direction (**X-axis tilt**)
4. An image is projected with a magnification that may vary among the views (**Mag**)
5. The image is rotated during projection (the tilt axis is not vertical on the camera) and may also stretch along an axis during projection (**Rotation**, **Projection skew**)
6. The image is shifted due to variations in specimen position

Grouping of Views to Reduce Variables

When grouping is used, a variable is constrained to change linearly over a set of contiguous views, or to have the same value for all of the views in the set.

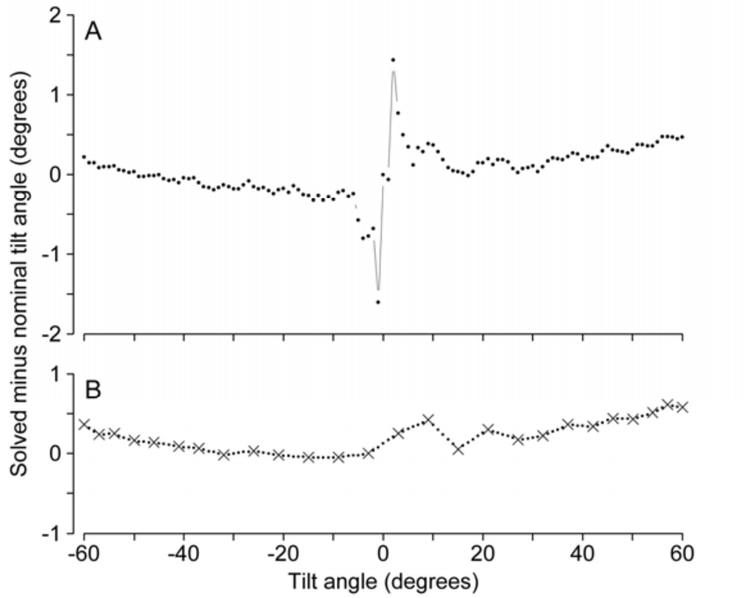


No grouping: Tiltalign solves for an independent tilt angle at each view



Grouping by 4: Tiltalign solves for tilt angle only at a subset of views (circles)
At other views, the tilt angle is constrained to be a linear combination of two variables
Fiducial positions from all views are used to find these tilt angles

Benefits of Grouping for Reducing Random Errors and Giving an Accurate Tilt Angle Solution



Grouping

- Grouping can dramatically reduce the number of variables being solved for.
- Grouping provides more averaging over errors in fiducial positions and keeps the solution from accommodating to random errors. (Mean residual may be higher but the solution should be more accurate.)
- Grouping is appropriate for slowly changing variables, especially for ones that are hard to solve for.

Key Measures in Alignment Output

- Mean of residual errors (distances between actual and predicted positions)
- Ratio of measurements to values being solved for (reflects amount of averaging over random position errors)
  - Measurements = X/Y positions (2 numbers per marked point)
  - Unknowns = Rot, Tilt, Mag, Stretch, X/Y shifts, plus 3-D positions