FeT
Frontiers of Electron Tomography

Electron Tomography Theory

- *Peter Ercius*
- *Frontiers of Electron Tomography Short Course 2017*
- *Molecular Foundry, Lawrence Berkeley National Laboratory, Berkeley, CA, USA*
- *October 25th, 2017*

UCLA STROBE NSF SCIENCE AND TECHNOLOGY CENTER MOLECULAR FOUNDRY BERKELEY LAB

Structure Directly Affects Functionality

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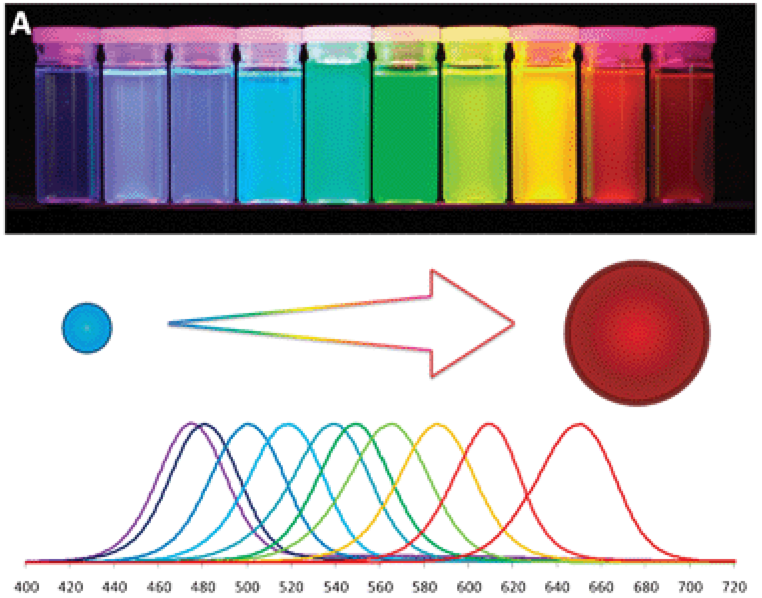
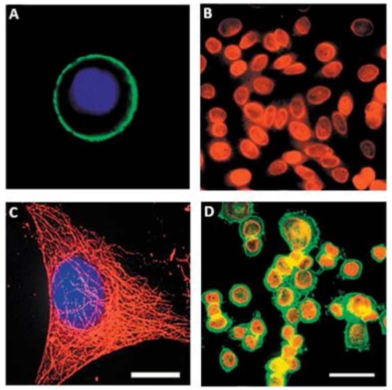
Quantum dots

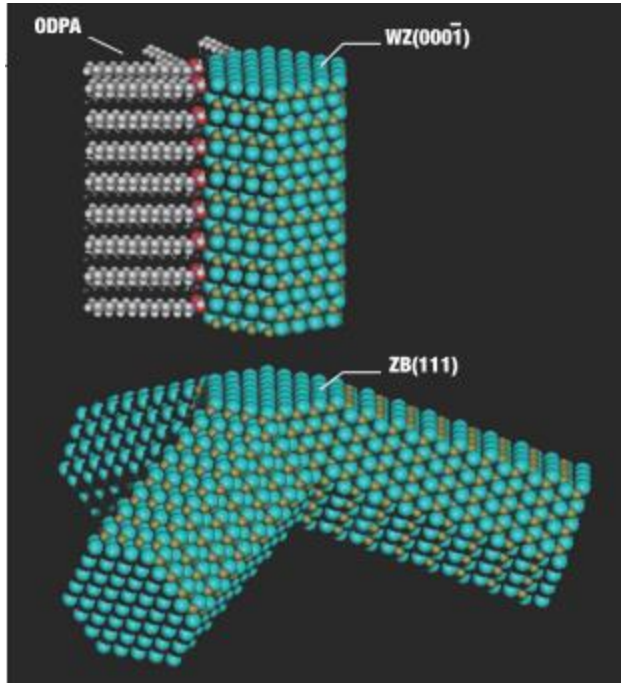


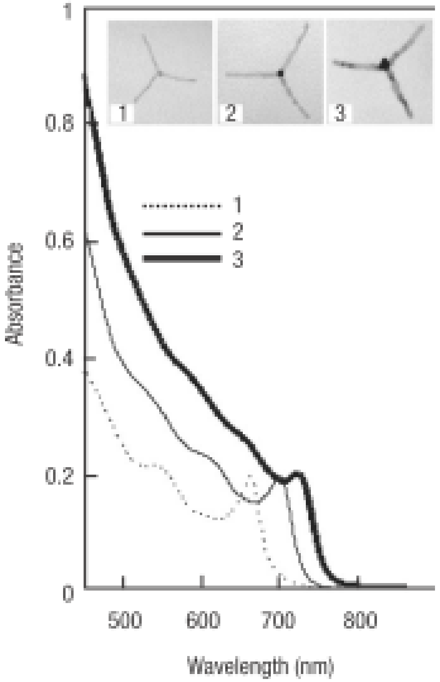


Medical imaging

P Zrazhevskiy *et al*, Chem So Rev (2010)

Tetrapods





L Manna et al, Nature (2003)

- Quantum dot size affects their optical emission color
- Tetrapod shape affects their optical absorption
- Need to measure morphology to determine shape mediated functionality

Projections Are Misleading

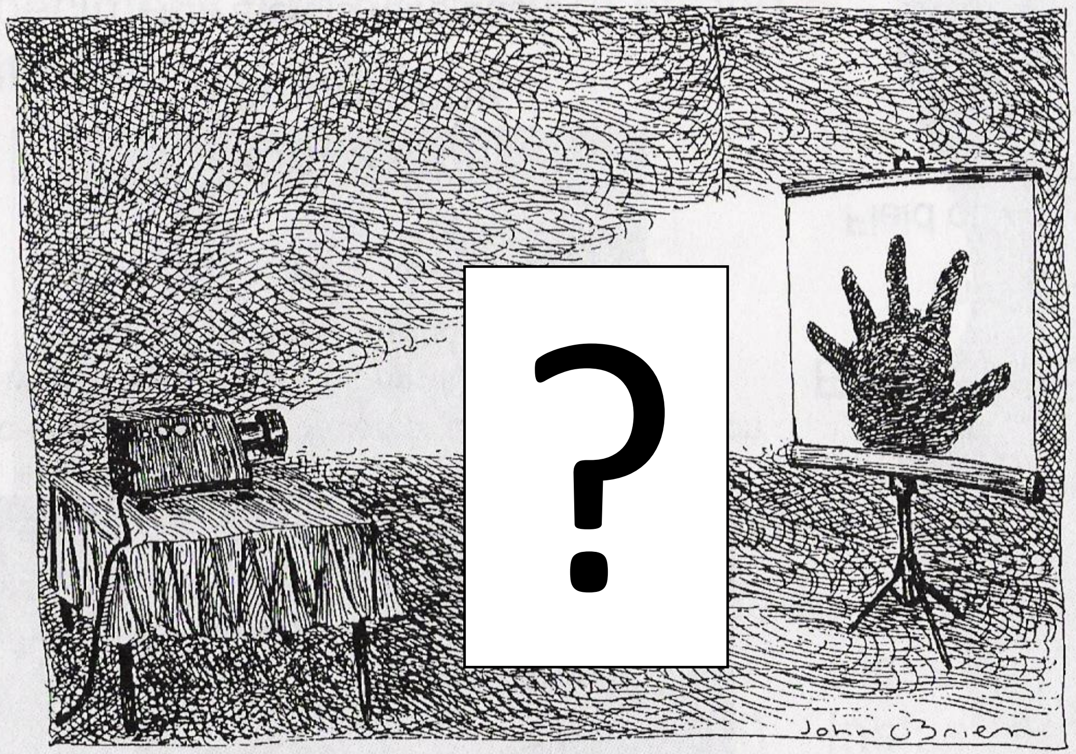


Fig. 5.1. A single projection image is plainly insufficient to infer the structure of an object. Drawing by John O'Brien; © 1991 The New Yorker Magazine.

Microscopists study the shadows on the wall because they do not have access to the objects that create them.

Hetero-/Homo-geneous Structures

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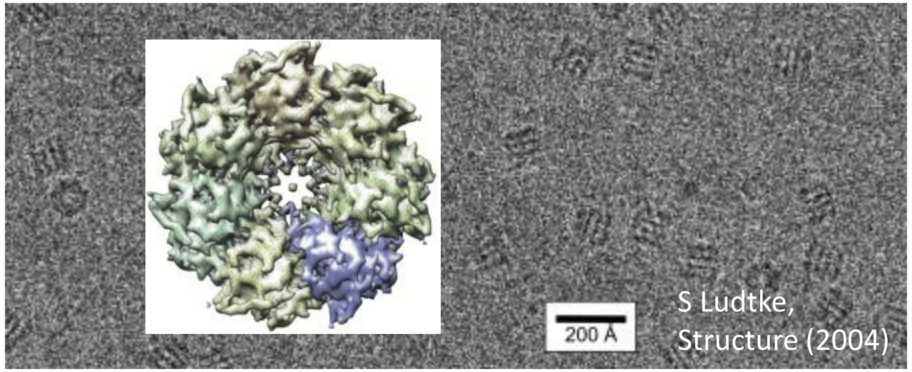
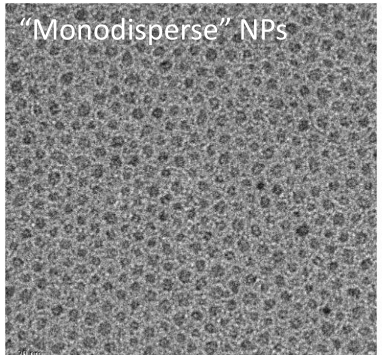
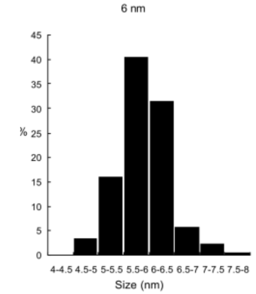
- Many biological structures can be copied and purified
  - Averaging improves SNR, reduces damage
  - 3D tertiary form gives molecular functionality and interactions
- Physical science nanomaterials are different on the atomic scale
  - ~~Averaging~~
- Must resolve local atomic structure directly to measure:
  - Defects, compositional anti-phase boundaries, amorphous structure, dopant atoms

> "If you can measure it, you can make it."
>
> \- A Liddle (NIST)

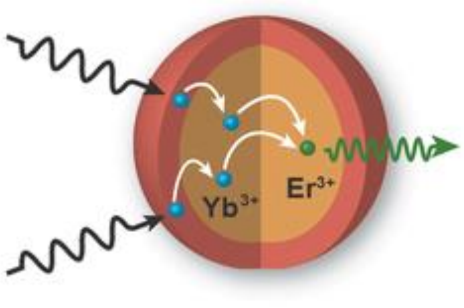


S Ludtke, Structure (2004)

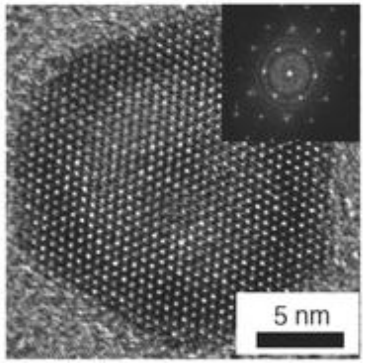




J. Park, Angew Chemi (2005)

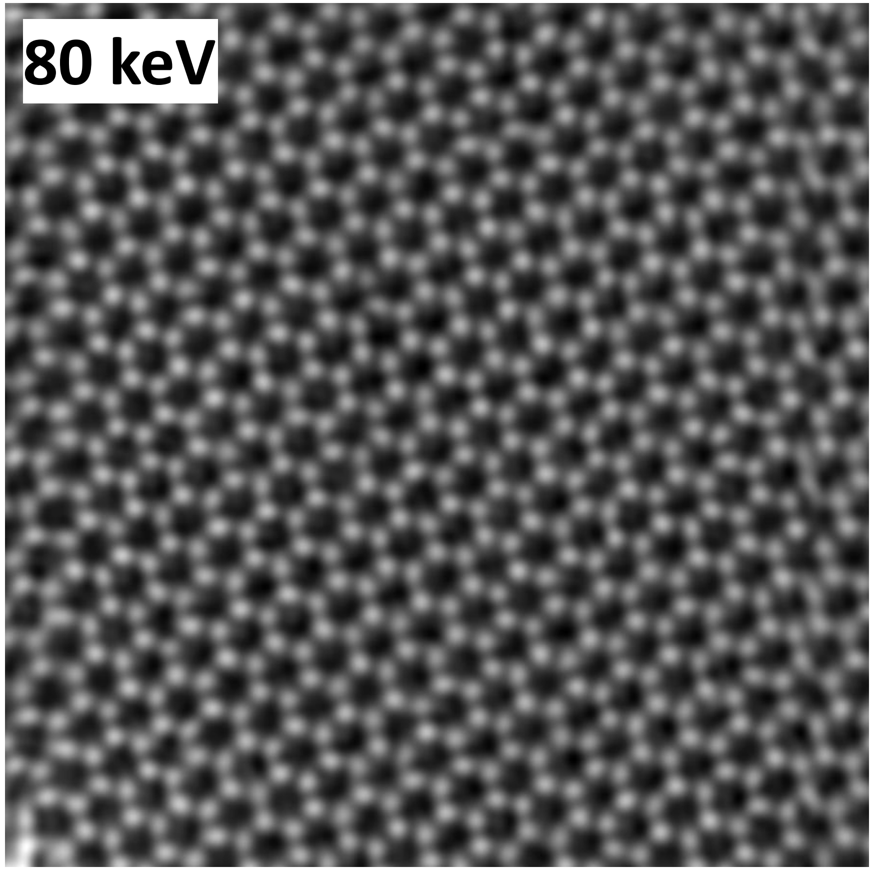


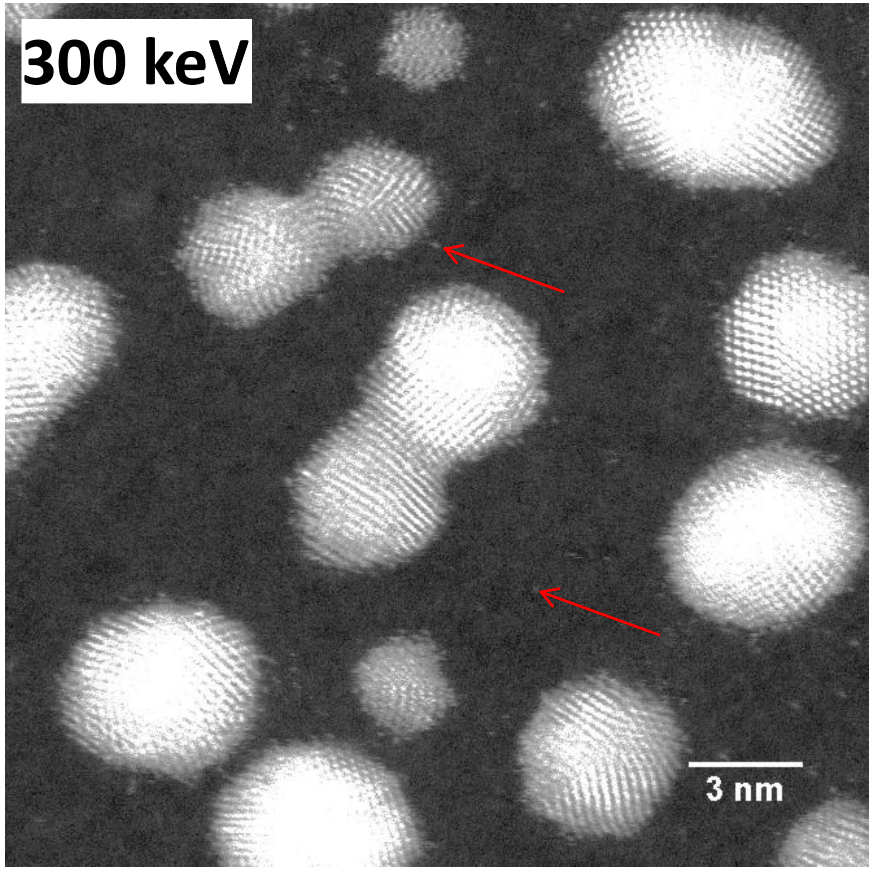
active core/active shell



E. Chan, Adv Mat (2015)

Aberration-corrected STEM

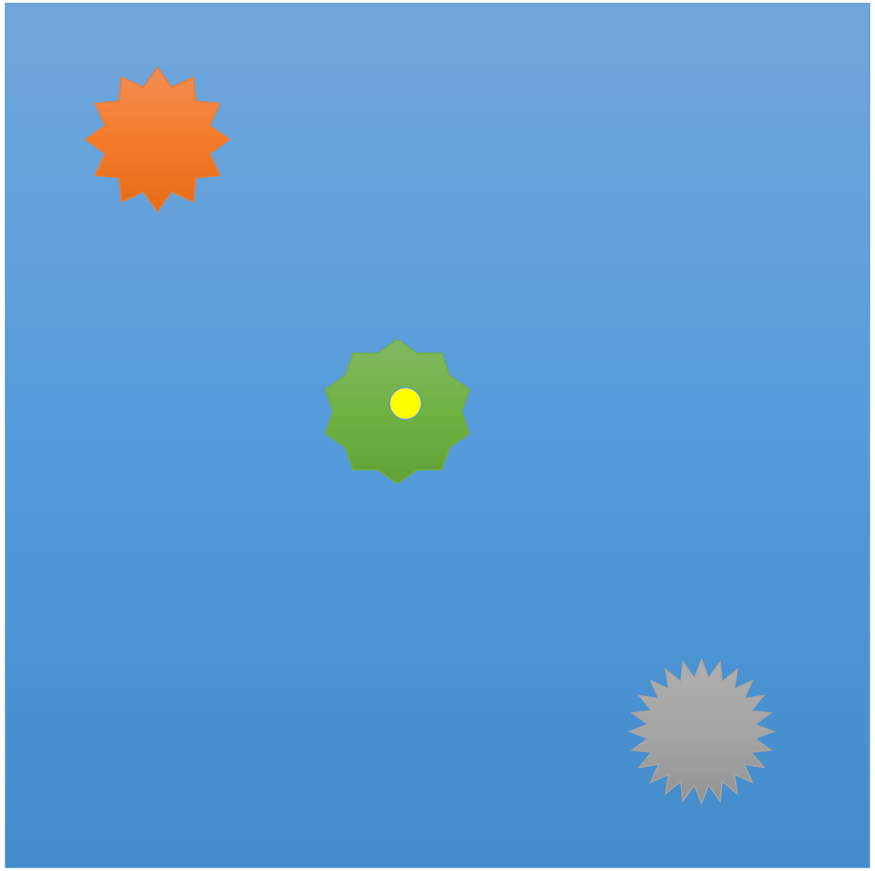




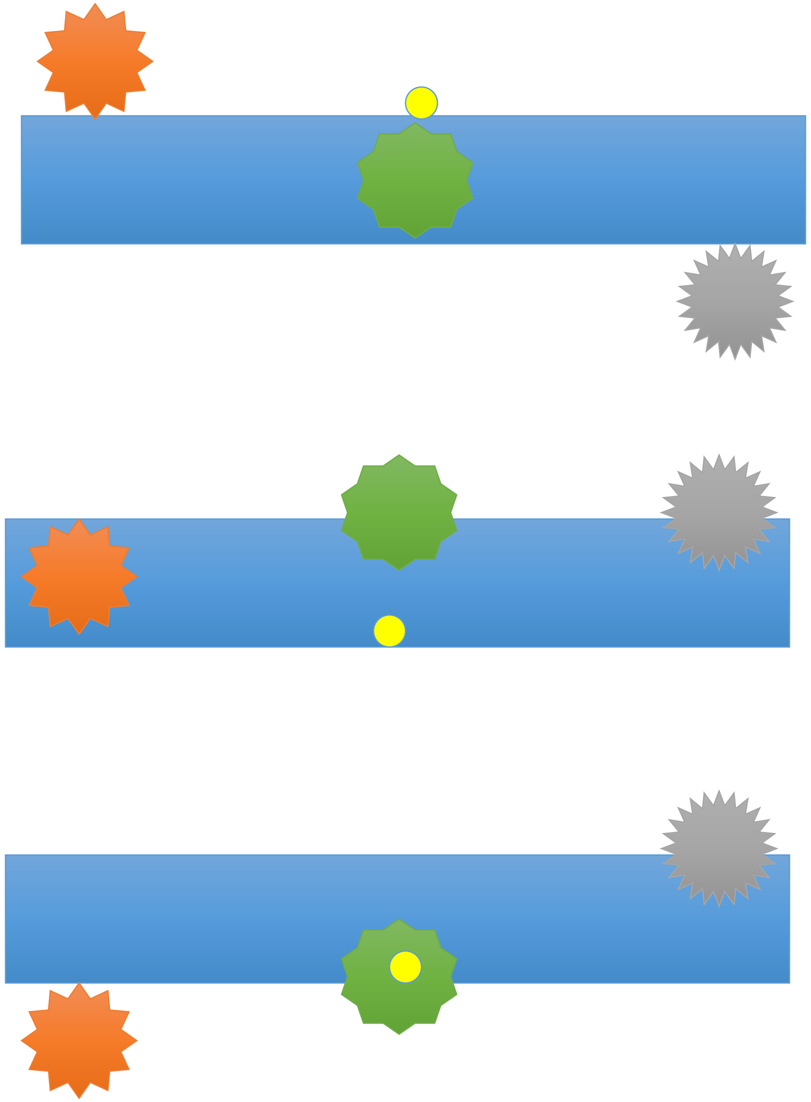
- Atomic resolution imaging with a focused probe in TEAM
- Single atom sensitivity for heavy and light elements
- 2D → 3D: No material is perfect! Grains, defects, etc.

Projection Problem for TEMs

Top View Projection



Side View



Techniques for 3D Structural Analysis

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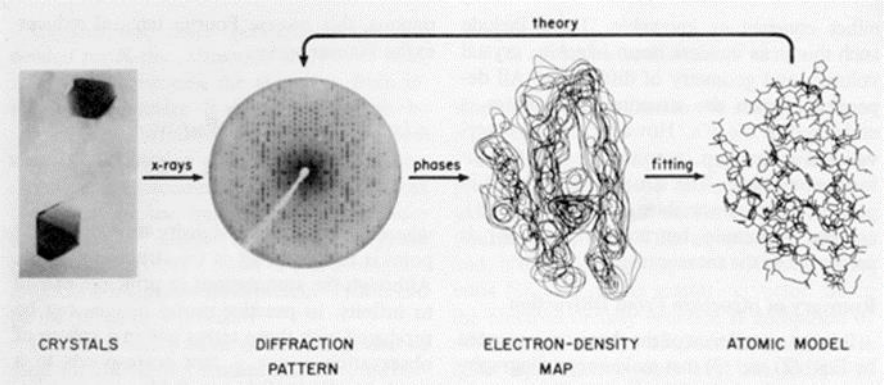
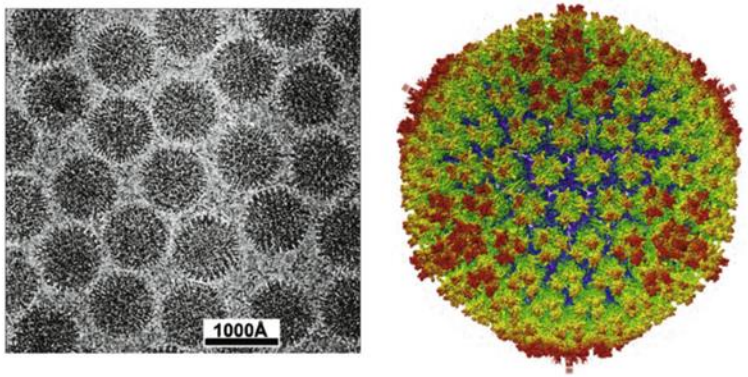
X-ray/Electron crystallography



- Homogeneous 3D crystal

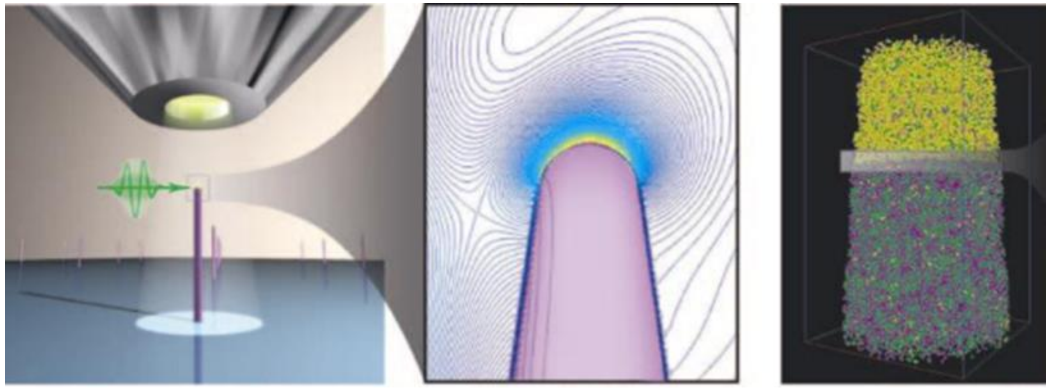
Cryo-EM



- Homogeneous, randomly oriented

Henderson, Arch Biochem Biophys (2015)

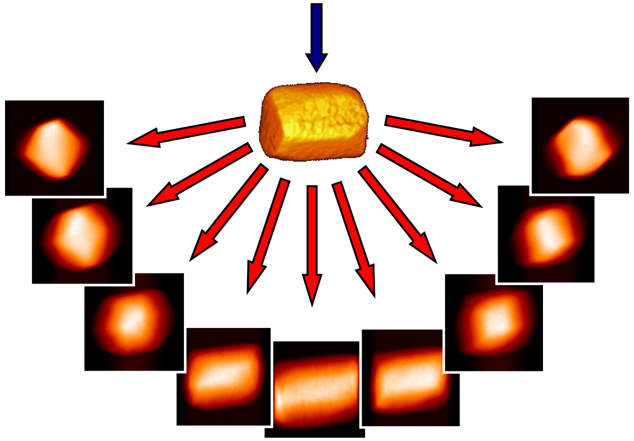
Atom Probe Tomography



- Only 30% – 60% of atoms

Seidman, et al, Ann Rev Mater Res (2007)

Electron tomography



- Single unique object from projections

M Weylend, P Midgley, Ultramicroscopy (2003)

Techniques for 3D Structural Analysis

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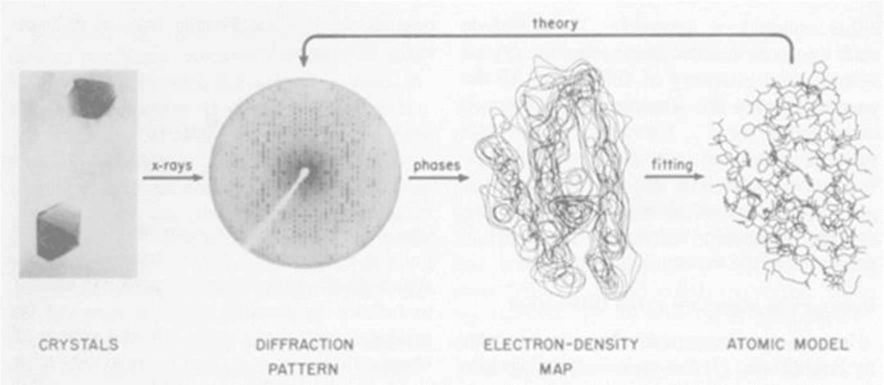
X-ray/Electron crystallography



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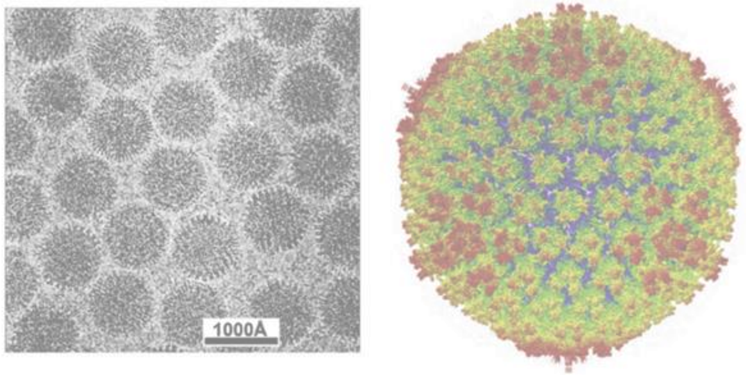
Cryo-EM



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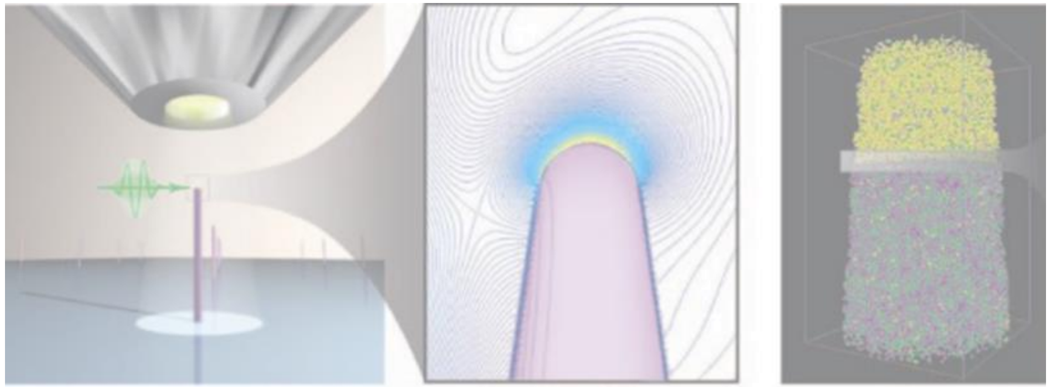

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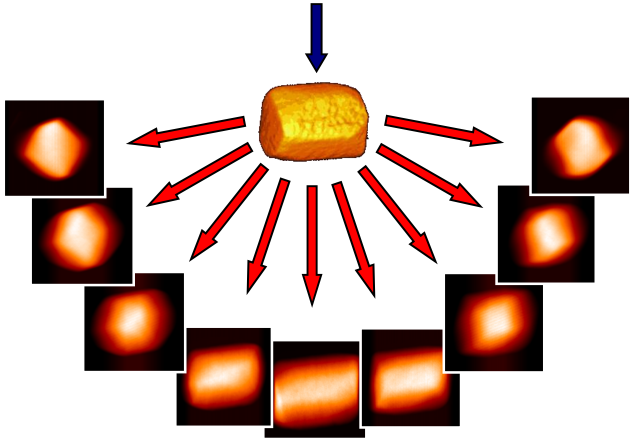
Electron tomography



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M Weylend, P Midgley, Ultramicroscopy (2003)

Electron Tomography

Tomography is a method in which a higher dimensional structure is reconstructed from a series lower dimensional projections (usually by sampling the structure from many different directions).

- Original description of projections are in Radon's 1917 paper:
  Radon, *Ber. Verh. K. Sachs. Ges. Wiss. Leipzig, Math.-Phys. Kl.* 69, 262 (1917)
- Originally developed for astronomy and more commonly used in medical "CAT-scans"
  - Computer Aided Tomography (CAT)
- Any series of projection images can be utilized
  - Look for inspiration in other fields (bio-, astro-, etc.)

An Atomic Electron Tomography Experiment

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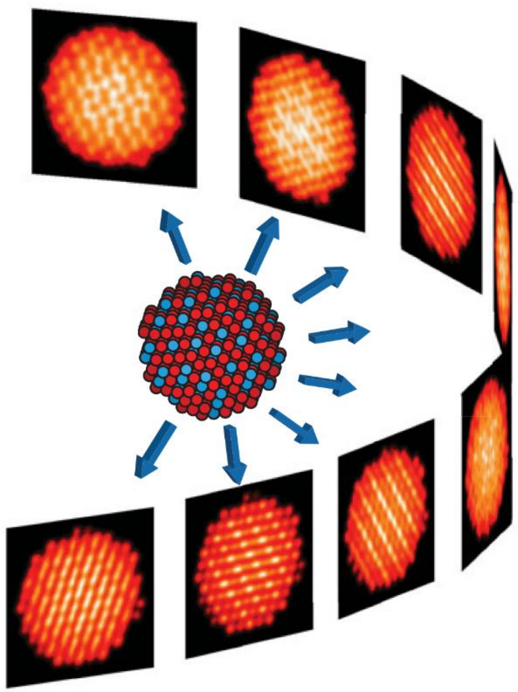

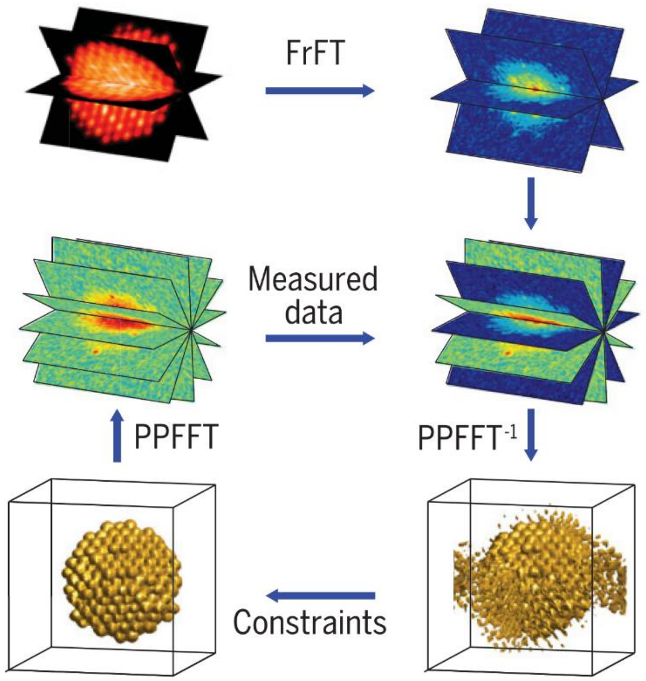

Acquisition

- Acquire HR images from many different angles
- Drift, stability, SNR, monotonic intensity

Reconstruction

- Reconstruct 3D density
- Accurate spatial alignment
- Determination of viewing directions

J Miao, P Ercius, SJL Billinge, *Science* (2016)

Projection Requirement

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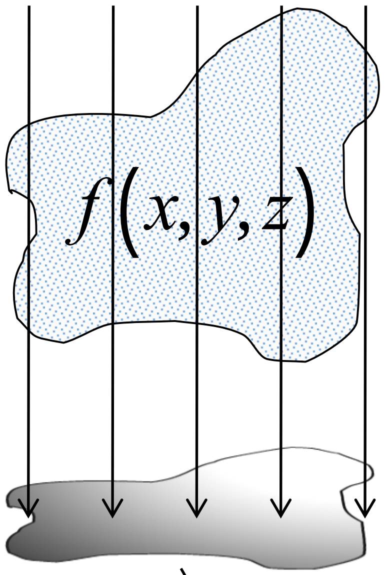
- Projected intensity must be a monotonic function of some property of the the object
    - Mass, thickness, electric-potential, etc.
- Beer's law for scattering is exponential with thickness:

$$I = I_o \exp^{-t/L_{\mathrm{el}}}$$

- TEMs are in fact structure projectors under certain conditions
    - TEM objective aperture enhances amplitude contrast
    - ADF-STEM produces incoherent Z contrast

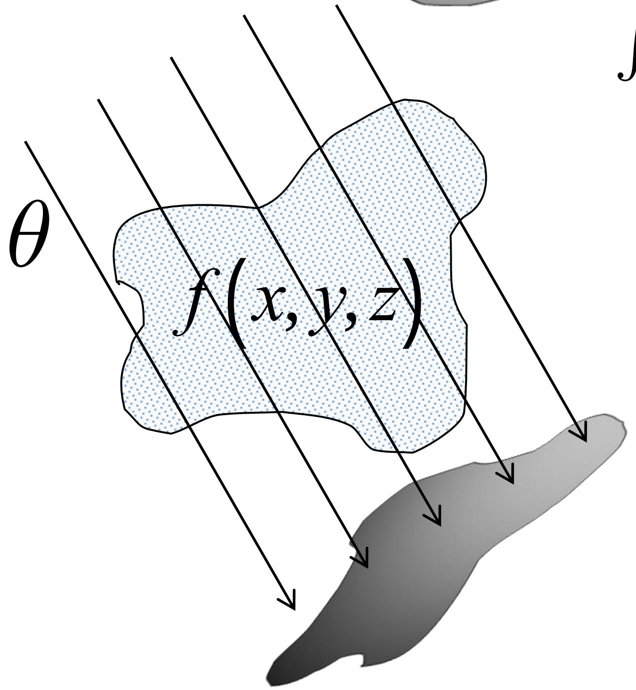
Linear Projection Operation



An object's density can be discretized as a function *f*(*x*,*y*,*z*)

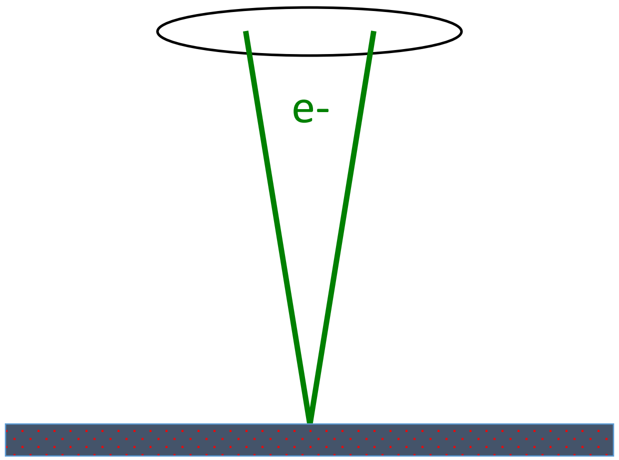
Projection is similar to summation along a given direction:

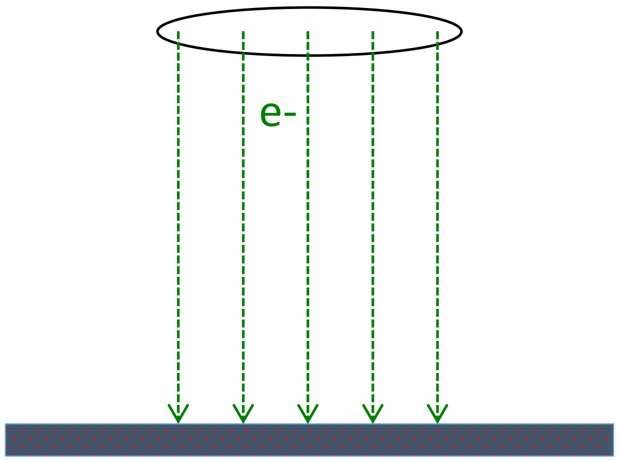
$$\int f(x,y,z)dz \equiv \sum_z f(x,y,z) = f(x,y)$$



$$\int f(x,y,z)d\theta \equiv \sum_\theta f(x,y,z) = f_\theta(x,y)$$

STEM vs. TEM Tomography





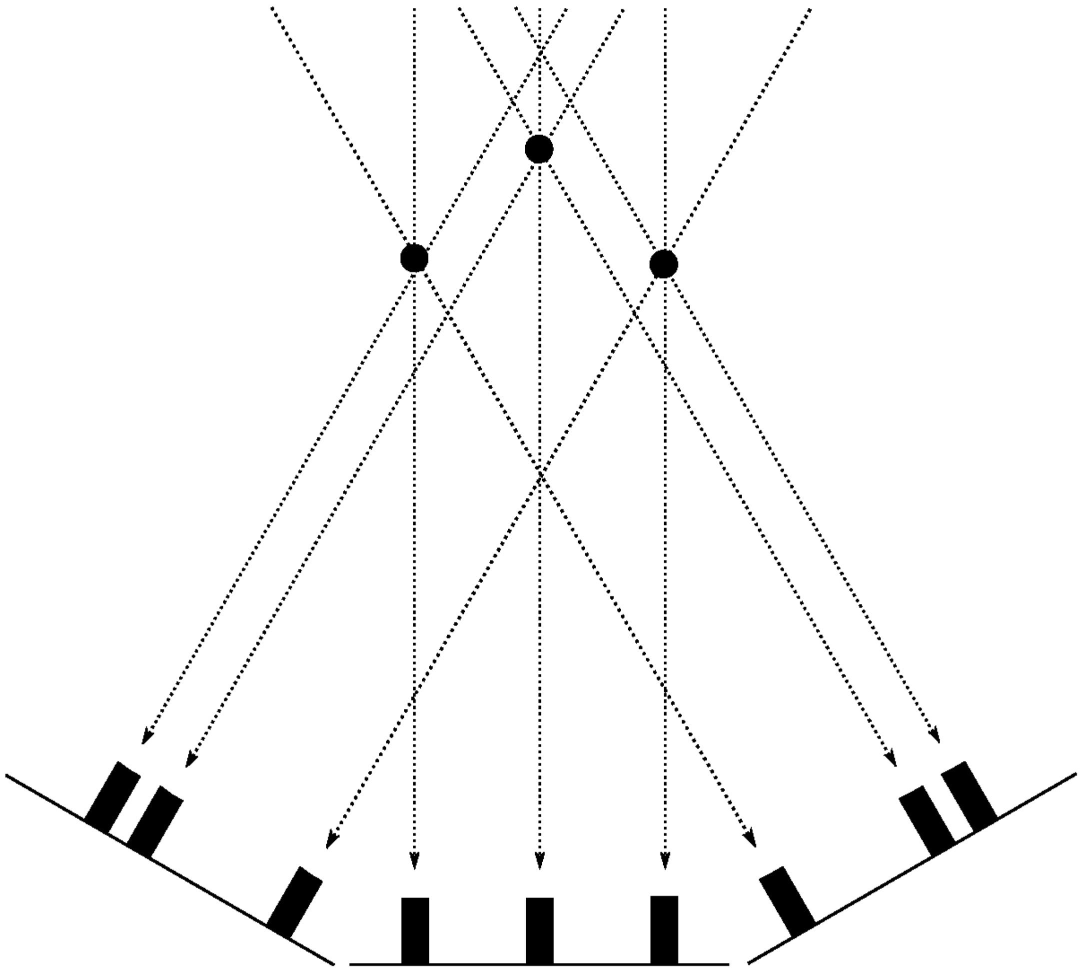
- ✗ Less ubiquitous
- ✗ Scanned image (30 sec)
- ✗ Sensitive to defocus
- ✗ Contamination, high dose
- ✓ Incoherent imaging method
- ✓ High contrast for heavy materials
- ✓ Multimodal (EELS, EDX)

------- Hard Materials -------

- ✓ Common parallel illumination
- ✓ Single-shot image (1 sec)
- ✓ Insensitive to defocus
- ✓ Low dose
- ✗ Complex phase information transfer function
- ✗ Low contrast → Large defocus
- ✗ May require CTF correction

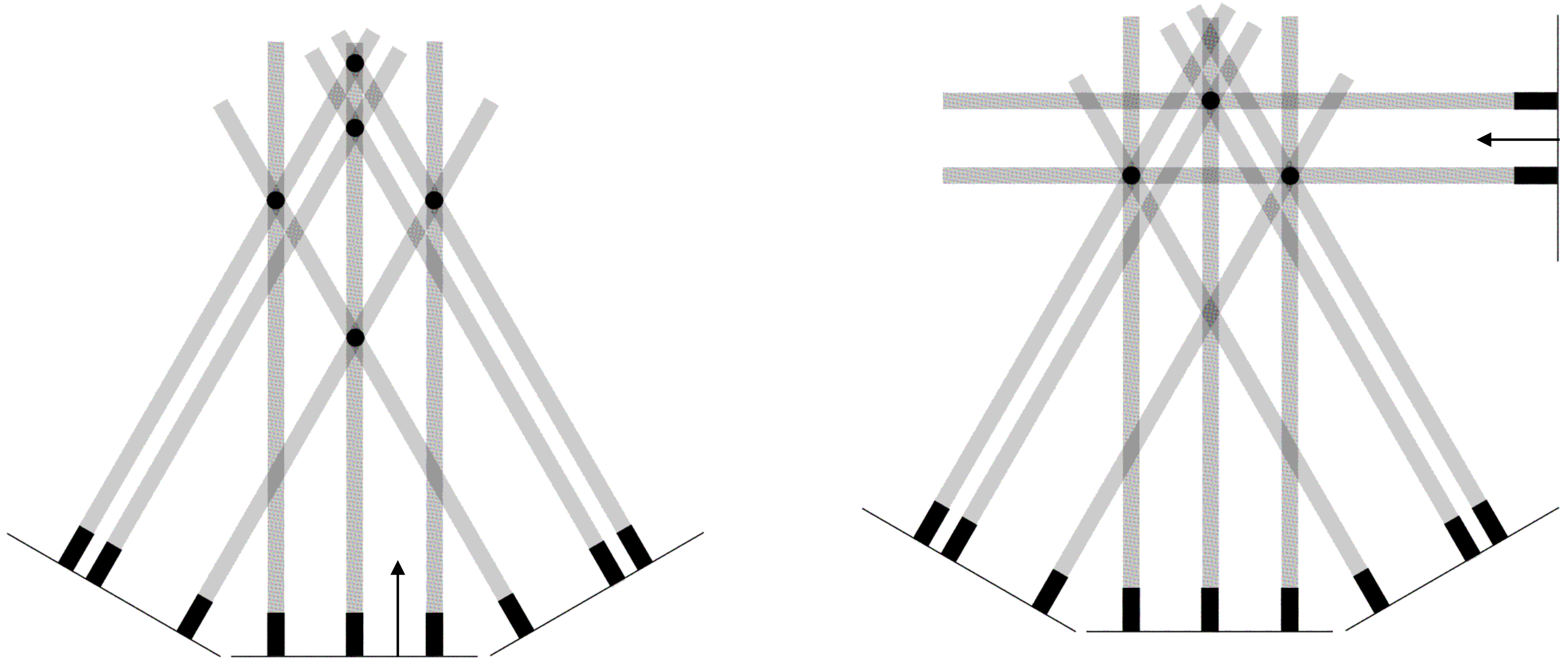
------- Soft Materials -------

3 Linear Projections



- A simple sum of the mass at each tilt-angle projected onto a line
- Each tilt tells us a little more about the shape and distribution

Tomographic Backprojection



- Real space transform
- Only 3 projections produce a clear artifact
- Many projections allow reconstruction of complicated objects

Multi-dimensional data: Image Stack vs Volume

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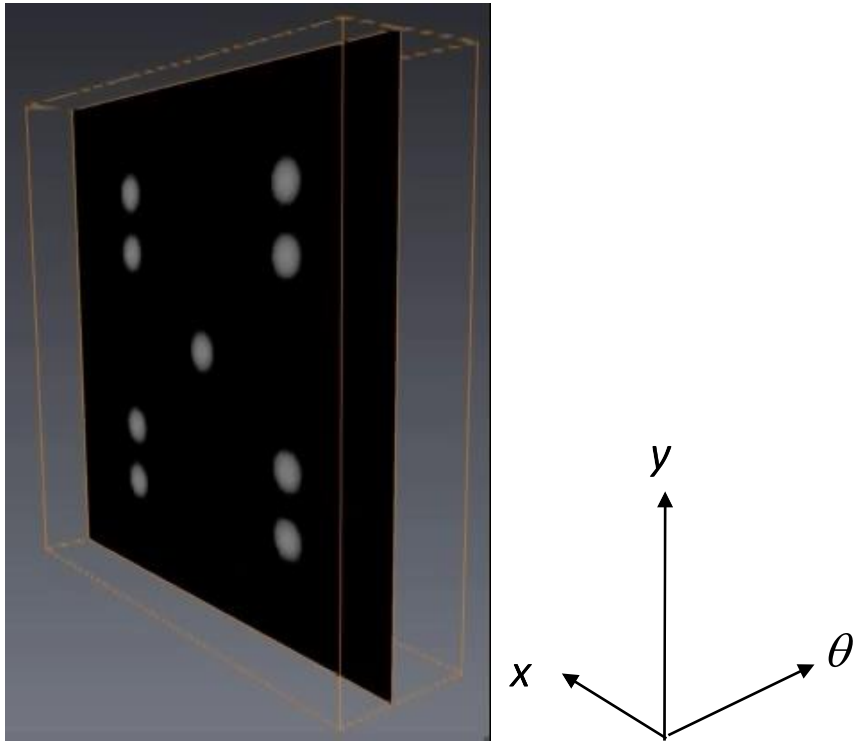
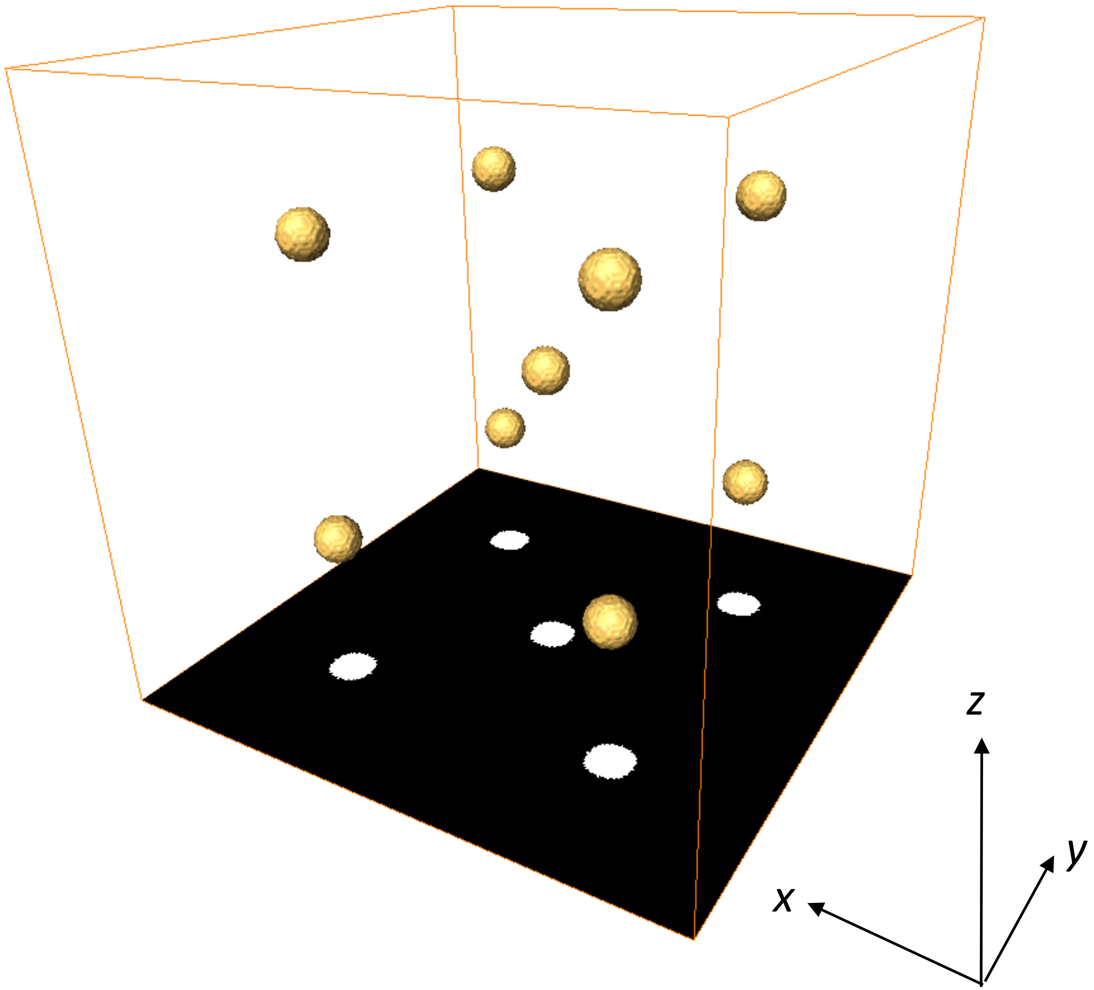
Image stack



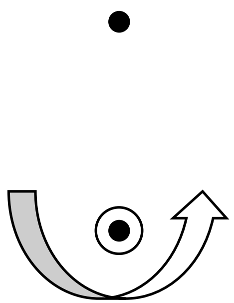
XY = images
Yθ = sinograms

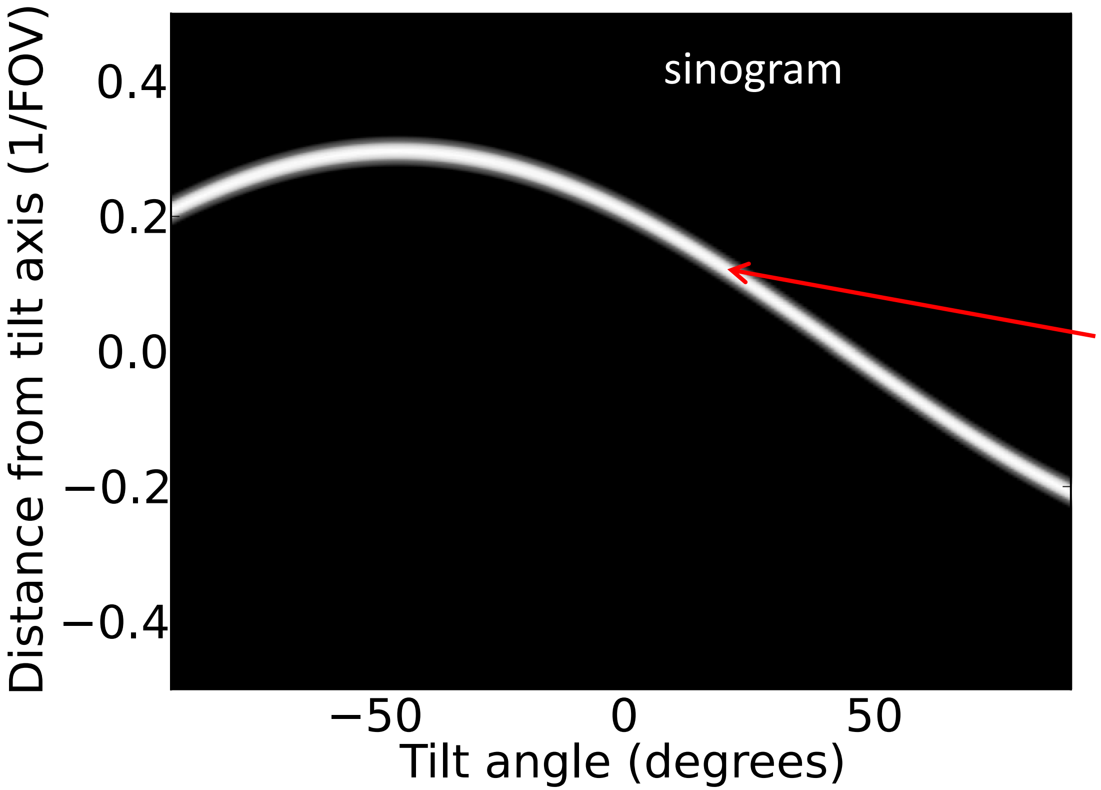
3D volume



Meaning of a Sinogram

Point object path

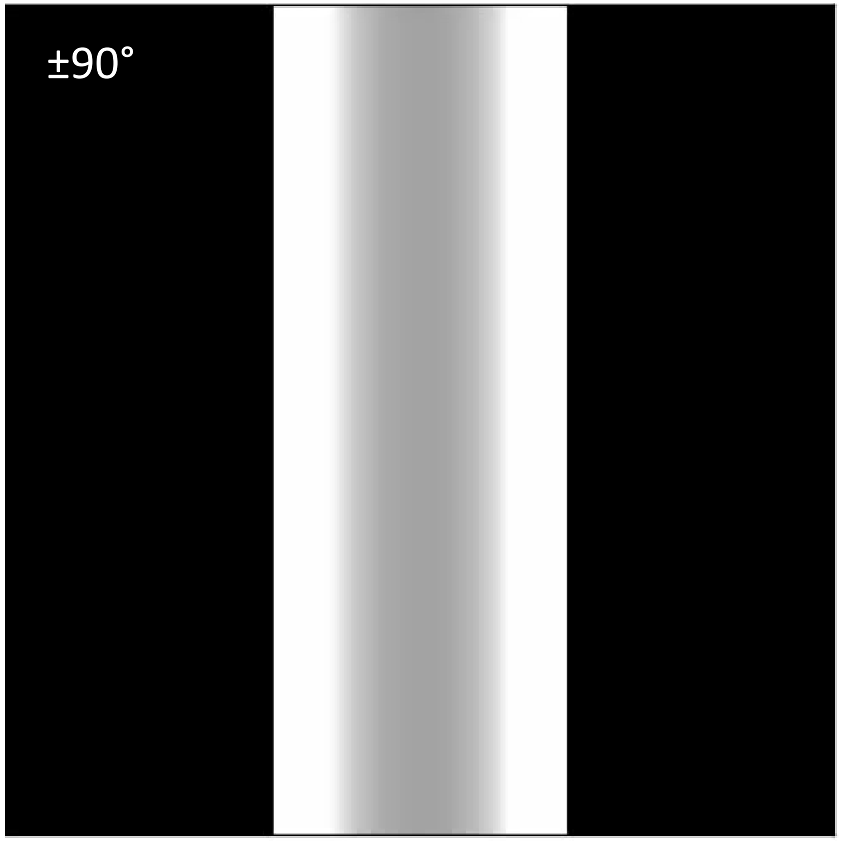




$R(p, \theta) = 1.00$

- A Sinogram shows the projected density a distance from the tilt axis at each tilt angle
  - Equivalent to Yθ in previous image stack video
- Reconstruct algorithms interpolate on the sonogram to fill a volume

Backprojection: Building Up a Reconstruction



- Simple Radon Backprojection
- Most common reconstruction method
- ±90°, 60 projections

Backprojection: Building Up a Reconstruction

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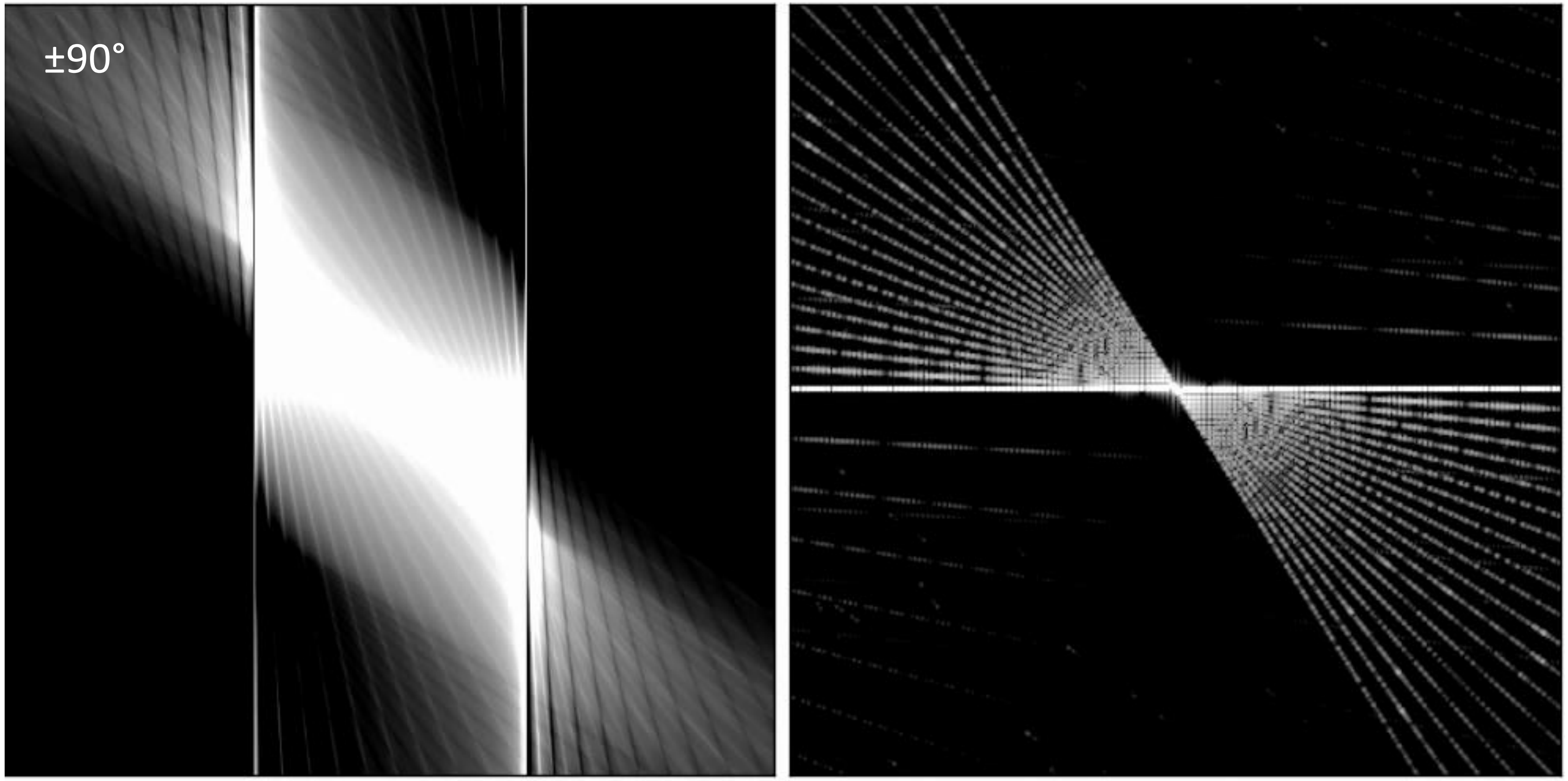

- Simple Radon Backprojection and corresponding FFT
- FFT shows how more information is filled in with each additional angle
- ±90°, 60 projections

Limited Tomography Reconstruction

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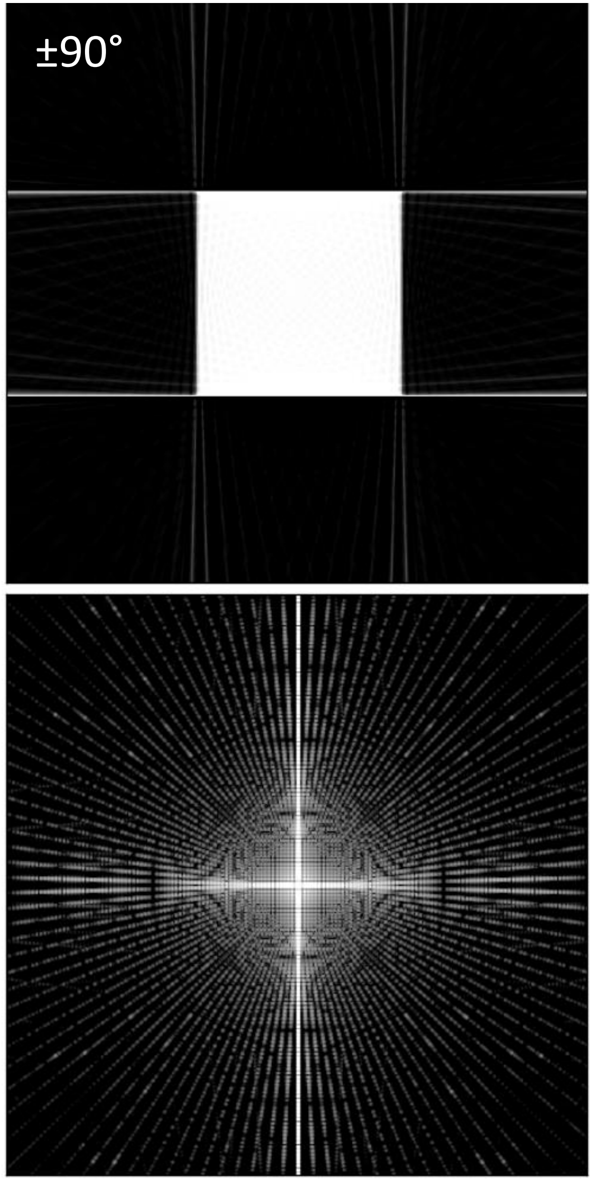
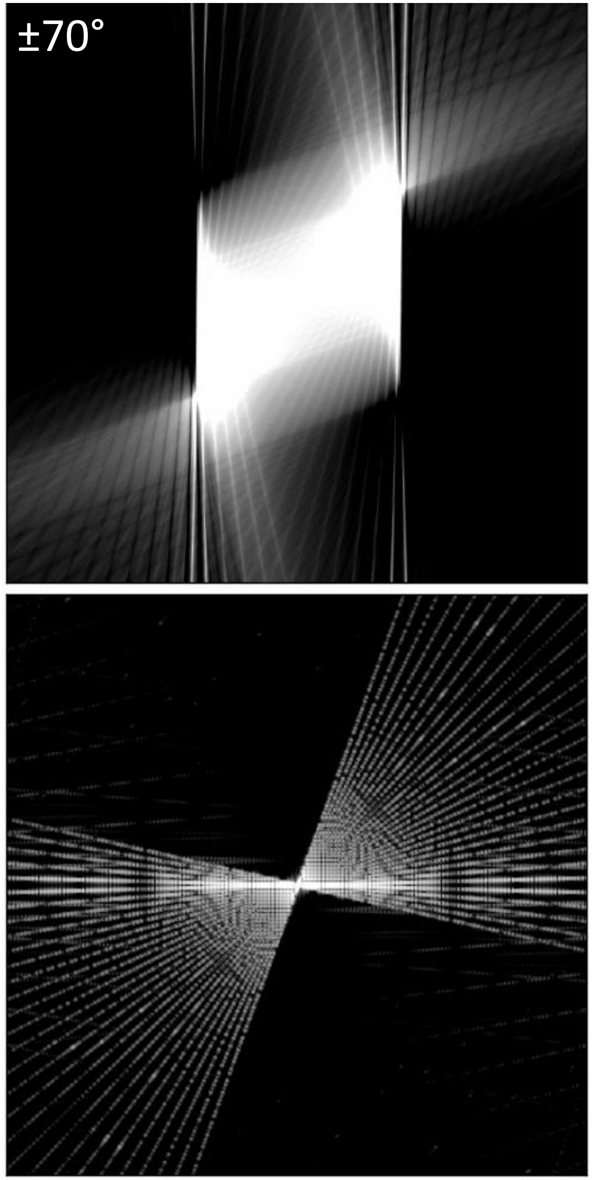
<u>Full reconstruction</u>



<u>±30° missing wedge</u>



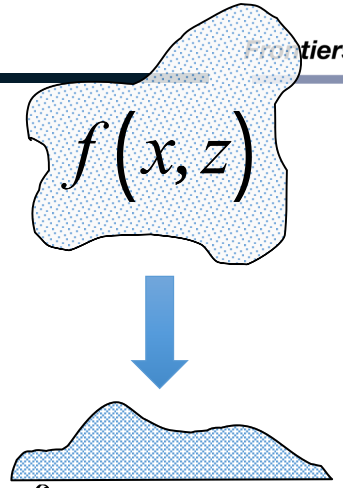
Radon backprojection
±70°, 50 projections

FFT of
Reconstruction

(2D) Fourier Slice Theorem

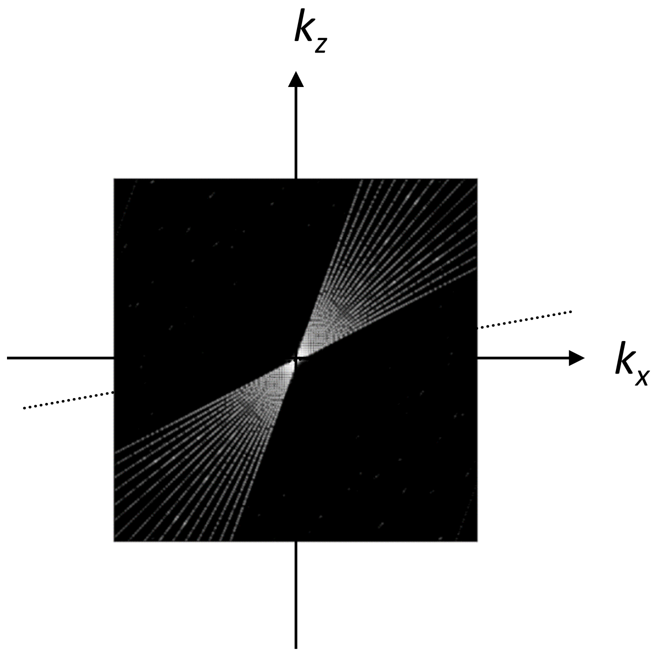
- Object has mass-density $f(x,z)$

$$F_{2D}\left[f(x,z)\right] = \int\int f(x,z)e^{i2\pi(k_x x, k_z z)}dxdz$$



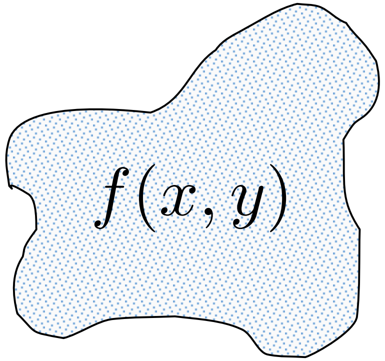
$$\int f(x,z)dz$$

A projection



- Acquire many projections to sample the object's information
- Possibility to invert Fourier space to retrieve full 3D information of the object

1D → 2D → 3D Fourier Transform



2D object with density function *f*(x,y) can also be represented in reciprocal space

$$\begin{aligned} F_{2D}[f(x,y)] &= F_x[F_y[f(x,y)]] \\ &= \underline{F}(k_x,k_y) \end{aligned}$$

3D object with denstiy function *f*(x,y,z) can also be represented in reciprocal space

$$\begin{aligned} F_{3D}[f(x,y,z)] &= F_x[F_y[F_z[f(x,y,z)]]] \\ &= \underline{F}(k_x,k_y,k_z) \end{aligned}$$

Fourier Slice Theorem

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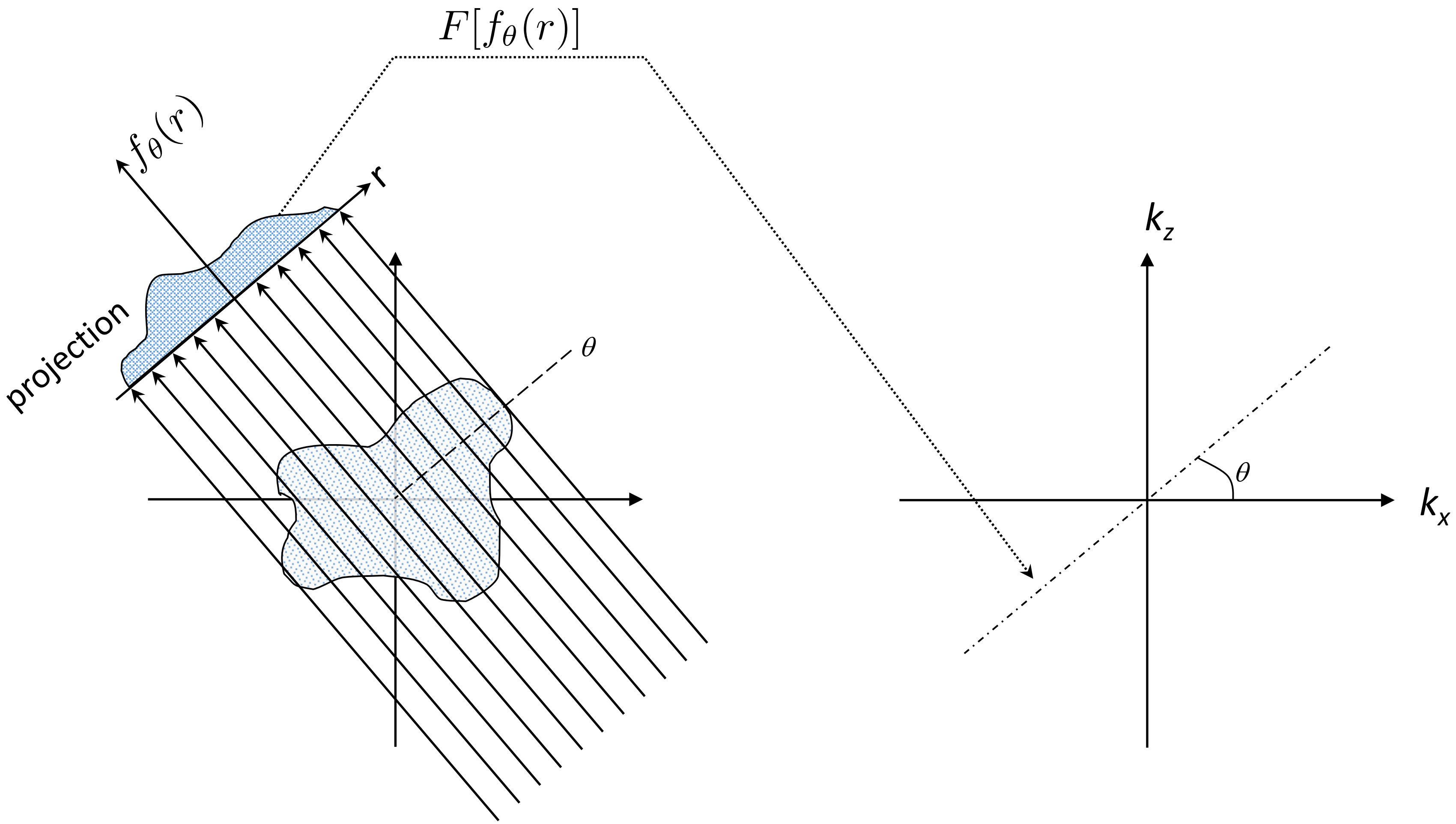
“A projection of an object is equivalent to a central slice of the object’s Fourier transform at the viewing angle”



Real space

Fourier (reciprocal) space

Connection Between Real and Fourier Space

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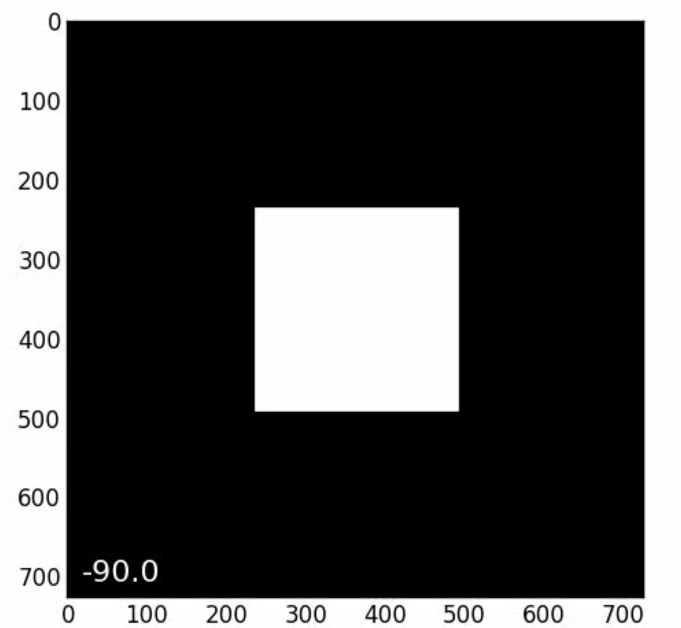
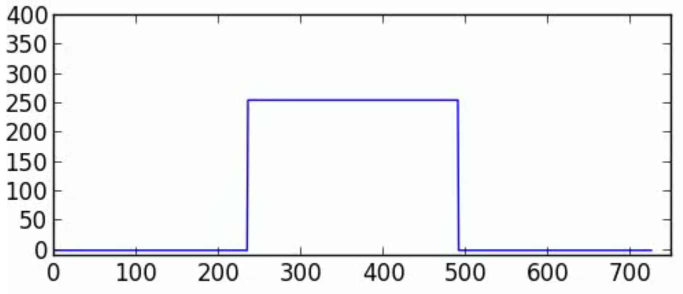
Real Space

2D object



1D projection



Projection image every 1.8° from ±90°

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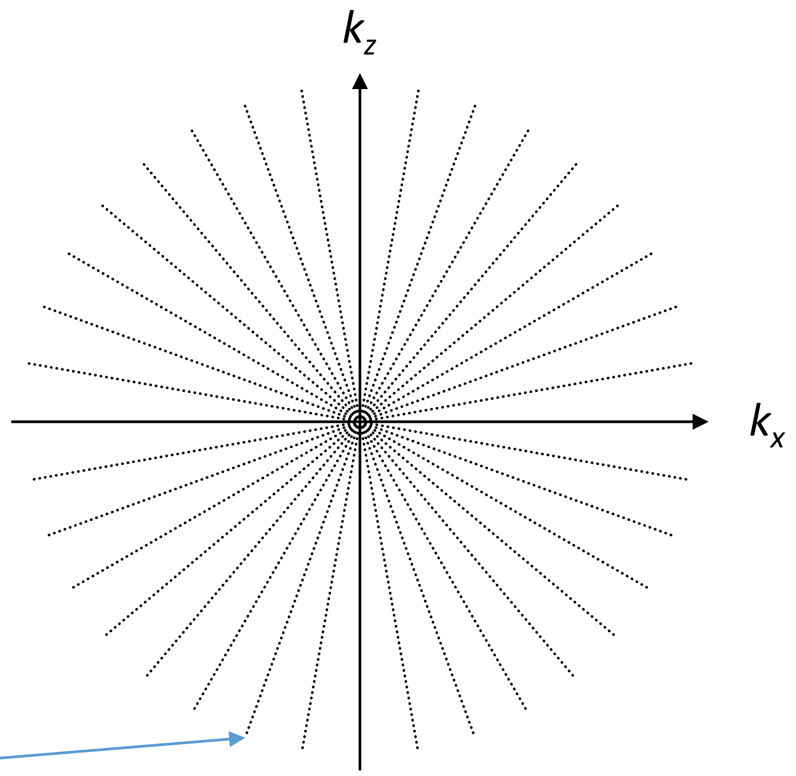
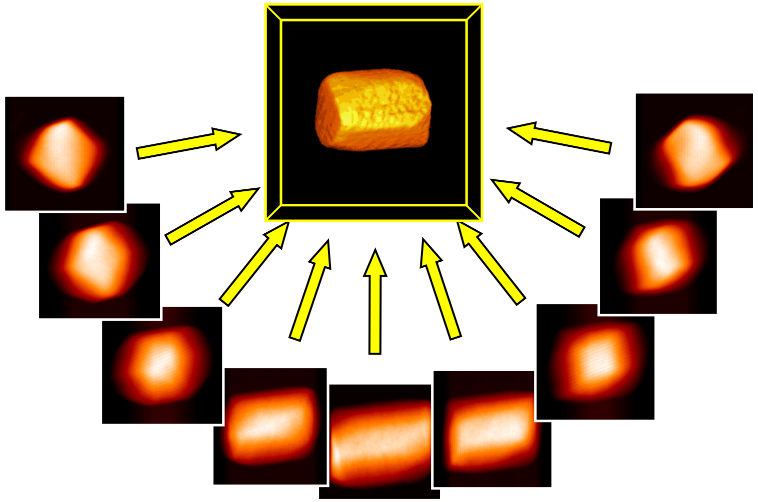
Fourier Space

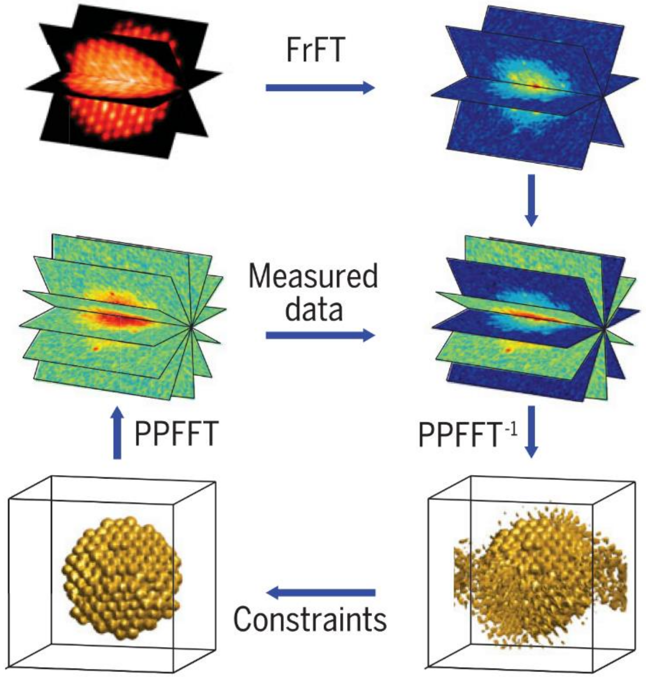


Central sections through Fourier space at each tilt-angle

Tomographic Reconstruction

Electron Tomography Reconstruction

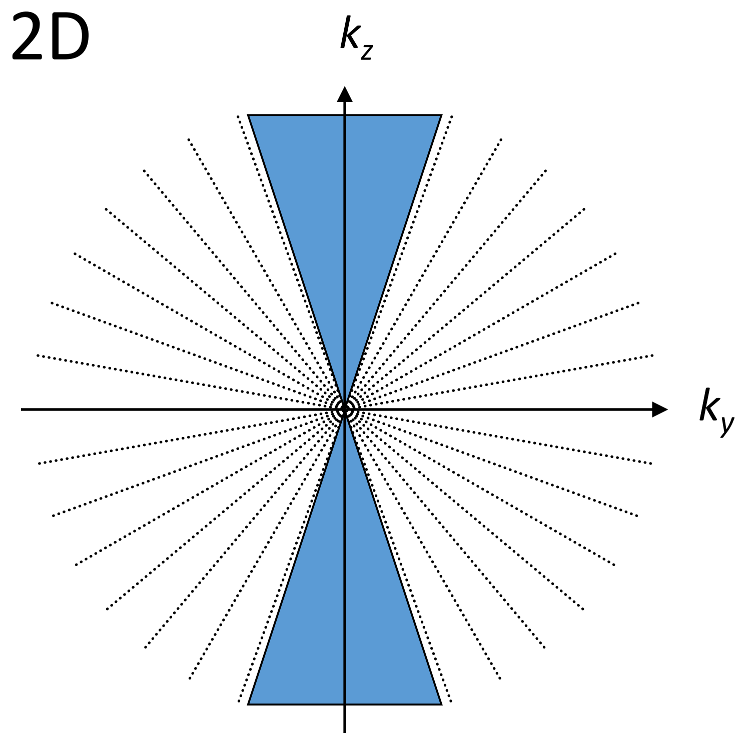


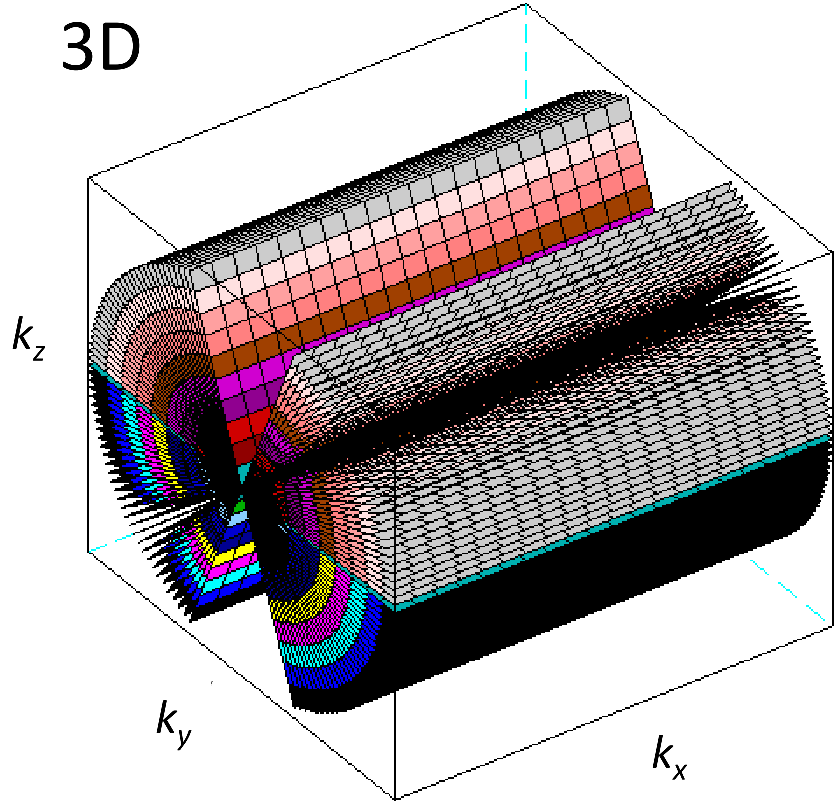


Requires:

- Projection requirement
- Accurate spatial alignment
- Determination of tilt axis
- Accurate angular increments

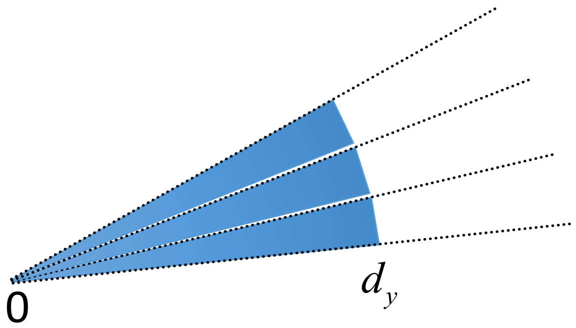
3D Fourier Information Sampling





- Missing information extends along the tilt-axis (X)
  - “Missing wedge of information”
- Artifacts and resolution reduction introduced along Y and Z due to missing information
- Resolution → X:image sampling, Y:tilt angles, Z:max tilt angle

Tomogram Resolution Estimation (*d*)



$$d_y = \frac{pD}{N}$$

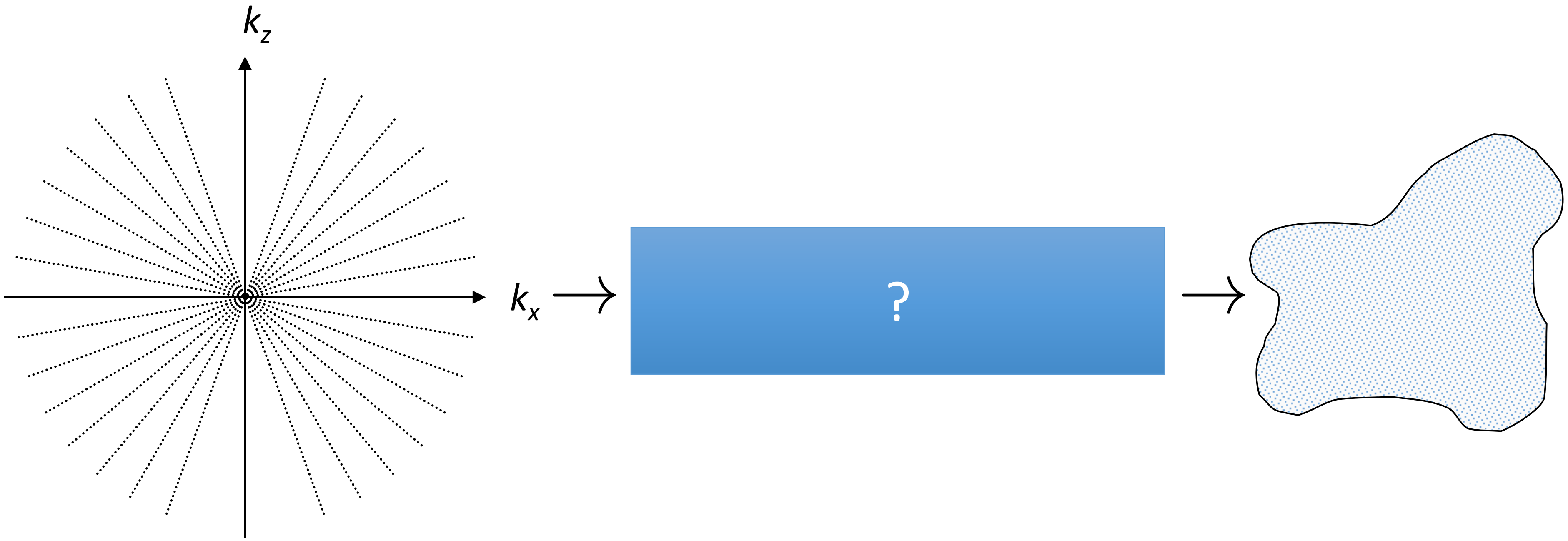
Discrete sampling of a continuous function

$$d_z = d_y e_{zy} = d_y \sqrt{\frac{a + \sin(a)\cos(a)}{a - \sin(a)\cos(a)}}$$

Elongation (e_{zy}) due to missing wedge

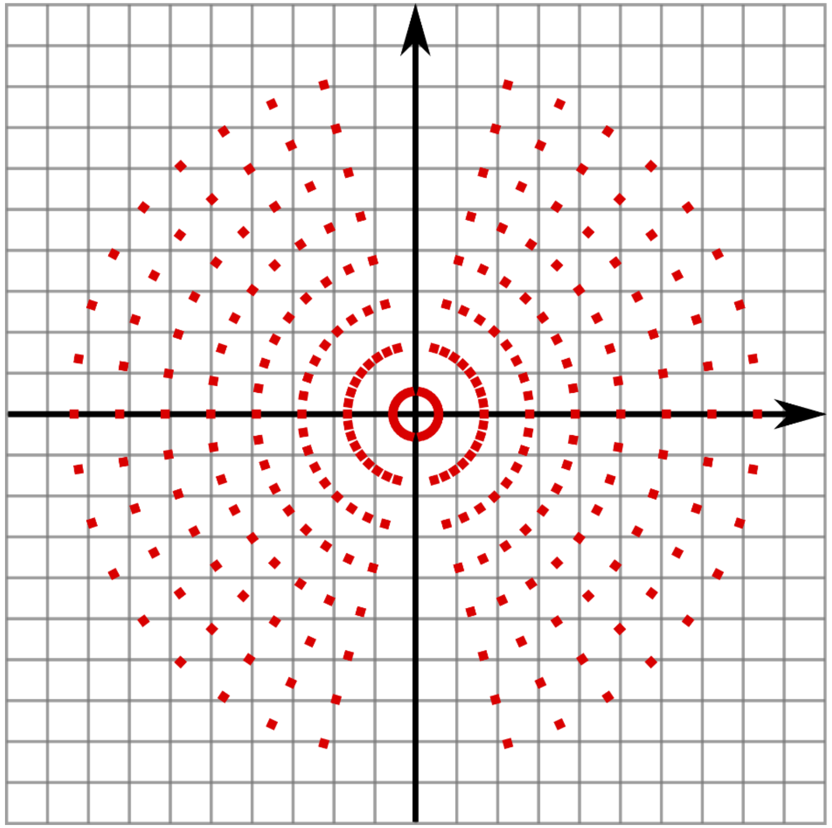
- Resolution along tilt-axis (x) is equivalent to the experimental resolution with perfect alignment
- Y-axis is diminished due to limited # tilt angles
  - 1° tilt steps provides a good sampling
- Z-axis is further diminished due to the missing wedge
  - d_z elongation is ≤1.3× for ±70° maximum tilt
- Electron dose limits applications for sensitive materials

Direct Fourier Inversion



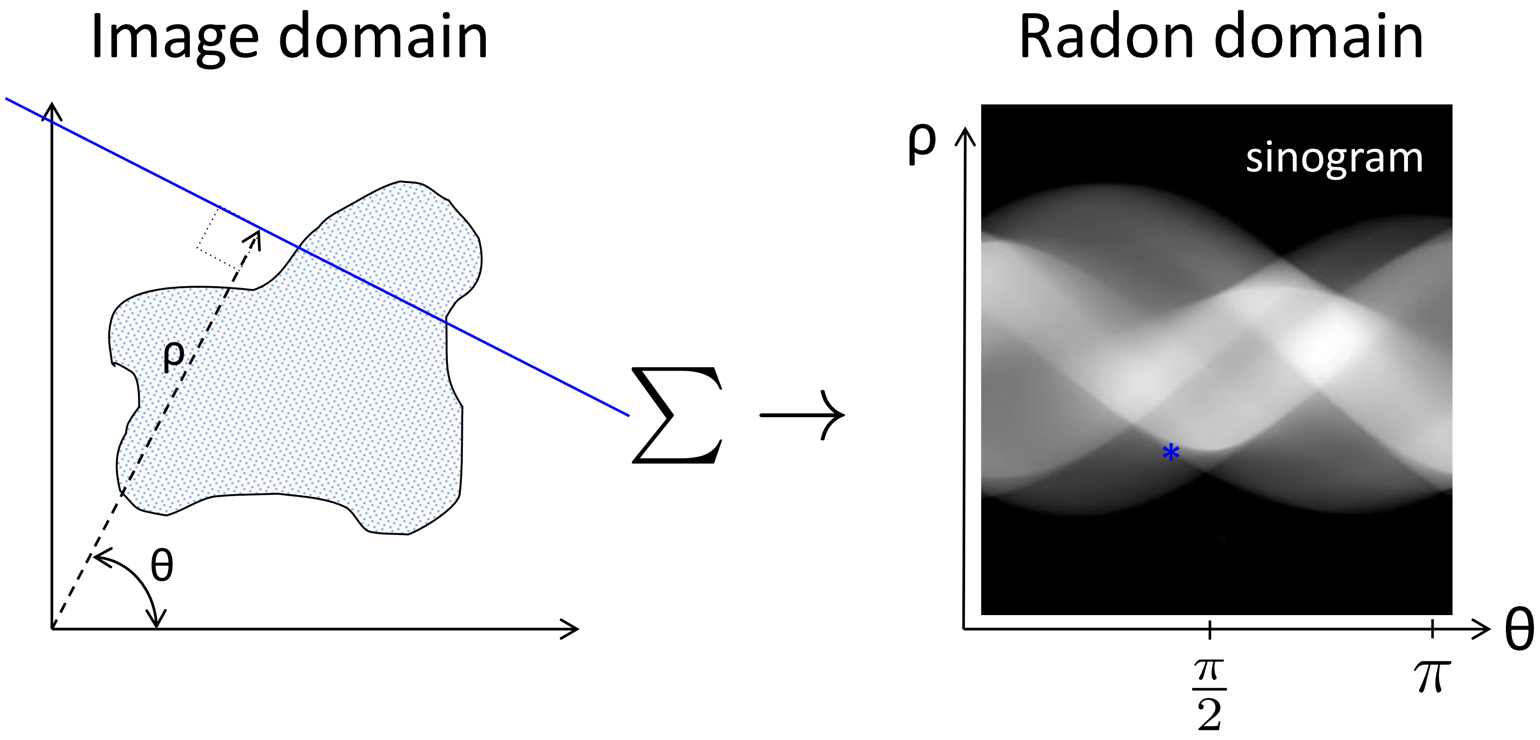
- We've acquired the data
- We've aligned and post-processed the data
- Does applying the inverse FFT produce our object?

Problems with Direct FT Inversion



- Acquisition: Radial
- Inverse FT: Cartesian
  - Inversion requires interpolation
- Some newer methods solve this problem (ex. GENFIRE)

The Radon Transform

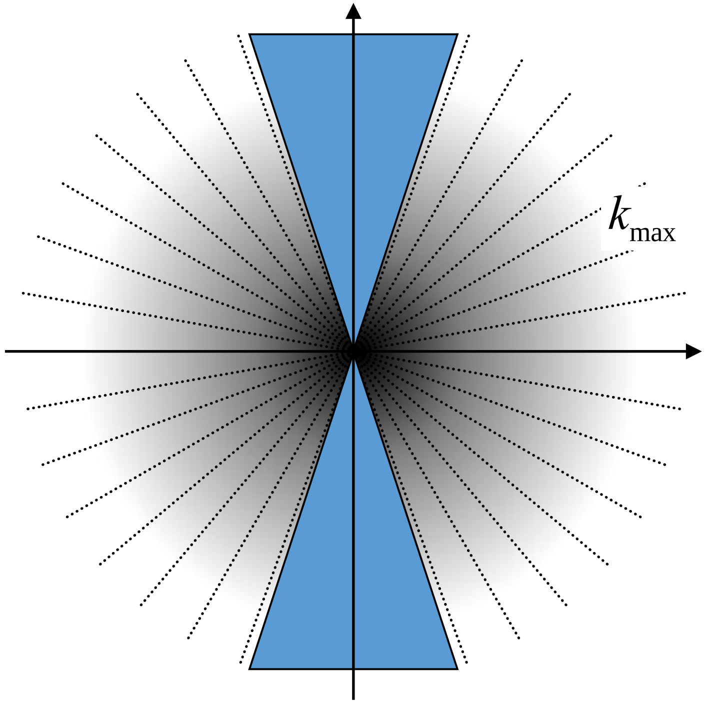


- Radon transform is a sum along a line at angle θ
- No Fourier transform is used
  - Avoids reciprocal space amplitude / phase
  - Simpler interpolation

Adapted from: IDL Reference Guide, Radon() transform

Weighted Backprojection

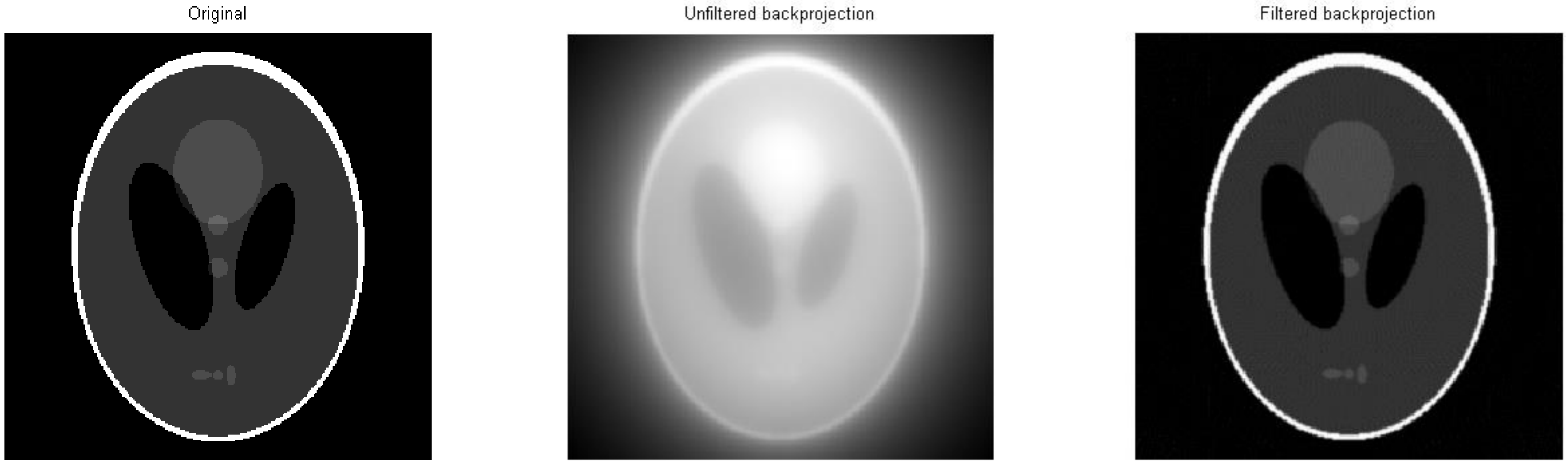
Weighted Backprojection Filter



$$w = r\Big|_0^{k_{\text{max}}}$$

- Low-frequency (large objects) over-sampled
- High-frequency (small objects) under-sampled
- Apply r-weighted filter *w* in F-space after backprojection

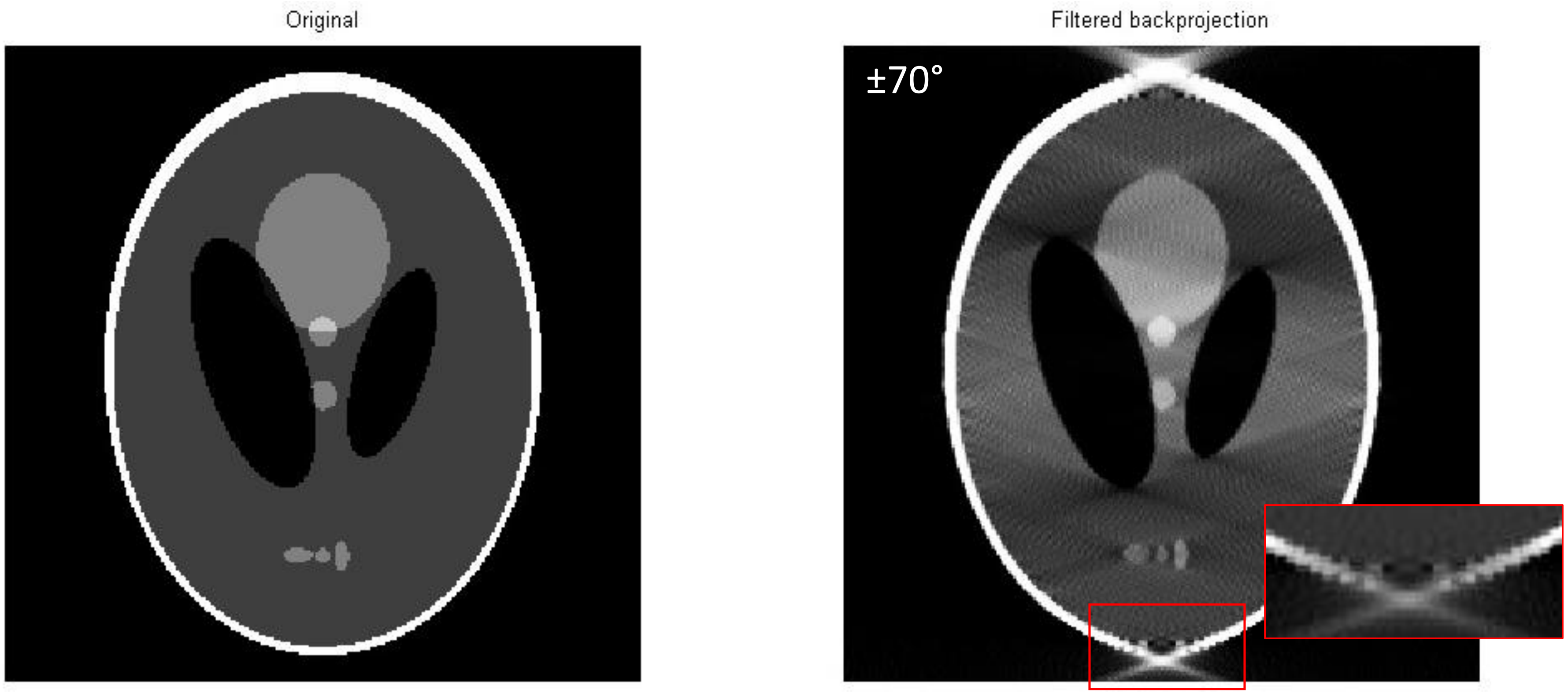
Effect of r-Weighted Backprojection



Original   Unfiltered   Filtered

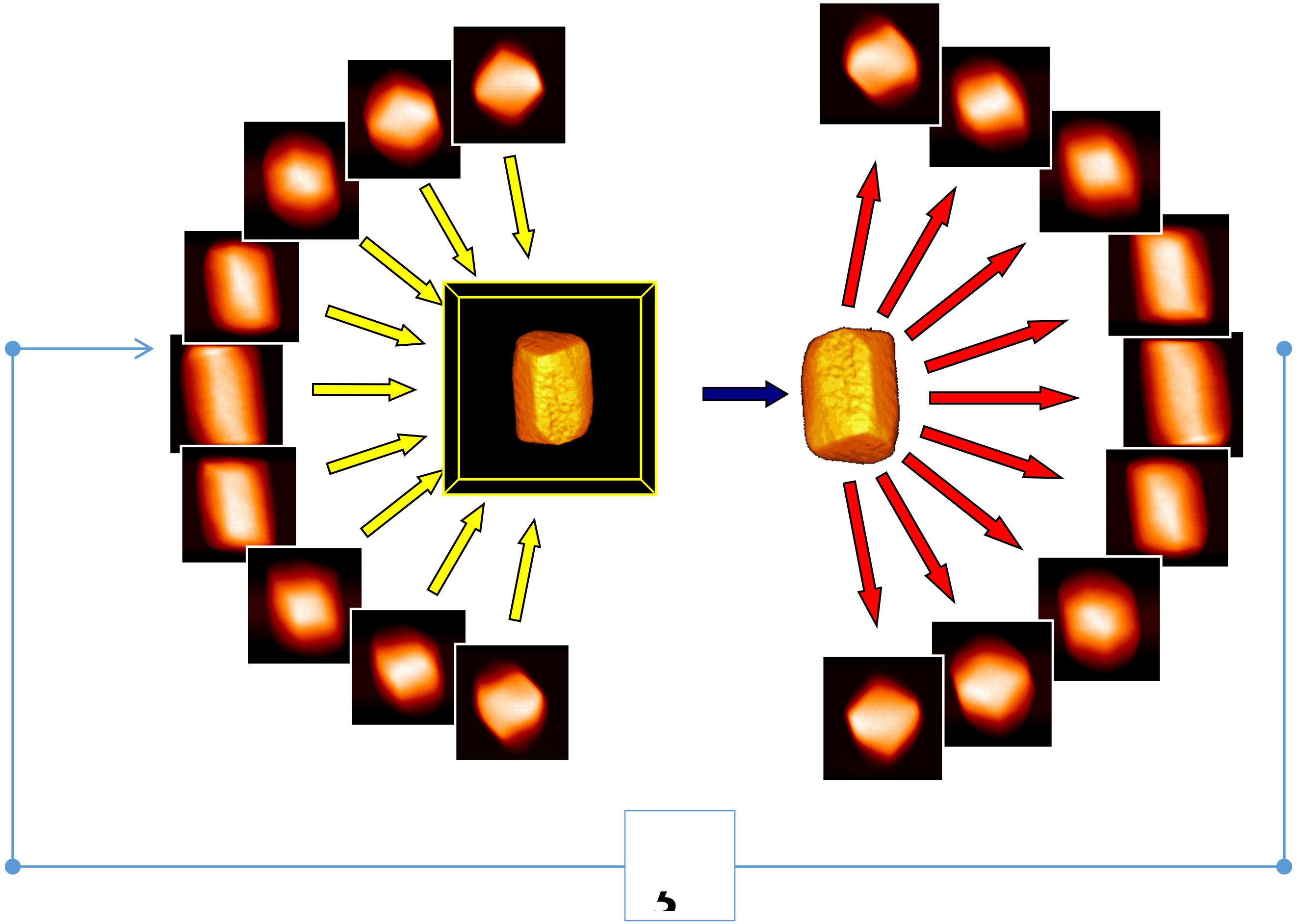
- Reconstruction with full tilt (±90° rotation)
- Unfiltered reconstruction dominated by low frequencies → blurred
- Filter faithfully reproduces the original

Artifacts in Filtered Backprojections



- Missing information introduces artifacts not seen in the original projections
- Can use the original projections as a reference and comparatively iterate to remove artifacts

The Iterative Process



- RE-project the model and compare with the original projections to remove artifacts
  - Interpolation occurs on the sinograms

Iterative Algorithms

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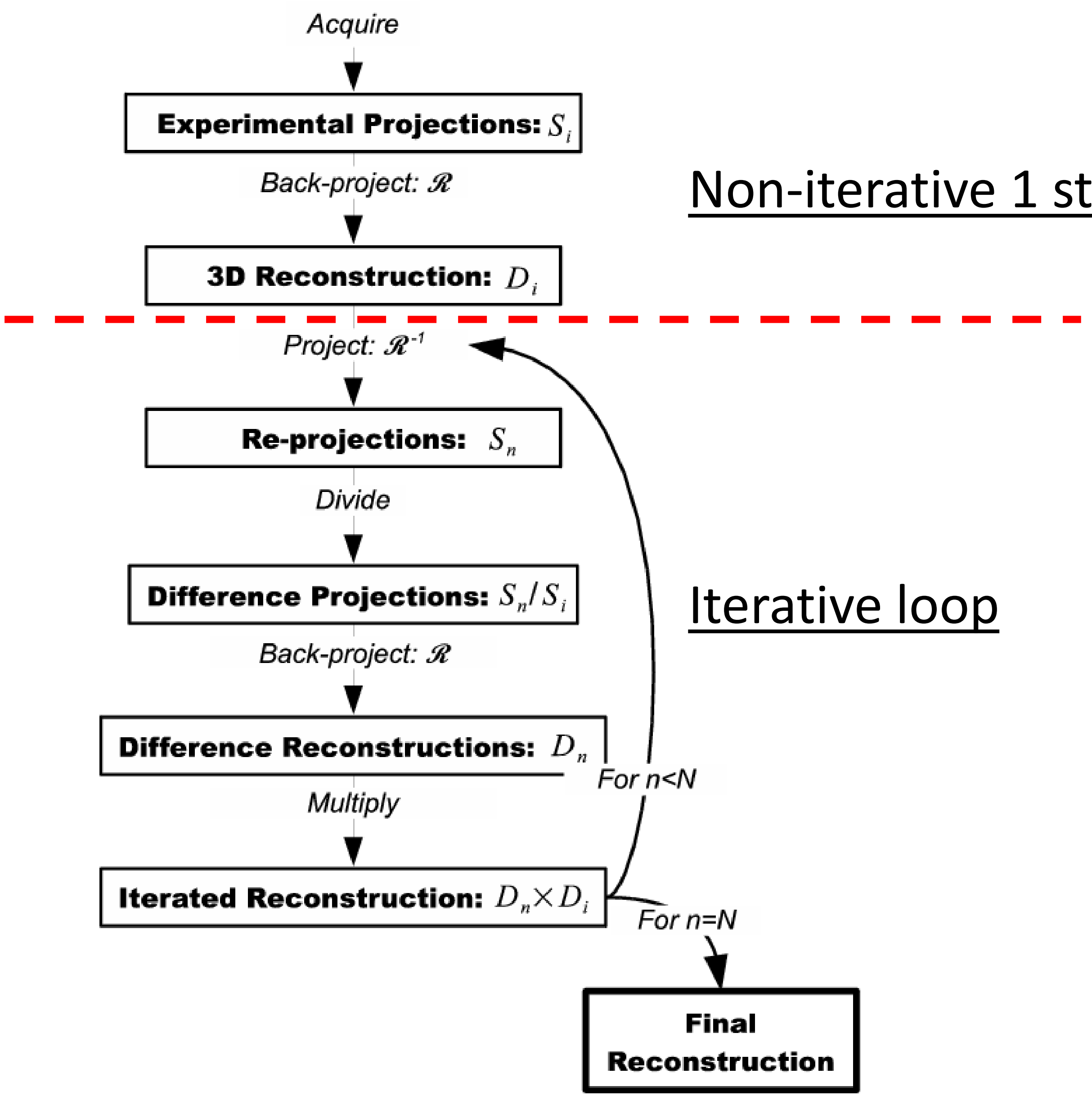
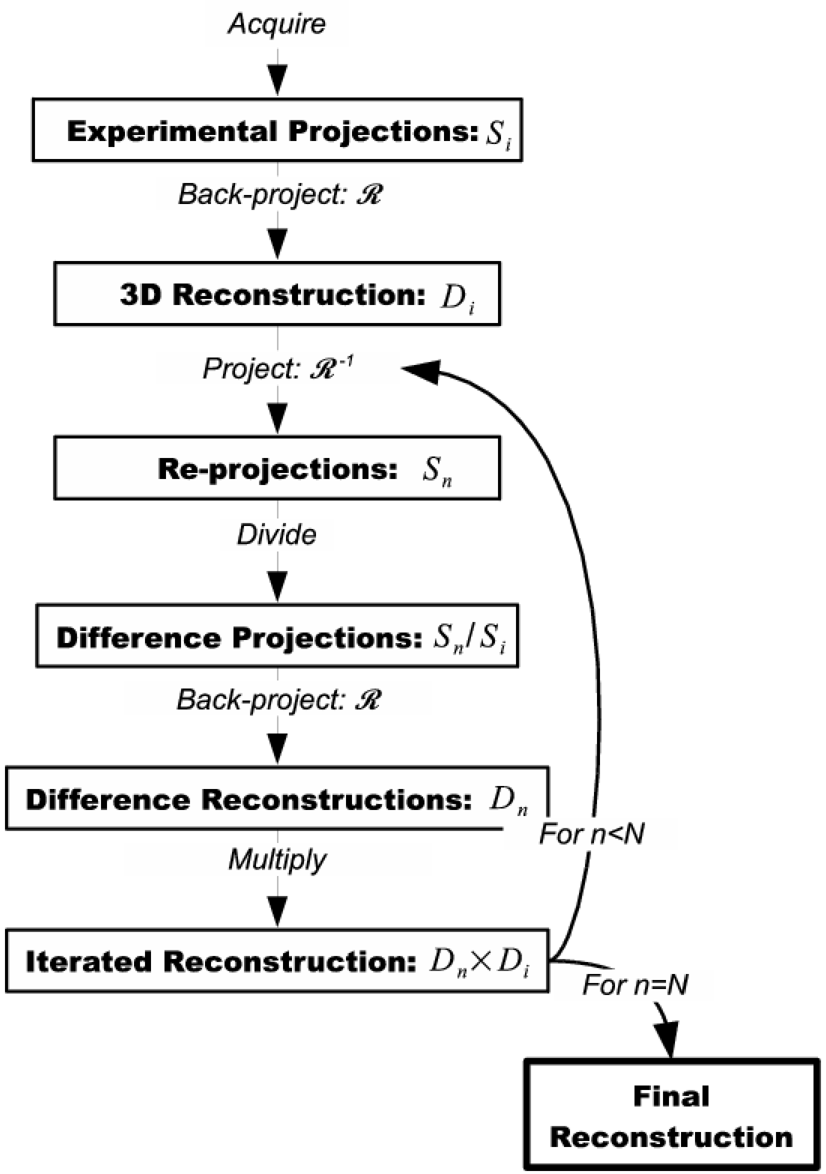


Non-iterative 1 step

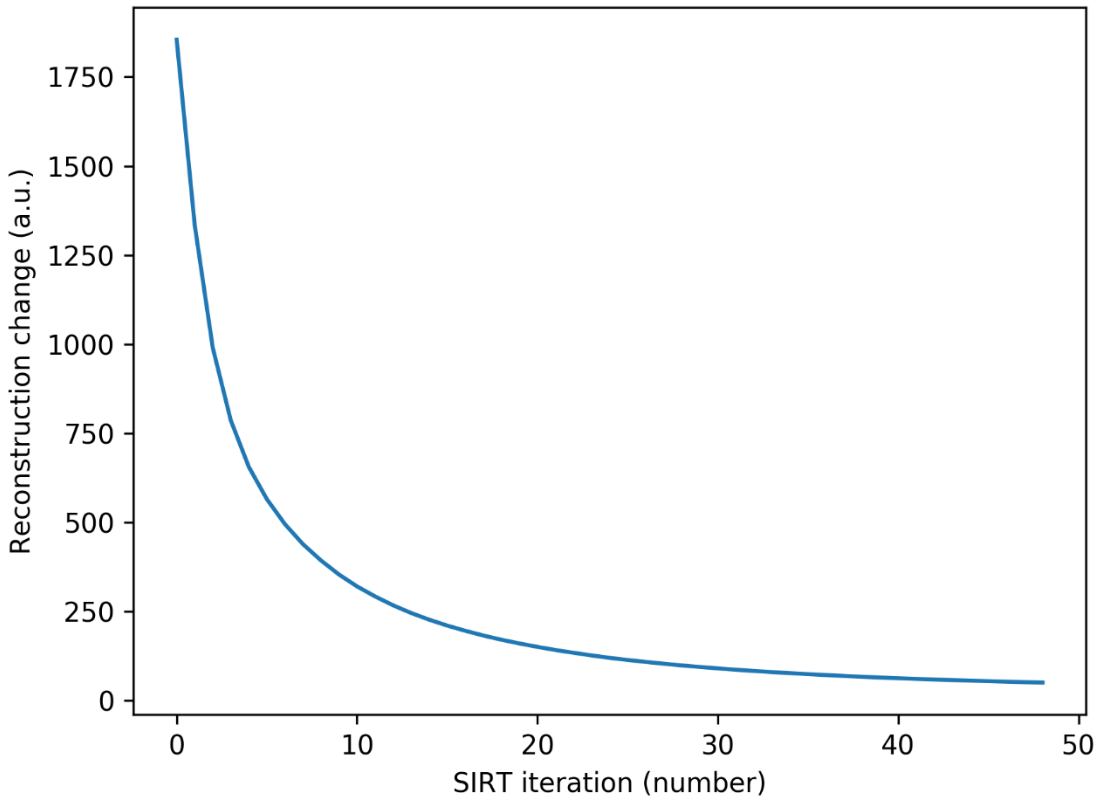
Iterative loop

Simultaneous Iterative Reconstruction Technique (SIRT)



1 - 20 SIRT iterations

SIRT Convergence



- Generally, the reconstruction converges after 20 or 30 iterations
- There are only very small changes in the reconstruction beyond this

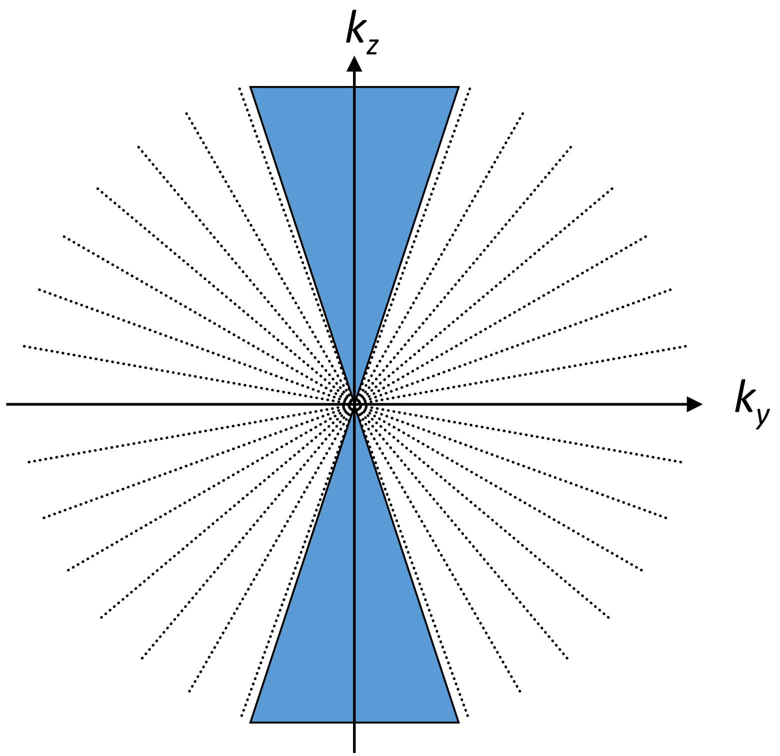
Advanced Iterative Fourier Algorithms

Frontiers of Electron Tomography

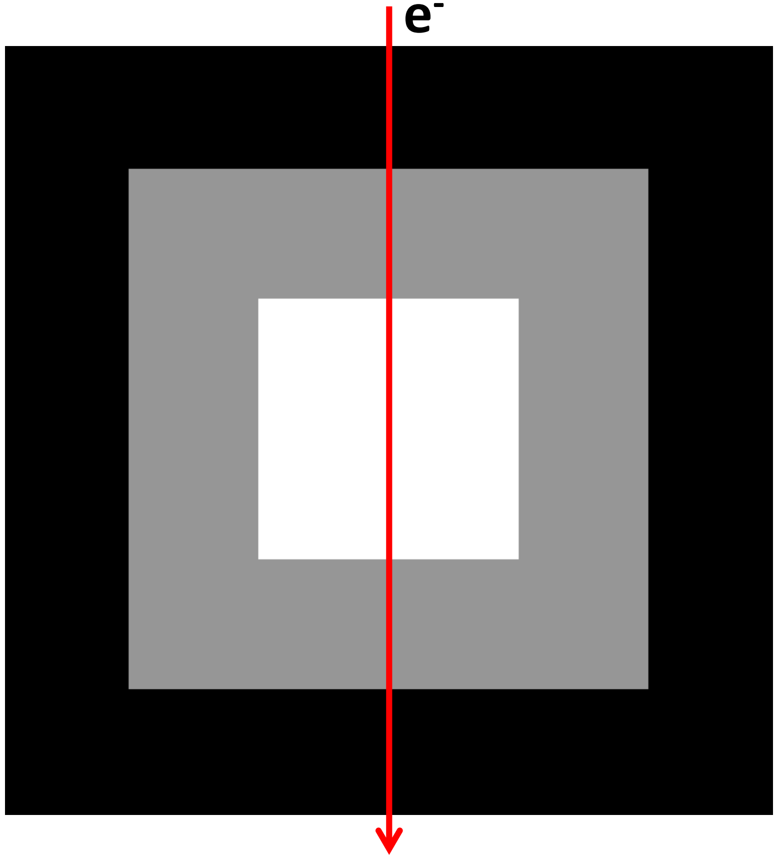
- EST is one type of iterative Fourier algorithm
  - Acquire data on a linogram grid to allow direct, iterative Fourier inversion
  - Incorporate constraints into reconstruction (all voxels > 0)
  - Attempts to calculate the best match between the reconstruction and experimental data
- Other newer algorithms provide similar or even improved fidelity of the reconstruction
  - GENFIRE: angle requirement is relaxed and allows for optimized angular alignment (http://www.physics.ucla.edu/research/imaging/FePt/)
  - Compressed sensing: improved reconstruction from noisy or fewer projections. Requires a sparsity condition:
    - L_1- or L_2-norm penalization (differences or square differences)
    - Total variation minimization (enforcing smoothness but edge preserving)

Reconstruction Review

- Back-projection is a real space operation
  - Simpler interpolation
- Weighted back-projection
  - r-weighted in Fourier space
- Iterative techniques compare each reconstruction against the original “perfect” projections
- More advanced algorithms are now available
  - Direct iterative Fourier inversion
  - Discrete tomography and atom counting
  - Compressed sensing (TV minimization)

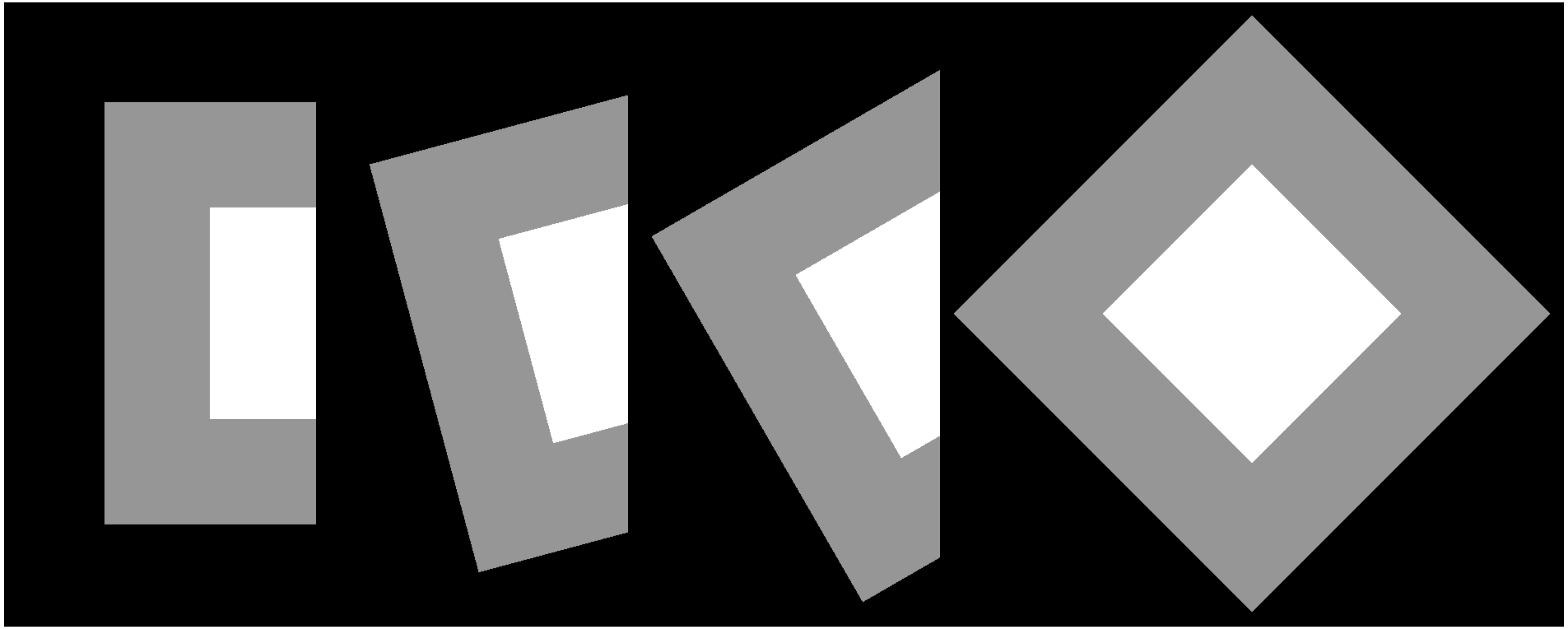


The Missing Wedge Effect



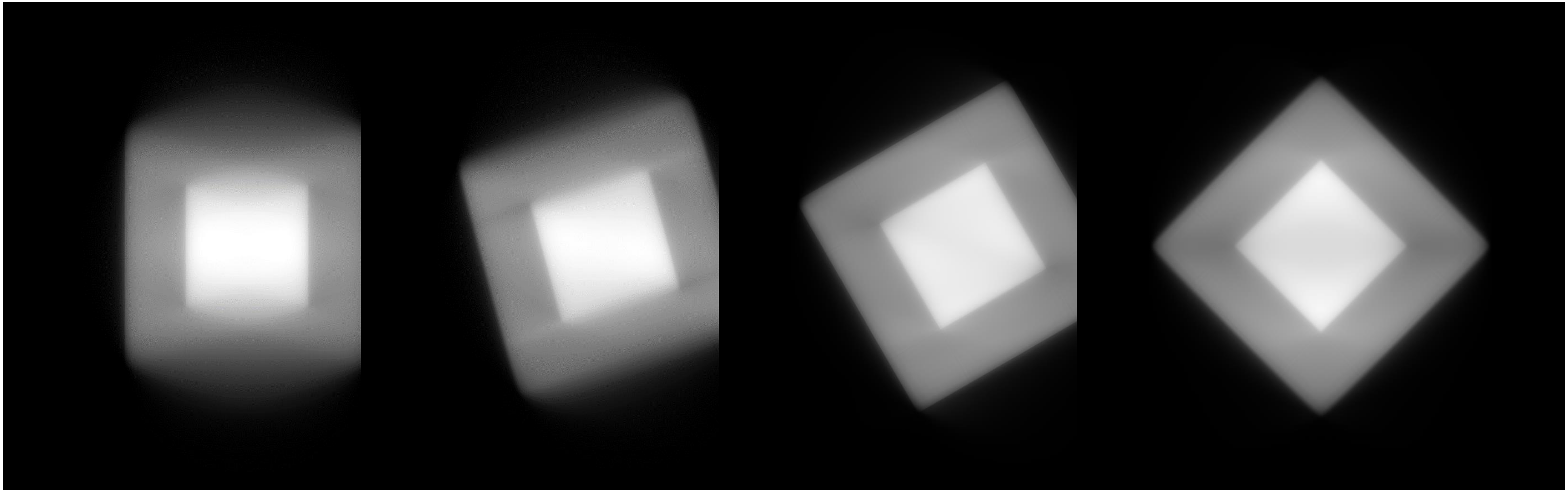
- Missing wedge causes elongation e_{zy} along e$^-$ beam direction
- High tilts are necessary for a faithful reconstruction
- Simulate linear projections of a faceted nanoparticle

Simulate Particle Reconstructions



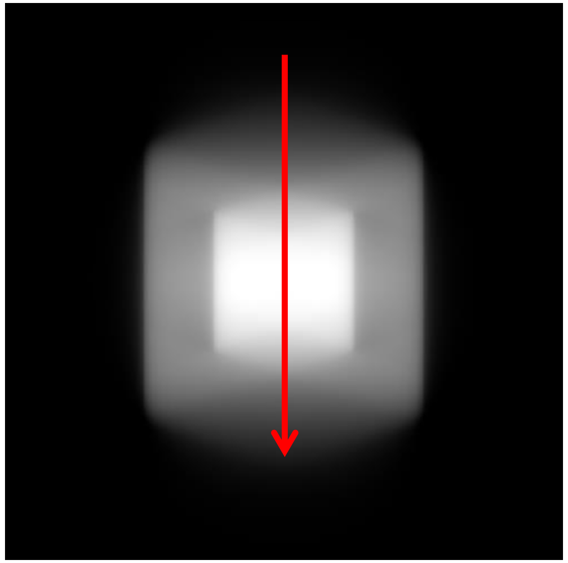
- Test reconstruction of the same particle at 4 different orientations: 0°, 15°, 30°, 45°
- All linear projections are along the vertical axis

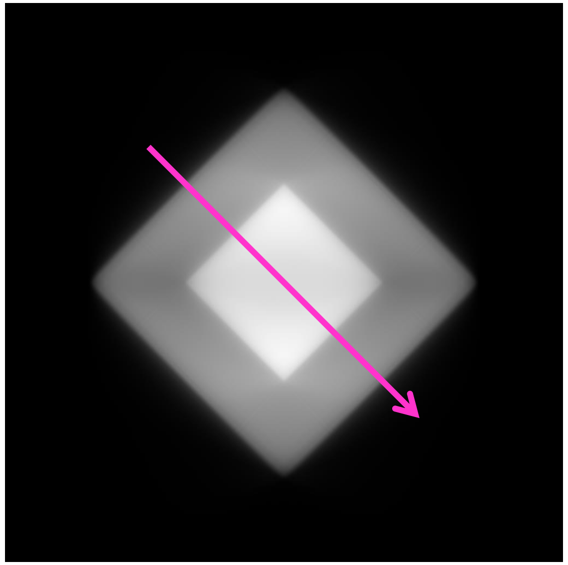
Binary Particle Test Reconstructions

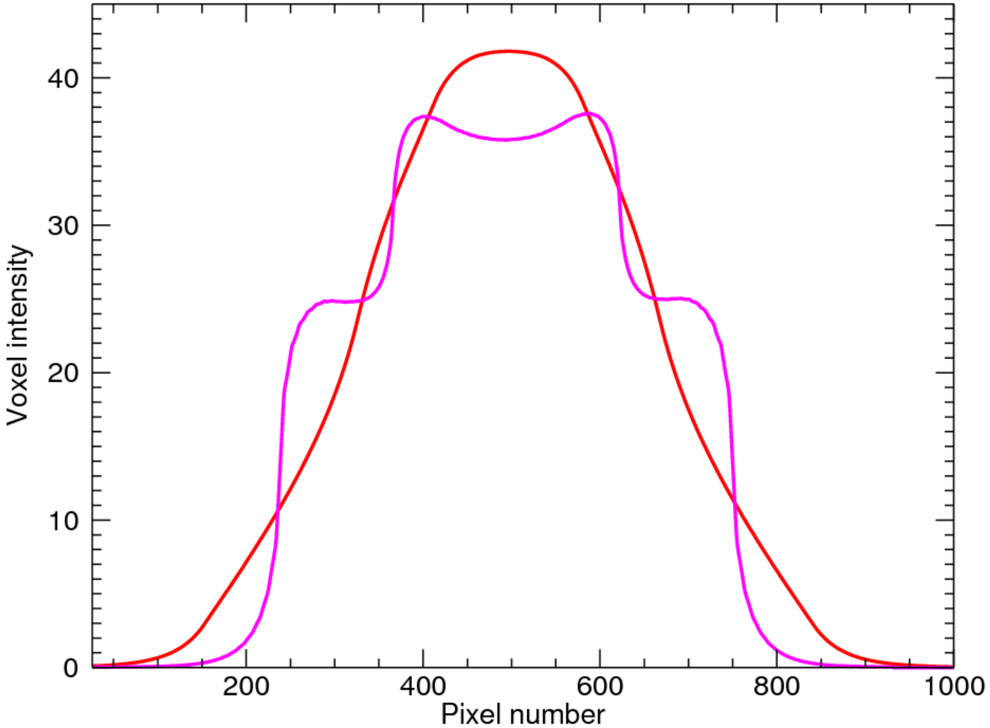


- Missing wedge is most prevalent for edges perpendicular to the projection direction
- 45° rotated object gives best results for this shape

Binary Particle Test Reconstructions

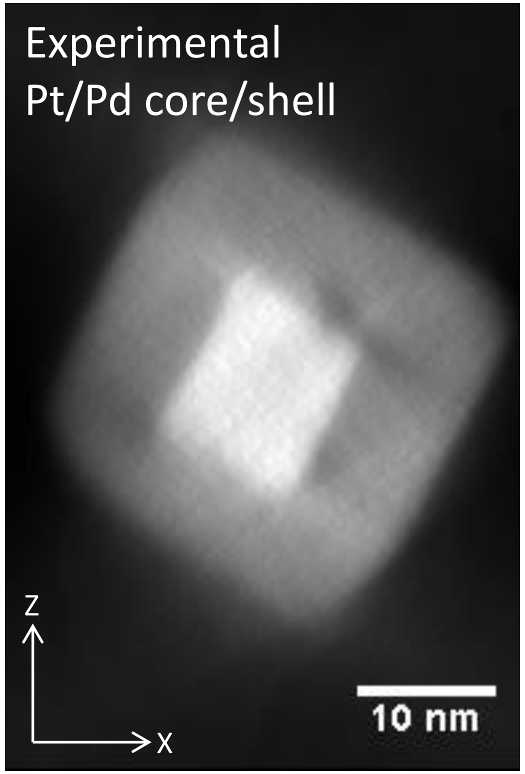


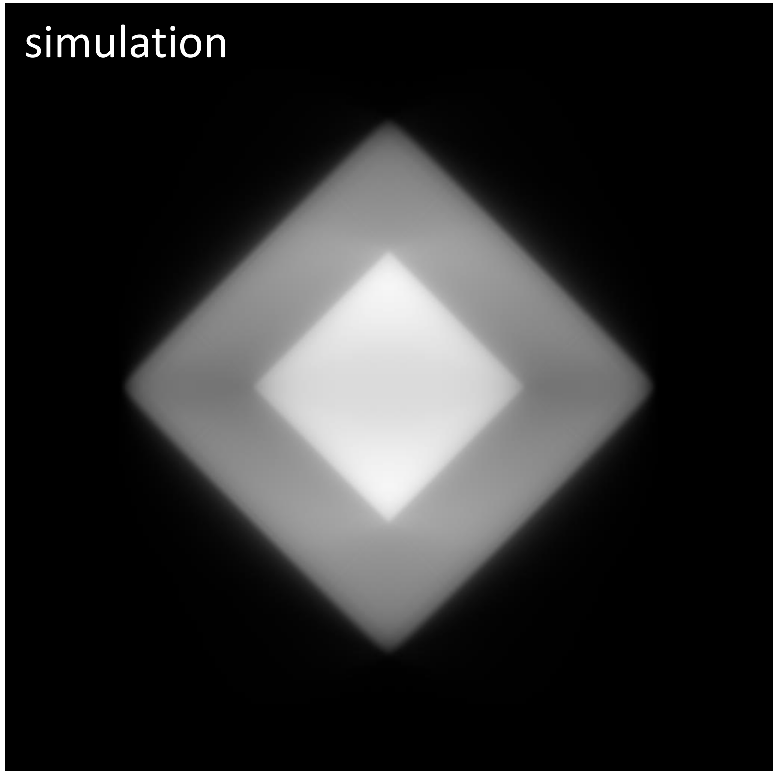




- Missing wedge is most prevalent for edges perpendicular to the projection direction
- 45° rotation gives good results for this faceted object

Experiment vs Simulation





- On-edge particles are more faithfully reconstructed with minimal artifacts
- Always keep in mind the direction of the missing wedge artifacts

Grand Challenge: 3D Atom Coordinates

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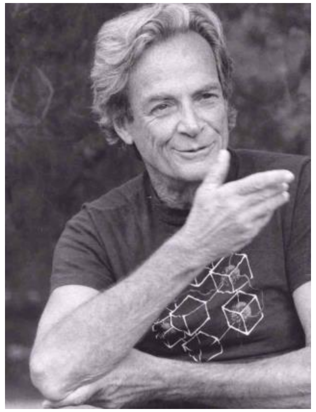
"It would be very easy to make an analysis of any complicated chemical substance; all one would have to do would be to look at it and see where the atoms are...



– Richard P. Feynman, 1959, "There's Plenty of Room at Bottom"

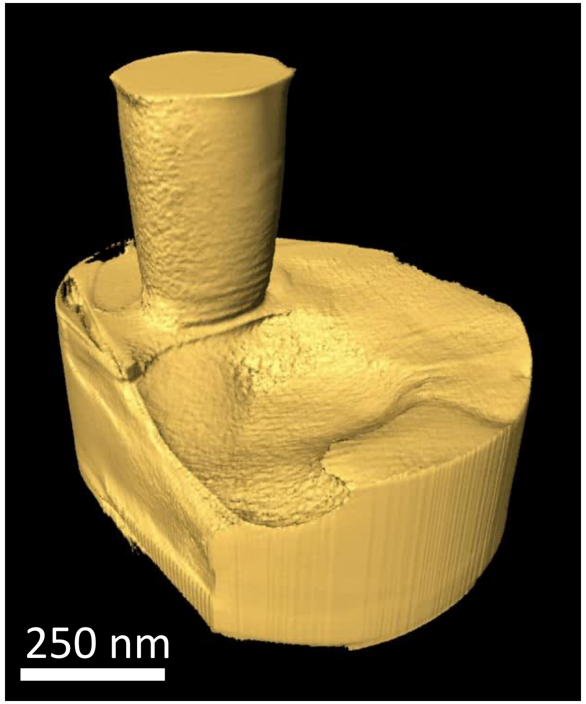
A grand challenge of materials science

- Image defects in materials
  - Dislocations, point defects
- Image atomic species in 3D
  - Dopant atoms, core-shell
- Ultimately: amorphous materials
  - Glass transition

Tomography Advancement

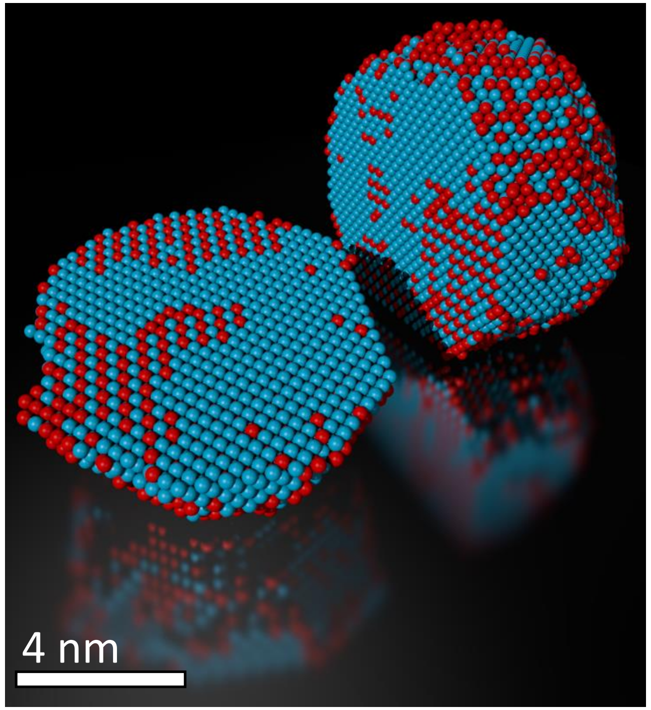
Frontiers of Electron Tomography

Copper interconnect



P Ercius, et al, Appl. Phys. Lett. **88**, 243116 (2006)

FePt Nanoparticle



Y Yang, et al, Nature, **542**, 7639 (2016)

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Frontiers of Electron Tomography

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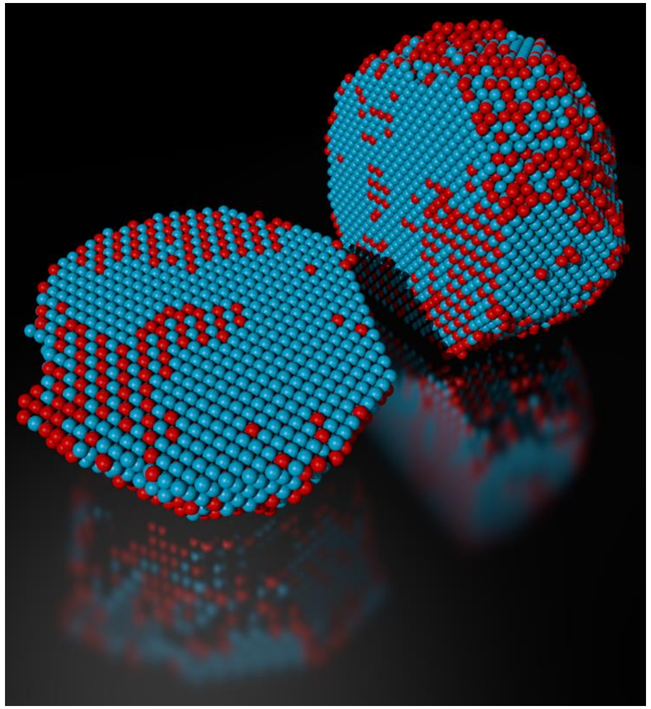
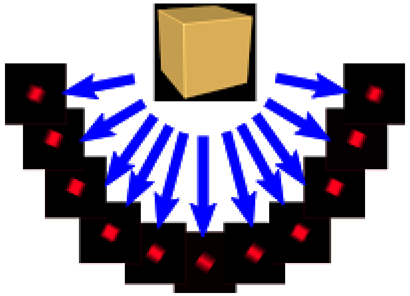
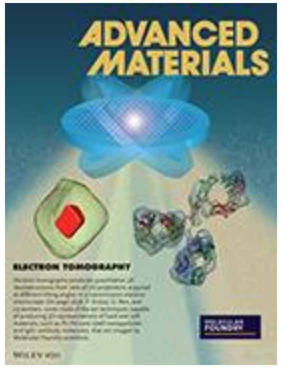


Resources

Frontiers of Electron Tomography





- tomviz: Open source 3D volumetric visualization and analysis (tomviz.org)
  - Volumetric, orthogonal slicing, rotation, animation
- Other reconstruction packages (parallelized):
  - Tomopy, astra-toolbox, IMOD, TomoJ, many others
- Recent review article with lots of background and applications (hard and soft materials)
  - P Ercius *et al*, "Electron Tomography: A Three-Dimensional Analytic Tool for Hard and Soft Materials Research," *Advanced Materials* **27:**38, pp. 5638–63 (2015). doi: [10.1002/adma.201501015](https://doi.org/10.1002/adma.201501015)
- International Workshop and Short Course on the Frontiers of Electron Tomography (www.electron-tomo.com)
  - October 23-27, 2017 in Berkeley, CA
  - Registration open now!