THE UNIVERSITY of TEXAS
HEALTH SCIENCE CENTER AT HOUSTON
SCHOOL of HEALTH INFORMATION SCIENCES

Sampling and Reconstruction

For students of HI 5323
“Image Processing”

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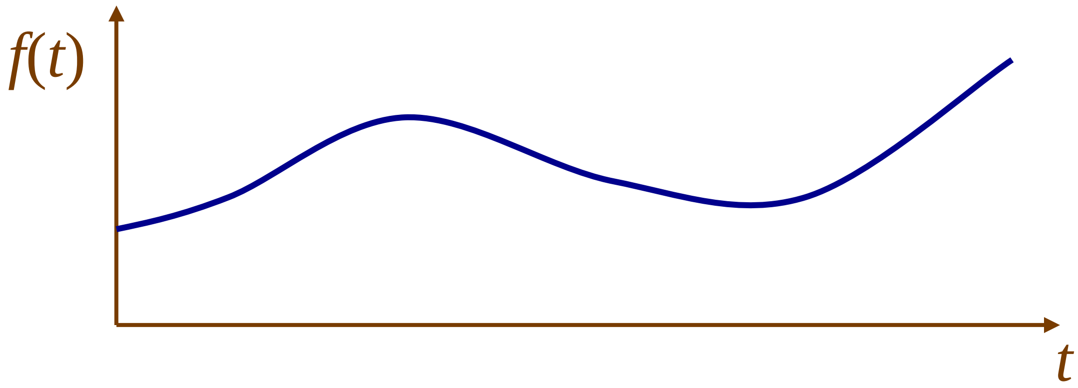
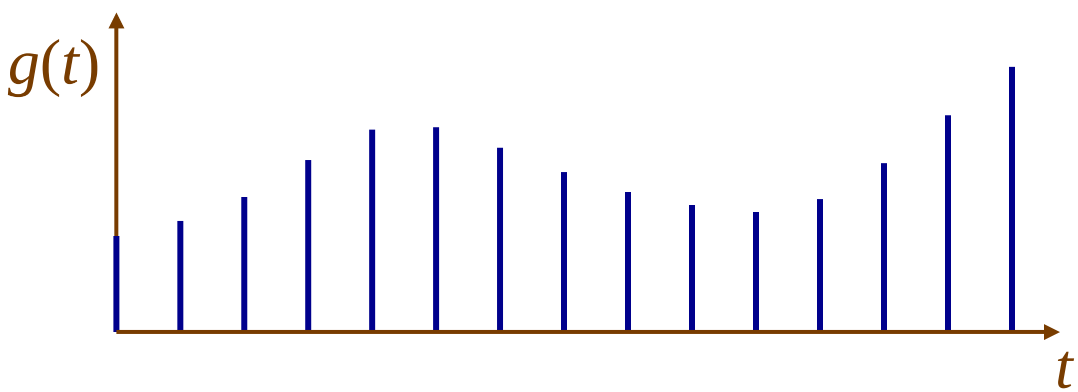
http://biomachina.org/courses/processing/10.html

Sampling

Continuous



Discrete



Sampling: Spatial/Temporal Domain

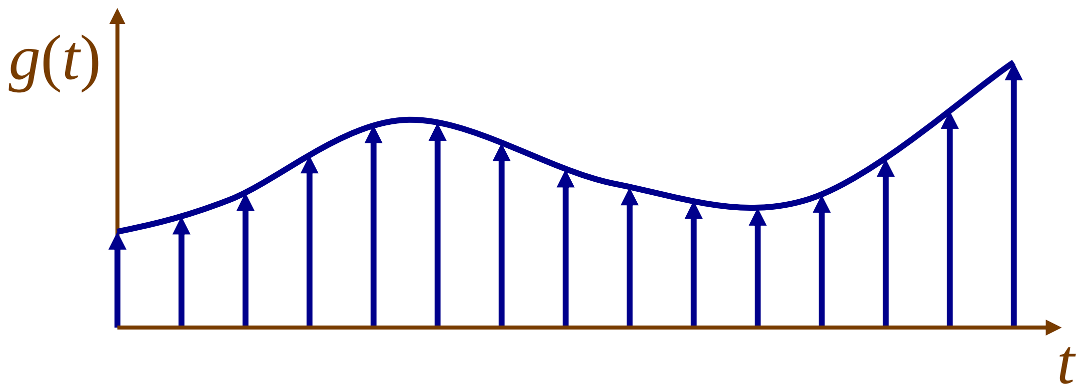
Sampling a continuous function f at time/space interval Δt to produce a discrete function g

$$g[n] = f(n\Delta t)$$

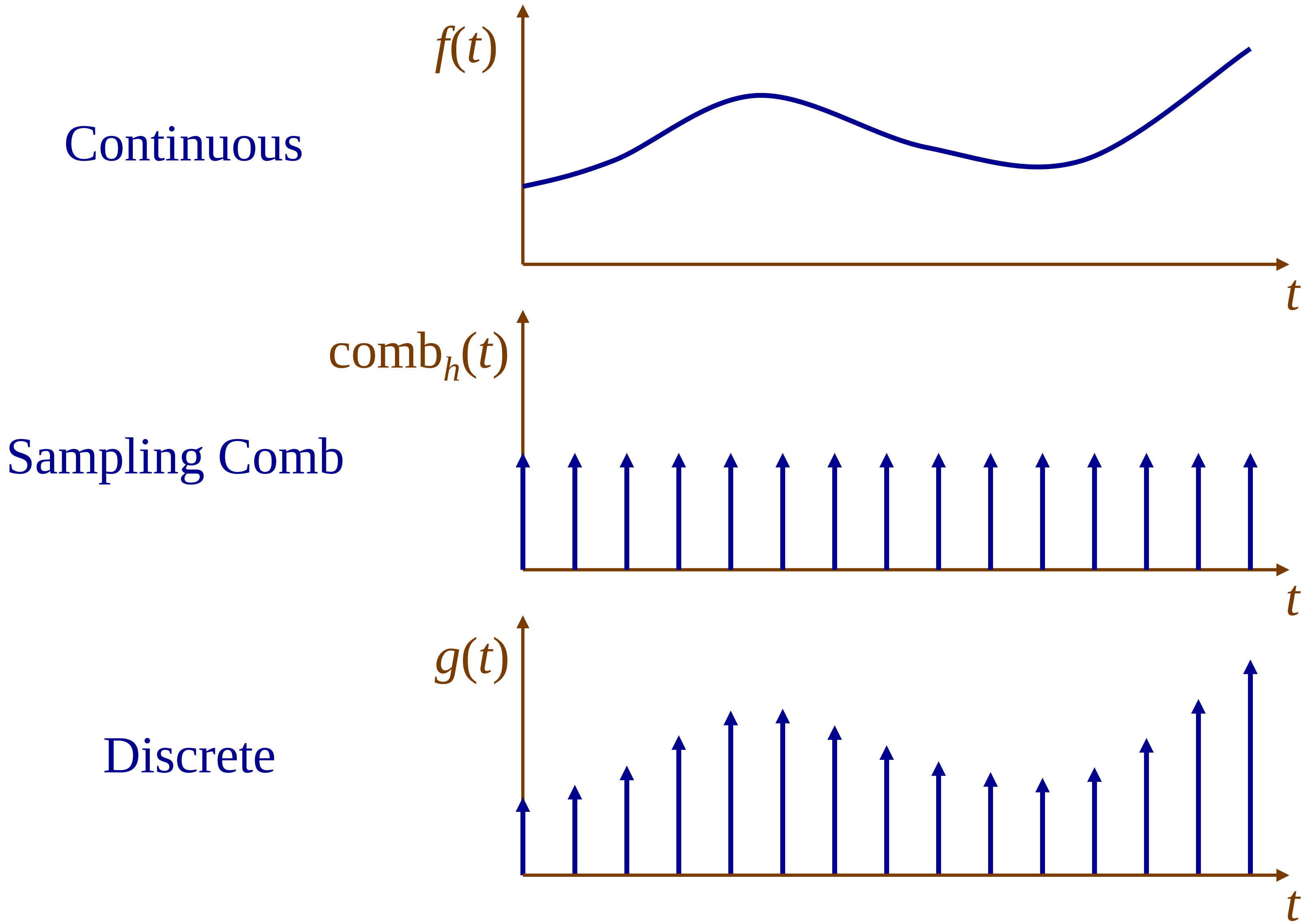
is the same as multiplying it by a comb:

$$g = f\,\mathrm{comb}_h$$

where $h = \Delta t$



Sampling: Spatial/Temporal Domain



Sampling: Frequency Domain

Sampling in the spatial/temporal domain by multiplying with comb_h

$$g = f\,\text{comb}_h$$

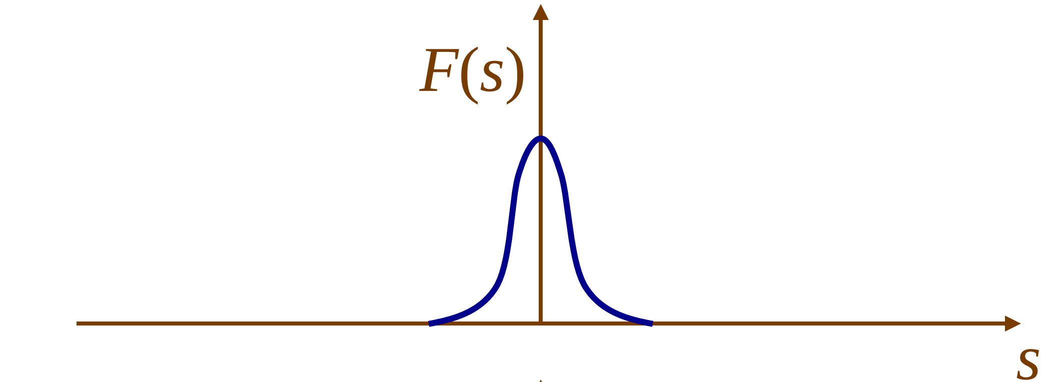
is the same as convolution in the frequency domain with the transform of comb_h:

$$G = F * \text{comb}_{1/h}$$

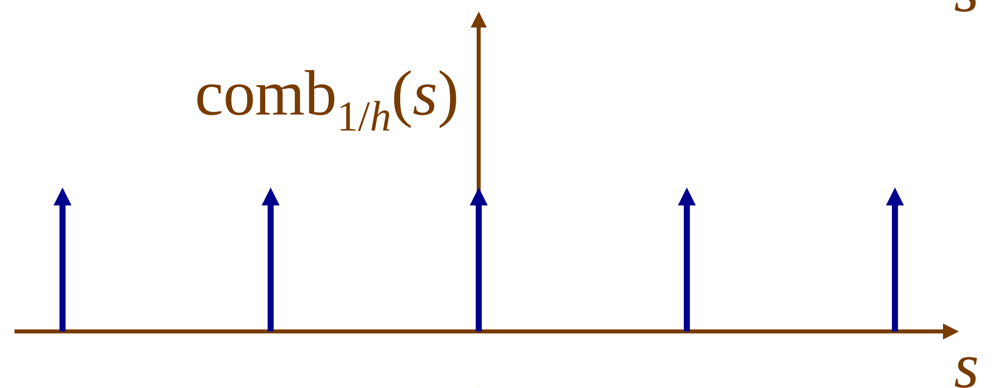
Convolution of a function and a comb causes a copy of the function to "stick" to each tooth of the comb, and all of them add together

Sampling: Frequency Domain

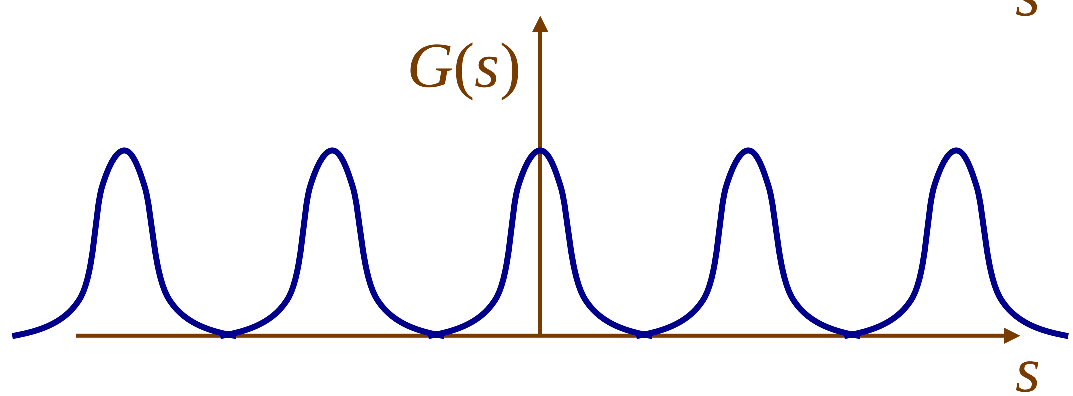
Spectrum



Comb's Spectrum



Spectrum of
Discrete Signal



Reconstruction

In theory, we can reconstruct the original continuous function by removing all of the extraneous copies of its spectrum created by the sampling process:

$$F(s) = G(s)\, \Pi_{1/h}(s)$$

In other words, keep everything in the frequency domain

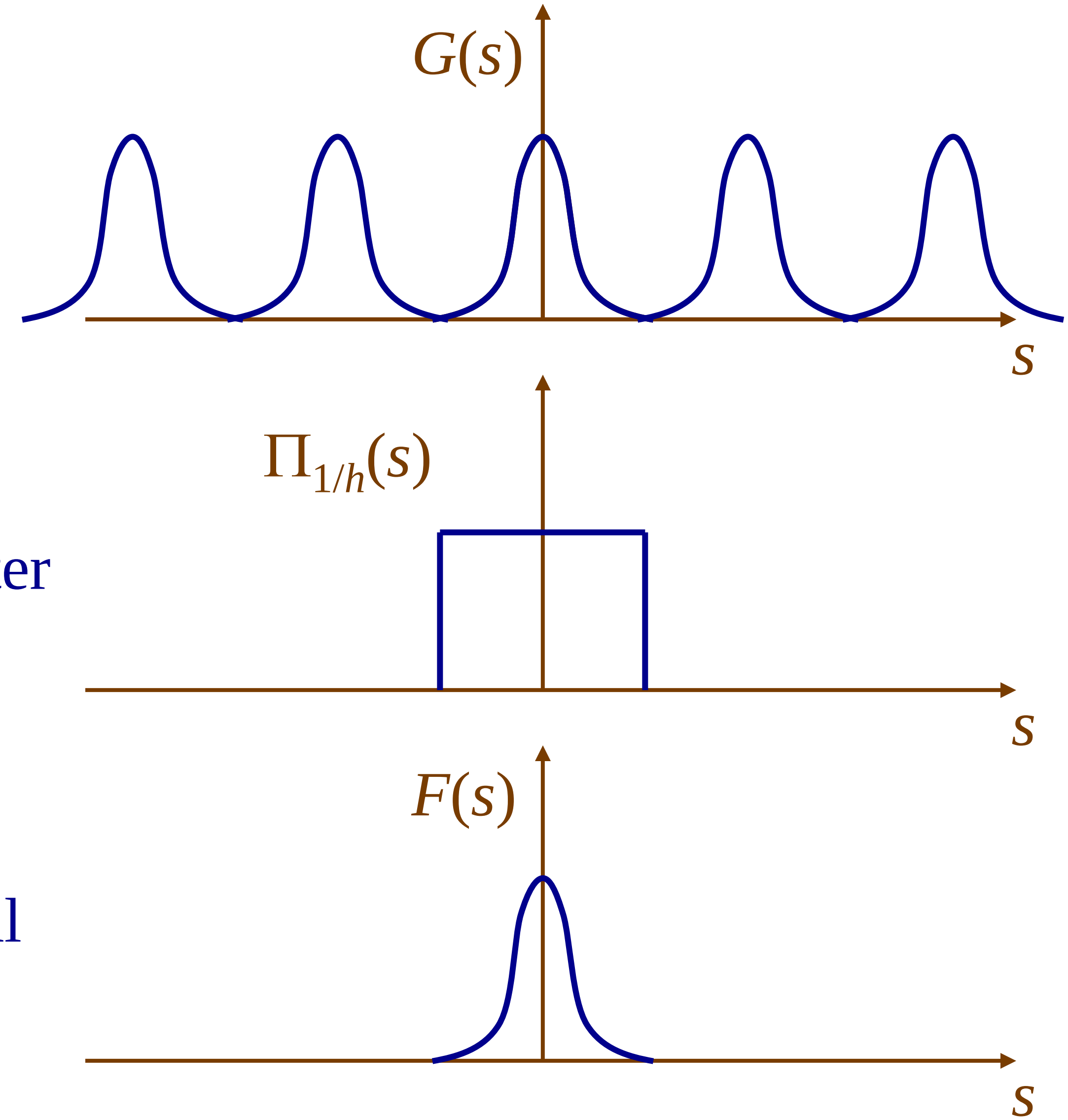
between $-\frac{1}{2h} \leq s \leq \frac{1}{2h}$ and throw the rest away

Reconstruction: Graphical Example

Spectrum of Discrete Signal

Rectangular (Box) Filter

Reconstructed Signal Spectrum



The Sampling Theorem

We can only do this reconstruction *if the duplicated copies do not overlap*

They do not overlap iff:

1. The signal is band limited, and
2. The highest frequency in the signal is less than $\frac{1}{2h}$

In other words, the sampling rate $1/h$ must be twice the frequency of the highest frequency in the image

This is called the *Nyquist rate*

Aliasing

What if the duplicated copies in the frequency domain do overlap?

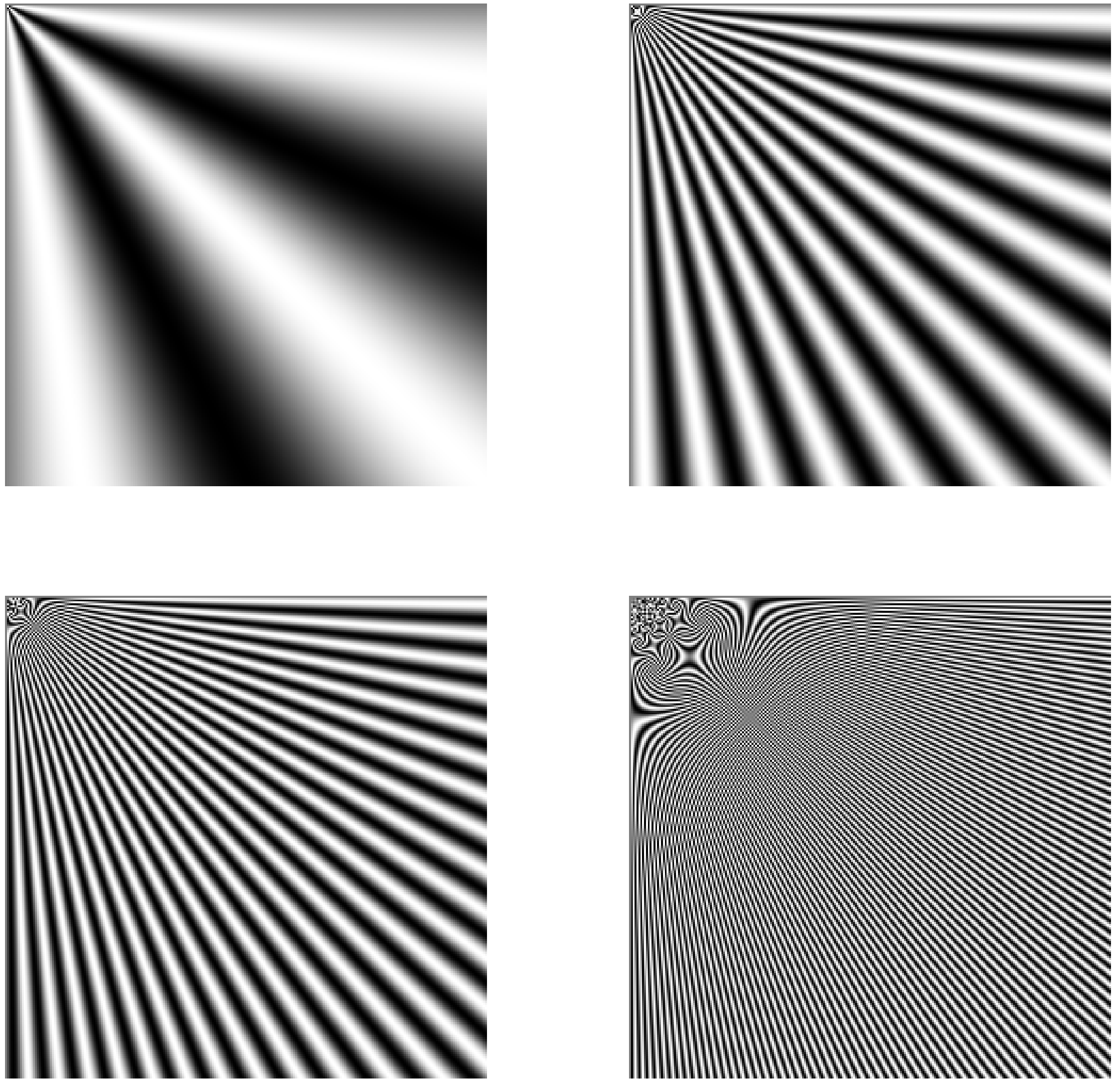
High frequency parts of the signal (those higher than $\frac{1}{2h}$) intrude into neighboring copies

The higher the frequency, the *lower* the point of overlap in the adjacent copy

These high frequencies masquerading as low frequencies is called *aliasing*

False low-frequency patterns are called *Moiré patterns*

Moiré Patterns

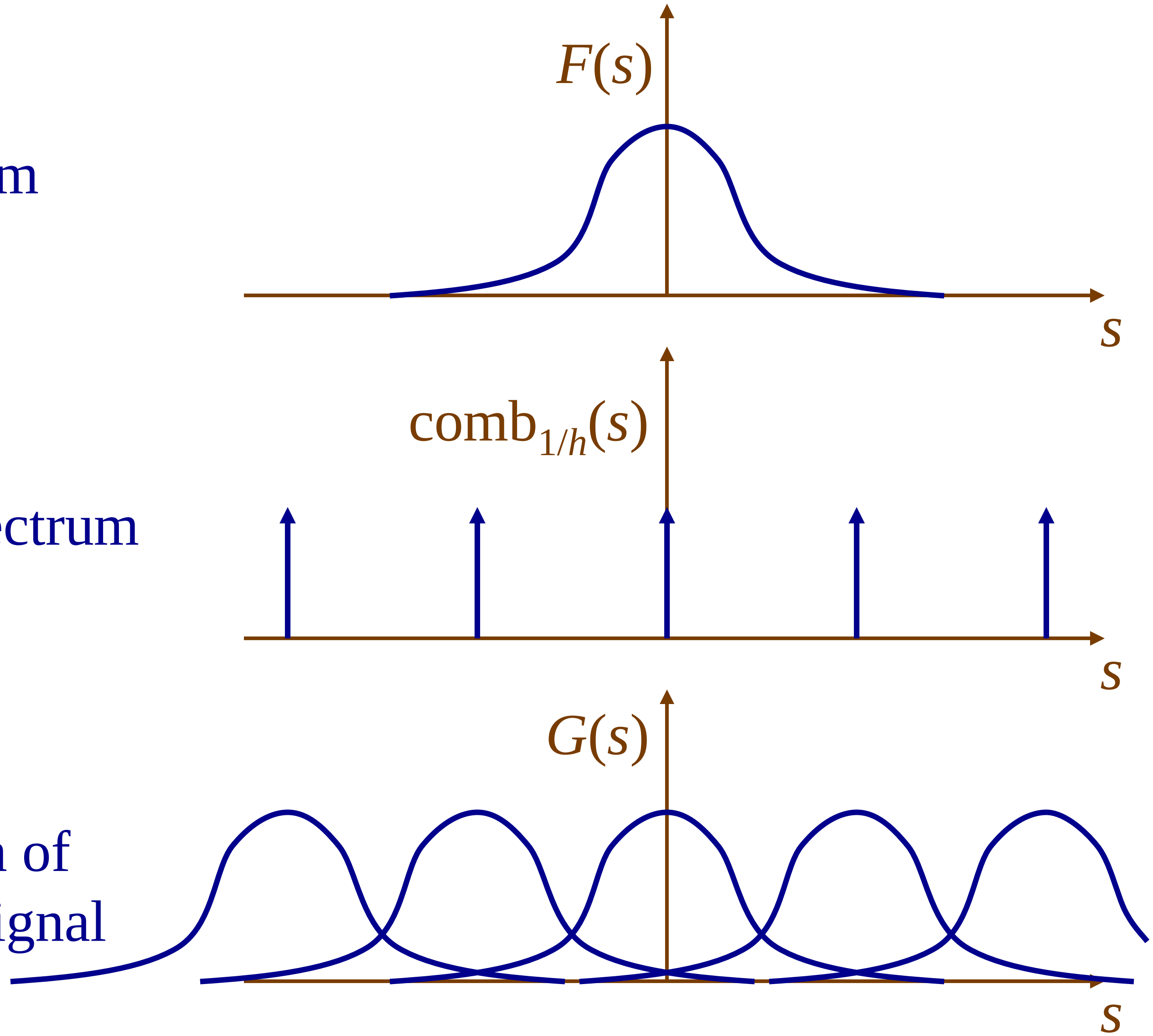


Sampling: Frequency Domain

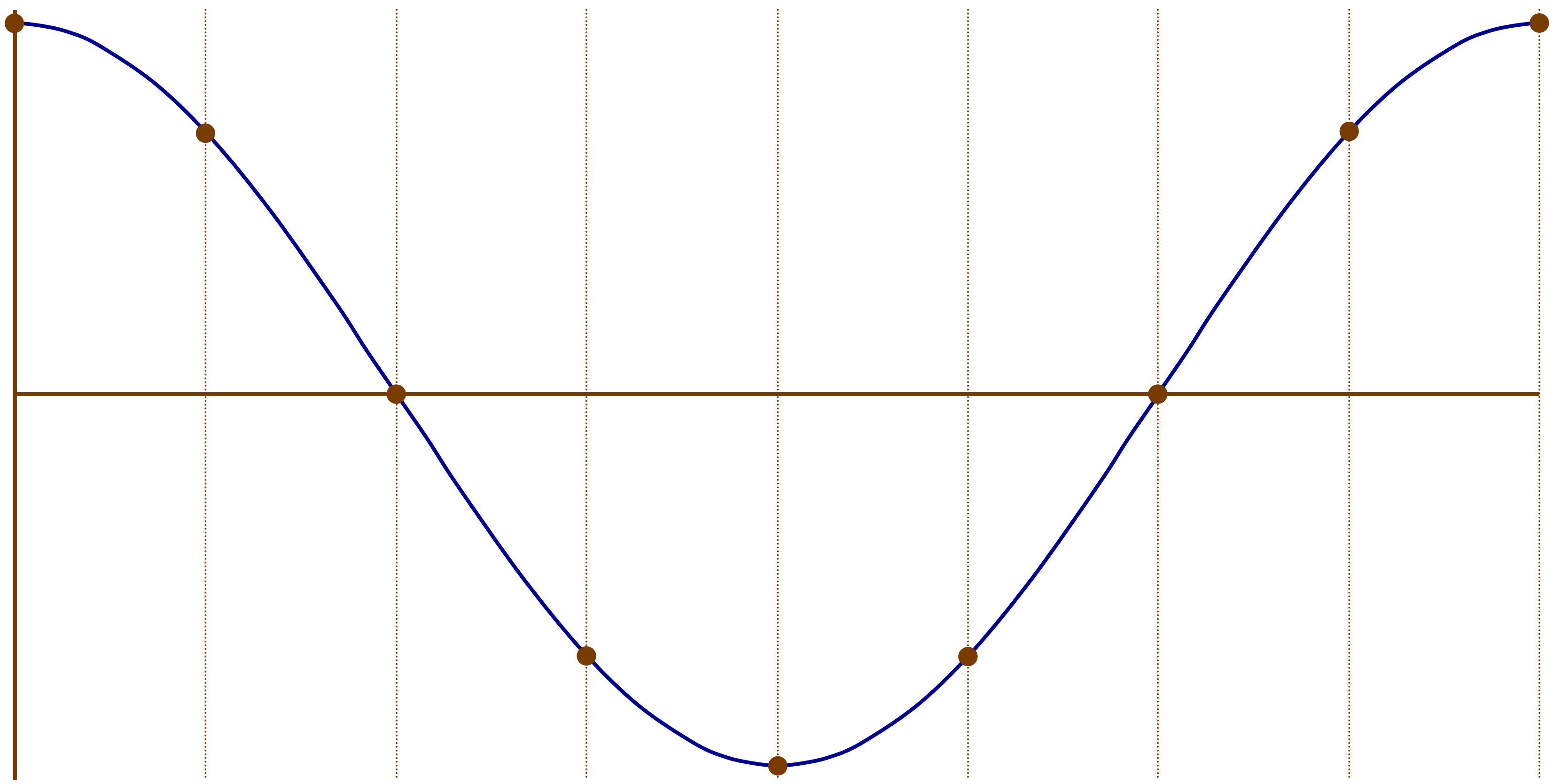
Spectrum

Comb's Spectrum

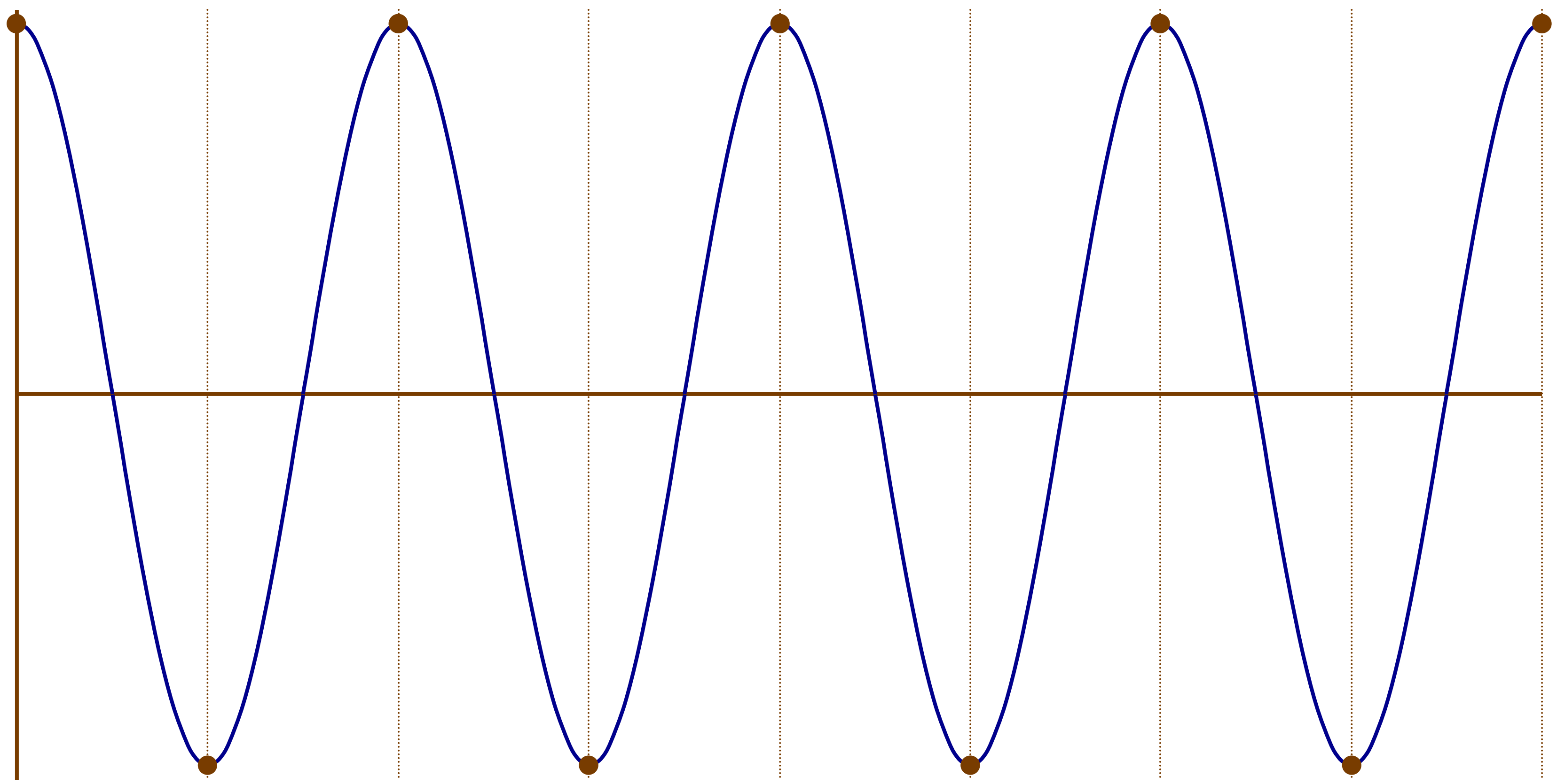
Spectrum of
Discrete Signal



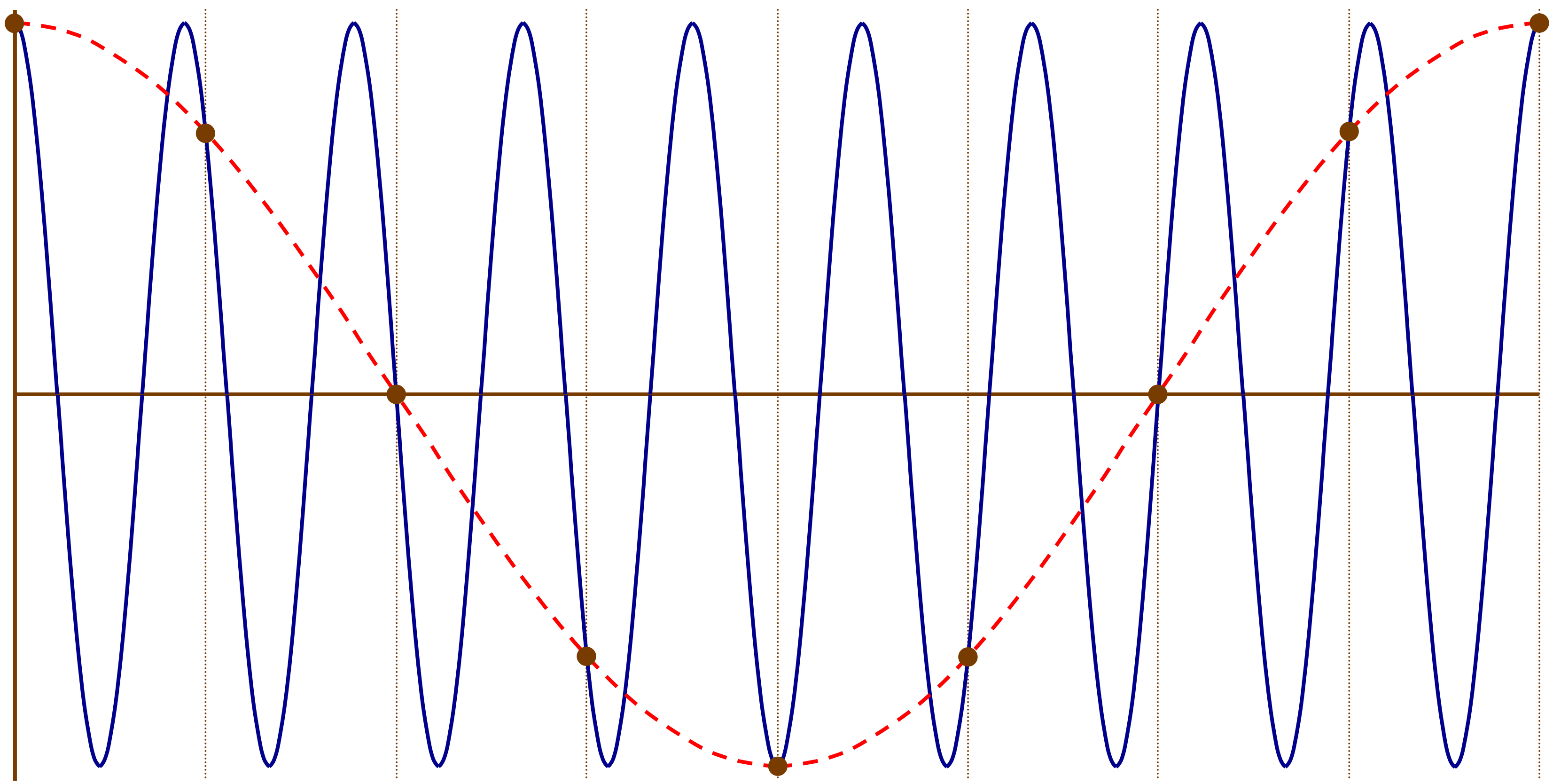
Sampling: Above the Nyquist Rate



Sampling: At the Nyquist Rate



Sampling: Below the Nyquist Rate



Preventing Aliasing

You have two choices:

1. Increase your sampling

2. Decrease the highest frequency in the signal *before sampling*

Reconstruction

Reconstruction was

$$F(s) = G(s)\ \Pi_{1/h}(s)$$

But in the time/spatial domain this is equivalent to

$$f(t) = g(t) * \mathrm{sinc}(2\pi t/h)$$

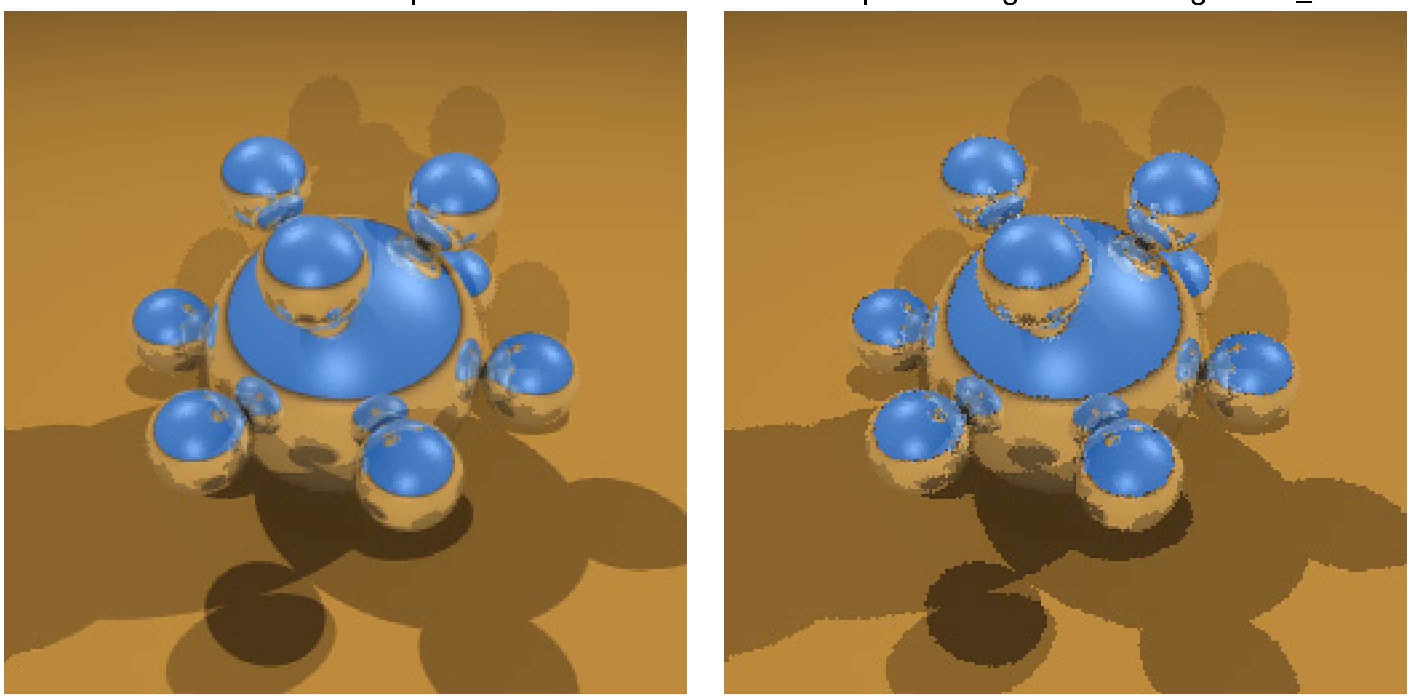
So, convolve your discretely-sampled (non-aliased) image with a sinc function and you can reconstruct the original continuous image

Imperfect Reconstruction

Problem: not perfect — the sinc function has infinite extent

By not perfectly clipping in the frequency domain, the duplicate copies now look like false high frequencies

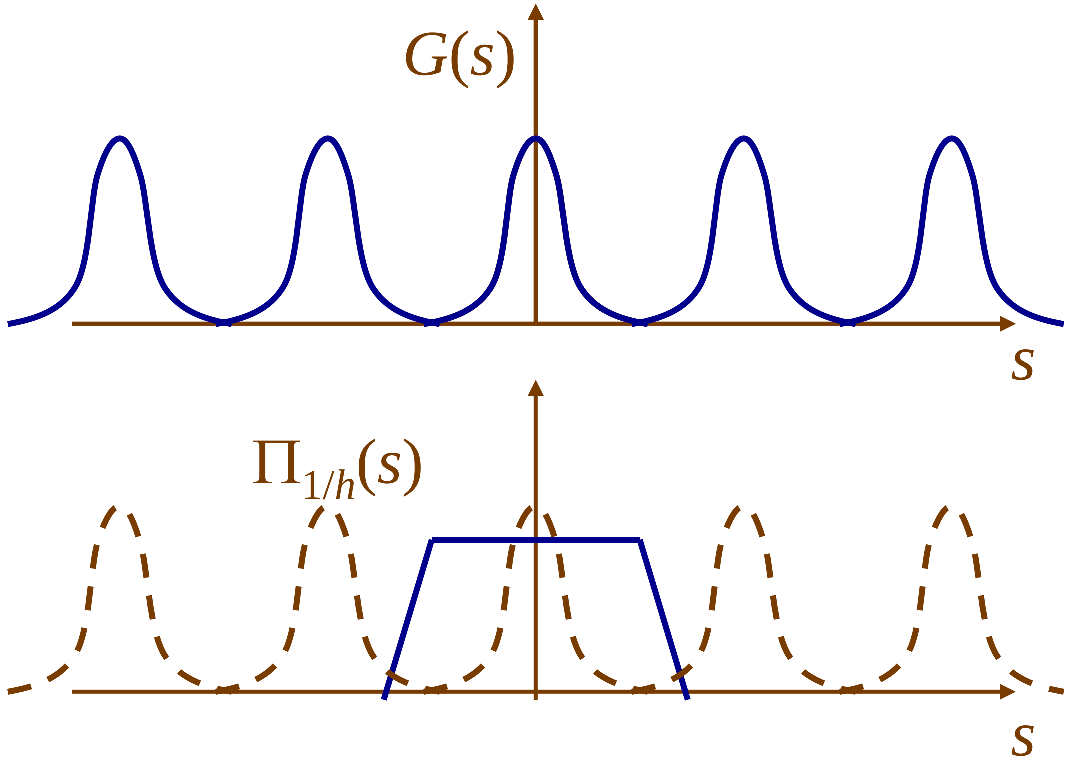
"Jaggies" in graphics: false high frequencies caused by poor reconstruction:



Imperfect Reconstruction

Spectrum of Discrete Signal

Imperfect Reconstruction



Correcting Imperfect Reconstruction:

1. Sample well above the Nyquist rate
2. Low-pass filter after reconstruction

Typical Processing Pipeline

1. Low-pass filter to reduce aliasing
2. Sample
3. Do something with the digitized signal/image
4. Reconstruct
5. Low-pass filter to correct for imperfect reconstruction

Statistical Reconstruction

Statistical Reconstruction

Statistical image reconstruction is the process of attempting to recreate the original (2D or 3D) signal given possibly noisy or corrupted 2D images

Terms:

- Scene: the 2D or 3D “real world”
- Images: (possibly corrupted) 2D pictures of a scene

Image reconstruction attempts to recreate the scene from images.

Required Knowledge

Reconstruction algorithms usually use one or more of

- Knowledge about the image formation process
- Knowledge about properties of the original scene

Examples:

- Deconvolution requires knowledge of the point spread function
- Wiener filtering requires an estimate of the strength of the noise (see Russ textbook).

Knowledge About Image Formation

Knowledge about image formation puts limits on reconstruction

Usually though "fitting the data": the reconstructed image can't vary too much from the original corrupted image

Example:

- Assuming white noise with standard deviation σ, the probability of getting noisy image g from scene f is:

$$p(g \mid f) = \prod_i e^{-(f_i - g_i)^2 / \sigma^2}$$

Use this info to find the most probable model for f.

Knowledge About Scene Properties

Possible general properties:

- Generally smooth
- A few scattered rapid transitions

Possible specific properties:

- Known scene contents (subject, anatomy, etc)
- Other related images/scenes

$p(f)$ can be determined for all scenes f

Knowledge About Scene Properties

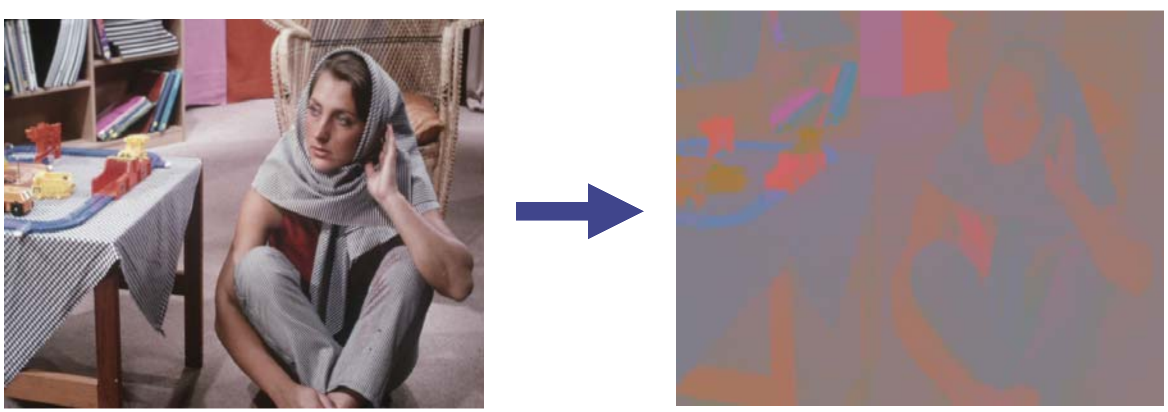
Example: Penalize unsmooth images

$$p(f) = \sum_{i,k \in N(i)} e^{-(f_i - f_k)}$$

where $N(i)$ denotes the "neighborhood" of i

Notice that one large discontinuity in intensity is more likely than several smaller discontinuities

Results in piecewise-constant images with infrequent but rapid discontinuities. Extreme case: use in color space conversion:



Statistical Reconstruction

Goal: for all possible reconstructed scenes f, find the one that maximizes $p(f \mid g)$ for image g

Problem: your knowledge of the imaging process tells you $p(g \mid f)$, but how do you determine $p(f \mid g)$?

Really big problem: How big is the space of all possible scenes f ?

Bayesian Reconstruction

$$p(f \mid g) = \frac{p(g \mid f)\,p(f)}{p(g)}$$

$p(g|f)$ is the data term

$p(f)$ is the a priori knowledge (prior)

$p(g)$ is usually assumed to be uniform

$p(f \mid g)$ is called the “a posteriori estimate” or “likelihood”

This is often called “maximum a posteriori” (MAP) or “maximum likelihood” estimation.

Bayesian Reconstruction

If $p(g \mid f)$ and $p(f)$ are negative exponentials, the process usually boils down to minimizing some function

$$\text{data}(f, g) + \lambda \, \text{prior}(f)$$

where $\text{data}(f, g)$ penalizes reconstructions f that don't agree with the data g, and $\text{prior}(f)$ penalizes reconstructions that are a priori unlikely (knowledge about scene properties).

The weight λ controls the relative importance of the two.

Balancing the Data and Prior Terms

$$\text{data}(f, g) + \lambda\ \text{prior}(f)$$

If λ is set too low, the data term dominates and there is little improvement

If λ is set too high, the prior term dominates and the reconstruction may not be true to the original

Optimization

Since the space of all f to search is far too large, non-exhaustive functional minimization techniques must be used:

- Gradient-descent
- Simulated annealing
- Neural networks
- Graduated non-convexity
- Etc.

Other Reconstruction Methods

There are many other reconstruction methods, but nearly all

- Use knowledge about the process that corrupted the image/signal
- Use knowledge about properties of the original scene/data
- Attempt to optimize some form of likelihood function

Questions?

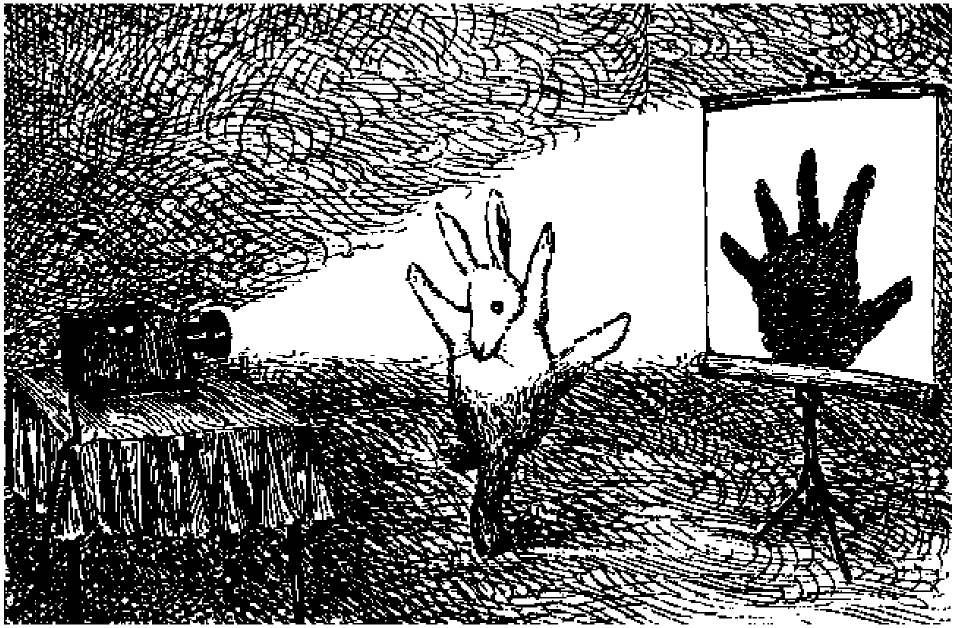
Reconstruction Concepts

Suggested Reading:

Kenneth R. Castleman, Digital Image Processing, Chapter 12, 16

3D Reconstruction

3D Reconstruction

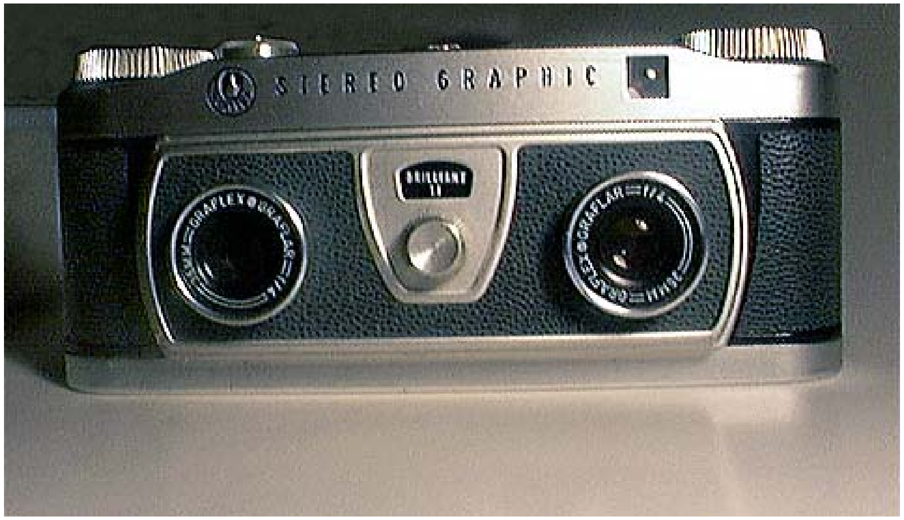


Frank J., Electron Tomography, Plenum, New York, 1992

•2D views are often not sufficient to recover the 3D object

3D Photography

- "3D stereoscopic imaging"
  - been around as long as cameras have
  - Use camera with 2 or more lenses (or stereo attachment)
  - Use stereo viewer to create impression of 3D



Motivation

- Digitizing real world objects
- Sometimes called "3D scanning"
- Getting realistic 3D models

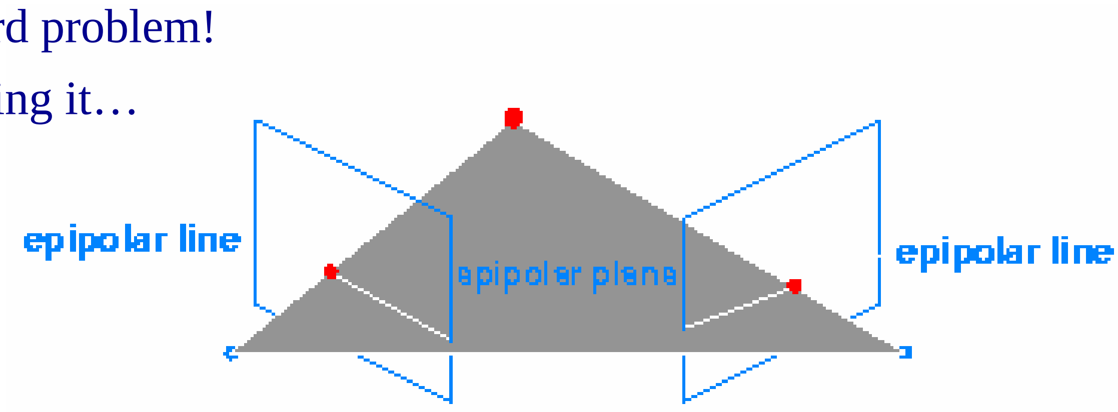
places

objects

humans

Stereo Matching

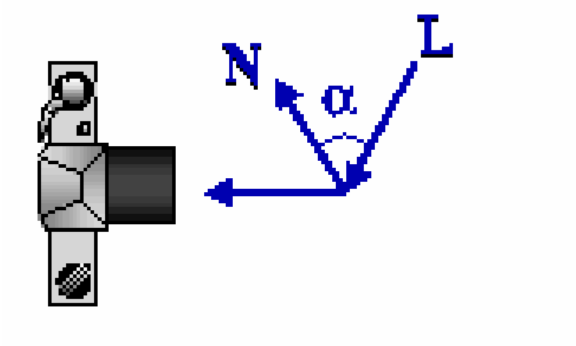
- Stereo Matching Basics
  - Needs two images, like stereoscopy
  - Given correspondence between points in 2 views, we can find depth by triangulation
  - But correspondence is hard problem!
  - A lot of literature on solving it…



- Stereo Matching Output
  - 3D point cloud
  - Remove outliers and pass through surface reconstructor

Using Shades

- Shape from Shading, [Horn]
  - Invert Lambert's Law (L=I k cos α) knowing the intensity at image point to solve for normal
- Photometric stereo [Woodham]
  - An extension of the above
  - Two or more images under different illumination conditions.
  - Each image provides one normal
  - Three images provide unique solution for a pixel.



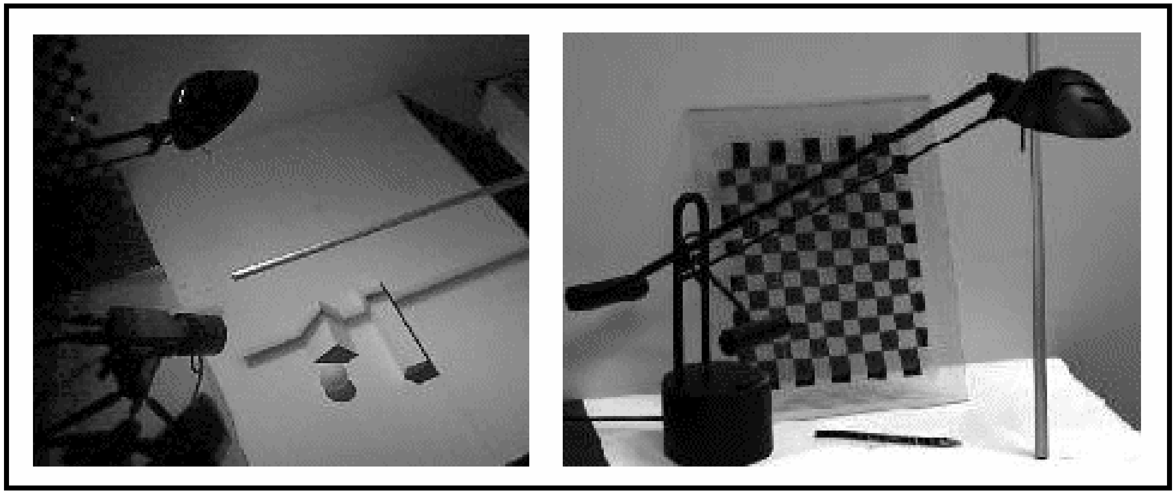
Active Sensing

- Passive methods (eg. stereo matching) suffer from ambiguities - many similar regions in an image correspond to a point in the other.
- Project known / regular pattern (“structured light”) into scene to disambiguate
- get precise reconstruction by combining views
  - Laser rangefinder
  - Projectors and imperceptible structured light

Desktop 3D Photography

Jean-Yves Bouguet, Pietro Perona

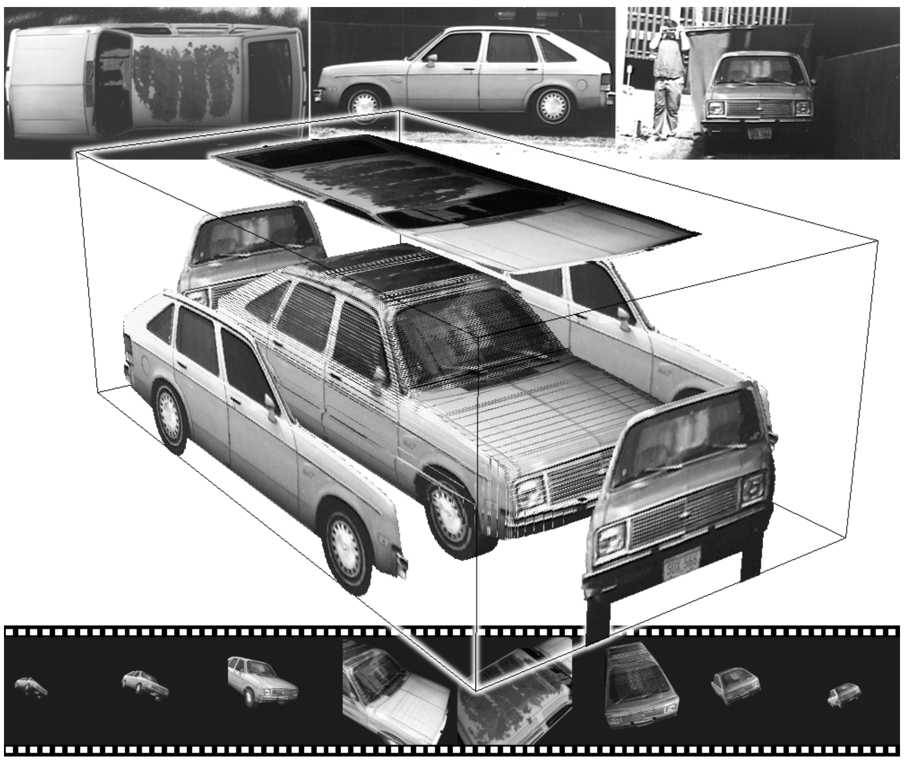
- Light object with lamp & aim camera at it
- Move stick around & capture shadow sequence
- Use image of deformed shadow to calc 3D shape



- Computation of 3d position from the plane of light source, stick and shadow

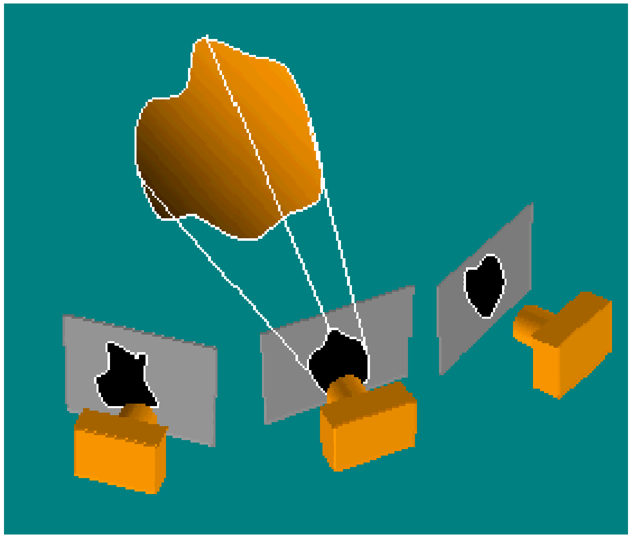
Volumetric Methods

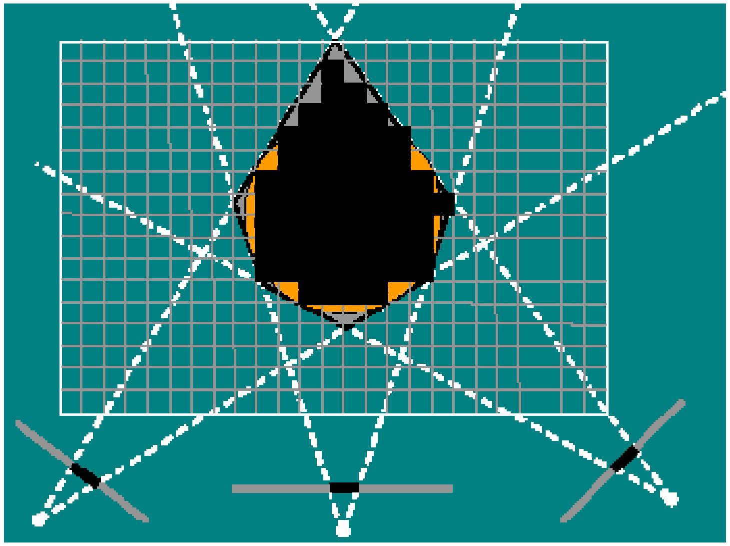
Chevette Project, Debevec, 1991



Voxel Models from Images

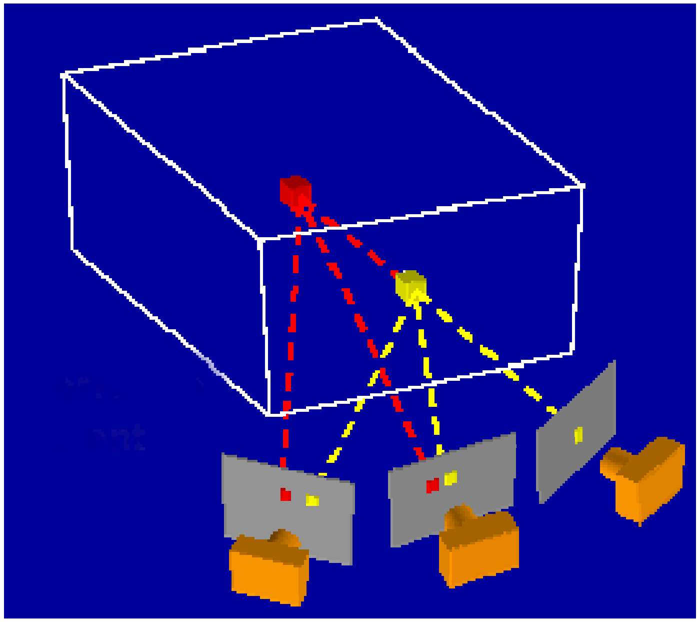
- When there are 2 colors in the image - use volume intersection [Szeliski 1993]
  - Back-project silhouettes from camera views & intersect





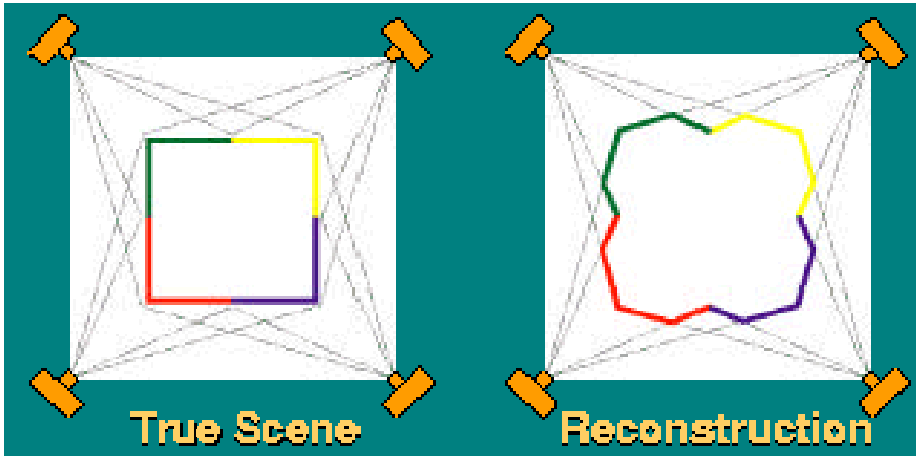
Voxel Models from Images

- With more colors but constrained viewpoints, we use voxel coloring [Seitz & Dyer, 1997]
  - Choose a voxel & project to it from all views
  - Color if enough matches
  - Prob - determining visibility of a point from a view
  - Solution - depth ordered traversal using a “view independent distance from separating plane”



Space Carving

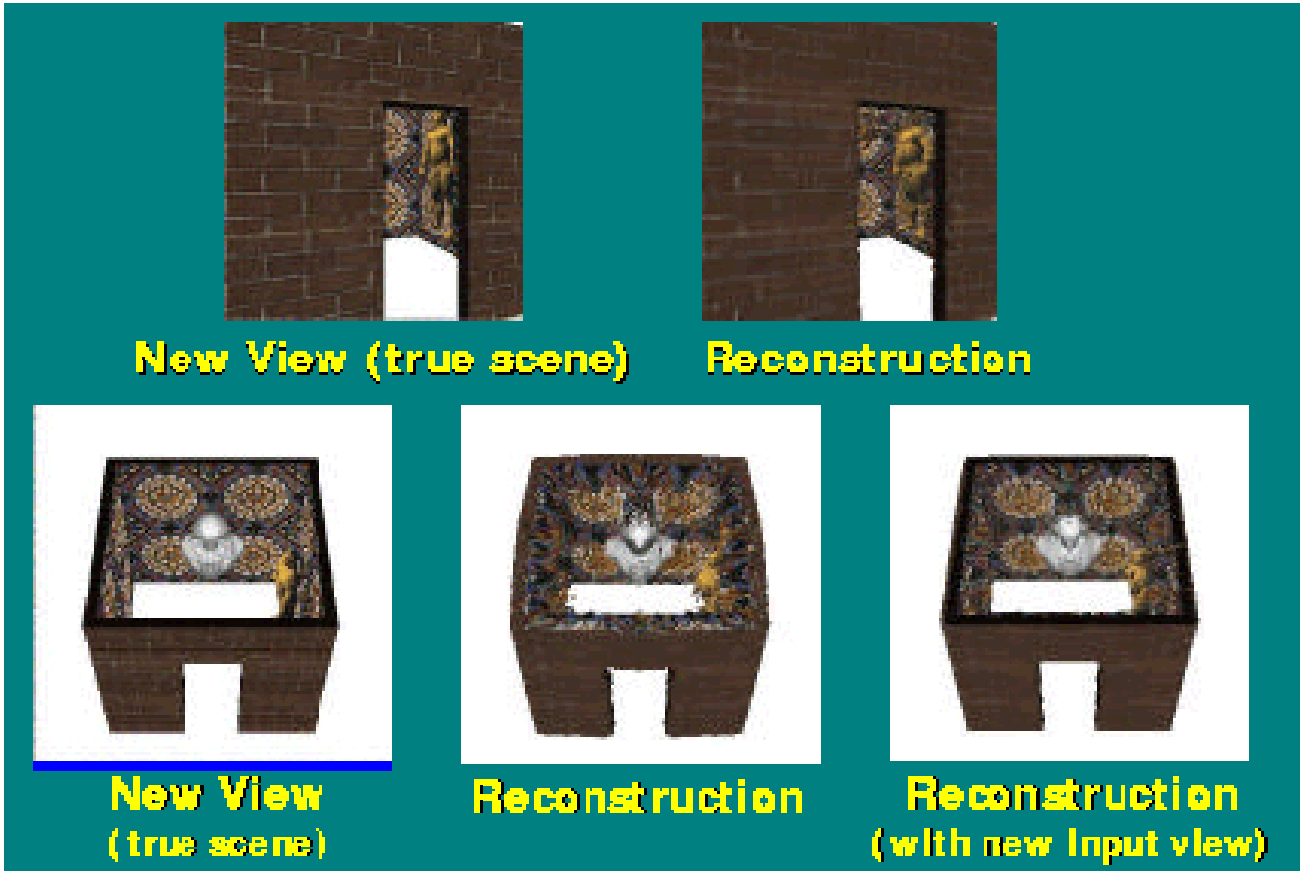
- Algorithm [Kutulakos & Seitz, 1998] :
  a) Initialize V to volume containing true scene
  b) For each voxel,
    - check if photo-consistent
    - if not, remove ("carve") it.



- Can be shown to converge to maximal photo-consistent scene (union of all photo-consistent scenes).

Space Carving : Results

- House walkthru - 24 rendered input views
- Results best as seen from one of the original views



Modeling from a Single View
(Criminisi et al, 1999)

- Compute 3D affine measurements of the scene from single perspective image
- Use minimal geom info
  - vanishing line for a pencil o planes || to reference plane
  - vanishing point of parallel lines along a direction outside reference plane

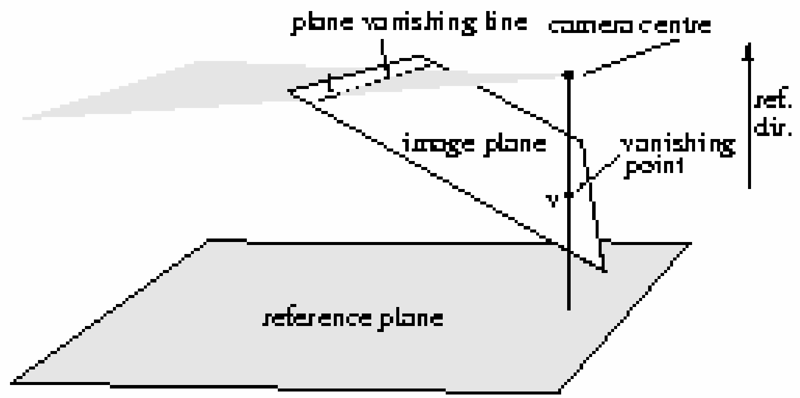


Figure 1: Basic geometry: The plane's vanishing line **l** is the intersection of the image plane with a plane parallel to the reference plane and passing through the camera centre. The vanishing point **v** is the intersection of the image plane with a line parallel to the reference direction through the camera centre.

Case Study - Façade
Debevec, Taylor & Malik, 1996

- Modeling architectural scenes from photographs
- Not fully automatic (user inputs blocky 3D model)
  - Using blocks leads to fewer params in architectural models
- User marks corresponding features on photo
- Computer solves for block size, scale, camera rotation by minimizing error of corresponding features
- Reprojects textures from the photographs onto the reconstructed model

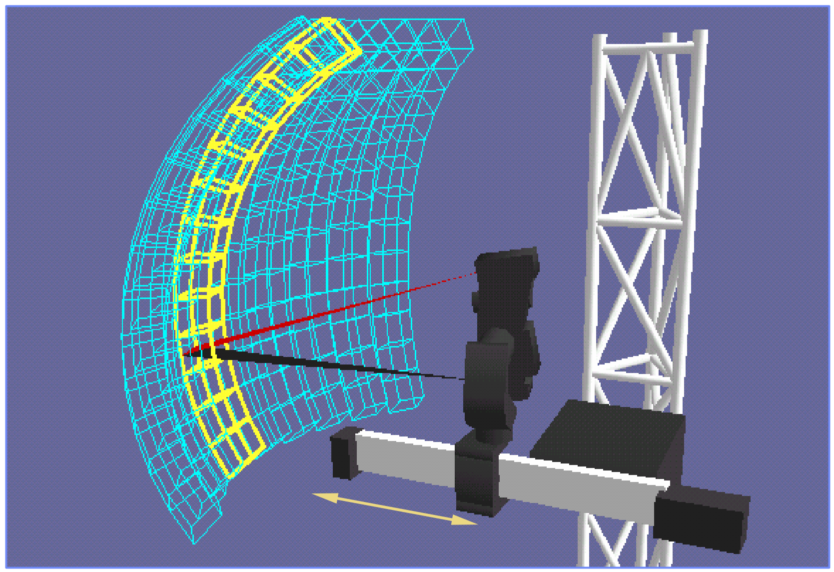
Arches and Surfaces of Revolution

Taj Mahal
modeled from
one photograph

Case Study - Digital Michelangelo Project

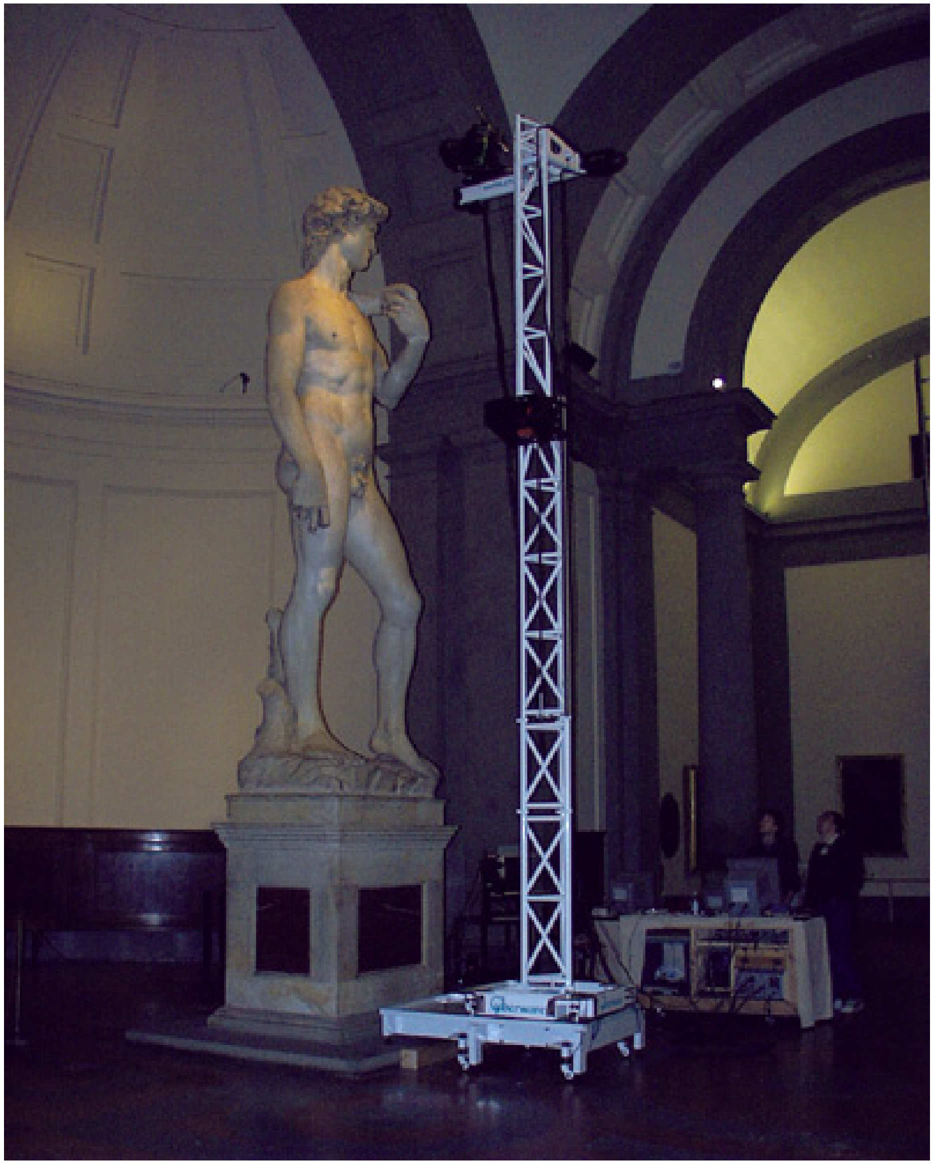
- 3D scanning of large statues (Levoy et al, 2000)
- Separate geometry and color scans
  - custom rig : laser scanner & camera mounted concurrently
- Range scan post-processing
  - Combine range scans from different positions
    - Use volumetric modeling methods (Curless, Levoy 1996)
  - Fill holes using space carving

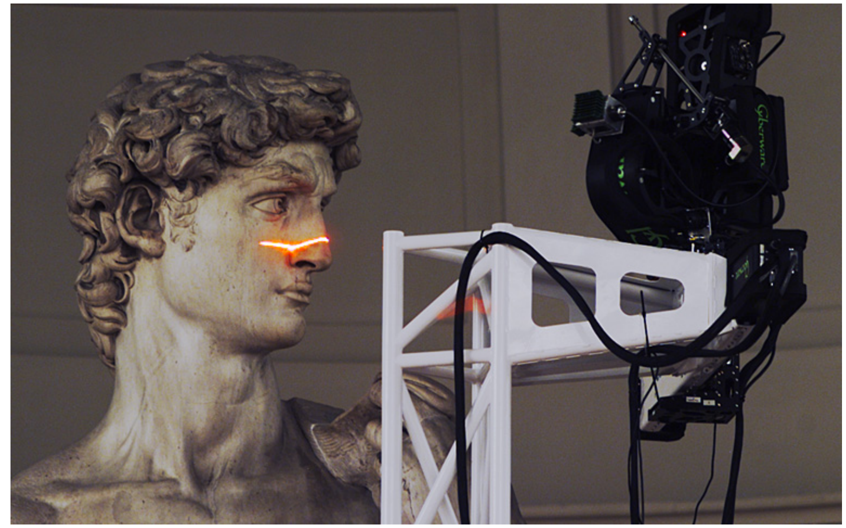
Digital Michelangelo - Scanning a Large Object

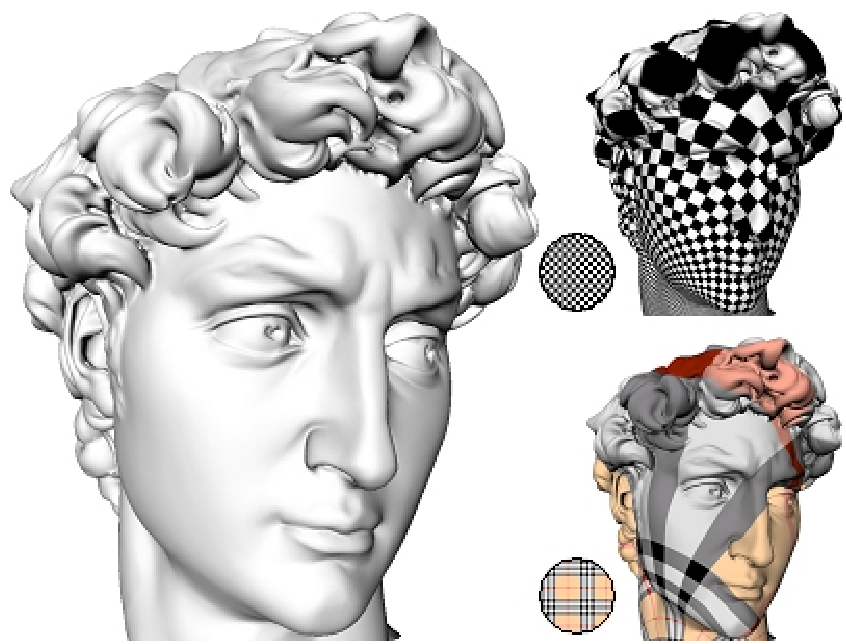


- calibrated motions
  - pitch (yellow)
  - pan (blue)
  - horizontal translation (orange)
- uncalibrated motions
  - vertical translation
  - remounting the scan head
  - moving the entire gantry

Digital Michelangelo







References – 3D Photography

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- Criminisi, A., I. Reid and A. Zisserman. Single View Metrology. In Proc. ICCV, pp 434-442, September 1999
- Curless, B. and M. Levoy. A Volumetric Method for Building Complex Models from Range Images. In Proc. SIGGRAPH 1996
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- Kutulakos, K. N. and S. Seitz. A Theory of Shape by Space Carving. URCS TR#692, May 1998, appeared in Proc. ICCV 1999.
- Levoy, M., Pulli, K., Curless, B. et al. The Digital Michelangelo Project - 3D Scanning of Large Statues. In Proc. SIGGRAPH 2000.
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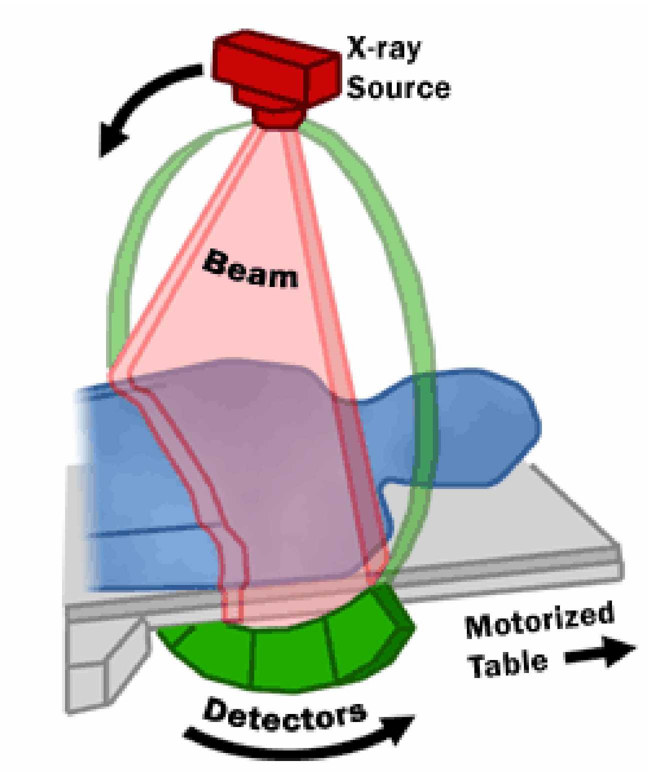
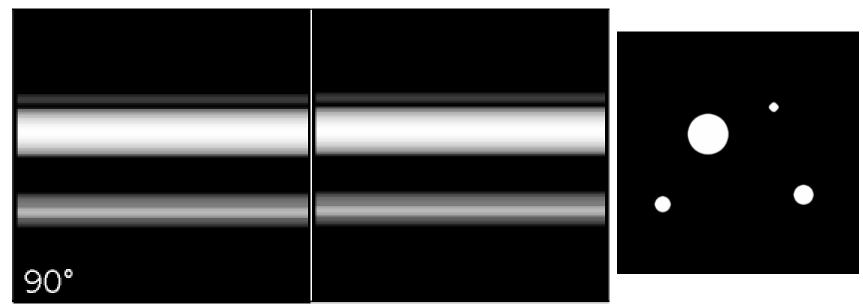
3D Reconstruction in Tomography

3D Reconstruction in Tomography

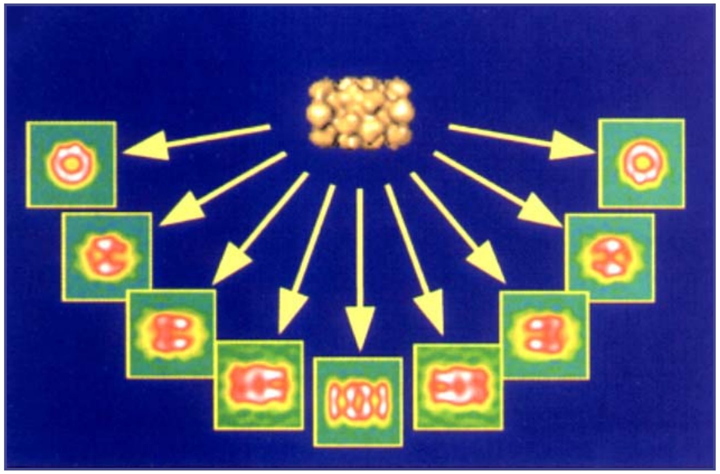


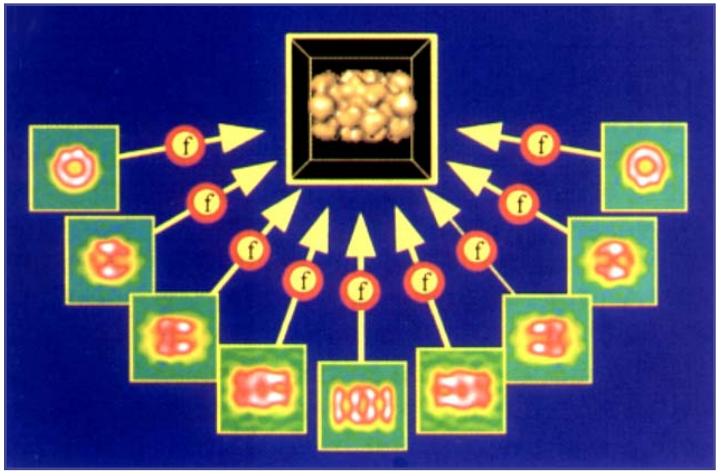
Computer reconstructs the 3D volume



- X-Ray source rotates around the opening
- Detector records different projections of the body
  (PC adds 1D projections to form the 2D slices, which form the 3D volume)

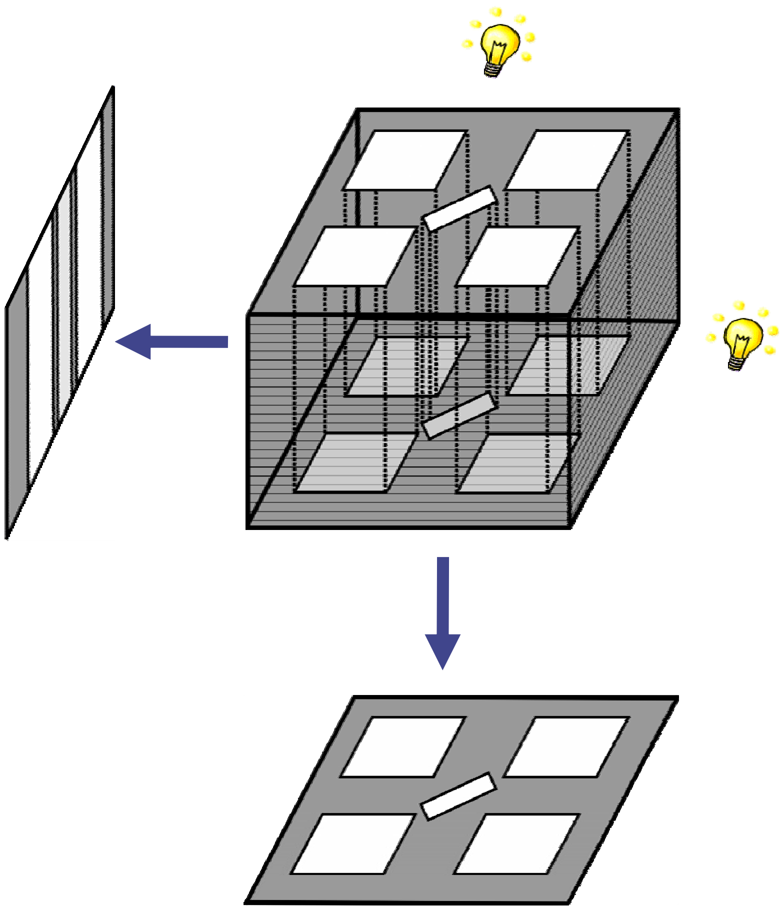
Back Projection



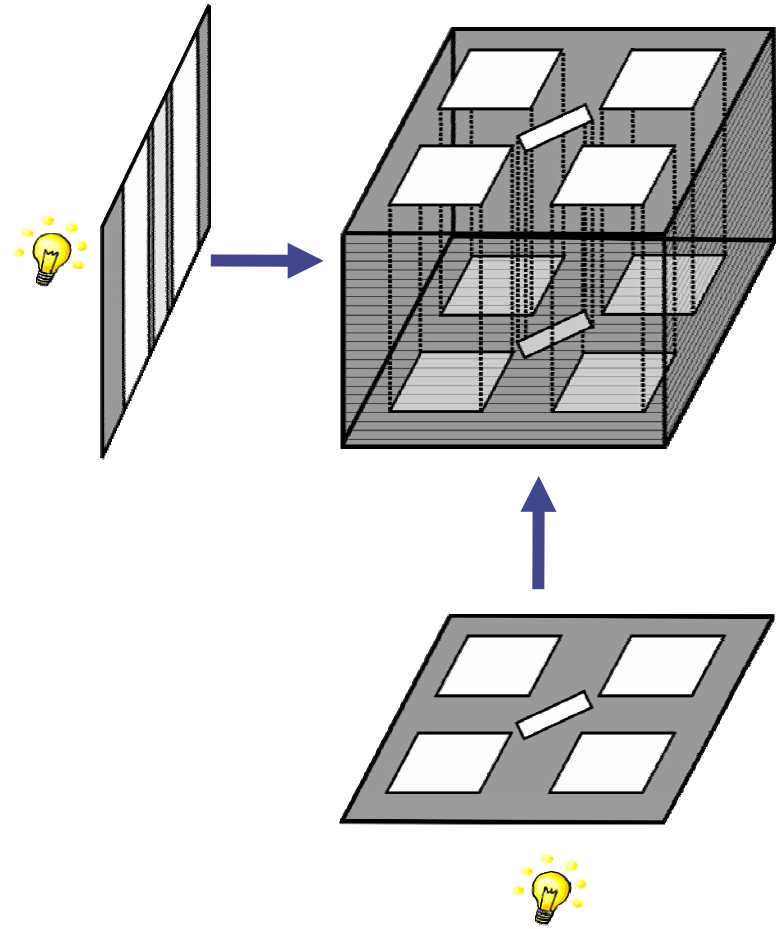


1. Recording of projections (2-D) at the various tilt angles
2. Backprojection of the 3-D object from the 2-D images

Back Projection

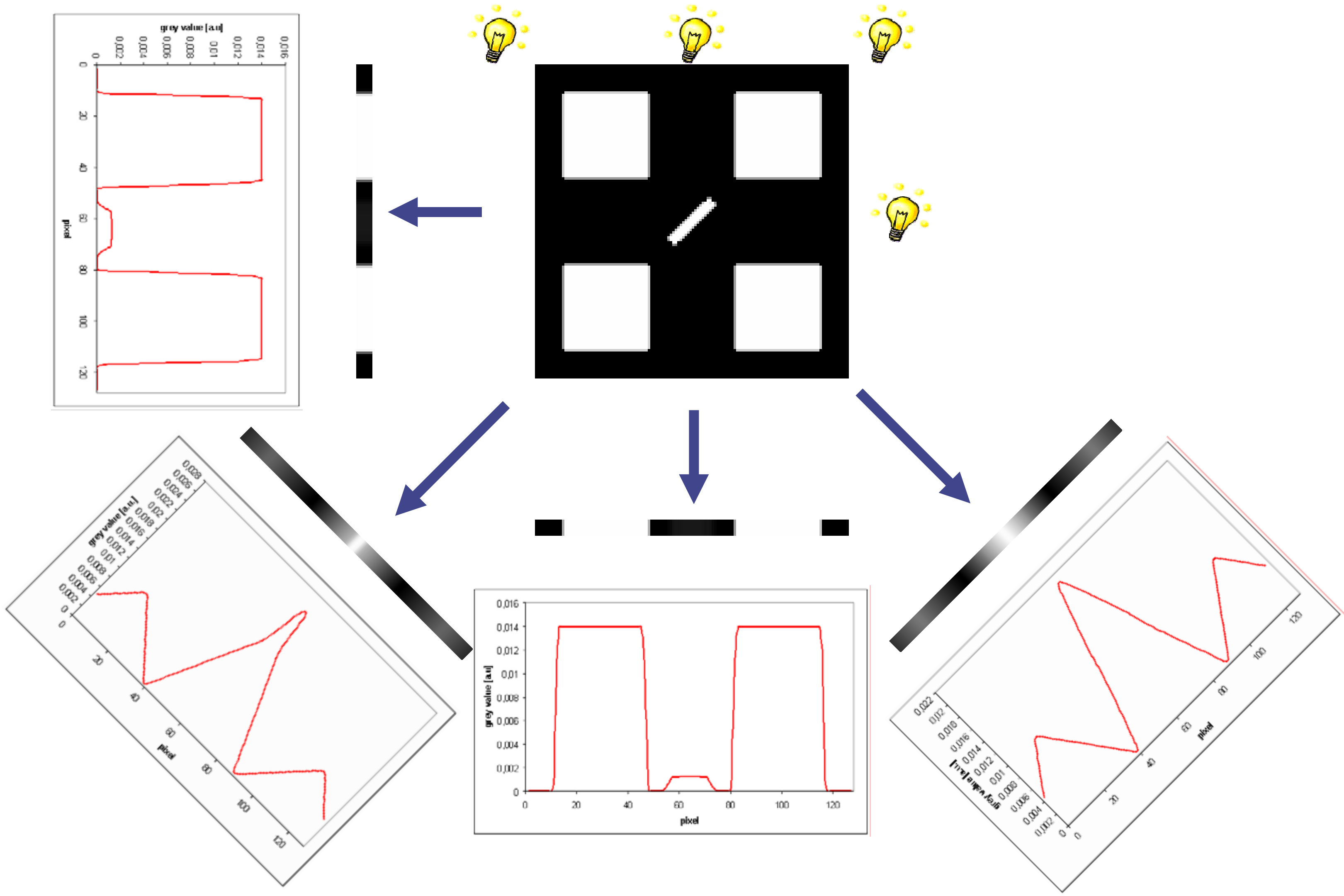


1. Projection

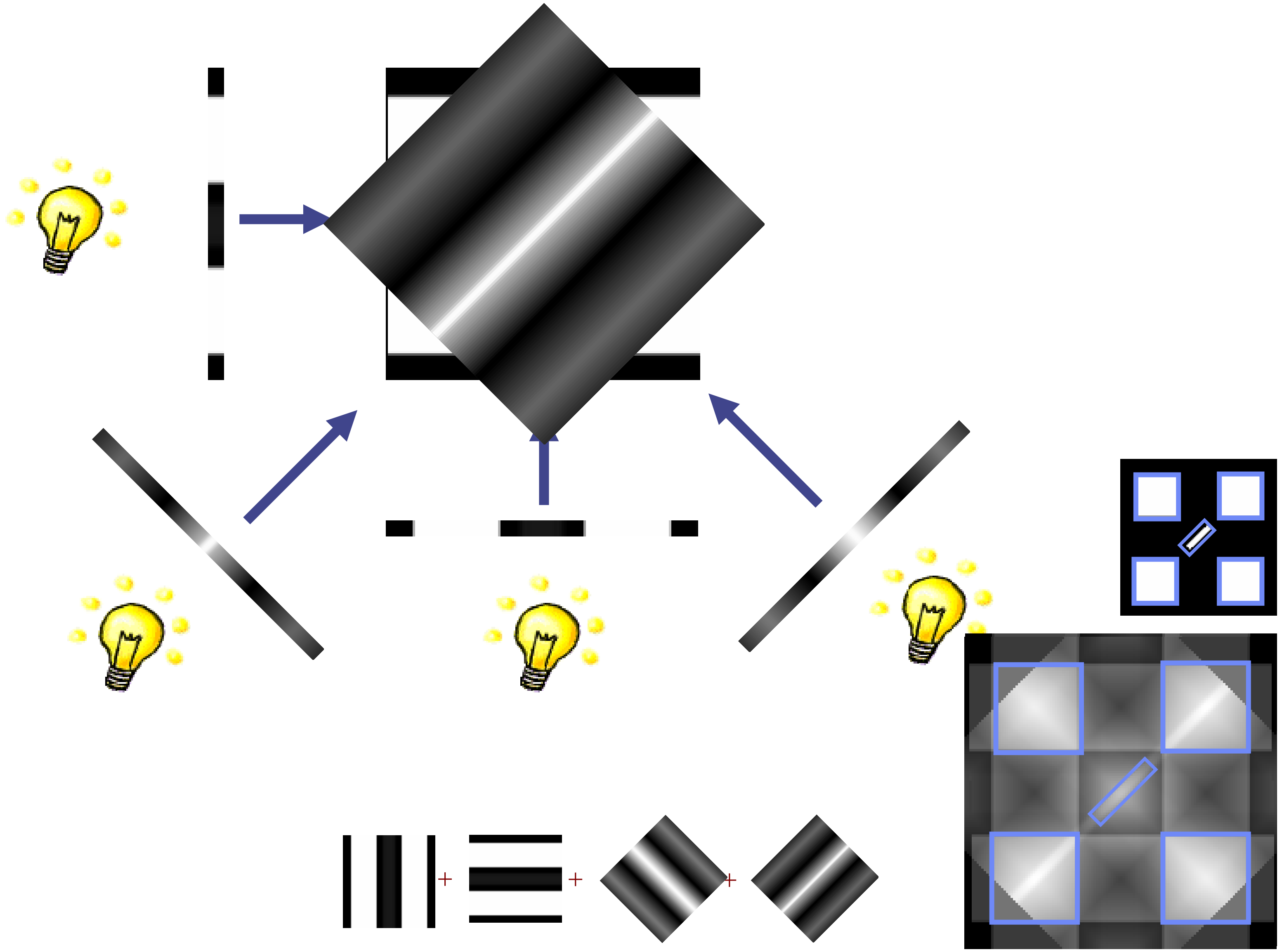


2. Backprojection

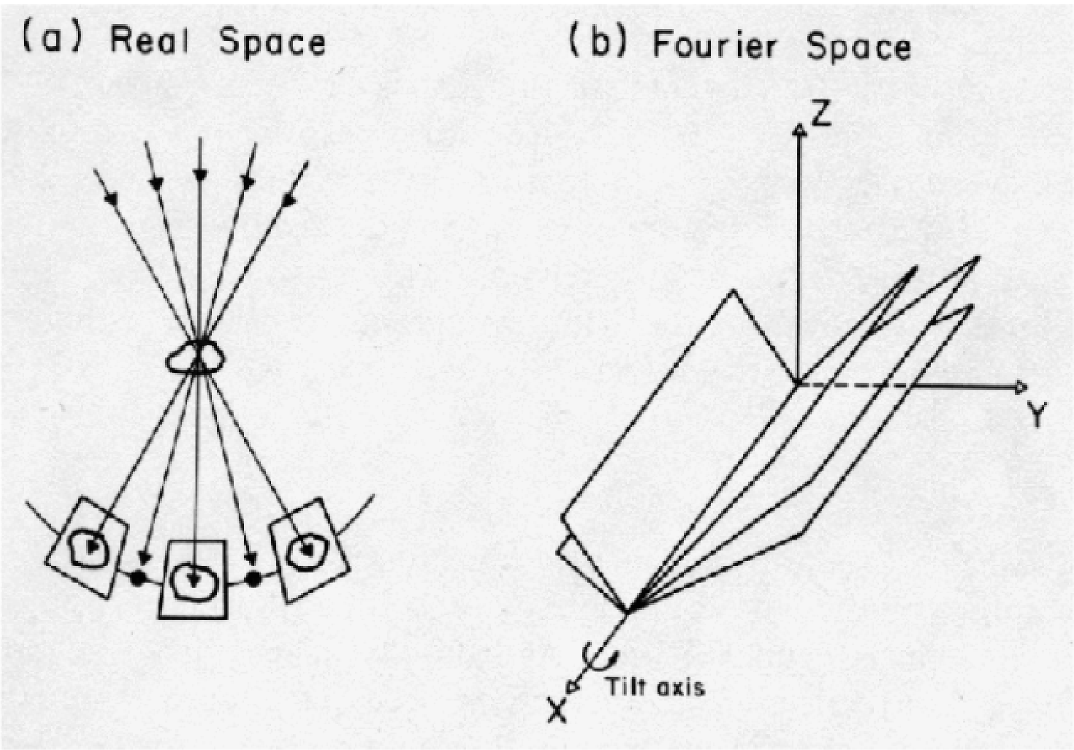
Example: 1D ➔ 2D



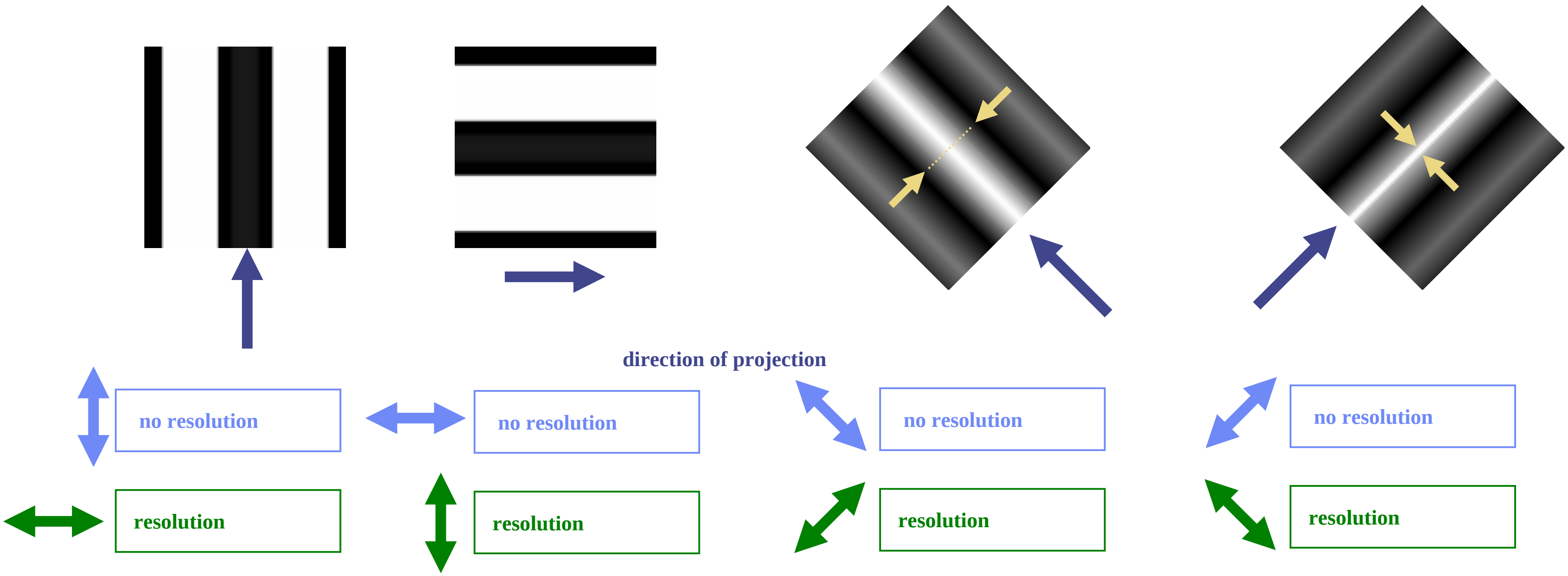
Example: 1D ➔ 2D



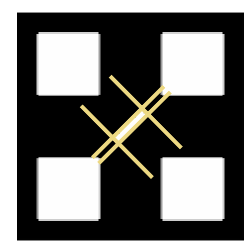
Under and Over Sampling in Fourier Space



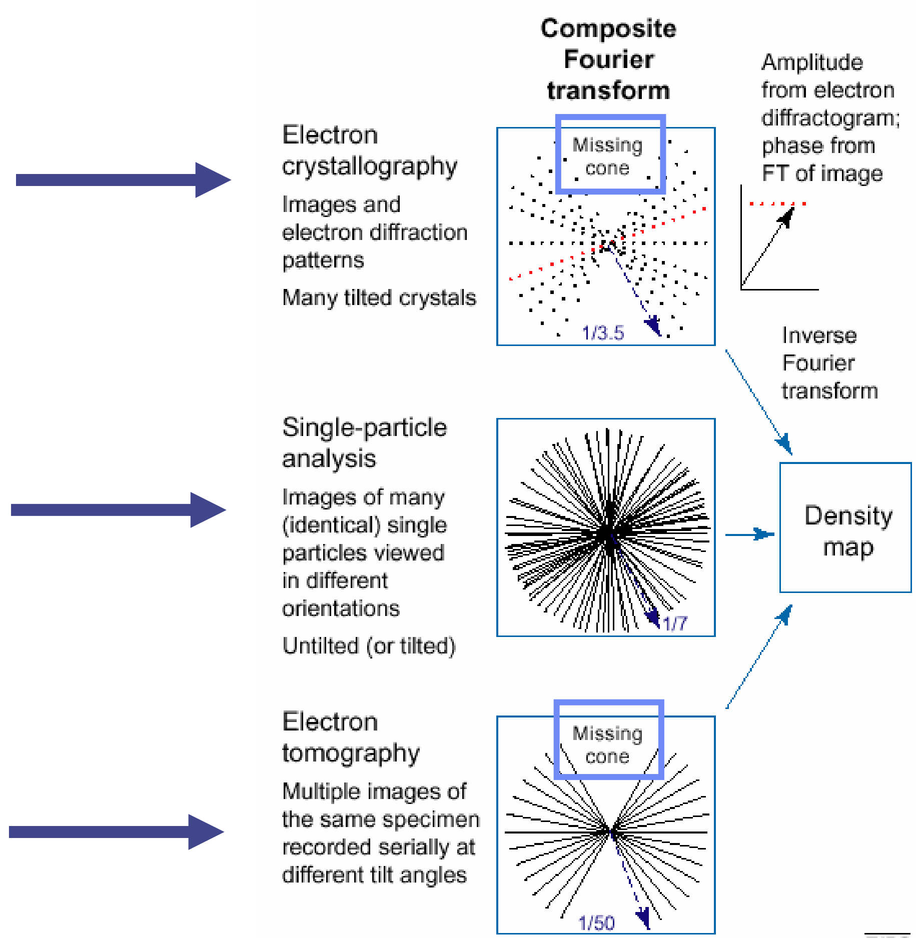
Anisotropic Resolution



→along direction of projection: no resolution



Electron Tomography ➔ 3D Structure



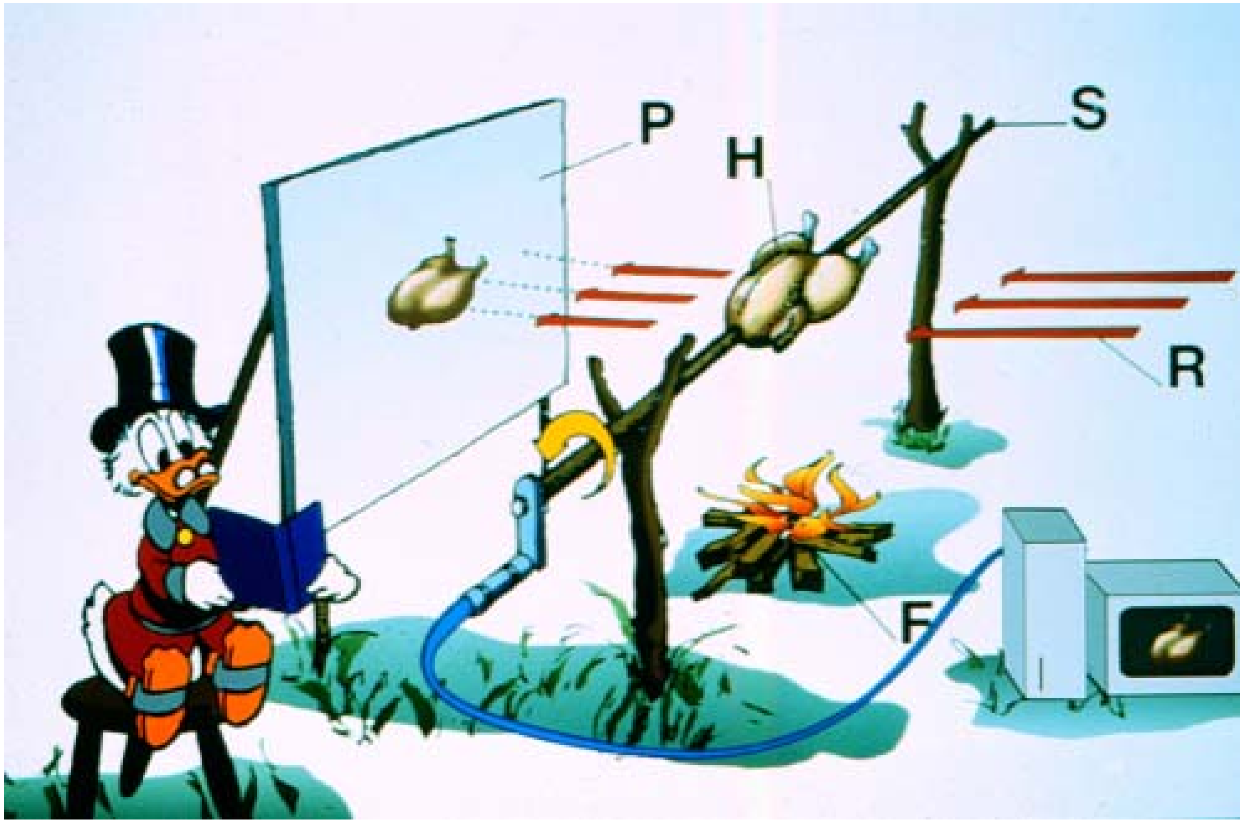
differences in

particle size,
particle preparation,
resolution,....

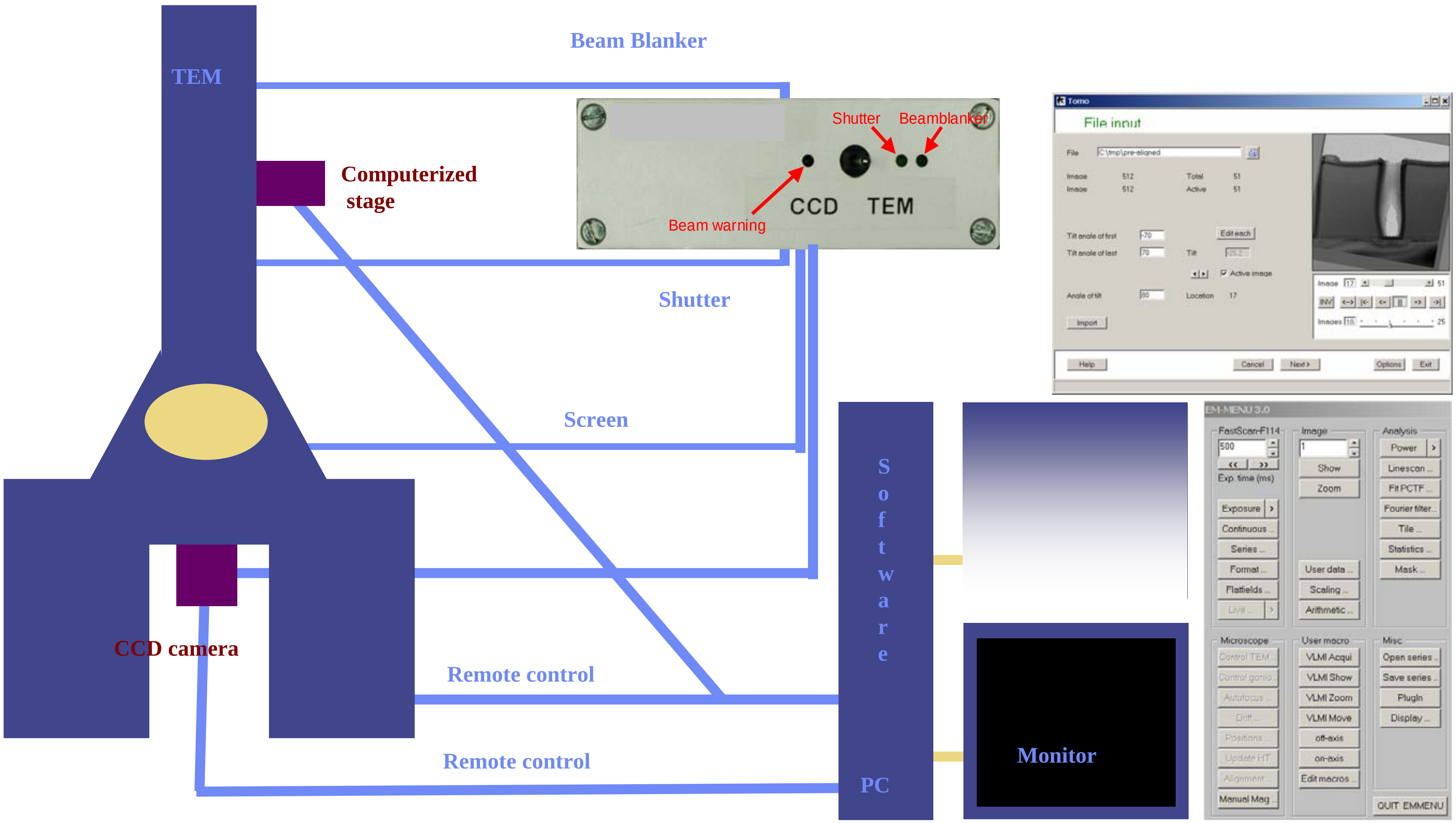
Baumeister & Steven, TIBS **25**, 2000

Electron Tomography ...

... manual ... automated

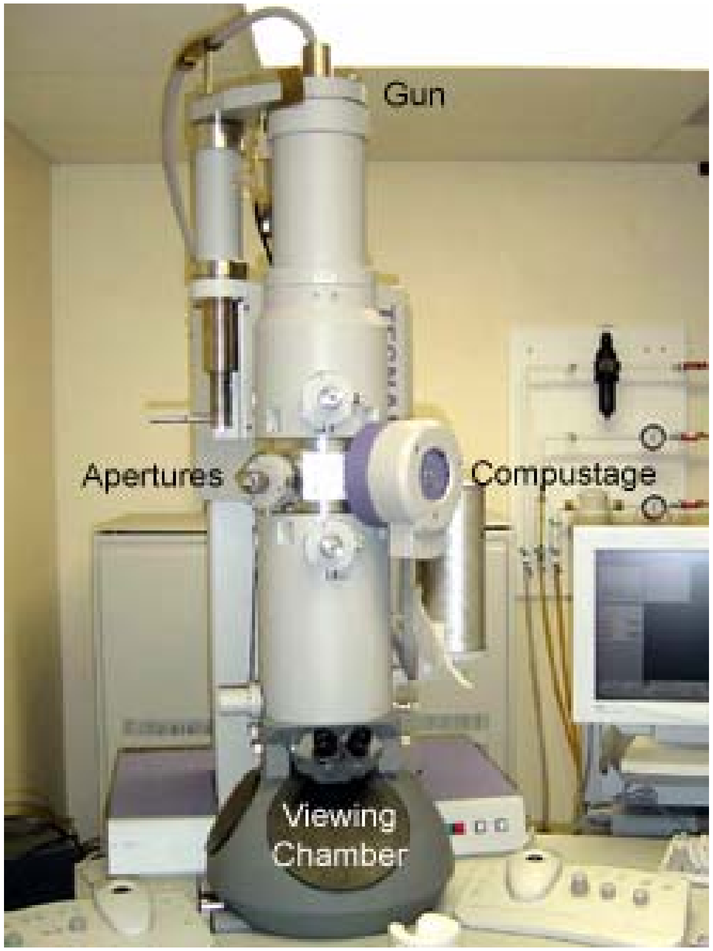


Electron Tomography Setup



The Electron Microscope

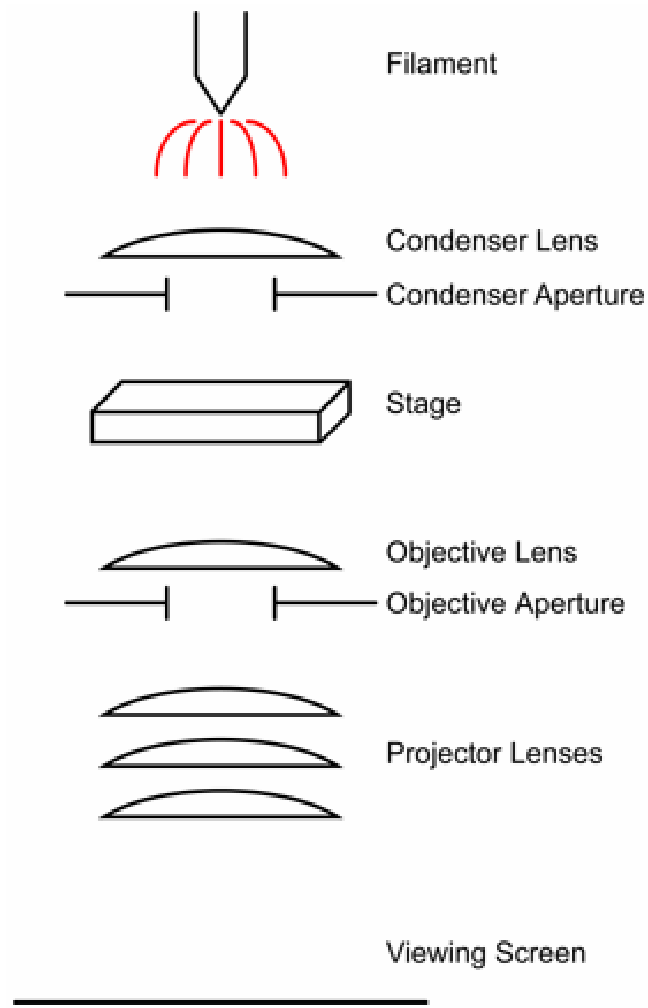
- Transmission Electron Microscope (TEM)
  - Detects internal structure of sample
  - Thin samples, so beam is not entirely absorbed
  - Cryo-EM: mostly phase object
  - Projection of 3D structure onto 2D screen (actually, projection of electrostatic potential)



http://cryoem.berkeley.edu/~nieder/em_for_dummies
http://www.udel.edu/chem/bahnson/chem645/presentations/Bianco.pdf

The Electron Microscope

- Electron gun: superheated metal filament emit electrons, collated into beam by thermionic and field emission gun
- Lenses: magnetic coils tuned to focus electron beam, to magnify image and to sharpen contrast
- Stage: holds sample, may be tilted by goniometer
- Aperture: limits size of electron beam.
  - Condenser aperture: maintains size of electron beam.
  - Objective aperture: controls contrast



http://cryoem.berkeley.edu/~nieder/em_for_dummies

http://www.udel.edu/chem/bahnson/chem645/presentations/Bianco.pdf

3 Phases of Electron Tomography

Data Collection	3-D Reconstruction	Display & Analysis
• Set of projections of the tilted structure • Correction of imperfection of the stage	• Alignment of the various projections • Tomographic reconstruction	• Surface and volume rendering • Merging and comparison with other structures
• Time: 20 minutes to hours	• Time: 15 minutes to several hours	• Time: 15 minutes to several weeks or months

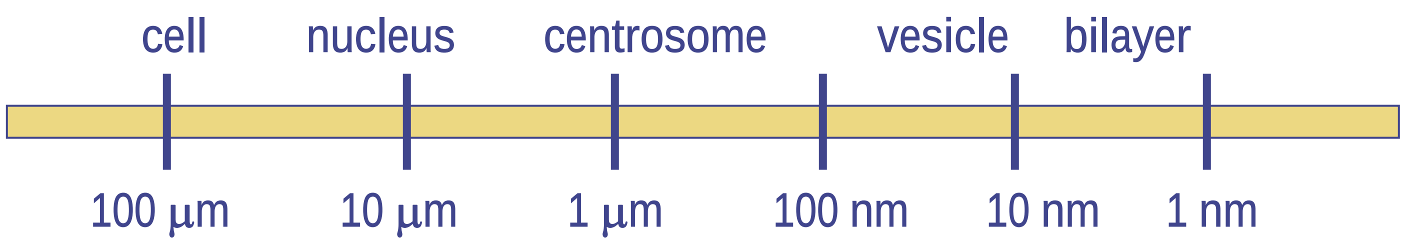
Attainable Resolution

d: resolution for ET reconstruction

D: object diameter

N: number of tomographic images

$d = \pi (D/N)$ Crowther et al., 1970



Projections equally distributed over full angular range (±90º)

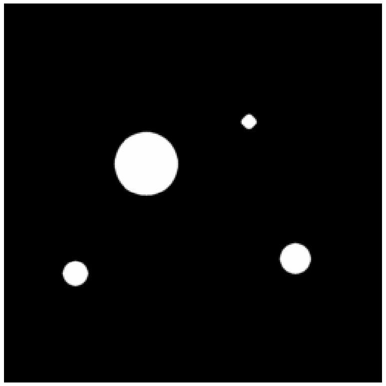
→ *6 nm resolution using 150 images of a 300 nm structure*

specimen shrinkage

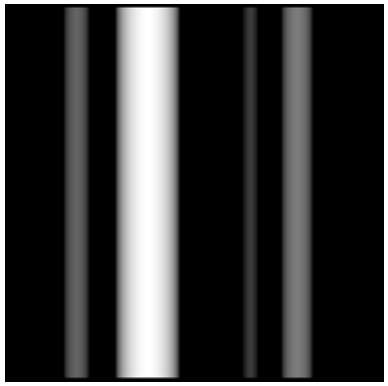
anisotropic resolution: tilt range (<)< 90°

Back-Projection Artifacts

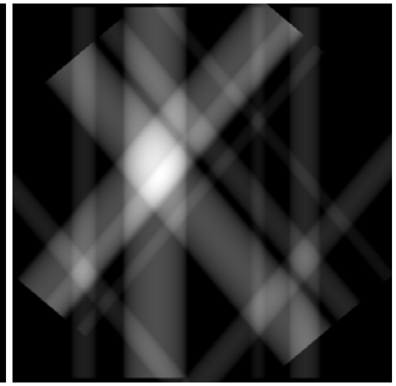
in direction of projection: no resolution → anisotropic resolution



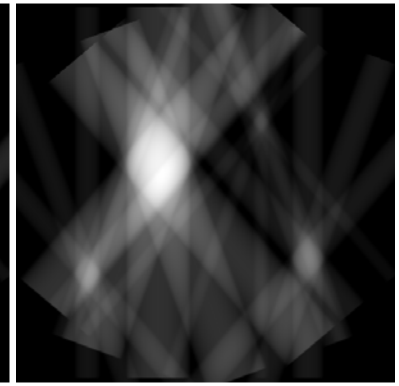
object



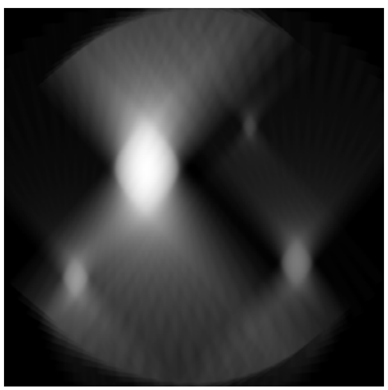
0°



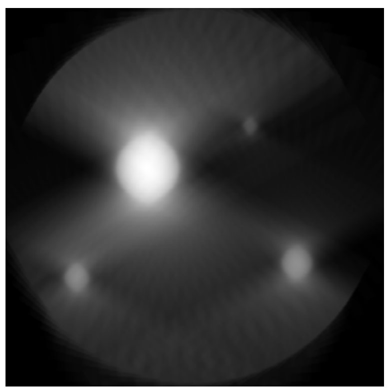
incr. 40° @ +/-40°



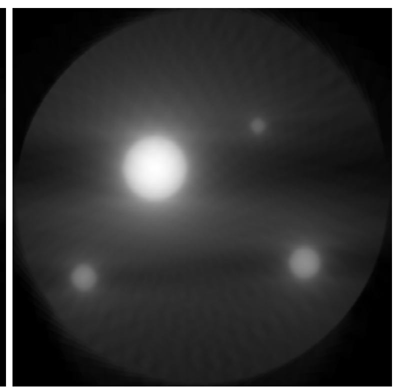
incr. 20° @ +/-40°



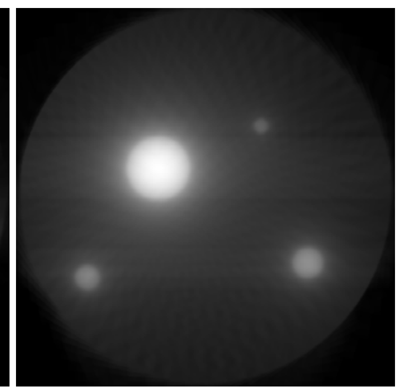
incr. 5° @ +/-40°



incr. 5° @ +/-60°



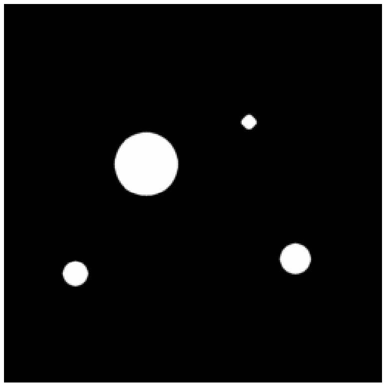
incr. 5° @ +/-80°



incr. 5° @ +/-90°

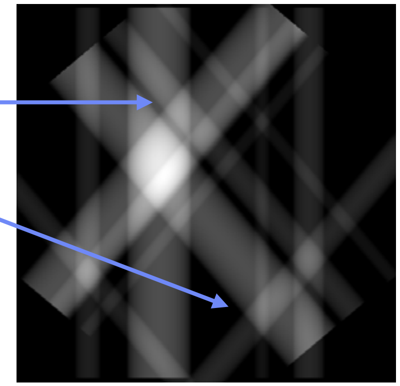
Back-Projection Artifacts

in direction of projection: no resolution → anisotropic resolution

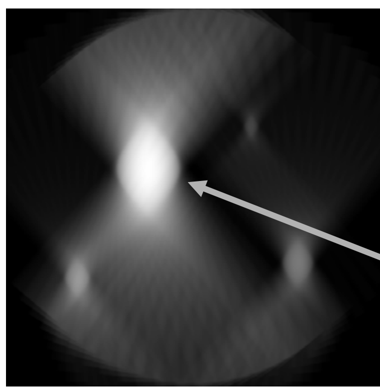


object

creation of
object artifacts



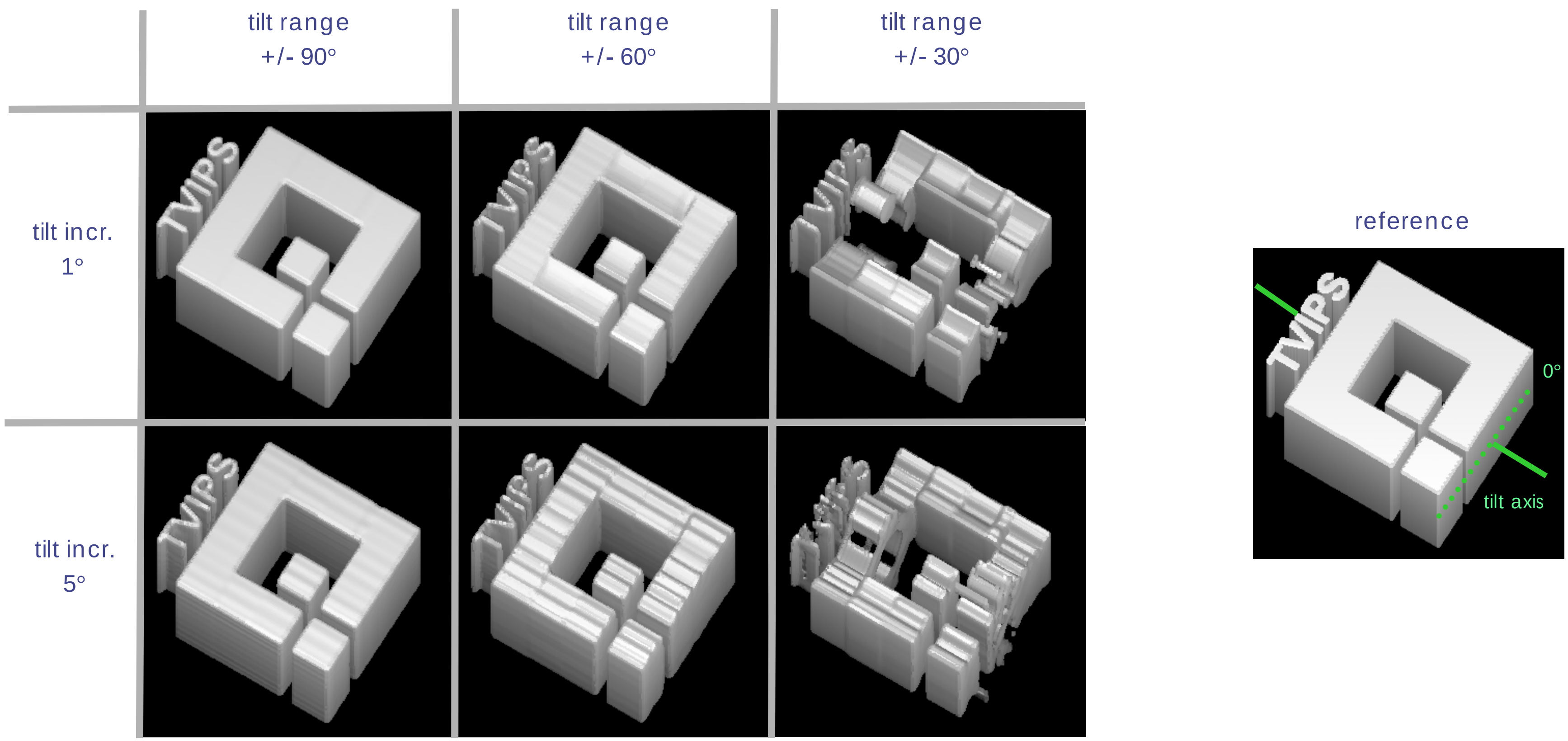
incr. 40° @ +/-40°



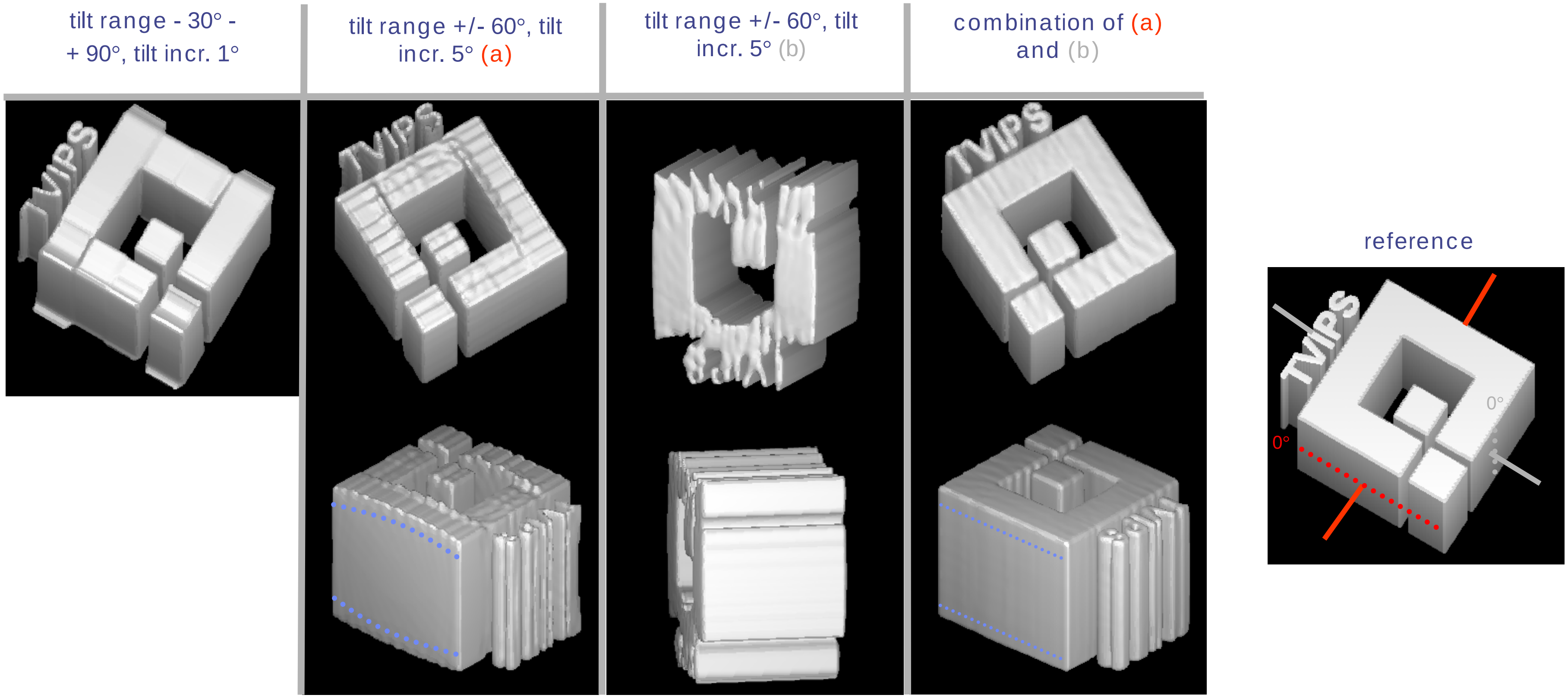
incr. 5° @ +/-40°

along direction of projection:
object elongation

Missing Wedge Effect

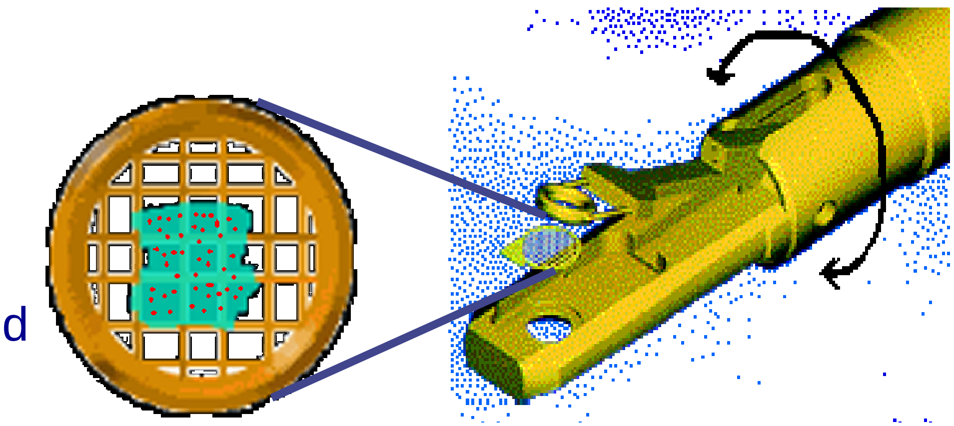


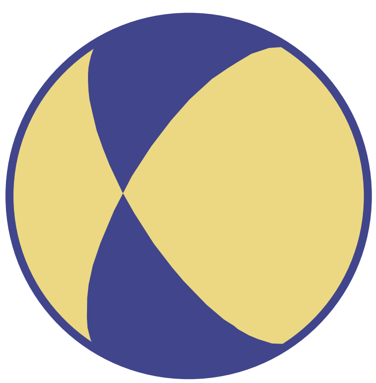
Missing Wedge Effect



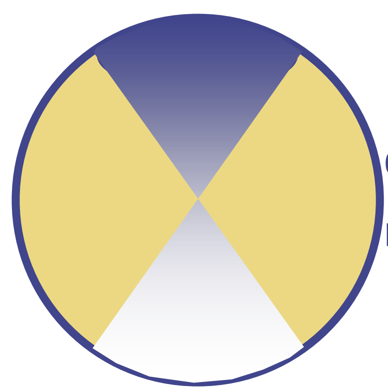
Missing Wedge Effect

- Optimal : ± 90° tilt
- At high tilt:
  - limitations of holder
  - grid and sample projected
  - increased path length





single tilt:
missing wedge



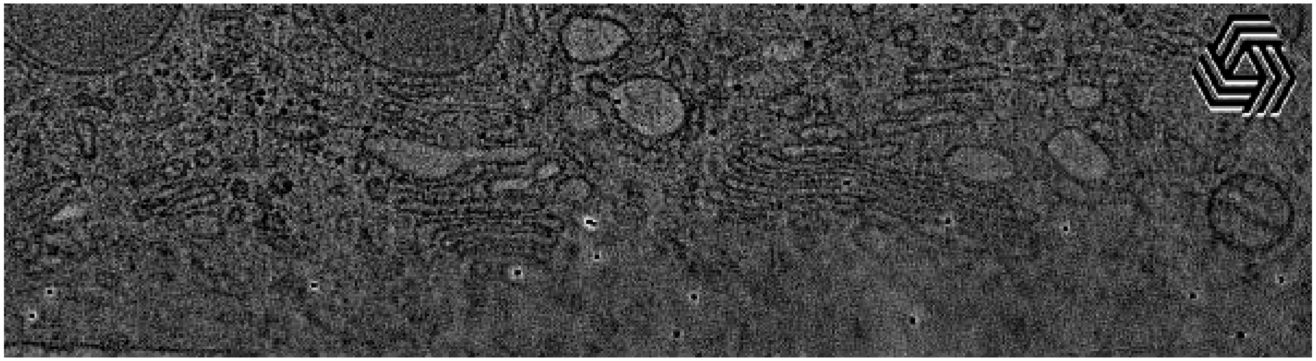
double tilt: missing pyramid

multiple tilt ➔ missing cone

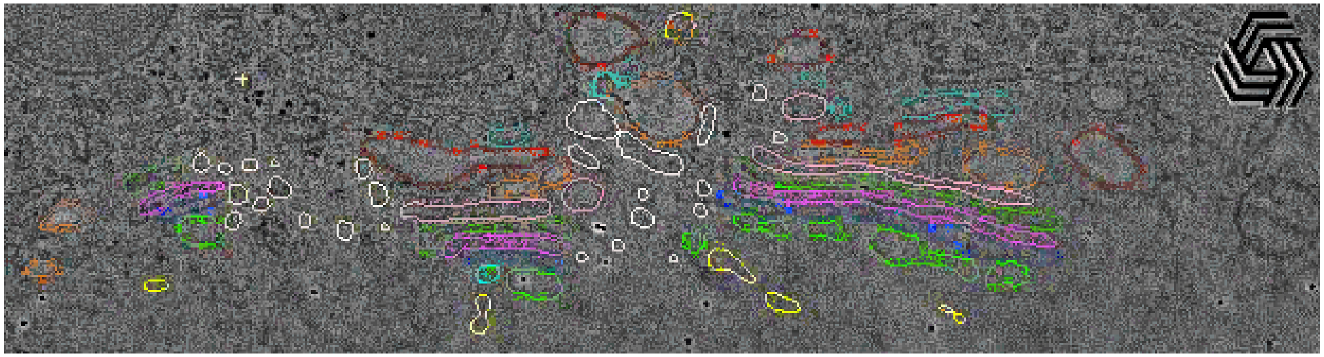
Tilt Series of a Mammalian Cell

University of Colorado
Marsh, B.J., see also http://bio3d.colorado.edu

tomogram



interpretation of the tomogram with segmentation and denoising

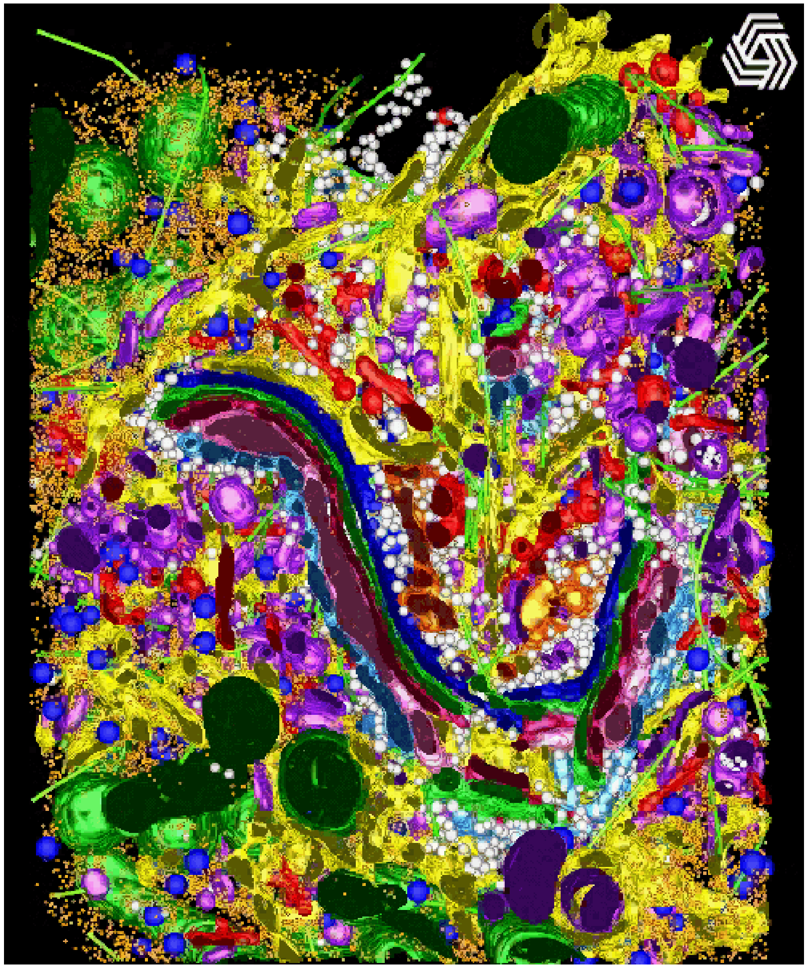


Tomography permits the viewing of computer-generated "slices" that are much thinner than could ever be cut physically with a microtome.

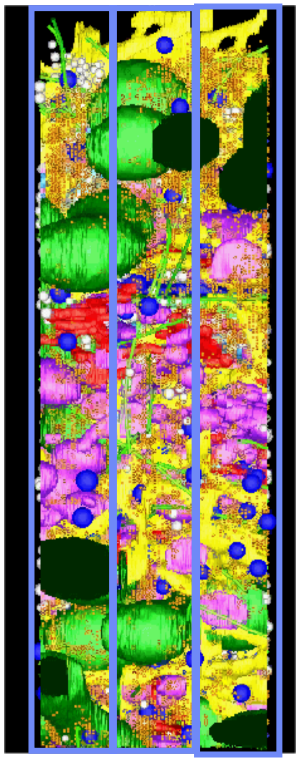
Tilt Series of a Mammalian Cell

University of Colorado

Marsh, B.J., see also http://bio3d.colorado.edu



surface representation:
4μm x 4μm x 1μm



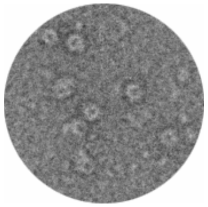
IMOD: Kremer et al., *J. Struc. Biol.*, **116**, 71-76, 1996

3D Reconstruction in High Resolution Electron Microscopy

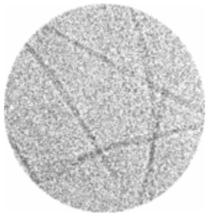
Tomography (tilt series): 6-10nm
EM (particle averaging): 0.5-3nm

Goal: Single View of Many Particles

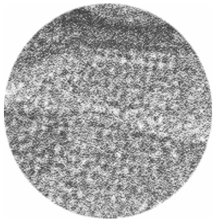
Types of Specimens



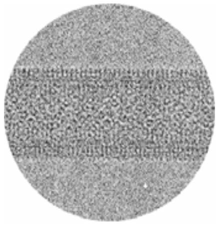
- Single Particles (Proteins, Ribosome)
  - No crystallization
  - Weak amplitude, no diffraction, alignment ambiguity, particle flexibility
  - ~7 angstroms



- Fibers and filaments (tubulin, collagen)
  - No crystallization, 2D distortion corrections, phase restrictions
  - Weak amplitude, no diffraction
  - ~9 angstroms



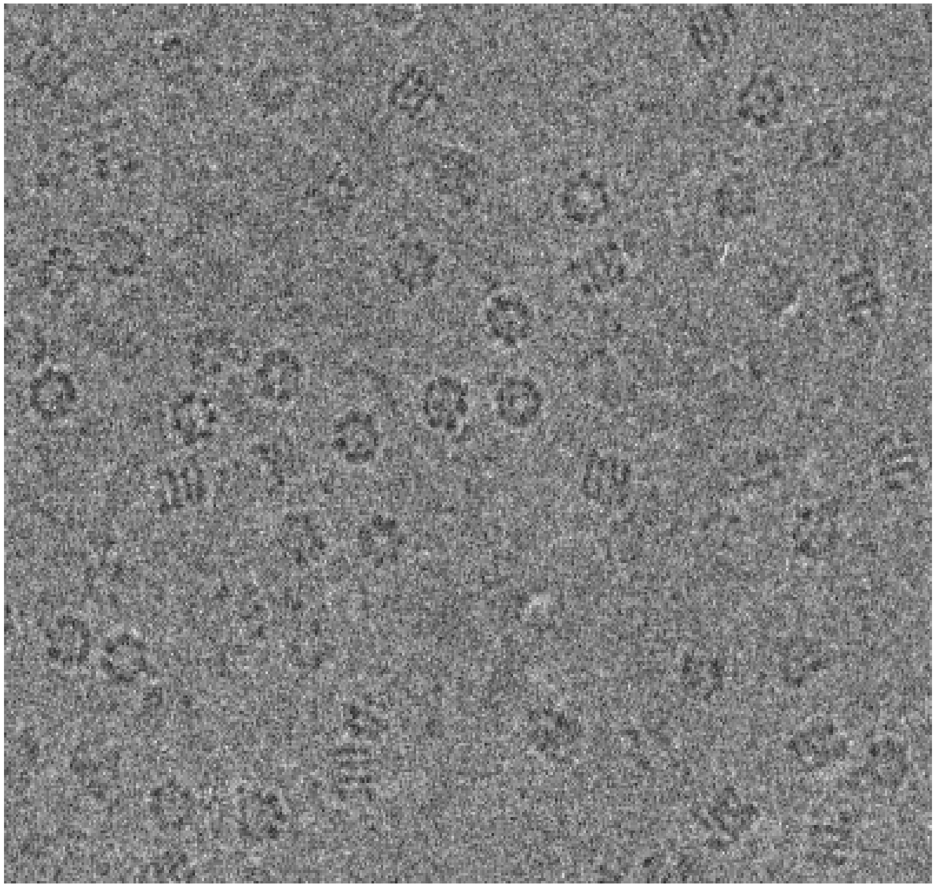
- 2D crystals (BR, AQP, LHCII)
  - Diffraction amplitudes, 2D distortion corrections, crystallographic methods
  - Crystallization, many tilts required, anisotropic data
  - ~3 angstroms



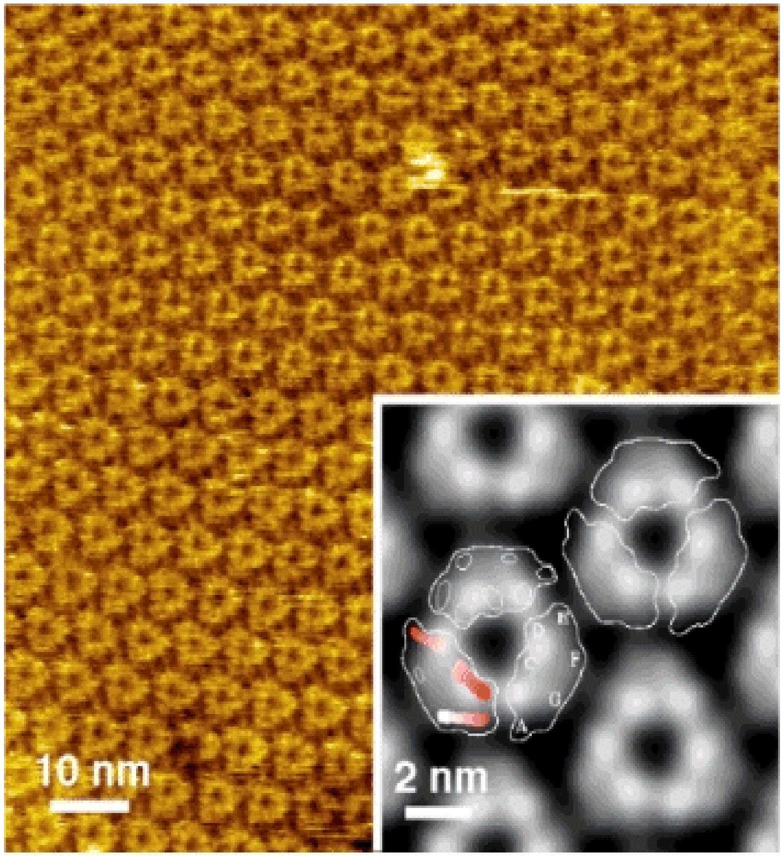
- Tubular crystals (AchR, Ca^{++}-ATPase)
  - Crystallization, No diffraction
  - Isotropic data, 3D distortion corrections, phase restrictions
  - ~5 angstroms

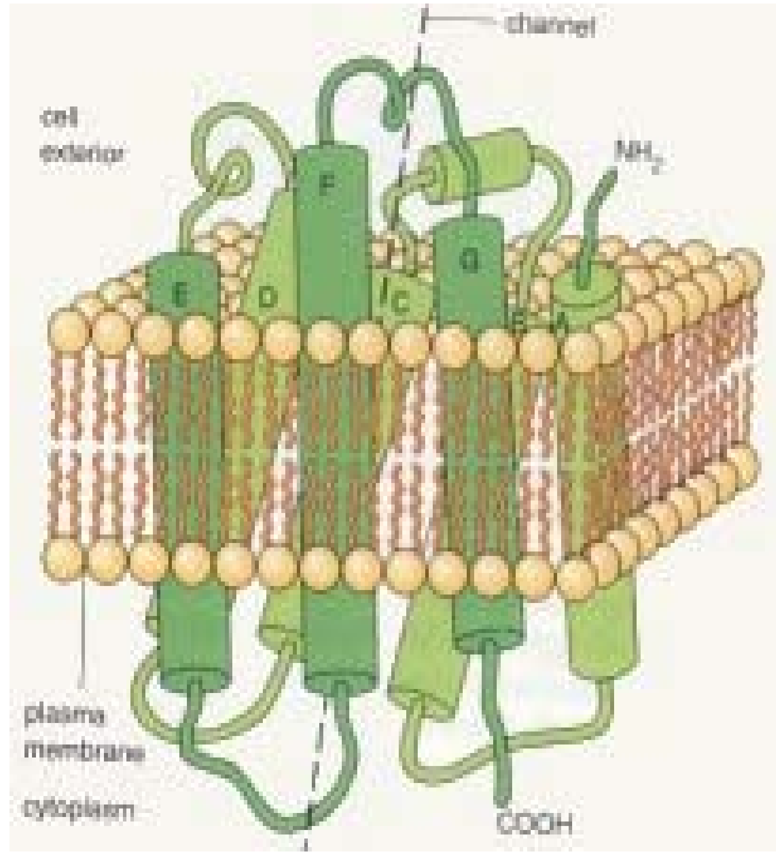
Single Particles

- Applicable to any protein or protein complex > 200kD
- Most common sample
- Number of software suites available
- Resolution ~9A (<7 with symmetry)



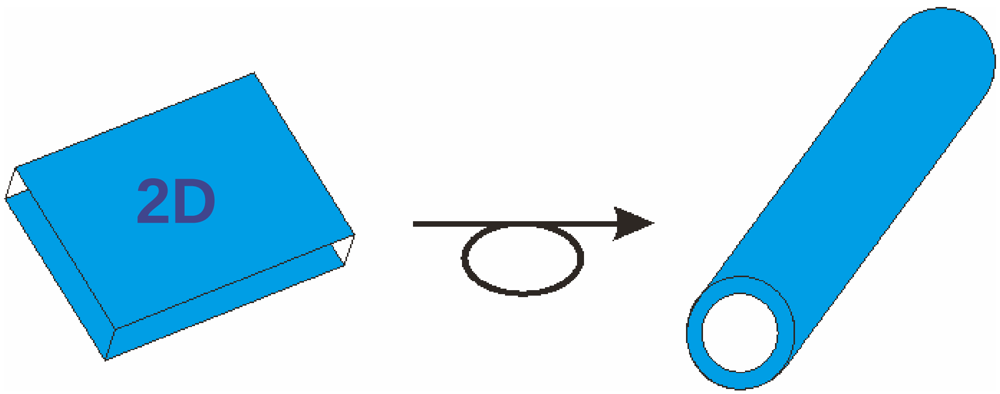
2D Crystals

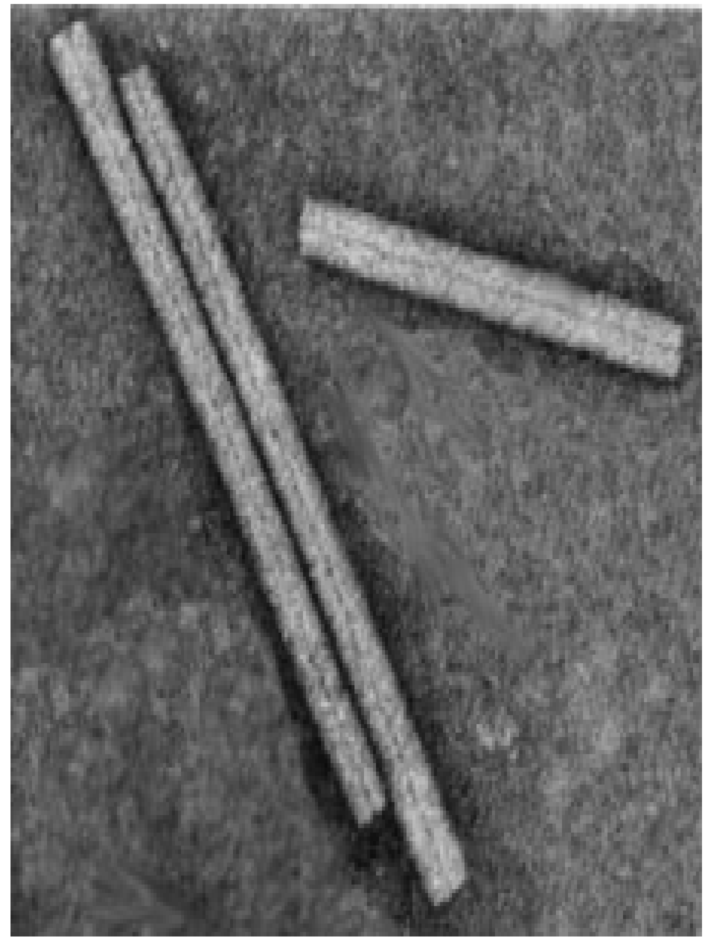




Henderson and Unwin

Tubular Crystals





Tubular vs. 2D or 3D Crystals

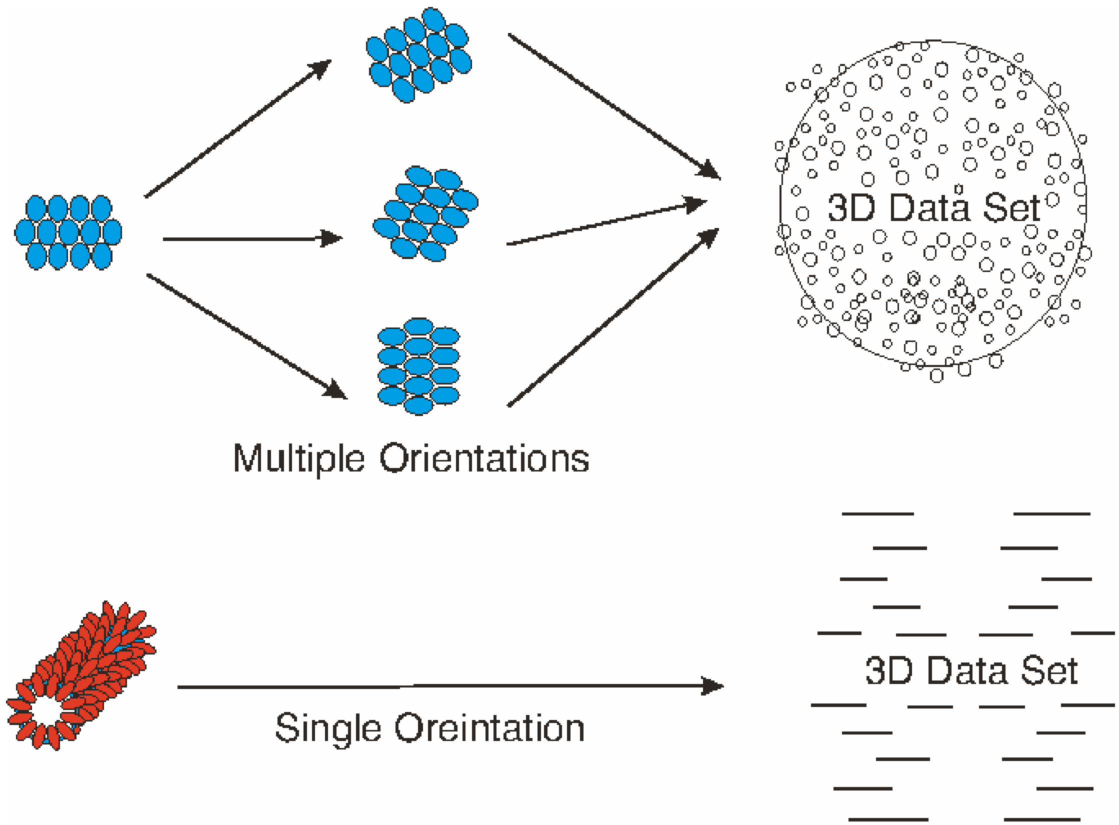
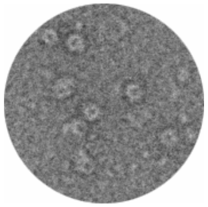


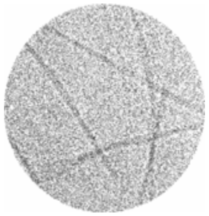
Image Recording

- Film
  - High density content (~20kx16k pixels)
  - Slow (development time, drying)
  - Requires digitization (scanning takes hours)
- CCD
  - Low density content (4kx4k pixels)
  - Fast (ms to sec)
  - Direct digital

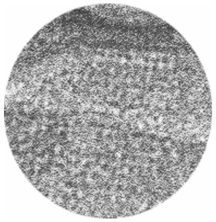
Processing Data



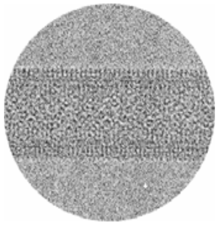
- Single Particles (Proteins, Ribosome)
  - Pick particles
  - Align
  - Classify, average and reconstruction



- Fibers and filaments (tubulin, collagen)
  - Pick segments determine symmetry
  - Align/rotate
  - Average



- 2D crystals (BR, AQP, LHCII)
  - Process images to achieve phases
  - Process diffraction data for amplitudes
  - Combine and refine as in X-ray

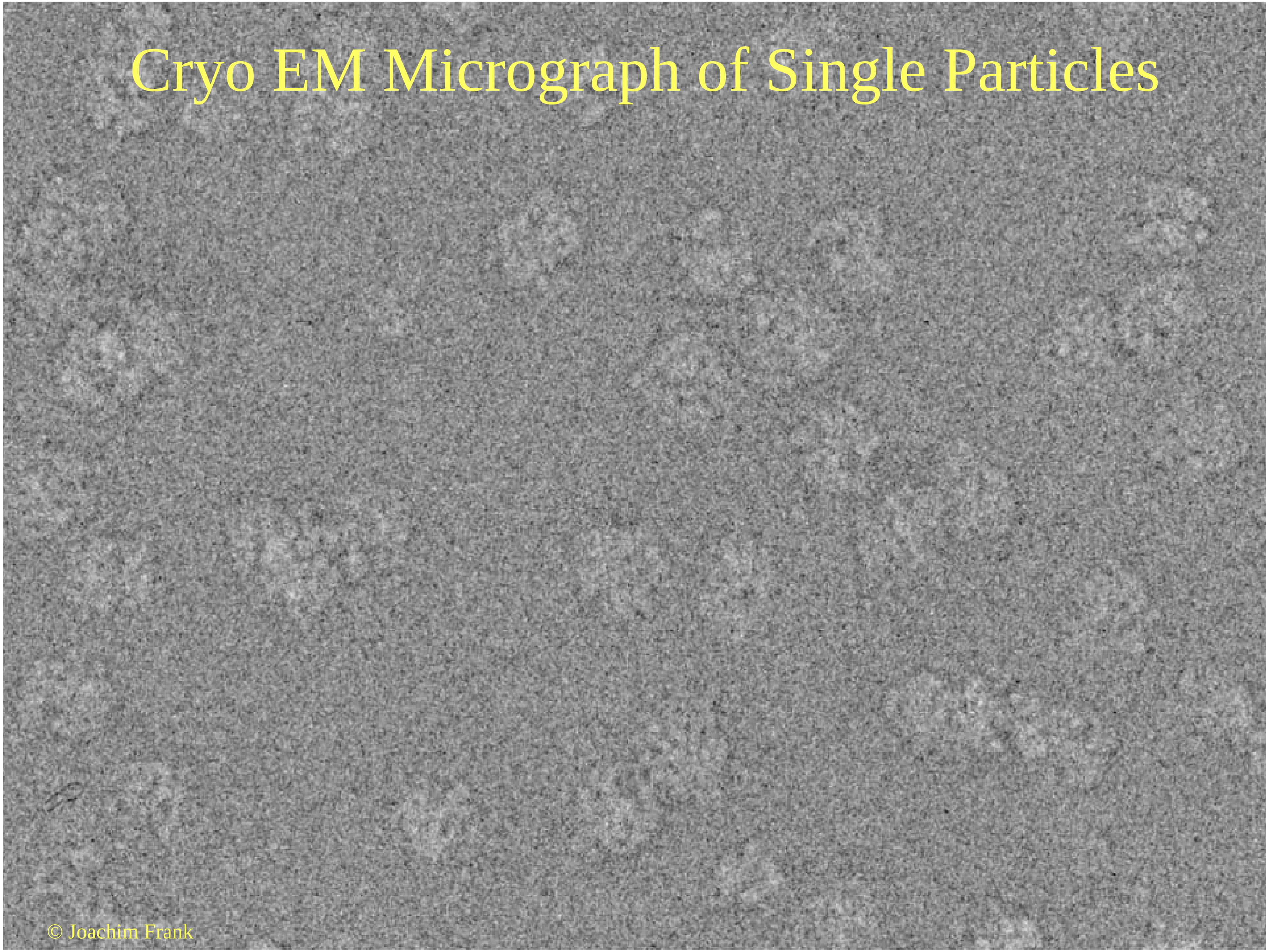


- Tubular crystals (AchR, Ca^{++}-ATPase)
  - Determine tube symmetry
  - Pick segments and distortion correction
  - Average and sum segments

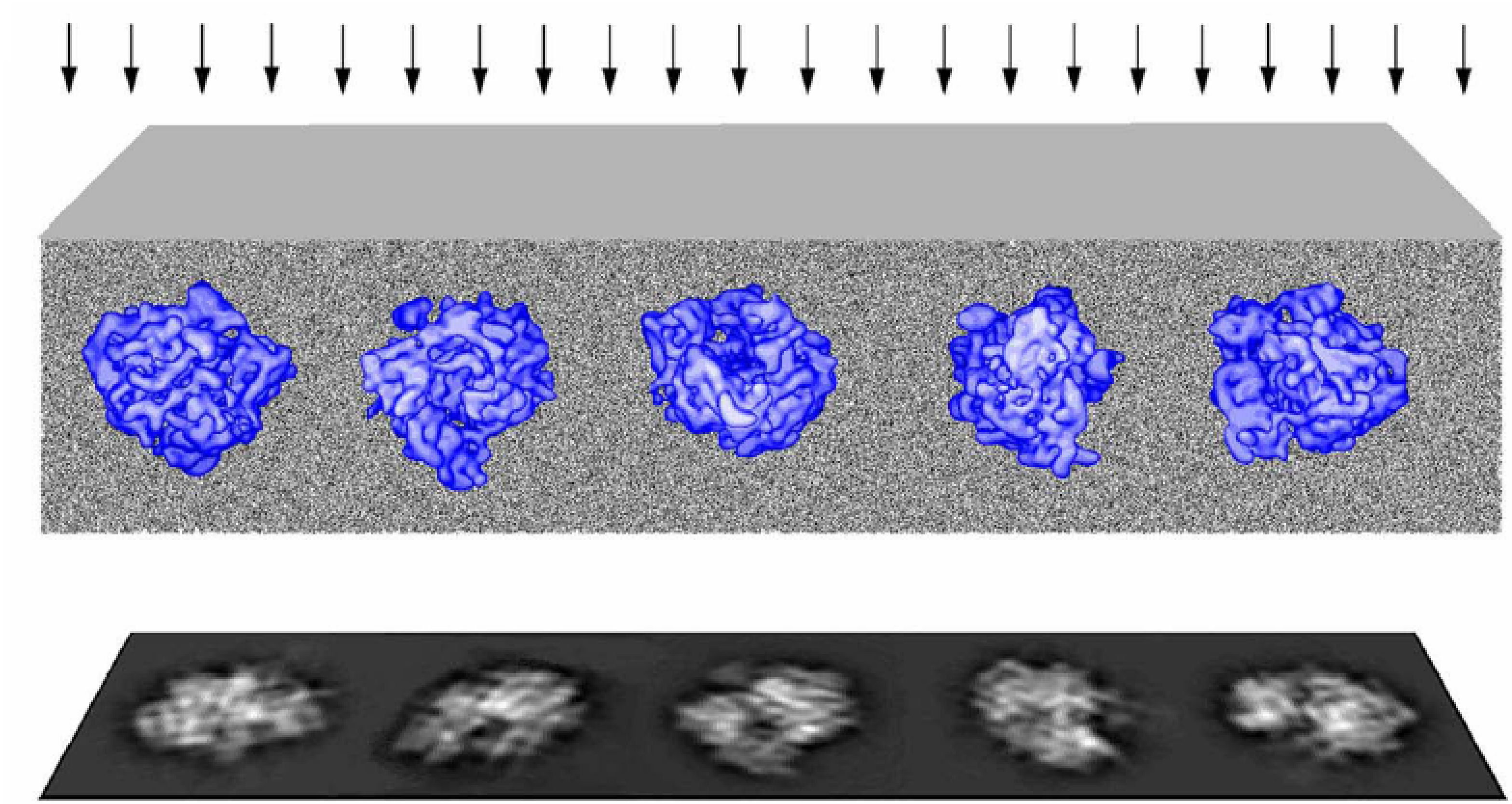
History of Electron Microscopy and 3D Reconstruction Methods

- 1950s: membrane topology of cellular structures, e.g. mitochondria
- 1950s: (Crick, Klug *et al*) FT of helical structures, selection rules
- 1964: (Parson and Martius) high resolution electron diffraction on fibers
- 1968: (DeRosier and Klug) first 3D structure determination of T4 Bacteriophage tail based on helical reconstruction
- 1970: (Crowther *et al*) first icosahedral viruses
- 1972 (Matricardi *et al*), 1974 (Taylor and Glaeser), 1975 (Unwin and Henderson): 2D crystals
- 1983 (Knauer *et al*): ribosome 3D reconstruction (asymmetric single particle)
- 1990 (Henderson *et al*): atomic resolution of bacteriorhodopsin (2D crystal)

Cryo EM Micrograph of Single Particles



What is Observed



Main Assumptions

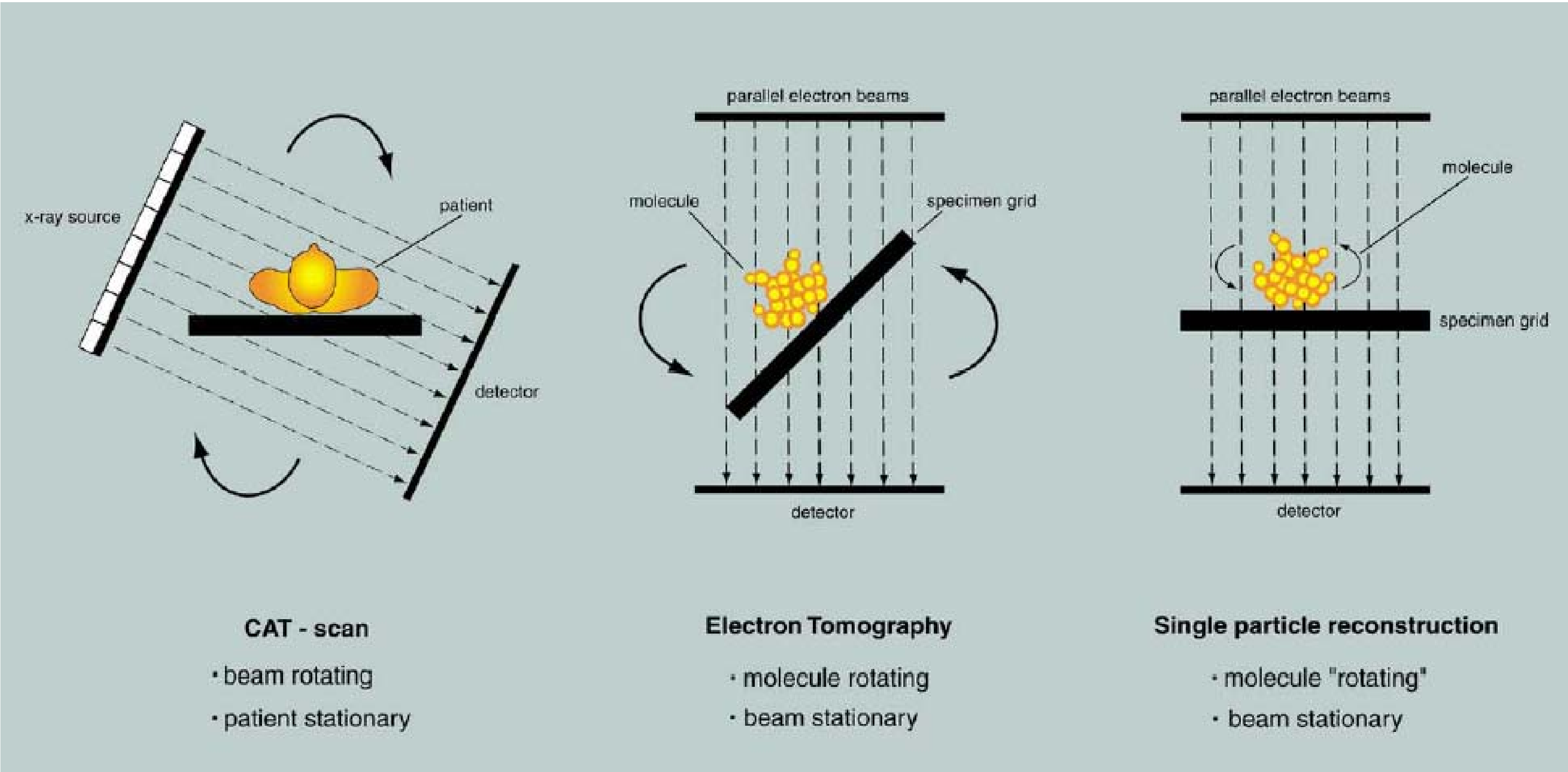
1) All particles in the specimen have identical structure

2) All are linked by 3D rigid body transformations (rotations, translations)

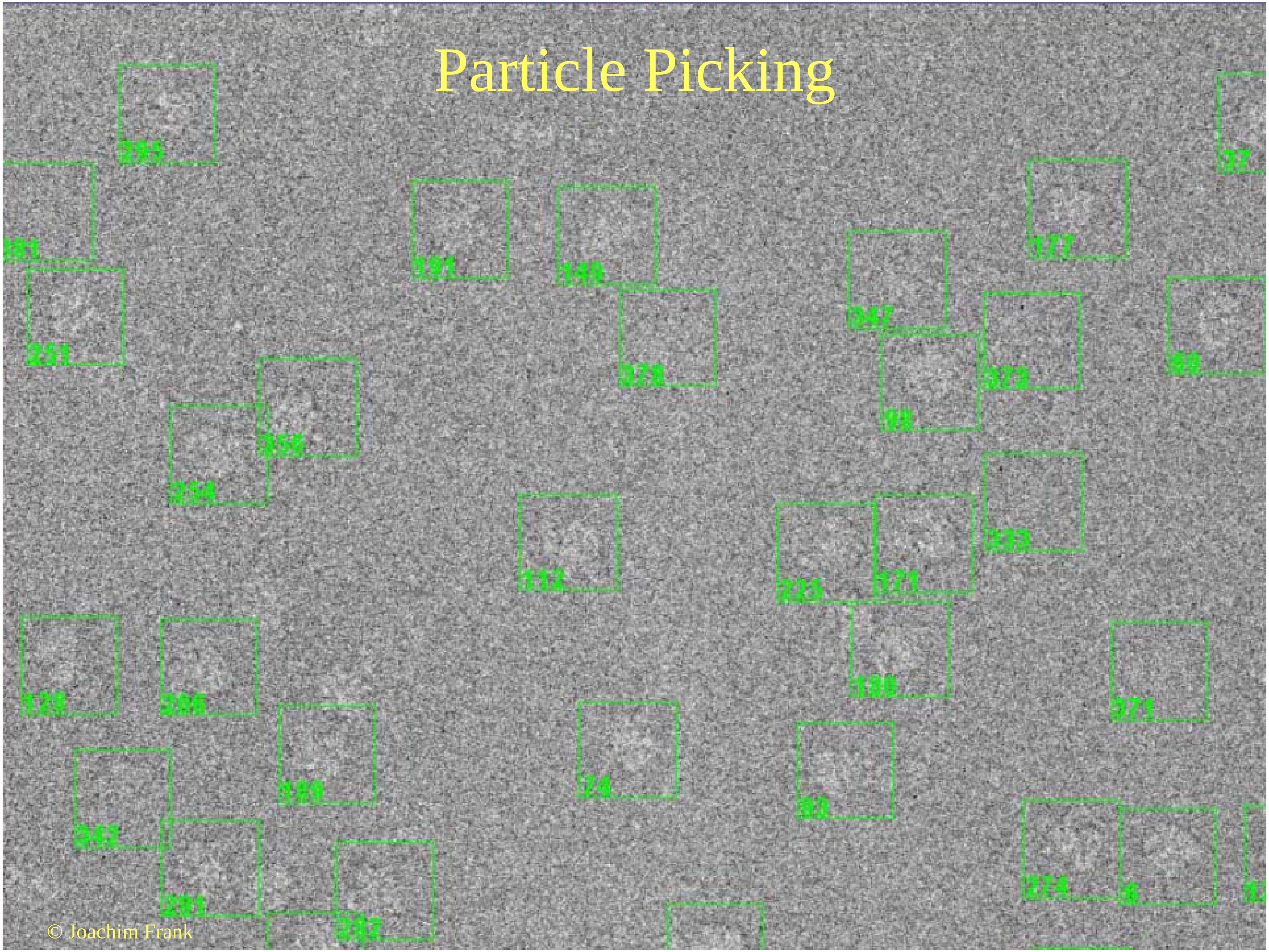
3) Particle images are interpreted as a “signal” part (= the projection of the common structure) plus “noise”

Important requirement:
even angular coverage, without major gaps.

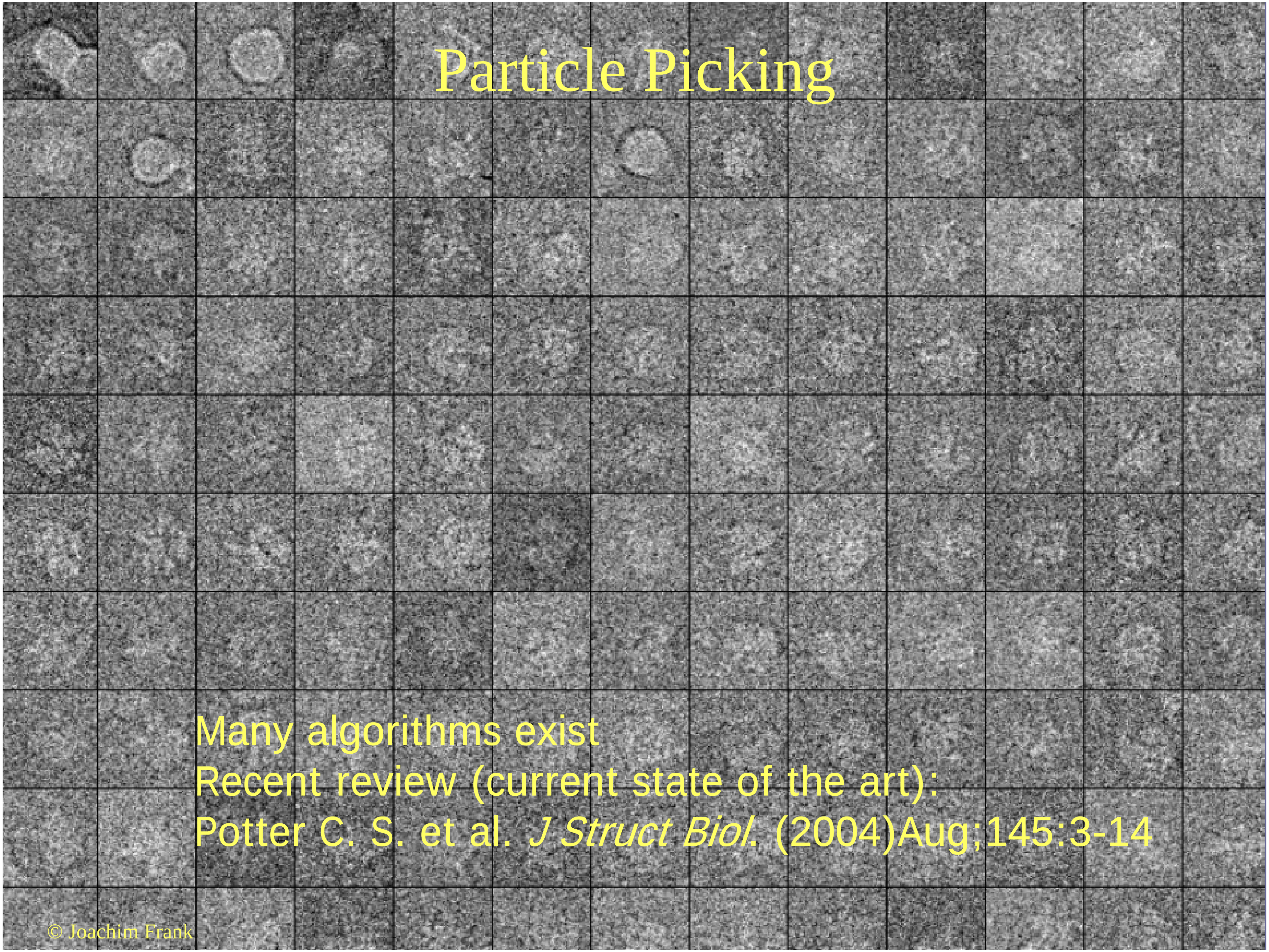
How to Get Even Angular Coverage



Particle Picking



Particle Picking

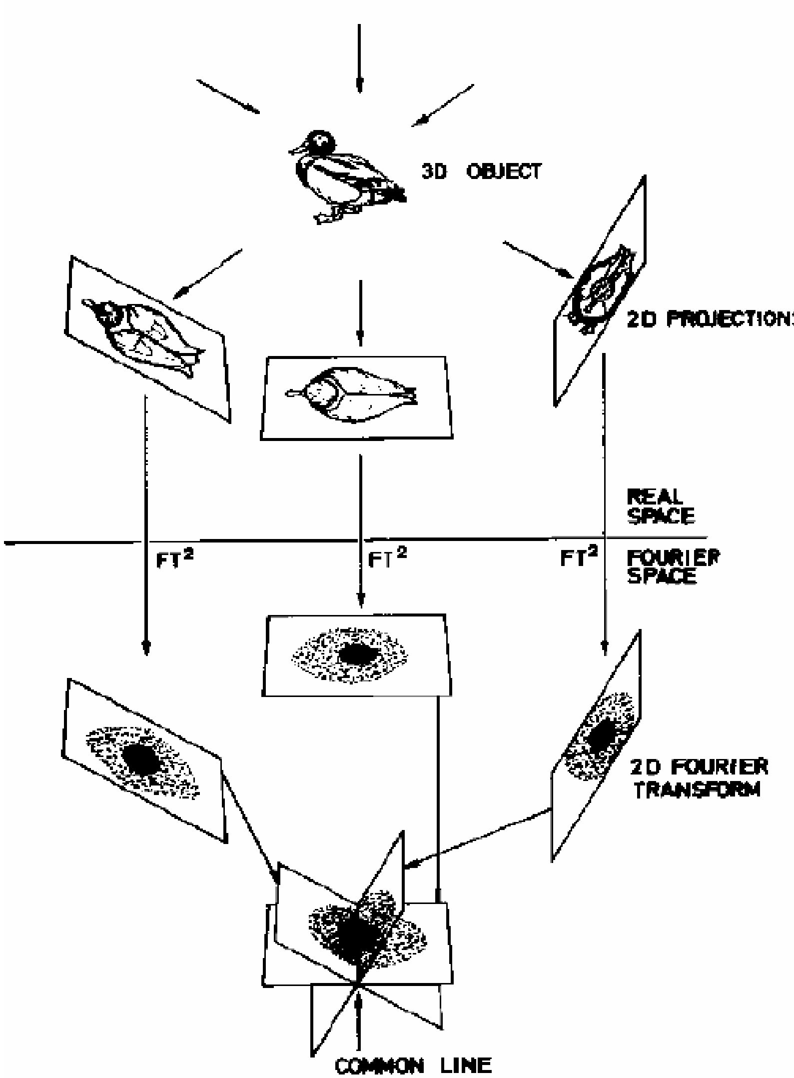


Many algorithms exist

Recent review (current state of the art):

Potter C. S. et al. *J Struct Biol*. (2004)Aug;145:3-14

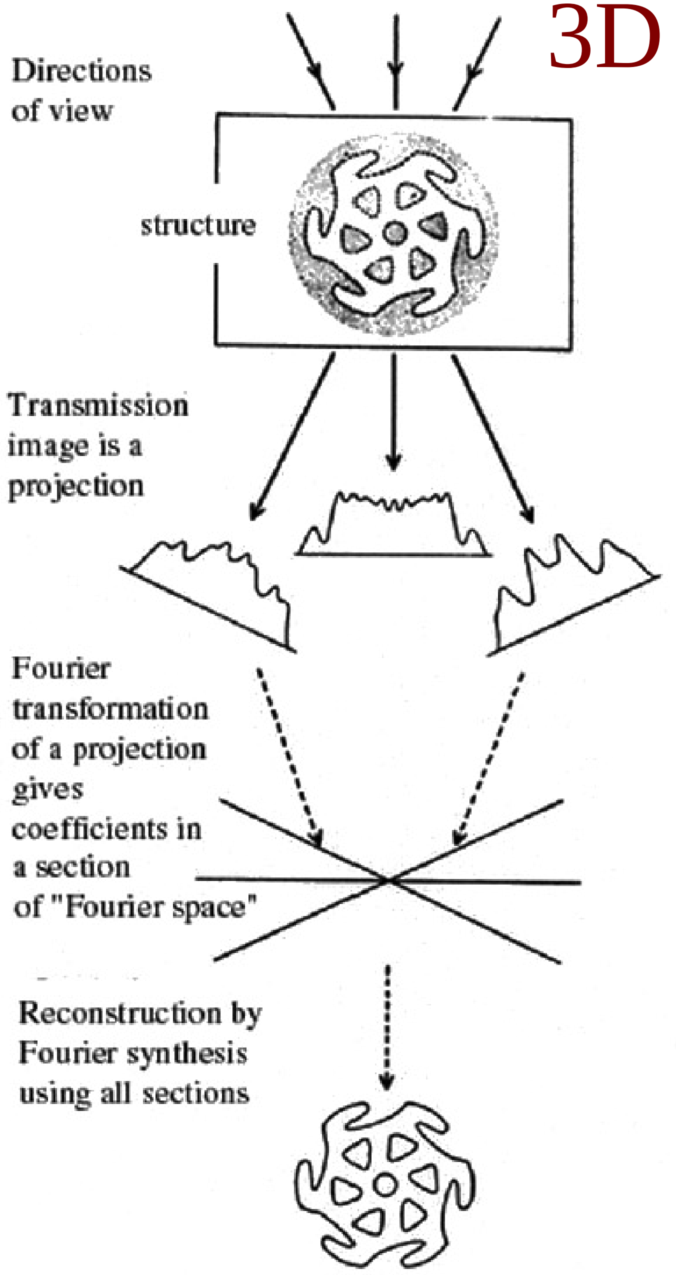
3D Reconstruction



Projection Theorem:

The 2D Fourier transform of the 2D projection of a 3D density is a central section of the 3D Fourier transform of the density perpendicular to the direction of projection.

3D Reconstruction



DeRosier & Klug, Nature 217 (1968) 133

Projection Theorem:

The 2D Fourier transform of the 2D projection of a 3D density is a central section of the 3D Fourier transform of the density perpendicular to the direction of projection.

This holds in Fourier Space.

Angular Reconstitution

Real Space:

Common Line Projection Theorem

Two different 2D projections of the same 3D object always have a 1D line projection in common.

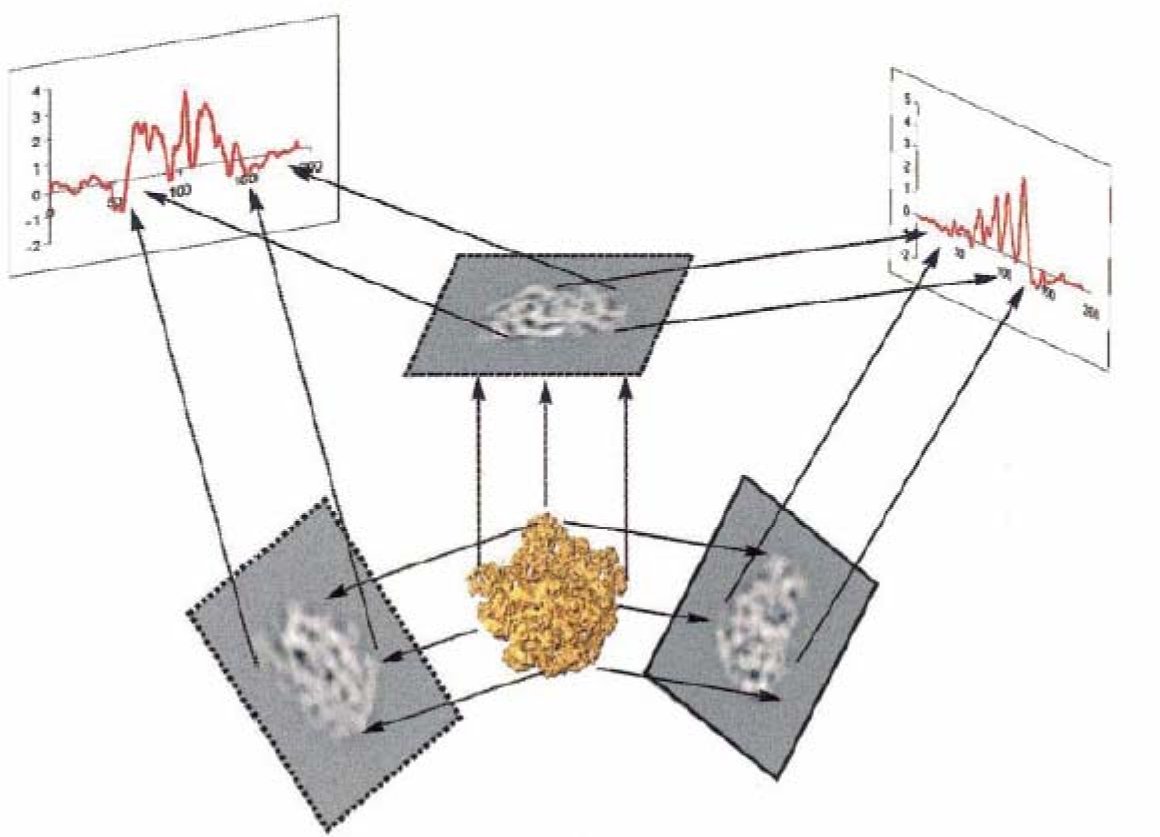


Fig. 11. The angular reconstitution technique is based on the common line projection theorem stating that two different two-dimensional (2D) projections of the same 3D object always have a one-dimensional (1D) line projection in common. From the angles between such common line projections, the relative Euler-angle orientations of set projections can be determined *a posteriori* (van Heel, 1987). For an entirely asymmetric particle like this 50S ribosomal subunit, at least three different projections are required to solve the orientation problem. For details see main text.

van Heel et al, Quarterly Reviews of Biophysics **33**, 4 (2000), pp. 307–369.

Sinograms

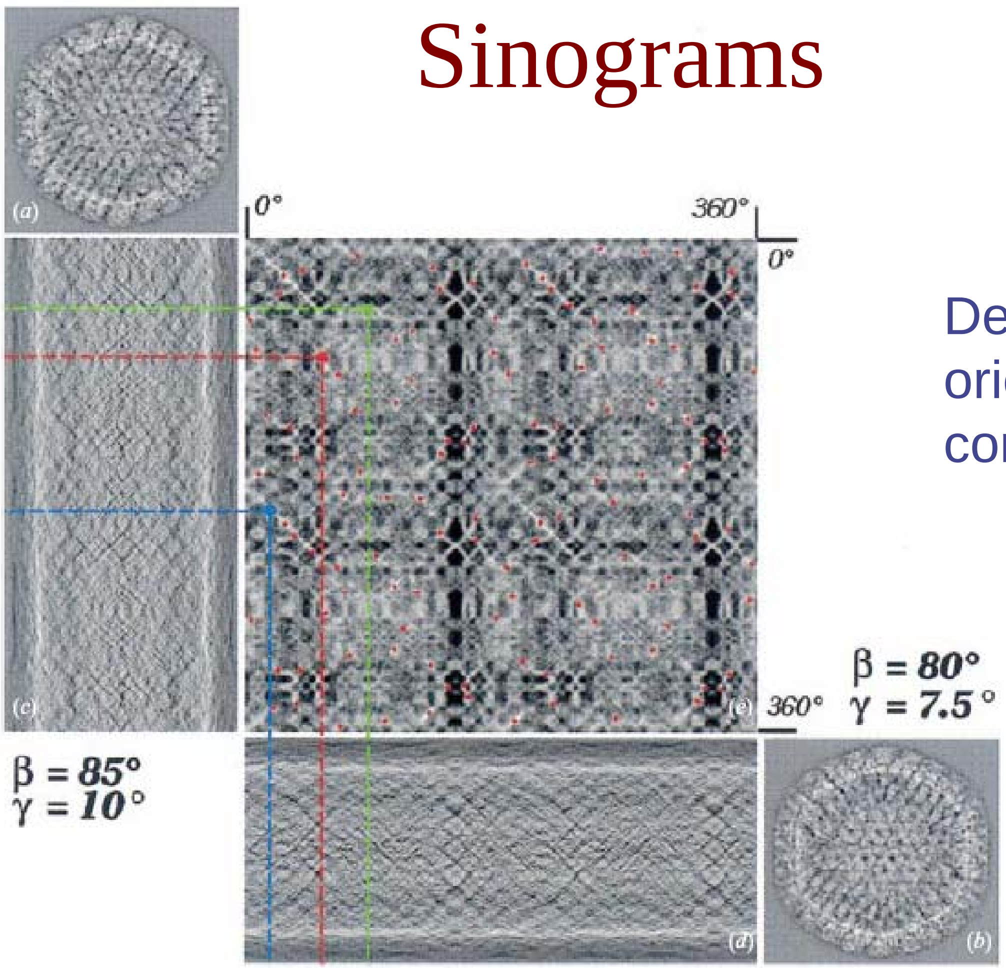
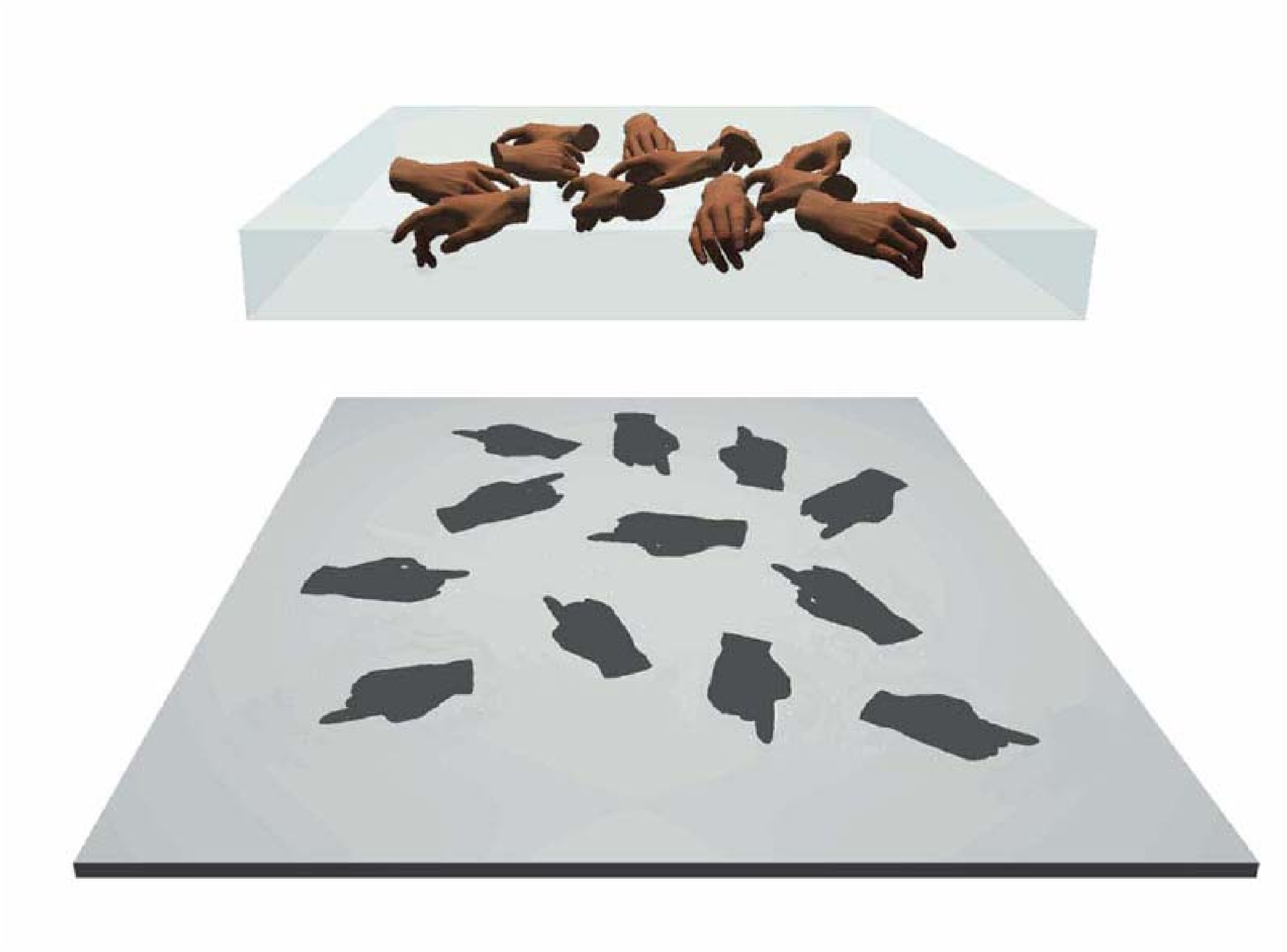


Fig. 13. Sinograms and sinogram correlation functions. This illustration provides a graphical overview of the relations between a 2D class average (noise-reduced projection images), their 'sinograms', and the sinogram correlation function between two sinograms. The images shown here (*a*, *b*) are class averages deduced a large data set of Herpes Simplex Virus Type 1 (HSV1) cryo-EM images. Each line of the sinogram images (*c*, *d*) is generated from the 2D projection image by summing all 1D lines of the 2D images, from top to bottom, after rotation of the image over angles ranging from 0° to 360°. Equivalently, the lines of the sinograms are 1D projections of the 2D images in all possible directions ranging from 0° to 360°. Each point of the sinogram correlation function contains the correlation coefficient of two lines of the two sinograms one is comparing (*e*).

Determine relative orientations with common lines!

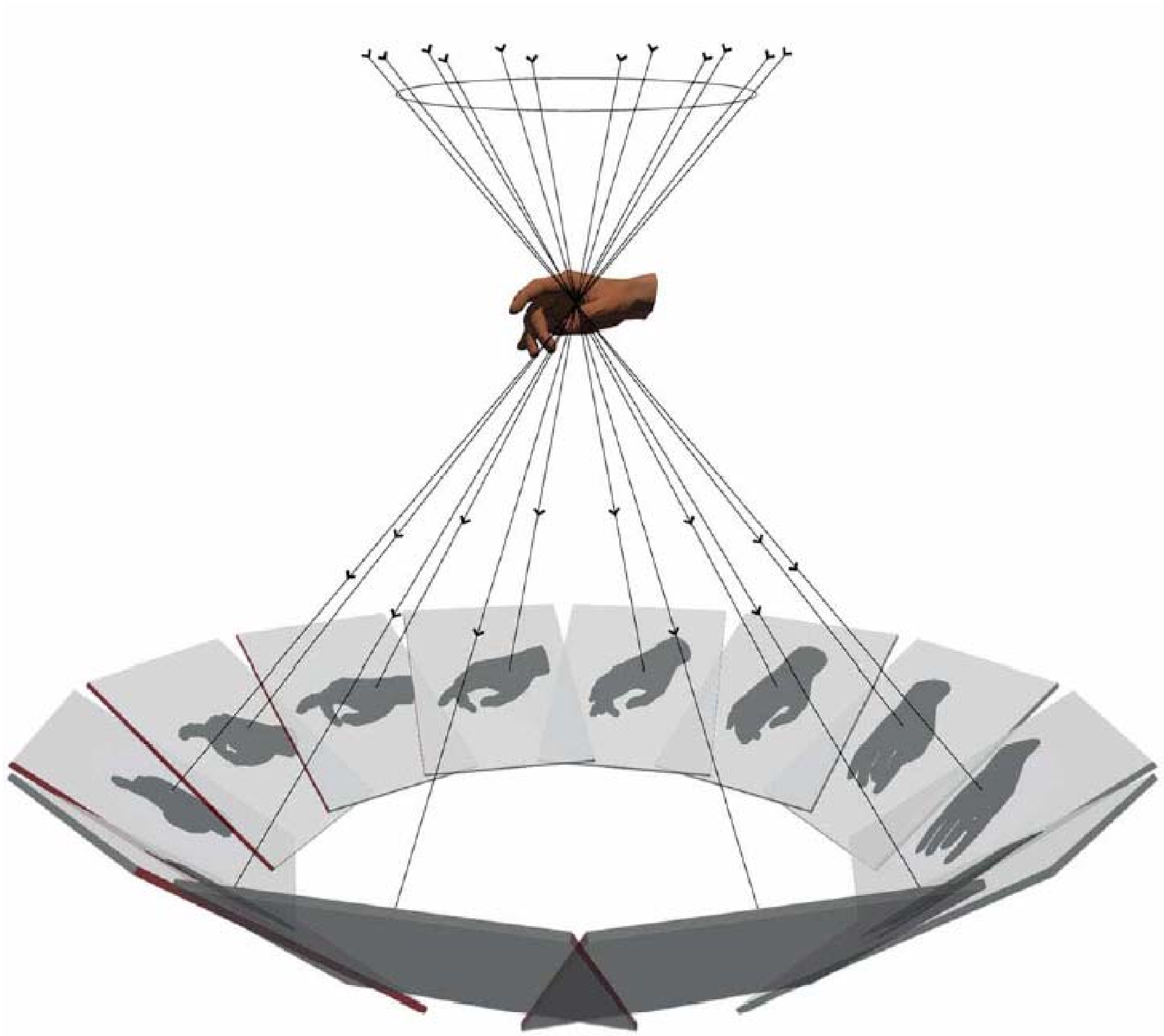
van Heel et al, Quarterly Reviews of Biophysics **33**, 4 (2000), pp. 307–369.

What if Particles are Aligned with Grid?

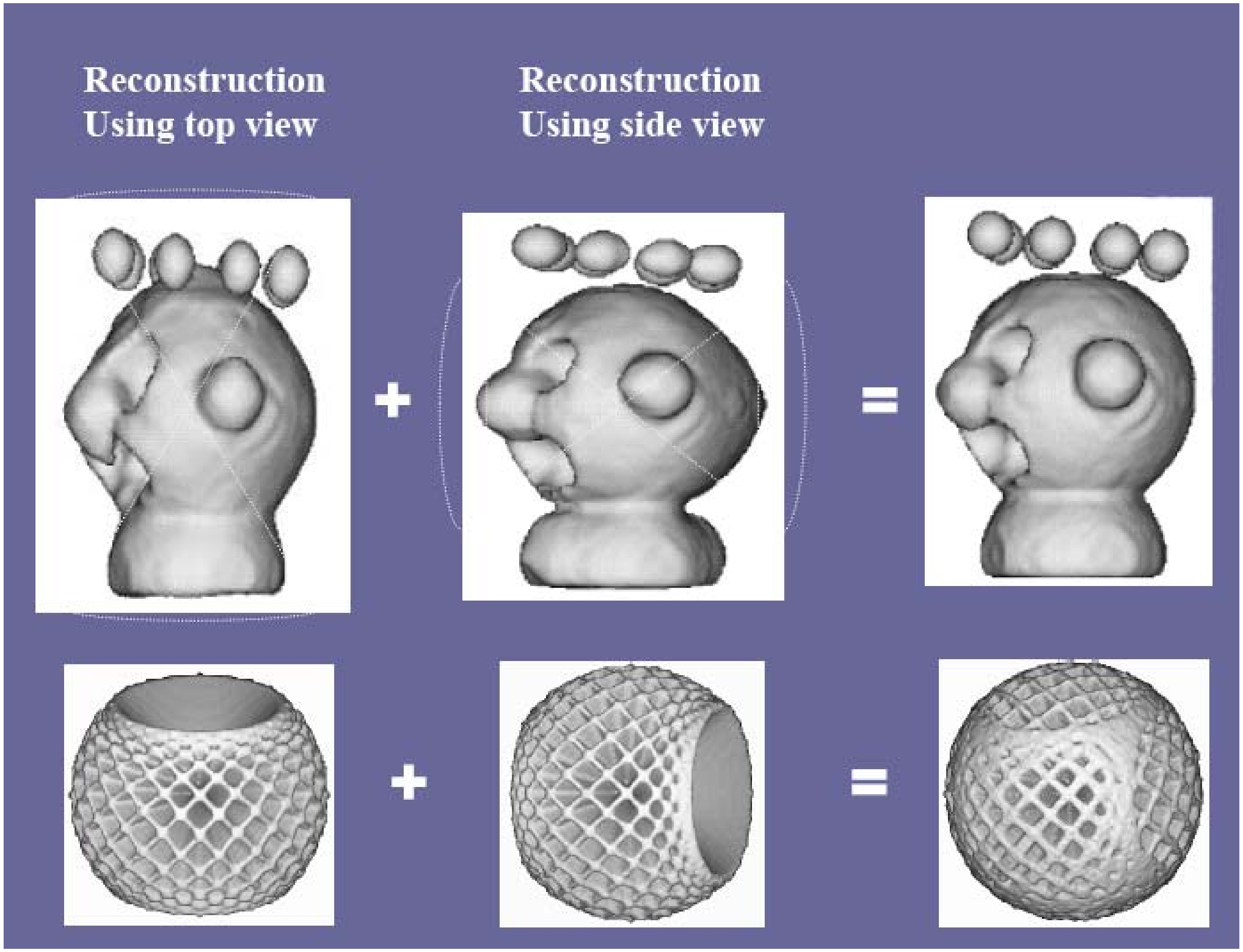


Solution: Tilt of Specimen

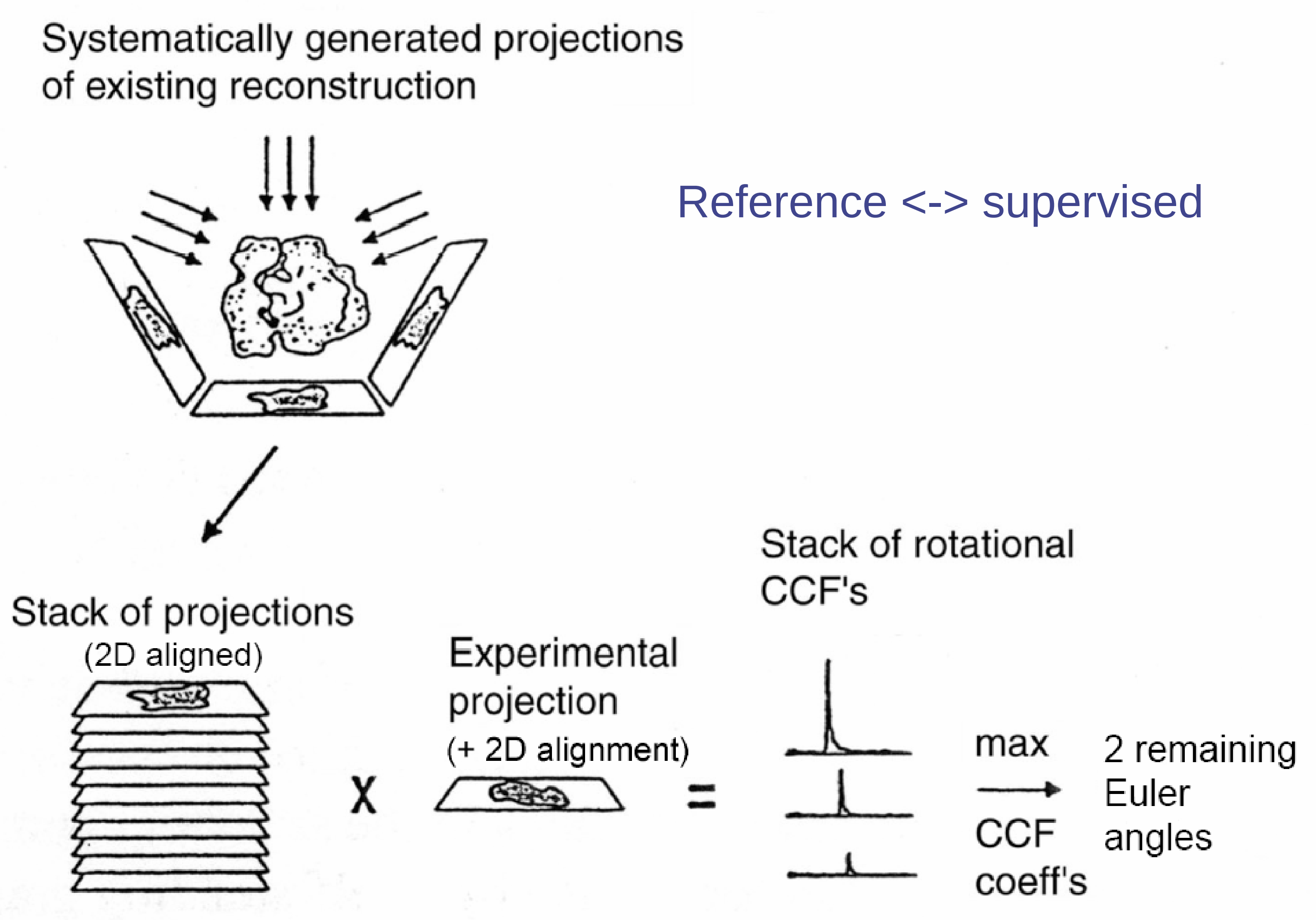
Random Conical Tilt



Missing Cone Artifacts



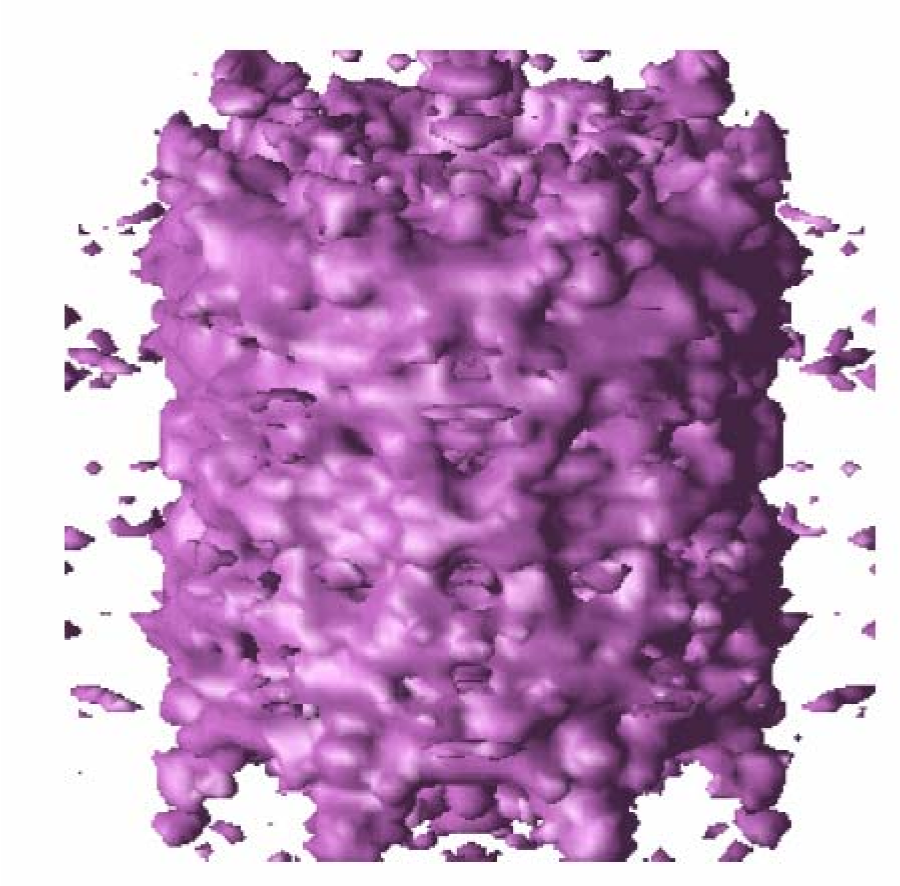
Reference-Based Projection Matching



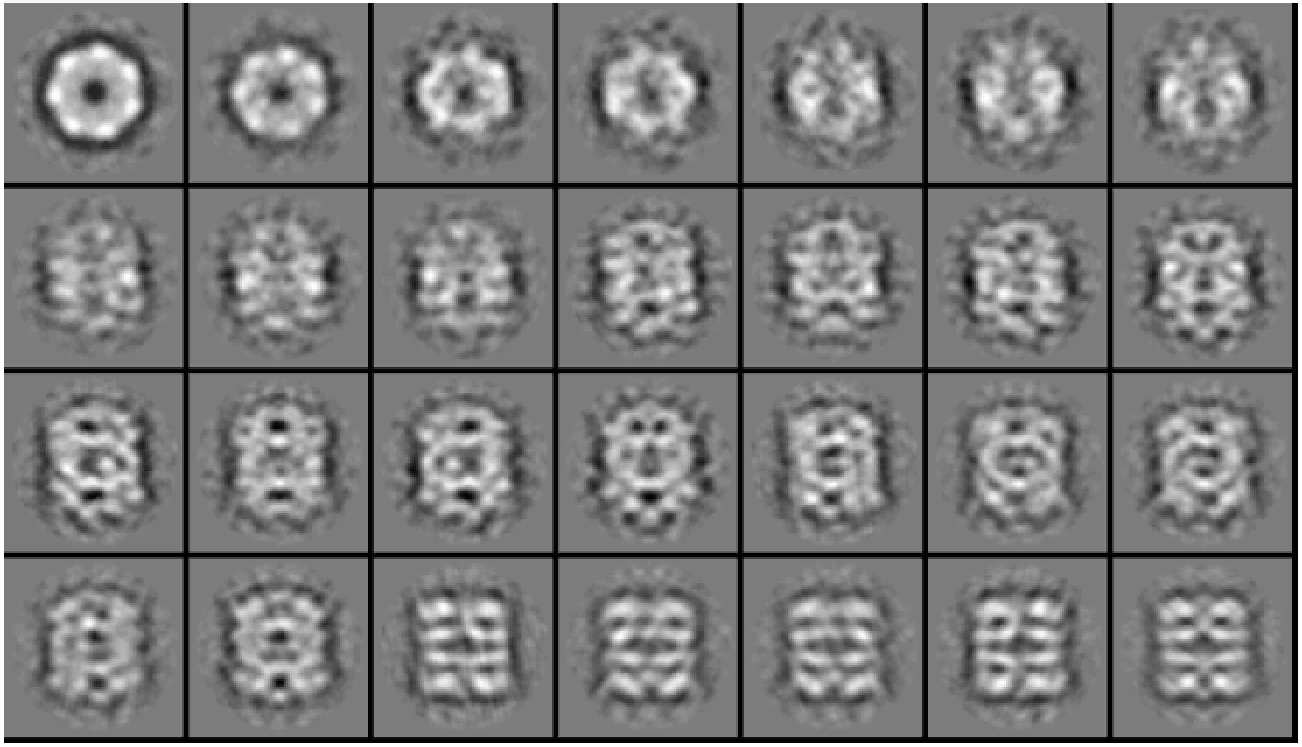
Reference <-> supervised

adapted from an image by Joachim Frank

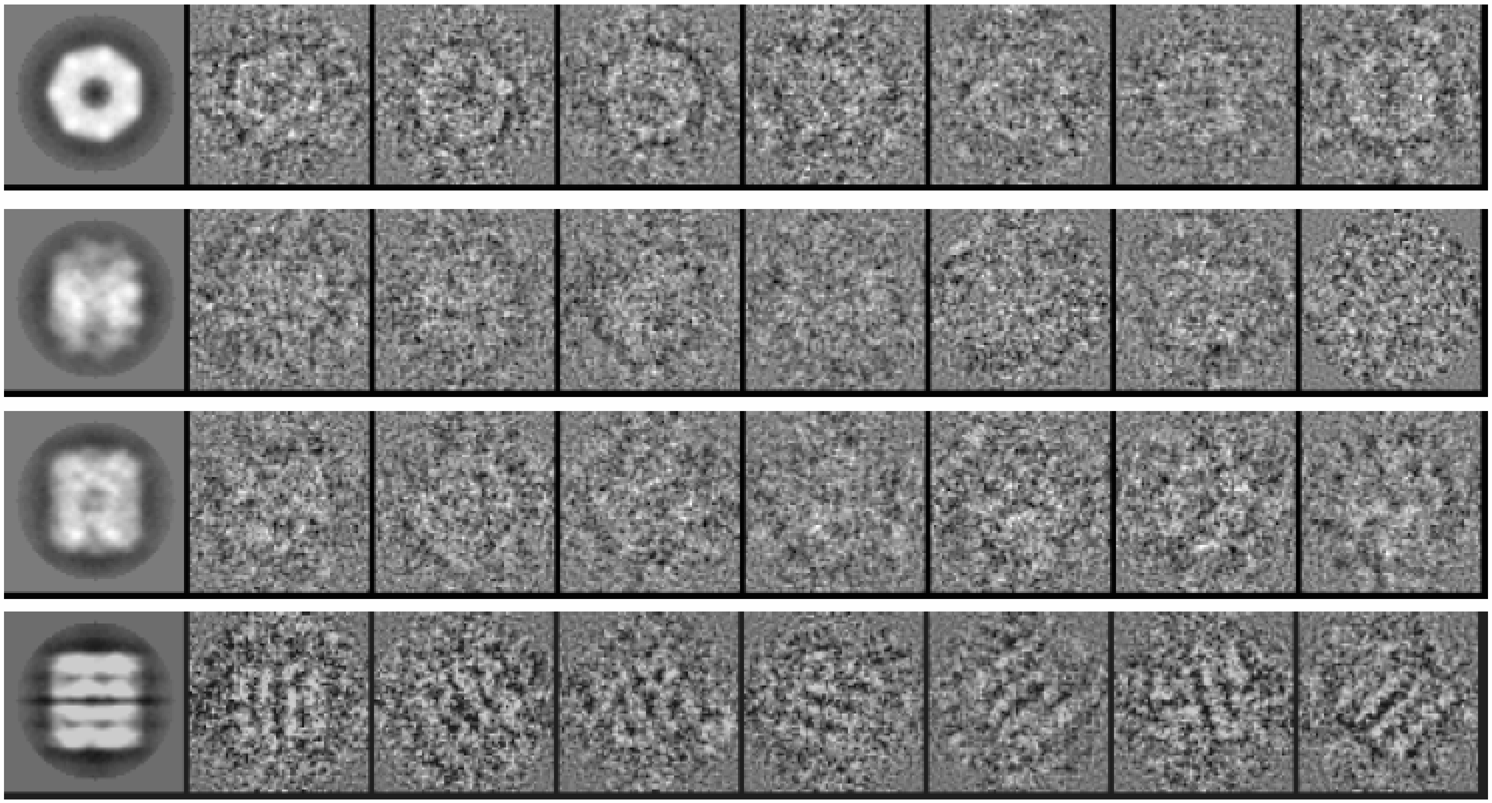
Preliminary Model



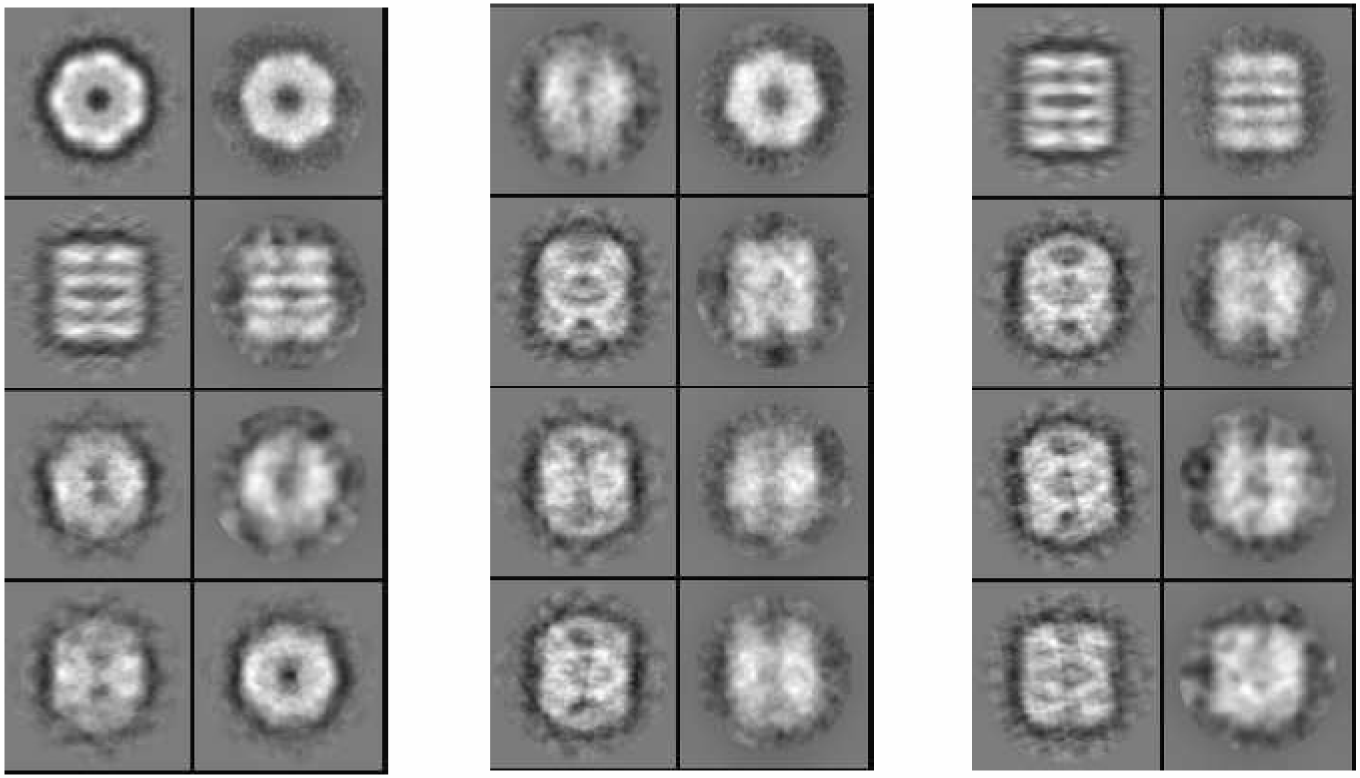
Projections



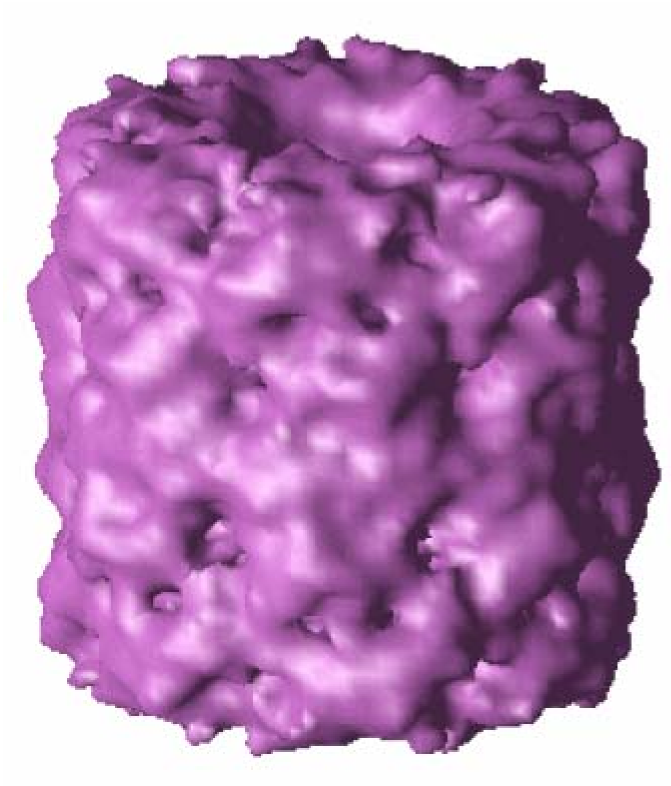
Classification



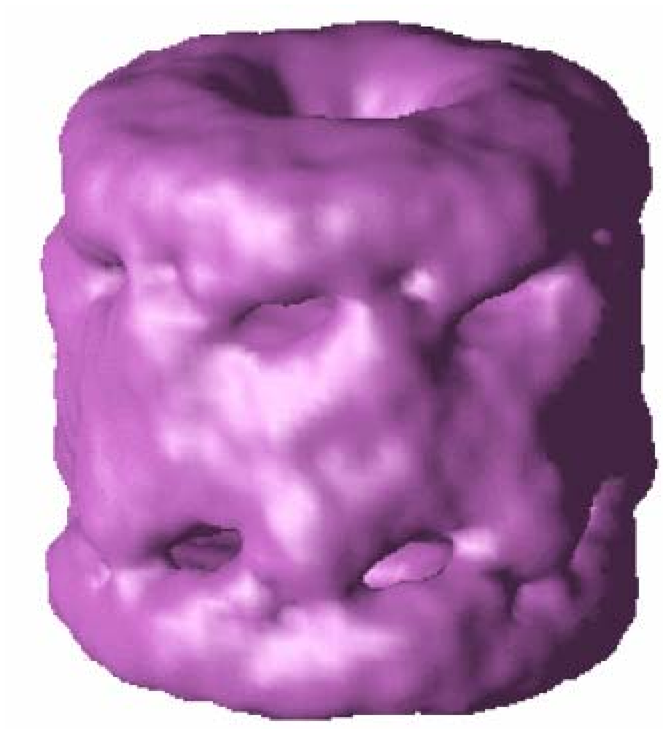
Class Averages



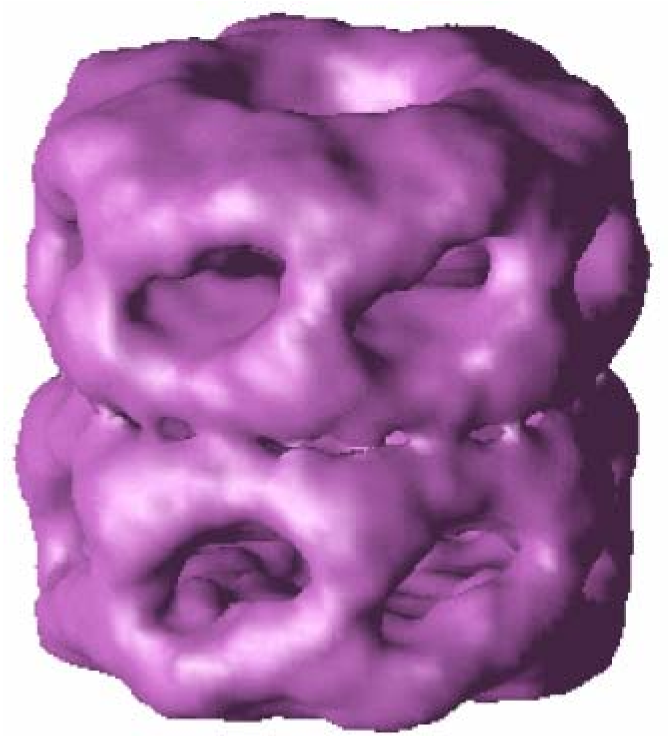
Iteration 1



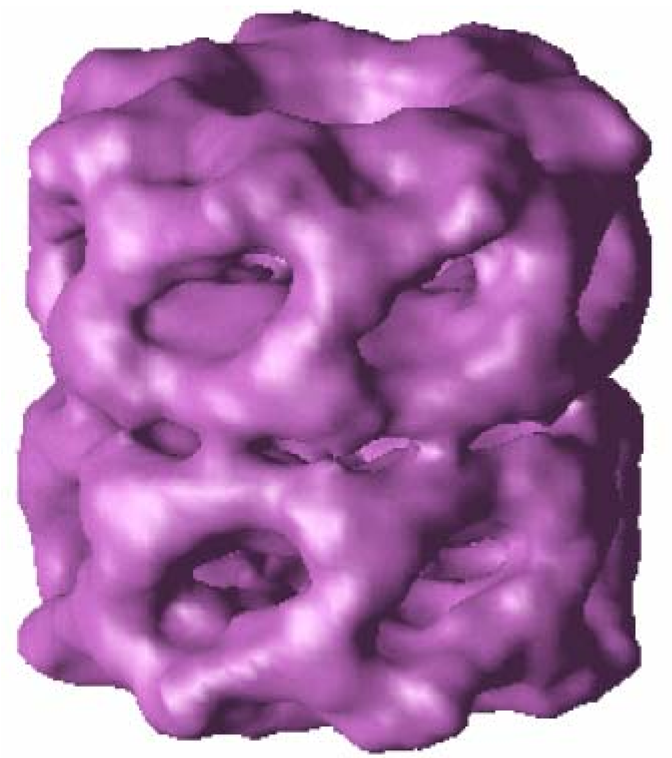
Iteration 2



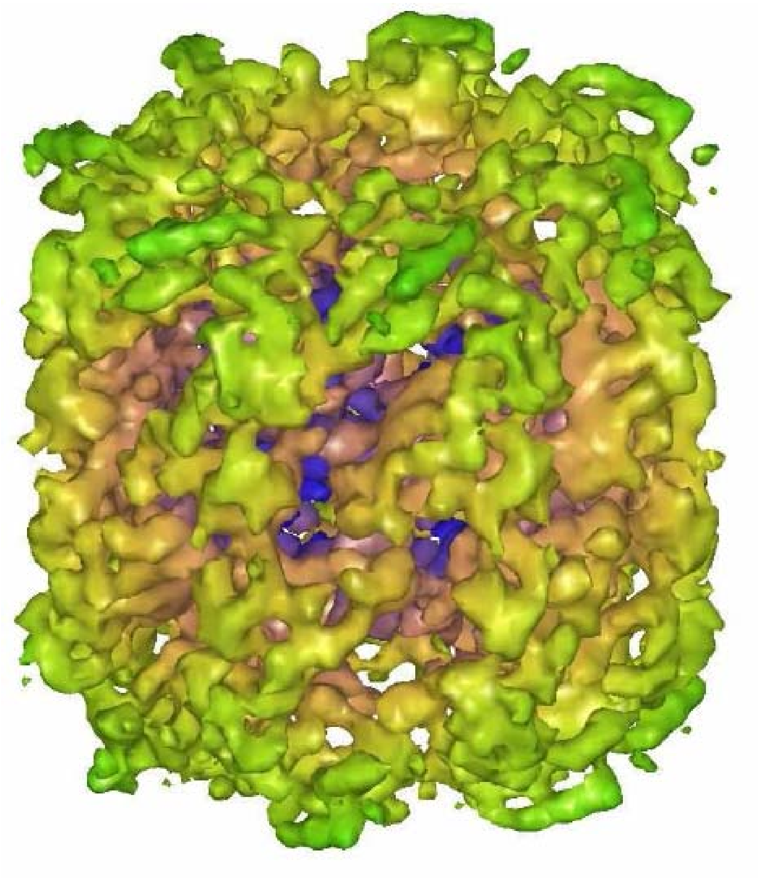
Iteration 3



Iteration 4



GroEL Reconstruction at 6.5Å



Reference - Electron Microscopy

Chapters 3,4,5 in: Joachim Frank, Three-Dimensional Electron Microscopy of Macromolecular Assemblies (1996, Academic Press)