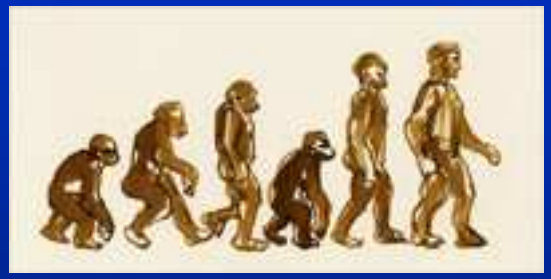
Quantitative Biology Lectures

January 13-14, 2008

Single-Particle Electron Microscopy

James Z. Chen

Seeing Is Believing

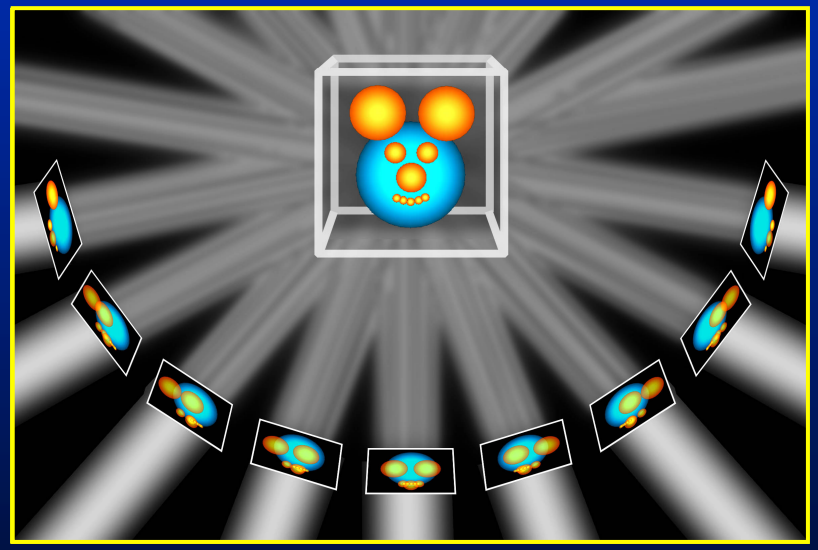


m mm μm nm Å

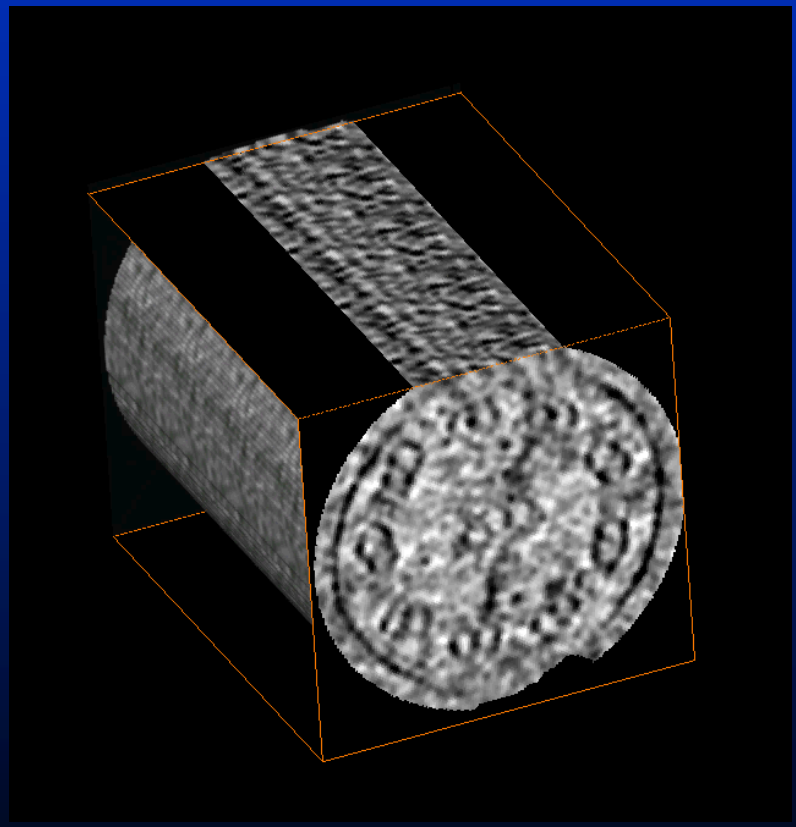
Direct Observation	Optical Microscope	Electron Microscope

Electron Tomography

Set of 2D projections
$\Rightarrow$ 3D reconstruction

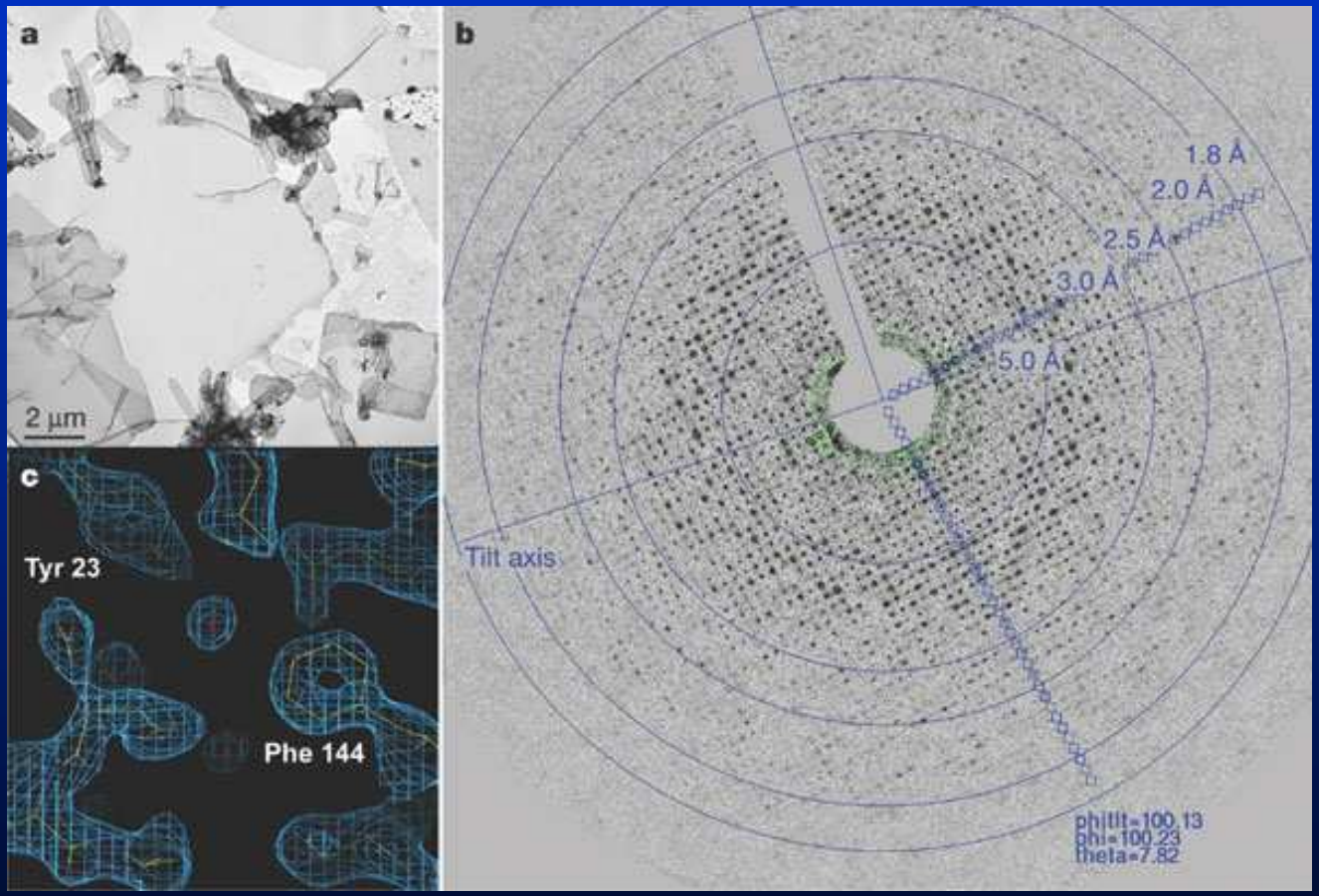


Eukaryotic Flagella (Axonemes)



By courtesy of Dr. Daniela Nicastro

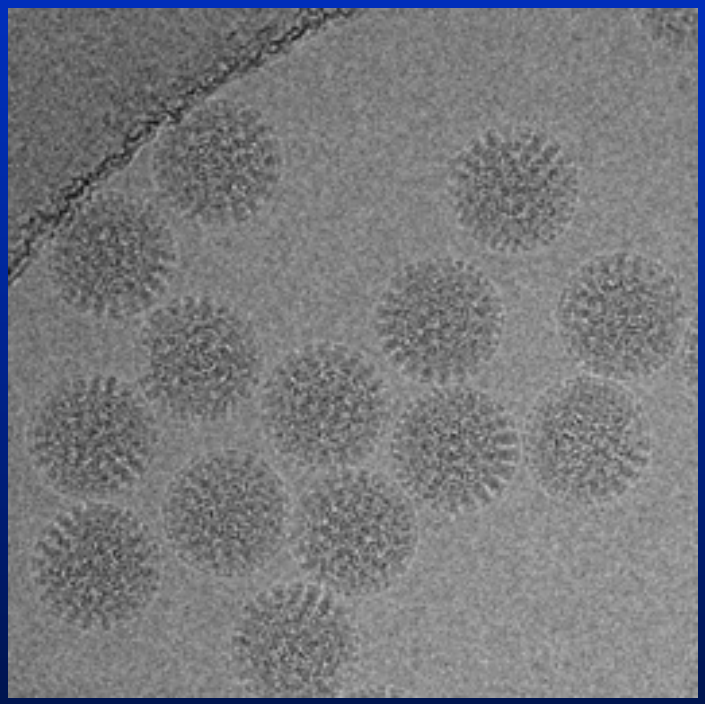
Electron Crystallography

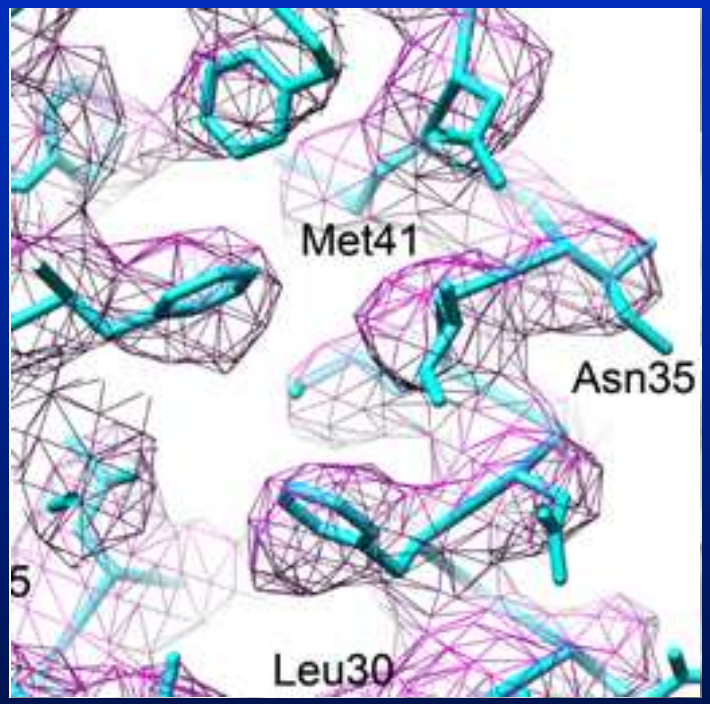


AQP0

Gonen, et al, Nature 438: 633-638.

Single-Particle EM





Rotavirus, DLP

Zhang, et al, PNAS, in press.

Single-Particle Electron Microscopy

Data Collection

- Electron microscope
- Image formation
- Specimen preparation
- Data acquisition

Data Processing

- Particle screening
- Particle alignment
- Particle classification
- Model reconstruction

Structure Analysis

- PDB-map docking
- Structure prediction
- Dynamic modeling
- High-resolution EM

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Single-Particle Electron Microscopy

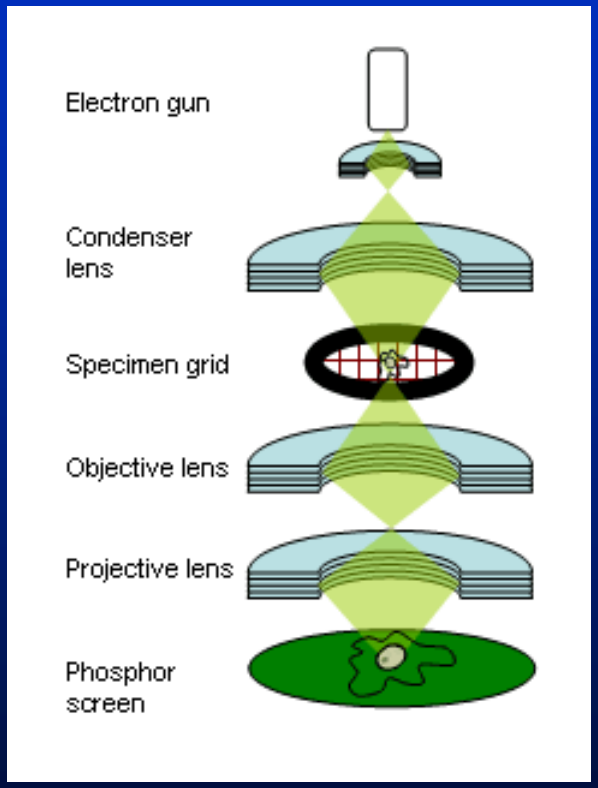
Instrument and Data Collection

James Z. Chen

Topics

- **Electron microscope**
- **EM image formation**
- **Specimen preparation**
- **Data acquisition**

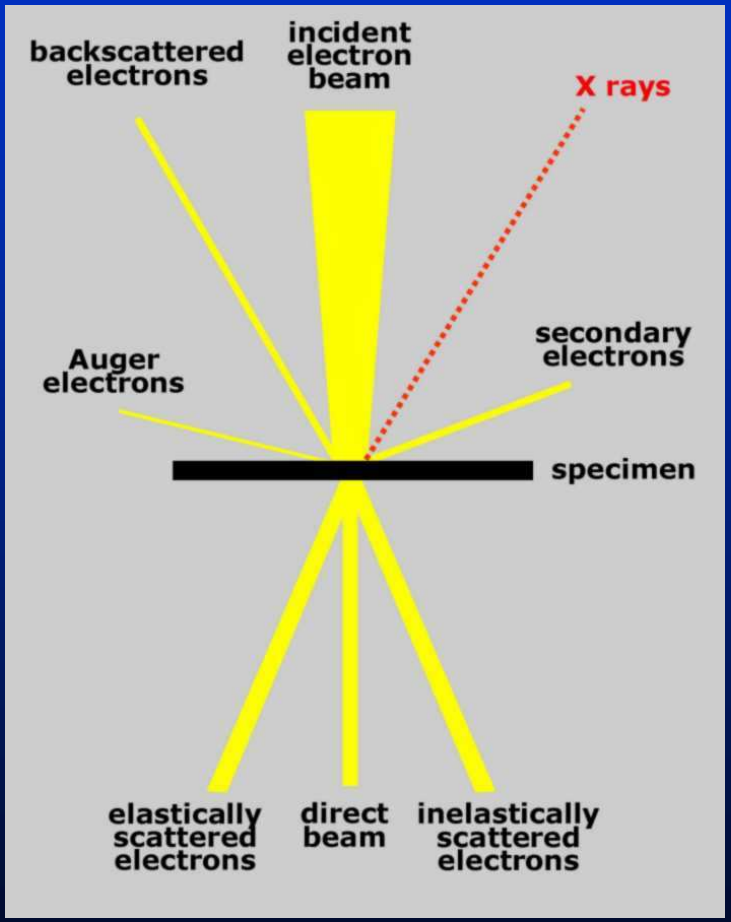
Electron Microscope



Transmission Electron Microscopy (TEM)

Beam-Specimen Interaction

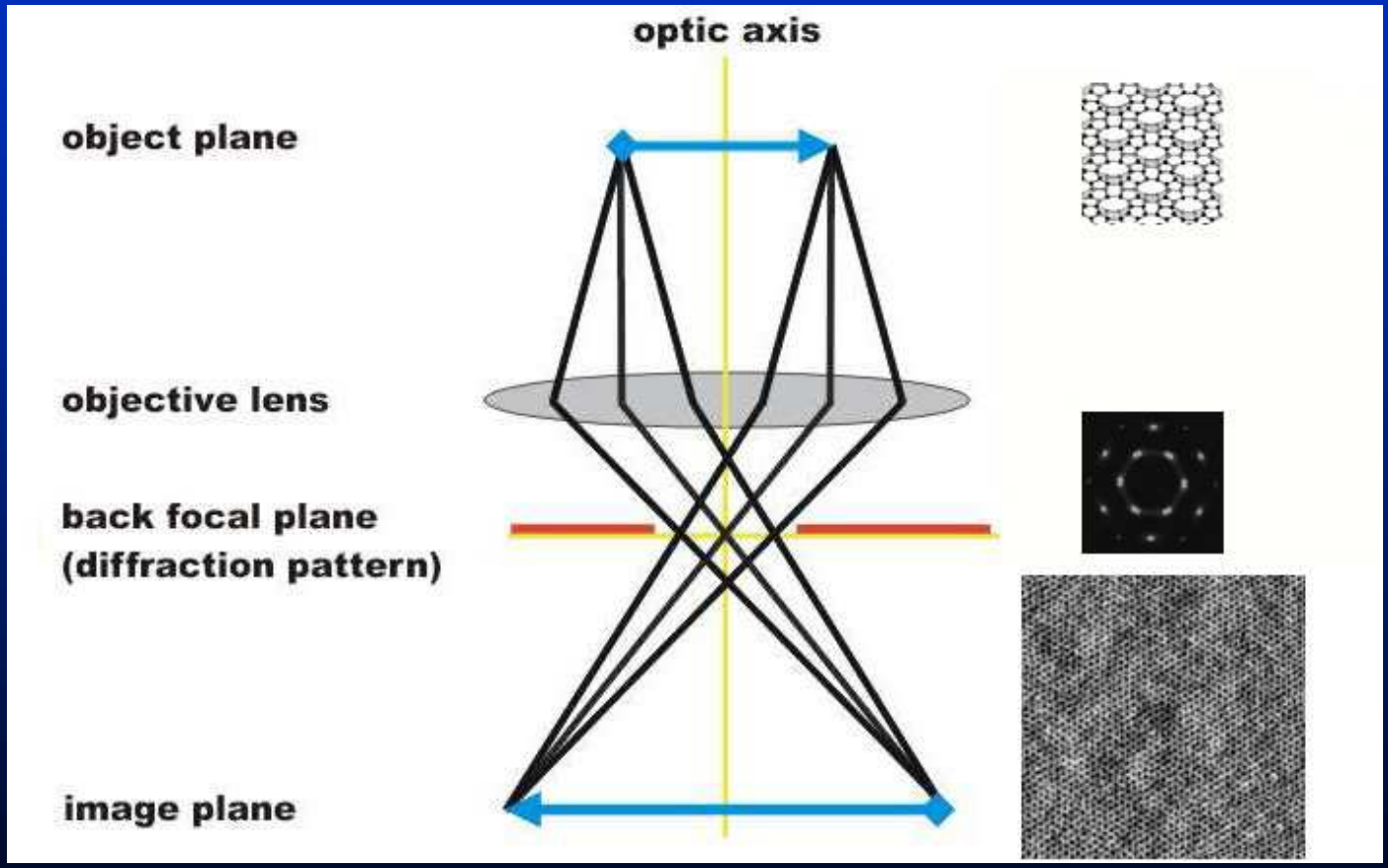
Analytical EM



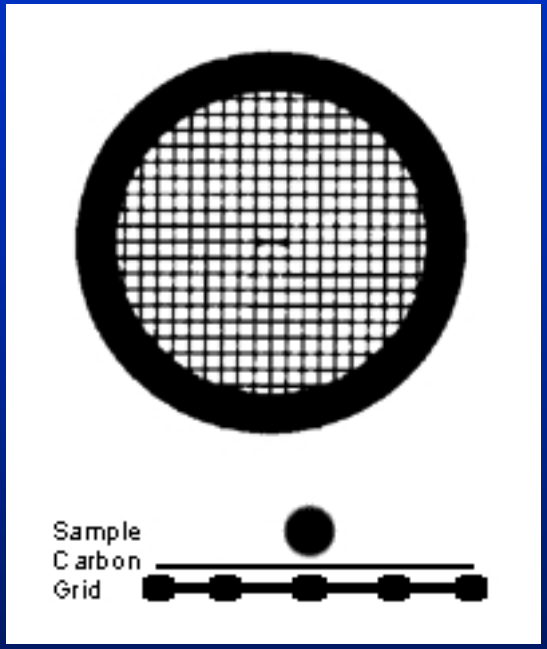
SEM

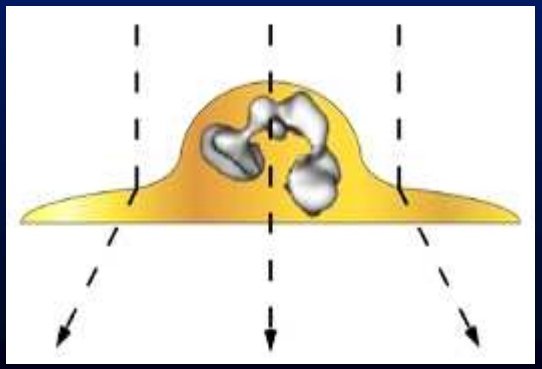
TEM

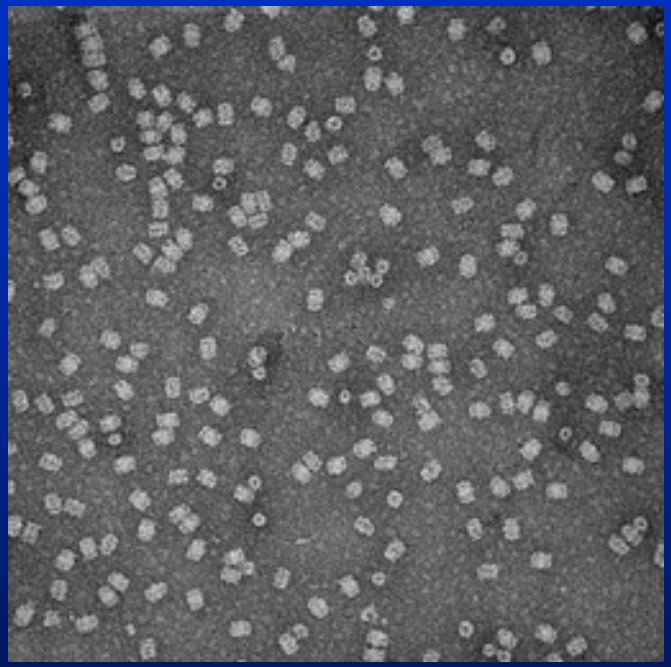
TEM Image Formation



Specimen Preparation: Negative Staining







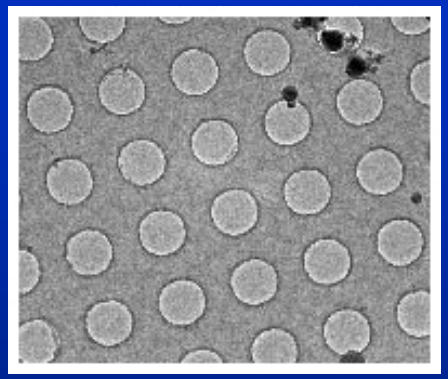
+ quick to make

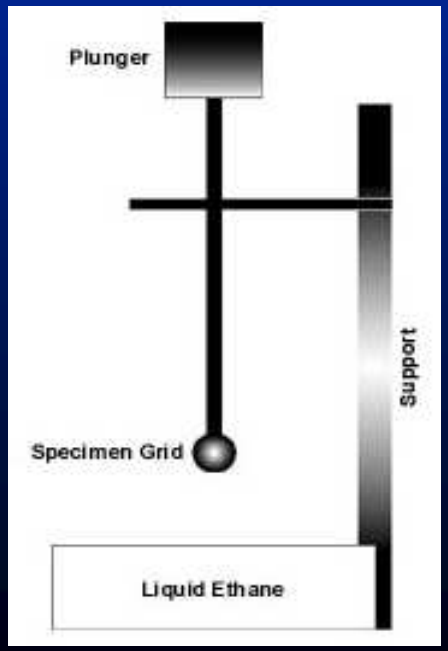
+ high dose-tolerance

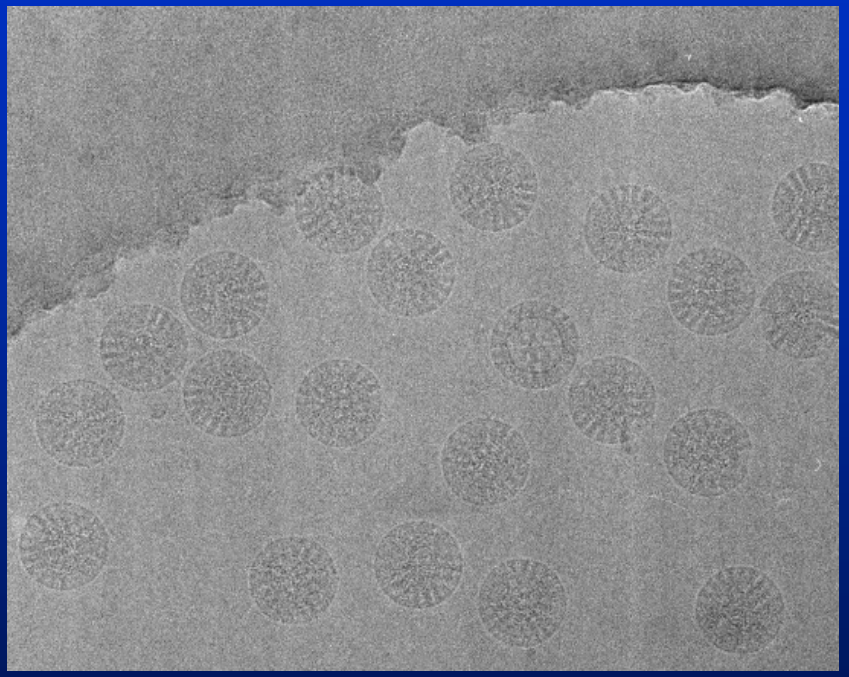
- preferred orientation

- lower resolution (nm)

Specimen Preparation: Cryo-plunging







+ higher resolution (Å)

- low dose-tolerance

- must work under LN_2

Mount Specimen

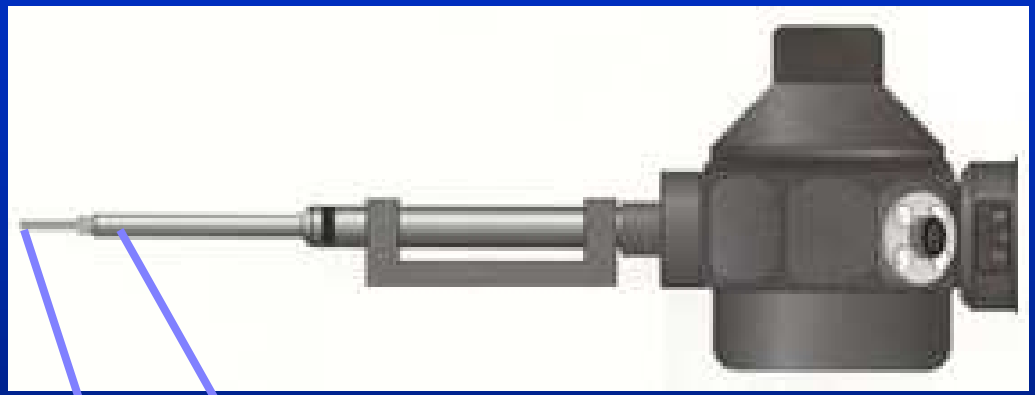
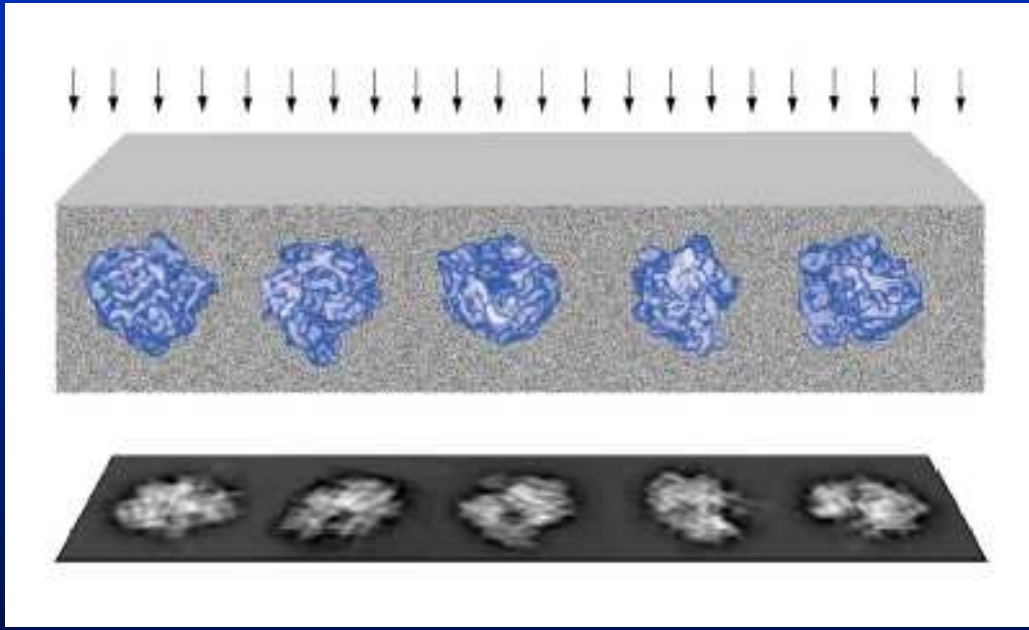
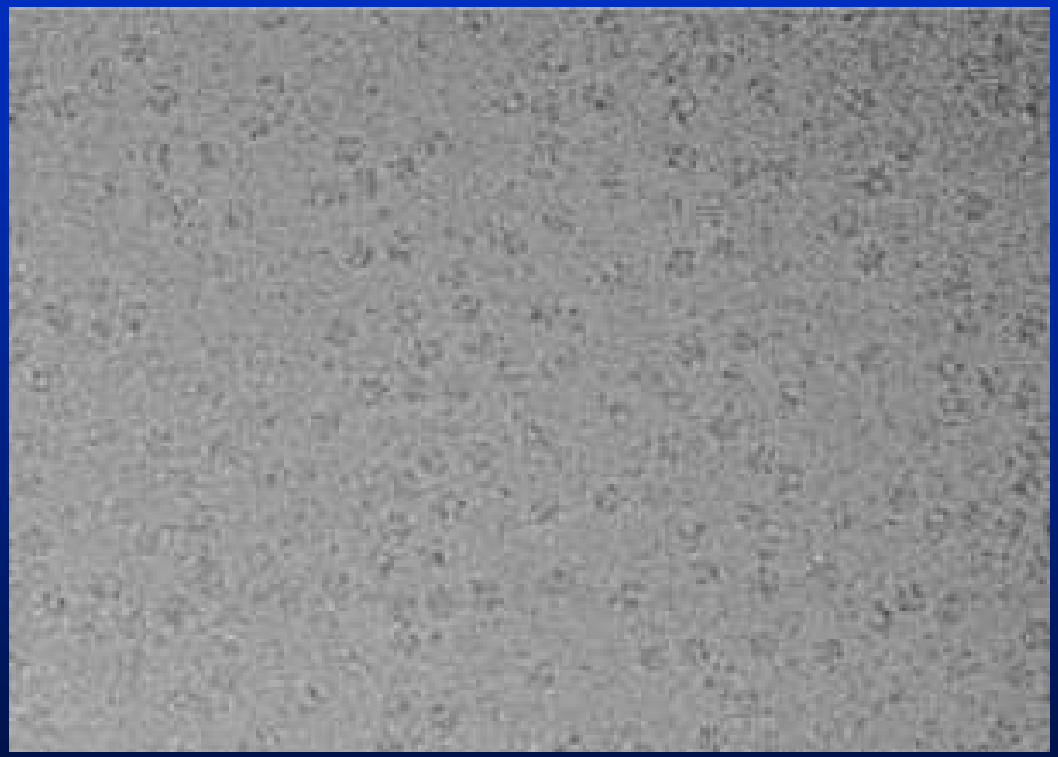


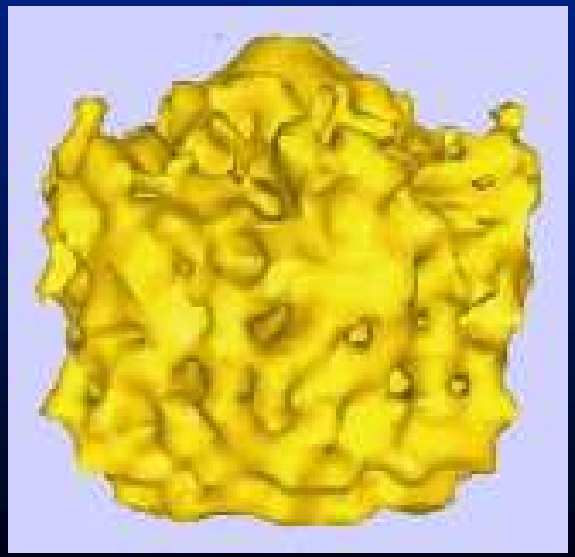
Image Acquisition



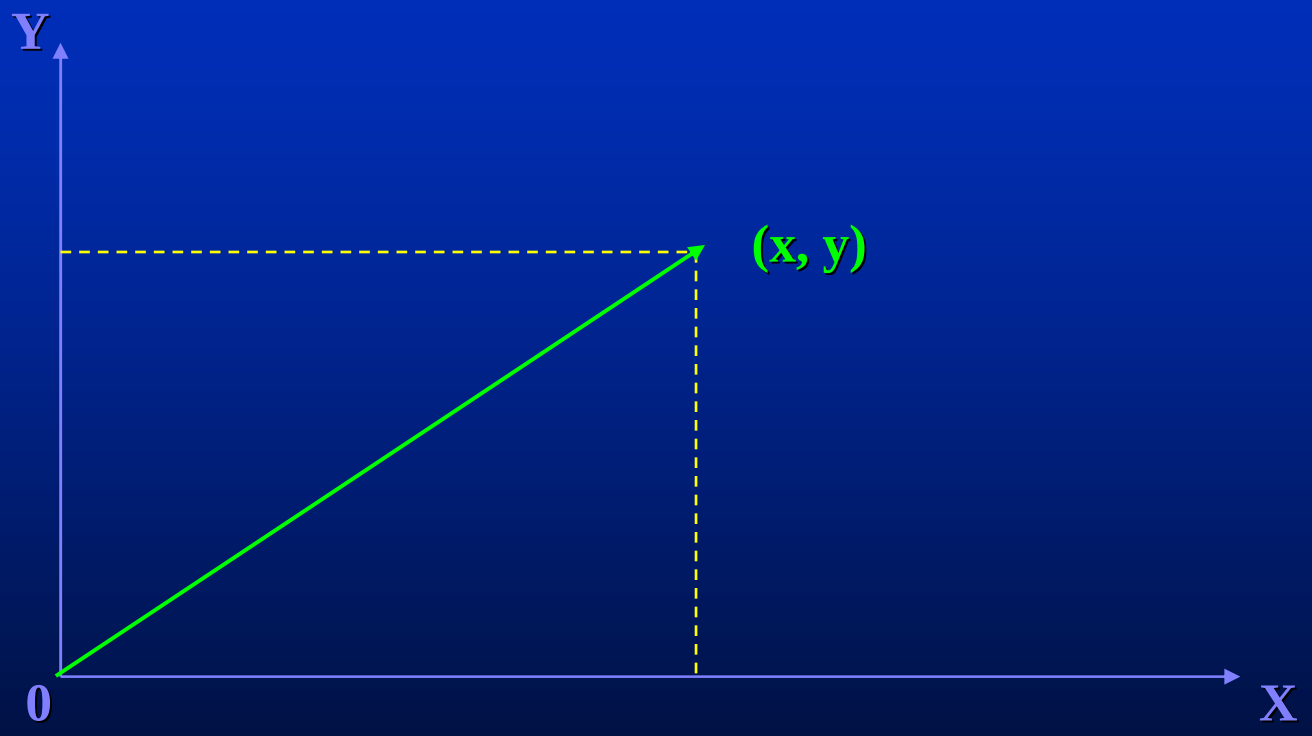
- **emulsion film**
- **CCD**
- **pixel detector**

Data Analysis



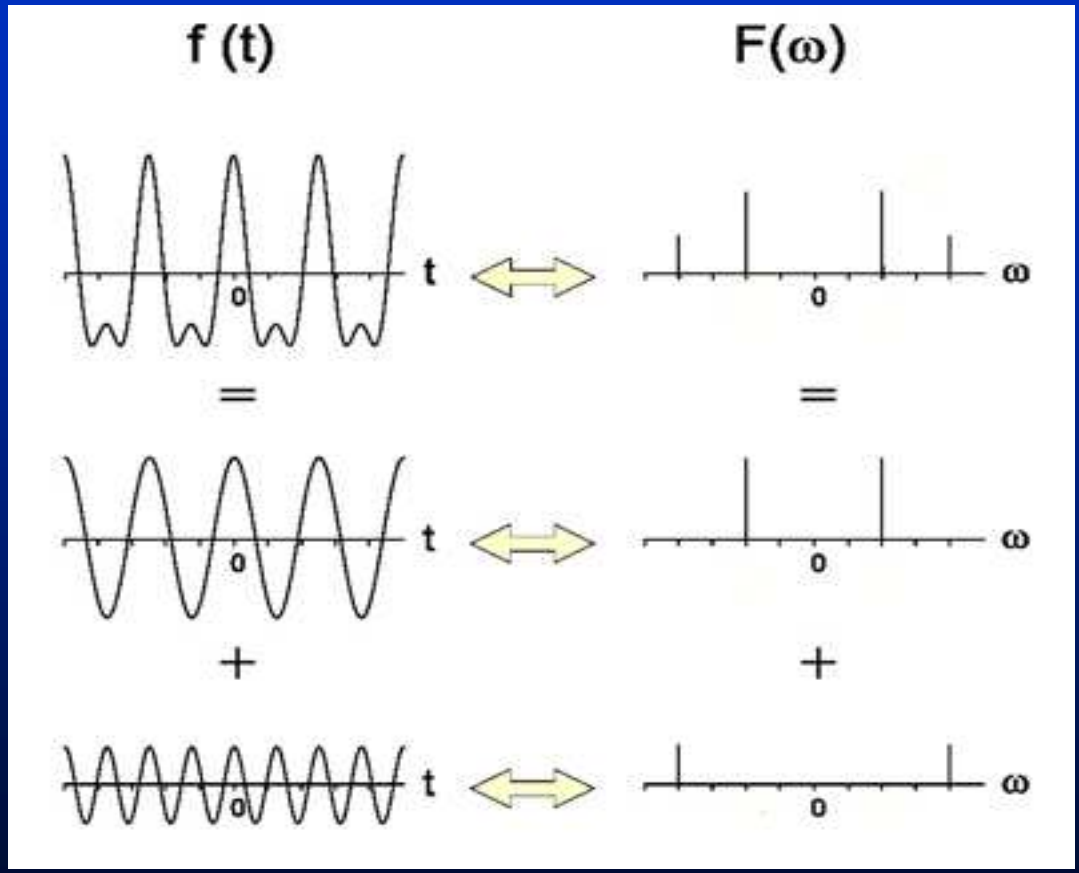


What is Fourier Transform?



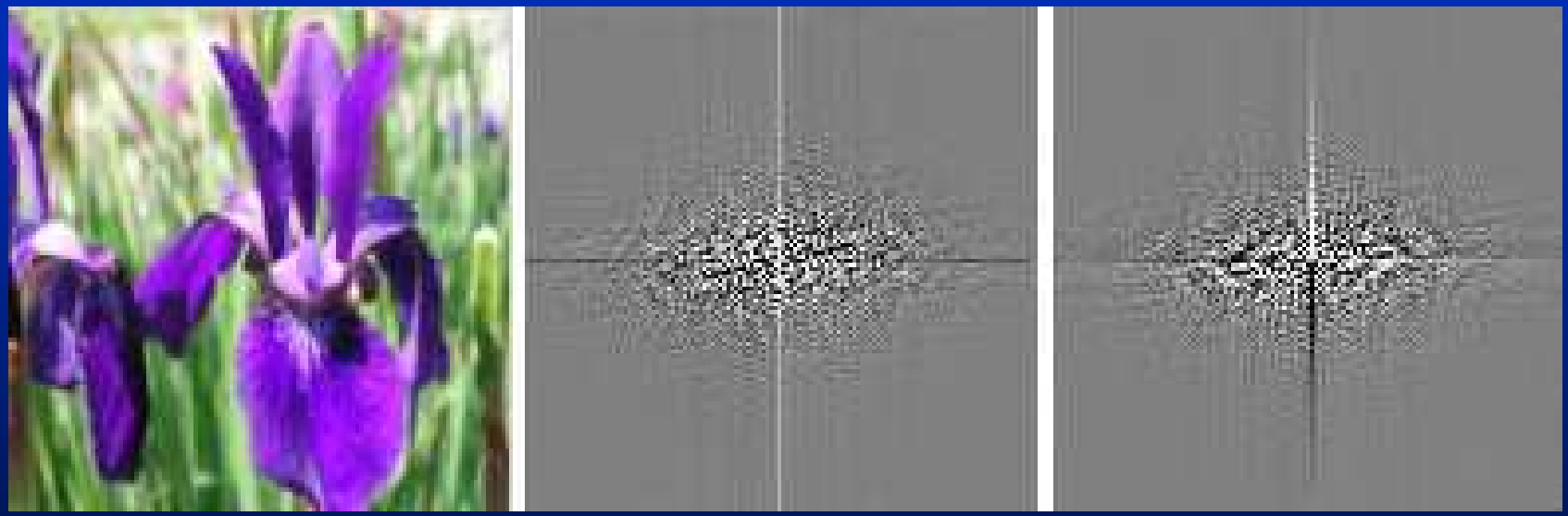
Vector in Cartesian Space

1D Fourier Transform



Real-space **Fourier-space**

2D Fourier Transform



2D Image Fourier Transform