

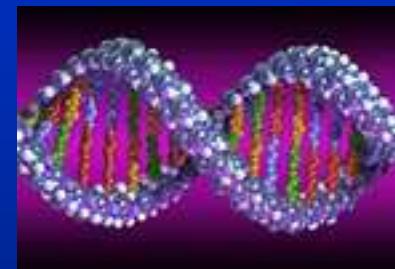
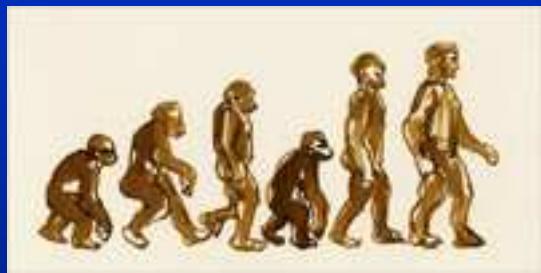
Quantitative Biology Lectures

January 13-14, 2008

# Single-Particle Electron Microscopy

*James Z. Chen*

# *Seeing Is Believing*



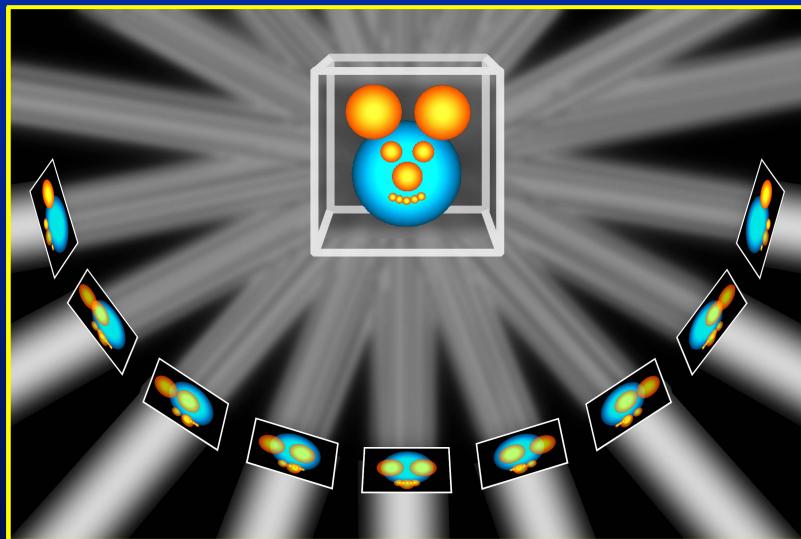
**Direct  
Observation**

**Optical  
Microscope**

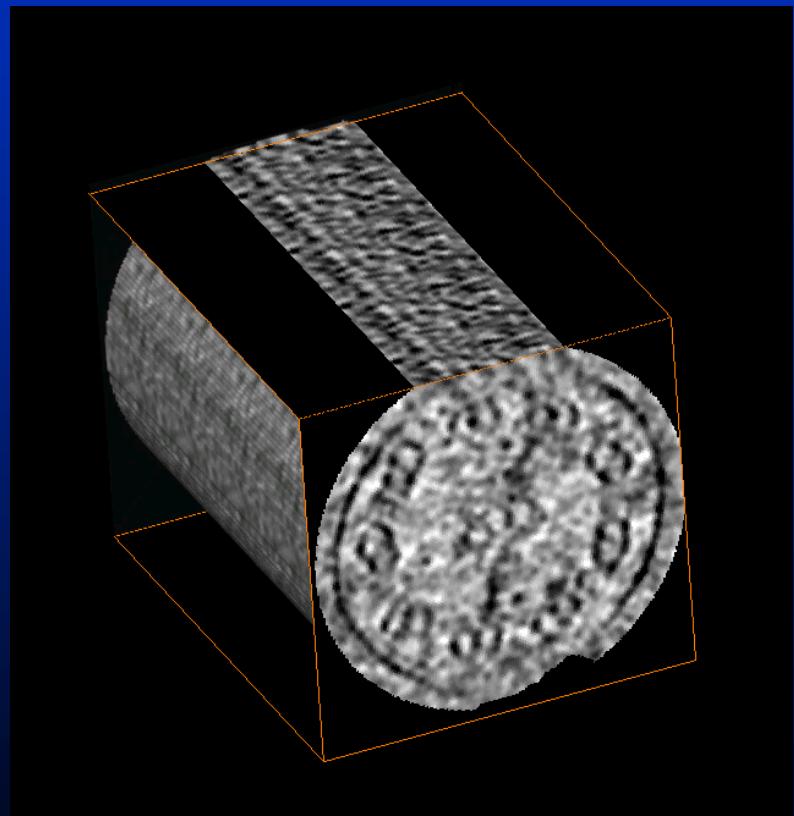
**Electron  
Microscope**

# *Electron Tomography*

**Set of 2D projections  
⇒ 3D reconstruction**

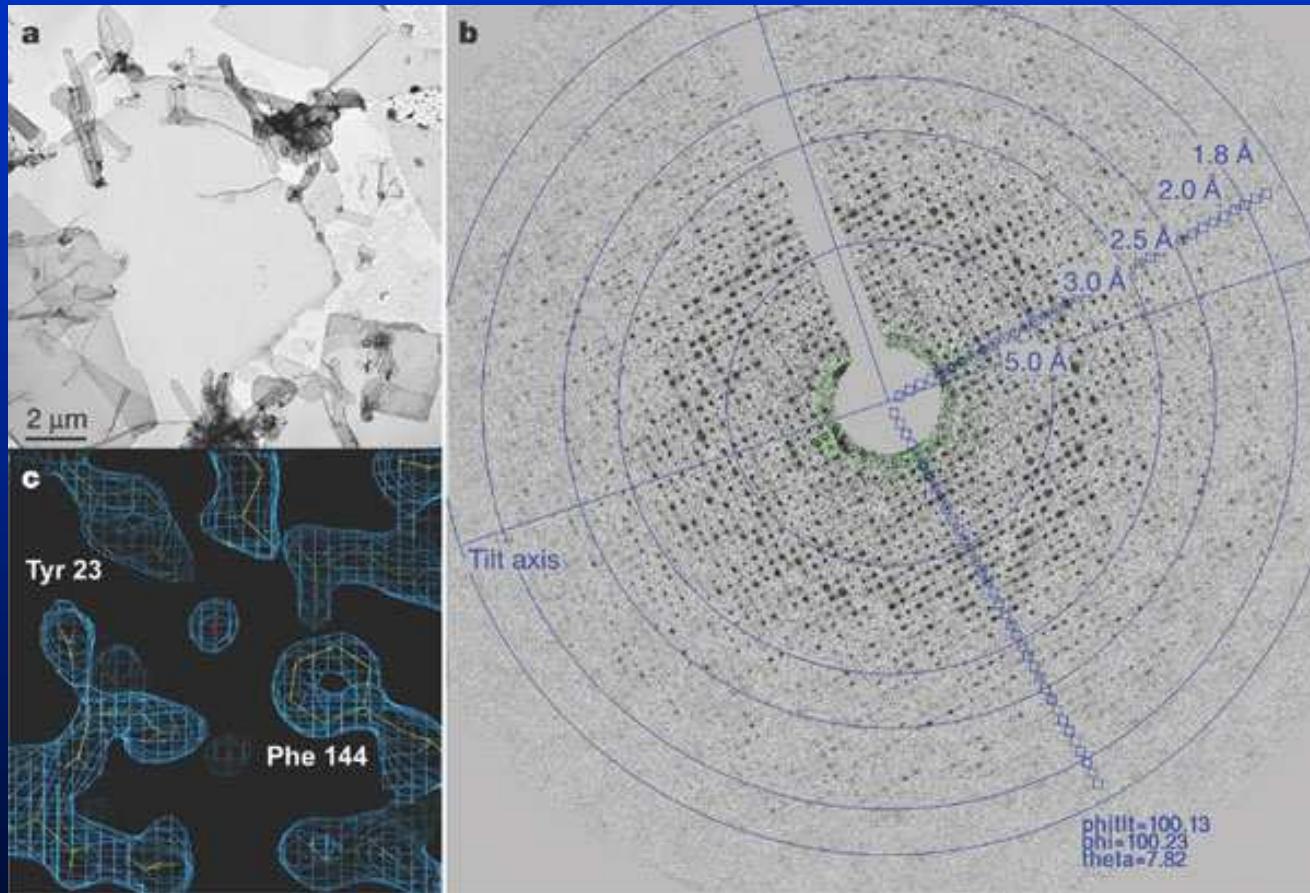


**Eukaryotic Flagella (Axonemes)**



*By courtesy of Dr. Daniela Nicastro*

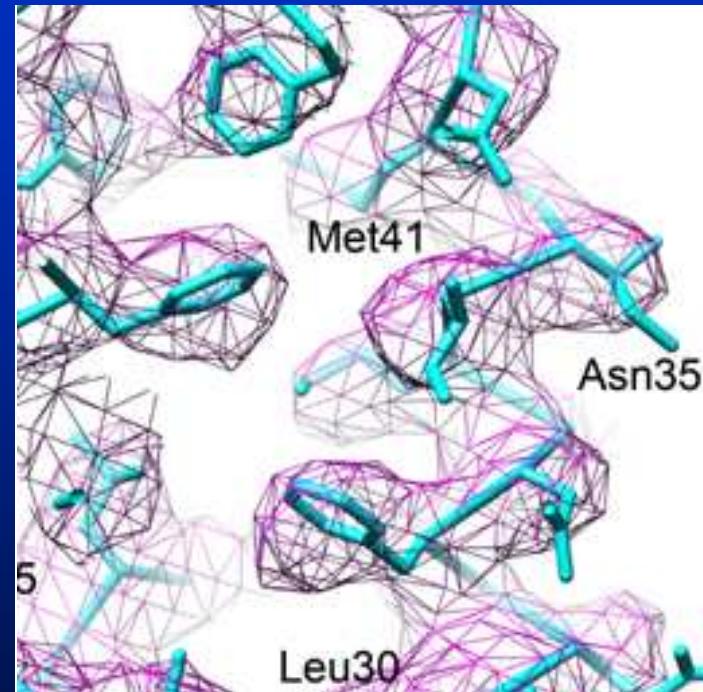
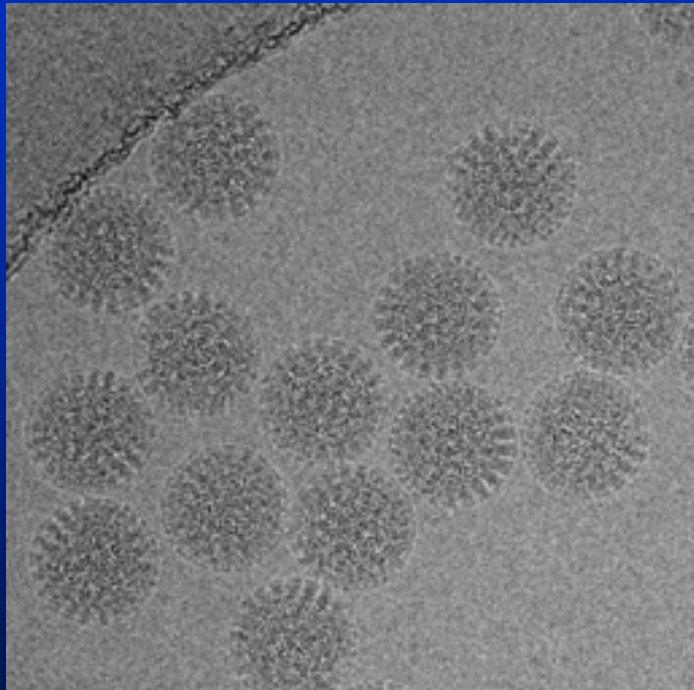
# *Electron Crystallography*



*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

Quantitative Biology Lectures

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# Single-Particle Electron Microscopy

*Instrument and Data Collection*

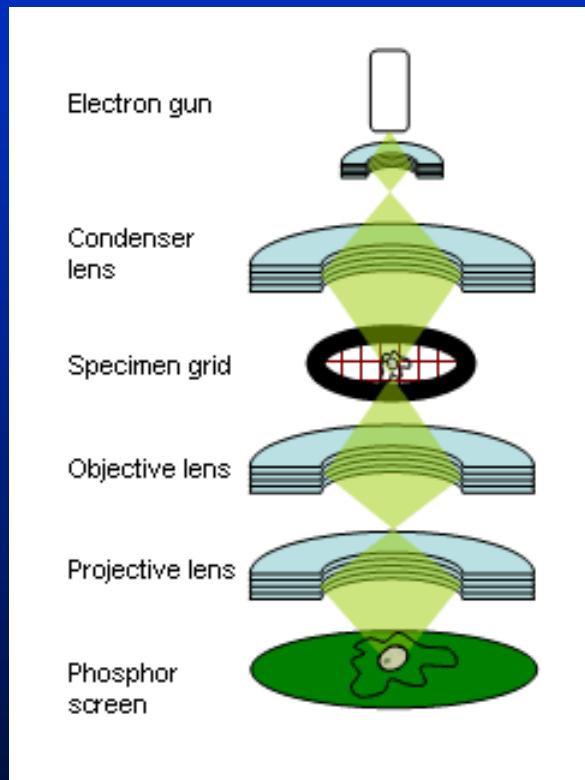
*James Z. Chen*

# *Topics*

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

# *Electron Microscope*



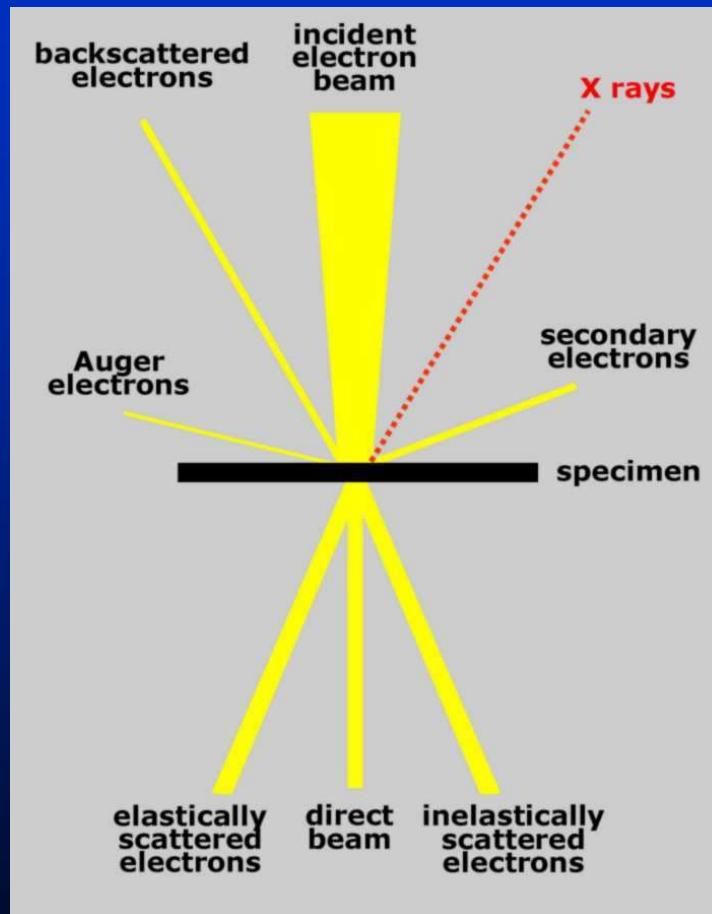
**Transmission Electron Microscopy  
(TEM)**

# *Beam-Specimen Interaction*

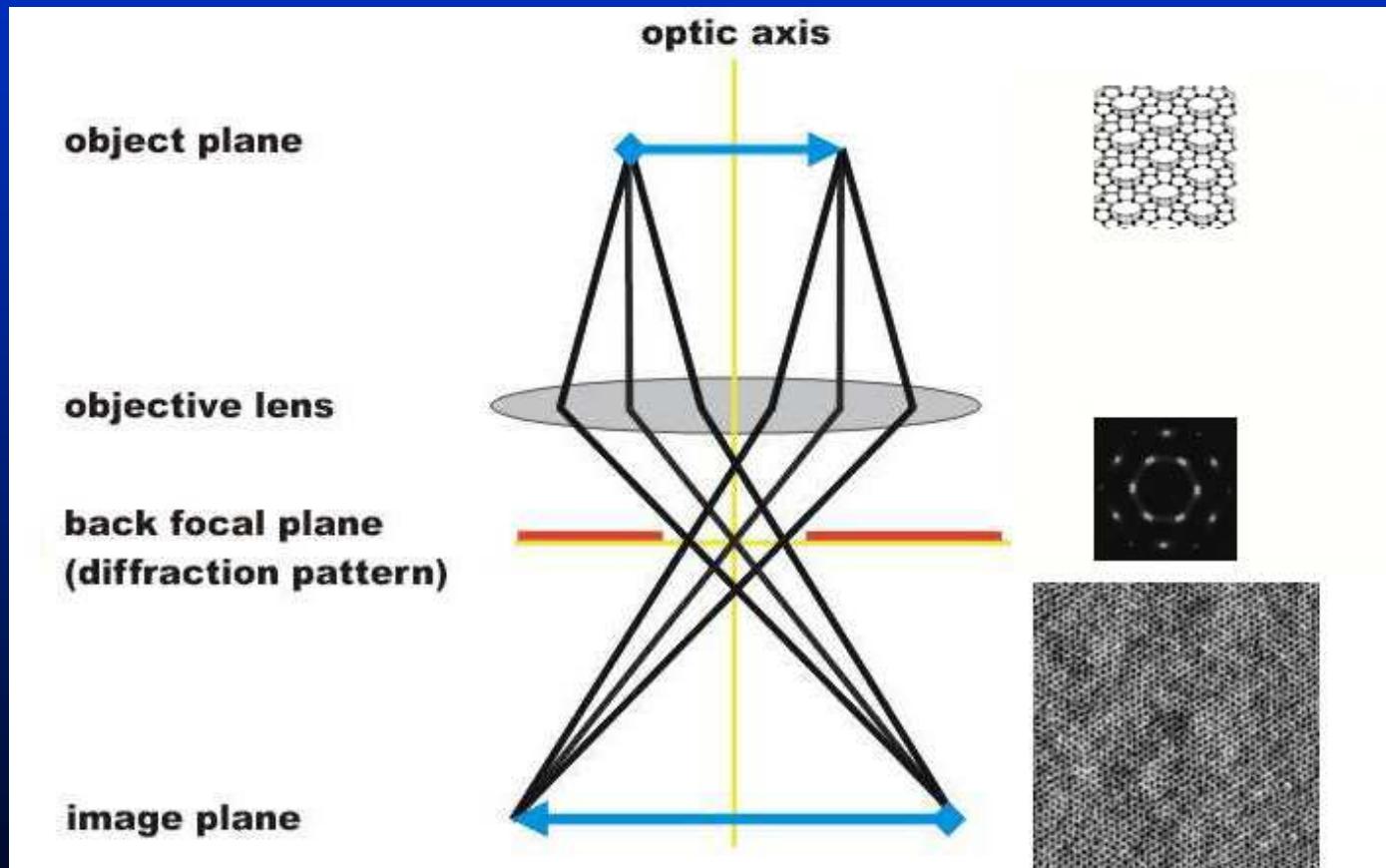
Analytical  
EM

SEM

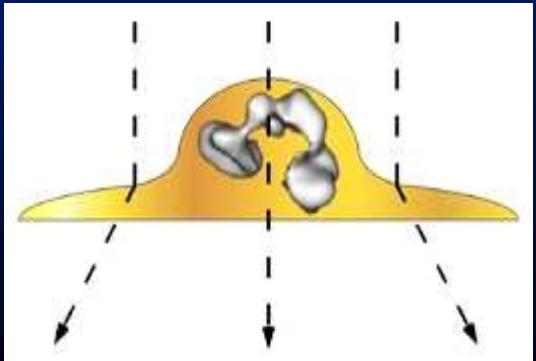
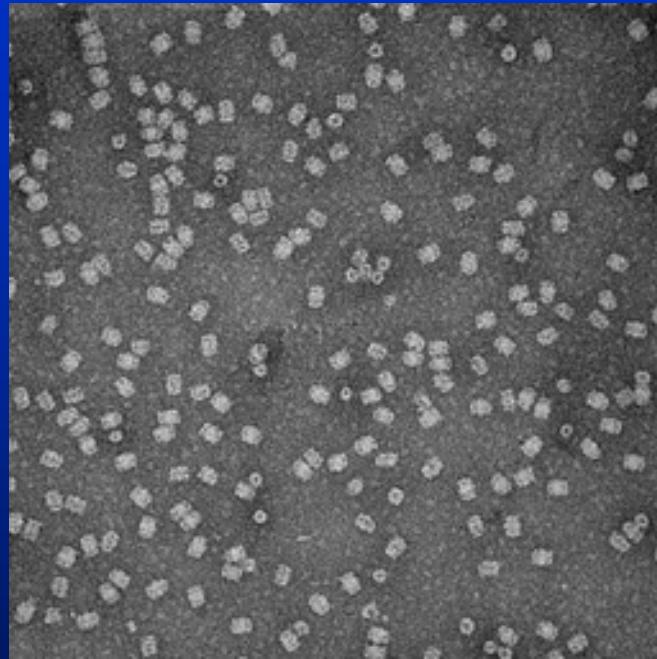
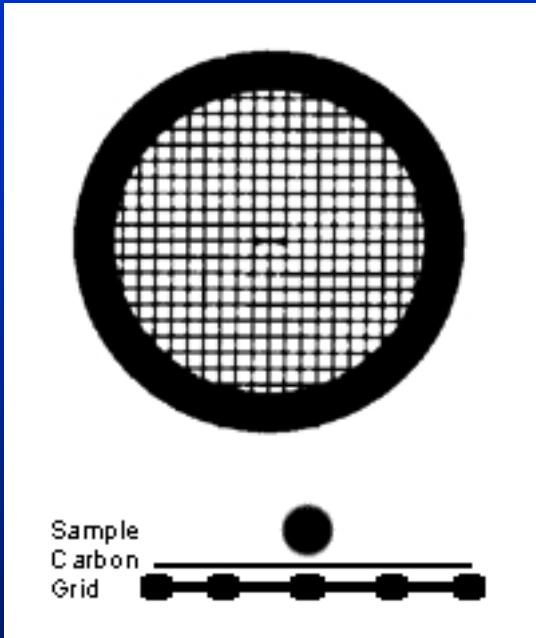
TEM



# *TEM Image Formation*

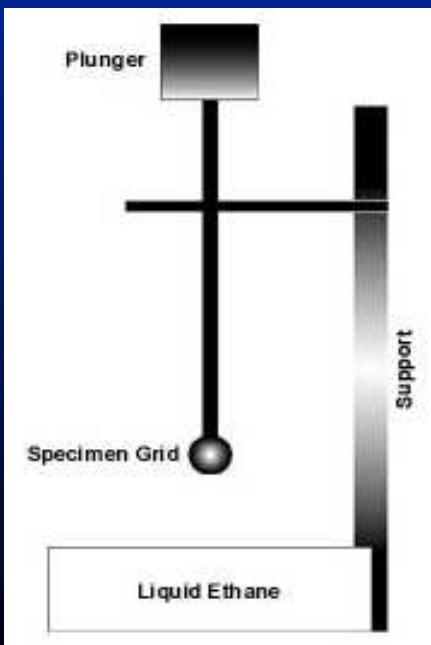
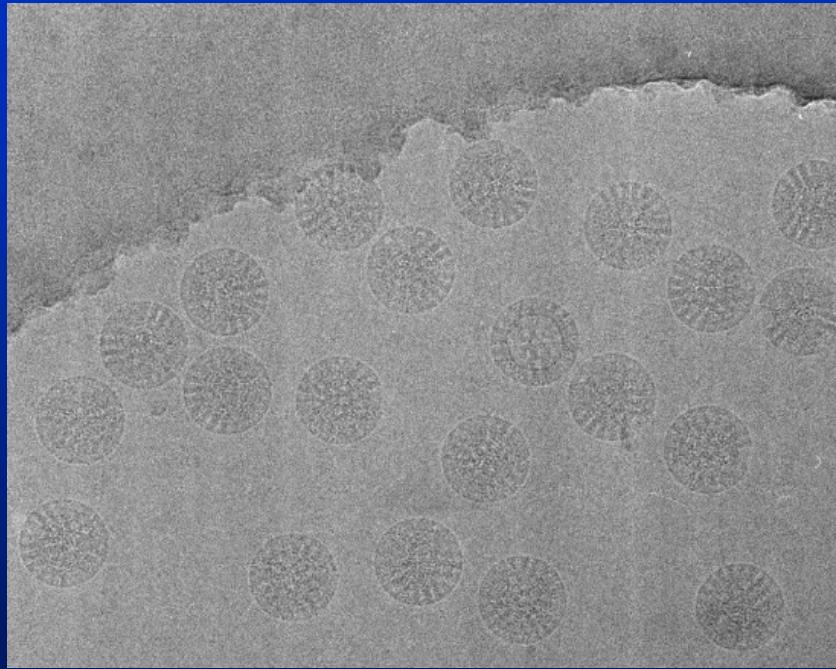
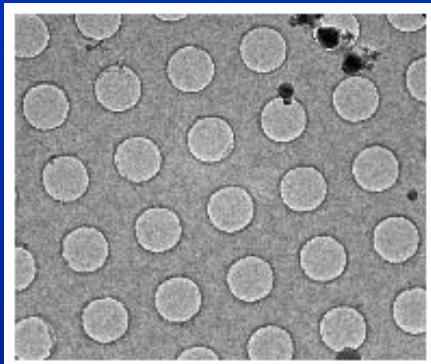


# *Specimen Preparation:* Negative Staining



- + quick to make
- + high dose-tolerance
- preferred orientation
- lower resolution (nm)

# *Specimen Preparation:* Cryo-plunging

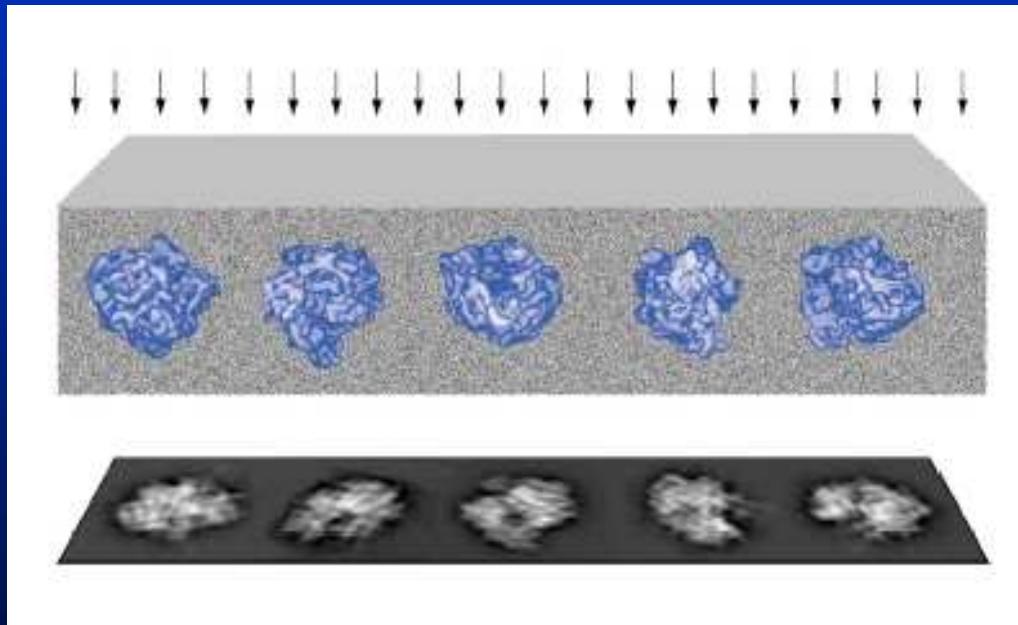


- + higher resolution ( $\text{\AA}$ )
- low dose-tolerance
- must work under  $\text{LN}_2$

# *Mount Specimen*



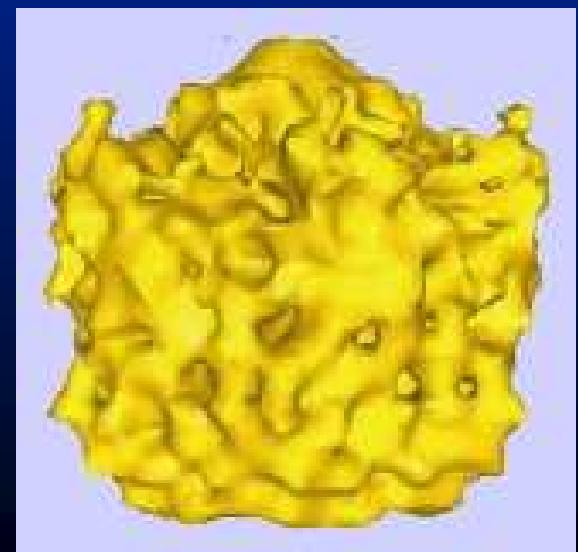
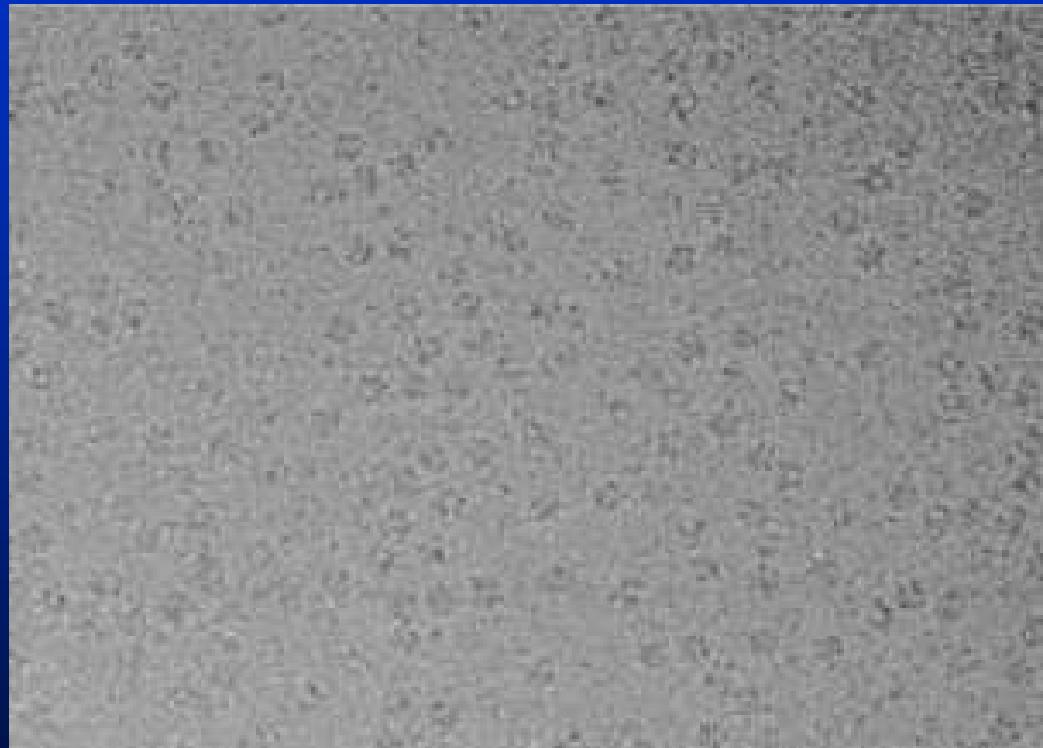
# *Image Acquisition*



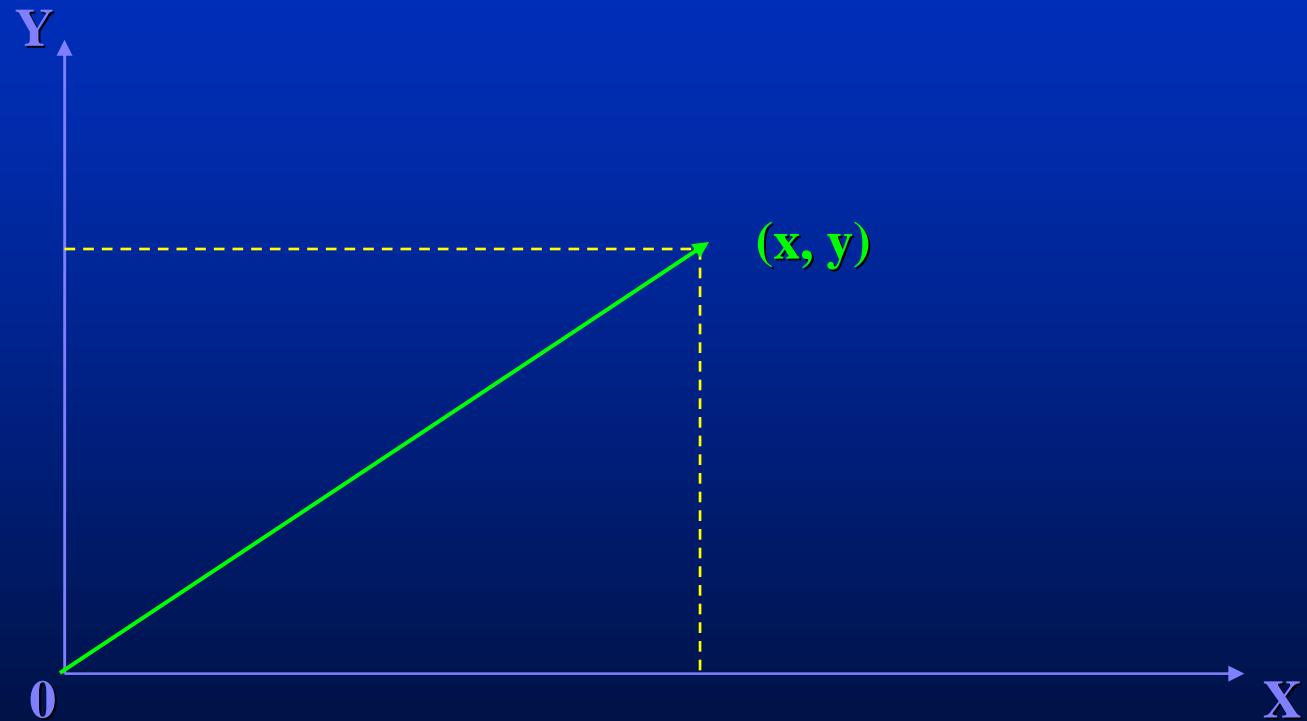
- emulsion film
- CCD
- pixel detector

# *Data Analysis*

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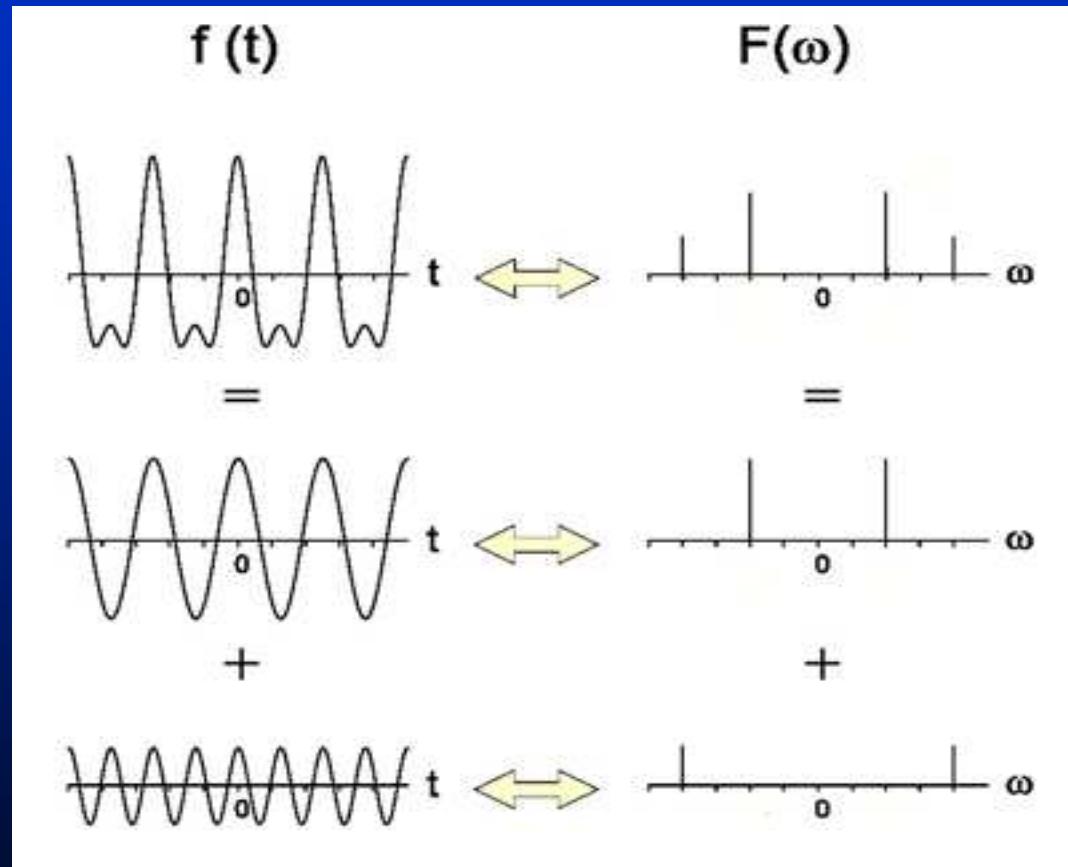


# *What is Fourier Transform?*



**Vector in Cartesian Space**

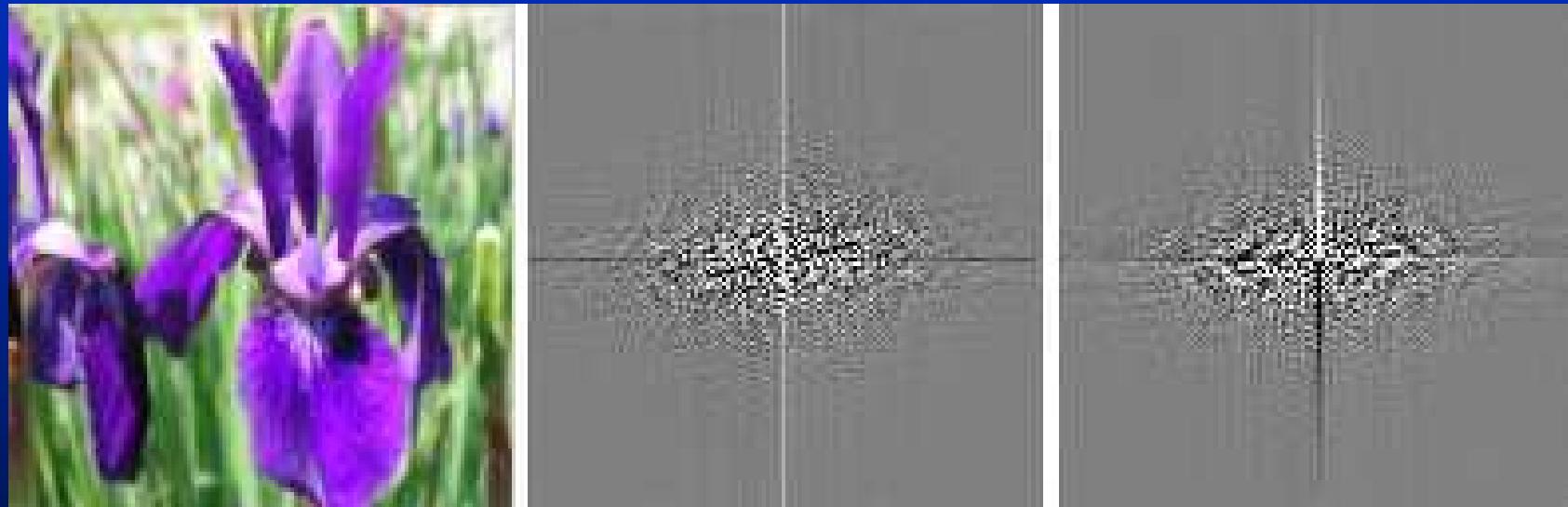
# *1D Fourier Transform*



Real-space

Fourier-space

# *2D Fourier Transform*



**2D Image Fourier Transform**