

Quantitative Biology Lectures

January 13-14, 2008

# Single-Particle Electron Microscopy

*Data Analysis and Model Reconstruction*

*James Z. Chen*

# *Topics*

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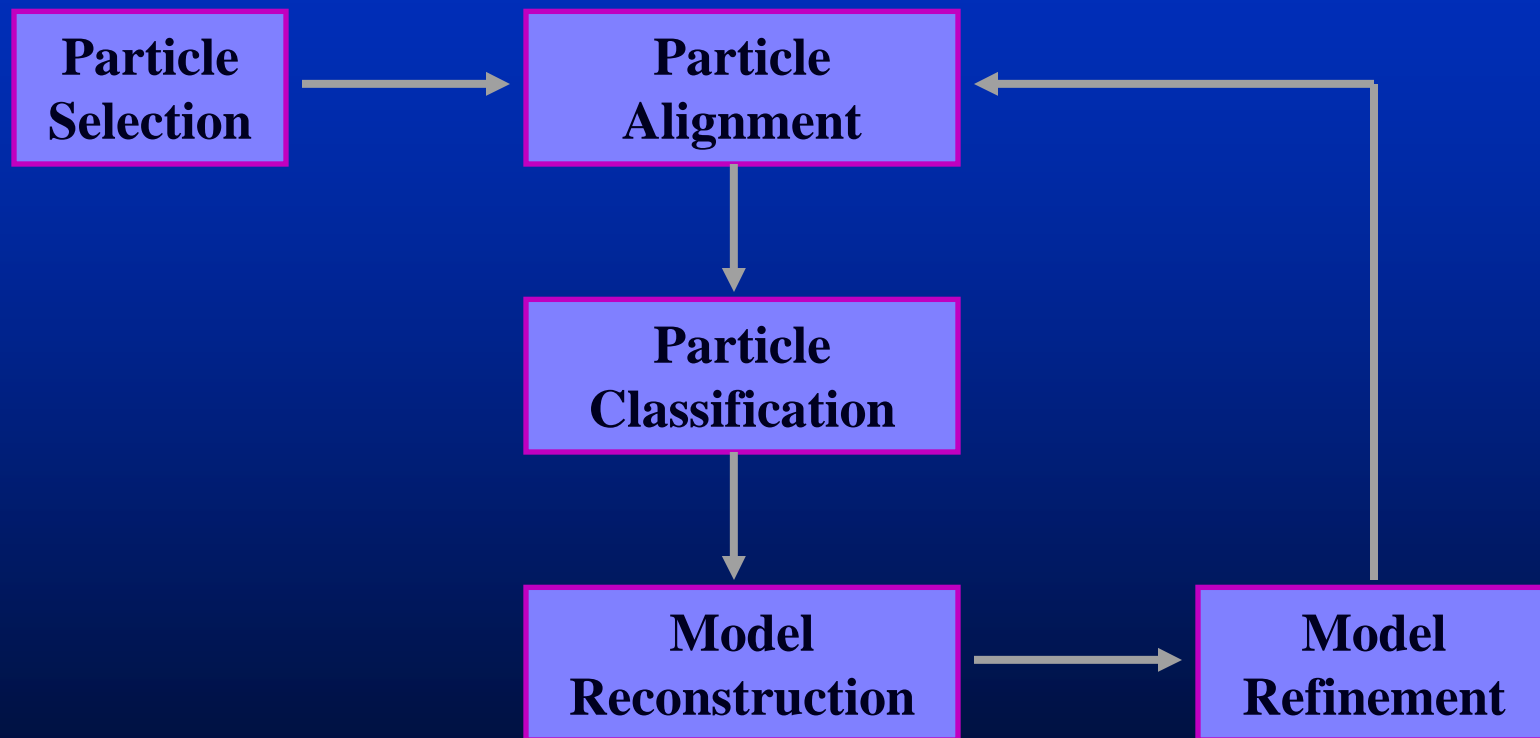
## **Common-Line Method**

- **particle selection**
- **particle alignment & classification**
- **model reconstruction & refinement**

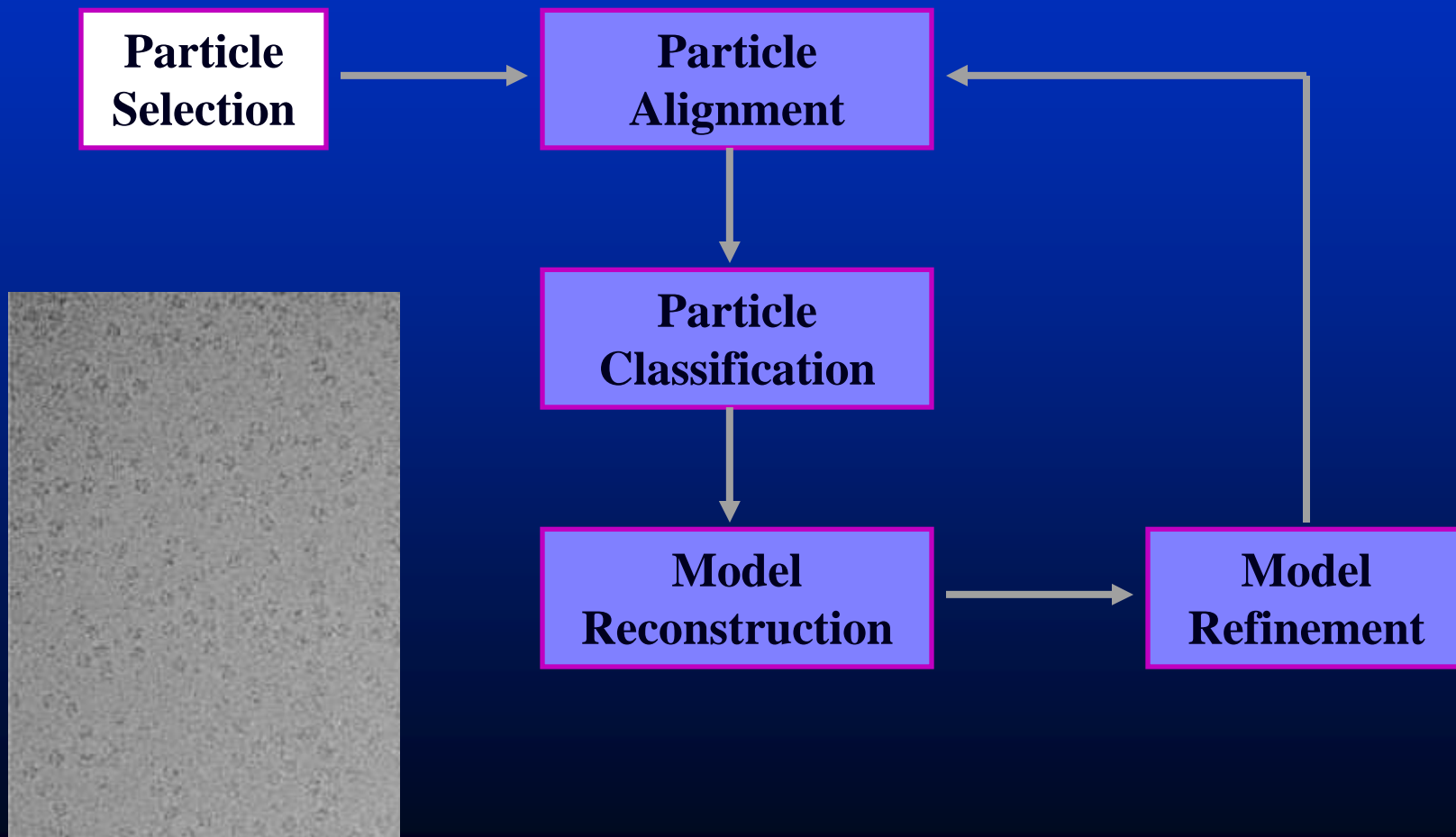
## **Random Conical Tilt**

- **tilt-pair data collection**
- **direct reconstruction**

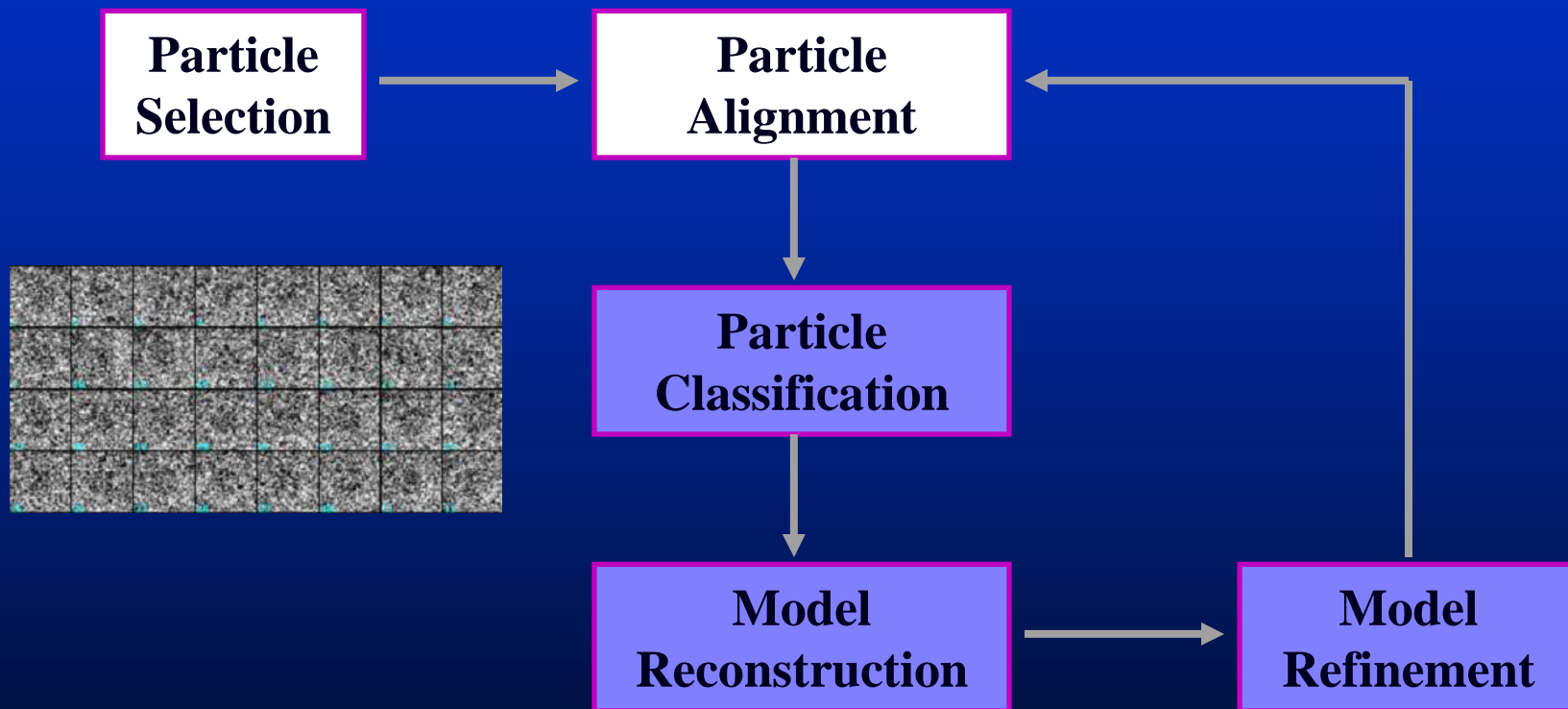
# *Data Processing Pipeline*



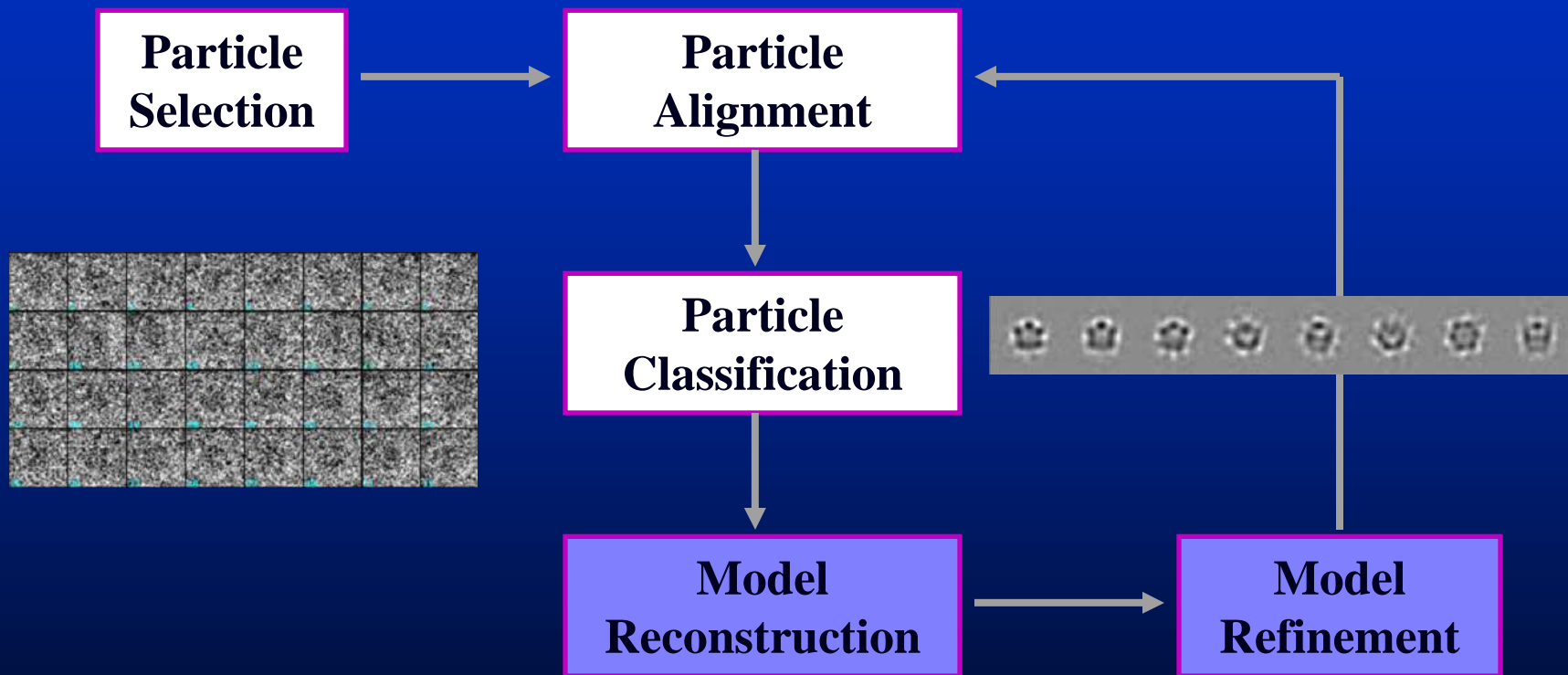
# *Data Processing Pipeline*



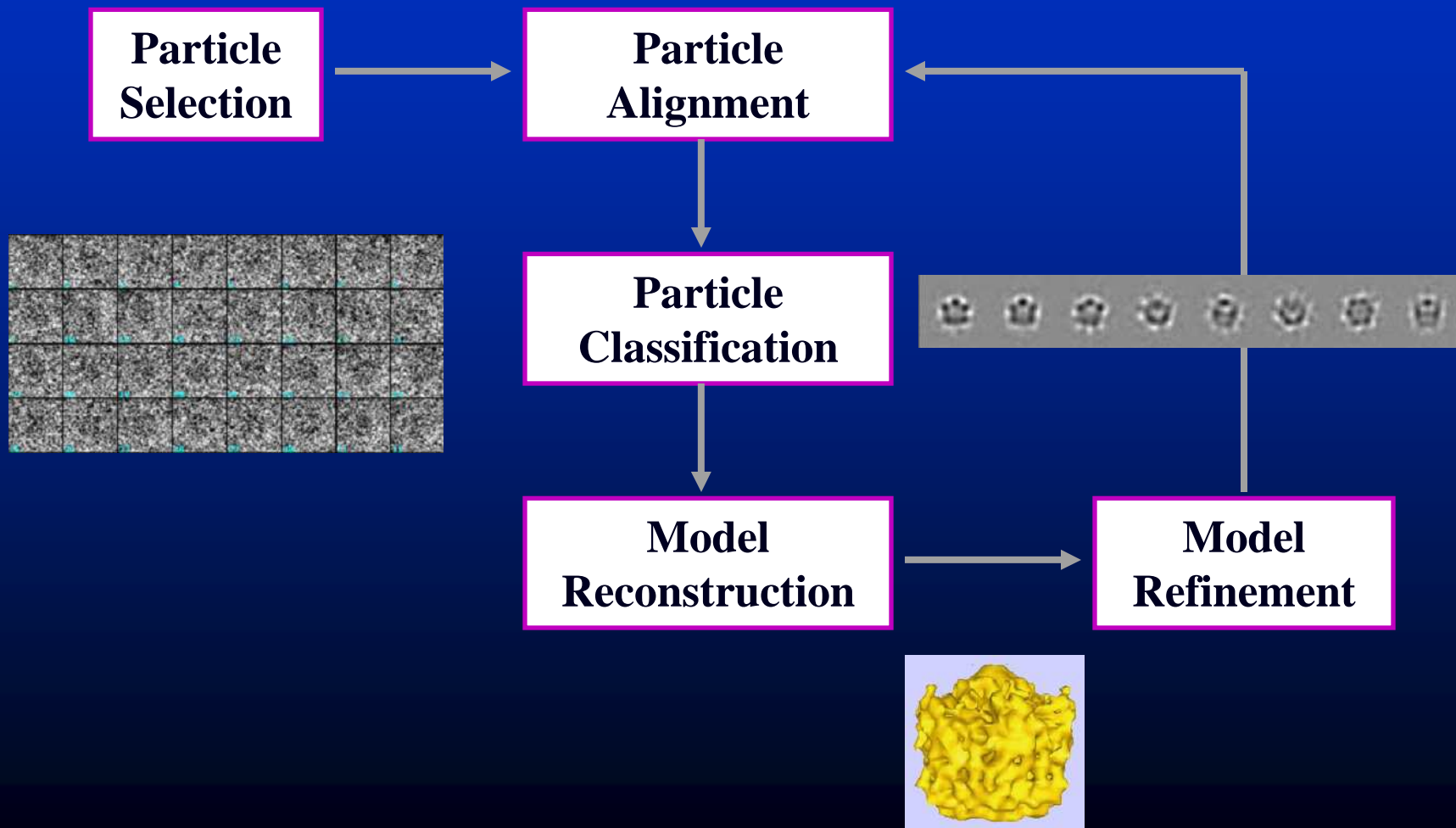
# *Data Processing Pipeline*



# *Data Processing Pipeline*



# *Data Processing Pipeline*



# *Particle Selection*

- **manual annotation**
- **template matching**
- **computer vision**
- **neural network**
- **and more ...**



# Template Matching

## Local Correlation Function (LCF) for image intensity matching

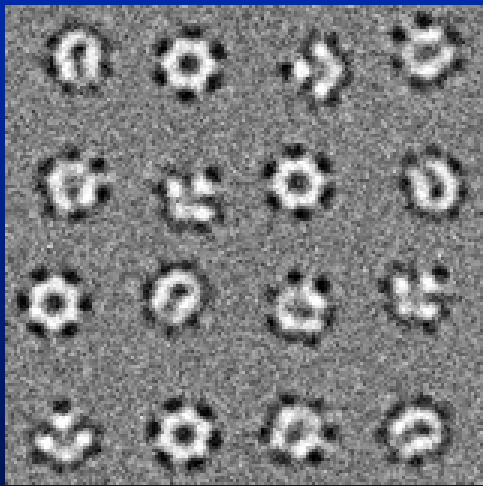
$$LCF(x) = \frac{1}{N_T \sigma(I_x)} \langle M_T \otimes T, I \rangle_x$$

## Spectra Correlation Function (SCF) for overall shape matching

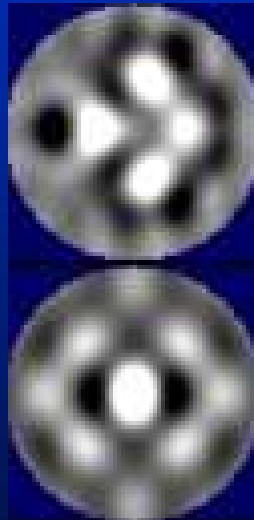
$$SCF(x) = \frac{1}{N_S \sigma(LCF_x)} \langle M_S \otimes ACF, LCF \rangle_x$$

$I$  is a template image,  $M_T$  and  $M_S$  are template masks,  $N_T$  and  $N_S$  are pixels under the respective masks.  $ACF$  is the auto-correlation function of  $I$ , and  $\sigma(\ )$  is the square-root of variance.

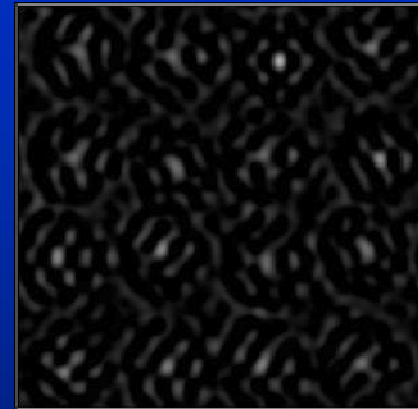
# *LCF & SCF Functions*



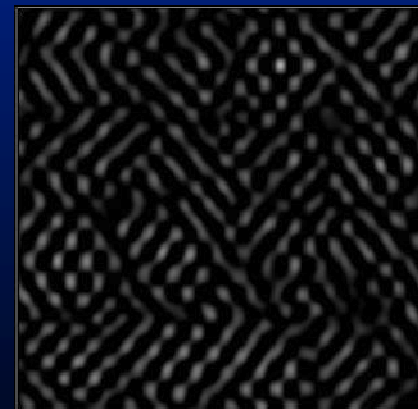
**Micrograph**



**Template**



**LCF**

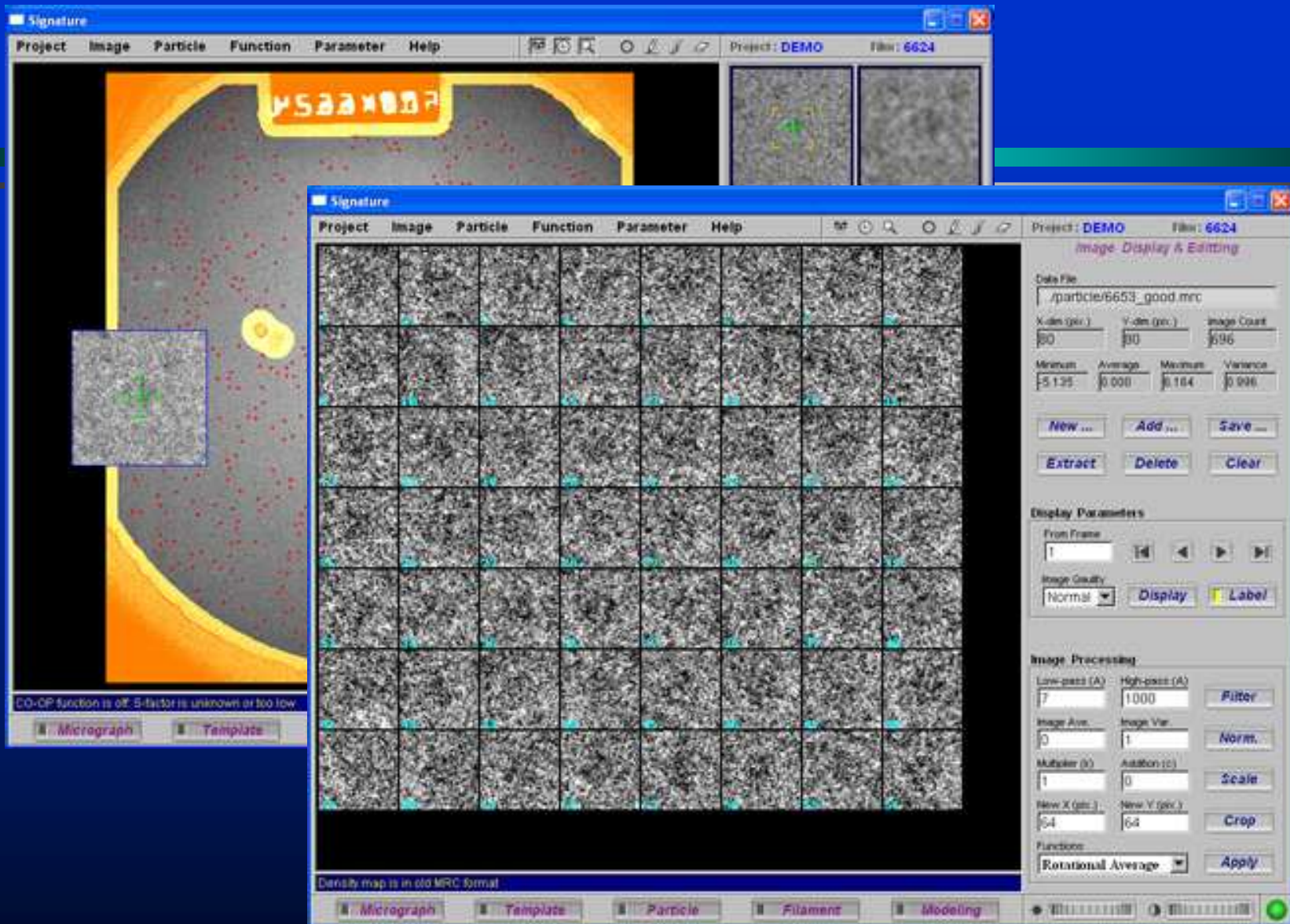


**SCF**

# *Sourceless of Templates*

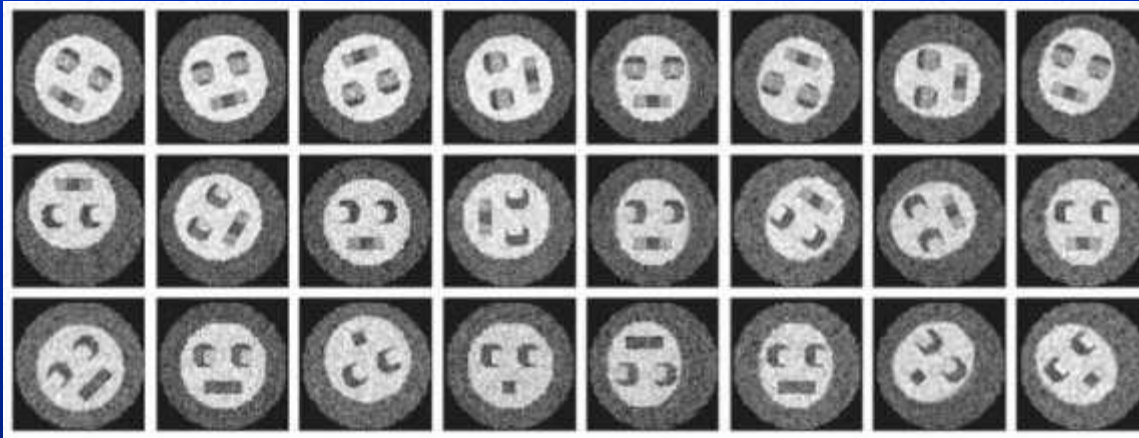
Depending on the stage of development, template images may come from

- ✓ Particle images cropped directly from a micrograph
- ✓ Class averages from a sizable particle dataset
- ✓ 2D projections from a 3D density model



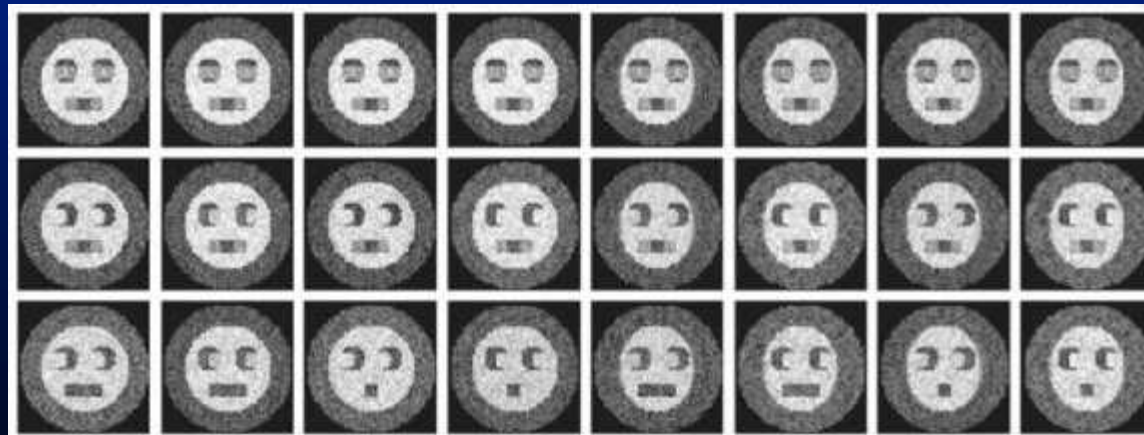
*[emlab.rose2.brandeis.edu/grigorieff/downloads/signature](http://emlab.rose2.brandeis.edu/grigorieff/downloads/signature)*

# Particle Alignment



## 2D Alignment

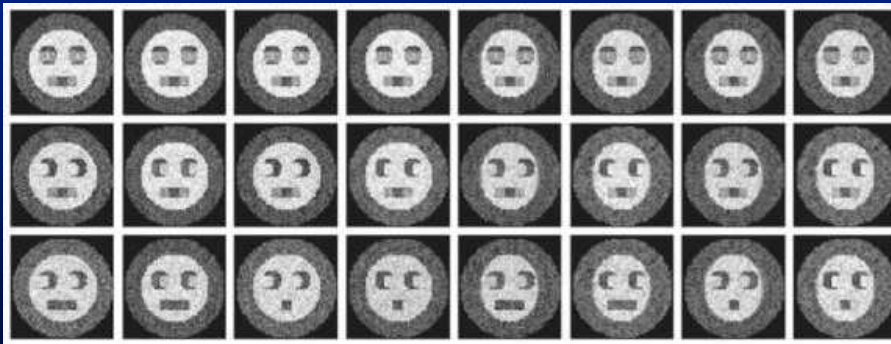
- Translation
- Rotation



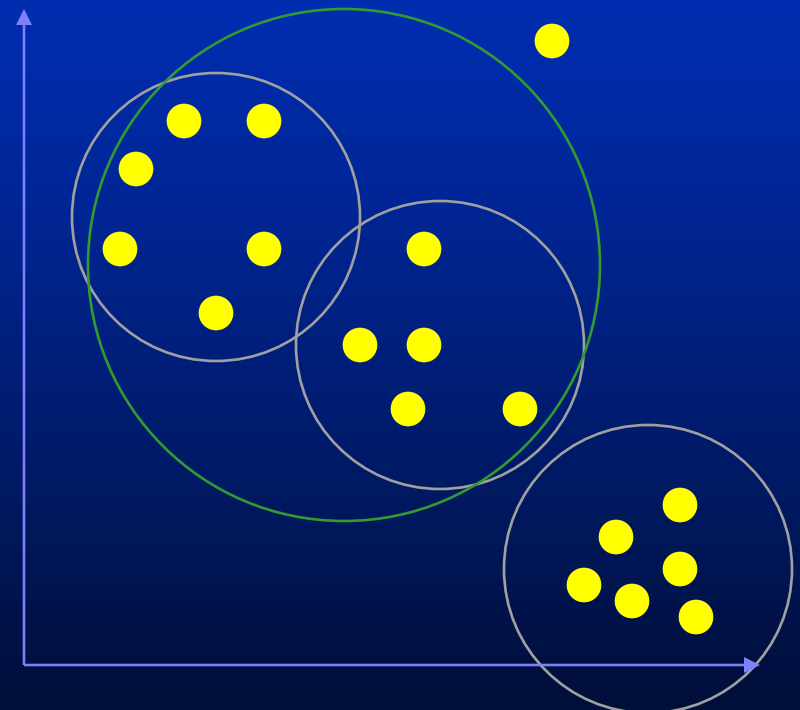
# Particle Classification

**Goal: improve SNR by class averaging**

- **PCA to identify eigen images (modes)**
- **decompose images in the eigen-space**
- **find point clusters  $\Rightarrow$  classes**

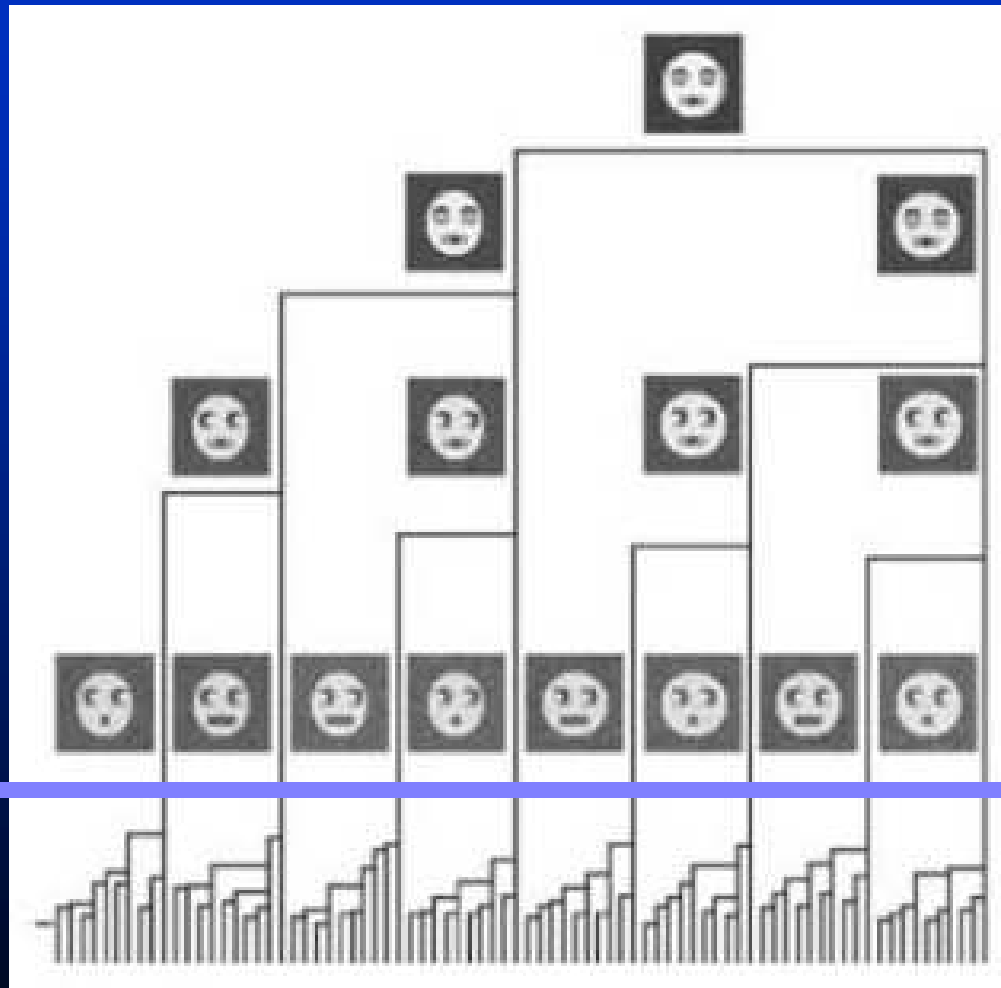


Feature 2

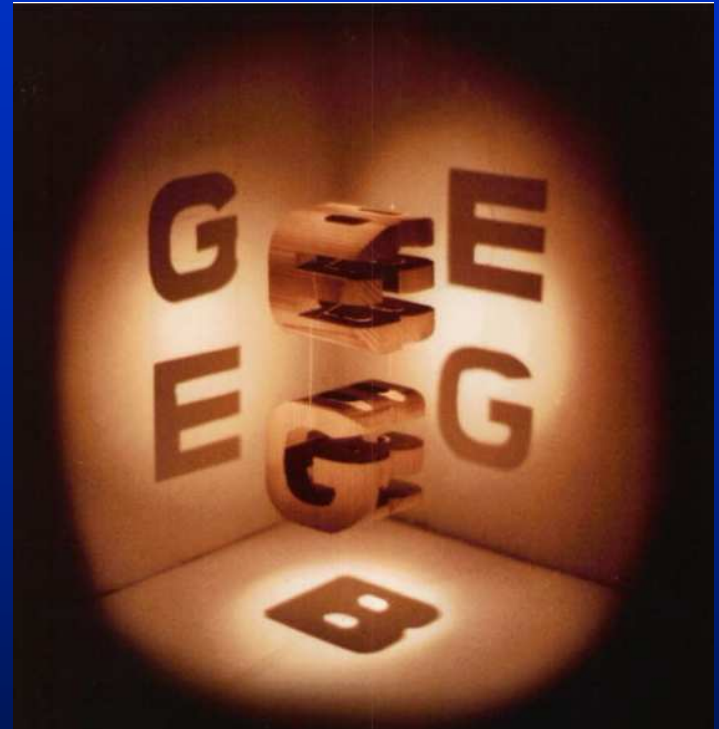
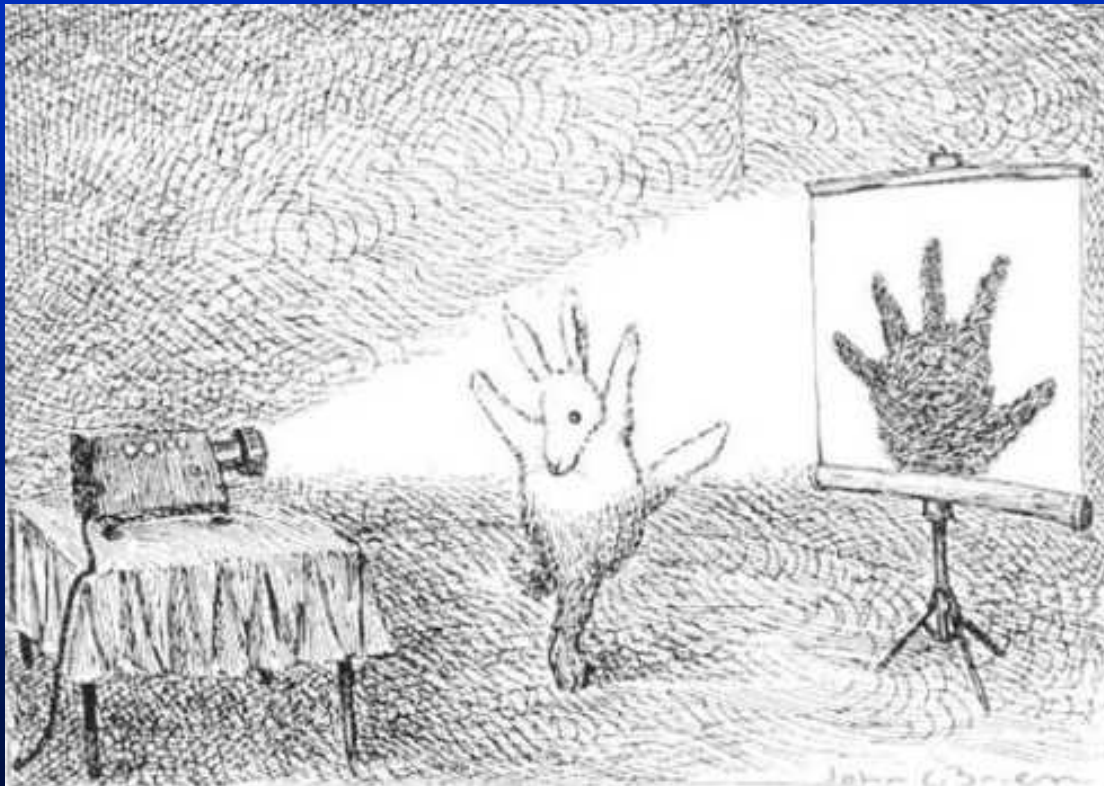


Feature 1

# *Particle Classification*



# *Model Reconstruction*



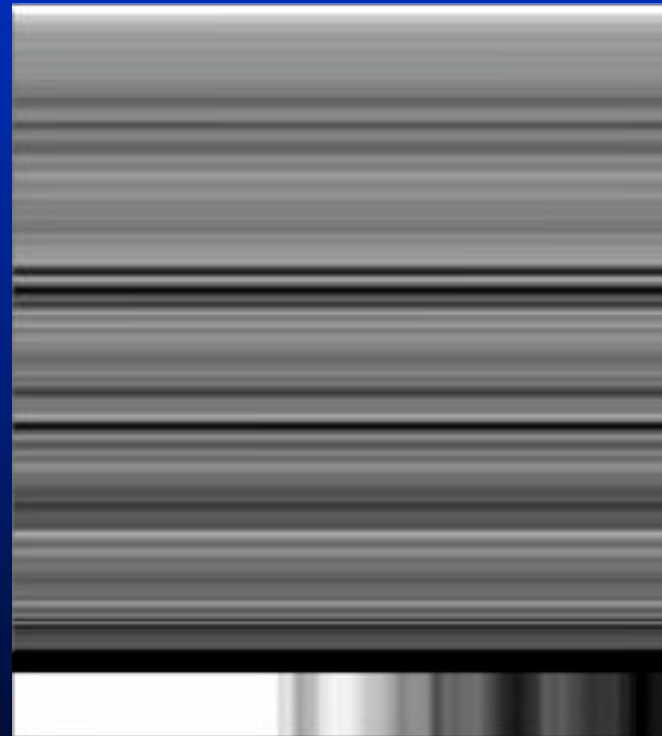
GÖDEL, ESCHER, BACH:

||||| *an Eternal Golden Braid* |||||

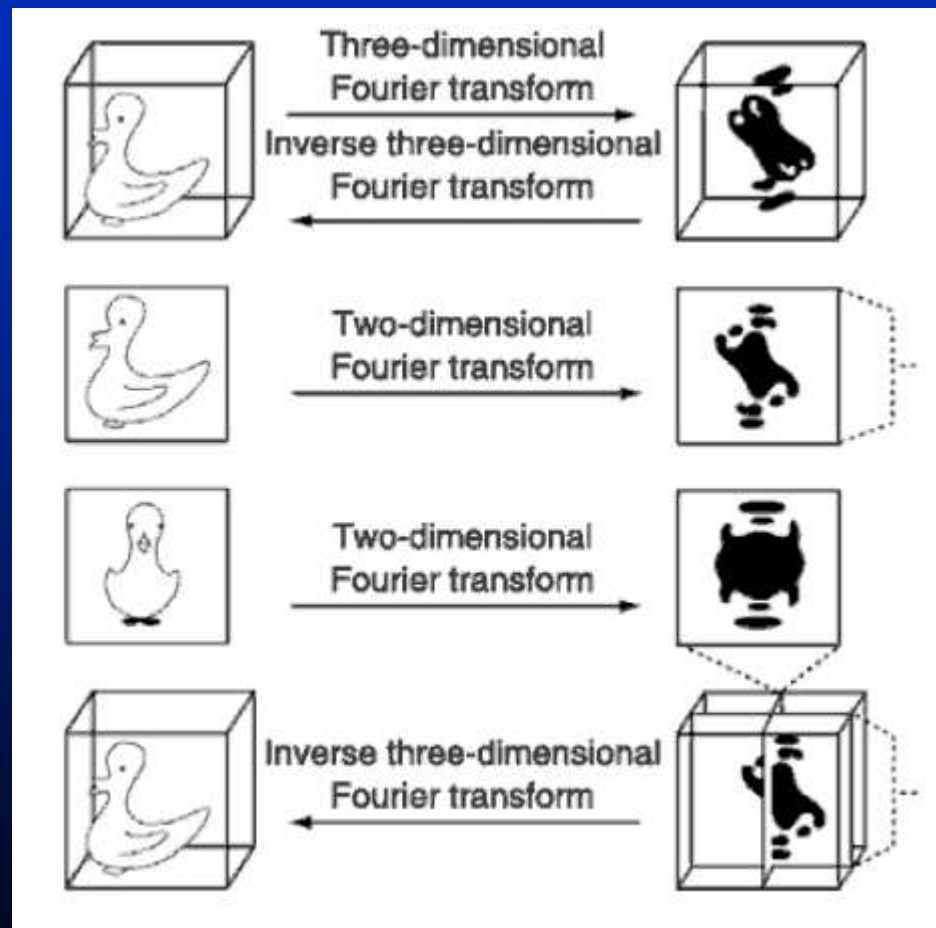
DOUGLAS R. HOFSTADTER



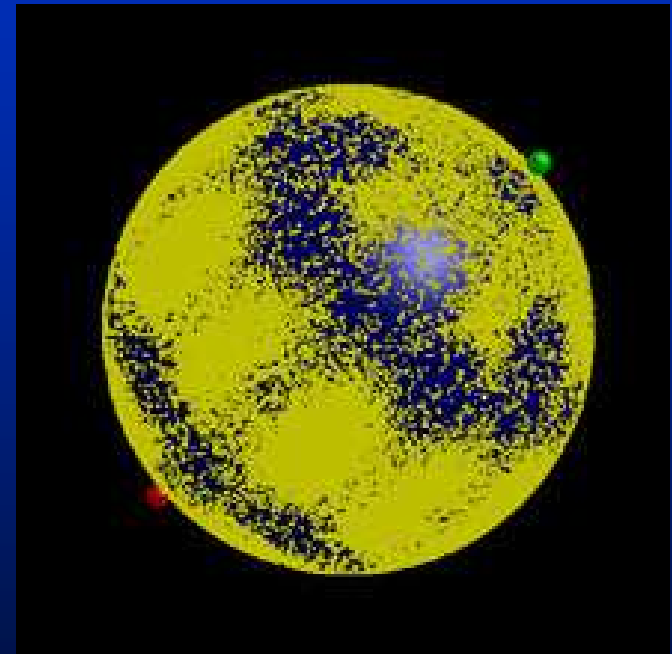
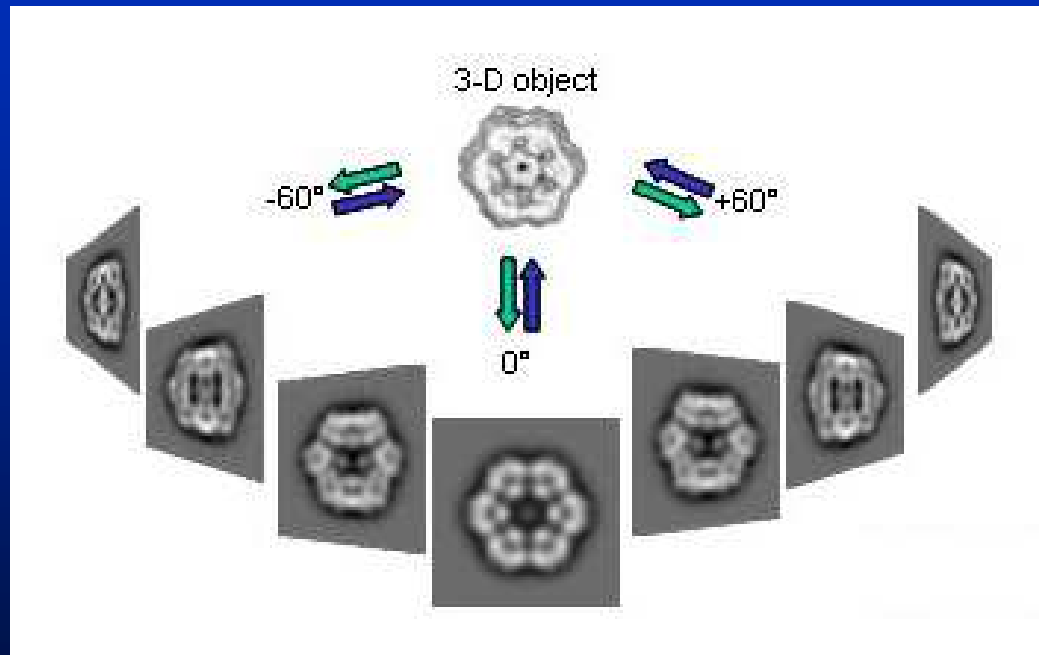
# *Back Projection*



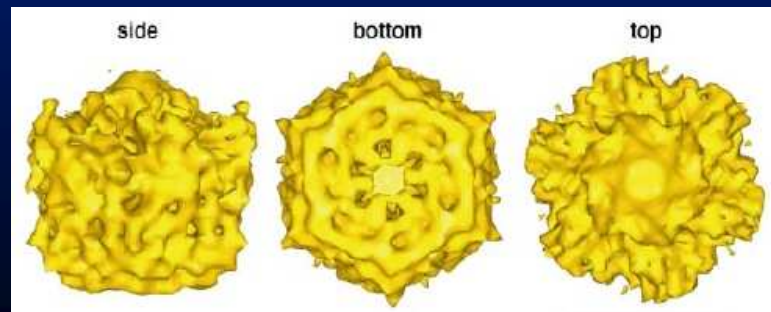
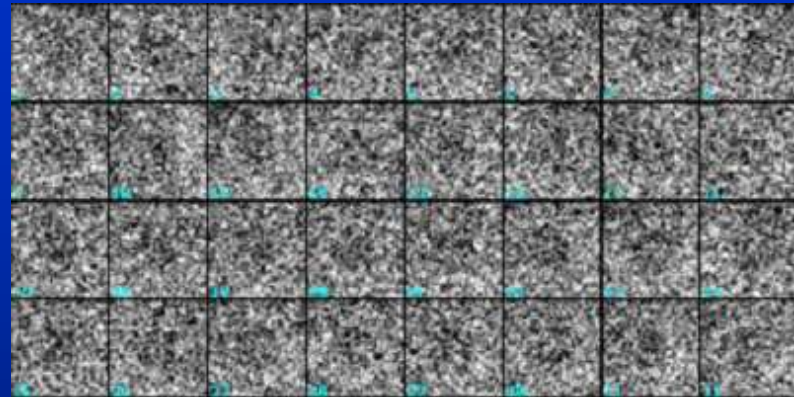
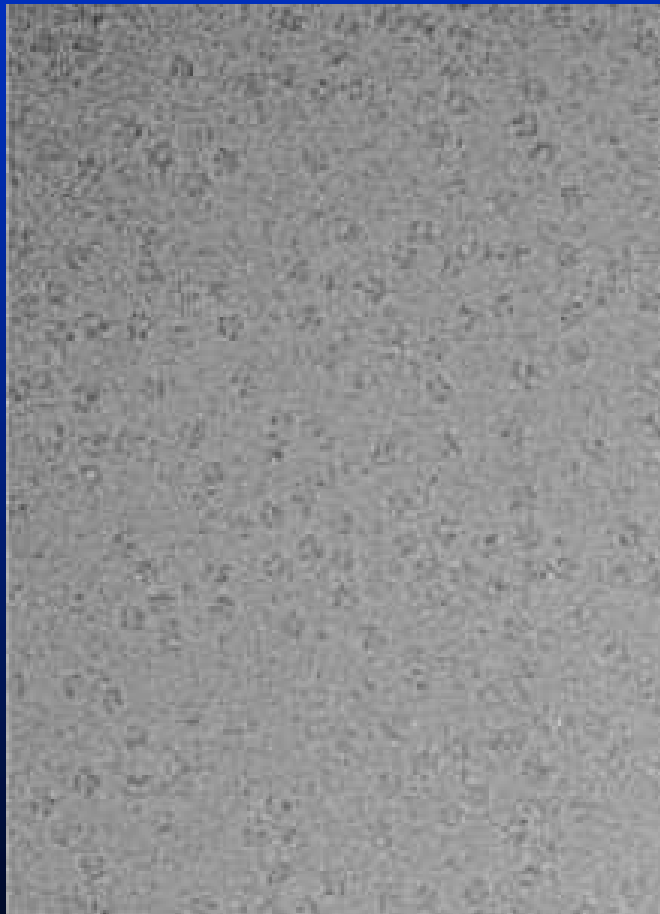
# *Common-Line Principle*



# *Model Reconstruction*



# *N-ethyl Maleimide Sensitive Factor*



# *Structural Analysis*

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