3D Single Particles Approaches to 2D Crystals

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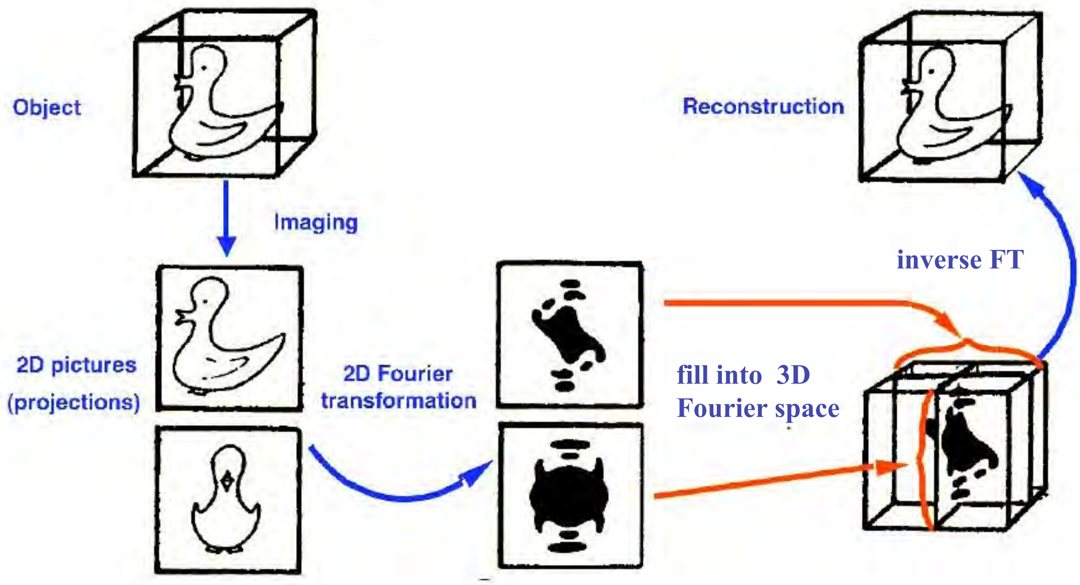
3D Reconstruction: Assign the Euler angles to projections, and "fill in" the 3D Fourier space.



Challenge: extremely low signal-to-noise ratio (SNR)

- Crystal images: apply Fourier filtration to **ordered** crystals

The average structure is contained in the diffraction spots (as sharp as possible) in the Fourier transform of the image.

- Single particle images: calculate the average of **aligned** particles in the same orientation

Crystallography method: Unbending process

Single particle method: Alignment process

Crystal imperfections translate into misalignments of particles

Concerns about Unbending:

- Unbending is in 2D space: sample support is lack of flatness

  (tilt angle will be slightly different for each unit cell)

- Error sources:
  - Displacement accuracy: displacement obtained by cross-correlation of the reference and the image
  - Deformation process

Single Particle Processing:

- Unbending can be done in 3D space: tilt angle can be different for each unit cell

- Error sources:
  - Alignment: cross-correlation, heuristic optimization, maximum likelihood

- No deformation in the unit cells

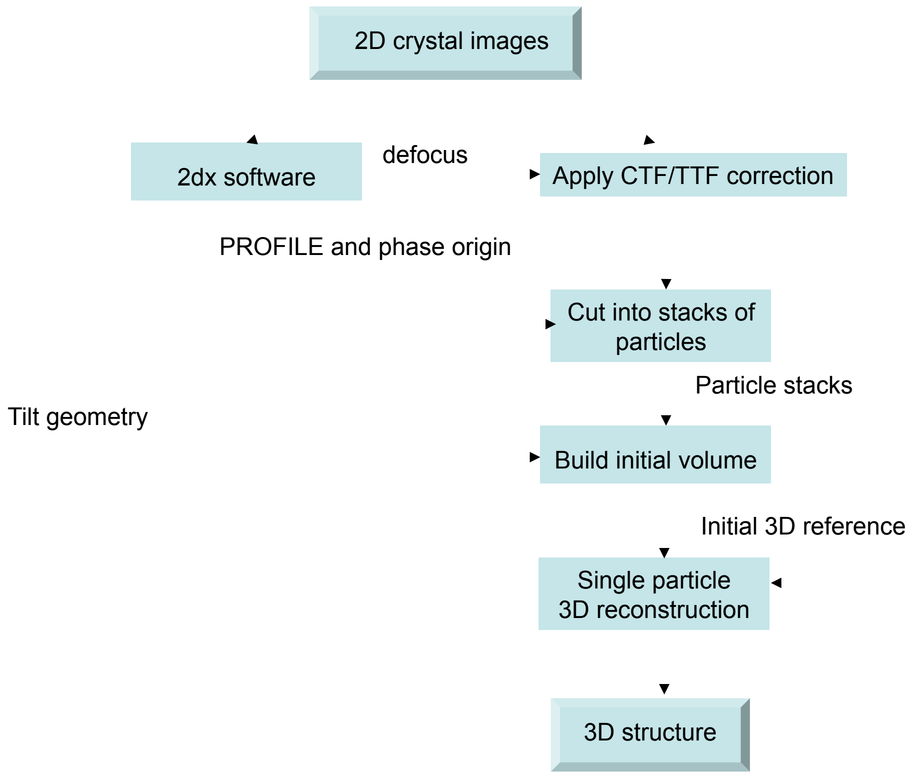
Single Particle Approaches to Single Particles

- Classify particles by orientations
- Align and average particles within classes
- Build 3D model
- Refine 3D model

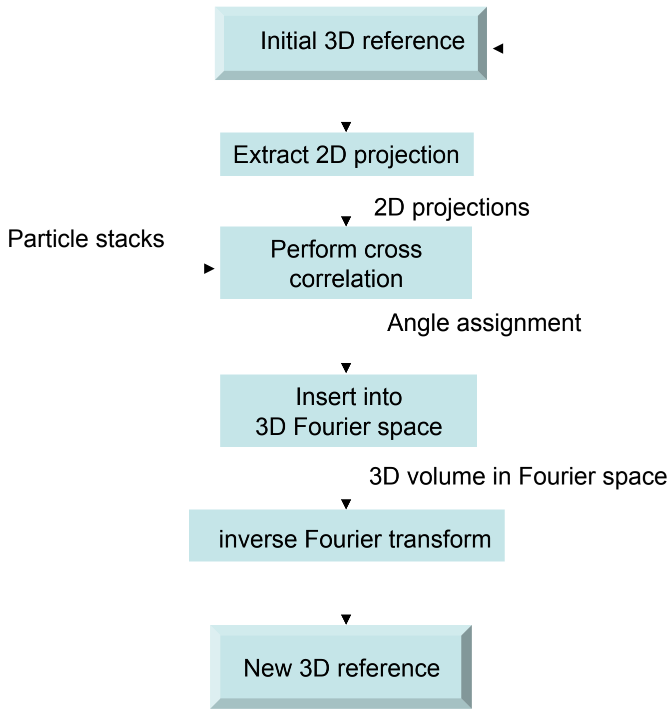
No crystal information.   Not easy to get initial 3D model.

Single Particles Approaches Applied to 2D Crystals

1. Make the best use of available information: crystal line, tilt geometry
2. Tackle the issue of lack of flatness by refining
   - Phase origin (the center of the particle)
   - Tilt geometry



Single Particle 3D Reconstruction



Cross-correlation alignment: $\phi = (\varphi, \theta, \psi, x, y)$

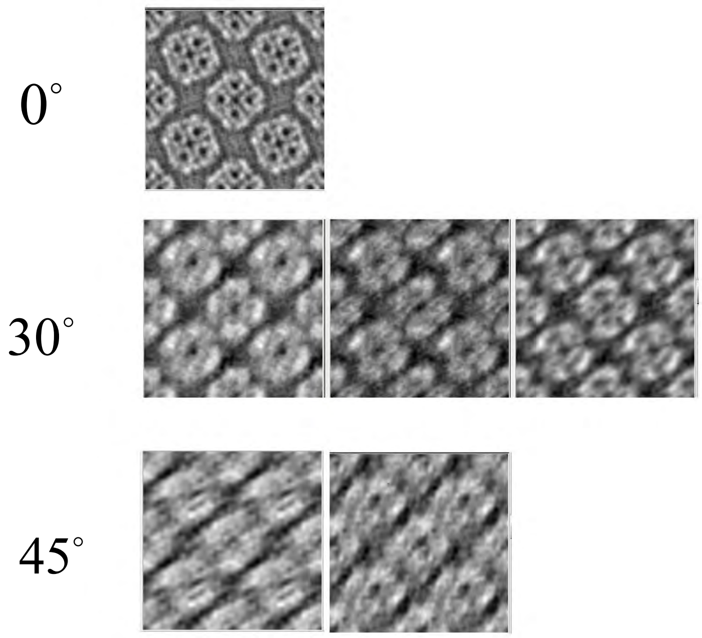
Reference volume A and particle X:

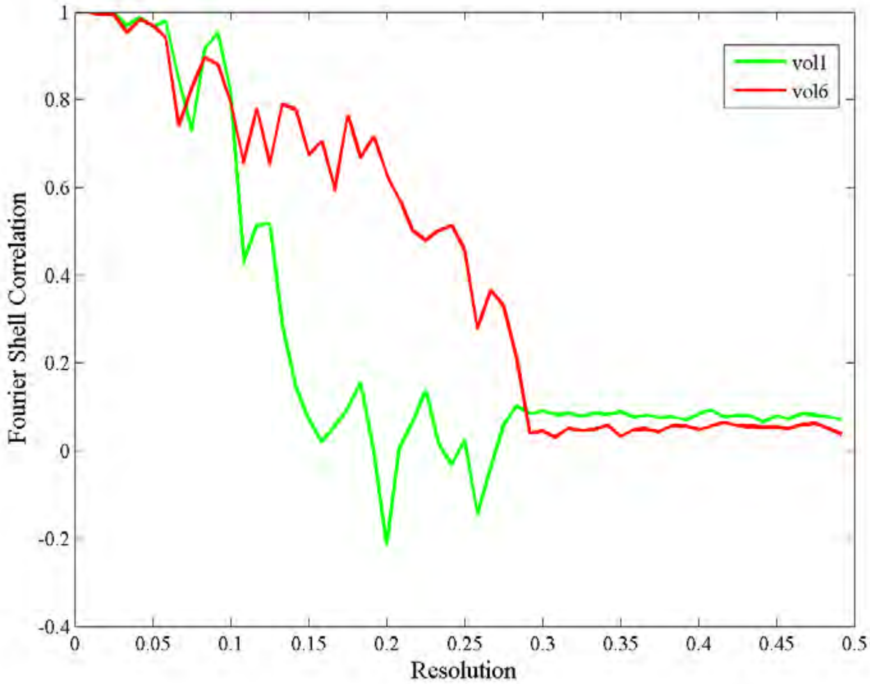
$$\phi_i^{(n+1)} = \arg\max_{\phi} \left\{ X_i \cdot P_{\phi_i}(A)^{(n)} \right\} \qquad (1)$$

$$A^{(n+1)} = \frac{1}{N} \sum_{i=1}^{N} I_{\phi_i}(X_i)^{(n+1)}) \qquad (2)$$

<u>3D Reconstruction Results:</u>

glycerol facilitator of Escherichia coli





Heuristic Optimization Algorithms: **locally** search for the translation and orientation parameters

$$\phi = (\varphi, \theta, \psi, x, y)$$

- Powell minimization search in Frealign
- Simulated annealing search

Using Large Number of Particles

- Accuracy of individual particle is trivial
- SNR is determined by alignment accuracy of large population of particles

The importance of data statistics

Maximum Likelihood (ML) Approach

- No alignment set $\phi_i = (\varphi_i, \theta_i, \psi_i, x_i, y_i)$ is determined. It is treated as random variables with some statistical distribution.
- Find the statistical parameters which best describe the observed data.

Method: Find the most likely parameters $\Theta = (A, \sigma, \phi)$ which results in the observed particles

Noise: Gaussian distribution with zero mean and std

Translation and orientation: Gaussian distribution with zero mean and std

3D ML for 2D Crystals

Each particle contributes to all **possible** projections with a probability:

$$\gamma_i(\phi;\Theta) = \left(\frac{1}{\sqrt{2\pi\sigma_k}} \right)^M \exp\left(-\sum_k \frac{\left| X_i - CTF_i \cdot P_\phi(A) \right|^2}{2\sigma_k{}^2} \right) \times \quad pdf(\phi \mid \Theta)$$

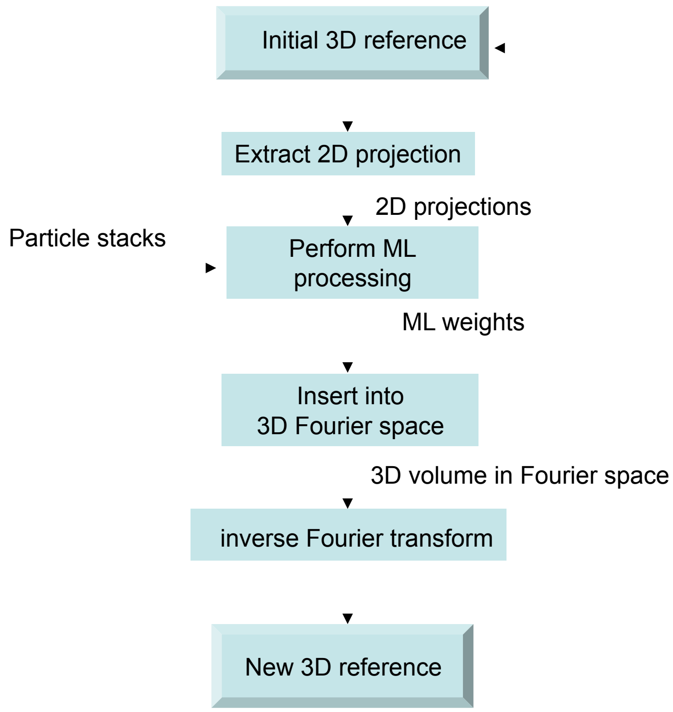
Quality of particles

Advantages of ML

- Free choice of initial reference, robust to low SNR.
- In addition to the weight representing the quality of the images, *pdf* of angles and shifts dominants the probability when SNR is very low or when reference is wrong.

The assumption of angles distribution is critical for the success of ML.

ML 3D Reconstruction



3D Reconstruction Results

Conclusion and Future Work

- Single particle approaches can improve the resolution by dealing with the unflatness problem of sample support

❖ Explore different 3D alignment approaches

❖ Investigate the errors introduced by overlapping projections and
develop algorithms to reduce the errors

❖ Incorporate the algorithms into 2dx software package

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