

Basel, Switzerland

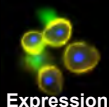


Third International Workshop on Electron Crystallography of Membrane Proteins.

August 1-7, 2010.

We acknowledge generous support from:





Expression



Purification



2D Crystallization



Sample Prep



Imaging



Image Processing



Model Building

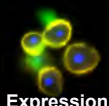
Electron Crystallography

1985

1995

2007

2010



Expression

Prokaryotic

Eukaryotic

Cell-Free



Purification

FPLC

Detergent Drop-Box



2D Crystallization

Dialysis Machine

Robotic Toolchain



Sample Prep

Sugar embedding

Carbon Sandwich



Imaging

FEG

Stable Stages

High-quality Vacuum

Automation

New Detectors



Image Processing

MRC

2dx, IPLT

ML, PCO



Model Building

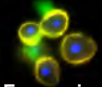
Modeling

MD refinement

Docking

Multi-Resolution

Electron Crystallography Bottlenecks



Expression



Purification



2D Crystallization



Sample Prep



Imaging



Image Processing



Model Building

Samples: "PHS" (Pure, Homogeneous, Stable).
 "Is a gel filtration profile still perfect after 1 week at 4°C?"

2D crystals: Automation.

Grid preparation: New sample supports (TiSi? Graphene?).

Images: Automation? Phase contrast STEM?

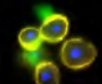
E-diff: Automated electron diffraction in TEM.

MRC, 2dx, IPLT: Throughput, User-friendliness, Automation,
 Maximum Likelihood for badly ordered 2D crystals.

Missing Cone: PCO (Projective Constraint Optimization)

Electron Crystallography

is still too slow...



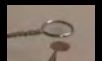
Expression



Purification



2D Crystallization



Sample Prep



Imaging



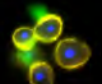
Image Processing



Model Building

was in the past	Should be in 2014
2 years	4 6 months
1 years	0 3 months
} 4 years	3 12 months
	1 3 months
6 months	

XRD



Expression



Purification



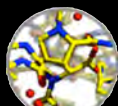
3D Crystallization



Data Collection



Data Processing



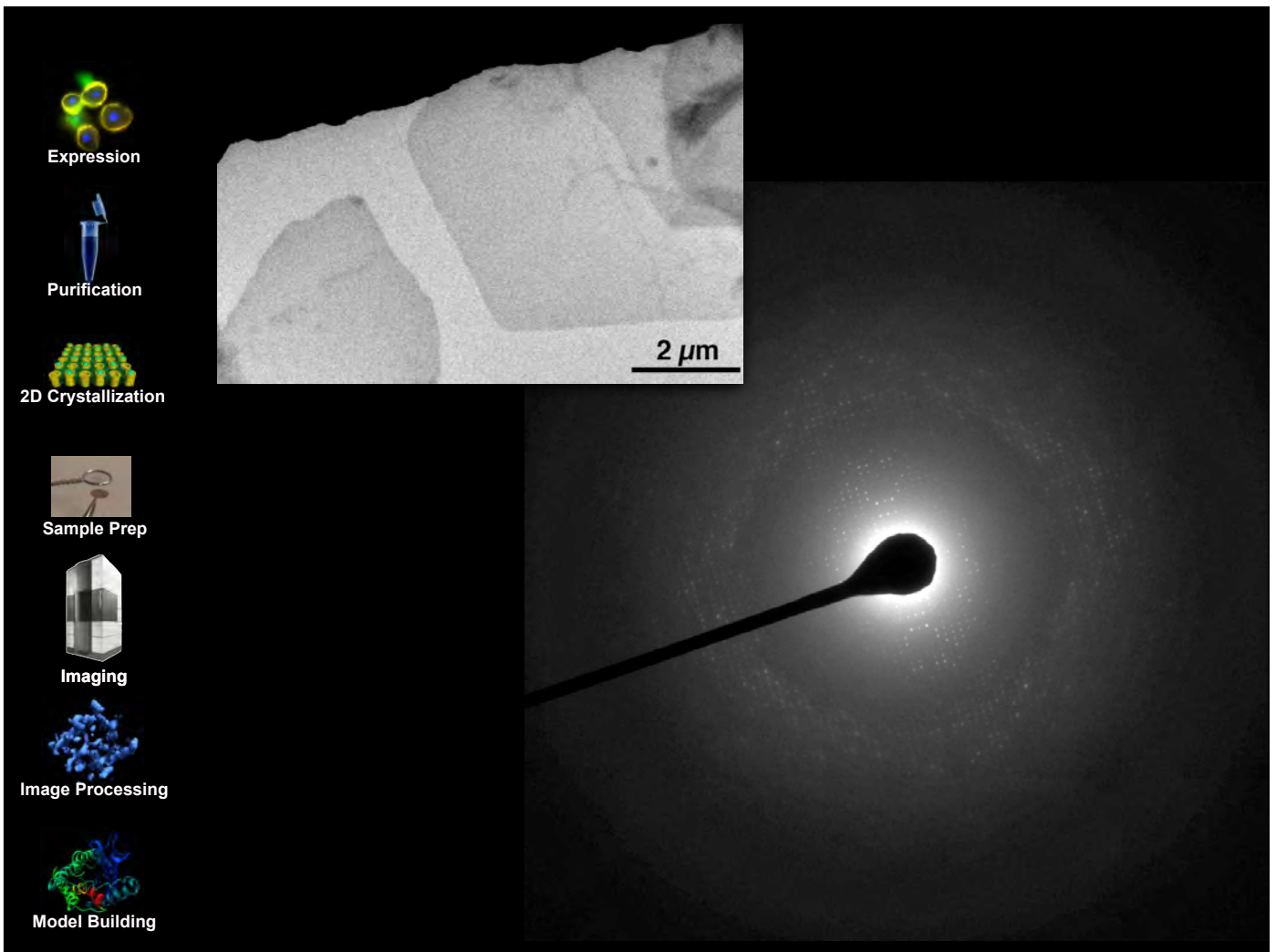
Model Building

3 months (or never)

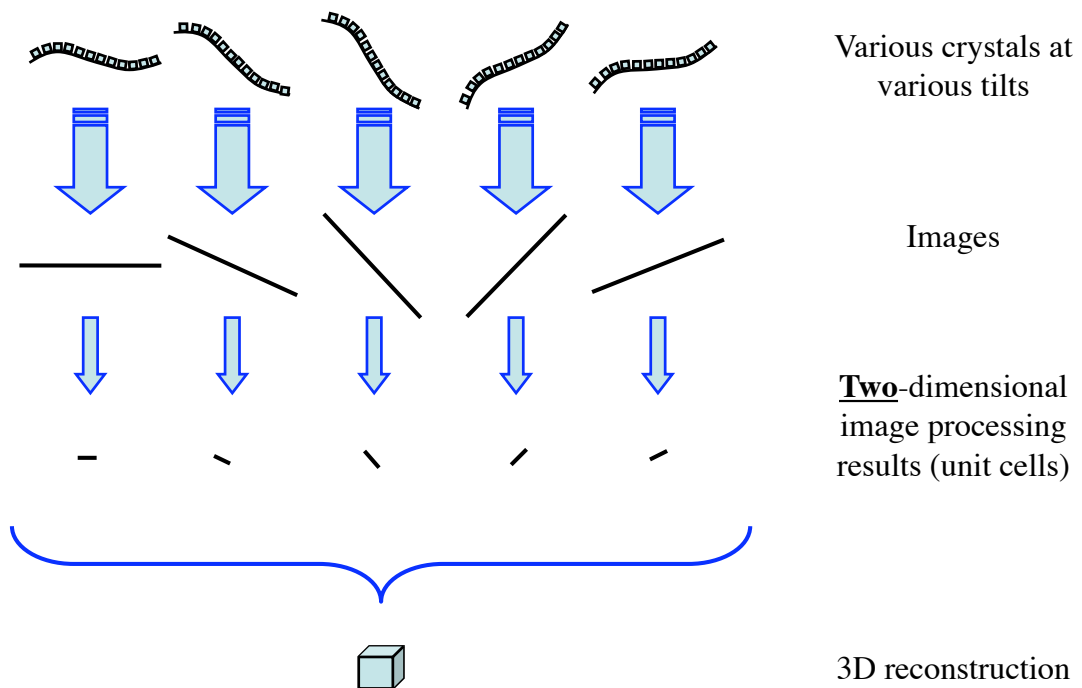
15 minutes

15 minutes

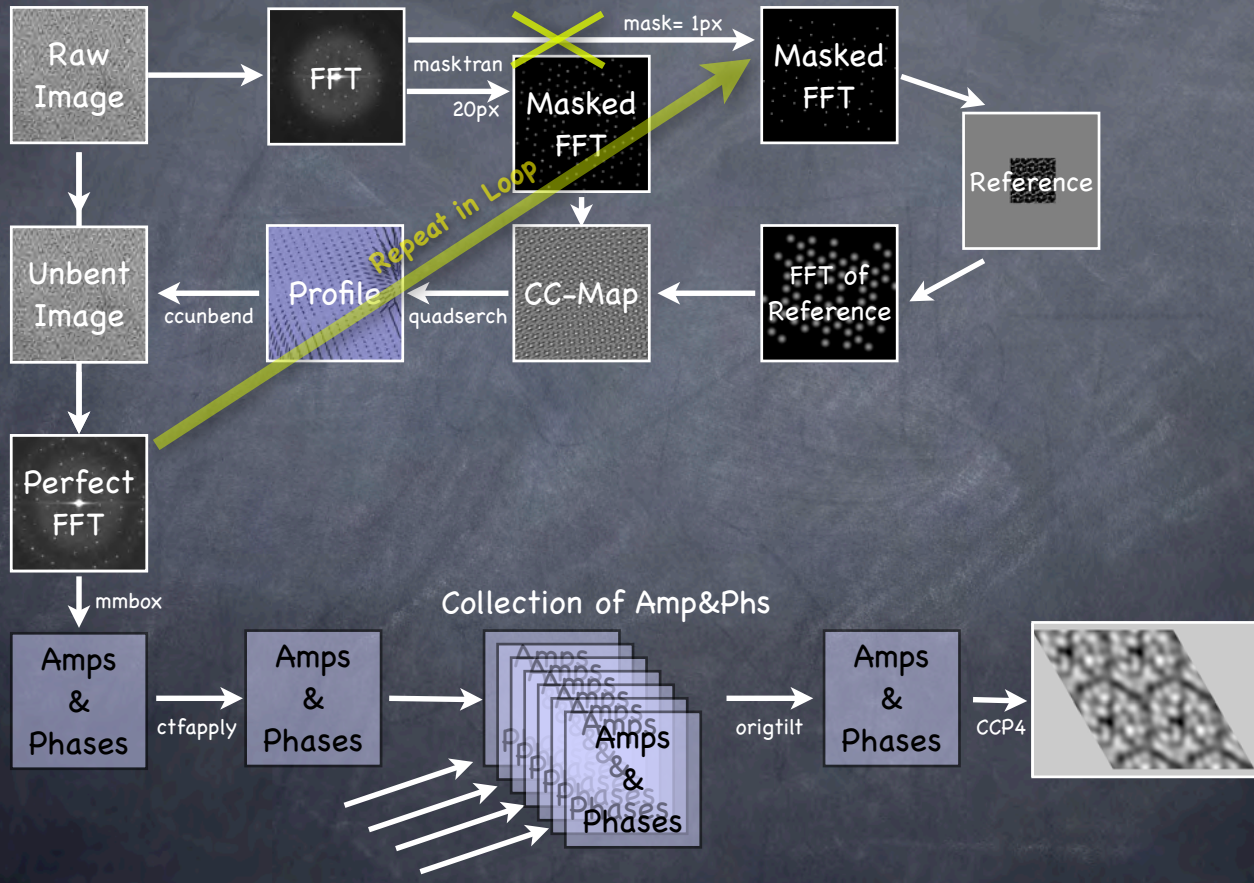
2 weeks



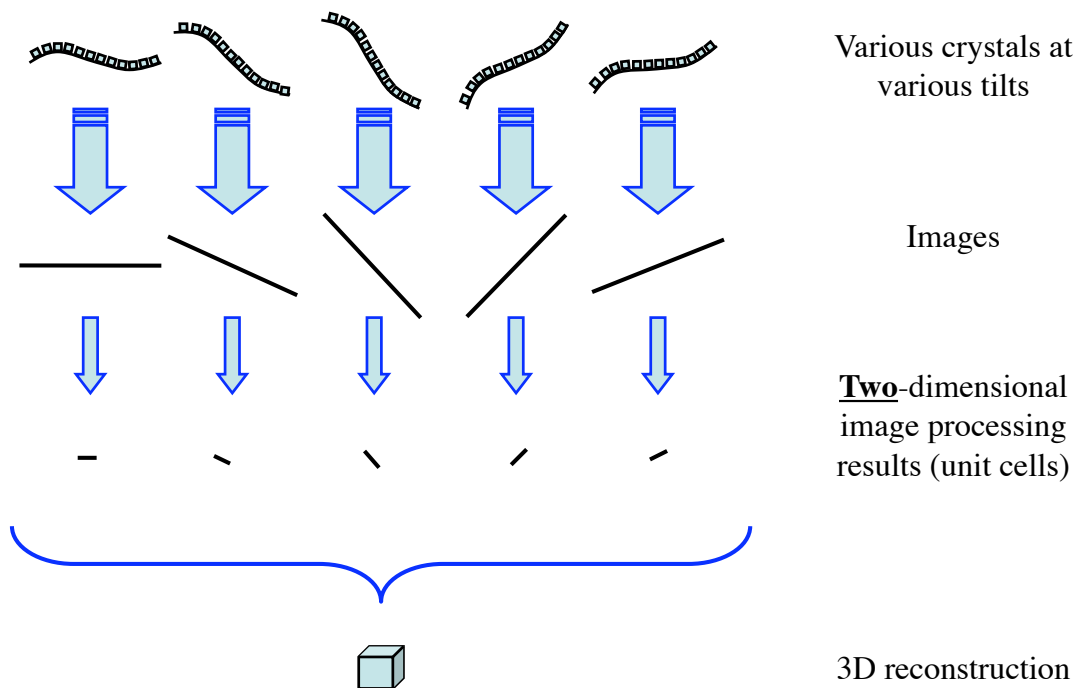
Common Electron Crystallography Algorithm



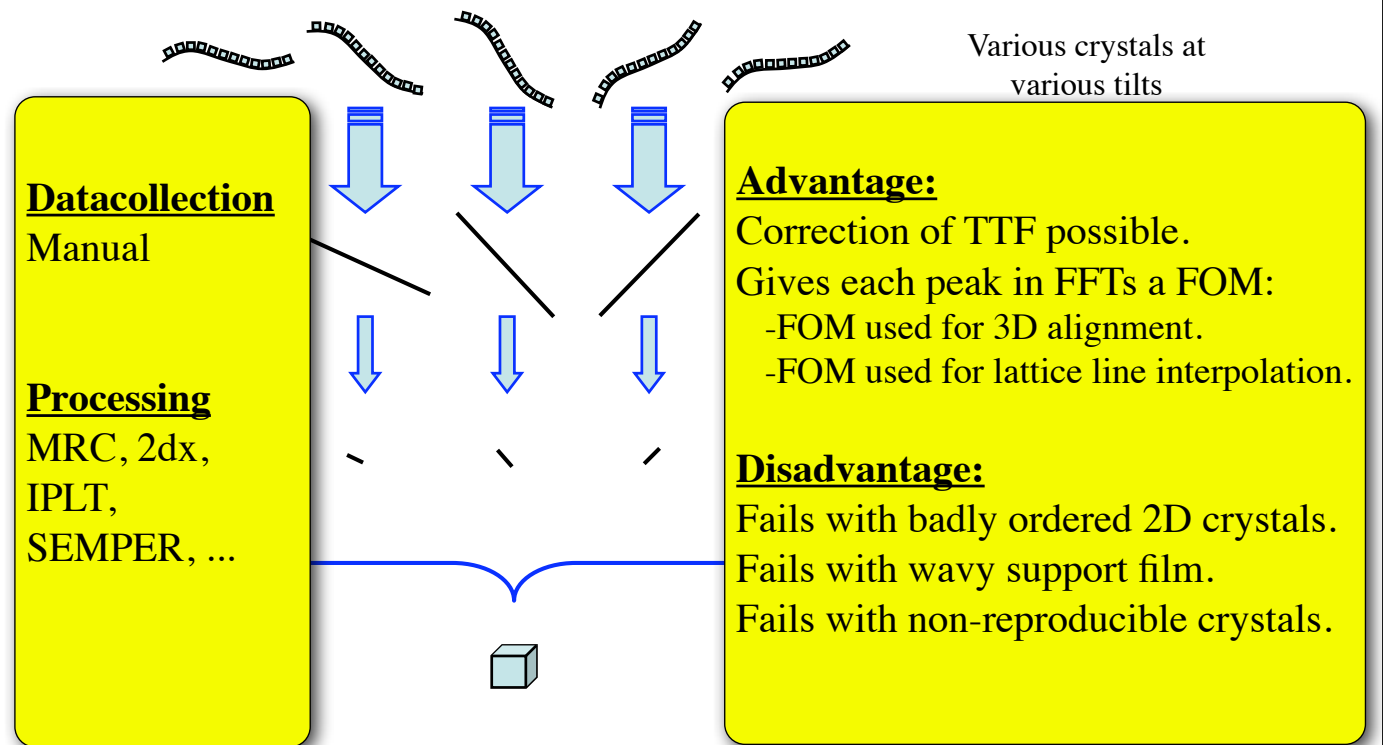
Unbending Algorithm



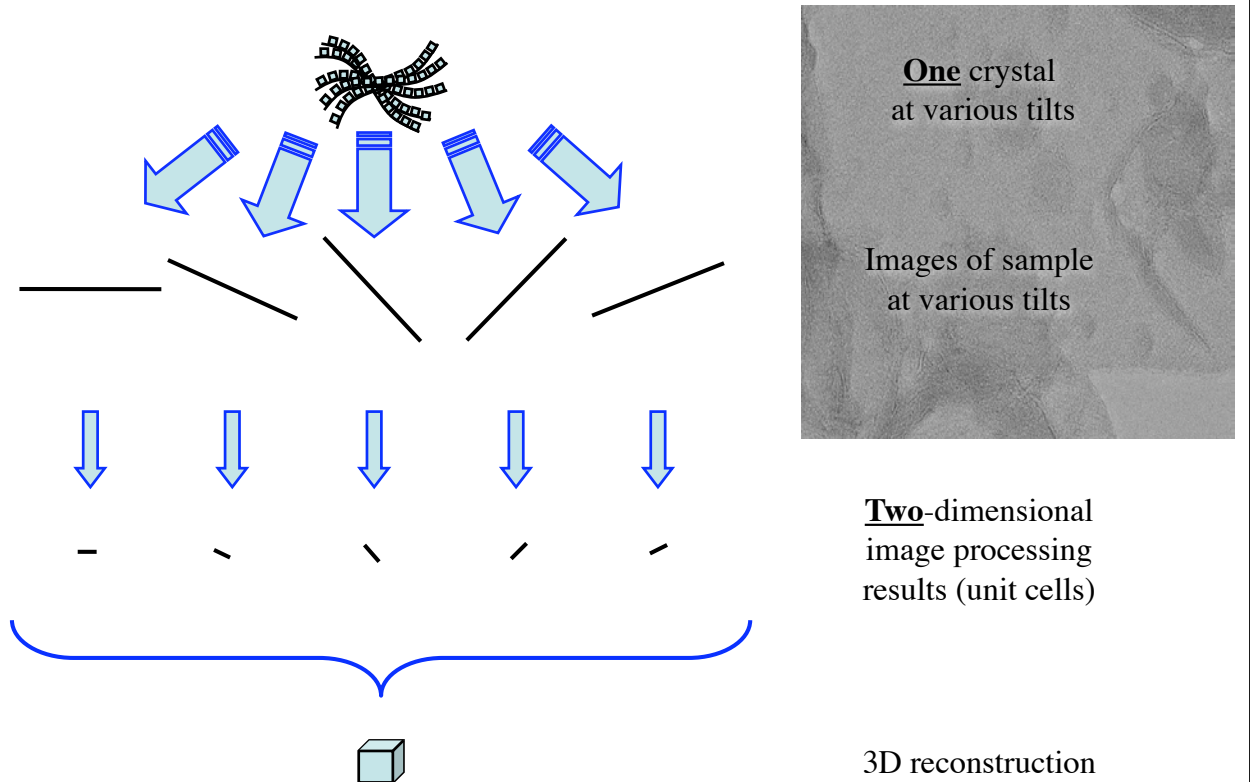
Common Electron Crystallography Algorithm



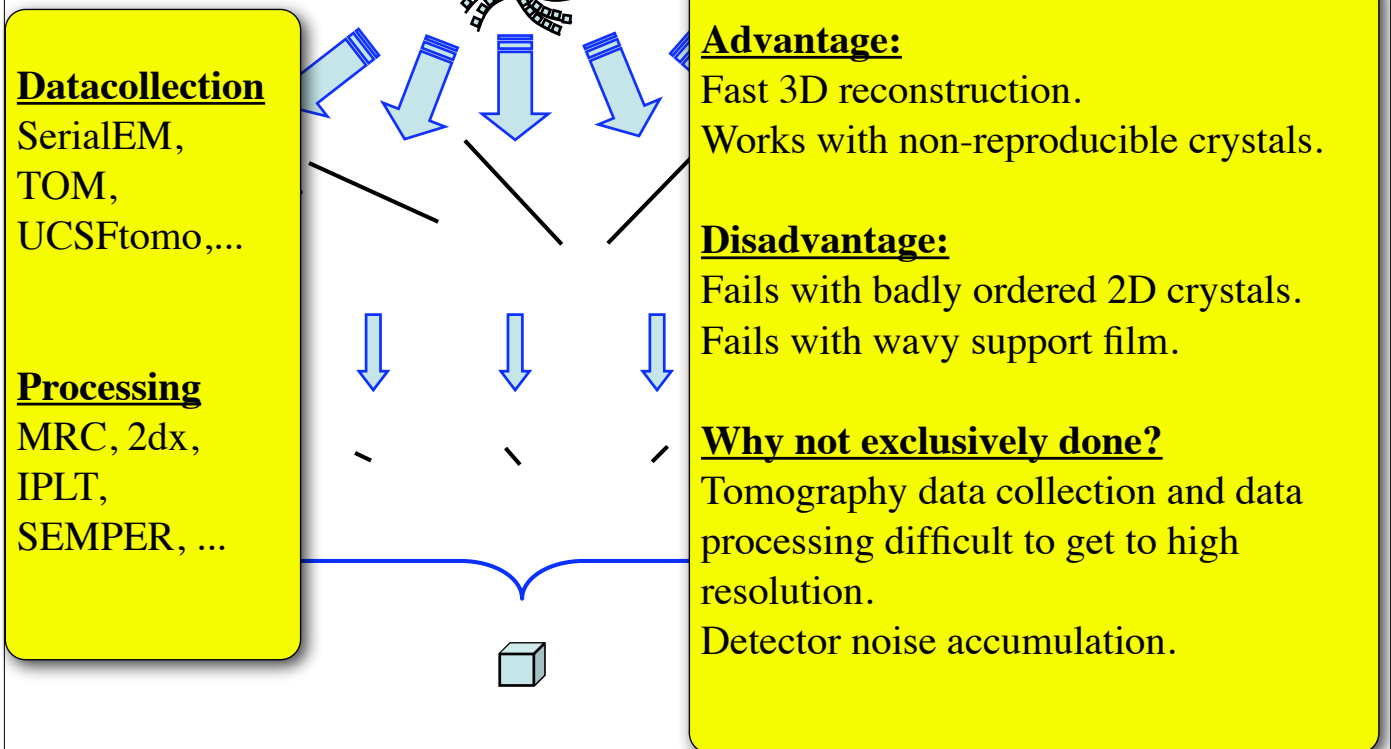
Common Electron Crystallography Algorithm



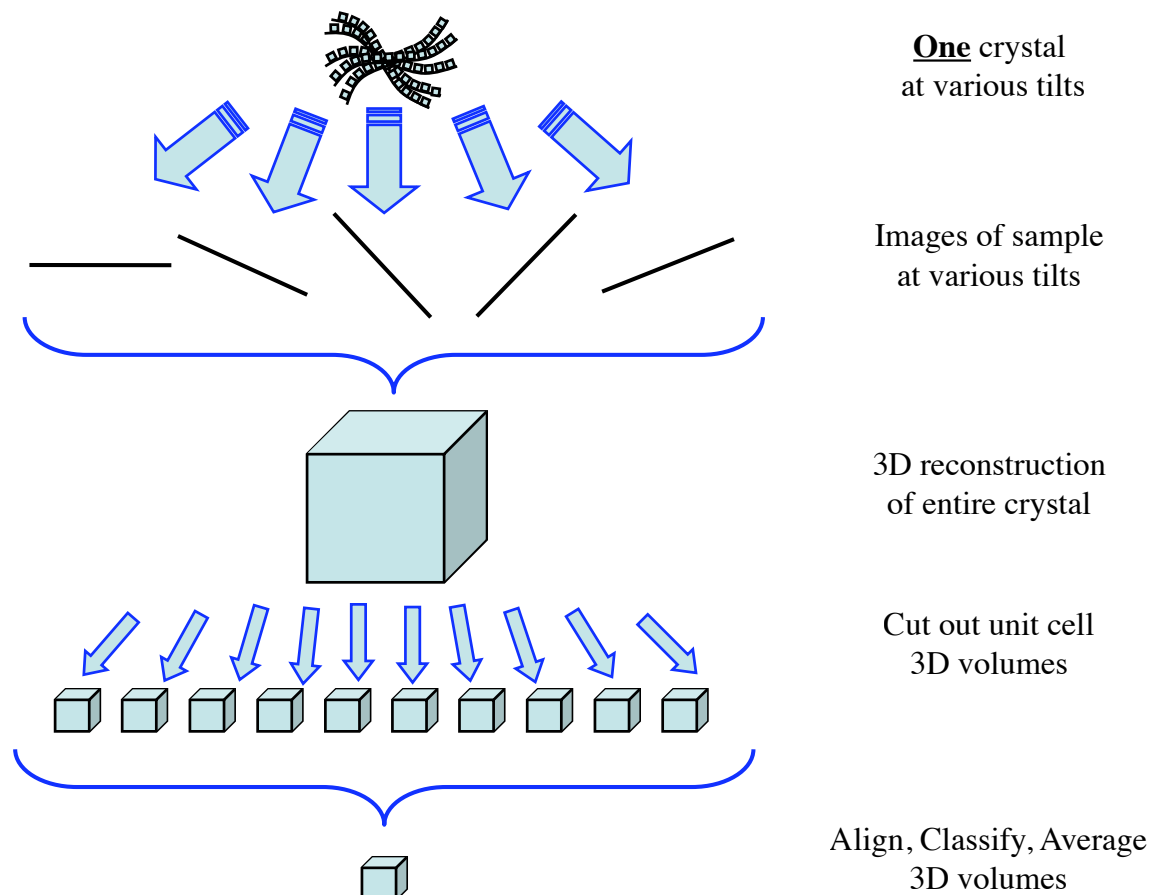
Electron Tomography Crystallography Algorithm



Electron Tomography Crystallography Algorithm



Common Electron Tomography Algorithm



Common Electron Tomography Algorithm

Datacollection

SerialEM,
TOM,
UCSFtomo,...

Processing

iMod,
TOM,...

Advantage:

Works with badly ordered 2D crystals.
Works with wavy support film.
Works with non-reproducible crystals.

Disadvantage:

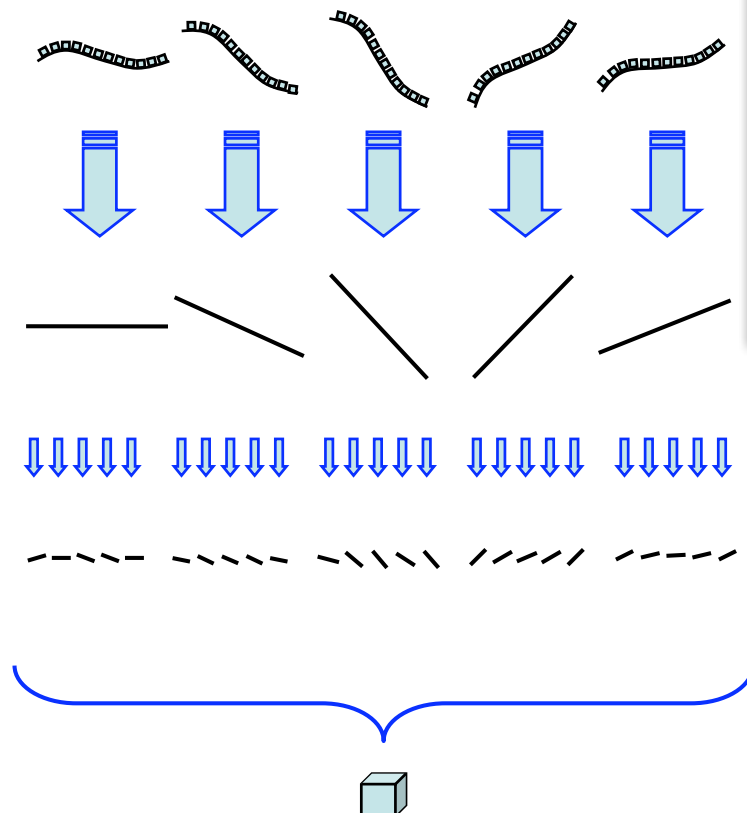
No TTF correction, but local CTF correction.
No FFT peak weights, but real-space classification.

Why not exclusively done?

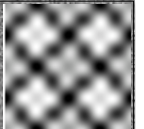
Tomography data collection and data processing at high resolution difficult.
Accumulation of detector noise.

3D volumes

Common Single Particle Algorithm



Crystallography



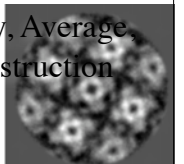
One or several crystals
at various tilts

Images of samples
at various tilts

Single particle
unit cell images



Align, Classify, Average,
get 3D reconstruction



CC-Single Particle

Common Single Particle Algorithm

Datacollection

Manual

Processing

Eman, Spider,
xmipp, ...

2dx:

(Xiangyan
Zeng's talk on
Friday)

Advantage:

Works with badly ordered 2D crystals.
Works with wavy support film.
Works with non-reproducible crystals.

Disadvantage:

No TTF correction, but local CTF correction.
No FFT peak weights, but real-space classification.

Why not exclusively done?

Software tools not yet at maturity of MRC programs?



Greetings from Single Particles

2D:

Alignment,
Classification,
Weighted Averaging

3D:

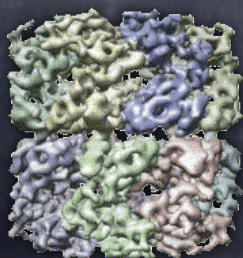
Angular Assignment

Real space:

ART/SIRT
Maximum Likelihood

Fourier space:

Backprojection
FREALIGN
(different measure)
Maximum Likelihood



Eman: Frequency Band Weighting
(Envelope fitting, dynamic spectral S/N ratio)