



SIMONS
ELECTRON
MICROSCOPY
CENTER

NYSBC 

Winter-Spring 2016 EM Course

Single-particle analysis (part II)

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Sample preparation

- Sample
 - Gel filtration
 - Gaussian peak
 - Concentration
 - 0.1 to 10uM for cryo-EM
 - Buffer
 - Ideally in water
 - Low salt content
 - No carbon source (glycerol, sucrose, free detergent...)
 - Fixation
 - GraFix

Sample preparation

- Grid preparation
 - Negative stain
 - Choice of stain
 - Staining methods
 - Grid blotting
 - Manual
 - FEI Vitroblot
 - Gatan CP3
 - Spraying
 - Time-resolved spray-plunge
 - Spotiton
 - Grid types
 - Hole size
 - Carbon vs gold substrate
 - Affinity grids

Screening and data collection

- Generate 2D Class averages and possibly 3D image reconstruction in stain to demonstrate that the particles are worthy of pursuit with cryo-EM (unless your samples have already been crystallized)
- Generate 2D class averages of particles in ice to demonstrate that you can see your particles and can attain thin ice
- Collect > 100,000 particle in ice using a 200+kV EM and a direct electron detector

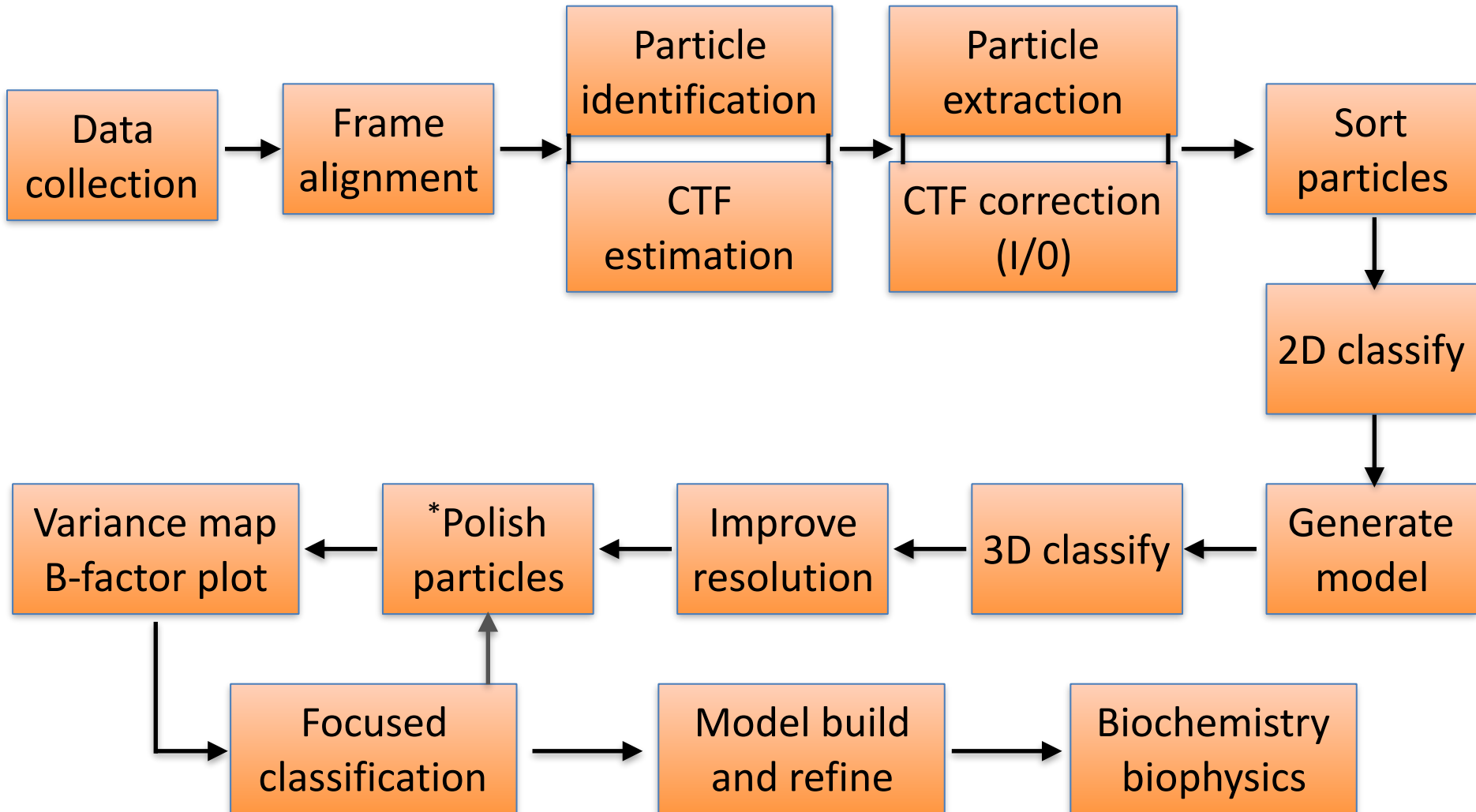
Screening and data collection

- Data collection strategy
 - Manual vs automated data collection
 - Low dose kit
 - Legikon
 - SerialEM
 - UCSFImage4
 - FEI EPU
 - JEOL JADAS

Screening and data collection

- Data collection strategy
 - One image per hole vs several
 - Dose rate
 - Total dose
 - Number of movie frames
 - Wait time between moves
 - Frequency of focusing

SPA Image analysis



Data processing

- Movie frames processing
 - Full frame processing
 - UCSF Motioncorr (Cheng lab)
 - UCSF MotionCor2 (Agard and Cheng lab)
 - Unblurr (Grigorieff lab)
 - Dose compensation
 - Per particle frame alignment
 - Relion movie mode (Sjors Scheres)
 - Measured dose compensation
 - Alignparts_Imbfgs (John Rubinstein)
 - Standard radiation damage compensation
 - XMIPP Optical flow

Data processing

- CTF estimation
 - CTF estimation
 - CTFFIND4
 - ACE
 - SPARX
 - EMAN
 - GCTF
 - Correction on aligned sums or on frames?

Data processing

- Particle picking
 - Manual (Forget about it!)
 - rkhat suggests, for new projects, that you pick >1000 particles by hand to familiarize yourself with the data so that you can assess its quality (e.g. particles share features or look like ink blots).
 - Gaussian based
 - DoG picker
 - EMAN2
 - Gautomatch
 - Template-based
 - EMAN
 - Relion
 - Gautomatch
 - FindEM (Appion)

Data processing

- Particle sorting
 - Image statistics (XMIPP's sort function)
 - Image classification

Data processing

- Image classification
 - 2D classification
 - Spider
 - IMAGIC (\$\$\$)
 - EMAN Iterative MSA (Multivariate Statistical Analysis)
 - Relion 2D
 - SPARX ISAC (Iterative Stable Alignment and Clustering)
 - XMIPP regular/rotational kernel SOM
 - Topology representing networks
 - Cluster affinity propagation
 - ... your own brew

Data processing

- Reference generation
 - Common lines
 - EMAN, EMAN2
 - Sparx
 - Simple/Simple2
 - IMAGIC (\$\$\$)
 - Bsoft
 - Random Conical Tilt
 - EMAN2
 - Sparx
 - Spider
 - XMIPP
 - Tomography
 - IMOD
 - EMAN2
 - XMIPP
 - Bsoft
 - Tilt pair test
 - Machine learning
 - CryoSPARC

Data processing

- Image classification
 - 3D classification
 - Xmipp ML3D
 - Relion 3D
 - Frealign
 - Bsoft

Data processing

- 3D map generation
 - Refinement
 - Xmipp ML3D
 - Relion 3D
 - Frealign
 - EMAN
 - Sparx
 - Bsoft
 - CryoSPARC

Data processing

- 3D map generation
 - Resolution estimation
 - FSC between 2 half-maps
 - Independent refinement of the two halves (gold standard)
 - Independent high resolution shells
 - Local resolution
 - Resmap
 - Bsoft LocRes
 - Masking individual domains
 - Variance estimation
 - Bootstrapping
 - Resmap

SPA Image analysis

