



Winter-Spring 2018 EM Course

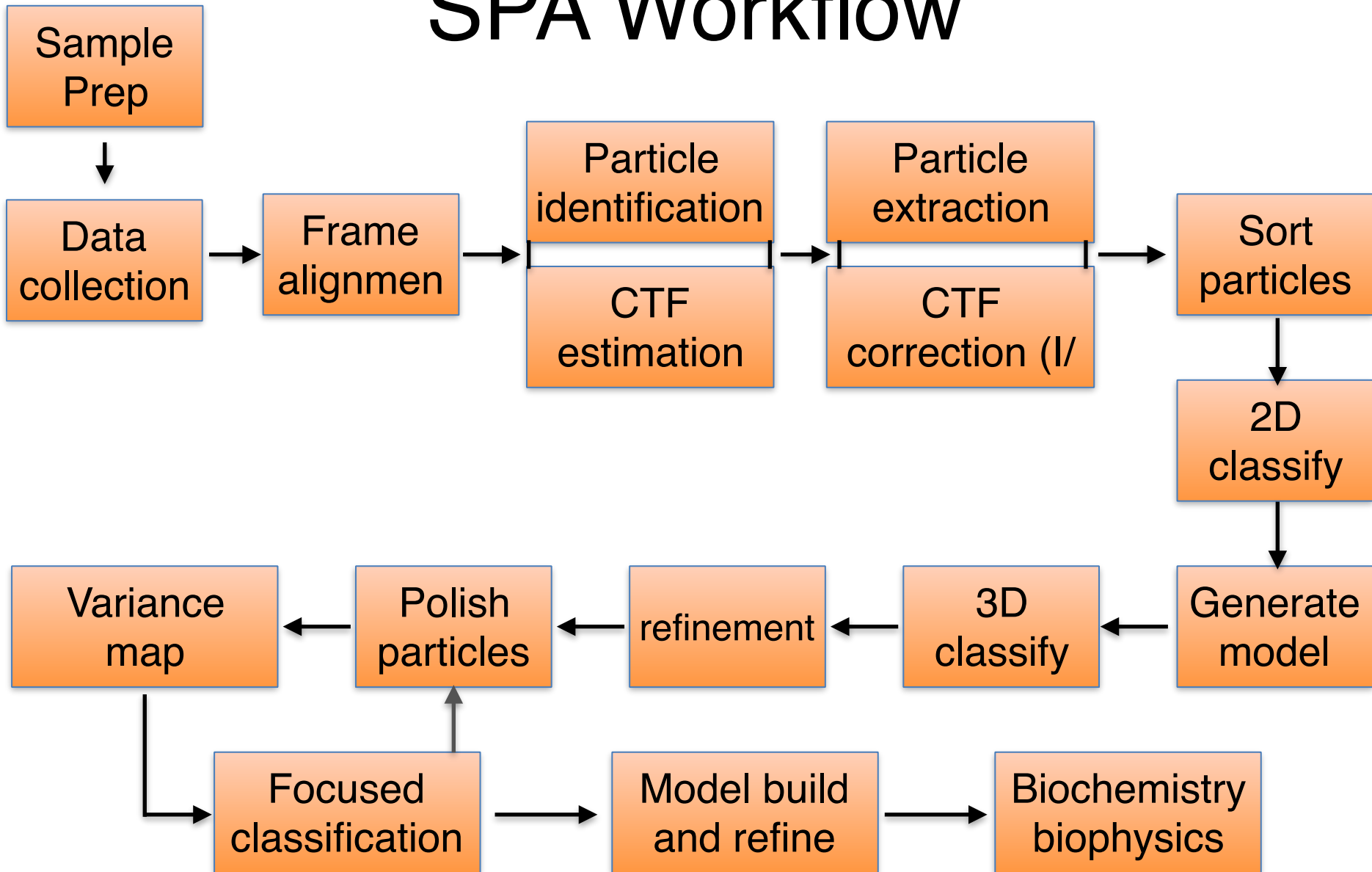
SIMONS
ELECTRON
MICROSCOPY
CENTER

Single-particle analysis (part II)

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NYSBC 

SPA Workflow



Sample preparation

- Sample
 - Gel filtration
 - Concentration
 - Buffer
 - 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

- Choice of microscope
 - FEG vs Lab6 emission chamber
 - Source coherence
 - Electron lenses
 - Stability and hysteresis
 - Condenser system
 - Electron beam parallelism
 - Sample holder
 - Stability and throughput
 - Phase plate
 - Energy filter

Screening and data collection

- Data collection strategy
 - Manual vs automated data collection
 - Low dose kit
 - Legimon
 - 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
 - Tilting
 - Phase plate

Data processing

- Movie frames processing
 - Full frame processing
 - UCSF Motioncorr (Yifan Chen 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
 - UCSF MotionCor2

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! ... or almost)
 - Gaussian based
 - DoG picker
 - EMAN2
 - Template-based
 - EMAN
 - Relion
 - Gautomatch
 - FindEM (Appion)
 - Etc...

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
 - IMAGIC (\$\$\$)
 - Bsoft
 - cryosparc
 - Random Conical Tilt
 - EMAN2
 - Sparx
 - Spider
 - XMIPP
 - Tomography
 - IMOD
 - EMAN2
 - XMIPP
 - Bsoft
 - Tilt pair test

Data processing

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

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 Workflow

