Winter-Spring 2016
EM Course

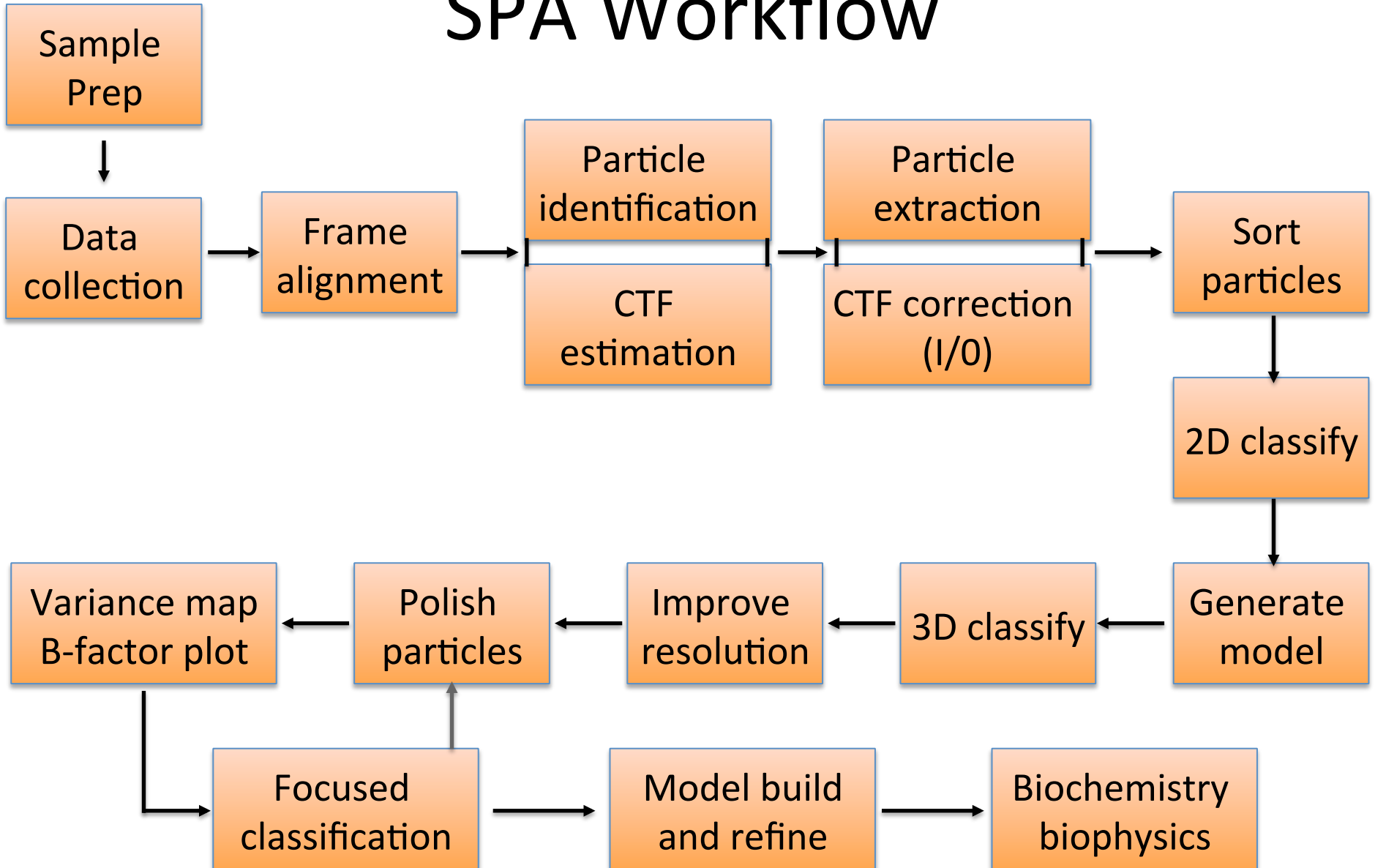## Single-particle analysis (part II)

Reza Khayat & Amedee des Georges

SIMONS
ELECTRON
MICROSCOPY
CENTER

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

Screening and data collection

- Data collection strategy

  - Manual vs automated data collection

    - Low dose kit
    - Leginon
    - 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

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_lmbfgs (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! … 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 kerden SOM
    - Topology representing networks
    - Cluster affinity propagation
    - … your own brew

Data processing

- Reference generation
  - Common lines
    - EMAN, EMAN2
    - Sparx
    - Simple
    - IMAGIC ($$$)
    - Bsoft
  - 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

Data processing

- 3D map generation

  - Refinement

    - Xmipp ML3D
    - Relion 3D
    - Frealign
    - EMAN
    - Sparx
    - Bsoft

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