Winter-Spring 2018 EM Course

Single-particle analysis (part II)

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SPA Workflow

Sample Prep

Data collection

Frame alignment

Particle identification

Particle extraction

CTF estimation

CTF correction (I/

Sort particles

2D classify

Variance map

Polish particles

refinement

3D classify

Generate model

Focused classification

Model build and refine

Biochemistry biophysics
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
    - 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
  – 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_lmbfgs (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 kerden 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

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Particle identification

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Particle extraction

CTF correction (I/

Sort particles

2D classify

Variance map

Polish particles

Improve resolution

3D classify

Generate model

Focused classification

Model build and refine

Biochemistry biophysics