SIMONS
ELECTRON
MICROSCOPY
CENTER

NYSBC

Winter-Spring 2019
EM Course

Single-particle analysis (part II)

Reza Khayat & Amedee des Georges

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
- 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

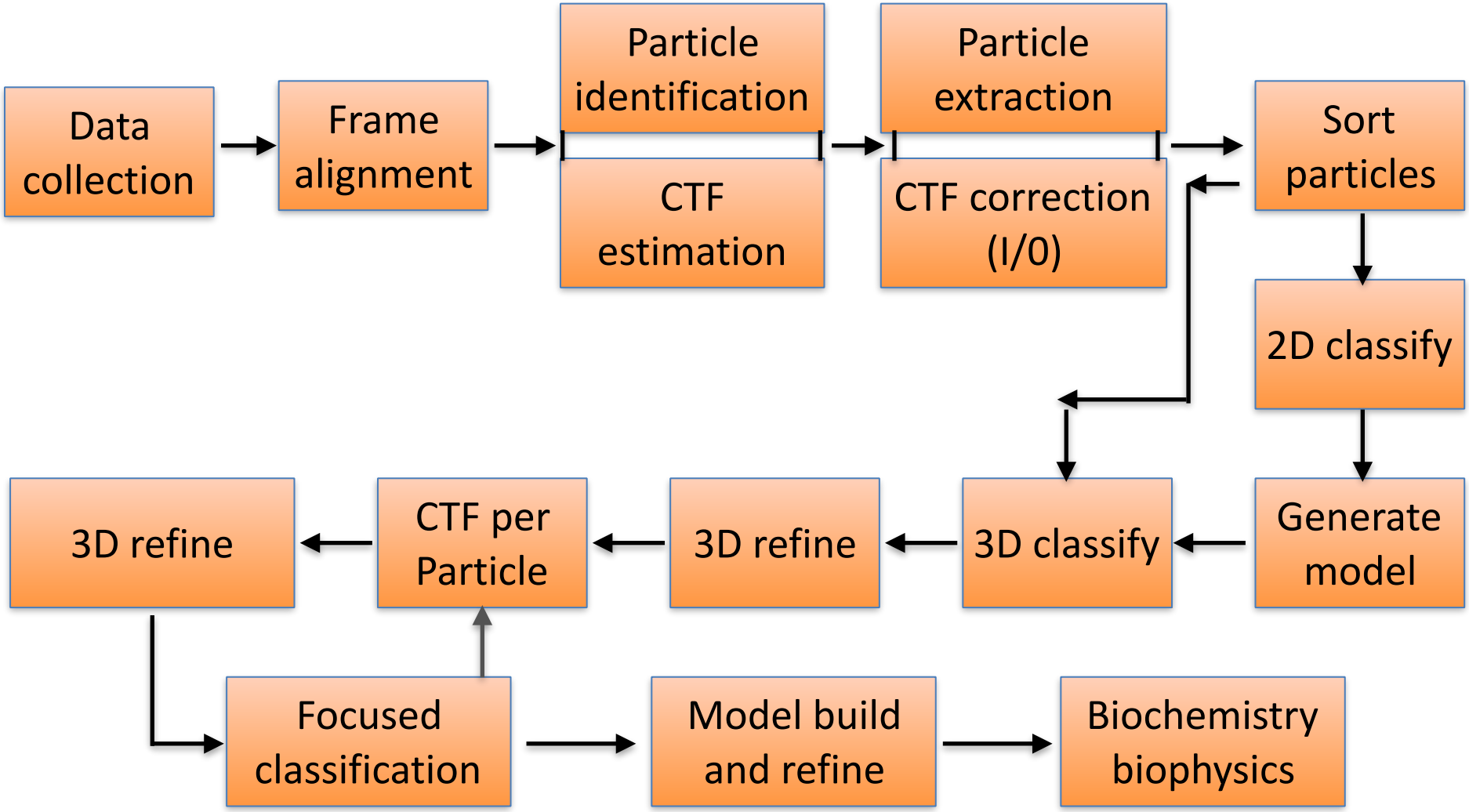
Screening and data collection

- Data collection strategy
  - Manual vs automated data collection
    - Low dose kit
    - 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

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_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

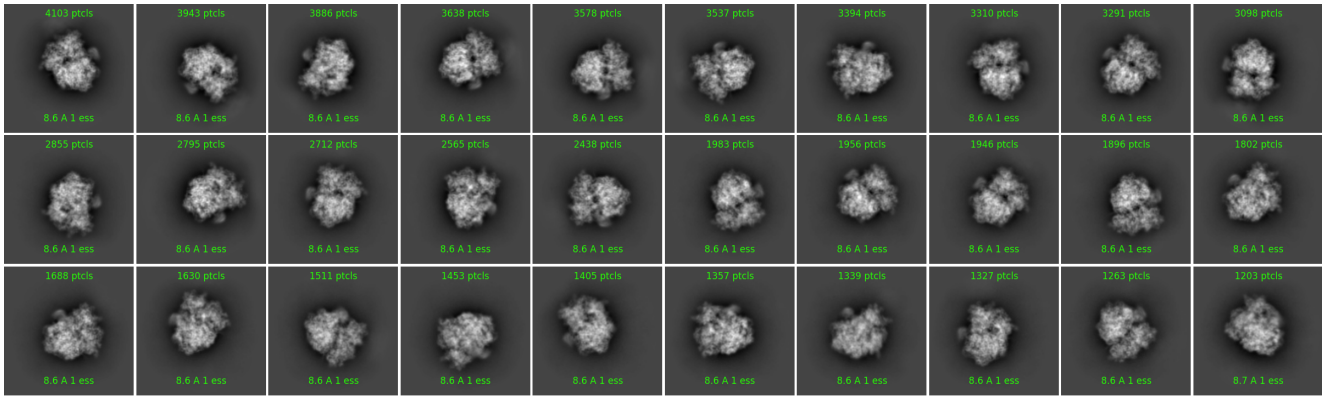
- Particle picking
  - Manual (Forget about it!)
    - rkhayat suggests, for new projects, that you pick >1000 particles by hand to familiarize yourself with the data so that you can assess its quality (e.g. particles share features or look like ink blots).
  - Gaussian based
    - DoG picker
    - EMAN2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

Data processing

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

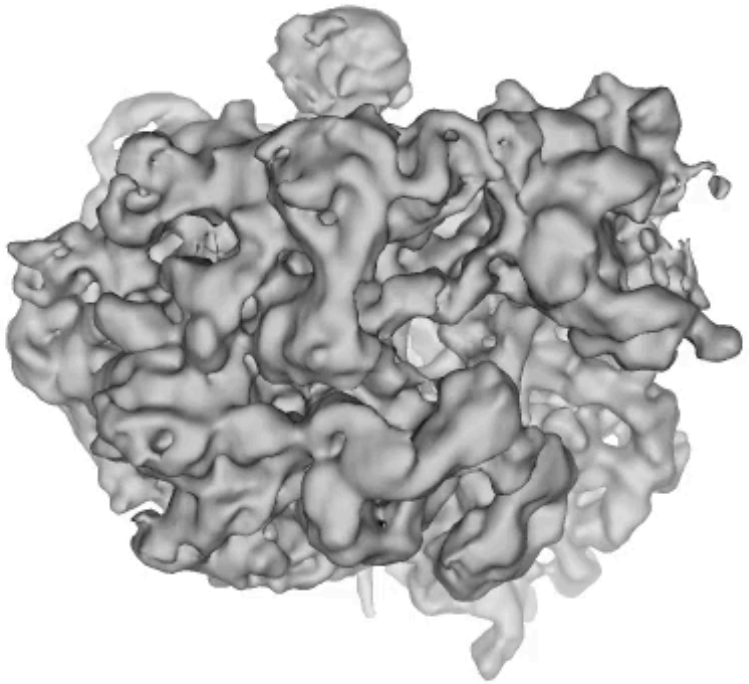
Data processing

- Image classification
  - 2D classification
    - Spider
    - IMAGIC ($$$)
    - EMAN(2) Iterative MSA (Multivariate Statistical Analysis)
    - Relion
    - SPARX ISAC (Iterative Stable Alignment and Clustering)
    - XMIPP
    - cryoSPARC



Data processing

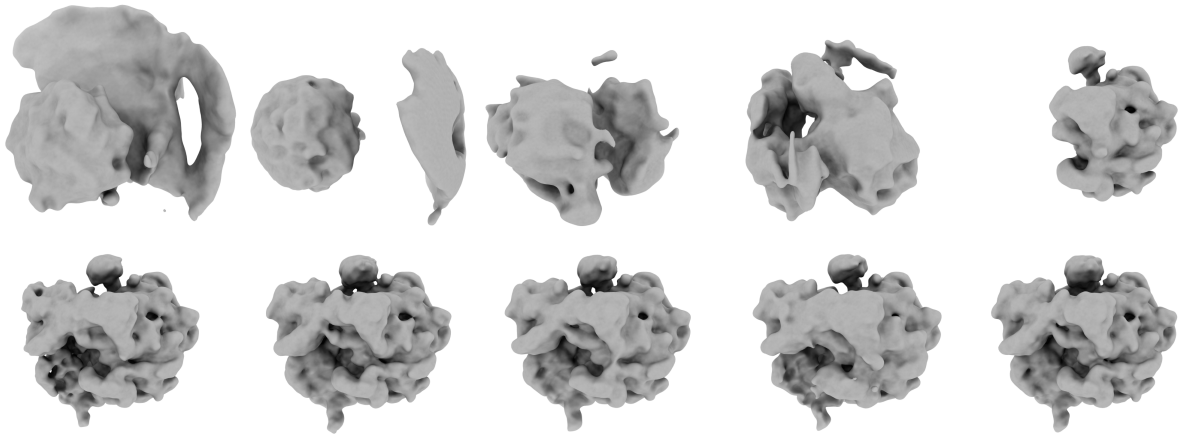
- Reference generation
  - Common lines
    - EMAN, EMAN2
    - Sparx
    - Simple/Simple2
    - IMAGIC ($$$)
    - Bsoft
  - Random Conical Tilt
    - EMAN2
    - Sparx
    - Spider
    - XMIPP
  - Tomography
    - IMOD
    - EMAN2
    - XMIPP
    - Bsoft
  - Tilt pair test
  - Machine learning
   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



Generated with CryoSPARC

Data processing

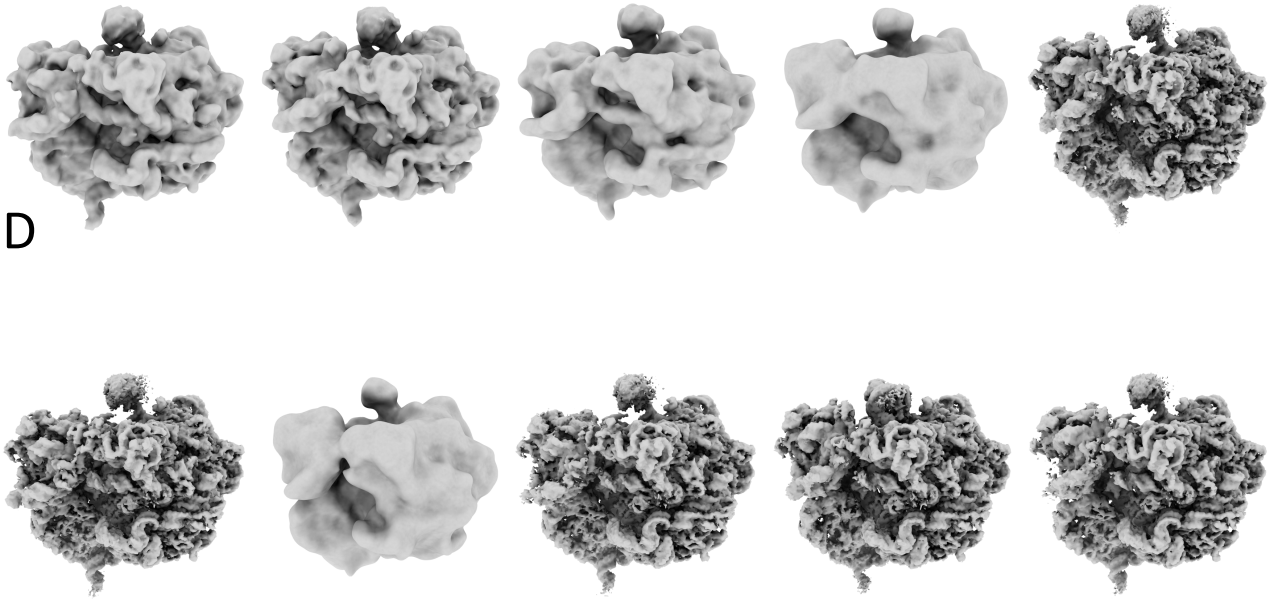
- Image classification
  - 3D classification



    - Xmipp ML3D
    - Relion 3D
    - Frealign and *cis*TEM
    - Bsoft
    - Sparx

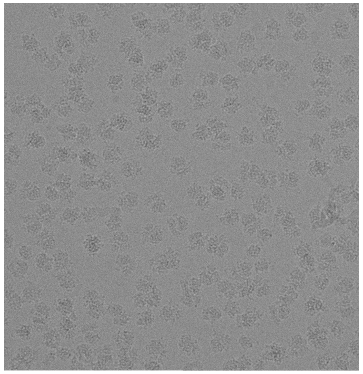
Data processing

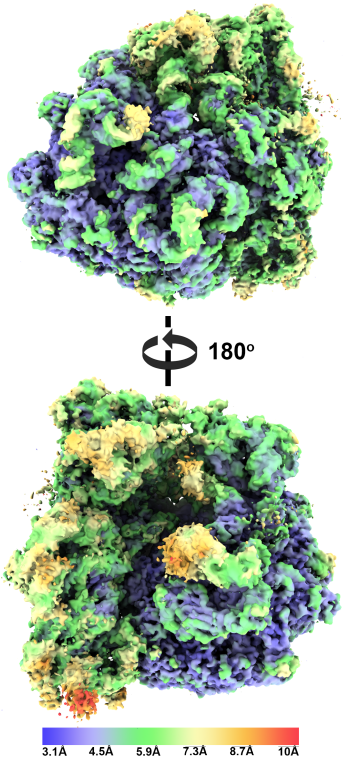
- 3D map generation
  - Refinement
    - Xmipp ML3D
    - Relion 3D
    - Frealign
    - EMAN
    - Sparx
    - Bsoft
    - CryoSPARC

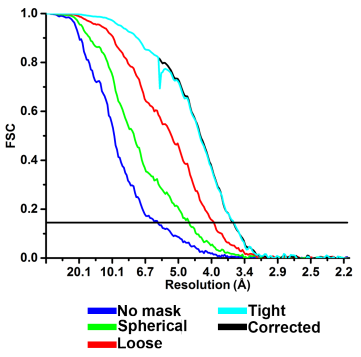


Data processing

- 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
      - LocRes (Bsoft)
      - MonoRes (XMIPP)
    - Variance estimation
      - Frealign
      - Sphire

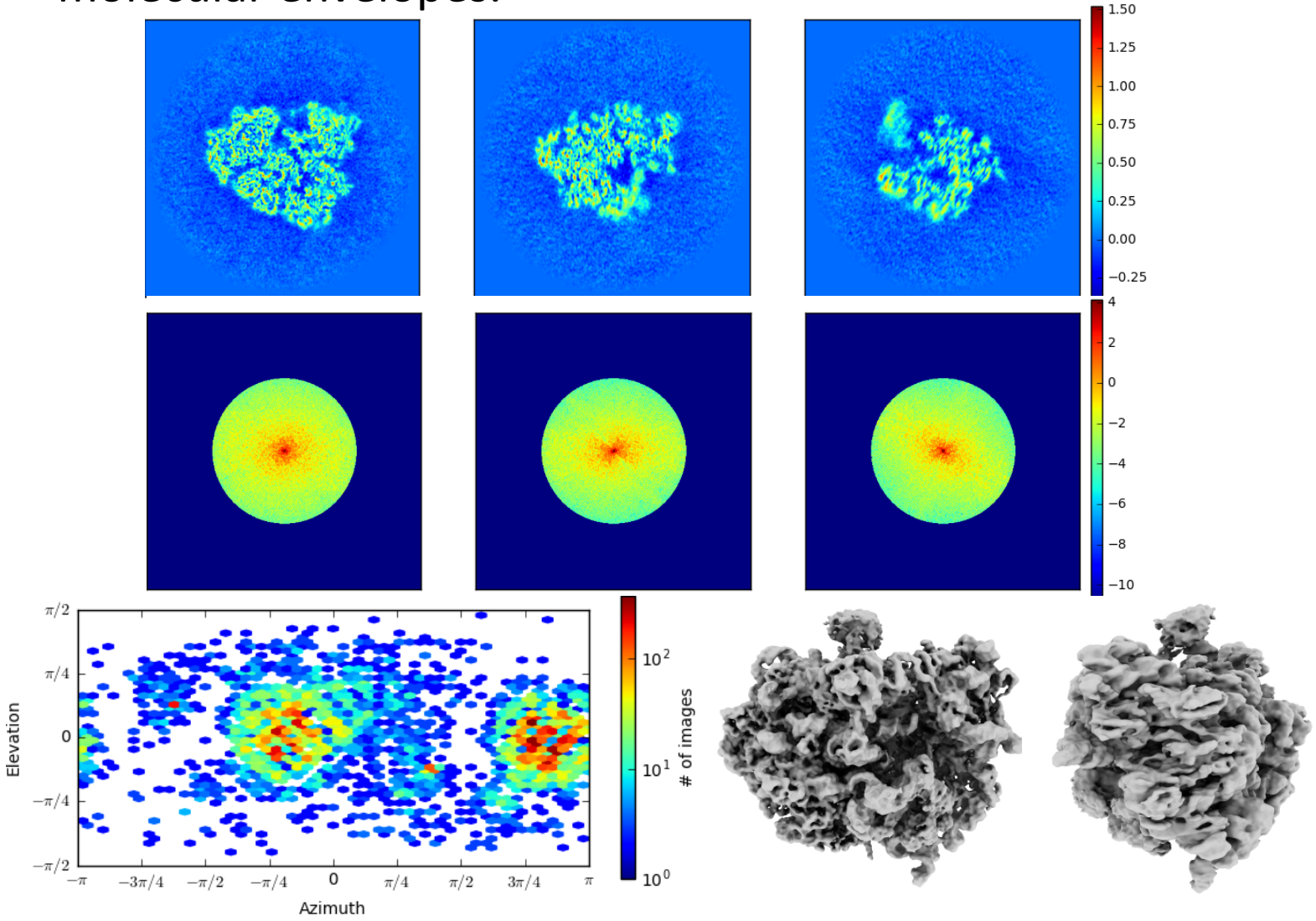






Data processing

- Anisotropy (cryoSPARC results)
  - Preferred orientation will give rise to elongated/stretched molecular envelopes.



SPA Image analysis