



---

SIMONS  
ELECTRON  
MICROSCOPY  
CENTER

---

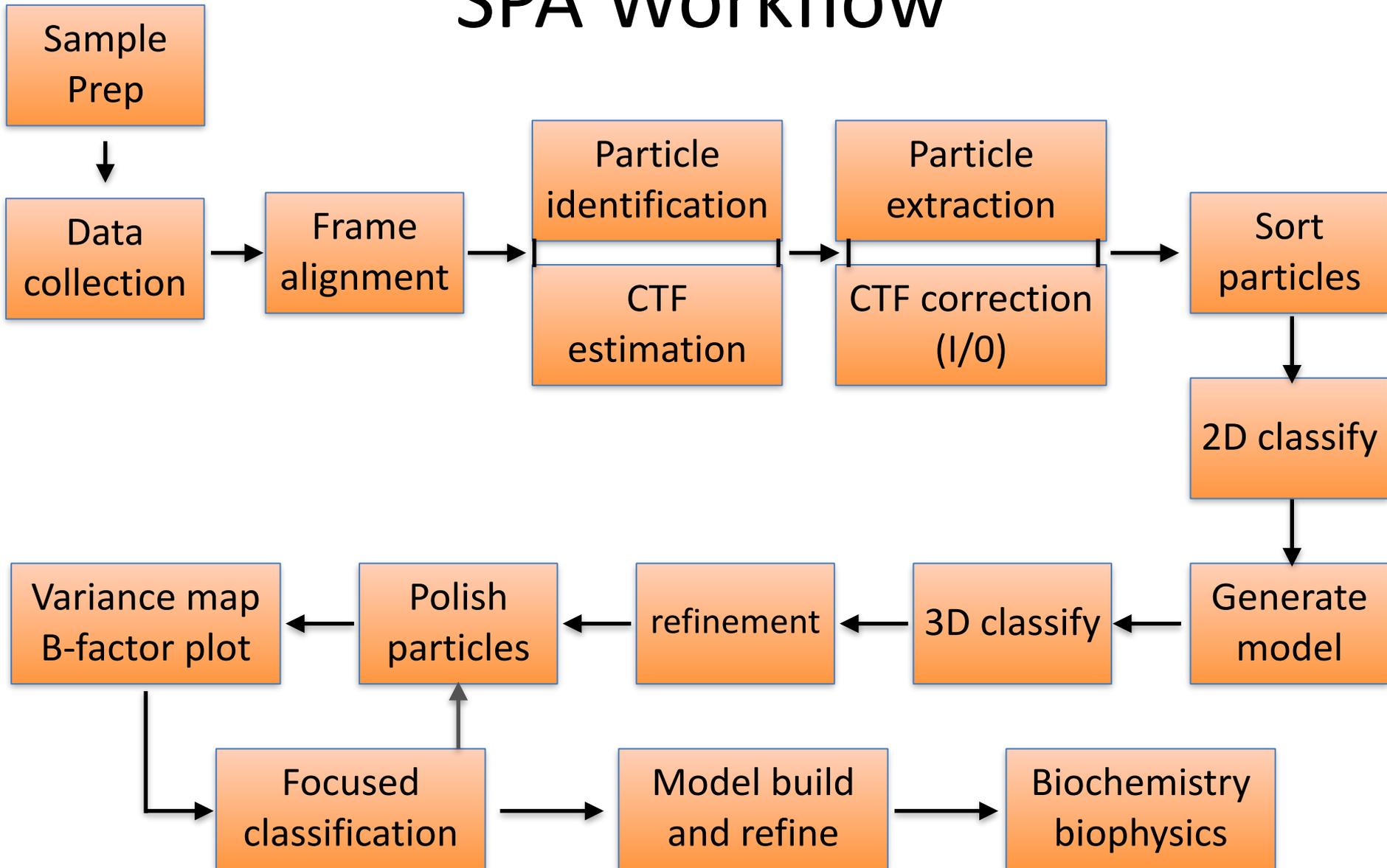
NYSBC 

# 2022 EM Course

Single-particle analysis (part II)

Reza Khayat & Amedee des Georges

# SPA Workflow



# Data processing

- Movie frames processing

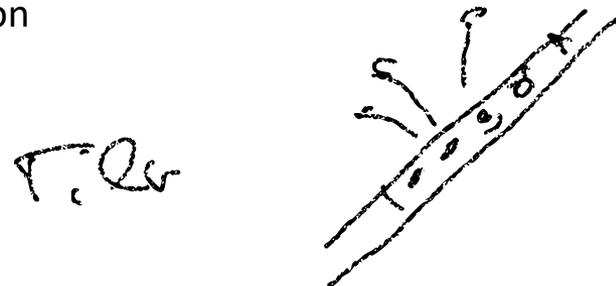
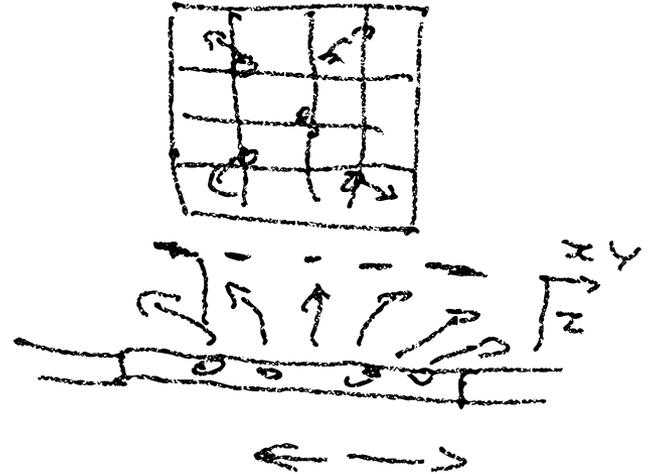
- Full frame processing

- 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) - with faster implementation in cryoSPARC
      - Standard radiation damage compensation
    - XMIPP Optical flow
    - UCSF MotionCor2

→ WARP.



# Data processing

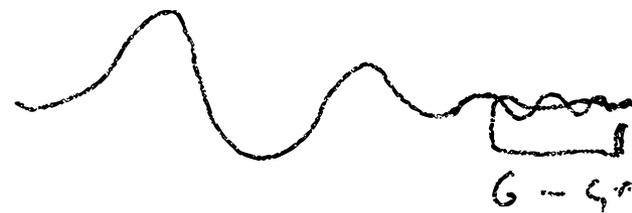
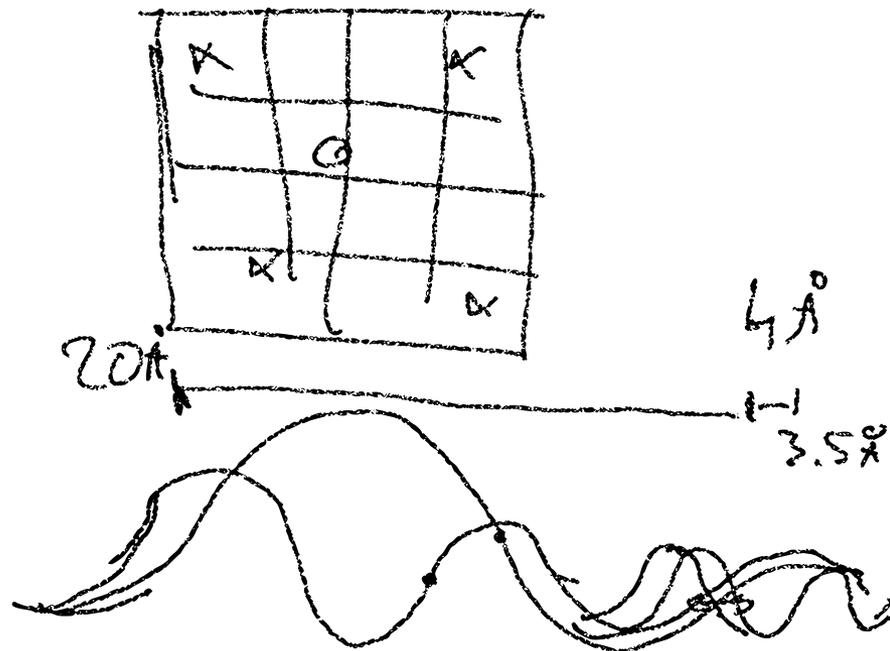
- CTF estimation

- CTF estimation

- CTFFIND4 ←
    - ACE
    - SPARX
    - EMAN
    - GCTF ←
    - cryoSPARC patch CTF
    - Warp patch CTF

- Correction on aligned sums or on frames?

- *Relion per particle.*



# Data processing

- Particle picking

- Manual (Forget about it! ... or almost)

- Gaussian based

- DoG picker
      - EMAN2

- Template-based

- EMAN
      - Relion
      - Gautomatch
      - FindEM (Appion)
      - Etc...

- Neural network-based picking

- Warp
      - cryolo
      - Topaz



# Data processing

- Particle sorting
  - Image statistics (XMIPP's sort function)
  - Image classification
    - 2D classification
    - 3D classification
    - Common lines sorting
    - Machine learning methods
      - cryoDRGN
      - ROME
      - Manifold embedding

# 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

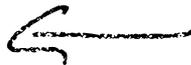
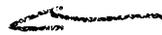
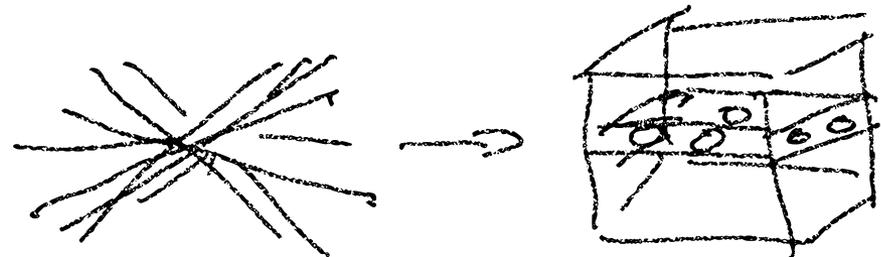
- Random Conical Tilt

- EMAN2
    - Sparx
    - Spider
    - XMIPP

- Tomography

- IMOD
    - EMAN2
    - XMIPP
    - Bsoft

- Tilt pair test



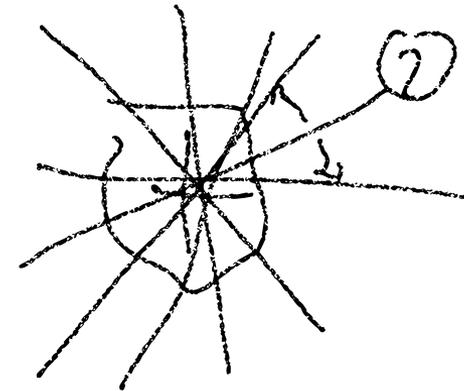
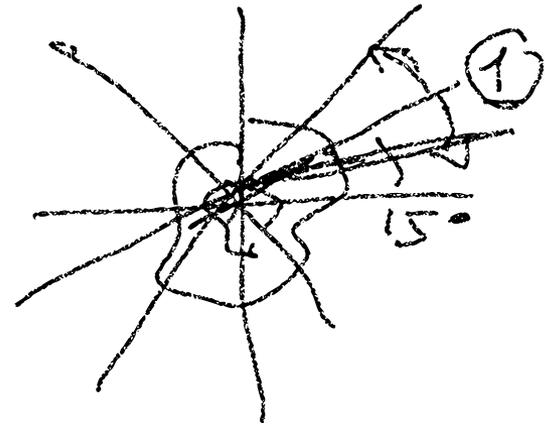
# Data processing

- Image classification

- 3D classification

- Xmipp ML3D —
    - Relion 3D —→
    - Frealign —
    - Bsoft
    - Cryosparc —

*Fast*



# 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

