Winter 2020
EM Course

Single-particle workflow

Amedee des Georges
Movie alignment

• Alignment of all frames to their average

Movie alignment

- Sub-frame alignment
Movie alignment

• Sub-frame alignment
Movie alignment

- Per-particle alignment
  - Relion “polishing”
    - alignment to a reference
    - estimation of contrast loss per frame

- alignparts_lmbfgs (Rubinstein/cryoSPARC):
  - alignment to self.
  - No re-estimation of contrast loss per frame.
Movie alignment

- Per-particle alignment.
Movie alignment

- Contrast loss and radiation damage correction
CTF estimation

• Contrast transfer function correction
  ➢ Estimating the defocus value of a micrograph
CTF estimation

• Contrast transfer function correction
  ➢ Estimating the defocus value of a micrograph
CTF estimation

- Contrast transfer function correction
  - Critical for resolution!

Fig. 5 Defocus spread envelope functions at 300kV. Envelope functions calculated according to Frank (1973) and Wade and Frank (1977) with the SPIDER command TF D.
CTF estimation

- Contrast transfer function correction
  - Estimating defocus per particle
CTF estimation

• Contrast transfer function correction

Estimating defocus per particle

Tegunov and Cramer, Bioxiv 2018
CTF estimation

• Contrast transfer function correction
  ➢ Correction of higher order aberrations
    o At the stage of 3D refinement.
Particle picking

• Deep learning algorithms win over all.
Particle picking

- Deep learning algorithms win over all.

Tegunov and Cramer, Biorxiv 2018
Particle picking

- Deep learning algorithms win over all.
  - Warp
  - crYOLO
  - Topaz

Tegunov and Cramer, BioRxiv 2018
Initial cleaning of particles

• Many ways of doing it

  ➢ Sorting based on statistics
  ➢ 2D classification
  ➢ Multi-reference ab-initio
  ➢ 3D classification
Initial cleaning of particles

• Many ways of doing it

- Sorting based on statistics
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Initial cleaning of particles

• Many ways of doing it
  - Sorting based on statistics
  - 2D classification
  - Multi-reference ab-initio
  - 3D classification
Aligning particles in 3D

- Precision and accuracy -> critical for resolution AND classification.
Aligning particles in 3D

- Precision and accuracy -> critical for resolution AND classification.

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Table 2 Angular sampling necessary to obtain a given resolution according to the Shannon theorem.

Des Georges et al., 2013. Applied and Numerical Harmonic Analysis
Aligning particles in 3D

- Precision and accuracy -> critical for resolution AND classification.
Aligning particles in 3D

• Local refinement
Aligning particles in 3D

- Local refinement

Oliver Clarke et al., Unpublished
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