SIMONS ELECTRON MICROSCOPY CENTER

NYSBC

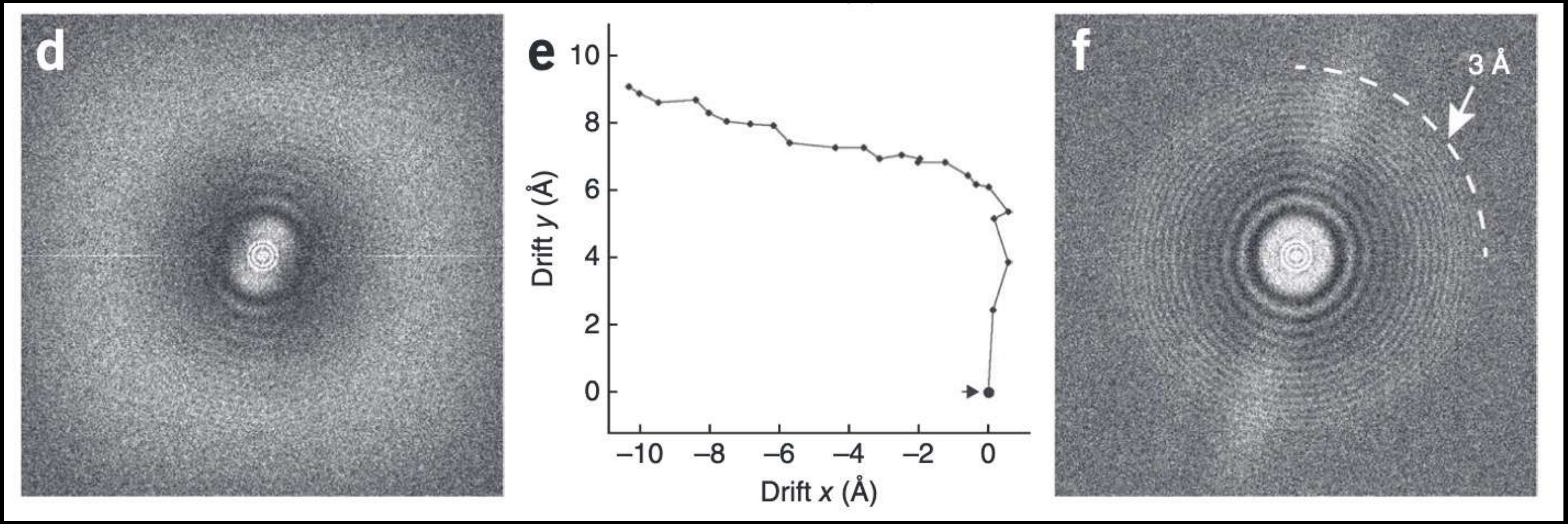
Winter 2023 EM Course

Single-particle workflow

Amedee des Georges

Movie alignment

- Alignment of all frames to their average

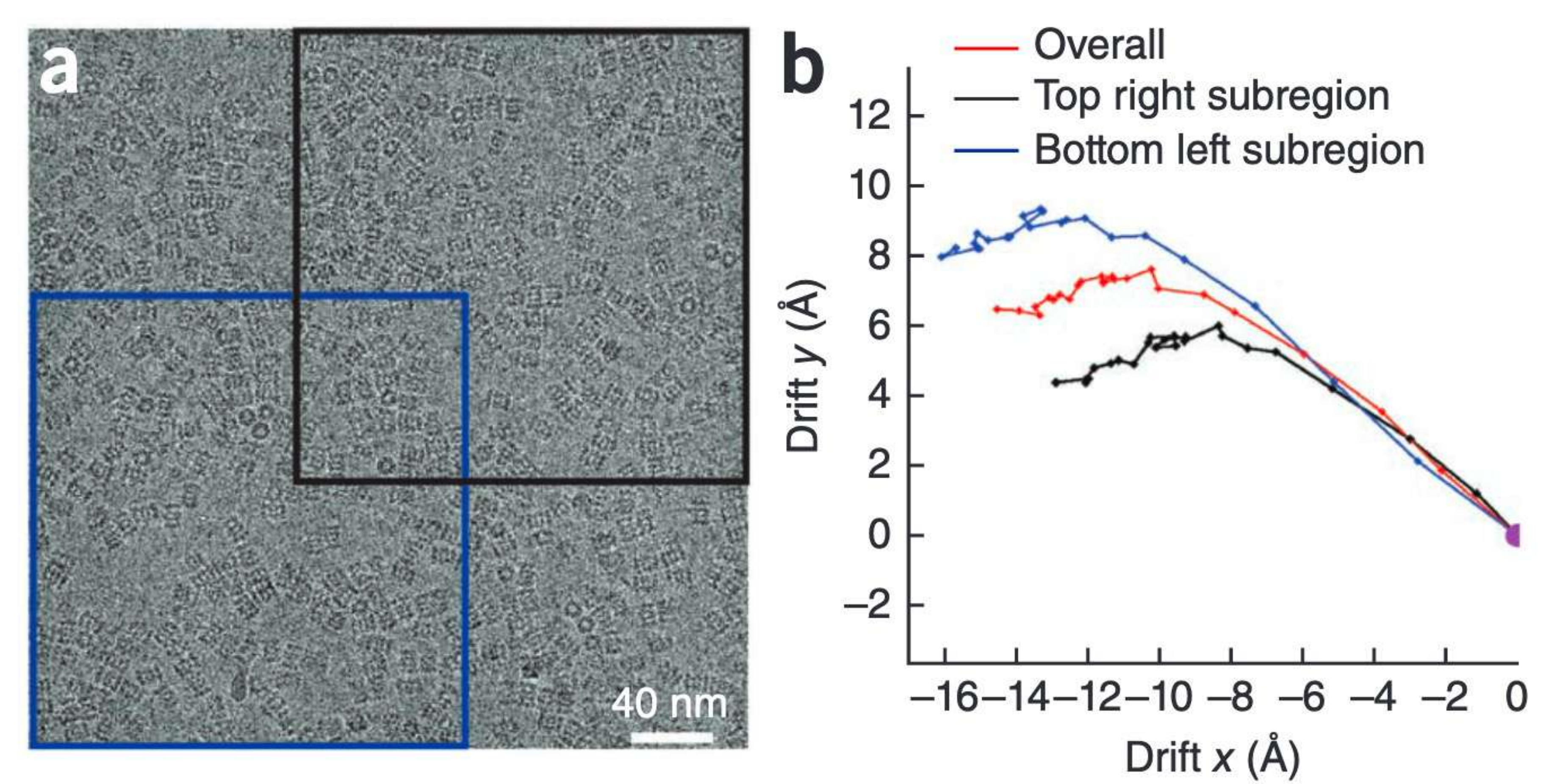


Li, X. et al. Nat. Methods 10, 584–590 (2013).

Li, X. et al. Nat. Methods 10, 584–590 (2013).

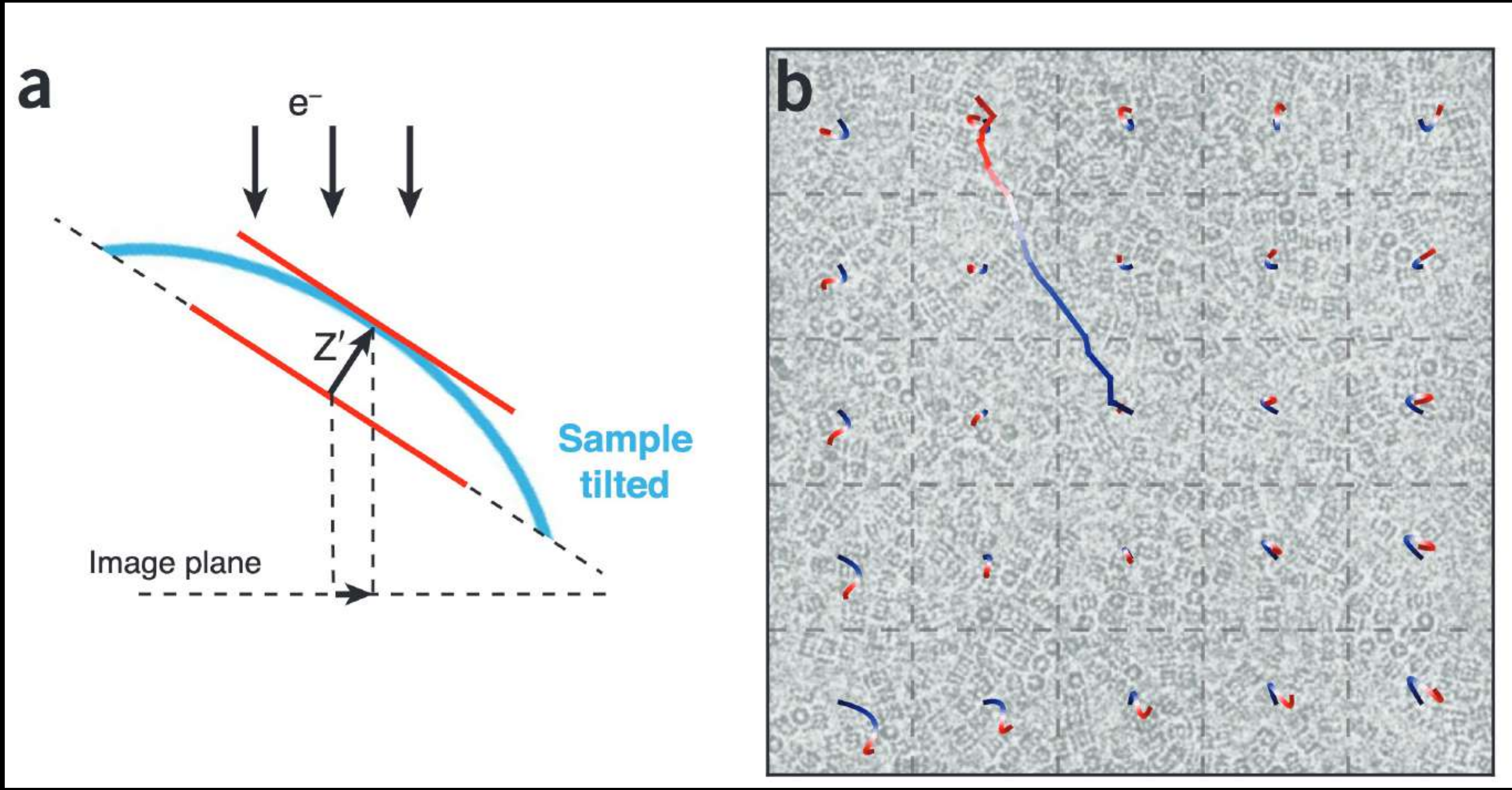
Movie alignment

- Sub-frame alignment



Movie alignment

- Sub-frame alignment



Zheng et al. N. Meth 2019

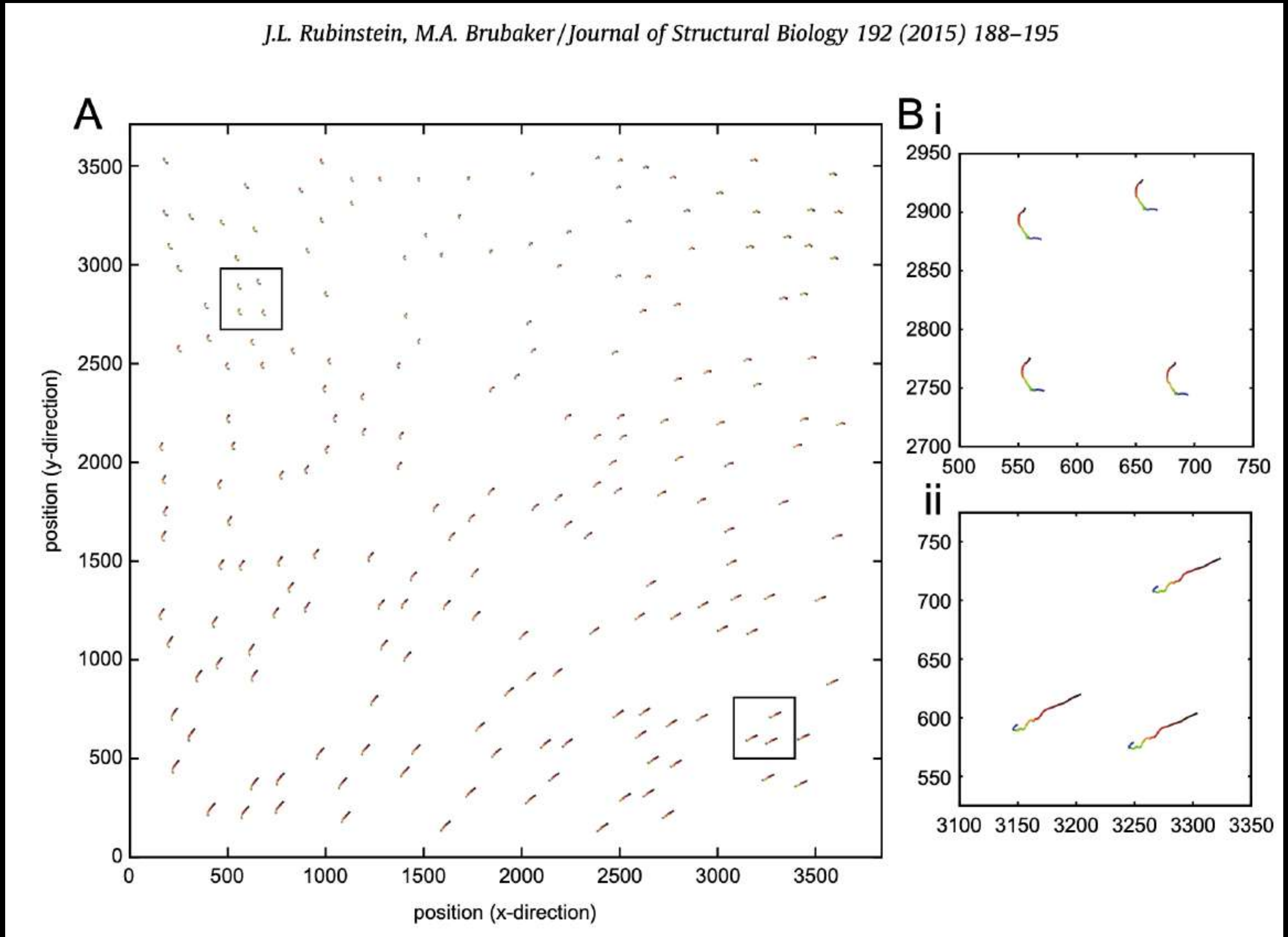
Movie alignment

- 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.

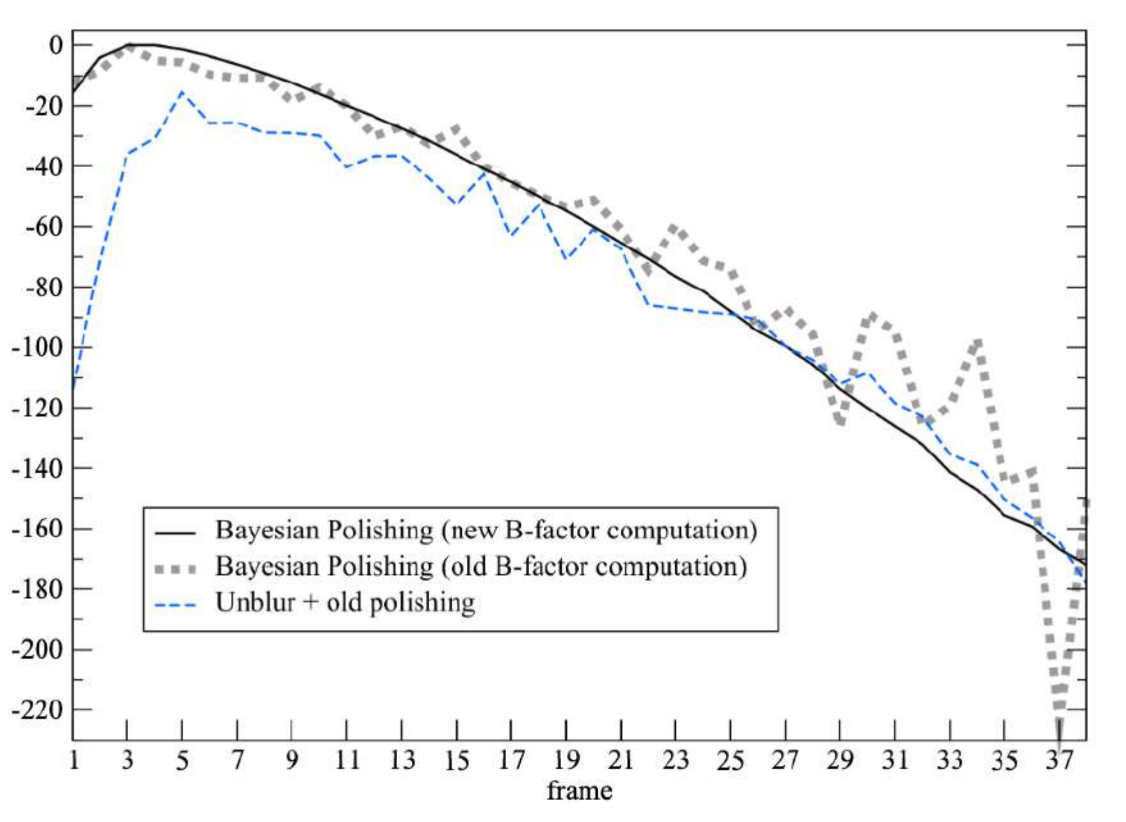
Movie alignment

- Per-particle alignment.



Movie alignment

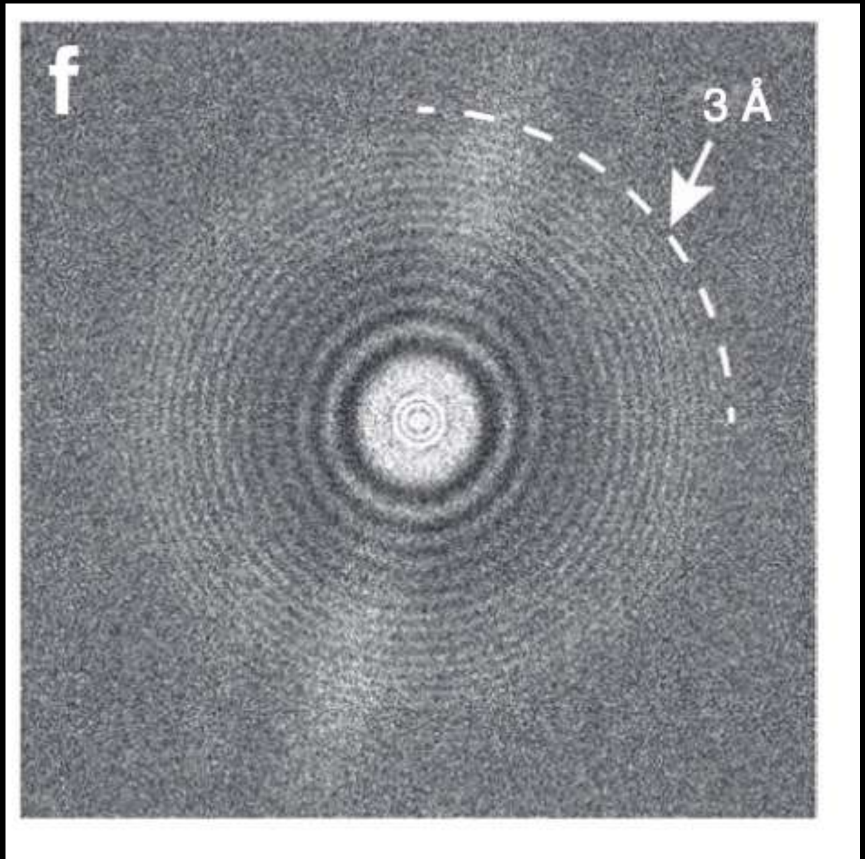
- Contrast loss and radiation damage correction



ZIVANOV, TANAKE AND SCHERES, IUCRJ 2019

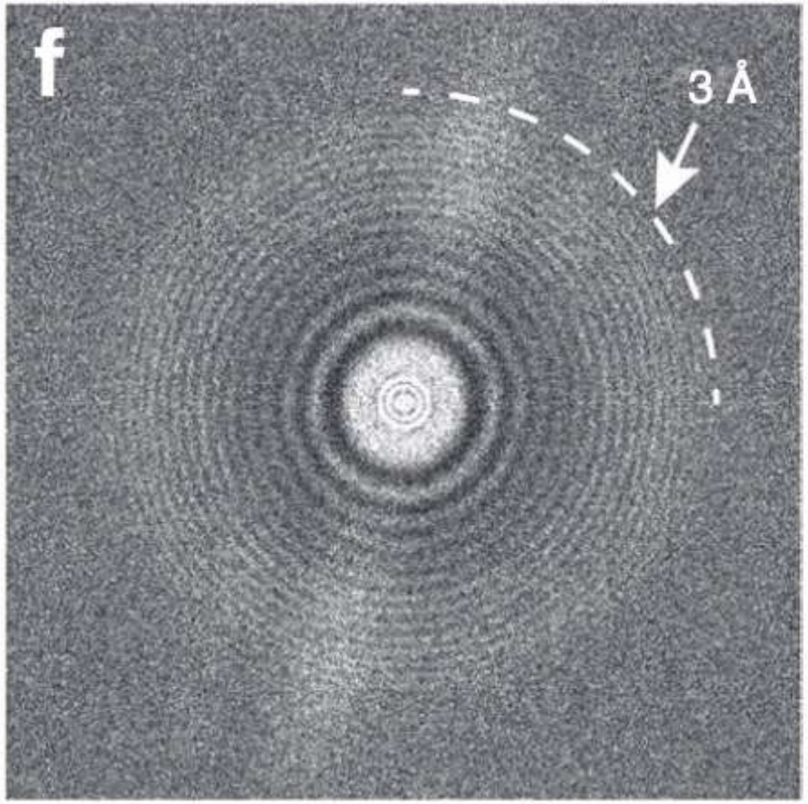
CTF estimation

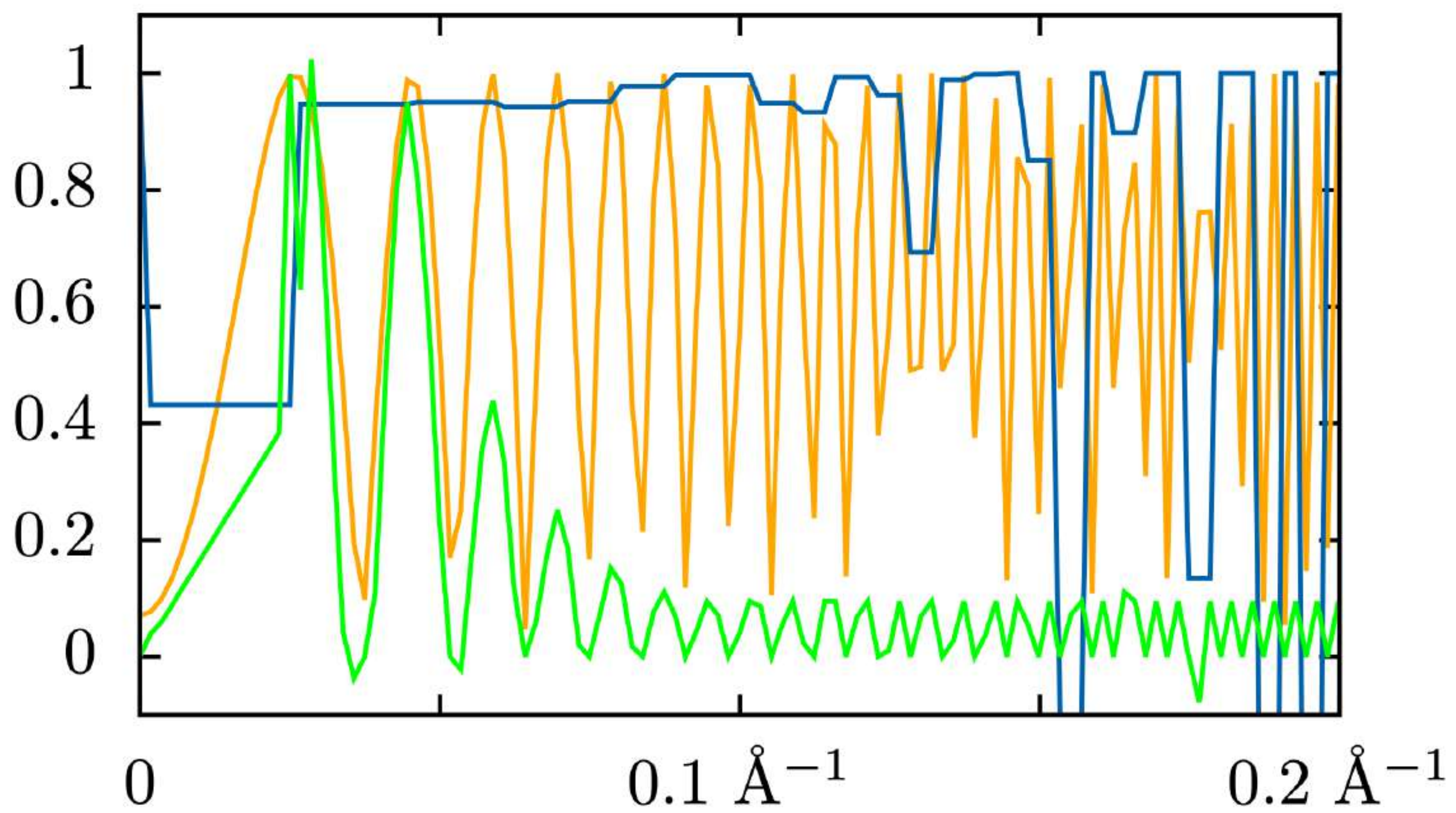
- Contrast transfer function correction
  - Estimating the defocus value of a micrograph



CTF estimation

- Contrast transfer function correction
  - ➢ Estimating the defocus value of a micrograph





CTF estimation

- Contrast transfer function correction
  - ➢Critical for resolution!

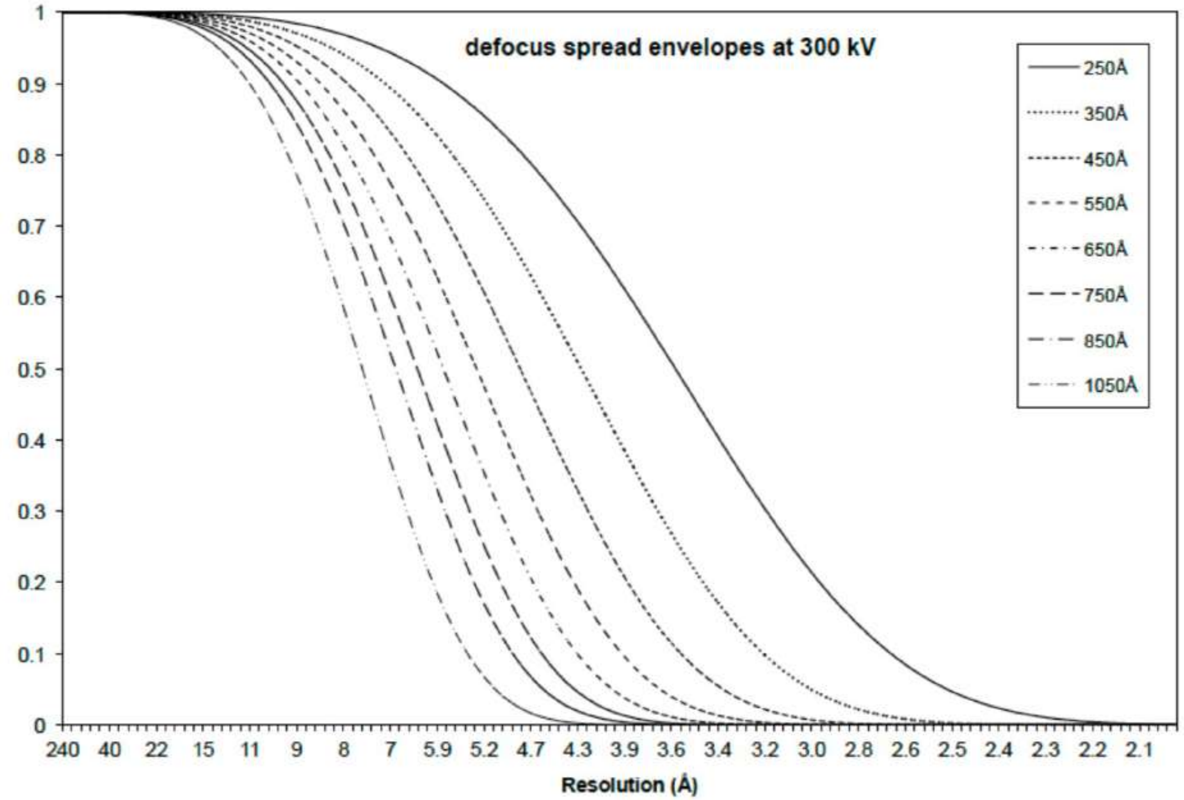
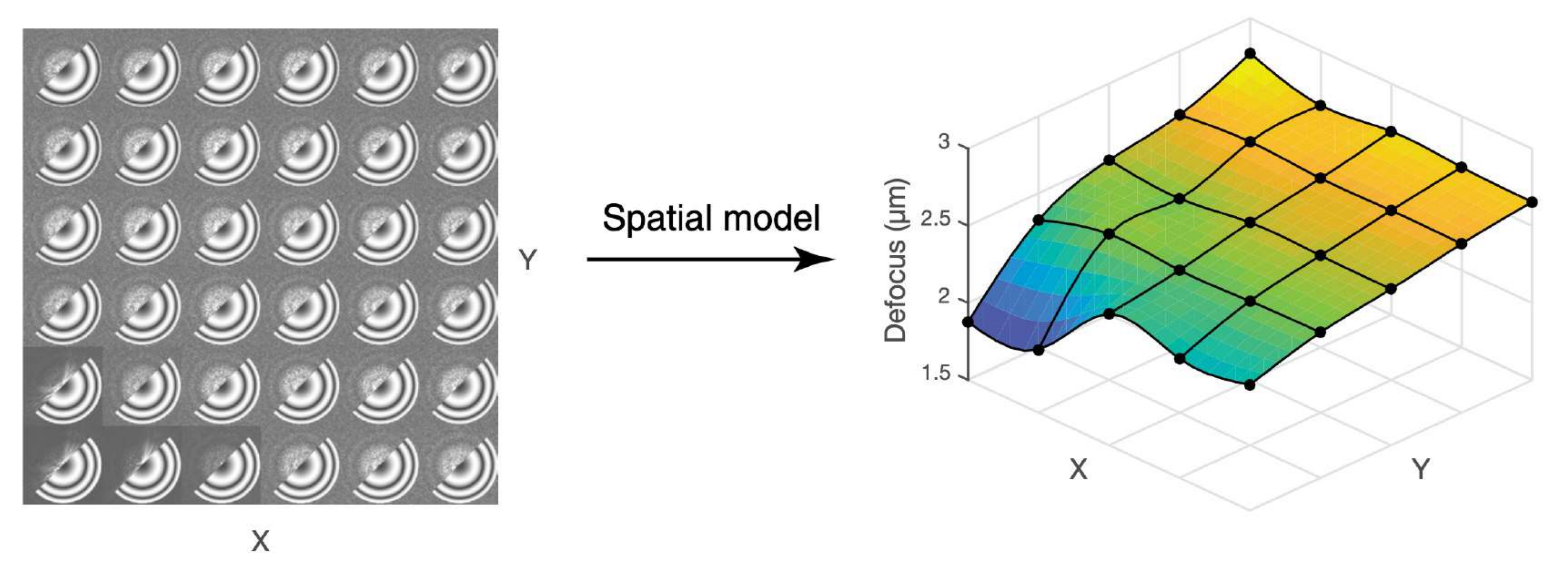


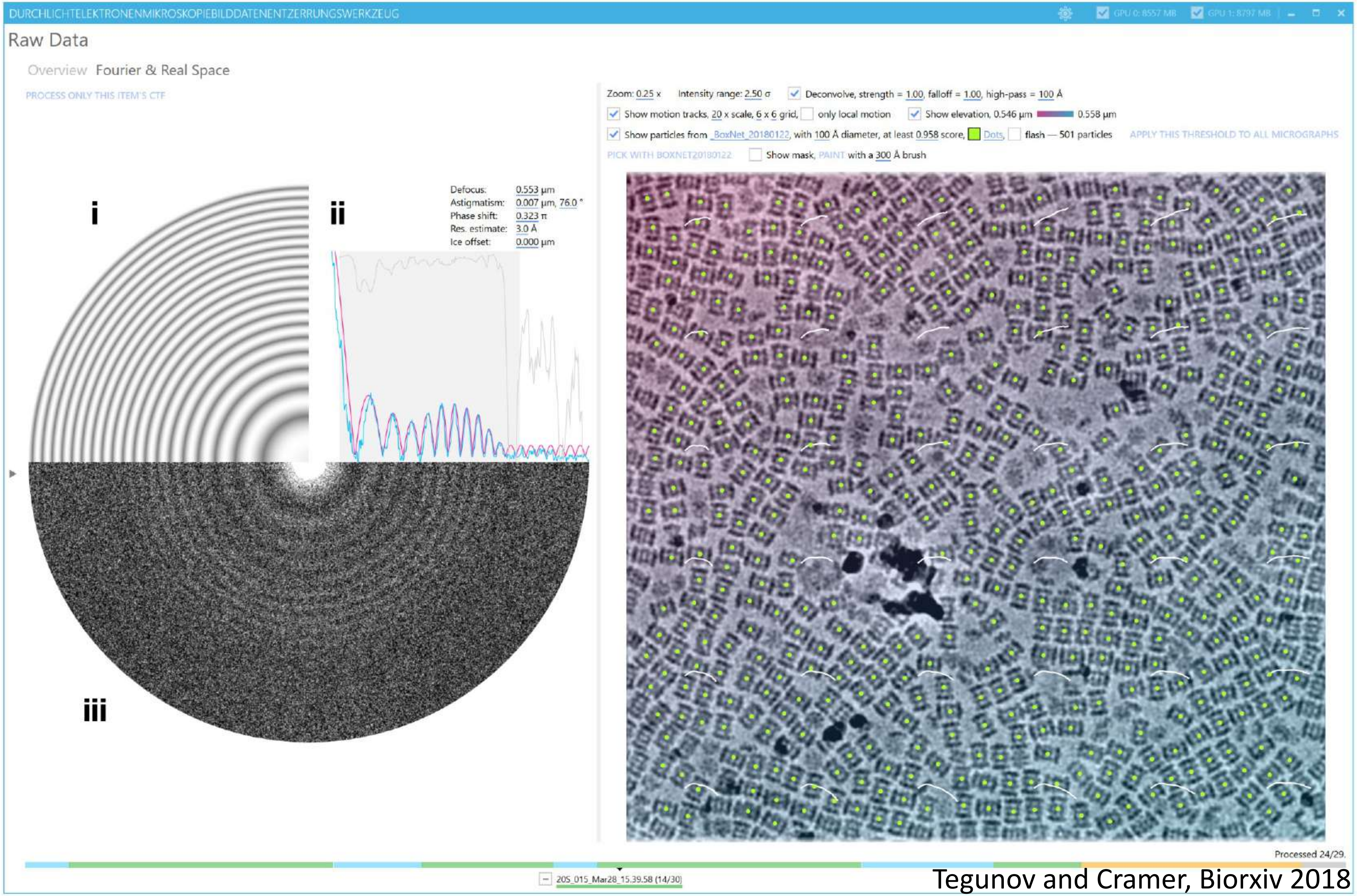
Fig. 5 Defocus spread envelope functions at 300kV. Envelope functions calculated according to Frank (1973) and Wade and Frank (1977) with the SPIDER command TF D.

CTF estimation

- Contrast transfer function correction
  - ➢Estimating defocus per particle



Tegunov and Cramer, Biorxiv 2018



Tegunov and Cramer, Biorxiv 2018

CTF estimation

- Contrast transfer function correction
  - Correction of higher order aberrations
    - At the stage of 3D refinement.

Particle picking

- Deep learning algorithms win over all.

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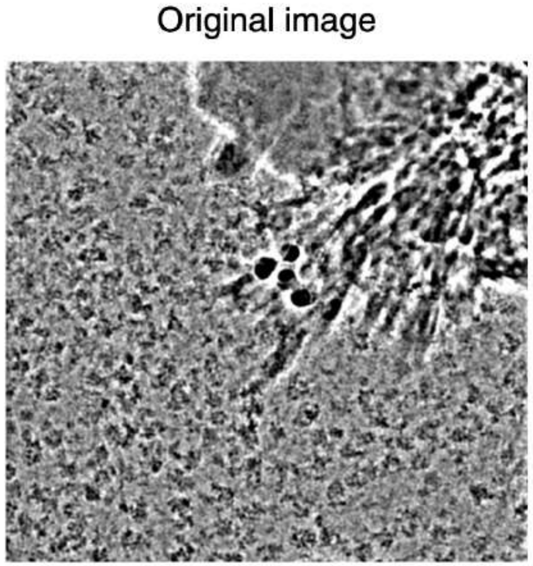
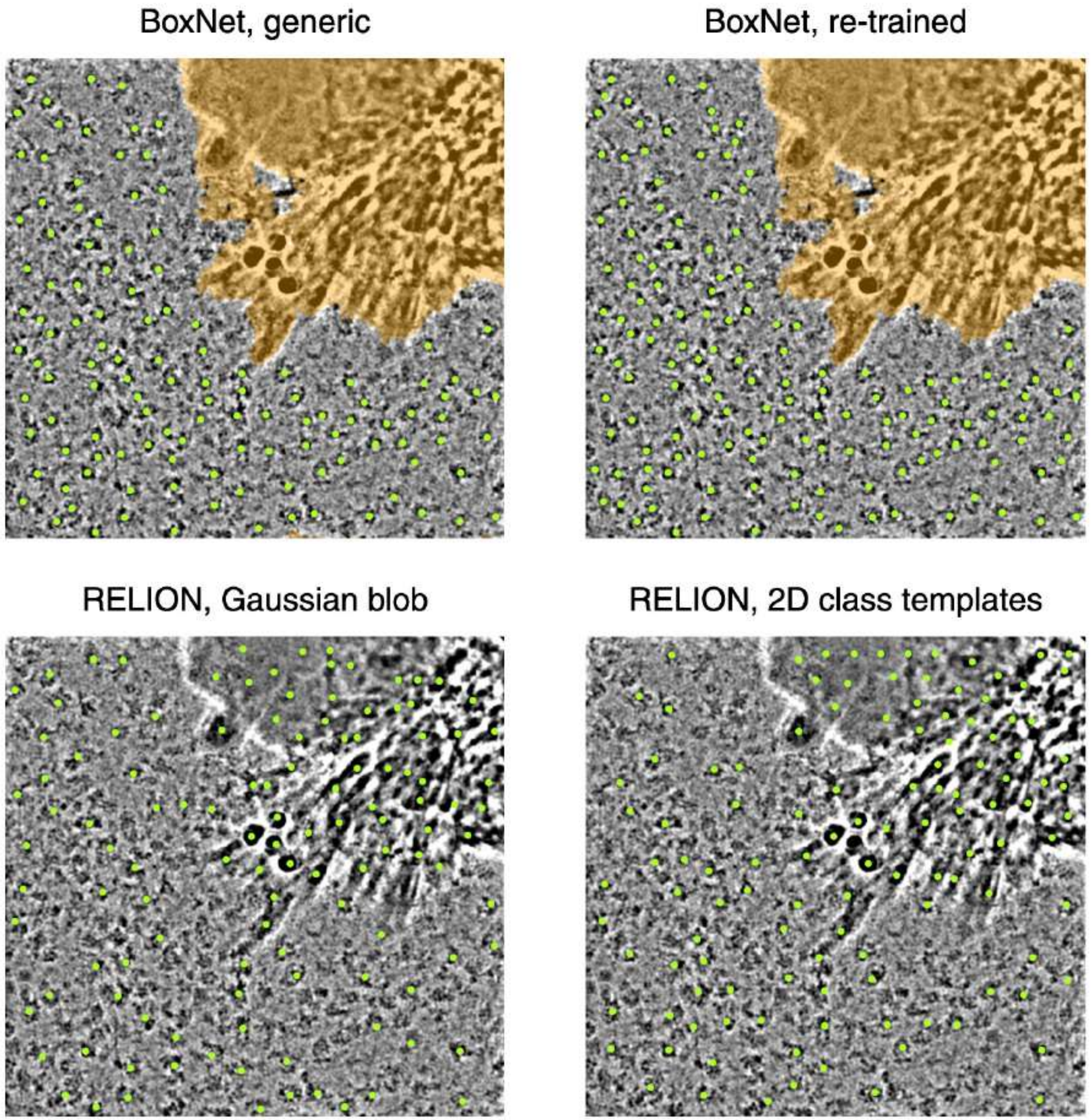
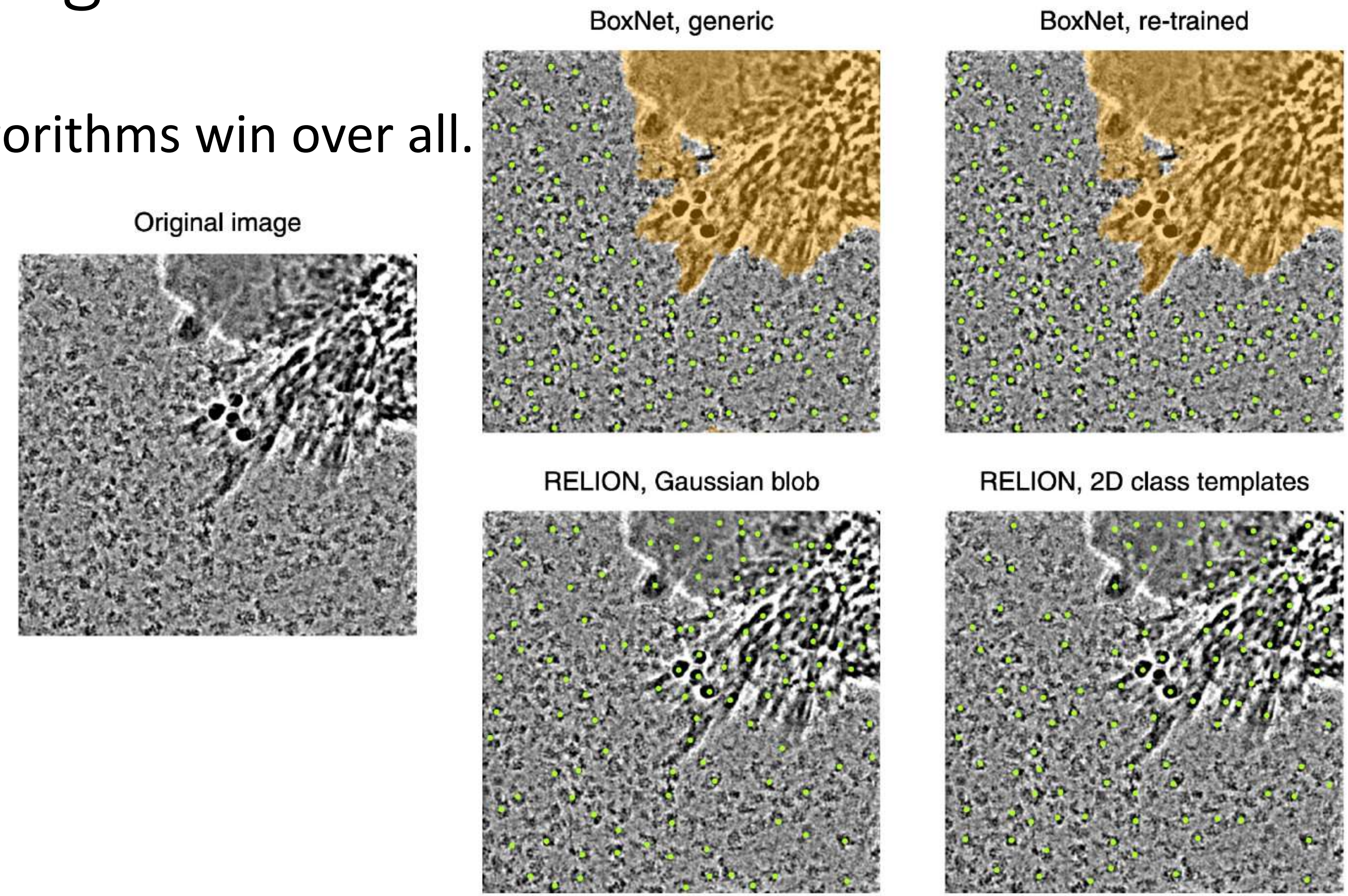




Tegunov and Cramer, Biorxiv 2018

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

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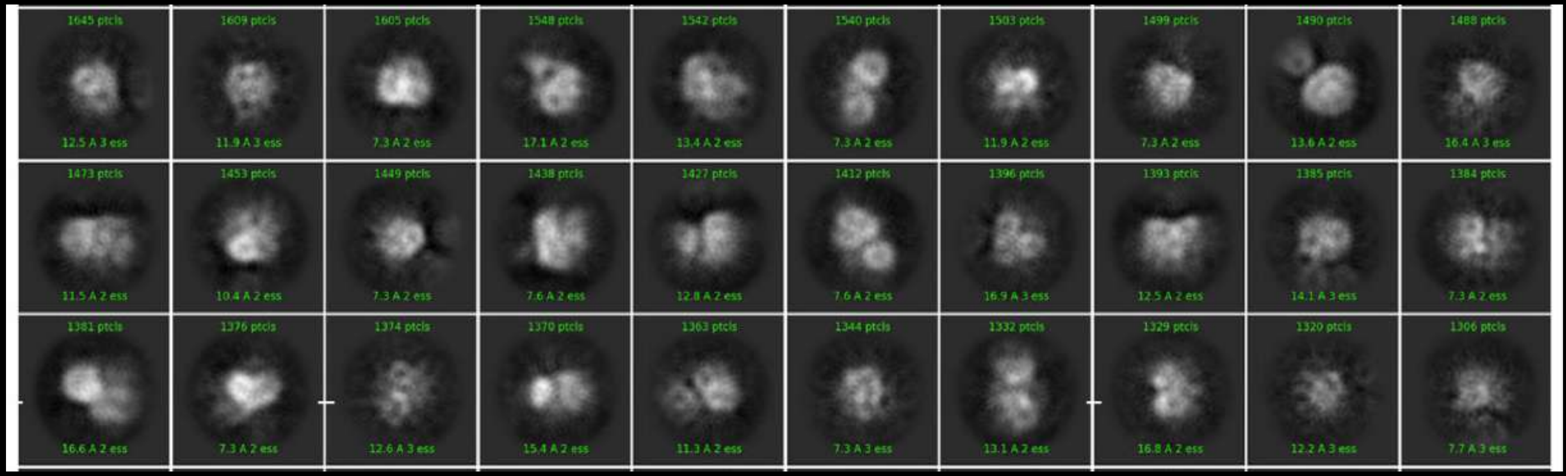

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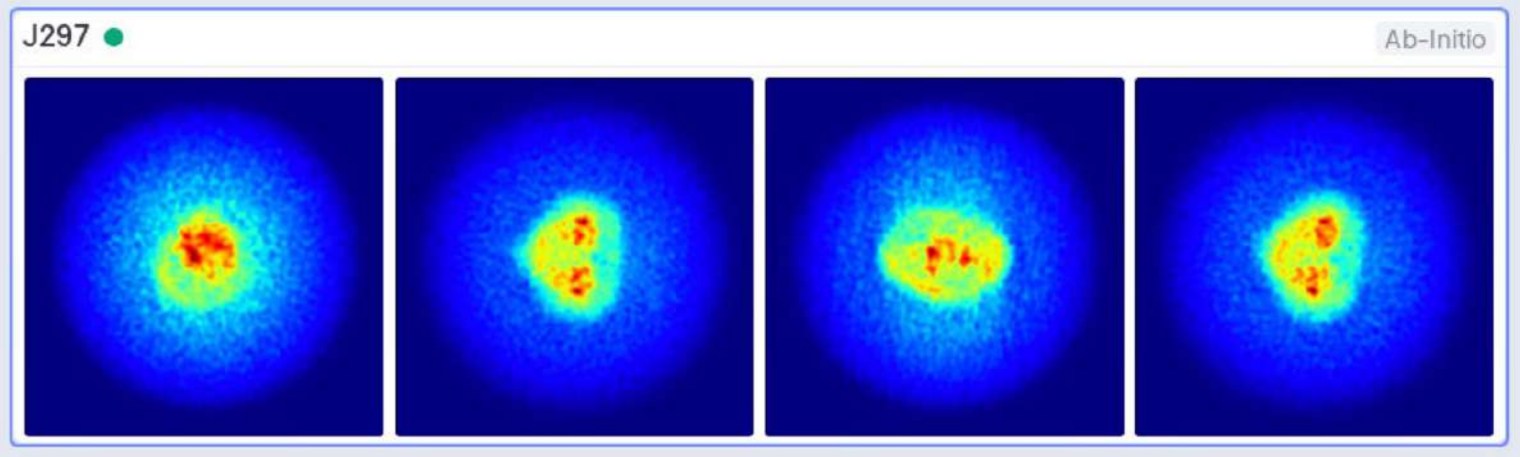

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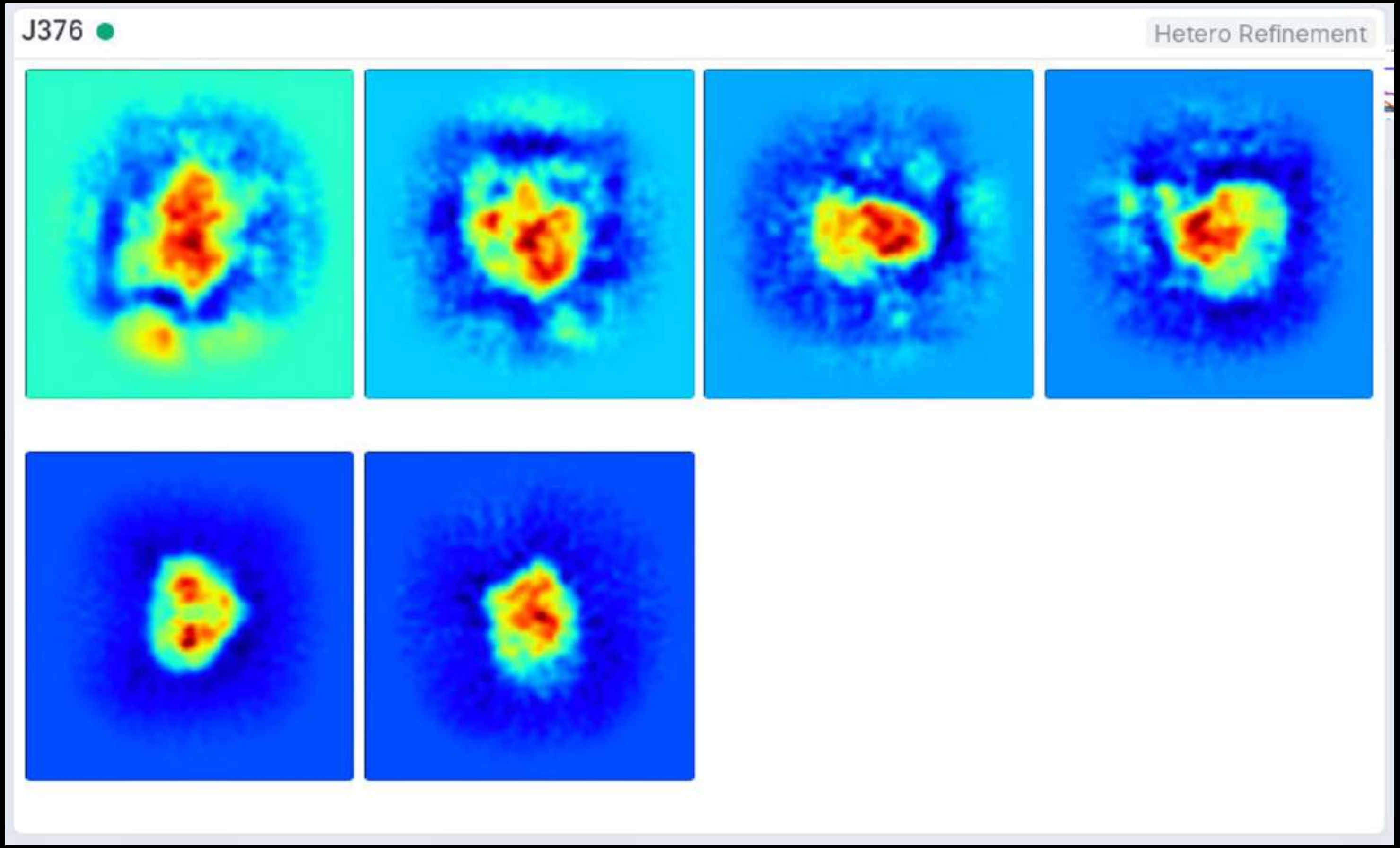
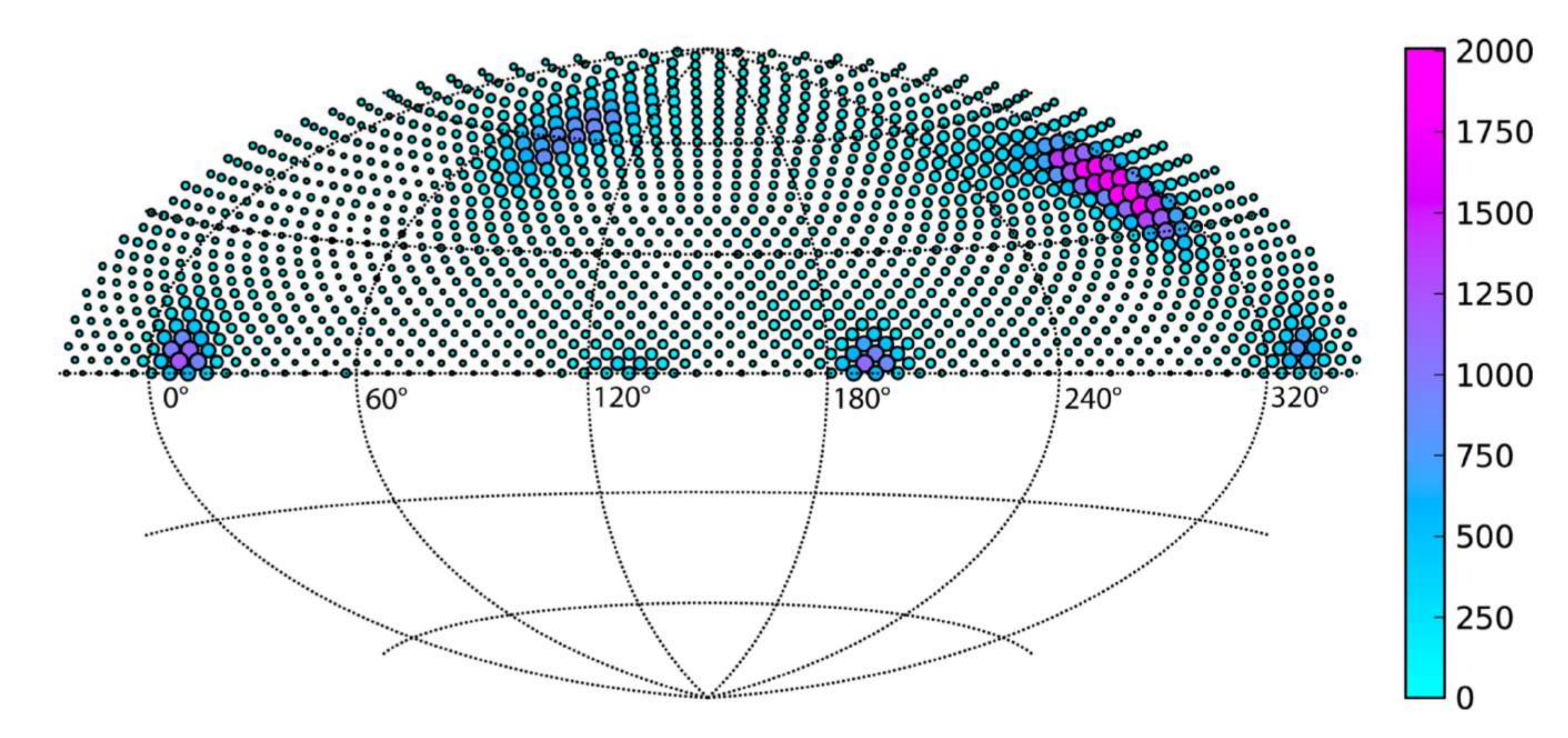


Aligning particles in 3D

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



Des Georges et al., 2013. *Applied and Numerical Harmonic Analysis*

Aligning particles in 3D

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

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

Resolution (Å)	Sampling (°)
50	8.1
30	4.9
20	3.3
15	2.5
12	2.0
10	1.6
8	1.3
6	1.0
5	0.82
4	0.65
3	0.49
2	0.33
1	0.16

Des Georges et al., 2013. *Applied and Numerical Harmonic Analysis*

Aligning particles in 3D

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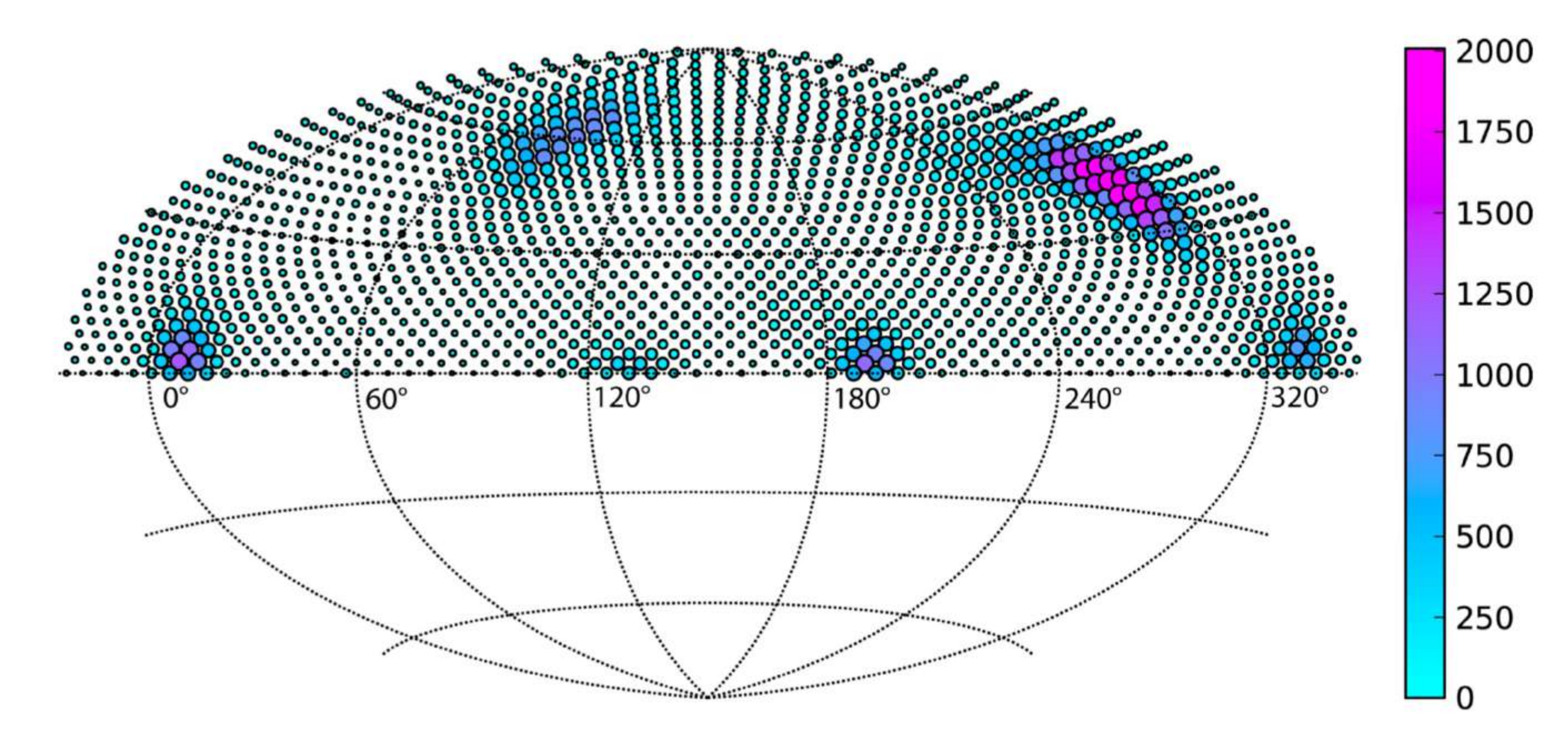
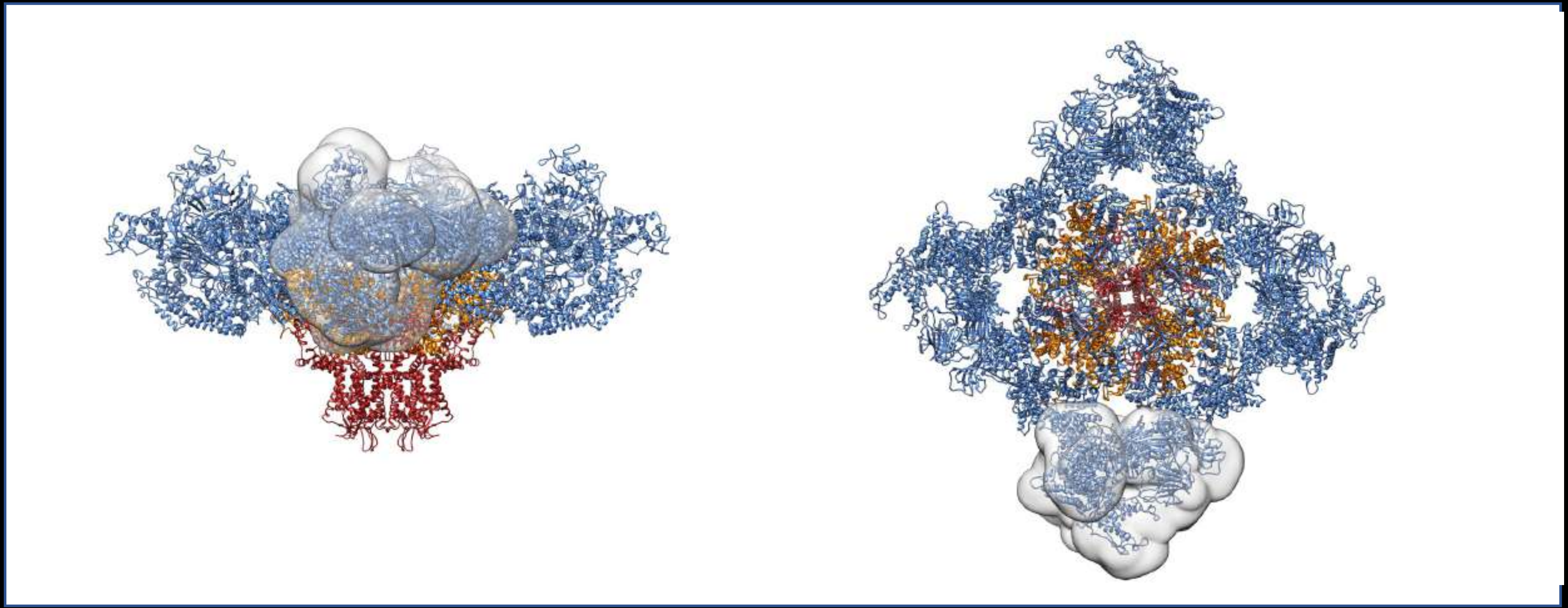


Des Georges et al., 2013. *Applied and Numerical Harmonic Analysis*

Aligning particles in 3D

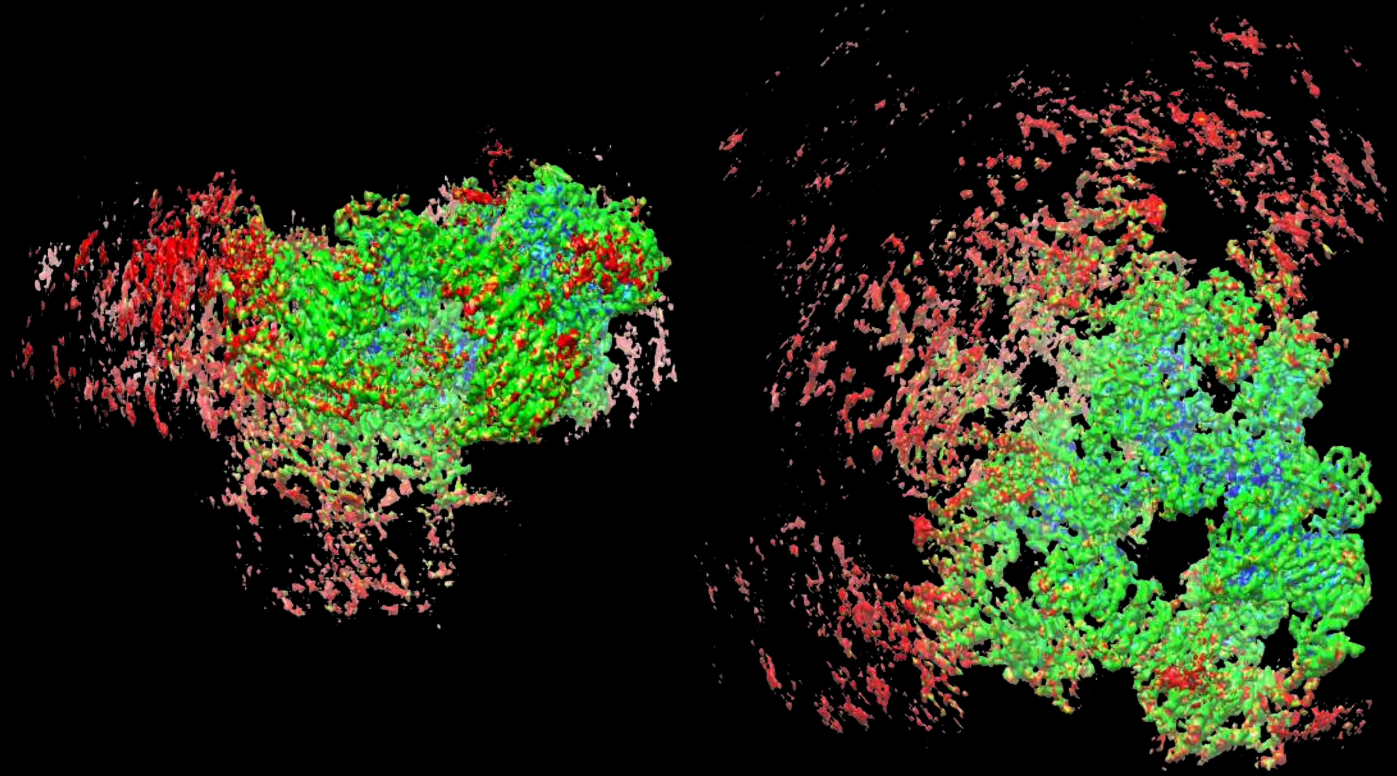
- Local refinement



Oliver Clarke, et al., Unpublished

Aligning particles in 3D

- Local refinement



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Winter 2020 EM Course

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Amedee des Georges