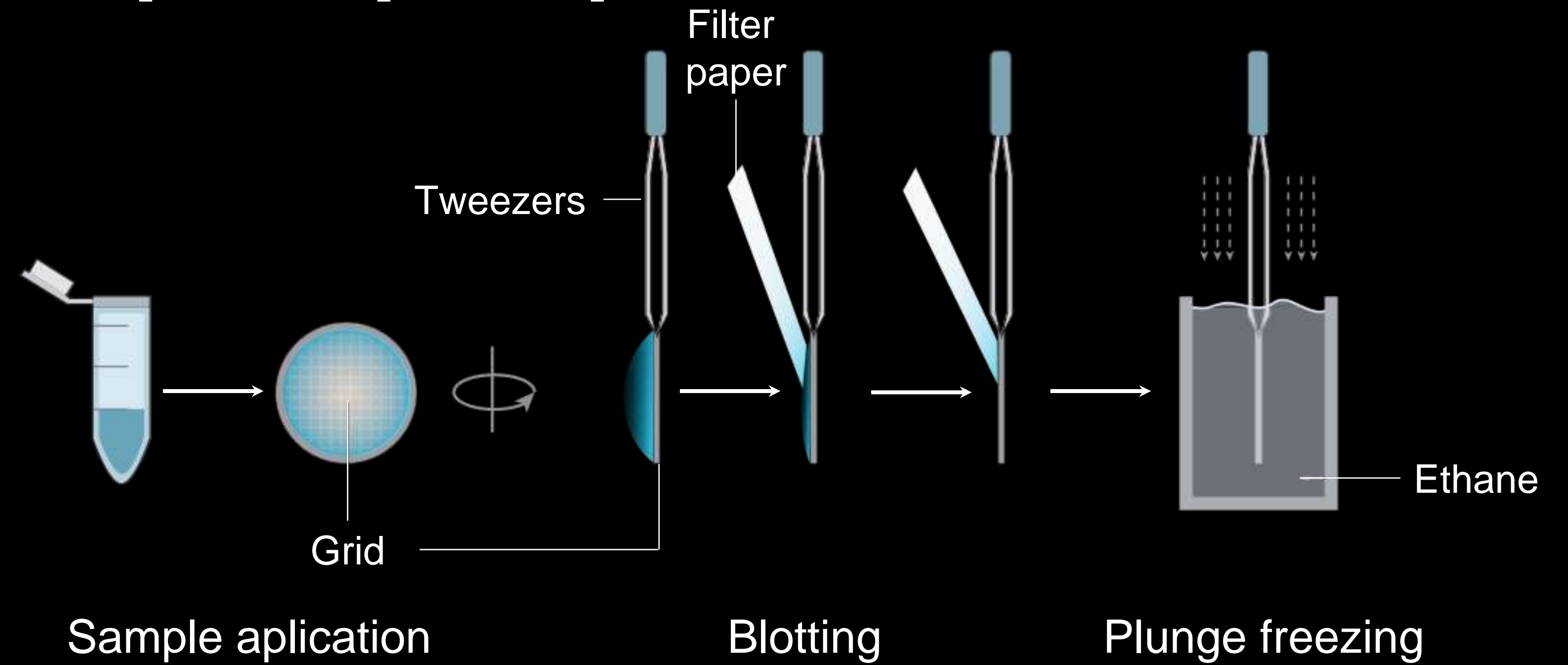


Cryo-electron microscopy of 70S ribosome

Electron Microscopy Lab, NRC Kurchatov insitute
2016

Sample preparation



Volume: 3ul

Blot force: -6; 0
Blot time: 2.5 s
Wait: 0 s
Hum: 95%
Temp: 10 C

Microscope setup

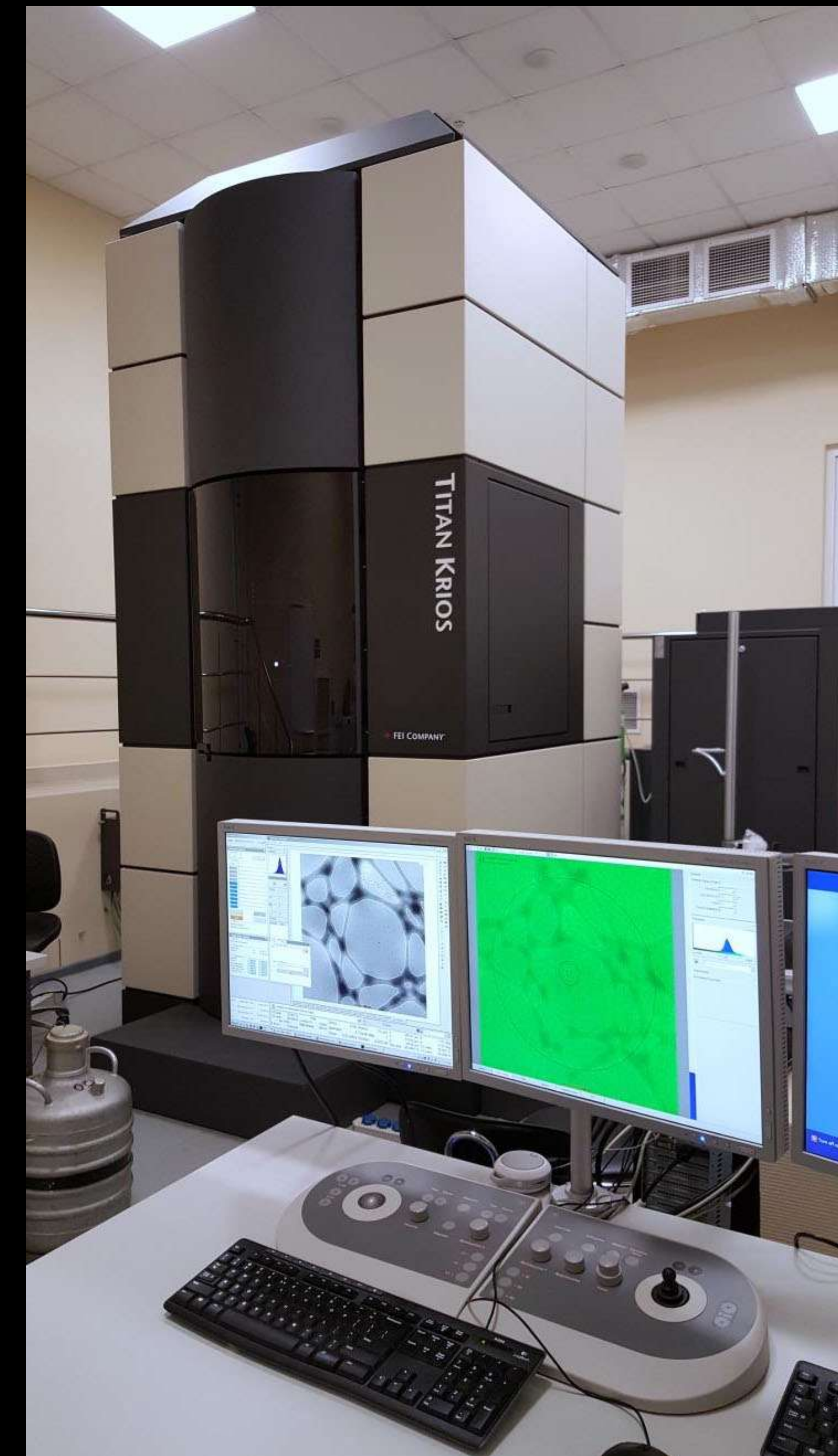
Magnification: 75 000x ; pixel size: 0.859Å

$C_1=2000$, $C_2 = 50$ (70), Obj = 100

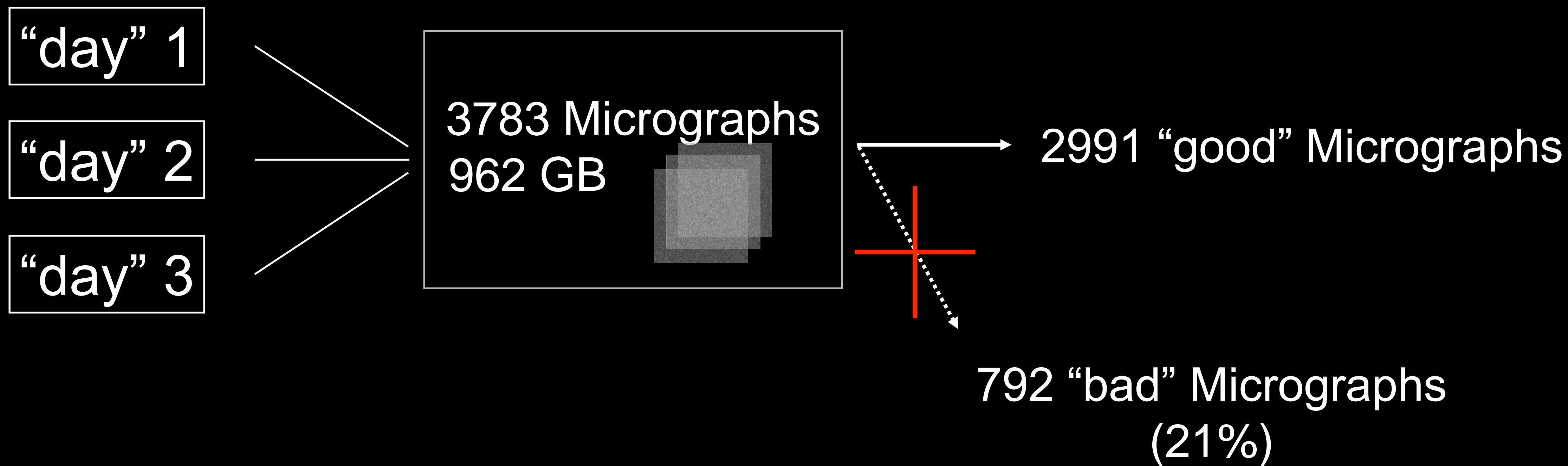
defocus range: [-0.4;-3.6:0.2]

Cs-corrector: on

dose ~ 56 e/Å²s, recorded (12 frames) ~ 44 e/Å²s



Data acquisition



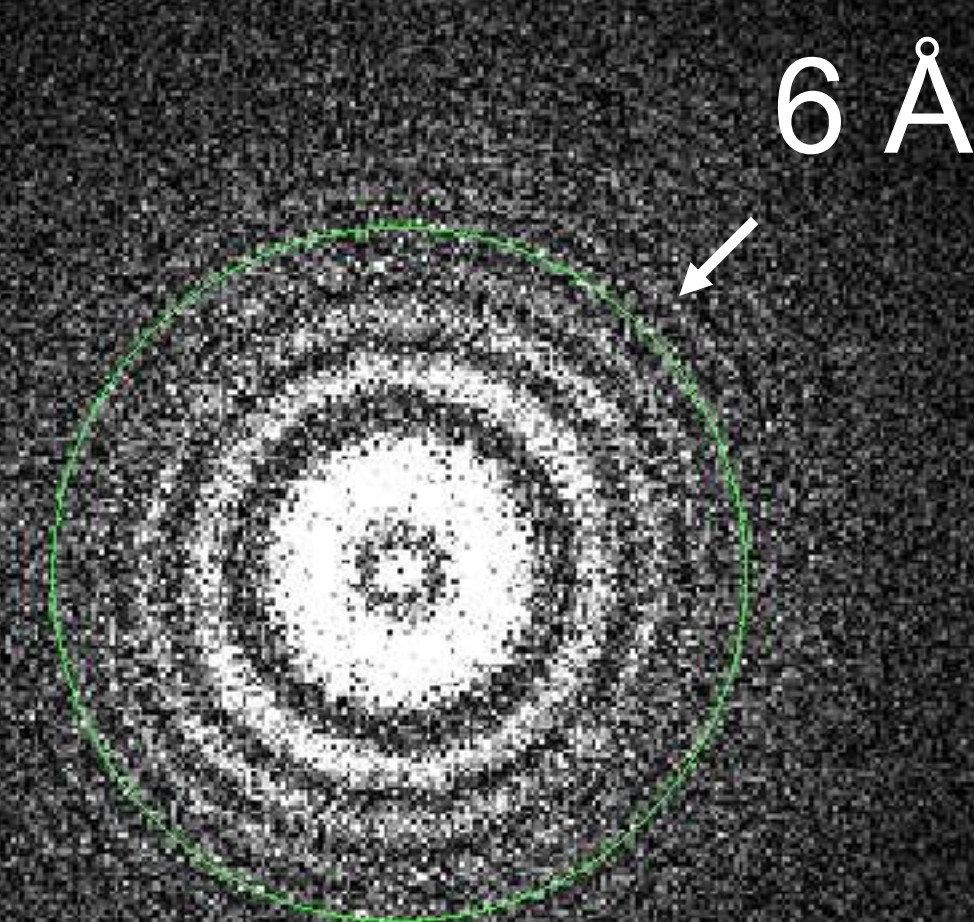
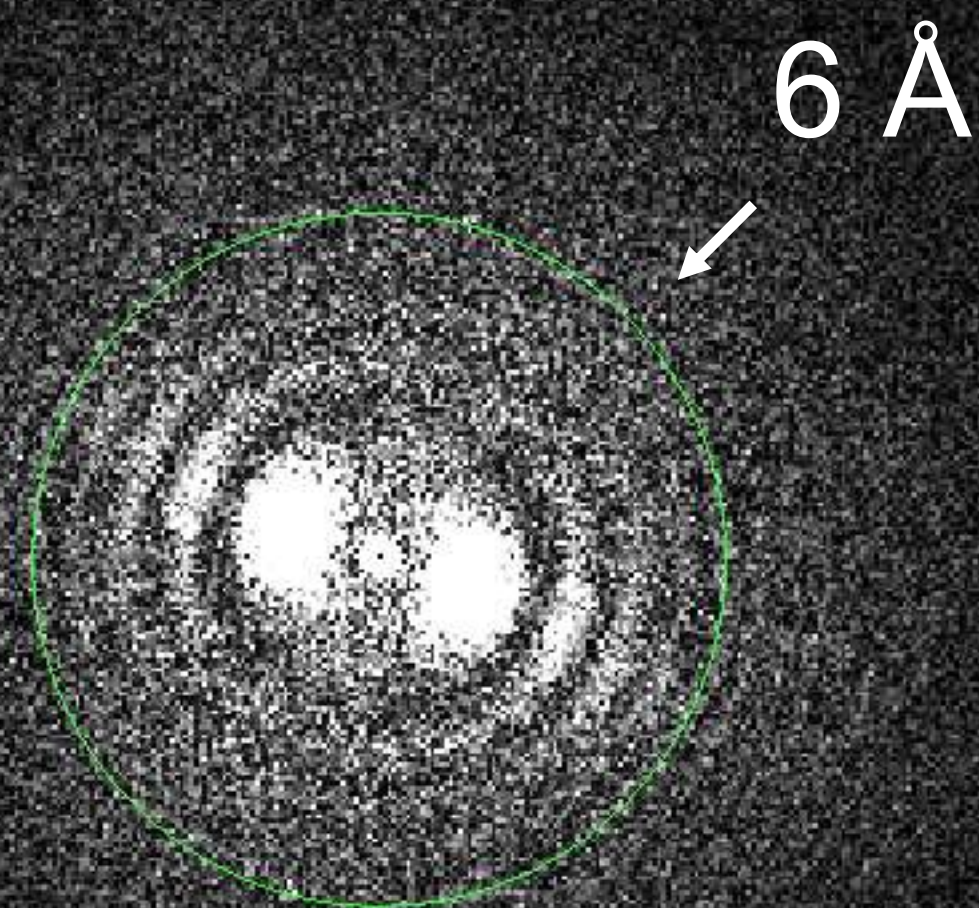
Micrograph: 7 frames in single stack, ~235MB each

Preprocessing / Motion correction

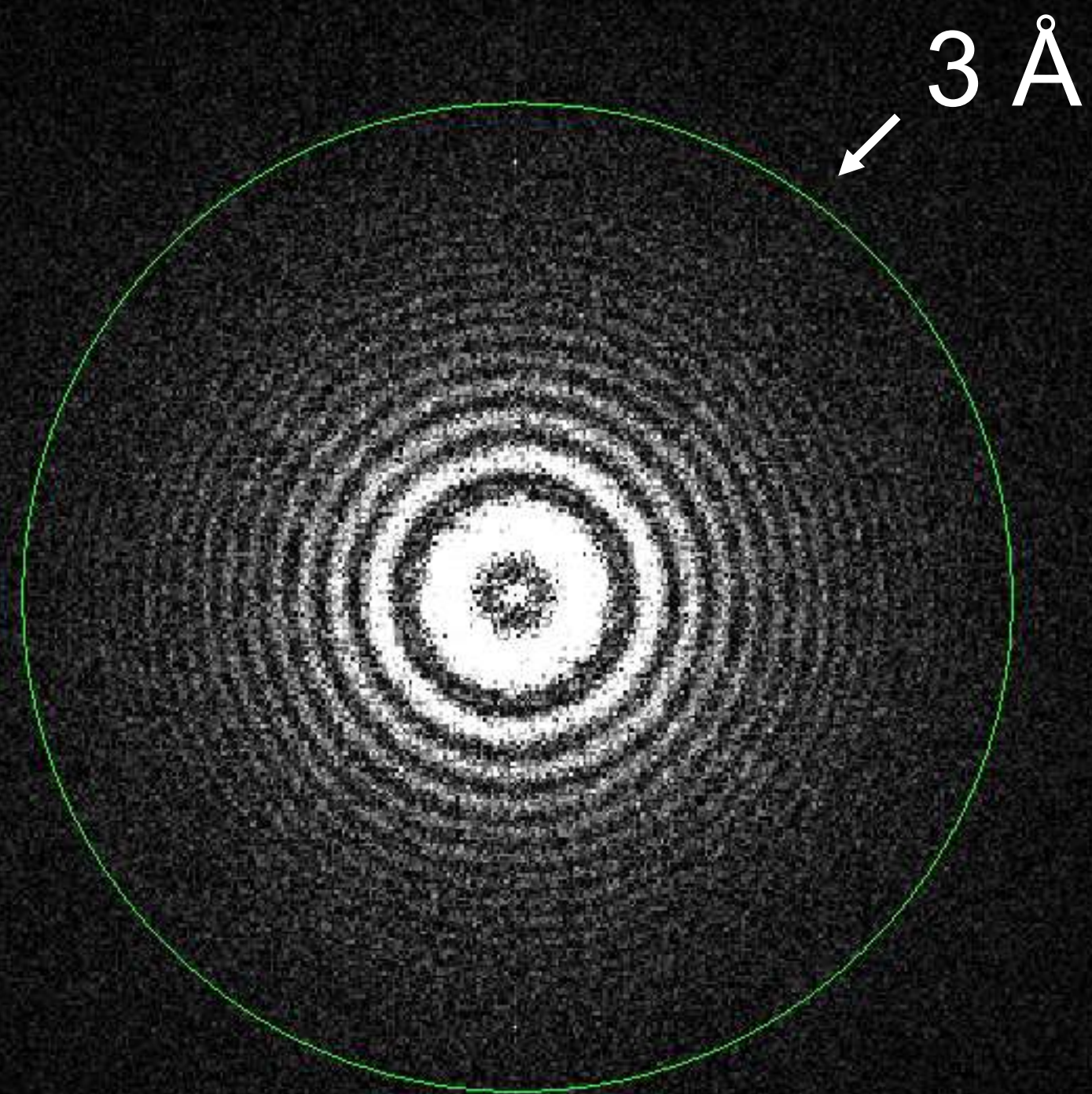
before

MotionCor2

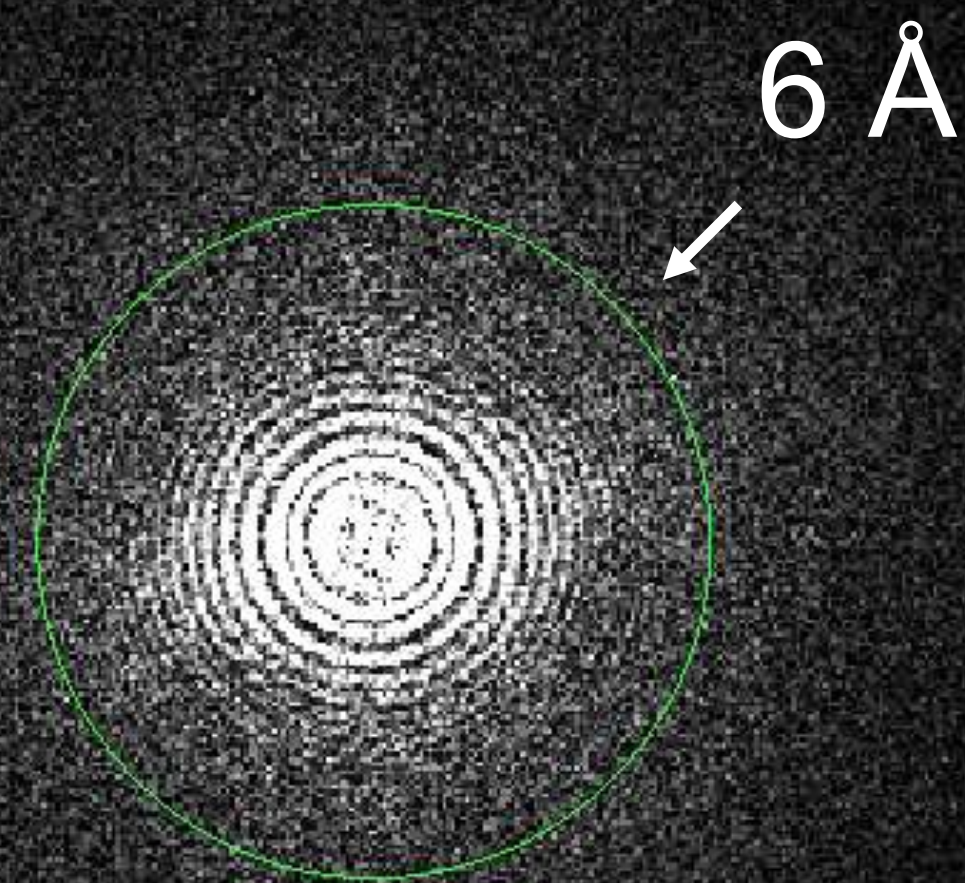
after



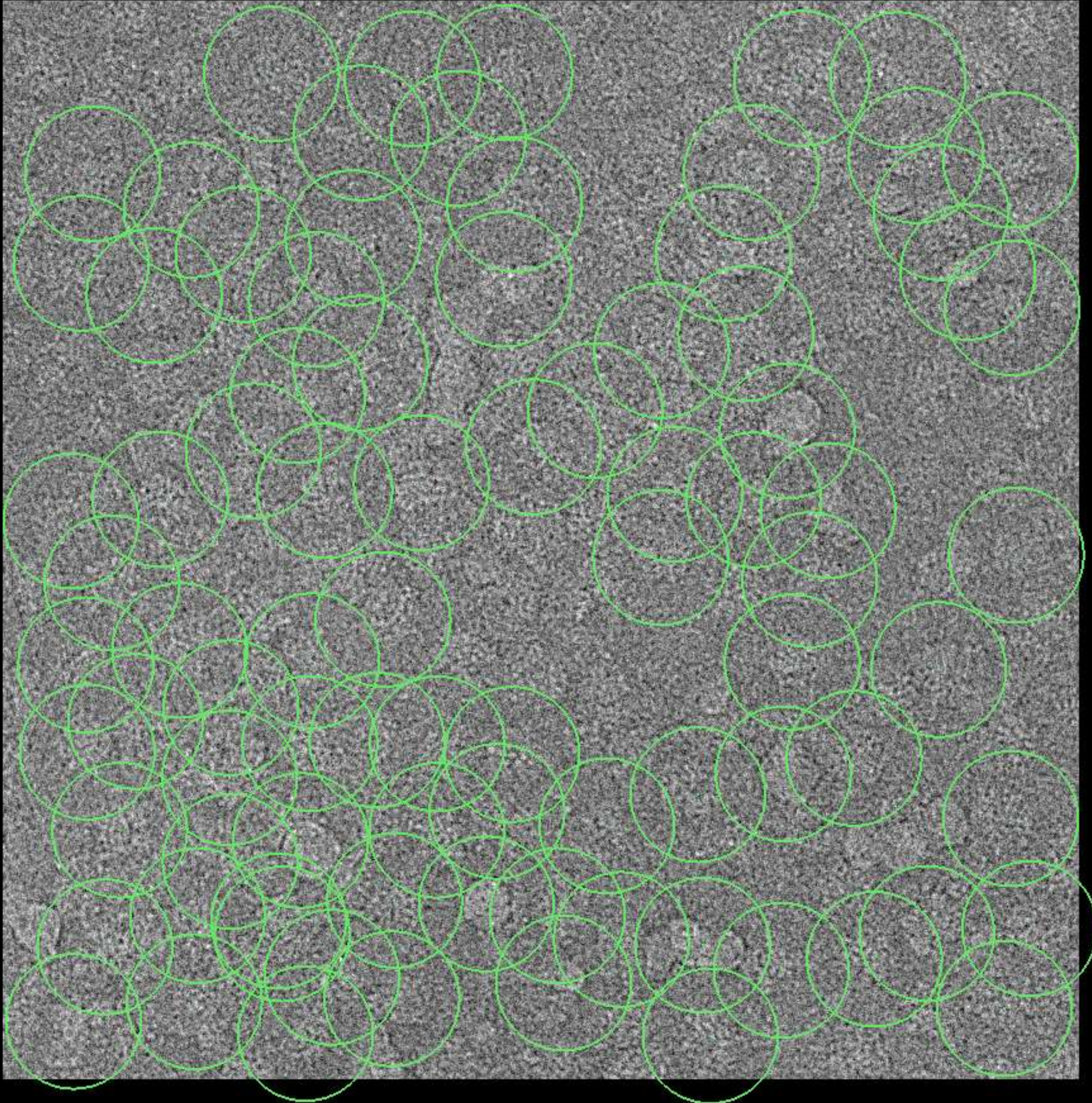
Preprocessing / Manual sorting



OK



Preprocessing / Semi-automated picking



[e2boxer.py](#)

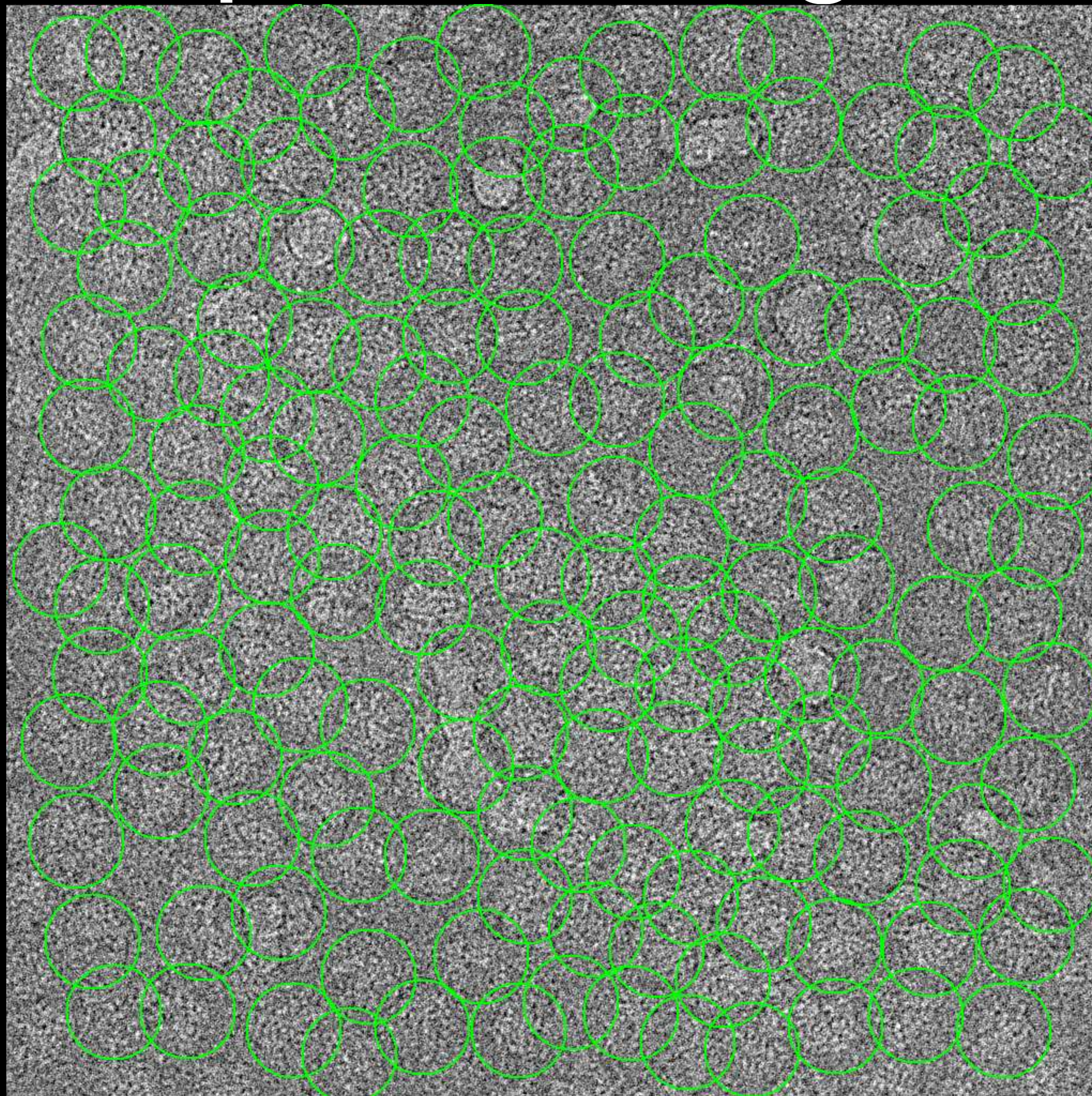
Swarm tool

1-3k particles

2D Class

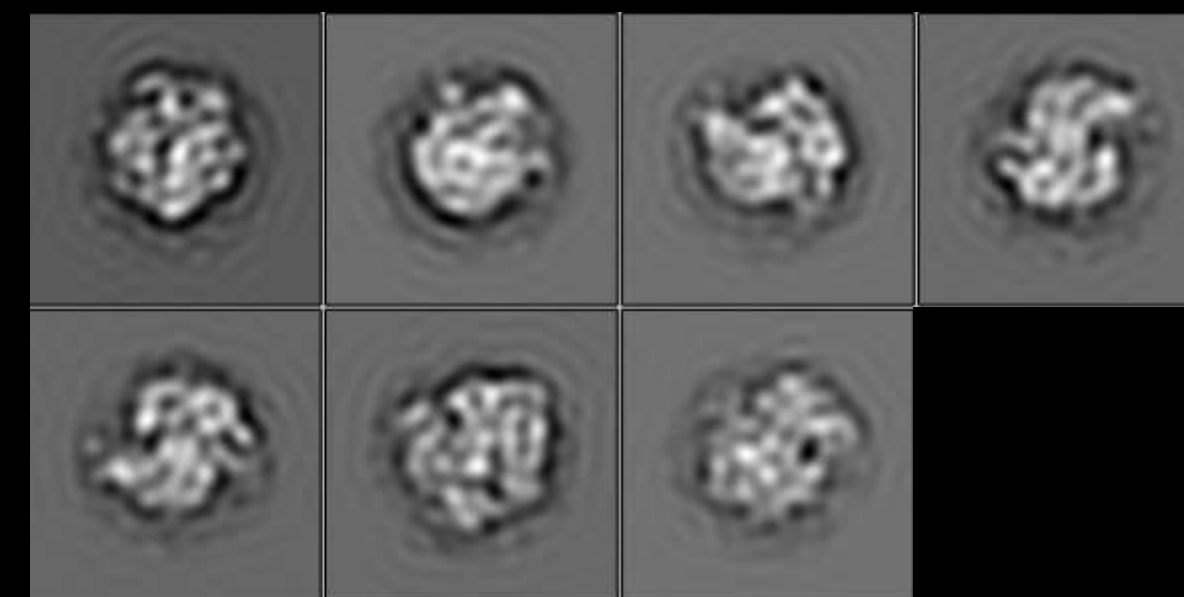
references for [Gautomatch](#)

Preprocessing / Auto picking



Gautomatch

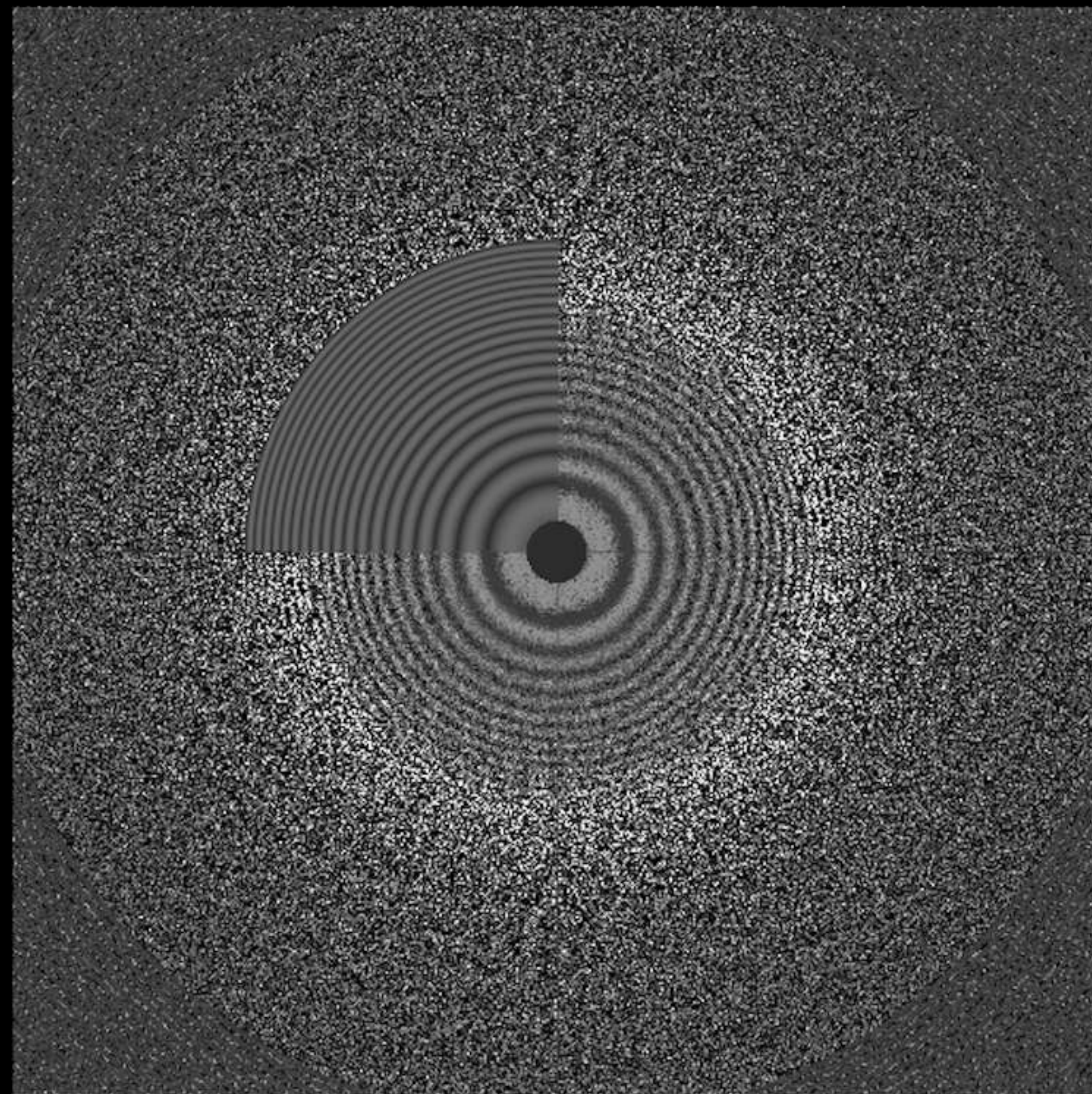
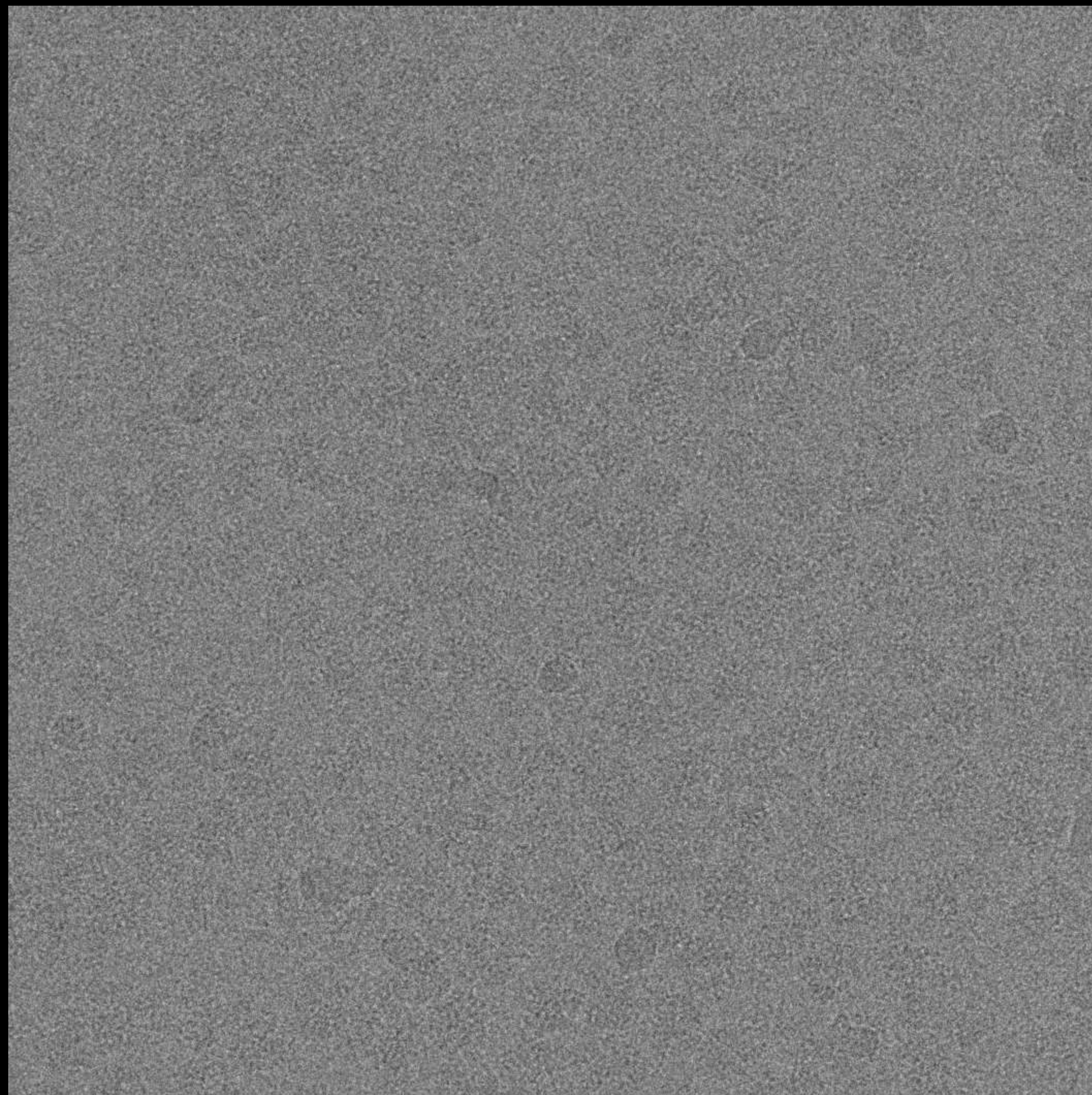
references



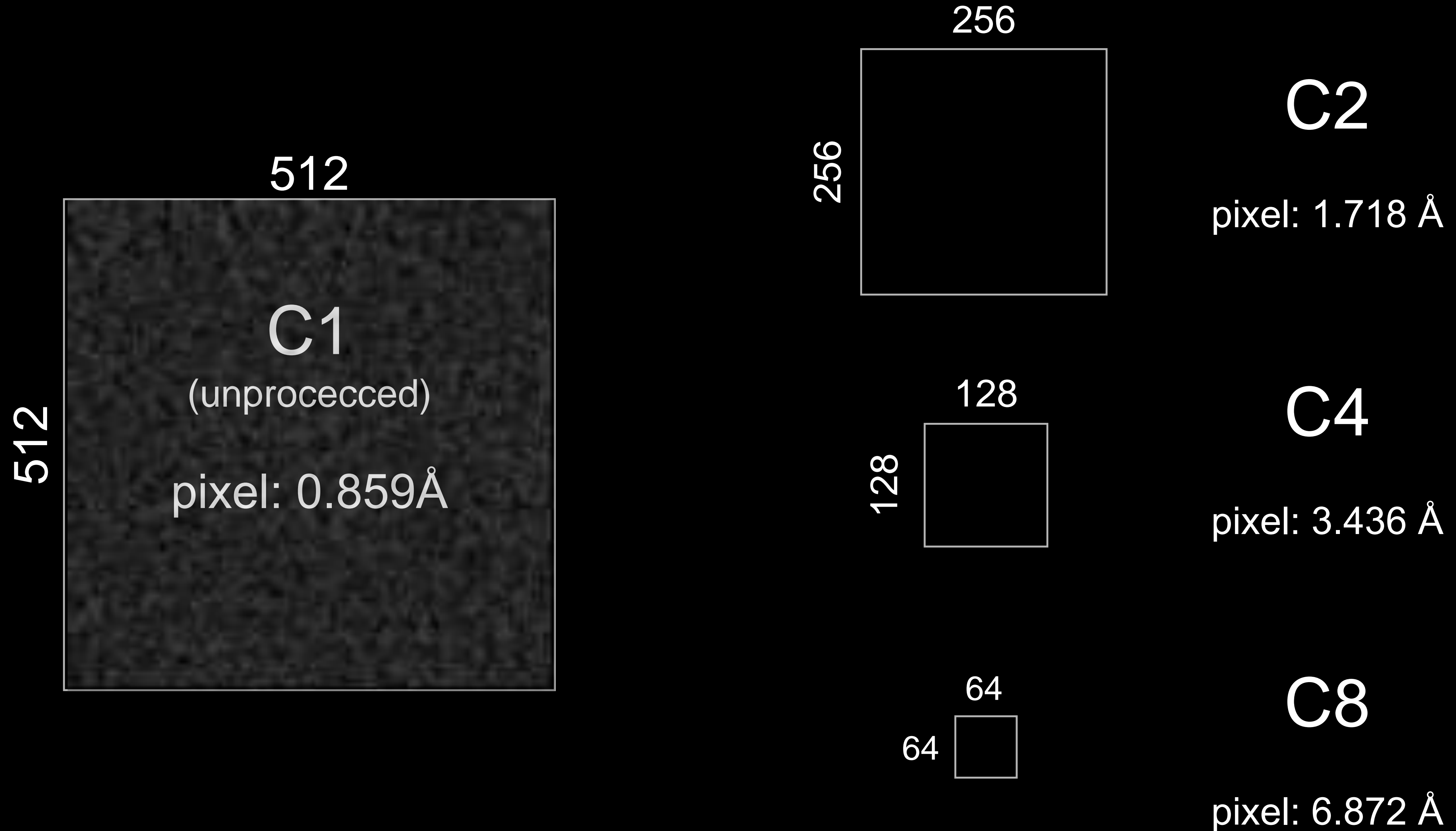
>500k particles

Preprocessing / CTF estimation

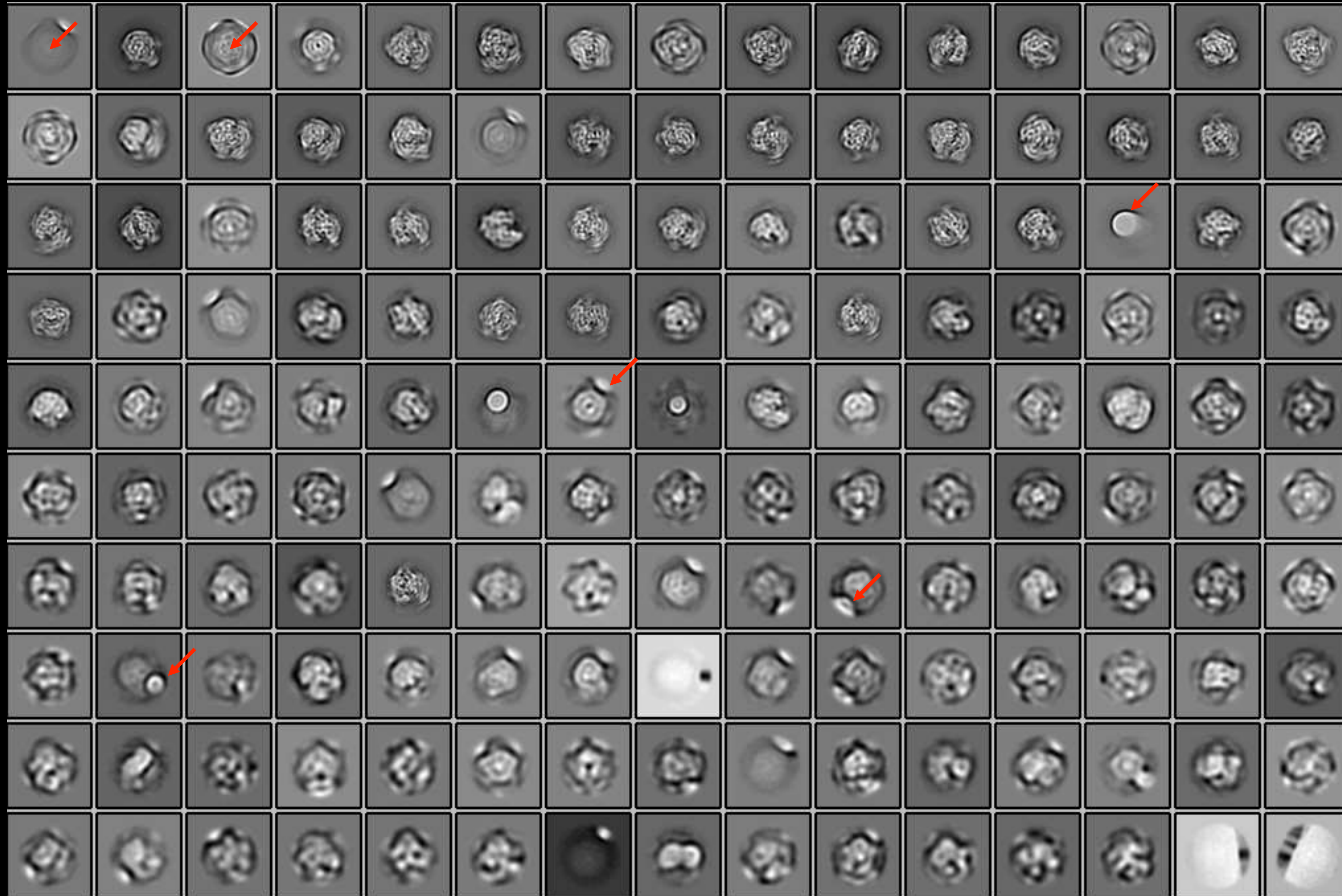
CTFFIND4



Relion / Particle extraction & binning



Relion / 2D classification: run1 (C8)



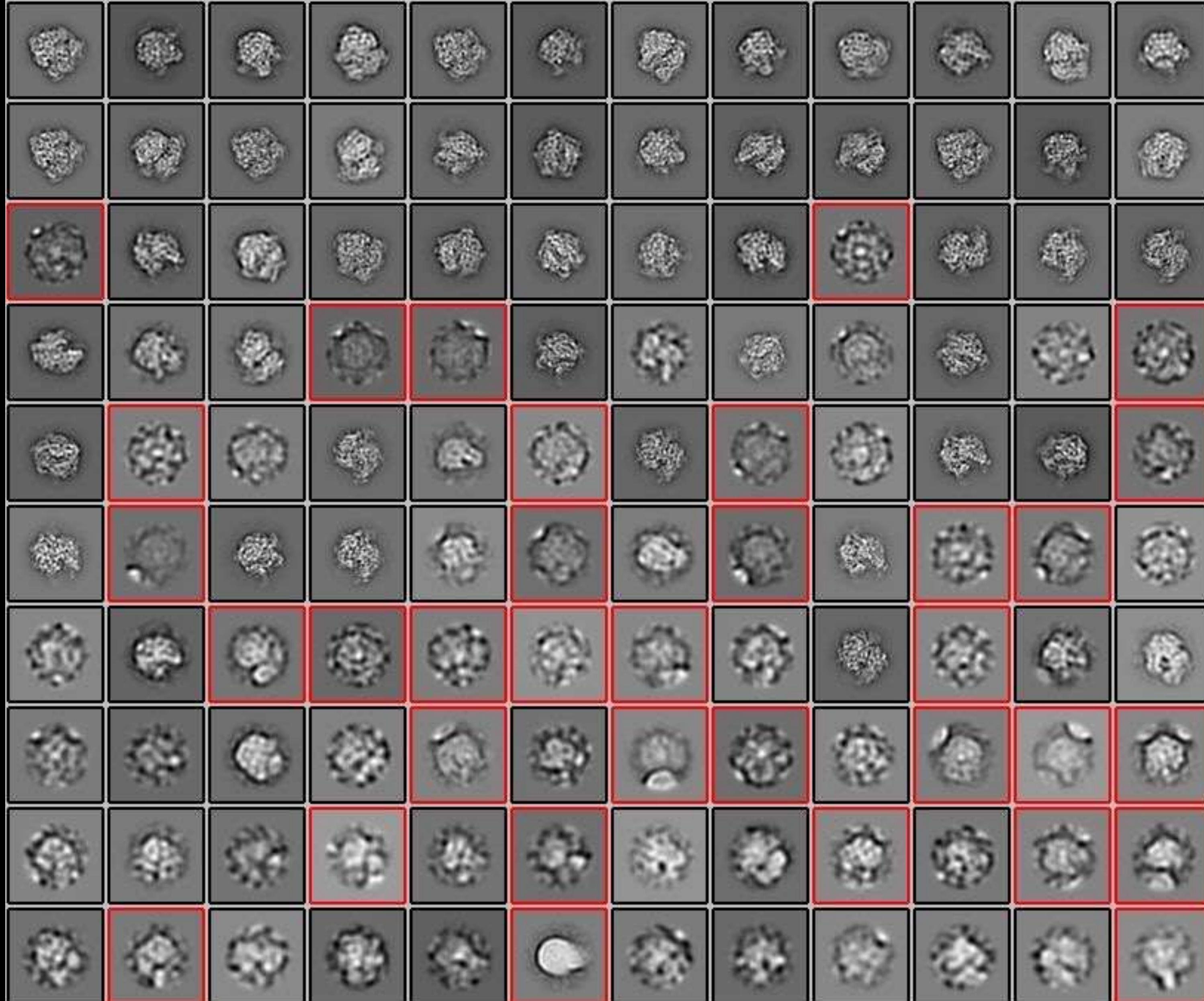
150 classes

20 it

~~ice/empty classes~~

443k particles

Relion / 2D classification: run2 (C8)



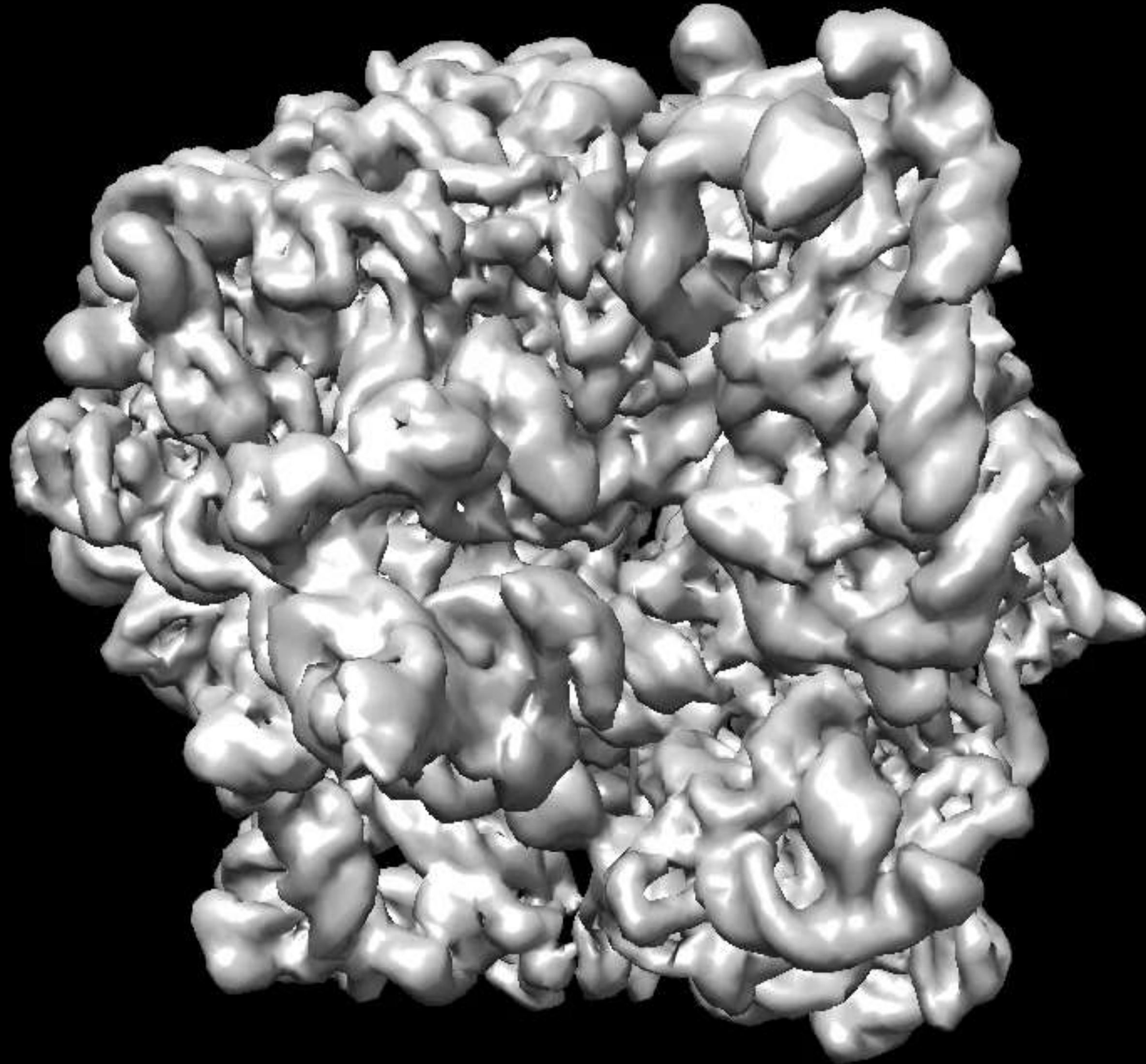
120 classes

20 it

~~ice/empty classes~~

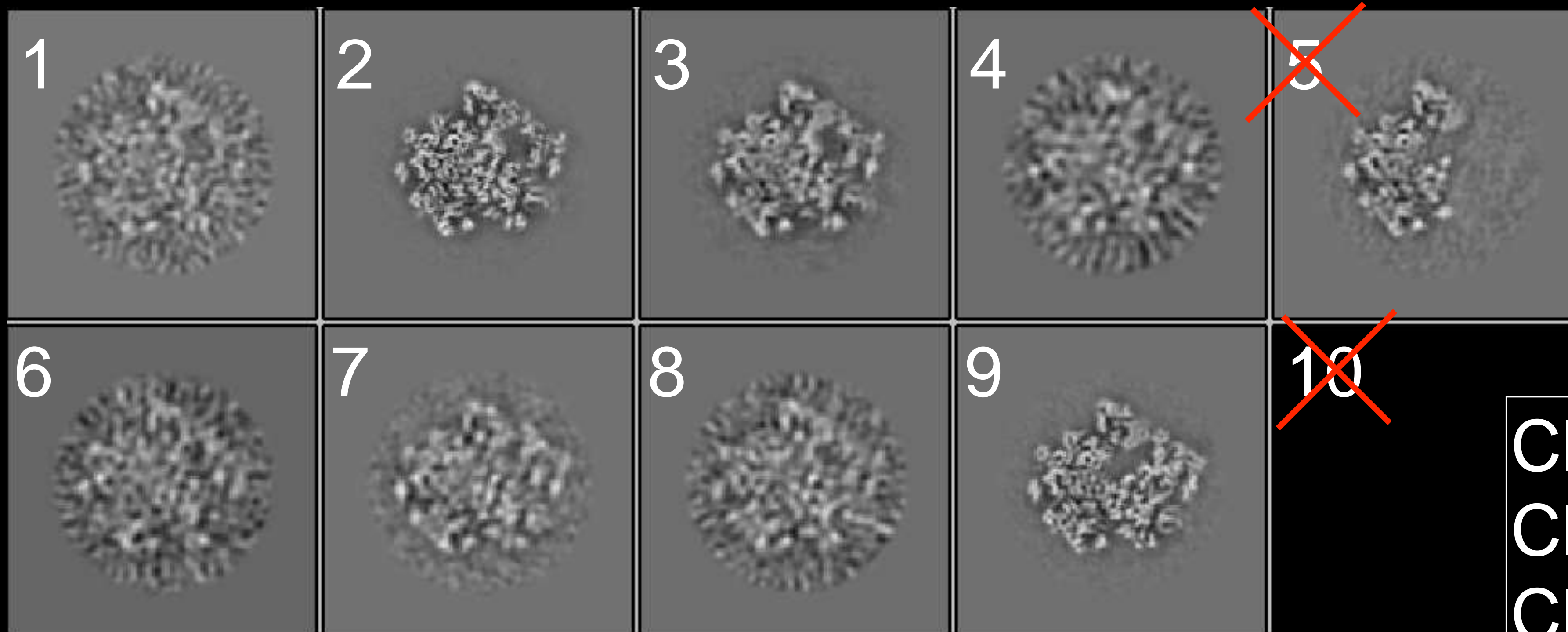
334k particles

Relion / 3D refinement (C4)



Reported res: 6.9 Å

Relion / 3D classification (C4)



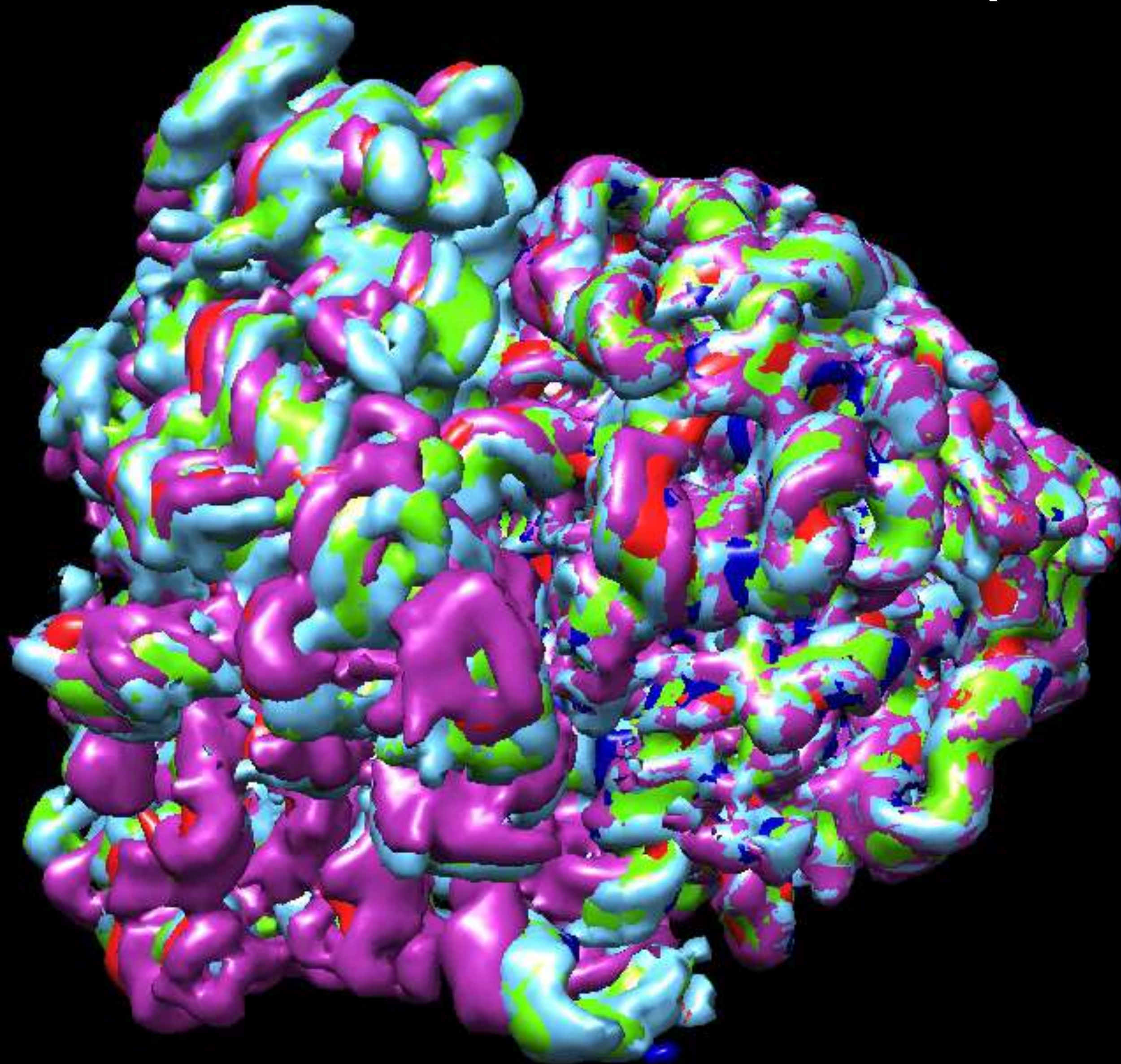
10 classes

20 it

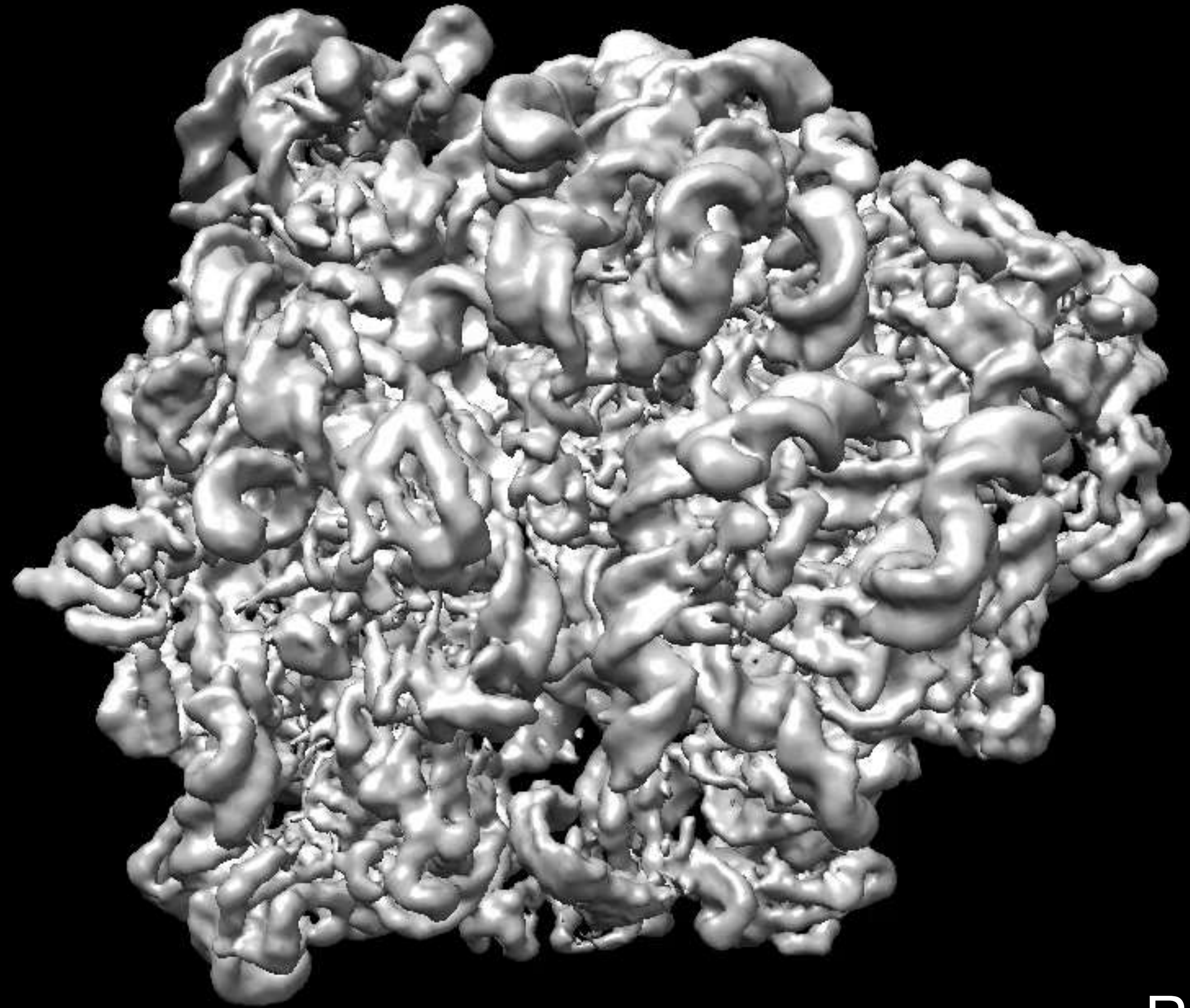


Class 1 - #1 (36k)
Class 2 - #2,3 (161k)
Class 3 - #4,6,8 (85k)
Class 4 - #7 (1.8k)
Class 5 - #9(41k)

Relion / 3D classification (C4)

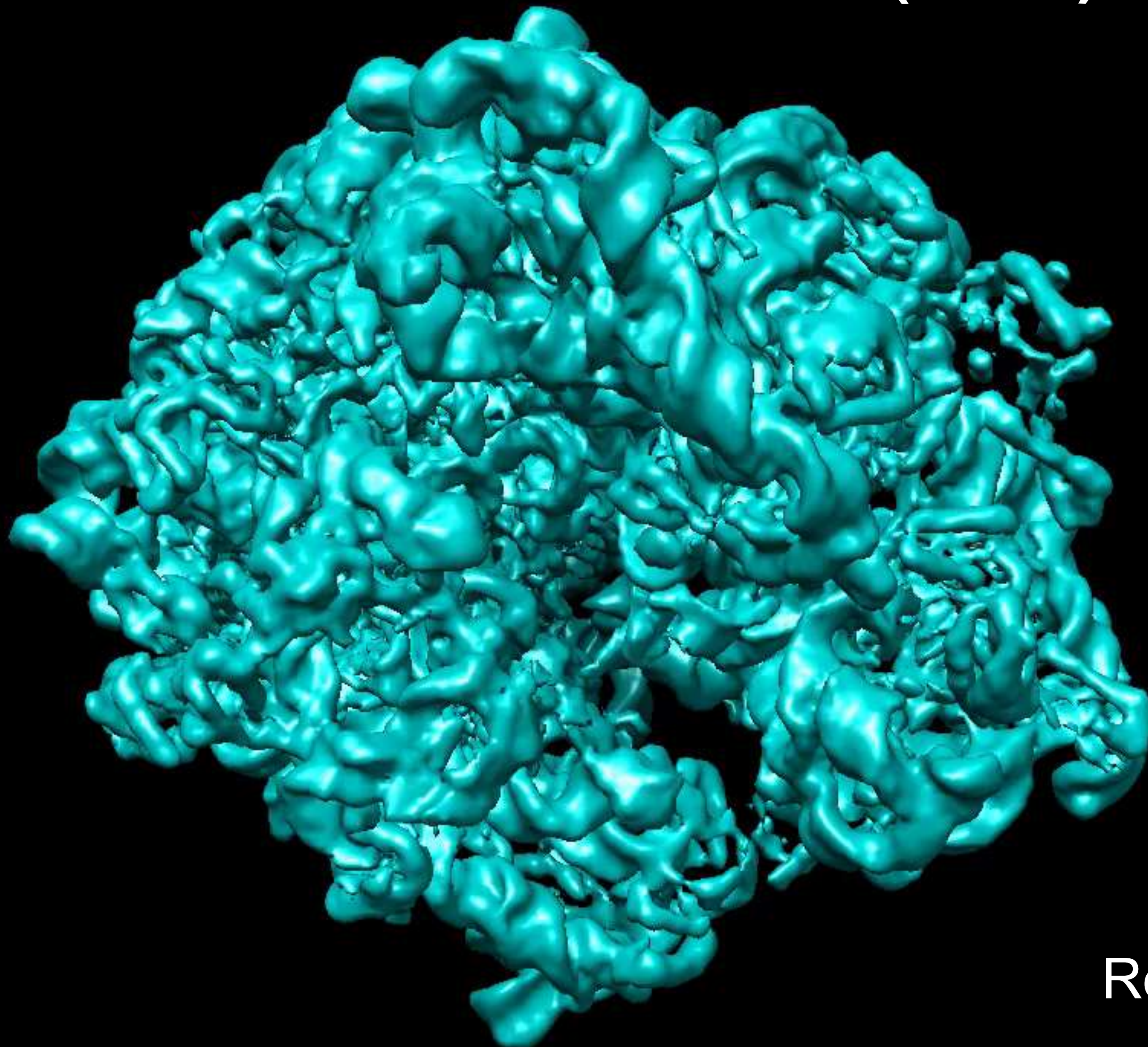


Relion / 3D refinement (C2). Class 2



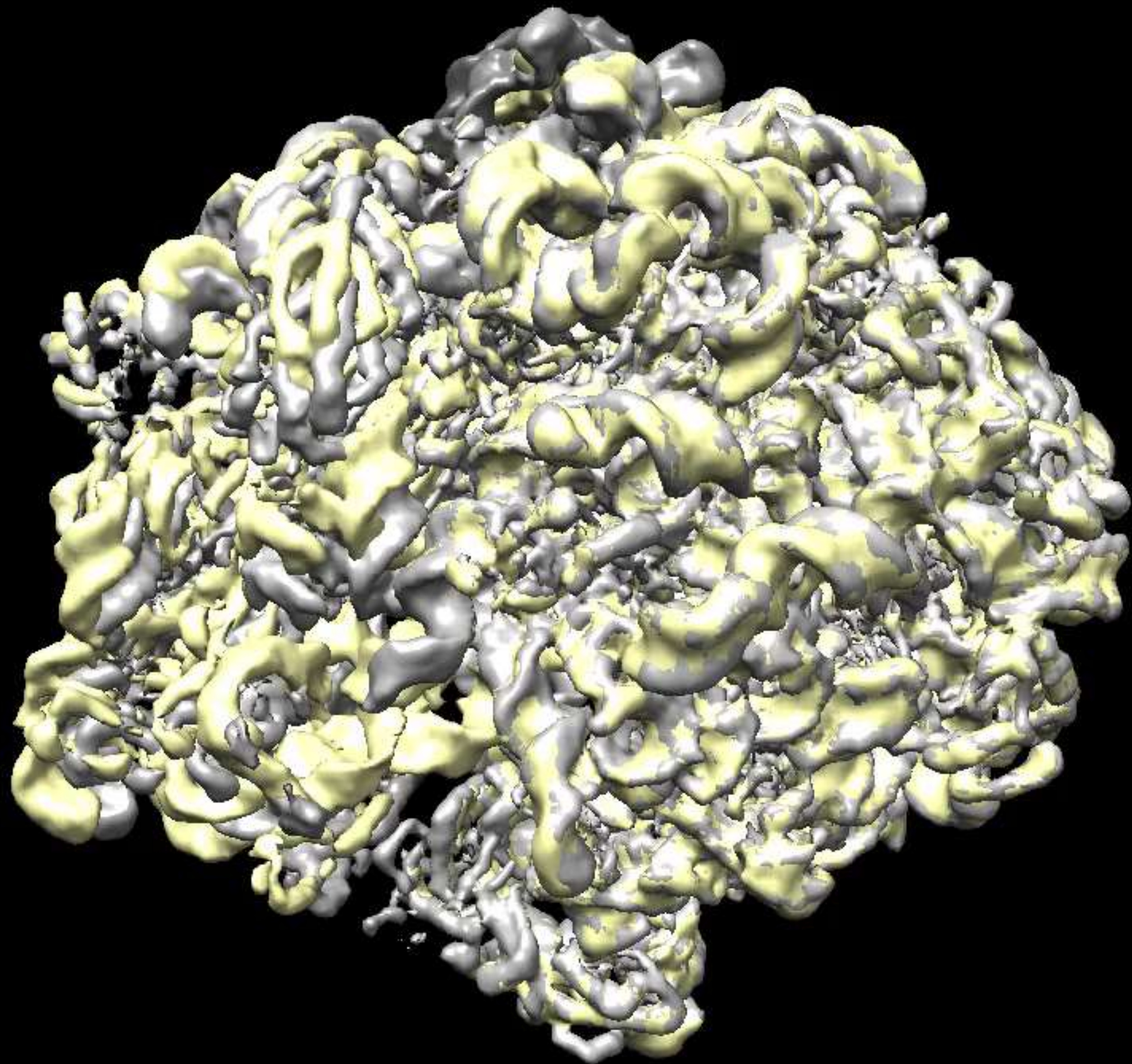
Reported res: 3.89 Å

Relion / 3D refinement (C2). Class 5



Reported res: 4.99 Å

2&5



Relion / Postprocessing

