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Image processing and 3D reconstruction

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Protocol status: Working
We use this protocol and it's working

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Abstract

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Troubleshooting

Image processing

1 Use cryoSPARC for the following steps except those particularly mentioned.

2 Do motion correction by [Patch Motion Correction]
Bin 2x in fourier cropping for super-resolution video stacks
Bin 1x in fourier cropping for regular video stacks

3 Do contrast transfer function determination by [Patch CTF Estimation]
Remove the outlier micrographs base on the estimated defocus and resolution value.

4 Do particle picking by [Topaz]
Manually pick 10 micrographs as learning dataset
Optimize the 'picking threshold' with the 10 mics
Apply the parameter to the entile dataset

5 Particle extraction
Use the box size 1.5 times larger than the target particles
Bin 4x to facilitate the following classification jobs

6 2D classification
Set 50-100 classes dependent on the data size
Remove the obvious junk particles

7 Obtain an initial model
[1] Use Ab-initial (Optional) only select the 2D classes that show high-resolution features
[2] Use previously determined structure if it's available
[3] Create a new medel by AlphaFold

8 Do 3D classification by [Reterogeneous Refinement]
Low-pass your model to 15-20 Å
Run the job with 2-3 junk models
Run multiple times (typically 2-4 rounds) until the result converges

9 Re-extract the particles with
bin 2x for super-resolution video stacks
bin 1x for regular video stacks

3D reconstruction

10 Do 3D reconstruction by [Homogeneous Refinement]
Repeat 2-3 times until the resolution converges
Check whether the FSC curve is healthy

11 (Optional)
Do CTF refiment followed by homogeneous refinement.
Check whether the resolution get improved

12 (Optional)
Do local refinement if the map contains multiple rigid sub-regions
Decide the masks based on [3D Variability] or [3D Flex]
Use [ChimeraX] to create the maps
Use [EMAN2] to compose the final maps at the end