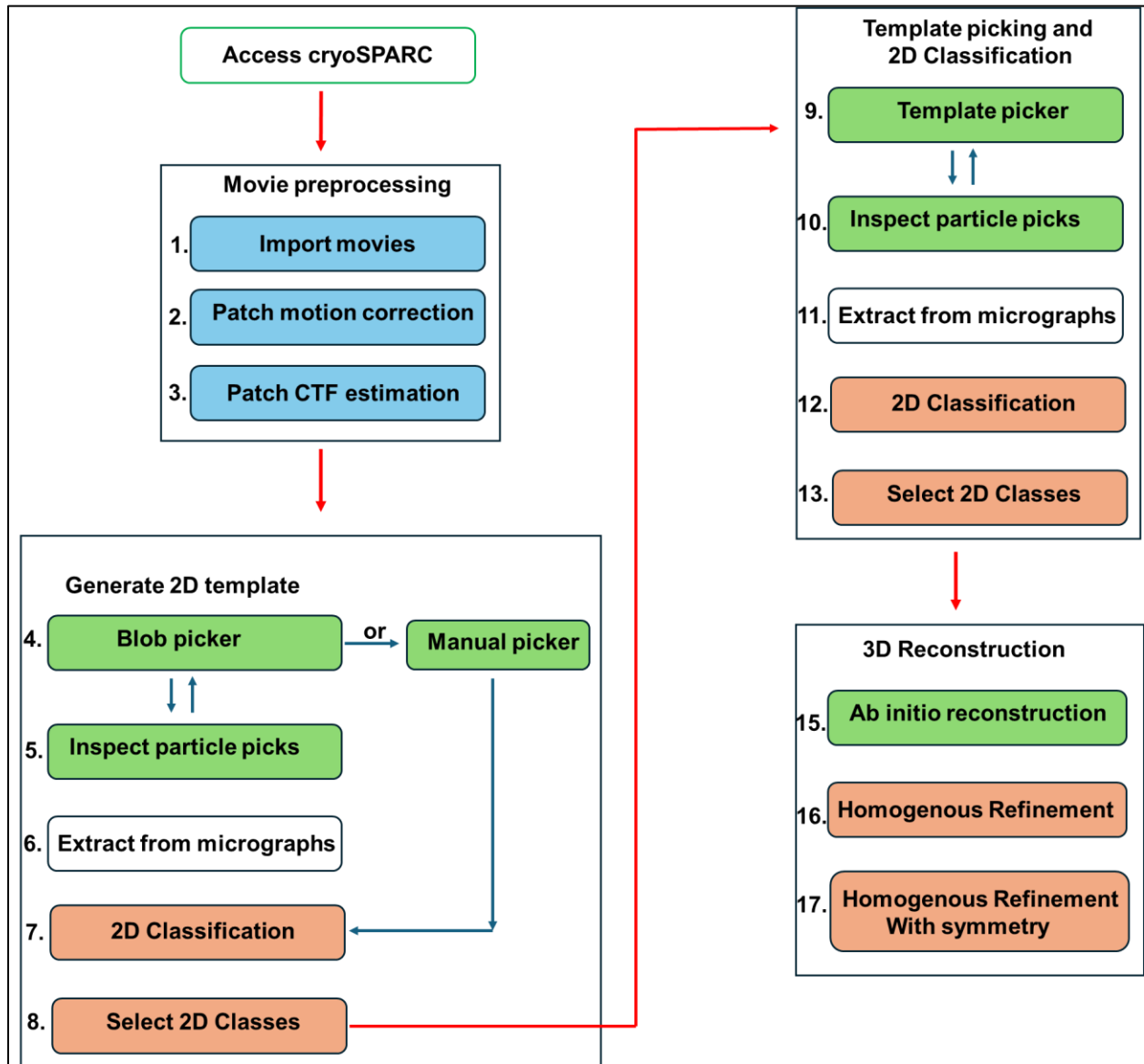


Cryo-EM Single Particle (SPA) Processing in cryoSPARC v4.0+

From movies to 3D consensus refinement



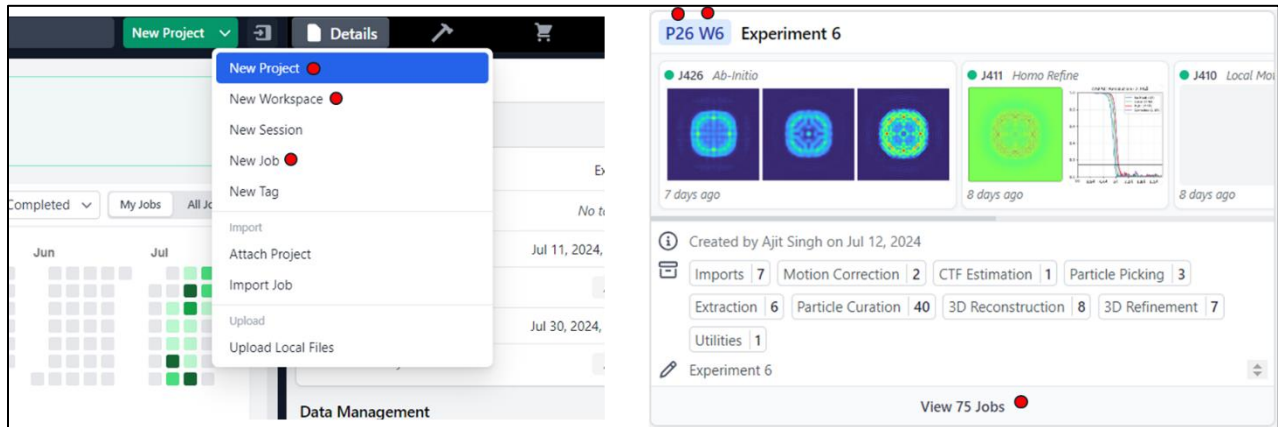
Accessing a Project and Workspace:

The organizational hierarchy in cryoSPARC is Project -> Workspace -> Job.

Projects (P) are often used for different users, samples, or biological systems.

Workspaces (W) are often used for separate datasets. Each project can contain multiple workspaces.

Jobs (J) are the basic processing operations of cryoSPARC and must be performed within a specific workspace. You can link jobs between workspaces, but you cannot link jobs between projects.



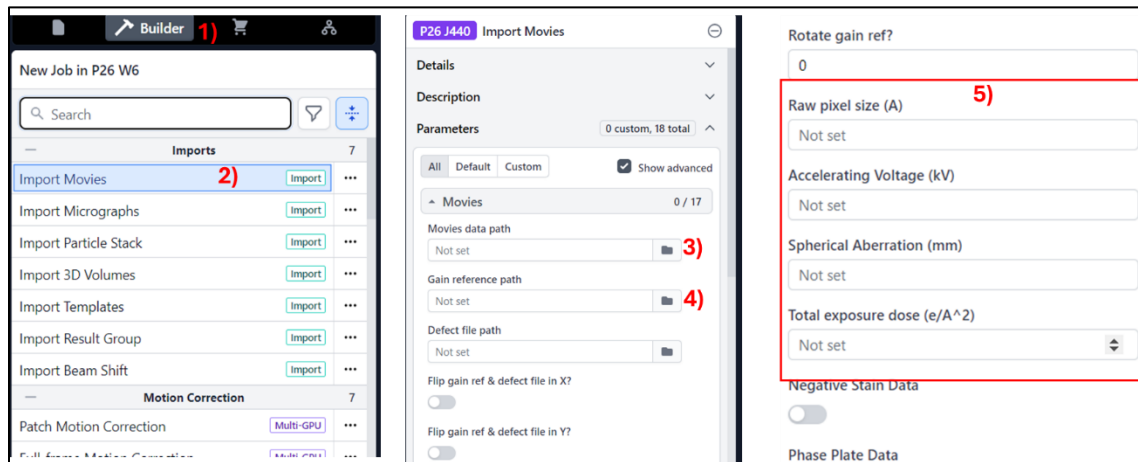
Note: Blue color in jobs indicates the job is running, red means the job has an error, and green means the job is complete.

Movie preprocessing: 1) Import movies, 2) Patch motion correction and 3) Patch CTF estimation

1. Import movies:

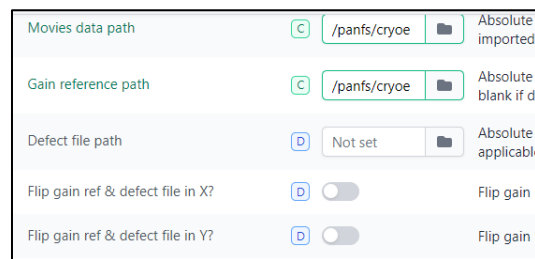
Steps to follow:

- 1) Click “Builder” button
- 2) Click “Import Movies” at the top. A new job is built, and you should have access to the “Details” and “Parameters” pages on the right.
- 3) Select Movies data path: Click the folder icon for Movies data path & the Select movies data path page opens. From here you can navigate directories to point cryoSPARC to your movies.
- 4) Select Gain reference path
- 5) **Set parameters:** Raw pixel size (Å), Accelerating voltage (kV), Spherical aberration (mm) and Total exposure dose ($e^-/\text{Å}^2$)
- 6) Queue job



Note:

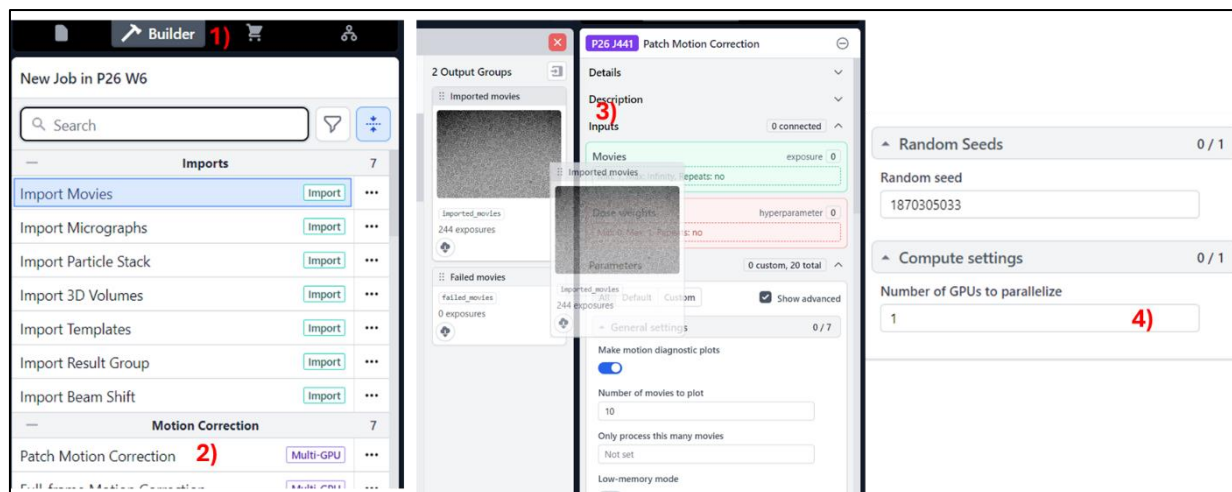
- All others set to default. The text for parameters that have been **changed from default turns green.**



2. Patch Motion Correction:

Steps to follow:

- 1) Click builder button
- 2) Click “Patch motion correction” job and a new job is built.
- 3) Open the previously run Import Movies job by clicking it. Drag “Imported movies” from the right side “output groups section of import movies” to the “inputs” Micrographs section of the Patch Motion Correction job builder.
- 4) Parameters to change: Only change the Compute Setting “Number of GPUs to parallelize” from default of 1 to 2 or more if available
- 5) Queue job



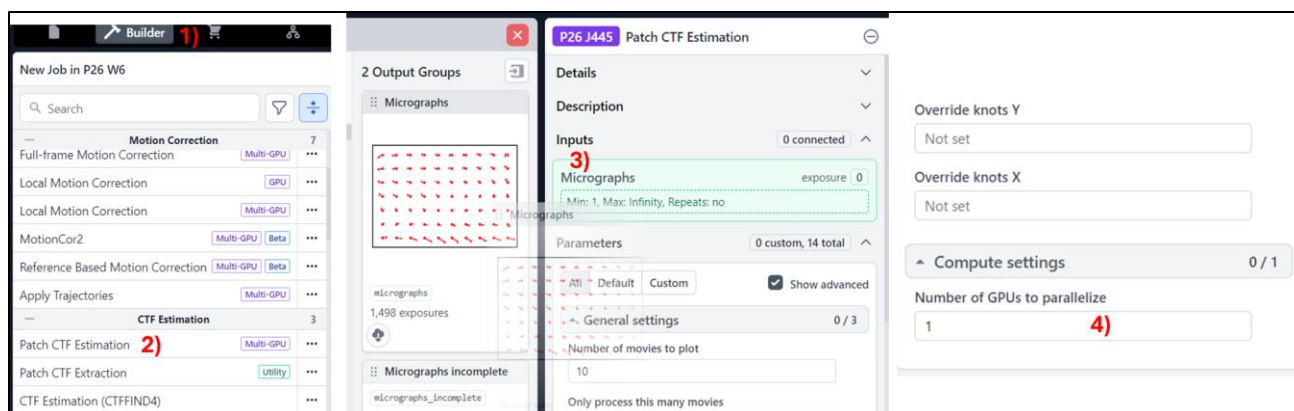
Note:

- All others set to default. The text for parameters that have been **changed from default turns green**.
- To speed up the motion correction job, we can adjust the Output F-crop factor to $\frac{1}{2}$. This specifies the amount of binning to apply. If the movies were collected at a pixel size of approximately 0.56 \AA , the Nyquist limit would be 1.12 \AA without binning. By binning by a factor of 2, our aligned micrographs will have a pixel size of 1.12 \AA , with a Nyquist limit of 2.24 \AA . If refinements approach this 2.24 \AA limit, we can repeat this step with an F-crop factor of 1 (no binning) to attempt to achieve higher resolution.

3. Patch CTF estimation:

Steps to follow:

- 1) Click “Builder” button
- 2) Click “Patch CTF Estimation” and the job builder opens.
- 3) Open the previously run Patch Motion Correction job by clicking it. Drag “Micrographs” from the right side “output groups section of the Patch motion correction” section to the “inputs” Micrographs section of the Patch CTF Estimation job builder.
- 4) Parameters to change: Only change the Compute Setting “Number of GPUs to parallelize” from default of 1 to 2 or more if available
- 5) Queue job



Note: All others set to default. The text for parameters that have been **changed from default** turns green.

Generate 2D template using Blob picker: 4) Blob picker, 5) Inspect particle picks, 6) Extract from micrograph, 7) 2D classification and 8) Select 2D classes

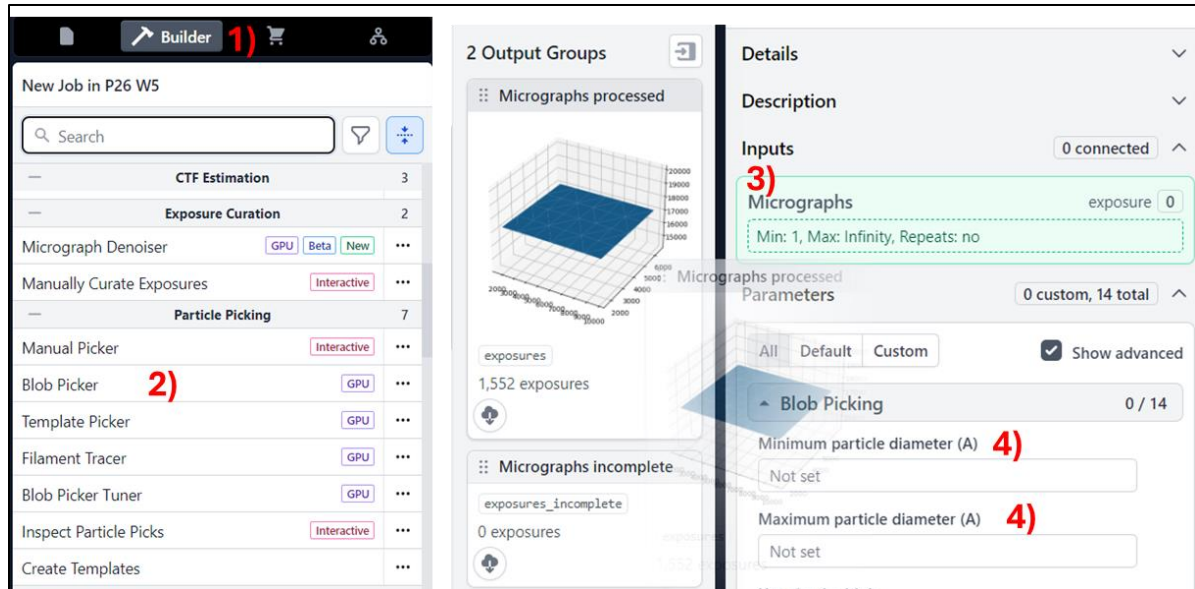
4. Blob Picker:

Steps to follow:

- 1) Click "Builder" button
- 2) Click "Blob Picker" and the job builder opens
- 3) Open the previously run "Patch CTF Estimation" job so the output groups are accessible. Then, drag "Micrographs Processed" into the Blob Picker input labeled "Micrographs."
- 4) Parameters to change: Minimum particle diameter (Å) (recommended to underestimate the minimum diameter), Maximum particle diameter (Å) (recommended to overestimate the maximum diameter).

All parameters are kept at their default settings, except if needed for the "Pick on denoised micrograph" option (Enabling this parameter will pick particles using the denoised micrographs, which can be helpful if the contrast is very poor. However, particles will ultimately be extracted from the raw micrographs, regardless of this setting.) and the "Maximum number of local maxima to consider" (< 500).

- Compute considerations: This job can only use one GPU (no parallelization option).
- 5) Queue job



Note:

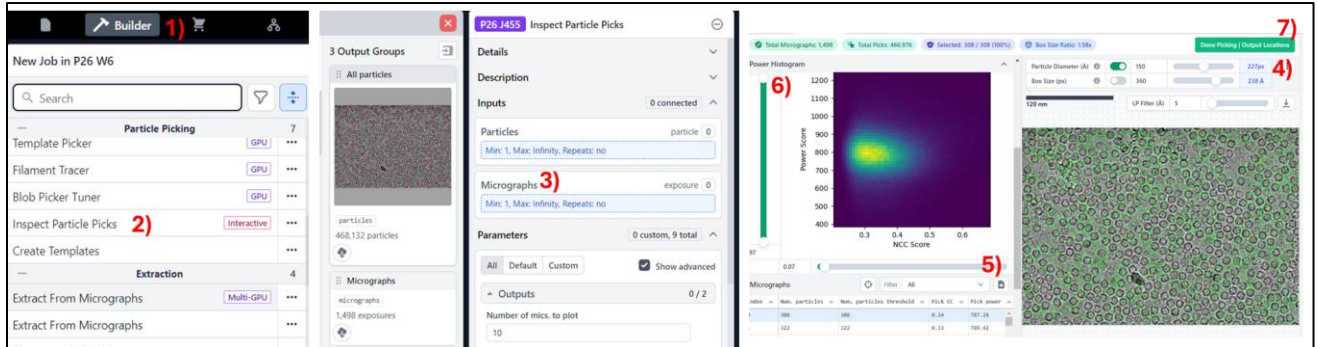
- All others set to default. The text for parameters that have been **changed from default turns green**.
- As the job runs, you can follow the progress in the event log and see the red dot blob picks for each micrograph. Once the first 10 micrographs have been processed, diagnostic plots will stop being displayed. You can revisit these plots by clicking "Show from top" to view the first 10 micrograph particle picks. The template is used for the particle picking.

5. Inspect Particle Picks:

Steps to follow:

- 1) Click "Builder" button
- 2) Click "Inspect particle picks" and the inspect particle pick job opens
- 3) Open the previously run "Blob picker" job so the output groups are accessible. Then, drag "All particles and Micrographs" into the Inspect particle pick input labeled "Particles and Micrographs." Queue the job (its interactive job)
- 4) Adjust Particle Diameter: The green circles depicting particle picks can get in the way of viewing the micrograph. Drag the "Particle diameter" slider to put a small green circle on each particle pick.
- 5) Optimize NCC Score: Drag the NCC score slider to the right and observe how many off-target picks are removed. Optimize this over 5 to 10 micrographs to remove off-target picks while maintaining on-target picks.
- 6) Adjust Power Histogram: Adjust the power histogram sliders. Optimize this over 5 to 10 micrographs to remove off-target picks while maintaining on-target picks.
- 7) Finalize Picks: Click "Done Picking Output Locations" in the top right.

8) Queue job



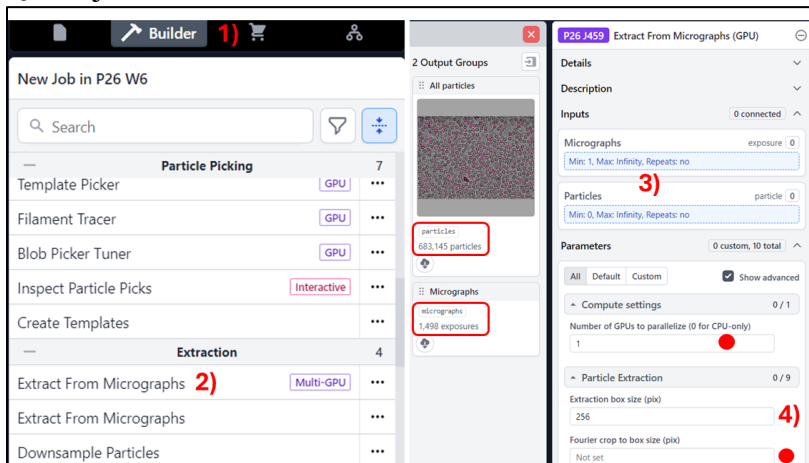
Note:

- All others set to default. The text for parameters that have been **changed from default turns green**.
- Iterate for Best Results: Iterate Step 4 and Step 5 until results are acceptable.
- It is better to overpick than to underpick because you can filter out many off-target picks with 2D classification, but unpicked particles must be recovered by some particle picking mechanism, or they will remain unused.

6. Extract from micrograph:

Steps to follow:

- 1) Click “Builder” button
- 2) Click “Extract from micrograph” the details and parameters panel opens
- 3) Open the previously run “Particle Inspection” job so the output groups are accessible
- 4) Drag “all accepted particles” and “all accepted micrograph” from the completed inspect particle pick job
- 5) **Parameters to change:** Extraction box size (pixel): xxx
- 6) Queue job



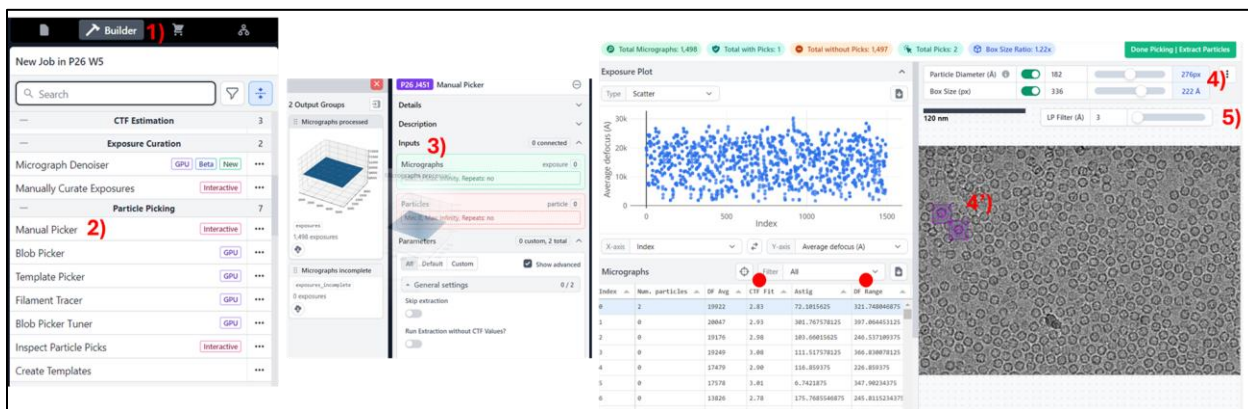
Note:

- All others set to default. The text for parameters that have been **changed from default turns green**.
- To speed up data processing in the initial stages, initiate the Fourier crop (Fourier Crop to box size (pix): xxx), which down samples the particles.

Or Manual picker:

Steps to follow:

- 1) Click “Builder” button
- 2) Click “Manual Picker” and the job builder opens
- 3) Open the previously run "Patch CTF Estimation" job so the output groups are accessible. Then, drag "Micrographs Processed" into the Manual Picker input labeled "Micrographs."
- 4) Once the job is started, you can manually adjust the particle diameter and Box size (using slider bar adjacent) in the job inspector/stream log. (The change of the value can be observed in the micrograph, particle diameter in green circle and box size in red square.
- 5) When viewing a micrograph, you may also use the Low pass filter (LP) slider to manually specify the contrast bounds in the job inspector/stream log.
- 6) Queue job



Note:

- All others set to default. The text for parameters that have been **changed from default turns green**.
- As a general rule of thumb, box sizes should be approximately 50% larger than the longest view of the particle (e.g., a particle with a ~ 200 Å diameter at its longest view should use a box size that covers ~ 300 Å). The reasons for this requirement are twofold: first, there needs to be sufficient solvent area for downstream processing steps to distinguish the particle from noise; second, the delocalization effects associated with the CTF may extend beyond the visible components of each particle image. For practical reasons, box sizes also needn't be

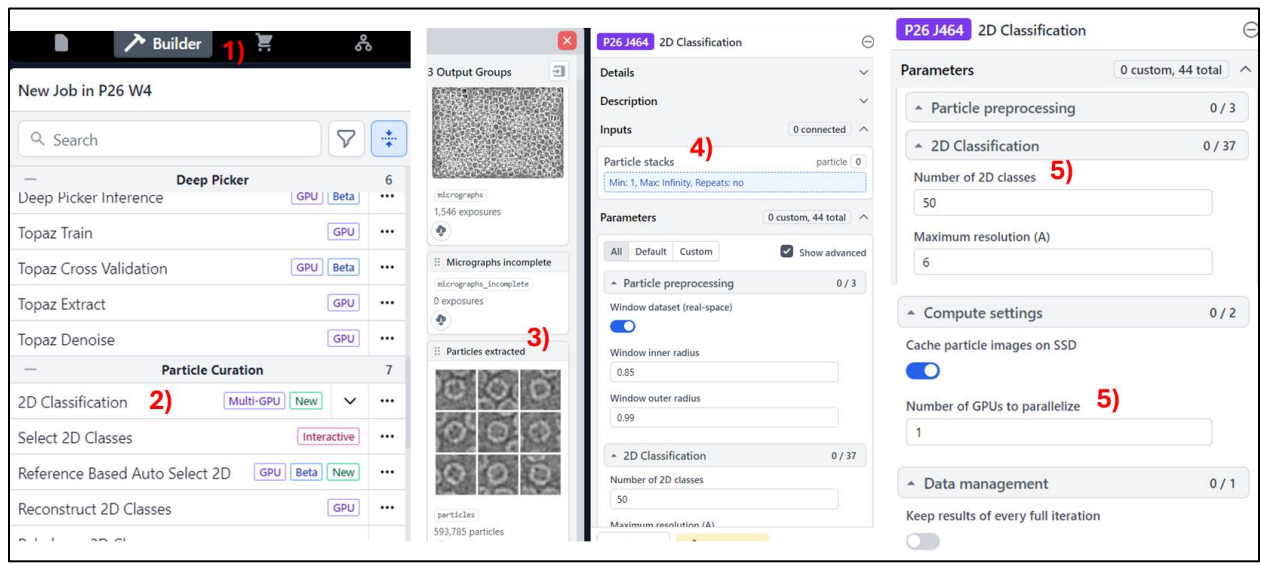
overly large as it would add unnecessary computation burden to downstream image processing steps.

- Link to choose good box size: <https://blake.bcm.edu/emanwiki/EMAN2/BoxSize>
- It is recommended to pick particles from multiple micrographs with a range of defocus and CTF fit values to ensure a comprehensive template.

7. 2D Classification:

Steps to follow:

- 1) Click “Builder” button
- 2) Then click “2D Classification job” & the details and parameters panel opens
- 3) Open the previously run “Extract from Micrographs” job so the output groups are accessible
- 4) Drag the “Particles Extracted” from the completed Extract from Micrographs job into the 2D Classification “Particle Stacks” input
- 6) **Parameters to change:** Number of 2D classes: xxx, Compute Setting “Number of GPUs to parallelize” from default of 1 to 2 or more if available
- 7) Queue job



Note:

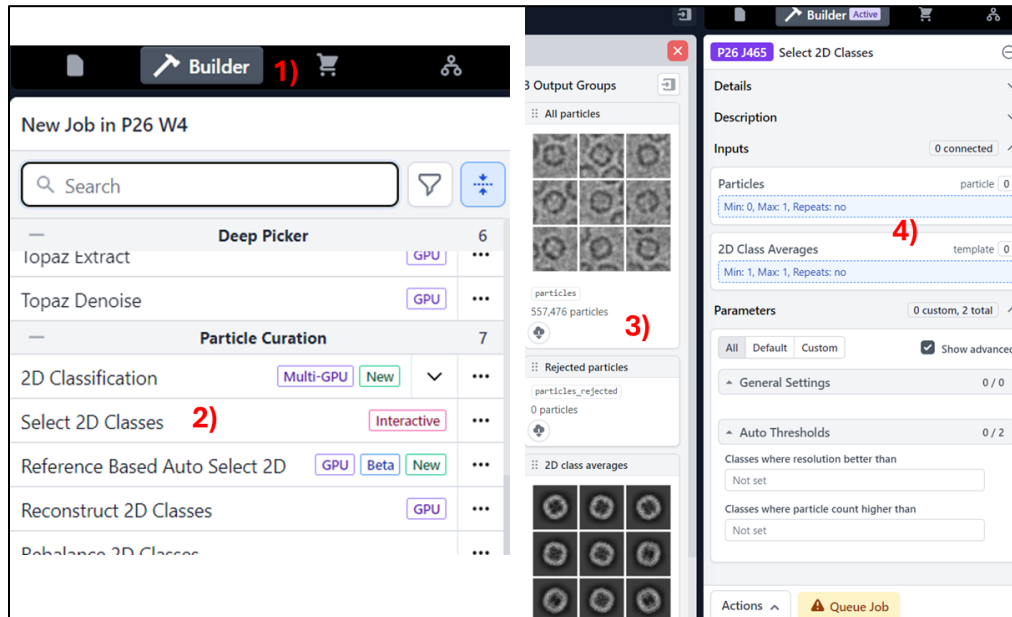
- All others set to default. The text for parameters that have been **changed from default turns green.**

8. Select 2D classes:

Steps to follow:

- 1) Click “Builder” button
- 2) Then click “Select 2D class job” & the details and parameters panel opens
- 3) Open the previously run “2D classification job” so the output groups are accessible

- 4) Drag “All particles” and “2D class averages” from the completed 2D classification job into the select 2D classes input
- 5) Queue job



Note:

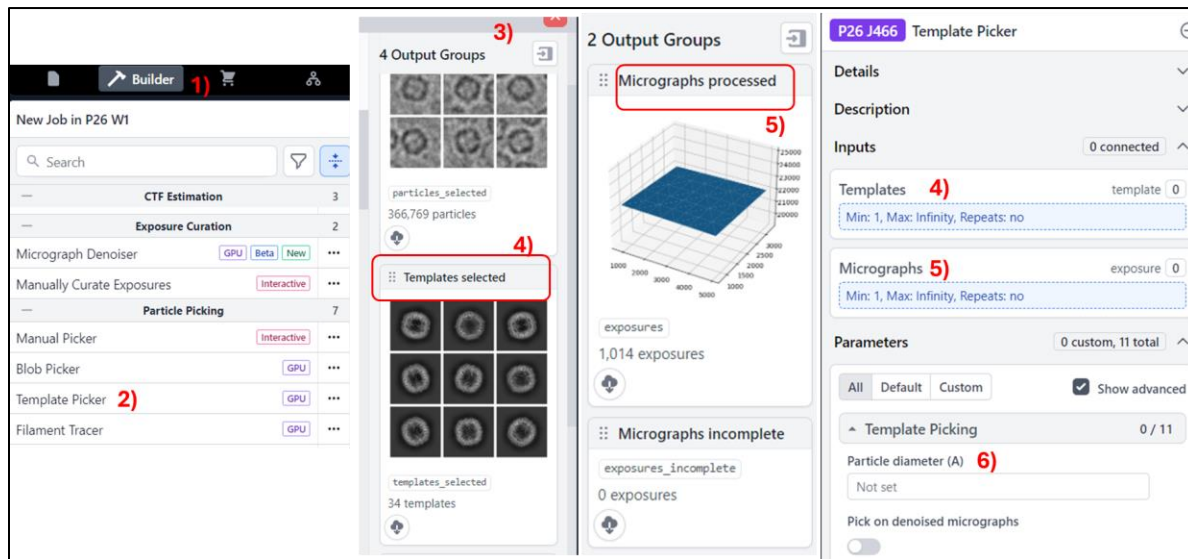
- All others set to default. The text for parameters that have been **changed from default turns green.**

Template picking and 2D classification: 9) Template picker 10) Inspect particle picks 11) Extract from micrograph 12) 2D classification 13) Select 2D classes

9. Template Picker:

Steps to follow:

- 1) Click “Builder” button
- 2) Then click “Template picker job” & the details and parameters panel opens
- 3) Open the previously run “select 2D class job” so the output groups are accessible
- 4) Drag the “Templates Selected” output from the completed “Select 2D Classes” job and drag it to the Templates input in the Template Picker builder
- 5) Open the previously run Patch CTF job Drag the “Micrographs Processed” output from the completed “Patch CTF” job and drag it to the Micrographs input in the Template Picker builder
- 6) **Parameters to change:** Particle diameter (Å): xx, Compute considerations – this job requires 1 GPU and cannot be parallelized
- 7) Queue job



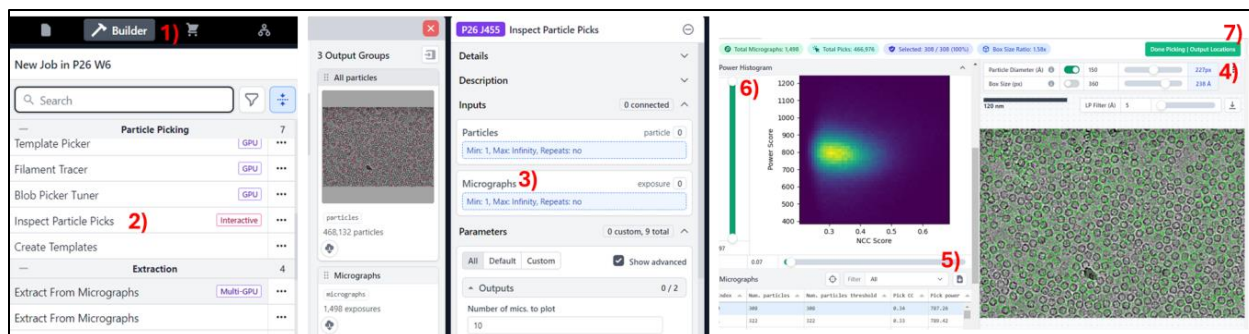
Note:

- All others set to default. The text for parameters that have been **changed from default turns green**.

10. Inspect particle picks:

Steps to follow:

- 1) Click “Builder” button
- 2) Click “Inspect particle picks” and the inspect particle pick job opens
- 3) Open the previously run "**Template picker**" job so the output groups are accessible. Then, drag "All particles and Micrographs " into the Inspect particle pick input labeled "Particles and Micrographs." Queue the job (its interactive job)
- 4) Adjust Particle Diameter: The green circles depicting particle picks can get in the way of viewing the micrograph. Drag the “Particle diameter” slider to put a small green circle on each particle pick.
- 5) Optimize NCC Score: Drag the NCC score slider to the right and observe how many off-target picks are removed. Optimize this over 5 to 10 micrographs to remove off-target picks while maintaining on-target picks.
- 6) Adjust Power Histogram: Adjust the power histogram sliders. Optimize this over 5 to 10 micrographs to remove off-target picks while maintaining on-target picks.
- 7) Finalize Picks: Click “Done Picking Output Locations” in the top right.
- 8) Queue job



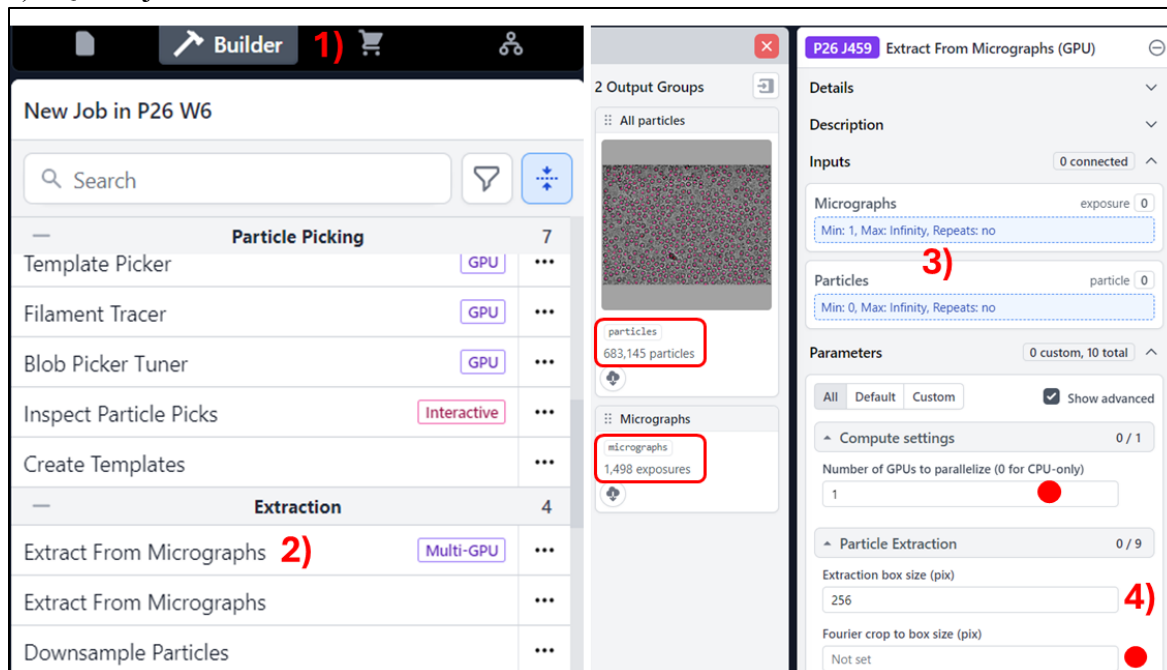
Note:

- All others set to default. The text for parameters that have been **changed from default turns green**.

11. Extract from micrograph:

Steps to follow:

- 1) Click “Builder” button
- 2) Click “Extract from micrograph” the details and parameters panel opens
- 3) Open the previously run “Particle Inspection” job so the output groups are accessible
- 4) Drag “all accepted particles” and “all accepted micrograph” from the completed inspect particle pick job
- 5) **Parameters to change:** Extraction box size (pixel): xxx
- 6) Queue job



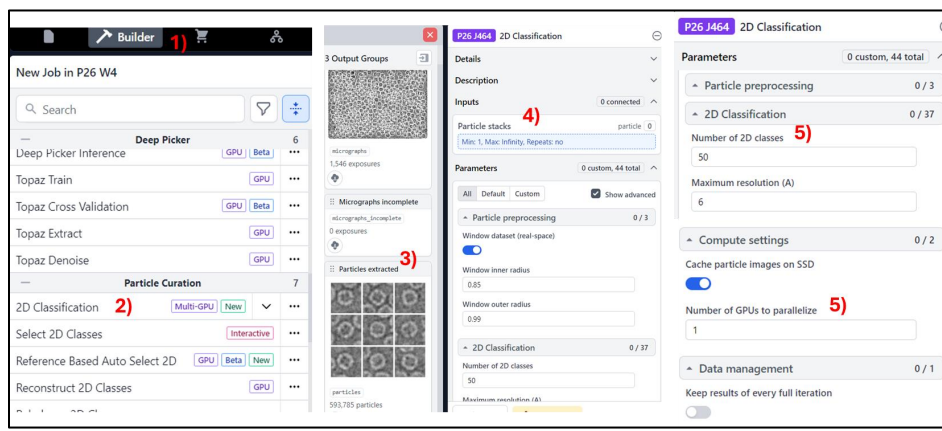
Note:

- All others set to default. The text for parameters that have been **changed from default turns green**.
- To speed up data processing in the initial stages, initiate the Fourier crop (Fourier Crop to box size (pix): xxx), which down samples the particles.

12. 2D Classification:

Steps to follow:

- 1) Click “Builder” button
- 2) Then click “2D Classification job” & the details and parameters panel opens
- 3) Open the previously run “Extract from Micrographs” job so the output groups are accessible
- 4) Drag the “Particles Extracted” from the completed Extract from Micrographs job into the 2D Classification “Particle Stacks” input
- 5) **Parameters to change:** Number of 2D classes: xxx, Compute Setting “Number of GPUs to parallelize” from default of 1 to 2 or more if available
- 6) Queue job



Note:

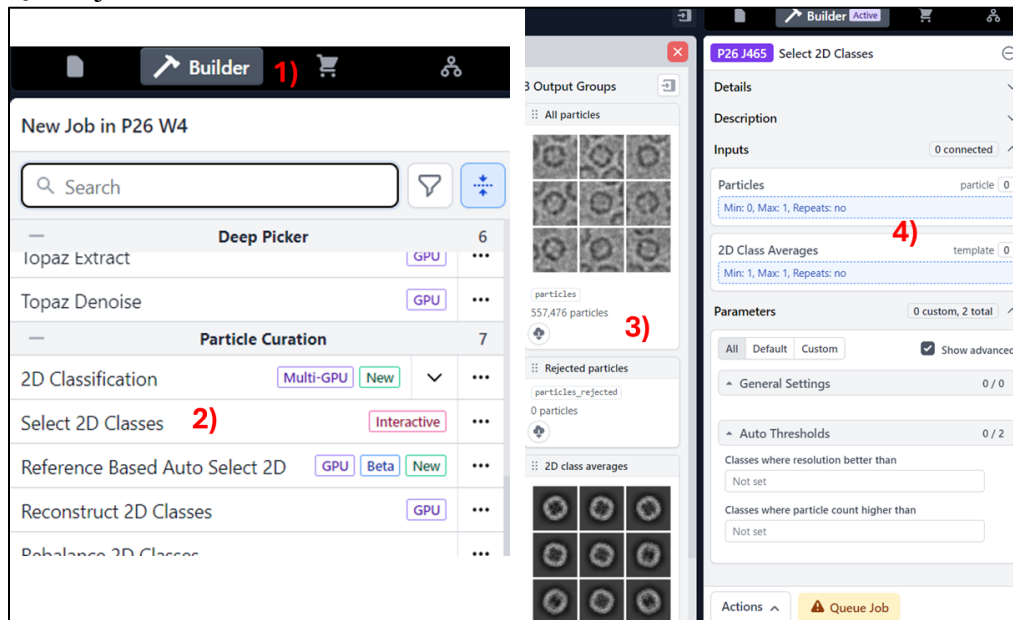
- All others set to default. The text for parameters that have been **changed from default turns green**.

13. Select 2D classes:

Steps to follow:

- 1) Click “Builder” button
- 2) Then click “Select 2D class job” & the details and parameters panel opens
- 3) Open the previously run “2D classification job” so the output groups are accessible
- 4) Drag “All particles” and “2D class averages” from the completed 2D classification job into the “select 2D classes job” input

5) Queue job



Note:

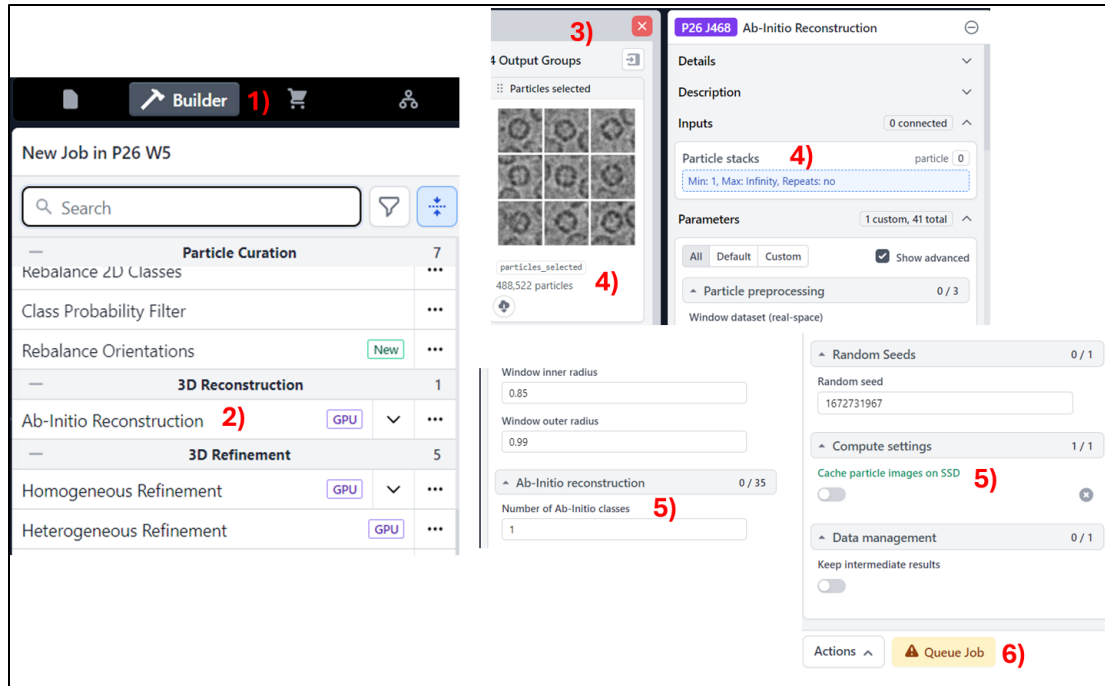
- All others set to default. The text for parameters that have been **changed from default turns green**.

3D Reconstruction: 14) *Ab-initio* reconstruction, 15) Homogenous refinement and 16) Homogenous refinement with symmetry enforced

14. *Ab-initio* reconstruction:

Steps to follow:

- 1) Click “Builder” button
- 2) Then click “*Ab-initio* reconstruction job” & the details and parameters panel opens
- 3) Open the previously run “select 2D class job” so the output groups are accessible
- 4) Drag “Particles selected” from the completed select 2D class job into the particle stack input of the “*Ab-initio* reconstruction job”
- 5) **Parameters to change:** Number of *Ab-initio* classes: xxx, cache particle images on SSD: off
- 6) Queue job



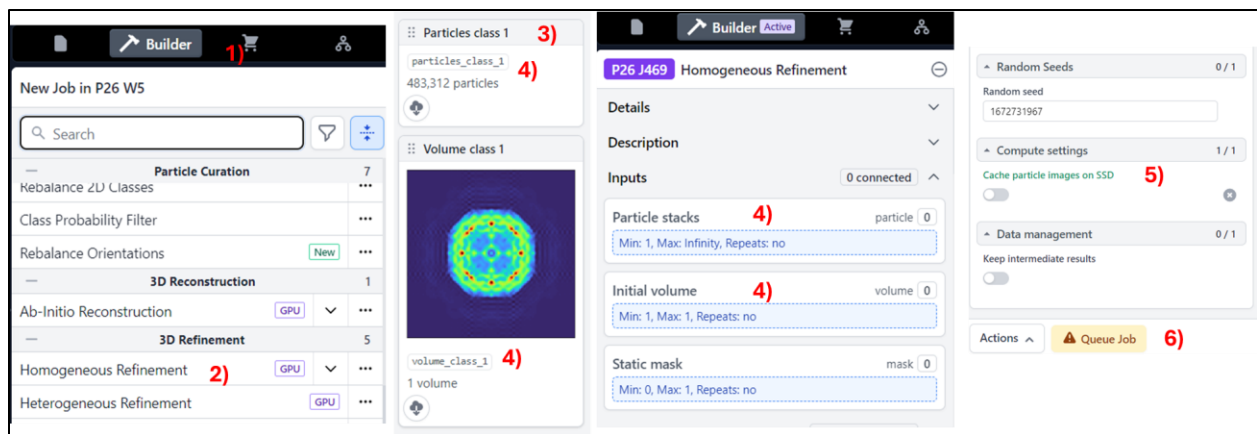
Note:

- All others set to default. The text for parameters that have been **changed from default turns green**.

15. Homogenous Refinement:

Steps to follow:

- 1) Click “Builder” button
- 2) Then click “Homogenous refinement job” & the details and parameters panel opens
- 3) Open the previously run “*Ab-initio* reconstruction job” so the output groups are accessible
- 4) Drag “the best desired *Ab-initio* class and the particles of the best class selected” from the completed *Ab-initio* reconstruction job into the particle stack and volume input of the “Homogenous refinement job”
- 5) **Parameters to change:** cache particle images on SSD: off
- 6) Queue job



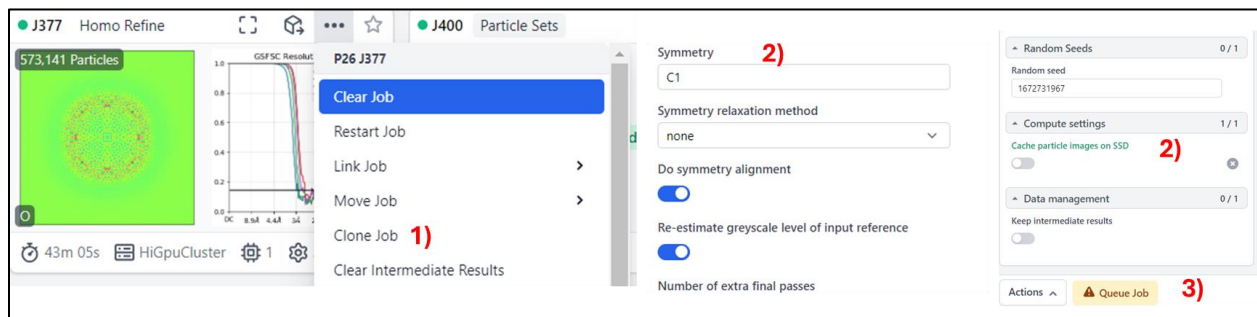
Note:

- All others set to default. The text for parameters that have been **changed from default turns green**.

16. Homogenous Refinement:

Steps to follow:

- 1) Right click the homogeneous refinement job from step 16 and click “clone”
- 2) Parameters to change: Symmetry: xx, Cache particles on SSD: OFF
- 3) **Queue Job**



Note:

- All others set to default. The text for parameters that have been **changed from default turns green**.