

Practical work: "3D alignment and classification"

Josué Gomez
Roberto Melero

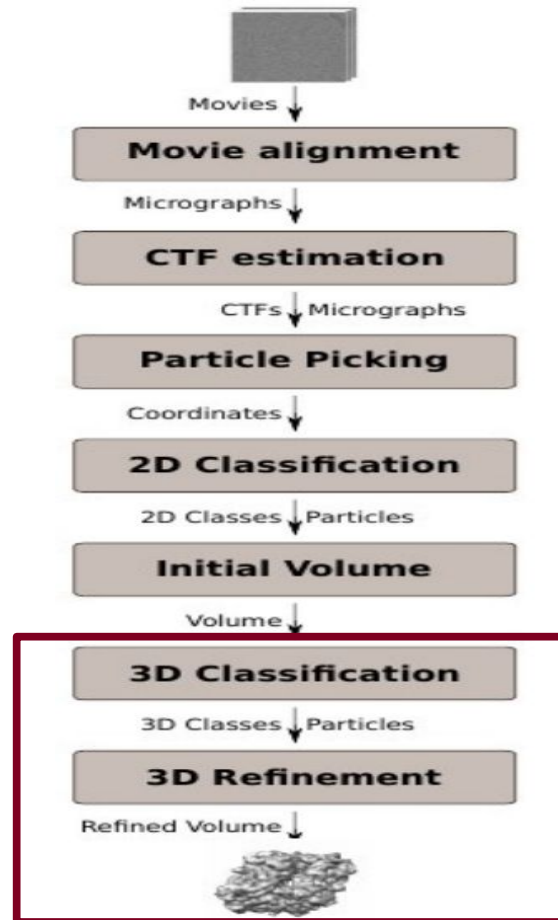
Centro Nacional de Biotecnología-CSIC
jgomez@cnb.csic.es



Overall workflow

A Single-Particle -Analysis
workflow:

From Movies to 3D Model



Our objective
in this
presentation

3D Classification

3D

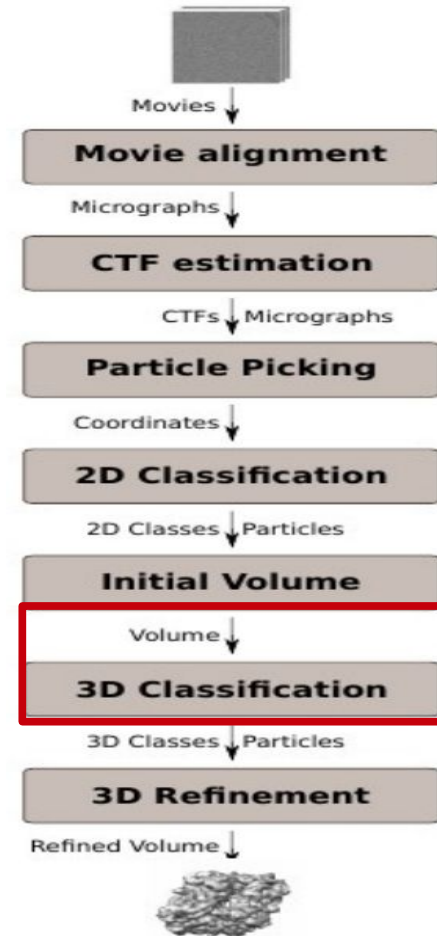
- ▶ **C** Initial volume
- ▶ **C** Preprocess
- ▶ **C** Refine

▶ **C** Classify

P relion - 3D classification

P grigoriefflab - frealign classify

- ▶ **C** Analysis
- ▶ **C** Reconstruct



3D Classification: parameters

Protocol Run: ProtRelionClassify3D

RELION Protocol: relion - 3D classification Cite Help

Run

Run name: relion - 3D classification ✎ Comment: ✎

Run mode: Continue Restart ? Host: localhost ▼

Parallel Threads: 1 MPI: 24 ? Use queue?: Yes No ?

Expert Level: Normal Advanced

Input CTF Optimisation Sampling Additional

Input

Continue from a previous run? Yes No ?

Input particles: Extract Best Particles.outputParticle 🔍 🗑️ 👁️ ?

Particle mask diameter (A): 290 ✎ ?

Number of classes: 4 ?

Reference 3D map

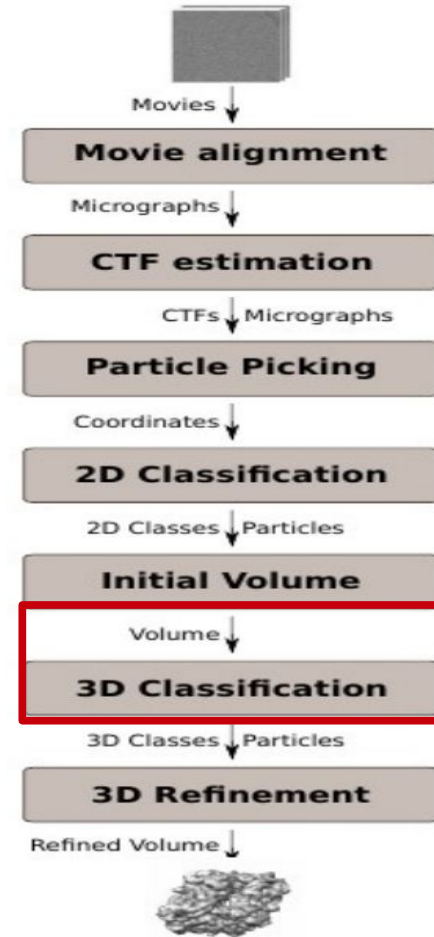
Input volume: Resize Volume.outputVol 🔍 🗑️ 👁️ ?

Is initial 3D map on absolute greyscale? Yes No ?

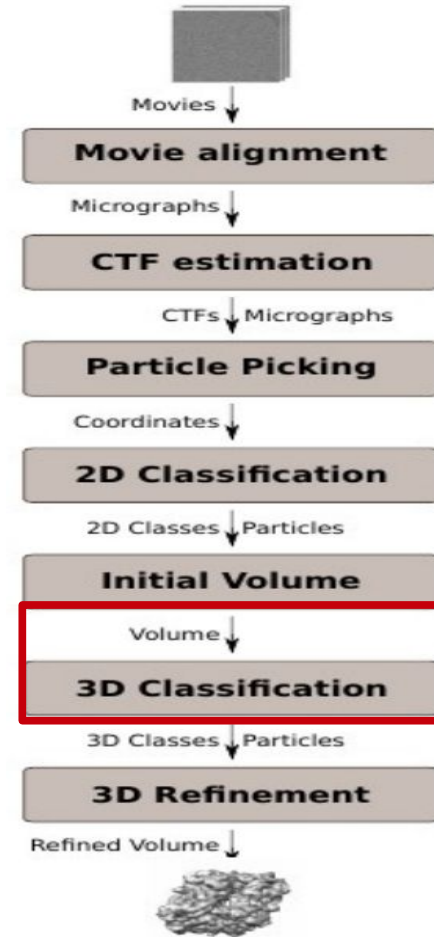
Symmetry: d2 ?

Initial low-pass filter (A): 40.0 ✎ ?

✕ Close 💾 Save ⚙️ Execute



3D Classification: results

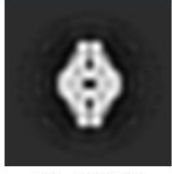





3D Classification: results

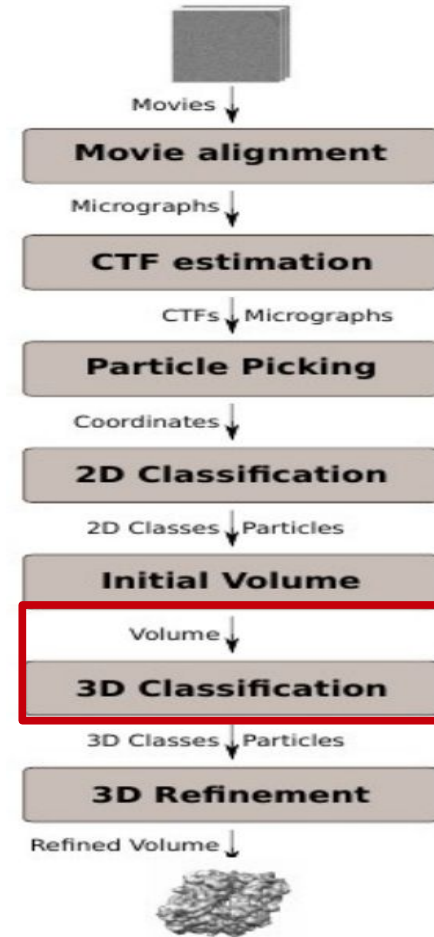
Metadata: relion_it025_classes_scipion.sqlite 4 items (100 x 100) (on finlay)

File Display Tools Help

Block Classes3D

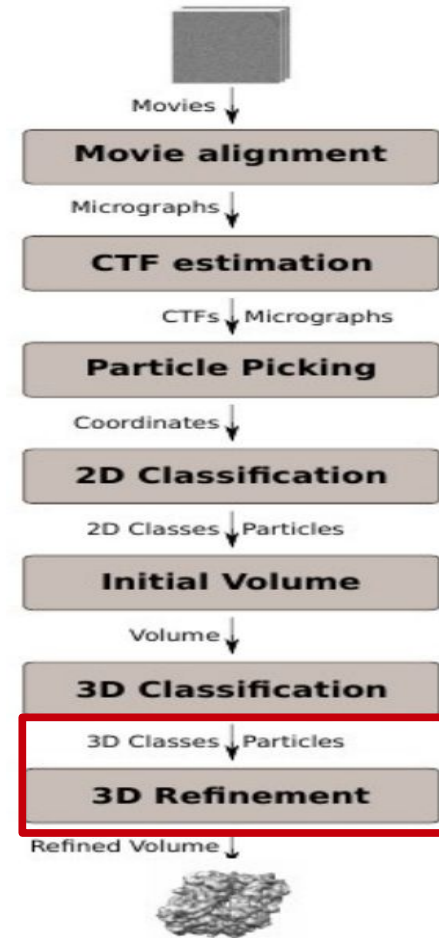
enabled	id	_size	_representative_filename	_rlnClassDistribution	_rlnAccuracyRotations	_rlnAccuracyTranslations
<input checked="" type="checkbox"/>	4	3262	 _size=3262, id=4	0.5374	4.6050	0.7830
<input checked="" type="checkbox"/>	2	1130	 _size=1130, id=2	0.1880	5.0500	1.3360
<input checked="" type="checkbox"/>	3	985	 _size=985, id=3	0.1653	5.2200	0.8980
<input checked="" type="checkbox"/>	1	643	 _size=643, id=1	0.1093	4.6450	0.9030

Close Particles Volumes Classes



3D Refinement

- ▼ 3D
 - ▷ **C** Initial volume
 - ▷ **C** Preprocess
 - ▼ **C** **Refine**
 - P** relion - 3D auto-refine
 - P** grigoriefflab - frealign
 - P** xmipp3 - projection matching
 - P** eman2 - refine easy
 - P** spider - refinement
 - ▷ more
 - ▷ **C** Classify
 - ▷ **C** Analysis
 - ▷ **C** Reconstruct
- ▷ **Tools**



3D Refinement: parameters

Protocol Run: ProtRelionRefine3D

RELION Protocol: relion - 3D auto-refine

Run

Run name: relion - 3D auto-refine

Run mode: Continue Restart

Host: localhost

Parallel Threads: 1 MPI: 24 Use queue? Yes No

Expert Level: Normal Advanced

Input CTF Optimisation Auto-Sampling Movies Additional

Input

Continue from a previous run? Yes No

Input particles: Extract Best Particles.outputParticle

Particle mask diameter (A): 290

Reference 3D map

Input volume: relion - 3D classification.outputVolun

Is initial 3D map on absolute greyscale? Yes No

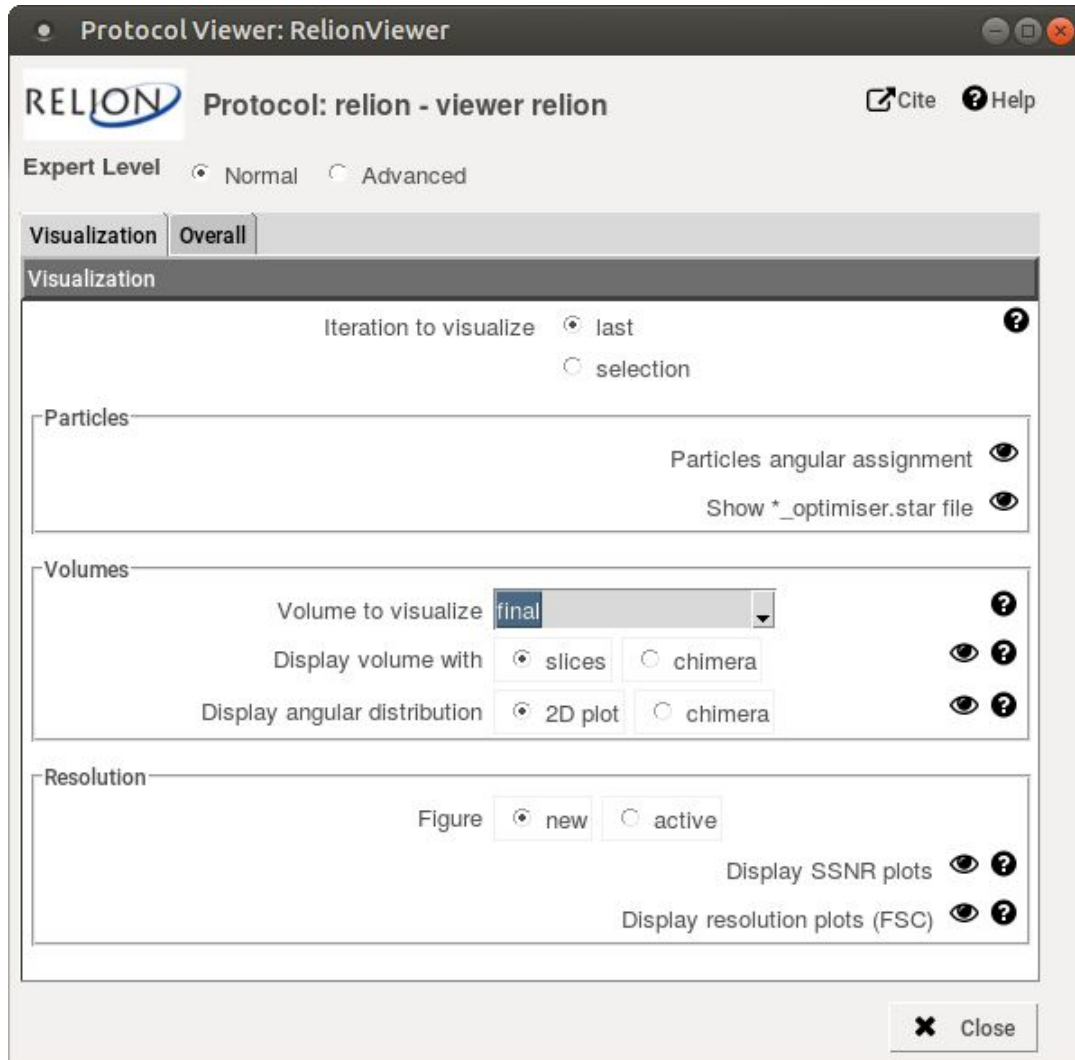
Symmetry: d2

Initial low-pass filter (A): 40.0

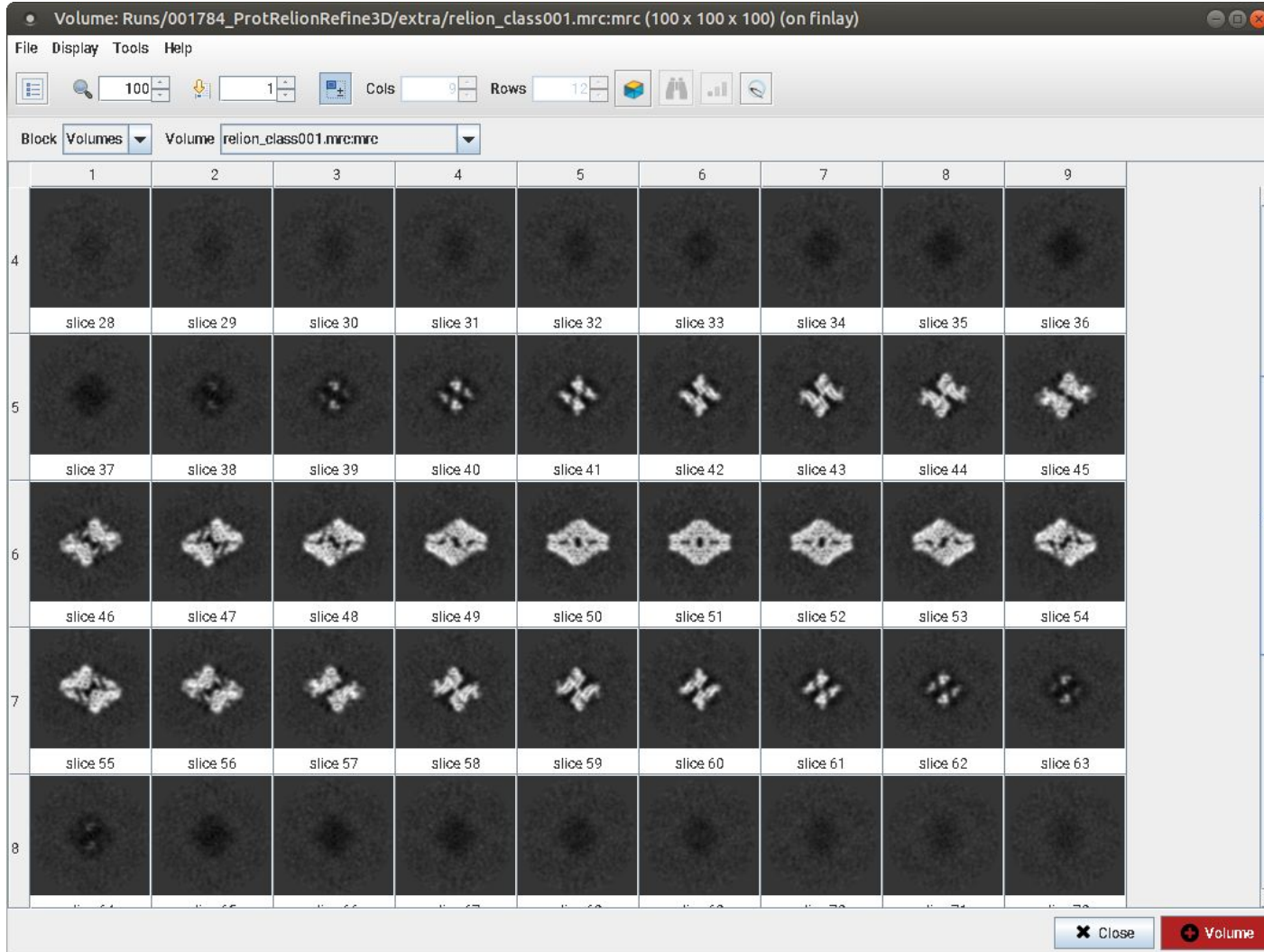
Close Save Execute



3D Refinement: results



3D Classification: results



3D Classification: results

