

An online curriculum and software platform for hands-on learning in single-particle cryo-EM and cryo-ET

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A step-by-step guide to accessing & using RELION simulator

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1. Access the cloud desktop from web browser

Recommended browser: Google Chrome

<u>URL to access desktop: http://132.249.229.224:8080/cryoedu/#/</u>

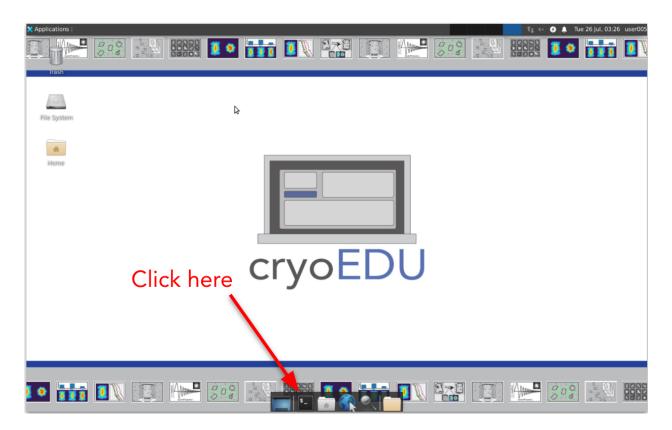
-> When successful, you should see the landing page



Input username & password provided

2. Open terminal on cloud desktop

On the desktop, open the terminal by clicking on the Terminal icon.



Useful Linux commands:

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- cd change directory. *Example*: move into the directory named 'Tutorial':
 - o \$ cd Tutorial
- mkdir makes a new directory. *Example*: creates a directory names 'test':
 - \$ mkdir test

3. Open RELION & import movies

🗙 Applications : 💼 RELION-3.1.3-commit-5 🔄 user005@ip-172-31-15 📷	Thunar	-	t⊧ ↔ ⊖ ♦	Tue 26 Jul, 19:01 user00
user005@ip-172-31-15-86: ~/Tutor		.1.3-commit-554e0e: /bigdisk/userhome/	user005/Tutorial	^ - × 🚽 🚺
File Edit View Search Terminal Heip user005gip:12-31-15-66:-5 15 Documents Music Public Tutorial gry mike-tes user005gip:12-31-15-65:-5 cd Tutorial/ user005gip:12-31-15-65:-5 cd Tutorial/ user005gip:12-31-15-65:-5 cd Tutorial/ user005gip:12-31-15-65:-/Tutorial/ user005gip:12-31-55-65:-/Tutorial/ user005-55-55-55-55-55-55-55-55-55-55-55-55-	File Jobs Schedules FileSt Middin correction CTF estimation Annual picking Particle extraction Subset selection 2D classification 3D anticla moden 3D auto-refine 3D auto-r		C-Imb.cam.ac.uk/relion Schedule Input to this job Output from this job	0
		his window to open stdout in a separate wind	w	

In project directory (e.g., 'Tutorial'), open RELION by typing: relion.

Click 'Import' and then input movie path into box with green browse button.

RELIO	N-3.1.3-commit-554e0e: /bigdisk/userhome/user001/Tutorial
File Jobs Schedules	Movies/mics Others Running
Import Motion correction CTF estimation Manual picking Auto-picking Particle extraction 2D classification 3D initial model 3D classification 3D auto-refine 3D multi-body CTF refinement Bayesian polishing Mask creation Join star files	Import raw movies/micrographs? Yes Raw input files: movies_sel/*frames.mrc Raw input files: movies? Yes Optics group name: opticsGroup1 MTF of the detector: Pixel size (Angstrom): 0.455 Voltage (kV): 200 Spherical aberration (mm): 2.7 Amplitude contrast: 0.1 Beamtilt in X (mrad): 0
Particle subtraction Post-processing Local resolution External	Beamtilt in Y (mrad): 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
Finished jobs	Running jobs Input to this job
009: Import/job009/ 008: MationCorr/job008/ 007: MationCorr/job007/ 006: MatonCorr/job006/ 005: AutoPick/job005/ 004: AutoPick/job003/ 003: CtfFind/job003/ 002: MationCorr/job002/ 001: Import/job001/	Scheduled jobs Output from this job
importing Written Import/job009/movie done!	is.star with 6 items (6 new items)

4. Run motion correction & visualize results

- a. Specify your Import job (e.g., Import/job001) as the Input in the I/O tab.
- b. Change parameters related to gain reference application. Selecting the gain reference file from the movies_sel/ directory: norm-gatandata-1.mrc
- c. Click Run when ready to get results from precalculation database

	RELIO	N-3.1.	.3-comm	it-554e0e: /bigdisk/userhome	user001/Tut	orial	^ _
ile Jobs	Schedules	I/O	Motion	Running			
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I/O view	Job actions	Cu	rrent: G	iive_alias_here	Displa	ay:	
Finished jo	bs		F	lunning jobs	Inpu	ut to this job	
002: MotionC 001: Import/			S	cheduled jobs	Out	put from this job	,

After job finishes, it will move to the 'Finished jobs' list and you can click on Display outputs. Clicking on

le Jobs Schedules	I/O Motion Running		
mport	Bfactor:	150 -	2
Aotion correction CTF estimation	Number of patches X, Y	5 5	2
lanual picking	Group frames:		
uto-picking			
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ubset selection D classification	Gain-reference image: r	n-test/norm-gatandata-1.	mrc 7 Browse
D classification D initial model	Gain rotation:	No rotation (0)	A 8
D classification			
D auto-refine		No flipping (0)	÷ 1
D multi-body	Defect file:		? Browse
TF refinement			
ayesian polishing	Use RELION's own implementation?		\$?
lask creation vin star files	MOTIONCOR2 executable:	EM/MOTIONCOR2/MotionC	Cor2 ? Browse
article subtraction	Which GPUs to use:		2
ost-processing			
ocal resolution	Other MOTIONCOR2 arguments		Y
xternal –			
I/O view Job actions	Current: 002: MotionCorr/job002	Schedule Check com	Continue!
inished jobs	Current: 002: MotionCorr/job002/	Dicplay: out: cor Input t out: log	rected_micrographs. file.pdf
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Finished jobs D08: MotionCorr/job008/ D07: MotionCorr/job008/ D07: MotionCorr/job006/ D05: AutoPick/job005/ D04: AutoPick/job003/ D03: Cffrind/job003/ D02: MotionCorr/job001/ * movies sel/17/jan09d_p_0001 Correcting beam-induced motio	Running jobs Scheduled jobs Scheduled jobs	Dicolay: Input t out: cor 001: Import/job0 Output from th 003: CtfFind/job0	rected_micrographs. file.pdf 01/ is job

corrected-micrographs.star will allow you to look at the micrographs whereas logfile.pdf will display log outputs.

5. Displaying micrographs

<u>Choice #1</u>: After motion correction: Select corrected micrographs.star as the output

• Make sure to change the scaling factor to 0.1 or 0.2 otherwise because the full micrograph size is very large

<u>Choice #2:</u> Using relion_display lets you lowpass filter the the micrographs for easier viewing

From the command line, list the output micrographs directory from Motion Correction:

```
$ ls MotionCorr/job002/movies_sel/
```

This will show you all files in the output motion correction folder. Using one of the filenames in this directory, type the following command:

```
$ relion_display --i
MotionCorr/job002/movies_sel/17jan09d_p_00014gr_00014sq_v01_00009hl16
_00002edhi_frames.mrc --gui
```

Then specify a lowpass filter value (e.g., 20) and a scaling factor (0.1).

6. Useful RELION commands

 $\verb"relion_image_handler" - A general tool for manipulating and interrogating images$

• Adding the option --stats will show the image/stack pixel dimensions

relion_display - Displays particle stacks, micrographs, slices through 3D volumes

• Adding the option --gui will allow interactive choice of filtering and scaling