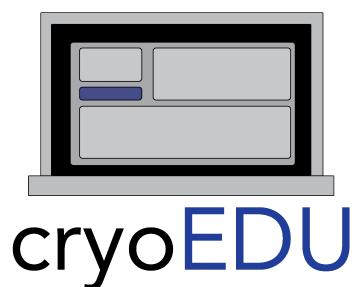
### Introduction to the RELION Simulator





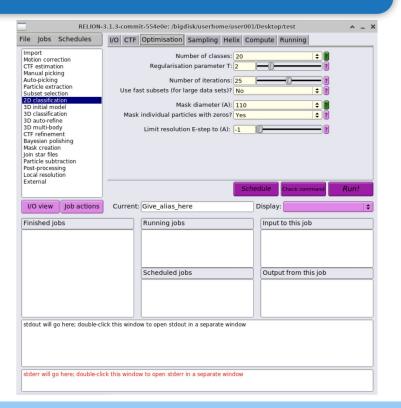




- 1. Logging onto and opening the RELION simulator on a cloud desktop environment
- 2. Practical Guide to the RELION simulator

## **RELION** in a nutshell

- RELION is a graphical user interface (GUI) that takes user input parameters and runs the job on the command line.
- RELION outputs data into automatically named & numbered directories
- The output and error from jobs is display in the 'stdout' and 'stderr' windows
- The simulator does *not* take into account any of the 'Running' tab





### How to use the simulator



### With RELION open, you will modify all parameters that are **GREEN**

RELION	-3.1.3-commit-554e0e: /bigdisk/userhome/user001/Desktop/test	RELION	-3.1.3-commit-554e0e: /bigdisk/userhome/user001/Desktop/test	^ _ ×
File Jobs Schedules	Movies/mics Others Running	File Jobs Schedules	I/O CTF Optimisation Sampling Helix Compute Running	
Import Motion correction CTF estimation Manual picking Auto-picking Particle extraction Subset selection 2D classification 3D initial model 3D classification 3D auto-refine 3D multi-body CTF refinement Bayesian polishing Mask creation Join star files Particle subtraction Post-processing Local resolution External	Import raw movies/micrographs? Yes       ?         Raw input files:       Micrographs/*.tif       ?         Raw input files:       Yes       ?         Are these multi-frame movies?       Yes       ?         Optics group name:       opticsGroup1       ?         MTF of the detector:       ?       ?         Pixel size (Angstrom):       0.455       ?         Voltage (kV):       200       ?         Spherical aberration (mm):       2.7       ?         Beamtilt in X (mrad):       0       ?         Beamtilt in Y (mrad):       0       ?         Schedule       Check command       Run!	Import Motion correction CTF estimation Manual picking Auto-picking Particle extraction Subset selection 2D classification 3D classification 3D auto-refine 3D multi-body CTF refinement Bayesian polishing Mask creation Join star files Particle subtraction Post-processing Local resolution External	Number of classes: 20 + 2 Regularisation parameter T: 2 - 2 Number of iterations: 25 - 2 Use fast subsets (for large data sets)? No + ? Mask diameter (A): 110 + 2 Mask individual particles with zeros? Yes + ? Limit resolution E-step to (A): 1 2 - 7 Schedule Check command R	← ←
I/O view Job actions	Current: Give_alias_here Display:	I/O view Job actions	Current: Give_alias_here Display:	\$

# Accessing the cloud desktop from web browser



Open a web browser (ideally Chrome) and navigate to this URL:

http://100.20.93.108:8080/cryoedu

SINGLEPARTICLE CRYOEDU Username		
CRYOEDU Username		
Password	Username	9
	Password	9

Username & password provided separately

# The cloud desktop

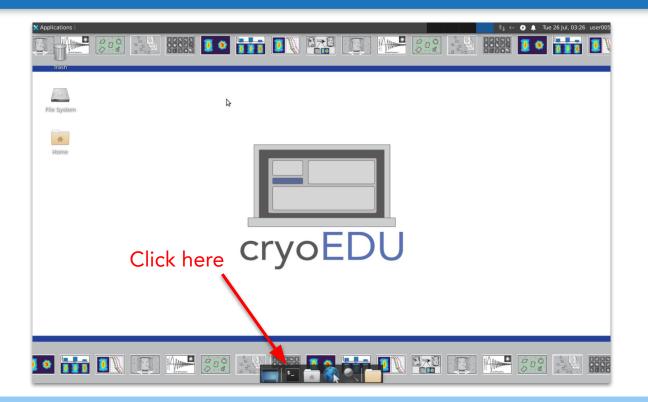


After logging in, you'll see the following desktop:

🗙 Applications 🗄												tį e	😉 🌲 Tu	e 26 Jul, 03:26	user005
Irash				1									10		
File System				\$											
# Home								_							
							оE	Đ	U						
						<b>J</b>									
	_	_	_	_	_	_	_	_	_	_	_	_	_	_	
<b>IO III</b>					a a a	5- 5-		dr		178 10					

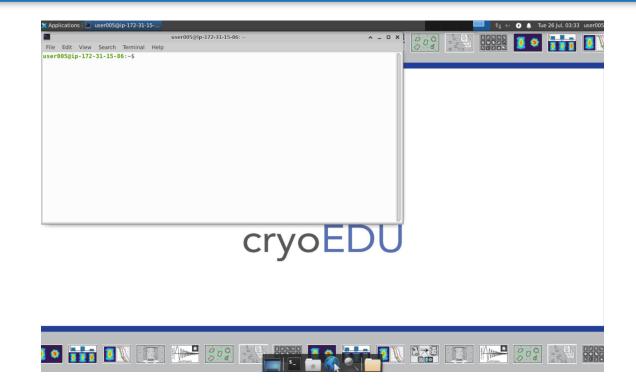
# Opening the terminal





# Opening the terminal





### Navigate to your home directory



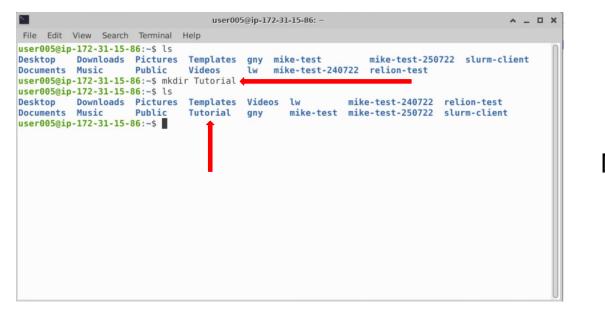
```
user008@ip-172-31-15-86:/$ pwd
/
user008@ip-172-31-15-86:/$ cd
user008@ip-172-31-15-86:~$ pwd
/bigdisk/userhome/user008
user008@ip-172-31-15-86:~$
```

Check to see which directory you're in: pwd

If you are in the top directory, enter: cd

# Making a directory





In the command line: mkdir [name of directory]

Navigate to your directory: cd [name of directory]

## Create a source environment to display PDFs



Create a source environment:

### source /etc/profile.d/singleparticle.sh

2.				user001@ip	-172-31-15-86:	~/Tutorial	^	-	×
File Ec	lit Vie	w Searc	h Terminal	Help					
user001	@ip-1	2-31-15	-86:~\$ ls						
Desktop	D		Pictures	Templates	Videos	slurm-client			
Documen	ts M	isic	Public	Tutorial	relion-test				
user001	@ip-1	2-31-15	-86:~\$ cd 1	-					
bash: c	d: T:	No such	file or di	rectory					
			-86:~\$ cd 1						
	~ .		-86:~/Tutor						
						e.star <b>movies_sel</b> track.txt			
					/etc/profil	e.d/singleparticle.sh 🔶 🚽 🚽			+
user001	@ip-1	2-31-15	-86:~/Tutor	ial\$					

### Import dataset



Import dataset:

## In -s /bigdisk/sptrain/fulldataset/movies\_sel

<b>1</b>					use	r005@ip-172-3	1-15-86: ~	~				^	-	×
File	Edit	View	Search	Terminal	Help									
user00	)5@ip	-172-3	31-15	86:~\$ ls										ſ
Deskto	op	Extra	act	Public	Videos	mike-test		relion	1-test					
Docume	ents	Musi	с	Templates	gny	mike-test-	240722	slurm-	-client					
Downlo	bads	Picto	ures	Tutorial	lw	mike-test-	250722							
user00	)5@ip	-172-3	31-15	-86:~\$ rmd	ir -r Ext	ract								
rmdir:	: inva	alid (	option	n 'r'										
Try 'r	rmdir	he	lp' fo	or more in	formation									
user00	)5@ip	-172-3	31-15	-86:~\$ rmd	ir r Extr	act								
rmdir:	fai	led to	o remo	ove 'r': N	o such fi	le or direc	tory							
rmdir:	fai	led to	o remo	ove 'Extra	ct': Not	a directory								
user00	)5@ip	-172-3	31-15	-86:~\$ rm	-r Extrac	t								
user00	5@ip	-172-3	31-15	-86:~\$ ls										
Deskto	op	Down	loads	Pictures	Templat	es Videos	lw	mi	ike-test-	240722	relion-test			
Docume	ents	Musi	с	Public	Tutoria	l gny	mike-t	est mi	ike-test-	250722	slurm-client	Ł		
user00	)5@ip	-172-3	31-15	-86:~\$ ln	-s /bigdi	sk/sptrain/	fulldat	aset/mo	ovies_sel					ł
user00	5@ip	-172-3	31-15	-86:~\$										1

# Dataset for the cryoEDU RELION simulator

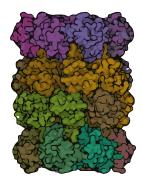


Published: 09 October 2017

### Achieving better-than-3-Å resolution by single-particle cryo-EM at 200 keV

Mark A Herzik Jr, Mengyu Wu & Gabriel C Lander 🖂

Nature Methods 14, 1075–1078 (2017) | Cite this article 10k Accesses | 90 Citations | 47 Altmetric | Metrics



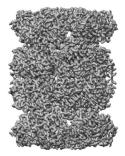
#### EMPIAR-10185

T. acidophilum 20S proteasome core movies obtained using Talos Arctica operating at 200 kV equipped with a K2 – stage position used for exposure target navigation

Publication:	Achieving better-than-3-Å resolution by single-particle cryo-EM at 200 keV <u>Herzik Jr MA (a), Wu M (b), Lander GC (b)</u> <i>Nat. Methods</i> <b>14</b> 1075-1078 (2017) PMID: <u>28991891</u> DOI: <u>10.1038/nmeth.4461</u>
Related PDB entry:	5vy3
Related EMDB entry:	EMD-8741 (3.1Å)
Deposited:	2018-05-03
Released:	2018-05-11
Last modified:	2018-05-11
Dataset size:	1.1 TB
Dataset DOI:	10.6019/EMPIAR-10185
Experimental metadata:	

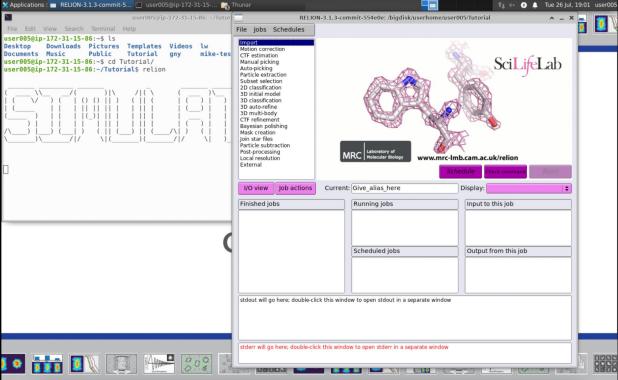






^ \_ X

### Start up RELION! 🕻 Applications 🗄 💼 RELION-3.1.3-commit-5... 🕥 user005@ip-172-31-15-. Thunar



# In your directory: relion

If the following pop-up appears hit yes to start a new project

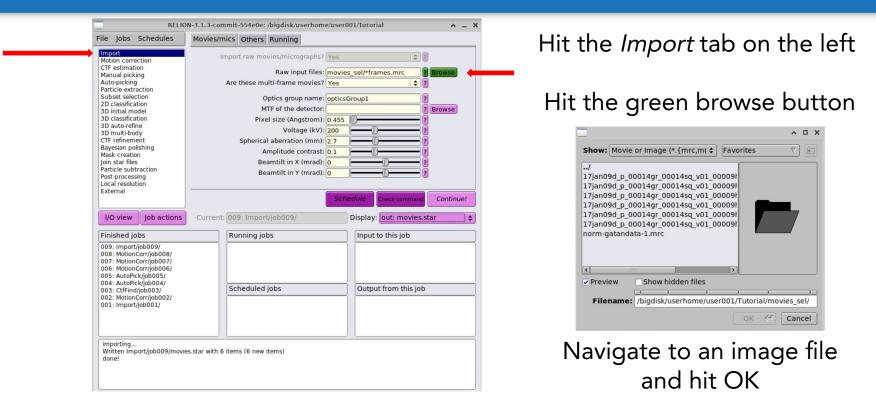
Your current directory does not look like a RELION project directory. Only run the RELION GUI from your project directory. Do you want to start a new project here? Yes 🖉 No

?



### Import movies





### Import movies



RELIC	ON-3.1.3-commit-554e0e: /bigdisk/userhome/user001/Tutorial
ile Jobs Schedules	Movies/mics Others Running
Import Motion correction	Import raw movies/micrographs? Yes
CTF estimation Manual picking	Raw input files: movies_sel/*frames.mrc 7 Browse
Auto-picking	Are these multi-frame movies? Yes
Particle extraction Subset selection	Optics group name: opticsGroup1
2D classification	MTF of the detector: 7 Browse
3D initial model 3D classification	Pixel size (Angstrom): 0.455
3D auto-refine	Voltage (kV): 200
3D multi-body CTF refinement	Spherical aberration (mm): 2,7
Bayesian polishing	Amplitude contrast: 0.1
Mask creation Join star files	Beamtilt in X (mrad): 0
Particle subtraction	Beamtilt in Y (mrad): 0
Post-processing Local resolution	
External	
	Schedule Check command Continue!
I/O view Job actions	Current: 009: Import/job009/ Display: out: movies.star \$
Finished jobs	Running jobs Input to this job
009: Import/job009/ 008: MotionCarr/job008/ 007: MotionCarr/job007/ 006: MotionCarr/job006/ 005: AutoPick/job005/ 003: CttFind/job003/ 003: CttFind/job003/ 002: MotionCarr/job002/ 001: Import/job001/	Scheduled jobs Output from this job
importing Written Import/job009/movi done!	es.star with 6 items (6 new items)

To grab all the movies, change the *Raw input files* field to: *movies\_sel/\*frames.mrc* 

\*: Allows targeting of *all* files with a given suffix

Run!

# Completed jobs will be shown on the left

### File structure within the simulator



user005@ip-172-31-15-86:~/Tutorial\$ ls
Import MotionCorr default\_pipeline.star movies\_sel track.txt
user005@ip-172-31-15-86:~/Tutorial\$

#### Directories created for each job type

user001@ip-172-31-15-86:~/Tutorial\$ ls Import MotionCorr default_pipeline.star movies_sel track.txt user001@ip-172-31-15-86:~/Tutorial\$ cd Import/	
user001@ip-172-31-15-86:~/Tutorial/Import\$ ls	<ul> <li>Directory created for each job</li> </ul>
job001 user001@ip-172-31-15-86:~/Tutorial/Import\$ cd job001/	Directory created for each job
<pre>user001@ip-172-31-15-86:~/Tutorial/Import/job001\$ ls</pre>	
RELION_JOB_EXIT_SUCCESS job.star note.txt scheduler_stderrclass.txt	<ul> <li>Metadata contained within</li> </ul>
done.txt movies.star run.out	each job
user001@ip-172-31-15-86:~/Tutorial/Import/job001\$	



Import Motion correction CTF estimation Manual picking Auto-picking Particle extraction 20 classification 30 initial model 30 classification 30 auto-refine 30 auto-refine Bayesian polishing Mask creation join star files Particle subtraction Post-processing Local resolution External	Input movies STAR file: First frame for corrected sum: Last frame for corrected sum: Dose per frame (e/A2): Pre-exposure (e/A2): EER fractionation: Do dose-weighting? Save non-dose weighted as well? Save sum of power spectra? Sum power spectra every e/A2:	1         7           1         7           32         7           Yes         7           No         7           No         7
VO view Job actions Finished Jobs 001: Import/job001/	Current: Give_alias_here Running jobs Scheduled jobs	Check command     Display:     Input to this job     Output from this job

Navigate to the Motion Correction job

### Input movies STAR file: Import/job001/movies.star

### Navigate to the Motion tab

#### 19

### **STAR Files**

Self-defining Text Archive and

Retrieval (STAR)

- First proposed in 1991
- Text-based file format for structured data
- Widely used in molecular-structural sciences but not exclusive

		user001@ip	-172-31-15-86: ~/	Futorial/MotionCori	/job002			^	_ 0	)
File Edit View Search 1	ferminal	Help								
version 30001										
ata optics										
oop_ rlnOpticsGroupName #1										
rlnOpticsGroup #2										
rlnMicrographOriginalP	ixelSiz	ze #3								
rlnVoltage #4										
rlnSphericalAberration										
<pre>rlnAmplitudeContrast #</pre>										
rlnMicrographPixelSize										
pticsGroup1	1	0.455000	200.000000	2.700000	0.100000	0.910000				
version 30001										
ata_micrographs										
000										
rlnMicrographName #1										
rlnMicrographMetadata :	#2									
rlnOpticsGroup #3										
<pre>rlnAccumMotionTotal #4 rlnAccumMotionEarly #5</pre>										
rlnAccumMotionEarly #5										
lotionCorr/job002/movie	s sel/1	17ian09d p	00014gr 00014	sa v01 00009hl	16 00002edhi	frames.mrc Mo	tionCor			
/job002/movies sel/17j							1 4			
.188346 5.990562	37.19			_	-					
lotionCorr/job002/movie										
/job002/movies_sel/17j			00014sq_v01_00	009hl16_00003e	dhi_frames.st	ar	1 3			
.648989 4.210024 otionCorr/job002/movies	26.43		00014ac 0001	ca v01 00000b1	16 00004odbi	framos mrs Mo	tionCor			
/job002/movies sel/17j							1 2			
.976145 1.480148	25.49		001104_001_00	00001120_000010						
lotionCorr/job002/movie	s sel/1	17jan09d p	00014gr 00014	sq v01 00009hl	16 00005edhi	frames.mrc Mo	tionCor			
/job002/movies_sel/17j			00014sq_v01_00	009hl16_00005e	dhi_frames.st	ar	1 3			
.052950 2.152053	30.90									
			00014gr 00014			frames mrc Mo	tionCor			
lotionCorr/job002/movie										
		00014gr_0					1 2			



### Motion correction

RELION-3.1.3-c	ommit-554e0e: /bigdisk/userhome/	user001/Tutorial	^ _ ×
File Jobs Schedules I/O Mo	otion Running		
Import Motion Correction CTF estimation Manual picking Auto-picking Particle extraction 2D classification 2D classification 2D classification 3D auto-refine	Gain rotation: A Gain flip: M Defect file: se RELION's own implementation? Y	IS -test/norm-gatandata-1.mc. to rotation (0) ÷ to flipping (0) ÷ es ÷ M/MOTIONCOR2/MotionCar2	7 Browse
ixternal		Schedule Check command	Run!
I/O view Job actions Curren	nt: Give_alias_here	Display:	+
Finished jobs	Running jobs	Input to this job	
002: MotionCorr/job002/ 001: Import/job001/			
	Scheduled jobs	Output from this job	,
stdout will go here; double-click this win	dow to open stdout in a separate wind	ow	
stderr will go here; double-click this win	dow to open stderr in a separate windo	w	

# Gain-reference image: movies\_sel/norm-gatandata-1.mrc

	^ 🗆 X
Show: Movie or Image (*.{mrc,m( \$	
/ 17jan09d_p_00014gr_00014sq_v01_00009ł 17jan09d_p_00014gr_00014sq_v01_00009ł 17jan09d_p_00014gr_00014sq_v01_00009ł 17jan09d_p_00014gr_00014sq_v01_00009ł 17jan09d_p_00014gr_00014sq_v01_00009ł norm-gatandata-1.mrc	7
Preview Show hidden files	
Filename: /bigdisk/userhome/user001/Tutorial/movi	es_sel/
ОК 🐔	Cancel

RELIO	N-3.1.3-commit-554e0e: /bigdisk/userhome/user001/Tutorial
File Jobs Schedules	I/O Motion Running
Import Motion correction CTF estimation Manual picking Auto-picking Particle extraction 2D classification 3D classification 3D auto-refine 3D multi-body CTF refinement Bayesian polishing Mask creation Join star files Particle subtraction Post-processing Local resolution External	Bfactor: 150 7 Number of patches X, Y 5 5 7 Group frames: 1 7 Binning factor: 2 7 Gain-reference image: (n-test/norm-gatandata-1.mrc) Browse Gain rotation: No rotation (0) + 7 Gain flip: No flipping (0) + 7 Defect file: 7 Browse Use RELION's own implementation? Yes + 7 MOTIONCOR2 executable: EM/MOTIONCOR2/MotionCor2 Browse Which GPUs to use: 0 Other MOTIONCOR2 arguments 7 Schedule Check command Run!
I/O view Job actions	Current: Give_alias_here Display:
Finished jobs 002: MotionCorr/job002/ 001: Import/job001/	Running jobs       Input to this job         Scheduled jobs       Output from this job

Try different gain orientations and visualize effect on motion correction

Computational demand circumvented by precalculated results

### Run!

# Visualizing/Assessing motion correction results

RELIC File Jobs Schedules	N-3.1.3-commit-554e0e: /bigdisk/userhome/user001/Tutorial A _ X A	
Import Motion correction CTF estimation Manual picking Particle extraction Subset selection 2D classification 3D initial model 3D classification 3D auto-refine 3D multi-body CTF refinement Bayesian polishing Mask creation Join star files Particle subtraction Post-processing Local resolution External	Bfactor: 150 2 Number of patches X, Y 5 5 7 Group frames: 1 2 Binning factor: 2 Gain-reference image: In-test/norm-gatandata-1.mrc 7 Gain-reference image: In-test/norm-gatandata-1.mrc 7 Gain filp: No rotation (0) 0 0 Gain filp: No filpping (0) 0 0 Defect file: 7 Browse Use RELION's own implementation? Yes 0 MOTIONCOR2 executable: EM/MOTIONCOR2/MotionCor2 8 Which GPUs to use: 0 Other MOTIONCOR2 arguments 7 Continue!	Select the job to view in
Correcting beam-induced m 4.67/4.67 min Generating logfile.pdf 1/ 1 sec	Current: 002: MotionCorr/job002/       Display:       •         Running jobs       Input out: corrected_micrographs.star         001: Import/job001/       001: Import/job001/         Scheduled jobs       Output from this job         0014gr_00014sq_v01_00009h116_00008edhi.frames.mrc       003: CtfFind/job003/         0014gr_00014sq_v01_00009h116_00008edhi.frames.mrc       •         0014gr_00014sq_v01_00009h116_00008edhi.frames.mrc       •         0014gr_00014sq_v01_00009h10_00002/corrected_micrographs.star       •	Hitting <i>Display</i> m

felect the job whose results you want to view in the *Finished jobs* panel

Hitting *Display* will show a drop-down menu of viewable results

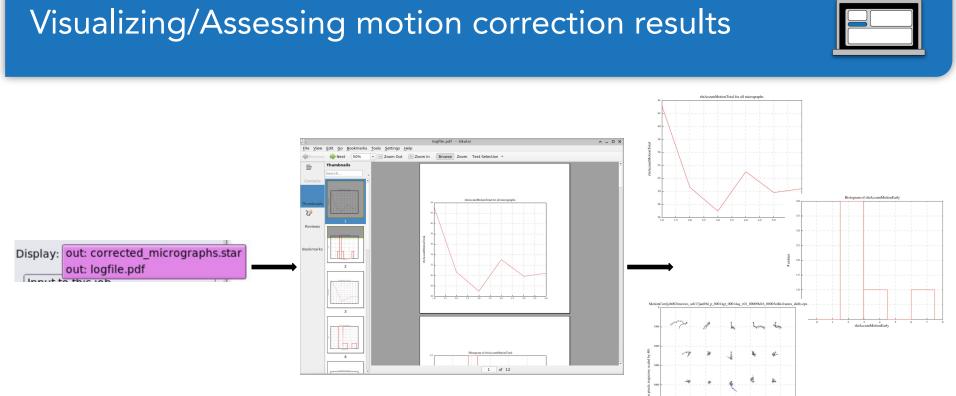
# Visualizing/Assessing motion correction results

	Relion display GUI	
Display: out: corrected_micrographs.star out: logfile.pdf	corrected_micrographs.star     Scale:   0.2   Min:   0   Max:   0   Sigma contrast:   0   Color:   greyscale   \$     Display:   rInMicrographName   \$   Osort images on:   rInOpticsGroup   \$   Reverse sort?   Apply orientations?   Read whole stacks?	
	Nr. columns: 1 Ori scale: 1 Max. nr. images: 1000 Display!	

Change *Scale* and *Nr. columns* to optimize visualization



MotionCorr/job002/corrected micrograp 🔺



Log files provide useful quantitative information on motion

### **CTF** estimation



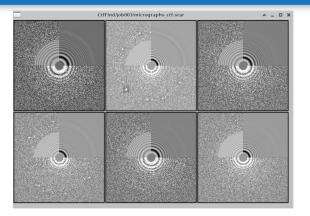
	I/O CTFFIND-4.1 Gctf Running			
Import Motion correction	Input micrographs STAR file	e: 2/corrected_microgram	ohs.star 7 Brown	
CTF estimation	Use micrograph without dose-weighting	1? No	÷ 7	
Manual picking				
Auto-picking	Estimate phase shifts	? No	\$ 2	
Particle extraction Subset selection	Phase shift - Min, Max, Step (deg	g) 0 180 1	0 7	
2D classification				
3D initial model	Amount of astigmatism (A	.): 100 <b>-</b> 🗍 -	?	
3D classification				
3D auto-refine 3D multi-body				
CTF refinement				
Bayesian polishing				
Mask creation				
Join star files Particle subtraction				
Post-processing				
Local resolution				
External				
		Schedule Check	command Rt	un!
I/O view Job actions	Current: Give_alias_here	Display:		\$
inished jobs	Running jobs	Input to this	job	
002: MotionCorr/job002/				
001: Import/job001/				
	Scheduled jobs	Output from	n this job	
	Scheduled jobs	Output from	n this job	
	Scheduled jobs	Output from	n this job	
	Scheduled jobs	Output from	n this job	
	Scheduled jobs	Output from	n this job	

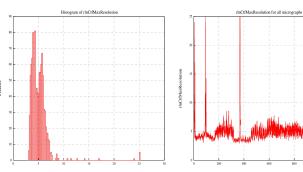
### Select the STAR file from your motion corrected micrographs: *corrected\_micrographs.star*

Run!

# Visualizing/Assessing CTF estimation results

RELIO	0N-3.1.3-commit-554e0e: /bigdisk/userhome	e/user001/Tutorial
File Jobs Schedules	I/O CTFFIND-4.1 Gctf Running	
Import Motion correction CTF estimation Manual picking Auto-picking Particle extraction 2D classification 3D classification 3D auto-refine 3D auto-refine 3D auto-refine 3D auto-refine system Dishing Mask creation Join star files Particle subtraction Post-processing Local resolution External	Input micrographs STAR file: Use micrograph without dose-weighting? Estimate phase shifts? Phase shift - Min, Max, Step (deg) Amount of astigmatism (A):	No ¢?
I/O view Job actions	Current: 003: CtfFind/job003/	Display: out: micrographs_ctf.sta ¢
Finished jobs 008: MotionCorr/job008/ 007: MotionCorr/job007/ 006: MotionCorr/job006/ 005: AutoPick/job005/ 004: AutoPick/job004/	Running jobs	Input t <sub>out:</sub> micrographs_ctf.star
003: CtfFind/job003/ 002: MotionCorr/iob002/	Scheduled jobs	Output from this job
001: Import/job001/		004: AutoPick/job004/ 005: AutoPick/job005/
Estimating CTF parameters 0 5/ 5 sec Generating logfile.pdf 0/ 0 sec	se/l7jan09d_p_00014gr_00014sq_v01_00009 using Niko Grigorieff's CTFFIND ~~("> > >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>	





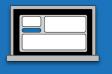
Data shown from a much larger dataset











RELION-3.1	.3-comm	it-554e0e: /bigdisk/userhome/user008/Mark 🔹 🗕 🗙
File Jobs Schedules I/O	Display	Colors Running
Save job settings           Motities         Load job settings           Order alphabetically         Order chronologically           Aute Order chronologically         Partities           Subities         Subities           Subities         Run scheduled jobs           3D c         Export scheduled job(s)           3D r         Import scheduled job(s)           3D r         Export scheduled job(s)           Cfff         Gently clean all jobs           Join star files         Particle subtraction           Post-processing         Post-processing	Alt+G	Particle diameter (A) 100 Scale for micrographs: 0.2 Sigma contrast: 3 White value: 0 Black value: 0 Lowpass filter (A) 20 Highpass filter (A) -1 Pixel size (A) -1 Scale for CTF image: 1 Pixel size (A) -1 Pixel s
Local resolution External I/O view Job actions Curr Finished jobs		Schedule     Check command     Run!       re_alias_here     Display:     \$       nning jobs     Input to this job
003: CtFind/ob003/ 002: MotionCorr/job002/ 001: Import/job001/		heduled jobs

To ensure Autopick results display...

Navigate to the Manual picking job

Particle diameter (Å): 100 Jobs  $\rightarrow$  Save job settings

No need to Run job

### Autopicking



RELION	-3.1.3-commit-554e0e: /bigdisk/userhome/user001/Tutorial	^ _ ×
File Jobs Schedules	I/O Laplacian References autopicking Helix Running	
Import Motion correction CTF estimation	Input micrographs for autopick: <mark>1/job003/micrographs_ctf.star</mark>	Browse
Manual picking Auto-picking	2D references:	Browse
Particle extraction	OR: provide a 3D reference? No \$	
Subset selection 2D classification	3D reference:	Browse
3D initial model		BIOWSE
3D classification	Symmetry: C1 ?	
3D auto-refine 3D multi-body	3D angular sampling: 30 degrees 💠 🏹	
CTF refinement Bayesian polishing Mask creation Join star files Particle subtraction Post-processing Local resolution External	Schedule Check command	Run!
I/O view Job actions	Current: Give_alias_here Display:	\$
Finished jobs	Running jobs Input to this job	
003: CtfFind/job003/ 002: MotionCorr/job002/ 001: Import/job001/	Scheduled jobs Output from this job	

Select the STAR file from your CTF estimation job: *micrographs\_ctf.star* 

Navigate to the Laplacian tab

### Autopicking

RELIO	N-3.1.3-commit-554e0e: /bigdisk/userhome/user001/Tutorial	^ _ ×
File Jobs Schedules	I/O Laplacian References autopicking Helix Running	
Import Motion correction CTF estimation Manual picking Auto-pickIng Particle extraction Subset selection 2D classification 3D initial model 3D classification 3D auto-refine 3D multi-body CTF refinement Bayesian polishing Mask creation Join star files Particle subtraction Post-processing Local resolution External	Max. diameter for LoG filter (A) 180 Are the particles white? No Maximum resolution to consider (A) 20	Te
I/O view Job actions	Current: Give_alias_here Display:	¢
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### est out different parameters to optimize autopicking!

Run!

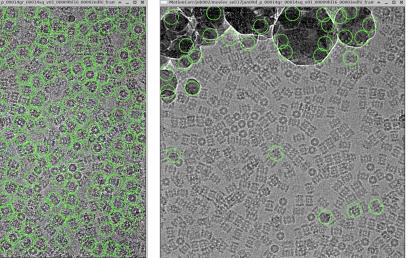
Computational demand circumvented by precalculated results



# Visualizing/Assessing Autopicking results



File Jobs Schedules	I/O Laplacian References autopicking	Helix Running	
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Mask creation			and the second s
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External	· · · · · · · · · · · · · · · · · · ·	Schedule Check command Continue!	A DE LA DE LA DE LA DE LA DELLA DELLA DELLA DELLA D
		in: micrographs_ctf.star	
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		out: logfile.pdf	
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007: MotionCorr/job007/			the second s
006: MotionCorr/job006/			
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003: CtfFind/job003/			的。""我们就是我们的。""你们的你们,我们们不能的?""我,我们没有我们
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Total number of particles from	n 6 micrographs is 1415		Changes
i.e. on average there were 23	6 particles per micrograph		gee
Done! Written: AutoPick/job0	05/loafile.pdf		•



Changes to particle diameters and thresholding significantly alter particle picking

### Lunch Break!



Try out different parameters from

- Motion correction
- CTF estimation
- Autopicking

To optimize your particle picks!

### Particle extraction



Jobs Schedules	I/O extra	ct Helix	Running						
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### STAR file from your CTF estimation: *micrographs\_ctf.star*

### Particle extraction



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ile Jobs Schedules	I/O extract Helix Running			
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Auto-picking	OD as automatical as tiples			
Particle extraction Subset selection	OR re-extract refined particles		<b>÷</b> ?	
2D classification	Refined particles STAR file		? Browse	
3D initial model	Reset the refined offsets to zero?	No No	\$ ?	
D classification	OR: re-center refined coordinates	2 No	\$ 7	
3D auto-refine	Recenter on - X, Y, Z (pix	): 0 0 0		
3D multi-body CTF refinement	Necencer on - A, 1, 2 (pix			
Bayesian polishing Mask creation Join star files				
Particle subtraction				
Post-processing Local resolution				
Local resolution		Schedule Check	command Run!	
Local resolution	Current: Give_alias_here	Schedule Check	command Run!	•
Local resolution External	Current: Give_alias_here			

STAR file from your CTF estimation: *micrographs\_ctf.star* 

Input coordinates: coords\_suffix\_autopick.star

Navigate to the *extract* tab

### Particle extraction



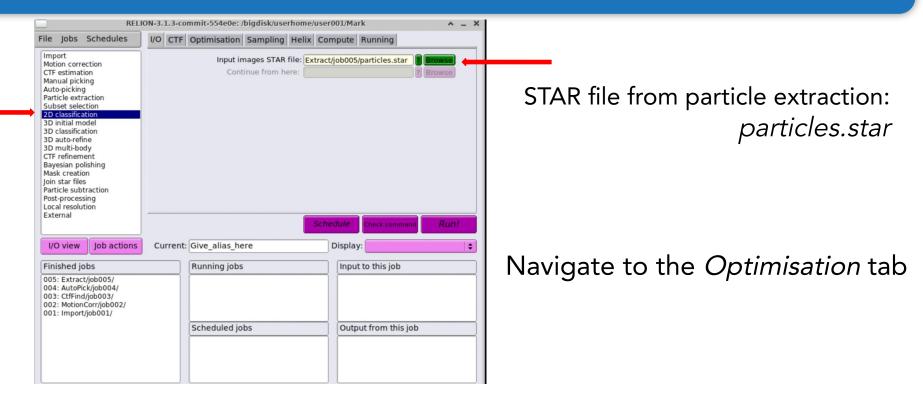
REL	ION-3.1.3-commit-554e0e: /bigdisk/userhome/user001/Mark	
File Jobs Schedules	I/O extract Helix Running	
Import Motion correction CTF estimation Manual picking Auto-picking Particle extraction Subset selection 2D classification 3D classification 3D classification 3D classification 3D auto-refine 3D multi-body CTF refinement Bayesian polishing Mask creation Join star files Particle subtraction Post-processing Local resolution External	Particle box size (pix): 300 Invert contrast? Yes Normalize particles? Yes Diameter background circle (pix): -1 Stddev for white dust removal: -1 Stddev for black dust removal: -1 Rescale particles? Yes Re-scaled size (pixels): 76 Schedule Check command Run!	Τe
I/O view Job actions	Current: Give_alias_here Display:	
Finished jobs 004: AutoPick/job004/ 003: CtfFind/job003/ 002: MotionCorr/job002/ 001: Import/job001/	Running jobs     Input to this job       Scheduled jobs     Output from this job	

### est out different extraction box and scaling parameters!

Run!

### 2-D Classification





### 2-D Classification

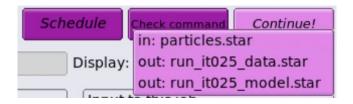
RELION-3.1.3-commit-554e0e: /bigdisk/userhome/user001/Mark ^ _ X		
ile Jobs Schedules	I/O CTF Optimisation Sampling Helix Compute Running	
Import Motion correction CTF estimation Manual picking Auto-picking Particle extraction Subset selection <b>2D</b> classification 3D initial model 3D classification 3D auto-refine 3D multi-body CTF refinement Bayesian polishing Mask creation Join star files Particle subtraction Post-processing Local resolution External	Number of classes:   Regularisation parameter T:   2   Number of iterations:   25   7   Use fast subsets (for large data sets)?   No   Mask diameter (A):   110   Mask individual particles with zeros?   Limit resolution E-step to (A):     Schedule     Check command     Run!	
I/O view Job actions	Current: Give_alias_here Display:	
Finished jobs 005: Extract/job005/	Running jobs Input to this job	
004: AutoPick/job004/ 003: CtfFind/job003/ 002: MotionCorr/job002/ 001: Import/job001/	Scheduled jobs Output from this job	

# Test out different 2-D classification parameters!

Run!

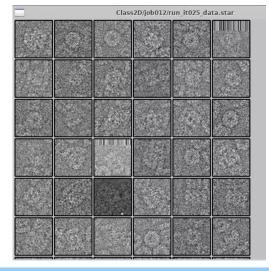


# Visualizing/Assessing 2-D classification results



data.star

Individual particles

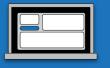


model.star

Class averages Class2D/job006/run\_it025\_model.star

## Go forth and simulate!

cryoEM



### More precalculated results coming soon!

### Modules

