

# Joint Group Meeting

(Loic Carrique, postdoc in Grimes group)

# EMBO course resume

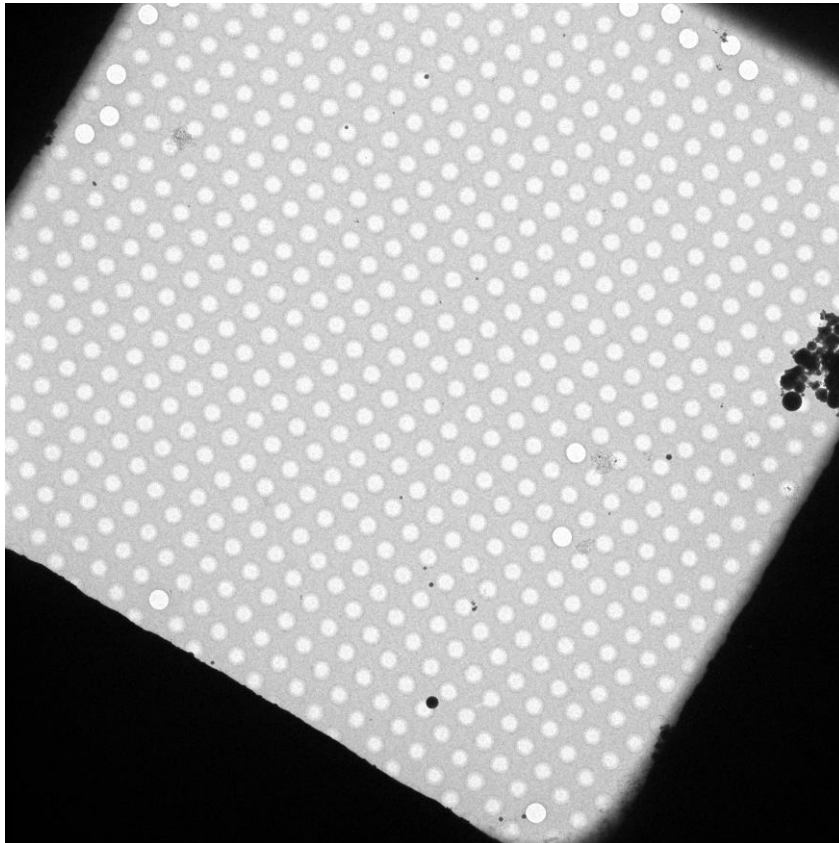
<b>Lecture 2</b> Basic concepts of Fourier analysis <b>Giulia Zanetti, BBK</b> <a href="#">(pdf)</a>	<b>Lecture 3</b> Concepts of convolution, point spread, and cross-correlation <b>Elena Orlova, BBK</b> <a href="#">(pdf)</a>	Lunch	<b>Practical 1</b> Images and transforms, filtering, CTF, diffraction spacings, filter design, EMAN 2 Initial steps in processing, Scipion/motion corr/dose fractionation ( <a href="#">eman</a> )( <a href="#">scipion</a> ) <b>Helen Saibil, BBK ; Jose Miguel de la Rosa Trevin, Marta Carroni, Stockholm, Guendalina Marini, Scott Gardner BBK</b>	
<b>Lecture 4</b> Principles of TEM image formation <b>Chris Russo, MRC-LMB</b> <a href="#">(pdf)</a>	<b>Lecture 5</b> Contrast transfer & CTF correction (phase contrast) (eBIC) <b>Dan Clare, Diamond</b> <a href="#">(pdf)</a>	Lunch	<b>13:30-14:00 B62</b> <b>Lecture Bart Marzec, JEOL</b> Think Cold: Pushing the SPA Resolution Limit with CryoARM 300	<b>Practical 2</b> Scipion preprocessing pipeline cont'd: CTF find4, phase contrast, Gautomatch, particle picking, examples of good and bad images, effects of dose and filtering <b>Jose Miguel de la Rosa Trevin, Marta Carroni, Stockholm. Dan Clare, Diamond</b> ( <a href="#">scipion</a> )
<b>Lecture 6</b> Tomography and CLEM <b>Kay Grünewald, Hamburg</b> <a href="#">(pdf)</a>	<b>Lecture 7</b> Subtomogram averaging, 3D CTF correction <b>John Briggs, MRC-LMB</b> <a href="#">(pdf)</a>	Lunch	<b>13:30-14:00 B62</b> <b>Lecture Andreas Förster, Dectris</b> Micro ED <a href="#">(pdf)</a>	<b>Practical 3</b> Tomography and segmentation <b>Daven Vasishtan, Oxford. Mauro Maiorca, Guendalina Marini, Josh Hutchings, Claudine Bisson, BBK</b> ( <a href="#">imod</a> )( <a href="#">segmentation</a> )
<b>Lecture 8</b> FIB milling /phase plate imaging <b>Julia Mahamid, EMBL-HD</b> <a href="#">(pdf)</a>	<b>Lecture 9</b> detectors, noise, frame alignment principles, particle detection <b>Helen Saibil, BBK</b> <a href="#">(pdf)</a>	Lunch	<b>13:30-14:00 B62</b> <b>Lecture Ieva Drulyte, Thermo Fisher</b> Micro ED: EPU-D results and perspectives <a href="#">(pdf)</a>	<b>Practical 4</b> Subtomogram averaging and 3D CTF correction, Dynamo <b>Daniel Castano, Giulia Zanetti, Daven Vasishtan, Josh Hutchings, Claudine Bisson</b> ( <a href="#">dynamo</a> )
<b>Lecture 10</b> Single particle analysis, reconstruction principles <b>John Rubinstein, Toronto</b> <a href="#">(pdf)</a>	<b>Lecture 11</b> Using symmetry, viruses, helices, extracting asymmetric parts <b>Doryen Bubeck, IC</b> <a href="#">(pdf)</a> <a href="#">(mov)</a> <a href="#">(ppt)</a>	Lunch	<b>Practical 5</b> Single particle analysis: cryosparc: initial model by SGD, 2D & 3D classification, refinement. Map inspection with Chimera <b>John Rubinstein, Ali Punjani, Marina Ivanova, Scott Gardner, BBK</b> ( <a href="#">cryosparc</a> ) <a href="#">(pdf)</a>	

# Why collect with a large pixel size is an advantage for screening

<b>Lecture 12</b> Single particle (Bayesian methods, particle classification ( <a href="#">pdf</a> , <a href="#">ppt</a> ) <b>Sjors Scheres, MRC-LMB</b>	<b>Lecture 13</b> Microscopes and automated data collection <b>Bridget Carragher, NYSBC</b> <a href="#">(pdf)</a>	Lunch	<b>13:30-14 :00 B62</b> <b>Lecture</b> <b>Paul Mooney,</b> <b>Gatan</b> Direct electron detectors <a href="#">(pdf)</a>	<b>Practical 6</b> Relion advanced topics in single particle analysis: 3D refinement with focussed classification, signal subtraction, postprocessing, sharpening, local filtering <b>Sjors Scheres, Kat Toropova, Giulia</b> <b>Zanetti, Scott Gardner</b> ( <a href="#">relion</a> )
<b>Lecture 14</b> Validation of 3D EM reconstructions, post processing <b>Peter Rosenthal, Crick</b> ( <a href="#">pdf</a> )	<b>Lecture 15</b> Interpretation of maps, docking of atomic structures <b>Maya Topf, BBK</b> ( <a href="#">pdf</a> )	Lunch	<b>Practical 7</b> Fitting of structures, flexible fitting (Flex- EM), model validation (TEMPy) ( <a href="#">pdf</a> ) <b>Tom Burnley, Maya Topf, Colin Palmer,</b> <b>Agnel Praveen Joseph, Sony Malhotra, Tristan</b> <b>Cragnolini, Aaron Sweeney</b>	
<b>Lecture 16</b> High resolution refinement, model assessment <b>Arjen Jakobi, Delft</b> ( <a href="#">pdf</a> )	<b>Lecture 17</b> Cryo EM in the pharmaceutical industry <b>Claudio Ciferri - Genentech</b> ( <a href="#">pdf</a> ) <b>Ilaria Ferlenghi, GSK</b>	Lunch	<b>Practical 8</b> local sharpening (LocScale), de novo structure building (CCP-EM, REFMAC) ( <a href="#">locscale.refmac</a> , <a href="#">buccaneer</a> ) <b>Arjen Jakobi, Tom Burnley, Colin Palmer,</b> <b>Sony Malhotra, Tristan Cragnolini, Aaron Sweeney</b>	
<b>Lecture 18</b> EMDB Data deposition, EMPIAR, eBIC <b>Gerard Kleywegt</b> ( <a href="#">pdf</a> )& <b>Osman Salih,</b> <b>EMDB</b> ( <a href="#">pdf</a> )	<b>Guest lecture 6</b> Future prospects for cryo EM <b>Richard Henderson, MRC LMB</b> <a href="#">(pdf)</a>	Lunch	Course review and departure	

# How to get good grids for Single Particles Analysis (SPA)

- **Which type of grids use?**
  - ✓ Gold/copper Quantifoil 200 mesh, R2/1
- **These settings are working for Strubi VitroRobot (II)**
  - ✓ 3s blotting, 3.5  $\mu$ L sample, 20°C, blotting force -25



# What to expect from one day on a Glacios microscope

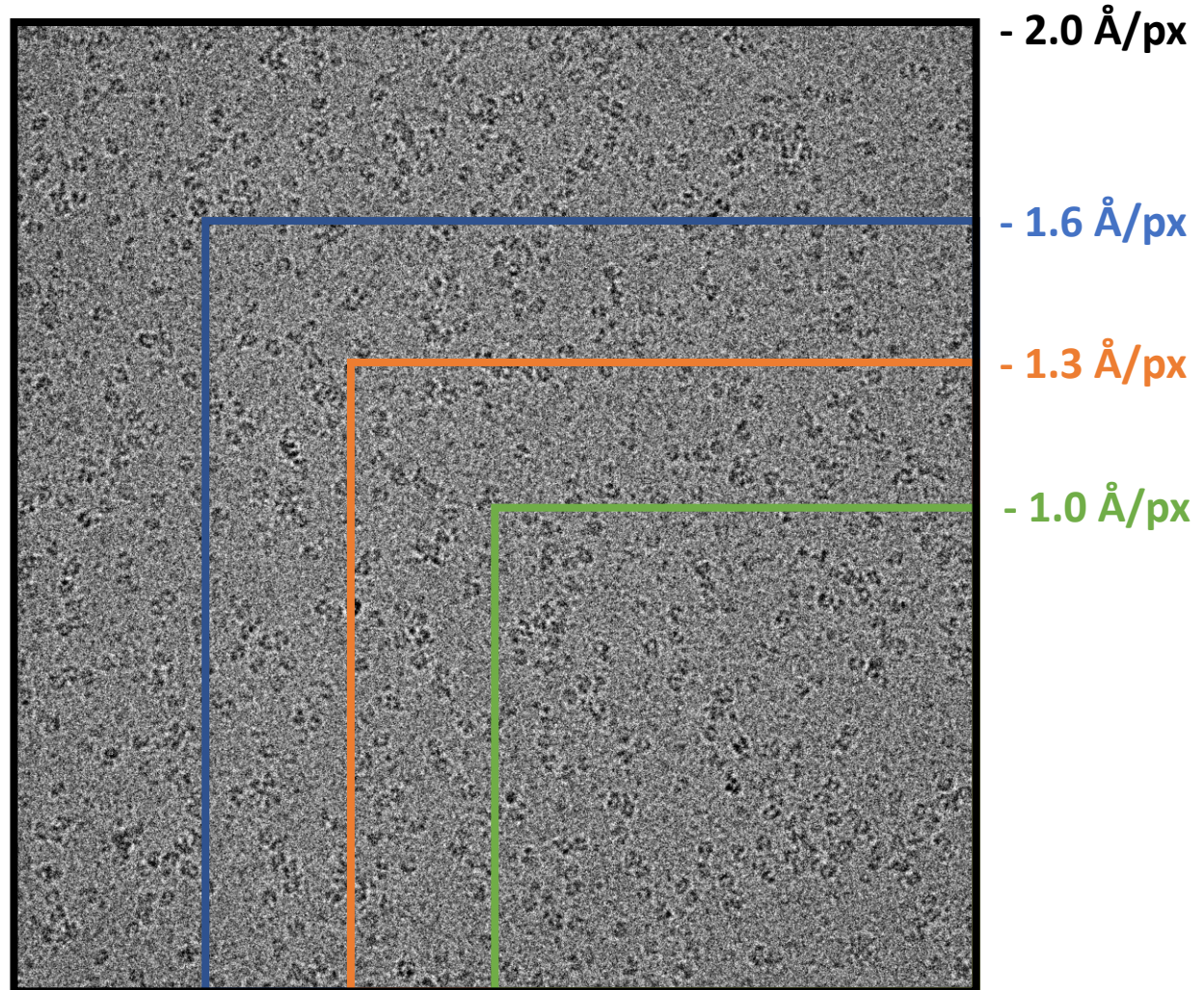
- **How many grids can we check per 24h Glacios session:**
  - ✓ Most of the time it's 2 x 12 grids
  - ✓ This correspond to 6 – 12 different samples/conditions
- **What we are checking when we screen:**
  - ✓ If there is sample on the grid
  - ✓ If the sample is going into the holes
  - ✓ If the distribution is correct or can be improve
- **Yes, but real data are better to check sample quality!!!**

How many dataset can I get?



# Why collect with a large pixel size is an advantage for screening

- ✓ Low mag (bigger pixel size) decrease the SNR in your particles
- ✓ Resolution is limited to two time the pixel size (Nyquist)
- ✓ But low mag mean bigger field of view!



# What to expect from one day on a Glacios microscope

- **How many grids can we check per 24h Glacios session:**
  - ✓ Most of the time it's 2 x 12 grids
  - ✓ This correspond to 6 – 12 different samples/conditions
- **What we are checking when we screen:**
  - ✓ If there is sample on the grid
  - ✓ If the sample is going into the hole
  - ✓ If the distribution is correct or can be improve
- **What we expect from a screening dataset:**
  - ✓ Have an idea of sample quality (Complex assembly, particles orientations, binding partner, conformational changes...)
  - ✓ Get an initial map at reasonable resolution (Between 4Å and 10Å) that could answer these questions

What is the minimal amount of data that I need to do that?



# Glacios data collection parameters

- ✓ Glacios microscope, Falcon III detector, linear mode and 92,000x mag (Pixel size 1.6 Å<sup>2</sup>)
- ✓ Total dose ~60 e<sup>-</sup>/Å<sup>2</sup>, 2s exposure, defocus range -1 to -2.5 μm → ~100 movies/h
- ✓ 1400 movies o/n for this dataset (Stage shift 10s, Fast beta, autofocus every 15 μm)



How many time to process the data?

2 days

Aligned movie, Full frames  
-1 μm defocus

Aligned movie, Full frames  
-2.2 μm defocus



# Cryosparc V2 – Motion correction and CTF estimation

- ✓ Motion Correction with Motioncor2 or CS full frame motion correction
- ✓ CTF estimation using GCTF and EquiPhase Averaging (EPA)
- ✓ Manual curation of micrographs

The screenshot displays the cryoSPARC V2 web interface. At the top, the cryoSPARC logo is on the left, and navigation tabs for 'P24: 20191009\_CPFpolyA\_35RNA' and 'W1: processing' are in the center. On the right, there are buttons for 'Details' and 'Job Builder'. Below these, the 'P24 → W1 DETAILS' section shows the current job is 'processing' with 'No description set'. A 'JOBS' list on the right includes 'J1: New Job J1', 'J2: New Job J2', 'J3: New Job J3', 'J4: New Job J4', and 'J5: New Job J5'. Below the jobs list, it shows 'CREATED BY' as '5d95efdfb831e71b0de7ddb3' and 'CREATED' as 'Thu Oct 10, 19 09:15:10 AM +01:00'. An 'ACTIONS' section contains a 'Delete' button.

The main workflow area shows a sequence of jobs:

- J1: Completed** (Import Movies)
- J2: Completed** (Full-frame motion correction) - This job includes two graphs: 'Curve: 2.79nm=17' and 'Curve: 4.24nm=17'.
- J3: Completed** (CTF Estimation (Gctf) (EPA)) - This job includes two micrograph images.
- J15: Waiting** (Curate Exposures) - This job is currently in a 'Waiting' state.
- J4: Completed** (Curate Exposures) - This job is currently in a 'Completed' state.

At the bottom, there are navigation tabs for 'Dashboard', 'Projects', and 'Resource Manager'. In the bottom right corner, there is a user profile icon and the name 'Loic'.

# Cryosparc V2 – Manual curation

- ✓ Allow to easily assess data quality based on metric (motion, defocus, CTF...)
- ✓ Check that no problems occurred during data collection
- ✓ Discard bad micrographs

P24: 20191009\_CPFpolyA\_35RNA

W1: processing

J15

Interactive

Overview

Inputs and Parameters

Outputs

Metadata

Total:1410

Accepted:0

Rejected:0

Selected:1410

#	DF Avg	Astigm	Phase	CTF Fit	CTF CC	Motion dist.	Mot cur
0	14076.19	323.95	0.00	3.52	0.00	4.23	1.9
1	26671.88	164.04	0.00	3.71	0.00	7.94	2.4
2	27833.99	136.56	0.00	3.82	0.00	7.19	2.2
3	19039.07	242.06	0.00	3.49	0.00	11.79	2.6
4	15386.72	347.66	0.00	3.95	0.00	9.63	2.3
5	23412.12	206.95	0.00	3.45	0.00	7.84	1.9
6	28843.75	140.60	0.00	3.51	0.00	5.67	2.2
7	18244.16	281.27	0.00	3.55	0.00	6.20	2.7
8	15119.14	339.84	0.00	3.85	0.00	8.17	2.8
9	21662.15	203.10	0.00	3.45	0.00	11.99	3.6
10	14441.41	402.27	0.00	3.54	0.00	9.13	2.6
11	8710.94	441.33	0.00	3.45	0.00	10.18	2.9
12	12996.09	398.42	0.00	3.56	0.00	9.53	2.2
13	19388.67	394.44	0.00	4.44	0.00	10.76	3.0
14	19652.36	288.96	0.00	3.62	0.00	10.18	2.9
15	27828.15	167.36	0.00	3.53	0.00	10.66	2.6
16	17759.77	311.81	0.00	3.45	0.00	8.96	2.0
17	9595.72	425.62	0.00	3.86	0.00	7.37	2.8
18	14203.14	421.76	0.00	3.64	0.00	12.45	3.6
19	11503.91	11.72	0.00	4.01	0.00	10.97	2.5
20	21757.81	273.23	0.00	3.45	0.00	8.24	2.3
21	18240.25	316.45	0.00	3.45	0.00	7.15	2.0
22	22548.83	277.26	0.00	3.45	0.00	7.44	2.5
23	14734.38	324.15	0.00	3.45	0.00	9.16	2.5
24	19748.06	238.24	0.00	3.68	0.00	7.79	1.8
25	14179.70	331.97	0.00	4.66	0.00	9.81	2.5
26	18065.06	601.65	0.00	6.73	0.00	12.77	3.1

Overview

Individual

Average defocus (Å)

Index

Colors

Index

Type

# of bins (X)

# of bins (Y)

0.00

1409.00

3971.01

54418.02

0.00

2206.81

Dashboard

Projects

Resource Manager

Details

Job Builder

JOB DETAILS

J15

WAITING

Manually Curate Exposures

New Job J15

Enter a description.

CLONED FROM

J4

CREATED BY

5d95f061b831e71b0de922ee

INTERACTIVE

Yes

CREATED

Thu Oct 10, 19 12:11:53 PM +01:00

QUEUED

Fri Oct 11, 19 10:12:55 AM +01:00

LAUNCHED

Fri Oct 11, 19 10:12:55 AM +01:00

WAITING

Fri Oct 11, 19 10:13:07 AM +01:00

STARTED

Fri Oct 11, 19 10:12:59 AM +01:00

KILLED

Thu Oct 10, 19 06:19:10 PM +01:00

PARENTS

J3

SIZE

83.97 KB

ACTIONS

Queue Job on default

Queue Job on compG000-lane

Kill Job

Clear Job

Clear Intermediate Results

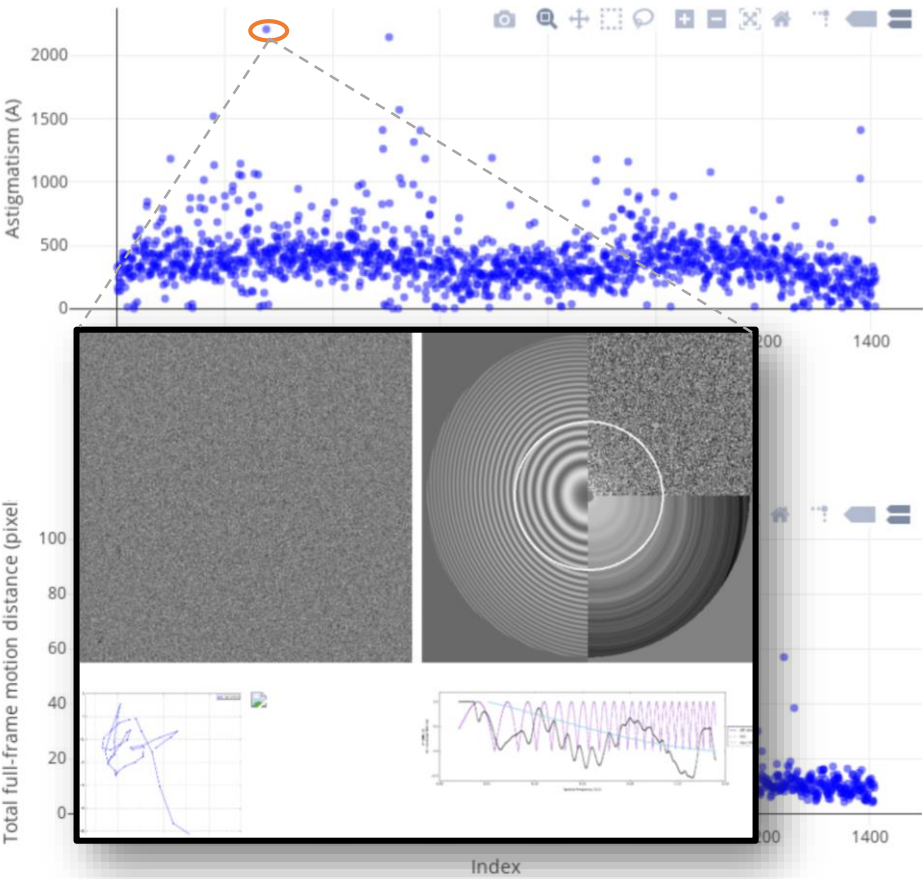
Export Job

Loic

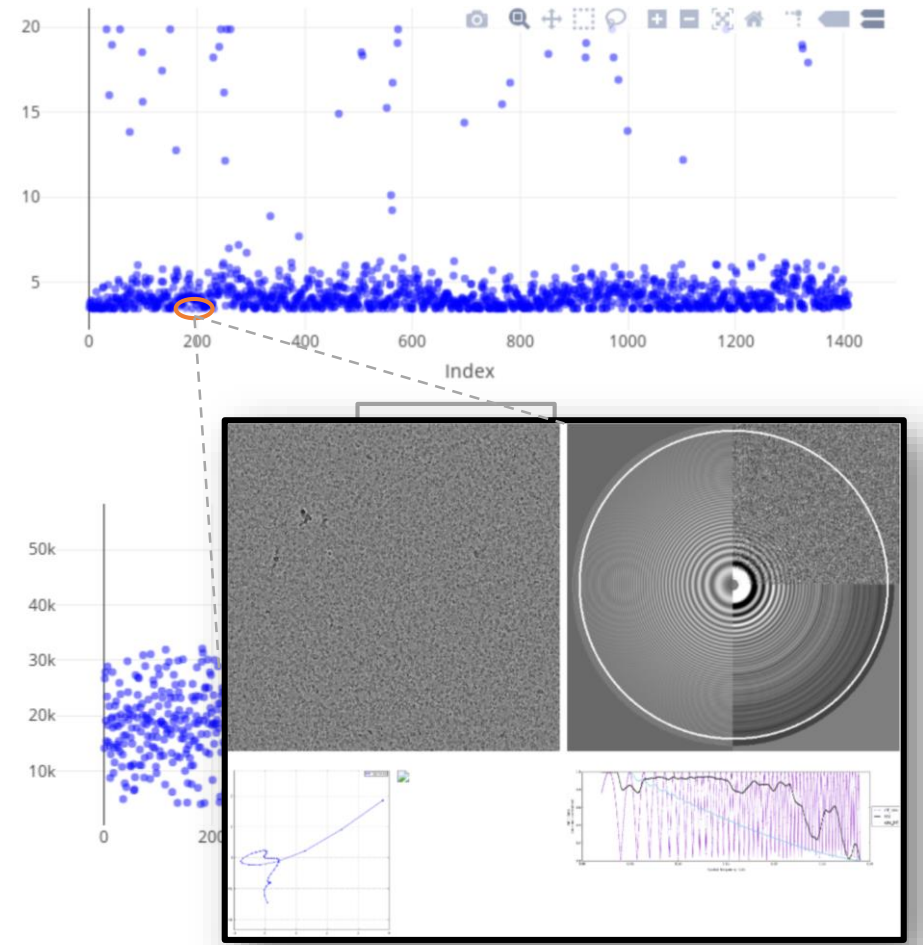
# Cryosparc V2 – Manual curation

- ✓ Plots of the different metrics

Astigmatism



CTF estimated Max Resolution



# Cryosparc V2 – Particles picking Step 1

- ✓ Blob picker with a wild range of sizes to capture most of the views
- ✓ Particles extraction at bin 2 or bin 4 (Depending on the pixel size used)
- ✓ 2D classification with 50 classes (Just to get the main views)

The screenshot displays the cryoSPARC V2 web interface. At the top, the project name is 'P24: 20191009\_CPFpolyA\_35RNA' and the current job is 'W1: processing'. The main workspace shows a workflow diagram with the following jobs:

- J29** (Completed): Patch CTF (M)
- J5** (Completed): Blob pick (M)
- J6** (Completed): Inspect Picks
- J7** (Completed): Extract Mic
- J8** (Completed): 2D Class

The right sidebar provides details for the current job, 'P24 → W1 DETAILS'. It shows the job is 'processing' with 'No description set'. Below this, there is a list of jobs (J1 to J5) and a 'Delete' button. The bottom of the interface includes navigation links for 'Dashboard', 'Projects', and 'Resource Manager', along with a user profile icon for 'Loic'.



# Cryosparc V2 – Particles picking Step 2

- ✓ Template picking based on previous selected 2D classes
- ✓ Particles extraction at bin 2 or bin 4
- ✓ 2D classification with 50 classes (To remove all the junks classes)
- ✓ 2<sup>nd</sup> 2D classification with 200 classes and high uncertainty score

cryoSPARC

P24: 20191009\_CPFpolyA\_35RNA

W1: processing

Details

Job Builder

P24 → W1 DETAILS

processing

No description set

JOBS

J1: New Job J1

J2: New Job J2

J3: New Job J3

J4: New Job J4

J5: New Job J5

CREATED BY 5d95efdfb831e71b0de7ddb3

CREATED Thu Oct 10, 19 09:15:10 AM +01:00

ACTIONS

Delete

Dashboard

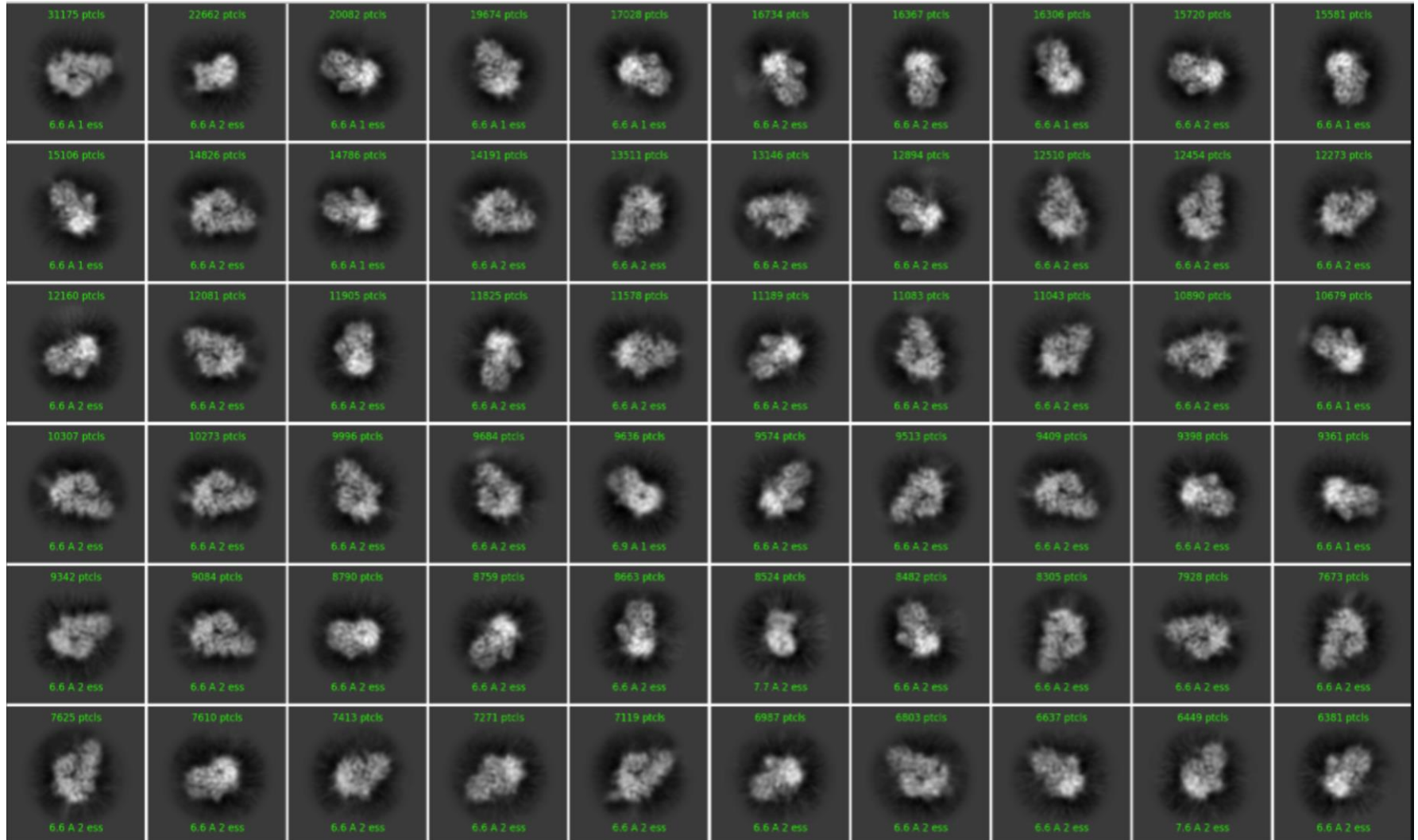
Projects

Resource Manager

Loic

# Cryosparc V2 – 2D classifications

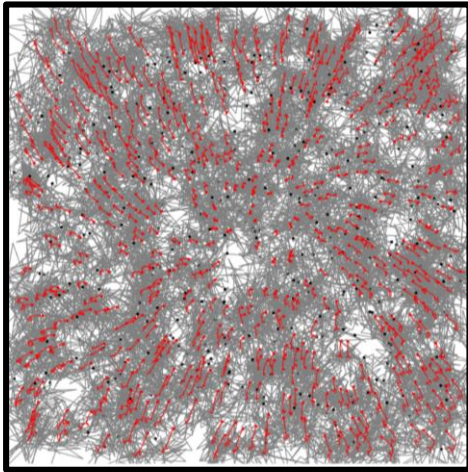
- ✓ Around 1,000,000 particles selected after 2D classification



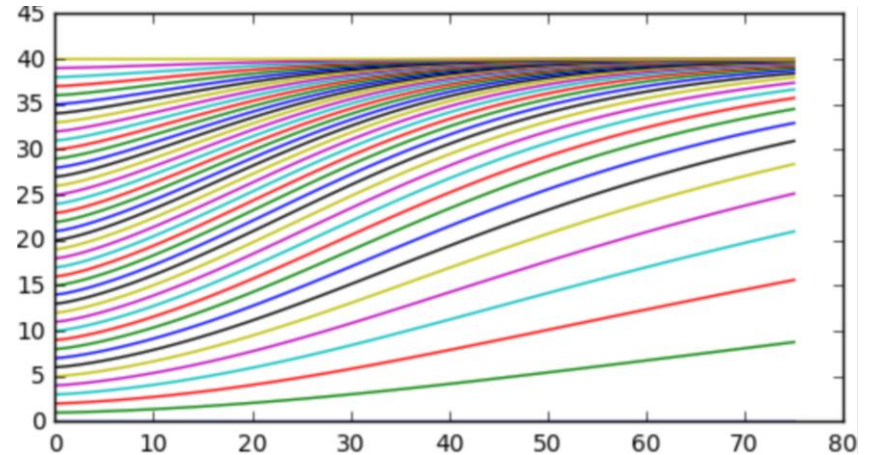
# Cryosparc V2 – Local motion correction and CTF

- ✓ Local motion correction is tracking the motion of each individual particles
- ✓ It can also re-extract particles unbinned or in a new box size at the same time

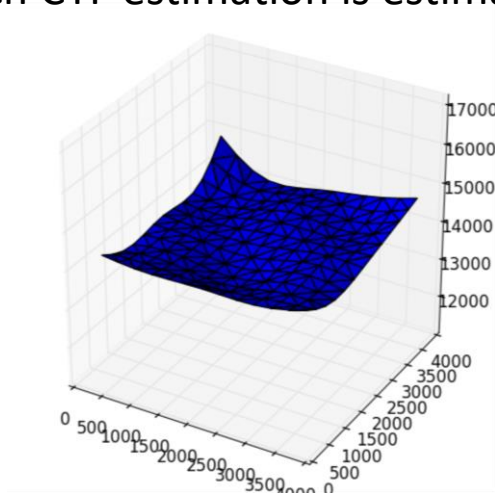
Local Motion



Exposure weights



- ✓ Patch CTF estimation is estimating local variation of defocus across your micrograph



# Cryosparc V2 – Ab initio model(s)

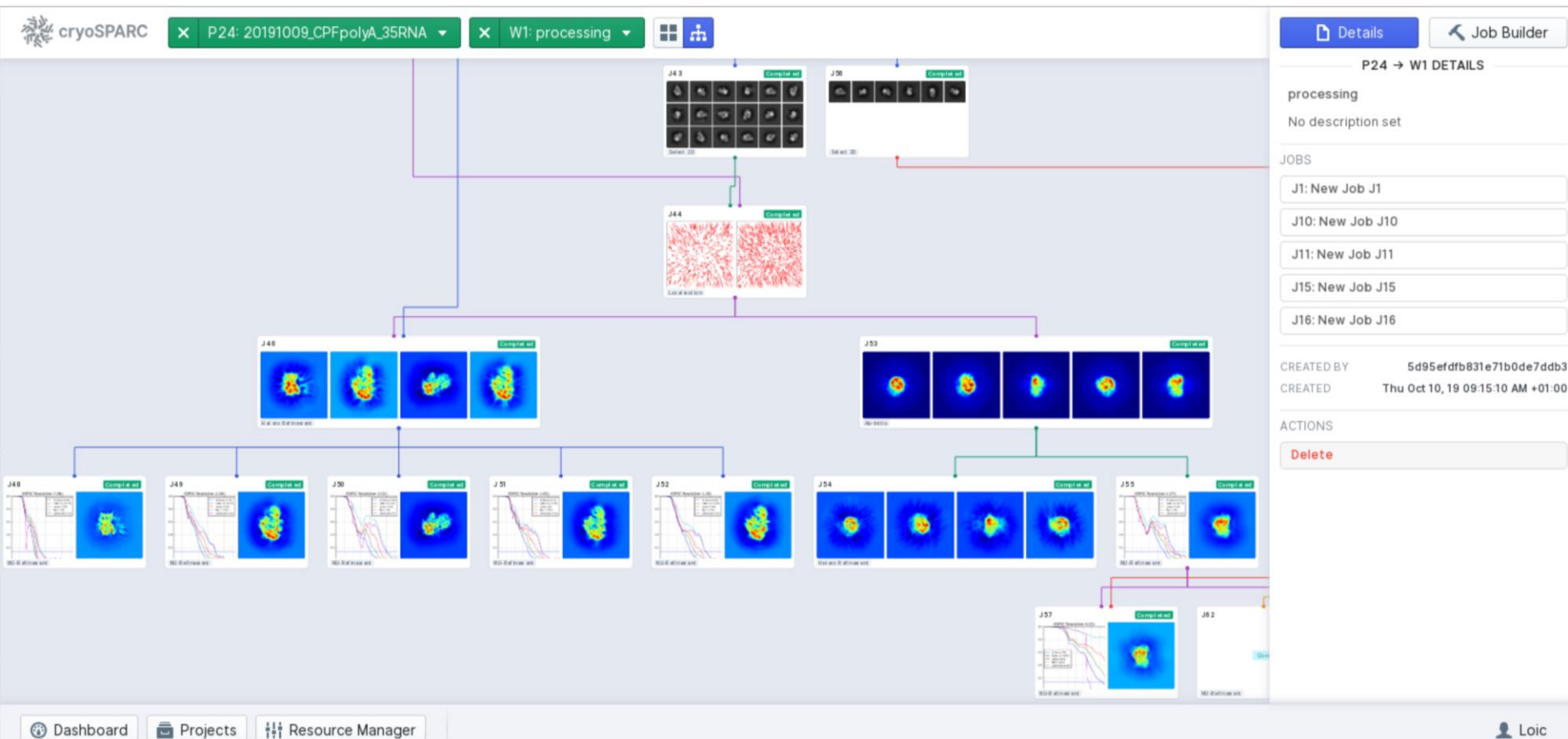
- ✓ SGD based model is one of the standard in SPA to get initial model
- ✓ 3 Ab initio models generated in parallel give good results as initial 3D classification
- ✓ Heterogenous refinement of these model is an extra step

The screenshot displays the cryoSPARC V2 web interface. At the top, the cryoSPARC logo is visible. Below it, there are tabs for 'P24: 20191009\_CPFpolyA\_35RNA' and 'W1: processing'. The main workspace shows a workflow diagram with jobs J14, J18, J22, J23, J24, J25, J26, J27, and J28. Job J22 is marked 'Completed' and shows a 3D classification grid. Job J23 is marked 'Completed' and shows a 3D classification map. Job J24 shows three 3D maps. Job J25 shows a 3D map. Jobs J26, J27, and J28 show 3D maps. The right sidebar contains a 'Details' tab and a 'Job Builder' button. Below these, it shows 'P24 → W1 DETAILS' with 'processing' and 'No description set'. A 'JOBS' list includes 'J1: New Job J1', 'J2: New Job J2', 'J3: New Job J3', 'J4: New Job J4', and 'J5: New Job J5'. Below this, it shows 'CREATED BY' as '5d95efdfb831e71b0de7ddb3' and 'CREATED' as 'Thu Oct 10, 19 09:15:10 AM +01:00'. An 'ACTIONS' section contains a 'Delete' button. The bottom navigation bar includes 'Dashboard', 'Projects', and 'Resource Manager'. The user 'Loic' is logged in.



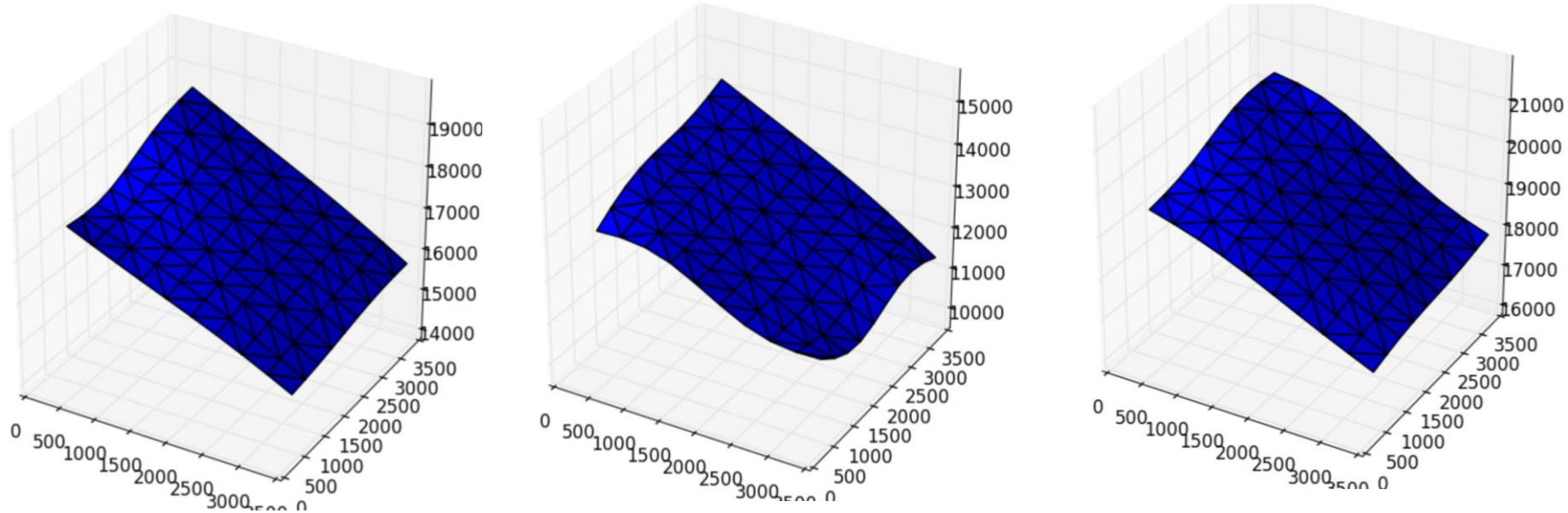
# Cryosparc V2 – Homogenous Refinement

- ✓ Each map is then refined independently
- ✓ Global sharpening, local resolution estimation and local filtering



# Cryosparc V2 – Good to process tilt dataset

- ✓ Dataset collected using a tilt angle of 30°



- **Cryosparc is bad for:**

- ✓ Focus classification
  - ✓ Symmetry mismatch
  - ✓ Virus particles with icosahedral symmetry
- 
- ✓ Using a script (PyEM from D. Asarnow, UCSF) you can transfer your data to relion

# Relion 3.1

- ✓ New fancy pink colour
- ✓ Bayesian polishing
- ✓ CTF refinement
- ✓ Beam tilt correction
- ✓ High-order aberration correction
- ✓ Merge dataset with  $\neq$  pixel size
- ✓ Refinement with particles that have  $\neq$  box size and/or pixel size
- ✓ Magnification anisotropy correction
- ✓ ....

