

PART I : SINGLE PARTICLE ANALYSIS

SEMC Appion workshop series

July 29-30, 2020

NATIONAL CENTER FOR
CRYOEM ACCESS & TRAINING



New York Structural
Biology Center

SIMONS ELECTRON
MICROSCOPY CENTER





NMR
CoMD/
NMR



X-ray
NYX
@NSLS-II



Protein Production
COMPPA



NRRMM

photo by Swapnil Bhatkar

SCHEDULE

JULY 29-30, 2020 2PM-4PM

PRACTICAL WORKSHOP WHERE STUDENTS WILL WORK IN PAIRS ON A TEST DATASET. AFTER EACH LECTURER GIVES AN OVERVIEW OF THE TOPIC THE ATTENDEES WILL HAVE HANDS-ON TIME TO USE THE INTERFACE.

1. Workshop intro

- Overview of Leginon/Appion
- Krios workflow
- Leginon targeter

2. Micrograph evaluation / CTF Estimation

3. Object Selection

4. Stack creation / Particle Alignment

[3 (cont). Object Selection with templates after initial class averages]

5. Other features: Pro tips / Direct Detector Tools / Import/Export Tools

6. Closing

HOW DO WE USE AUTOMATION?

NRAMM

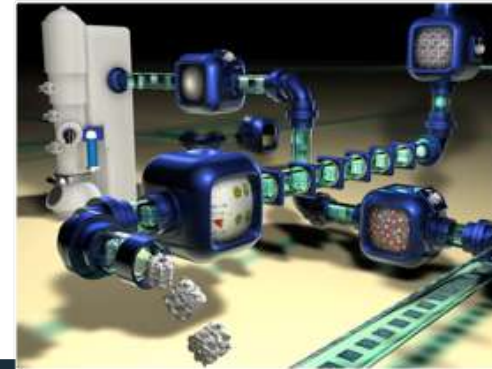
The overall mission of NRAMM is to develop, test and apply technology for automating and streamlining cryo-electron microscopy (cryoEM) for structural biology.



Specimen preparation



Image acquisition



Data processing

HOW DO WE USE AUTOMATION?

Leginon is a system designed for automated collection of images from a transmission electron microscope.



Appion is a "pipeline" for processing and analysis of EM images.

SIMONS ELECTRON MICROSCOPY CENTER

Acknowledgements

Some of this work was performed at the National Center for CryoEM Access and Training (NCCAT) and the Simons Electron Microscopy Center located at the New York Structural Biology Center, supported by the NIH Common Fund Transformative High Resolution Cryo-Electron Microscopy program (U24 GM129539,) and by grants from the Simons Foundation (SF349247), NYSTAR, and the NIH National Institute of General Medical Sciences (GM103310), Agouron Institute (F00316), NIH OD019994 and RR029300 and NY State Assembly.

SEMC OPERATIONS TEAM

EM Directors



E M G @nysbc.org

Staff



SEMC APPION DEVELOPMENT TEAM



**Anchi
Cheng**



Sargis Dallakyan



Swapnil Bhaktar









**Shaker
Krit**



Alex Noble

Tomography

SEMC TRAINING

Type	Frequency	Title	Description
 feedback	yearly	General user meeting	State of the center
 knowledge	yearly	SEMC EM Course	Theory behind EM <i>Graduate level course</i>
 computation	quarterly	SEMC Appion workshop	Data processing workshops
 access	monthly	SEMC New User Orientation	Facility use and Safety Training Sample preparation [neg-stain & cryo] <i>Leginon intro/use of screening microscopes</i>
 troubleshooting	weekly	User Project Discussion Meetings	<i>Appointments: Tue @3pm, Thurs @3pm & @3:30pm</i>
 instrumentation	daily	Advanced Leginon use / 1-1 training appointments	Training for independent use of the microscopes and other SEMC resources

NCCAT TRAINING DOCUMENTATION

<https://nccat.nysbc.org/activities/nccat-cross-training/>

<https://nccat.nysbc.org/activities/nccat-remote-learning/>



National Center for CryoEM Access and Training

HOME

ABOUT

NEWS

ACCESS

TRAINING

ACKNOWLEDGEMENTS

JOBS

TRAINING

NCCAT Workshops
and Short courses

> SPA Short course
2020

NCCAT Cross-training
Programs

NCCAT Remote
Learning

> Remote Office
Hours

> Online Classroom

> Previous EM
Courses

> Curriculum
Partners

Workshops and Cross-training

NCCAT provides access to state-of-the-art equipment, including high-end microscopes and direct detectors, as well as specimen preparation robots, screening microscopes and all the other ancillary equipment required to solve structures to the highest possible resolution using cryo electron microscopy (cryoEM) methods.

An already established cross-training program provides training across a wide variety of skill levels and career goals. Our workshops and forums provide opportunities to target specific areas of education and professional development.



Workshops & Courses

[Learn with us. >](#)



Embedded cross-training

[Join our community. >](#)



Remote Learning Central

[Distance education. >](#)



LEGINON & APPION TRAINING DOCUMENTATION

leginon.org | appion.org



Leginon Search: Leginon

Overview Activity Roadmap Issues News Documents **Wiki** Forums Files Repository

Leginon Main Page

Overview

Leginon is a system designed for automated collection of images from a transmission electron microscope.

Contact information:

Report bugs and request features as [New issues](#) at this site.

Use [Leginon user forum](#) for asking questions and to see answers to previous questions.

Current Release: 3.4

Software Installation Manual and User Guide:

- To learn all the details, start with [An Introduction to Leginon](#)
- To install Leginon, see [Getting Started as a System Administrator](#)
- To learn how to use Leginon for everyday work, see [Getting Started as a basic Leginon user](#)
- [Full Leginon Manual Table of Contents](#)

Developer Guide:

- [Leginon Developer Guide](#)

Publications:

Primary Publication to Cite:

- "Automated Molecular Microscopy: The New Leginon System" (2005), C Suloway, J Pulokas, D Fellmann, A Cheng, F Guerra, J Quispe, S Stagg, CS Potter and B Carragher. Journal of Structural Biology 151 (1): pp. 41-60.

Additional References:

- "Rapid, routine structure determination of macromolecular assemblies using electron microscopy: Current progress and remaining challenges", B. Carragher, D. Fellmann, F. Guerra, R.A. Milligan, F. Mouche, J. Pulokas, J. Quispe, C. Suloway, Y. Zhu and C.S. Potter, J. of Synchrotron radiation. 11, pp 83-85 (2004)
- "A relational database for cryoEM: Experience at one year and 50,000 Images", D. Fellmann, J. Pulokas, R.A. Milligan, B. Carragher and C.S. Potter, J. of Structural Biology, 137, p. 273-282 (2002)

Appion Search: Appion

Overview Activity Roadmap Issues Spent time News Documents **Wiki** Forums Files Repository

Appion Main Page

Overview

Appion is a "pipeline" for processing and analysis of EM images. Appion is integrated with [Leginon](#) data acquisition but can also be used stand-alone after uploading images (either digital or scanned micrographs) or particle stacks using a set of provided tools. Appion consists of a web based user interface linked to a set of python scripts that control several underlying integrated processing packages. All data input and output within Appion is managed using tightly integrated SQL databases. The goal is to have all control of the processing pipeline managed from a web based user interface and all output from the processing presented using web based viewing tools.

The underlying packages integrated into Appion include [MotionCor2](#), [Gctf](#), [EMAN](#), [Spider](#), [FREALIGN](#), [IMAGIC](#), [XMIPP](#), [IMOD](#), [ProtoTom](#), [ACE](#), [CTFFIND](#) and [CTFTILT](#), [findEM](#), [DogPicker](#), [TiltPicker](#), [RMeasure](#), [EM-BFACTOR](#), and [Chimera](#). These packages must be acknowledged by appropriate citations when used within Appion. Appropriate citations are provided on the individual pages in Appion as well as [here](#).

Current Release: 3.3

Download Appion

Follow the Appion [installation instructions](#) to download and install Appion.

If you download Appion we strongly encourage you [register as an Appion user](#). This will allow us to keep you informed of new releases, bug fixes, and other useful information, and also allow us to keep track of the user base which is important to ensure future support of the software.

Appion User Manual

The **Appion Manual** includes:

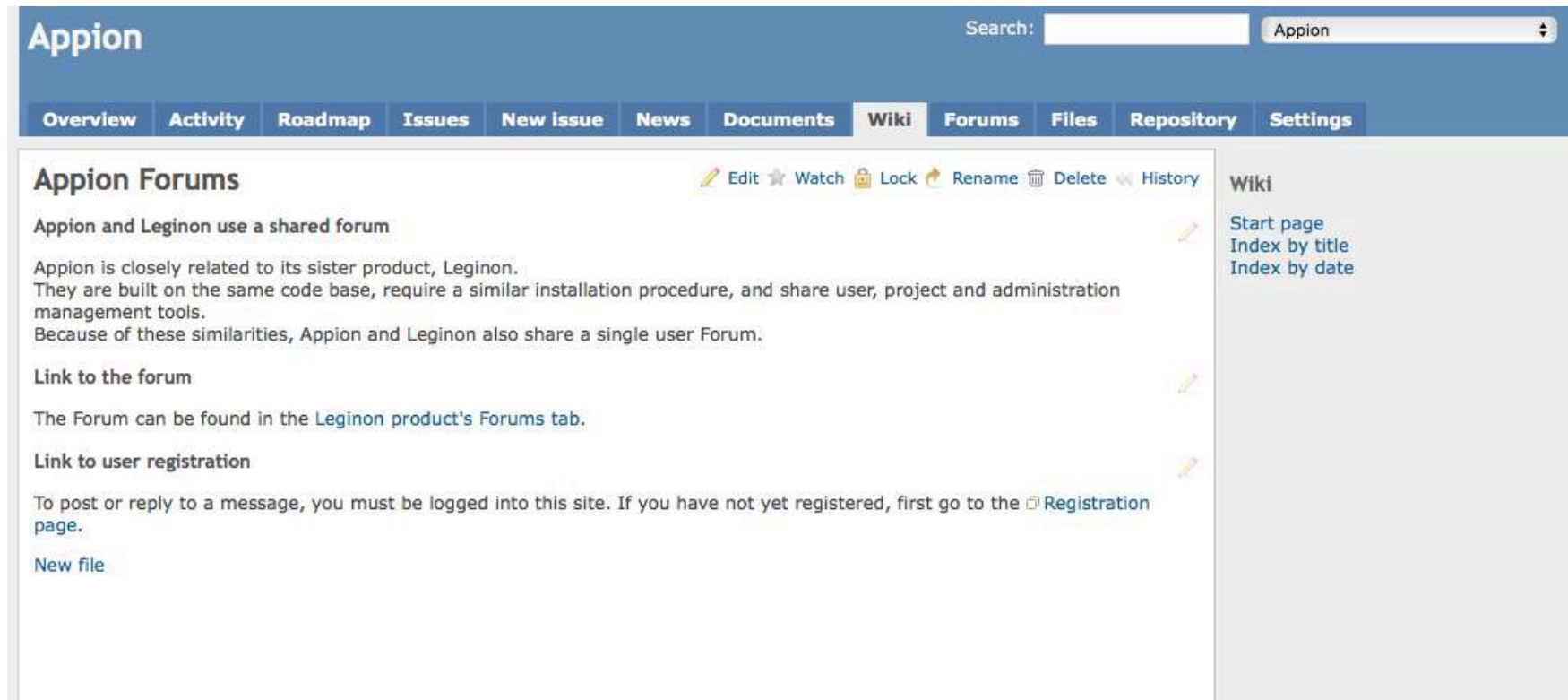
- a description of Appion,
- version change logs,
- upgrade instructions,
- installation instructions and
- daily usage instructions

Appion Developer's Guide

The [developers guide](#) is the primary resource for getting started with code development. Appion is an open source project. You are free to contribute to it.

APPION.ORG

Register an account



The screenshot shows the Appion website interface. At the top, there is a blue header with the 'Appion' logo on the left, a search bar in the center, and a dropdown menu on the right showing 'Appion'. Below the header is a navigation bar with tabs: Overview, Activity, Roadmap, Issues, New issue, News, Documents, Wiki, Forums, Files, Repository, and Settings. The 'Forums' tab is selected. The main content area is titled 'Appion Forums' and contains the following text: 'Appion and Leginon use a shared forum', 'Appion is closely related to its sister product, Leginon. They are built on the same code base, require a similar installation procedure, and share user, project and administration management tools. Because of these similarities, Appion and Leginon also share a single user Forum.', 'Link to the forum', 'The Forum can be found in the [Leginon product's Forums tab](#).', 'Link to user registration', 'To post or reply to a message, you must be logged into this site. If you have not yet registered, first go to the [Registration page](#).', and 'New file'. On the right side of the main content area, there is a 'Wiki' section with links: 'Start page', 'Index by title', and 'Index by date'.

Appion

Search: Appion

Overview Activity Roadmap Issues New issue News Documents Wiki Forums Files Repository Settings

Appion Forums

Appion and Leginon use a shared forum

Appion is closely related to its sister product, Leginon. They are built on the same code base, require a similar installation procedure, and share user, project and administration management tools. Because of these similarities, Appion and Leginon also share a single user Forum.

Link to the forum

The Forum can be found in the [Leginon product's Forums tab](#).

Link to user registration

To post or reply to a message, you must be logged into this site. If you have not yet registered, first go to the [Registration page](#).

New file

Wiki

- Start page
- Index by title
- Index by date

APPION.ORG

<http://emg.nysbc.org/redmine/projects/nysbc-semc-user-group/issues>

Appion

Search: Appion

Overview

Activity

Roadmap

Issues

New Issue

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Repository

Settings

Issues

Filters

☒ Status

open

☒ Tracker

is

Bug

Add filter

Options

Apply

Clear

Save

	#	Project	Tracker	Status	Priority	Subject	Author	Assignee
<input type="checkbox"/>	3811	Appion	Bug	Assigned	Normal	dd alignment with motioncorr fails with larger movies or larger shifts	Anchi Cheng	Anchi Cheng
<input type="checkbox"/>	3809	Appion	Bug	New	Normal	When users are identified in the path, upload image is put into base leginon directory, not user directory if leginon.cfg is not set in user home directory	Anchi Cheng	
<input type="checkbox"/>	3808	Appion	Bug	Assigned	Normal	uploadImages.py does not set "frame path" field in SessionData	Anchi Cheng	Anchi Cheng
<input type="checkbox"/>	3804	Appion	Bug	Assigned	Normal	DogPicker fails to display result images when testing through job submission	Carl Negro	Carl Negro
<input type="checkbox"/>	3802	Appion	Bug	New	Normal	slow aligned particle insertion caused time out in uploadMaxlikeAlignment.py	Anchi Cheng	

Issues

View all issues

Summary

Custom queries

Appion 2.2 Test Matrix

Appion 3.2 bug fixes

Appion 3.2 feature

Appion Known Bugs

Appion Test Suite

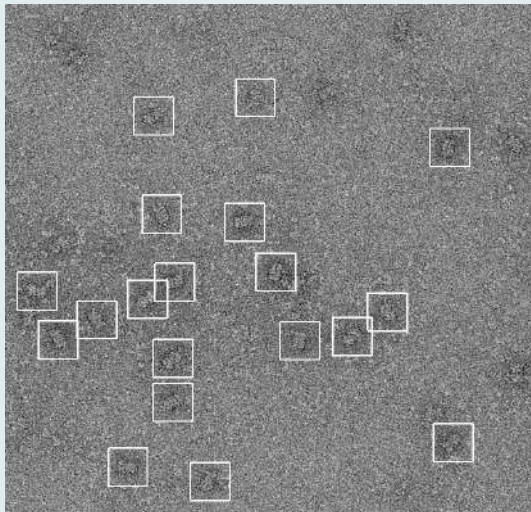
Unassigned issues

APPION WORKSHOPS

Single particle

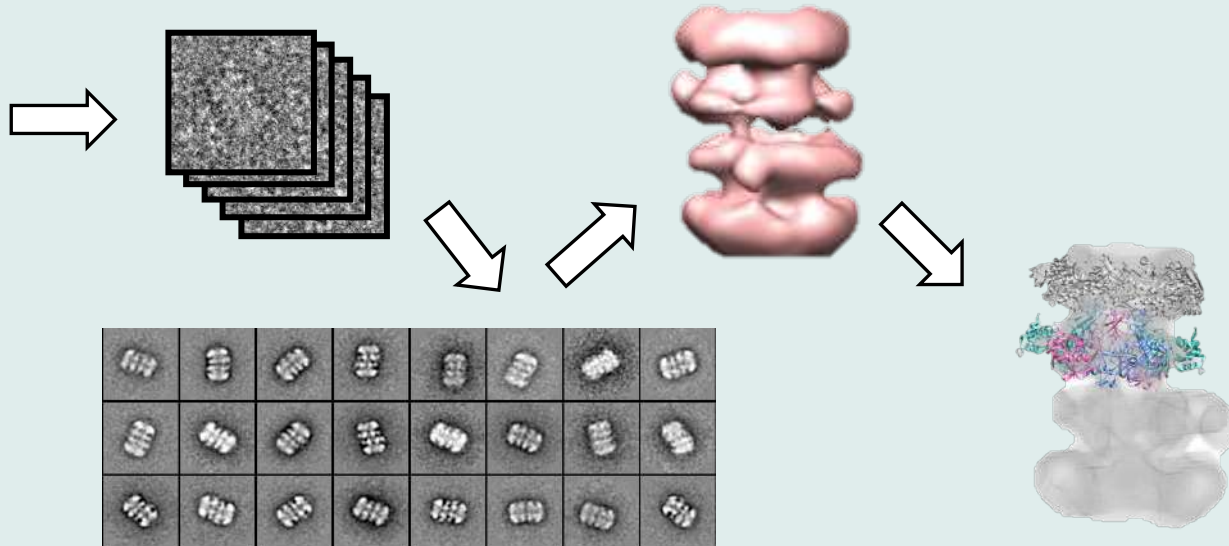
Part I

Tools to generate a particle stack from micrographs and obtain an initial assessment of the sample.



Part II

**3D reconstruction,
refinement and
validation.**

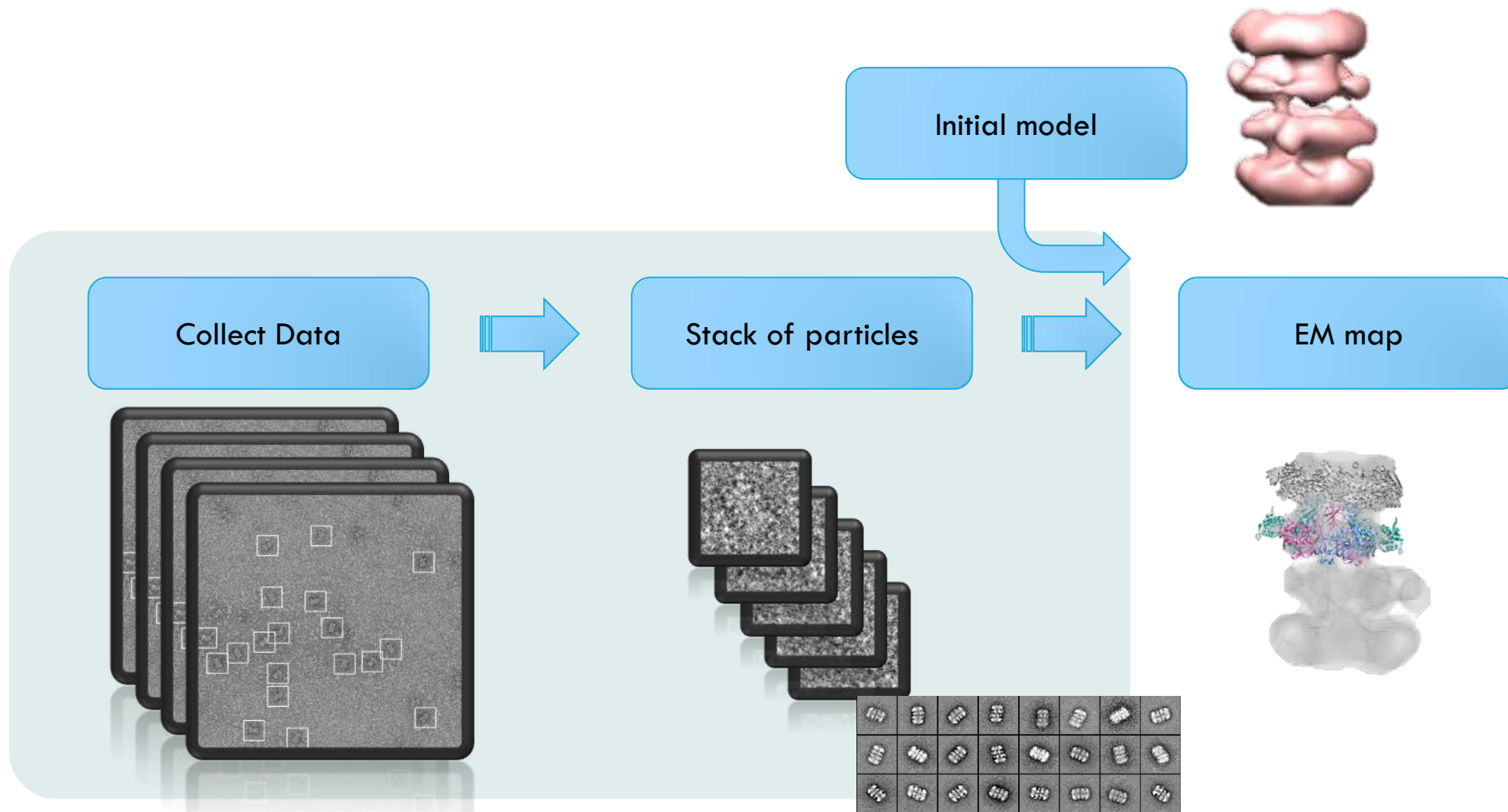


Tomography

Part III

Tomography tools.

SINGLE PARTICLE ANALYSIS



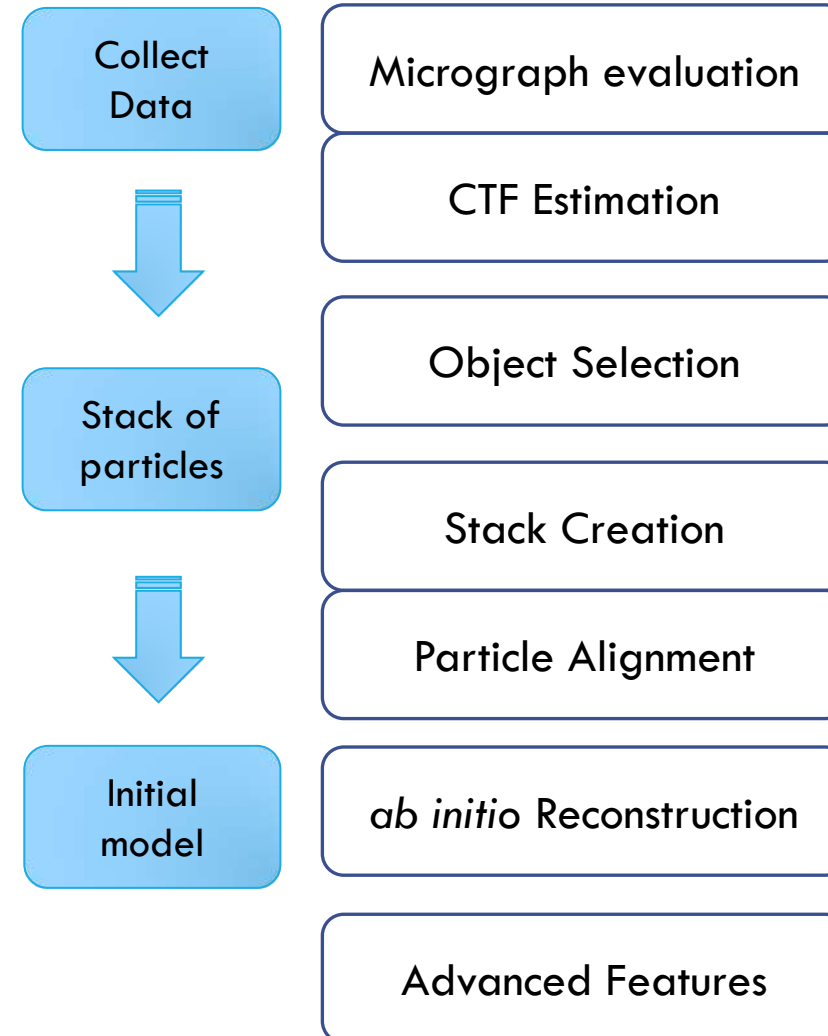
Schedule

July 29-30 2pm-4pm

Practical workshop where students will work in pairs on a test dataset.

After each lecturer gives an overview of the topic the attendees will have hands-on time to use the interface.

SINGLE PARTICLE ANALYSIS



DATA ACQUISITION AND PROCESSING PLATFORMS

EM instrumentation

**FEI Titan Krios#1 / #2 / #3
#4 / #5 / #6
GIF/K3**



**FEI Tecnai F20
DE20
TVIPS 4K CMOS**



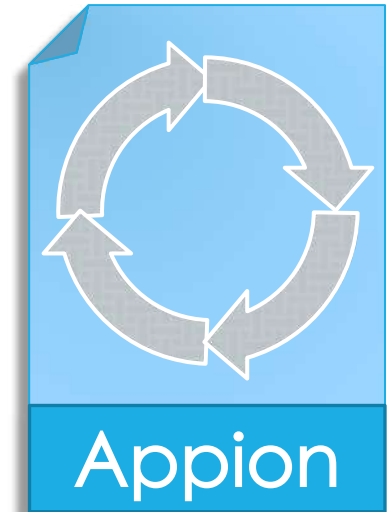
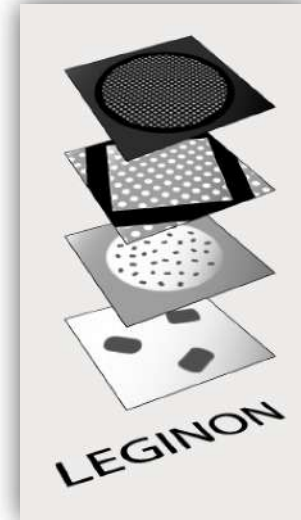
**FEI Tecnai Biotwin
TVIPS 4K CMOS**



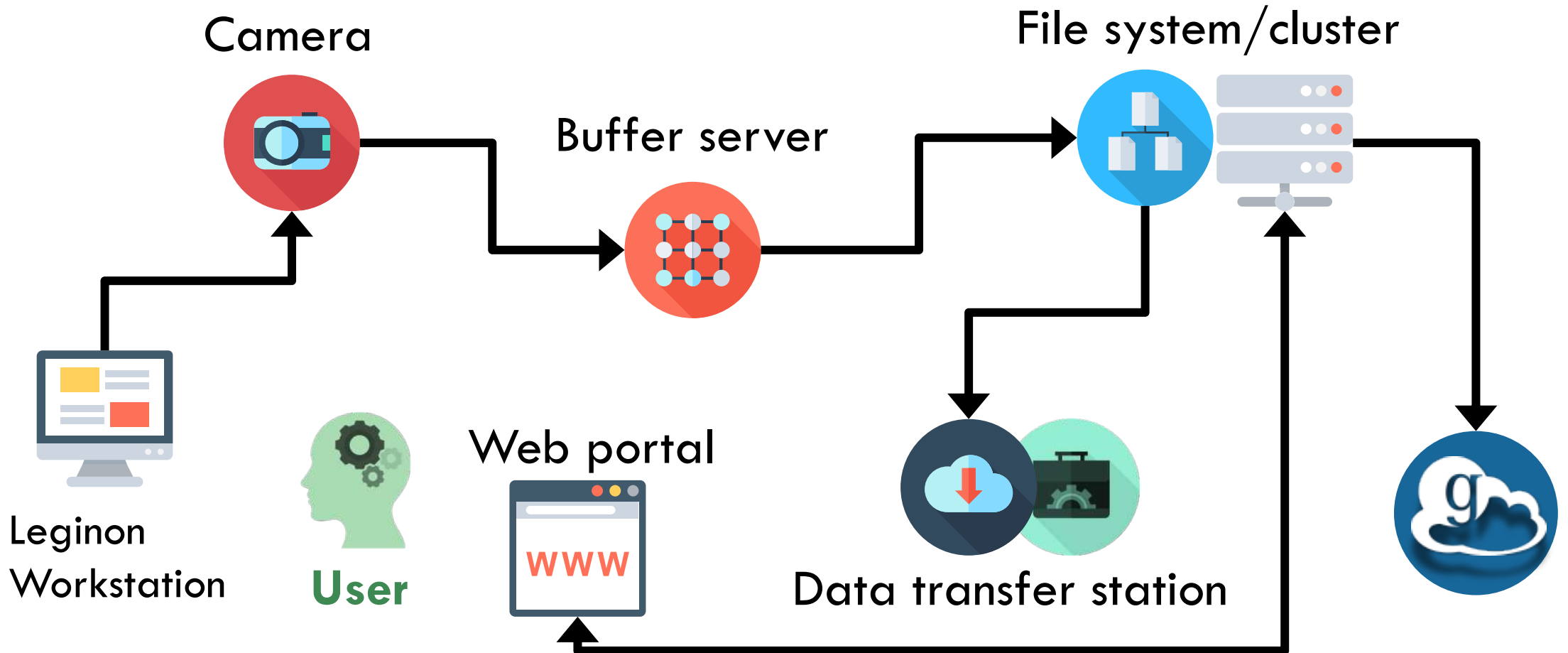
**JEOL 1230
Gatan US4000 CCD**



**FEI Helios 650
Quorum cryostage**

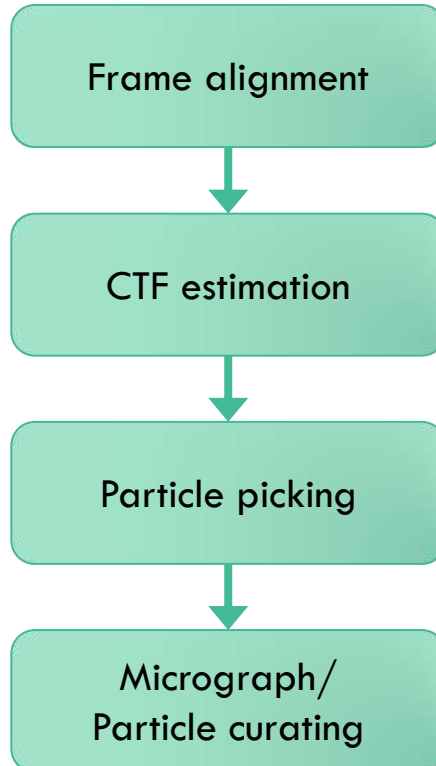
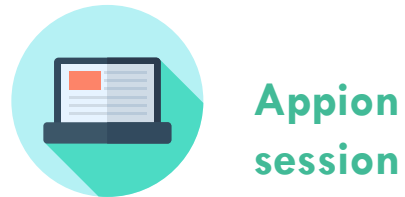
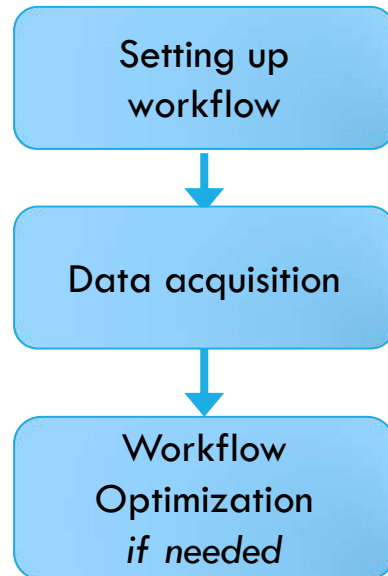


ON THE FLY DATA PIPELINE

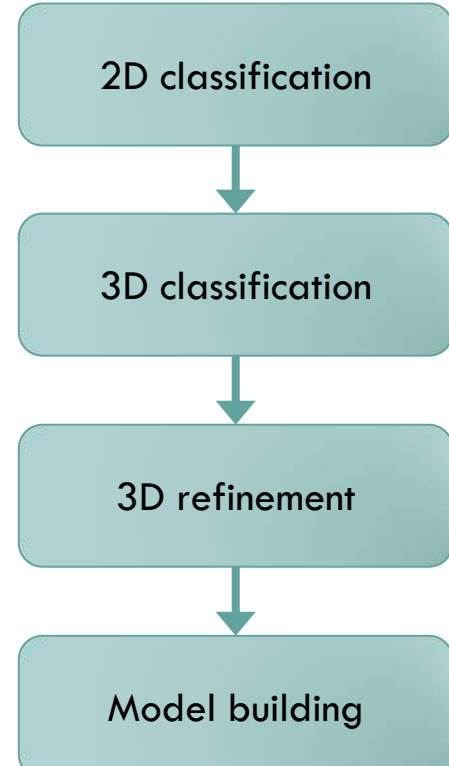
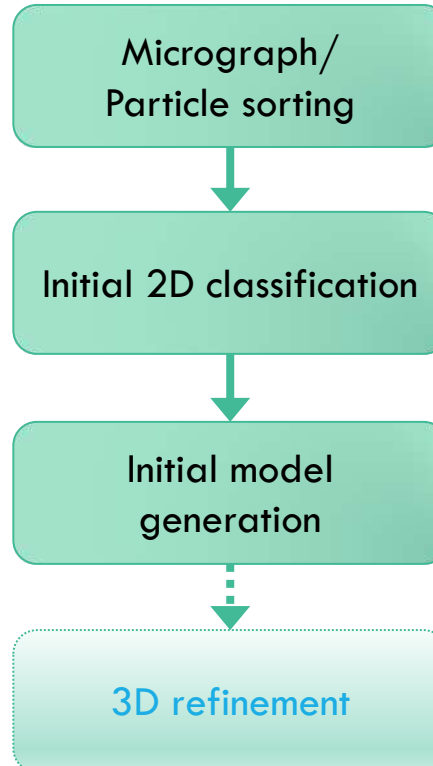


EXAMPLE: SINGLE-PARTICLE WORKFLOW

During EM session



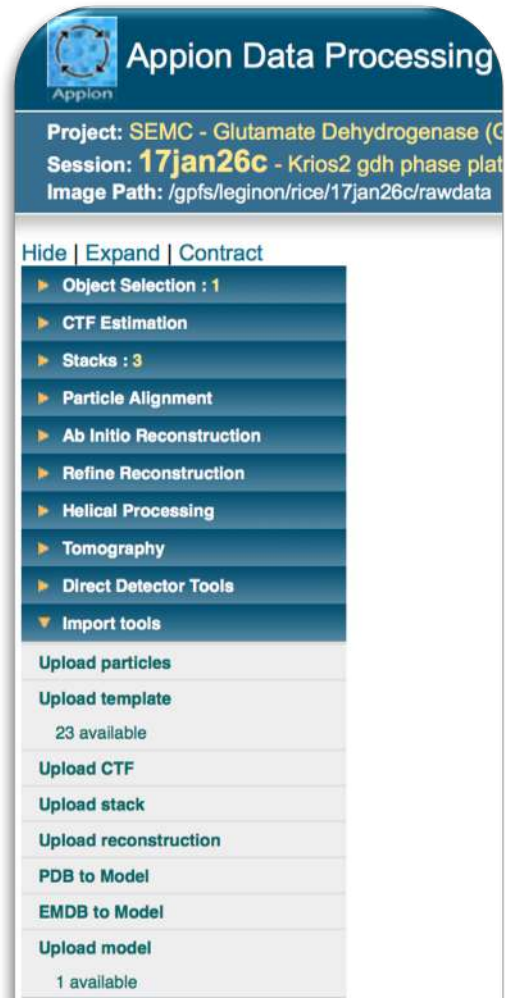
After EM session



PROCESSING WORKFLOW



During EM session



Appion
session

Frame alignment

CTF estimation

Particle picking

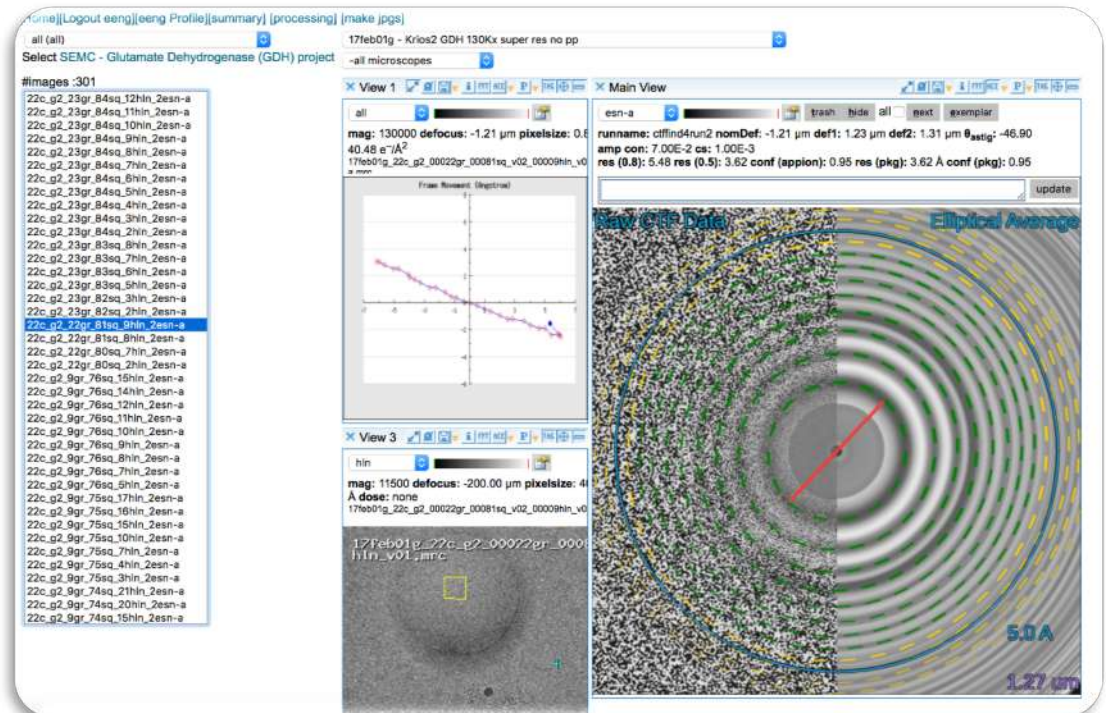
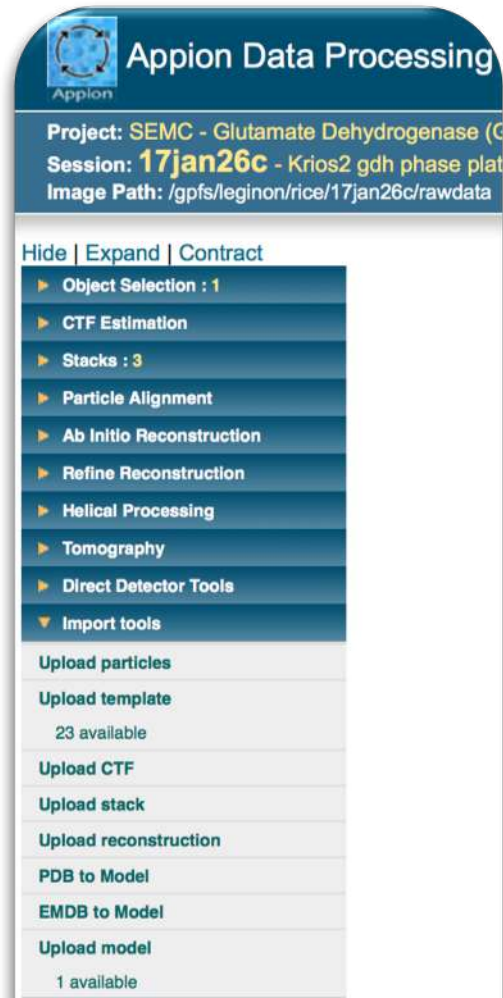
Micrograph/
Particle curating

PROCESSING WORKFLOW

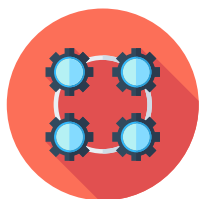


During EM session


Leginon / Appion web interface



PACKAGES OFFERED



After EM session



CTF Estimation Selection Page


Project: SEMC - Glutamate Dehydrogenase (GDH) (256)
Session: **16dec06e** - 1.68 mg/mL GDH + 0.001% DDM (1.3/1.4 UltrAu 300mesh)
Image Path: /gdfs/leginon/ikim/16dec06e/rawdata

Hide | Expand | Contract

- ▼ Object Selection : 3
 - Select Particle Picker...
4 complete
 - Repeat an image loop run
- ▼ CTF Estimation
 - Estimate the CTF...
5 complete
1 running
 - Transfer results to another preset
 - Repeat an image loop run
- ▼ Stacks : 9
 - Stack creation
11 complete
 - more stack tools
- ▼ Particle Alignment
 - Select Particle Alignment...
2 complete
 - Run Feature Analysis...
3 complete
 - Run Particle Clustering...
4 complete
 - Run MaskItOn
 - Template Stacks
- ▼ Ab Initio Reconstruction
 - OptiMod Common Lines
 - EMAN Common Lines
 - SIMPLE Common Lines
 - ▼ Refine Reconstruction


CTF Estimation Procedures

During CTF estimation the goal is to fit the standard CTF equation ([wikipedia](#)) to the power spectra of the electron micrographs




CTFFIND v4

CTFFIND uses a robust grid search algorithm to find the optimal CTF parameters. Please see the [Grigorieff lab website](#) for more information.




GCTF v1.06

This is a GPU accelerated program for real-time CTF determination, refinement, evaluation and correction. Please see the [Dynein lab website](#) for more information.



ACE 2

ACE 2 is an unpublished re-implementation of ACE1, but written in objective-C ACE2 make several improvements over ACE1 including a several speed enhancements and a robust astigmatism estimate.
Note: It was designed around FEI Tecnai FEG data and other have reported problems using this program



ACE 1

ACE1 is the original edge detection program for finding the CTF parameters. Astigmatism estimation never worked quite right in ACE1 and it has a tendency to give false positives,

Appion web interface



Particle Alignment Selection Page

Project: SEMC - Glutamate Dehydrogenase (GDH) (256)
Session: **16dec06e** - 1.68 mg/mL GDH + 0.001% DDM (1.3/1.4 UltrAu 300mesh)
Image Path: /gdfs/leginon/ikim/16dec06e/rawdata

Hide | Expand | Contract

- ▼ Object Selection : 3
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4 complete
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 - Estimate the CTF...
5 complete
1 running
 - Transfer results to another preset
- ▼ Stacks : 9
 - Stack creation
11 complete
 - more stack tools
- ▼ Particle Alignment
 - Select Particle Alignment...
2 complete
 - Run Feature Analysis...
3 complete
 - Run Particle Clustering...
4 complete
 - Run MaskItOn
- ▼ Ab Initio Reconstruction
 - OptiMod Common Lines
 - EMAN Common Lines
 - SIMPLE Common Lines
 - ▼ Refine Reconstruction
- ▼ Helical Processing
 - Helical Image Processing (PHOELIX)

Particle Alignment Procedures


Particle alignment consists of shift, rotation, and mirror transformations for all particles in a stack to a common orientation or template. The reference free methods are great methods to create a template for particle picking or reference-based alignments.



Xmipp Maximum Likelihood Alignment


this method is the most robust, but takes some time to complete. It uses the [Xmipp ml_align2d](#) program to perform alignments.

This method is unbiased and very thorough, but also the slowest of the methods (~days). While it produces excellent templates, it only does a coarse search (integer pixels shifts and large angle increments), so it is best to use ref-based alignment to get better alignment parameters



Xmipp 2 Clustering 2D Alignment

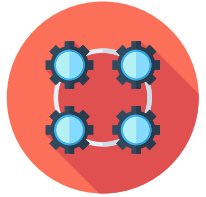
this method builds a hierarchical classification of particles It uses the [Xmipp 2 c12d](#) program to perform alignments. It is a relatively fast method that aligns and classify the images at the same time. The method starts by estimating a few classes that are further subdivided till the desired number of classes is reached. Every time an image is compared to the class averages it is aligned before-hand. **NOTE: in Xmipp 2.4 the alignment parameters are not saved in the database, and therefore this method cannot be used for RCT / OTR reconstructions.**



Xmipp 3 Clustering 2D Alignment

this method builds a hierarchical classification of particles It uses the [Xmipp 3 c12d](#) program to perform alignments. It is a relatively fast method that aligns and classify the images at the same time. The method starts by estimating a few classes that are further subdivided till the desired number of classes is reached. Every time an image is compared to the class averages it is aligned before-hand.

WHO DOES THE PROCESSING WORKFLOW?



After EM session

Appion web interface

SEMC computing

**Home institution
computing/cluster**

Micrograph/
Particle sorting

2D classification

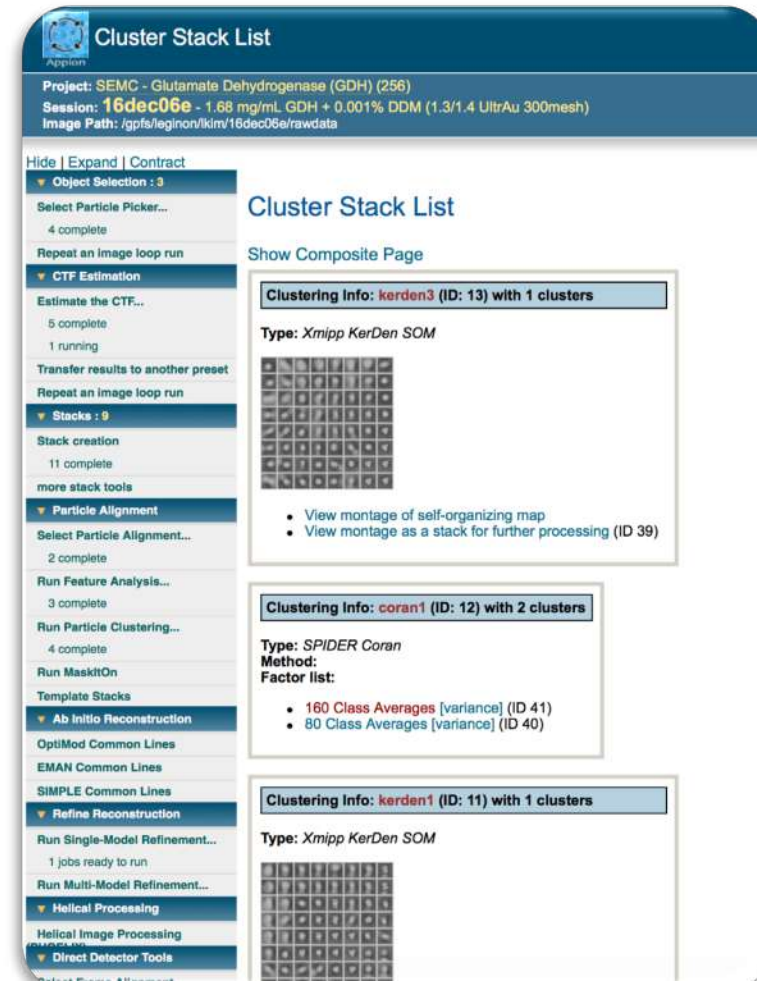
Initial 2D classification

3D classification

Initial model
generation

3D refinement

Model building



REMOTE DATA TRANSFER

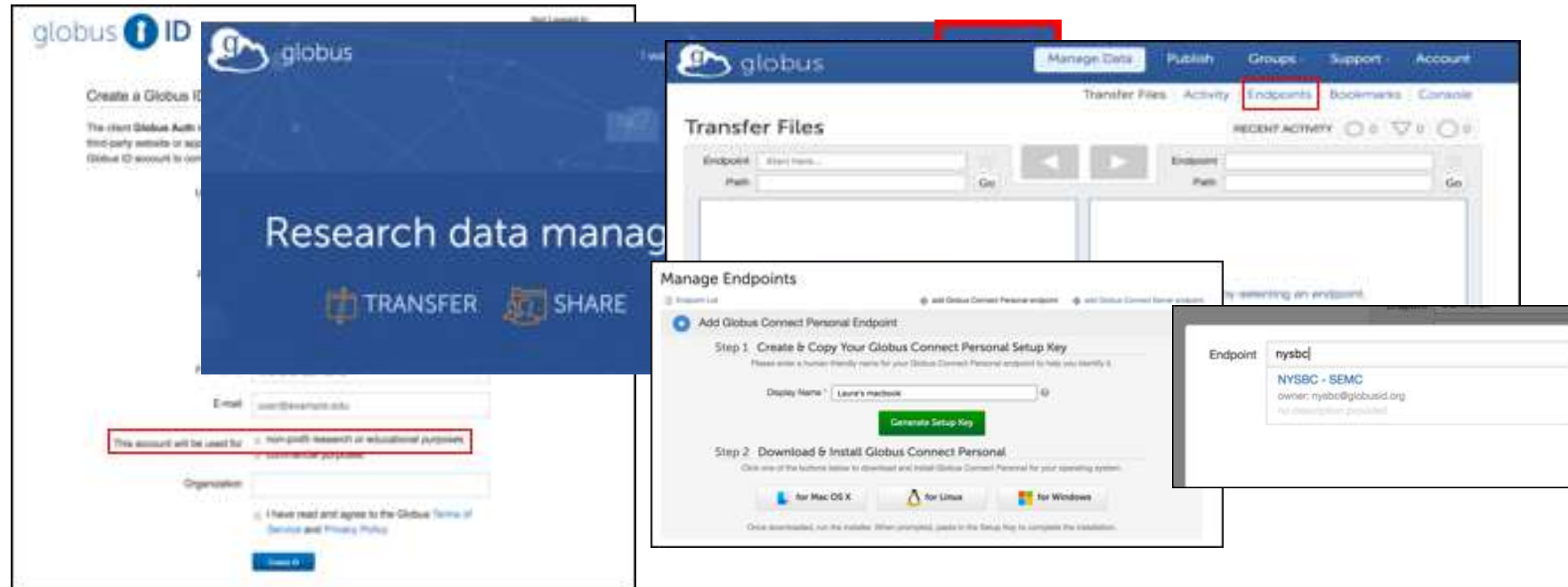


Remote
data
transfer

Create an account at
globusid.org

Login at
globus.org

Transfer files

The image displays three overlapping screenshots of the Globus web interface. The leftmost screenshot shows the 'Create a Globus ID' page with a red box highlighting the account type selection: 'non-profit research or educational purposes' and 'commercial purposes'. The middle screenshot shows the 'Transfer Files' page with a red box highlighting the 'Endpoints' tab in the top navigation bar. The rightmost screenshot shows the 'Manage Endpoints' page with a red box highlighting the 'Add Globus Connect Personal Endpoint' section, which includes a 'Generate Setup Key' button and download links for Mac OS X, Linux, and Windows. A small inset window on the right shows a table of endpoints with one entry: 'nysbc' with owner 'nysbc@globusid.org'.

REMOTE DATA TRANSFER



Remote
data
transfer

/beegfs/frames/[username]/[session name]/

/beegfs/legion/[username]/[session name]/

/beegfs/appion/[username]/[session name]/

SCHEDULE

JULY 29-30 2PM-4PM

PRACTICAL WORKSHOP WHERE STUDENTS WILL WORK IN PAIRS ON A TEST DATASET. AFTER EACH LECTURER GIVES AN OVERVIEW OF THE TOPIC THE ATTENDEES WILL HAVE HANDS-ON TIME TO USE THE INTERFACE.

1. Workshop intro

- Overview of Leginon/Appion
- Krios workflow
- Leginon targeter

2. Micrograph evaluation / CTF Estimation

3. Object Selection

4. Stack creation / Particle Alignment

[3 (cont). Object Selection with templates after initial class averages]

5. Other features: Pro tips / Direct Detector Tools / Import/Export Tools

6. Closing

Start your web browsers

nccatweb.nysbc.org

Login: nworkshop

Password: appion2020

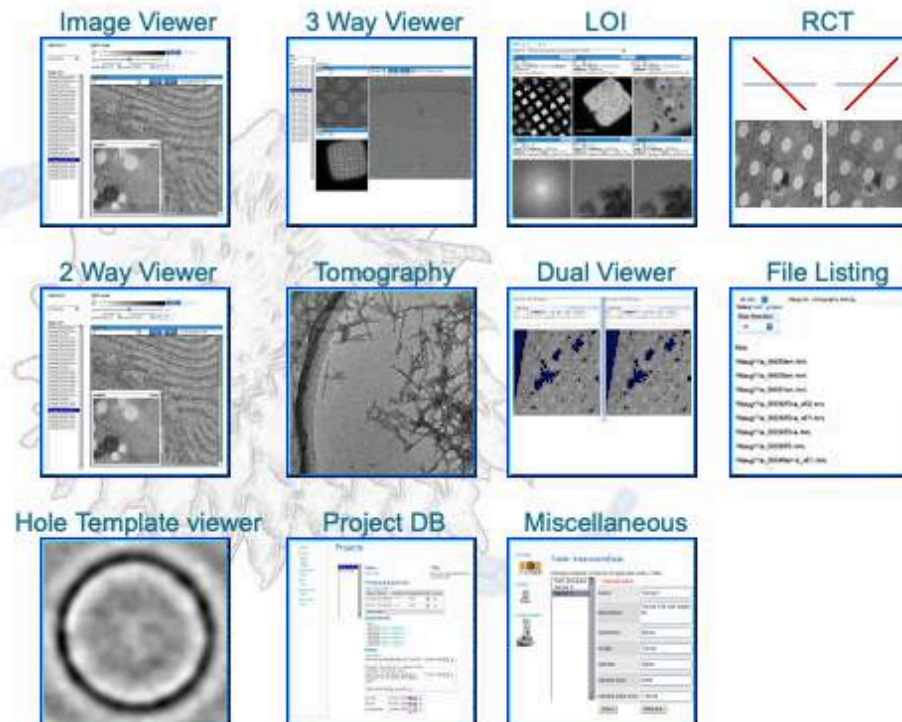
APPION & LEGINON WEBTOOLS

nccatweb.nysbc.org

[Logout nworkshop][nworkshop Profile]

Appion and Leginson DB Tools

[test Dataset]



APPION & LEGINON WEBTOOLS

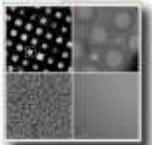
nccatweb.nysbc.org

[Home][Logout nworkshop][nworkshop Profile]



Project: Workshop - Workshop A

View Projects



Project Status

Statistics

<Detailed view> <edit>

Short Description

Workshop A

owners: NCCAT Workshop [edit](#)

processing db: nccatap64 [unlink](#) Note: db won't be deleted

1 Experiments [show all](#)

[upload images to new session](#)

Name	Session Id	User	Description	Total images	Total Duration	Reconstructions	Processing Runs	Last Run	Image Path	Sharing	
20workshopA	403	NCCAT Workshop	workshop A	10	00m				/beegfs/leginon/nworkshop/20workshopA/rawdata	No [->]	summary»

[Home][Logout nworkshop][nworkshop Profile]



Projects

View Projects



Project Status
Statistics

Workshop - Workshop A	NCCAT Workshop	nccat	Workshop A	1 experiment last: 20workshopA
Workshop - Workshop B	NCCAT Workshop	nccat	Workshop B	1 experiment last: 20workshopB
Workshop - Workshop C	NCCAT Workshop	nccat	Workshop C	1 experiment last: 20workshopC
Workshop - Workshop D	NCCAT Workshop	nccat	Workshop D	1 experiment last: 20workshopD
Workshop - Workshop E	NCCAT Workshop	nccat	Workshop E	1 experiment last: 20workshopE
Workshop - Workshop F	NCCAT Workshop	nccat	Workshop F	1 experiment last: 20workshopF
Workshop - Workshop G	NCCAT Workshop	nccat	Workshop G	2 experiments last: 20workshopI
Workshop - Workshop H	NCCAT Workshop	nccat	Workshop H	1 experiment last: 20workshopH
Workshop - Workshop I	NCCAT Workshop	nccat	Workshop I	1 experiment last: 20Instructors

APPION & LEGINON WEBTOOLS

nccatweb.nysbc.org

[\[Home\]](#)[\[Logout eeng\]](#)[\[eeng Profile\]](#)[\[summary\]](#) [\[processing\]](#) [\[make jpgs\]](#)

all (all)

16dec31d - proteasome grid screening and possible collection 135k krio...

Select SEMC - Proteasome test project

-all microscopes

#images :485

cflat0.5-2.0-1.3gr_30sq_5hln_7enn
cflat0.5-2.0-1.3gr_30sq_5hln_6enn
cflat0.5-2.0-1.3gr_30sq_5hln_5enn
cflat0.5-2.0-1.3gr_30sq_5hln_4enn
cflat0.5-2.0-1.3gr_30sq_5hln_3enn
cflat0.5-2.0-1.3gr_30sq_5hln_2enn

cflat0.5-2.0-1.3gr_30sq_4hln_7enn

cflat0.5-2.0-1.3gr_30sq_4hln_6enn

cflat0.5-2.0-1.3gr_30sq_4hln_5enn

cflat0.5-2.0-1.3gr_30sq_4hln_4enn

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cflat0.5-2.0-1.3gr_30sq_3hln_5enn

cflat0.5-2.0-1.3gr_30sq_3hln_4enn

cflat0.5-2.0-1.3gr_30sq_3hln_3enn

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cflat0.5-2.0-1.3gr_30sq_2hln_7enn

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cflat0.5-2.0-1.3gr_30sq_2hln_5enn

cflat0.5-2.0-1.3gr_30sq_2hln_4enn

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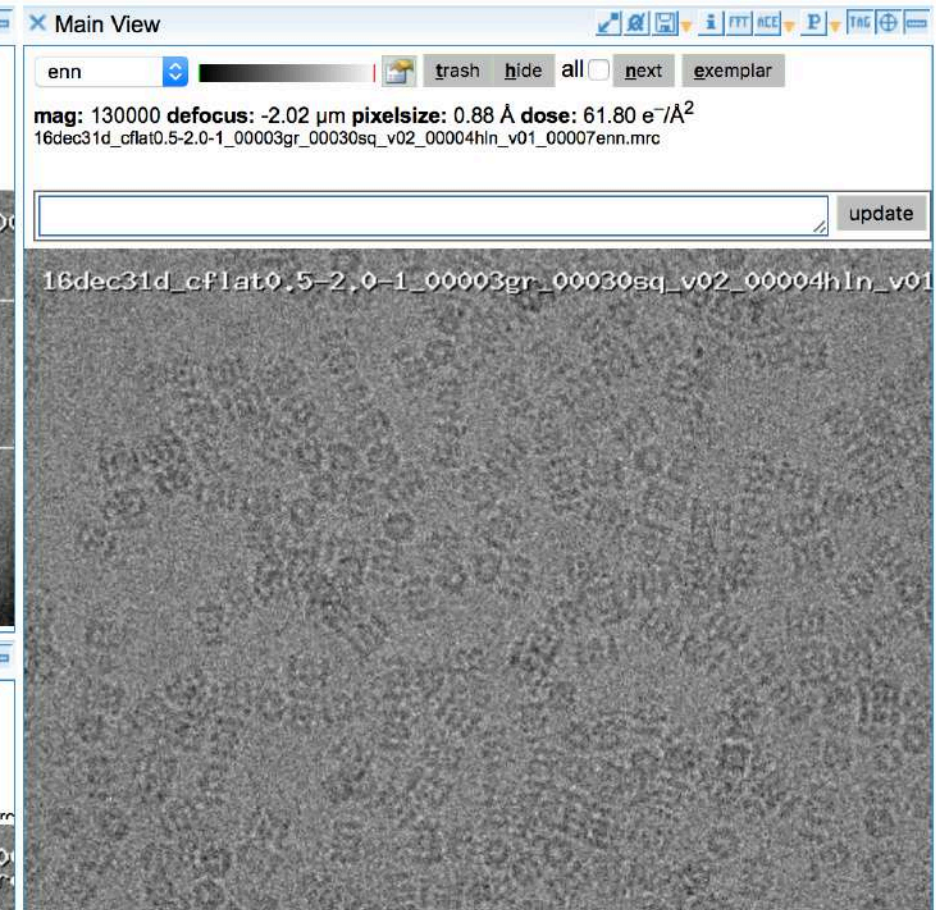
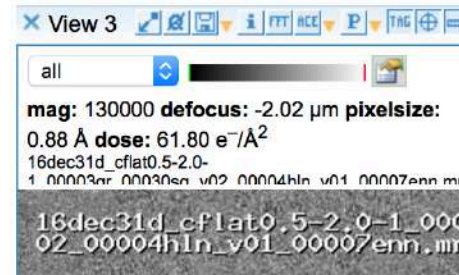
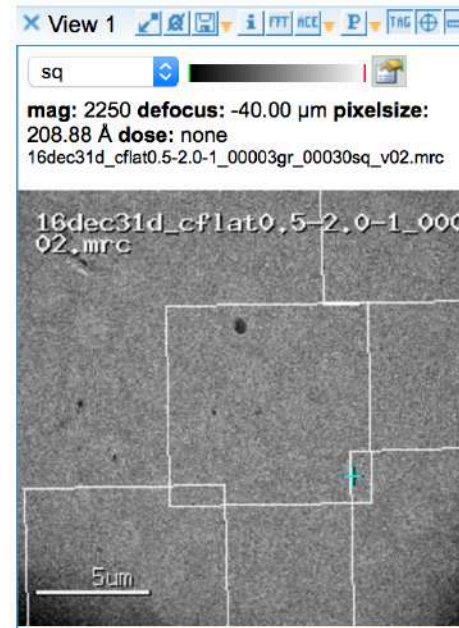
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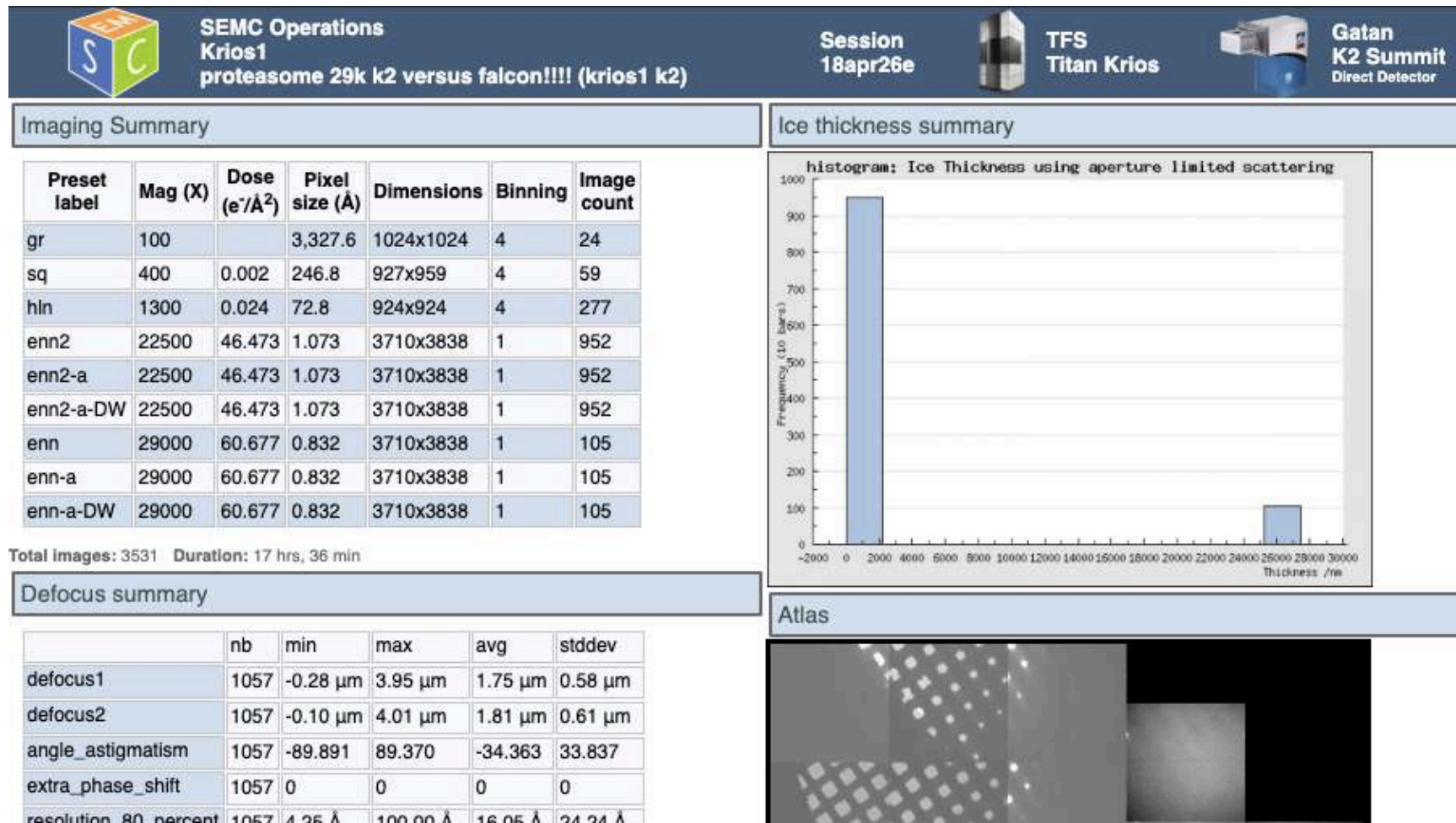
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cflat0.5-2.0-1.23gr_26sq_3hln_2enn



APPION & LEGINON WEBTOOLS

nccatweb.nysbc.org



COMPUTATIONAL RESOURCES



Web resources

nccatweb.nysbc.org

[emg.nysbc.org/
redmine](http://emg.nysbc.org/redmine)

nccat.nysbc.org



Cluster resources

NCCAT
workq cluster



On site resources

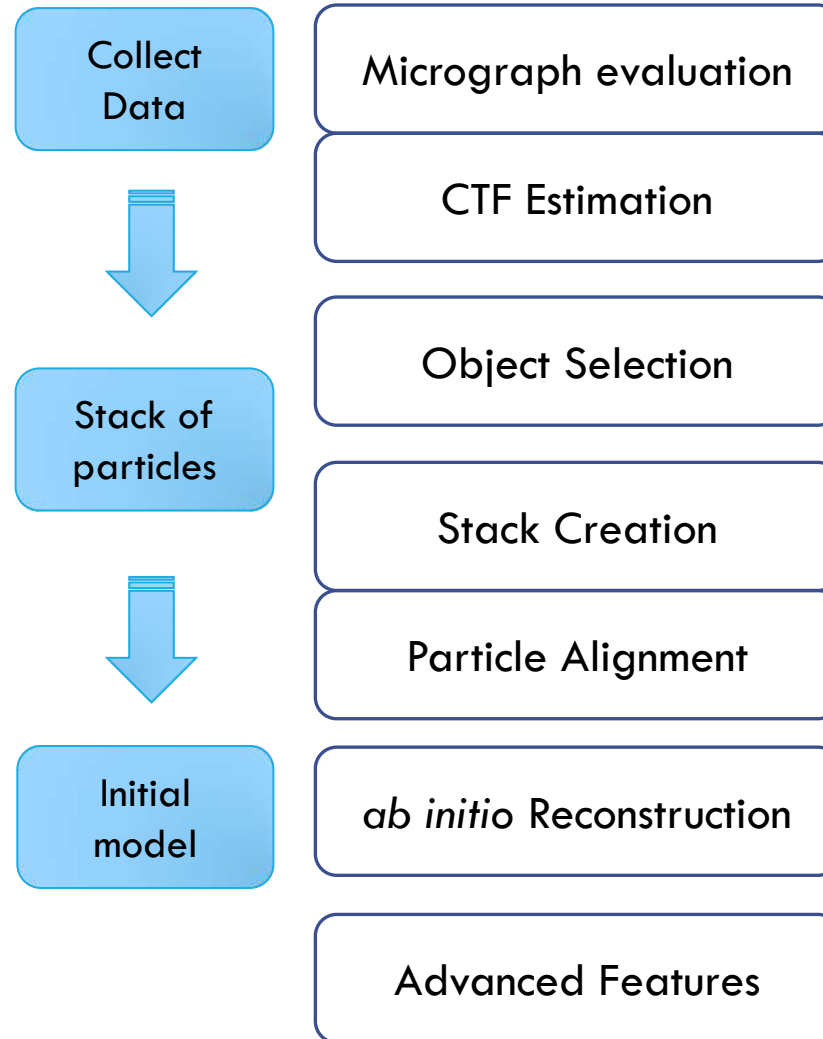
EM session user

Leginon / support
workstations

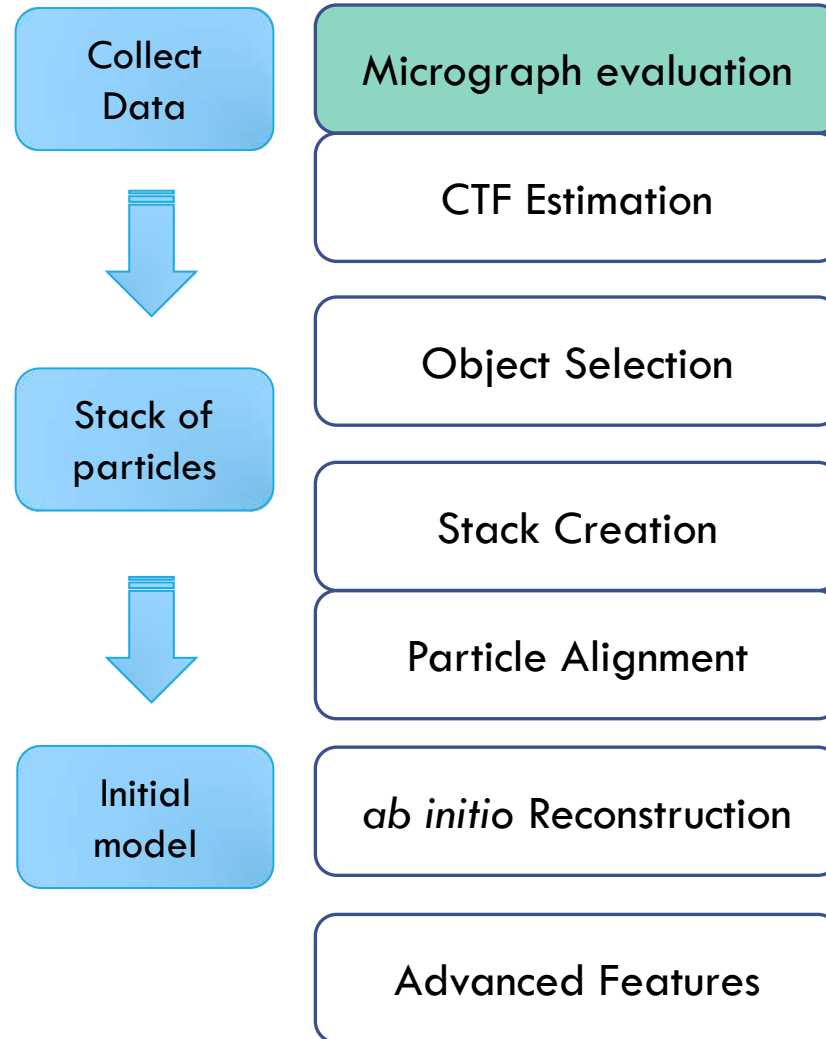
GPU workstations

Data transfer
computers

SCHEDULE



SCHEDULE



MICROGRAPH EVALUATION

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all (all)

Select SEMC - Proteasome test project

#images :485

cflat0.5-2.0-1.3gr_30sq_5hln_7enn
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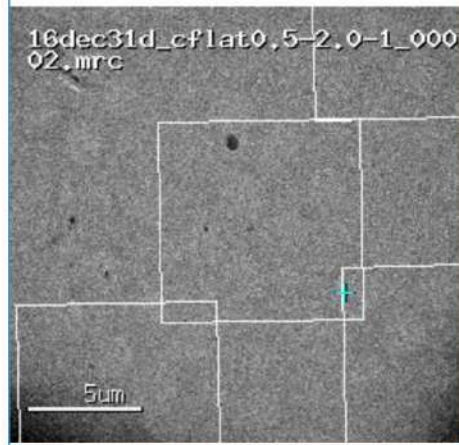
16dec31d - proteasome grid screening and possible collection 135k krio...

-all microscopes

View 1

sq

mag: 2250 defocus: -40.00 μm pixelsize:
208.88 Å **dose: none**
16dec31d_cflat0.5-2.0-1_00003gr_00030sq_v02.mrc



View 3

all

mag: 130000 defocus: -2.02 μm pixelsize:
0.88 Å **dose: 61.80 $\text{e}^-/\text{\AA}^2$**
16dec31d_cflat0.5-2.0-1_00003gr_00030sq_v02_00004hln_v01_00007enn.mrc

16dec31d_cflat0.5-2.0-1_00003gr_00030sq_v02_00004hln_v01_00007enn.mrc

Main View

enn trash hide all next exemplar

mag: 130000 defocus: -2.02 μm pixelsize: 0.88 Å dose: 61.80 $\text{e}^-/\text{\AA}^2$
16dec31d_cflat0.5-2.0-1_00003gr_00030sq_v02_00004hln_v01_00007enn.mrc

update

16dec31d_cflat0.5-2.0-1_00003gr_00030sq_v02_00004hln_v01_00007enn.mrc

MICROGRAPH EVALUATION

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all (all)

Select SEMC - Proteasome test project

#images :485

cflat0.5-2.0-1.3gr_30sq_5hln_7enn
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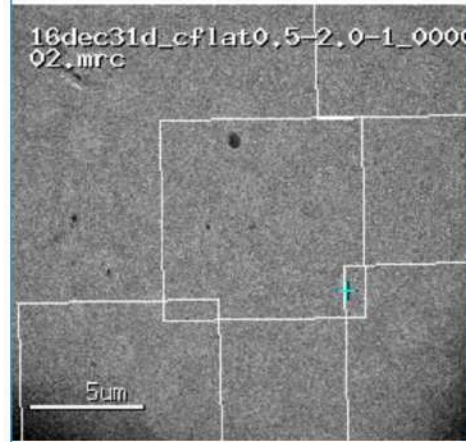
16dec31d - proteasome grid screening and possible collection 135k krio...

-all microscopes

View 1

sq

mag: 2250 defocus: -40.00 μm pixelsize:
208.88 Å dose: none
16dec31d_cflat0.5-2.0-1_00003gr_00030sq_v02.mrc



View 3

all

mag: 130000 defocus: -2.02 μm pixelsize:
0.88 Å dose: 61.80 $\text{e}^-/\text{\AA}^2$
16dec31d_cflat0.5-2.0-1_00003gr_00030sq_v02_00004hln_v01_00007enn.mrc

16dec31d_cflat0.5-2.0-1_00003gr_00030sq_v02_00004hln_v01_00007enn.mrc

Main View

enn

mag: 130000 defocus: -2.02 μm pixelsize: 0.88 Å dose: 61.80 $\text{e}^-/\text{\AA}^2$
16dec31d_cflat0.5-2.0-1_00003gr_00030sq_v02_00004hln_v01_00007enn.mrc

16dec31d_cflat0.5-2.0-1_00003gr_00030sq_v02_00004hln_v01_00007enn.mrc

MICROGRAPH EVALUATION

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all (all)

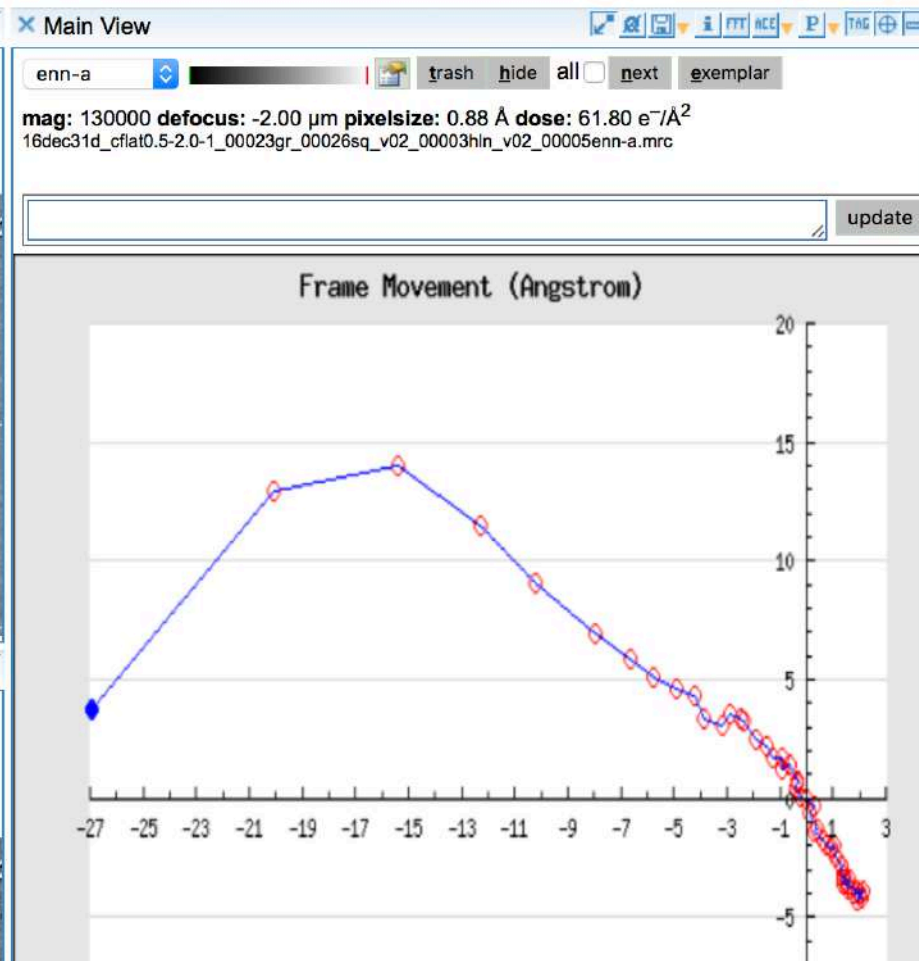
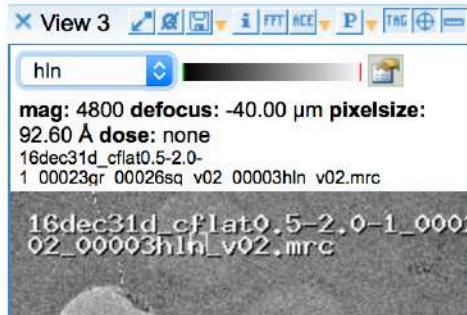
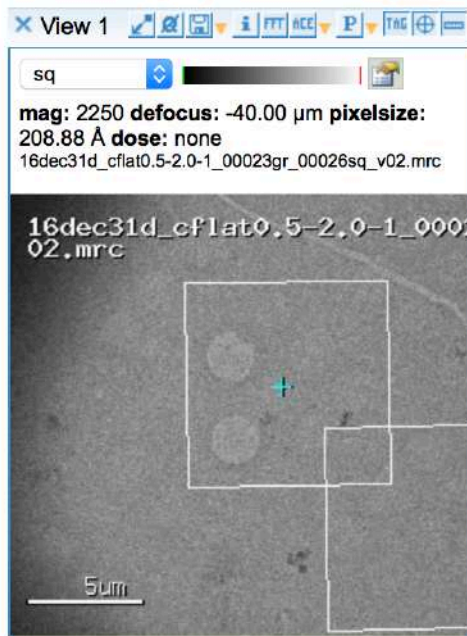
Select SEMC - Proteasome test project

#images :427

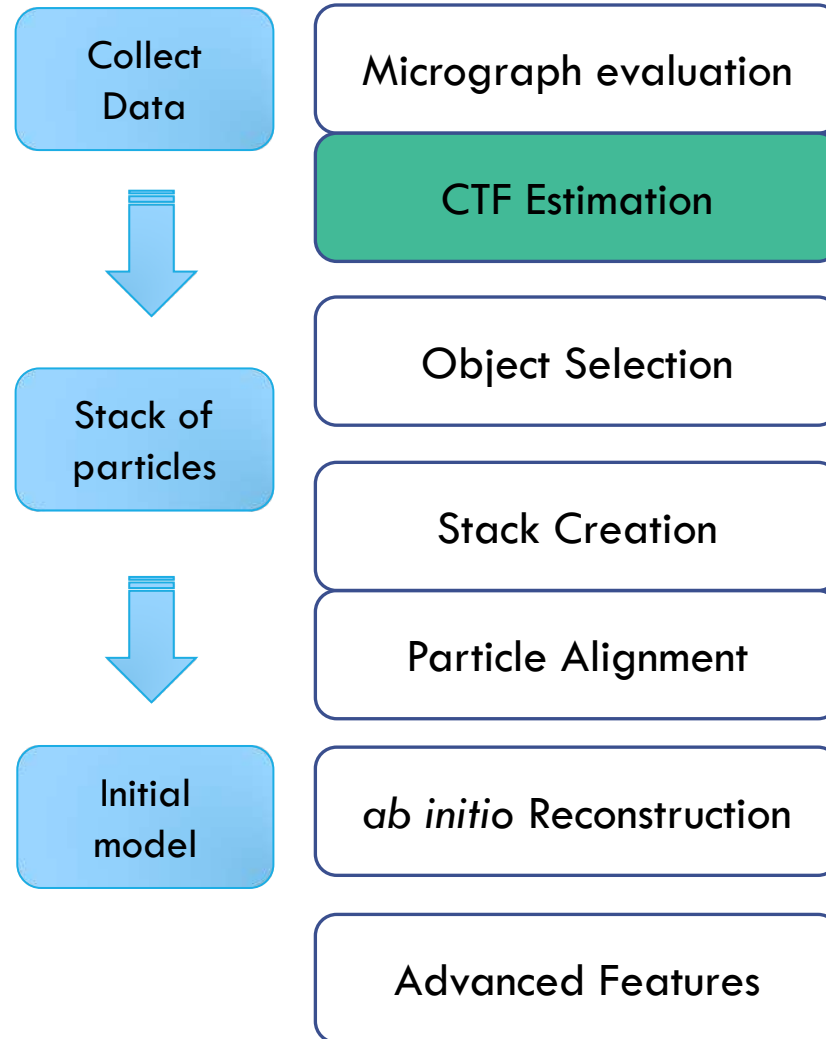
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16dec31d - proteasome grid screening and possible collection 135k krio...

-all microscopes



SCHEDULE



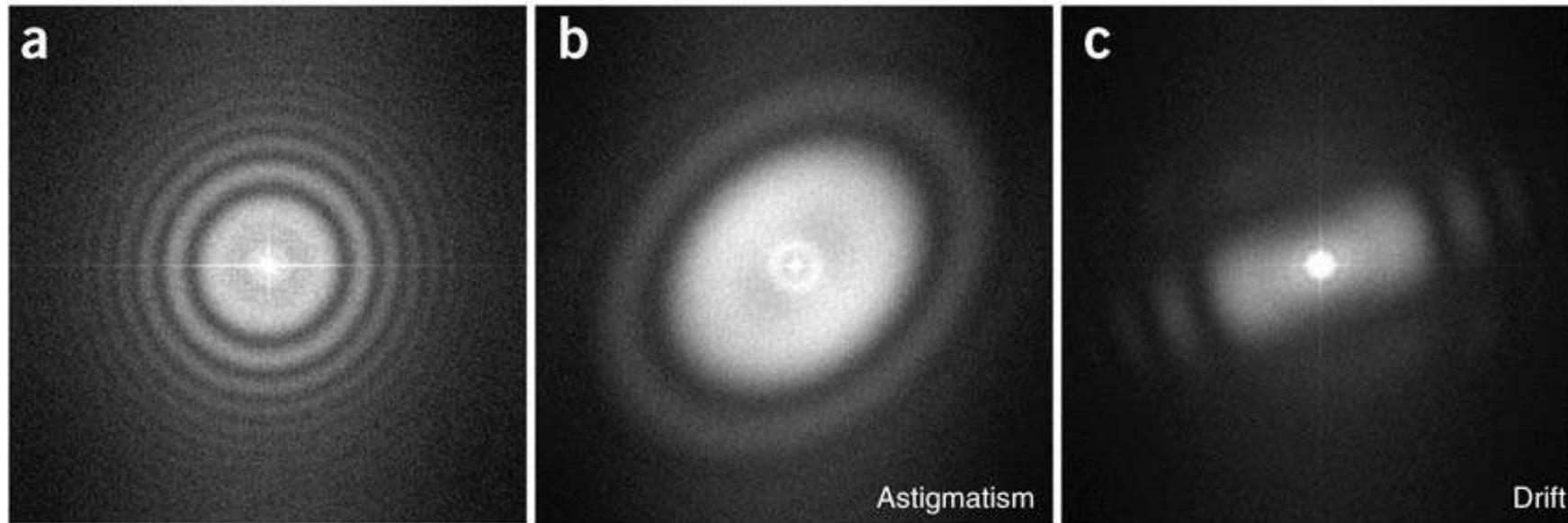
CTF

Contrast**T**ransfer**F**unction

CTF is the function which modulates the amplitudes and phase of an electron beam in a TEM

A recorded image can be regarded as a CTF degraded true object and allows for CTF correction to restore that image

CTF: WHY IS MONITORING THE CTF IMPORTANT IN OUR DATA COLLECTION?



CTF: WHY IS MONITORING THE CTF IMPORTANT IN OUR DATA COLLECTION?

all (all) Select SEMC - Proteasome test project

#images :427

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- cflat0.5-2.0-1_3gr_30sq_4hln_7enn-a
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- cflat0.5-2.0-1_3gr_30sq_2hln_7enn-a
- cflat0.5-2.0-1_3gr_30sq_2hln_3enn-a
- cflat0.5-2.0-1_3gr_30sq_2hln_2enn-a
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- cflat0.5-2.0-1_23gr_26sq_2hln_2enn-a
- cflat0.5-2.0-1_23gr_26sq_2hln_1enn-a

16dec31d - proteasome grid screening and possible collection 135k krio...

-all microscopes

View 1

sq

mag: 2250 defocus: -40.00 μm pixelsize: 208.88 Å dose: none
16dec31d_cflat0.5-2.0-1_00003gr_00030sq_v02.mrc

16dec31d_cflat0.5-2.0-1_00003gr_00030sq_v02.mrc

5 μm

View 3

hln

mag: 4800 defocus: -40.00 μm pixelsize: 92.60 Å dose: none
16dec31d_cflat0.5-2.0-1_00003gr_00030sq_v02_00004hln_v01.mrc

16dec31d_cflat0.5-2.0-1_00003gr_00030sq_v02_00004hln_v01.mrc

Main View

enn-a

trash hide all next exemplar

mag: 130000 defocus: -2.02 μm pixelsize: 0.88 Å dose: 61.80 $\text{e}^-/\text{Å}^2$
16dec31d_cflat0.5-2.0-1_00003gr_00030sq_v02_00004hln_v01_00007enn-a.mrc

16dec31d_cflat0.5-2.0-1_00003gr_00030sq_v02_00004hln_v01_00007enn-a.mrc

update

CTF: WHY IS MONITORING THE CTF IMPORTANT IN OUR DATA COLLECTION?

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all (all)

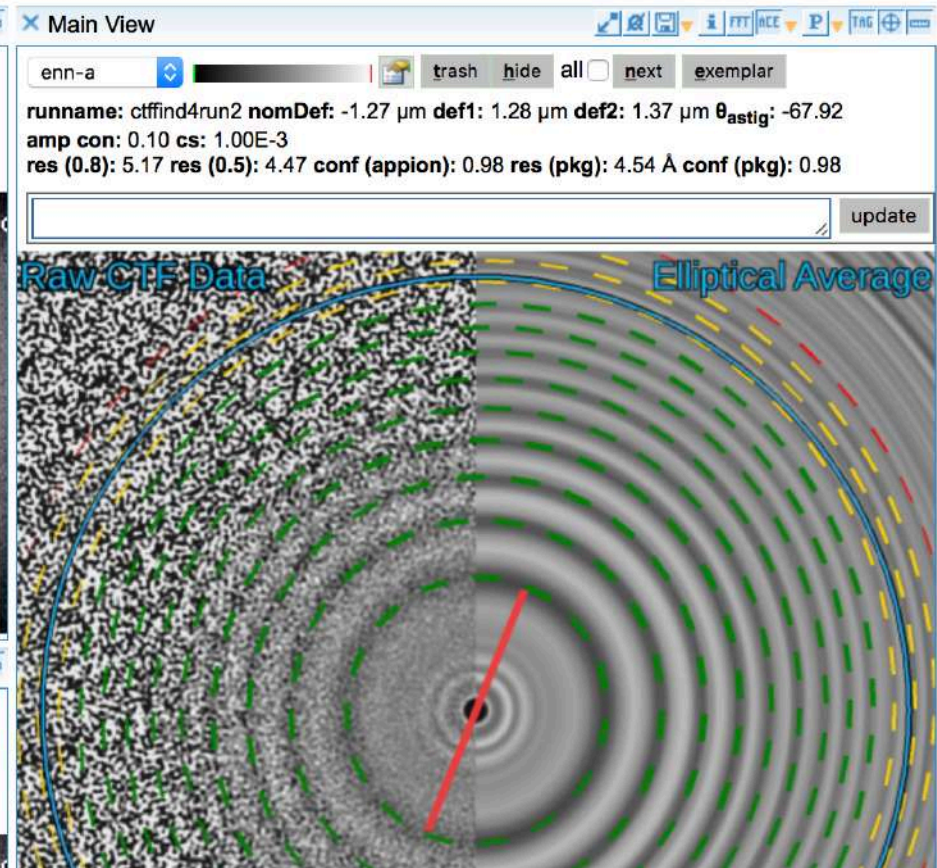
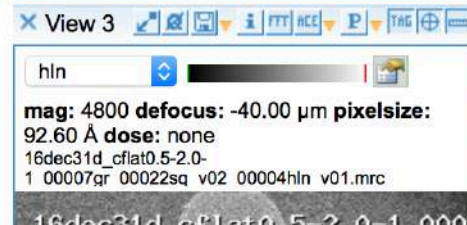
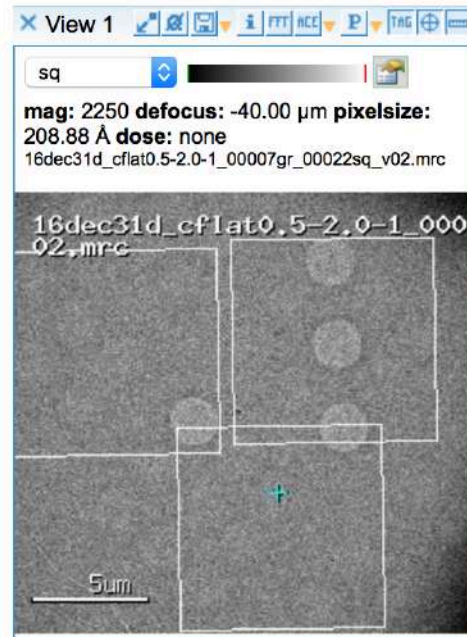
16dec31d - proteasome grid screening and possible collection 135k krio...

Select SEMC - Proteasome test project

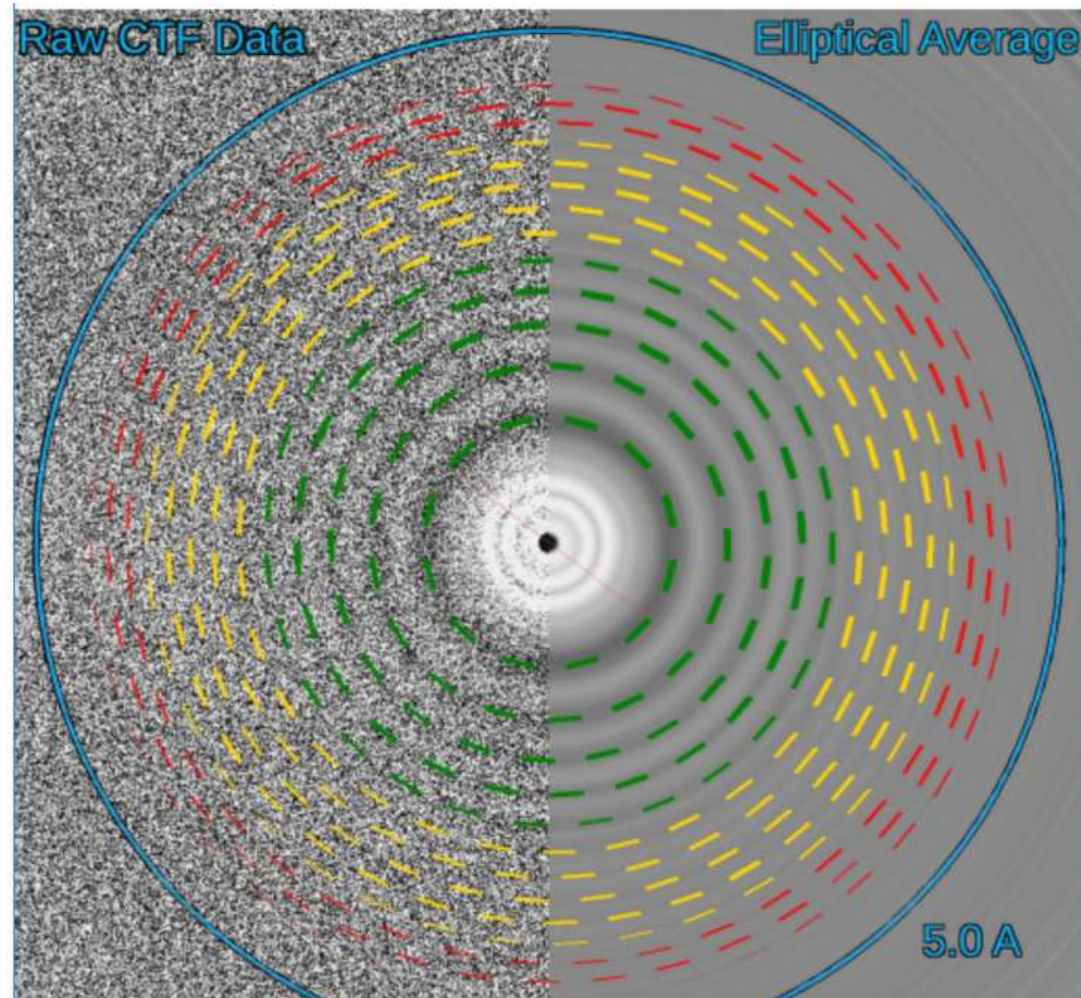
-all microscopes

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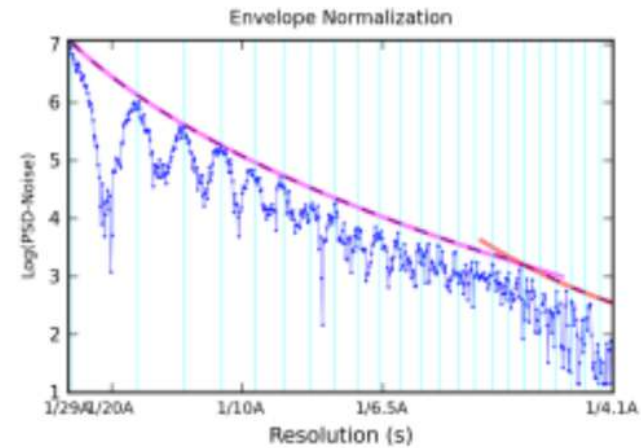
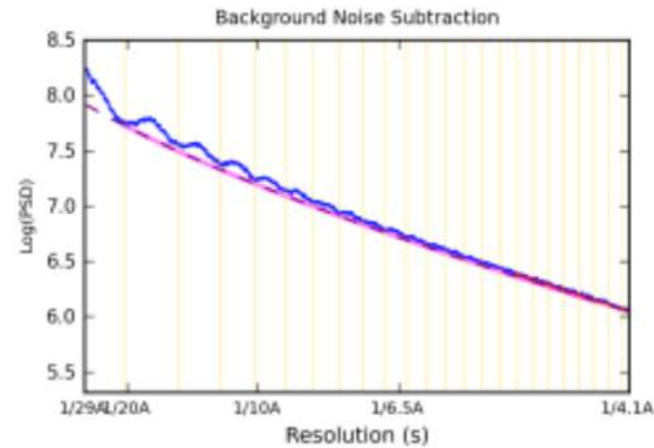
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cflat0.5-2.0-1.12gr_23sq_2hln_5enn-a
cflat0.5-2.0-1.12gr_23sq_2hln_4enn-a
cflat0.5-2.0-1.12gr_23sq_2hln_3enn-a
cflat0.5-2.0-1.12gr_23sq_2hln_2enn-a
cflat0.5-2.0-1.7gr_22sq_4hln_7enn-a
cflat0.5-2.0-1.7gr_22sq_4hln_6enn-a
cflat0.5-2.0-1.7gr_22sq_4hln_5enn-a
cflat0.5-2.0-1.7gr_22sq_4hln_4enn-a
cflat0.5-2.0-1.7gr_22sq_4hln_3enn-a
cflat0.5-2.0-1.7gr_22sq_4hln_2enn-a
cflat0.5-2.0-1.7gr_22sq_3hln_7enn-a
cflat0.5-2.0-1.7gr_22sq_3hln_6enn-a
cflat0.5-2.0-1.7gr_22sq_3hln_5enn-a
cflat0.5-2.0-1.7gr_22sq_3hln_4enn-a
cflat0.5-2.0-1.7gr_22sq_3hln_3enn-a
cflat0.5-2.0-1.7gr_22sq_3hln_2enn-a
cflat0.5-2.0-1.7gr_22sq_2hln_7enn-a
cflat0.5-2.0-1.7gr_22sq_2hln_6enn-a
cflat0.5-2.0-1.7gr_22sq_2hln_5enn-a
cflat0.5-2.0-1.7gr_22sq_2hln_4enn-a
cflat0.5-2.0-1.7gr_22sq_2hln_3enn-a
cflat0.5-2.0-1.7gr_22sq_2hln_2enn-a
cflat0.5-2.0-1.7gr_21sq_5hln_6enn-a
cflat0.5-2.0-1.7gr_21sq_5hln_5enn-a
cflat0.5-2.0-1.7gr_21sq_5hln_4enn-a
cflat0.5-2.0-1.7gr_21sq_5hln_3enn-a
cflat0.5-2.0-1.7gr_21sq_5hln_2enn-a



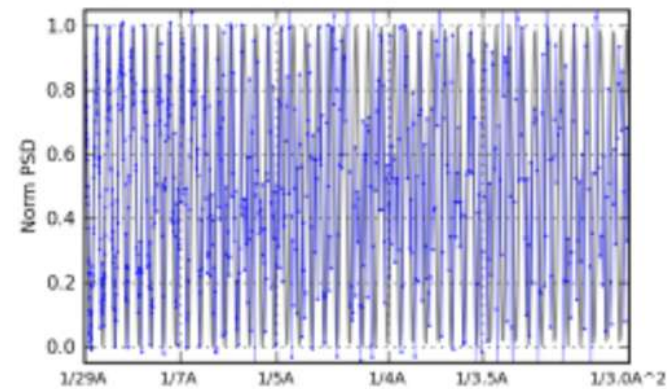
CTF: WHY IS MONITORING THE CTF IMPORTANT IN OUR DATA COLLECTION?



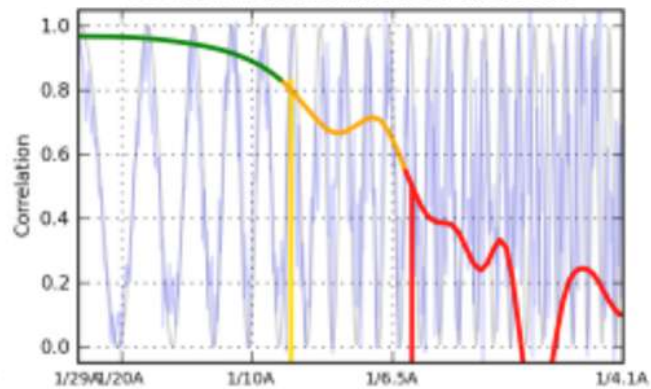
CTF: WHY IS MONITORING THE CTF IMPORTANT IN OUR DATA COLLECTION?



of CTF data (30-10Å 0.948 / 5-peak 0.928) Def1 = 2.064e-06 / Def2 = 2.101e-06



Resolution limits: 8.70Å at 0.8 and 6.20Å at 0.5



CTF: WHY IS MONITORING THE CTF IMPORTANT IN OUR DATA COLLECTION?

[\[Home\]](#)[\[Logout eeng\]](#)[\[eeng Profile\]](#)[\[summary\]](#) [\[processing\]](#) [\[make jpgs\]](#)

all (all)

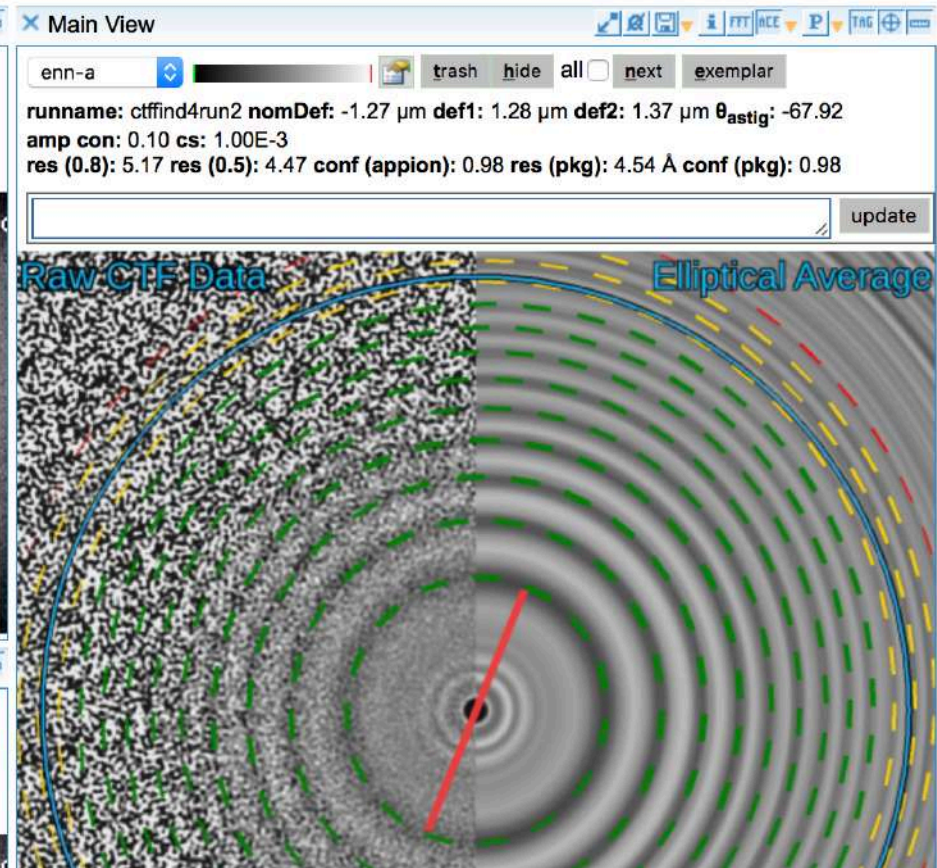
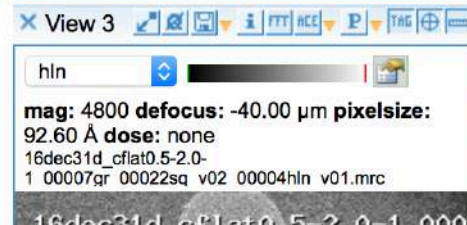
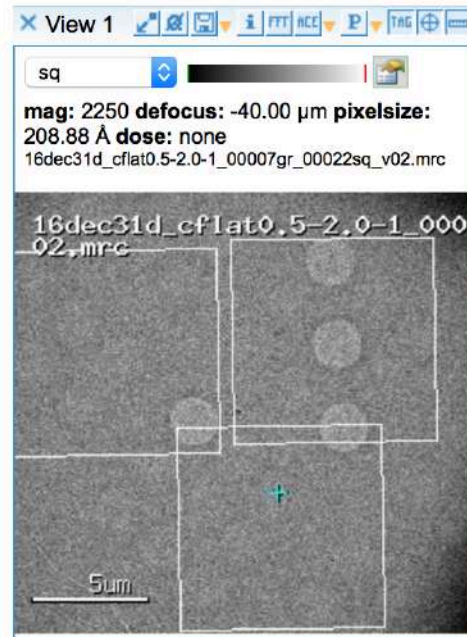
16dec31d - proteasome grid screening and possible collection 135k krio...

Select SEMC - Proteasome test project

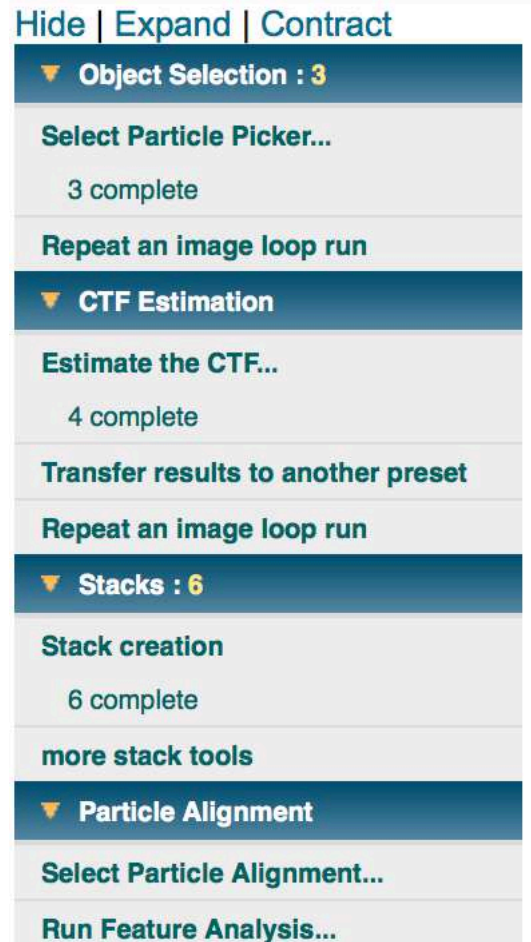
-all microscopes

#images :427

cflat0.5-2.0-1.12gr_23sq_3hln_4enn-a
cflat0.5-2.0-1.12gr_23sq_3hln_3enn-a
cflat0.5-2.0-1.12gr_23sq_3hln_2enn-a
cflat0.5-2.0-1.12gr_23sq_2hln_8enn-a
cflat0.5-2.0-1.12gr_23sq_2hln_7enn-a
cflat0.5-2.0-1.12gr_23sq_2hln_6enn-a
cflat0.5-2.0-1.12gr_23sq_2hln_5enn-a
cflat0.5-2.0-1.12gr_23sq_2hln_4enn-a
cflat0.5-2.0-1.12gr_23sq_2hln_3enn-a
cflat0.5-2.0-1.12gr_23sq_2hln_2enn-a
cflat0.5-2.0-1.7gr_22sq_4hln_7enn-a
cflat0.5-2.0-1.7gr_22sq_4hln_6enn-a
cflat0.5-2.0-1.7gr_22sq_4hln_5enn-a
cflat0.5-2.0-1.7gr_22sq_4hln_4enn-a
cflat0.5-2.0-1.7gr_22sq_4hln_3enn-a
cflat0.5-2.0-1.7gr_22sq_4hln_2enn-a
cflat0.5-2.0-1.7gr_22sq_3hln_7enn-a
cflat0.5-2.0-1.7gr_22sq_3hln_6enn-a
cflat0.5-2.0-1.7gr_22sq_3hln_5enn-a
cflat0.5-2.0-1.7gr_22sq_3hln_4enn-a
cflat0.5-2.0-1.7gr_22sq_3hln_3enn-a
cflat0.5-2.0-1.7gr_22sq_3hln_2enn-a
cflat0.5-2.0-1.7gr_22sq_2hln_7enn-a
cflat0.5-2.0-1.7gr_22sq_2hln_6enn-a
cflat0.5-2.0-1.7gr_22sq_2hln_5enn-a
cflat0.5-2.0-1.7gr_22sq_2hln_4enn-a
cflat0.5-2.0-1.7gr_22sq_2hln_3enn-a
cflat0.5-2.0-1.7gr_22sq_2hln_2enn-a
cflat0.5-2.0-1.7gr_21sq_5hln_6enn-a
cflat0.5-2.0-1.7gr_21sq_5hln_5enn-a
cflat0.5-2.0-1.7gr_21sq_5hln_4enn-a
cflat0.5-2.0-1.7gr_21sq_5hln_3enn-a
cflat0.5-2.0-1.7gr_21sq_5hln_2enn-a



CTF: HOW TO RUN A JOB.



nccatweb.nysbc.org

Login:

nworkshop



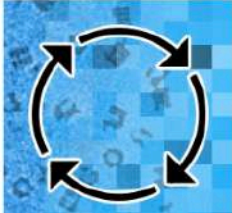
Password:

appion2020

CTF: HOW TO RUN A JOB.

CTF Estimation Procedures

During CTF estimation the goal is to fit the standard CTF equation [\(wikipedia\)](#) to the power spectra of the electron micrographs

	<h3>CTFFIND v4</h3> <p>CTFFIND uses a robust grid search algorithm to find the optimal CTF parameters. Please see the Grigorieff lab website for more information.</p>
	<h3>GCTF v1.06</h3> <p>This is a GPU accelerated program for real-time CTF determination, refinement, evaluation and correction. Please see the Dynein lab website for more information.</p>
	<h3>ACE 2</h3> <p>ACE 2 is an unpublished re-implementation of ACE1, but written in objective-C ACE2 make several improvements over ACE1 including a several speed enhancements and a robust astigmatism estimate.</p>

CTF: HOW TO RUN A JOB.

Run name

ctffind4run3

Output directory

/gpfs/appion/test/16dec31d/ctf/

☐ Use DD Stack

Select an aligned framestack

ddstack1

enn-a

Preset

☐ Wait for more images after finishing

Max number of images to process

Images to process:

☒ Do not process hidden or rejected images

☐ All images independent of status

☐ Exemplar and keep images only

Image order:

☒ Forward

☐ Reverse

☐ Shuffle

Continuation:

☒ Continue unfinished run (default)

☐ Reprocess all images

☒ Commit results to database

Optional Parameters

0.07

Amplitude Contrast

1024

Field Size

50

Minimum Resolution (in Å)

4

Maximum Resolution (in Å)

0.1

Defocus Step (in µm)

25

Number of Steps to Search

0.05

Expected Astigmatism (in µm)

☒

Use best values from Database

☐

Override Nominal Value (in µm)

☒

Allow parallel runs

☐

Use phase plate

10

Minimum Phase Shift in search (degrees)

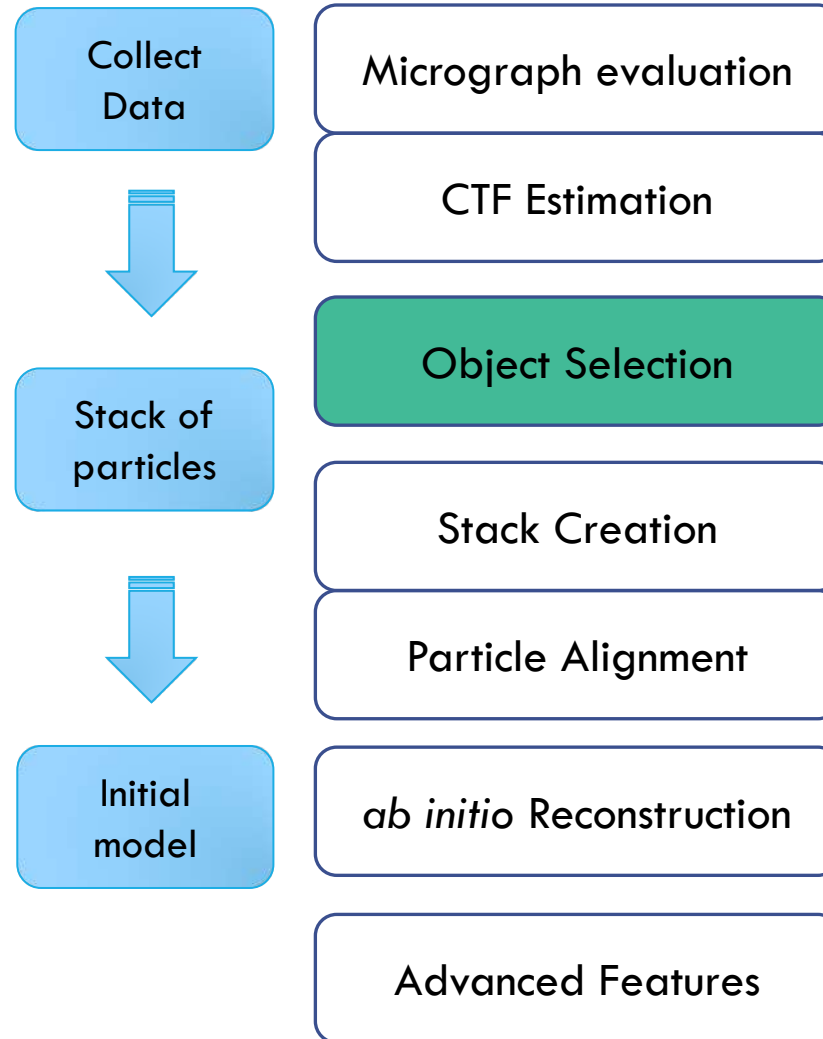
170

Maximum Phase Shift in search (degrees)

10

Initial Phase Shift Search Step (degrees)

SCHEDULE



PARTICLE PICKING

1. Initial Picking

DoG Picking

- Unbiased and rough

Manual Picking

- Time consuming, possible bias, can be more accurate

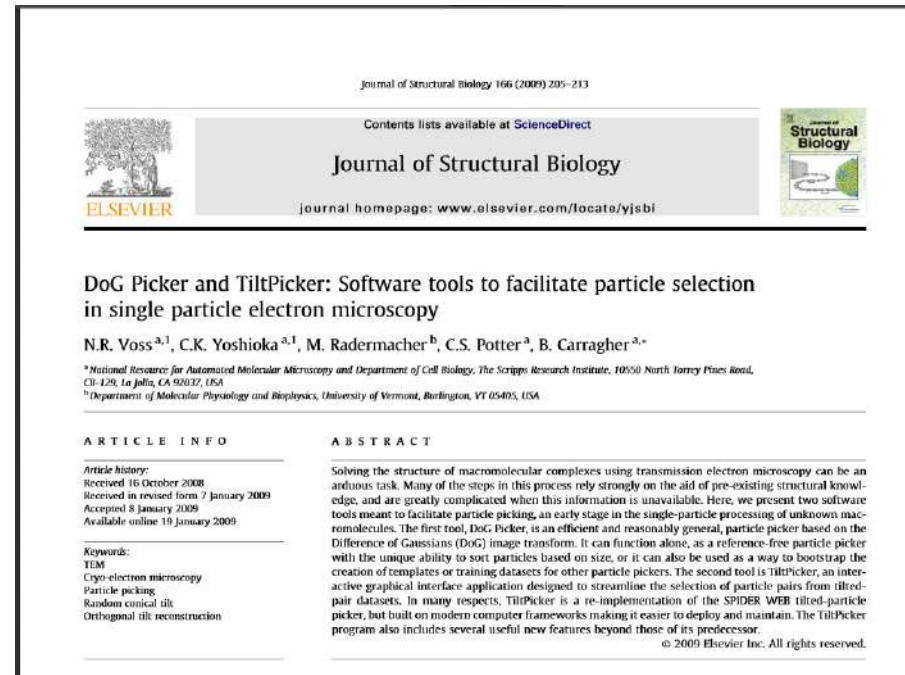
2. Make a stack, 2D alignment, generate good class averages

3. Template Picking

- Based on generated class averages

PARTICLE PICKING

Difference of Gaussian (DoG) Picker



PARTICLE PICKING

Difference of Gaussian (DoG) Picker



Run name

dogrun1

Output directory

/gpfs/appion/test/15workshopH/extract

Preset: upload

☐ Wait for more images after finishing

Max number of images to process

Images to process:

- ☒ Do not process hidden or rejected images
- ☐ All images independent of status
- ☐ Exemplar and keep images only

Image order:

- ☒ Forward
- ☐ Reverse
- ☐ Shuffle

Continuation:

- ☒ Continue unfinished run (default)
- ☐ Reprocess all images

☒ Commit results to database

☐ Test these settings on image:

Just Show Command

Particle Diameter:

Particle diameter for filtering (in Angstroms)

Peak thresholds:

Minimum threshold

Maximum threshold

Max number of particles allowed per image

Filter Values:

Low Pass (in Angstroms; 0 = off)

High Pass (in Angstroms; 0 = off)

Median (in pixels; 0 = off)

Pixel Limit (in Standard Deviations; 0 = off)

Binning (power of 2)

☒ Plane regression

Defocal pairs:

☐ Calculate shifts for defocal pairs

Advanced options:

Maximum peak area multiple

Minimum peak overlap distance multiple

Peak extraction type

☒ Center of mass (default)

☐ Maximum position

☐ Pick only doubles

☐ Invert image density

K-factor (sloppiness)

Multi-scale dogpicker:

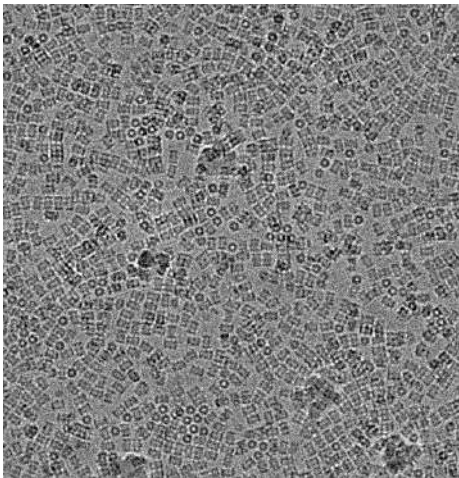
Number of Slices (number of sizes)

Size Range (in Angstroms)

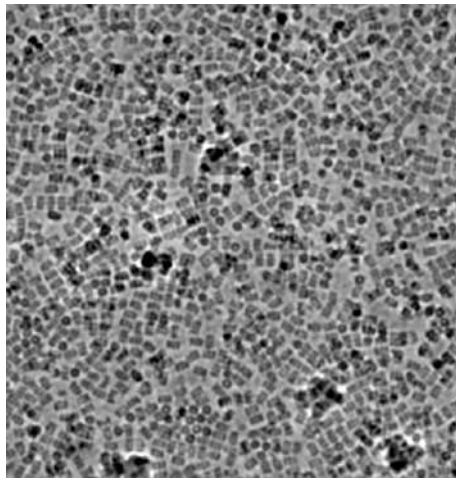
PARTICLE PICKING

*Difference of
Gaussian (DoG) Picker*

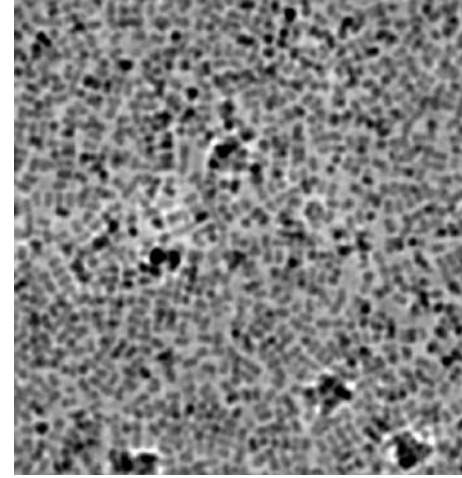
Low Pass Filtering



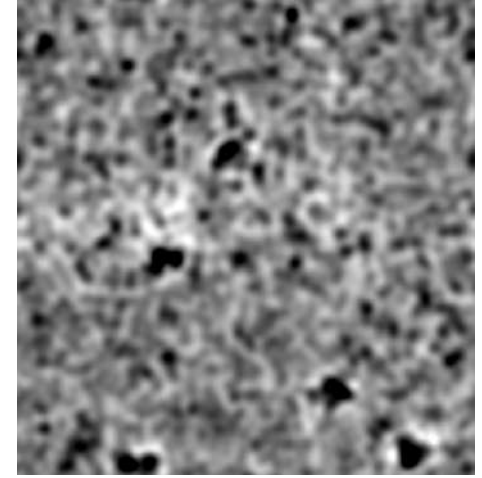
0 Å



20 Å



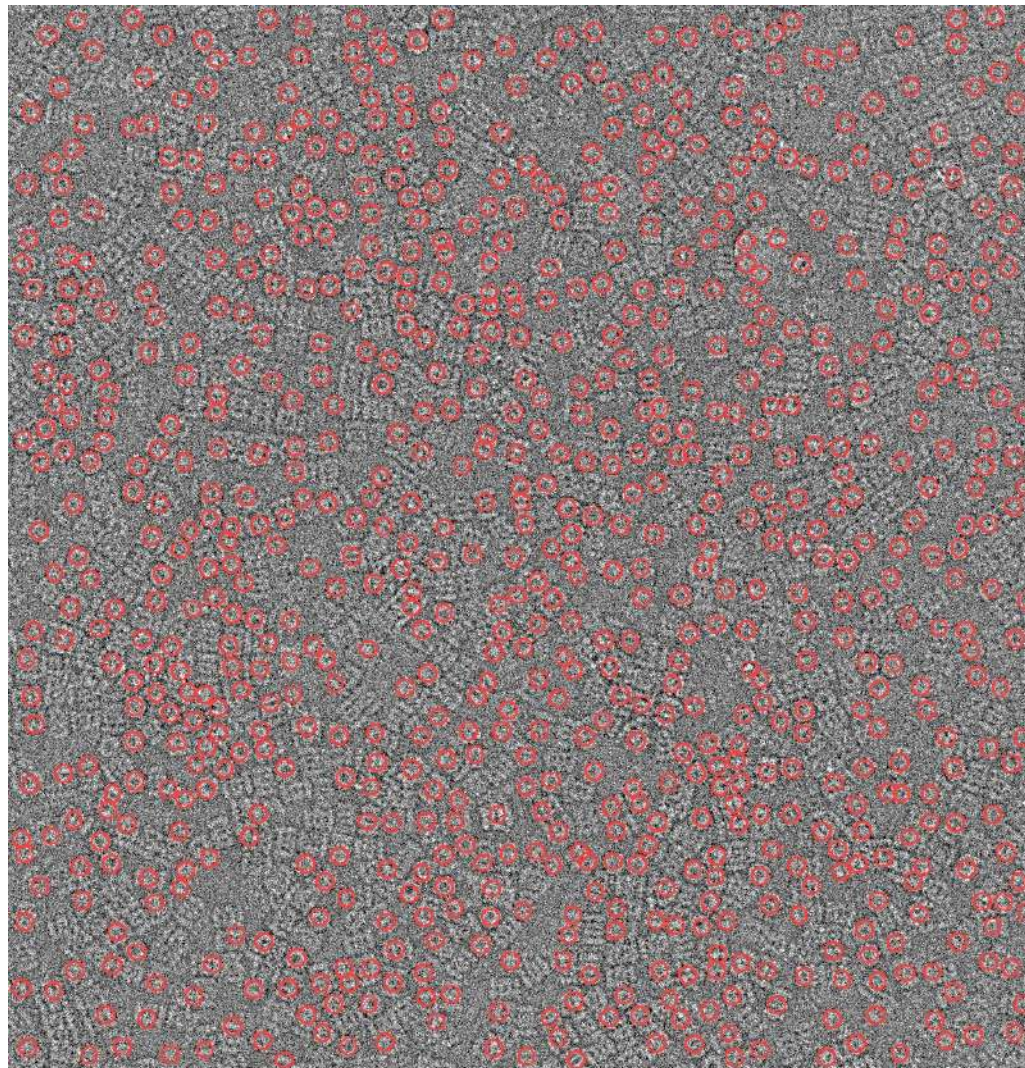
40 Å



60 Å

PARTICLE PICKING

*Difference of
Gaussian (DoG) Picker*



Particle Diameter:

80 [Particle diameter for filtering \(in Angstroms\)](#)

Peak thresholds:

0.4 [Minimum threshold](#)

1.5 [Maximum threshold](#)

1500 [Max number of particles allowed per image](#)

4 [Binning \(power of 2\)](#)

Filter Values:

15 [Low Pass \(in Angstroms; 0 = off\)](#)

0 [High Pass \(in Angstroms; 0 = off\)](#)

2 [Median \(in pixels; 0 = off\)](#)

4 [Pixel Limit \(in Standard Deviations; 0 = off\)](#)

☒ [Plane regression](#)

Defocal pairs:

☐ [Calculate shifts for defocal pairs](#)

Image Contrast:

☐ [White Particles \(i.e. negatively stained\)](#)

☒ [Black Particles \(i.e. ice embedded\)](#)

Peak extraction type:

☒ [Center of mass \(default\)](#)

☐ [Maximum position](#)

☐ [Do NOT create summary peak pairs](#)

☐ [Pick only doubles](#)

1.2 [Maximum peak area multiple](#)

1.5 [Minimum peak overlap distance multiple](#)

1.2 [K-factor \(slopes\)](#)

Multi-scale dogpicker:

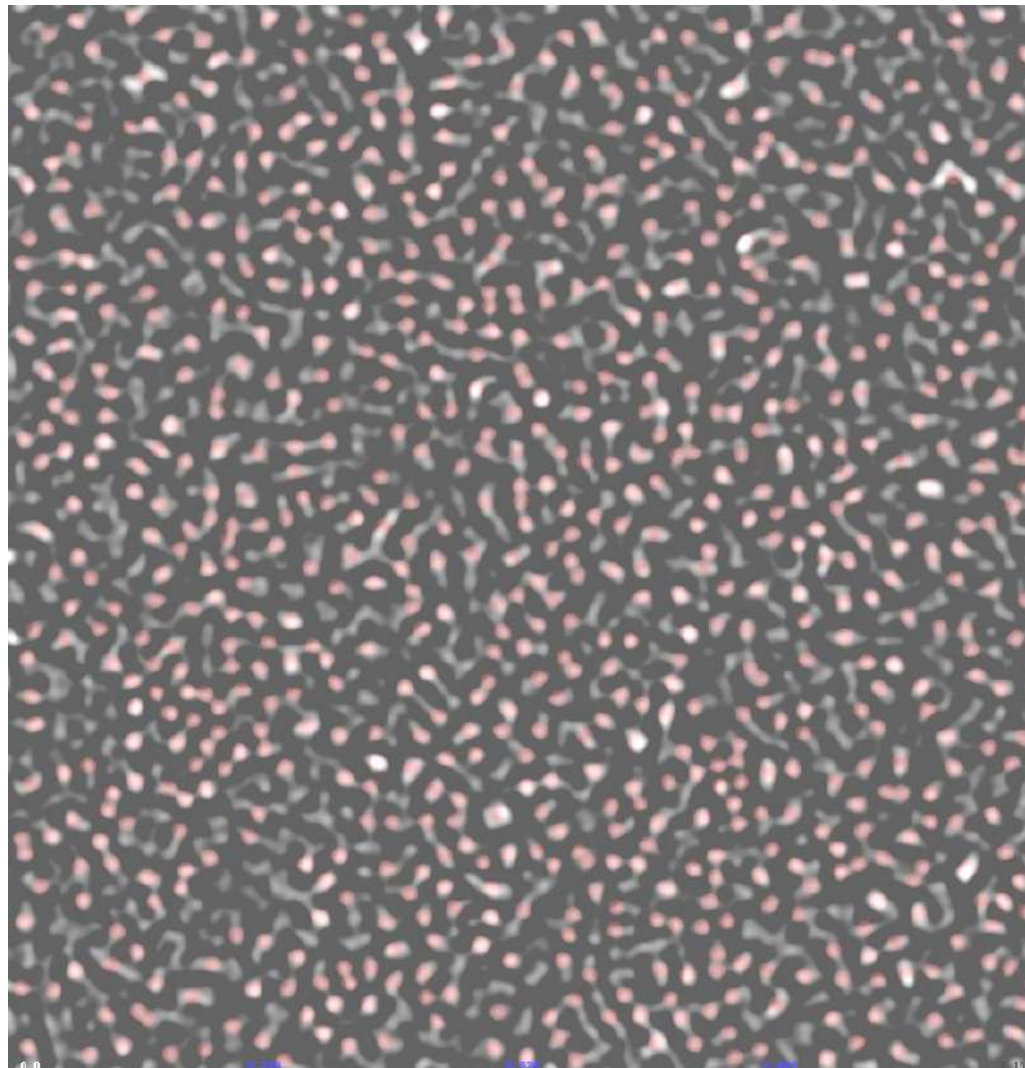
[Number of Slices \(number of sizes\)](#)

[Size Range \(in Angstroms\)](#)

PARTICLE PICKING

*Difference of
Gaussian (DoG) Picker*

DoG Map



Particle Diameter:

80 Particle diameter for filtering (in Angstroms)

Peak thresholds:

0.4 Minimum threshold

1.5 Maximum threshold

1500 Max number of particles allowed per image

4 Binning (power of 2)

Filter Values:

15 Low Pass (in Angstroms; 0 = off)

0 High Pass (in Angstroms; 0 = off)

2 Median (in pixels; 0 = off)

4 Pixel Limit (in Standard Deviations; 0 = off)

☒ Plane regression

Defocal pairs:

☐ Calculate shifts for defocal pairs

Image Contrast:

☐ White Particles (i.e. negatively stained)

☒ Black Particles (i.e. ice embedded)

Peak extraction type:

☒ Center of mass (default)

☐ Maximum position

☐ Do NOT create summary peak pairs

☐ Pick only doubles

1.2 Maximum peak area multiple

1.5 Minimum peak overlap distance multiple

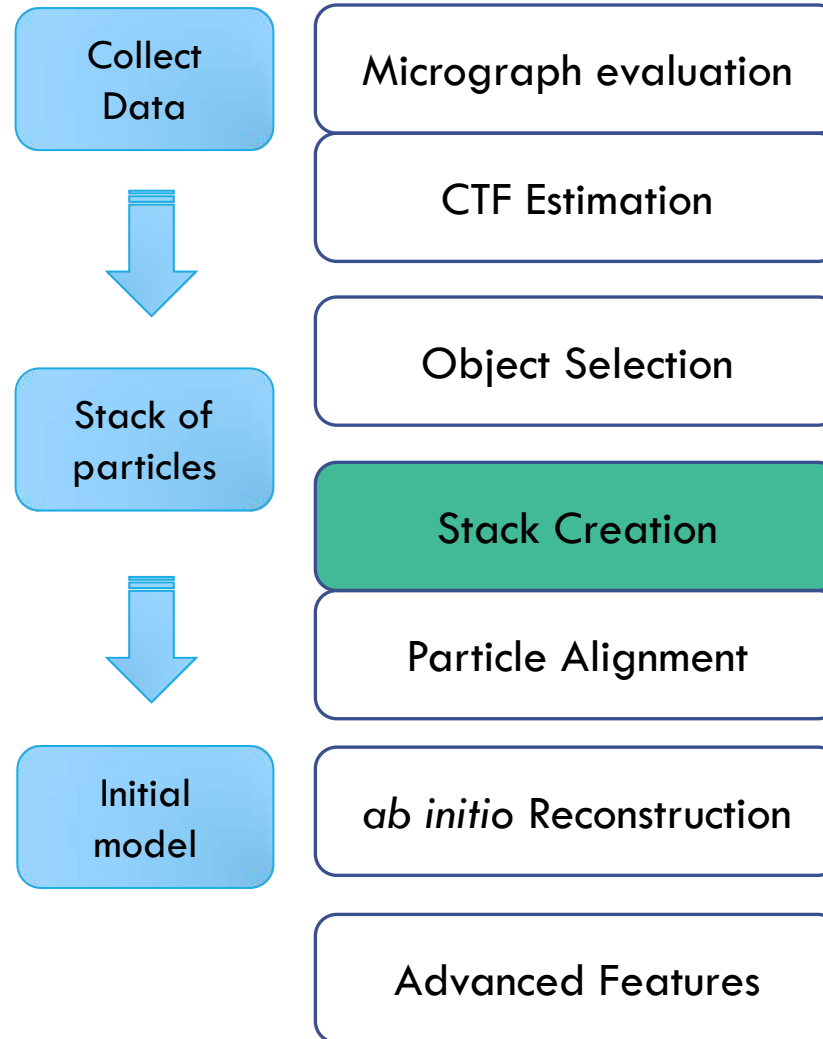
1.2 K-factor (slopiness)

Multi-scale dogpicker:

Number of Slices (number of sizes)

Size Range (in Angstroms)

SCHEDULE



STACK CREATION AFTER PARTICLE PICKING

Box size: should be at least 1.5 X particle size

Should be a multiple of small primes (2,3,5)

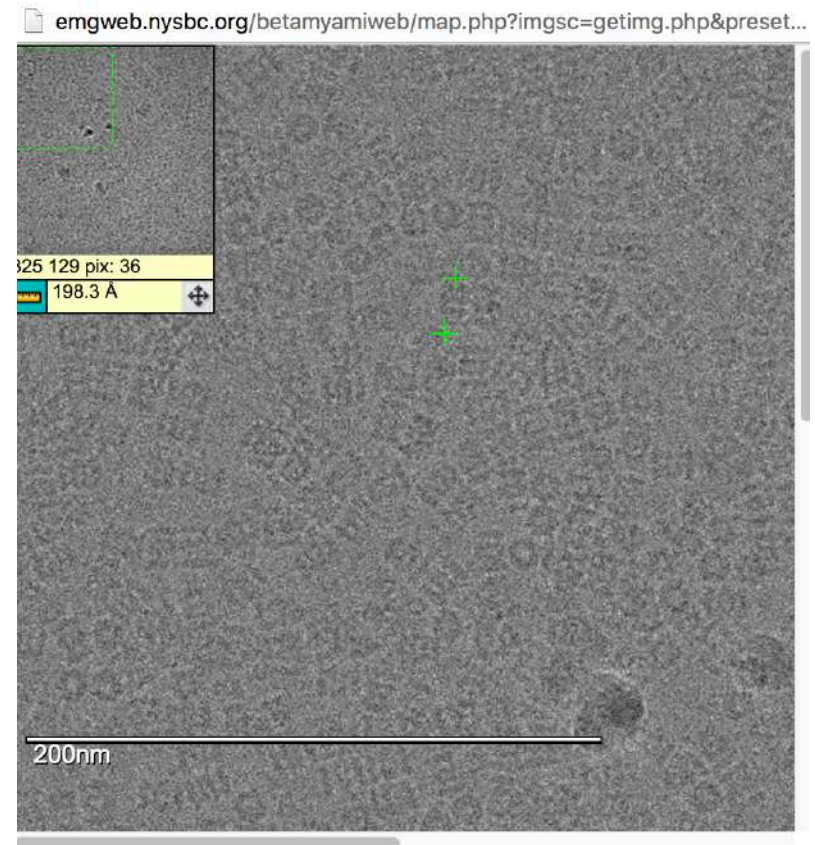
Measure approximate particle size in Leginon using Ruler tool

Convert to pixels

Today's sample:

- Diameter $\sim 200\text{\AA}$
- Pixel size $\sim 1.5\text{\AA}$

Box size 192 pixels



STACK CREATION FOR TEMPLATES / QUICK ANALYSIS

Run name: stack1

Output directory: /gpfs/appion/test/15workshop/stacks

Description: stack from dogpicker

Preset: upload
☐ Wait for more images after finishing

Show Advanced Options

Output file format: IMAGIC

Density modifications:
☒ Invert image density
Show Advanced_Stack_Options_1

Particles: ddogrun1 (1,131 parts)

192 Box Size (Unbinned, in pixels)
☐ (override boxsize)

3 Binning

0 Pixel Limit (in Standard Deviations; 0 = off)

☒ Fast Insert

☒ Ctf Correct Particle Images

☐ CTF Confidence Cutoff
Use Values Above: 0.8 (between 0.0 - 1.0)

~~Hide Advanced_Ctf_Options~~
CTF Correction Method
Ace 2 PhaseFlip Whole Image

CTF Sorting Method
Resolution 0.8 criteria
-OR- choose a ctf run: all (0)
-OR- ☐ Only use CTFFIND values

☐ CTF Resolution Cutoff
Resolution range at 0.8 criteria:
between and (in Angstroms)
Resolution range at 0.5 or software package criteria:
between and (in Angstroms)

Defocus Limits
Min -1.2e-6 & Max -2.83e-6

Initial Stack: After dogpicking, goal is to make templates

Bin as much as possible

- Ideally boxes 72 pixels or fewer in dimension

CTF correct by phase flipping

Invert image density for Cryo!

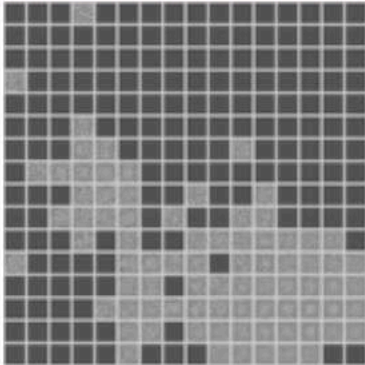
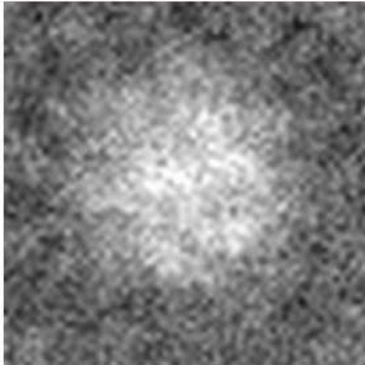
For large sets, can choose only exemplar images

EXAMINE STACKS

Hide | Expand | Contract

- ▼ Object Selection : 2
 - Select Particle Picker...
2 complete
 - Repeat an image loop run
- ▼ CTF Estimation
 - Estimate the CTF...
4 complete
 - Transfer results to another preset
 - Repeat an image loop run
- ▼ Stacks : 5
 - Stack creation
7 complete
 - more stack tools
- ▼ Particle Alignment
 - Select Particle Alignment...
4 complete
 - Run Feature Analysis...
2 complete
 - Run Particle Clustering...
8 complete
 - Run MaskItOn
 - Template Stacks
- ▼ Ab Initio Reconstruction
 - OptiMod Common Lines
2 complete
 - EMAN Common Lines
 - SIMPLE Common Lines
- ▼ Refine Reconstruction
 - Run Single-Model Refinement...
 - Run Multi-Model Refinement...
- ▼ Helical Processing
 - Helical Image Processing (PHOELIX)
- ▼ Direct Detector Tools

Stack Info: stack1 (ID: 1) [hide](#) [delete](#) ([download particles data](#)) [list per-particle CTF info](#) [bake recipe](#)



averaged stack image
[\[Center Particles\]](#)
[\[Learning Stack Cleaner\]](#)
[\[Sort Junk\]](#)
[\[Create Substack\]](#)
[\[Mask Particles with Box\]](#)

mean/stddev montage
[\[Filter by Mean/Stdev\]](#)

date time: 2017-01-08 16:59:24

session id: 15workshop1

description: stack from dogpicker [edit](#)

particles: 798

images used: 4

file size: 12.5 MB

path: /gpfs/appion/test/15workshop1/stacks/stack1

stack file: [start.hed](#) [hed](#) [img](#)

box size: 64 pixels

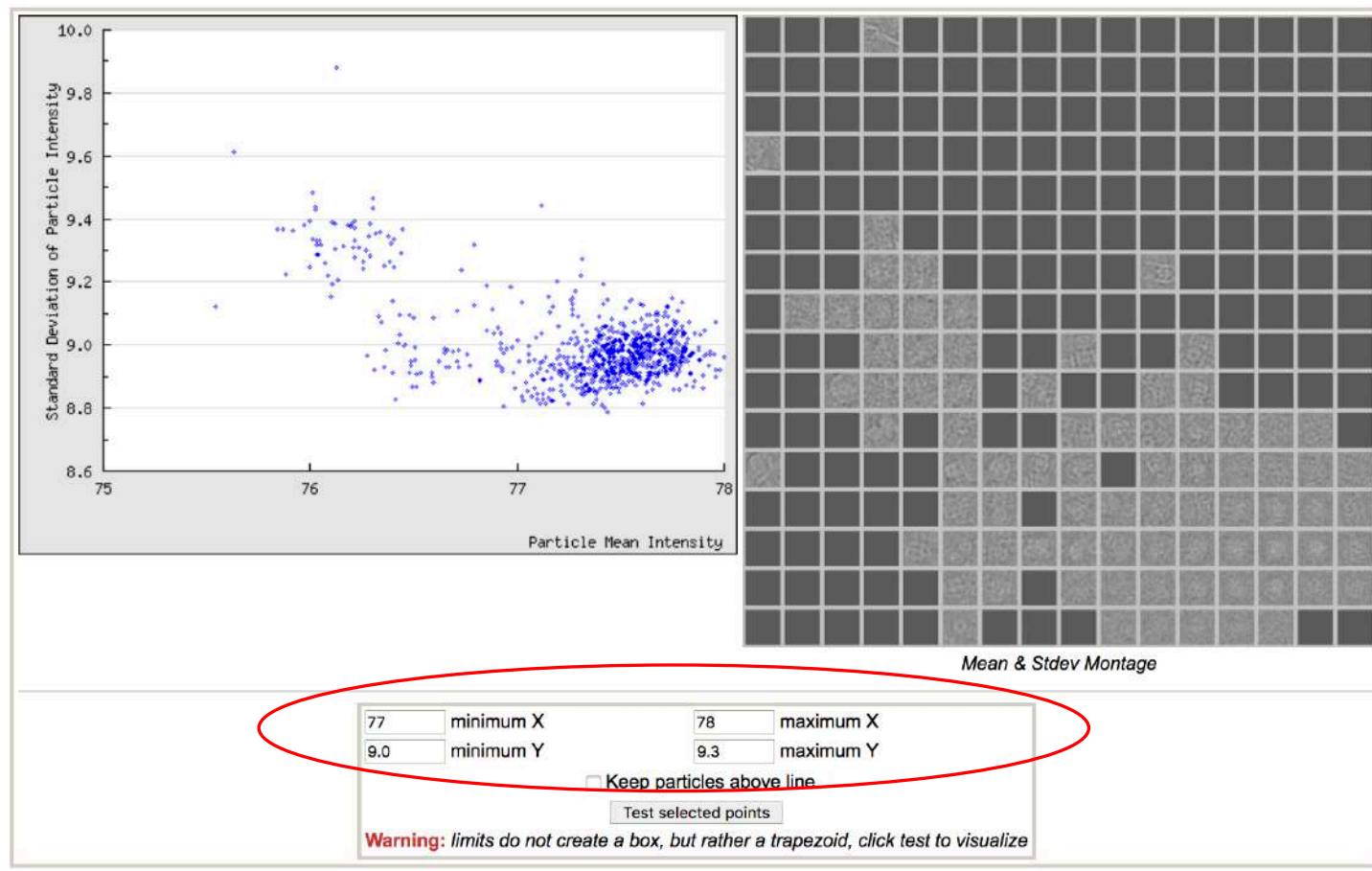
pixel size: 4.41 Å/pixel

ctf correct: yes, ace2imagephase

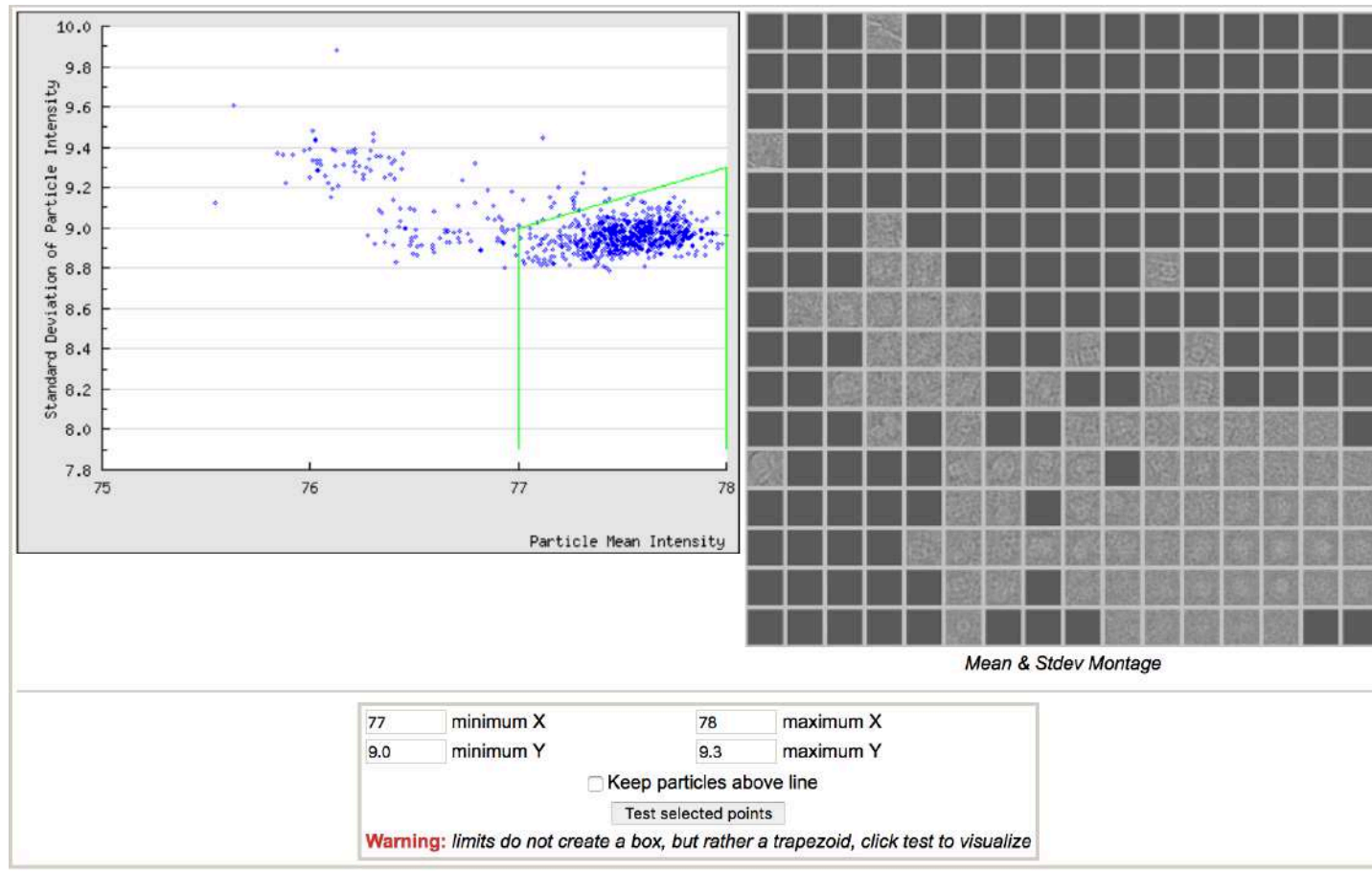
selection run: [ddogrun1](#) (1)

[\[Show original stack summary page\]](#)

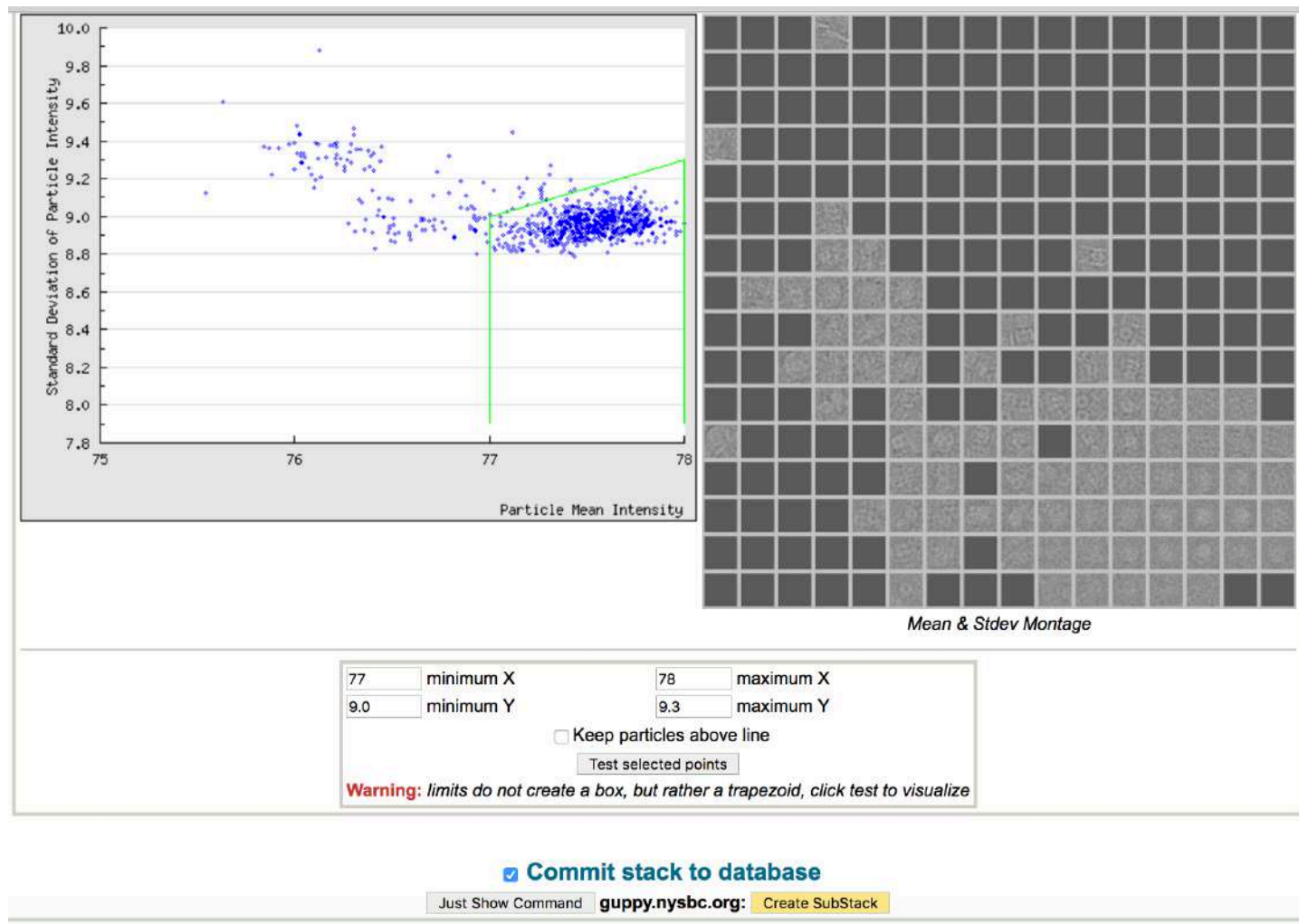
CLEAN UP BY MEAN / SD FILTER



TEST POINTS TO DRAW TRAPEZOID



CREATE SUBSTACK



Hide | Expand | Contract

▼ Object Selection : 2

Select Particle Picker...

2 complete

Repeat an image loop run

▼ CTF Estimation

Estimate the CTF...

4 complete

Transfer results to another preset

Repeat an image loop run

▼ Stacks : 5

Stack creation

7 complete

more stack tools

▼ Particle Alignment

Select Particle Alignment...

4 complete

Run Feature Analysis...

2 complete

Run Particle Clustering...

8 complete

Run MaskItOn

Template Stacks

▼ Ab Initio Reconstruction

OptiMod Common Lines

2 complete

EMAN Common Lines

SIMPLE Common Lines

▼ Refine Reconstruction

Run Single-Model Refinement...

Run Multi-Model Refinement...

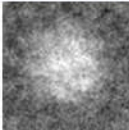
▼ Helical Processing

Helical Image Processing (PHOELIX)

▼ Direct Detector Tools

[Show original stack summary page]

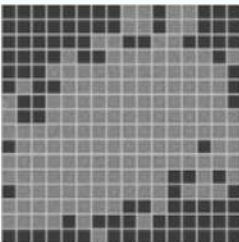
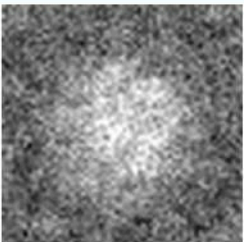
Stack Info: **stack1** (ID: 1) [hide](#) [delete](#) ([download particles data](#)) [list per-particle CTF info](#) [bake recipe](#)



average
[\[Sort Junk\]](#)

date time: 2017-01-08 16:59:24
session id: **15workshop1**
descript: stack from dogpicker [edit](#)
size: 798 / 12.5 MB
path: /gpfs/appion/test/15workshop1/stacks/stack1/start.hed [hed](#) [img](#)
box/apix: 64 pixels / 4.41 Å/pixel

Stack Info: **stack1-meanfilt1** (ID: 2) [hide](#) [delete](#) ([download particles data](#)) [list per-particle CTF info](#)



substack
→

date time: 2017-01-08 17:06:30
session id: **15workshop1**
description: 77 78 9.0 9.3 ... 654 particle substack of stackid 1 [edit](#)
particles: 654 (144 bad)
images used: 4
file size: 10.2 MB
path: /gpfs/appion/test/15workshop1/stacks/meanfilt1
stack file: [start.hed](#) [hed](#) [img](#) ([bad.hed](#))
box size: 64 pixels
pixel size: 4.41 Å/pixel
ctf correct: yes, ace2imagephase
selection run: **ddogrun1** (1)

averaged stack image
[\[Center Particles\]](#)
[\[Learning Stack Cleaner\]](#)
[\[Sort Junk\]](#)
[\[Create Substack\]](#)
[\[Mask Particles with Box\]](#)

mean/stdev montage
[\[Filter by Mean/Stdev\]](#)

[Show original stack summary page]

PARTICLE ALIGNMENT AND CLASSIFICATION

Hide | Expand | Contract

▼ Object Selection : 1

Select Particle Picker...

1 complete

Repeat an image loop run

▼ CTF Estimation

Estimate the CTF...

4 complete

Transfer results to another preset

Repeat an image loop run

▼ Stacks : 2

Stack creation

2 complete

more stack tools

▼ Particle Alignment

Select Particle Alignment...

Template Stacks

▼ Refine Reconstruction

Run Single-Model Refinement...

Run Multi-Model Refinement...

▼ Helical Processing

Helical Image Processing (PHOELIX)

▼ Direct Detector Tools

Select Frame Alignment

Launch Alignment Catchup

Select Per Particle Alignment

Launch Frame Transfer

▼ Import tools

Upload more images

Upload particles

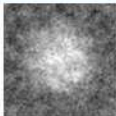
Upload template

Upload CTF

Upload stack

[Show original stack summary page]

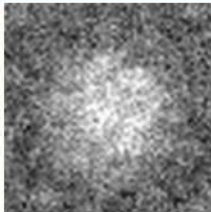
Stack Info: **stack1 (ID: 1)** hide delete (download particles data) list per-particle CTF info bake recipe

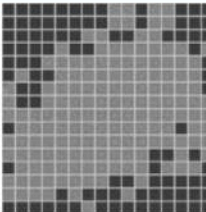
 **average**
[Sort Junk]

date time: 2017-01-08 16:59:24
session id: 15workshop1
descript: stack from dogpicker edit
size: 798 / 12.5 MB
path: /gfps/appion/test/15workshop1/stacks/stack1/start.hed hed img
box/apix: 64 pixels / 4.41 Å/pixel

substack
→

Stack Info: **stack1-meanfilt1 (ID: 2)** hide delete (download particles data) list per-particle CTF info

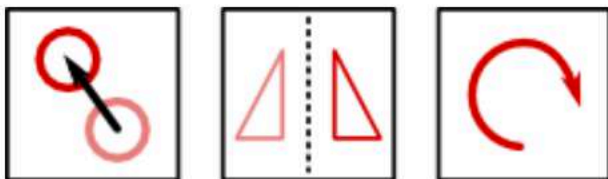
 **averaged stack image**
[Center Particles]
[Learning Stack Cleaner]
[Sort Junk]
[Create Substack]
[Mask Particles with Box]

 **mean/stdev montage**
[Filter by Mean/Stdev]













date time: 2017-01-08 17:06:30
session id: 15workshop1
description: 77 78 9.0 9.3 ... 654 particle substack of stackid 1 edit
particles: 654 (144 bad)
images used: 4
file size: 10.2 MB
path: /gfps/appion/test/15workshop1/stacks/meanfilt1
stack file: start.hed hed (bad.hed)
box size: 64 pixels
pixel size: 4.41 Å/pixel
ctf correct: yes, ace2imagephase
selection run: ddogrun1 (1)

[Show original stack summary page]

Particle Alignment Procedures



Particle alignment consists of shift, rotation, and mirror transformations for all particles in a stack to a common orientation or template. The reference free methods are great methods to create a template for particle picking or reference-based alignments.

 <p>Xmipp Maximum Likelihood Alignment</p> <p>this method is the most robust, but takes some time to complete. It uses the Xmipp ml_align2d program to perform alignments.</p> <p>This method is unbiased and very thorough, but also the slowest of the methods (~days). While it produces excellent templates, it only does a course search (integer pixels shifts and large angle increments), so it is best to use ref-based alignment to get better alignment parameters</p>	 <p>Relion 2D Maximum Likelihood Alignment</p> <p>this method is the most robust, but takes some time to complete. It uses the Relion Refine 2d program to perform alignments.</p> <p>Like Xmipp Maximum Likelihood (from the same author), this method is unbiased and very thorough, but also the slowest of the methods (~days). While it produces excellent templates, it only does a course search (integer pixels shifts and large angle increments), so it is best to use ref-based alignment to get better alignment parameters</p> <p>Note: does not upload alignment parameters to database. Thinks it is Xmipp Max Like run when finished eventhough upload will not work.</p>	 <p>Xmipp Reference Based Maximum Likelihood Alignment</p> <p>similar to reference-free but you select templates first. It uses the Xmipp ml_align2d program to perform alignments.</p> <p>Still untested as to how much bias the reference gives you, but this may be useful in some cases. Also, when no particles align to a particular template, it goes black and unused in further iterations.</p>
 <p>Xmipp 2 Clustering 2D Alignment</p> <p>this method builds a hierarchical classification of particles It uses the Xmipp 2 cl2d program to perform alignments. It is a relatively fast method that aligns and classify the images at the same time. The method starts by estimating a few classes that are further subdivided till the desired number of classes is reached. Every time an image is compared to the class averages it is aligned before-hand. NOTE: in Xmipp 2.4 the alignment parameters are not saved in the database, and therefore this method cannot be used for RCT / OTR reconstructions.</p>	 <p>IMAGIC Multi Reference Alignment (MRA)</p> <p>this method uses the IMAGIC m-r-a command to align your particles to the templates within a specified template stack</p>	 <p>Spider Reference-free Alignment</p> <p>this method uses the Spider AP SR command to align your particles.</p> <p>WARNING: this method is very quick (~few minutes), but also very sloppy and does not always do a great job. The only way to obtain decent results is to run several times and compare the results.</p>
 <p>Xmipp 3 Clustering 2D Alignment</p> <p>this method builds a hierarchical classification of particles It uses the Xmipp 3 cl2d program to perform alignments. It is a relatively fast method that aligns and classify the images at the same time. The method starts by estimating a few classes that are further subdivided till the desired number of classes is reached. Every time an image is compared to the class averages it is aligned before-hand.</p>	 <p>Iterative Stable Alignment and Clustering (ISAC)</p> <p>Initial version. More information about ISAC is available from: sxisac - SPARX</p>	 <p>Ed's Iteration Alignment</p> <p>this method uses the Spider AP SR and the Spider AP SH commands to align your particles through multiple iterations of ref-free and ref-based alignments.</p> <p>WARNING: report all problems to Ed Brignole</p>
 <p>Relion 2.0 GPU-Powered 2D Maximum Likelihood Alignment</p> <p>this method is the most robust, but takes some time to complete. It uses the Relion Refine 2d program to perform alignments.</p> <p>Like Xmipp Maximum Likelihood (from the same author), this method is unbiased and very thorough, but also the slowest of the methods (~days). While it produces excellent templates, it only does a course search (integer pixels shifts and large angle increments), so it is best to use ref-based alignment to get better alignment parameters</p>	 <p>Iterative MSA/MRA</p> <p>Fast & easy 2D ref-free alignment by iterative MSA/MRA. Classification can be performed using a topology-representing network or IMAGIC MSA. Multi-reference alignment can be performed using IMAGIC or EMAN.</p> <p>Spider Reference-based Alignment</p> <p>first you select template and then this method uses the Spider AP MQ command to align your particles to the selected templates. Multiprocessing additions has made this extremely fast (~1 hour).</p> <p>This method is broken on SPIDER version 18 or newer, see bug report</p>	 <p>EMAN Refine 2d Reference-free Alignment</p> <p>Fast and easy 2D ref-free alignment using EMAN's refine2d.py program</p>

INITIAL FAST ALIGNMENT BY MAXIMUM LIKELIHOOD

- Binned Stack
- Few References (classes)
- Decent No. Processors
- Monitor time estimate
- If too long
 - Bin more
 - More CPU's
 - Fewer Classes

The screenshot shows the GUPPY Max Like Alignment web interface. Several fields are circled in red:

- MaxLike Run Name:** maxlike1
- Output Directory:** /gpts/appion/test/15workshop1/align/
- Limiting numbers:**
 - Unbinned Clip diameter (pixels): 64
 - Particle binning: 1
 - Number of Particles: 654
- Filters:**
 - Low Pass Filter Radius (Angstroms): 10
 - High Pass Filter Radius (Angstroms): 2000
- Job parameters:**
 - Number of References: 8
 - Angular Increment: 5
 - Maximum number of iterations: 15
 - ☒ Use Mirrors in Alignment
 - ☒ Save memory by checking less shifts
 - ☒ Fast Mode Setting
- Search space reduction criteria:** Normal search
- Convergence stopping criteria:** Normal search
- Noise distribution type:** ☒ Gaussian ☐ Student's T
- Time estimate for first iteration:** 2.06 minutes
- Buttons:** Just Show Command, guppy.nysbc.org, Run Max Like Alignment

OOPS, NEED TO ADD DESCRIPTION

ERROR: Enter a brief description of the particles to be aligned

MaxLike Run Name: maxlike1

Output Directory:
/gpfs/appion/test/15workshop/align/

Description of Max Like Alignment:
maxlike align 8 ref

Select a stack of particles to use
stack1-meanfilt1 ID: 2 (654 particles, 4.41 Å/pix, 64x64)

☒ **Commit to Database**

4 **Number of Processors**

Limiting numbers
64 **Unbinned Clip diameter (pixels)**
1 **Particle binning**
654 **Number of Particles**

Filters
10 **Low Pass Filter Radius (Angstroms)**
2000 **High Pass Filter Radius (Angstroms)**

Job parameters
8 **Number of References**
5 **Angular Increment**
15 **Maximum number of iterations**
☒ **Use Mirrors in Alignment**
☒ **Save memory by checking less shifts**
☒ **Fast Mode Setting**
Search space reduction criteria
Normal search
Convergence stopping criteria
Normal search
Noise distribution type:
☒ **Gaussian** ☐ **Student's T**

Time estimate for first iteration: 2.06 minutes

Just Show Command guppy.nysbc.org Run Max Like Alignment

DISPLAY ALIGNED STACKS

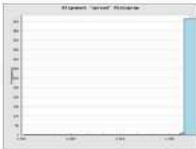

Hide | Expand | Contract

- ▼ Object Selection : 1
 - Select Particle Picker...
 - 1 complete
 - Repeat an image loop run
- ▼ CTF Estimation
 - Estimate the CTF...
 - 4 complete
 - Transfer results to another preset
 - Repeat an image loop run
- ▼ Stacks : 2
 - Stack creation
 - 2 complete
 - more stack tools
- ▼ Particle Alignment
 - Select Particle Alignment...
 - 1 complete
 - Run Feature Analysis...
 - Run Particle Clustering...
 - Run MaskItOn
 - Template Stacks
- ▼ Ab Initio Reconstruction
 - EMAN Common Lines
 - SIMPLE Common Lines
- ▼ Refine Reconstruction
 - Run Single-Model Refinement...
 - Run Multi-Model Refinement...
- ▼ Helical Processing

Alignment Stack List

Show Composite Page

Align Stack Info: maxlike1 (ID: 1) hide delete



avg image spread distribution

[mask particles with box]

date time: 2017-01-08 17:27:59

description: maxlike align 8 ref edit

size: 654 particles (10.2 MB)

original stack: 2

of classes: 8

reference stack: [part17jan08r24_average.hed](#)

pixel / box size: 4.41 Å/pixel and 64 pixels

stack file: /gpfs/appion/test/15workshop1/align/maxlike1/alignstack.hed

Run Feature Analysis On Align Stack Id 1

Show Composite Page

EXAMINE CLASSES

stack: /gpfs/appion/test/15workshopI/align/maxlike1/part17jan08r24_average.hed

#images: 8

from: 0 to: 7 binning: 1 quality: jpeg 50 info: ☒ scale bar: ☐ Load

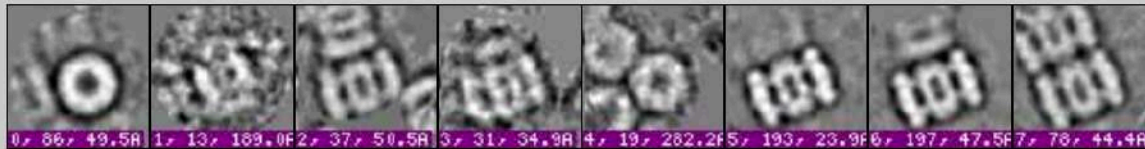
Selection mode: exclude

Selected images:

Select: View Raw Particles View Aligned Particles Create SubStack Create Templates Include Particles Create Template Stack Run Common Lines

Excluded images:

Exclude: Create SubStack



Note: With this particle, we basically have only 2 classes: a top view and a side view

GO TO SELECT MODE

stack: /gpfs/appion/test/15workshopI/align/maxlike1/part17jan08r24_average.hed

#images: 8

from: 0 to: 7 binning: 1 quality: jpeg 50 info: ☒ scale bar: ☐ Load

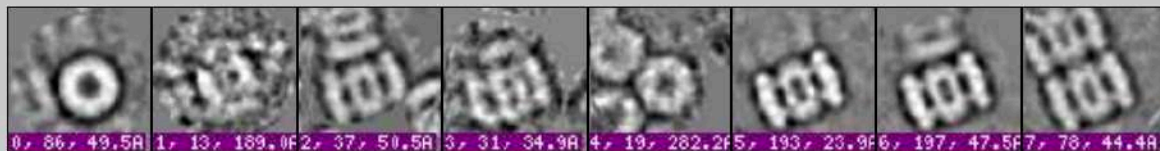
Selection mode: select

Selected images:

Select: View Raw Particles View Aligned Particles Create SubStack Create Templates Include Particles Create Template Stack Run Common Lines

Excluded images:

Exclude: Create SubStack



PICK CLASSES FOR TEMPLATES

stack: /gpfs/appion/test/15workshopI/align/maxlike1/part17jan08r24_average.hed

#images: 8

from: 0 to: 7 binning: 1 quality: jpeg 50 info: ☒ scale bar: ☐ Load

Selection mode: select

Selected images: 0,5

Select: View Raw Particles View Aligned Particles Create SubStack Create Templates Include Particles Create Template Stack Run Common Lines

Excluded images: 1,2,3,4,6,7

Exclude: Create SubStack





Templates: Used for template-based picking

Template Stack: Used for ab initio reconstruction

Substack: Make a new stack using only particles from good classes

UPLOAD TEMPLATES

Align Stack Info: maxlike1 (ID: 1) [hide](#) [delete](#)



date time: 2017-01-08 17:27:59

description: maxlike align 8 ref [edit](#)

size: 654 particles (10.2 MB)

original stack: [2](#)

of classes: 8

reference stack: [part17jan08r24_average.hed](#)

pixel / box size: 4.41 Å/pixel and 64 pixels

stack file: [/gpfs/appion/test/15workshop/align/maxlike1/alignstack.hed](#)

avg image spread distribution

[\[mask particles with box\]](#)

Selected Image Numbers: 0,5

Output directory:

Template Description:

Particle Diameter:
 (In Angstroms)

☒ **Commit to Database**

[Just Show Command](#) [guppy.nysbc.org](#) [Upload Template](#)

PARTICLE PICKING: TEMPLATE PICKER

FndEM
A Template Correlator

Run name
tmplrun4

Output directory
/gpfs/appion/test/15workshop1/extract

Preset: upload
☐ Wait for more images after finishing

Max number of images to process

Images to process:
☒ Do not process hidden or rejected images
☐ All images independent of status
☐ Exemplar and keep images only

Image order:
☒ Forward
☐ Reverse
☐ Shuffle

Continuation:
☒ Continue unfinished run (default)
☐ Reprocess all images

☒ Commit results to database

☒ Use multi-processor threading
☐ Do not delete .dwn.mrc files after finishing

Mask Diameter:
143 Mask diameter for template(s) (in Angstroms)
☐ Use template mirrors

Peak thresholds:
0.5 Minimum threshold
Maximum threshold
1500 Max number of particles allowed per image

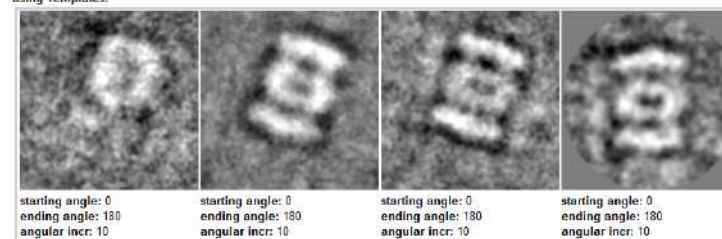
Filter Values:
15 Low Pass (in Angstroms; 0 = off)
0 High Pass (in Angstroms; 0 = off)
2 Median (in pixels; 0 = off)
4.0 Pixel Limit (in Standard Deviations; 0 = off)
4 Binning (power of 2)
☒ Plane regression

Defocal pairs:
☐ Calculate shifts for defocal pairs

Advanced options:
1.0 Maximum peak area multiple
1.5 Minimum peak overlap distance multiple

Peak extraction type
☒ Center of mass (default)
☐ Maximum position
☐ Pick only doubles
☐ Invert image density

Using Templates:



FndEM If you find this software useful please cite:
"FndEM—a fast, efficient program for automatic selection of particles from electron micrographs." (2004) Roseman AM. J Struct Biol. v145(1-2). PMID: 15065677
A Template Correlator

☐ Test these settings on image: mrc file name

Just Show Command SEMC-head shortq Run Correlator

MAKE LARGER STACK FROM TEMPLATES

The screenshot shows a software interface for creating a stack from templates. Several settings are highlighted with red circles:

- Run name:** stack4
- Output directory:** /gifs/apion/test/15workshop/stacks
- Description:** template picked bin 2
- Particles:** tmprun1 (3,881 parts)
- Particle labels:** templ1
- Stacks:** None
- Box Size (Unbinned, in pixels):** 216
- Binning:** 2
- Preset:** upload
- Wait for more images after finishing:** ☐
- Hide Advanced_Loop_Options:** ☐ Max number of images to process
- Images to process:**
 - ☐ Do not process hidden or rejected images
 - ☐ All images independent of status
 - ☒ Exemplar and keep images only
- Image order:**
 - ☒ Forward
 - ☐ Reverse
 - ☐ Shuffle
- Continuation:**
 - ☒ Continue unfinished run (default)
 - ☐ Reprocess all images
- Commit results to database:** ☒
- Output file format:** IMAGIC
- Density modifications:**
 - ☒ Invert image density
- Show Advanced_Stack_Options_1:** ☐
- CTF Correct Particle Images:** ☒
- CTF Confidence Cutoff:** ☐ Use Values Above: 0.8 (between 0.0 - 1.0)
- Hide Advanced_Ctf_Options:** ☐
- CTF Correction Method:** Ace 2 PhaseFlip Whole Image
- CTF Sorting Method:** Resolution 0.8 criteria
- OR- choose a ctf run:** all (0)
- OR- Only use CTFIND values:** ☐
- CTF Resolution Cutoff:** ☒ Resolution range at 0.8 criteria: between 2 and 6 (in Angstroms)
- Resolution range at 0.5 or software package criteria:** between 2 and 6 (in Angstroms)
- Defocus Limits:** Min -1.2e-6 & Max -2.83e-6

OVERRIDE SIZE IF NECESSARY

ERROR: Bad prime number in boxsize, try using 192 or 224 instead or check 'override box size' to force

Run name
stack4

Output directory
/gpfs/appion/test/15workshop1/stacks

Description
"template picked bin 2"

Preset: upload
☐ Wait for more images after finishing

[Show Advanced Options](#)

Output file format: IMAGIC

Density modifications:
☒ Invert image density
[Show Advanced Stack Options](#)

Particles: tmplrun1 (3,881 parts)
☐ Particle labels: templ1

Stacks: None

216 **Box Size** (Unbinned, in pixels)
☒ (override boxsize)

2 **Binning**

0 **Pixel Limit** (in Standard Deviations; 0 = off)

☒ Fast Insert

☒ Ctf Correct Particle Images

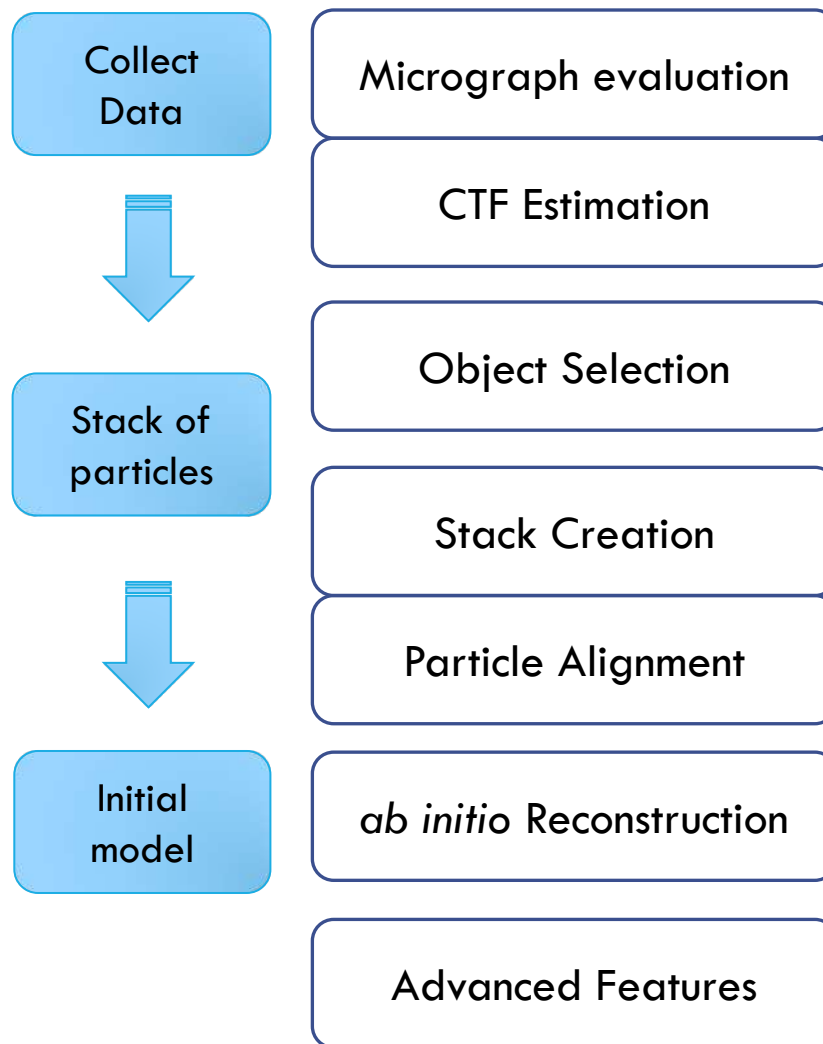
☐ CTF Confidence Cutoff
Use Values Above: 0.8 (between 0.0 - 1.0)
[Show Advanced CTF Options](#)

[Show Advanced Stack Options](#)

[Just Show Command](#) [guppy.nysbc.org](#) [Make Stack](#)

$216/2=108$
 $108/2=54$
 $54/2=27$
 $27=3*3*3$

SCHEDULE



SCHEDULE

JULY 29-30, 2020 2PM-4PM

PRACTICAL WORKSHOP WHERE STUDENTS WILL WORK IN PAIRS ON A TEST DATASET. AFTER EACH LECTURER GIVES AN OVERVIEW OF THE TOPIC THE ATTENDEES WILL HAVE HANDS-ON TIME TO USE THE INTERFACE.

1. Workshop intro

- Overview of Leginon/Appion
- Krios workflow
- Leginon targeter

2. Micrograph evaluation / CTF Estimation

3. Object Selection

4. Stack creation / Particle Alignment

[3 (cont). Object Selection with templates after initial class averages]

5. Other features: Pro tips / Direct Detector Tools / Import/Export Tools

6. Closing