

NEW YORK STRUCTURAL BIOLOGY CENTER



ANNUAL USER MEETING 2018

September 20, 2018

SIMONS ELECTRON MICROSCOPY CENTER



GENERAL USER MEETING

SCHEDULE

- Update on instrumentation, facility, access and policies.
- General discussion of user needs
- Picnic @ 3pm

ANUAL RE-CERTIFICATION

USER ACCOUNT RENEWAL

<https://www.surveymonkey.com/r/SEMC-user-certification>

On-line account renewal.

Users will confirm status, acknowledge basic rules and SOPs.

Should have completed surveys by Sept 20.

Will deactivate accounts starting in mid-October.

SAFETY

<http://semc.nysbc.org/sops/>

STANDARD OPERATING PROCEDURES

General SOPs

Each instrument has an SOP

ACCOUNTS AND IDENTIFICATION

User accounts

NYSBC ID

24 hr access

SEMC OPERATIONS TEAM

2018

Directors



Bridget



Clint

 @nysbc.org

Staff



Bill



Ed



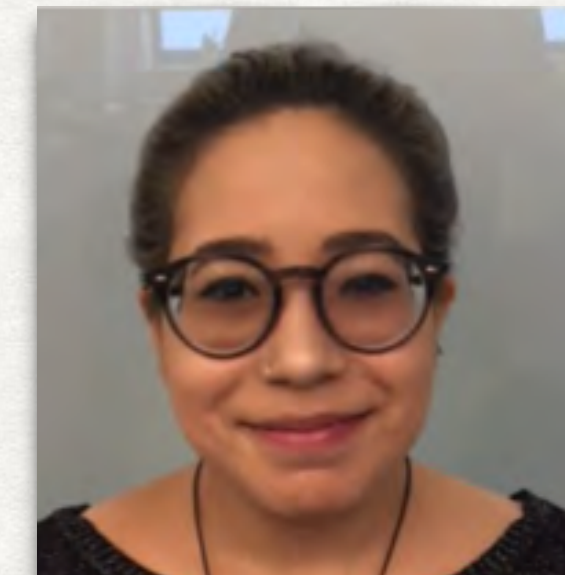
Laura



Misha



Ashleigh



Daija



Carolina



Renee

INSTRUMENTATION

2018 DATA GENERATION AT SEMC

EMs available

FEI Titan Krios#1 / #2 / #3

Falcon3 x3

K2 x3

FEI Tecnai F20

DE20

TVIPS 4K CMOS

FEI Tecnai Biotwin

TVIPS 4K CMOS

JEOL 1230

Gatan US4000 CCD

FEI Helios 650

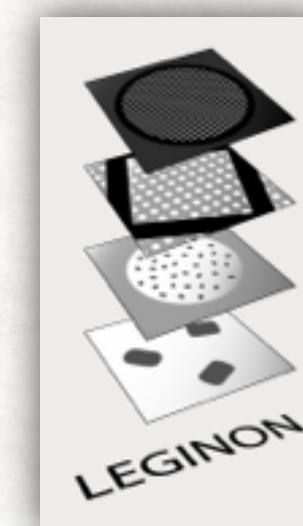
Quorum cryostage



Cameras

7 direct detectors on 4
TEMs

4 CMOS/CCDs on 3
TEMs +
1 SEM



LEGINON & APPION USE

Central MySQL Database and web server

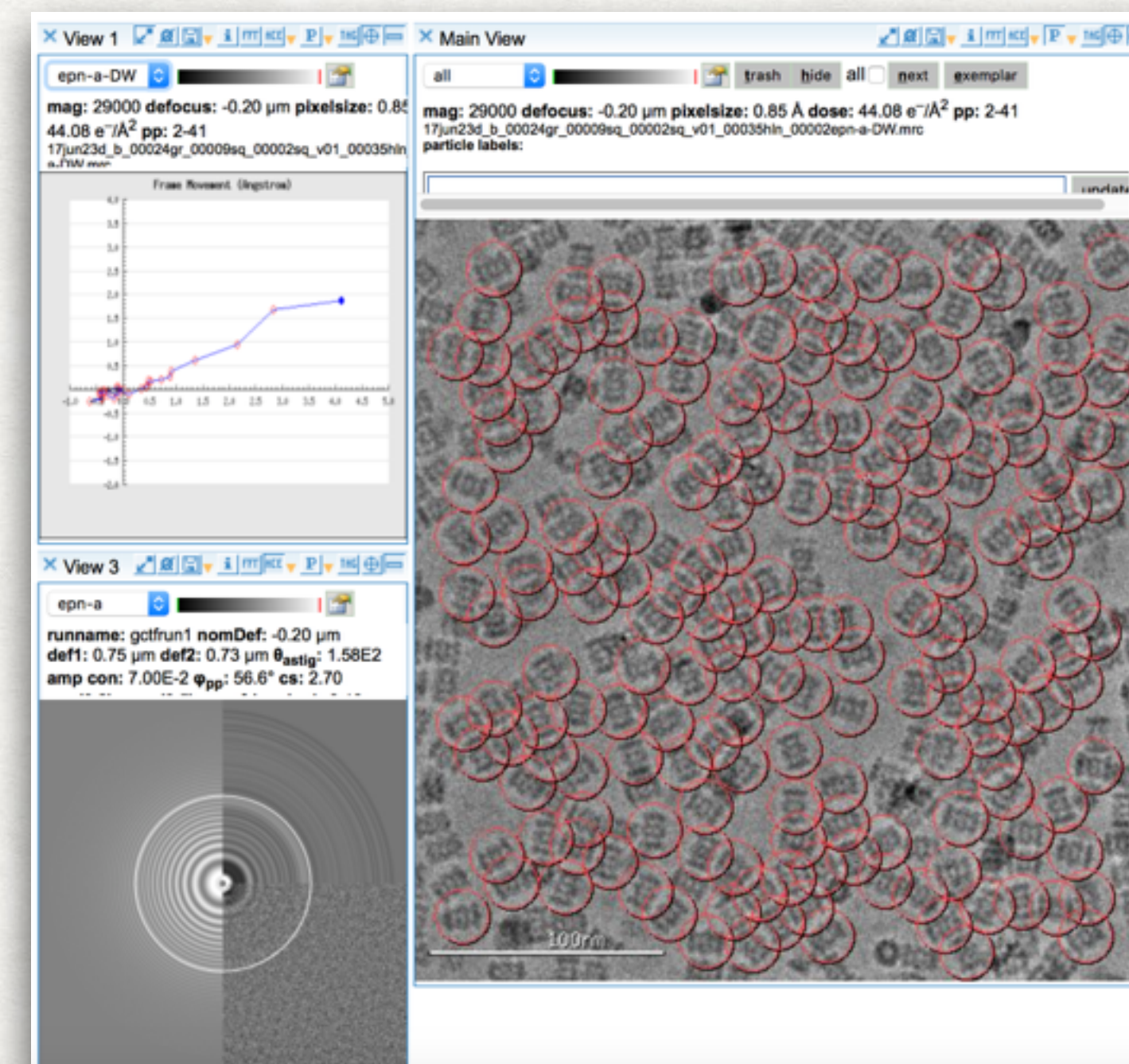
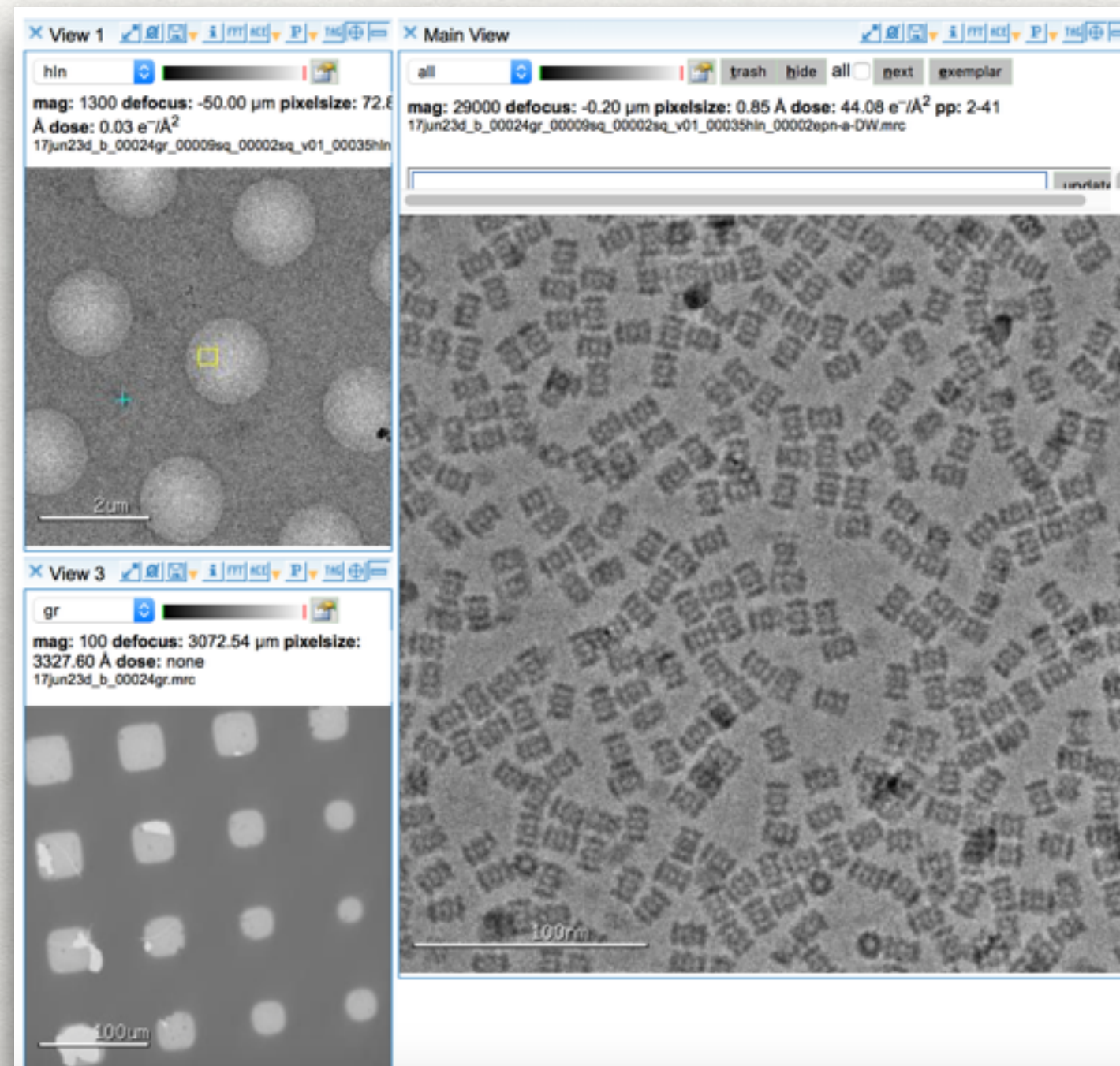
Size of images: 393.51 TB

DB records: 1,425,659,999

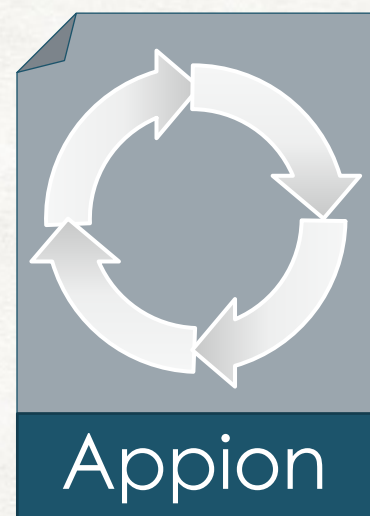
Size of database: 14.34 GB



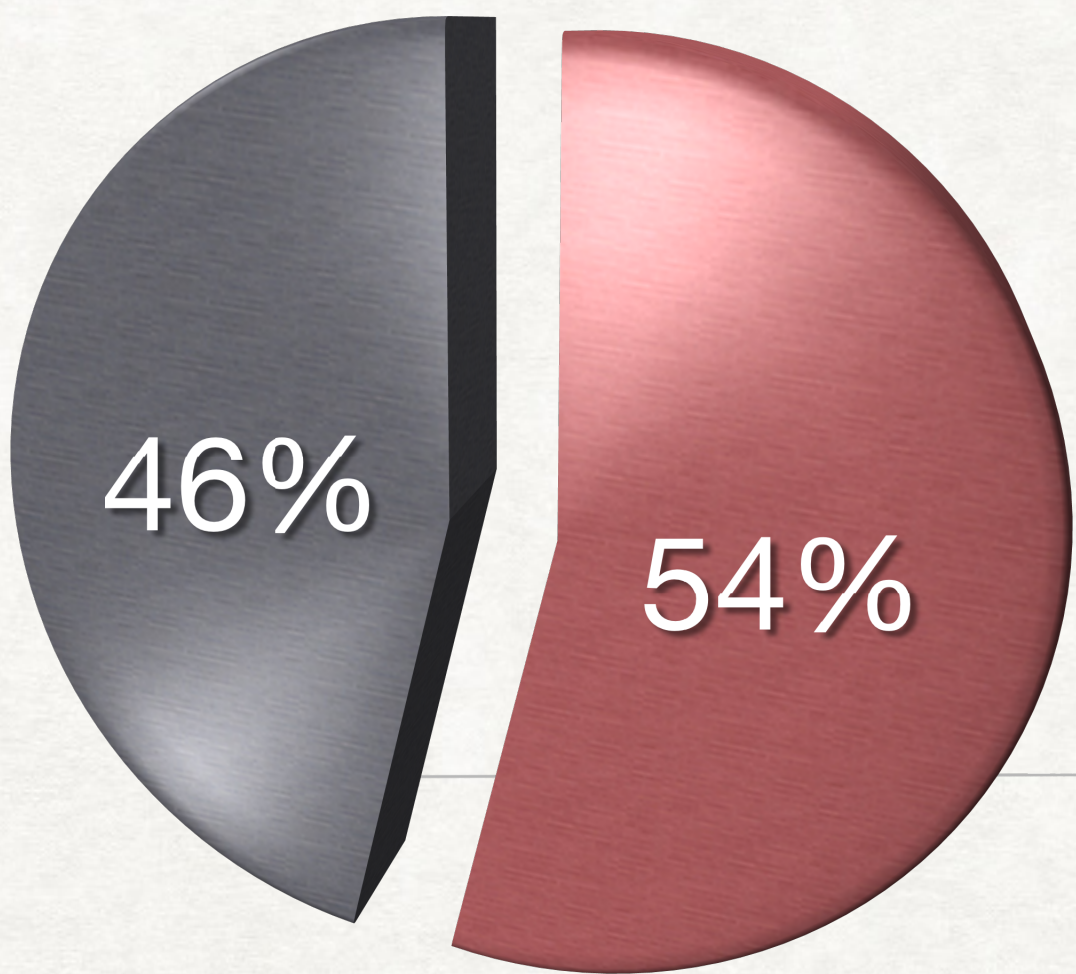
*3/4 users who use Leginon
also have Appion sessions*



LEGINON & APPION USE



Other scopes &
CMOS/CCD



Krios &
DD cameras

TEM Exposure images in
2015 & 2016:

1,069,315

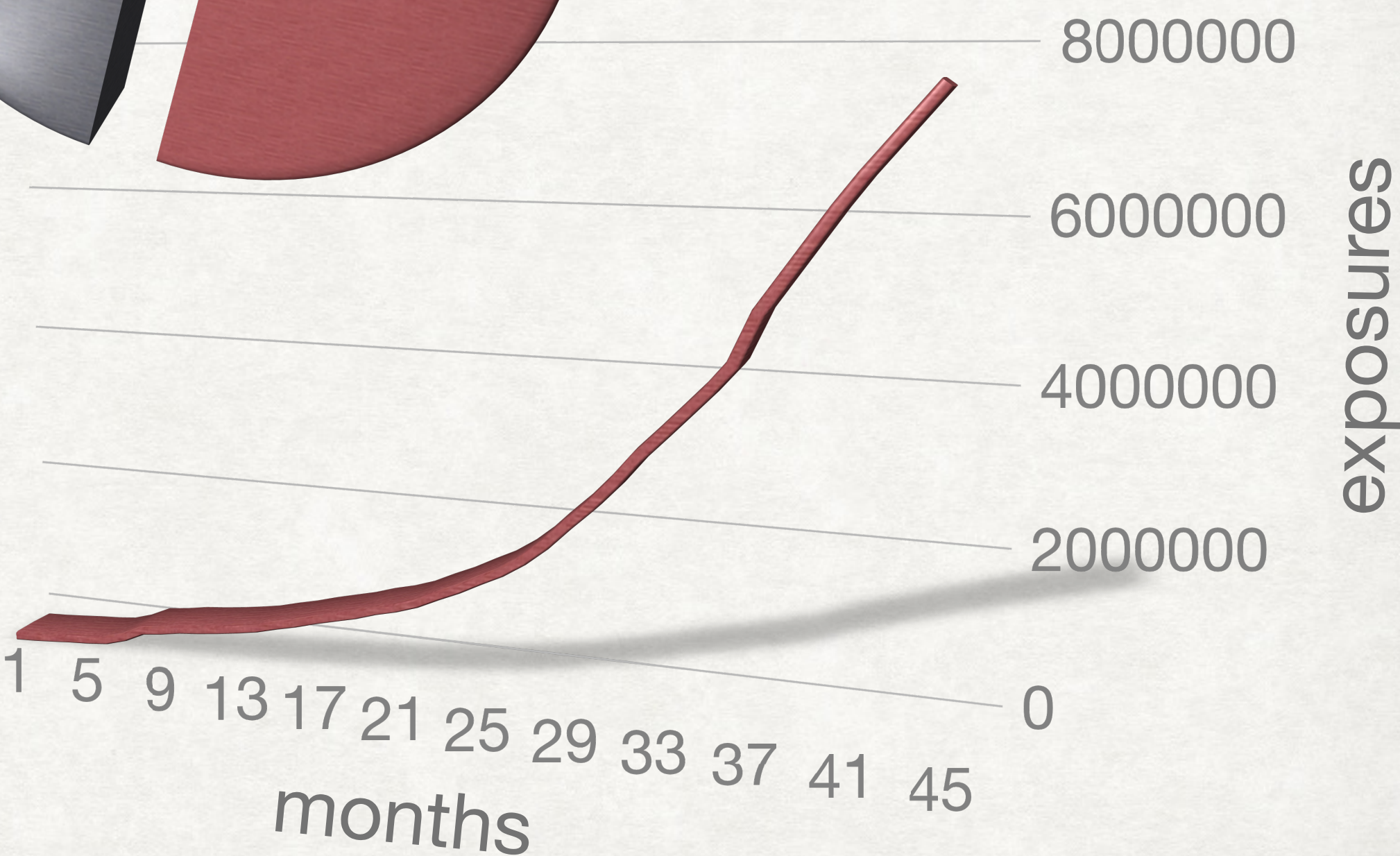
#TEM Exposure images
in 2017:

2,954,507

TEM Exposure images:

7,559,093*

353,5271 high mag images Jan-today 2018



*Total number of saved images since 2015: 1,425,659,999

LEGINON & APPION USE



Backup is the user's responsibility.

Leginon database older than 1 yr will be converted from MRC to JPEG.

Also, MRCs will be deleted.

COMPUTING: BUG REPORTING

APPION.ORG

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

SEMC Appion development team
Nullam subire

Appion

Search: Appion

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

Issues

Filters

☒ Status Add filter

☒ Tracker

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	
<input type="checkbox"/>	3765	Appion	Bug	In Test	Normal	commit doesn't uncheck when testing image in dogpicker	Dmitry Lyumkis	Sargis Dallakyan
<input type="checkbox"/>	3752	Appion	Bug	Assigned	Normal	Generating missing virtual substack from virtual substack gives header nz is None error	William Rice	William Rice
<input type="checkbox"/>	3422	Appion	Bug	In Test	Urgent	Fix magnification in Appion generated FREALIGN par file	Melody Campbell	Melody Campbell
<input type="checkbox"/>	3758	Appion	Bug	In Code Review	Normal	uploaded signle direction tilt series causes error in	Anchi Cheng	Alex Noble

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



Swapnil
Bahktar



Shaker
Krit



Anchi Cheng



Sargis Dallakyan

INSTRUMENTATION

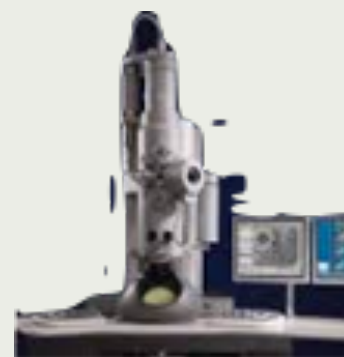
Open request EMs

120 kV



JEOL 1230
+
Gatan US4000
4K CCD

120 kV



FEI Tecnai 12
+
TVIPS F416
4K CMOS

200 kV
FEG



FEI Tecnai F20
+
TVIPS F416
4K CMOS
Direct Electron
DE-20
Direct Detector

Request pending staff availability

30 kV
FEG



FEI Helios 650
+
4K ETD, TLD, ICE

Application required to use

300 kV
FEG



FEI Titan
Krios1
+
FEI
Falcon 3
Direct Detector

Gatan
K2 Summit
Direct Detector

300 kV
FEG



FEI Titan
Krios2
+
FEI
Falcon 3
Direct Detector

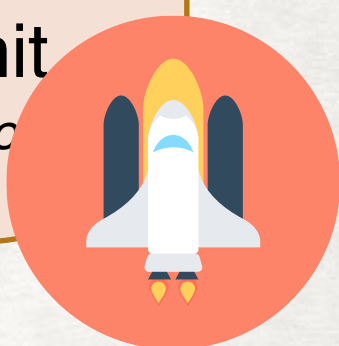
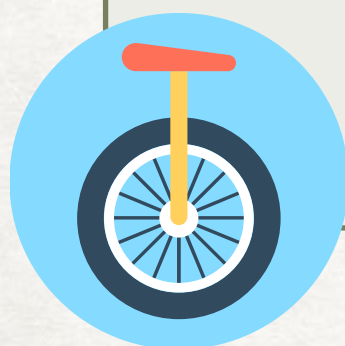
Gatan
K2 Summit
Direct Detector

300 kV
FEG



FEI Titan
Krios3
+
FEI
Falcon 3
Direct Detector

Gatan
K2 Summit
Direct Detector



Minimal staff assistance
Independent operation training program

Full staff assistance
Training program focused on data collection

CANCELLATION POLICY

Waitlist:

You may request to be put on the waitlist to access unused time or last minute cancellations. However, you must be ready at a moment's notice (less than 24 h notice).

Consecutive Sessions:

You may not request 3 consecutive sessions during weekday-daytime (9am-5pm) hours.

No Shows:

If you find for any reason, that you will not use a session you have booked – YOU MUST CANCEL 36 HOURS IN ADVANCE or Friday morning if you are cancelling your Monday session. Otherwise your institutions will still be counted as using that session.

Lateness:

If you are more than 30 minutes late, you will forfeit your session, if others wish to use it. If you know you will be late in advance notify the SEMC Staff 24 hours in advance.

KRIOS USAGE : 2.5 INSTRUMENTS

Krios01



Regular operations: VPP, extended image shift
FalconIII or K2 option

Krios02



Regular operations: VPP, Cs corrector, Energy Filter
FalconIII or K2 option

Krios03



Regular operations: VPP, Energy Filter, extended image shift
FalconIII or K2 option
-Time split between NYSBC & Columbia

KRIOS USAGE : STANDARD SETTINGS

Single particle set up

K2 Summit in counting mode:

Pixel size: $\sim 1.1 \text{ \AA}/\text{px}$

Dose: $8 \text{ e}^-/\text{px}/\text{s}$

Frame rate: 200-250 ms

Exposure time: 8-10 sec

MotionCor2

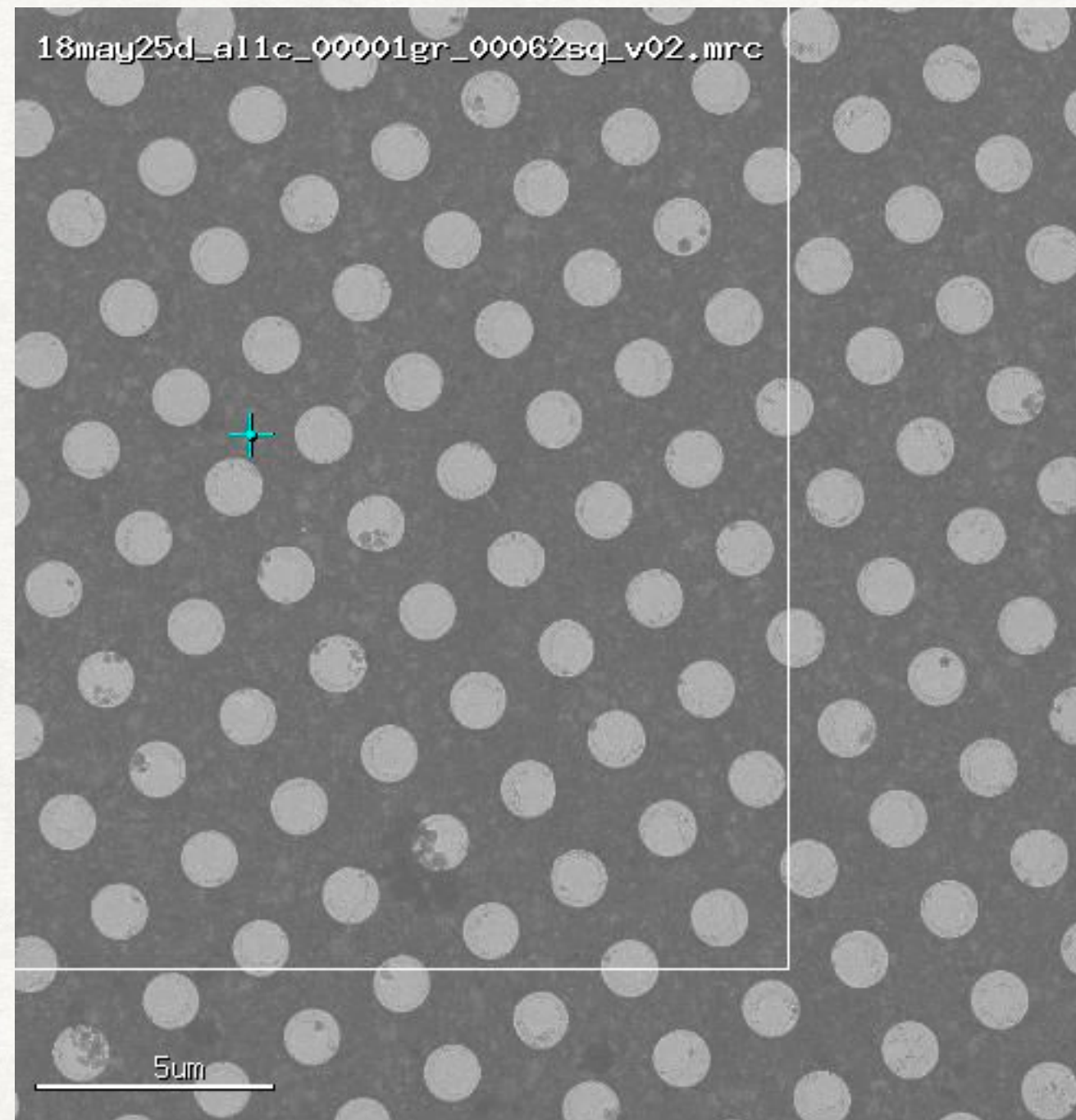
CTFFIND4

Ice thickness



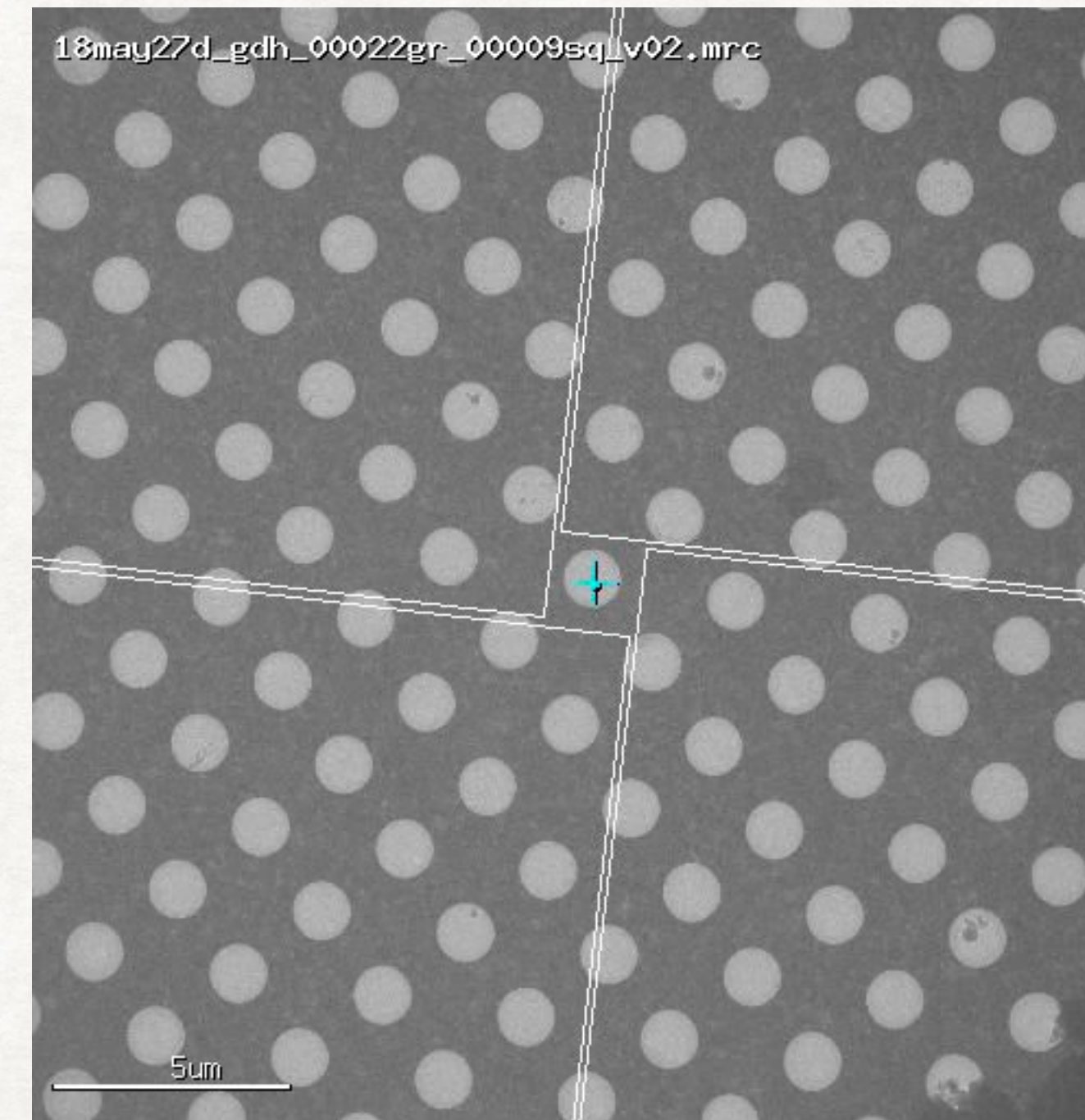
KRIOS USAGE : EXTENDED IMAGE SHIFT

Krios01



Single hl target 400x per sq

Hole targeting can be used to re-center the square or to avoid obviously bad areas

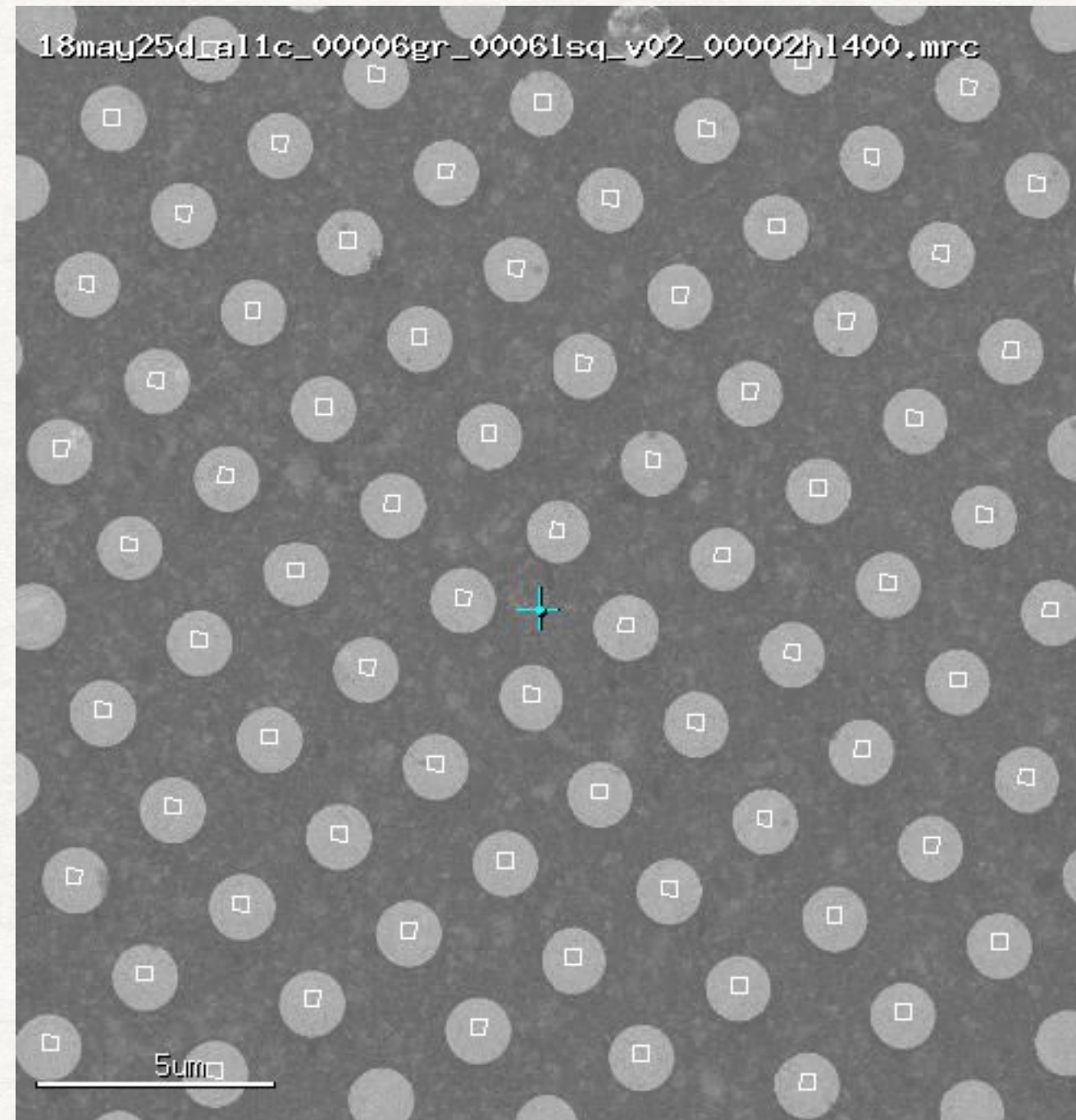


Up to four hl 570x targets per sq

Hole targeting can be used to choose preferred areas and extend the sq coverage

KRIOS USAGE : EXTENDED IMAGE SHIFT

Krios01

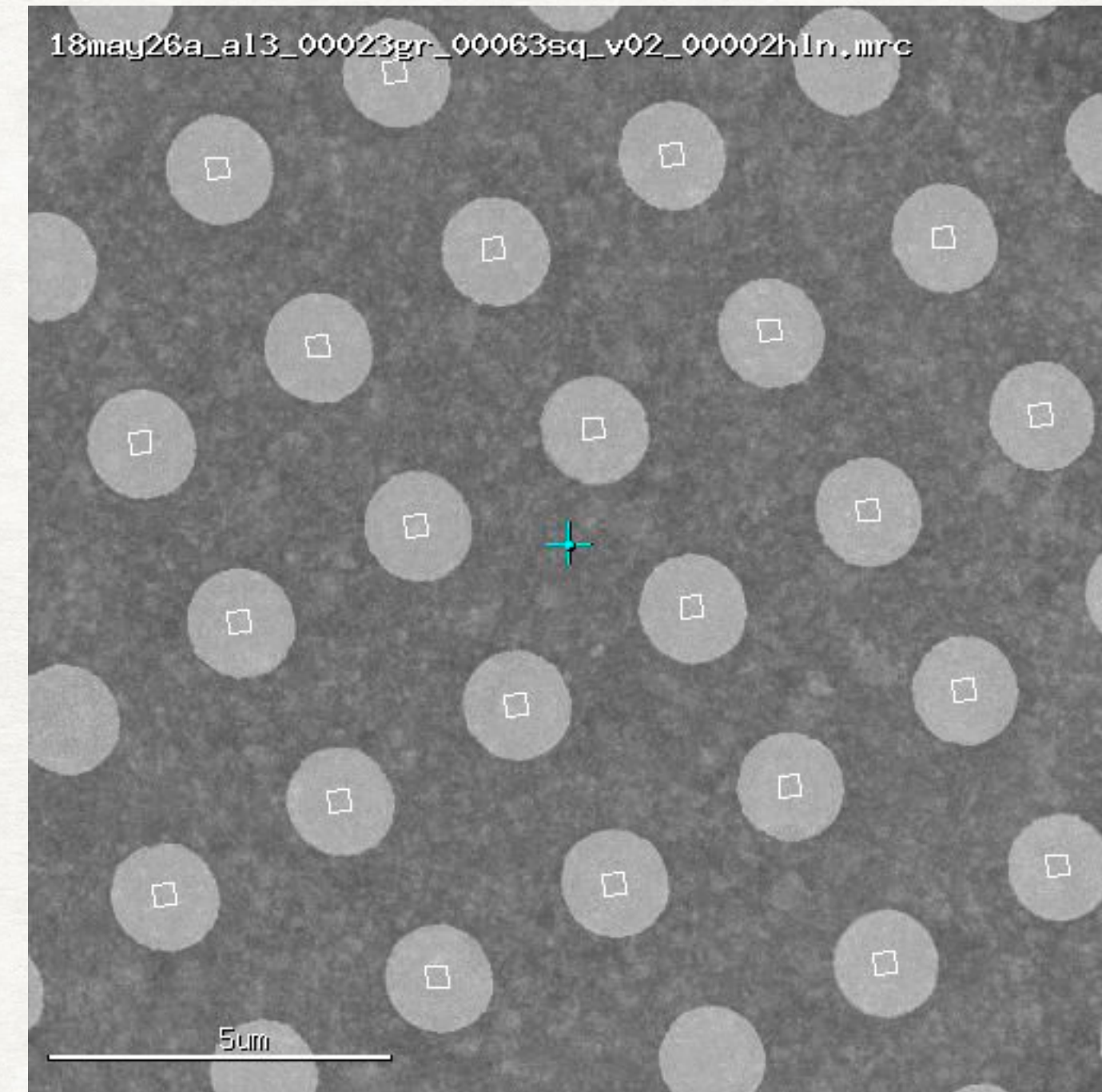


70 avg targets per hole image

Speed_{enn} = **4200/day**

Speed_{hl} = 60.5/day

← 60 squares (1hl per 1 sq)!



22 avg targets per hole image

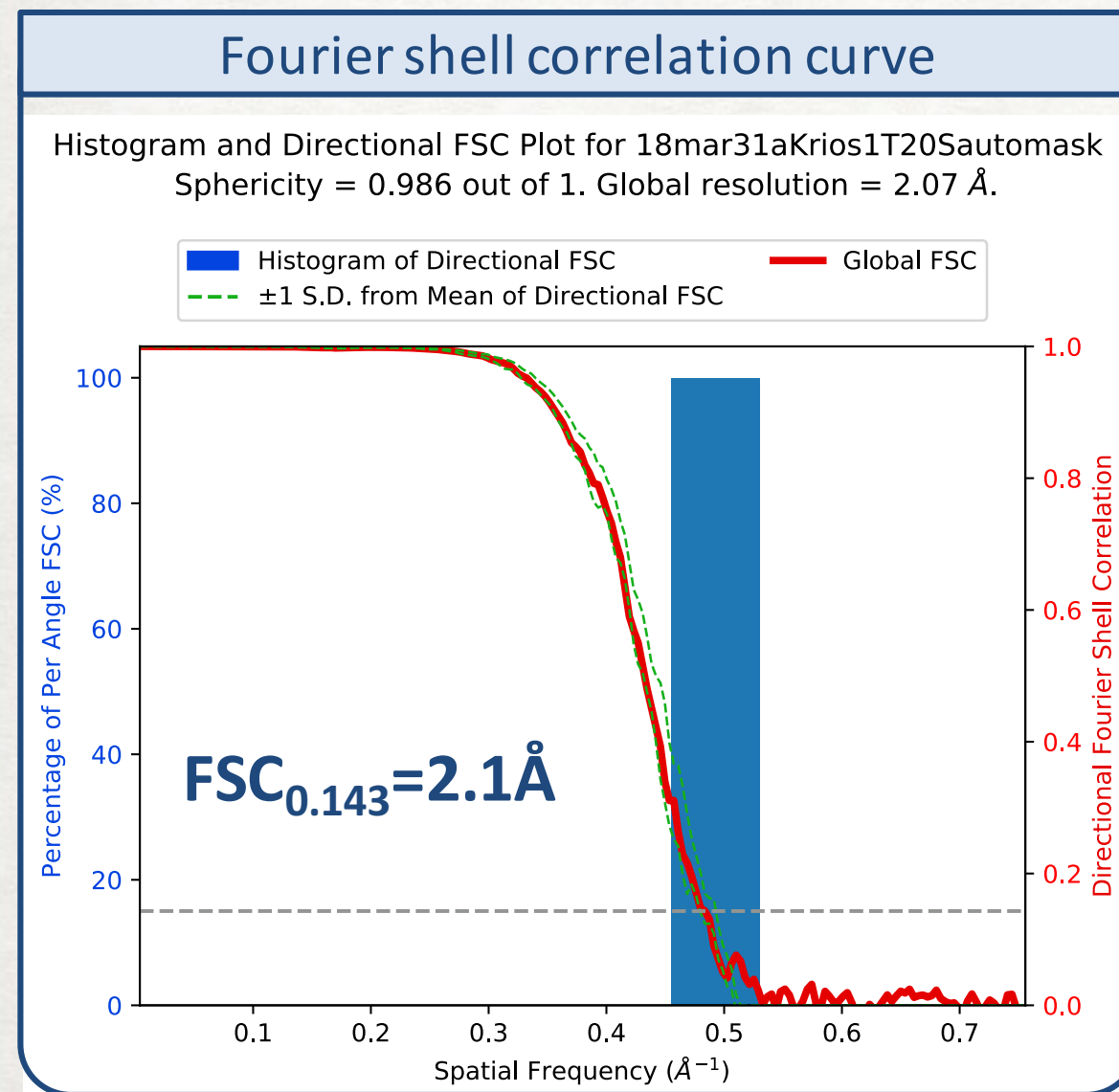
Speed_{enn} = **3500/day**

Speed_{hl} = 158/day

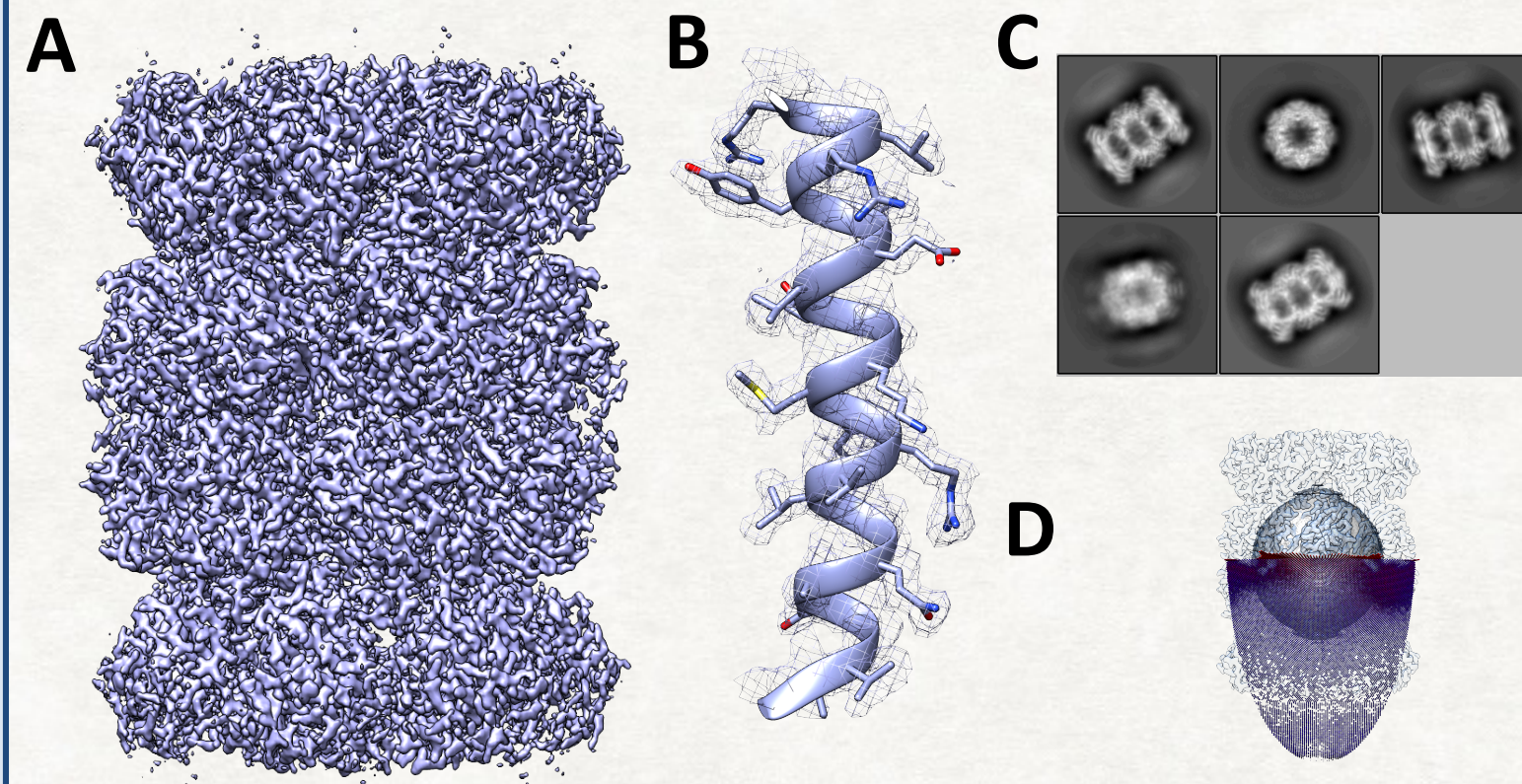
← ~40 squares (4hl per 1 sq)!

KRIOS TESTING

Workflow validation/testing

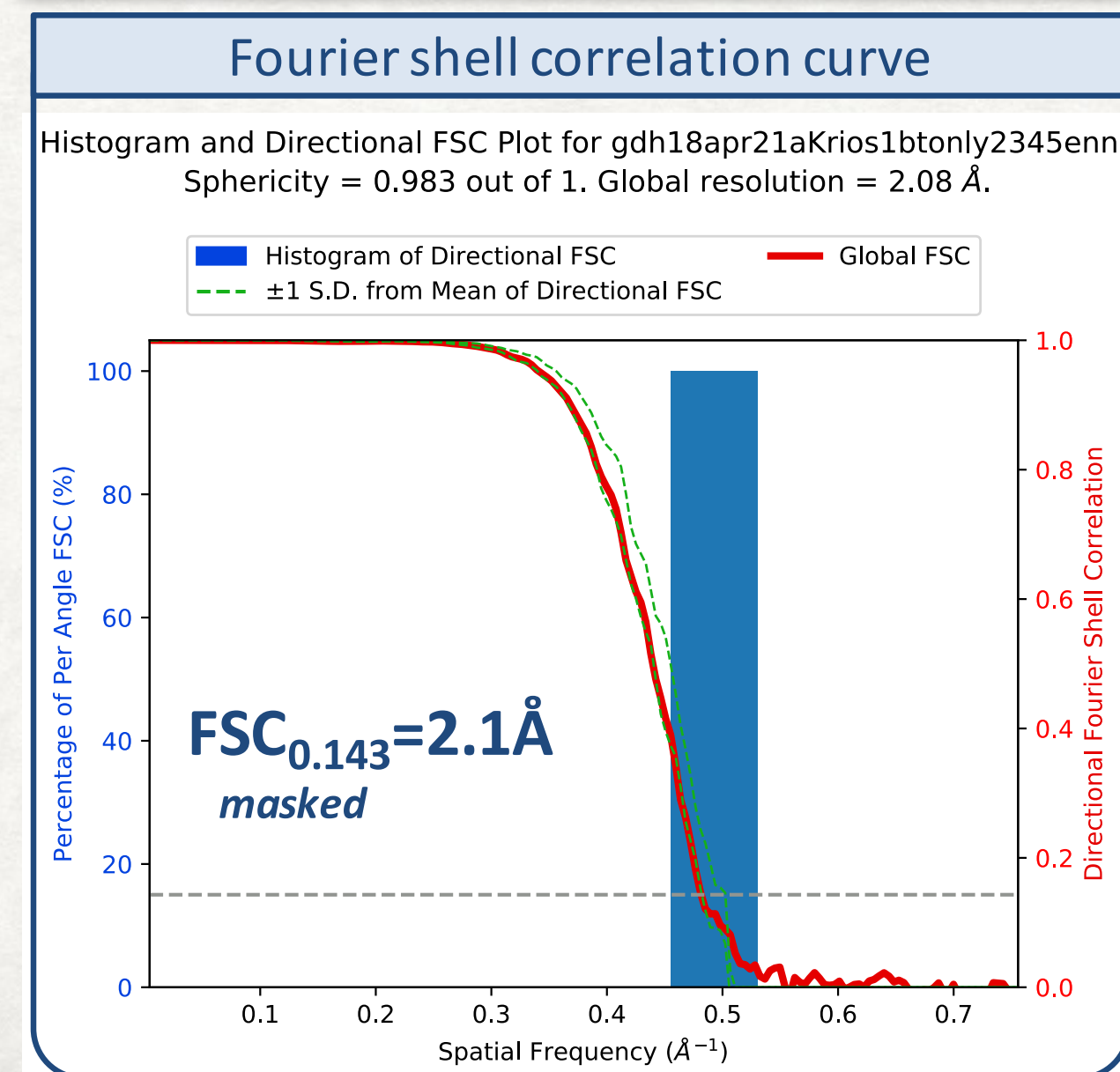


CryoEM reconstruction of the T20S

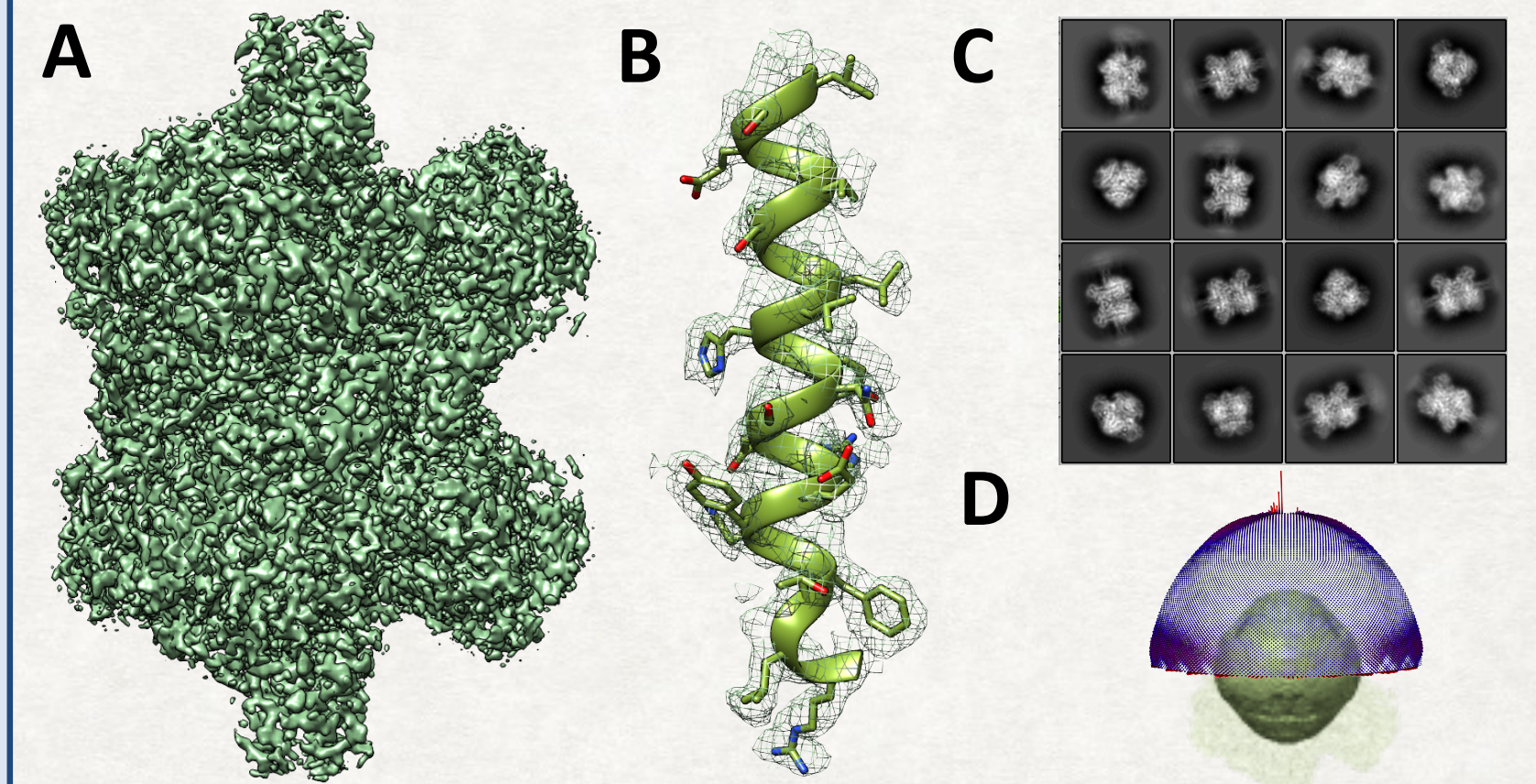


(A) Isosurface representation of the T20S map. (B) An α -helical segment from one β subunit (PDB: 6BDF) is shown in ribbon representation docked into the corresponding region of the reconstruction. (C) Representative 2D class-averages (box size 26.5x26.5nm). (D) 3D-FSC in grey with respect to T20S orientation shown in (A).

Quarterly
reporting



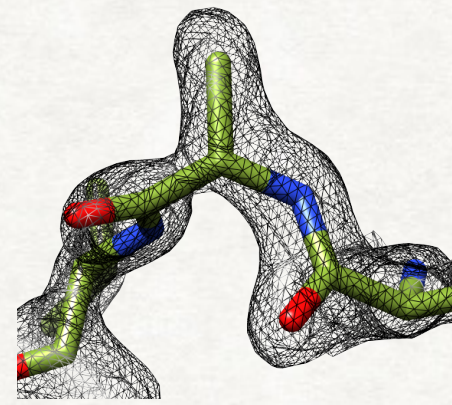
CryoEM reconstruction of GDH



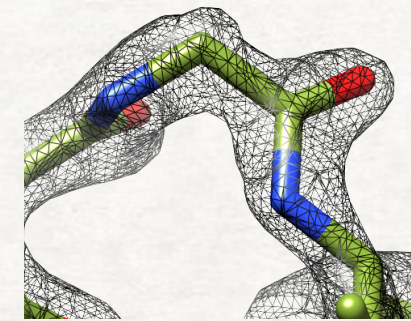
(A) Isosurface representation of the GDH map. (B) An α -helical segment from one β subunit (PDB: Kotaro) is shown in ribbon representation docked into the corresponding region of the reconstruction. (C) Representative 2D class averages. Box size 23.3x23.3nm. (D) 3D-FSC in grey shown with respect to GDH orientation shown in (A).

KRIOS TESTING

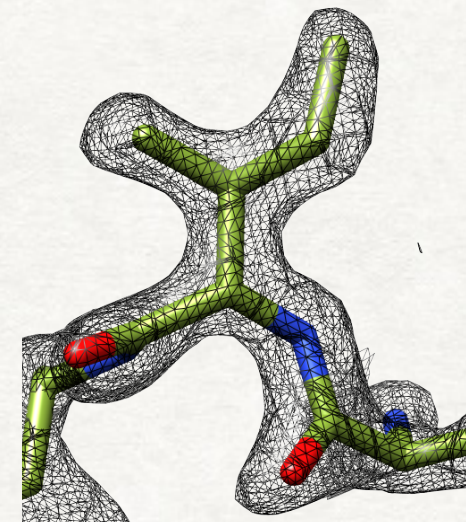
Workflow validation/testing



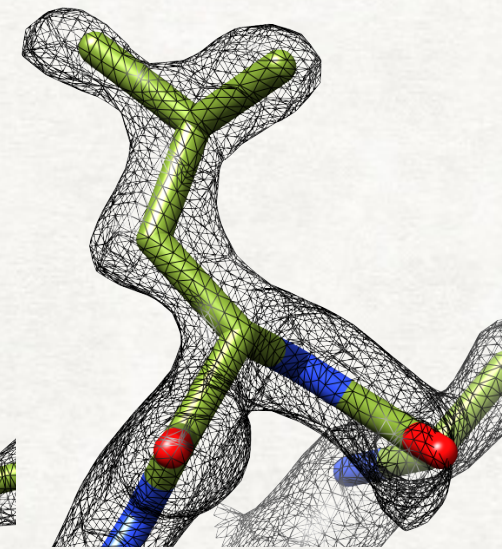
Alanine



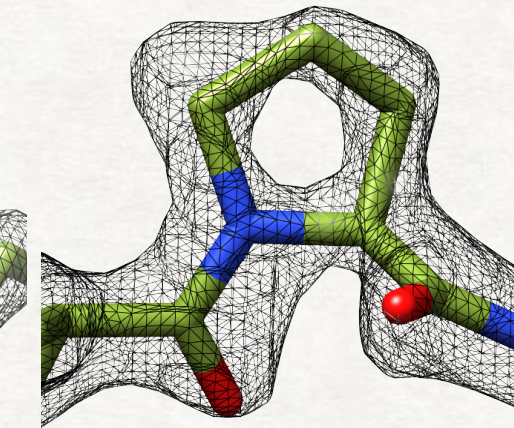
Glycine



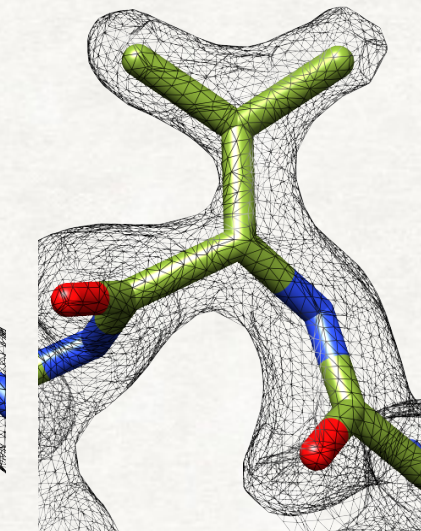
Isoleucine



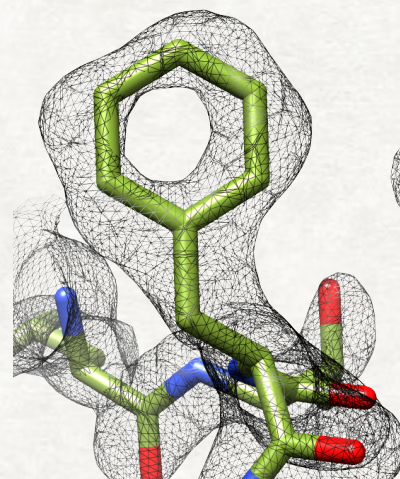
Leucine



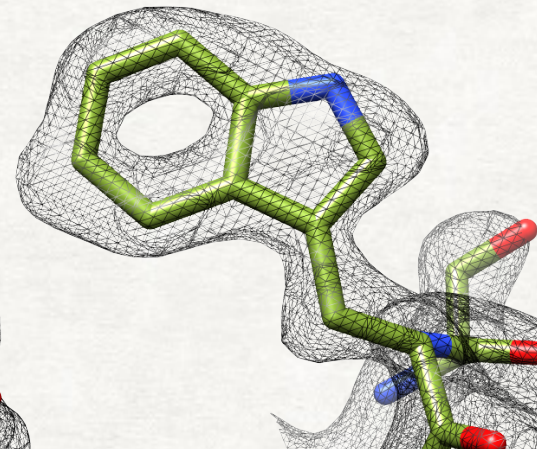
Proline



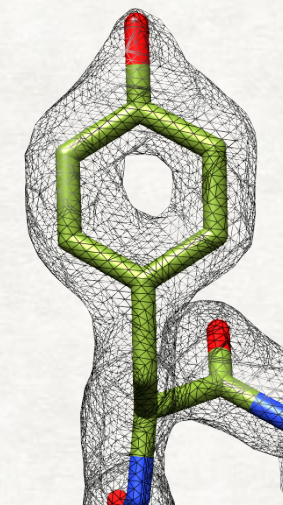
Valine



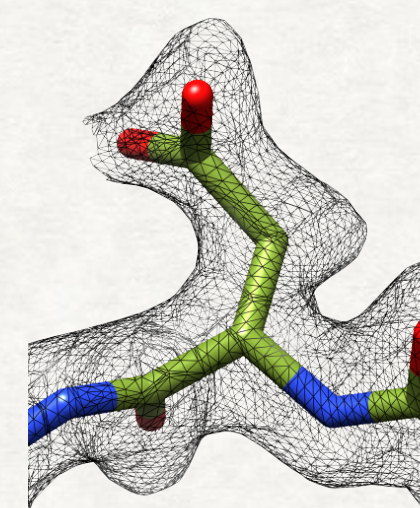
Phenylalanine



Tryptophan



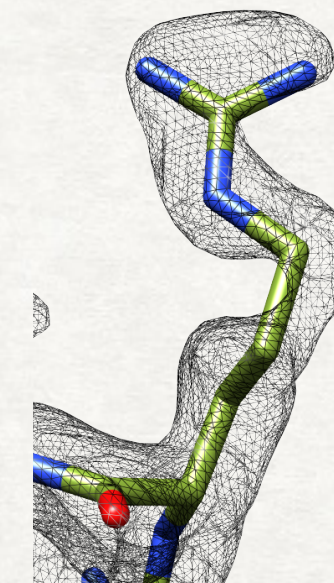
Tyrosine



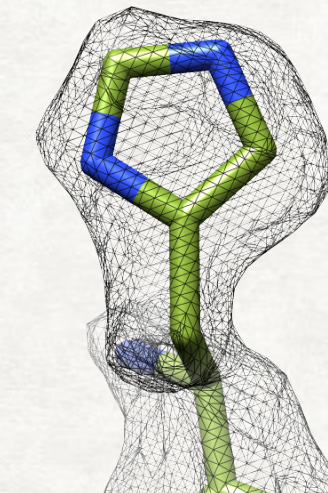
Aspartic acid



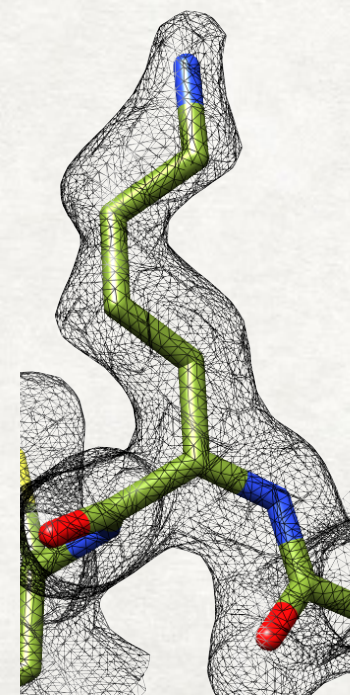
Glutamic Acid



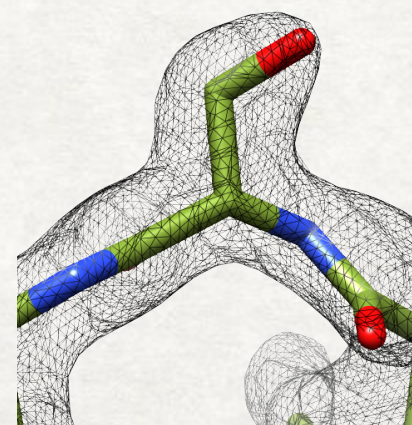
Arginine



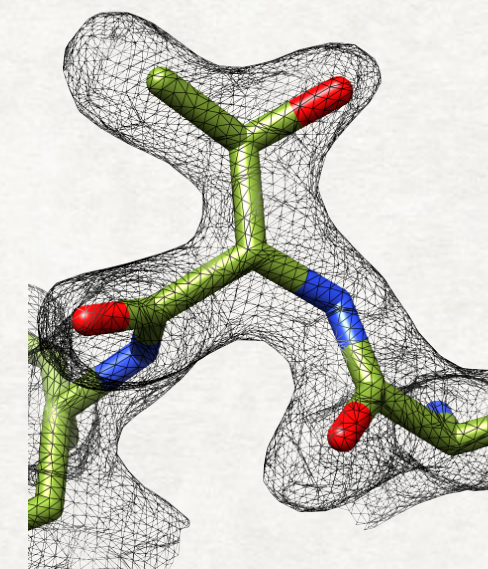
Histidine



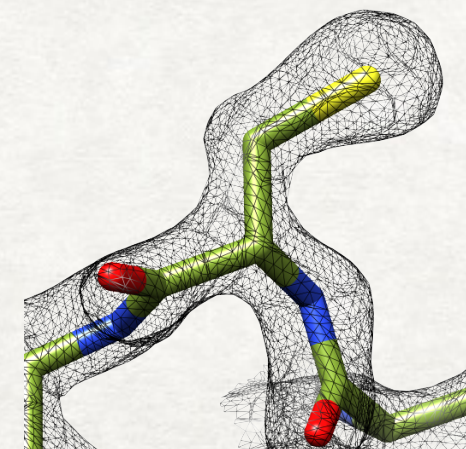
Lysine



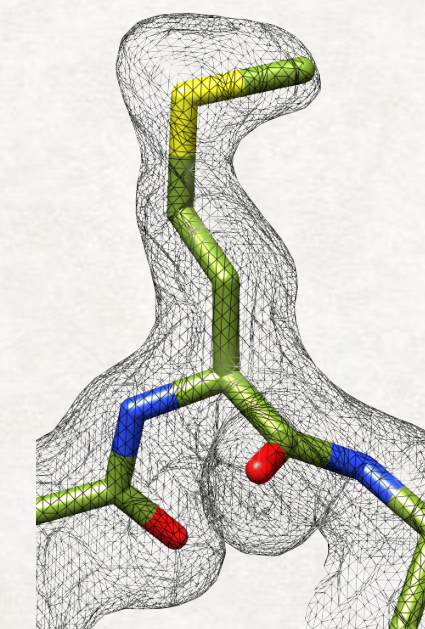
Serine



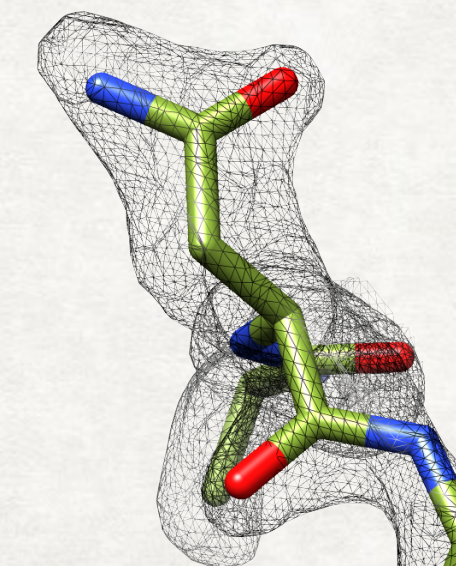
Threonine



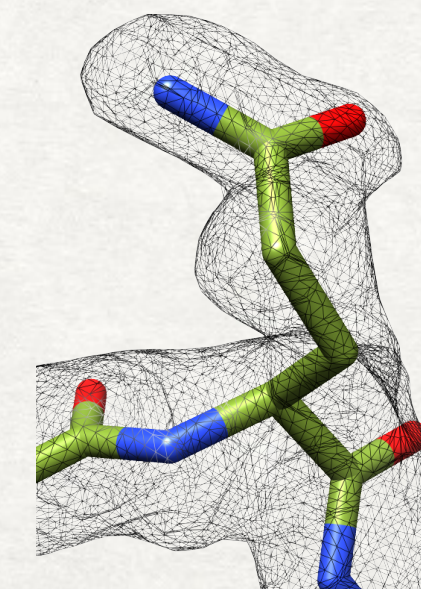
Cysteine



Methionine



Asparagine



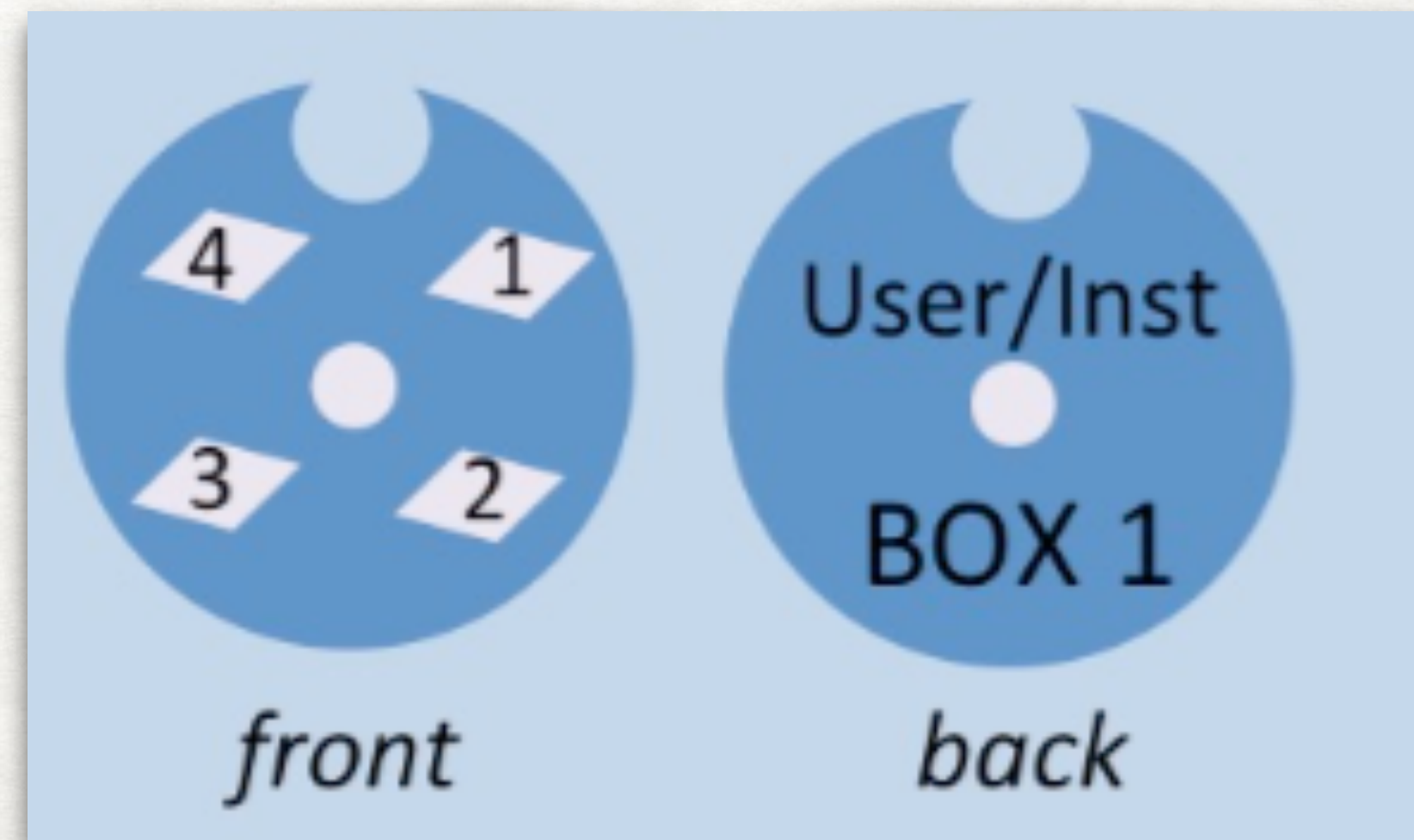
Glutamine

KRIOS USAGE : GRID STORAGE

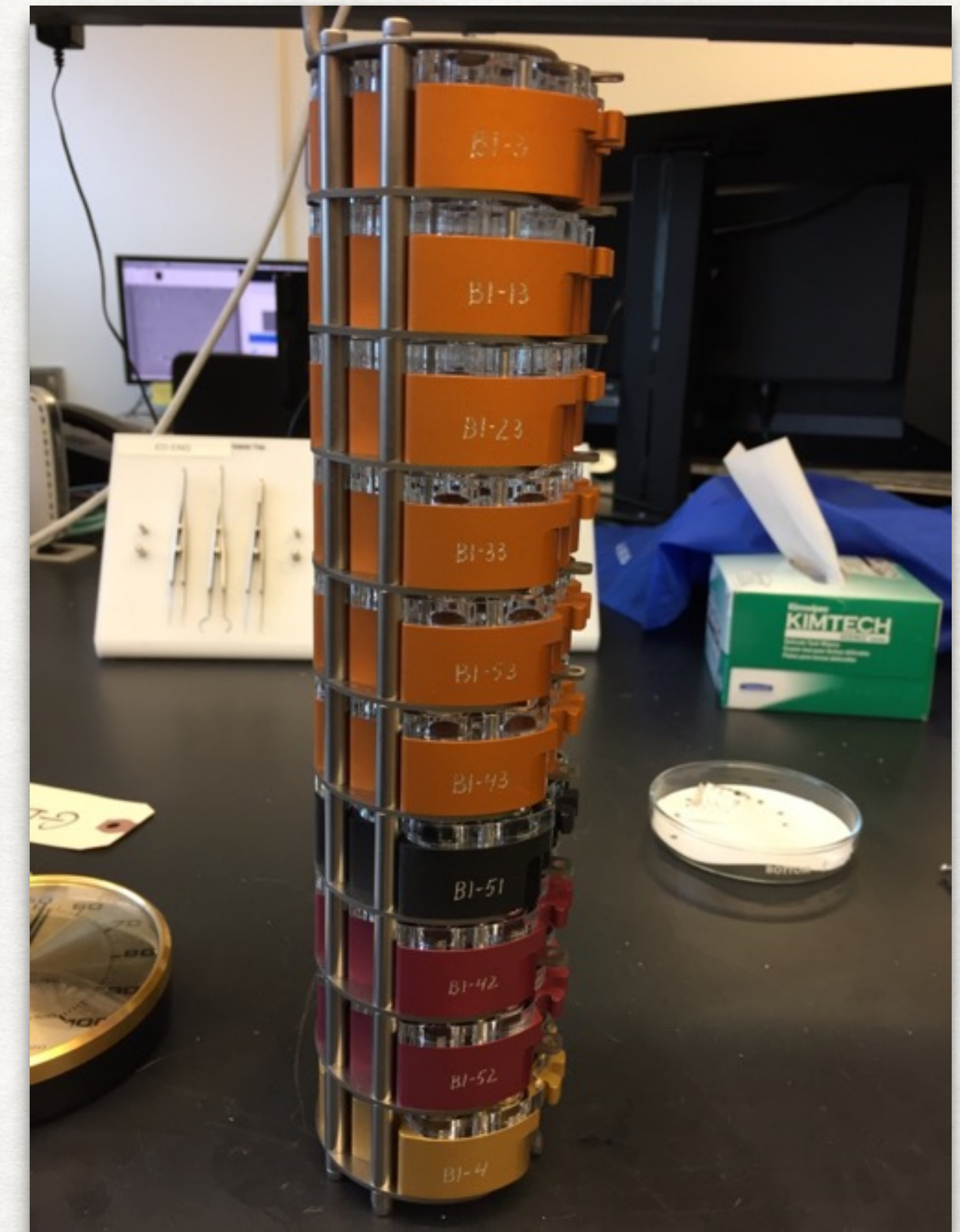
NON TATAM CUM TECHNICA PERITI



Krios



Grid drop off and pickup
Business days 2pm-4pm



KRIOS USAGE : RAPID ACCESS



There are 2 types of rapid access:

A) Moments notice

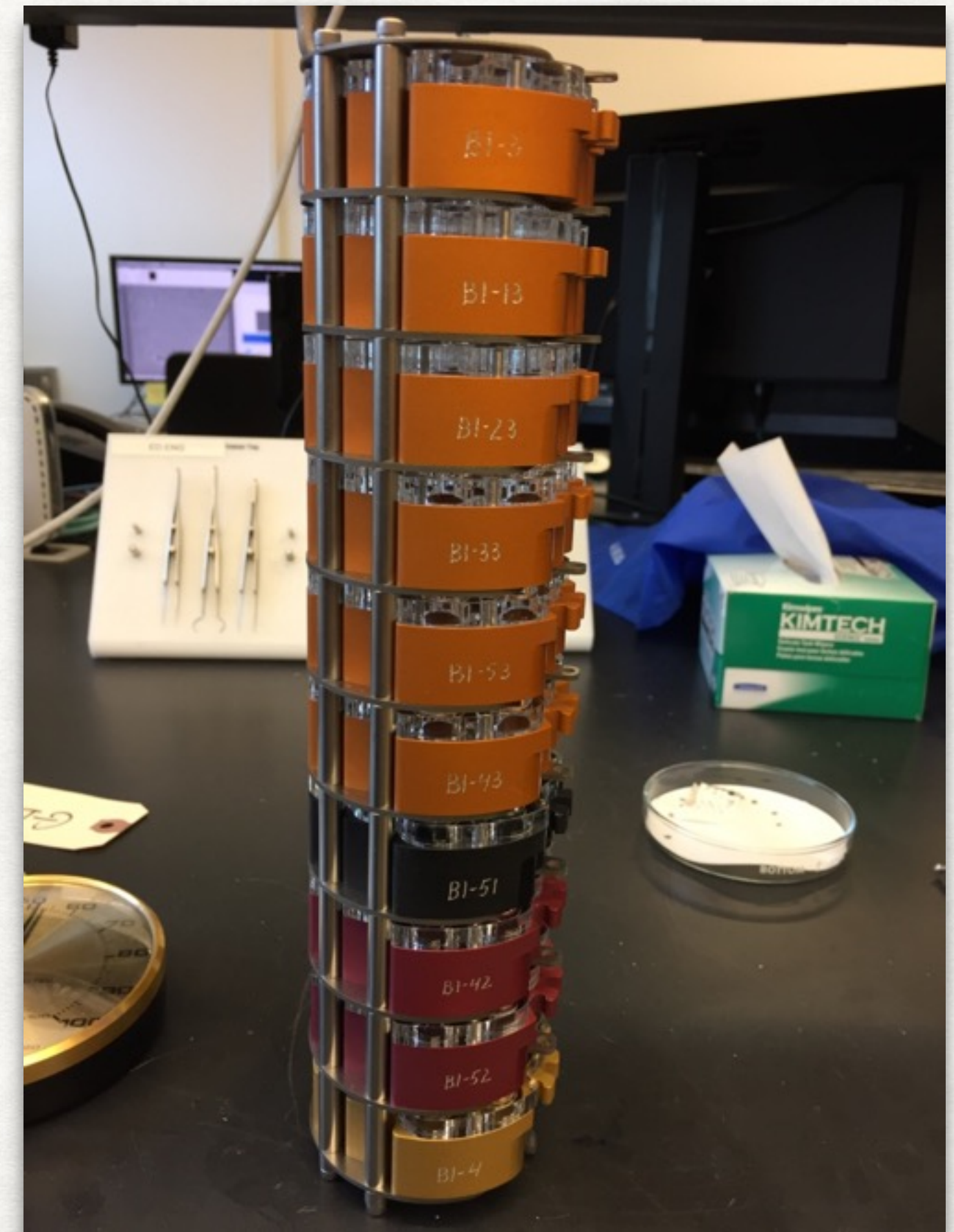
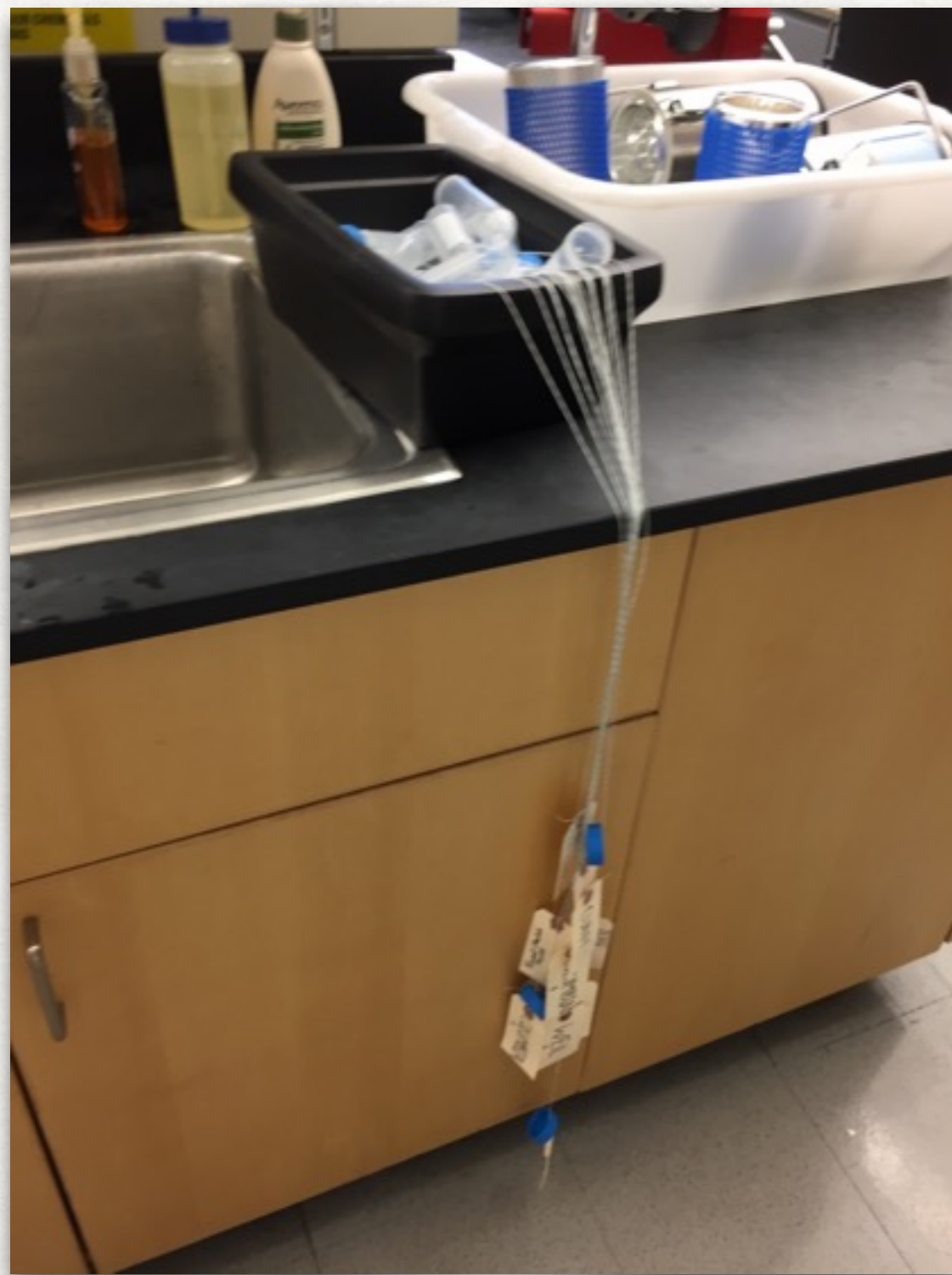
Time available due to an un-expected opening in the schedule. Grids should be pre-clipped and stored at the center, or the user should be able to bring it to the center at a moment's notice for immediate cassette loading.

B) Scheduled rapid access

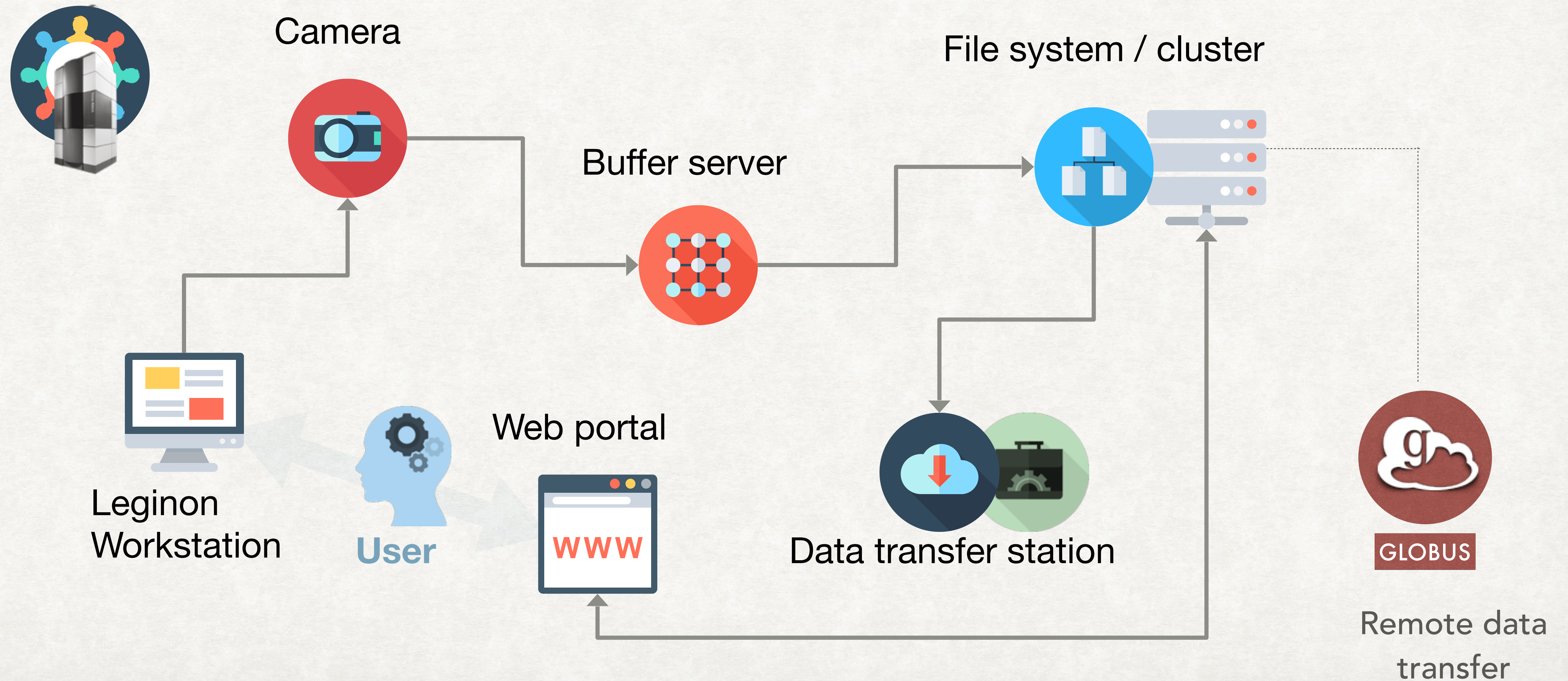
In between cycles we have system time for maintenance and development. Unused time during this period may be re-allocated as pre-scheduled rapid access time.

TEMPORARY : GRID STORAGE

Annual dewar defrost
Oct



DATA PIPELINE



REMOTE TRANSFER

MAKE SOMETHING CLEVER IN LATIN



Remote data

Create an account at
globusid.org

Login at globus.org

Transfer files

globus ID

Not Logged-In

globus

I want to... Pricing News Support Log In

Manage Data Publish Groups Support Account

Transfer Files Activity **Endpoints** Bookmarks Console

RECENT ACTIVITY 0 0 0

Endpoint List

+ add Globus Connect Personal endpoint + add Globus Connect Server endpoint

Add Globus Connect Personal Endpoint

Step 1 Create & Copy Your Globus Connect Personal Setup Key

Please enter a human-friendly name for your Globus Connect Personal endpoint to help you identify it.

Display Name *

Generate Setup Key

Step 2 Download & Install Globus Connect Personal

Click one of the buttons below to download and install Globus Connect Personal for your operating system.

for Mac OS X

for Linux

for Windows

Once downloaded, run the installer. When prompted, paste in the Setup Key to complete the installation.

Endpoint

nysbc



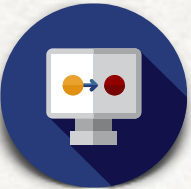
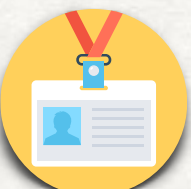


NYSBC - SEMC

owner: nysbc@globusid.org

no description provided


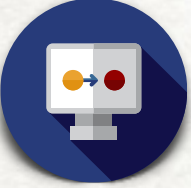
TRAINING AND EDUCATION

[HTTP://SEMC.NYSBC.ORG/TRAINING.HTML](http://semc.nysbc.org/training.html)

Type	Frequency	Title	Description
 feedback	yearly	General user meeting	State of the center
 knowledge	yearly	SEMC EM Course	Theory behind EM Graduate level course
 computation	quarterly	SEMC Appion workshop	Data processing workshops
 access	monthly	SEMC New User Orientation	Facility use and Safety Training Leginon intro/use of screening microscopes
 troubleshooting	weekly	User Project Discussion Meetings	Appointments: Tue @ 3:00pm, Thurs @3:00pm
 instrumentation	daily	Advanced Leginon use / 1-1 training	Training for independent use of the microscopes and other SEMC resources

TRAINING AND EDUCATION

<http://semc.nysbc.org/training.html>

Type	Frequency	Title	Description
 knowledge	yearly	SEMC EM Course	Theory behind EM Graduate level course
	Jan 2019	https://www.surveymonkey.com/r/SEMCcourse18	
 computation	quarterly	SEMC Appion workshop	Data processing workshops
Appion Part 1: Basics & 2D SPA	Sept 27	Registration: https://www.surveymonkey.com/r/JS6XLDS	
Appion Part 2: 3D SPA	Oct 9	Registration: https://www.surveymonkey.com/r/BQYG8C6	
Appion Part 3: Tomography	Dec 6	Registration: https://www.surveymonkey.com/r/BQYYXN3	
Leginon (for Krios data collection)	Nov 8	Registration: https://www.surveymonkey.com/r/66395S3	

NATIONAL CENTER *FOR* CRYOEM ACCESS *AND* TRAINING

NCCAT



New York
Structural
Biology
Center

OPEN FORUM

QUESTIONS