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

EMG@nysbc.org

## Staff

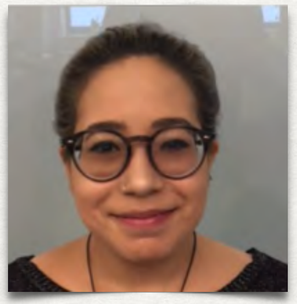
Bill

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

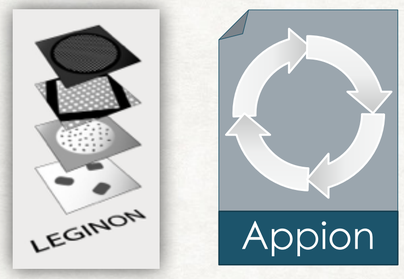
# LEGINON & APPION USE

## Central MySQL Database and web server

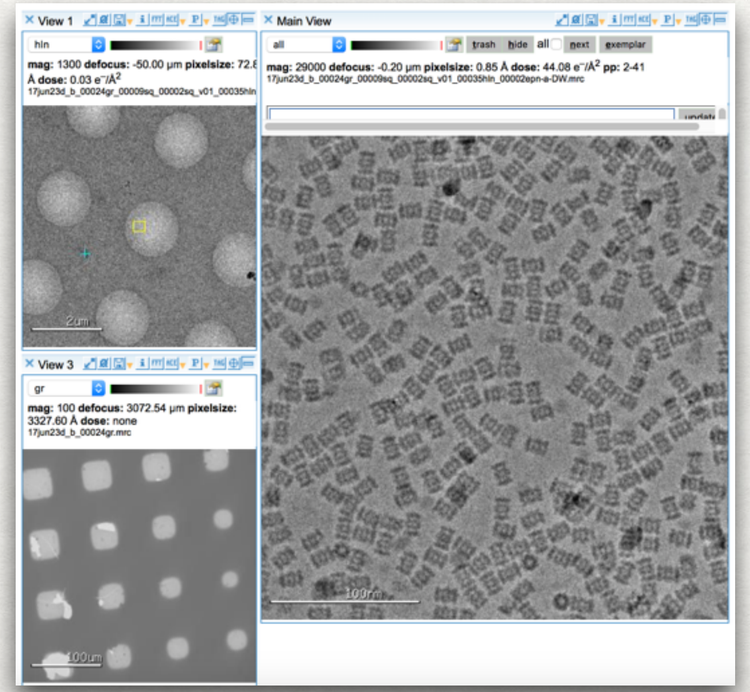
**Size of images:** 393.51 TB

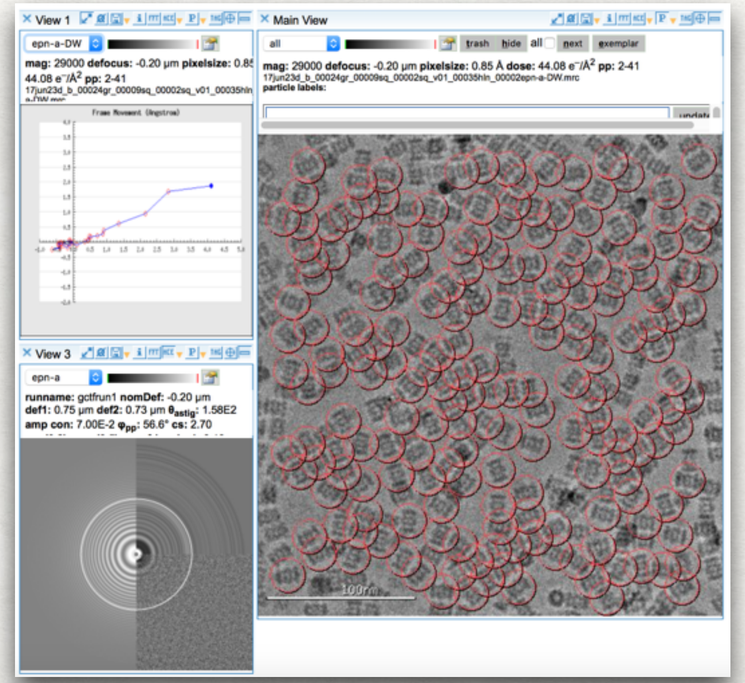
**# DB records:** 1,425,659,999

**Size of database:** 14.34 GB




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

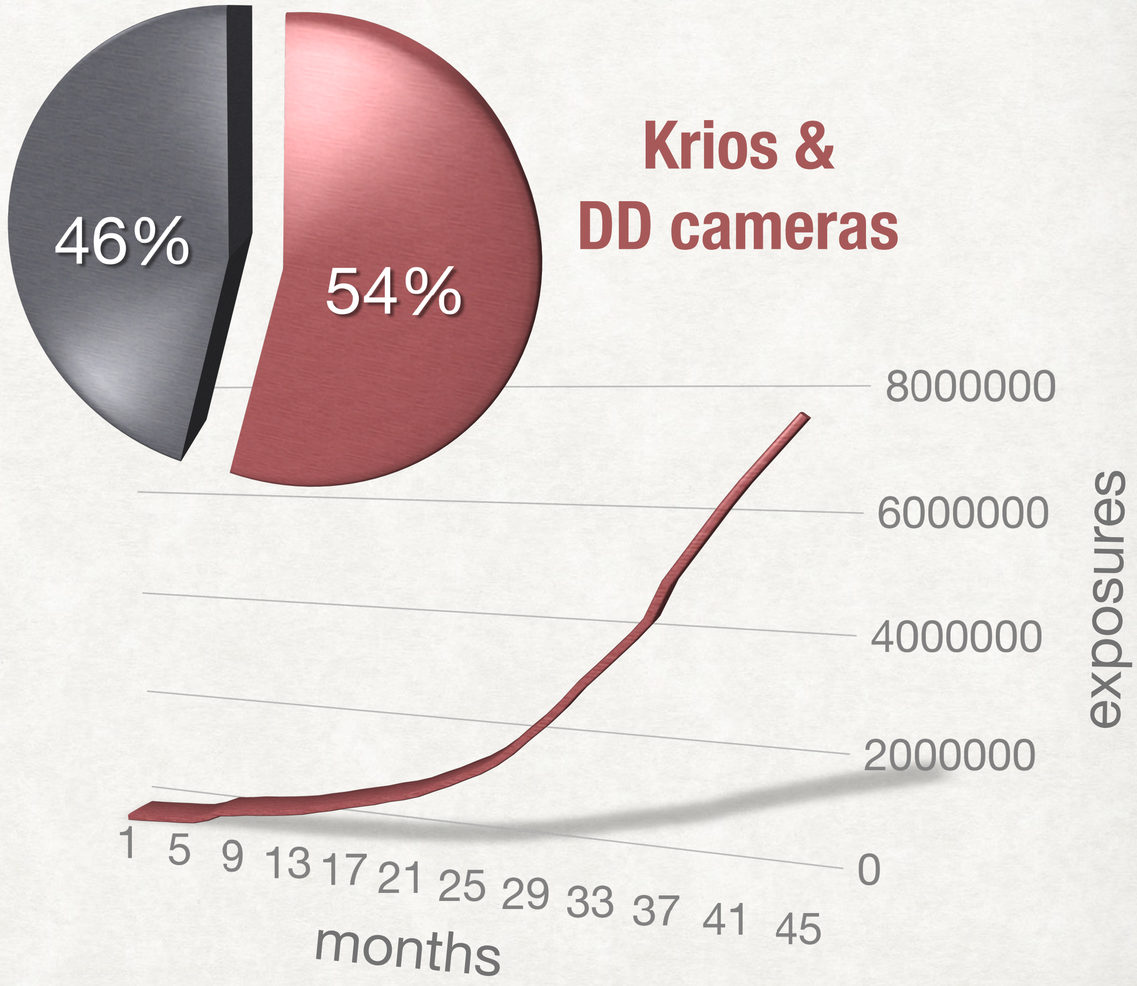


# LEGINON & APPION USE

| | |
|---|---|
| *# 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.**

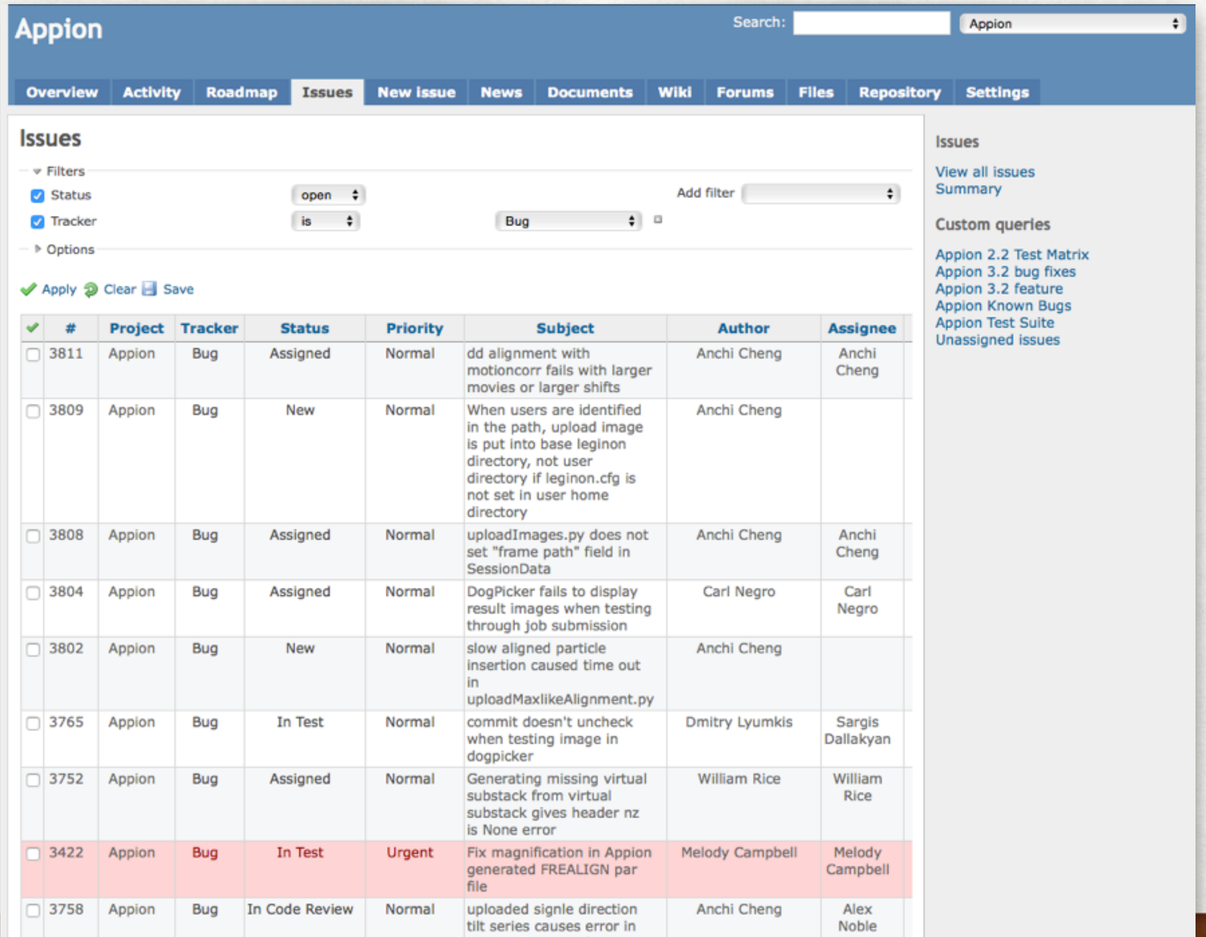
Leginon database older than 1 yr will be converted from MRC to JPEG.

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*

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 Detecto*

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: ~1.1Å/px

Dose: 8e-/px/s

Frame rate: 200-250 ms

Exposure time: 8-10 sec

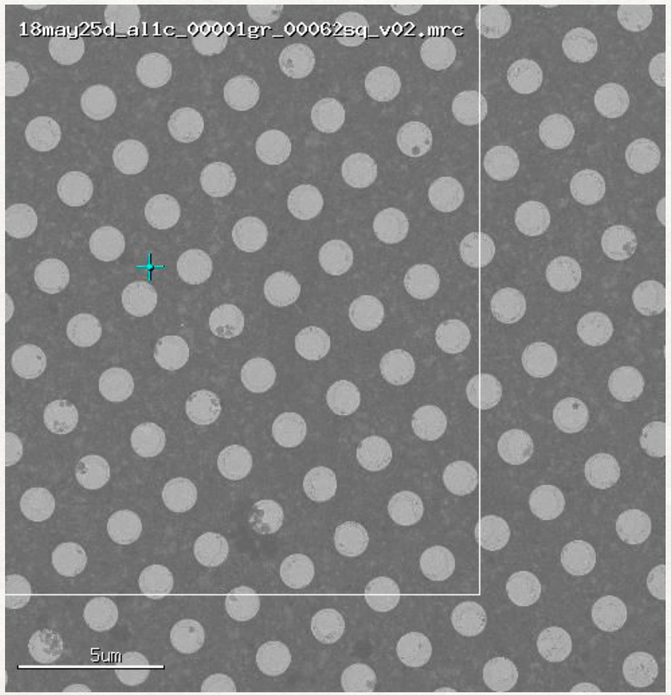
MotionCor2

CTFFIND4

Ice thickness

# KRIOS USAGE : EXTENDED IMAGE SHIFT

Krios01




Single hl target 400x per sq

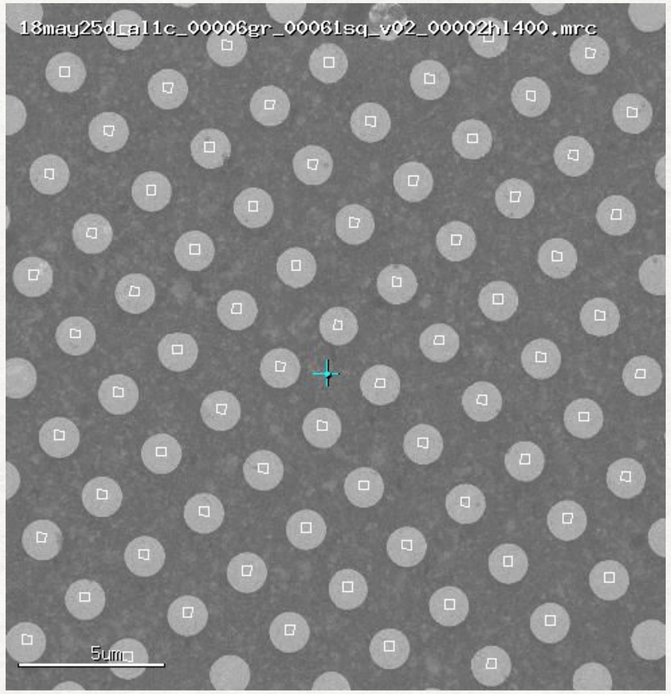
Hole targeting can be used to re-center the square or to avoid obviously bad areas

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

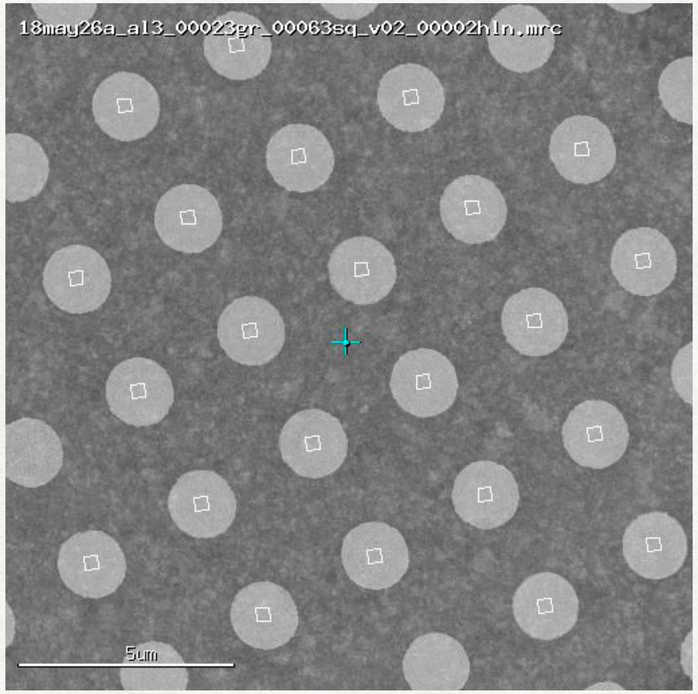



70 avg targets per hole image

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

$Speed_{hl}$ = 60.5/day

← 60 squares (1hl per 1 sq)!




22 avg targets per hole image

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

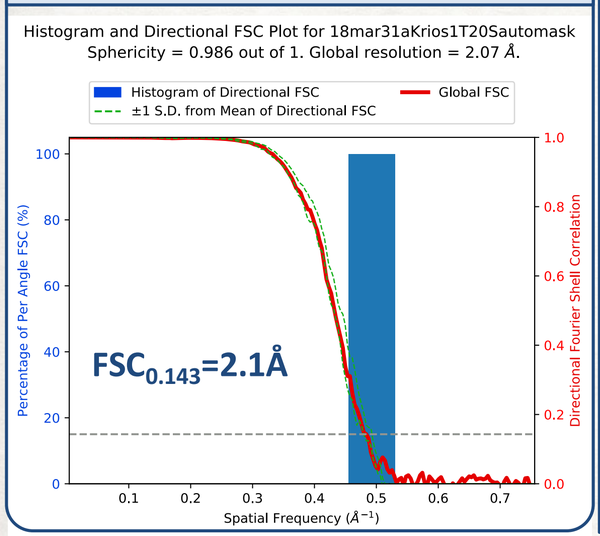
$Speed_{hl}$ = 158/day

← ~40 squares (4hl per 1 sq)!

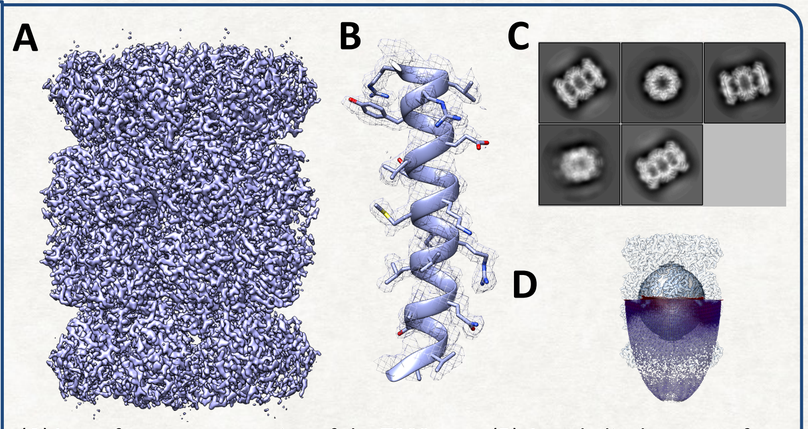
# KRIOS TESTING

## Workflow validation/testing

### Fourier shell correlation curve



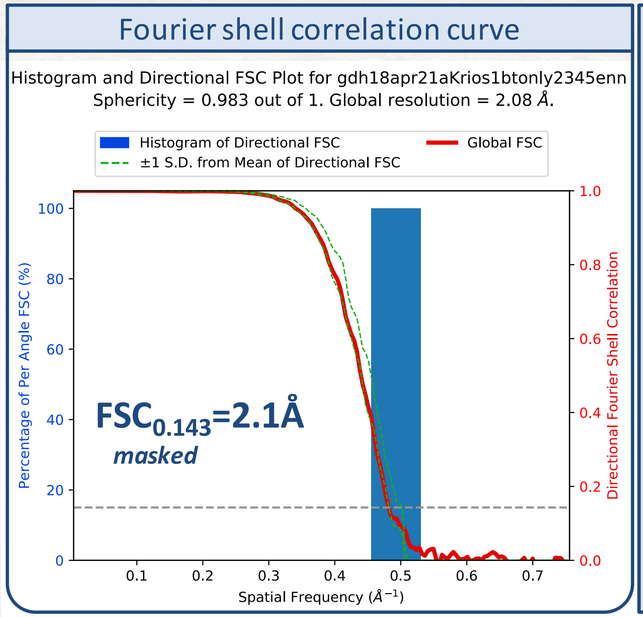

### CryoEM reconstruction of the T20S



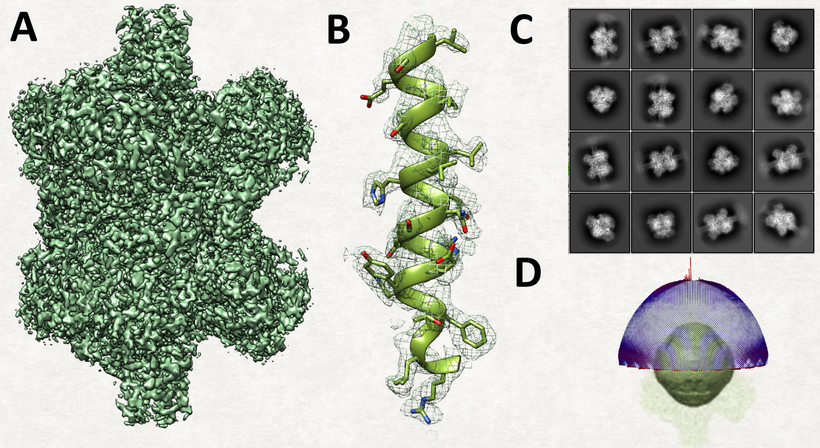

(**A**) Isosurface representation of the T20S map. (**B**) An α-helical segment from one β subunit (PDB: 6BDF) is shown in ribbon representation docked into the corresponding region of the reconstruction. (**C**) Representative 2D class-averages (box size 26.5x26.5nm). (**D**) 3D-FSC in grey with respect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

# Quarterly reporting

### Fourier shell correlation curve



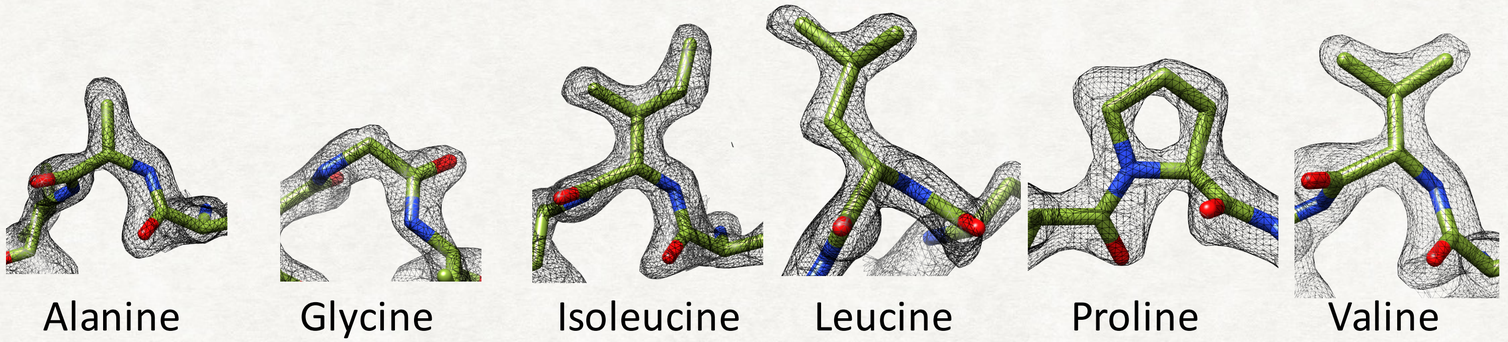

### CryoEM reconstruction of GDH

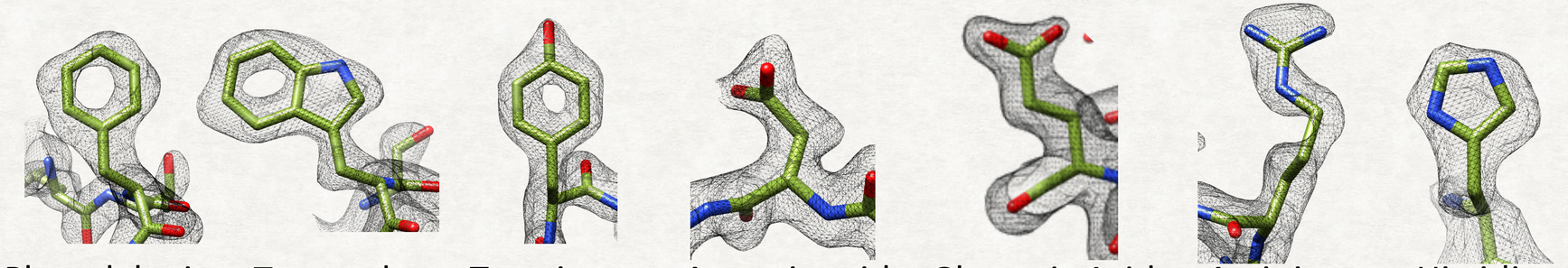


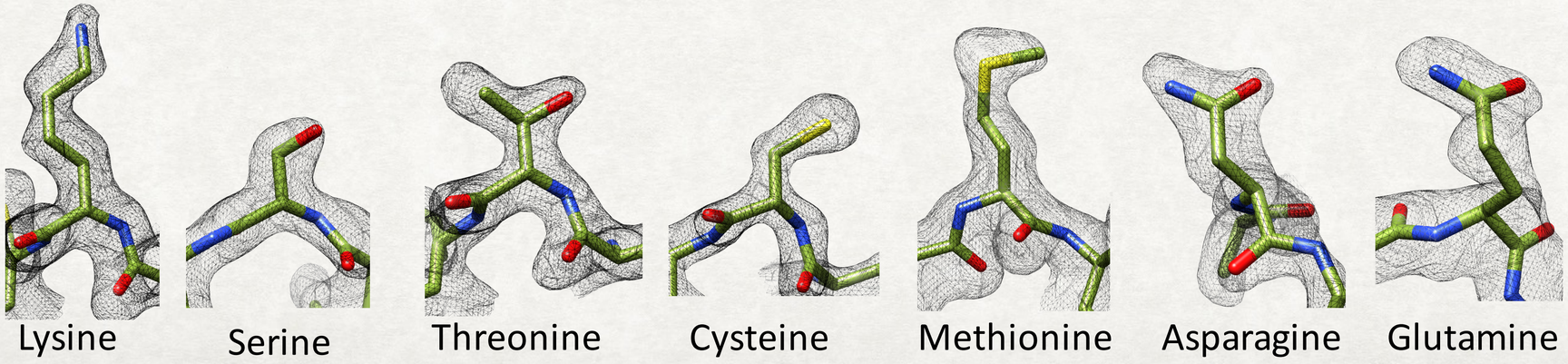

(**A**) Isosurface representation of the GDH map. (**B**) An α-helical segment from one β subunit (PDB: Kotaro) is shown in ribbon representation docked into the corresponding region of the reconstruction. (**C**) Representative 2D class averages. Box size 23.3x23.3nm. (**D**) 3D-FSC in grey shown with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

# KRIOS TESTING

## Workflow validation/testing



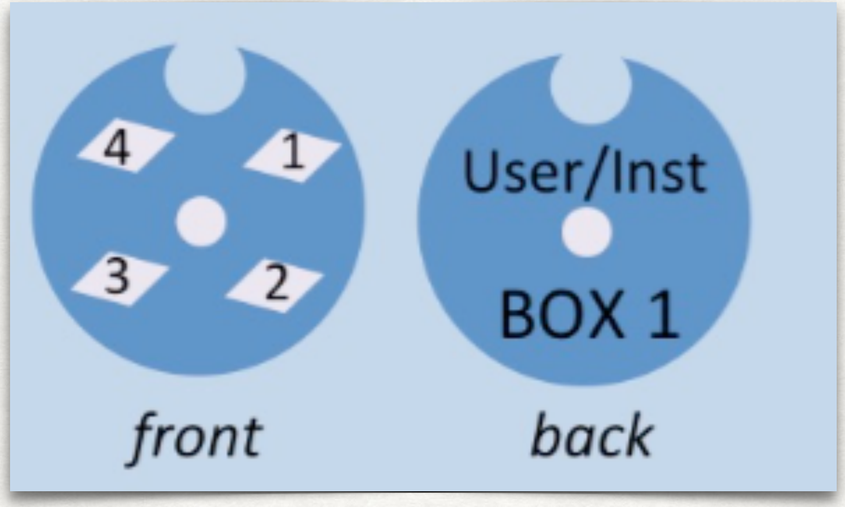



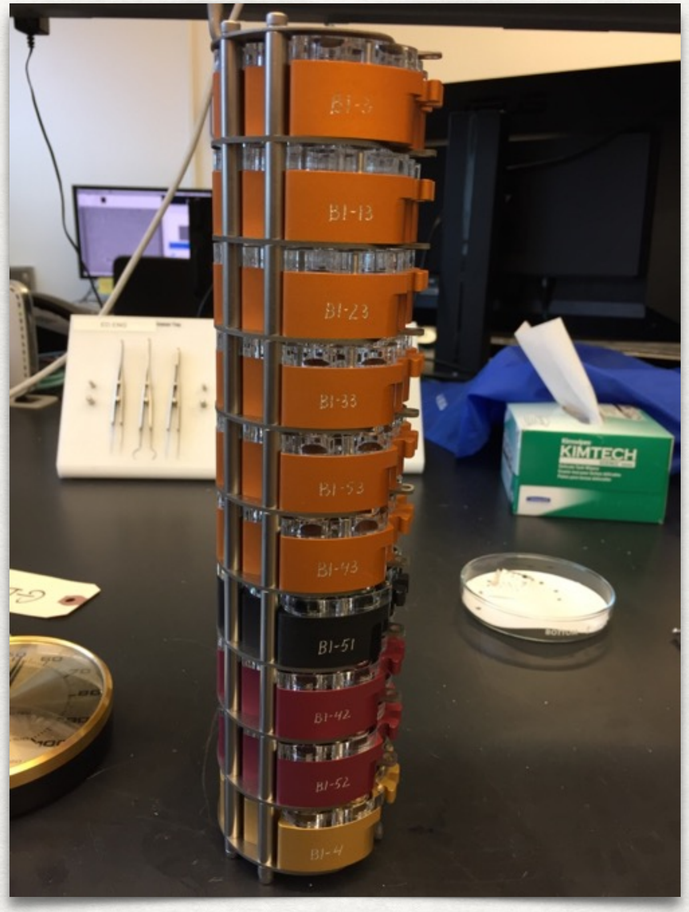
# KRIOS USAGE : GRID STORAGE

## NON TATAM CUM TECHNICAE PERITI

Krios




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



# KRIOS USAGE : RAPID ACCESS

There are 2 types of rapid access:

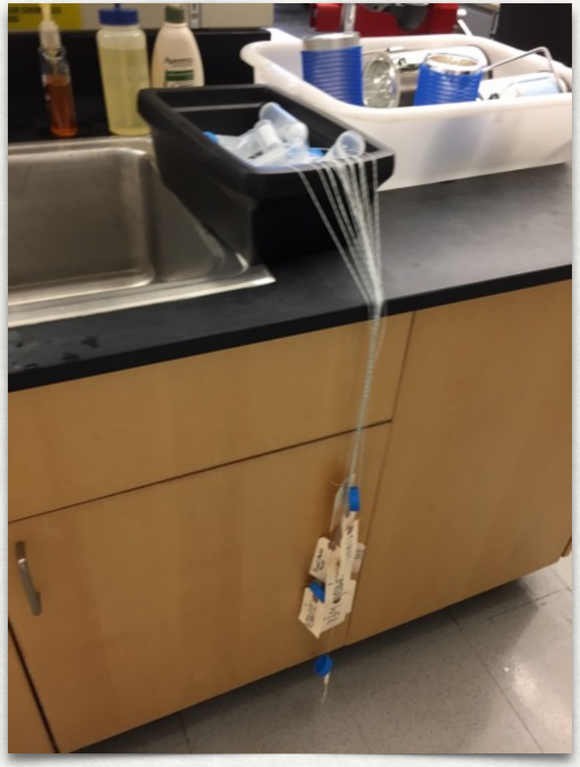
**A) Moments notice**
Time available due to an un-expected opening in the schedule. Grids should be pre-clipped and stored at the center, or the user should be able to bring it to the center at a moment's notice for immediate cassette loading.

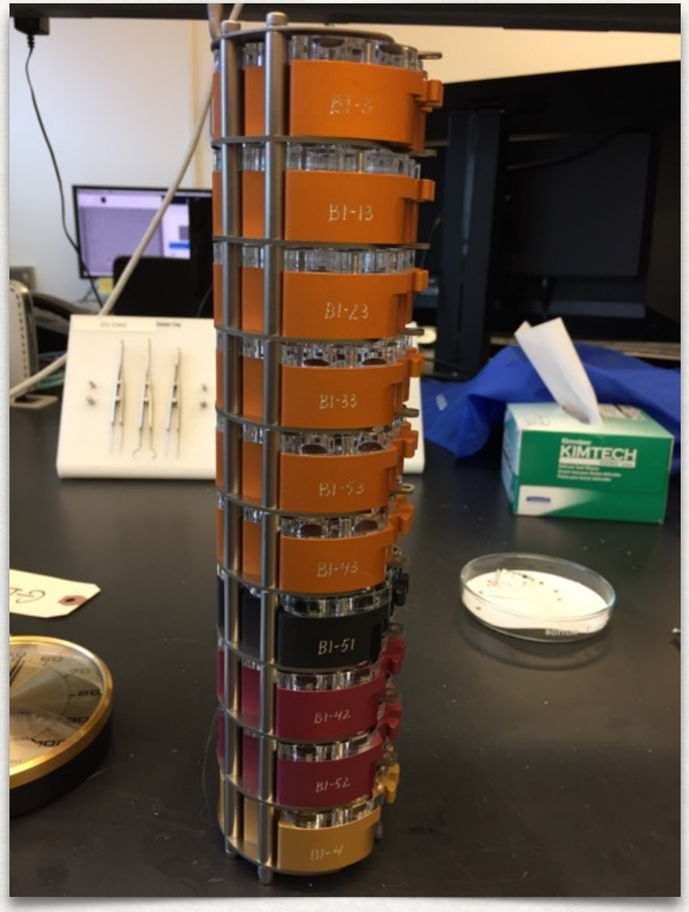
**B) Scheduled rapid access**
In between cycles we have system time for maintenance and development. Unused time during this period may be re-allocated as pre-scheduled rapid access time.

# TEMPORARY : GRID STORAGE

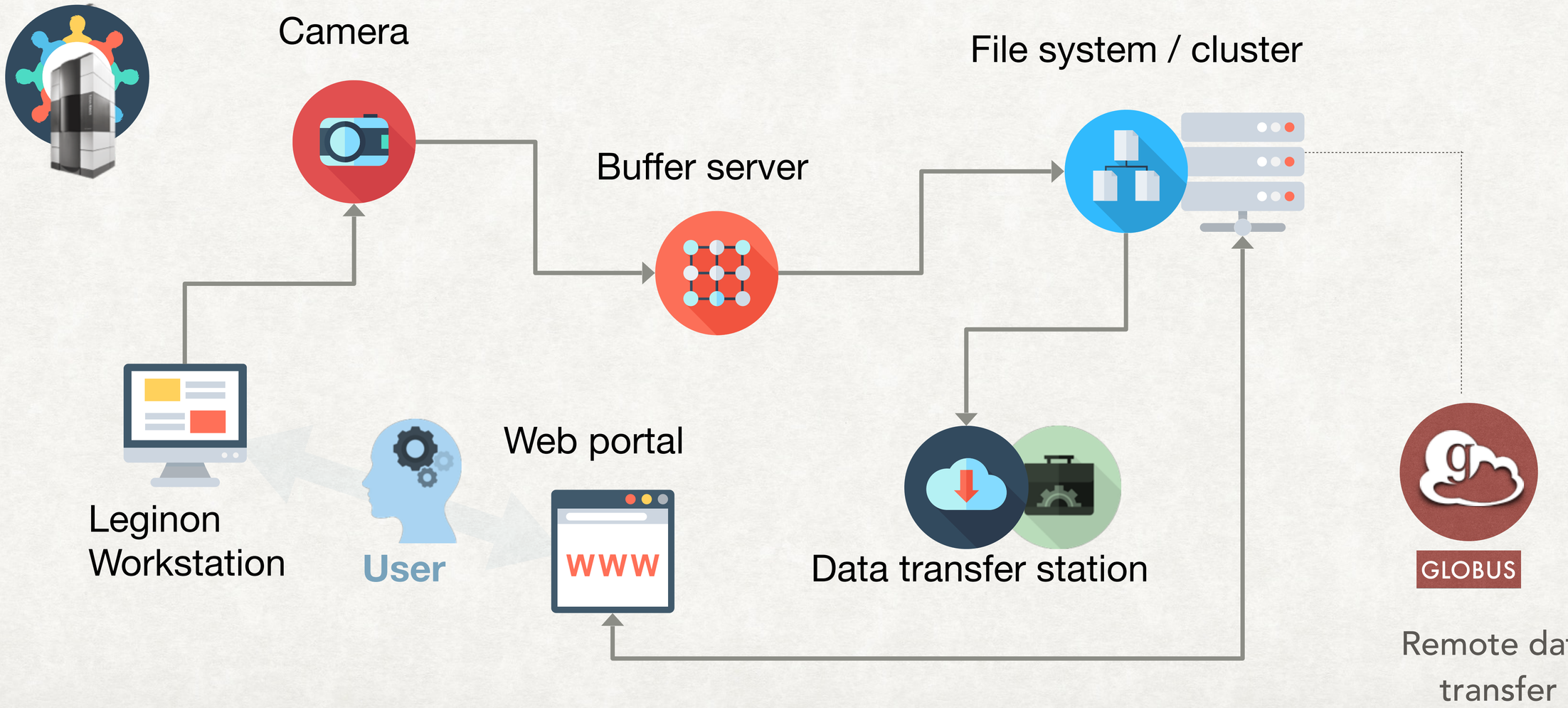
Annual dewar defrost

Oct





# DATA PIPELINE



# REMOTE TRANSFER

## MAKE SOMETHING CLEVER IN LATIN

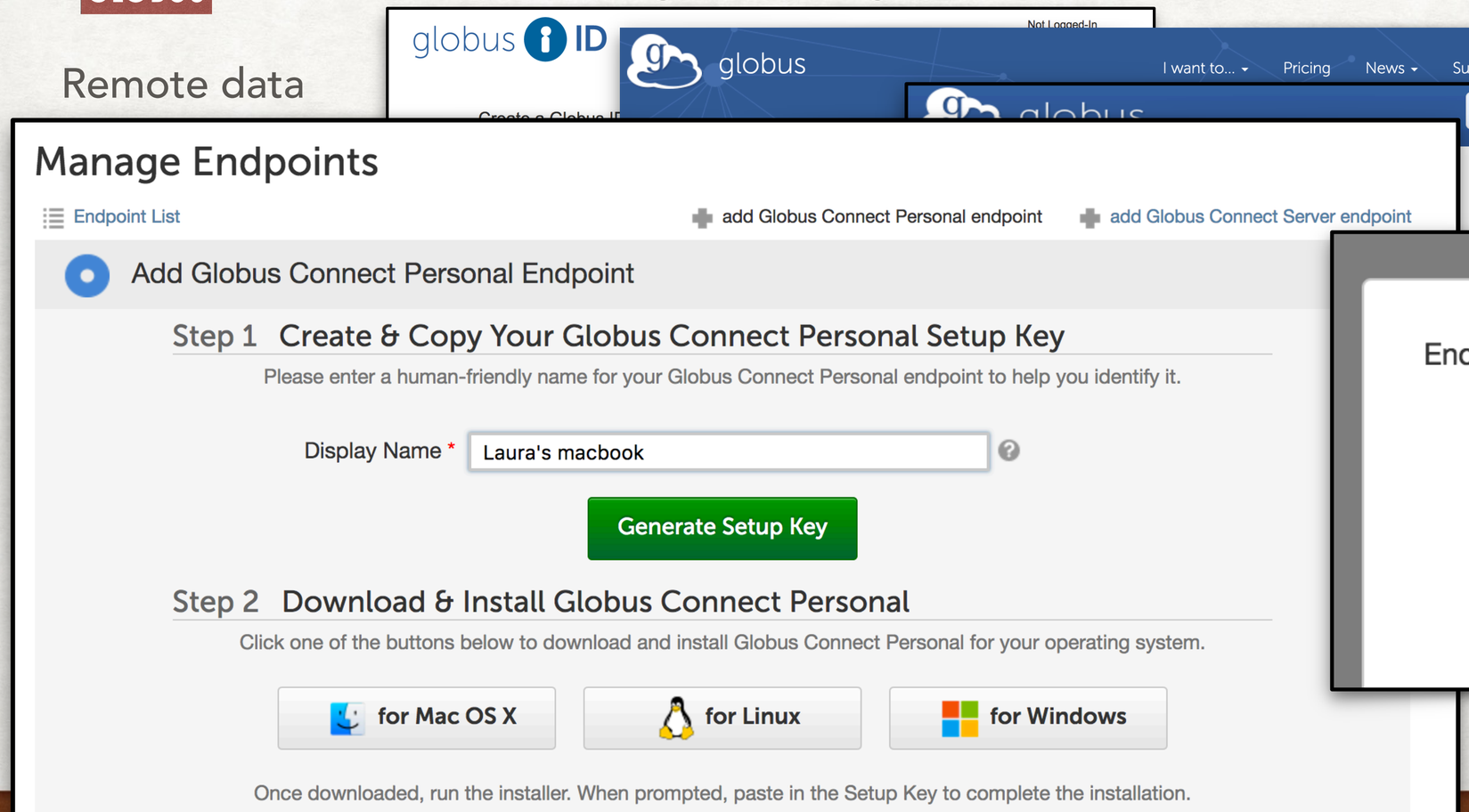
GLOBUS

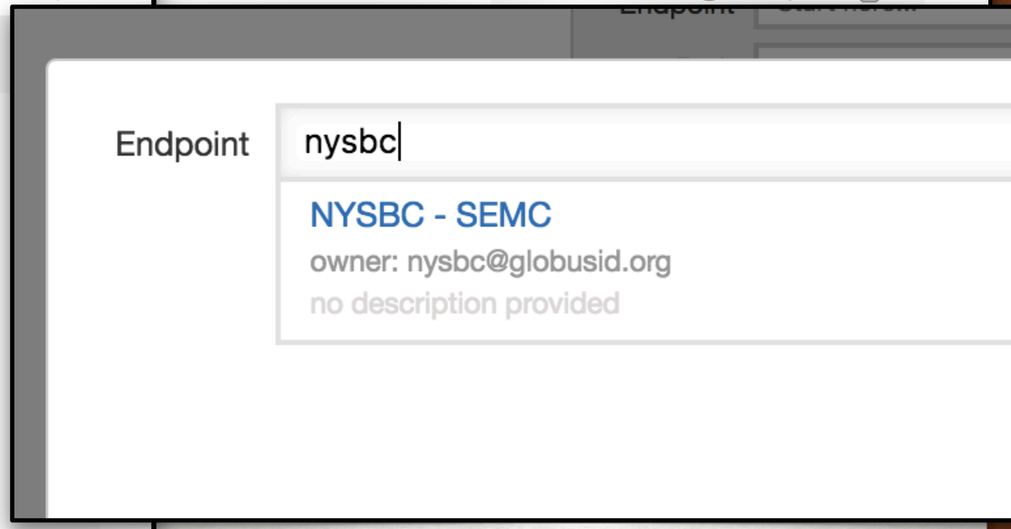
Remote data

Create an account at globusid.org

Login at globus.org

Transfer files



# TRAINING AND EDUCATION

## 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<br>Graduate level course |
| computation | quarterly | SEMC Appion workshop | Data processing workshops |
| access | monthly | SEMC New User Orientation | Facility use and Safety Training<br>Leginon intro/use of screening microscopes |
| troubleshooting | weekly | User Project Discussion Meetings | Appointments: Tue @ 3:00pm,<br>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<br>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