CRYOEM 001 :
INTRO TO CRYOEM

NCCAT Embedded Training – Master Class series

June 9-10, 2021

NATIONAL CENTER FOR
CRYOEM ACCESS & TRAINING

New York Structural
Biology Center

SIMONS ELECTRON
MICROSCOPY CENTER

NCCAT Cross-training program

Daniela Fera

Swarthmore College, PA

NCCAT-TP1-DF200401

Daniela is an Assistant Professor in the Department of Chemistry and Biochemistry at Swarthmore College. She is interested in viruses, the adaptive immune response to viruses, and the signaling pathways important for antibody development. Her laboratory uses biochemistry and structural biology approaches to analyze antibody-spike and kinase-kinase complexes.

Daniela received her Ph.D. in Chemistry from the University of Pennsylvania, where she screened and characterized small molecules that might inhibit the cancer-causing activities of human papillomavirus oncoproteins, to identify therapies for those who have already been infected with the virus. She then went on to do a postdoctoral fellowship at Boston Children's Hospital/Harvard Medical School in the laboratory of Stephen C. Harrison, doing structural biology on HIV spikes to guide vaccine design.

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NCCAT Cross-training program

Eric Gibbs

Case Western Reserve University, OH

NCCAT-TP1-EG200929

Eric is a postdoctoral scholar in Sudha Chakrapani's lab at Case Western Reserve University. He has been there since 2018 after receiving his PhD from Duke University in the lab of Chunlei Liu. His current focus is on pentameric ligand-gated ion channels and is more broadly interested in mechanisms that govern synapse formation and regulation.

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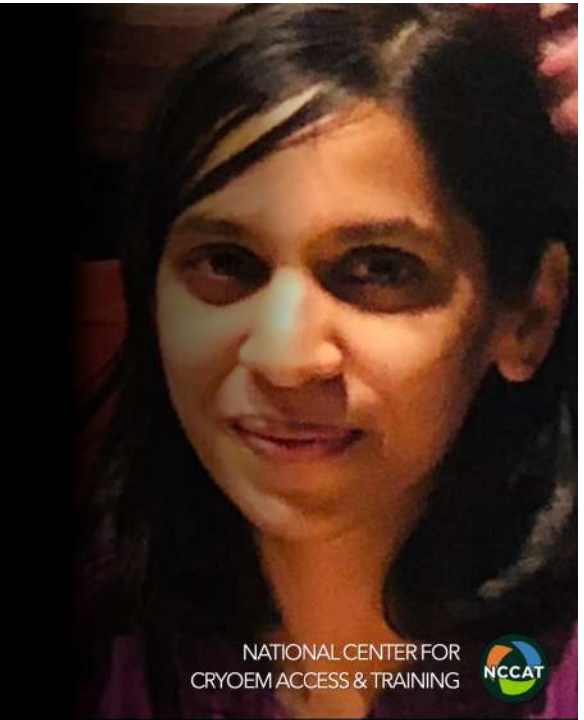
NCCAT Cross-training program

Tilini Wijeratne

University of California, Santa Cruz, CA

NCCAT-TP1-EG200929



Tilini's research career began as an undergraduate at California State University in Dr. Paul Weers' laboratory. In particular, Tilini's research in structural biology began with studying lipoprotein-bound nano-disks of apolipophorin III through negative-stain EM. After her undergraduate degree in Biochemistry, she joined University of California, Santa Cruz in 2017 as a PhD candidate and currently works in Dr. Seth Rubin's laboratory studying the oncoprotein B-Myb and how it regulates the cell cycle. Tilini uses other techniques like NMR, X-ray crystallography and negative-stain EM to probe the structural aspects of B-Myb. Since joining the lab, she has made progress towards isolating the B-Myb-nucleosome complex which she will study its structural basis through Cryo-EM. Tilini has completed a workshop series on Cryo-EM techniques conducted by Stanford SLAC Cryo-EM center.

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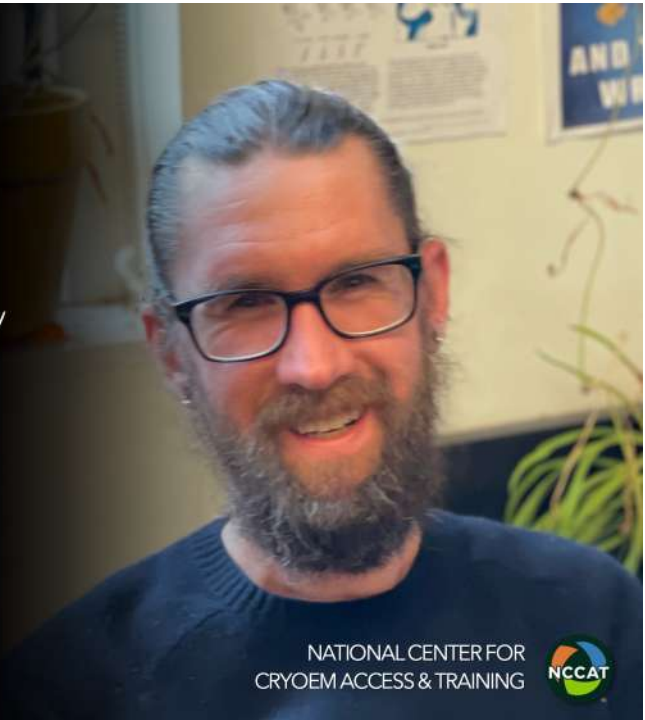
NCCAT Cross-training program

Justin Finley Acheson

University of Virginia, VA

NCCAT-TP1-JA181201



Justin's doctoral training is in structural biology using X-ray crystallography to understand protein-protein interactions and investigation of reaction mechanisms *in crytsallo*. His drive for science is understanding of these mechanisms in large macromolecular complexes. To that end he joined Dr. Jochen Zimmer's lab as a postdoctoral researcher to study membrane protein complexes. Recently, he as shifted to cryoEM to expand his structural biology toolbox. Mostly self-taught he hopes to gain further understanding and skills though the NCCAT cross-training program.

NATIONAL CENTER FOR CRYOEM ACCESS & TRAINING

WEEK 1
JUNE 6/09-10

Wed 6/9 3-5pm

- Overview of what's happening in June
- Introduction to merit badges
- Practical: NS-EM demo with F20 to spot check and iterate

Thur 6/10 2-5pm

- Screening microscope (F20) in detail
- Screening with Leginon

NCCAT CROSS-TRAINING

knowledge

capability

capacity

productivity

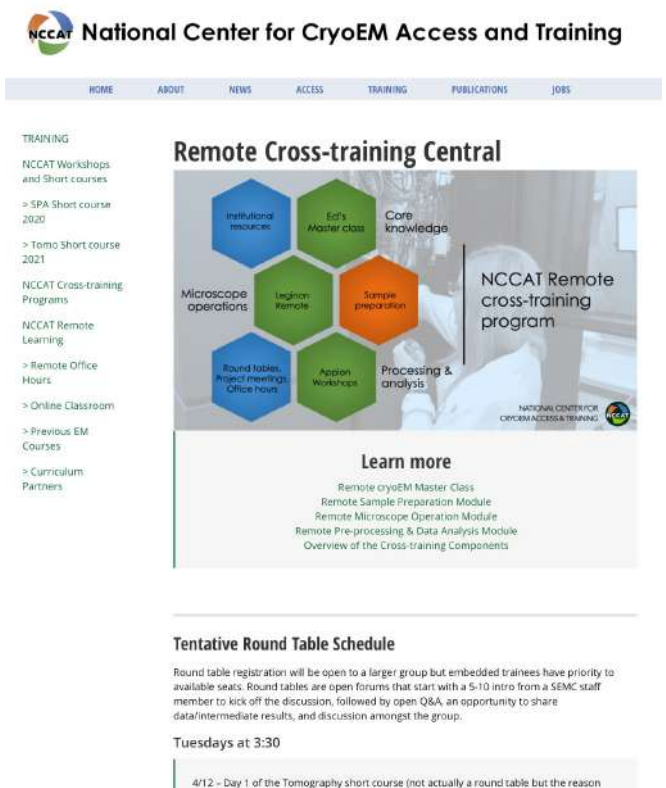
performance

Training is teaching, or developing in oneself or others, any skills and knowledge that relate to specific useful competencies. Training has specific goals of improving one's capability, capacity, productivity and performance.

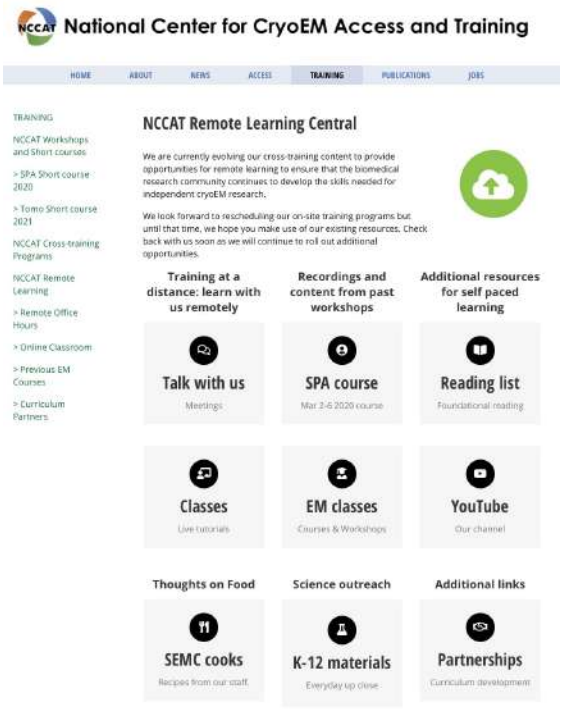
https://en.wikipedia.org/wiki/Training

NCCAT CROSS-TRAINING RESOURCES

Remote Cross-training Program



Remote Learning Central



SEMC meetings

Lab meetings

Project meetings

Office hours

NCCAT workshops

Roundtables

Appion

Remote learning modules

Core knowledge

Sample preparation

Microscope operations

Data collection

Data processing

CRYOEM 001 : SINGLE PARTICLE MASTERCLASS

Introduction to cryoEM: SPA

Building a cryoEM toolkit

EM compatible samples

EM support films and grids

Sample preparation

Tools of the trade:

- microscopes and detectors

Microscope operations

Data collection strategies

Data assessment & QC

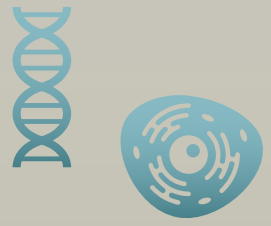
Data processing:

- cryoEM IT infrastructure
- On-the-fly feedback
- 3D Reconstruction

Visualization and validation

NCCAT CROSS-TRAINING FOCUS ON 4 AREAS

Core knowledge



Sample preparation

Microscope operations & Data collection

Processing & Data analysis

Core knowledge

Sample preparation

Microscope operations & Data collection

Processing & Data analysis

SCHEDULE

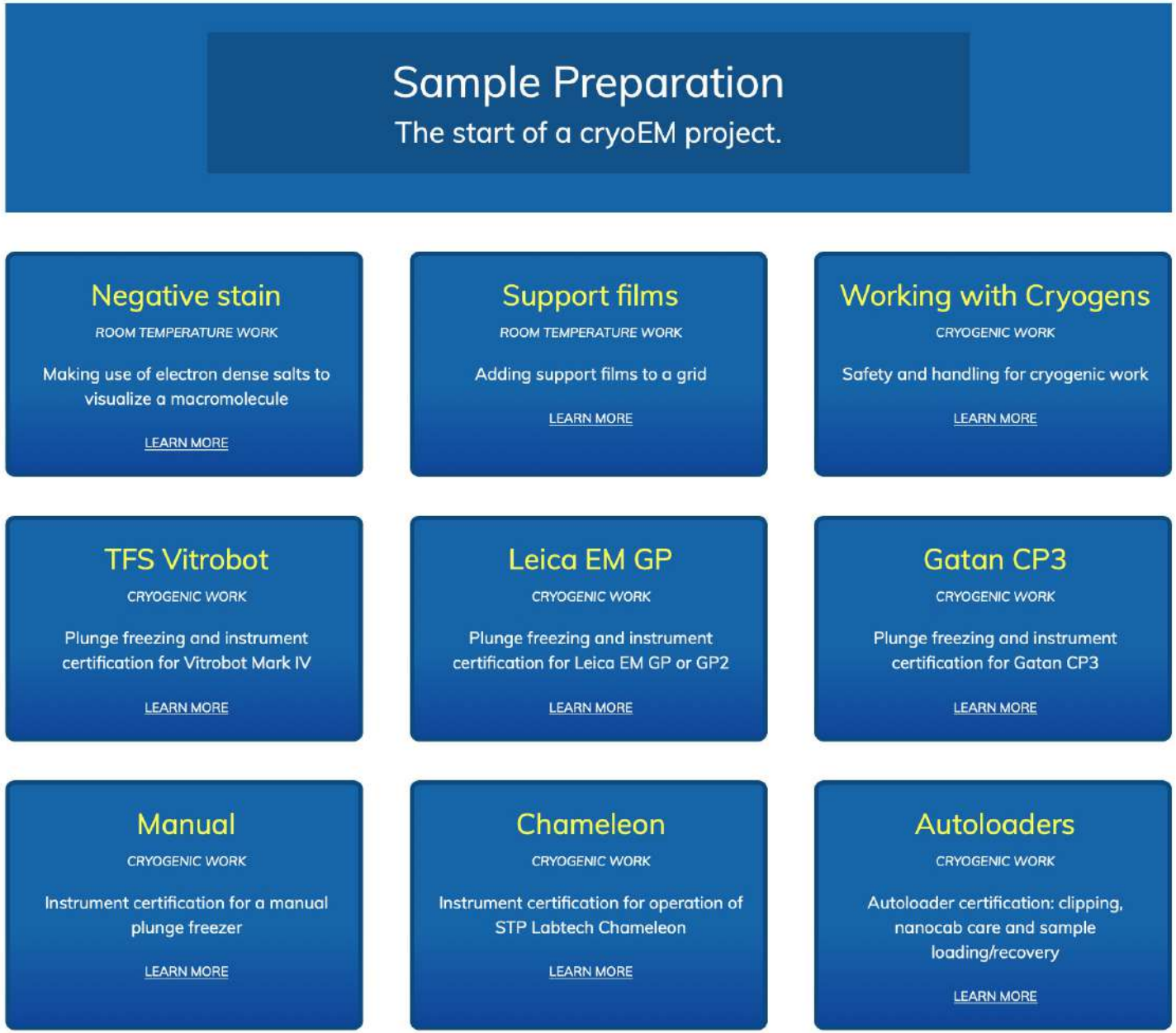
I. Sample purification and grid preparation
   a) cryoEM merit badges
   b) Chameleon demo
overview of blot free vitrification vs plunge freezing methods

II. Grid screening & evaluation
   a) Sample holders
   -Side entry systems: Gatan 626/Elsa holder and loading
   -Autoloader systems: autogrid clipping and loading
   b) F20 setup and demo of screening with Leginon

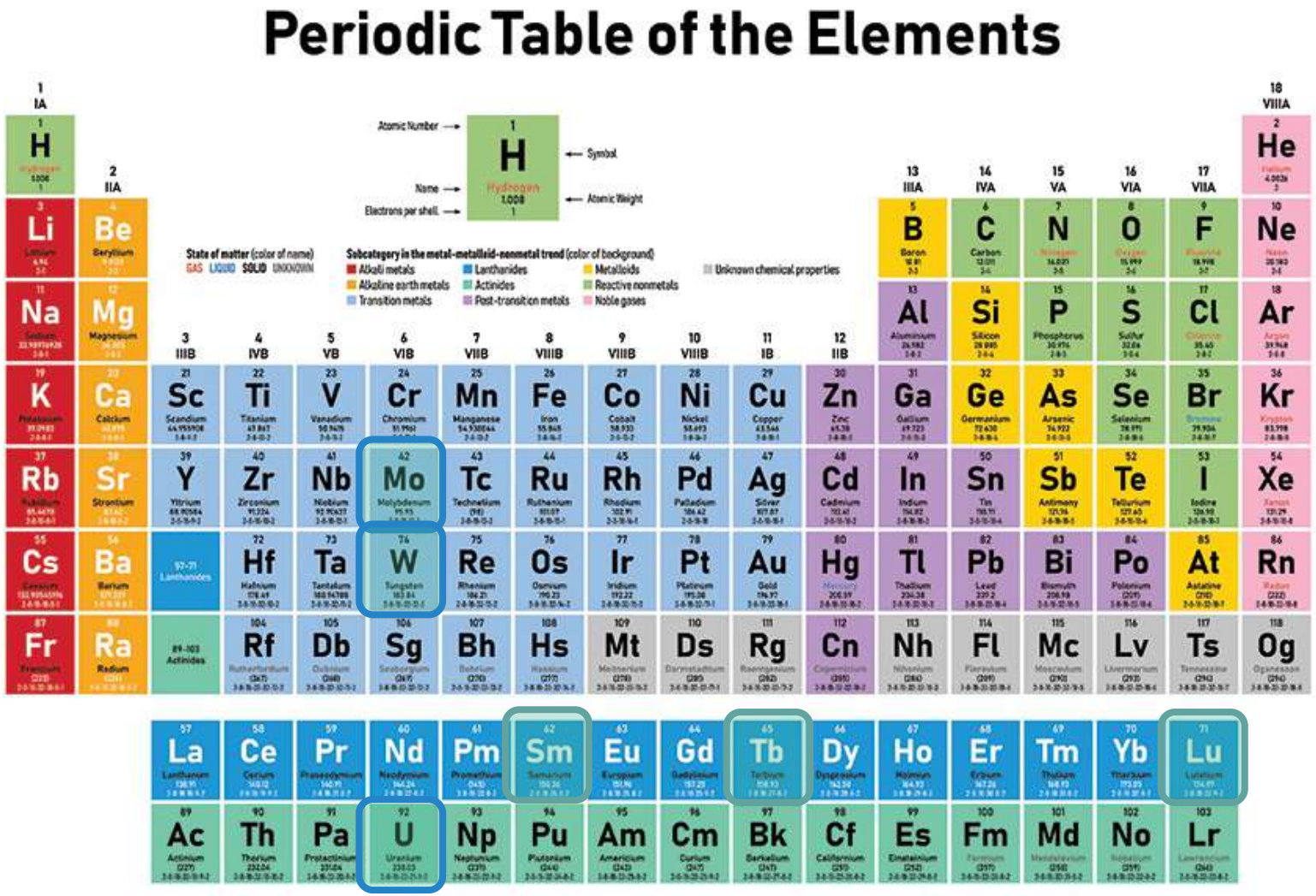
III. Cryo-EM data collection
   a) Glacios setup and advanced sample screening/preliminary data collection with Leginon
   b) Krios high res data collection with Leginon

IV. Image (pre)-processing
   a) On the fly feedback cryoSPARC live
   b) Working with your own data

THE STARTING POINT – COHORT3



NEGATIVE STAIN



WHAT IS YOUR NEGATIVE STAIN USE?

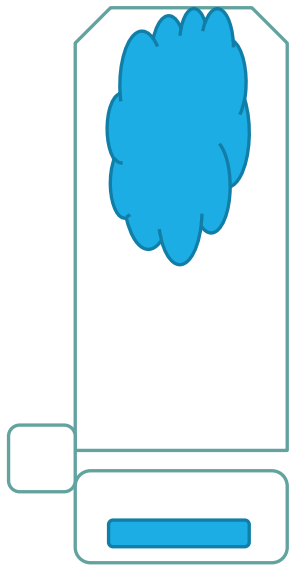
Daniela	Screening samples 2D classes 3D models	0.7% Uranyl Formate
Eric	Spot check	0.7% Uranyl Formate
Tilini	Spot check	0.7% Uranyl Formate/Acetate
Justin	Spot check Moving along SEC profile	0.7% Uranyl Formate/Acetate

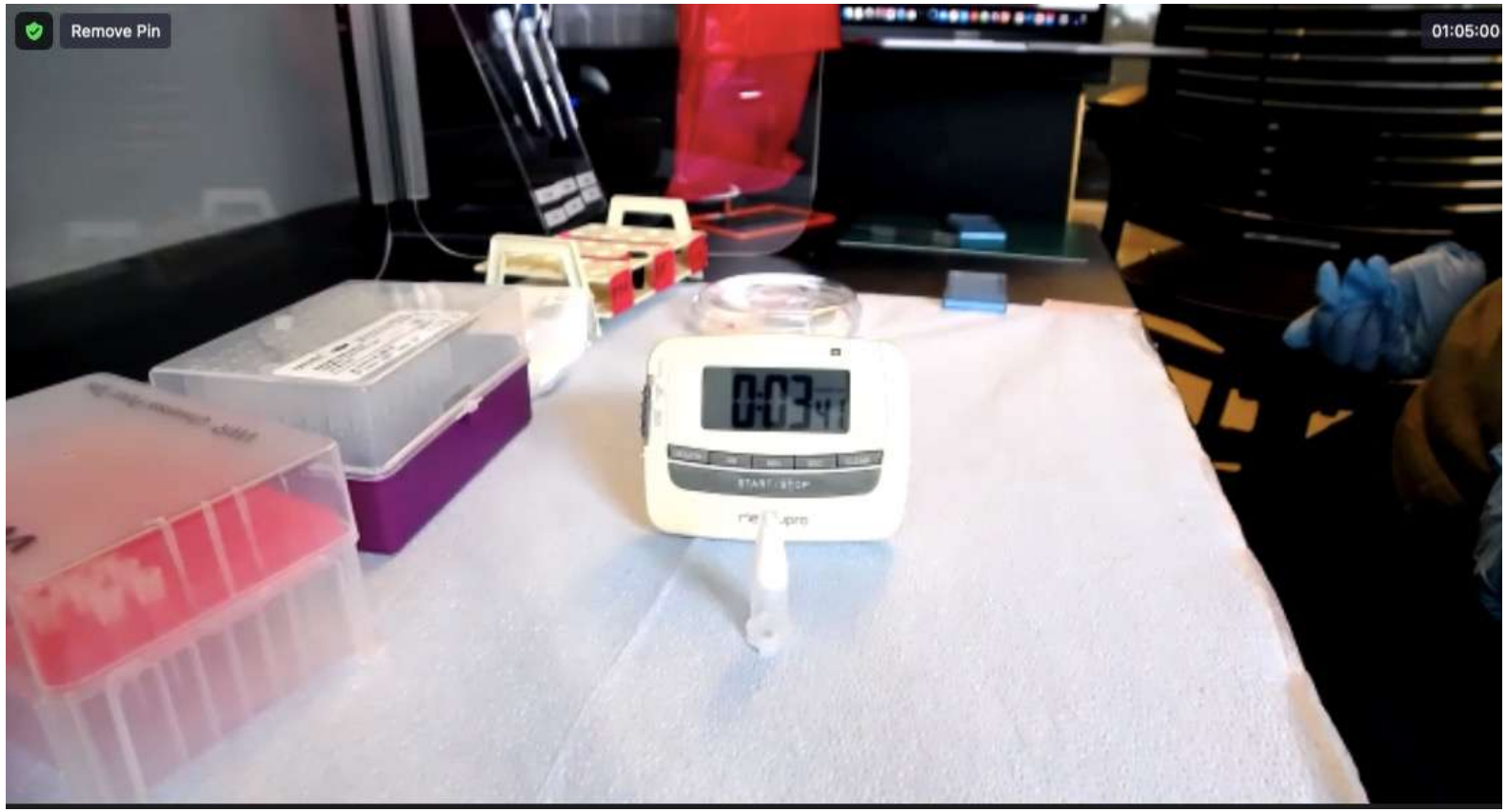
WHICH SIDE TO PUT SAMPLES ON?

Shiny / polished
Dull / rough

Glow discharge parameters
-Power / Current
-O2, H2, Ar, Air

NEGATIVE STAIN INCUBATION IN A MICROFUGE TUBE

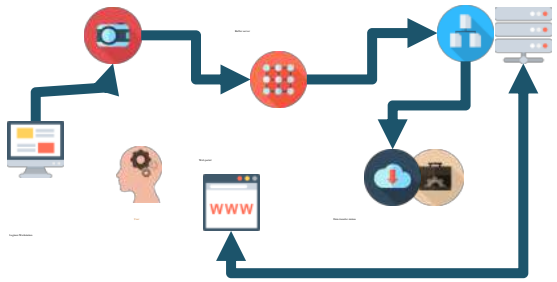




WHERE ARE THE BOTTLENECKS?

NRAMM

technology aimed towards completely automating the processes involved in solving macromolecular structure using cryo-electron microscopy (cryoEM)



Sample preparation

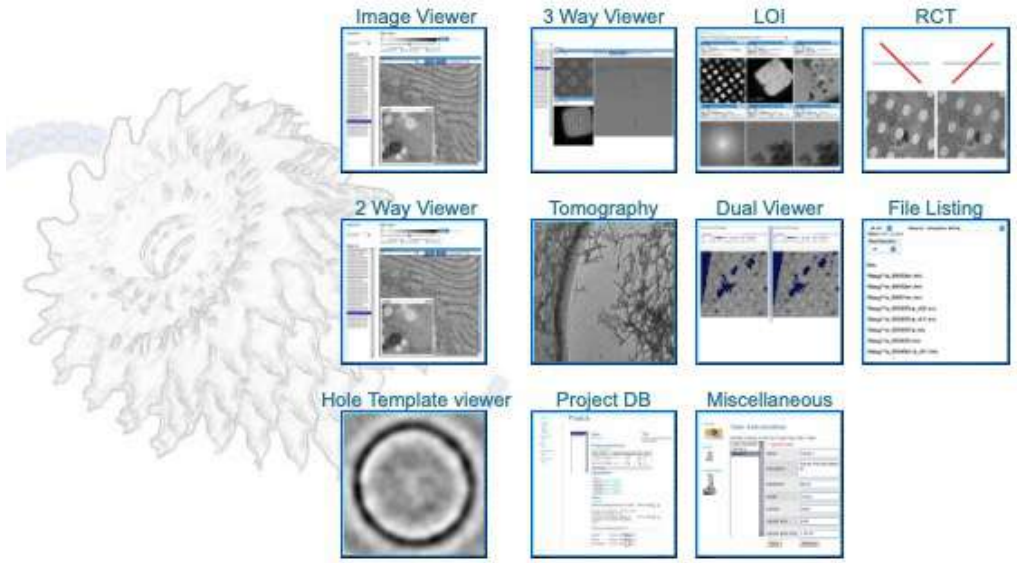
Data collection

(pre-)Processing

Spotiton

Appion

EMGWEB.NYSBC.ORG Appion and Leginon Tools



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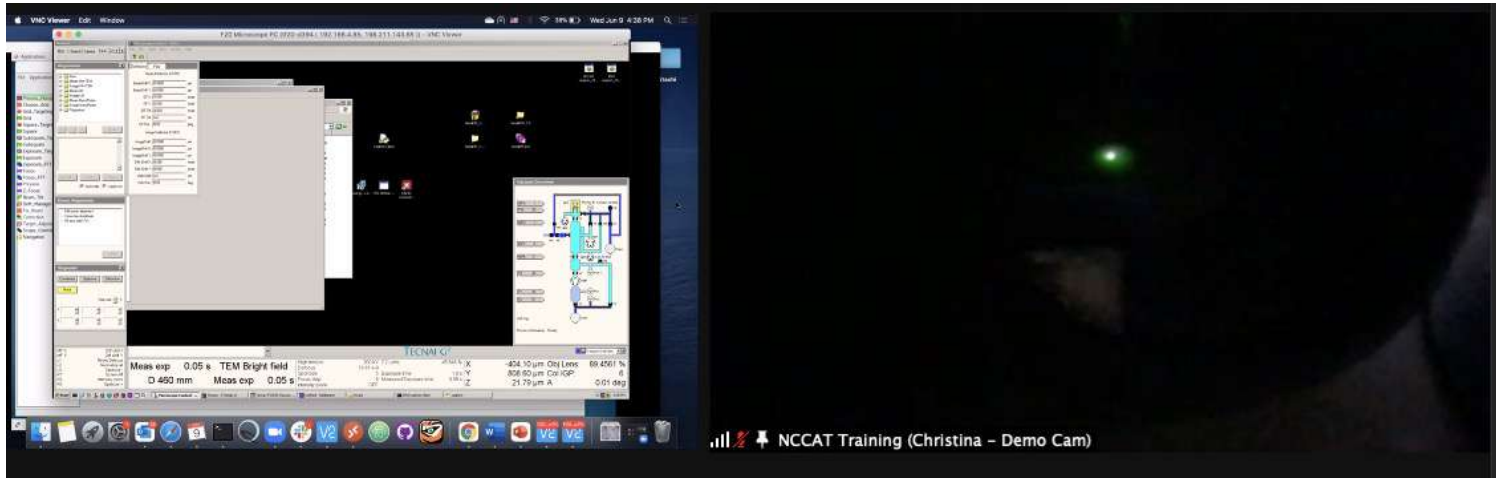
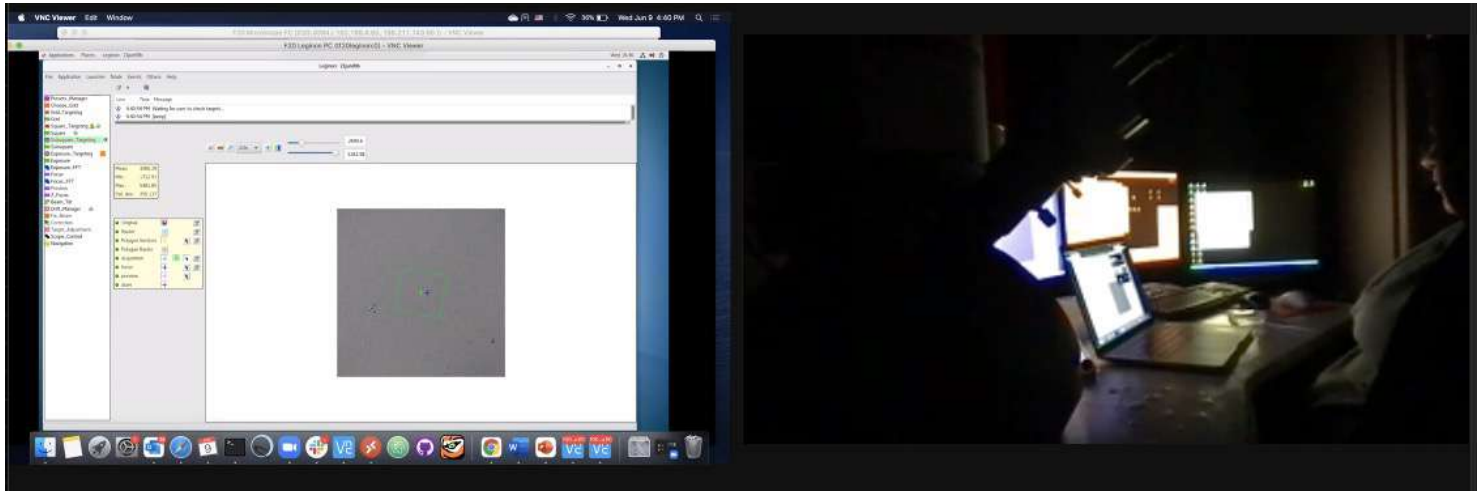
ninfo / nccat032020

ON THE F20





WEEK 1
JUNE 6/09-10

WEEK 2
JUNE 6/16-17

Wed 6/9 3-5pm

Overview of what's happening in June

Introduction to merit badges

Practical: NS-EM demo with F20 to spot check and iterate

Thur 6/10 2-5pm

Screening microscope (F20) in detail

Screening with Leginon

SCREENING

- Grid handling
- Holders
- Screening microscopes
- Cameras
- Strategy – Leginon
- Pre-processing
- Optimize

HOLDERS

Daniela – TF20 | Polara
RT holder – Gatan 626

Eric – TF20
RT holder – Gatan 626 – Gatan Elsa

Tilini – Autoloaders all the way

Justin – TF20 | Spirit
RT holder – Gatan 626

Fomblin – O rings

MICROSCOPE ALIGNMENTS

- Factory alignments
- Engineer/Service alignments
- User alignments

-Alignment tab

-Direct alignments

Gun tilt

Gun shift

Pivot points

Beam tilt

Rotation center

Stigmators

Aperature

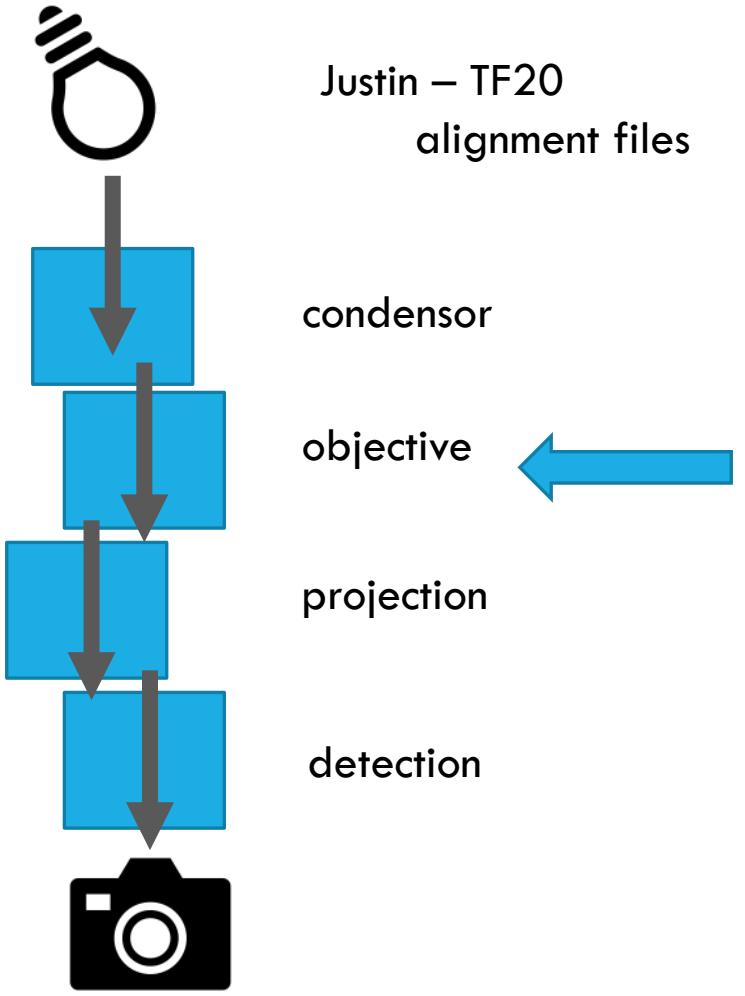
What is important for screening?

Eric – TF20

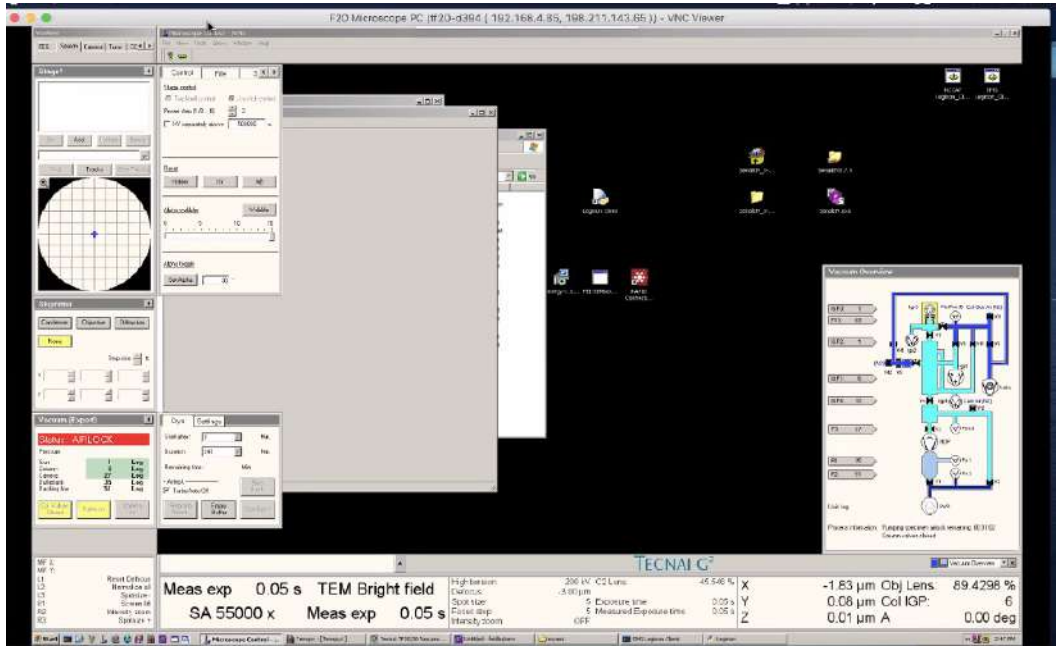
beam shift | beam tilt | stigmators

Justin – TF20

alignment files

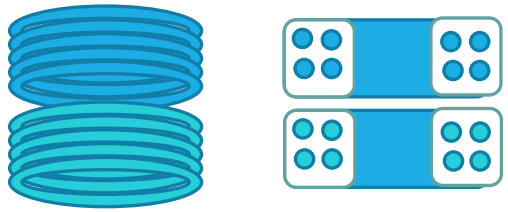


ON THE F20



Microscope considerations

Tungsten filament
LaB6
FEG



C2
Objective
SA

Diffusion
PVP
Turbo
IGP

LEGINON

Leginon allows for automated data collection using object oriented interface.

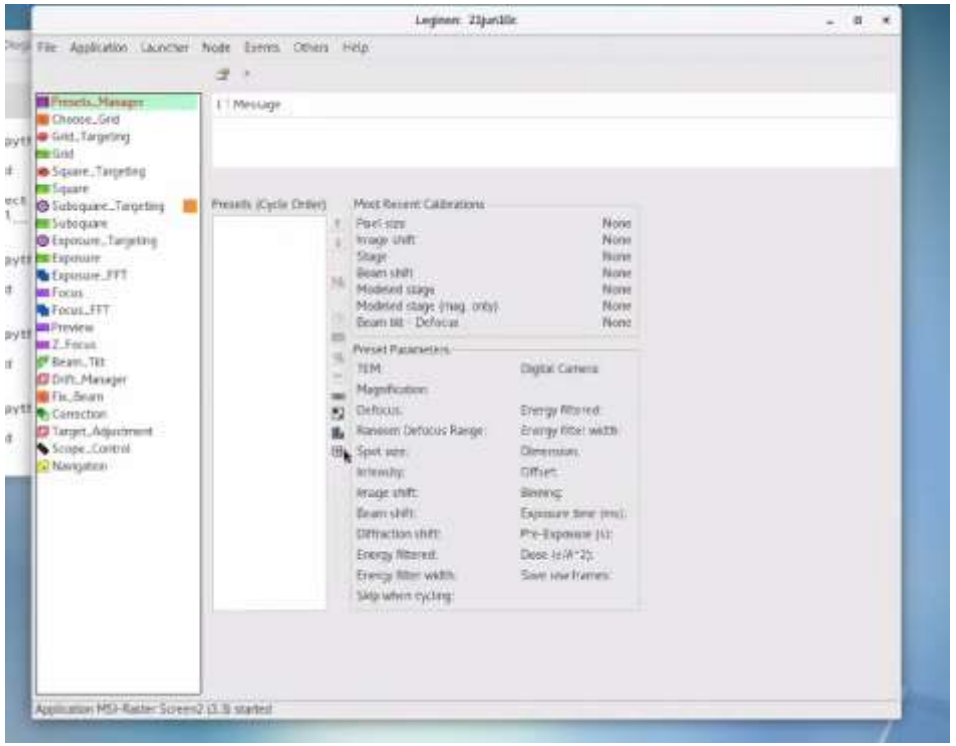
Microscope client

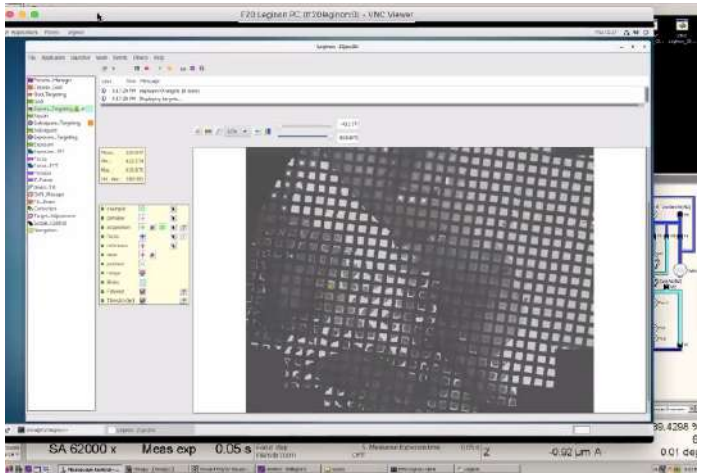
Camera client

Leginon server

Database Storage server

Web interface





LEGINON

leginon.org

21jun10c_G1_00017gr_00003sq_v01_00003hl_00002en.mrc

Presets

gr - grid

sq - square

hl - hole

fa/fc -focus

en - exposure

Applications

MSI-T

Multi-scale imaging

-low dose imaging

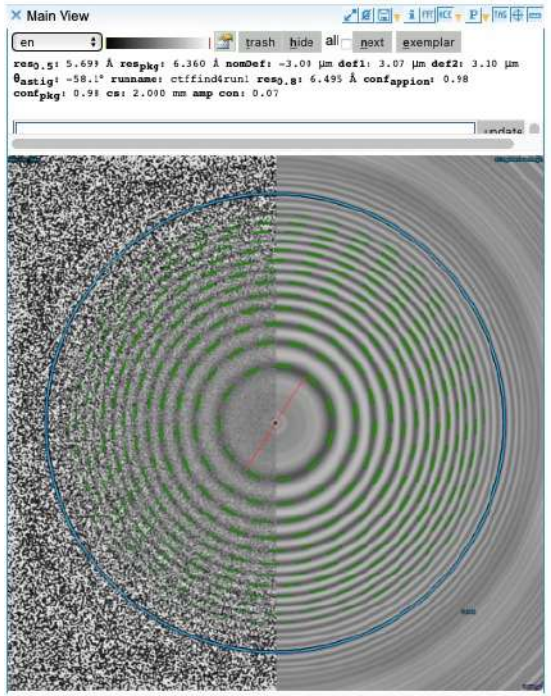
WHAT ABOUT PRE-PROCESSING?

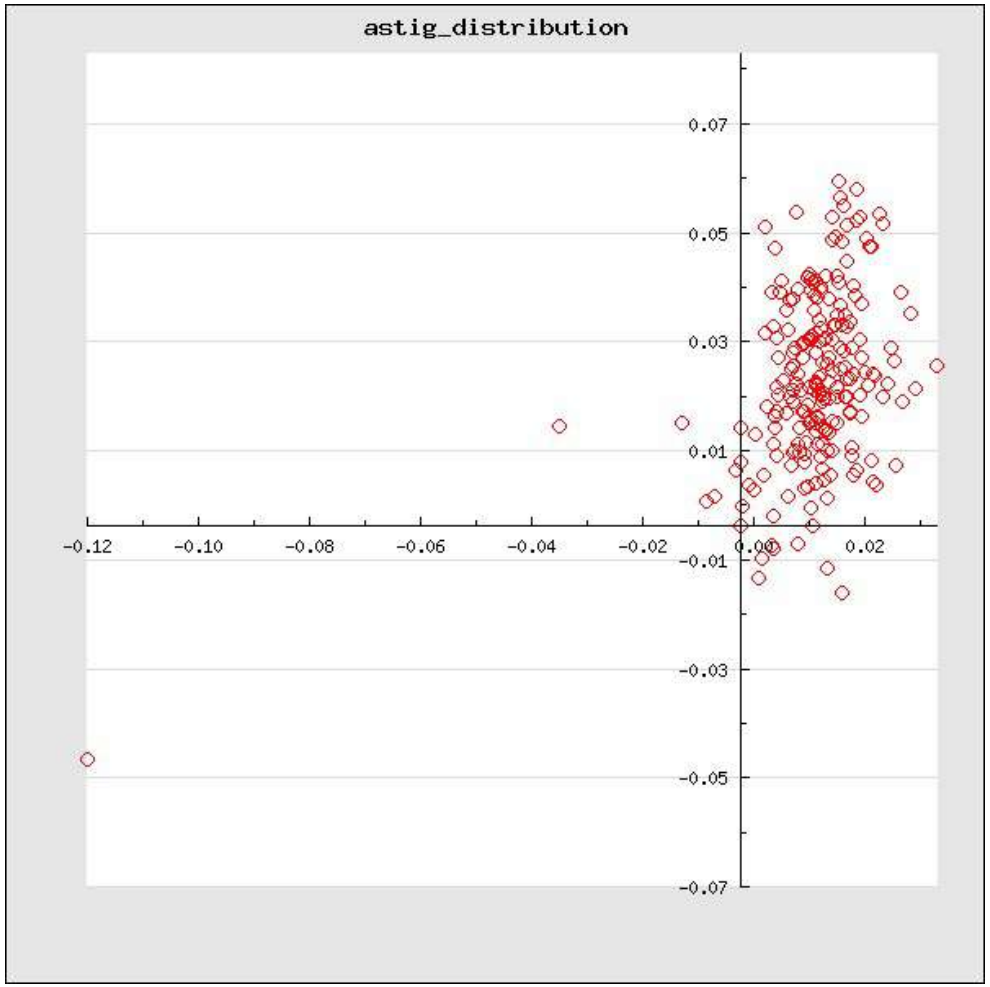
CTF estimation

Overall Summary for 216 CTF estimates

Overall Stats: (download ctf data)

	nb	min	max	avg	stddev
defocus1	216	2.81 µm	3.39 µm	3.09 µm	0.10 µm
defocus2	216	2.91 µm	3.43 µm	3.16 µm	0.10 µm
angle_astigmatism	216	-89.760	81.551	-53.180	26.050
extra_phase_shift	216	0	0	0	0
resolution_80_percent	216	5.492 Å	28.165 Å	6.807 Å	1.629 Å
resolution_50_percent	216	4.916 Å	11.084 Å	6.059 Å	0.643 Å
package resolution	216	5.796 Å	12.047 Å	6.662 Å	0.638 Å





WHAT ABOUT PRE-PROCESSING?

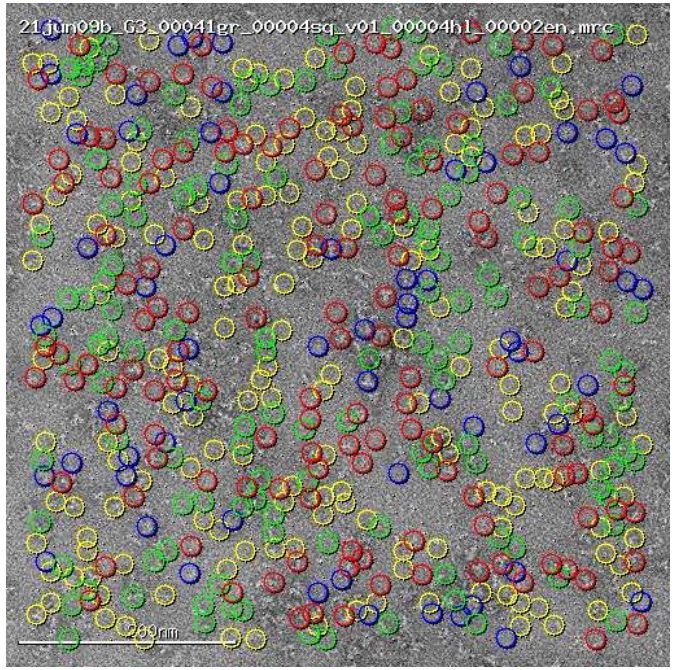
Particle picking / Object selection

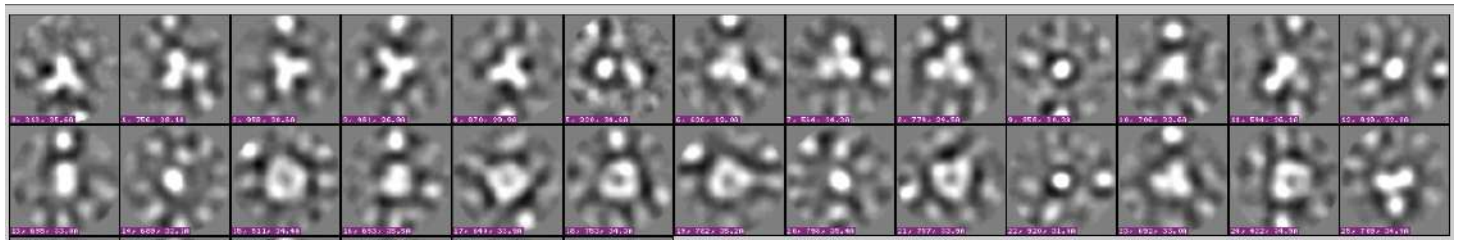
Particle Selection Info: tmplrun1 (ID: 2) hide delete

date time:	2021-06-10 11:51:56
method:	Template Correlator
preset:	en
path:	/gpfs/appion/dfera/21jun09b/extract/tmplrun1
# particles:	198,876 (download coordinates)
# images:	216 (920.7 part/img)
view picks:	view picks in multi-assessor

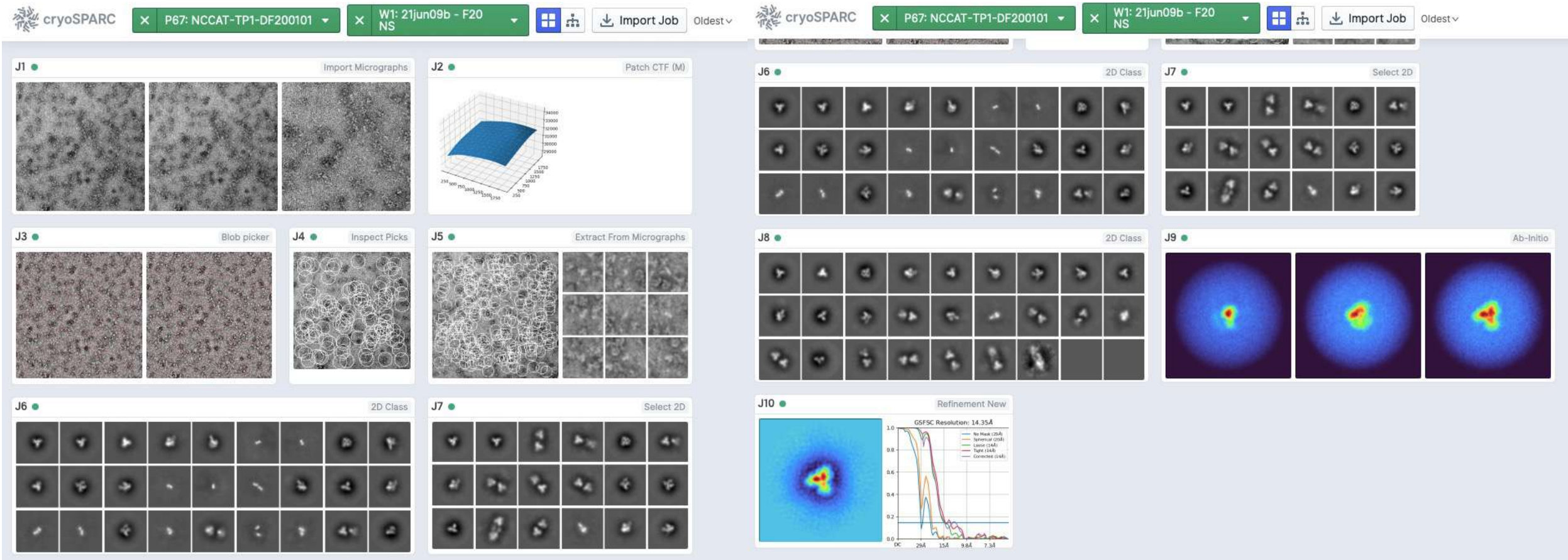
Particle Selection Info: dogrun1 (ID: 1) hide delete

date time:	2021-06-09 17:24:43
method:	DOG Picker
preset:	en
path:	/gpfs/appion/dfera/21jun09b/extract/dogrun1
# particles:	58,067 (download coordinates)
# images:	216 (268.8 part/img)
view picks:	view picks in multi-assessor





CRYOSPARC PRE-PROCESSING



SCREENING CONSIDERATIONS ON THE MICROSCOPE

Do you take an Atlas?

How do you spot check?

How much data needs to be collected?

How many grids do you need to screen?

Can you save grids?

Record keeping?

WHAT'S NEXT?

WEEK 2
JUNE 6/16-17

Wed 6/16 3-5pm

Overview of cryo sample prep

Introduction to merit badges

Practical: Vitrobot Mark IV and Glacios

Thur 6/17 2-5pm

Screening microscope (Glacios) in detail

Screening with Leginon