

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 apolipoprotein 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 through the NCCAT cross-training program.

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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

The screenshot shows the NCCAT Remote Cross-training Central webpage. At the top is the NCCAT logo and the text "National Center for CryoEM Access and Training". Below this is a navigation bar with links: HOME, ABOUT, NEWS, ACCESS, TRAINING, PUBLICATIONS, and JOBS. The main content area is titled "Remote Cross-training Central" and features a central graphic with seven hexagons representing different training components: Institutional resources, Eo's Master class, Core knowledge, Microscope operations, Legion Remote, Sample preparation, Round tables, Project meetings, Office hours, Andon Workshops, and Processing & analysis. To the right of the graphic is the text "NCCAT Remote cross-training program". Below the graphic is a "Learn more" section with links to: Remote cryoEM Master Class, Remote Sample Preparation Module, Remote Microscope Operation Module, Remote Pre-processing & Data Analysis Module, and Overview of the Cross-training Components. On the left side of the page, there is a "TRAINING" section with links to: NCCAT Workshops and Short courses, SPA Short course 2020, Tomo Short course 2021, NCCAT Cross-training Programs, NCCAT Remote Learning, Remote Office Hours, Online Classroom, Previous EM Courses, and Curriculum Partners.

Tentative Round Table Schedule

Round table registration will be open to a larger group but embedded trainees have priority to available seats. Round tables are open forums that start with a 5-10 intro from a SEMC staff member to kick off the discussion, followed by open Q&A, an opportunity to share data/intermediate results, and discussion amongst the group.

Tuesdays at 3:30

4/12 - Day 1 of the Tomography short course (not actually a round table but the reason

Remote Learning Central

The screenshot shows the NCCAT Remote Learning Central webpage. At the top is the NCCAT logo and the text "National Center for CryoEM Access and Training". Below this is a navigation bar with links: HOME, ABOUT, NEWS, ACCESS, TRAINING, PUBLICATIONS, and JOBS. The main content area is titled "NCCAT Remote Learning Central" and features a central graphic with a green circle containing a white cloud icon. To the right of the graphic is the text "NCCAT Remote Learning Central". Below the graphic is a "Learn more" section with links to: Remote cryoEM Master Class, Remote Sample Preparation Module, Remote Microscope Operation Module, Remote Pre-processing & Data Analysis Module, and Overview of the Cross-training Components. On the left side of the page, there is a "TRAINING" section with links to: NCCAT Workshops and Short courses, SPA Short course 2020, Tomo Short course 2021, NCCAT Cross-training Programs, NCCAT Remote Learning, Remote Office Hours, Online Classroom, Previous EM Courses, and Curriculum Partners.

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

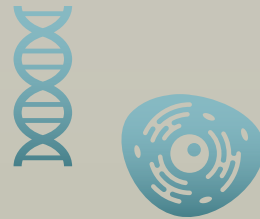
3D Reconstruction

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

Sample Preparation

The start of a cryoEM project.

Negative stain

ROOM TEMPERATURE WORK

Making use of electron dense salts to visualize a macromolecule

[LEARN MORE](#)

Support films

ROOM TEMPERATURE WORK

Adding support films to a grid

[LEARN MORE](#)

Working with Cryogenics

CRYOGENIC WORK

Safety and handling for cryogenic work

[LEARN MORE](#)

TFS Vitrobot

CRYOGENIC WORK

Plunge freezing and instrument certification for Vitrobot Mark IV

[LEARN MORE](#)

Leica EM GP

CRYOGENIC WORK

Plunge freezing and instrument certification for Leica EM GP or GP2

[LEARN MORE](#)

Gatan CP3

CRYOGENIC WORK

Plunge freezing and instrument certification for Gatan CP3

[LEARN MORE](#)

Manual

CRYOGENIC WORK

Instrument certification for a manual plunge freezer

[LEARN MORE](#)

Chameleon

CRYOGENIC WORK

Instrument certification for operation of STP Labtech Chameleon

[LEARN MORE](#)

Autoloaders

CRYOGENIC WORK

Autoloader certification: clipping, nanocab care and sample loading/recovery

[LEARN MORE](#)

Periodic Table of the Elements

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

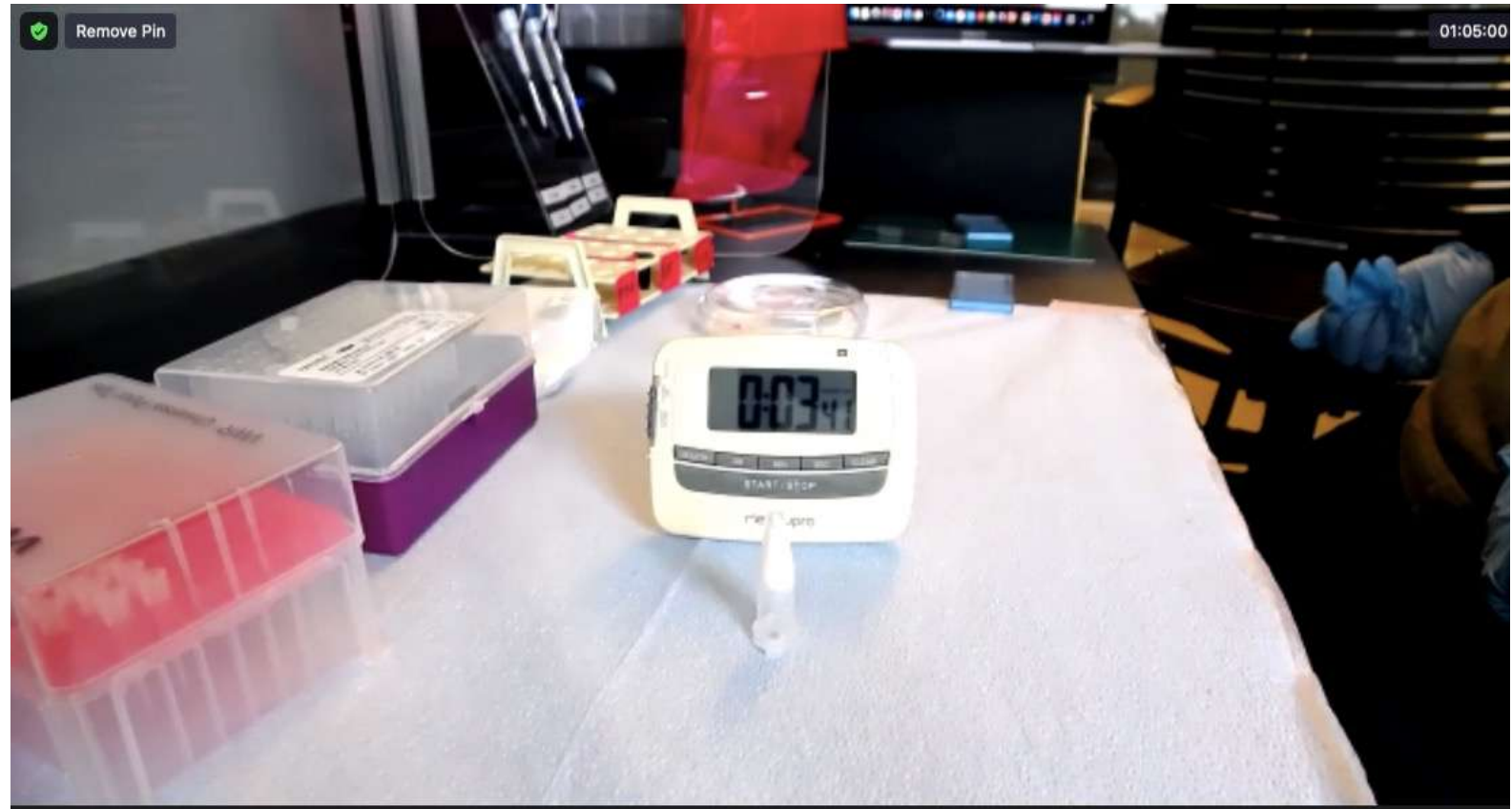
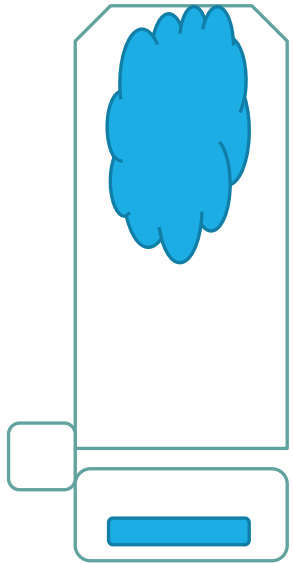
WHICH SIDE TO PUT SAMPLES ON?



Shiny / polished
Dull / rough

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

NEGATIVE STAIN INCUBATION IN A MICROFUGE TUBE



WHERE ARE THE BOTTLENECKS?

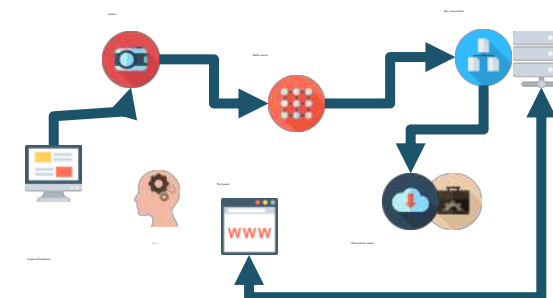
technology aimed towards completely automating the processes involved in solving macromolecular structure using cryo-electron microscopy (cryoEM)



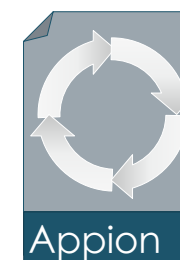
Sample preparation



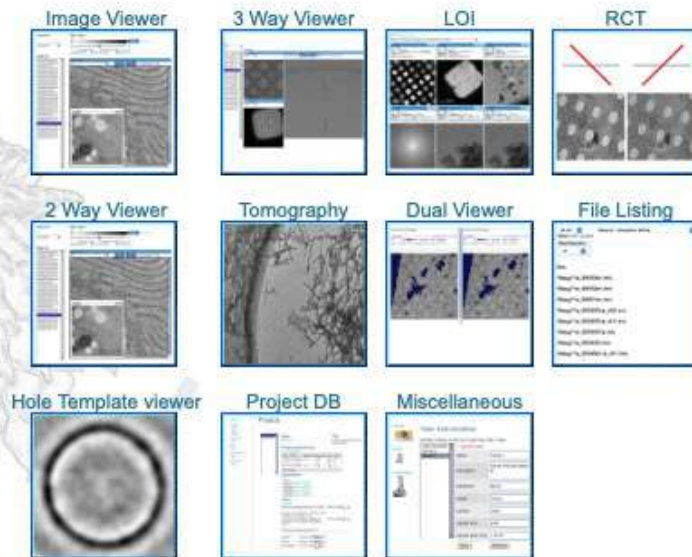
Data collection



(pre-)Processing



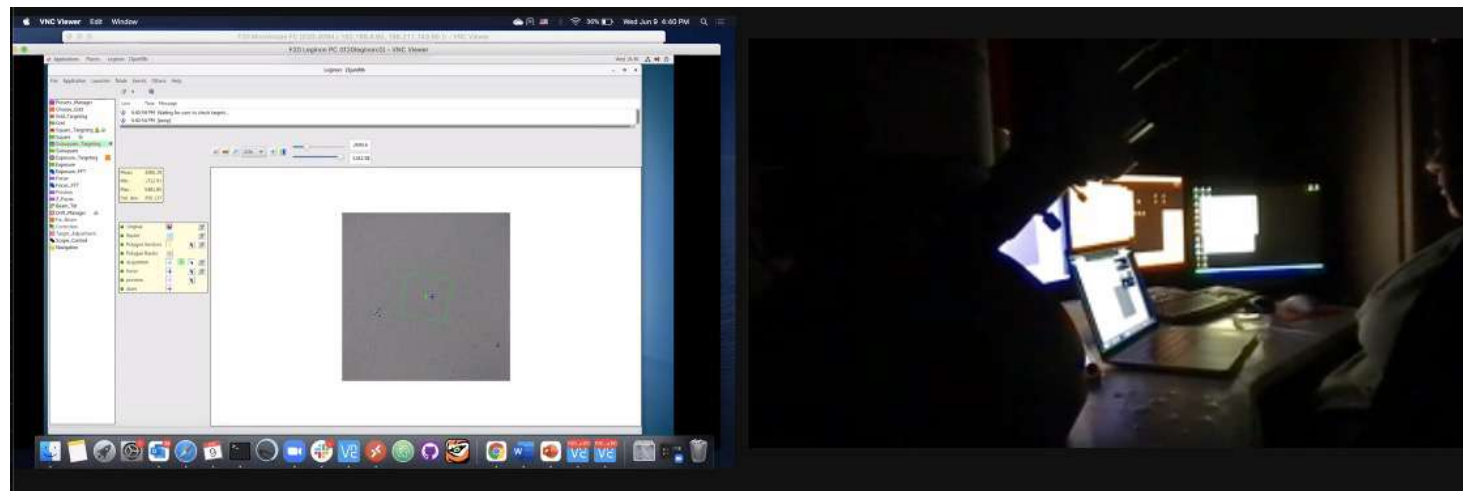
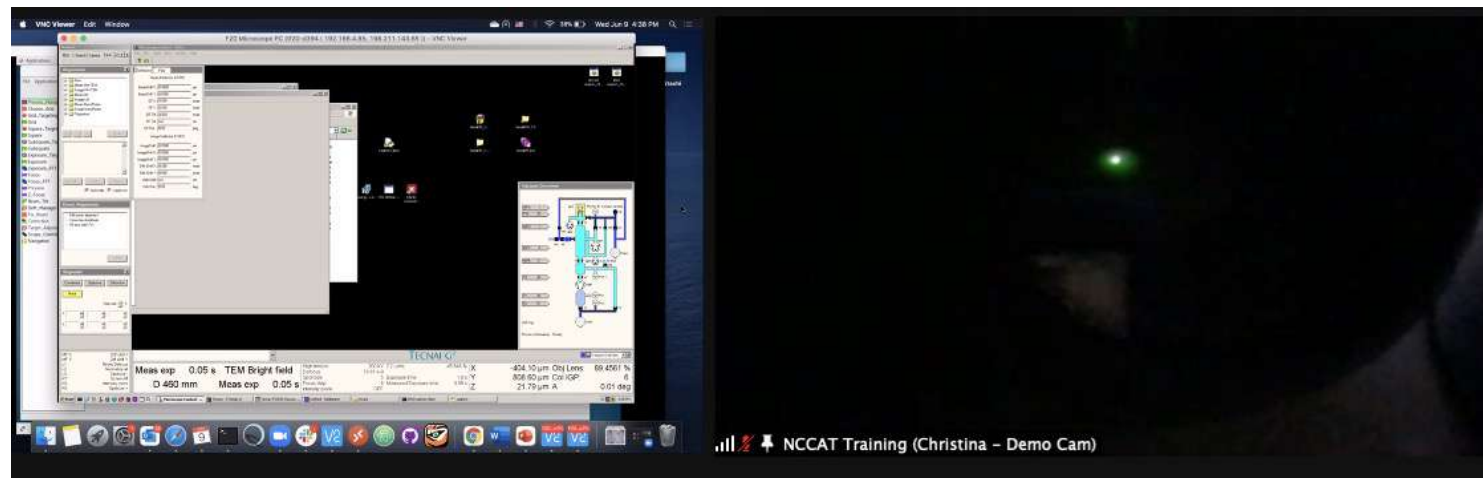
EMGWEB.NYSBC.ORG Appion and Leginon Tools



EMGWEB.NYSBC.ORG

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

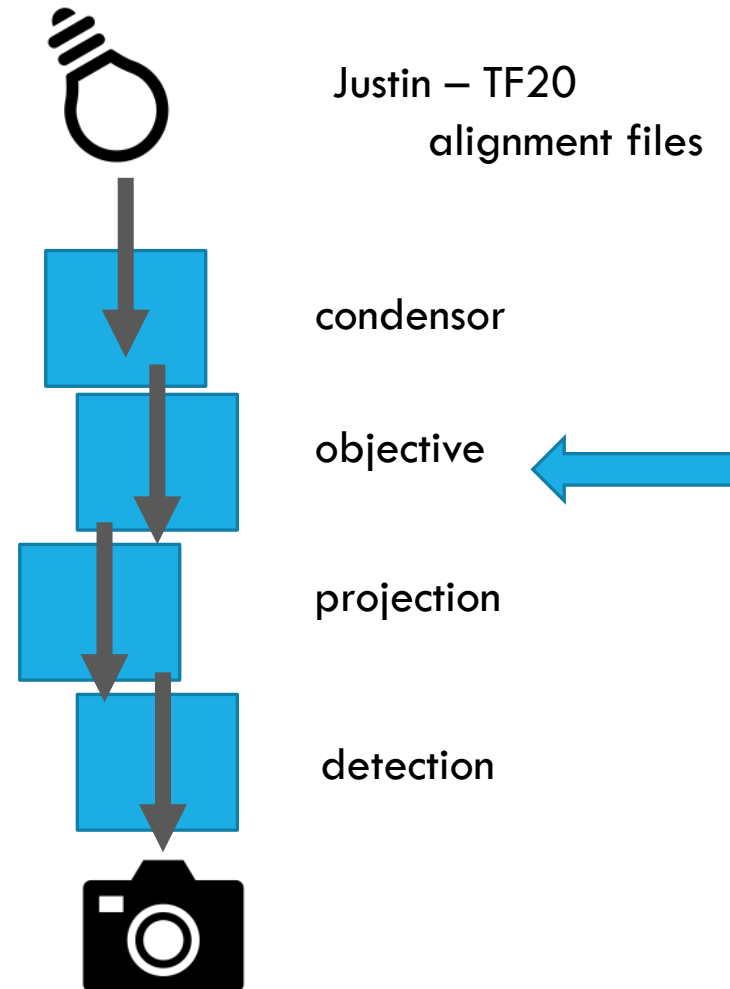
What is important for screening?

Eric – TF20

beam shift | beam tilt | stigmators

Justin – TF20

alignment files





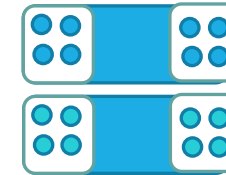
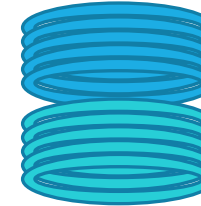
Microscope considerations



Tungsten filament

LaB6

FEG



C2

Objective

SA

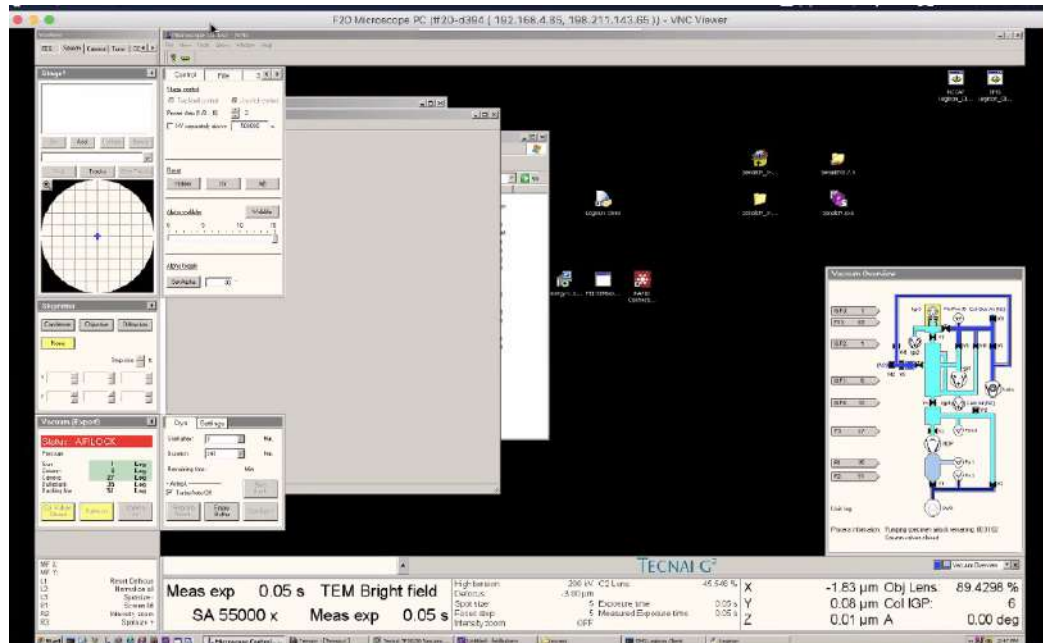


Diffusion

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

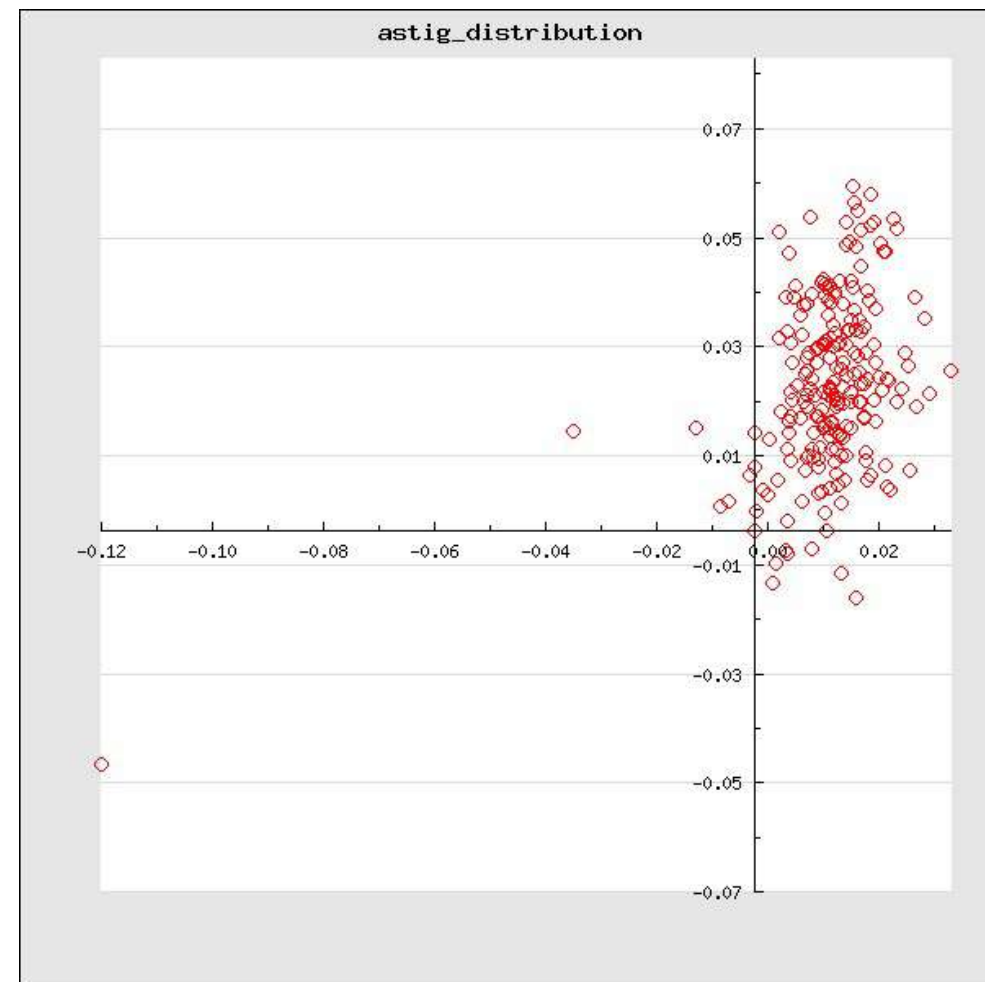
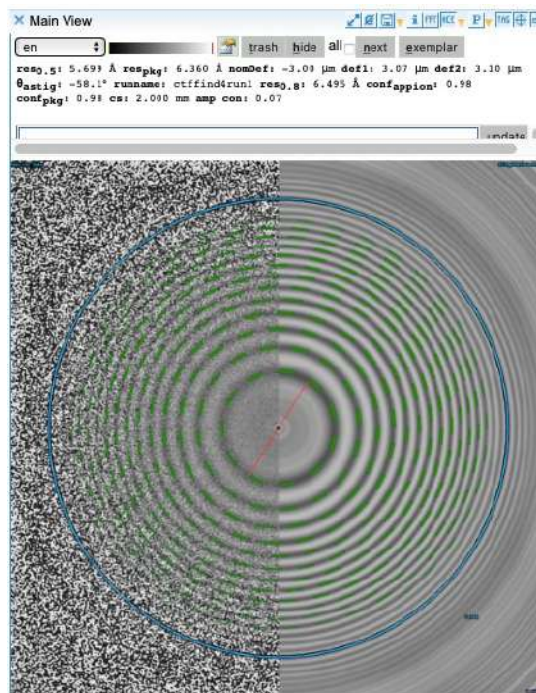
WHAT ABOUT PRE-PROCESSING?

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?

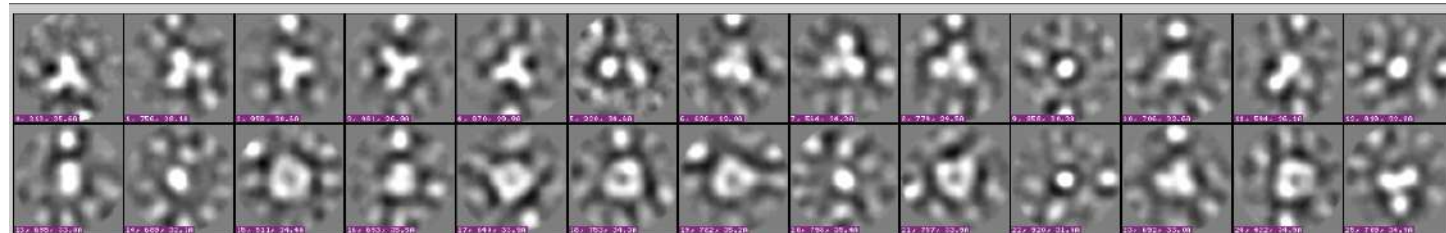
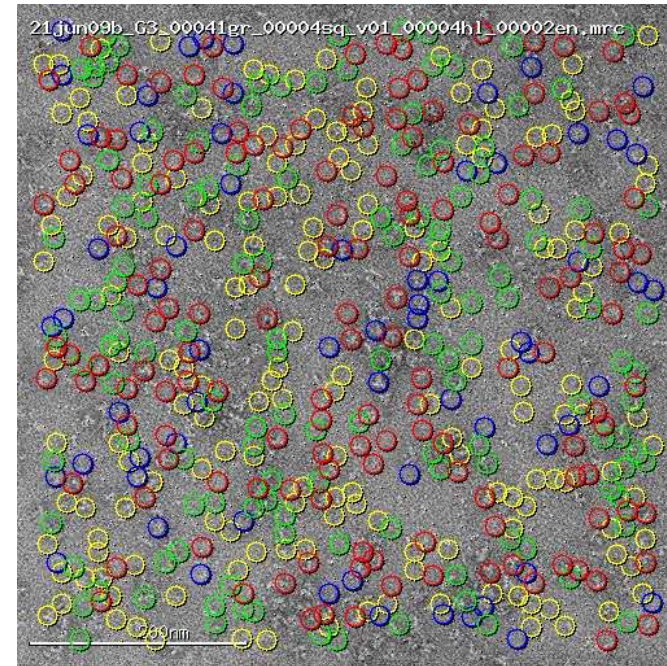
Particle picking / Object selection

Particle Selection Info: **tmplrun1** (ID: 2) [hide](#) [delete](#)

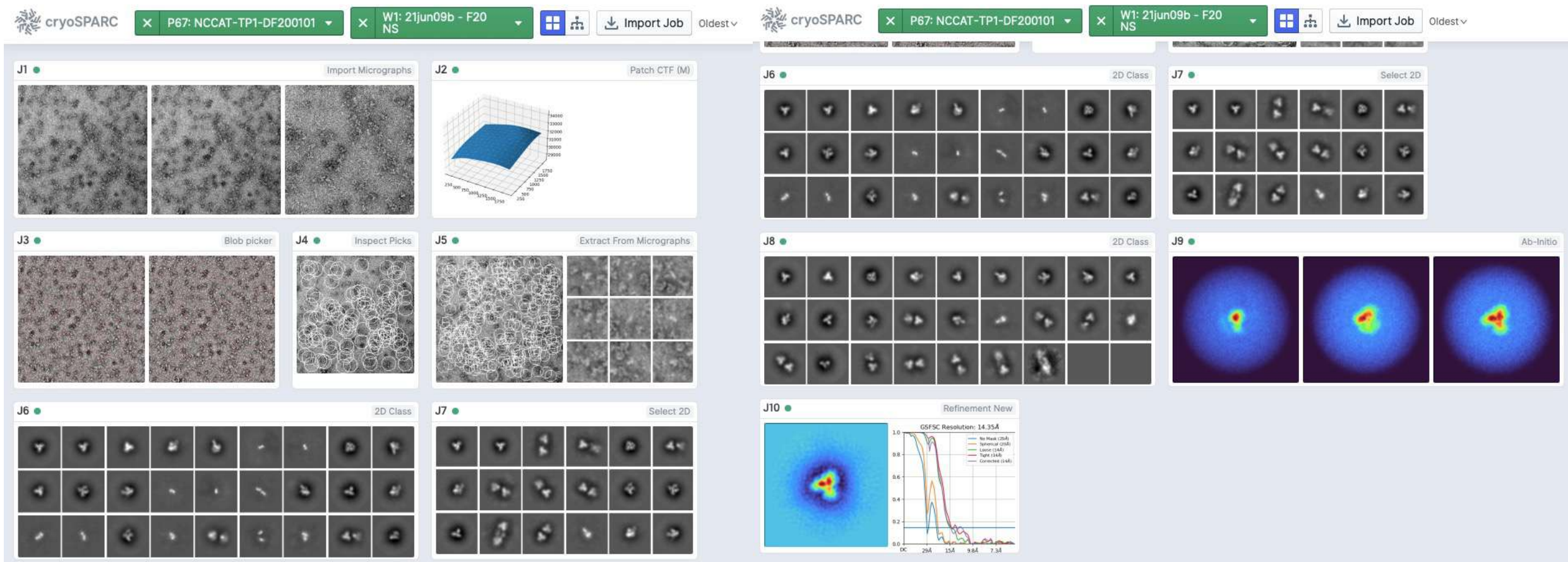
date time: 2021-06-10 11:51:56
method: Template Correlator
preset: en
path: /gpfs/appion/dfera/21jun09b/extract/tmplrun1
particles: 198,876 ([download coordinates](#))
images: 216 (920.7 part/img)
view picks: [view picks in multi-assessor](#)

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