Cryo-electron microscopy at Leeds

Approaches and challenges for high resolution data acquisition

Dr Rebecca Thompson

ASTBURY BIOSTRUCTURE LABORATORY

UNIVERSITY OF LEEDS


Quiet
Rehearsal
Recording


Astbury
Biostructure
Laboratory

Officially opened January 2017..


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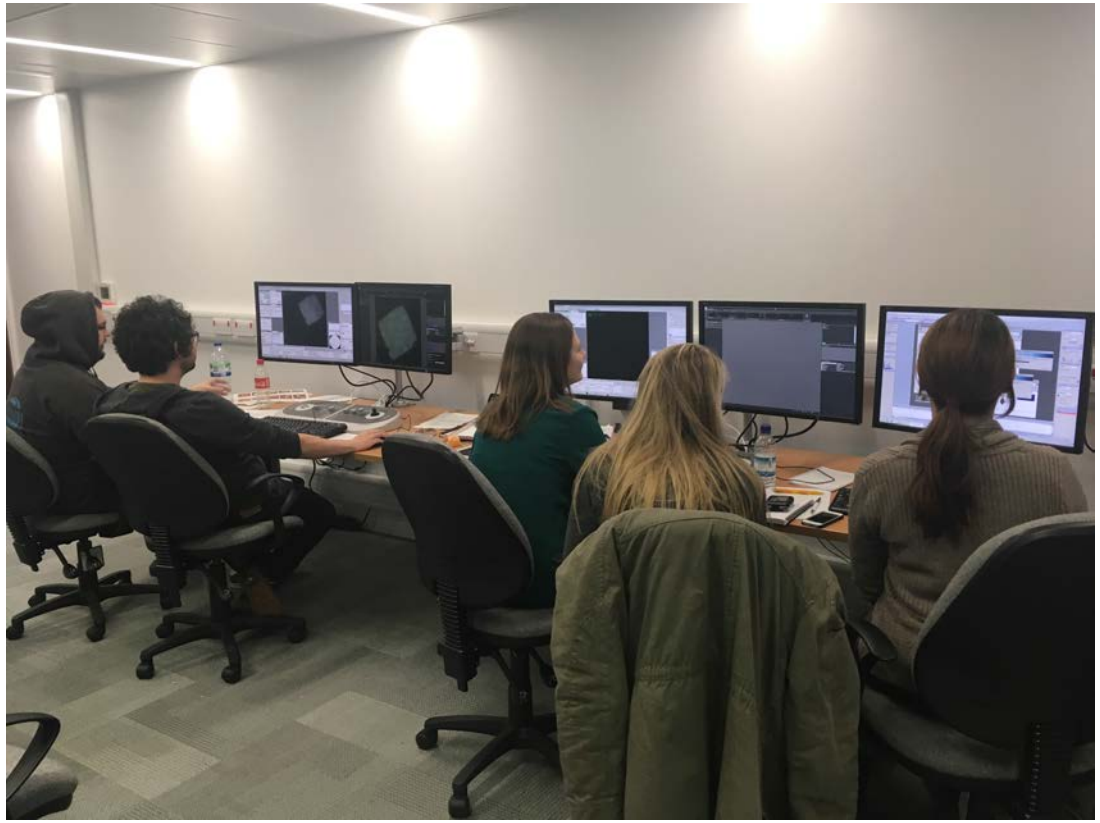
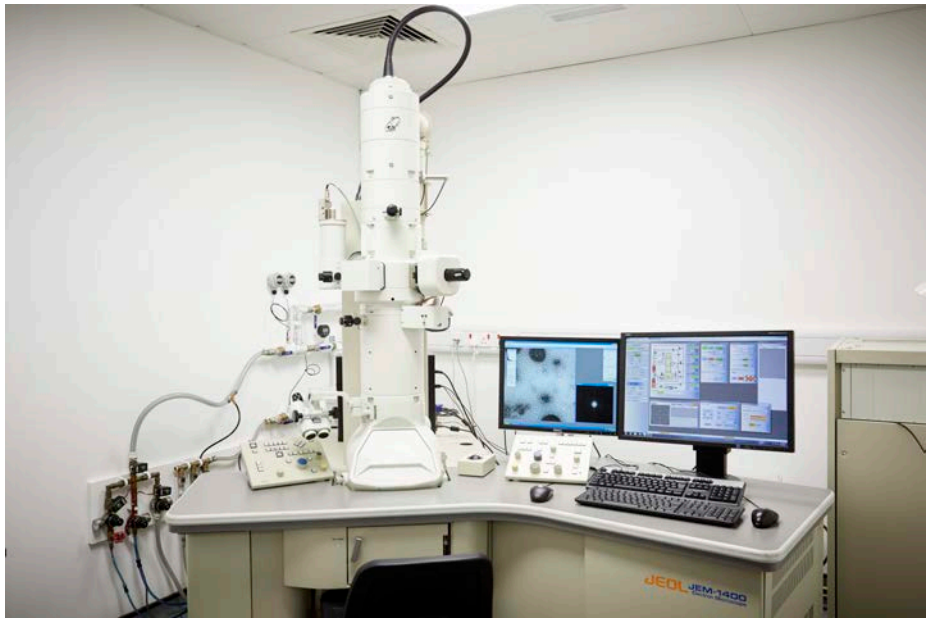
X-FEG





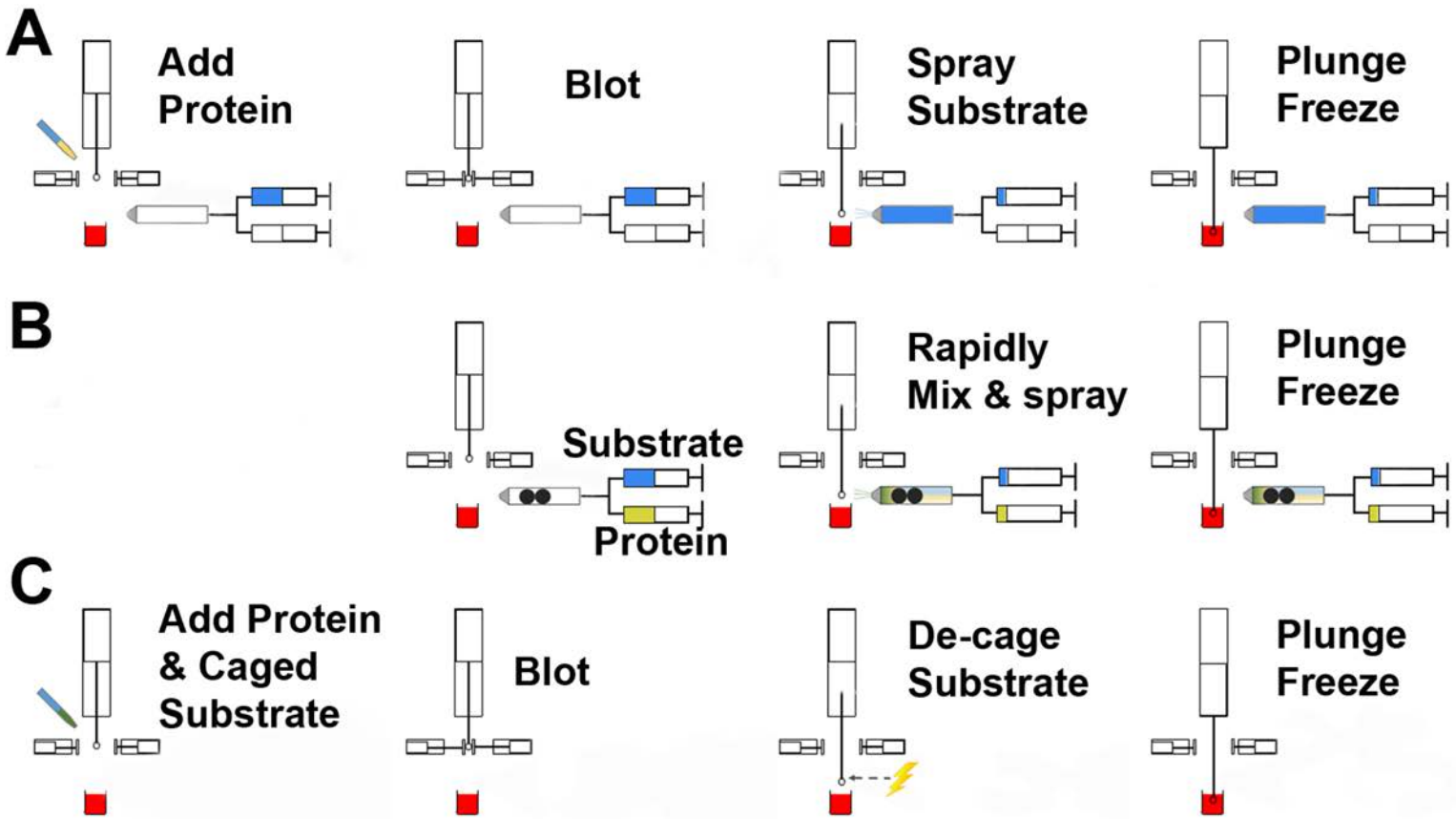
Transmission electron microscopes

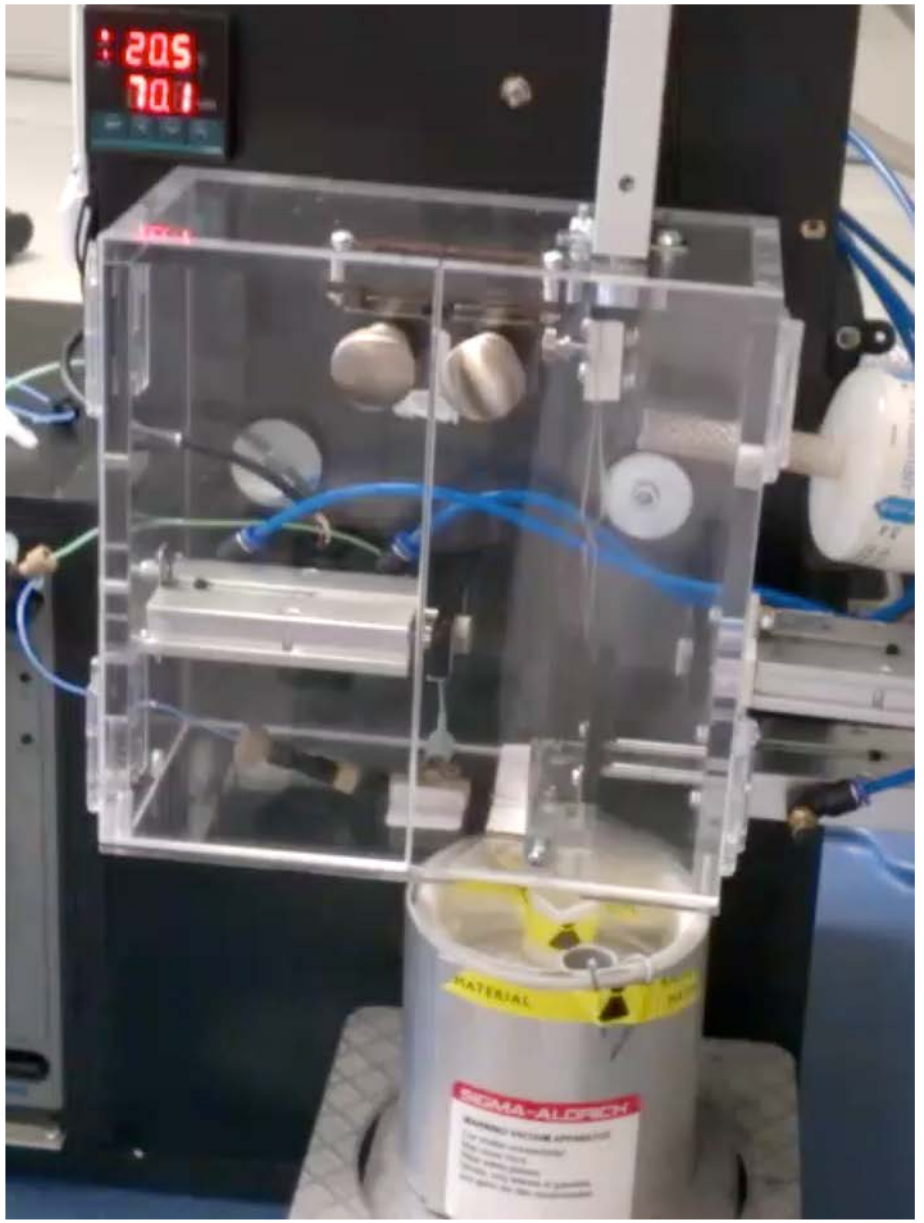
- JEOL 1400- Negative stain/ambient sections
- FEI T12- Negative stain/ambient sections
- FEI F20- Negative stain/ambient sections/ cryo-EM screening/tomography

Sample preparation of macromolecular complexes

- Leica EM-GP- adherent cells/macromolecular complexes
- Vitrobot Mk 4- macromolecular complexes
- Housed in < 20 % RH sample preparation room

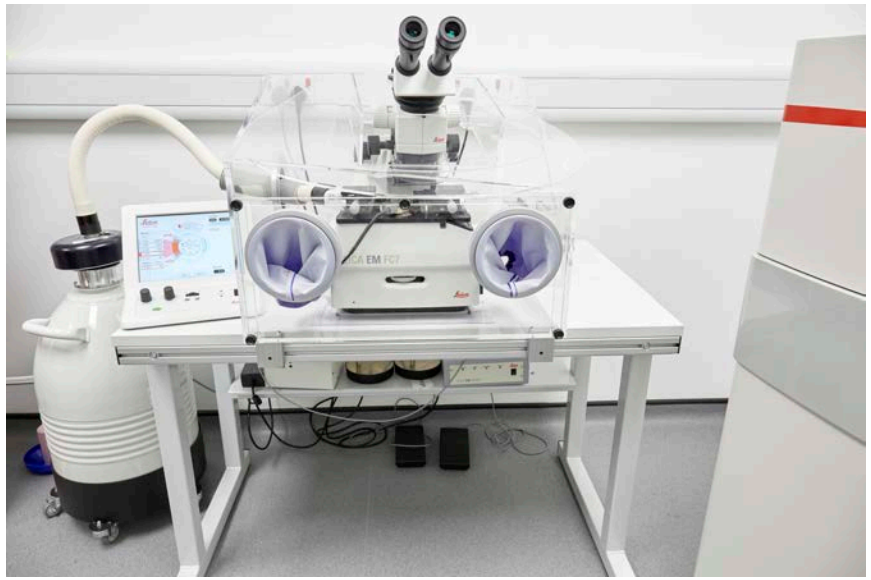
Spraying approaches and time resolved EM





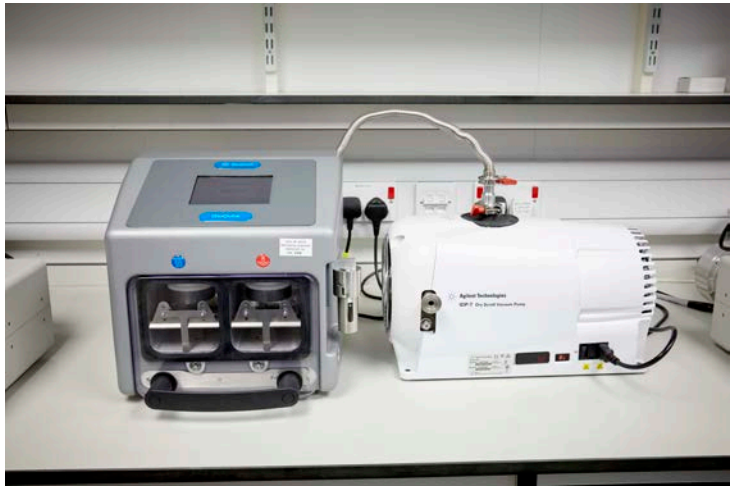
Stephen Muench, Howard White

Sample preparation of cells, tissues and organisms

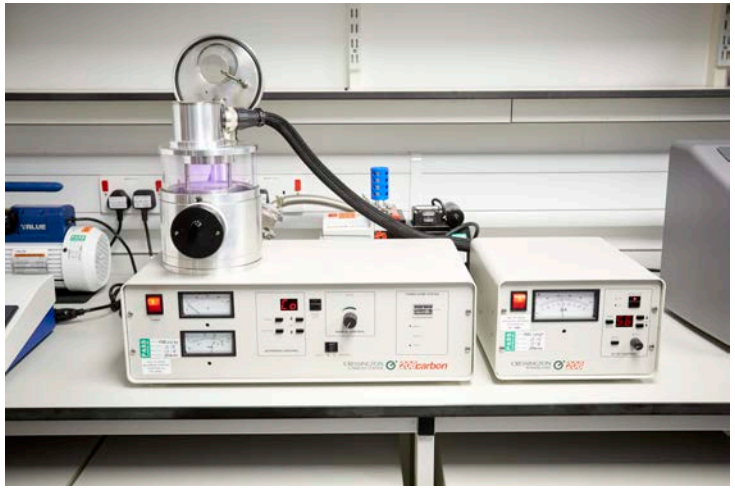


- New this year!
- Leica EM ICE High pressure freezer- for vitrification of cells, tissues and organisms like *C. Elegans*
- Leica AFS2 for freeze substitution of high pressure frozen specimens
- Leica UC7 cryo/ultramicrotome for sectioning of ambient/Tokyasu/vitreous (CEMOVIS) samples

Glow discharge



Quorum GloQube



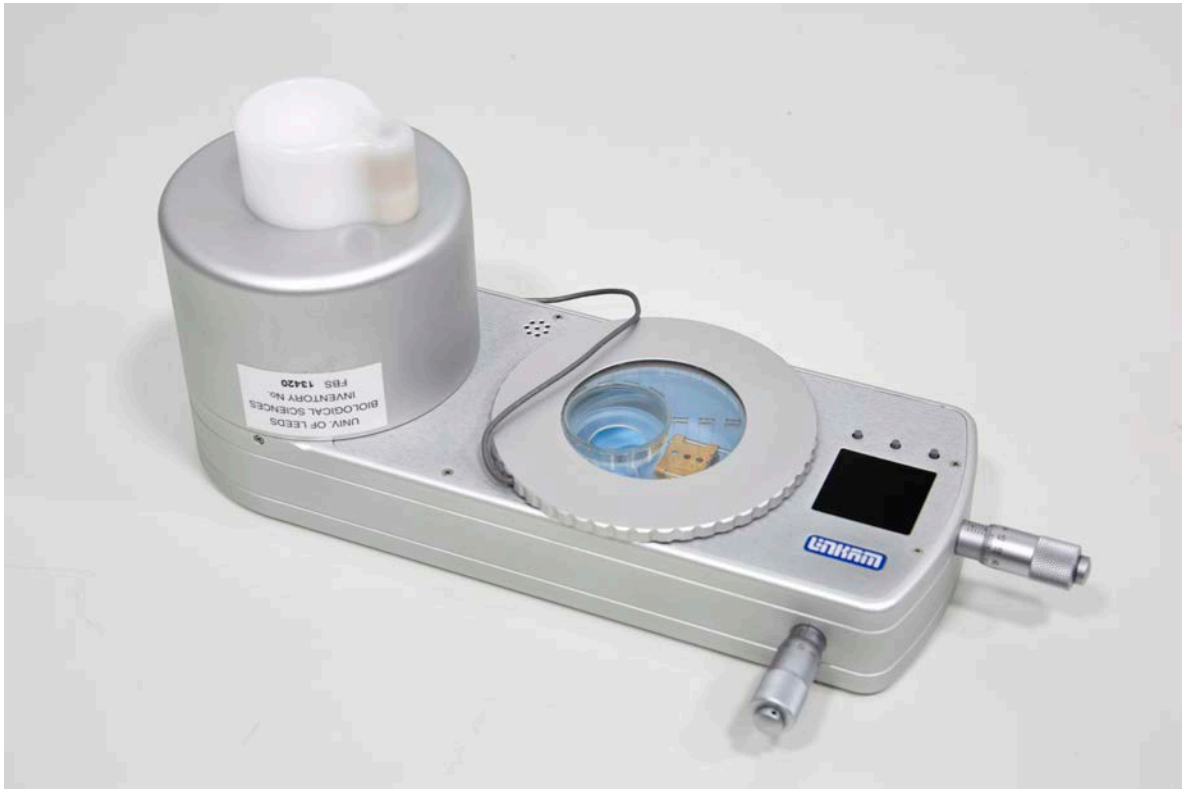
Cressington 208

PELCO easiGlow

Considering purchase of a plasma cleaner- opinions welcome!

Cryo-Fluorescence

Leica CryoCLEM system



Linkam cryo fluorescence stage
(Can be fitted onto Zeiss LSM 880 with Airyscan)

Facility operates at BSL2

The Team

Rebecca Thompson
Facility Manager/ Senior cryo-EM scientist

Dan Maskell
Cryo-EM support scientist
Training Lead

Emma Hesketh
Cryo-EM support scientist
IT lead

Martin Fuller
EM technician
JEOL1400/T12 and Ultramicrotomy lead

The User Base

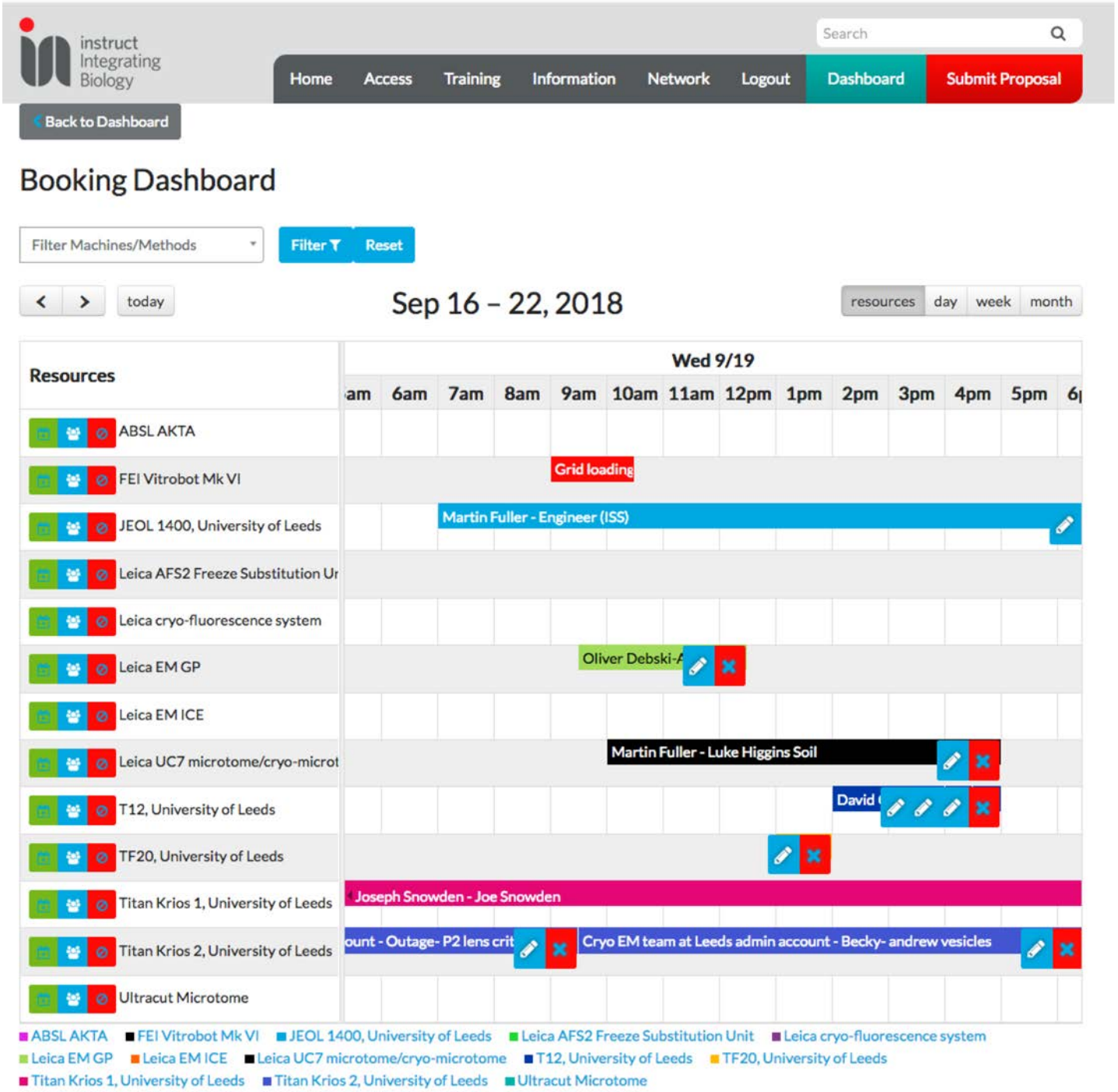
- 134 ‘active’ users from across the University
- ~75 % internal users Faculty Biological Sciences, with remainder from Chemistry, Physics, Medicine, Food Science, Engineering/Materials Science, and external collaborators.
- ~ 25-30 use cryo-EM as primary tool of research
- On Titan Krioses, ~ 20 % available time used by external service users

External Users

- Mix of industry and academia
- Shortly to offer access through Instruct-ERIC
- ~ 75 % attend site for collection (repeat customers most likely not to attend
- Remote customers can see files for inspection during collection, other feedback done via skype and email. Plans to implement remove viewing.
- Do not offer remote control (and no plans for users)

Equipment Access

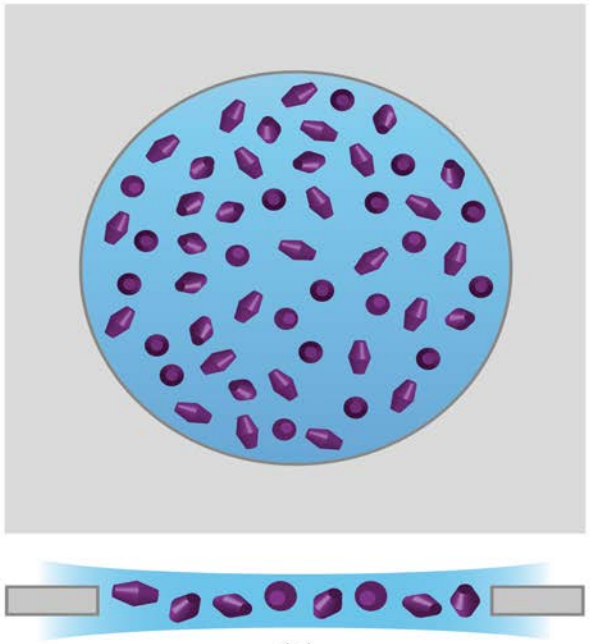
- Managed through Instruct's 'Aria' booking system
- Titan Krios bookings managed by facility staff
- Balance of internal/external, projects and unfunded currently managed by Facility staff with oversight from Director



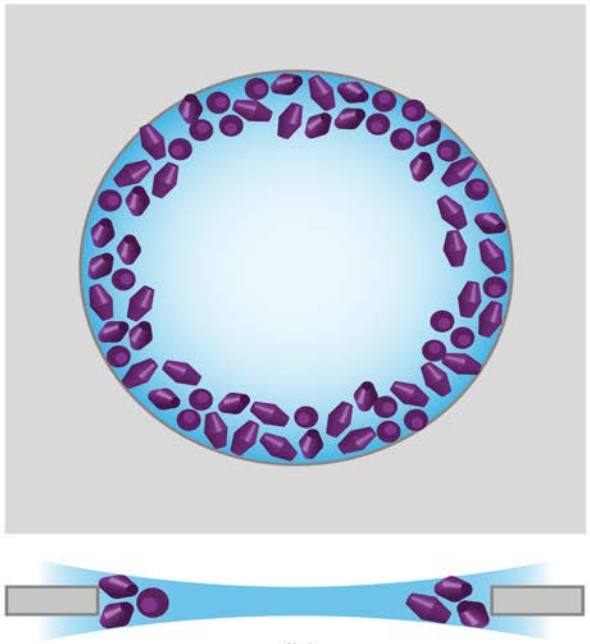
Standard Single Particle Pipelines

- Sample optimization and screening is a massive bottleneck
- We end up using Krioses for (most) screening
- Aim to make this as efficient as possible- 2/3 loads per screening day (22-33 grids) plus ‘overnight’ short runs for borderline projects

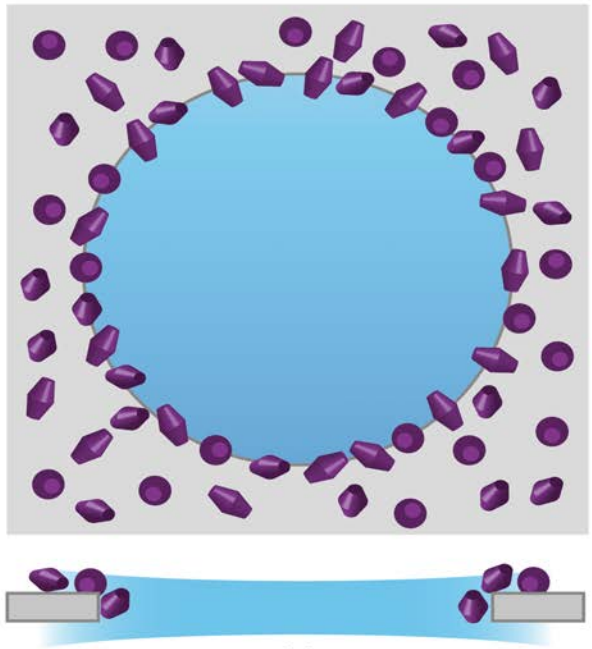
Well distributed



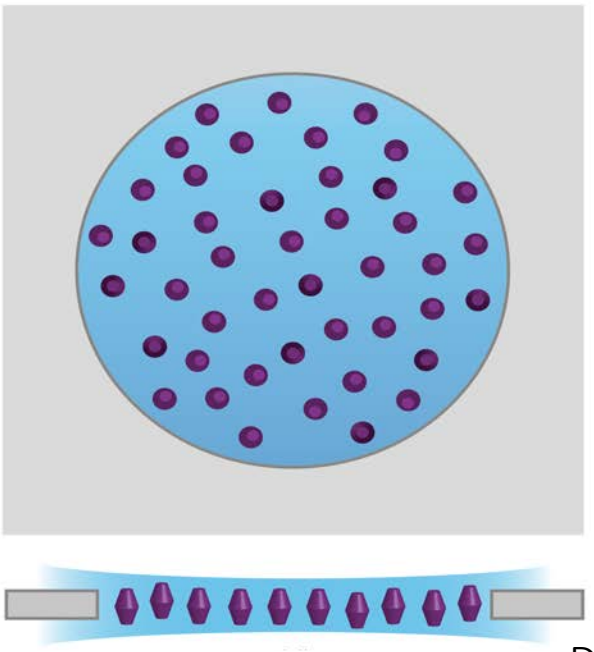
Thin ice in center of hole pushing particles to the hole edge



Affinity for the support



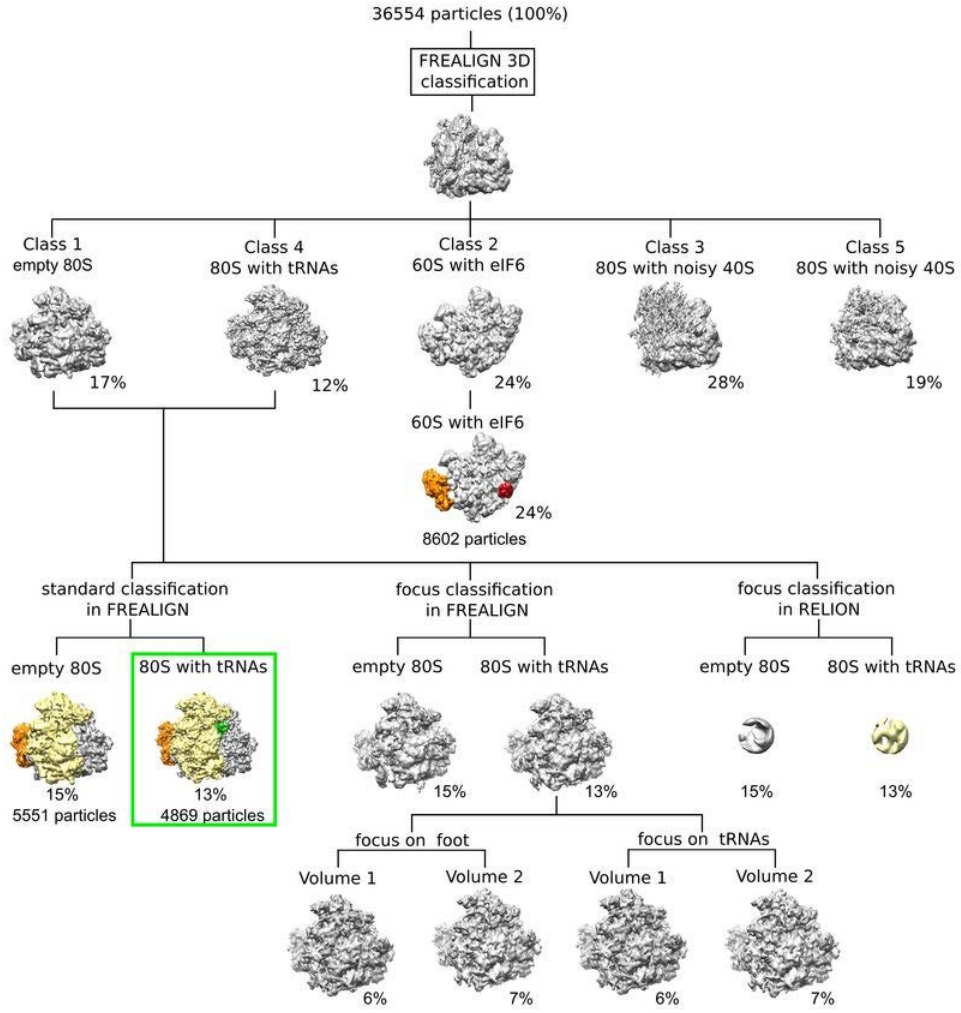
Preferred orientation at air water interface



Drulyte *et al* 2018

Data collection

E Plubirus Paucioribus?
(Out of Many, Fewer)

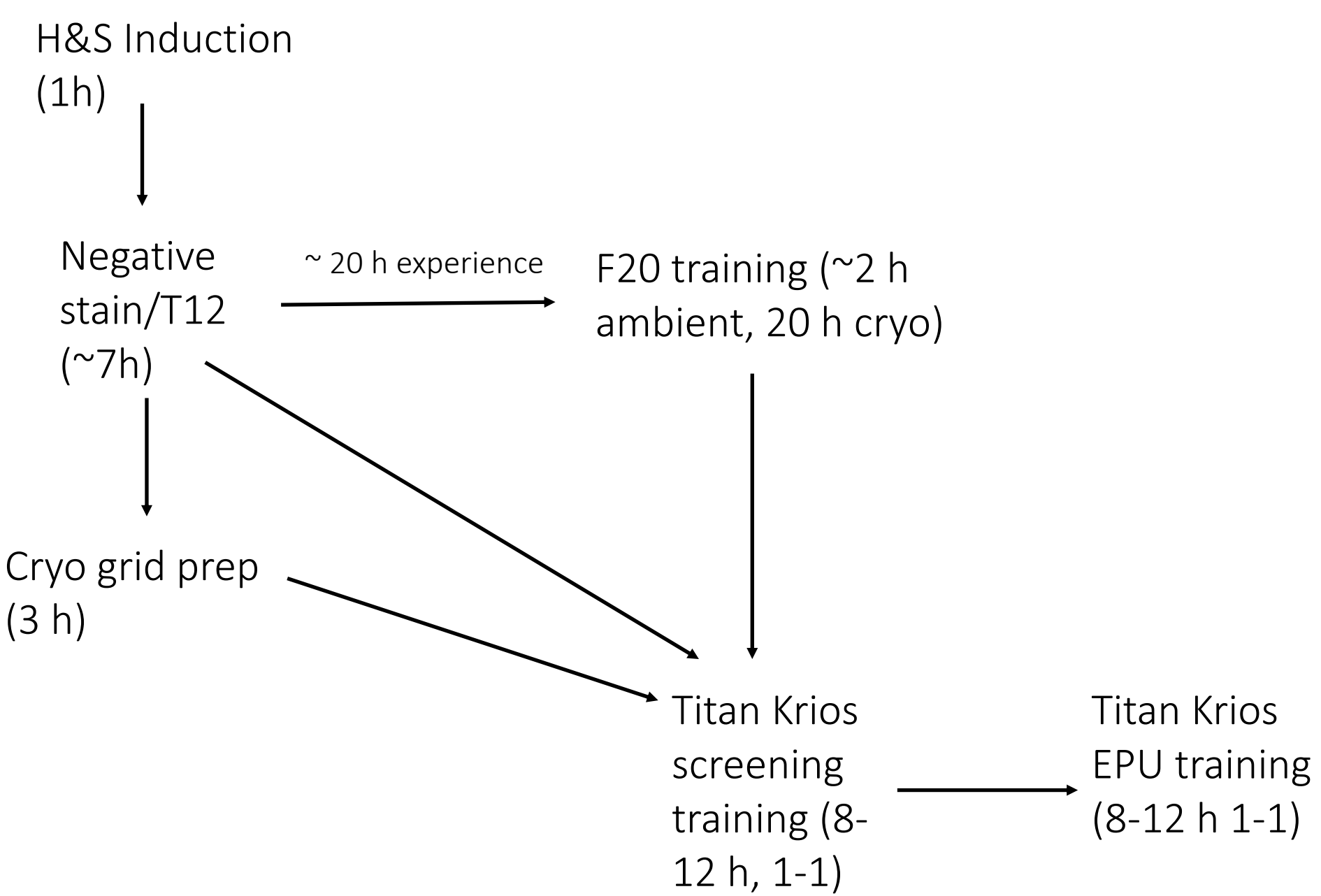


Sarkar *et al*, NSBM 2017

Data collection- Facility staff

- How can we as Support Scientists provide best possible service?
  - Monitoring of equipment and environment (26 parameters measured and alarmed)
  - Work to an SOP (but when providing service will deviate if requested)
  - Checklists
- How can we best monitor performance of the microscopes over time?
  - Periodic collection of ‘standard’ samples?
- How do you (continually) train cryo-EM support staff?

Training Module	Module Code
Inductions	**000**
Induction - Service lab	001
Induction - Cryo lab	002
Grid Prep - Stain	**100**
Carbon coating "Collodion method"	101a
Carbon coating "floating"	101b
Glow discharge Cresington	102a
Glow discharge Ted Pella	102b
Glow discharge GloCube	102c
Negative staining	103
Grid prep - cryo	**200**
Vitrobot	201a
Leica GP	201b
Microscope Training	**300**
JEOL	301
T12 - beginners	302
T12 - advanced	303
F20	304
Krios 1 - EPU	305a
Krios 2 - EPU	305b
Krios 1 - Advanced users	306a
Krios 2 - Advanced users	306b
Computing	**400**
Starter pack	401
Basic Unix commands	401a
General computing guide	401b
Relion starters guide	401c
cryo sparc	401d
Using Arc3	401e
Working from home	401f
Access screening data - Krios	401g
OTF - internal	402



Data collection- internal users

Which detector?

Dose per frame?

Total dose?

How many images will I get?

Detector mode?

Mag?

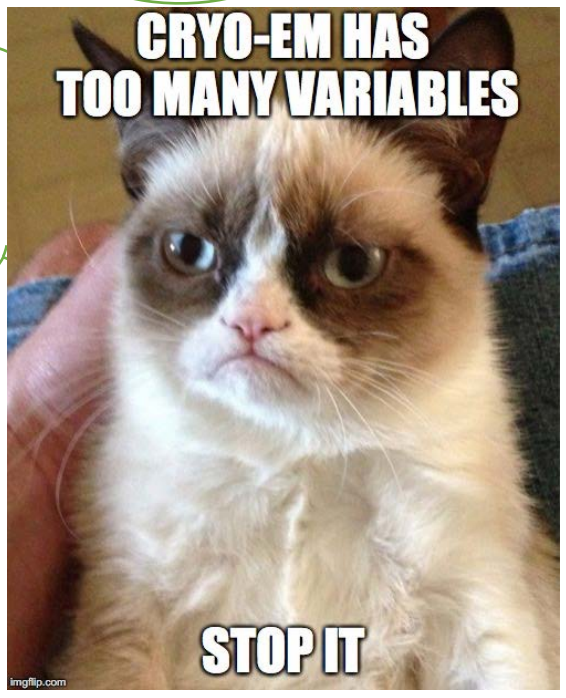
Phase plate?

How many do I need?

Acquisitions per hole?

Energy filter?

<u>Just HOW do I do it?</u>



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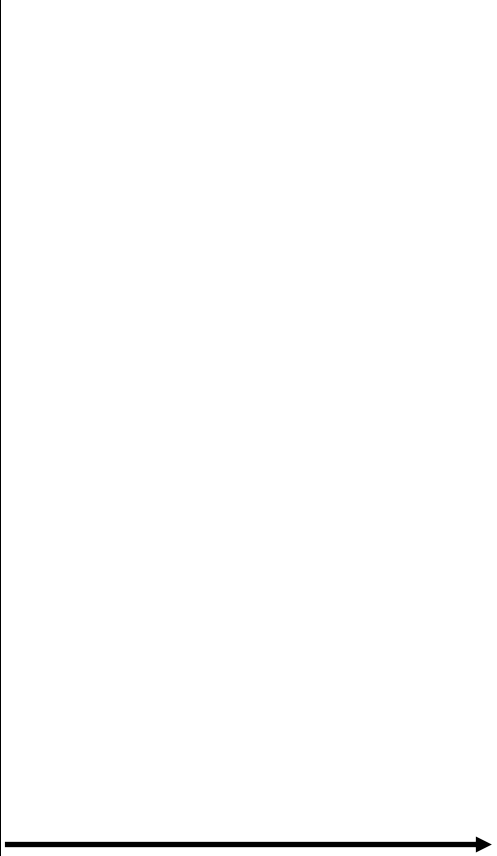

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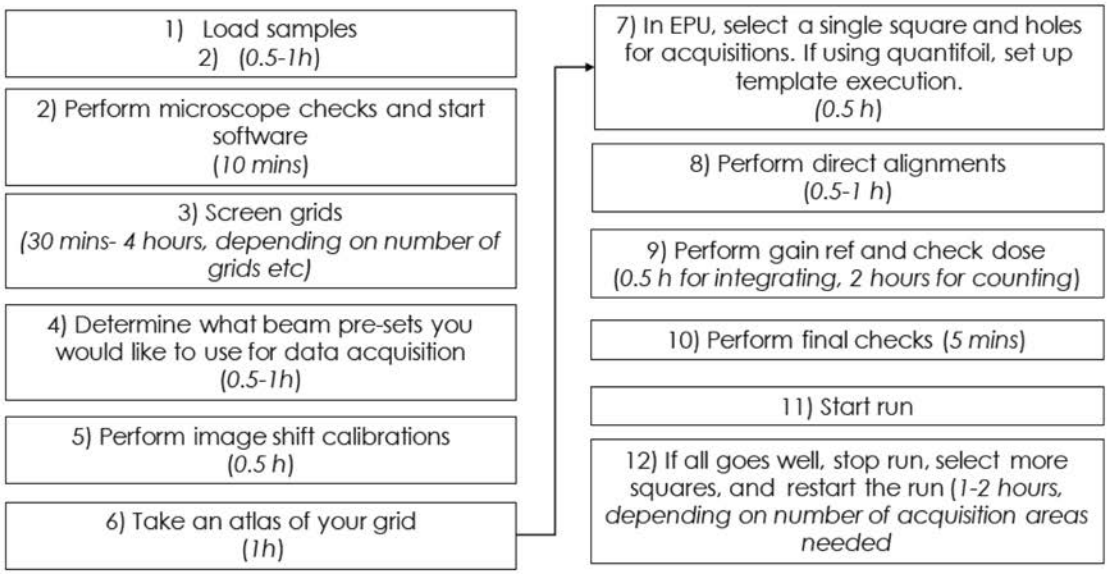
Krios 1- EPU guide

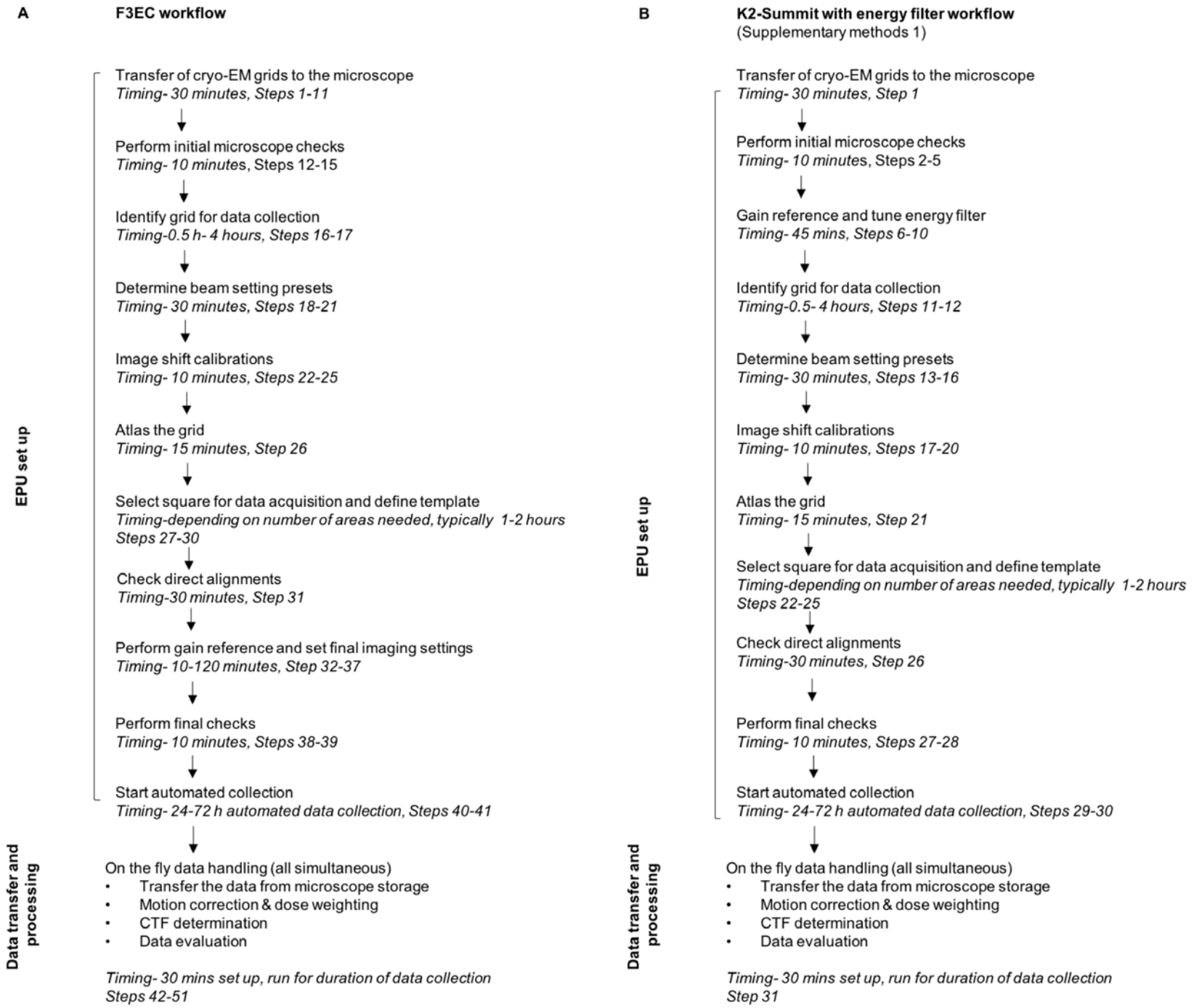
Microscope software v 2.7, EPU version 1.9



The aim of this guide is to guide you through the process of collecting data using the EPU software. This guide is split into sections according to the flow chart above. Although it is possible to do some of the steps in different orders, if you are unsure follow the flow chart as it minimises chances of errors.

Please note, the Krios software is not fool proof, and users can cause serious issues if certain actions are performed. To minimise chance of damage to the microscope, we ask users request facility help if they want to change any of the following:

- Any settings in the FEG control.
- Any settings in the gun alignments (main alignment menu or direct alignments)
- Any column alignments.
- Move anything around in the user account. There is one user account for everyone, do not change the arrangement without explicit permission.
- Align the condenser apertures.
- C2/C3 lens adjustment.



- Teaching aid
- Resource for external users
- Improves reproducibility
- Reduces sessions 'lost' for preventable reasons
- Helps with trouble shooting
- Users require less expert knowledge- but still need to understand why they are making choices!
- Reduced appetite for becoming a cryo-EM 'expert'- learning on a 'need to know' basis

In Press Nature Protocols

- User data sheet provided for every session
- Improves tracking of parameters
- Reduces users errors during processing

Data acquisition report

Date: 12th October 2018

Hardware	
Microscope	Krios 2
Detector (mode)	K2 (counted)
Accelerating voltage (keV)	300
Pixel size (A)	1.065
Data acquisition parameters	
Nominal magnification	130 000x
Spot size	7
Illuminated area	1.3 um
Dose	
Square pixel (A^2)	1.13
Dose per physical pixel per second	5.3
Dose per A^2/sec	4.6
Exposure time (seconds)	12
Total dose (e/A^2)	55.2
Number of fractions	48
Dose per fraction (e/A^2)	1.15
EPU parameters	
Defocus range (-µm)	-1.3--3.1
Autofocus	Every 10 um using objective
Drift measurement	Once per grid square 0.05nm/s
Delay after stage shift	5s
Delay after image shift	5s
Exposures per hole	2
Apertures (size in microns)	
C1	2000
C2	70
C3	2000
Objective	100

General information
Physical pixel size of Falcon III: 14 µm
Physical pixel size of K2: 5 µm
Cs of microscope: 2.7 mm

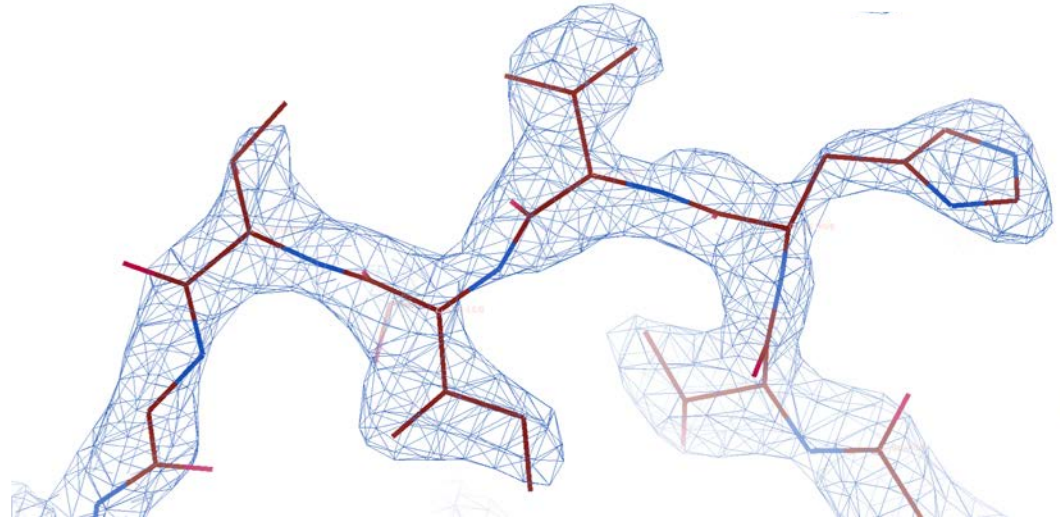
Detector choice

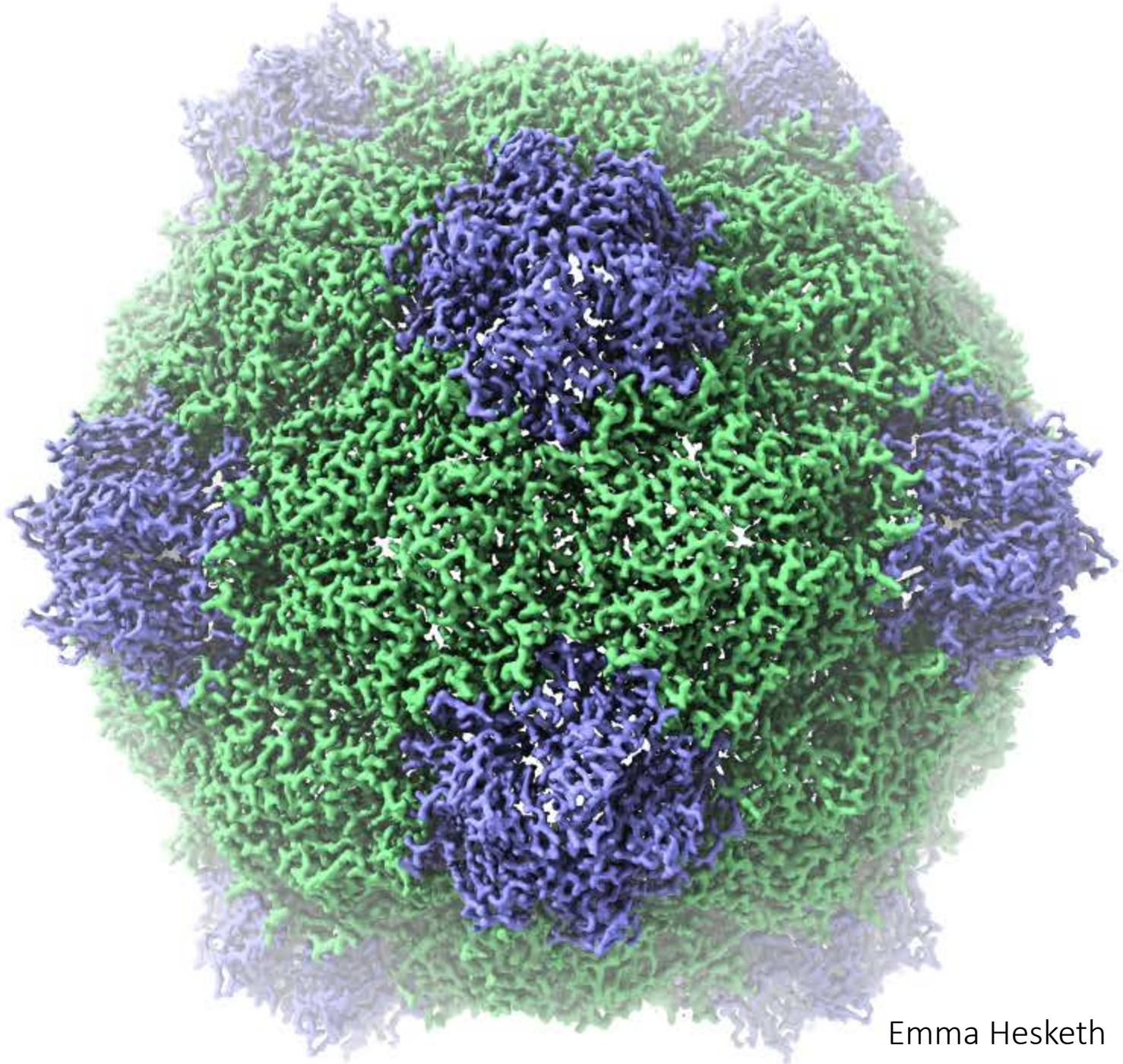
Which Detector?

- Internal users split pretty evenly across F3EC and K2
- F3EC used mostly in integrating mode
- Tomography/sub ~120-150 kDa single particle projects K2
- Internal users never really use K2 super resolution
- External users preference for K2 counting

2.6 Å structure from Falcon III in integrating mode

- 48 h data collection
- 5619 Micrographs (117/hou
- Autopicked ~300,000
- 280,000 particles in final

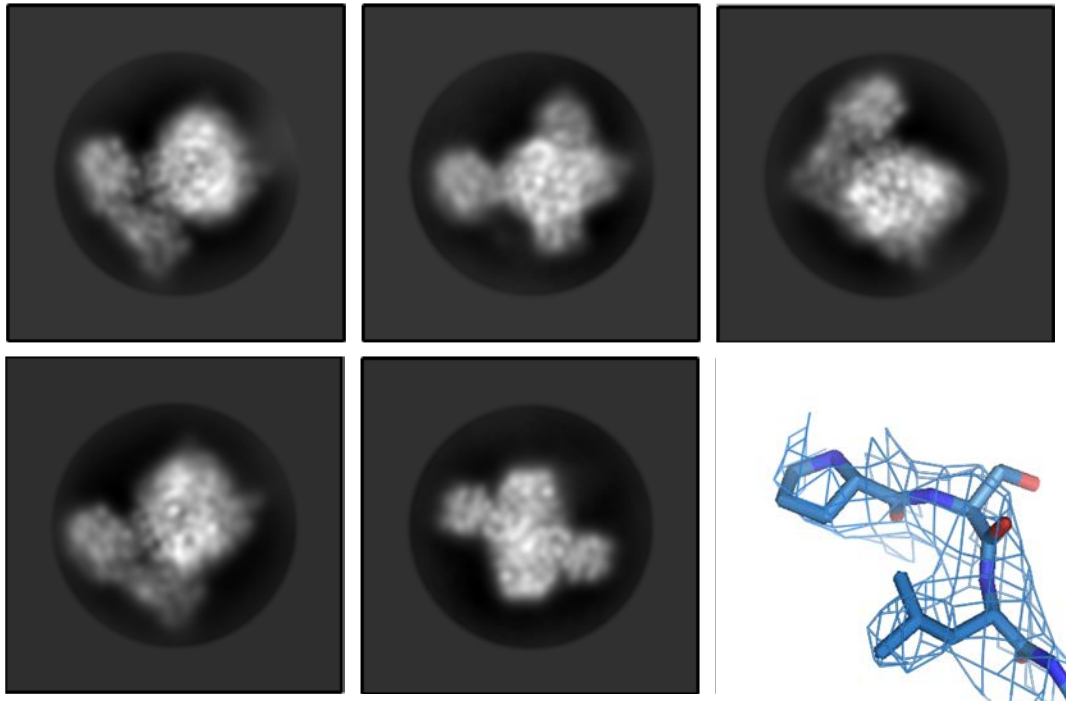


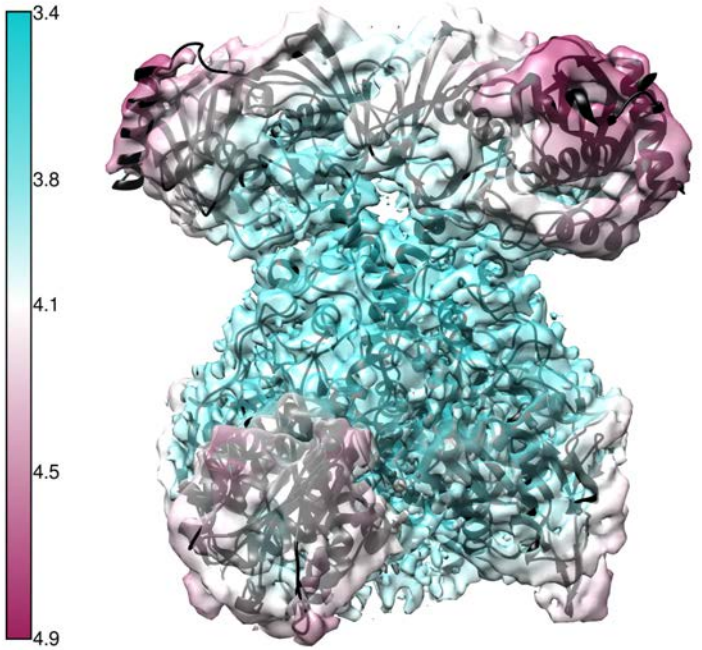


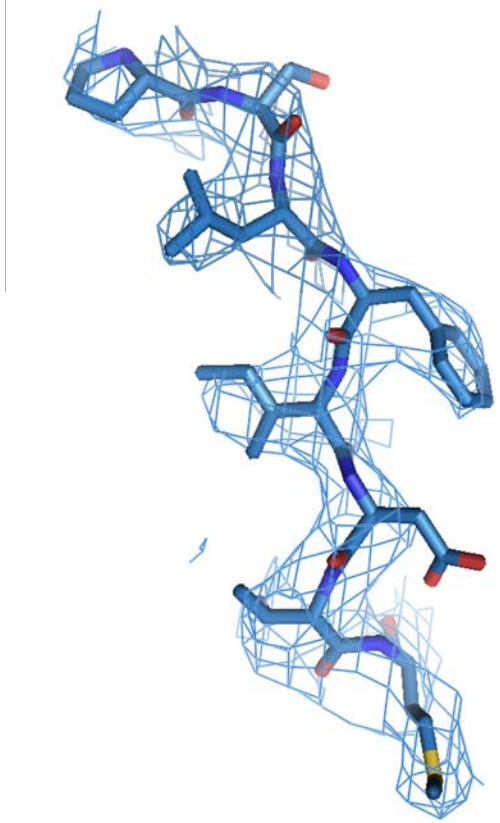
Emma Hesketh

Falcon 3 in integrating mode

- 200 kDa soluble protein
- 4267 micrographs
- Autopicked 1.6 million particles
- 110 e/A^2 so you can see the particles!
- 3.5 Å structure



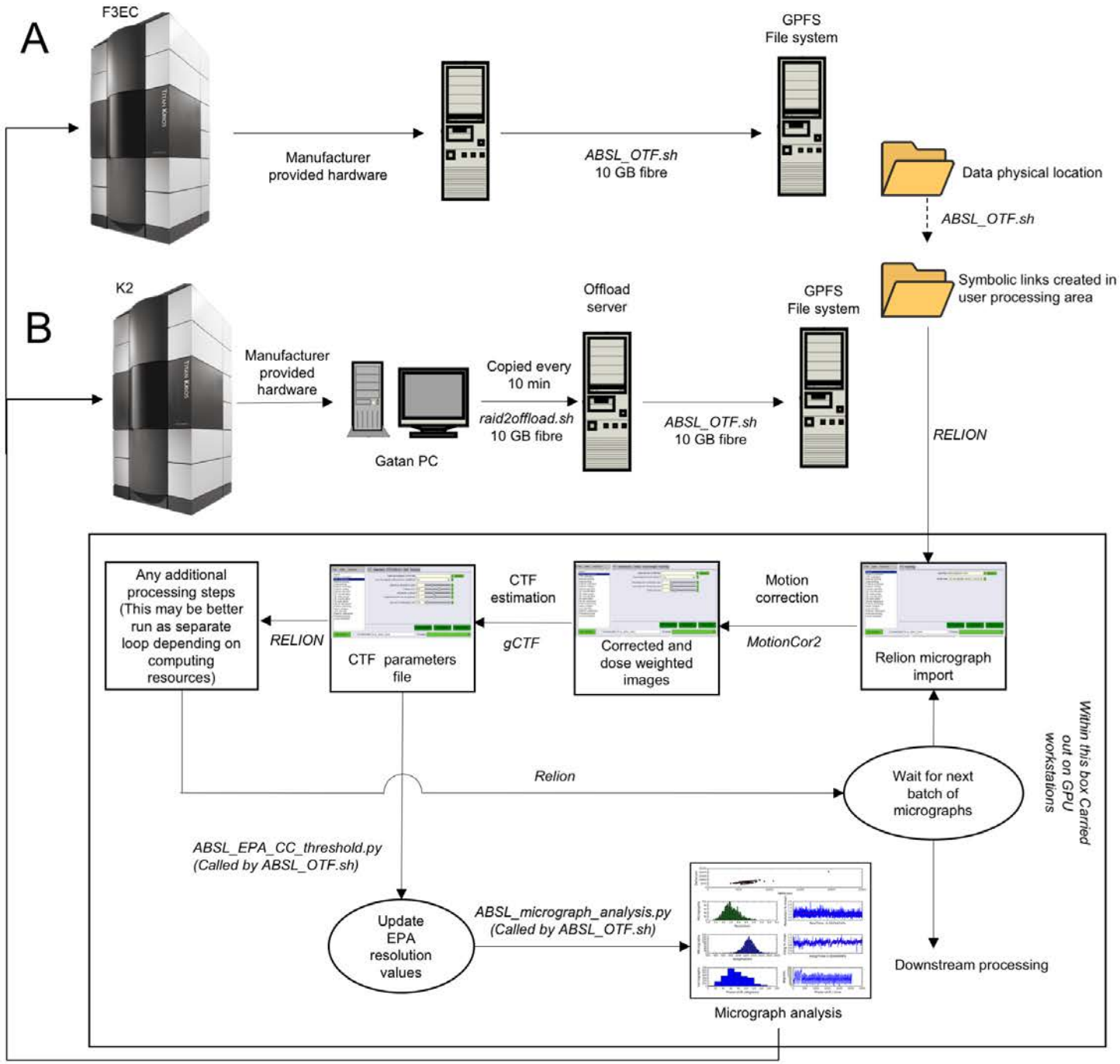




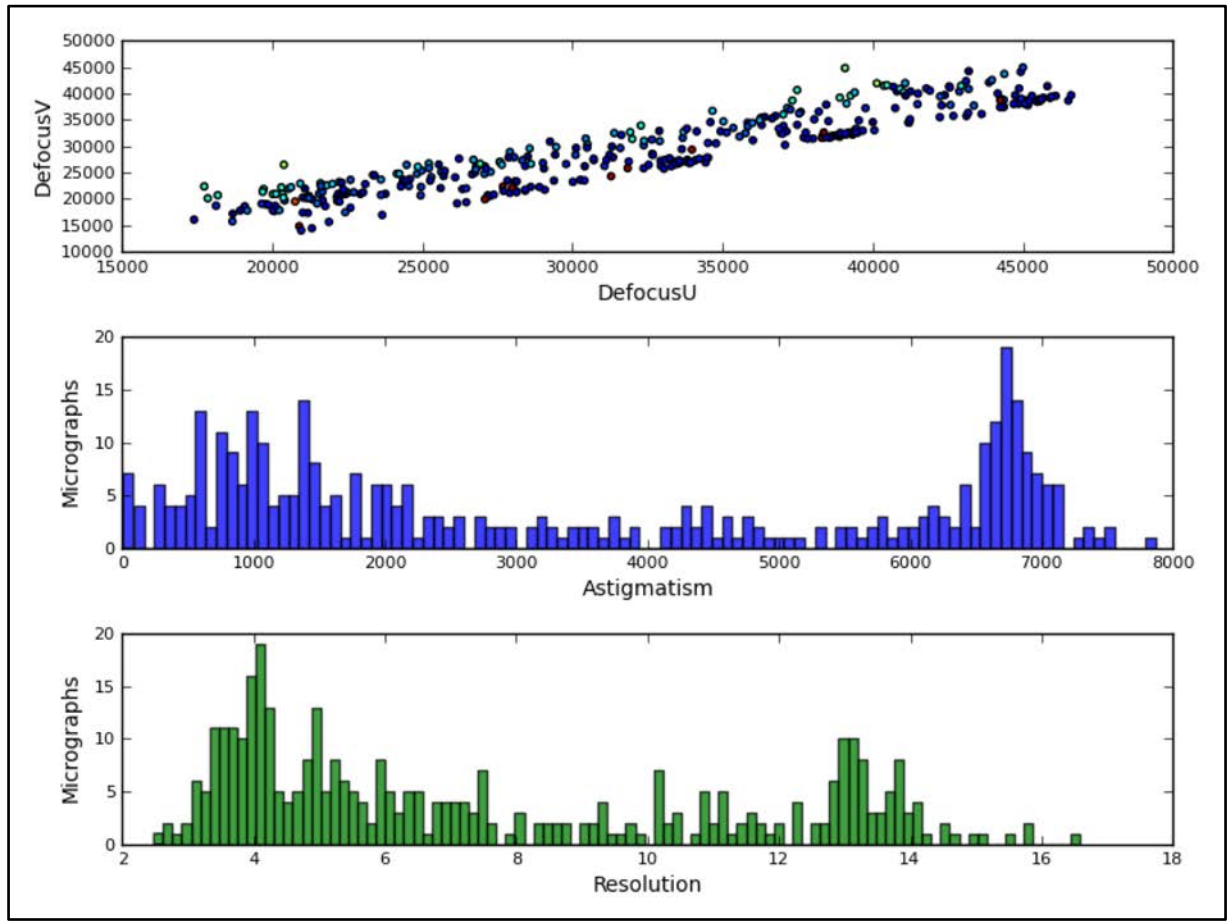
Ieva Drulyte

Dealing with the Data Deluge

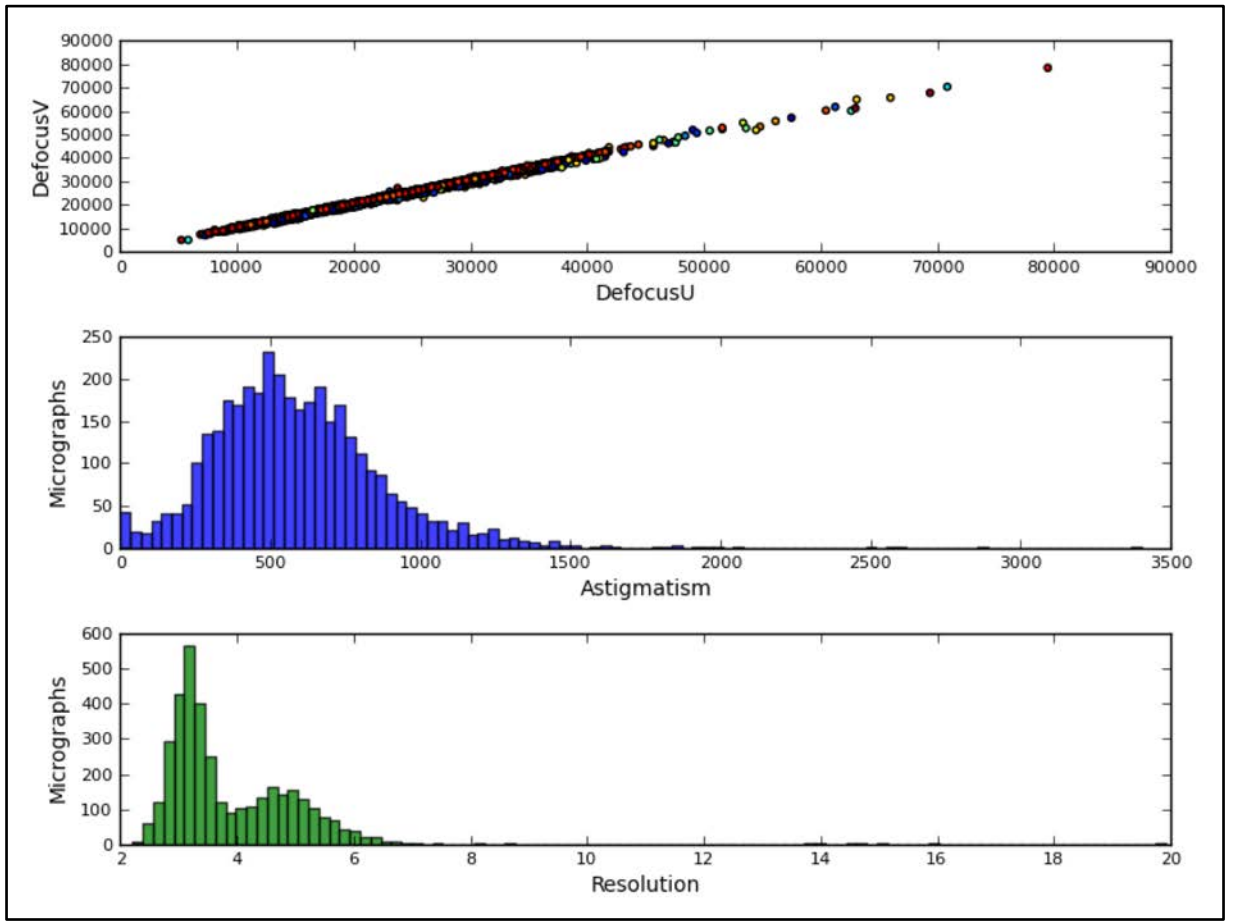
‘Current’ OTF image processing



Assessing Micrograph Quality

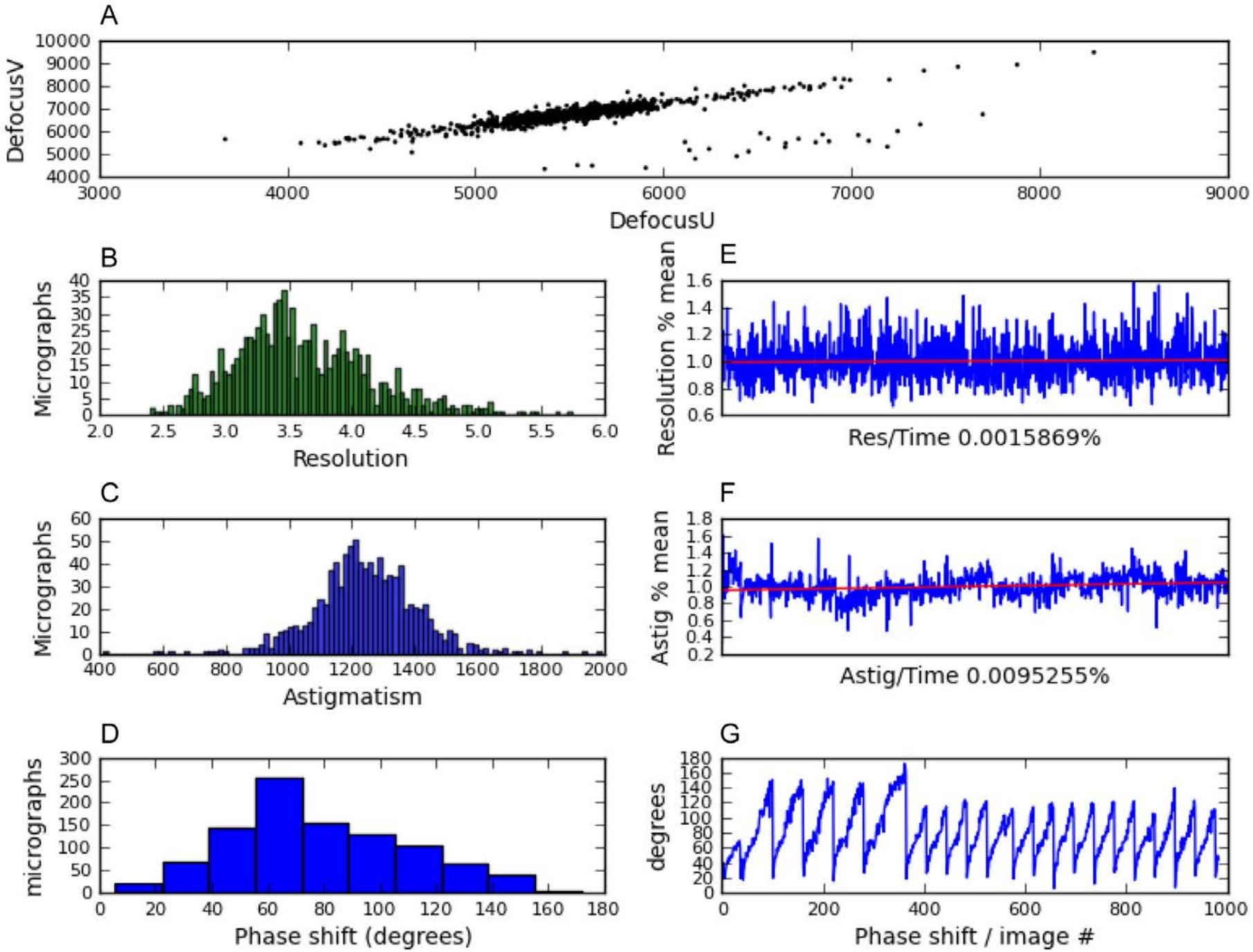


Bad dataset



‘Requires investigation’ dataset

Matt Iadanza


A
DefocusV
DefocusU
B
Micrographs
Resolution
C
Micrographs
Astigmatism
D
micrographs
Phase shift (degrees)
E
Resolution % mean
Res/Time 0.0015869%
F
Astig % mean
Astig/Time 0.0095255%
G
degrees
Phase shift / image #

Outlook

- Efficient screening continues to be a challenge- Focused screening days and overnight collections for ‘borderline’ projects. New hardware may help..
- Training and democratization of knowledge- how can we share best practice?
- Single OTF processing changing rapidly- challenge to constantly adapt systems
- Expect tomography work to increase- implementing OTF pipelines for assessing data (especially PP)
- Implementation of other software for routine collection (SerialEM) of tomography data, not just dose-symmetric schemes.

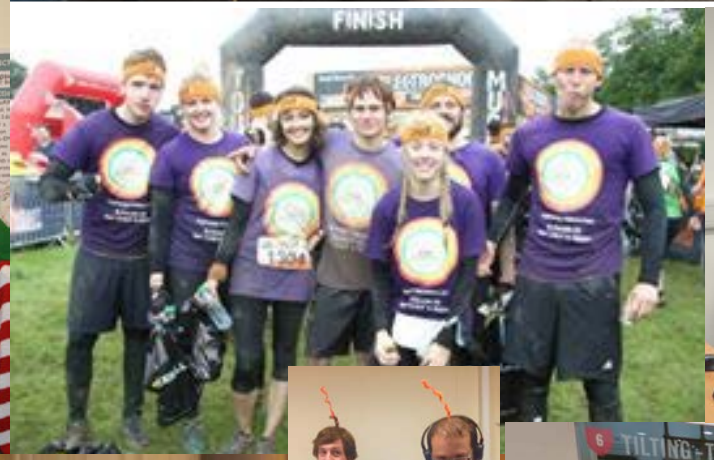
Acknowledgements

Neil Ranson

Emma Hesketh
Martin Fuller
Dan Maskell

Matt Iadanza
Shaun Rawson

Ieva Drylute



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Any questions?

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