- How do users use the microscopes?
- How are users scheduled?
- Data collection strategies (software, protocols).
- Are sessions aborted if samples are not good?
- How is throughput measured?
- What is throughput?
- Any other issues that you feel are relevant.

European Molecular Biology Laboratory

Heidelberg, Germany
- Main laboratory

Hinxton, UK
- European Bioinformatics Institute (EMBL-EBI)

Grenoble, France
- Research and services for structural biology

Hamburg, Germany
- Research and services for structural biology

Monterotondo, Italy
- Mouse biology

EMBL

EMBL electron microscopy

CBB unit, Cell Biology and Biophysics

- Yannick Schwab

EM core facility

- Yannick Schwab

SCB unit, Structural and Computational Biology

- Christoph Mueller
- Martin Beck
- Carsten Sachse
- ~~John Briggs~~ → ??????

EMBL outstations

- Grenoble (France)
- Hamburg (Germany)

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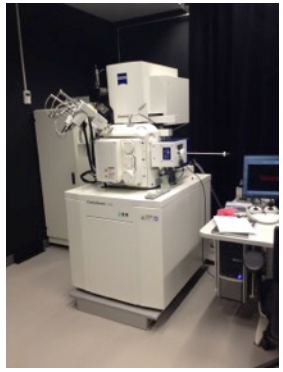
MORIGANI


TECNAI




Tenaio


TITAN KRIOS


FEI
Scios


FEI
Talos Arctica


TITAN KRIOS


TITAN KRIOS


TITAN KRIOS


TITAN KRIOS


TITAN KRIOS

Cryo-EM training/usage

Internal users:

Training “how to collect data” → guided sessions → autonomous user with booking rights.

Actual electron microscopy training on voluntairy basis, no guaranteed data collection during these training sessions!

- Tecnai12: Two sessions per day.
- Polara: One to two day sessions.
- Krios: Two to four day sessions.

External users/guests:

- Outstations.
- iNext (http://www.inext-eu.org/), currently scheduling two slots per month.
- Sessions run by me (until I get help in April).

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EMBL facilities booking system

PPMS Start Page

Search ...

Facilities available in EMBL:

- Advanced Light Microscopy Facility (ALMF) details
- Chemical Biology Core Facility (CB) details
- Electron Microscopy Core Facility (EMCF)

  Instruments
  - IT resources (3)
  - Light Microscope (1)
  - Microtomes (6)
  - Sample prep (8)
  - SCB - Cryo-EM equipment (4)
  - SEM - Scanning Electron Microscopes (2)
  - TEM - Transmission Electron Microscopes (3)

- Flow Cytometry Core Facility (Flow) details
- Genomics Core Facility (GCF) details
- Metabolomics Core Facility (MCF) details
- Protein Expression and Purification Core Facility (PepCore) details
- Proteomics Core Facility (PCF) details

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Fixed recipes for all cryo microscopes:

- Acquire grid maps

Single particle:

- Set up Low Dose
- Screen grid squares
- Acquire grid square maps
- Tune/align/calibrate
- Setup grid squares
- Run automated acquisition

Tomography/correlative:

- Acquire grid square maps
- Find targets/do correlation
- Set up Low Dose
- Tune/align/calibrate
- Setup targets
- Run automated acquisition

Current software of choice: AutoCTF & SerialEM.

One day setup on Krios.

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Alignments
CompuStage Height
Gun
Align NanoProbe
Align HM-TEM
Align LM
Condenser
Stigmators
Calibrate NanoProbe
Calibrate HM-TEM
Calibrate LM
Done
Auto help
Direct Alignments
Gun Tilt
Gun Shift
nP Beam tilt pp X
nP Beam tilt pp Y
Beam shift
Tomo Beam shift
Center C2 aperture
Condenser center TEM
Done
Auto help

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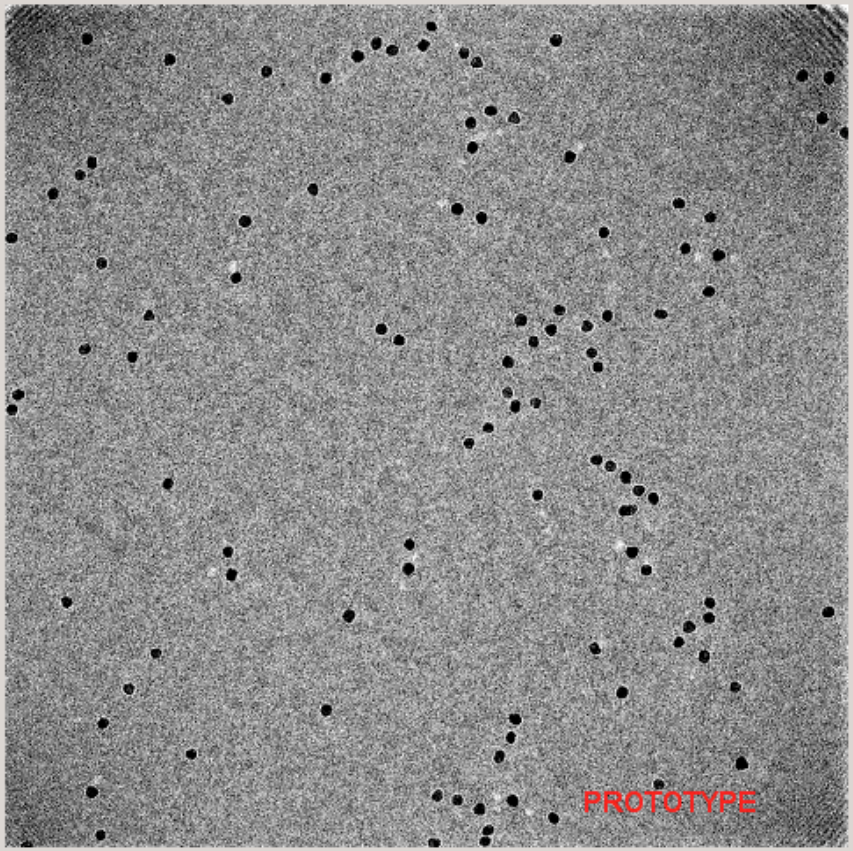
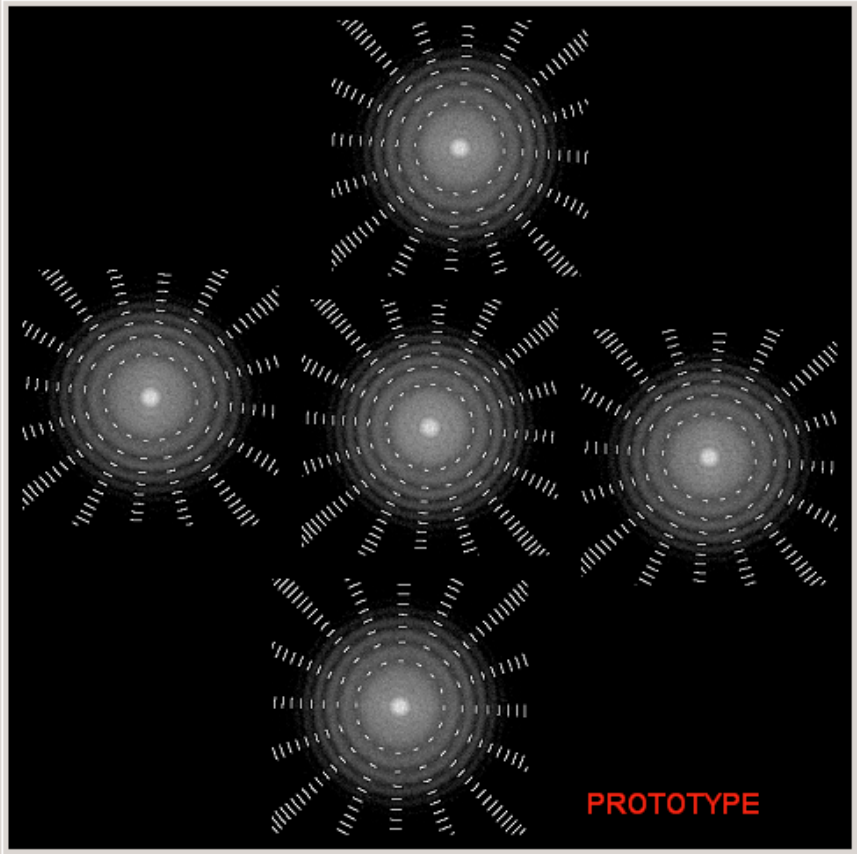
FEI AutoCTF - Copyright 2015 FEI Company - PROTOTYPE - DO NOT DISTRIBUTE





Power spectrum | 1D profile

AutoCTF | Calibrations | Settings

Exp. time 2

Binning 2

Readout area Full

Phase plate inserted

Measure

Auto-Stigmate

Condition Phase Plate + Auto-Stigmate

Make tableau

Auto-coma

Stop

Auto-focus to -1 µm

Target phase shift -0.5 pi rad

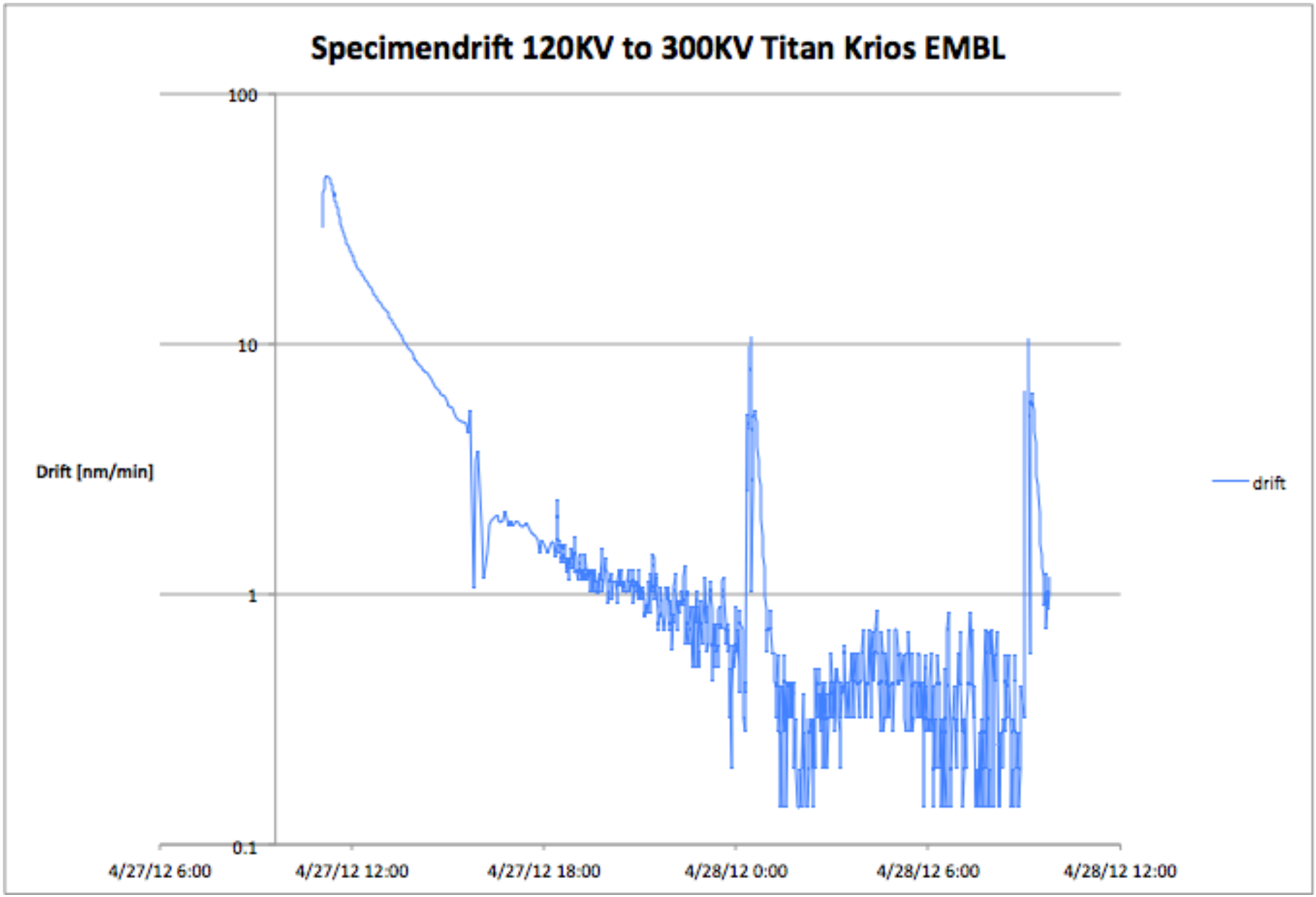
Status

ZemlinTableauTask succeeded in 2 iterations

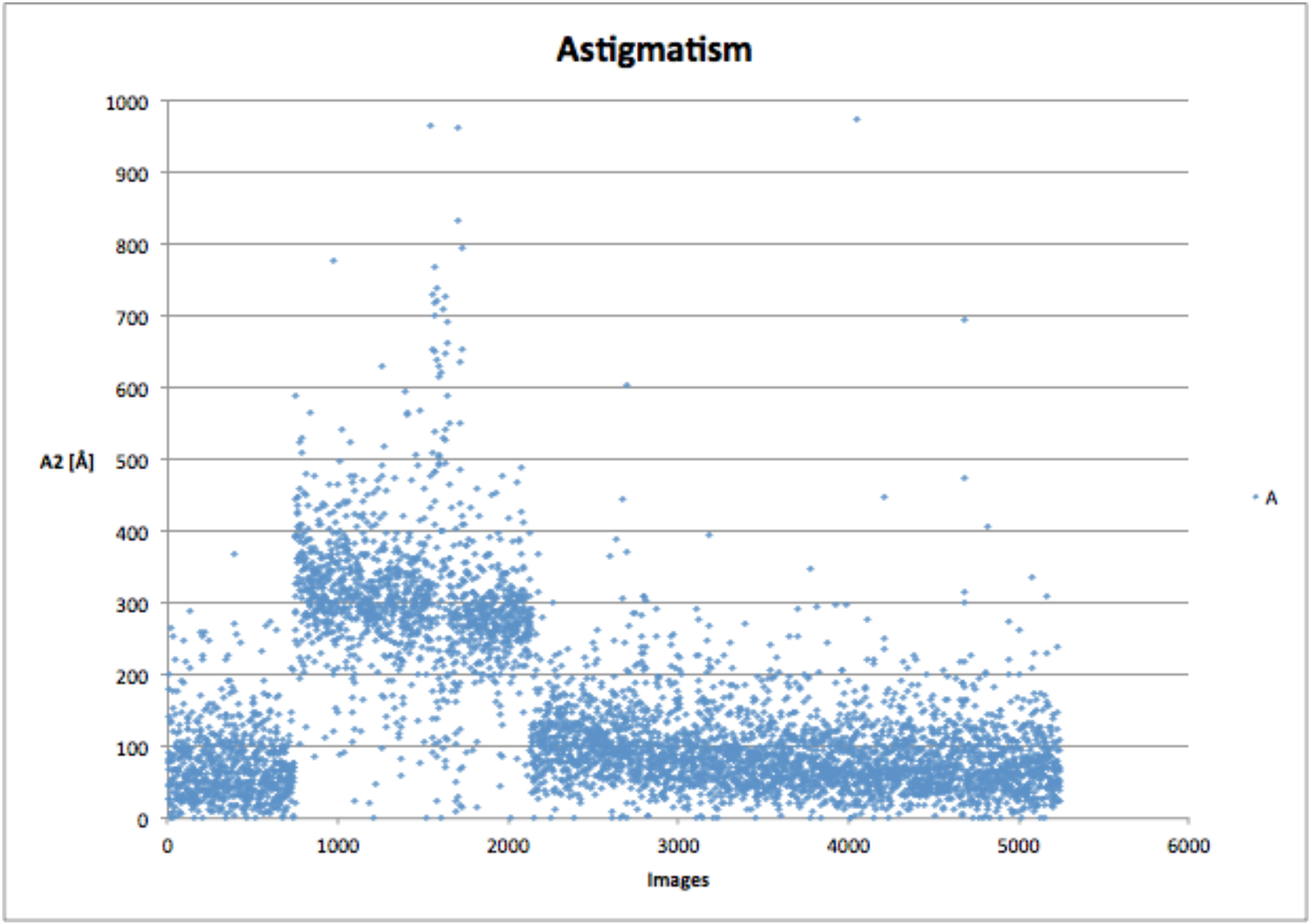
Aberration	Magnitude	Angle
defocus	-2.15 um	
astigmatism	26.62 nm	-9.5 deg
coma	5.08 um	132.2 deg
Spherical aberration	2.70 mm	
FIT ERROR	6.66 nm	
Tableau auto-coma iteration 2		
defocus	-2.15 um	
astigmatism	27.35 nm	-4.5 deg
coma	0.29 um	-66.8 deg
Spherical aberration	2.70 mm	
FIT ERROR	7.94 nm	

AutoCTF version '2015-03-27 (PROTOTYPE)'

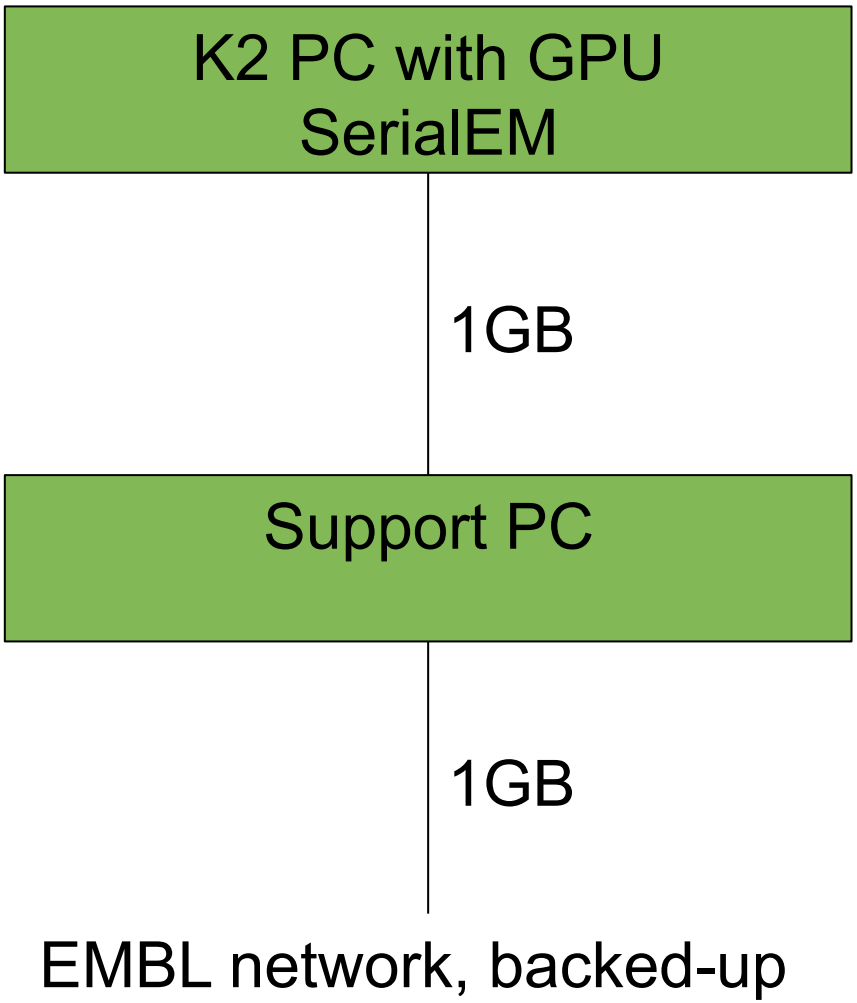
Only align column when things are stable!



Tune every session and after major trouble like water failure or runaway focus.



Throughput Krios Quantum/K2 Tomo



- Align frames on-the-fly using GPU in K2 pc.
- Also save raw (uncorrected) superresolution data.

One to five tilt series per hour

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Throughput Krios Quantum/K2 SPA

K2 PC, SerialEM

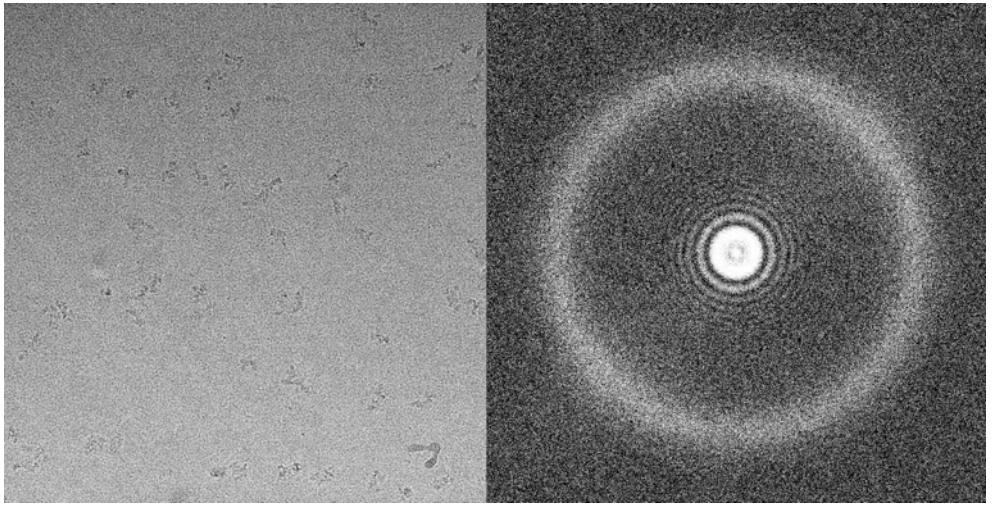
1GB

Support PC, GPU, IMOD

1GB

EMBL network, backed-up

- EarlyReturnNextShot in SerialEM.
- No rotation/flip.
- Uncorrected LZW compressed TIFF, <400MB stacks of 40 superresolution frames.
- IMOD FrameWatcher grabs frames.
- IMOD FrameAlign output:
  - 4K aligned image
  - Raw data
  - JPEG with image & power spectrum.



30 to 80, stacks per hour (40 superresolution frames)

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EMBL internal online EM-help

temhelpembl | Krios

em-help.structures.embl.de/Krios/main.html

Search

Load & map grids

Loading
Map grids

Map grid squares

Map grid squares

K2 setup

Tune GIF & gain reference

K2 Tomo

Low Dose tomography
Microscope tuning
Setup positions
Run

K2 single particle

Setup grid squares
Low Dose single particle
Microscope tuning
Check dose rate and dose
Run

On-the-fly frame alignment on IMOD pc

Support PC & data transfer

Microscope status

Goal:

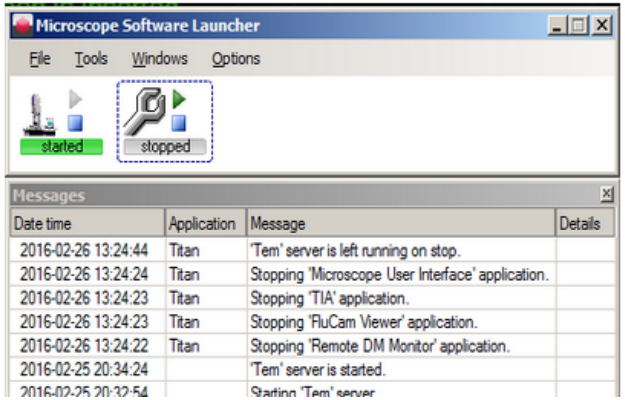
- Is everything running as expected?

Titan pc

Supervisor
Superuser#1

Check the Microscope Launcher, Applications (bottom window), these applications should be running:

1. Microscope User Interface.
2. TIA.
3. FluCam Viewer.
4. Remote DM monitor.
5. FeiBBox.exe (Tem).



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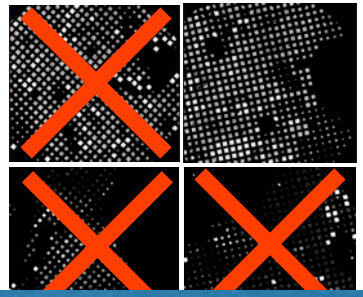

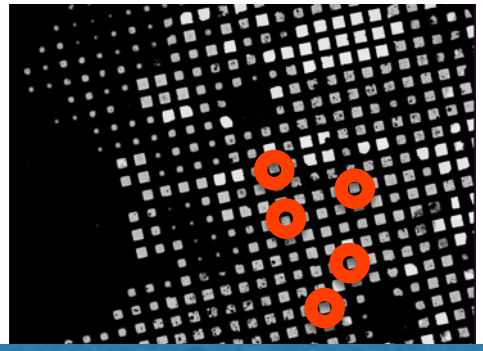

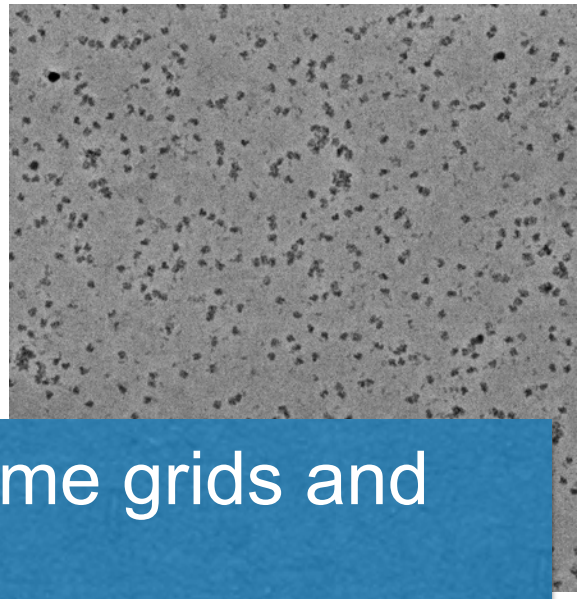

Current screening means checking some grids and assuming the other grids are similar:

- 6–8 hours Titan screening on “screened” samples.
- 1 hour setup if sample is ok!

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