Unified EM setup: the benefits of SerialEM for facilities

Wim Hagen

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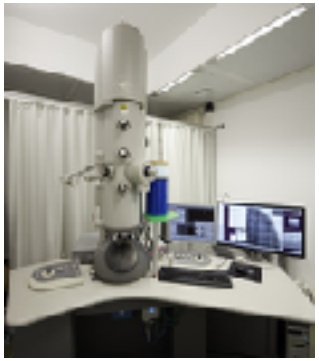
CBB unit / EM core facility

Morgagni
MegaView

CM120
SIS

JEOL 2100+
Ruby camera



Tecnai F30
Gatan OneView

Zeiss Crossbeam

- Yannick Schwab, Team Leader and Head of Electron Microscopy Core Facility
- Mandy Boermel, Scientific Officer
- Pedro Machado, Technical Officer
- Rachel Mellwig, EM Facility Operations Manager
- Giulia Mizzon, Scientific Officer
- Paolo Ronchi, Scientist in EM Facility
- Martin Schorb, Application Engineer

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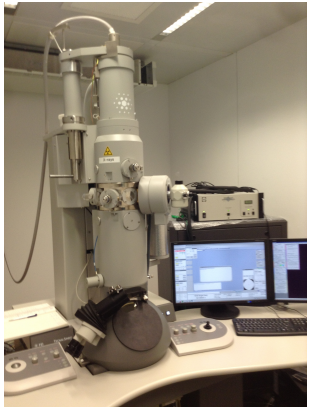
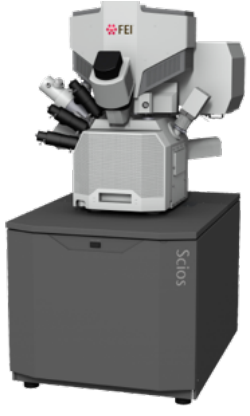
SCB unit / cryo-EM service platform



Tecnai 12
US 4000

Polara
Falcon 2
GIF2002



Arctica
Falcon 3

Krios
Quantum K2

- Felix Weis, Cryo-Electron Microscopy Specialist
- Wim Hagen, Senior Engineer in Electron Microscopy

One third guests over iNext and Direct Access.

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EMBL European Molecular Biology Laboratory

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EMBL Heidelberg hosts a cryo-electron microscopy (cryo-EM) service platform, available for use by external scientists with both single particle and tomography projects.

The platform gives access to state-of-the-art cryo-EM equipment for structure determination projects using the latest technology and methods. The platform hosts a FEI Titan Krios G3 equipped with a phase plate and a Quantum-K2 camera and a 200 kV FEI Talos Arctica with an electron counting Falcon 3 camera. Both microscopes are equipped with an automatic sample loading system and configured for automatic data acquisition.

Experts are on hand to help and support researchers during microscope handling, data acquisition and optimisation of imaging conditions.

Thanks to a continuous test program with Thermo Fisher, we ensure the microscopes have the latest hardware and software features.

Titan Krios G3 equipped with phase plate and Quantum-K2 camera.

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EMBL Imaging Center

Why SerialEM?

Tecnai 12

- Windows XP, US4000, SerialEM.
- Windows 7, US4000, SerialEM.

Polara

- Windows 2000, GIF2002/film, SerialEM, UCSF Tomo.
- Windows XP, GIF2002/US4000, SerialEM, EPU, FEI Tomo.
- Windows 7, GIF2002/Falcon 2, SerialEM, ~~EPU, FEI Tomo.~~

Talos Arctica

- Windows 7, Falcon 3, SerialEM, EPU.

Krios

- Windows XP, GIF2002/US4000/film, SerialEM.
- Windows XP, GIF2002/US4000/film, SerialEM, EPU, FEI Tomo.
- Windows XP, GIF2002/Falcon 2, SerialEM, EPU, FEI Tomo.
- Windows XP, Quantum K2, SerialEM, ~~EPU, FEI Tomo.~~
- Windows 7, Quantum K2, SerialEM, EPU, FEI Tomo.
- 2019: Quantum K3, SerialEM -> EPU, FEI Tomo?

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Latest FEI EPU/Tomo
- Only Thermo Fischer microscopes with supported camera's on Windows 7 software.

Latest Gatan Latitude(S)
- Only Gatan camera's supported by GMS3 Windows 7 software, regardless of microscope type.

Leginon

SerialEM
- Windows 2000, Windows XP, Windows 7
- Thermo Fischer Tecnai, Titan, Talos, Glacios.
- JEOL
- Hitachi

- Gatan
- TVIPS
- FEI
- AMT
- DirectElectron
- EMSIS (formerly Olympus)
- JEOL Ruby
- STEM from Gatan's DigiScan or Thermo Fischer's STEM imaging.

FEI Maps?????

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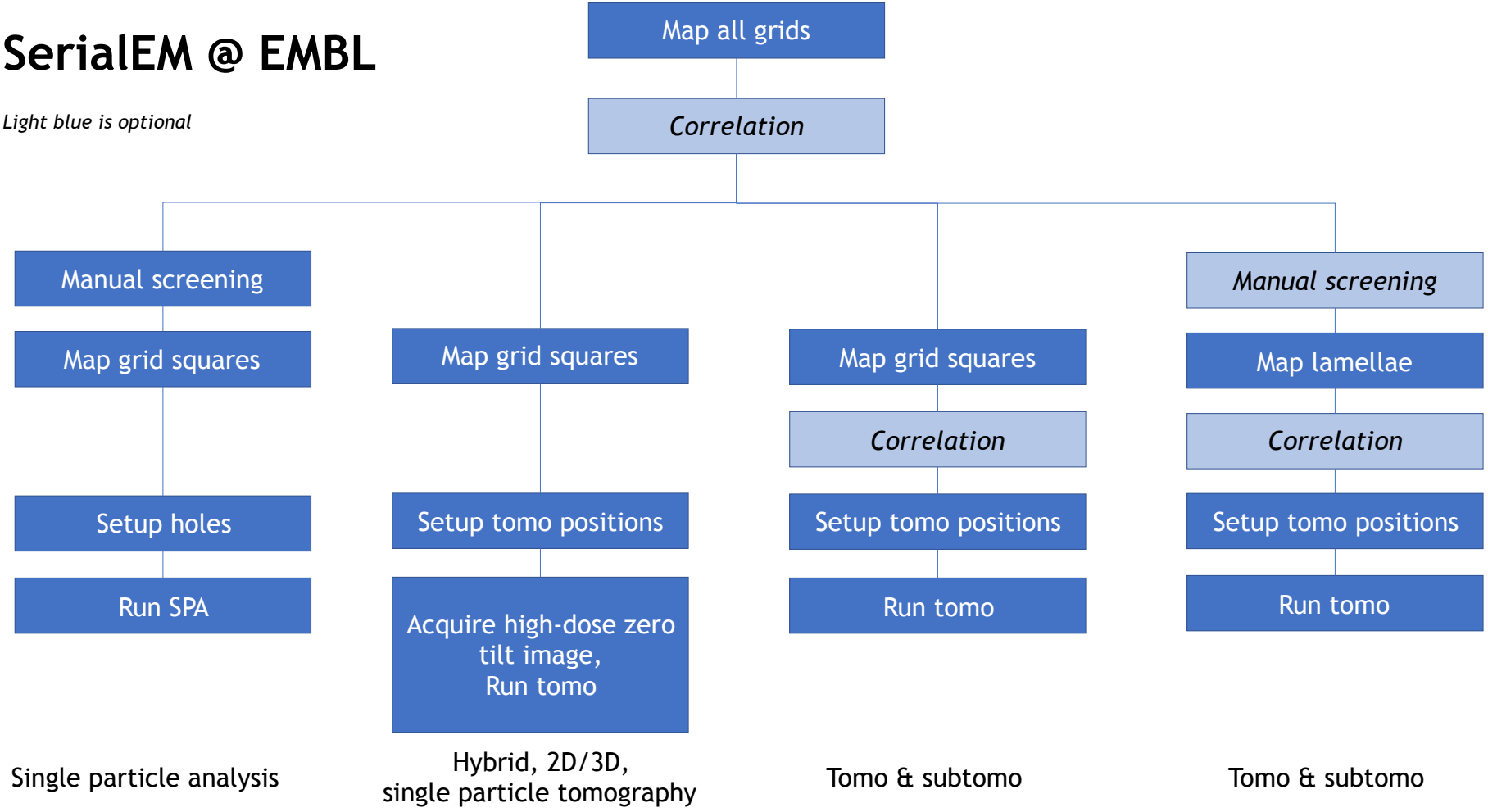
SerialEM @ EMBL

Light blue is optional



Any session

- Load grids, map all grids in lowest usable LM mode -> email.

SPA

- Screen for good squares.
- Select squares/lamellae, map montage in SA mode -> email.
- Setup acquisition.

Tomo

- Select squares/lamellae, map montage in SA mode -> email.
- Search for targets, setup acquisition.

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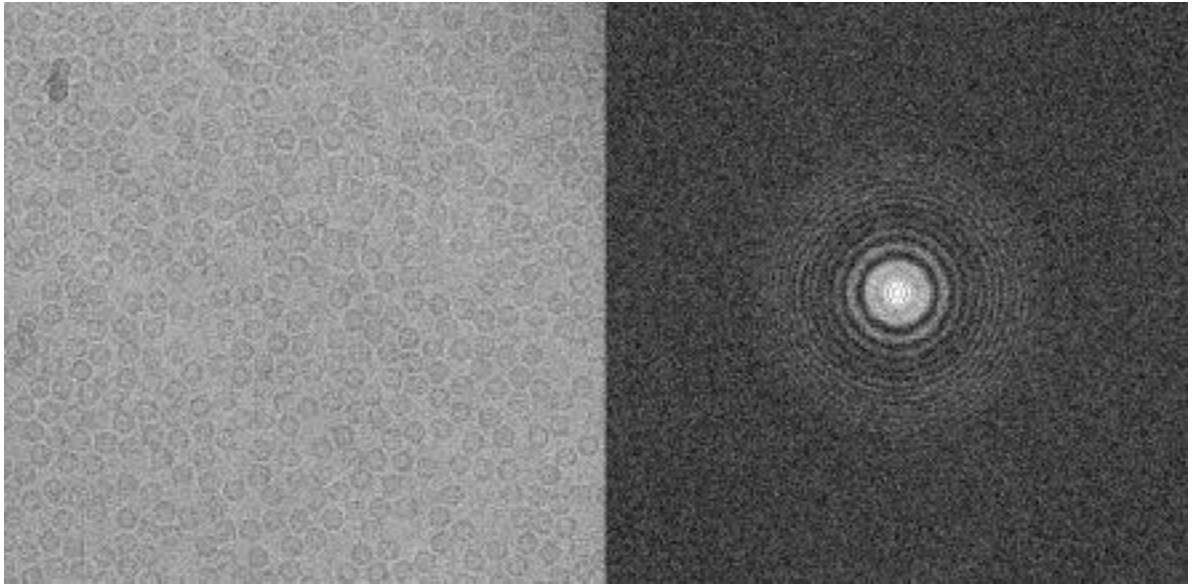
SPA acquisition

Gatan pc SerialEM:

- Post-actions.
- EarlyReturnNextShot.
- Save uncorrected LZW compressed tif.



Support pc with GPU:

- IMOD FrameWatcher.
- IMOD AlignFrames.
- Data stored on EMBL network.

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Raw Data

Overview | Fourier Space | Real Space

Input

Preprocessing

CTF

Motion

Models

Pick Particles

Output

STOP PROCESSING

Processing Status

Astigmatism

Defocus

Estimated resolution

Tomo

ELSEVIER

Journal of Structural Biology

Volume 197, Issue 2, February 2017, Pages 191-198

Implementation of a cryo-electron tomography tilt-scheme optimized for high resolution subtomogram averaging

Wim J.H. Hagen, William Wan, John A.G. Briggs

⊞ **Show more**

https://doi.org/10.1016/j.jsb.2016.06.007

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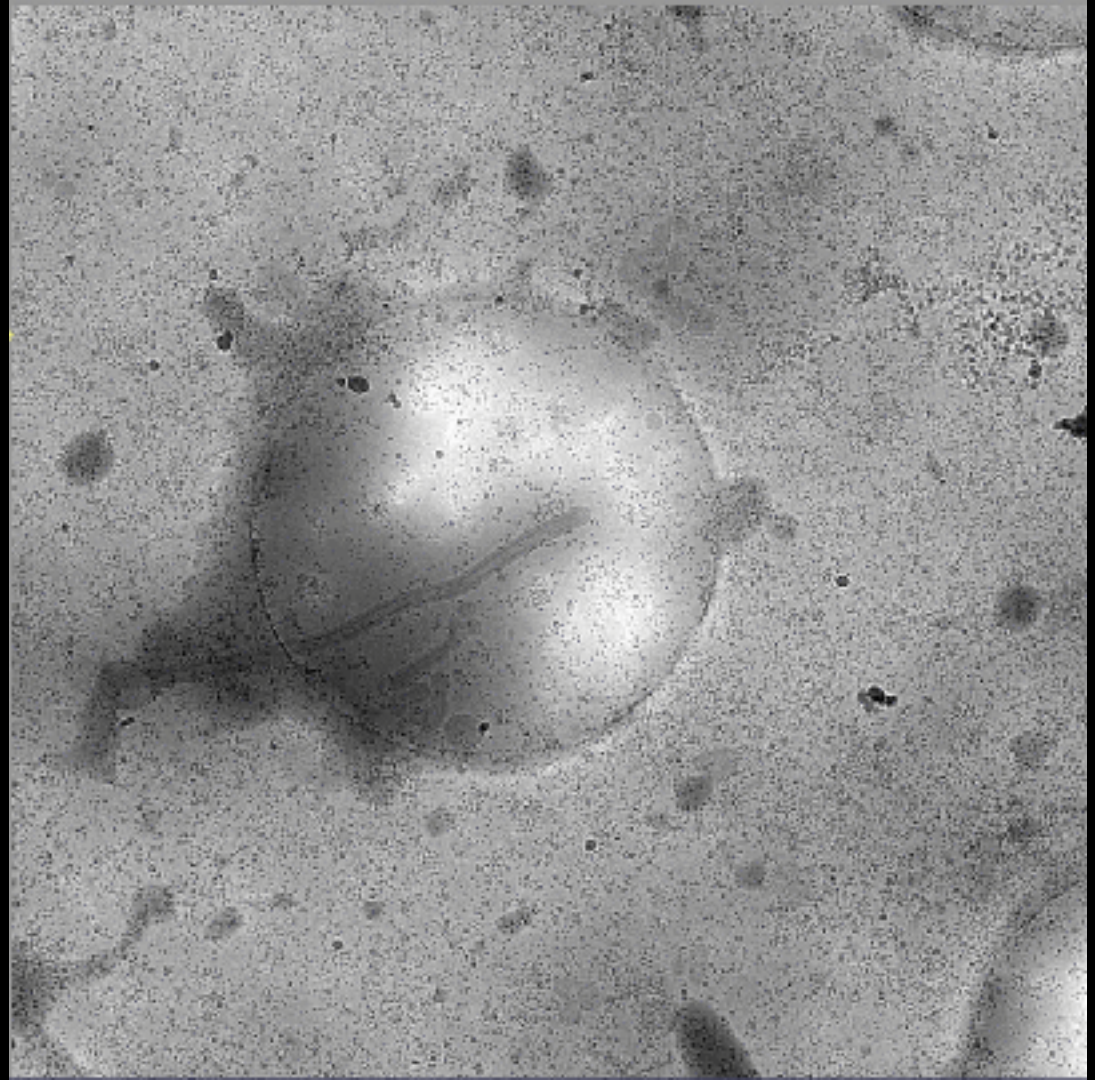

SerialEM Tomo acquisition

Gatan pc SerialEM:

- Gatan pc has GPU.
- Dose-symmetric tilt scheme.
- On-the-fly frame alignment.
- Also save raw frames as uncorrected LZW compressed tif.
- Currently +/- 60°, 3° steps, 41 images, 27 minutes.

- IMOD FrameWatcher & AlignFrames also possible.

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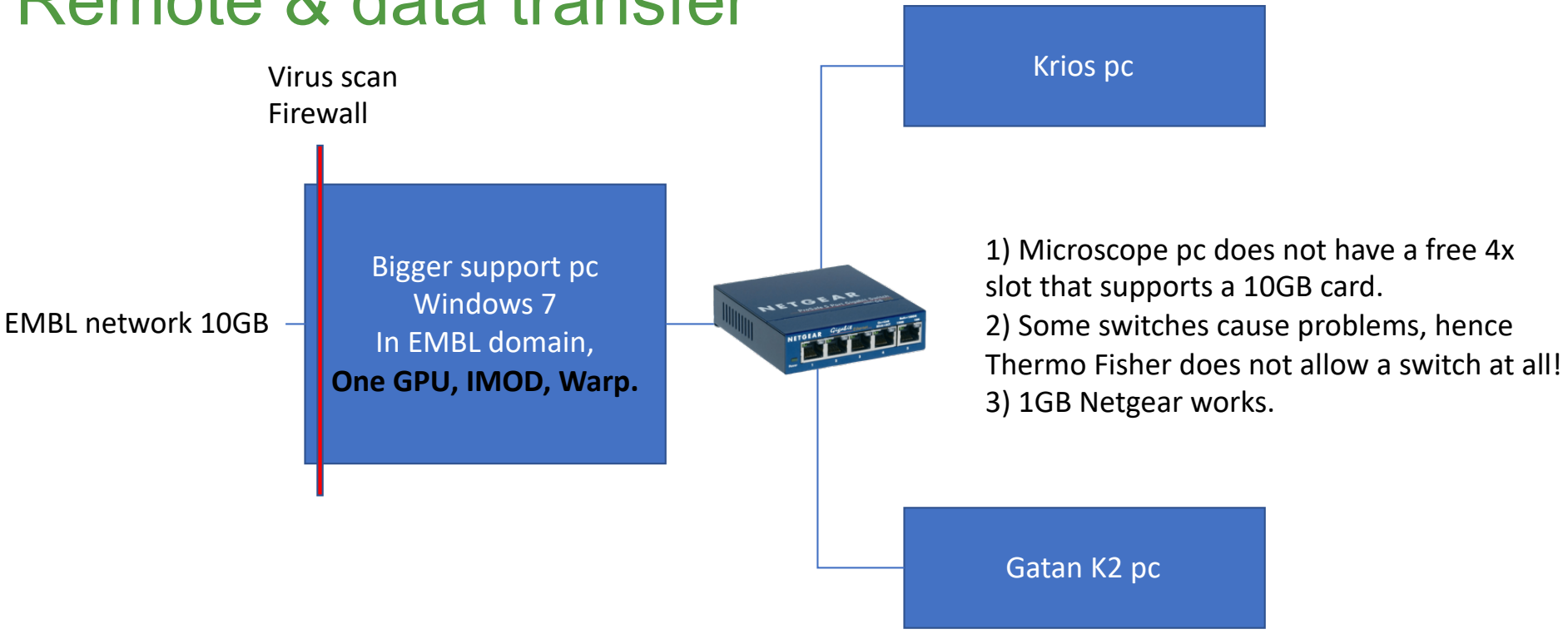
Remote & data transfer



1) Microscope pc does not have a free 4x slot that supports a 10GB card.
2) Some switches cause problems, hence Thermo Fisher does not allow a switch at all!
3) 1GB Netgear works.

Advantage: Remote to Microscope AND Gatan pc by VNC through Support pc with port forwarder.

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Remote & data transfer

- Internal users store data on group shares.
- No storage space, no data acquisition!

- 10Tb storage for external users.
- Typically for three months.
- Data transfer over EMBL Aspera server.

- Warp data?

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Internal training

- Data collection training only.
- EM training on request.

Internal & external sessions

- Minimise need for operator help.

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SerialEM

- Maps, EPU, Tomo, Latitude and LatitudeS.
- Built-in AutoStigmate, AutoComa (uses CTFFIND4).
- Built-in active beam tilt compensation (so far SPA only).

- Very flexible.
- Fast support.
- Works with everything.

- **Steep learning curve!**

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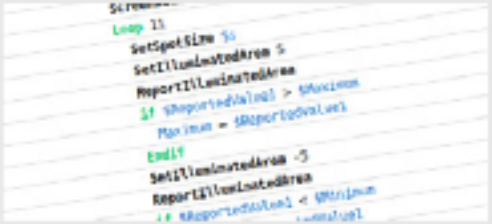
nexperion

Expertise Software Contact

SerialEM Script Repository



A repository for sharing SerialEM scripts (macros) allowing automation of TEM data acquisition is being provided by Nexperion.

> Read more

Solutions for Electron Microscopy

SerialEM and IMOD

We provide support and training for SerialEM and IMOD, a freely available solution for acquisition and processing of (cryo)electron tomography data.

> Read more

www.nexperion.net

www.youtube.com/playlist?list=PLGggUwWmzvs-DV4jCap

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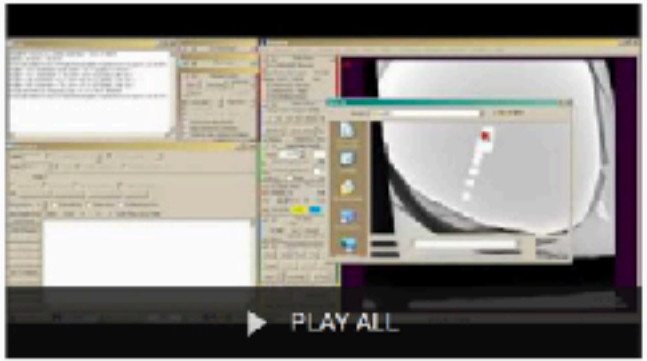

SerialEM Lectures and Tutorials

23 videos • 14,694 views • Last updated on Nov 28, 2012

1 

SerialEM Introduction Lecture Part 1

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SerialEM Introduction Lecture Part 2

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Acquiring a gain reference with SerialEM

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SerialEM Introduction Lecture Part 4

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SerialEM Note: Installation and Calibration

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Date_Created:	2017-11-12
Last_Updated:	2018-08-28

Abstract

When I helped a few sites to install and calibrate SerialEM, I had impression that most new users felt this process was very hard. I felt the same way when I initially learned to install and calibrate SerialEM by myself. I even got frustrated and had to call David for a few times. When I think back about all the troubles I had to install and calibrate SerialEM, I believe I would have an easier time if I had a brief guideline document for what steps to follow in order, and what to do in each step. The helpfile from SerialEM is very complete to provide almost all information needed, but it is perhaps a lot to read and not clear where to start for a beginner.

I wanted to list some steps here to guide you through this initial installation and calibration phase. It is like a crush list. For more detailed information, you should always find it from helpfile.

Installation

Here are steps to follow

1. Ask David for the initial system file. Normally you would fill out a "questionnaire" available at the ftp server - http://bio3d.colorado.edu/ftp/SerialEM/questionnaire.txt and send it to David. David will then create a framework file on the same ftp server for you to download. This framework file is a zip file, you can download it to local like Desktop and unzip it by double clicking on the file. Beside a sub-folder "Admin" created under C:\ProgramData\SerialEM, the most important file in the framework is one initial system file called "SerialEMproperties.txt". You must have this file to get started. Please refer to the SerialEM webpage for the latest information regarding this.

Acknowledgements:
- David Mastronarde
- Chen Xu
- The SerialEM community
- Felix Weis

Questions?

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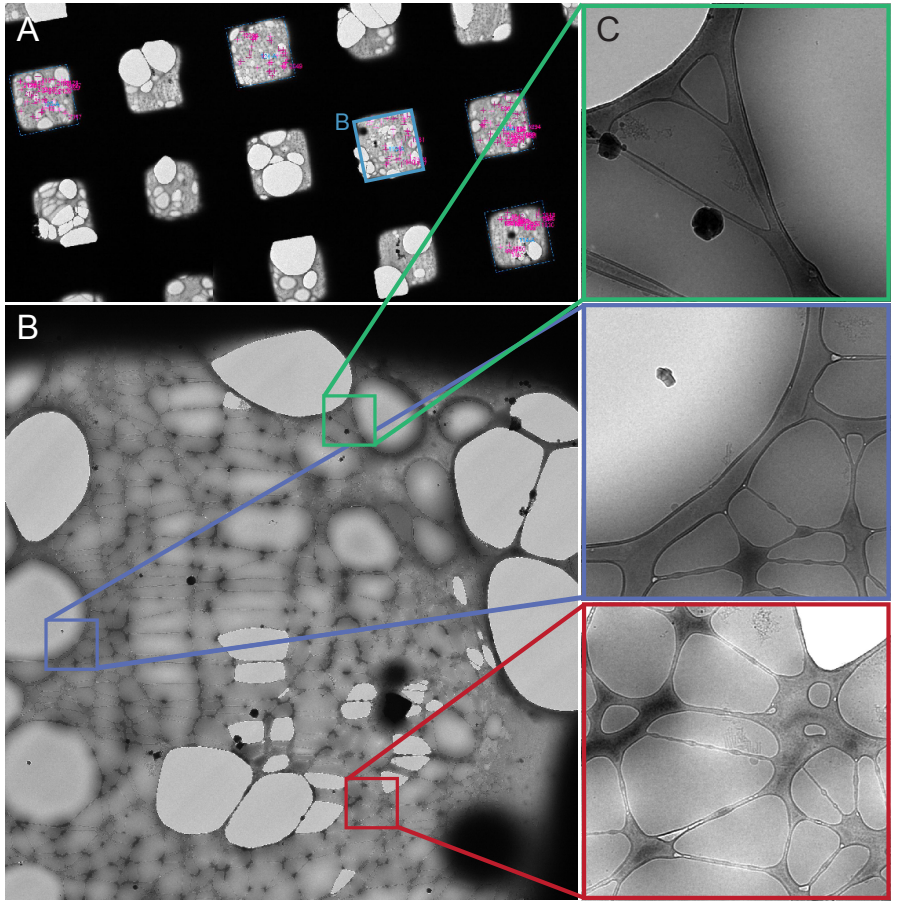
Latest development

EM-Tools

- Map squares at 2250x.
- Mark positions.
- Cut positions and scale to 15Kx Low Dose View image: virtual maps.
- Align to virtual maps at 15Kx.

Why?

- E.g. fibrils, a flock here and there…
- Standard approach:
  2000 images per day, 10% yield.
- EM-Tools approach:
  1000 images per day with 100% yield.
- Selection can be done during grid square mapping.



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