

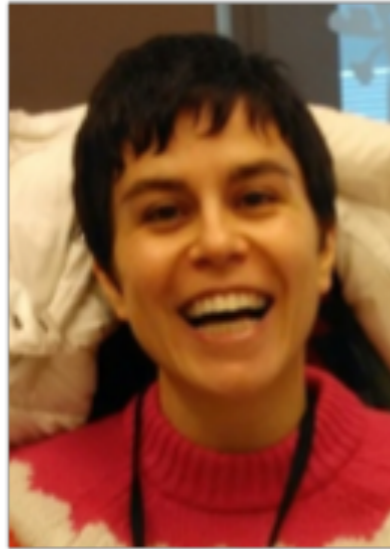
# Swedish cryo-EM national facility Stockholm node



# The people



**Gunnar von Heijne**  
Director



**Marta Carroni**  
Facility Manager  
Head of facility



**Julian Conrad**  
Facility Manager



**Stefan Fleischmann**  
System  
Administrator



**Jose Miguel  
de la Rosa Trevin**  
IT Manager



**Karin Walldén**  
Research assistant

**Stockholm Node**



**Alexey Amunt's  
group**



**Erik Lindhal's  
group**



**Bernt Eric Uhlin**



**Linda Sandblad**



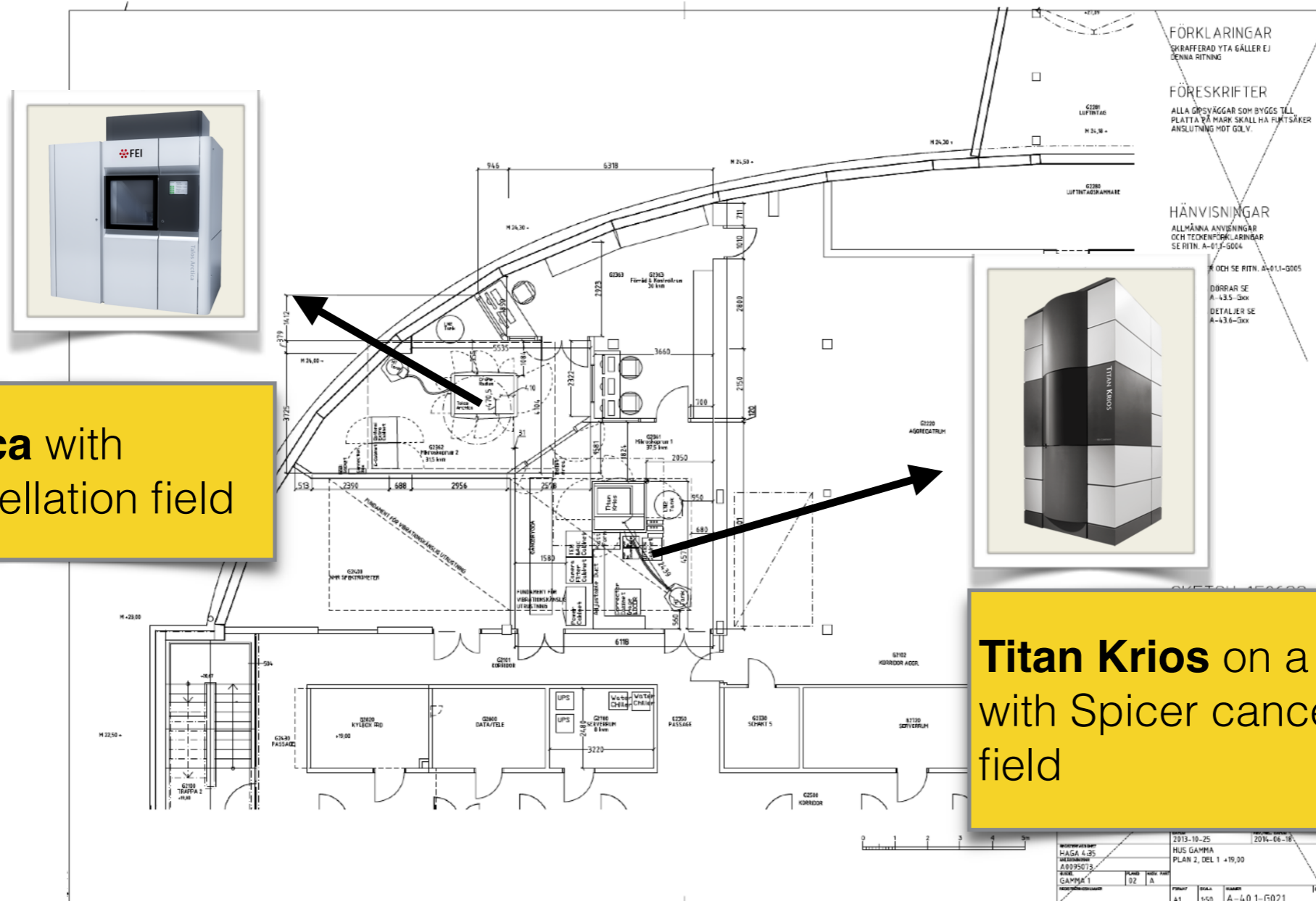
**Michael Hall**



**Camilla Homlund**

**Umeå Node**

# Planning of the room



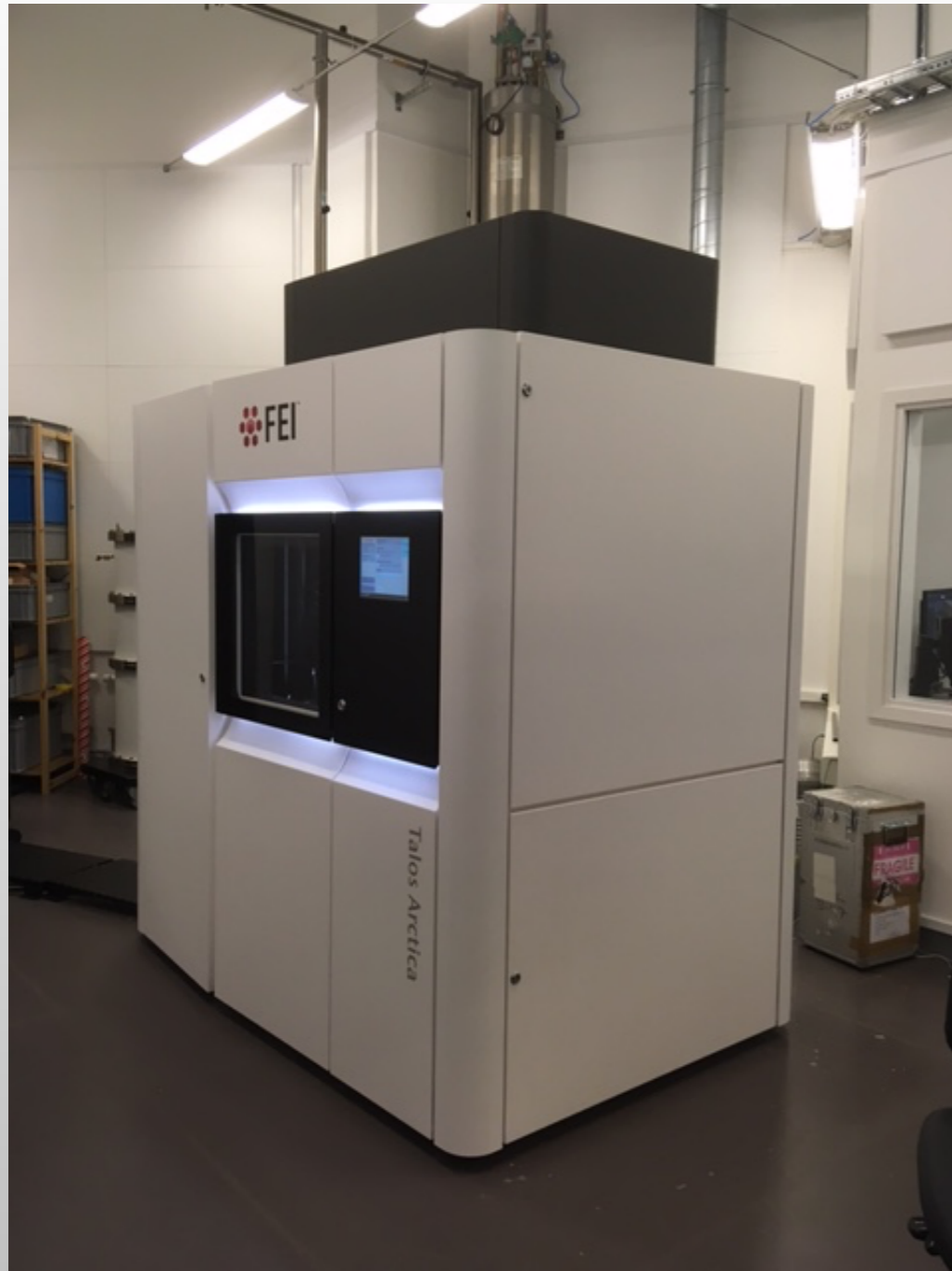
**Talos Arctica** with Spicer cancellation field

**Titan Krios** on a slab with Spicer cancellation field

## CryoEM lab requirements:

- Water pipes for microscopes chillers
- Temperature and humidity control (20°C, 30%)
- Hood for handling ethane (?)
- Oxygen and ventilation system
- Smoke sensors, fire extinguishers and sprinkles
- *Nitrogen supply for the microscopes*

# The microscopes... construction time



**Arctica ready in March 2016**



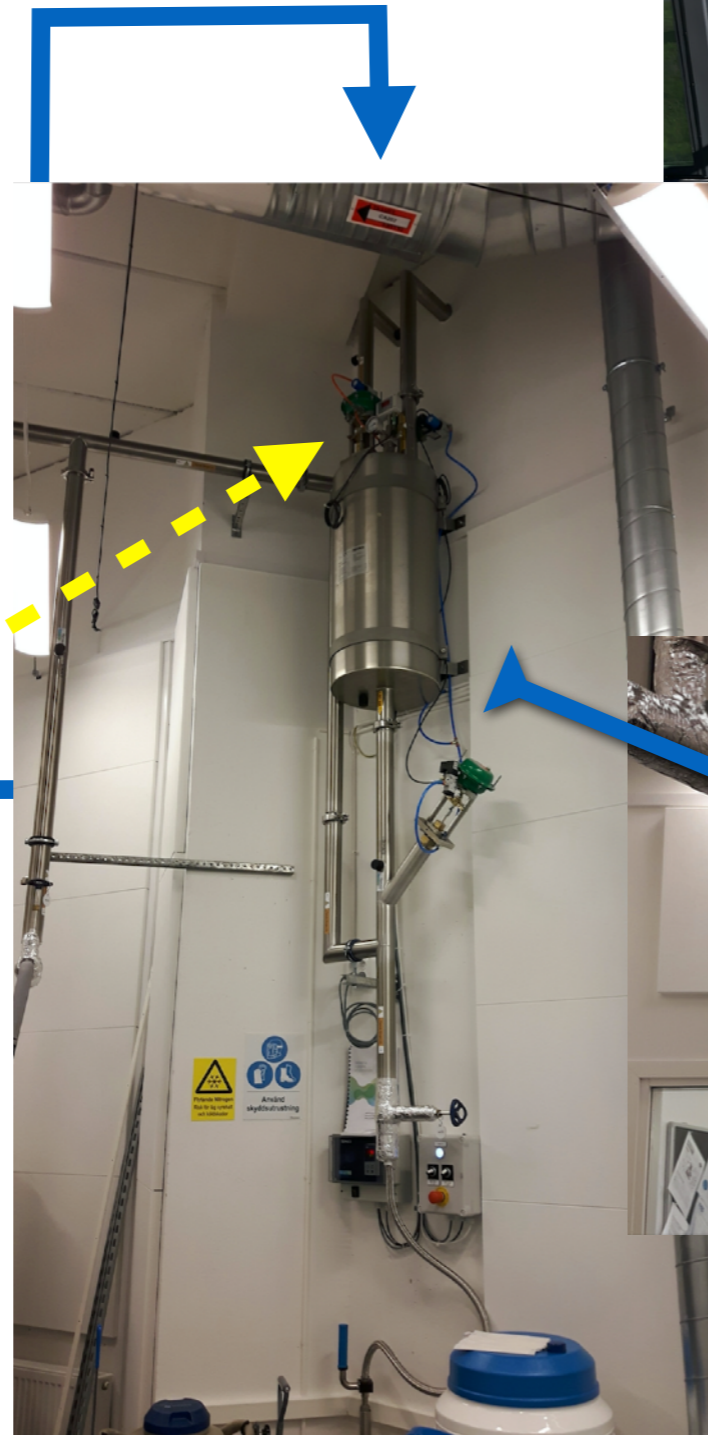
**Krios ready in June 2016**

# The LN<sub>2</sub> refilling system

outside: big LN<sub>2</sub> tank



outside: exhaust for N gas



inside: LN<sub>2</sub> straight into the microscopes and tap for daily usage

inside: 60 litres LN<sub>2</sub> tank

# Microscopes



	Talos Arctica	Titan Krios
e <sup>-</sup> energy	200kV	300kV
electron source	X-FEG	X-FEG
condenser lens system	C1+C2+ minicondenser	constant current C1+C2+C3+ minicondenser
objective lens system	constant current	constant current
Volta phase plate	yes	yes
specimen stage	Autoloader for 12 cartridges + single tilt stage	Autoloader for 12 cartridges + dual tilt stage
software for microscope operation	TEM 2.11.1 + SerialEM	TEM 2.11.1+ SerialEM
cameras, detectors and filter	CCD Ceta + <b>Falcon III</b>	CCD Ceta + <b>Falcon III + K2 summit post GIF</b>
softwares for data acquisition and analysis	EPU + SerialEM + Tomo Inspect3D, Scipion box	EPU + SerialEM + Tomo + DM3 .23 + Inspect3D, Scipion box

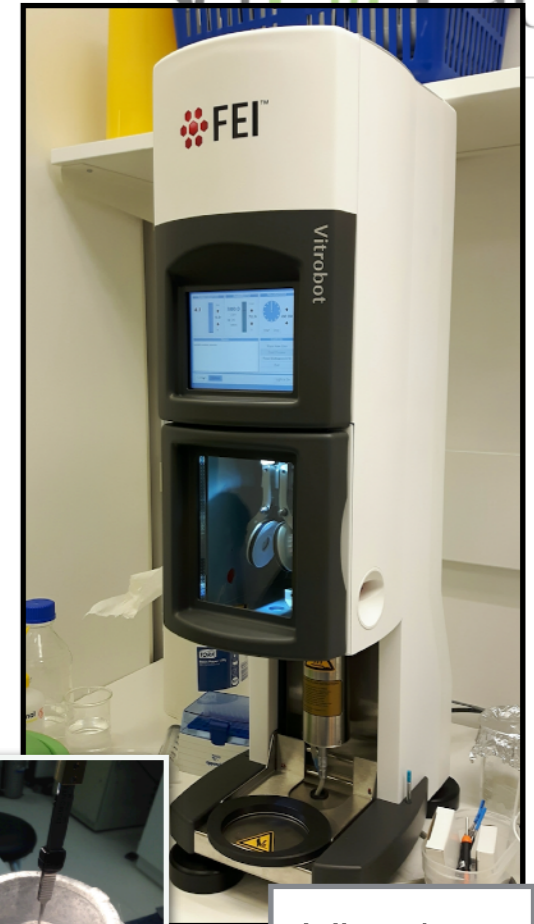
# Some auxiliary equipment



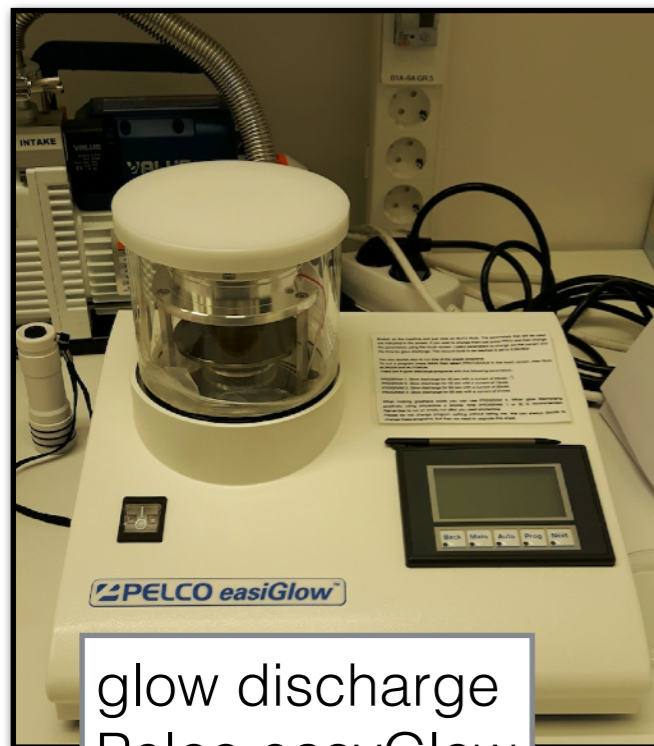
Manual plunger



Carbon coater Q150T E



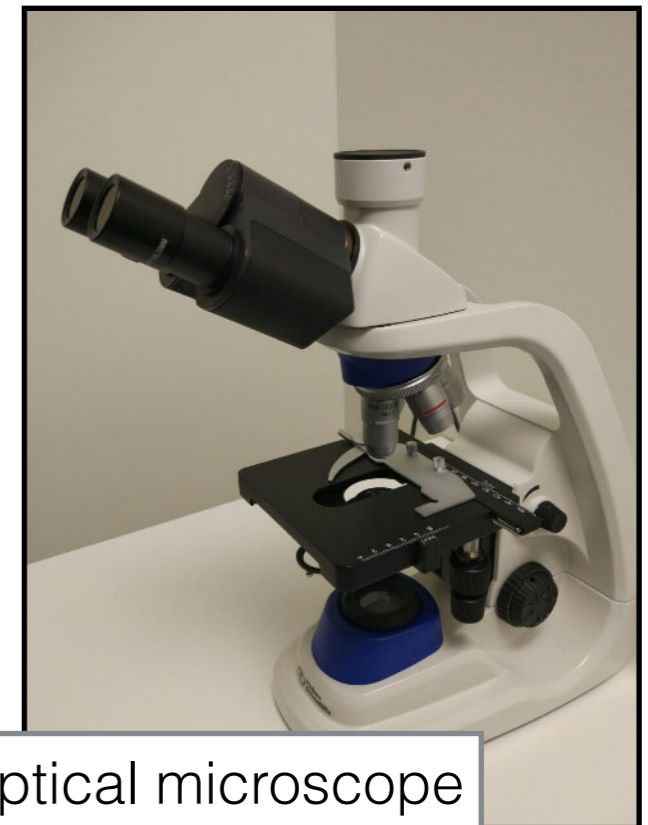
Vitrobot



glow discharge  
Pelco easyGlow



glow discharge  
Q globe



Optical microscope

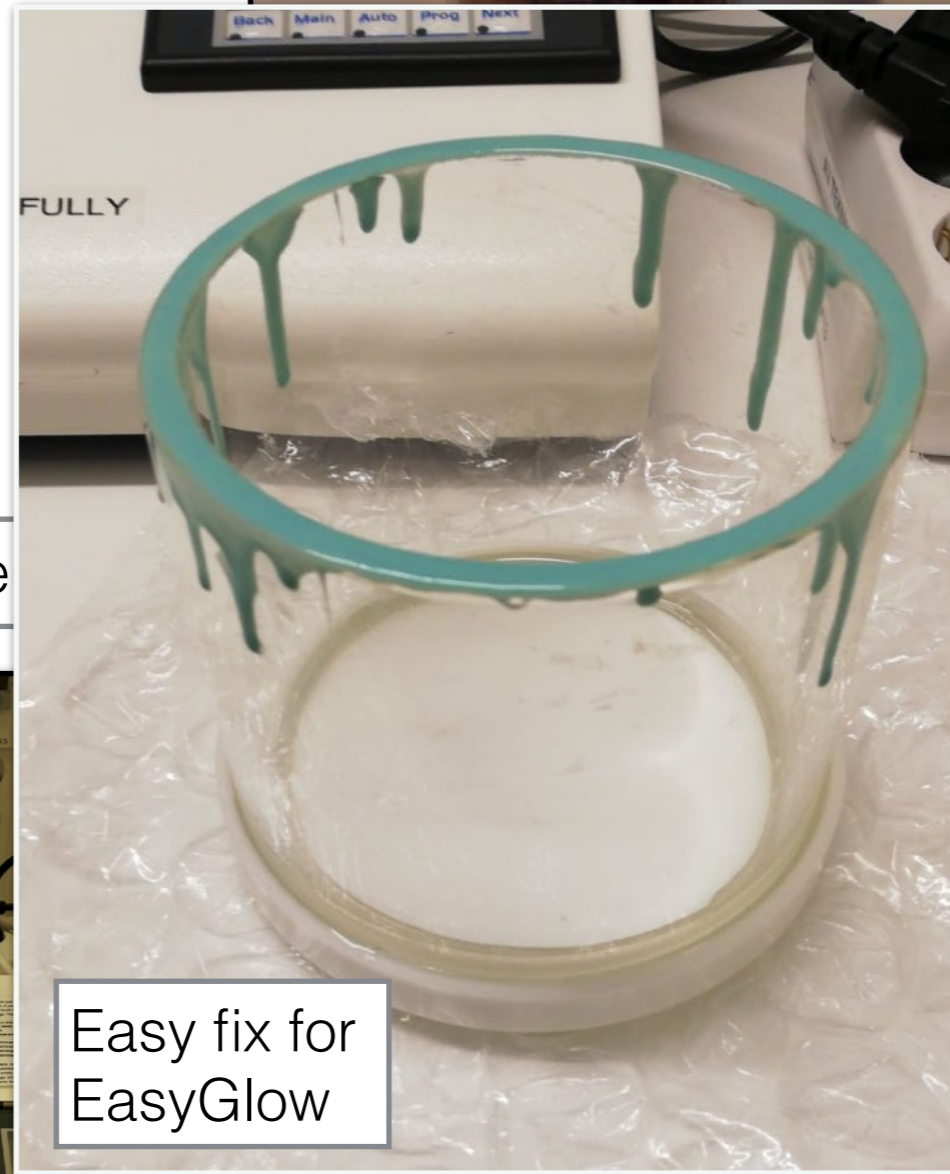
# Some auxiliary equipment



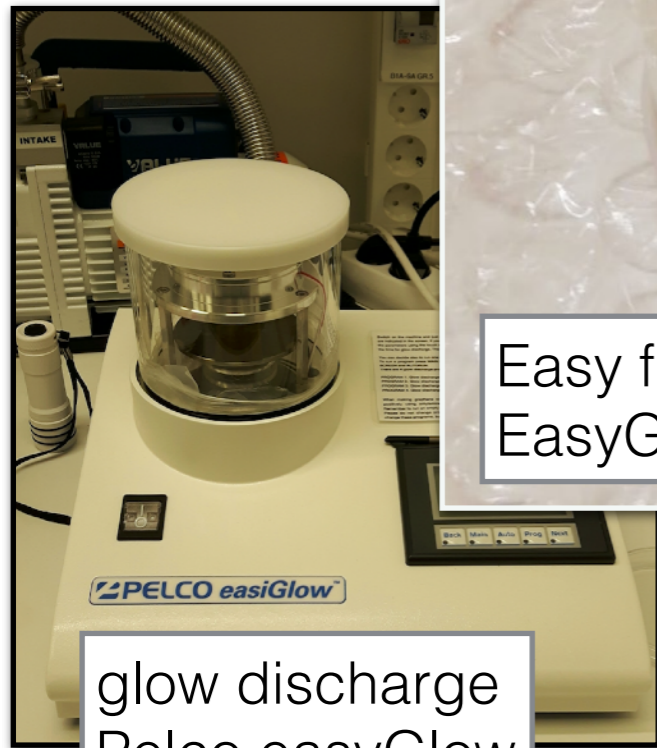
Manual plunge



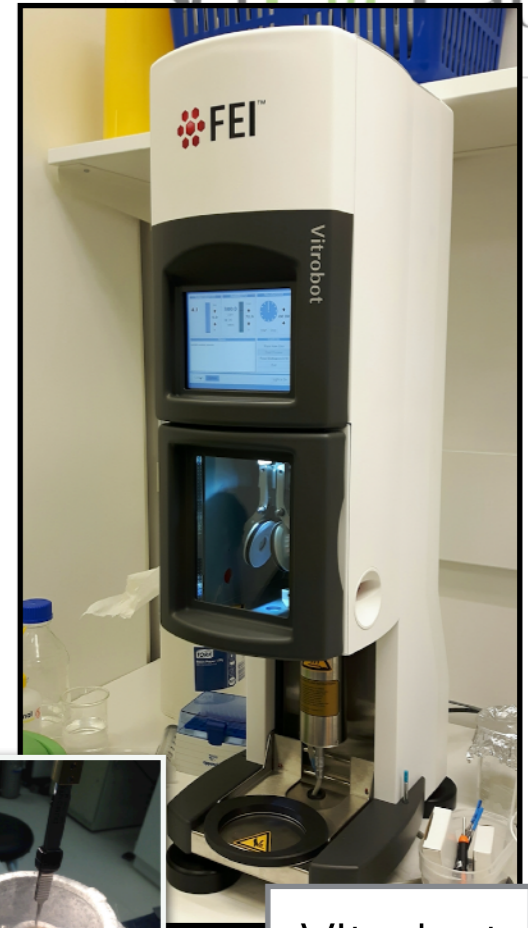
glow discharge  
Q globe



Easy fix for  
EasyGlow



glow discharge  
Pelco easyGlow



Vitrobot



Optical microscope



# A hybrid setup

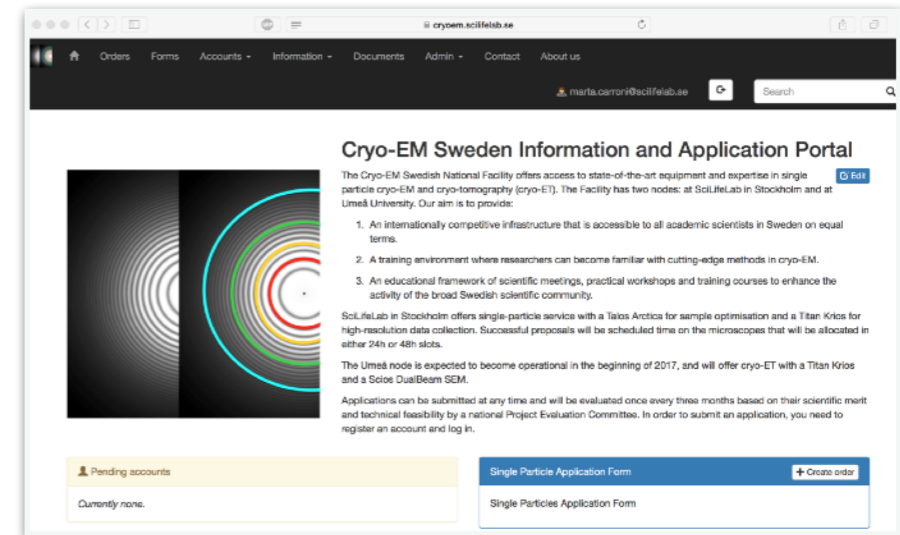
🚶 Internal users 🚲

🚚 External national users ✈️

## Training



## National portal

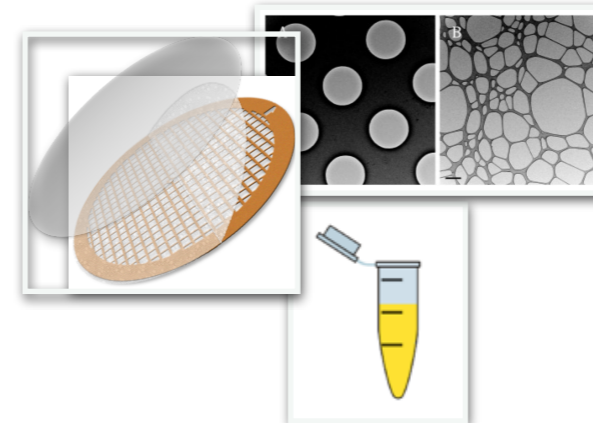


## Booking system

Date	9:00a	9:00a	9:00a	9:00a
6	Marta Carroni cryo-cycle Talos Arctica	Marta Carroni downtime power shut recovering Talos Arctica	Juni Andreil Titan Krios	A. Amunts Titan Krios
7	Marta Carroni cryo-cycle Titan Krios	Marta Carroni methods Titan Krios	Rei Matsuoka Vitrobot	Vivek Singh Carbon coater
8	Yuzuru Itoh Vitrobot	Dari Kimanus Vitrobot	Rei Matsuoka Vitrobot	Vivek Singh Vitrobot
9	Alexander Mühlip Rasmus Kok with Alex Titan Krios	Yuzuru Itoh Titan Krios	Pascal Meier Iven Winkelmann Titan Krios	A. Amunts Vitrobot
10	Yuzuru Itoh Talos Arctica	Markel Martínez Vitrobot	Markel Martínez Talos Arctica	Dari Kimanus Vitrobot
11	Marta Carroni cryo-cycle Titan Krios	Marta Carroni BAG KI Titan Krios	Geoffrey Masuyer Talos Arctica	Yuzuru Itoh Talos Arctica
12	Marta Carroni cryo-cycle Titan Krios	Marta Carroni BAG LU Talos Arctica	Rei Matsuoka Vitrobot	Vivek Singh Titan Krios
13	Marta Carroni cryo-cycle Titan Krios	Fiei Matsuoka Talos Arctica	Marta Carroni BAG UU Titan Krios	Karin Waldén Karin Waldén Titan Krios
14	Marta Carroni cryo-cycle Titan Krios	Marta Carroni BAG LU Talos Arctica	Annemarie Perez Boerema Course Vitrobot	Björn Forsberg Talos Arctica
15	Marta Carroni cryo-cycle Titan Krios	Marta Carroni BAG UU Titan Krios	Rebecca Howard Talos Arctica	Mikaela Rapo Vitrobot
16	Marta Carroni cryo-cycle Titan Krios	Marta Carroni BAG KI Titan Krios	Juni Andreil Talos Arctica	Victor Toblasson Victor Toblasson Titan Krios
17	Marta Carroni cryo-cycle Titan Krios	Markel Martínez Talos Arctica	Rebecca Howard Talos Arctica	Andreas Carlström Talos Arctica
18	Marta Carroni cryo-cycle Titan Krios	Markel Martínez Talos Arctica	Juni Andreil Talos Arctica	Victor Toblasson Victor Toblasson Titan Krios
19	Marta Carroni cryo-cycle Titan Krios	Markel Martínez Talos Arctica	Marta Carroni BAG UU Titan Krios	Dari Kimanus Talos Arctica
20	Marta Carroni cryo-cycle Titan Krios	Marta Carroni BAG LU Talos Arctica	Marta Carroni BAG KI Titan Krios	Dari Kimanus Talos Arctica
21	Marta Carroni cryo-cycle Titan Krios	Marta Carroni BAG LU Talos Arctica	Marta Carroni BAG UU Titan Krios	Dari Kimanus Talos Arctica
22	Marta Carroni cryo-cycle Titan Krios	Marta Carroni BAG LU Talos Arctica	Marta Carroni BAG UU Titan Krios	Dari Kimanus Talos Arctica
23	Marta Carroni cryo-cycle Titan Krios	Marta Carroni BAG LU Talos Arctica	Marta Carroni BAG UU Titan Krios	Dari Kimanus Talos Arctica
24	Marta Carroni cryo-cycle Titan Krios	Marta Carroni BAG LU Talos Arctica	Marta Carroni BAG UU Titan Krios	Dari Kimanus Talos Arctica
25	Marta Carroni cryo-cycle Titan Krios	Marta Carroni BAG LU Talos Arctica	Marta Carroni BAG UU Titan Krios	Dari Kimanus Talos Arctica
26	Marta Carroni cryo-cycle Titan Krios	Marta Carroni BAG LU Talos Arctica	Marta Carroni BAG UU Titan Krios	Dari Kimanus Talos Arctica
27	Marta Carroni cryo-cycle Titan Krios	Marta Carroni BAG LU Talos Arctica	Marta Carroni BAG UU Titan Krios	Dari Kimanus Talos Arctica

Screening 😜

Data acquisition 😊



## Sample preparation

- How to choose grids, make carbon, make graphene oxide, glow discharge grids
- How to plunge freeze specimens with the Vitrobot
- How to do negative stain if required
- How to load grids and cassette into the scope

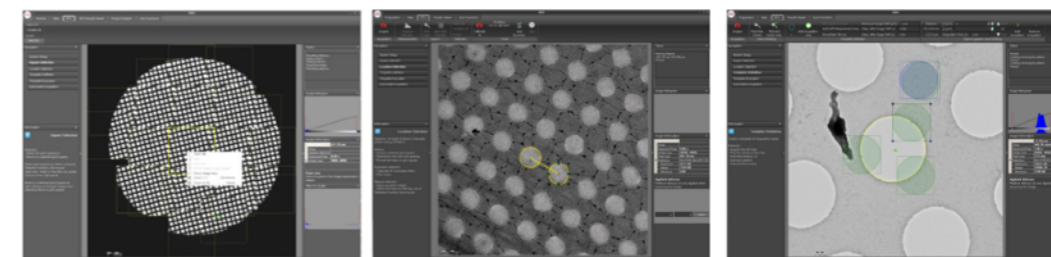
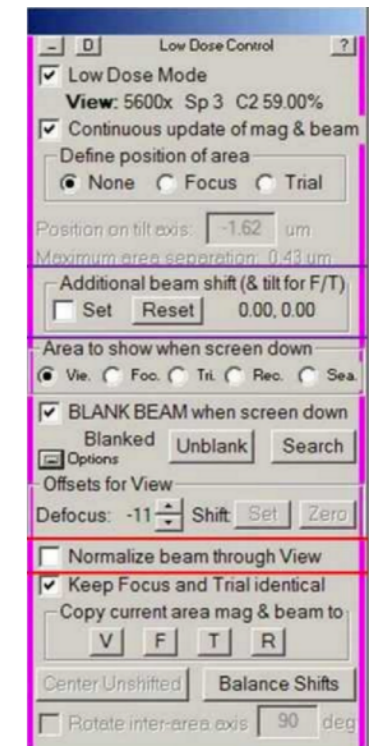
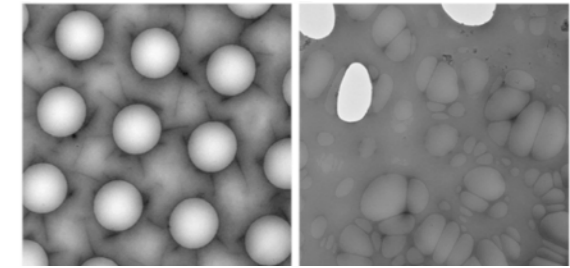
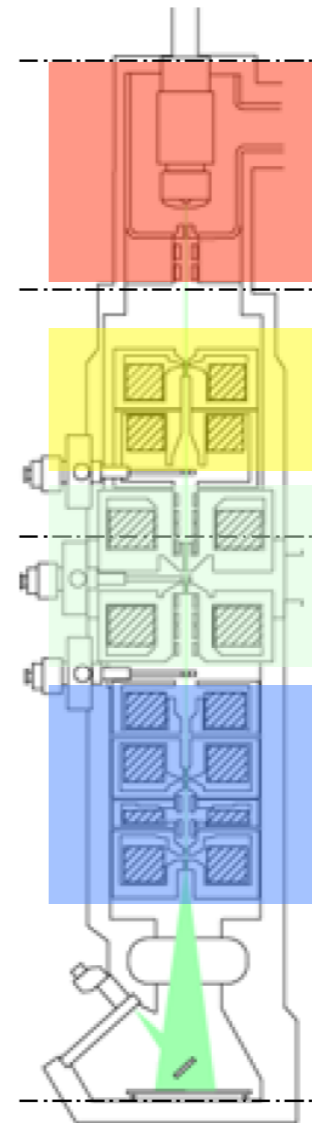
## First microscope operation

- Microscope parts and basic direct alignments (AutoCTF)
- Low-dose concepts and EPU (or SerialEM) usage for both screening and data collection

## Usage of energy filter and K2 detector

- Tomography acquisition (SerialEM and Tomo)
- Setup of EPU for K2 and first data processing steps

## Annual courses and workshops for image processing



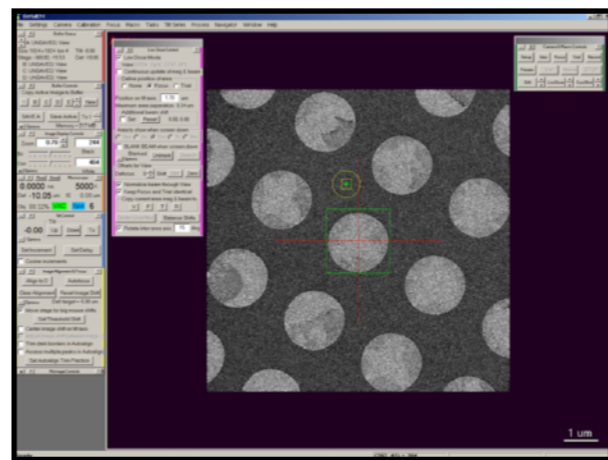
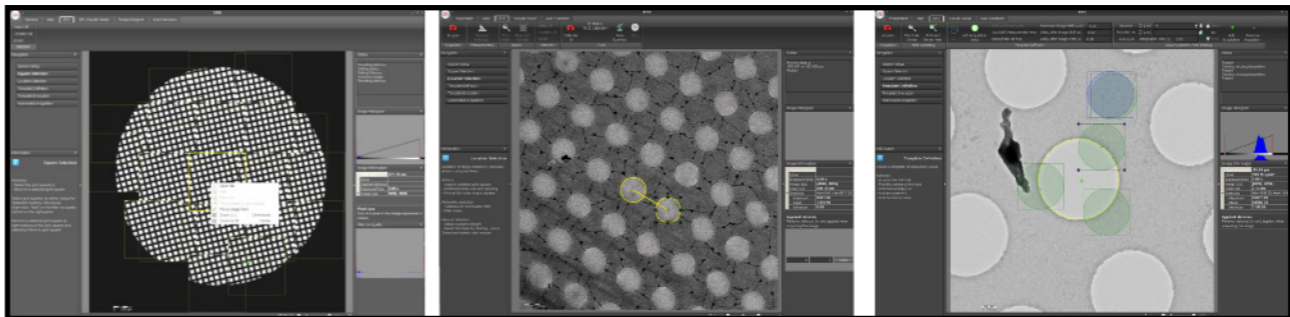
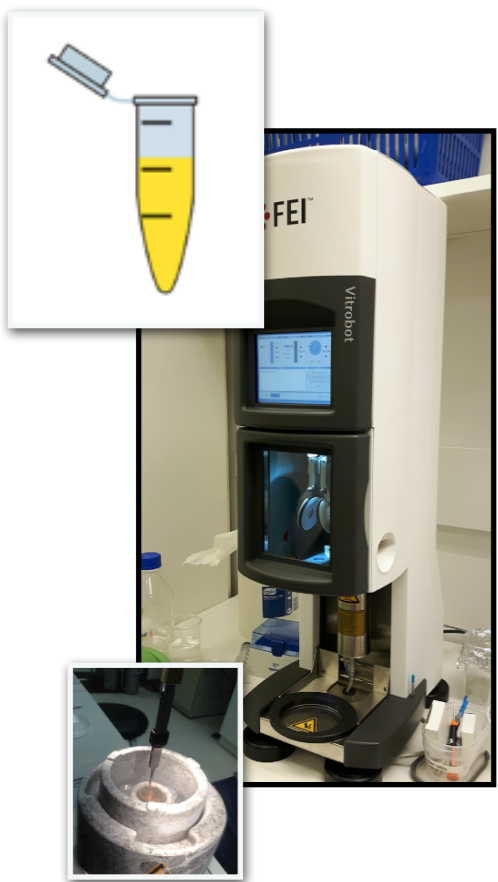


# Portal for national facility applications

- Attach evidence of the sample quality, homogeneity, monodispersity (SEC-MALLS, DLS)
- Justify why is it suitable for cryo-EM
- Include negative stain image if available and preliminary reconstruction if possible
- Include information on image processing knowledge
- BAG (95% time) and Rapid access (5%) applications
- Charge for users 5000SEK (~500€) per 24h slot
- Evaluation by a scientific committee of academics appointed by each Swedish University



# Screening day... and months...



- 2-3 different concentrations
- 8-12 grids
- grids type (to start R2/2 or Lacey)
- support (carbon/graphene oxide)
- pentylamine glow

- Screen until lunch time
- We use EPU to quickly move and change optics
- Only focus once per grid square
- Check 4-5 different areas/ice thickness

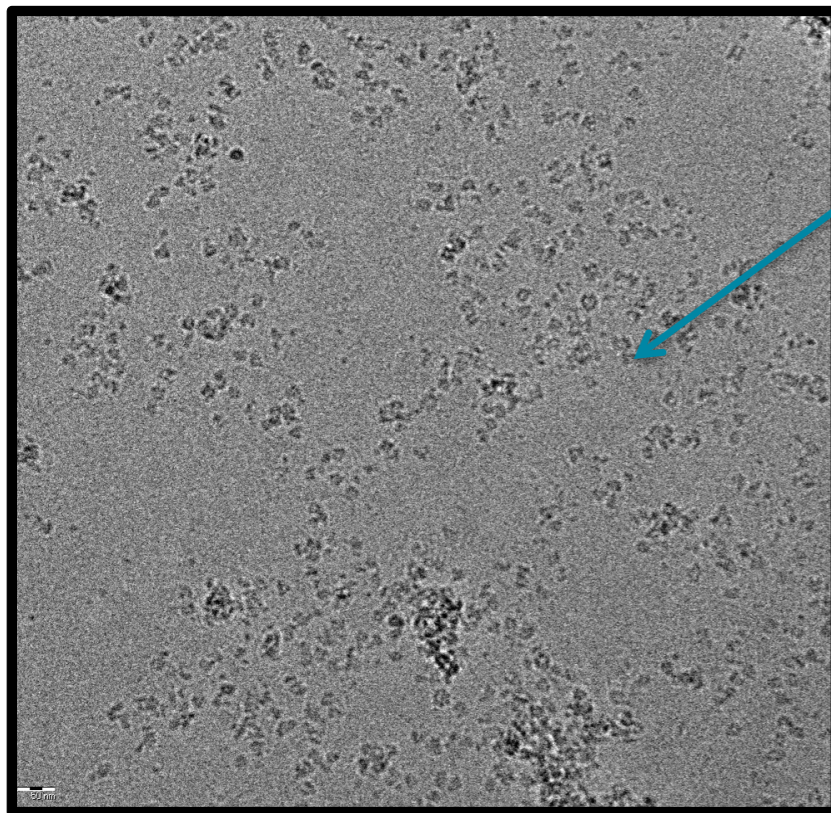
- Start a data collection if a good grid is found
- If not, re-freeze and check max 6 grids
- For tomography all with serialEM. If promising collect one or two manually. Next set-up some o/n.



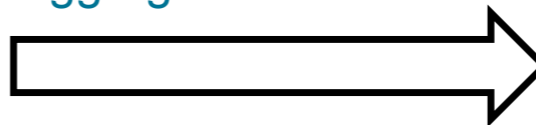
# A screening example

## 1.5 month screening

Final grid condition obtained after **5 screening session on the Talos Arctica**.

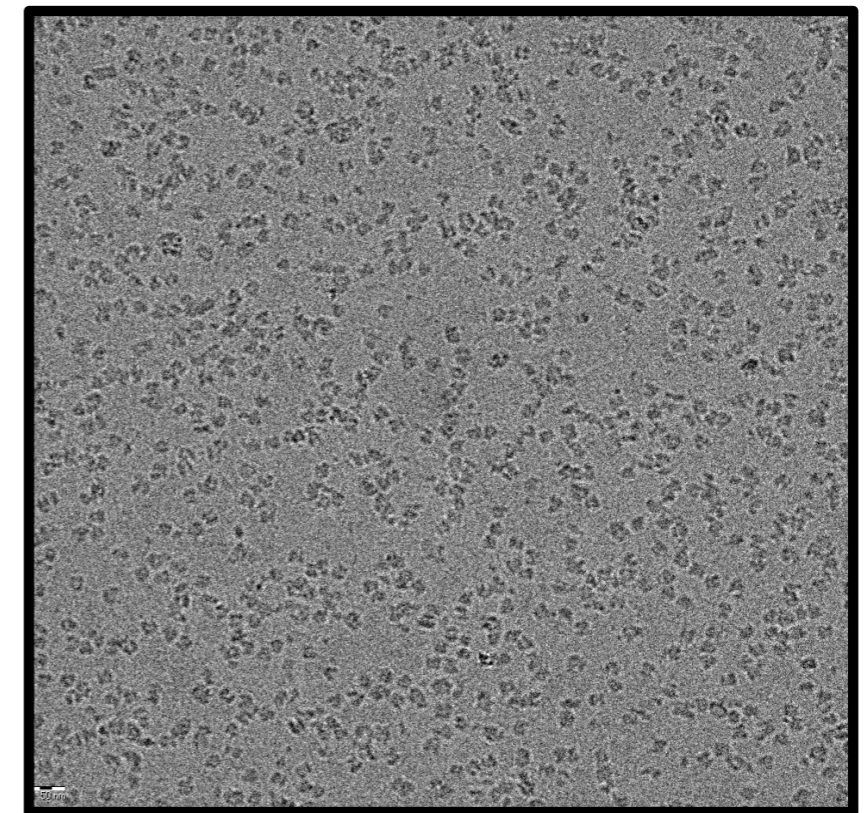


Uneven particle spread  
aggregation



### Optimization:

1. Grid type
2. Glow discharge conditions
3. Ice thickness

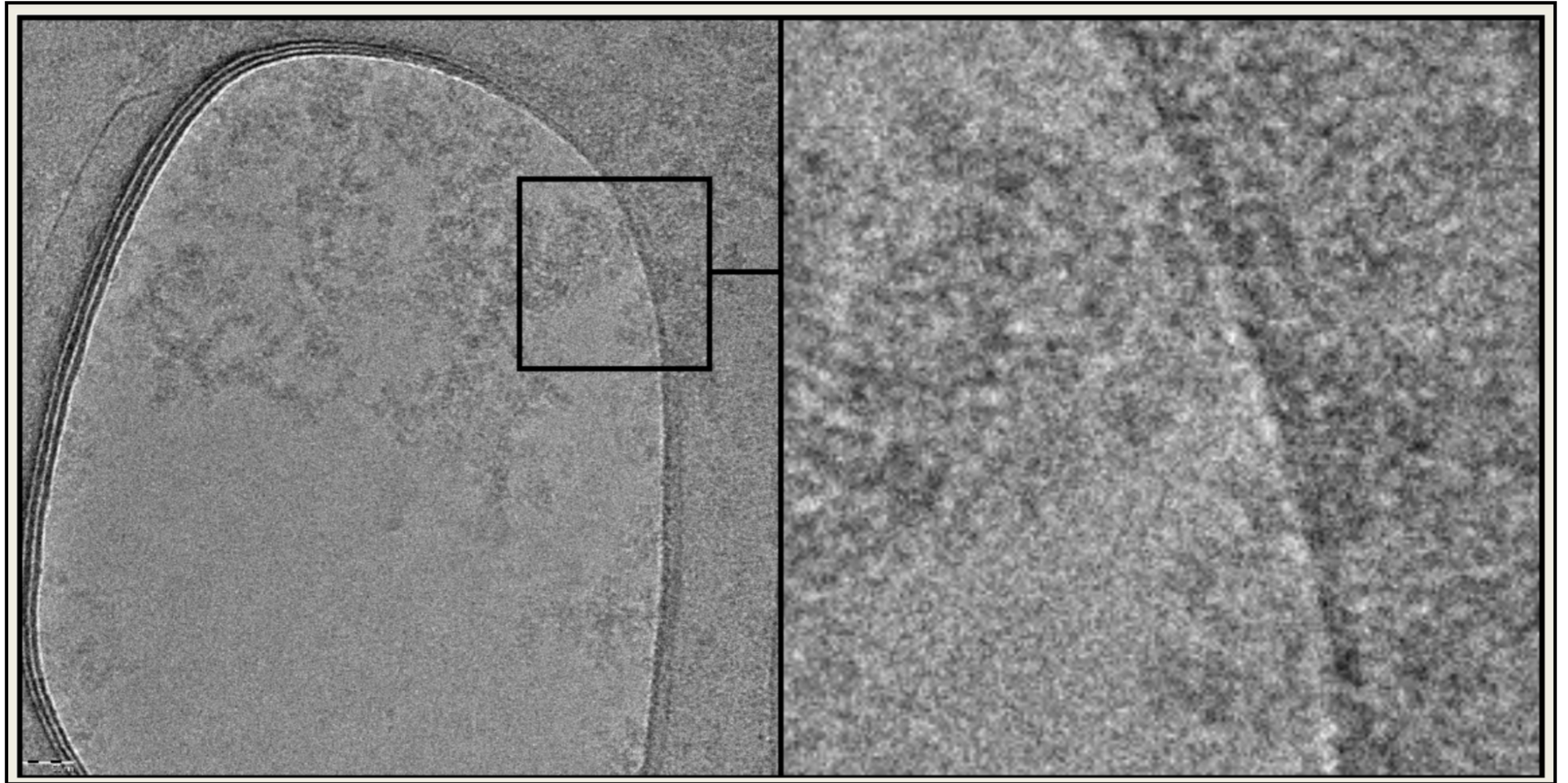


300Å

Sample: 0.8  $\mu\text{M}$ , 3 $\mu\text{l}$  Grids: C-Flat 1/1, 400#  
Blotting conditions: 3s, 30s wait, 0 offset  
Glow discharge conditions: 40s, 20mA  
Imaging conditions: Talos, 92K, 30 electrons,  
 Falcon II

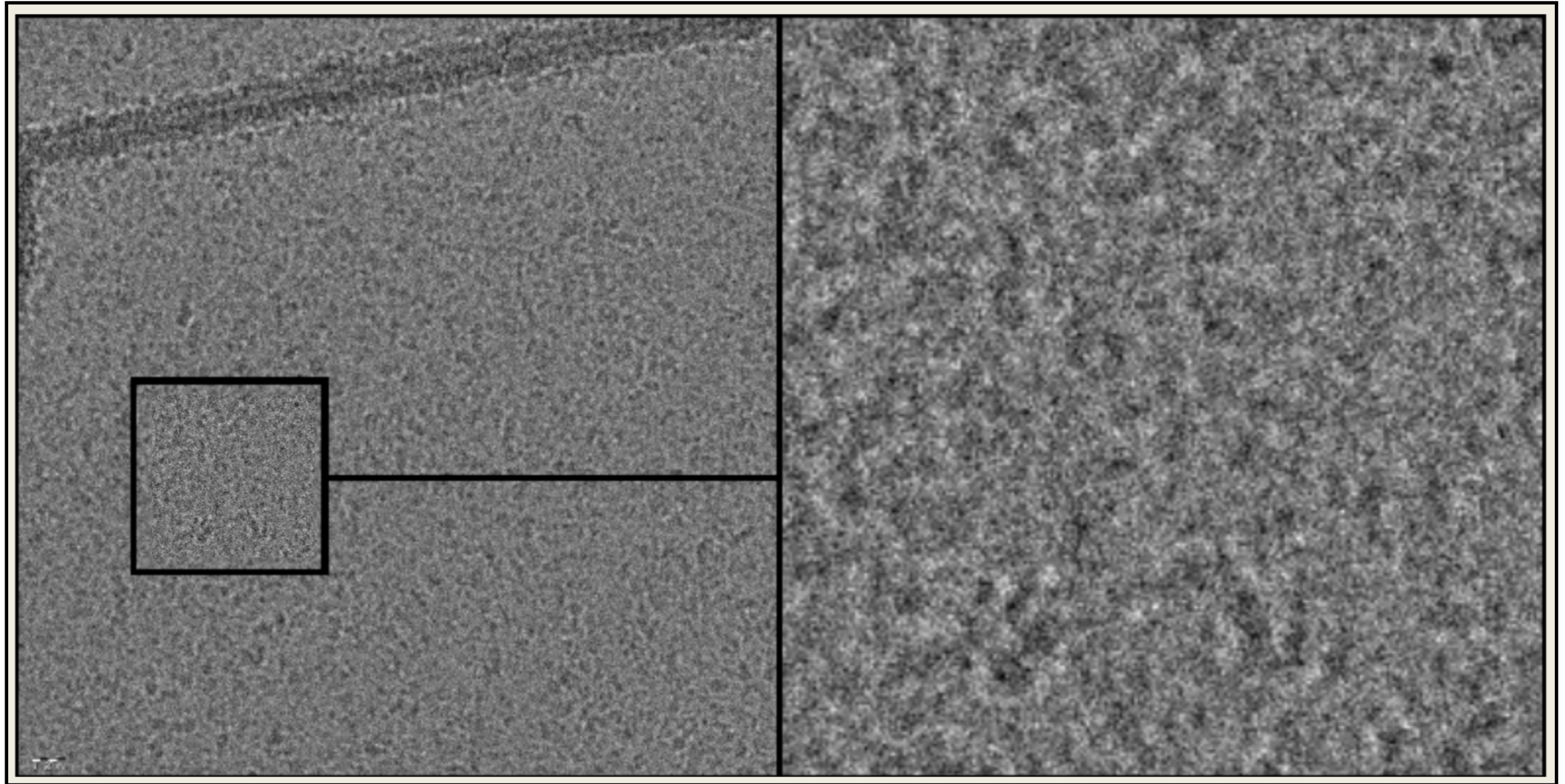
Sample: 1.25  $\mu\text{M}$ , 3 $\mu\text{l}$  Grids: Q 1.2/1.3, 300#  
 400#mesh Blotting conditions: 3s, 30s wait  
Glow discharge conditions: 60s, 20mA  
Imaging conditions: Krios, 130K, 28 electrons,  
 K2 counting

# Another screening example



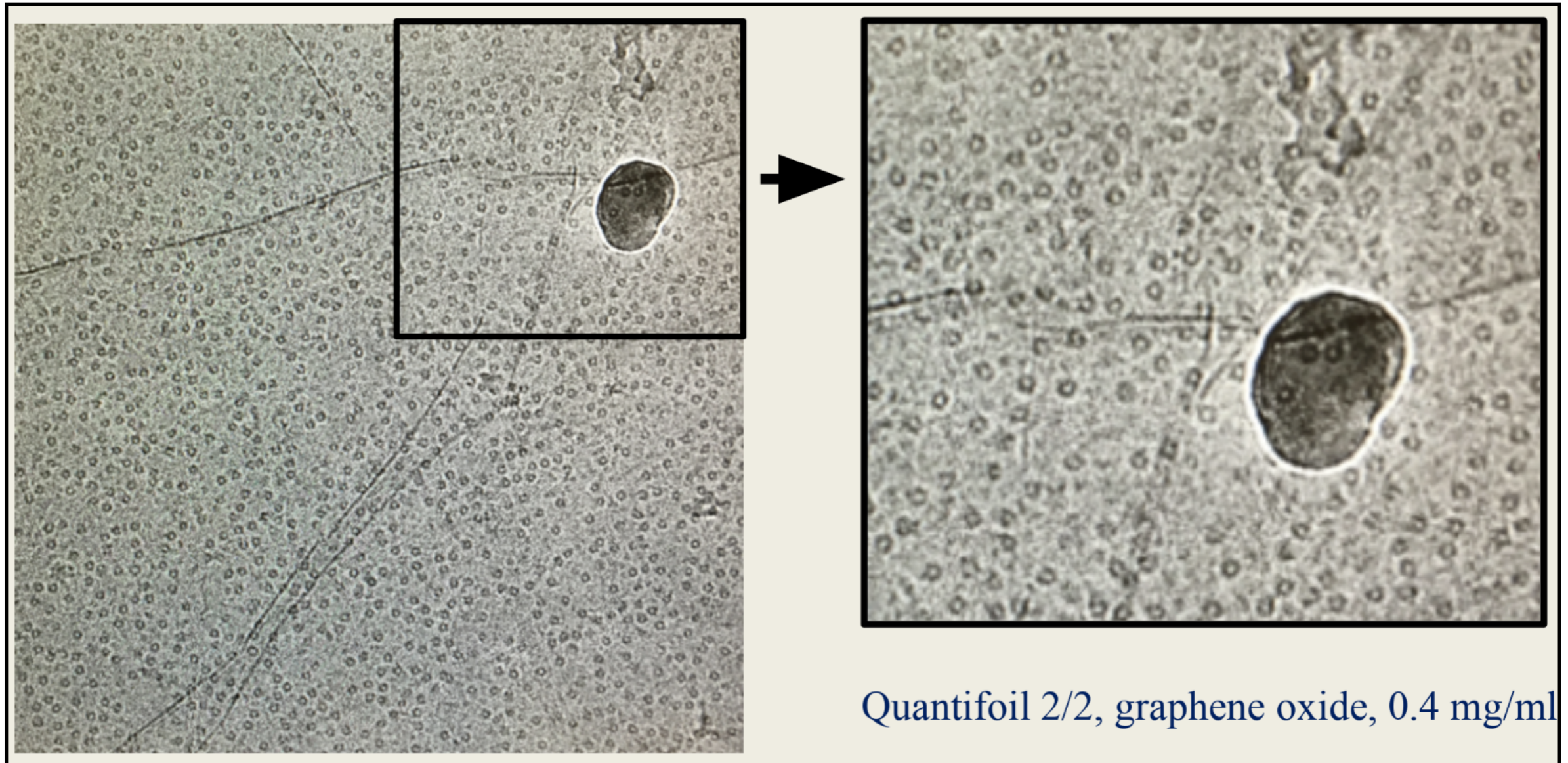
Aggregation on ice holes

# Another screening example



On **carbon** particles aggregate less but the contrast is very poor

# Another screening example

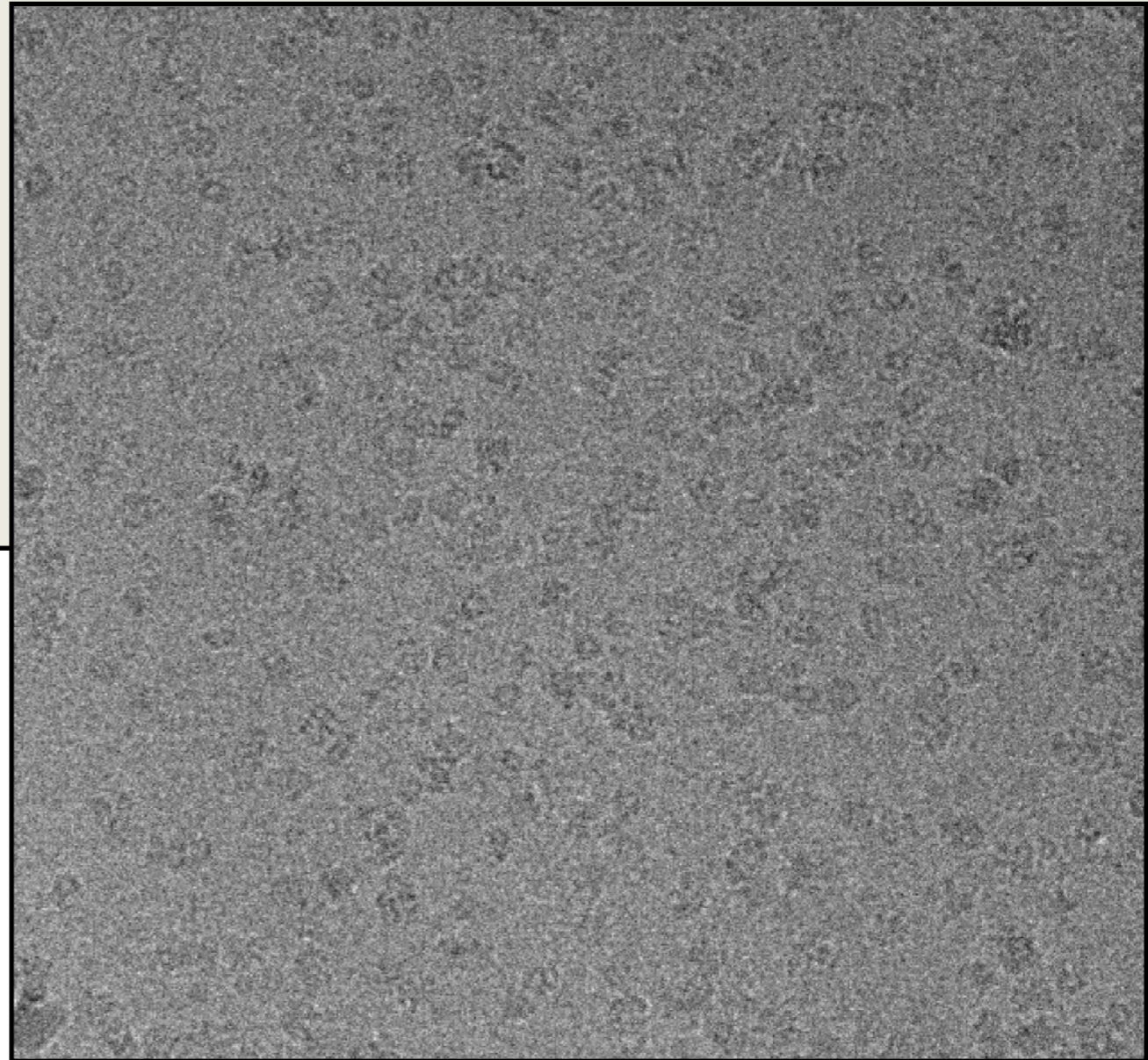
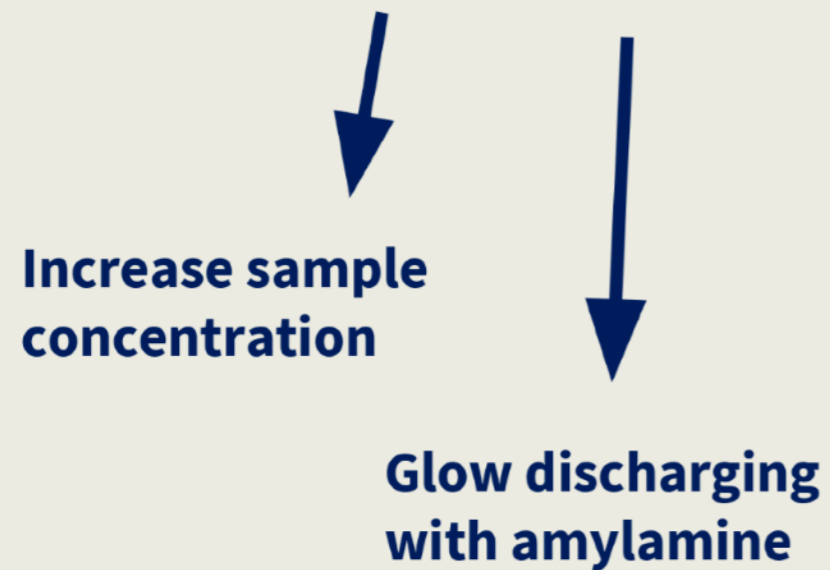


On **graphene oxide** particles are nicely separated, but preferentially oriented



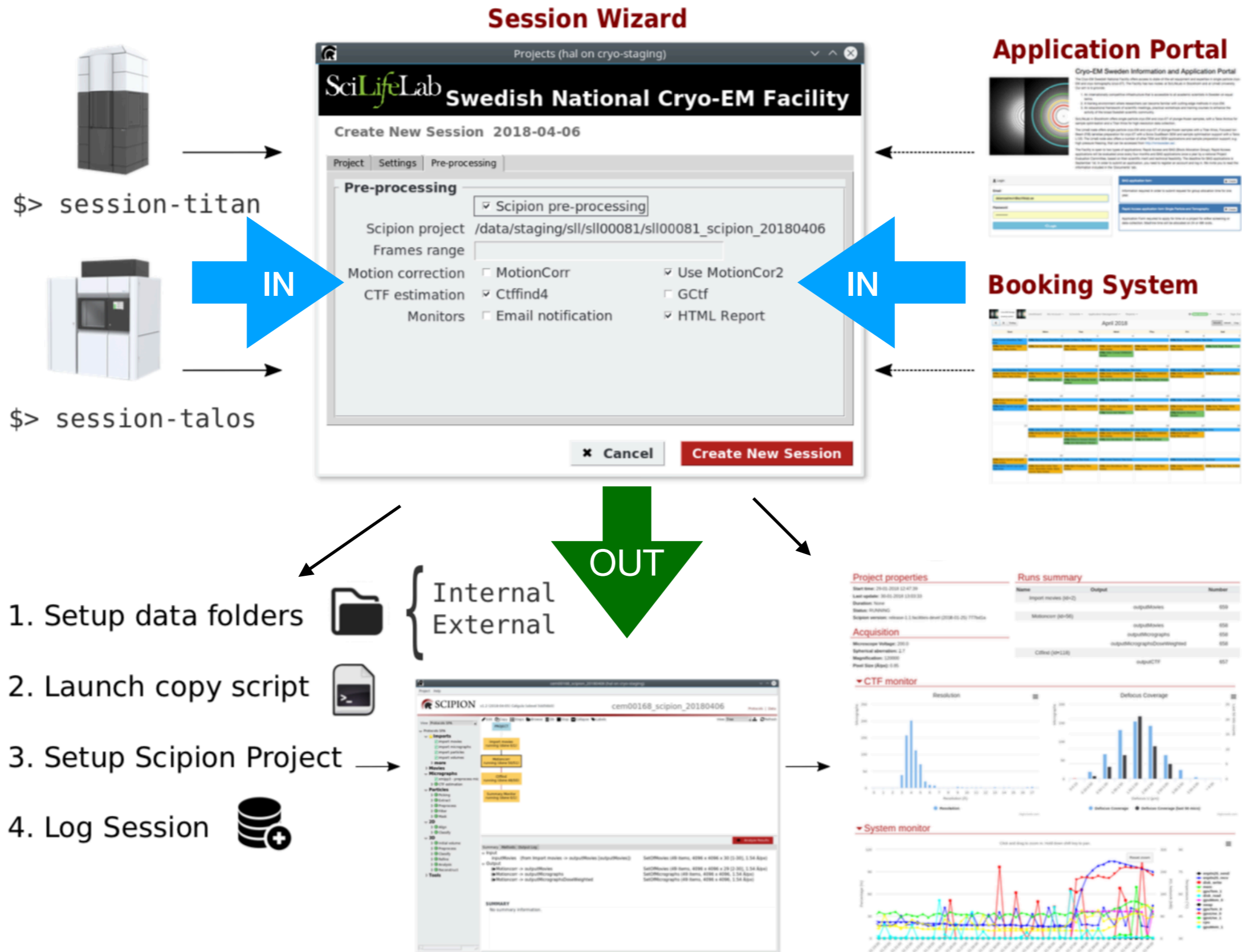
# Another screening example

- ~~a) Polylysine~~
- b) Capture sample in pure ice grids

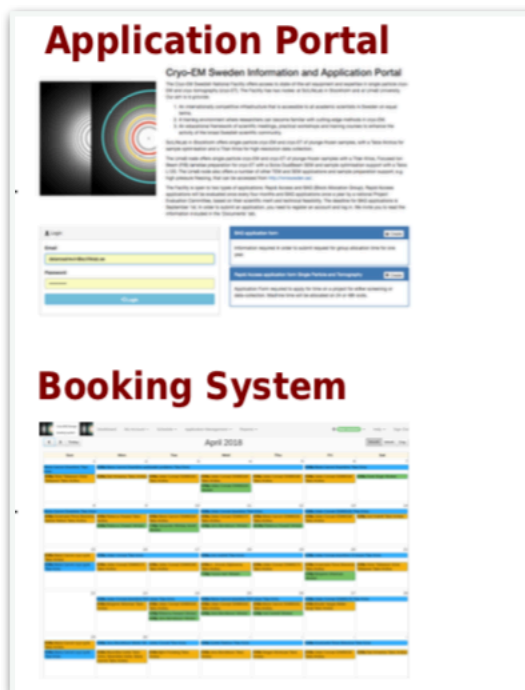
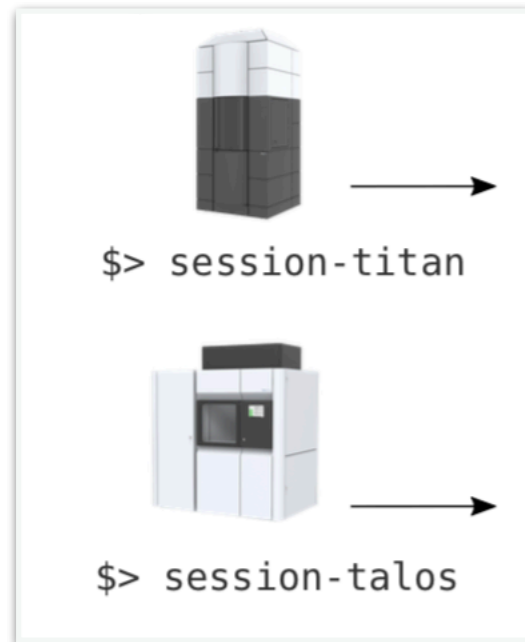


Back to pure ice grids....

# Traceability and on-the-fly preprocessing



# The Wizard



SciLifeLab
Swedish National Cryo-EM Facility

Create New Session 2018-10-09

**Microscope**  Krios 1  Talos

**Camera** K2 Falcon 3

**Project** National Internal Facility

**CEM code** CEM00263

**PI** Sebastian Westhoff -- westenho@chem.gu.se

**User**

**Operator** Julian

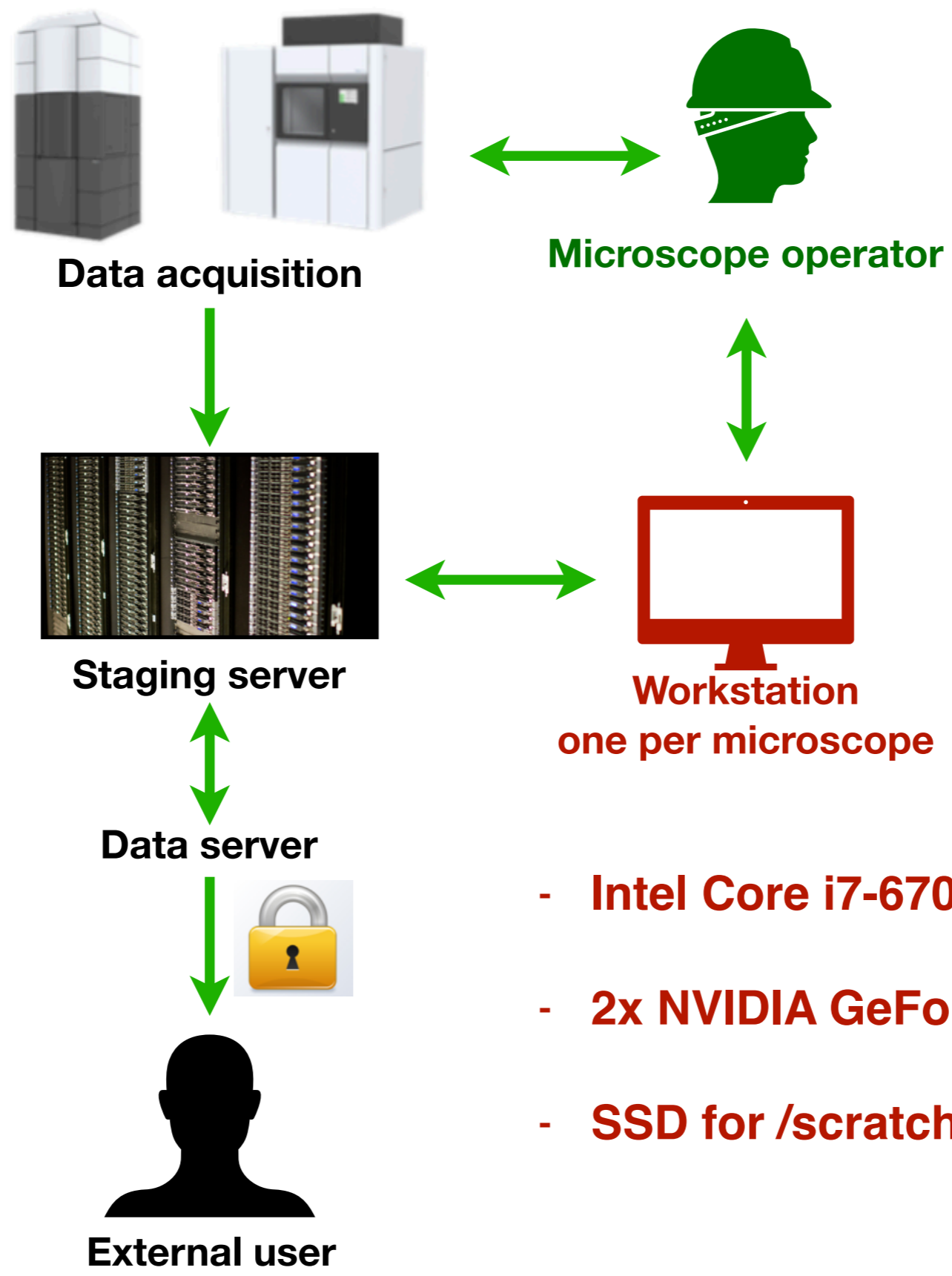
**Session ID**

**Pre-processing**  Scipion  None

✕ Cancel
Create New Session

Not true, the user can also choose to run Warp on a Windows partition

# Data preprocessing and access/download



- Intel Core i7-6700K (4 cores, 4,0 GHz)
- 2x NVIDIA GeForce 1070
- SSD for /scratch



# Traceability and on-the-fly preprocessing

The image shows a screenshot of the SciLifeLab web interface. The main window is titled "Projects (hal on cryo-staging)" and displays the "Create New Session" form for the date "2017-07-19". The interface is divided into two panes. The left pane shows the "Project" settings, including:

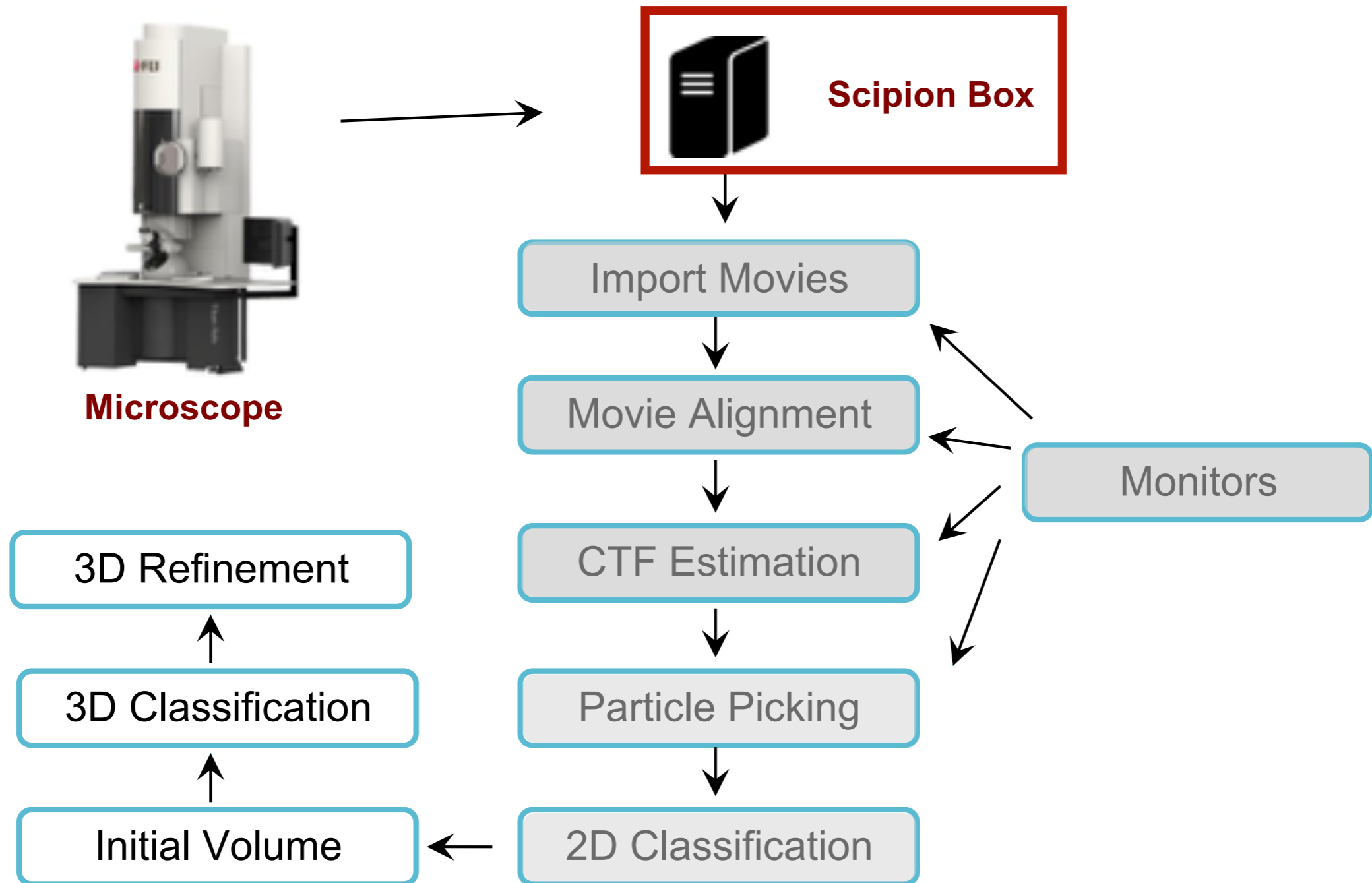
- User name: Marta Carroni
- Project Type: National Facility
- Project ID: cem00065
- Project folder: /data/staging

The right pane shows the "Pre-processing" settings, which are currently selected. The settings include:

- Scipion pre-processing
- Scipion project: /data/staging/cem/cem00065/cem00065\_scipion\_20170719
- Frames range: (empty text box)
- Motion correction:  MotionCorr,  Use MotionCor2
- CTF estimation:  Ctffind4,  GCTf
- Monitors:  Email notification,  HTML Report

At the bottom of the dialog, there are two buttons: "Cancel" and "Create New Session".

# On-the-fly preprocessing



# On-the-fly preprocessing

**SCIPION** (2018-05-23) (sdevel 84e67ed) fac00088\_scipion\_20181012 [Protocols](#) | [Data](#)

View: **Protocols SPA** Copy Delete Export Export & upload Labels View: Tree Refresh

- Protocols SPA
  - Imports
    - import movies
    - import micrographs
    - import particles
    - import volumes
    - more
  - Movies
  - Micrographs
    - xmipp3 - preprocess mic
    - CTF estimation
  - Particles
    - Picking
    - Extract
    - Preprocess
    - Filter
    - Mask
  - 2D
    - Align
    - Classify
  - 3D
    - Initial volume
    - Preprocess
    - Classify
    - Refine
    - Analysis
    - Reconstruct
  - Tools

**PROJECT**

```
graph TD; PROJECT --> Import[Import movies saved]; Import --> Motion[Motioncorr saved]; Motion --> Ctffind[Ctffind saved]; Motion --> Gctf[Gctf saved]; Motion --> Xmipp3_1[xmipp3 - supervised saved]; Ctffind --> Summary[Summary Monitor saved]; Gctf --> Summary; Xmipp3_1 --> Xmipp3_2[xmipp3 - supervised saved]; Xmipp3_2 --> Relion[relion - extract particles saved]; Relion --> Relion_2d[relion 2d - TEMPLATE saved]; Relion_2d --> Scipion[scipion - 2d streamer saved];
```

[Analyze Results](#)

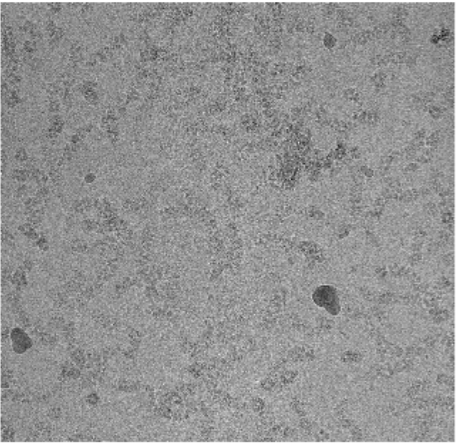
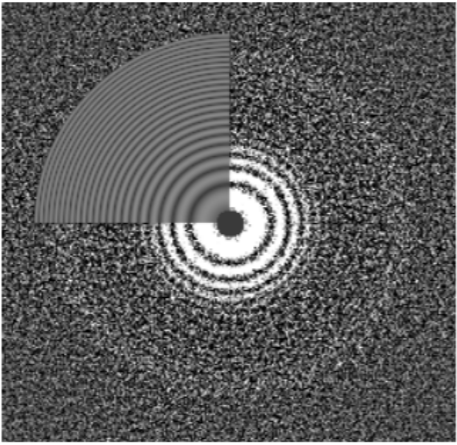
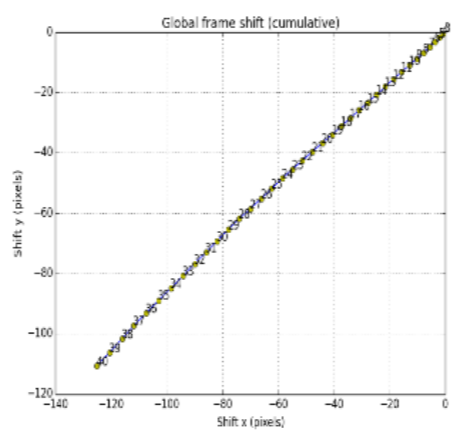
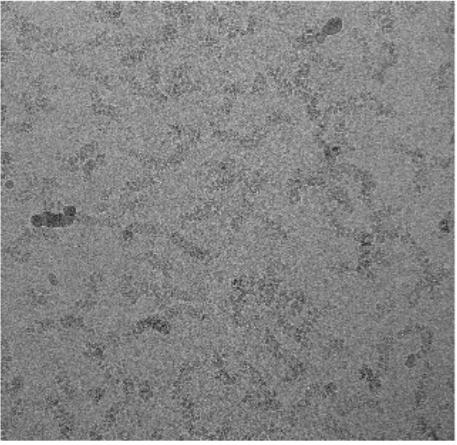
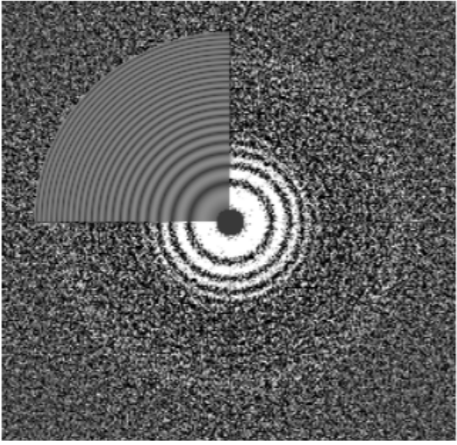
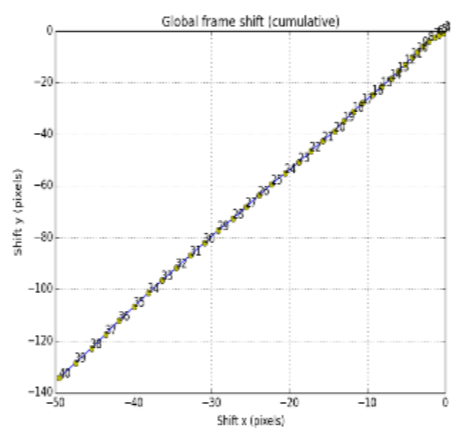

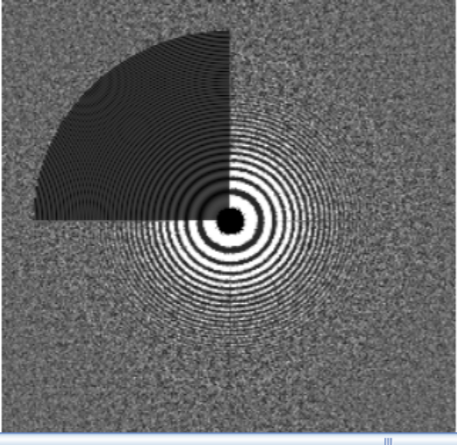
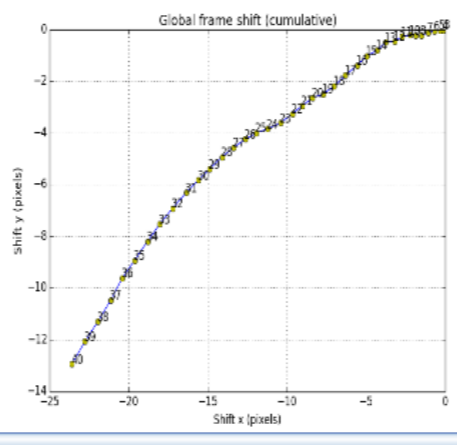
[Summary](#) [Methods](#) [Output Log](#)



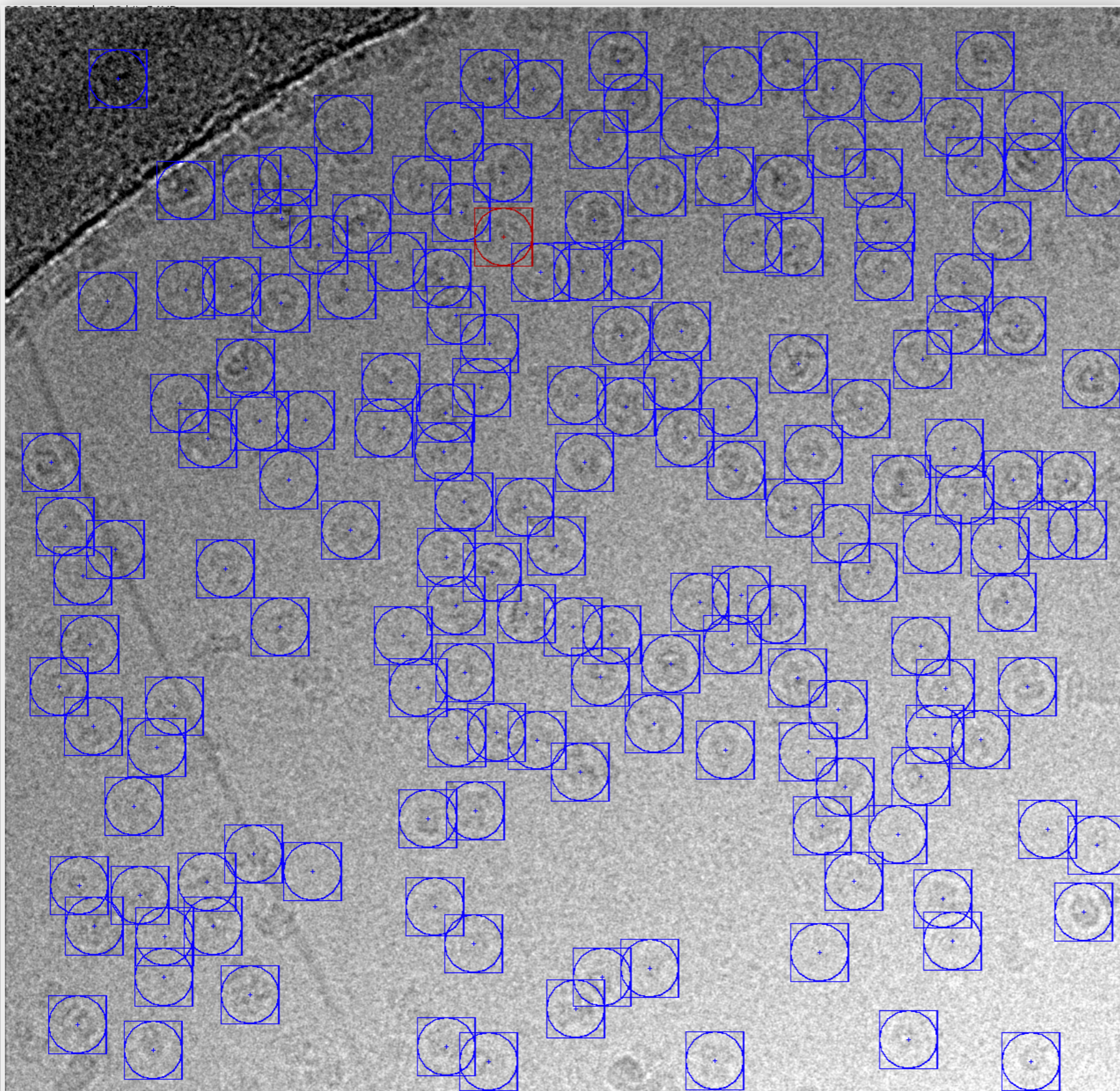
# On-the-fly preprocessing

Metadata: ctf5.sqlite 2354 items (639 x 618)

Block CTF

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2	<input checked="" type="checkbox"/>				12031.7832	11920.0654	36.7149	1.0094	3.9026	Runs/000055_ProtMotionCorr/extr
3	<input checked="" type="checkbox"/>				20984.0508	20795.8164	92.3815	1.0091	4.5448	Runs/000055_ProtMotionCorr/extr

# On-the-fly preprocessing



x=3801 y=1113 value=21.50

File Filters Window Help

Size (px): 200 Shape:   + Color:  Center

Autopick

Deactivate Training Threshold: 0 Explore (%): 50

Micrographs

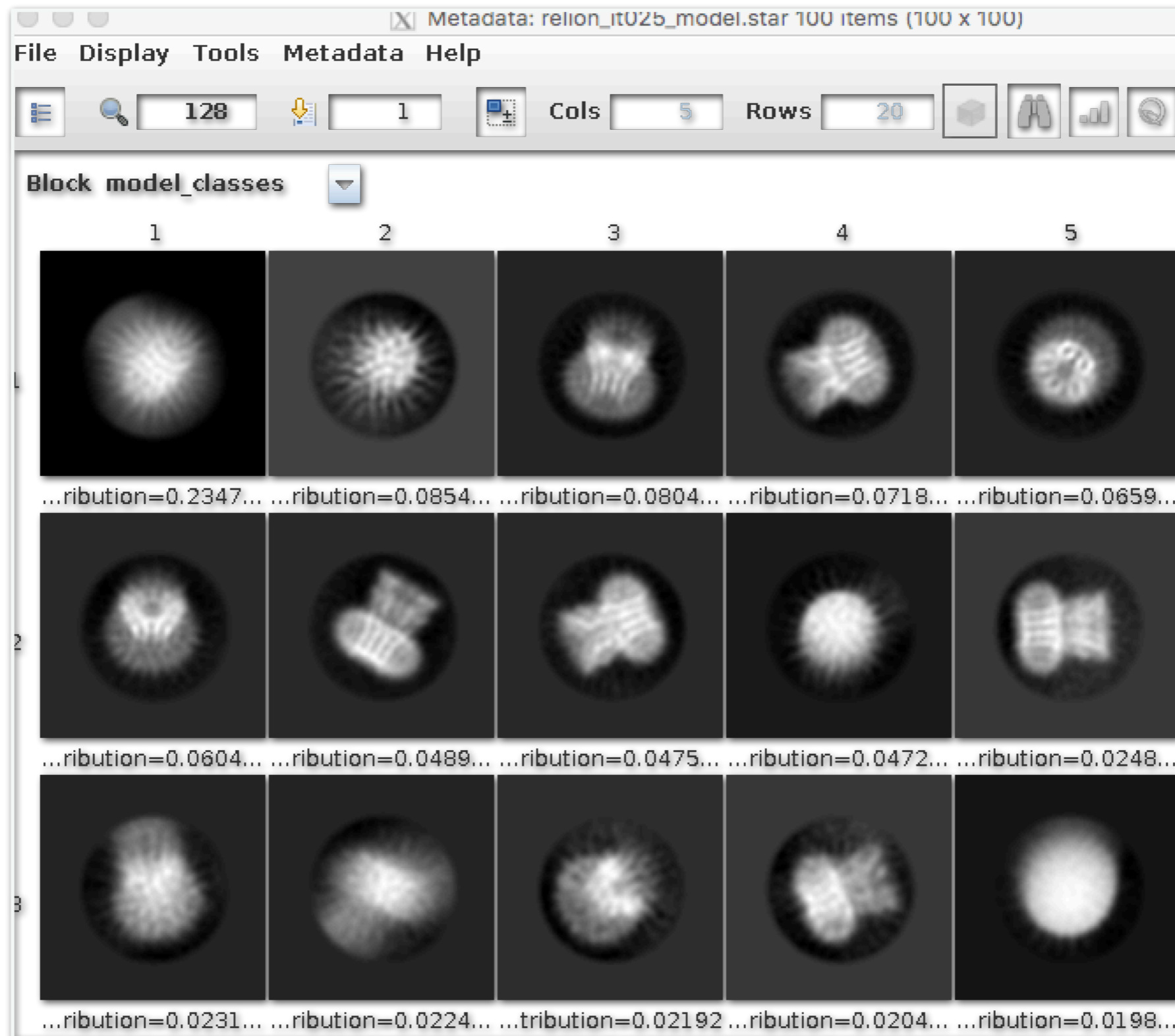
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2	Images-Disc1_GridSquare_7902564_Data_FoilHole_7906393_Data_7...	21	Manual
3	Images-Disc1_GridSquare_7902564_Data_FoilHole_7906393_Data_7...	44	Manual
4	Images-Disc1_GridSquare_7902564_Data_FoilHole_7906393_Data_7...	13	Manual
5	Images-Disc1_GridSquare_7902564_Data_FoilHole_7906394_Data_7...	16	Manual
6	Images-Disc1_GridSquare_7902564_Data_FoilHole_7906394_Data_7...	1	Manual
7	Images-Disc1_GridSquare_7902564_Data_FoilHole_7906394_Data_7...	13	Manual
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9	Images-Disc1_GridSquare_7902564_Data_FoilHole_7906395_Data_7...	4 + 4	Supervised
10	Images-Disc1_GridSquare_7902564_Data_FoilHole_7906395_Data_7...	0 + 10	Supervised
11	Images-Disc1_GridSquare_7902564_Data_FoilHole_7906395_Data_7...	0 + 29	Supervised
12	Images-Disc1_GridSquare_7902564_Data_FoilHole_7906395_Data_7...	0 + 5	Supervised
13	Images-Disc1_GridSquare_7902564_Data_FoilHole_7906396_Data_7...	0 + 0	Supervised
14	Images-Disc1_GridSquare_7902564_Data_FoilHole_7906396_Data_7...	0 + 6	Supervised
15	Images-Disc1_GridSquare_7902564_Data_FoilHole_7906396_Data_7...	2 + 16	Supervised
16	Images-Disc1_GridSquare_7902564_Data_FoilHole_7906396_Data_7...	0 + 0	Supervised
17	Images-Disc1_GridSquare_7902564_Data_FoilHole_7906397_Data_7...	0 + 6	Supervised
18	Images-Disc1_GridSquare_7902564_Data_FoilHole_7906397_Data_7...	0 + 19	Corrected
19	Images-Disc1_GridSquare_7902564_Data_FoilHole_7906397_Data_7...	0 + 29	Supervised

Manual: 583 Automatic: 3541

Reset Micrograph 0.33

Close Save Coordinates

# On-the-fly preprocessing



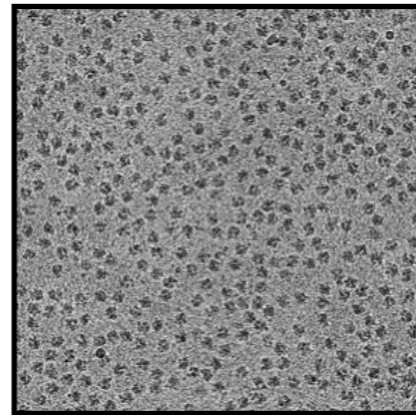
# On-the-fly preprocessing: initial model

The screenshot displays a software interface for cryo-EM data processing. At the top, a window title reads "sll00126\_scipion\_20180808 (hal on cryo-staging)". Below this is a grid of 12 columns and 4 rows of image slices, labeled from "slice 25" to "slice 72". The interface includes a top menu bar with "Project", "Help", and "Protocols SPA". A left sidebar contains a "Particles" section with a "Pickings" sub-section, and a "3D" section with steps: "Initial volume", "Preprocess", "Classify", "Refine", and "Analysis". The "Classify" step is currently selected. The main area shows a workflow diagram with green boxes indicating completed steps, such as "align 2d - TEMPLATE (copy 2) failed (done 2/3)", "align 2d - TEMPLATE (copy 4) finished", "align 2d - TEMPLATE (copy 5) finished", "align 2d - TEMPLATE (copy 6) finished", "align 2d - TEMPLATE (copy 7) finished", "align 2d - TEMPLATE (copy 8) failed (done 2/3)", "align variousviews\_good picks variousviews\_good clean\_sides\_views\_only finished", "align\_sides\_views\_only finished", "relion - 3D auto-refine startc5eman5vonly finished", "relion - 3D auto-refine startc5eman5vonly 05 finished", and "relion - 3D auto-refine finished". A red button labeled "Analyze Results" is located in the bottom right corner. The bottom of the interface shows tabs for "Summary", "Methods", and "Output Log", with "SUMMARY" currently selected.

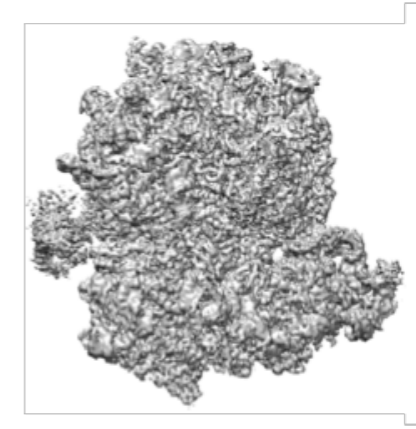


# More applications

## Single-particle

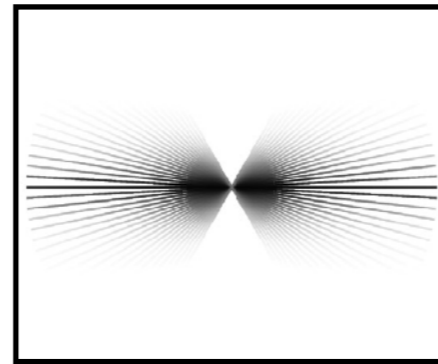


2D-projections

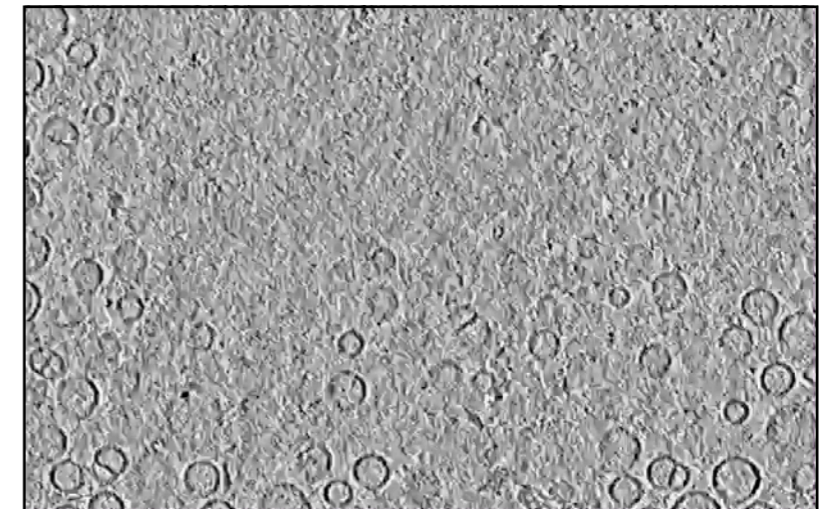


3D-reconstruction

## Tomography

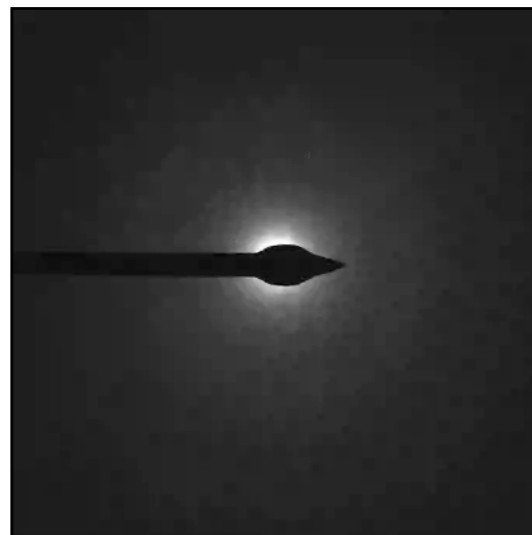


2D-projections tilt-series

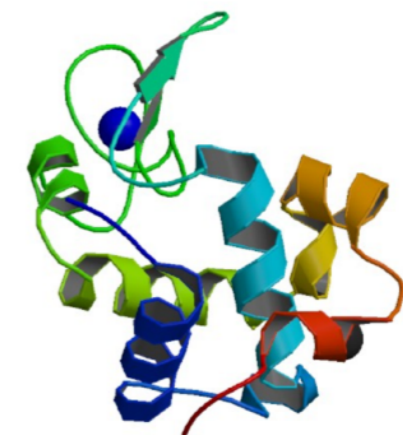


tomo reconstruction

## MicroED (with X.Zou and H.Xu)

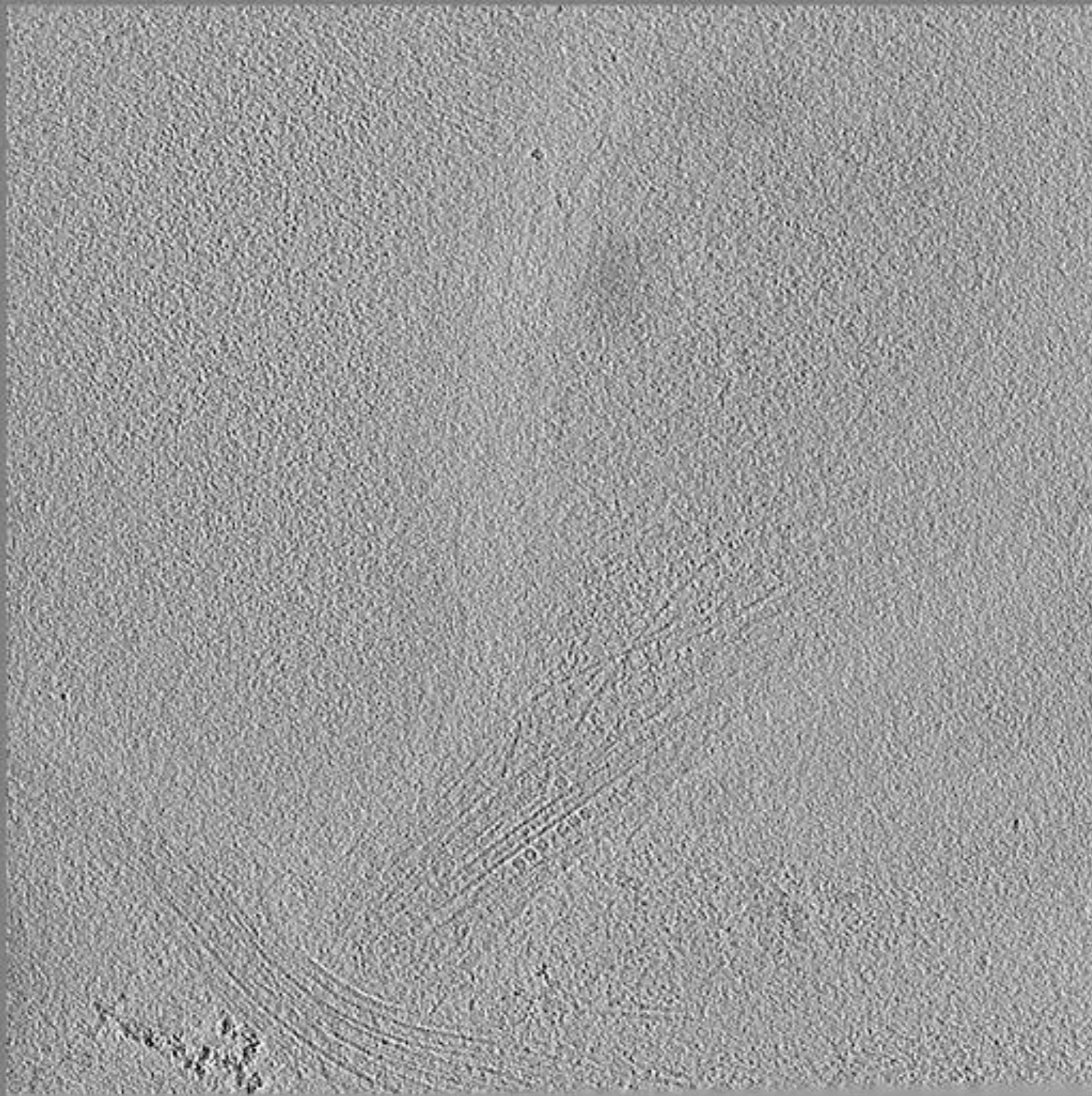


electron-diffraction pattern



structure determination

# Tomo: an example



# The Stockholm node operation so far


- In Nov 2018 we will celebrate two years of full operation
- Inaugural symposium in May 2017
- Full usage of the machines with ~100 national projects and ~80 internal, run also on holidays
- Involvement of our staff in a lot of TRAINING
  - 40-45 internal users trained at the microscopes
  - annual image processing training workshops available to the whole country
- Development of SOFTWARE TOOLS for cryoEM data processing (Scipion, Relion3.0)



# Publications

**10 publications plus 3 under review to our knowledge**

## Year 2018


 [Extracellular nanovesicles released from the commensal yeast \*Malassezia sympodialis\* are enriched in allergens and interact with cells in human skin](#)

Johansson HJ, Vallhov H, ..., Lehtiö J, Scheynius A

*Sci Rep* **8** (1) - [2018-12-00; online 2018-06-15]

**Cryo-EM [Service]**

 DOI

 Crossref


 [Structure of the chloroplast ribosome with chl-RRF and hibernation-promoting factor](#)

Boerema AP, Aibara S, ..., Lindahl E, Amunts A

*NPLANTS* **4** (4) 212-217 [2018-04-00; online 2018-04-02]

**Cryo-EM [Service]**

 DOI

 Crossref

## Year 2017

 [The cryo-EM structure of hibernating 100S ribosome dimer from pathogenic \*Staphylococcus aureus\*](#)


Matzov D, Aibara S, ..., Amunts A, Yonath AE

*Nat Commun* **8** (1) - [2017-12-00; online 2017-09-28]

**Cryo-EM [Service]**

 PubMed

 DOI

 Crossref


 [Mechanistic Insights into Autoinhibition of the Oncogenic Chromatin Remodeler ALC1](#)

Lehmann LC, Hewitt G, ..., Boulton SJ, Deindl S

*Molecular Cell* **68** (5) 847-859.e7 [2017-12-00; online 2017-12-00]

**Cryo-EM [Service]**

 DOI

 Crossref


 [Regulatory coiled-coil domains promote head-to-head assemblies of AAA+ chaperones essential for tunable activity control](#)

Carroni M, Franke KB, ..., Bukau B, Mogk A

*Elife* **6** (-) - [2017-11-22; online 2017-11-22]

**Cryo-EM [Collaborative]**

 DOI

 Crossref

# Cryo-Net

Umeå

Stockholm

Aarhus

Copenhagen

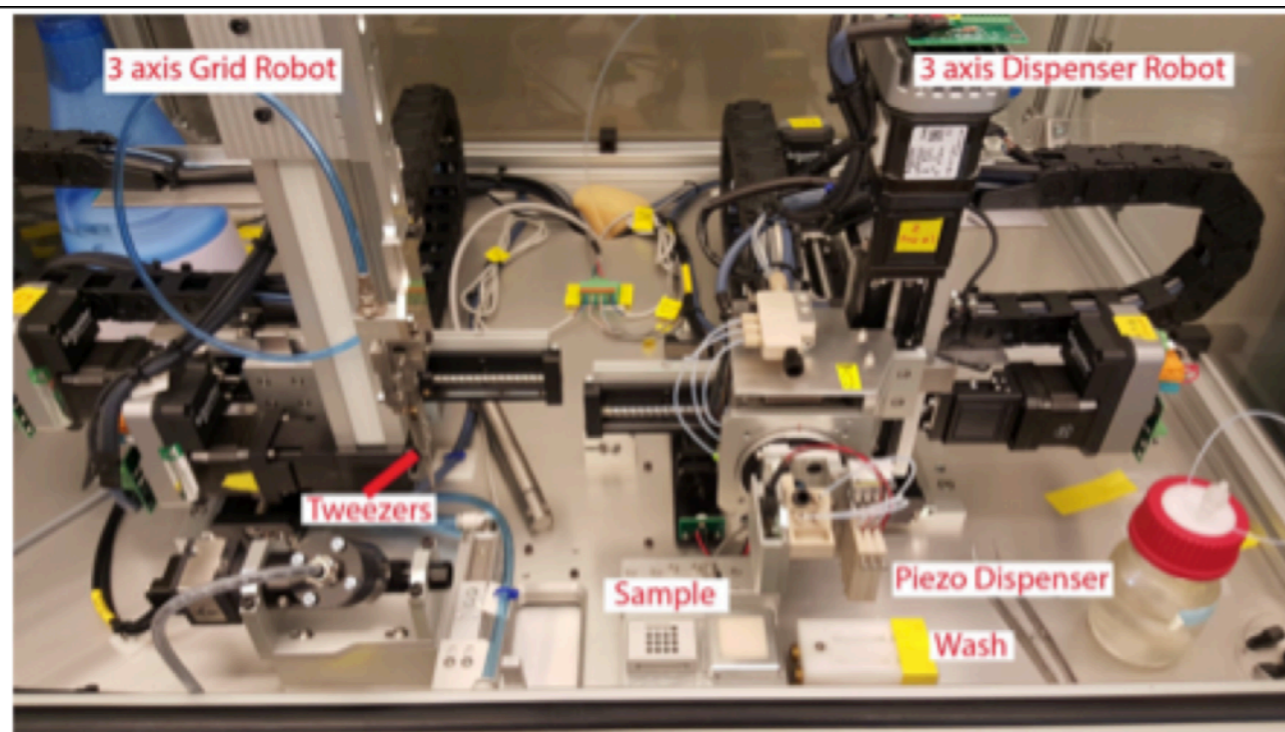
*Knut och Alice  
Wallenbergs  
Stiftelse*

ново  
nordisk  
fonden

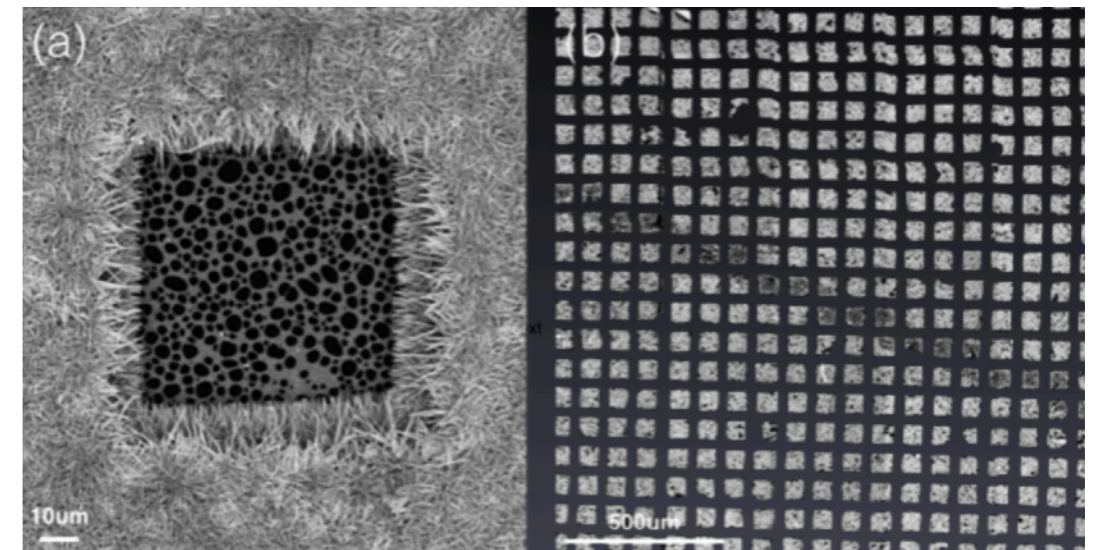
- Initiative bridging the cryo-EM communities of Sweden and Denmark, with the idea of enlarging to the rest of the Nordics
- Annual international symposiums. First one two weeks ago in Copenhagen and another to follow next year in Stockholm
- Funded research projects between facilities funded
- Workshops with international speakers/teachers

# Future improvement

- Improvement with acquisition of a new tool for grid preparation



Spotiton 1.0 is a novel robotic device for preparing vitrified specimens using inkjet dispensing.



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Wallenbergs  
Stiftelse*

*Kempestiftelserna*

SciLifeLab



Stockholms  
universitet



FAMILJEN ERLING-PERSSONS STIFTELSE

**Thanks to all research groups at the Department of  
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**Thanks to Masanori Mori and Dennis Lorentzen  
(Our TF microscope engineers)**