The 5th CryoEM Course at LBMS, BNL

In situ sample preparation tutorial & demonstration

Jianfeng Lin

Yale CryoEM Resource

6-5-2025

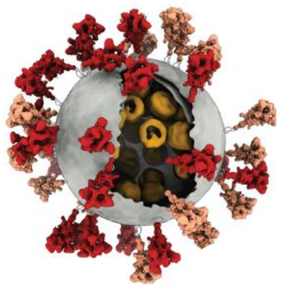
Main contents

1. *In situ* specimens accessible by cryoEM

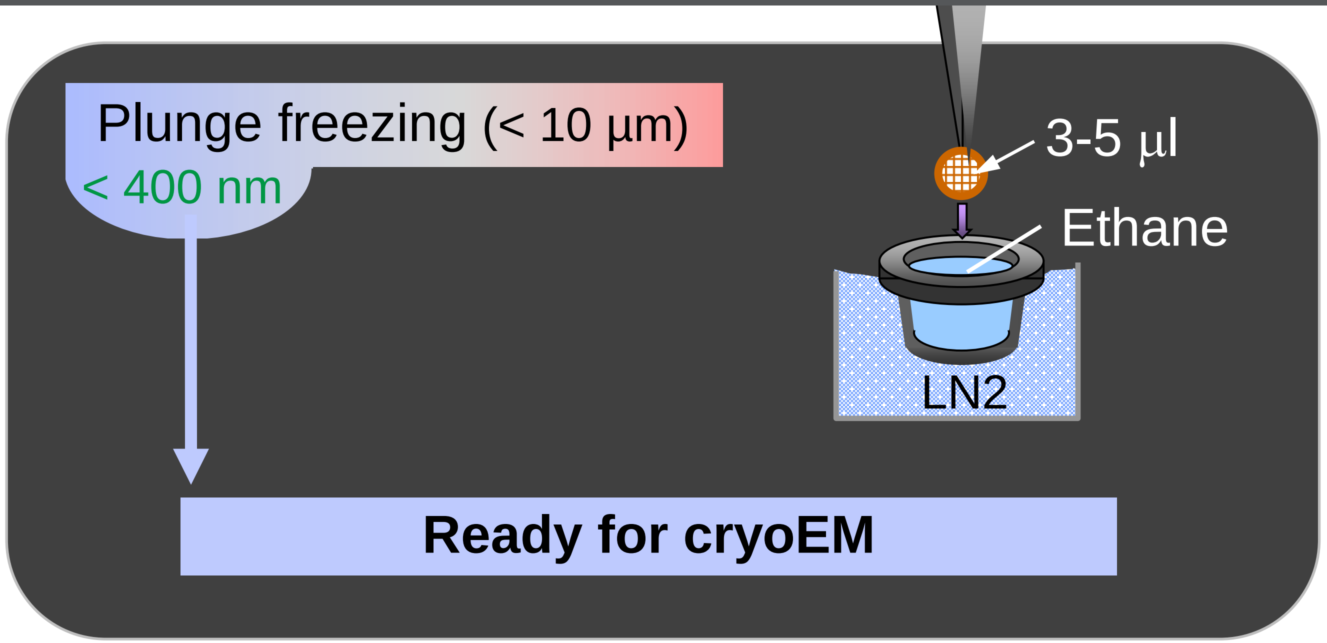
2. Five considerations for *in situ* sample preparation

3. Tutorial of major steps of cryo lamella preparation

1. *In situ* specimens accessible by cryoEM

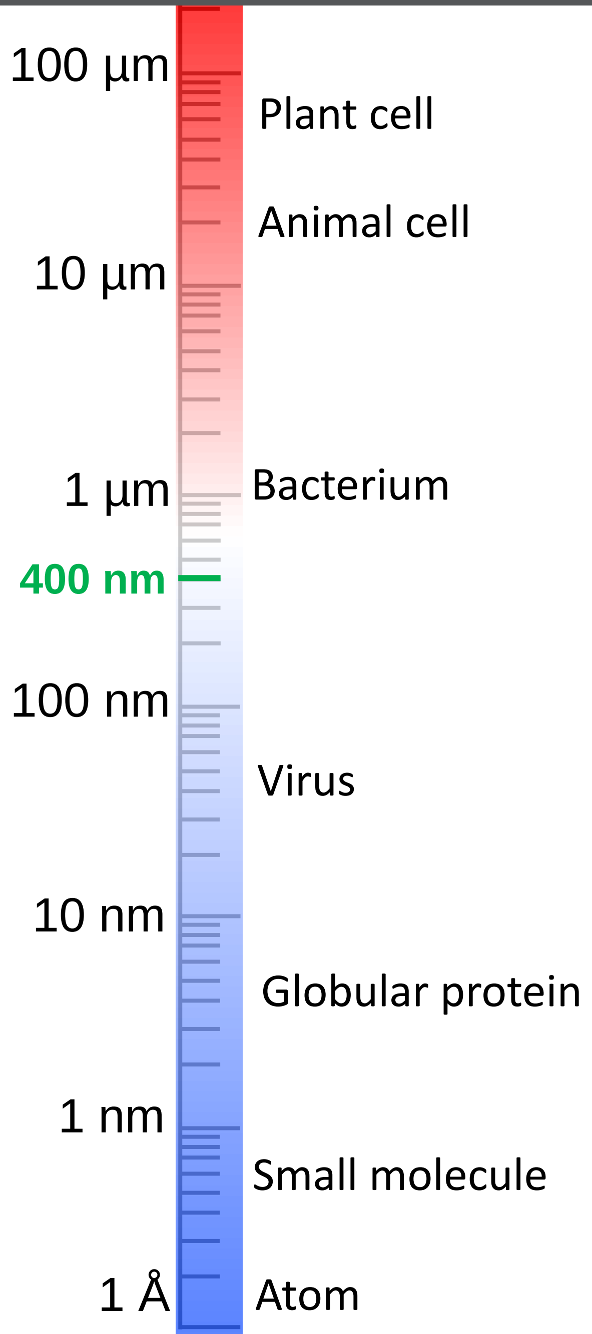


Yao et al., Cell 2020



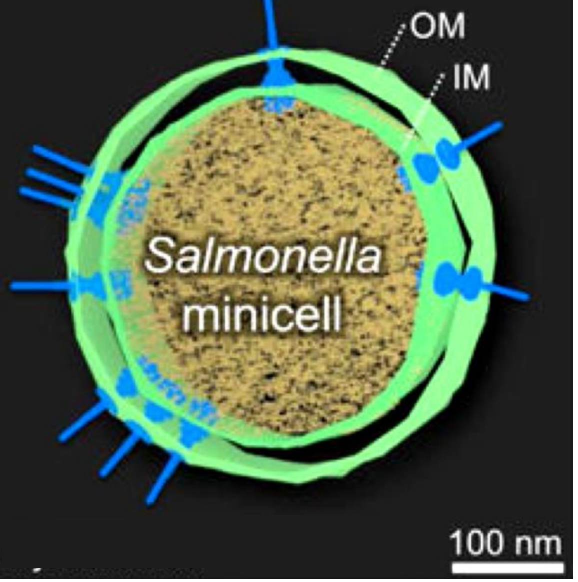
- Virus: e.g., Covid-19
- Isolated or reconstituted systems: e.g., ciliary axoneme

Lin & Nicastro, Science 2018

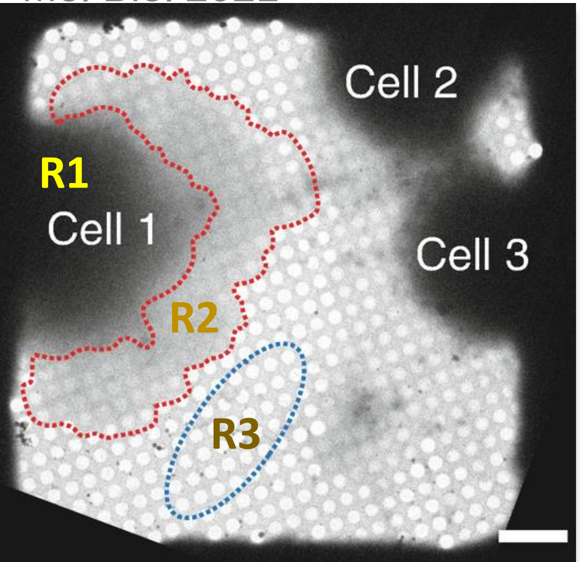


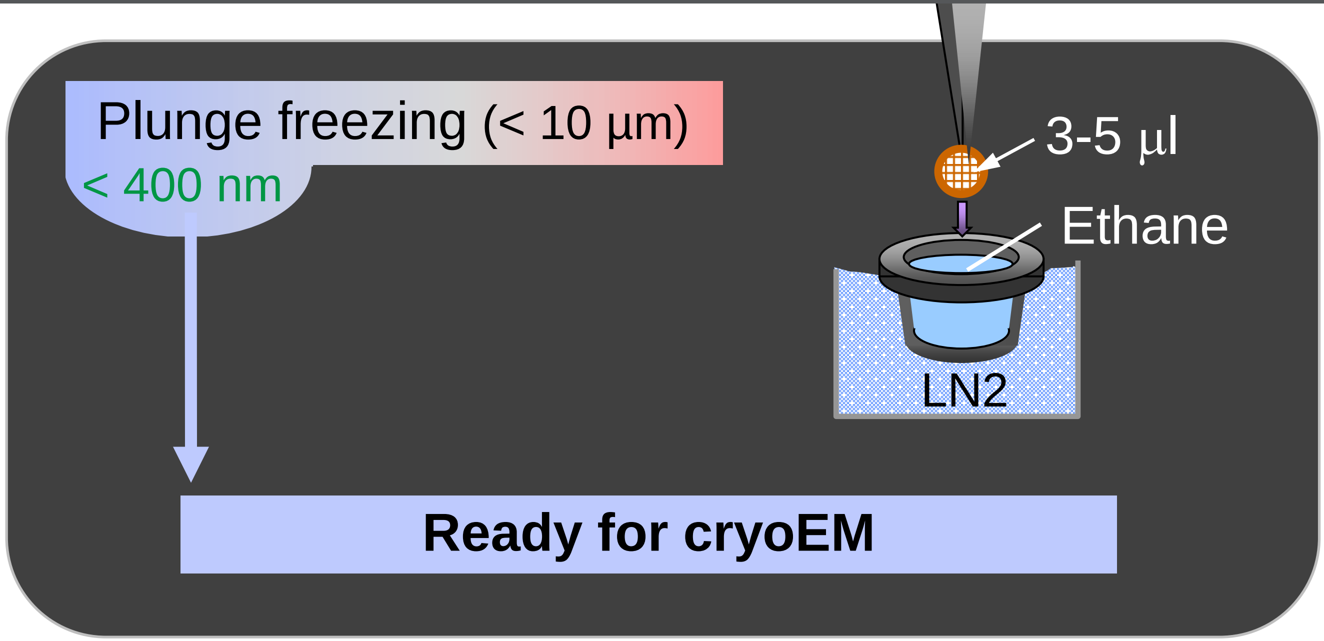
1. *In situ* specimens accessible by cryoEM

Hu et al., Cell 2017

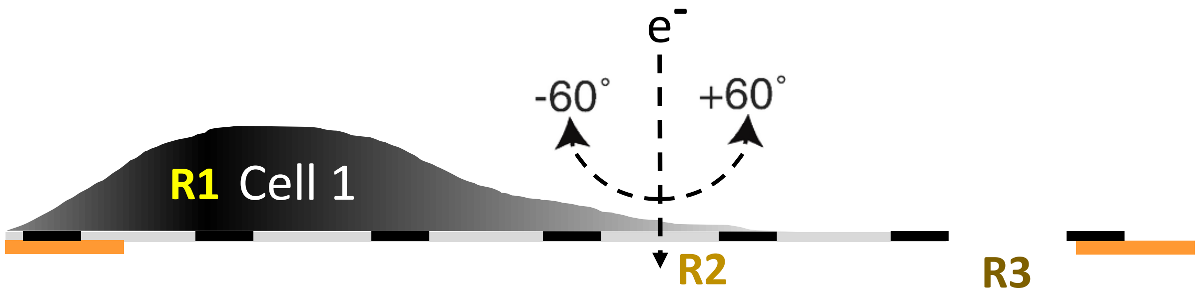


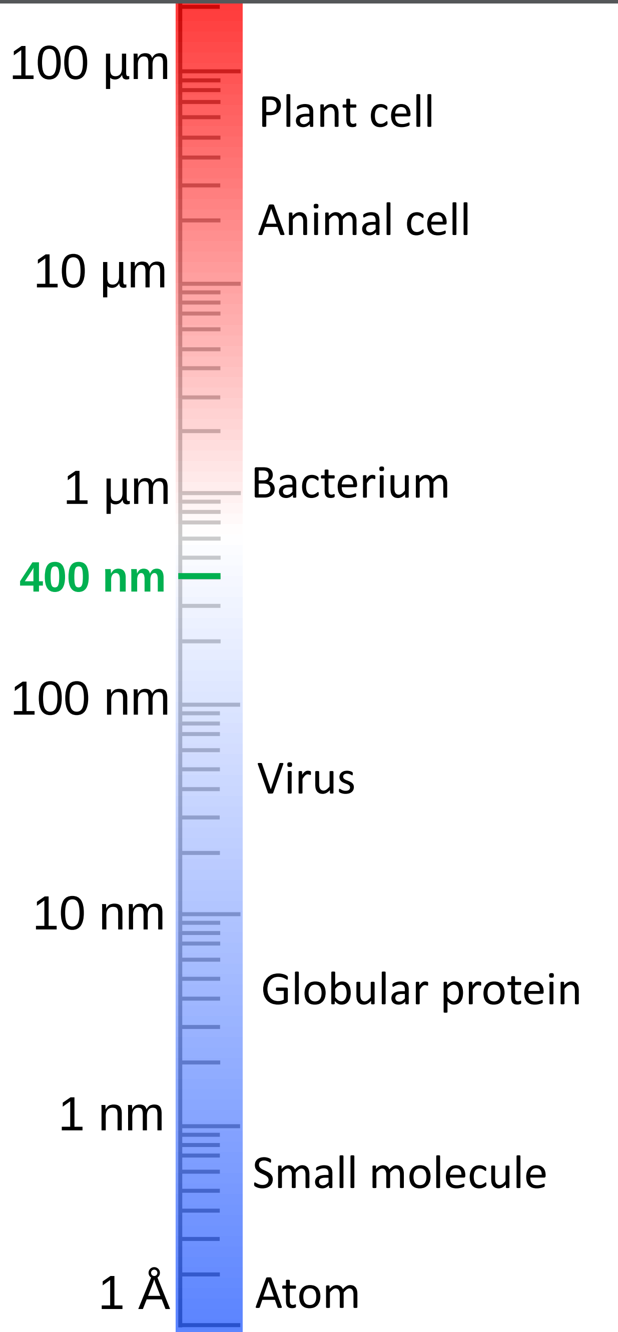
Serwas & Davies. Methods Mol Biol 2021





- Virus: e.g., Covid-19
- Isolated or reconstituted systems: e.g., ciliary axoneme
- Small/thin cells: e.g., minicells
- Peripheral regions of cells: e.g., mammalian cells





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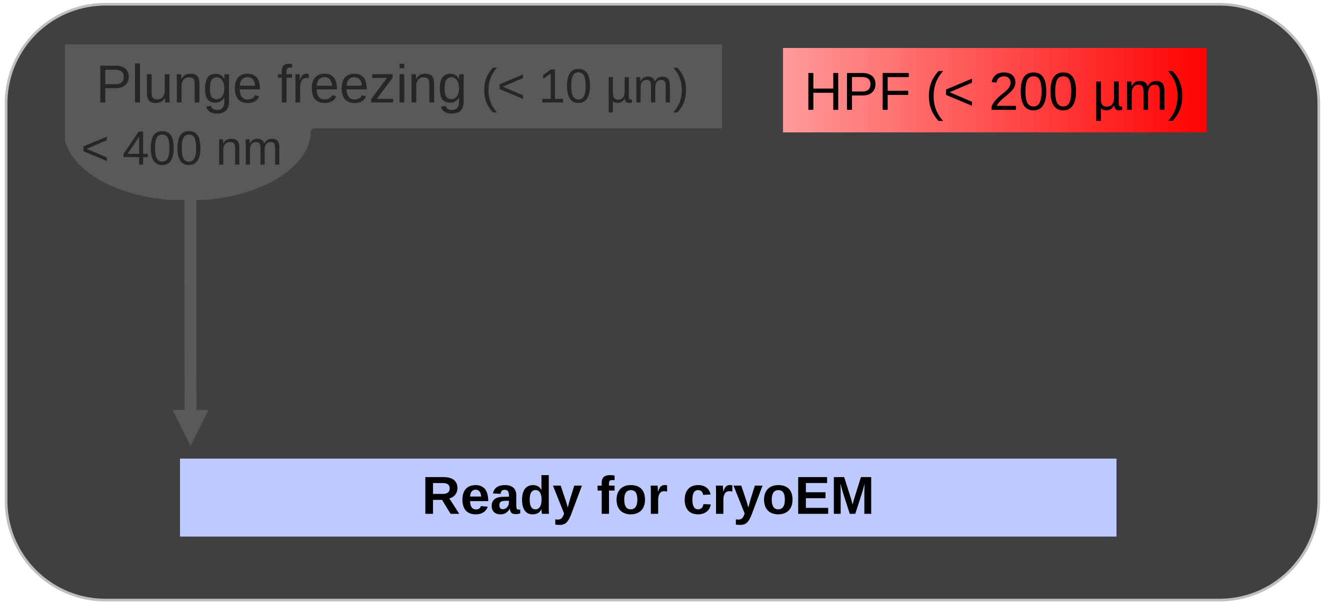

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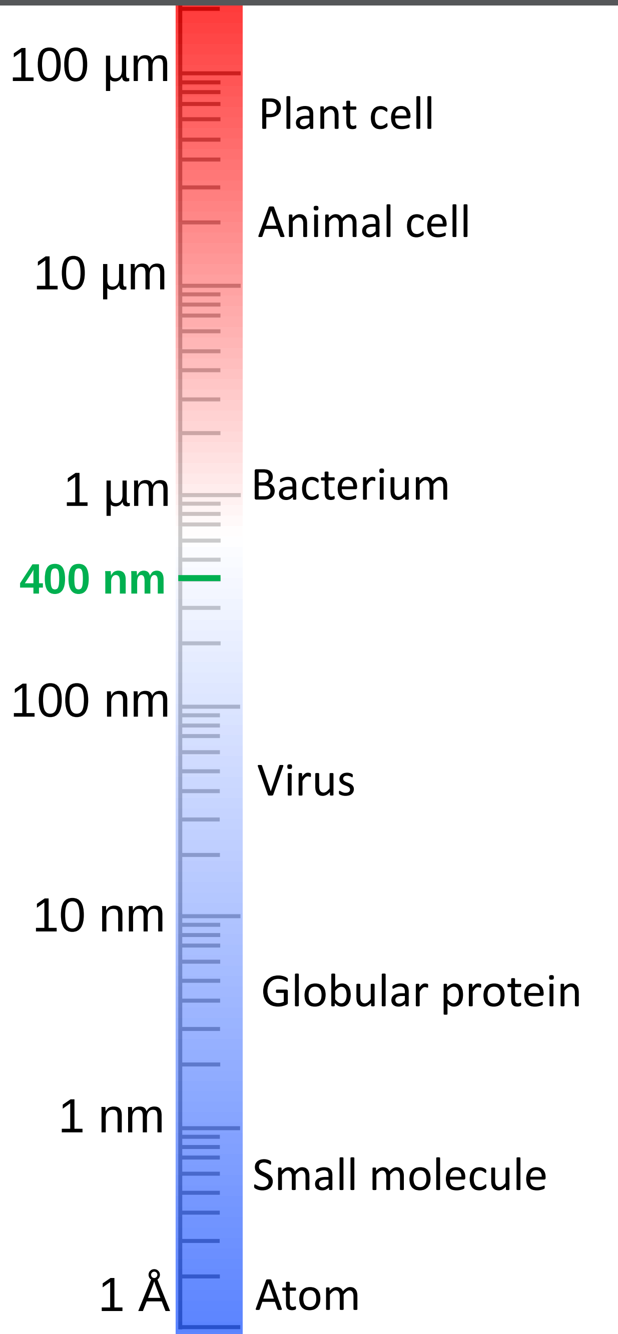
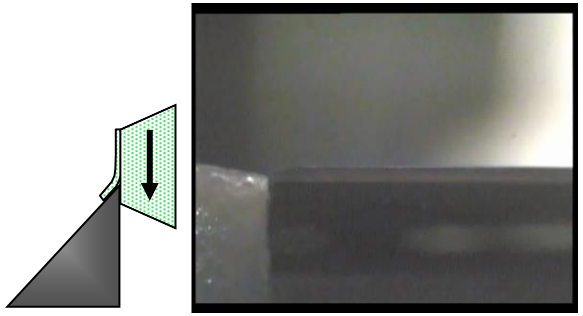
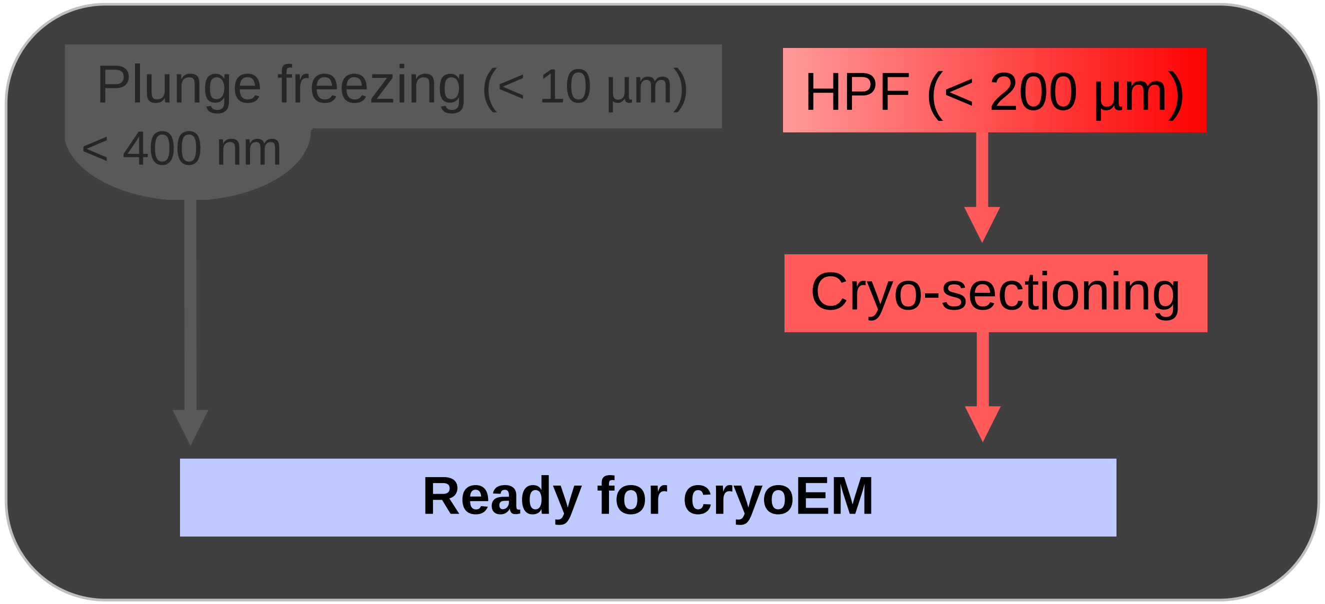


1. *In situ* specimens accessible by cryoEM

CEMOVIS





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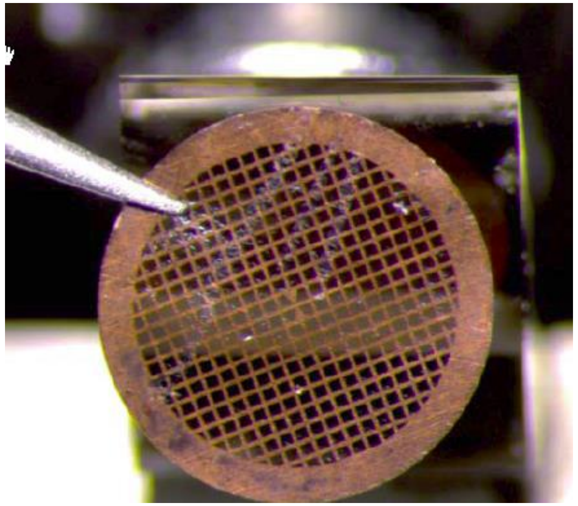
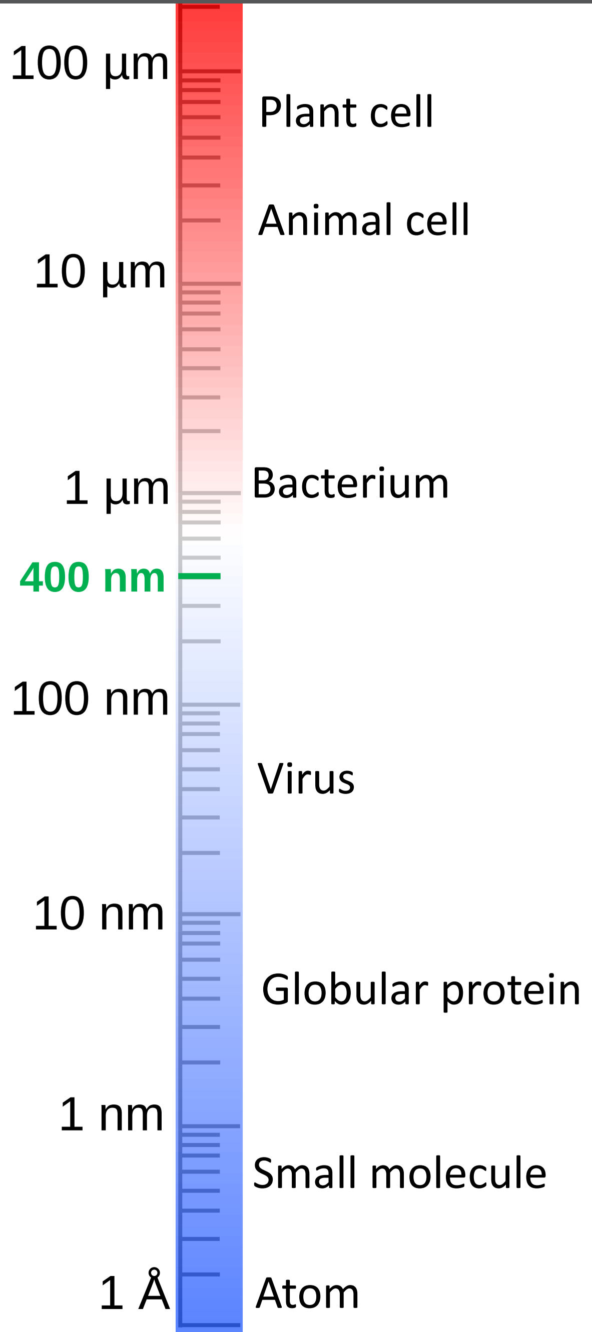
- Cryo-sections



Adapted from Thermo Fisher Scientific (TFS)



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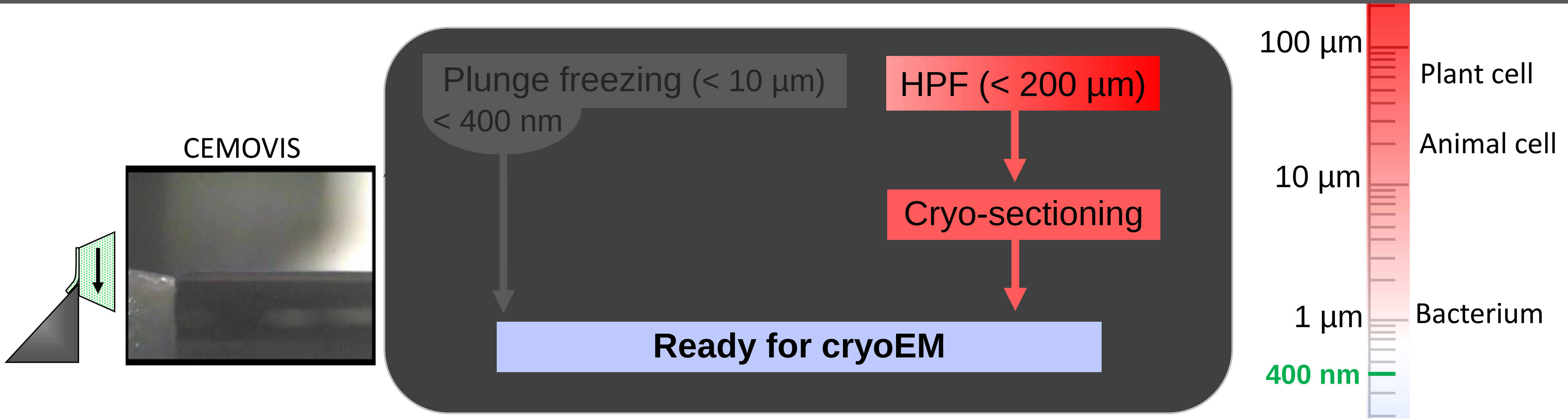

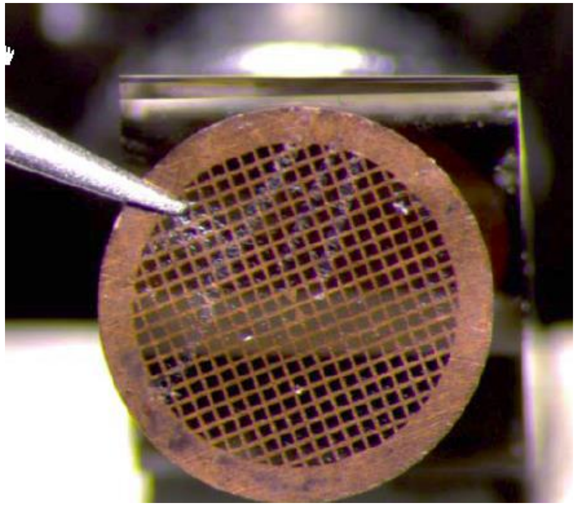

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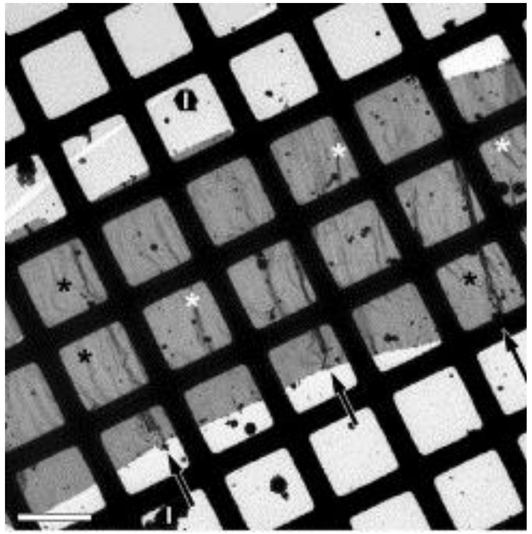
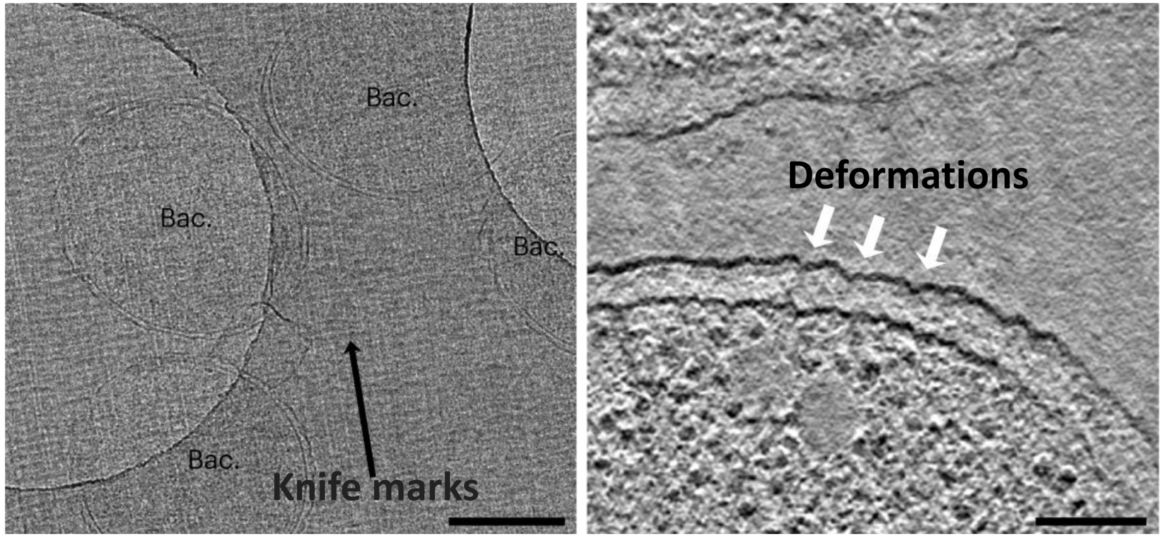


Al-Amoudi et al., J Struct Biol 2005



Berger et al., Nature Methods 2023

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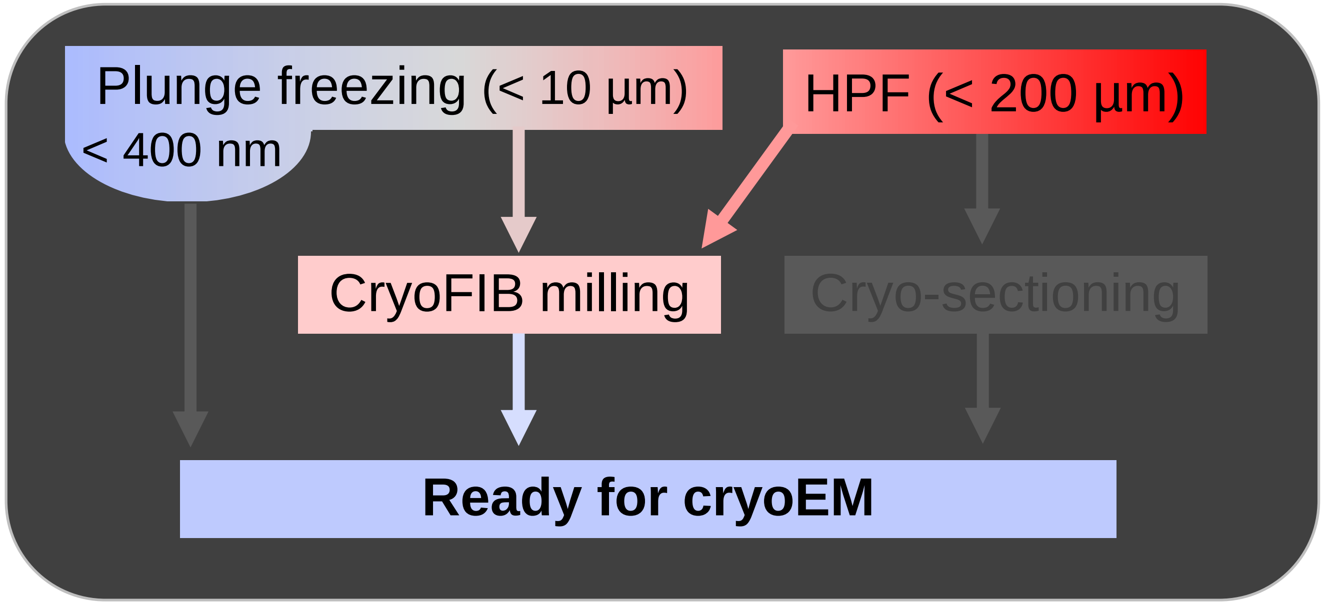

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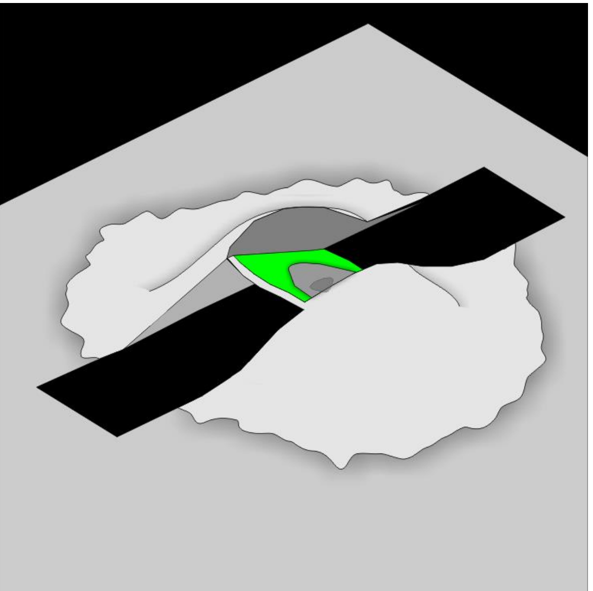
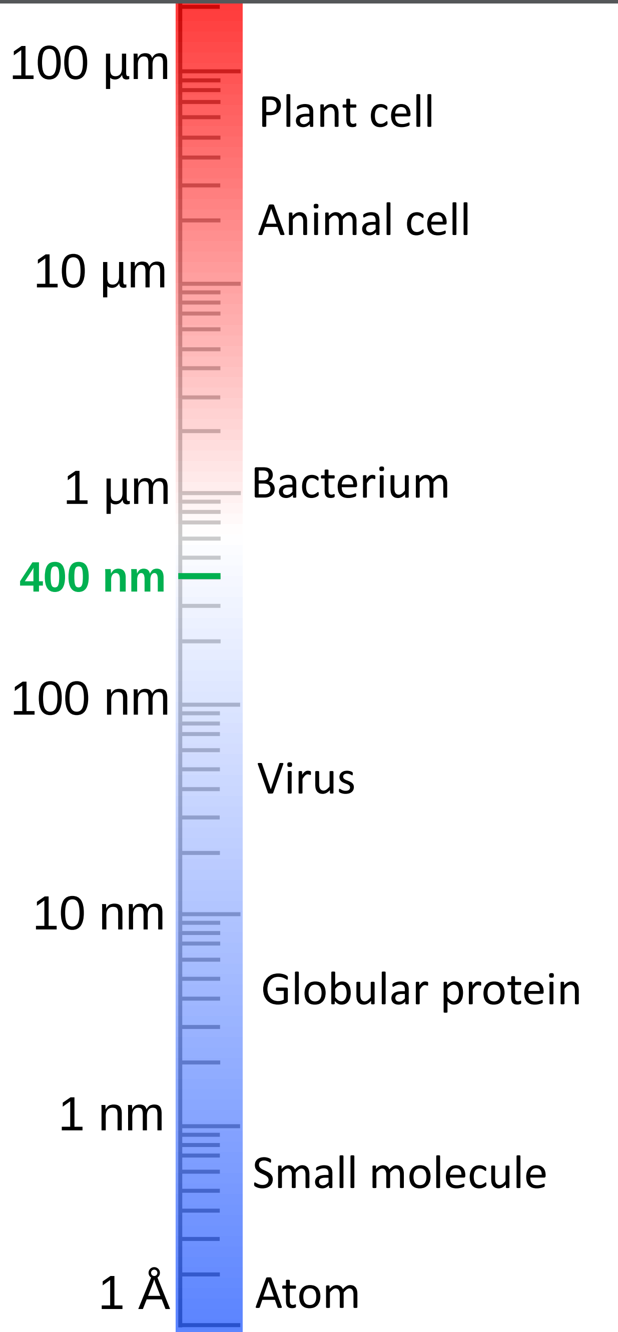
- Cryo-sections
- Cryo-lamellae





Main contents

1. *In situ* specimens accessible by cryoEM

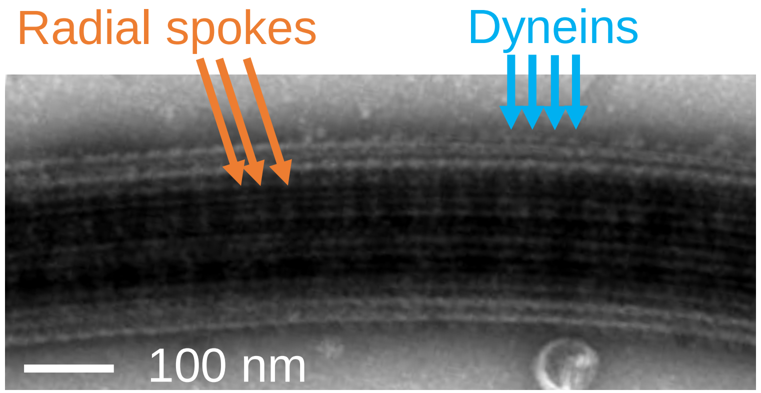
2. Five considerations for *in situ* sample preparation

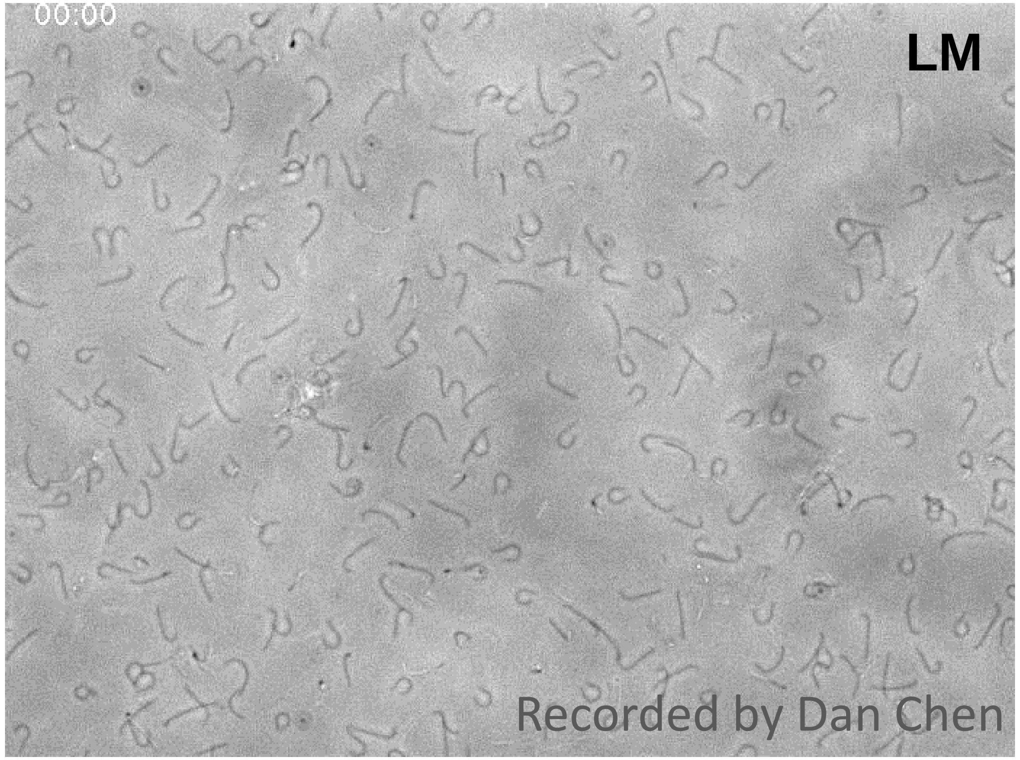
3. Tutorial of major steps of cryo lamella preparation

2. Five considerations for *in situ* sample preparation

2.1 Validation of the sample quality e.g., Negative stain EM & Reactivation of flagellar axoneme

Negative stain EM

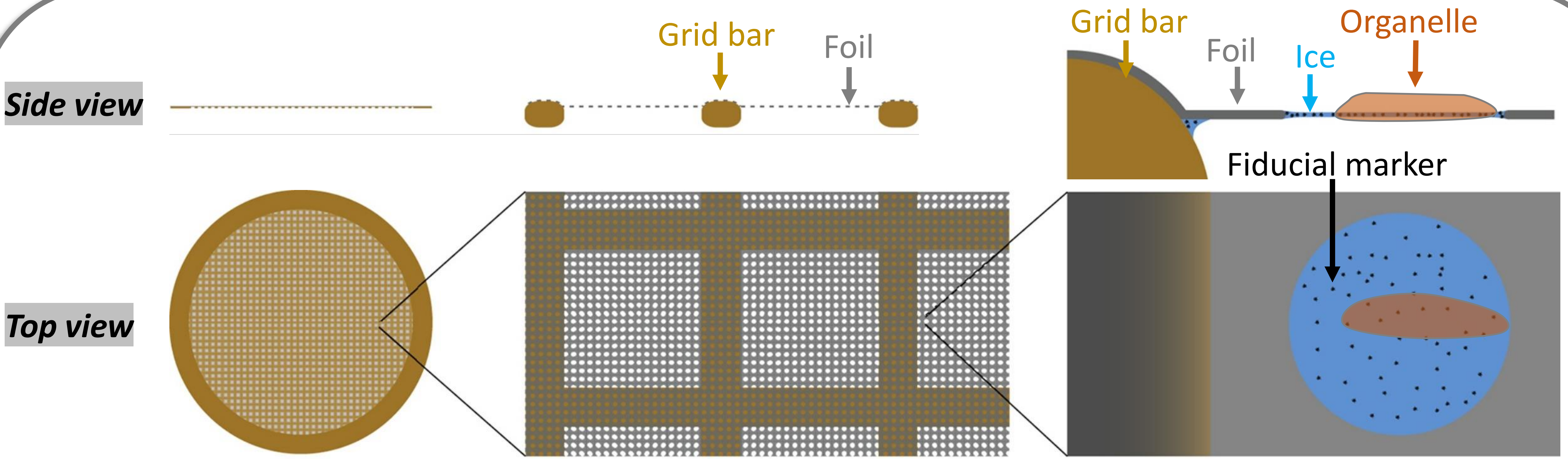




2. Five considerations for *in situ* sample preparation

2.1 Validation of the sample quality

2.2 EM grid => Grid / Foil materials



Grid materials

Copper	**Gold**
Nickel	CuRh
Titanium	Molybdenum
Silicon	Aluminum
	Tungsten

Foil materials

Amorphous carbon

Gold

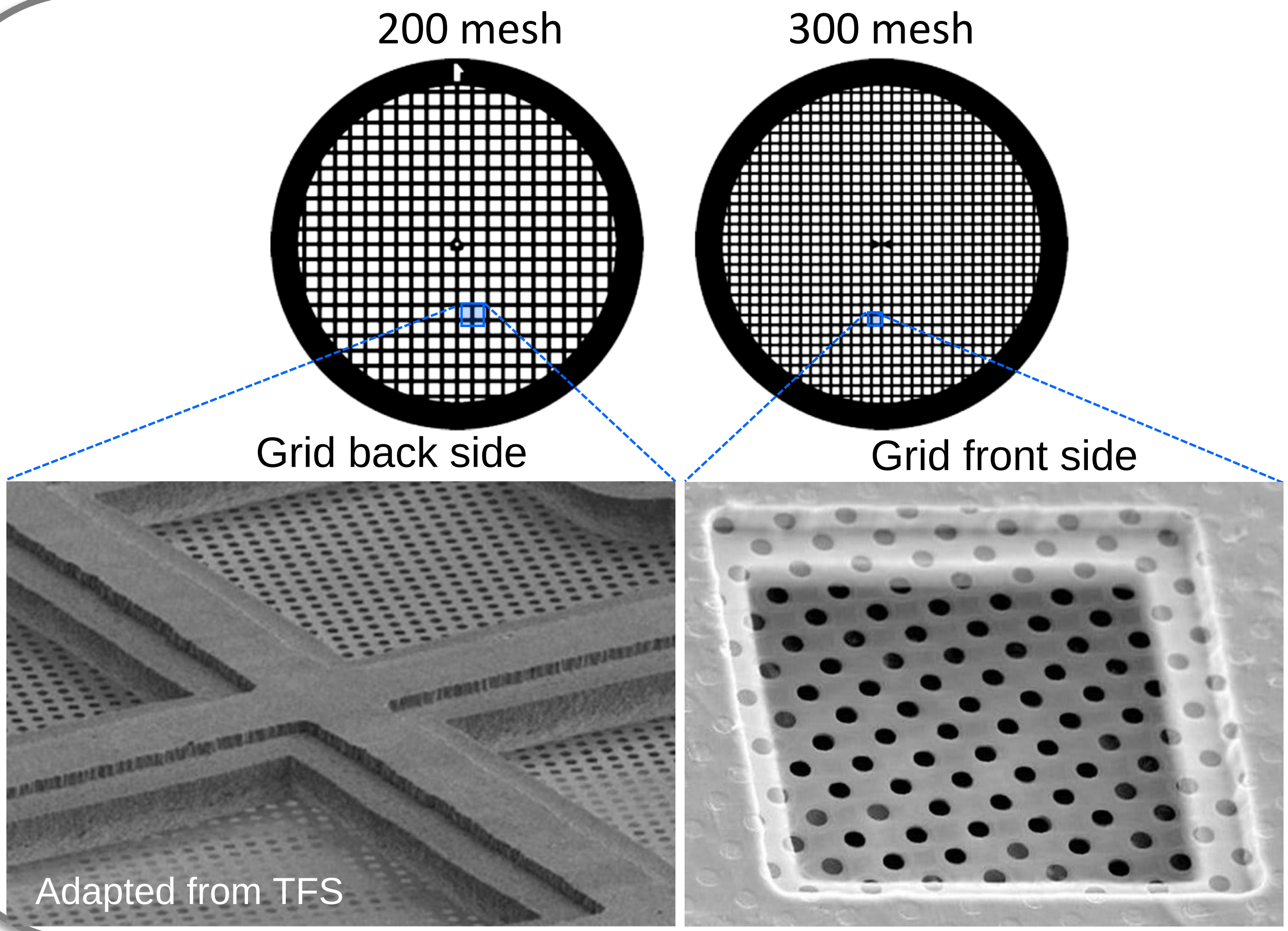
TiSi	SiN
SiO_2	SiC

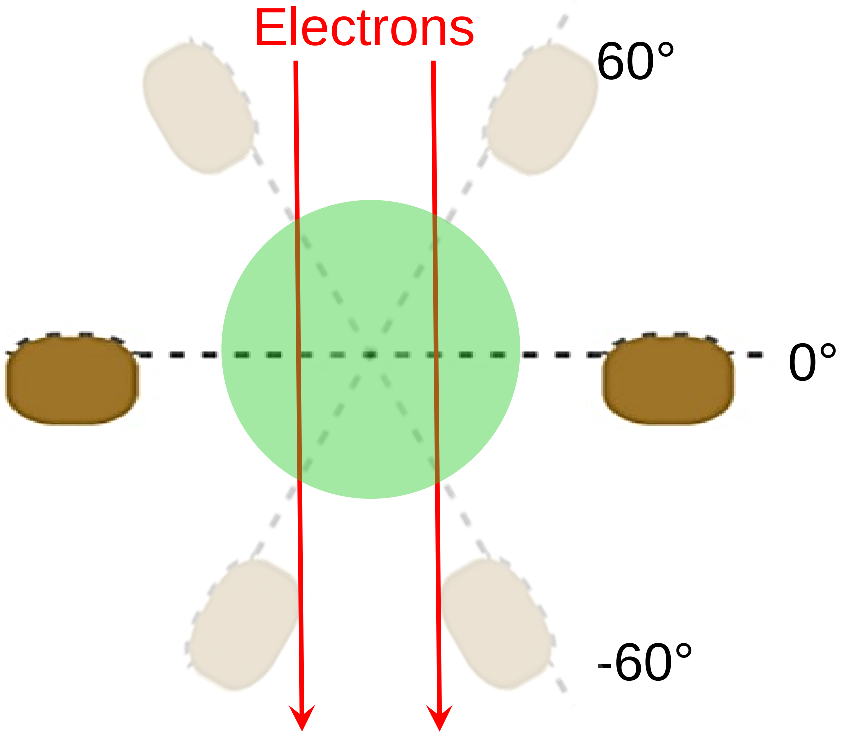
Modified from Russo & Passmore, Curr Opin Struct Biol 2016

2. Five considerations for *in situ* sample preparation

2.1 Validation of the sample quality

2.2 EM grid => Mesh / Hole size





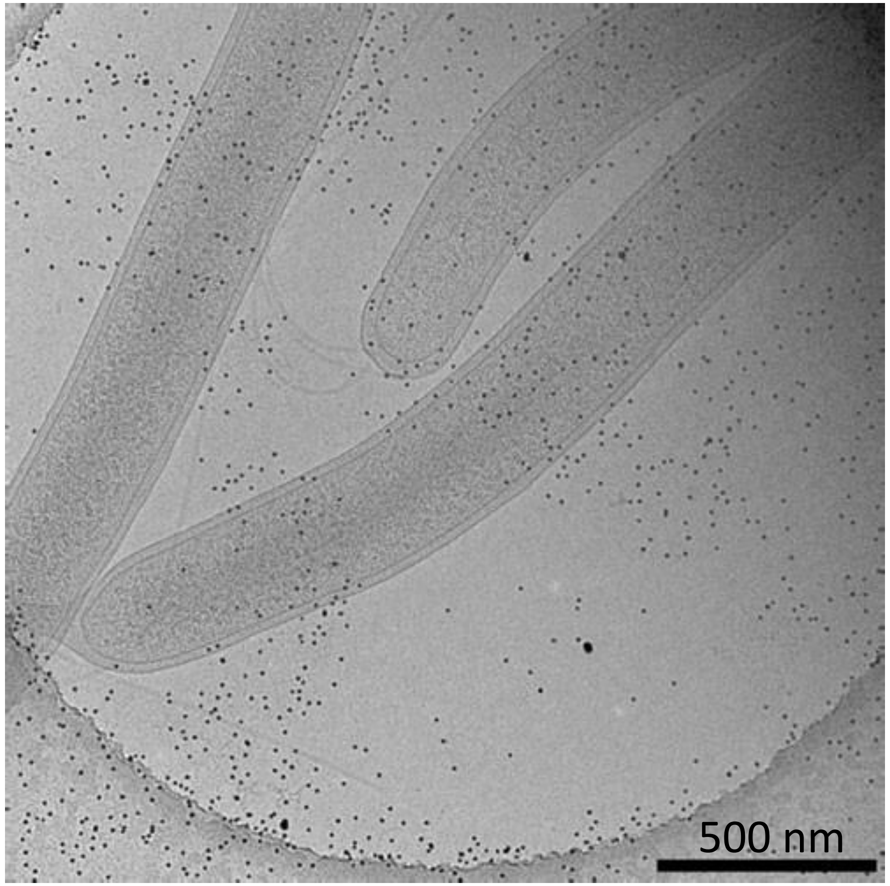
2. Five considerations for *in situ* sample preparation

2.1 Validation of the sample quality

2.2 EM grid

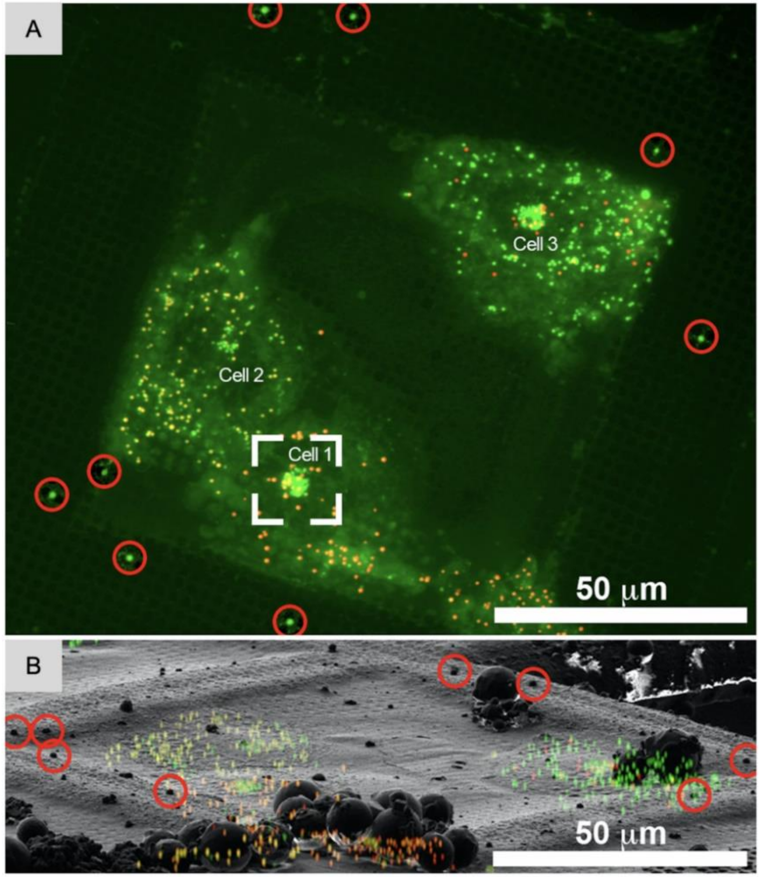
2.3 Fiducial markers

e.g., 10-nm BSA-treated colloidal gold for tilt series alignments



Iancu et al., Nature Protocols 2006

e.g., 1-µm Magnetic beads for FLM and SEM/FIB microscopy.



Arnold et al., Biophysical Journal 2016

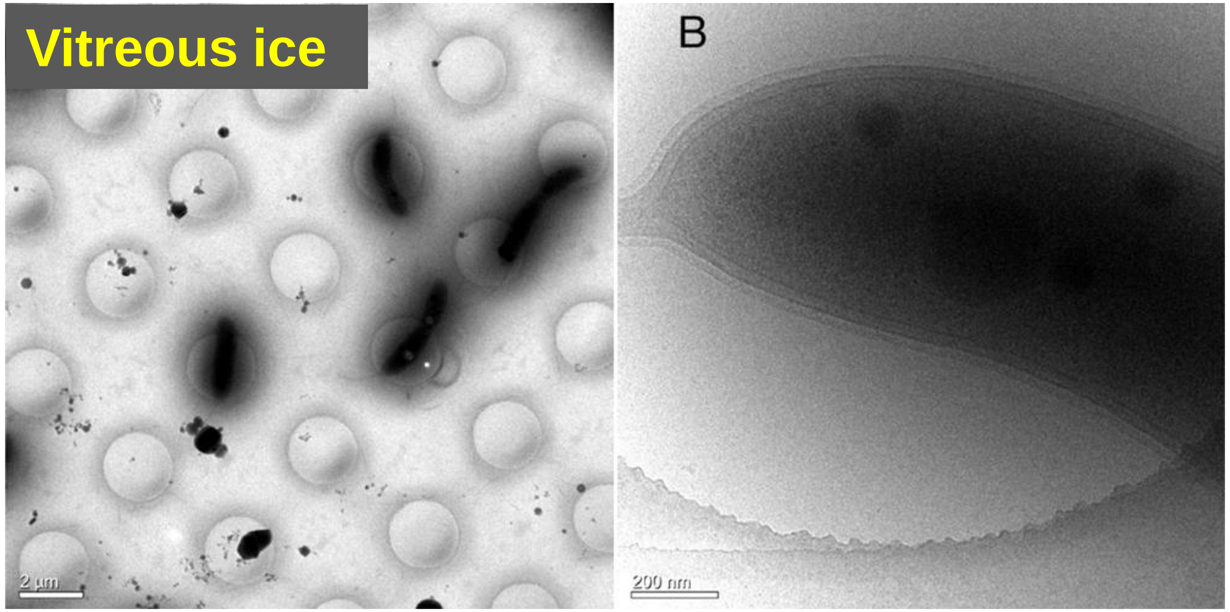
2. Five considerations for *in situ* sample preparation

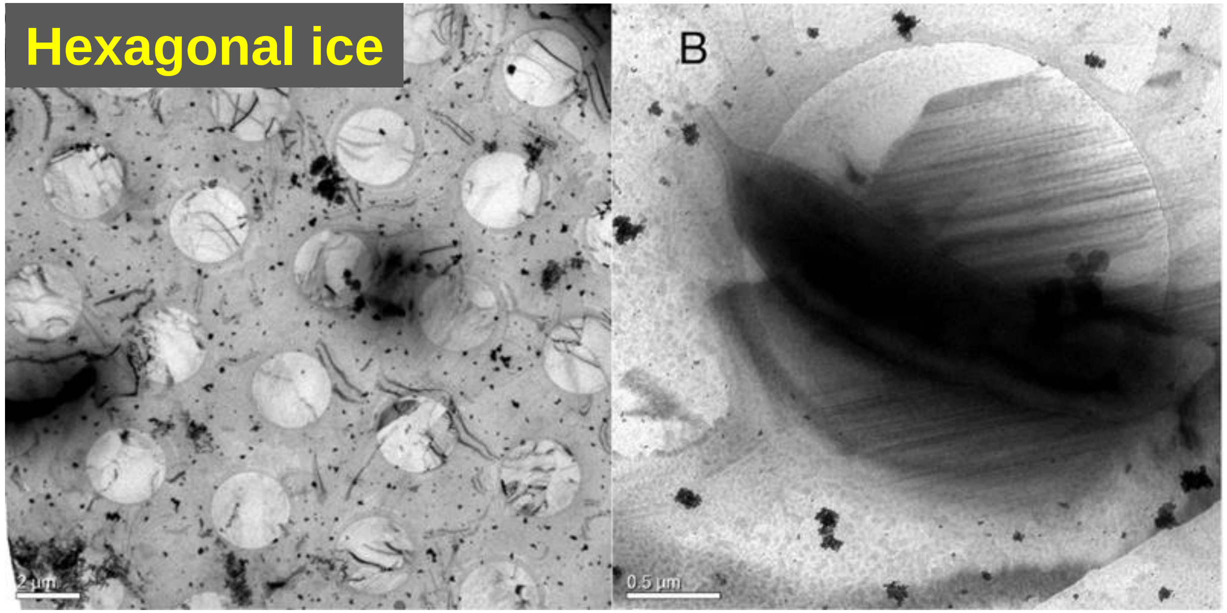
2.1 Validation of the sample quality
2.2 EM grid
2.3 Fiducial markers
2.4 Cryogen e.g., LE or 37% LE-63% LP mixture

T_{LE}= -182.8 ℃ ~ -88.6 ℃

T_{LP}= -189.7 ℃ ~ -42.2 ℃

T_{LN2}=-210 ℃ ~-195.8 ℃





Tivol et al., Microsc Microanal 2008

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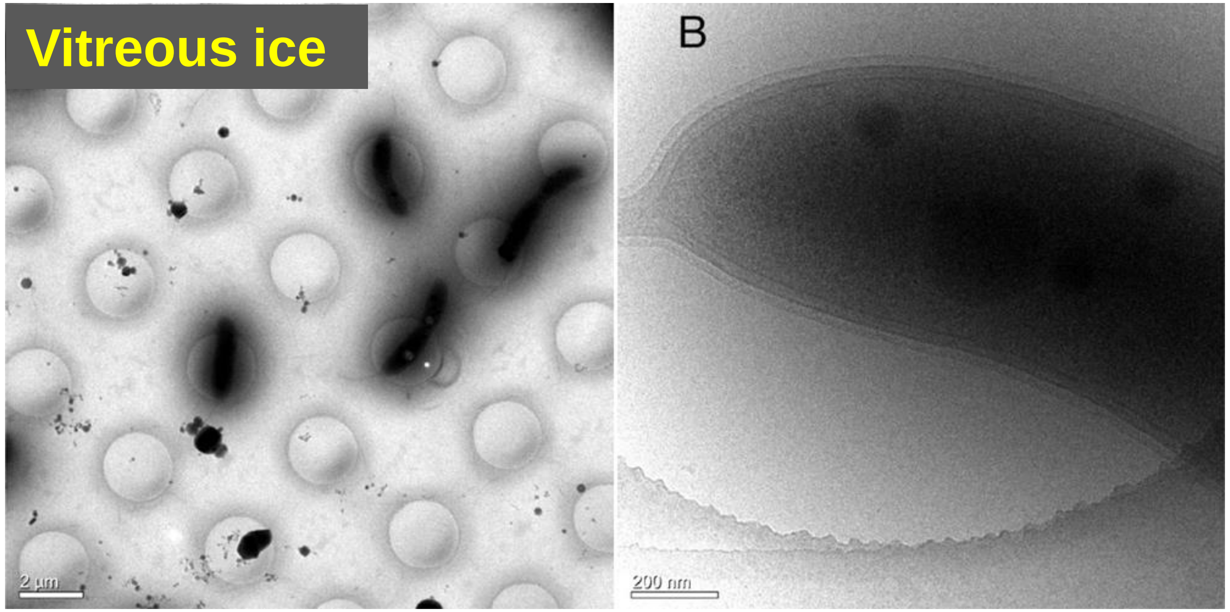
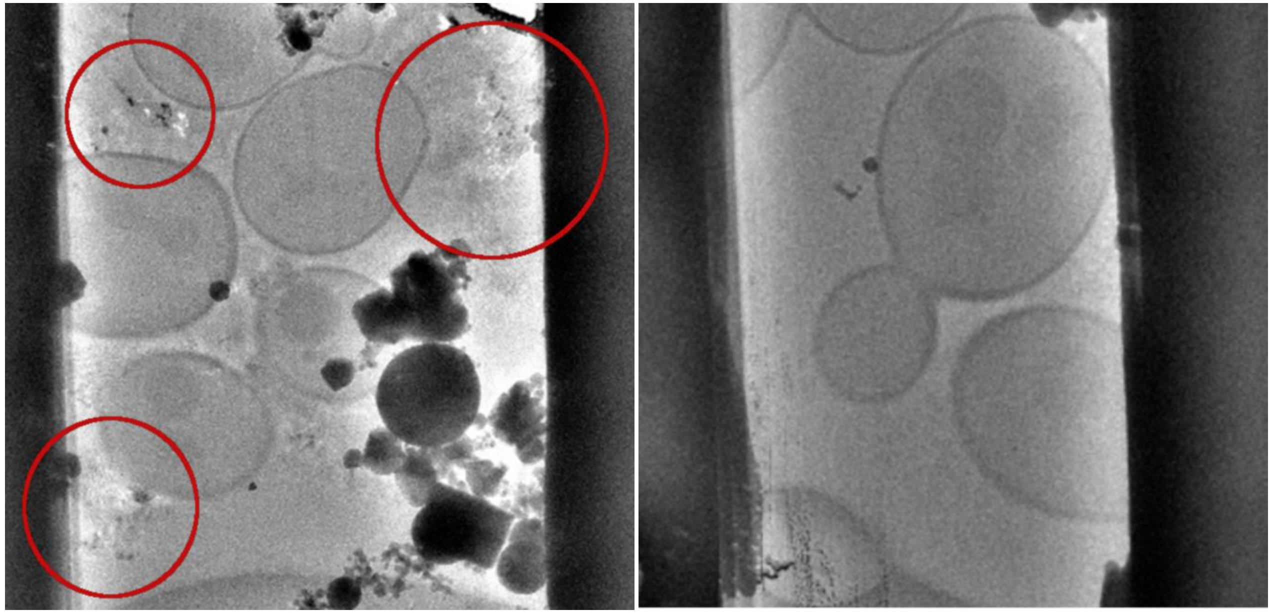


Without glycerol

5% glycerol



Tivol et al., Microsc Microanal 2008

Moravcová et al., J Vis Exp 2021

2. Five considerations for *in situ* sample preparation

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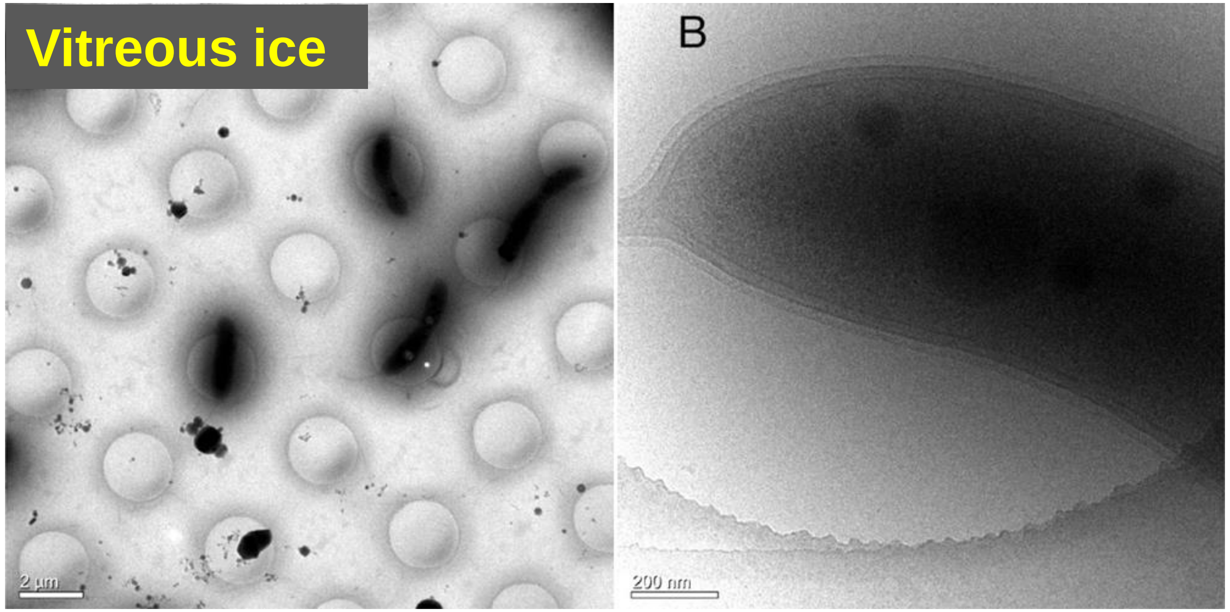
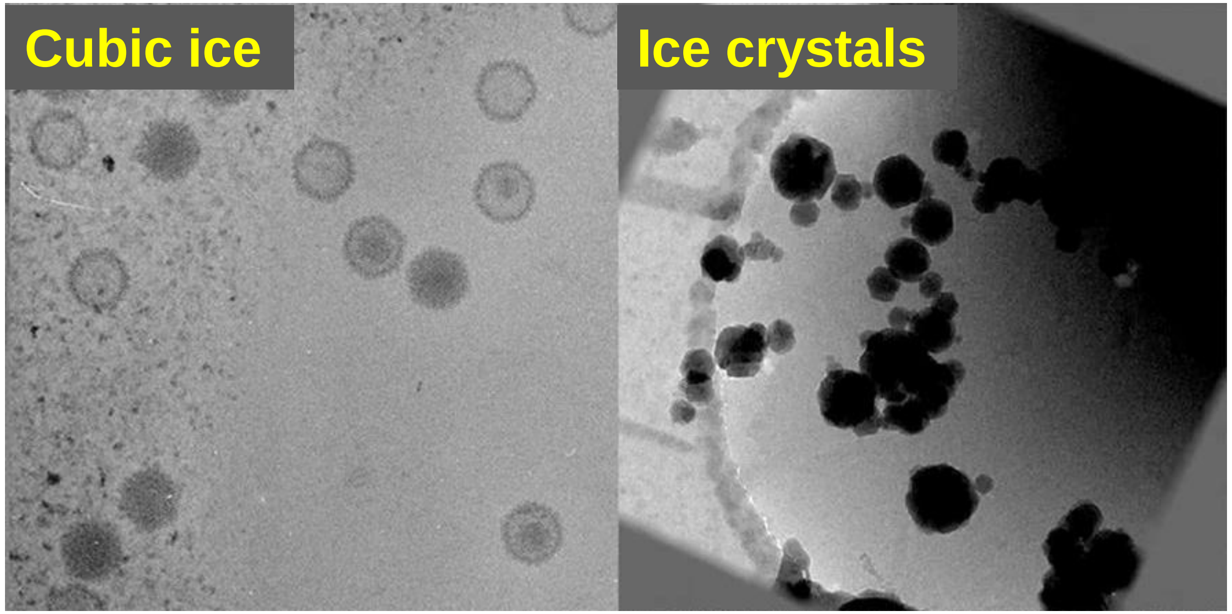




Tivol et al., Microsc Microanal 2008

Bhella D, Biophysical Reviews 2019

2. Five considerations for *in situ* sample preparation

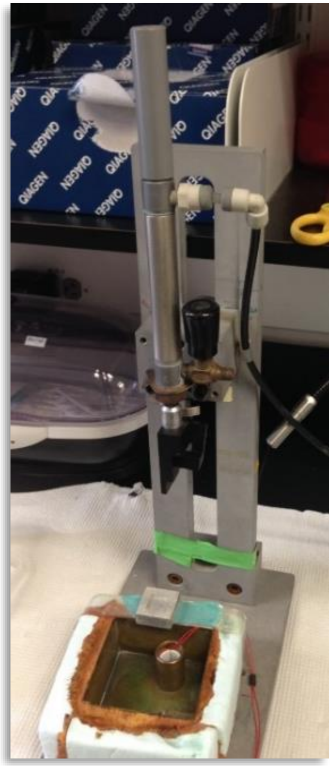
2.1 Validation of the sample quality

2.2 EM grid

2.3 Fiducial markers

2.4 Cryogen

2.5 Plunger



Homemade

EMS-002 (EMS)

EM GP2 (Leica)

Vitrobot Mark IV (TFS)

Cryoplunge™3 (Gatan)

Main contents

1. *In situ* specimens accessible by cryoEM

2. Five considerations for *in situ* sample preparation

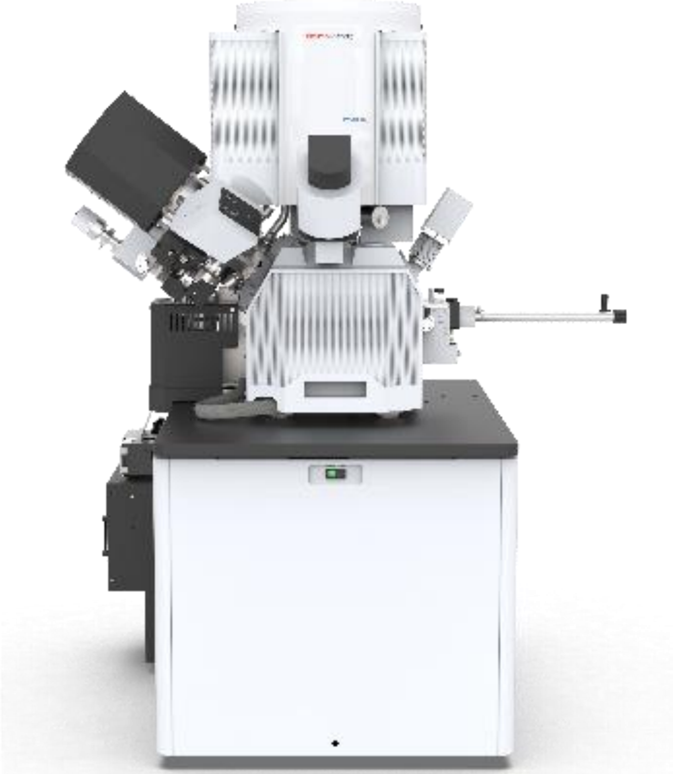
3. Tutorial of major steps of cryo lamella preparation

Examples of cryoFIB milling instruments

Crossbeam
(ZEISS)

Scios, Aquilos 1/2
(Thermo Fisher Scientific)

Hydra Bio Plasma-FIB
(Thermo Fisher Scientific)



Arctis Cryo-Plasma-FIB
(Thermo Fisher Scientific)

Main contents

1. *In situ* specimens accessible by cryoEM

2. Five considerations for *in situ* sample preparation

3. Tutorial of major steps of cryo lamella preparation (with Vitrobot & Aquilos 2)

3.1 Prepare frozen-hydrated cells on an EM grid

Vitrification

T

CryoFIB

Sample screening
Atlas & lamella sites

iFLM (Optional)
Target selection

Pt sputter
Sample conductivity

Pt GIS
Protective coating

Pt sputter (Optional)
Sample conductivity

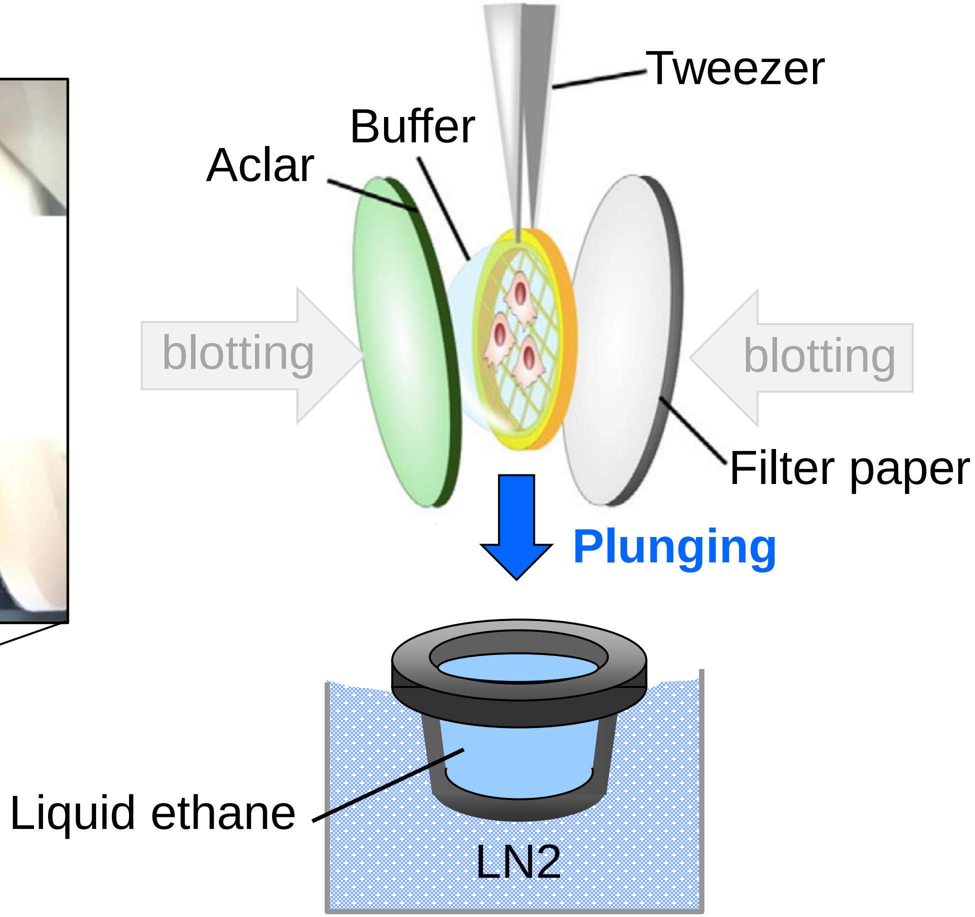
Lamella milling
Preparation, Milling, & thinning

iFLM (Optional)
Target confirmation

Pt sputter (Optional)
Lamella conductivity

T

CryoET



Schaffer et al., JSB 2017; Medeiros et al., Curr Opin Microbiol 2018; Wagner et al., Nature Protocols 2020

3.2 Transfer the grids to Aquilos 2

Vitrification

↓ **T**

CryoFIB

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Sample conductivity

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Preparation, Milling,
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iFLM (Optional)
Target confirmation

Pt sputter (Optional)
Lamella conductivity

↓ **T**

CryoET

1. Prepare the Aquilos 2.
2. Prepare the grids.
3. Transfer the grids to the Aquilos 2.

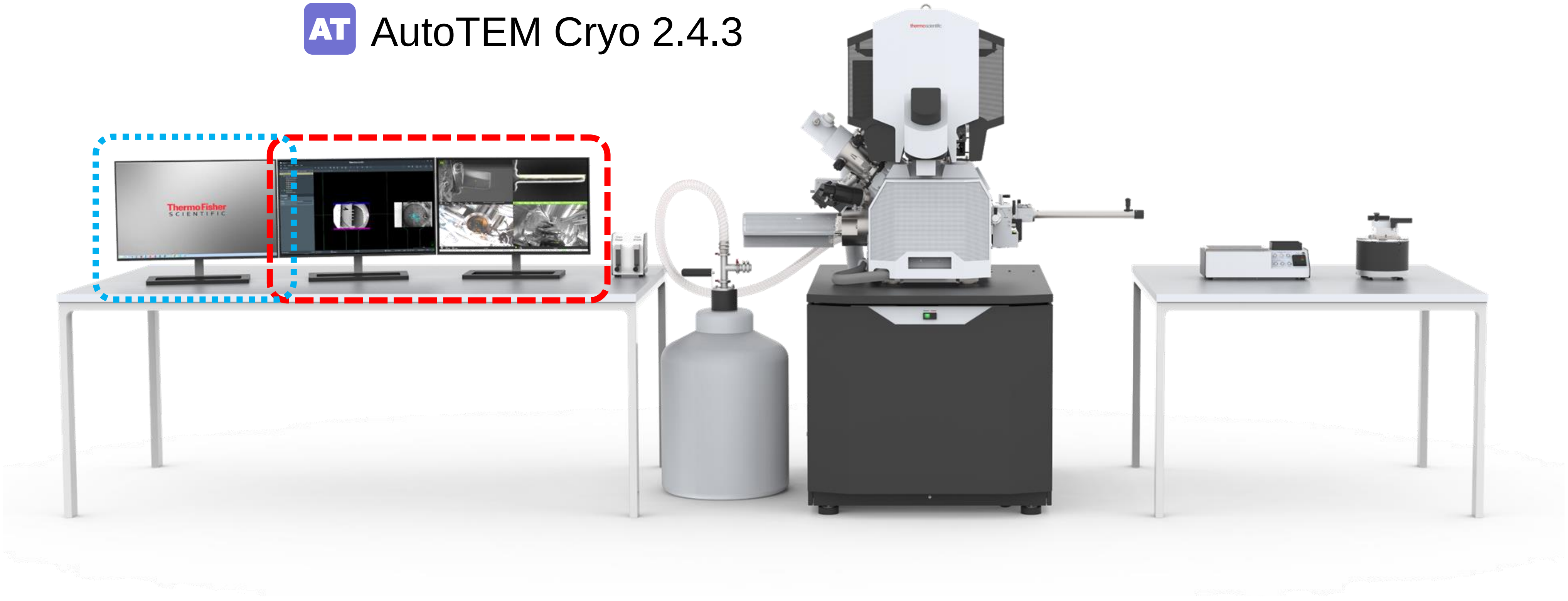
Aquilos 2 & Software used in this tutorial

In Support PC

- Flow DDE
- FlowView

In Aquilos 2 PC

- Microscope Control v32.1.1
- Maps 3.22
- Fluorescence Microscope Control 1.3.0
- AutoTEM Cryo 2.4.3



3.2.1 Prepare the system _Select the Shuttle type

(xT Microscope Control)

Vitrification

T

CryoFIB

Sample screening
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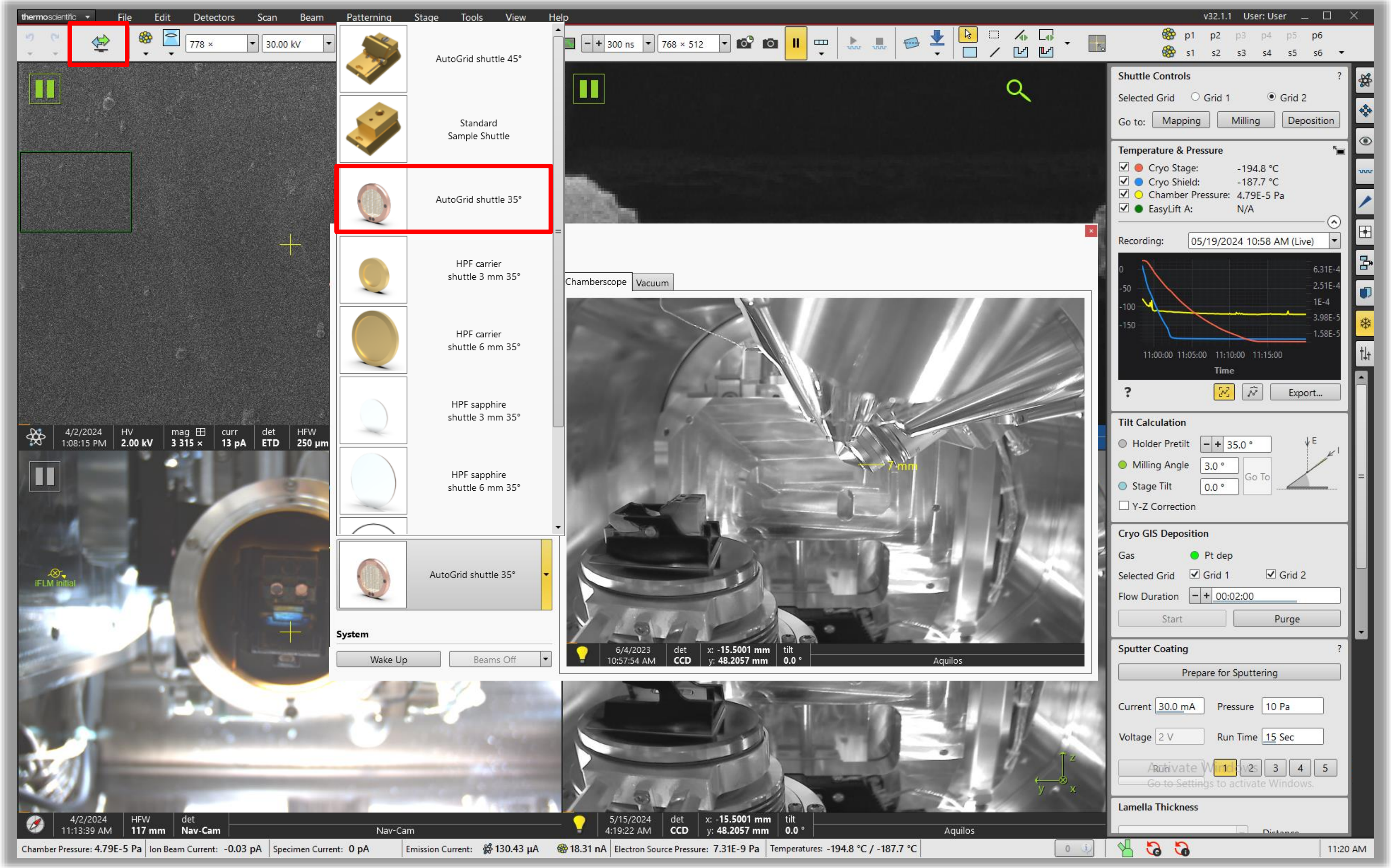
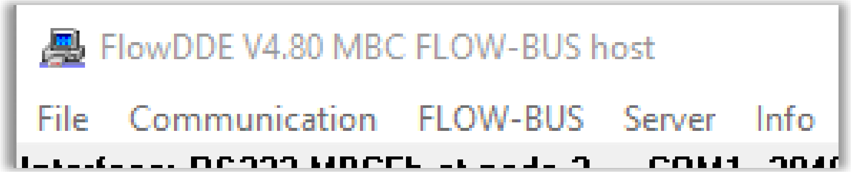
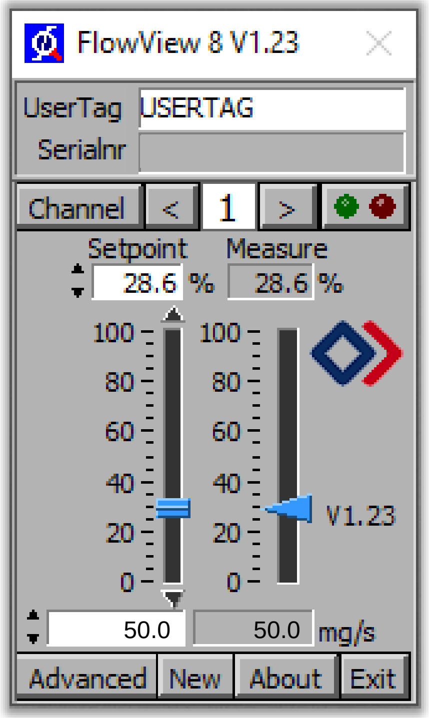
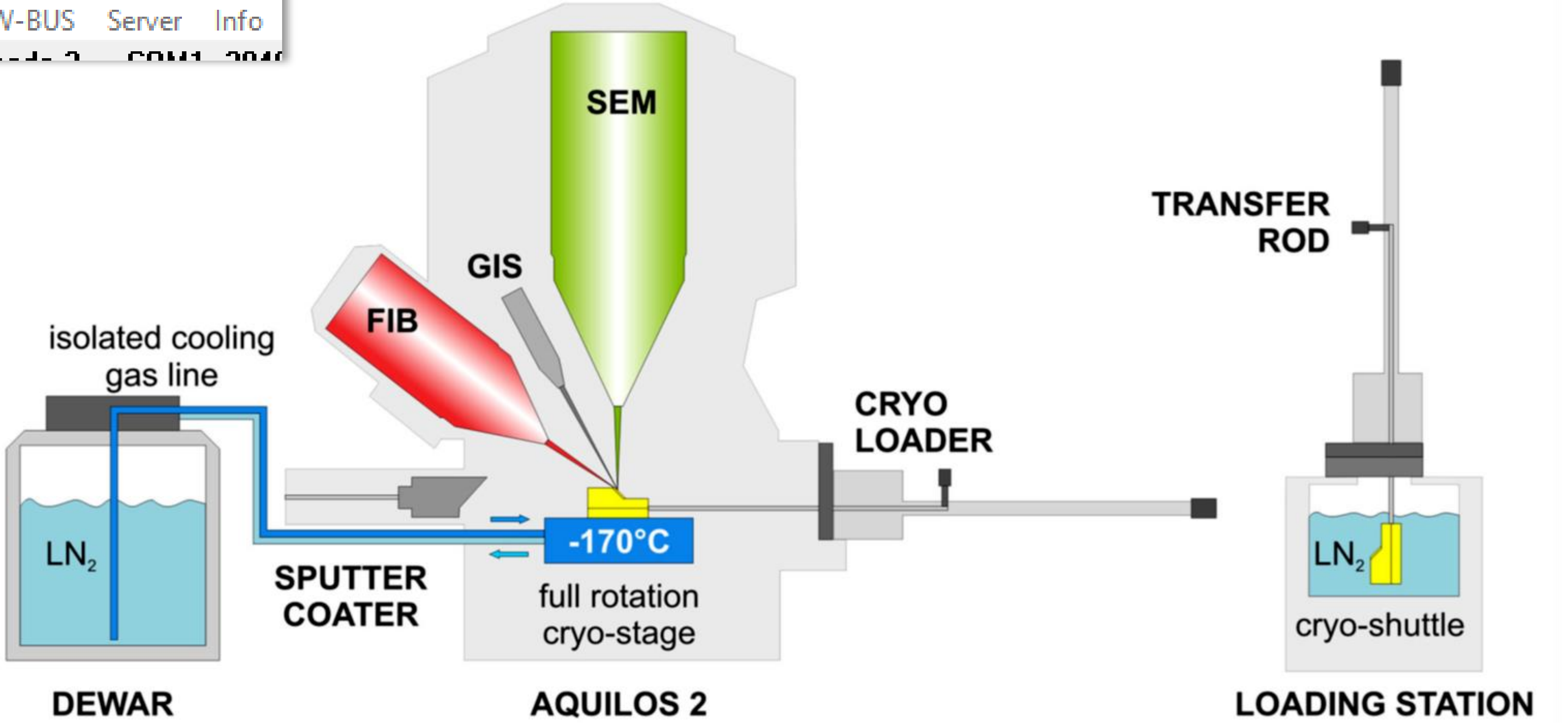


3.2.1 Prepare the system _Purge the system

- Cooling-gas line, Loading station (>0.5h)





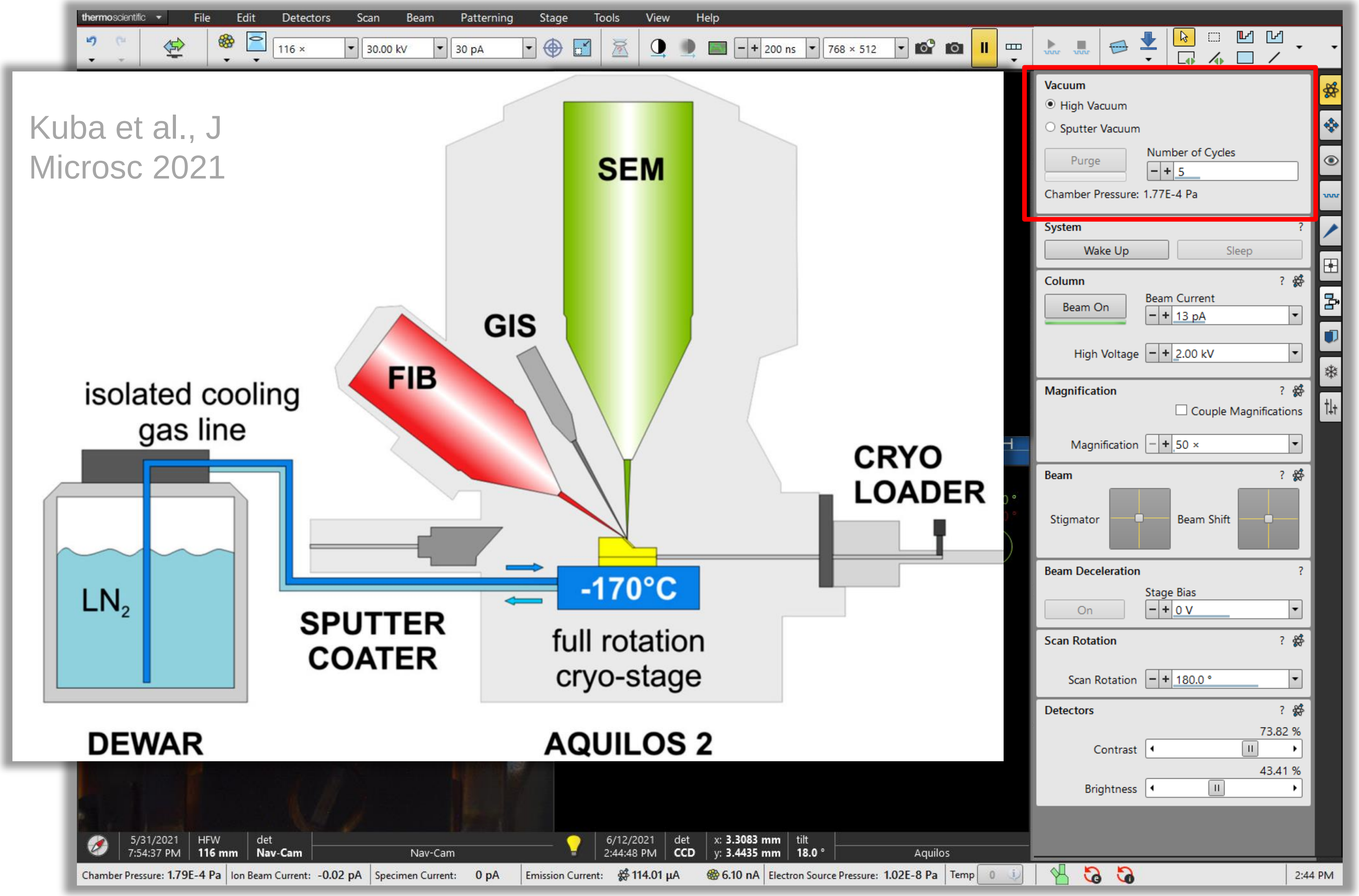


Kuba et al., J Microsc 2021

3.2.1 Prepare the system _Purge the system

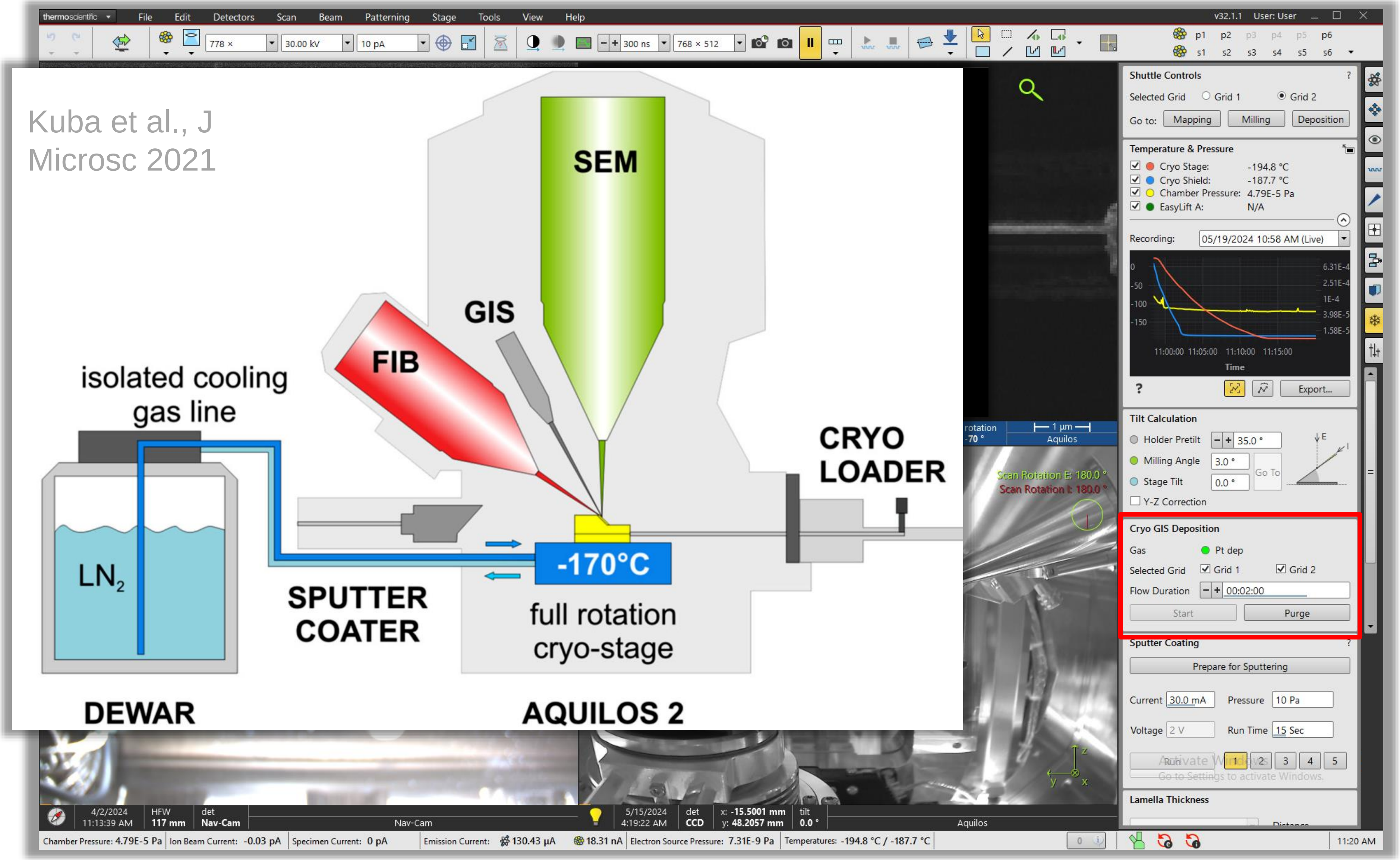
(xT Microscope Control)

- Cooling-gas line, Loading station, Argon line (5 cycles)

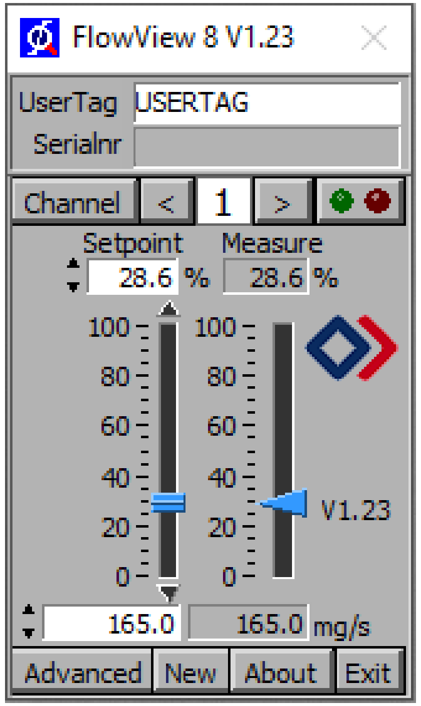


3.2.1 Prepare the system _Purge the system (xT Microscope Control)

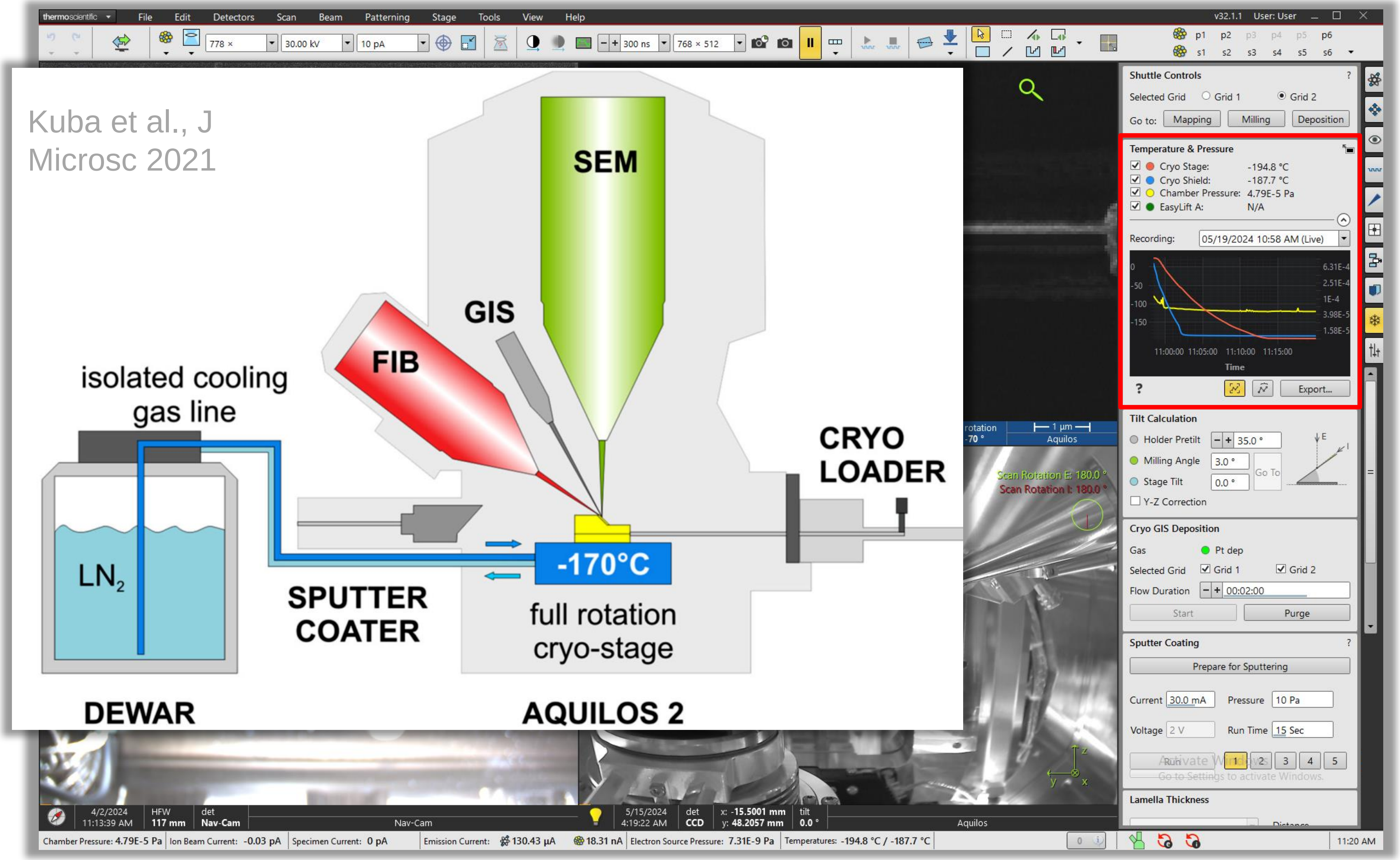
- Cooling-gas line, Loading station, Argon line, GIS (2 mins).



3.2.1 Prepare the system _Cool down the system (xT Microscope Control)



Varies by instruments



3.2.2 Prepare the grids _Clip the grids

Vitrification

T

CryoFIB

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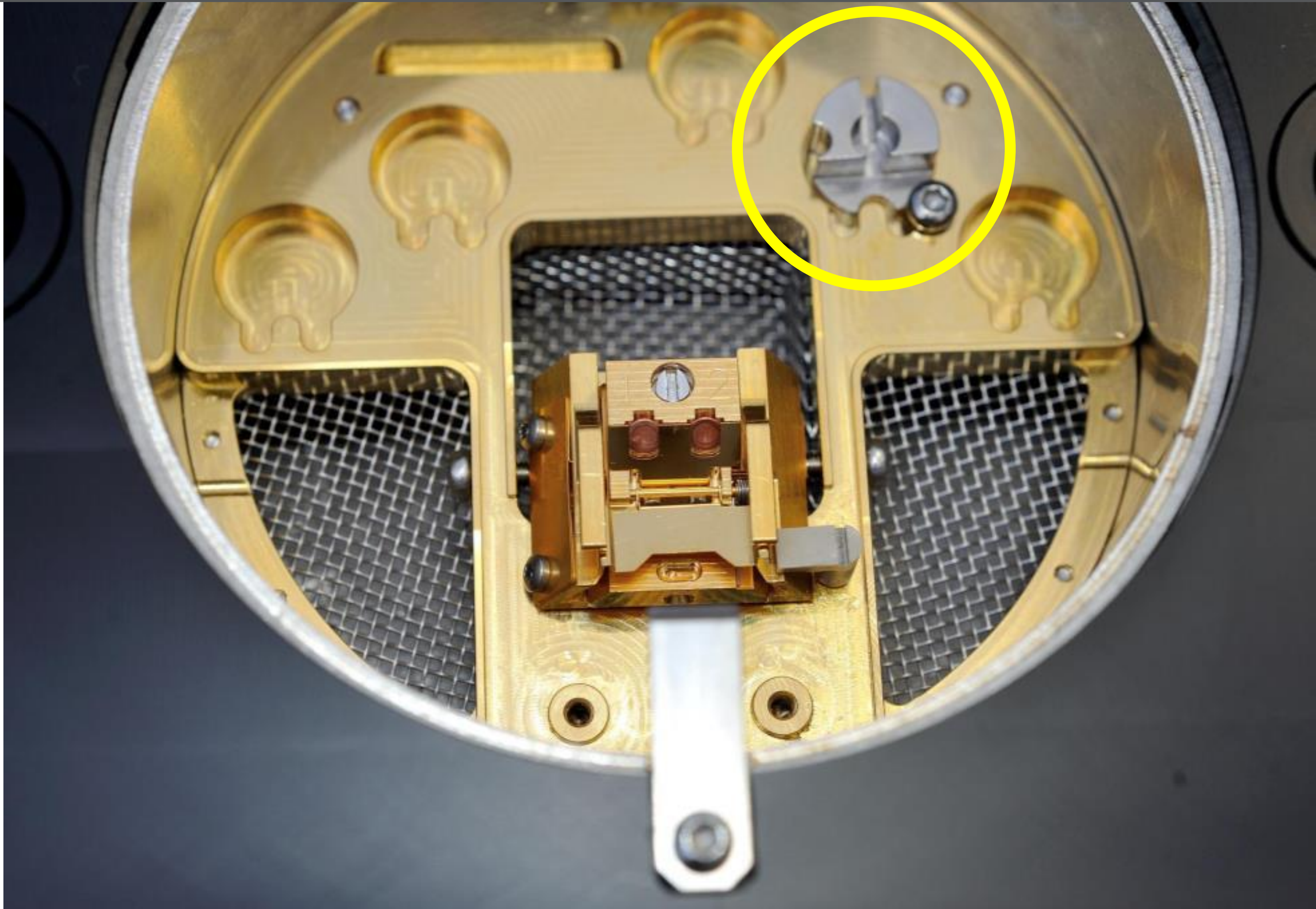

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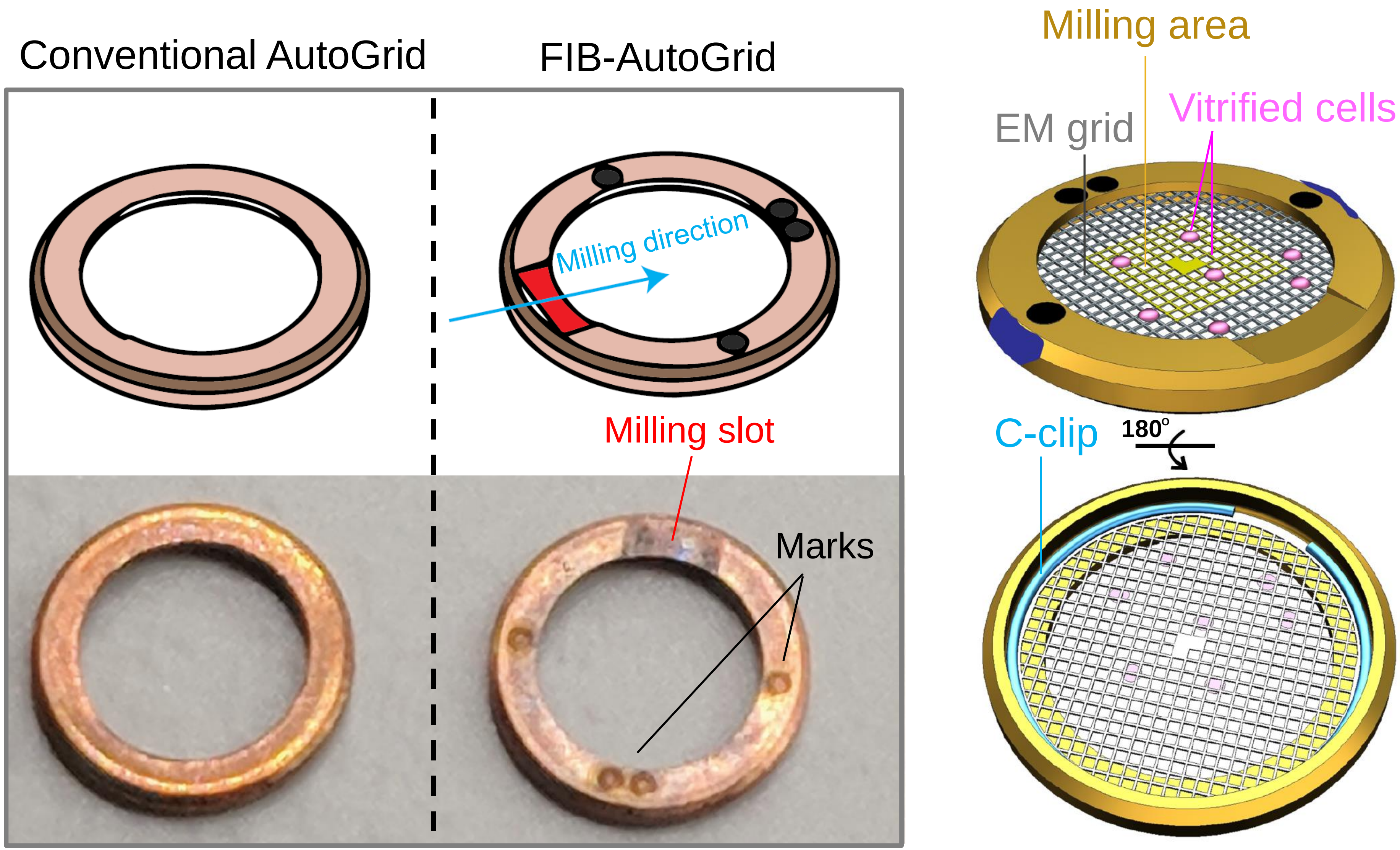

Adapted from Wagner, et al., Nature Protocols 2020

Mark the AutoGrid rim to facilitate orienting the lamellae during grid loading

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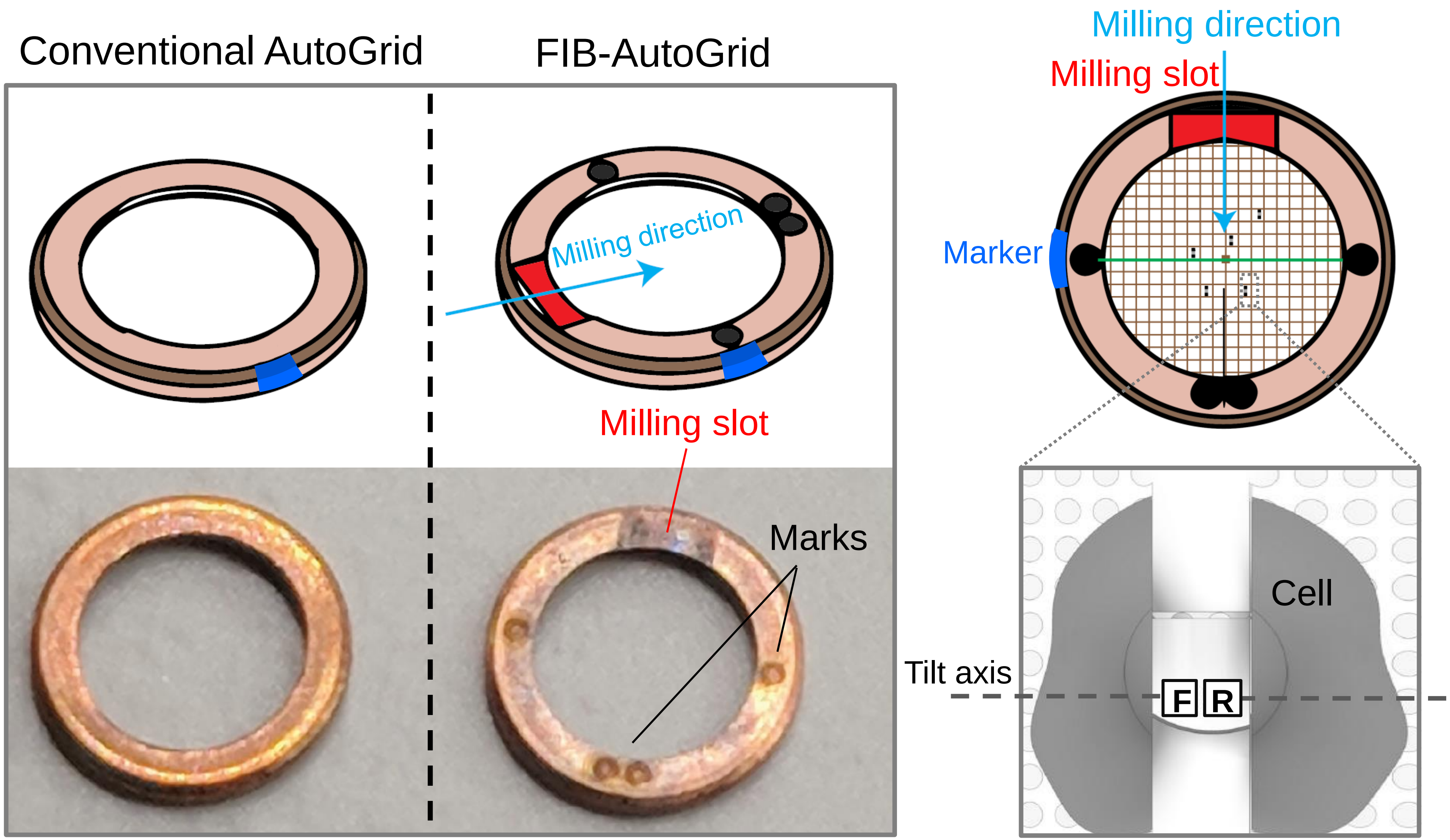

Adapted from Wagner, et al., Nature Protocols 2020; Schaffer et al., JSB 2017

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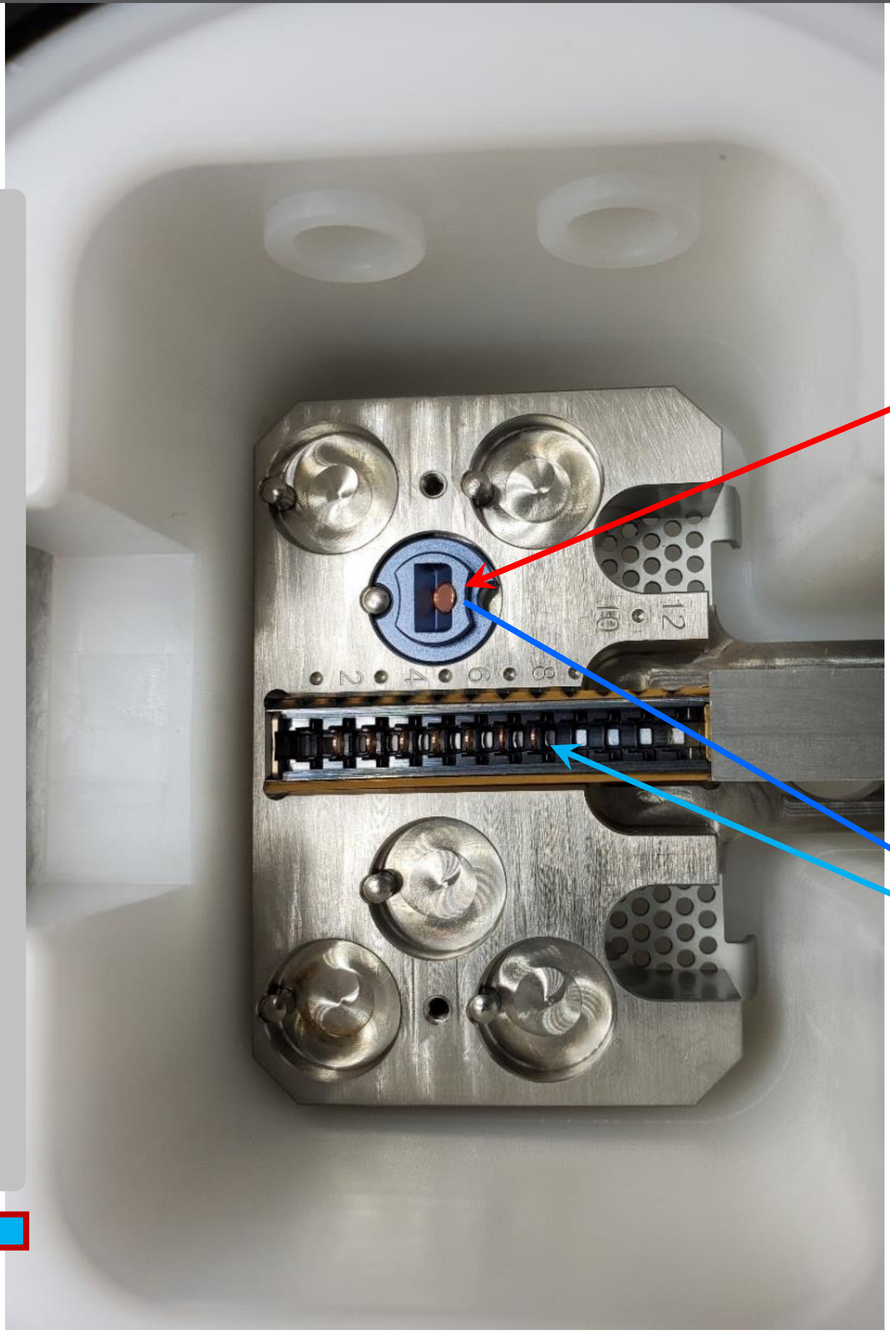
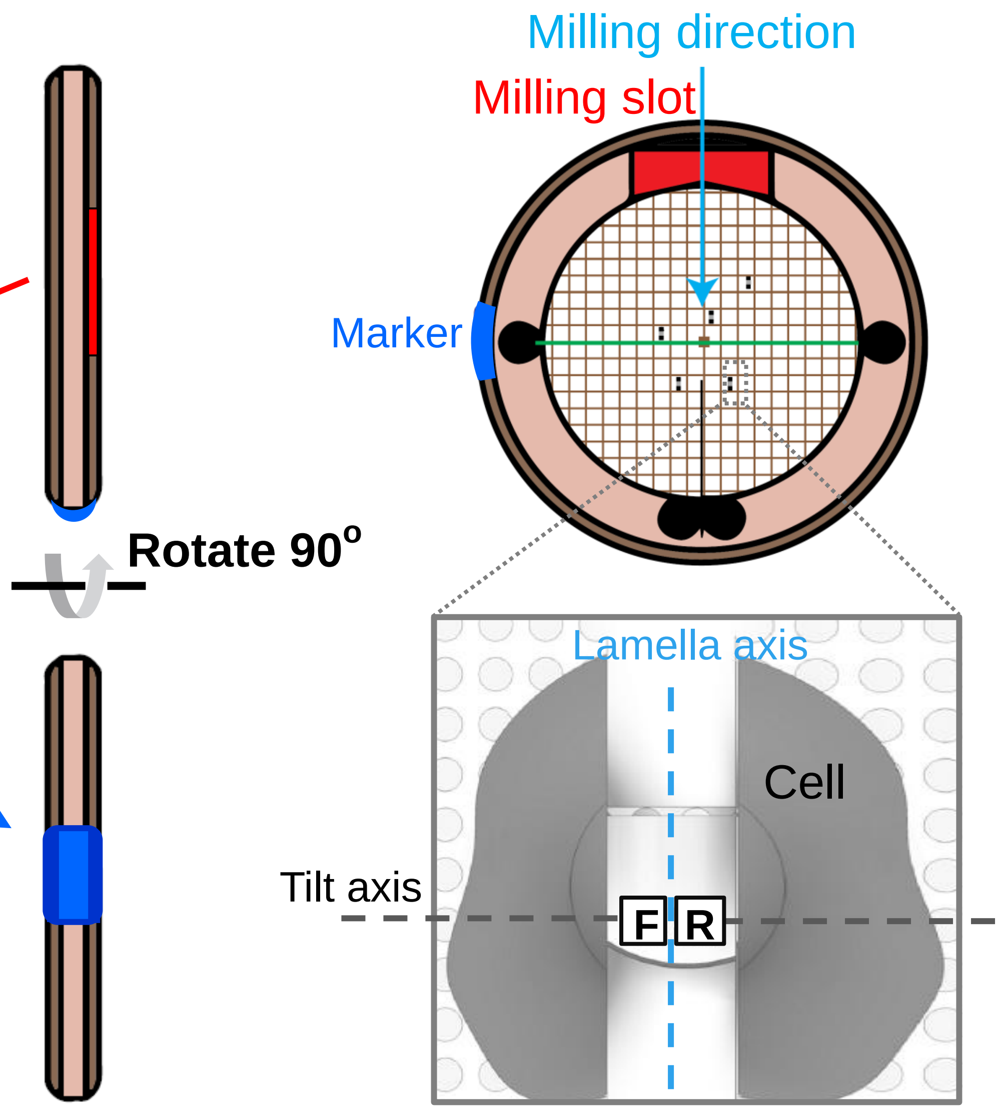
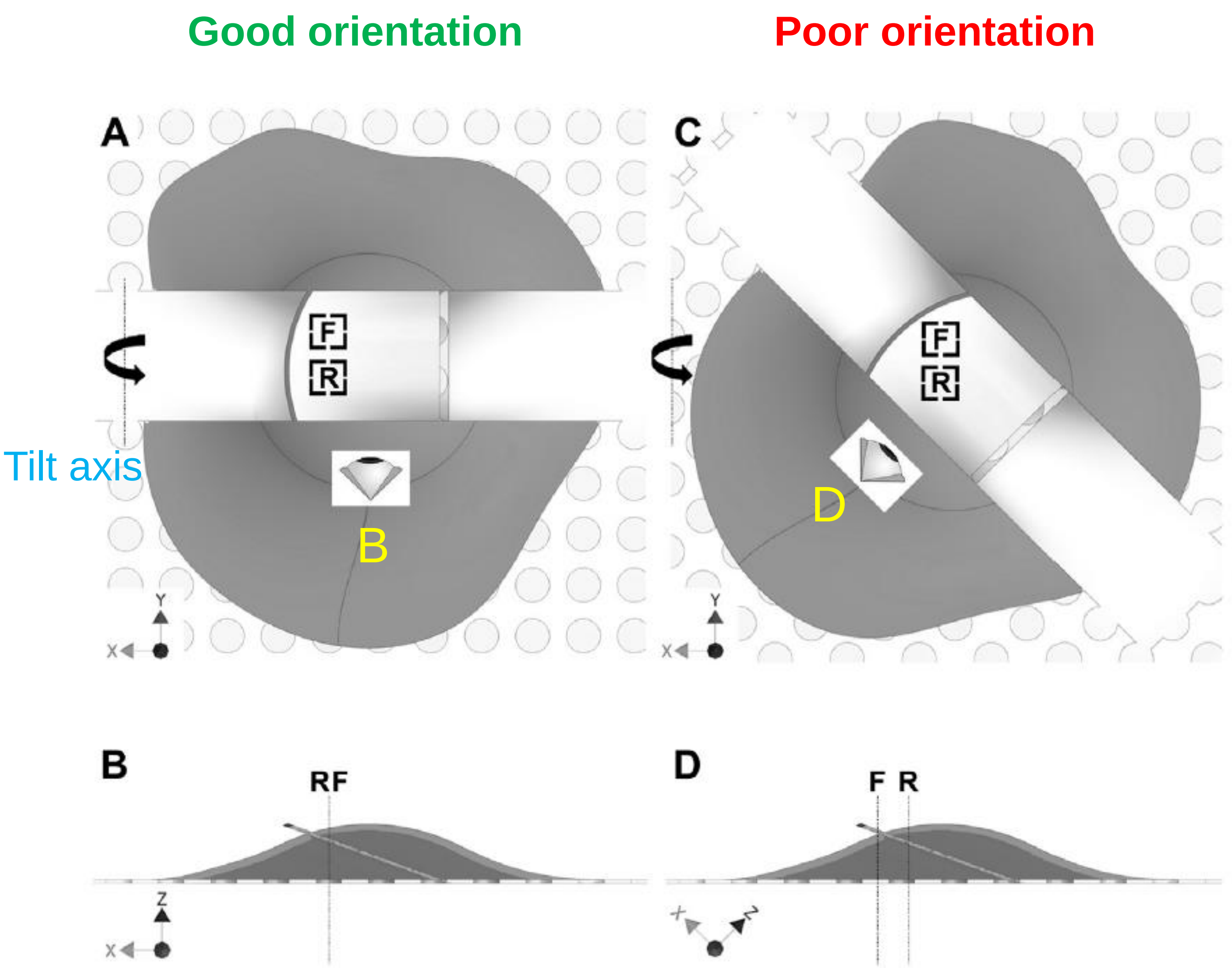
CryoET





Adapted from Wagner, et al., Nature Protocols 2020; Schaffer et al., JSB 2017

Poor orientation causes occlusion at high tilt angles & inaccurate focusing



al., JSB 2017

3.2.3 Transfer the grids _Load grids to Autogrid shuttle

Vitrification

↓ **T**

CryoFIB

Sample screening
Atlas & lamella sites

iFLM (Optional)
Target selection

Pt sputter
Sample conductivity

Pt GIS
Protective coating

Pt sputter (Optional)
Sample conductivity

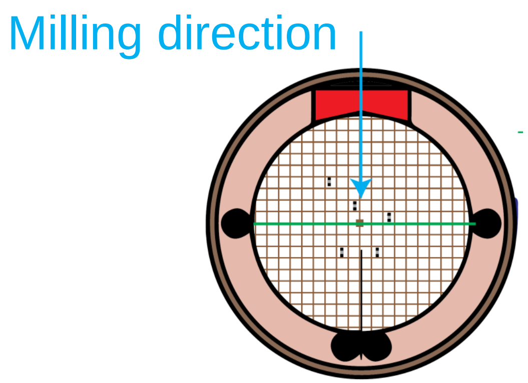
Lamella milling
Preparation, Milling, & thinning

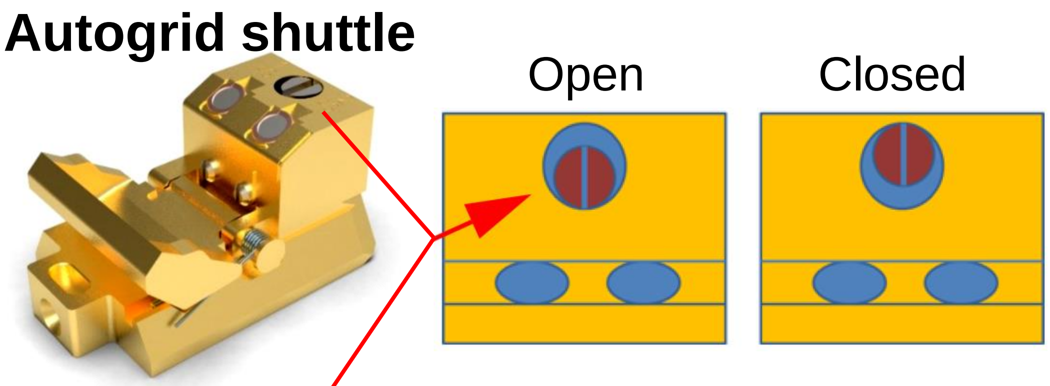
iFLM (Optional)
Target confirmation

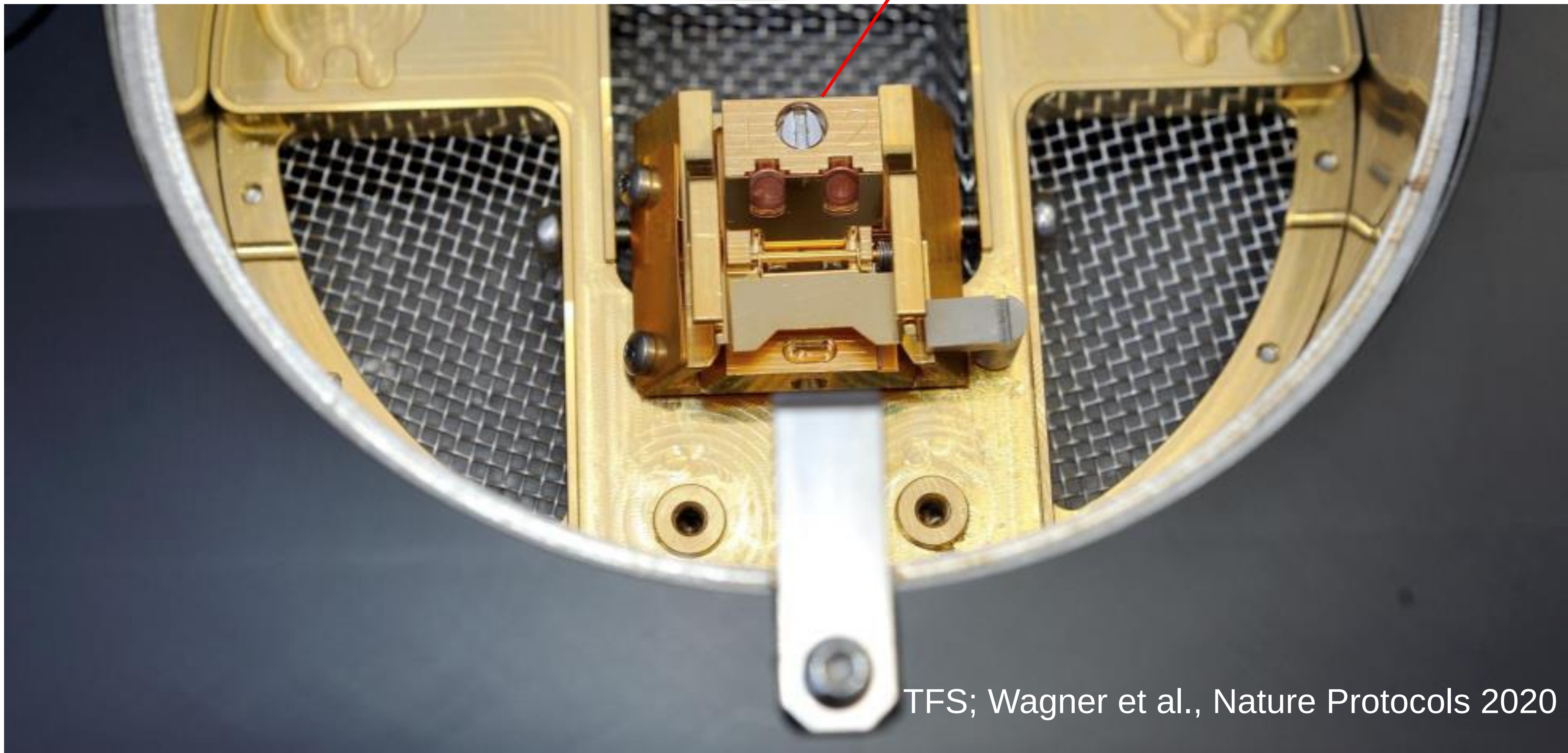
Pt sputter (Optional)
Lamella conductivity

↓ **T**

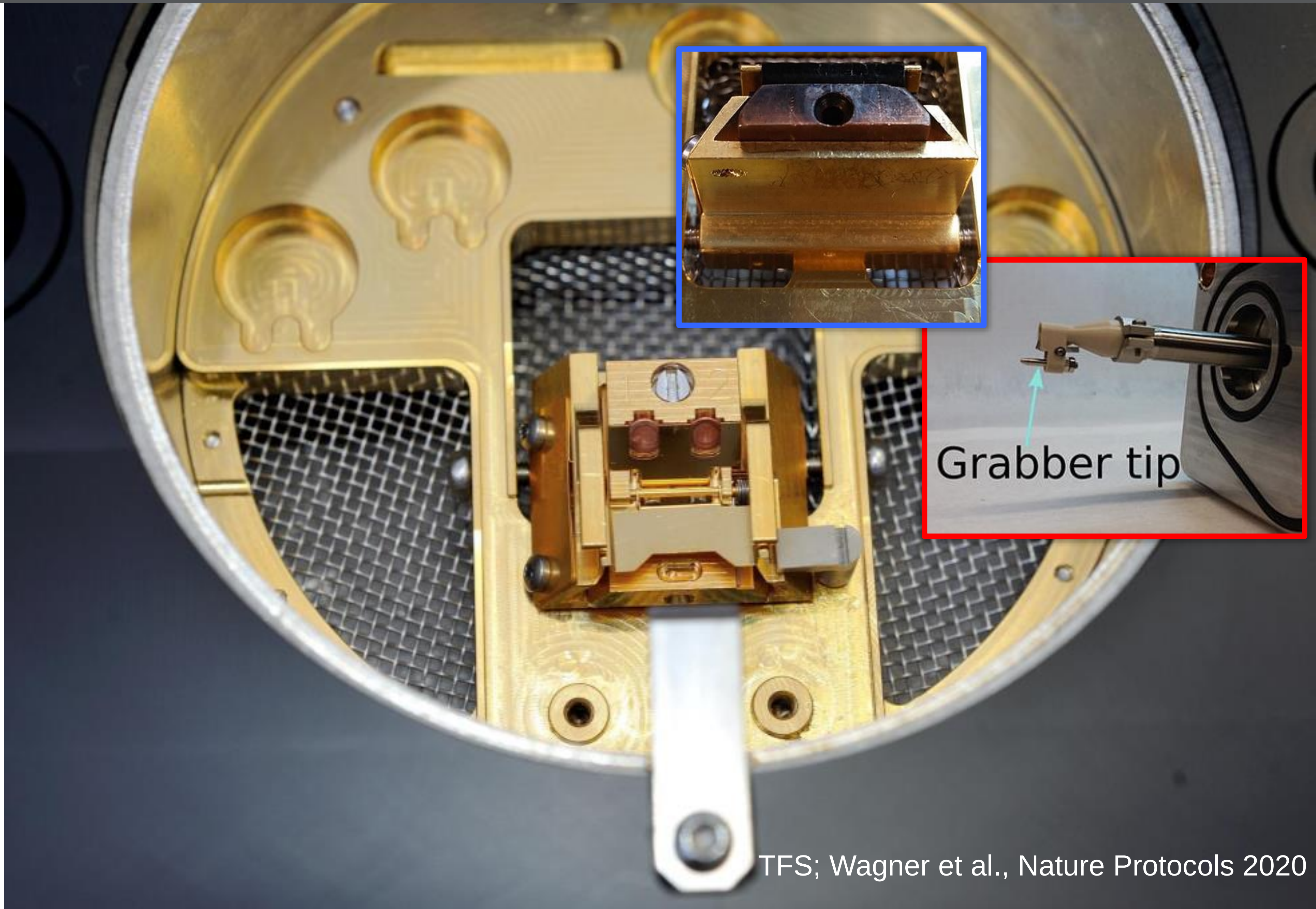
CryoET





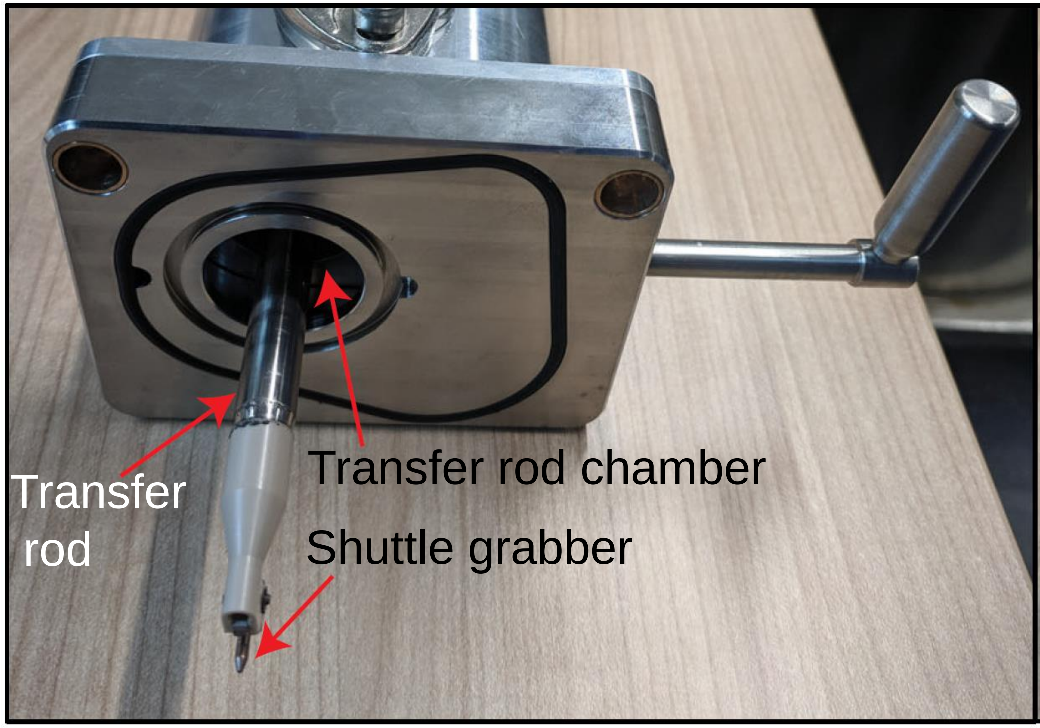


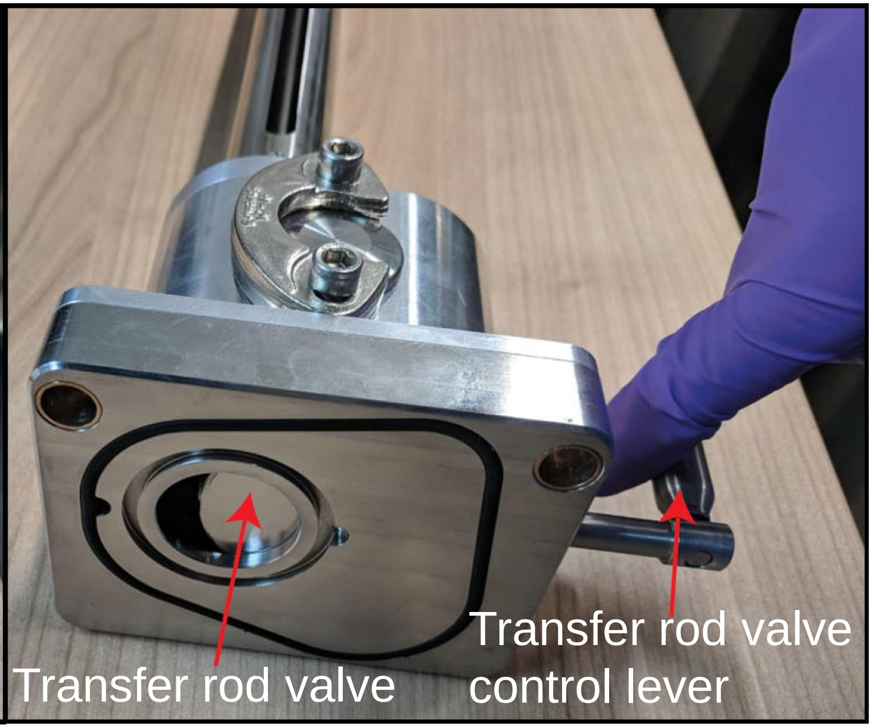
3.2.3 Transfer the grids _Load shuttle to Transfer rod



TFS; Wagner et al., Nature Protocols 2020

3.2.3 Transfer the grids _Load shuttle to Transfer rod





TFS; Lam & Villa, Methods Mol Biol 2021; Wagner et al., Nature Protocols 2020

3.2.3 Transfer the grids to the Aquilos 2

(Video from TFS)

3.3 Sample screening _Setup E-beam

(xT Microscope Control)

Vitrification

↓

CryoFIB

Sample screening

Atlas & lamella sites

iFLM (Optional)

Target selection

Pt sputter

Sample conductivity

Pt GIS

Protective coating

Pt sputter (Optional)

Sample conductivity

Lamella milling

Preparation, Milling, & thinning

iFLM (Optional)

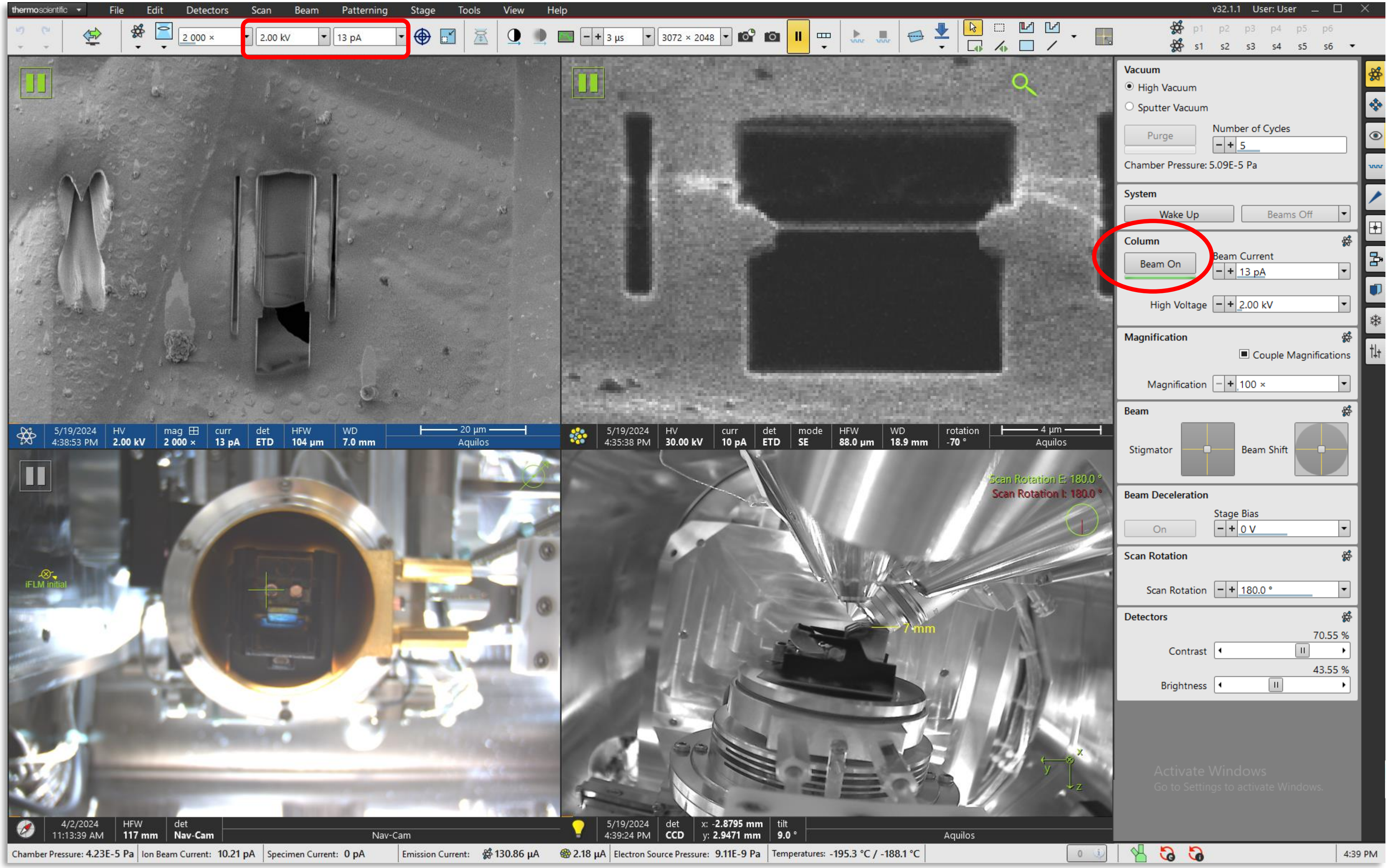
Target confirmation

Pt sputter (Optional)

Lamella conductivity

↓

CryoET



3.3 Sample screening _Link Z to FWD

(xT Microscope Control)

Vitrification

CryoFIB

Sample screening

Atlas & lamella sites

iFLM (Optional)

Target selection

Pt sputter

Sample conductivity

Pt GIS

Protective coating

Pt sputter (Optional)

Sample conductivity

Lamella milling

Preparation, Milling, & thinning

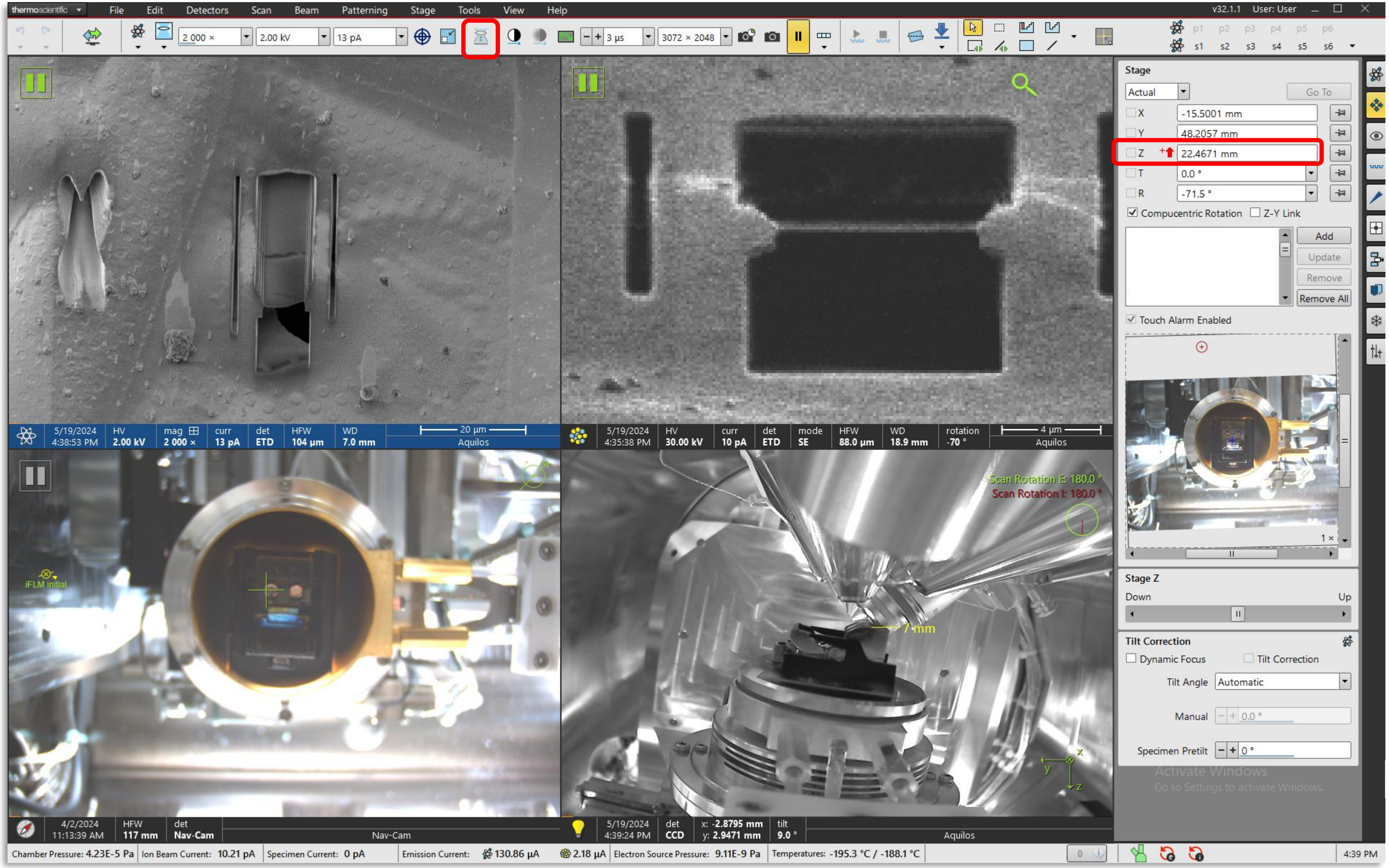
iFLM (Optional)

Target confirmation

Pt sputter (Optional)

Lamella conductivity

CryoET



3.3 Sample screening _Quickly check the grids

(xT Microscope Control)

Vitrification

CryoFIB

Sample screening

Atlas & lamella sites

iFLM (Optional)

Target selection

Pt sputter

Sample conductivity

Pt GIS

Protective coating

Pt sputter (Optional)

Sample conductivity

Lamella milling

Preparation, Milling, & thinning

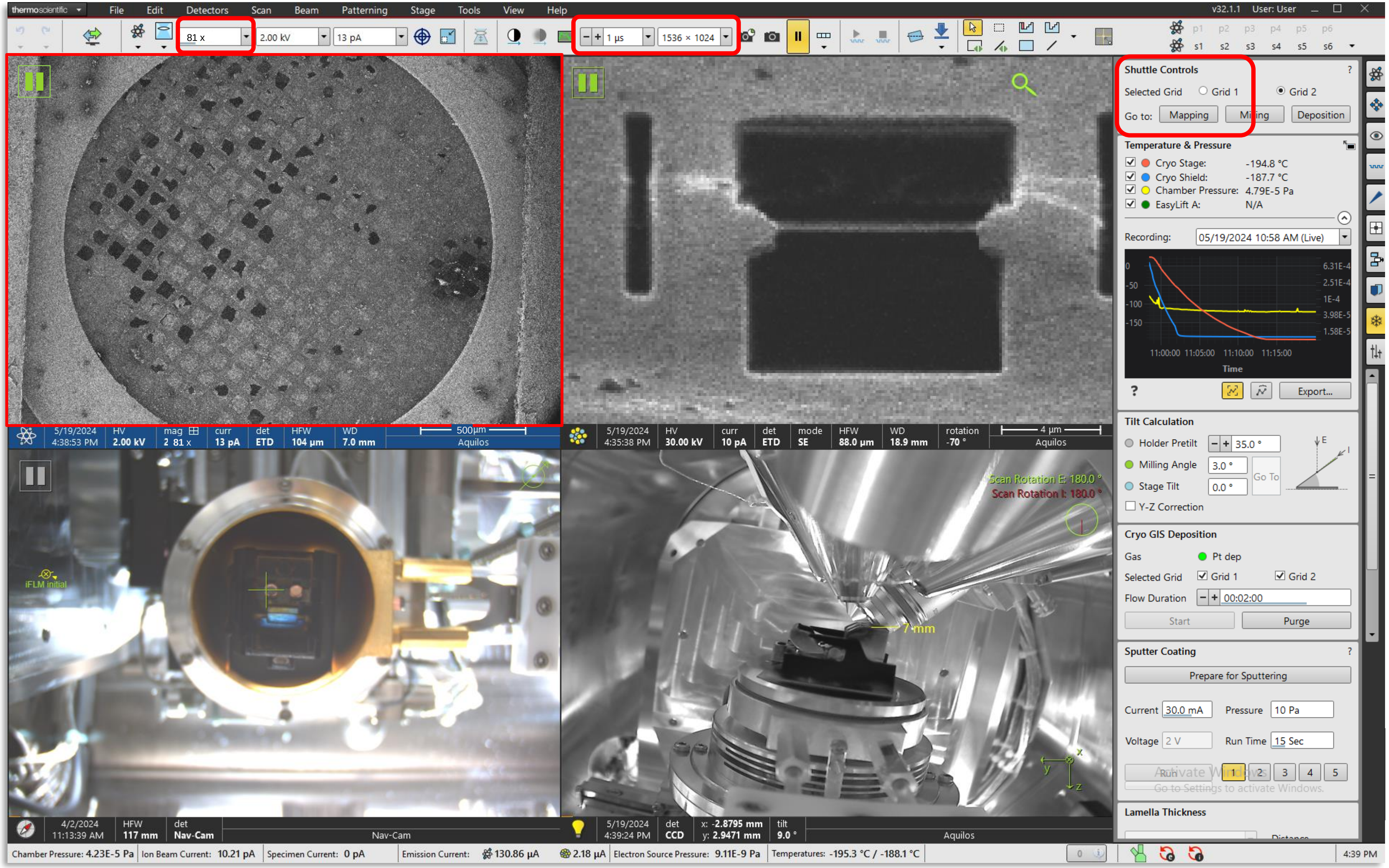
iFLM (Optional)

Target confirmation

Pt sputter (Optional)

Lamella conductivity

CryoET



3.3 Sample screening _Create a Maps Project

(Ma Maps)

Vitrification

↓ T

CryoFIB

Sample screening
Atlas & lamella sites

iFLM (Optional)
Target selection

Pt sputter
Sample conductivity

Pt GIS
Protective coating

Pt sputter (Optional)
Sample conductivity

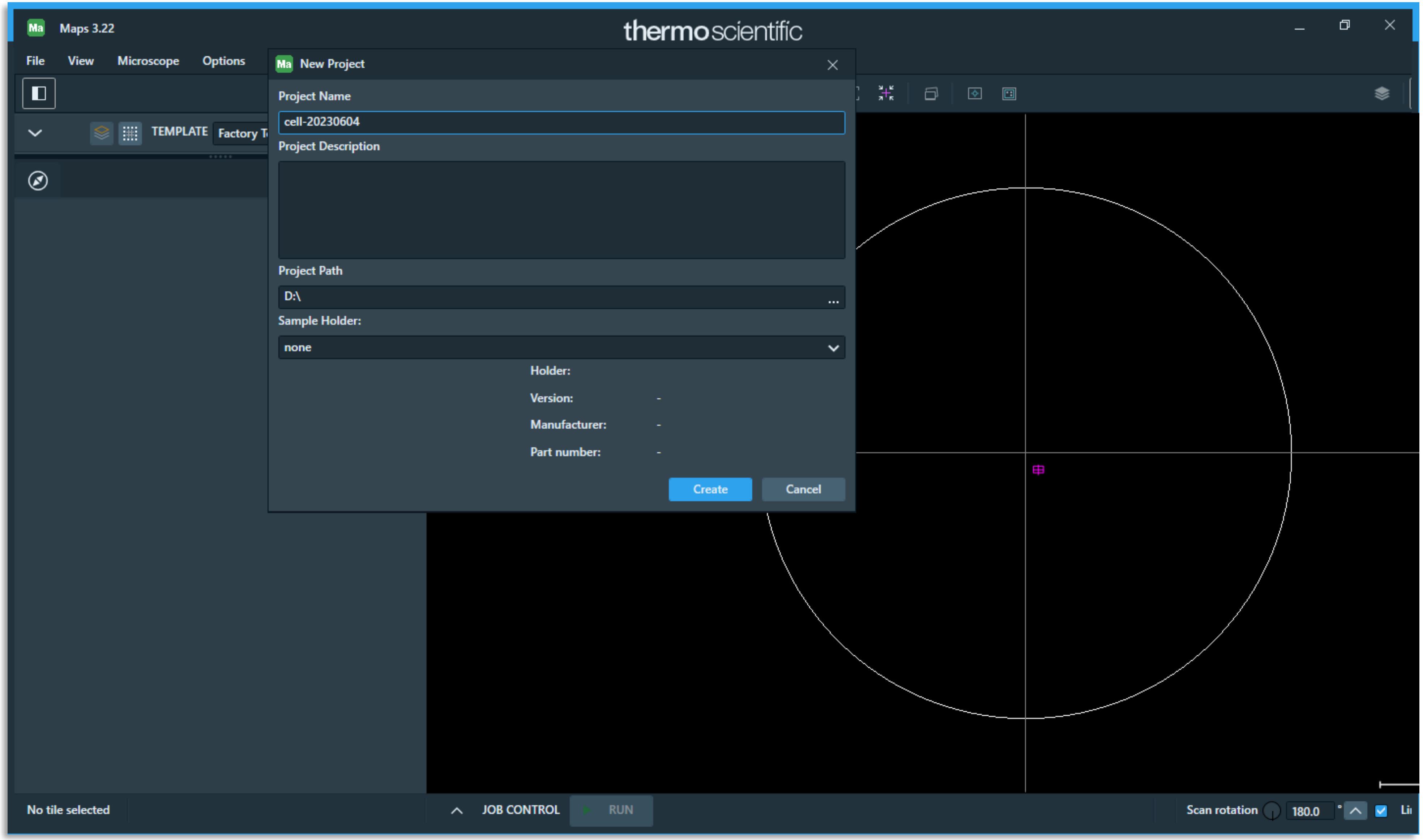
Lamella milling
Preparation, Milling, & thinning

iFLM (Optional)
Target confirmation

Pt sputter (Optional)
Lamella conductivity

↓ T

CryoET



3.3 Sample screening _Take a snapshoot of the grid

(Ma Maps)

Vitrification

↓

CryoFIB

Sample screening
Atlas & lamella sites

iFLM (Optional)
Target selection

Pt sputter
Sample conductivity

Pt GIS
Protective coating

Pt sputter (Optional)
Sample conductivity

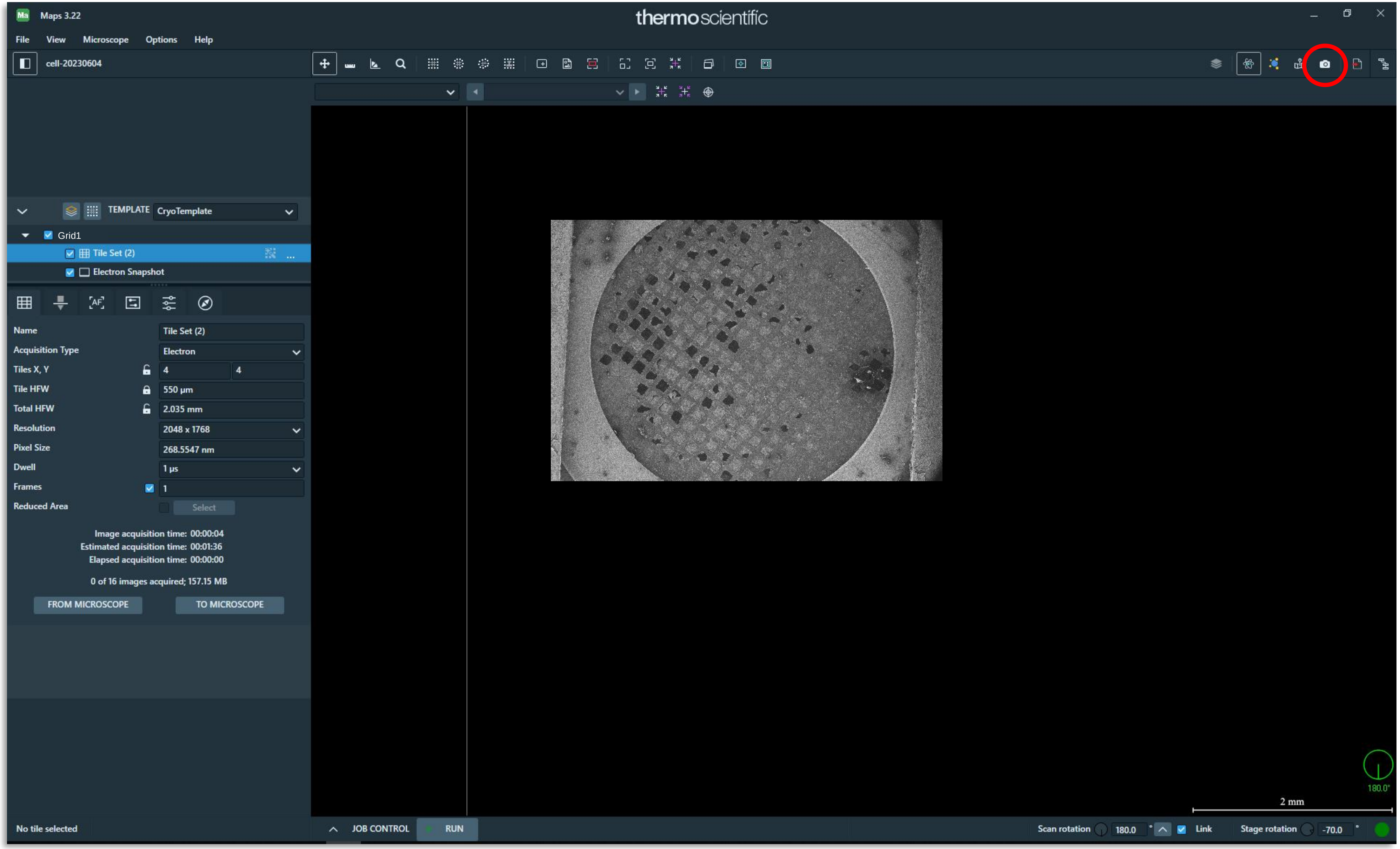
Lamella milling
Preparation, Milling, & thinning

iFLM (Optional)
Target confirmation

Pt sputter (Optional)
Lamella conductivity

↓

CryoET



3.3 Sample screening _Set up Tile Set & Run atlas acquisition (Ma Maps)

Vitrification

T

CryoFIB

Sample screening
Atlas & lamella sites

iFLM (Optional)
Target selection

Pt sputter
Sample conductivity

Pt GIS
Protective coating

Pt sputter (Optional)
Sample conductivity

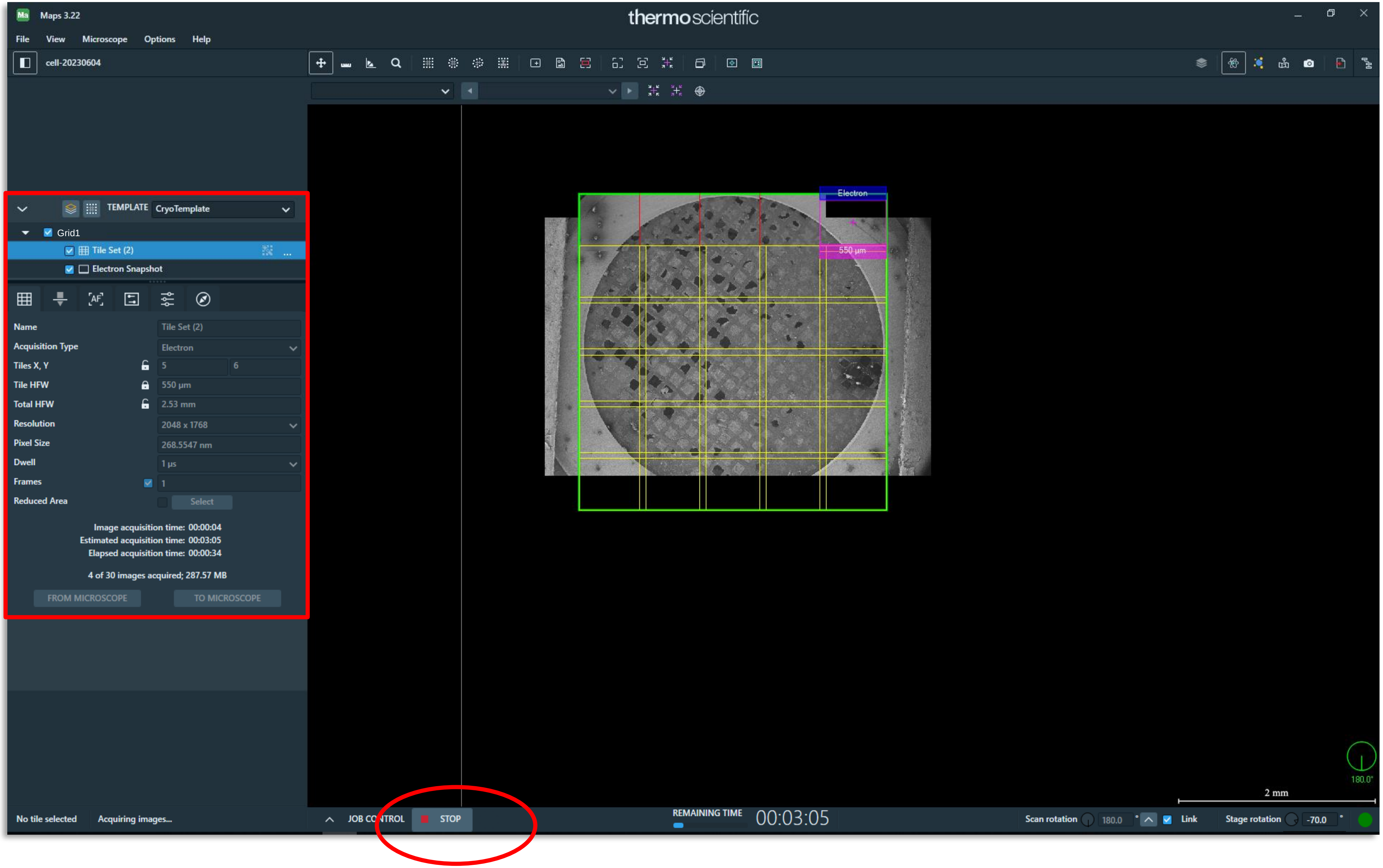
Lamella milling
Preparation, Milling, & thinning

iFLM (Optional)
Target confirmation

Pt sputter (Optional)
Lamella conductivity

T

CryoET



3.3 Sample screening _Add candidate lamella sites

(Ma Maps)

Vitrification

CryoFIB

Sample screening

Atlas & lamella sites

iFLM (Optional)

Target selection

Pt sputter

Sample conductivity

Pt GIS

Protective coating

Pt sputter (Optional)

Sample conductivity

Lamella milling

Preparation, Milling, & thinning

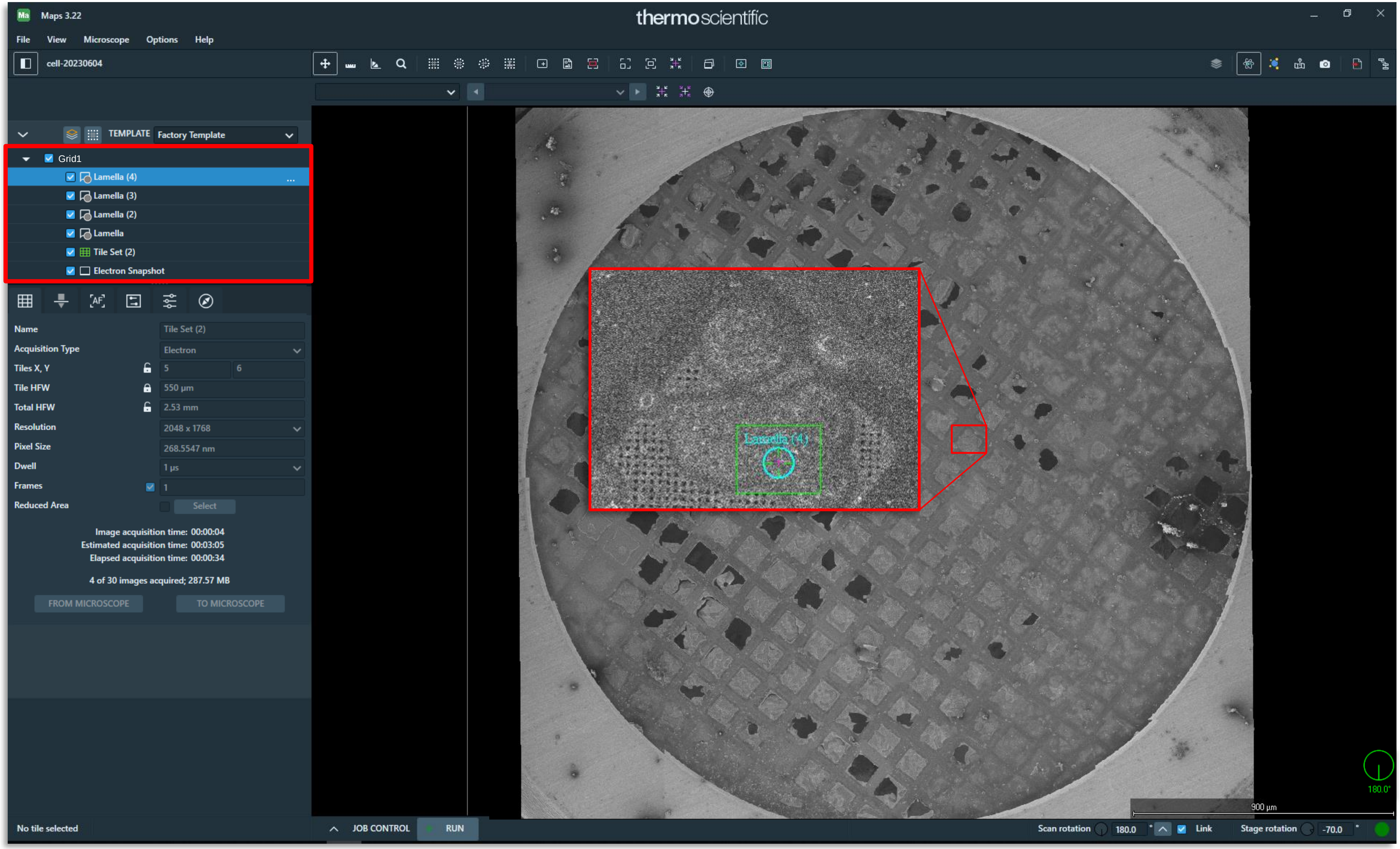
iFLM (Optional)

Target confirmation

Pt sputter (Optional)

Lamella conductivity

CryoET



3.4 CryoFLM for target selection

Vitrification

↓ T

CryoFIB

Sample screening
Atlas & lamella sites

iFLM (Optional)
Target selection

Pt sputter
Sample conductivity

Pt GIS
Protective coating

Pt sputter (Optional)
Sample conductivity

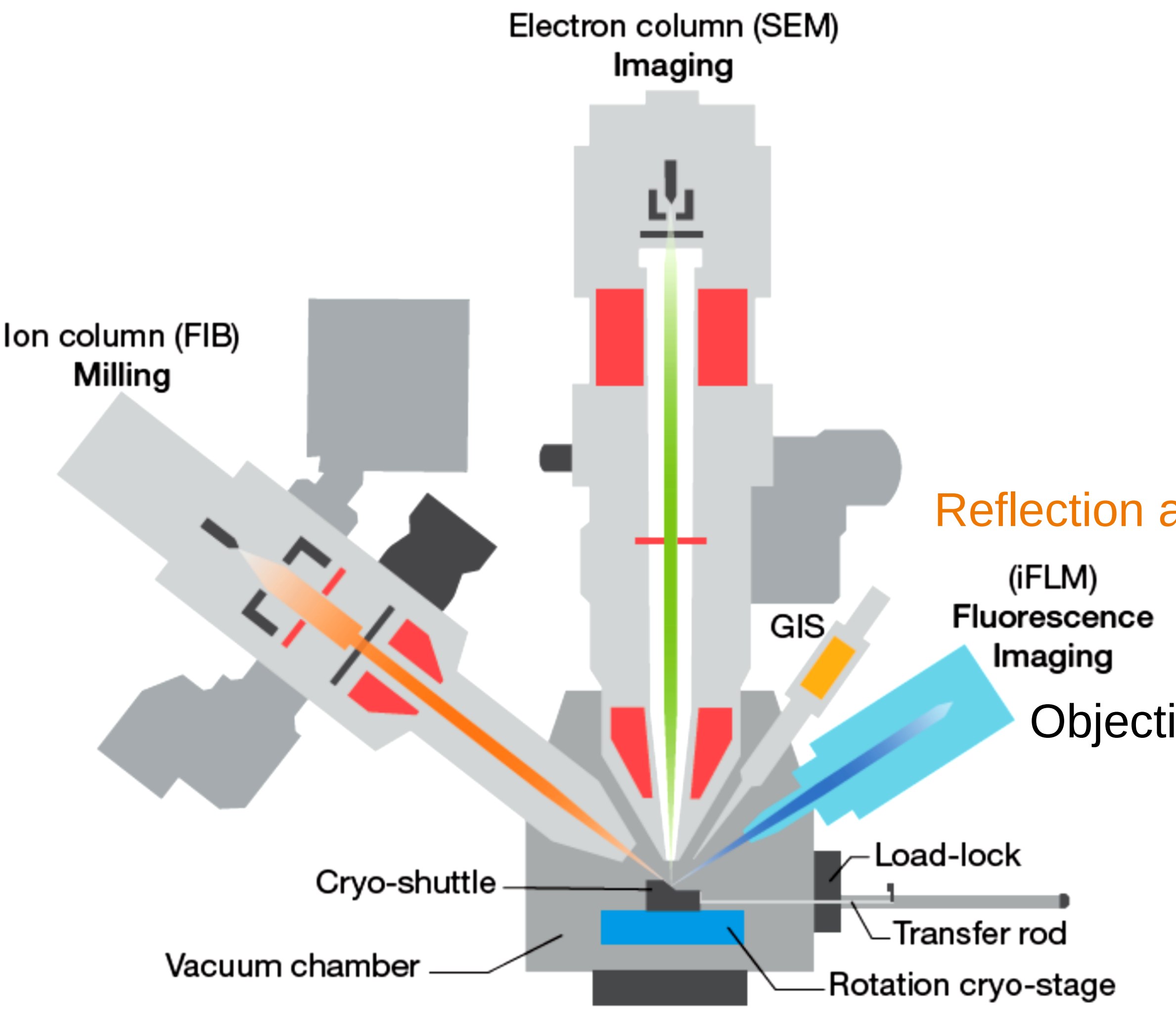
Lamella milling
Preparation, Milling, & thinning

iFLM (Optional)
Target confirmation

Pt sputter (Optional)
Lamella conductivity

↓ T

CryoET



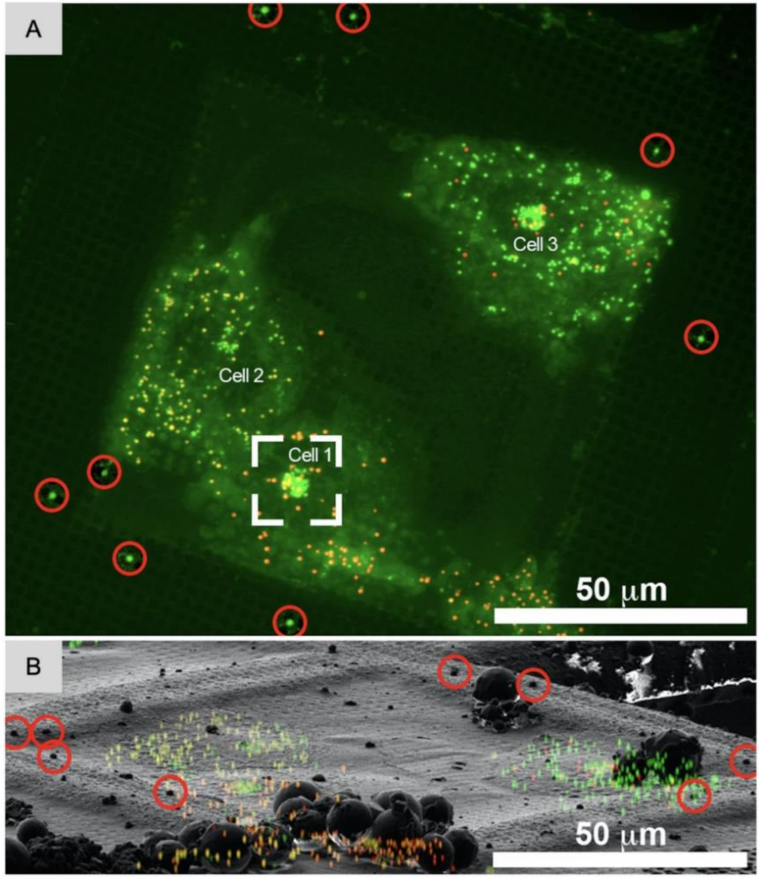
Reflection and fluorescence imaging

Objective: Piezo-driven, 20×, 0.7 N.A., minimum FOV ~350 μm

Adapted from TFS

Fiducial markers & X-Fiducials for accurate FLM-SEM alignment and target positioning

1-µm Magnetic beads

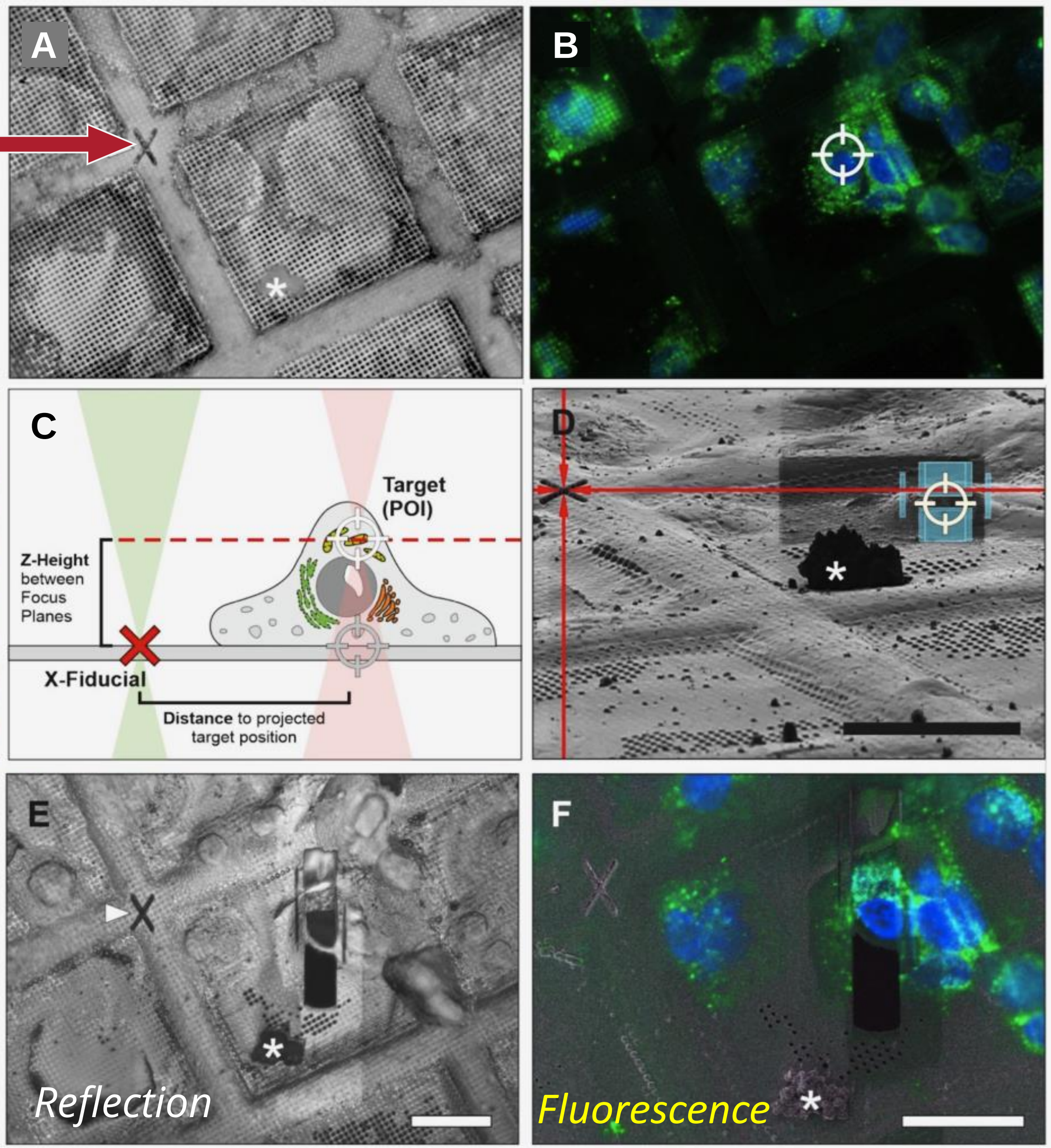


Arnold et al., Biophys J 2016

X-Fiducial

Before milling

After milling



Adapted from https://cryoem101.org/chapter-2-et/

3.4 CryoFLM _Focus with Objective

(Fluorescence Microscope Control)

Vitrification

↓ T

CryoFIB

Sample screening
Atlas & lamella sites

iFLM (Optional)
Target selection

Pt sputter
Sample conductivity

Pt GIS
Protective coating

Pt sputter (Optional)
Sample conductivity

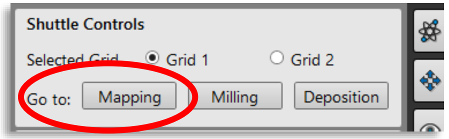
Lamella milling
Preparation, Milling, & thinning

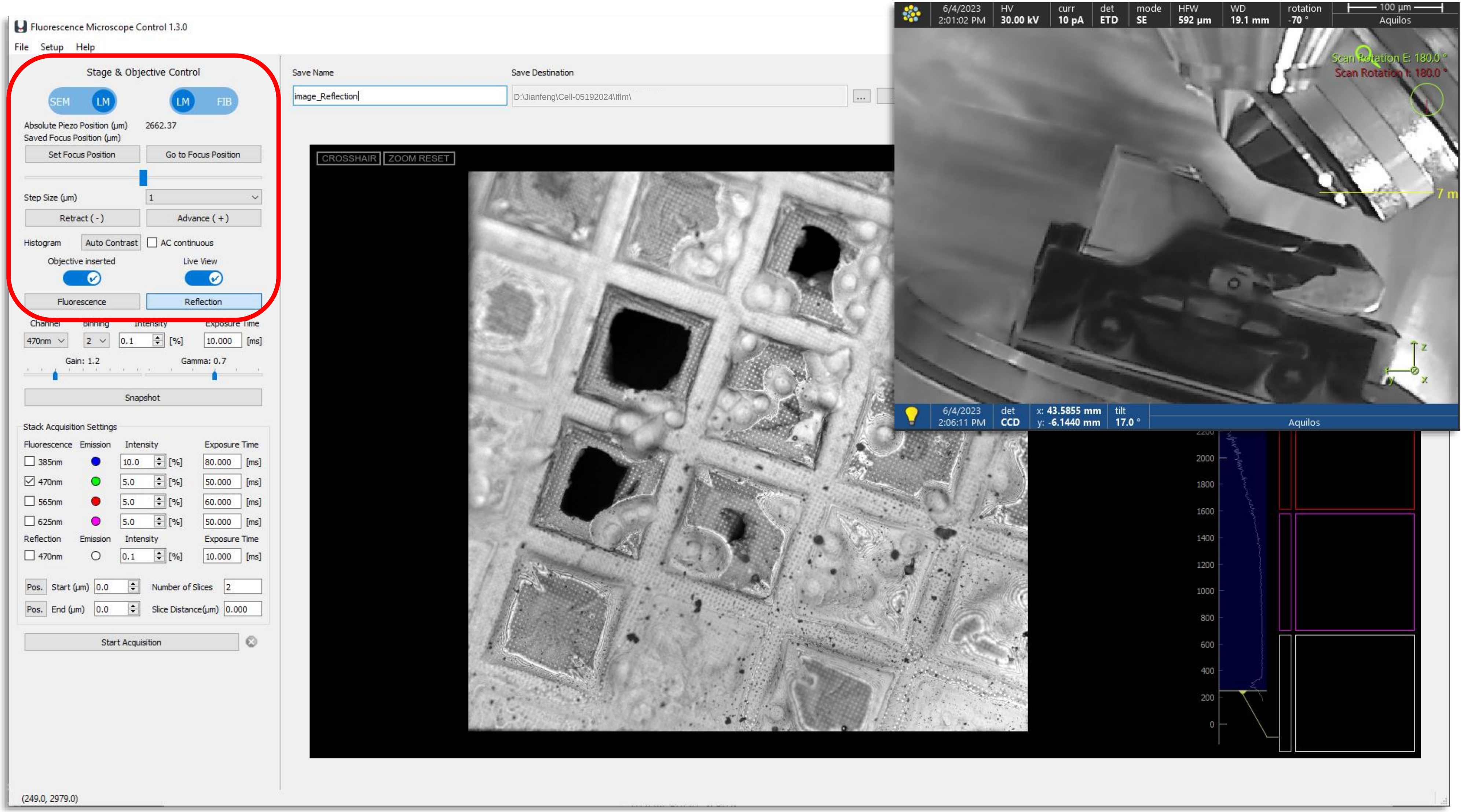
iFLM (Optional)
Target confirmation

Pt sputter (Optional)
Lamella conductivity

↓ T

CryoET





3.4 CryoFLM _Setting up imaging parameters (Fluorescence Microscope Control)

Vitrification

↓ T

CryoFIB

Sample screening
Atlas & lamella sites

iFLM (Optional)
Target selection

Pt sputter
Sample conductivity

Pt GIS
Protective coating

Pt sputter (Optional)
Sample conductivity

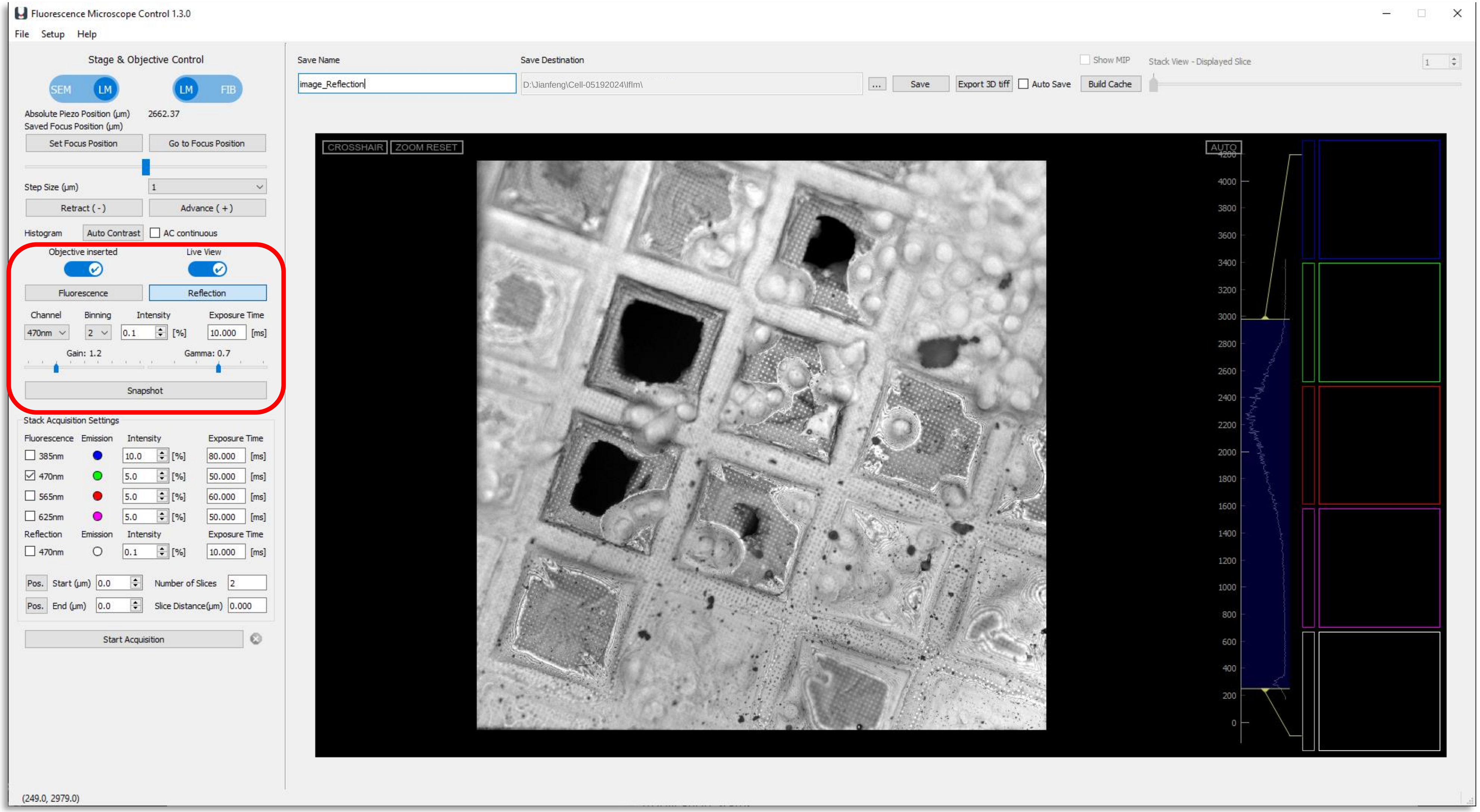
Lamella milling
Preparation, Milling,
& thinning

iFLM (Optional)
Target confirmation

Pt sputter (Optional)
Lamella conductivity

↓ T

CryoET



3.4 CryoFLM _Setting up imaging parameters (Fluorescence Microscope Control)

Vitrification

T

CryoFIB

Sample screening
Atlas & lamella sites

iFLM (Optional)
Target selection

Pt sputter
Sample conductivity

Pt GIS
Protective coating

Pt sputter (Optional)
Sample conductivity

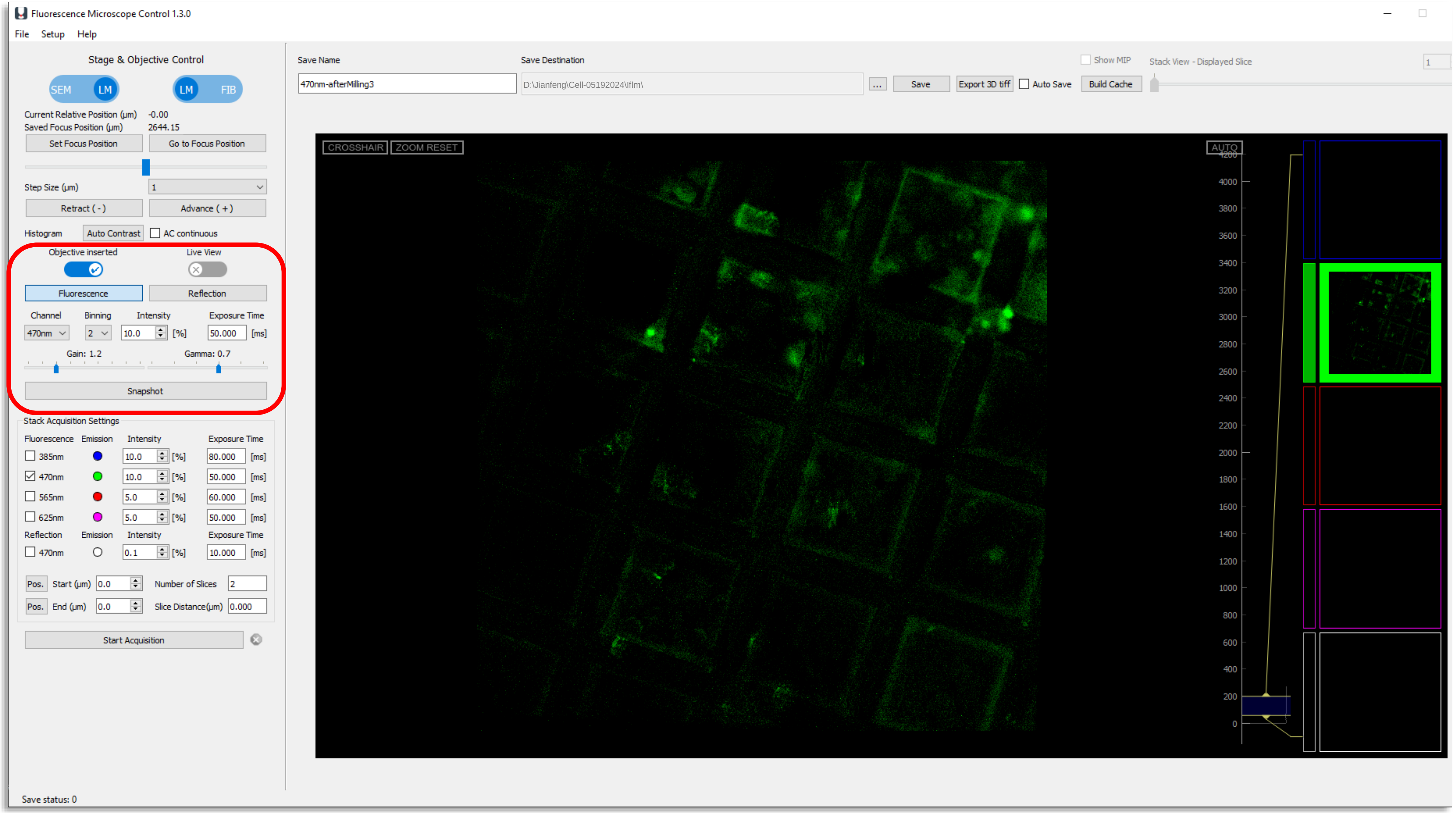
Lamella milling
Preparation, Milling, & thinning

iFLM (Optional)
Target confirmation

Pt sputter (Optional)
Lamella conductivity

T

CryoET



3.4 CryoFLM _Setting up Z-stack

(Fluorescence Microscope Control)

Vitrification

T

CryoFIB

Sample screening

Atlas & lamella sites

iFLM (Optional)

Target selection

Pt sputter

Sample conductivity

Pt GIS

Protective coating

Pt sputter (Optional)

Sample conductivity

Lamella milling

Preparation, Milling, & thinning

iFLM (Optional)

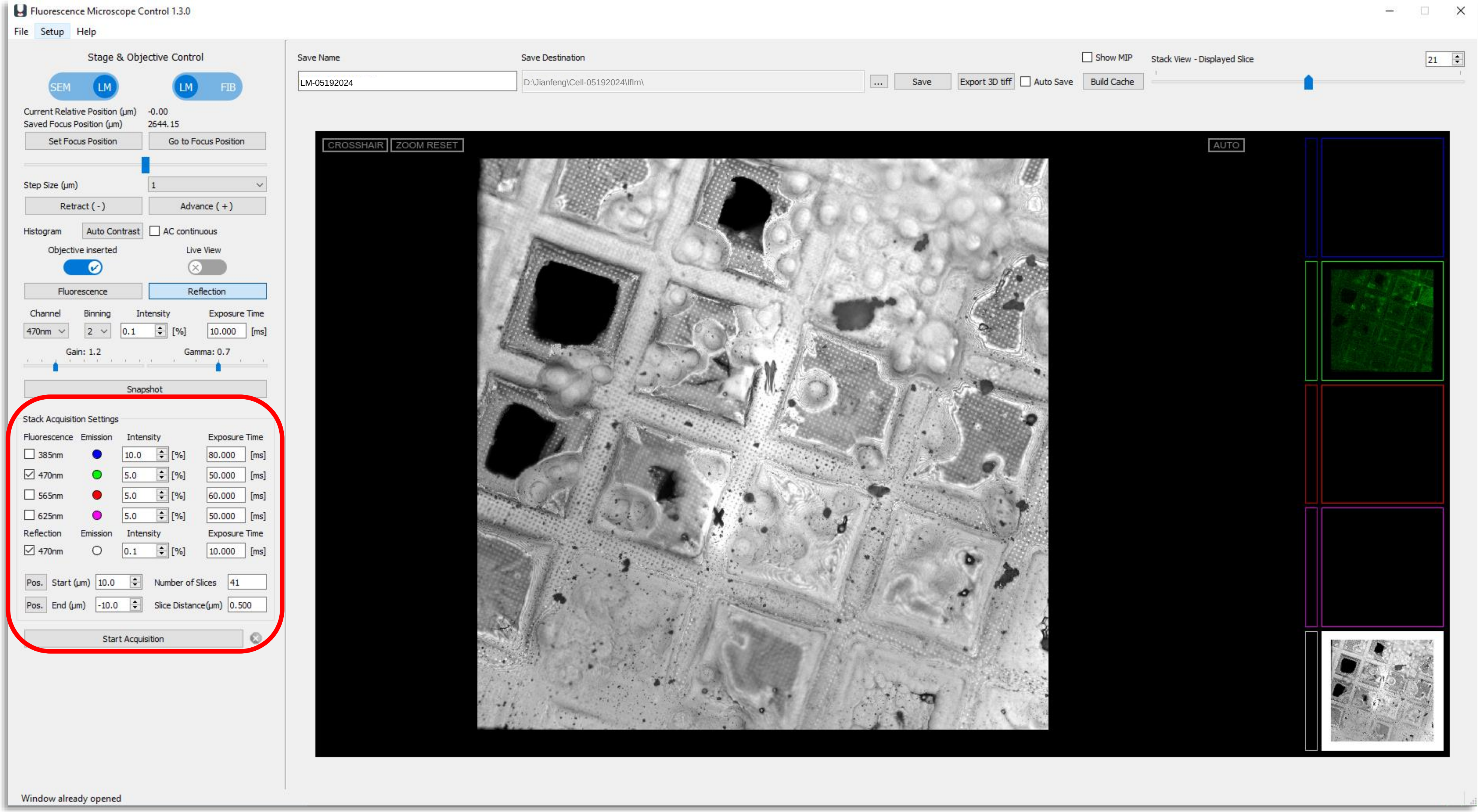
Target confirmation

Pt sputter (Optional)

Lamella conductivity

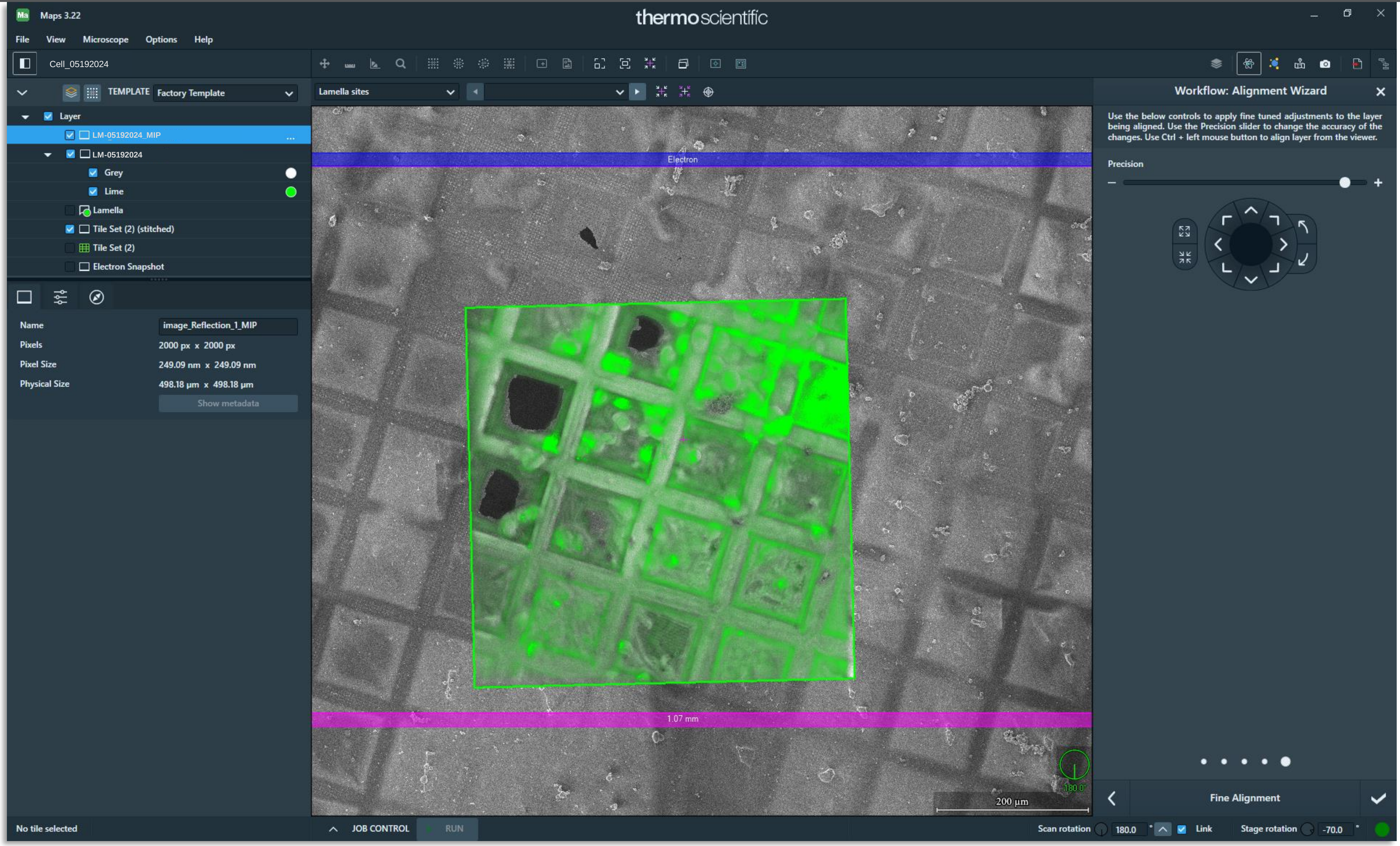
T

CryoET



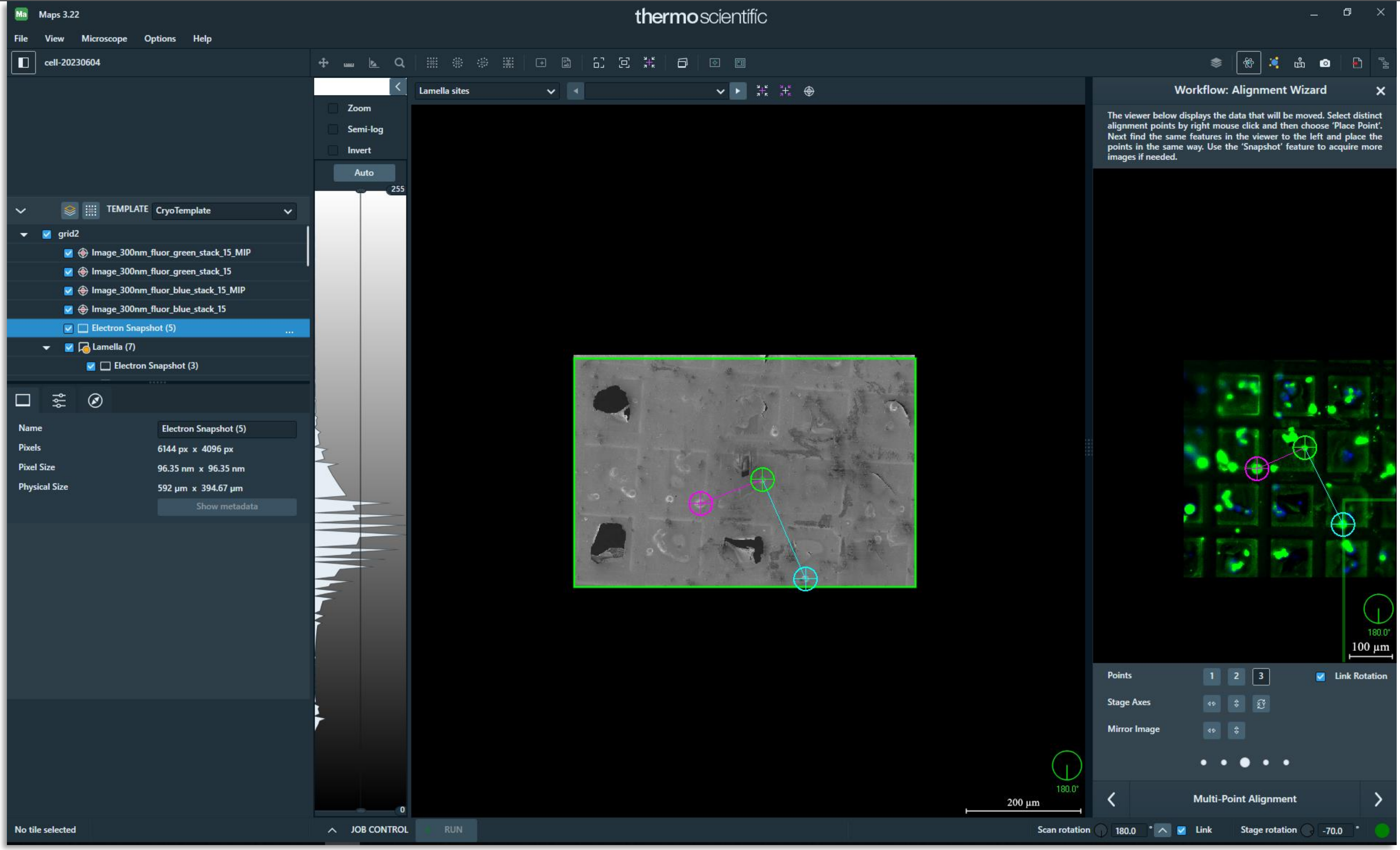
3.4 CryoFLM _FLM-SEM alignment by Fine Alignment

(Maps)



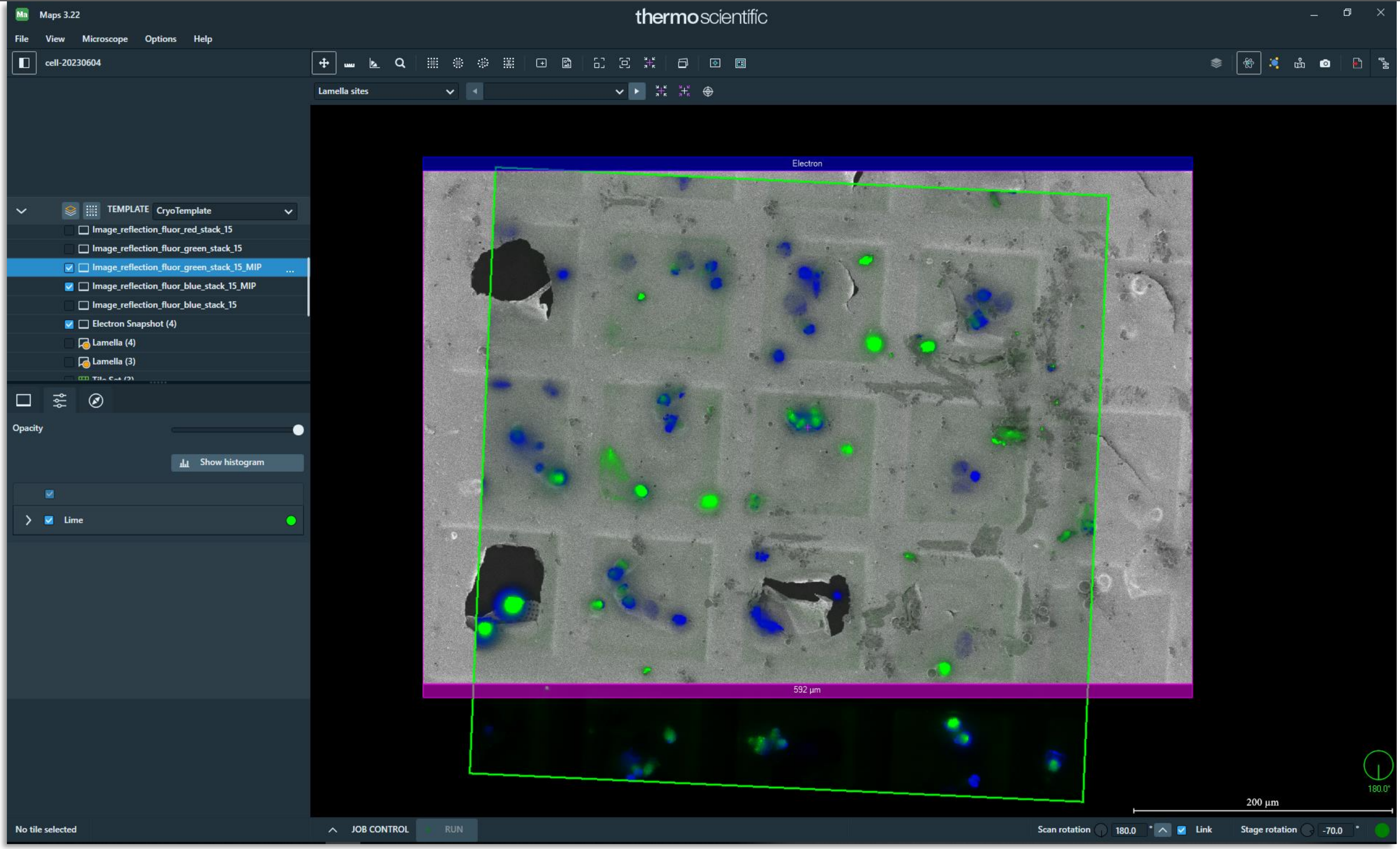
3.4 CryoFLM _FLM-SEM alignment by Multi-Point Alignment

(Ma Maps)



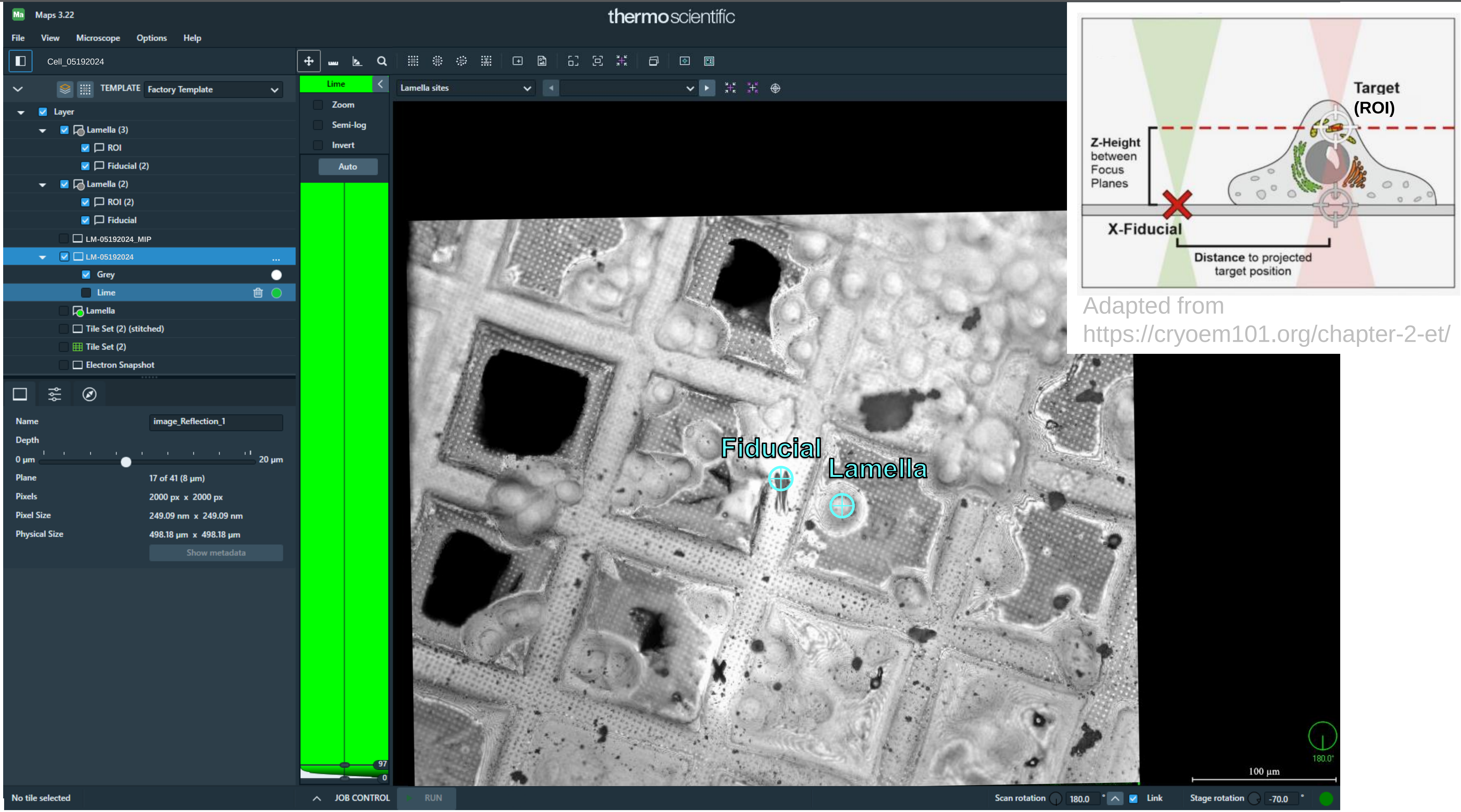
3.4 CryoFLM _FLM-SEM alignment by Multi-Point Alignment

(Ma Maps)



3.4 CryoFLM _Define Fiducial & ROI markers for each lamella

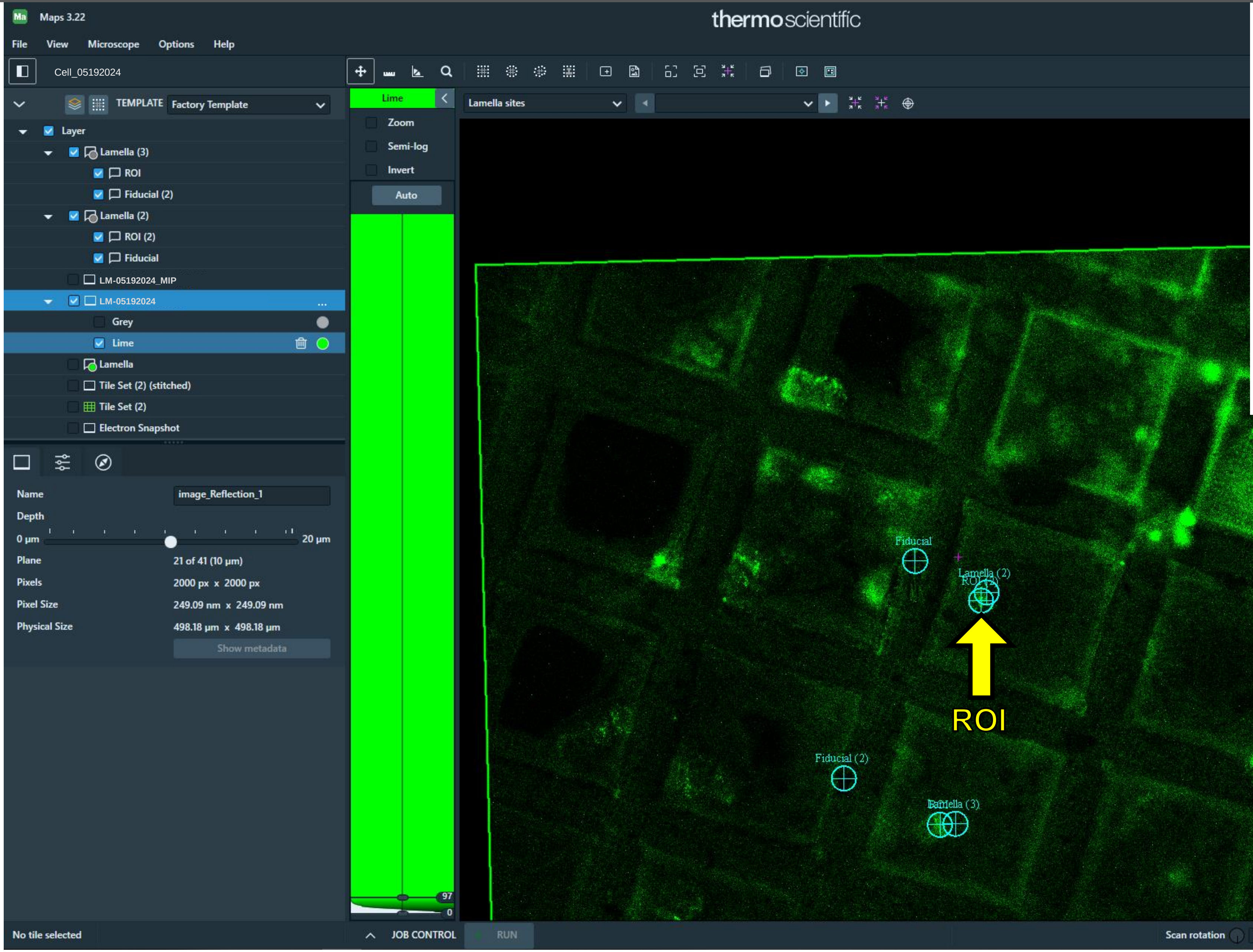
(Maps)

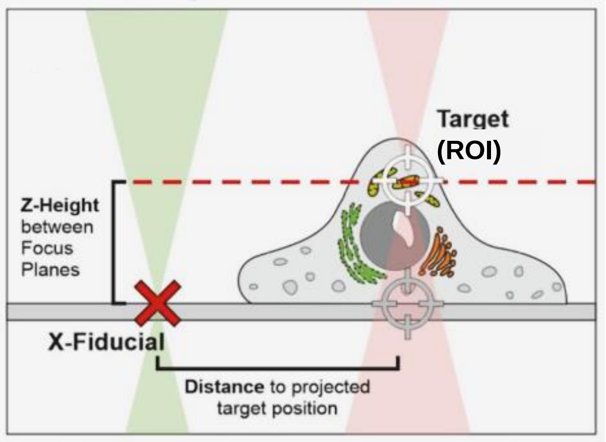


Adapted from
https://cryoem101.org/chapter-2-et/

3.4 CryoFLM _Define Fiducial & ROI markers for each lamella

(Ma Maps)





Adapted from
https://cryoem101.org/chapter-2-et/

3.5 Pt sputter

Vitrification

↓ **T**

CryoFIB

Sample screening
Atlas & lamella sites

iFLM (Optional)
Target selection

Pt sputter
Sample conductivity

Pt GIS
Protective coating

Pt sputter (Optional)
Sample conductivity

Lamella milling
Preparation, Milling, & thinning

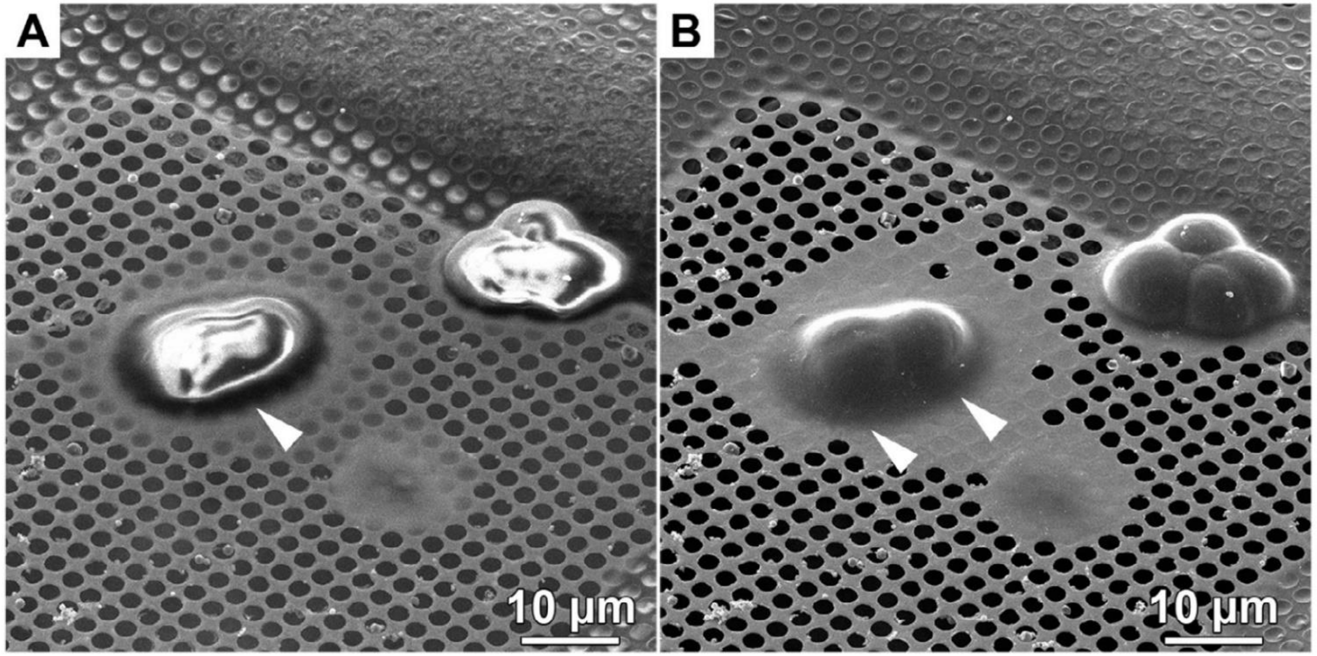
iFLM (Optional)
Target confirmation

Pt sputter (Optional)
Lamella conductivity

↓ **T**

CryoET

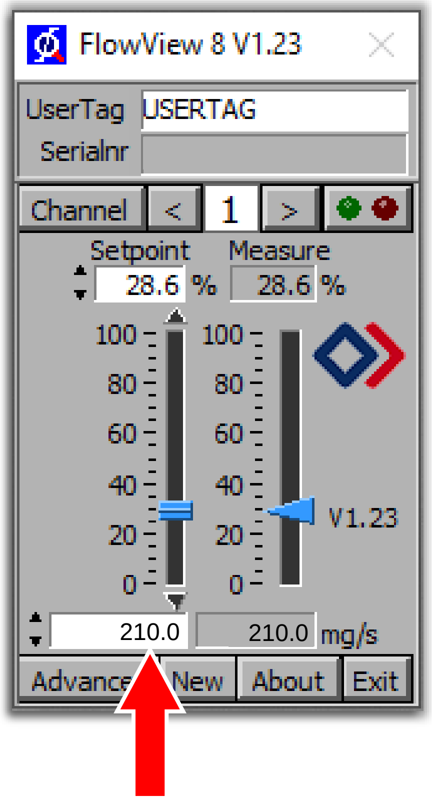
(Optional) Inorganic Pt minimizes charging, ensure targeting and precise milling.



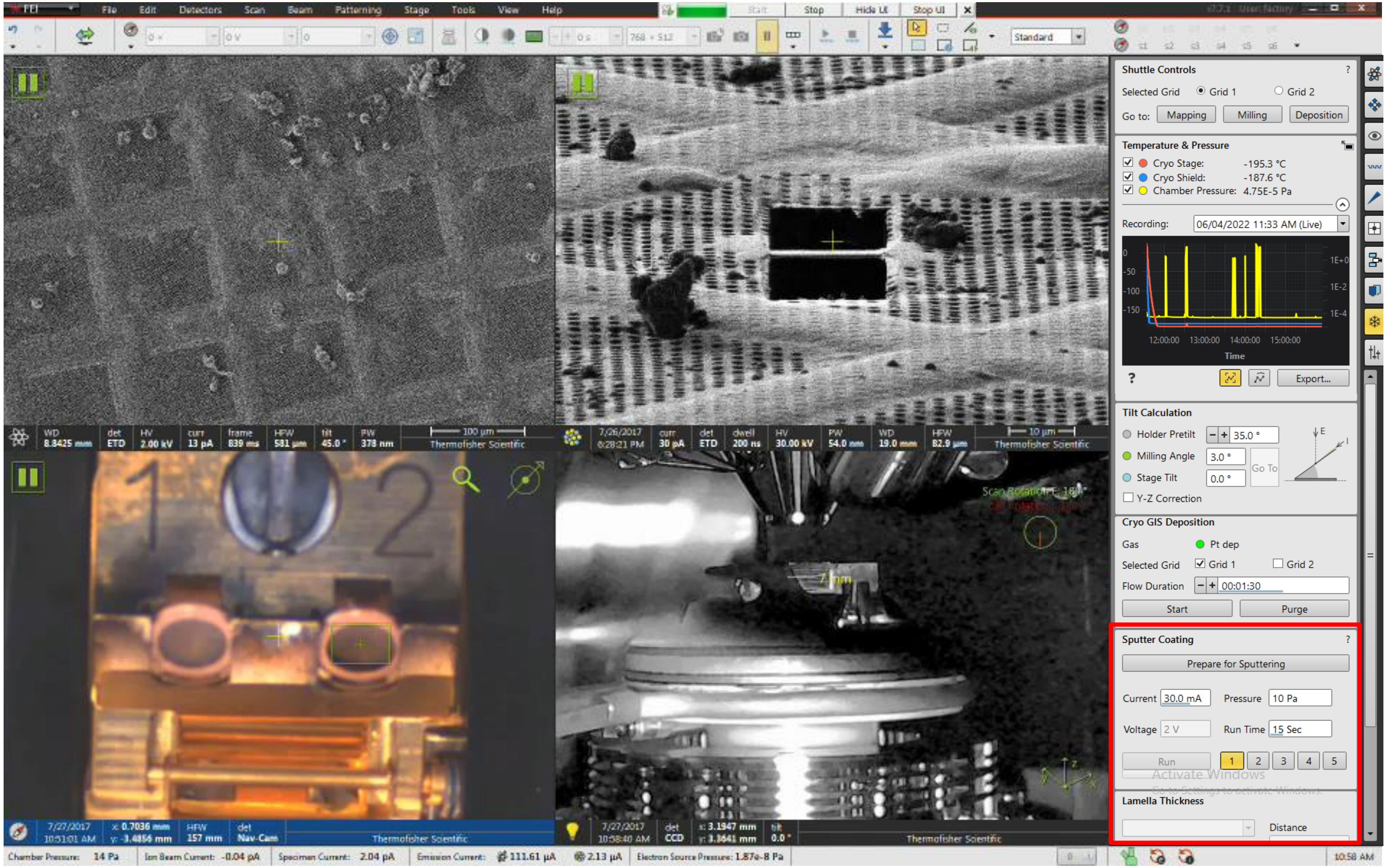
Schaffer et al., JSB 2017

3.5 Pt sputter

(Video from TFS; xT Microscope Control)



Increase to 210 mg/s
(varies by instruments)



3.5 Pt GIS

(xT Microscope Control)

Vitrification

T

CryoFIB

Sample screening
Atlas & lamella sites

iFLM (Optional)
Target selection

Pt sputter
Sample conductivity

Pt GIS
Protective coating

Pt sputter (Optional)
Sample conductivity

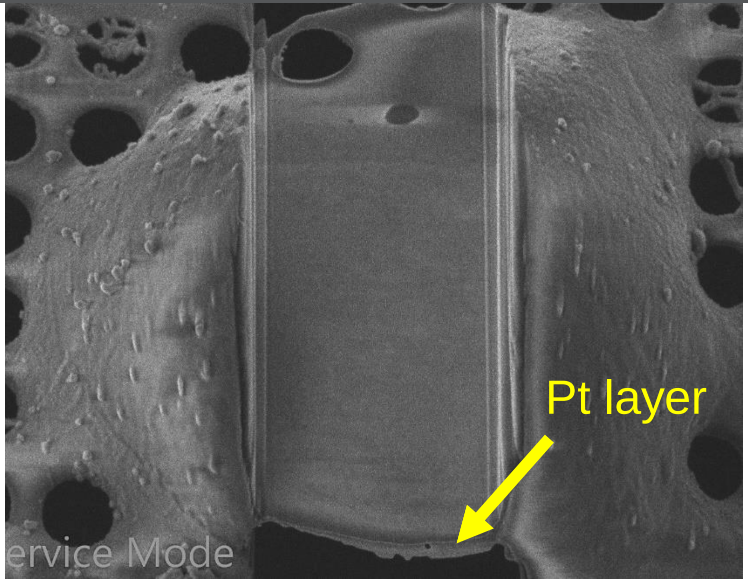
Lamella milling
Preparation, Milling, & thinning

iFLM (Optional)
Target confirmation

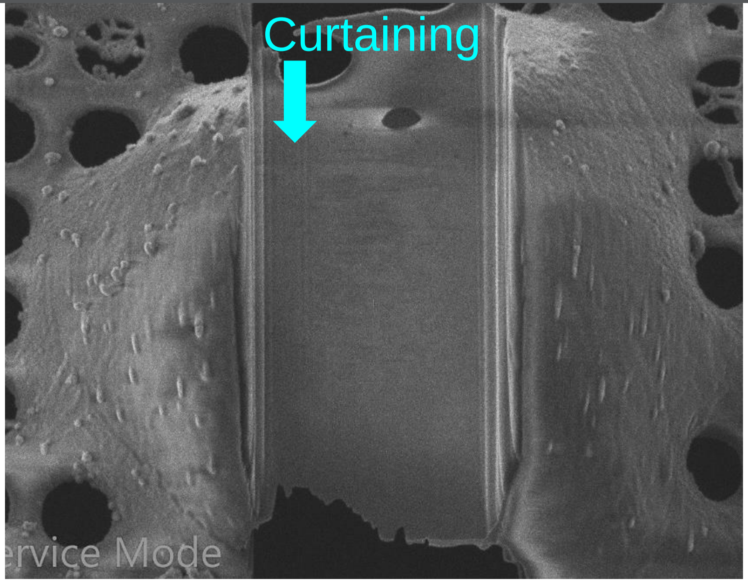
Pt sputter (Optional)
Lamella conductivity

T

CryoET

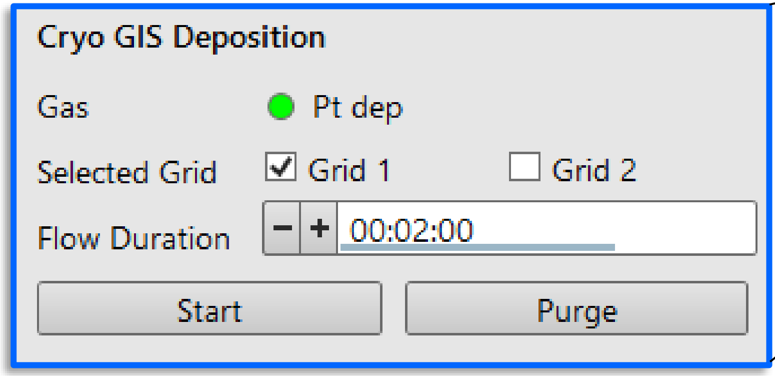


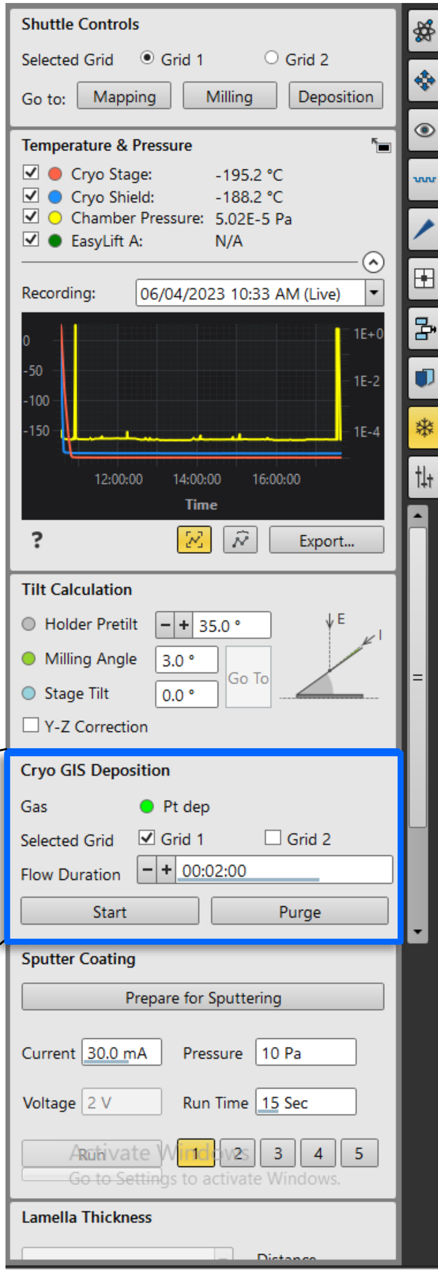
With an intact Pt layer



With a broken Pt layer

Organometallic Pt protects sample surface during milling, minimize curtaining and redeposition.





Duration varies by samples / instruments

3.6 Pt sputter (Optional)

(xT Microscope Control)

Vitrification

T

CryoFIB

Sample screening
Atlas & lamella sites

iFLM (Optional)
Target selection

Pt sputter
Sample conductivity

Pt GIS
Protective coating

Pt sputter (Optional)
Sample conductivity

Lamella milling
Preparation, Milling, & thinning

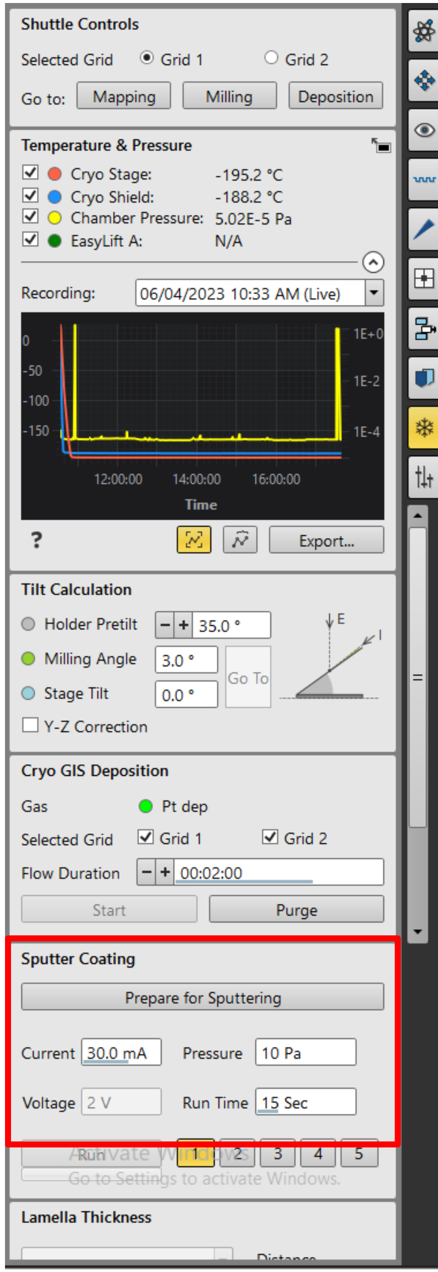
iFLM (Optional)
Target confirmation

Pt sputter (Optional)
Lamella conductivity

T

CryoET

> (Optional) Inorganic Pt minimizes charging, ensure targeting and precise milling.



Vitrification

T

CryoFIB

Sample screening
Atlas & lamella sites

iFLM (Optional)
Target selection

Pt sputter
Sample conductivity

Pt GIS
Protective coating

Pt sputter (Optional)
Sample conductivity

Lamella milling
Preparation, Milling, & thinning

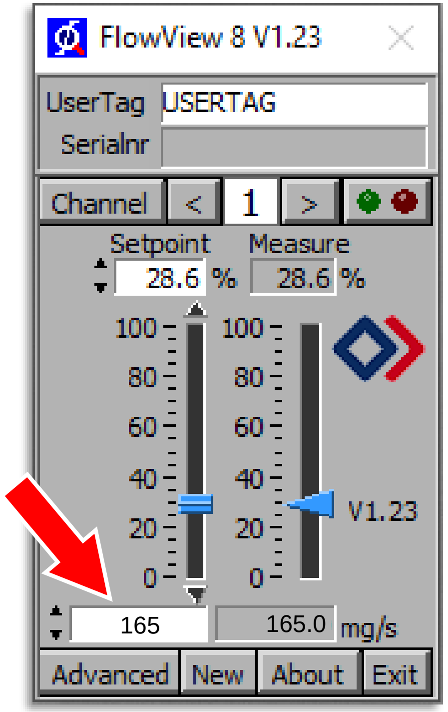
iFLM (Optional)
Target confirmation

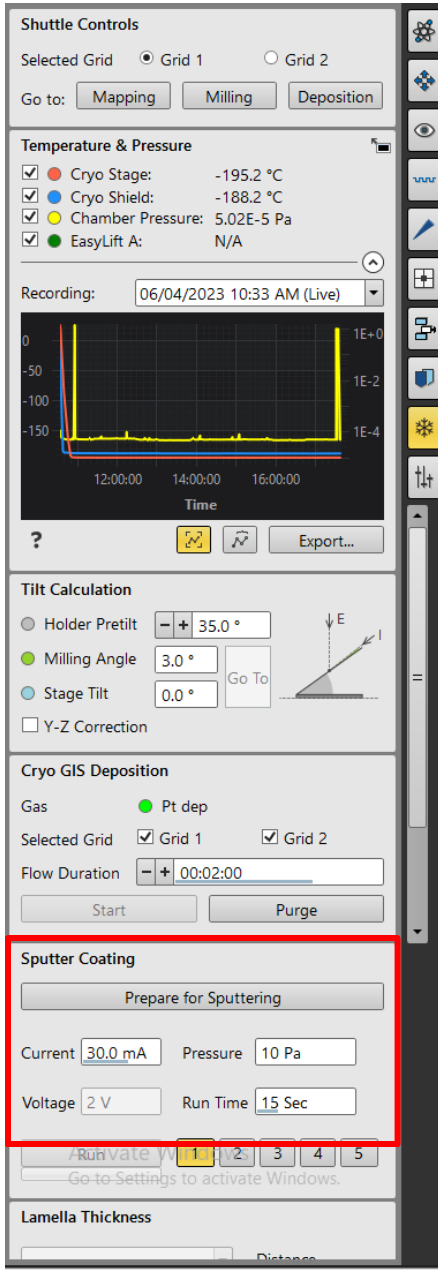
Pt sputter (Optional)
Lamella conductivity

T

CryoET

Upon completion, reduce to 165 mg/s. (varies by instruments)





3.7 Automated lamella milling using AutoTEM Cryo AT

Vitrification

T

CryoFIB

Sample screening
Atlas & lamella sites

iFLM (Optional)
Target selection

Pt sputter
Sample conductivity

Pt GIS
Protective coating

Pt sputter (Optional)
Sample conductivity

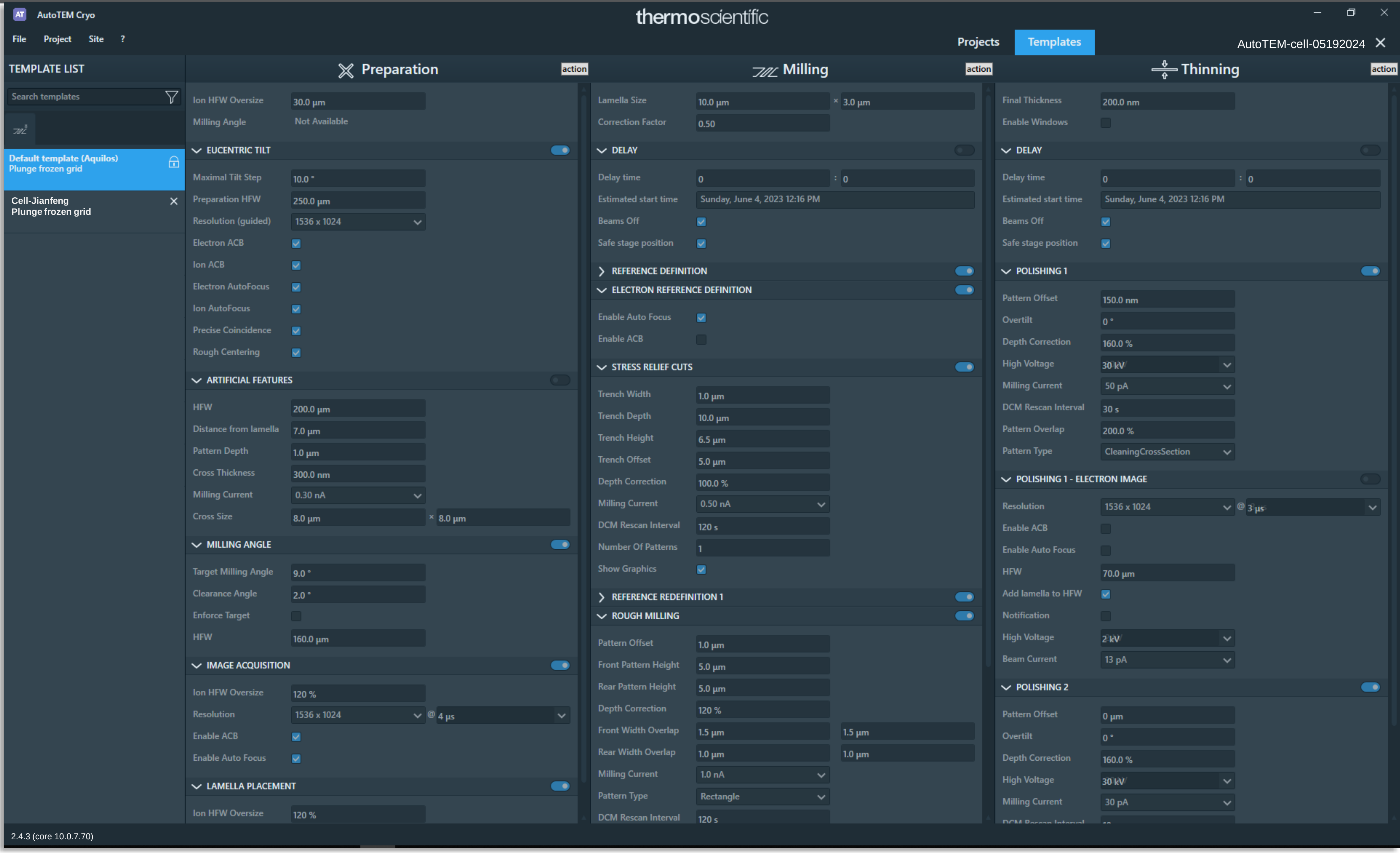
Lamella milling
Preparation, Milling, & thinning

iFLM (Optional)
Target confirmation

Pt sputter (Optional)
Lamella conductivity

T

CryoET



Eucentric height & tilt calculation

(AT AutoTEM Cryo)

Vitrification

T

CryoFIB

Sample screening

Atlas & lamella sites

iFLM (Optional)

Target selection

Pt sputter

Sample conductivity

Pt GIS

Protective coating

Pt sputter (Optional)

Sample conductivity

Lamella milling

Preparation, Milling, & thinning

iFLM (Optional)

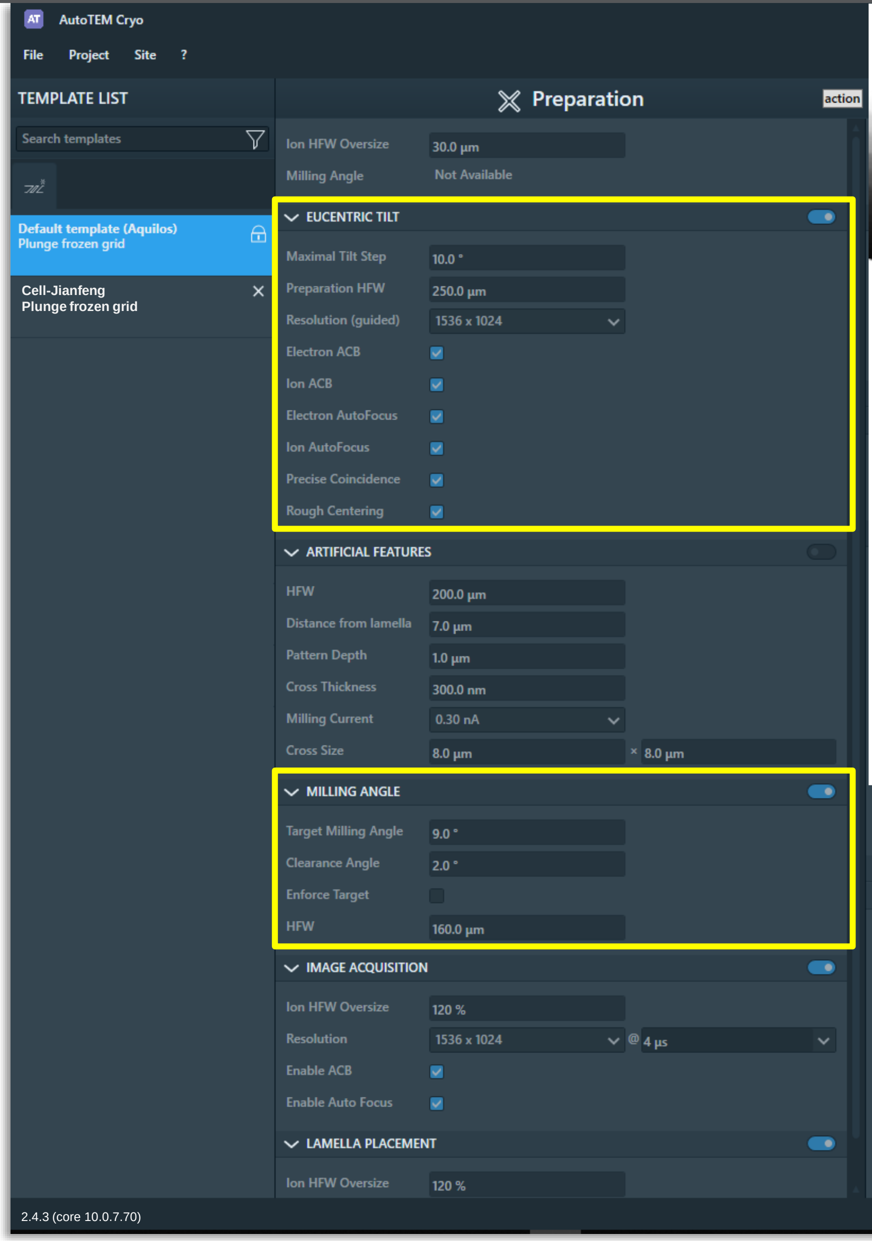
Target confirmation

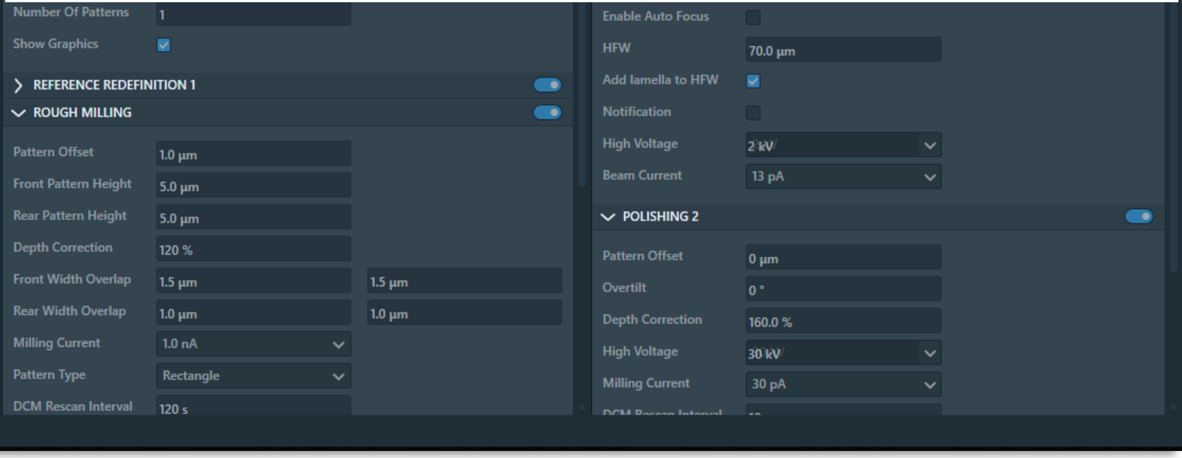
Pt sputter (Optional)

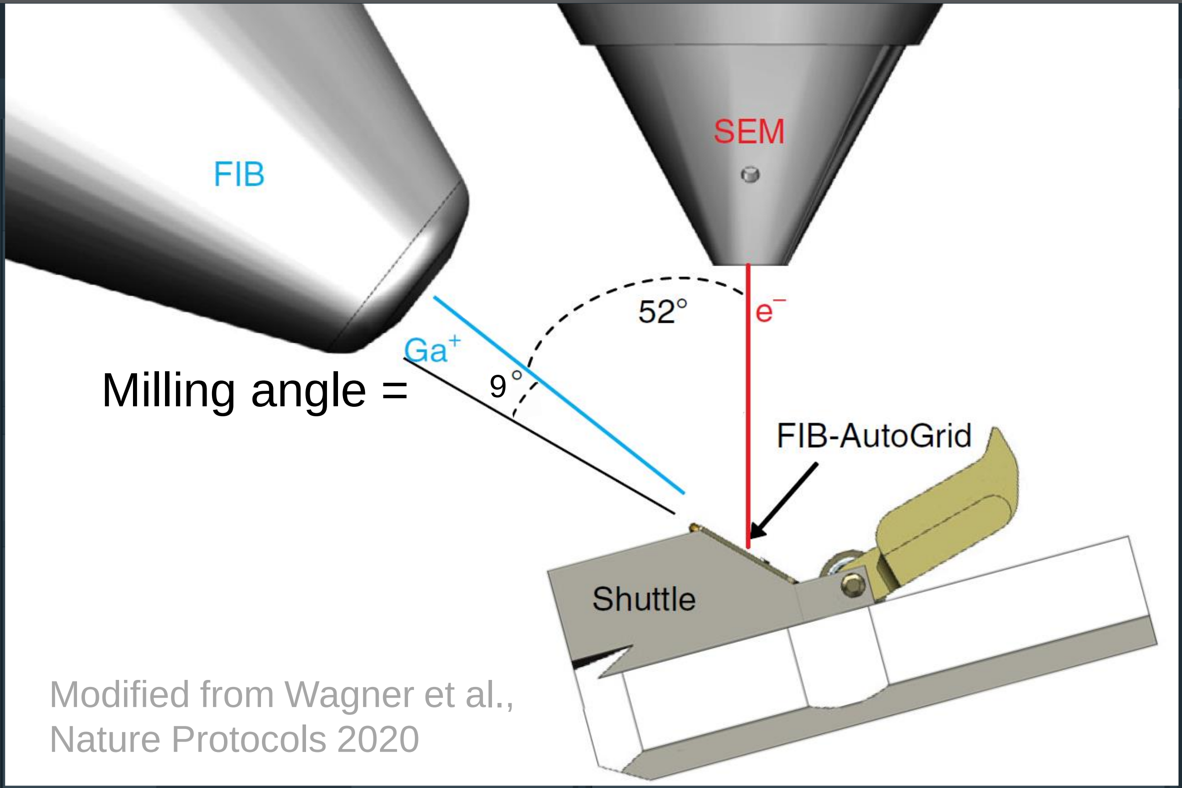
Lamella conductivity

T

CryoET







Modified from Wagner et al., Nature Protocols 2020

Artificial Features (Optional)

(AT AutoTEM Cryo)

Vitrification

↓ T

CryoFIB

Sample screening
Atlas & lamella sites

iFLM (Optional)
Target selection

Pt sputter
Sample conductivity

Pt GIS
Protective coating

Pt sputter (Optional)
Sample conductivity

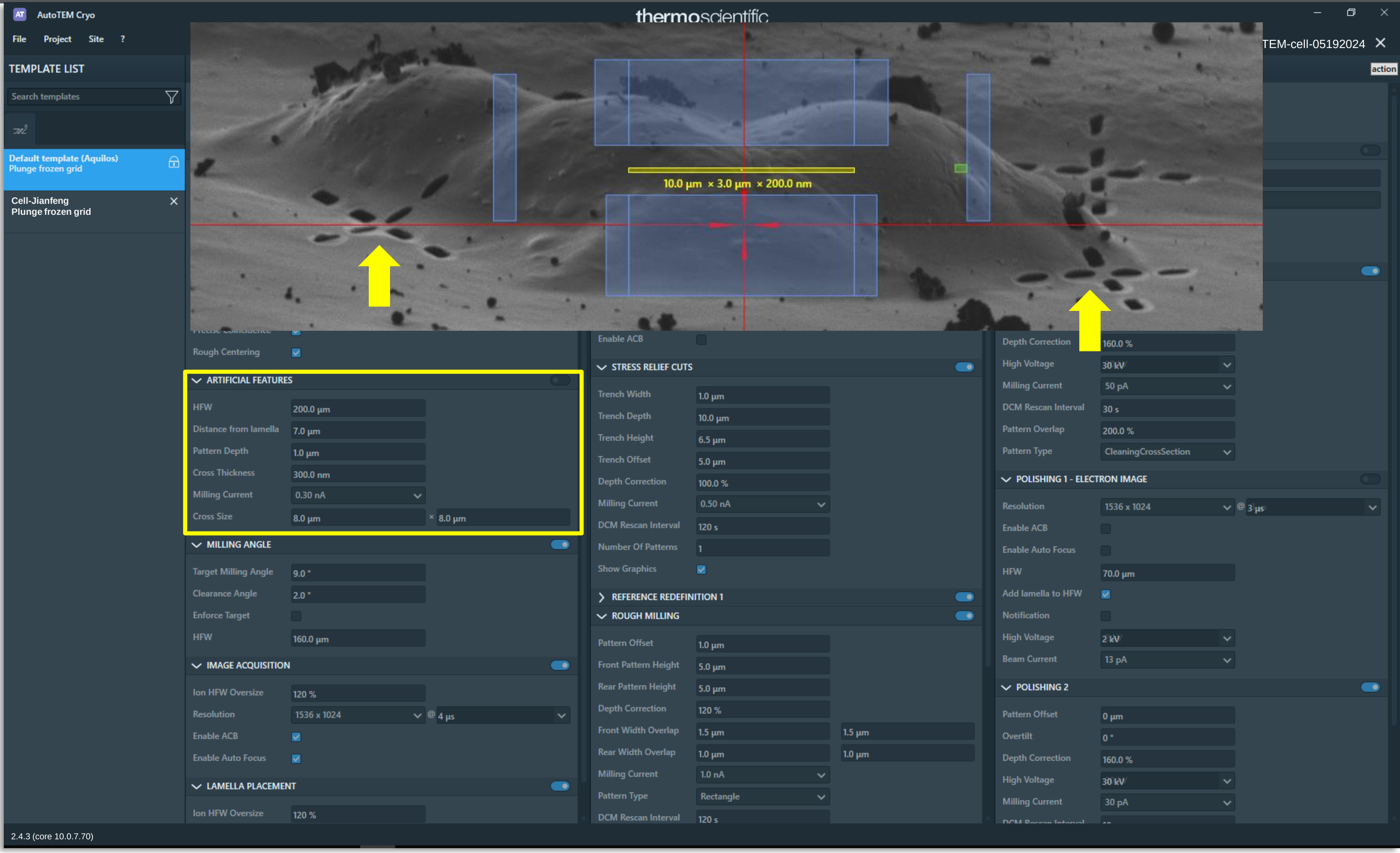
Lamella milling
Preparation, Milling,
& thinning

iFLM (Optional)
Target confirmation

Pt sputter (Optional)
Lamella conductivity

↓ T

CryoET



Micro-expansion joints

(AT AutoTEM Cryo)

Vitrification

T

CryoFIB

Sample screening

Atlas & lamella sites

iFLM (Optional)

Target selection

Pt sputter

Sample conductivity

Pt GIS

Protective coating

Pt sputter (Optional)

Sample conductivity

Lamella milling

Preparation, Milling, & thinning

iFLM (Optional)

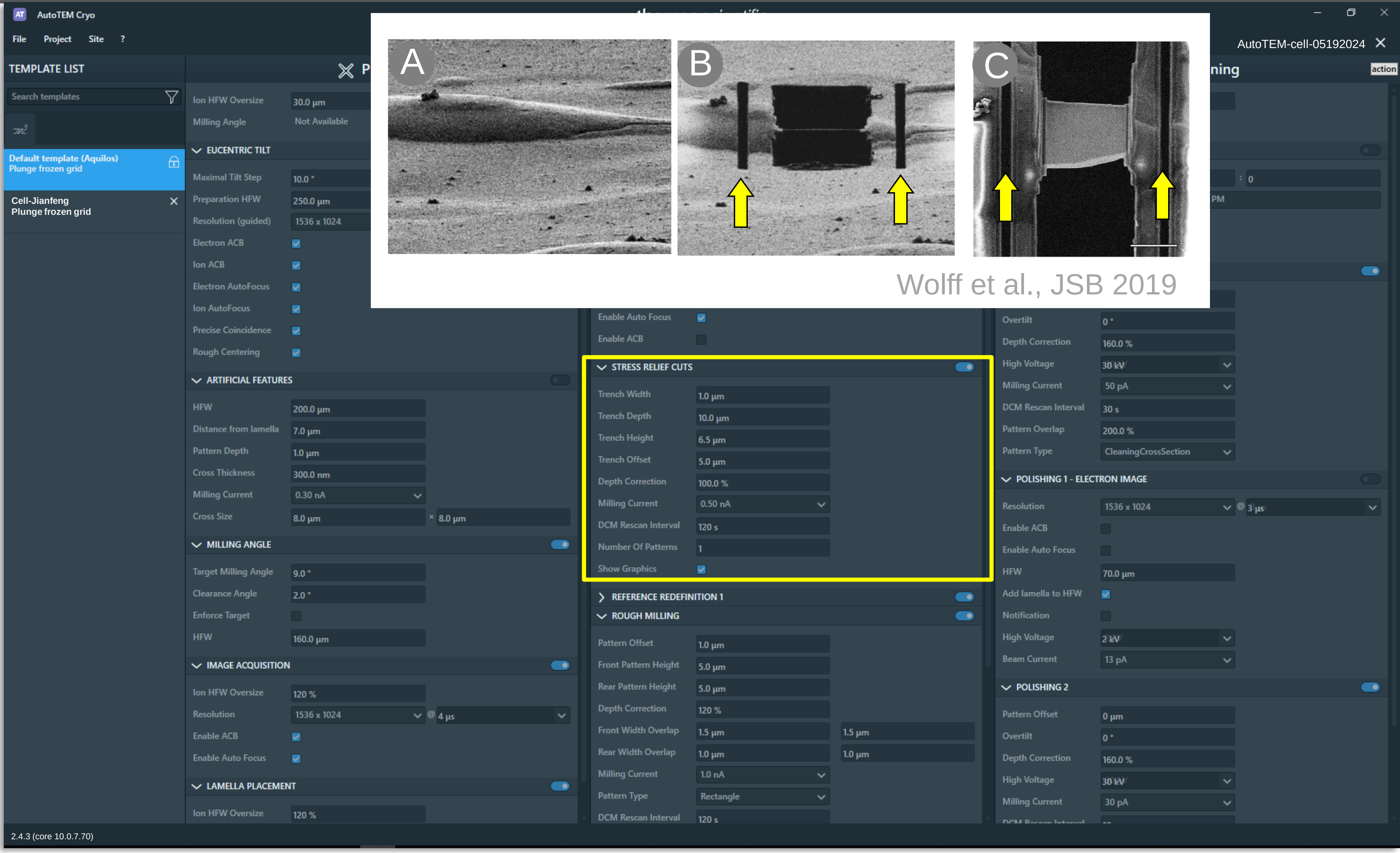
Target confirmation

Pt sputter (Optional)

Lamella conductivity

T

CryoET



Wolff et al., JSB 2019

Stepwise milling procedure

(AT AutoTEM Cryo)

Vitrification

↓ T

CryoFIB

Sample screening
Atlas & lamella sites

iFLM (Optional)
Target selection

Pt sputter
Sample conductivity

Pt GIS
Protective coating

Pt sputter (Optional)
Sample conductivity

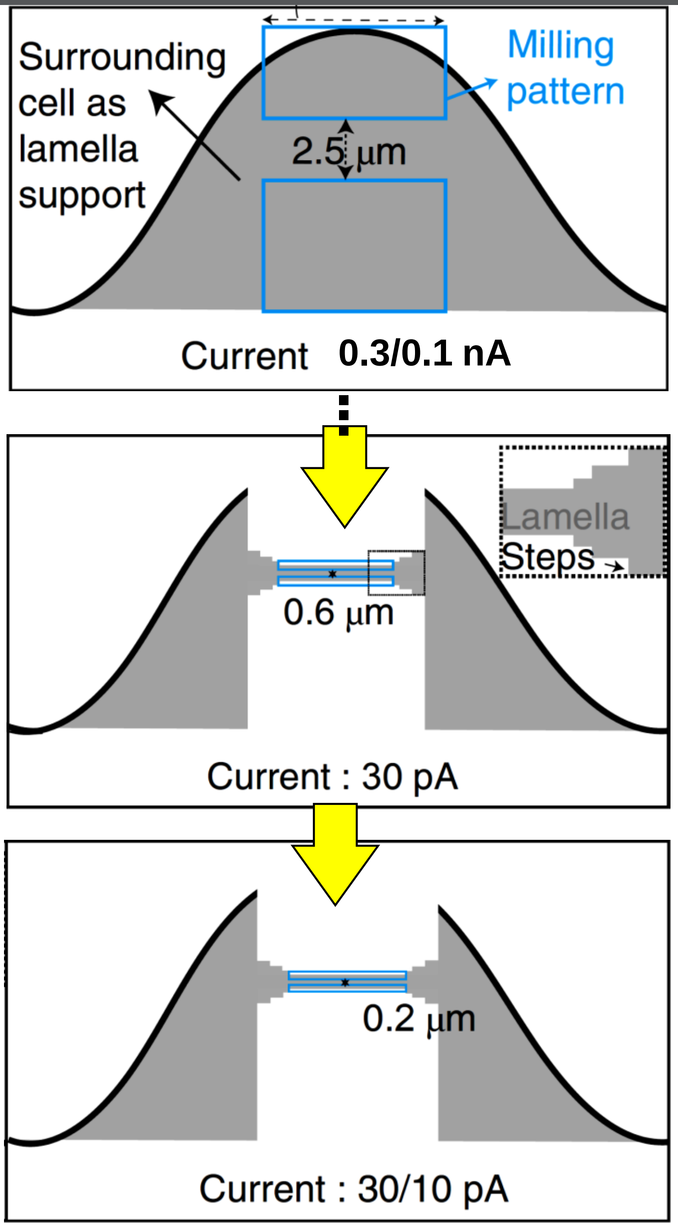
Lamella milling
Preparation, Milling, & thinning

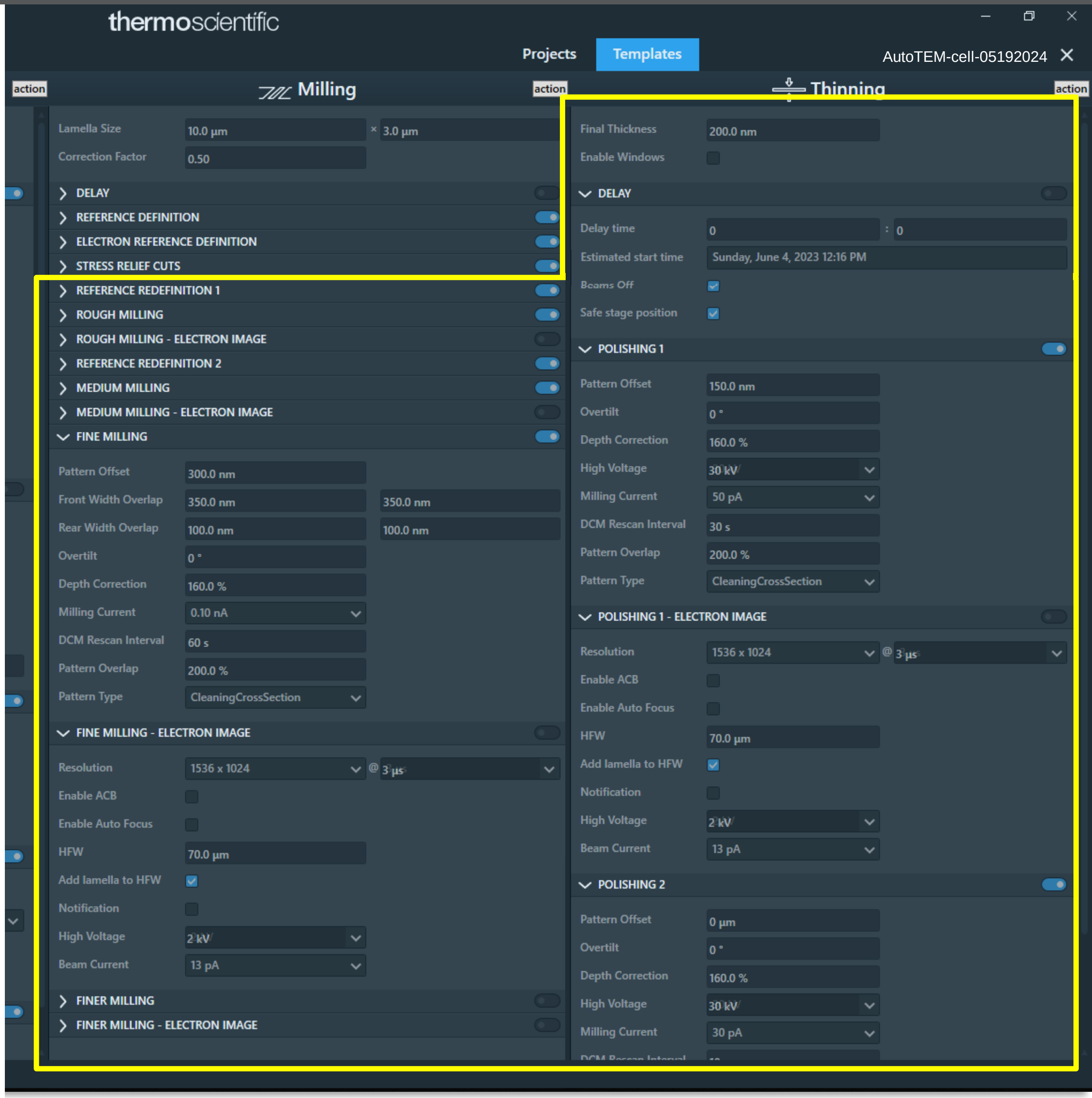
iFLM (Optional)
Target confirmation

Pt sputter (Optional)
Lamella conductivity

↓ T

CryoET

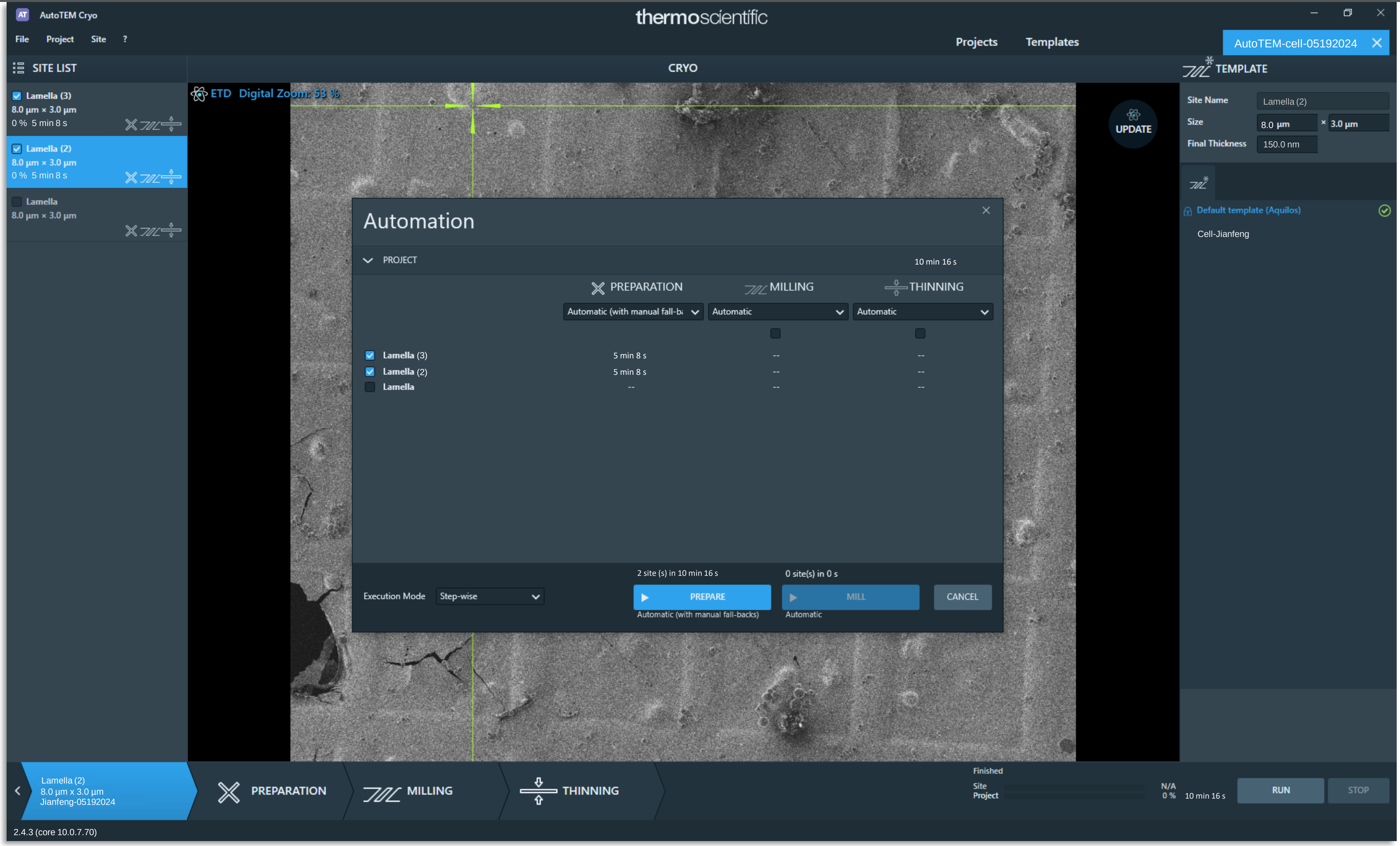




Modified from Wagner et al., Nature Protocols 2020

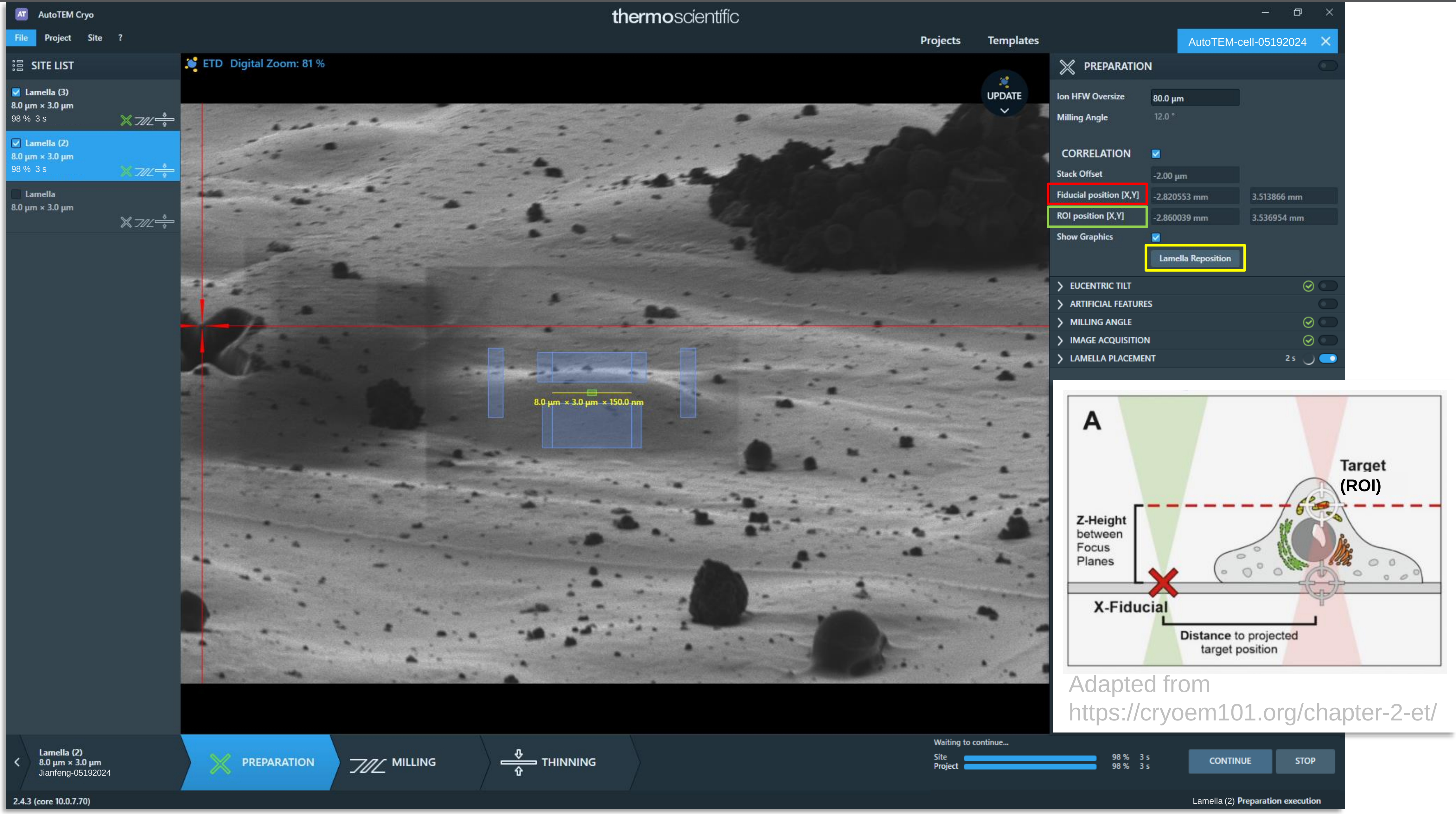
3.7.1 Automated lamella milling _Preparation

(AT AutoTEM Cryo)



3.7.1 Automated lamella milling _Preparation

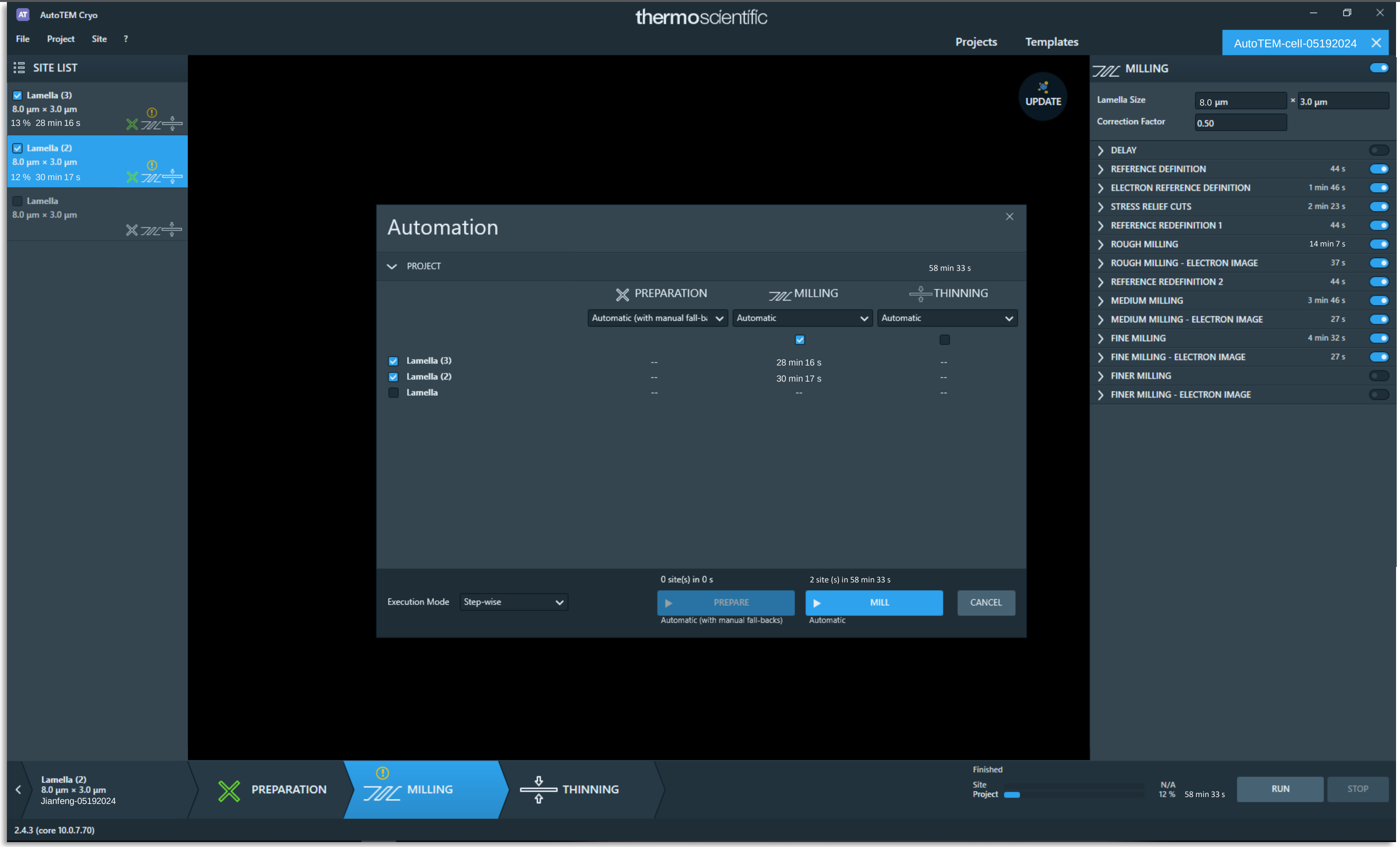
(AT AutoTEM Cryo)



Adapted from
https://cryoem101.org/chapter-2-et/

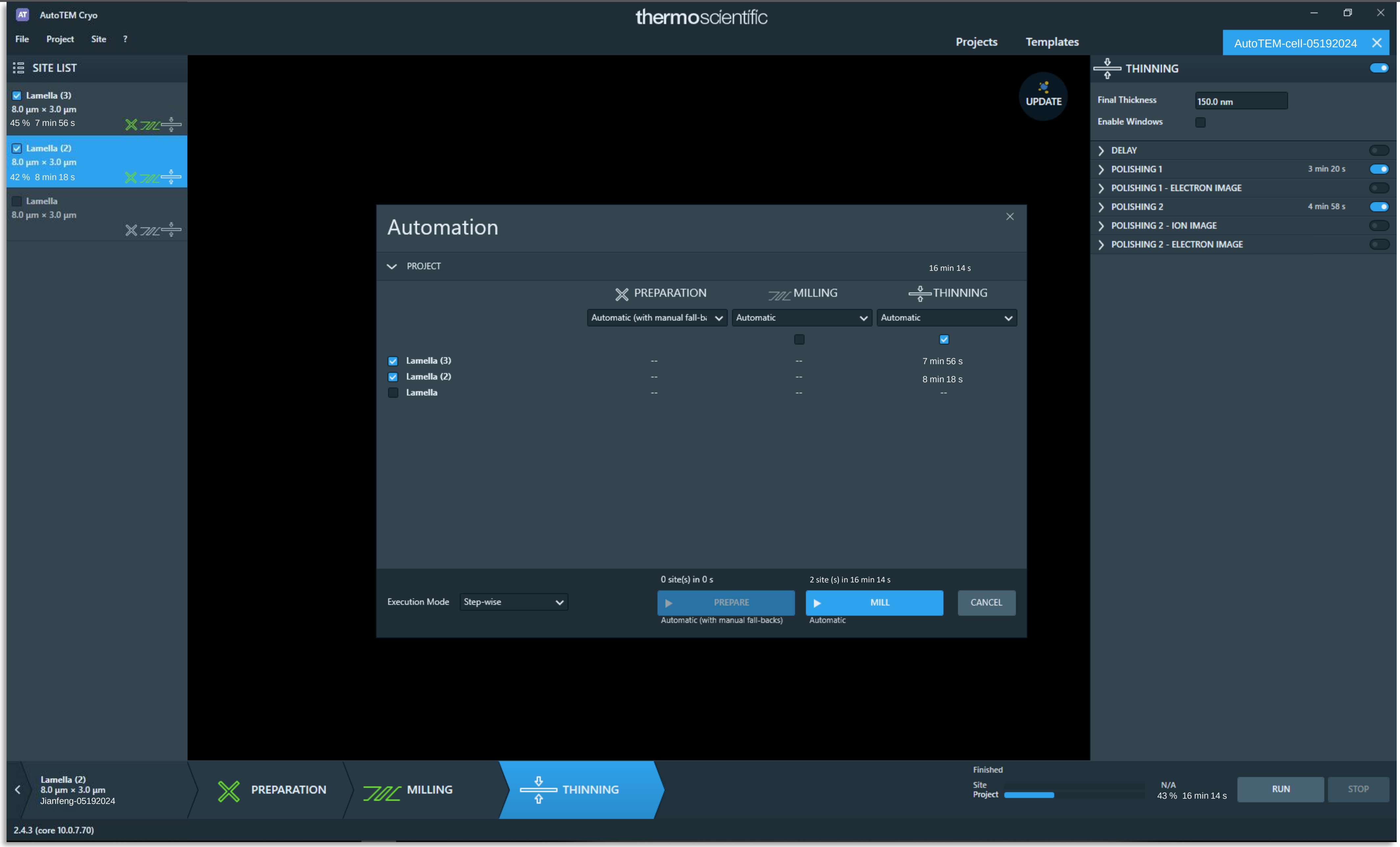
3.7.2 Automated lamella milling _Milling

(AT AutoTEM Cryo)



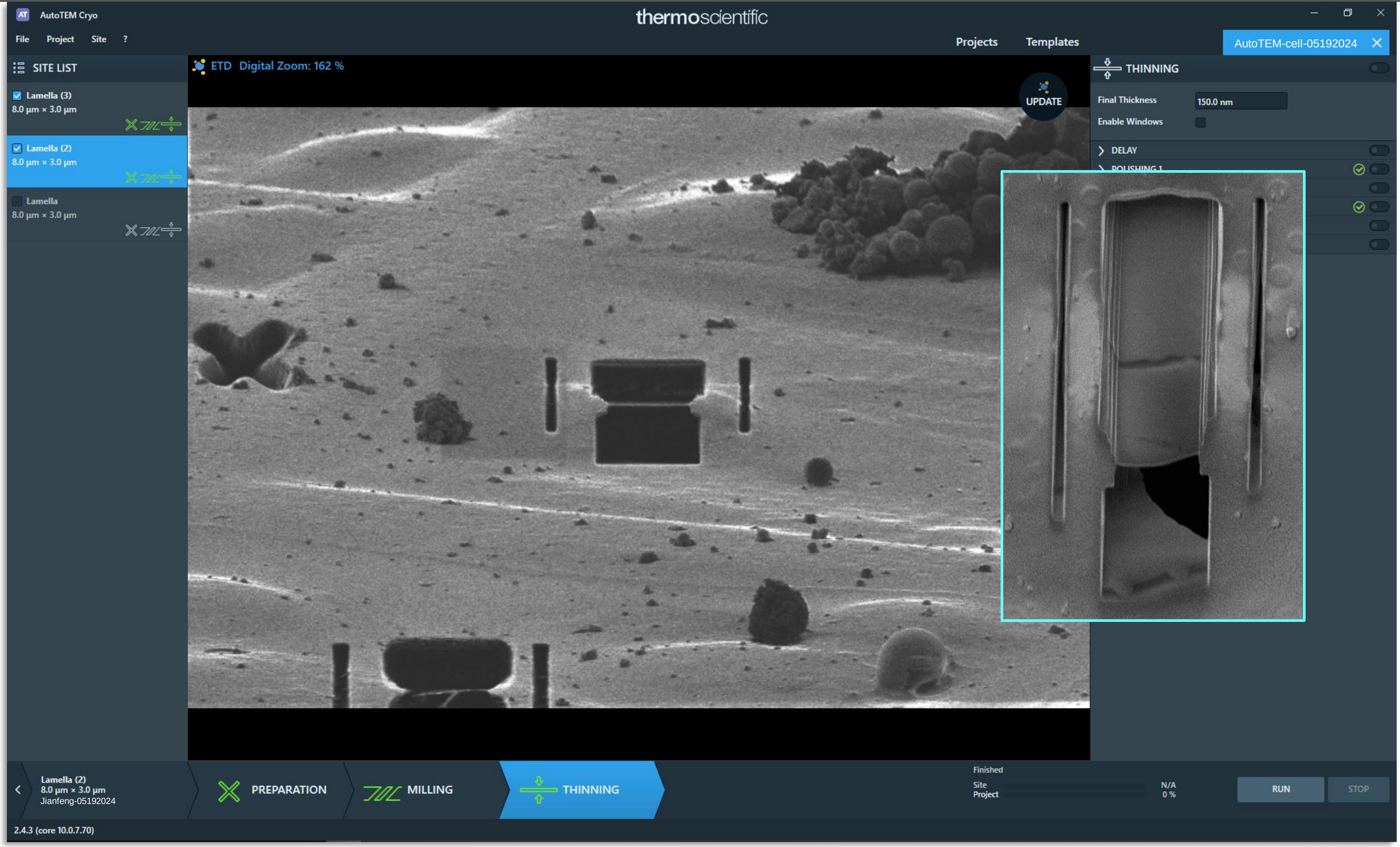
3.7.3 Automated lamella milling _Thinning

(AT AutoTEM Cryo)



3.7.3 Automated lamella milling _Thinning _example-lamella 1

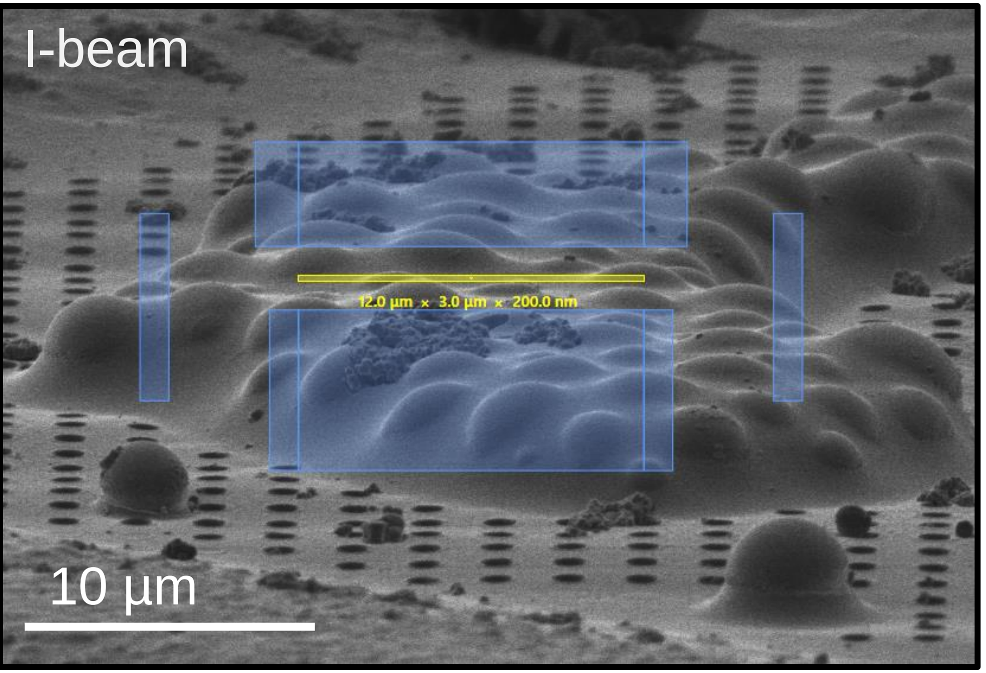
(AT AutoTEM Cryo)



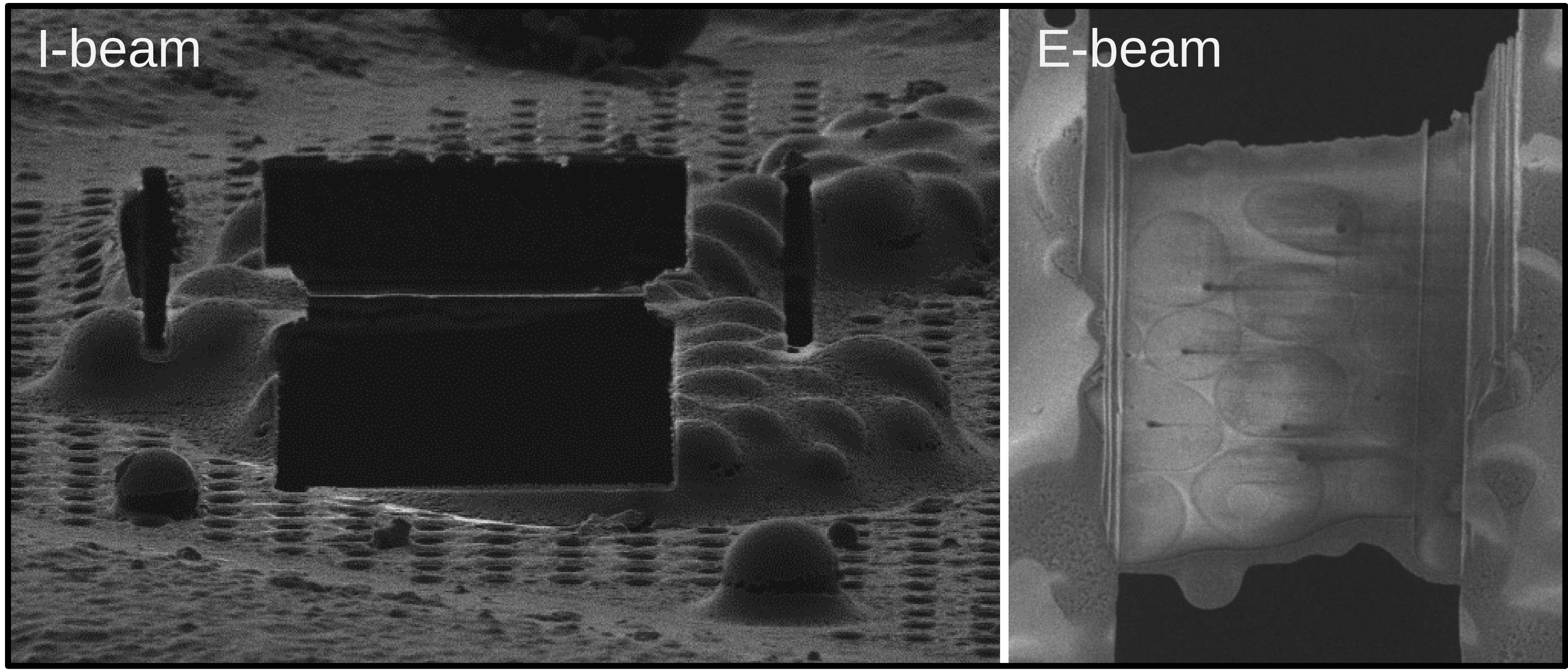
3.7.3 Automated lamella milling _example-lamella 2

(xT Microscope Control)

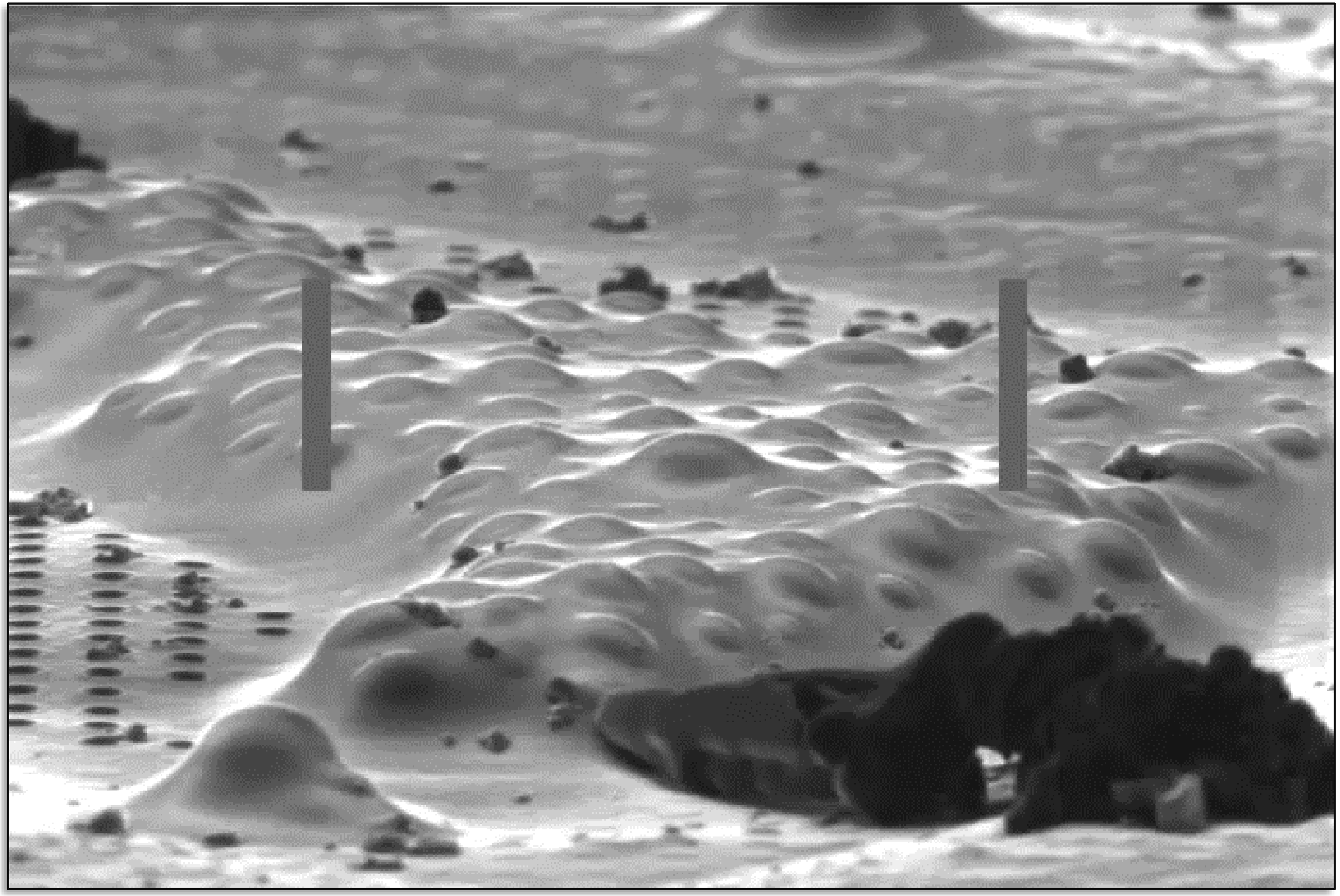
Before milling



After milling



3.7.3 Automated lamella milling _example-lamella 3 (30x accelerated video)



3.8 CryoFLM target confirmation _example-lamella 4

Vitrification

T

CryoFIB

Sample screening
Atlas & lamella sites

iFLM (Optional)
Target selection

Pt sputter
Sample conductivity

Pt GIS
Protective coating

Pt sputter (Optional)
Sample conductivity

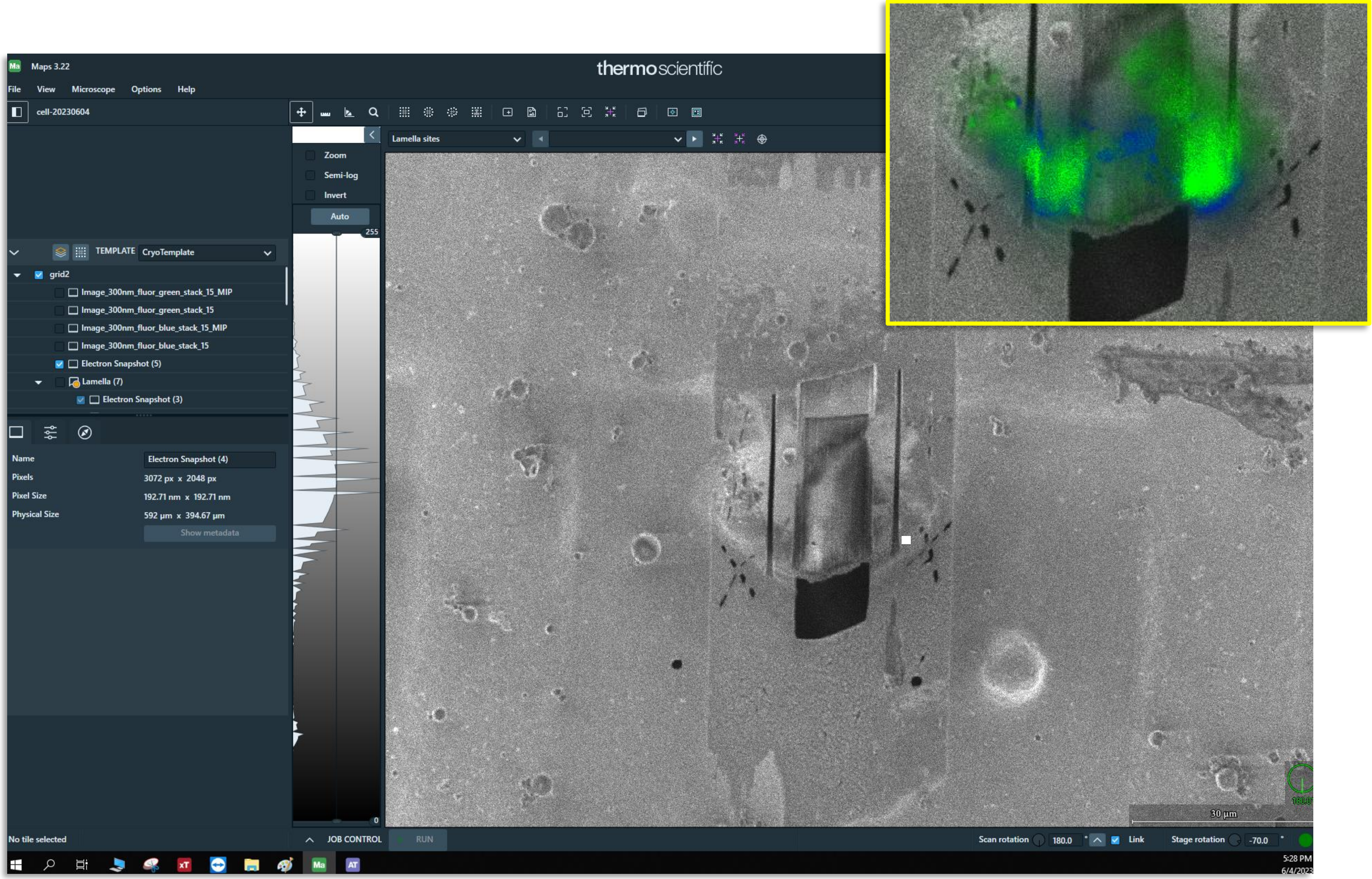
Lamella milling
Preparation, Milling,
& thinning

iFLM (Optional)
Target confirmation

Pt sputter (Optional)
Lamella conductivity

T

CryoET



3.9 Pt sputter (optional)

Vitrification

↓ T

CryoFIB

Sample screening
Atlas & lamella sites

iFLM (Optional)
Target selection

Pt sputter
Sample conductivity

Pt GIS
Protective coating

Pt sputter (Optional)
Sample conductivity

Lamella milling
Preparation, Milling, & thinning

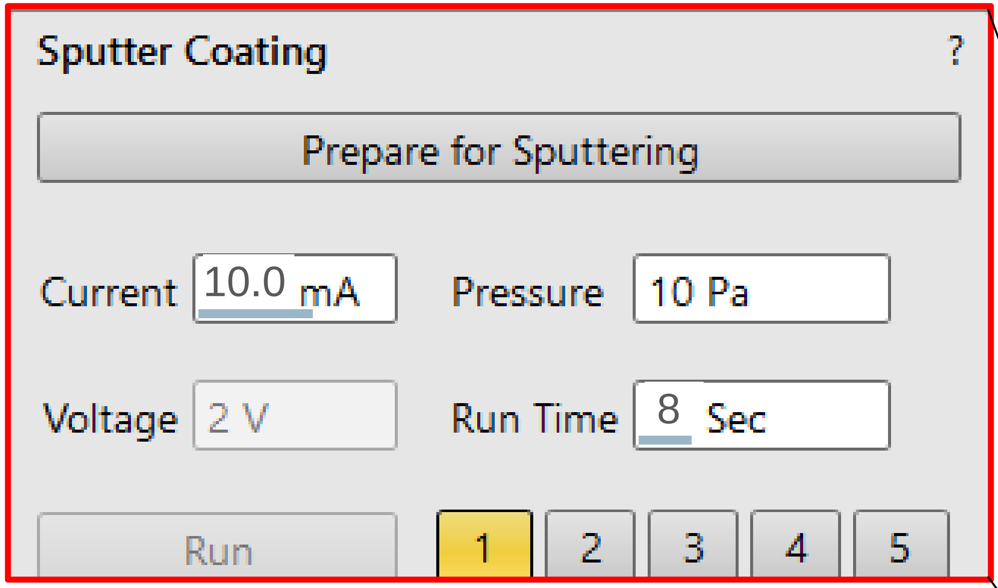
iFLM (Optional)
Target confirmation

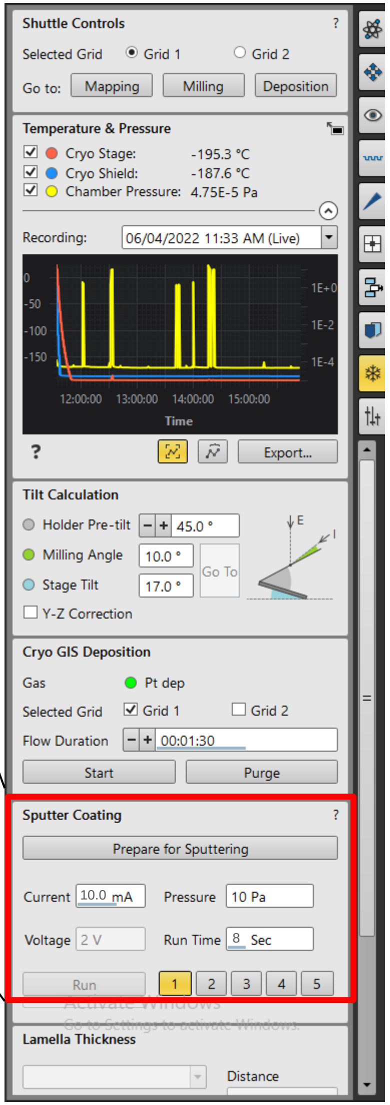
Pt sputter (Optional)
Lamella conductivity

> (Optional) minimize charging; ensure low beam-induced movement and using of VPP.

↓ T

CryoET





3.10 Unloading grids

(Video from TFS)

Vitrification

↓ **T**

CryoFIB

Sample screening
Atlas & lamella sites

iFLM (Optional)
Target selection

Pt sputter
Sample conductivity

Pt GIS
Protective coating

Pt sputter (Optional)
Sample conductivity

Lamella milling
Preparation, Milling,
& thinning

iFLM (Optional)
Target confirmation

Pt sputter (Optional)
Lamella conductivity

↓ **T**

CryoET

Some common cryoFIB workflow variants

CryoFIB

Sample screening
Atlas & lamella sites

iFLM (Optional)
Target selection

Pt sputter
Sample conductivity

Pt GIS
Protective coating

Pt sputter (Optional)
Sample conductivity

Lamella milling
Preparation, Milling, & thinning

iFLM (Optional)
Target confirmation

Pt sputter (Optional)
Lamella conductivity

e.g., for example-lamella 4

CryoFIB

Sample screening
Atlas & lamella sites

Pt sputter
Sample conductivity

Pt GIS
Protective coating

Pt sputter (Optional)
Sample conductivity

iFLM (Optional)
Target selection

Lamella milling
Preparation, Milling, & thinning

iFLM (Optional)
Target confirmation

Pt sputter (Optional)
Lamella conductivity

e.g., for example-lamella 1

CryoFIB

Sample screening
Atlas & lamella sites

Pt sputter
Sample conductivity

Pt GIS
Protective coating

Pt sputter (Optional)
Sample conductivity

Lamella milling
Preparation, Milling, & thinning

Pt sputter (Optional)
Lamella conductivity

If CLEM is unnecessary

CryoFIB

Sample screening
Atlas & lamella sites

Pt sputter
Sample conductivity

Pt GIS
Protective coating

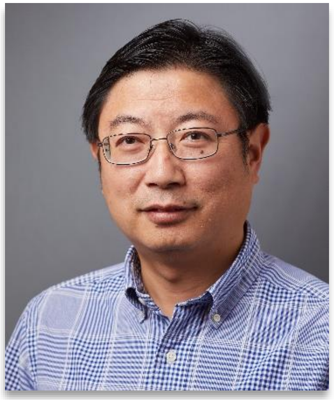
Lamella milling
Preparation, Milling, & thinning

If CLEM is unnecessary & no charging problems

e.g., for example-lamellae 2&3

(Video from TFS)

Acknowledgment



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