CryoEM Course at LBMS, BNL (June 11-14th, 2024)

CryoET sample preparation tutorial & demonstration

Jianfeng Lin

Yale CryoEM Resource

6-13-2024

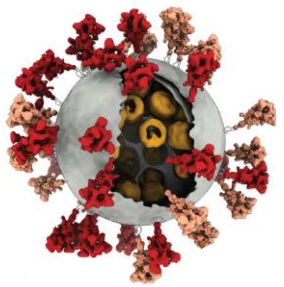
Main contents

1. Specimens accessible by cryoET

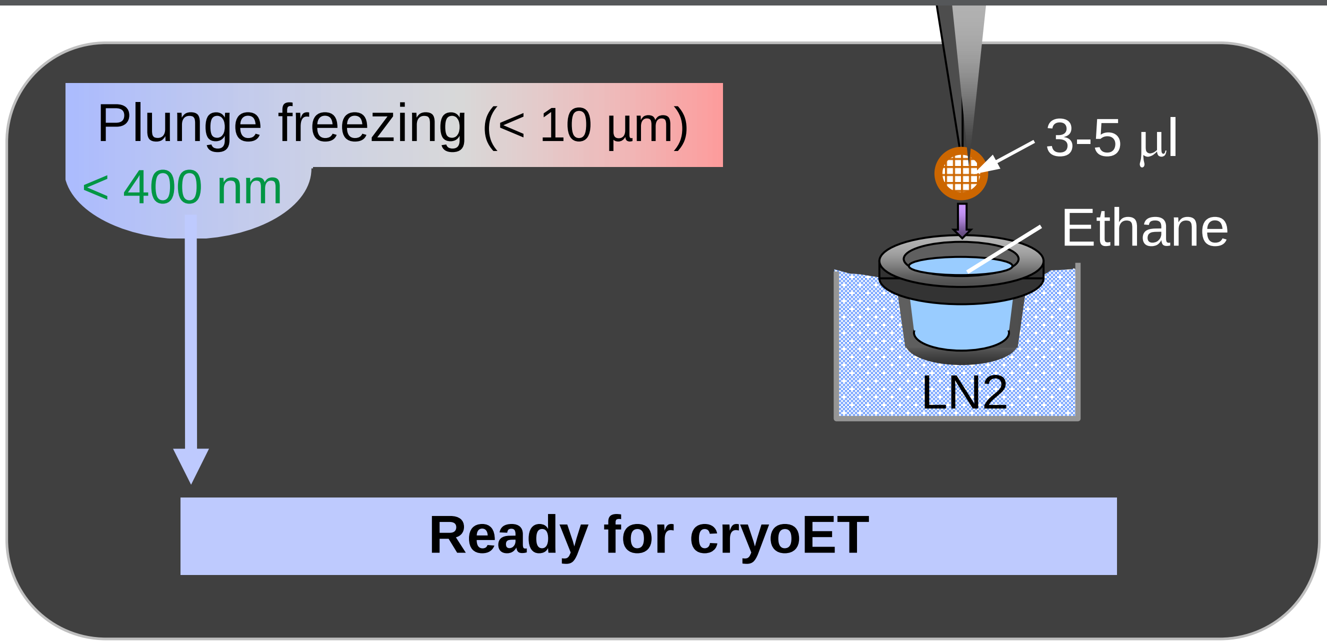
2. Five considerations for cryoET sample preparation

3. Tutorial of major steps of cryo lamella preparation

1. Specimens accessible by cryoET

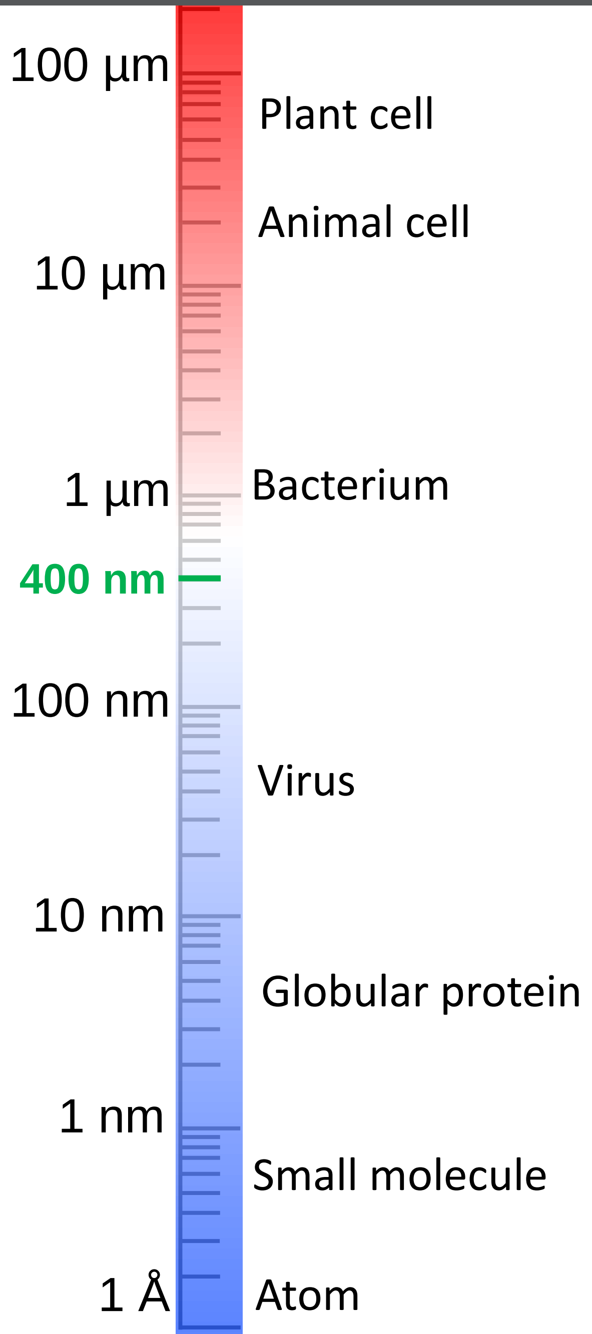


Yao et al., Cell 2020



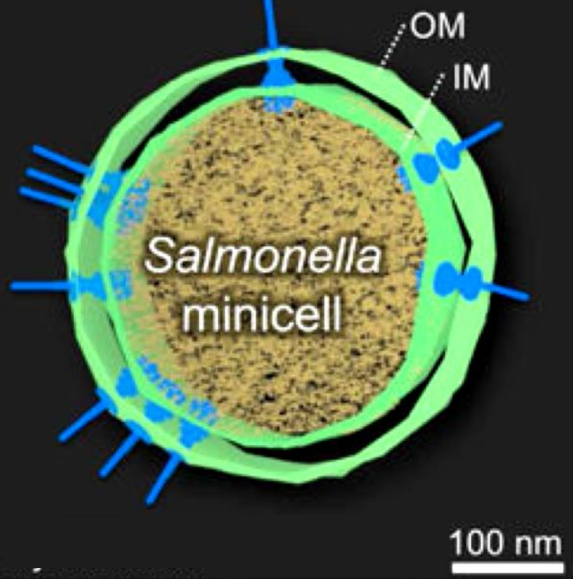
- Virus: e.g., Covid-19
- Isolated or reconstituted systems: e.g., ciliary axoneme

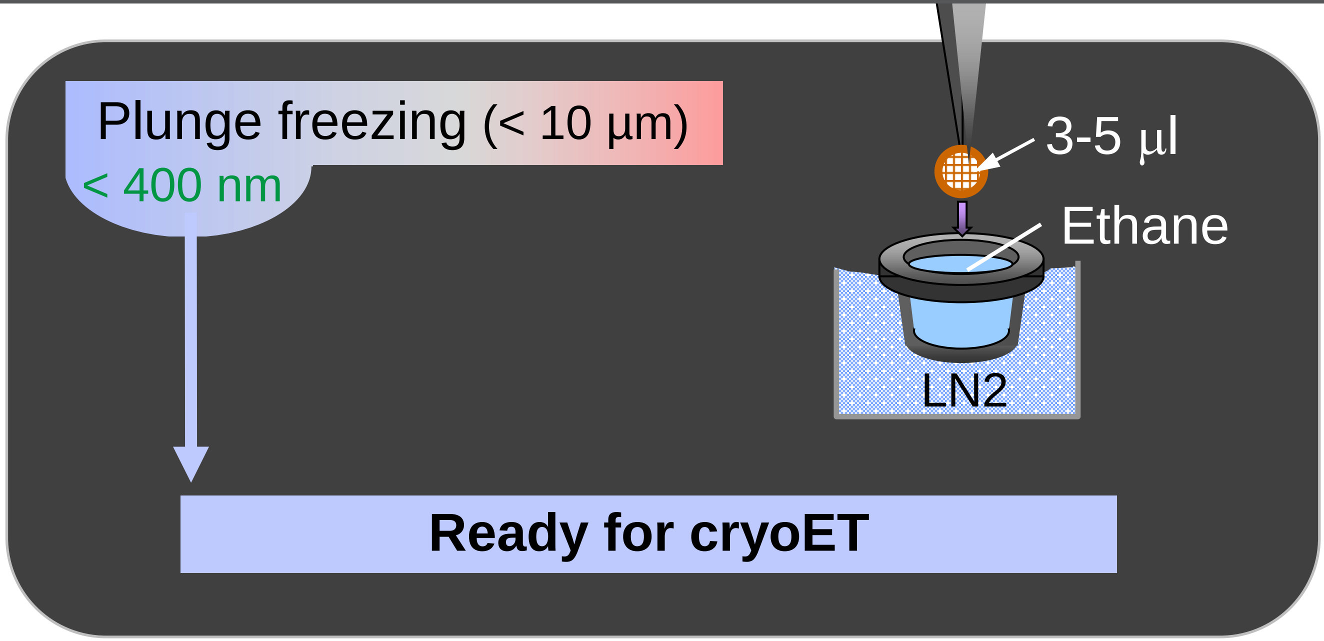
Lin & Nicastro, Science 2018

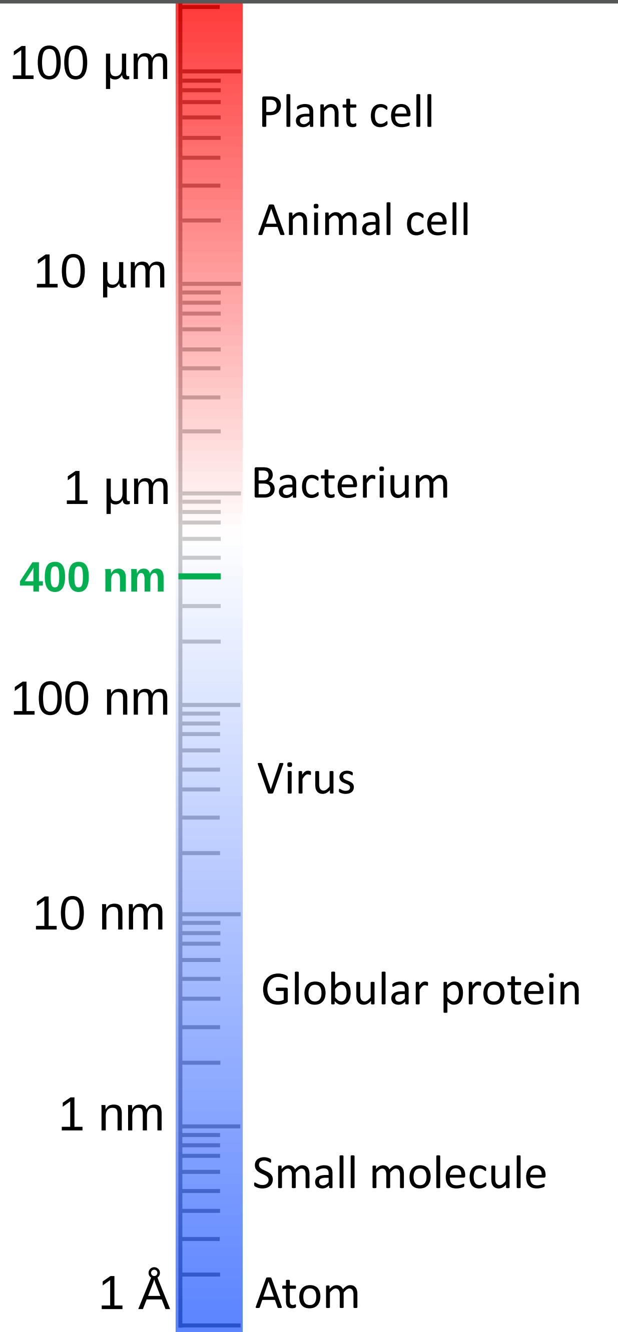


1. Specimens accessible by cryoET

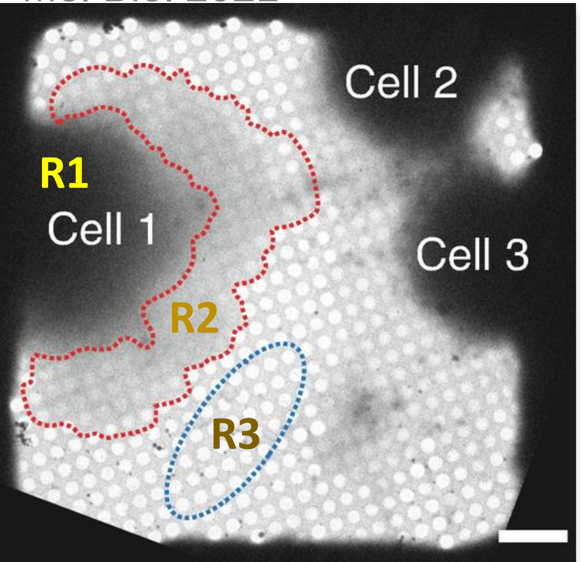
Hu et al., Cell 2017



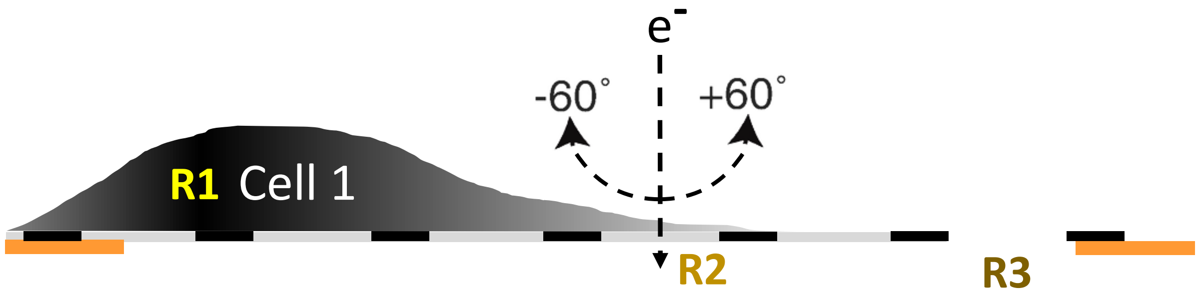




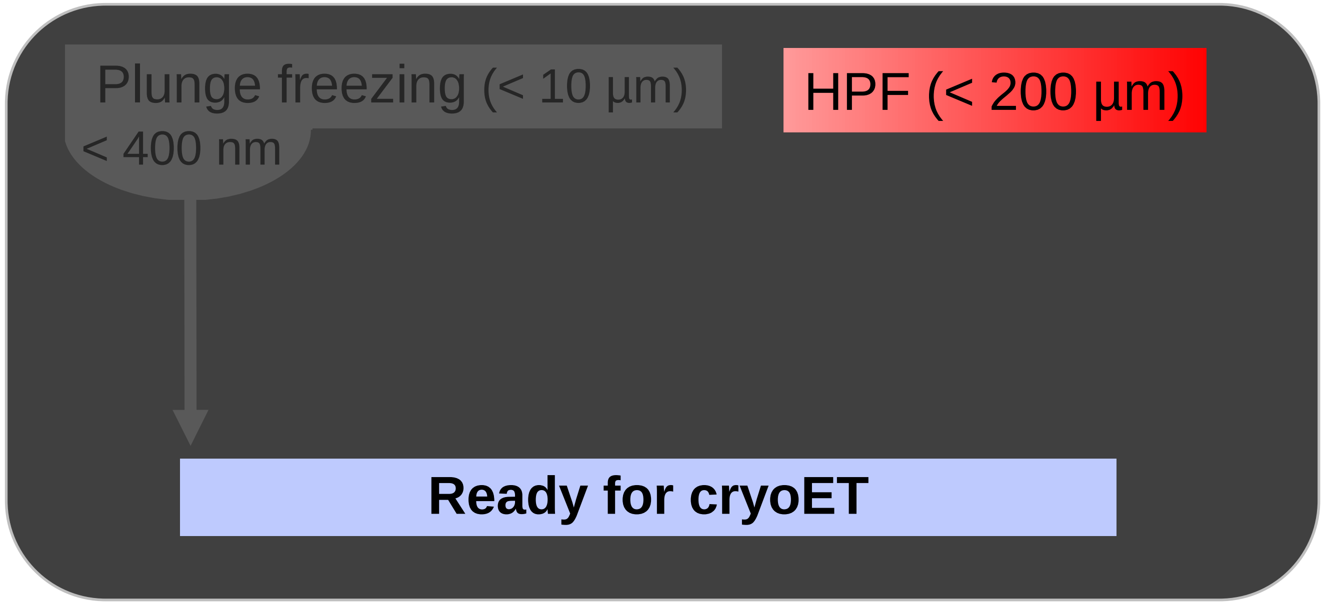
Serwas & Davies. Methods Mol Biol 2021



- 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



1. Specimens accessible by cryoET



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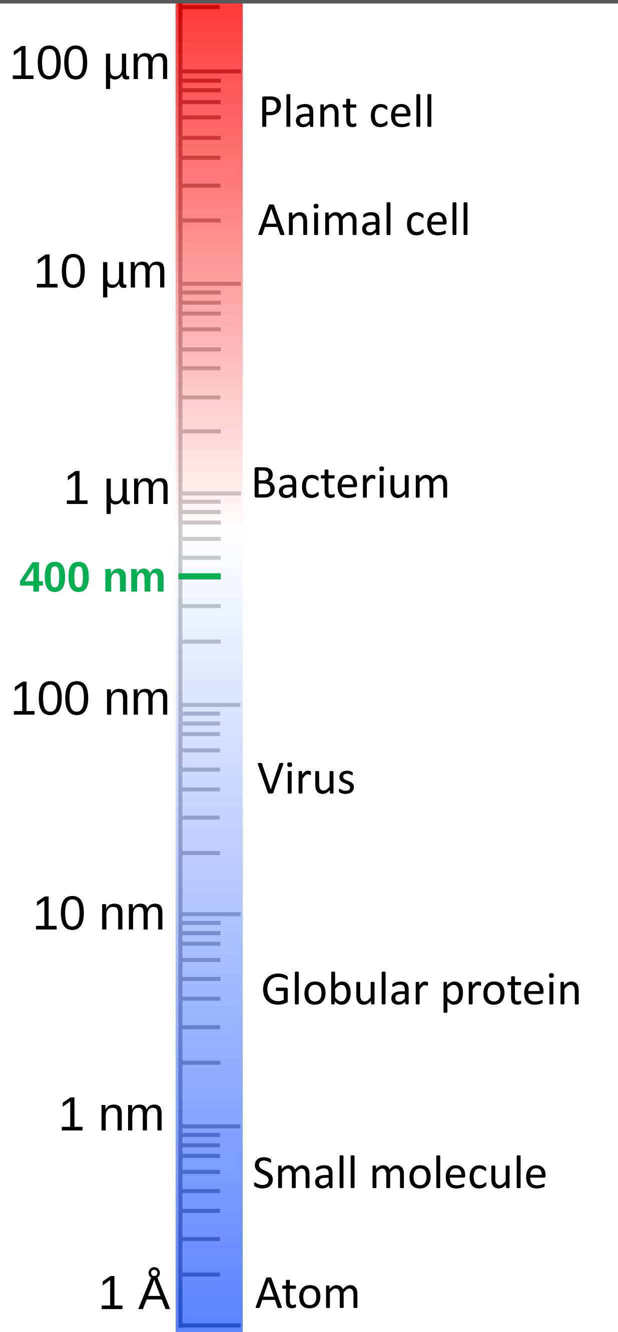
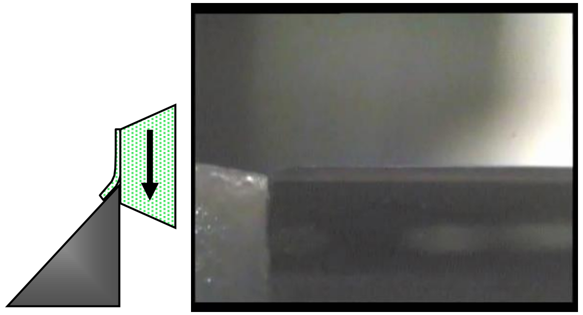
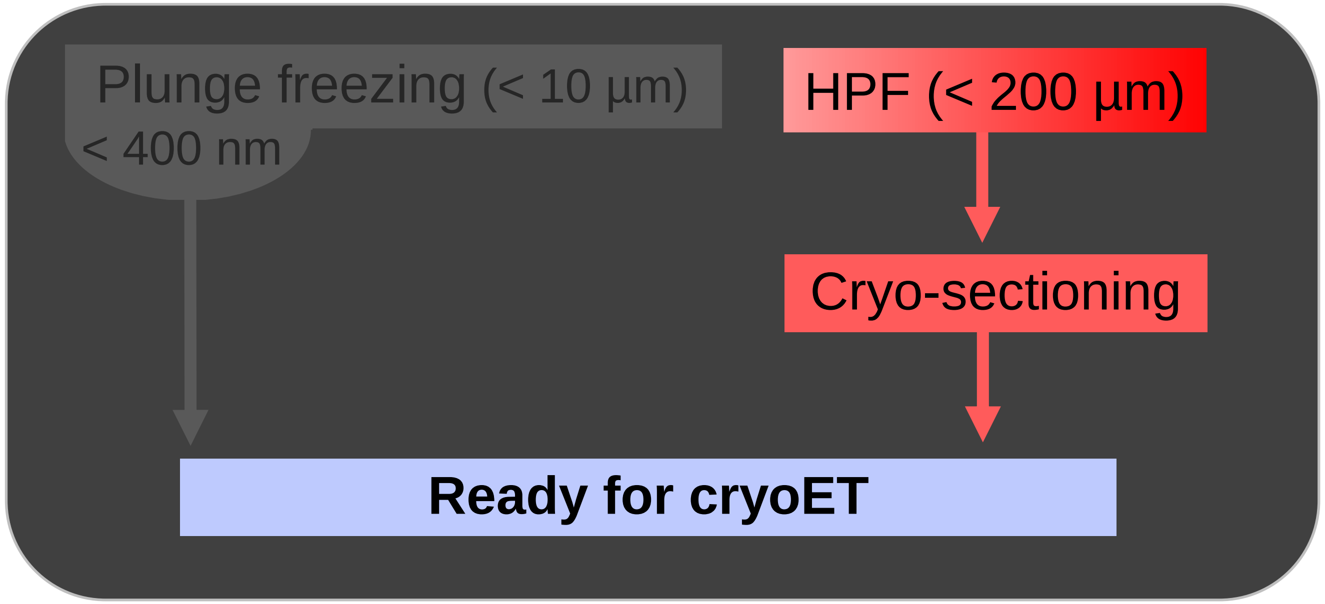
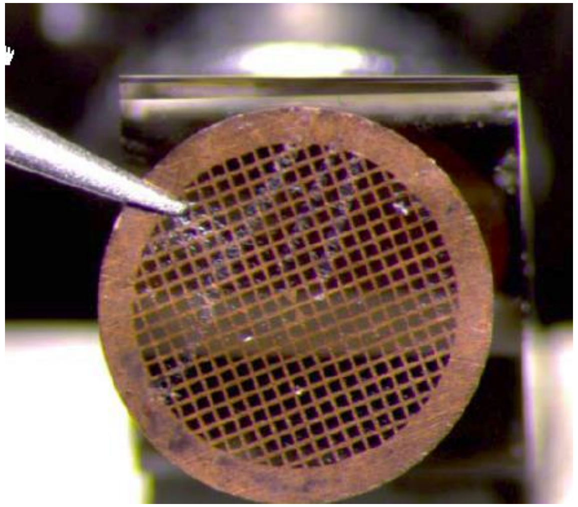


1. Specimens accessible by cryoET

CEMOVIS

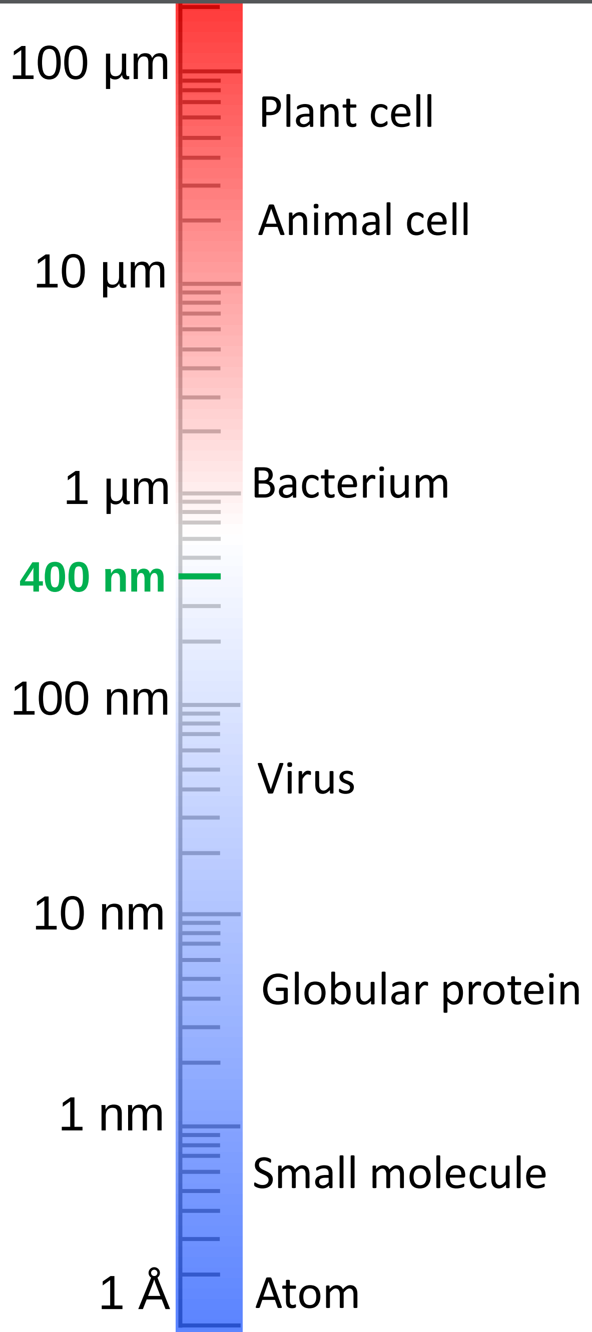






Adapted from Thermo Fisher Scientific (TFS)

- 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
- Cryo-sections



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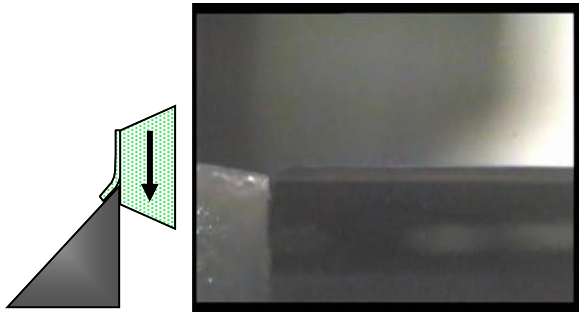


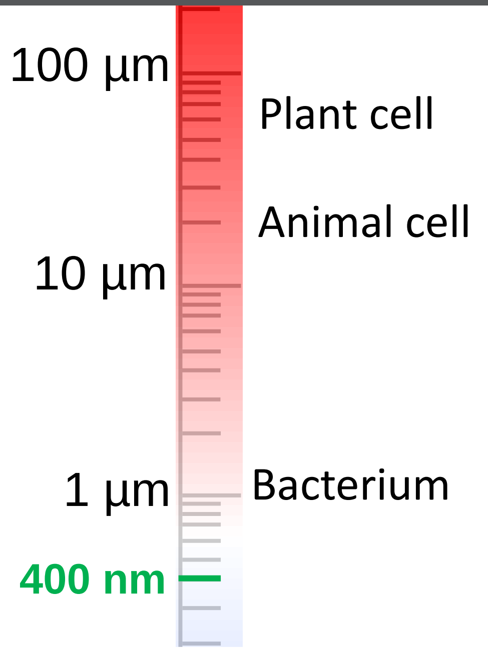
Plunge freezing (< 10 µm)

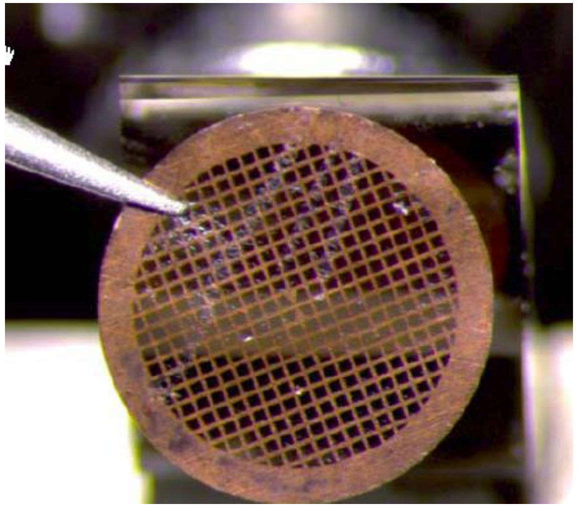
< 400 nm

HPF (< 200 µm)

Cryo-sectioning

Ready for cryoET





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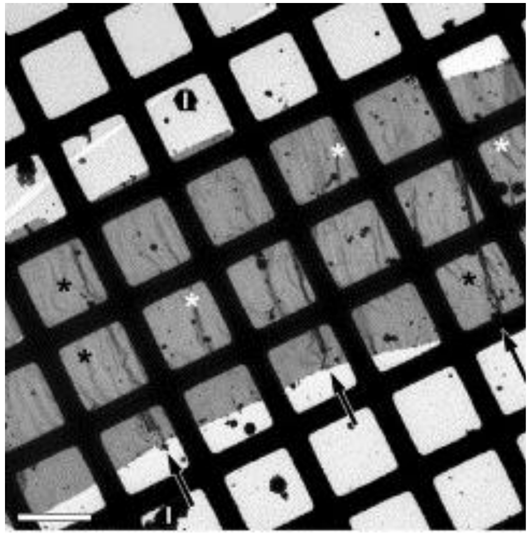
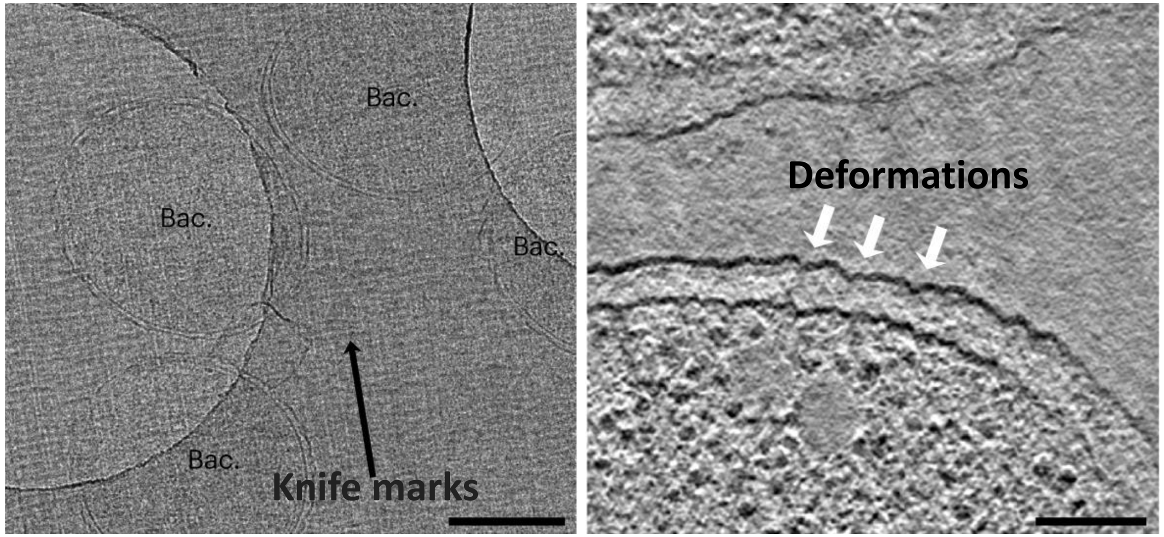


Al-Amoudi et al., J Struct Biol 2005



Berger et al., Nature Methods 2023

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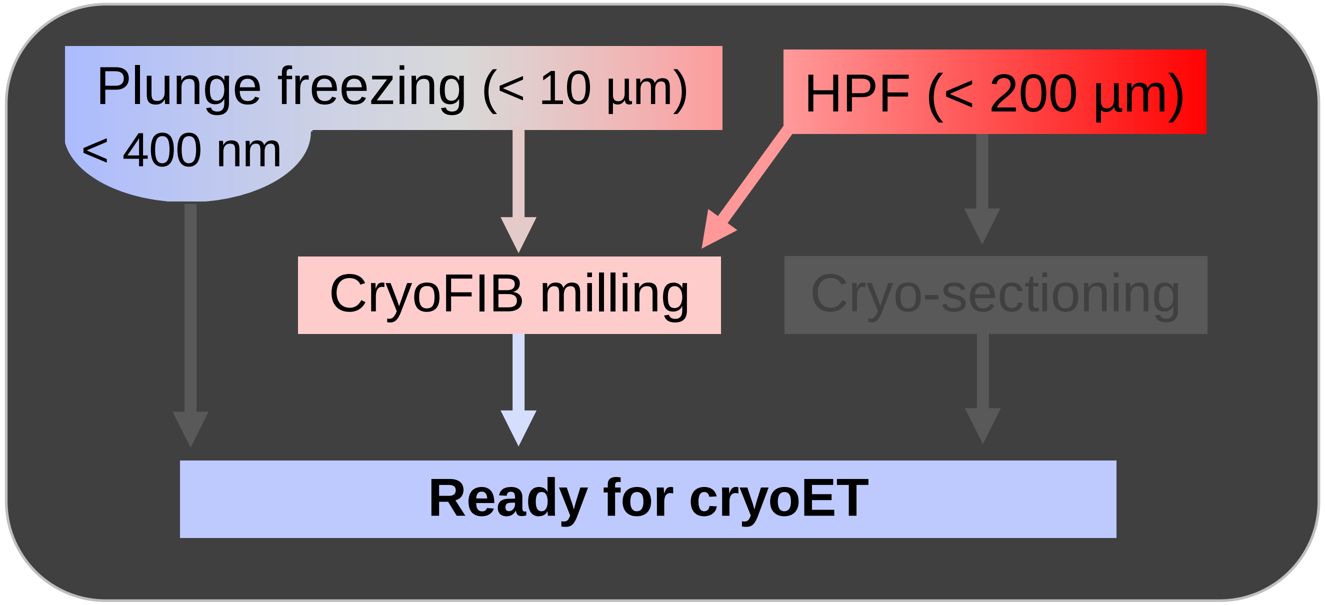

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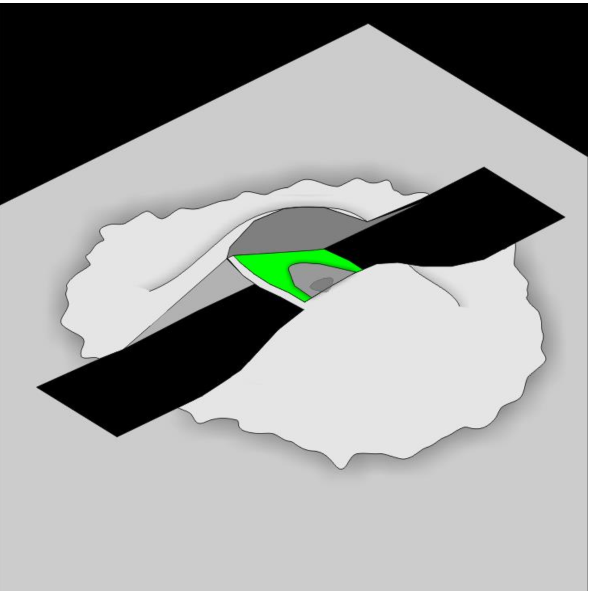
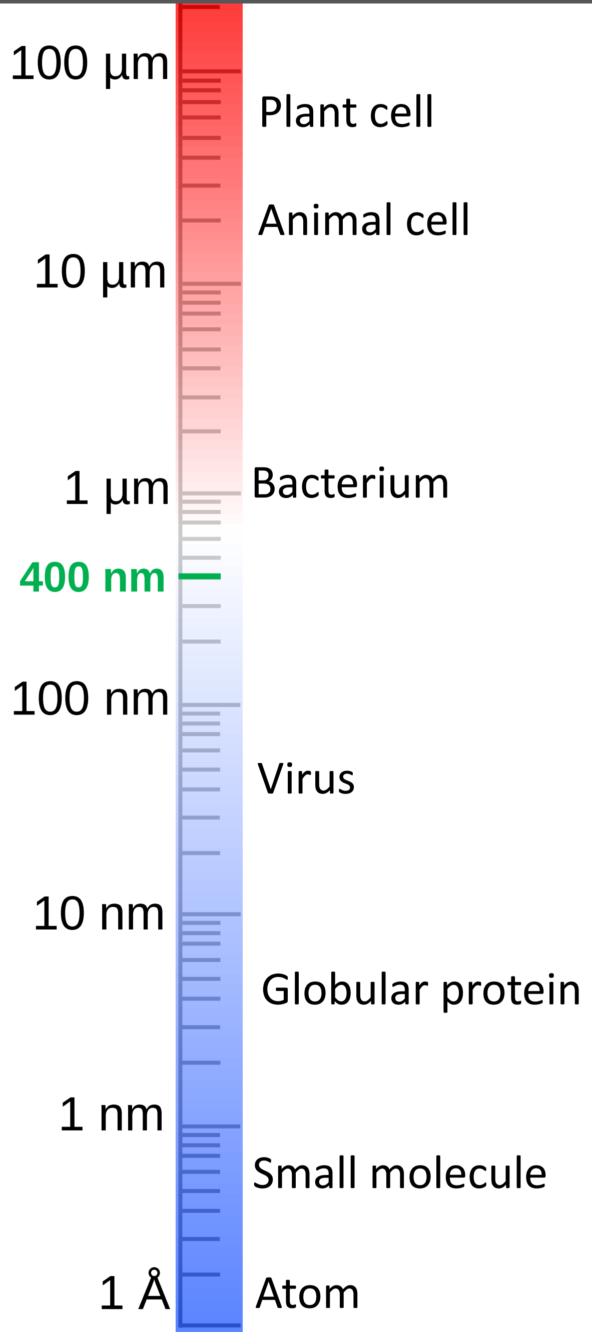
- Peripheral regions of cells: e.g., mammalian cells
- Cryo-sections
- Cryo-lamellae





Main contents

1. Specimens accessible by cryoET

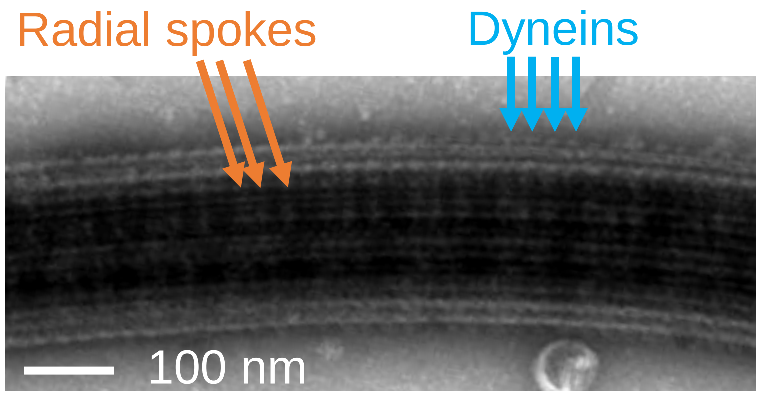
2. Five considerations for cryoET sample preparation

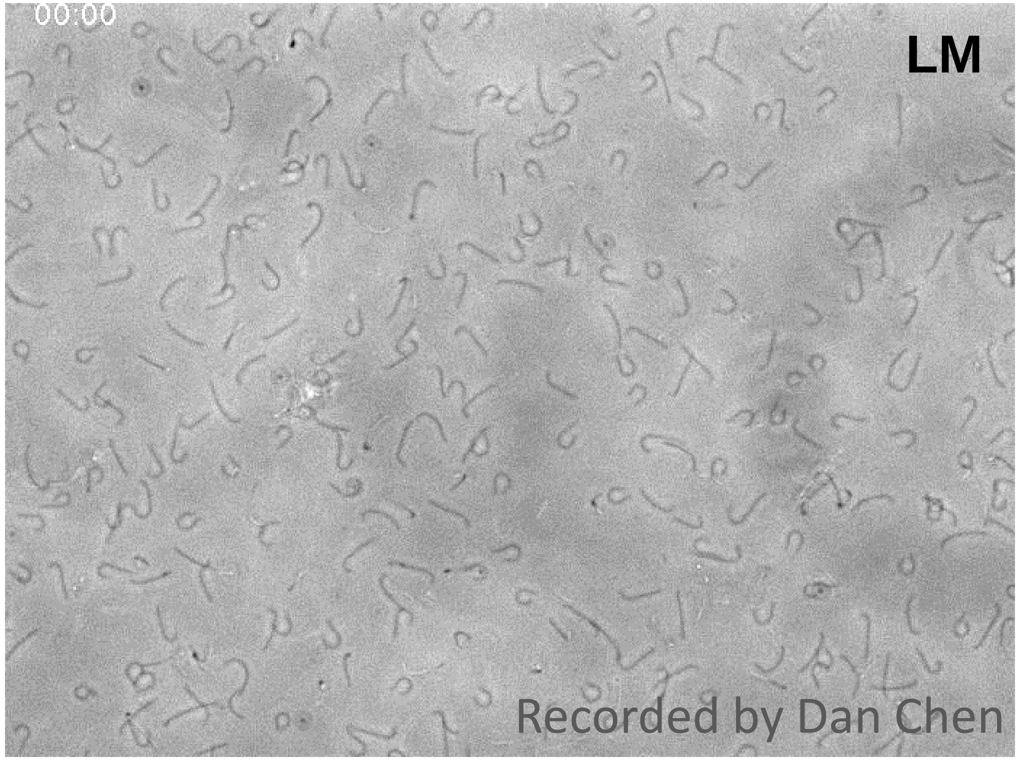
3. Tutorial of major steps of cryo lamella preparation

2. Five considerations for cryoET sample preparation

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

Negative stain EM

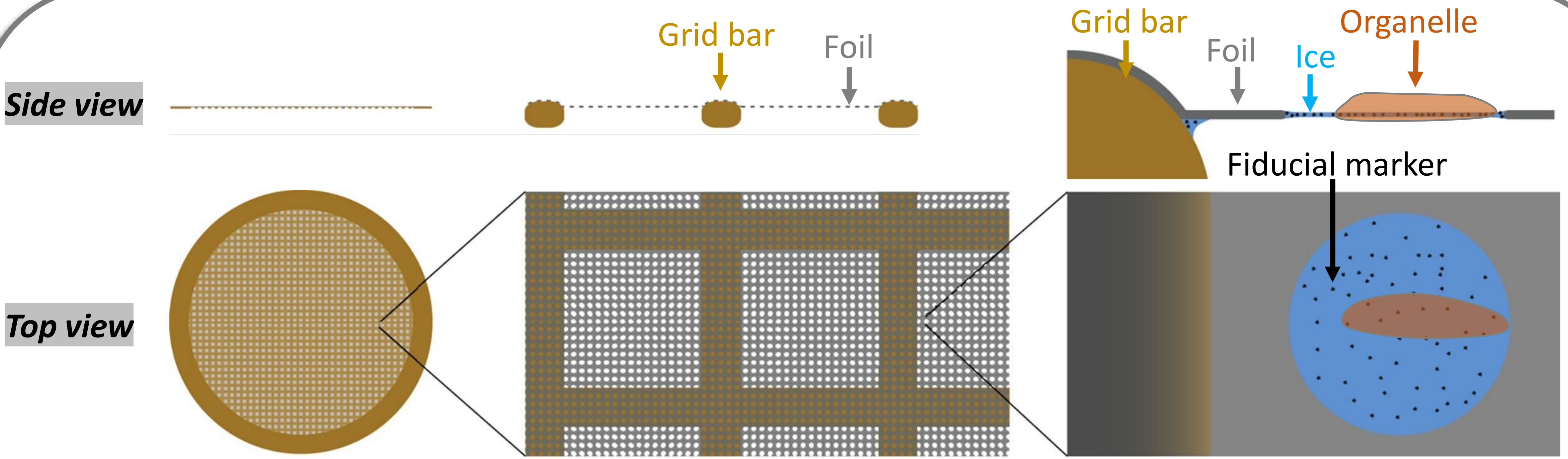




2. Five considerations for cryoET sample preparation

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

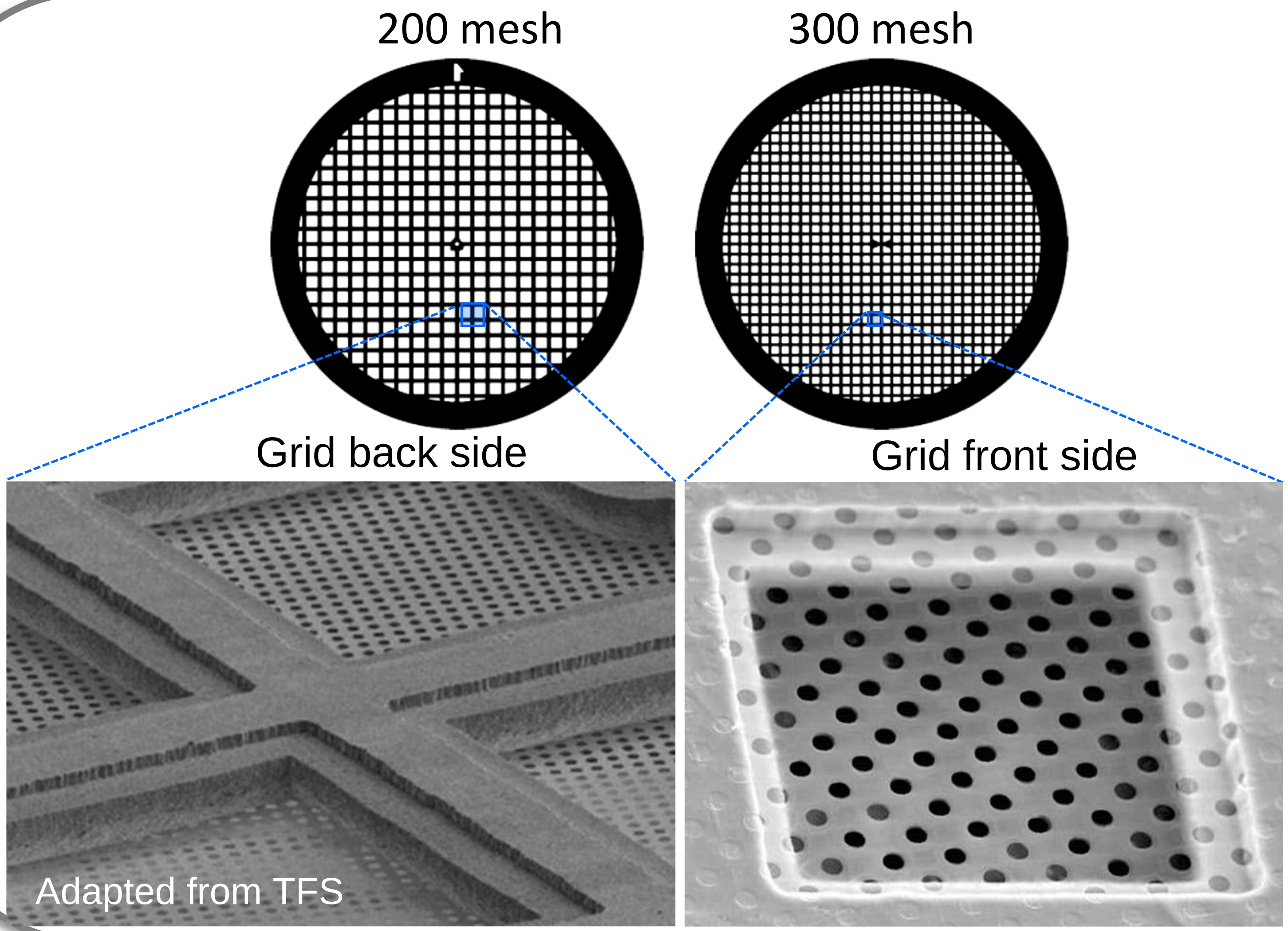
TiSi	SiN
SiO_2	SiC

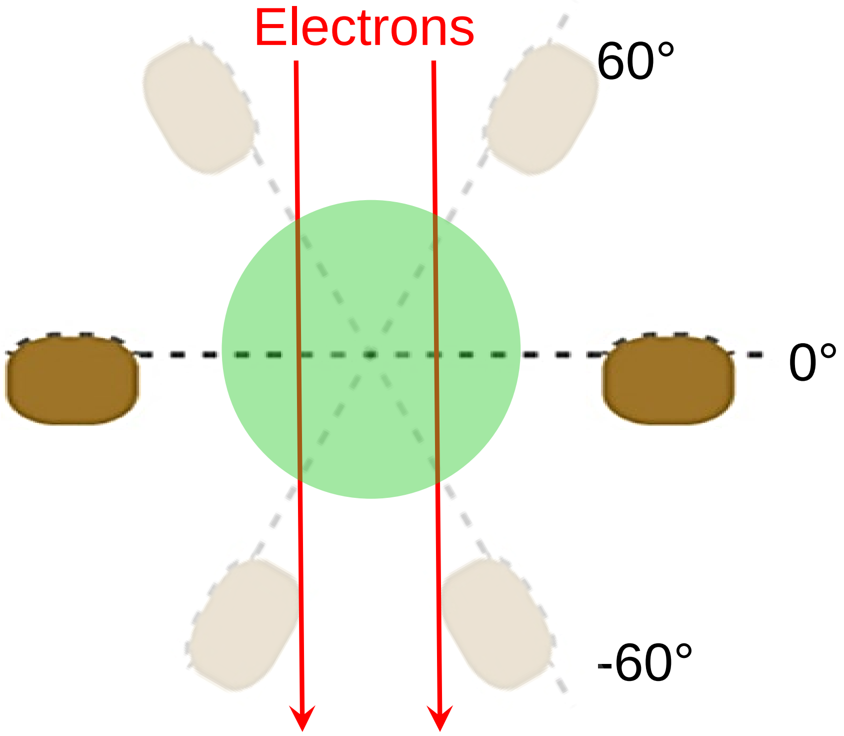
Modified from Russo & Passmore, Curr Opin Struct Biol 2016

2. Five considerations for cryoET sample preparation

2.1 Validation of the sample quality

2.2 EM grid => Mesh / Hole size





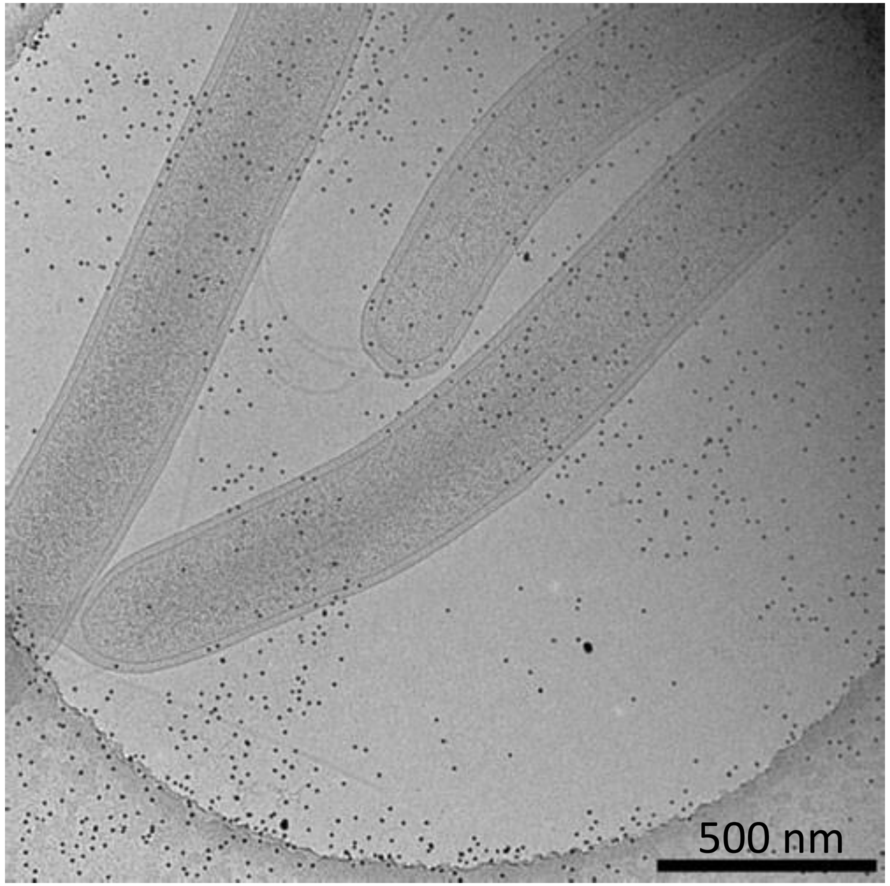
2. Five considerations for cryoET sample preparation

2.1 Validation of the sample quality

2.2 EM grid

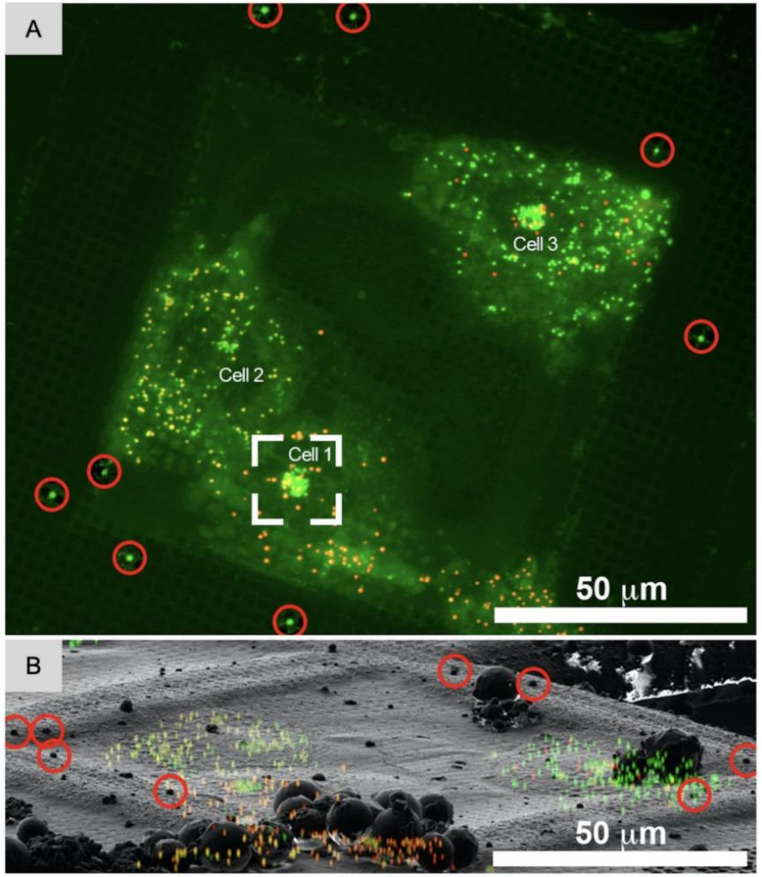
2.3 Fiducial markers

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 cryoET sample preparation

2.1 Validation of the sample quality

2.2 EM grid

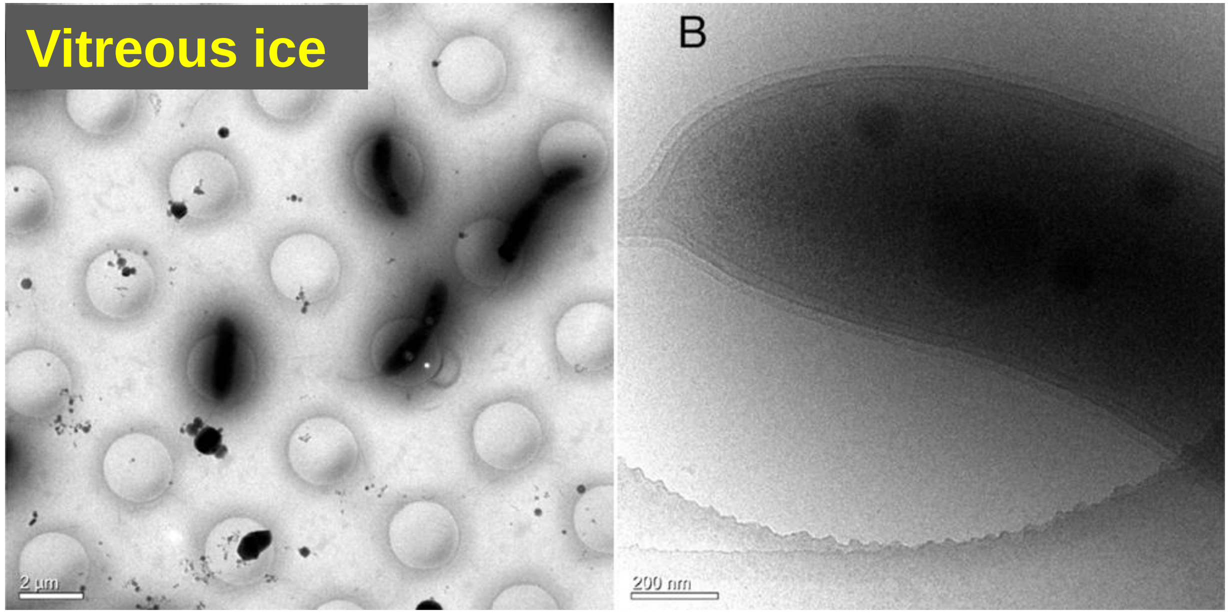
2.3 Fiducial markers

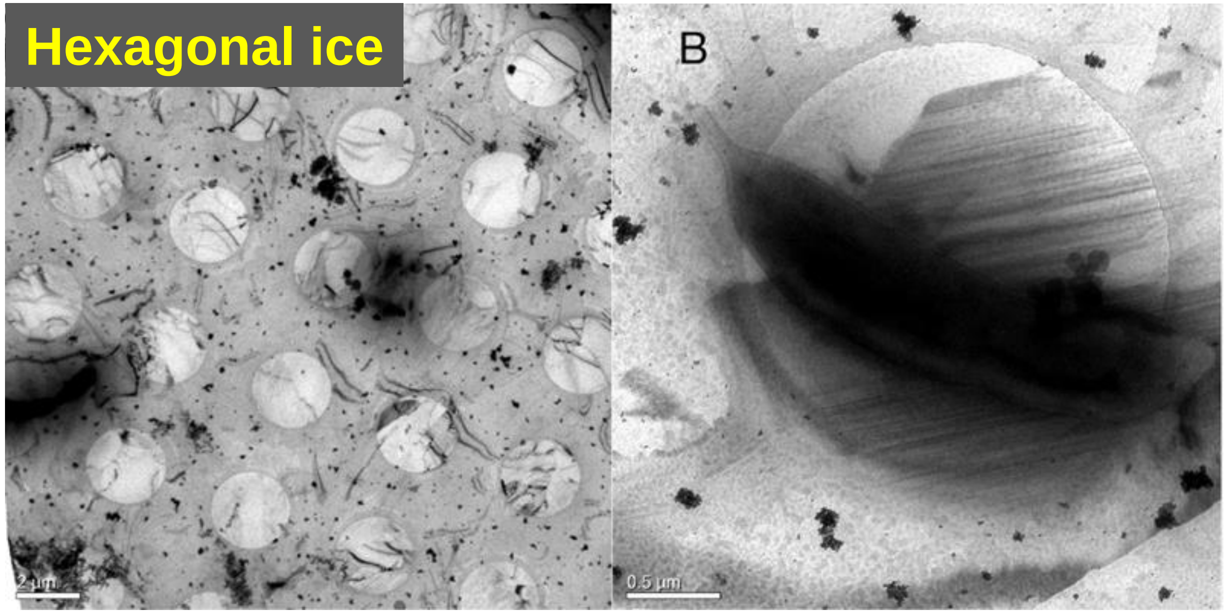
2.4 Cryogen e.g., LE or 37% LE-63% LP mixture

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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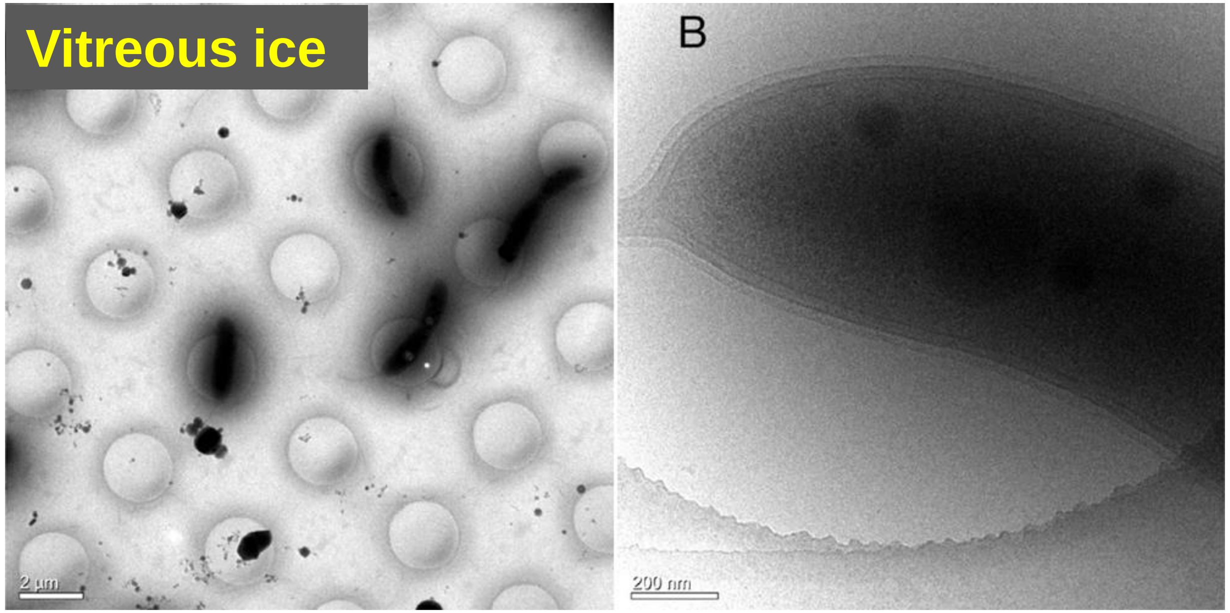
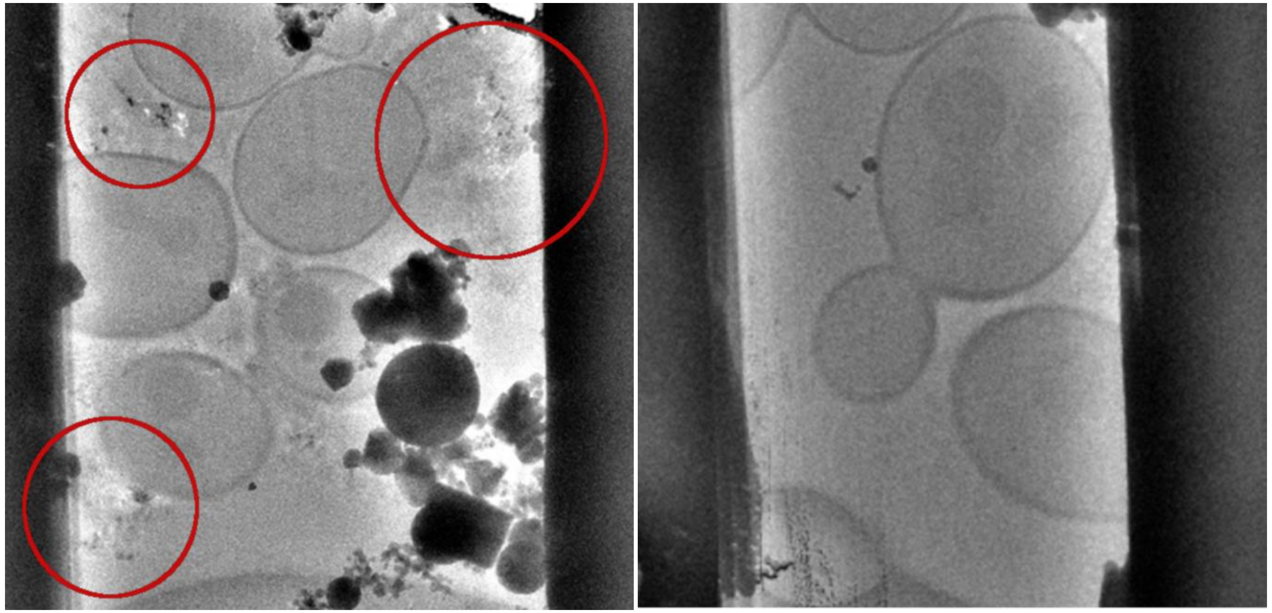


Tivol et al., Microsc Microanal 2008

Without glycerol

5% glycerol



Moravcová et al., J Vis Exp 2021

2. Five considerations for cryoET sample preparation

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2.2 EM grid

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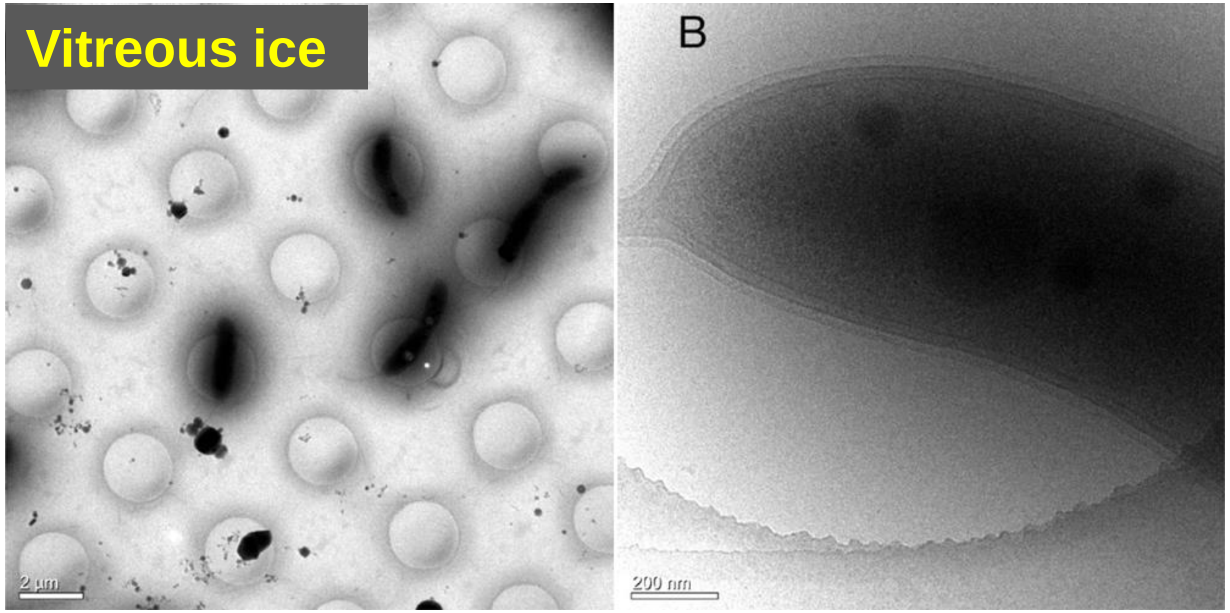

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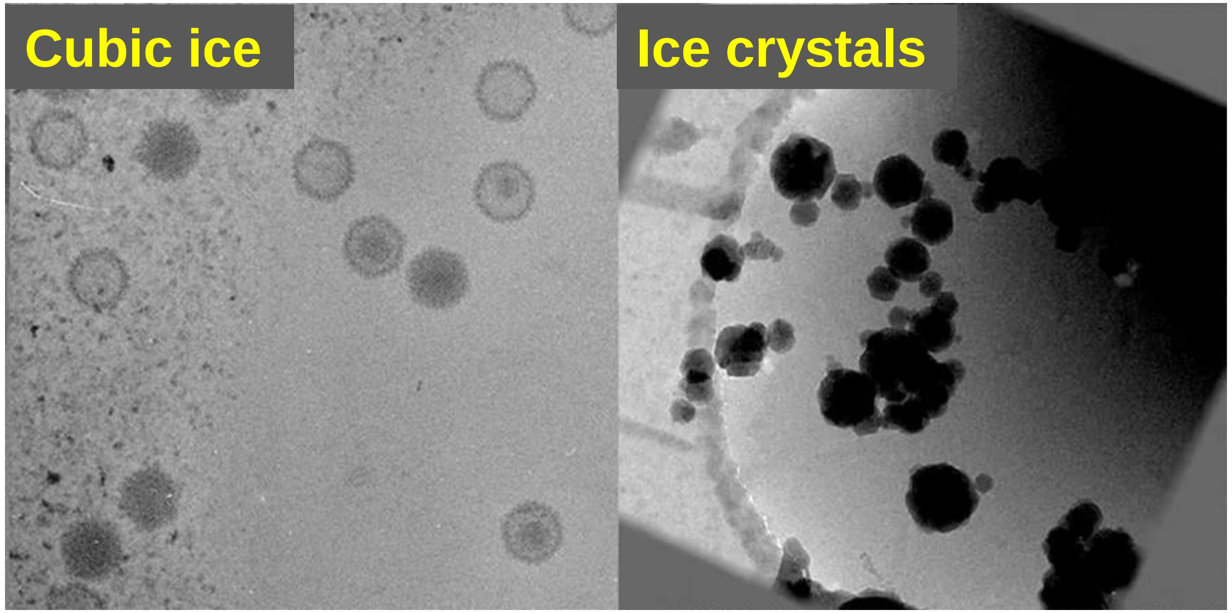


Bhella D, Biophysical Reviews 2019

2. Five considerations for cryoET sample preparation

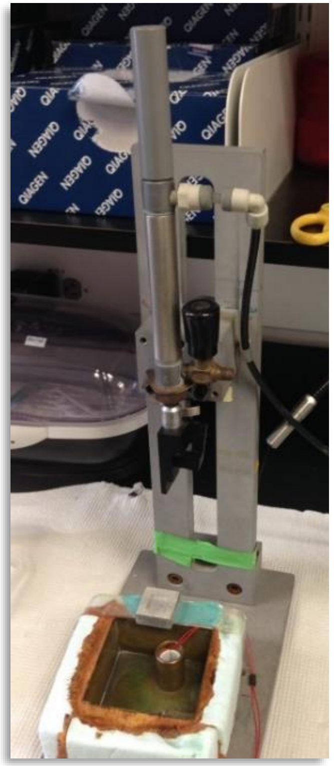
2.1 Validation of the sample quality

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. Specimens accessible by cryoET

2. Five considerations for cryoET sample preparation

3. Tutorial of major steps of cryo lamella preparation

Examples of cryoFIB milling instruments

Crossbeam (ZEISS)

Arctis
(Thermo Fisher Scientific)

Scios, Aquiclos 1/2
(Thermo Fisher Scientific)

Main contents

1. Specimens accessible by cryoET

2. Five considerations for cryoET 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

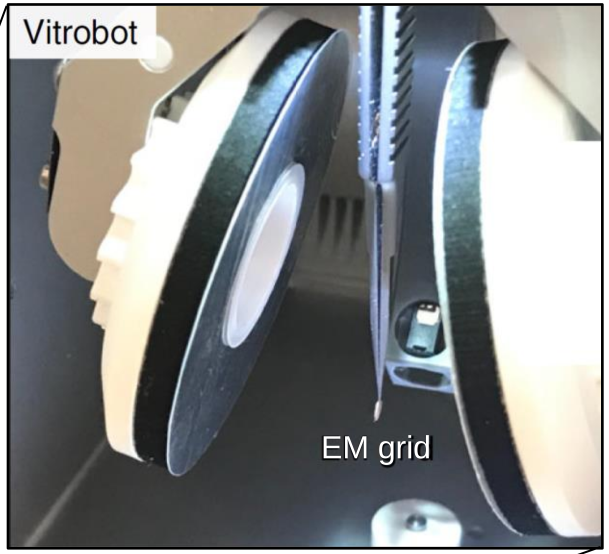
Lamella milling
Preparation, Milling,
& thinning

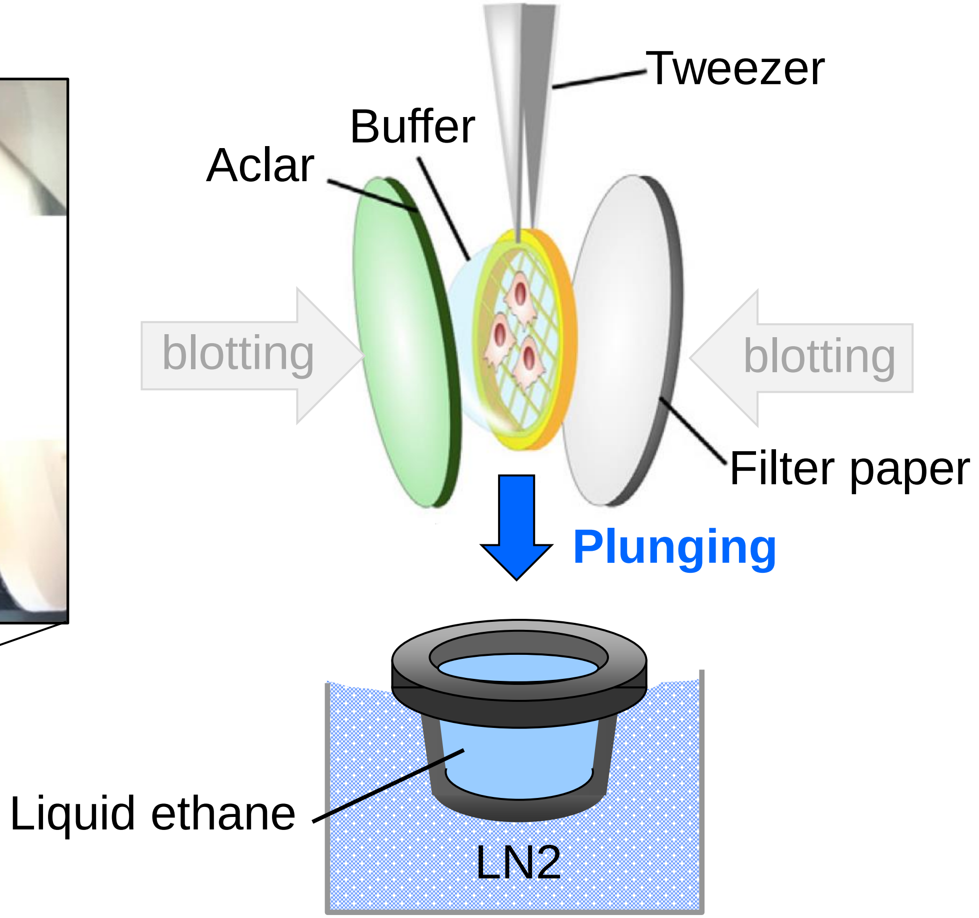
iFLM (Optional)
Target confirmation

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

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

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

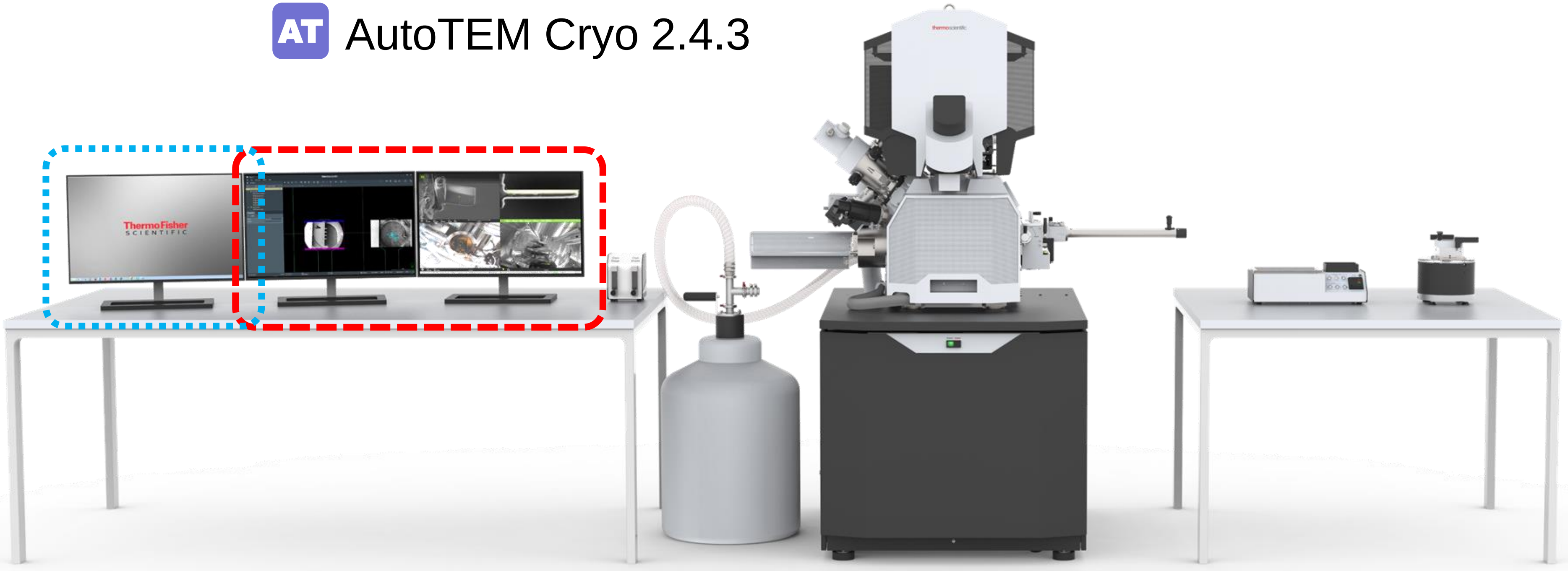
Aquilos 2 & Software used in this tutorial

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
Atlas & lamella sites

iFLM (Optional)
Target selection

Pt sputter
Sample conductivity

Pt GIS
Protective coating

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Lamella milling
Preparation, Milling, & thinning

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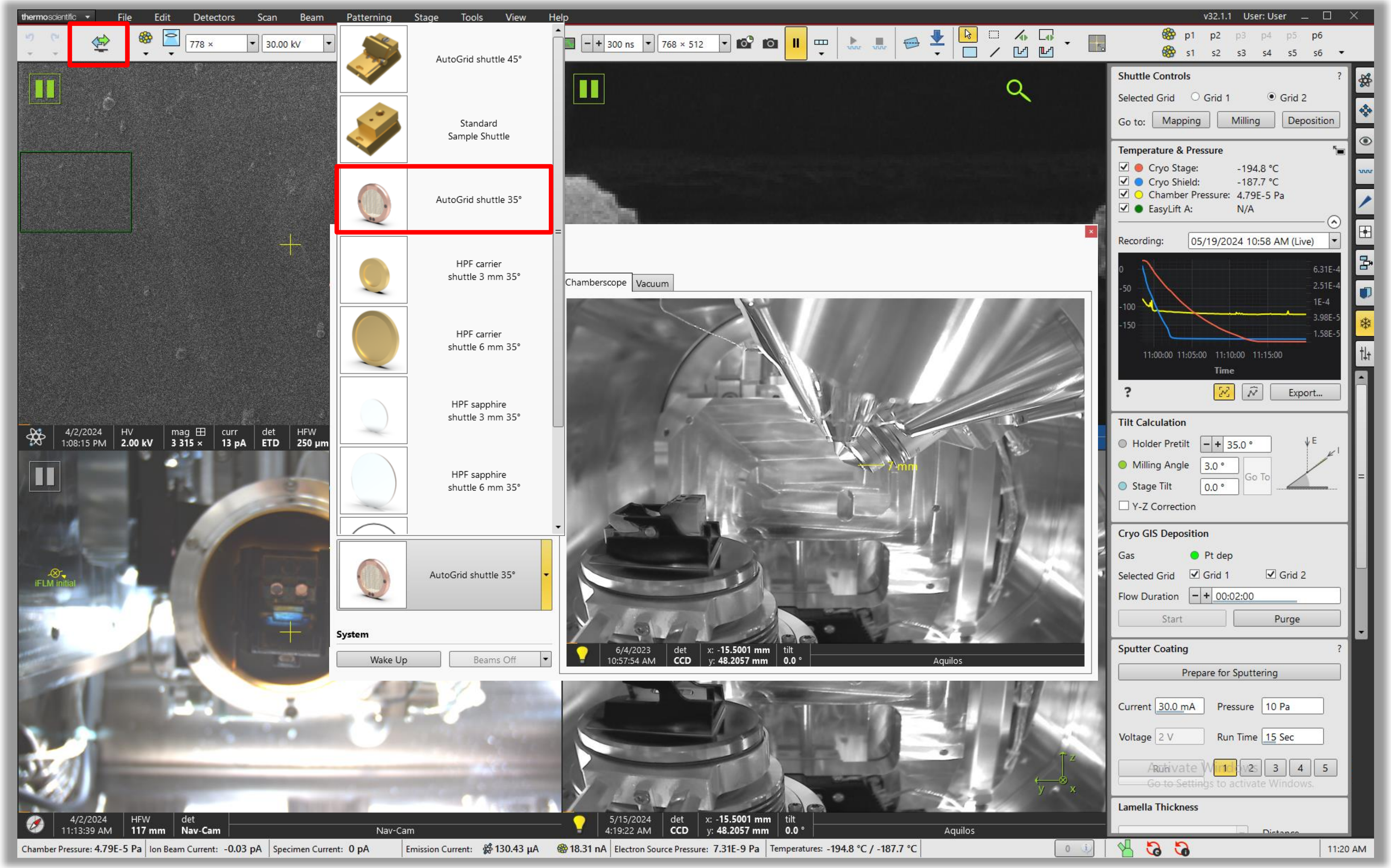
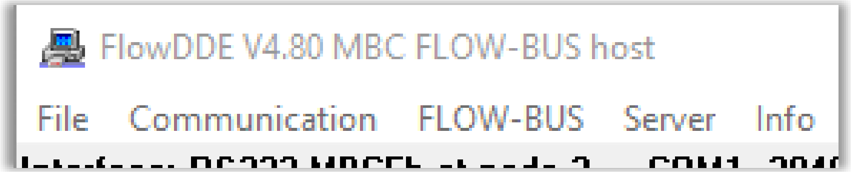
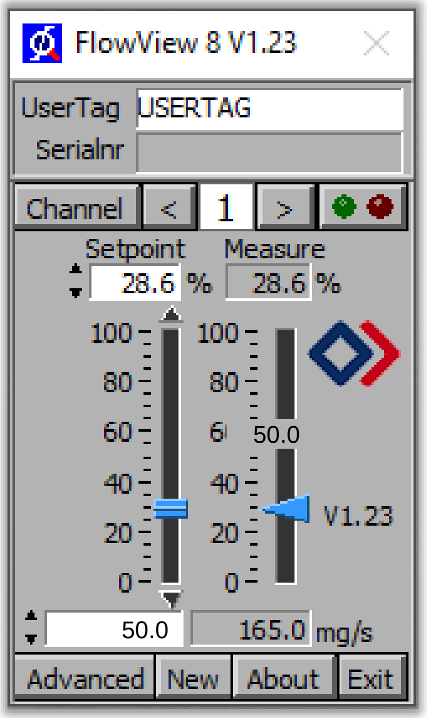
CryoET

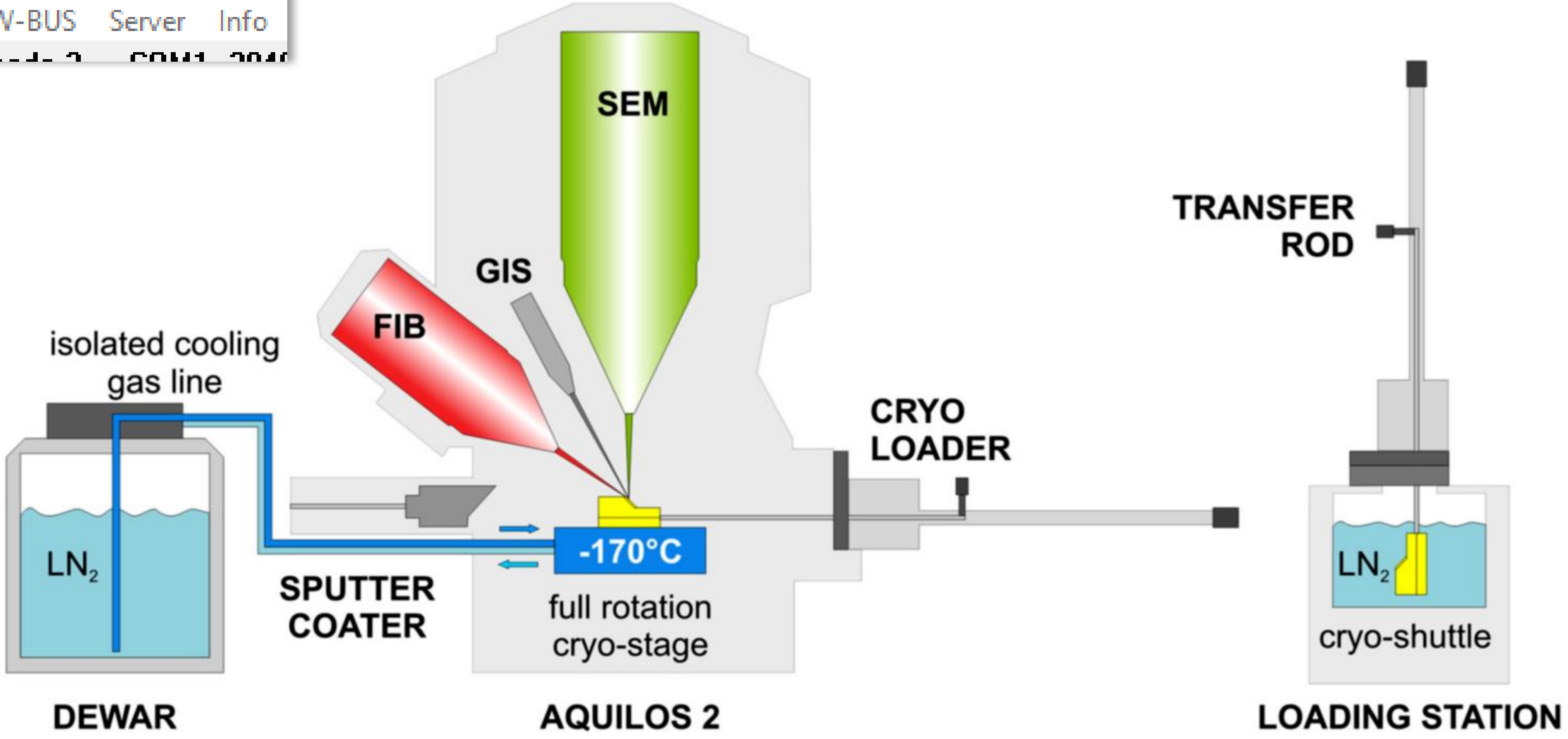


3.2.1 Prepare the system _Purge the system

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





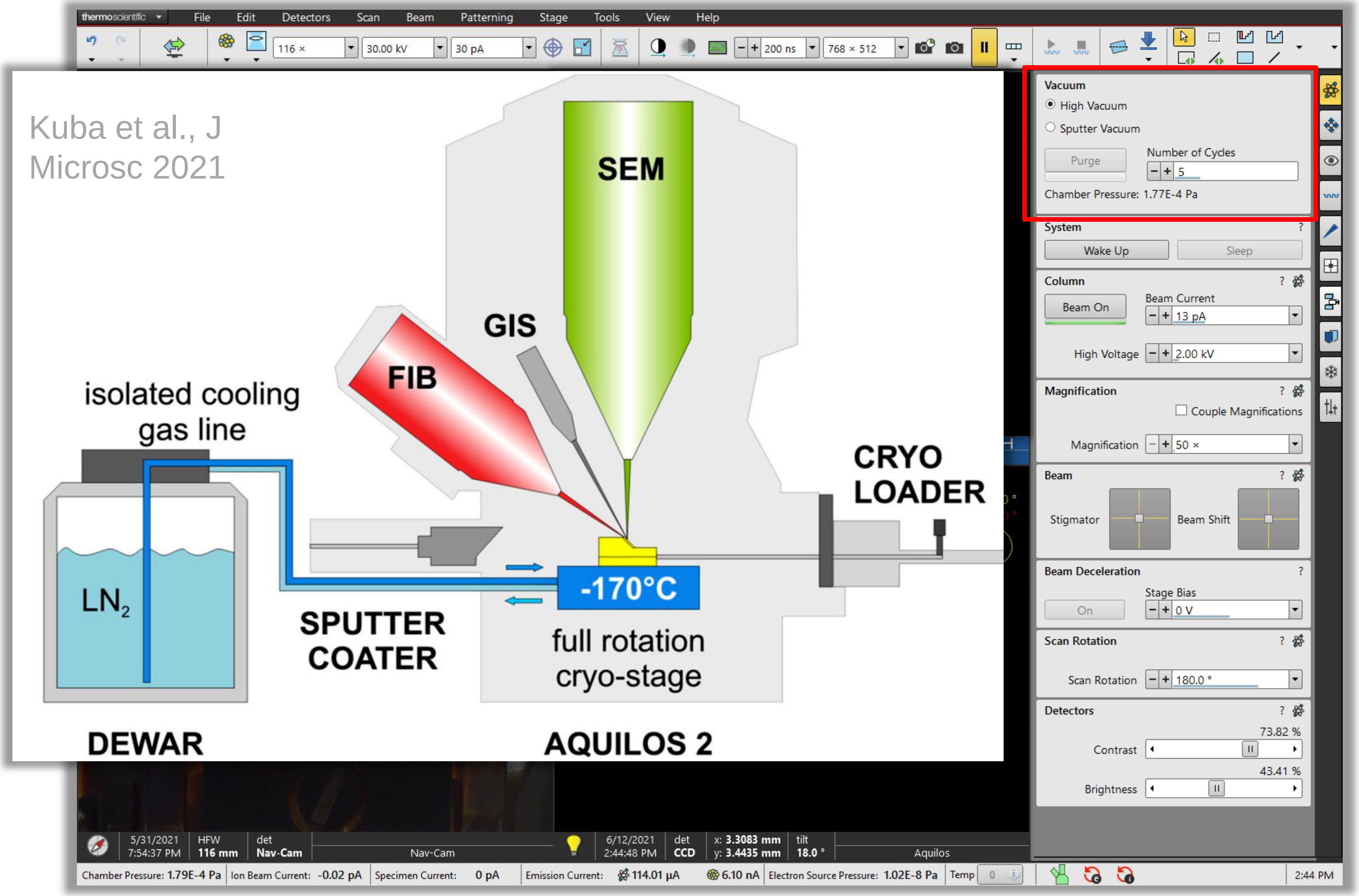


Kuba et al., J Microsc 2021

3.2.1 Prepare the system _Purge the system

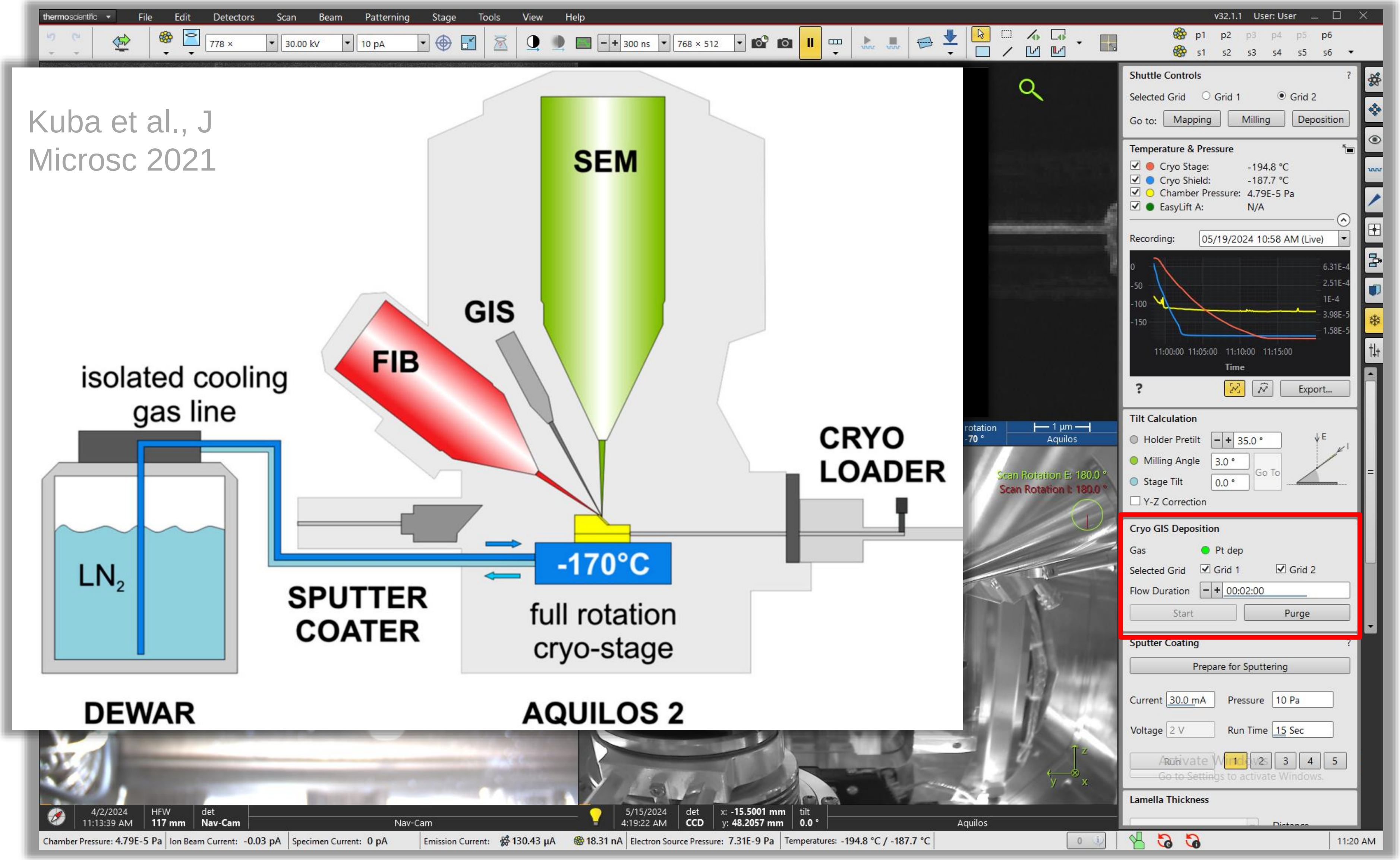
(xT Microscope Control)

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

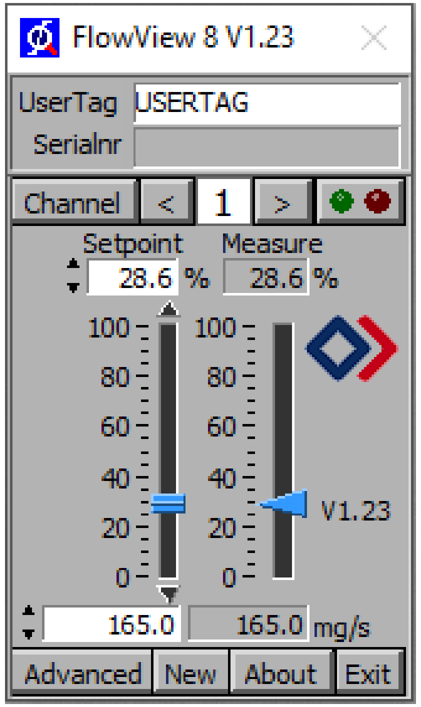


3.2.1 Prepare the system _Purge the system

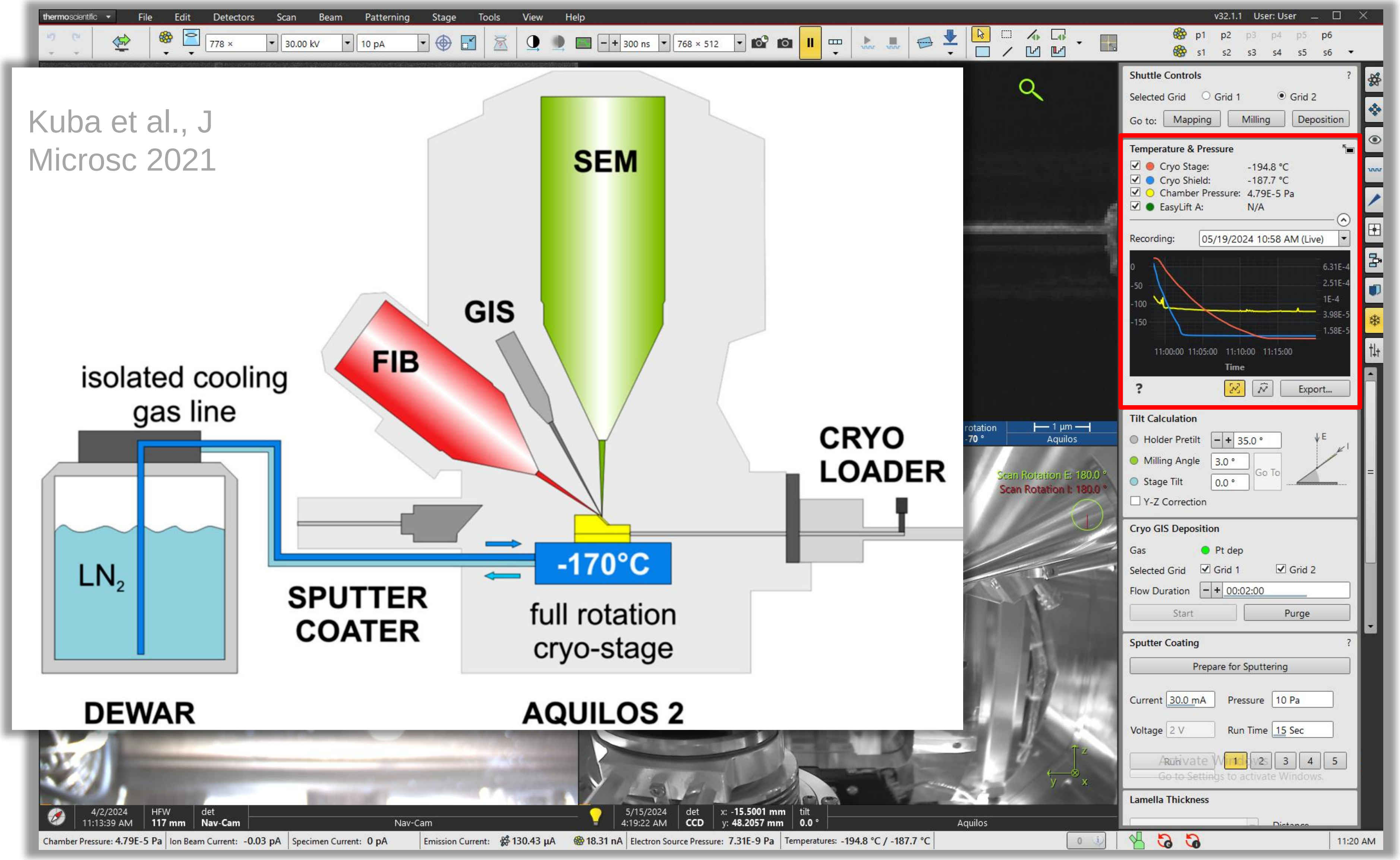
- Cooling-gas line, Loading station, Argon line, GIS (2 mins).



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

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,
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Target confirmation

Pt sputter (Optional)
Lamella conductivity

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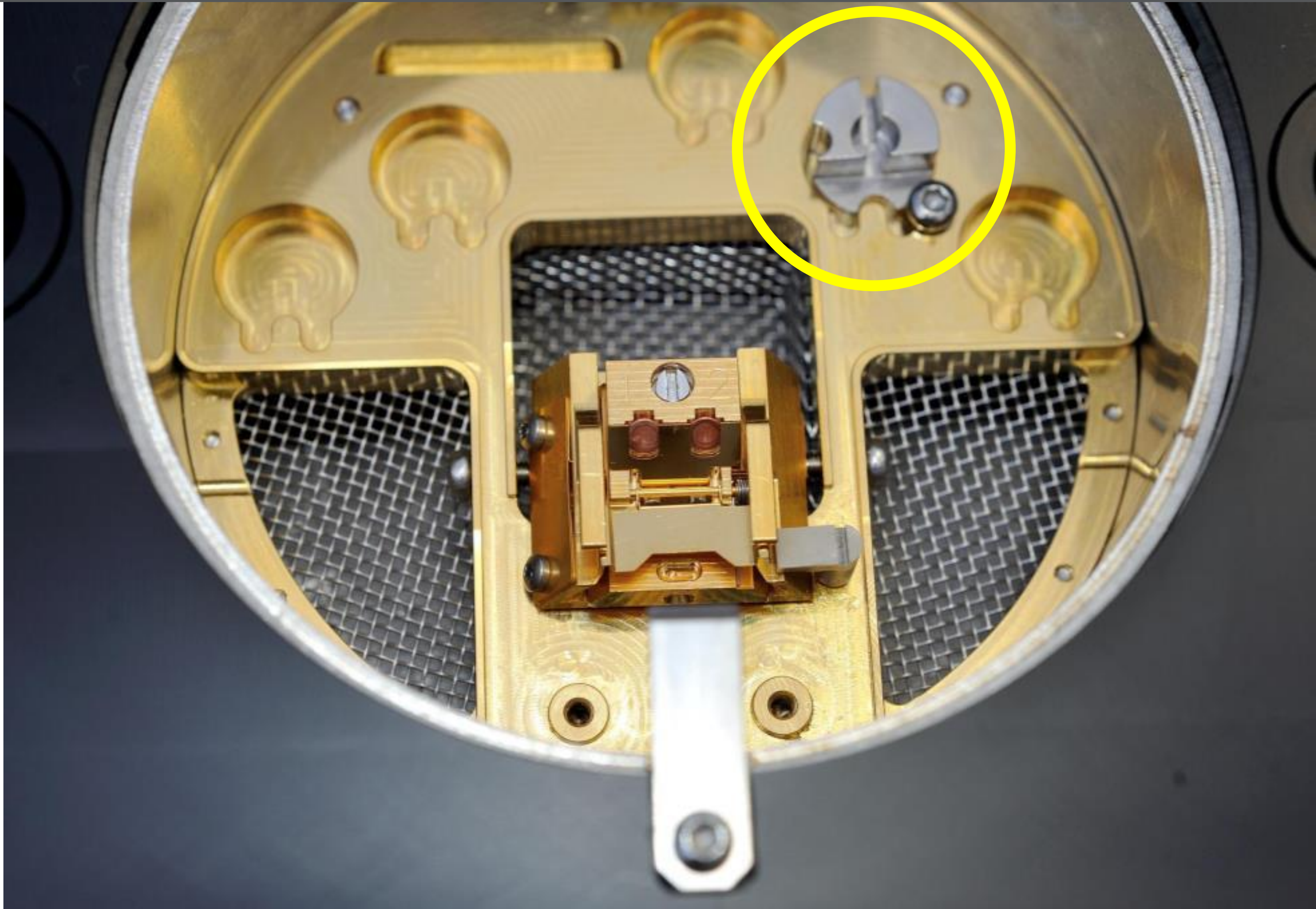
CryoET



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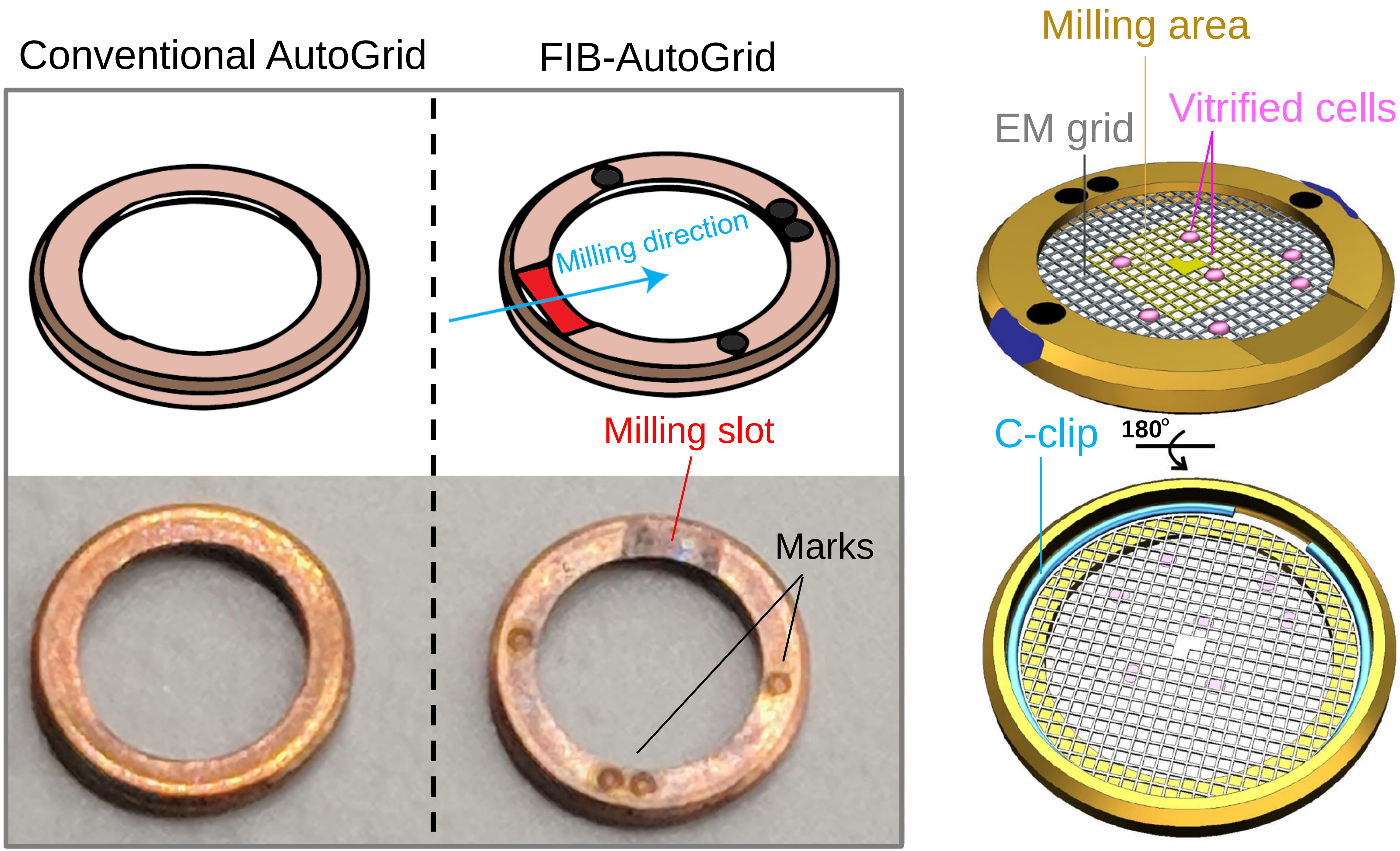

Adapted from Wagner, et al., Nature Protocols 2020

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

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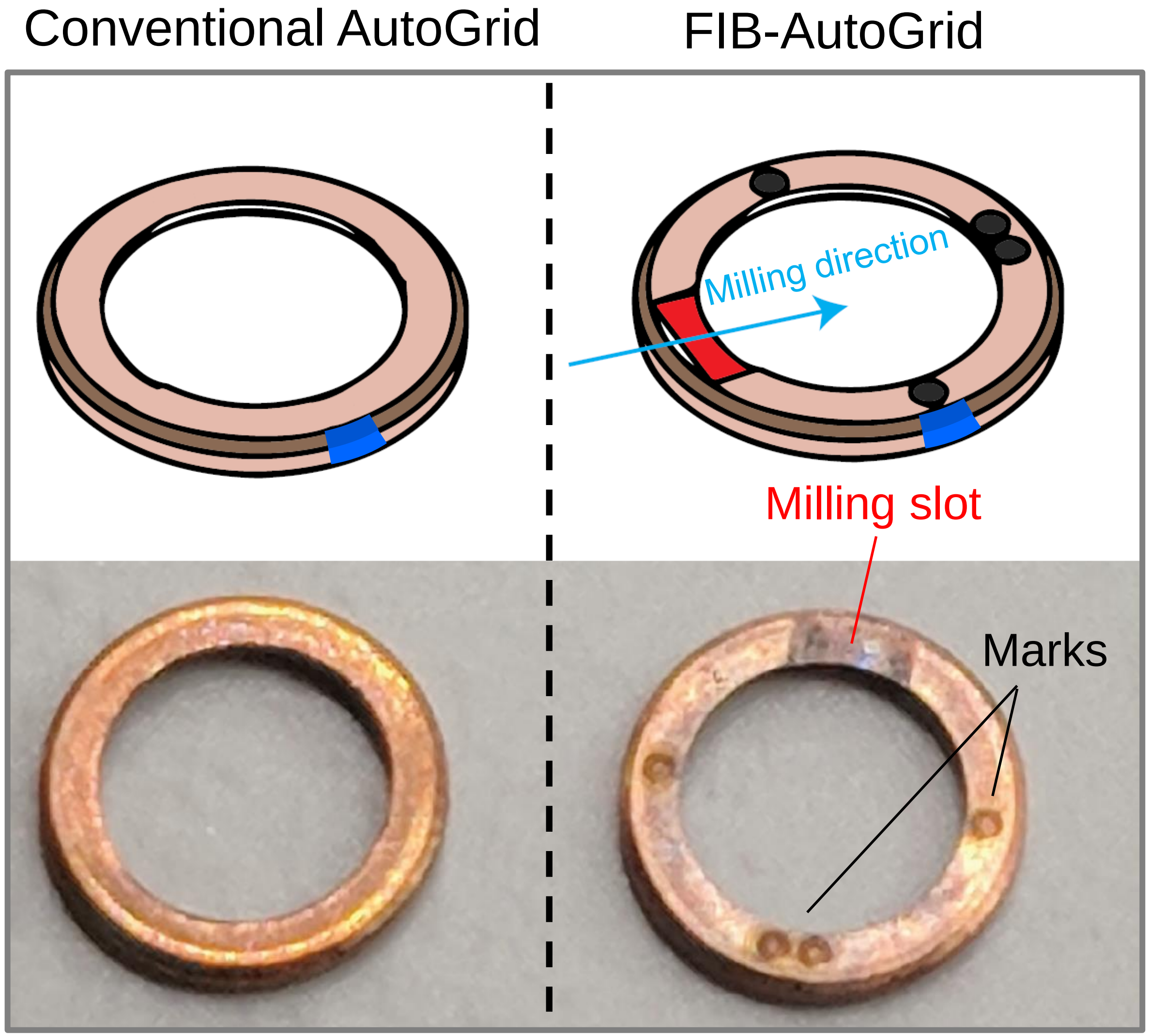

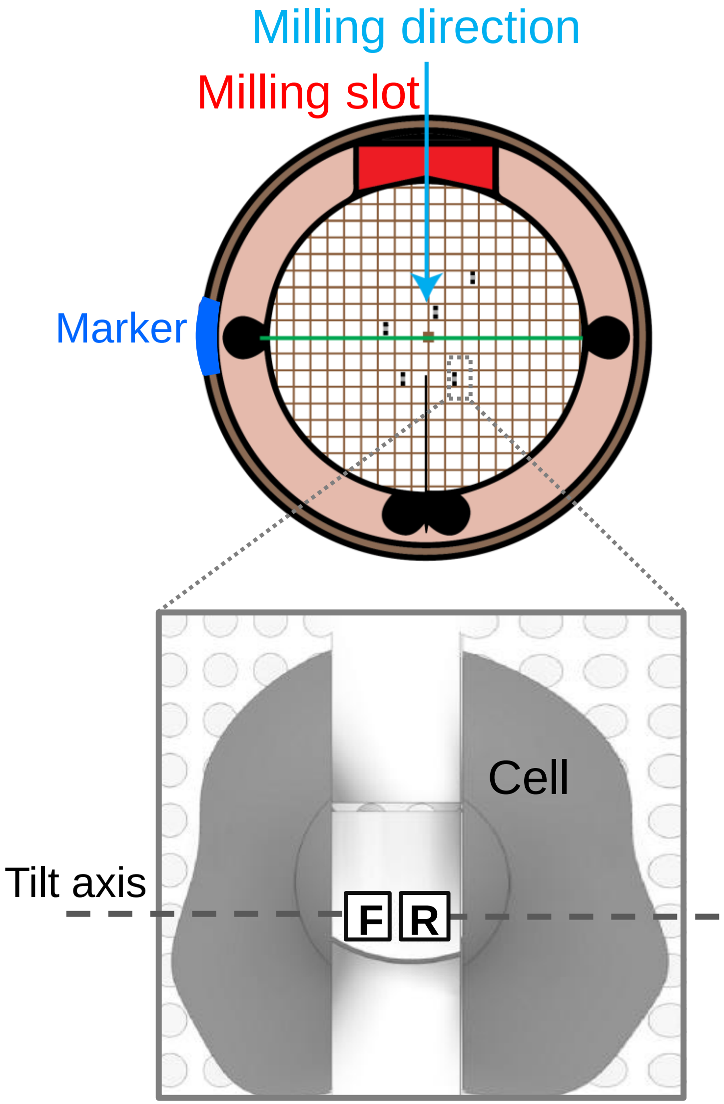

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Sample conductivity

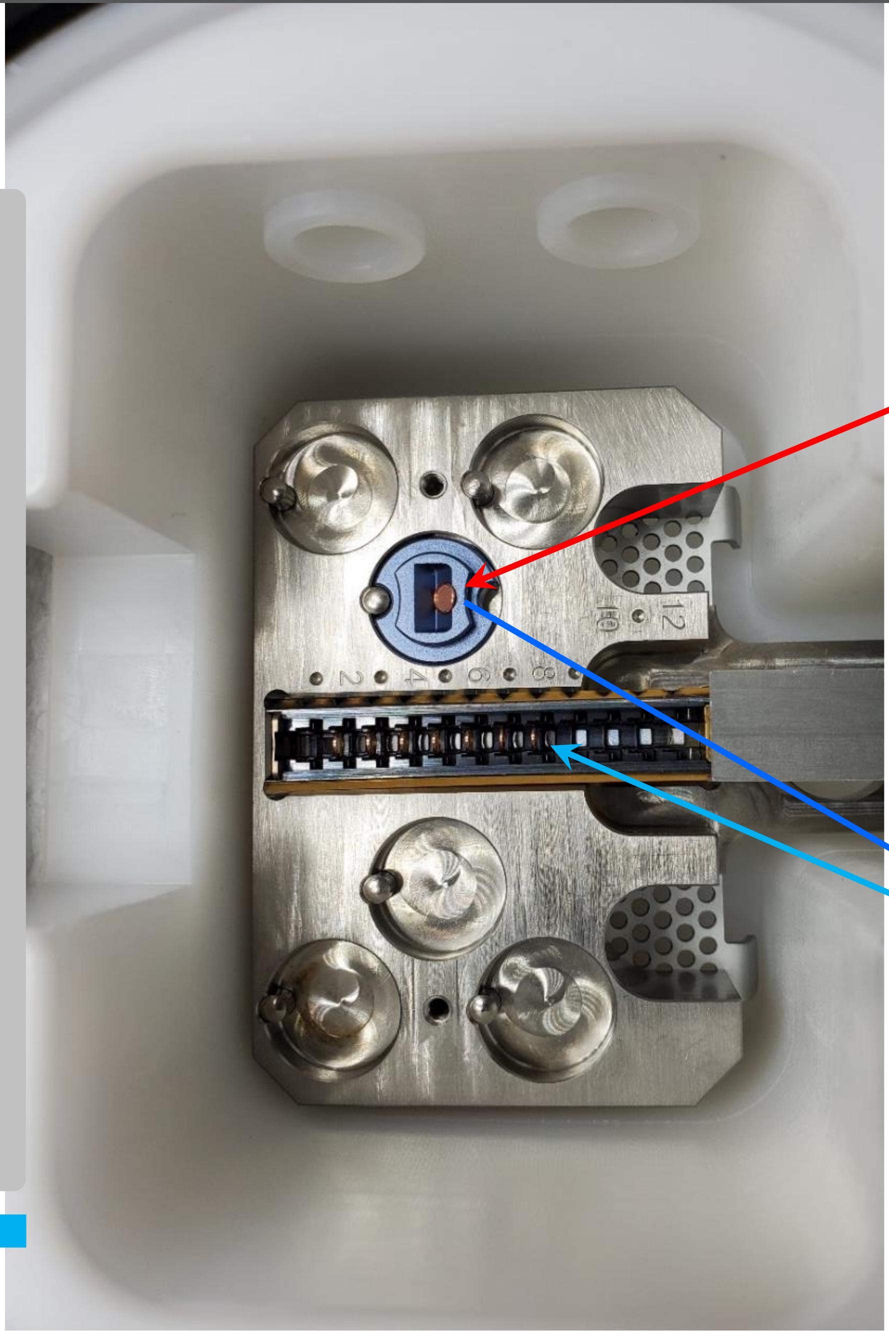
Lamella milling
Preparation, Milling, & thinning

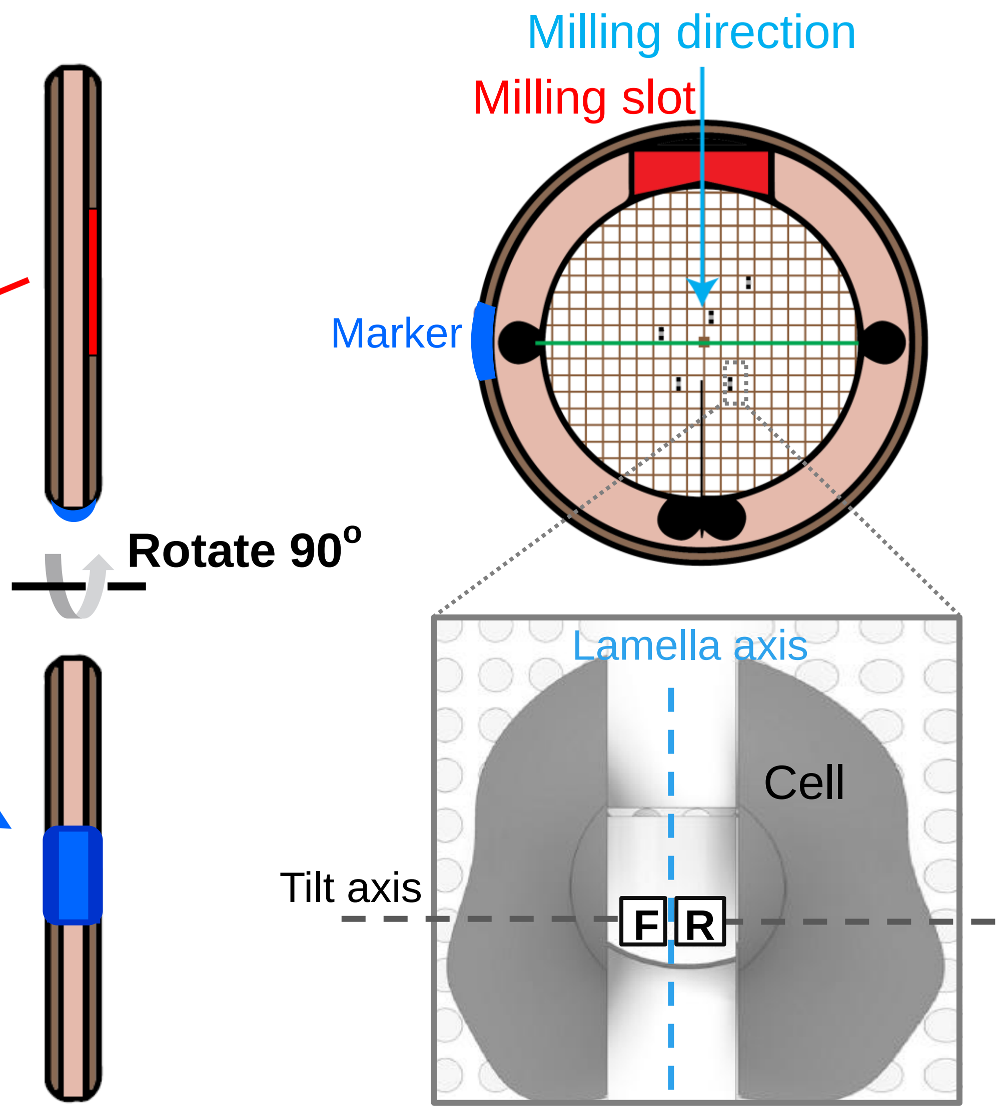
iFLM (Optional)
Target confirmation

Pt sputter (Optional)
Lamella conductivity

↓ T

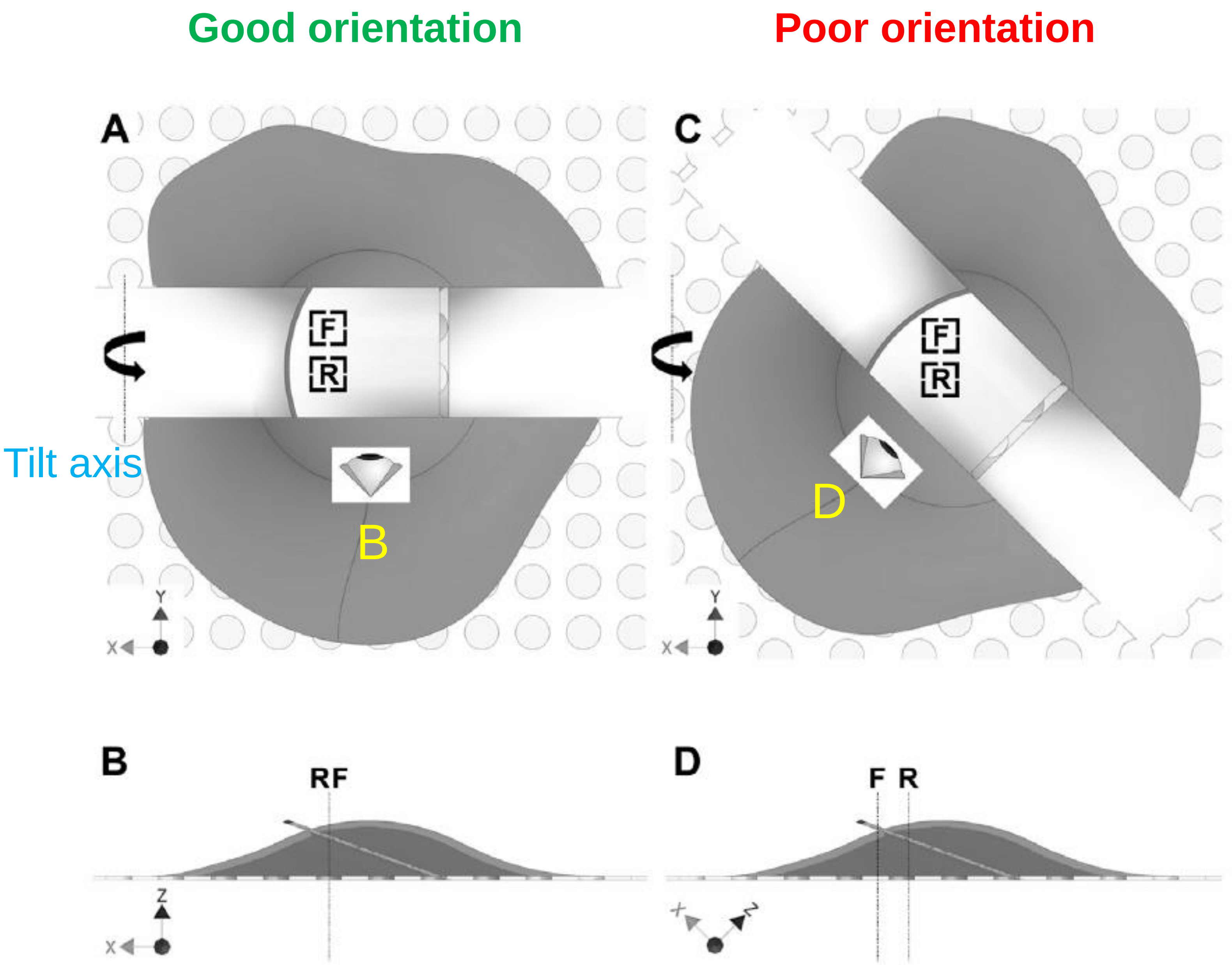
CryoET





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

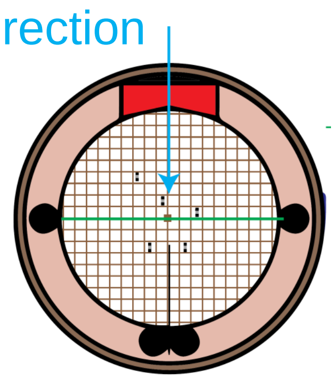
Lamella milling
Preparation, Milling,
& thinning

iFLM (Optional)
Target confirmation

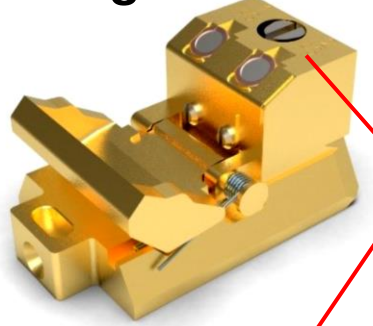
Pt sputter (Optional)
Lamella conductivity

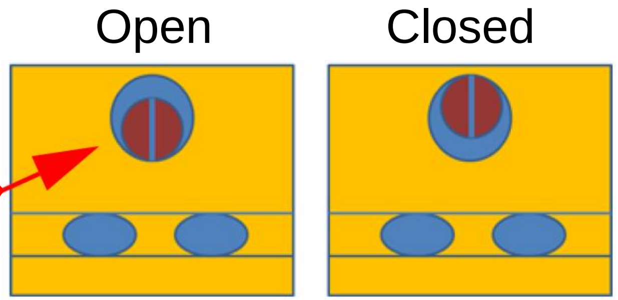
↓ **T**

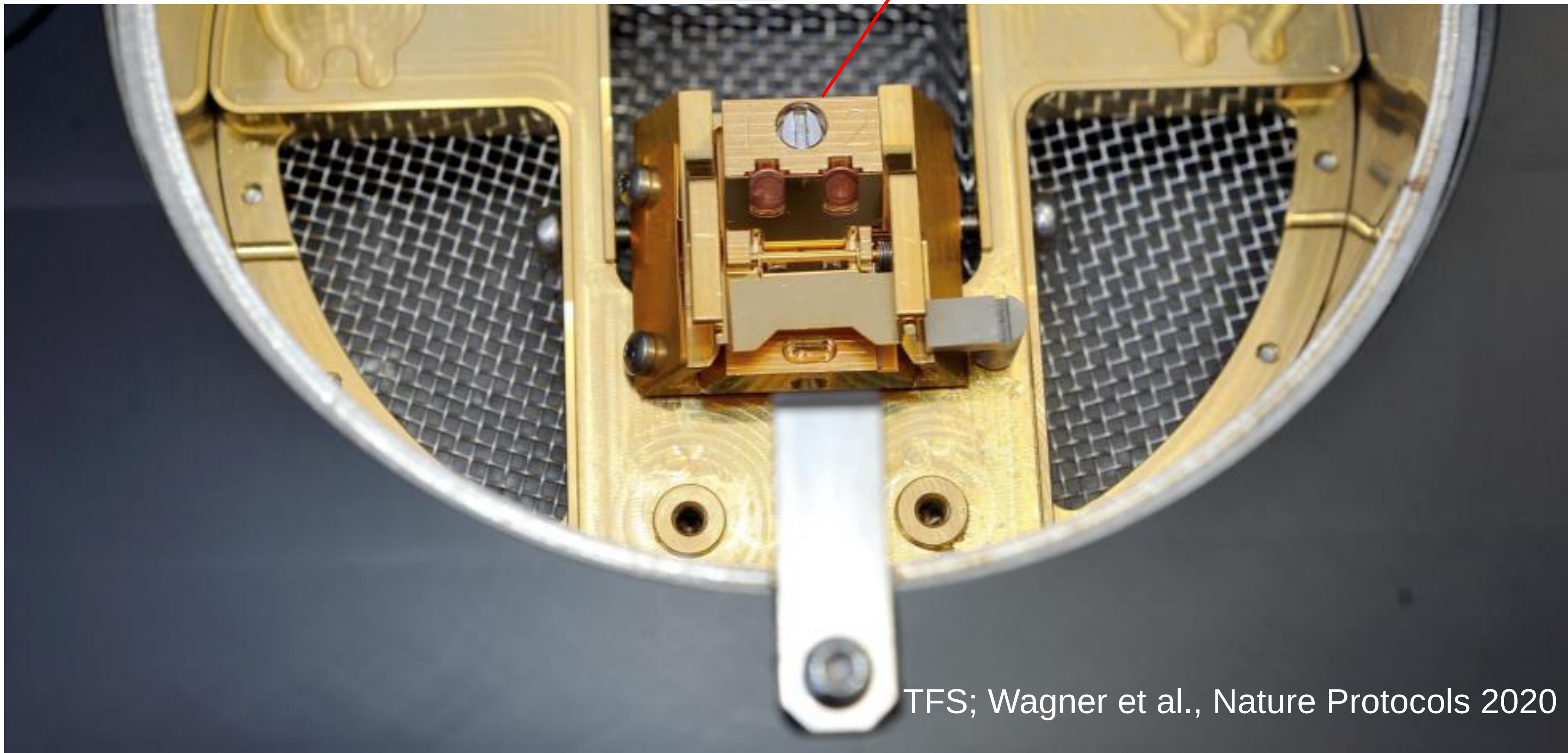
CryoET



Autogrid shuttle

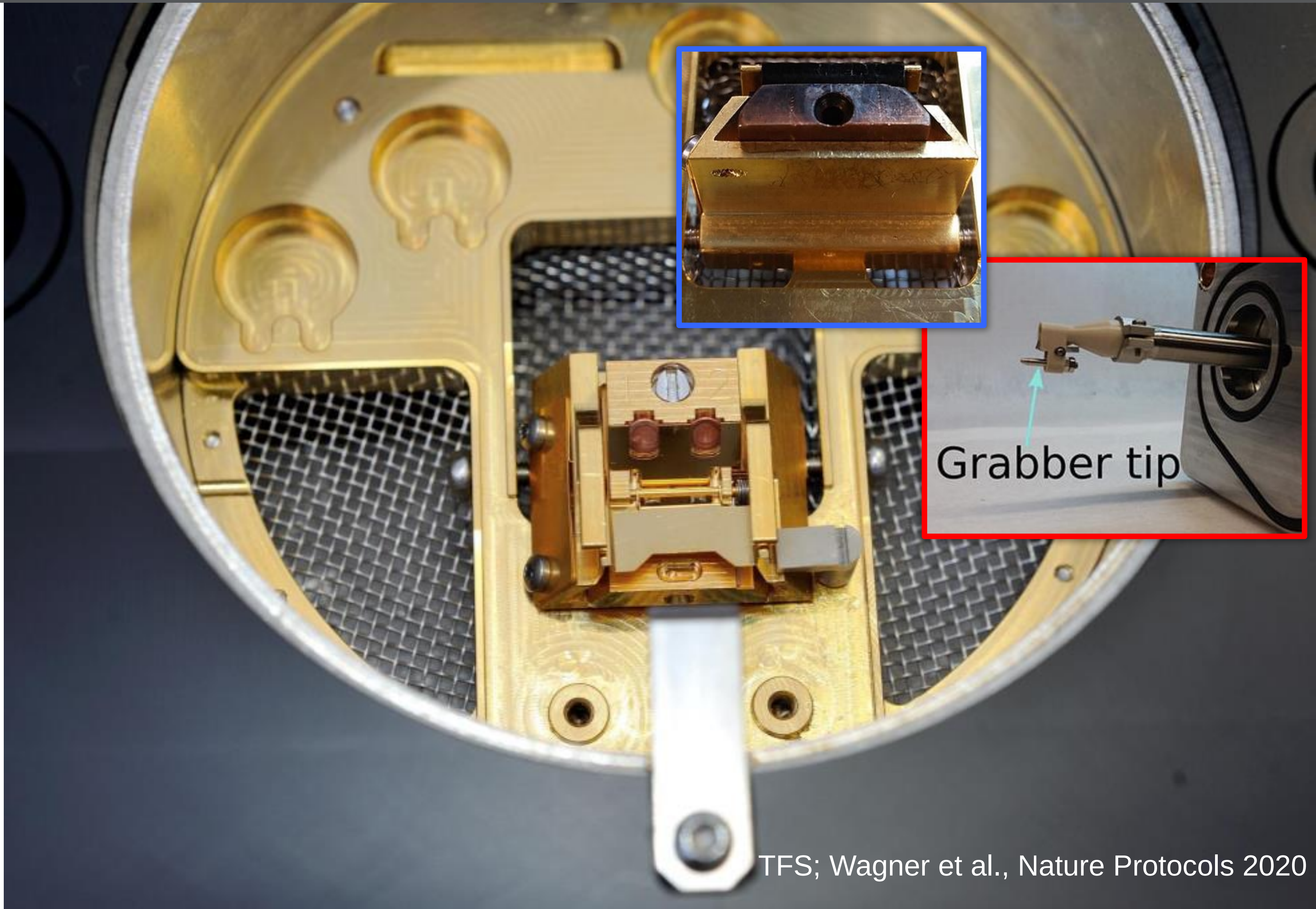






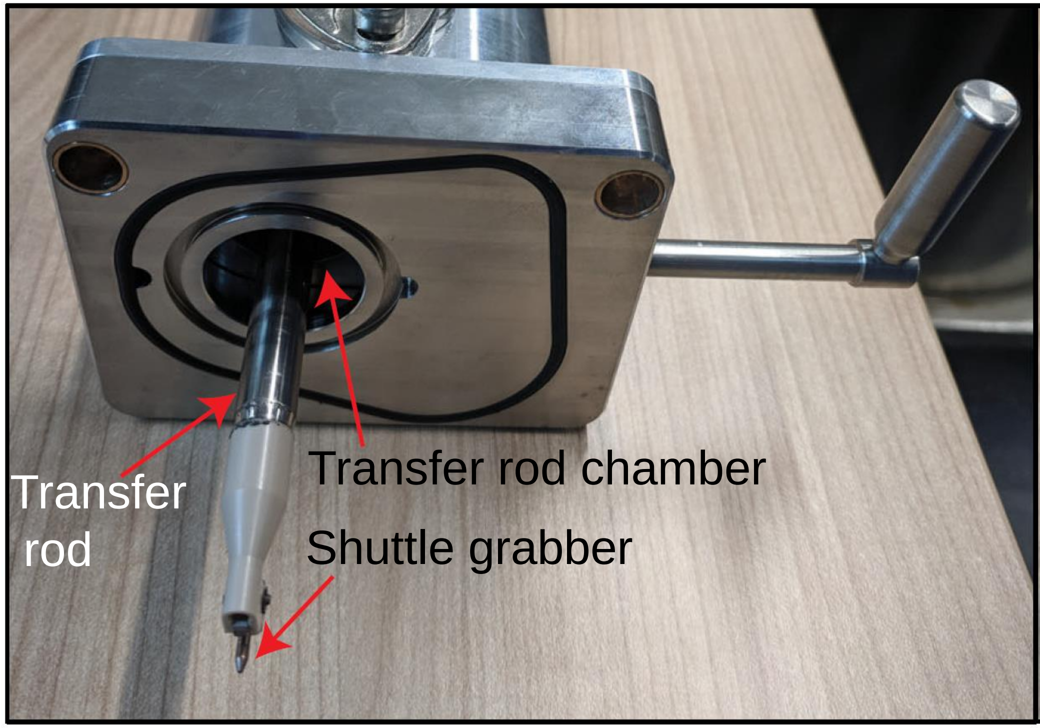
TFS; Wagner et al., Nature Protocols 2020

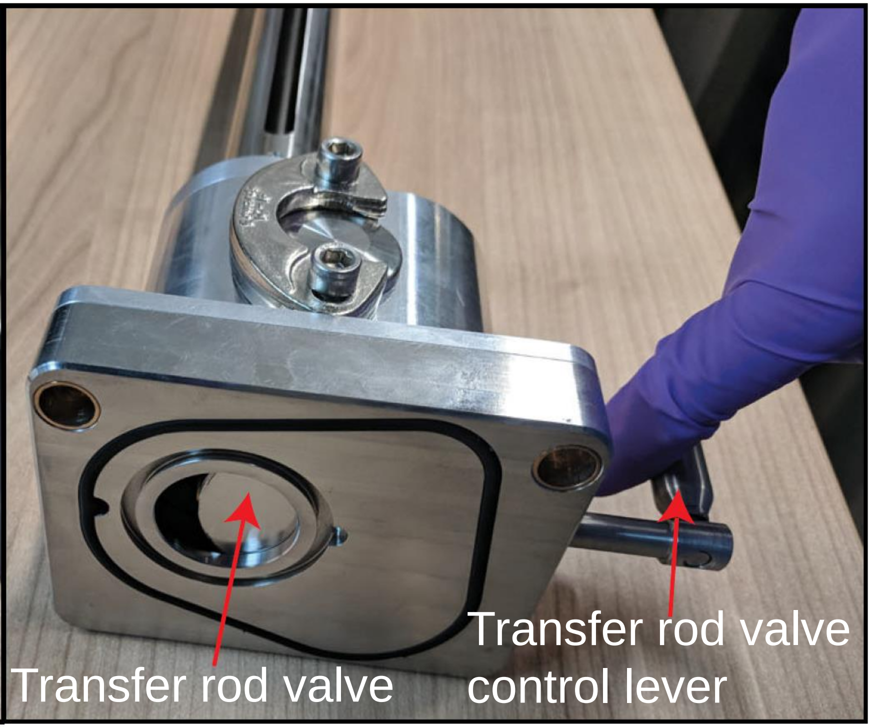
3.2.3 Transfer the grids _Load shuttle to Transfer rod



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

↓ 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)

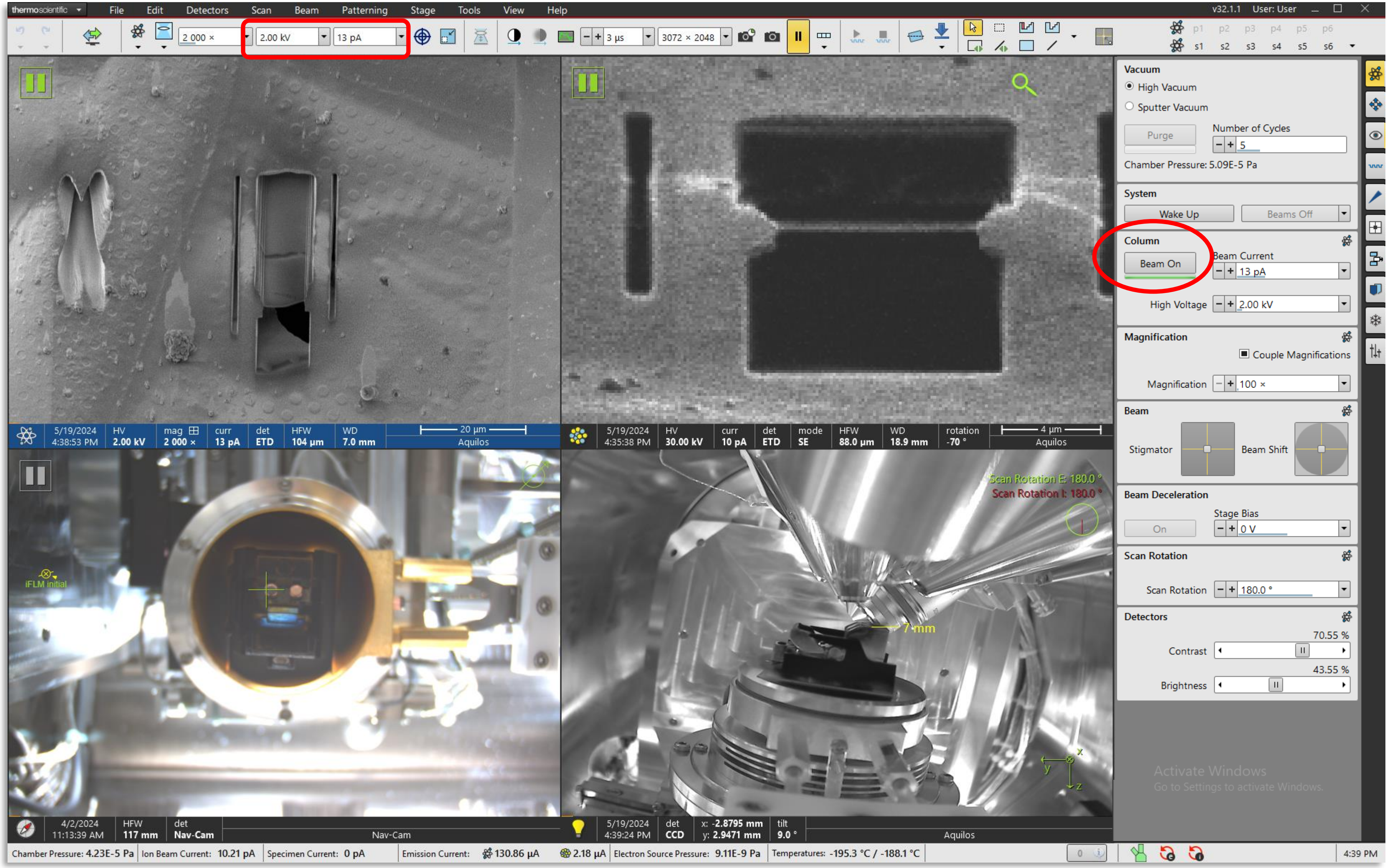
Target confirmation

Pt sputter (Optional)

Lamella conductivity

↓ T

CryoET



3.3 Sample screening _Link Z to FWD

(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)

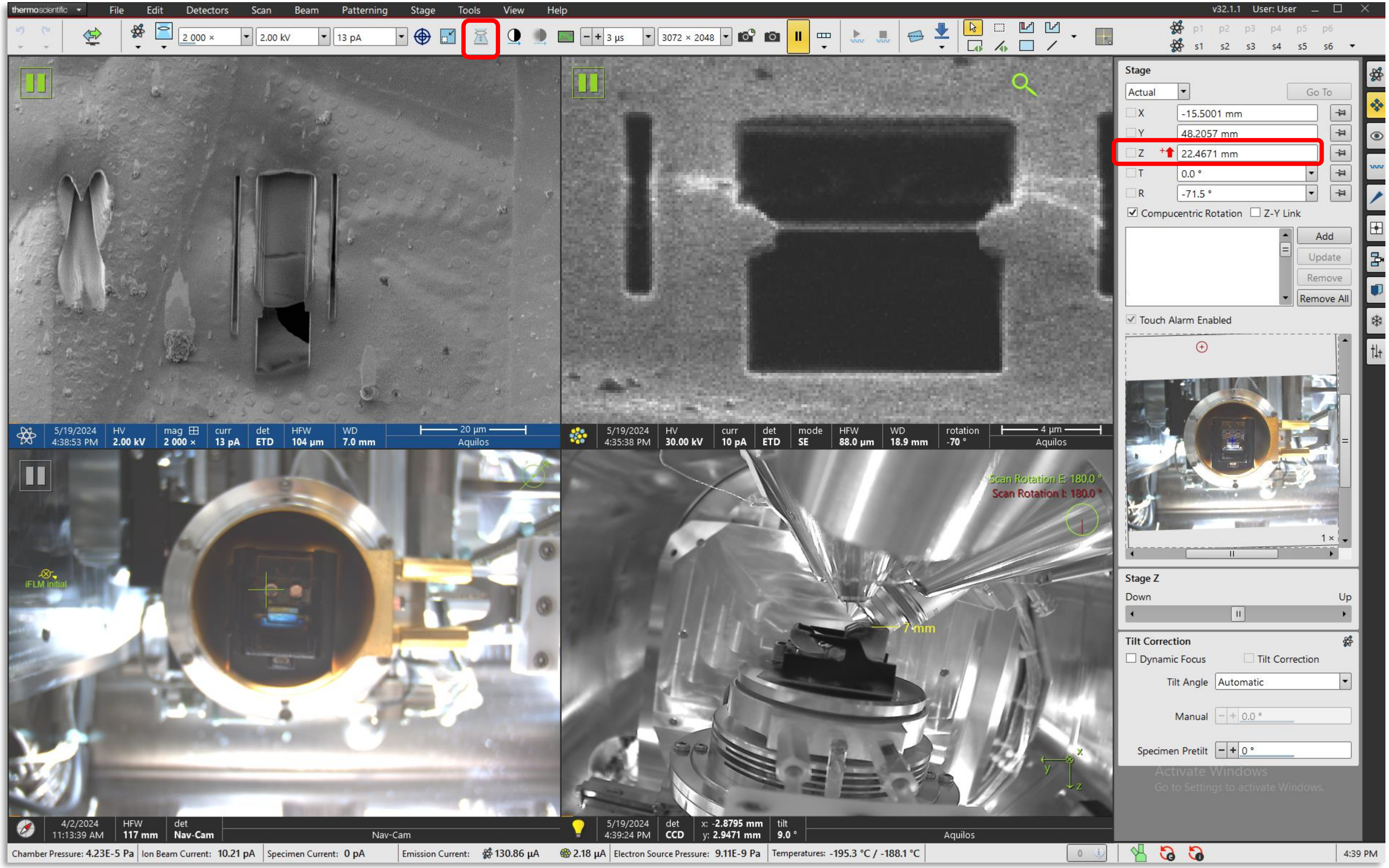
Target confirmation

Pt sputter (Optional)

Lamella conductivity

↓ T

CryoET



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)

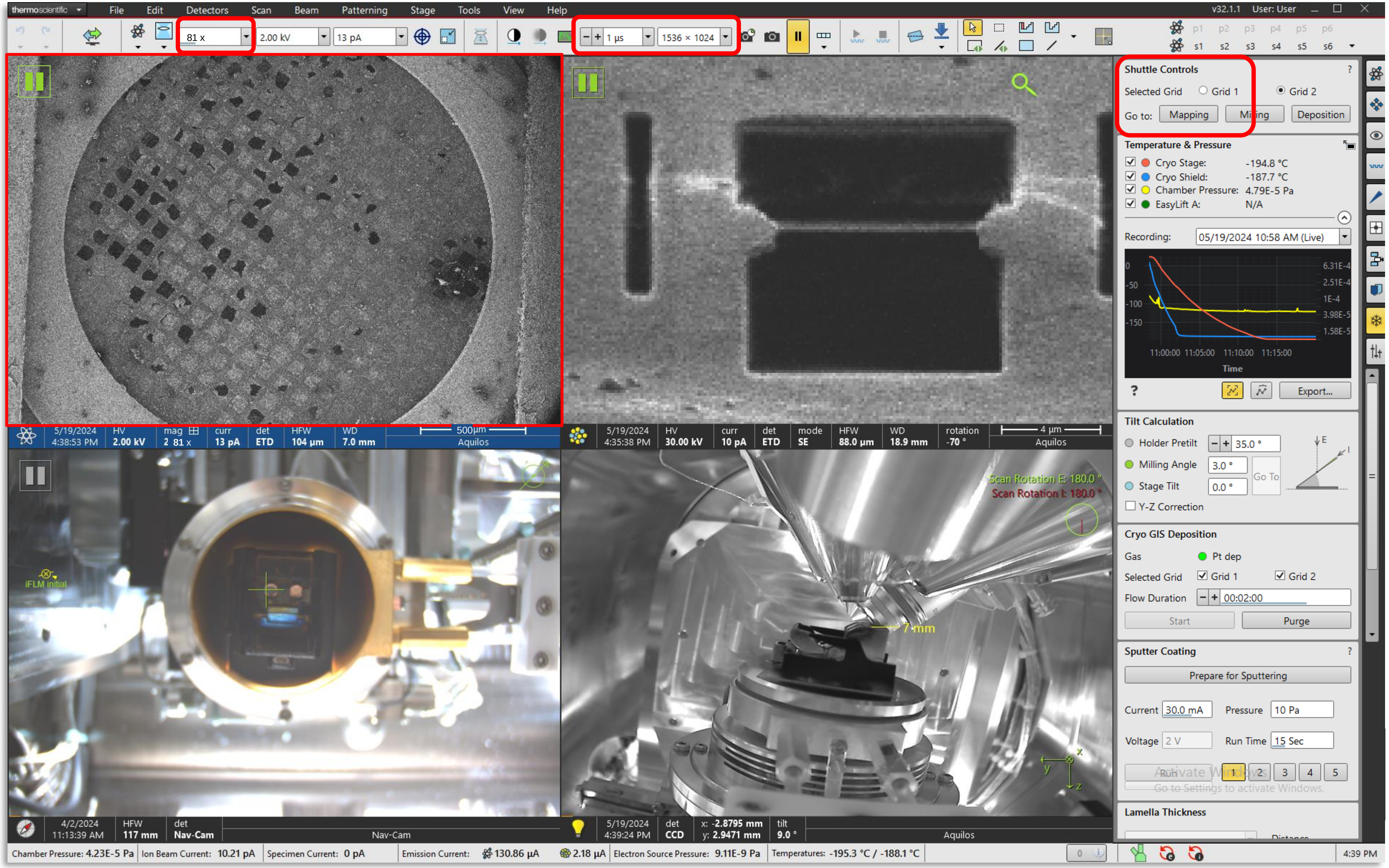
Target confirmation

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)

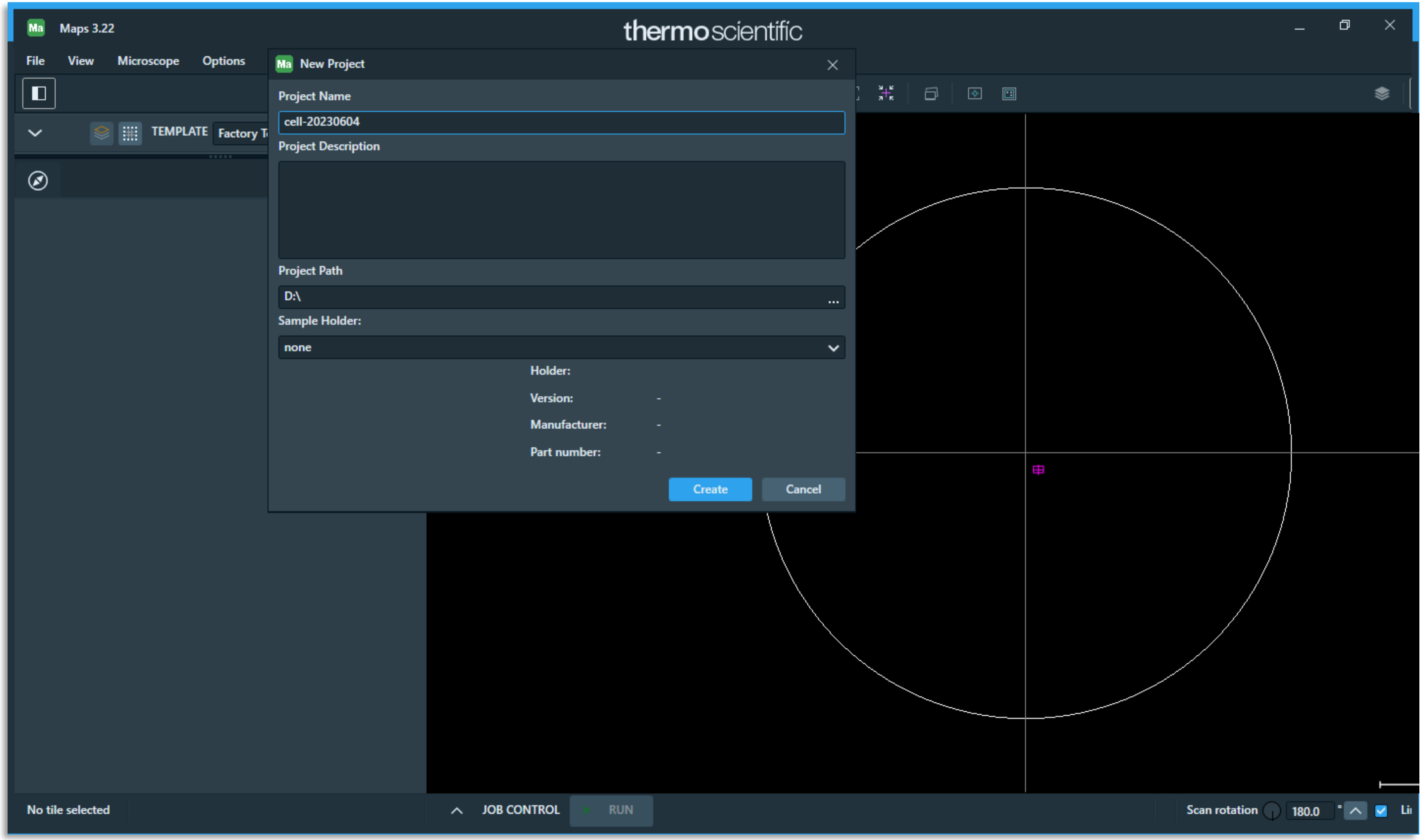
Target confirmation

Pt sputter (Optional)

Lamella conductivity

T

CryoET



3.3 Sample screening _Take a snapshoot of the grid

(Ma Maps)

Vitrification

↓ 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)

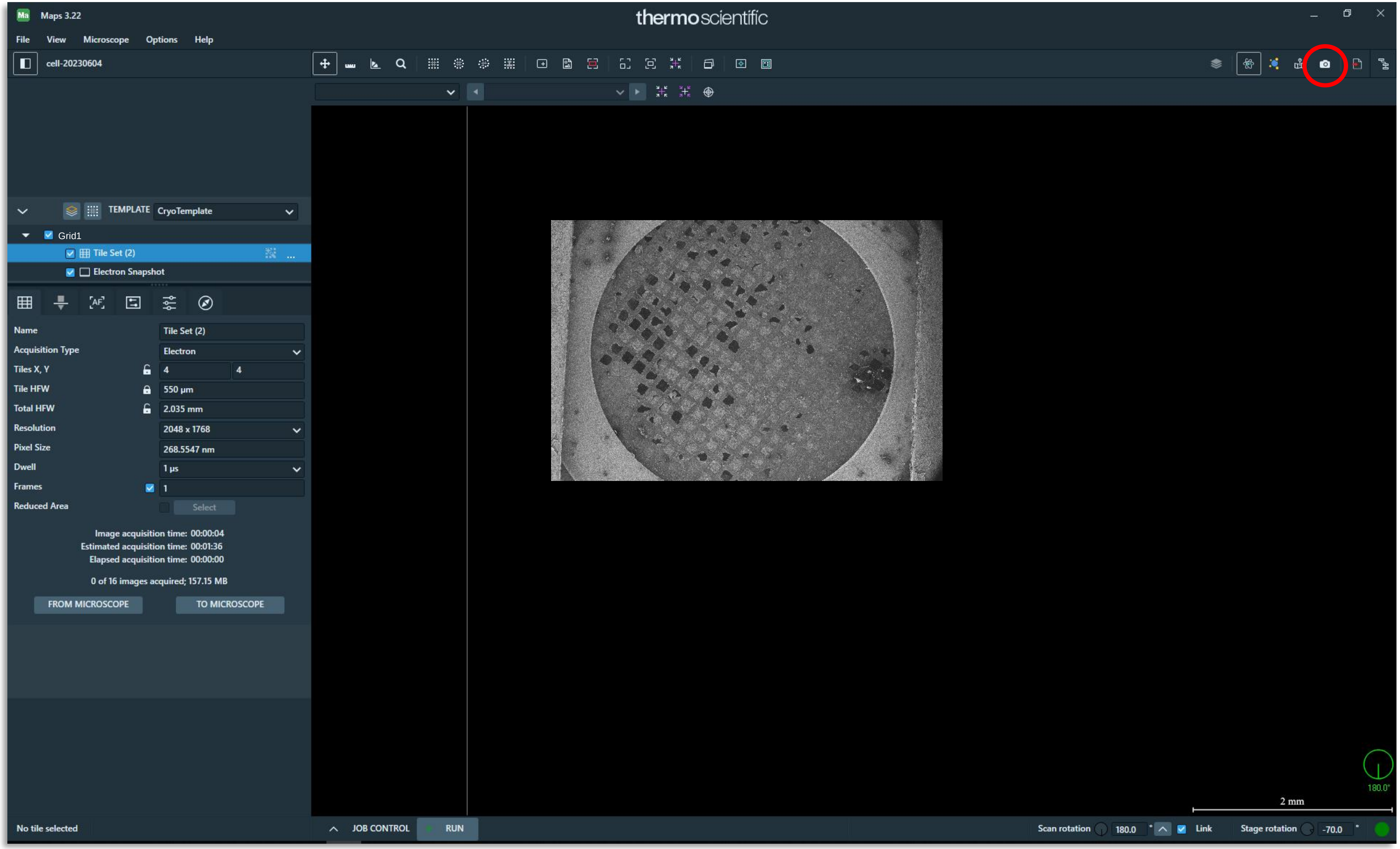
Target confirmation

Pt sputter (Optional)

Lamella conductivity

↓ T

CryoET



3.3 Sample screening _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)

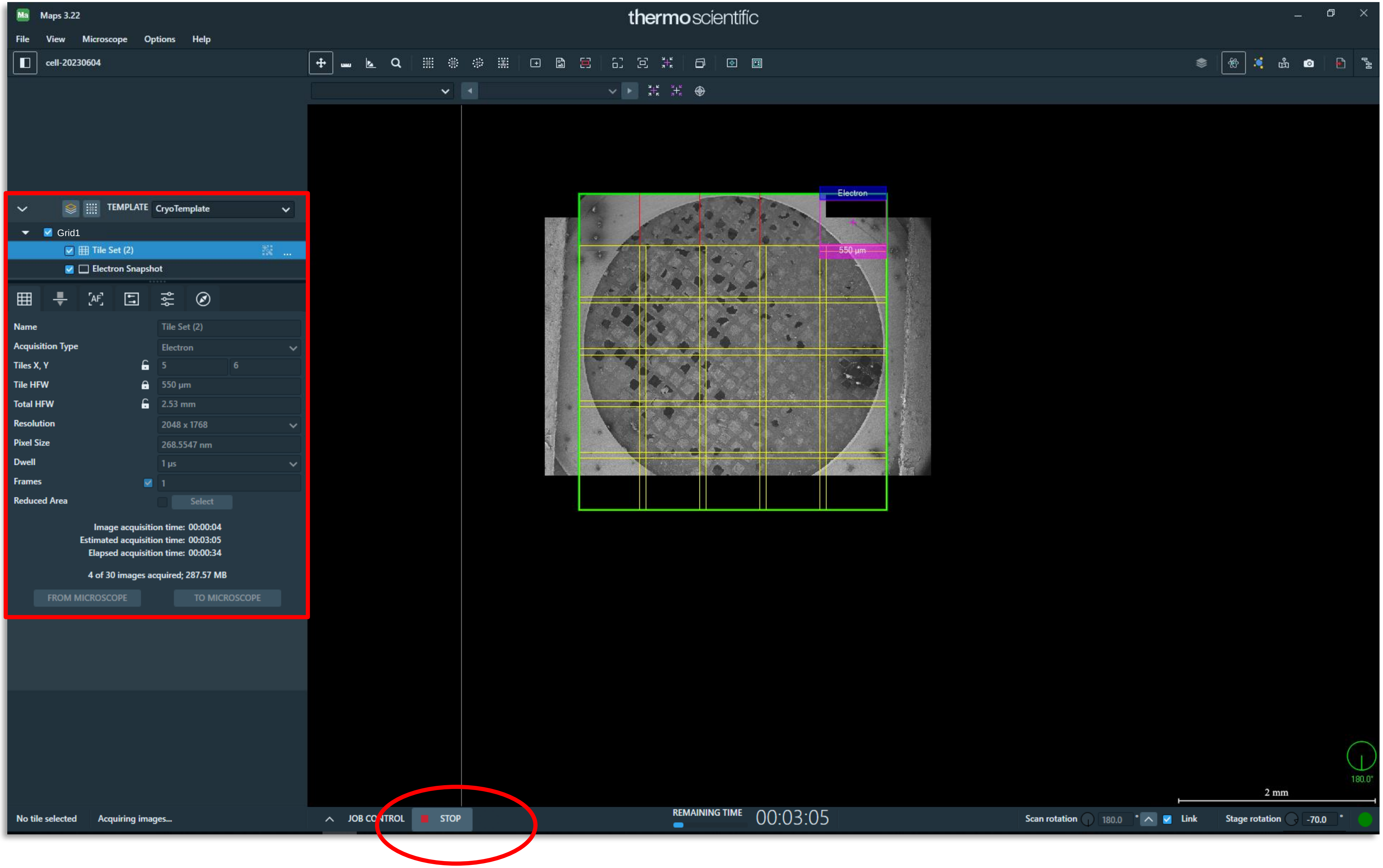
Target confirmation

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

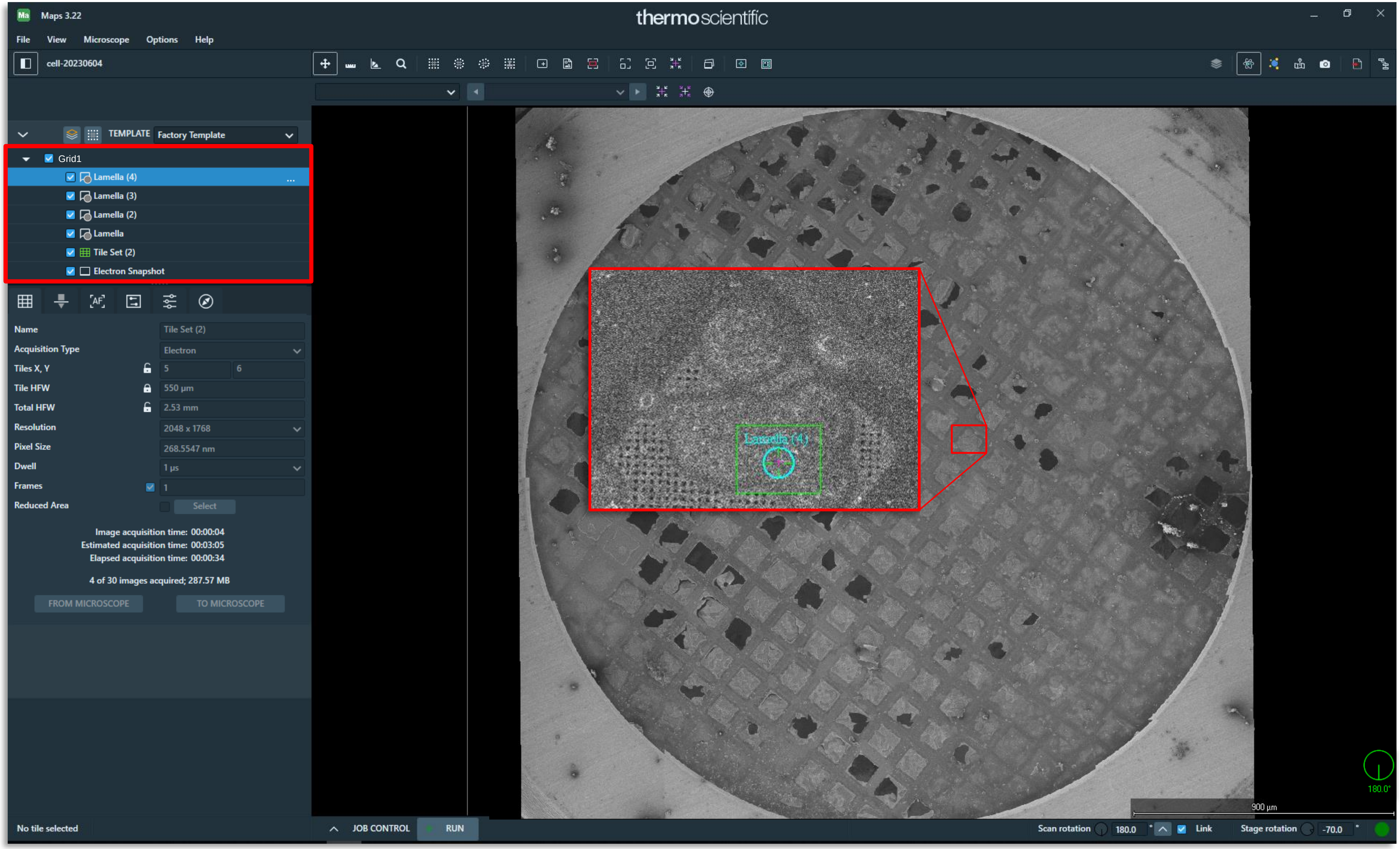
Lamella milling
Preparation, Milling, & thinning

iFLM (Optional)
Target confirmation

Pt sputter (Optional)
Lamella conductivity

↓

CryoET



3.4 CryoFLM for tart 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

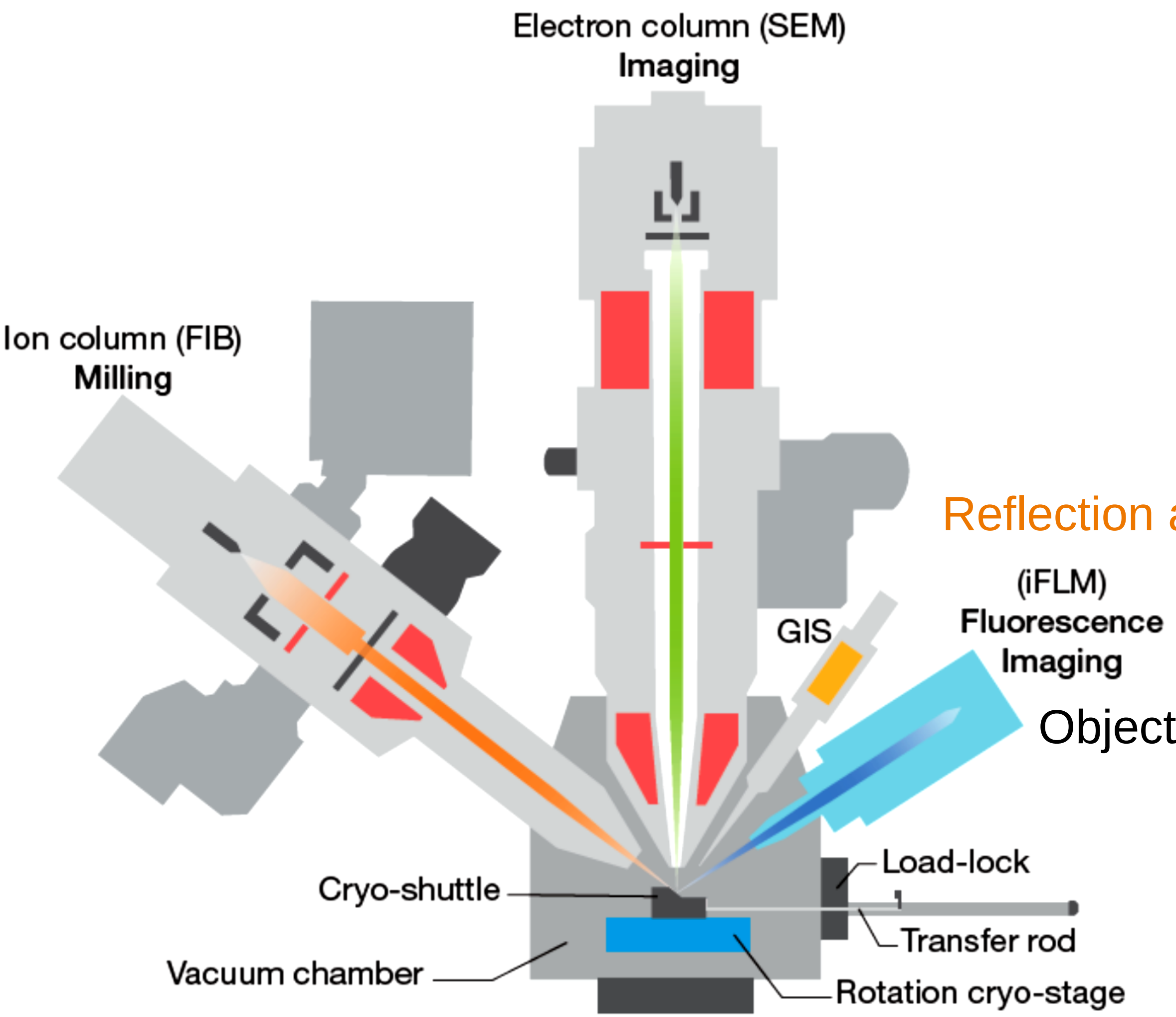
Lamella milling
Preparation, Milling, & thinning

iFLM (Optional)
Target confirmation

Pt sputter (Optional)
Lamella conductivity

↓ T

CryoET



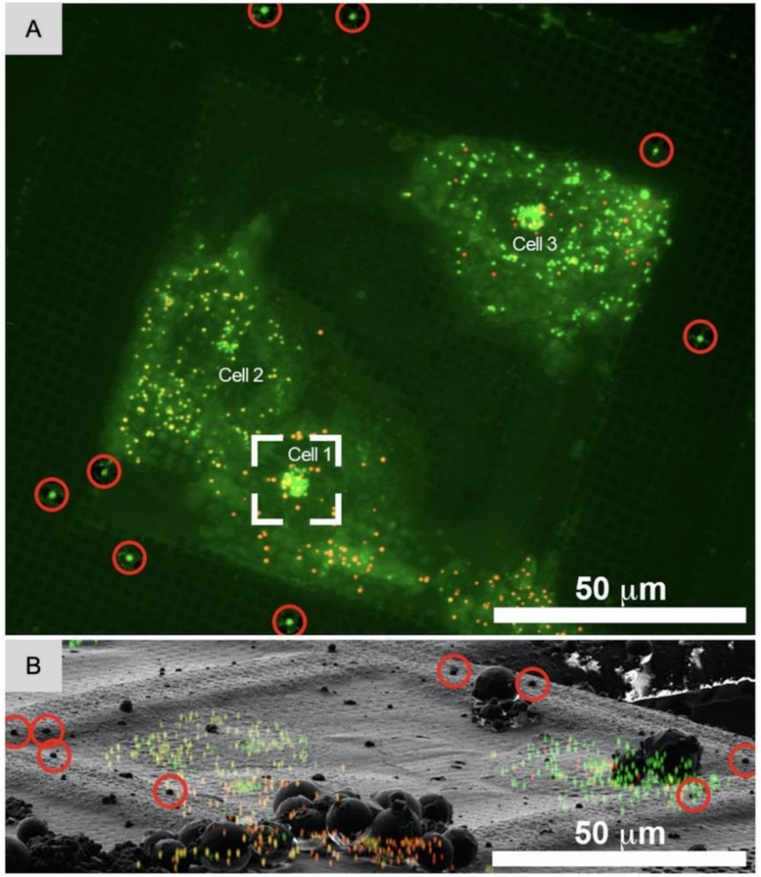
Reflection and fluorescence imaging

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

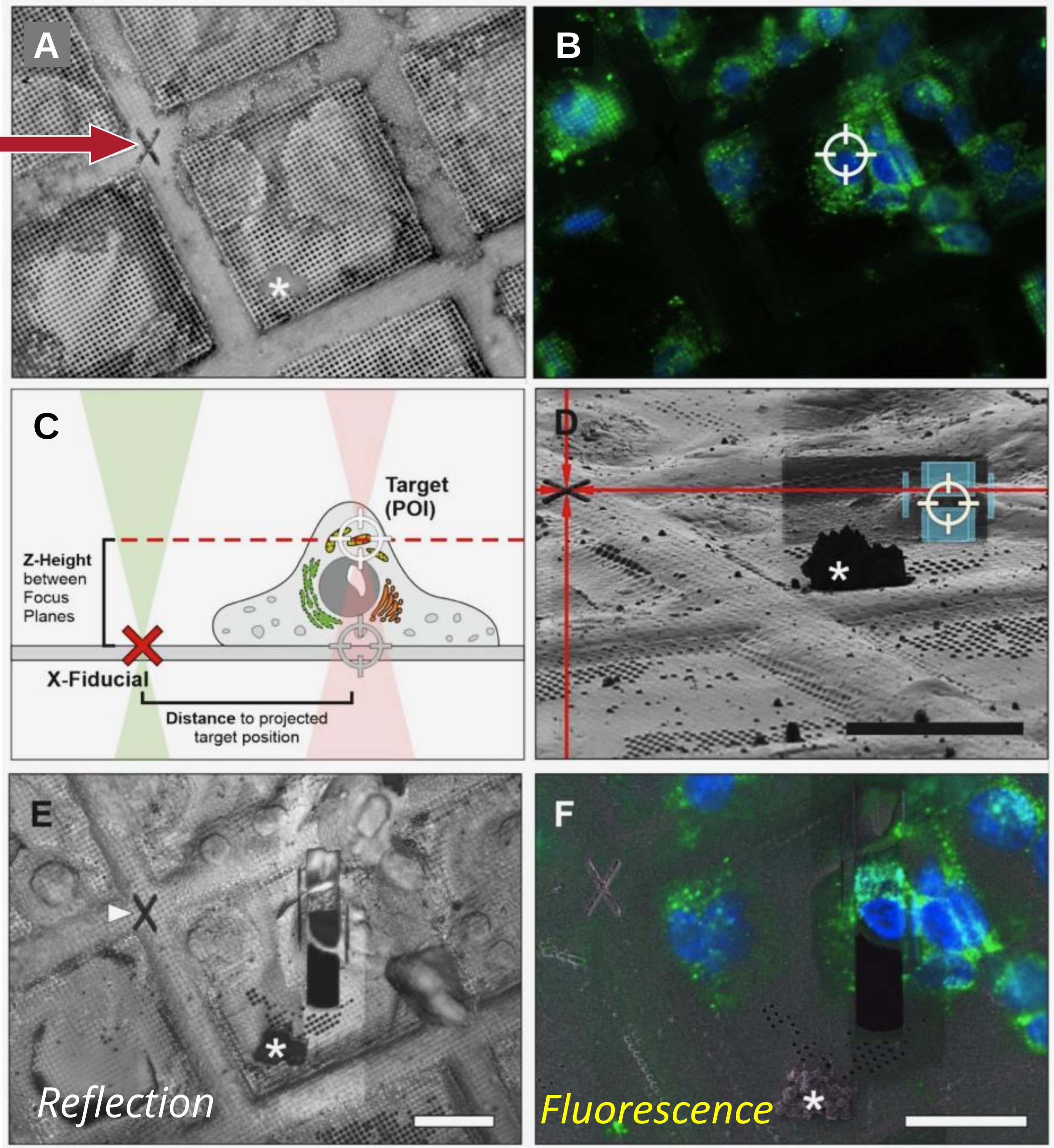


Arnold et al., Biophys J 2016

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

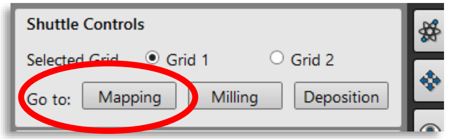
Lamella milling
Preparation, Milling, & thinning

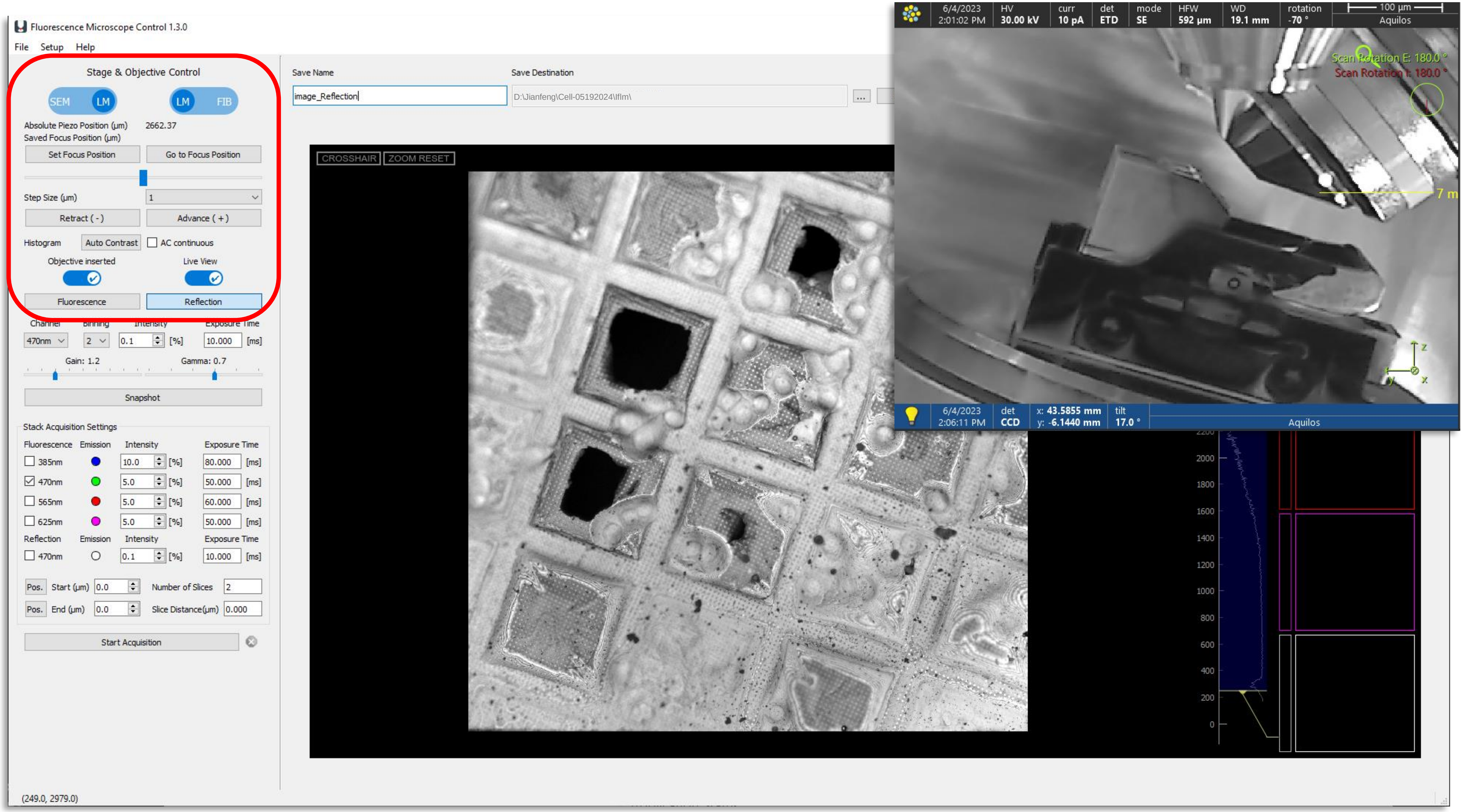
iFLM (Optional)
Target confirmation

Pt sputter (Optional)
Lamella conductivity

T

CryoET





3.4 CryoFLM _Setting up imagining 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

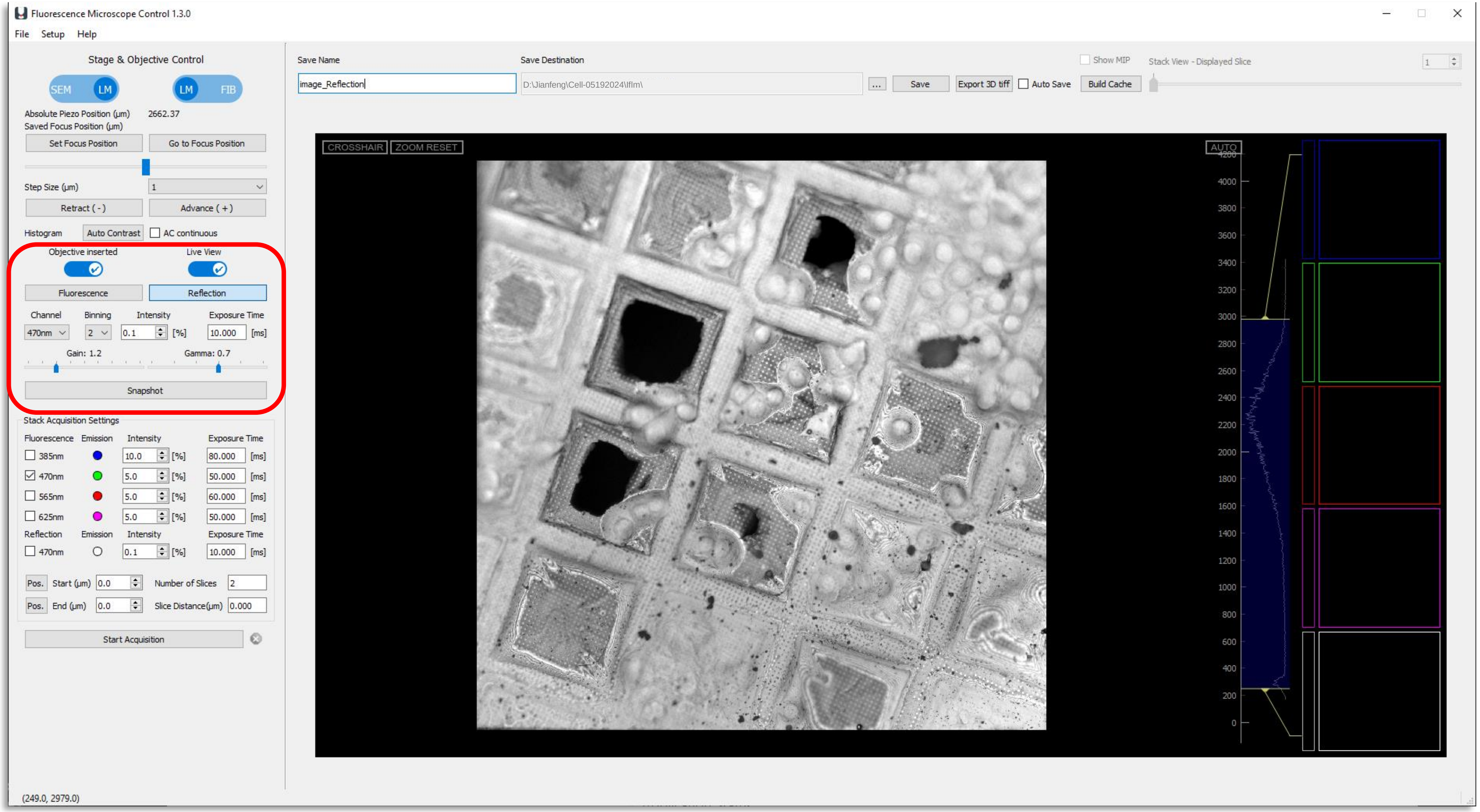
Lamella milling
Preparation, Milling, & thinning

iFLM (Optional)
Target confirmation

Pt sputter (Optional)
Lamella conductivity

T

CryoET



3.4 CryoFLM _Setting up imagining 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

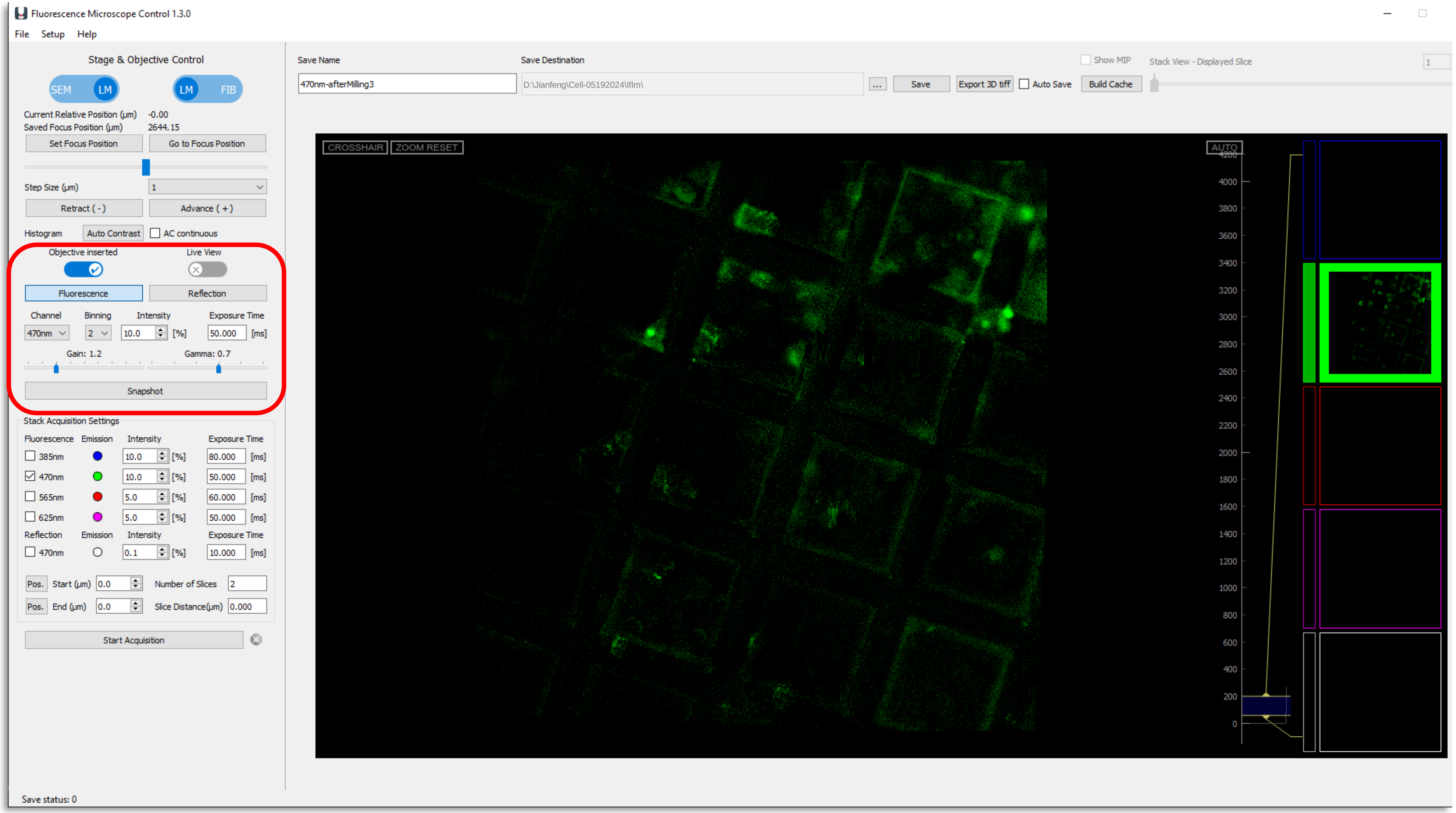
Lamella milling
Preparation, Milling, & thinning

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

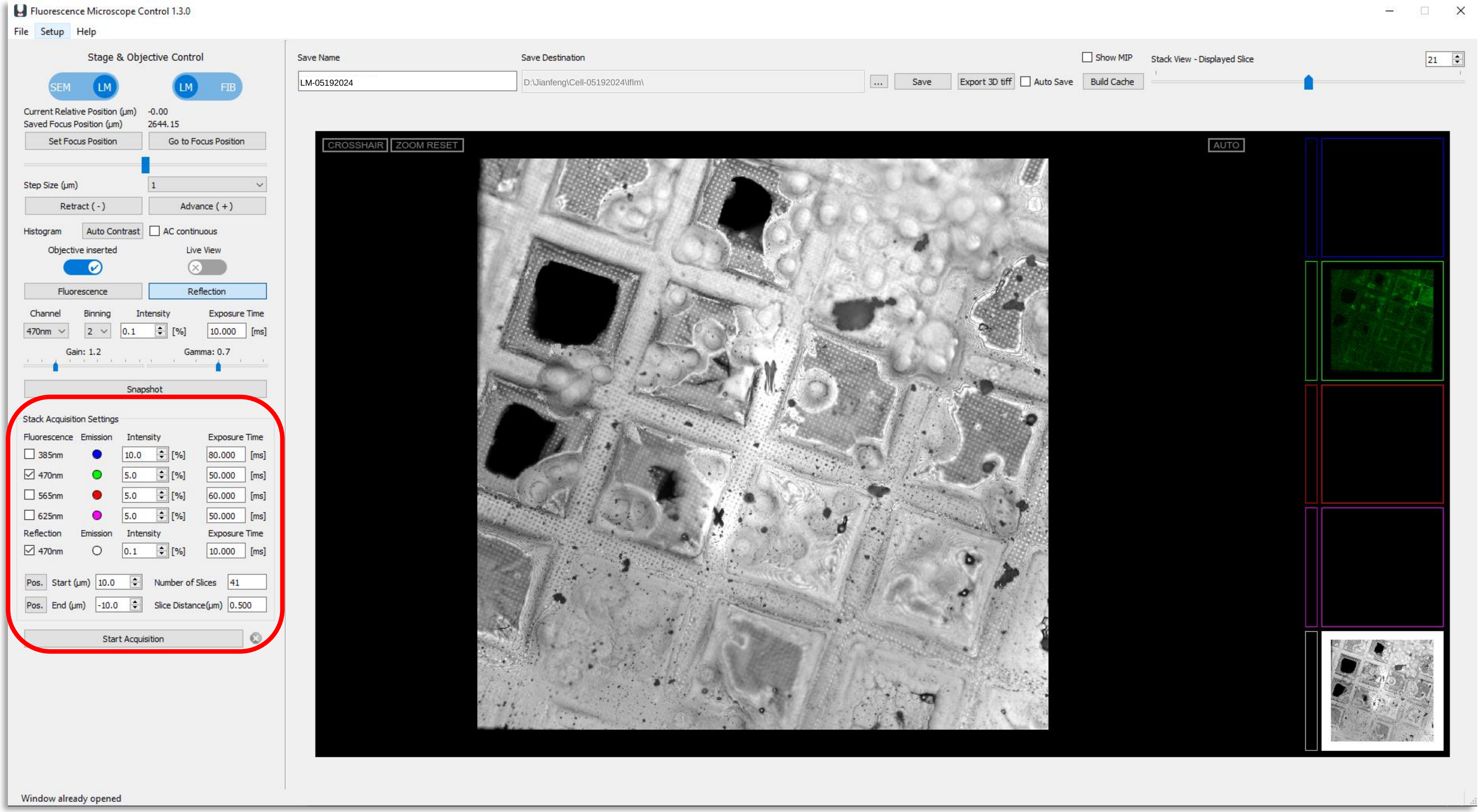
Lamella milling
Preparation, Milling, & thinning

iFLM (Optional)
Target confirmation

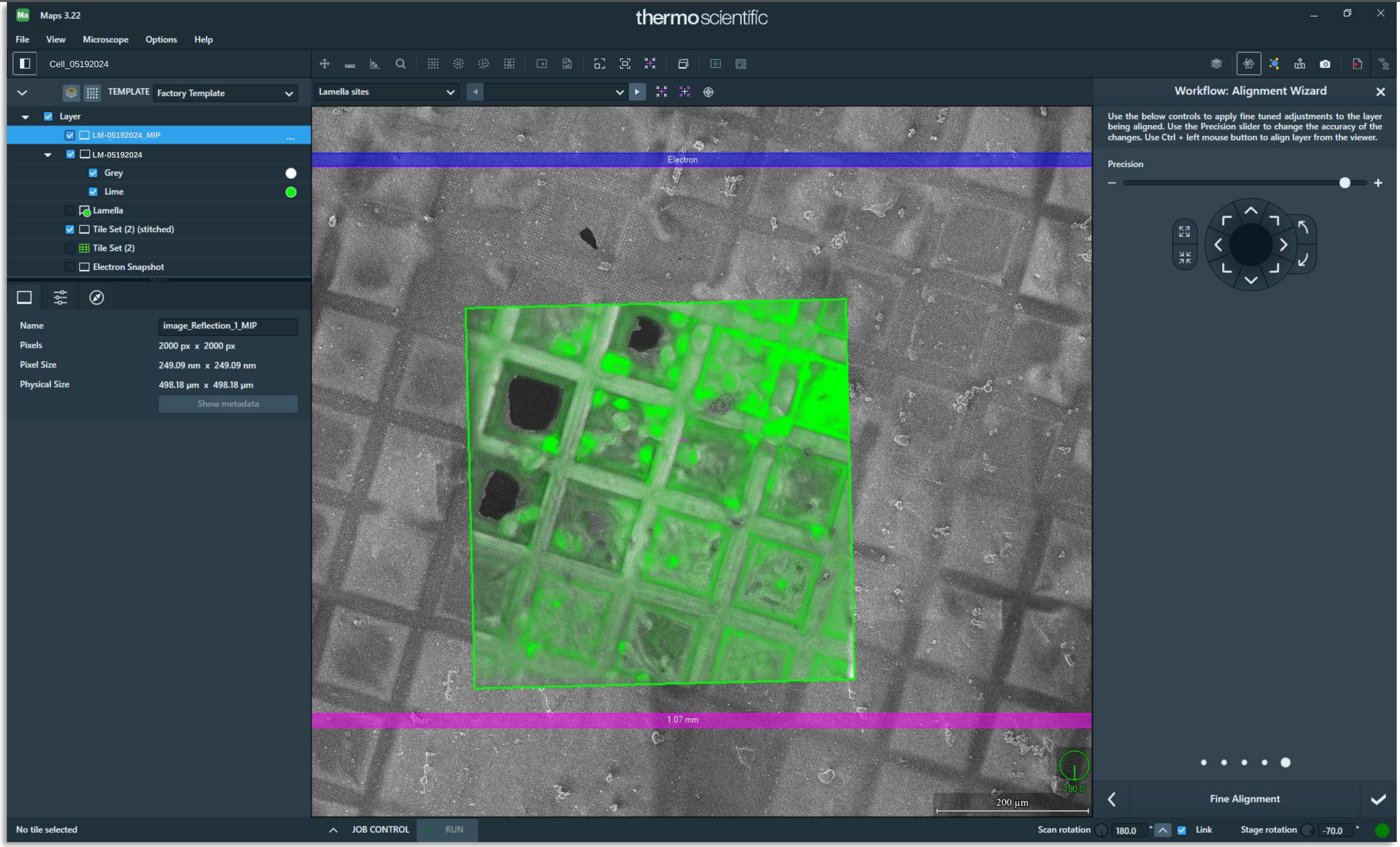
Pt sputter (Optional)
Lamella conductivity

T

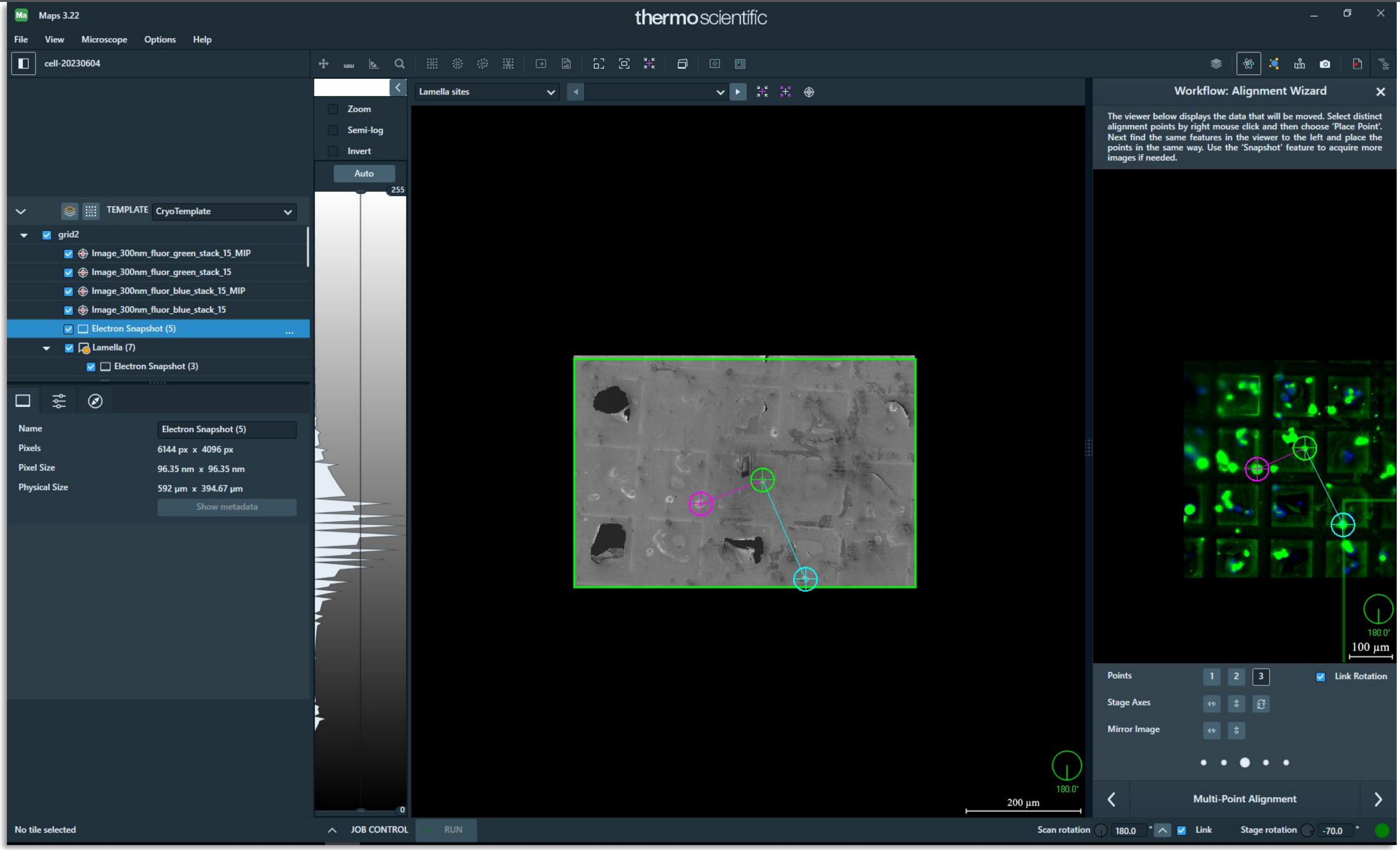
CryoET



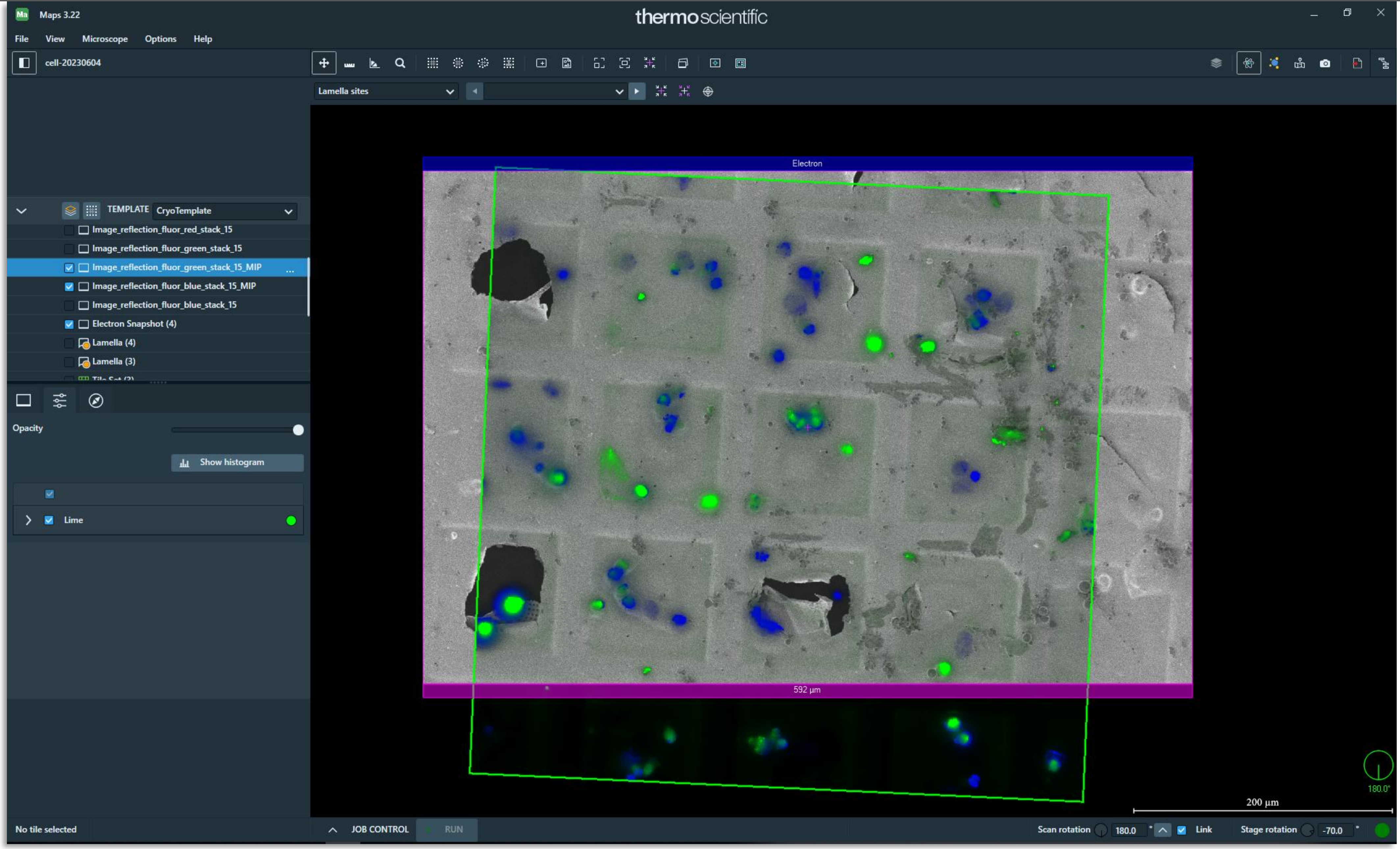
3.4 CryoFLM _FLM-SEM alignment by Fine Alignment (Ma Maps)



3.4 CryoFLM _FLM-SEM alignment by Multi-Point Alignment (Maps)

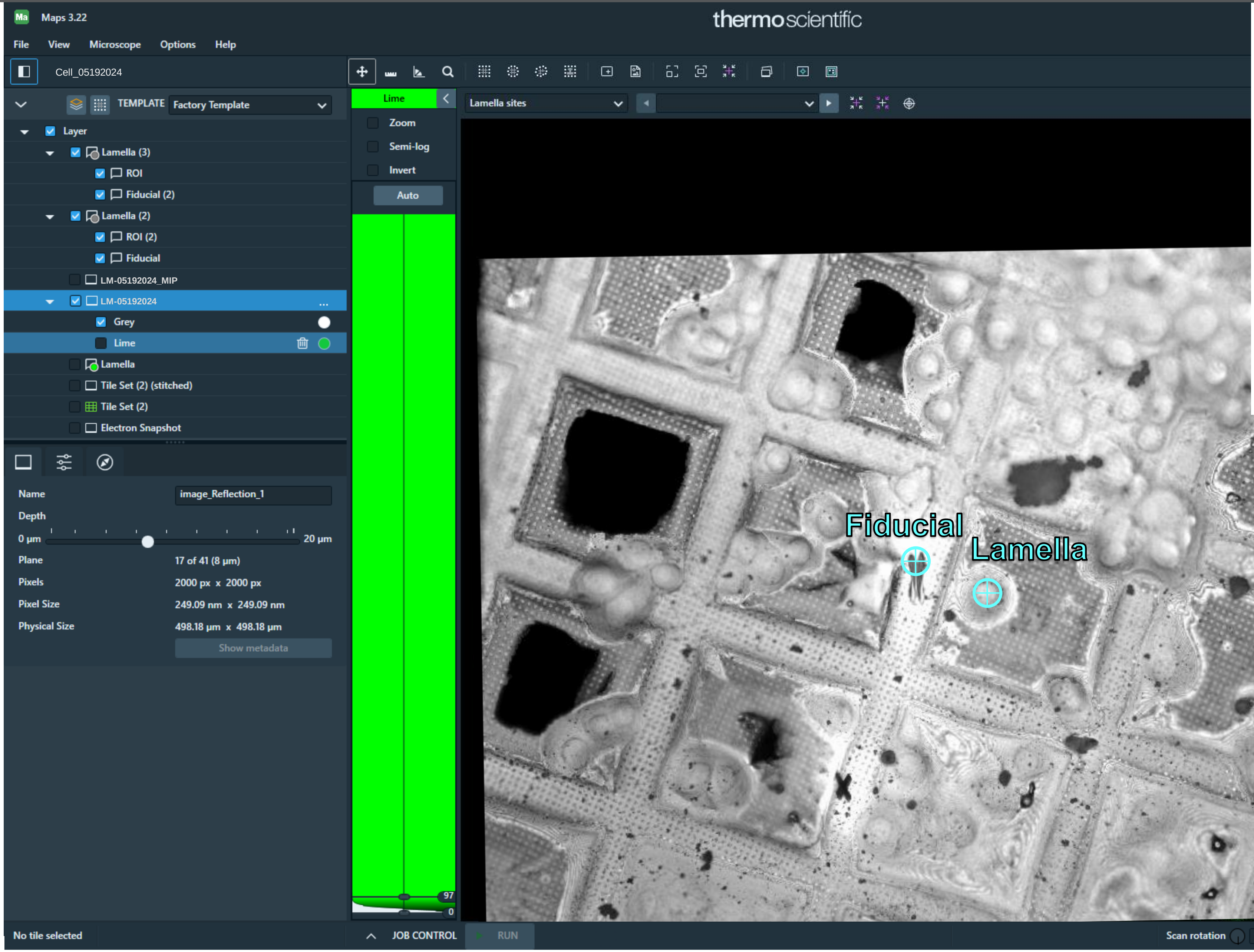


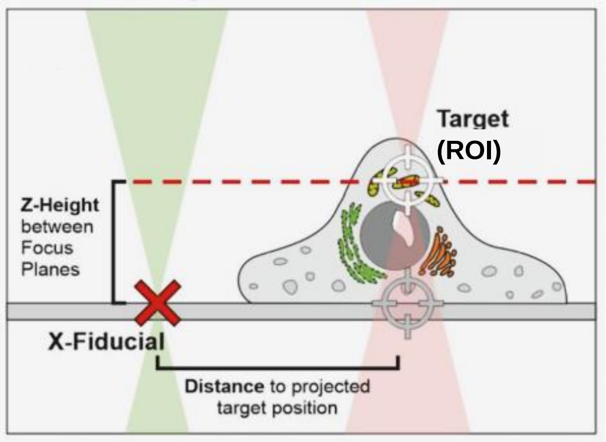
3.4 CryoFLM _FLM-SEM alignment by Multi-Point Alignment (Ma Maps)



3.4 CryoFLM _Define Fiducial & ROI markers for each lamella

(Ma Maps)

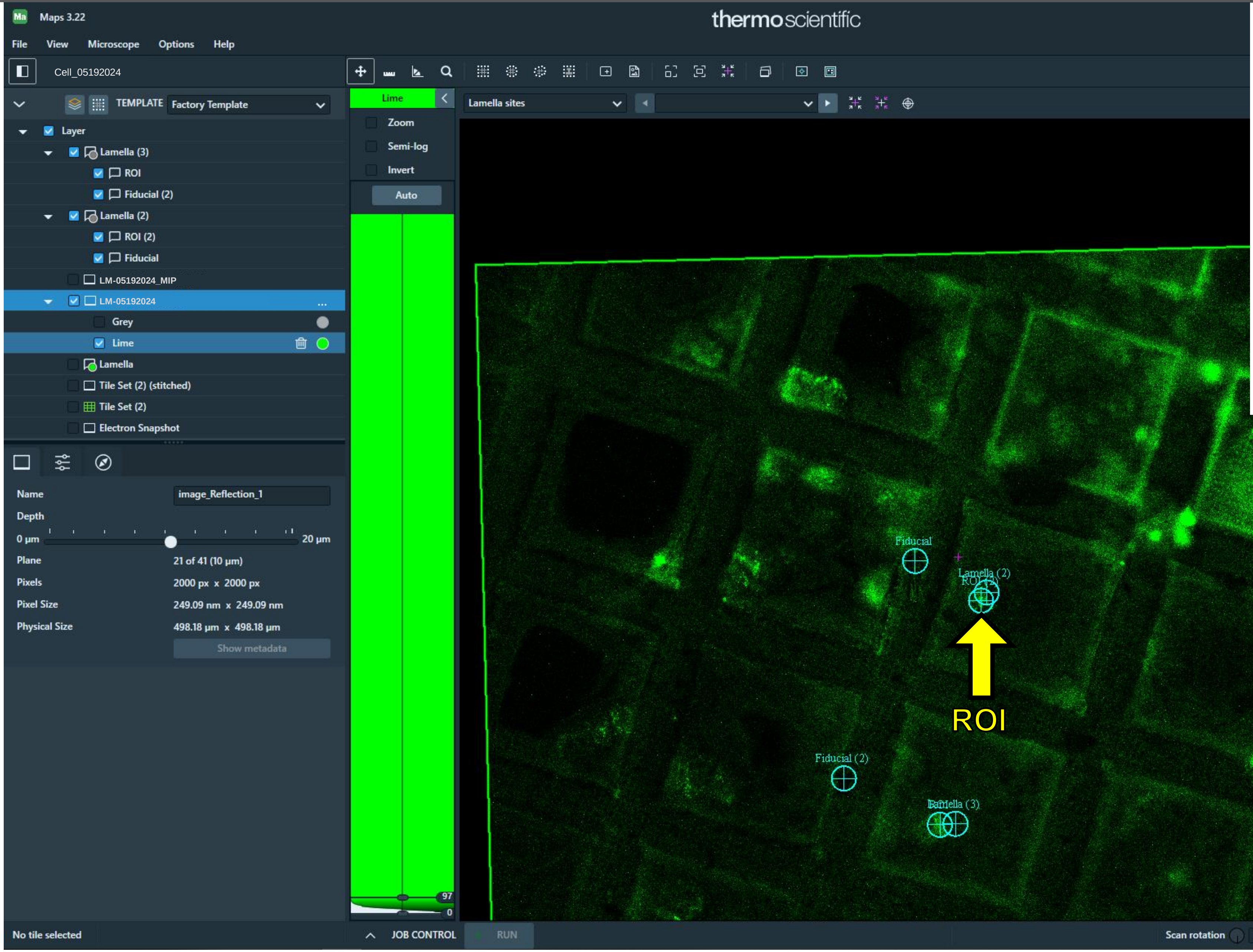


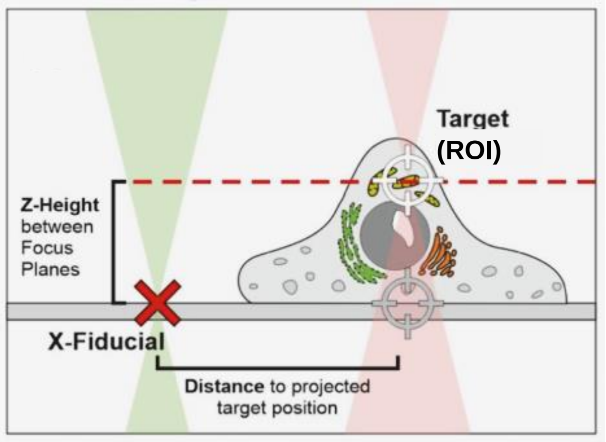


Adapted from
https://cryoem101.org/chapter-2-et/

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

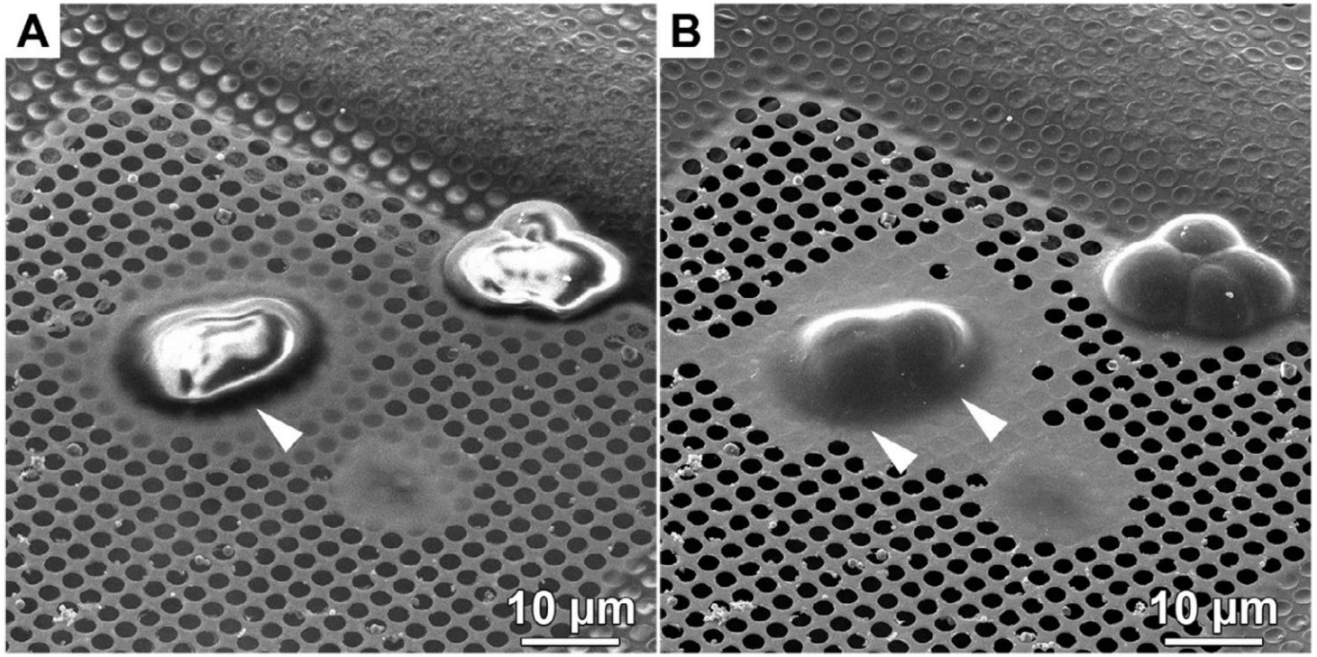
iFLM (Optional)
Target confirmation

Pt sputter (Optional)
Lamella conductivity

↓ T

CryoET

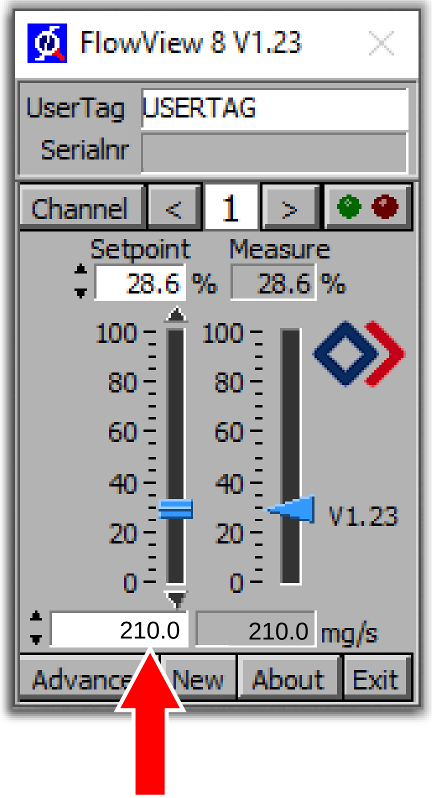
> (Optional) Inorganic Pt minimizes charging, ensure targeting and precise milling.



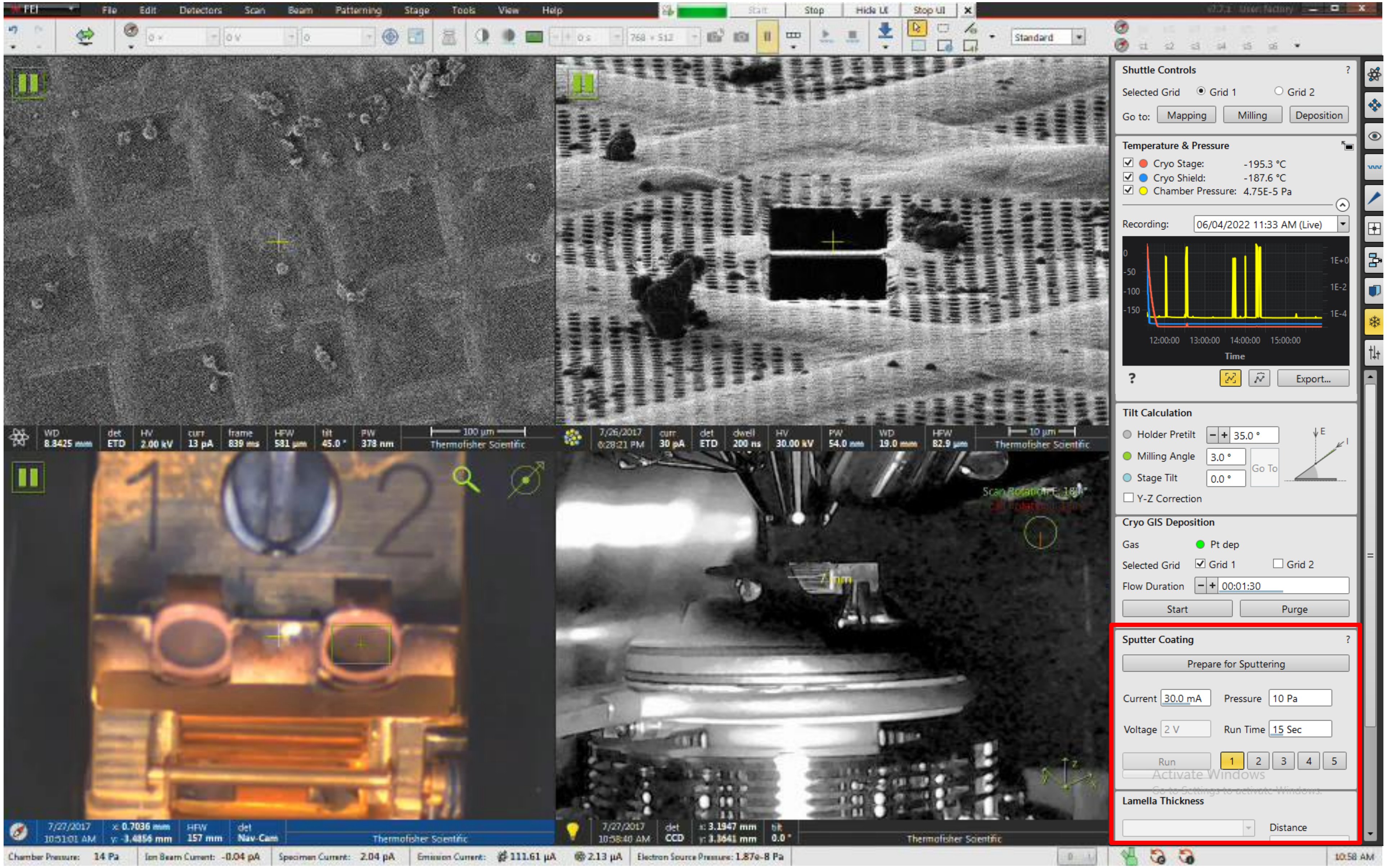
Schaffer et al., JSB 2017

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

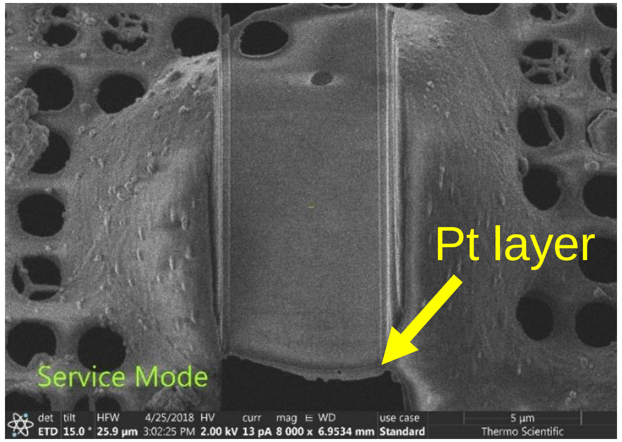
Lamella milling
Preparation, Milling, & thinning

iFLM (Optional)
Target confirmation

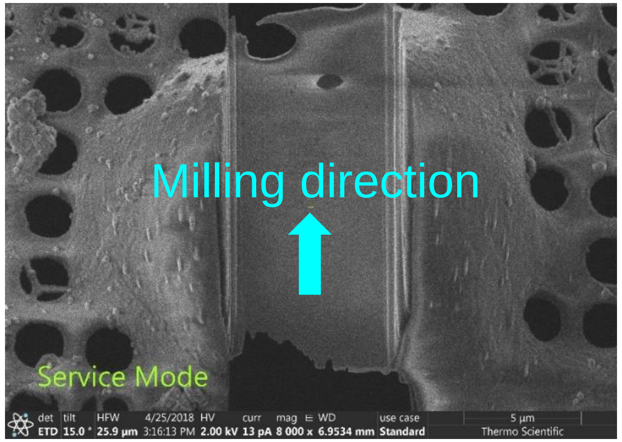
Pt sputter (Optional)
Lamella conductivity

↓ T

CryoET

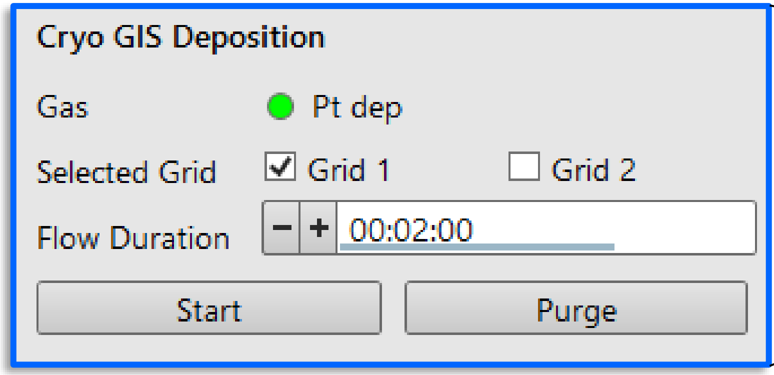


With an intact Pt layer

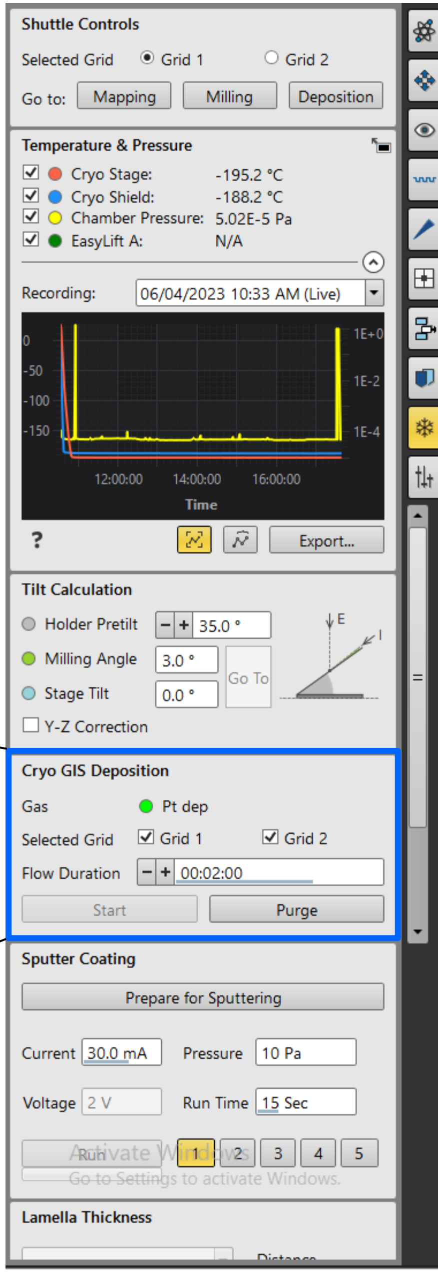


With a broken Pt layer

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

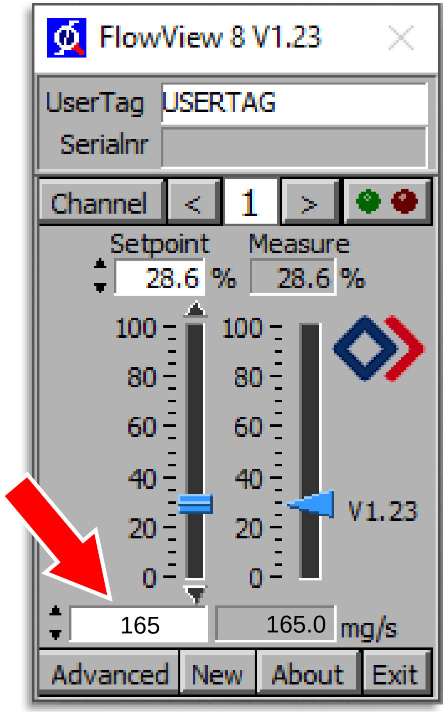
iFLM (Optional)
Target confirmation

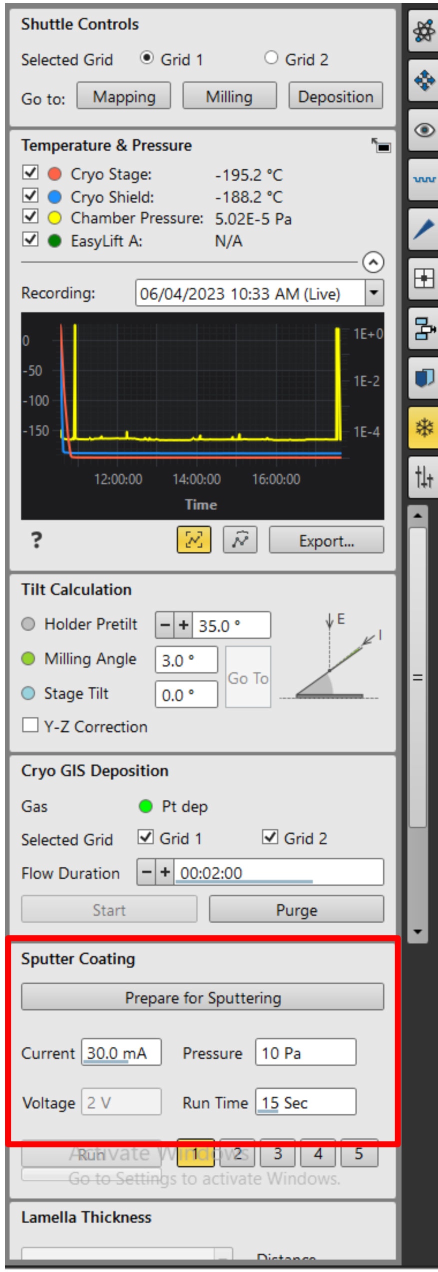
Pt sputter (Optional)
Lamella conductivity

T

CryoET

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

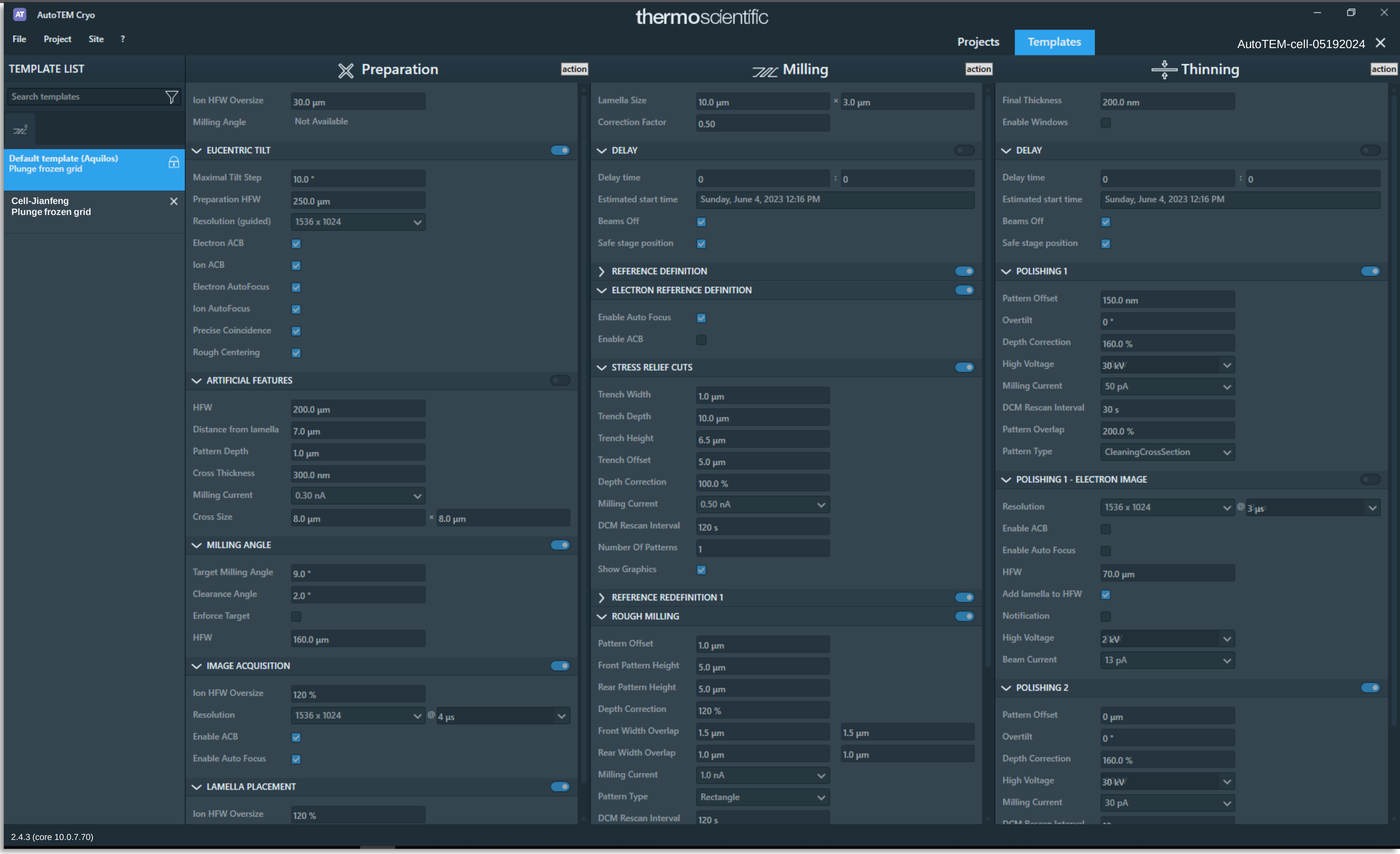
Lamella milling
Preparation, Milling, & thinning

iFLM (Optional)
Target confirmation

Pt sputter (Optional)
Lamella conductivity

↓ T

CryoET



Eucentric height & tilt calculation

(AT AutoTEM Cryo)

Vitrification

↓

CryoFIB

Sample screening
Atlas & lamella sites

iFLM (Optional)
Target selection

Pt sputter
Sample conductivity

Pt GIS
Protective coating

Pt sputter (Optional)
Sample conductivity

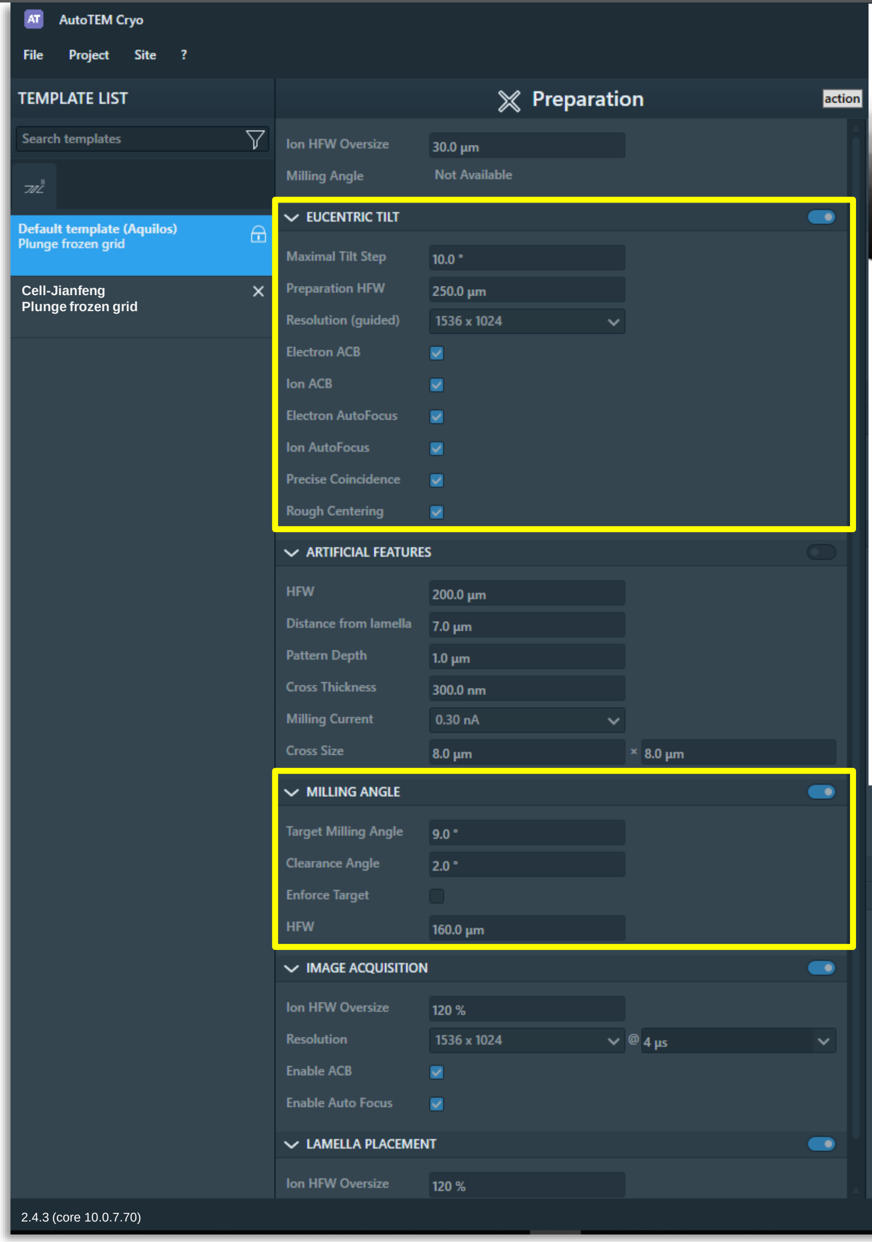
Lamella milling
Preparation, Milling, & thinning

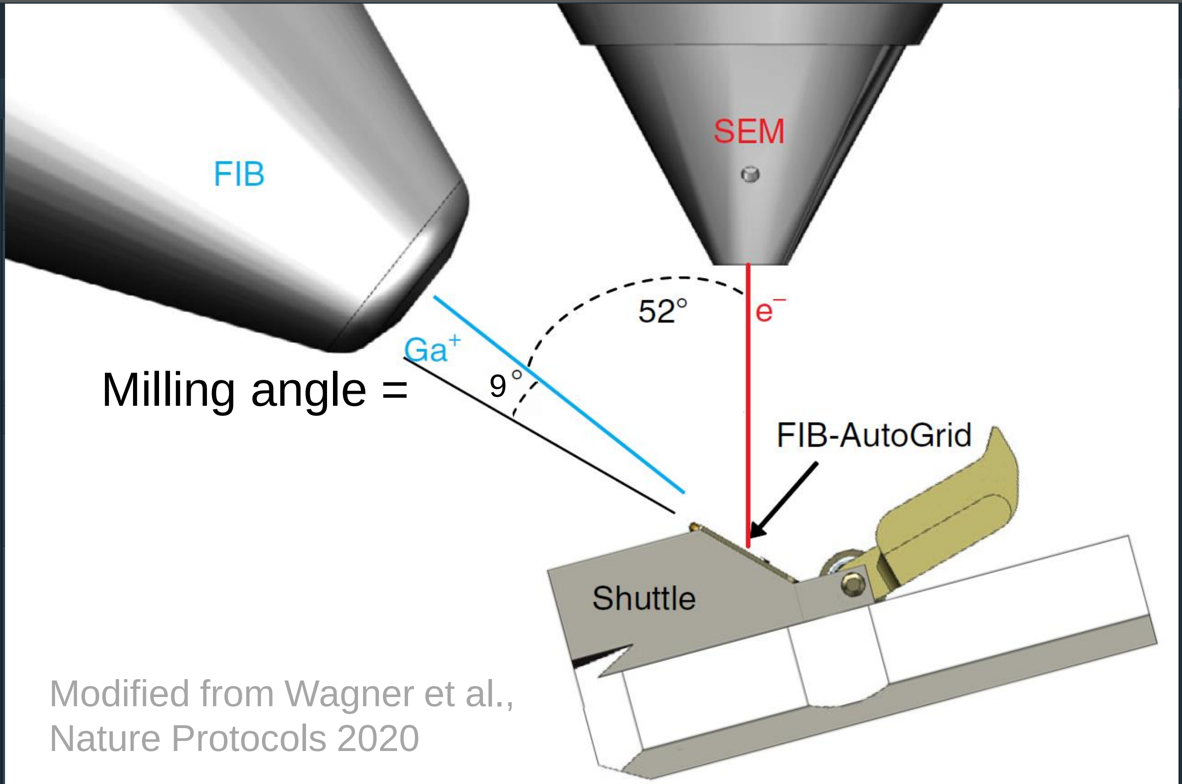
iFLM (Optional)
Target confirmation

Pt sputter (Optional)
Lamella conductivity

↓

CryoET





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

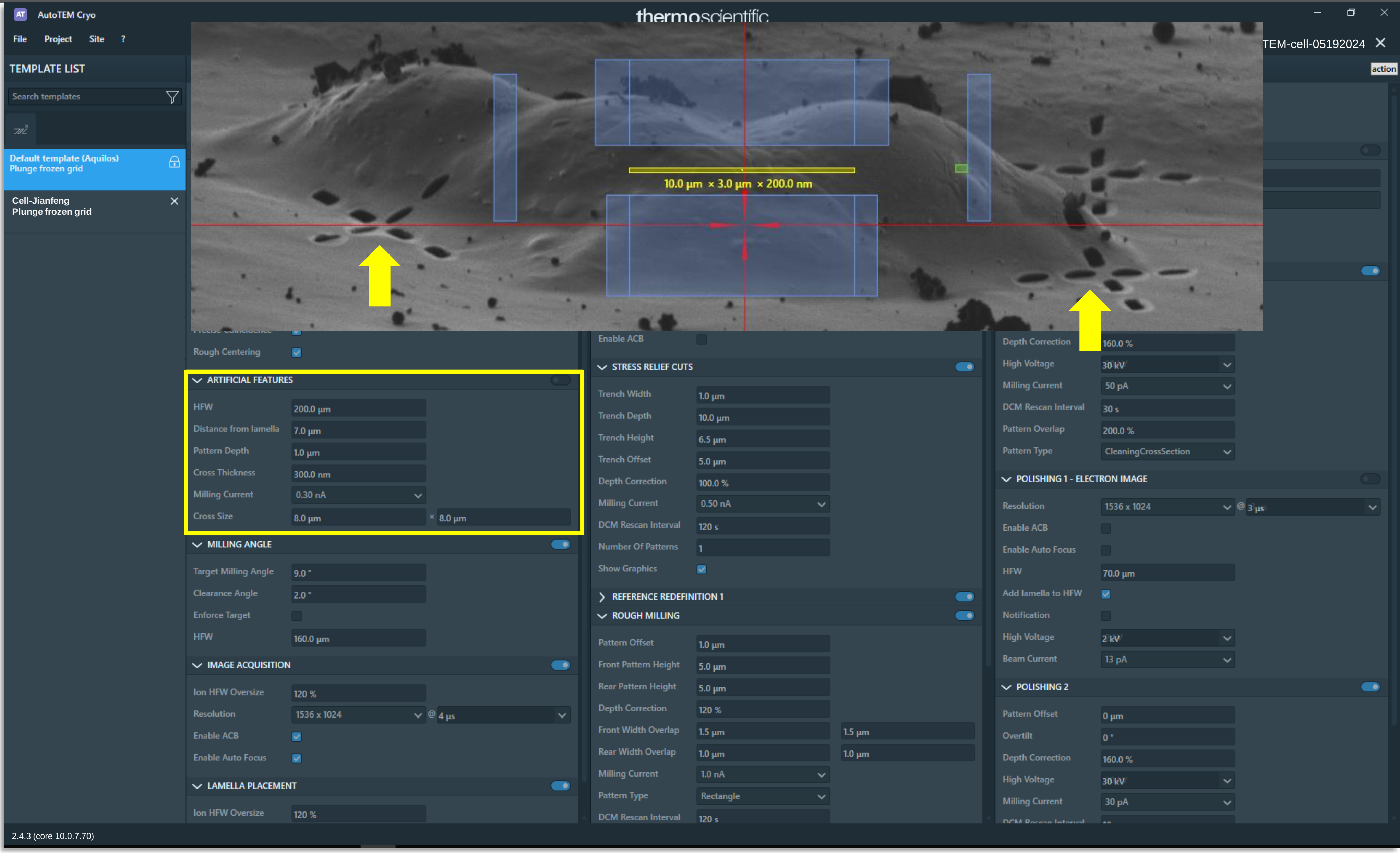
Lamella milling
Preparation, Milling,
& thinning

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

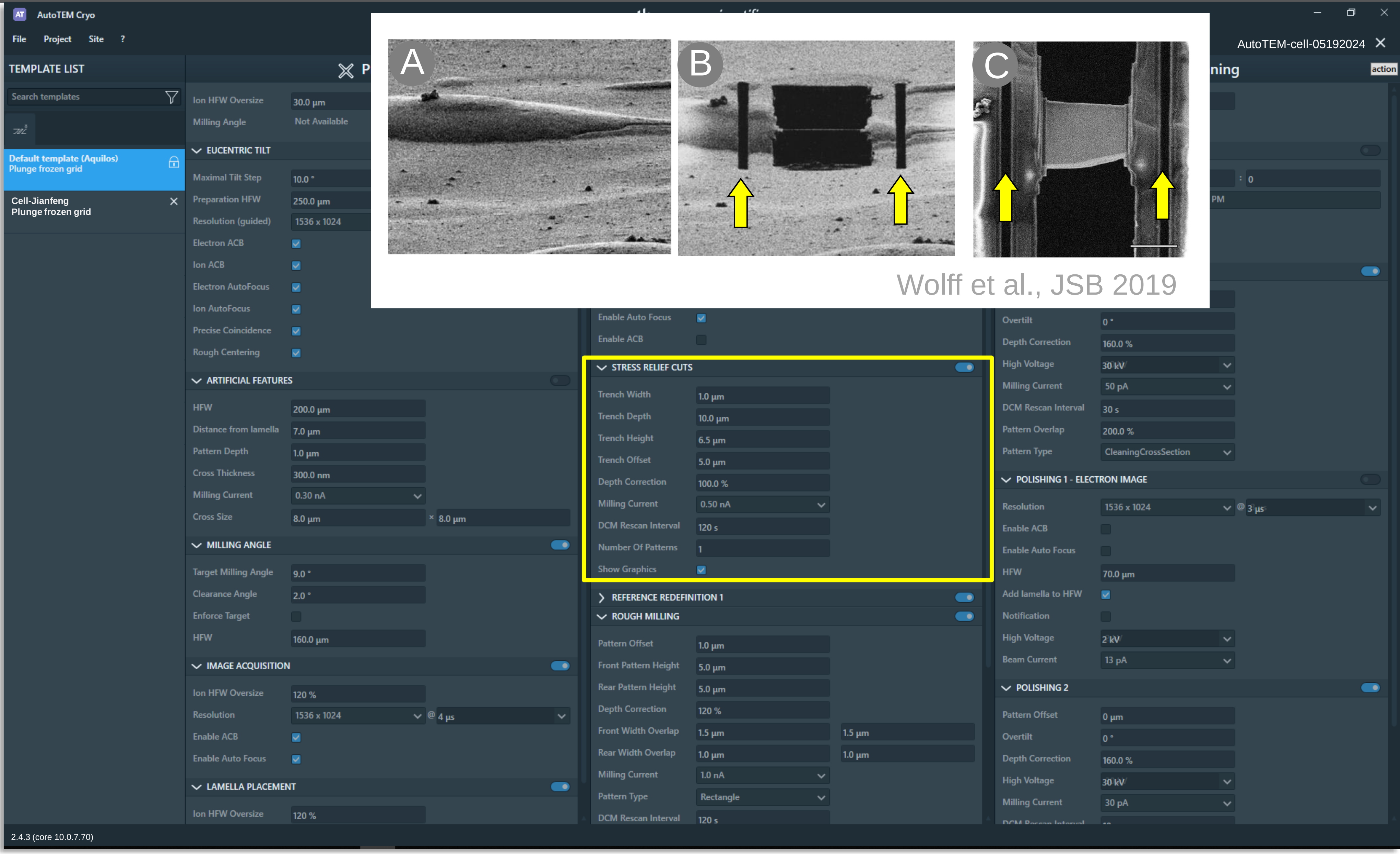
Lamella milling
Preparation, Milling, & thinning

iFLM (Optional)
Target confirmation

Pt sputter (Optional)
Lamella conductivity

↓ T

CryoET



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

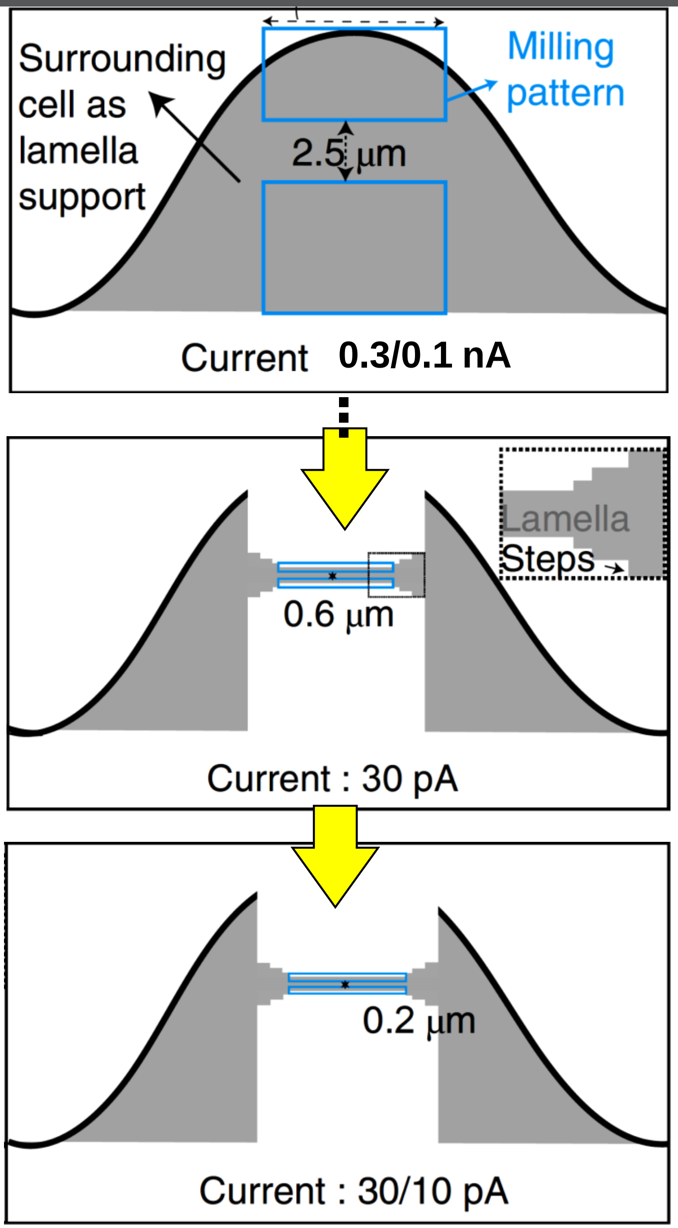
Lamella milling
Preparation, Milling, & thinning

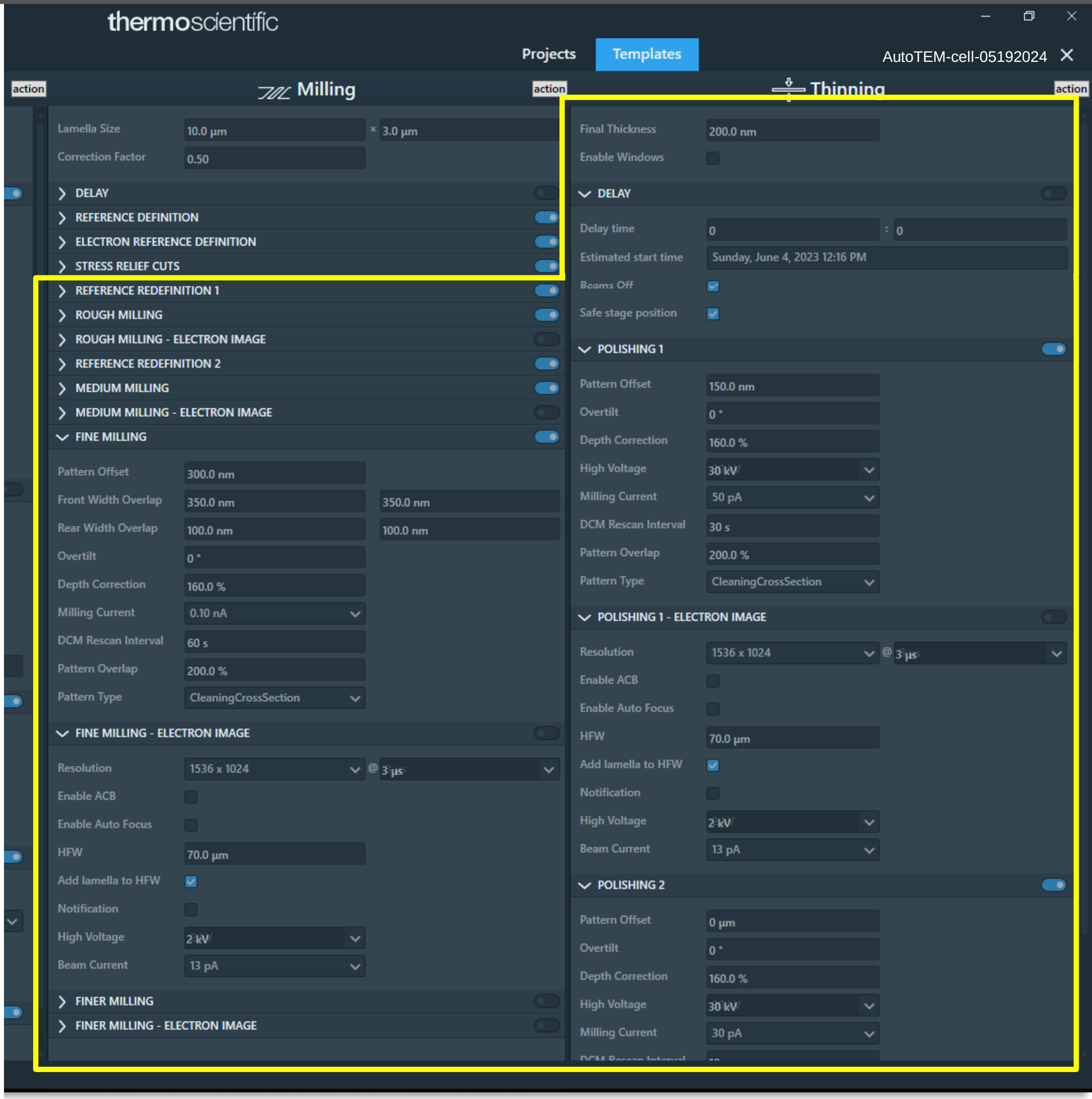
iFLM (Optional)
Target confirmation

Pt sputter (Optional)
Lamella conductivity

↓ T

CryoET

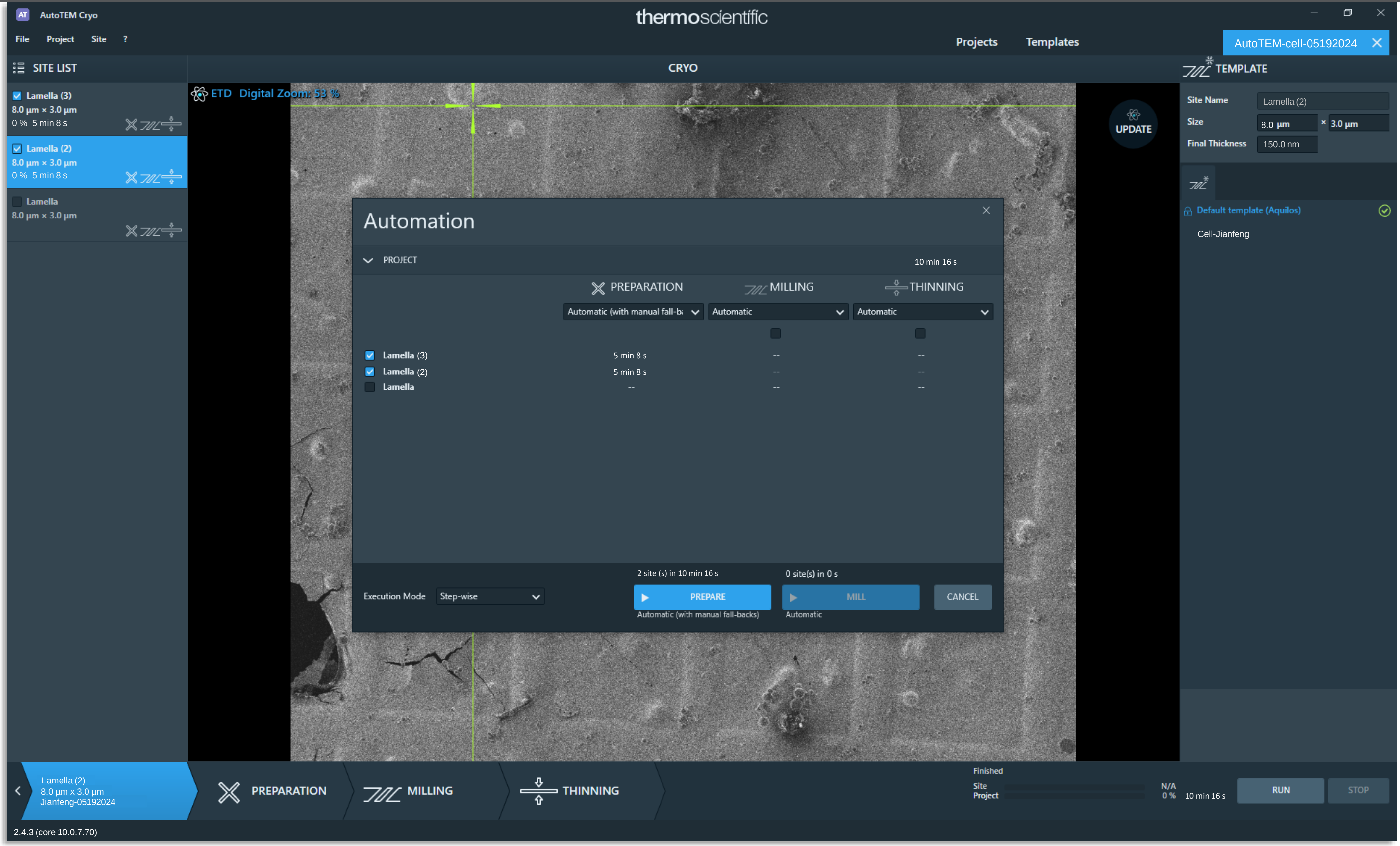




Modified from Wagner et al., Nature Protocols 2020

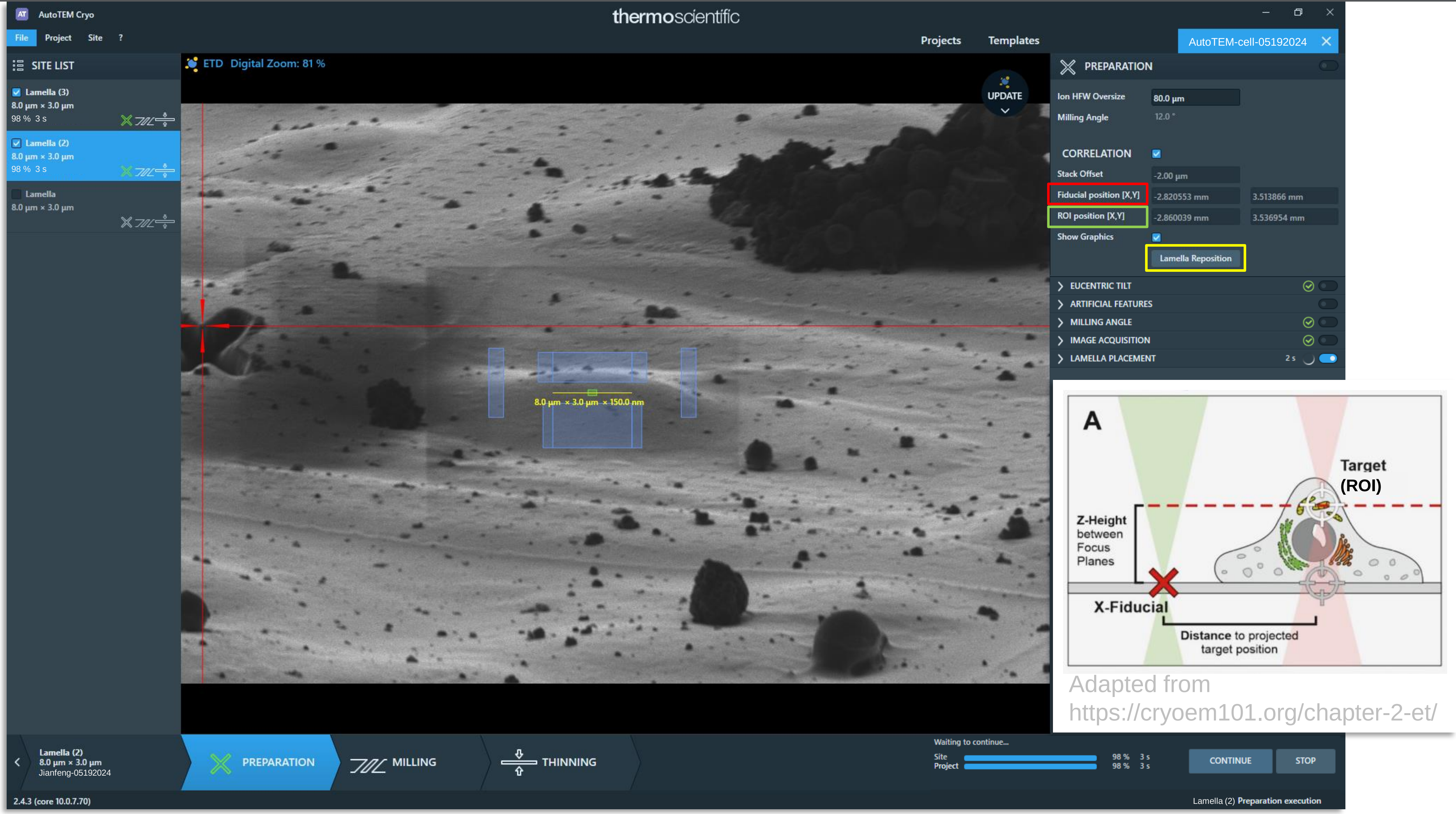
3.7.1 Automated lamella milling _Preparation

(AT AutoTEM Cryo)



3.7.1 Automated lamella milling _Preparation

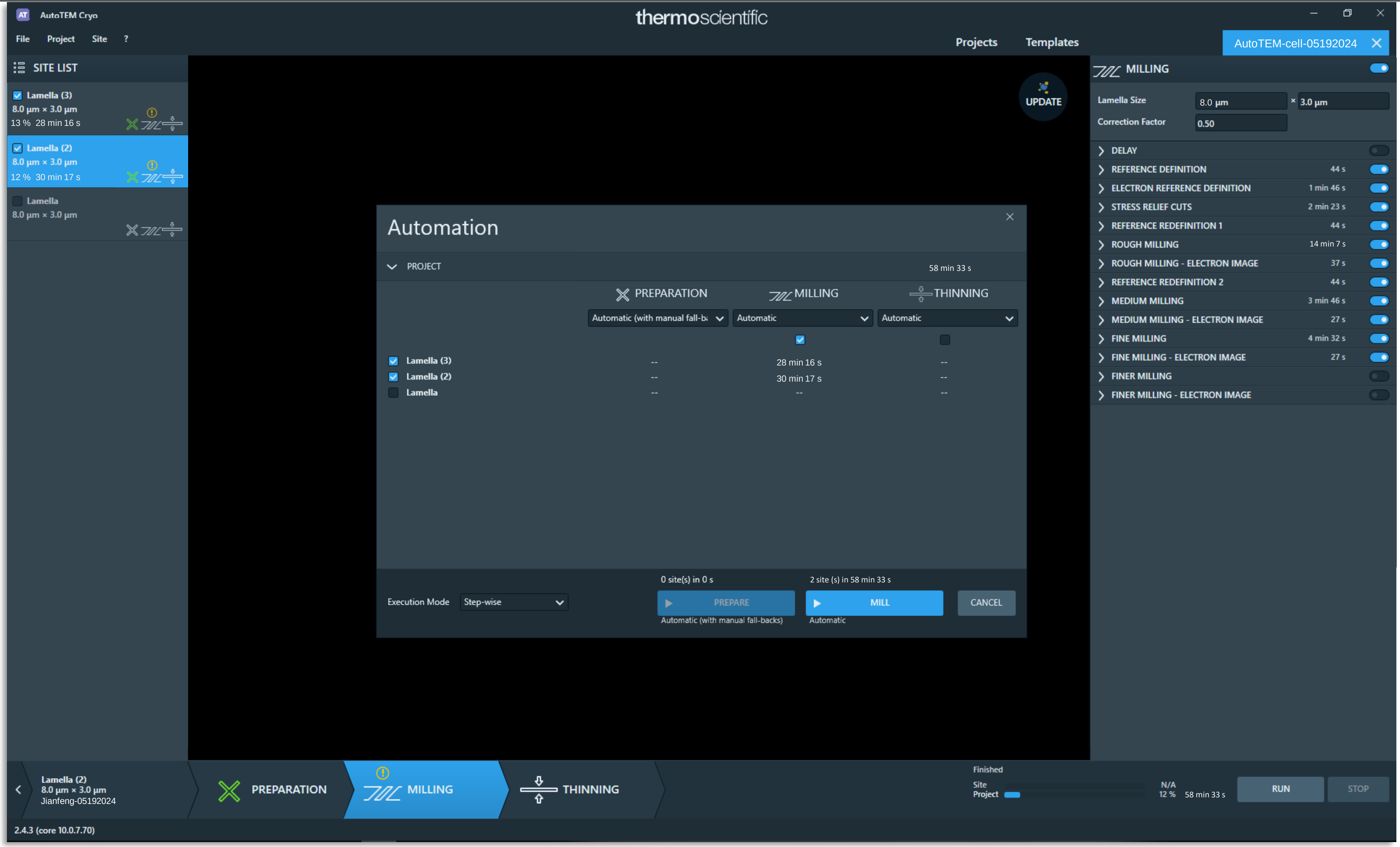
(AT AutoTEM Cryo)



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

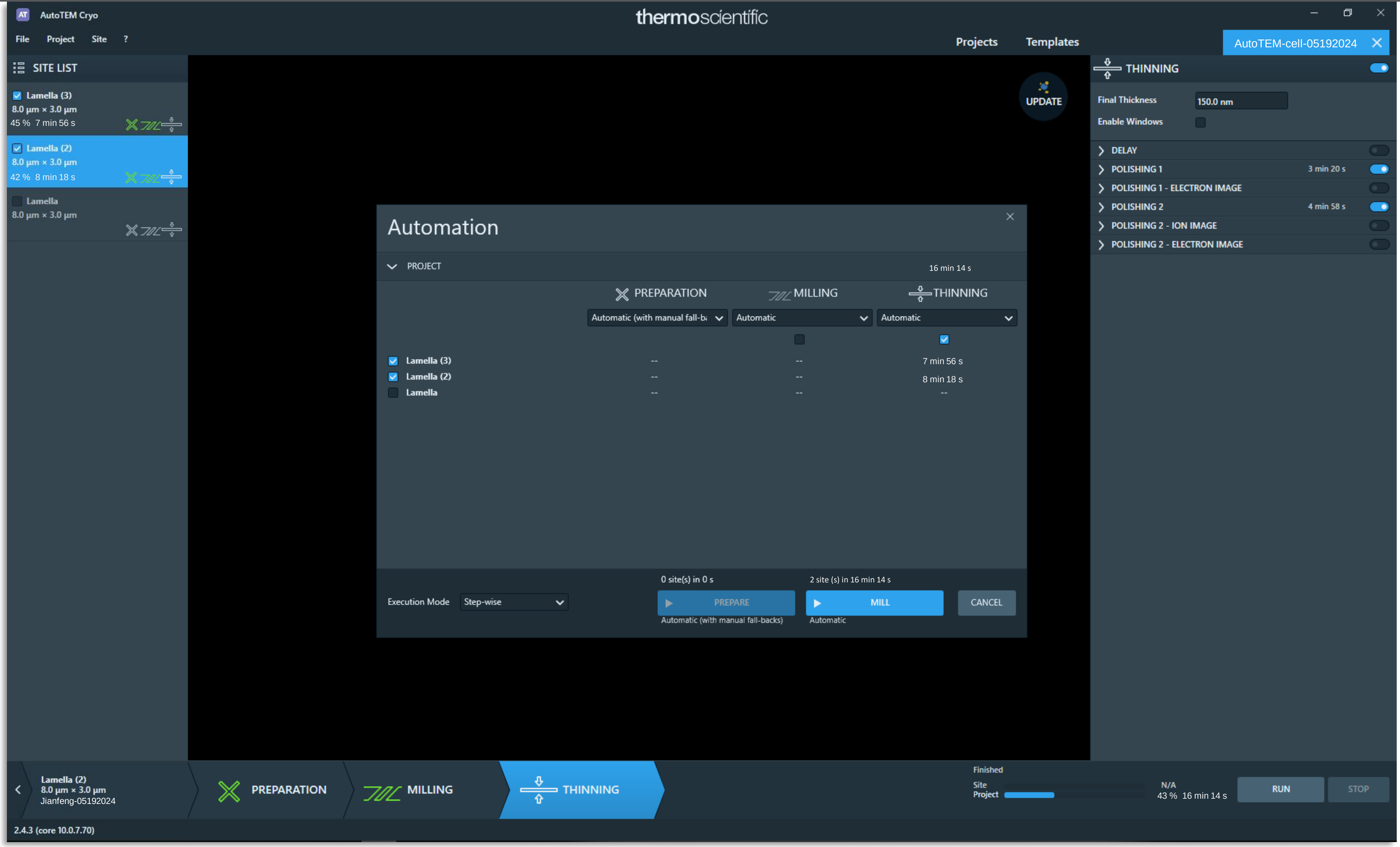
3.7.2 Automated lamella milling _Milling

(AT AutoTEM Cryo)



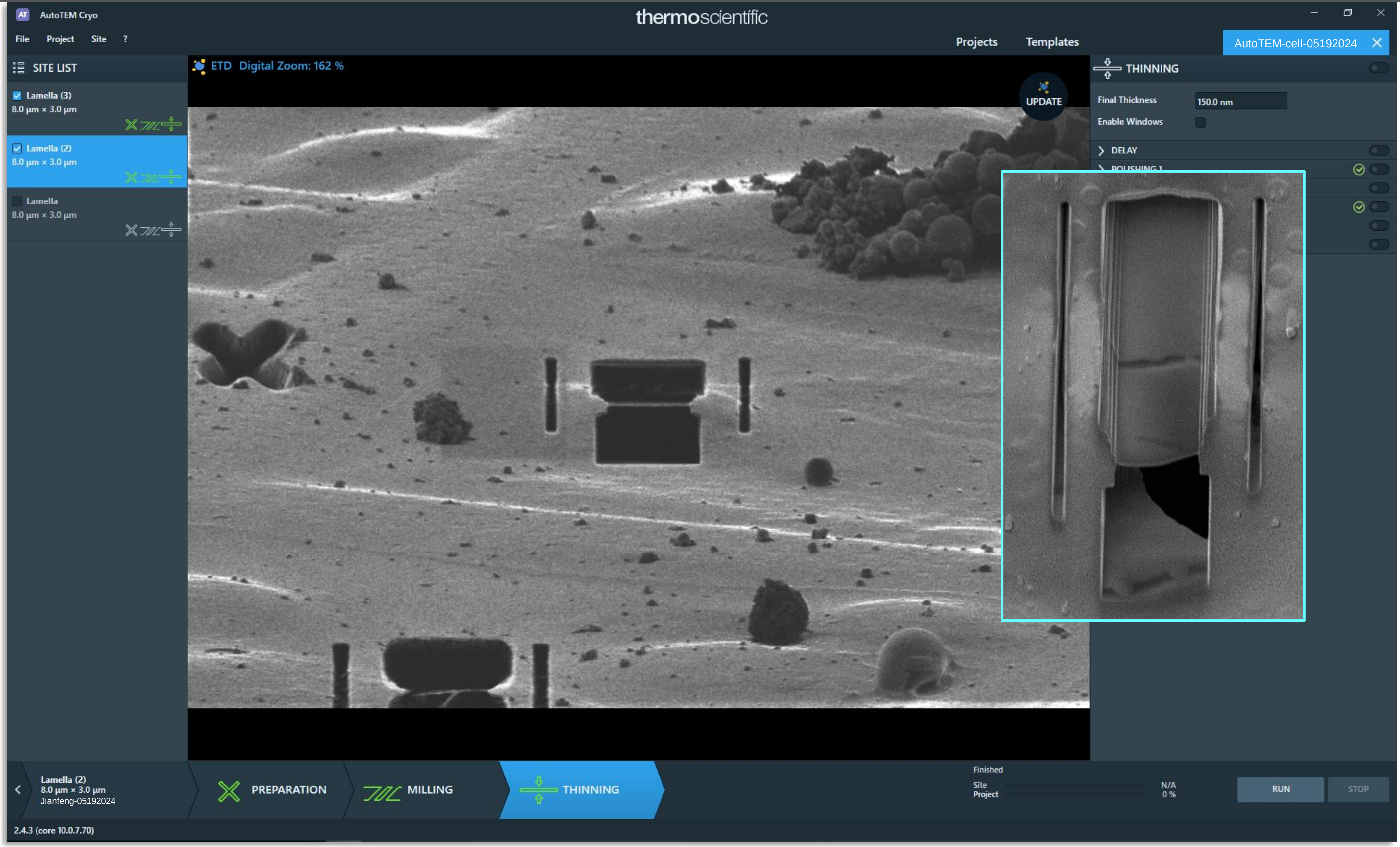
3.7.3 Automated lamella milling _Thinning

(AT AutoTEM Cryo)



3.7.3 Automated lamella milling _Thinning _example-lamella 1

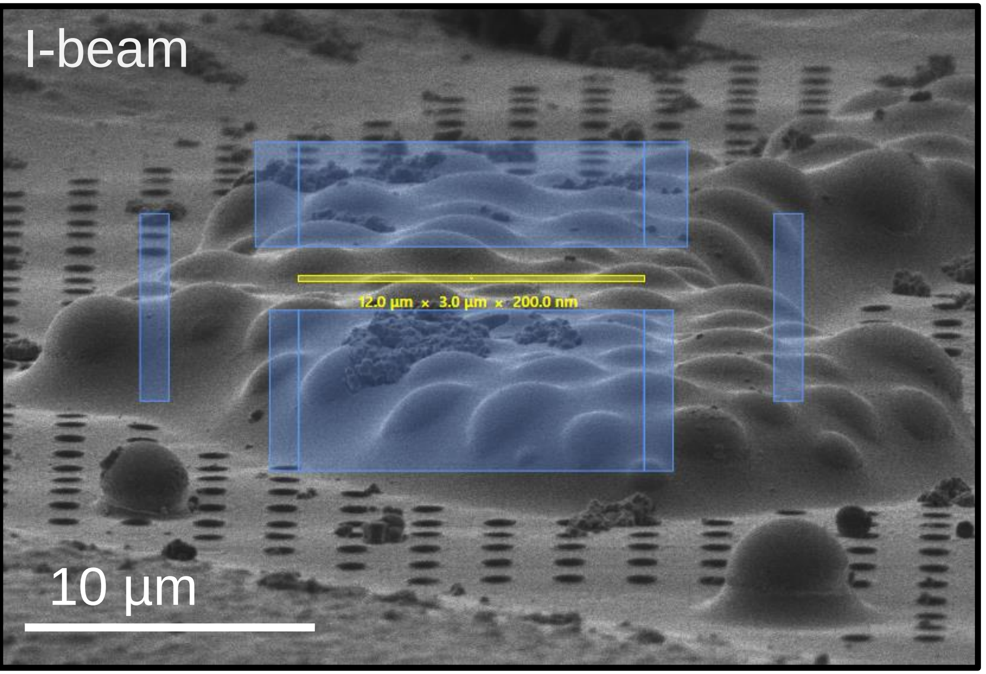
(AT AutoTEM Cryo)

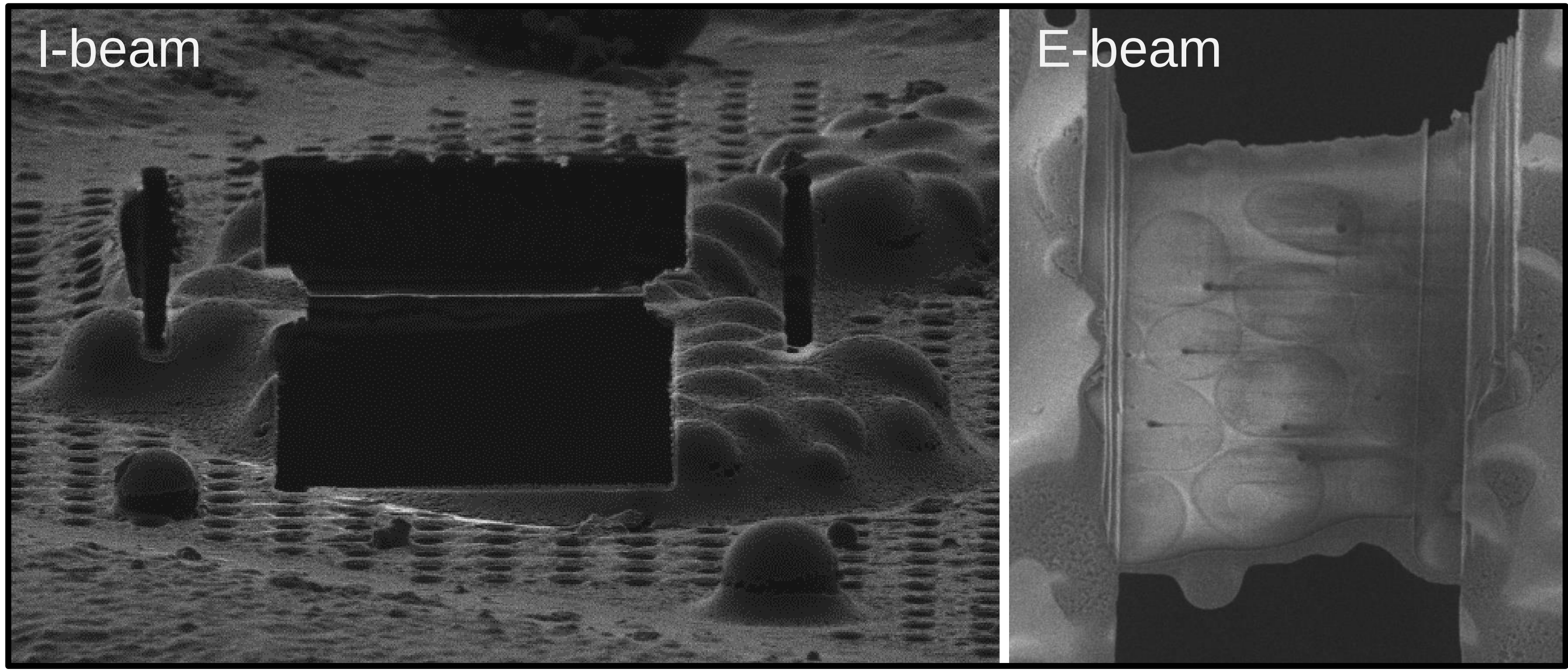


3.7.3 Automated lamella milling _example-lamella 2

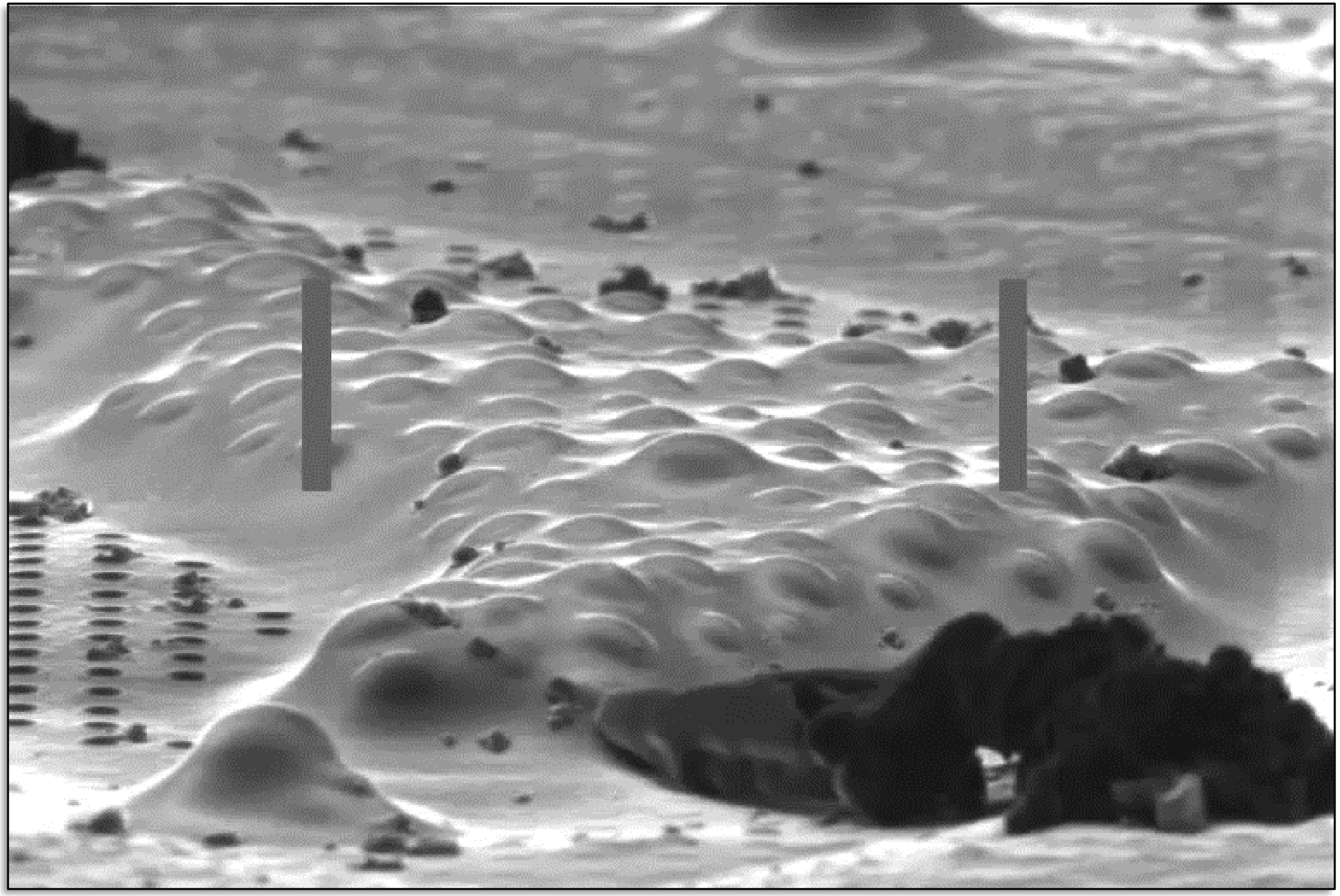
(xT Microscope Control)

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

CryoFIB

Sample screening
Atlas & lamella sites

iFLM (Optional)
Target selection

Pt sputter
Sample conductivity

Pt GIS
Protective coating

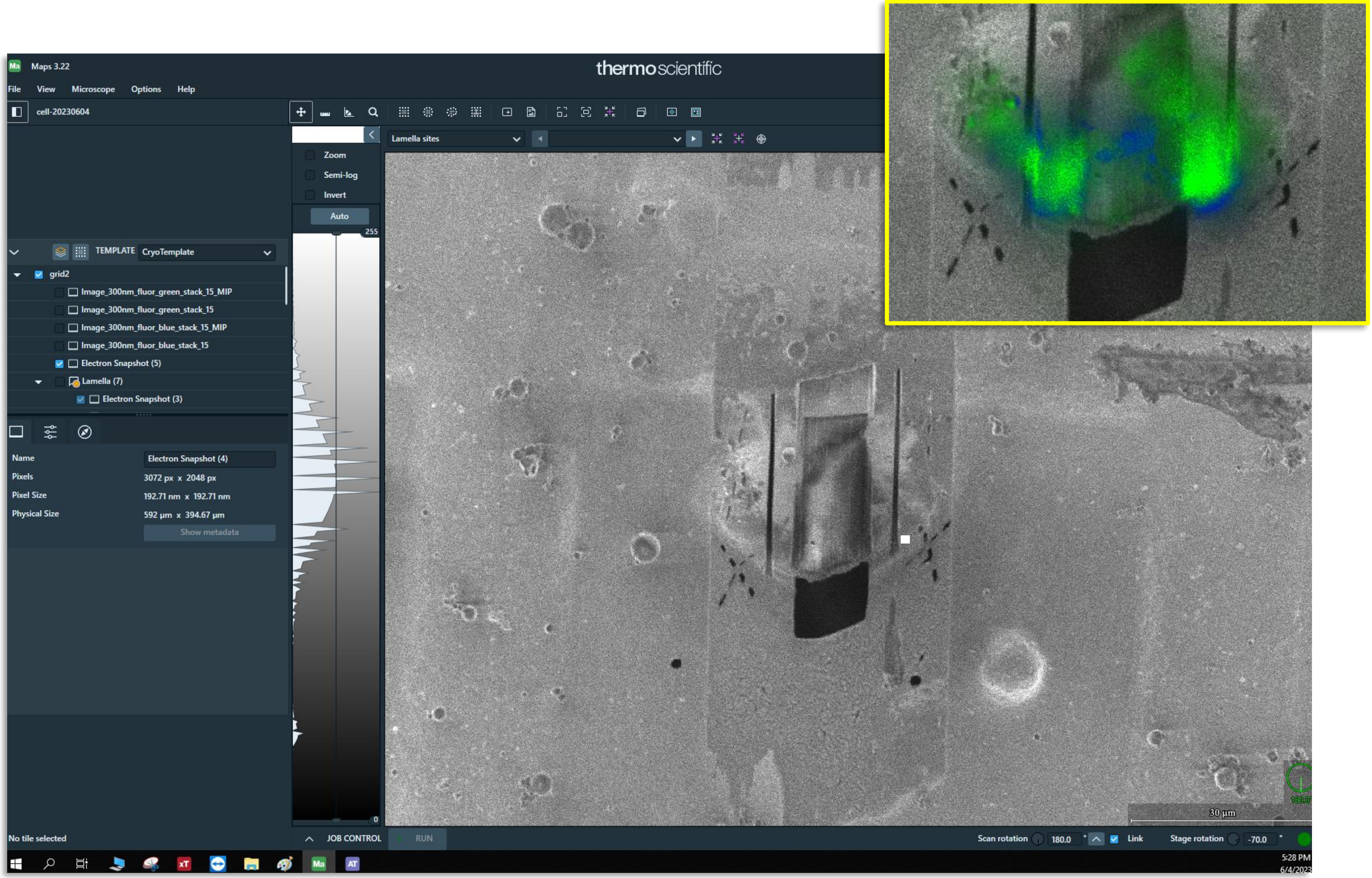
Pt sputter (Optional)
Sample conductivity

Lamella milling
Preparation, Milling, & thinning

iFLM (Optional)
Target confirmation

Pt sputter (Optional)
Lamella conductivity

CryoET



3.9 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

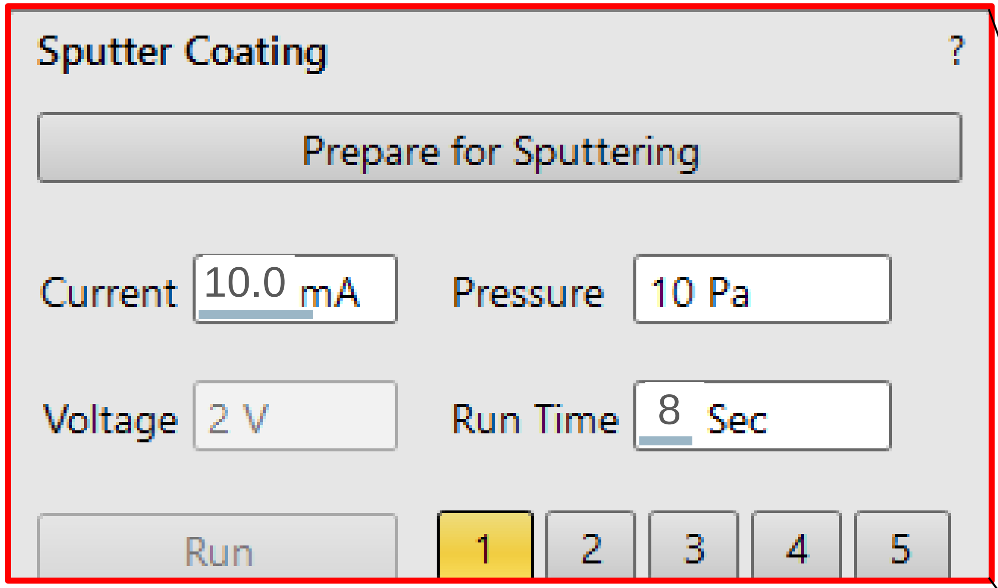
Lamella milling
Preparation, Milling, & thinning

iFLM (Optional)
Target confirmation

Pt sputter (Optional)
Lamella conductivity

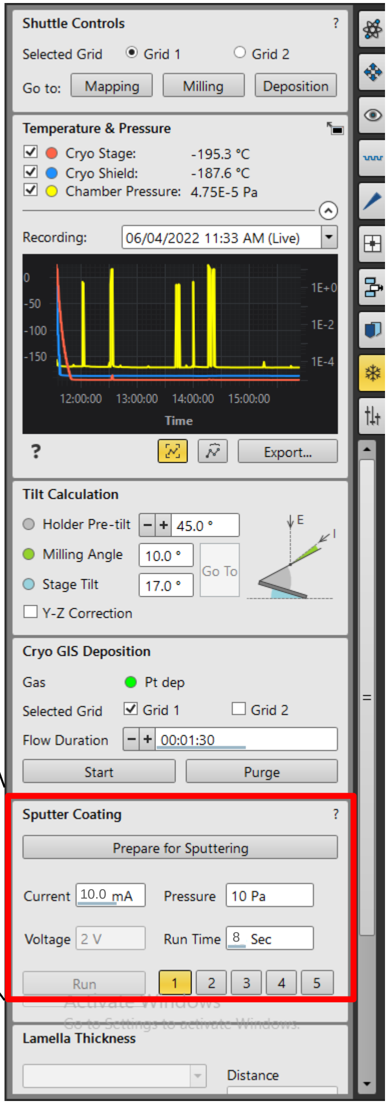
↓ T

CryoET



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

xT



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

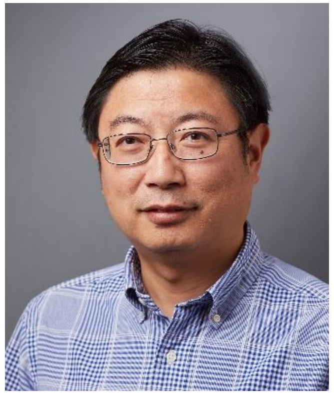
Lamella milling
Preparation, Milling, & thinning

If CLEM is unnecessary & no charging problems

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

(Video from TFS)

Acknowledgment



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