Webinar Series

Small Crystals, Big Insights: How Electron Diffraction is Transforming Materials, Life Science, and Chemistry Research

Episode 3 – Simple Electron Diffraction Workflow from Sample Prep to Structural Solutions

Presenter: Jessica Burch, PhD

Wednesday, May 7, 9 am CST

- *You will be muted during the workshop*
- *You can ask questions using the Q&A tool.*
- *You should hear music if your sound is working*

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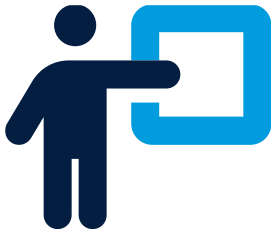
We are starting now



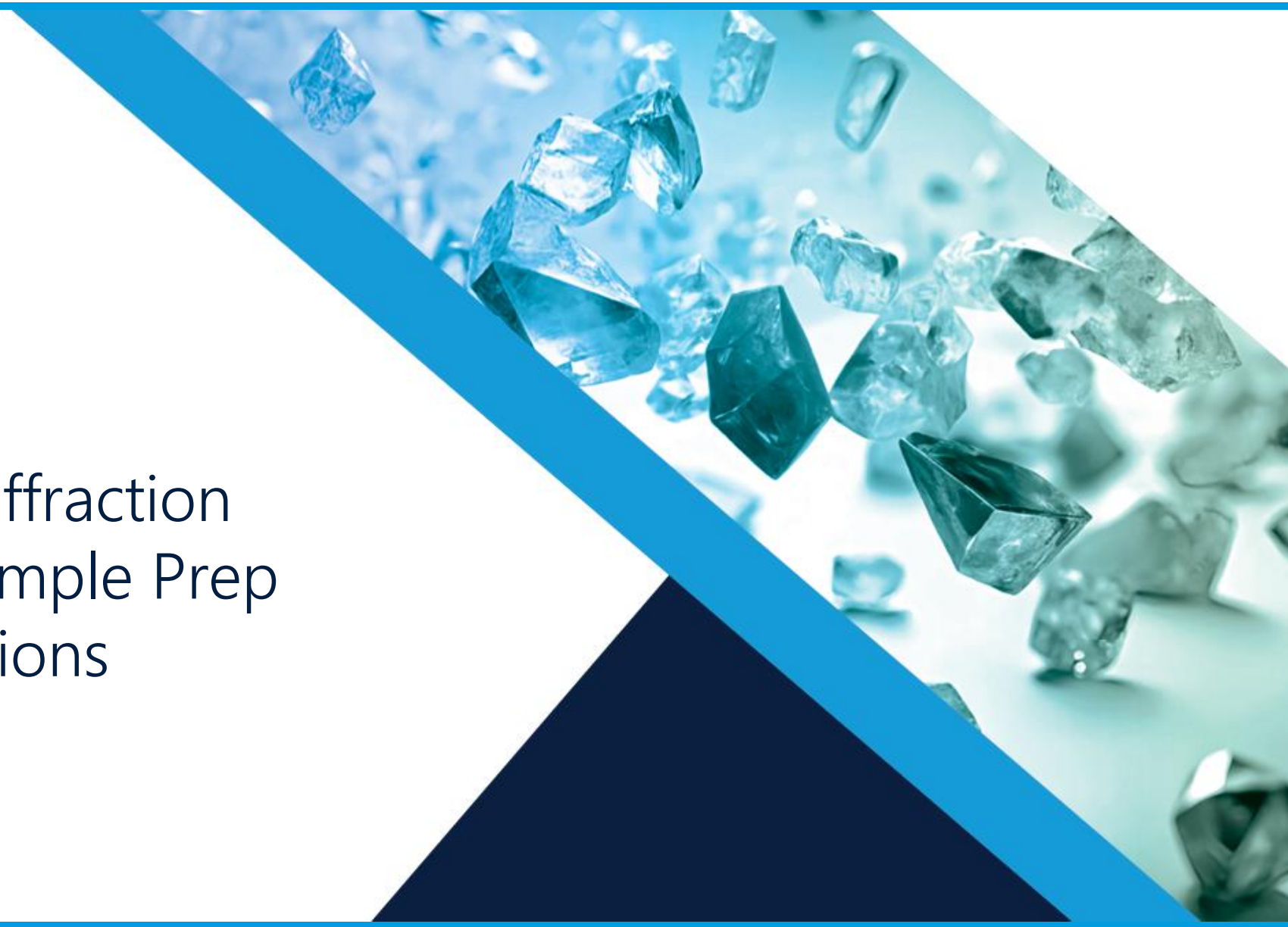
Presenter:
Jessica Burch
Application Scientist

Host:
Joseph Ferrara
CSO, Rigaku Americas

You can ask questions during the presentation. Please use the Q&A to ask questions.

Recording will be available tomorrow.

Simple Electron Diffraction Workflow from Sample Prep to Structural Solutions



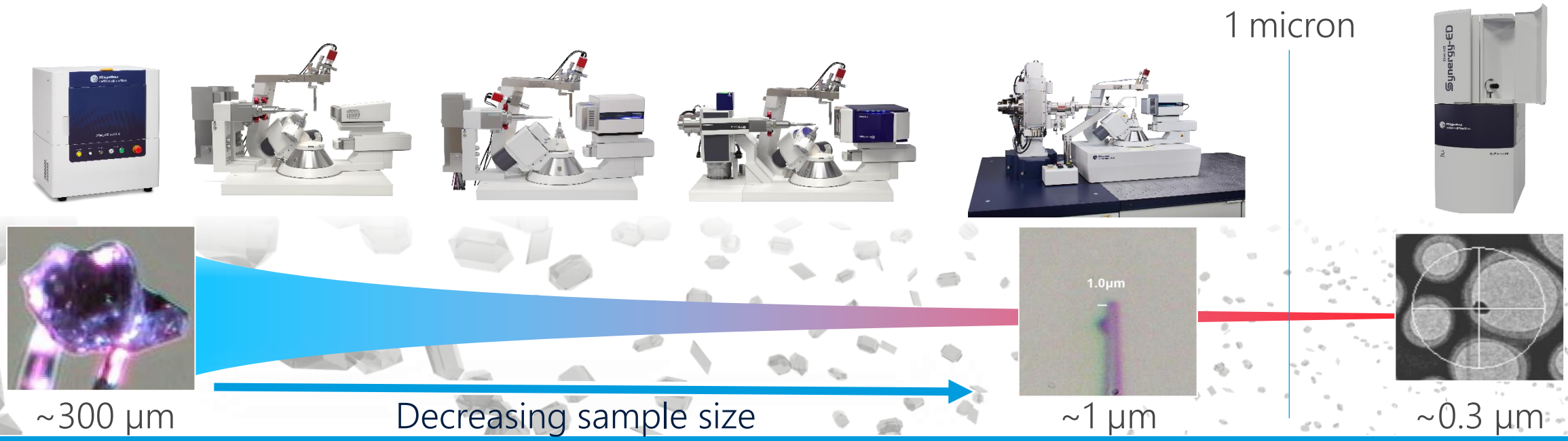
Agenda

1. Overview of 3D ED / microED
2. Sample preparation strategies
3. 3D ED data collection and processing with the Synergy-ED
4. Use of automation
5. Summary

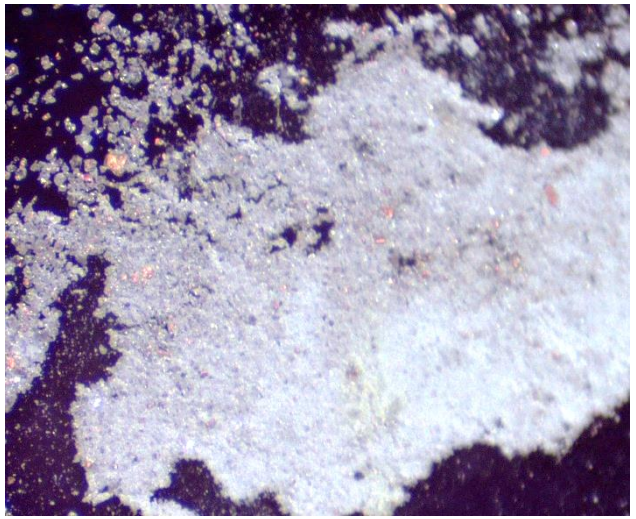
1. Overview of 3D ED / microED

Breaking the 1 Micron Barrier

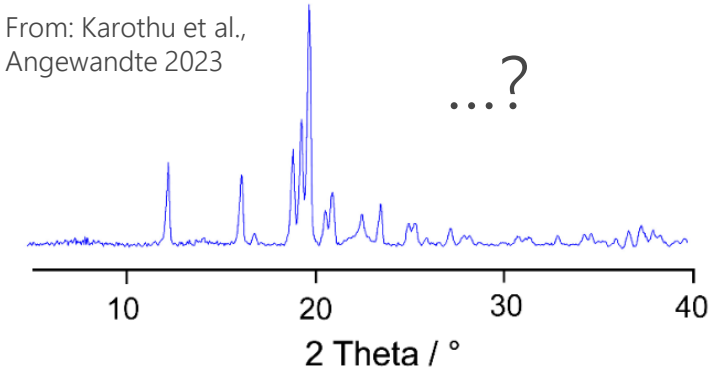
- X-rays reach down to micron size crystals
- Electrons reach *up* to micron sized samples.
- X-ray and electron diffraction are **complementary** techniques, together allowing study of samples from several hundred microns all the way down to the nanoscale.

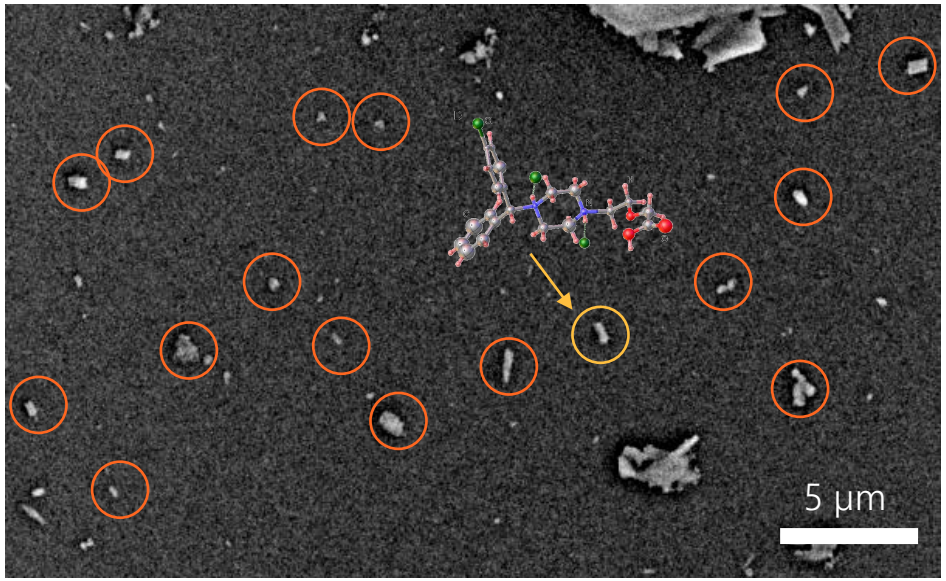


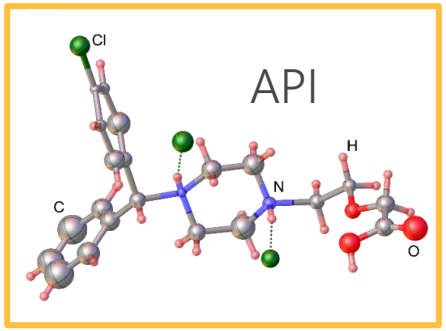
Powders Grain-by-Grain

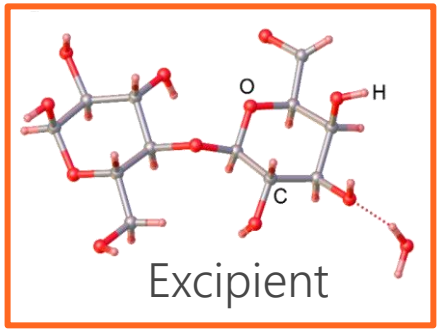


From: Karothu et al., Angewandte 2023



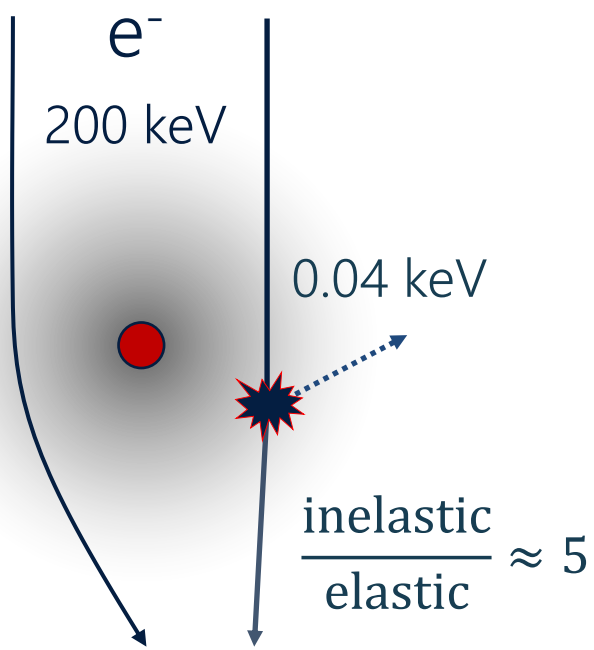




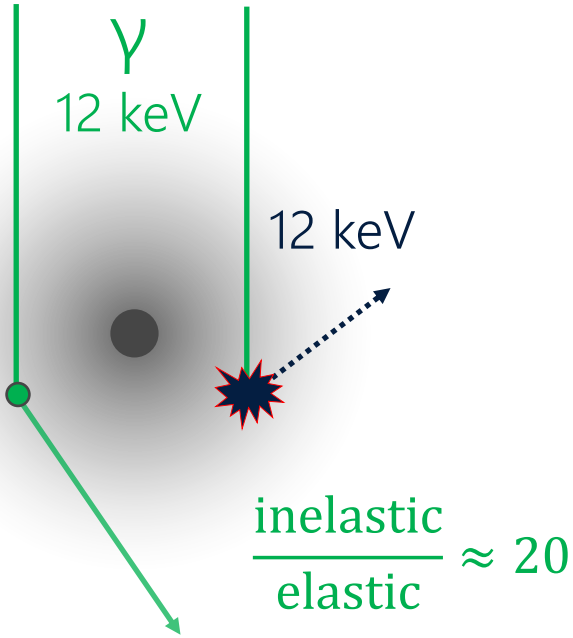


Electrons vs. X-rays

Elastic scattering from Coulomb potential



Elastic scattering from electron density



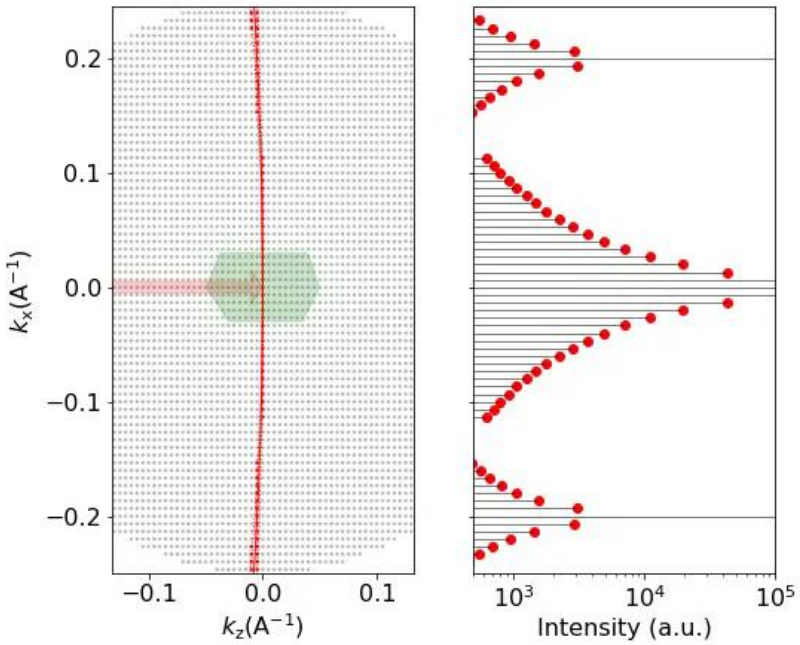
Electrons interact 10^6 times stronger
→ sub-µm crystals are possible *and required*

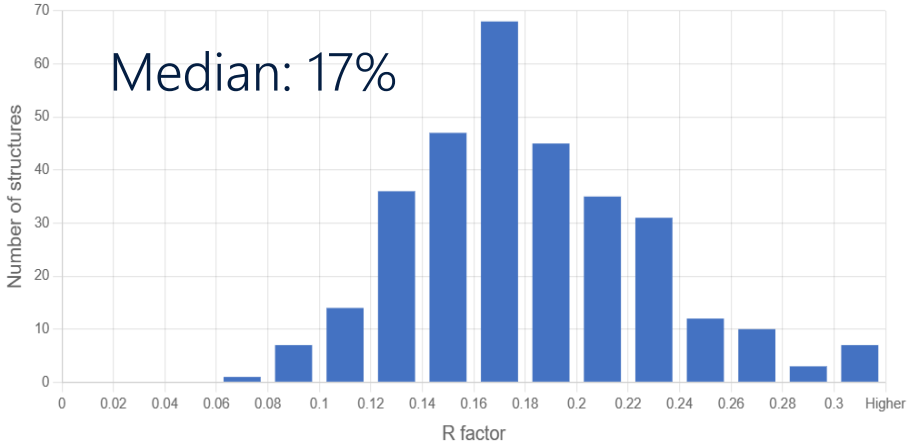
10^3x lower energy deposit per elastic event
→ less radiation damage per information

Electron Diffraction - Data

- Very strong interaction: dynamical diffraction due to multiple scattering
  - $I_{hkl} \neq |F_{hkl}|^2$

- New data collection approach *unlike* "classical" ED
  - Dynamical diffraction gets suppressed!
  - Refinement in **kinematical** approximation: *systematically* high R-values, but good structures!





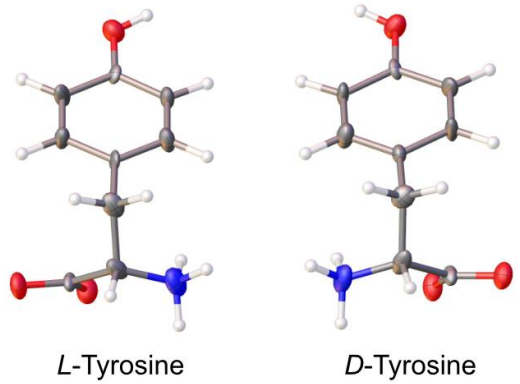
Spence, J. C. H. (2006). High-resolution electron microscopy for materials science.

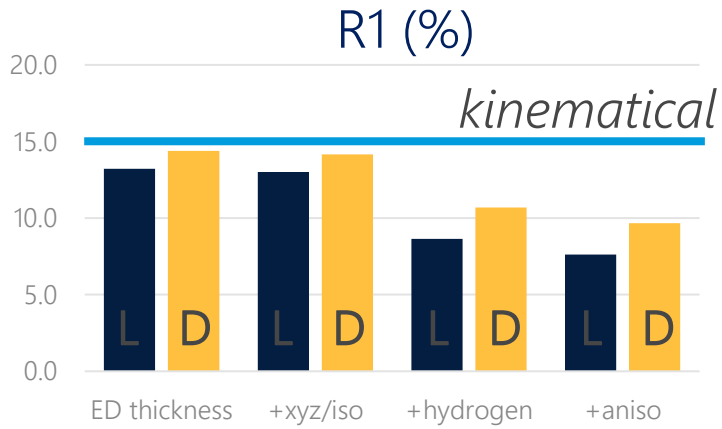
Electron Diffraction - 3D ED / microED

- Replace X-rays in single-crystal diffraction by electrons! Invented from 2007 by several groups independently: 3D ED, microED, RED, cRED…

- Full **dynamical** refinement: heavy computation, but **improved R-values and maps, absolute structure!**

- Conducted in adapted TEMs by specialized research groups





Palatinus, L.; Petříček, V.; Corrêa, C.A., *Acta Cryst. A* **2015**, *71*, 235–244.
Brázda, P.; Palatinus, L.; Babor, M., *Science* **2019**, *364*, 667–669.
Klar, P.B.; Krysiak, Y.; Xu, H.; Steciuk, G.; Cho, J.; Zou, X.; Palatinus, L., *Nat. Chem.* **2023**, *15*, 848.
Truong, K.-N. *et al. Symmetry* **2023**, 15(8), 1555.

Crystallography Meets Electron Microscopy

Transmission Electron Microscope

- Wide variety of measurement types
- Frequent manual alignments required
- Often qualitative/image analysis

Single Crystal X-ray Diffractometer

- Specific measurement modalities
- Fully automated and integrated
- Quantitative and (reduced) data-driven

→ Differing expectations for instrumentation and analysis

XtaLAB Synergy-ED

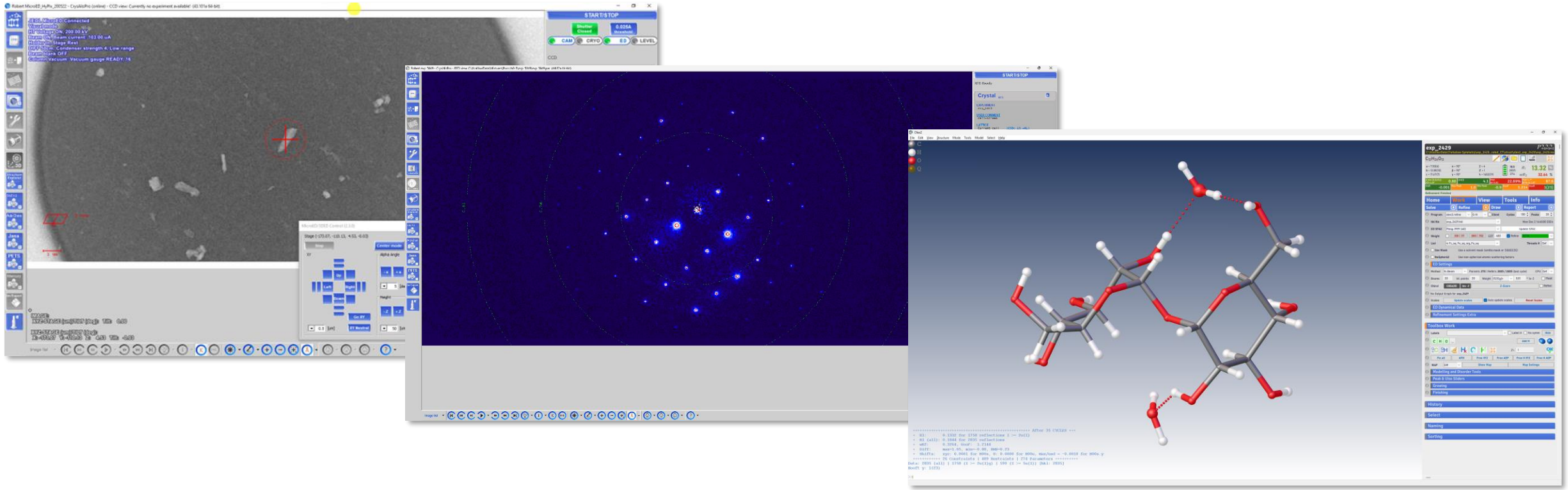
A dedicated electron diffractometer,
made for non-specialists

Highly optimized and integrated hardware and software design for microED / 3D ED

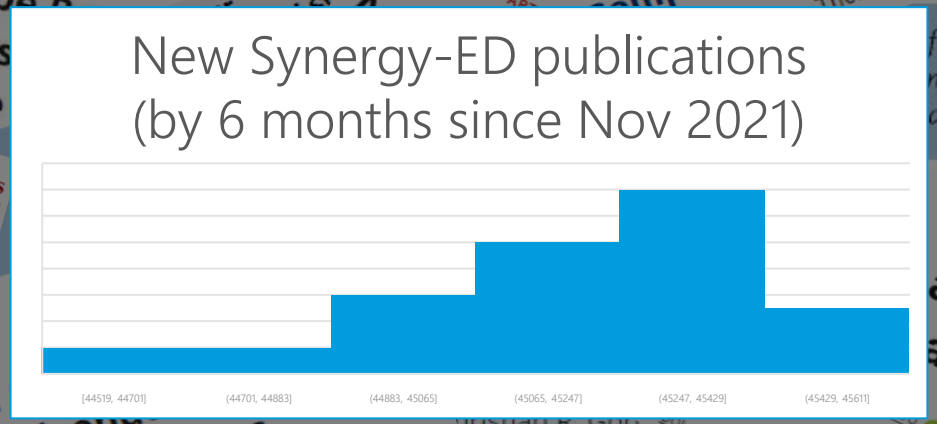
Seamless workflows, from instrument control to structure solution

XtaLAB Synergy-ED: An Integrated Experience

Screen, collect, automate, process, solve, manage,… in *one* seamless interface



Screen for crystals → Collect diffraction data → Solve and refine structure



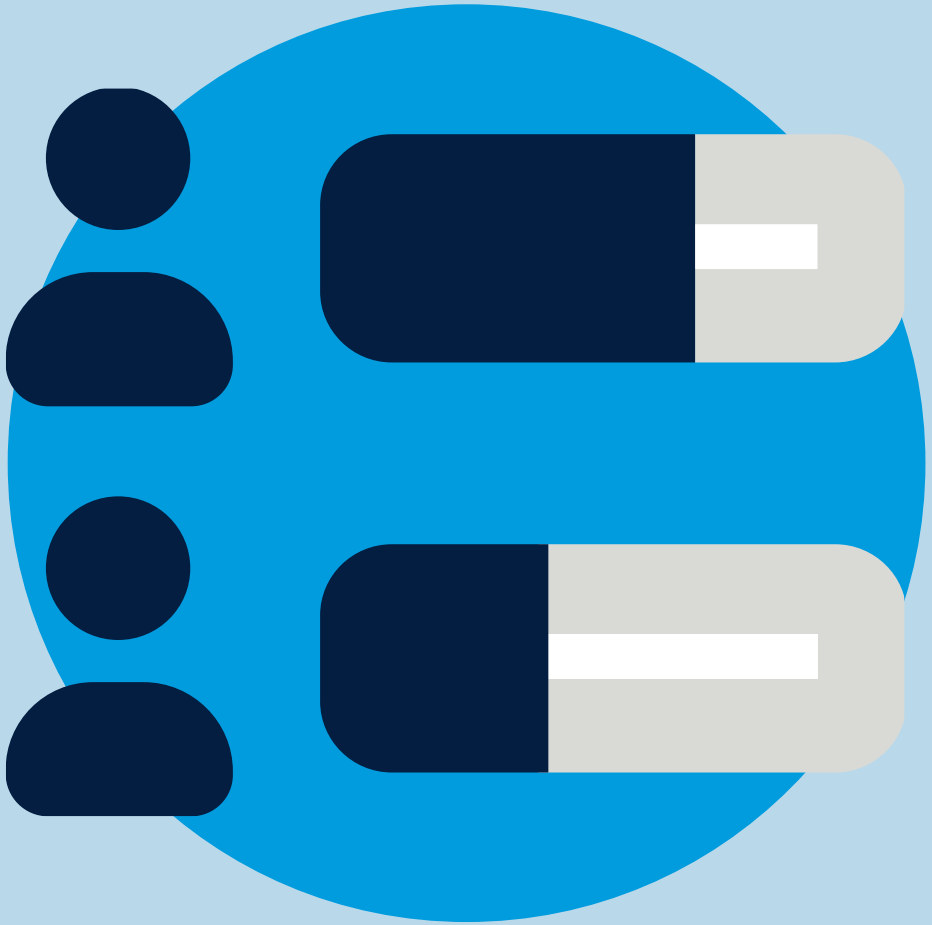
https://rigaku.com/resources/publication-library

>50 Synergy-ED peer-reviewed publications as of December 2024

..many more in preparation

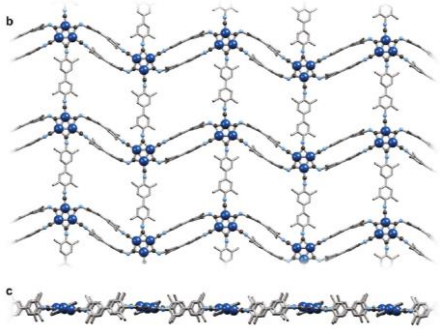
Polling Question

#1

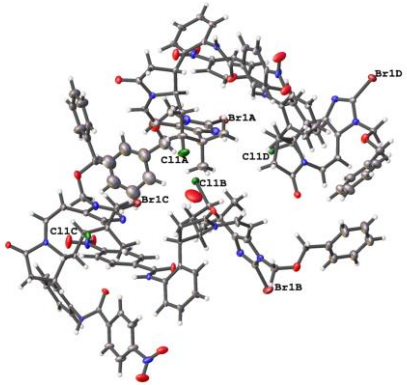


2. Sample preparation strategies

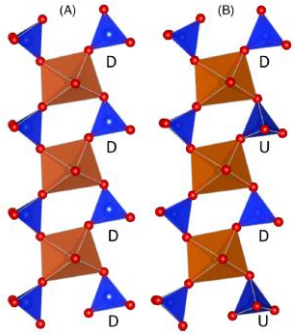
Sample Preparation: Types of Samples



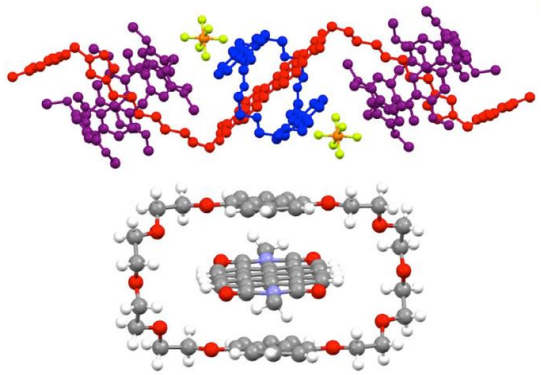
MOFs/COFs/ZIFs



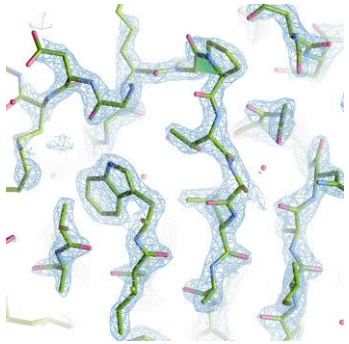
Natural products



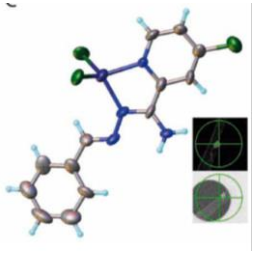
Ceramics



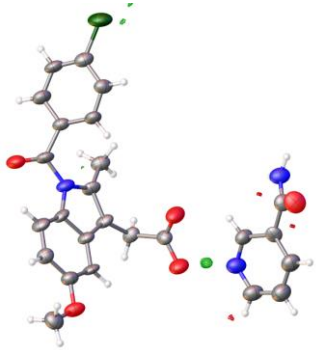
Macromolecular complexes



Proteins



Organometallics

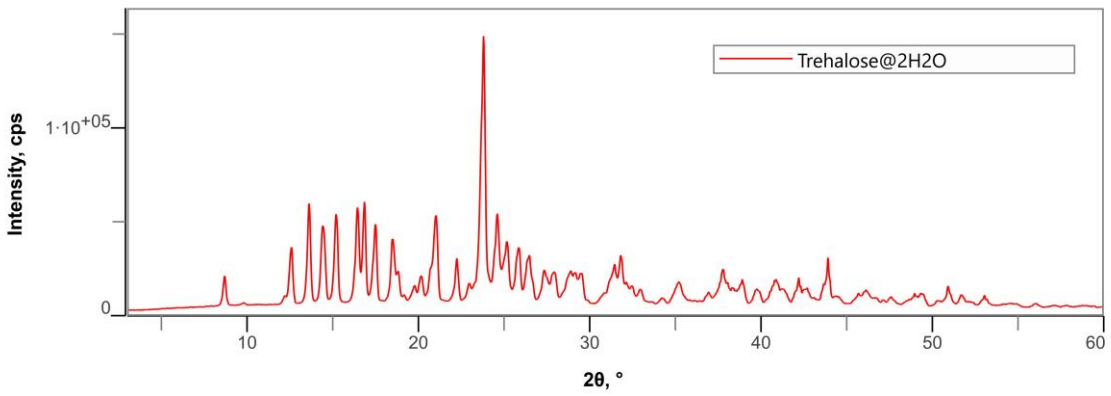


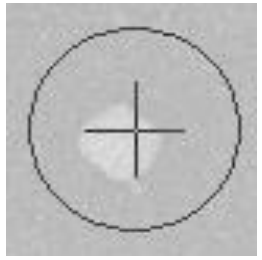
Pharmaceuticals

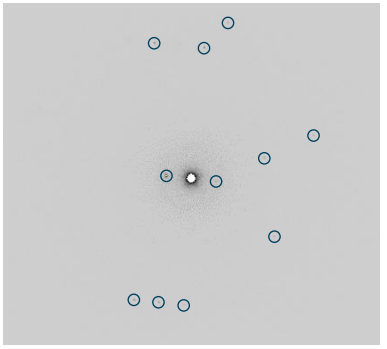
- Mixtures/impure
- Air-sensitive
- Sub-milligram quantities
- Require solvation

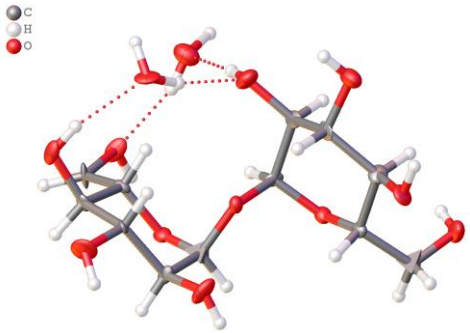
Sample Requirements: Crystallinity

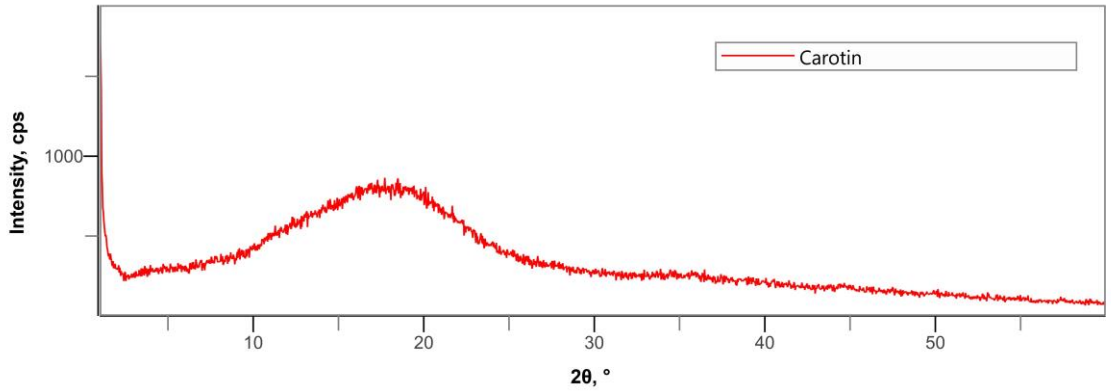
Powder pattern | Particle | Electron diffraction | Structure solution

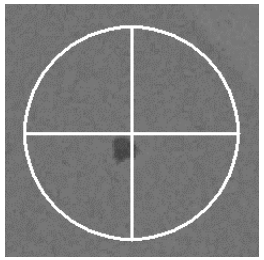


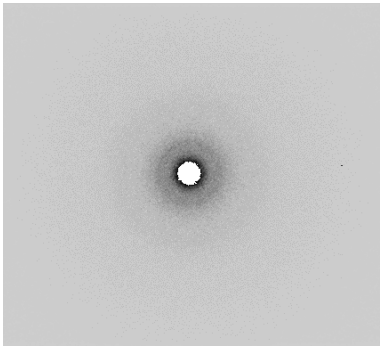












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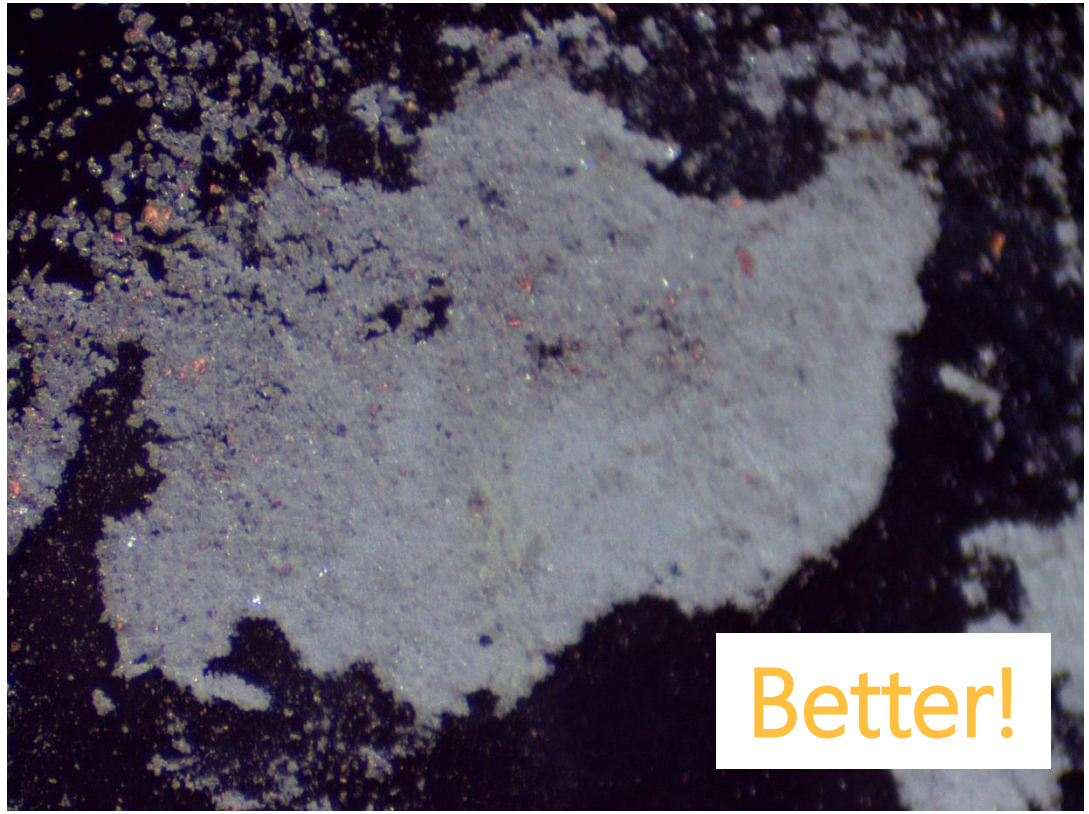
Crystallinity is a prerequisite for electron diffraction

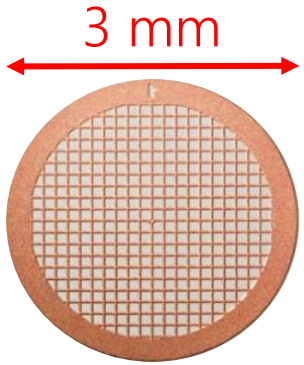
Sample Requirements: Particle Size

Particle size < 1 µm?

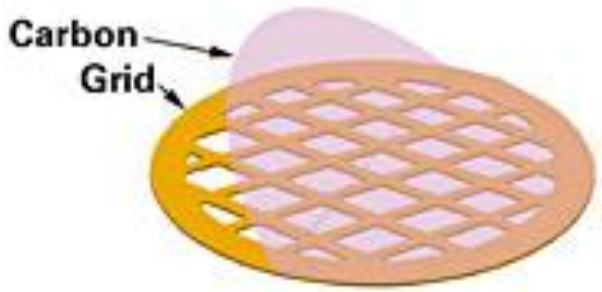


If crystals are visible by an optical microscope, they are too big for 3D ED. Crush or try SCXRD instead!

Sample Grids



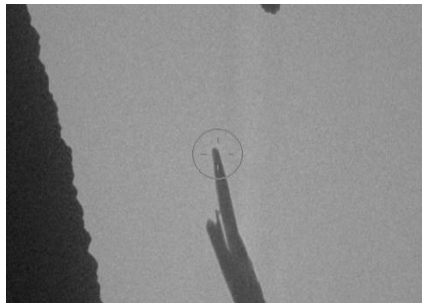
200 mesh Cu TEM grid



Can purchase grids with varying:

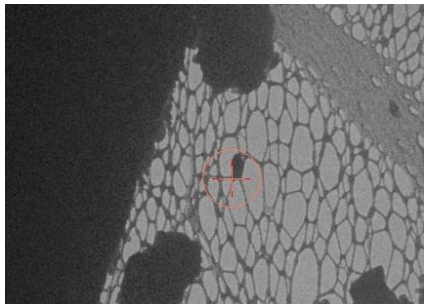
- Mesh size
- Metal grating
- Carbon / polymer supports

Continuous Carbon



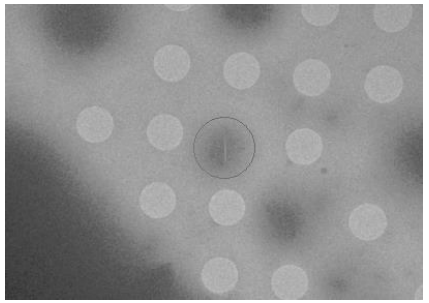
A good starting point

Lacey Carbon



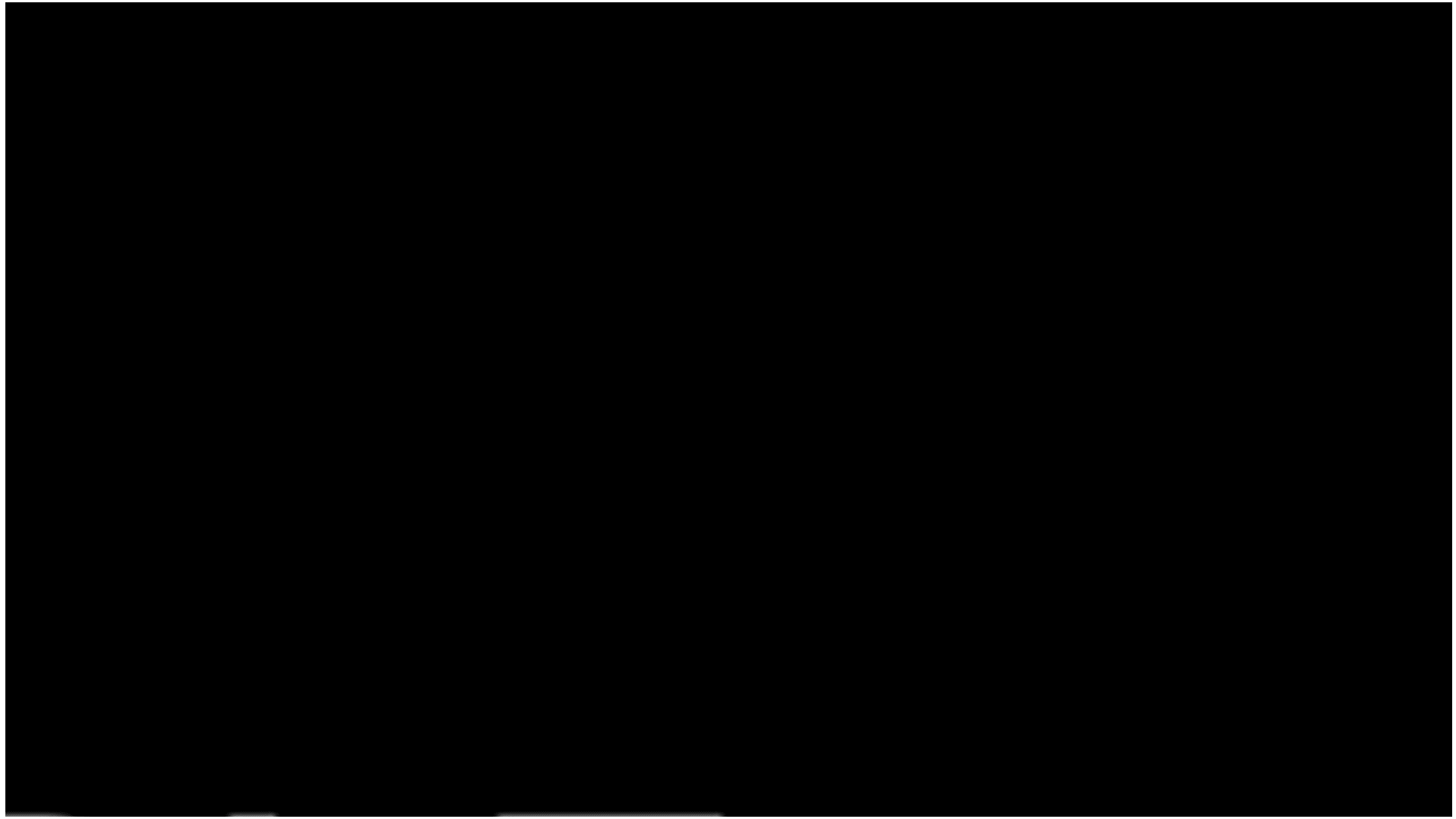
Can help with preferred orientation

Hole Array (Quantifoil)



Useful when freezing samples within solvent

Grid Preparation

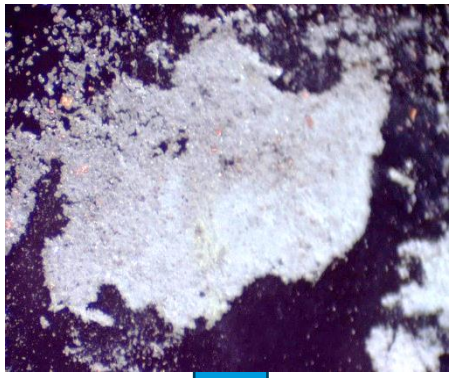


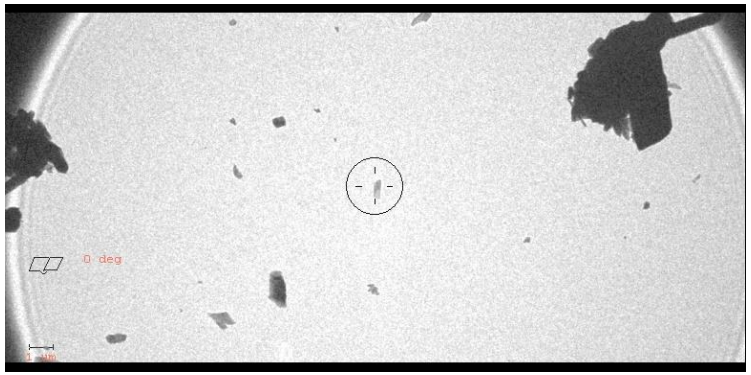
Grid Preparation

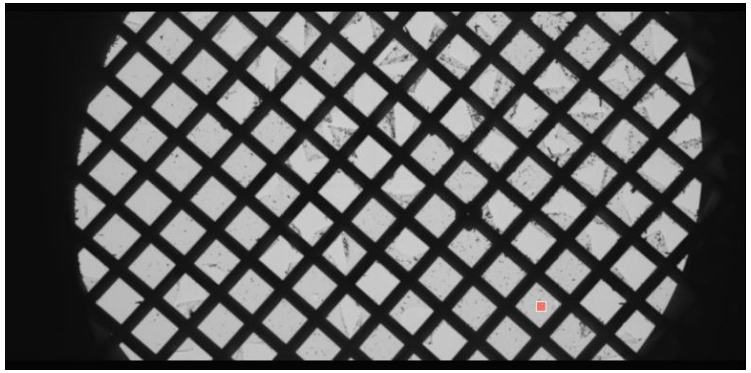
What makes a good sample grid?

- Crystallite size <1 µm
- No preferred orientation of crystallites
- Crystallite spacing of few µm

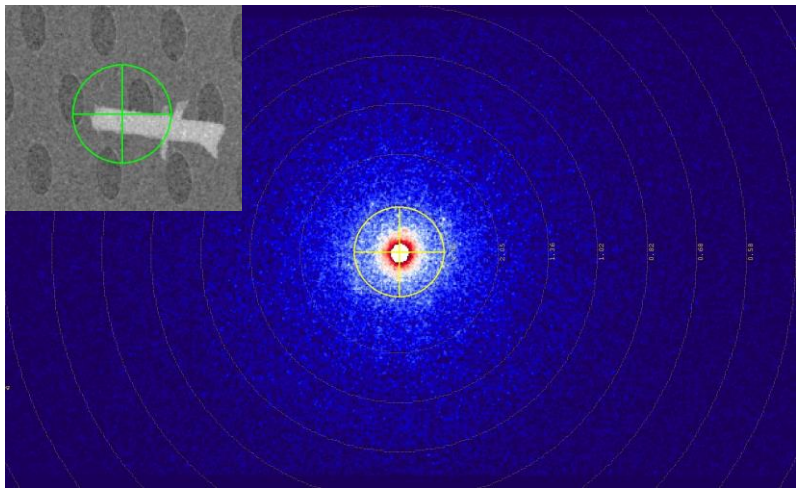
Grid is much larger than required sample volume: **it's fine, if only parts are well loaded. The optimal regions can be identified during data collection.**







Cryogenic Preparation



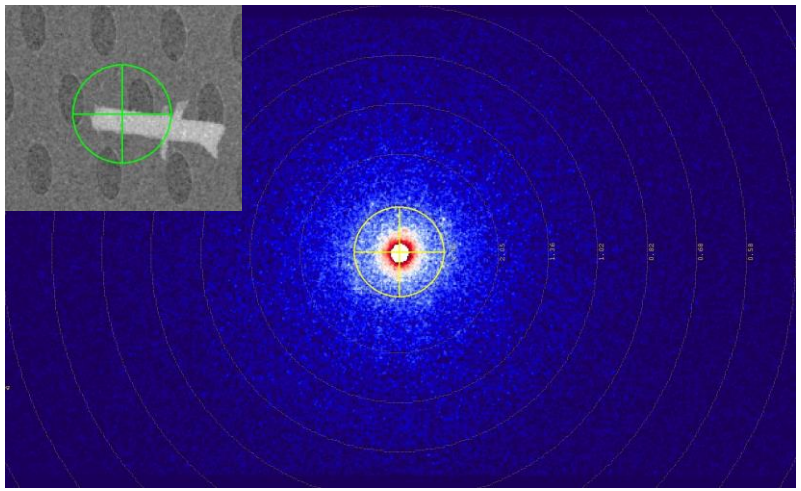
Vacuum

RT
(297 K)

No useful diffraction data

See strong diffraction >30 degrees in 2θ by powder, but nothing by ED?

Cryogenic Preparation

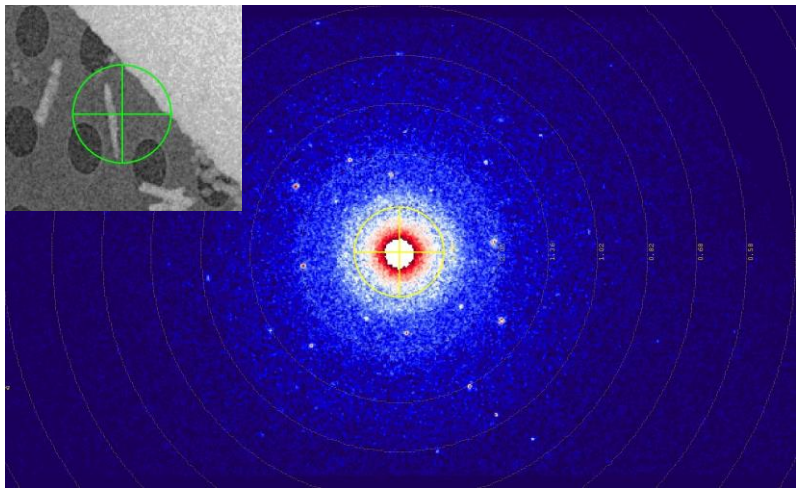


Vacuum

RT
(297 K)

Vacuum

Cryo-Transfer
(100 K)



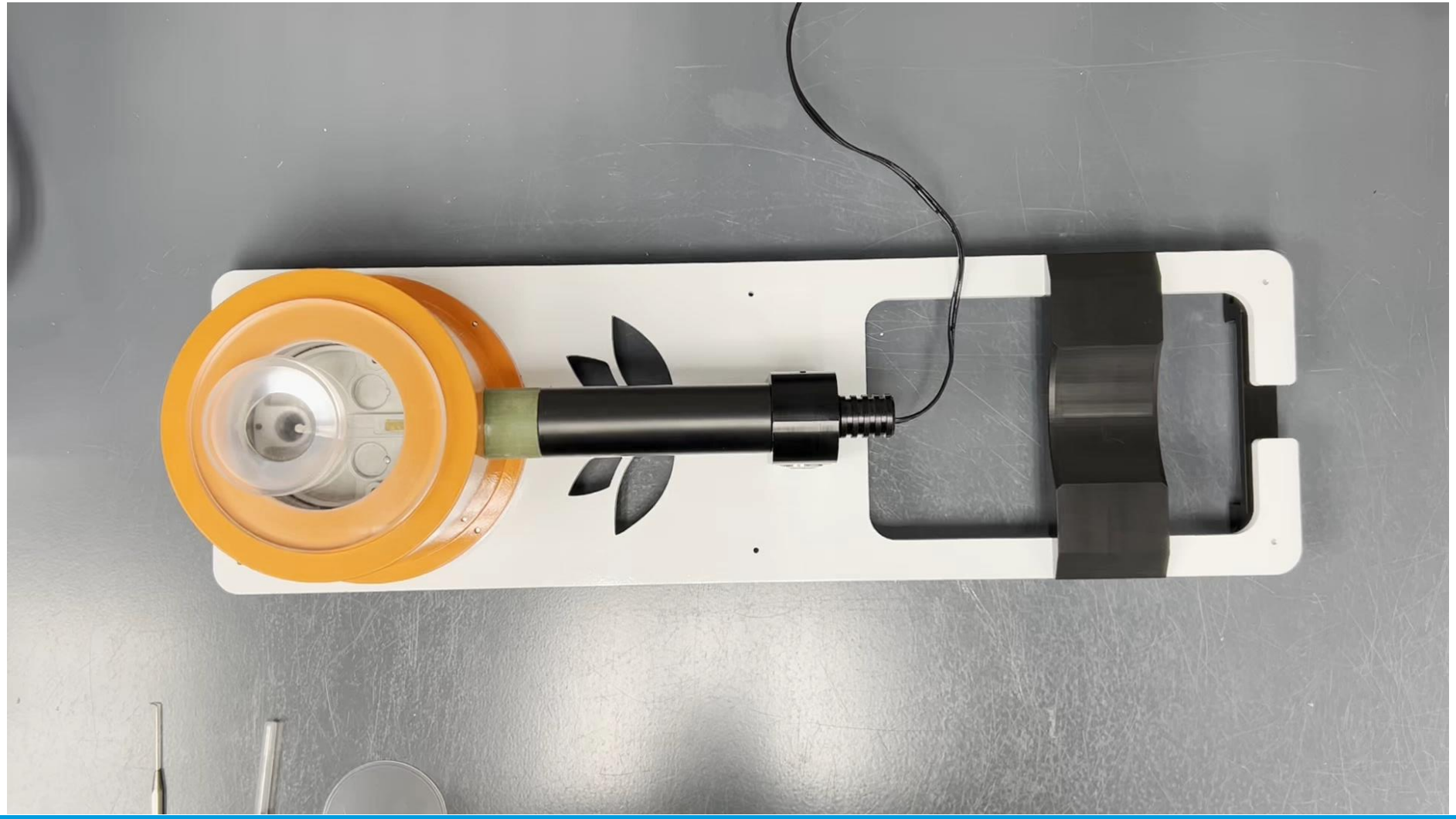
No useful diffraction data

0.60 Å data, immediate structure

See strong diffraction >30 degrees in 2θ by powder, but nothing by ED?

It could be due to the strong vacuum!
A cryo transfer can address vacuum instability

Cryogenic Sample Loading

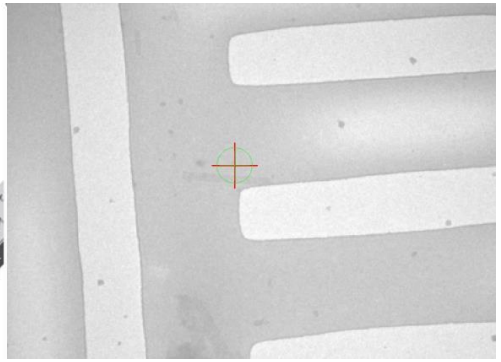


Sample Loading

Sample Preparation: The Possibilities

Multi-specimen holders

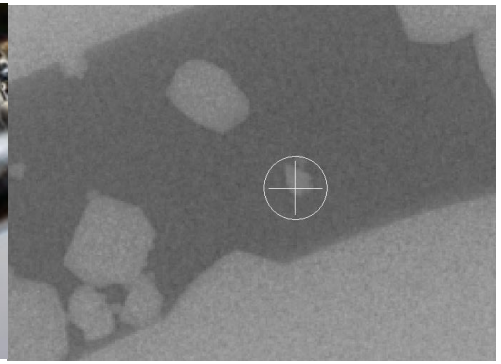
Liquid/gas cells



API crystal in solvent

Air-free holders

Heating holders

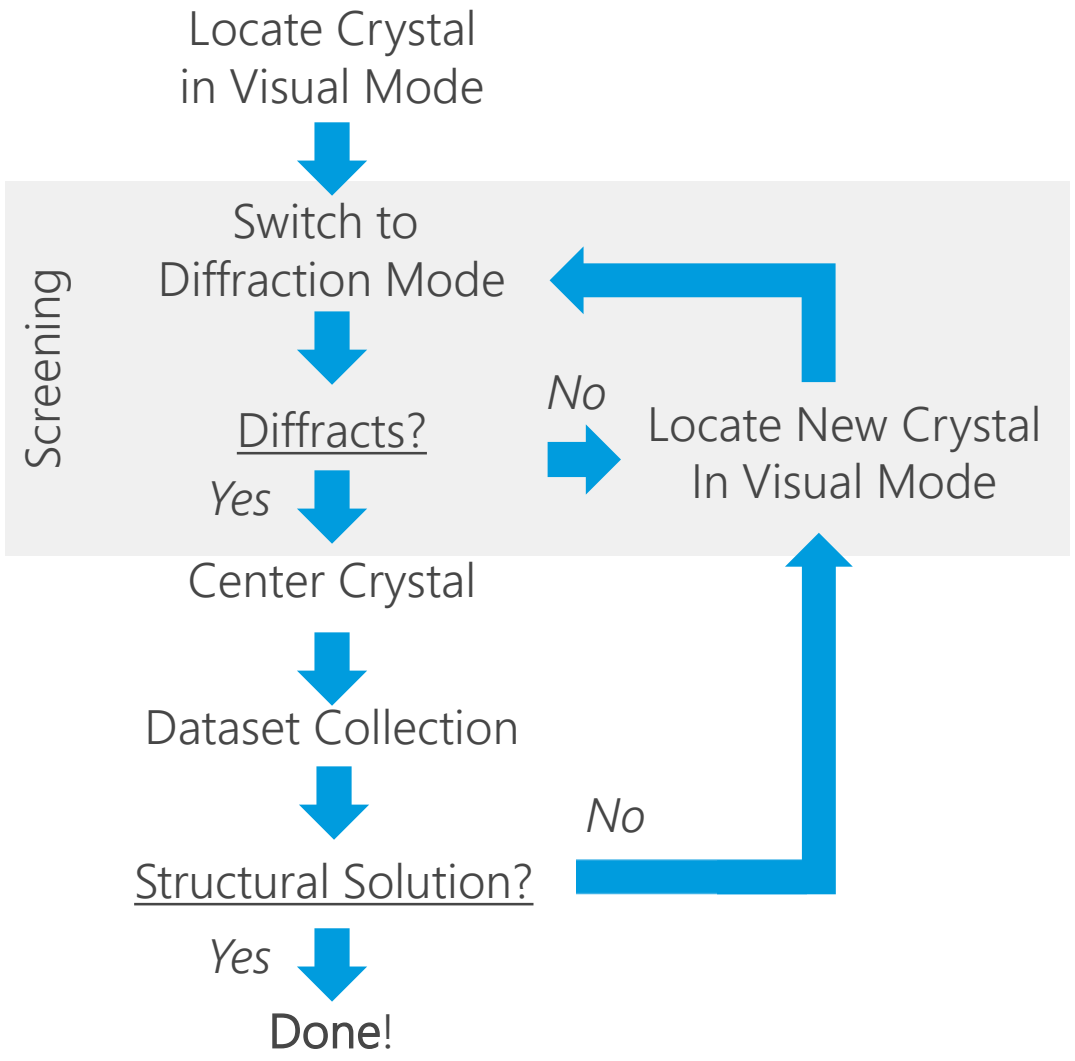


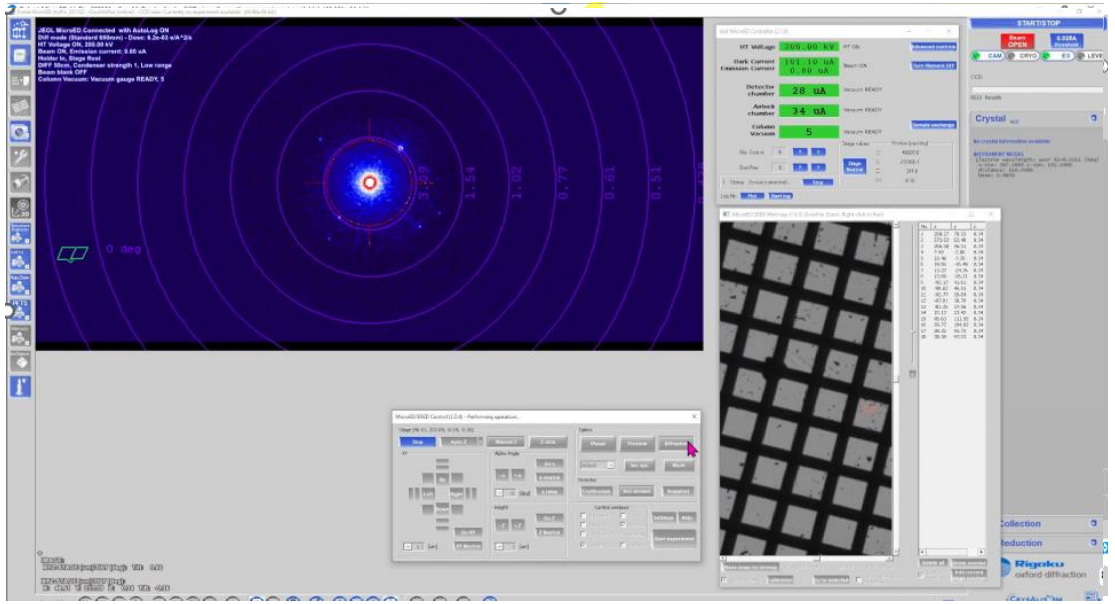
MOF heated to 200˚C

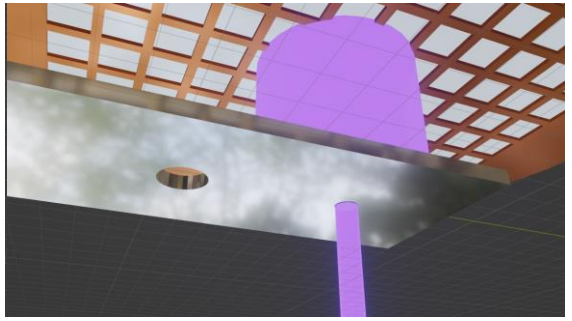
Questions?

3. 3D ED data collection and processing with the Synergy-ED

Manual Data Collection Workflow

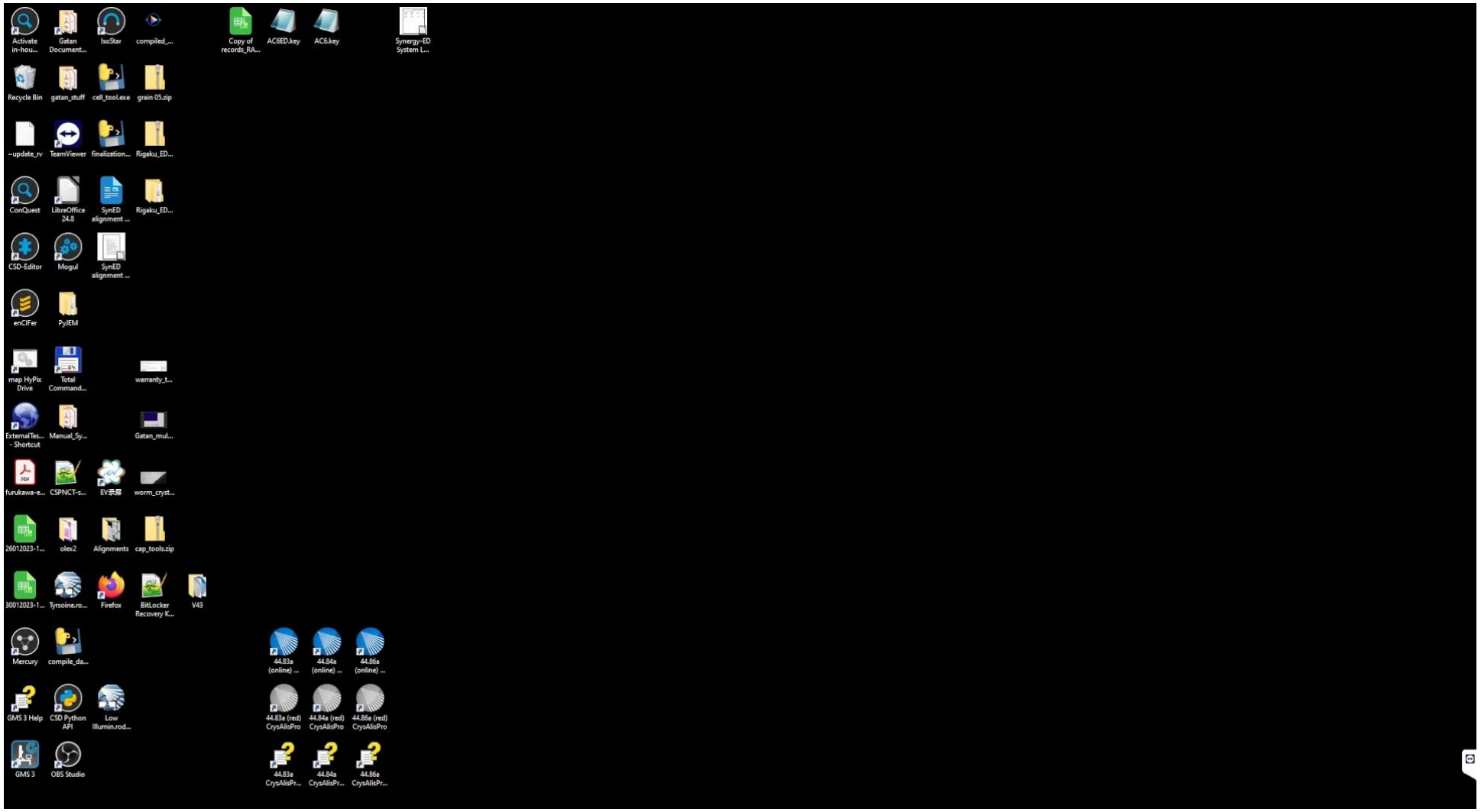




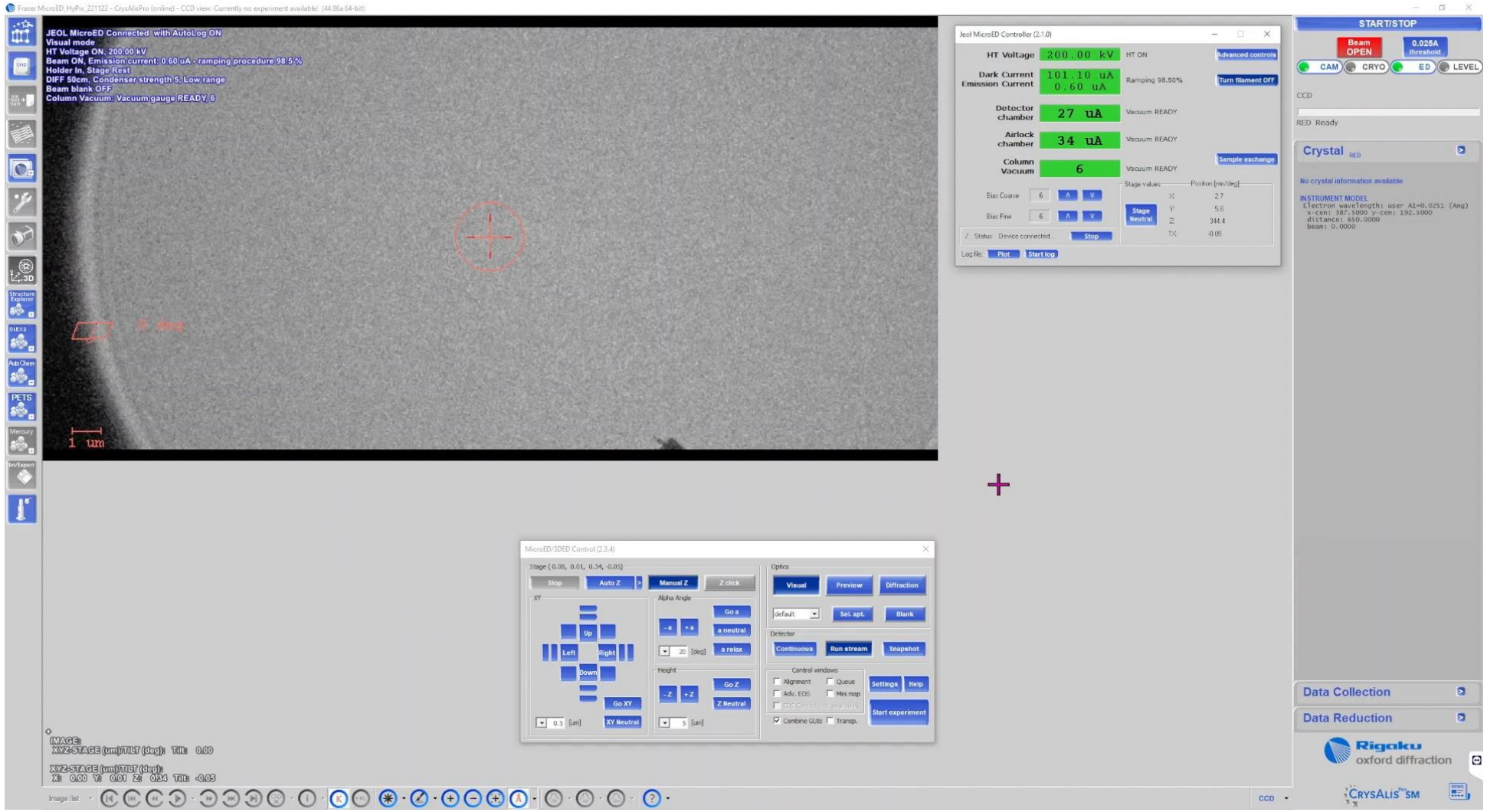


Selection
Aperture

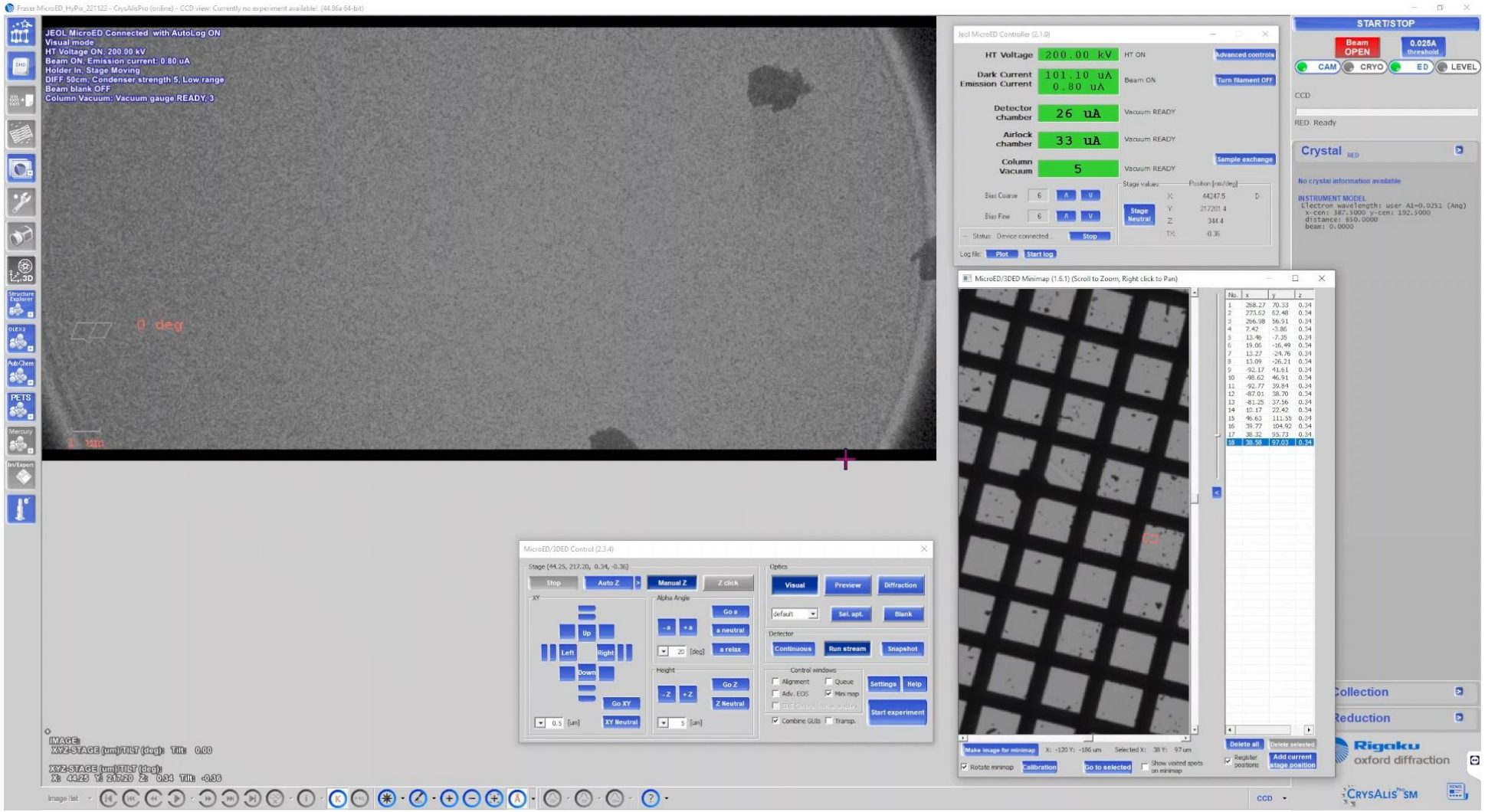
Getting Started



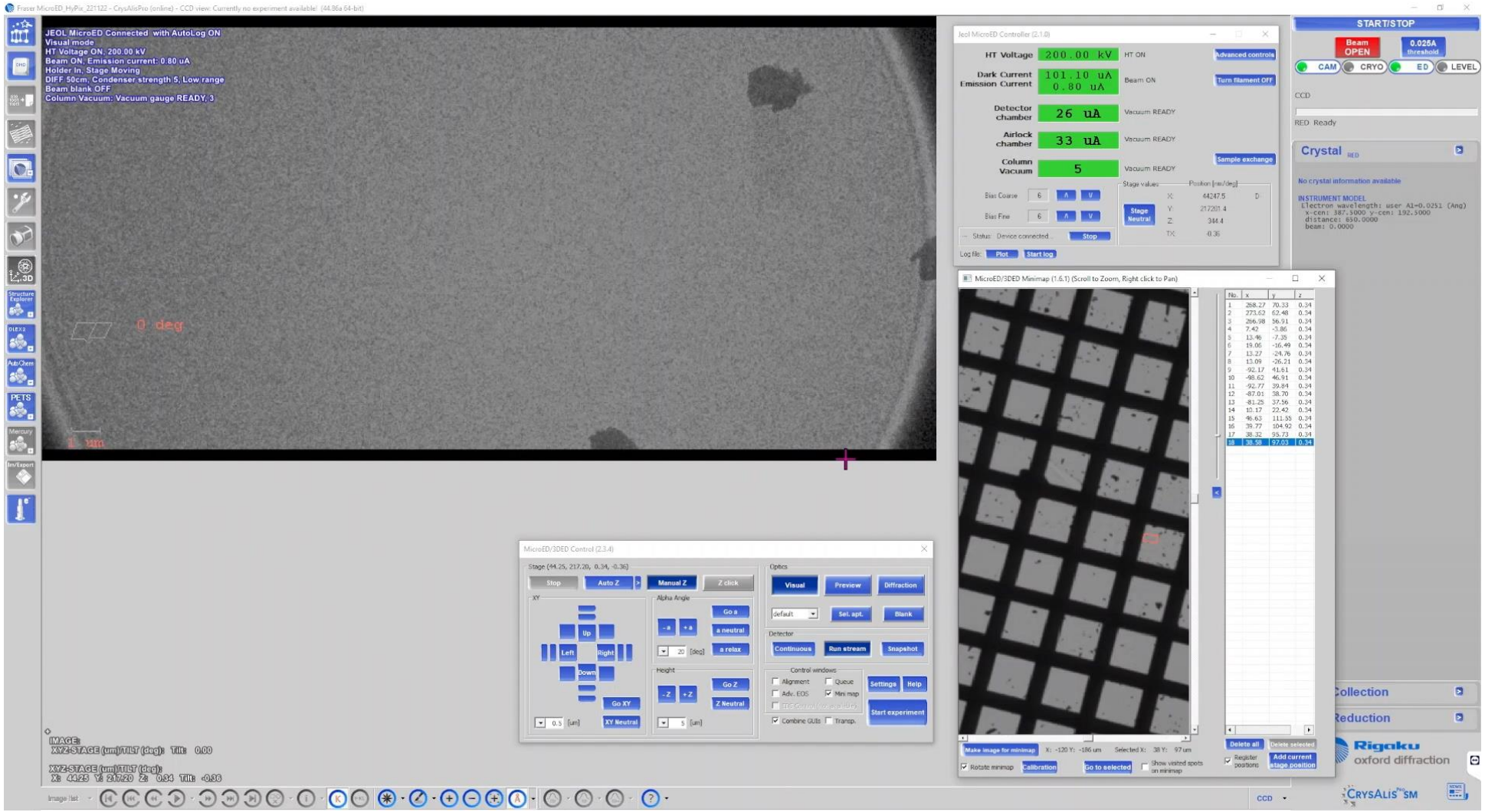
Minimap Collection



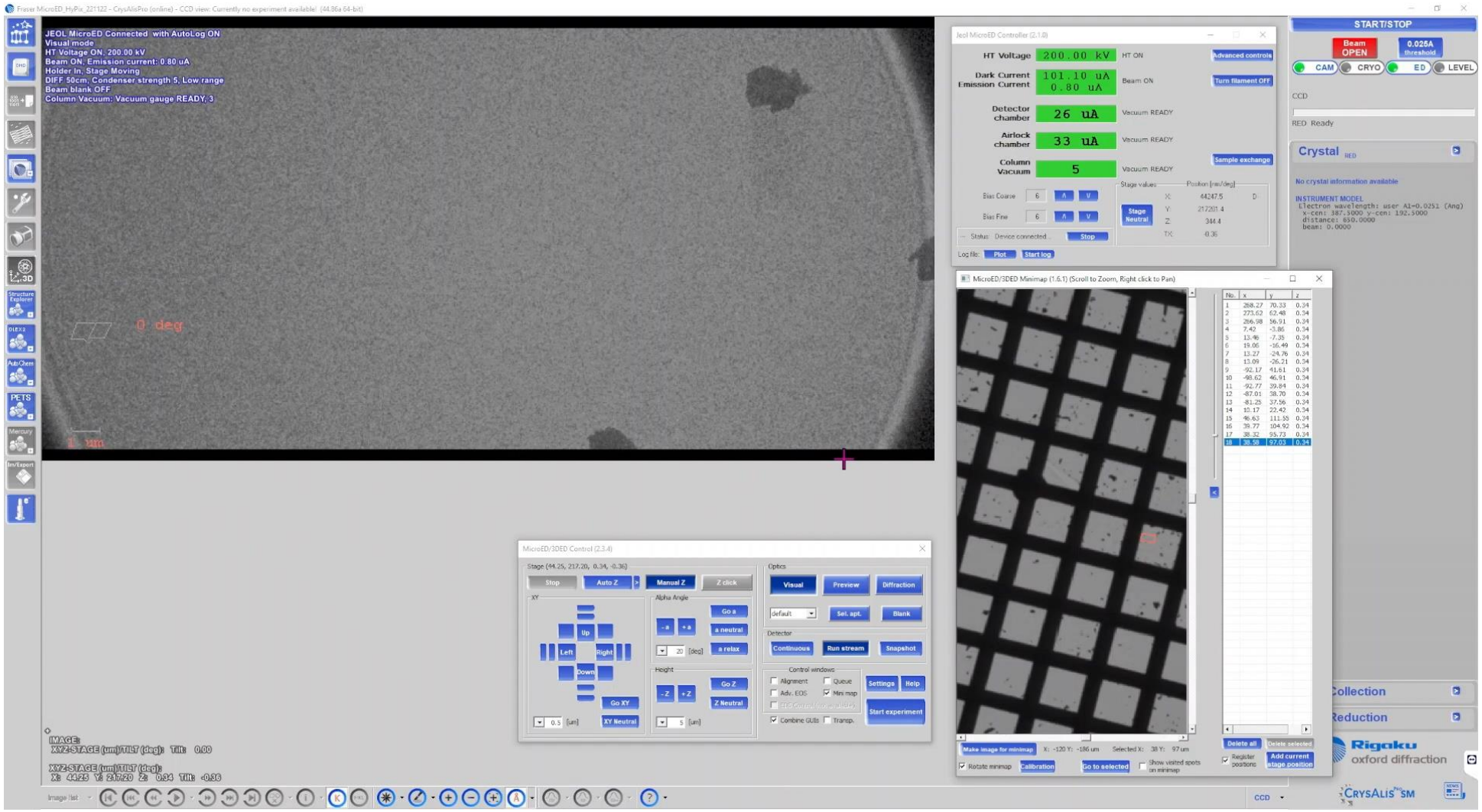
Particle Screening



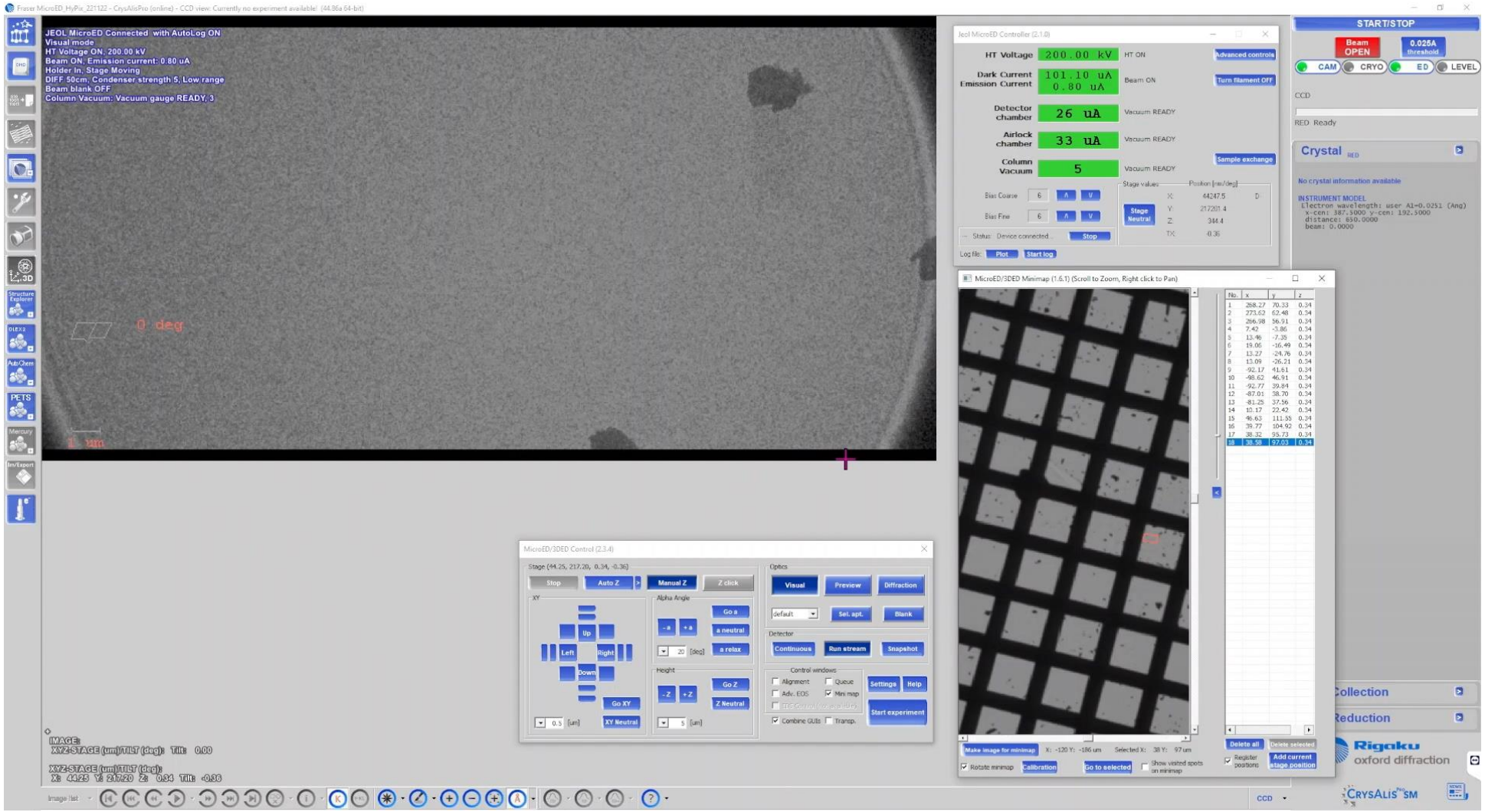
Particle Screening: Thickness



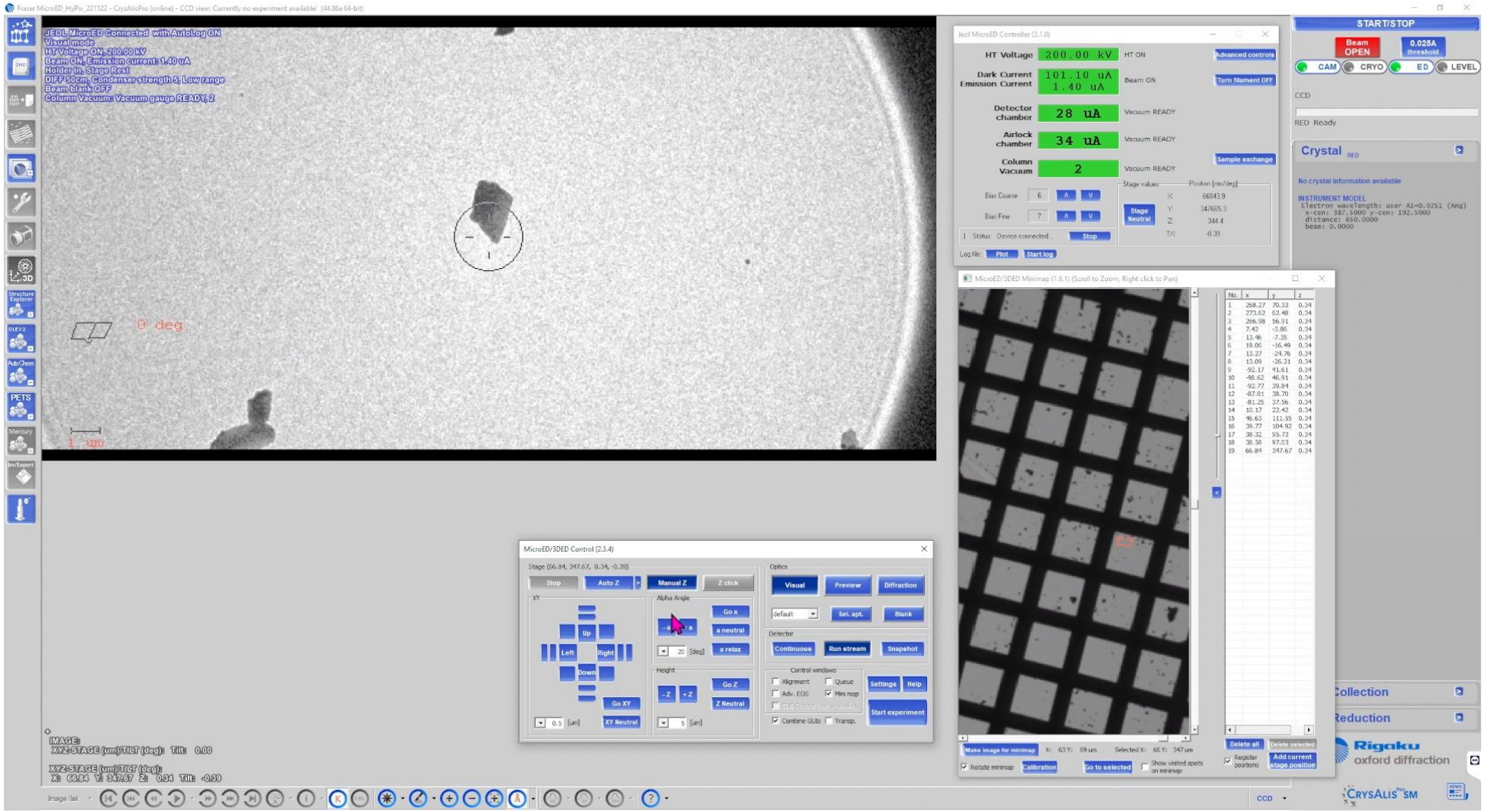
Particle Screening: Thickness



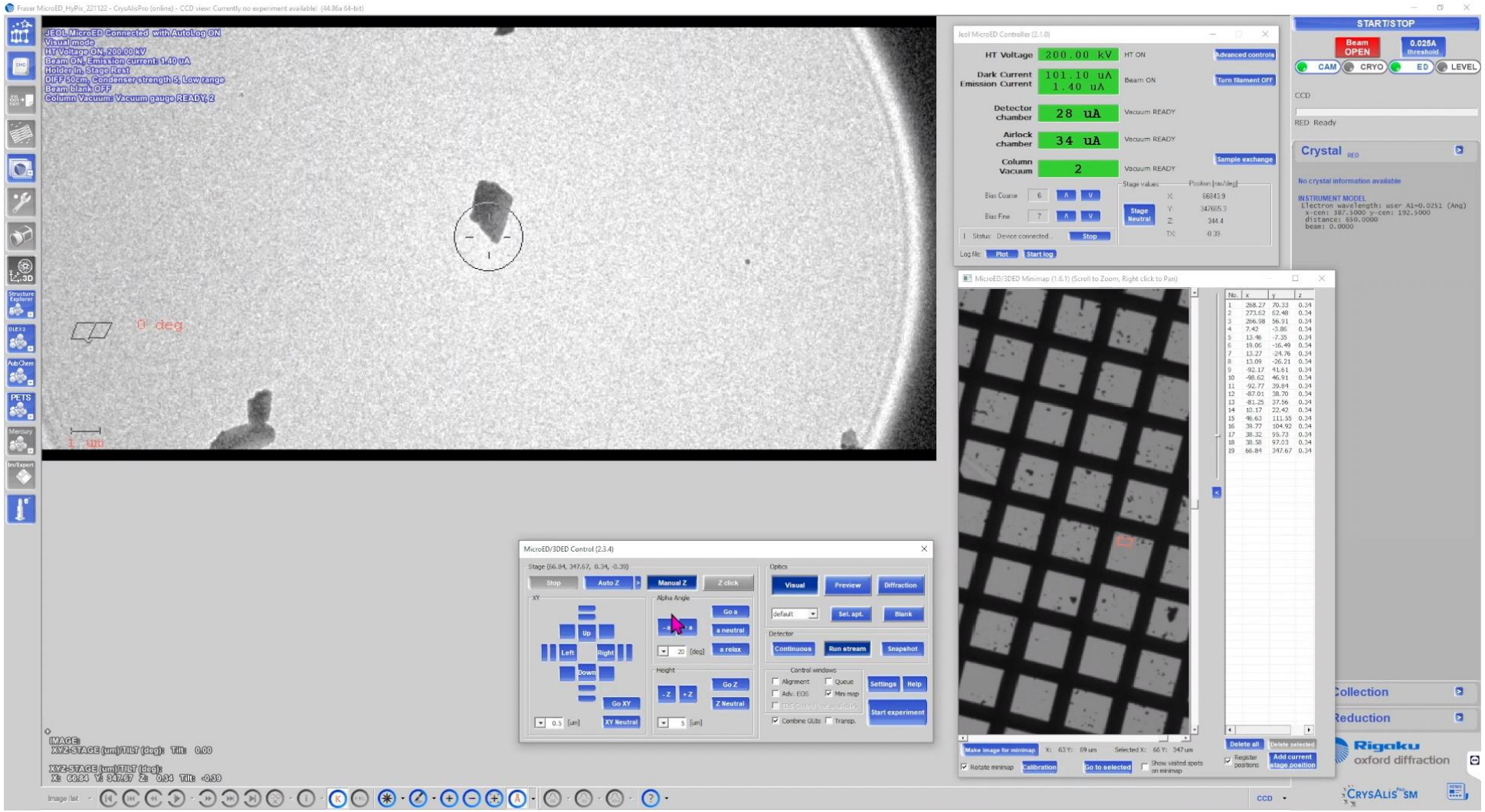
Particle Screening: Thickness



Data Collection Through Structural Solution

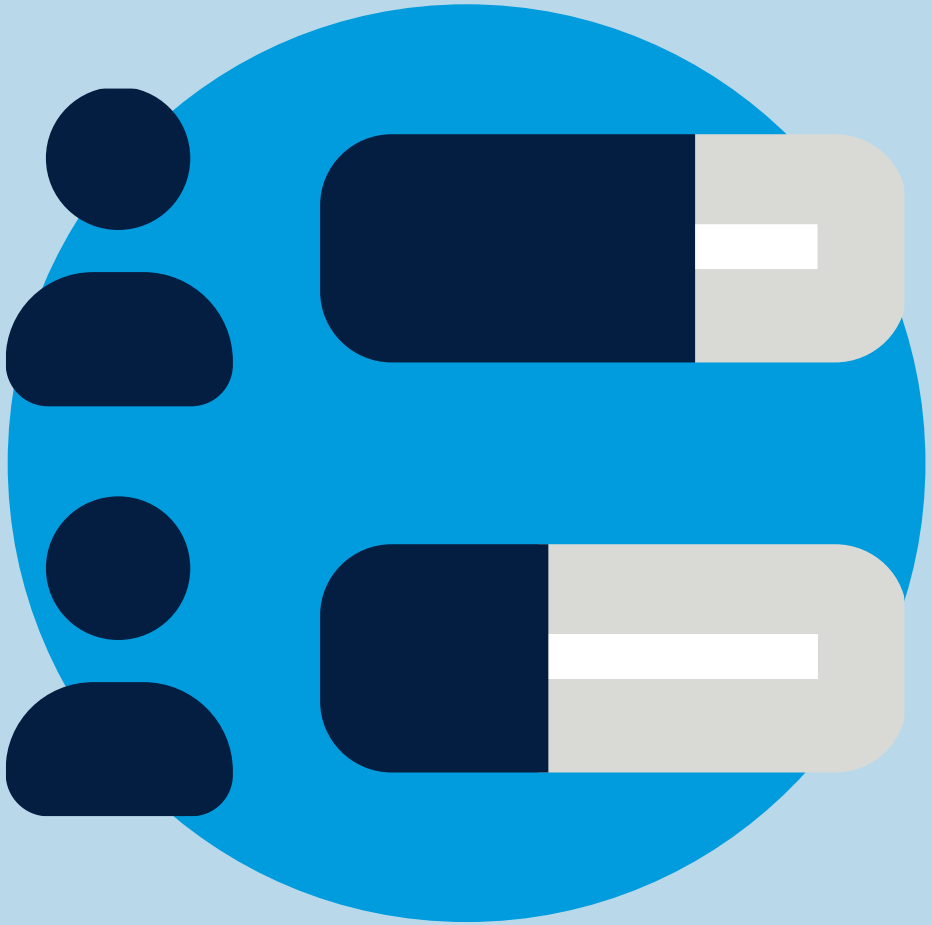


Data Collection Through Structural Solution



Polling Question

#2



4. Use of automation

Workflow with Automation

Locate Crystal in Visual Mode

Pre-experiment

Switch to Diffraction Mode

Diffracts?

No → Locate New Crystal In Visual Mode → Switch to Diffraction Mode

Yes

Experiment

Center Crystal

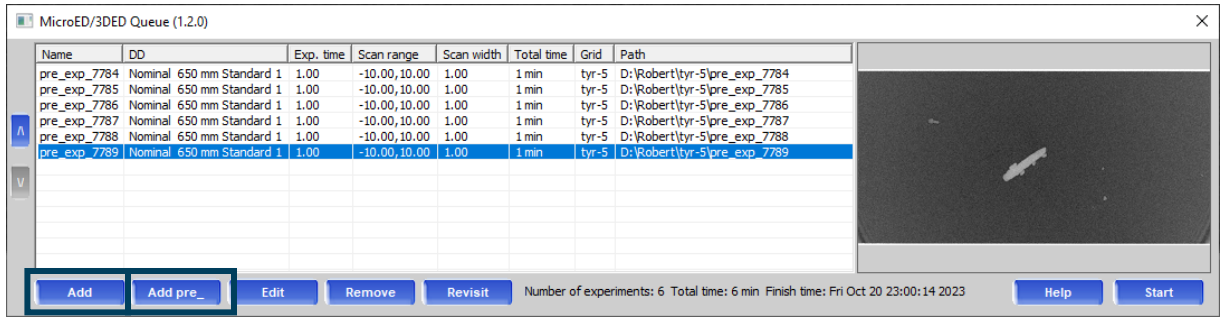
Dataset Collection

Structural Solution?

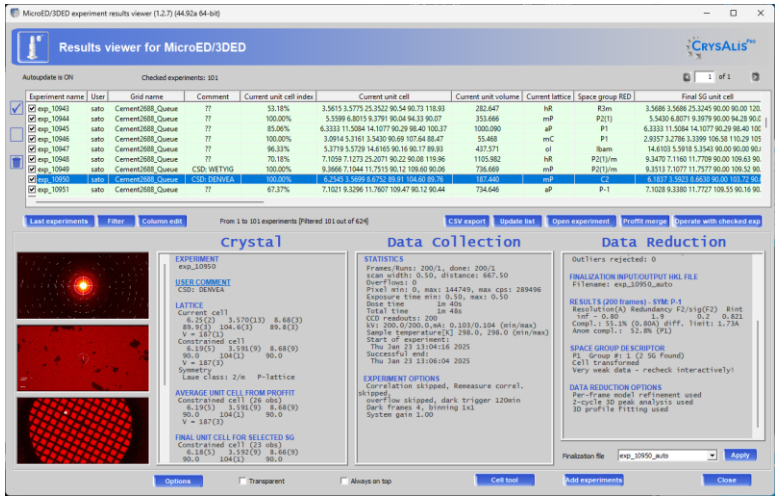
No → Center Crystal

Yes

Done!

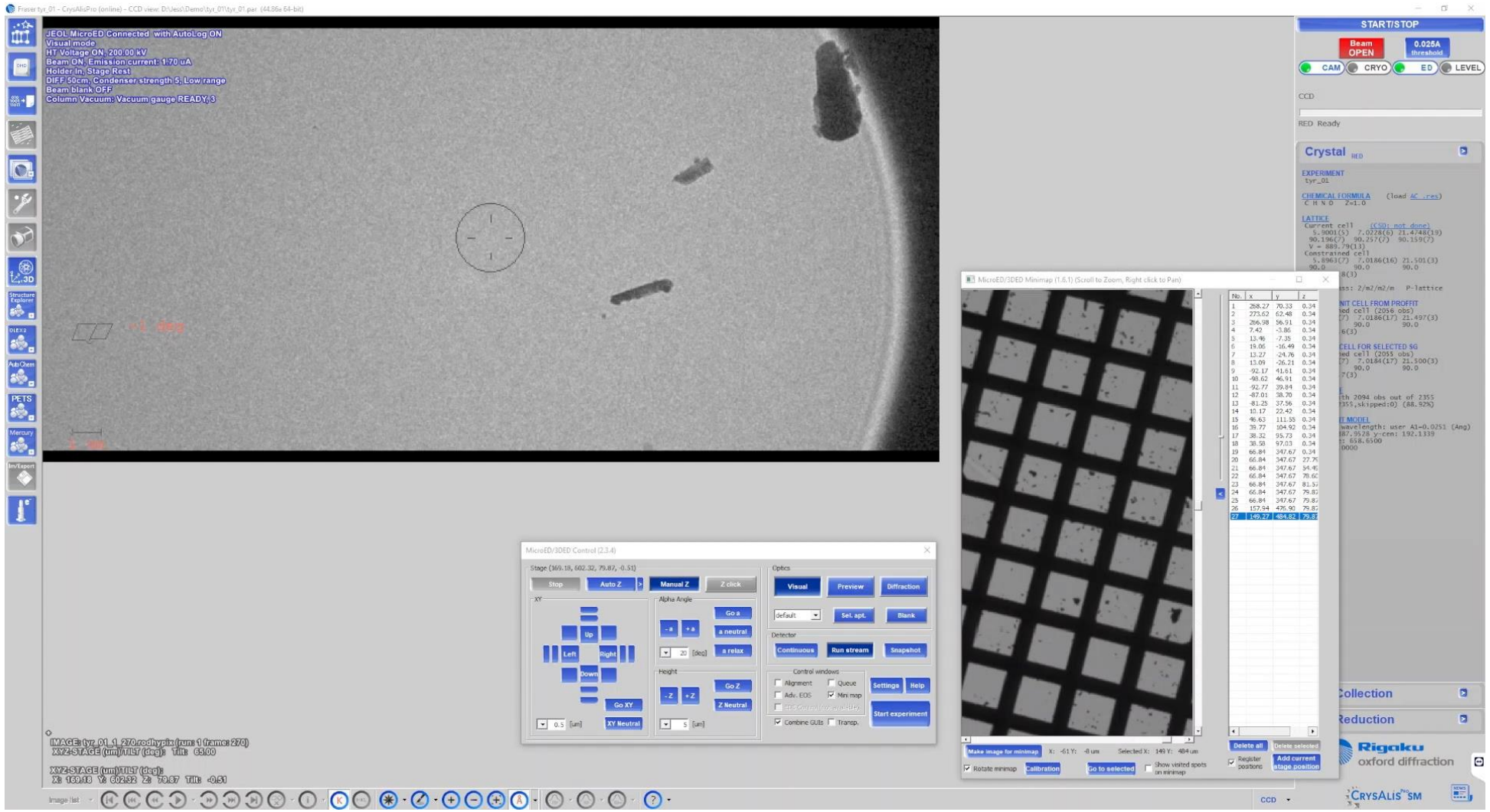


Queuing allows for unattended data collection

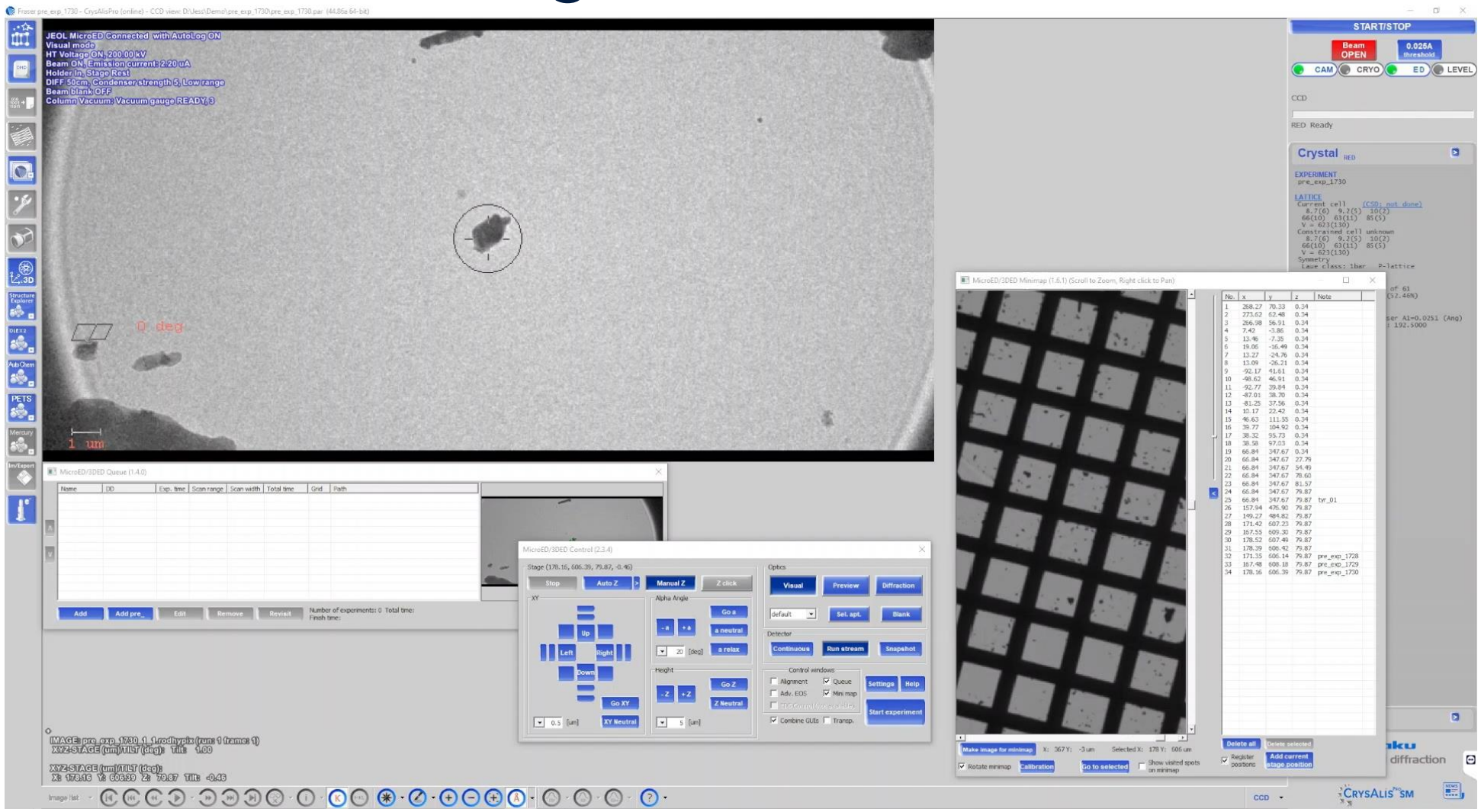


Results viewer streamlines decisions about data quality

Automation Workflows: Particle Screening



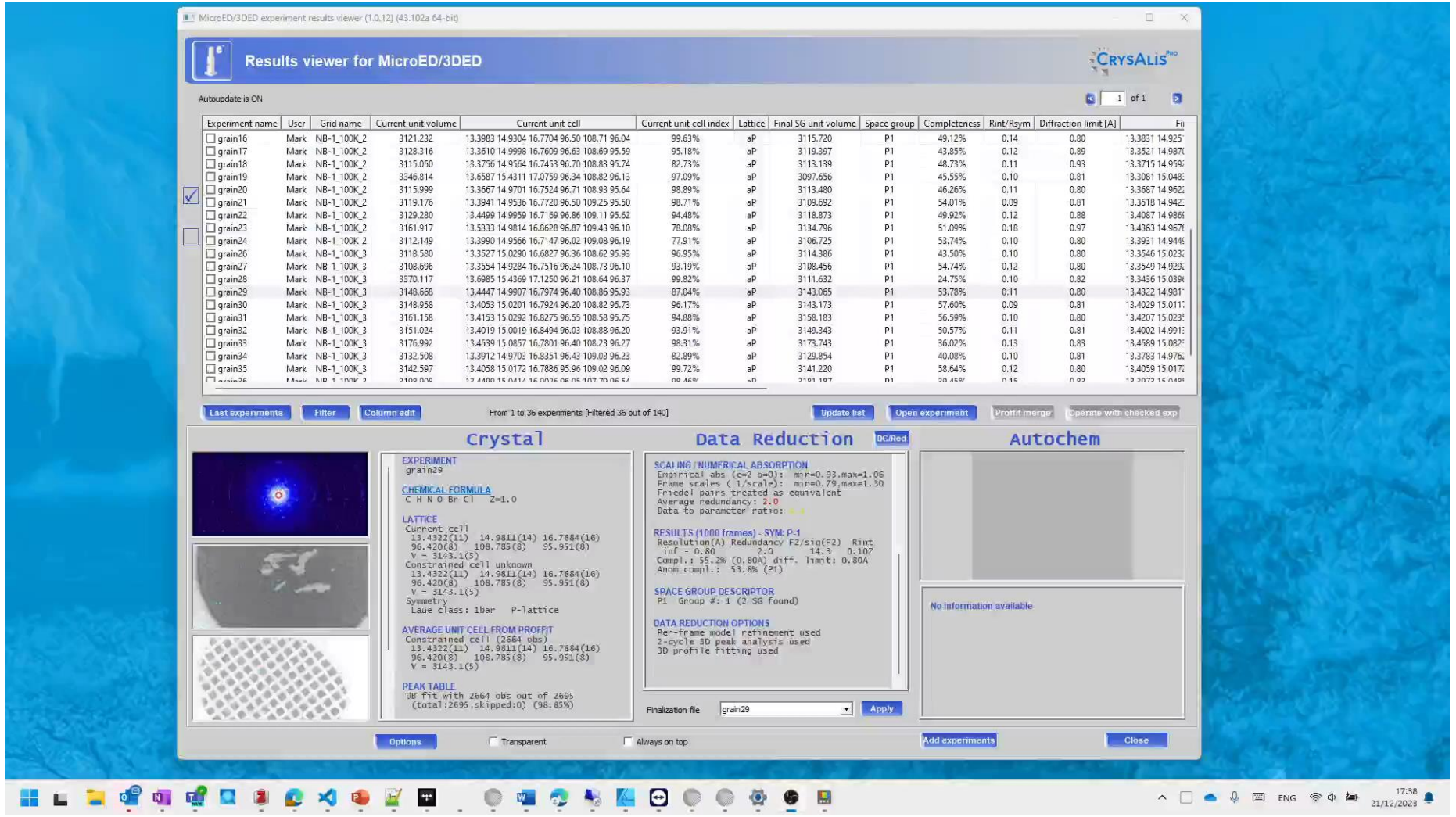
Automation: Centering and Data Collection



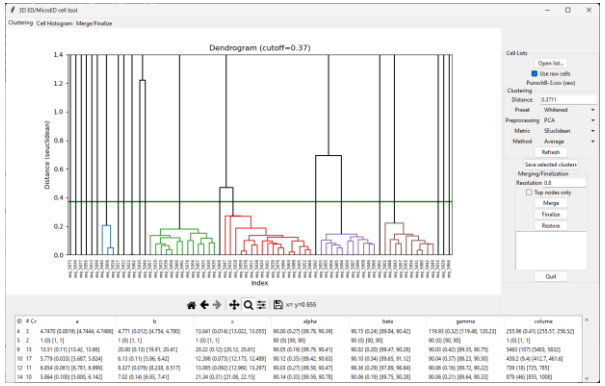
Results Viewer

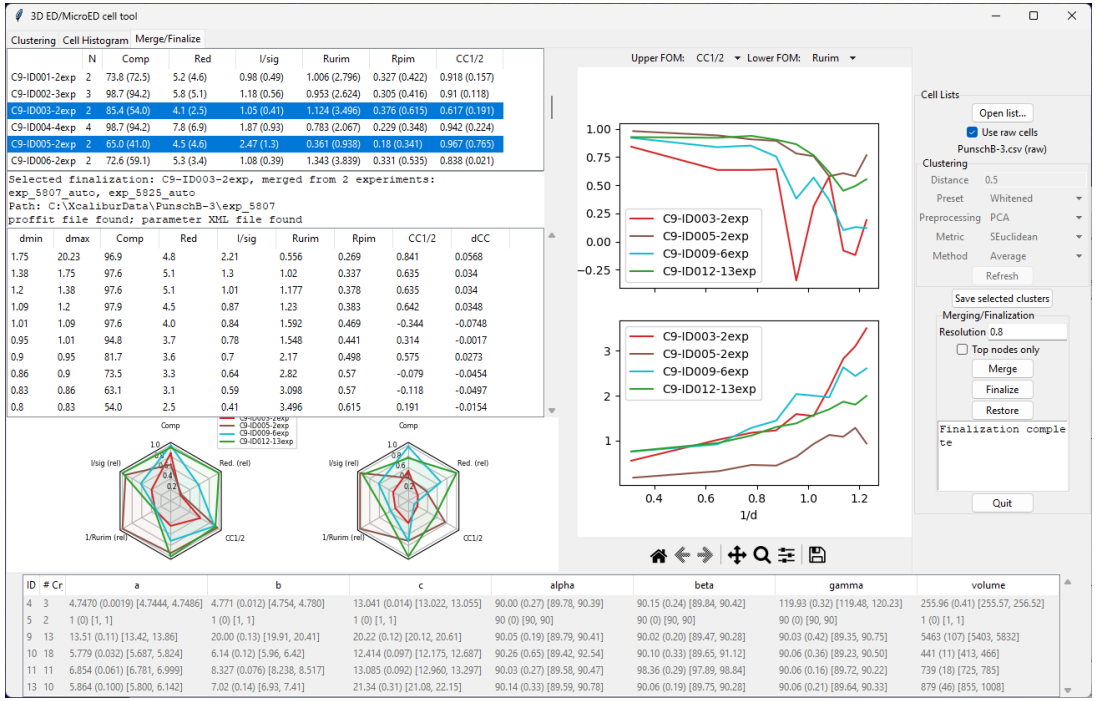
Results Viewer

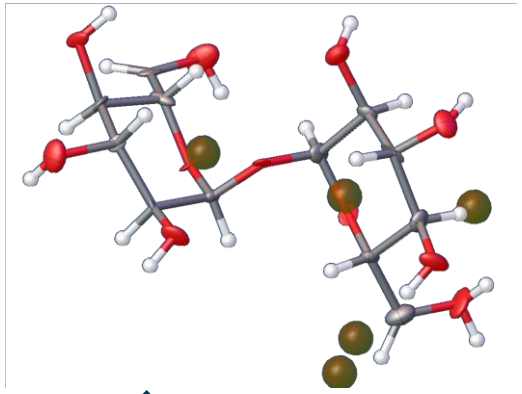
Structures from Merging



Structures from Merging







Cluster #11
11 grains

Automatic merging and scaling for each clustering tree node

Automatic structure solution with AutoChem from optimal merged solution

Workflow with Automation

Locate Crystal in Visual Mode

Pre-experiment

Switch to Diffraction Mode

Diffracts?

No → Locate New Crystal In Visual Mode → Switch to Diffraction Mode

Yes

Experiment

Center Crystal

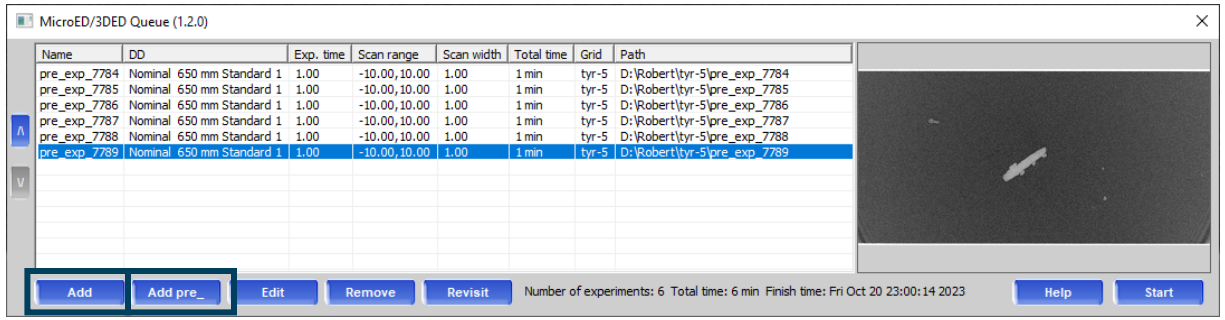
Dataset Collection

Structural Solution?

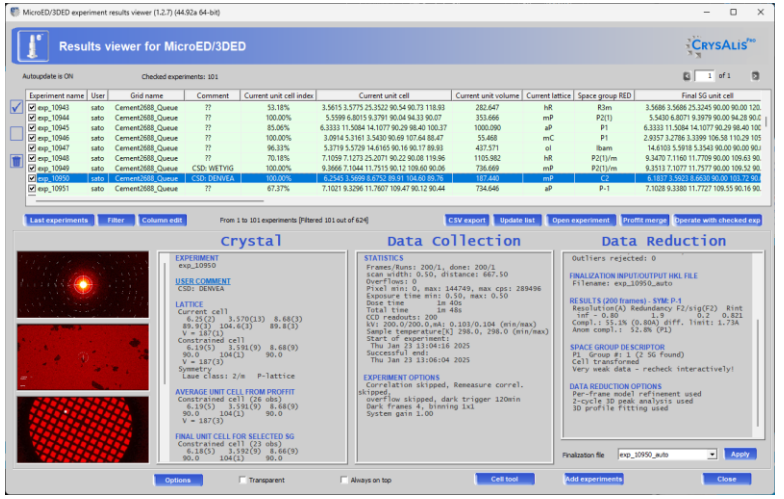
No → Center Crystal

Yes

Done!

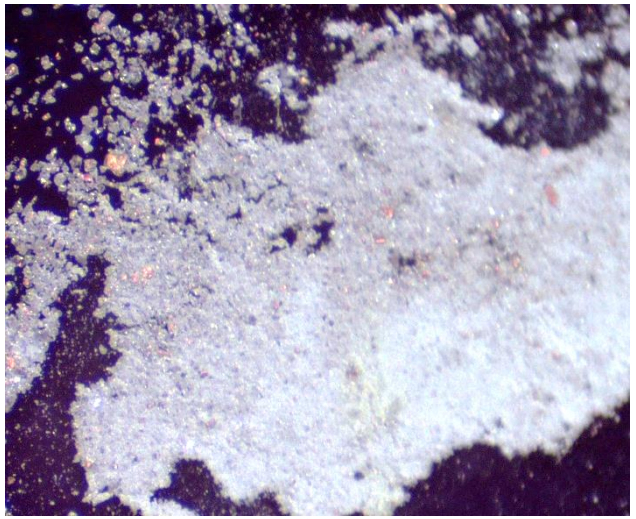


Queuing allows for unattended data collection

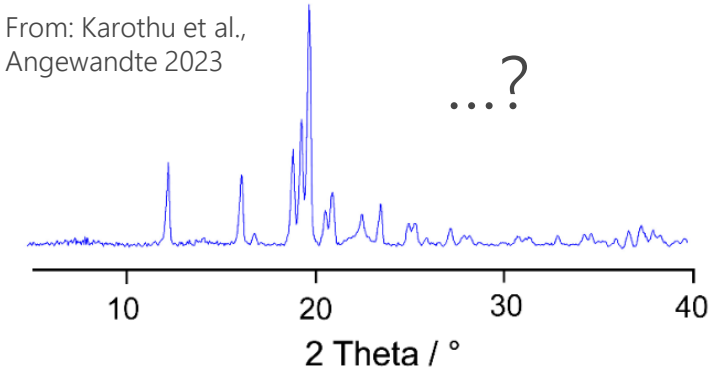


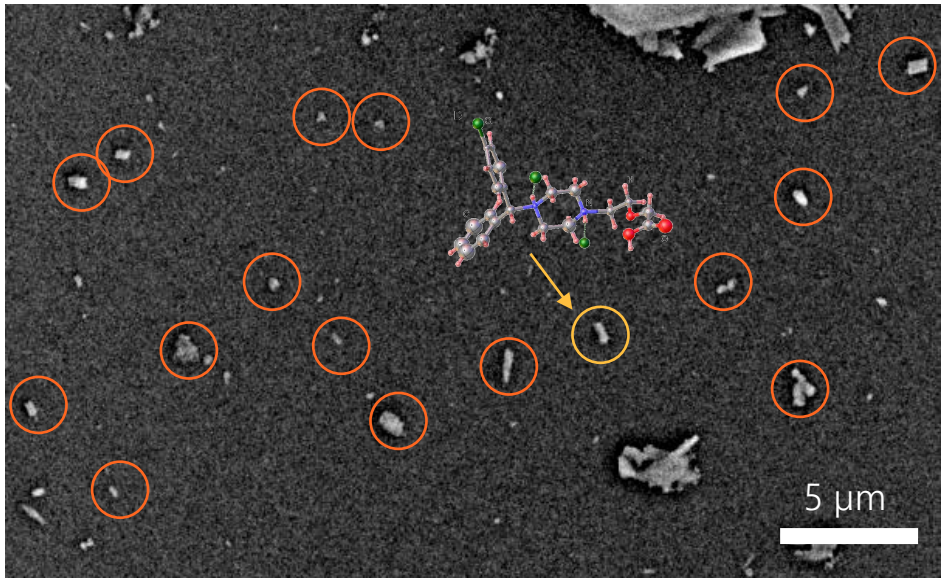
Results viewer streamlines decisions about data quality

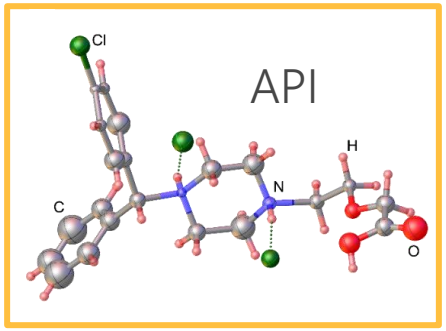
Powders Grain-by-Grain

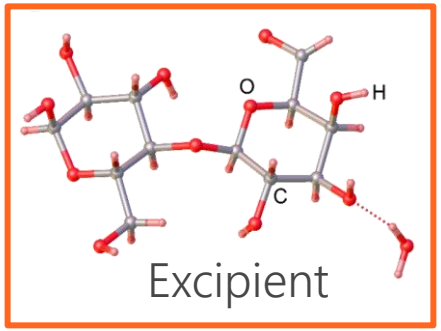


From: Karothu et al., Angewandte 2023





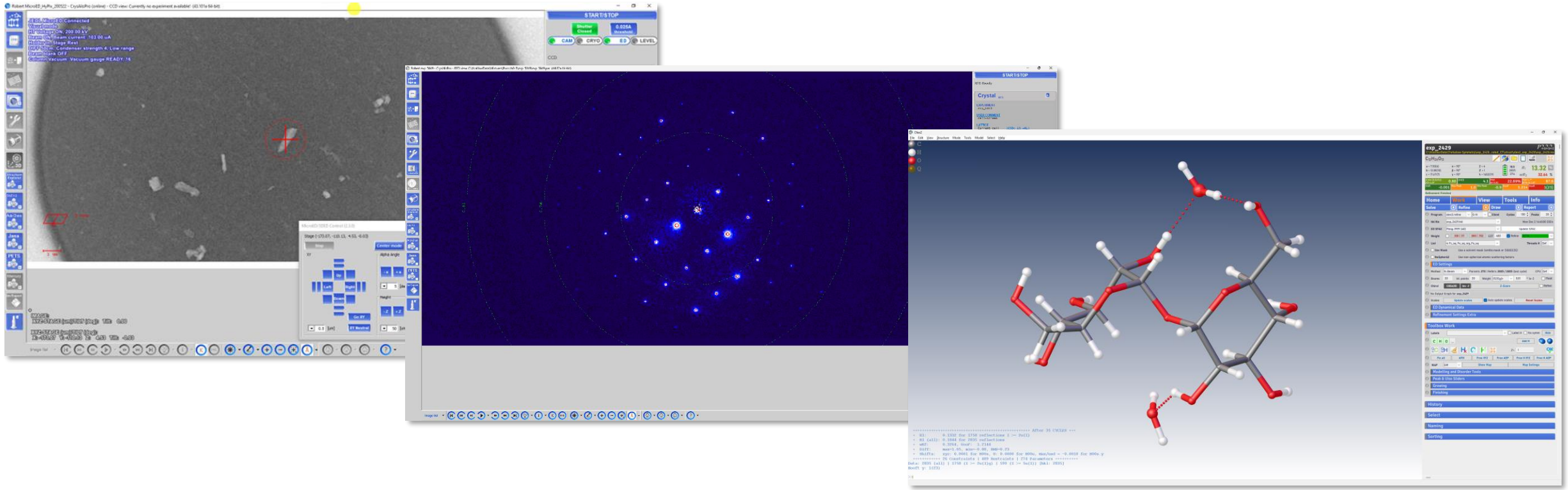




5. Summary

XtaLAB Synergy-ED: An Integrated Experience

Screen, collect, automate, process, solve, manage,… in *one* seamless interface



Screen for crystals → Collect diffraction data → Solve and refine structure

XtaLAB Synergy-ED

A dedicated electron diffractometer,
made for non-specialists

Highly optimized and integrated hardware and software design for microED / 3D ED

Seamless workflows, from instrument control to structure solution

Questions?

We'll follow up with your questions.

Recording will be available tomorrow.

Register for webinar.

Webinar Series

Small Crystals, Big Insights: How Electron Diffraction is Transforming Materials, Life Science, and Chemistry Research

Episode 4 – Solving Pharma's Toughest Solid Form Challenges with Electron Diffraction

Presenter: Simon Bates, PhD

Wednesday, June 4, 9 am CST

Don't forget to register for the next episode!