thermoscientific

Fast structure determination for proteins and small molecules – a MicroED solution

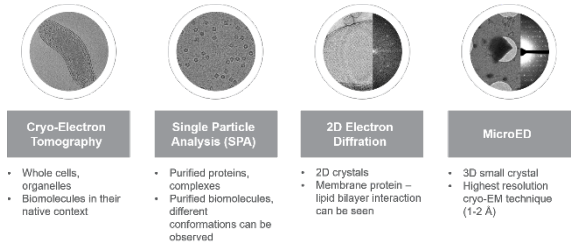
Abhay Kotecha, Bart Buijsse, Michael Janus, Lingbo Yu, Hans Raaijmakers

ABSTRACT

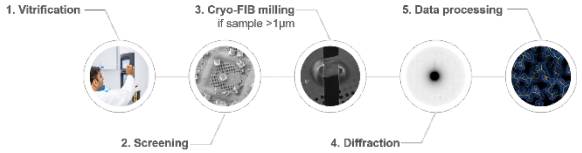
In this session, you will learn how micro electron diffraction (MicroED) allows fast, high-resolution 3D structure determination of small chemical compounds and biological macromolecules. To efficiently collect diffraction datasets of nano-crystals, a cryo-TEM is equipped with a specially designed diffraction camera (Ceta-D) and a MicroED package. The latter combines the necessary hardware components, as well as optimized optical settings and specialized EPU-D Software for automated data collection. Combined with the intrinsic microscope performance, the data collection is fully automated and can be realized in a matter of minutes.

INTRODUCTION

MicroED is a new technique for structure determination of biological macromolecules and small molecules:

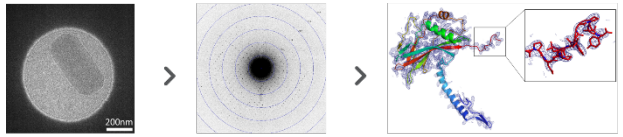


The MicroED workflow consists of four or five steps depending on the size of the crystals under investigation:



EXAMPLES

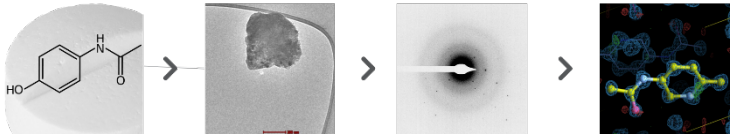
Figure 1. MicroED of nanocrystals - Granulovirus



	X-ray	XFEL	MicroED CetaD
Expression	Recombinant	Native	Native
Size (µm)	5x5x5	0.2x0.2x0.4	0.2x0.2x0.4
# Crystals	21	83000	5
Resolution (Å)	1.7	2	2.8

Granulin, is a small protein that forms the coat of granulovirus. This protein is of size 29.4 kDa (245 amino acids) and forms nanocrystals (200-400nm in size) containing on average 9000 unit cells, with cubic symmetry and a unit cell of 103-Å. This corresponds with a volume of the diffracting body of less than 0.02 um3. This is an order of magnitude smaller than what was possible so far using protein nanocrystals in electron diffraction and has only been achieved using XFEL or large recombinant crystals by X-rays.

Figure 2. MicroED of small molecules and natural compounds



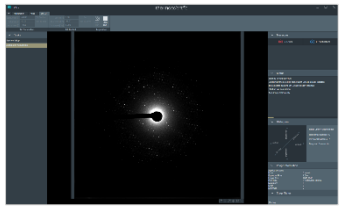
MicroED of small molecules is fast, orthogonal to NMR and mass spectrometry, and sample prep is very easy (~10^{-12}g of sample, even mixtures). Shown here, the structure of paracetamol was obtained from a ground-up tablet. The contained crystals, diffracted to 0.8 Å and were collected in less than 2 min. The structure was obtained by direct phasing .

EQUIPMENT

Table 1. Existing microscopes can be upgraded easily

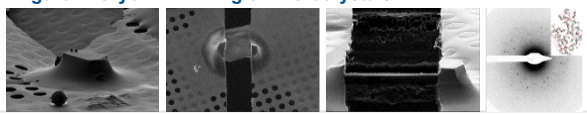
		Glacios/Talos	Krios
❶ Ceta-D camera			
• Optimized for diffraction application: increased accuracy and sensitivity		✓	✓
• Compatible with SPA screening requirements			
• Compatible with bottom-mount filter (retractable)			
❷ MicroED package	Data acquisition SW	✓	✓
	Modified beam stop, optimized for MicroED application	✓	✓
	Optimized C2/SA aperture set	✓	
	MicroED lens series	✓	✓
	90° rotation projection system	✓	

Figure 3. EPU-D software for MicroED



Dedicated MicroED software with a focus on automation and through-put with the same ease-of-use EPU is known for.

Figure 4. Cryo-FIB milling of microcrystals



Intermediated-sized crystals (1-50 µm) can be thinned to make them suitable (150-600 nm) for MicroED while preserving the internal order of the crystal lattice. Lysozyme, used as a test system, resolves to 1.9 Å in cryo-FIB milled crystals.

CONCLUSIONS

- **Fast atomic-resolution 3D structural information**. Diffraction data from nanocrystals in minutes.
- **Instant productivity**. Nanocrystals as small as 100 nm can be readily analyzed, removing the burden of growing large crystals (as with X-ray crystallography). Also reduces the amount of sample material required. Mixtures of different polymorphs and compounds can be analyzed.
- **Complete turnkey solution**. Including hardware, software and support from one single vendor. Acquired data can be readily processed using established reconstruction packages for X-ray crystallography.
- **2-in-1 solution**. MicroED and single particle analysis (SPA) can be performed on the same cryo-electron microscope. This solution is compatible with new microscopes but is also retrofittable on existing units.

FURTHER INFORMATION

Please scan this QR-code to get to the website containing the MicroED introduction video as well as a download link for the PDF describing the MicroED package and the Ceta-D camera.

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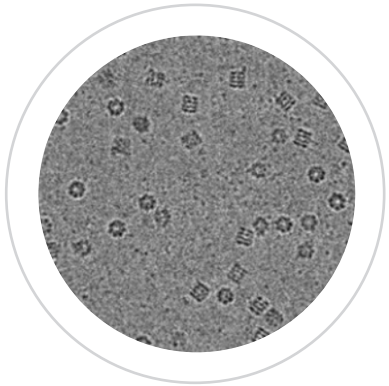
MicroED is a New Technique for Structure Determination of Biological Macromolecules

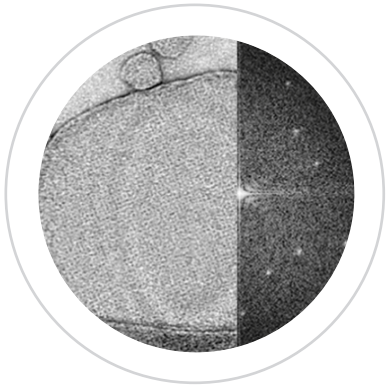
Cryo-Electron Tomography

- Whole cells, organelles
- Biomolecules in their native context



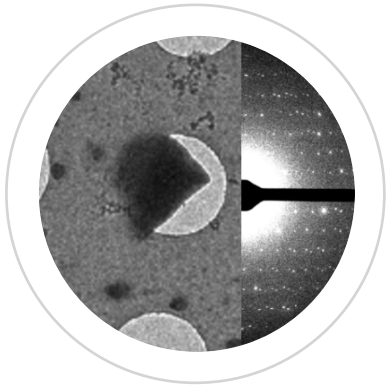
Single Particle Analysis (SPA)

- Purified proteins, complexes
- Purified biomolecules, different conformations can be observed



2D Electron Diffration

- 2D crystals
- Membrane protein – lipid bilayer interaction can be seen



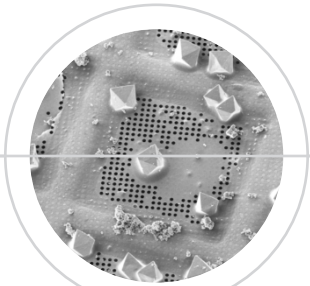
MicroED

- 3D small crystal
- Highest resolution cryo-EM technique (1-2 Å)

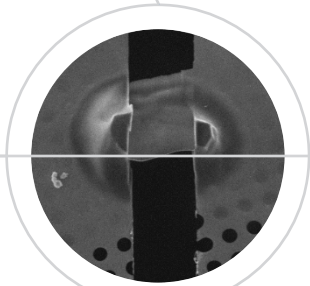
The MicroED Workflow

Vitrification

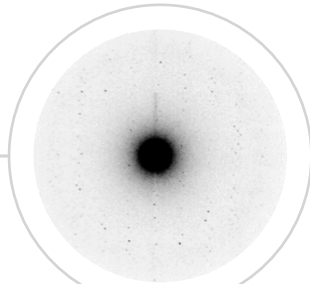
Screening



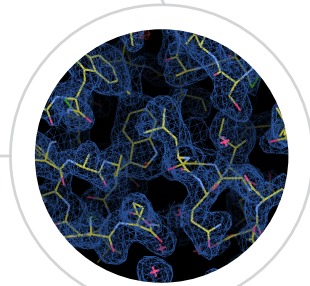
Cryo-FIB milling
if sample >1µm

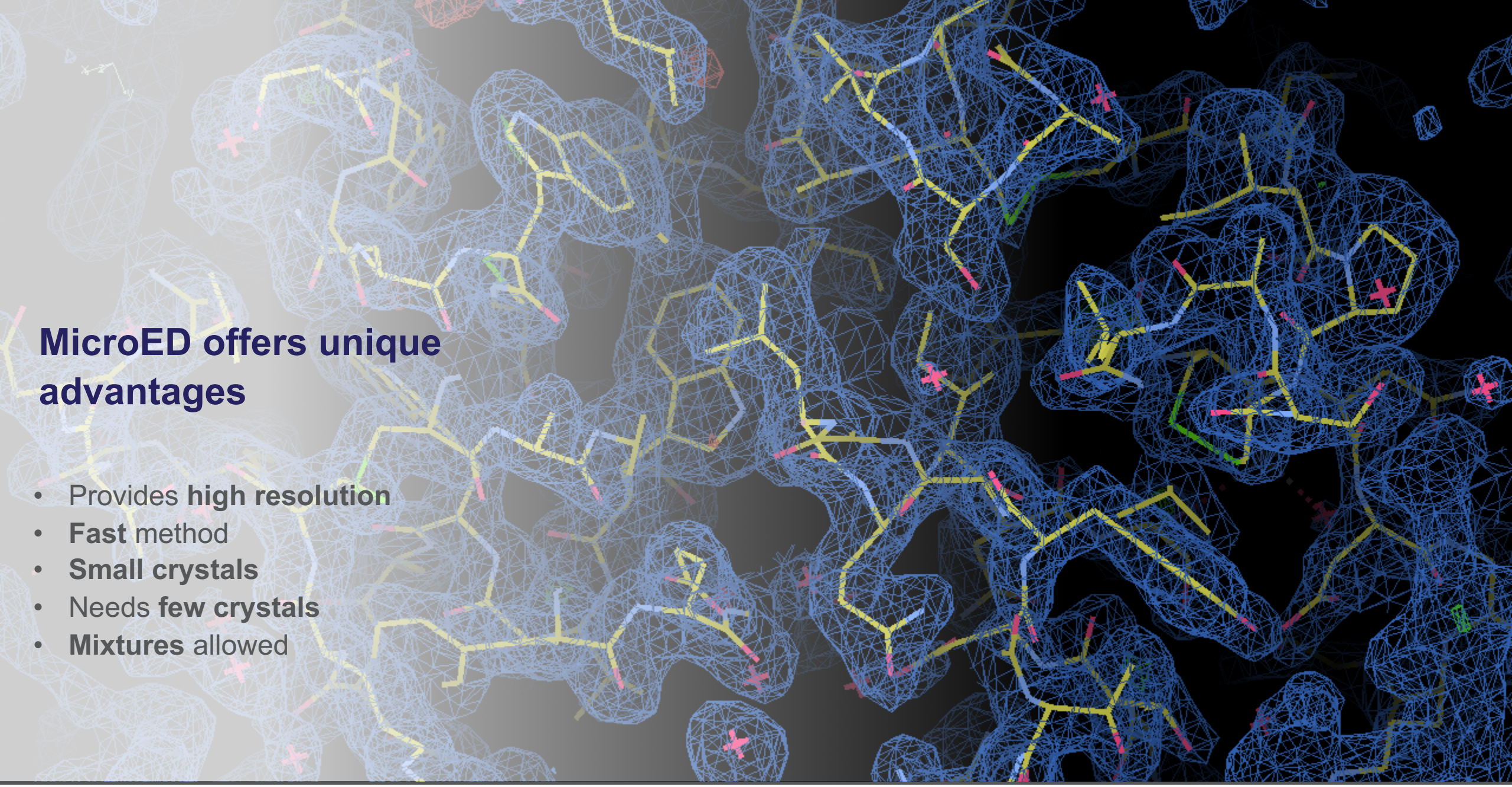


Diffraction



Data processing

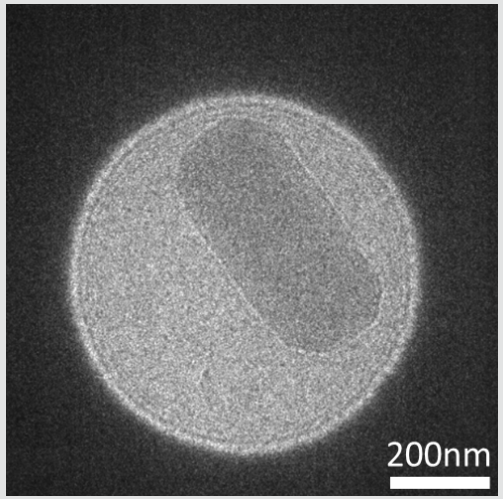


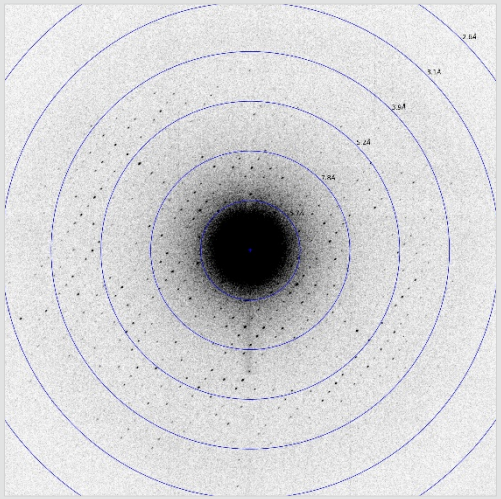


MicroED offers unique advantages

- Provides **high resolution**
- **Fast** method
- **Small crystals**
- Needs **few crystals**
- **Mixtures** allowed

Resolving Nano-crystals of Granulovirus





Granulin

Protective virus coat
29 kDa protein
9,000 unit cells/virion
0.5 hr data collection on
5 crystals

	X-ray	XFEL	MicroED CetaD
Expression	Recombinant	Native	Native
Size (µm)	5x5x5	0.2x0.2x0.4	0.2x0.2x0.4
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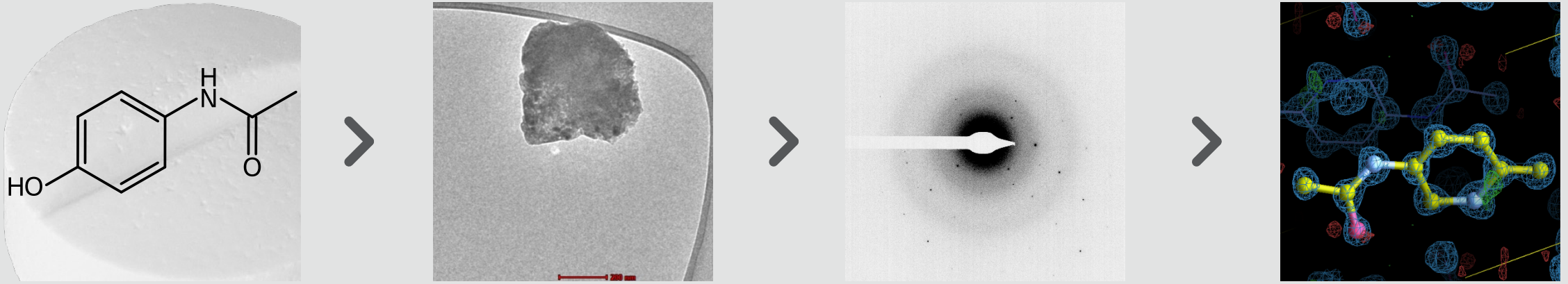
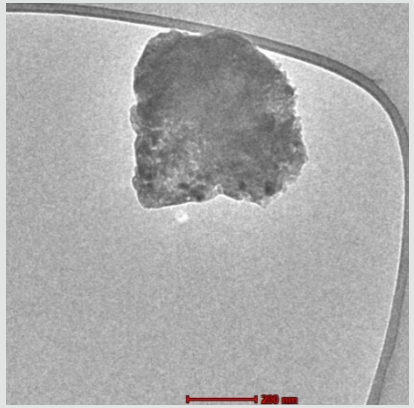
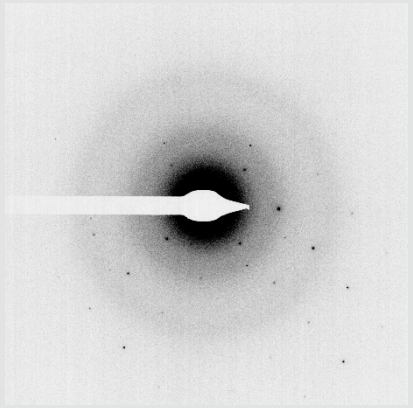
Structure Determination of Drugs and Natural Compounds Using MicroED

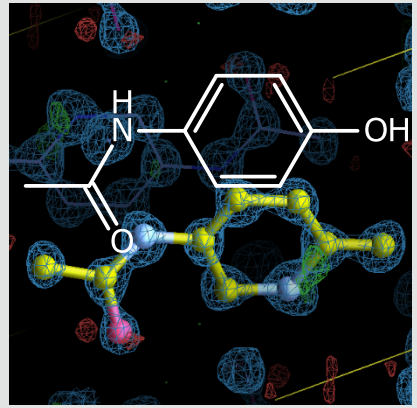


Sample	Prep.	Acquisition	Analysis
Grinded paracetamol	Lacey carbon grid, cryo temperature	160 x 1.0° x 1sec 0.88Å	Dials → shelxt 70% complete

Structure Determination of Drugs and Natural Compounds Using MicroED







Sample

Grinded paracetamol

Prep.

Lacey carbon grid,
cryo temperature

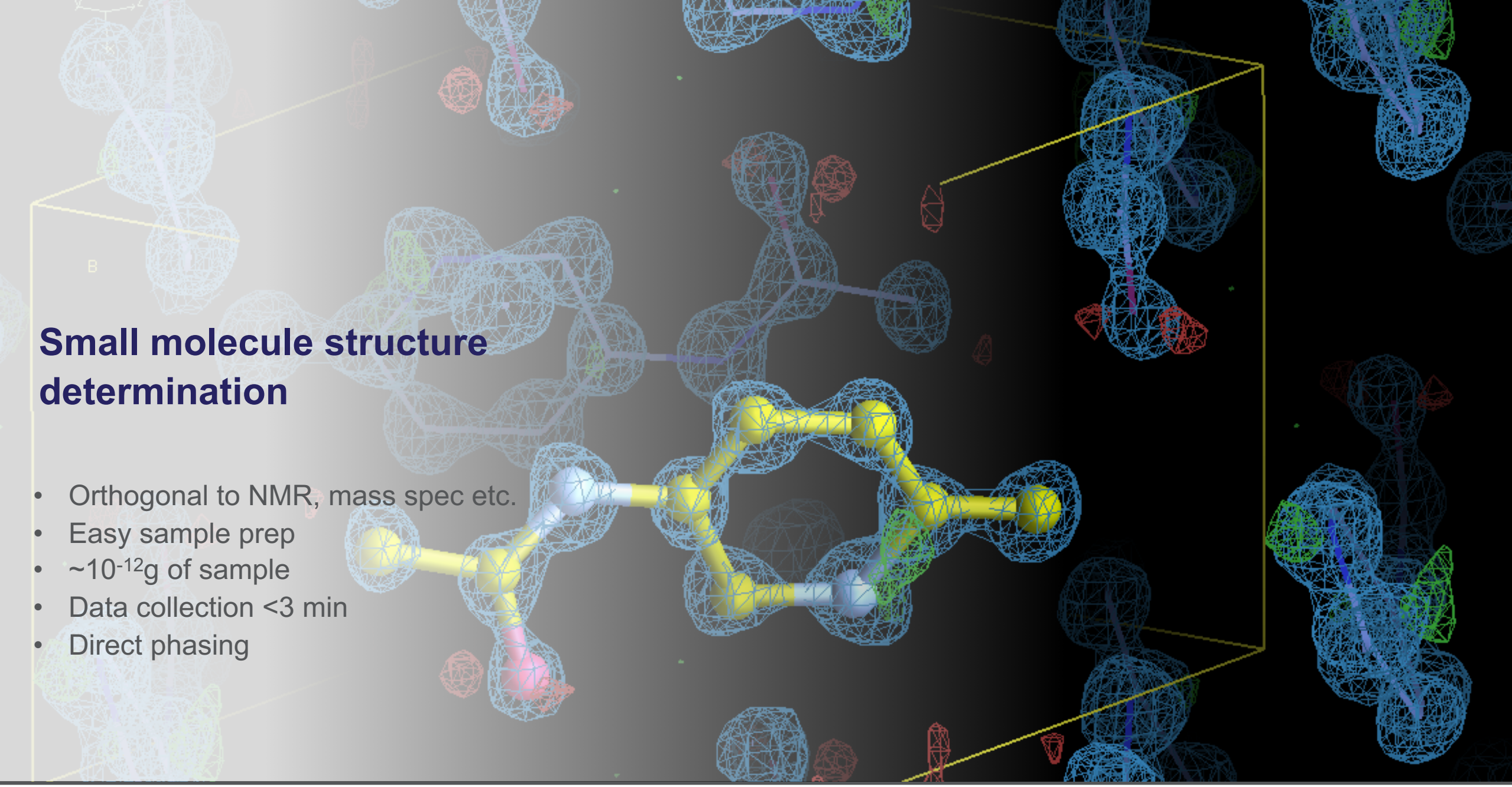
Acquisition

160 x 1.0° x 1sec
0.88Å

Analysis

Dials → shelxt
70% complete

Small molecule structure determination



- Orthogonal to NMR, mass spec etc.
- Easy sample prep
- ~10^{-12}g of sample
- Data collection <3 min
- Direct phasing

MicroED is Enabled by the Ceta-D Camera and the MicroED Package

Existing microscopes can be upgraded easily

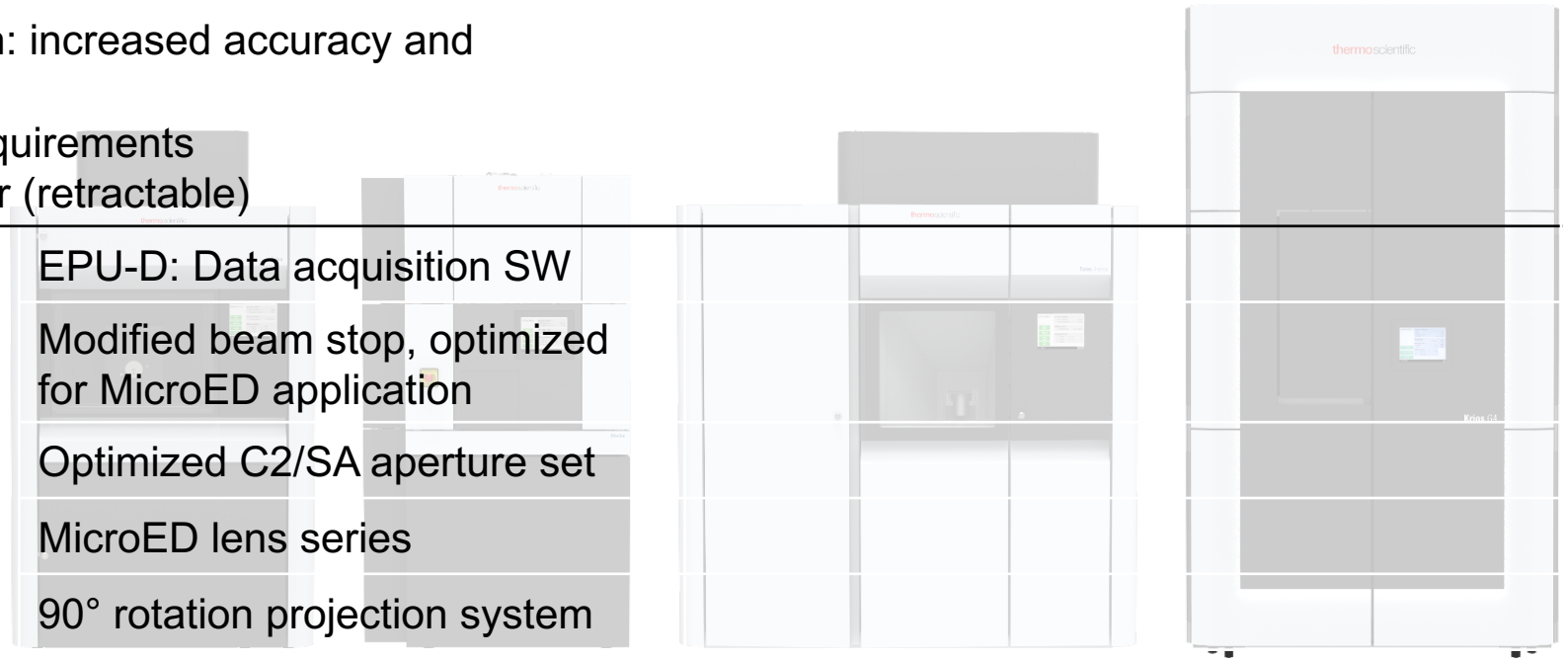
Glacios/Talos **Krios**

❶ Ceta-D camera

- Optimized for diffraction application: increased accuracy and sensitivity
- Compatible with SPA screening requirements
- Compatible with bottom-mount filter (retractable)

❷ MicroED package

- EPU-D: Data acquisition SW
- Modified beam stop, optimized for MicroED application
- Optimized C2/SA aperture set
- MicroED lens series
- 90° rotation projection system

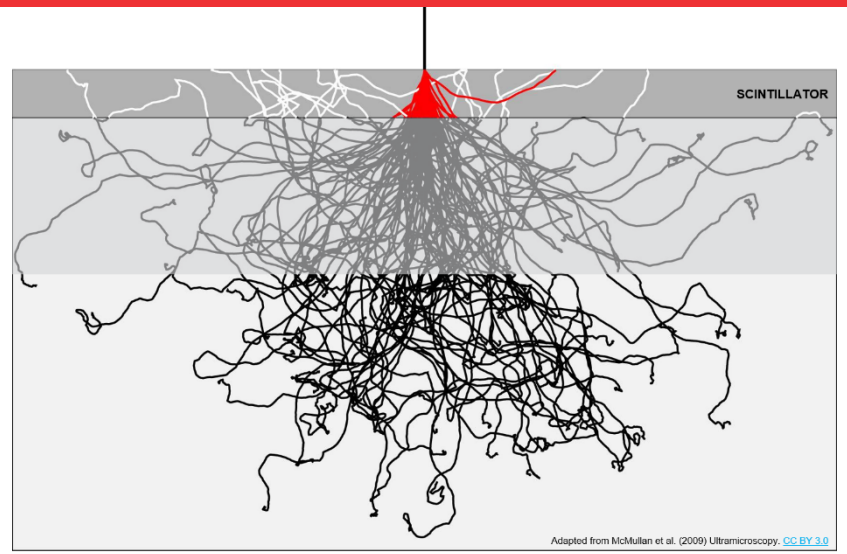


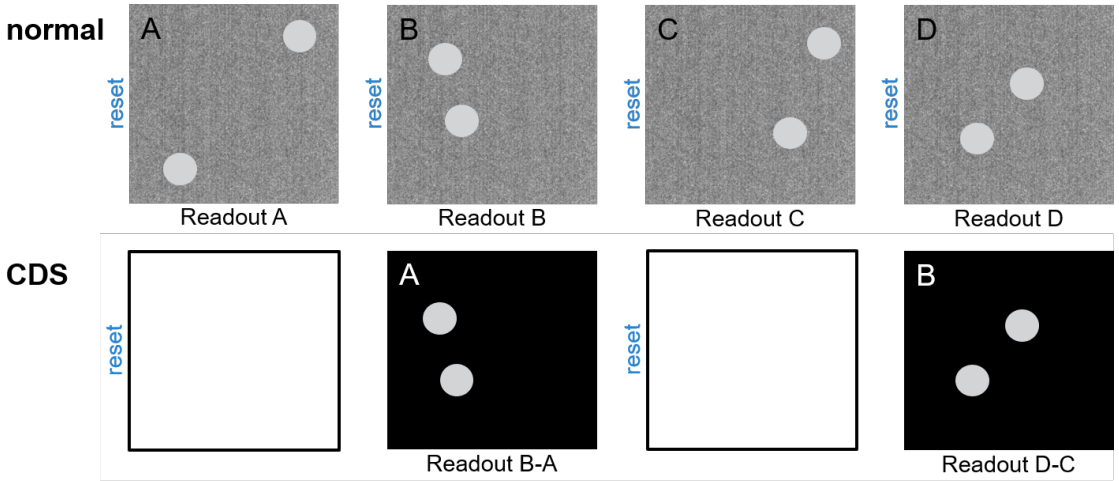
Ceta-D Camera

Scintillator-based detection with a CMOS sensor **designed for MicroED**

The Ceta-D offers improved signal-to-noise and enables continuous tilt diffraction

- **4x higher sensitivity compared to Ceta**: Optimized scintillator thickness for enhanced signal-to-noise ratio and improved DQE at low frequency.
- Movie mode is enabled for **continuous-tilt diffraction.**
- **Correlated Double Sampling** (CDS) in movie mode **decreases noise** 2.5x.





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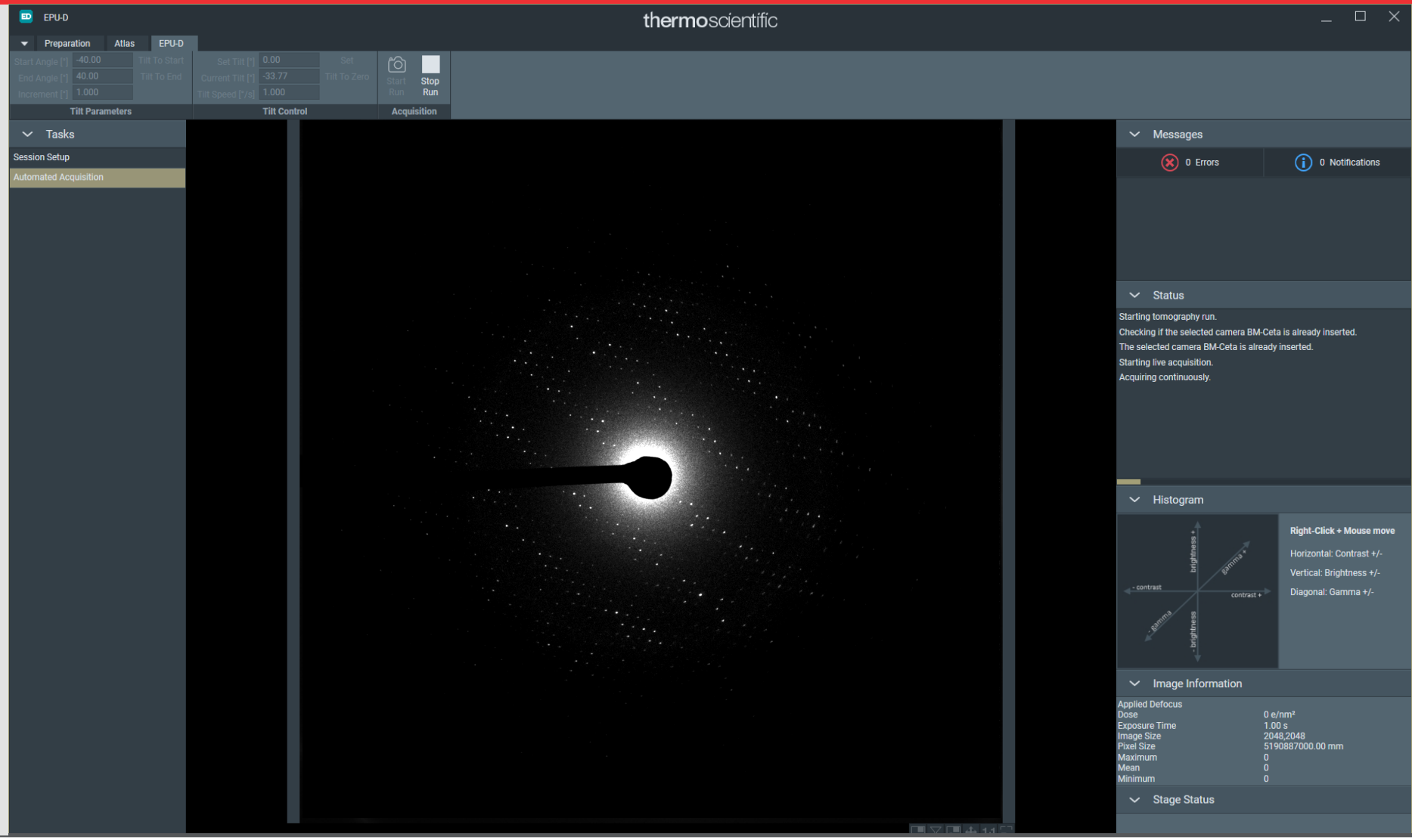
EPU-D Acquisition Software

Dedicated MicroED software with a focus on **automation, usability and throughput**

EPU-D facilitates MicroED with the same ease-of-use EPU is known for

- Intuitive workflow **GUI for navigation, set-up and acquisition**.
- **Fast and easy setup** of MicroED experiments
- **Continuous tilt series** acquisition.



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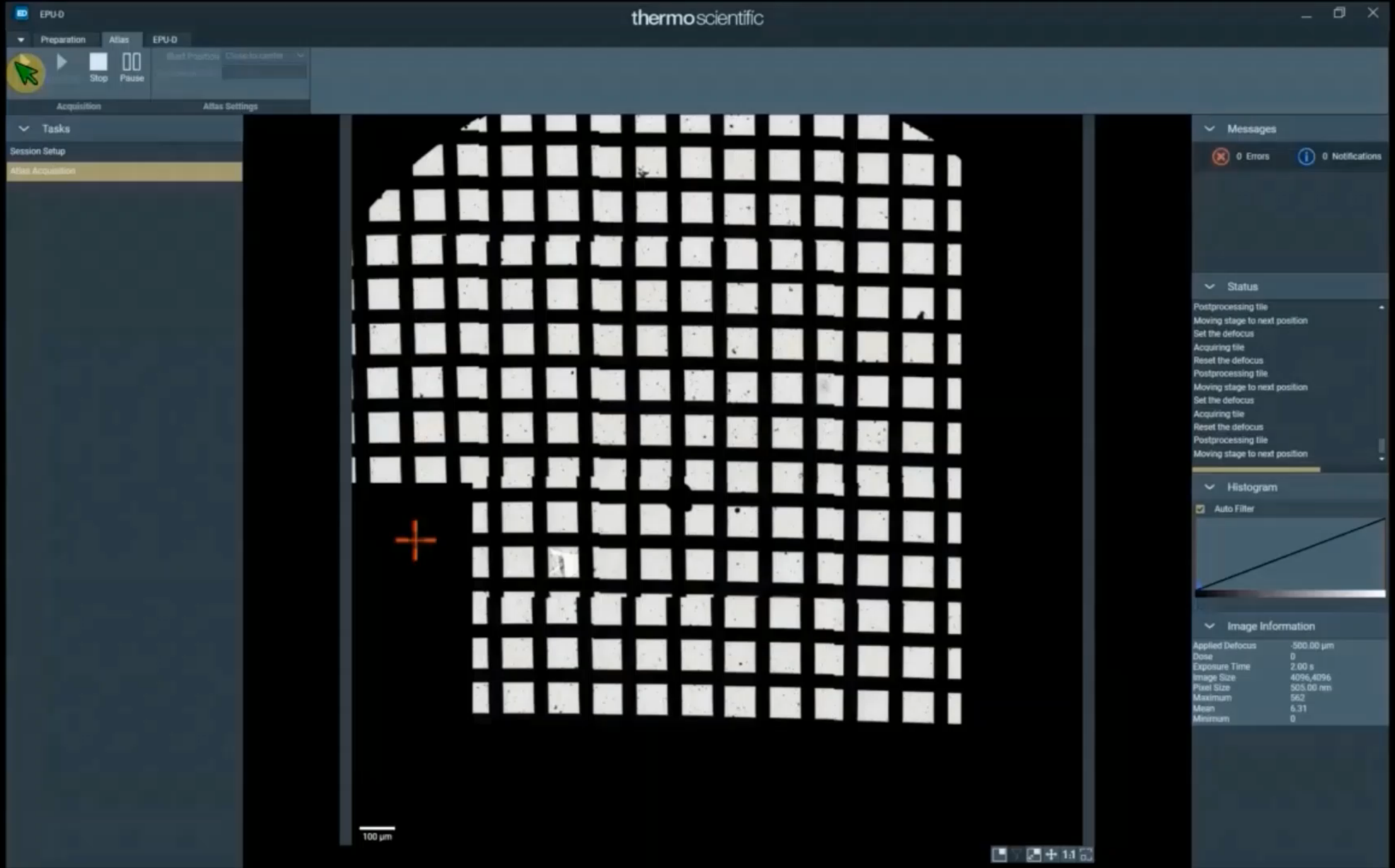
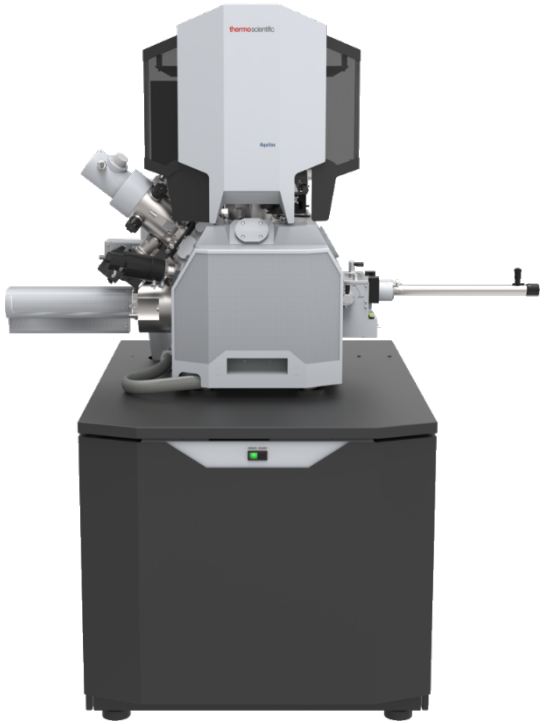

EPU-D
thermoscientific
Preparation
Atlas
EPU-D
Stop
Pause
Acquisition
Atlas Settings
Tasks
Session Setup
Atlas Acquisition
Messages
0 Errors
0 Notifications
Status
Postprocessing tile
Moving stage to next position
Set the defocus
Acquiring tile
Reset the defocus
Postprocessing tile
Moving stage to next position
Set the defocus
Acquiring tile
Reset the defocus
Postprocessing tile
Moving stage to next position
Histogram
Auto Filter
Image Information
Applied Defocus -500.00 µm
Dose 0
Exposure Time 2.00 s
Image Size 4096,4096
Pixel Size 505.00 nm
Maximum 562
Mean 6.31
Minimum 0
100 µm
1:1

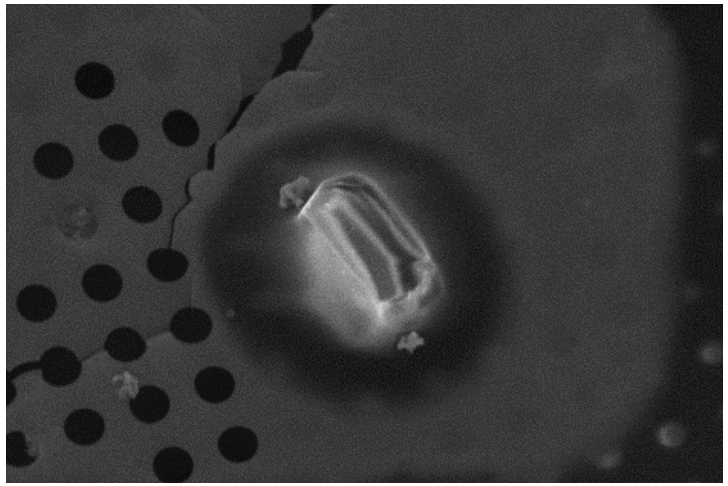
Aquilos Cryo-FIB

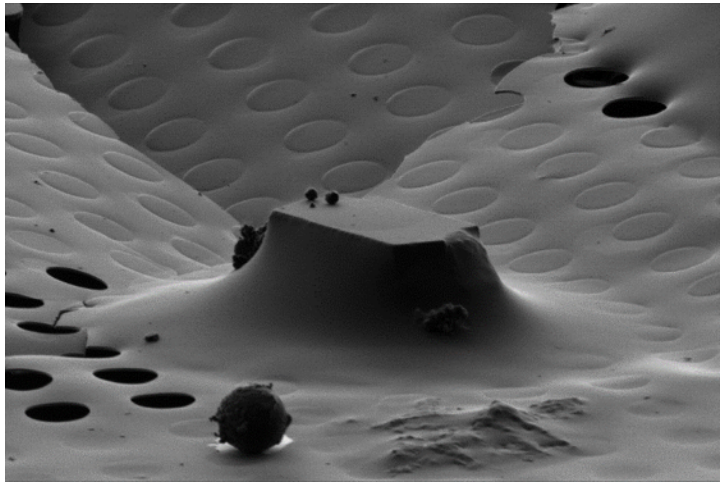
Cryo-FIB milling makes micro-crystals usable for MicroED

Intermediate-sized crystals can be thinned to make them suitable for MicroED while preserving the internal order of the crystal lattice.

- Crystal thickness for MicroED is limited to ~700nm.







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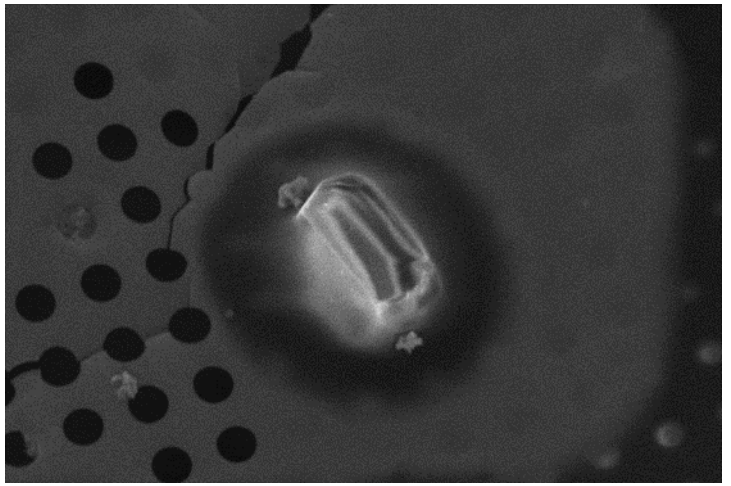

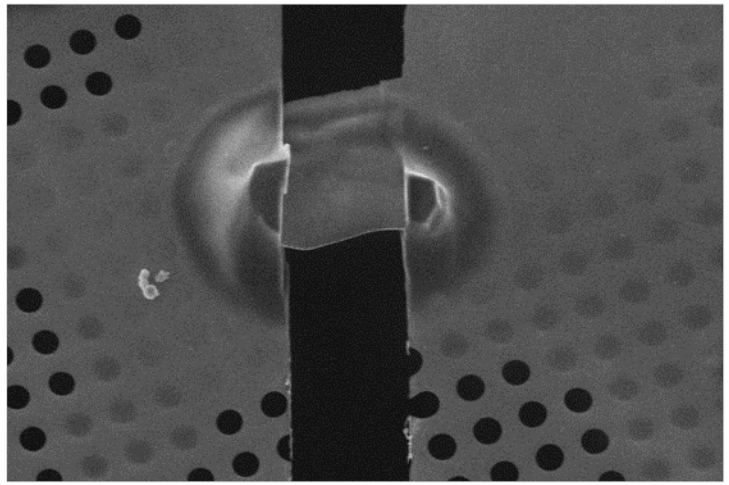

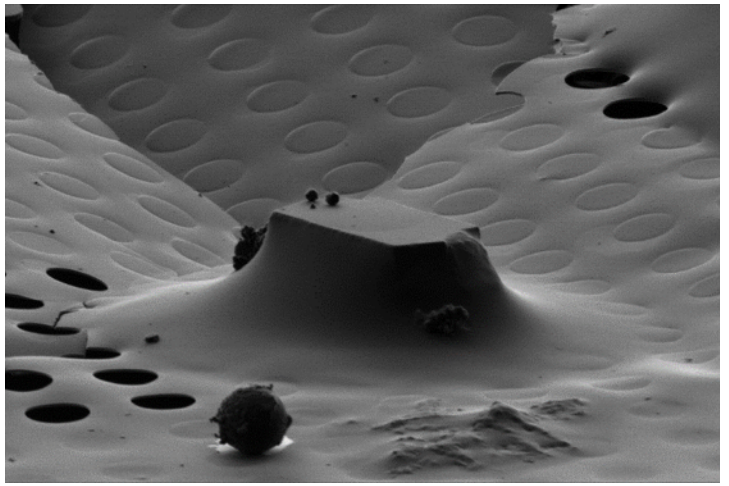

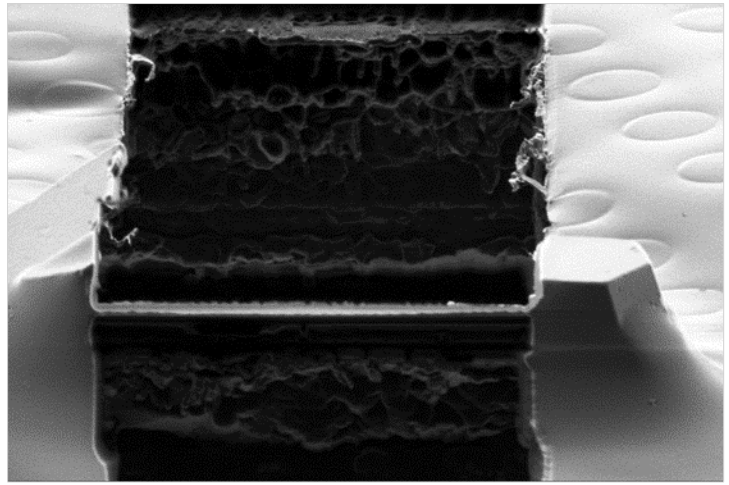

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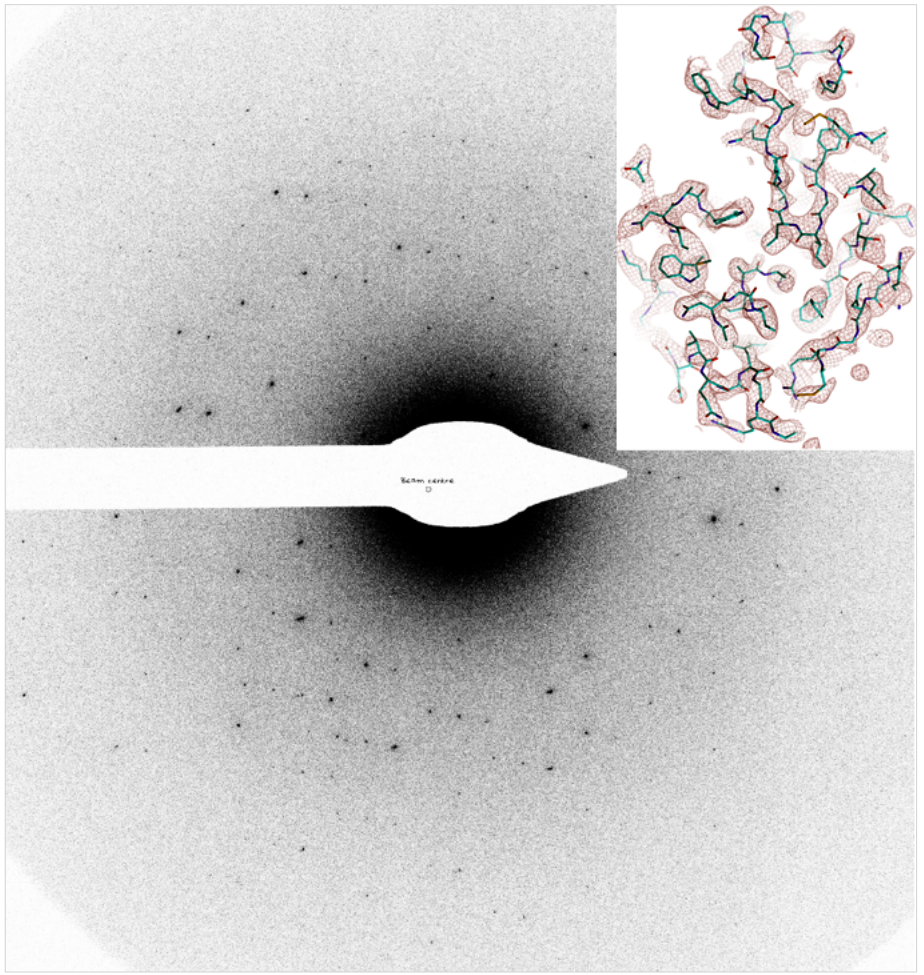

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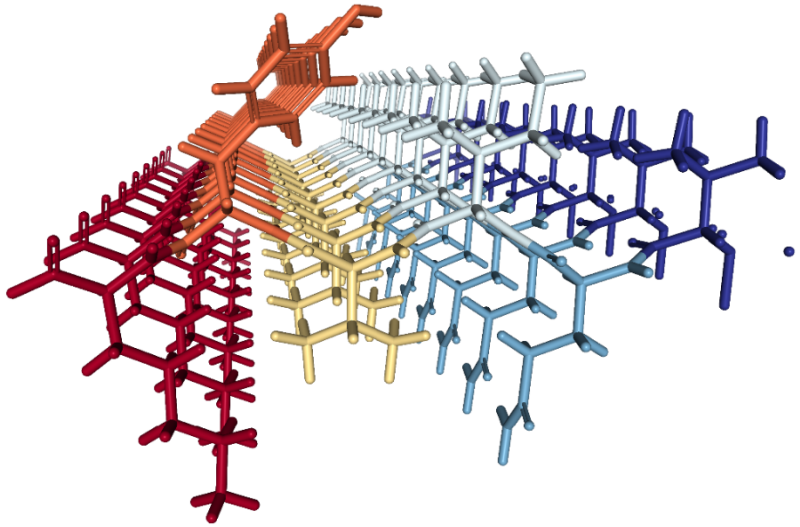
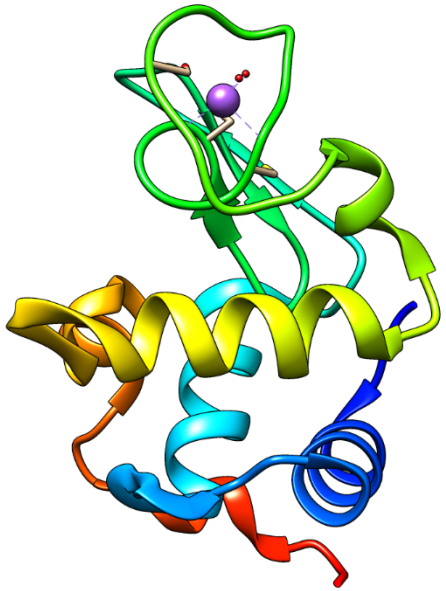
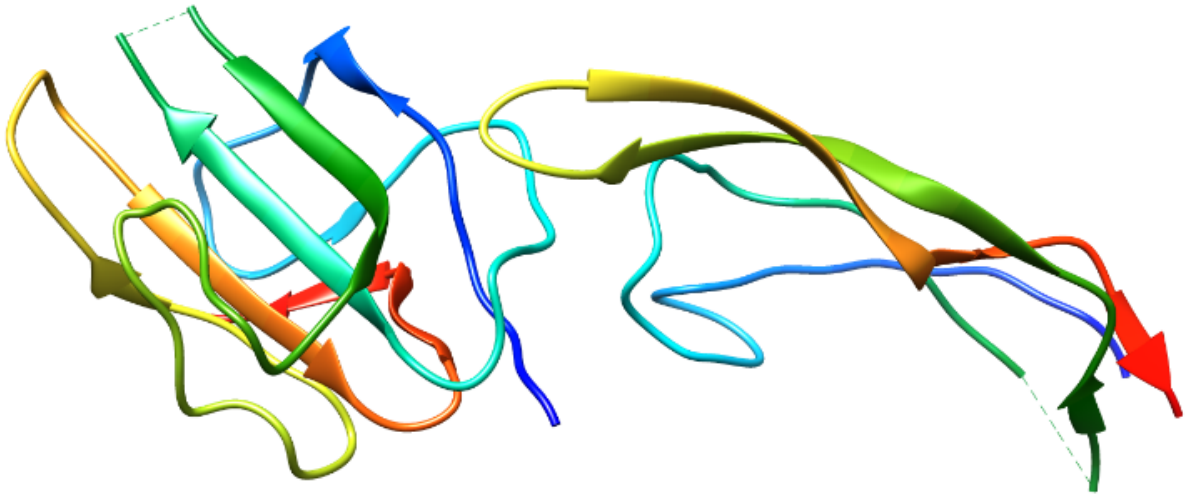
Summary



Tau peptide



Lysozyme



TGF-βm:TβRII

Images of PDB 5K7N; 5K7O; 5TY4; 5K7P; 5K7Q; 5K7R; 5K7S; 5K7T created with Chimera

- MicroED can make use of crystals too small for XRD
- Components can be retrofitted (depending on configuration)
- High resolution, high throughput technique
- Cryo-FIB opens a path for intermediate-sized microcrystals