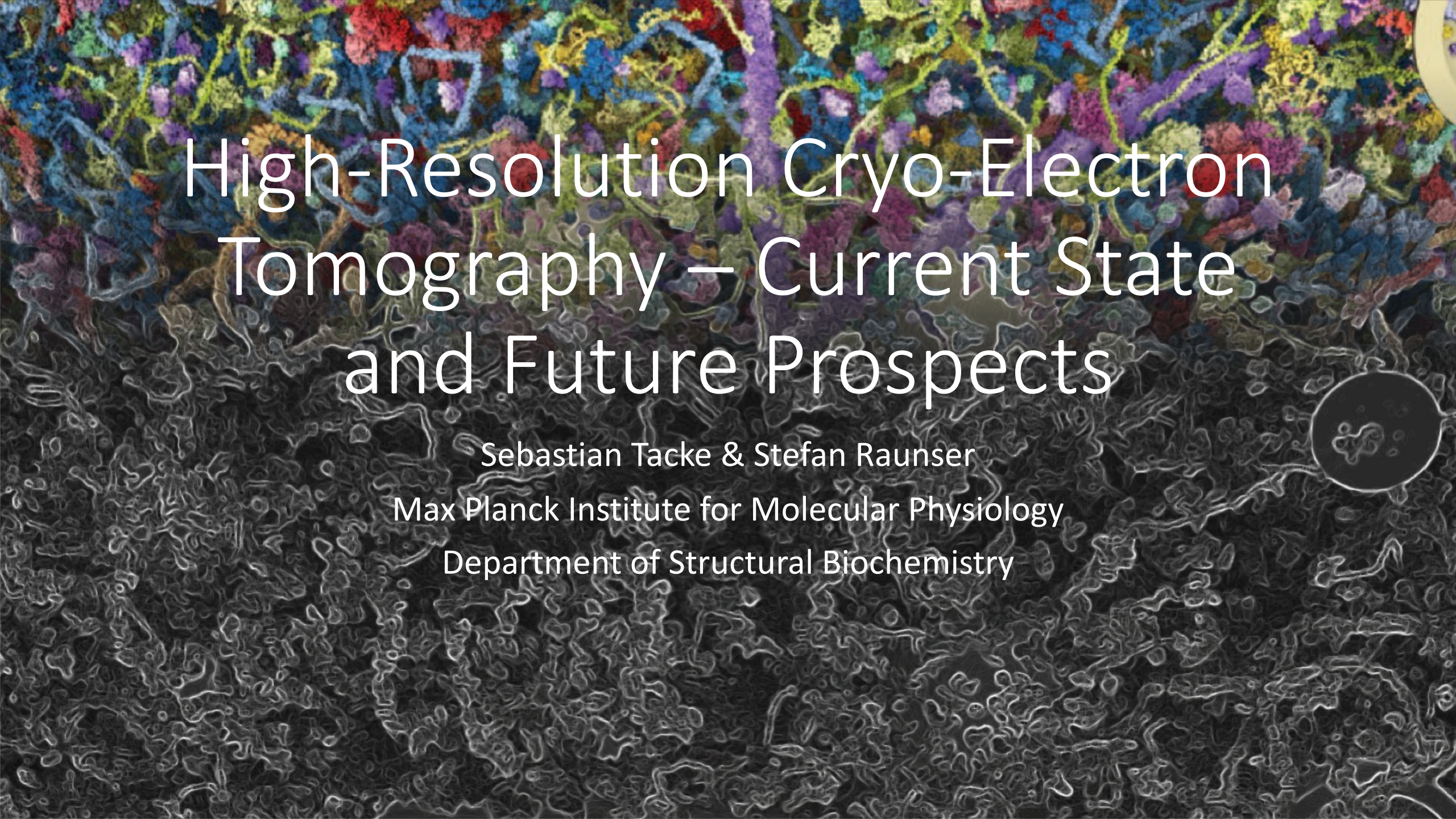

High-Resolution Cryo-Electron Tomography – Current State and Future Prospects

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Why Structural Biology?

- Structure and function of macromolecules are highly interdependent.
- Multiple structures of different functional states help to understand molecular mechanisms.
- *In situ* structures help to understand how molecules interact with their native environment.
- Structures can be used as starting point to design functional experiments (e.g. by guided design of mutations).

Common Methods in Structural Biology

X-Ray Crystallography

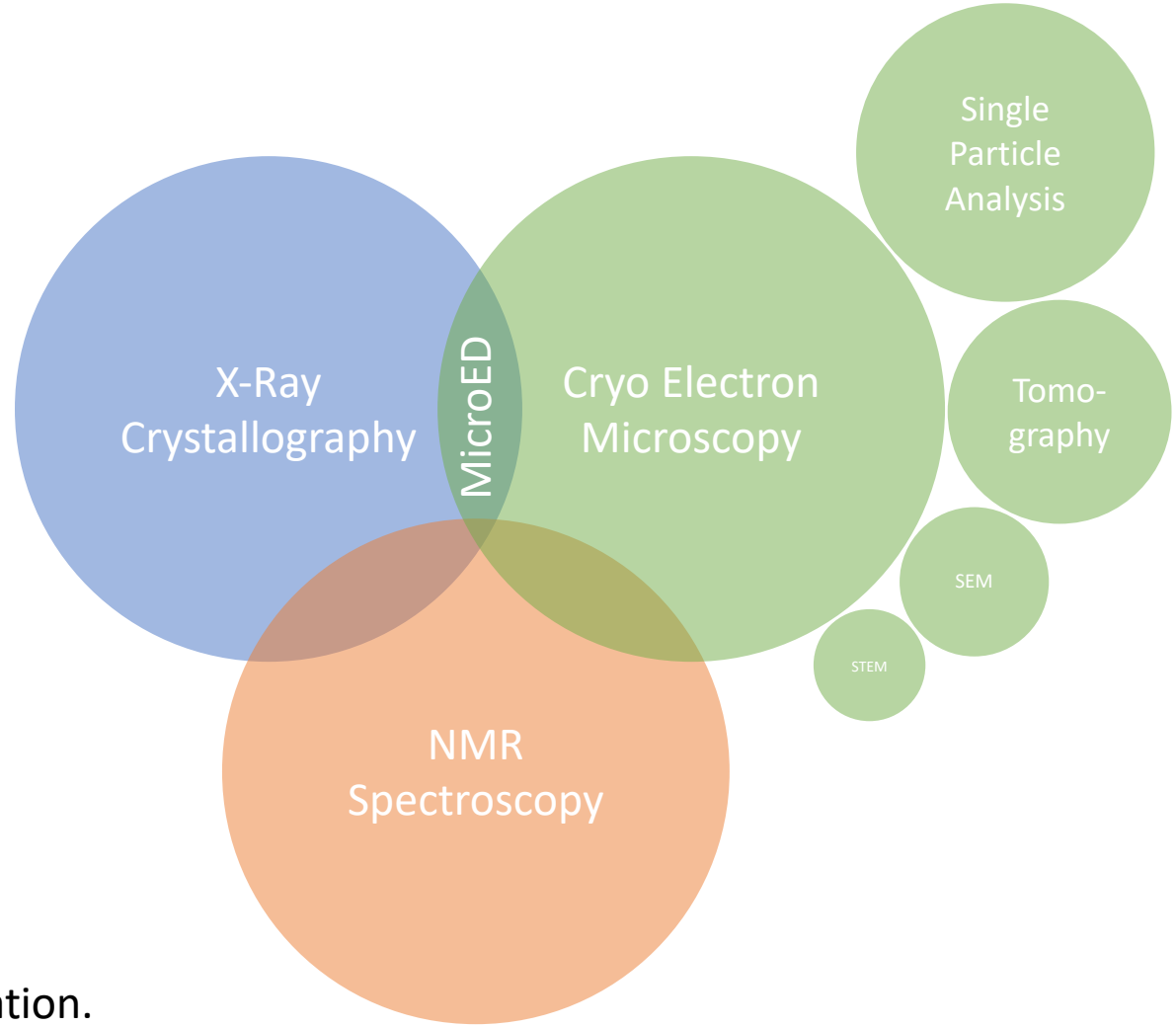
- Can be very quick and easy.
- No principle limitations for protein size etc.
- Large amounts of highly pure material necessary.
- Protein needs to form highly ordered crystals.
- Phase problem needs to be solved.

NMR Spectroscopy

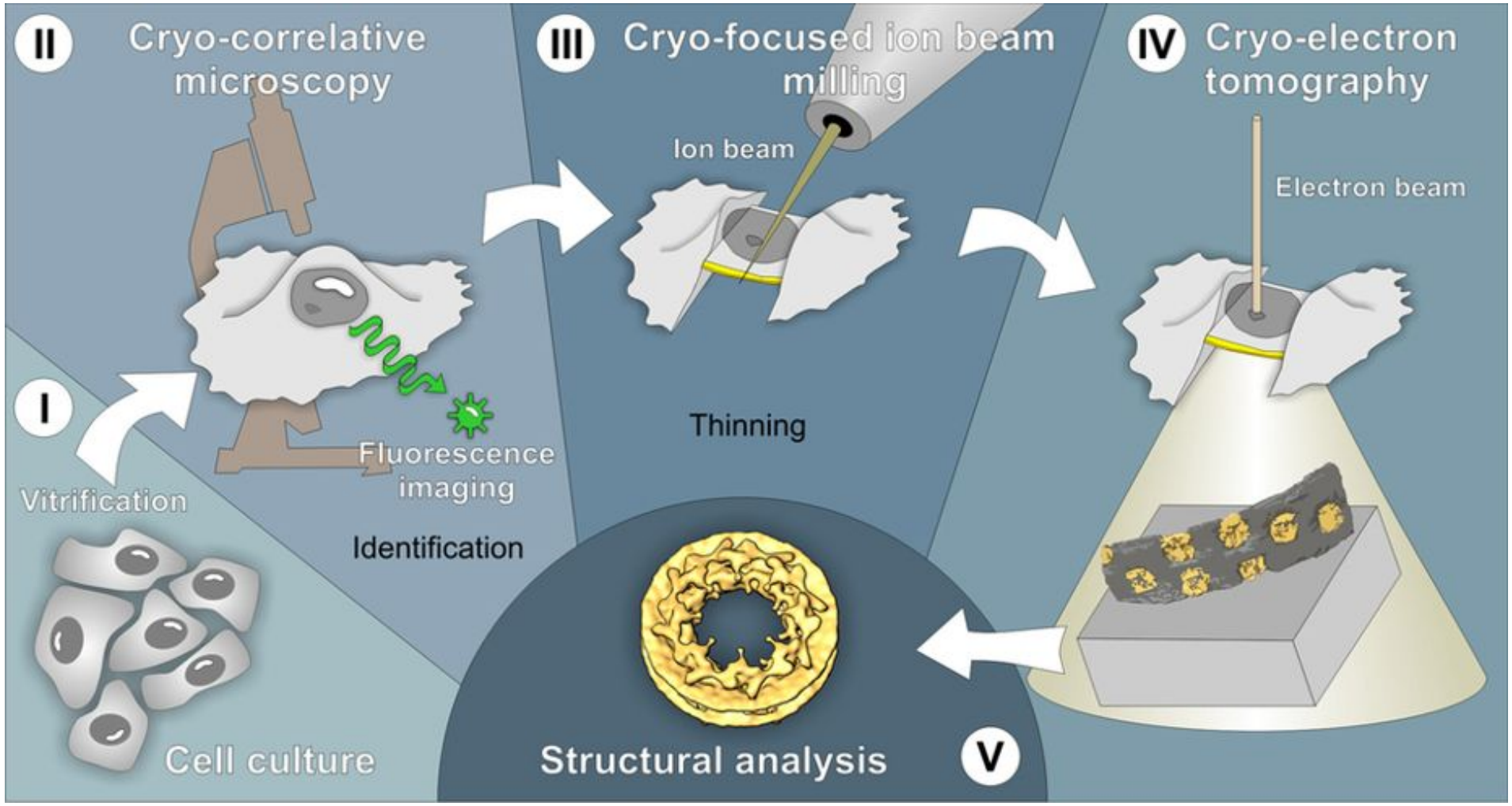
- True solution method.
- Accounts for dynamics in macromolecules.
- In-cell NMR is possible in some cases.
- Requires labelling with heavy nuclides (N2, C13, N15).
- Limited to small proteins or selective labelling.
- Sample needs to be extraordinarily stable.

Cryo Electron Microscopy (Single Particle Analysis)

- Lower sample amounts necessary.
- Proteins do not need to be crystallized.
- Not always easy to find good conditions for sample preparation.
- Sample preparation might be harmful for the protein.
- Difficult for smaller molecules.

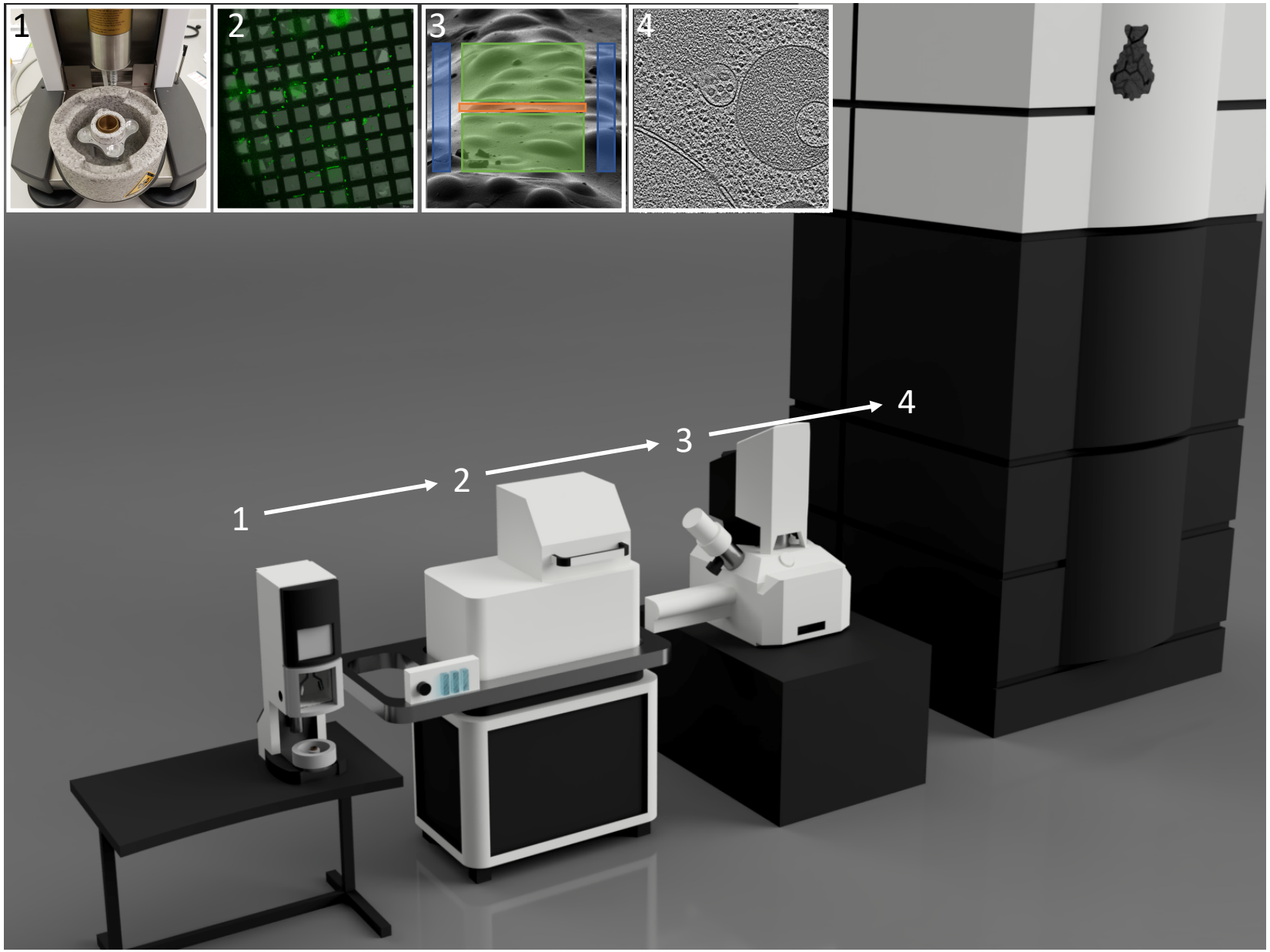


Cryo Electron Tomography

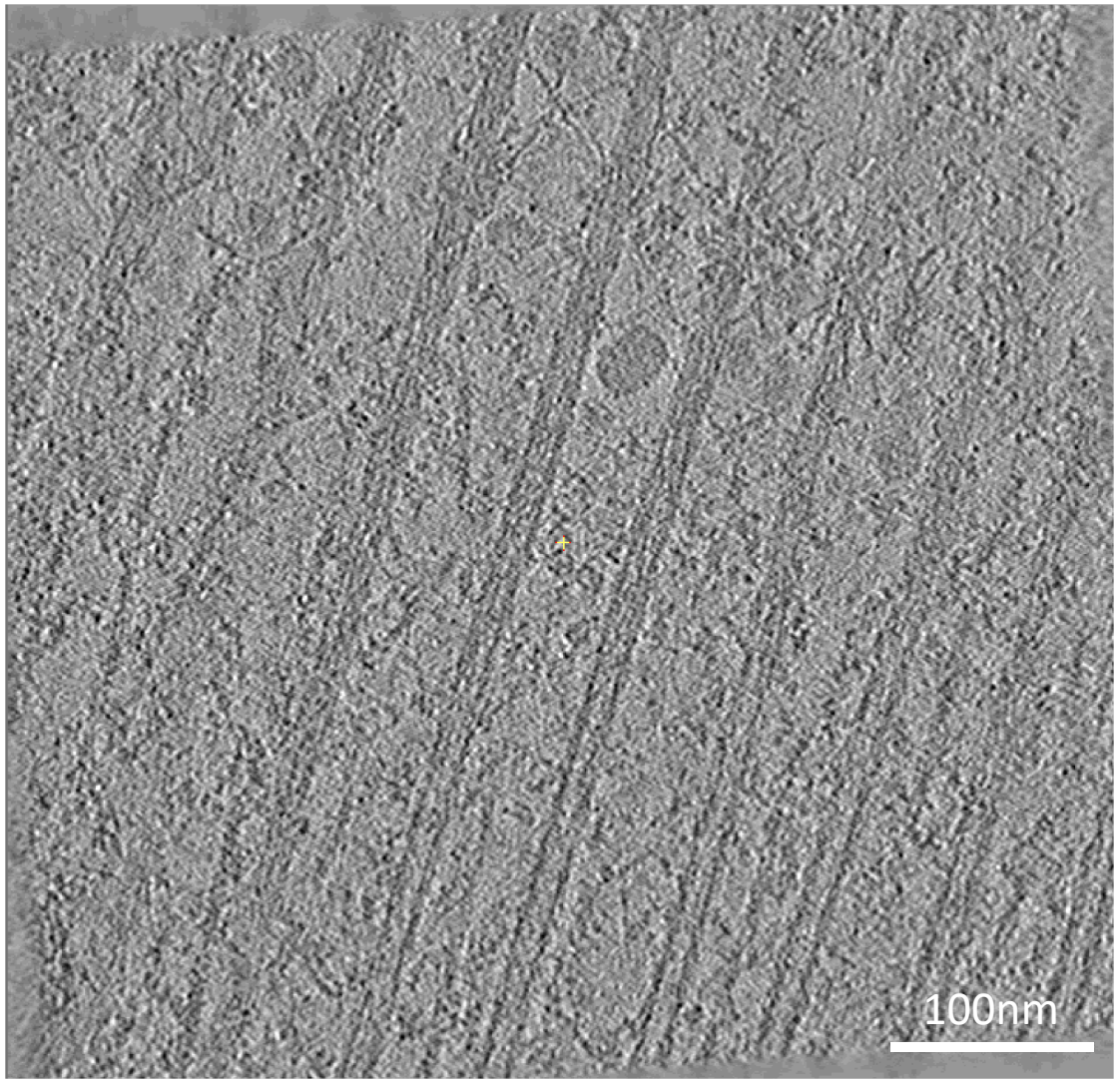


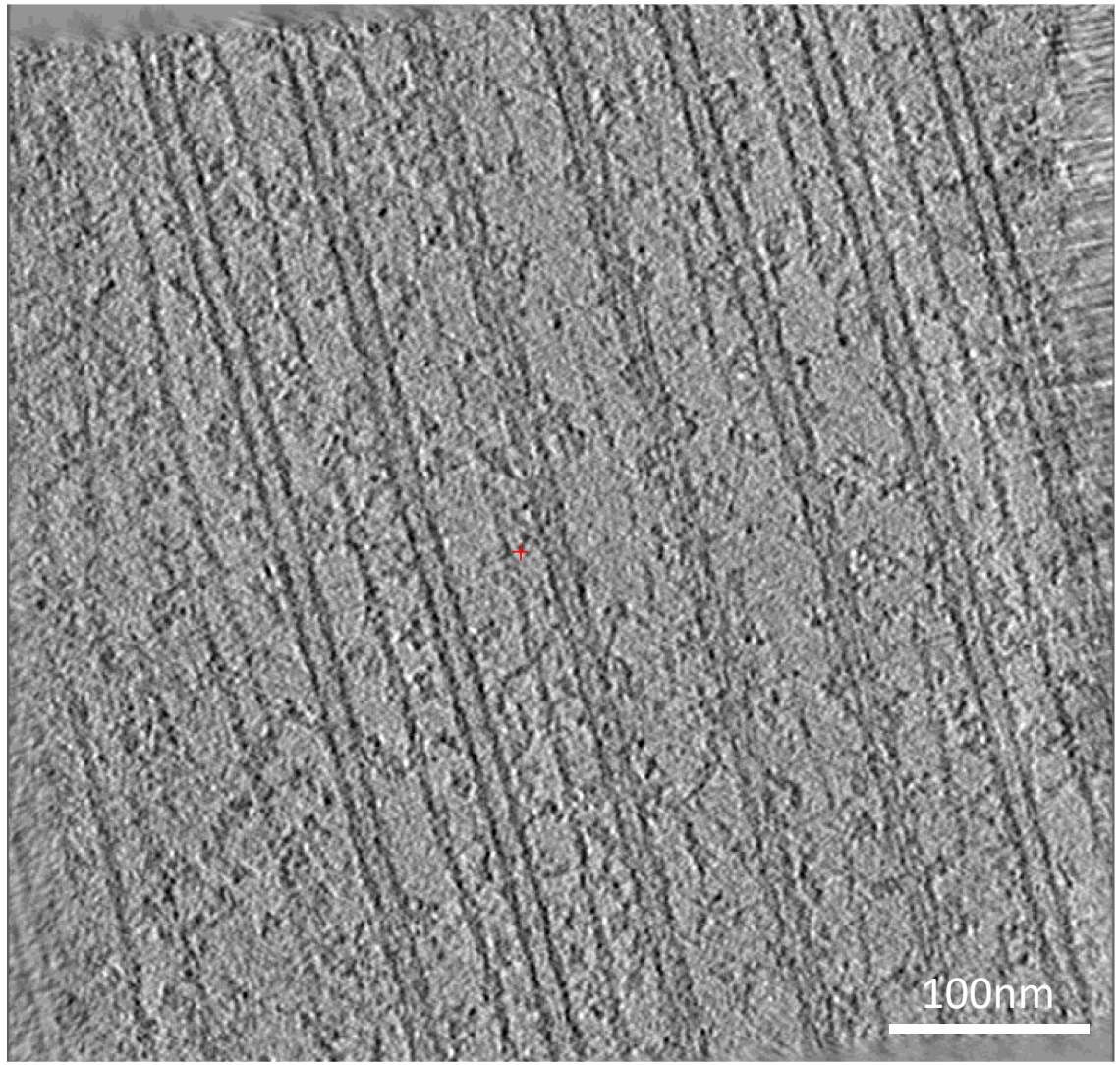
see also [1]

Cryo Electron Tomography



Cryo Electron Tomography





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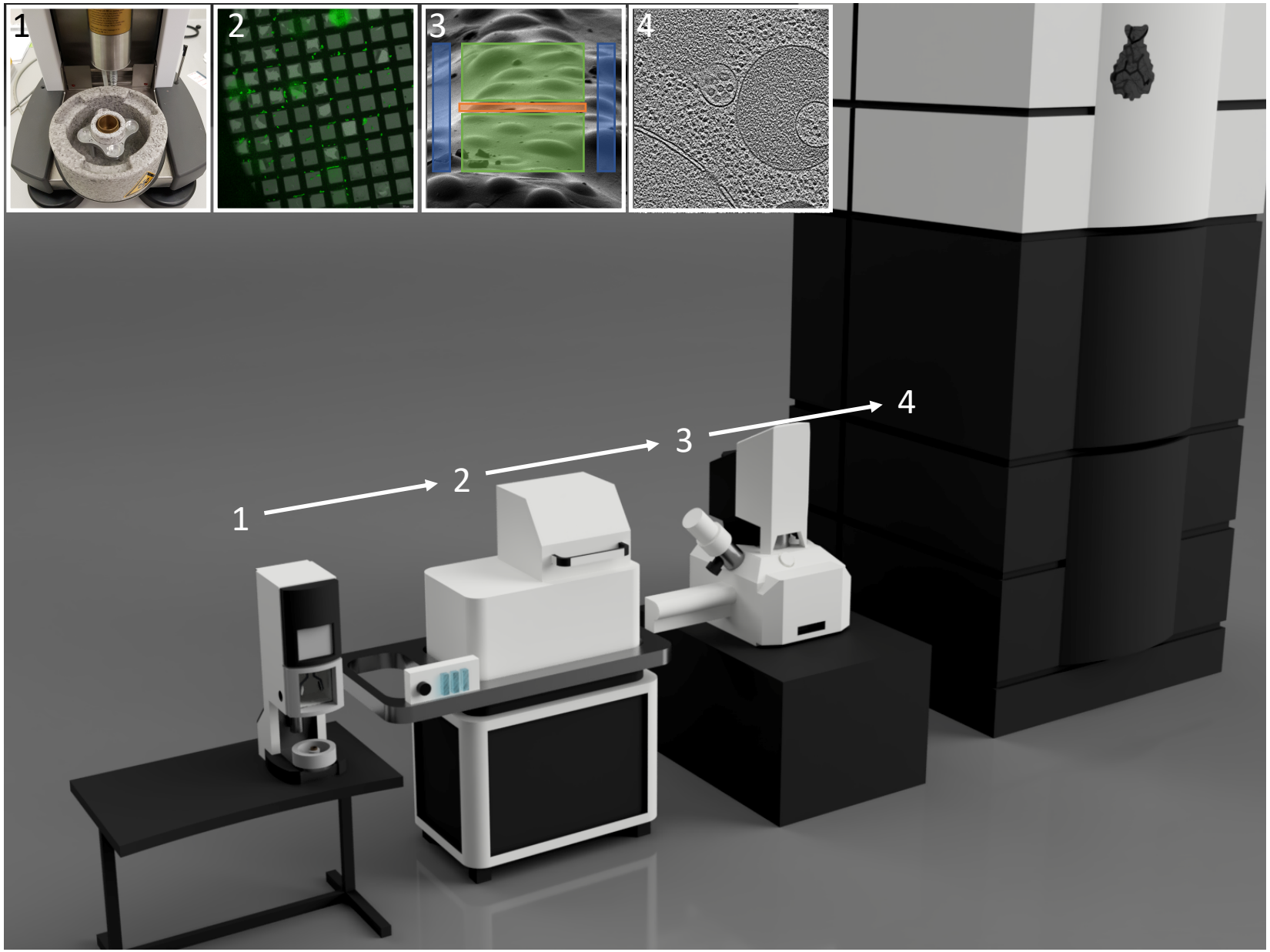
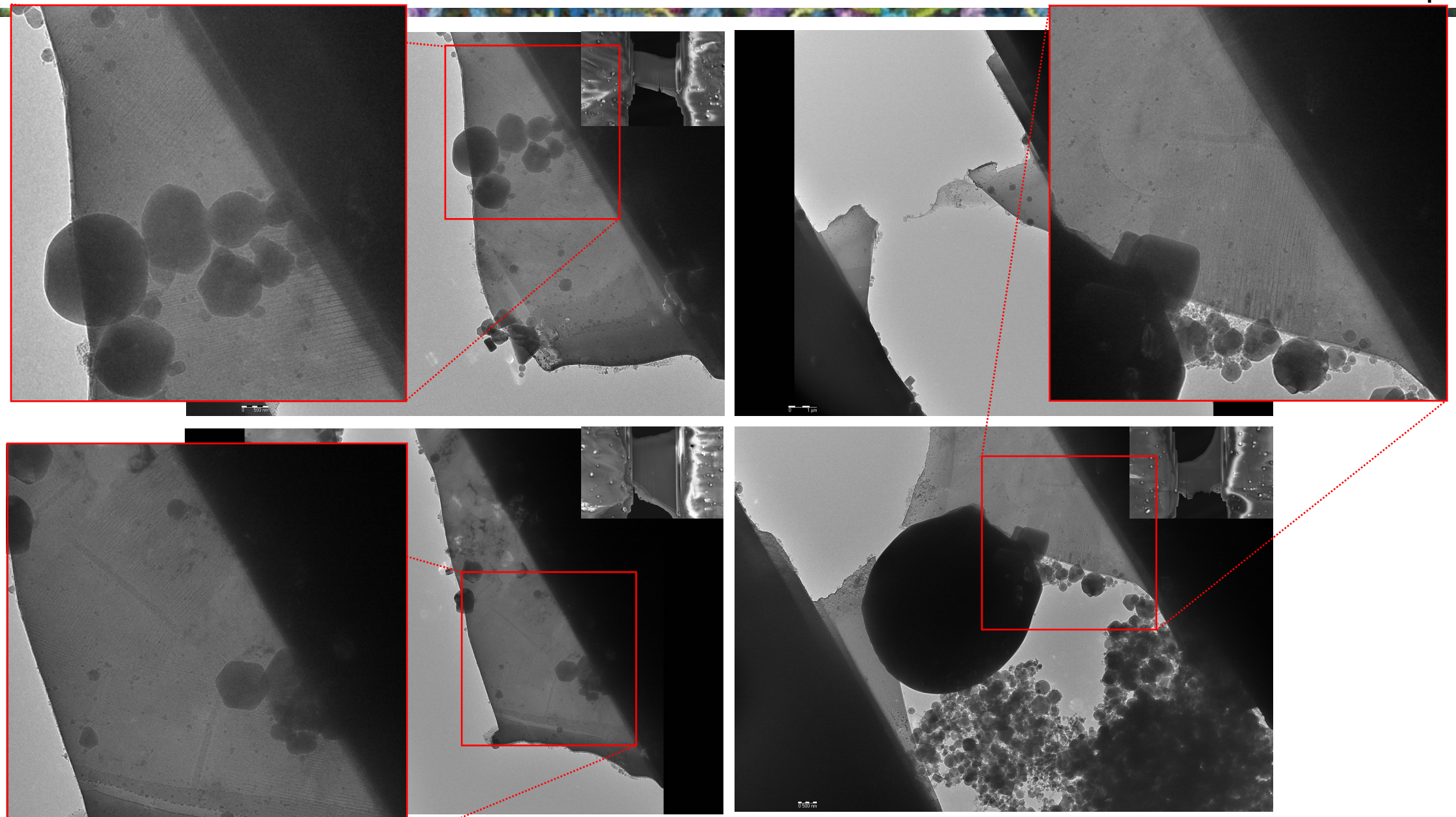


Artefacts



Requirements for High-Resolution Investigations

1500 tomograms with thickness below 150 nm.

Current performance

Approx. 10 lamellae per Day (8h)

↓

Approx. one month for one dataset

↓

Approx. two month of data acquisition

Target performance

30 lamellae per day

↓

Approx. 10 days for one dataset

↓

Approx. two weeks of data acquisition

Current limitations:

1. Reliable vitrification
2. Artefact free transfer.
3. Reduced contamination rate.
4. Automated milling missing.
5. Faster data acquisition.

References

[1] A. Rigort (2016). **Recent developments in FEI‘s in situ cryo-electron tomography workflow**. *Nature Methods*. 13, 958-960.