Chains (Veldhoven)
2019-12-10

KAVLI INSTITUTE of Nanoscience Delft

Bionanoscience Department
Think big about life at the smallest scale

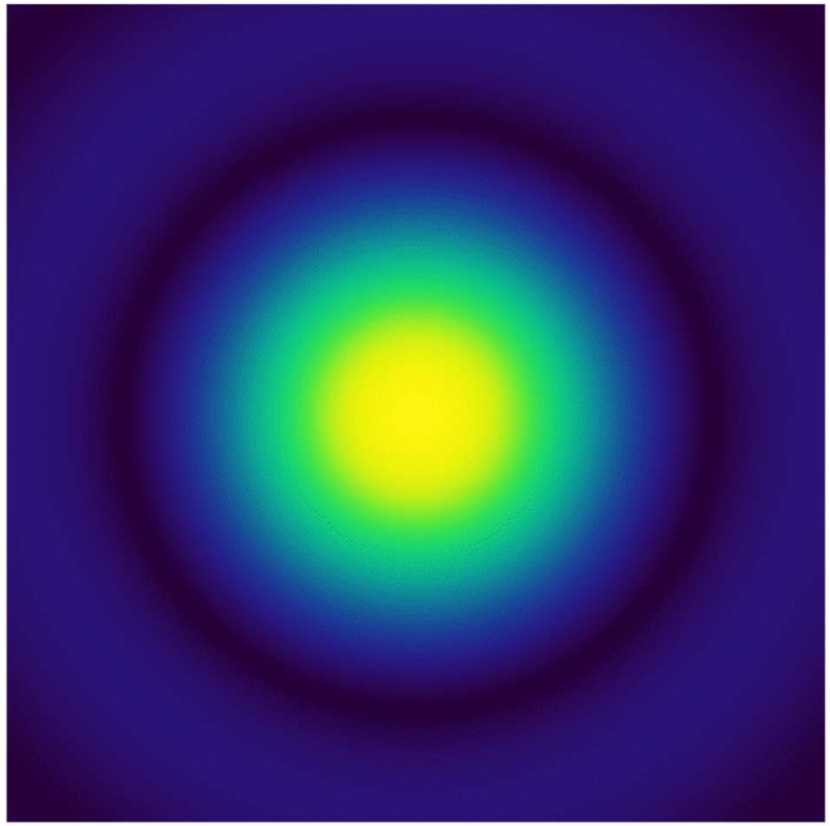
Towards automated serial electron diffraction for beam-sensitive materials

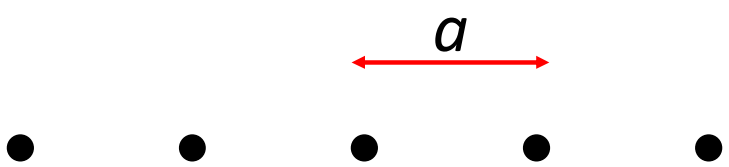
Stef Smeets

Kavli Institute of Nanoscience Delft

TU Delft

- Fourier Transform

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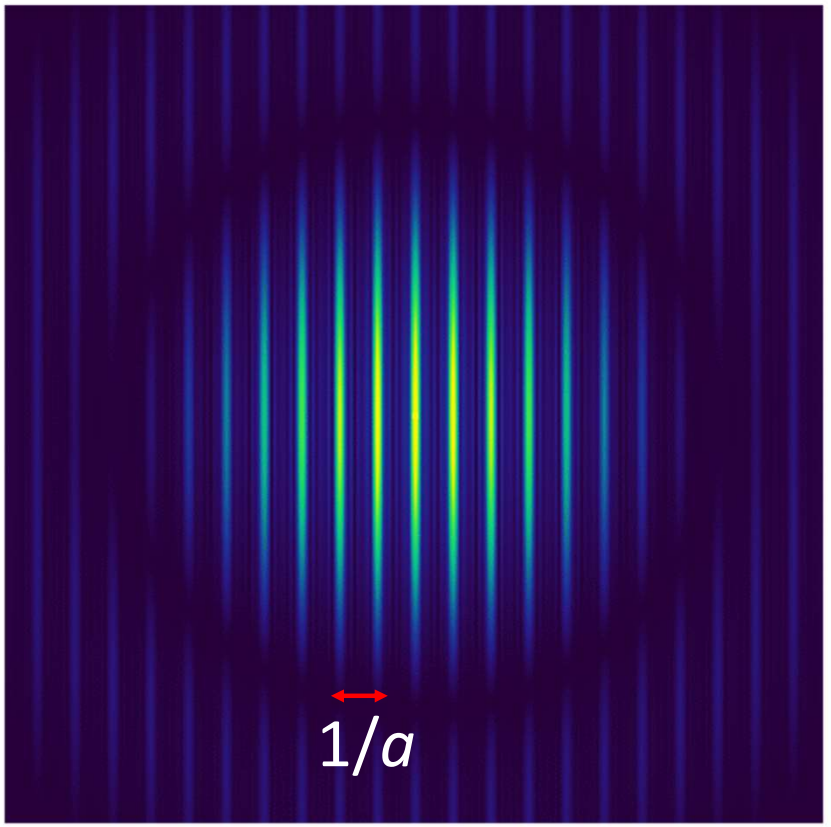
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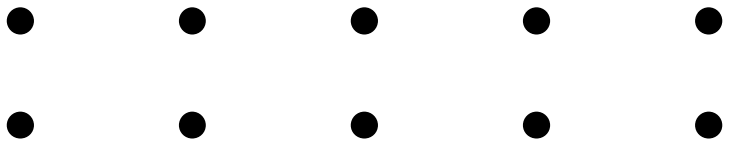

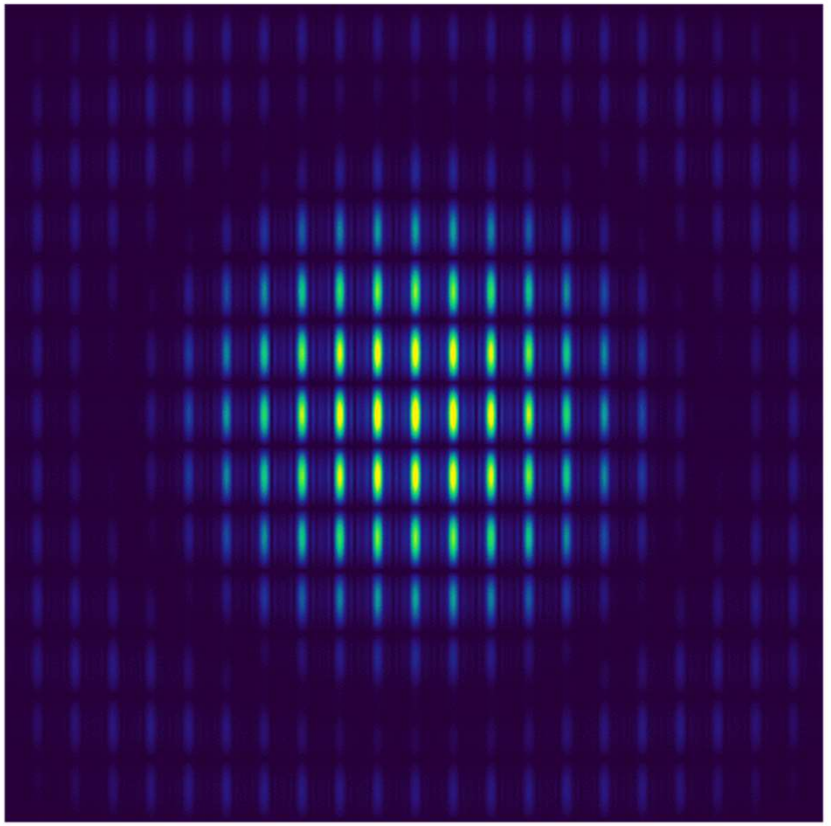

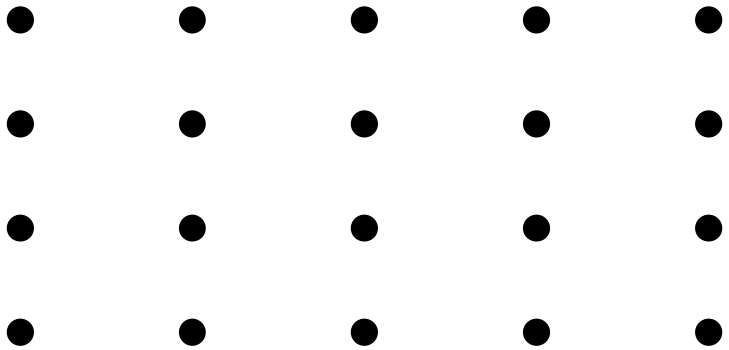

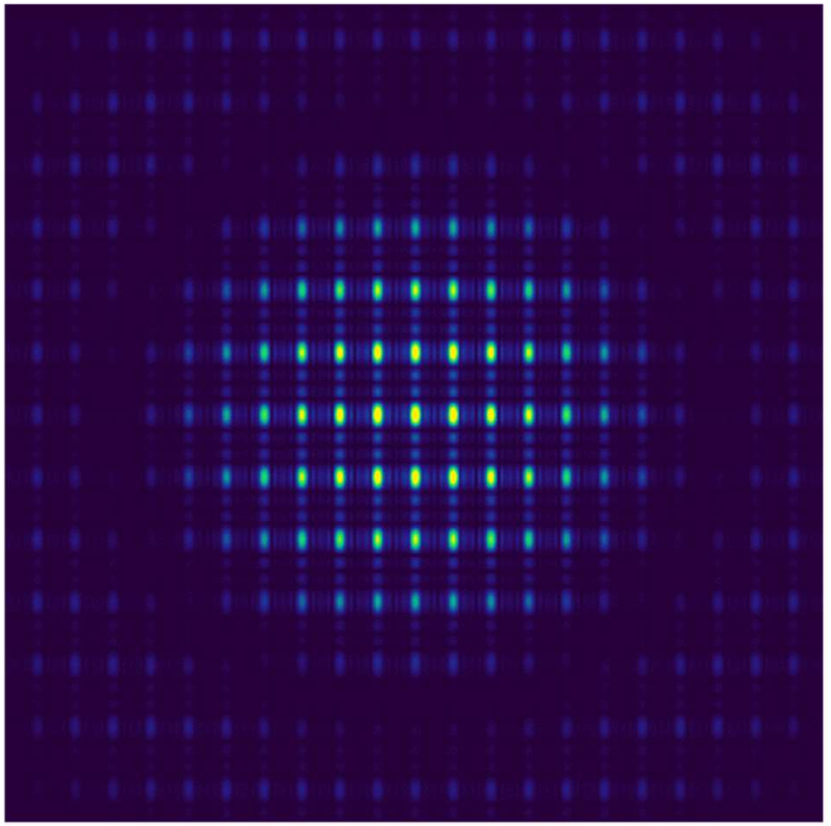

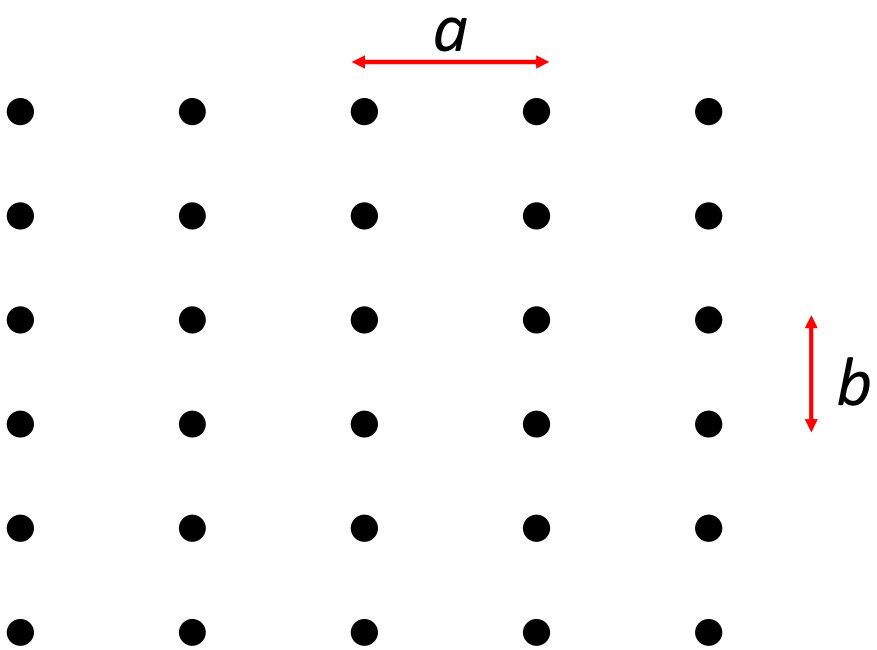
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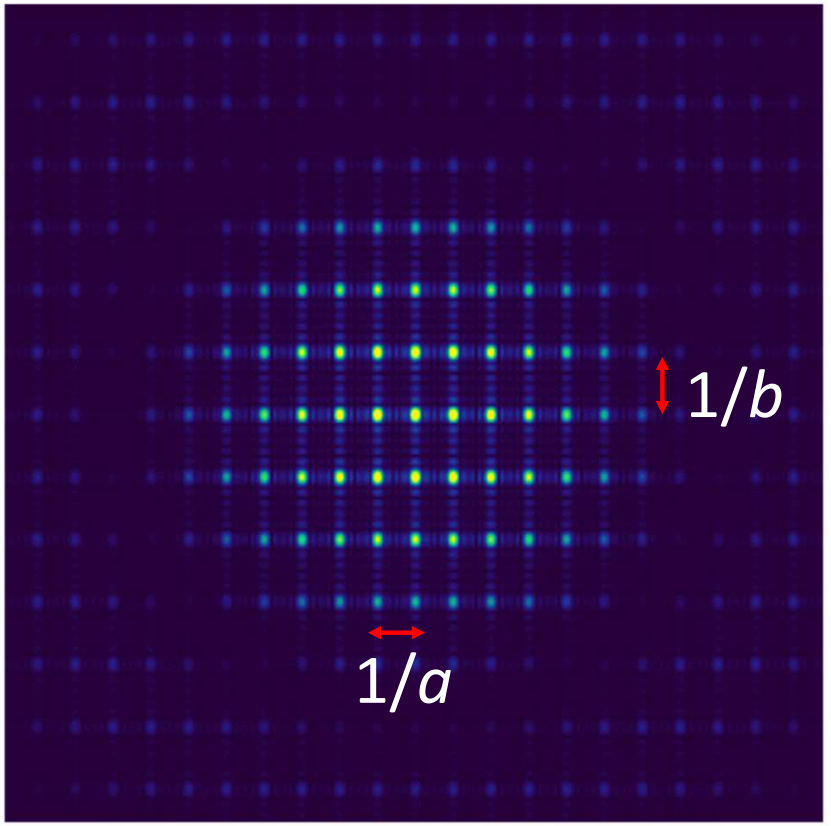
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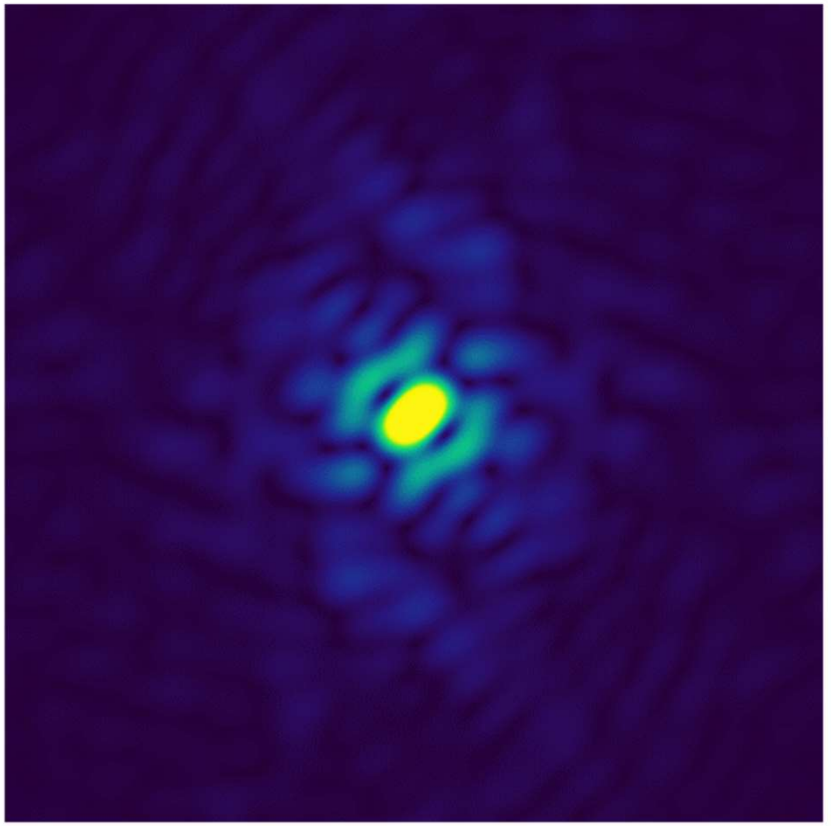
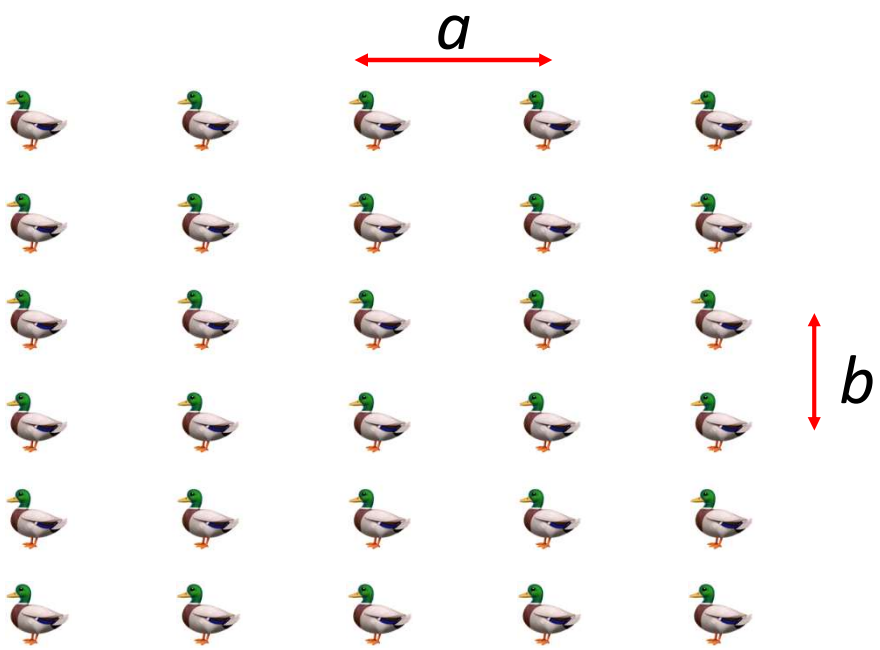
1/a



duck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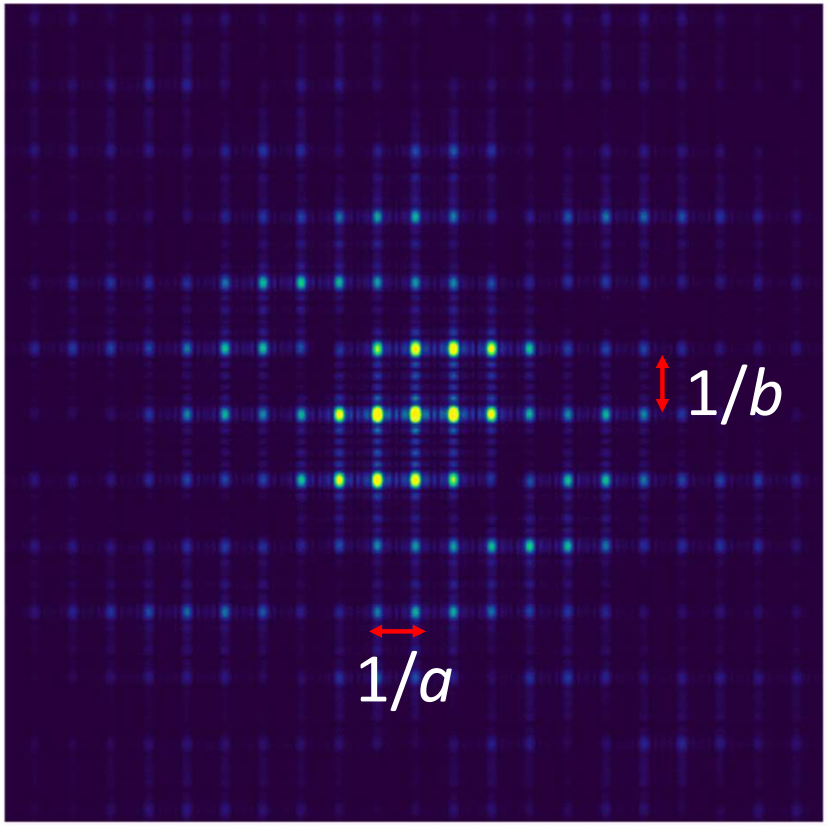
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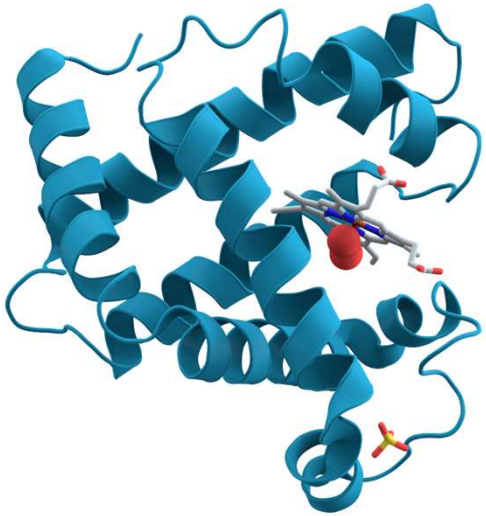
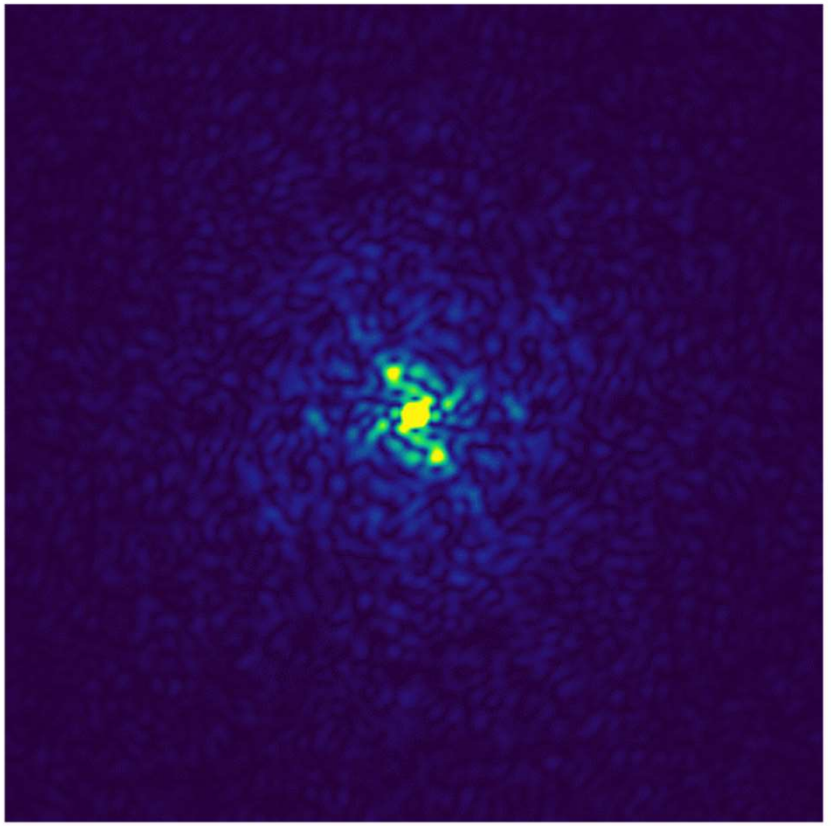
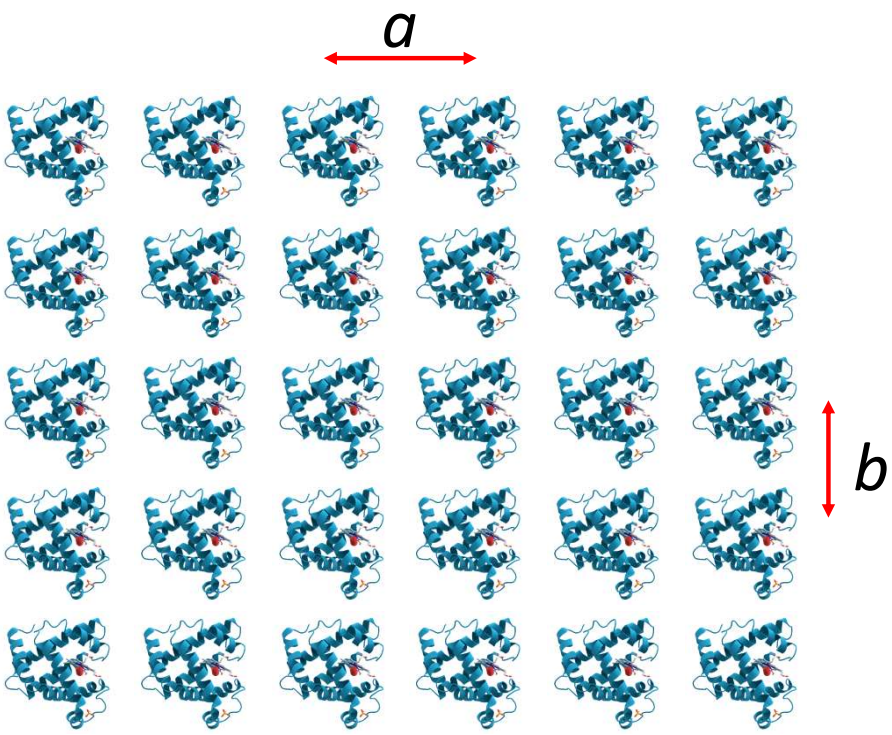
1/a



myoglobin

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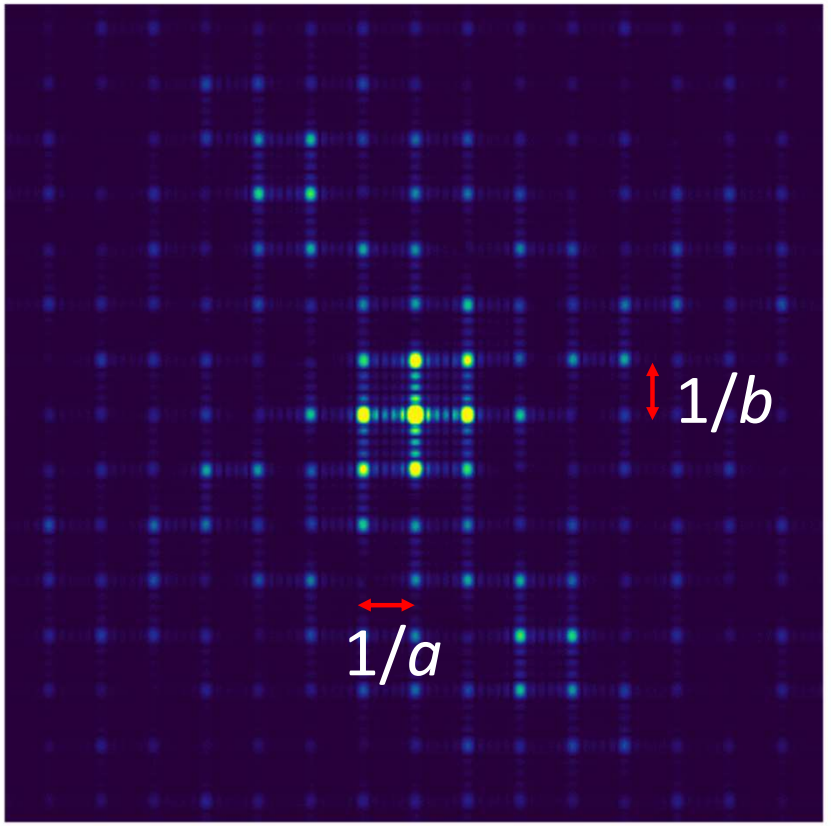
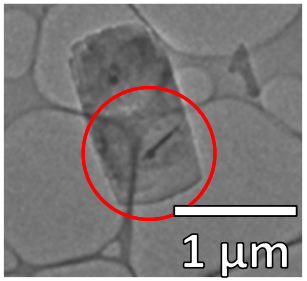
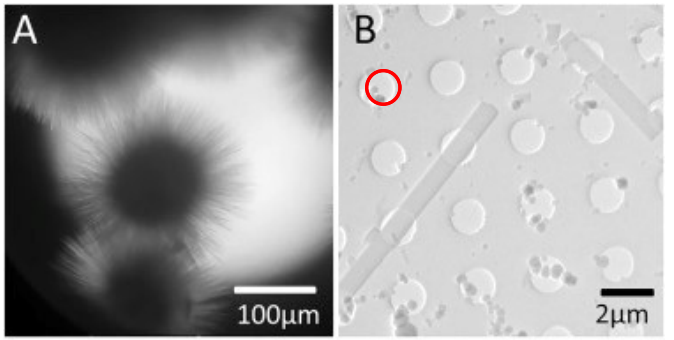
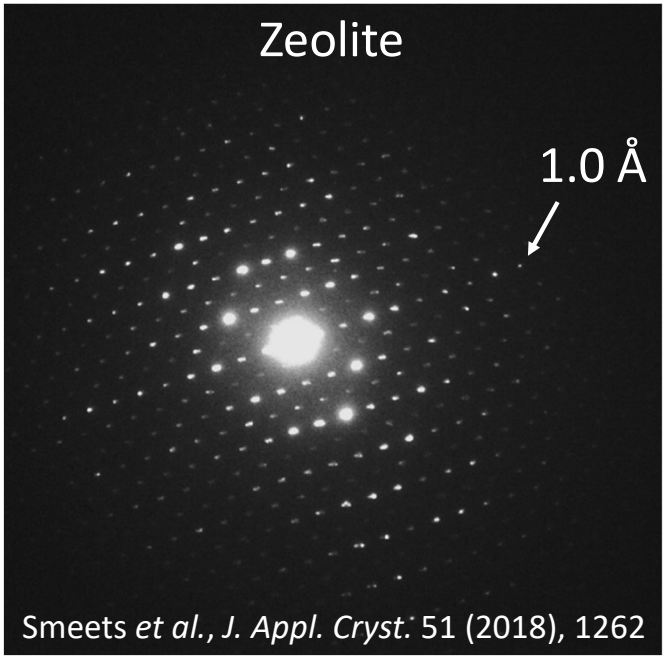

1/b
1/a



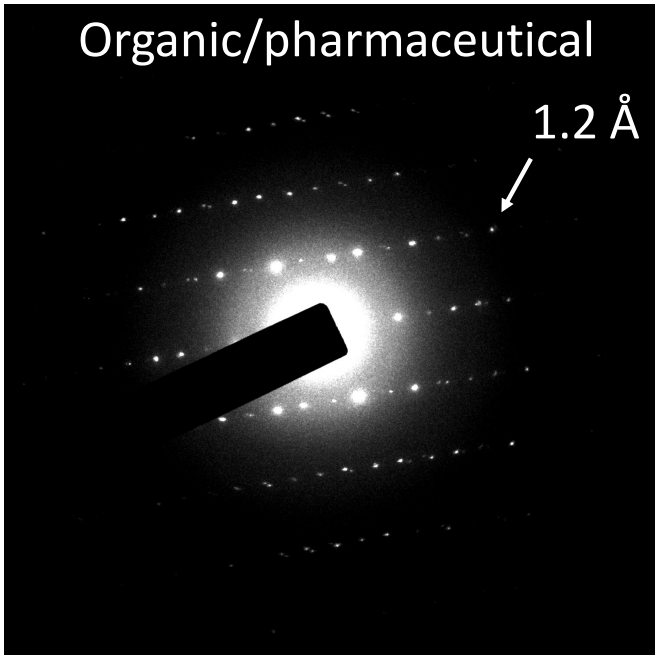
$$F_{hkl} = \sum_{j=1}^{N} f_j e^{i2\pi(hx_j+ky_j+lz_j)}$$

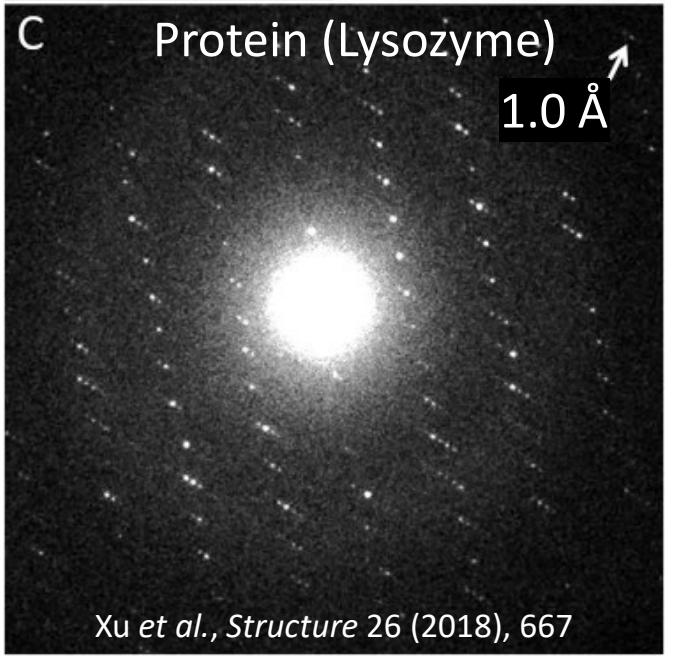
Diffraction spots — *Atomic model*



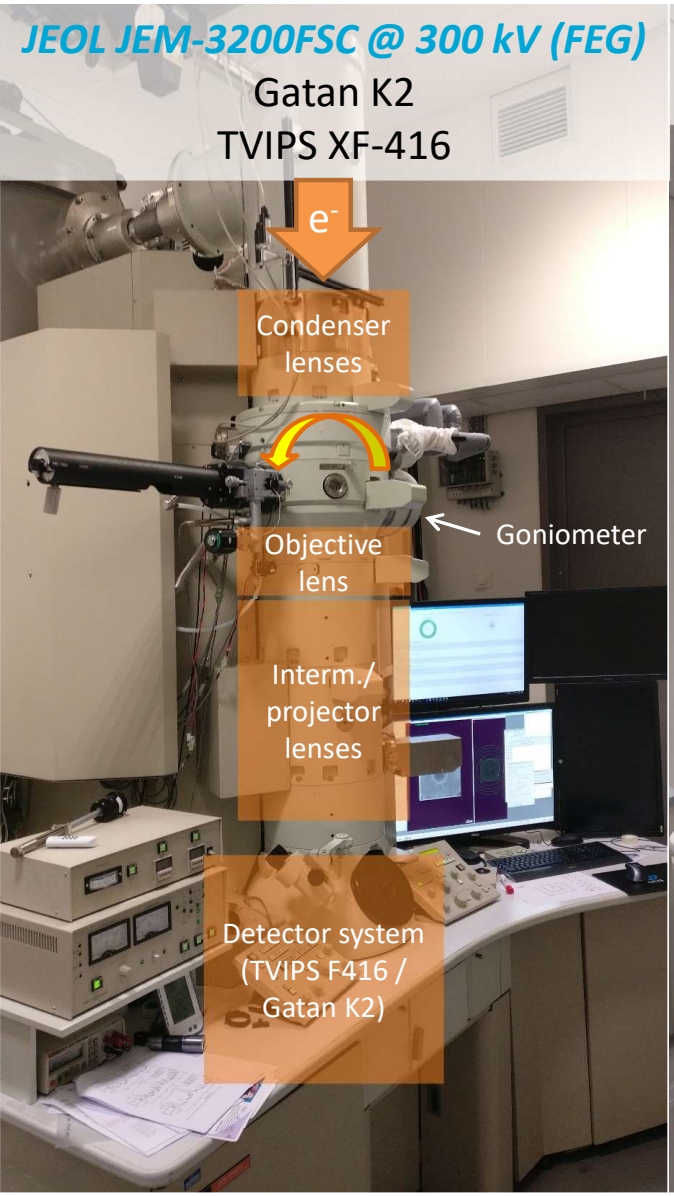


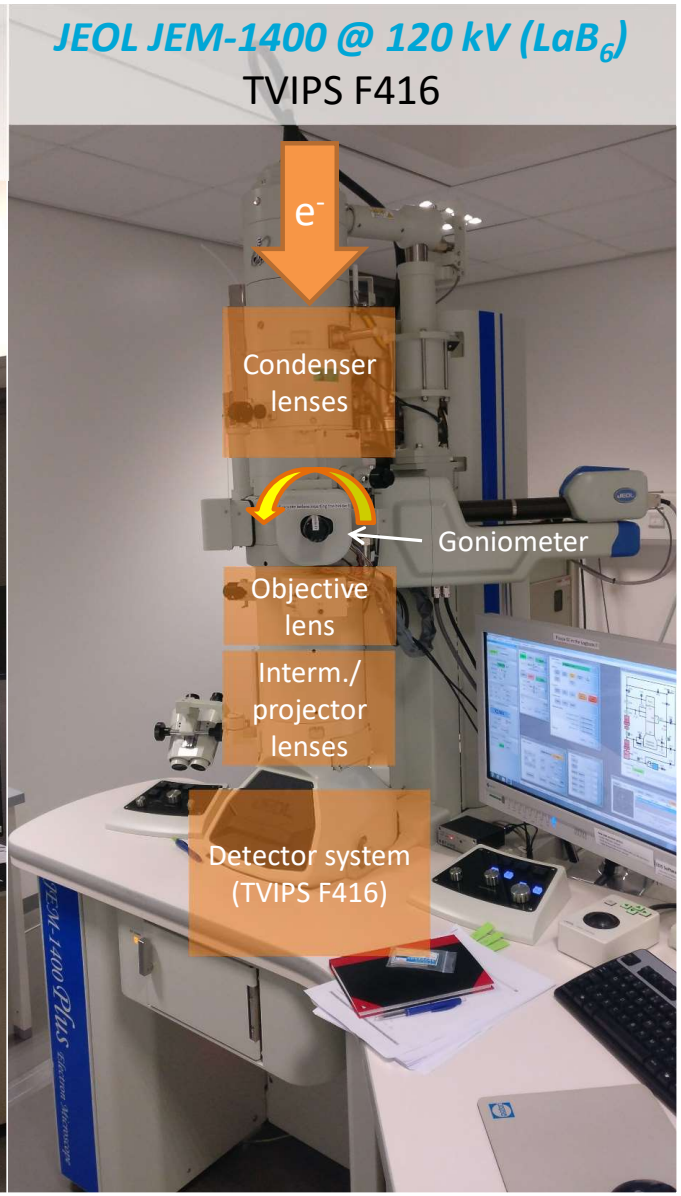
Smeets *et al.*, *J. Appl. Cryst.* 51 (2018), 1262



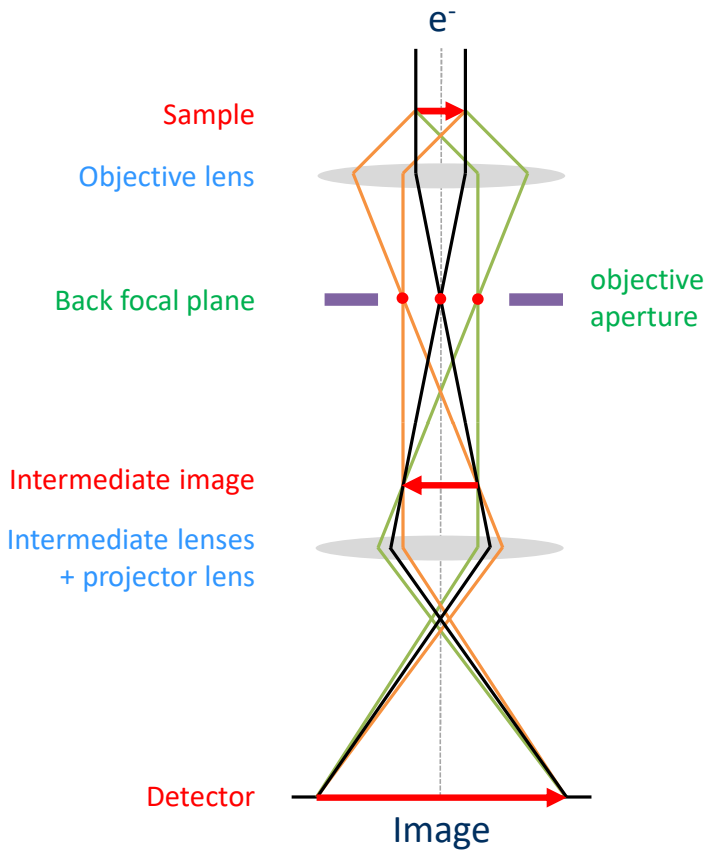


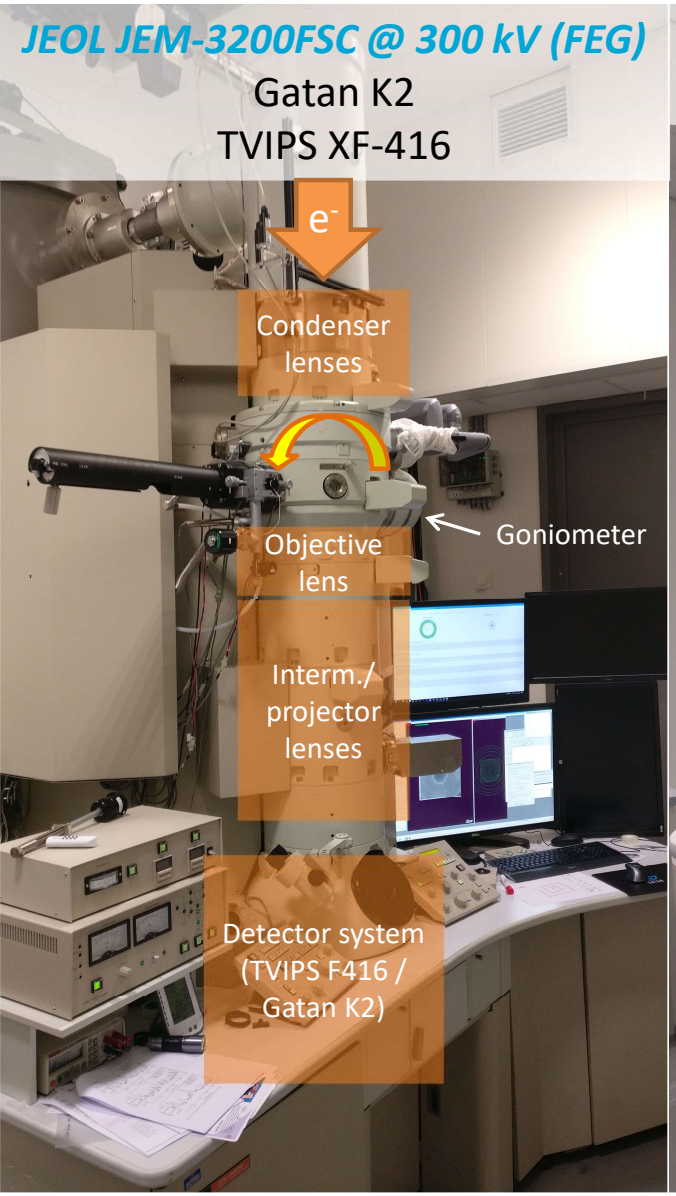
Xu *et al.*, *Structure* 26 (2018), 667

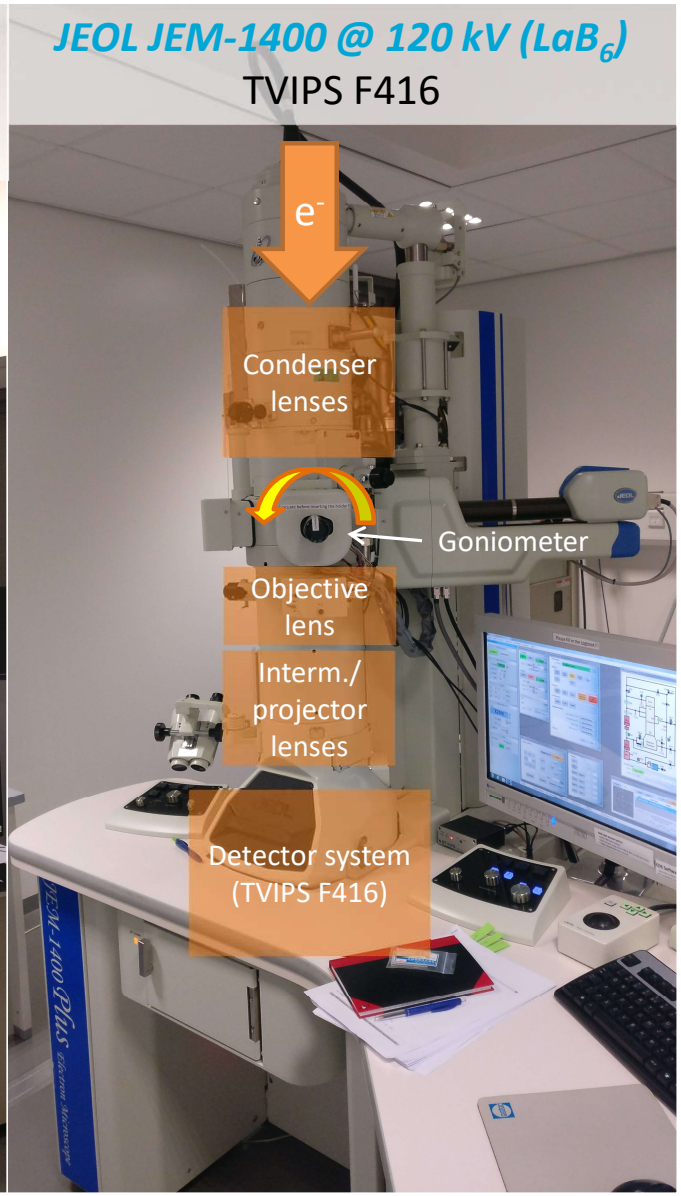




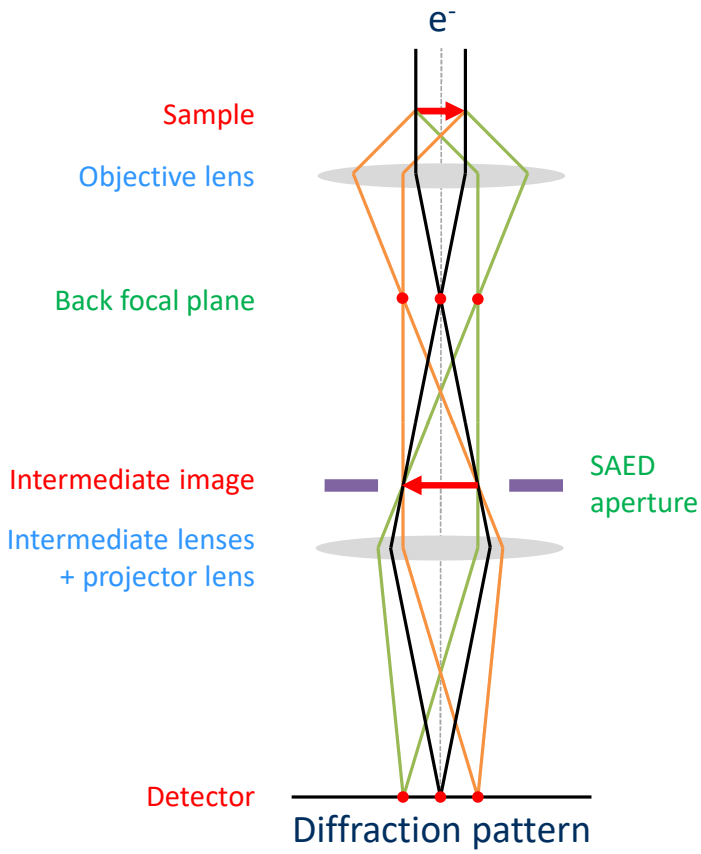
Electron microscope



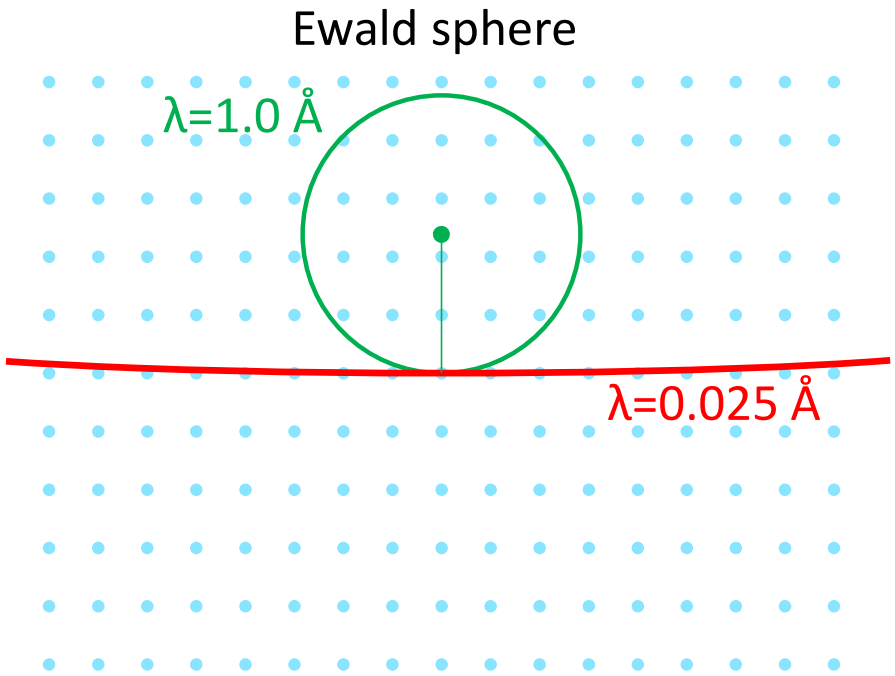




Electron 'diffractometer'

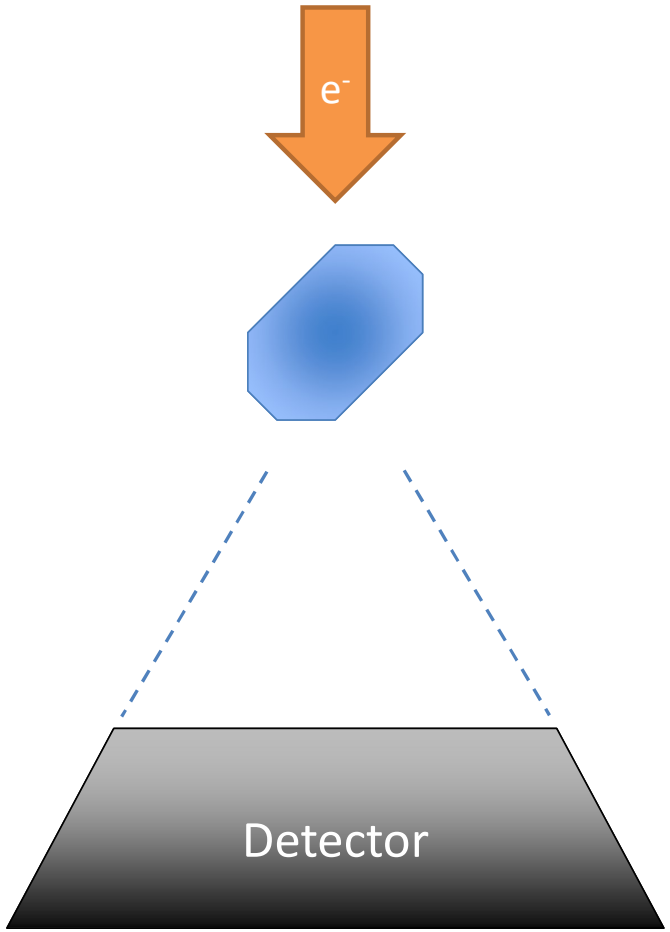


Electrons as a radiation source



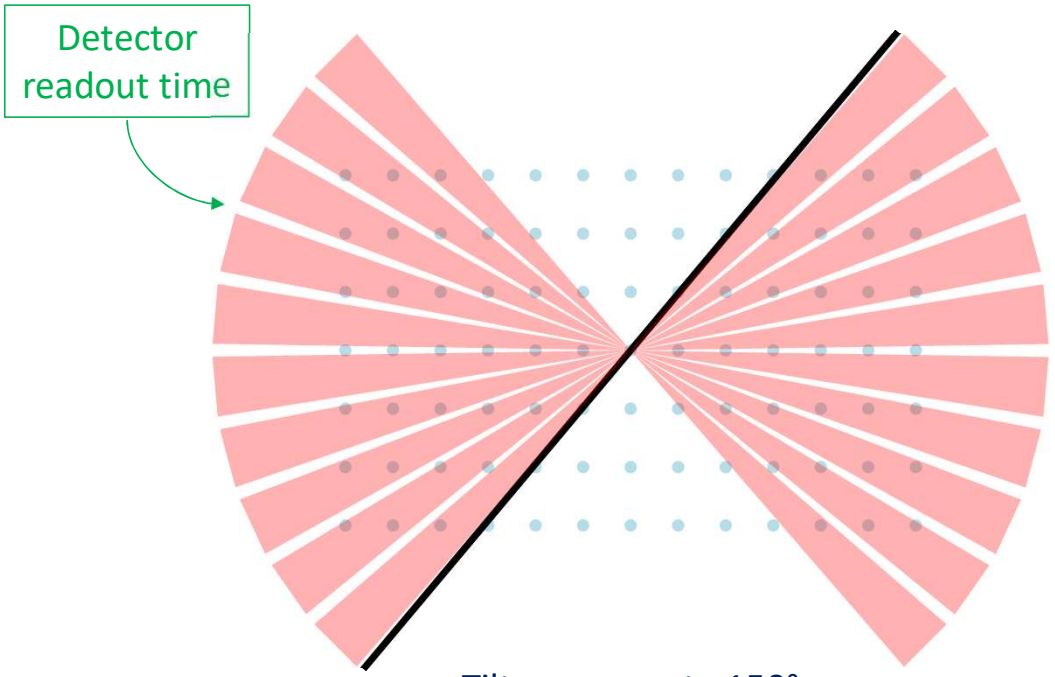
- Wavelength: ~0.025 Å @ 200 keV
- Probe electrostatic potential
- High vacuum (<10^{-3} mbar)
- Strong interaction (10^6 stronger than X-rays)
- Require small samples (< 1 µm)

3D Electron diffraction



Continuous rotation method

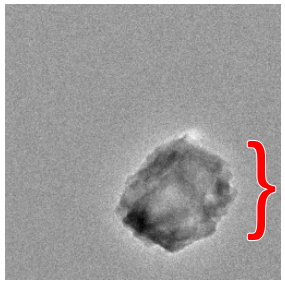
Nederlof *et al.*, *Acta Cryst. D* (2013), 69:1223
Nannenga *et al.*, *Nat. Methods* (2014), 11:927
Gemmi *et al.*, *J. Appl. Cryst.* (2015), 48:718
Cichocka *et al.*, *J. Appl. Cryst.* (2018), 51:1652

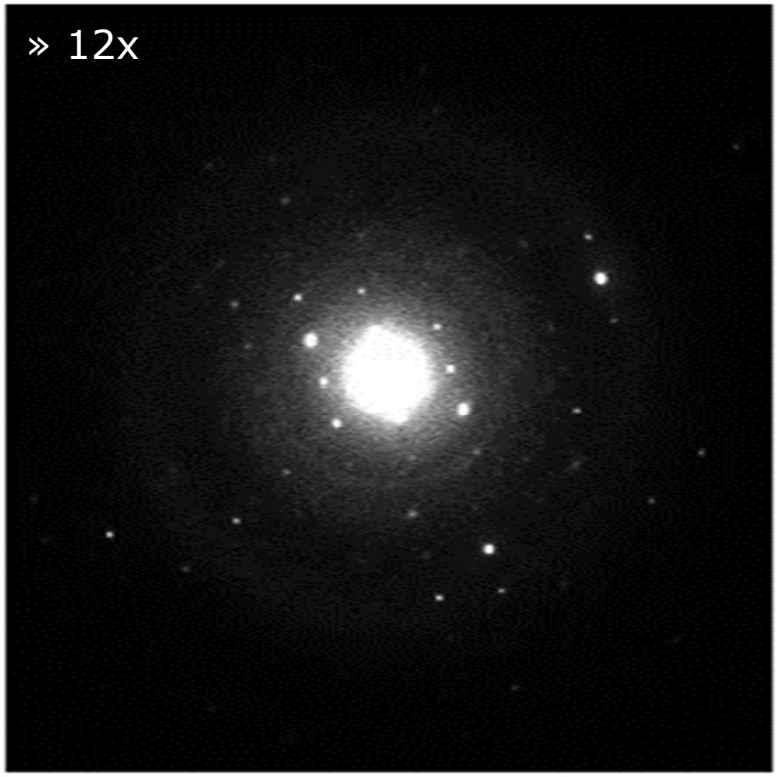


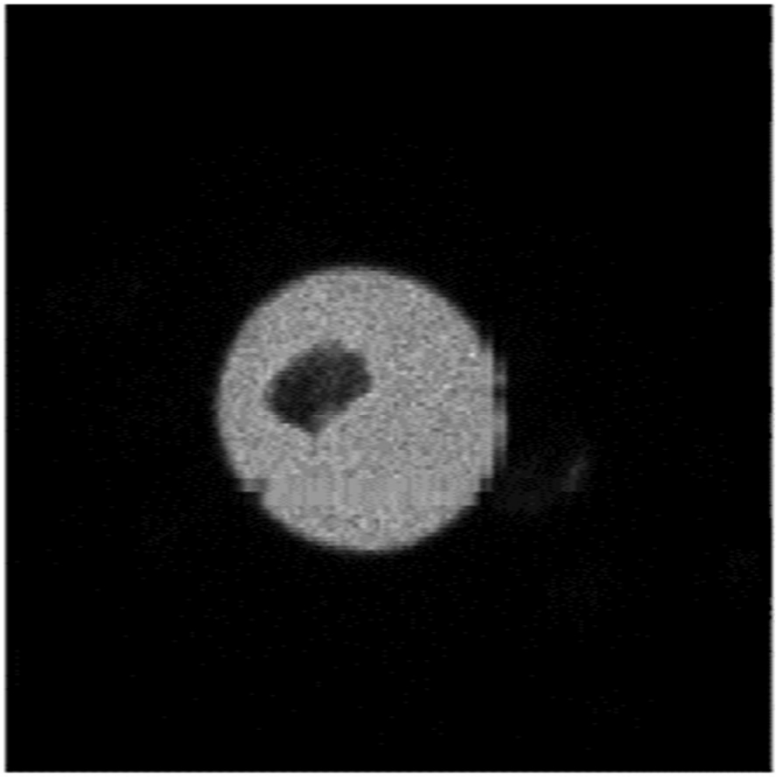
Tilt range: up to 150°
Oscillation angle: 0.1-0.5°
Rotation speed: 0.5-2.0°/s
<5 minutes

Zeolite mordenite

Rotate: -43.90° to 58.65° @ 0.45°/s (102.55°)
Exposure: 0.5 s, oscillation angle: 0.23°
JEOL 2100-LaB_6 @ 200 kV (Timepix camera)



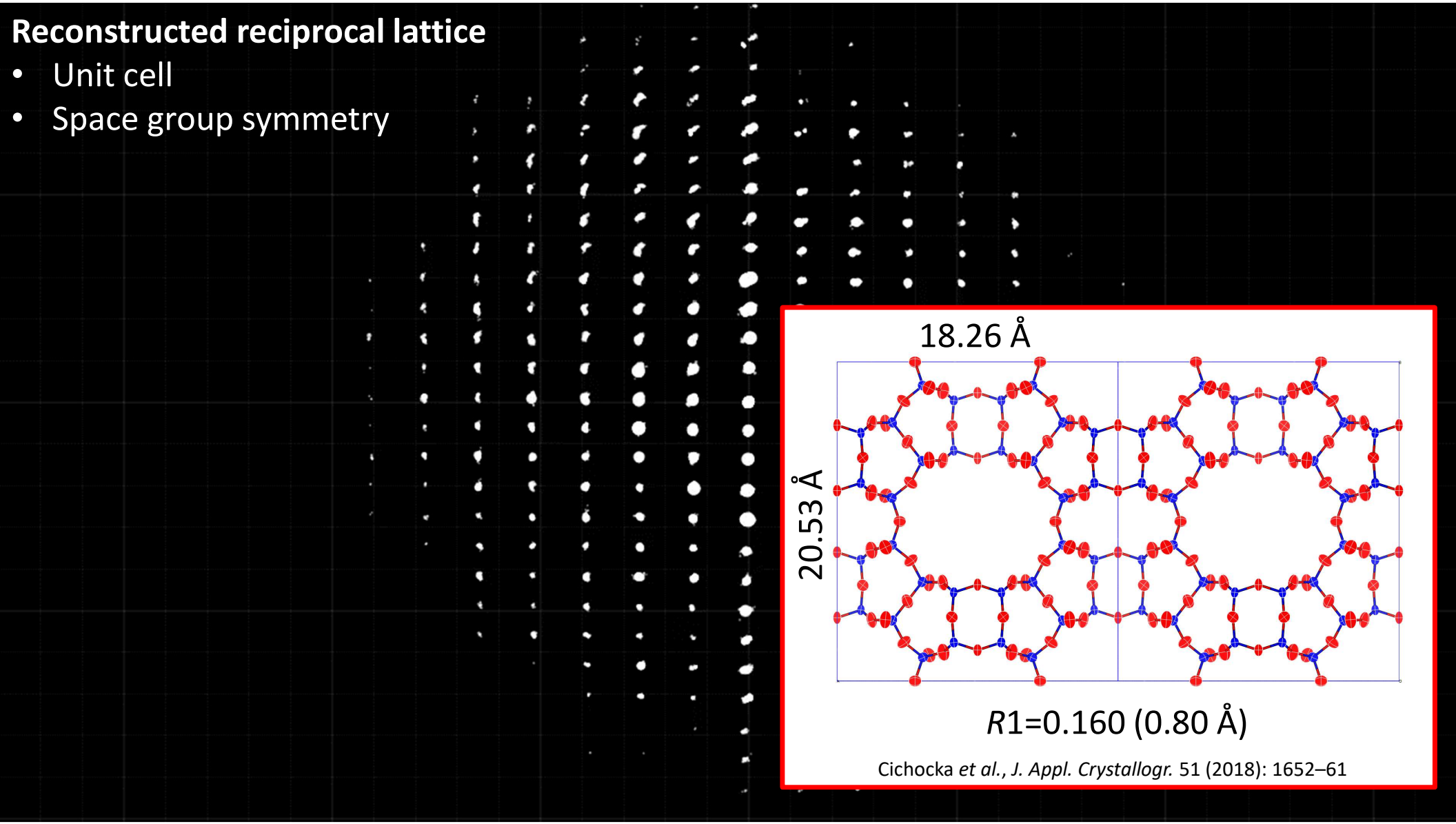




Cichocka *et al.*, *J. Appl. Crystallogr.* 51 (2018), 1652

Reconstructed reciprocal lattice

- Unit cell
- Space group symmetry

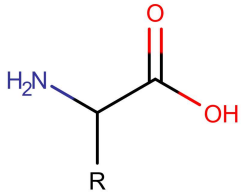


Cichocka *et al.*, *J. Appl. Crystallogr.* 51 (2018): 1652–61

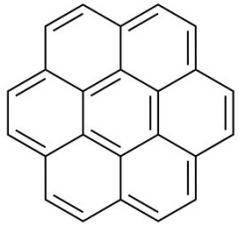
Radiation damage sensitivity

Material	Characteristic dose D_{ec} ($e^-/Å^2$)
Bacteriorhodopsin	0.5
Amino acid (glycine)	1.6
Polyethylene	6.0
Coronene	70.0
Phthalocyanine	120.0
Zeolite (ZSM-5)	300.0
Calcite (200 kV)	39000.0

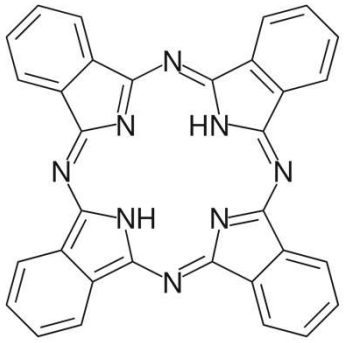
Egerton, Micron 119 (2019)



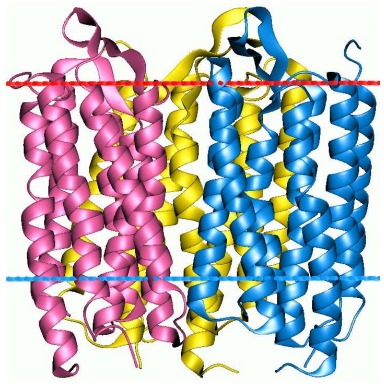
Amino acid



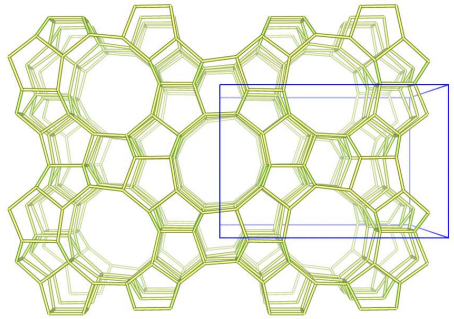
Coronene



Phthalocyanine

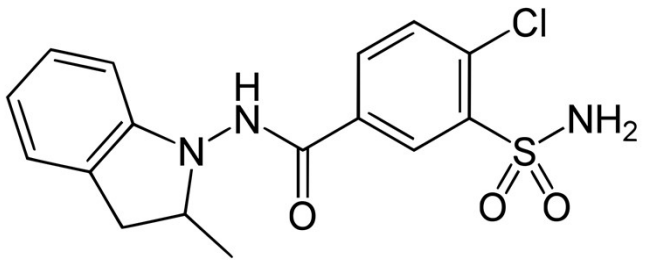


Bacteriorhodopsin

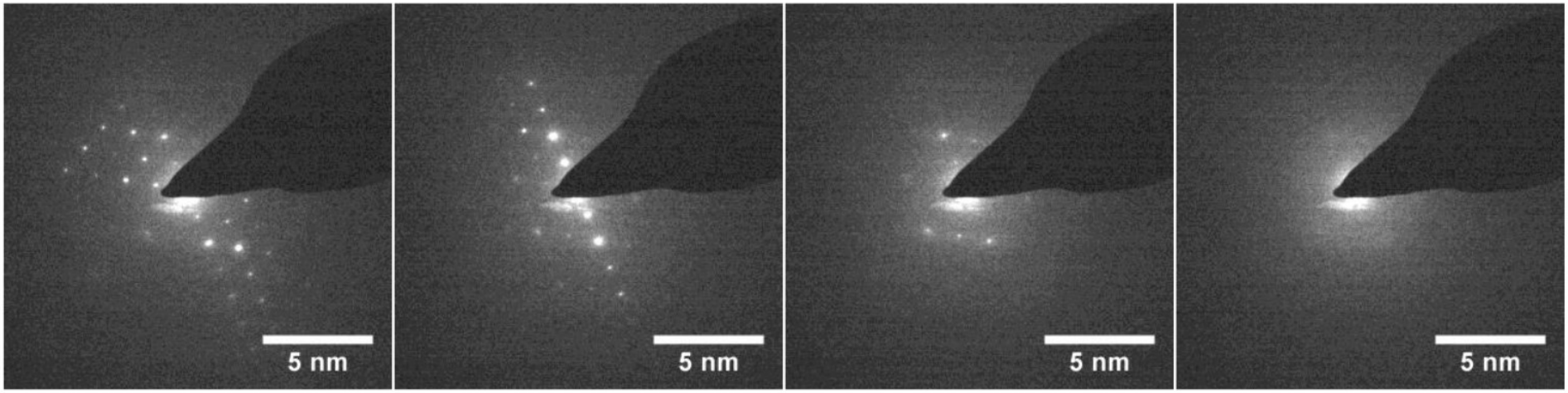


ZSM-5 zeolite

Radiation damage



Indapamide

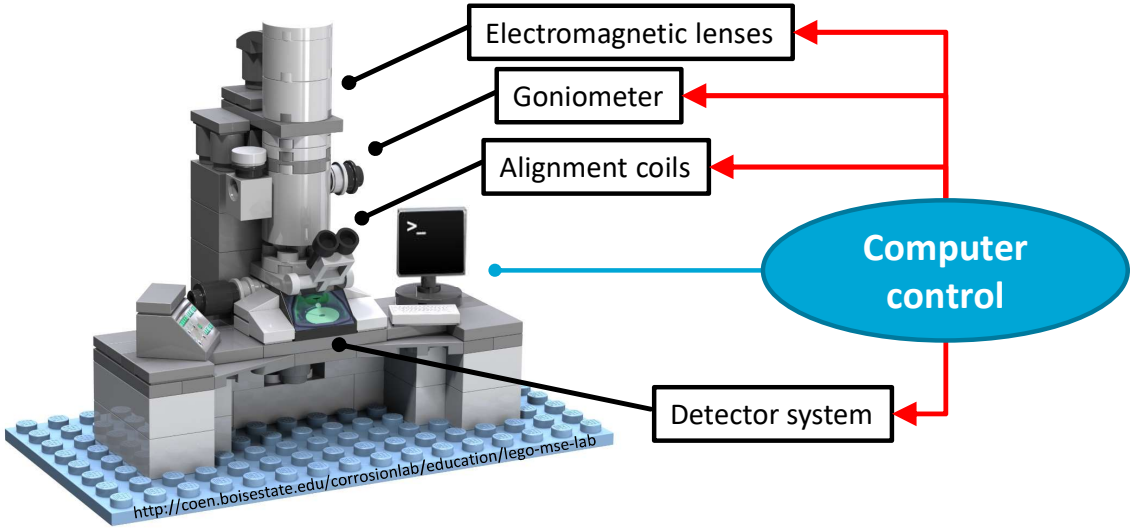


Δt =	45 s	120 s	170 s	250 s
D =	1.60 $e^-/Å^2$	4.31 $e^-/Å^2$	6.10 $e^-/Å^2$	8.98 $e^-/Å^2$

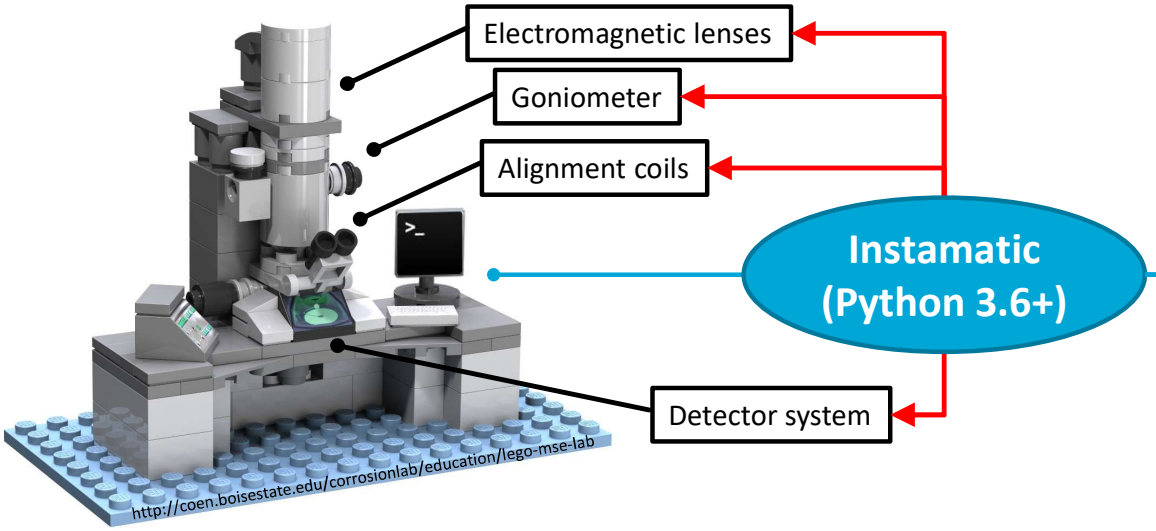
S'ari *et al., J. Phys. Conf. Ser.* 644 (2015)

No standard for data collection

- No software – many labs use ad hoc data collection protocols
- Manual data collection: tedious and not reproducible
- Lack of automation
  - Unnecessary dose accumulation
  - Low redundancy
  - Biased crystal selection



More automation

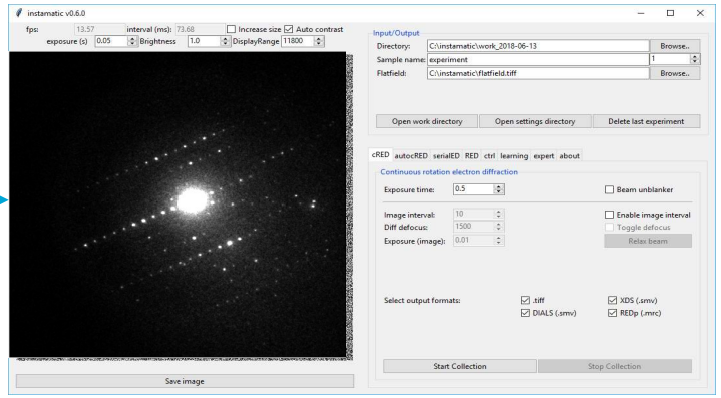


Tools

- Crystal finder/tracking
- Neural network
- Calibrations/alignments
- Reading/writing images
- Grid mapping/montaging

Automated experiments

- Electron diffraction
- Unsupervised data collection
- Screening



Microscope control

TFS Titan/Themis Z

JEOL 1400/2100/
3200/ARM200

Simulated

Camera interface

ASI Cheetah

Gatan

Simulated

TVIPS (X)F416

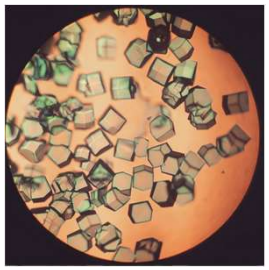
Source code:
http://github.com/stefsmeets/instamatic

Protocol for serial electron crystallography

Dose deposition

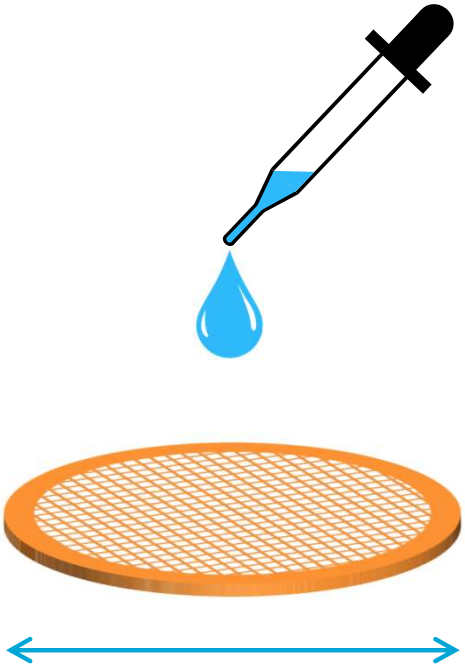
- Screen sample stage and generate global map ← Very low dose
- Select and image regions of interest ← Low dose
- For each region:
  - Identify and **localize** crystals of interest
  - For each crystal:
    - Move crystal to the electron probe
    - Collect diffraction data ← High dose

Sample preparation

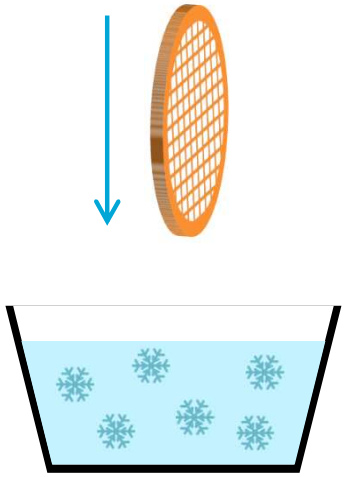


Protein crystals
(Mother licquor)

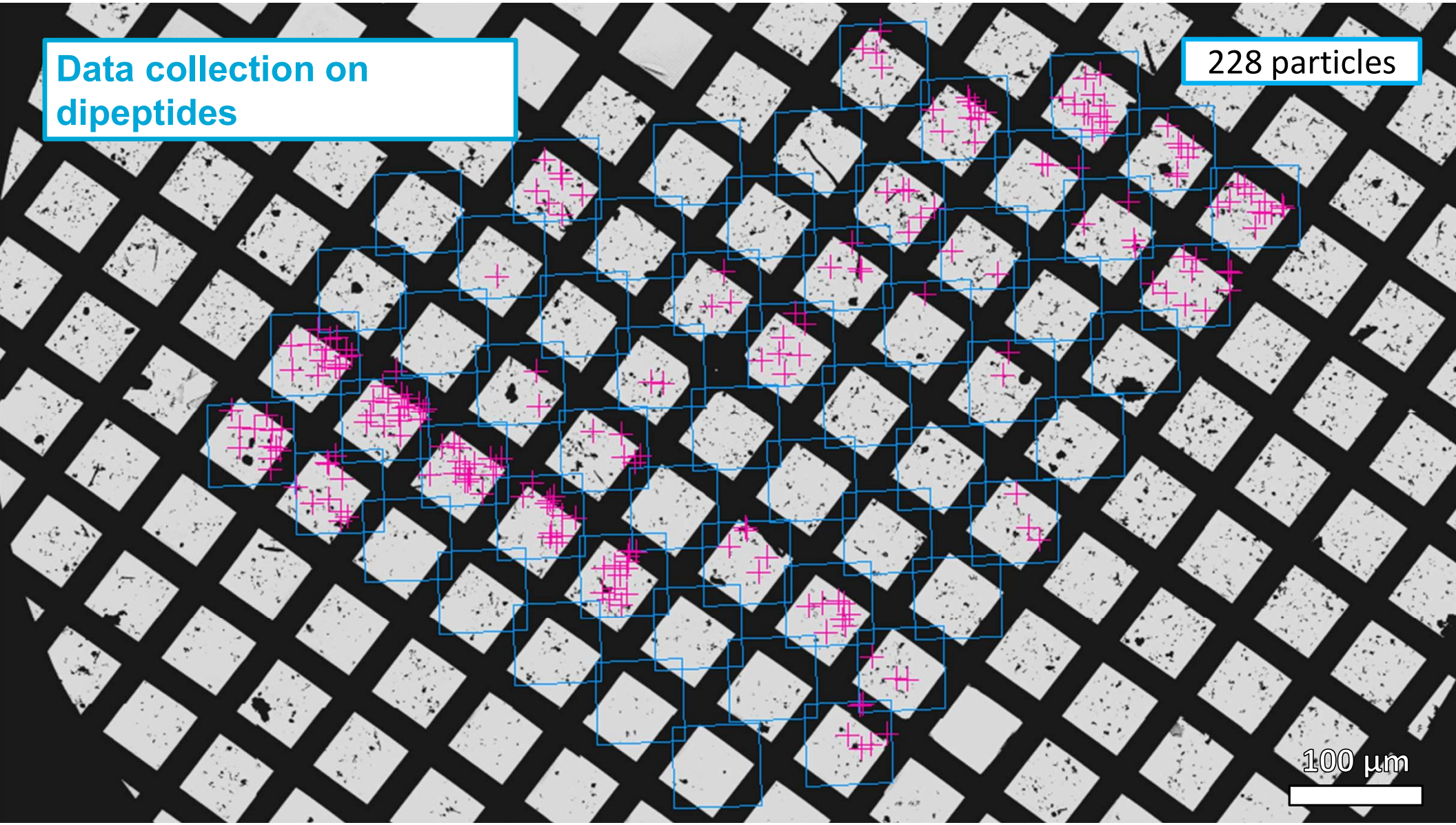
Crystalline powder
(Disperse in EtOH)



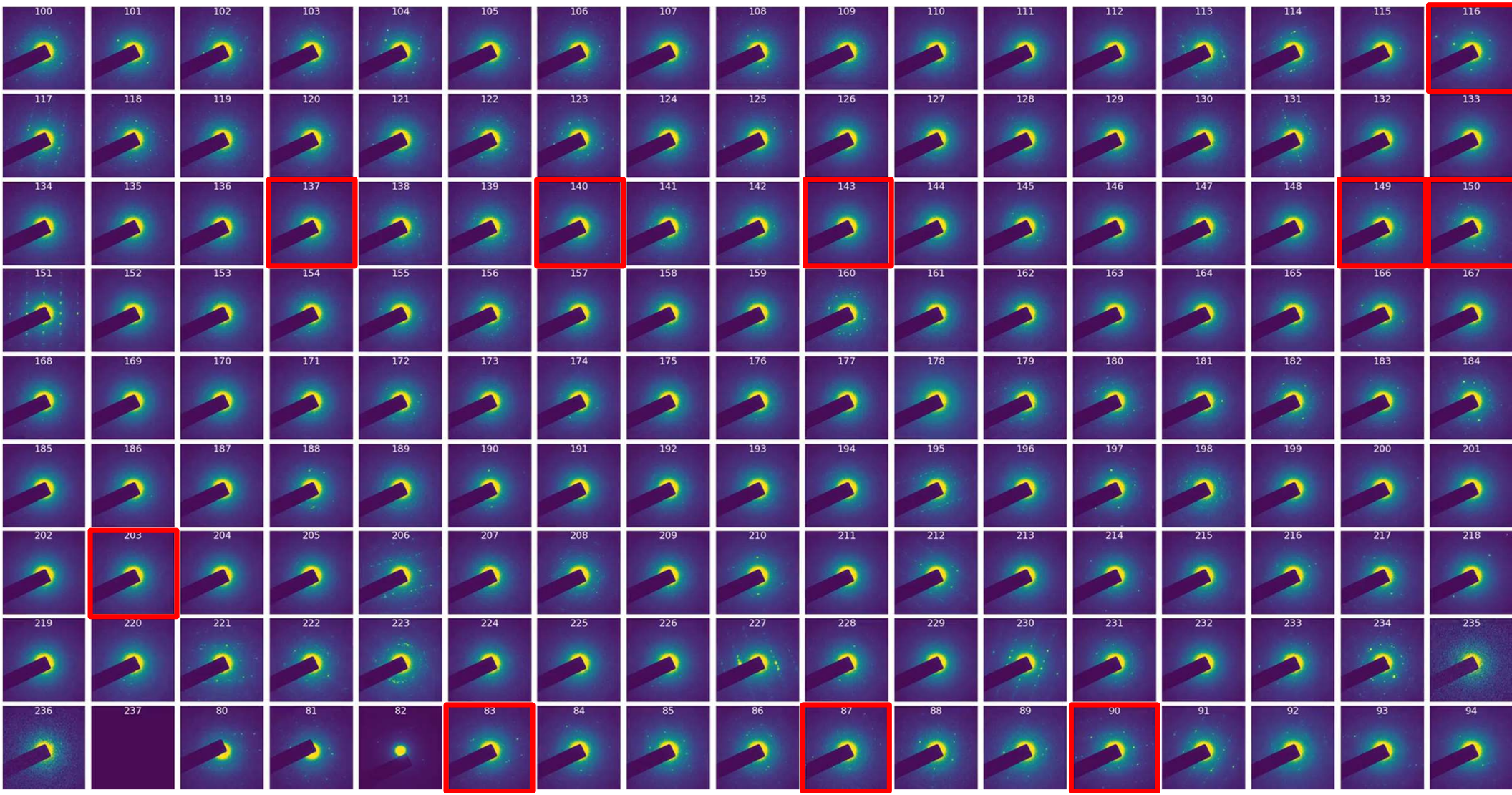
Optional

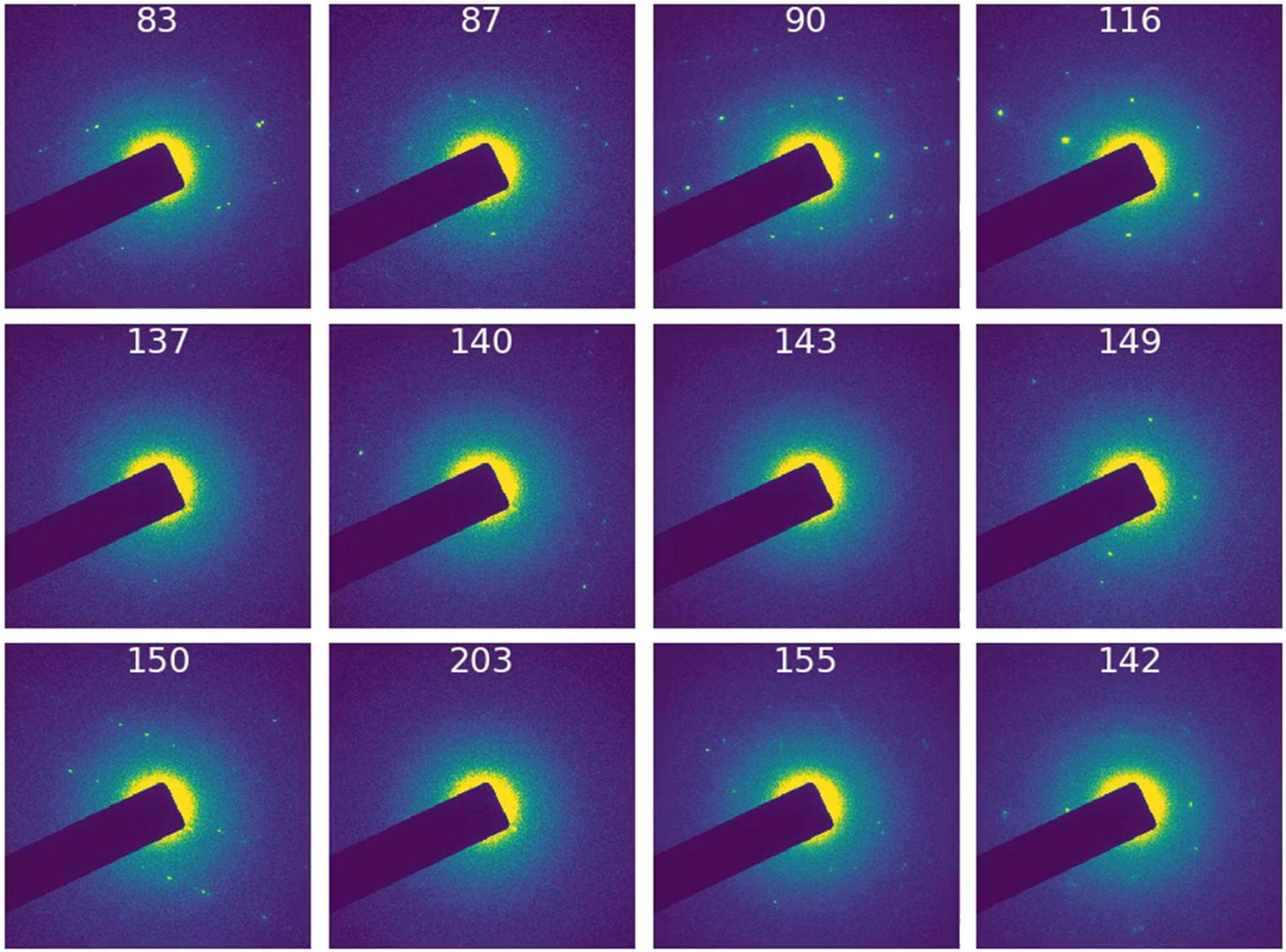


Plunge freezing
(liquid ethane)


Data collection on dipeptides
228 particles
100 µm

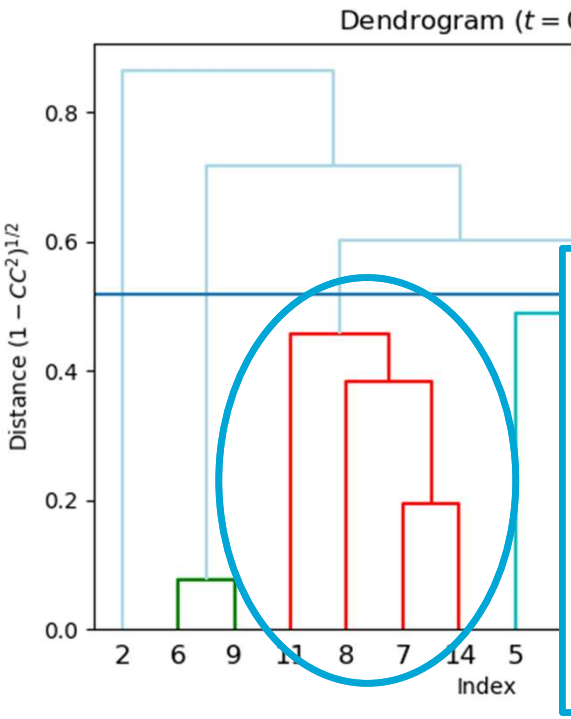
158 crystals rotated 20° @ 1.70°/s | Total exposure: 12 s/crystal | 14 indexed (XDS)




83
87
90
116
137
140
143
149
150
203
155
142

Indexing & cluster analysis

#	*a* (Å)	*b* (Å)	*c* (Å)	*β* (°)
1	13.10	23.84	5.09	92.8
2	13.98	18.12	5.53	102.4
3	13.37	19.03	4.87	92.9
4	13.07	78.54	4.88	90.7
5	13.18	61.85	4.86	90.1
6	12.58	22.76	4.96	90.7
7	12.82	21.34	4.90	91.2
8	12.76	20.35	4.94	91.7
9	11.62	27.18	5.12	93.7
10	12.97	95.24	4.86	90.7
11	12.73	20.40	4.97	88.9
12	13.11	18.59	4.94	90.3
13	12.97	78.12	4.91	91.5
14	12.99	20.51	5.27	93.5
X-ray ref.	13.111	21.380	5.113	90.624



Observed	2158
Unique	840
Compl.	58 %
Resolution	1.0 Å
$CC_{1/2}$	97.8
$I/\sigma(I)$	1.76
R_{meas}	0.387

Summary

- Automatically collect ED data on **very** beam-sensitive crystals
- Protocol for minimal dose deposition before acquisition
- Automation is key enabling high-throughput acquition and data analysis
- Methods are equally suitable for biological as materials science problems