DutchBioPhysics (Veldhoven)
2019-10-08

KAVLI INSTITUTE of Nanoscience Delft

Bionanoscience Department
Think big about life at the smallest scale

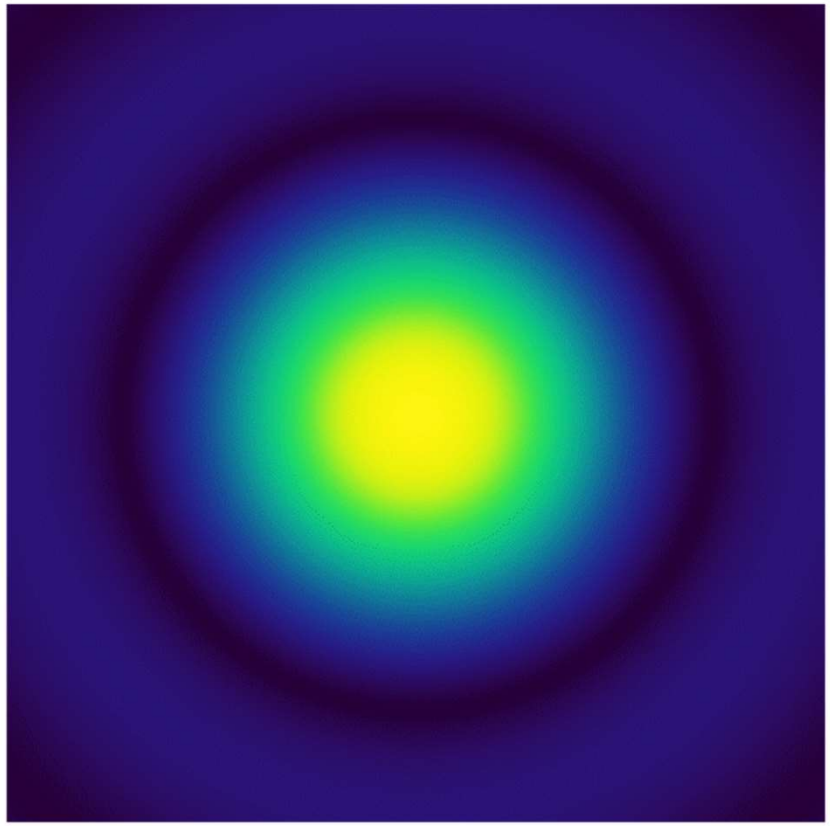
Towards automated serial electron diffraction for macromolecular crystallography

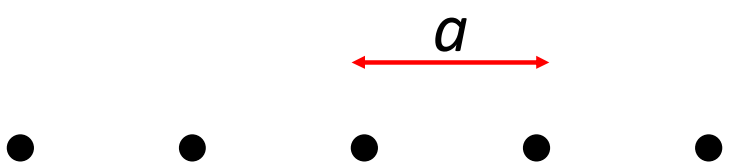
Stef Smeets

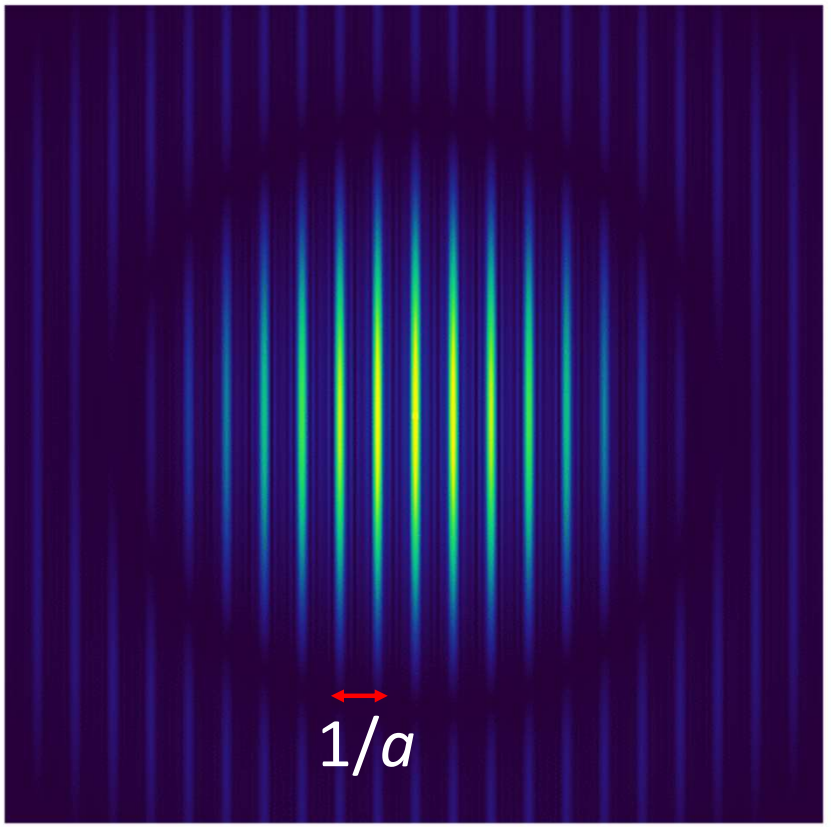
Kavli Institute of Nanoscience Delft

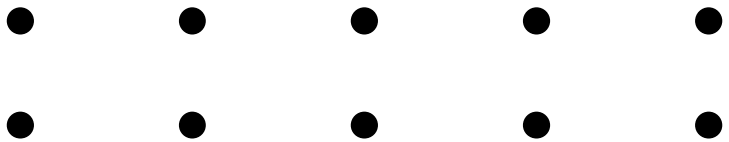
TU Delft

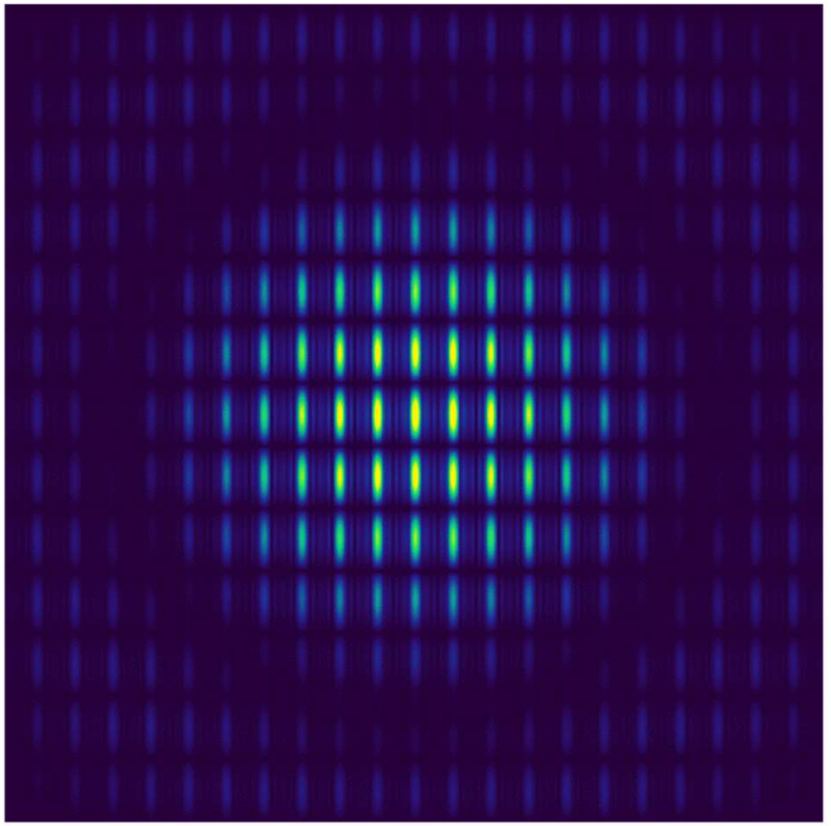
- Fourier Transform

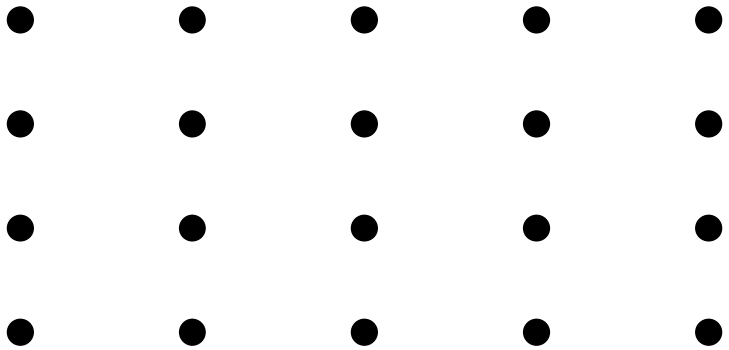


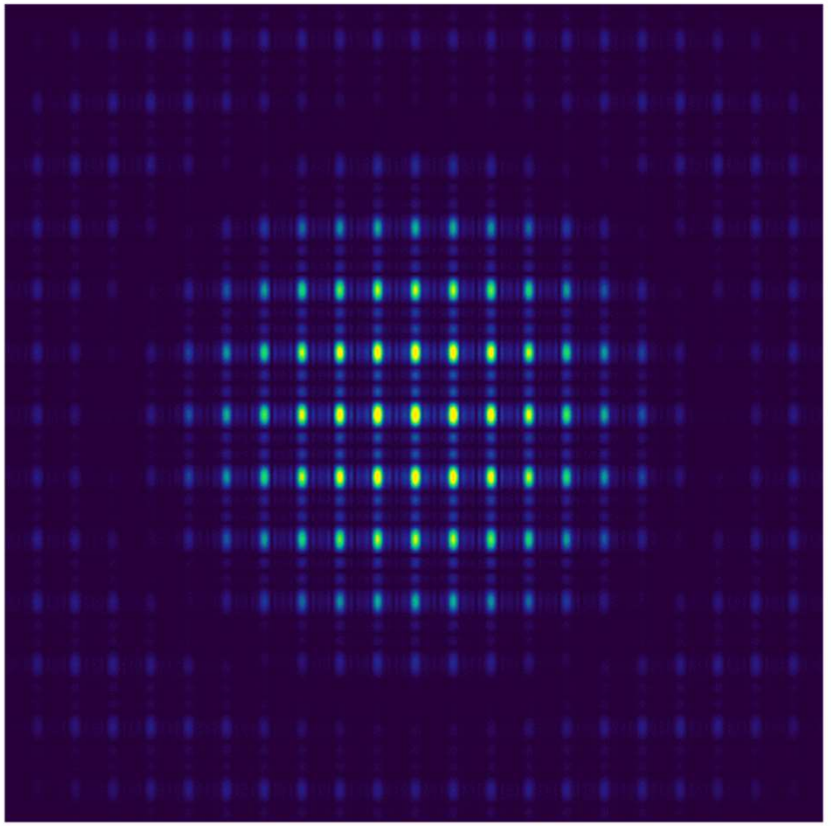

a

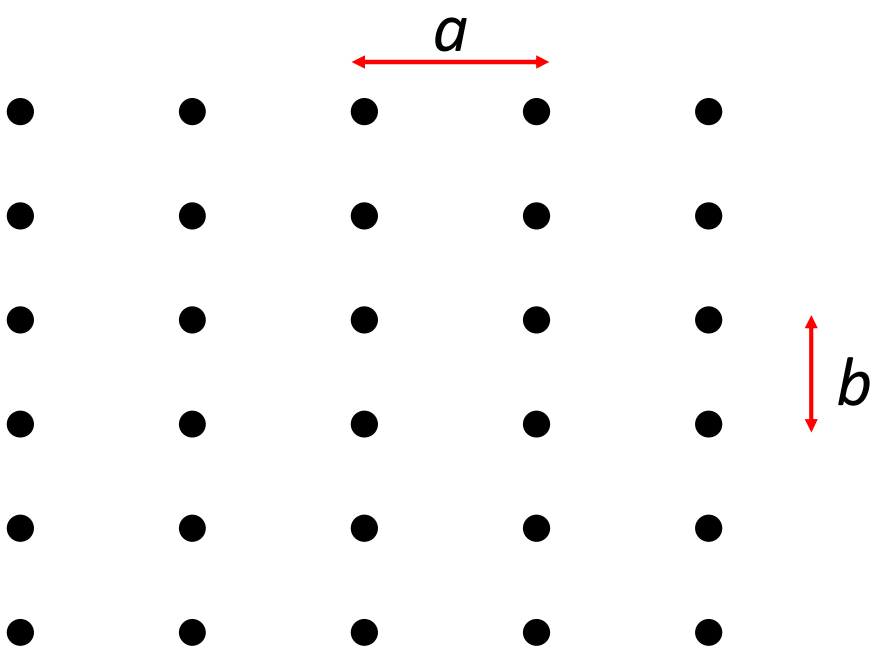

1/a

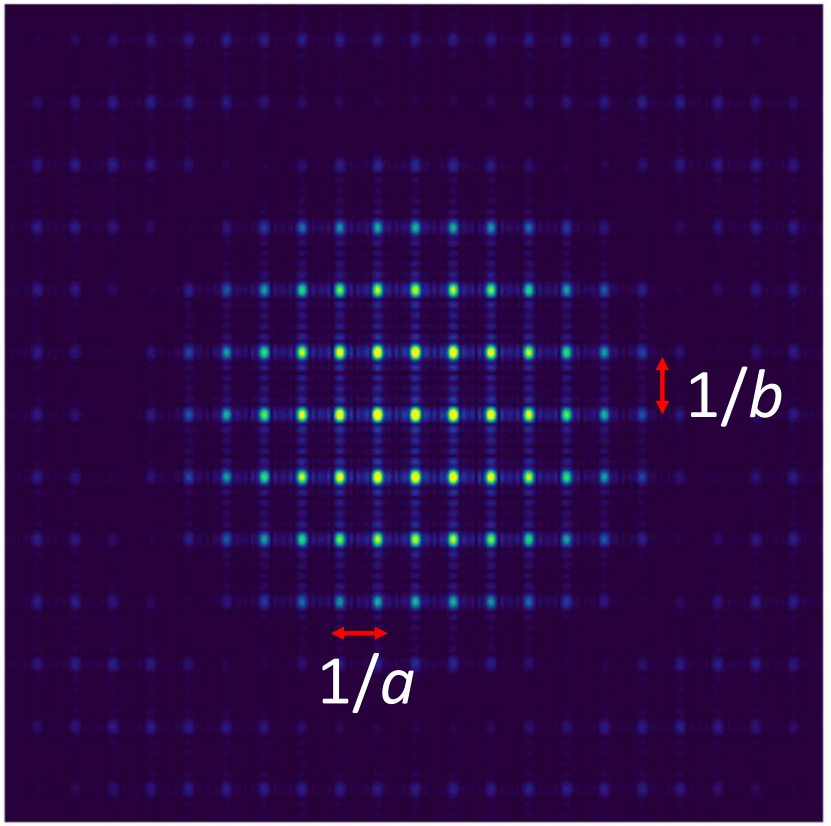


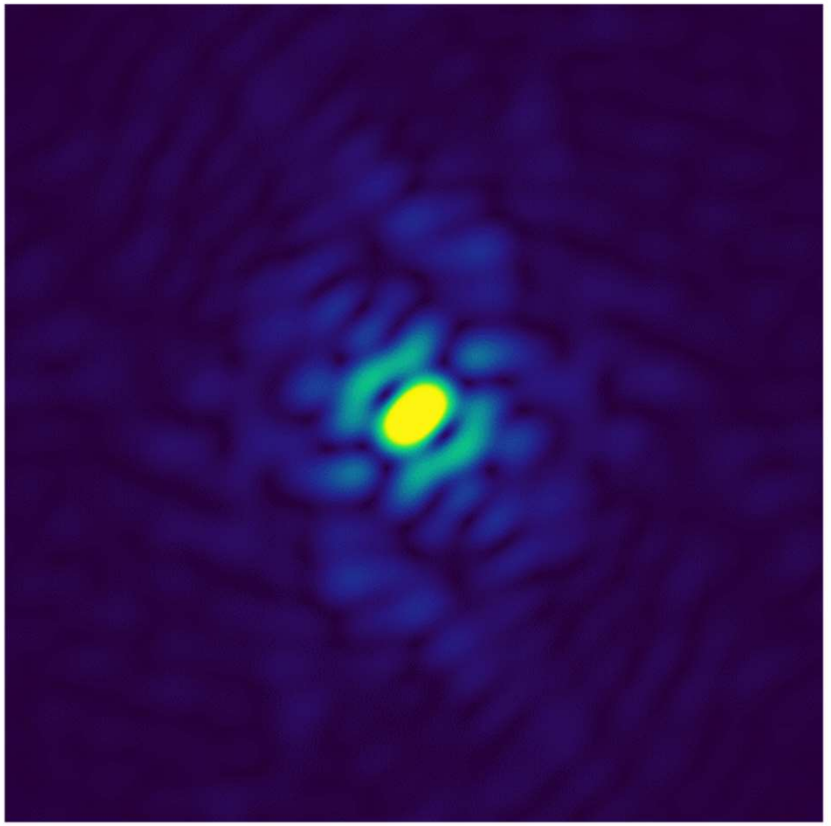




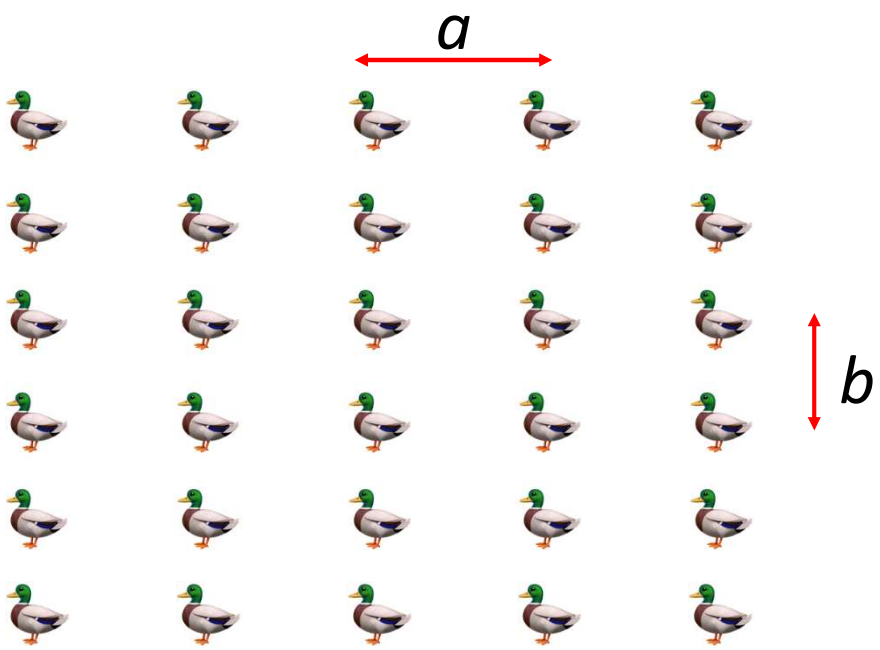


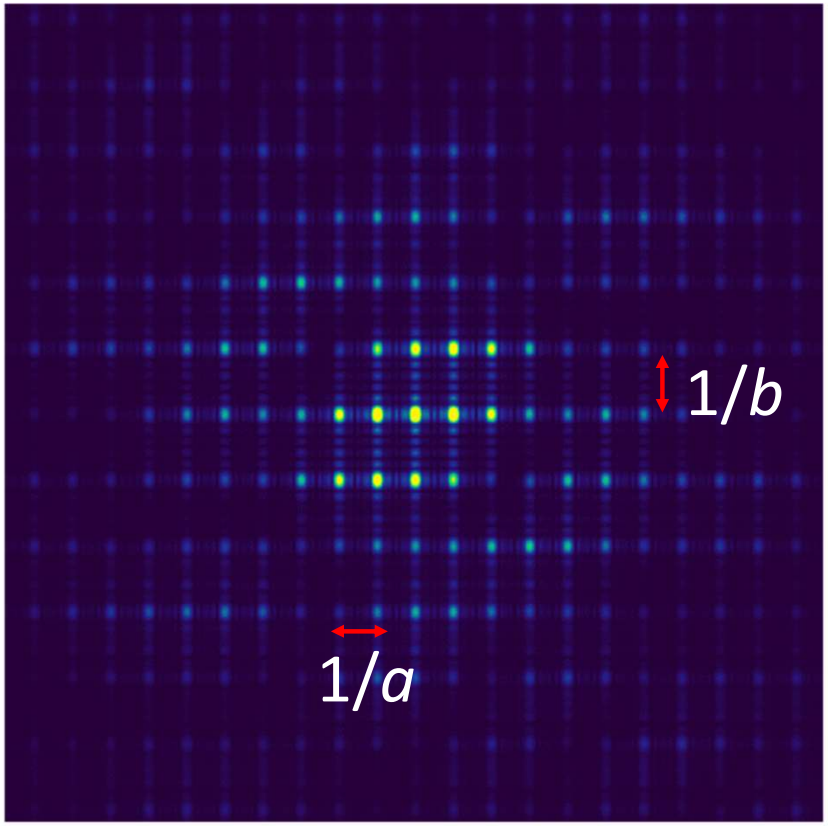

a
b

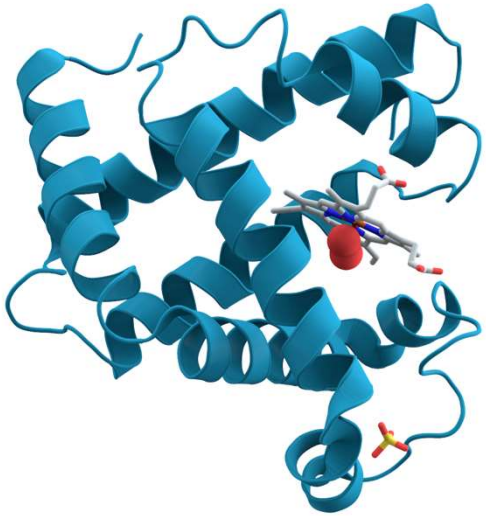

1/b
1/a



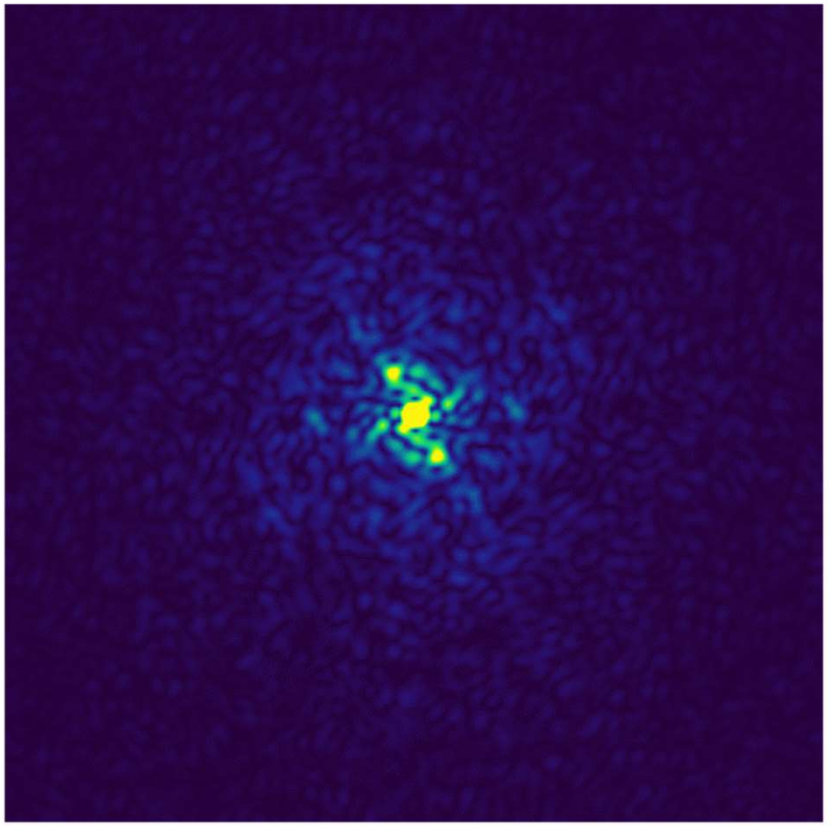
duck

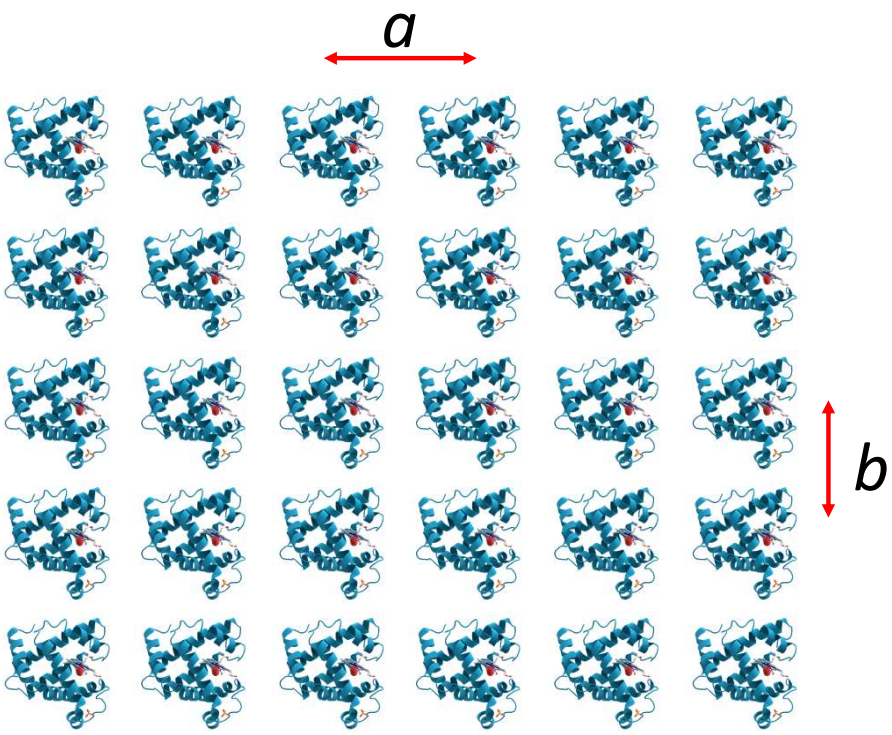

a
b

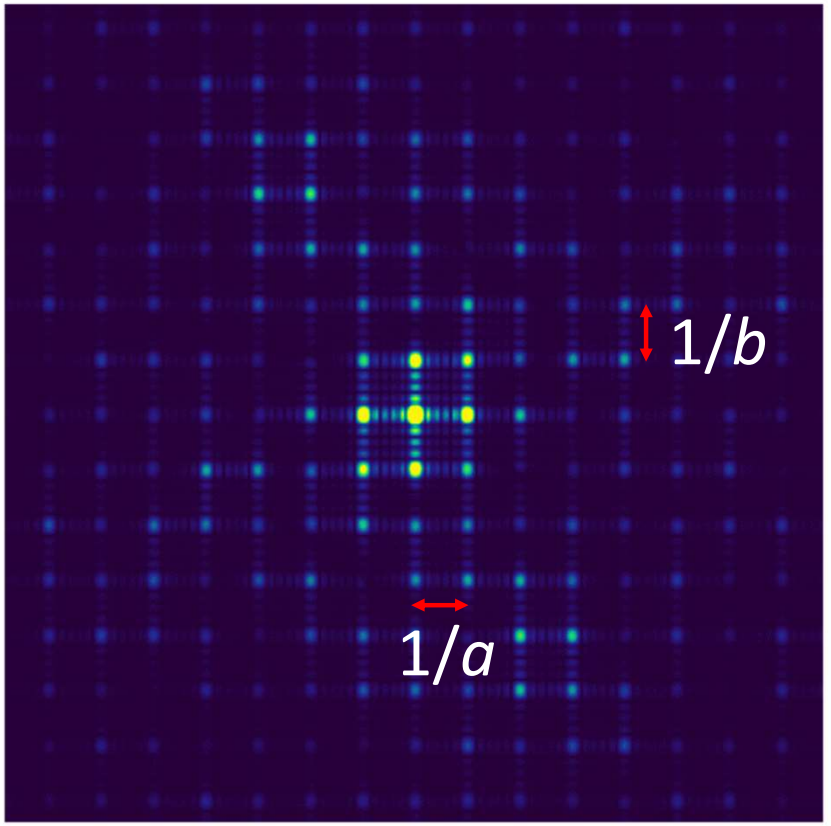

1/b
1/a

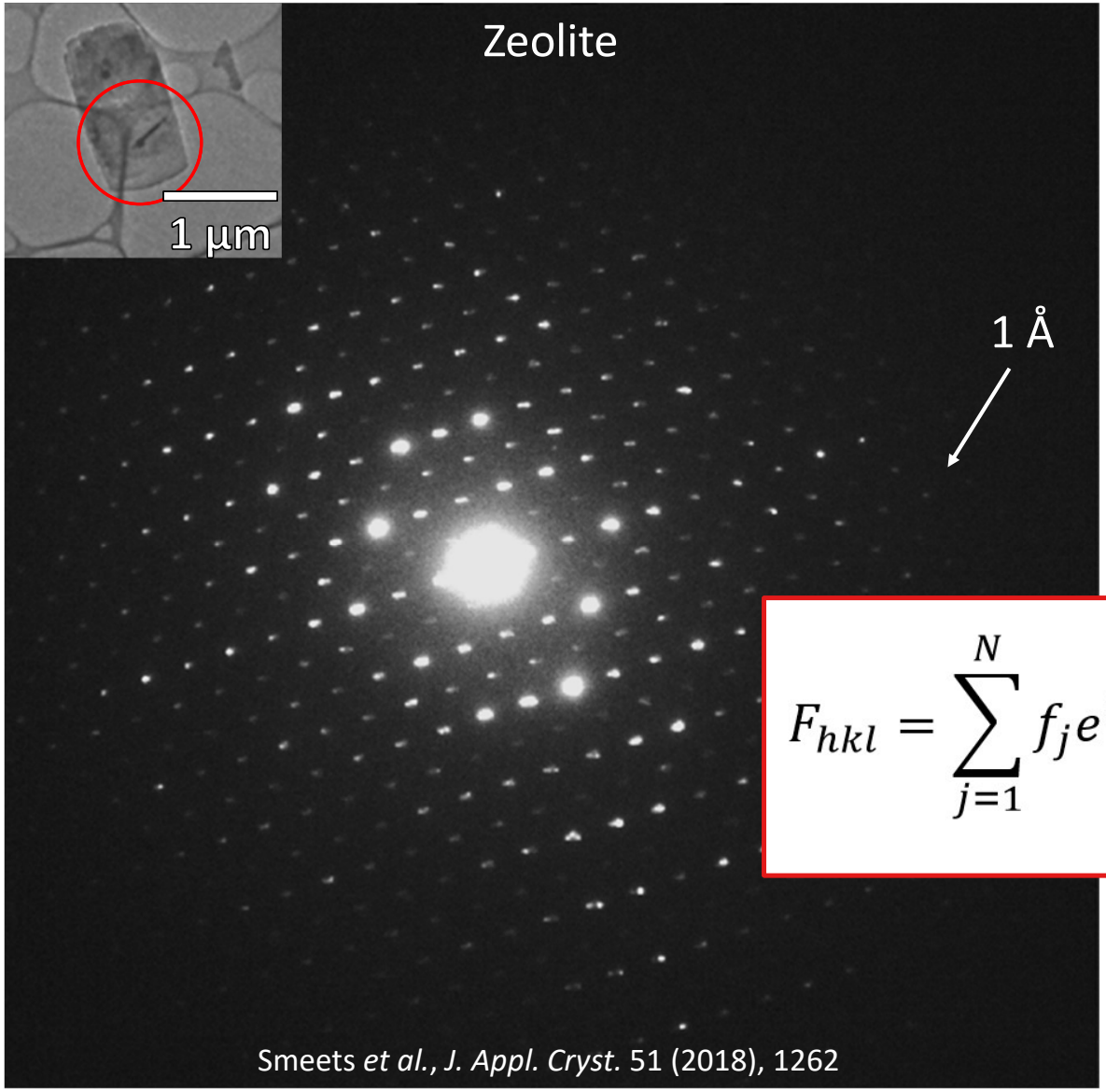


myoglobin

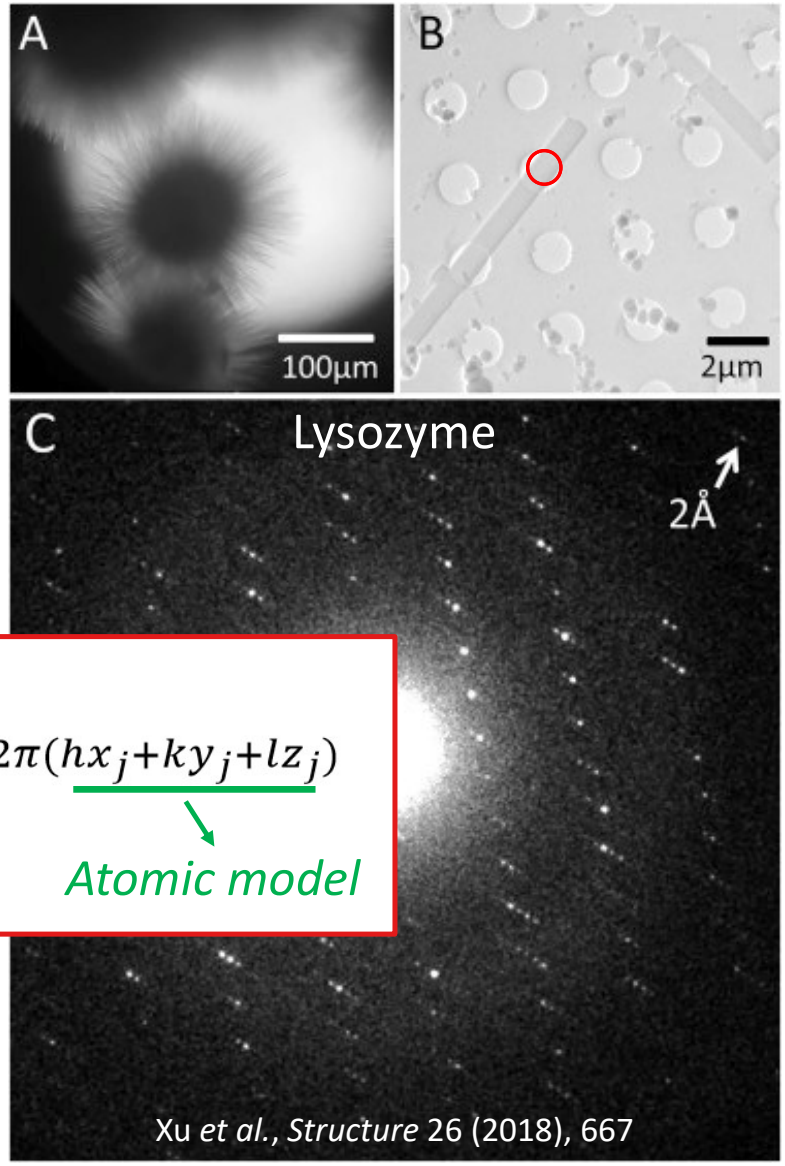



a
b


1/b
1/a



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

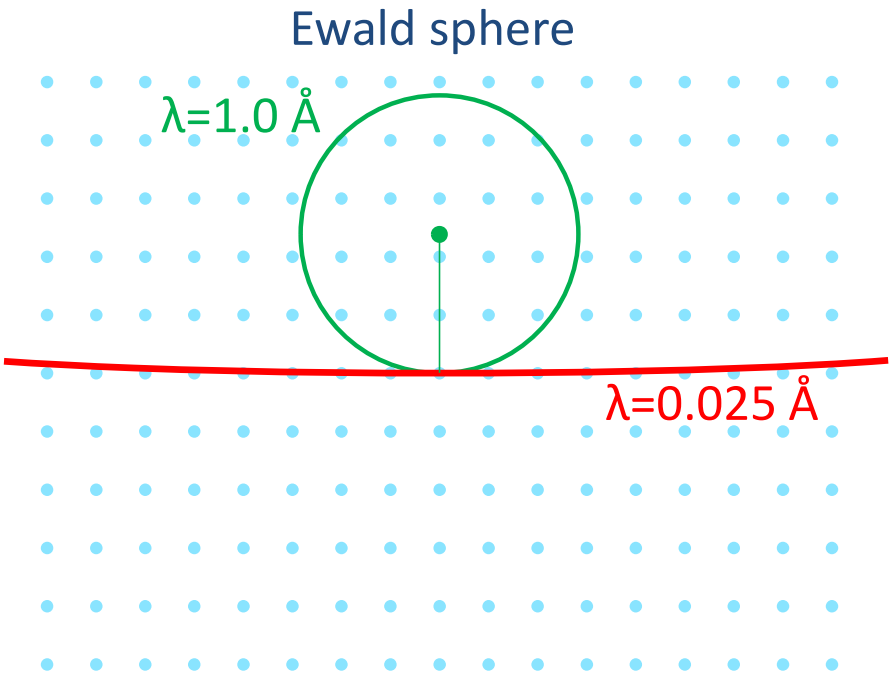


Xu *et al.*, *Structure* 26 (2018), 667

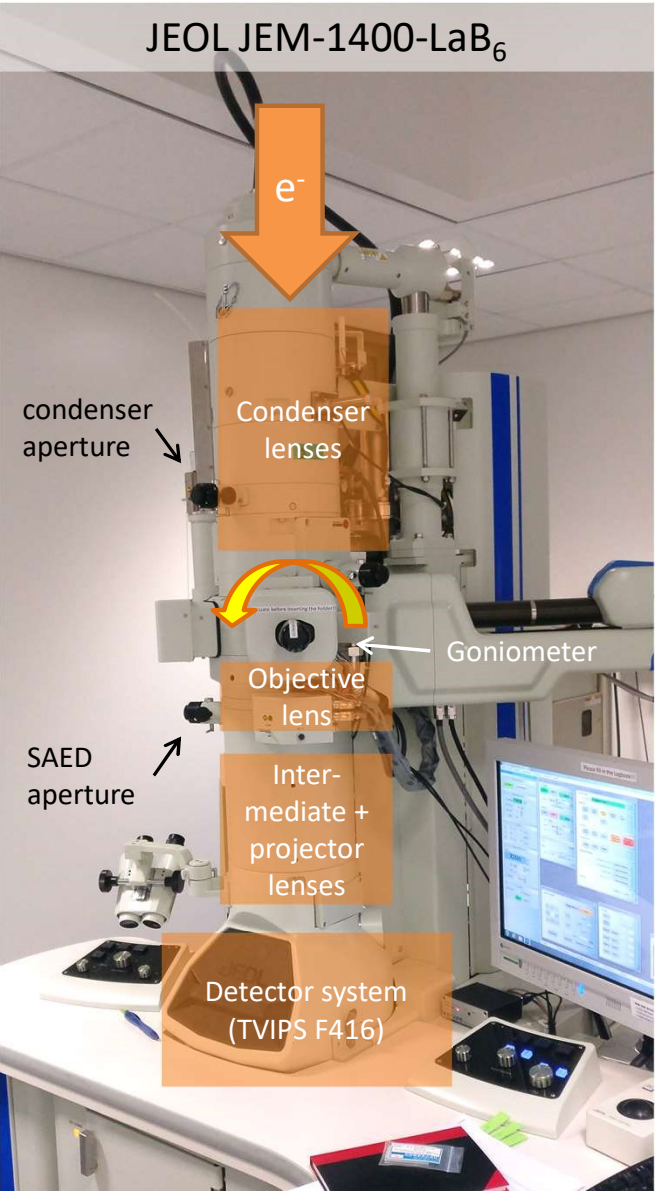
$$F_{hkl} = \sum_{j=1}^{N} f_j e^{i2\pi(hx_j+ky_j+lz_j)}$$

Atomic model

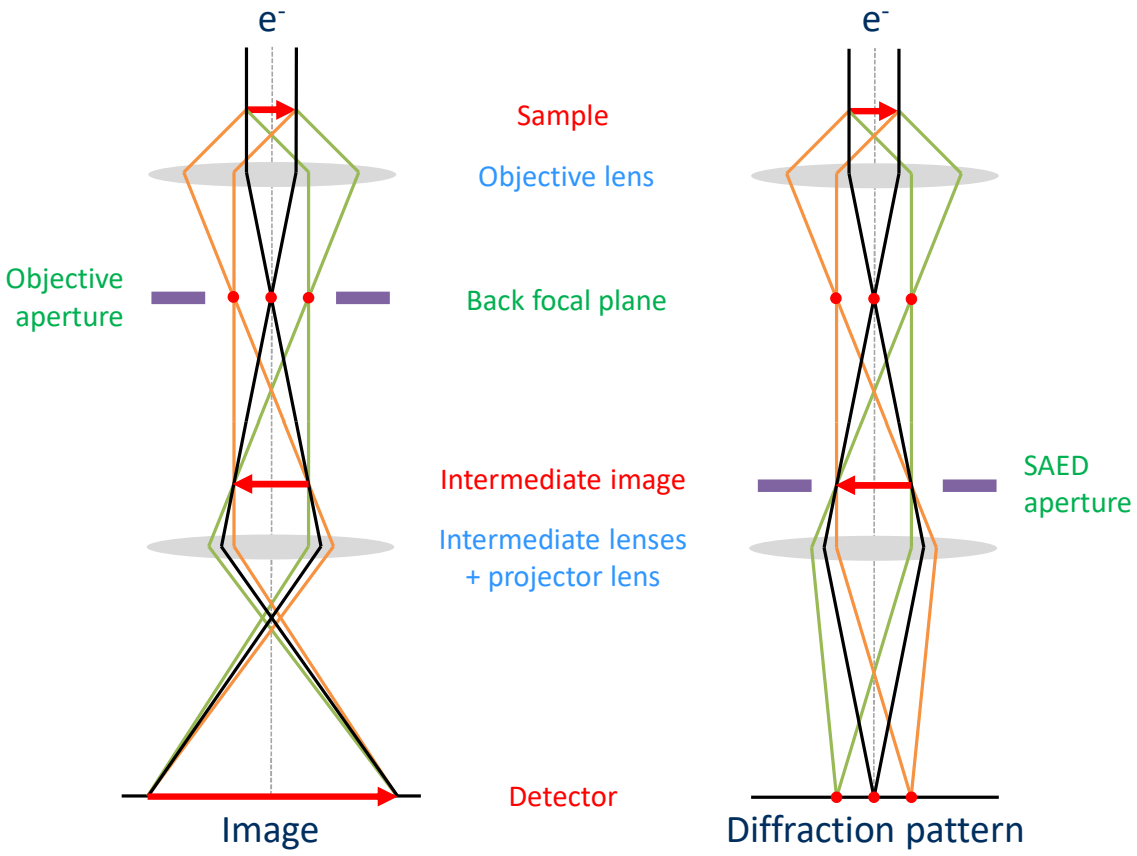
Electrons as a radiation source



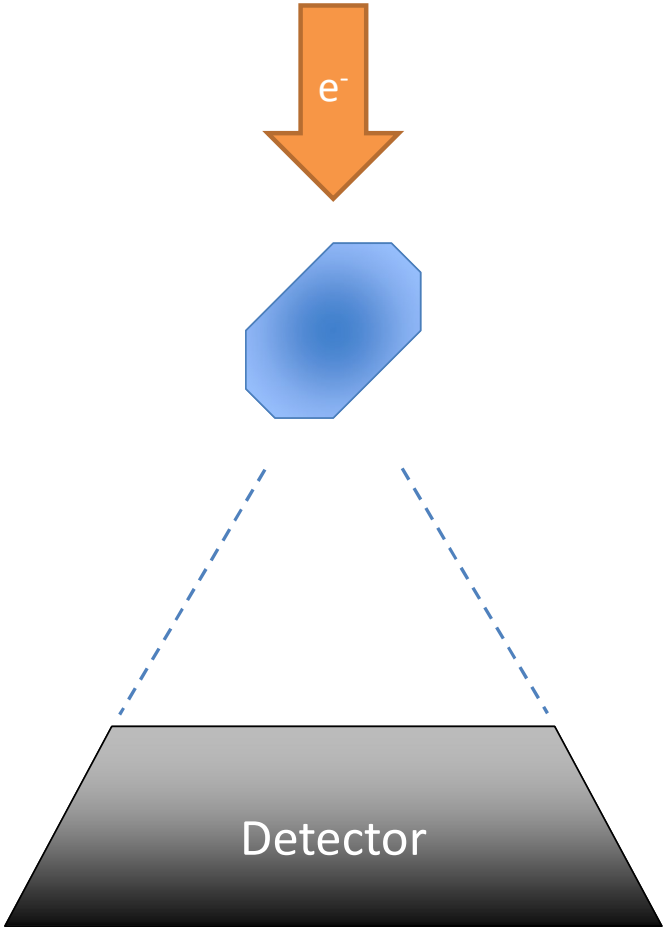
- Accelerating voltage: 100 to 300 keV
- Wavelength: 0.0251 Å @ 200 keV
- Probe electrostatic potential
- Strong interaction (10^6 stronger than X-rays)
- Require small samples (< 1 µm)
- High vacuum (<10^{-3} mbar)



Electron ‘diffractometer’

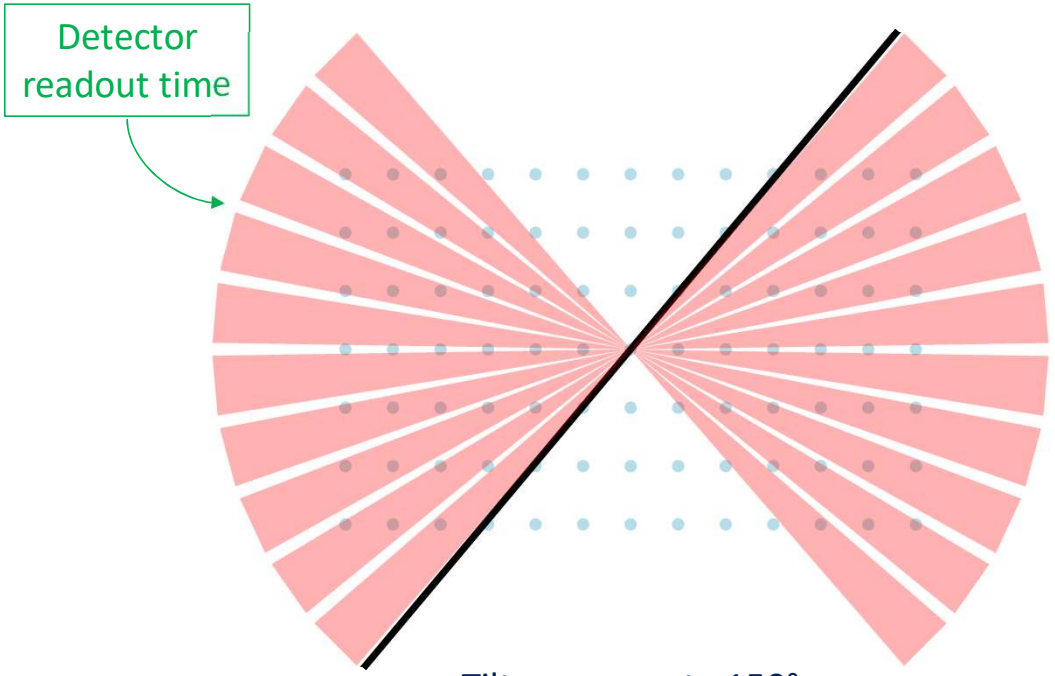


3D Electron diffraction



Continuous rotation method

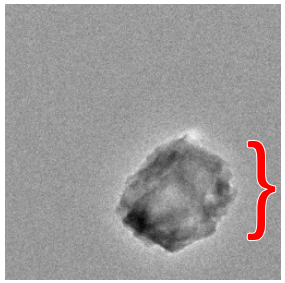
Nederlof *et al.*, *Acta Cryst. D* (2013), 69:1223
Nannenga *et al.*, *Nat. Methods* (2014), 11:927
Gemmi *et al.*, *J. Appl. Cryst.* (2015), 48:718
Cichocka *et al.*, *J. Appl. Cryst.* (2018), 51:1652

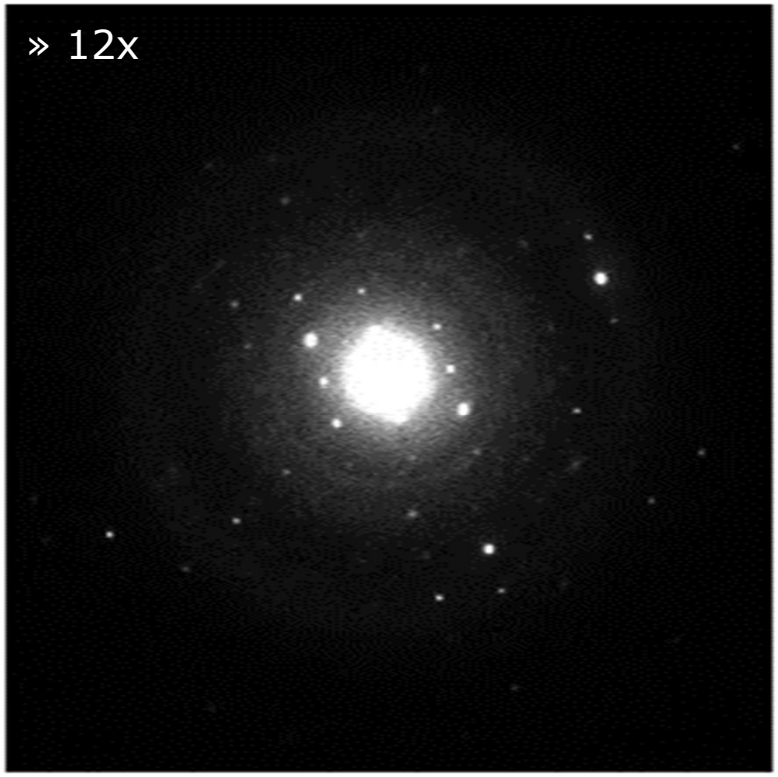


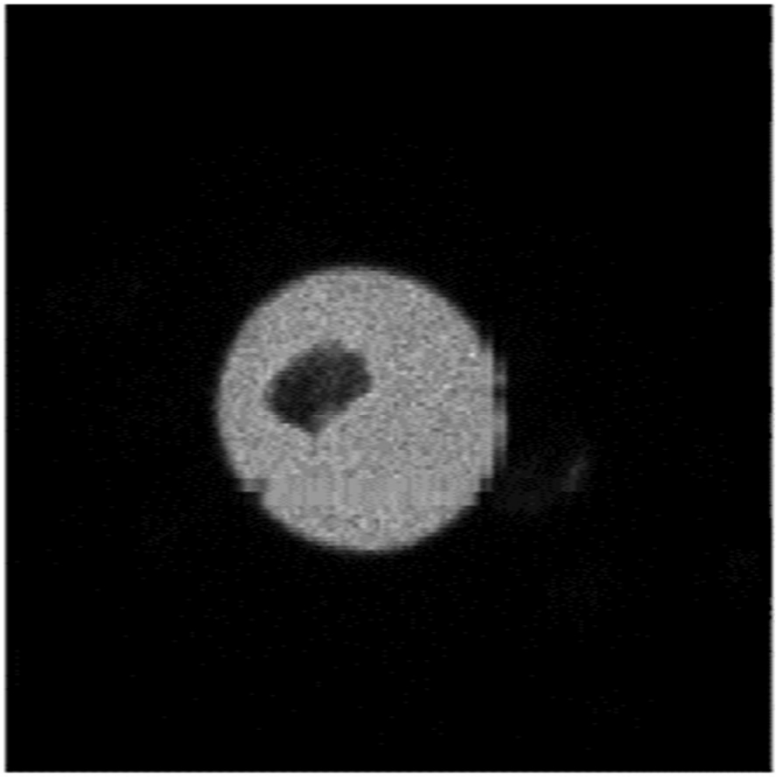
Tilt range: up to 150°
Oscillation angle: 0.1-0.5°
Rotation speed: 0.5-2.0°/s
<5 minutes

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)



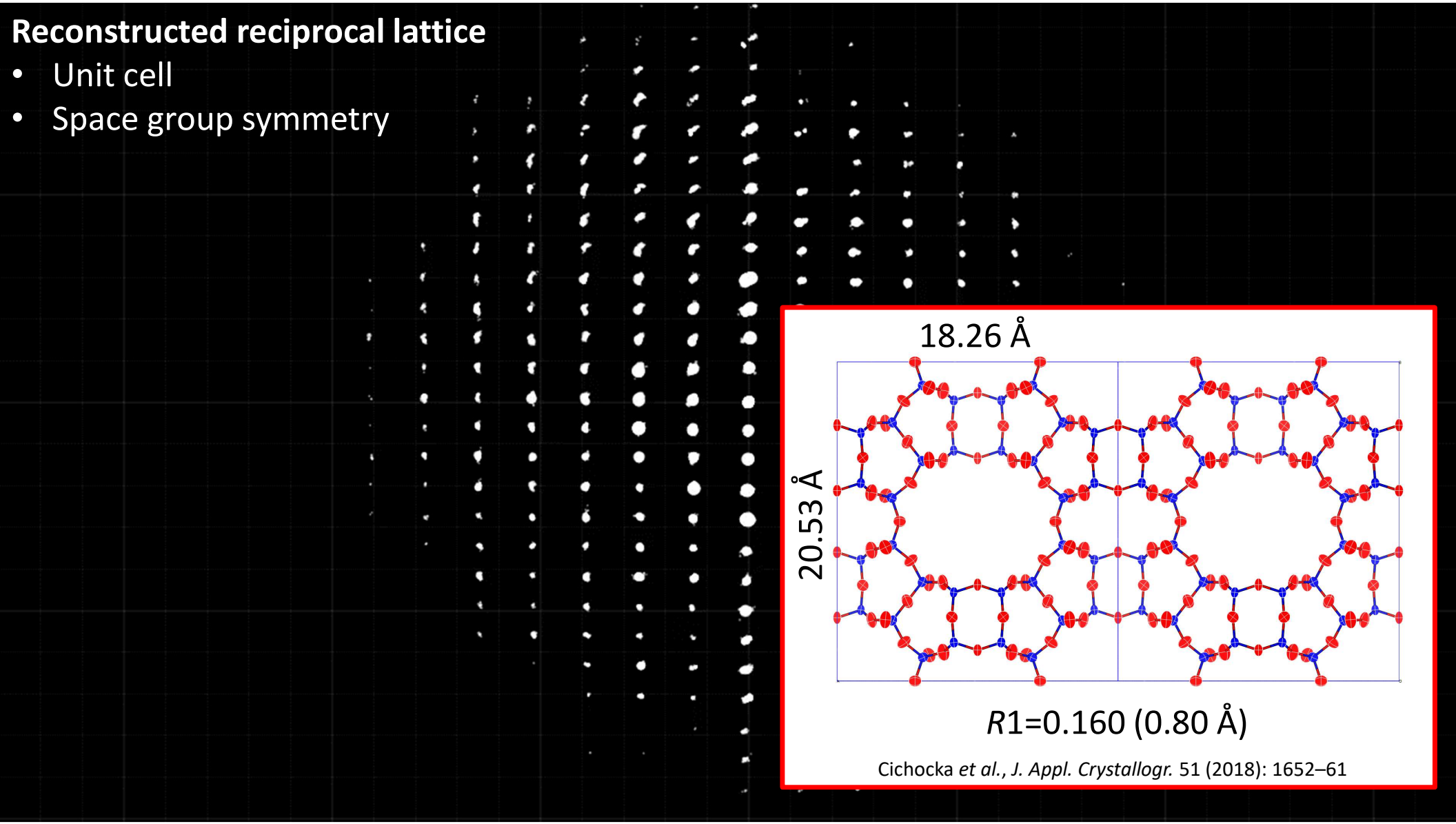




Cichocka *et al.*, *J. Appl. Crystallogr.* 51 (2018), 1652

Reconstructed reciprocal lattice

- Unit cell
- Space group symmetry

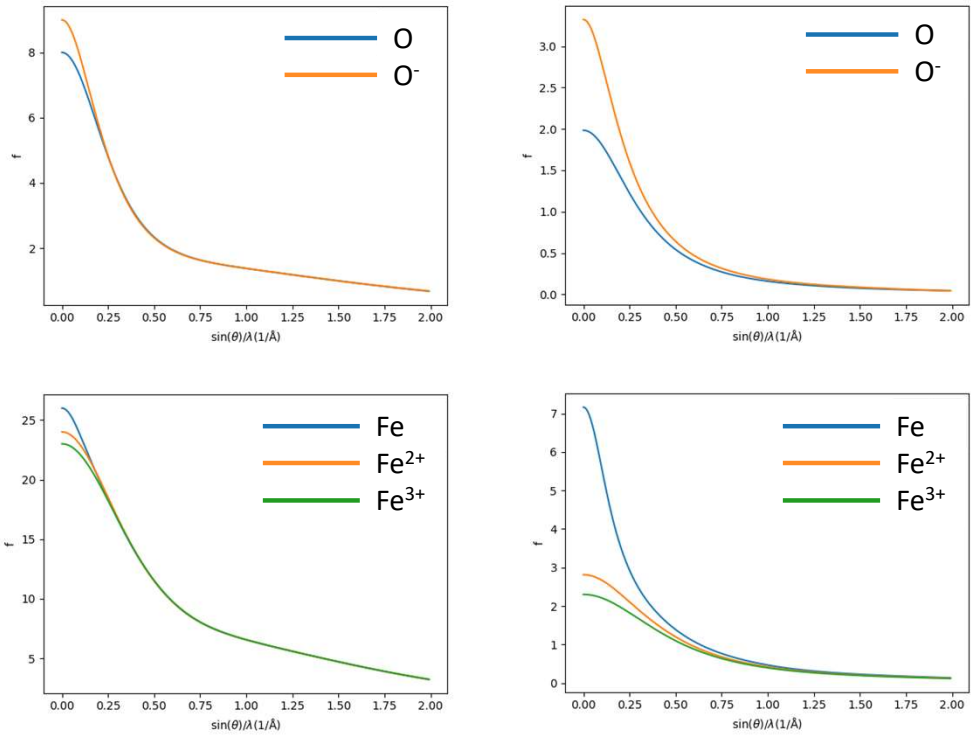


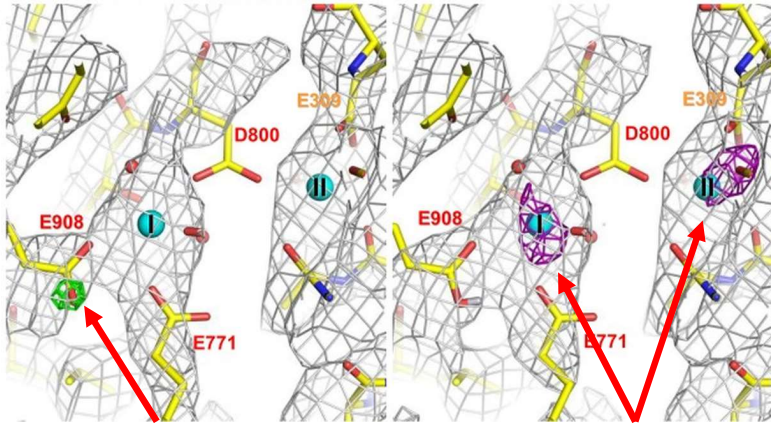
Cichocka *et al.*, *J. Appl. Crystallogr.* 51 (2018): 1652–61

Determine charge states

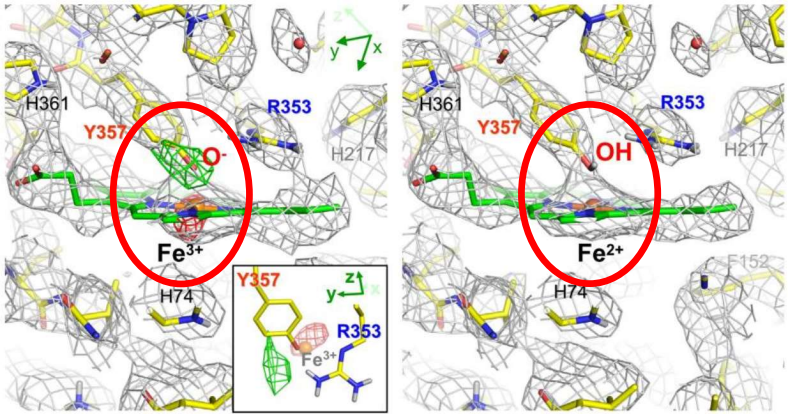
Enhanced constrast in scattering factors

X-rays → Electrons





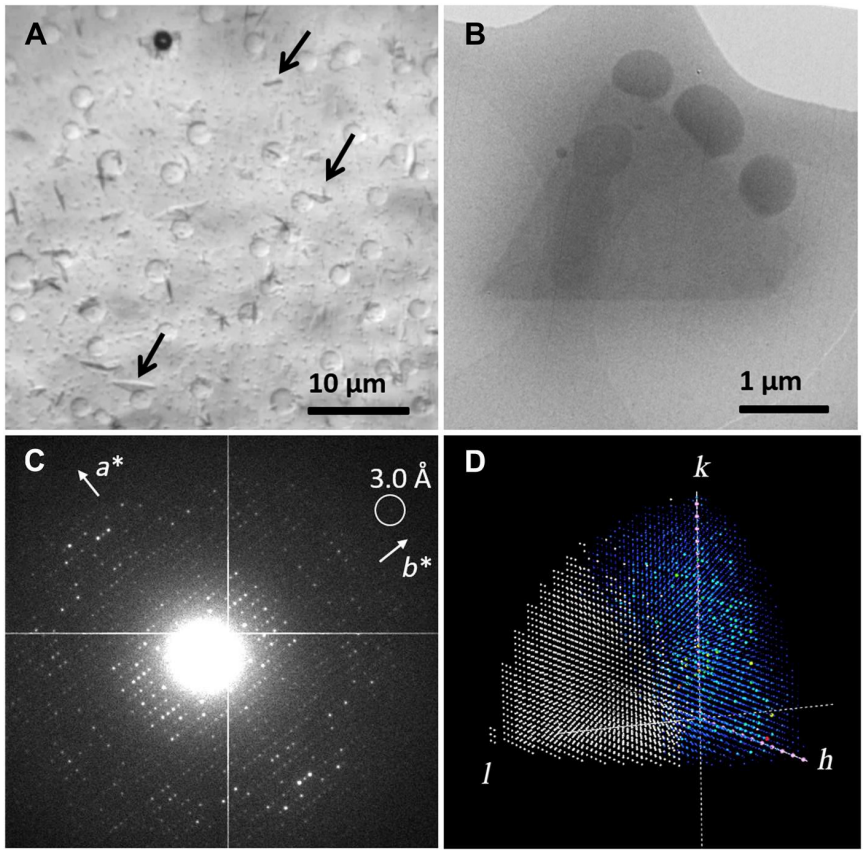
H+ ATPase Ca2+



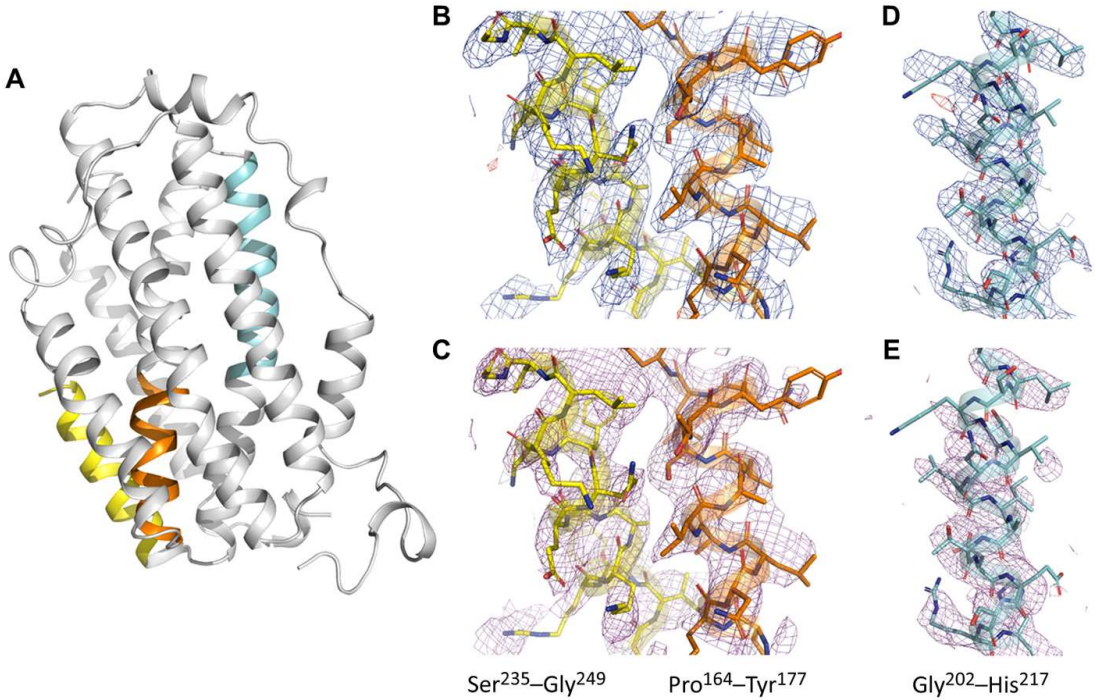
Catalase

Yonekura *et al.*, *PNAS* (2015), 112(11):3368

Structure determination of R2lox using ED



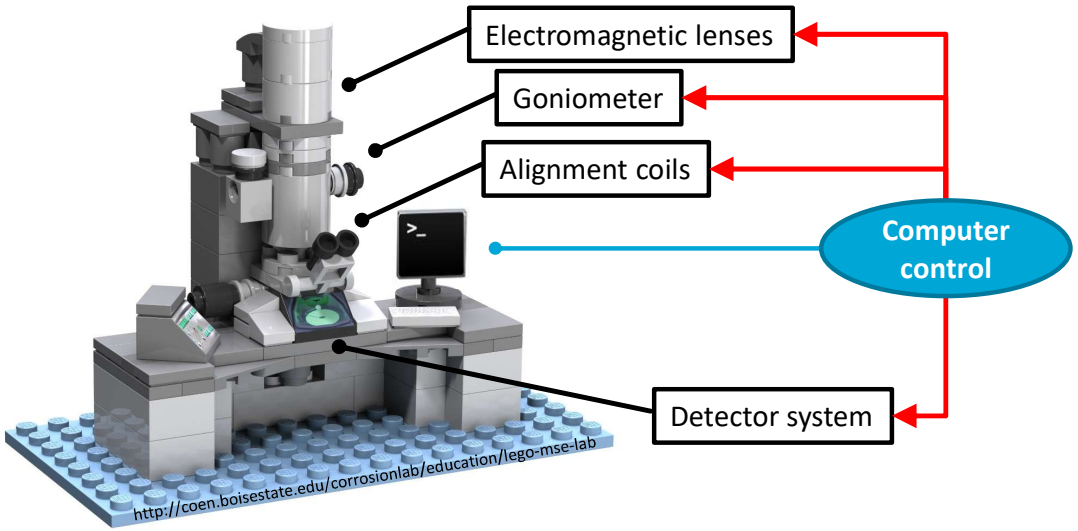
Xu *et al.*, *Sci. Adv.* (2019), 5(8):eaax4621

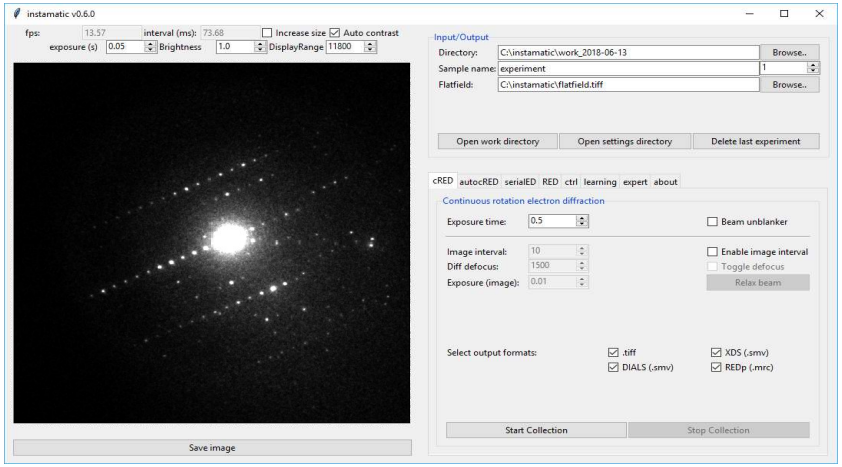


Merge 21 crystals
Solved with molecular replacement
Resolution: 3.0 Å
Completeness: 62.8 %

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





- Modular GUI
- Crystal finder/tracking
- Neural network
- Calibrations/alignments
- Automated experiments
- SerialEM integration

Instamatic **(Python3.6+)**

Serial rotation electron diffraction

Serial electron diffraction

Continuous rotation electron diffraction

Data reduction server

- .tiff (patterns + images)
- *REDp* (.ed3d, .mrc)
- *XDS* (XDS.INP, .smv)
- DIALS (.bat files)

- .hkl files
- Phase analysis
- Input files

Microscope control

TFS Titan/Themis Z

JEOL 1400/2100/3200

Simulated

Camera interface

ASI Cheetah

Gatan Orius

Simulated

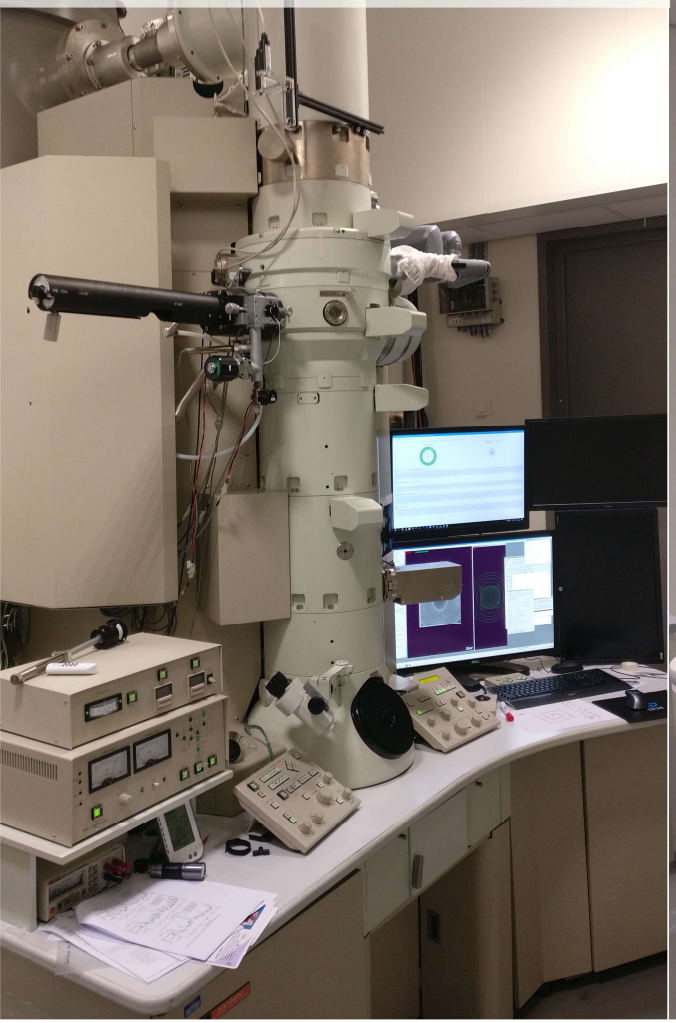
TVIPS (X)F416

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

JEOL JEM-3200FSC (FEG)

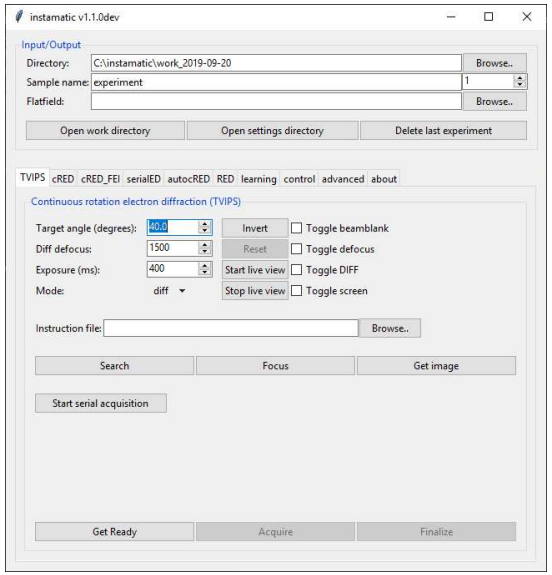
Gatan K2

~~TVIPS XF416~~



JEOL JEM-1400 (LaB_6)

TVIPS F416

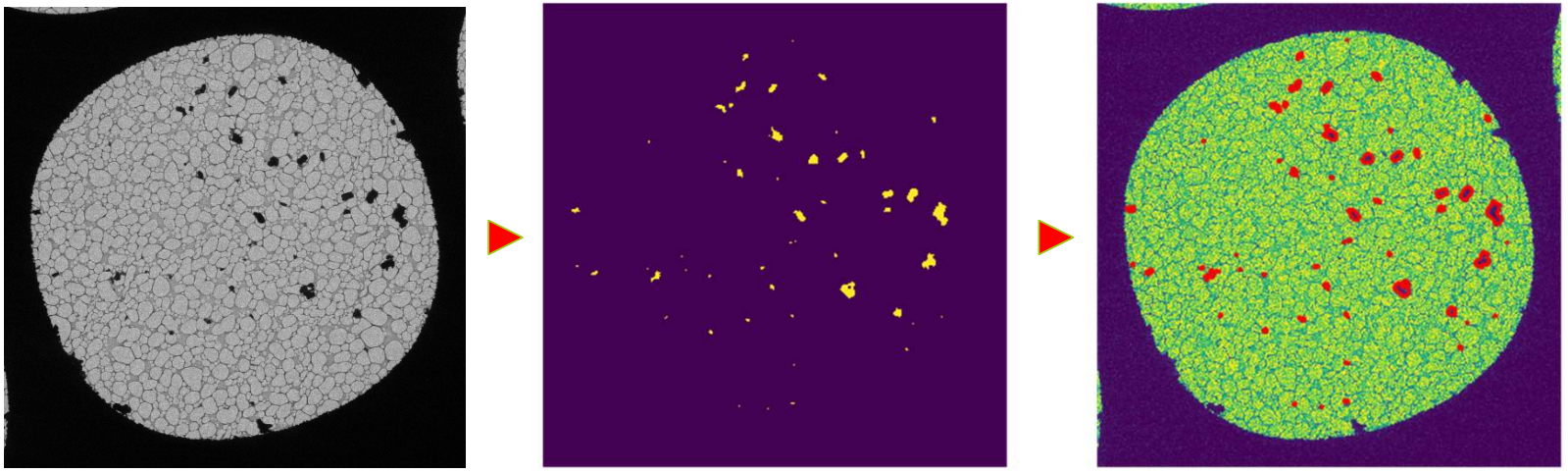


TVIPS (X)F416

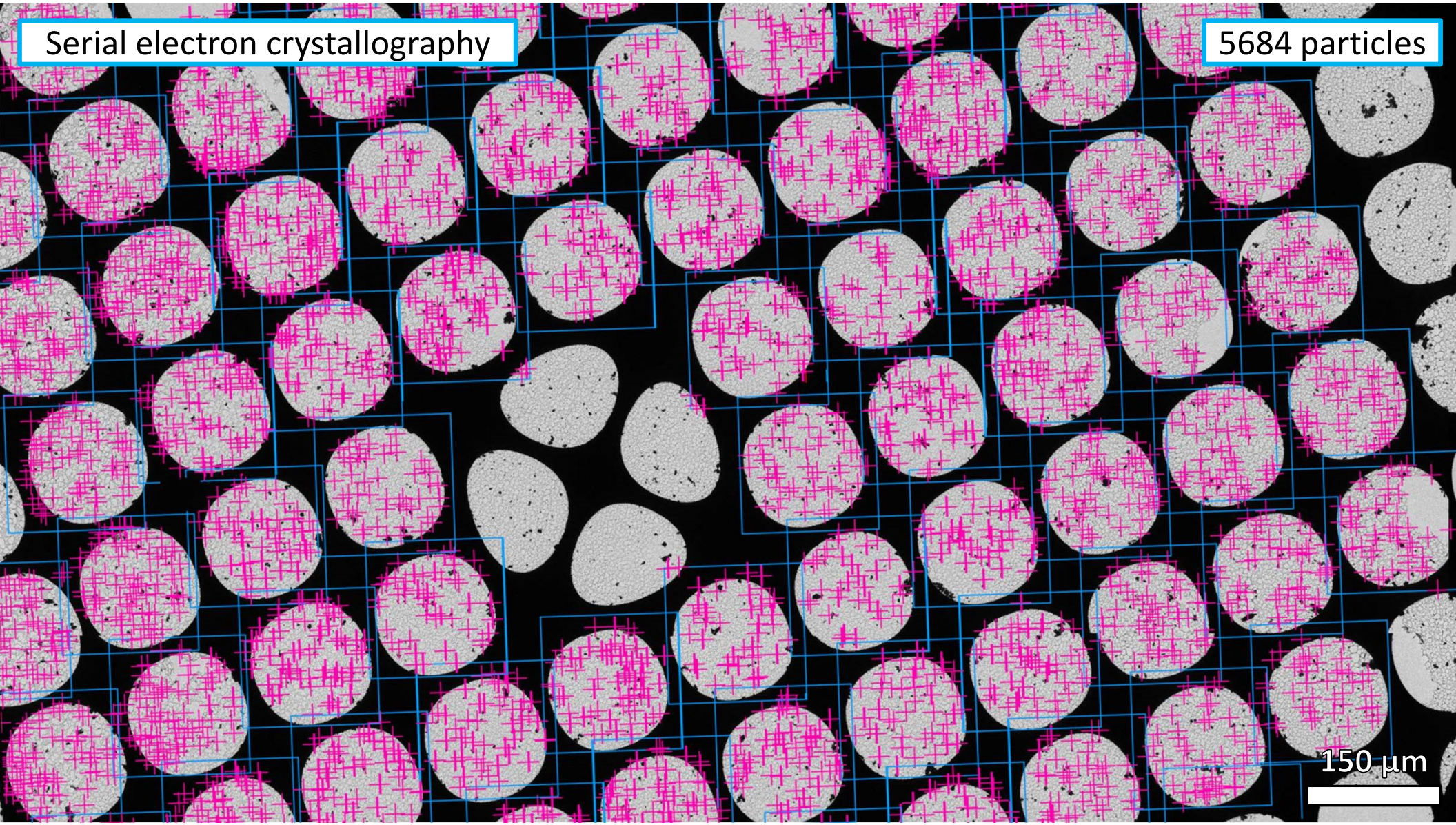
Serial electron crystallography

1. Global map (SerialEM)
2. Medium mag map (roi)
3. Segmentation
4. Data acquisition (instamatic)

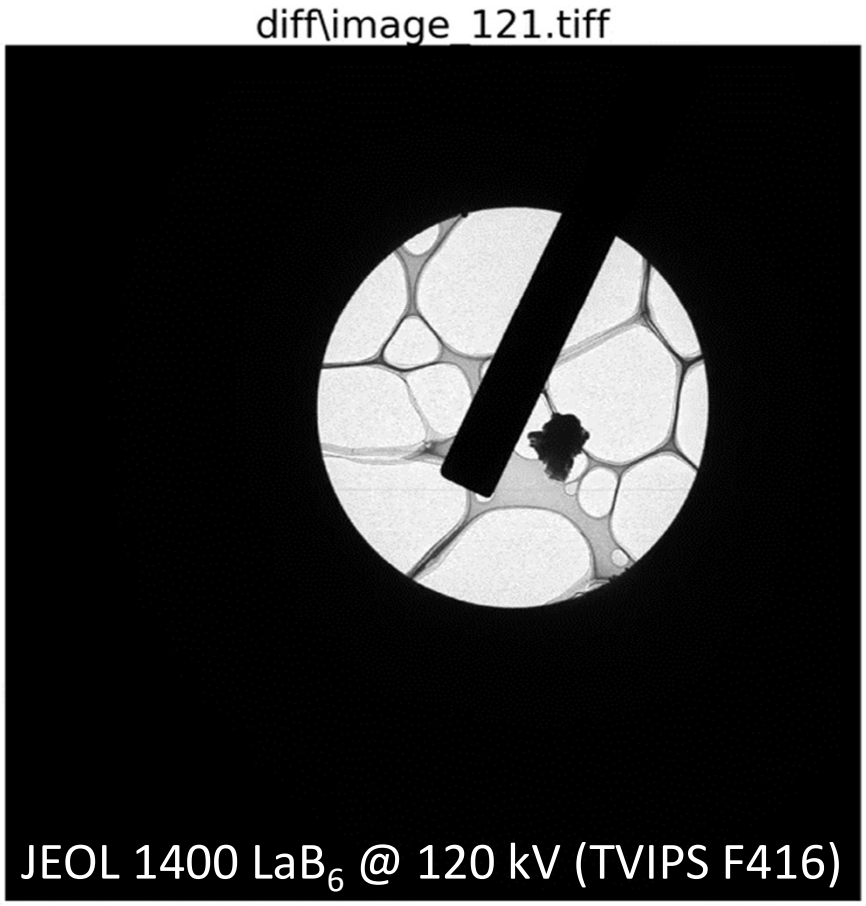
Segmentation



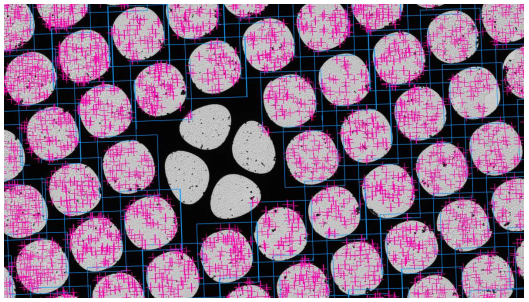
150 µm


Serial electron crystallography
5684 particles
150 µm

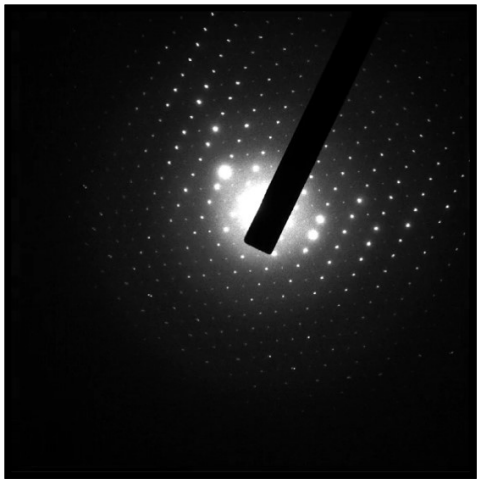
SerialED data collection (Zeolite SSZ-45)



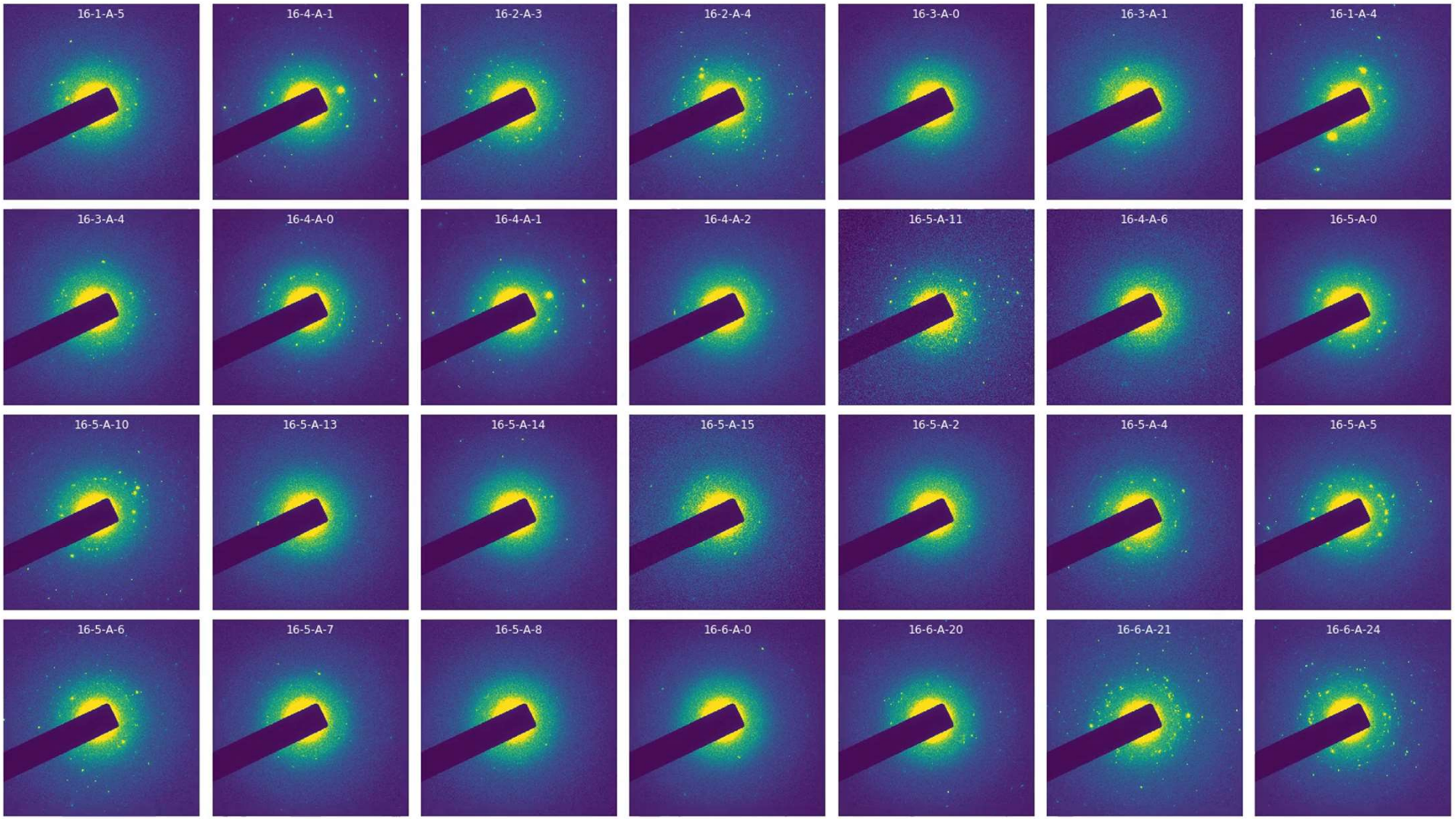
Python (instamatic)



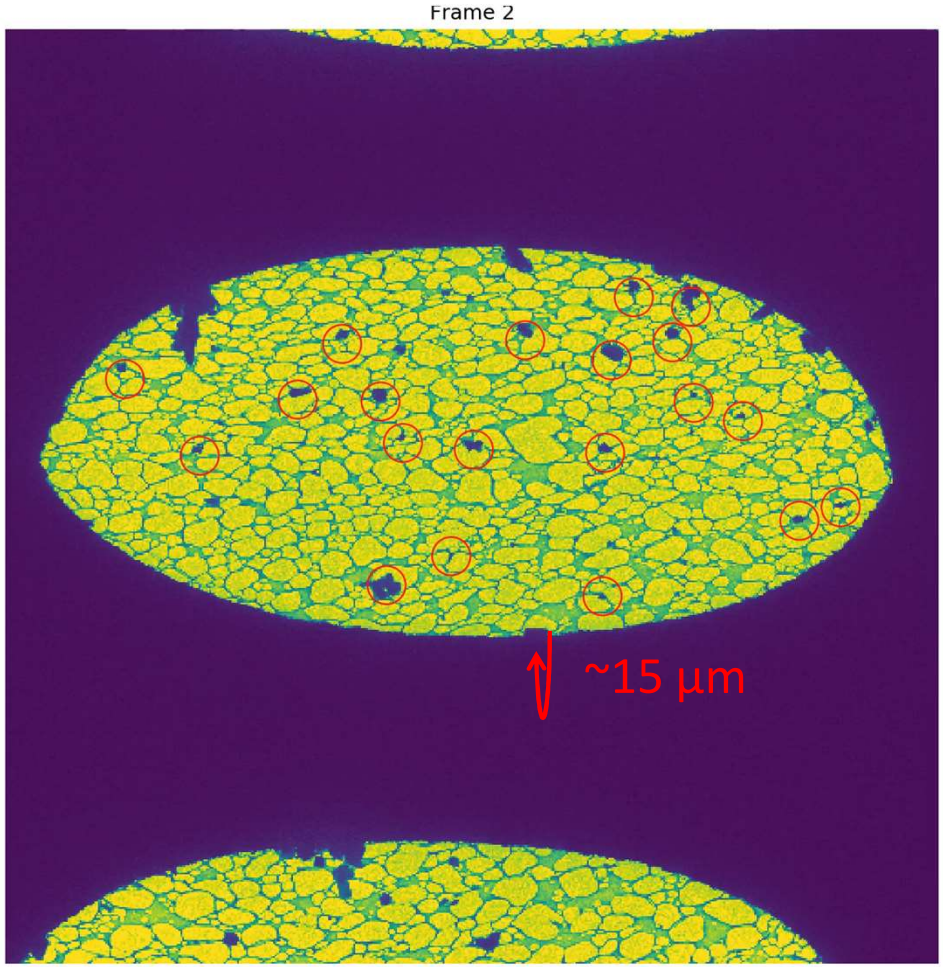
```python
from instamatic import TEMController
from instamatic.formats import write_tiff
from instamatic.serialem import read_nav_file

ctrl = TEMController.initialize()

markers = read_nav_file("nav.nav", acquire_only=True)

for i, marker in enumerate(markers):
    ctrl.stageposition.set_xy_with_backlash_correction(x=marker.stage_x,
                                                       y=marker.stage_y)

    img, h = ctrl.getImage(exposure=0.2)
    write_tiff(f"sed_diff_{i:4d}.tiff", img, header=h)

    ctrl.difffocus.defocus(offset=1500)

    img, h = ctrl.getImage(exposure=0.2)
    write_tiff(f"sed_image_{i:4d}.tiff", img, header=h)

    ctrl.difffocus.refocus()
```

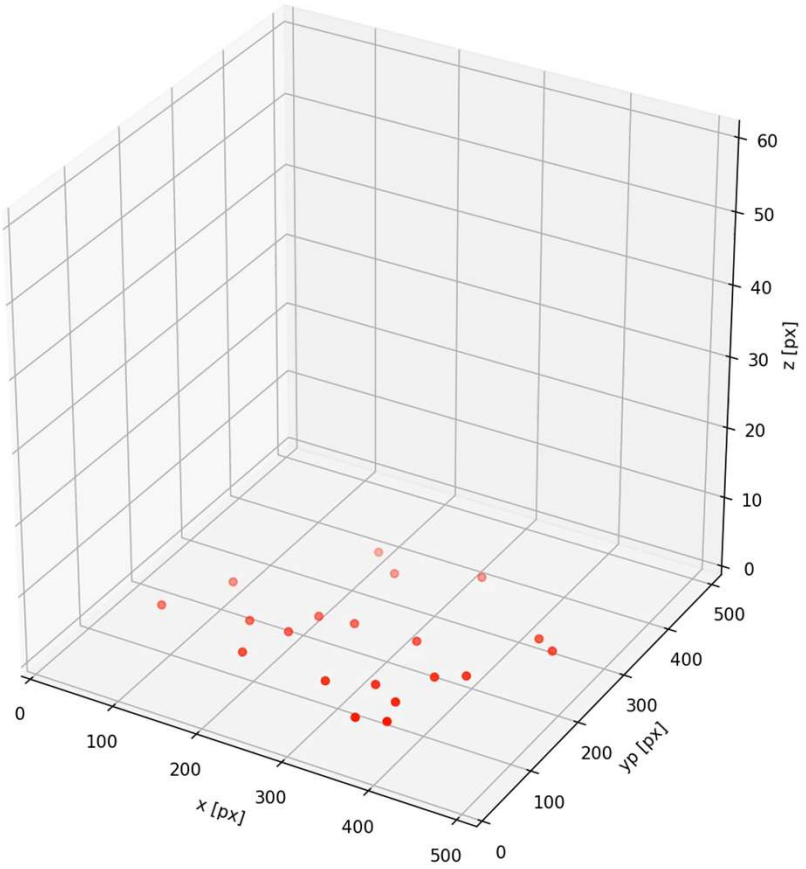


28 crystals rotated from -15 to 15°

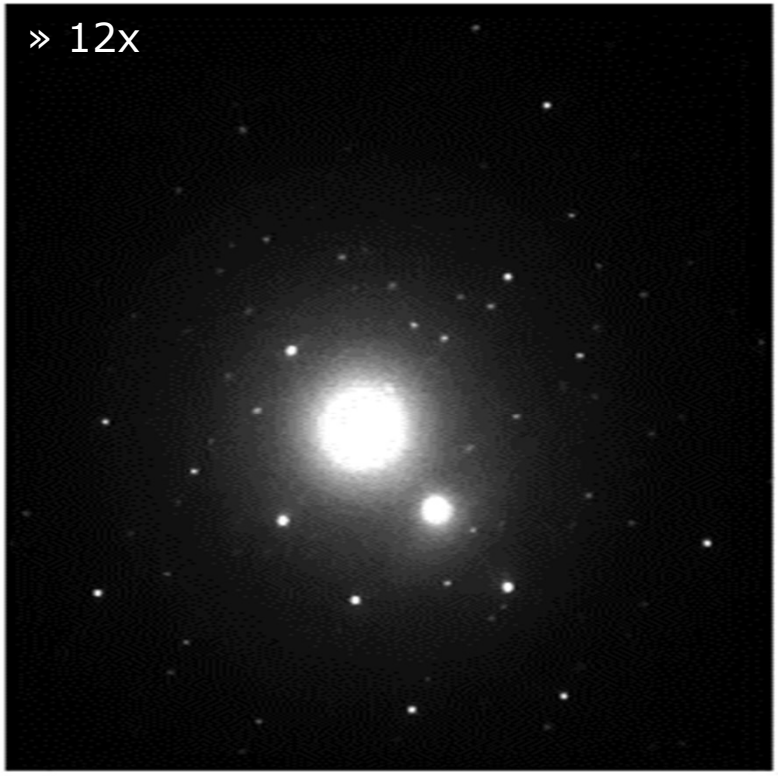


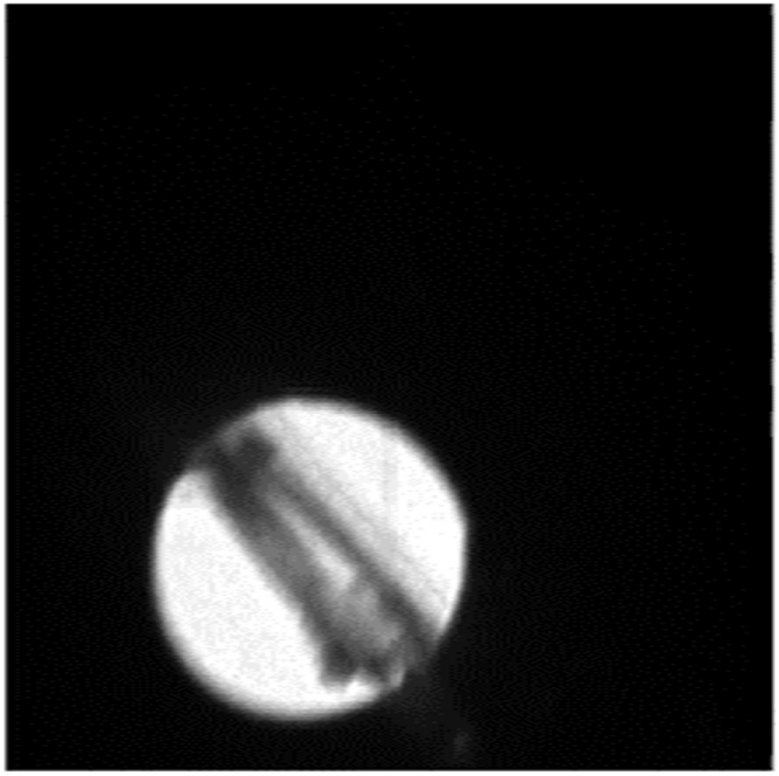
The crystal tracking problem





Automated crystal tracking via beam shift



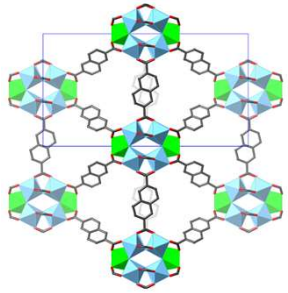


JEOL 2100-LaB_6 @ 200 kV (Timepix)
Rotation: -44.0 to 47.4° @ 0.76°/s (91.4°)
Exposure: 0.5 s, oscillation angle: 0.39°

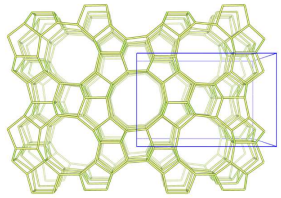
Wang *et al.*, *IUCrJ* 6 (2019), 854-867

Serial rotation electron diffraction

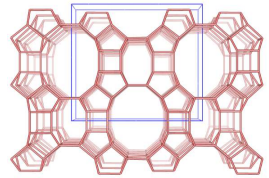
Sample	ZSM-5	PCN-416	ZSM-5 + mordenite	PST-20 + ZSM-25
Data collection time	6 h	2 h	2 h	4 h
Rotation (mean) /°	11.9	4.0	16.3	16.1
Rotation (max) /°	76.2	44.4	73.6	78.5
# crystals	250	139	123	148
# data sets > 5°	126	66	89	99
# data sets > 20°	43	15	33	42
# indexed data sets	47	27	42/11	31/19
Resolution /Å	0.77	0.90	0.76/0.81	1.46/1.46



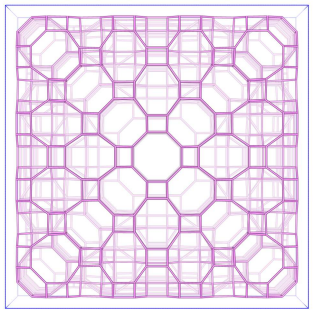
PCN-416 (100)



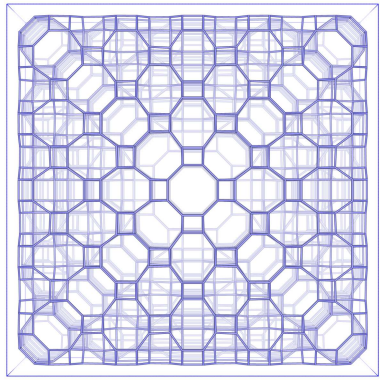
ZSM-5



Mordenite



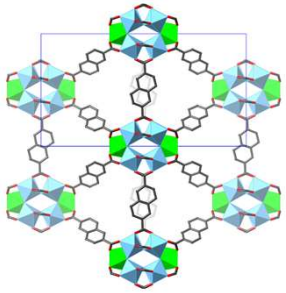
ZSM-25



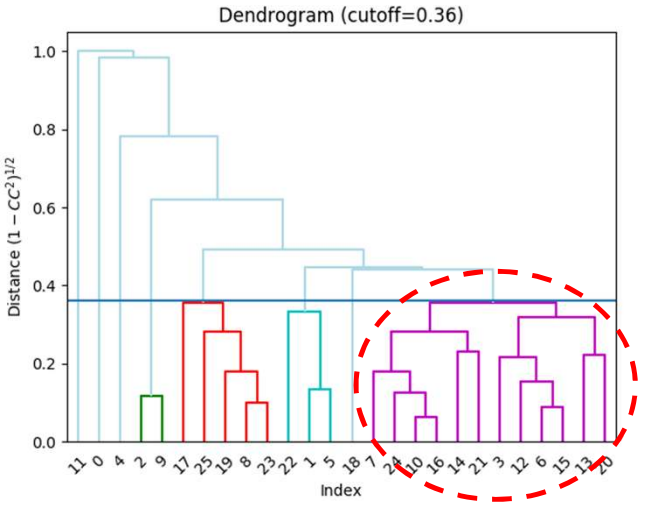
PST-20

Serial rotation electron diffraction

Sample	ZSM-5	PCN-416	ZSM-5 + mordenite	PST-20 + ZSM-25
Data collection time	6 h	2 h	2 h	4 h
Rotation (mean) /°	11.9	4.0	16.3	16.1
Rotation (max) /°	76.2	44.4	73.6	78.5
# crystals	250	**139**	123	148
# data sets > 5°	126	66	89	99
# data sets > 20°	43	15	33	42
# indexed data sets	47	27	42/11	31/19
Resolution /Å	0.77	0.90	0.76/0.81	1.46/1.46



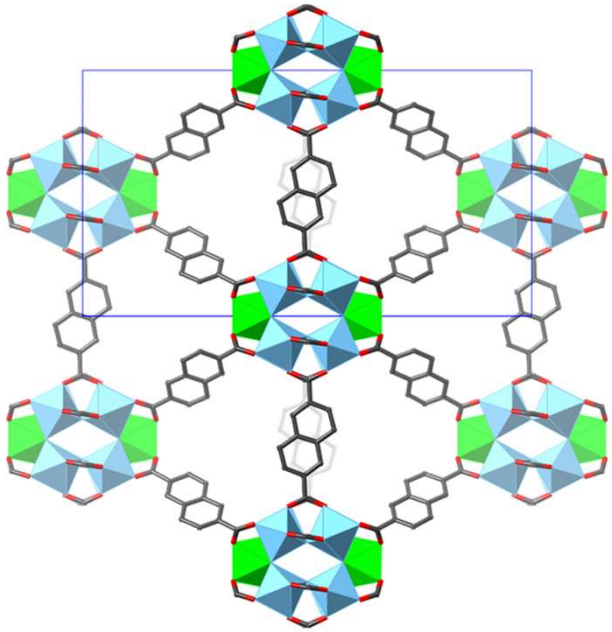
PCN-416 (100)



Hierarchical cluster analysis selects best matching data for merging

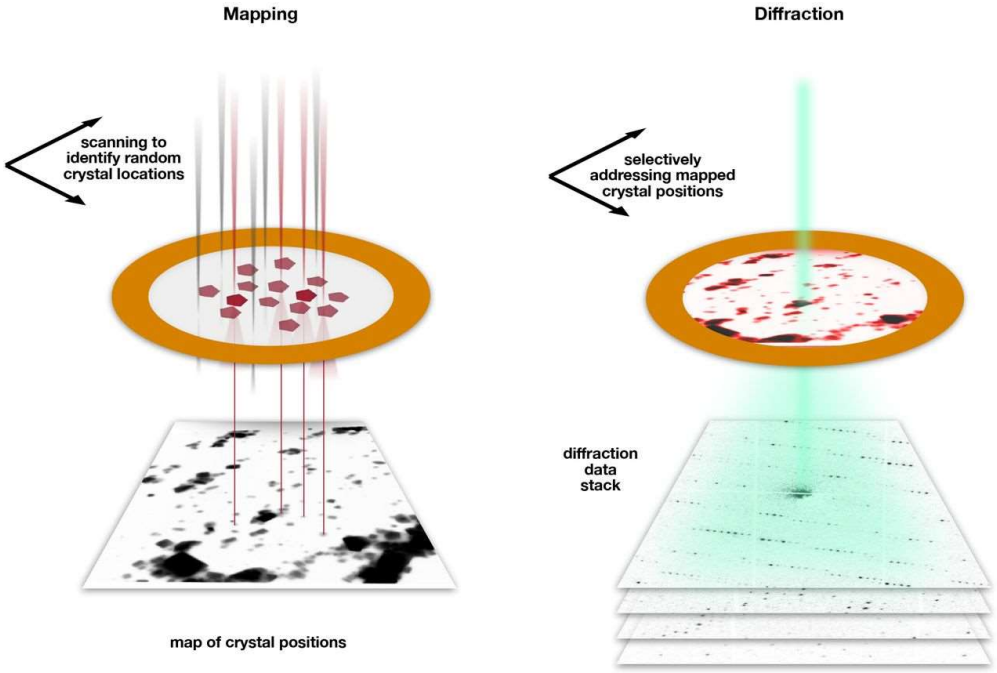
Serial vs. manual ED

Sample	Manual	SerialED
Space group	$I\bar{4}2m$	$I\bar{4}2m$
Cell /Å	16.7, 30.1	16.5, 29.8
# data sets	1	12
Resolution /Å	1.05	0.90
Completeness	100%	97%
# uniq. refls	1918	2825
# obs. refls.	912	1254
***R*1**	0.258	0.216



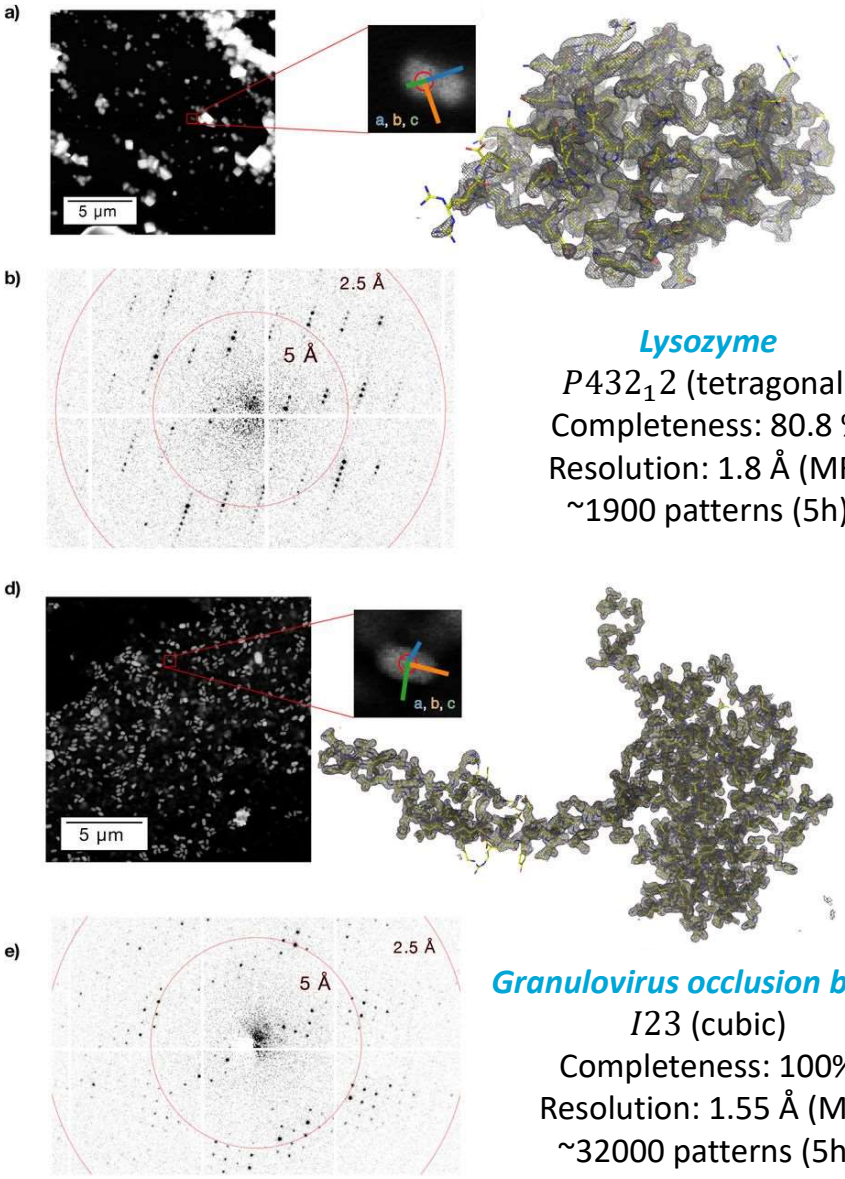
$I\bar{4}2m$
a=16.496(3) Å
c=29.947(5) Å

SerialED on proteins



Using STEM nanodiffraction

Bücker et al., bioRxiv; doi: https://doi.org/10.1101/682575



Lysozyme
$P43_12$ (tetragonal)
Completeness: 80.8 %
Resolution: 1.8 Å (MR)
~1900 patterns (5h)

Granulovirus occlusion bodies
$I23$ (cubic)
Completeness: 100%
Resolution: 1.55 Å (MR)
~32000 patterns (5h)

Summary

- Electron diffraction is very well suited for structure analysis
  - Reliable structures can be obtained routinely
- Small, but growing community
- Data collection (and processing!) protocols are being developed
- Automation for high-throughput data collection
- Equally useful for structural biology / materials science applications