Electron crystallography

3D electron diffraction for crystal structure determination

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Outline

- What is 3D ED
- From diffraction patterns to crystal structure
- Challenges in 3D ED:
  - Experimental techniques
  - Crystal tracking
  - Distortions in diffraction patterns
  - Dealing with dynamical diffraction effects
- Some examples and highlights

Electron crystallography

General definition: a scientific field which retrieves crystallographic information by using electrons as a radiation probe

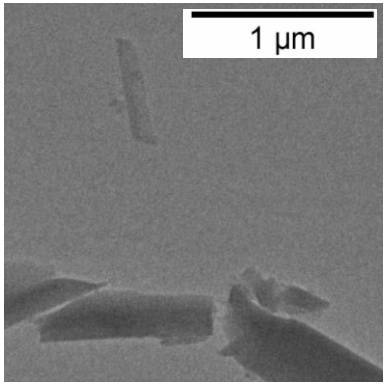
In a stricter sense: crystal structure determination predominantly by means of electron diffraction

Information obtainable (in principle) from crystallographic investigation:

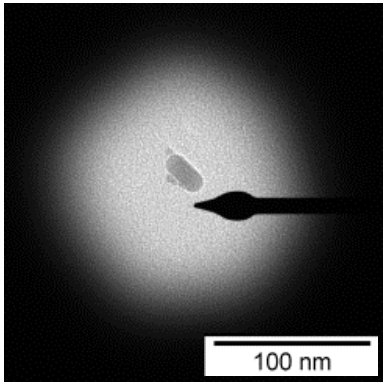
- crystal structure
- chemical composition
- polymorphism
- molecular connectivity
- molecular structure including absolute configuration
- nature of molecular species (salt/co-crystal)
- bonding ...

Electrons interact strongly with atoms
--> possibility to analyze small crystals.

For a crystallographer, this is small



…and this is really really small.



Diffraction and crystal structure

Basic relationship: diffracted intensity is proportional to the square of the amplitude of the diffracted wave:

$$I \propto A^2$$

The diffracted wave from a (small) crystal is proportional to the Fourier transform of the scattering power density in the unit cell of the crystal. This quantity is called the *structure factor:*

$$F_{\mathbf{h}} = \int_{V_{UC}} \rho(\mathbf{r}) \exp(2\pi i \mathbf{h}.\mathbf{r}) \mathrm{d}V$$

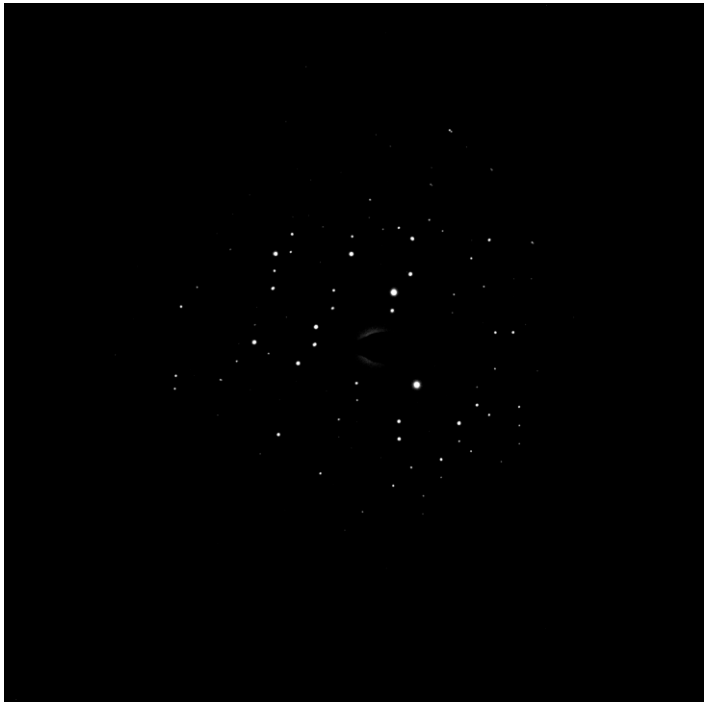
$$\mathbf{h} = h\mathbf{a}^* + k\mathbf{b}^* + l\mathbf{c}^*$$

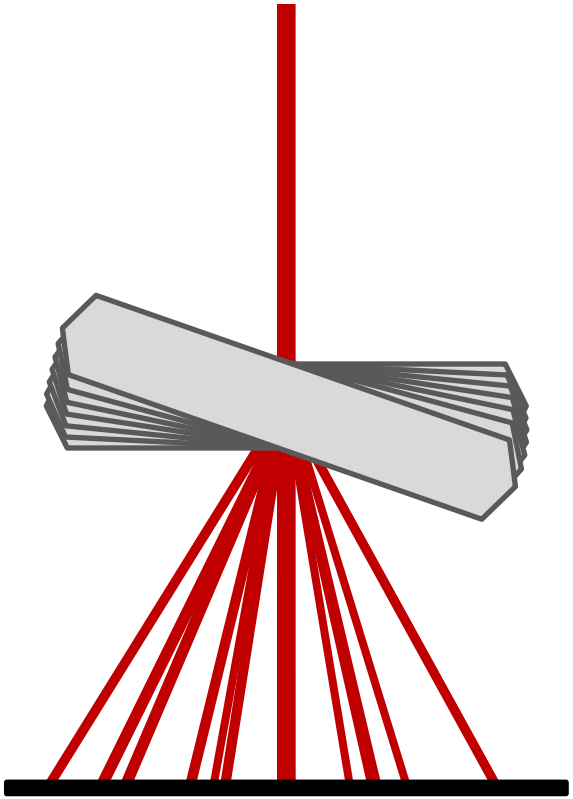
Hence: $I_{\mathbf{h}} \propto |F_{\mathbf{h}}|^2$

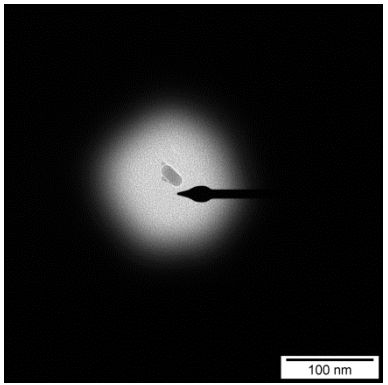
Basic assumption for the validity of the relationship: The crystal is very small. It is so small, that each quantum of radiation gets scattered at most once.

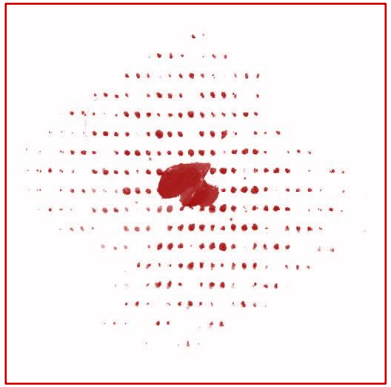
The theory based on this assumption is called the *kinematical diffraction theory*

3D electron diffraction (3D ED)

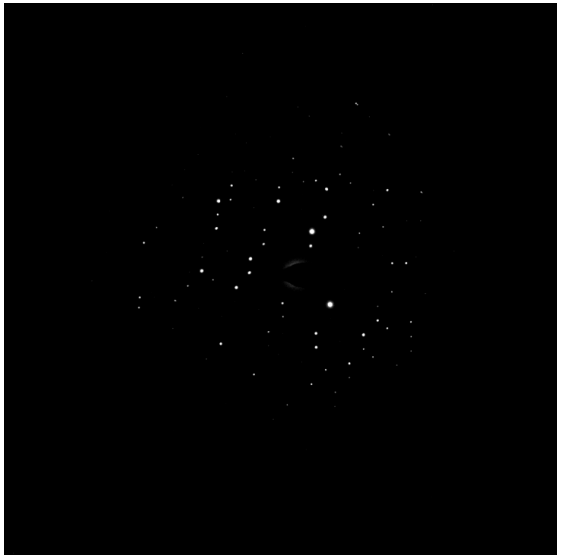


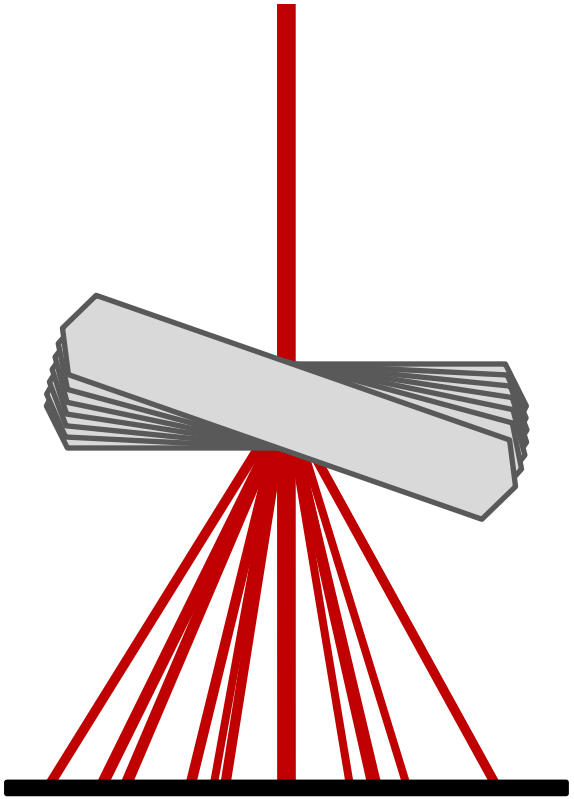


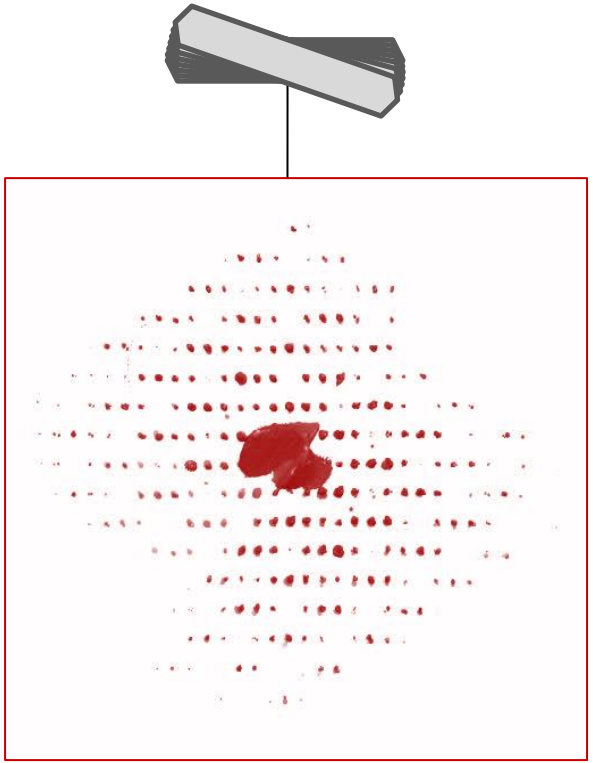




3D electron diffraction (3D ED)



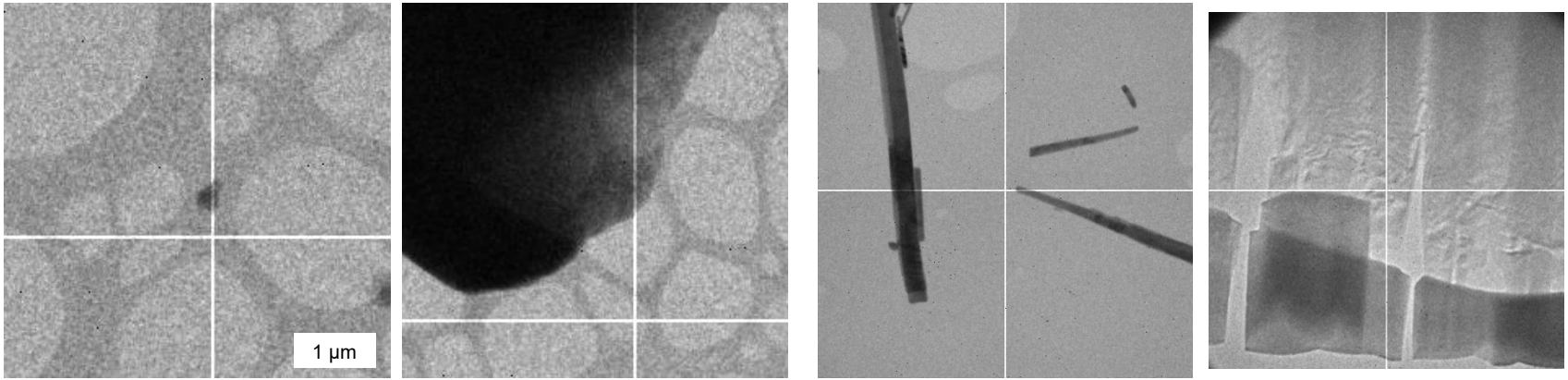




From sample to crystal structure

Step 0: prepare the sample

You need small, but especially THIN crystals. Ideally <100nm



From sample to crystal structure

Step 1: collect data

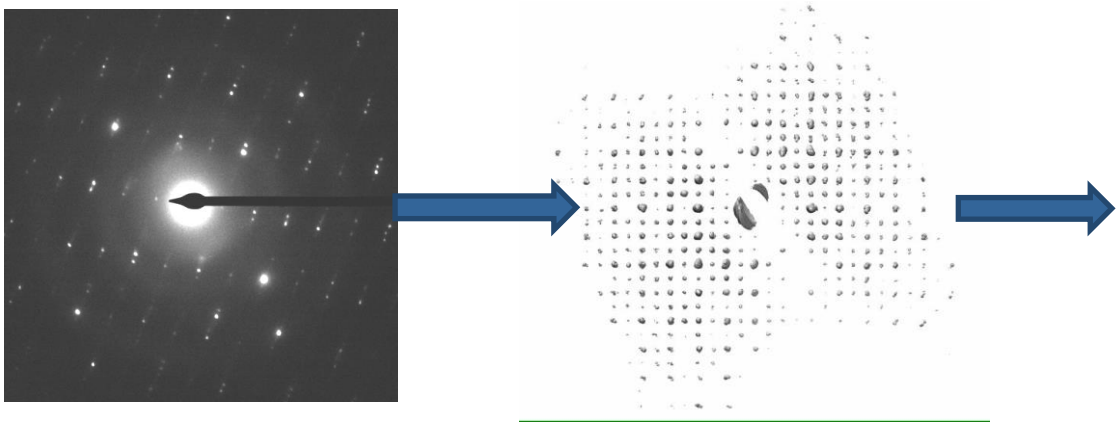
- option ***a***: you have an adapted microscope with suitable data collection software. Well, then use it!
- option ***b***: you have a dedicated electron diffractometer. Even better!
- option ***c***: No special microscope, no special software. You can still collect the data manually. Tedious, but works fine for beam-stable materials!

What microscope do you need:

- large tilt – at least ±40°
- precession capability useful, but not necessary
- radiation hard detector, ideally with fast readout (not 100% necessary)
- no need for special features, no high resolution, no aberration correction, monochromator or filter...

From sample to crystal structure

Step 2: process the data



1	0	6	398.55	1.61
2	0	6	31.94	1.70
0	1	6	111.23	1.72
-2	2	6	17.69	0.86
4	-1	7	0.21	0.30
5	-1	7	0.08	0.72
1	0	7	21.21	0.52
2	0	7	74.14	1.59
3	0	7	16.37	2.11

Several programs available for this purpose:

PETS2, XDS,
CrysAlis PRO
Dials, APEX4,
eADT

From sample to crystal structure

Step 3: solve the structure

1	0	6	398.55	1.61
2	0	6	31.94	1.70
0	1	6	111.23	1.72
-2	2	6	17.69	0.86
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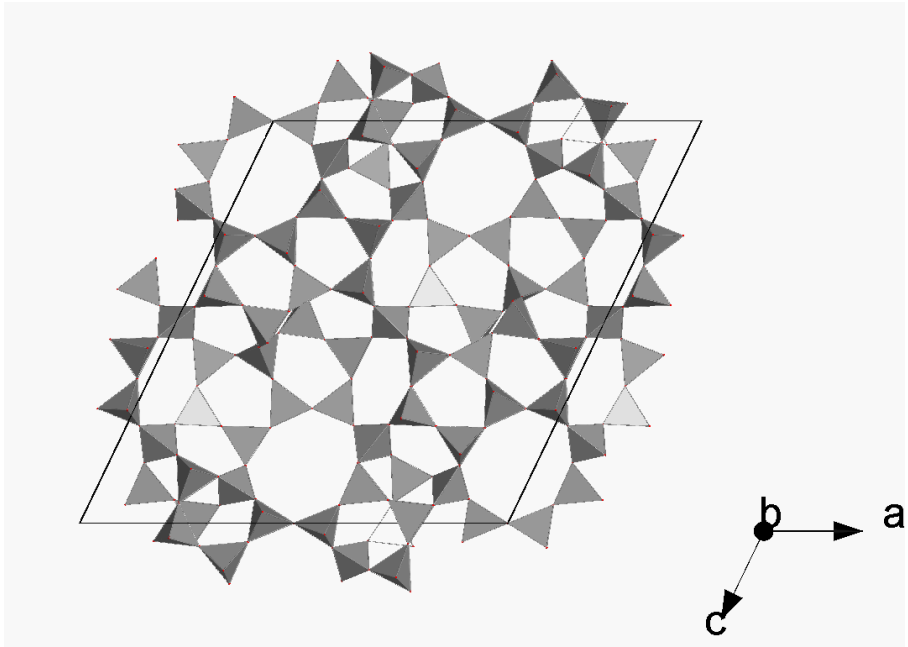


Available software: SHELXS/T/D
Superflip
SIR
XLENS

From sample to crystal structure

Step 4: refine the structure

Structure refinement = optimization of structure model against experimental data.
Performed by least-squares minimization

$$S = \sum_{\mathbf{h}} w_{\mathbf{h}} \left(I^{\mathbf{h}}_{obs} - I^{\mathbf{h}}_{calc}\left(s, \{x, y, z, U\}\right)\right)^2$$

$$\Delta p = \left((J^T [w] J)^{-1} J^T [w] \right) \Delta I$$

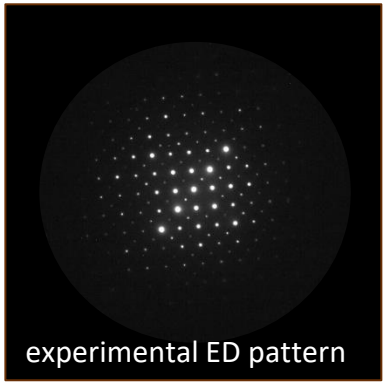
$$J_{ij} = \frac{\partial I_i (p_1 \ \ldots \ p_n)}{\partial p_j}$$

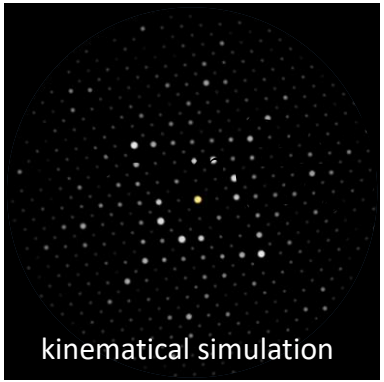
The key problem: Calculation of $I^{\mathbf{h}}_{calc}$!

Option 1: Ignore multiple scattering => use kinematical diffraction theory => *kinematical refinement*

Option 2: Include multiple scattering => use dynamical diffraction theory => *dynamical refinement*

History of structure analysis by ED = history of fight with multiple scattering


experimental ED pattern


kinematical simulation

Kinematical approximation:

$$I_{\mathbf{h}} \propto |F_{\mathbf{h}}|^2$$

Dynamical theory:

1) Find all reflections that contribute to diffraction

2) Build structure matrix A:

$$a_{ii} = 2KS_{\mathbf{g}_i}, i = 1, N_{beams}$$

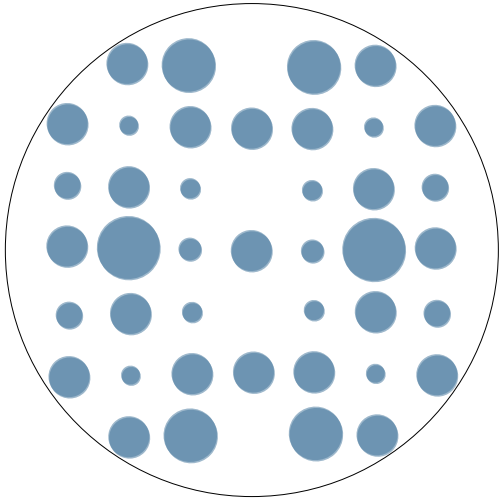
$$a_{ij} = U_{\mathbf{g}_i - \mathbf{g}_j}, i, j = 1, N_{beams}; i \neq j$$

3) Calculate scattering matrix S:

$$\mathbf{S} = \exp\left(\frac{2\pi i t \mathbf{A}}{2K_n}\right)$$

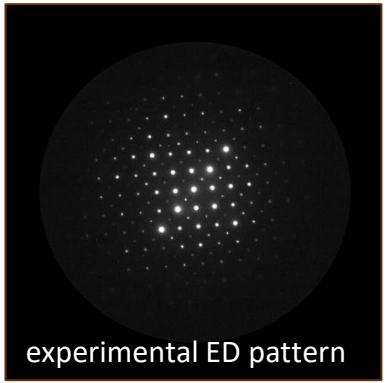
4) Calculate intensities from the first column of **S**:

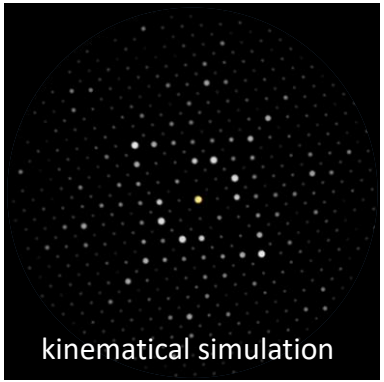
$$I_{\mathbf{h}_i} = |s_{i1}|^2$$



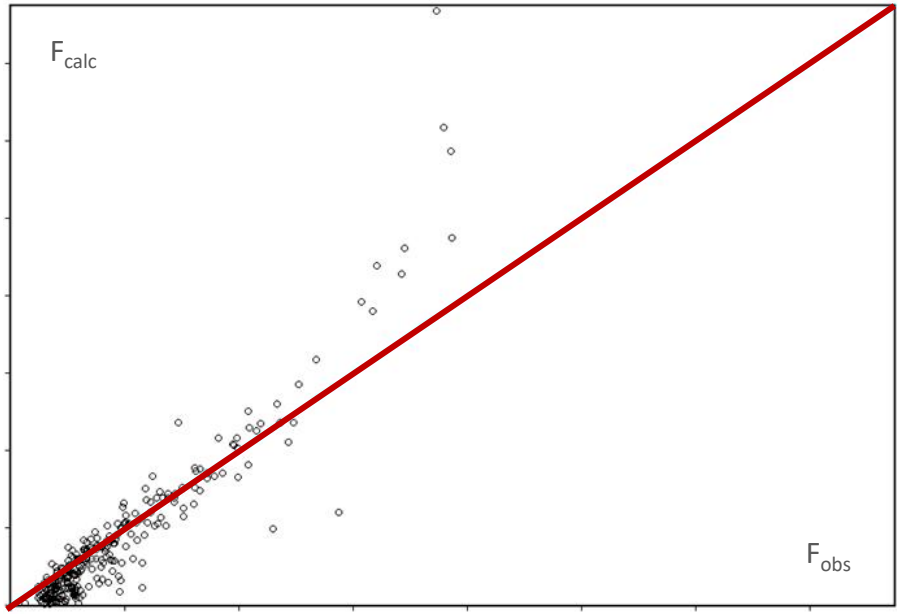
Dealing with the dynamical effects in the refinement

1) Ignore
2) Average out
3) Correct approximately
4) Take into account





The easiest aproach.
Leads to the highest figures of merit.
Potentially inacccurate structure models,
low sensitivity to details.



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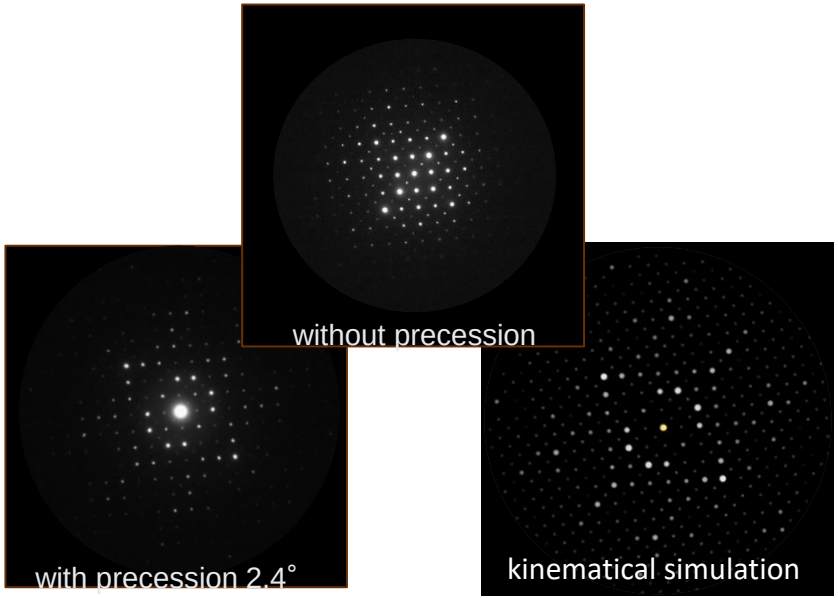
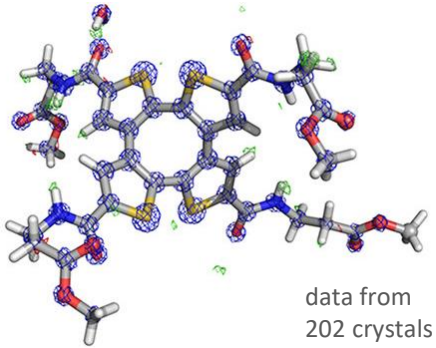


Averaging helps a lot.
Dynamical effects critically depend on the crystal orientation and can vary sharply.
However, averaging does not remove the effects completely, no matter how much data you average.

Averaging options:
During data collection: continuous rotation or precession
Post data collection: averaging several datasets.

Averaging a large number of crystals is particularly useful

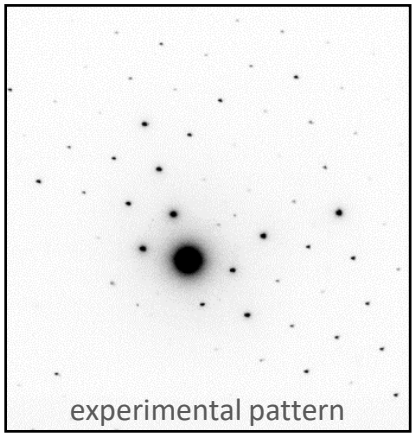


Grüne et al. (2021) Crystals 10, Smeets et al. (2019), Angew. Chem. Int. Ed. 58, Ueda et al. (2021), JACS 143

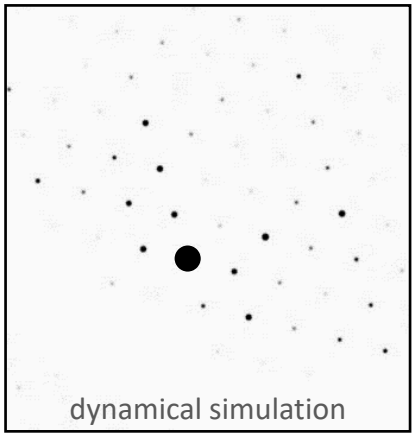
Dealing with the dynamical effects in the refinement

1) Ignore
2) Average out
3) Correct approximately
4) Take into account

This approach is called **dynamical refinement** for short and gives the best results in most cases.



experimental pattern



dynamical simulation

1) Refinement uses frame-based intensities
2) Filtering of reflections based on their proximity to Ewald sphere
3) I_{calc} is a function of thickness and crystal orientation
4) Integration still necessary to limit sensitivity to crystal imperfections

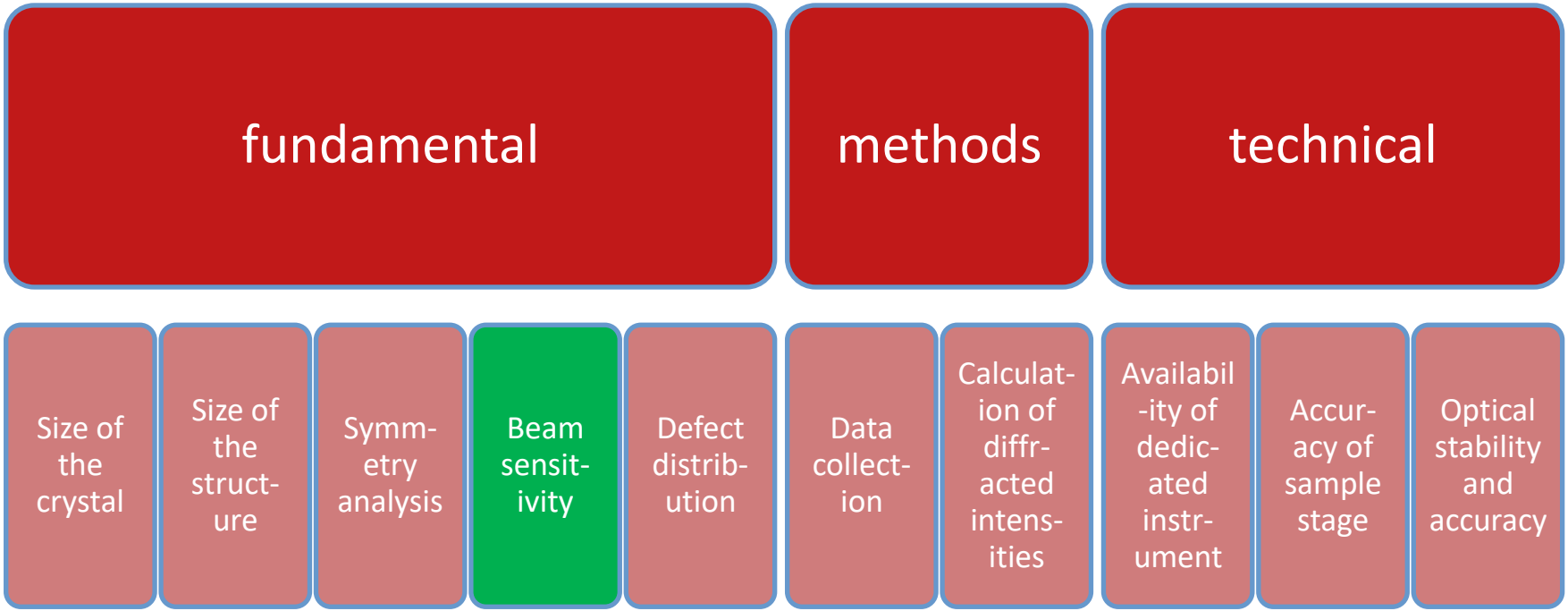
Downside: long(ish) computing times

Palatinus et al. (2015), Acta Cryst. A 71, Palatinus et al. (2019), Acta Cryst. B75 , Klar et al. (2023), Nat. Chem. 15

The main challenges

fundamental					methods		technical		
Size of the crystal	Size of the struct-ure	Symm-etry analysis	Beam sensit-ivity	Defect distrib-ution	Data collect-ion	Calculat-ion of diffr-acted intens-ities	Availabil-ity of dedic-ated instr-ument	Accur-acy of sample stage	Optical stability and accuracy

Beam sensitive materials



Beam sensitive materials

Electrons are less damaging than x-rays per single elastic event.

However, the probed volume is much smaller in electron diffraction.

Therefore, **radiation damage is a much bigger issue for electron crystallography!**

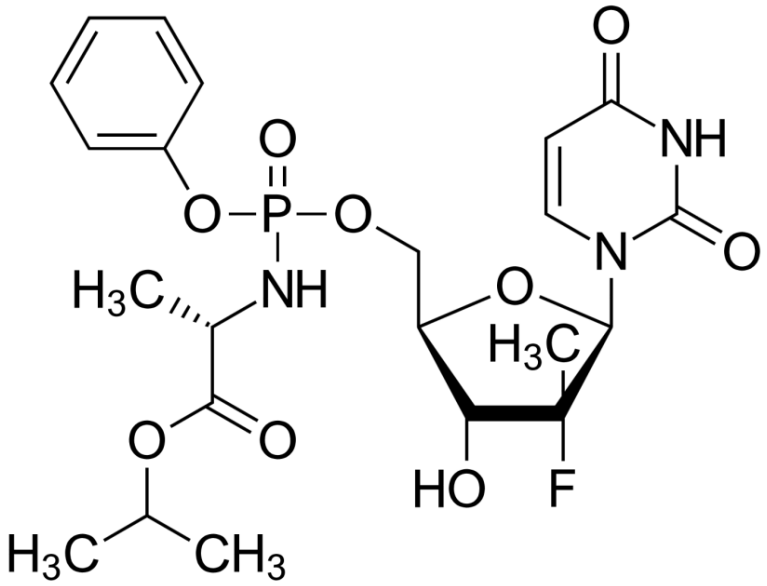
What is "beam sensitive"? Indicative limiting doses for the loss of crystallinity	
zeolites	>100 e/Å^2
MOFs	5-15 e/Å^2
protein crystals	1-10 e/Å^2
crystals of small organic molecules with hydrogen bonds	0.5-10 e/Å^2
crystals of small organic aliphatic molecules with van der Waals bonds only	0.01-0.5 e/Å^2

SOLUTION:

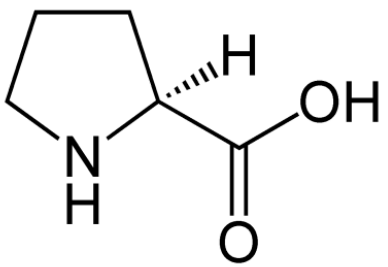
1) **Use fast data collection with the modern sensitive direct detection cameras**
2) **Collect data on different parts of a large(r) crystal or use serial electron crystallography**

Sofosbuvir L-prolin

a cocrystal of L-prolin and an anti-hepatitis drug (both chiral)



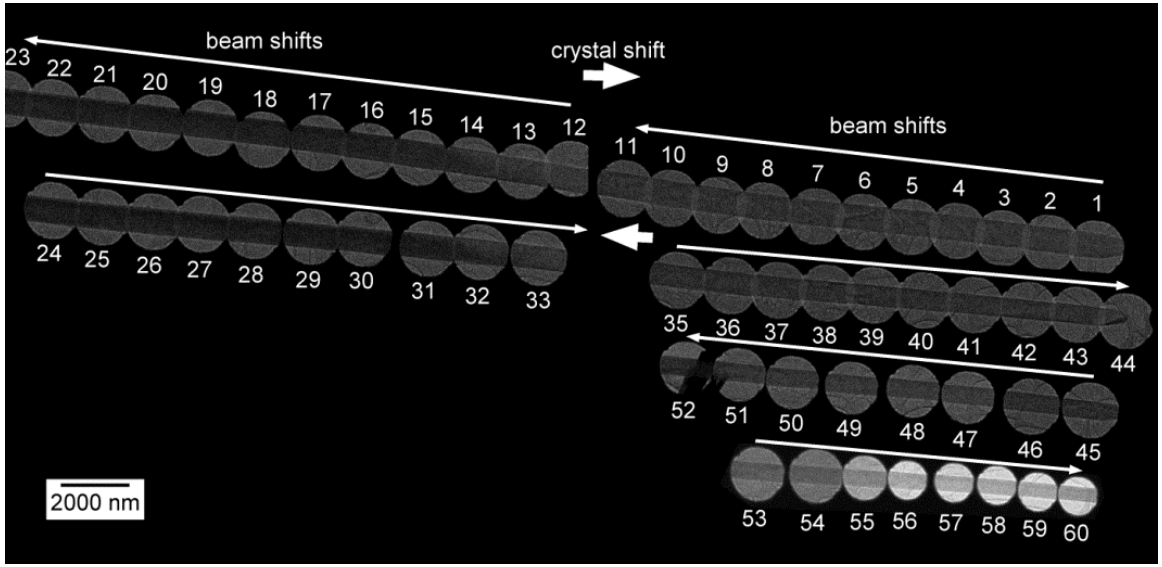
Sofosbuvir – antivirotic

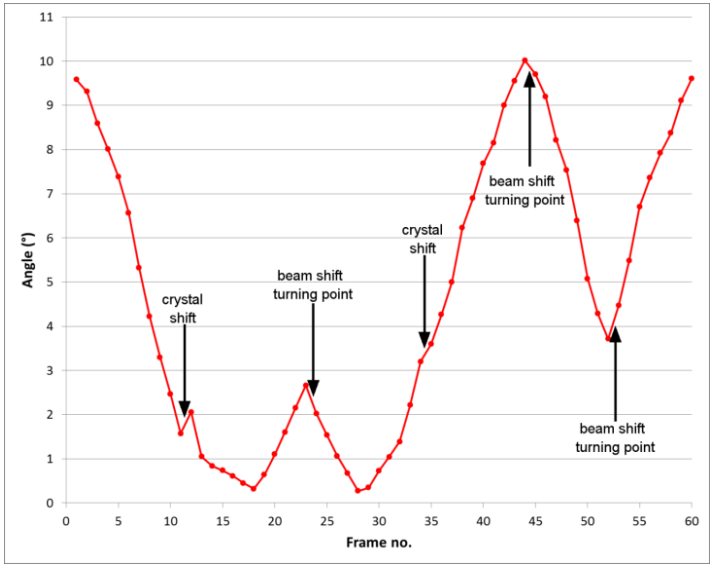


L-proline – amino acid

Sofosbuvir L-prolin

A cocrystal of L-prolin and an anti-hepatitis drug.
Extremely beam-sensitive, most crystals deteriorate after <0.08 e/Å^2.
Crystals form long ribbons.





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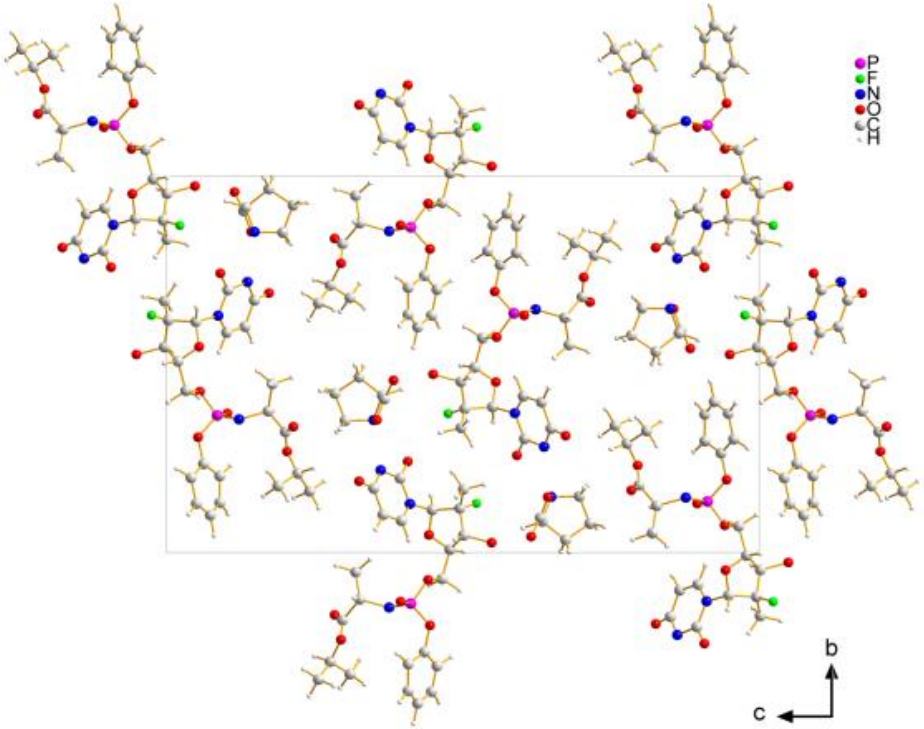
Sofosbuvir L-prolin



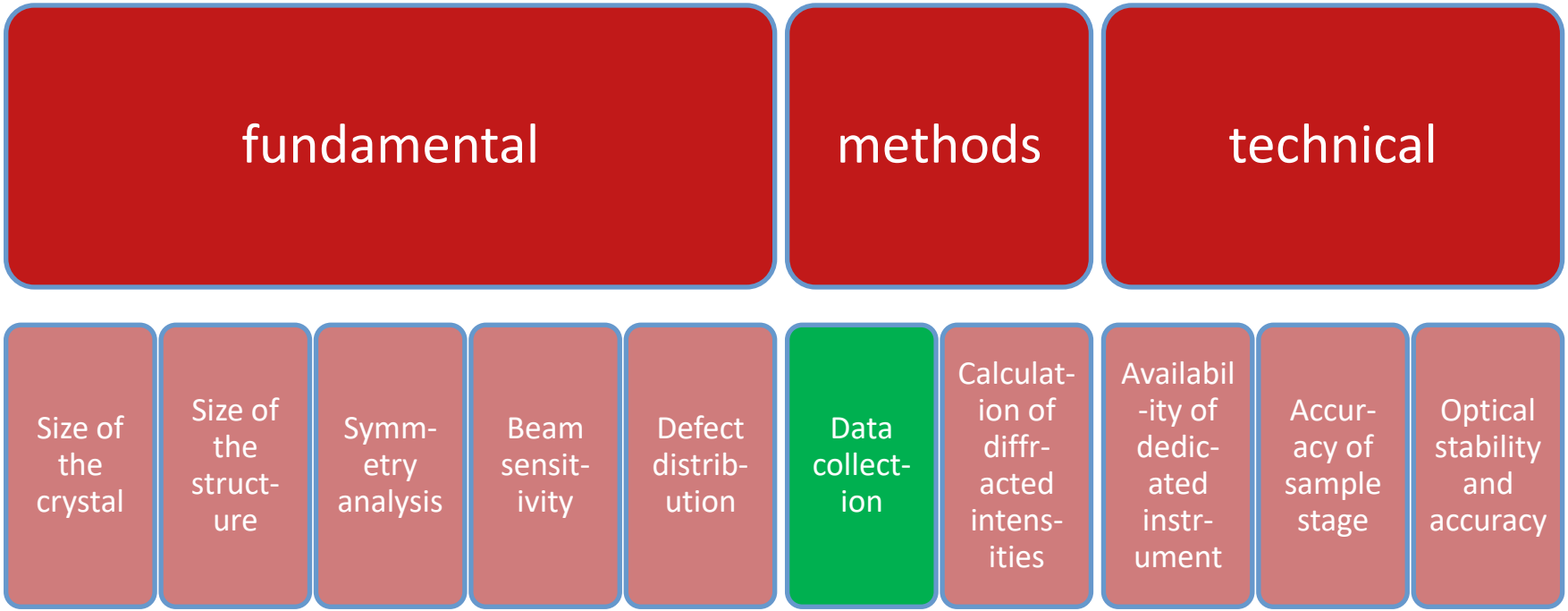
Formula: $C_{27}H_{38}N_4O_{11}FP$
44 independent non-H atoms

Space group $P2_12_12_1$

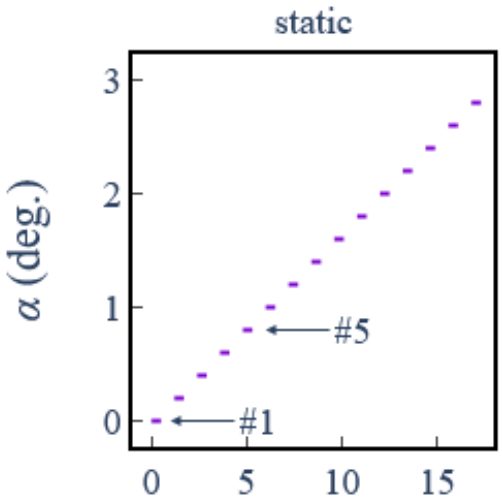
a=5.35Å, b = 19.60Å, c = 29.87Å
Unit cell volume: 3127 Å^3

Kinematical refinement	R_{obs} = 19.7%
Dynamical refinement	R_{obs} = 9.7%

Data collection techniques

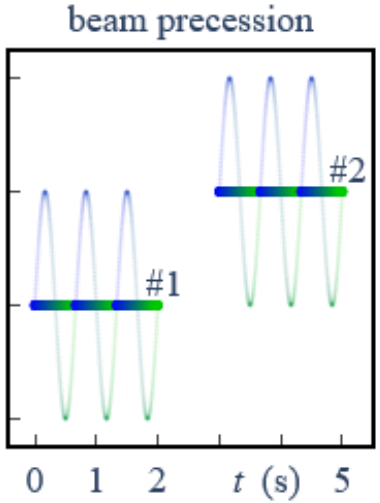


3D ED variants



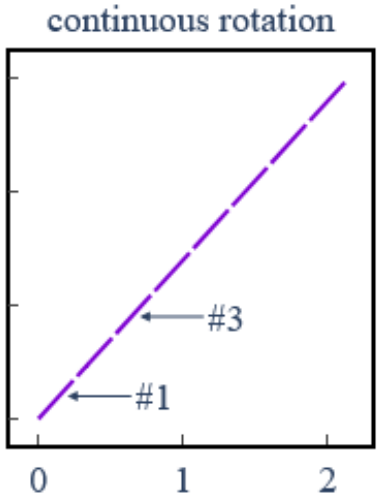
static-beam 3D ED

ADT/EDT
(Automated/Electron Diffraction Tomography)
MicroED (version from 2013)



precession-assisted 3D ED

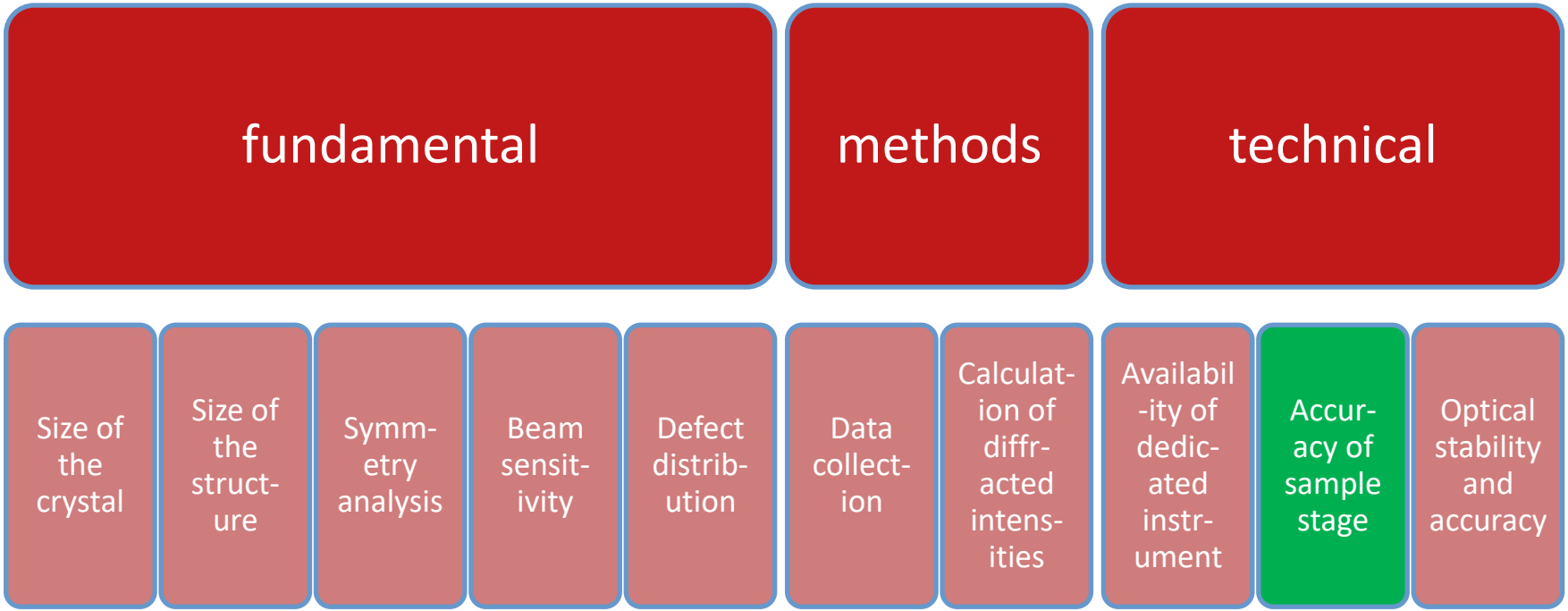
PEDT
(Precession El. Diffr. Tomography)
precession ADT



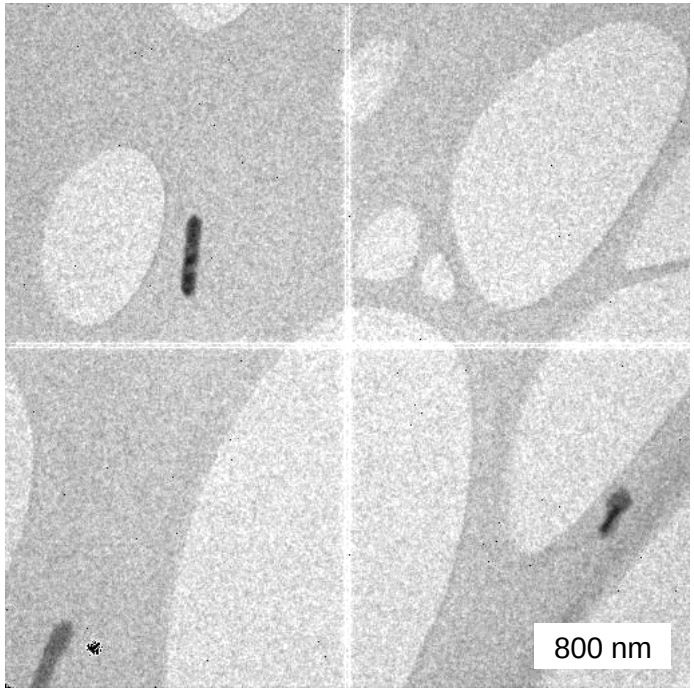
continuous-rotation 3D ED

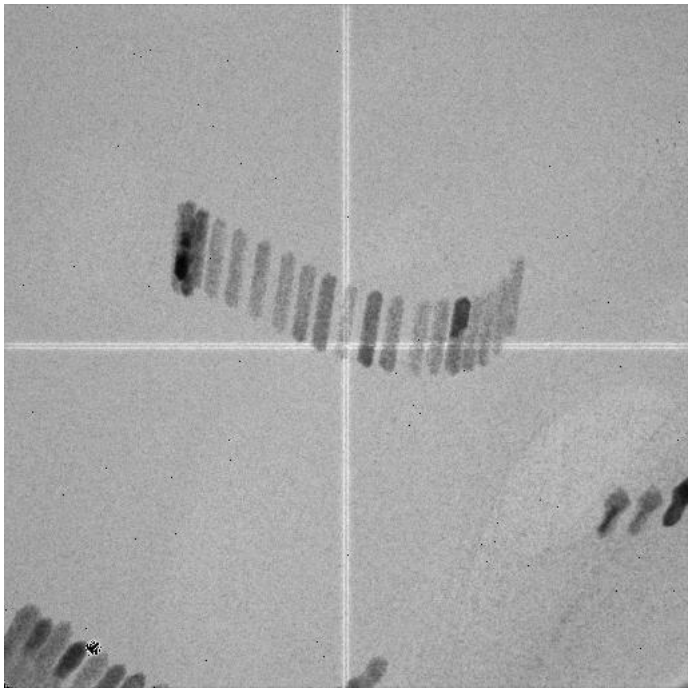
cRED (continuous RED)
IEDT (integrated EDT)
MicroED (current version)

Crystal tracking

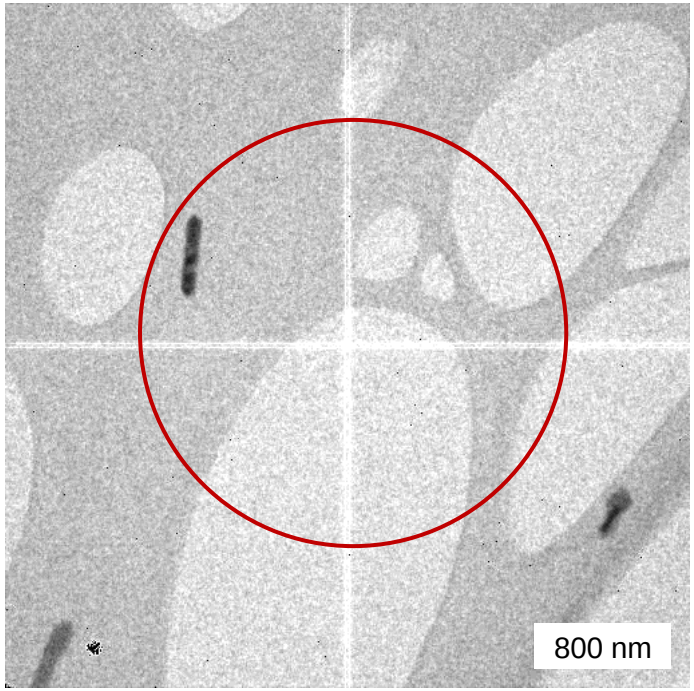


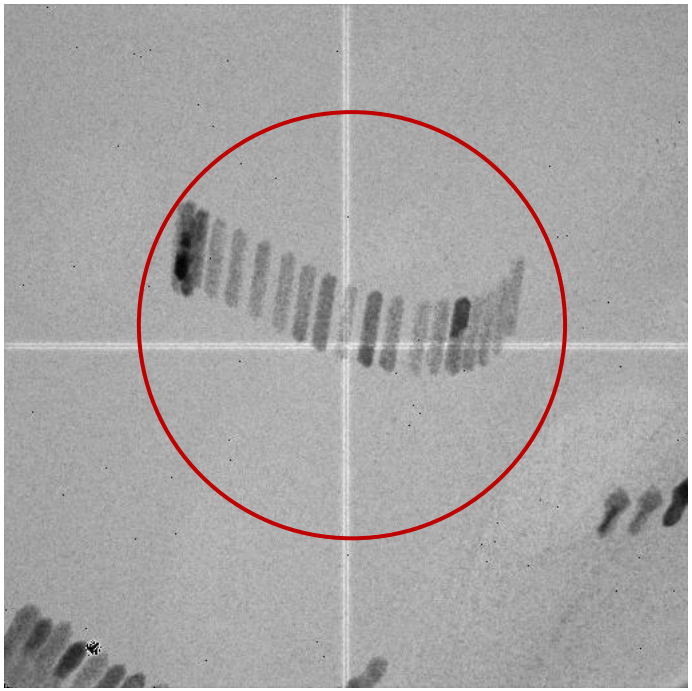
Crystal tracking





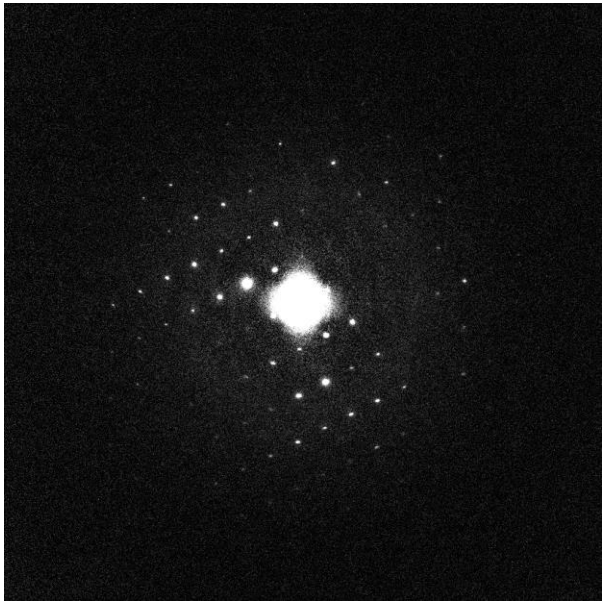
Crystal tracking

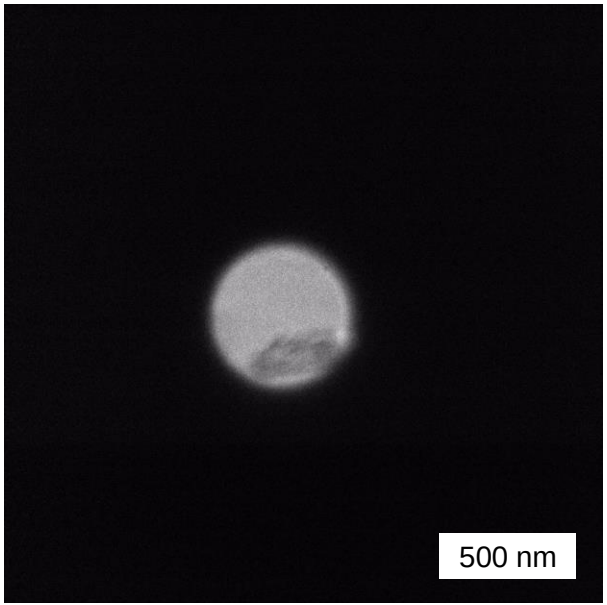




Option 1: use a large beam. Downside: high background, illumination of more than one crystal, varying illumination of large crystals

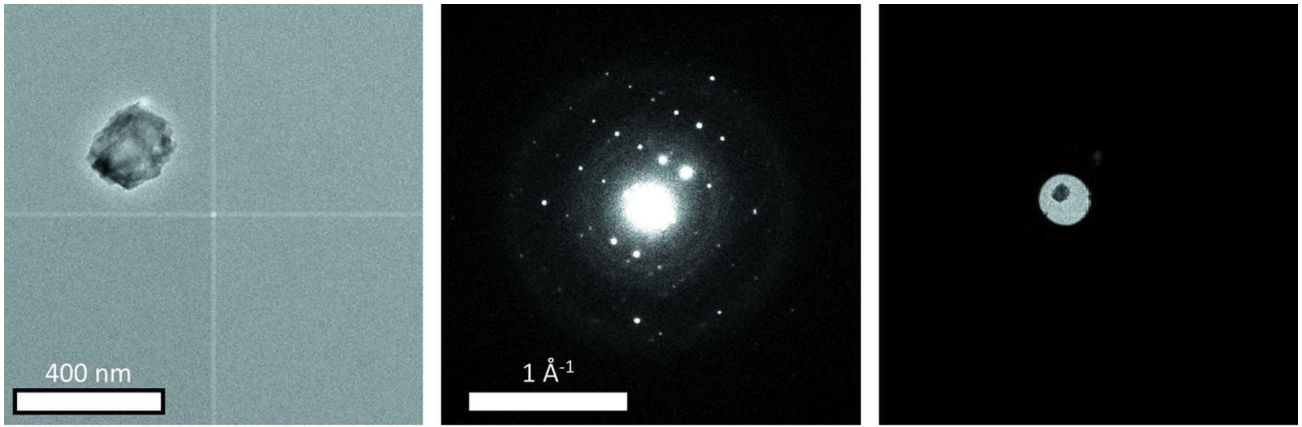
Crystal tracking





Option 2: switch from diffraction to imaging to track the crystal. Downside: lens hysteresis, incompatible with continuous rotation

Crystal tracking



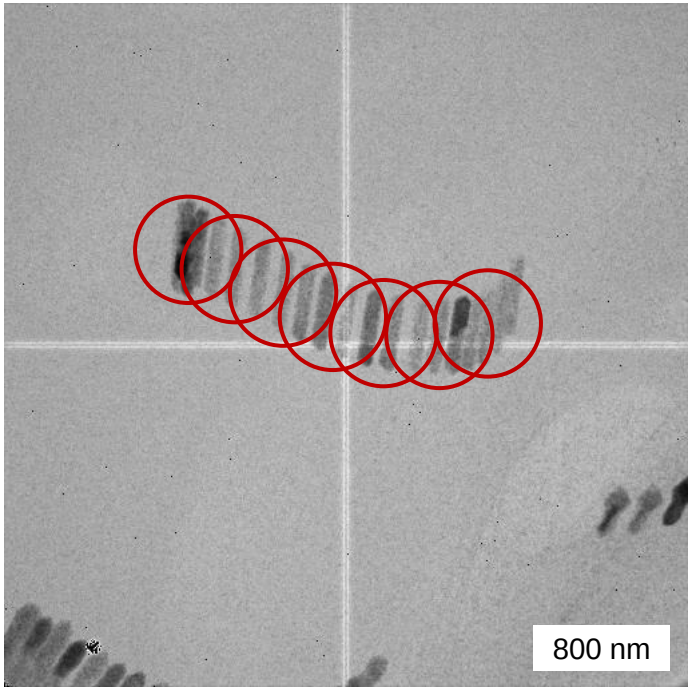
JOURNAL OF APPLIED CRYSTALLOGRAPHY

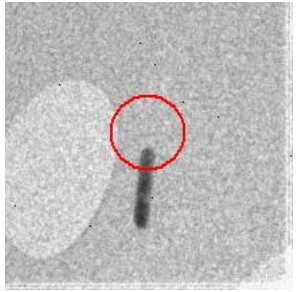
Cichocka *et al.*

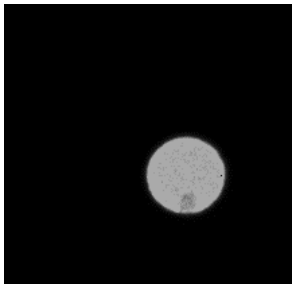
Volume 51 | **Part 6** | December 2018 | Pages 1652–1661 | 10.11107/S1600576718015145

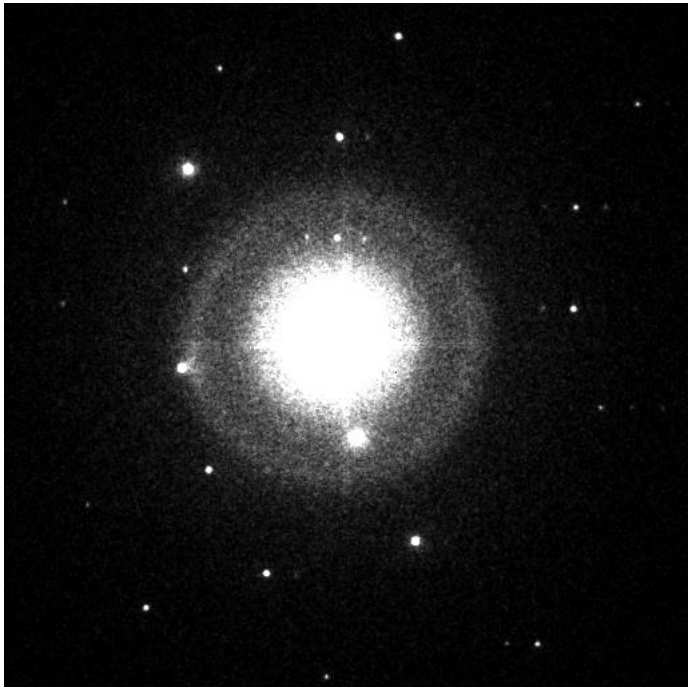
Option 3: defocus the diffraction to see the crystal in the central beam. Downside: lens hysteresis, gaps in the data

Crystal tracking





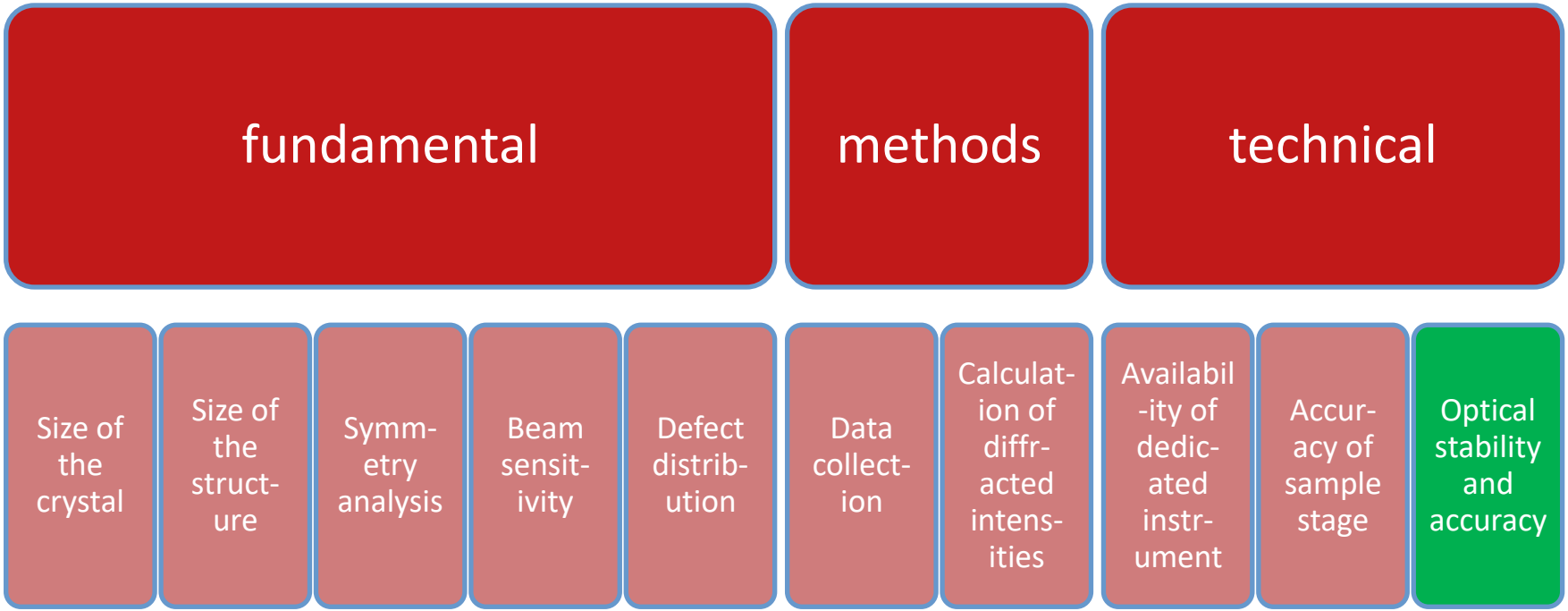




Option 4: pre-record the path of the crystal, then track it using the pre-recorded path. Downside: additional time (not much), additional illumination of the crystal (not much, may be avoided), potentially not perfectly reproducible path

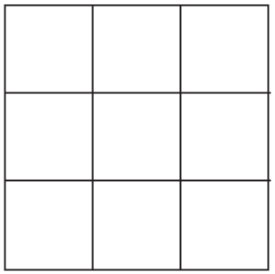
Gemmi et al. (2015), J. Appl. Cryst. 48; Plana-Ruiz et al. (2020), Ultramicroscopy 211

Optical distortions and lattice parameters

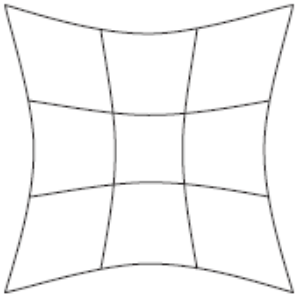


Optical distortions and lattice parameters

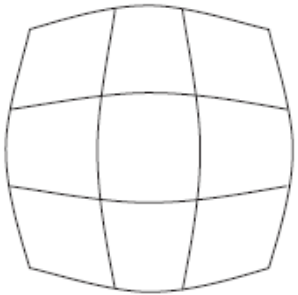
undistorted image



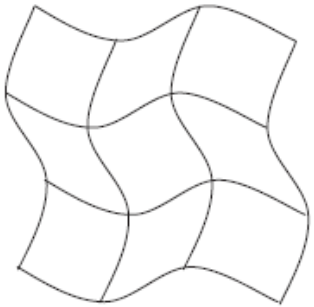
pincushion distortion



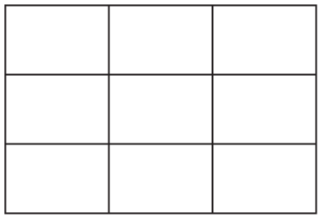
barrel distortion



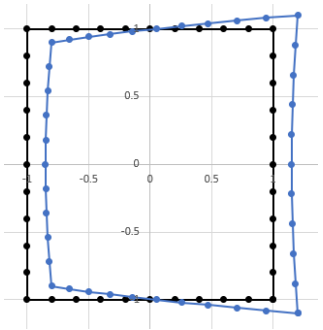
spiral distortion



elliptical distortion



parabolic distortion



Optical distortions and lattice parameters

Example:

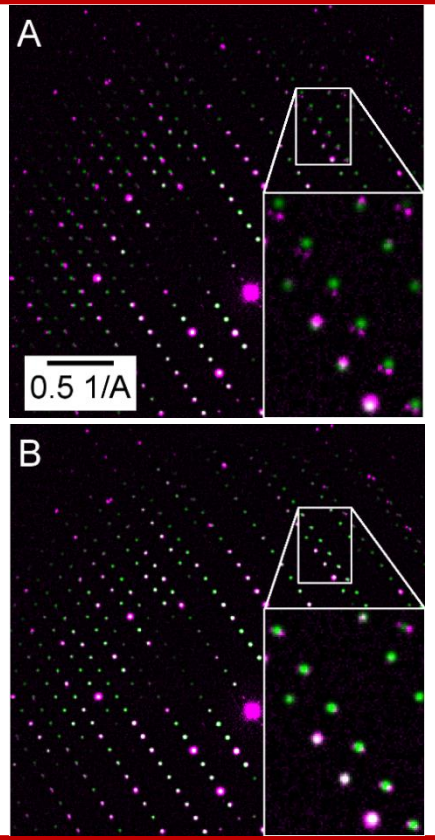
- Lutetium aluminium garnet, Bravais clas cF, a=11.9084 Å
- Main reason for distorted unit cell: elliptical distortion

Without distortion correction:

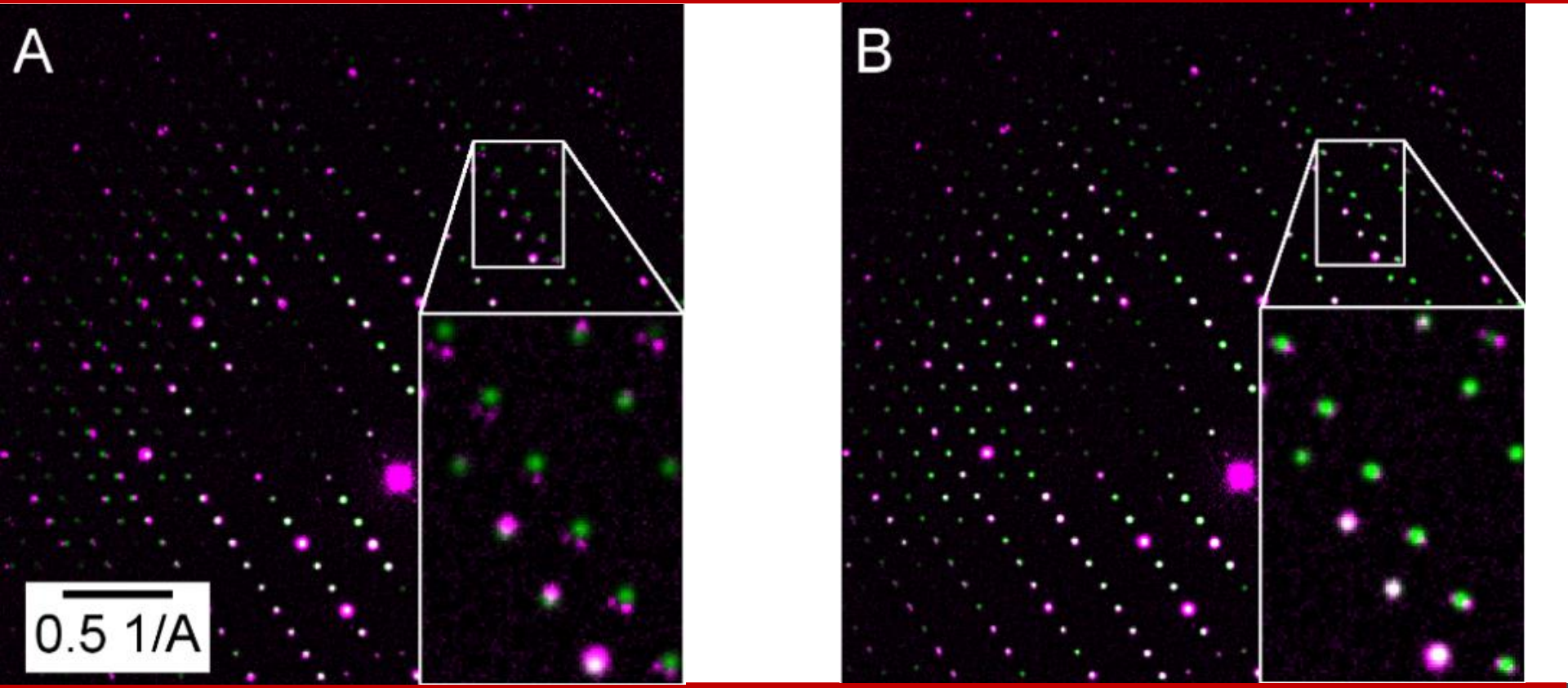
	a (Å)	b (Å)	c (Å)	alpha (°)	beta (°)	gamma (°)
DS1	11.848(1)	**11.858(3)**	11.907(1)	90.21(1)	**90.33(1)**	89.97(1)
DS2	11.842(1)	**11.881(2)**	11.888(2)	89.77(2)	**89.75(1)**	89.75(1)
DS3	11.854(1)	**11.863(3)**	11.890(1)	89.80(1)	**90.40(1)**	90.11(1)

With distortion correction:

	a (Å)	b (Å)	c (Å)	alpha (°)	beta (°)	gamma (°)
DS1	11.909(1)	**11.910(2)**	11.908(1)	90.00(1)	**90.00(1)**	90.02(1)
DS2	11.908(1)	**11.914(2)**	11.908(2)	90.03(2)	**90.00(1)**	89.98(1)
DS3	11.907(1)	**11.912(2)**	11.909(1)	90.001(3)	**90.00(1)**	90.00(1)

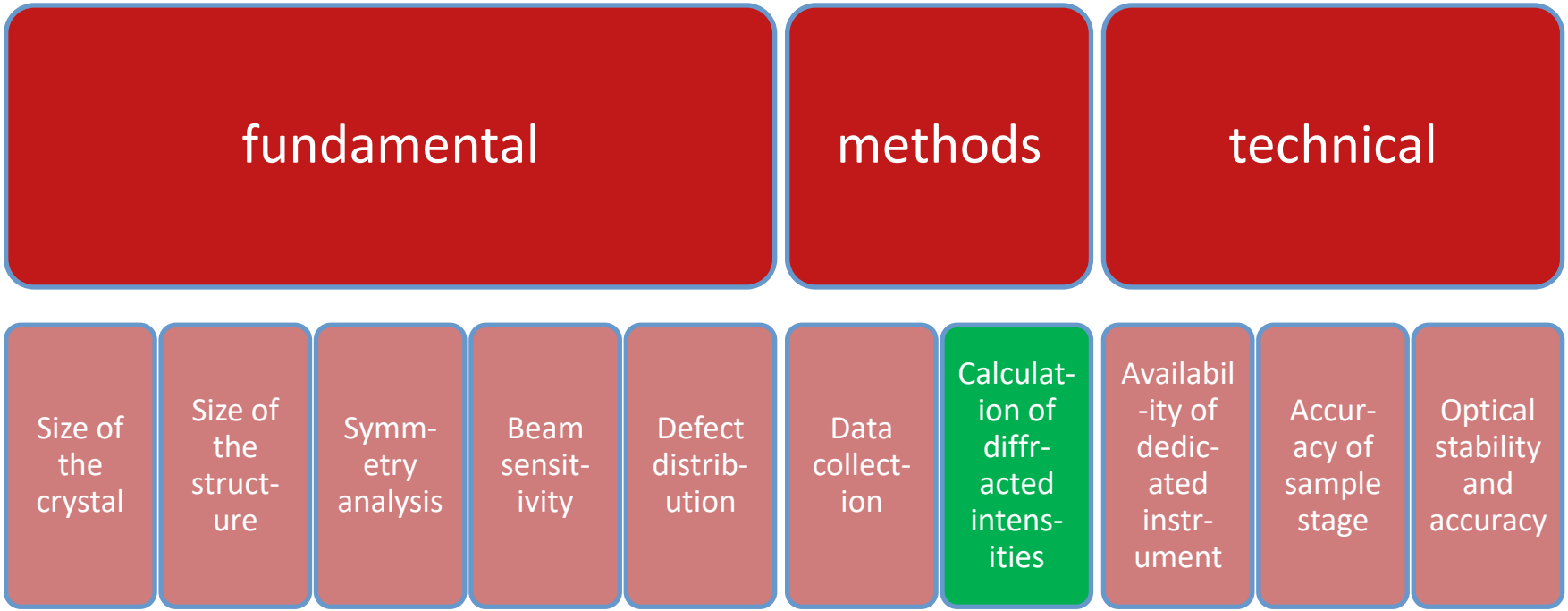


Optical distortions and lattice parameters



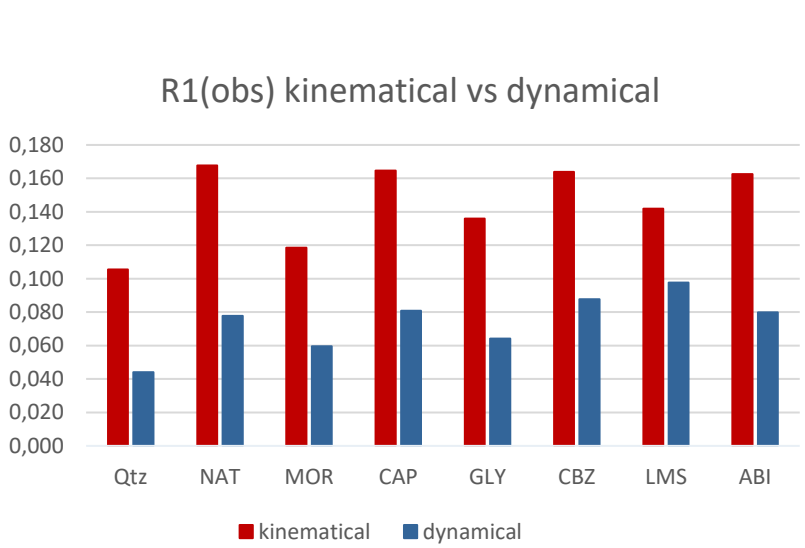
Brazda et al. (2023), IUCrJ

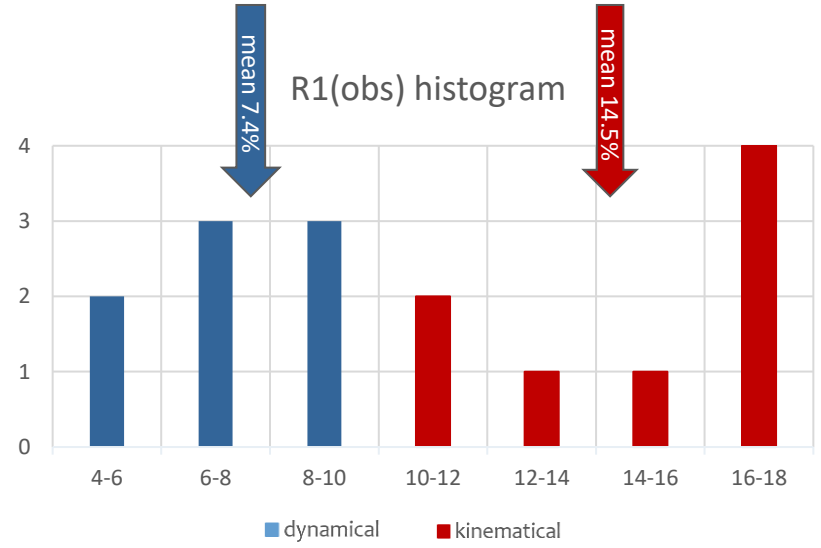
Calculation of intensities



The R-factor gap in elecron crystallography

8 structures carefully refined kinematically and dynamically from the same data





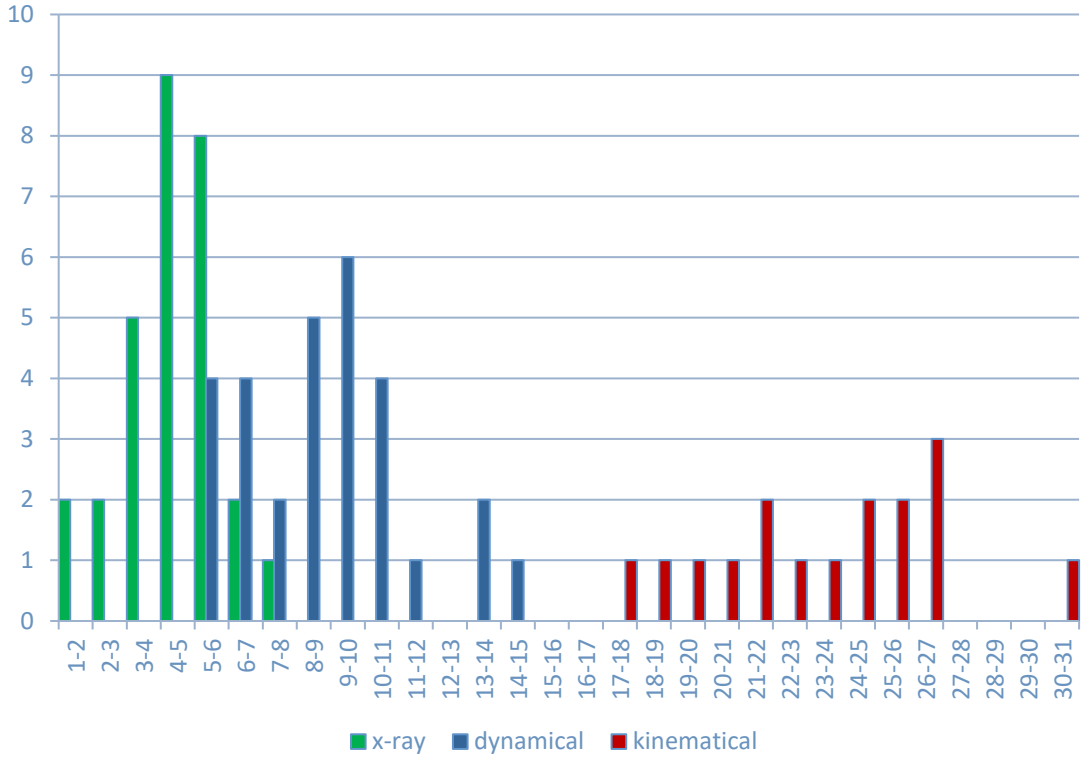
Dynamical: refined against unmerged data, but R-factors calculated on merged data for comparison with the kinematical factors.

The R-factor gap in elecron crystallography

The fit to 3DED data is not as good as for x-ray data even for dynamical refinement.

Reason: diffraction comes from imperfect, often irregular crystals, but theory assumes perfect crystals

Solution: modify the refinement to account for crystal imperfections



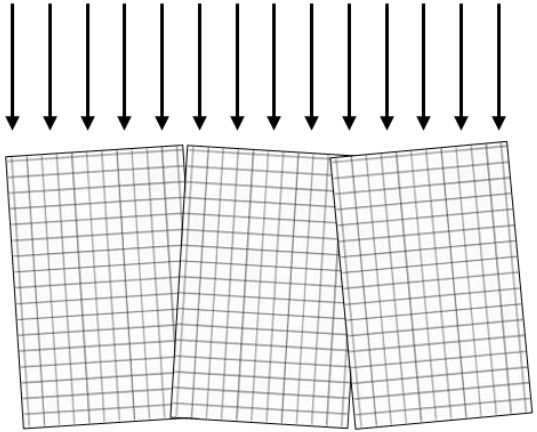
Mosaicity in the dynamical case

Aspects to consider:

- The intensity depends on the crystal orientation
- Electrons passing from one mosaic block to the next „know“their history
- Column apoproximation may come to the rescue

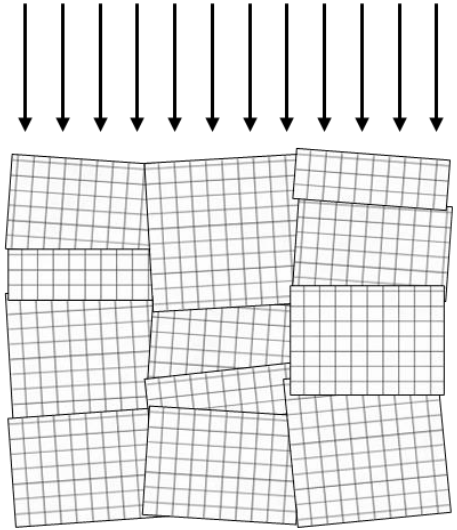
Case 1: large blocks

Incoherent superposition of diffraction from individual blocks



Case 2: small blocks

Coherent superposition of diffraction from individual blocks in each column + incoherent superposition of individual columns

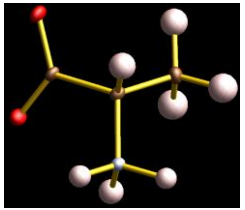


Mosaicity in the dynamical case

L-alanine: simple organic molecule

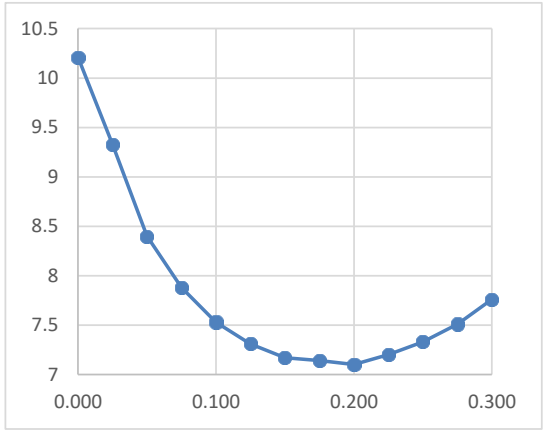
orthorhombic, $P2_12_12_1$

relatively high mosaicity of 0.29° (from data reduction)



Test 1: Incoherent isotropic mosaicity

0.000	10.21
0.025	9.33
0.050	8.4
0.075	7.88
0.100	7.53
0.125	7.31
0.150	7.17
0.175	7.14
0.200	7.1
0.225	7.2
0.250	7.33
0.275	7.51
0.300	7.76

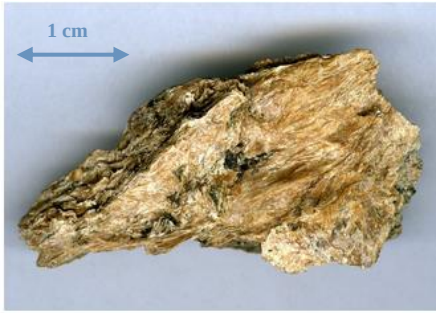


Test 2: Combined coherent and incoherent mosaicity

μ_o \ μ_i	0.000	0.025	0.050	0.075	0.100	0.125	0.150	0.175	0.200	0.225	0.250
0.00	10.18	9.33	8.40	7.95	7.58	7.36	7.25	7.18	7.27	7.32	7.51
0.05	10.02	9.15	8.33	7.90	7.54	7.32	7.22	7.16	7.24	7.29	7.49
0.10	9.62	8.84	8.15	7.78	7.49	7.28	7.18	7.13	7.21	7.26	7.46
0.15	9.07	8.48	7.96	7.66	7.41	7.22	7.14	7.09	7.16	7.23	7.42
0.20	8.57	8.16	7.78	7.54	7.32	7.16	7.10	7.08	7.14	7.23	7.42
0.25	8.24	7.94	7.63	7.42	7.24	7.11	7.08	7.07	7.12	7.24	7.44
0.30	7.94	7.71	7.47	7.32	7.17	7.09	7.09	7.10	7.15	7.30	7.52
0.35	7.69	7.54	7.38	7.29	7.19	7.16	7.16	7.18	7.25	7.43	7.67
0.40	7.56	7.47	7.37	7.31	7.28	7.31	7.31	7.34	7.43	7.64	7.85

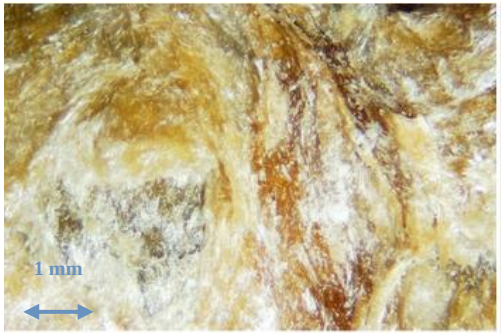
$\mu_{comb} = \mu_i^2 + \mu_o^2 = 0.31°$, very close to the experimental value

Example – eveslogite



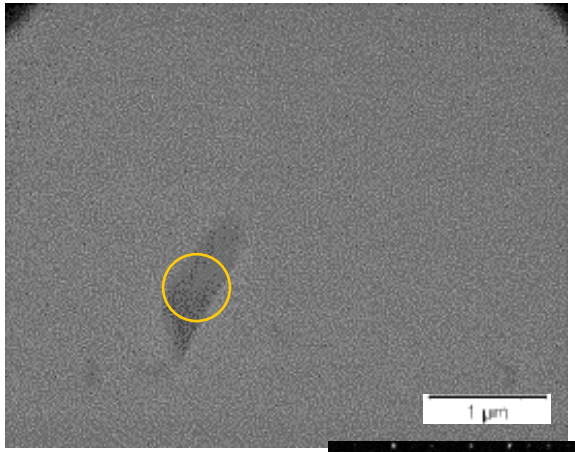
Eveslogite, etc.

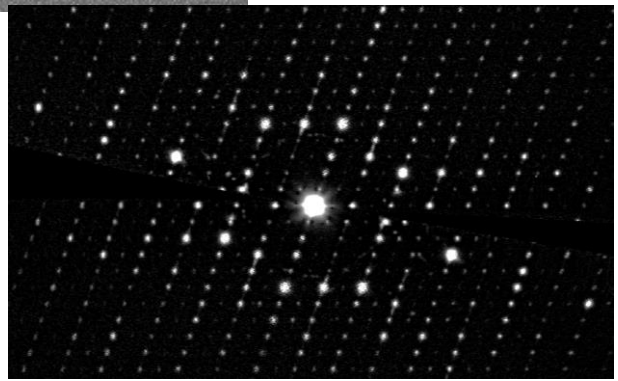
Fersman Gorge, Eveslogchorr Mt, Khibiny Massif, Murmansk Oblast, Russia



Eveslogite

Eveslogchorr Mt, Khibiny Massif, Murmansk Oblast, Russia





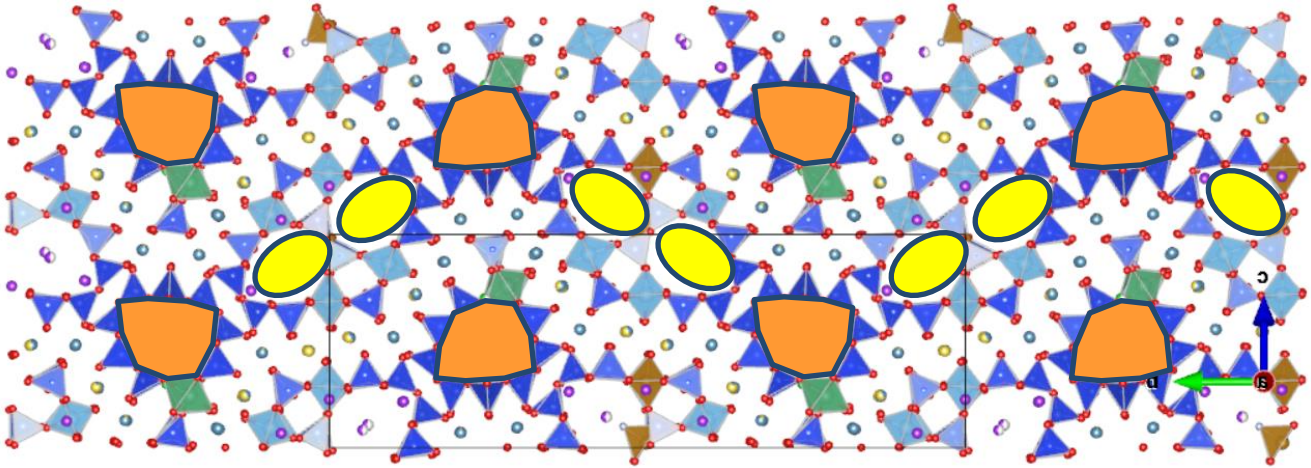
Chemical Properties of Eveslogite

Formula: $(Na,K,Ca,Sr,Ba)_{48}\,[(Ti,Nb,Mn,Fe^{2+})_{12}Si_{48}O_{144}(OH)_{12}](F,OH,Cl)_{14}$

IMA Formula: $(Ca,K,Na,Sr,Ba)_{48}(Ti,Nb,Fe,Mn)_{12}(OH)_{12}Si_{48}O_{144}(OH,F,Cl)_{14}$

mindat.org

Example – eveslogite



space group	$P2_1$
a	14.1898 Å
b	44.7704 Å
c	15.9111 Å
b	109.4677°
V_{UC}	9530.171 Å^3

~ 360 atoms in the asymmetric unit

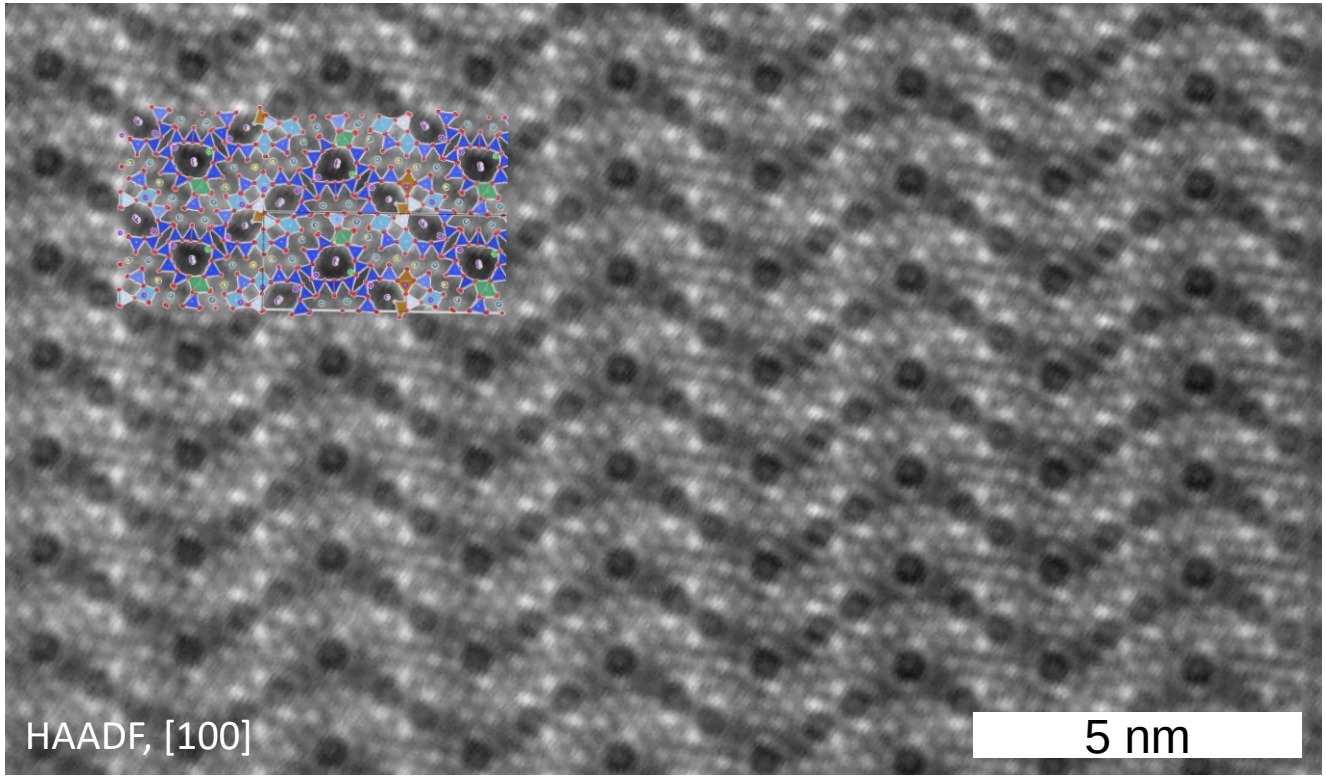
115 842 reflections R_{obs} = 19.82

R_{int} = 17.24 R_{all} = 24.70

Na	
K	
Ca	
Nb	
Ti	
Si	

E. Götz, U. Kolb (TU Darmstadt), S.V. Krivovichev (University of Saint Petersburg), Marco Schowalter (University of Brehmen)

Example – eveslogite



Example – CO_2-loaded zeolite

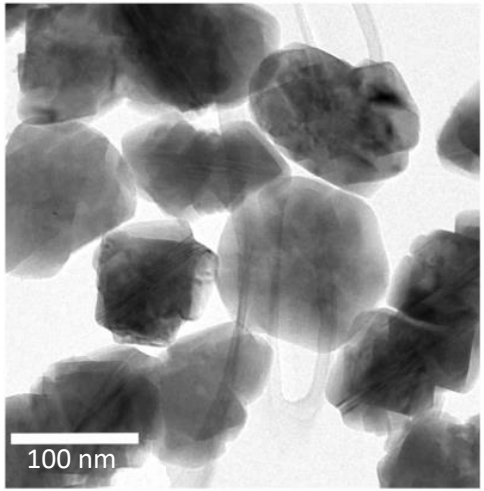
CO_2-loaded CHABAZITE

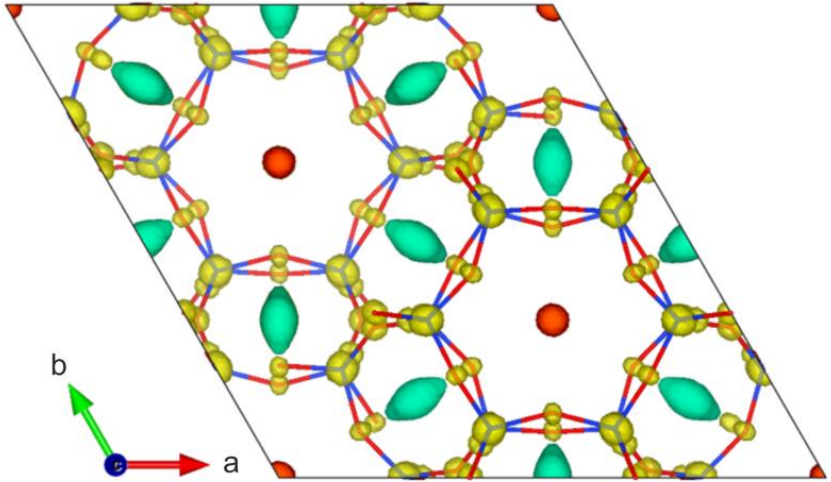
Synthesis of an industrially important zeolite in nanocrystalline form without OSDA.

Extra-framework cations: Na+, K+, Cs+.

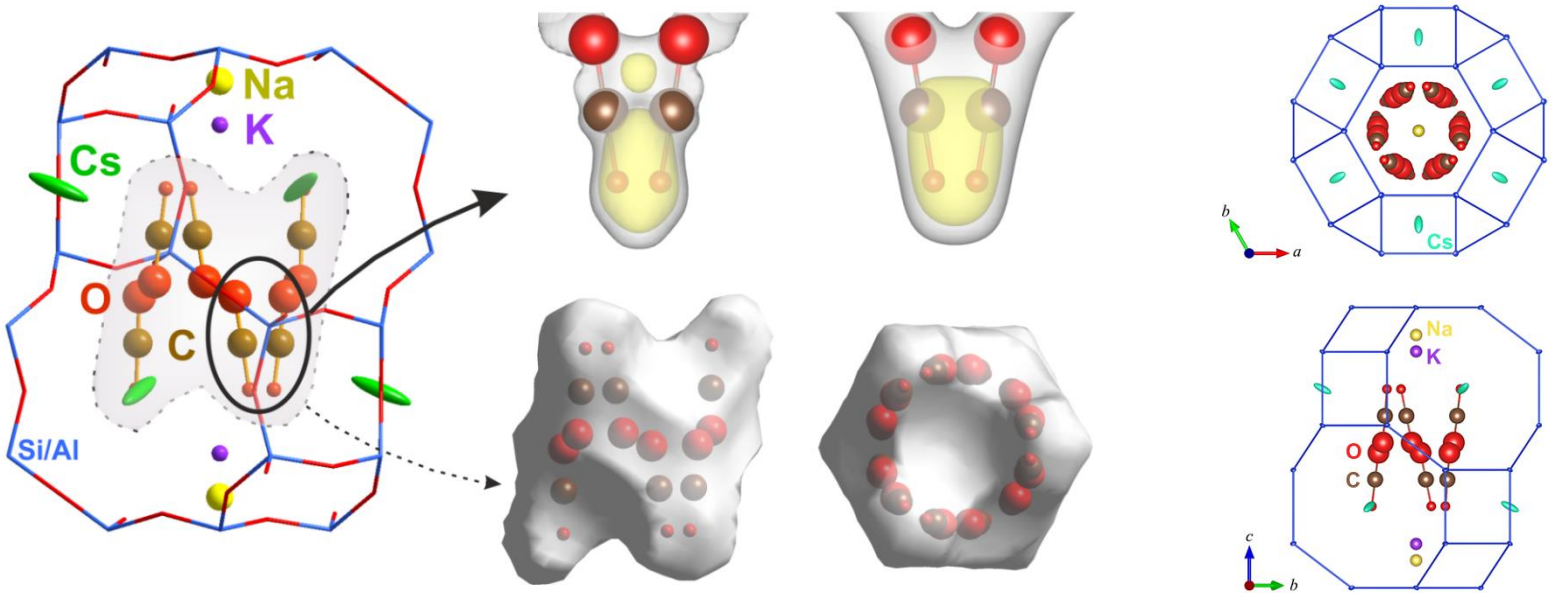
Chabazite has a very good CO_2 adsorption and selectivity towards CH_4.

Crystallographic question:
Can we locate the CO_2 molecules in the chabazite structure?





Example – CO_2-loaded zeolite



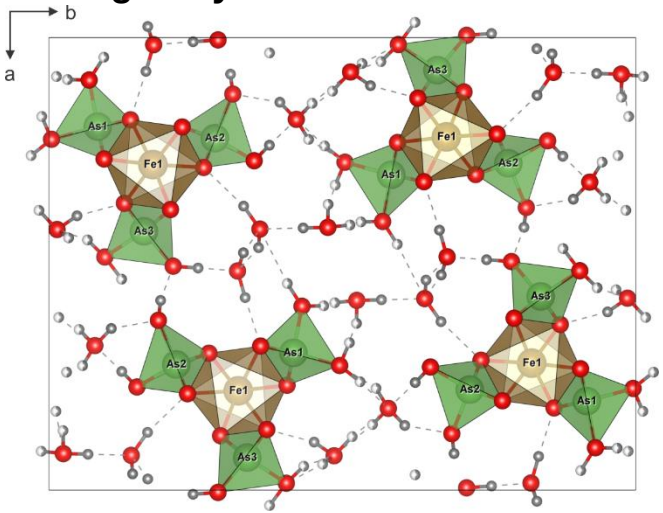
Theoretical maximum adsorption capacity: 9 CO_2 molecules per unit cell
Experimental adsorption capacity: 8 CO_2 molecules per unit cell

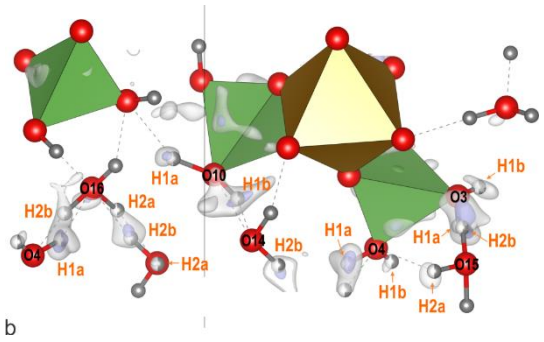
Cationic composition is crucial for the capacity and selectivity of CO_2 adsorption

Example – disordered hydrogen atoms

Hydrogen disorder in kaatialaite $Fe[AsO_2(OH)_2]\ 5H_2O$

The structure of synthetic kaatialaite known (Boudjada & Guitel, 1981) but the hydrogen sites remained **undetected from X-ray single-crystal data**.





b

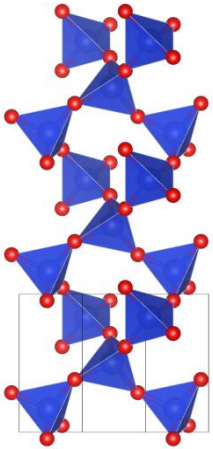
Map after dynamical refinement of the structure including the non-disordered hydrogen

22 independent hydrogen positions, out of them 12 disordered

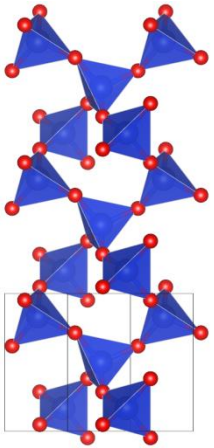
Absolute structure and absolute configuration

Absolute structure is a specification of the orientation of a non-centrosymmetric crystal structure under the operation of inversion (Online dictionary of Crystallography)

A non-centrosymmetric crystal may or may not be composed of chiral species.

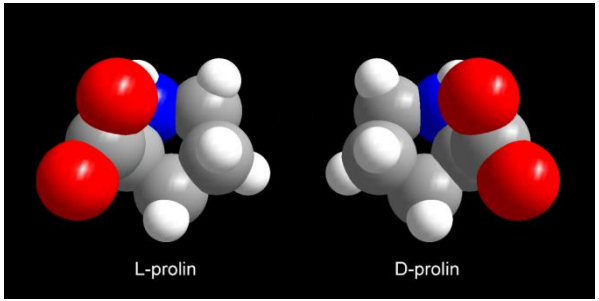


left quartz ($P3_121$)



right quartz ($P3_221$)

Absolute configuration is a specification of the spatial arrangement of atoms in a molecule containing *chiral centers.* Such molecules are not superimposable onto their mirror images. Different absolute configuration may mean (and often means) different biological function of the molecule.



Absolute configuration of molecules is most reliably and most often determined by determining the **absolute structure** of crystals containing the molecule.

Absolute structure and absolute configuration

Breaking Friedel's law

Friedel's law: In kinematical approximation, opposite structure factors have equal amplitudes: $|F_{-h}| = |F_h|$
Consequence: It is impossible to determine absolute structure from kinematical diffracted intensities

X-rays:

Resonant scattering shifts the phase of scattered photons from atoms -> Friedel's law does not hold exactly.

Strength of resonant scattering depends on the degree of non-centrosymmetricity, on the wavelength and atomic number.

Light atoms have very low resonant scattering -> difficulties in determination of absolute structure of organic species.

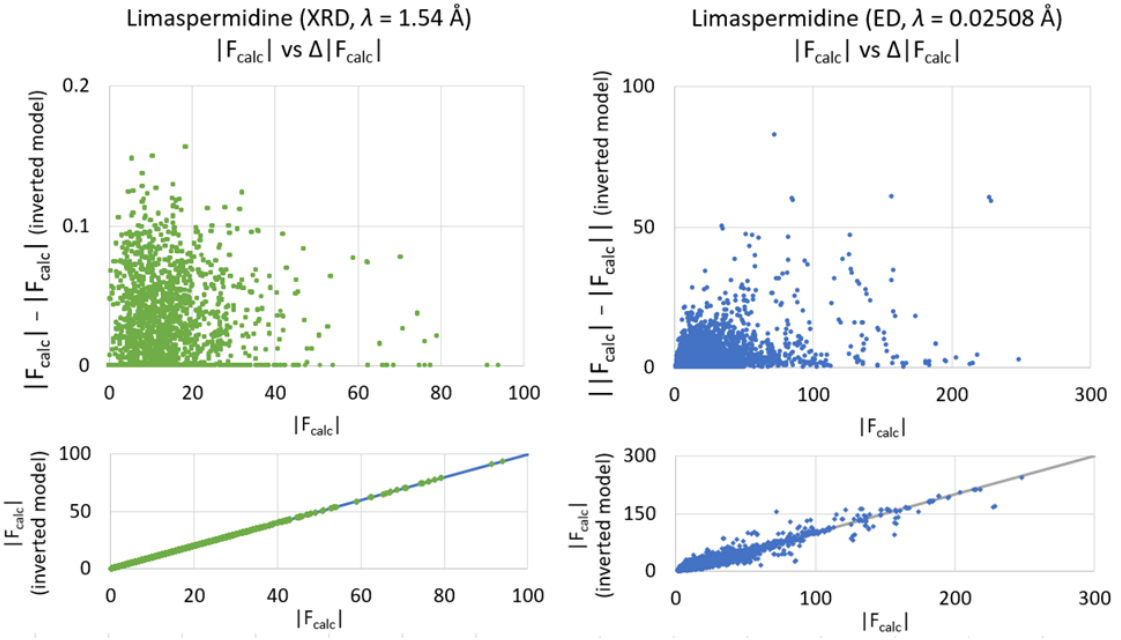
Electrons:

Electron difraction is dynamical = coherent interference of more that one diffracted beam -> Friedel's law does not hold. In three-beam approximation:

$$I_h - I_{-h} \propto F_h F_g F_{h-g} \sin\varphi$$

where φ is the sum of structure factor phases

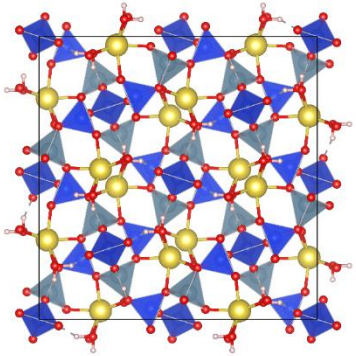
Strength of the breaking of Friedel's law depends only on the degree of non-centrosymmetricity (deviation of $\sin\varphi$ from 0) not on the atomic number. Absolute structure is equally easily determined for light and heavy atoms.



Absolute structure and absolute configuration

Absolute structure determination

Natrolite
$Na_2[Al_2Si_3O_{10}]\cdot 2H_2O$
*Fdd*2
V = 2252 Å³
T = 293 K



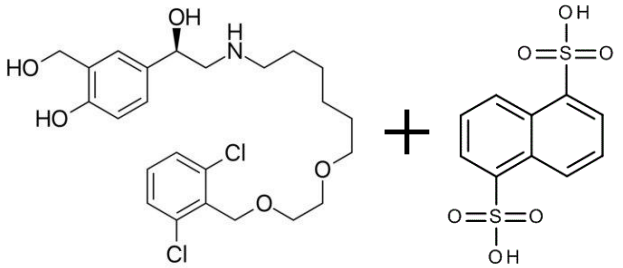
Dynamical refinement:

Correct enantiomorph: Robs = 5.95%

Wrong enantiomorph: Robs = 15.81%

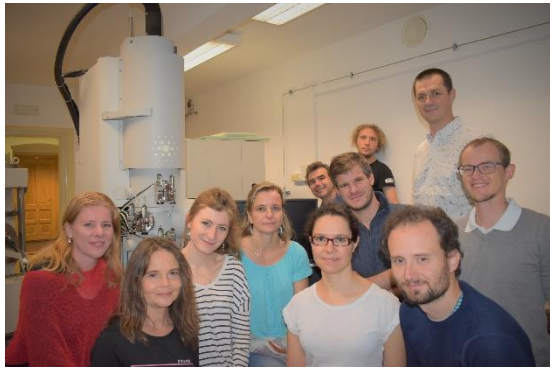
z-score (confidence level): **36.13σ**

vilanterol + 1,5-naphthalenedisulfonic acid



Dataset	R(obs) (%) (R-form)	R(obs) (%) (S-form)	Confidence sigma level (z-score)	Probability of *R*-form (%)
1	10.93	12.48	5.809	100.000
2	11.21	12.71	8.970	100.000
3	11.16	12.17	7.727	100.000
4	10.82	12.06	8.471	100.000
5	11.91	13.04	6.931	100.000
6	12.18	12.57	5.011	100.000
Comb.			**17.336**	100.000

Acknowledgment



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Summary

- ✓ Structure determination by 3D electron diffraction methods has become an almost routine method applicable to all classes of materials
- ✓ Many applications can be done on almost any commercial TEM
- ✓ Optimized TEM setup and software customizations and needed for the best results
- ✓ Dedicated instruments are available on the market, making the method much more easily accessible
- ✓ Many challenges remain. There is a potential for further significant improvement of the method
- ✓ Take-home-message: 3D ED can solve many hard problems in material science. Do not lose time attacking these problems with „familiar“ methods, spend it rather on learning 3D ED (or contact someone to help you)…