H2020-MSCA ITN
Grant n. 956099

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DI TECNOLOGIA

WP1 – 3D ED techniques

Mauro Gemmi – Istituto Italiano di Tecnologia

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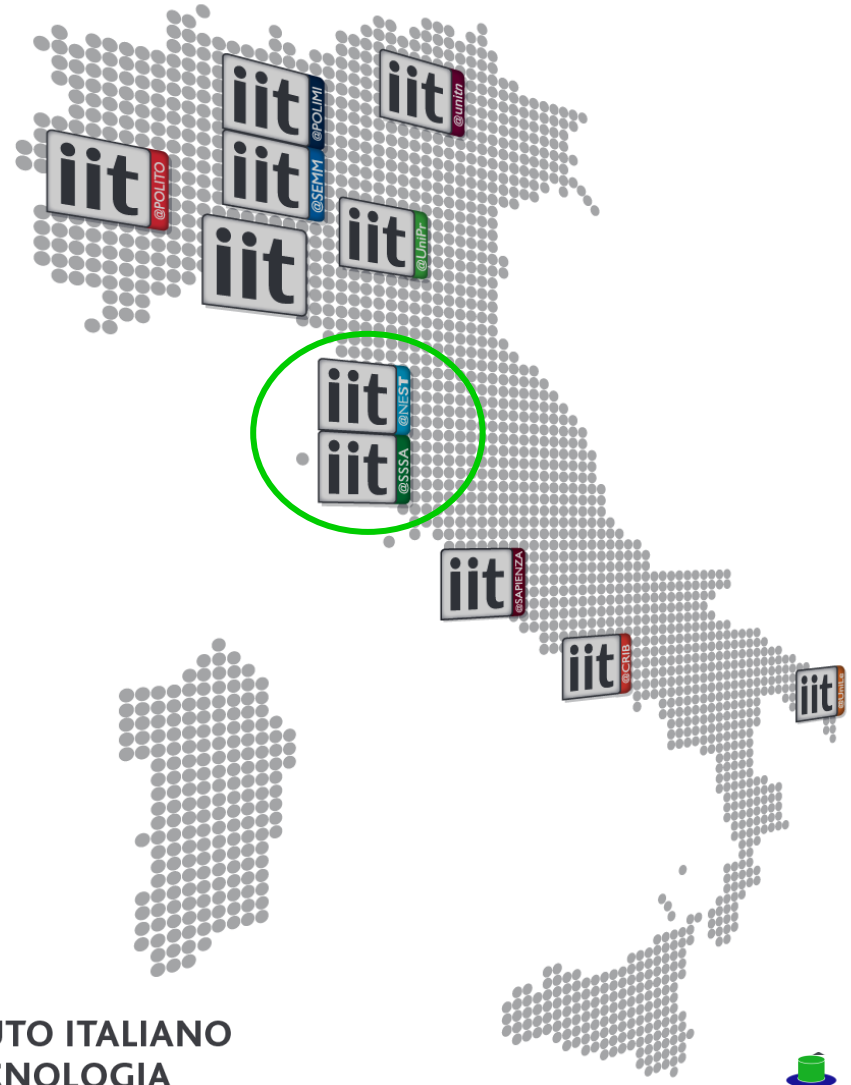
“is intended to promote Italy's technological development and advanced education, consistent with national policies for scientific and technological development, thus strengthening the national production system”



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ELECTRON CRYSTALLOGRAPHY

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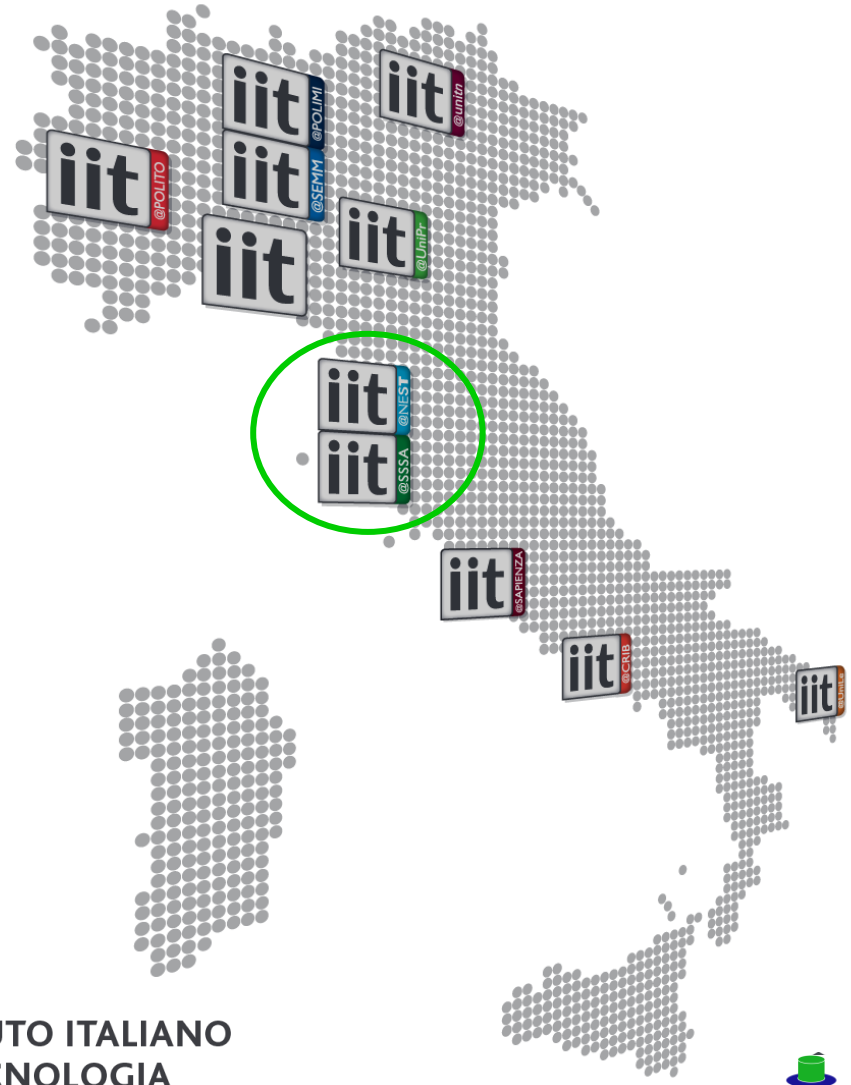

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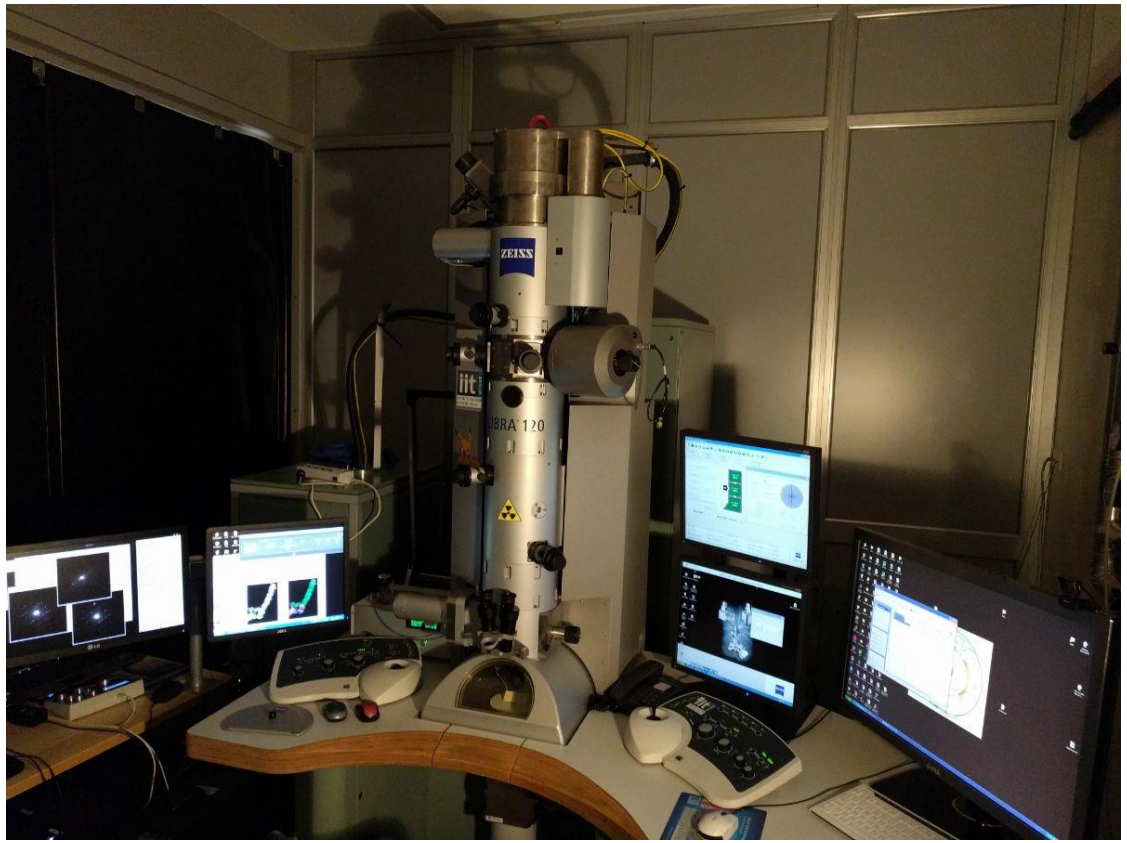
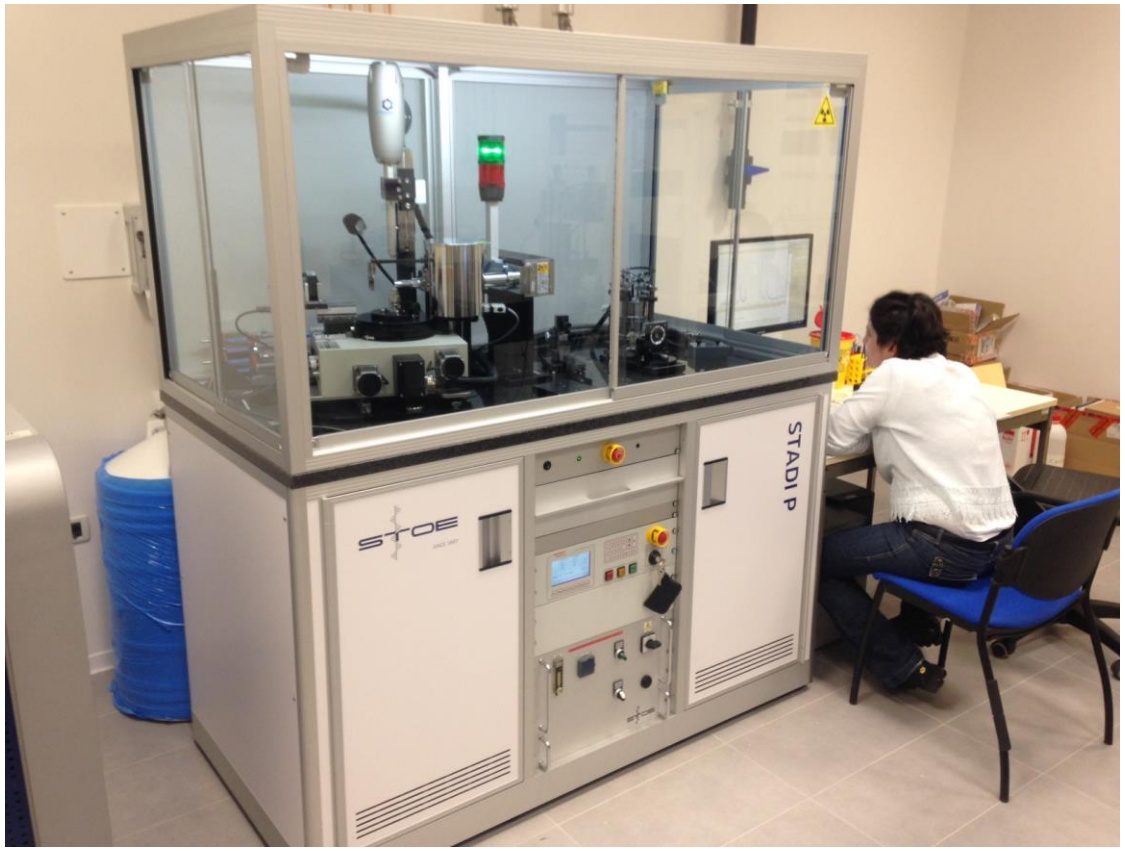
INSTRUMENTS



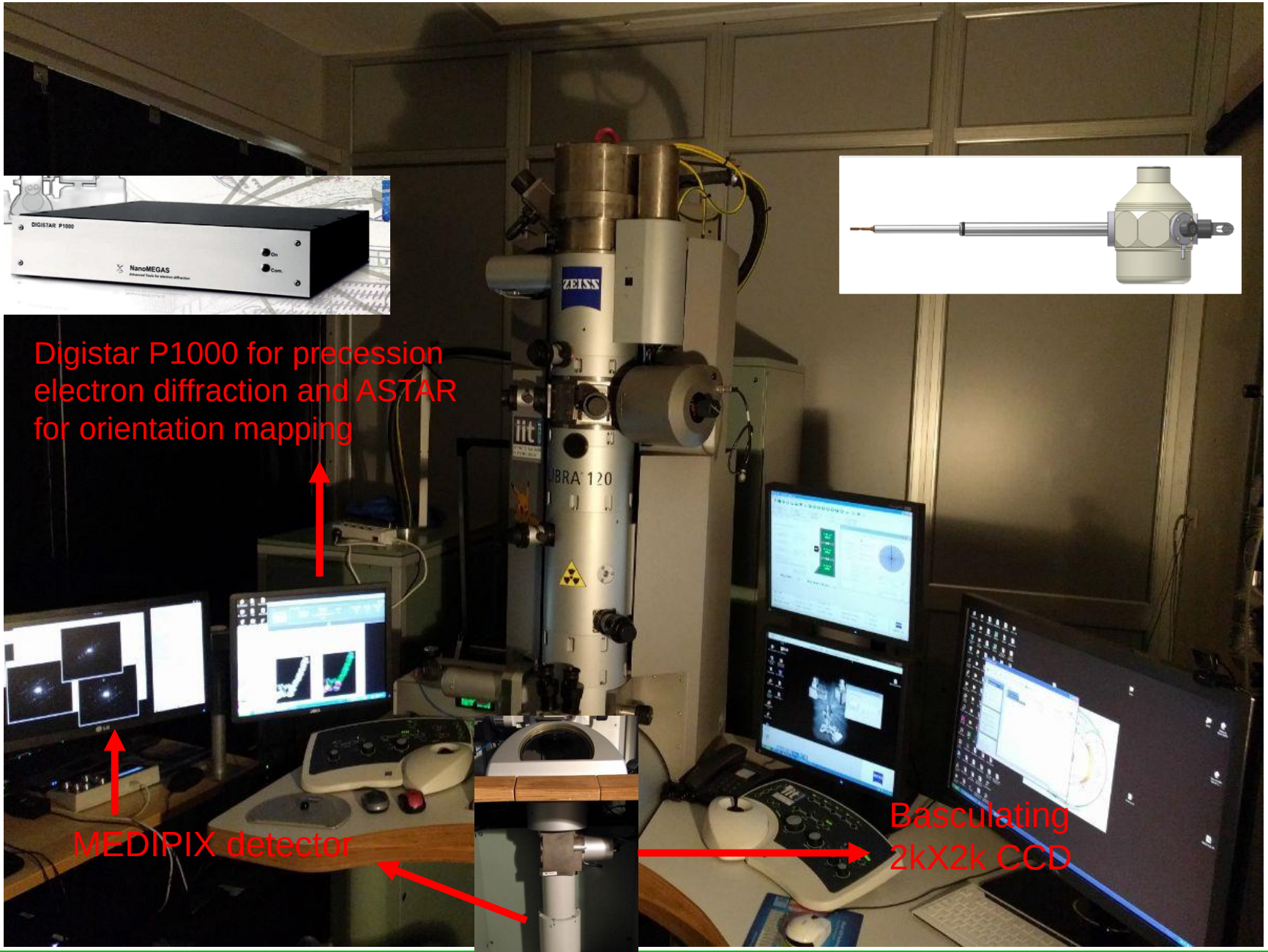
Zeiss Libra 120 TEM

120 kV instrument

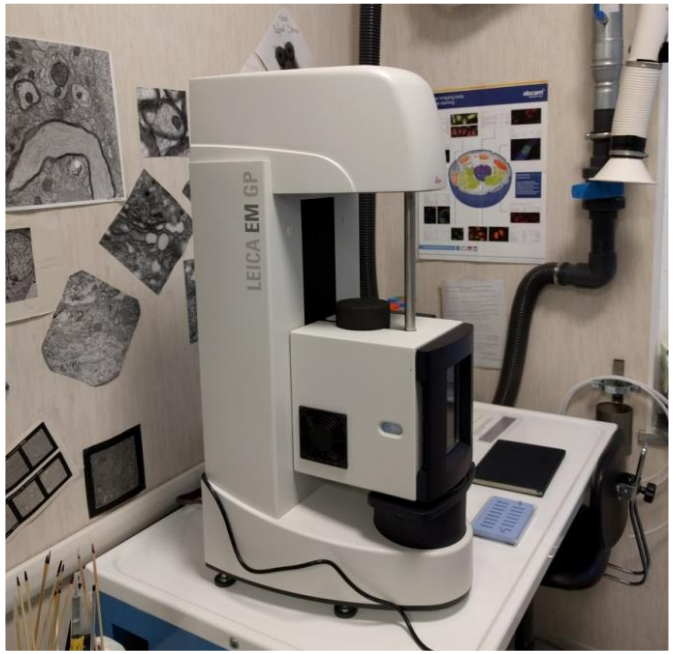
with in column energy filter



Stoe Stadi P diffractometer


DIGISTAR P1000
NanoMEGAS
Digistar P1000 for precession electron diffraction and ASTAR for orientation mapping
MEDIPIX detector
Basculating 2kX2k CCD

Sample Preparation



Leica Cryo plunging system

Leica UC7 Cryo ultramicrotome

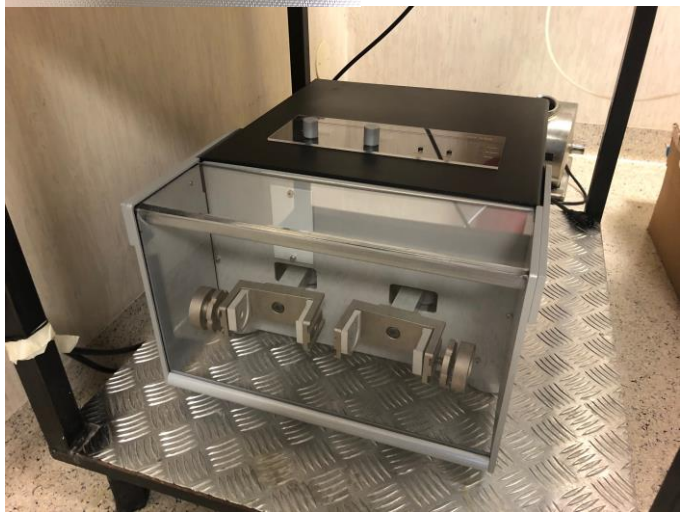
Synthesis Facility

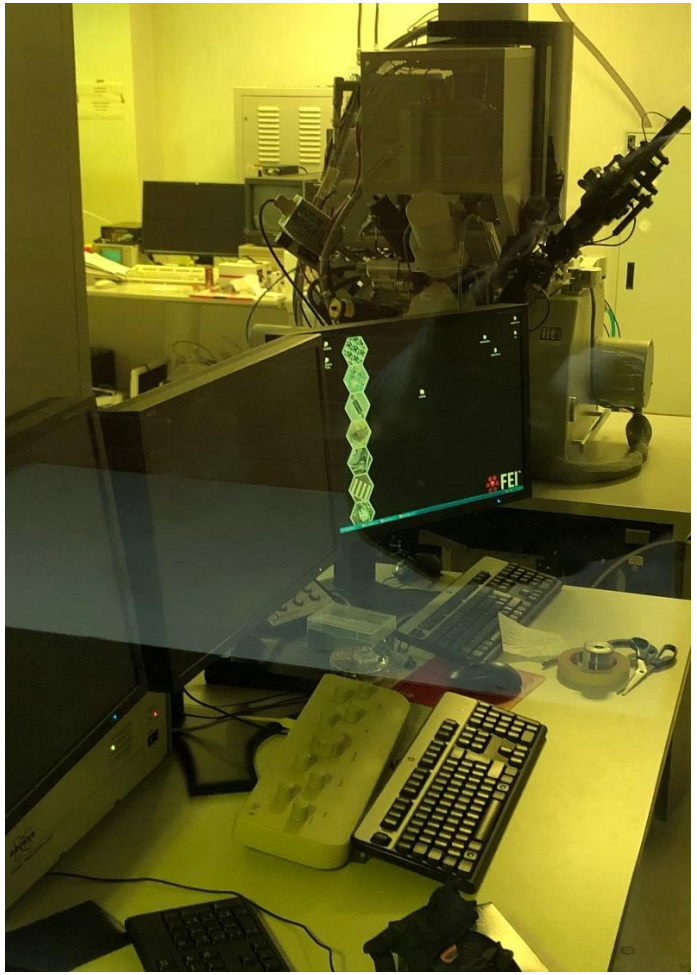
Owen
1 HT Owen maximum T=1650°C
1 medium T Owen T=650°C

Shaker for Mechanochemical synthesis

Chemical synthesis facility

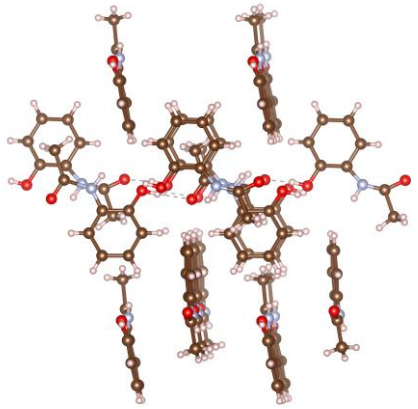
Chemical characterization facility





FIB ThermoFisher Helios 600

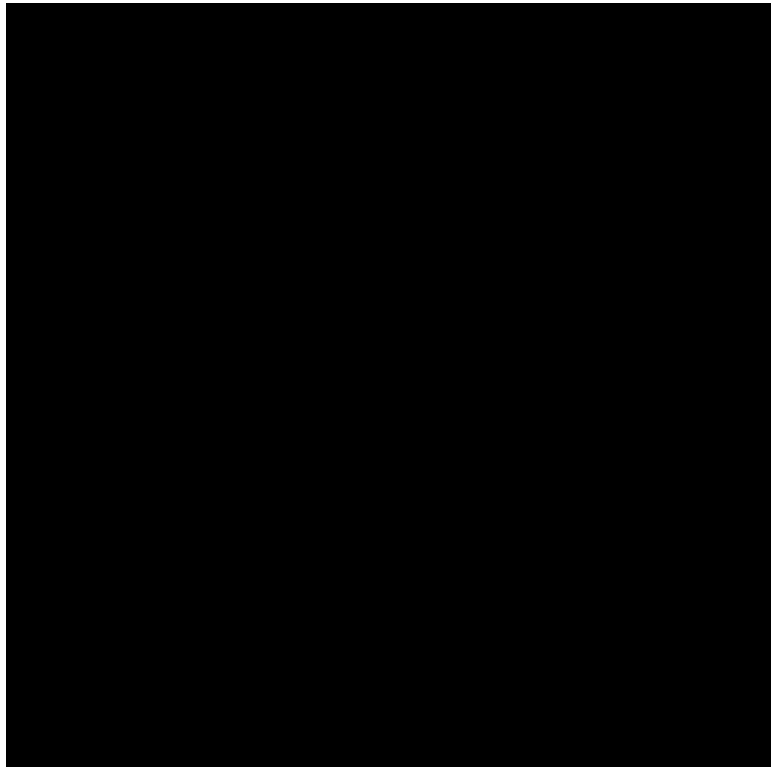
In the head quarter in Genova we will have access to a ThermoFisher Spectra 300 fully equipped.



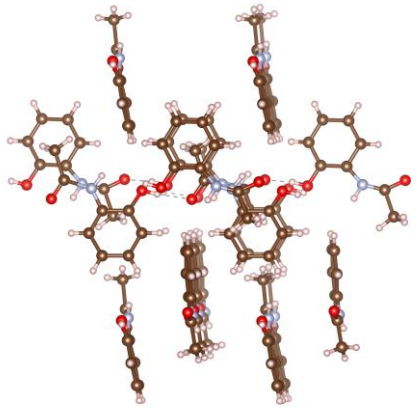
WP1 Beam and vacuum sensitive materials

IIT

WP1 aims at the seting-up of sample preparation, data acquisition and data reduction strategies for an efficient structure characterisation of beam and vacuum sensitive materials.



- ❑ Fast and low dose data collection
- ❑ Fast crystal search strategies
- ❑ Special strategies of sample preparation to protect beam and vacuum sensitive samples



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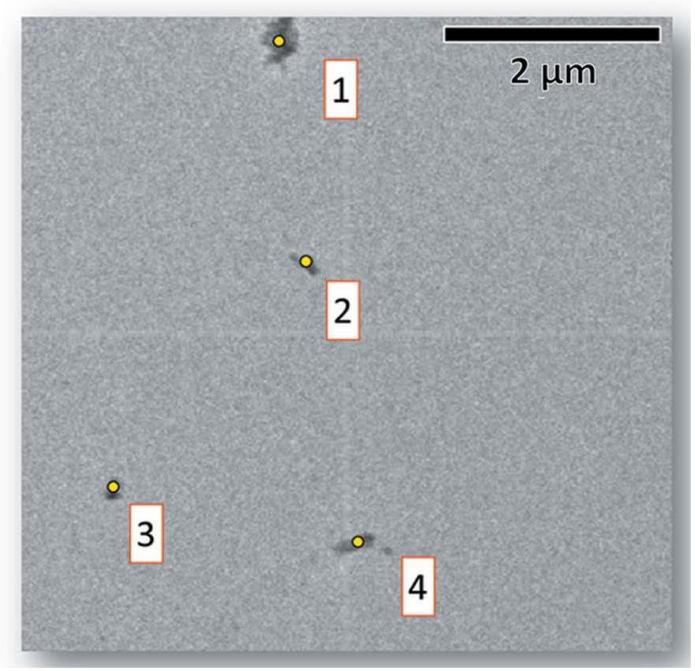

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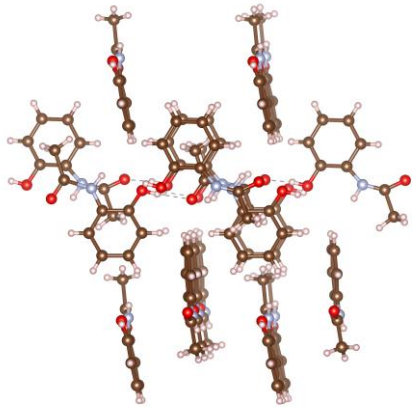

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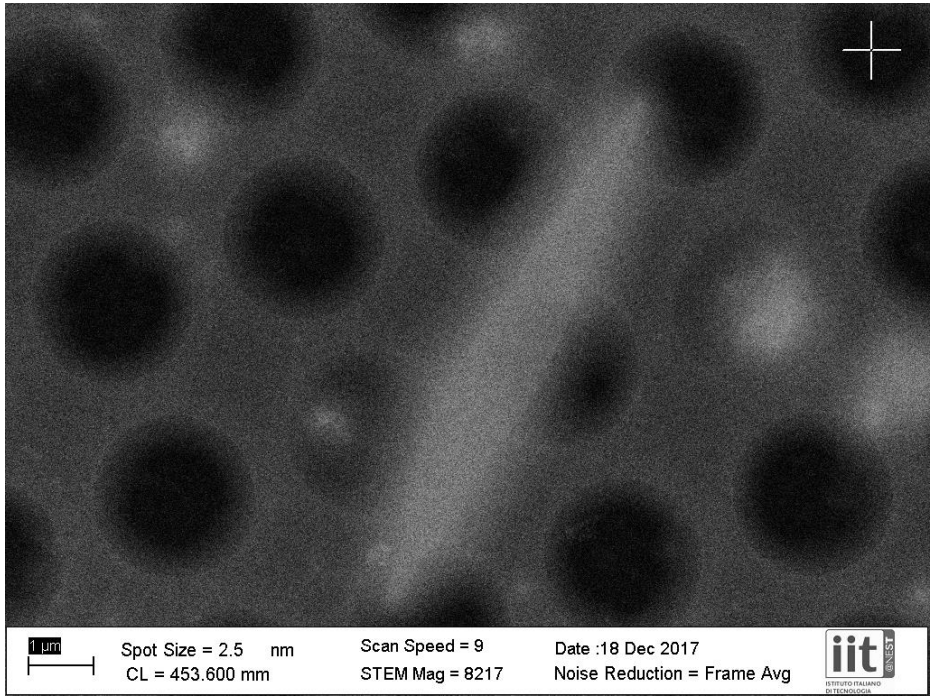


- ❑ Fast and low dose data collection
- ❑ Fast crystal search strategies
- ❑ Special strategy of sample preparation to protect beam and vacuum sensitive samples

WP1 Beam and vacuum sensitive materials

T1.1: Low-dose data collection protocols (**ESR1/IIT, ESR2/IIT, ESR8/JGU, ESR11/SU**)

T1.2: Reducing vacuum and beam sensitivity through sample preparation (**ESR1/IIT, ESR8/JGU**)

T1.3: Structure solution, polymorphism determination and high throughput data collection for pharmaceutical and other beam sensitive compounds (**ESR1/IIT, ESR2/IIT, ESR11/SU**)

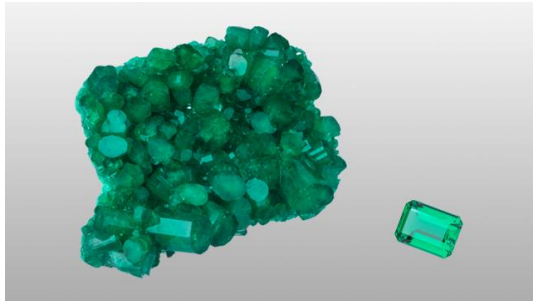
T1.4: Standardization of 3D ED data collection protocols (**All ESR**)

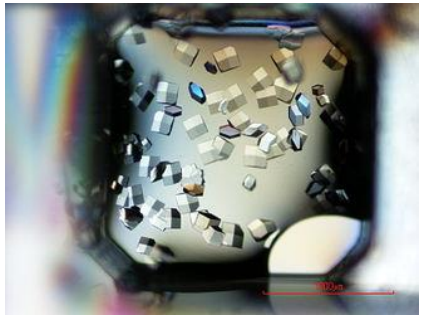
In collaboration with IUCr we need to create specific entries for structure solved and refined with 3D ED data that will be inserted in the CIF vocabulary

The final goal is a CIF fully compatible and able to store all the deatils of data collection and analysis in case of 3D ED data.

What is all about?

Natural or synthetic unknown crystal

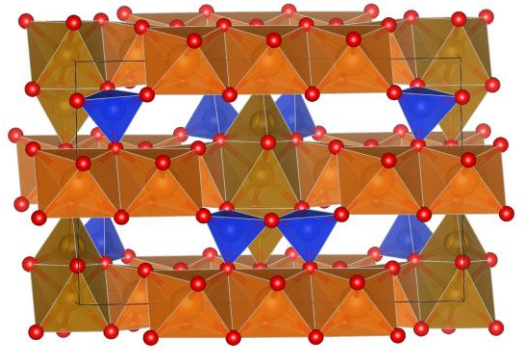


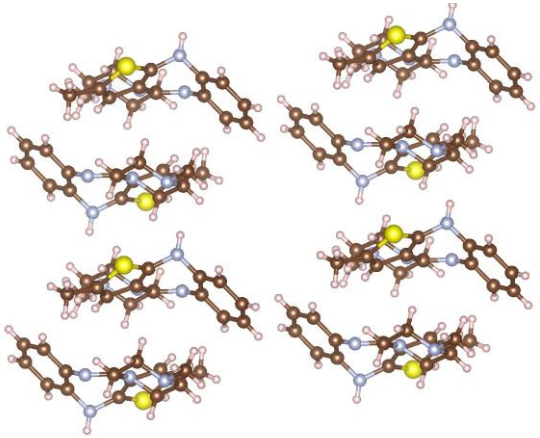


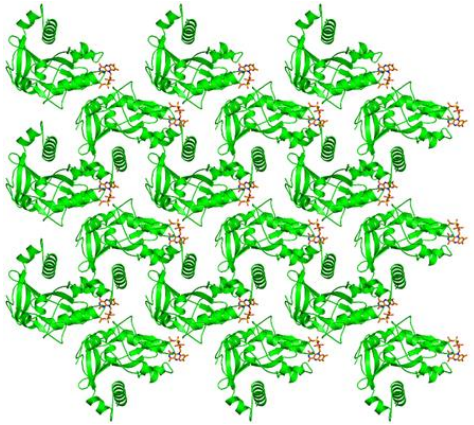
THE CRYSTALLOGRAPHIC PROBLEM

Having a minimal knowledge:
Tentative chemical composition
Maybe we have the molecule…..

When the crystals get nano!!!!!

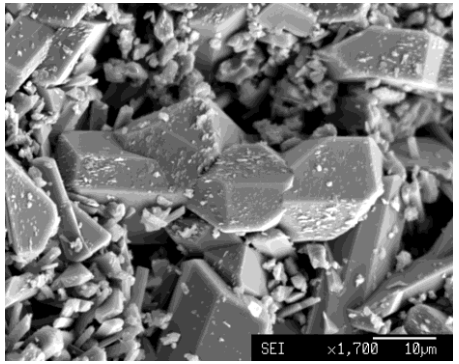


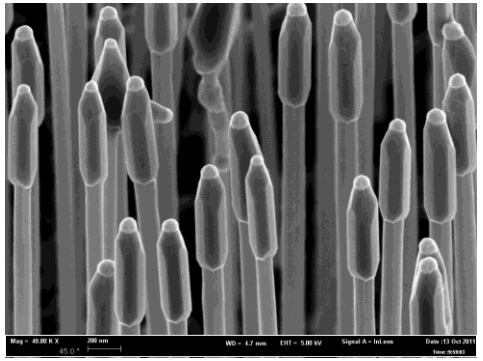




Electron diffraction for investigating a crystal structure

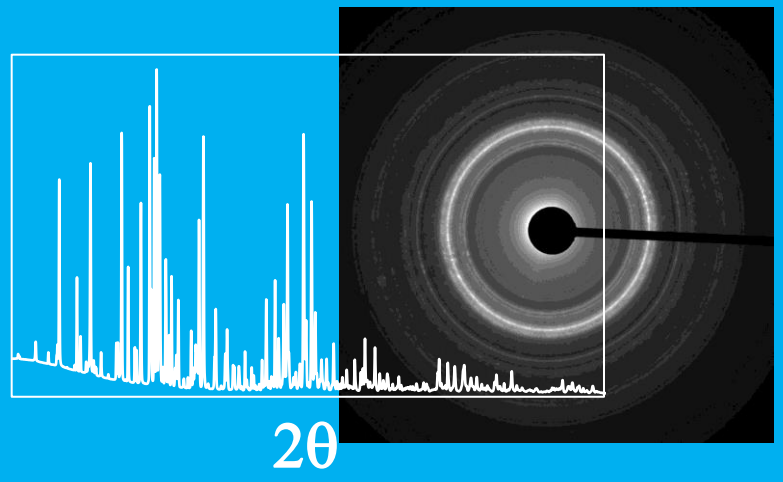
S
I
Z
E





Micro → Nano

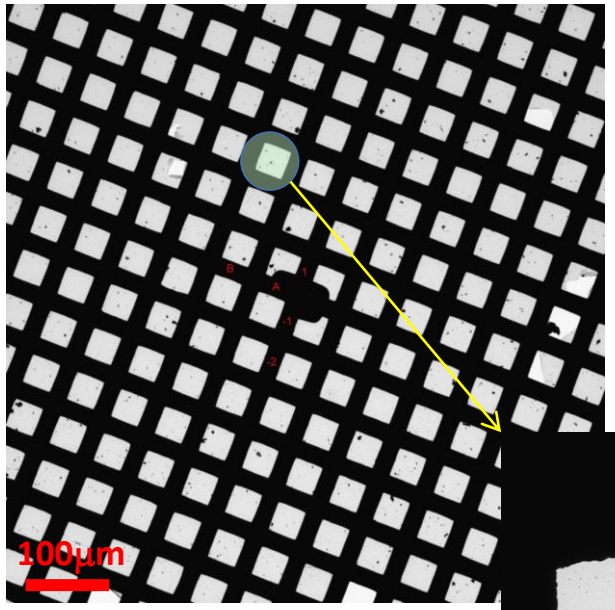
h	k	l	Intensity	Sigma
0	8	1	700.91	2.65
0	10	1	936.4	3.06
1	-10	-1	233.79	1.53
1	-9	-2	318.47	1.78
1	-9	0	16.54	0.41
1	-8	-1	381.32	1.95
1	-8	0	18.05	0.42
1	-7	-2	381.29	1.95

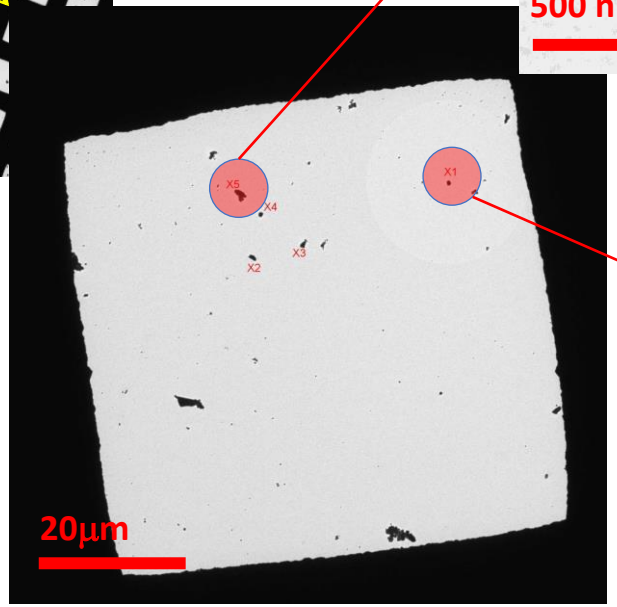


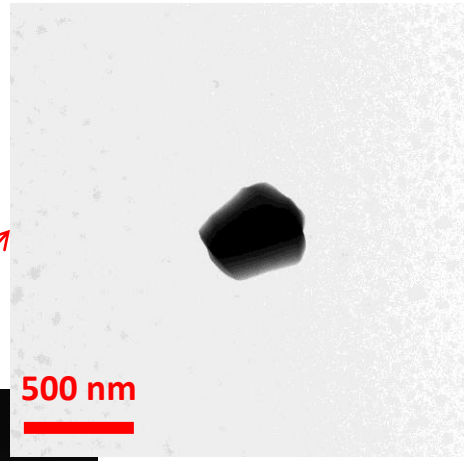
Single crystal Diffraction → Powder Diffraction

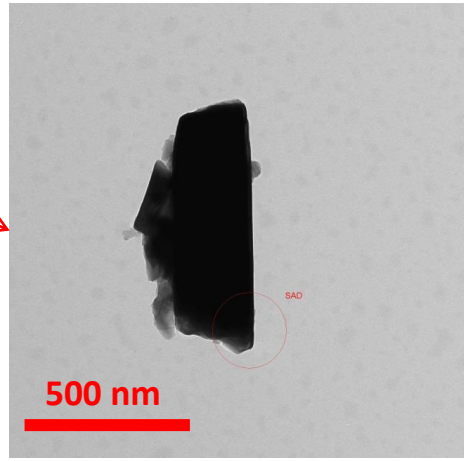
X-ray Lab	30 μm
Synchrotron	1μm
Electrons	100 nm

In a tem we can analyse crystal by crystal……minimum size for an ADT exp is in the range of 100nm



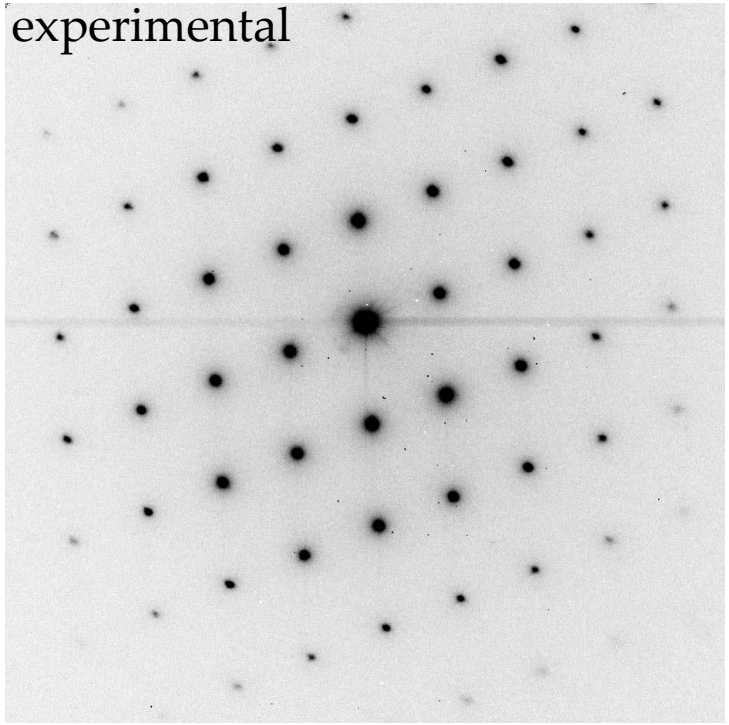


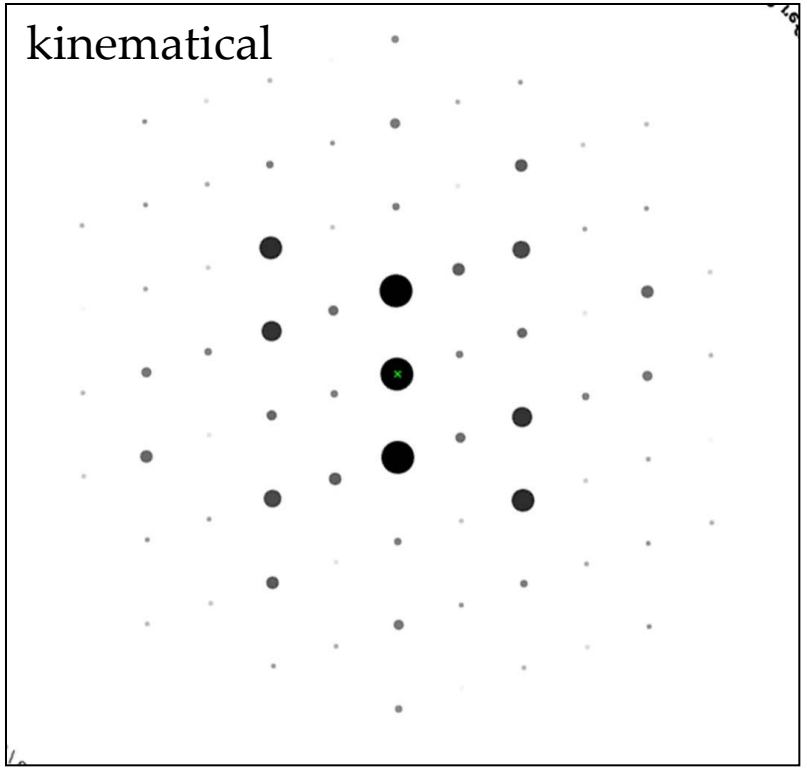




We have a single crystal diffractometer for nanometric crystals

Dynamical effects (no first order Born Aprrox.)

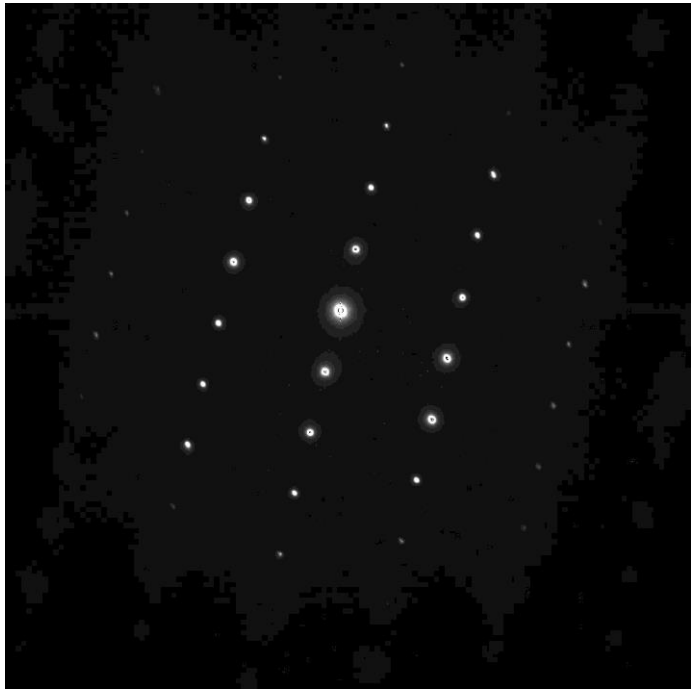




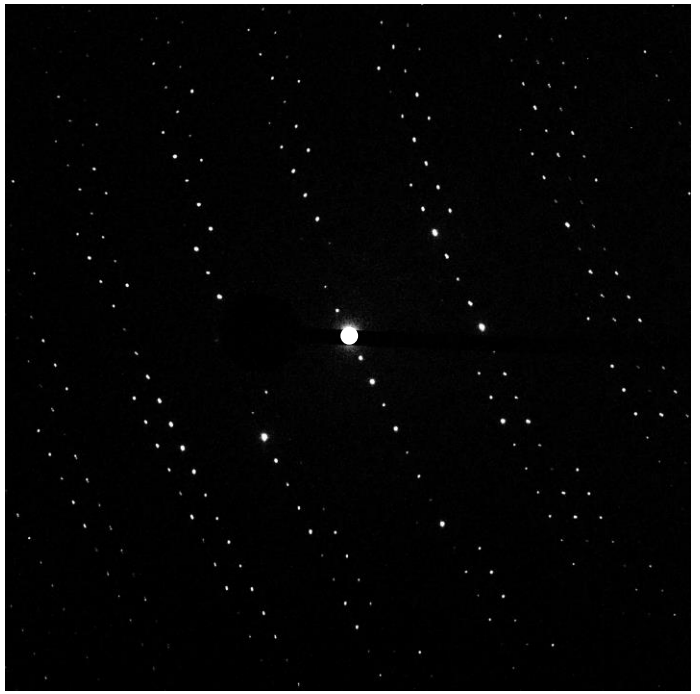
[100] Calcite ($CaCO_3$)

- distribution of intensities completely altered
- the reflections intensities become very similar

$$I(hkl) = \left|Fourier(V(\vec{r}))\right|^2$$

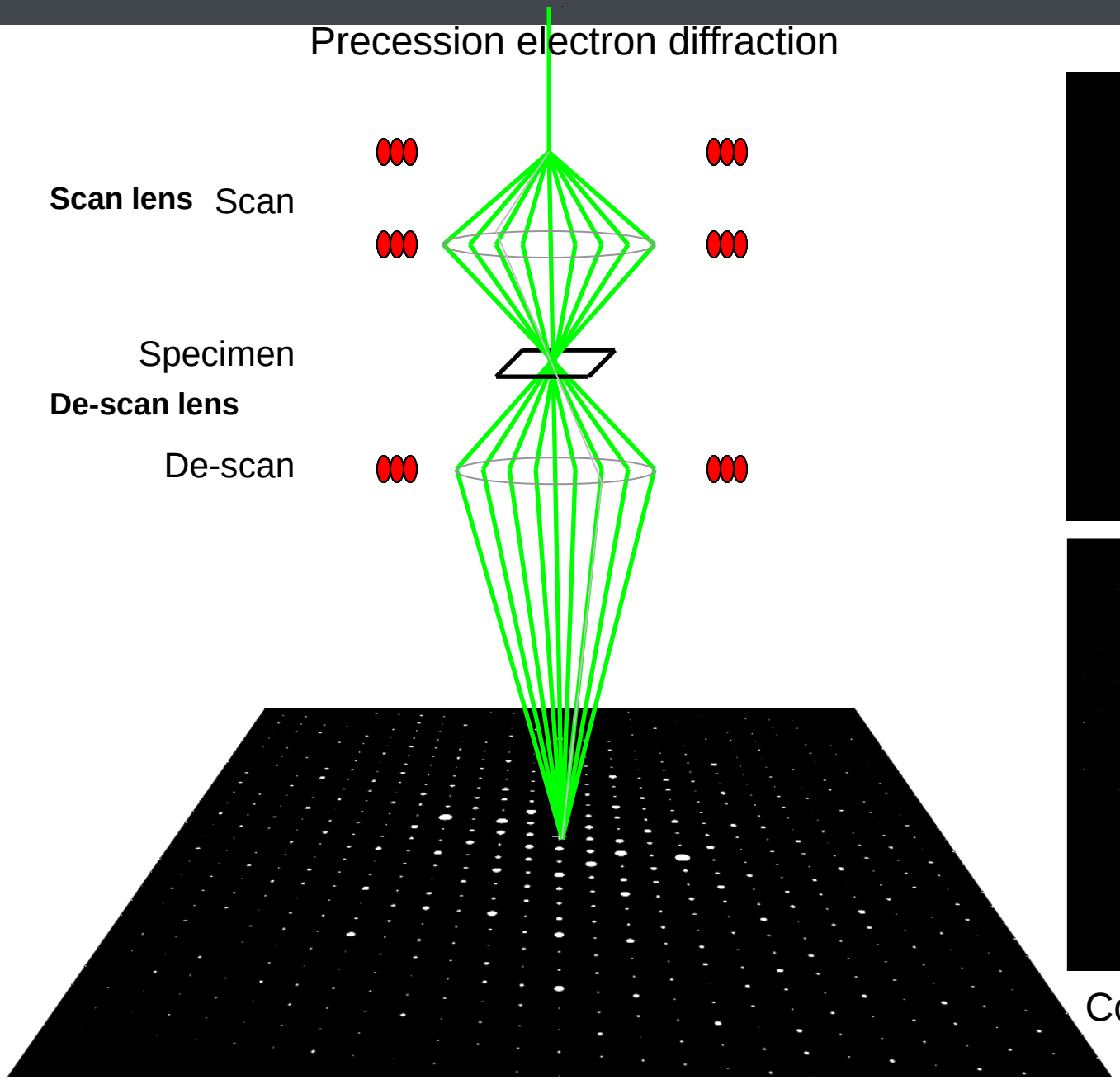


Oriented Zone axis

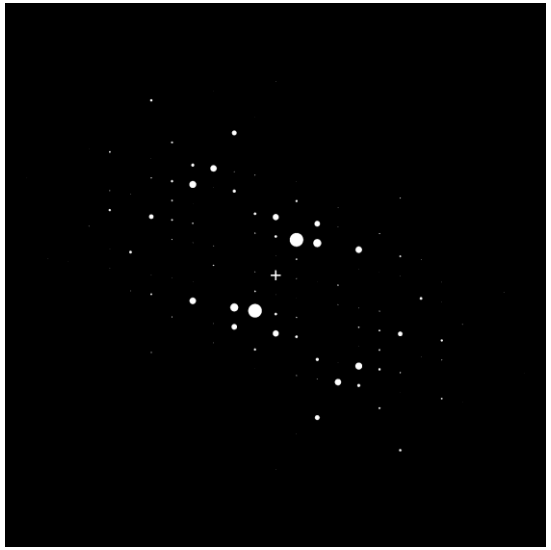


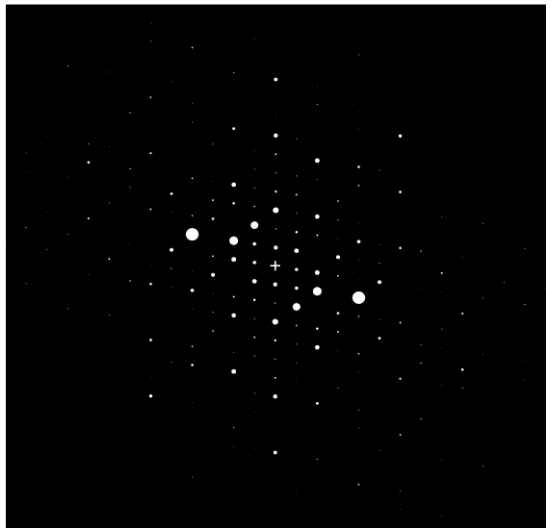
Not oriented

Precession electron diffraction



Reference : C.Own PhD thesis



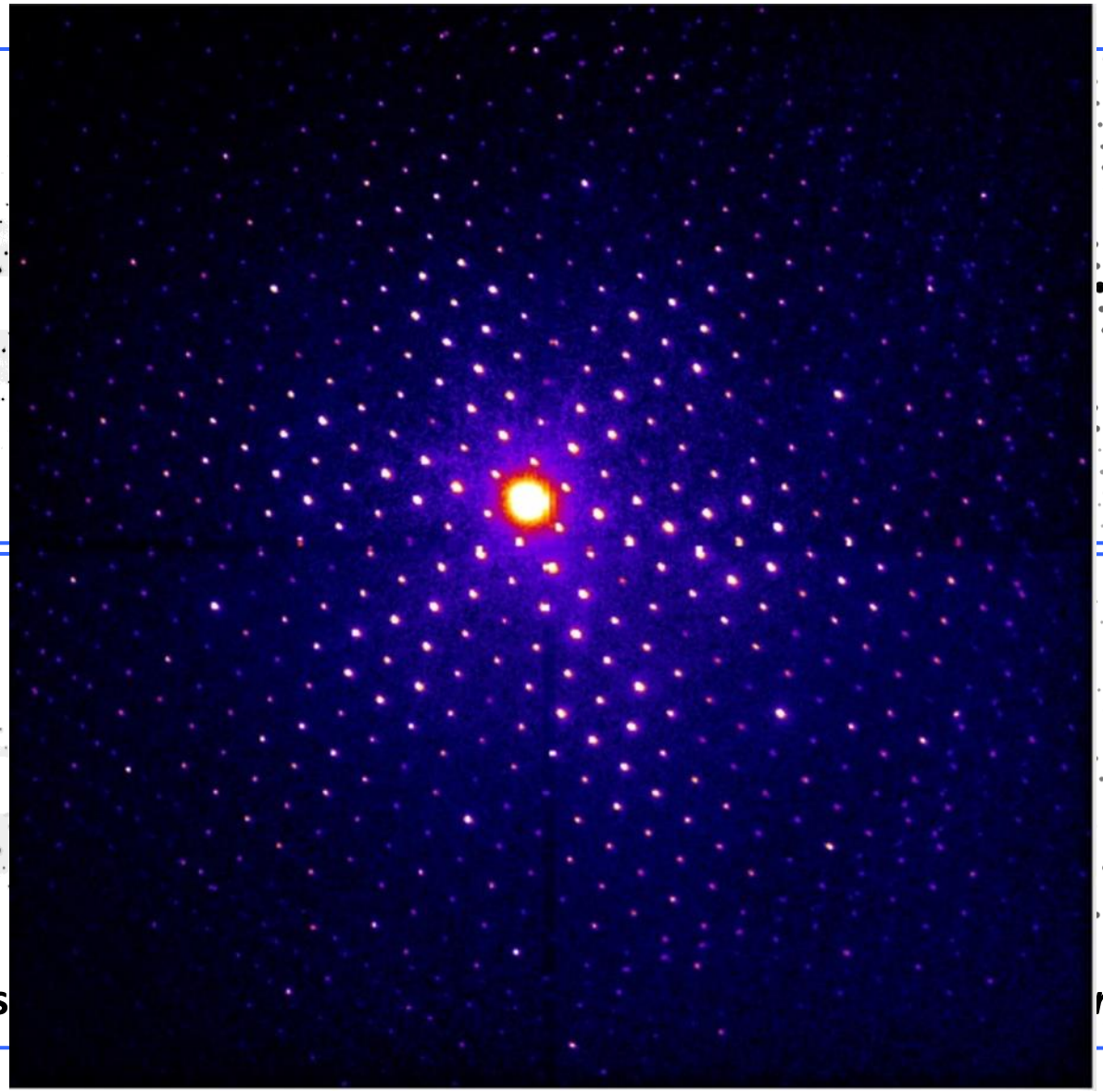


Co… Precession…

Vincent and Midgley Ultramicroscopy 53 (1994) 271.

From Standard SAED to PED

[111]

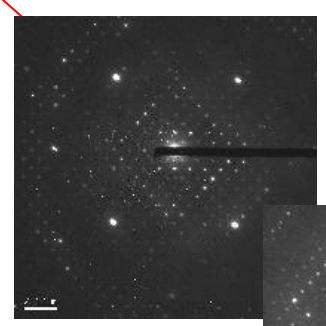


[100]

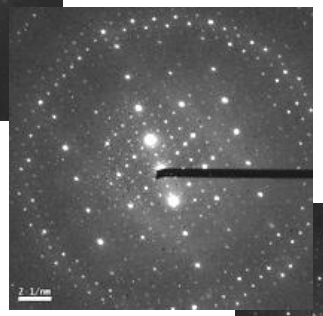
Precess

ray like)

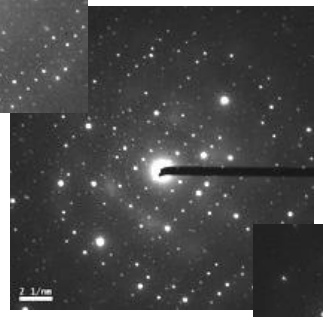
Zone Axis Patterns



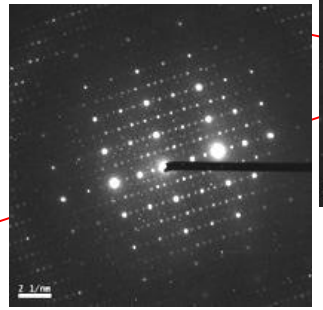
diff18_25: **28,5°**



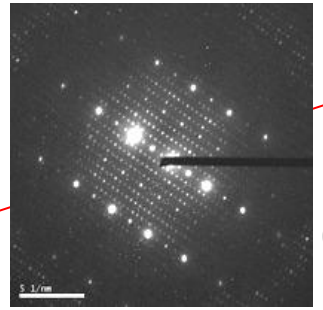
diff21_26: **22,2°**



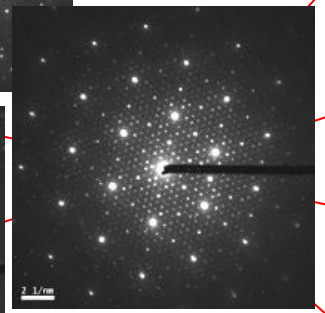
diff18_26: **15,1°**



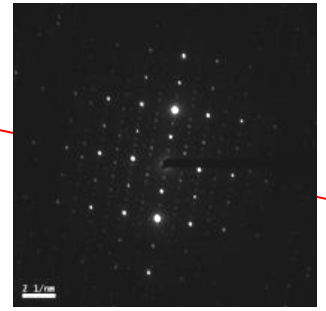
diff16_26: **19,5°**



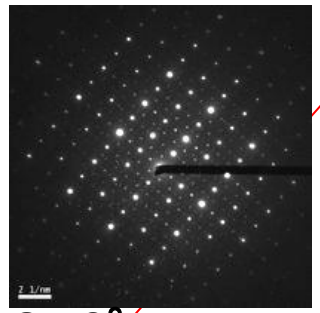
diff3_26: **29,0°**



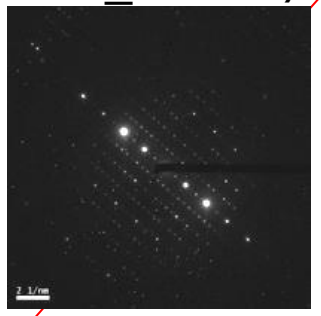
diff19_26: **0°**



diff2_27: **-11,6°**



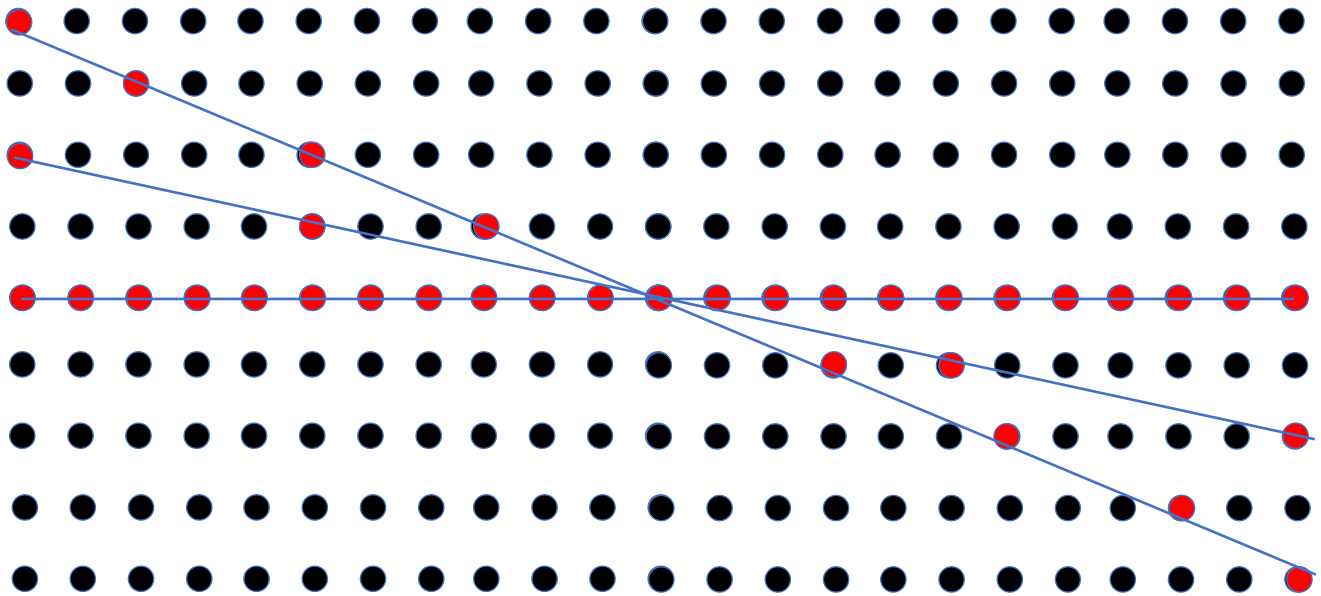
diff20_26: **-33,5°**



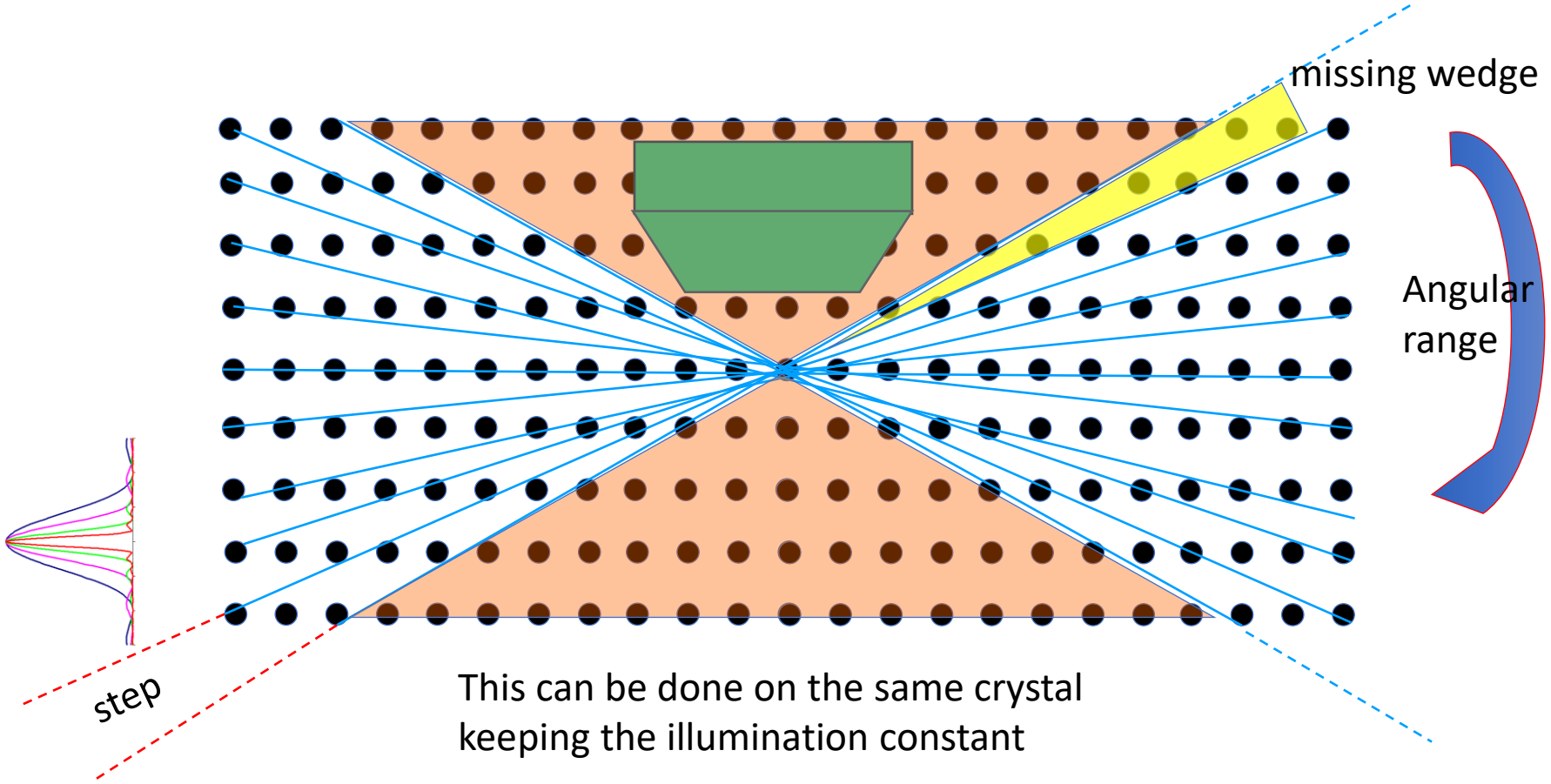
diff17_26: **-21,0°**

Limitations:
Reciprocal space coverage
Rescaling

How to scan the reciprocal space



Electron Diffraction Tomography



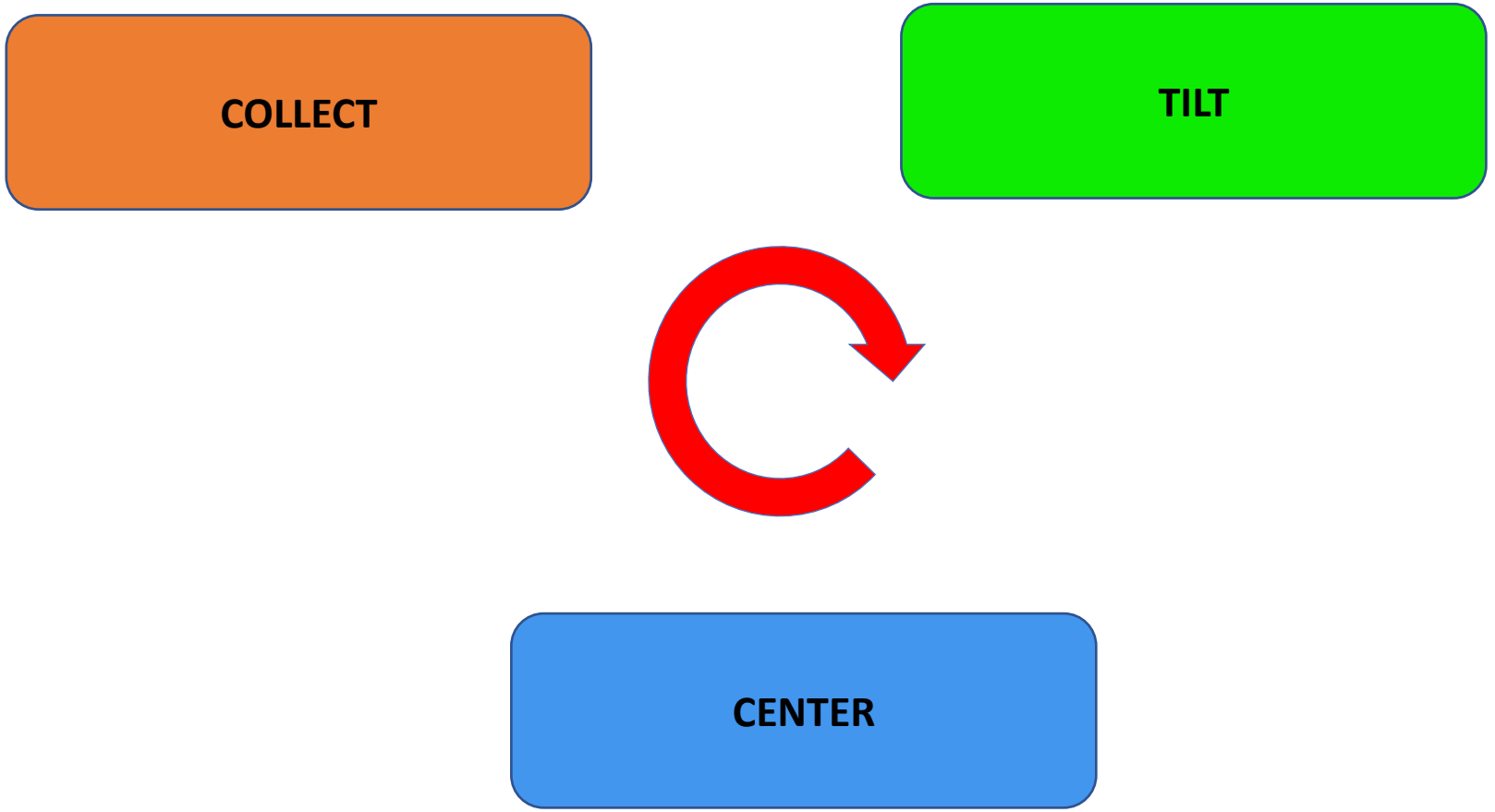
This can be done on the same crystal keeping the illumination constant

Dynamical effect ok
Excitation error to be solved

Angular range to maximize
Crystal moves

Kolb et al Ultramicroscopy 107 (2007) 507.

EDT FLOW CHART



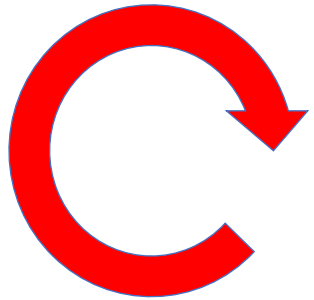
EDT FLOW CHART

COLLECT

Collect a diffraction Pattern	SAED Nano diffraction	Precession mode Stationary beam

TILT

Tilt the crystal	Mechanical Electrical	1°-2° 0.01°

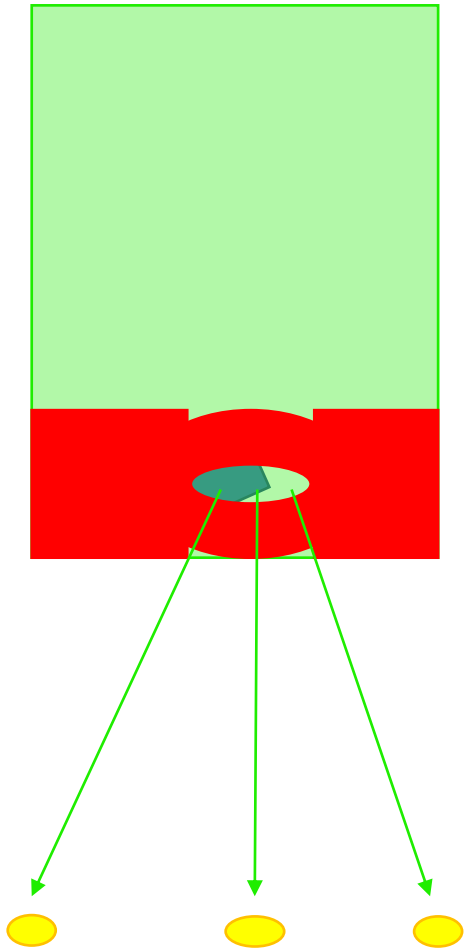


CENTER

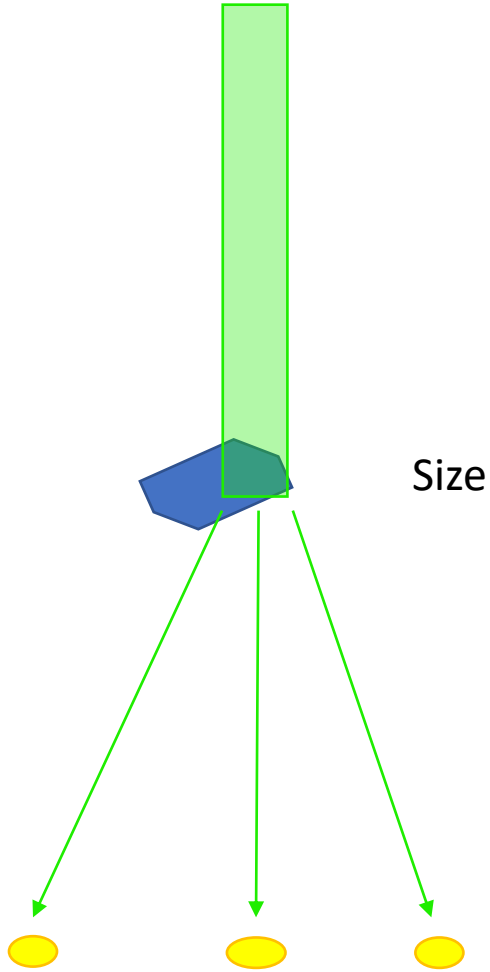
Center the crystal	Manually Automatically	TEM STEM

SAED vs. NANODIFFRACTION

Size > 100nm



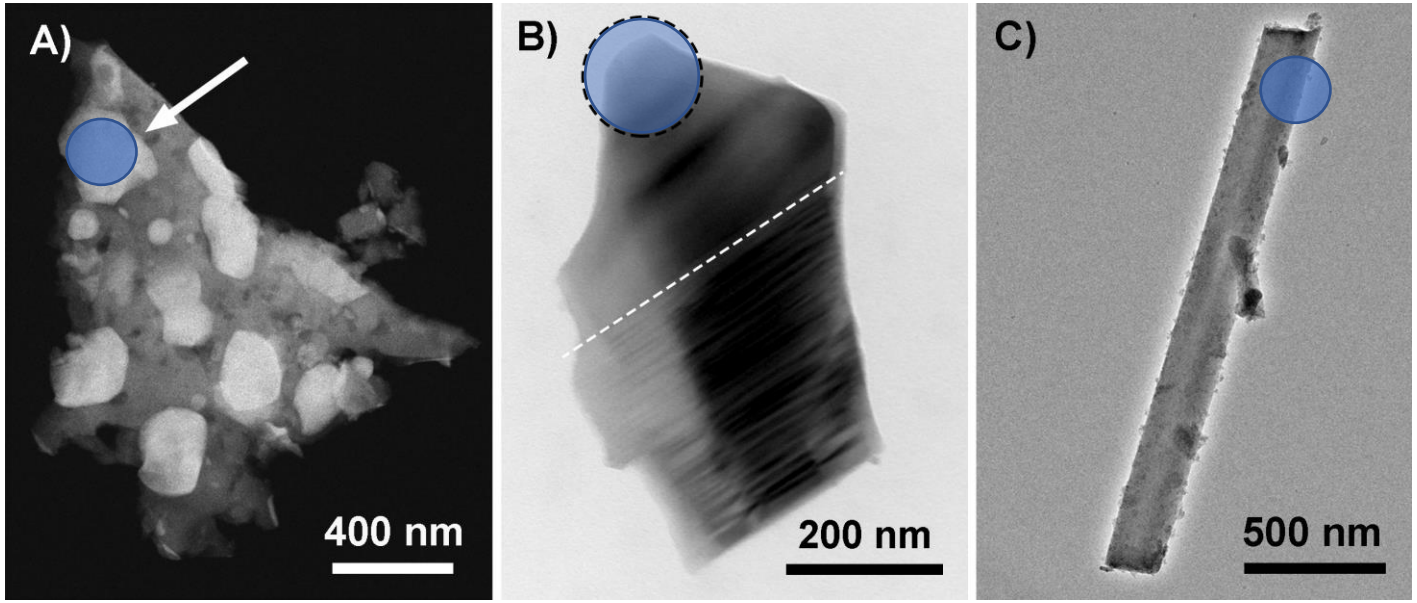
Effect of spherical aberration (aperture delocalization)
Illumination of a larger area



Size > 50 nm or less

Imaging difficult to handle in TEM mode

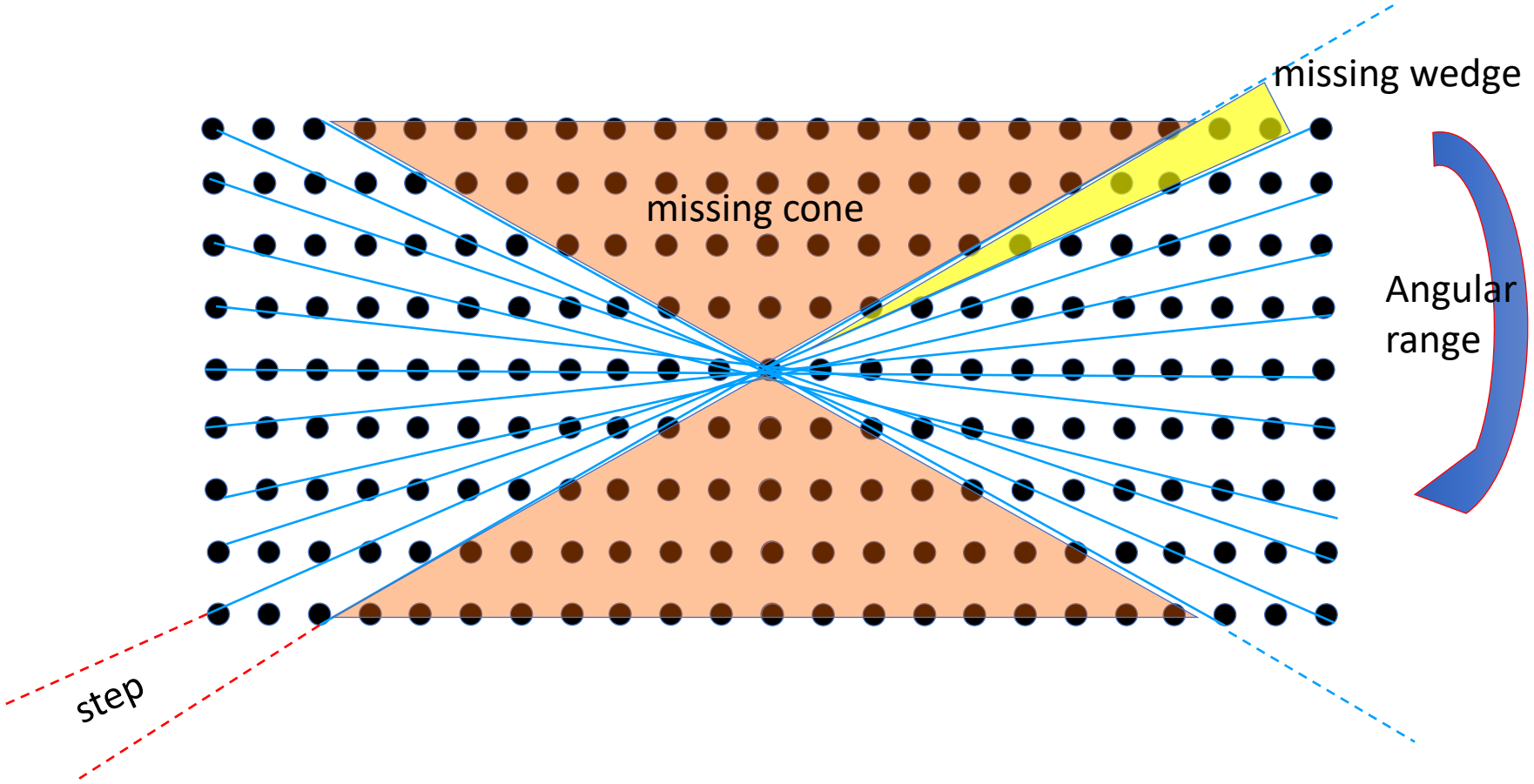
3D Nanodiffraction



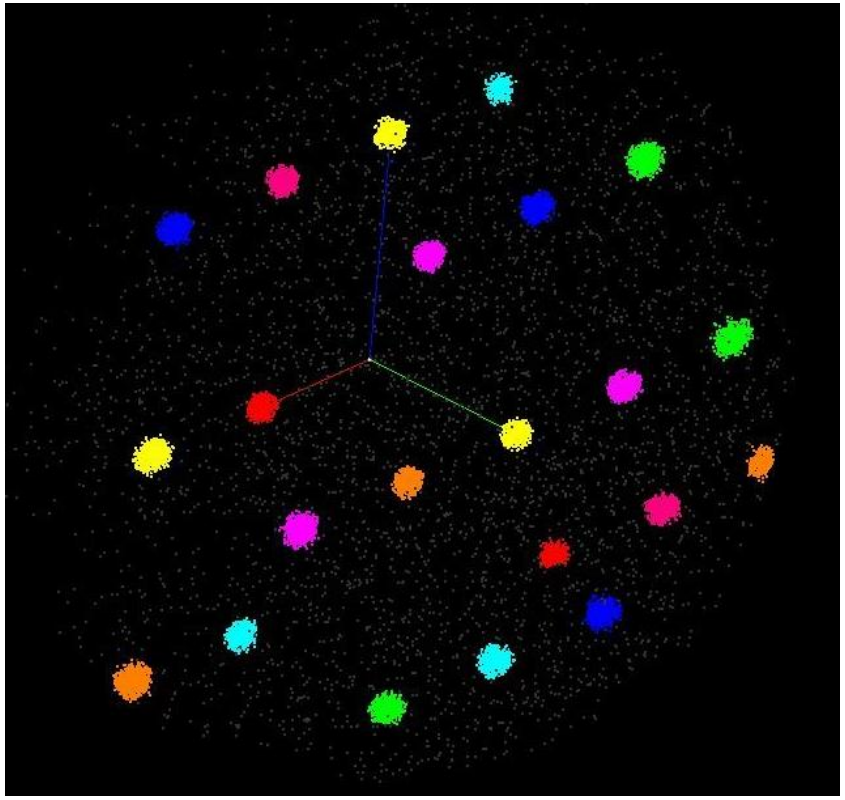
Local crystallography

Intergrowth – Twinning – Order disorder – Polytipism

Electron Diffraction Tomography

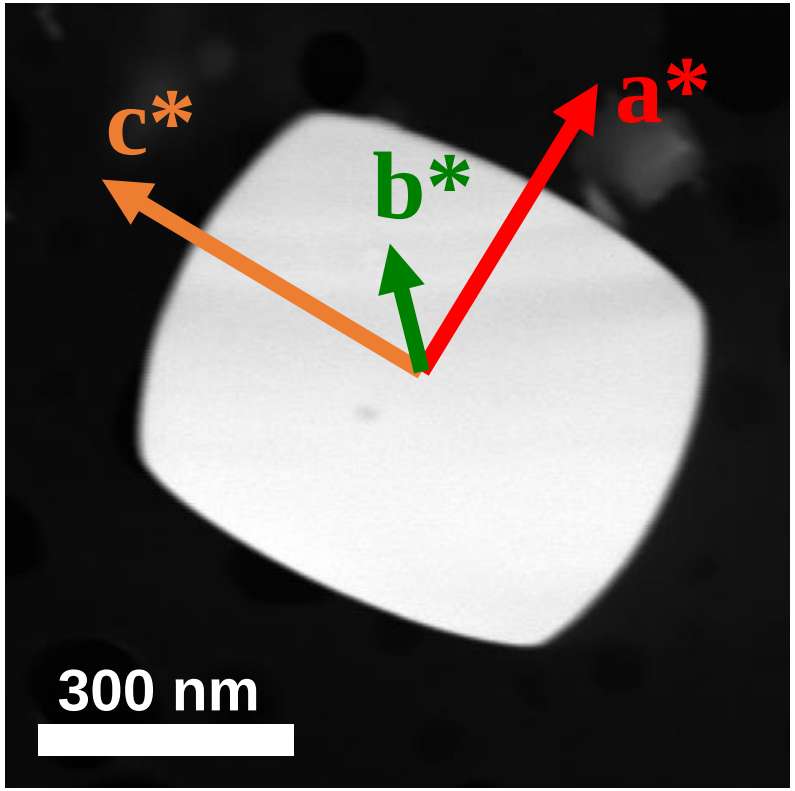


Cell parameters & Orientation



Cell parameters

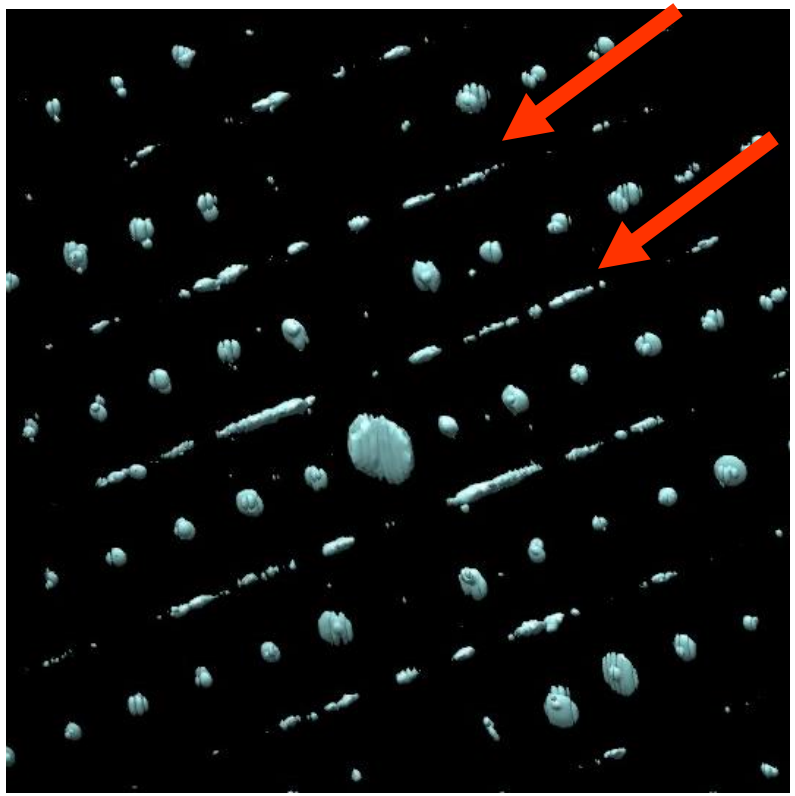
manual selection or clustering
in difference vector space



Orientation matrix

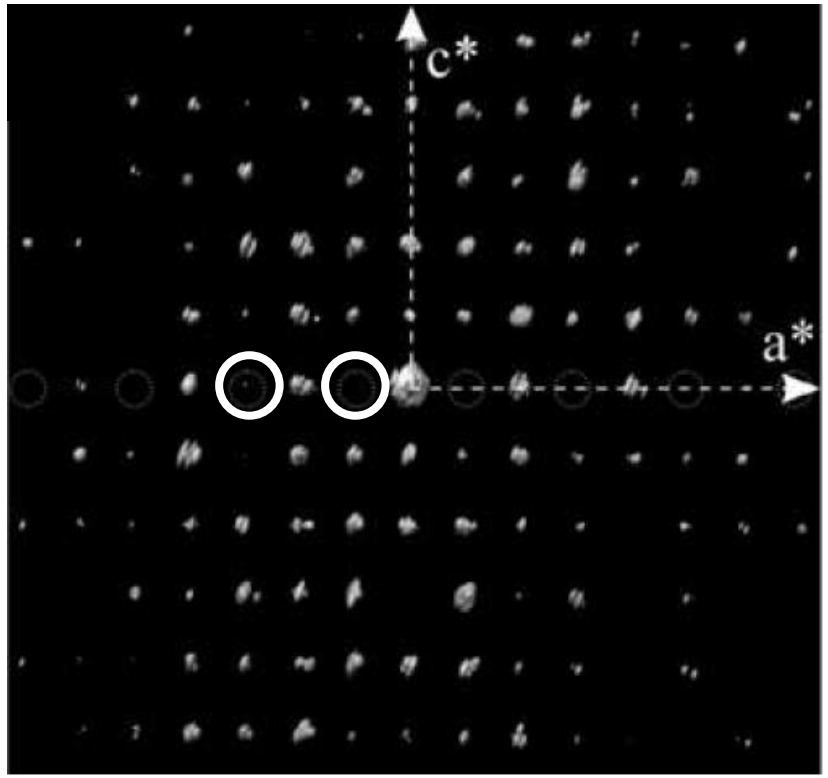
correlation with crystal shape for
determination of direction of
growth and facets

Disorder & Symmetry



Disorder

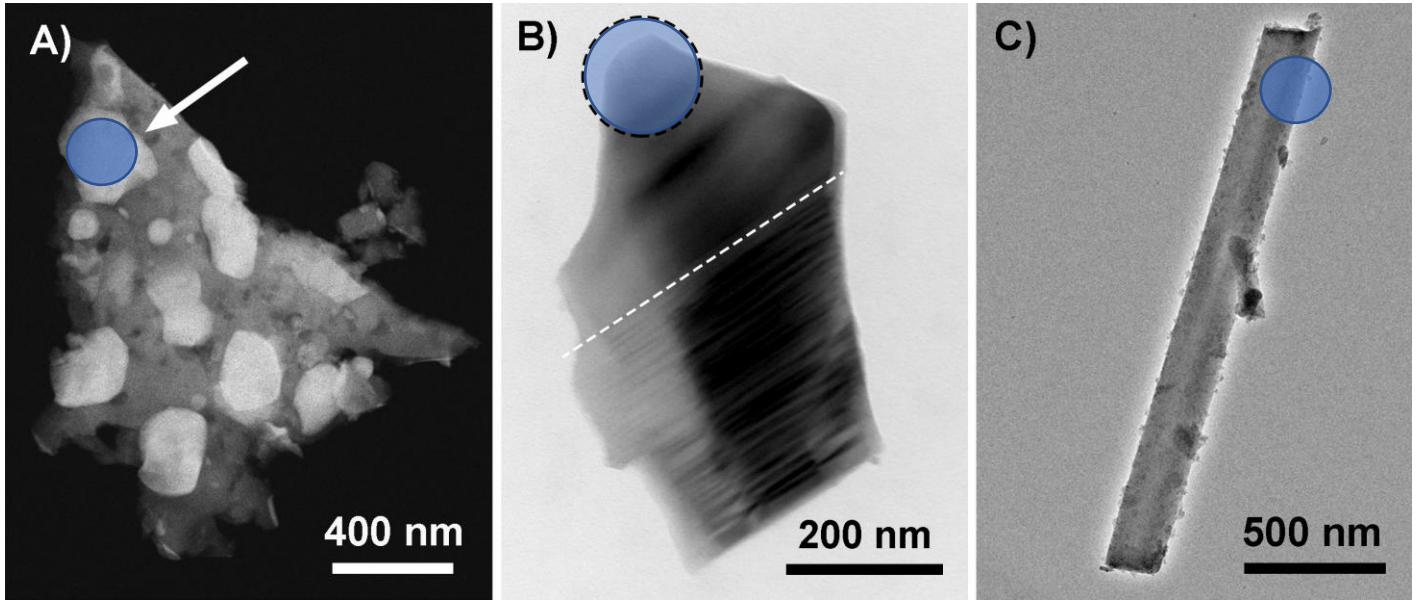
$0kl : k = 2n+1$



Extinctions

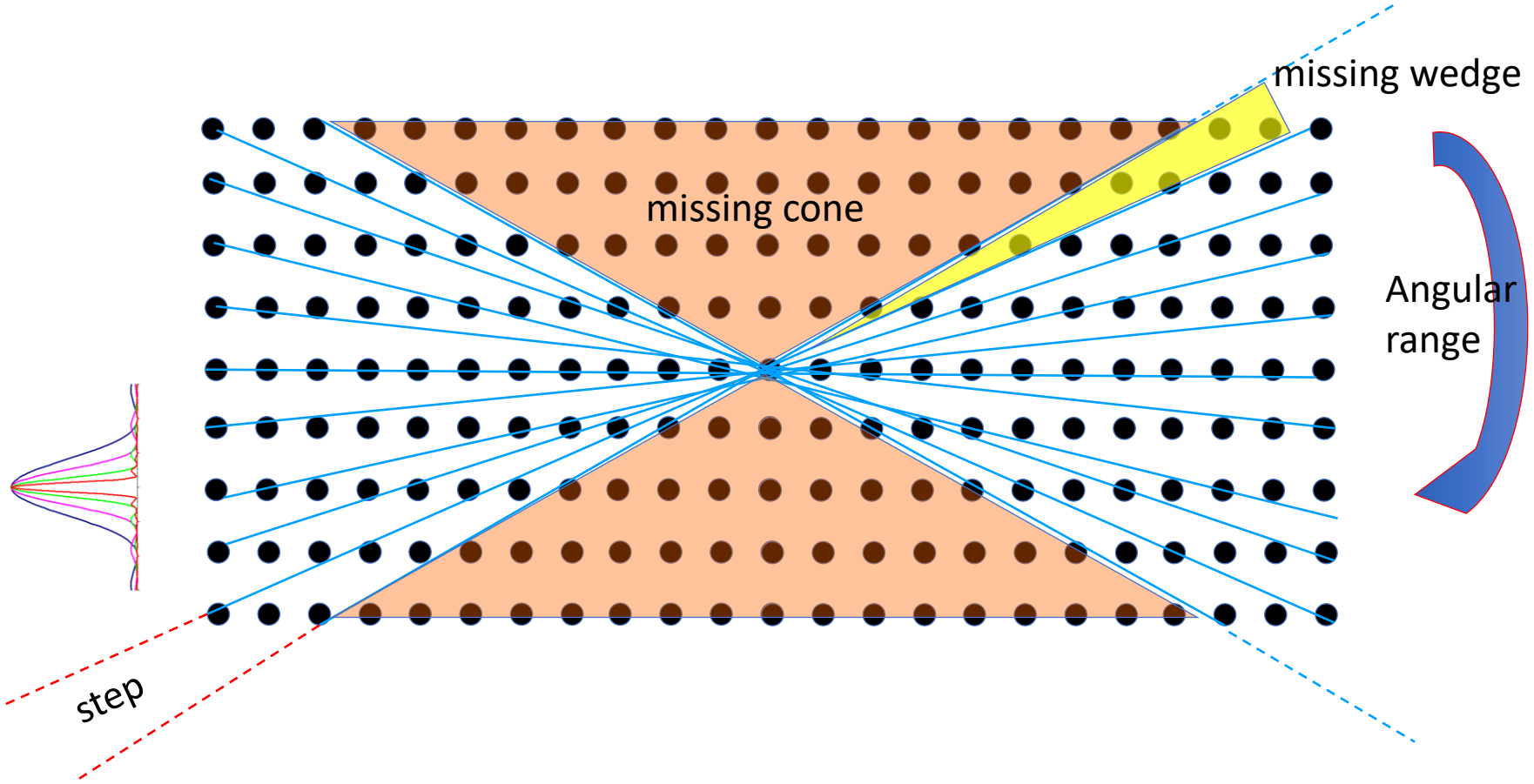
$hk0 : h = 2n$

All these 3D info



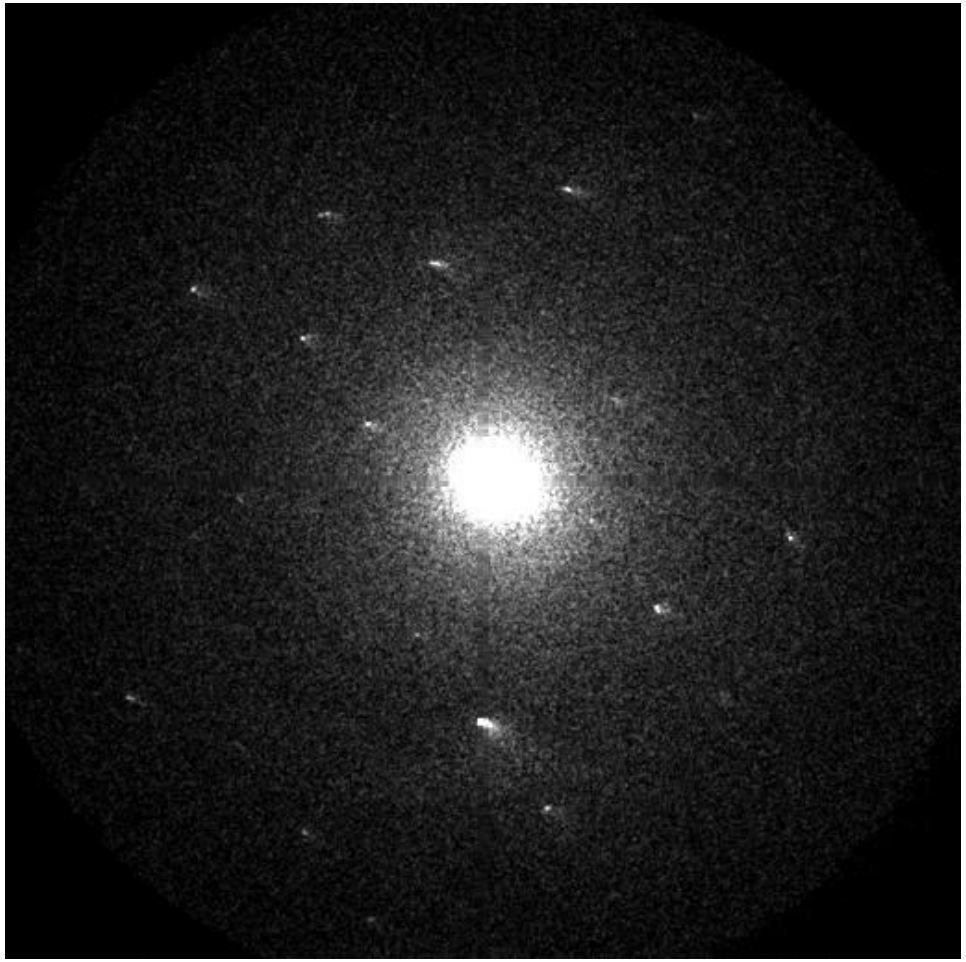
From a very small area

Electron Diffraction Tomography



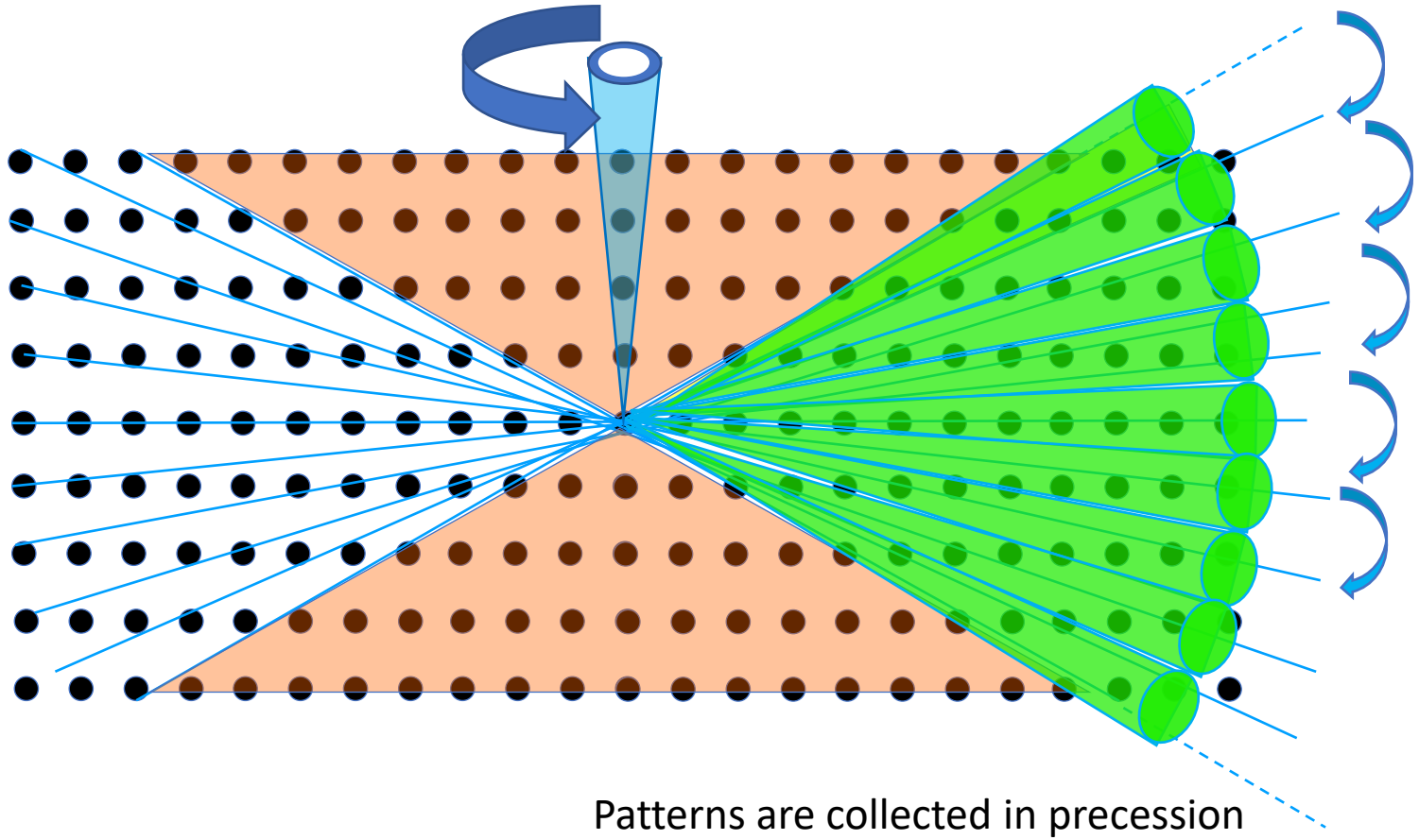
The missing wedge affetcs mainly the quality of the intensities:

Structure solution possible but not gurantee……



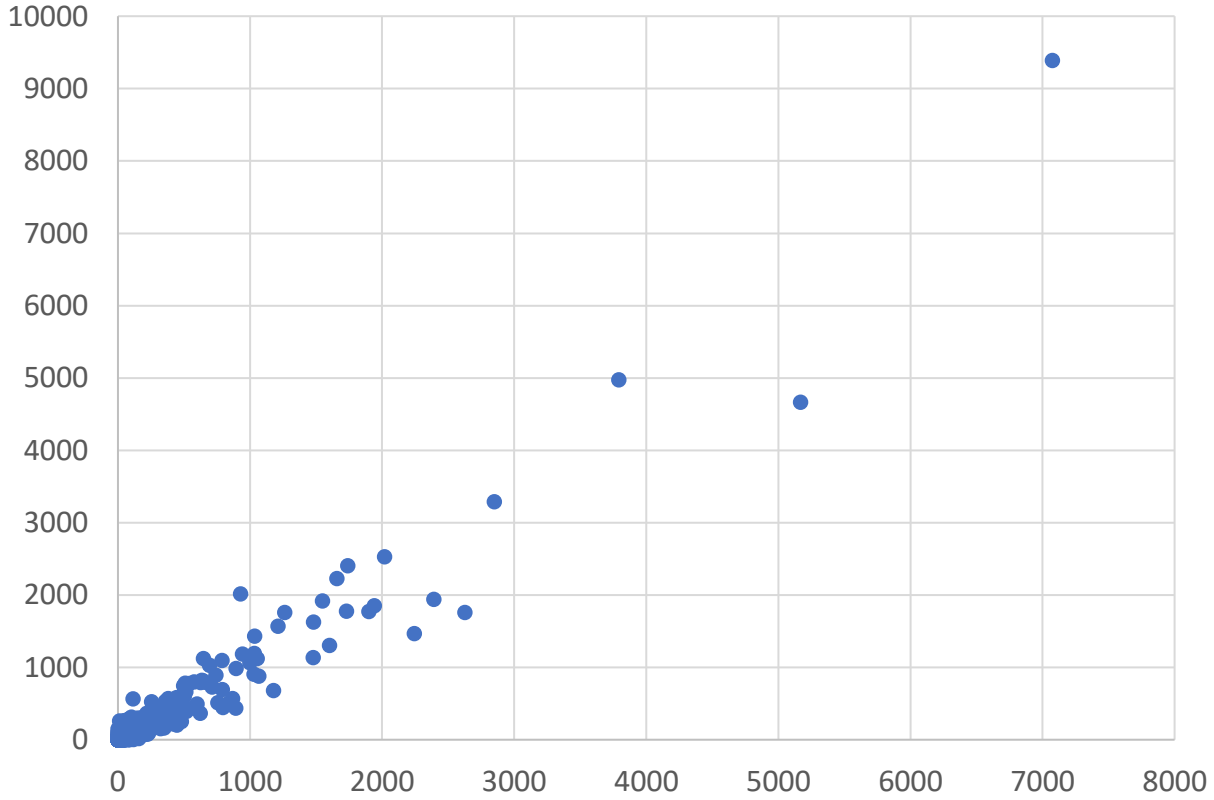
Angular step 0.15°

Precessed Electron Diffraction Tomography
PEDT



Mugnaioli et al. Ultramicroscopy 109 (2009) 758

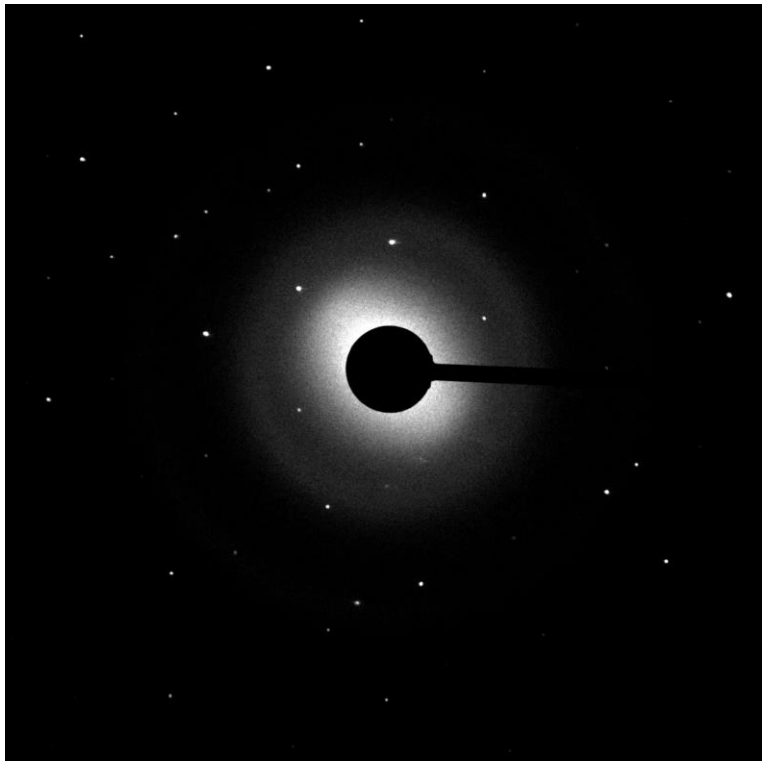
Patterns are collected in precession mode with an aperture semiangle comparable with the angular step
Angular step: 1°
Precession angle: 1° - 2°

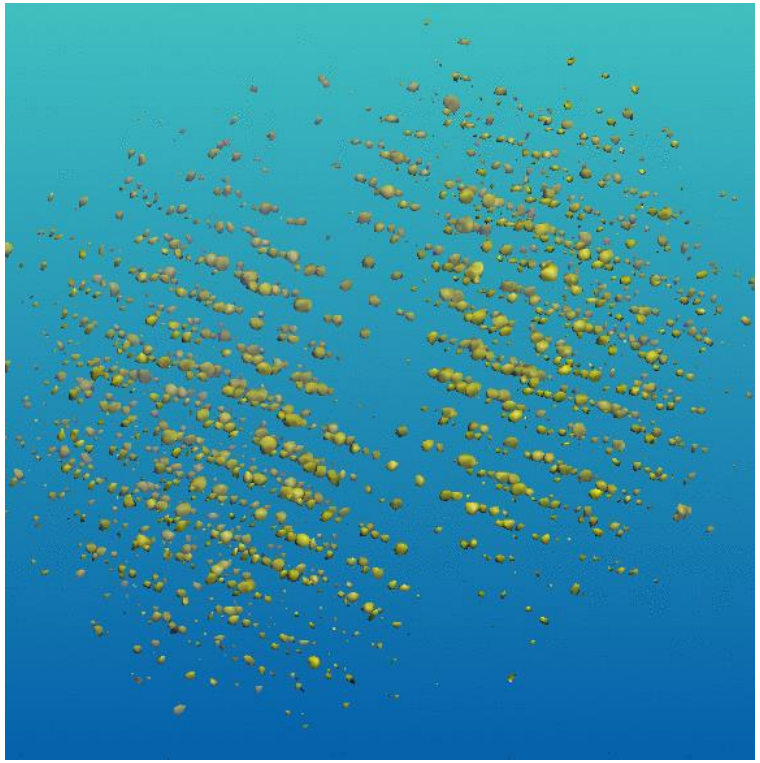


F_{obs} vs F_{calc}

- The intensity data are quasi-kinematical suitable for structure solution (direct methods, charge flipping,...), (SIR, SHELX, JANA)

Electron Diffraction Tomography

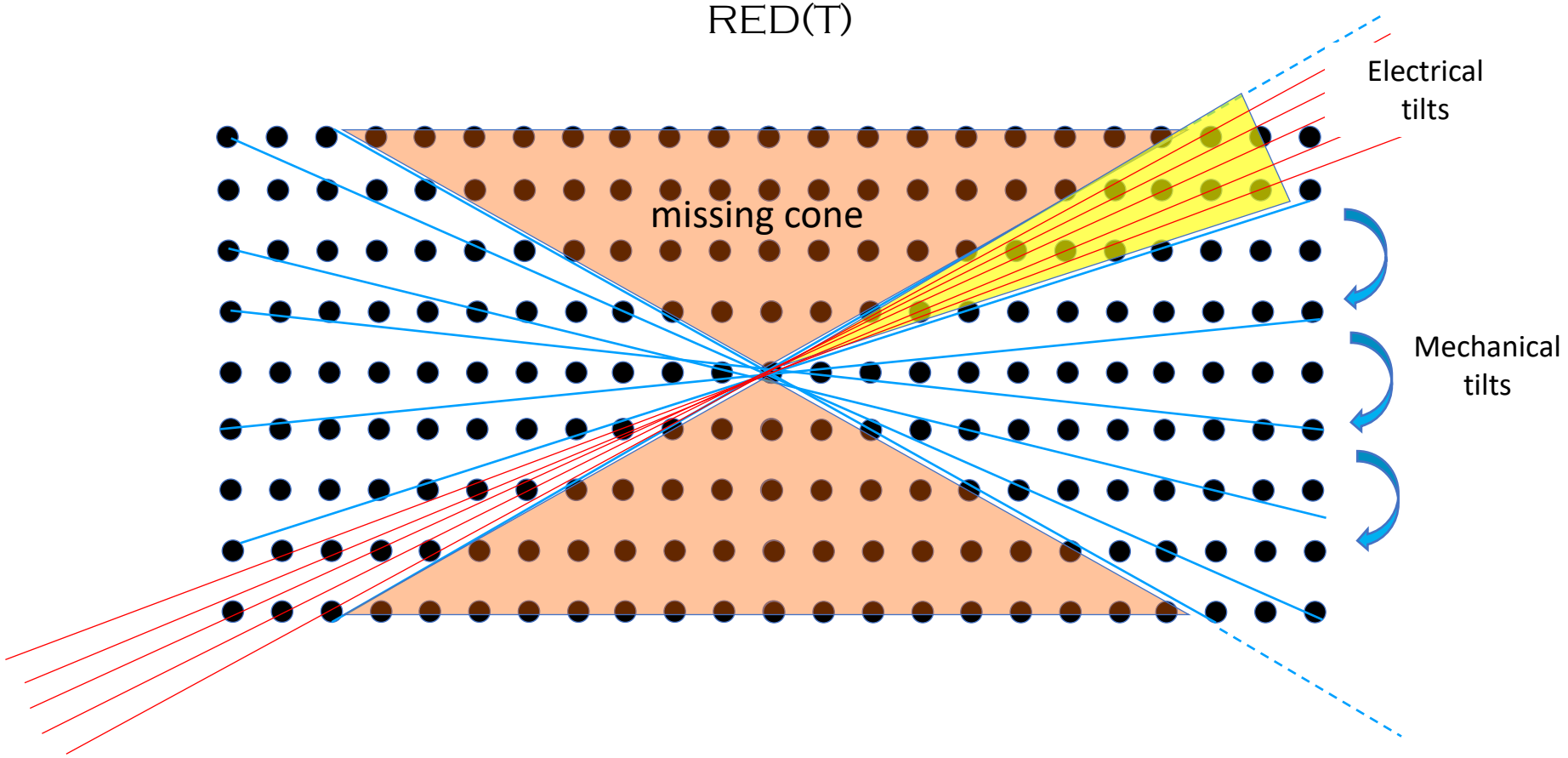




3D reconstruction of the reciprocal space

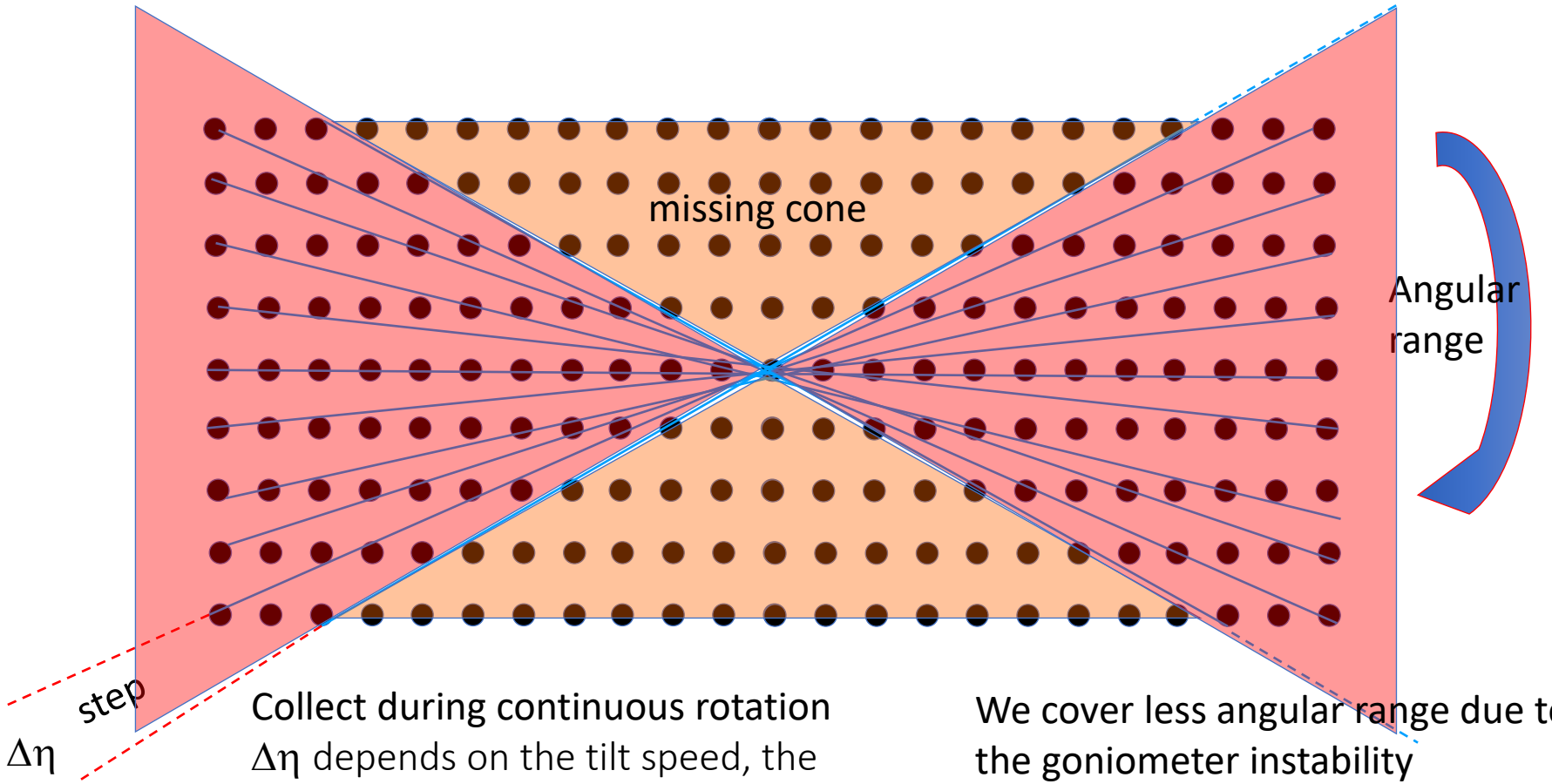
- Unit cell parameters, crystal symmetry
- From the reflection intensities get the crystal structure

Rotation Electron Diffraction Tomography
RED(T)



Zhang et al. Z. Kristallogr. 225 (2010) 94.

Fast Electron Diffraction Tomography (Continuous Rotation/MicroED)



Collect during continuous rotation

Δη depends on the tilt speed, the exposure time and the detector read out

We cover less angular range due to the goniometer instability

But we collect much faster 2-5 minutes or less.

Neederlof et al. Acta Cryst. D69 (2013) 1223.
Nannenga et al. Nat. Methods 11 (2014) 927.
Gemmi et al. Journal of Applied Crystallography 48 (2015) 718.

For the continuous rotation we need a fast detector

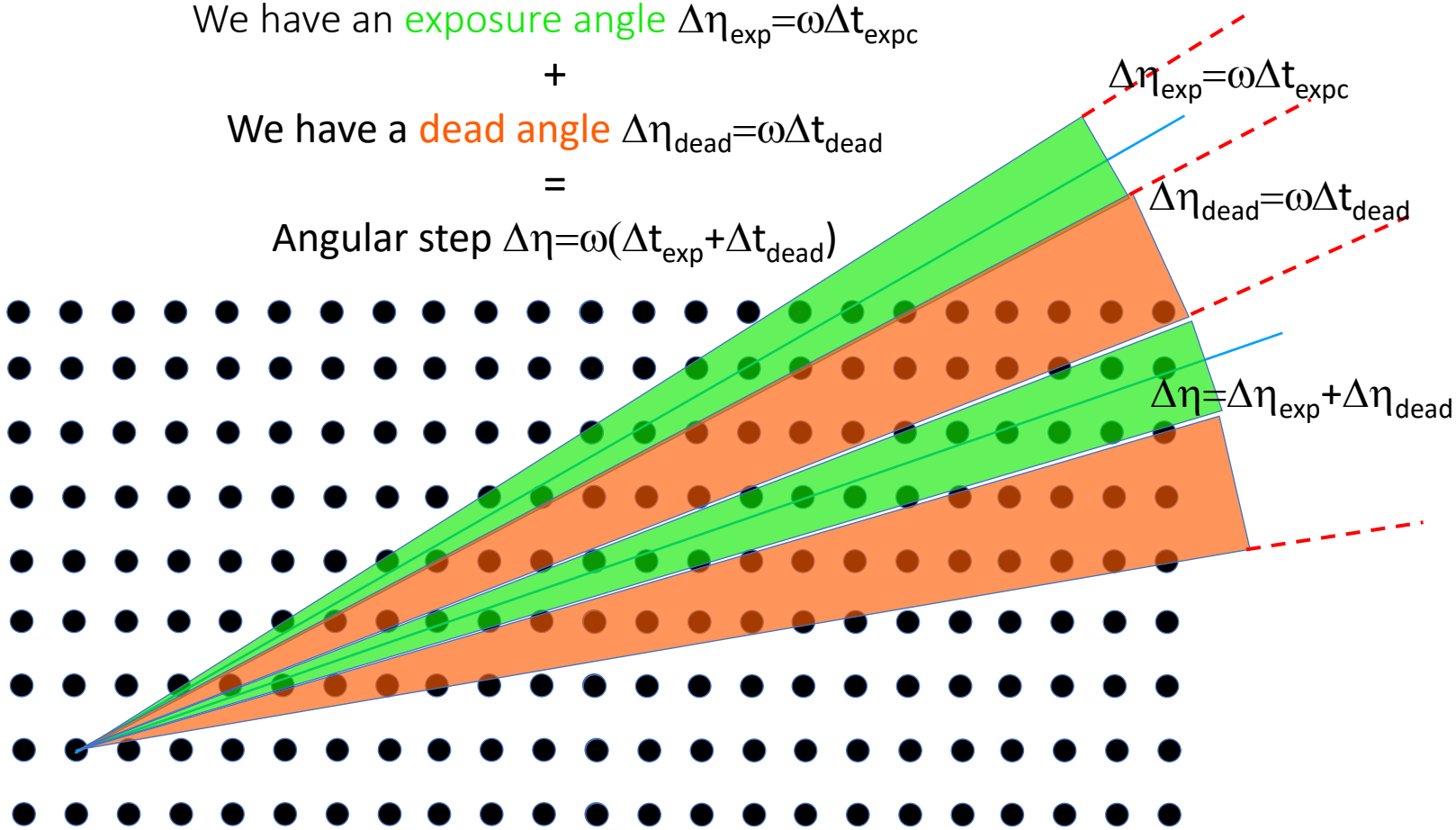
We have an exposure angle $\Delta\eta_{exp}=\omega\Delta t_{expc}$

+

We have a dead angle $\Delta\eta_{dead}=\omega\Delta t_{dead}$

=

Angular step $\Delta\eta=\omega(\Delta t_{exp}+\Delta t_{dead})$



We can tune $\Delta\eta$ by varying the rotation speed ω: in our TEM from 0.3° to 5° per second

$\Delta\eta_{dead}$ must be minimized → we need a fast detector

The crystal should not move during the continuous tilting

For the continuous rotation we need a fast detector

Fast and sensitive detector:

MEDIPIX single electron detection camera which has a read out time of 7 ms

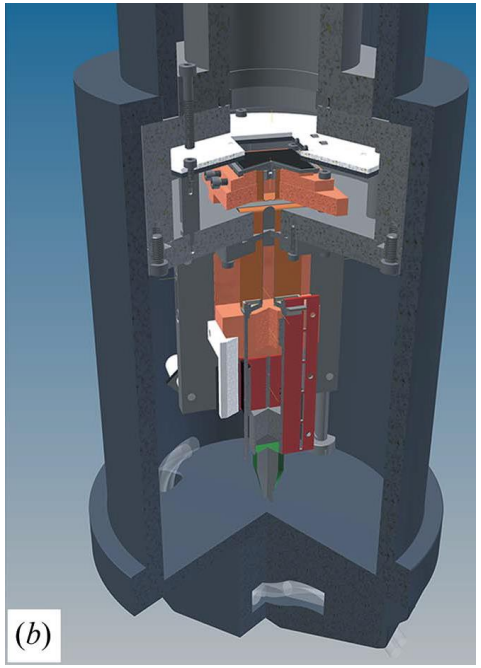
The fastest is the detector the fatest we can rotate the crystal the less time we illuminate the crystal: very low dose.

In our TEM we can vary the rotation speed in the interval

0.3°/s – 5°/s

With a dead time of 7 ms the lost reciprocal space angle varies between:

0.002° and 0.035° negligible



Genderen et al. Acta Cryst (2016) A72, 236-242.

Continuous (fast) acquisition

Timepix detector
512x512 px

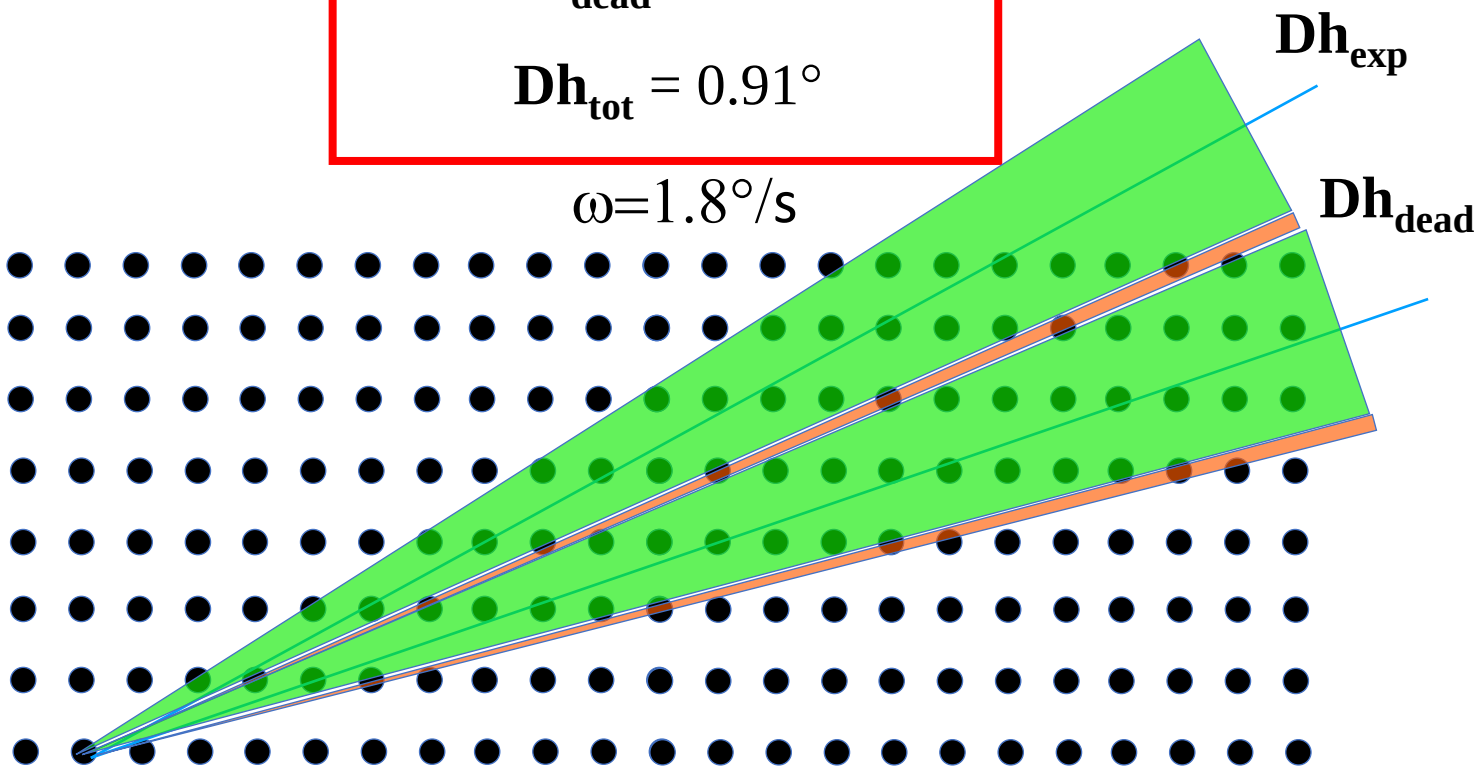
Exposure: 0.45 s

Dh_{exp} = 0.83°

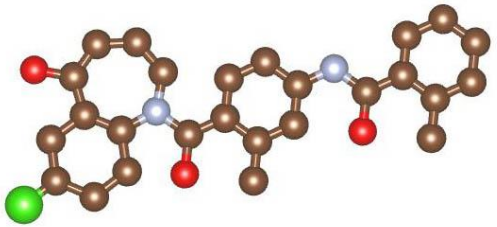
Dh_{dead} = 0.08°

Dh_{tot} = 0.91°

IEDT

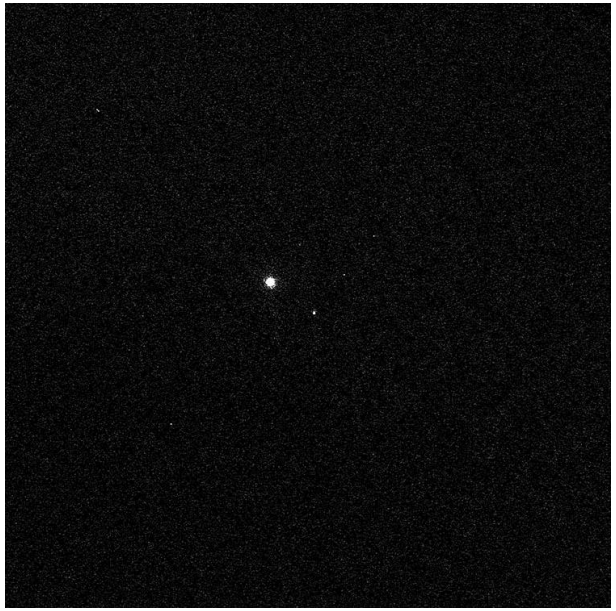


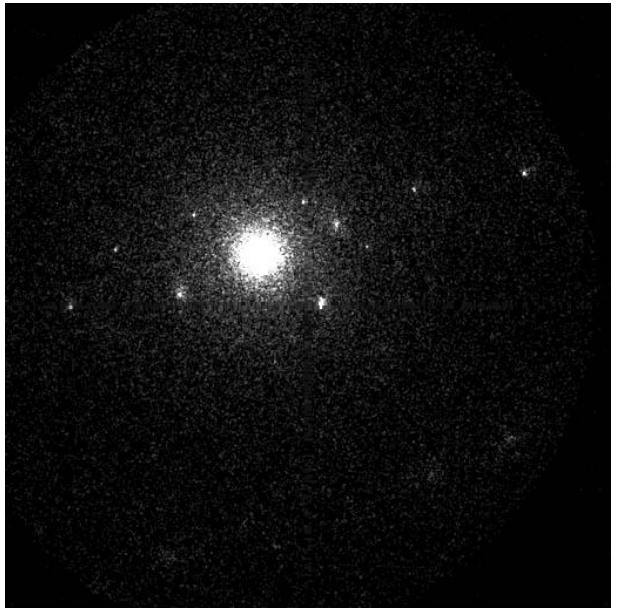
Low Dose

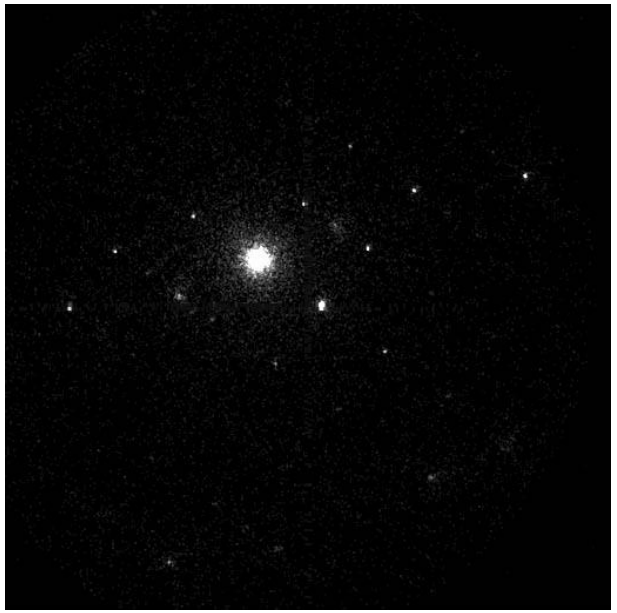


API1

MEDIPIX







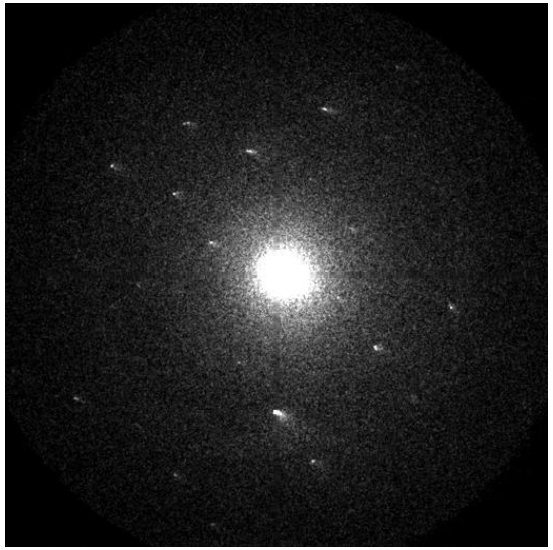
CCD + Energy Filter

MEDIPIX no Energy Filter

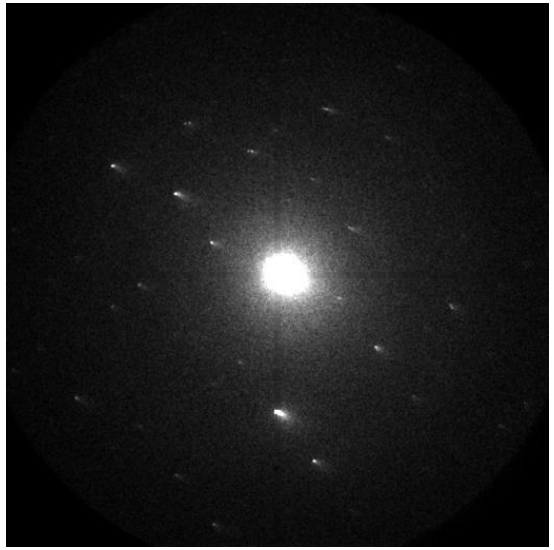
MEDIPIX + Energy Filter

The dose can be reduced <0.05 el $Å^{-2}s^{-1}$

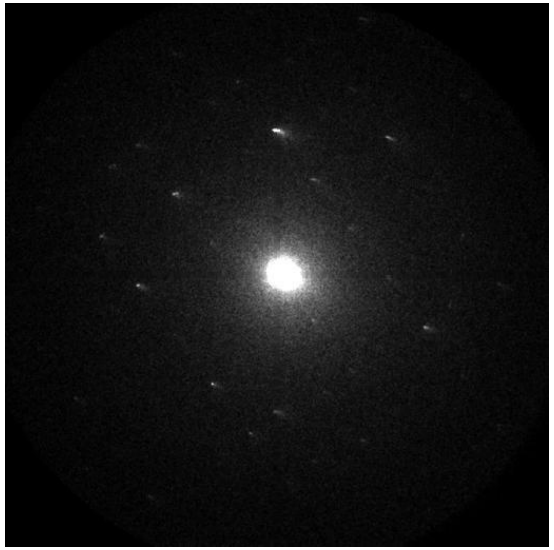
From REDT to IEDT



0.15°



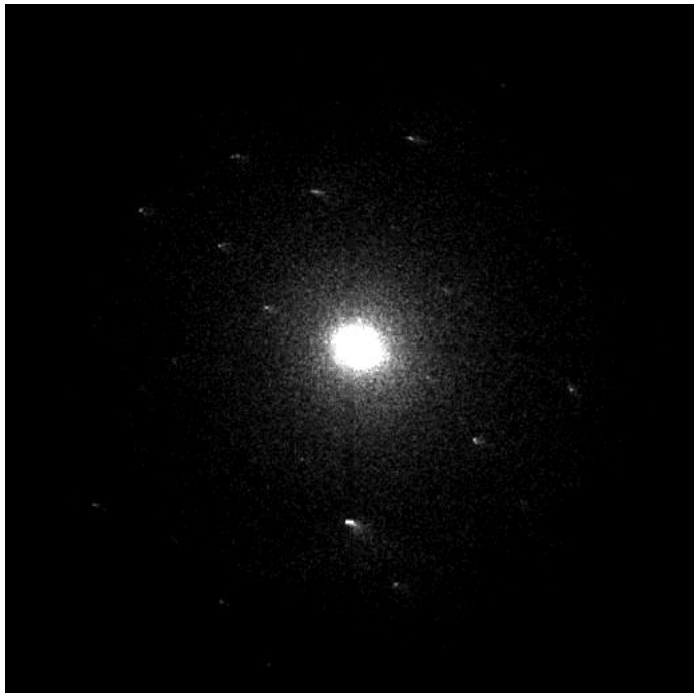
0.50°



1°

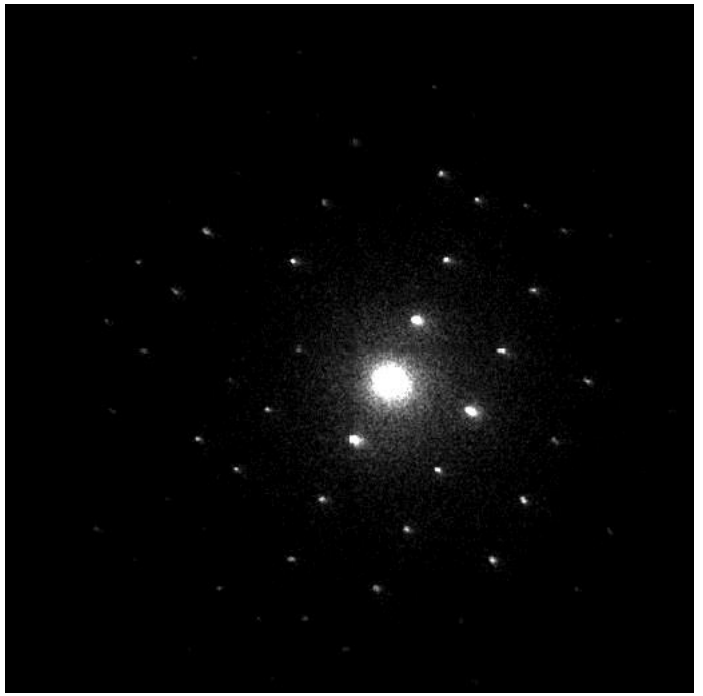
Angular integration

Continuous vs stepwise



IEDT or cRED

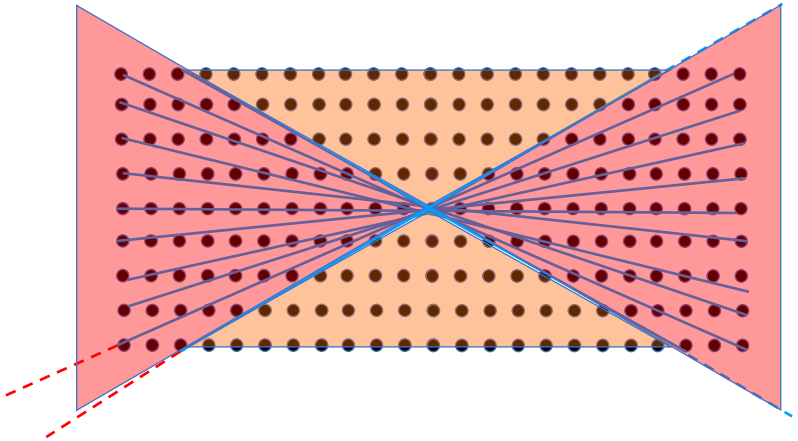
0.15° each frame



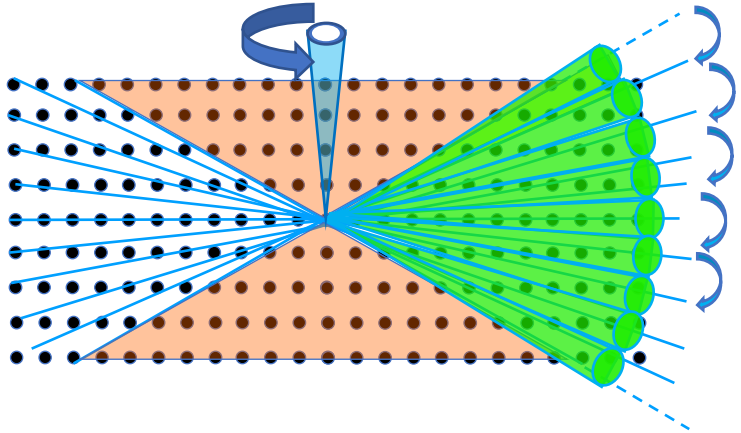
PEDT stepwise
1° prec
1° each frame

3D ED

Continuous



Step wise



- You can cover smaller angular range of the reciprocal space 40°-80°
- You can go faster 2-5 minutes, but also 30 seconds
- You can work in low dose

- You can cover large angular range of the reciprocal space 100-120°
- it is time consuming 15 min- 30 min

Different 3D ED

Nano diffraction Precession mode	Mechanical 1°	Automatically STEM
SAED Precession mode	Mechanical 1°	Semi automatic/ Manual TEM
SAED Stationary mode	Mechanical 1°	Manually TEM
SAED Stationary mode	Mechanical 2° Electrical 0.01°	Automatic TEM
SAED or Nanodiff Stationary mode	Continuous	Goniometer Stability

Automatic PEDT

Kolb et al. Ultram. 107 (2007) 507
Mugnaioli et al. Ultram. 109 (2009) 758

"Manual" PEDT

Palatinus et al. Inorg.Chem. 50 (2011) 3743
Gemmi et al. Actra Cryst B68 (2012) 15.

"Manual" EDT no prec

Fan et al. Inorg. Chem. 52 (2013) 11067.

Rotation

Singh et al. J. Appl. Cryst 47 (2014) 1
Mayence et al. Inorg. Chem. 53 (2014) 5067.

Continuous Rotation – IEDT - MICROED

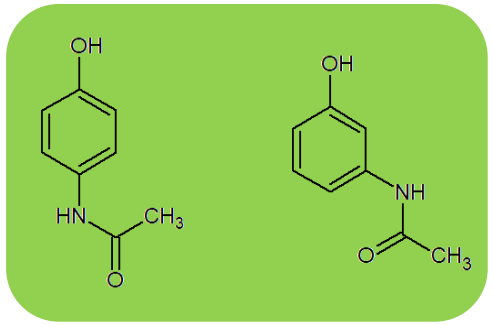
Neederlof et al. Acta Cryst. D69 (2013) 1223.
Nannenga et al. Nat. Methods 11 (2014) 927.
Gemmi et al. Journal of Applied Crystallography 48 (2015) 718.

ORTHOCETAMOL FROM VODES

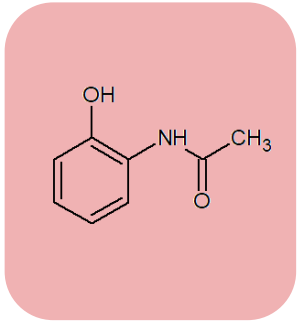
Orthocetamol

✓ demonstrates lower hepatoxicity than widely used Paracetamol

✓ promising as an anti-arthritic treatment

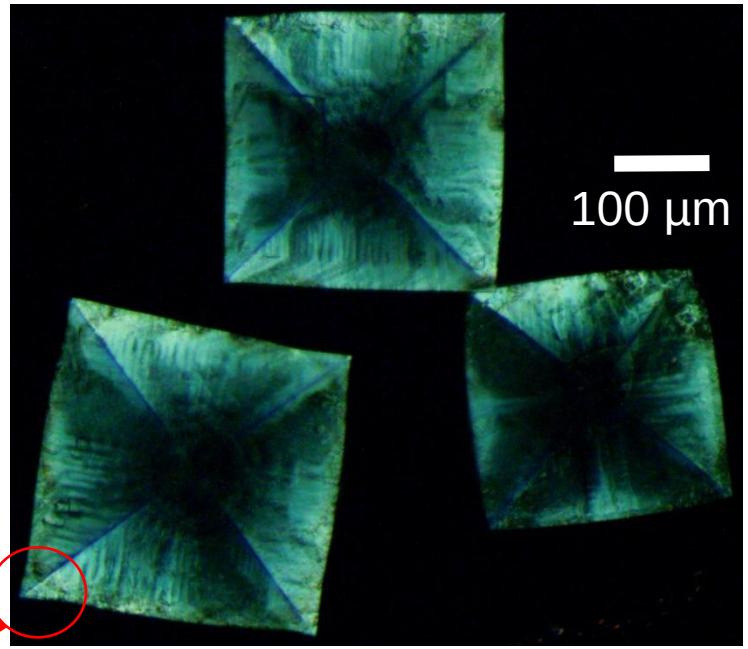


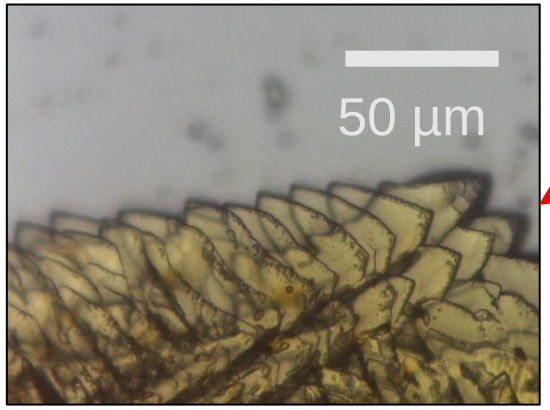
Paracetamol & Metacetamol are well known.



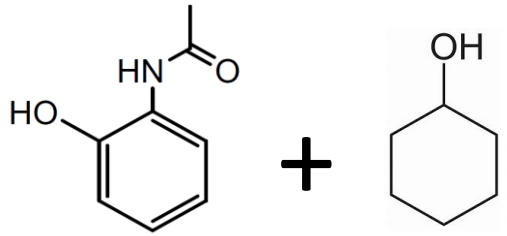
Orthocetamol was first reported in 1876, before the discovery of X-ray.

Structure is still unknown!



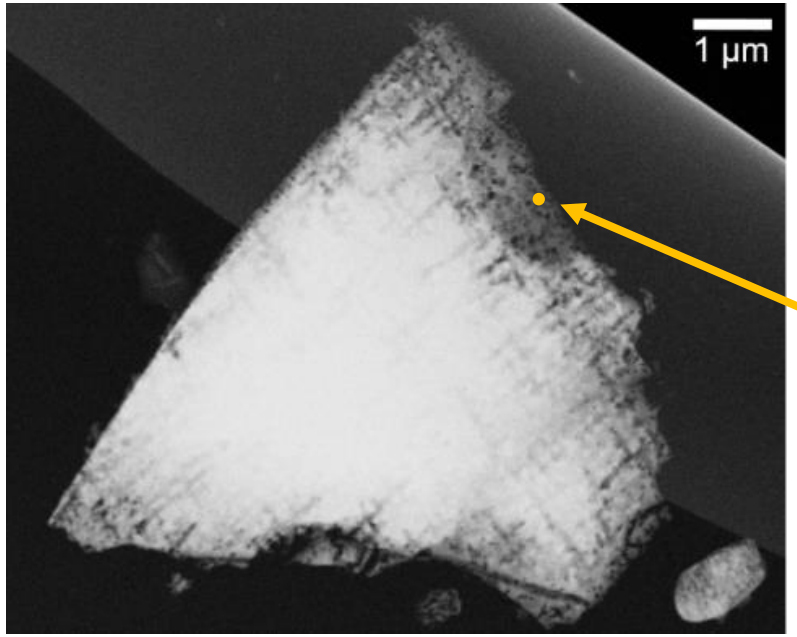


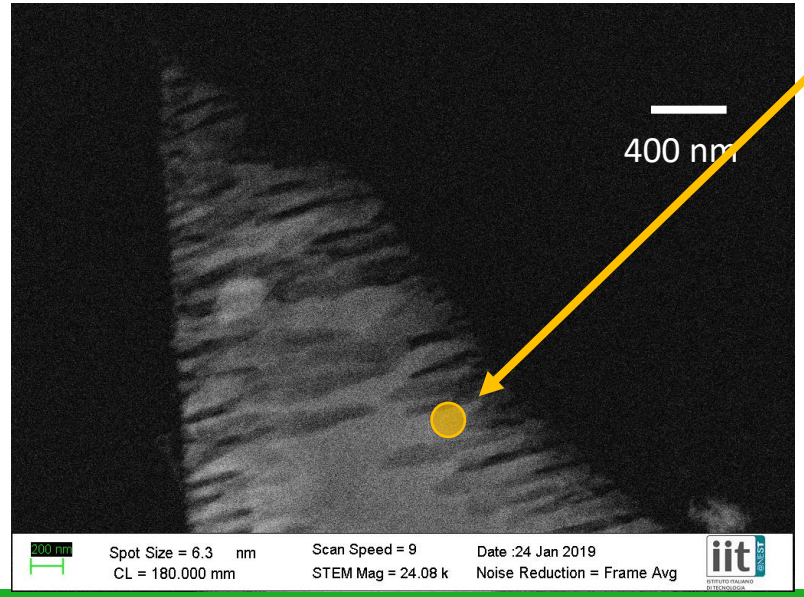
VODES



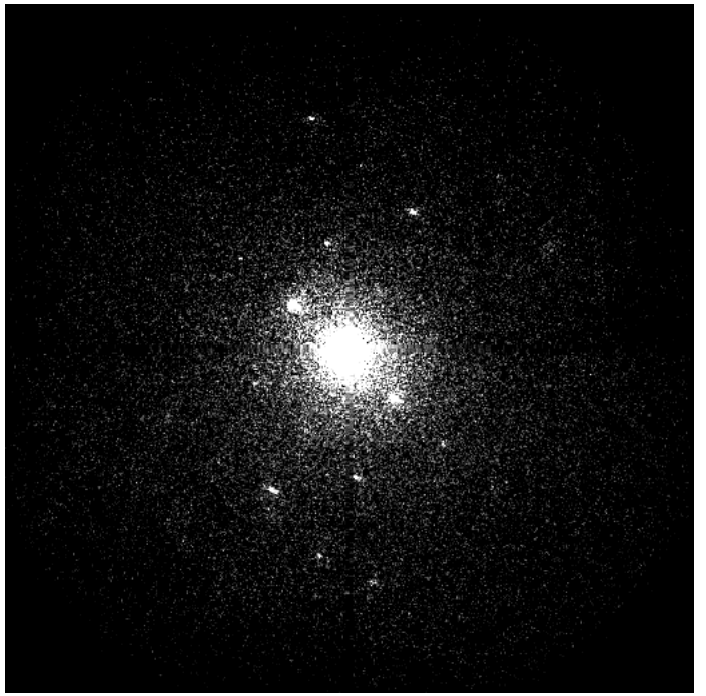
cyclohexanol

Orthocetamol from VODES





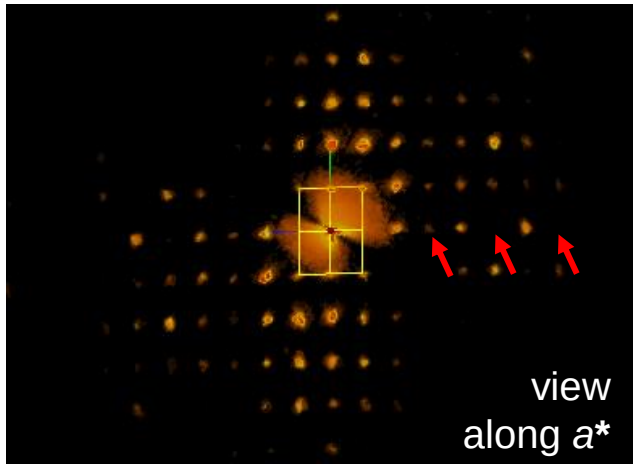
3D ED data collection in precession mode

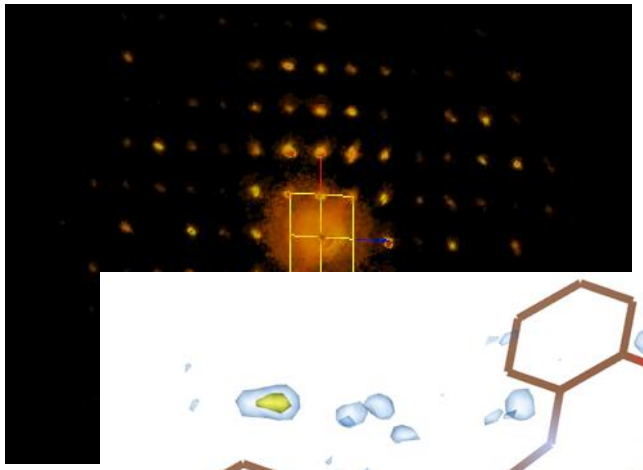


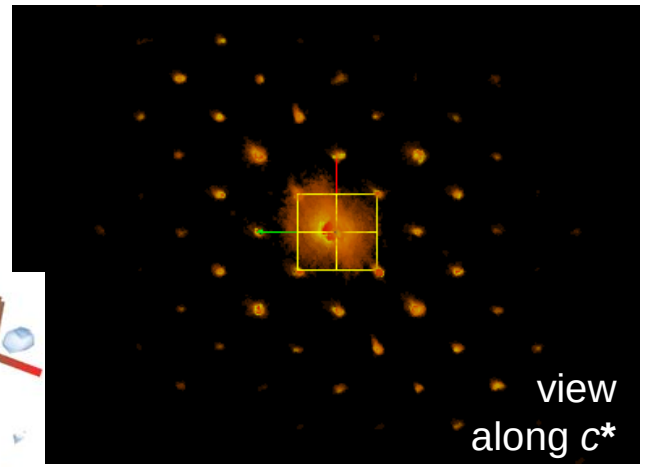
Multiple twinning single domains of few hundreds of nanometers

ISTITUTO ITALIANO DI TECNOLOGIA

Orthocetamol from VODES



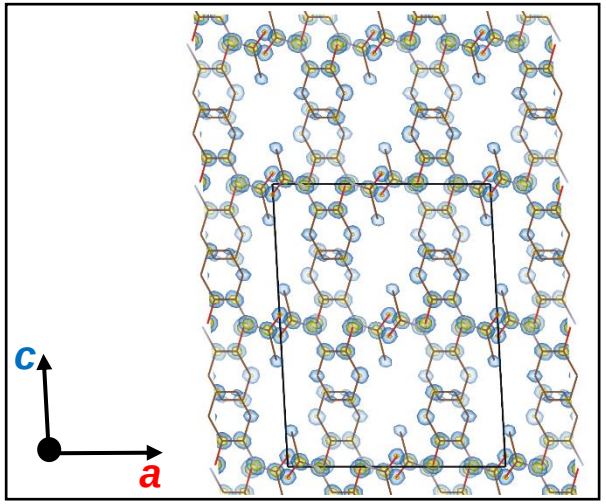


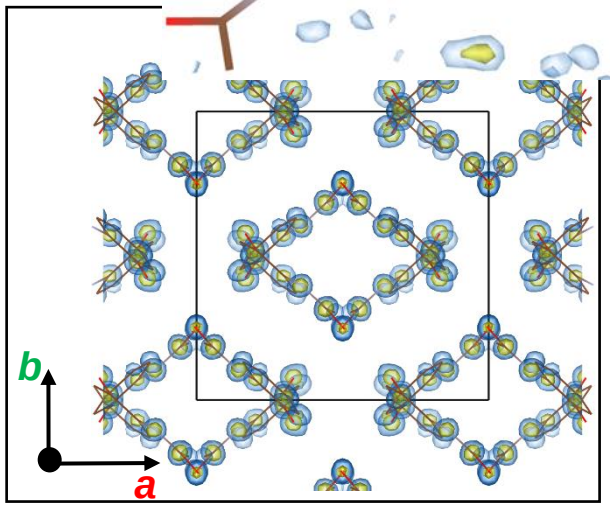


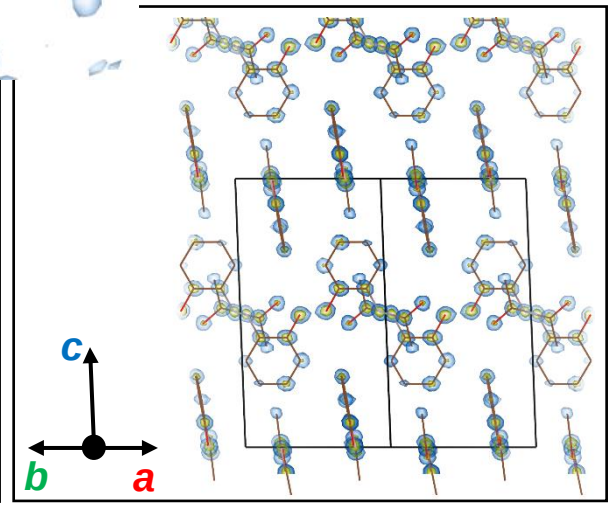
a = 10.56 Å
b = 10.39 Å
c = 13.72 Å
β = 93.113°

Tetr

C-centered monoclinic C2/c (pseudo-tetragonal)





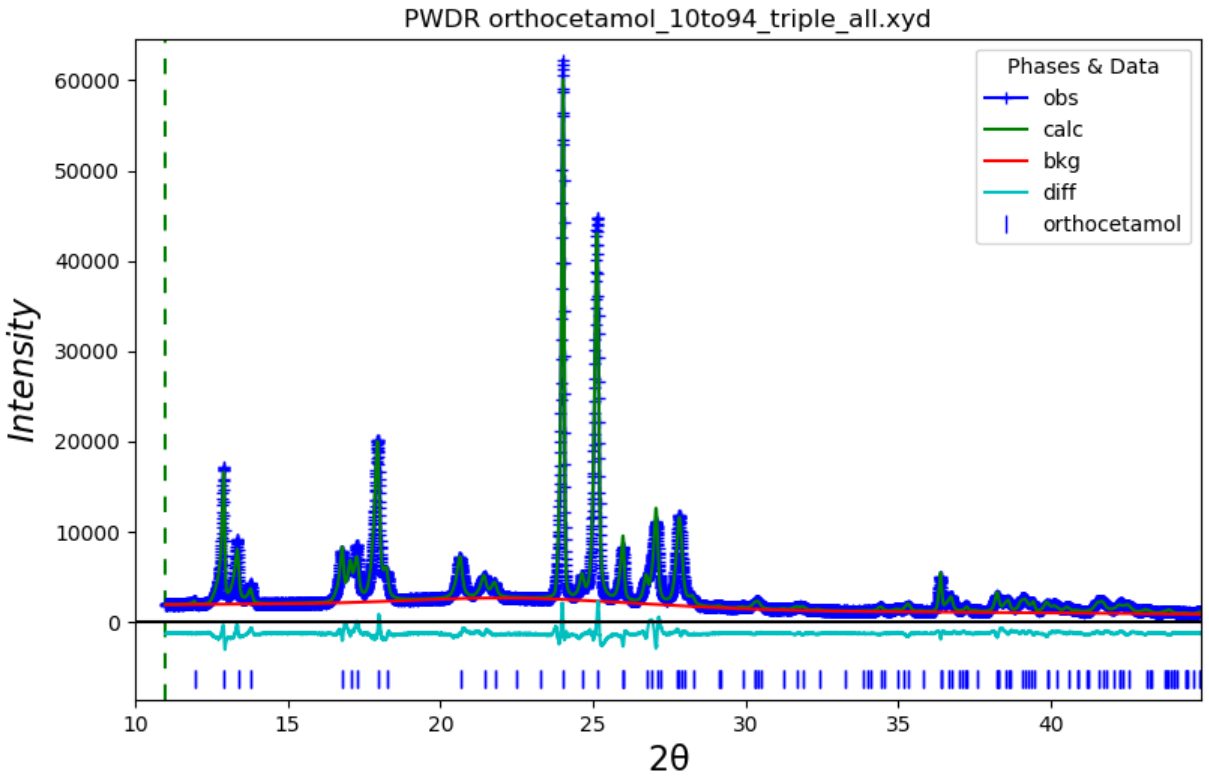


Solved ab-initio by direct methods in ***C2/c***.

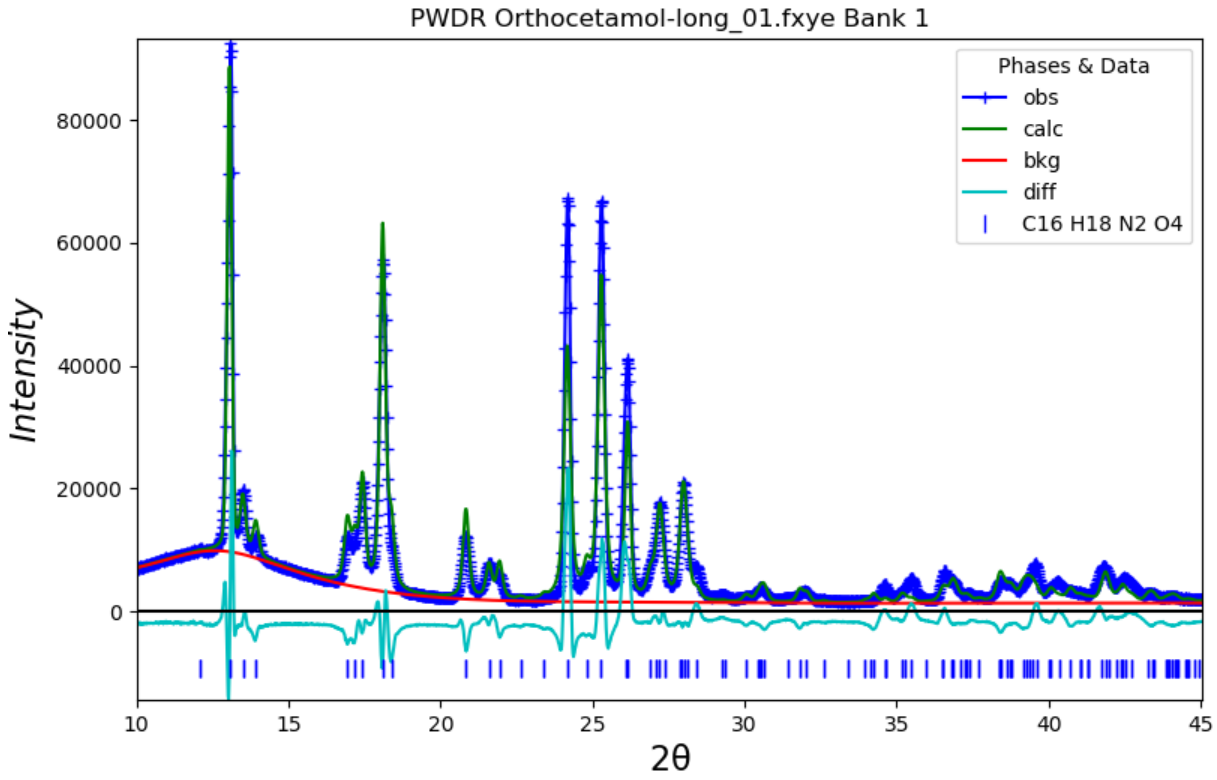
All 11 non-hydrogen atoms were clearly recognizable.

Andrusenko I. et al. (2019) Angewandte Chem. Int. Ed. **58**, 10919

Orthocetamol from VODES

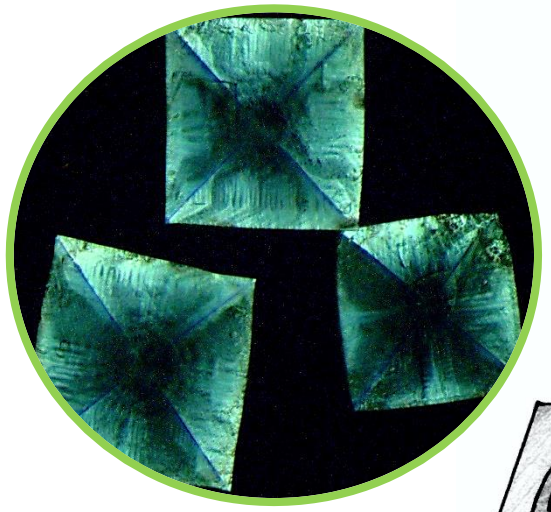


Debye-Scherrer data collection



Bragg-Brentano

Although it's a pure phase, automatic indexing from PXRD was not successful.
Problems: peak broadening, weak intensity above 30 ° 2theta (d= ca. 3 Å).



Thank you