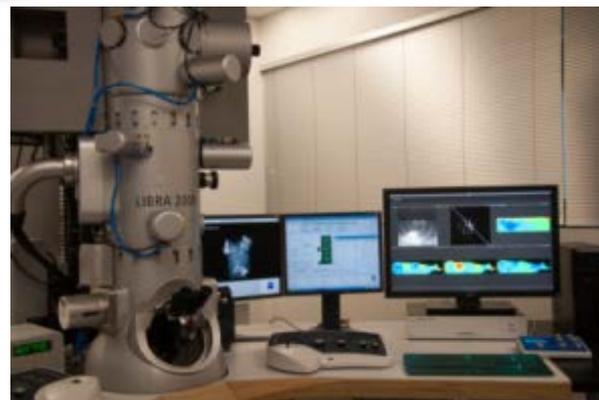
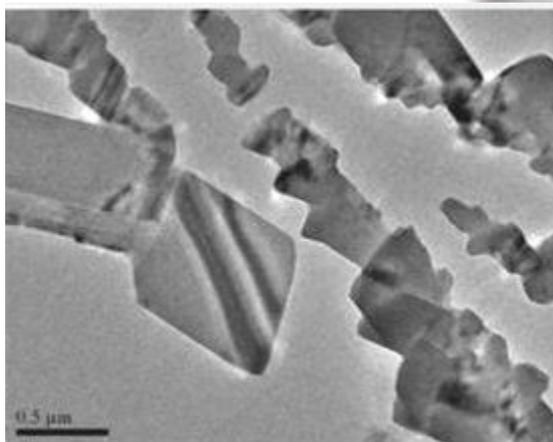


CRYSTAL STRUCTURE DETERMINATION OF PHARMACEUTICALS WITH ELECTRON DIFFRACTION



Dr. Partha Pratim Das
Application Specialist, NanoMEGAS SPRL, Belgium
pharma@nanomegas.com

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Sponsored by The International Centre for Diffraction Data

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Free transnational access

to the most advanced TEM equipment
and skilled operators for HR(S)TEM,
EELS, EDX, Tomography, Holography and
various in-situ state-of-the-art
experiments

X 2 SME participate

CEOS
NanoMEGAS

★ Academic
● SME



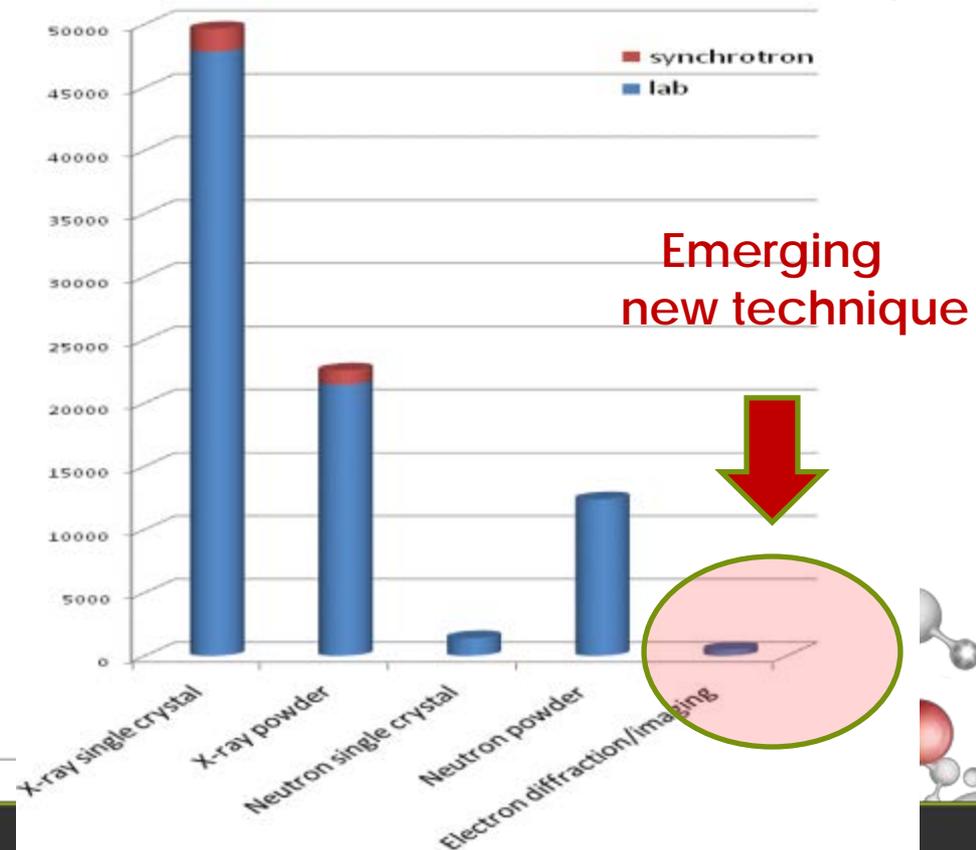
X-ray

Neutron

Electron

Crystallography

Why using electron crystallography ?



STRUCTURE ANALYSIS WITH ELECTRON DIFFRACTION

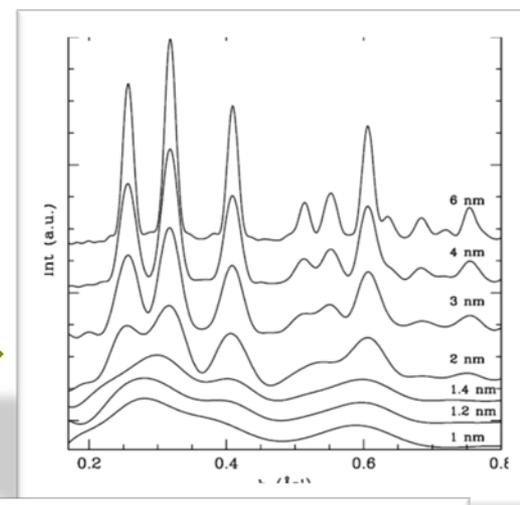
Why electrons?



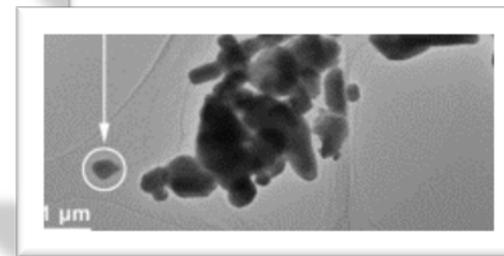
■ 10^{4-5} times stronger interaction with matter compared with X-ray

- *single crystal data on powder sample*
- *short data collection time*

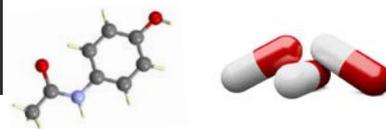
- X-Ray peaks broaden with crystals of nm range



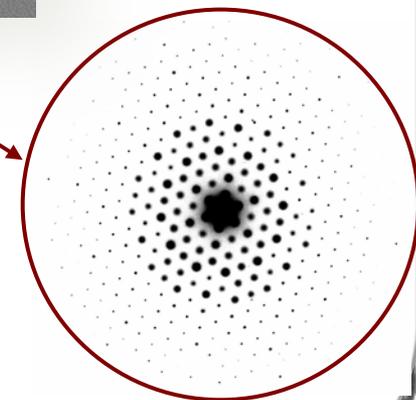
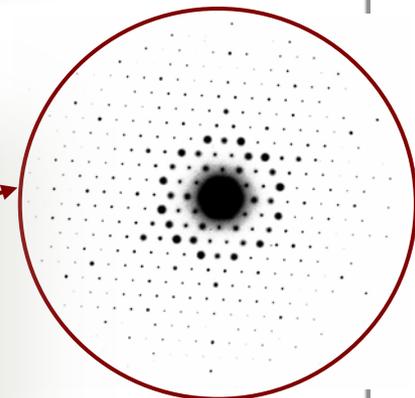
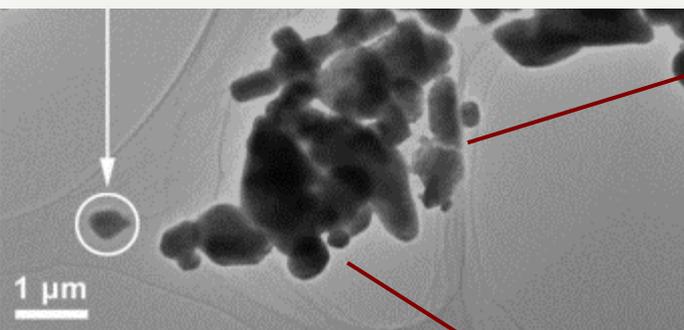
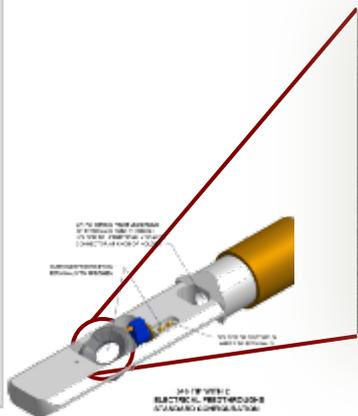
With Electron microscope we can study nm- and micro-sized crystals



STRUCTURE ANALYSIS WITH TEM



TEM : Electron diffraction advantages

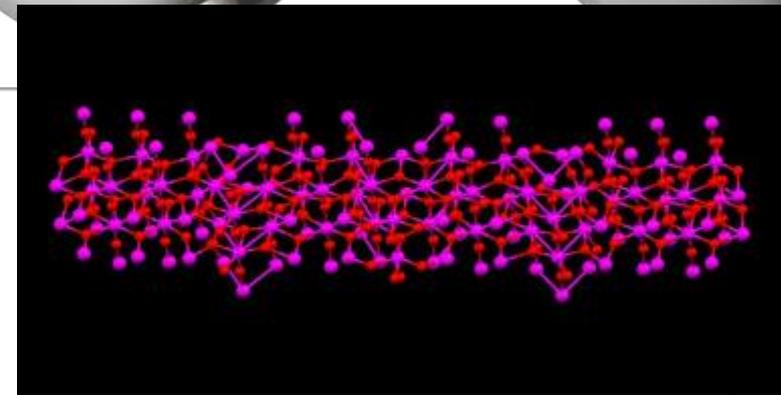
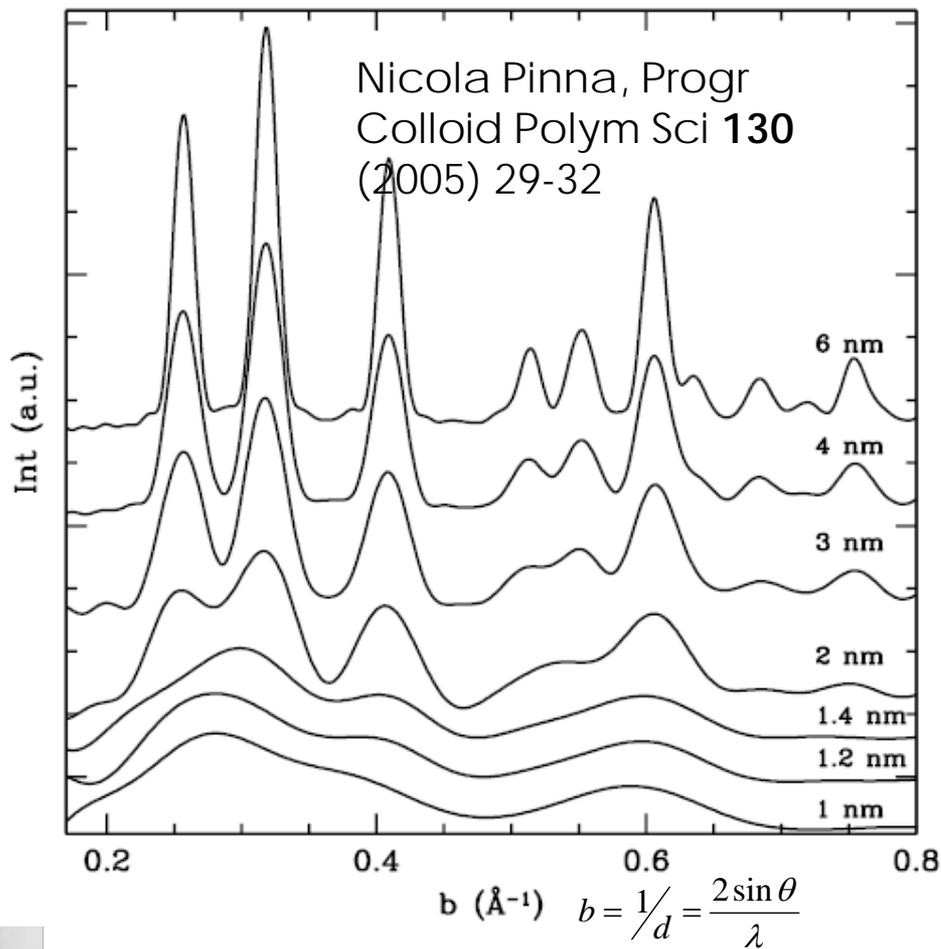


TEM goniometer

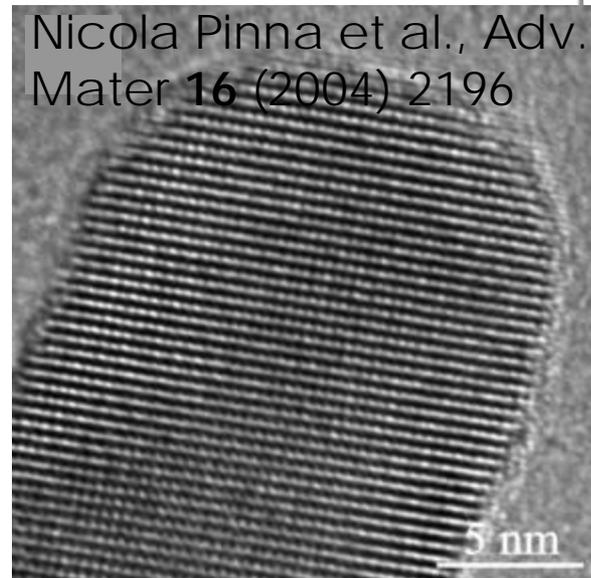
Every TEM (electron microscope) may produce ED patterns and HREM from individual single nanocrystals

ED information: Cell parameter and symmetry determination

Measuring intensity values leads to structure determination

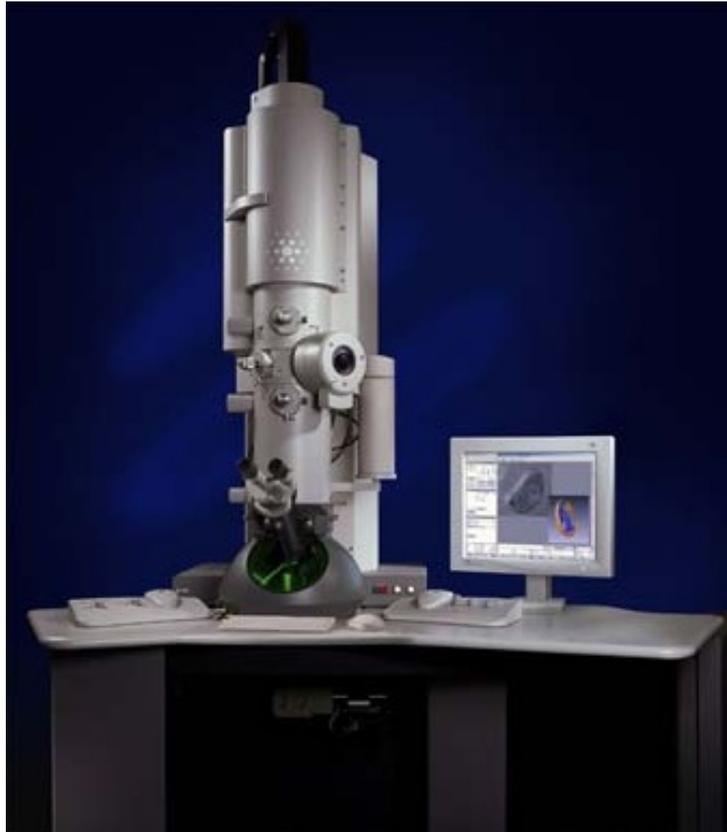


Ta_2O_5
tantite



**Crystals < 5 nm look like "X-Ray amorphous"
but we can see them in TEM!**

Transmission electron microscopy (TEM)



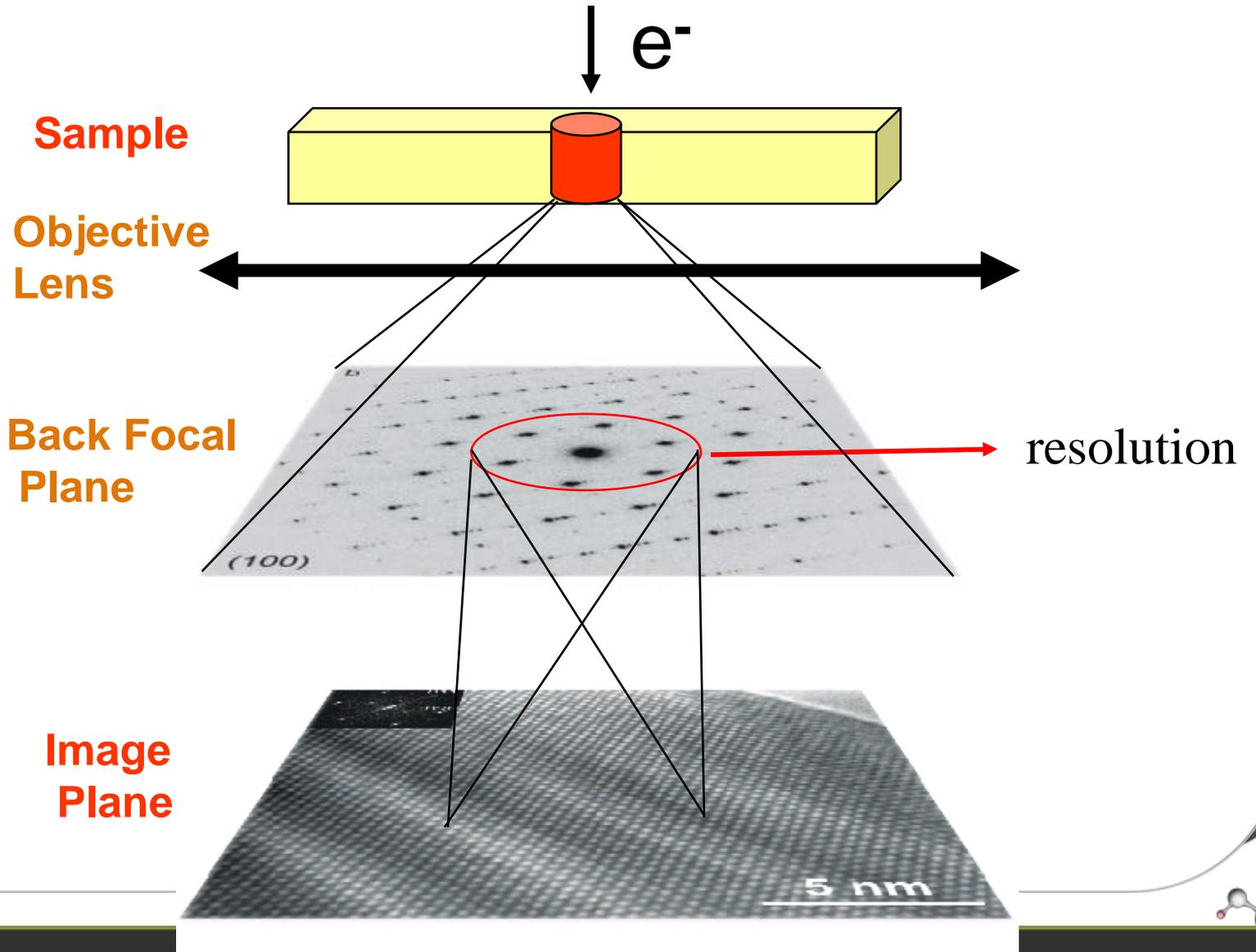
- **Diffraction:** selected area, nano- and convergent beam electron diffraction
- **Imaging:** conventional, high resolution
- **Chemical analysis:** EDS and EELS



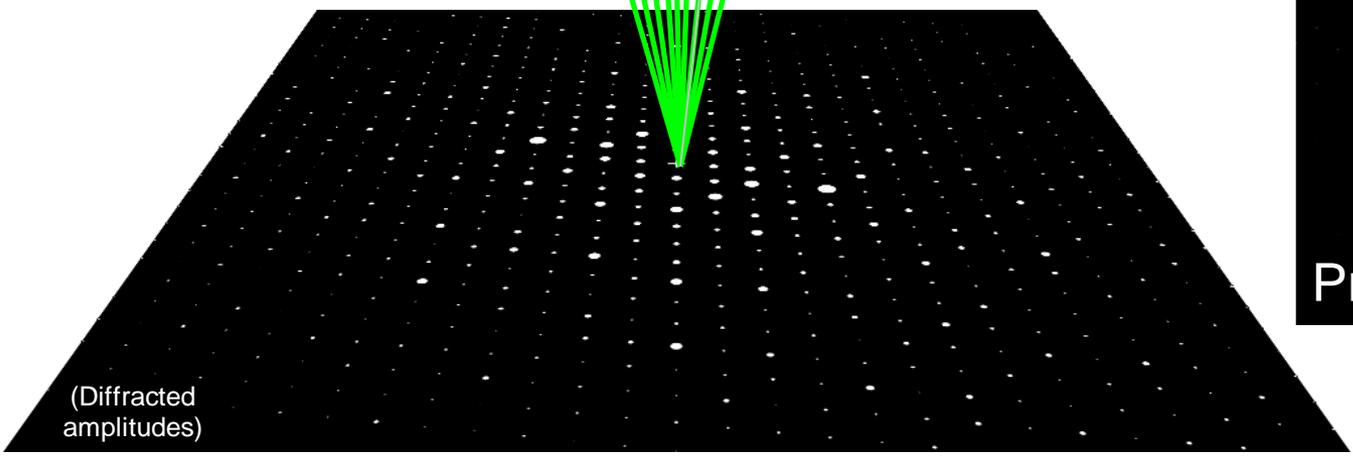
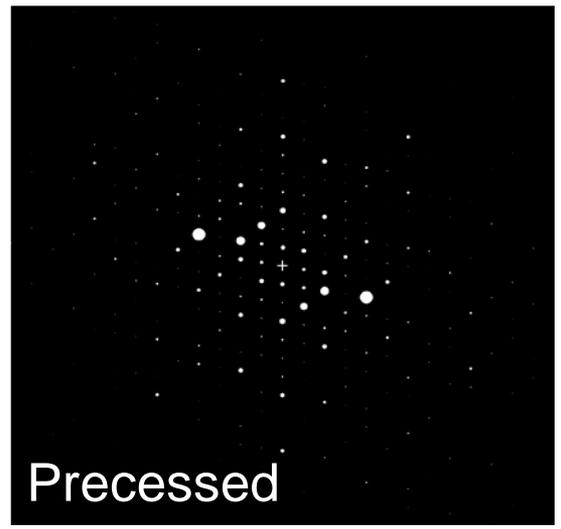
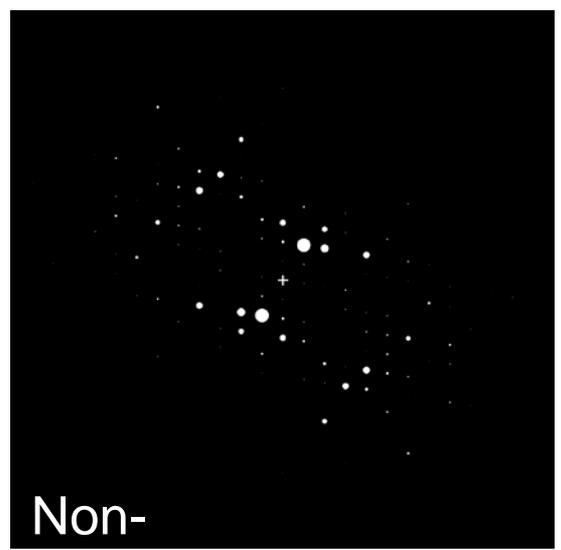
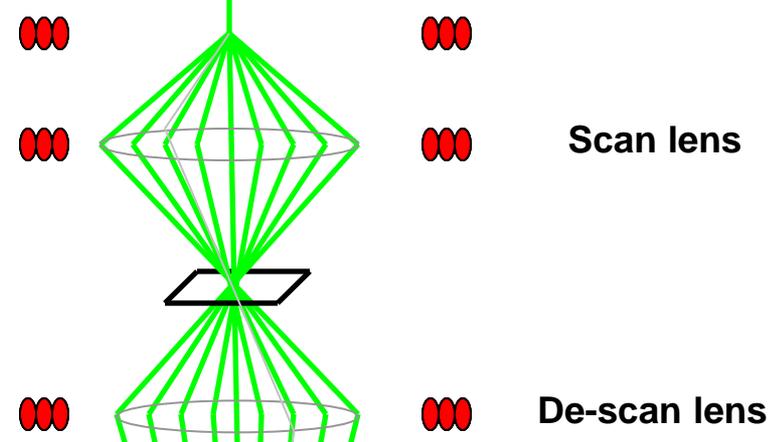
NanoMEGAS

Advanced Tools for electron diffraction

Image formation



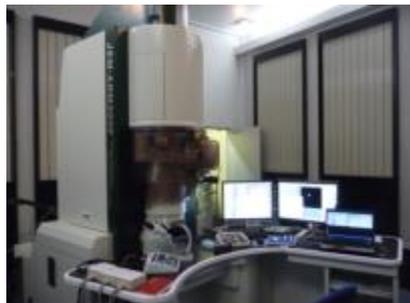
PRECESSION DIFFRACTION



(Diffracted amplitudes)

Precession...

Digital precession interface for advanced TEM



JEOL microscopes



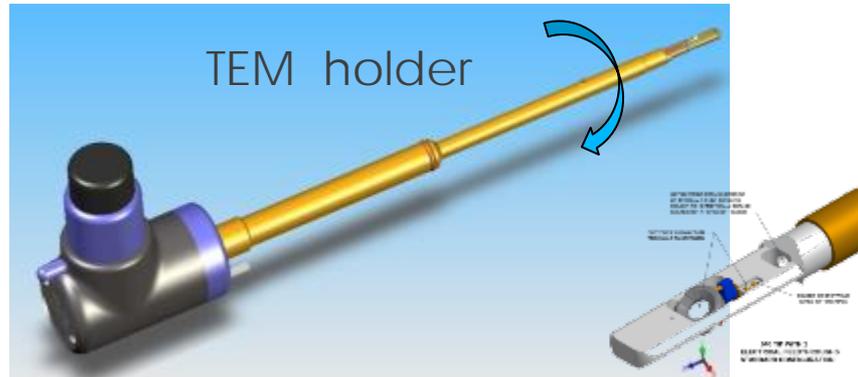
Zeiss microscopes



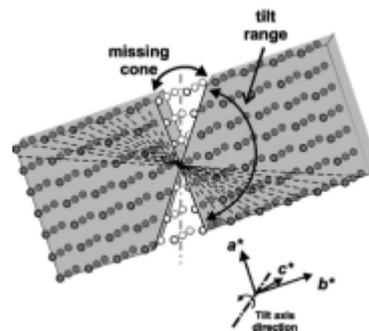
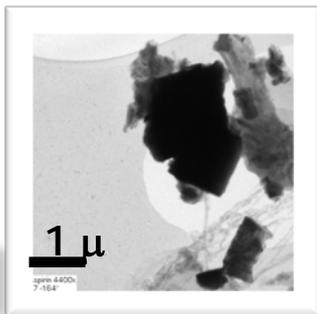
FEI microscopes



STRUCTURE ANALYSIS WITH TEM with Precession(PEDT)



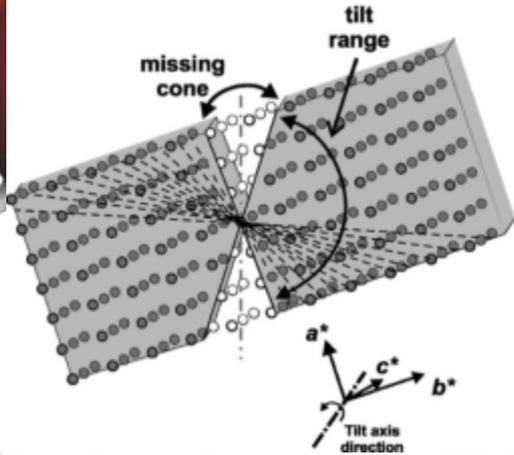
By tilting the TEM goniometer (single /double tilt, tilt rotation , tomography) we can reconstruct the reciprocal space of the crystal . Tilt angle may vary from -70 to $+70$ deg or less depending on TEM configuration and TEM goniometer specifications



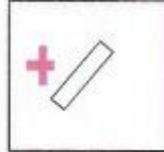
Organic samples degrade fast under the beam – cooling sample at Liquid Nitrogen is mandatory

TEM - PEDT : 3D sampling of reciprocal space

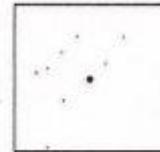
Select a crystal



Image



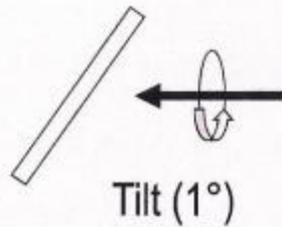
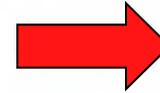
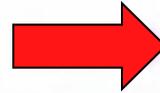
Diffraction



Tilt series

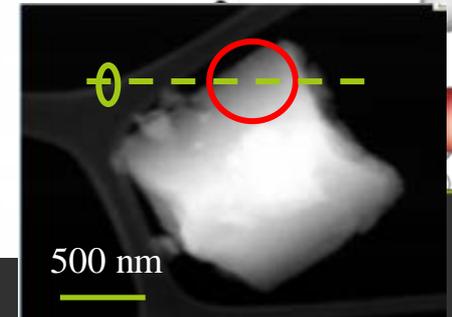
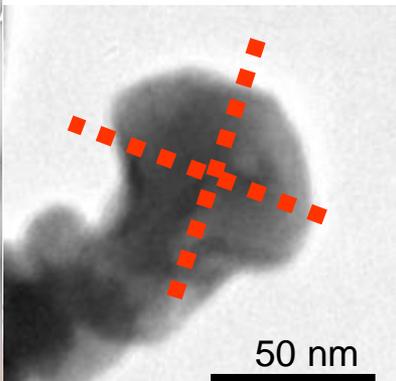


⋮

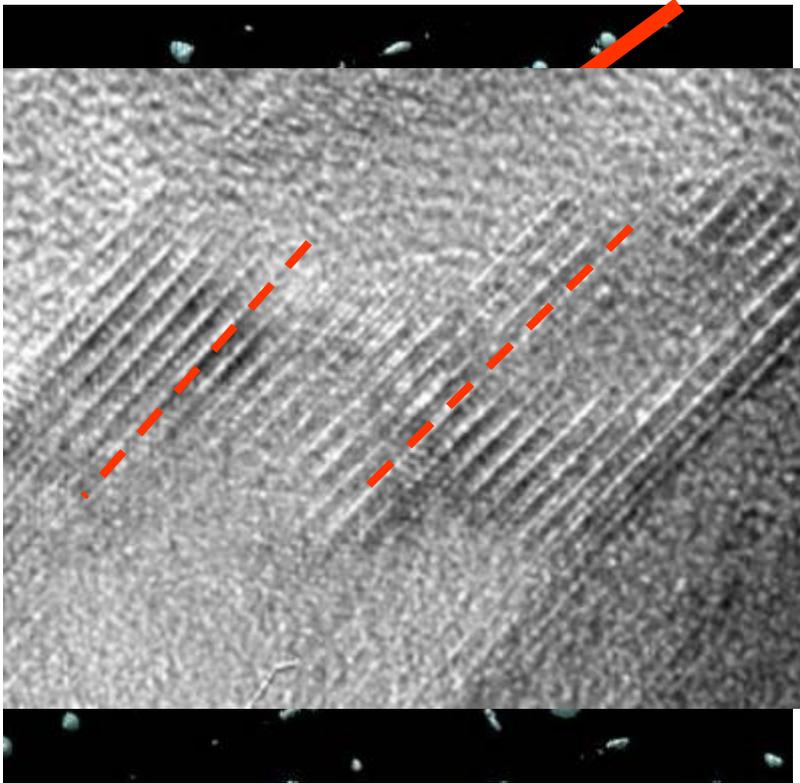


Arbitrary axis: Less dynamical effects,
More reflections
Easier to learn

Data collection: Any TEM using SAED or NED,
 $\sim 30^\circ$ for unit cell parameter
 $\geq 100^\circ$ for structure solution

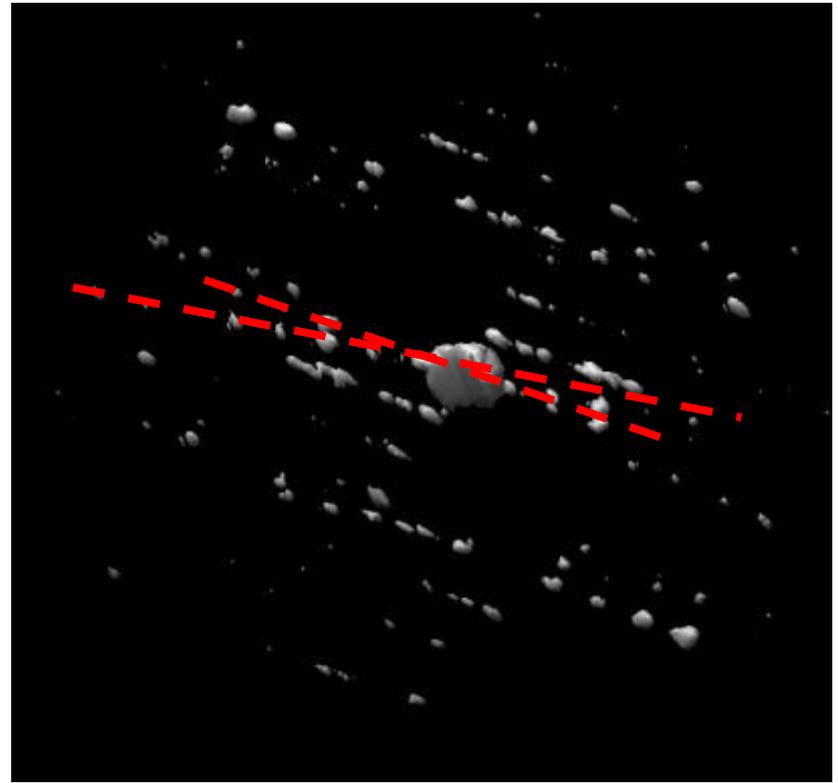


TEM ADT3D : Disorder & polycrystallinity



DISORDER

$0kl : k = 2N+1$

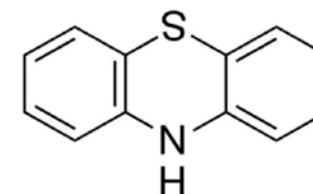


POLYCRYSTALS

c^* tilted $\sim 3^\circ$

Data Acquisition of Organics/Pharmaceuticals

Phenothiazine: anti-psychotic agent

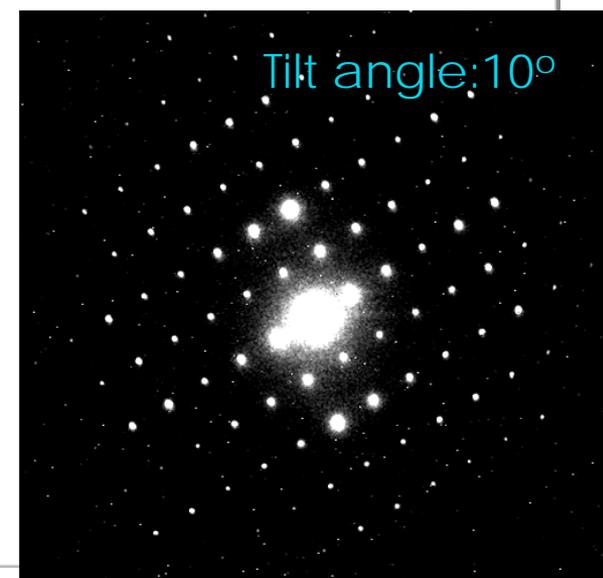
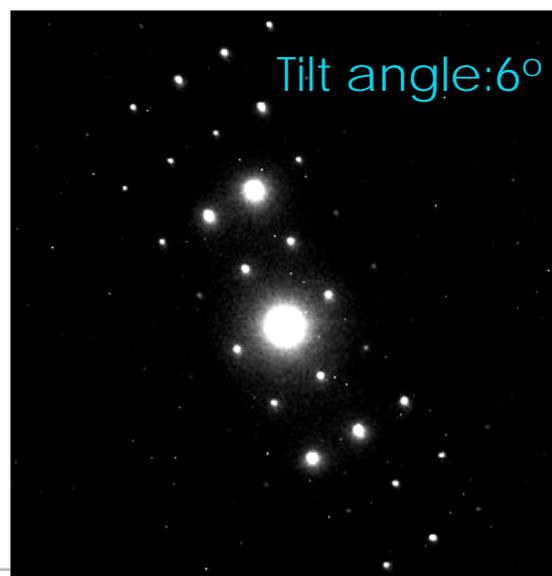
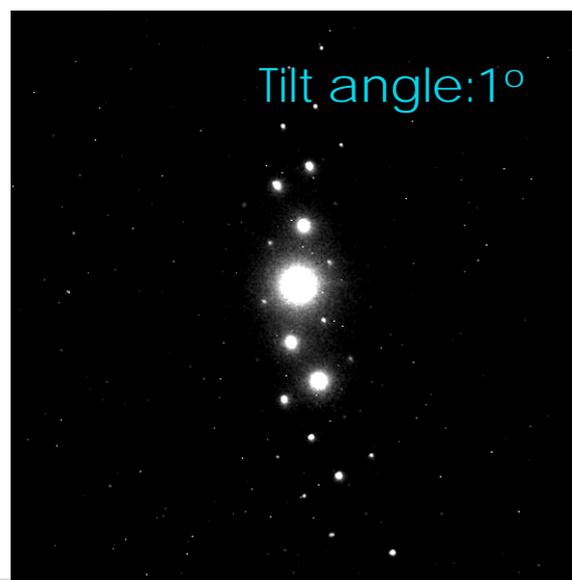


Precession Electron Diffraction Tomography

Tilt range: 40°

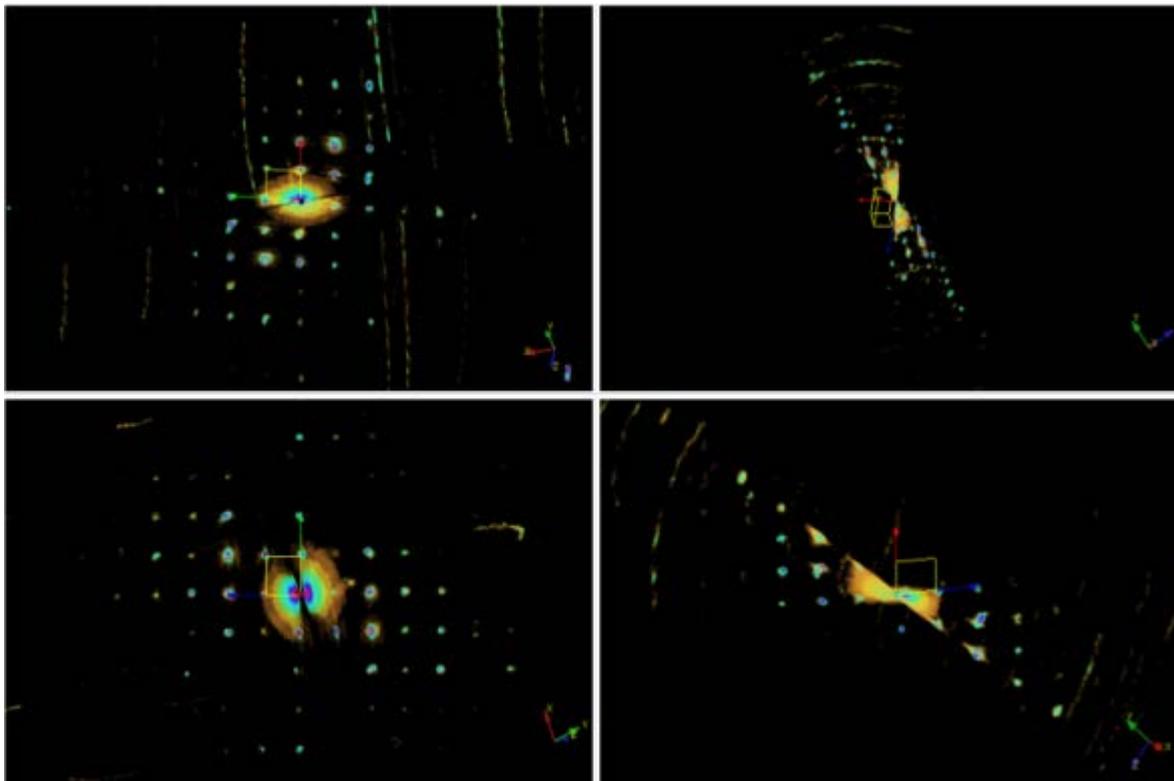
Tilt angle: 1°

Precession Angle: 1°



Data Acquisition of Organics/Pharmaceuticals

Phenothiazine: anti-psychotic agent



Experimental

$a = 9.03 \text{ \AA}$

$b = 9.01 \text{ \AA}$

$c = 12.04 \text{ \AA}$

$\beta = 94.21^\circ$

New Polymorph !!!!!

Timepix Detector in Barcelona

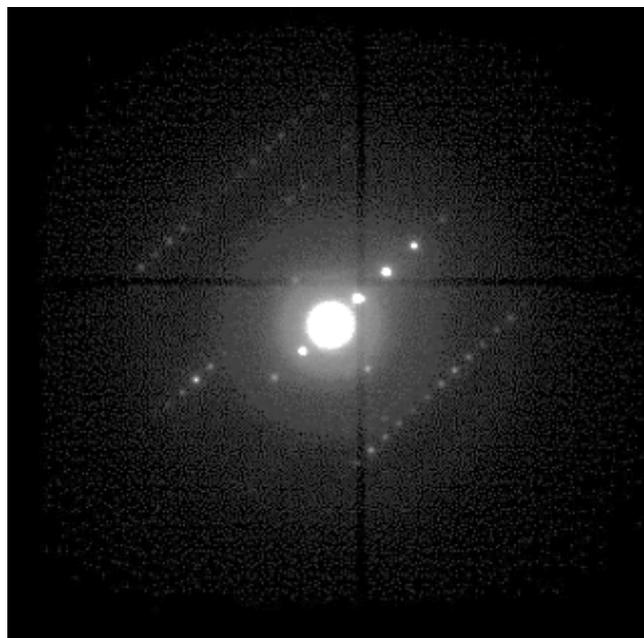


1000 times more sensitive than CCD

Collaboration NanoMEGAS - Univ Barcelona - Univ Leiden

Structure determination of pharmaceutical compounds using TEM electron diffraction without Cryo Cooling

Data Collected with MEDIPIX, CBZ Crystal

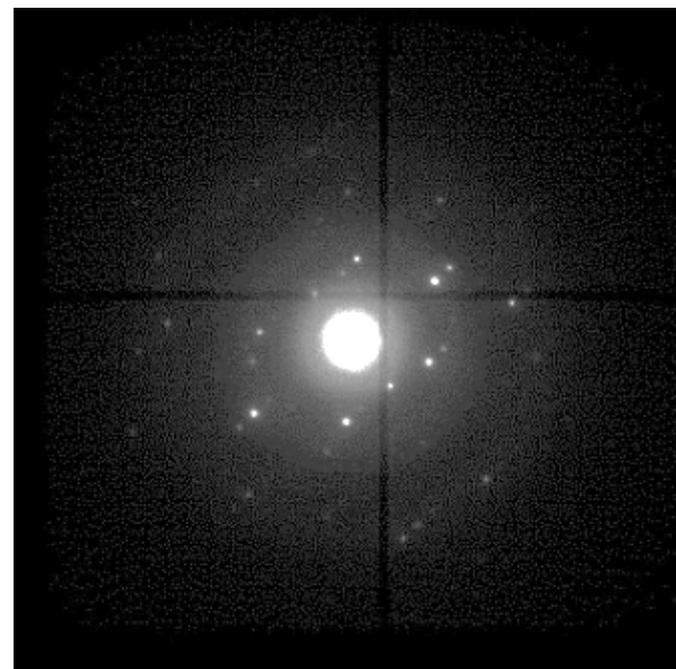


NO CRYO USED !!!

Collection time < 3 min

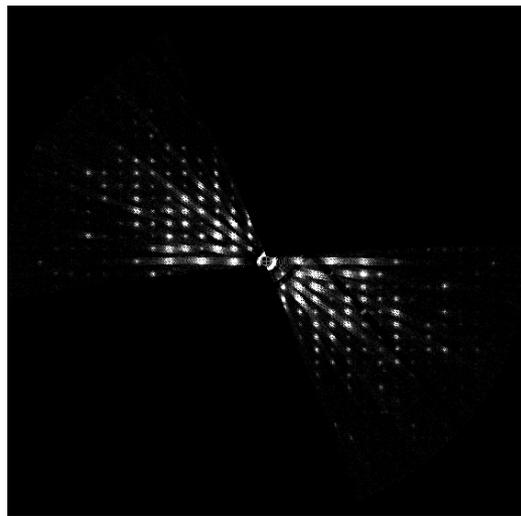
(Data collected -25° to 26°)

55 frames summed / 1°

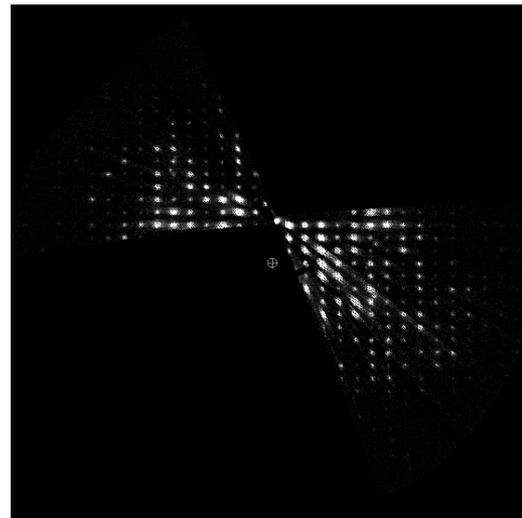


Resolution: 0.8 \AA

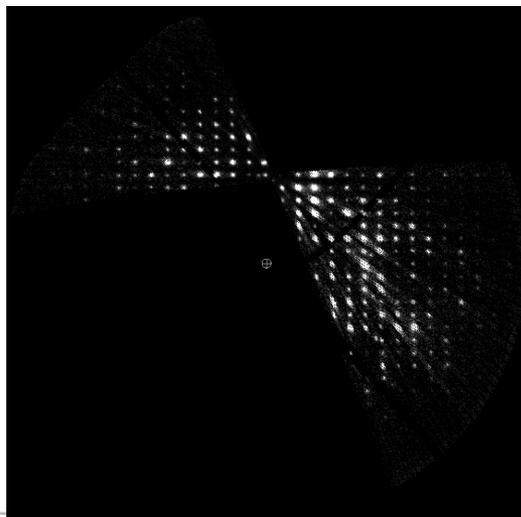
0kl



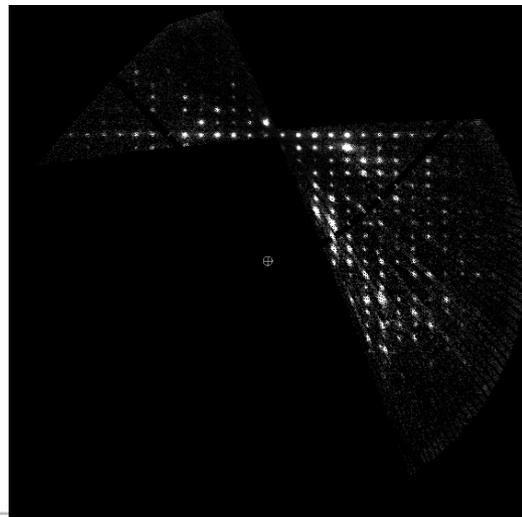
1kl



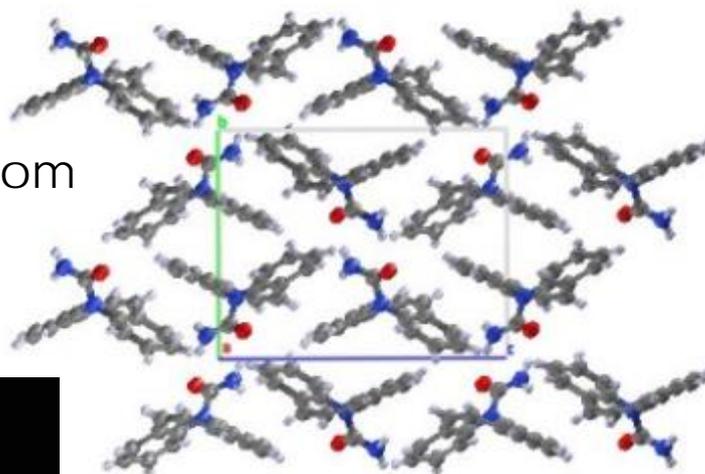
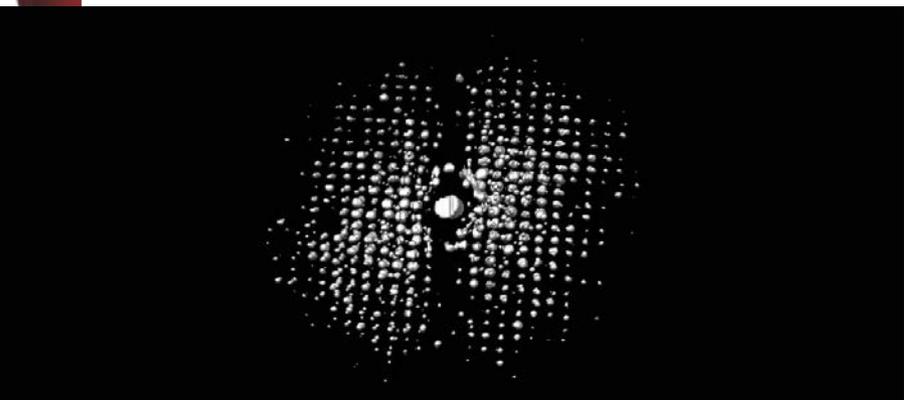
2kl



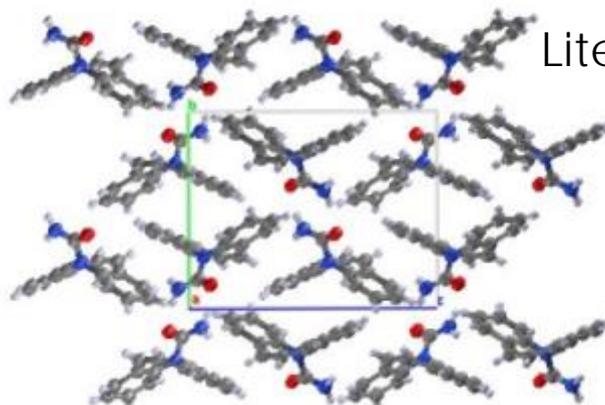
3kl



ab-initio solved Structure of CBZ from
ED Data



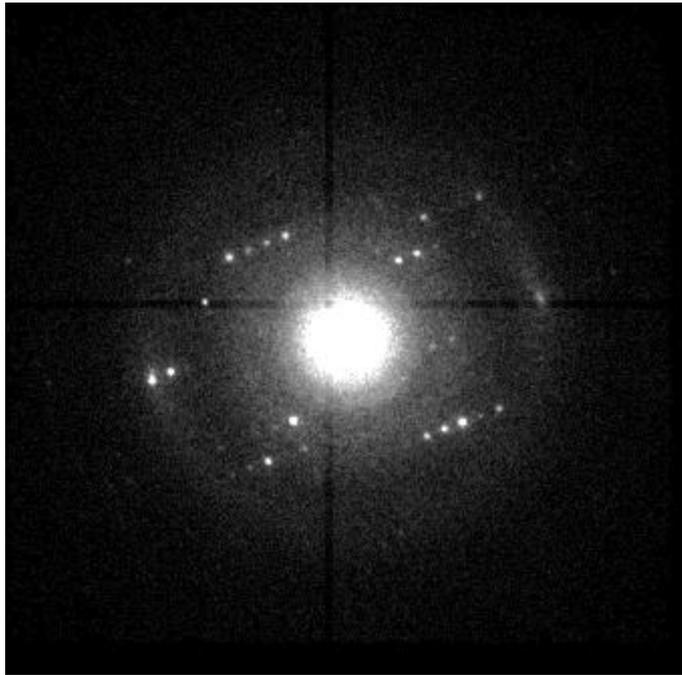
Experimental
 $a = 7.68 \text{ \AA}$
 $b = 11.44 \text{ \AA}$
 $c = 13.92 \text{ \AA}$
 $\beta = 91.22^\circ$



Literature Reported
 $a = 7.534 \text{ \AA}$
 $b = 11.150 \text{ \AA}$
 $c = 13.917 \text{ \AA}$
 $\beta = 92.94^\circ$

Literature Reported Structure of CBZ

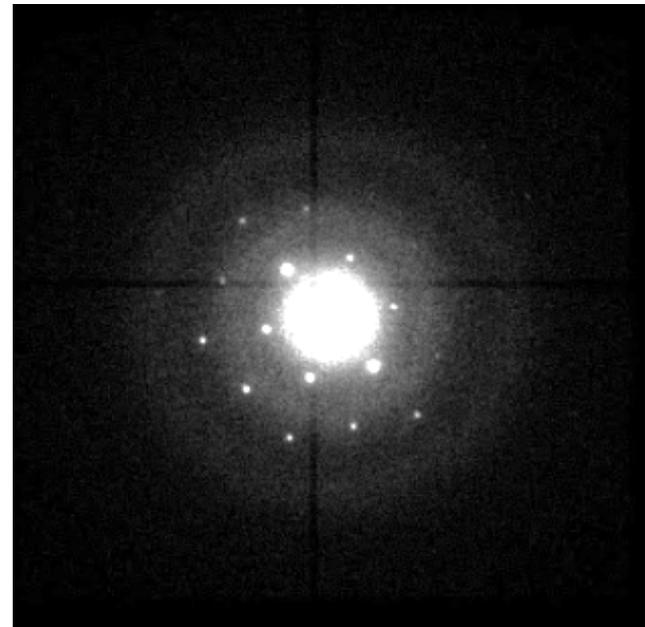
Data Collected with MEDIPIX, Nicotinic Acid Crystal



NO CRYO USED !!!

Collection time < 3 min

(Data collected -10^0 to 26^0)



Resolution: 0.8 \AA
20 frames summed / 1^0

Experimental

$$a = 7.19 \text{ \AA}$$

$$b = 11.74 \text{ \AA}$$

$$c = 7.28 \text{ \AA}$$

$$\beta = 112.65$$

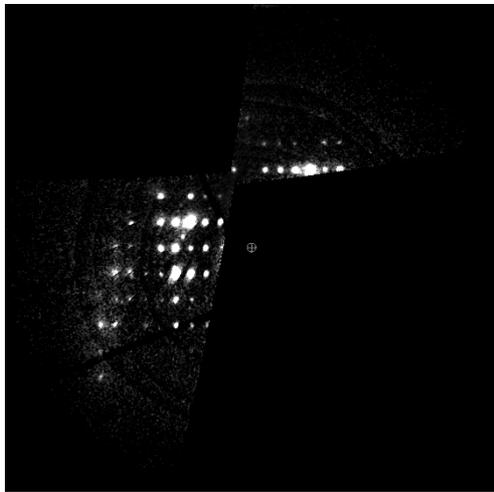
Literature Reported

$$a = 7.19 \text{ \AA}$$

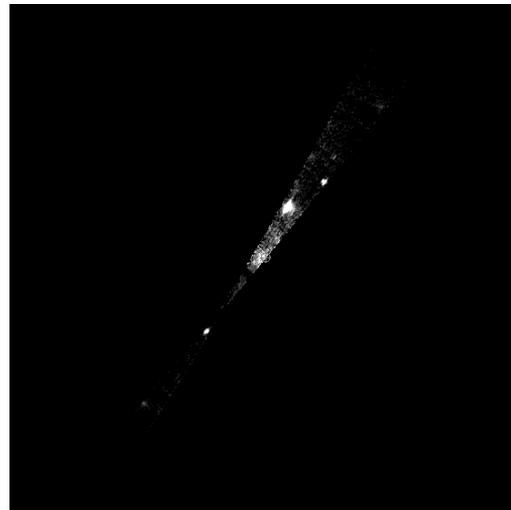
$$b = 11.69 \text{ \AA}$$

$$c = 7.23 \text{ \AA}$$

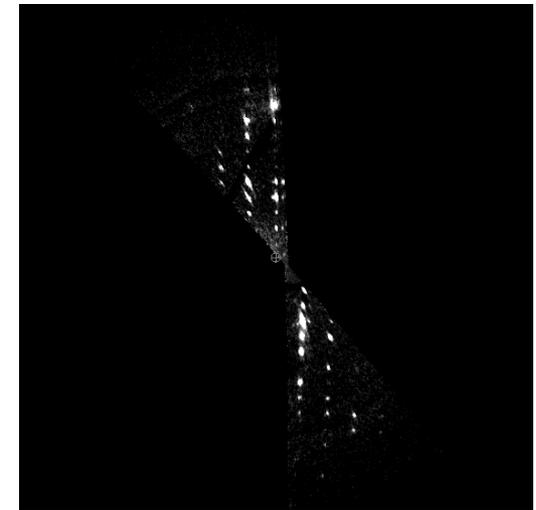
$$\beta = 113.55 \text{ \AA}$$



1kl



h1l



hk1



NanoMEGAS

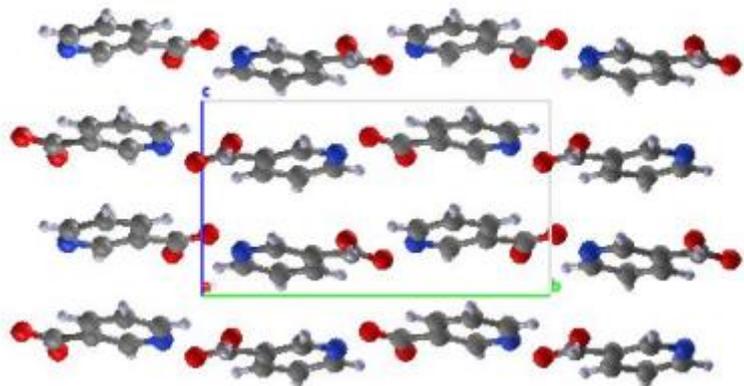
Advanced Tools for electron diffraction

MEGAS

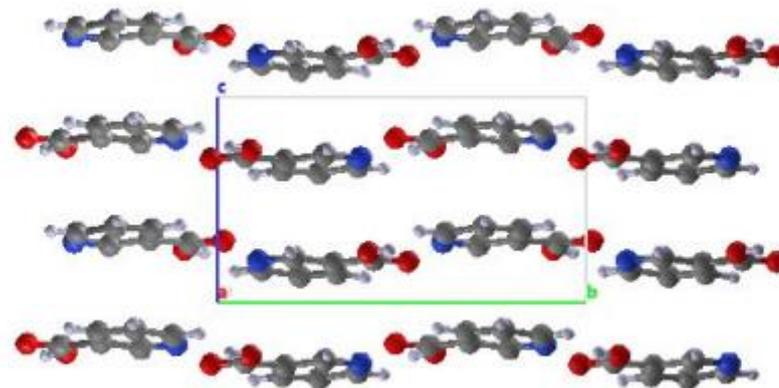
electron diffraction

SA Structure from ED Data

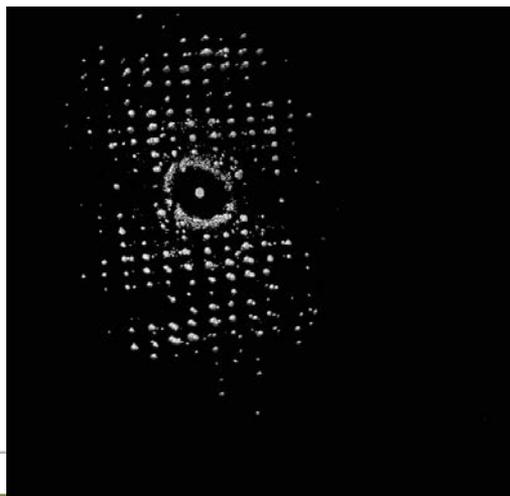
The Structure can not be solved by DM, due to low completeness



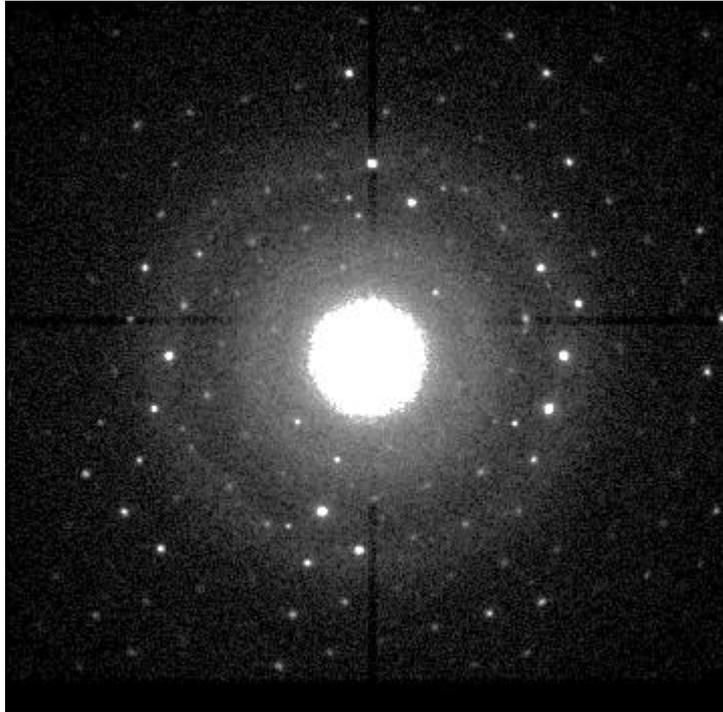
Experimental



Literature Reported



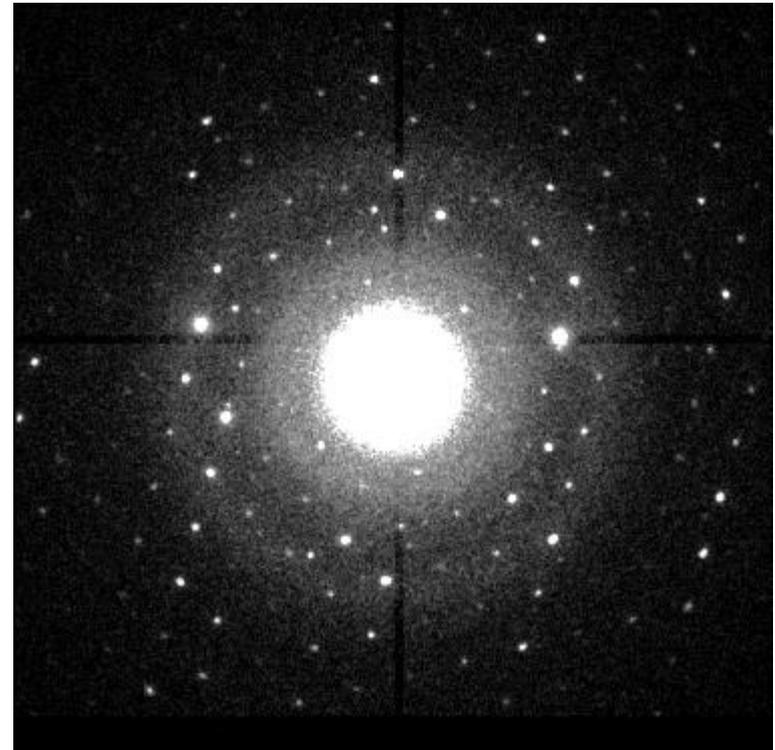
Data Collected with MEDIPIX, Salicylic Acid Crystal



NO CRYO USED !!!

Collection time < 3 min

(Data collected -16° to 20°)



Resolution: 0.8 \AA

38 summed / 1°

Experimental
Two Data sets

$$a = 5.14 \text{ \AA}$$

$$b = 8.99 \text{ \AA}$$

$$c = 7.49 \text{ \AA}$$

$$\beta = 106.01$$

Literature Reported

$$a = 4.89 \text{ \AA}$$

$$b = 11.20 \text{ \AA}$$

$$c = 11.24 \text{ \AA}$$

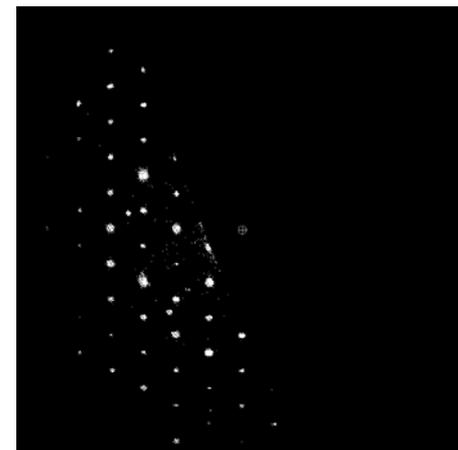
$$\beta = 92.49 \text{ \AA}$$



1kl



h1l



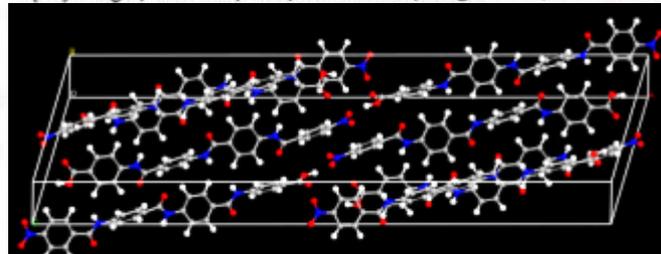
hk1

New Polymorph !!!!!

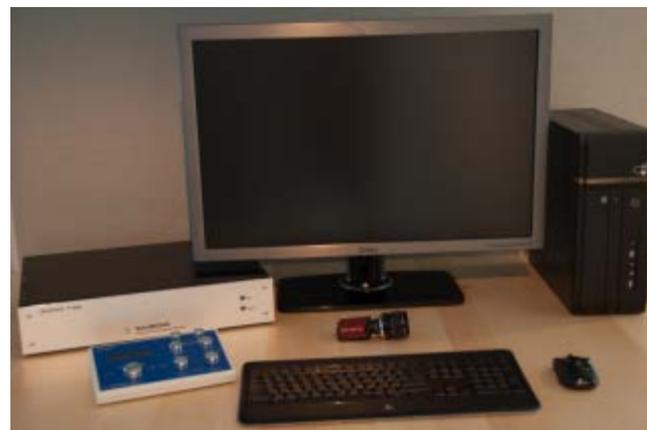
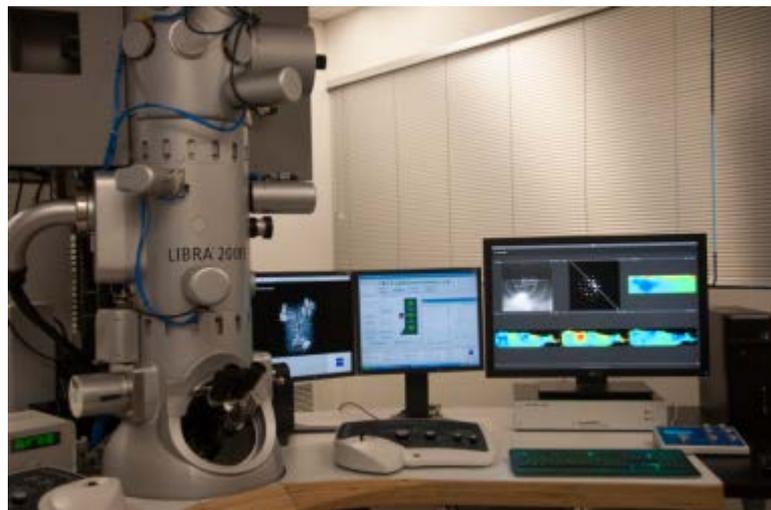
Crystal Structures solved from ADT^[1,2] data (new structures are marked with *)

	Space group	N* ind. reflections	N* ind. atoms	Volume (Å ³)	Completeness (%)
Phosphates					
SrP ₂ N ₅ O ^{[3]*}	Pnam	1790	25	1900	86
Ba ₈ P ₁₂ N ₁₇ O ₉ Br ₃ *	P6 ₃ /m	1343	11	1530	99
Tungstates					
Na ₂ W ₆ O ₁₃ ^[4]	P-1	738	10	262	69
Na ₂ W ₂ O ₇	Cmce	454	9	1264	91
K ₂₀ Al ₄ W ₂₄ O ₈₈ *	C2	1307	36	1983	84
Layered materials					
Sodium titanate (Na ₂ Ti ₆ O ₁₃) ^[5]	C2/m	517	11	510	72
Sodium titanate (NaTi ₃ O ₇ ·2H ₂ O) ^{[6]*}	C2/m	628	13	670	79
Hydrous silicate*	P-4m2	121	8	540	84
Ca-compounds					
Calcite (CaCO ₃) ^[7]	R-3c	106	3	120	97
Calcium silicate hydrate (Ca ₅ Si ₆ O ₁₇ ·5H ₂ O)*	Cm	689	19	930	69
High pressure phases					
Hydrous Al-pyroxene (Mg ₂ Al(OH) ₂ AlSiO ₆) ^{[8]*}	C2/c	498	8	560	87
Minerals					
Barite (BaSO ₄) ^[9]	Pnma	355	5	350	82
Mullite (Al ₆ Si ₂ O ₁₃)	Pbam	213	5	180	86
Charoite ₉₆ (K,Sr) ₁₅ (Ca,Na) ₃₂ [(Si ₇₀ (O,OH) ₁₈₀)](OH,F) ₄ ·nH ₂ O ^{[10]*}	P2 ₁ /m	3353	89	4430	96
Charoite ₉₀ (K,Sr) ₁₅ (Ca,Na) ₃₂ [(Si ₇₀ (O,OH) ₁₈₀)](OH,F) ₄ ·nH ₂ O ^{[11]*}	P2 ₁ /m	2878	90	4450	97
Metal Organic Frameworks (MOF)					
MFU_4large (Cl ₄ Zn ₂ N ₁₈ C ₃₆ O ₆ H ₁₂) ^{[12]*}	Fm-3m	655	8	32770	100
Basolite (C ₆ H ₈ CuO ₂)	Fm-3m	384	7	18640	99
Bi-MOF*	Pca2 ₁	1158	34	3560	67
Inorganic nanophasess					
Intermetallic nanoparticles (ZnSb) ^[13]	Pbca	106	2	440	70
Intermetallic nanoparticles (Zn ₈ Sb) ^{[13]*}	P-1	3651	30	1610	57
Intermetallic matrix (NiTe)	P6 ₃ /mmc	37	2	150	93
Intermetallic nanodomains (Ni ₃ Te ₂)*	P6 ₃ /mc	57	5	300	95
Semiconductor 6H-SiC	P6mm	52	6	130	100
Pseudo-spinel (Li ₂ Ti ₃ NiO ₈) ^[14]	P-3c1	187	11	720	91
Zeolites					
ZSM-5 (Na ₄ Al ₄ Si _{96-x} O ₁₉₂)	Pnma	2288	39	5490	79
IM-5 (Si ₂₈₈ O ₅₇₆)	Cmcm	2170	71	16380	68
Natrolite (Na ₄ Al ₃ Si ₁₃ O ₃₈ ·2H ₂ O) ^[15]	Fdd2	719	10	2250	92
ITO_43 (Si ₁₀₂ O ₁₈₄) ^{[15]*}	Cmmm	2735	39	14040	91
ECS-3 ((Na,K) ₃ Al ₃ Si ₄ C ₁₂ H ₃ O ₁₂ ·nH ₂ O) ^{[16]*}	Cc	4417	62	5040	72
Organic					
NLO-active material 10-CNBA (C ₂₉ NH ₁₇) ^[17]	P2 ₁ /c	1871	30	2000	90
Oligo p-benzamide OPBA3 ^[18]	P2 ₁ /c	3078	30	1755	81
Oligo p-benzamide OPBA4 ^{[18]*}	C2/c	3576	39	4545	77

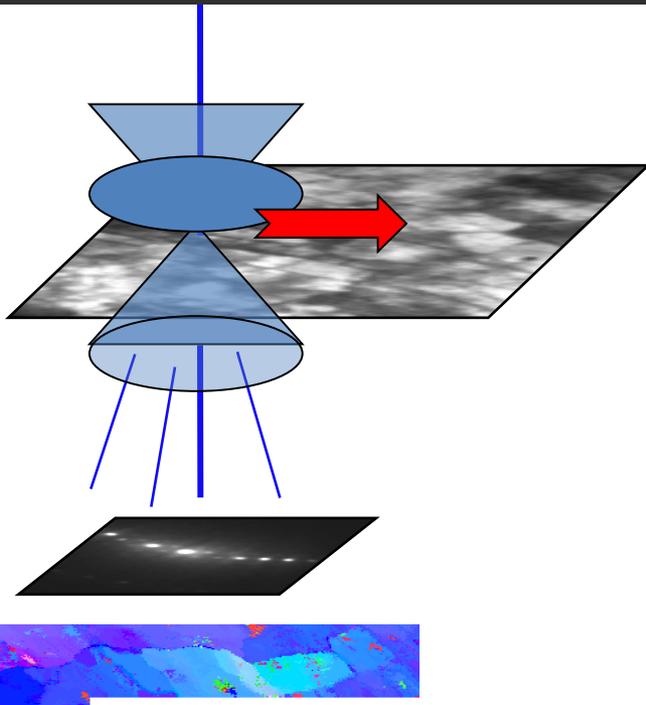
- [1] Kolb U., Gorelik T., Kübel C., Otten M. T., Hubert D. (2007) *Ultramicroscopy* **107**, 507.
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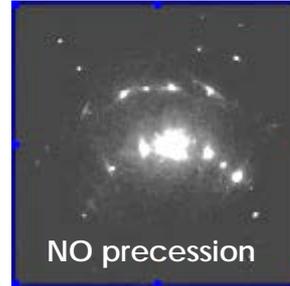
Find Crystal Texture & Amorphous Content Random Diffraction Tomography



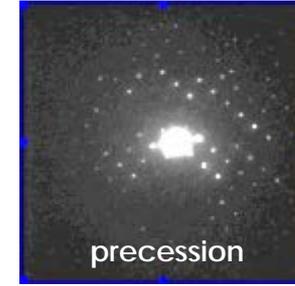
ASTAR (Orientation and phase imaging in TEM)



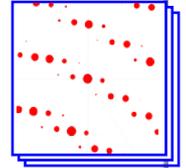
Orientation map



NO precession



precession

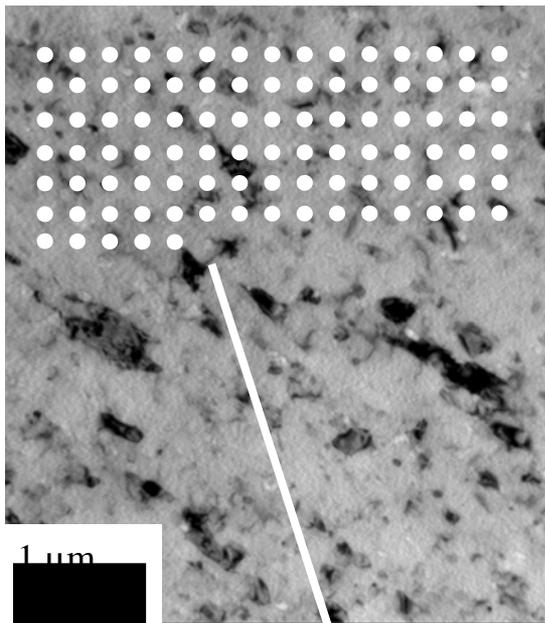


Using precession diffraction the number of ED spots observed increases (almost double) ; correlation index map becomes much more reliable when compared with templates

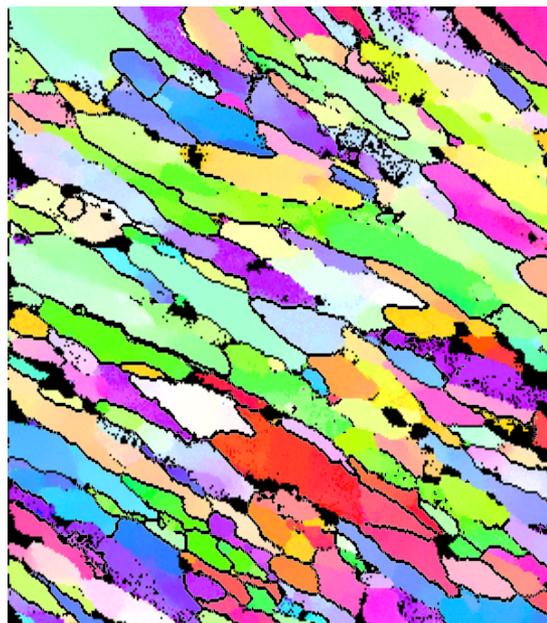
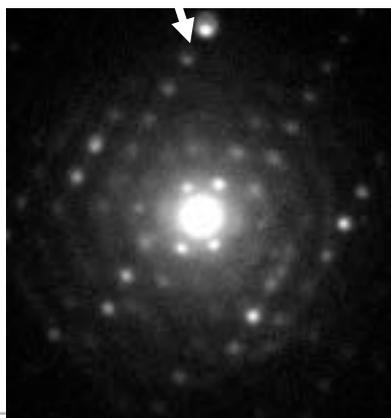


Scanning the TEM beam in precession mode
Step size 0.1 nm -100 nm
Dedicated CCD with > 100 frames /sec
Typical area 5 x 5 microns
Scanning times (typical) 5-10 min

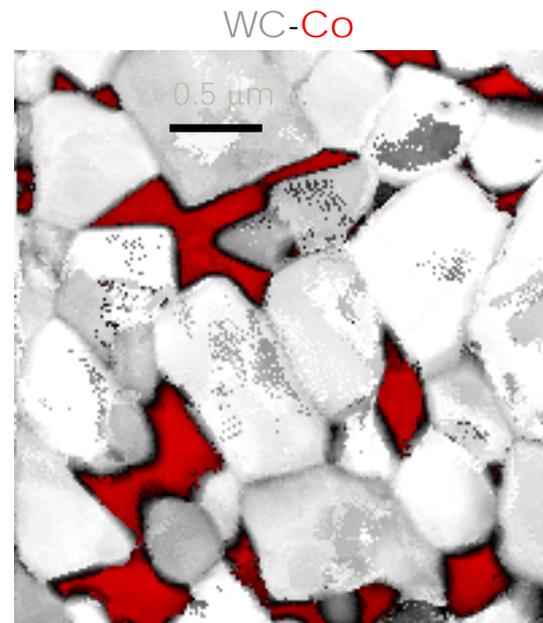
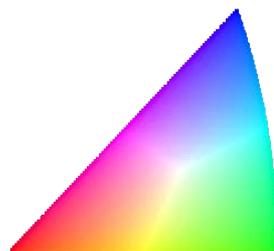
ASTAR : Automated Crystal Orientation Mapping



Severely deformed
7075 Al. Alloy



Orientation map



Phase map



What about the amorphous content in a sample ?

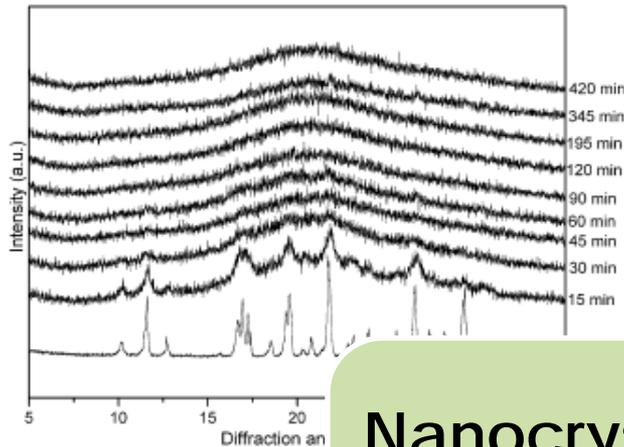
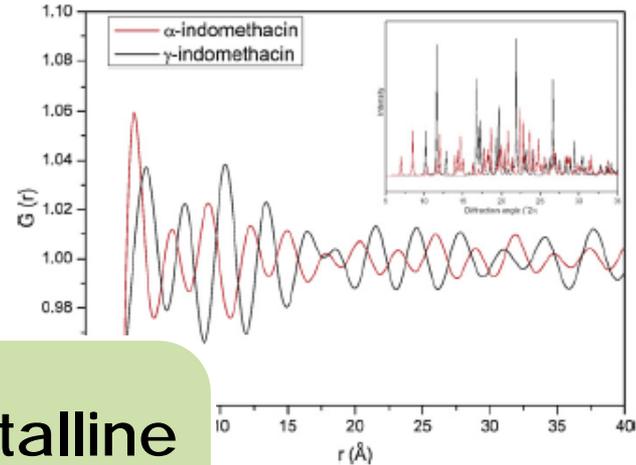


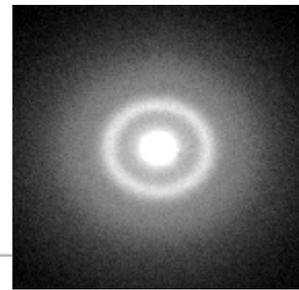
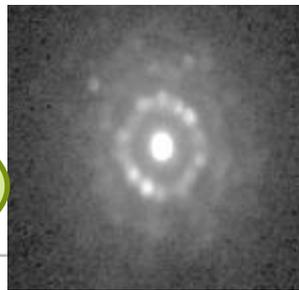
Fig. 2. XRPD diffractograms of the cryo-m for different times (time in the figure correct)



and α -form of indomethacin. XRPD diffractograms of γ -indomethacin are shown in the inset.

Nanocrystalline
or Amorphous
?

ED pattern from
overlapping nanocrystals
< 10 nm

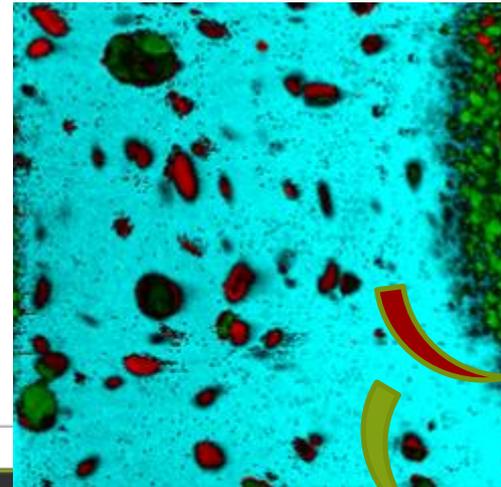
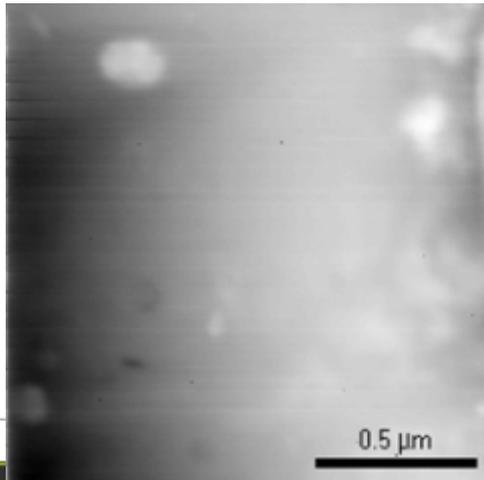
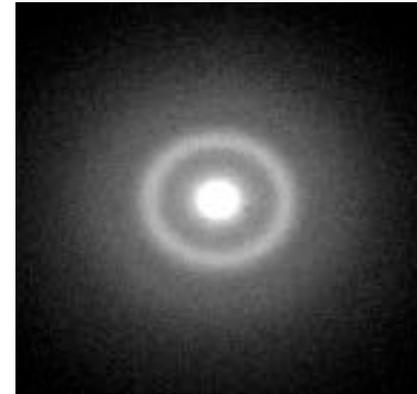
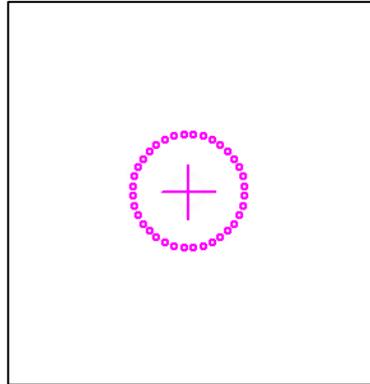
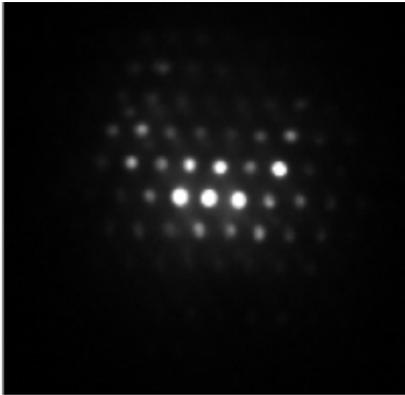


ED pattern from
amorphous area

both may show "X-ray amorphous" pattern

Nanocrystalline or Amorphous ?

Example : Mg-Cu-Gd partly recrystallized metallic
glass with Mg_2Cu and Cu_2Gd crystalline precipitates

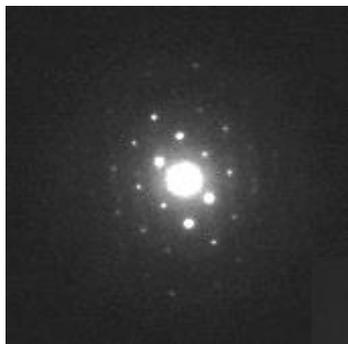


crystalline

amorphous

ORGANIC crystals : beam sensitive

*Only possible to collect several
(non ZA oriented ED patterns
from different crystals*



Sample	Crystal	Beam	Wavelength	Spot Size	Spot Intensity	Spot Position	Spot Color	Spot Shape	Spot Size	Spot Intensity	Spot Position	Spot Color	Spot Shape
1	1	1	1	1	1	1	1	1	1	1	1	1	1
2	2	2	2	2	2	2	2	2	2	2	2	2	2
3	3	3	3	3	3	3	3	3	3	3	3	3	3
4	4	4	4	4	4	4	4	4	4	4	4	4	4
5	5	5	5	5	5	5	5	5	5	5	5	5	5
6	6	6	6	6	6	6	6	6	6	6	6	6	6
7	7	7	7	7	7	7	7	7	7	7	7	7	7
8	8	8	8	8	8	8	8	8	8	8	8	8	8
9	9	9	9	9	9	9	9	9	9	9	9	9	9
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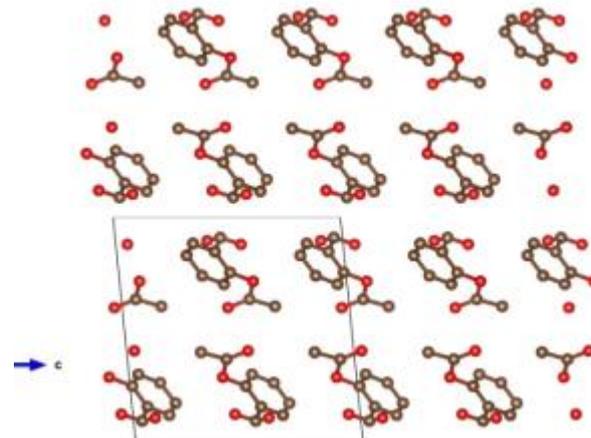
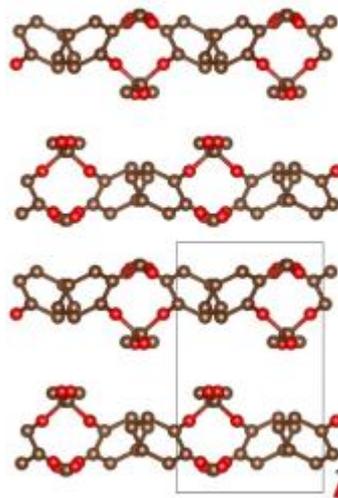
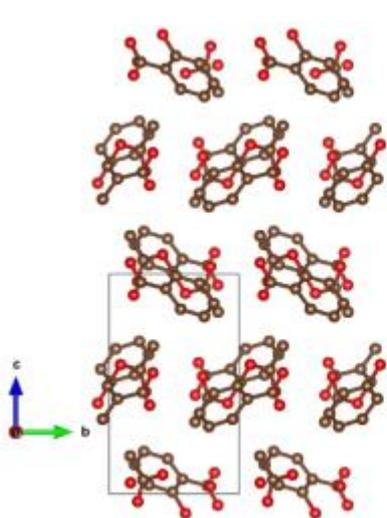
*CRYSTAL UNIT CELL can be calculated
from several patterns (oriented or not)*



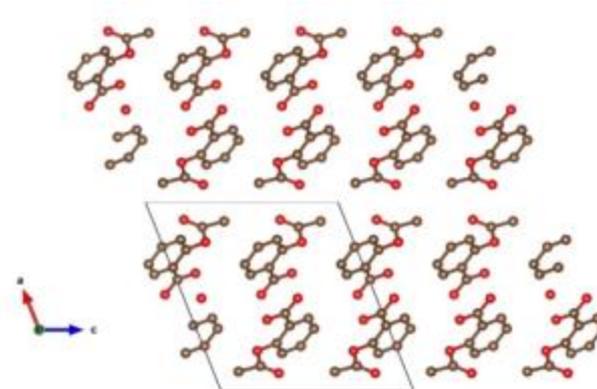
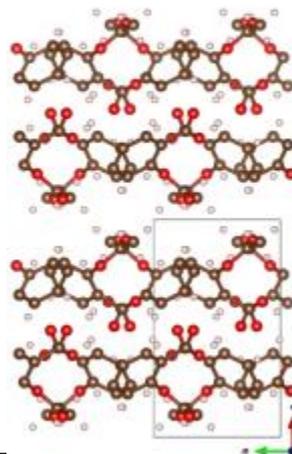
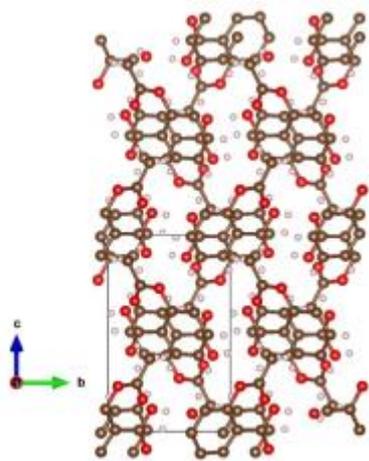


ASPIRIN

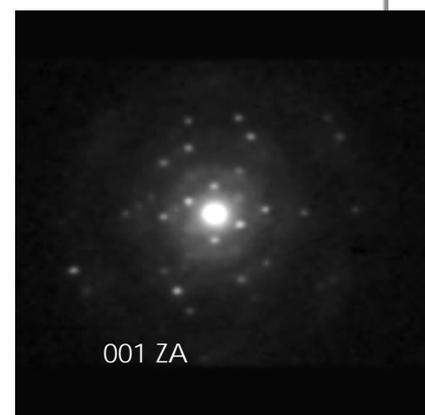
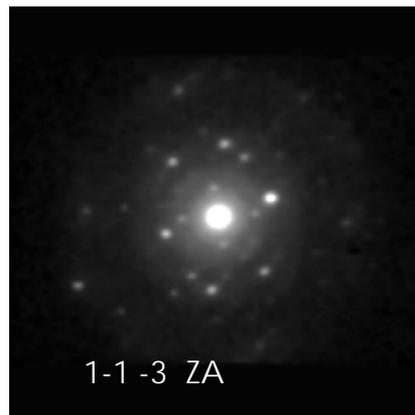
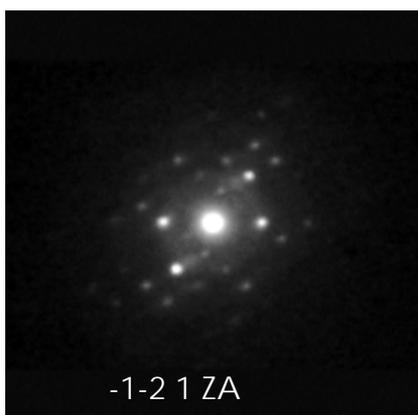
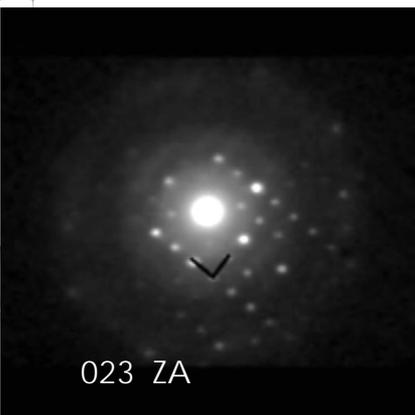
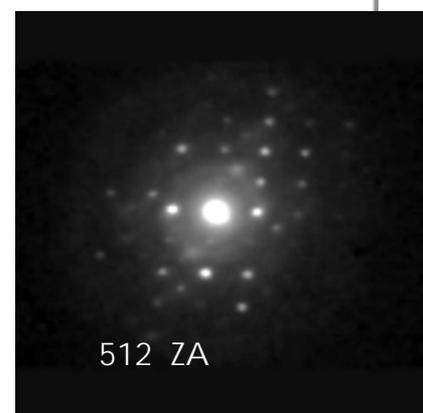
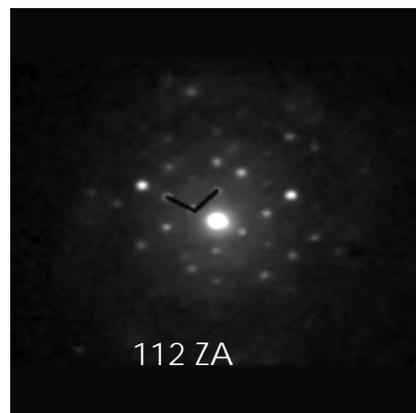
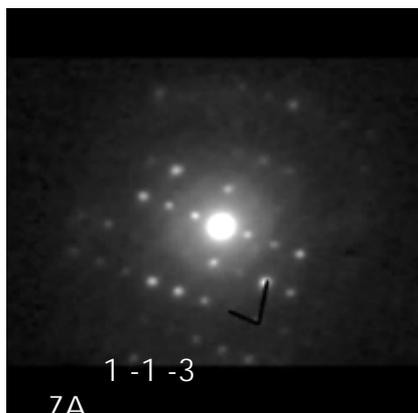
form I (P21/c): $a=11.233(3) \text{ \AA}$, $b=6.544(1) \text{ \AA}$, $c=11.231(3) \text{ \AA}$, $\hat{a} = 95.89(2)^\circ$



form II (P21/c): $a=12.095(7) \text{ \AA}$, $b=6.491(4) \text{ \AA}$, $c=11.323(6) \text{ \AA}$, $\hat{a}=111.509(9)^\circ$



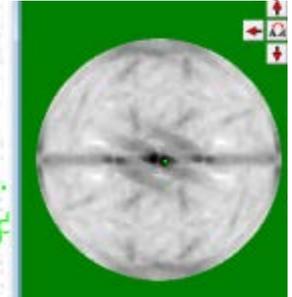
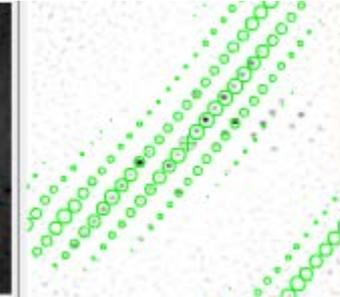
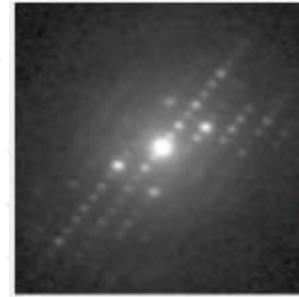
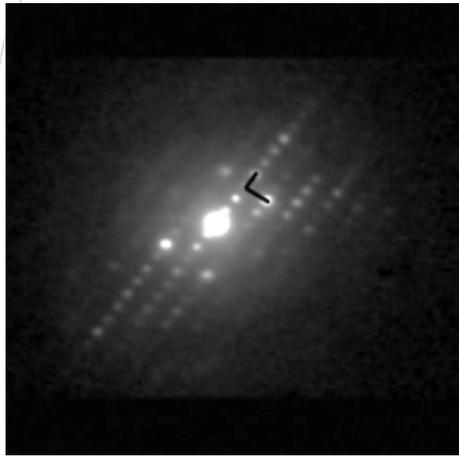
ASTAR : Random precession diffraction tomography



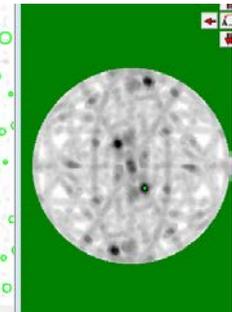
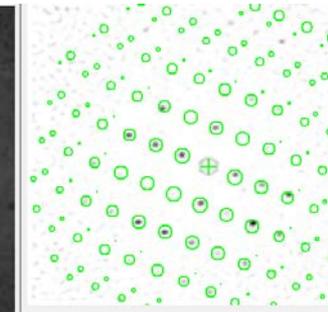
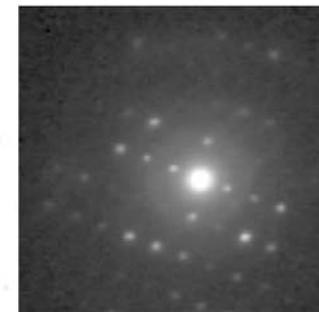
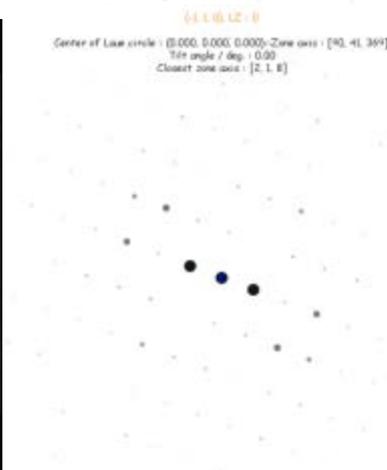
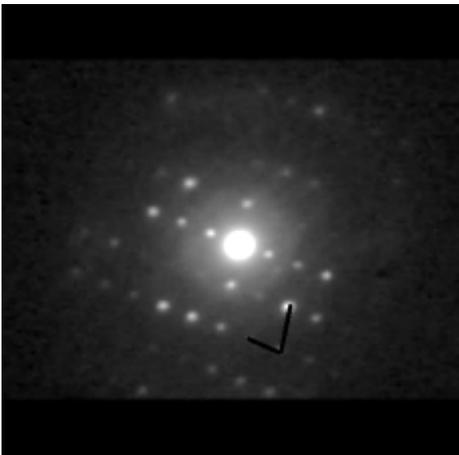
EDiff software : cell parameters are found from 11 random PED patterns

a	b	c	alpha	beta	gamma
10.68	6.663	11.75	90.0	95.0	90.0

ASTAR : Random 3D diffraction tomography



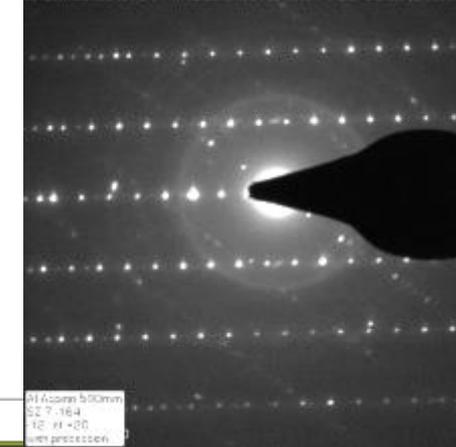
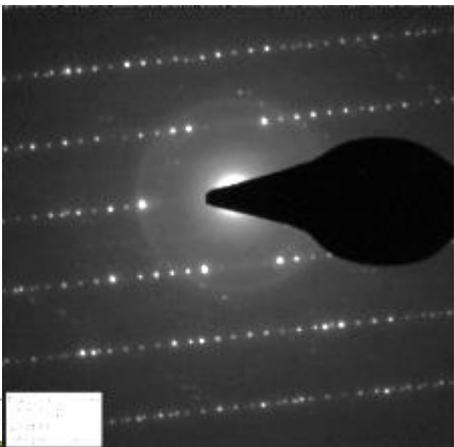
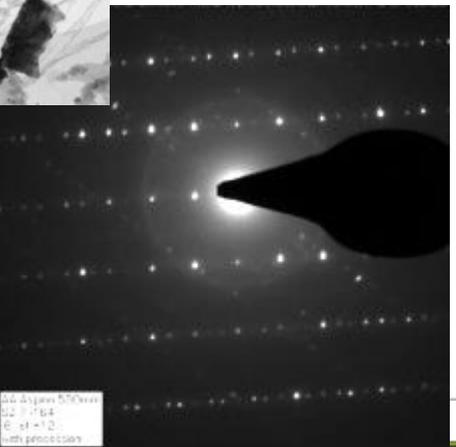
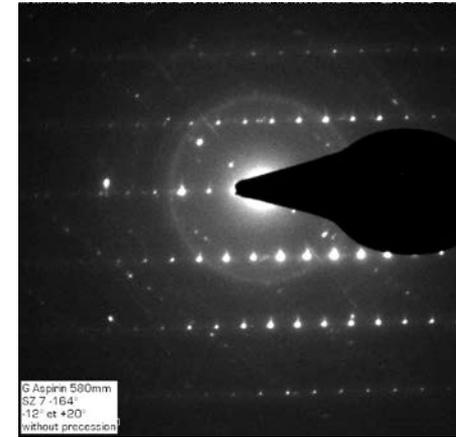
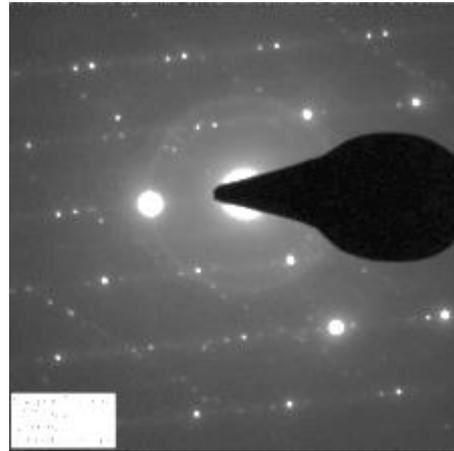
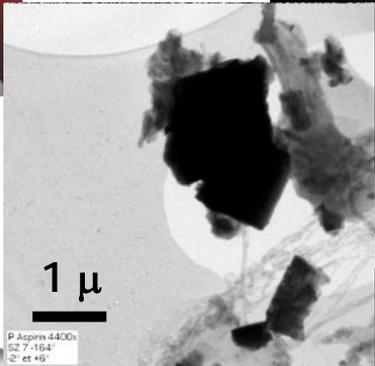
ZA 001 Best match



ZA 113 Best Match

ASPIRIN

PED patterns improvement with precession



CONCLUSIONS

Try electron diffraction !



NanoMEGAS

Advanced Tools for electron diffraction

CONCLUSIONS

Most important reasons to use Electron Diffraction

- ❑ Nanocrystalline samples that give poor X-Ray patterns
- ❑ Inconclusive cell determination /crystal structure from X-Ray
- ❑ Detailed overview over crystalline vs amorphous content
- ❑ Solve crystal structures ab-initio from < 50 nm crystals

How Electron Diffraction can be used ?

- Use any TEM (120, 200, 300 kv LaB6 or FEG)
- 3D precession diffraction tomography (ADT-3D) can help to find ab-initio the unit cell /crystal structure of any unknown crystal > 50 nm
- Random precession diffraction tomography (ASTAR) to find unit cell and crystal structure by reconstructing reciprocal space from quasi-oriented PED patterns
- Reveal detailed local amorphous vs crystalline part in samples

Collaborators

University of Lieden, Group of Prof. J. P. Abrahams

University of Cambridge, Group of Prof. Paul Midgley

Center for Nanotechnology Innovation@NEST , Dr. Mauro Gemmi

SIMAP-INP Grenoble-France, Dr. Edgar Rauch

Contact us at

pharma@nanomegas.com

www.nanomegas.com/Pharma

