TEM 3D Precession Electron Diffraction Tomography to Solve Pharmaceutical API Structures

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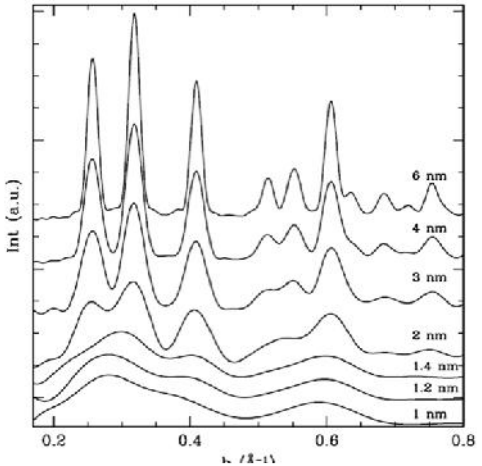
STRUCTURE ANALYSIS WITH ELECTRON DIFFRACTION

Why electrons?

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

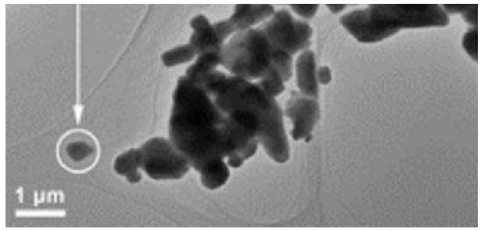
  - *single crystal data on powder sample*
  - *short data collection time*

- X- Ray peaks broaden with crystals of nm range

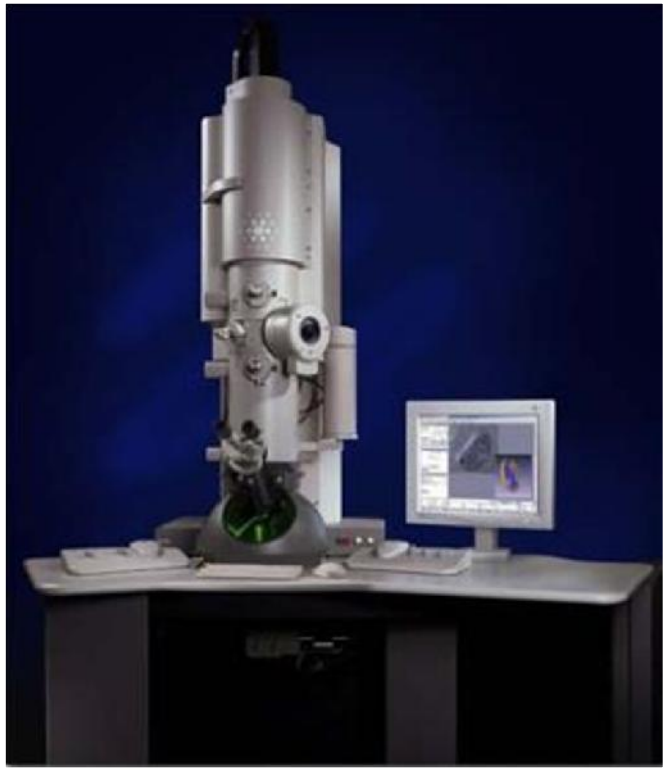


With Electron microscope we can study

nm- and micro-sized crystals



STRUCTURE ANALYSIS WITH TEM

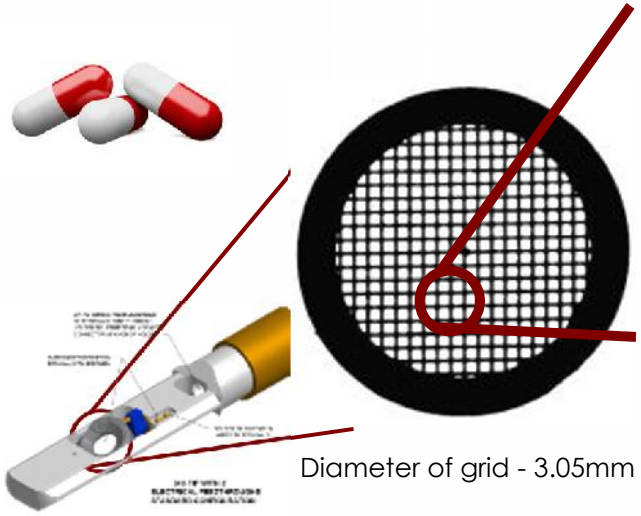


Transmission electron microscopy (TEM)

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

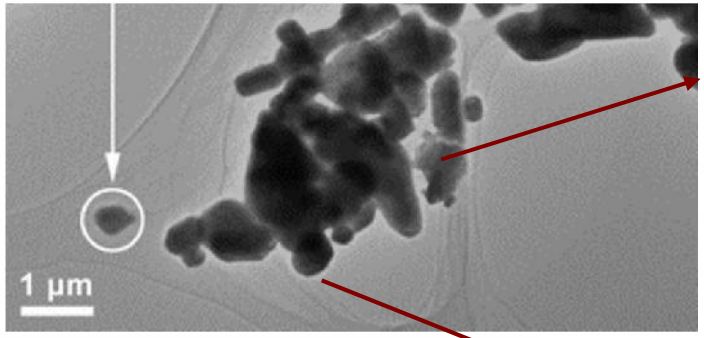
STRUCTURE ANALYSIS WITH TEM

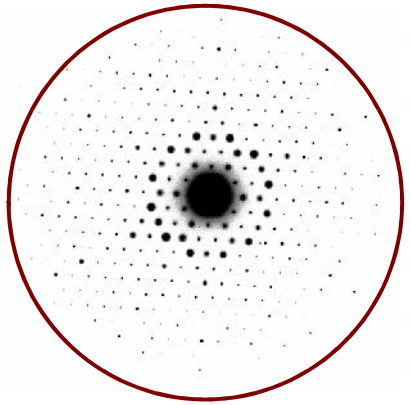
TEM : Electron diffraction advantages

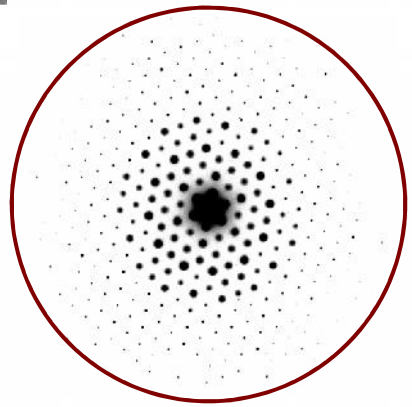


Diameter of grid - 3.05mm

TEM goniometer



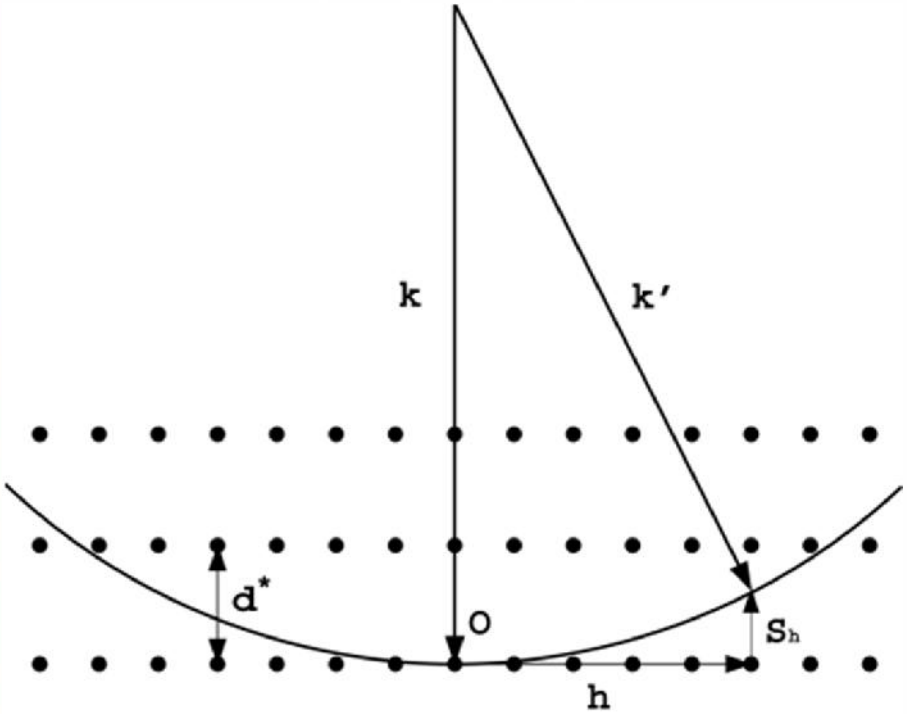




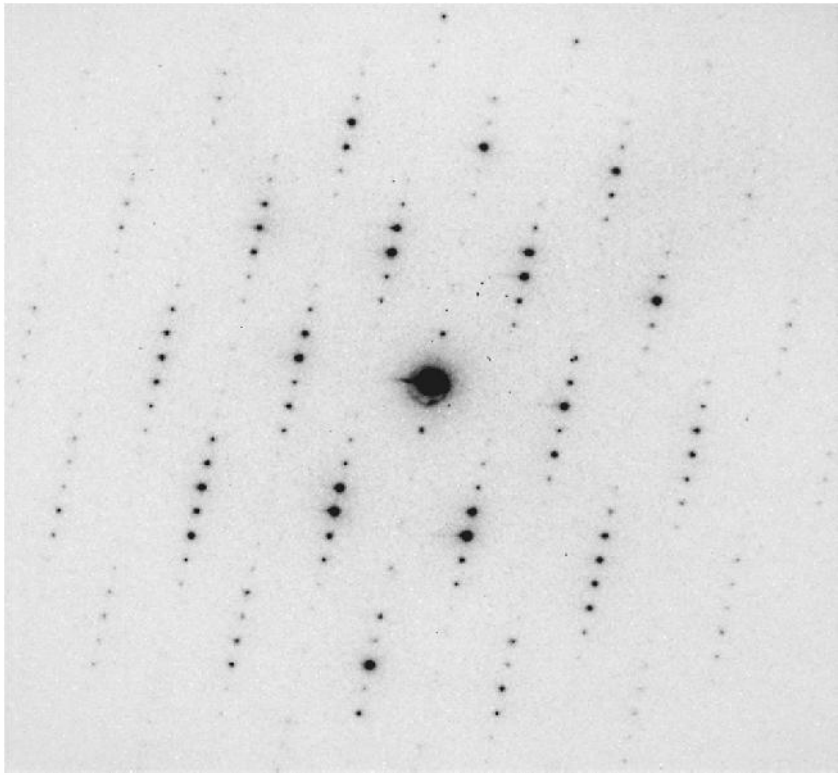
Every TEM (electron microscope) may produce ED patterns and HREM from individual single nanocrystals

ED information: Cell parameter and symmetry determination. Measuring intensity values leads to structure determination

Electron diffraction



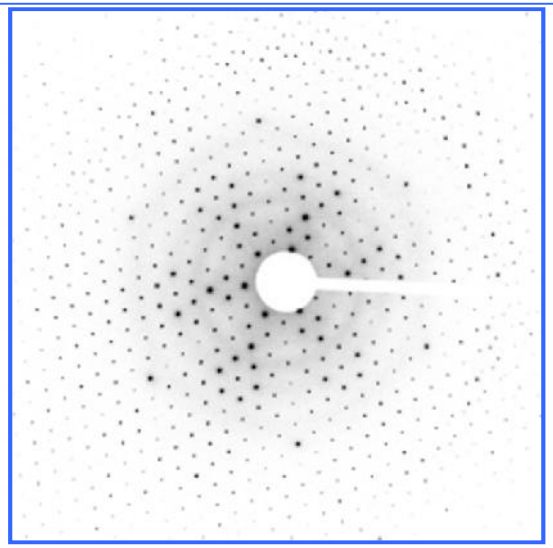
VOLTAGE IN KV	λ IN Å
100	0.0370
300	0.0197
1000	0.0087

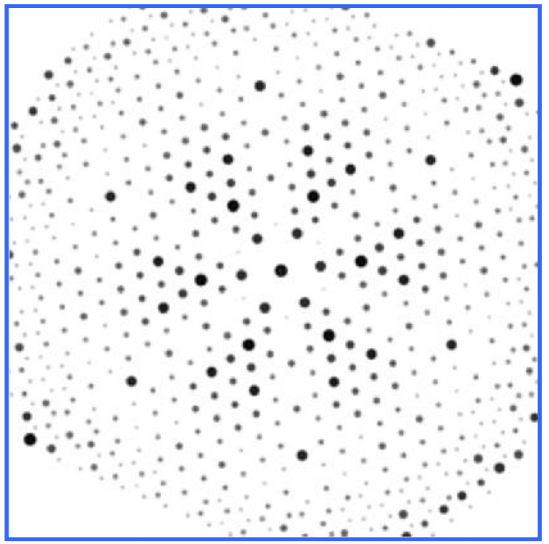


The Ewald sphere is flat! → **In one shot we record a reciprocal lattice plane**

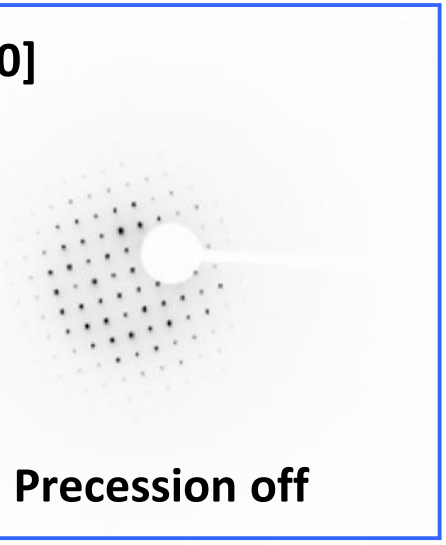
Zone axis PED on “standrard” mayenite

[111]

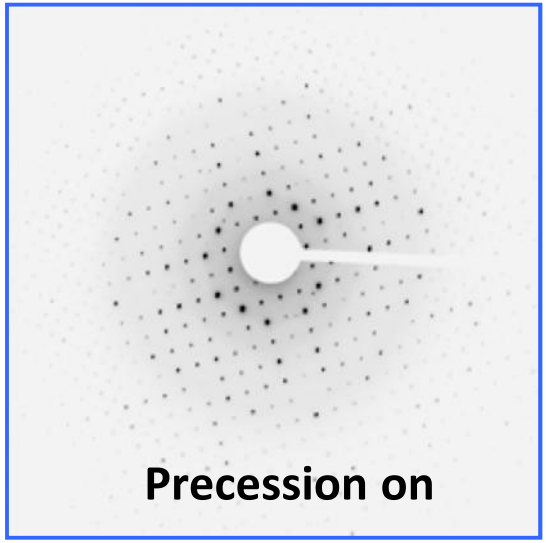




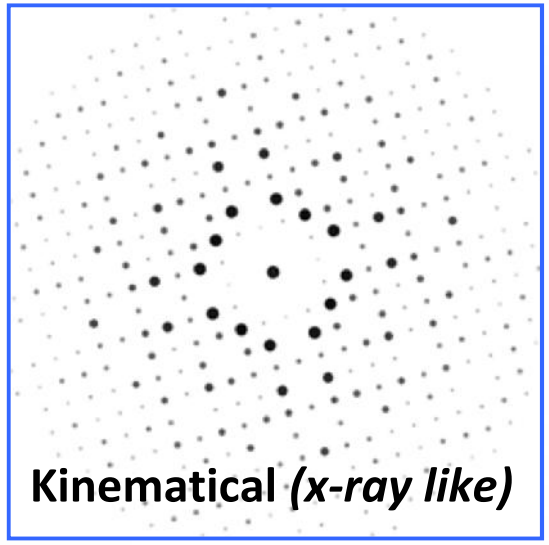
[100]



Precession off

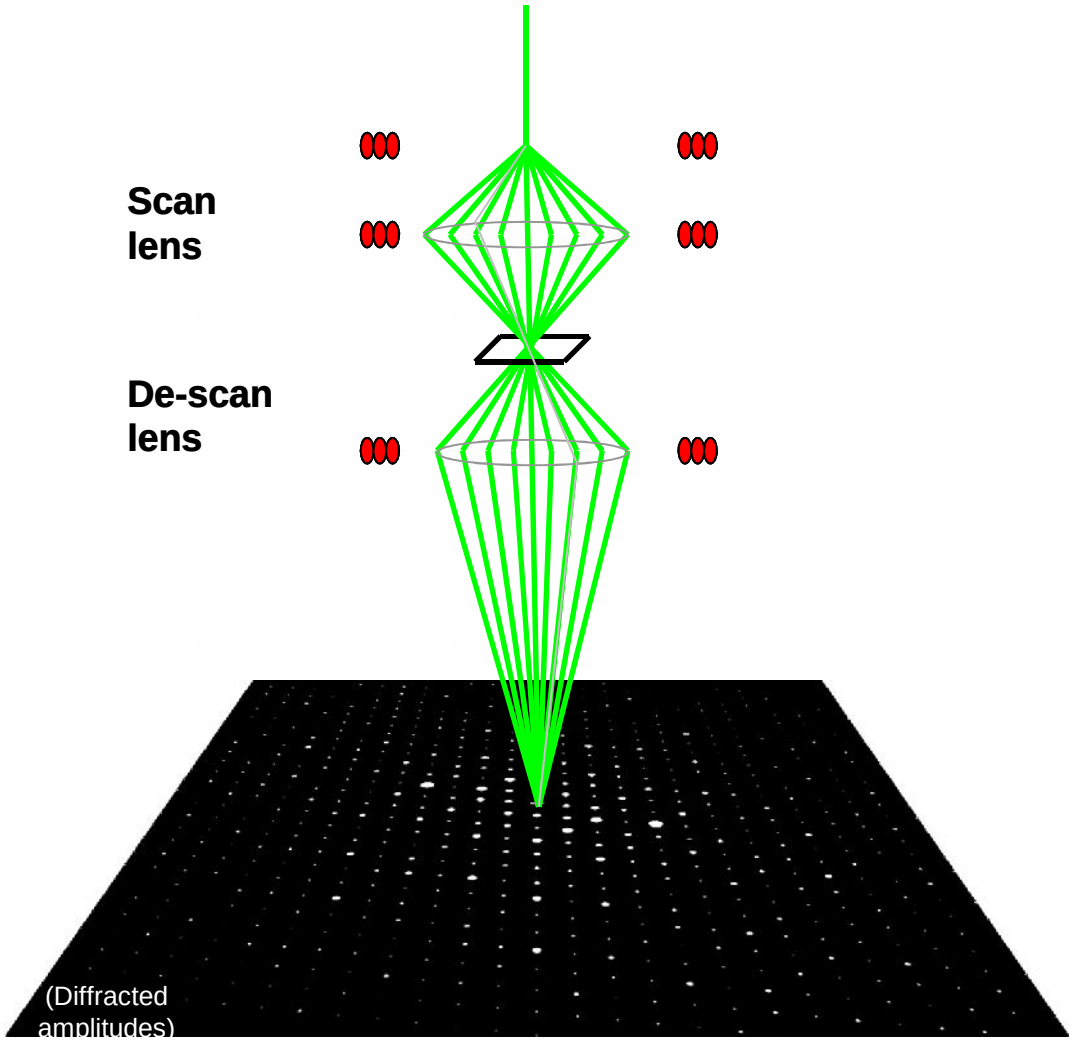


Precession on

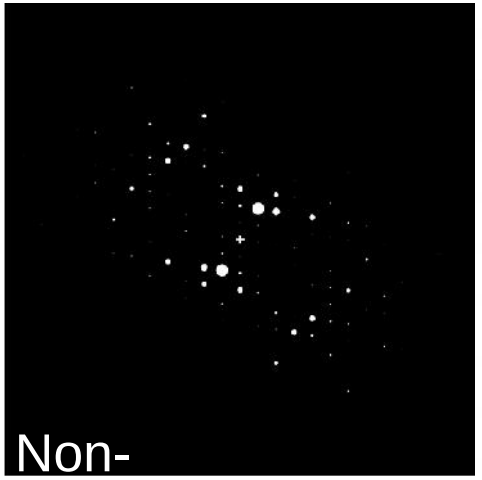


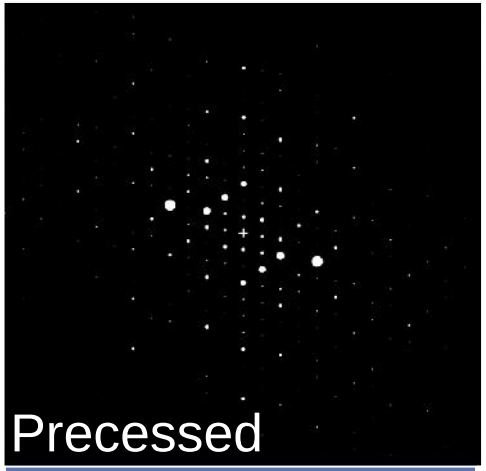
Kinematical *(x-ray like)*

Courtesy Dr. M. Gemmi IIT Pisa Italy



Reference : C.Own PhD thesis





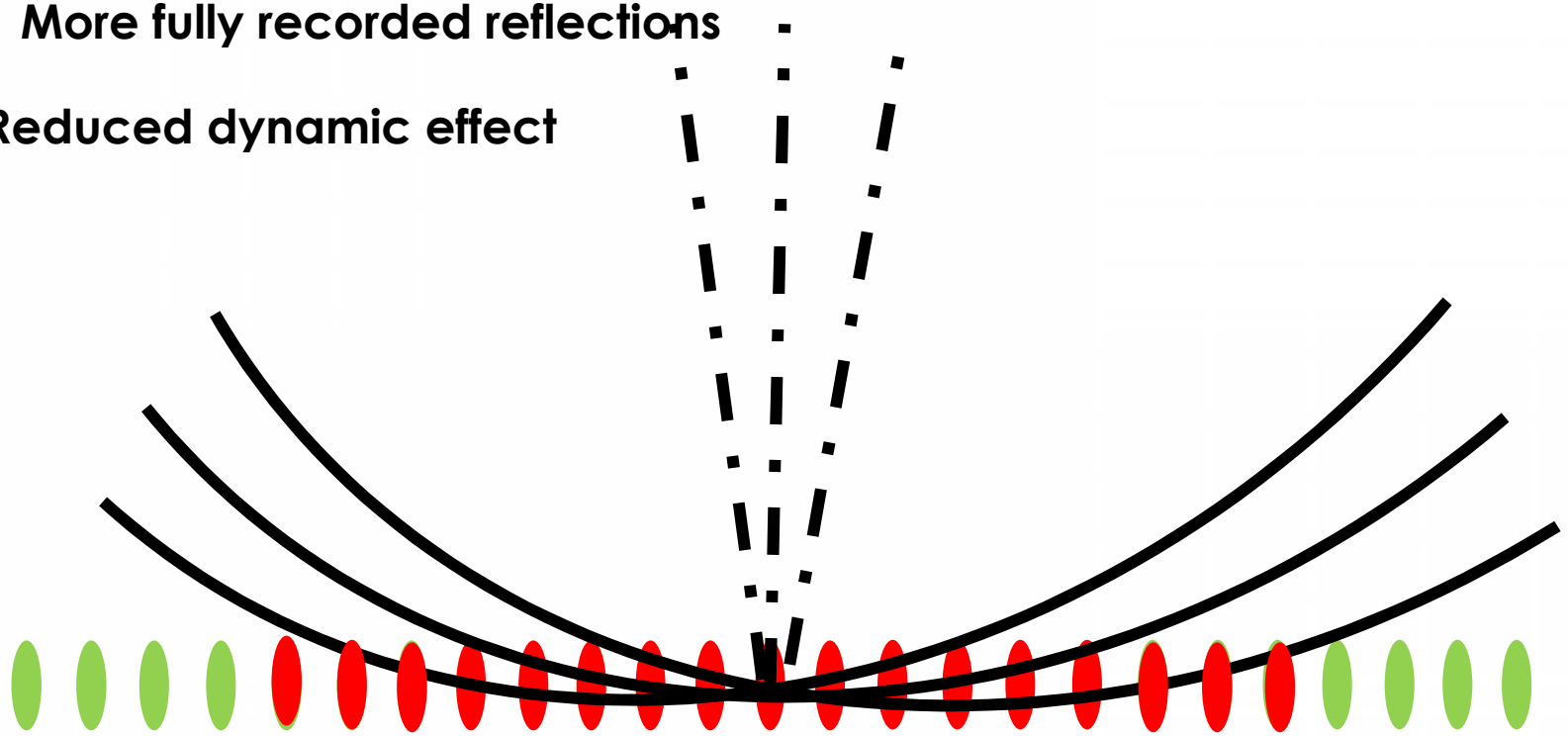
Precession…

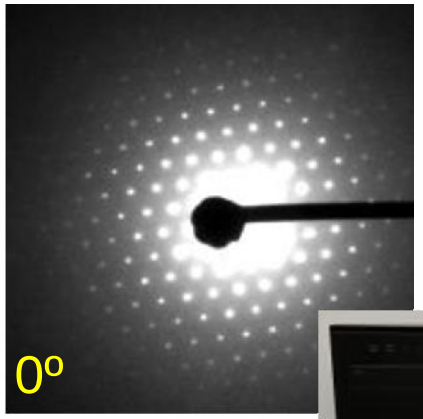
Advantages of PED

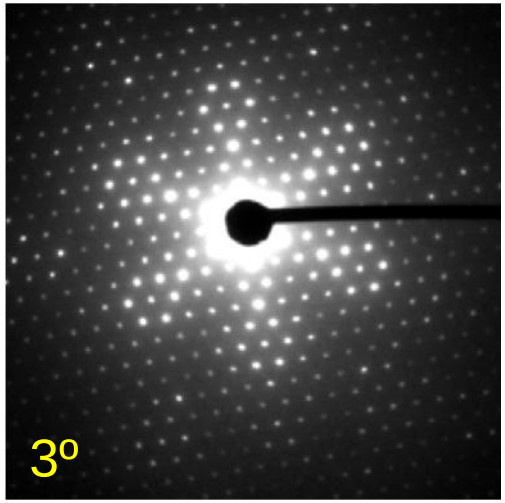
- increase of the number of diffraction spots intercepted by the Ewald sphere

- **More fully recorded reflections**

- **Reduced dynamic effect**

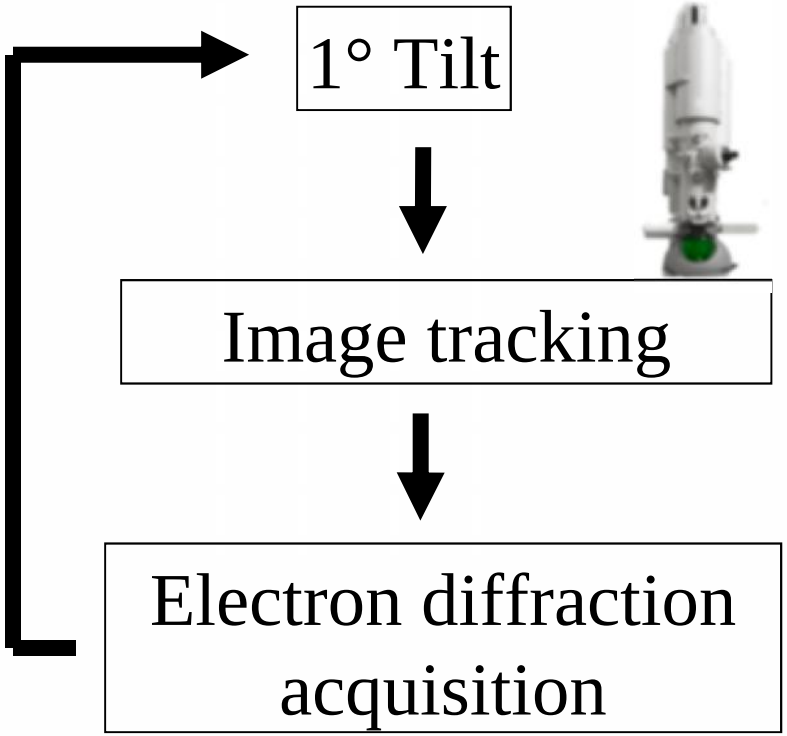


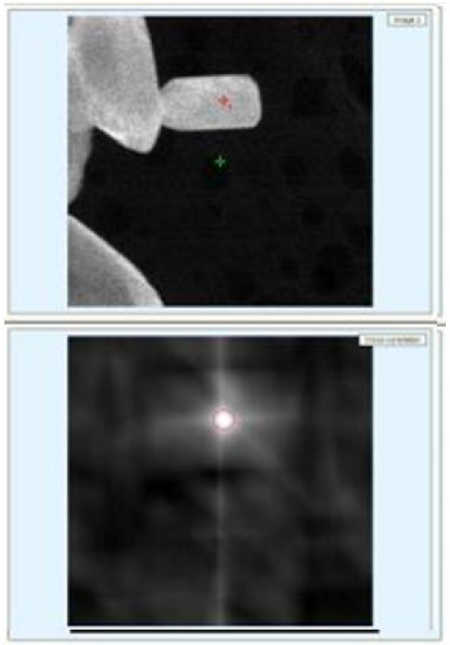


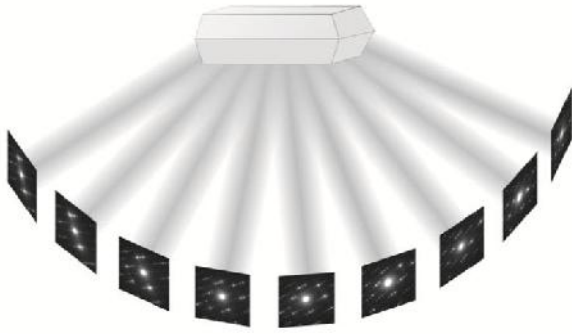


Precession unit Digistar
more kinematical ED intensities

3D electron diffraction tomography

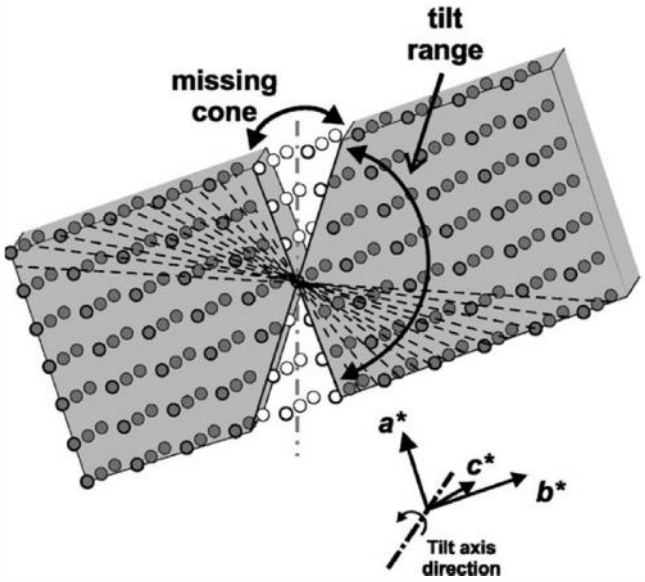


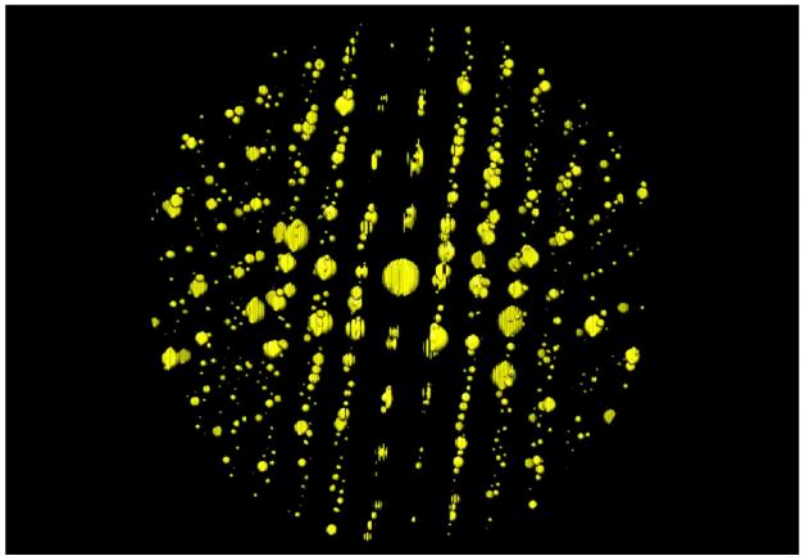




Acquisition is easy, fast and highly reproducible

3D electron diffraction tomography

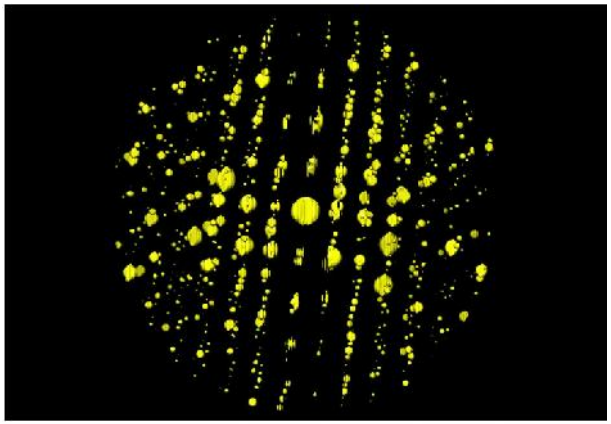




- ✓ complete or almost complete diffraction data to extract easily unit cell and crystal symmetry
- ✓ conceptually simple, data can be taken with any CCD camera
- ✓ easy solution of structures by direct methods or simulated annealing

R close to 25-35 % : reveal all 3D atomic positions with 5-30 pm precision !

3D TEM Diffraction Tomography for 3D atomic structure solution



Hemimorphite		**Reference structure (X-ray and neutron diffraction)**			**3D precession diffraction Tomography**			
Atoms	**Label**	**X**	**Y**	**Z**	**X**	**Y**	**Z**	**Δ(Å)**
Zn	Zn	0.2047(1)	0.1613(1)	0	0.205	0.160	0	0.02
Si	Si	0	0.1465(2)	0.5076(5)	0	0.141	0.529	0.12
O	O1	0.1604(8)	0.2055(1)	0.6363(4)	0.152	0.217	0.657	0.18
O	O2	0	0.1669(2)	0.1938(4)	0	0.156	0.204	0.12
OH	O3	0.3050(2)	0	0.0410(6)	0.289	0	0.073	0.21
O	O4	0	0	0.5912(6)	0	0	0.601	0.05
H_2O	O5	1/2	0	0.5195(13)	1/2	0	0.491	0.15
Mayenite		**Reference structure**			**Electron**			
Atoms	**Label**	**X**	**Y**	**Z**	**X**	**Y**	**Z**	**Δ(Å)**
Ca	Ca	0.89096(5)	0	3/4	0.902	0	3/4	0.14
Al	Al1	0.01866(3)	0.01866(3)	0.01866(3)	0.018	0.018	0.018	<0.02
Al	Al2	¼	7/8	0	¼	7/8	0	-
O	O1	0.18556(3)	0.18556(3)	0.18556(3)	0.184	0.184	0.184	0.03
O	O2	0.44182(4)	0.15035(3)	0.03677(4)	0.439	0.148	0.041	0.06
$Y_{0.8}Pr_{0.2}Ba_2Cu_3O_7$		**Reference structure**			**Electron**			
Atoms	**Label**	**X**	**Y**	**Z**	**X**	**Y**	**Z**	**Δ(Å)**
Ba	Ba	0.5	0.5	0.1850(2)	0.5	0.5	0.1874	0.05
Y/Pr	Y	0.5	0.5	0.5	0.5	0.5	0.5	-
Cu	Cu1	0	0	0	0	0	0	-
Cu	Cu2	0	0	0.3565(5)	0	0	0.355	<0.02
O	O1	0	0.5	0	0	0.5	0	-
O	O2	0	0	0.1566(23)	0	0	0.160	<0.02
O	O3	0.5	0	0.3776(21)	0.5	0	0.382	0.06
O	O4	0	0.5	0.3765(21)	0	0.5	0.383	0.06

Kinematical refinement : Find all 3D atomic positions with 2-15 pm accuracy !

Important points to remember

1) **Needs data of +- 30^{O} (minimum)for unit cell determination and Space group**

2) **Several datasets +- 60^{O} are merged for structure solution**

3) **Data sets are merged based on the strongest symmetry equivalent reflections to increase completeness for structure solution**

4) **Working under low dose condition to reduce beam damage for beam sensitive materials**

TEM - organic samples
What is the allowed maximum (critical) dose ?

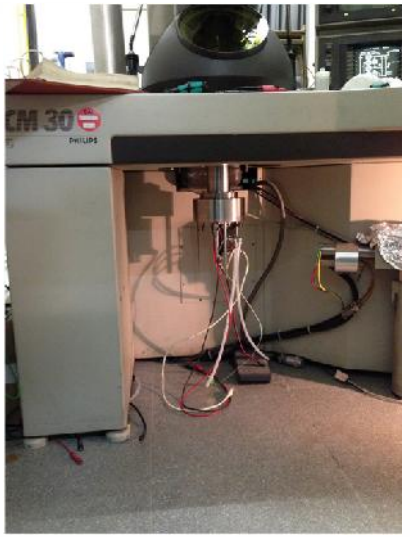
Most biological specimens (proteins) Glaeser, 1971	~ 6 e-/Å^2
Aromatic polymers (such as polystyrene)	~ 36 e-/ Å^2
Anthracence	~ 42 e-/ Å^2
Poly-xylene Kumar & Adams, 1990; Williams & Carter, 2004	~ 120 e-/ Å^2
Zeolites	~ 100 e-/Å^2
Ceramics Pan & Crozier, 1993	~ 600 e-/Å^2

Ideal detector for low dose Electron Microscopy

- Less radiation damage on sample
- For instance: more rotation information
- Very sharp images (No blurring effects from electron multiplication as in CCD)
- 1 Electron = 1 Count

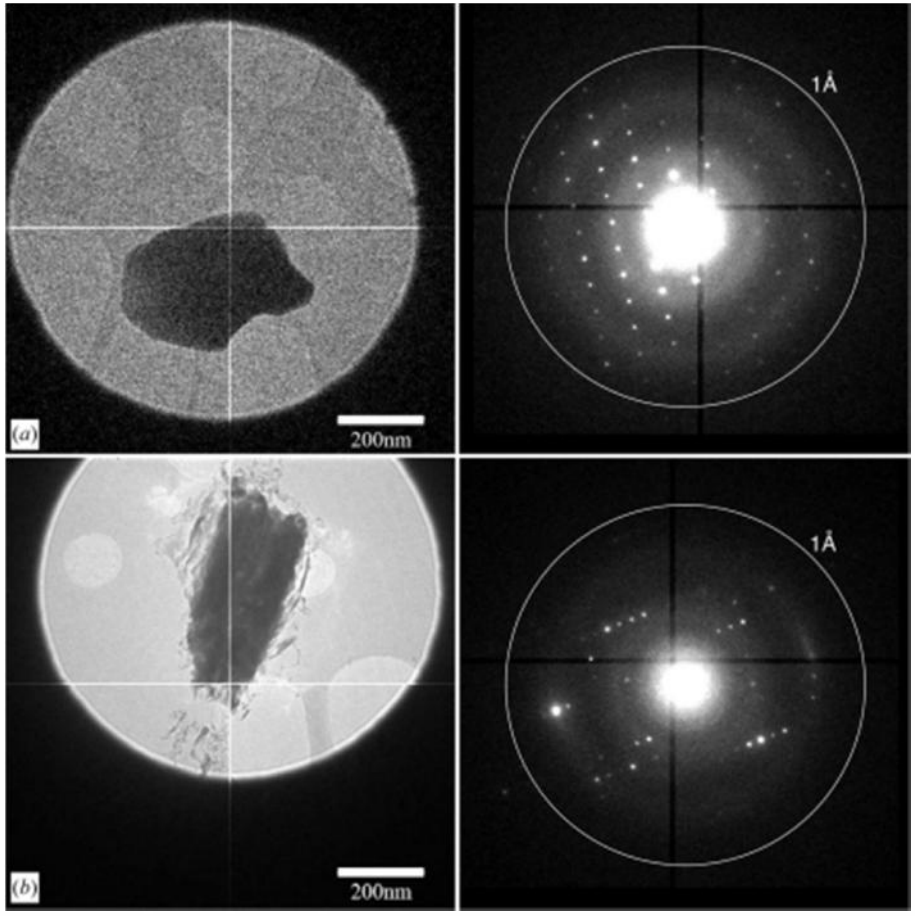
TimePix CMOS x 100 more sensitive than CCD

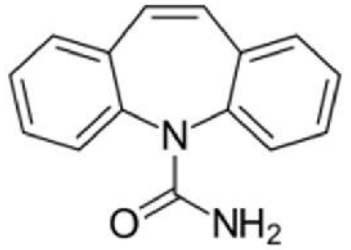
X 10 more sensitive than IP



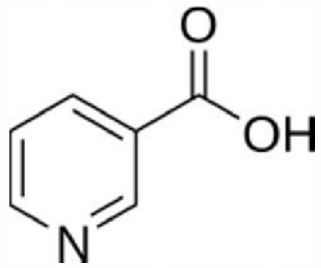
- **Medipix-Timepix**
  - 512 x 512 pixels
  - > 120 fps
  - dynamic range 13.5
  - 55 micron pixel

Total accumulated dose 4.0 e-/ Å^2 (CBZ) & 2 e-/ Å^2 (Nicotinic Acid)

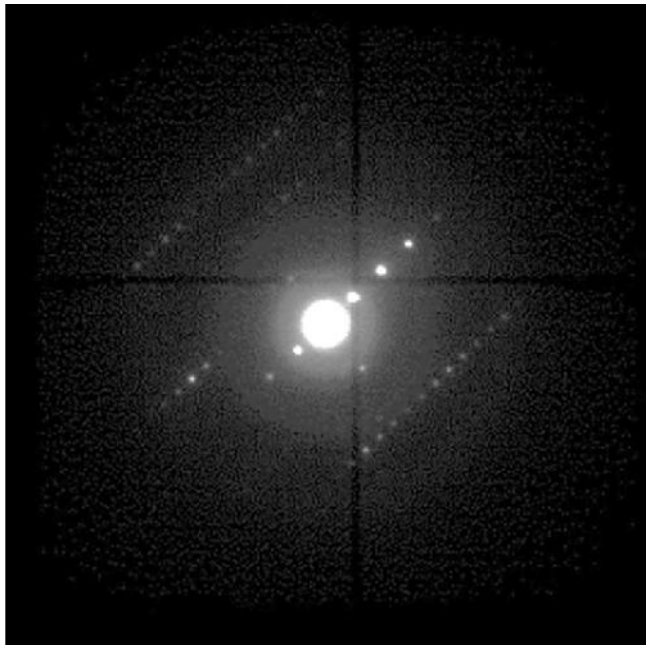




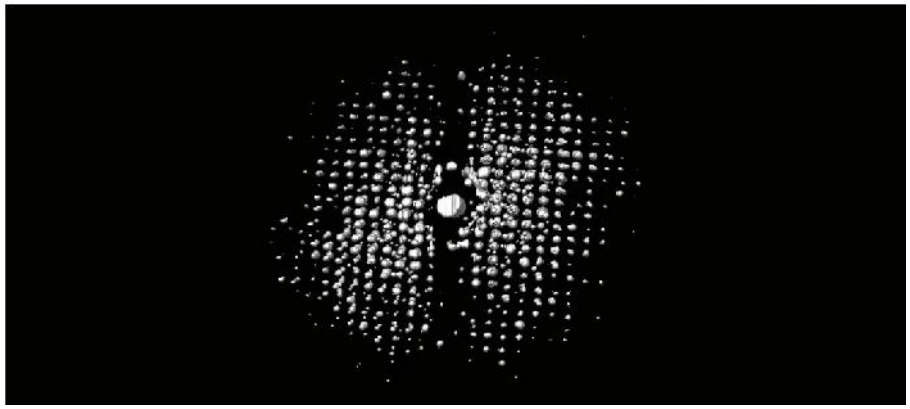
High Resolution Diffraction Pattern
fast tomography without Cryo

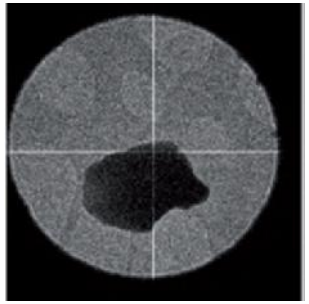


carbamazepine (CBZ) crystal



(Data collected -25^0 to 26^0)
with continuous rotation of the crystal



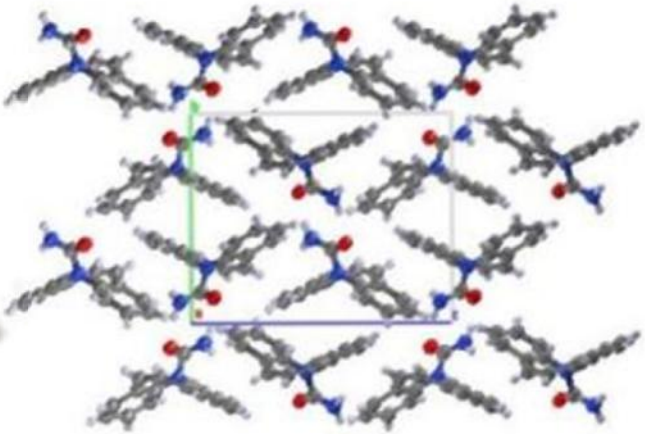


NO CRYO USED

Resolution: 0.8 Å

Ab-initio structure solution of carbamazepine(CBZ) crystal from ED Data

H atoms was fixed according to geometry

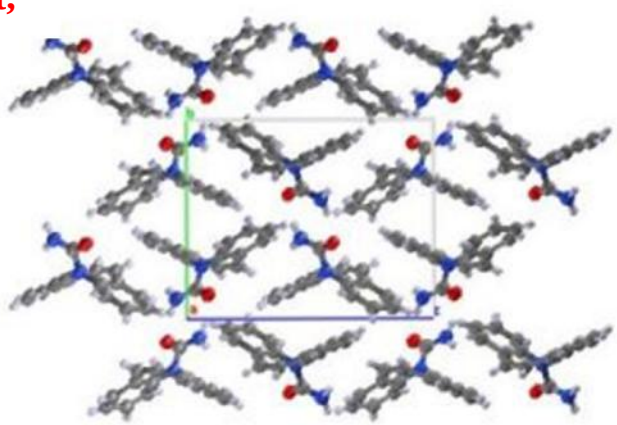


RT Measurement

ED Unit cell
a = 7.53 (1) Å
b = 11.139 (6) Å
c = 14.06(2) Å
β = 92.80(8)°

Structure was solved using a single dataset, but it was refined by merging 5 data sets

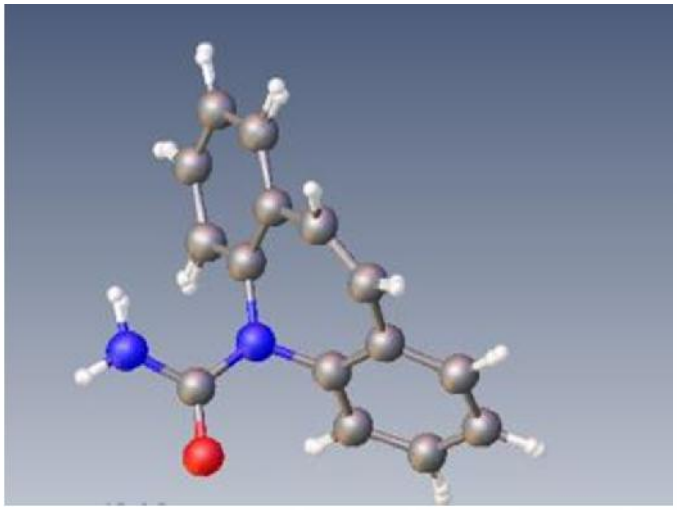
Literature X-ray Reported
a = 7.487 (1) Å
b = 11.041 (2) Å
c = 13.775 (3) Å
β = 92.94 (4) Å



CBZ

	CBZ
Resolution	**8.73 -0.8**
Completeness	**45.0**
Reflections	**2202**
Unique Reflections	**1071**
R merge	**8.4**
R1	**32.2**
wR2	**55.6**

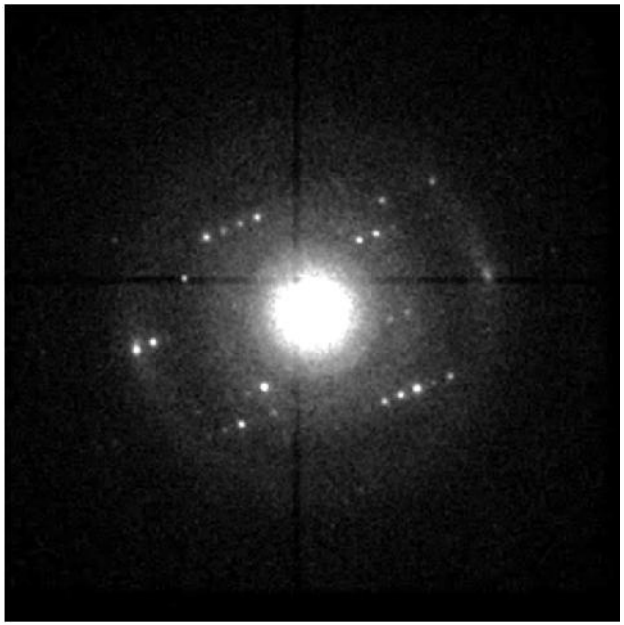
	El Hassan	*single*	*5x merged*
C1-N1-C14	117.13(3)	119(1)	116(1)
O1-C15-N2	122.26(3)	122(1)	120(1)
C7-C8-C9	126.25(3)	128(2)	129(2)
C6-C7-C8	127.75(3)	126(2)	126(2)
C8-C9-C14	123.04(3)	124(2)	124(2)
C1-C6-C7	123.44(3)	119(2)	125(2)

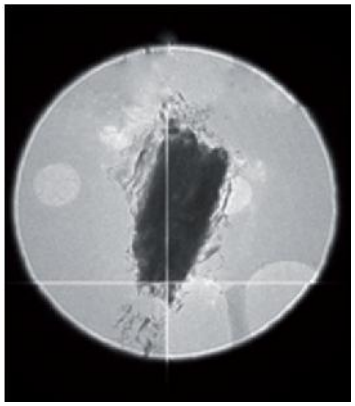


RMSD 0.11 Å between ED Structure and X-ray Structure of CBZ form III

Acta A72 (2016)
doi:10.1107/S2053273315022500

Nicotinic Acid Crystal





Resolution: 0.8 Å

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

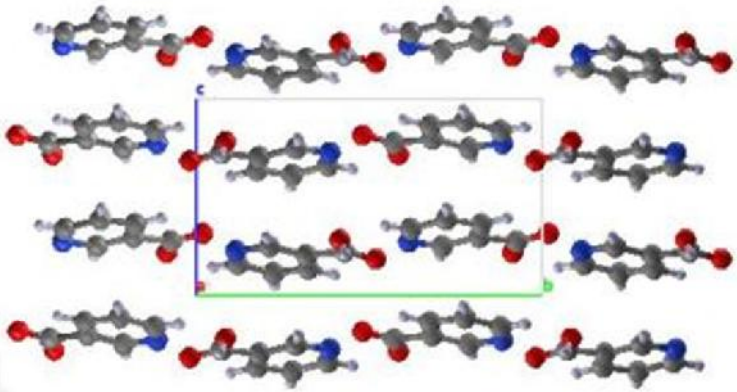
with continuous rotation of the crystal

NO CRYO USED

Structure was solved ab-initio by DM from ED Data

Solved from ED

RT Measurement



H atoms was fixed according to geometry

Unit cell from ED

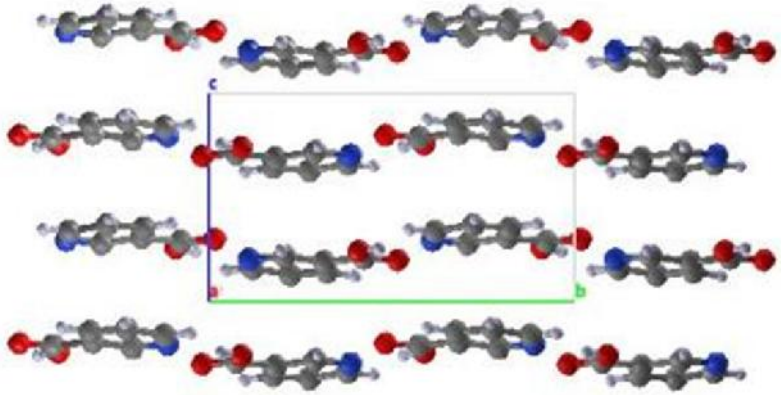
a = 7.30 (1) Å

b = 11.693 (2) Å

c = 7.33 (3) Å

β = 113.7 (1) °

Literature X-ray Reported Structure



Literature X-ray Reported

a = 7.186 (2) Å

b = 11.688 (3) Å

c = 7.231 (2) Å

β = 113.55 (6)°

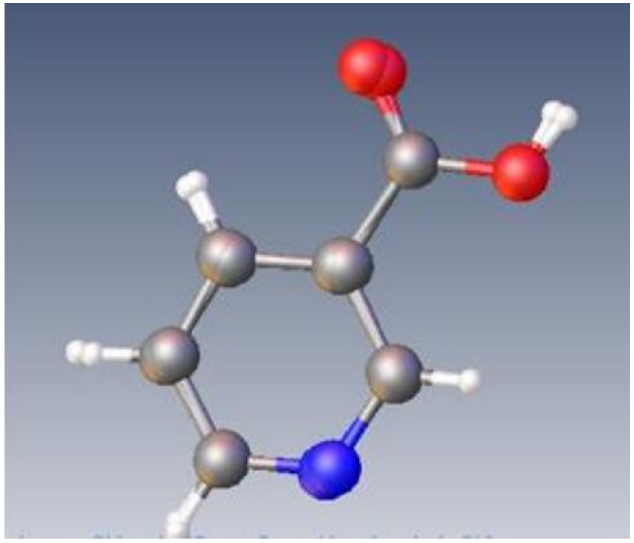
Structure was solved using a single dataset, but it was refined by merging 2 data sets

Acta Cryst. (2016). A72, 236–242

	Nicotinic Acid
Resolution	**5.82 -0.75**
Completeness	**35.6**
Reflections	**953**
Unique Reflections	**503**
R merge	**7.1**
R1	**35.6**
wR2	**63.9**

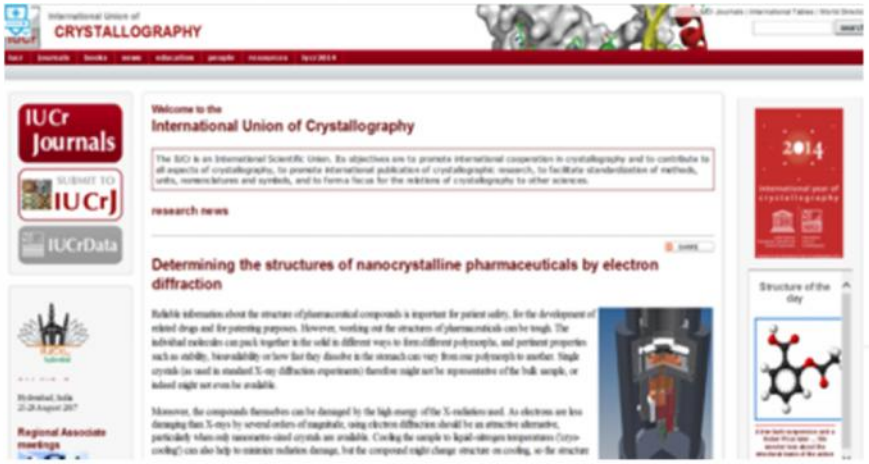
	Kutoglu	*single*	*2x merged*
N-C	1.348(4)	1.36(2)	1.41(3)
C1-C2	1.397(4)	1.41(1)	1.46(3)
C2-C3	1.406(4)	1.44(4)	1.50(5)
C3-C4	1.383(5)	1.42(3)	1.33(3)
C4-C5	1.393(5)	1.41(3)	1.36(4)
C5-N	1.342(4)	1.28(4)	1.26(5)
C2-C6	1.490(4)	1.46(2)	1.53(3)
C6-O1	1.308(4)	1.34(3)	1.32(5)
C6-O2	1.211(4)	1.16(2)	1.14(3)

	Kutoglu	*single*	*2x merged*
C1-N1-C14	117.13(3)	119(1)	116(1)
O1-C15-N2	122.26(3)	122(1)	120(1)
C7-C8-C9	126.25(3)	128(2)	129(2)
C6-C7-C8	127.75(3)	126(2)	126(2)
C8-C9-C14	123.04(3)	124(2)	124(2)
C1-C6-C7	123.44(3)	119(2)	125(2)



RMSD 0.11 Å between ED Structure and X-ray Structure of Nicotinic acid

Acta A72 (2016)
doi:10.1107/S2053273315022500



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Thanks for your attention !!!

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Electron Diffraction Workshop, 21st August 2017

HICC, MR. 1.05
Workshop Starts at 8.30 AM

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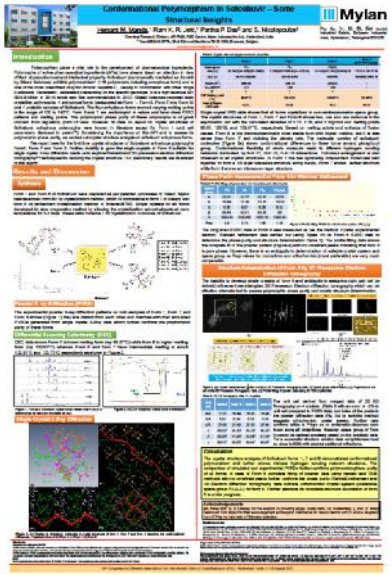

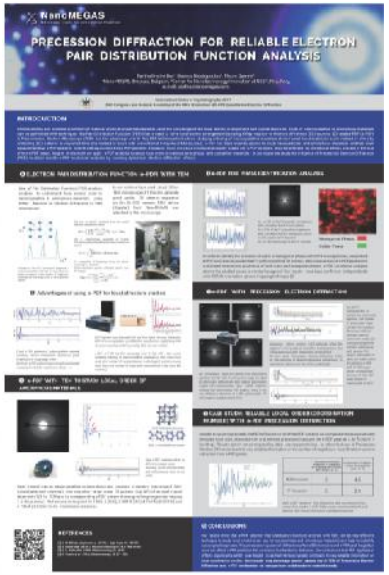

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