

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

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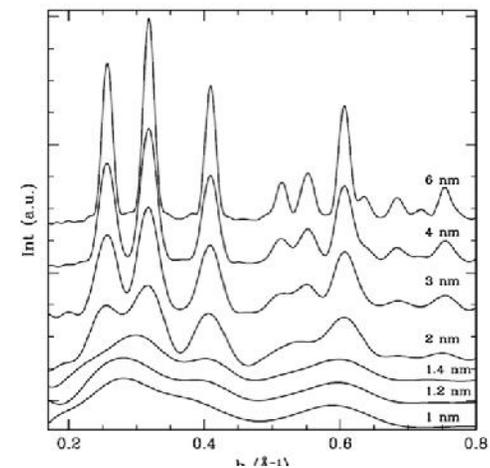
# STRUCTURE ANALYSIS WITH ELECTRON DIFFRACTION

## Why electrons?

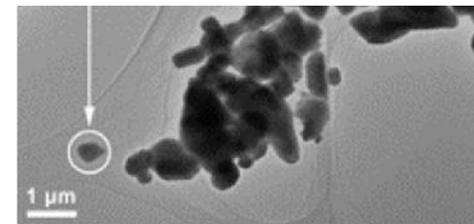
□  $10^{4-5}$  times stronger interaction with matter compared with X-ray

- *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)

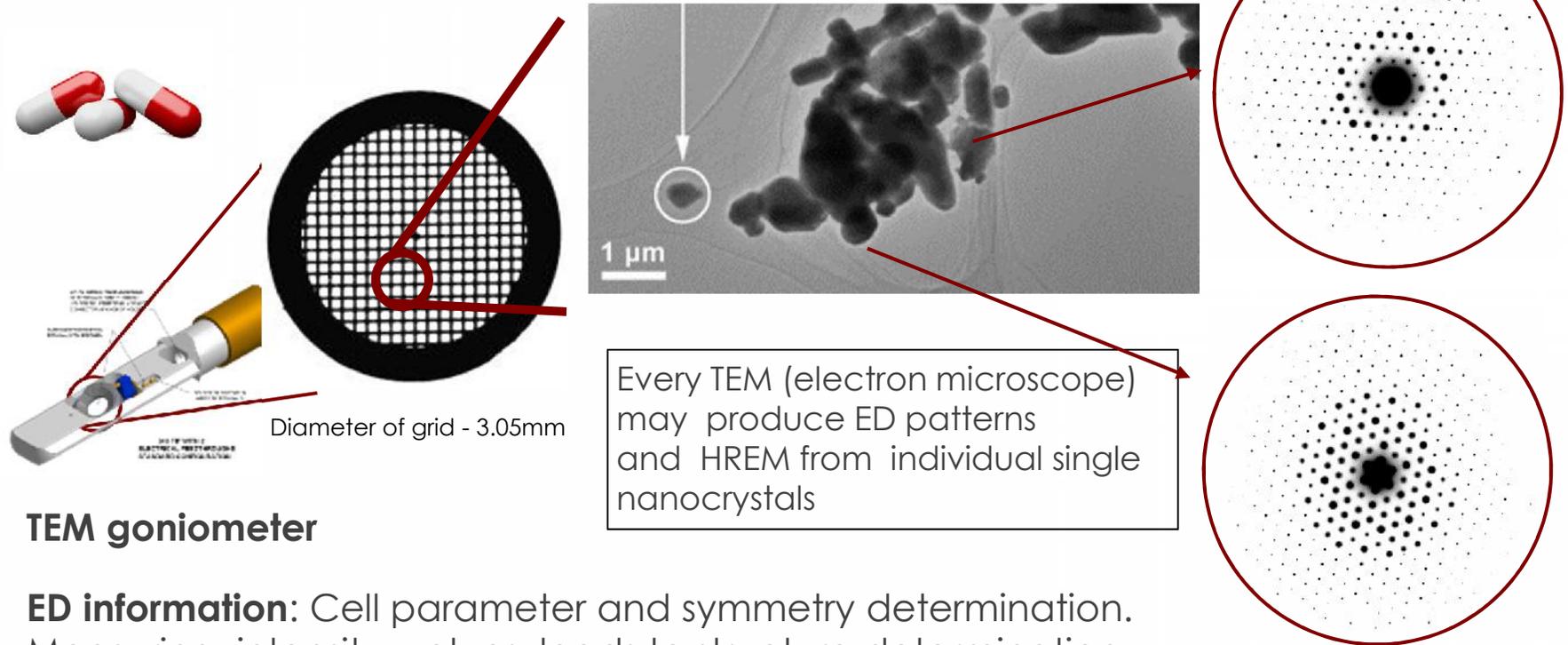


- **Diffraction** – selected area, nano- and convergent beam electron diffraction
- **Imaging** – conventional, high resolution (HREM)
- **Chemical analysis** – EDS and EELS



# STRUCTURE ANALYSIS WITH TEM

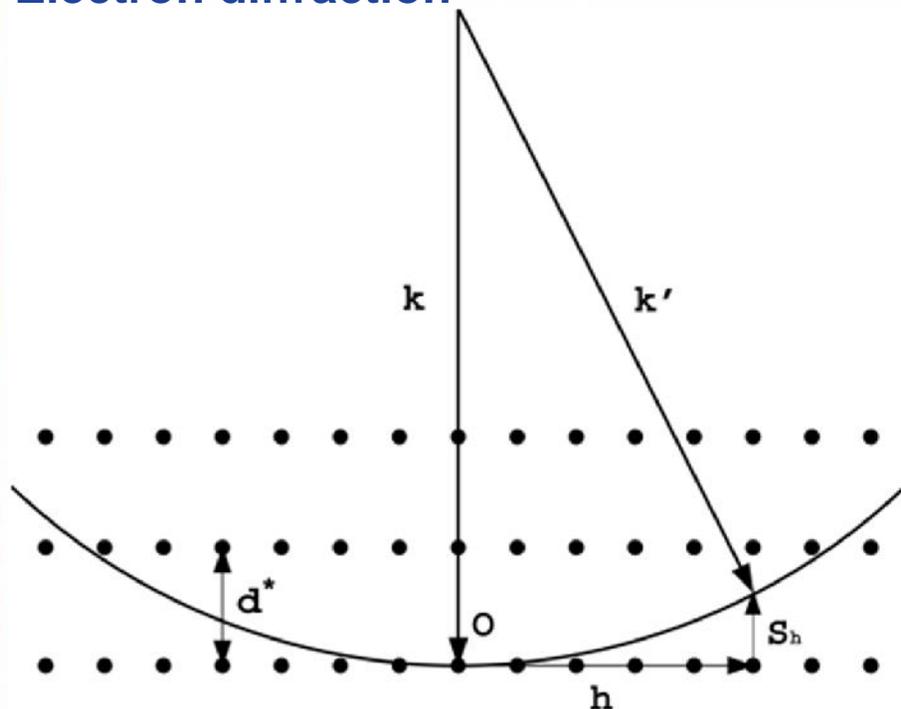
## TEM : Electron diffraction advantages



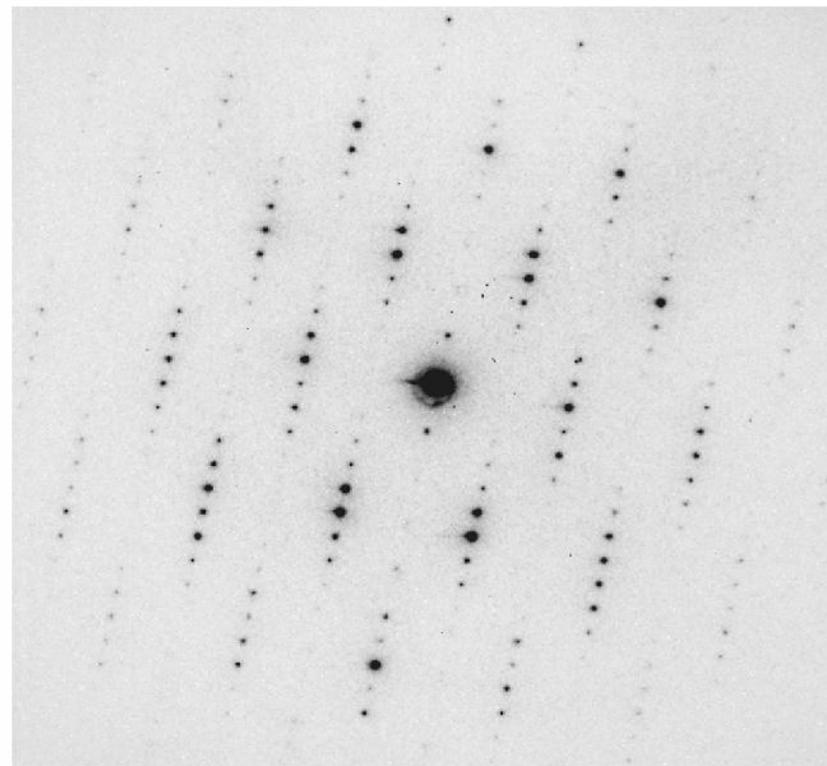
### TEM goniometer

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

## Electron diffraction



VOLTAGE IN KV	$\lambda$ IN Å
100	0.0370
300	0.0197
1000	0.0087



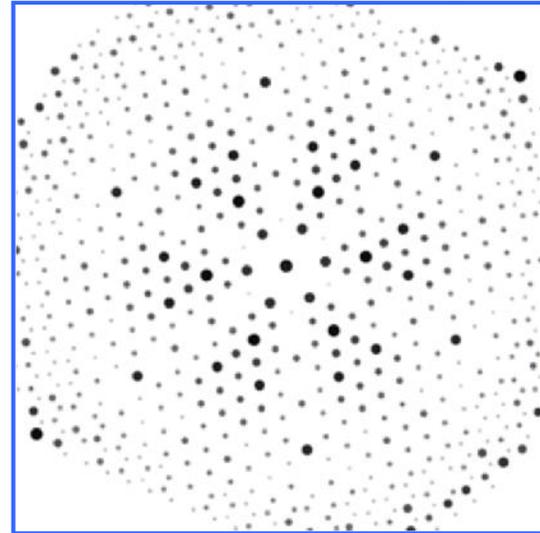
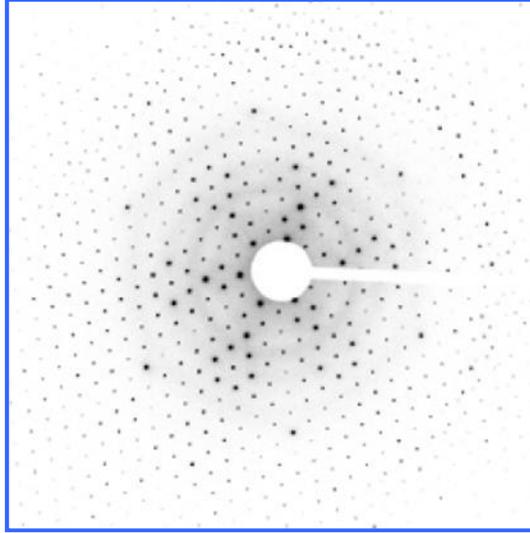
**The Ewald sphere is flat!**



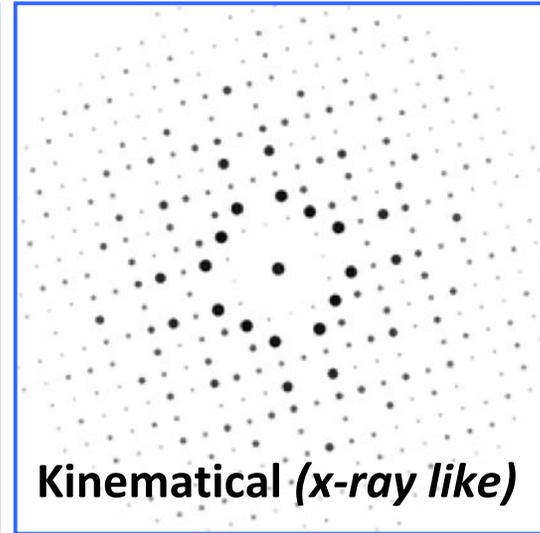
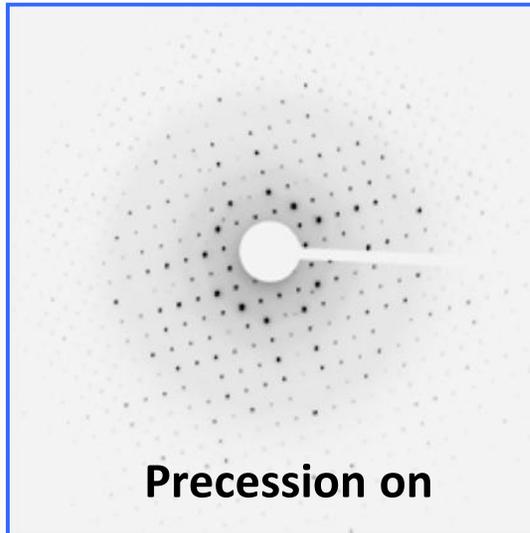
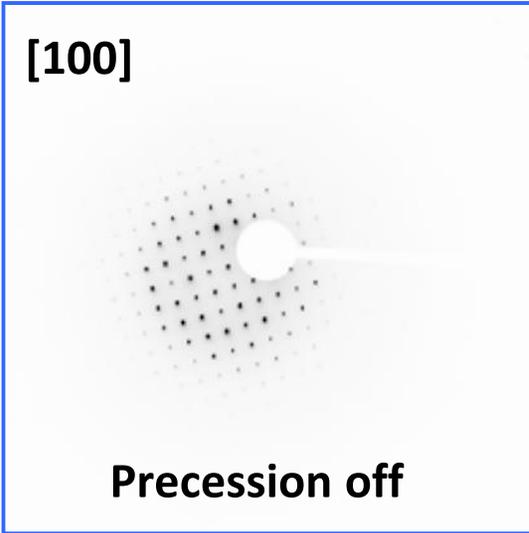
**In one shot we record a reciprocal lattice plane**

Zone axis PED on "standard" mayenite

[111]



[100]

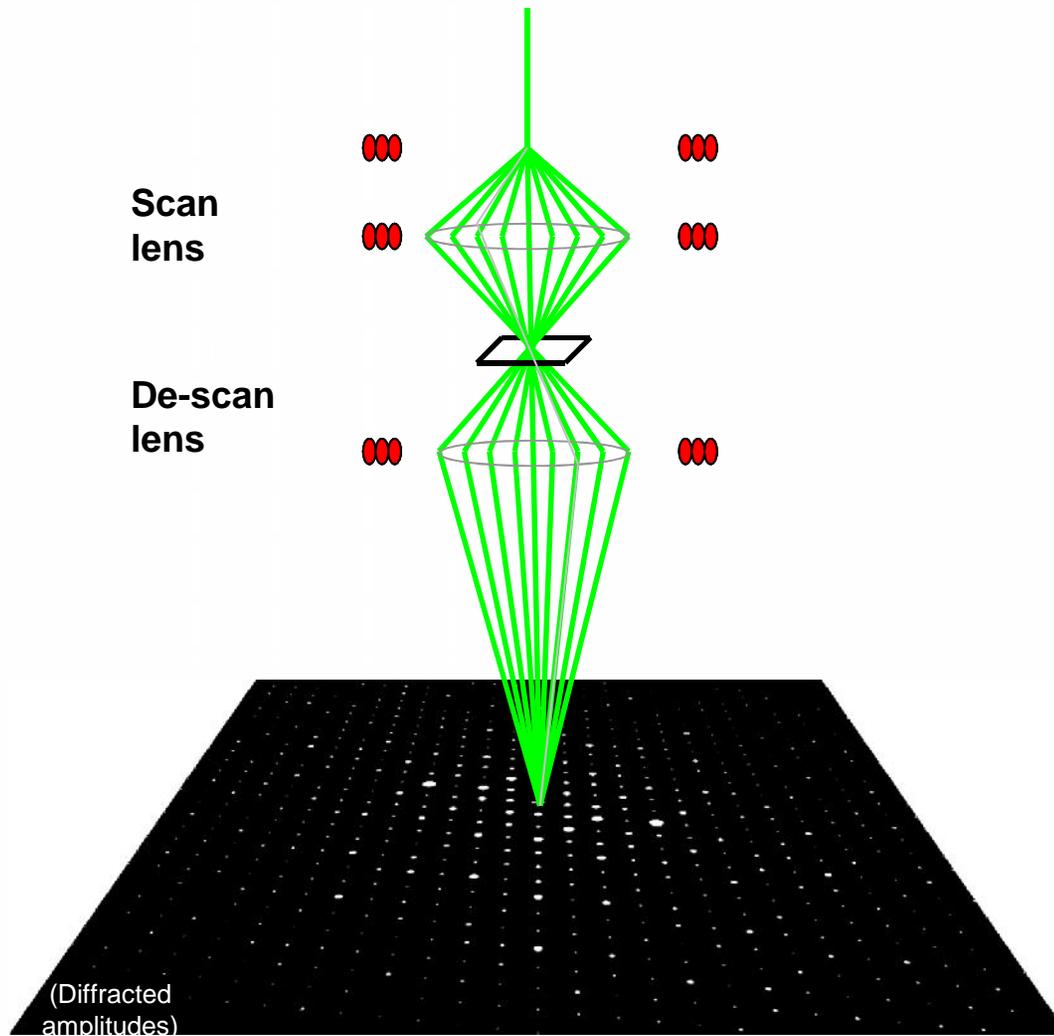


Precession off

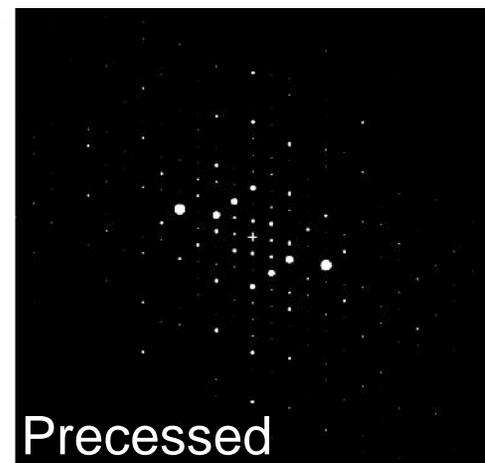
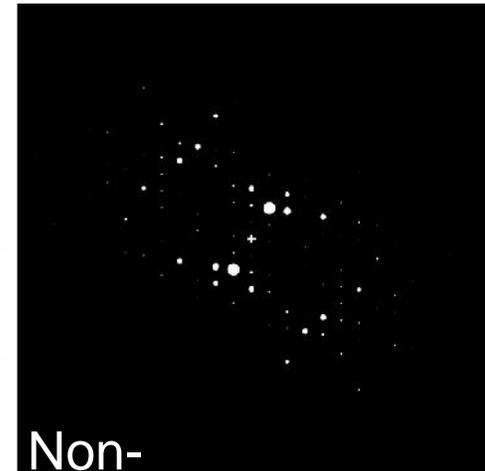
Precession on

Kinematical (*x-ray like*)

Courtesy Dr. M. Gemmi IIT Pisa Italy



Reference : C.Own PhD thesis

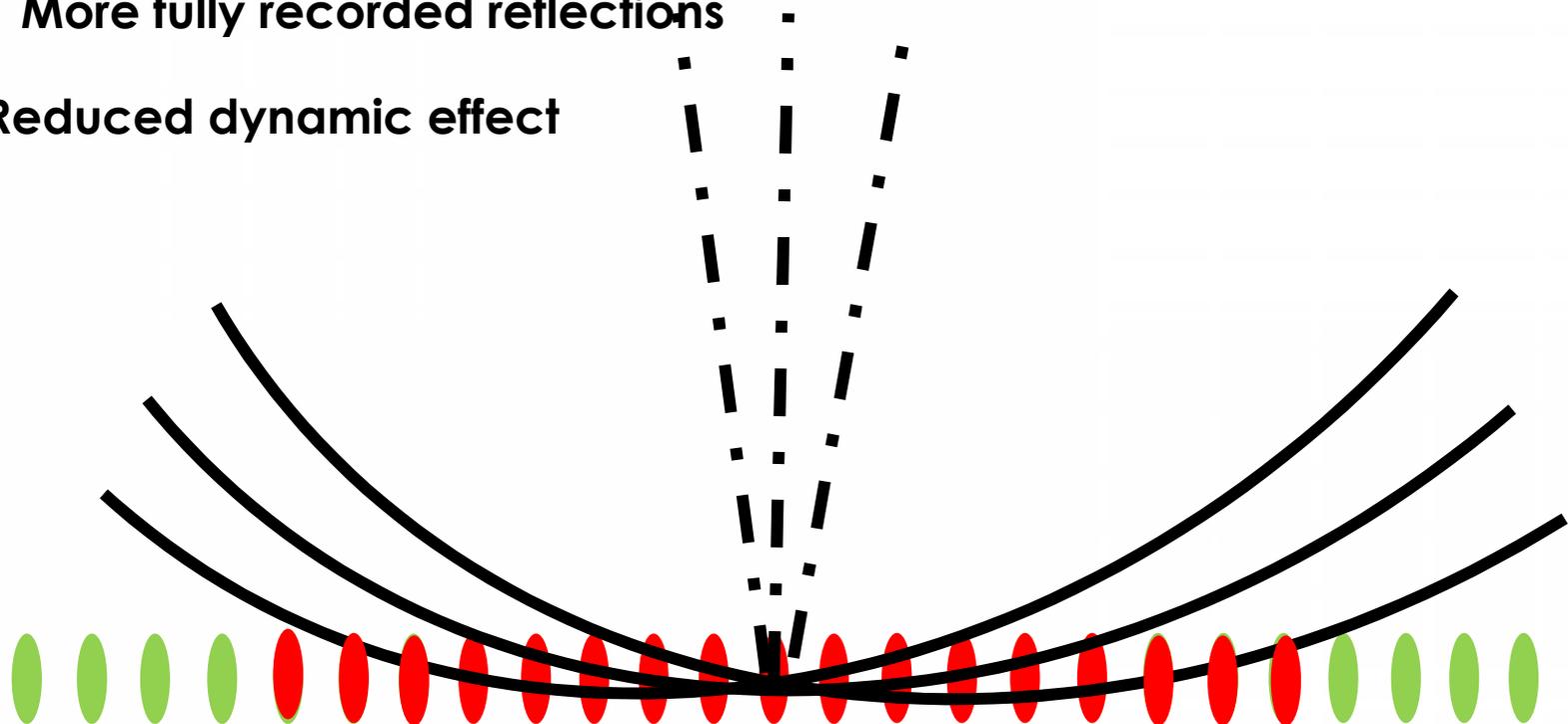


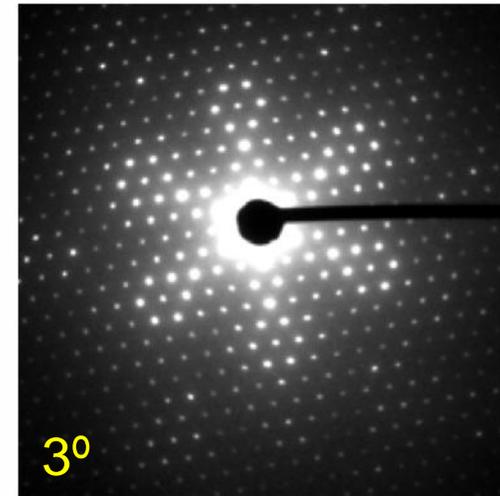
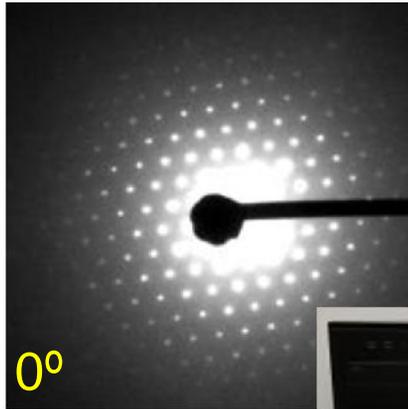
Precession...

THE UNIVERSITY OF TEXAS AT AUSTIN

# Advantages of PED

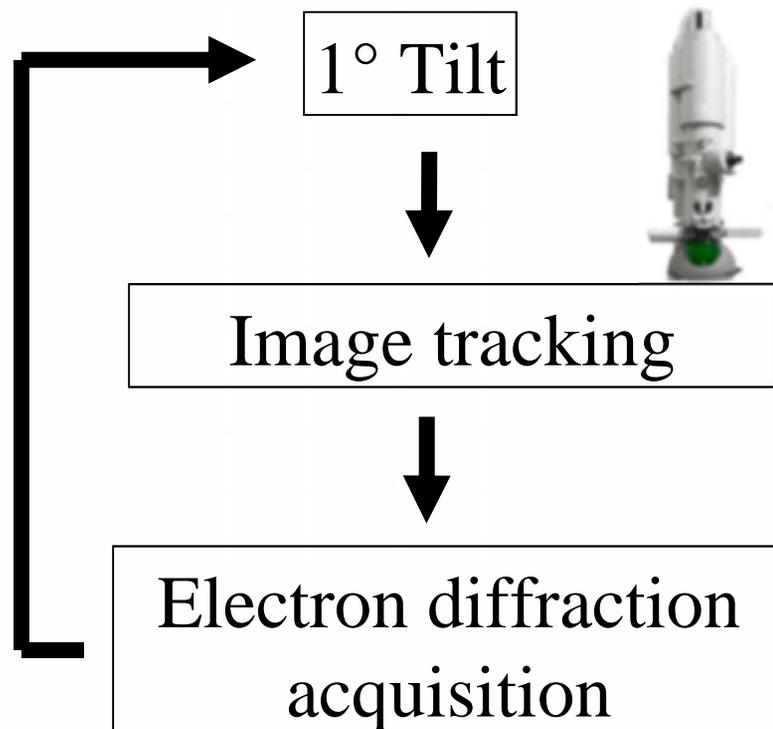
- increase of the number of diffraction spots intercepted by the Ewald sphere
- **More fully recorded reflections**
- **Reduced dynamic effect**



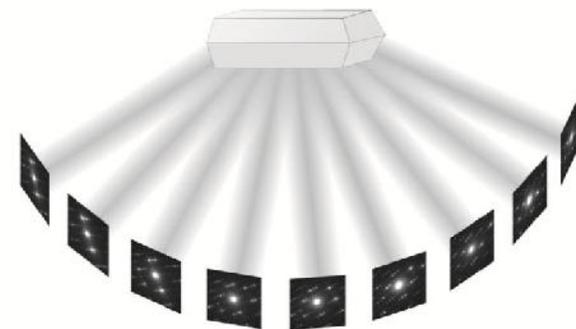
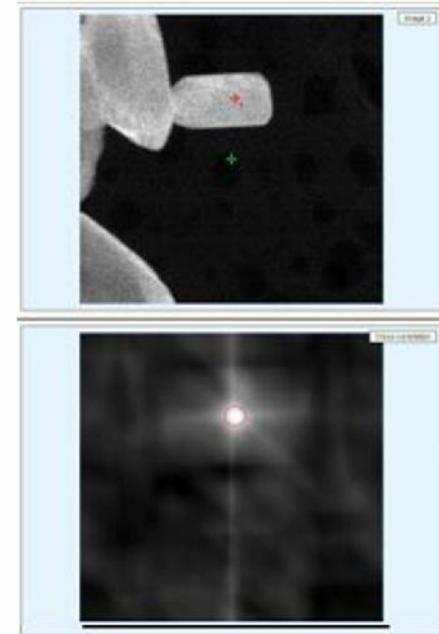


Precession unit Digistar  
more kinematical ED intensities

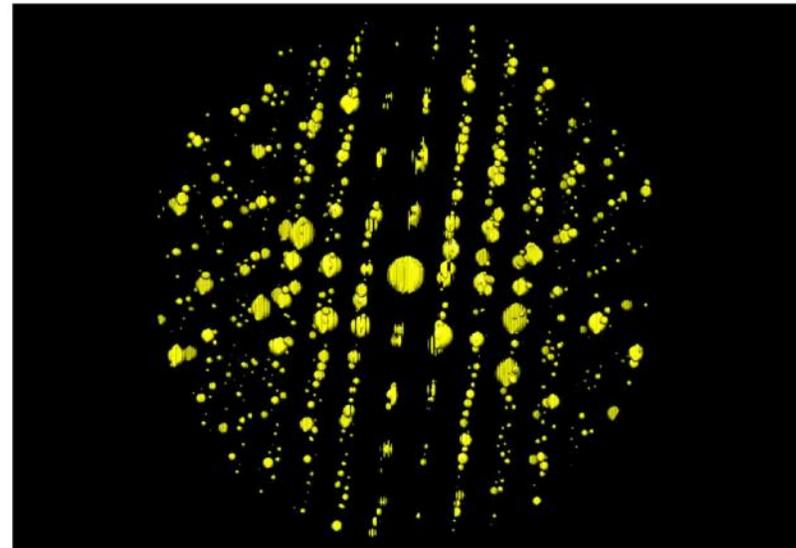
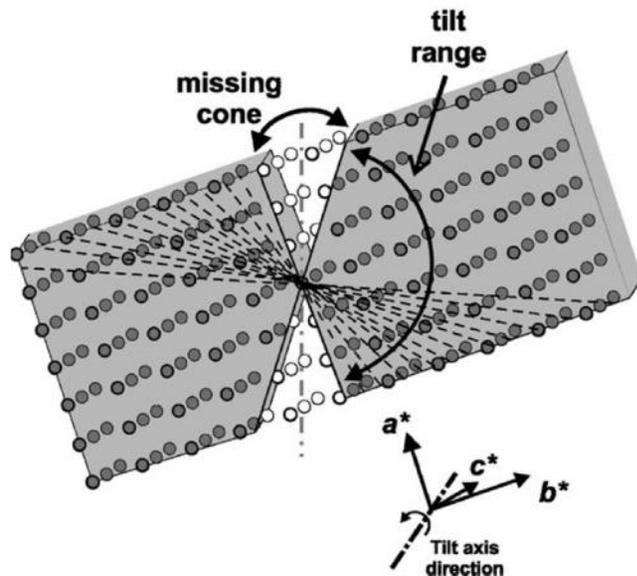
# 3D electron diffraction tomography



Acquisition is easy, fast and highly reproducible



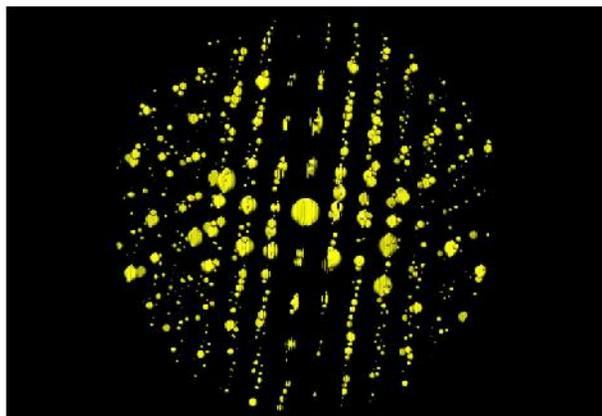
# 3D electron diffraction tomography



- ✓ 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	(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 <sub>2</sub> O	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	(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	1/4	7/8	0	1/4	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 <sub>0.8</sub> Pr <sub>0.2</sub> Ba <sub>2</sub> Cu <sub>3</sub> O <sub>7</sub>		Reference structure			Electron			
Atoms	Label	X	Y	Z	X	Y	Z	(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  $\pm 30^\circ$  (minimum) for unit cell determination and Space group
- 2) Several datasets  $\pm 60^\circ$  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	$\sim 6 \text{ e-}/\text{\AA}^2$
Aromatic polymers (such as polystyrene)	$\sim 36 \text{ e-}/\text{\AA}^2$
Anthracence	$\sim 42 \text{ e-}/\text{\AA}^2$
Poly-xylene	$\sim 120 \text{ e-}/\text{\AA}^2$
Kumar & Adams, 1990; Williams & Carter, 2004	
Zeolites	$\sim 100 \text{ e-}/\text{\AA}^2$
Ceramics	$\sim 600 \text{ e-}/\text{\AA}^2$
Pan & Crozier, 1993	

## □ 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

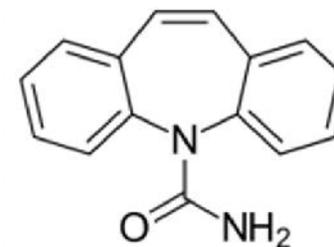
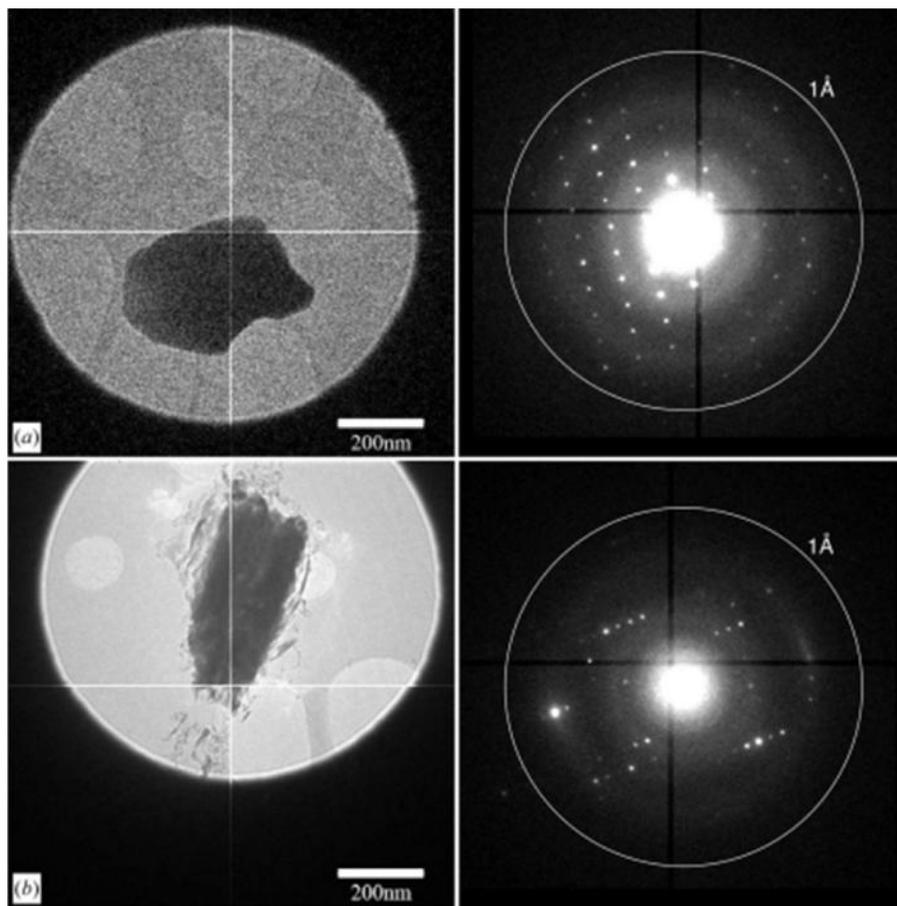
**TimePix CMOS x 100 more sensitive than CCD**

**X 10 more sensitive than IP**

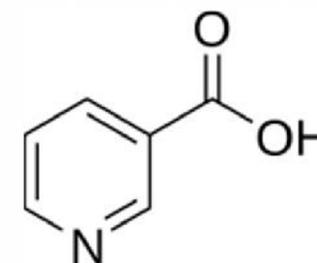
- **Medipix-Timepix**
  - 512 x 512 pixels
  - > 120 fps
  - dynamic range 13.5
  - 55 micron pixel



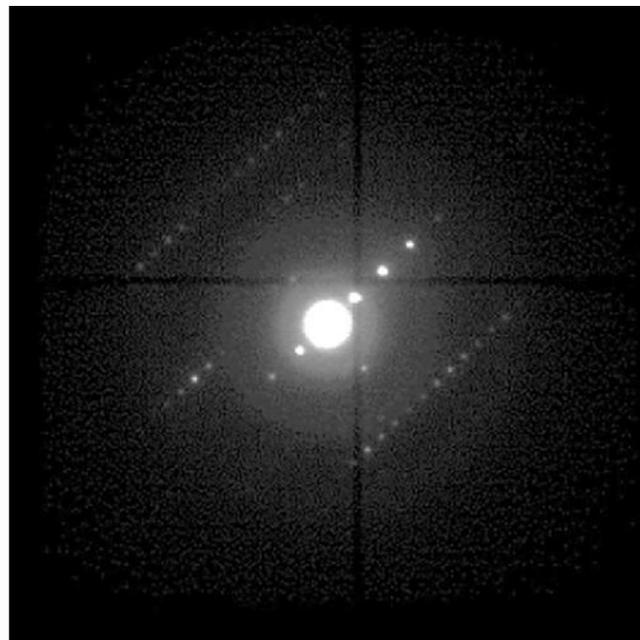
Total accumulated dose 4.0 e-/Å<sup>2</sup> (CBZ) & 2 e-/Å<sup>2</sup> (Nicotinic Acid)



**High Resolution Diffraction  
Pattern  
fast tomography without Cryo**



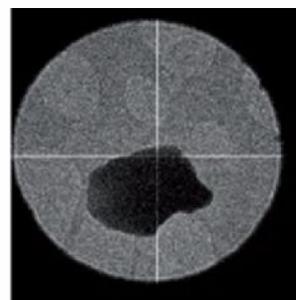
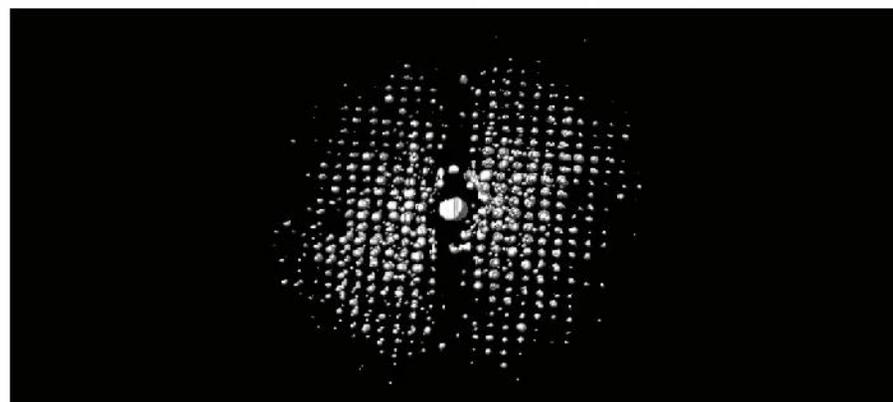
# carbamazepine (CBZ) crystal



NO CRYO USED



(Data collected  $-25^{\circ}$  to  $26^{\circ}$ )  
with continuous rotation of the crystal

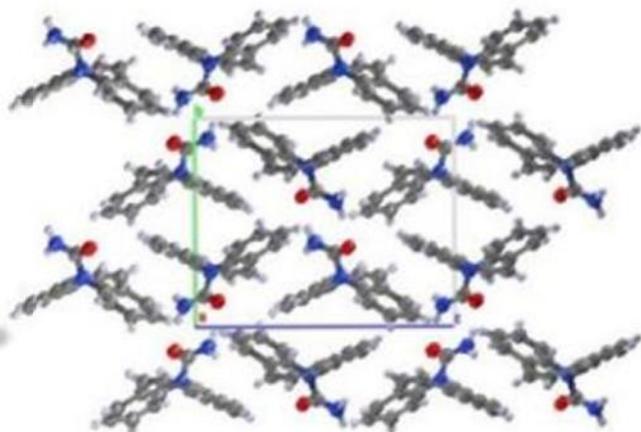


Resolution:  $0.8 \text{ \AA}$

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



H atoms was fixed according to geometry



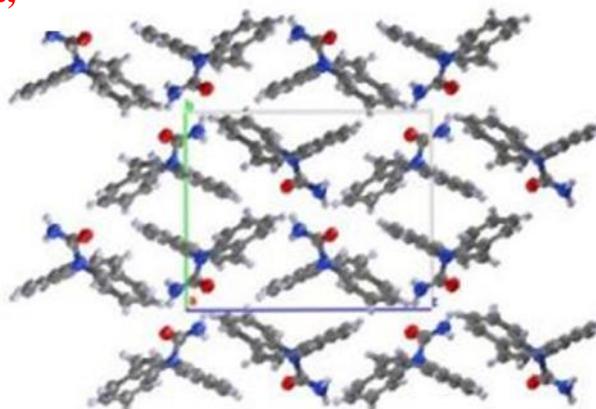
## RT Measurement

ED Unit cell  
 $a = 7.53 (1) \text{ \AA}$   
 $b = 11.139 (6) \text{ \AA}$   
 $c = 14.06(2) \text{ \AA}$   
 $\beta = 92.80(8)^\circ$

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

Literature X-ray Reported

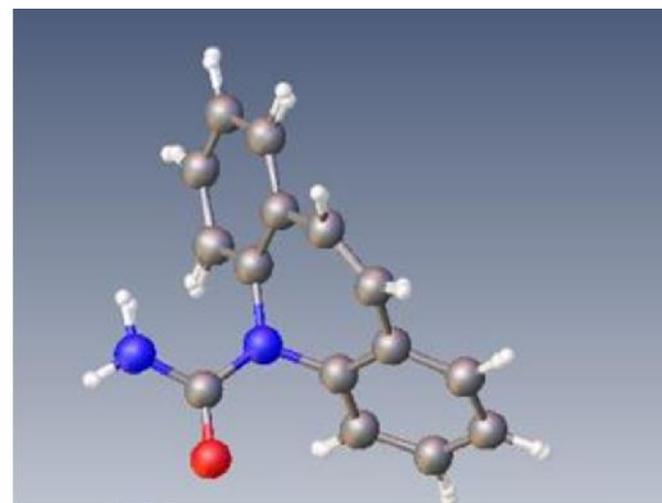
$a = 7.487 (1) \text{ \AA}$   
 $b = 11.041 (2) \text{ \AA}$   
 $c = 13.775 (3) \text{ \AA}$   
 $\beta = 92.94 (4) \text{ \AA}$



## 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

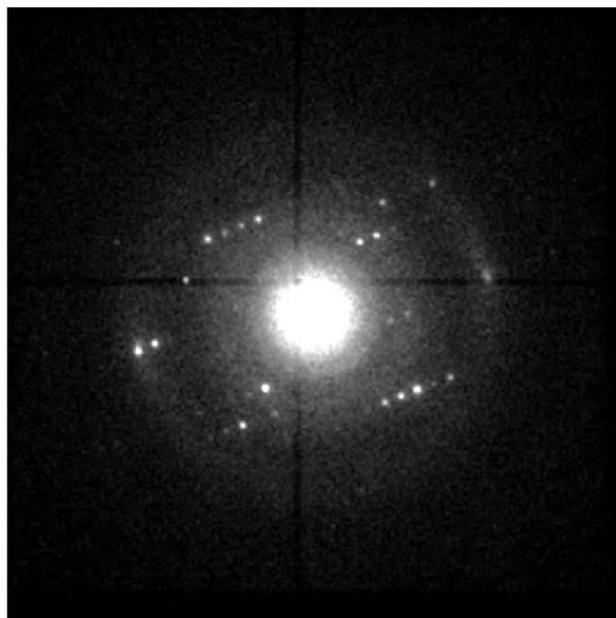
	<i>El Hassan</i>	<i>single</i>	<i>5x merged</i>
C1-N1-C14	117.13(3)	119(1)	116(1)
O1-C15-N2	122.26(3)	122(1)	120(1)
C7-C8-C9	126.25(3)	128(2)	129(2)
C6-C7-C8	127.75(3)	126(2)	126(2)
C8-C9-C14	123.04(3)	124(2)	124(2)
C1-C6-C7	123.44(3)	119(2)	125(2)



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

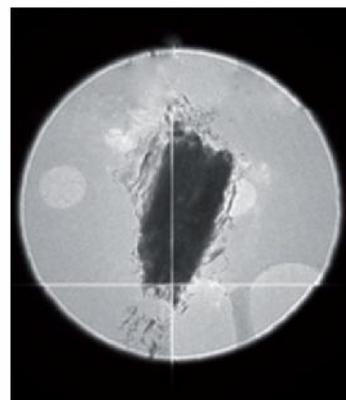
Acta A72 (2016)  
doi:10.1107/S2053273315022500

# Nicotinic Acid Crystal



Resolution: 0.8 Å

(Data collected  $-10^0$  to  $26^0$ )  
with continuous rotation of the crystal

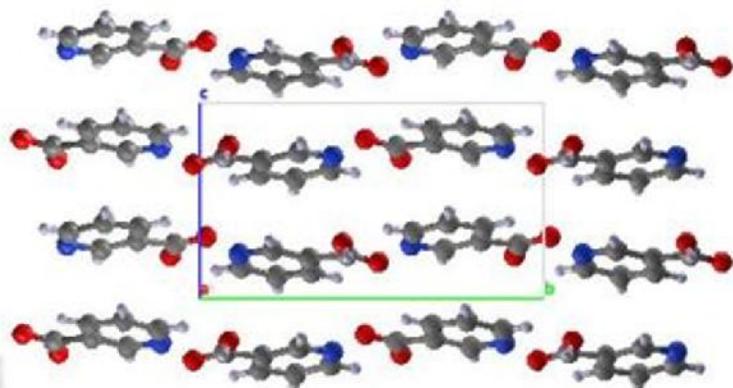


NO CRYO USED

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

$$a = 7.30 (1) \text{ \AA}$$

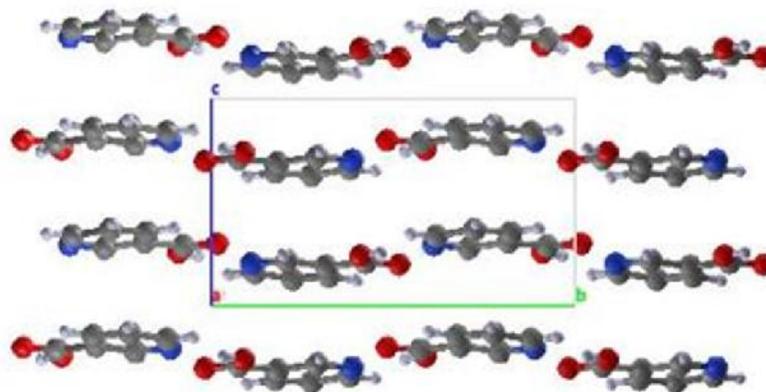
$$b = 11.693 (2) \text{ \AA}$$

$$c = 7.33 (3) \text{ \AA}$$

$$\beta = 113.7 (1)^\circ$$

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

Literature X-ray Reported Structure



Literature X-ray Reported

$$a = 7.186 (2) \text{ \AA}$$

$$b = 11.688 (3) \text{ \AA}$$

$$c = 7.231 (2) \text{ \AA}$$

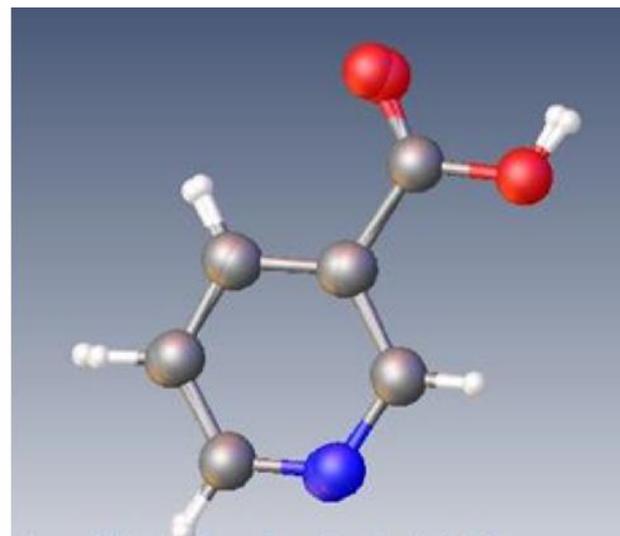
$$\beta = 113.55 (6)^\circ$$

Acta Cryst. (2016). A72, 236–242

	Nicotinic Acid
<b>Resolution</b>	<b>5.82 -0.75</b>
<b>Completeness</b>	<b>35.6</b>
<b>Reflections</b>	<b>953</b>
<b>Unique Reflections</b>	<b>503</b>
<b>R merge</b>	<b>7.1</b>
<b>R1</b>	<b>35.6</b>
<b>wR2</b>	<b>63.9</b>

	<i>Kutoglu</i>	<i>single</i>	<i>2x merged</i>
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)

	<i>Kutoglu</i>	<i>single</i>	<i>2x merged</i>
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



Acta A72 (2016)  
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research papers



## Ab initio structure determination of nanocrystals of organic pharmaceutical compounds by electron diffraction at room temperature using a Timepix quantum area direct electron detector

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**Keywords:** electron nanocrystallography; Timepix quantum area detector; carbon nanotubes; molecular weight; electron diffraction; structure determination.

CCDC reference: 1438802, 1438803, 1438804, 1438805, 1438806.

Supporting information: this article has supporting information at [journals.iucr.org](http://journals.iucr.org).

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Until recently, structure determination by transmission electron microscopy of beam-sensitive three-dimensional nanocrystals required electron diffraction tomography data collection at liquid nitrogen temperature, in order to reduce radiation damage. Here it is shown that the novel Timepix detector combines a high dynamic range with a very high signal-to-noise ratio and single-electron sensitivity, enabling ab initio phasing of beam-sensitive organic compounds. Low-dose electron diffraction data ( $\sim 0.025 \text{ e}^- \text{ \AA}^{-2} \text{ s}^{-1}$ ) were collected at room temperature with the rotation method. It was ascertained that the data were of sufficient quality for structure solution using direct methods using software developed for X-ray crystallography (XDS, SHELX) and for electron crystallography (ADTSD/PETS, SJR2014).

### 1. Introduction

**Microscopy**  
TODAY  
2011 Innovation Award

Presented to  
*NanoMEGAS SPRL,*  
*University of Grenoble,*  
*and*  
*CNRS Grenoble*

For development of  
**ASTAR,**  
an automated crystal orientation  
and phase mapping system  
for diffraction analysis  
in transmission electron microscopes



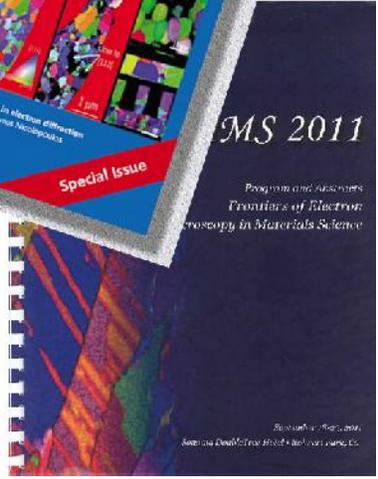
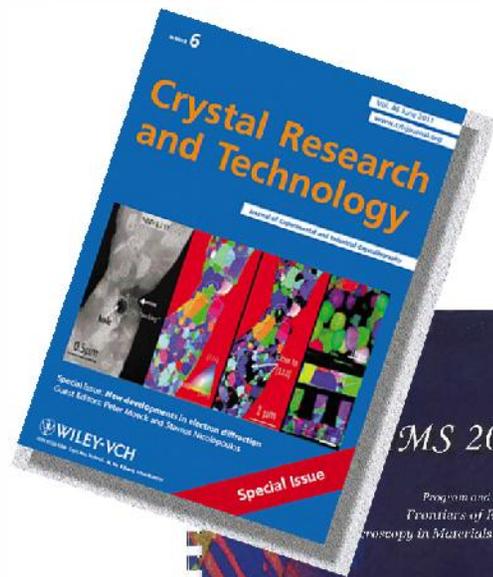
**NanoMEGAS**  
Advanced Tools for electron diffraction

**PRECESSION  
DIFFRACTION  
SOLUTIONS**

Orientation Mapping  
Phase Mapping  
Tensile Resolution  
topspin  
Strain Mapping  
EDX-EELS microscopy  
3D Diffraction Tomography

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> 400 PED related articles in 12 years





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Center for Nanotechnology Innovation@NEST , Italy, Dr. Enrico Mugnaoli

Teracystal, Romania, Mihaela Pop

Mylan laboratories Limited, India, Dr. Ram jetti, Dr. Hemant Mande

**Thanks for your attention !!!**

*Contact us*

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[www.nanomegas.com/Pharma](http://www.nanomegas.com/Pharma)

# Electron Diffraction Workshop, 21<sup>st</sup> August 2017



## IUCr Workshop

### Electron Diffraction for Materials Science and Pharmaceutical Applications

**Workshop Date:** 21st August, 2017  
**Venue:** Hyderabad International Convention Centre, Hyderabad, India.

**About the Workshop**  
This one day workshop will focus on applications of Electron Diffraction on Materials Science and Pharmaceuticals. The meeting will start with a basic introduction to transmission electron microscope (TEM) and electron diffraction (ED) and move to the latest state-of-the-art applications for crystal structure analysis using electron diffraction. This will give a unique opportunity for young and senior scientists from Industry (Pharmaceutical, Electronics, Aerospace etc.) and Academia to learn about exciting latest results on ED applications. The workshop will address useful case studies for amorphous and nanomaterials characterization, solving pharmaceutical crystal structures with LO, orientation microscopy at nm scale (EBSD-TEM like) for alloys, metals, semiconductors, organics etc.

**Number of participants:** 100 maximum  
The workshop will be in the form of lectures/presentations by experts in the area of electron Crystallography.

**Registration Fee:** There is no registration fee to attend the workshop provided the participant has registered in the main IUCR2017 meeting. Registration of this workshop should be done through the main IUCR2017 registration process.

Work program will be announced later.

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**Workshop Starts at 8.30 AM**

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**Combinatorial Polymers in Solubility - Some Structural Insights**

James M. Stupp, Ram K. K. Jha, Patrick D. Cal and S. M. Stupp

**Mylan**

**Introduction**

Polymers play a key role in the development of pharmaceutical products. The development of new polymers is a complex task that involves the synthesis of a large number of different polymers and the evaluation of their properties. This process is often accelerated by the use of combinatorial chemistry, which allows the synthesis of a large number of different polymers in a single experiment.

**Experimental**

The polymers were synthesized using a combinatorial approach. The synthesis was carried out in a 96-well plate, where each well contained a different polymer. The polymers were then characterized using a variety of techniques, including NMR, IR, and DSC.

**Results and Discussion**

The results show that the polymers exhibit a wide range of properties, including different solubilities and thermal stabilities. The data indicates that the combinatorial approach is a powerful tool for the discovery of new polymers.

**Conclusion**

The combinatorial approach is a powerful tool for the discovery of new polymers. It allows the synthesis of a large number of different polymers in a single experiment, which can then be evaluated for their properties.

**NanoMEGAS**

**RANDOM ELECTRON DIFFRACTION TOMOGRAPHY FOR STRUCTURE ANALYSIS OF PHARMACEUTICALS**

**Introduction**

Random Electron Diffraction Tomography (REDT) is a powerful technique for the structure analysis of pharmaceuticals. It allows the determination of the 3D structure of a sample from a series of 2D diffraction patterns. This technique is particularly useful for the analysis of complex, non-crystalline samples.

**Experimental**

The REDT experiment was carried out using a NanoMEGAS instrument. The sample was irradiated with a beam of electrons, and the resulting diffraction patterns were recorded. The data was then processed using a series of software tools to determine the 3D structure of the sample.

**Results and Discussion**

The results show that the REDT technique is a powerful tool for the structure analysis of pharmaceuticals. It allows the determination of the 3D structure of a sample from a series of 2D diffraction patterns, which can then be used to identify the sample.

**Conclusion**

The REDT technique is a powerful tool for the structure analysis of pharmaceuticals. It allows the determination of the 3D structure of a sample from a series of 2D diffraction patterns, which can then be used to identify the sample.

**NanoMEGAS**

**PRECISION DIFFRACTION FOR RELIABLE ELECTRON PAIR DISTRIBUTION FUNCTION ANALYSIS**

**Introduction**

Precision Diffraction (PD) is a powerful technique for the reliable analysis of electron pair distribution functions (PDFs). It allows the determination of the PDF of a sample from a series of diffraction patterns. This technique is particularly useful for the analysis of complex, non-crystalline samples.

**Experimental**

The PD experiment was carried out using a NanoMEGAS instrument. The sample was irradiated with a beam of electrons, and the resulting diffraction patterns were recorded. The data was then processed using a series of software tools to determine the PDF of the sample.

**Results and Discussion**

The results show that the PD technique is a powerful tool for the reliable analysis of electron PDFs. It allows the determination of the PDF of a sample from a series of diffraction patterns, which can then be used to identify the sample.

**Conclusion**

The PD technique is a powerful tool for the reliable analysis of electron PDFs. It allows the determination of the PDF of a sample from a series of diffraction patterns, which can then be used to identify the sample.

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