PAUL SCHERRER INSTITUT

PSI

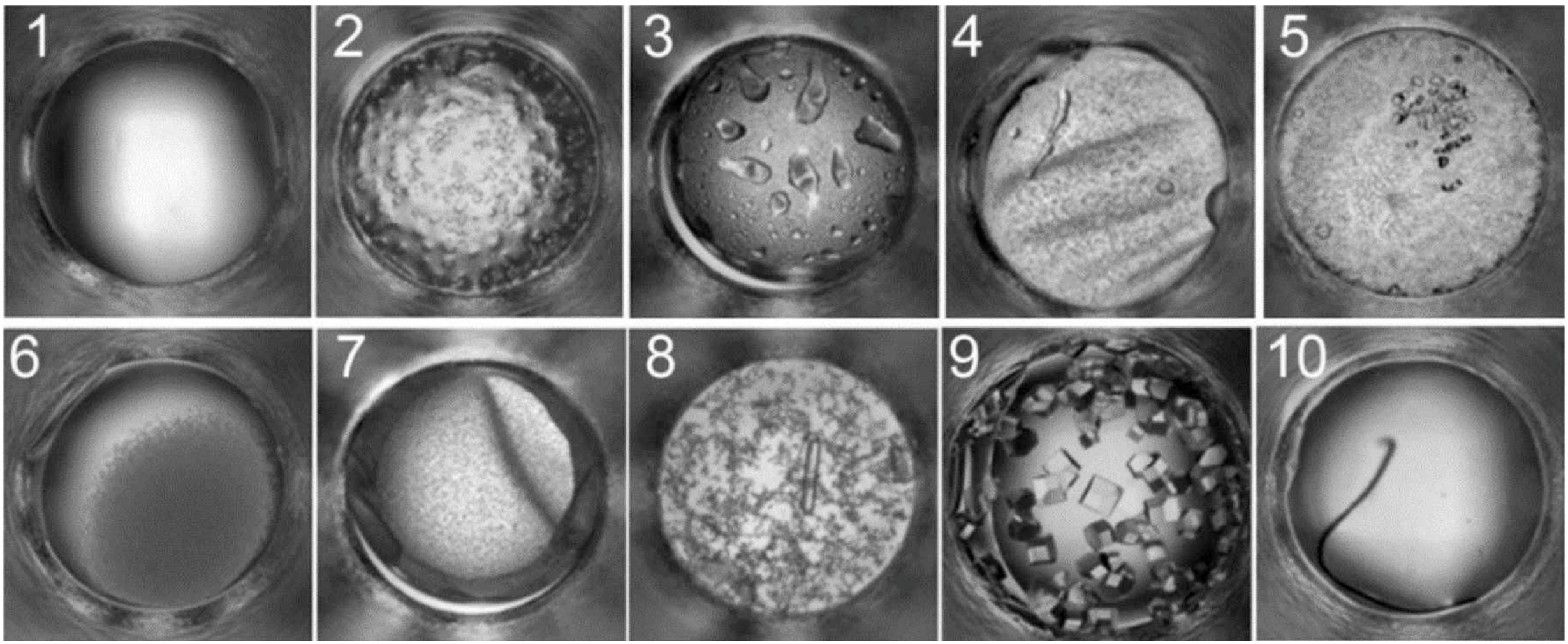
Dr. Tim Grüne :: Paul Scherrer Institut :: tim.gruene@psi.ch

Macromolecular Crystallography with Electron Diffraction

PSI MX Journal Club — 12^{th} September 2016

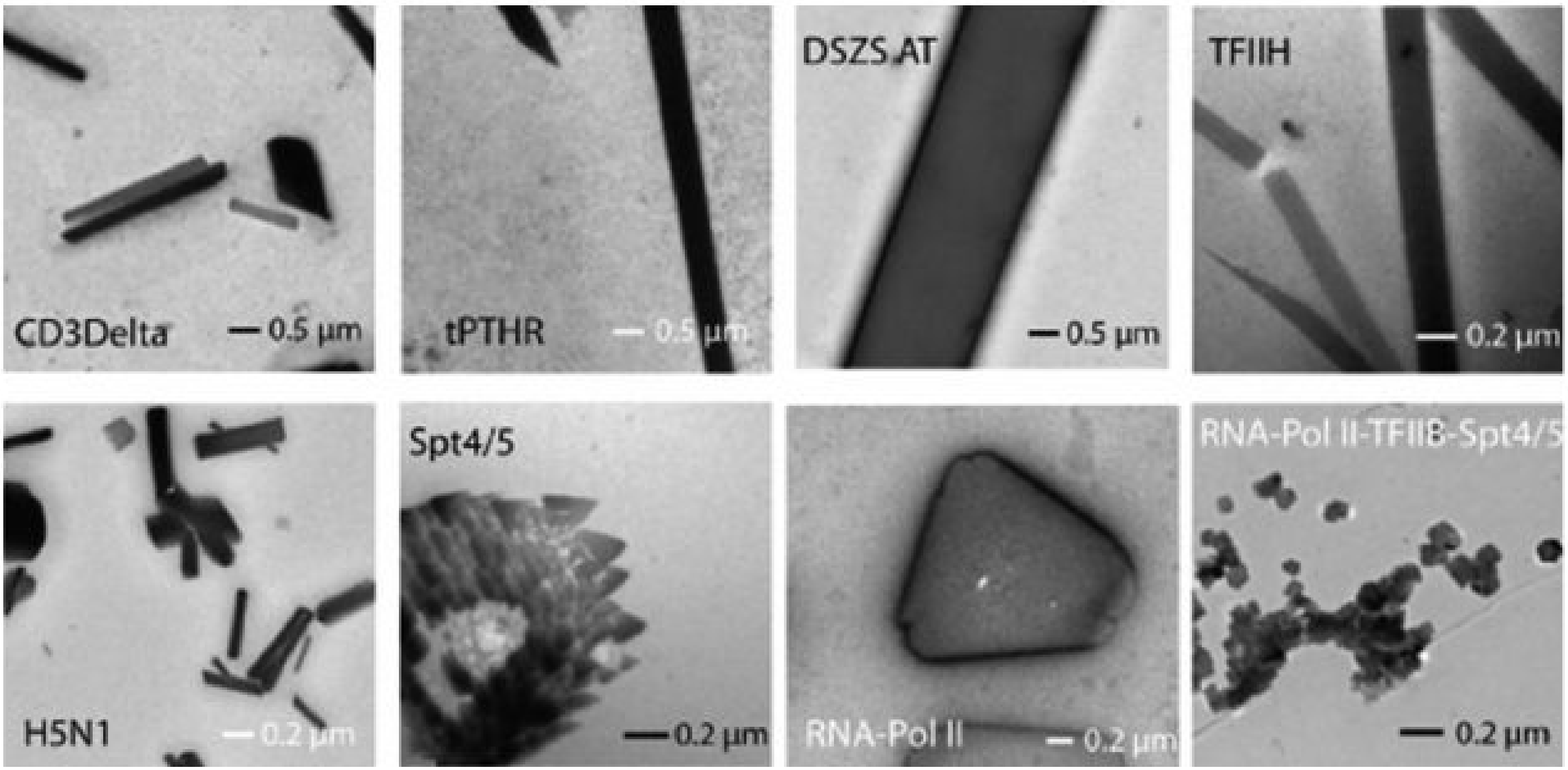
1 - Nanocrystals

The (seemingly) Empty Drop



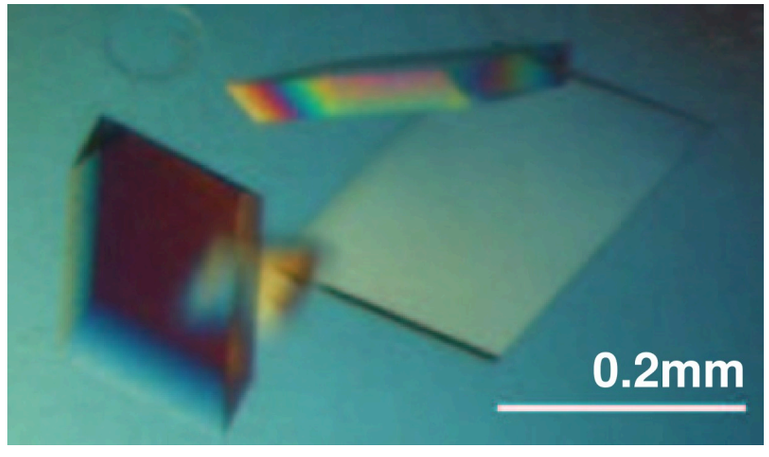
Luft, Wolfley, Snell, Crystal Growth& Design (2011), 11, 651–663

Drops viewed through TEM

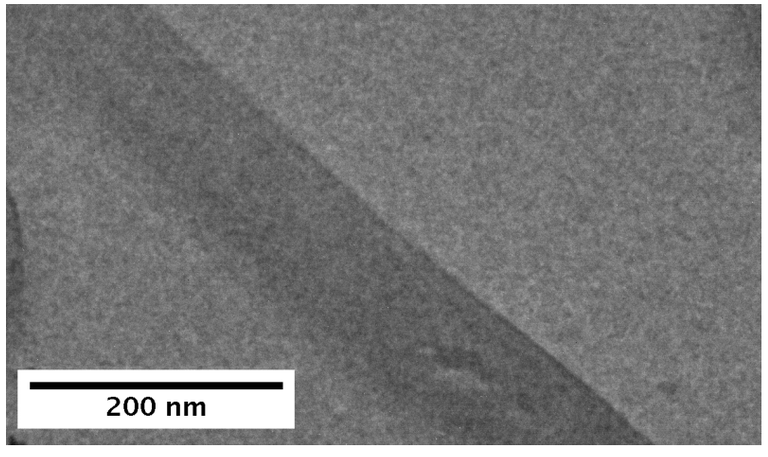


Stevenson,. . . , Calero, PNAS (2014) 111, 8470–8475 / Calero, . . . , Snell, Acta Cryst (2014) F70, 993–1008

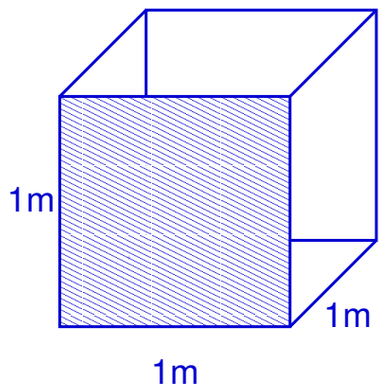
How small is “nano”?

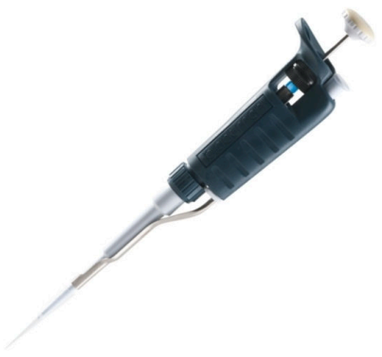


typical protein crystal size for X-rays



typical protein crystal size for electrons, 100x140x1,700 nm^3





volumes compare like 6 bath tubs of water *vs.* $10\mu l$

2 - How to react to Radiation Damage

Radiation Damage limits Diffraction

- Henderson / Garman limit: 20–50 MGy = 20–50 MJ/kg before half intensity is lost

- $m \propto V$: nanocrystal can take 10^{-9} photons compared to macrocrystal

- Same resolution requires same dose (number of counts on detector)

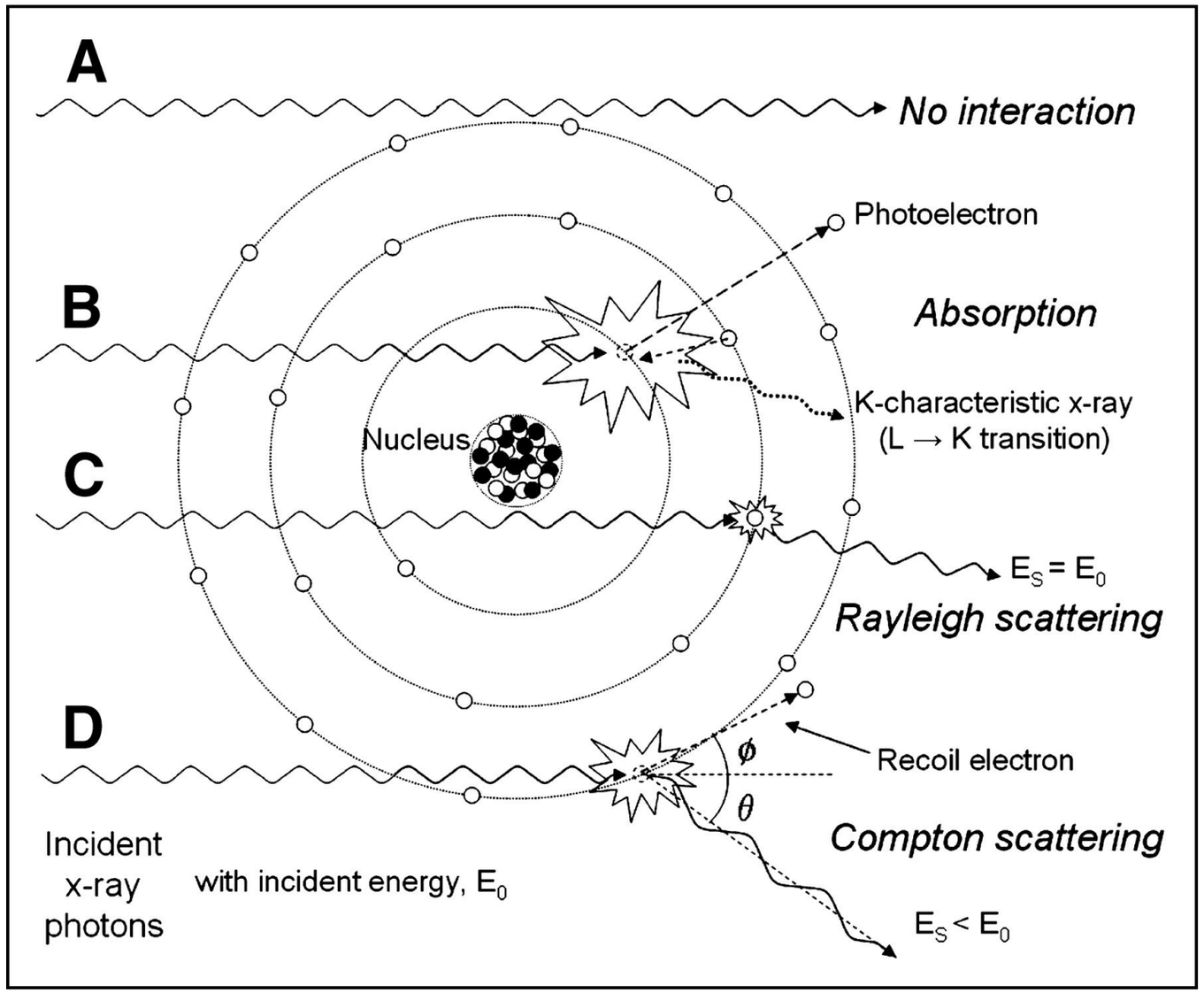
Means to overcome Radiation Damage

1. More sensitive detectors: hybrid pixel detectors like Pilatus close to ideal (single count reflections)

2. “Measure before destroy”: merge data from few to many individual crystals
   - manually since 1980’s: room temperature data, virus data
   - automated: high intensity free electron lasers, also minimises noise

3. Use electrons instead of X–rays and gain a factor of 1,000 in signal *vs.* damage

X–ray Interaction with Matter



Illustrative summary of X–ray and γ–ray interactions. JA Seibert & JM Boone, J. Nucl. Med. Technol. 2005;33:3-18

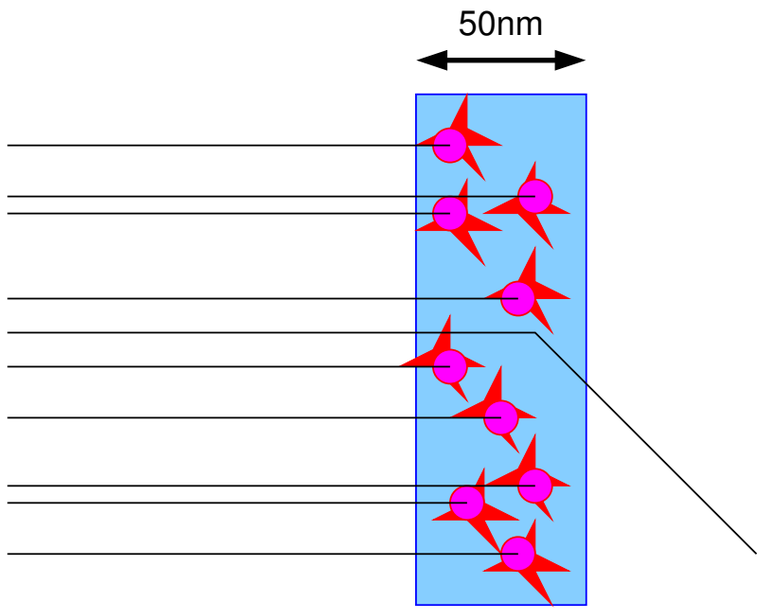
Interaction of X–rays at 12keV with $100\mu m$ soft tissue

A	Transmission	96.6%
B	Photo absorption	3.0%
C	Elastic Scattering	0.2%
D	Compton Scattering	0.2%

Red: Radiation damage Green: Diffraction

Every diffracting photon is accompanied by 16 damaging photons

X–rays Scattering and Electron Scattering

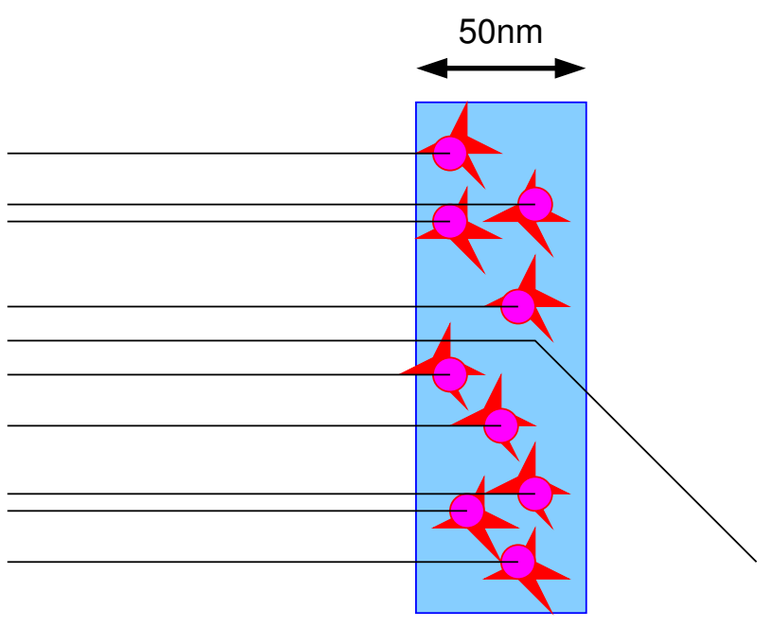


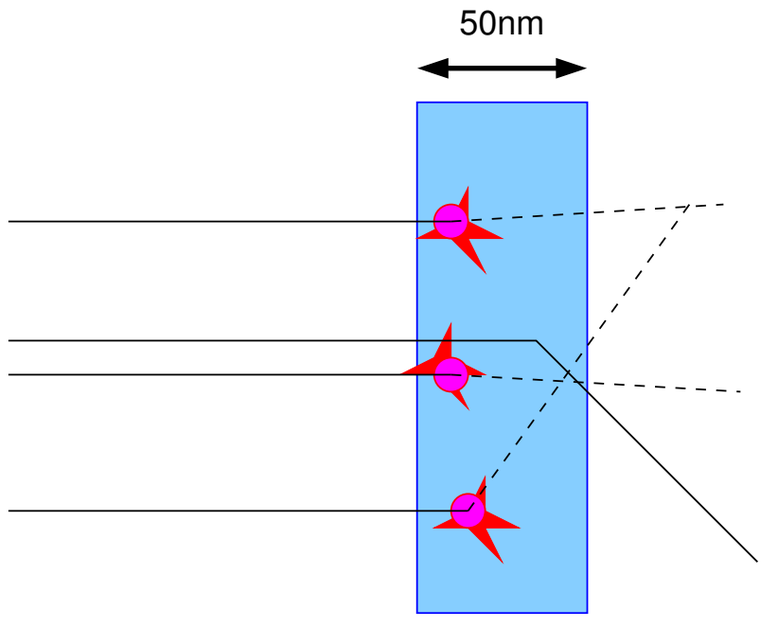
X–rays (10keV)

- Probability of inelastic scattering: 10^{-4}
- Deposited energy: 10keV
- Probability of elastic scattering: $10^{-5} = 10^{-4}/10$
- Damage per diffracted photon: 100keV

(Figures from R. Henderson, Q. Rev. Biophys. (1995), 28, 171–193)

X–rays Scattering and Electron Scattering





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- Damage per diffracted photon: 100keV

e^- (200keV)

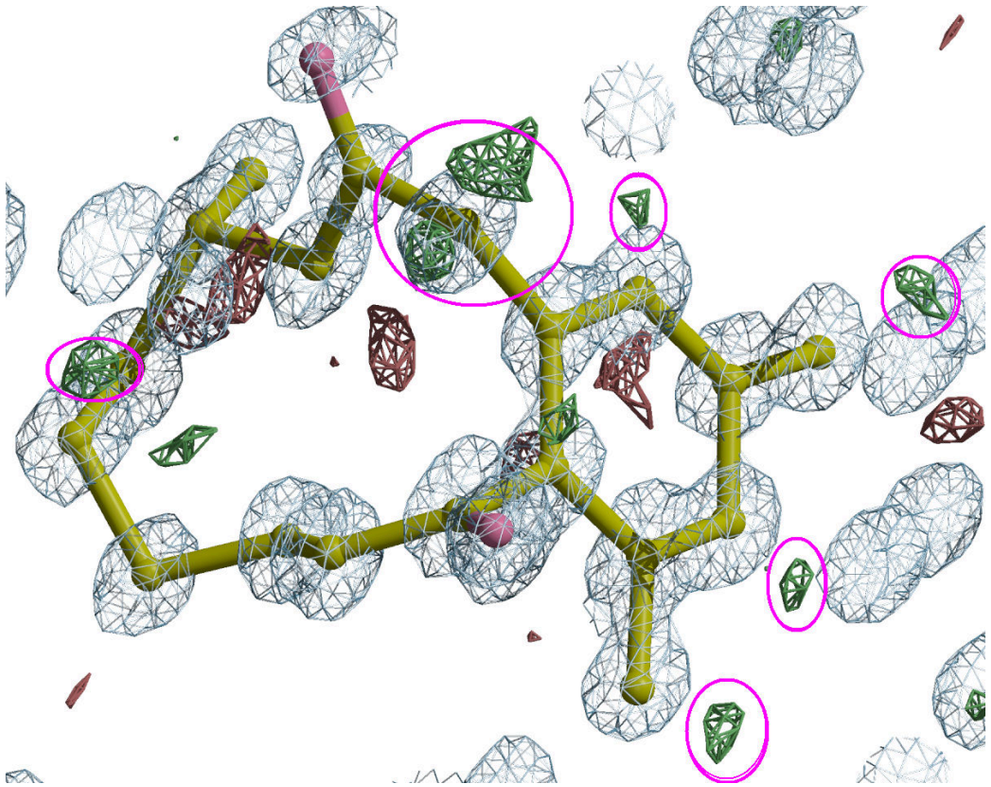
- Probability of inelastic scattering: 30%
- Deposited energy: 20eV
- Probability of elastic scattering: 10%
- Damage per diffracted electron: 60eV = 0.06keV

2,000 times more damage with X–rays

(Figures from R. Henderson, Q. Rev. Biophys. (1995), 28, 171–193)

Other Features of Electrons Scattering

- Small crystals may show less defects
- Hydrogen much better visible
- Map corresponds to electrostatic potential
- Powder consist of single nanocrystals



Sample courtesy Novartis, CSD code IRELOH

Electrons — Cure for Radiation Damage?

1. Limits in sample thickness (200keV electrons: 100% absorption for samples $> 1\mu m$, i.e. no signal)
2. Nanocrystals difficult to detect
3. 500nm = 50*100Å: “countable” number of unit cells
4. Cumbersome sample handling
5. No anomalous signal: phasing; chirality
6. Dynamic and multiple scattering obstruct refinement
7. Instruments not made for diffraction

3 - Electron Diffraction

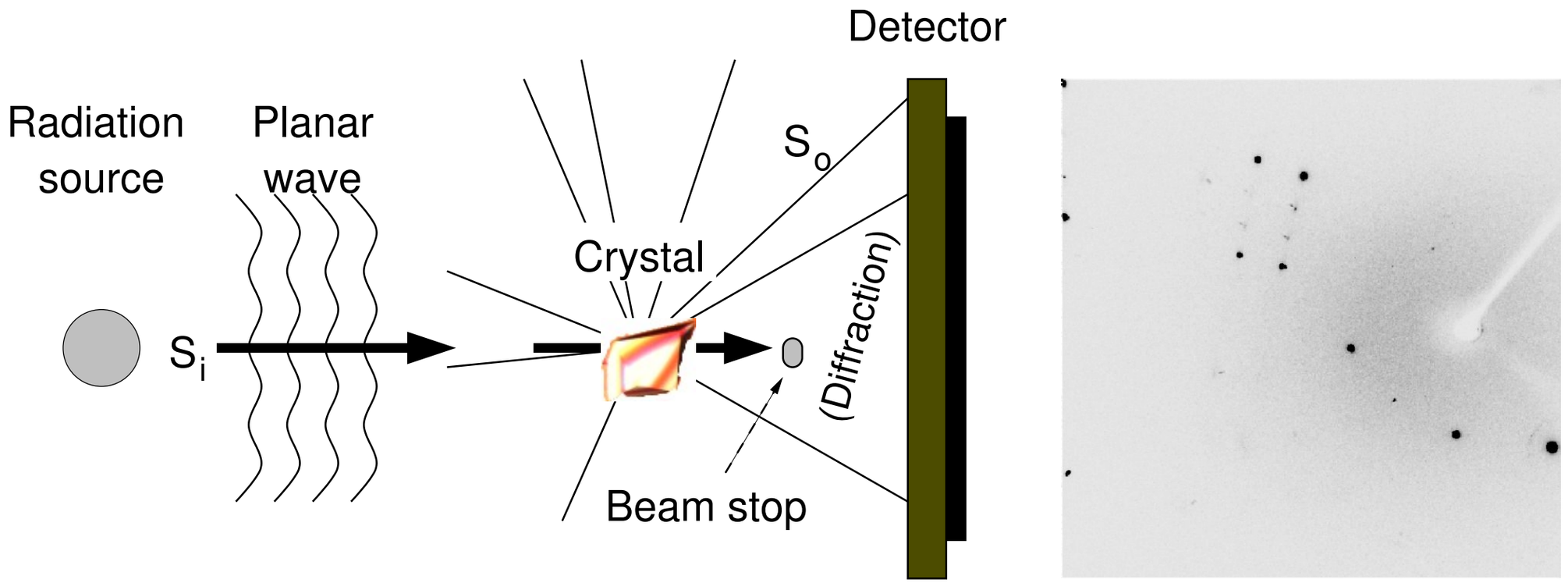
N.B.: 2D and 3D Crystals

The 2012 edition of International Tables F consider “Electron diffraction of protein crystals” (Chapter 19.2) only of 2D crystals.

Here, electron crystallography is the same as X–ray or neutron crystallography:

We measure the diffraction of a 3D crystal, i.e. a solid with periodic repeats in three dimensions, as result of an incoming planar wave.

Structure Determination by Single Crystal Diffraction



- Radiation: X–ray, neutrons, or electrons
- Diffraction spots: interaction between wave and **crystal**
- Position governed by Laue conditions $(\vec{S}_o - \vec{S}_i) \cdot \vec{a} = h \wedge (\vec{S}_o - \vec{S}_i) \cdot \vec{b} = k \wedge (\vec{S}_o - \vec{S}_i) \cdot \vec{c} = l$
- Experimental result: **Position** and **Intensity** for each spot

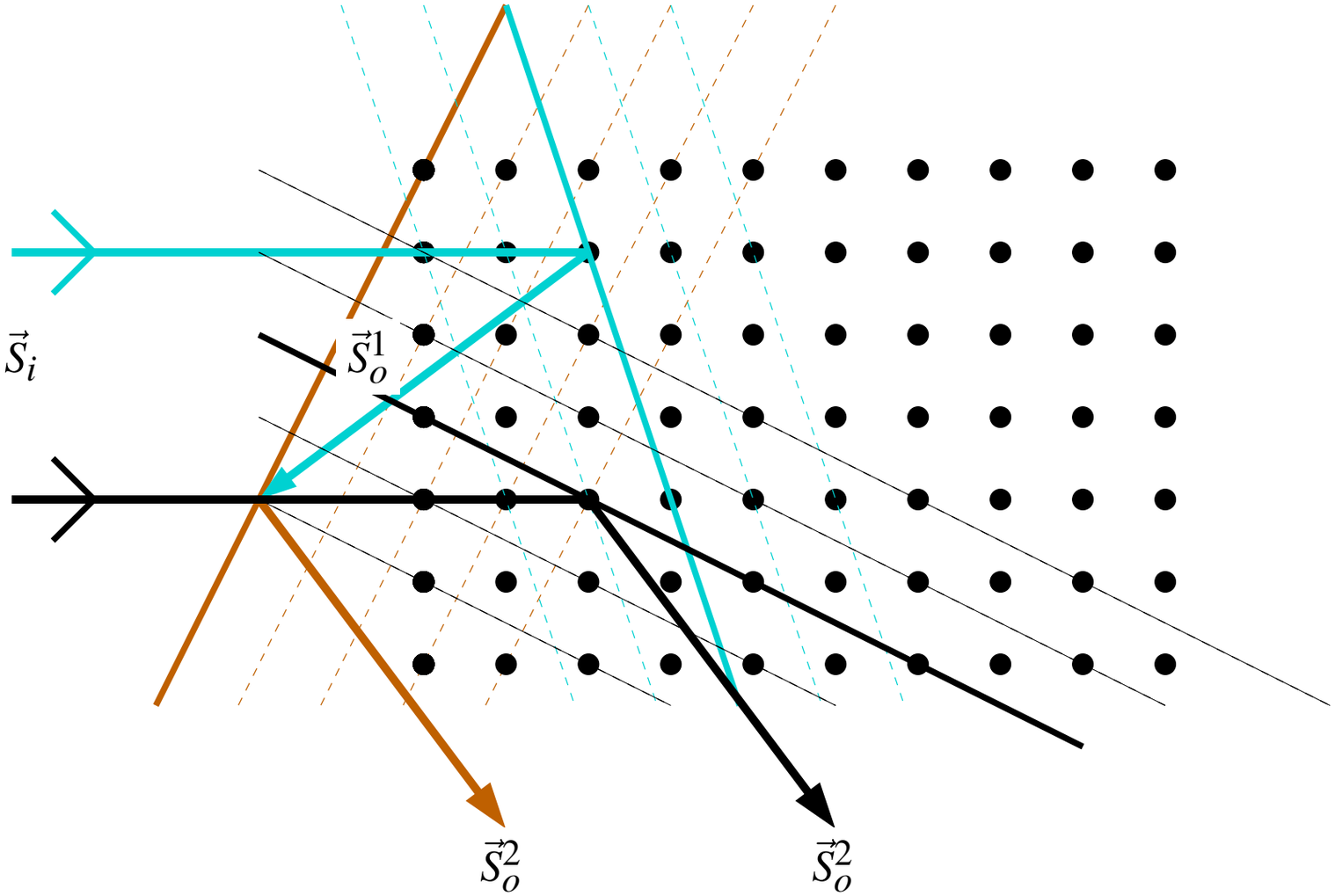
Electrons: Multiple Scattering

- electron interact strongly with matter = high scattering probability

- nice, as it enables small crystal volumes

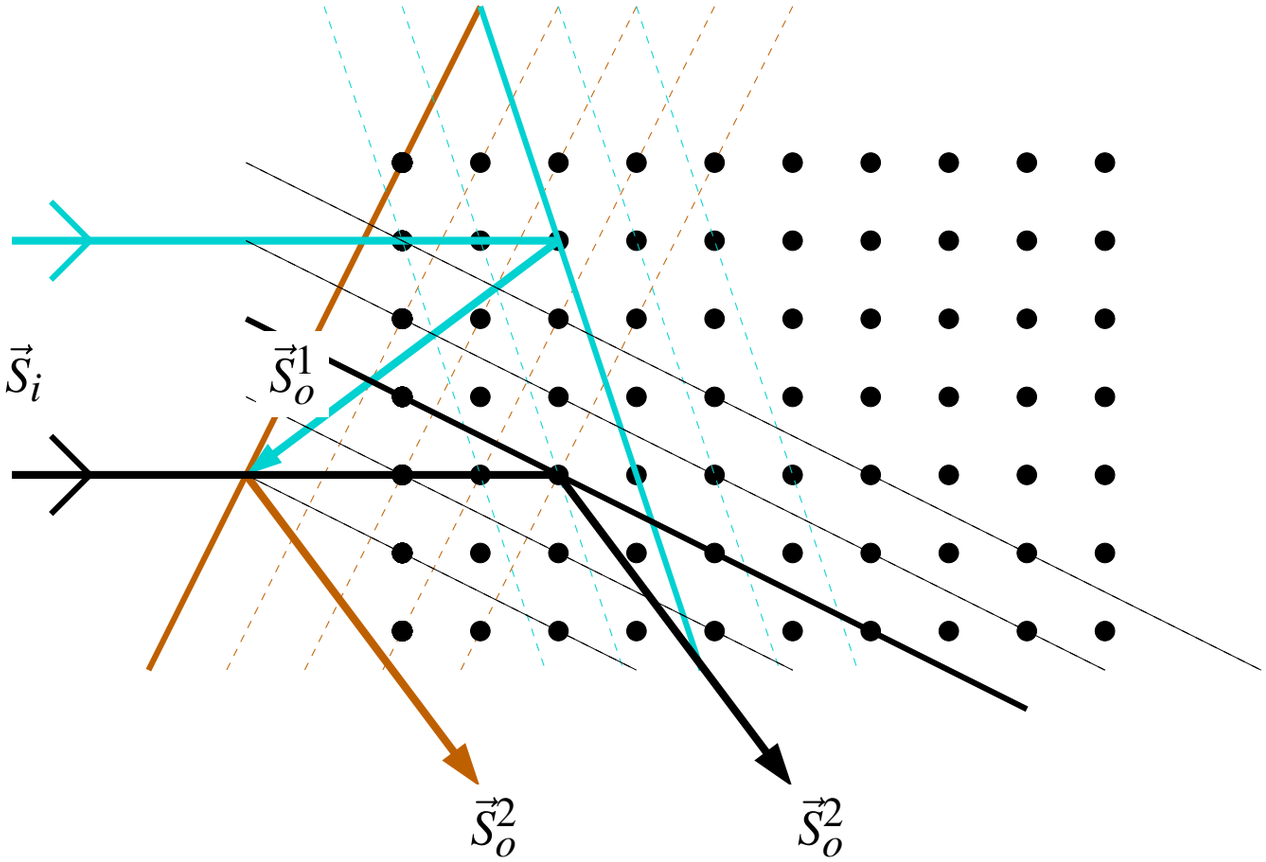
- effects that multiple scattering must be taken into account ($I \not\propto |F|^2$)

Multiple (Dual) Scattering



- Outgoing ray $\vec{S}_o^1$ acts as incoming ray for reflection $\vec{S}_o^2$.
- Re–reflection with 10% probability at 50 nm path length

Multiple (Dual) Scattering



Laue Conditions (accordingly $\vec{b}$ and $\vec{c}$):

$$\begin{aligned}(\vec{S}_o^1 - \vec{S}_i)\cdot\vec{a} &= h_1\\ (\vec{S}_o^2 - \vec{S}_o^1)\cdot\vec{a} &= h'\\ \hline (\vec{S}_o^2 - \vec{S}_i)\cdot\vec{a} &= h_1 + h'\end{aligned}$$

Simplest approximation:

$$I_{\text{exp}}(h_2k_2l_2) \propto |F_{\text{ideal}}(h_2k_2l_2) + \alpha F_{\text{ideal}}(h_1k_1l_1)|^2$$

- α: re–scattering of S_o^1
- $F(h_1k_1l_1)$ must be strong
- $F(h',k',l')$ must be strong
- $F(h_2k_2l_2)$ must be weak

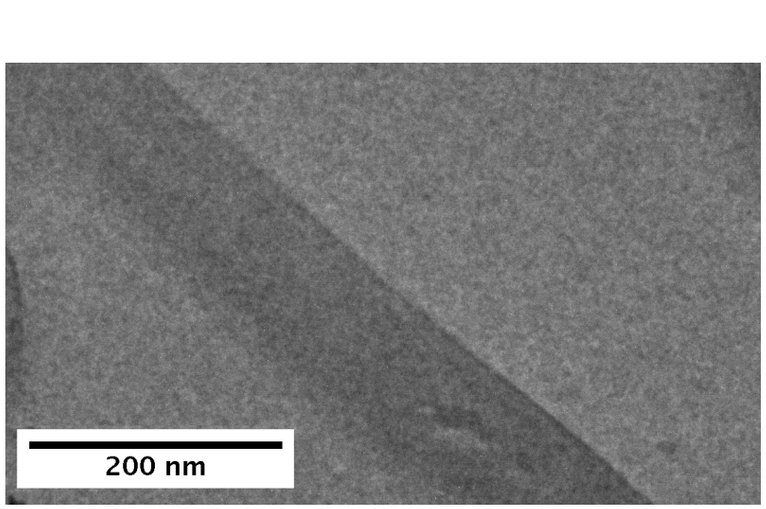
$\Rightarrow$ affects high resolution data

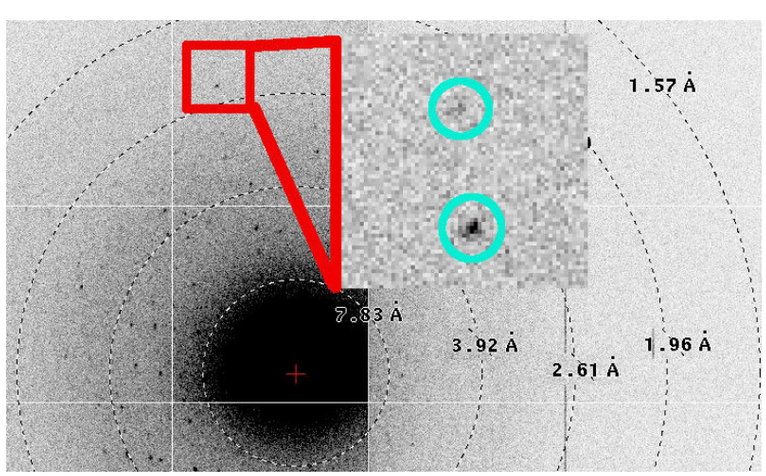
Protein crystallographers are used to enormous errors: multiple scattering may just add a bit.

4 - Electron Diffraction Structure of Hen Egg Lysozyme

The Crystal and Diffraction Experiments

Lysozyme nanocrystals measured at NeCEN (Netherlands), FEI Titan Krios microscope with Timepix camera





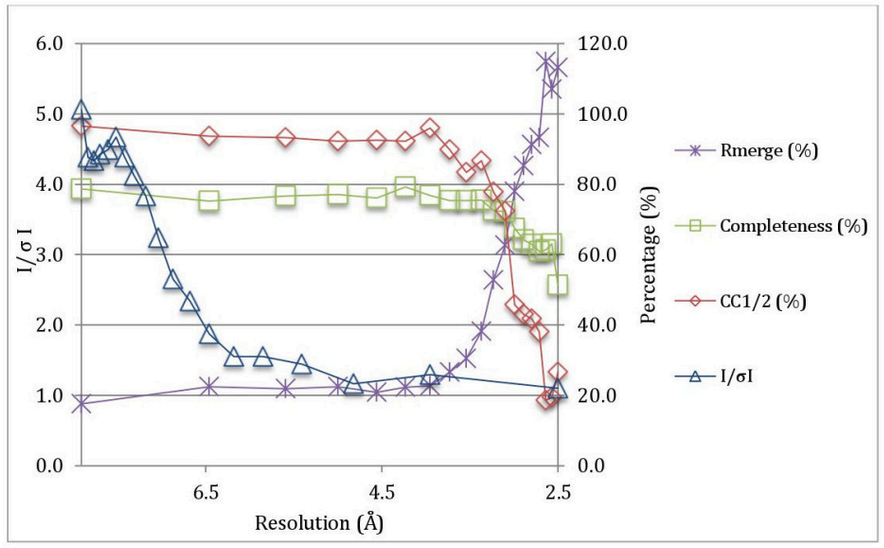
- 0.1615°/frame fast scan
- 0.048° / frame slow scan
- 11 e^-/Å^2 dose
- 10Hz read–out
- $\lambda = 0.02508$Å
- 2.078m (!) detector distance
- 40° largest wedge, mostly 20°
- few spots to 2.2Å (inset)

Data Processing with XDS

- “spots are spots”: XDS integrates electron diffraction data
- XDS feature per–pixel spatial corrections: covers a few lens distortions
- *caveat*: unset “REFLECTING_RANGE” refines to 20–40°
- default corrections model most likely inappropriate
- weak spots? determine background “along” pixel

Incomplete Data: Merging Data from six Crystals

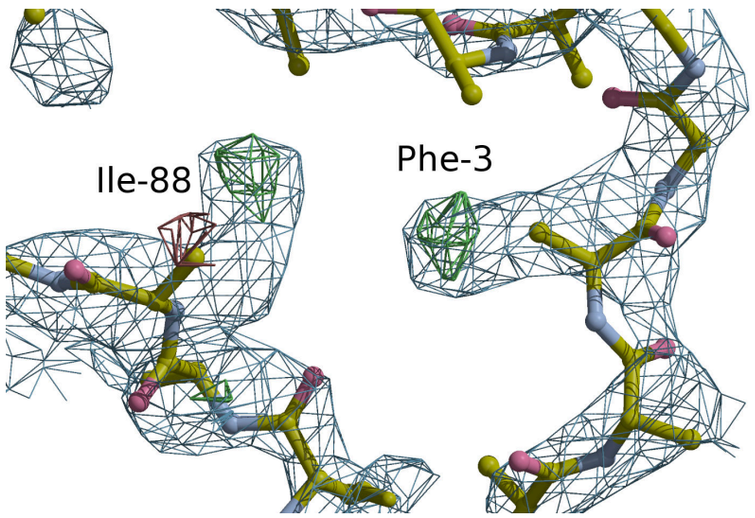
	Single crystal	Merged data
Data integration		
Space group	$P2_12_12$	
Unit cell dimensions		
a, b, c (Å)	104.56, 68.05, 32.05	
α, β, γ (°)	90.0, 90.0, 90.0	
Number of crystals	1	6
Resolution (Å) [1]	32.05-2.50 (2.57-2.50)	57.04-2.50 (2.57-2.50)
R_{merge} (%)	31.7 (107.3)	35.7 (113.2)
I/σI	2.92 (1.10)	2.87 (1.10)
Completeness (%)	0	69.0 (51.3)
Reflections	9518 (817)	25148 (1373)
Unique reflections	3445 (236)	5808 (299)
Redundancy	2.76 (3.46)	4.33 (4.59)
Refinement		
R1 (%)	25.90	23.54
$R_{complete}$ (%) [Luebben 2015]	32.49	27.21
<B> (Å^2)	33.08	36.49
RmsZ bonds	0.779	0.765
RmsZ angles	0.974	0.911



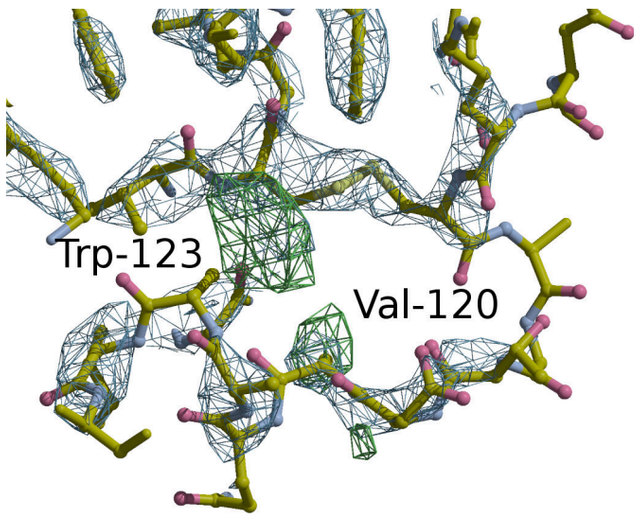
PDB–ID 4R0F: $P2_12_12$ with 104.63Å 66.49Å 31.65Å

Structure Solution

1. Molecular Replacement from poly Ala **monomer** with Phaser uniquely determines space group $P2_12_12$ (TFZ=19.8, LLG=335.3)
2. Side chain completion with Buccaneer all except 27 atoms
3. Refinement with refmac5
   - *"source electron MB"* scattering factors for electrons
   - *"mapc free exclude"* do not estimate missing reflections: avoid model bias at low completeness



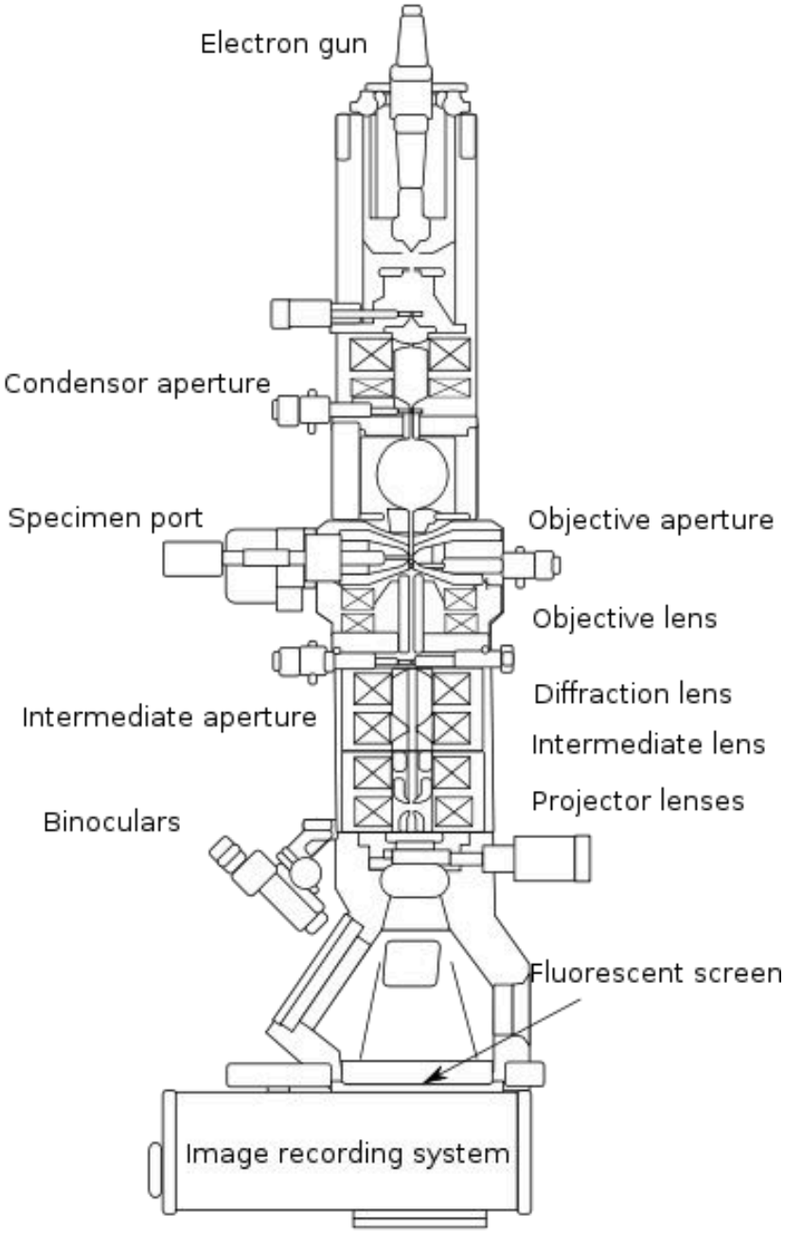
MR solution shows difference density for bulky side chains



Refined map guides model completion

5 - Instruments for Electron Diffraction

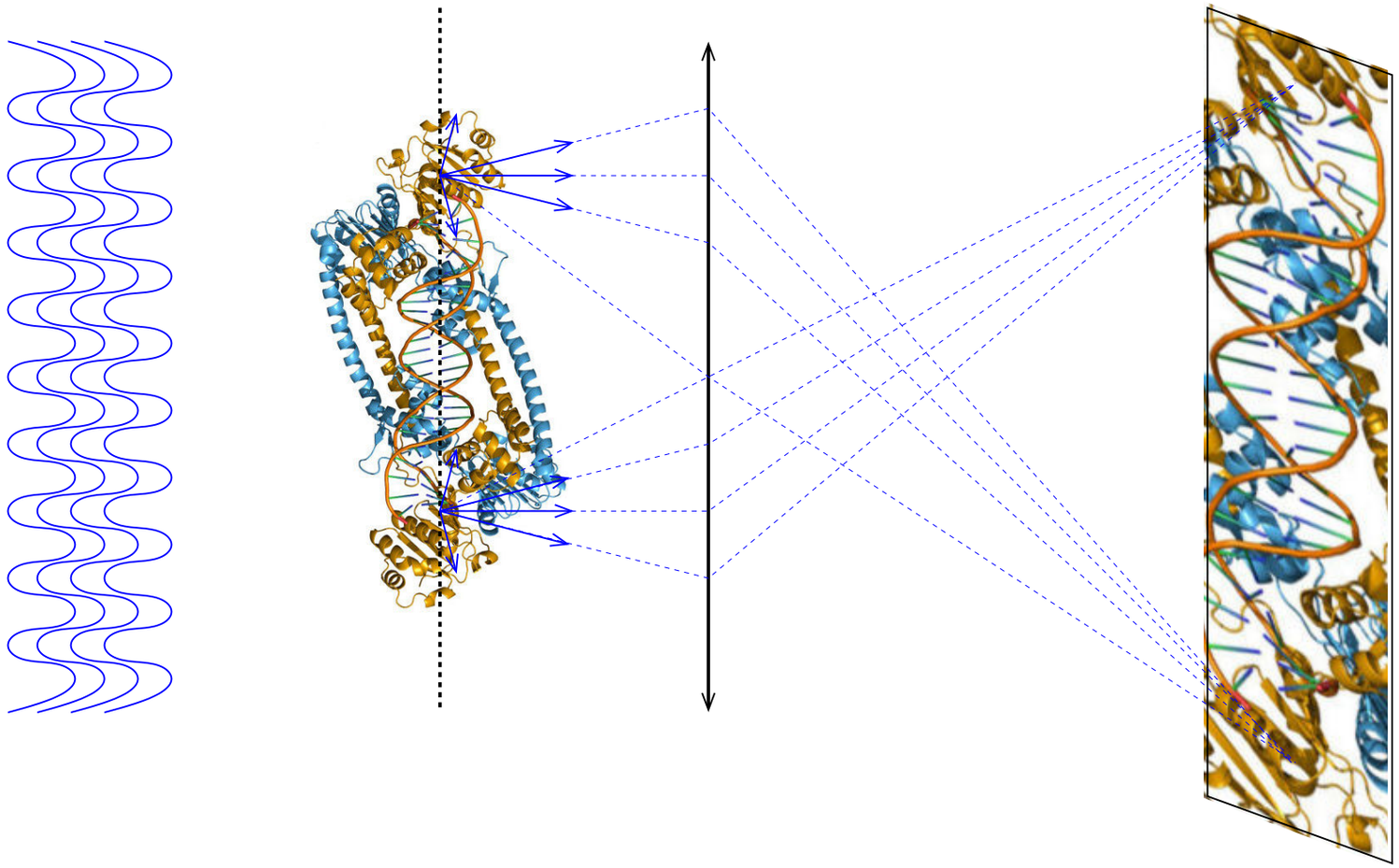
Electron Microscopes



(Wikipedia)

- Field emission gun: 100–300keV electrons
- 200kev: $\lambda = 0.02508$Å
- 2–3 lenses above sample: parallel, narrow beam
- ≥ 1 lens below sample: effective detector distance
- New: hybrid pixel detector (Timepix, Eiger, Jungfrau…)

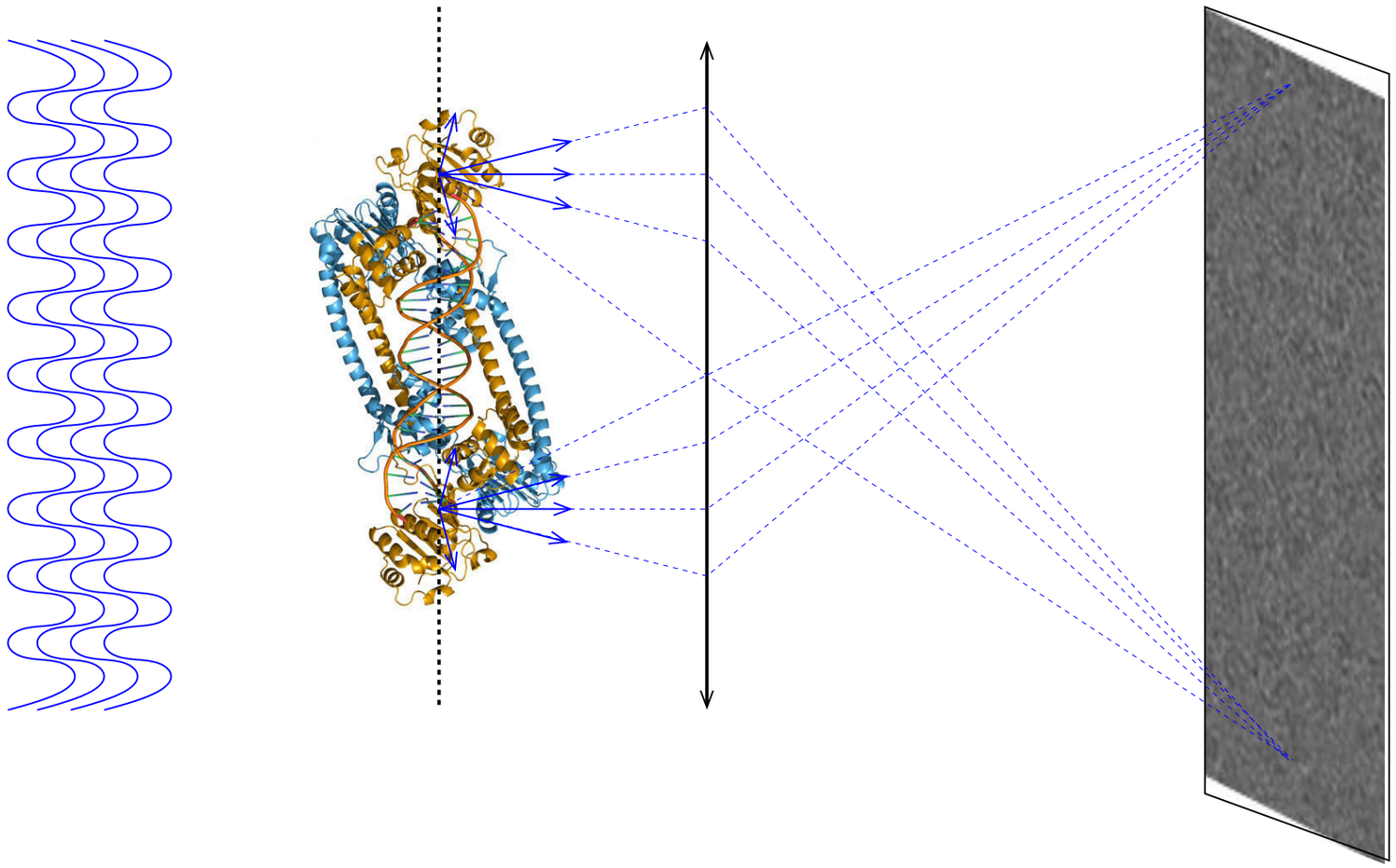
Electron Microscope: Imaging Mode



Plane Wave Object Diffraction Lens Image Plane (Detector)

Rays of **equal origin** focus on detector

Electron Microscope: Imaging Mode

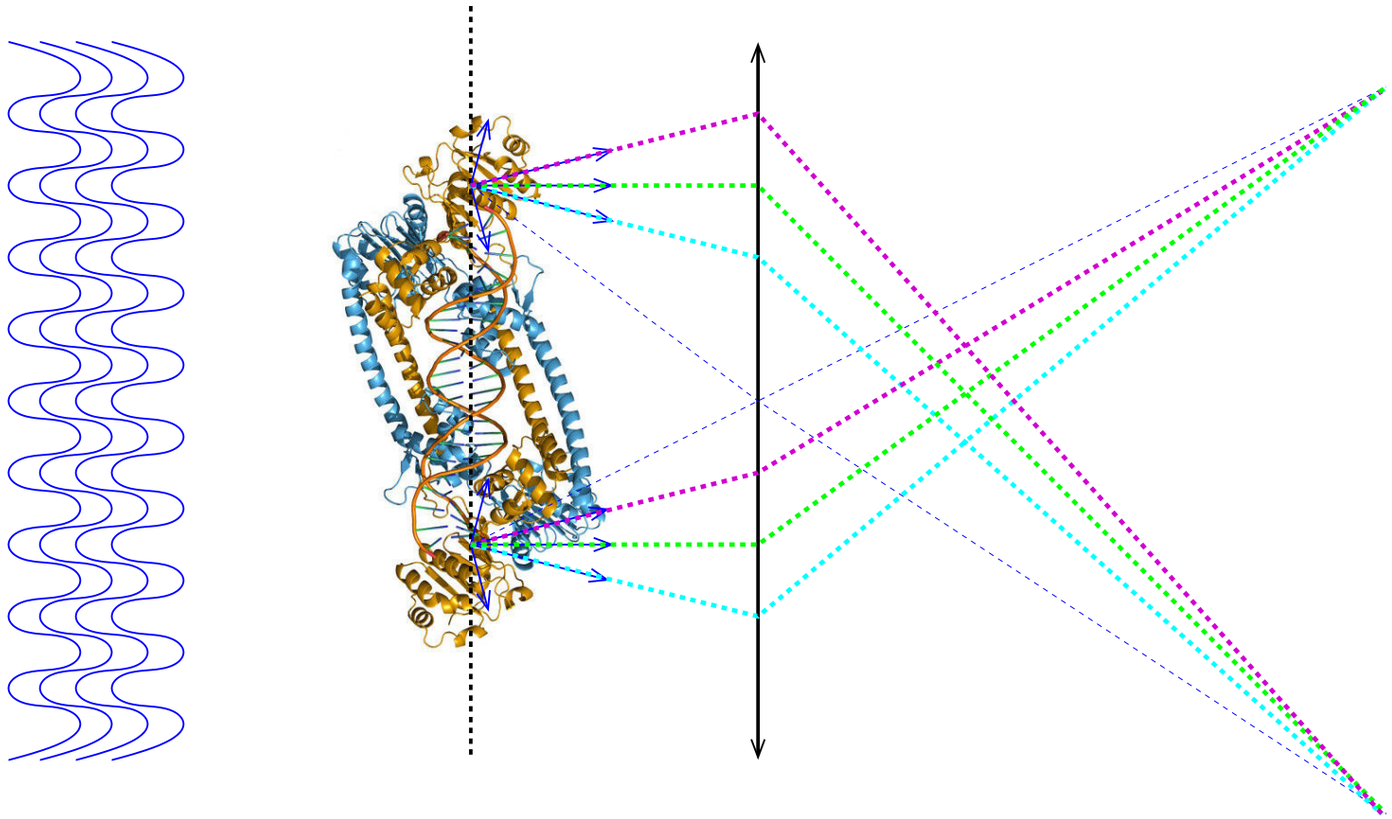


Plane Wave Object Diffraction Lens Image Plane (Detector)

Detector noise and radiation sensitivity require low contrast images

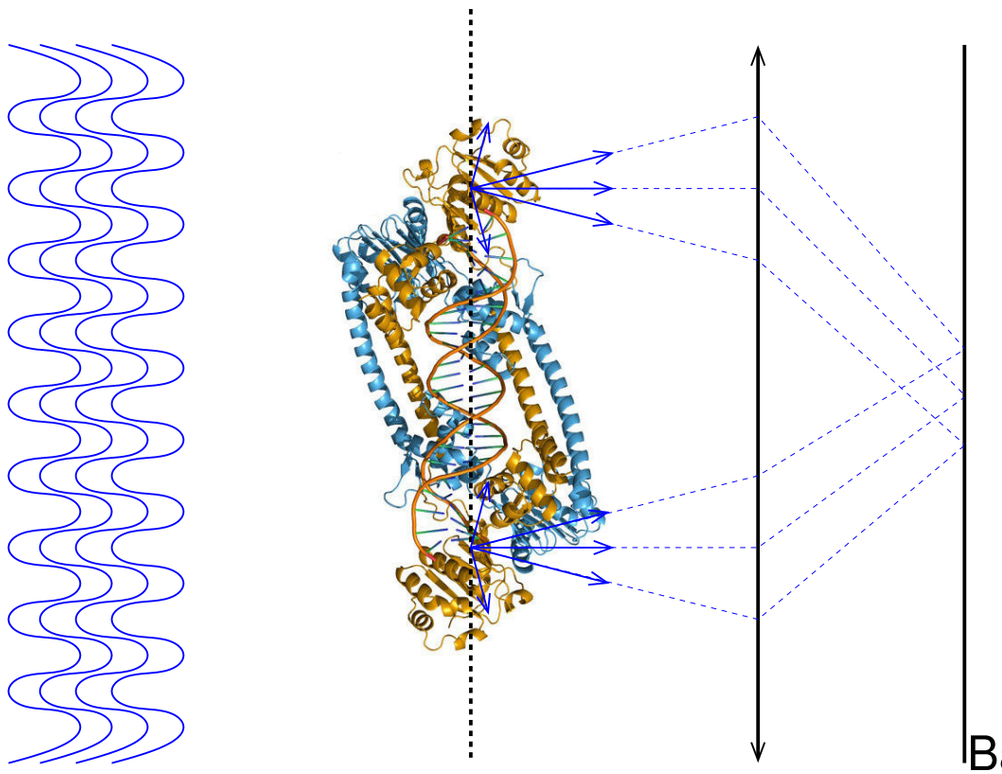
Martinez-Rucobo *et al.* Molecular Cell (2015) 58, 1079–1089

Electron Microscope: Diffraction Mode



Plane Wave Object Diffraction Lens

Electron Microscope: Diffraction Mode



Plane Wave Object Diffraction Lens

Image at *Back focal Plane* =
||Fouriertransform of object||

For crystals:

diffraction spots according to Laue conditions

Backfocal Plane

Rays of **equal direction** focus on detector

Summary

- Electron diffraction suitable for structural biology
- Ideal for 100–500nm crystal thickness
- Integration, Solution, Refinement: Methods and programs fit for electrons
- Our results direct for hardware improvements:
  - Goniometer precision and accuracy
  - Lens system optimisation for diffraction
  - Hybrid pixel detectors for 300–1,000 keV electrons

6 - Acknowledgements

- Jan Pieter Abrahams, Max Clabbers, Eric van Genderen, Wei Wan
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