PAUL SCHERRER INSTITUT

PSI

WIR SCHAFFEN WISSEN — HEUTE FÜR MORGEN

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

Electron Crystallography for Structure Solution — Latest Results from the PSI Electron Diffraction Group

Research Complex at Harwell Seminars

13th April 2017

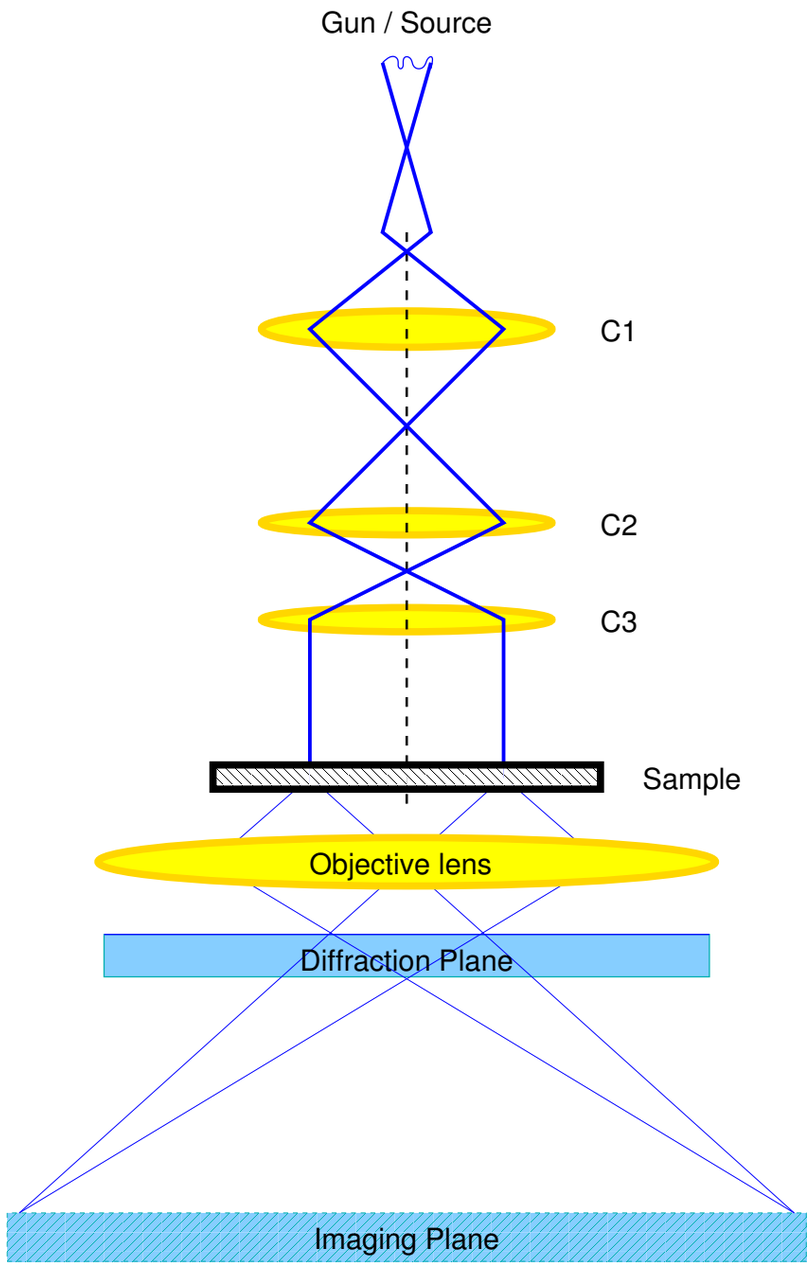
1 - Crystallography under the Microscope

Instrumentation available at C–CINA, University Basel

1. FEI Polara, 200keV, 512x512 Timepix detector, single axis $\pm 30^\circ$

2. FEI Talos, 200keV, 1024x1024 Timepix detector, single axis $\pm 40^\circ$

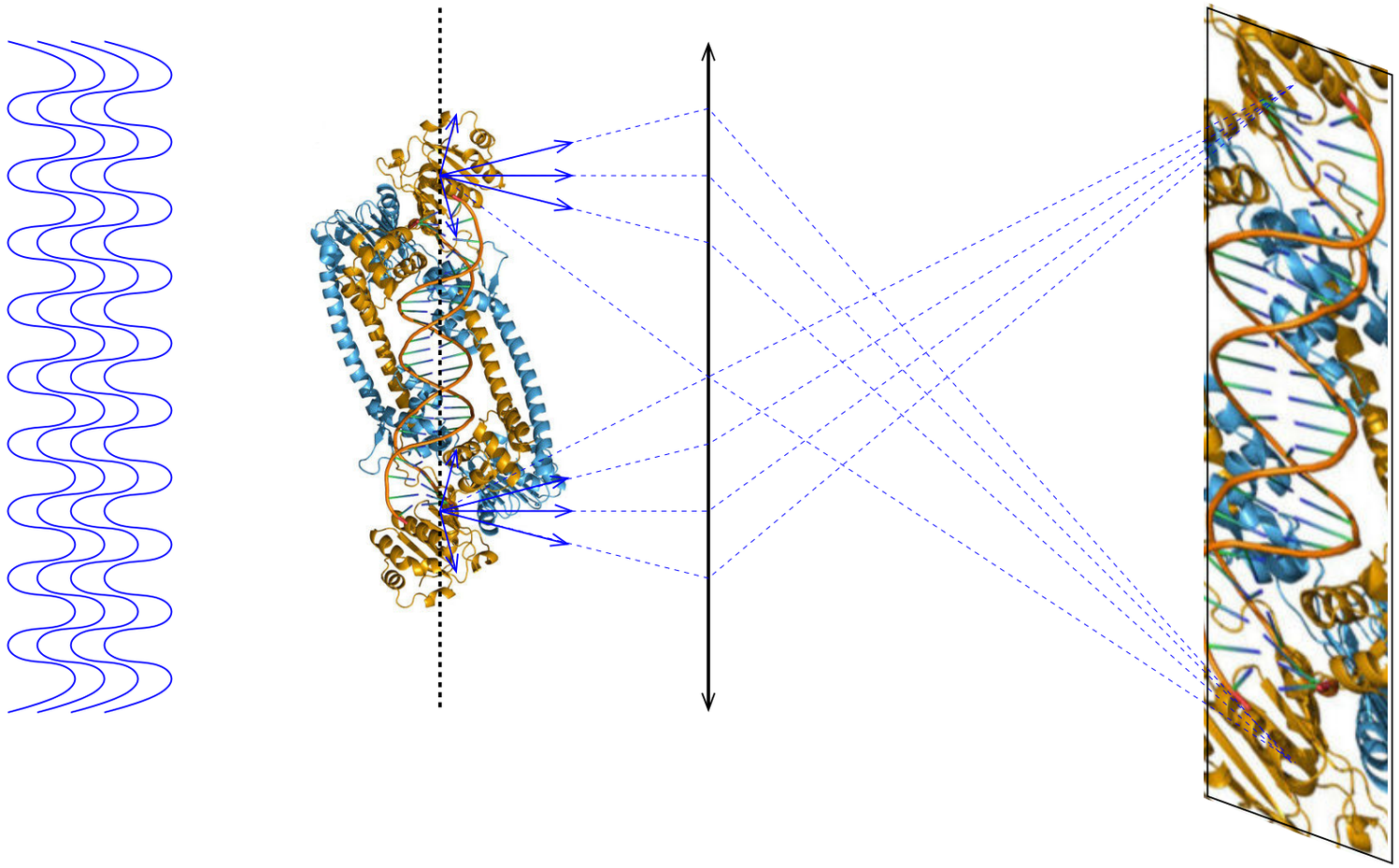
The Lens System



- Lenses C1–C3 shape beam
- Crystallography: Parallel beam
- Objective lens: sets effective detector distance to back-focal plane = diffraction mode
- C3 not present in all microscopes

Lenses cause distortions.

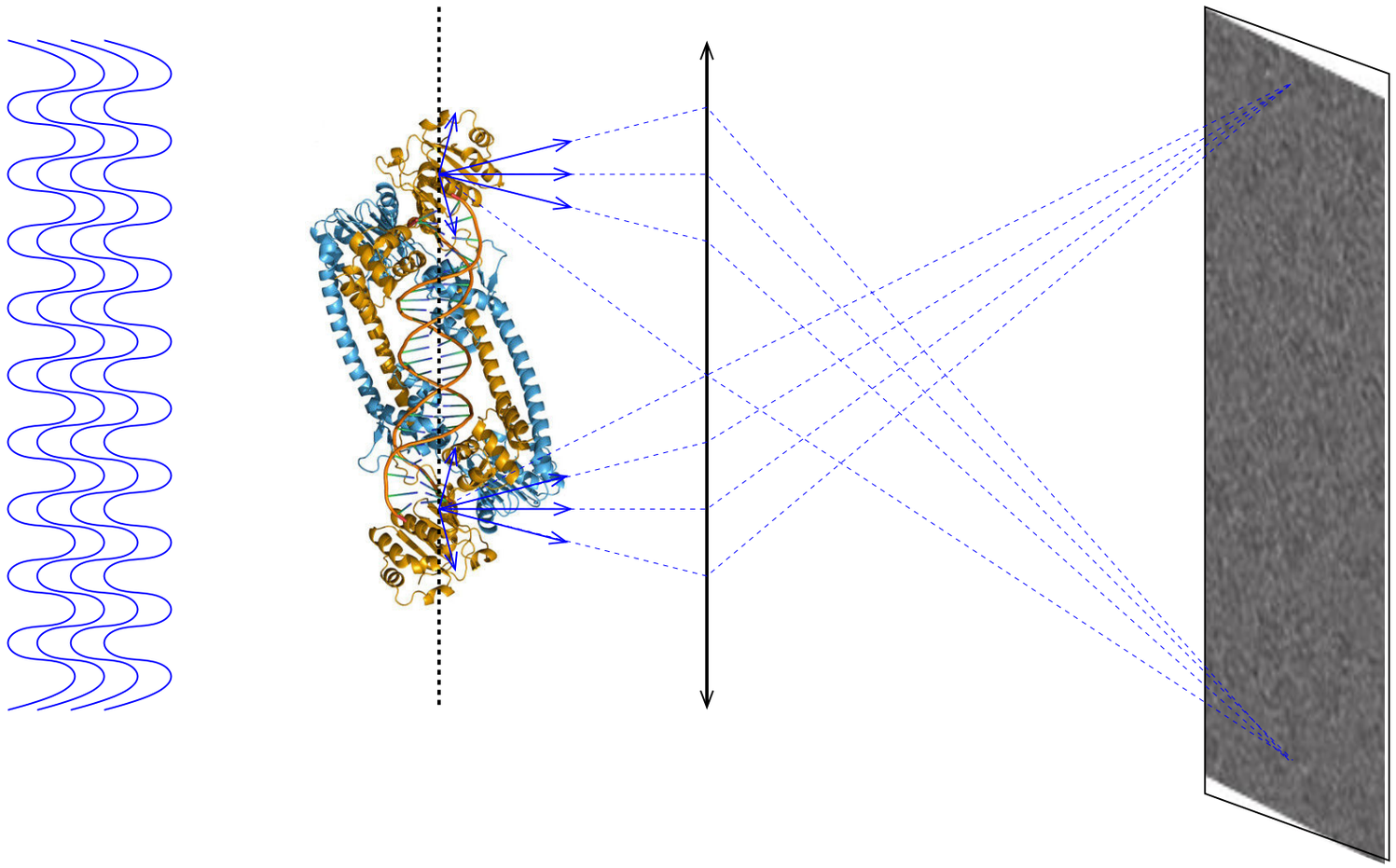
Electron Microscope: Imaging Mode



Plane Wave Object Lense Image Plane (Detector)

Rays of **equal origin** focus on detector

Electron Microscope: Imaging Mode

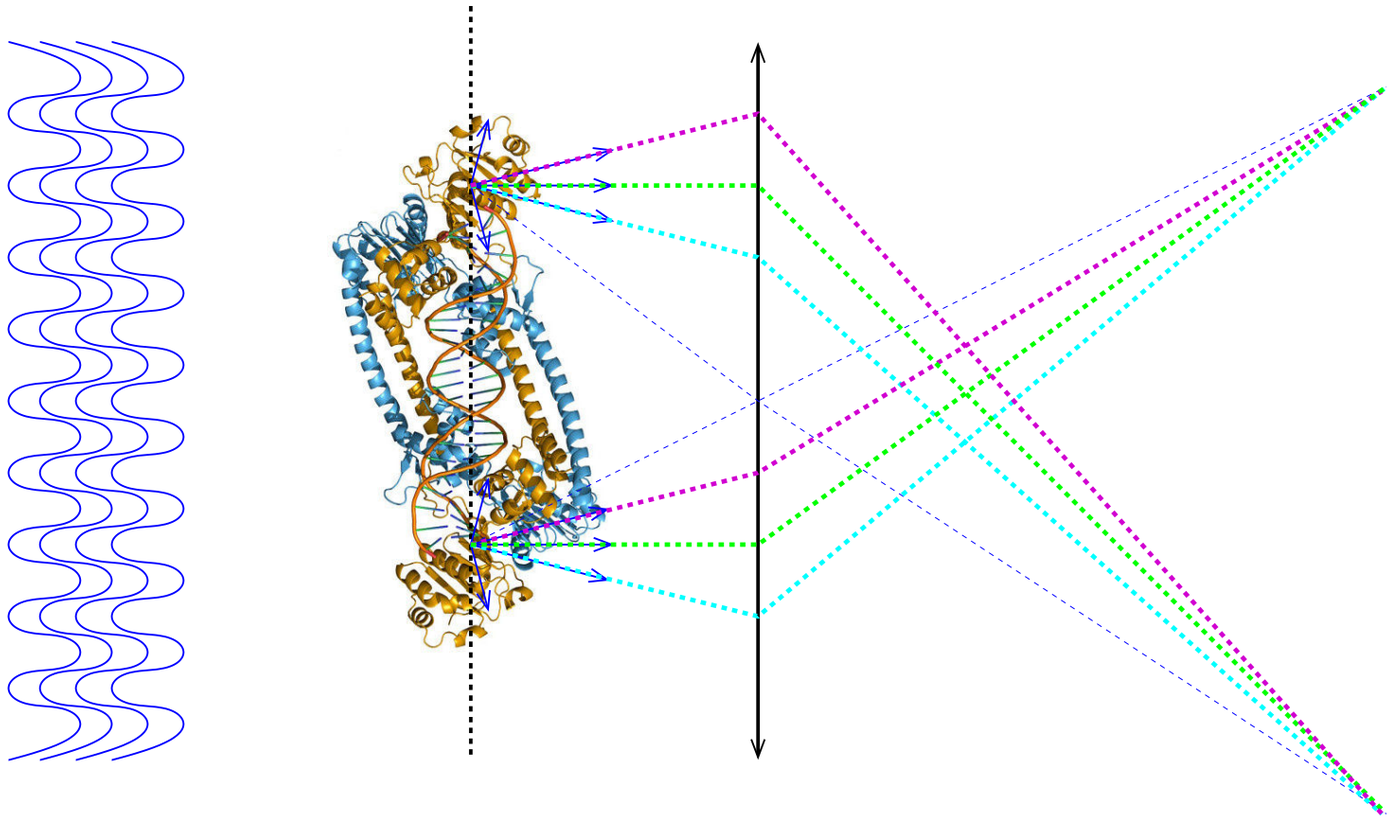


Plane Wave Object Lense Image Plane (Detector)

Detector noise and radiation senstivity require low contrast images

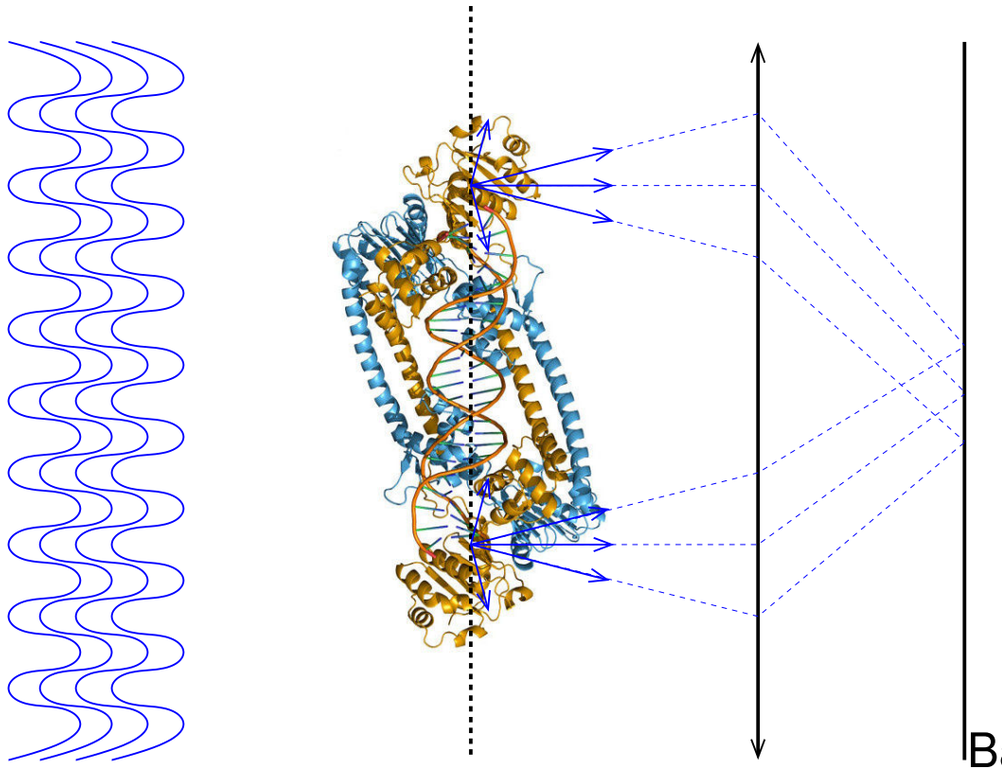
Martinez-Rucobo *et al.* Molecular Cell (2015) 58, 1079–1089

Electron Microscope: Diffraction Mode



Plane Wave Object Lense

Electron Microscope: Diffraction Mode



Plane Wave Object Lense Backfocal Plane

Image at Backfocal Plane =
||Fouriertransform of object||

If object = crystal:

diffraction spots according to Laue condition

Rays of **equal direction** focus on detector

2 - Experimental Considerations

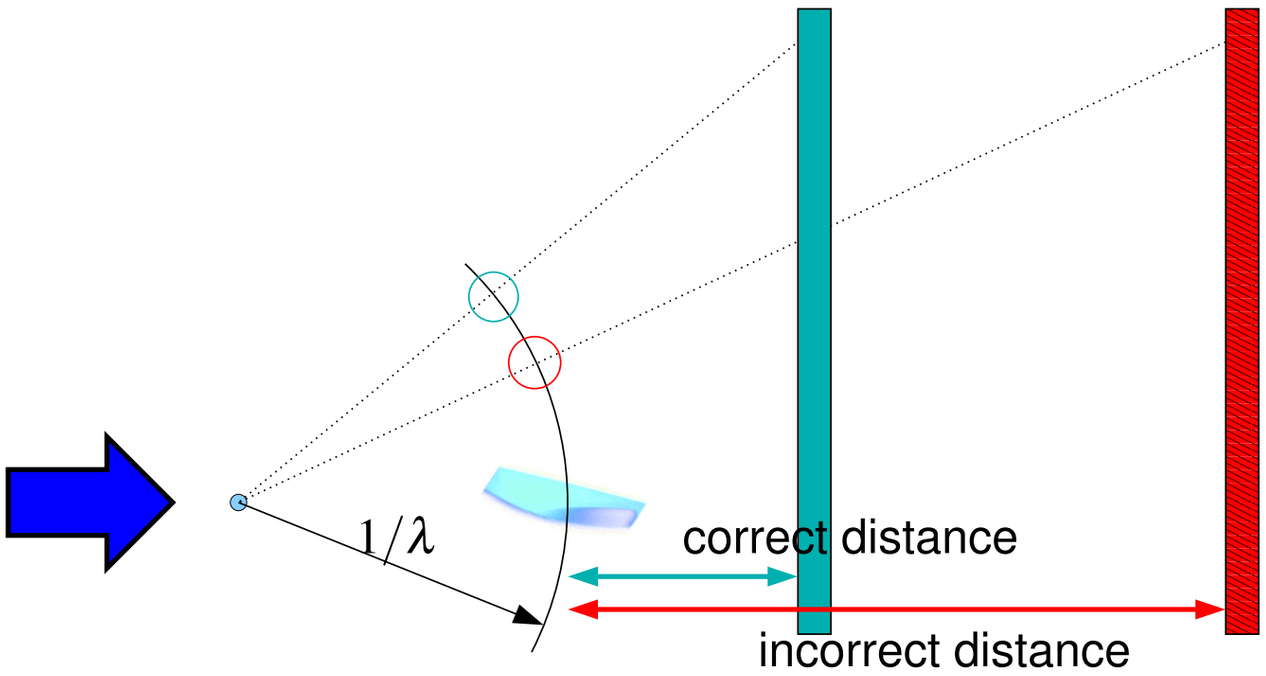
1. Ewald sphere: refinement stability

2. Dynamic scattering

3. Detectors

X–rays: The Ewald Sphere

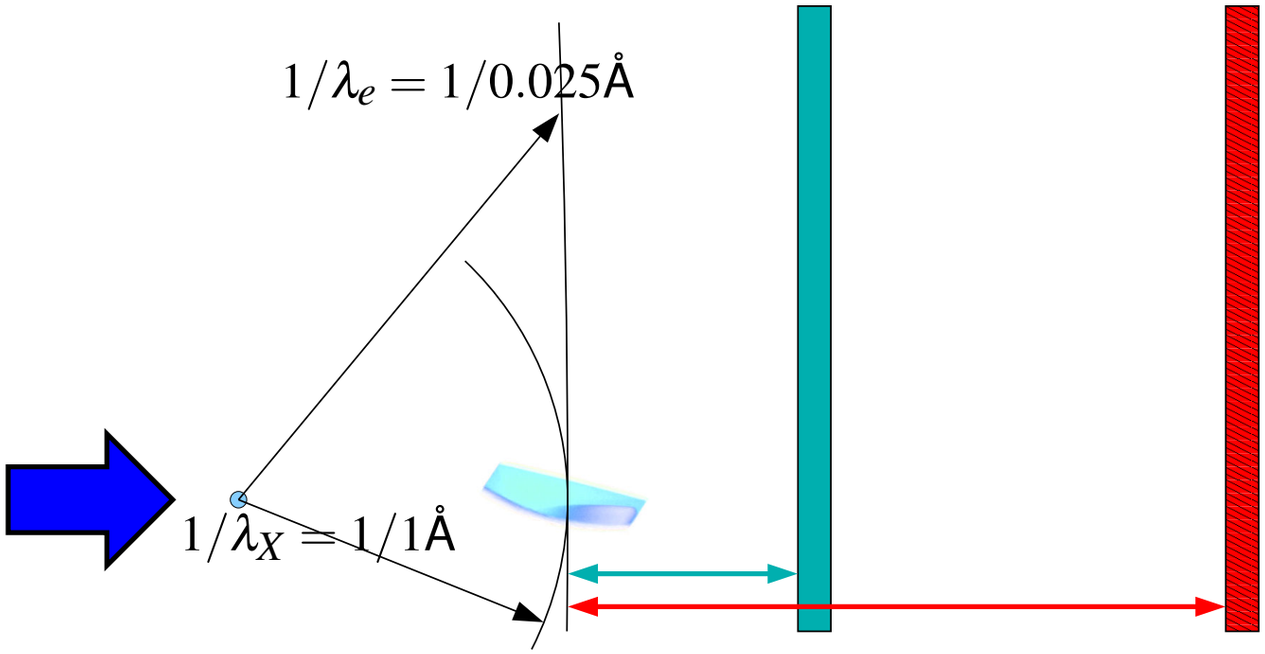
$\lambda = 1$Å, “normal” resolution: $2\theta_{\max} = 40°$



- Assume: wrong detector distance
- Diffraction spot calculated wrongly (red circle)
- Reciprocal lattice becomes distorted

Curvature of the Ewald sphere **gauges** the diffraction geometry

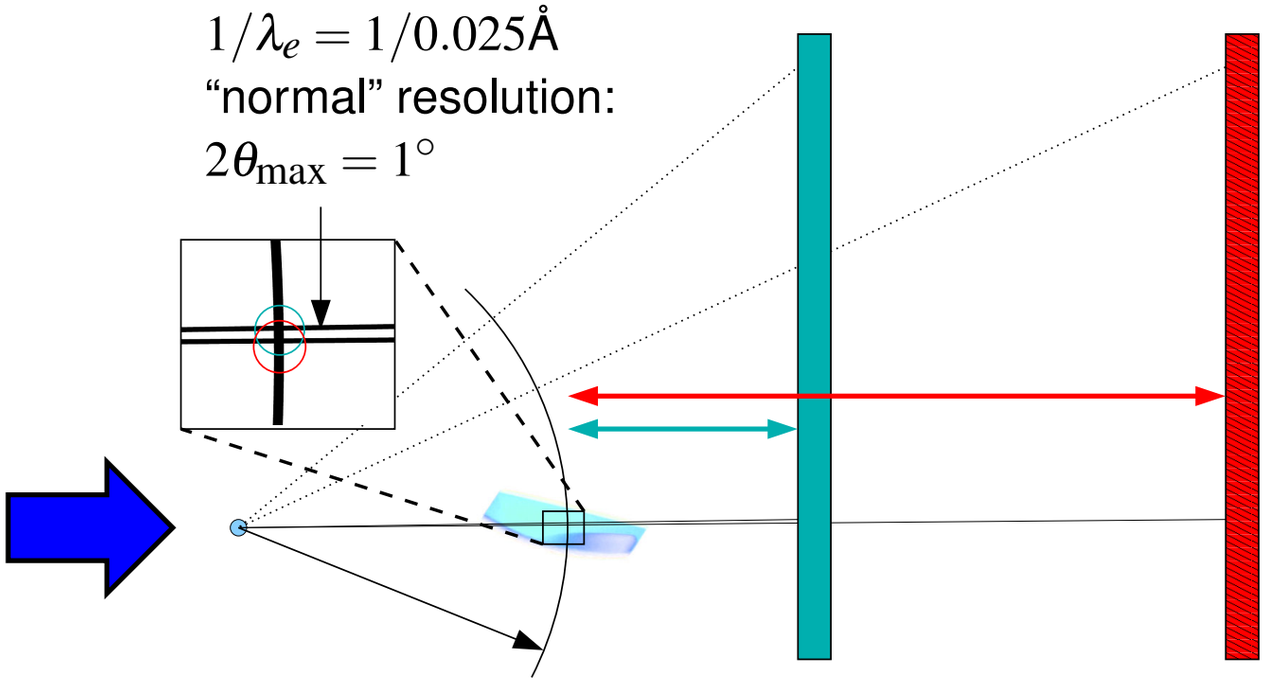
Electrons: The Ewald “Plane”



- Typical X–ray wavelength $\lambda_X = 1$Å
- Typical e^- wavelength $\lambda_e = 0.025$Å
- Radius of Ewald sphere 40x greater
- Ewald sphere nearly flat

Electrons: The Ewald “Plane”

$1/\lambda_e = 1/0.025$Å
“normal” resolution:
$2\theta_{\max} = 1°$



- typical wavelength with X–rays: 1Å
- typical wavelength with electrons: 0.025Å
- opening angle $\leq 1 - 4°$
- Ewald sphere virtually flat
- Without curvature: impossible to refine both detector distance and cell

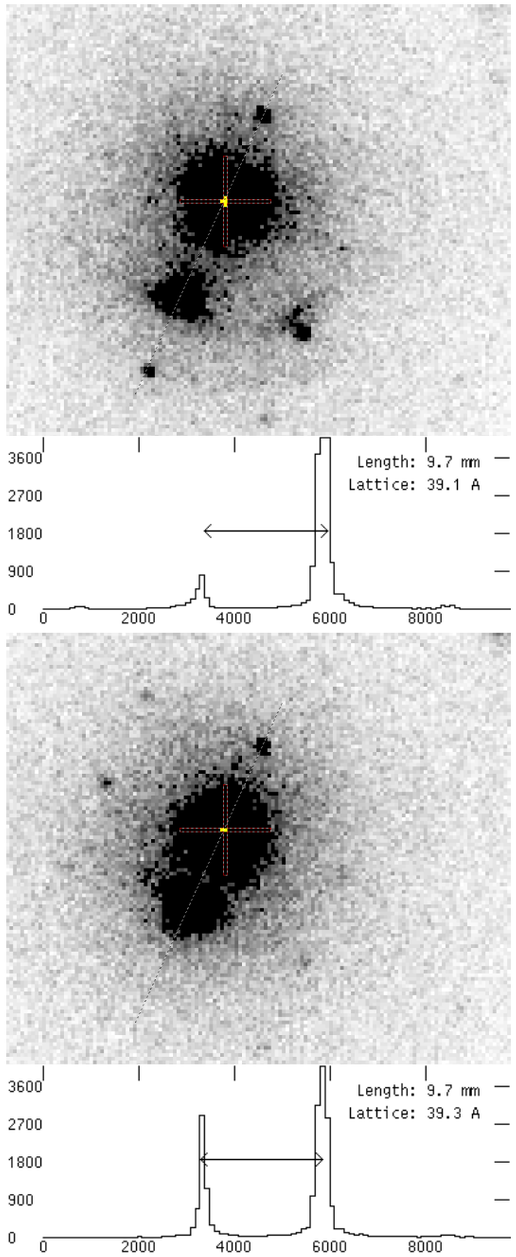
Summary: The Ewald “Plane”

- Detector distance and unit cell parameters are strongly related
- Wrongly set distance can lead to incorrect bond lengths
- Distance refinement with X–ray data routine
- Distance refinement with electron data = unstable
- good: Distance calibration from powder sample
- better: Distance calibration from chemical bond lengths

Dynamic Scattering

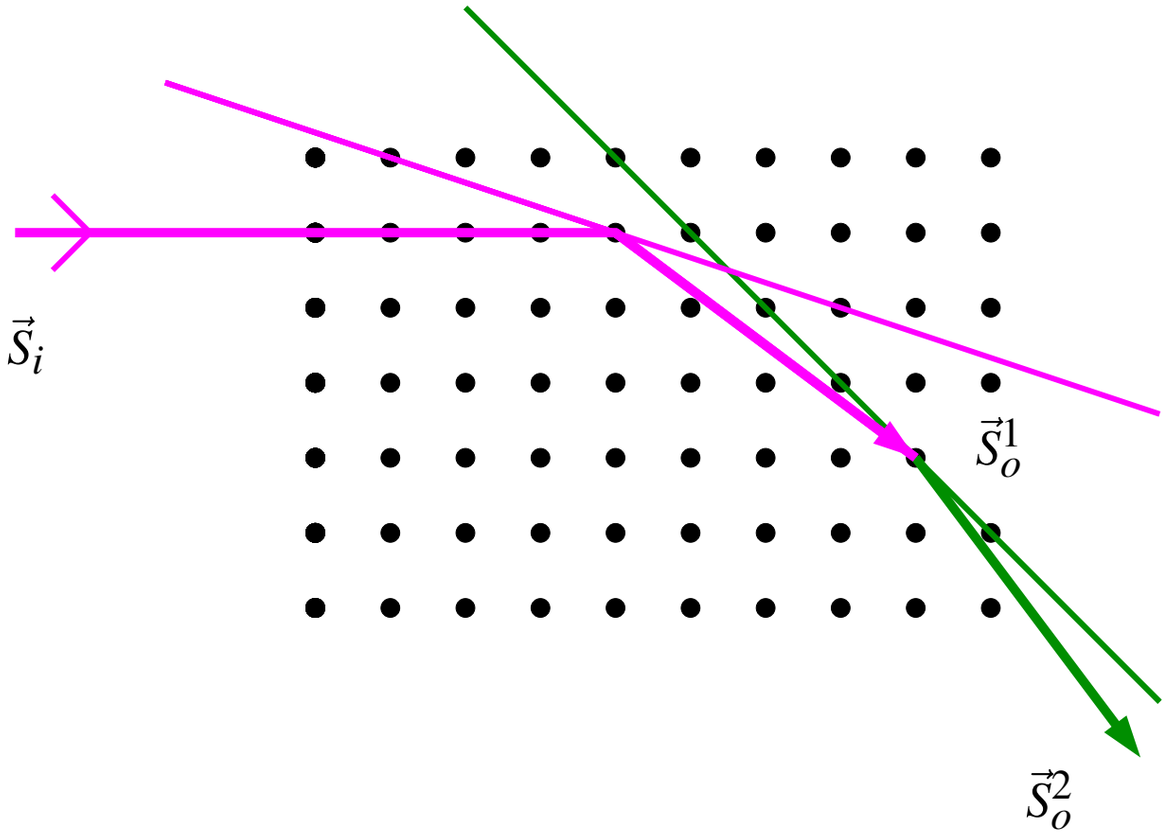
- Kinematic Theory of Diffraction: Every photon / electron / neutron scatters once in the crystal
- $|F_{\text{ideal}}(hkl)| \propto \sqrt{I_{\text{exp}}(hkl)}$
- Dynamic Scattering: Multiple Scattering events occur
- Electron Diffraction: Multiple Scattering occurs even with nanocrystals

Dynamic Scattering



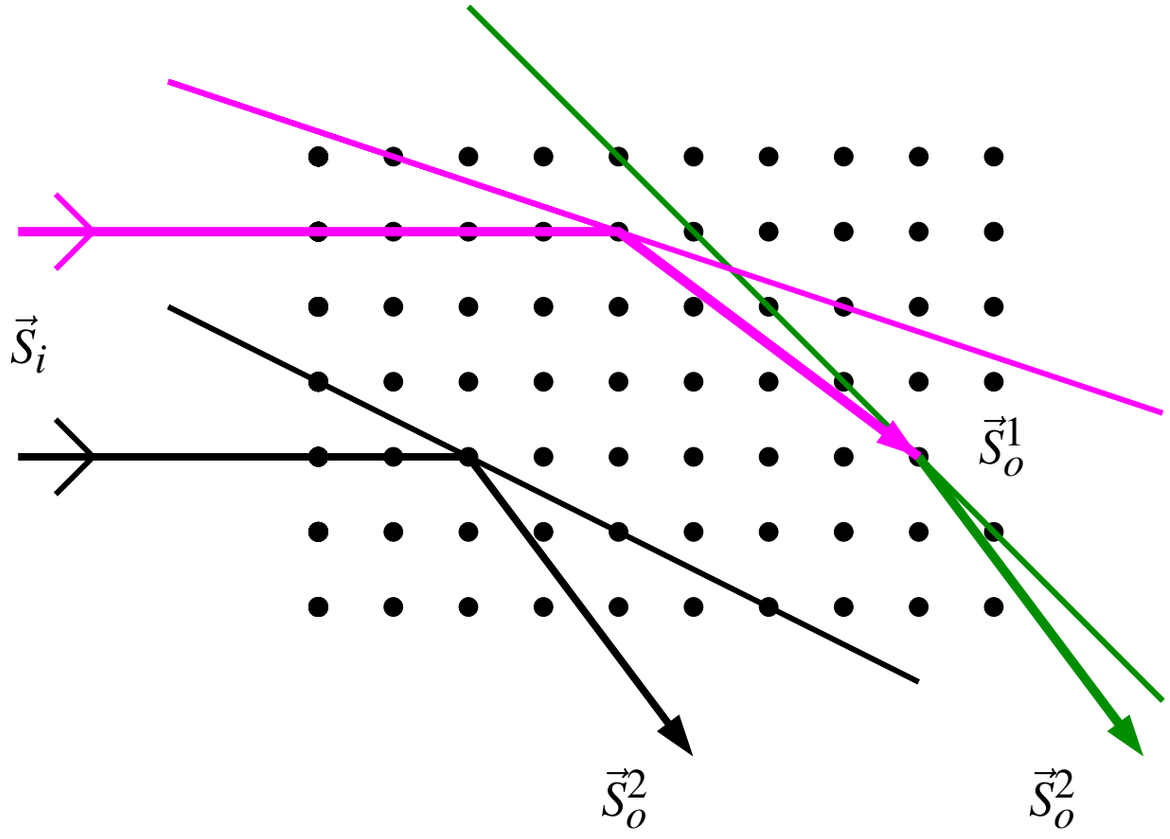
Data from SAPO–34: $I(-2,-1,1) > I_{\text{direct beam}}$ (Eiger chip, 256x256 px)

Multiple (Dual) Scattering



- Outgoing ray $\vec{S}_o^1$ acts as incoming ray for reflection $\vec{S}_o^2$.
- Probability of re–reflection thickness dependent

Multiple (Dual) Scattering



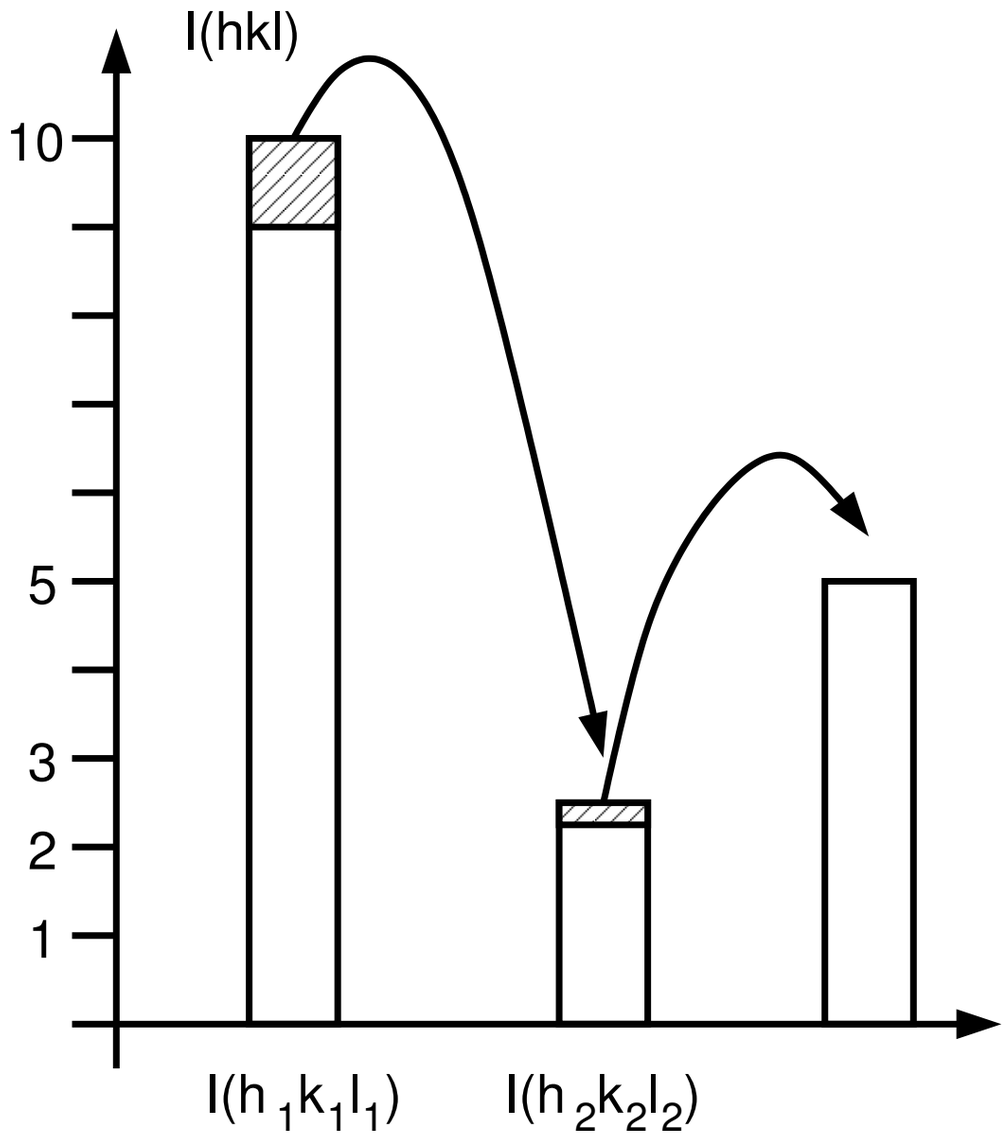
Laue Conditions (accordingly $\vec{b}$ and $\vec{c}$):

$$(\vec{S}_o^1 - \vec{S}_i) \cdot \vec{a} = h_1$$
$$(\vec{S}_o^2 - \vec{S}_o^1) \cdot \vec{a} = h'$$
$$(\vec{S}_o^2 - \vec{S}_i) \cdot \vec{a} = h_1 + h' = h_2$$

Requirement for detrimental effect on $I(h_2, k_2, l_2)$

- $I(h_1 k_1 l_1)$ must be strong
- $I(h', k', l')$ must be strong
- $I(h_2 k_2 l_2)$ must be weak
- $I(h_1 k_1 l_1)$ and $I(h_2 k_2 l_2)$ on same frame

Multiple (Dual) Scattering



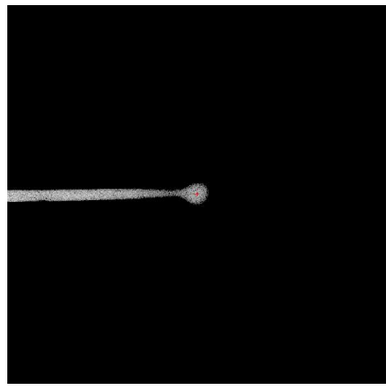
- Re-reflection more likely for thicker crystal(path)
- Percentage similar for all reflections on frame ($2\theta \approx 0$)
- 10% of strong reflection affects weak reflection

$\Rightarrow$ Measured intensities “shifted” from strong to weak

$\Rightarrow$ Low resolution reflection under–, high resolution reflections overestimated

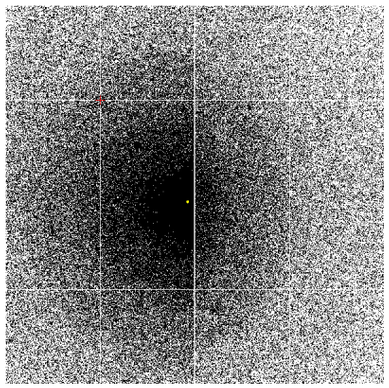
$\Rightarrow$ Covered during refinement by reduced B–factor: electron diffraction includes map–sharpening

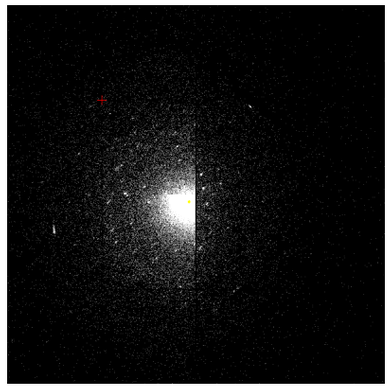
e^- Diffraction Studies with Timepix and Eiger



TVIPS CMOS

$20 \leq I \leq 21$ cts





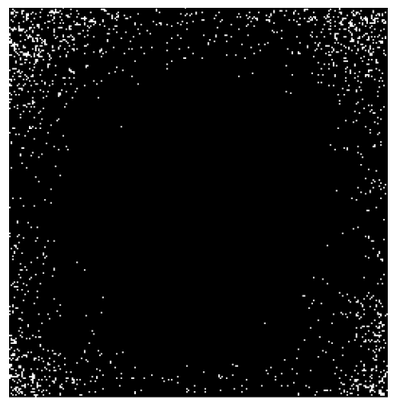
1024x1024 Timepix

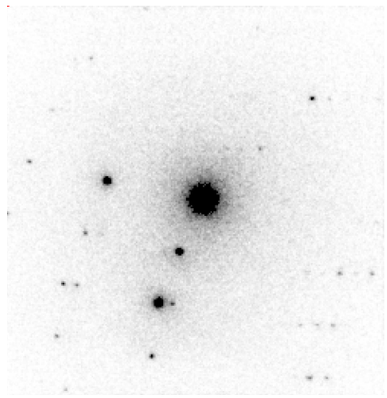
$0 \leq I \leq 1$ ct Lysozyme (inv[d])

$\approx 1kHz, 50\mu\text{m} \times 50\mu\text{m}$

cut–off: 11809

dead time $\approx 0.01s$





256x256 Eiger (PSI)

$0 \leq I \leq 1$ ct SAPO–34 crystal

$\leq 23kHz, 75\mu\text{m} \times 75\mu\text{m}$

cut–off: 16, 64, or 4096 (4, 8, 12 bit)

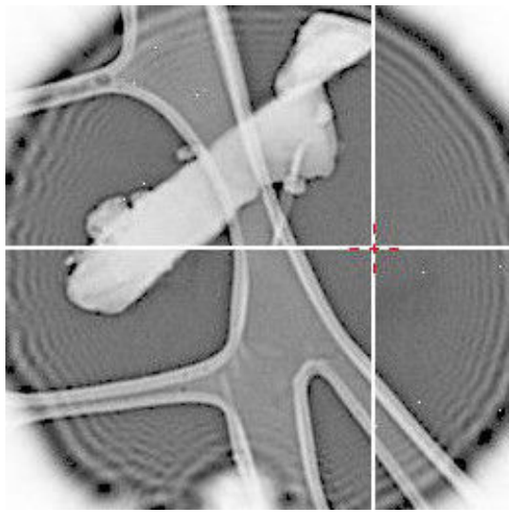
dead time 3μs

3 - Structures

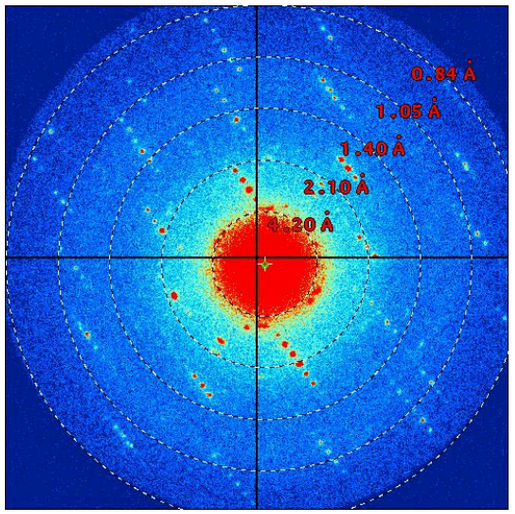
Pharmaceutical I: Visualisation of Hydrogen Atoms

H–atom positions can be refined against electron diffraction data
CCDC: IRELOH, Dai et al., Eur. J. Org. Chem (2010), 6928-6937

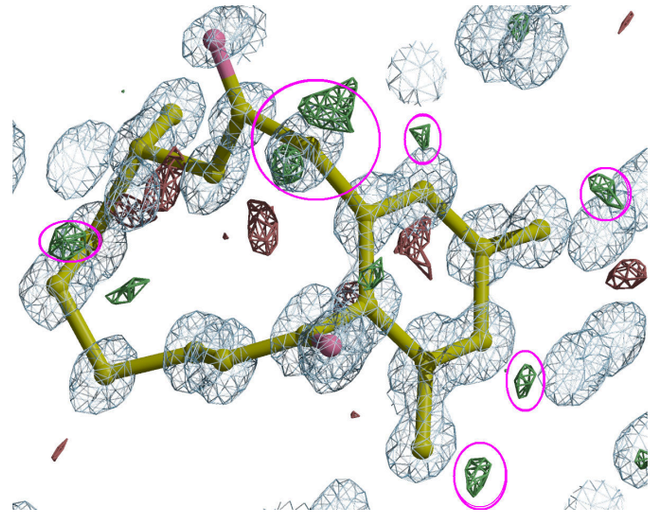
Sample courtesy Novartis



- Field of view: $3\mu m$
- Crystal: $1.6\mu m \times 400nm$



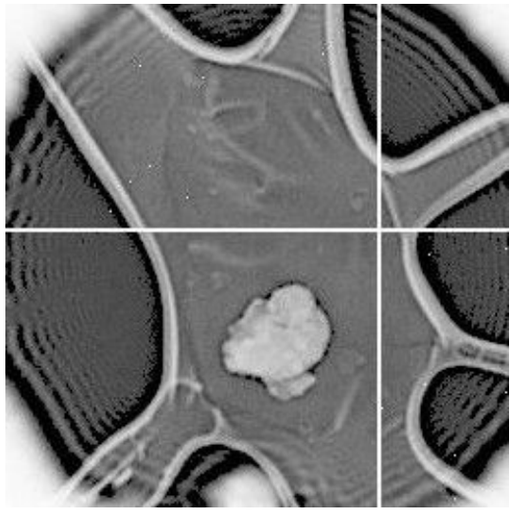
- $d_{\text{min}} < 0.8$Å
- $P2_12_12_1$: 85% completeness with 3 crystals
- a=8.06Å b=10.00Å c=17.73Å



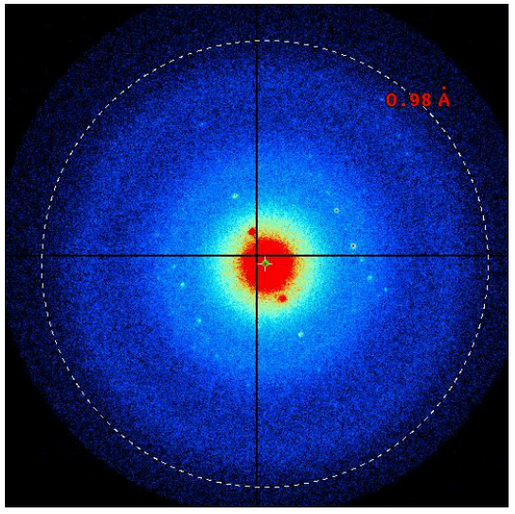
- **Hydrogen atoms** in difference map even with poor model
- 1334 reflections, 195 parameters, 156 restraints (RIGU)
- $R1 = 15.5\%, R_{\text{complete}} = 18.5\%$

Pharmaceutical II: Differentiation of Atom Types

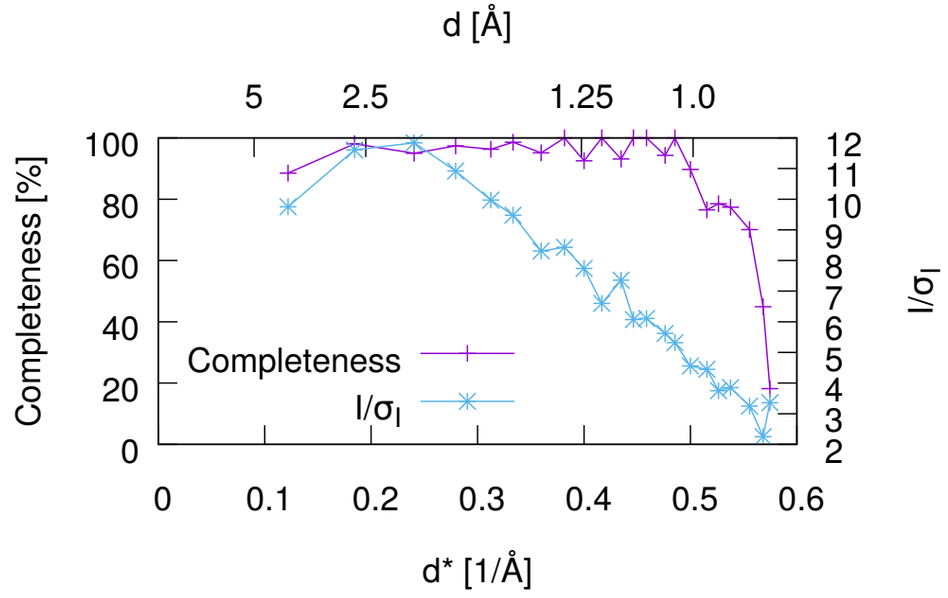
Data quality: recognition of atom types, C *vs.* O *vs.* N *etc.* (CCDC: EPICZA)



- Field of view: $3\mu m$
- Crystal: $400nm$ diameter

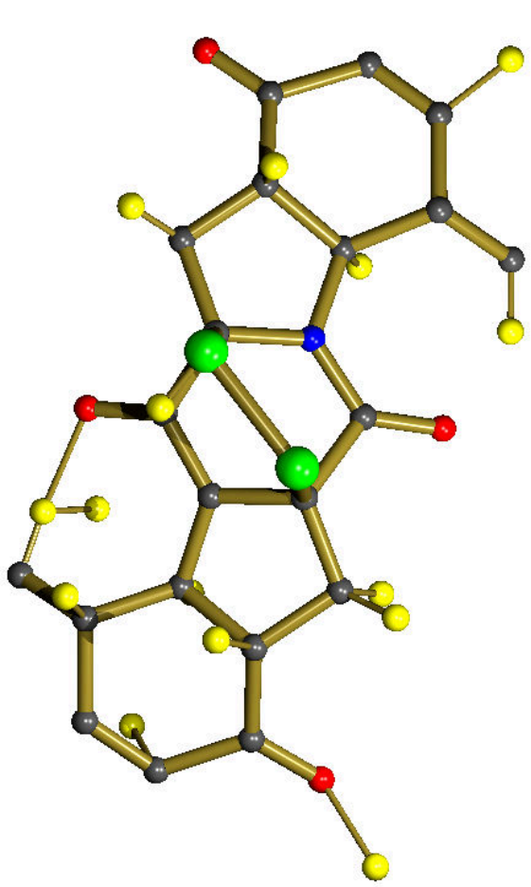


- $d_{\text{min}} = 0.87$Å
- a=11.35Å, b=12.7Å, c=13.0Å
- $P2_12_12_1$: completeness with 4 crystals: 86%

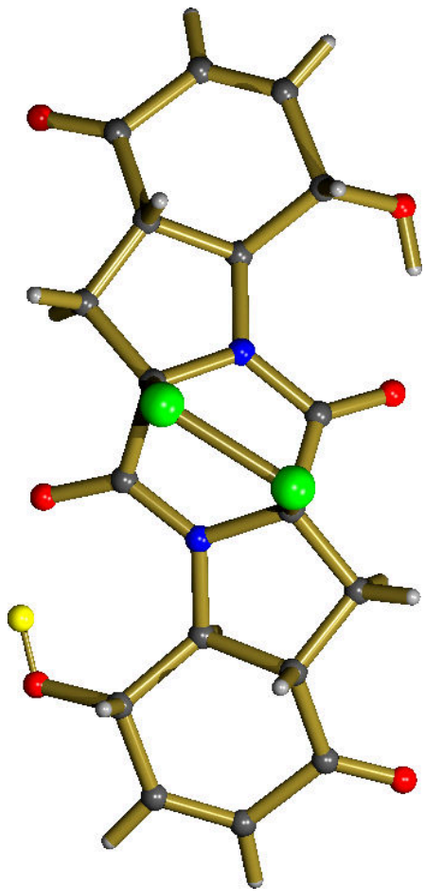


- 2545 refl., 258 param., 267 restraints (RIGU)
- all data: $R1 = 15.9\%, R_{\text{complete}} = 19.1\%$
- $R1 = 14.7\%, R_{\text{complete}} = 18.0\%$

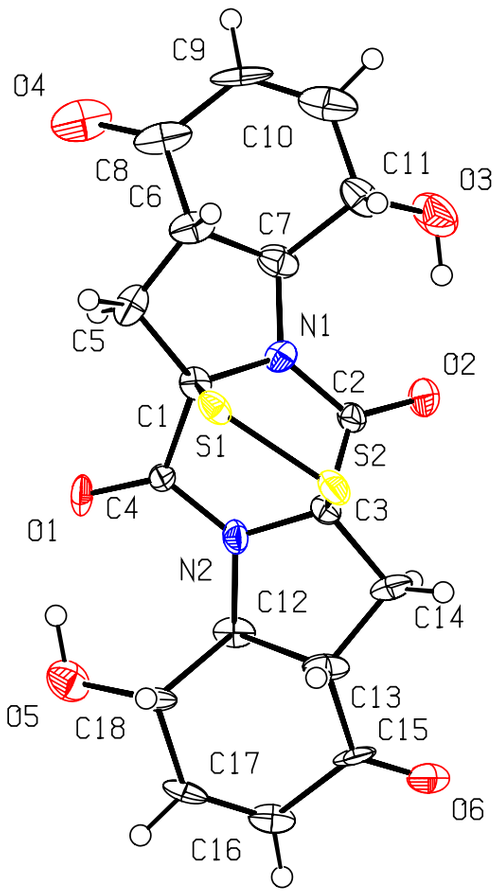
Pharmaceutical II (EPICZA): Structure Solution Process



Direct methods reveal H atoms
=data quality



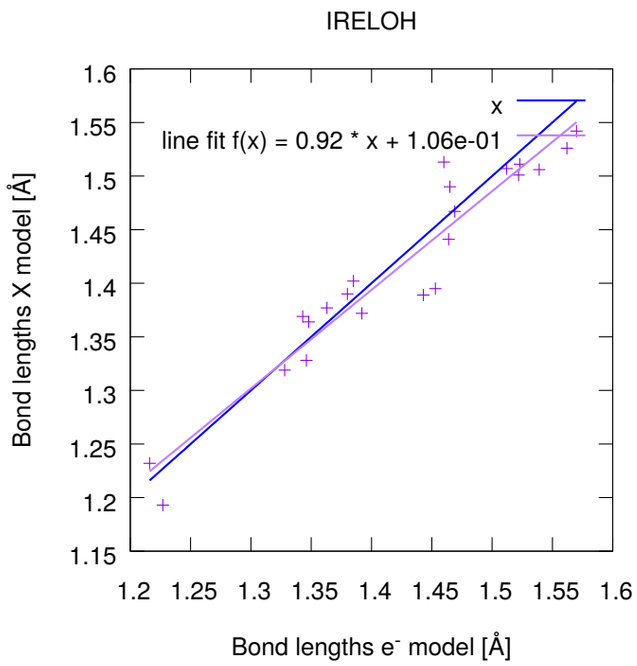
HFIX: all except 1 H
=model quality

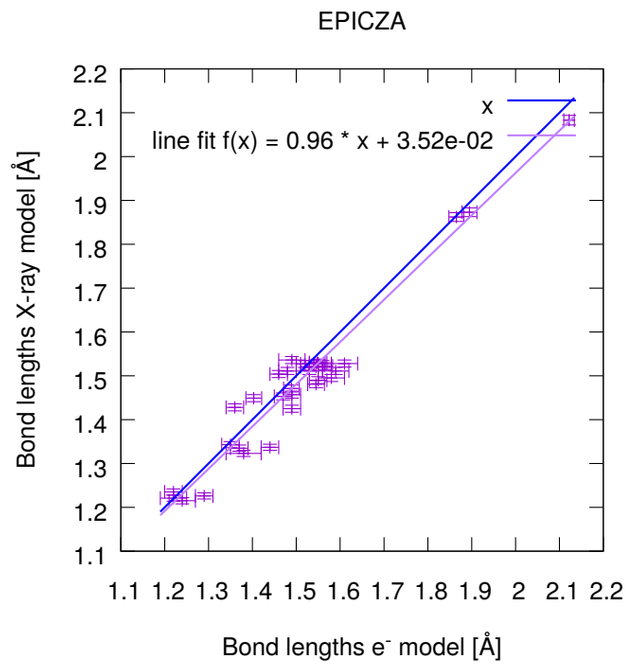


Final Structure

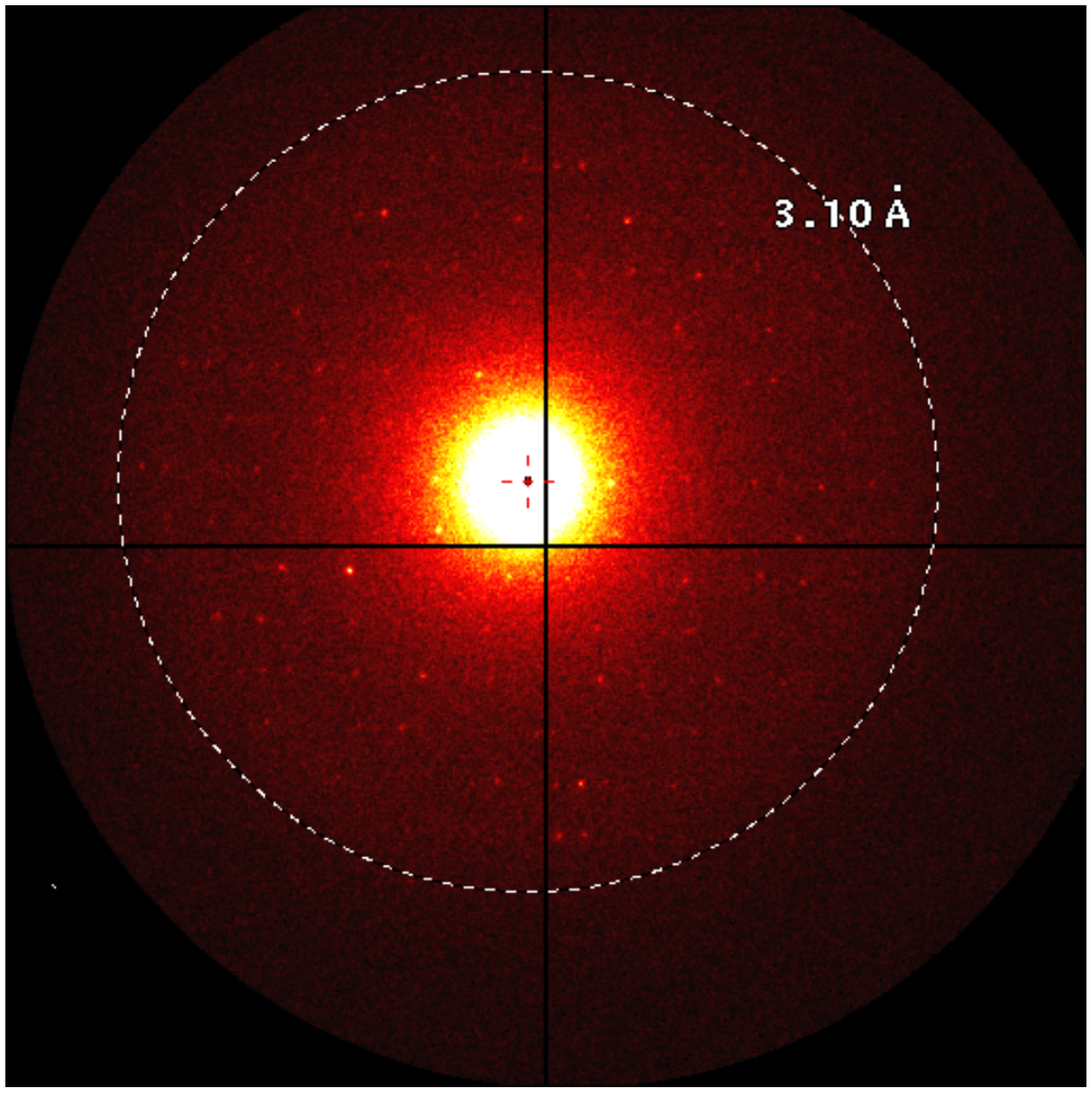
Summary: Electron Diffraction of Organic Compounds

- Structures can be solved with X–ray knowledge and methods.
- Radiation damage present, but not (always) limiting
- Kinematic approximation sufficient for high quality structures

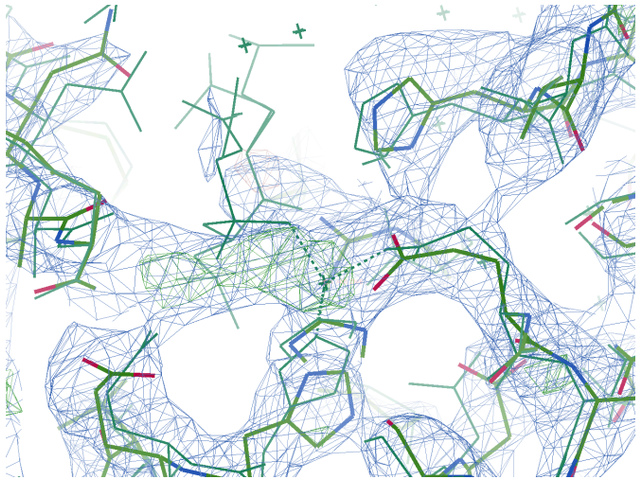




Thermolysin (sample courtesy Ilme Schlichting)



- Spacegroup $P6_122$
- Unit Cell 94.3 94.3 130.4 90° 90° 120°
- $d_{\text{min}} = 3.5$Å
- 72.4% completeness
- MR with 3DNZ poly Alanine: TFZ=26.4, LLG=433
- Buccaneer: side chain extension 315/316
- Refmac5: R1/“Rfree” = 28.0% / 29.9% (4N5P w/o water)



Summary: Electron Diffraction of Proteins

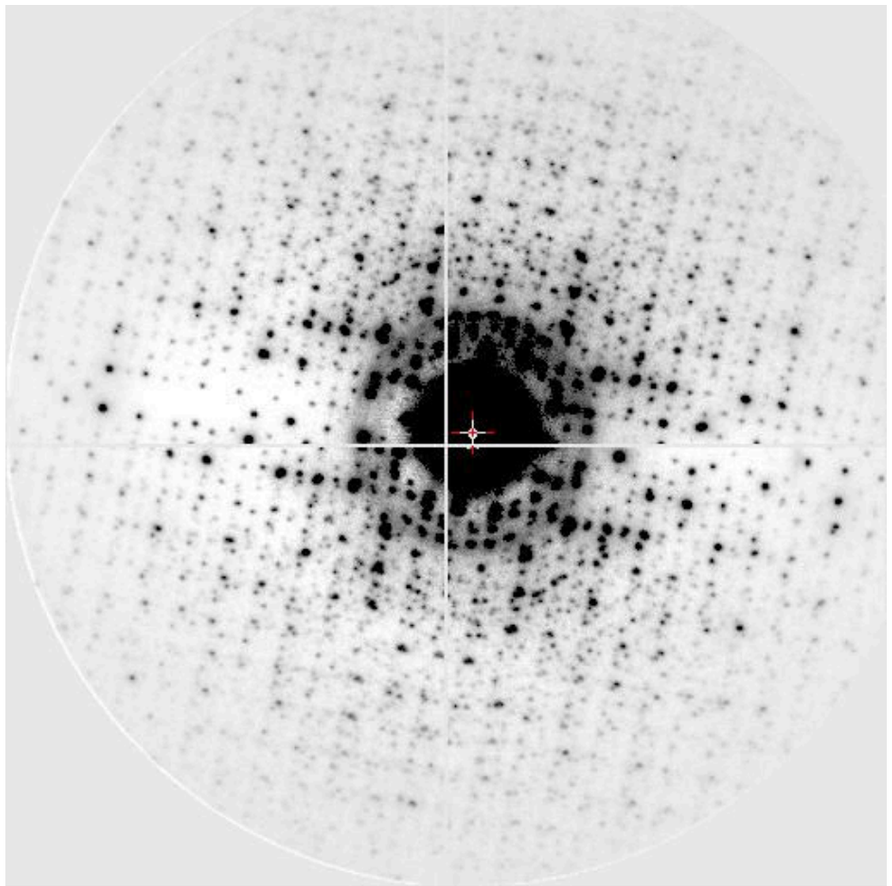
- Structures can be solved with X–ray knowledge and methods.
- Kinematic approximation sufficient for high quality structures
- Radiation damage major limit
- Possibility: “serial” electron crystallography

4 - Crystallographic Lens Corrections

Garnet Andradite

- The garnet Andradite, $Ca_3Fe_2{}^{3+}(SiO_4)_3$, radiation hard
- 2 grids courtesy Xiaodong Zou (Stockholm)
- Space group $Ia\bar{3}d$, $a = 12.06314(1)$Å (ICSD No. 187908)

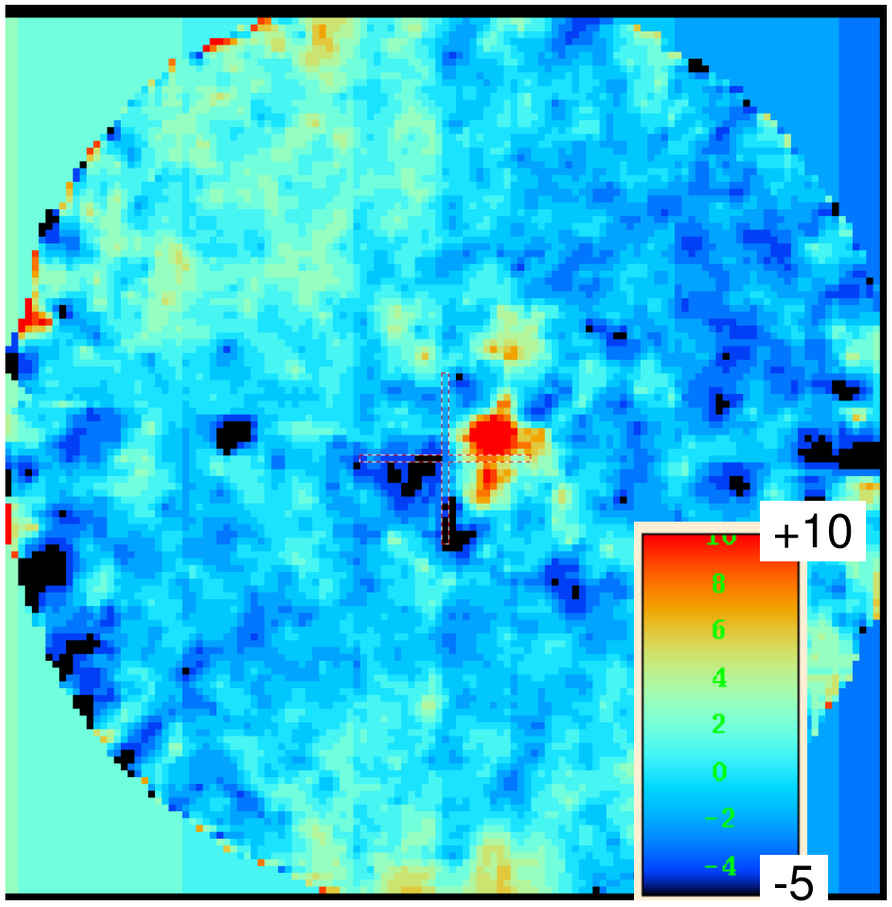
(Wikipedia)



- Summed images from Garnet (200keV)
- 66.8° rotation
- good coverage of detector surface

Spatial Correction for the Detector Surface

XDS Correction Table X–coordinate



- Spot positions determined through Laue Conditions

$$\vec{S}.\vec{a} = h$$
$$\vec{S}.\vec{b} = k$$
$$\vec{S}.\vec{c} = l$$

- Deviations between calculated and observed positions
- per–pixel look-up tables for X– and Y–coordinates
- **Independent** of Source of Error

Directly Visible Improvements

Garnet Data set processed before spatial correction:

BEAM_DIVERGENCE: 0.16°
REFLECTING_RANGE: 0.47°

Garnet Data set processed after spatial correction:

BEAM_DIVERGENCE: 0.15°
REFLECTING_RANGE: 0.28°

Improved Cell Accuracy with Look–up Tables

1. Collect data from garnet
2. Change as little as possible
3. Collect data from target sample
4. Process using garnet correction tables

Sample Courtesy Roche $C_{31}H_{29}Cl_2F_2N_3O_4$**, SG** $P2_1$

Data Collection and Processing: **Max Clabbers**

	a	b	c	α	β	γ
XRPD	6.405	18.206	25.829	90.000	92.180	90.000
XDS uncorrected	6.556	18.728	26.276	90.500	92.243	90.540
XDS corrected	6.564	18.721	26.254	90.064	92.171	90.137

5 - Data Processing

Integration with XDS

XDS (W. Kabsch, K. Diederichs) for data integration and scaling

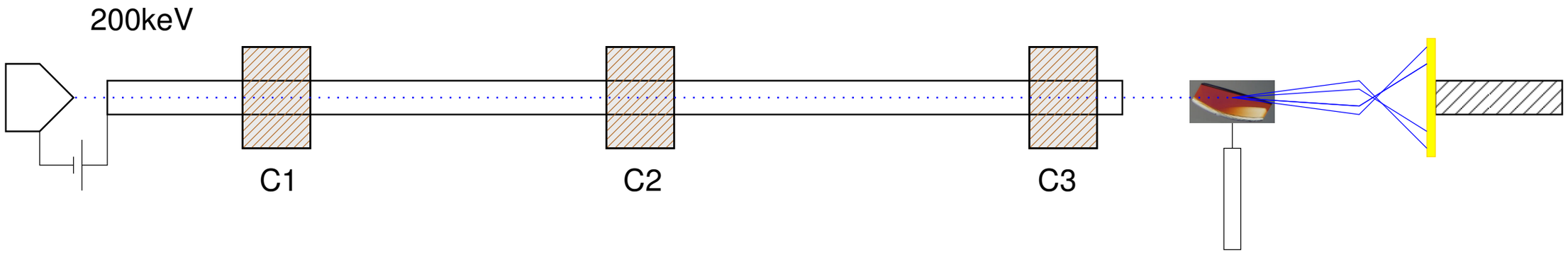
- Profile fitting: extraction of weak data
- Versatile geometry
- Detector segmentation
- Refinement of experimental params:
  1. Detector distance | Unit cell
  2. Cell orientation
  3. (Beam position)
  4. Rotation axis
  5. Reflecting range

```
REFINED VALUES OF DIFFRACTION PARAMETERS DERIVED FROM   1134 INDEXED SPOTS
REFINED PARAMETERS:   AXIS BEAM ORIENTATION CELL
STANDARD DEVIATION OF SPOT    POSITION (PIXELS)      0.89
STANDARD DEVIATION OF SPINDLE POSITION (DEGREES)     0.34
SPACE GROUP NUMBER      1
UNIT CELL PARAMETERS       8.175    17.501    10.047  90.545  89.139  89.205
E.S.D. OF CELL PARAMETERS  3.1E-02 2.0E-02 2.6E-02 1.9E-01 2.4E-01 1.3E-01
REC. CELL PARAMETERS   0.122354  0.057149  0.099544  89.443  90.869  90.804
COORDINATES OF UNIT CELL A-AXIS     4.389     0.564    -6.874
COORDINATES OF UNIT CELL B-AXIS    12.754    -9.682     7.061
COORDINATES OF UNIT CELL C-AXIS    -4.384    -8.268    -3.657
CRYSTAL MOSAICITY (DEGREES)     0.646
LAB COORDINATES OF ROTATION AXIS  0.998477  0.054854  0.005969
DIRECT BEAM COORDINATES (REC. ANGSTROEM)   0.003595  0.005733 39.872410
DETECTOR COORDINATES (PIXELS) OF DIRECT BEAM     256.80    257.27
DETECTOR ORIGIN (PIXELS) AT                      256.00    256.00
CRYSTAL TO DETECTOR DISTANCE (mm)       485.00
LAB COORDINATES OF DETECTOR X-AXIS  1.000000  0.000000  0.000000
LAB COORDINATES OF DETECTOR Y-AXIS  0.000000  1.000000  0.000000
```

Downstream of Processing

- Scaling: part of XDS (single data set)
- Merging: XSCALE (no overfitting of σ's), sadabs (higher outlier rejection)
- SHELXT / SHELXD unmodified
- Phaser / Buccaneer unmodified
- Refinement:
  - SHELXL (electron scattering factors *e.g.* Peng *et al.* (1996))
  - Refmac5: `SOURCE ELECTRON MB` (Mott-Bethe); `MAPC FREE EXCLUDE`
- in principle like D. Dorset (1992), but easier and faster

6 - Conclusions



- Horizontal tube: better space, more stable goniometer
- Fixed Voltage
- Thin tube
- C3–lens system: parallel beam
- 360° Vertical Goniometer (precision, contact cooling)
- Detector moveable

7 - Acknowledgements

- Prof. J. P. Abrahams, Dr. E. van Genderen, M. Clabbers, Dr. T. Blum, C. Borsa, J. Heidler, Dr. R. Pantelic
- Novartis (Compounds)
- Roche (Compounds)
- Dr. I. Nederlof, ASI (Medipix / Timepix)
- Dr. B. Schmitt, PSI Detector group
- Prof. K. Diederichs (XDS)
- Dr. W. Kabsch (XDS)
- Dr. D. Waterman (DIALS)
- Prof. J. van Bokhoven, ETH Zürich
- Prof. X. Zou and S. Hovmöller, University Stockholm (garnet grids)