PAUL SCHERRER INSTITUT

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

The Rotation Method in Electron Crystallography

DGK Meeting
29th March 2017

1 - The Rotation Method

- Zou, Hovmöller, Oleynikov, “Electron crystallography - Electron microscopy and electron diffraction”, IUCr texts on crystallography 16, Oxford University Press (2011)

- Methods: ADT3D (2007; Gorelik *et al.*, Acta Cryst. B68 (2012)), RED (2013) (Wan *et al.*, J Appl. Cryst 46 (2013))

- **here:** Arndt & Wonacott (1978), “The rotation method in crystallography”, Amsterdam Press (1977);
  - suitable for medium to large unit cells
  - no beam precession
  - single axis, continuous rotation
  - shutterless read–out

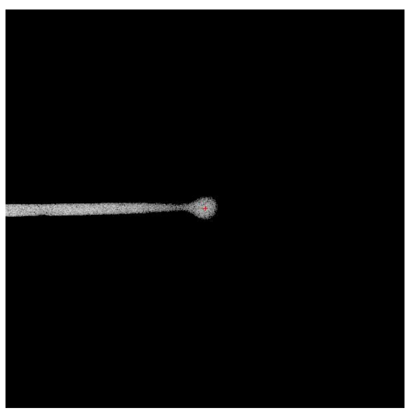
2 - Our Instrumentation

Instrumentation available at C–CINA, University Basel

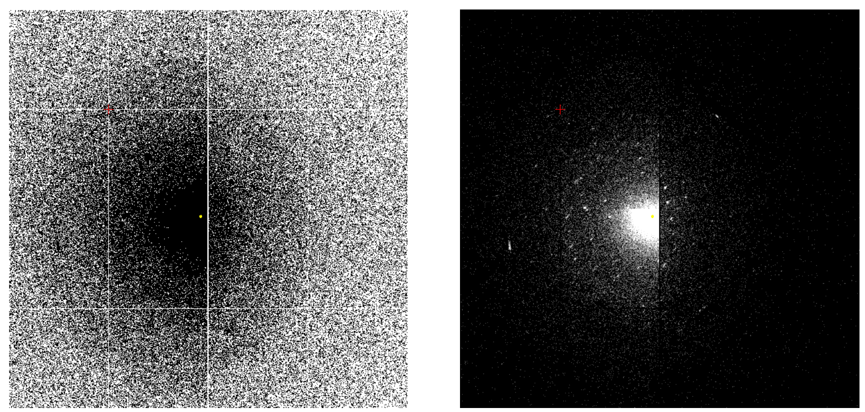
1. FEI Polara, 200keV, 512x512 Timepix detector, single axis $\pm 30^\circ$
2. FEI Talos, 200keV, 1024x1024 Timepix detector, single axis $\pm 40^\circ$

3 - Hybrid Pixel Detectors

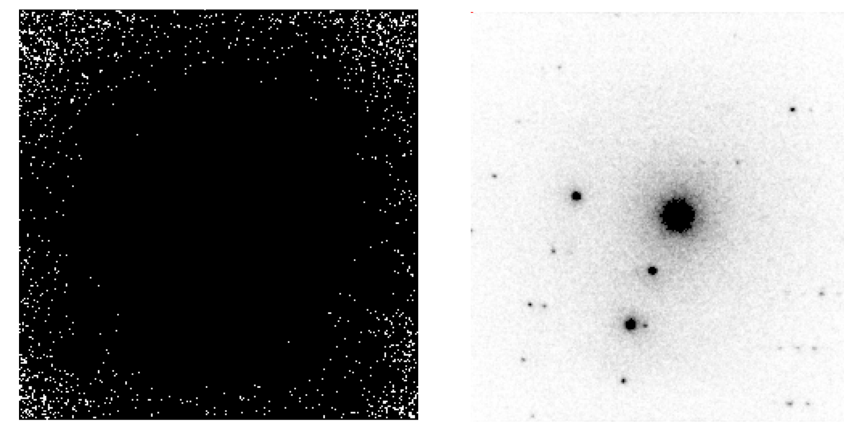
e^- Diffraction Studies with Timepix and Eiger



TVIPS CMOS
$20 \le I \le 21$ cts



1024x1024 Timepix
$0 \le I \le 1$ ct   Lysozyme (inv<u>d</u>)
$\approx 1kHz, 50\mu$m $\times$ 50μm
cut–off: 11809
dead time $\approx 0.01s$



256x256 Eiger (PSI)
$0 \le I \le 1$ ct   SAPO–34 crystal
$\le 23kHz, 75\mu$m $\times$ 75μm
cut–off: 16, 64, or 4096 (4, 8, 12 bit)
dead time 3μs

4 - 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

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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
```

Dowstream of Processing

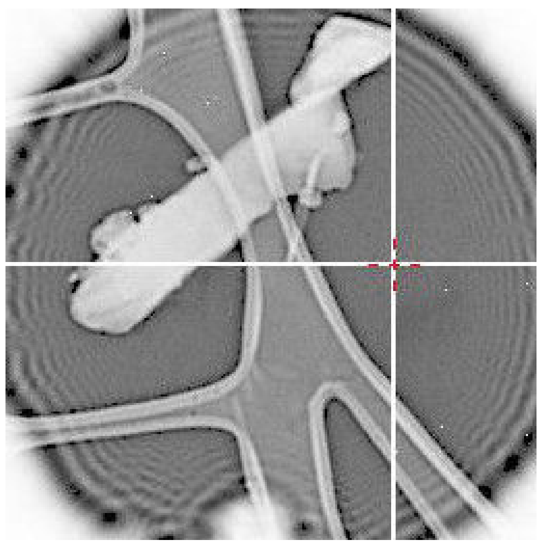
- Scaling: part of XDS (single data set)
- Merging: XSCALE (no overfitting of σ's), sadabs (higher outlier rejection)
- SHELXT / SHELXD unmodified
- Refinement: SHELXL (electron scattering factors *e.g.* Peng *et al.* (1996))
- in principle like D. Dorset (1992), but easier and faster

5 - Structures

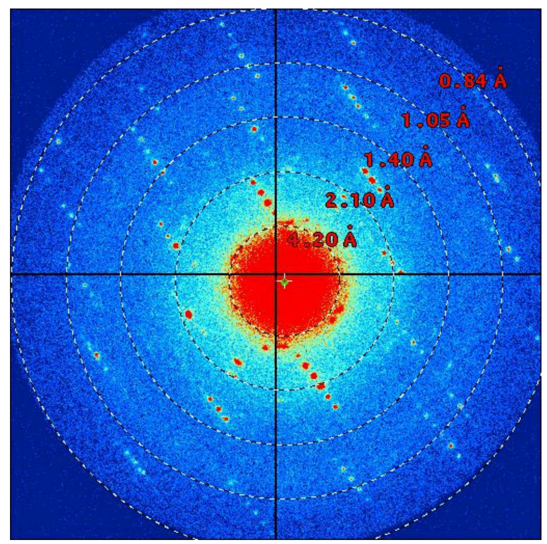
Pharmaceutical I: Visualisation of Hydrogen Atoms

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

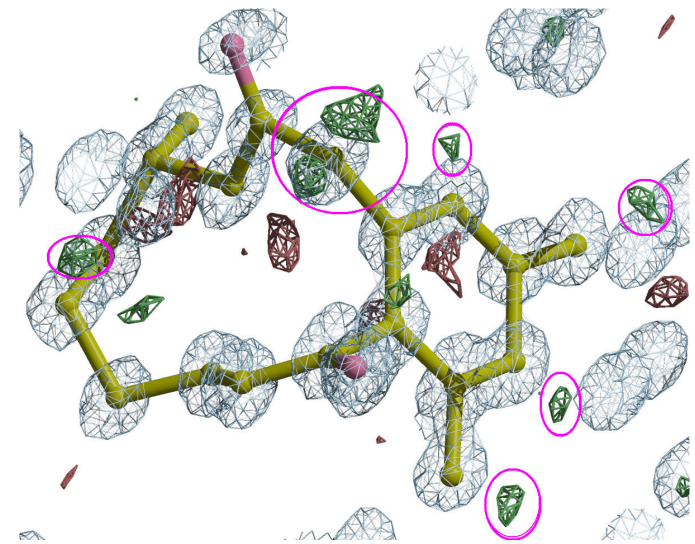
Sample courtesy Novartis



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



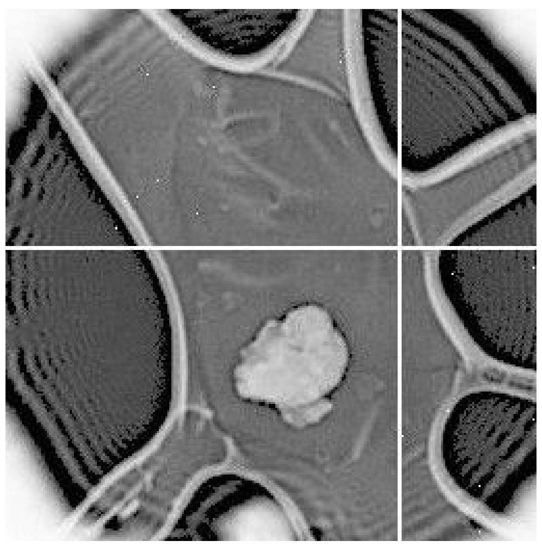
- $d_{\text{min}} < 0.8$Å
- $P2_12_12_1$: 85% completeness with 3 crystals
- a=8.06Å b=10.00Å c=17.73Å

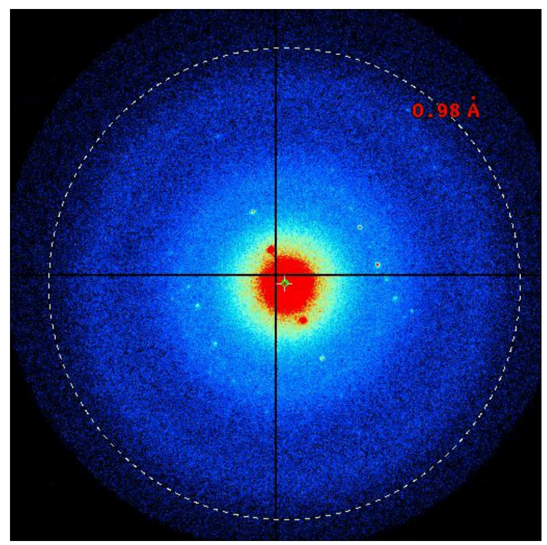


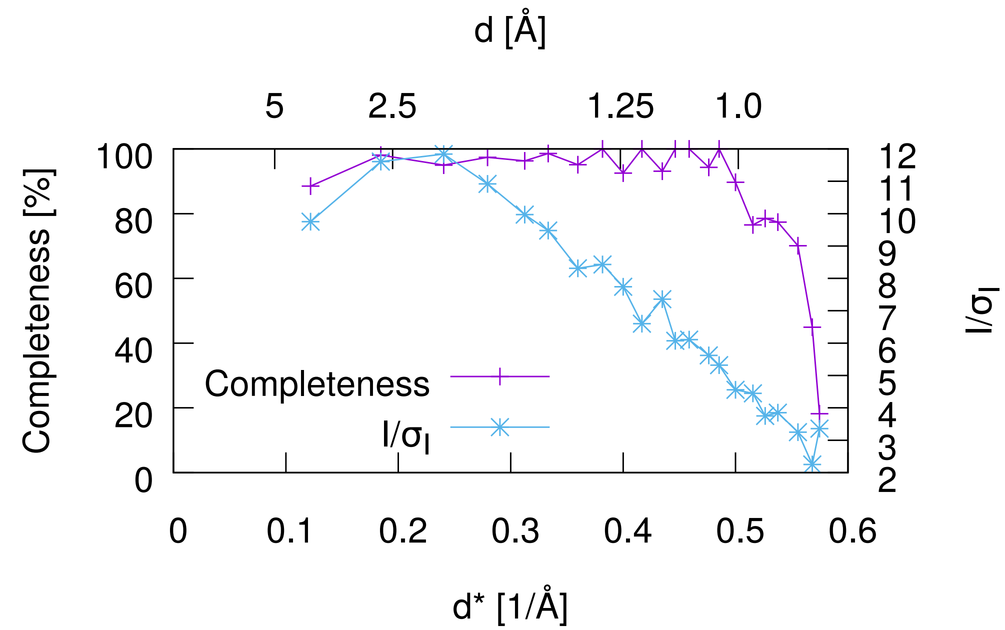
- **Hydrogen atoms** in difference map even with poor model
- 1334 reflections, 195 parameters, 156 restraints (RIGU)
- $R1 = 15.5\%, R_{\text{complete}} = 18.5\%$

Pharmaceutical II: Differentiation of Atom Types

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





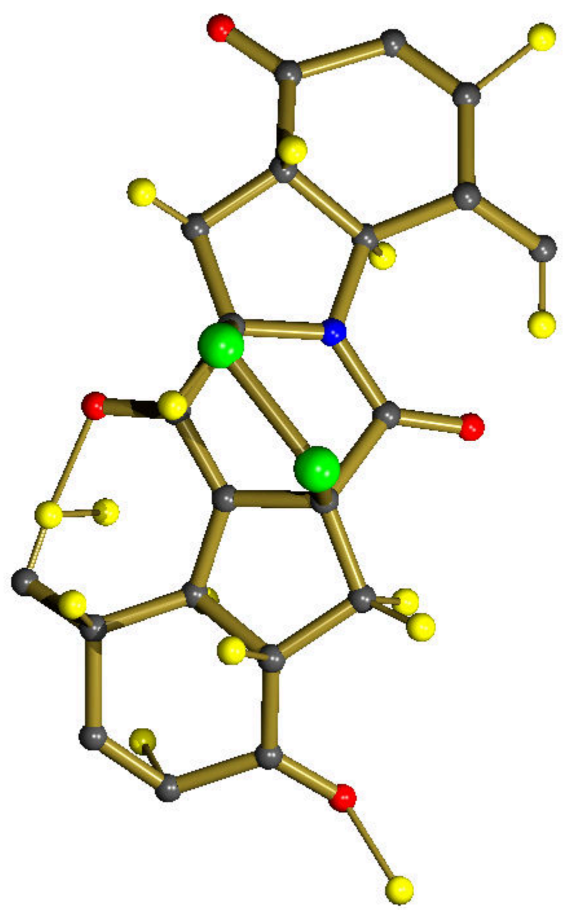


- Field of view: $3\mu m$
- Crystal: $400nm$ diameter

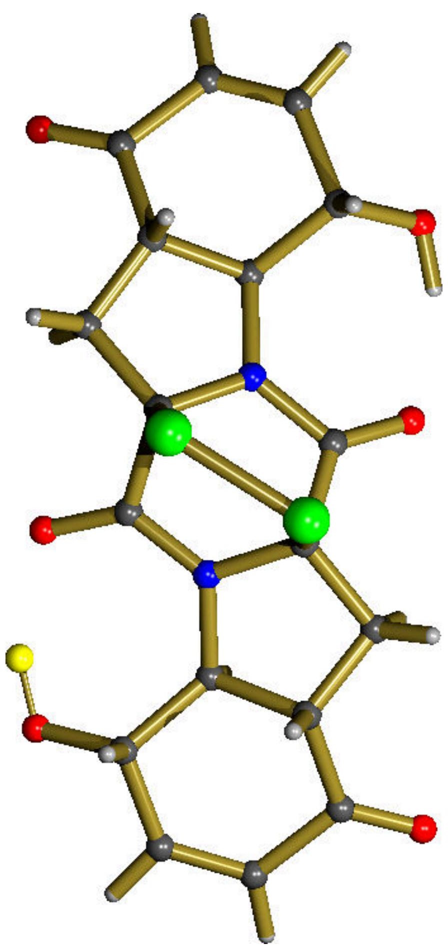
- $d_{\text{min}} = 0.87$Å
- a=11.35Å, b=12.7Å, c=13.0Å
- $P2_12_12_1$: completeness with 4 crystals: 86%

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

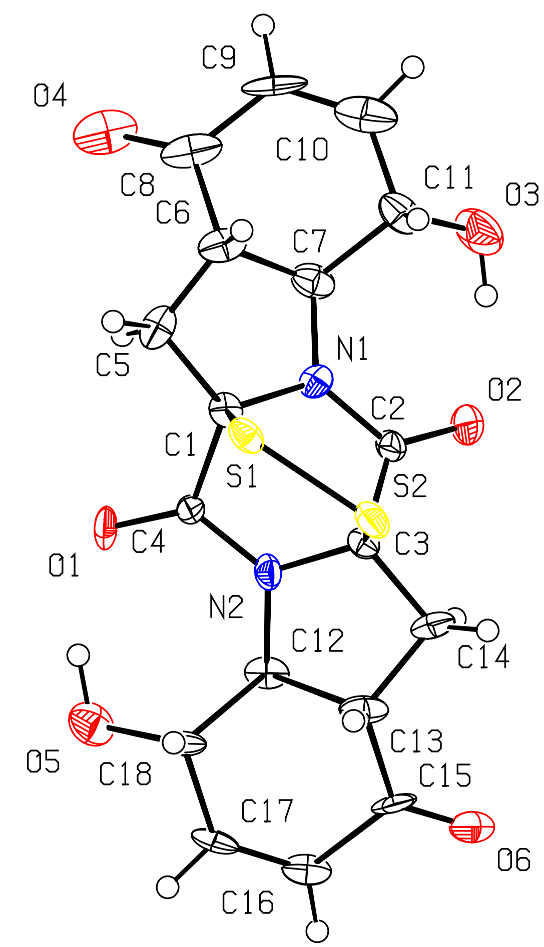
Pharmaceutical II (EPICZA): Structure Solution Process



Direct methods reveal H atoms
=data quality



HFIX: all except 1 H
=model quality



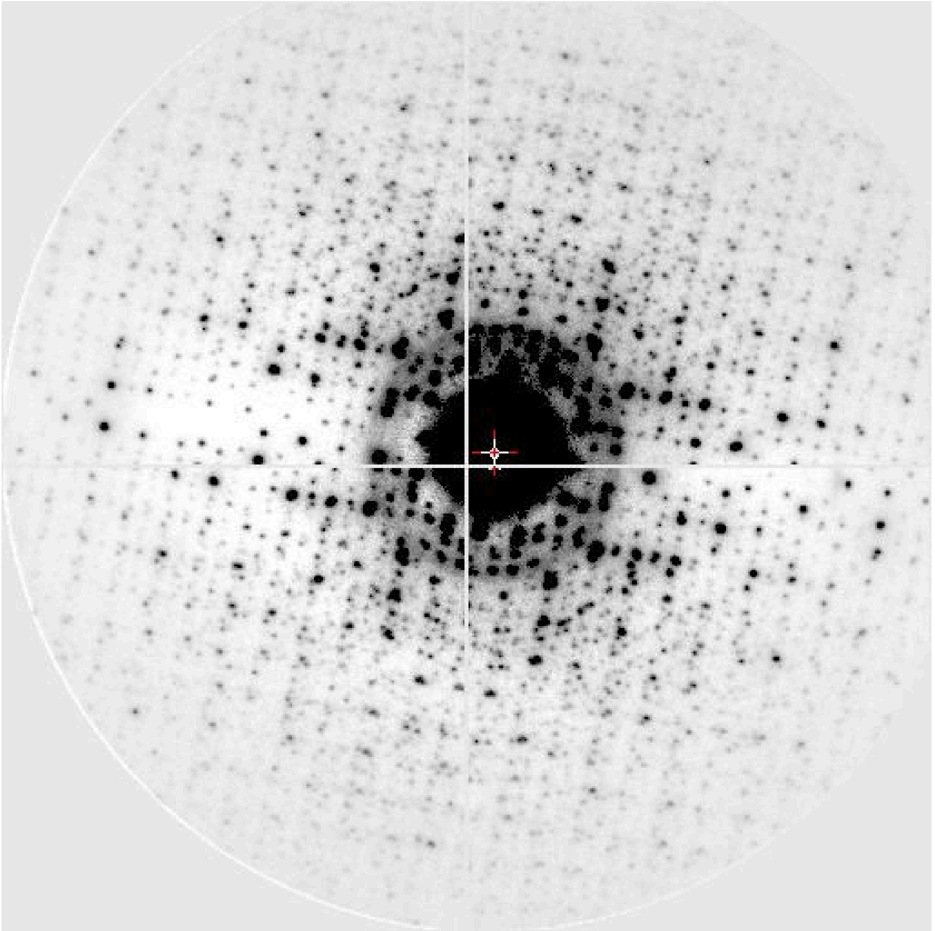
Final Structure

6 - 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)

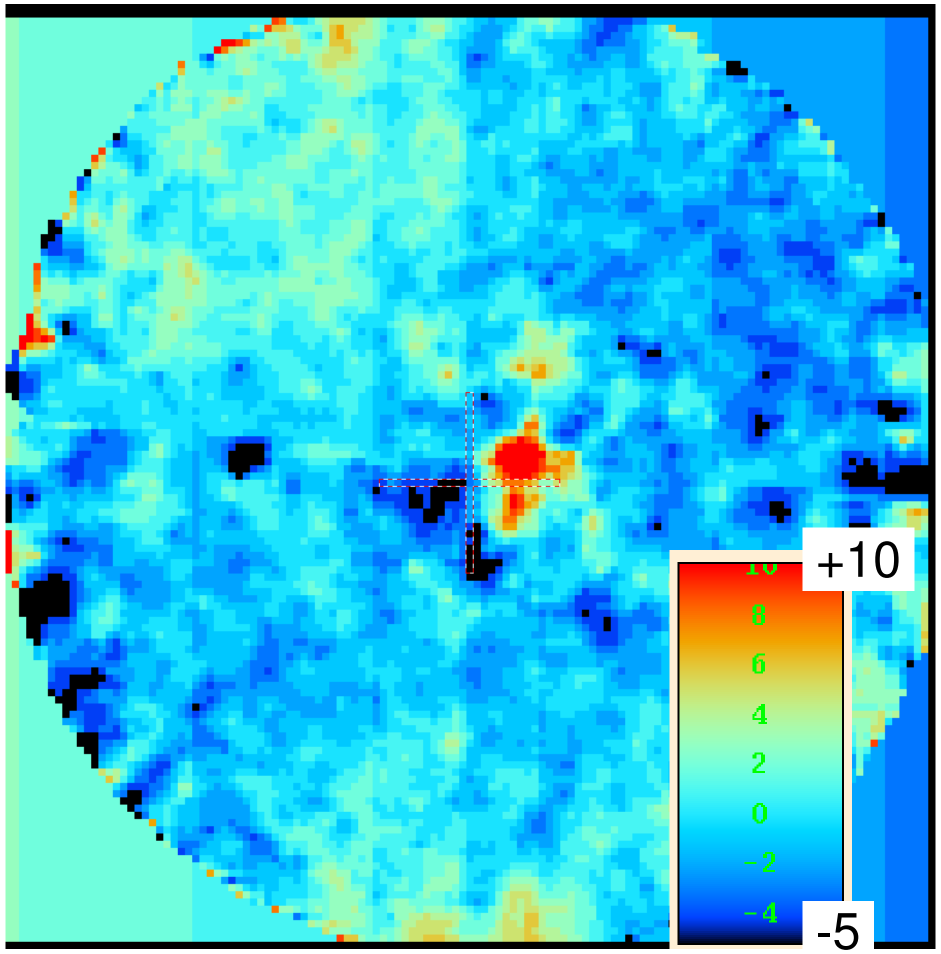
(Wikipedia)



- 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

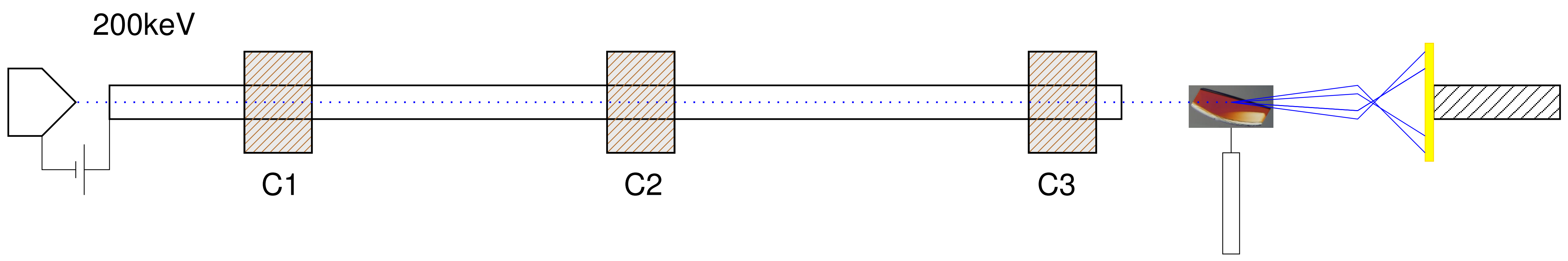
1. Collect data from garnet
2. Change as little as possible
3. Collect data from target sample
4. Process using garnet correction tables

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

7 - 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

8 - Acknowledgements

- Prof. J. P. Abrahams, Dr. E. van Genderen, M. Clabbers, Dr. T. Blum, C. Borsa, J. Heidler, Dr. R. Pantelic
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- Dr. W. Kabsch (XDS)
- Dr. D. Waterman (DIALS)
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