DIALS for ED

Data analysis and structure determination using DIALS and CCP4

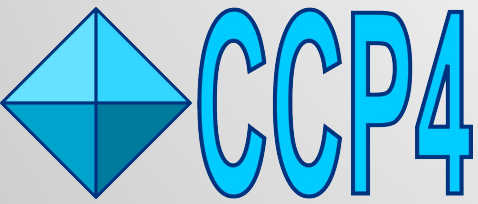
David Waterman

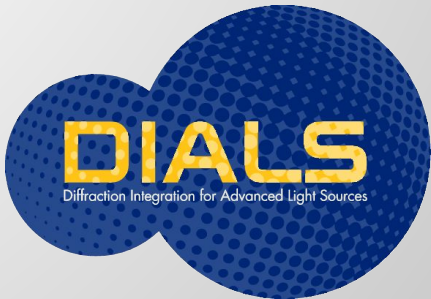
eBIC November 2019

CCP4

DIALS
Diffraction Integration for Advanced Light Sources

What is DIALS?

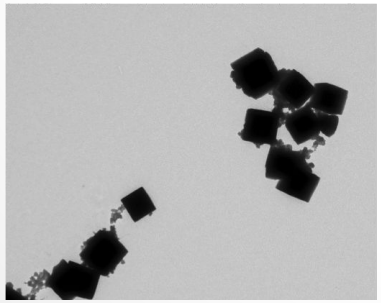


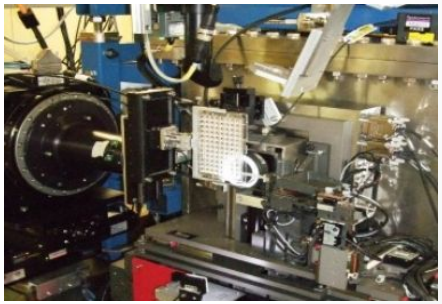


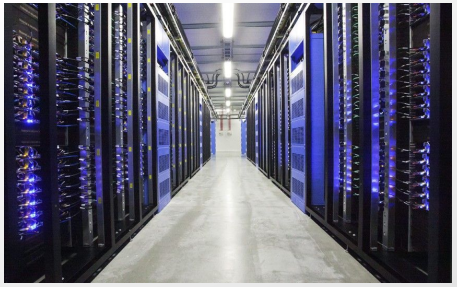
What is DIALS?

Diffraction **I**ntegration for **A**dvanced **L**ight **S**ources

- Project began in late 2011 at Diamond Light Source and CCP4
- Additional funding by BioStruct-X and now Wellcome Trust
- Aim to develop new data processing software to meet modern challenges
- Now an international collaborative development







CCP4

DIALS
Diffraction Integration for Advanced Light Sources

Legacy

Built upon the strengths of older projects

CCP4

Present and future

Novel features such as new indexing methods, multiple experiment joint refinement, smoothly varying models, improved data for weak diffraction...

CrossMark

research papers

Acta Crystallographica Section D
Biological Crystallography
ISSN 1399-0047

New methods for indexing multi-lattice diffraction data

Richard J. Gildea,[a] David G. Waterman,[b,c] James M. Parkhurst,[a] Danny Axford,[a] Geoff Sutton,[d] David I. Stuart,[a,d] Nicholas K. Sauter,[e] Gwyndaf Evans[a] and Graeme Winter[a]*

[a]Diamond Light Source Ltd, Harwell Science and Innovation Campus, Didcot OX11 0DE, England, [b]STFC Rutherford Appleton Laboratory, Didcot OX11 0QX, England, [c]CCP4, Research Complex at Harwell, Rutherford Appleton Laboratory, Didcot OX11 0FA, England, [d]Division of Structural Biology, The Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford OX3 7BN, England, and [e]Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA

Correspondence e-mail: graeme.winter@diamond.ac.uk

A new indexing method is presented which is capable of indexing multiple crystal lattices from narrow wedges of diffraction data. The method takes advantage of a simplification of Fourier transform-based methods that is applicable when the unit-cell dimensions are known *a priori*. The efficacy of this method is demonstrated with both semi-synthetic multi-lattice data and real multi-lattice data recorded from crystals of ~1 µm in size, where it is shown that up to six lattices can be successfully indexed and subsequently integrated from a 1° wedge of data. Analysis is presented which shows that improvements in data-quality indicators can be obtained through accurate identification and rejection of overlapping reflections prior to scaling.

1. Introduction

A fundamental limitation of conventional macromolecular crystallography is the necessity of obtaining one or more crystals of sufficient size and quality to record a reasonably complete data set. The development of microfocus beamlines has allowed data to be collected from smaller crystals than ever before [see the recent reviews of the history and capabilities of microfocus beamlines by Evans *et al.* (2011) and Smith *et al.* (2012)]. Frequently, particularly in the cases of viruses and membrane proteins, only small, poor-quality crystals may be available and it may only be possible to collect a highly incomplete data set over a small oscillation range for each individual crystal before the diffraction quality is affected by radiation damage.

research papers

CrossMark

Acta Cryst D STRUCTURAL BIOLOGY
ISSN 2059-7983

Diffraction-geometry refinement in the *DIALS* framework

David G. Waterman,[a,b]* Graeme Winter,[c] Richard J. Parkhurst,[c,d] Aaron S. Brewster,[e] Nicholas K. Sauter[e] a

[a]STFC Rutherford Appleton Laboratory, Didcot OX11 0QX, England, [b]CCP4, Research Complex at Harwell, Rutherford Appleton Laboratory, Didcot OX11 0FA, England, [c]Diamond Light Source Ltd, Harwell Science and Innovation Campus, Didcot OX11 0DE, England, [d]MRC Laboratory of Molecular Biology, Francis Crick Avenue, Cambridge CB2 0QH, England, and [e]Lawrence Berkeley National Laboratory, Berkeley, CA 94720, US
david.waterman@stfc.ac.uk, gwyndaf.evans@diamond.ac.uk

Received 7 October 2015
Accepted 4 February 2016

Edited by E. F. Garman, University of Oxford, England

Keywords: global refinement; *DIALS* framework; centroid refinement.

Rapid data collection and modern computing resources pro
to revisit the task of optimizing the model of diffractio
integration. A comprehensive description is given of new
upon established methods by performing a single global re
utilizing a smoothly varying model of the crystal lattice whe
global refinement technique extends to multiple data se
constraints to handle the problem of correlated paramet
small wedges of data. Examples of advanced uses of the so
the design is explained in detail, with particular emphasis
extensibility it entails.

1. Introduction

The successful integration of single-crystal diffraction da
depends on the accurate prediction of Bragg spot locations
area-detector images. An initial model for the diffracti
geometry may be constructed from metadata provided w
the diffraction images (Parkhurst *et al.*, 2014) or provided
the user. This starting model is completed by estimating crys
parameters, which are usually derived from data by an au
indexing procedure, such as that of Steller *et al.* (1997). T
model is rarely sufficient for accurate prediction throughou

research papers

J Appl Cryst JAC JOURNAL OF APPLIED CRYSTALLOGRAPHY
ISSN 1600-5767

Robust background modelling in *DIALS*

James M. Parkhurst,[a,b] Graeme Winter,[a] David G. Waterman,[c,d] Luis Fuentes-Montero,[a] Richard J. Gildea,[a] Garib N. Murshudov[b]* and Gwyndaf Evans[a]*

[a]Diamond Light Source Ltd, Harwell Science and Innovation Campus, Didcot OX11 0DE, UK, [b]Laboratory of Molecular Biology, Francis Crick Avenue, Cambridge CB2 0QH, UK, [c]STFC Rutherford Appleton Laboratory, Didcot OX11 0FA, UK, and [d]CCP4, Research Complex at Harwell, Rutherford Appleton Laboratory, Didcot OX11 0FA, UK.
*Correspondence e-mail: garib@mrc-lmb.cam.ac.uk, gwyndaf.evans@diamond.ac.uk

Received 1 June 2016
Accepted 24 August 2016

Edited by A. R. Pearson, Universität Hamburg, Germany

Keywords: integration; robust outlier rejection; generalized linear models; background modelling.

A method for estimating the background under each reflection during integration that is robust in the presence of pixel outliers is presented. The method uses a generalized linear model approach that is more appropriate for use with Poisson distributed data than traditional approaches to pixel outlier handling in integration programs. The algorithm is most applicable to data with a very low background level where assumptions of a normal distribution are no longer valid as an approximation to the Poisson distribution. It is shown that traditional methods can result in the systematic underestimation of background values. This then results in the reflection intensities being overestimated and gives rise to a change in the overall distribution of reflection intensities in a dataset such that too few weak reflections appear to be recorded. Statistical tests performed during data reduction may mistakenly attribute this to merohedral twinning in the crystal. Application of the robust generalized linear model algorithm is shown to correct for this bias.

1. Introduction

In macromolecular crystallography (MX), integration programs – such as *MOSFLM* (Leslie, 1999), *XDS* (Kabsch, 2010), *d*TREK* (Pflugrath, 1999) and *DIALS* (Waterman *et al.*, 2013) – are used to estimate the intensities of individual Bragg reflections from a set of X-ray diffraction images. Whilst details of the processing differ, these programs all follow the same basic procedure to calculate the intensity estimates. For each reflection, pixels in the neighbourhood of the predicted

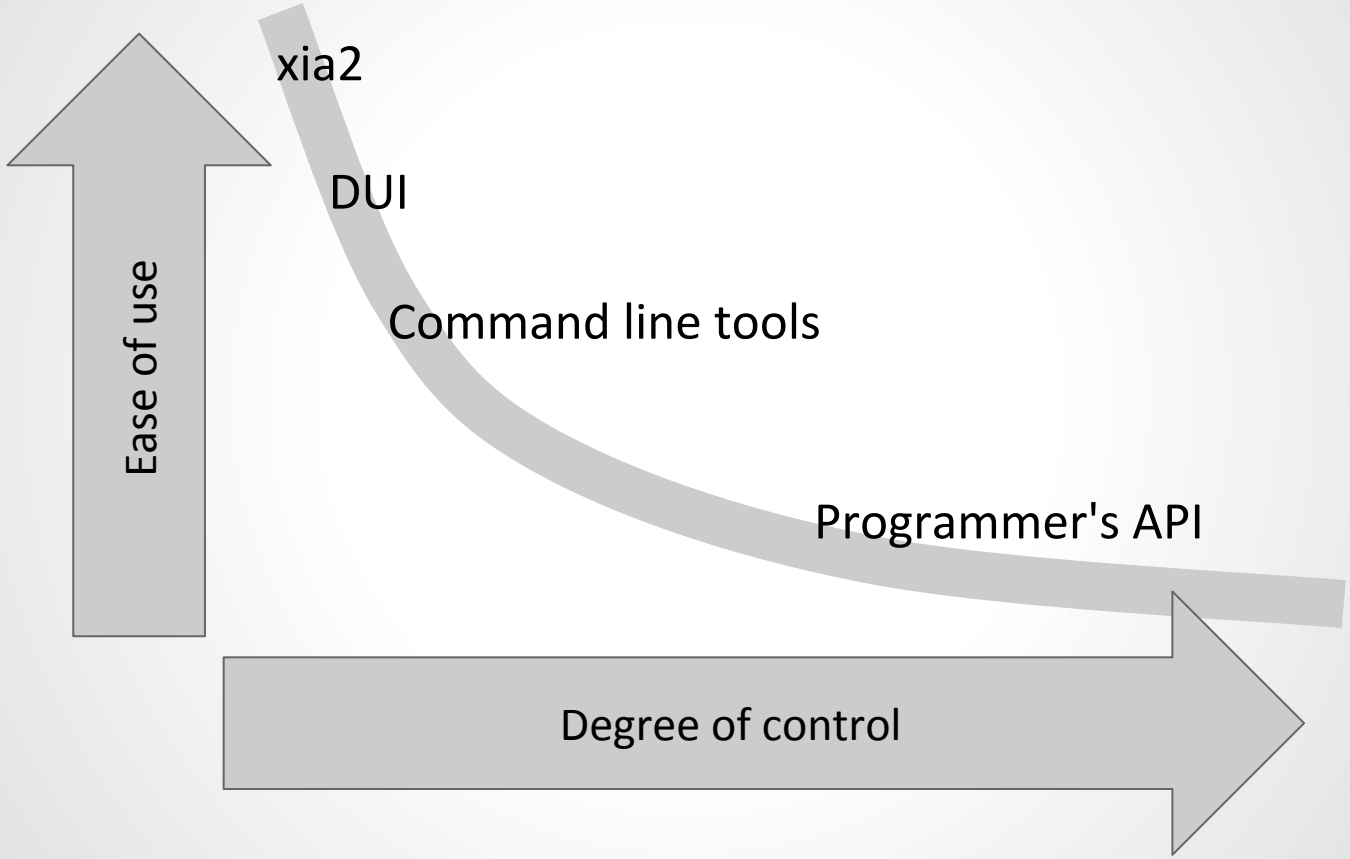
CCP4

DIALS
Diffraction Integration for Advanced Light Sources

Philosophy

Philosophy

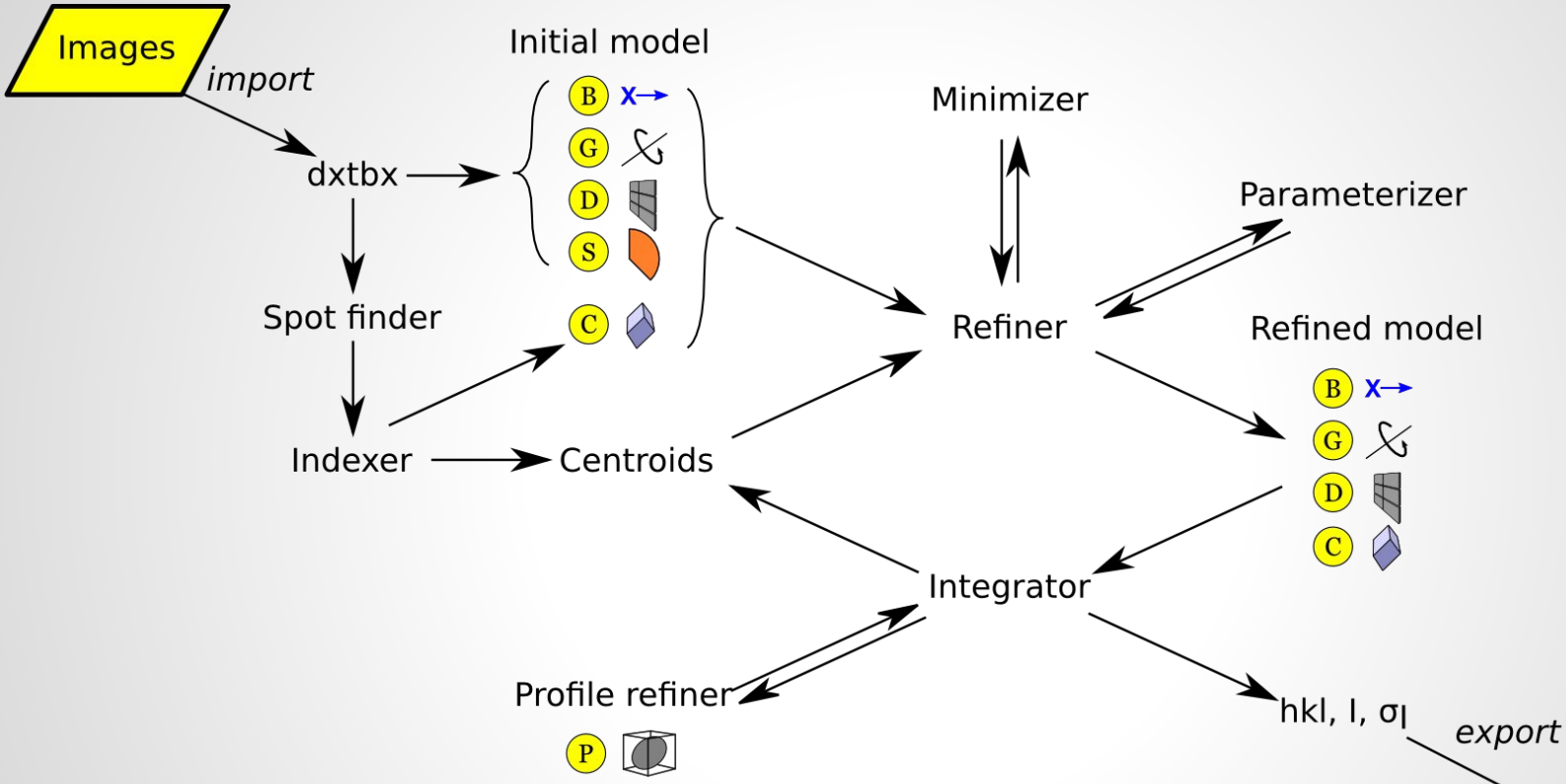
Levels of interaction



CCP4

DIALS
Diffraction Integration for Advanced Light Sources

Flowchart for data processing



CCP4

DIALS
Diffraction Integration for Advanced Light Sources

DIALS files

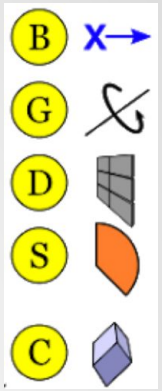
From v2.0.0 onwards:

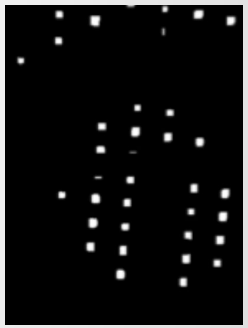
`".expt"` file (e.g. `imported.expt`)

- metadata for images - file location, experimental model details (beam orientation and wavelength, detector position and orientation etc.)

`".refl"` file (e.g. `strong.refl`)

- spot/reflection information - coordinates (centroids), intensity values, etc.





CCP4

DIALS
Diffraction Integration for Advanced Light Sources

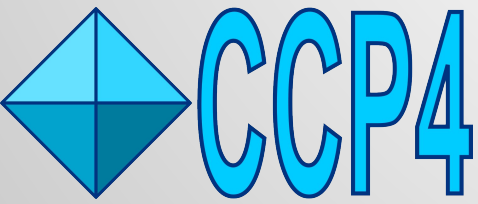
DIALS CLI

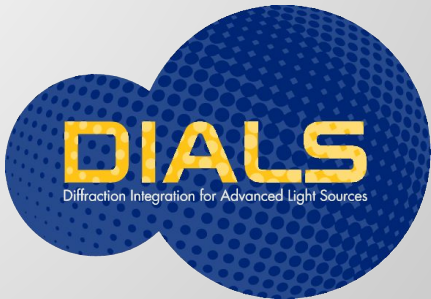
Main programs:

dials.import
dials.find_spots
dials.index
dials.search_beam_position
dials.refine_bravais_settings
dials.reindex
dials.refine
dials.integrate
dials.symmetry
dials.scale
dials.export

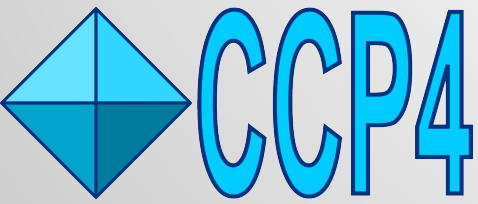
```
$ dials.find_spots datablock.json nproc=8

...

Histogram of per-image spot count for imageset 0:
71268 spots found on 200 images (max 1897 / bin)
*
*
**** *                                                 *       *
*********                                ** ******* ***** *  * *
************                         ** ****************************
**************** * ***************************************************
************************************************************************
************************************************************************
************************************************************************
************************************************************************
1                            image                                200

------------------------------------------------------------------------
Saved 71268 reflections to strong.pickle
Time Taken: 88.113627
```

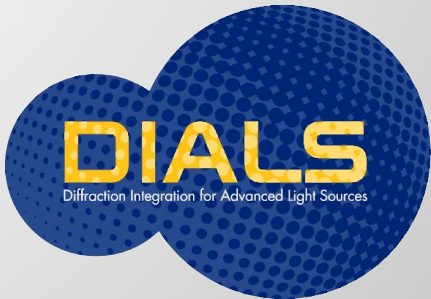
More than 70 **dials.*** commands in version 2.0



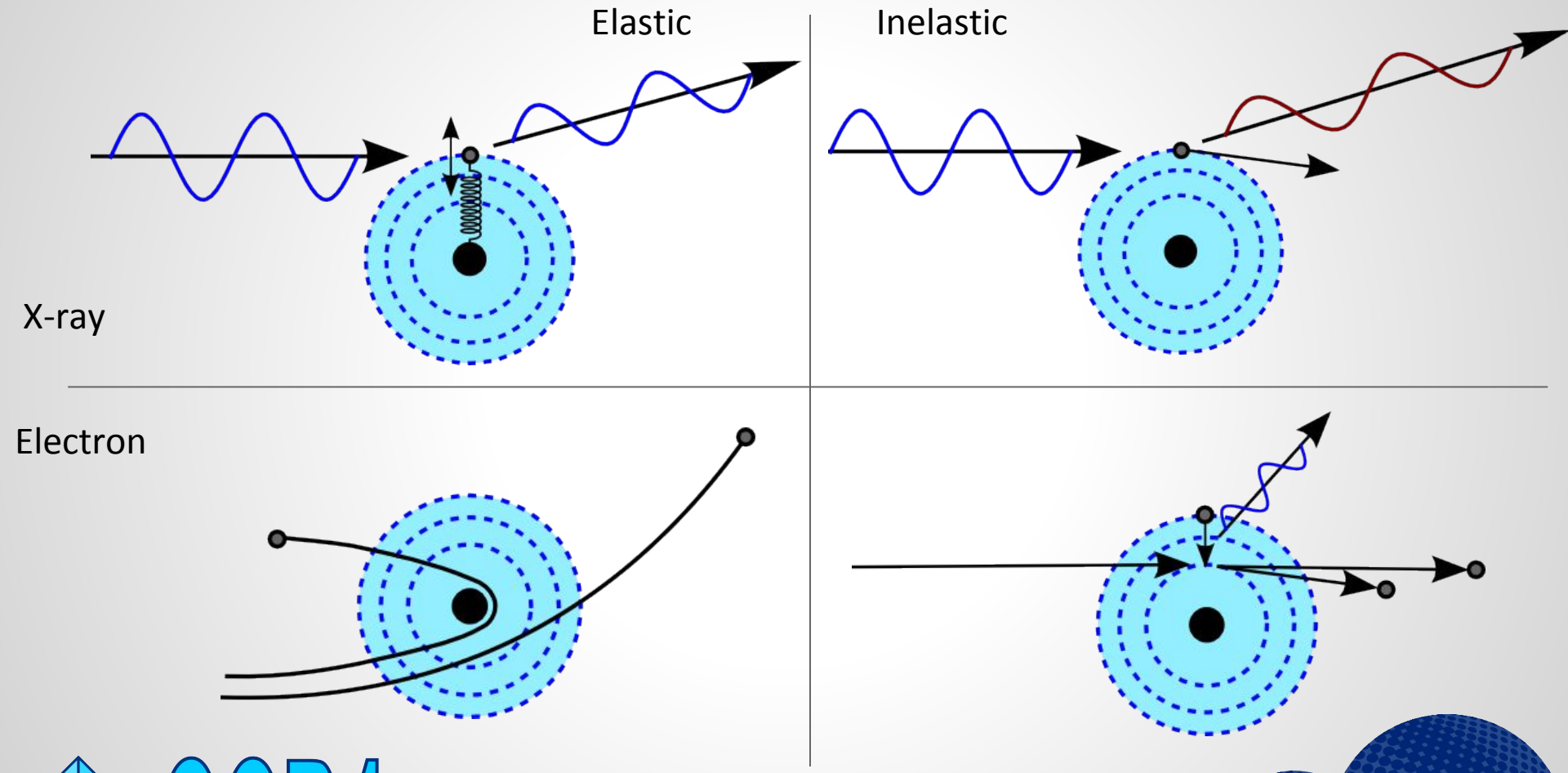


Electron diffraction basics





Scattering



CCP4

DIALS
Diffraction Integration for Advanced Light Sources

Scattering

X-ray scattering probes electron density

Electron scattering probes electrostatic potential

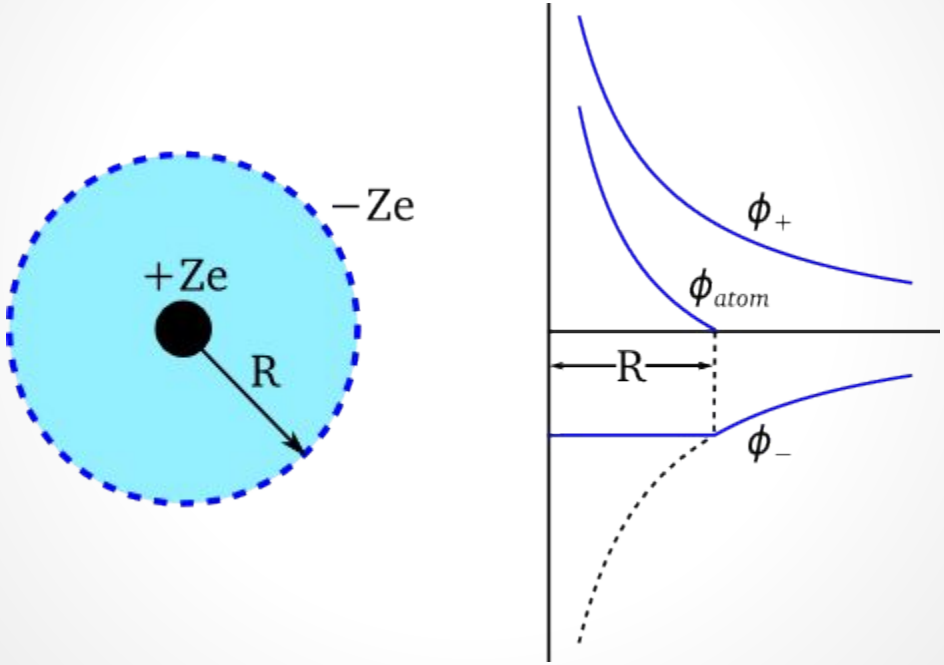


Figure adapted from Vainshtein 1964

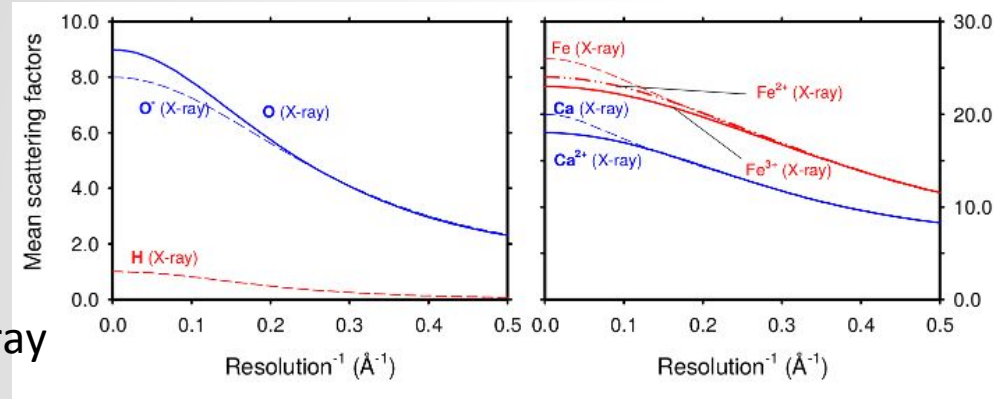
CCP4

DIALS
Diffraction Integration for Advanced Light Sources

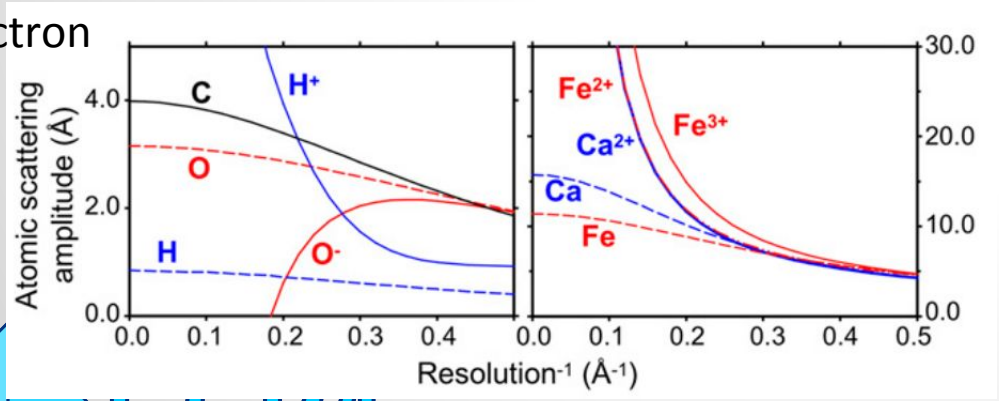
Charged state

Coulomb potential maps can reveal information about charges
(Yonekura *et al.* PNAS 2015)

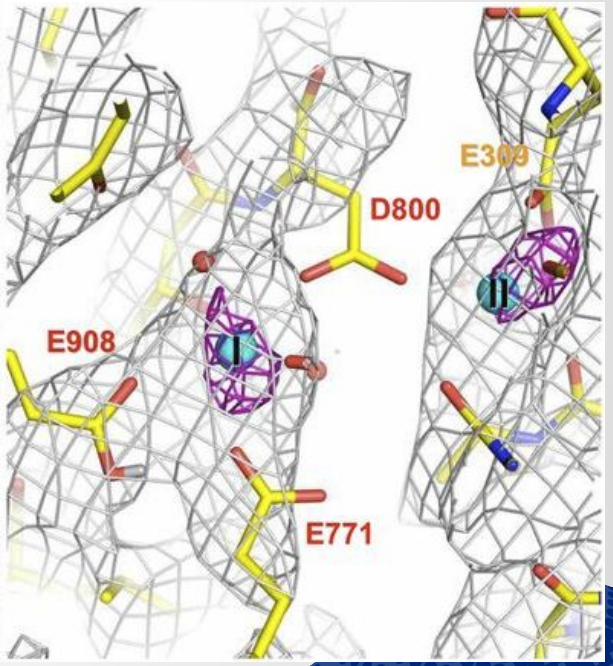
X-ray



Electron



Ca^{2+}-ATPase. When neutral Ca is assumed, difference map shows large positive peaks

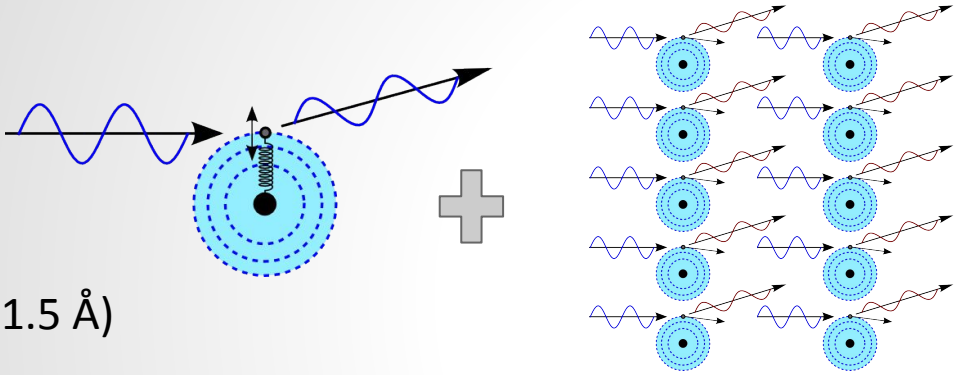


CCP4

DIALS
Diffraction Integration for Advanced Light Sources

Radiation damage

Damage accumulates alongside diffraction by the ratio of useful (elastic) to damaging (inelastic) events (Henderson, Quart. Rev. Biophys. 1995)

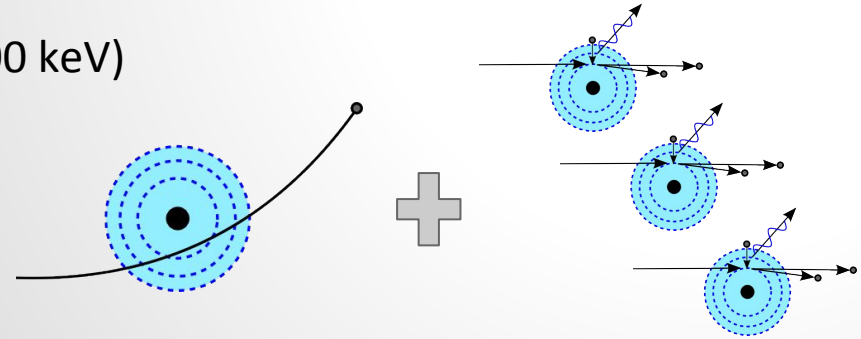


X-ray (1.5 Å)

× 8000 eV = **80'000 eV**

Enough to 'kill' a unit cell
(Sliz *et al.* Structure 2003)

Electron (200 keV)



× 20 eV = **60 eV**

Electrons better by >1000 times

CCP4

DIALS
Diffraction Integration for Advanced Light Sources

Crystal size

The radiation damage argument implies that a crystal for electron diffraction can be 1000 times smaller in volume than a crystal for X-ray diffraction

Small crystals or crystal fragments may be better ordered (de la Cruz 2017)

However, it is more complicated...

The strength of interaction implies multiple scattering unless the crystal thickness is much below the mean free path

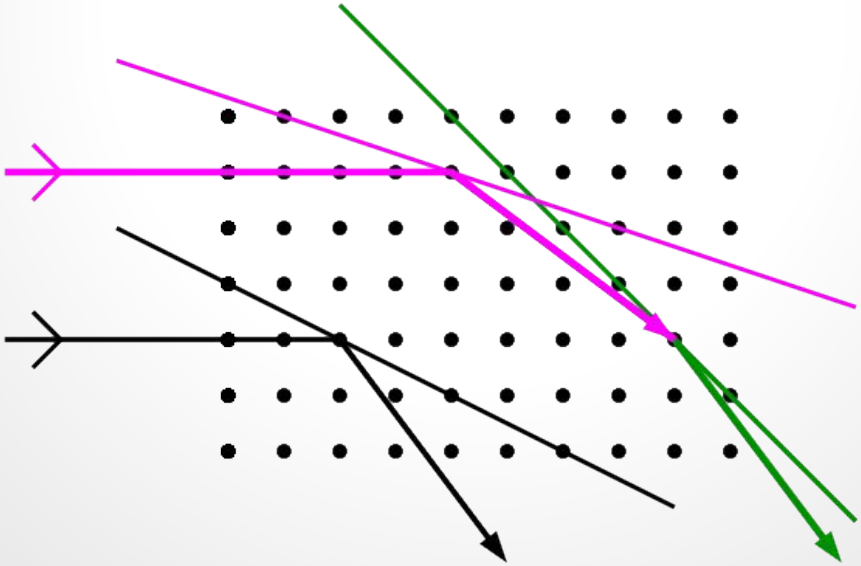


Figure credit: Tim Grüne

Crystal size

Thin samples are *obligatory*

But not as thin as theory suggests (100 nm @ 200 keV, Subramanian 2015)

For proteins, with continuous rotation, thickness < 400 nm seems to keep dynamic effects acceptably low (Hattne 2015)

Nevertheless, *vanishingly* thin plates may still be best (Yonekura 2015)

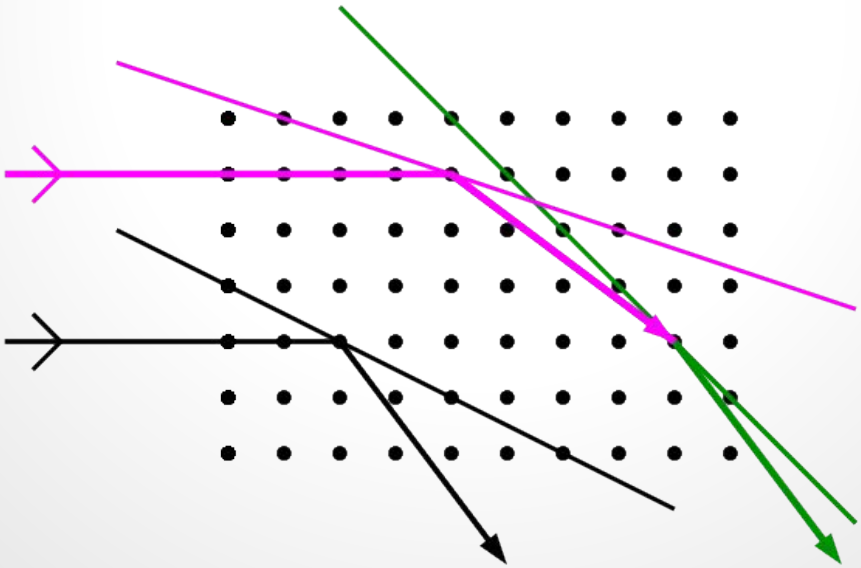
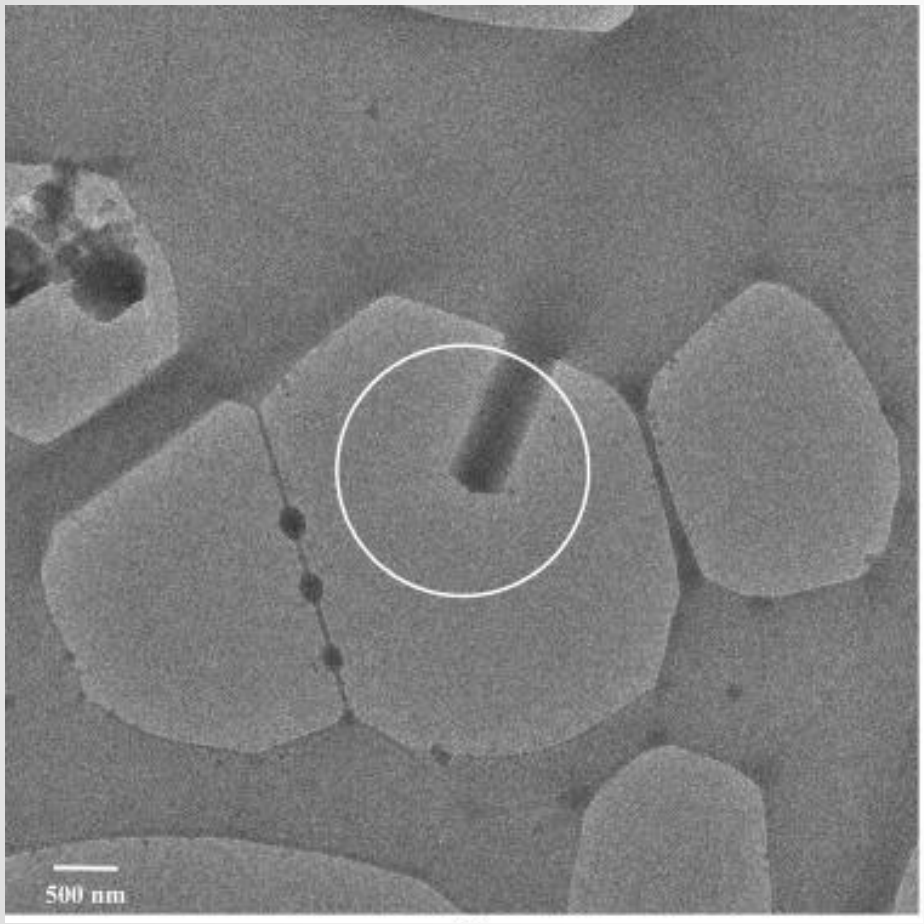


Figure credit: Tim Grüne

Crystal size



Lysozyme nanocrystal

Diffracting volume: 0.14 mm^3
(< 6×10^5 unit cells)

Clabbers *et al.* 2017

CCP4

DIALS
Diffraction Integration for Advanced Light Sources

FIB milling as sample preparation

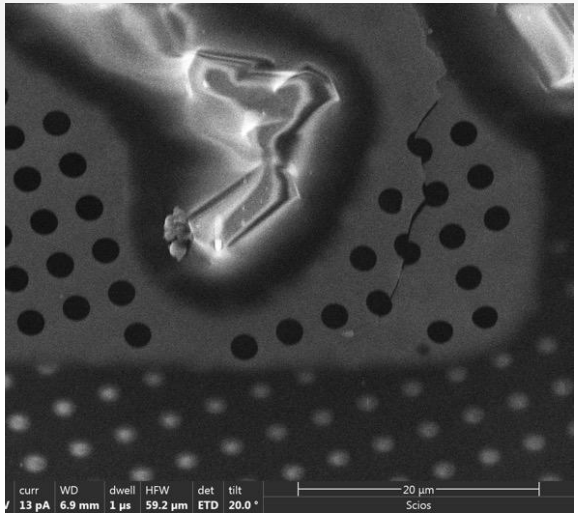
Image credits:

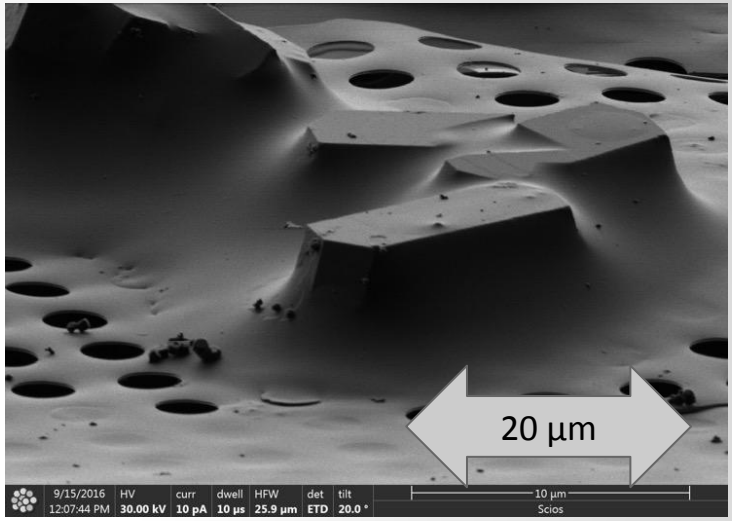
Emma Beale (DLS)

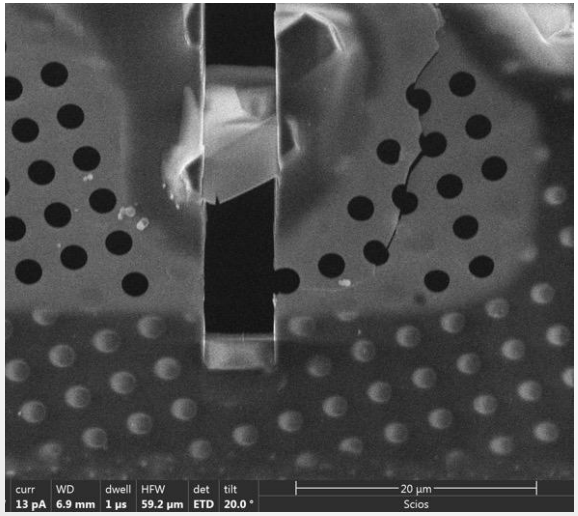
Corey Hecksel (eBIC)

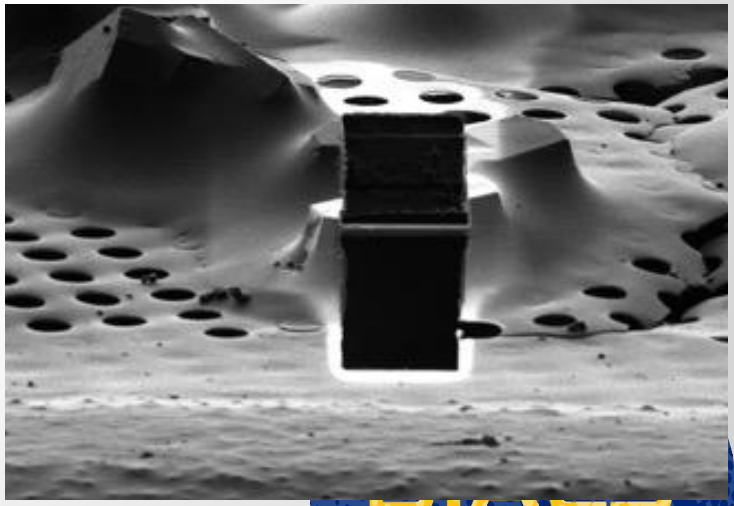
Abhay Kotecha (STRUBI)

Jose Trincao (DLS)









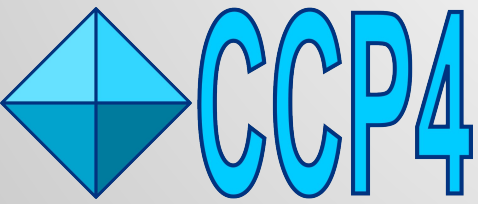
See also Duyvesteyn *et al.* PNAS 2018

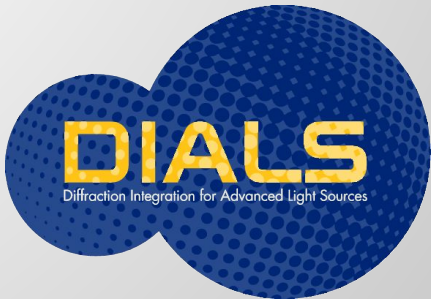
CCP4

DIAMOND
Diffraction Integration for Advanced Light Sources

Image formats and metadata

Boring but important





EM image formats

As with MX, various image formats exist, some manufacturer-specific

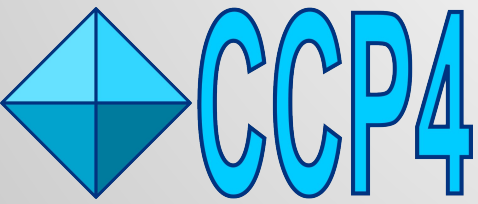
Independent from MX, except for generic formats (such as TIFF)

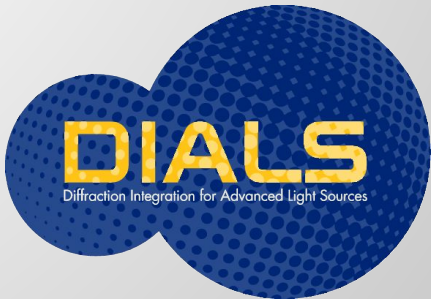
Three approaches to get the data into DIALS:

1. *External*: convert to standard MX format (SMV, CBF etc.)
2. *Internal*: write new format reading code to go into dxtbx
3. *Extension*: write new format reading code as a plug-in

```
dxtbx.install_format --user [/path/to/format/class.py] [URL]
```

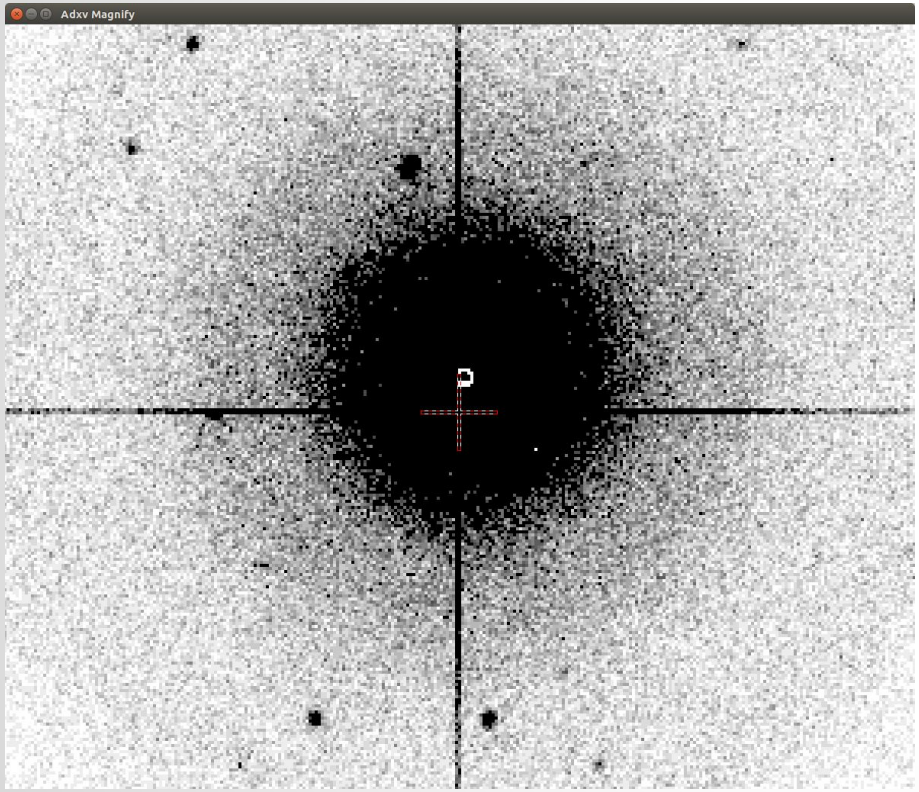
In any case, missing experiment geometry metadata may be a problem

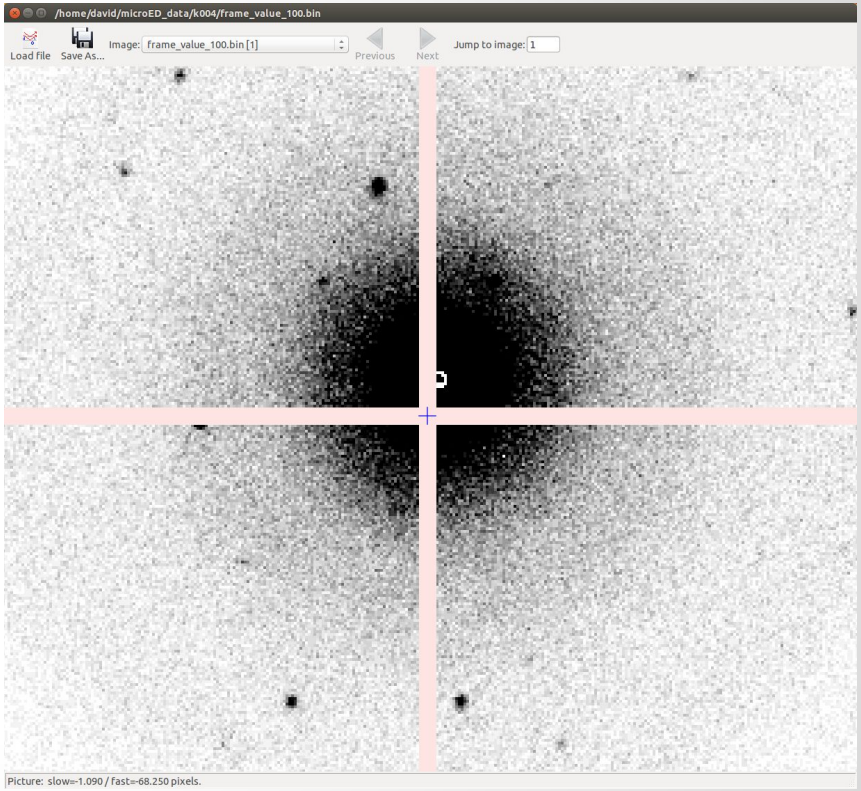




Extending dxtbx

Plug in a new Format class to recognise new image file formats





CCP4

DIALS
Diffraction Integration for Advanced Light Sources

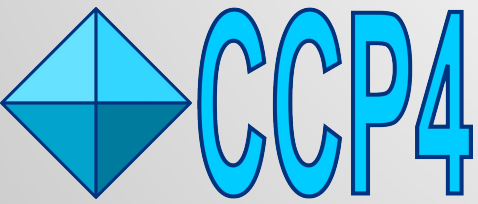
EM image formats

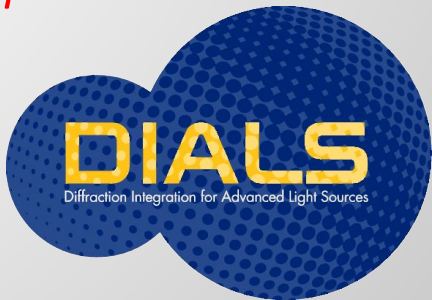
Explicit support for a handful of formats (in distribution or as plug-in), e.g.:

- Timepix quad raw, miniCBF, SMV
- Timepix quad^2 miniCBF
- ThermoFisher Falcon II raw image
- ThermoFisher Falcon III converted to SMV
- ThermoFisher Ceta 16M image stack (`.ser` format, no metadata)
- ThermoFisher extended header MRC
- Gatan DM4 stack (no metadata)
- Gatan OneView converted to miniCBF
- TVIPS SMV (from tvips2smv)
- DirectElectron DE-64 converted to TIFF

https://github.com/dials/dxtbx_ED_formats

Could add others, but we don't want to be image format zookeepers





Experimental geometry uncertainty

Without careful calibration there may be uncertainty about various things:

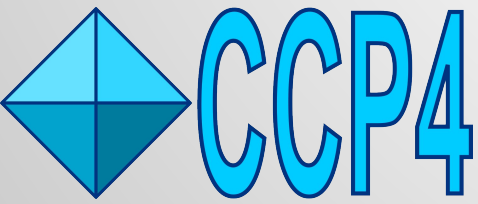
- Beam centre
- Detector (effective) distance
- Detector gain
- Multi-panel metrology
- Rotation axis handedness
- Rotation axis orientation

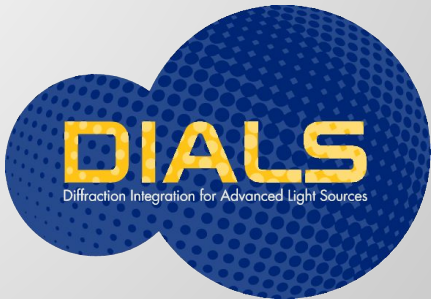
Relatively poor sample stages for rotation adds additional error:

- $\Delta\phi$ / image

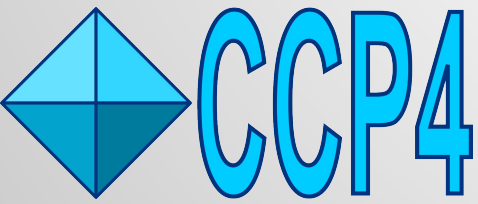
In addition, lenses mean questions about:

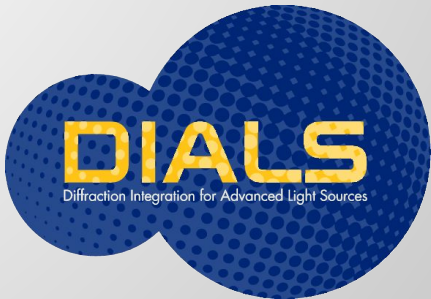
- Obliquity (parallax)
- Diffraction pattern distortion





Diffraction geometry





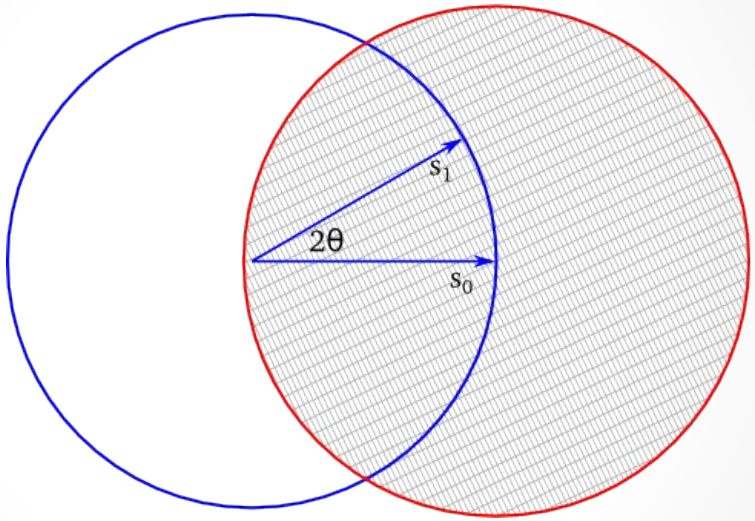
Ewald construction

X-ray diffraction

Photon energy 12 keV (λ = 1.03 Å)

Scattering vector at 2 Å

2θ = 29.9°



CCP4

DIALS
Diffraction Integration for Advanced Light Sources

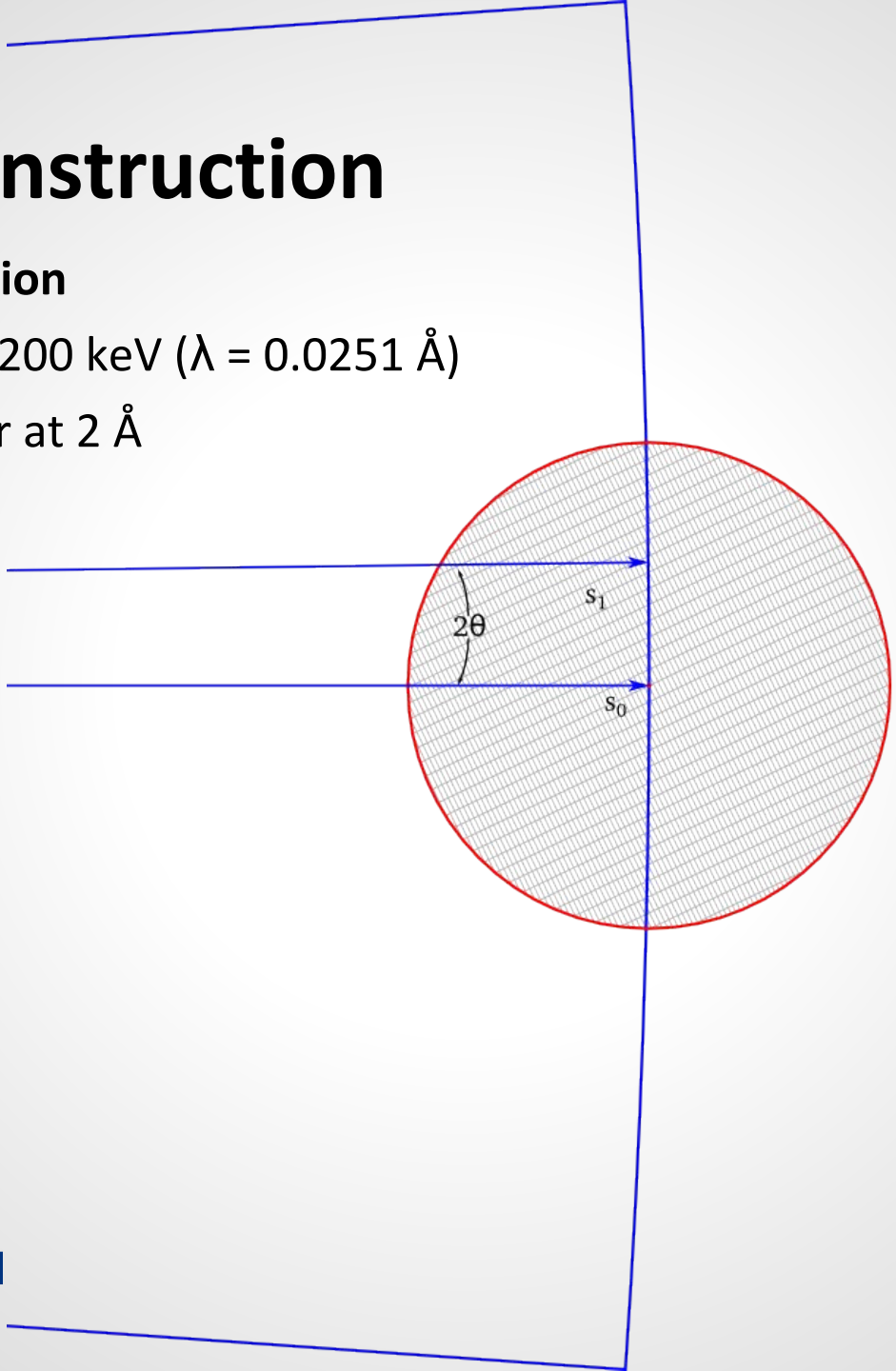
Ewald construction

Electron diffraction

Electron energy 200 keV (λ = 0.0251 Å)

Scattering vector at 2 Å

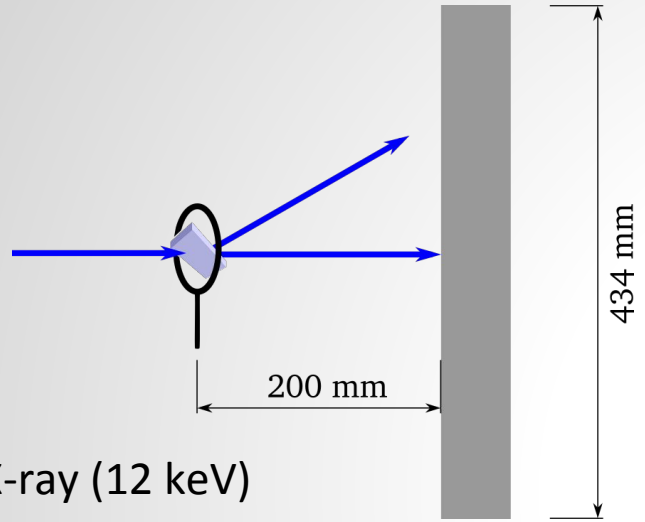
2θ = 0.72°



CCP4

DIALS
Diffraction Integration for Advanced Light Sources

Diffraction geometry



X-ray (12 keV)

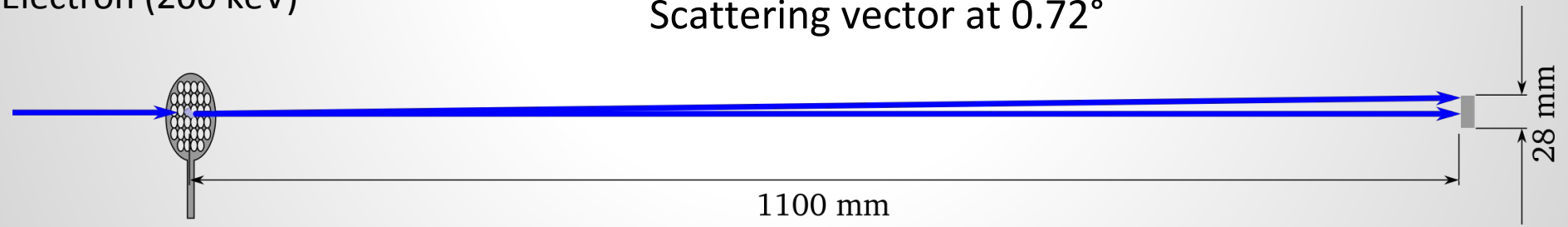
Typical MX geometry, Pilatus 6M detector

Scattering vector at 29.9°

Electron (200 keV)

Real ED example, Timepix quad

Scattering vector at 0.72°



CCP4

DIALS
Diffraction Integration for Advanced Light Sources

Diffraction geometry

Real detector distance actually fixed

Beam paths are complex, through lens system

We ignore this and use the *effective* detector distance

Might also ignore parallax? Depends on detector technology

But not image distortion, if present

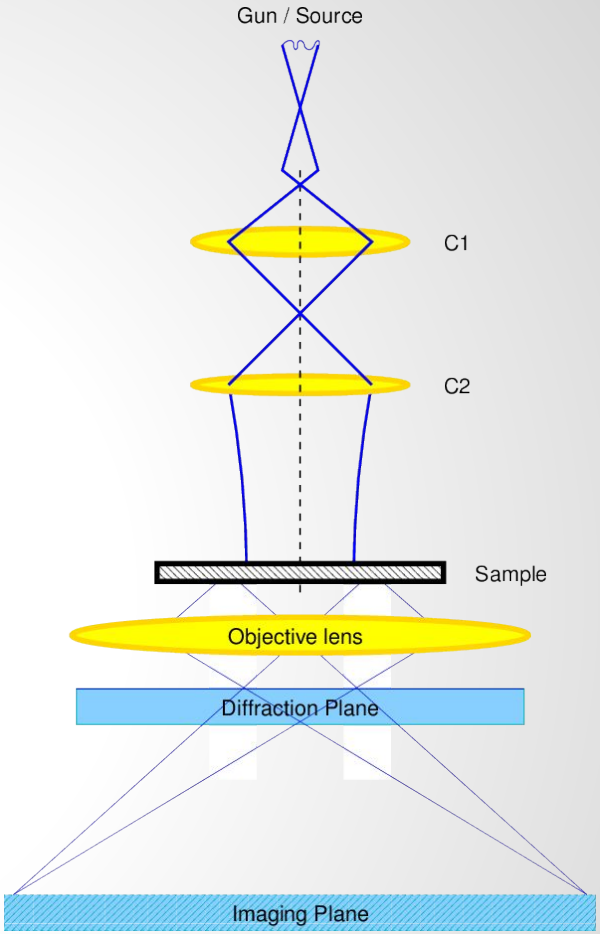
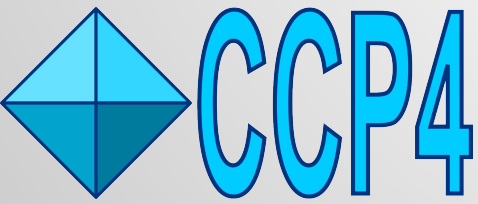


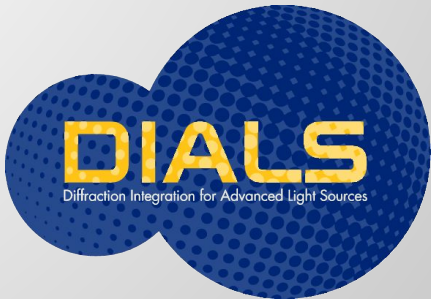
Figure credit: Tim Grüne

CCP4

DIALS
Diffraction Integration for Advanced Light Sources

Challenges

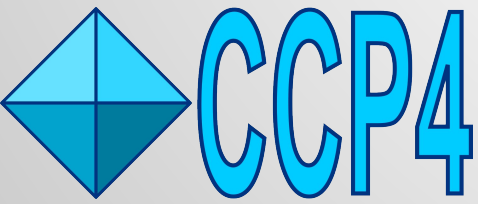

CCP4

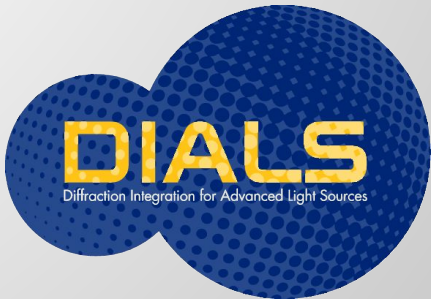

DIALS
Diffraction Integration for Advanced Light Sources

Challenges

Almost flat Ewald sphere, high detector distance and low diffraction angle

- Lens distortions may introduce systematic error in observed positions
- Indexing from a single image is challenging
- It may even be difficult to determine the direction of rotation
- Joint refinement of detector and unit cell may not be possible
- Refined detector and unit cell parameters may be poor
- The beam centre may drift

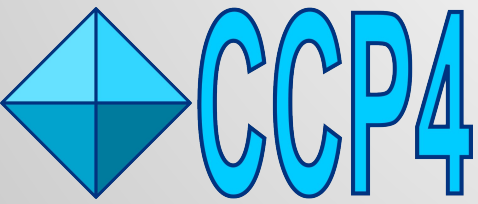


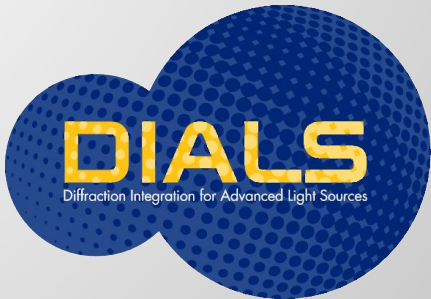


Challenges

Almost flat Ewald sphere, high detector distance and low diffraction angle

- Lens distortions may introduce systematic error in observed positions
- Indexing from a single image is challenging
- It may even be difficult to determine the direction of rotation
- Joint refinement of detector and unit cell may not be possible
- Refined detector and unit cell parameters may be poor
- The beam centre may drift



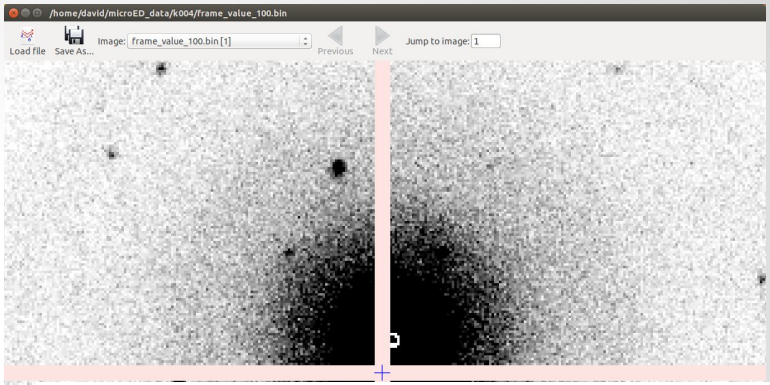


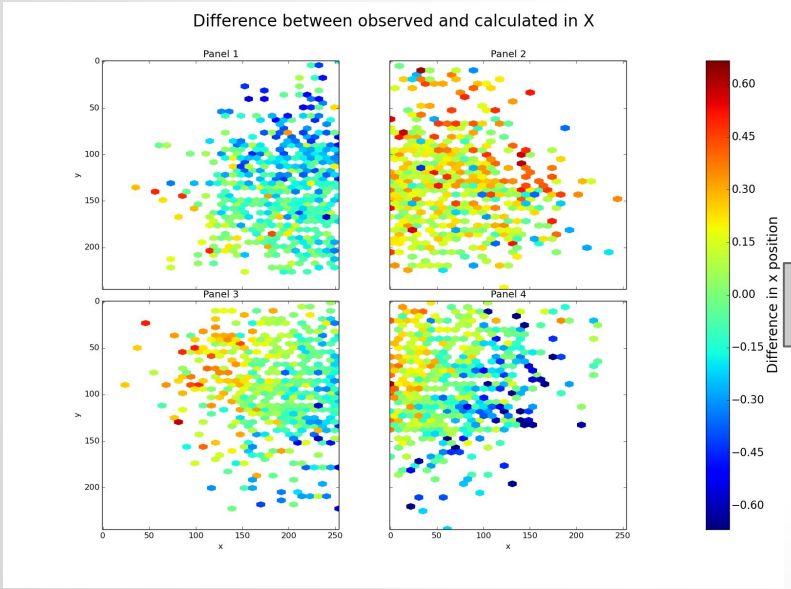
Lens distortions

Pentasil zeolite, 64° tilt, 200 keV

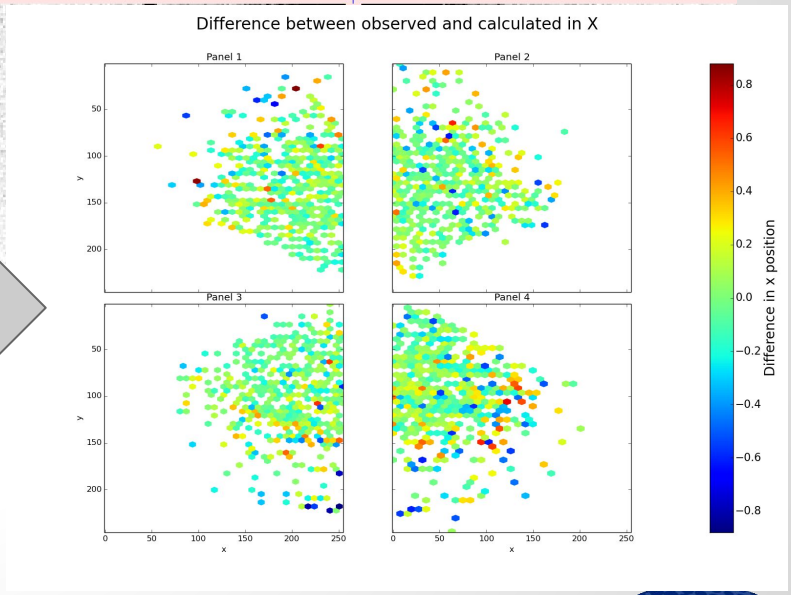
Timepix direct electron detector as a 4 panel quad

Some image distortion evident





panel refinement



CCP4

DIALS
Diffraction Integration for Advanced Light Sources

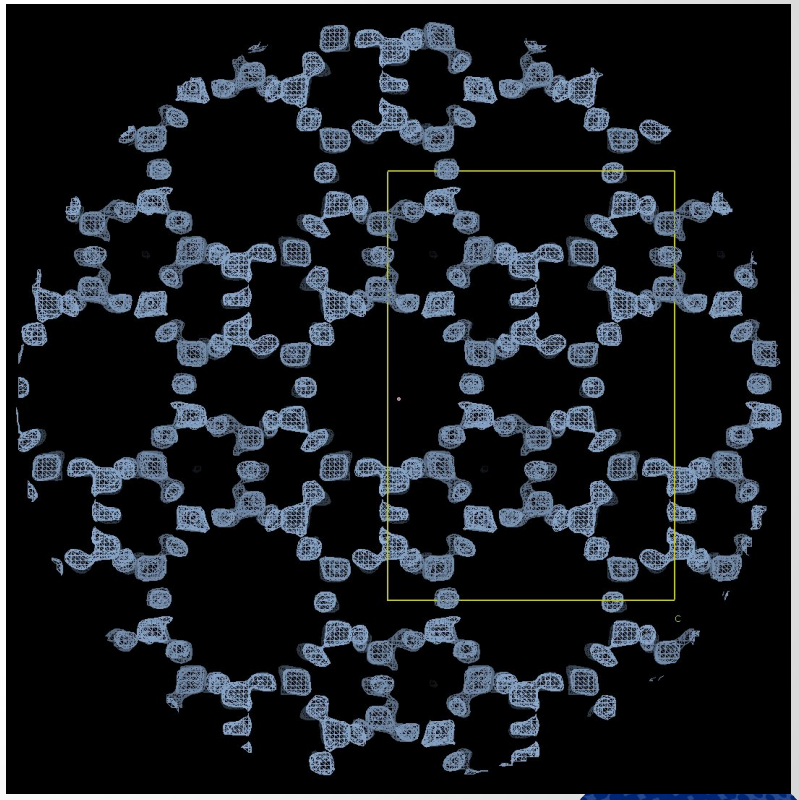
That gross 'fix' was sufficient to integrate this dataset with DIALS

Scaled with AIMLESS, structure solved *ab initio* by SHELXT

Refinement with SHELXL using electron scattering factors

Poor statistics typical for e- diffraction data, but maps are reasonable

Space group	Pnma
Cell dim (Å)	20.09, 19.96, 13.51
Resolution (Å)	14.16 - 0.70 [14.16 - 3.83] (0.71 - 0.70)
Rmerge (%)	21.9 [11.6] (53.4)
Rmeas (%)	26.0 [15.5] (64.0)
I/σ	1.90 [4.3] (0.8)
CC½	0.98 [0.97] (0.92)
Completeness (%)	70.7 [60.1] (70.4)
R1 (%)	20.7
wR2 (%)	48.7



CCP4

DIALS
Diffraction Integration for Advanced Light Sources

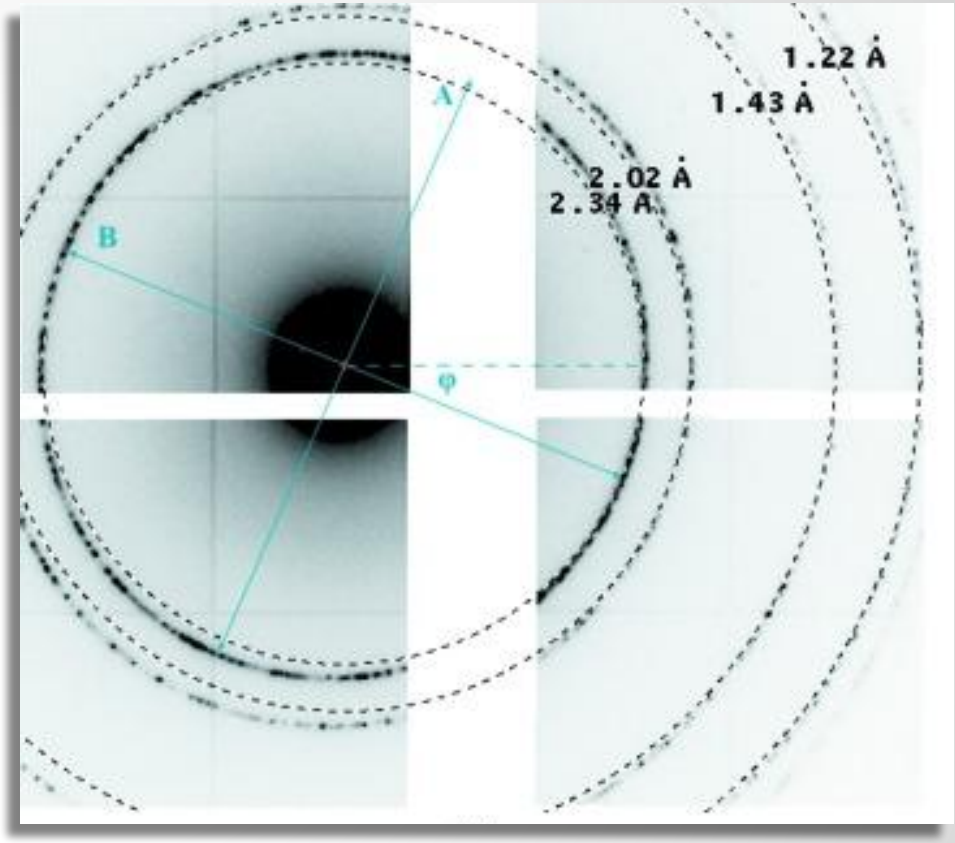
Elliptical distortion

A more general solution is to use pixel-wise distortion tables

Determine parameters from e.g. Al powder diffraction standard

Potentially refine parameters from diffraction data?

Best approach: *avoid distortion in the first place*



Clabbers *et al.* 2017

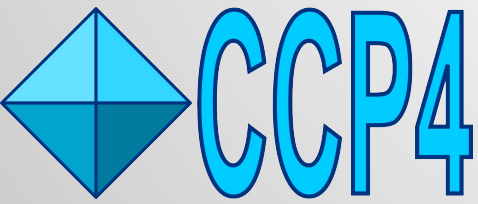
CCP4

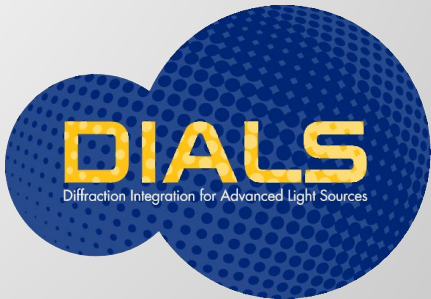
DIALS
Diffraction Integration for Advanced Light Sources

Challenges

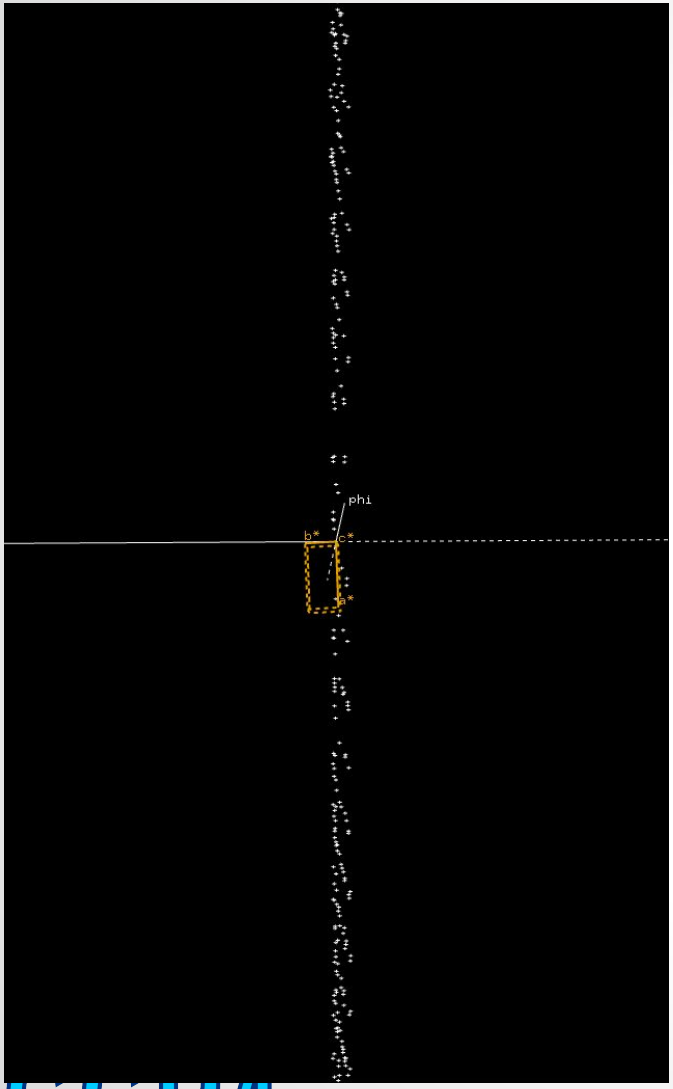
Almost flat Ewald sphere, high detector distance and low diffraction angle

- Lens distortions may introduce systematic error in observed positions
- Indexing from a single image is challenging
- It may even be difficult to determine the direction of rotation
- Joint refinement of detector and unit cell may not be possible
- Refined detector and unit cell parameters may be poor
- The beam centre may drift

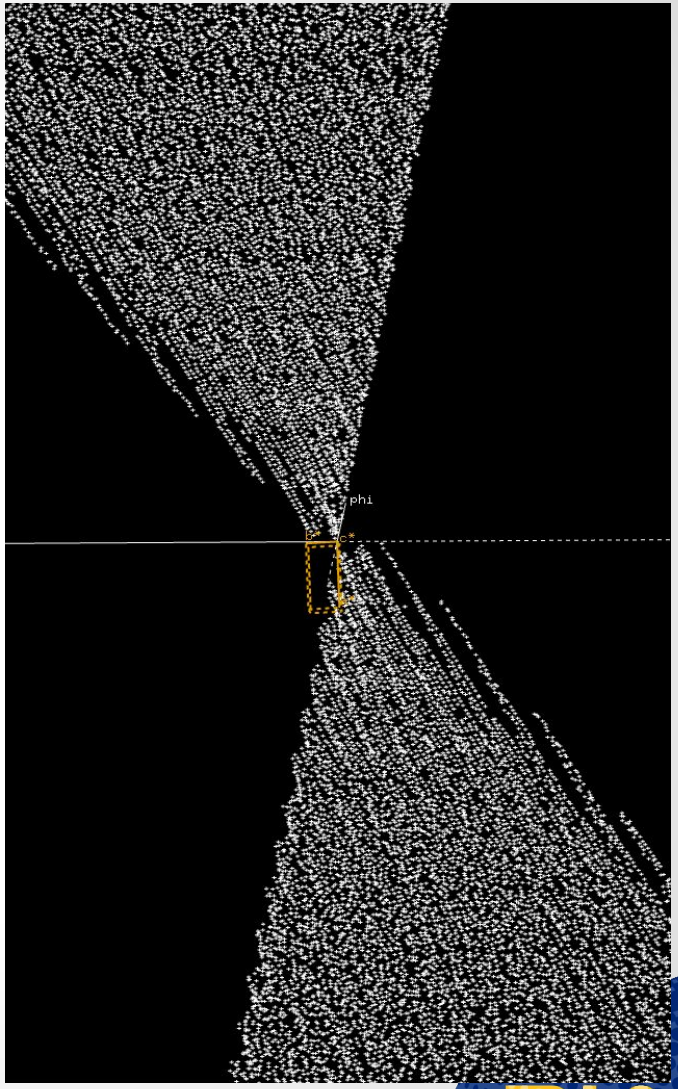




single image



56° wedge



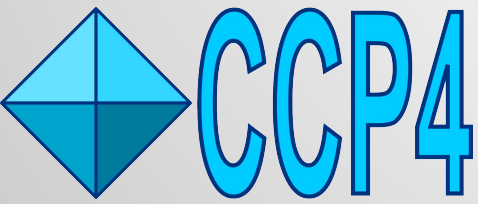
CCP4

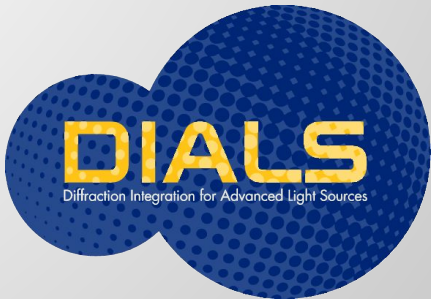
DIALS
Diffraction Integration for Advanced Light Sources

Challenges

Almost flat Ewald sphere, high detector distance and low diffraction angle

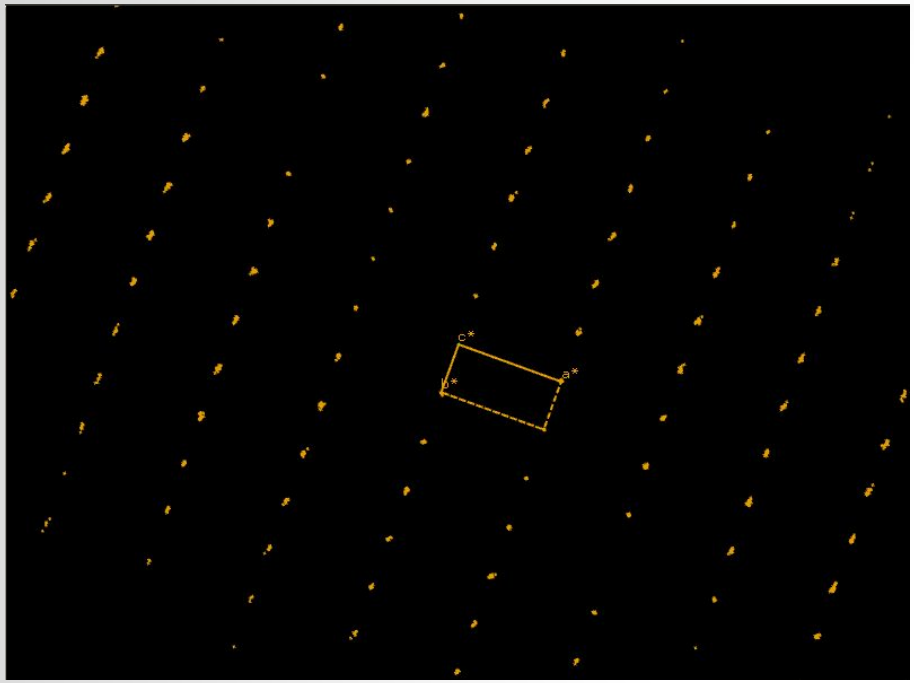
- Lens distortions may introduce systematic error in observed positions
- Indexing from a single image is challenging
- It may even be difficult to determine the direction of rotation
- Joint refinement of detector and unit cell may not be possible
- Refined detector and unit cell parameters may be poor
- The beam centre may drift



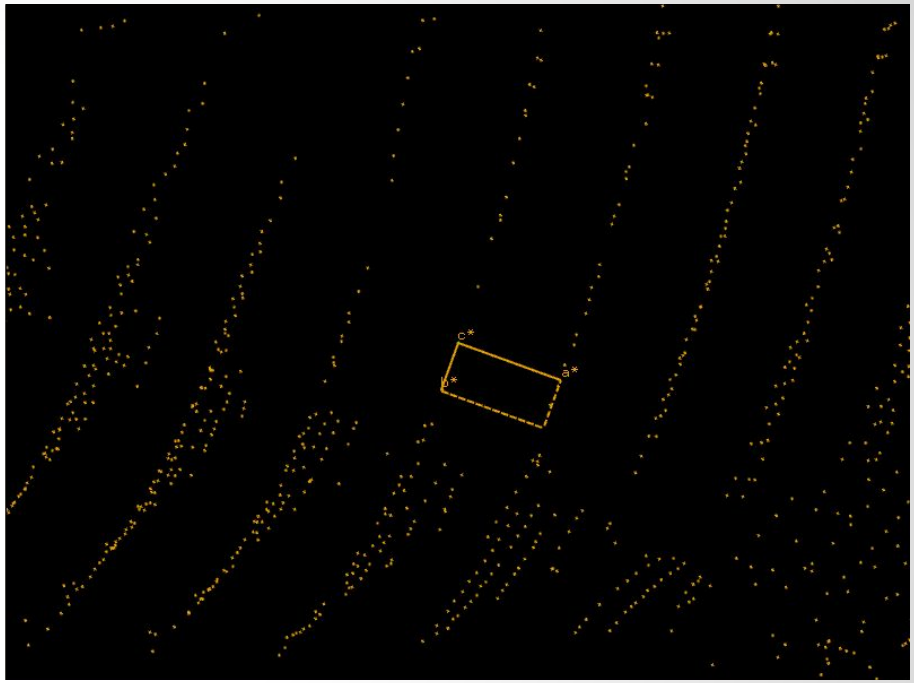


MX geometry

right rotation axis



inverted rotation axis

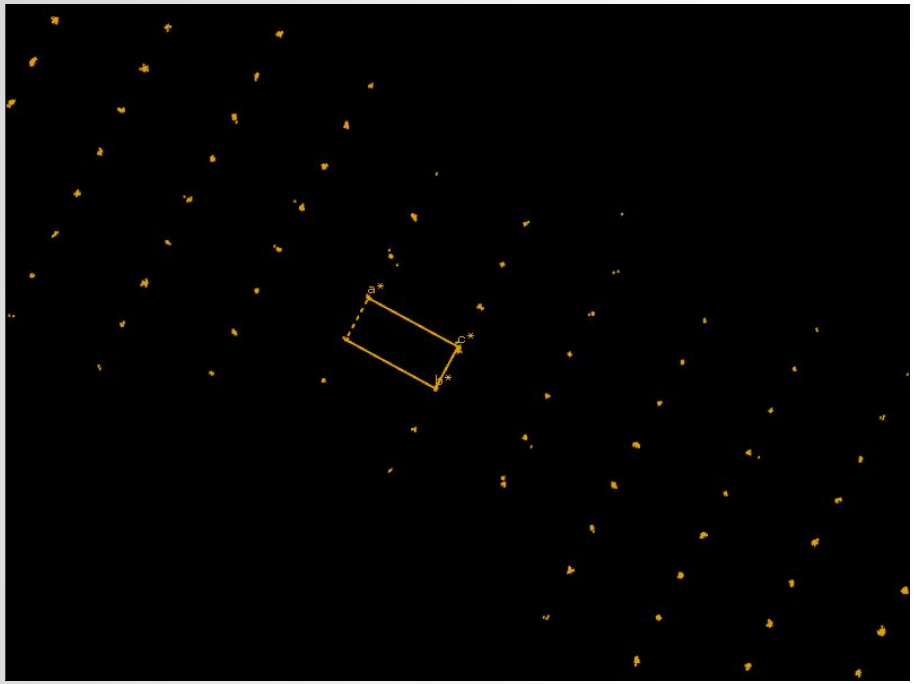


CCP4

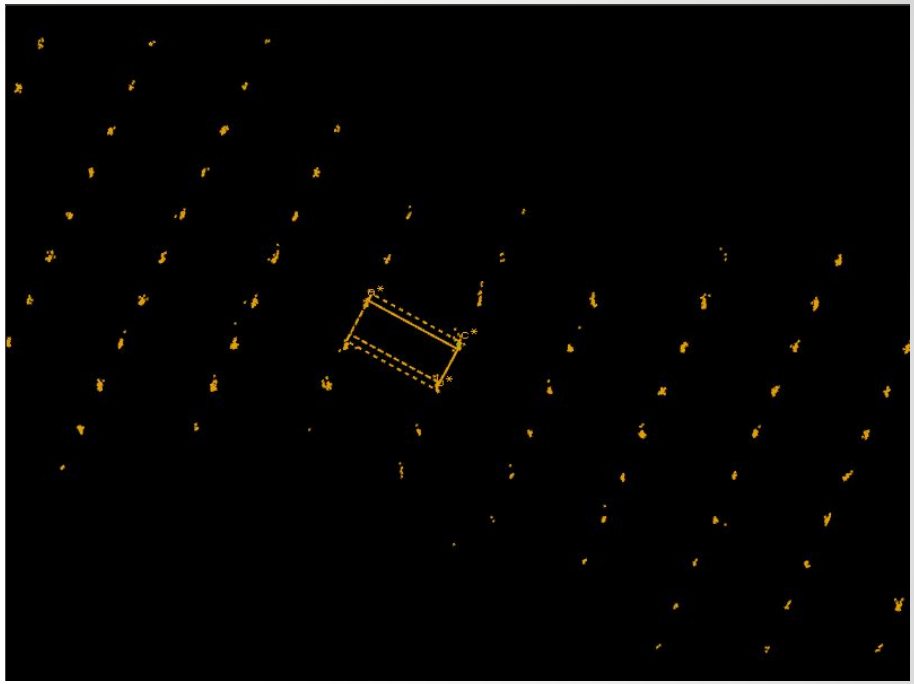
DIALS
Diffraction Integration for Advanced Light Sources

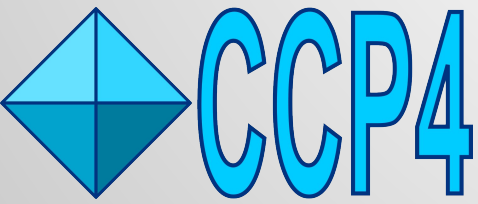
ED geometry

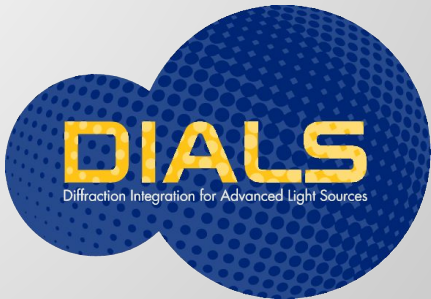
right rotation axis

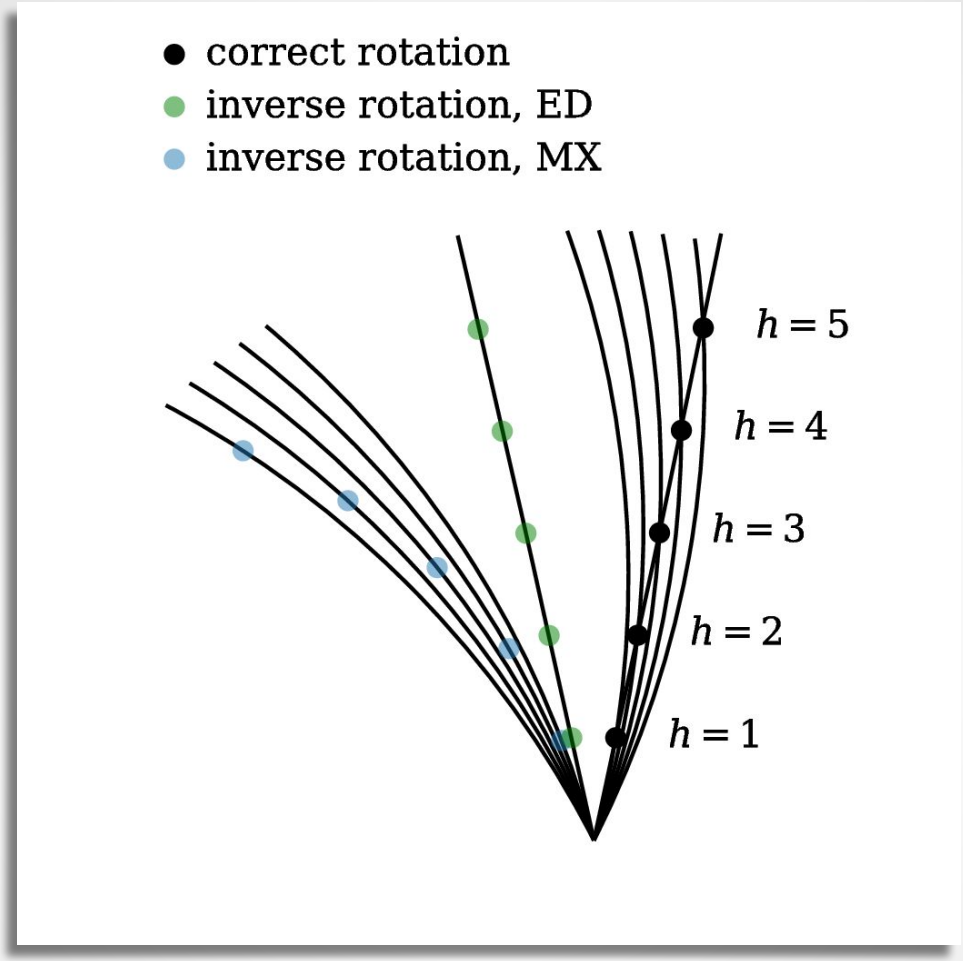


inverted rotation axis







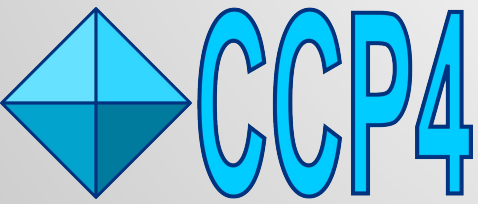


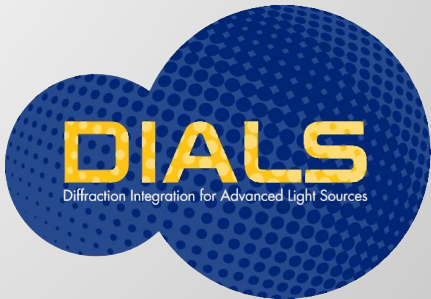
Clabbers *et al.* 2018

Challenges

Almost flat Ewald sphere, high detector distance and low diffraction angle

- Lens distortions may introduce systematic error in observed positions
- Indexing from a single image is challenging
- It may even be difficult to determine the direction of rotation
- Joint refinement of detector and unit cell may not be possible
- Refined detector and unit cell parameters may be poor
- The beam centre may drift

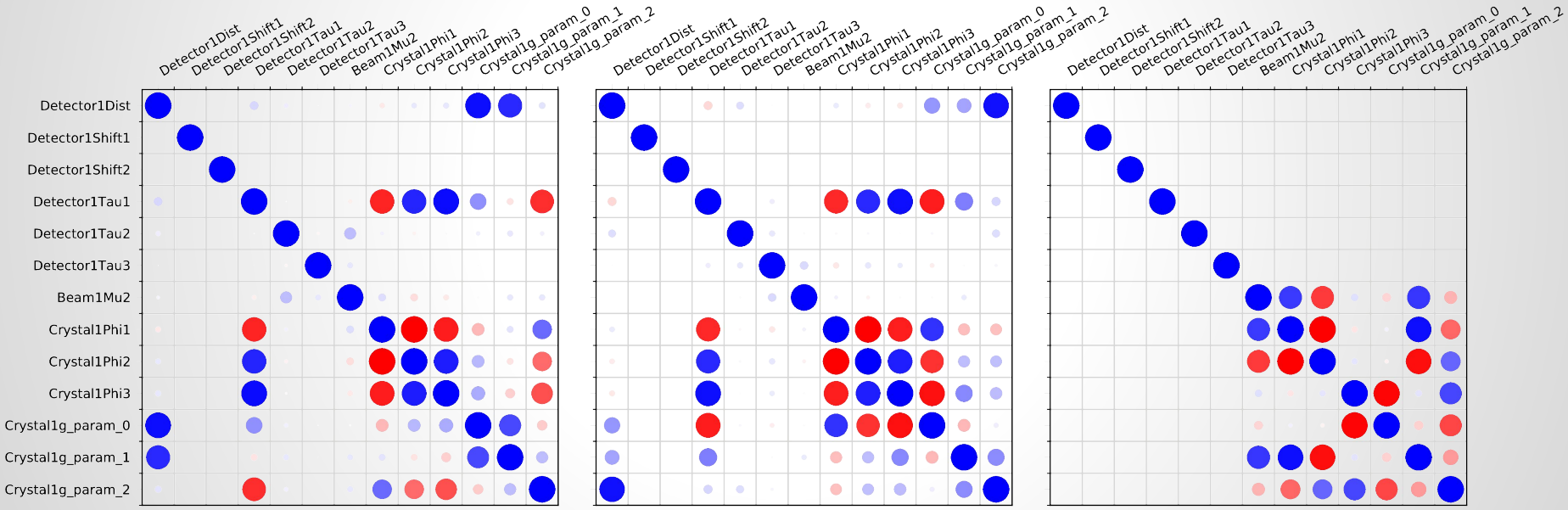




Diagnostics for problematic refinement

DIALS provides diagnostics using the Jacobian from non-linear least squares refinement of the diffraction geometry

1. **corrgrams** Simulated data, MX geometry (12 keV, 200 mm distance)



X residuals Y residuals ϕ residuals

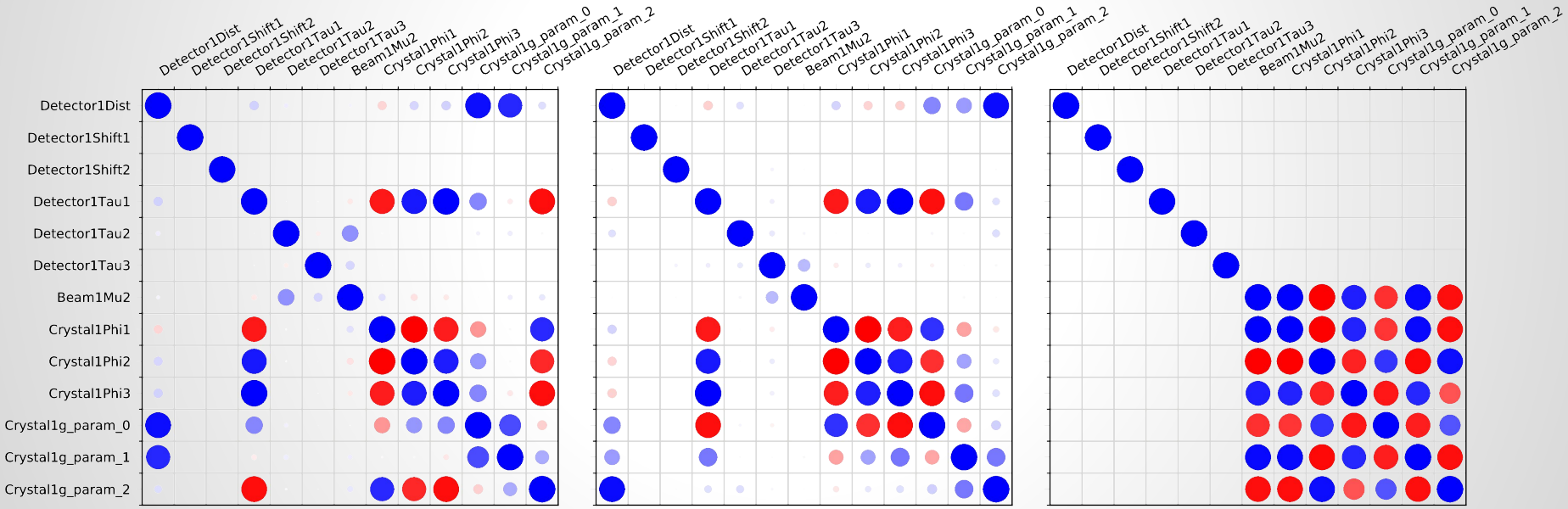
CCP4

DIALS
Diffraction Integration for Advanced Light Sources

Diagnostics for problematic refinement

DIALS provides diagnostics using the Jacobian from non-linear least squares refinement of the diffraction geometry

1. **corrgrams** Simulated data, ED geometry (200 keV, 1570 mm distance)



X residuals Y residuals ϕ residuals

CCP4

DIALS
Diffraction Integration for Advanced Light Sources

Diagnostics for problematic refinement

DIALS provides diagnostics using the Jacobian from non-linear least squares refinement of the diffraction geometry

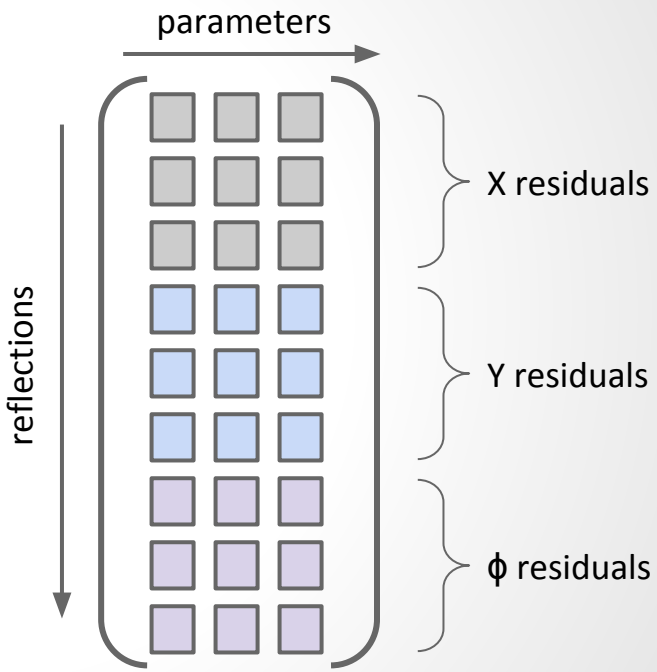
2. condition number

Linearised step: $\mathbf{J}\,\Delta\mathbf{p} = \Delta\mathbf{r}$

For the simulated examples shown:

$$\mathrm{cond}(\mathbf{J}_{MX}) \approx 2 \times 10^{3}$$

$$\mathrm{cond}(\mathbf{J}_{ED}) \approx 5 \times 10^{5}$$



CCP4

DIALS
Diffraction Integration for Advanced Light Sources

Stabilising refinement

General rule: stabilise refinement by constraints (fix) or restraints

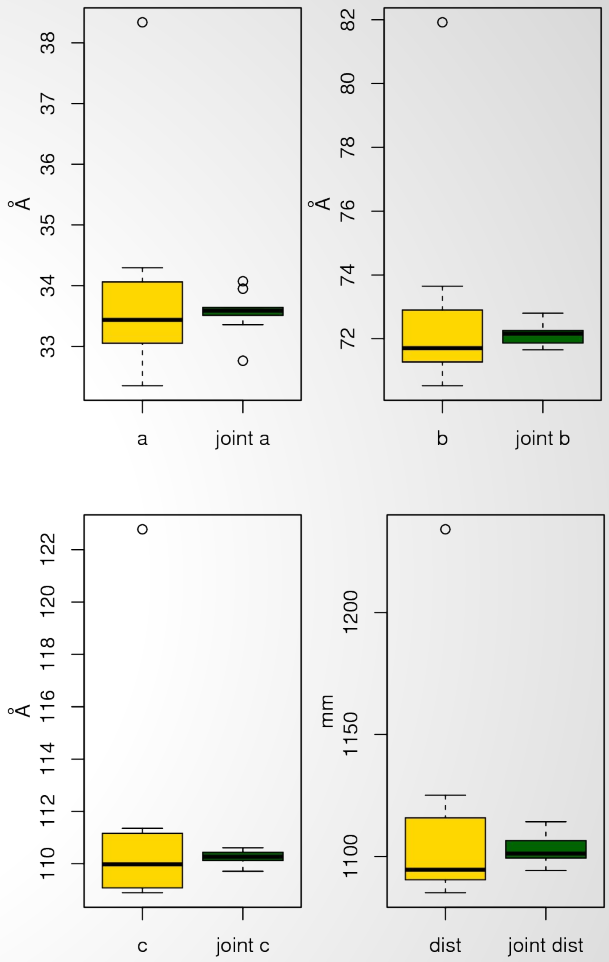
Diagnostics help to identify problematic parameters

Flat Ewald sphere → extremely high correlation between cell and detector distance

Best case scenario: *effective distance is well-calibrated, so fix it*

Other unstable parameters: detector 'tilt' and 'twist' rotations, and beam orientation

Restrain unit cell to a target, or group average in joint refinement



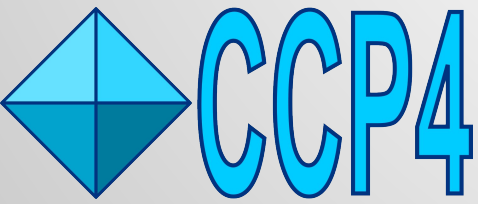
CCP4

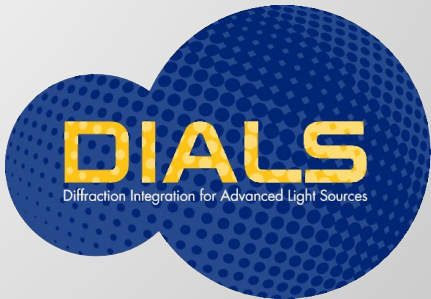
DIALS
Diffraction Integration for Advanced Light Sources

Challenges

Almost flat Ewald sphere, high detector distance and low diffraction angle

- Lens distortions may introduce systematic error in observed positions
- Indexing from a single image is challenging
- It may even be difficult to determine the direction of rotation
- Joint refinement of detector and unit cell may not be possible
- Refined detector and unit cell parameters may be poor
- The beam centre may drift





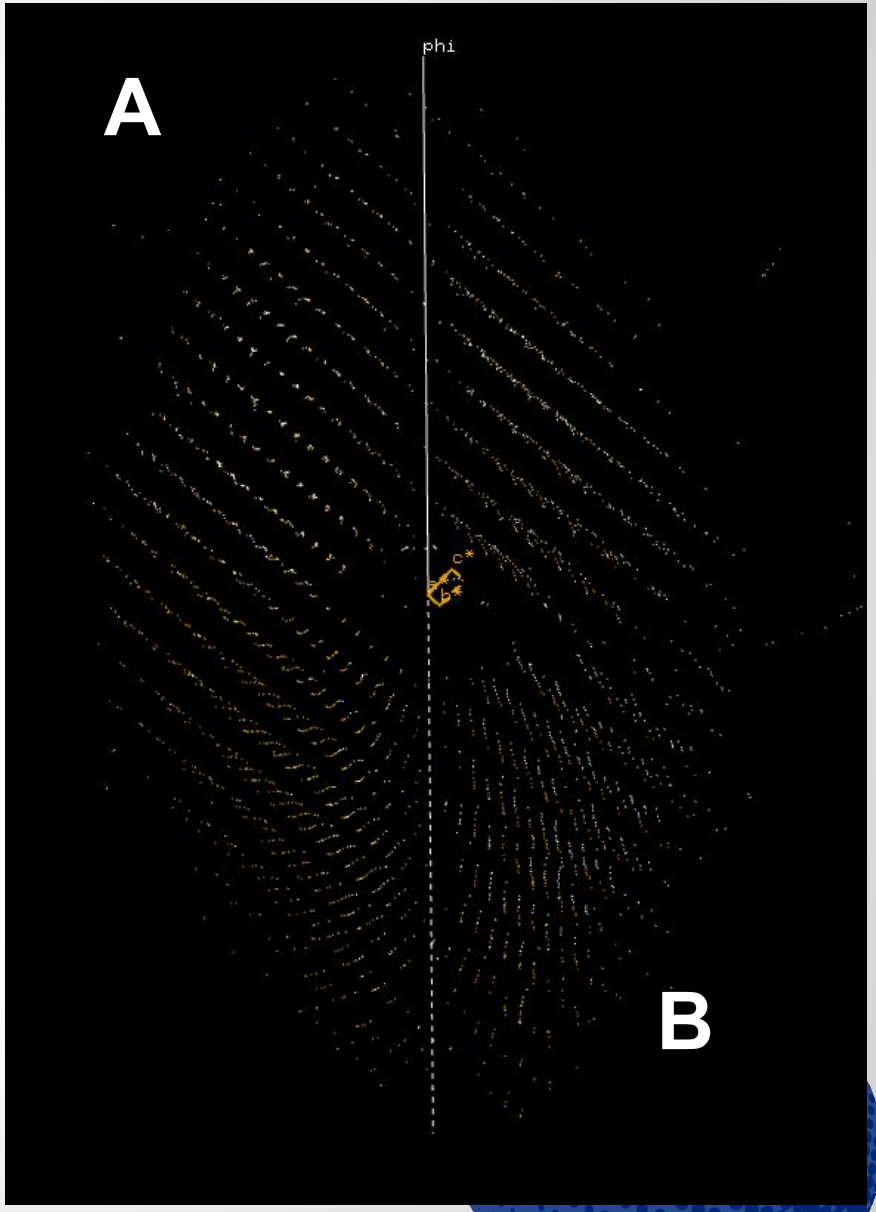
Beam drift

Example: FIB-milled lysozyme still tilt series from eBIC

Beam direction drift during the data collection causes severe distortion

Nevertheless, spots can be indexed by `dev.dials.creep_index`

The reciprocal lattice view is aligned along planes in the region marked **A**, however in other regions, such as **B**, the observations do not map to parallel planes.

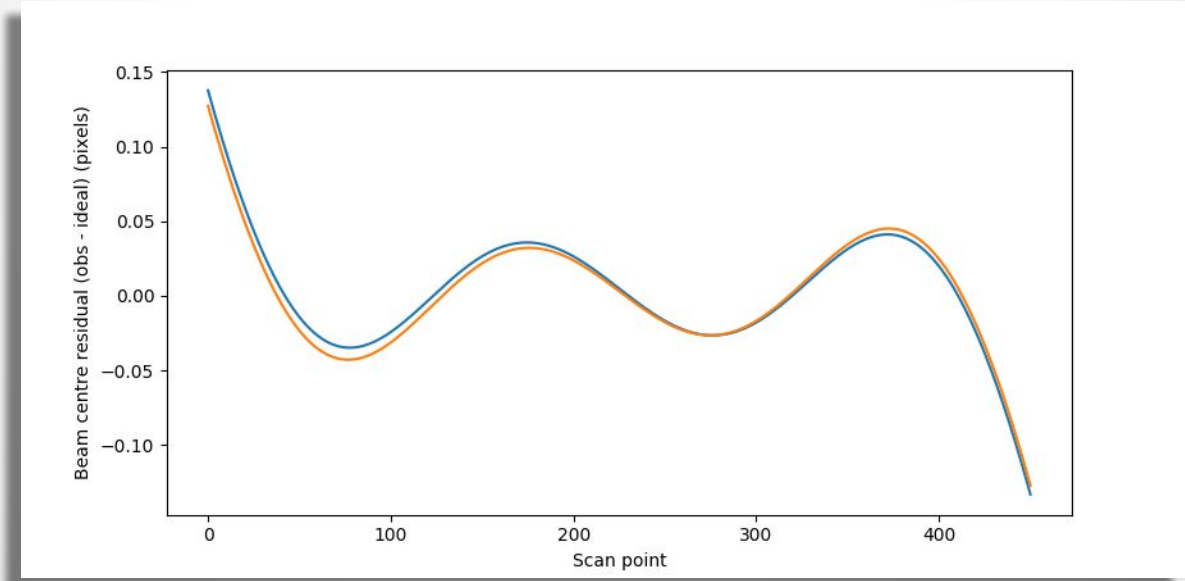


Beam drift

Less severe beam drift seems common even with continuous rotation

In order to process Max Clabbers' lysozyme nanocrystal data we added Gaussian-smoother scan-varying beam refinement to DIALS

Tested using simulated images created with simTBX

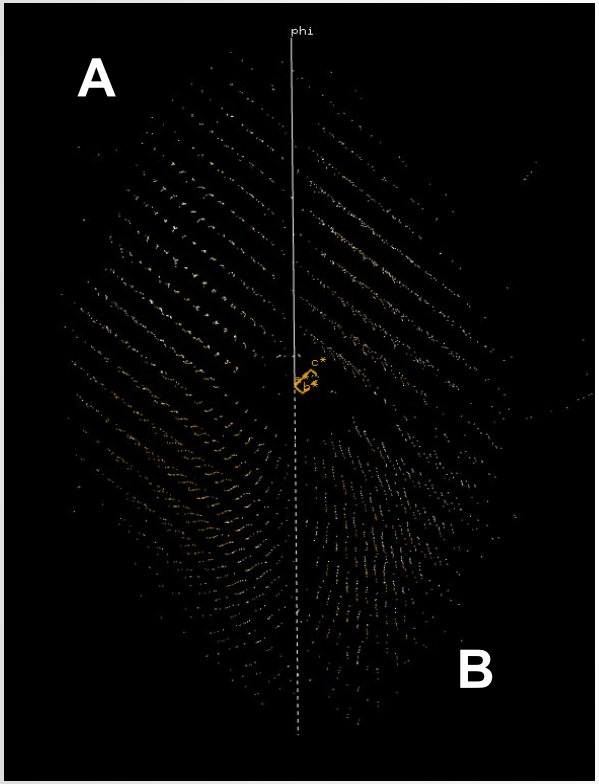


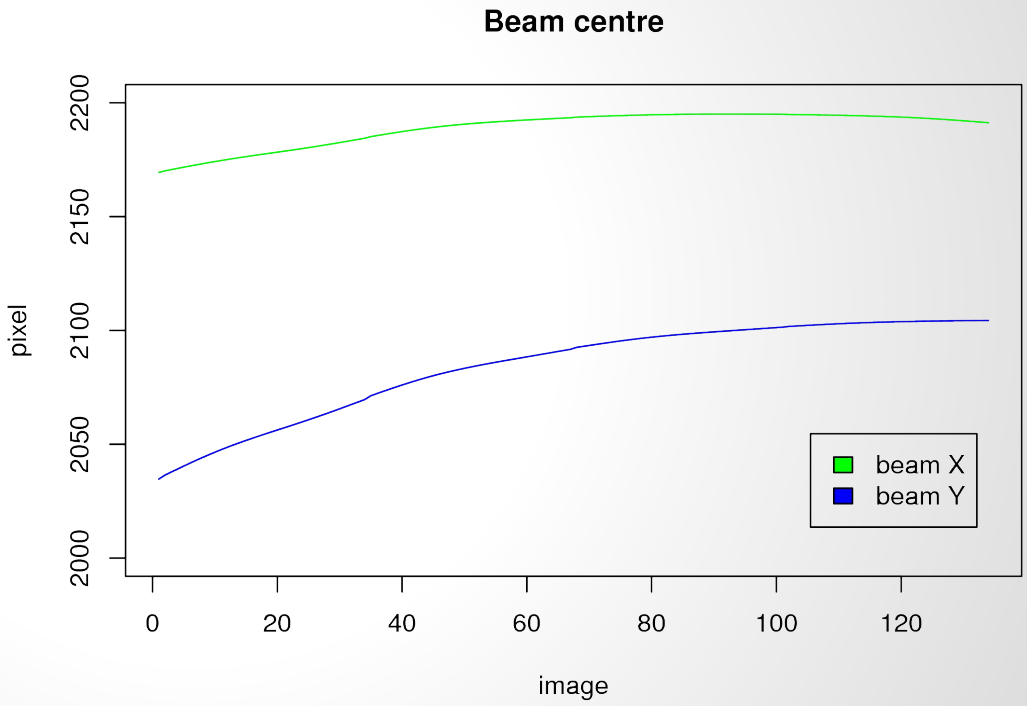
CCP4

DIALS
Diffraction Integration for Advanced Light Sources

Beam drift

Can track the beam drift of the lamella tilt series: about 70 pixels in Y!





Integration

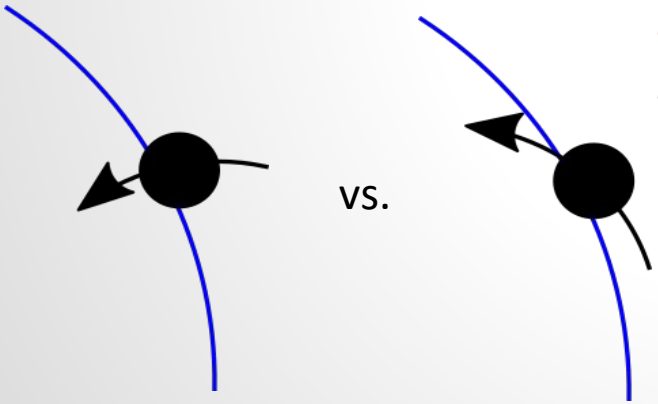
Most programs perform empirical profile modelling using local strong spots.

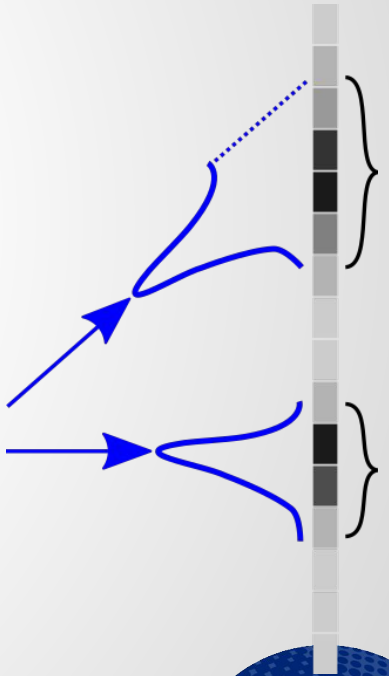
DIALS makes 3D profile models, like **XDS**.

The 3D method takes advantage of the Kabsch transform.

- **geometry**
- crystal mosaicity
- crystal shape
- crystal size

- beam size
- beam divergence
- beam spectral dispersion
- **obliquity**
- detector point spread





CCP4

DIALS
Diffraction Integration for Advanced Light Sources

Integration

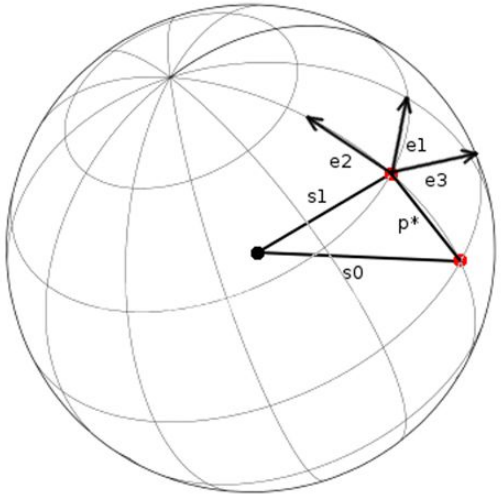
Kabsch's transform:

Spot shape is distorted by:

1. obliquity of rays on the detector
2. obliquity of the relp's passage through the Ewald sphere

1. is handled by mapping pixel values onto the Ewald sphere.

2. is handled by choosing a non-orthogonal third dimension along the relp's actual direction of travel.



Profile coordinate system

Use the Kabsch model of a normal distribution on the surface of the Ewald sphere

2 parameters:

σ_D - roughly "beam divergence"
σ_M - roughly "mosaicity"

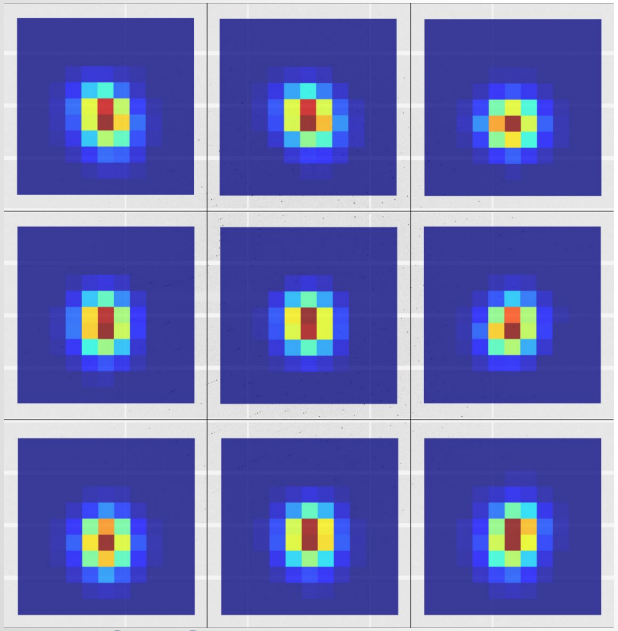
CCP4

DIALS
Diffraction Integration for Advanced Light Sources

Integration

3D profiles are formed for different regions of the detector and different blocks of the ϕ-scan.

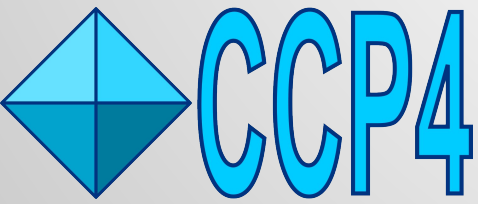
DIALS uses a rectangular grid on the detector, and overlapping blocks in ϕ, with size chosen so that each reflection is integrated fully in one block

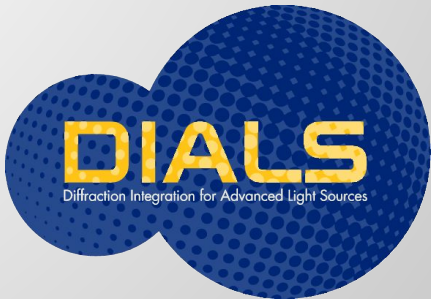


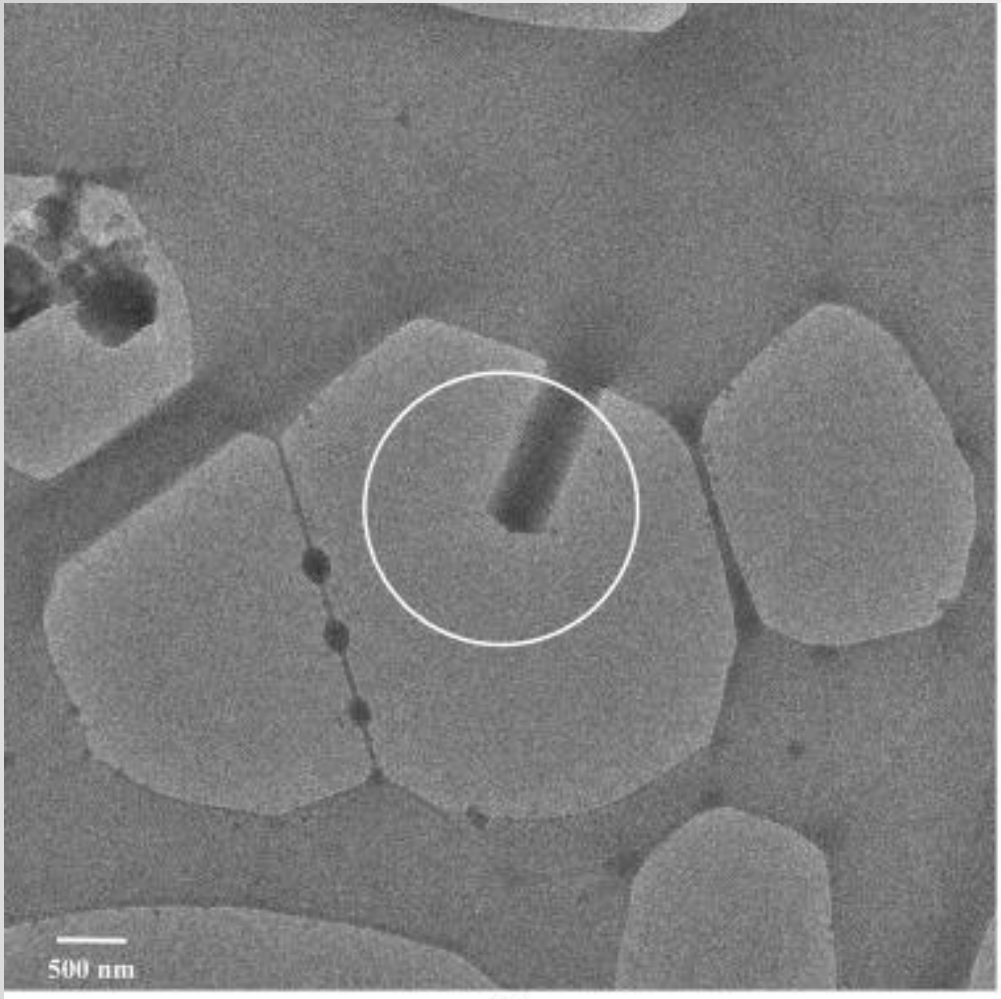
CCP4

DIALS
Diffraction Integration for Advanced Light Sources

Example processing



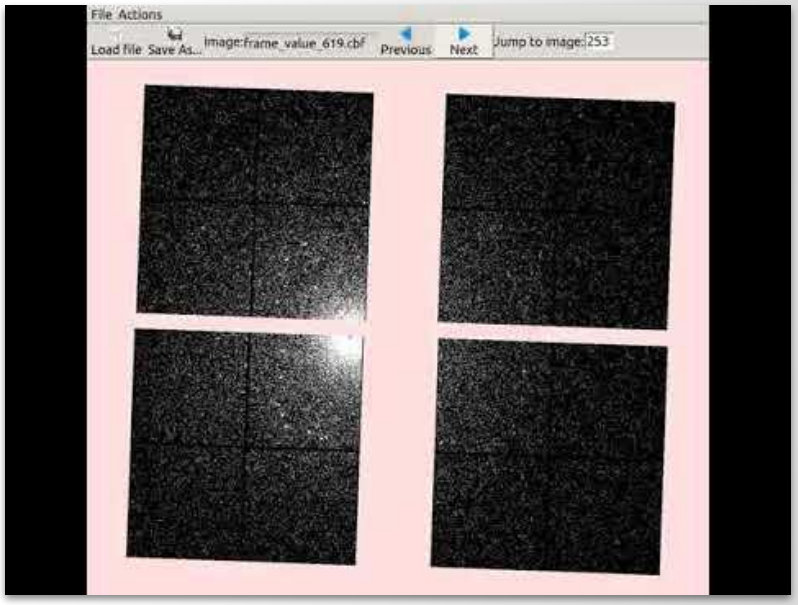




Lysozyme nanocrystal

Diffracting volume: 0.14 μm^3
(< 6×10^5 unit cells)

Clabbers *et al.* Acta Cryst D 2017; 2018



CCP4

DIALS
Diffraction Integration for Advanced Light Sources

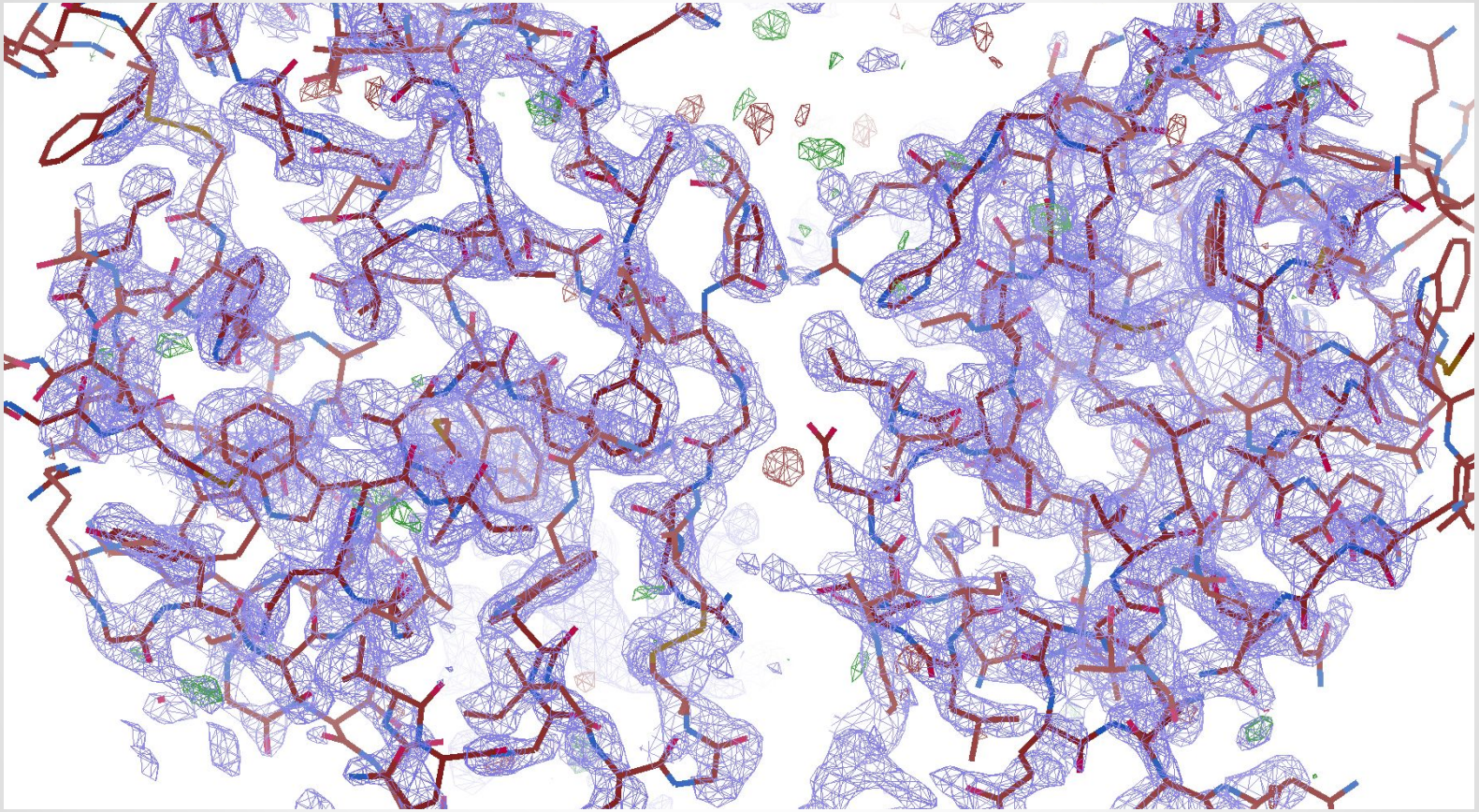
ED structure solution with CCP4

- 7 datasets integrated with **DIALS**, including elliptical distortion and beam drift corrections as described https://dials.github.io/documentation/tutorials/dials_for_ed.html
- Combined and scaled with **pointless** and **aimless** (~60% complete to 2.1 Å)
- Phased by MR with **phaser** using `FORMFACTORS ELECTRON`
- Model building by **buccaneer**
- Unit cell refinement performed by **Refmac5**
- Final model refinement by **Refmac5** and **PDB_REDO**, using SOURCE `ELECTRON MB; MAPC FREE EXCLUDE`
- Robust validation for incomplete data using *Rcomplete* rather than *Rfree*
- ***Rwork*=25.2%; *Rcomplete*=29.2%**
- Demonstrated equivalent data quality to that obtained by XDS (Clabbers et al., Acta Cryst D 2018)

CCP4

DIALS
Diffraction Integration for Advanced Light Sources

ED structure solution with CCP4

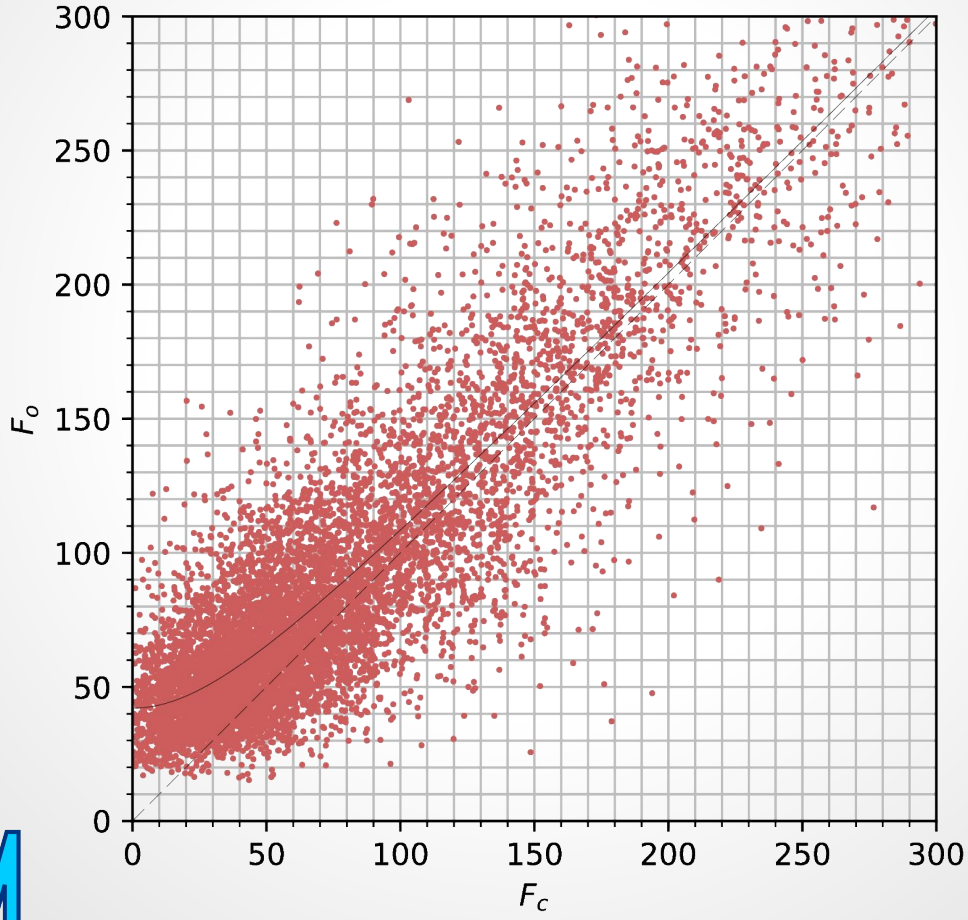


CCP4

DIALS
Diffraction Integration for Advanced Light Sources

Breakdown of the kinematic assumption

```
dials.plot_Fo_vs_Fc hklin=refined.mtz
```



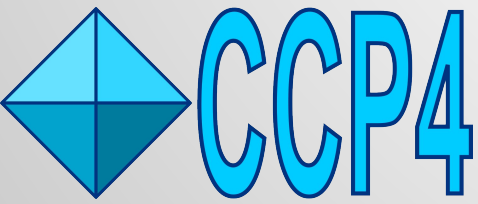
CCP4

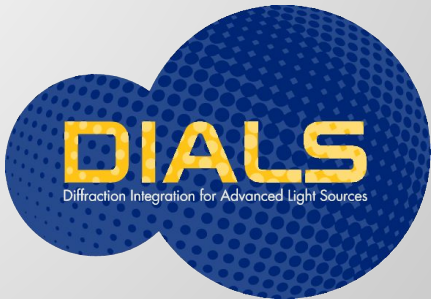
DIALS
Diffraction Integration for Advanced Light Sources

CCP4

DIALS
Diffraction Integration for Advanced Light Sources

Conclusions





Conclusions

Adapting DIALS for ED required some new features: e.g. image format readers, elliptical distortion correction and smoothly varying beam

If initial experiment metadata is good enough, indexing is usually okay

Modelling accurate diffraction geometry may be challenging

Refinement diagnostics plus trial and error to find the right protocol

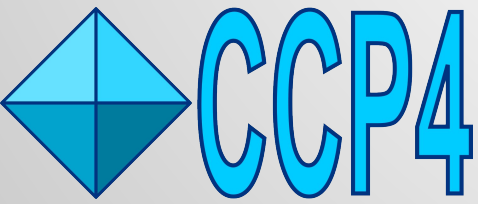
No special requirements for integration (found so far)

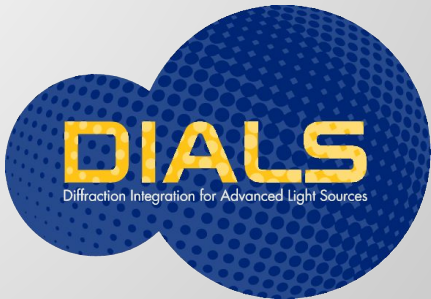
Phasing (MR) & refinement works, though room for optimisation

More appropriate models for electron scattering will help

Current hardware is sufficient but not ideal

Experience with (a limited number) of examples → immediate and largest gains likely to be in hardware and experiment rather than software





Acknowledgements

Development

James Parkhurst

Simulation

James Holton

Discussion

Andrew Leslie

Johan Hattne

Example datasets

Tim Grüne (PSI)

Ronan Keegan (CCP4)

Max Clabbers (Basel/PSI)

Jan Pieter Abrahams (Basel/PSI)

Taimin Yang (MMK)

Hongyi Xu (MMK)

Hugo Lebrette (DDB)

Martin Högbom (DDB)

Xiaodong Zou (MMK)

Lysozyme lamella

Emma Beale (DLS)

Helen Ginn (STRUBI)

Felix de Haas (FEI)

Corey Hecksel (eBIC)

Abhay Kotecha (STRUBI)

Jason van Rooyen (DLS)

Jose Trincao (DLS)

Gwyndaf Evans (DLS)

Peijun Zhang (eBIC)

Dave Stuart (DLS/STRUBI)

Funding

wellcome

diamond

UKRI Science and Technology Facilities Council

CCP4

DIALS Diffraction Integration for Advanced Light Sources