

# Software tools for the analysis of serial femtosecond crystallography (SFX)

Nadia Zatsepin, ASU

## Thank you

- Tom White, CFEL/DESY
- Anton Barty, CFEL/DESY
- Rick Kirian, CFEL/DESY, ASU
- John Spence, ASU, LBNL
- Henry Chapman, CFEL/DESY, U.Hamburg

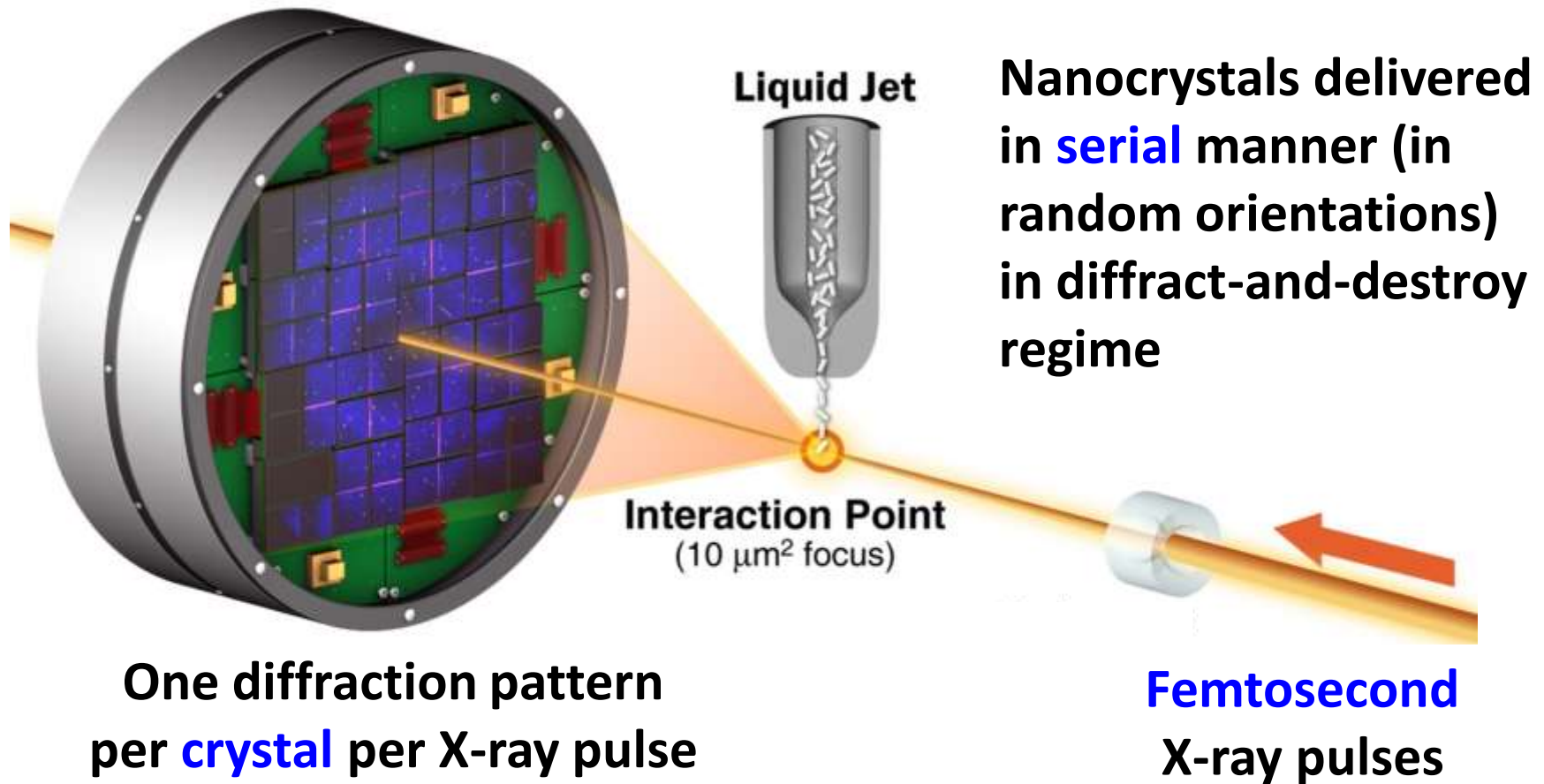
Crystallography at XFEL Sources  
Workshop, August 5<sup>th</sup>, 2014



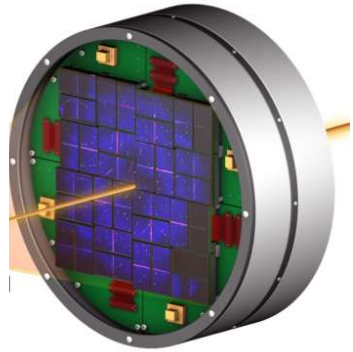
[bioxfel.org](http://bioxfel.org)

Software for serial crystallography  
Nadia Zatsepin – IUCr 2014, Aug 5<sup>th</sup>

# Serial femtosecond crystallography (SFX)



# SFX data analysis pipeline



CSPAD

DAQ: XTC files,  
containing X-ray  
pulse parameters,  
diagnostics, motor  
positions, ..

## Cheetah

1. Hit finding (data reduction)
2. Background estimation, removal
3. Clean diff. pattern & meta data → HDF5
4. Statistics & prelim. analysis

## CCP4, Phenix et al.

Phasing, building,  
refinement,  
validation

## CrystFEL

1. Indexing
2. Integration
3. Merging
4. Post refinement
5. Indexing ambiguity removal

RCSB  
**PDB**  
PROTEIN DATA BANK

# Why new software?

- New type of data
- Large amount of data
- New, complicated detectors

LCLS fires at **120 Hz**

CSPAD detector at CXI, LCLS:  $2.3 \times 10^6$  pixels, 16 bit

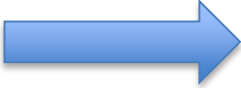
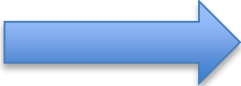
**4.6 MB / frame = 2 TB / hour**

→ Up to ~ **120 TB from a single experiment** (5 shifts) from one detector

European XFEL will run at 27,000 Hz...

# Data retention at LCLS

## Storage Classes



Space	Size	Backup	Lifetime	Storage class	Comment
xtc	Unlimited	Tape archive	6 months	Short-term	Raw data
usr	Unlimited	Tape archive	6 months	Short-term	Raw data from users' DAQ systems
hdf5	Unlimited	Tape archive	6 months	Short-term	Data translated to HDF5
scratch	Unlimited	None	6 months	Short-term	Temporary data
xtc/hdf5	10TB	n/a	2 years	Medium-term	Selected XTC and HDF5 runs
ftc	10TB	None	2 years	Medium-term	Filtered, translated, compressed
res	1TB	Tape	2 years	Medium-term	Analysis results
User home	20GB	Disk + tape	Indefinite		User code
Tape archive	Unlimited	Two copies	10 years	Long-term	Raw data

**Papers take ~2 years to publish:**

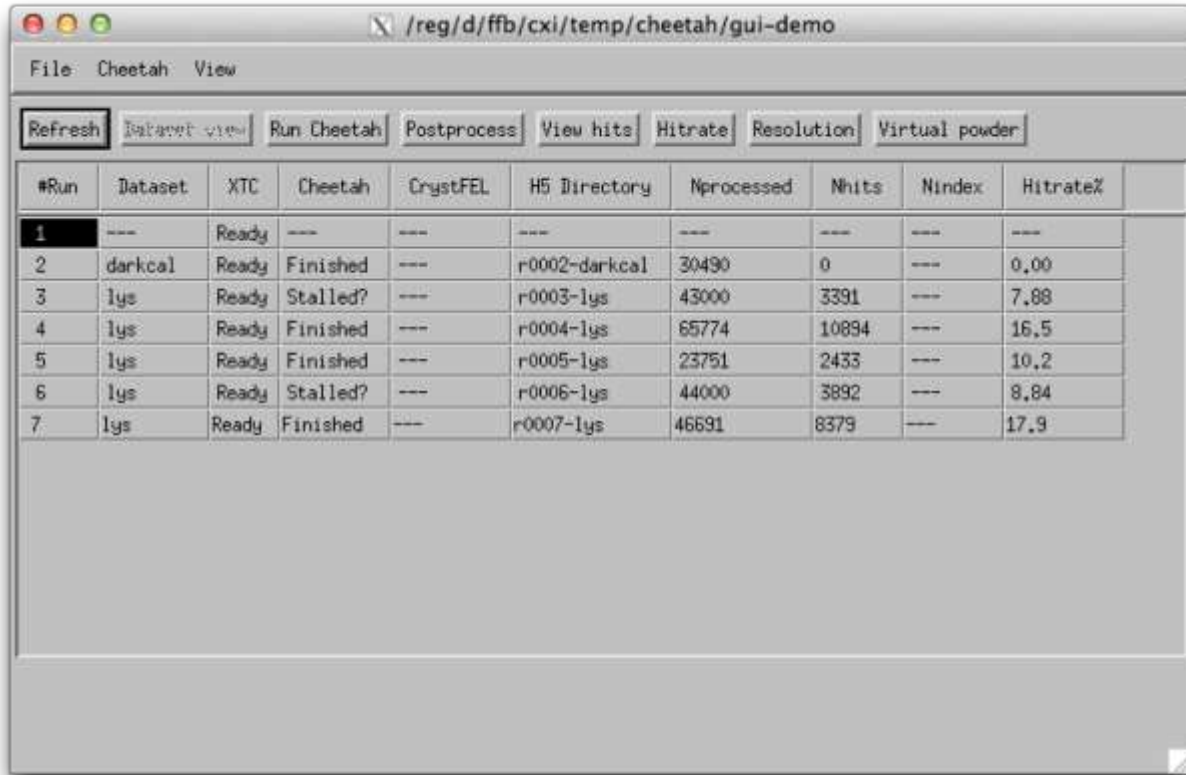
**6 months is very little time for deletion of raw data**

**Need reliable, high throughput data reduction software**

# Cheetah

[www.desy.de/~barty/cheetah](http://www.desy.de/~barty/cheetah)

- Raw data → diffraction frames
- Parallelized
- High throughput
- Live feedback during experiment
- Preinstalled at SLAC

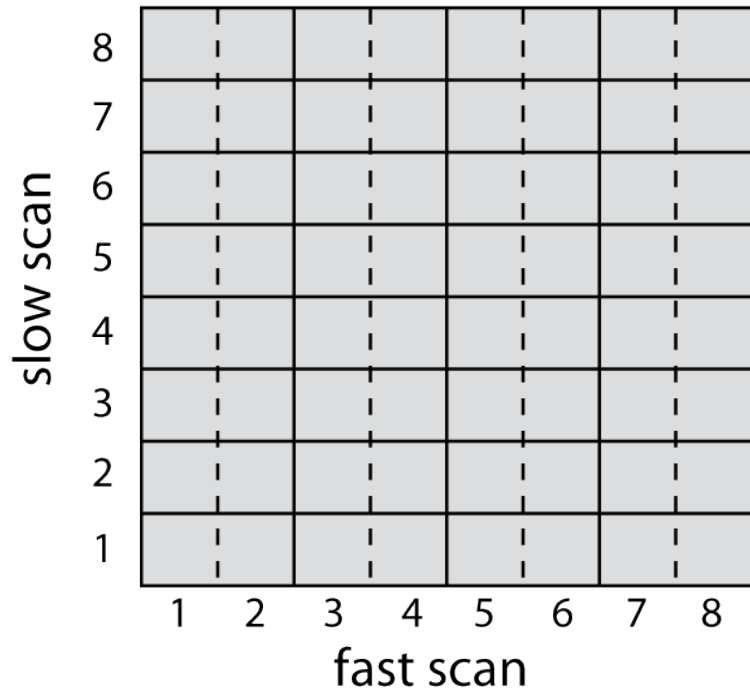


The screenshot shows a web browser window titled "/reg/d/ffb/cxi/temp/cheetah/gui-demo". The browser address bar shows the URL. The page has a menu bar with "File", "Cheetah", and "View". Below the menu bar is a toolbar with buttons: "Refresh", "Dataset view", "Run Cheetah", "Postprocess", "View hits", "Hitrate", "Resolution", and "Virtual powder". The main content is a table with the following columns: #Run, Dataset, XTC, Cheetah, CrystFEL, H5 Directory, Nprocessed, Nhits, Nindex, and Hitrate%. The table contains 7 rows of data.

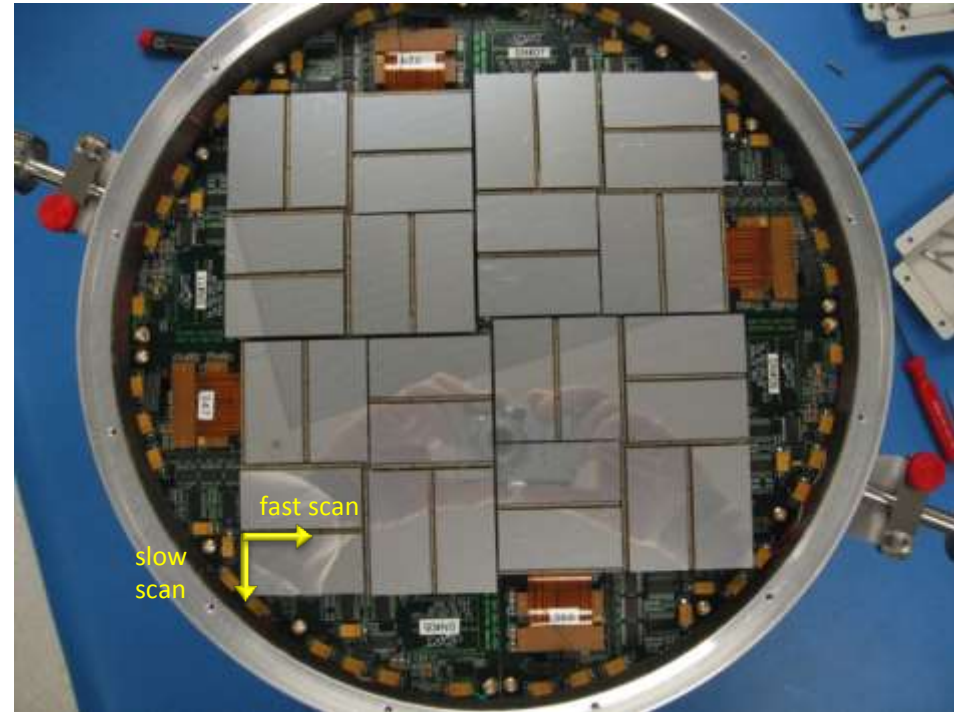
#Run	Dataset	XTC	Cheetah	CrystFEL	H5 Directory	Nprocessed	Nhits	Nindex	Hitrate%
1	---	Ready	---	---	---	---	---	---	---
2	darkcal	Ready	Finished	---	r0002-darkcal	30490	0	---	0,00
3	lys	Ready	Stalled?	---	r0003-lys	43000	3391	---	7,88
4	lys	Ready	Finished	---	r0004-lys	65774	10894	---	16,5
5	lys	Ready	Finished	---	r0005-lys	23751	2433	---	10,2
6	lys	Ready	Stalled?	---	r0006-lys	44000	3892	---	8,84
7	lys	Ready	Finished	---	r0007-lys	46691	8379	---	17,9

A. Barty, et al. (2014) Cheetah: software for high-throughput reduction and analysis of serial femtosecond X-ray diffraction data. *J Appl Cryst.* 47 1118.

# CSPAD geometry: non trivial



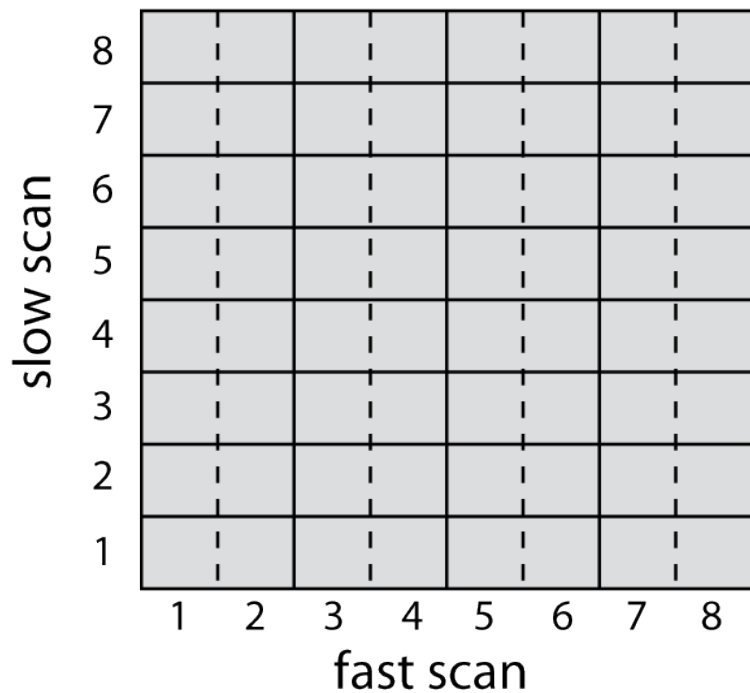
**Data layout**



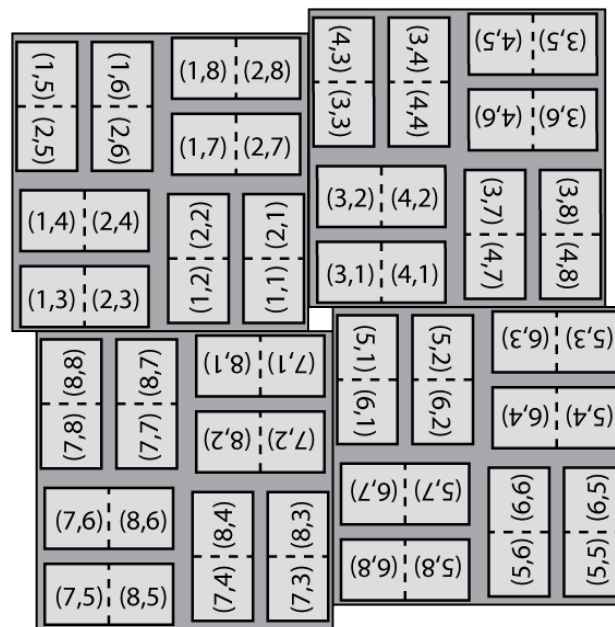
**Physical layout**

H. T. Philipp et al. (2011) *Pixel array detector for x-ray free electron laser experiments*. Nucl Instrum. Methods A **649**, 67.

# CSPAD geometry: non trivial



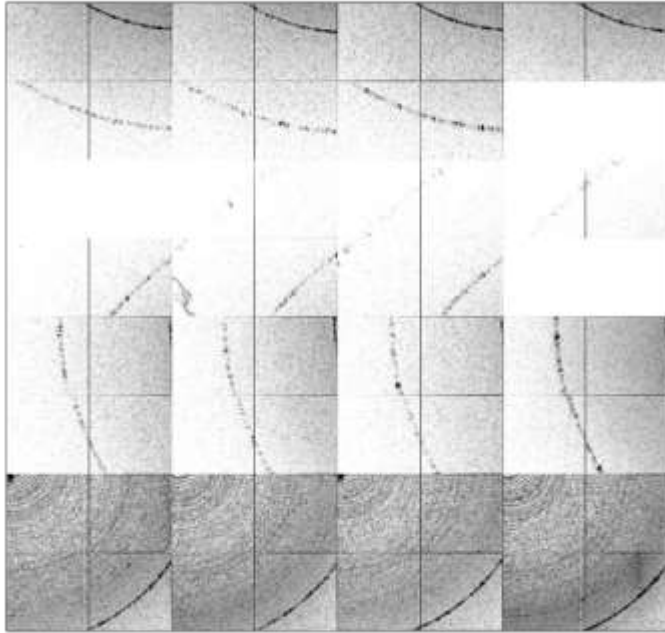
**Data layout**



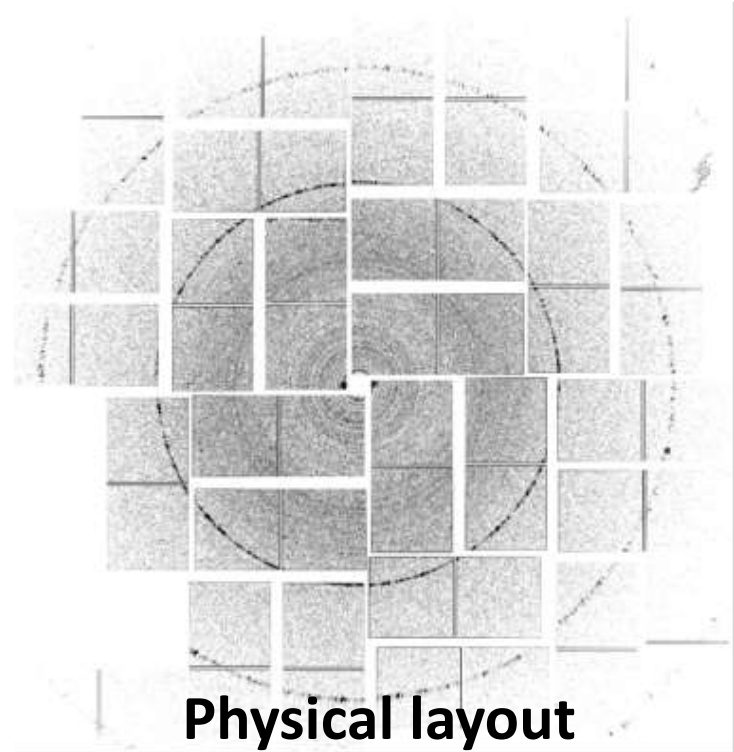
**Physical layout**



# CSPAD geometry: non trivial



**Data layout**  
**Virtual powder**



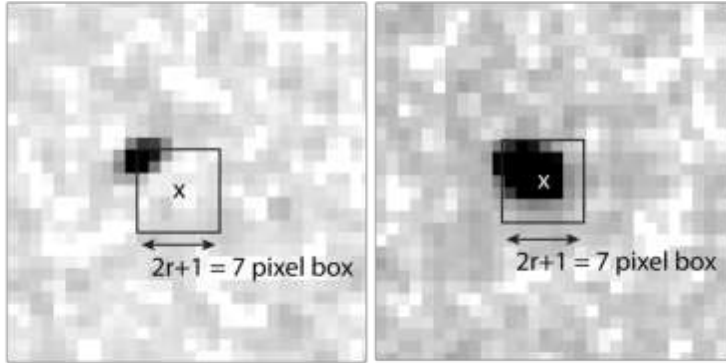
**Physical layout**  
**Virtual powder**

**Data quality is very sensitive to precise geometry.**

**Comparison of predicted and found peak locations can be used to refine the detector geometry**

# Cheetah: background subtraction

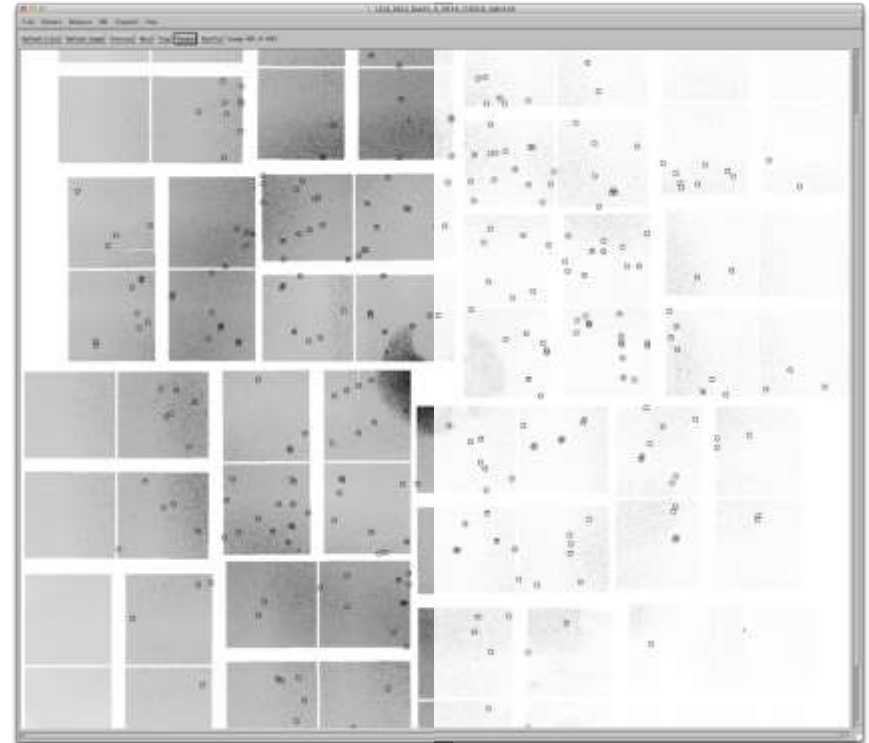
Use data from the current frame to estimate background in current frame



Background = median of pixels in the box

- Assumes peaks are small compared to background variations
- Requires more background pixels in box than peak-containing pixels

**Selection of local background radius is important**

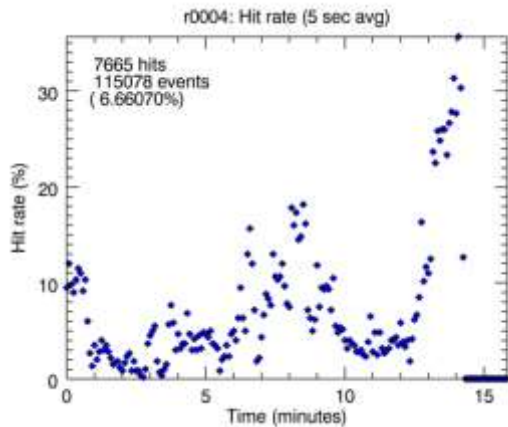


**(right) After local background subtraction**

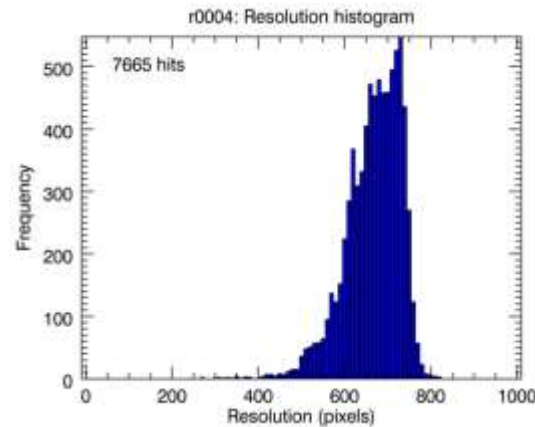
Figures adapted from Anton Barty

# Cheetah: useful diagnostic tool

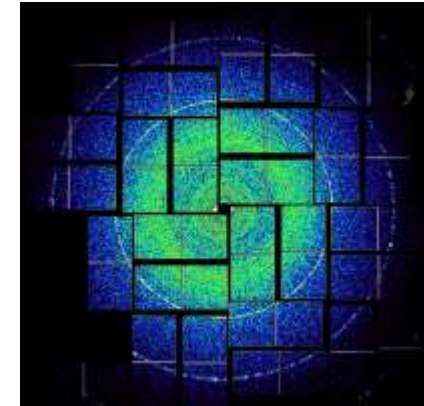
## Hit rates



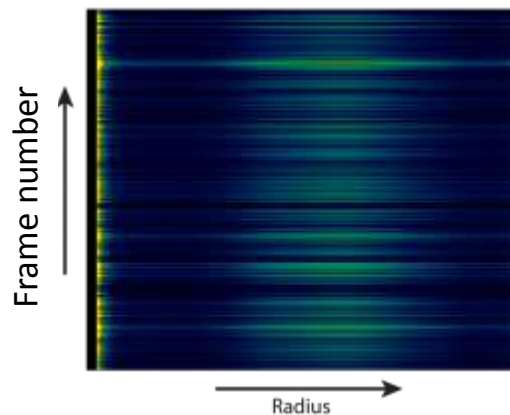
## Resolution



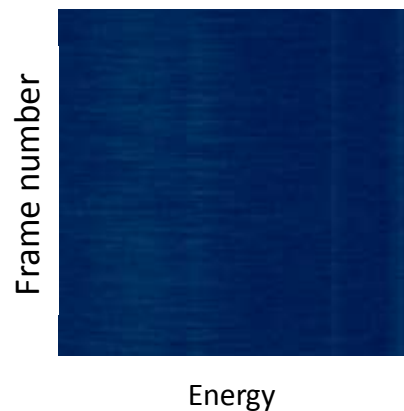
## Virtual powder



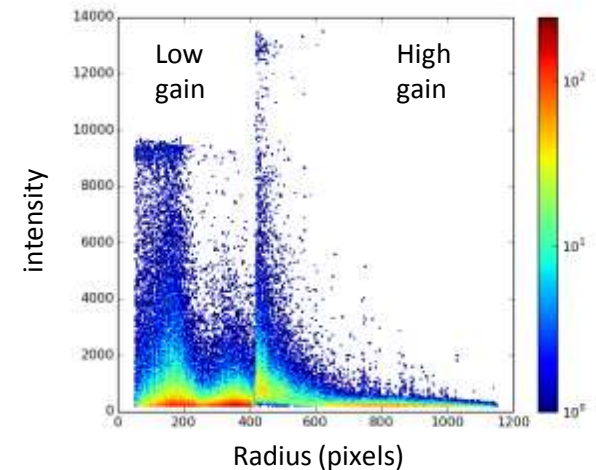
## Radial stacks



## Spectral stack



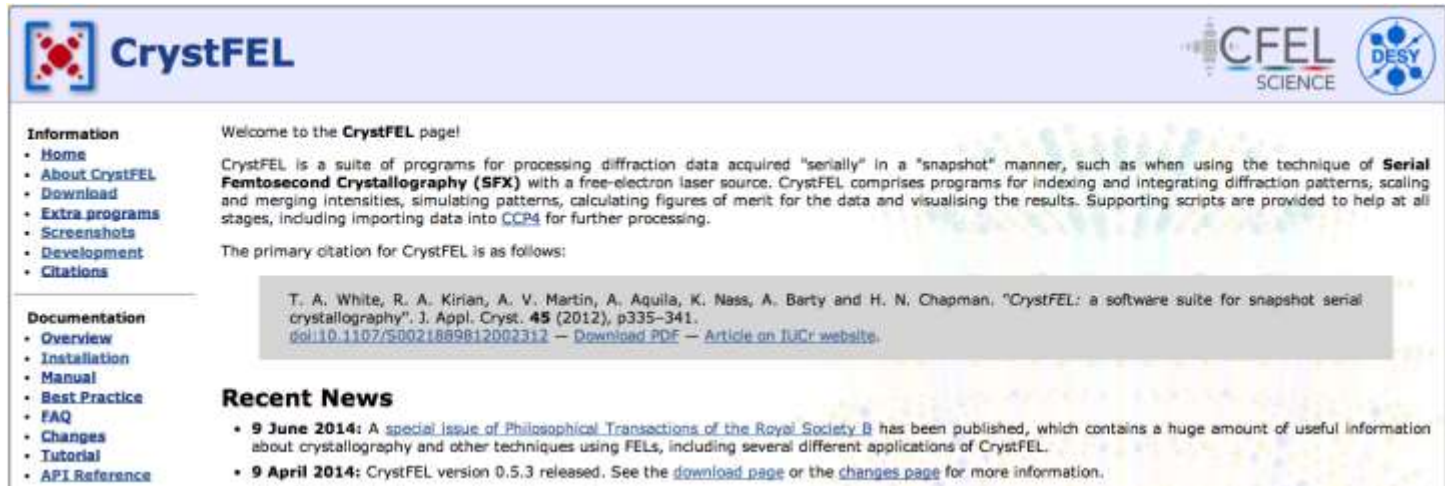
## Saturation plots



# CrystFEL

- Software for serial crystallography data analysis
- Designed for, but not limited to, working with XFEL data
- Free and open-source (GNU GPL3)

[www.desy.de/~twhite/crystfel](http://www.desy.de/~twhite/crystfel)



The screenshot shows the CrystFEL website homepage. At the top left is the CrystFEL logo, and at the top right are the CFEL SCIENCE and DESY logos. The main content area is divided into three sections: Information, Documentation, and Recent News. The Information section includes links for Home, About CrystFEL, Download, Extra programs, Screenshots, Development, and Citations. The Documentation section includes links for Overview, Installation, Manual, Best Practice, FAQ, Changes, Tutorial, and API Reference. The Recent News section features two entries: one from June 2014 about a special issue in the Philosophical Transactions of the Royal Society B, and another from April 2014 about the release of CrystFEL version 0.5.3. A central text block provides the primary citation for CrystFEL.

**Information**

- Home
- About CrystFEL
- Download
- Extra programs
- Screenshots
- Development
- Citations

**Documentation**

- Overview
- Installation
- Manual
- Best Practice
- FAQ
- Changes
- Tutorial
- API Reference

Welcome to the **CrystFEL** page!

CrystFEL is a suite of programs for processing diffraction data acquired "serially" in a "snapshot" manner, such as when using the technique of **Serial Femtosecond Crystallography (SFX)** with a free-electron laser source. CrystFEL comprises programs for indexing and integrating diffraction patterns, scaling and merging intensities, simulating patterns, calculating figures of merit for the data and visualising the results. Supporting scripts are provided to help at all stages, including importing data into [CCP4](#) for further processing.

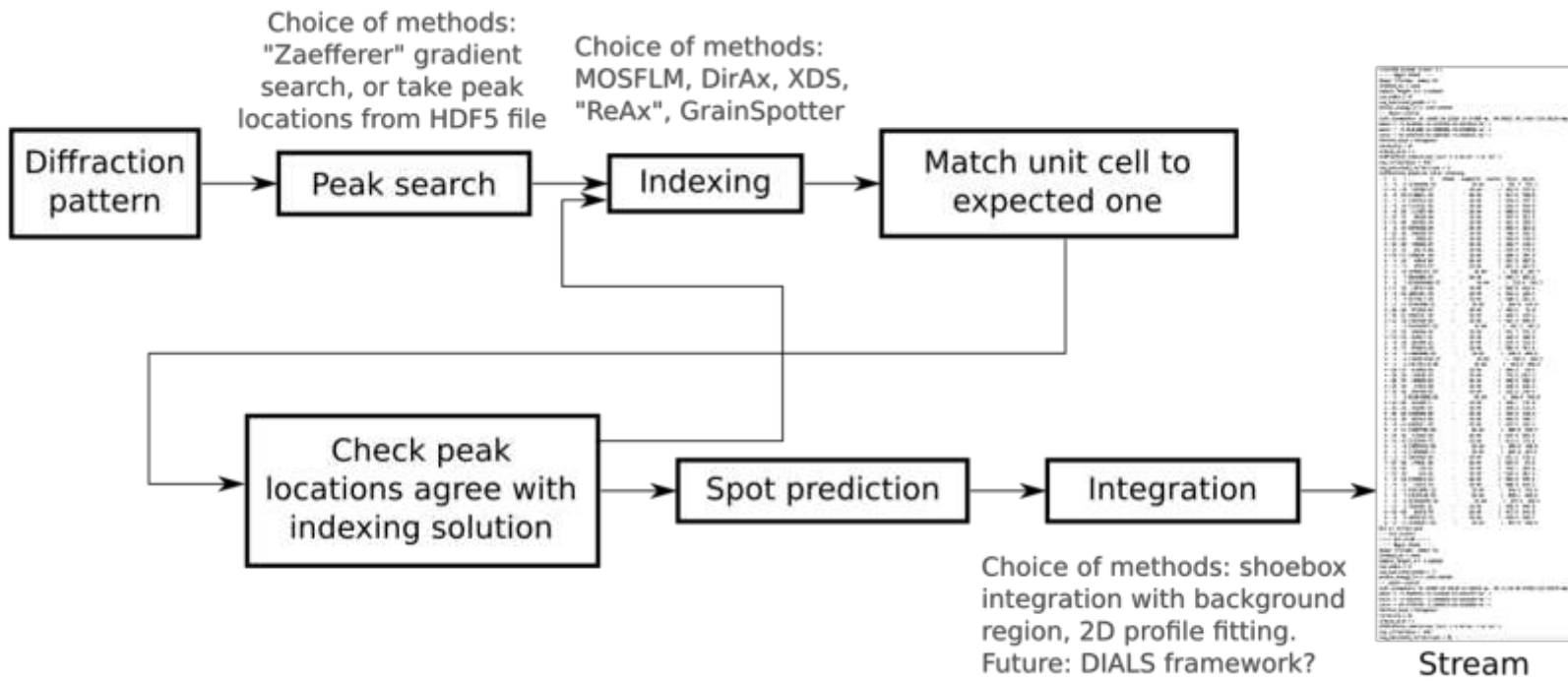
The primary citation for CrystFEL is as follows:

T. A. White, R. A. Kirian, A. V. Martin, A. Aquila, K. Nass, A. Barty and H. N. Chapman, "CrystFEL: a software suite for snapshot serial crystallography". *J. Appl. Cryst.* **45** (2012), p335–341.  
[doi:10.1107/S0021889612002312](https://doi.org/10.1107/S0021889612002312) — [Download PDF](#) — [Article on IUCr website](#).

**Recent News**

- **9 June 2014:** A [special issue of Philosophical Transactions of the Royal Society B](#) has been published, which contains a huge amount of useful information about crystallography and other techniques using FELs, including several different applications of CrystFEL.
- **9 April 2014:** CrystFEL version 0.5.3 released. See the [download page](#) or the [changes page](#) for more information.

# CrystFEL processing pipeline (each image)

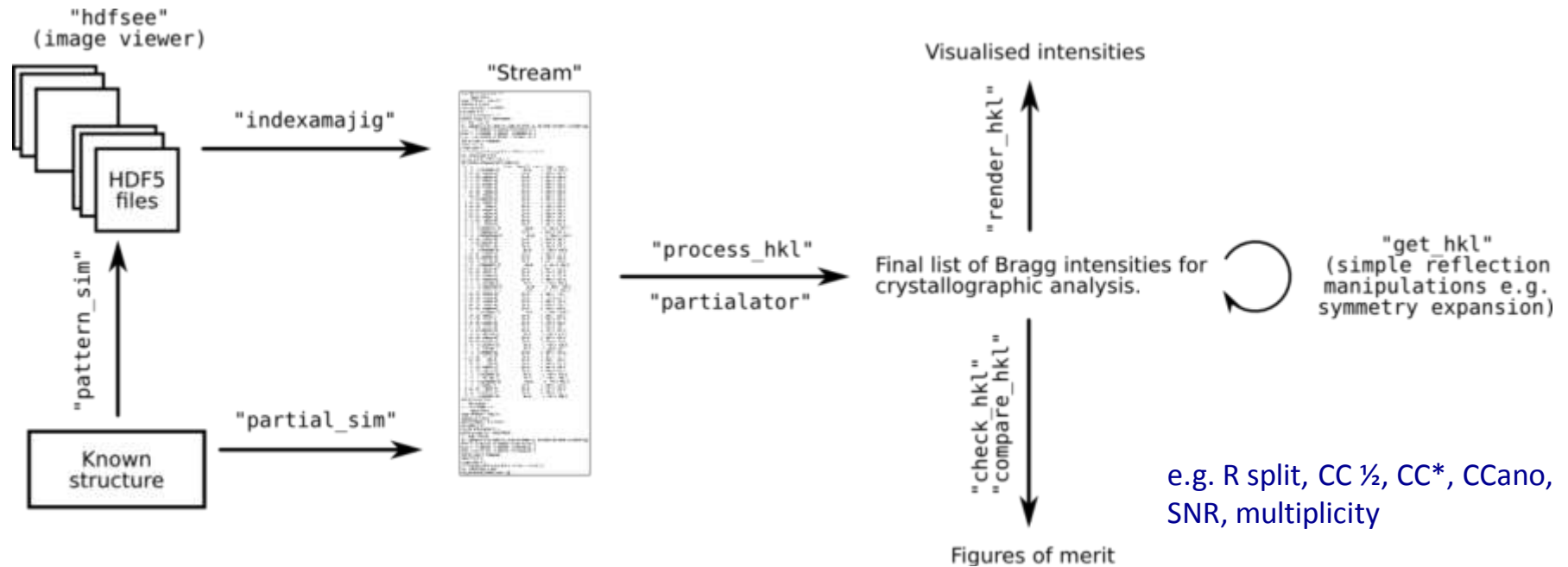


**Once parameters have been optimized, processing is completely automatic, and parallelized.**

Command line driven, easily called from scripts

Well documented; full tutorial online

# CrystFEL's core programs

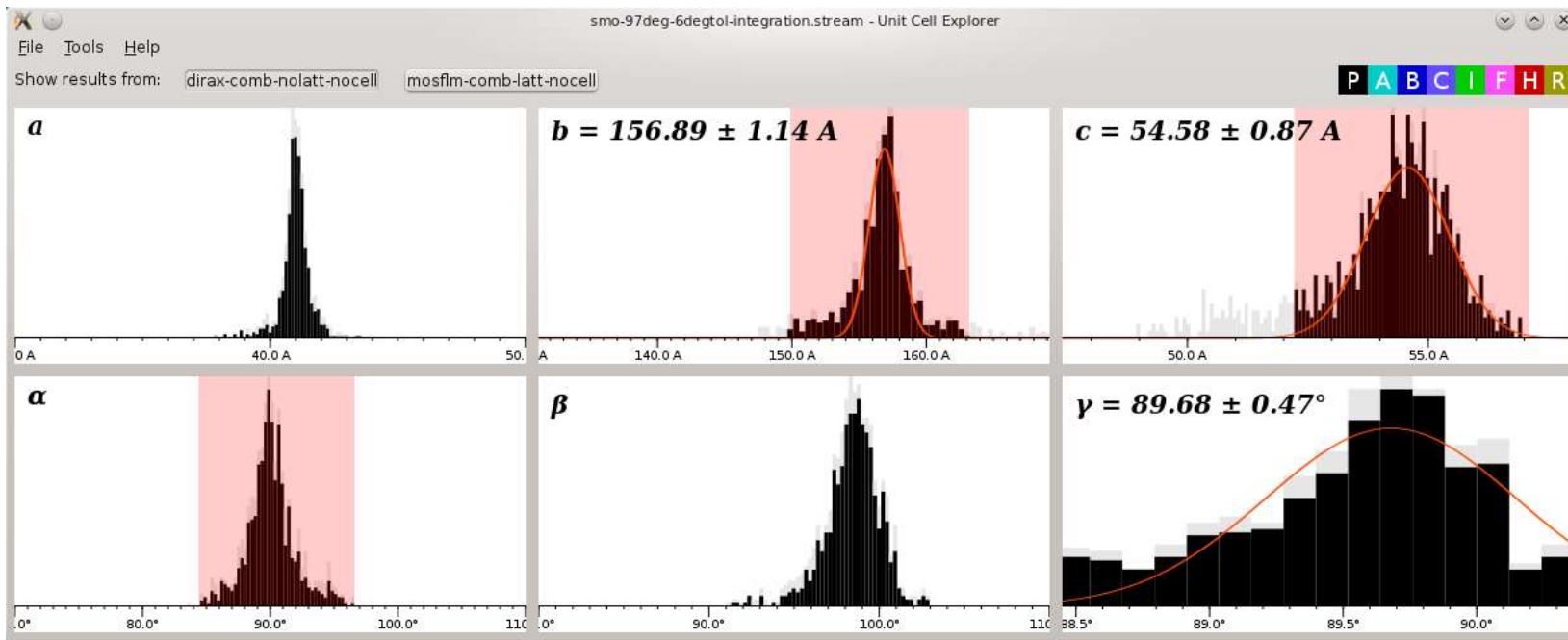


- File formats: HDF5's, everything else is text
- Stream file contains everything about each diffraction pattern: e.g. peaks positions (found; integrated), reflection intensities, cell parameters, indexing method
- Share library contains most of CrystFEL functionality
- External programs can use these functions too

Figure from  
Tom White

# Unknown unit cell?

## CrystFEL's unit cell explorer

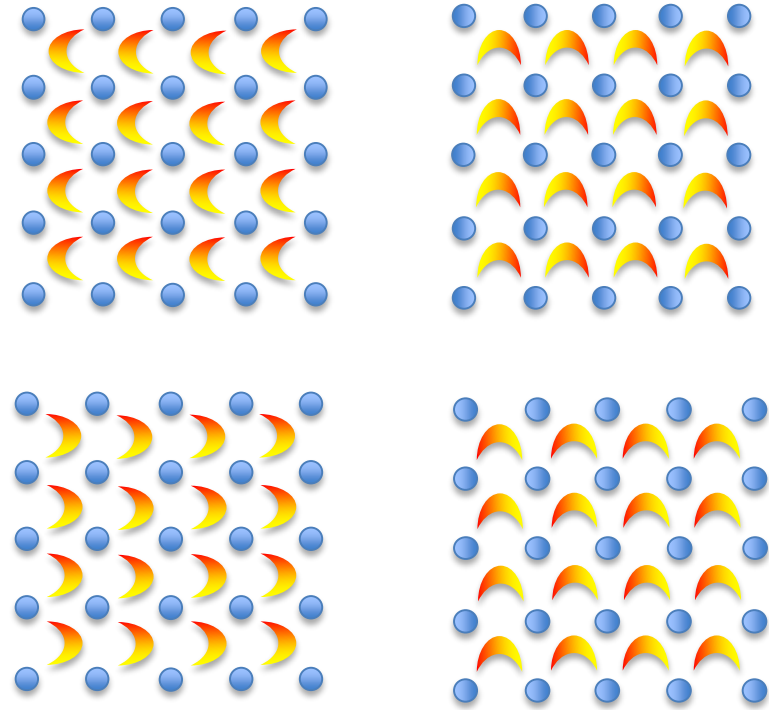


- Displays indexing results from different algorithms / space groups
- Fits Gaussians to calculate mean cell parameters

# Indexing ambiguities

If the lattice has higher symmetry than the unit cell, the diffraction pattern can be indexed in more than one orientation since the lattice has the same geometry in various orientations of the crystal

Autoindexing algorithms can't tell these apart, so for SFX data are (initially) merged in higher symmetry

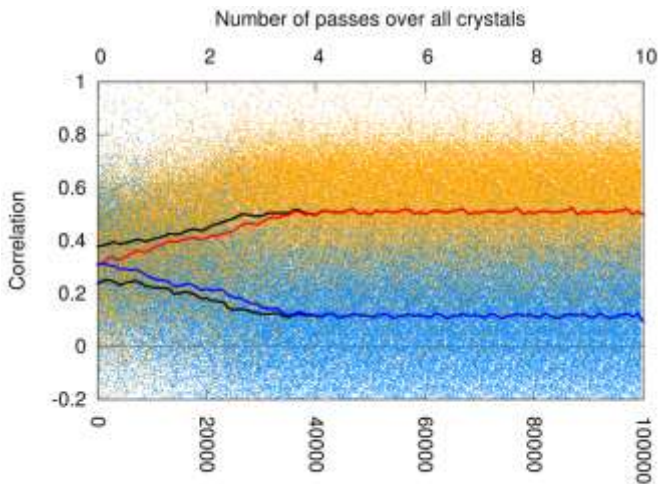


e.g. P63 will have          P6322 symmetry

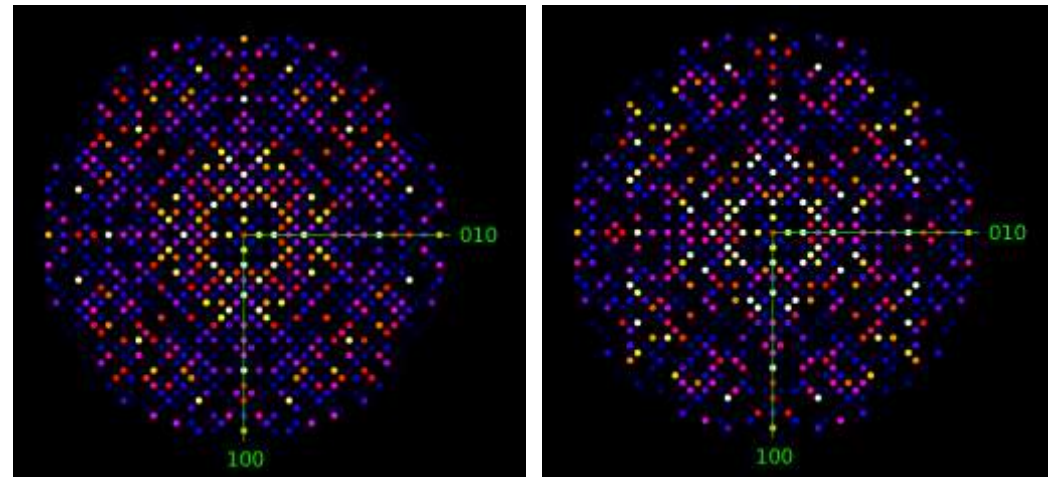


# Ambigator: removing the indexing ambiguity

- Large amounts of noise make it difficult to resolve the ambiguity by correlating intensities.
- Ambiguity can be resolved by a **clustering approach**:
- Brehm & Diederichs (2014) Breaking the indexing ambiguity in serial crystallography. Acta Cryst. D70, 101.



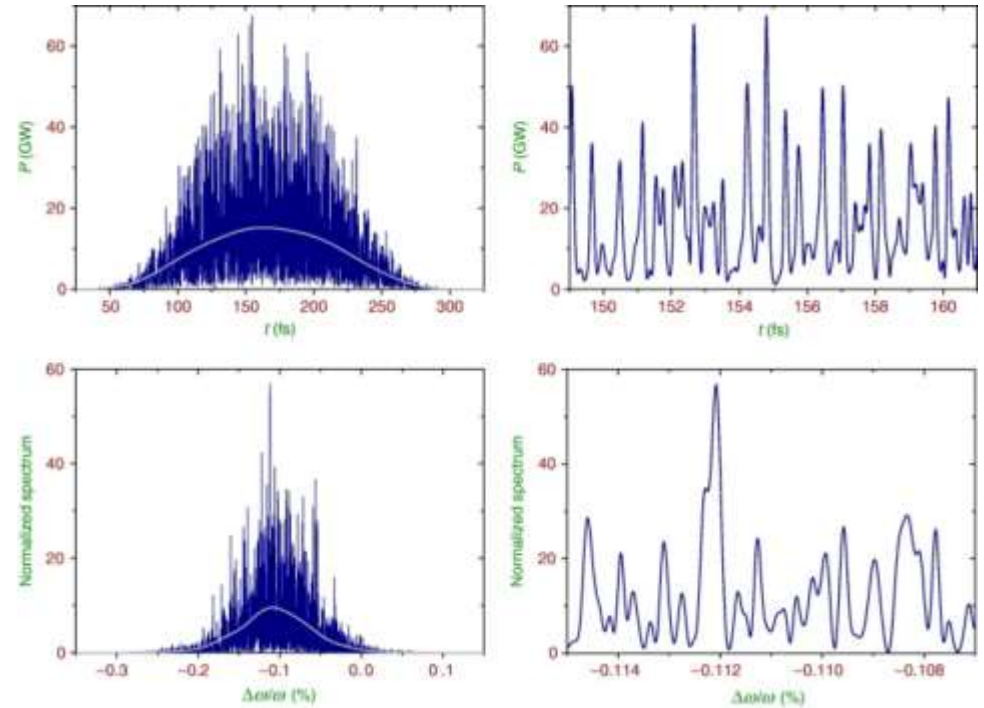
Mean correlation coefficients between individual crystals in two orientations, showing separation into clusters.



Reciprocal space sections before and after (right) the resolution procedure, showing that the artificial fourfold symmetry arising from the ambiguity has been eliminated.

# Why do we need so many patterns?

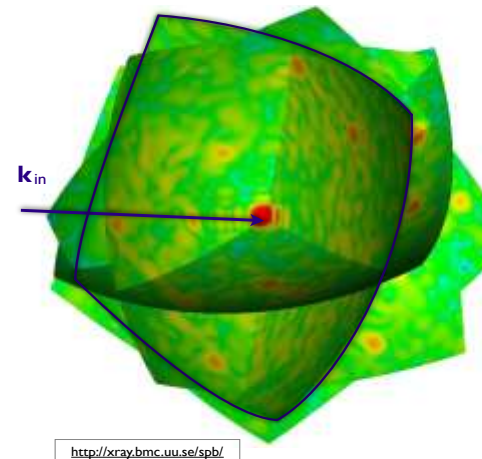
- Error sources in SFX are not well understood
- SASE: every pulse is like a new experiment
- Shot to shot variations need to be modeled or averaged over



Images: Geloni et al., New J. Phys. 12 (2010) 035021

# Why do we need so many patterns?

- Crystal size
- Crystal quality
- Crystal isomorphism
- X-ray pulse bandwidth
- X-ray pulse spectrum
- X-ray pulse intensity
- Position of crystal in the beam
- Partially recorded reflections
- ...



An angular integration over the Bragg spot is needed to obtain the structure factor.  
**One snapshot gives partial reflections.**

R. A. Kirian et al. (2010) Femtosecond protein nanocrystallography - data analysis methods. Opt. Exp. 18, 5713

# partiality

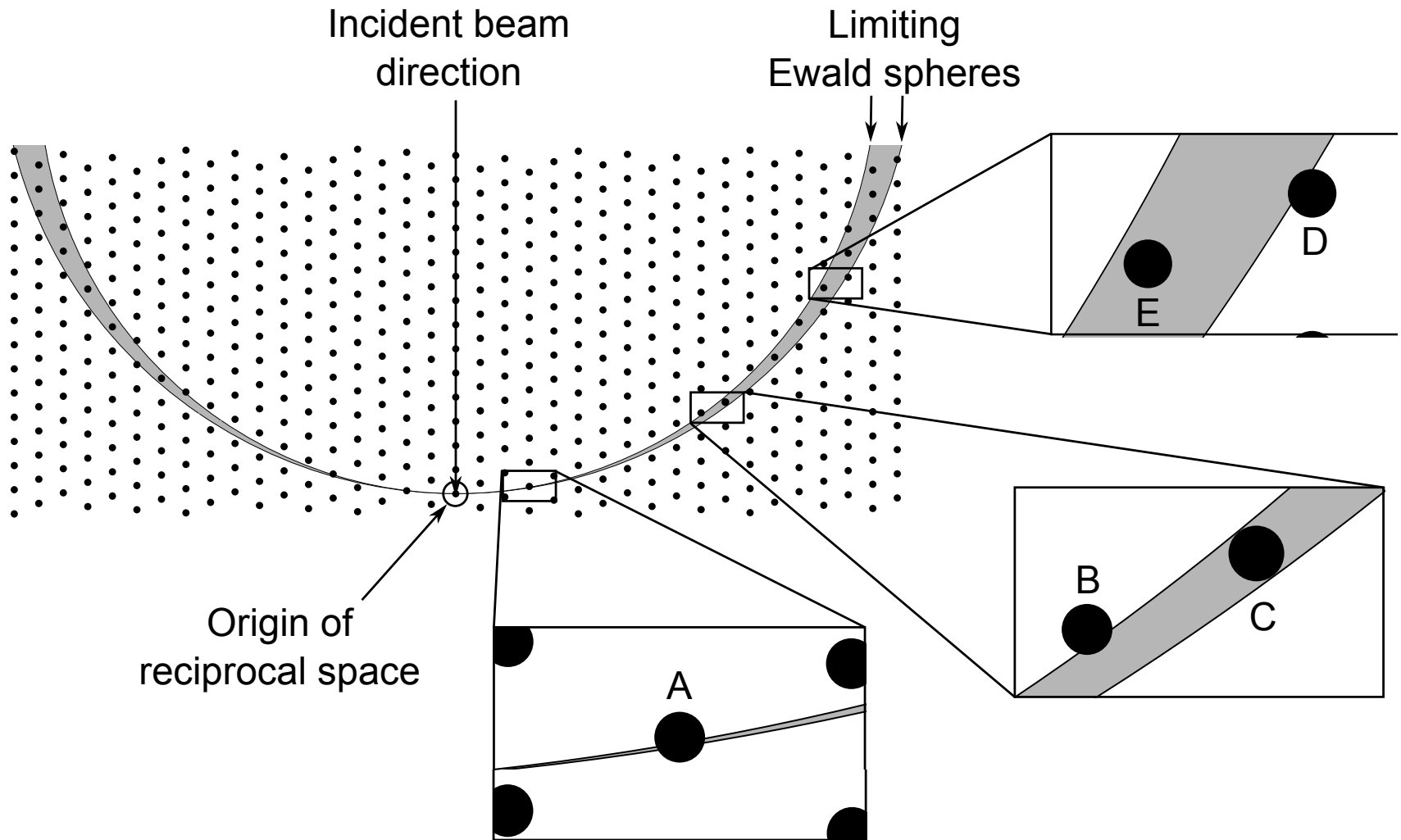
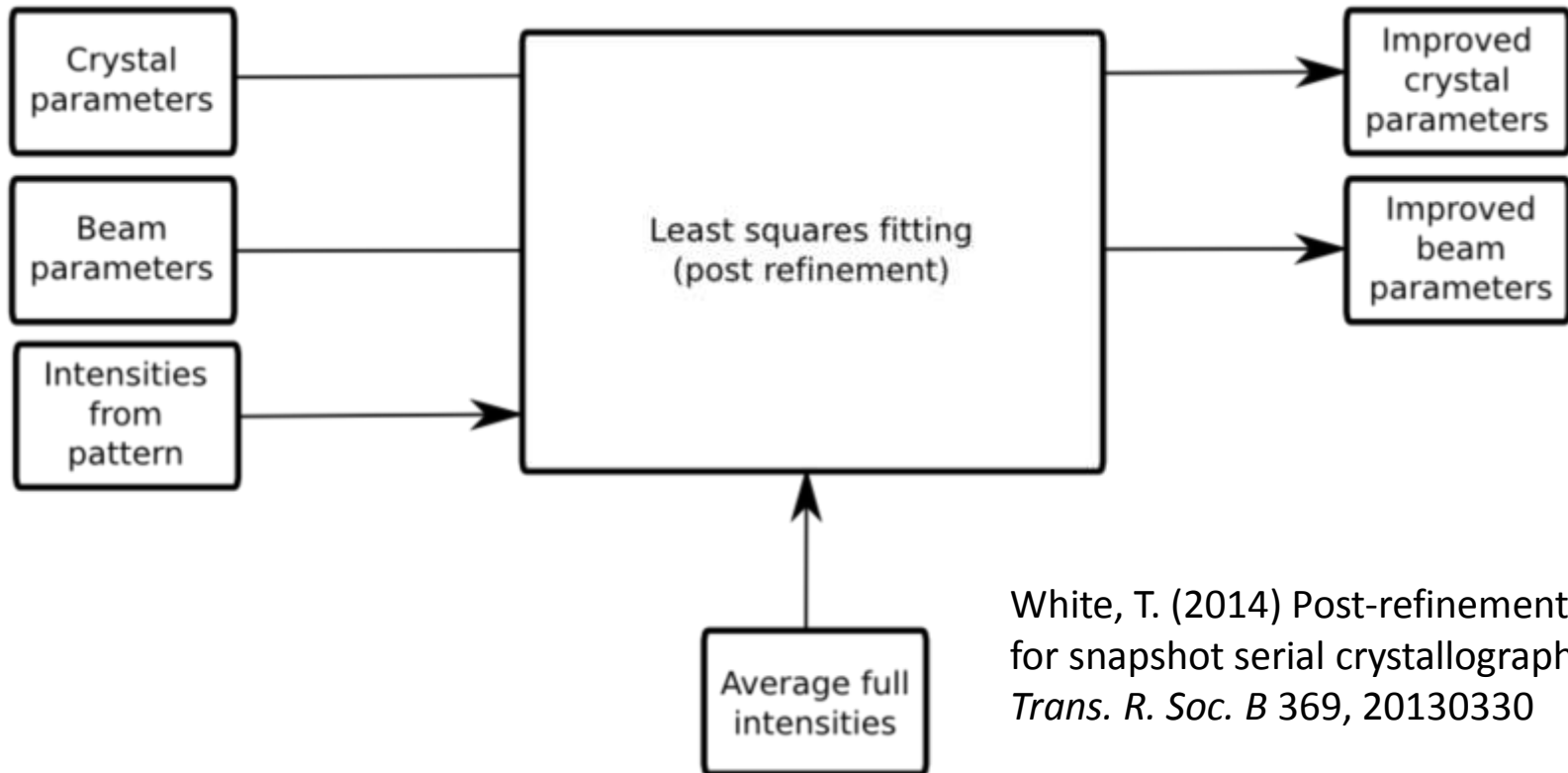


Figure from White, T. (2014) *Phil. Trans. R. Soc. B* 369, 20130330 .

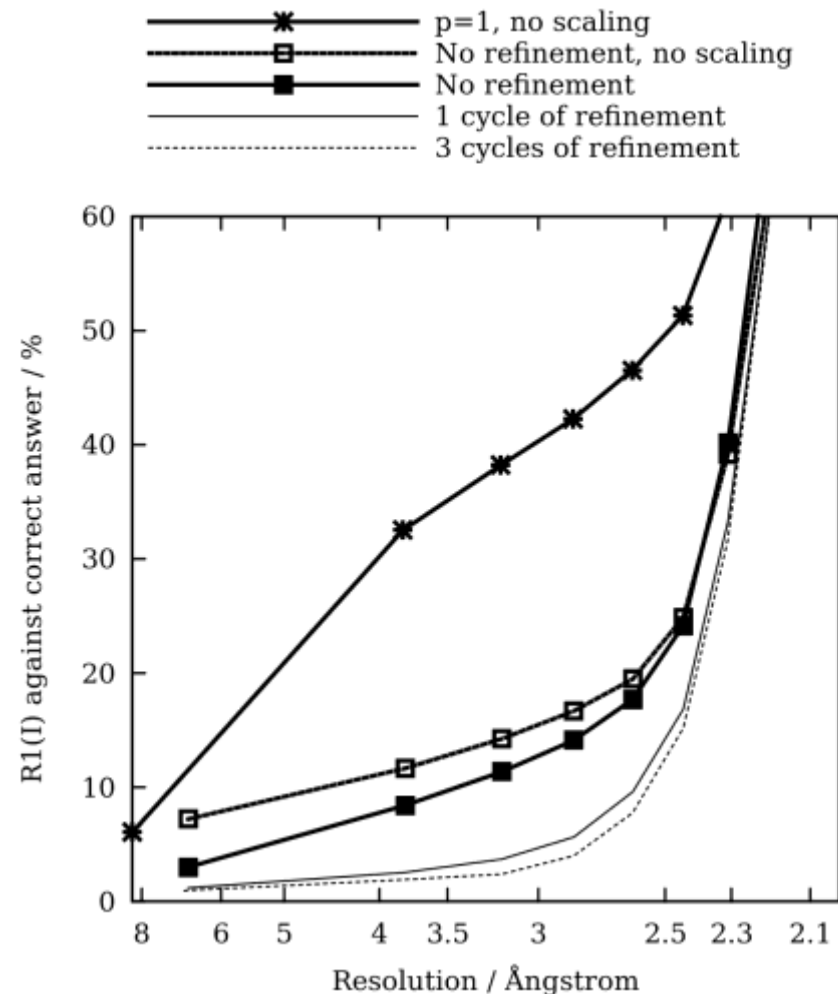
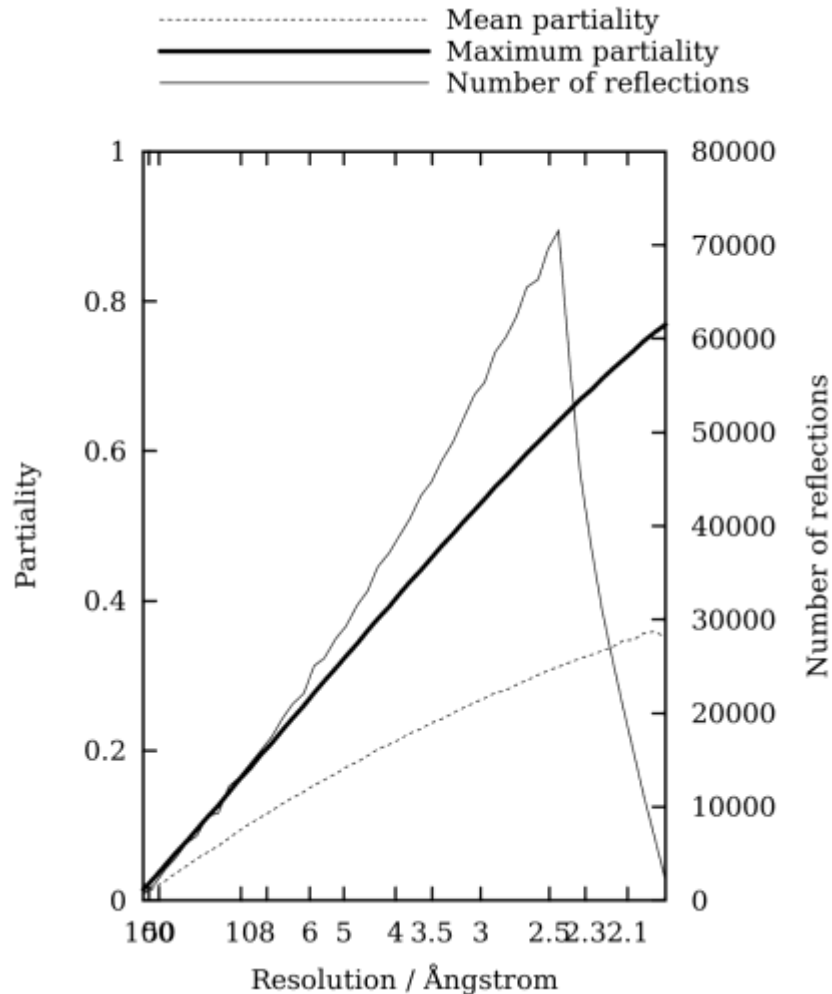
# Solving the partiality problem



White, T. (2014) Post-refinement method for snapshot serial crystallography. *Phil. Trans. R. Soc. B* 369, 20130330

Algorithm similar to: Kabsch, W. (2010) Integration, scaling, space-group assignment and post-refinement. *Acta Cryst D*. 66, 133.

# Solving the partiality problem

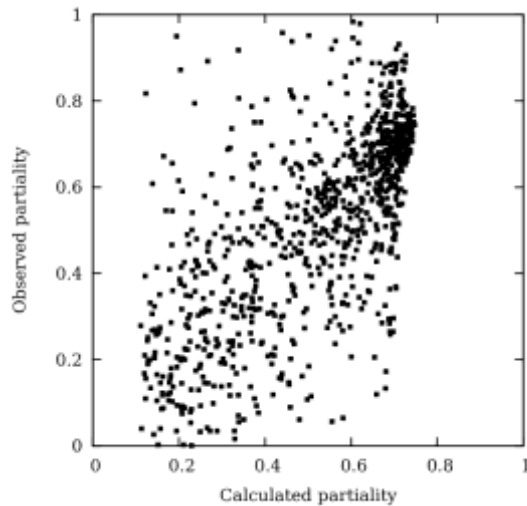


Figures from Tom White.

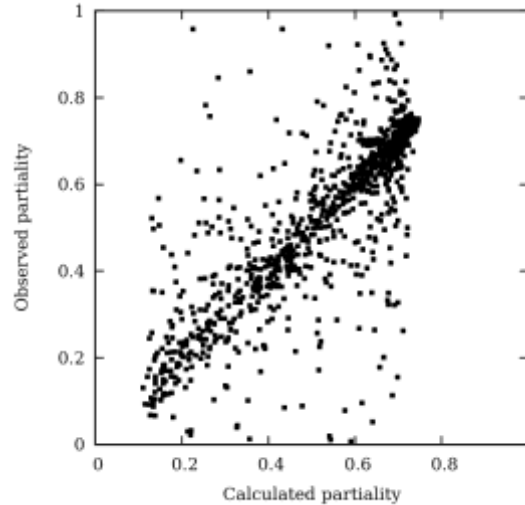
See: White, T. (2014) *Phil. Trans. R. Soc. B* 369, 20130330 .

Software for serial crystallography  
Nadia Zatsepin – IUCr 2014, Aug 5<sup>th</sup>

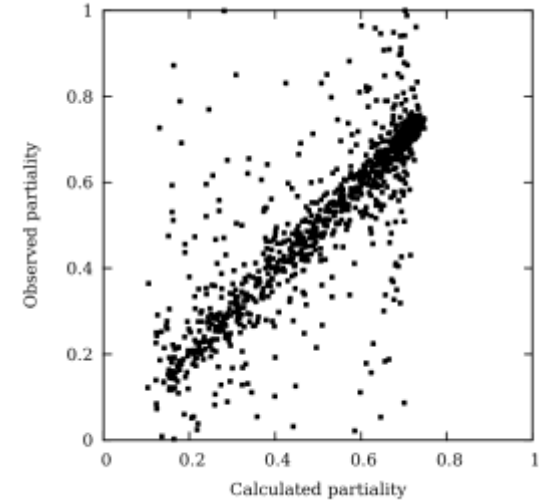
# Solving the partiality problem



Initial condition  
(0.1% cell error)



After 1 cycle of  
post refinement



After 3 cycles of  
post refinement

White, T. (2014) Post-refinement method for snapshot serial crystallography. *Phil. Trans. R. Soc. B* 369, 20130330 .

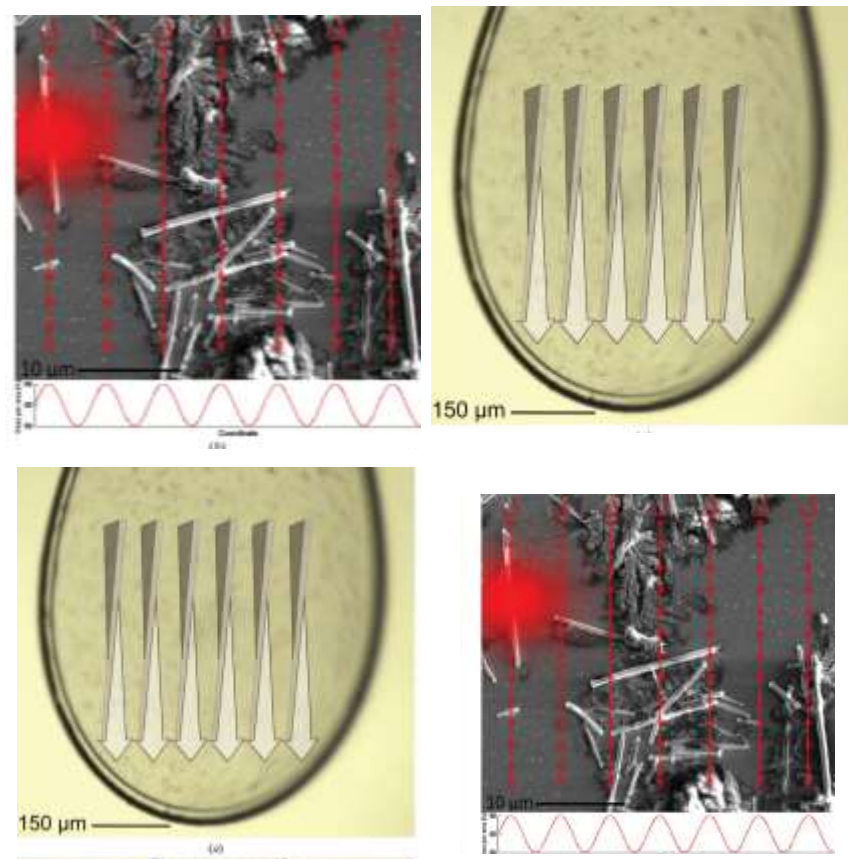
Algorithm similar to: Kabsch, W. (2010) Integration, scaling, space-group assignment and post-refinement. *Acta Cryst D.* 66, 133.

Figures from Tom White.

See: White, T. (2014) *Phil. Trans. R. Soc. B* 369, 20130330 .

# “Beyond” XFELs

- Serial crystallography has been successfully carried out at synchrotrons

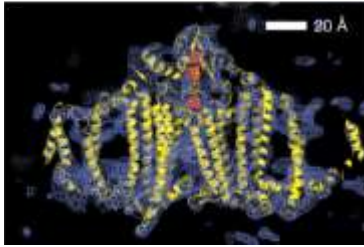


Figures from Gati, C. et al. Serial crystallography on in vivo grown microcrystals using synchrotron radiation. IUCrJ 2014.



# CrystFEL in the PDB

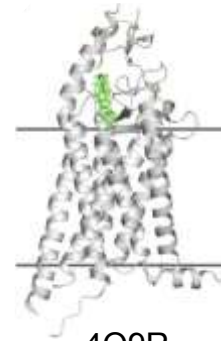
Some results from LCLS data processed with Cheetah and CrystFEL



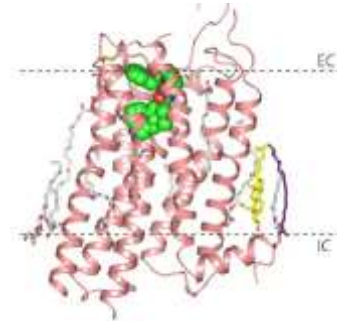
**3PCQ**  
Photosystem I (the first SFX experiment)  
Chapman et al., Nature 2011



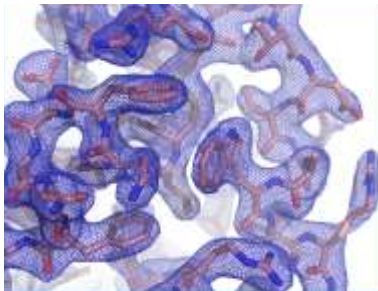
**4HWY**  
Natively inhibited Cathepsin B  
Redecke, Nass et al., Science 2013



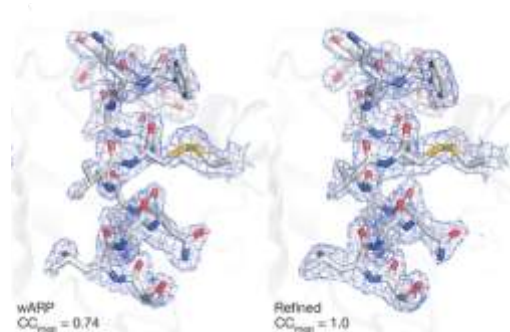
**4O9R**  
*Smoothed* receptor using LCP injector  
Weierstall et al., Nature Communications 2014



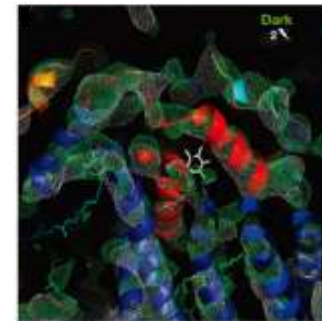
**4NC3**  
Serotonin receptor bound to ergotamine  
Liu et al., Science 2013



**4O34**  
Serial crystallography using a synchrotron beamline  
Stellato et al., IUCrJ 2014



**4N5R**  
Lysozyme (Gd derivative) ab initio phasing using SAD  
Barends et al., Nature 2013



**4Q54**  
Photosystem II in S<sub>3</sub> excited state  
Kupitz, Basu et al., Nature 2014

& more

# More serial crystallography software

- Psana - LCLS analysis frameworks. Python interface
- CASS – data reduction (no indexing etc)
  - Foucar, L. et al. CASS—CFEL-ASG software suite. (2012) Comp. Phys. Comm. 183 (10) 2207-2213.
- cctbx.xfel – data reduction and SFX processing
  - Built on Computational Crystallography ToolBox: same as Phenix and LABELIT
  - Sauter N. et al. Acta Cryst. (2013). D69, 1274
  - Future: post refinement
- nXDS – Kabsch, Acta D. 2014
  - Not Monte Carlo
  - Future: model X-ray spectrum; consider shape transforms from nanocrystals

Acta Crystallographica Section D  
**Biological  
Crystallography**  
ISSN 1399-0047

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Jahnstrasse 29, D-69120 Heidelberg, Germany

## Processing of X-ray snapshots from crystals in random orientations

A functional expression is introduced that relates scattered X-ray intensities from a still or a rotation snapshot to the corresponding structure-factor amplitudes. The new approach was implemented in the program *nXDS* for processing monochromatic diffraction images recorded by a multi-

Received 9 April 2014  
Accepted 10 June 2014

# Workshops: where to learn more

- Bio-XFEL data analysis workshop August 21-22, 2014
  - Organized by BioXFEL STC, LBNL and SLAC, at Berkeley.
  - [www.bioxfel.org/events/details/9](http://www.bioxfel.org/events/details/9)
  - There will be a Bio-XFEL data analysis workshop in 2015
- **SSRL/LCLS User meeting 2014: Oct 10, 2014**
  - Software for Serial Crystallography (1 day workshop)
  - Spaces available – book now.
  - <https://conf-slac.stanford.edu/ssrl-lcls-2014/welcome>
- **BioXFEL 2<sup>nd</sup> International Conference – Puerto Rico, Jan 14-16, 2015**
  - [www.bioxfel.org/events/details/6](http://www.bioxfel.org/events/details/6)
- **ACA 2015 (July, PA): SFX data analysis workshop (possibly)**

[www.desy.de/~barty/cheetah](http://www.desy.de/~barty/cheetah) & [www.desy.de/~twhite/crystfel/](http://www.desy.de/~twhite/crystfel/)

# CrystFEL citations

## 2014

- C. Kupitz, S. Basu, I. Grotjohann, R. Fromme et al. "Serial time-resolved crystallography of photosystem II using a femtosecond X-ray laser". *Nature* (2014). [doi:10.1038/nature13453](https://doi.org/10.1038/nature13453) and PDB entries [4PBU](https://www.rcsb.org/entry/4PBU) and [4Q54](https://www.rcsb.org/entry/4Q54).
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- O. Yefanov, C. Gati, G. Bourenkov, R. A. Kirian et al. "Mapping the continuous reciprocal space intensity distribution of X-ray serial crystallography". *Phil. Trans. Roy. Soc. B* 369 (2014) 20130333 [doi:10.1098/rstb.2013.0333](https://doi.org/10.1098/rstb.2013.0333) (**open access** - [download PDF](#)).
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## Useful references

Here are some papers describing CrystFEL or its algorithms:

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