Bio-XFEL Data Analysis Workshop
August 21, 2014, LBNL
Bldg 15-253

How good are my data?

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Software tutorial: http://cci.lbl.gov/xfel
Location at pslogin.slac.stanford.edu: /reg/g/cctbx

National Institutes of Health
National Institute of General Medical Sciences
BASIC DISCOVERIES FOR BETTER HEALTH

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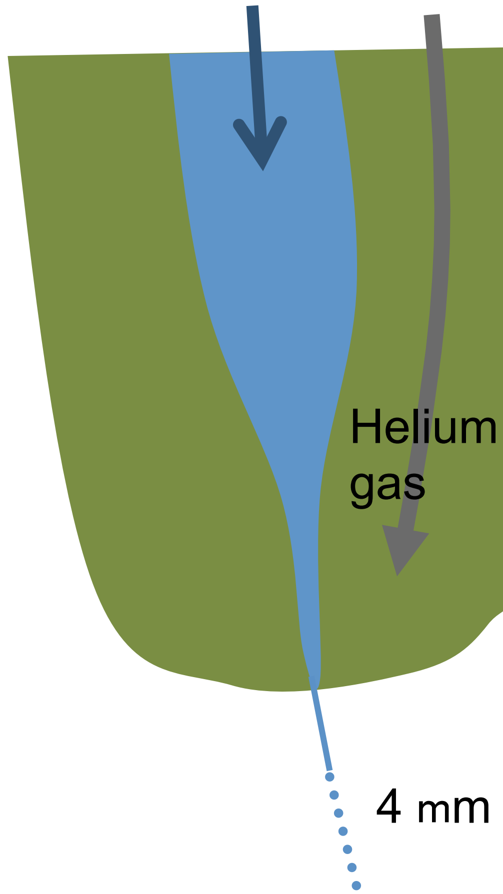
U.S. DEPARTMENT OF ENERGY

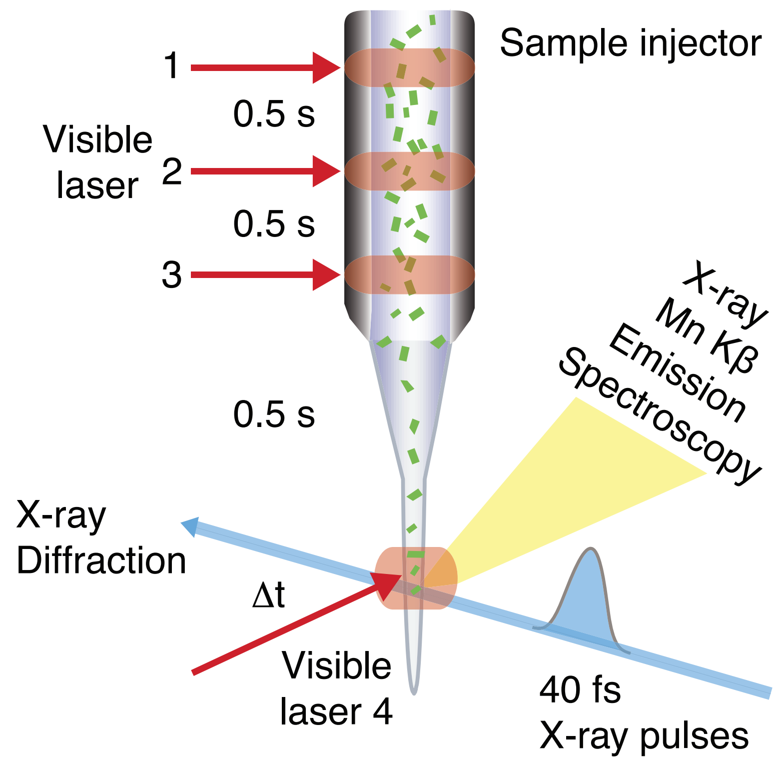
XFEL diffraction results processed with *cctbx.xfel*

Gas dynamic virtual nozzle



- L220 lysozyme (in tutorial)
- Gd-lysozyme (in tutorial)
- *Bacillus* Cry3A δ-endotoxin *in vivo* crystals
- Polyhedrin crystals (submitted)

Electrospun microjet



- Photosystem II redox states
- Thermolysin (in tutorial)

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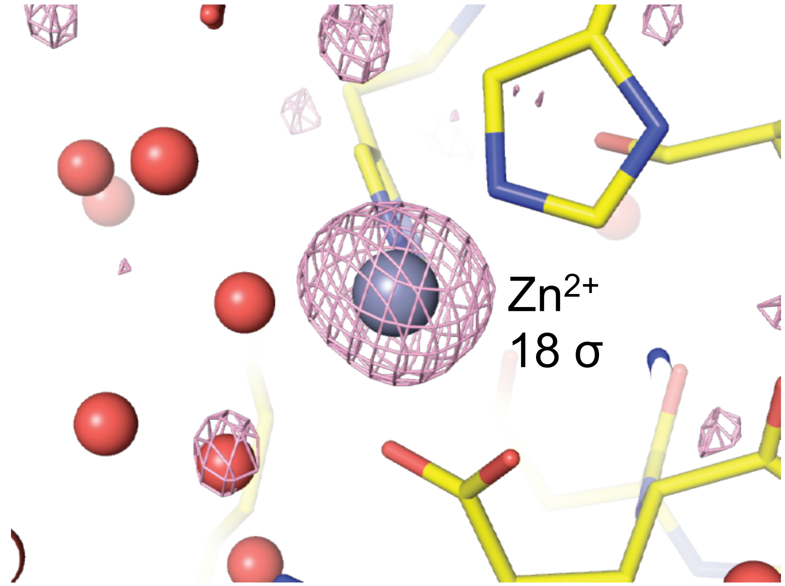
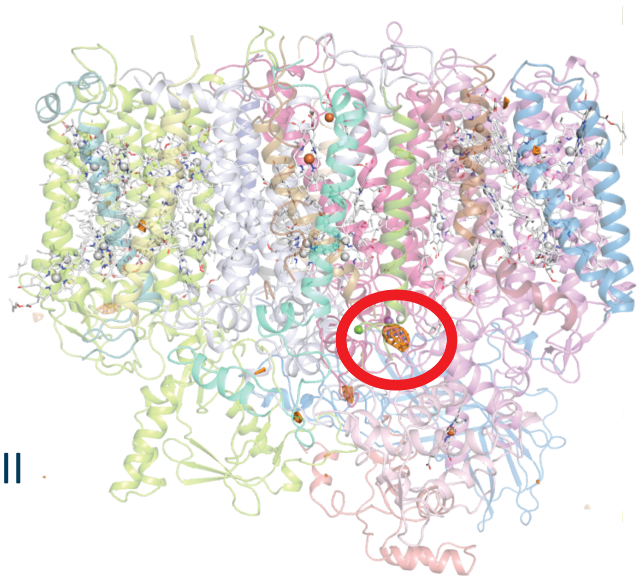
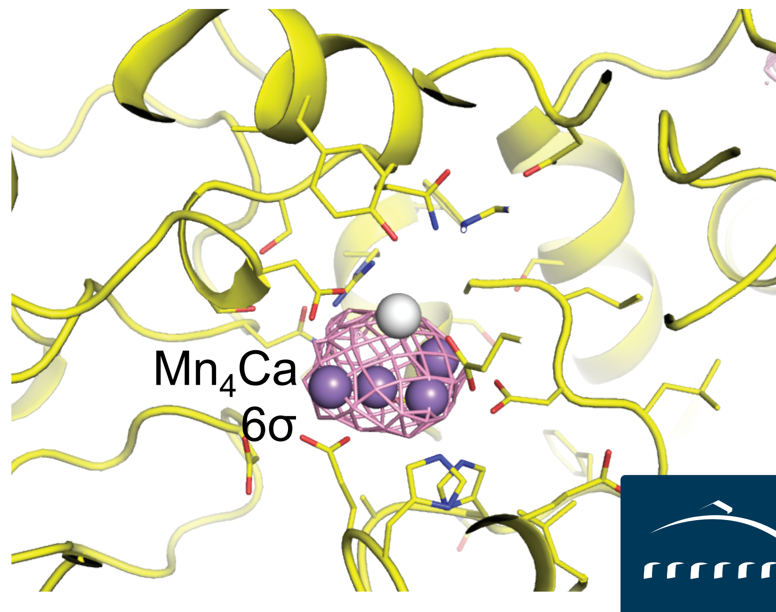
Anomalous difference maps detect Zn, Mn



thermolysin





photosystem II
S_0 state

Kern (2014) Nature Communications 5: 4371

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The *cctbx.xfel* workflow:

------------------------------------SLAC computing---------------——————----------------Laptop

pyana / cctbx module

```
~$USER/myrelease/cxi84914
  /*.cfg
  /*.phil
cxi.lsf → batch queues
```

Original data XTC format → *Index and integrate* → *Merging* → *Structure solution*

Original data:

```
/reg/d/psdm/cxi/cxi84914/xtc
  e157-r0021-s00-c00.xtc
                -c01.xtc
            -s01-c00.xtc
```

Index and integrate:

```
/reg/d/psdm/cxi/cxi84914/scratch
  /r0021/000/stdout/s00.log
            /out/idx-s00-*.pickle
            /integration/int-*.pickle
```

Merging:

```
cxi.merge
  → *.mtz
```

Structure solution:

```
phenix
```

cctbx standalone

```
any directory
cxi.index idx*.pickle *.phil
cctbx.image_viewer
```

Software tutorial: http://cci.lbl.gov/xfel

cctbx.xfel at pslogin.slac.stanford.edu: /reg/g/cctbx

This powerpoint: http://cci.lbl.gov/xfel/2014_workshop

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Cctbx.xfel goals for the Bio-XFEL workshop

- Work through L498 thermolysin data (or a subset)
  - First worked example from XTC to final maps
  - Detect anomalous signal?
- Try the Gd-lysozyme data
- Start tonight
  - 2-hour work session insufficient to actually work the data
  - Discover student questions not anticipated
  - Application to students' own data
  - Gd-lysozyme not yet documented on the wiki
- Extended topics
  - LCLS instruments CXI or XPP; SACLA (Aaron Brewster)
  - Space groups with a lattice ambiguity (Wolfgang Brehm)
  - Sparse data (Oliver Zeldin)
  - Migration to psana (Chris O'Grady)

Software tutorial: http://cci.lbl.gov/xfel

cctbx.xfel at pslogin.slac.stanford.edu: /reg/g/cctbx

This powerpoint: http://cci.lbl.gov/xfel/2014_workshop

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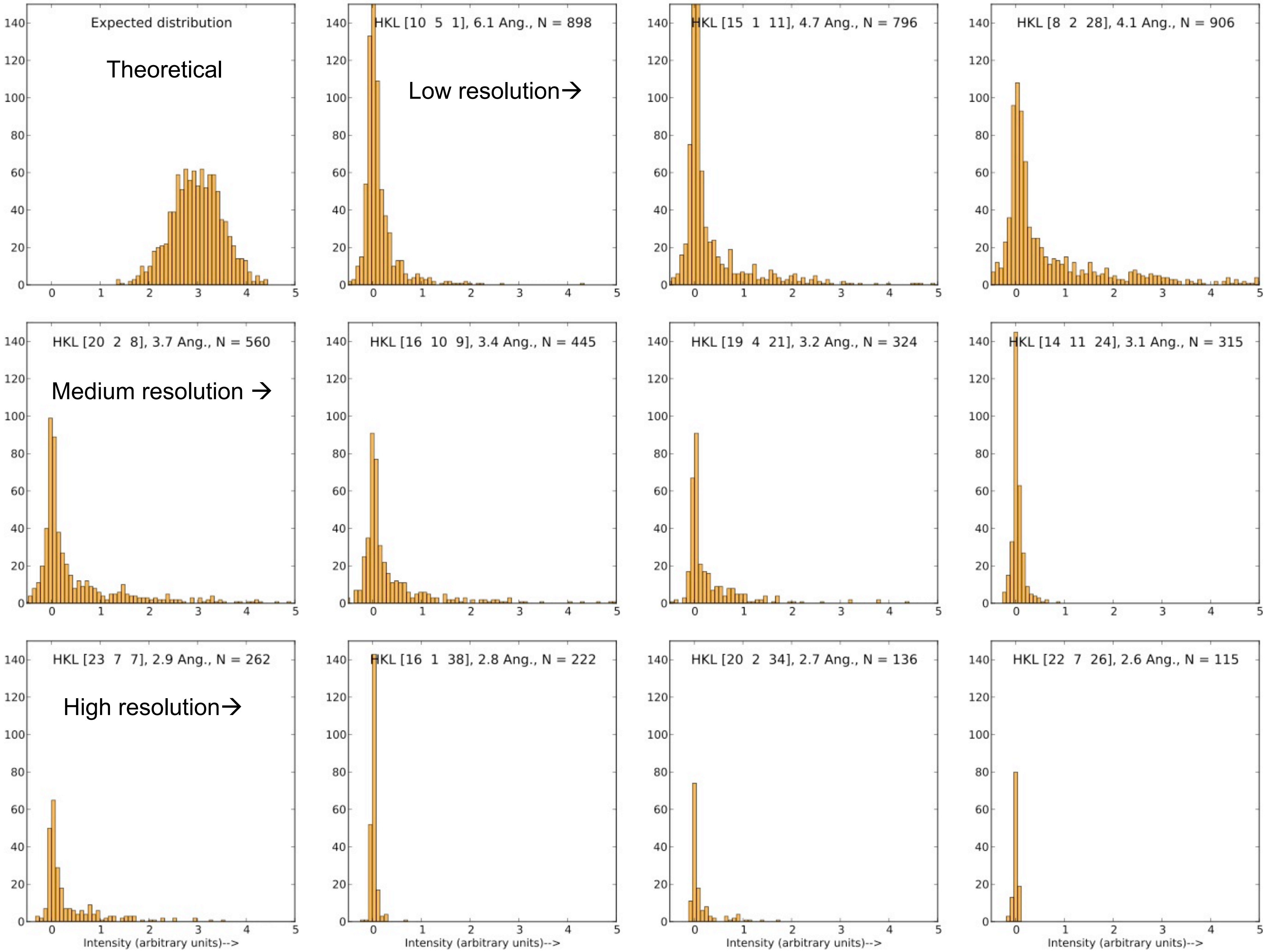
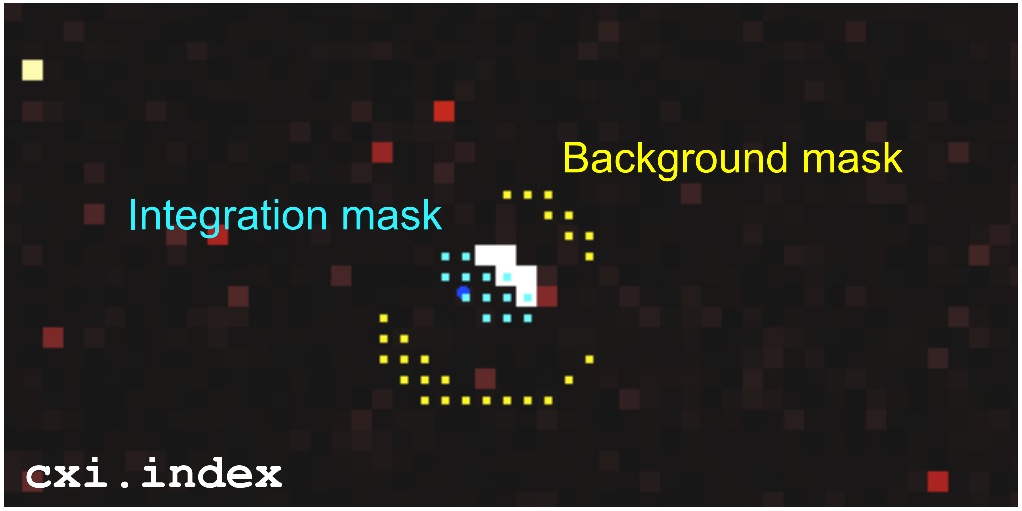
Ensemble distribution of measurements of single Bragg spots



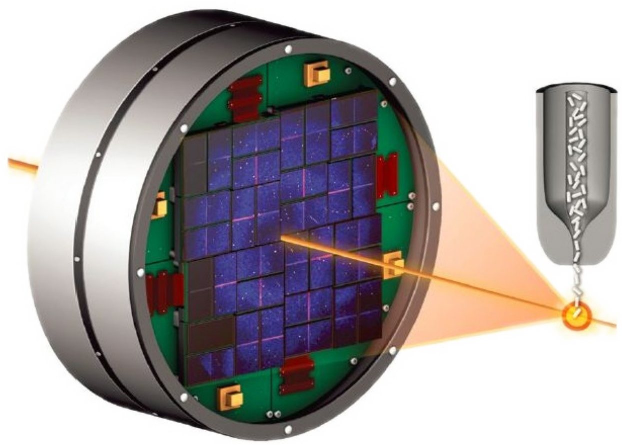
Signal to noise and model accuracy are major issues



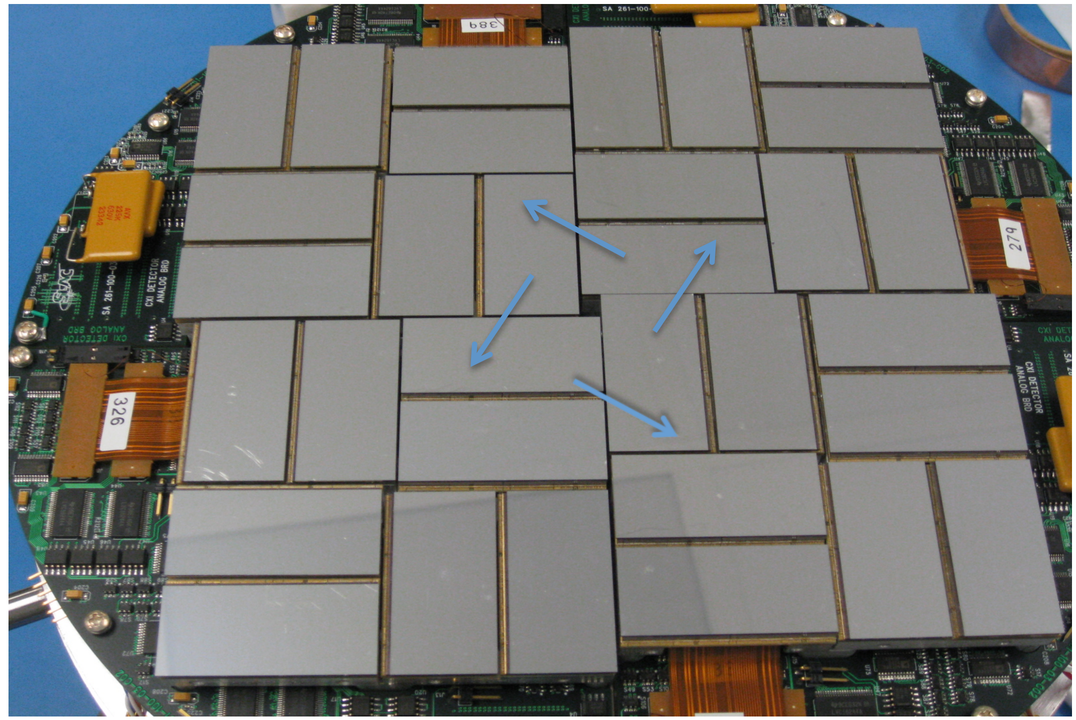
- Well conforming spot shape models intended to enhance signal to noise
- But are susceptible to positional error

Exact relationship of sensor tiles is crucial for modeling reflections

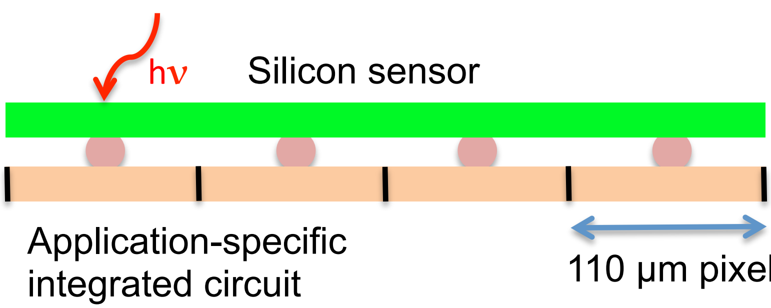
[Hart *et al.*, 2012, *Proc. of SPIE*]

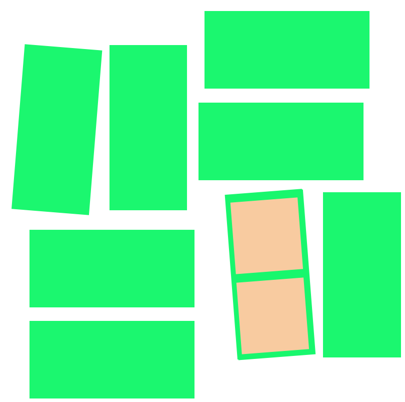


Cornell-SLAC pixel array detector (CS-PAD) at LCLS CXI endstation



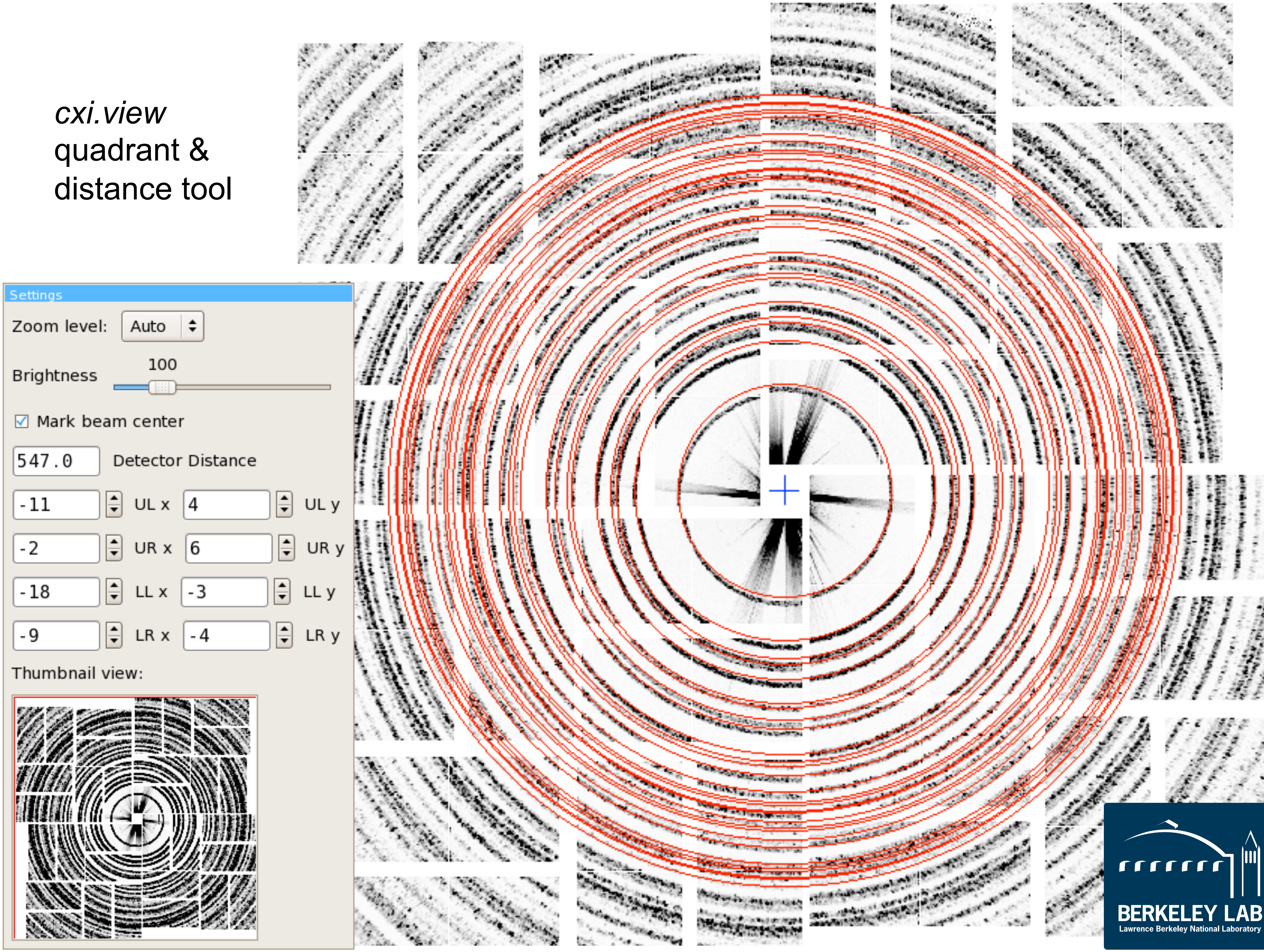
Quadrants are on movable rails



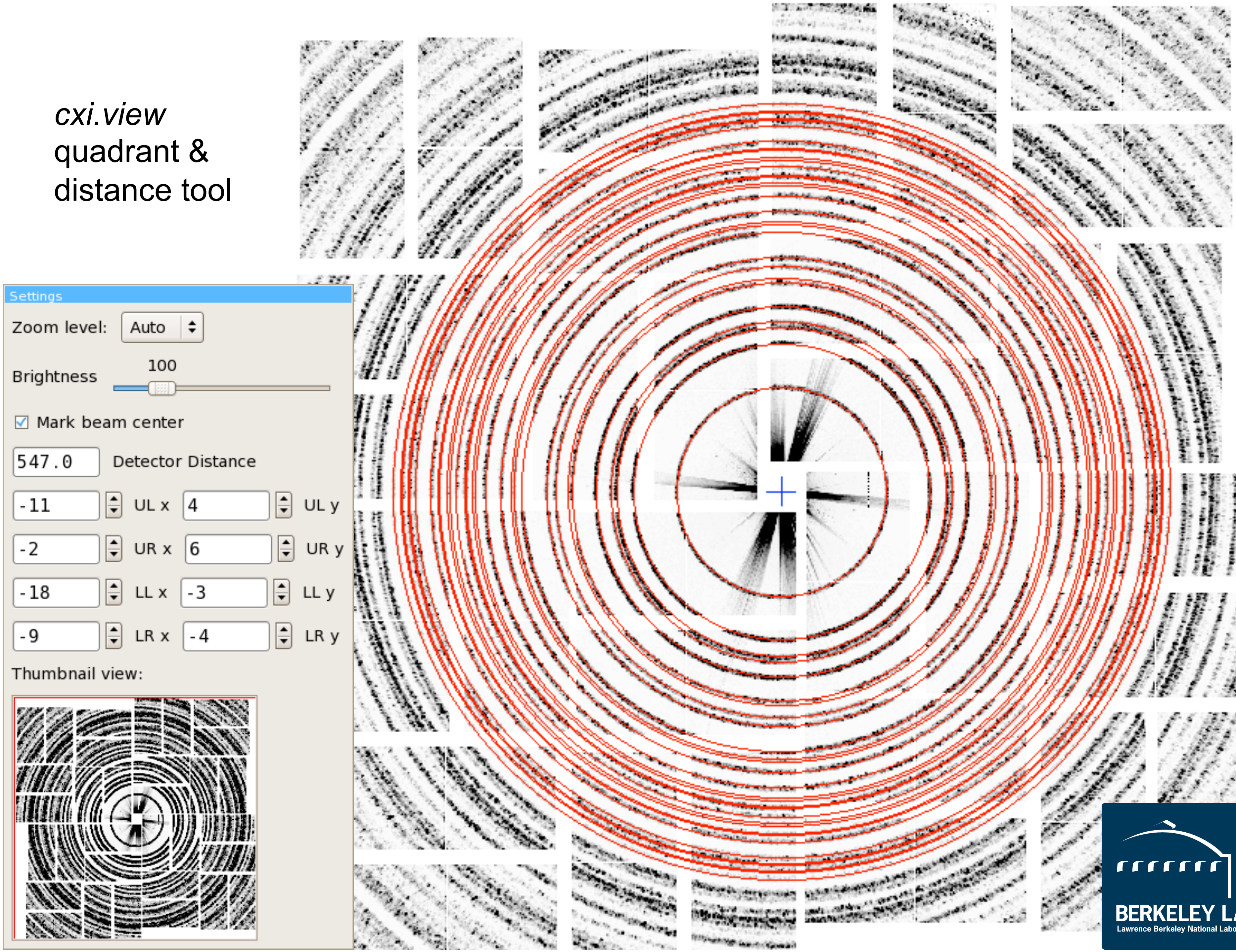


Sensors are field-serviceable

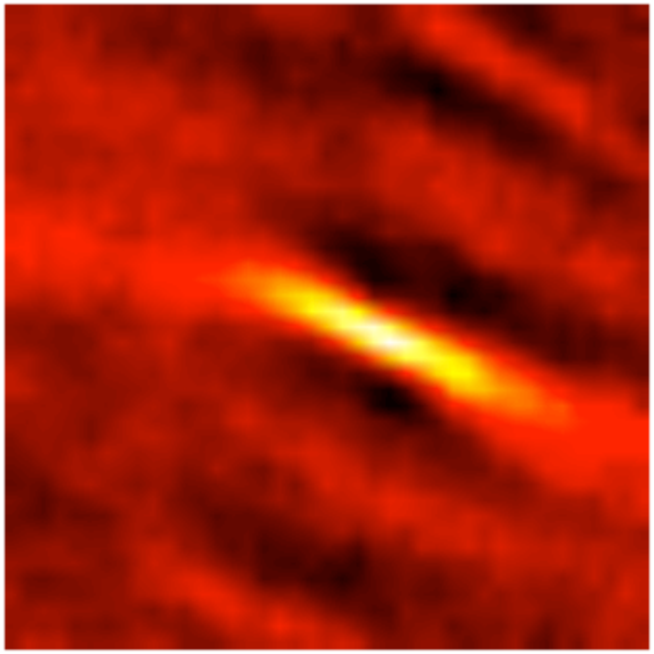
cxi.view
quadrant &
distance tool



cxi.view
quadrant &
distance tool



cspad.quadrants tool for automatic detection



- As a function of quadrant position (x & y), autocorrelation of the ring pattern with itself upon 45° rotation.
- Do the same with all 4 quadrants.

Toolbox is now refactored; the user now places the result x,y quadrant displacements directly in the phil file:

```
distl {
  quad_translations=3 -4 -3 -7 -12 0 -7 -5
}
```

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Detector versioning

The new system brings metrology under user rather than developer control.
Default detector geometries are still hard-coded based on date stamp.

```
> cxi.detector_format_versions

Format version   Det. address      Start time   End time
       CXI 3.2   CxiDs1-0|Cspad-0 Sep 2010     Mar 2011
       CXI 4.1   CxiDs1-0|Cspad-0 Mar 2011     Oct 2011
       CXI 5.1   CxiDs1-0|Cspad-0 Oct 2011     May 2012
       CXI 6.1   CxiDs1-0|Cspad-0 May 2012     Jan 2013
       CXI 7.1   CxiDs1-0|Cspad-0 Jan 2013     Aug 2013
       CXI 7.d   CxiDsd-0|Cspad-0 Jan 2013     Aug 2013
       CXI 8.1   CxiDs1-0|Cspad-0 Aug 2013     Jan 2014
       CXI 8.2   CxiDs1-0|Cspad-0 Jan 2014     Mar 2014
       CXI 8.d   CxiDsd-0|Cspad-0 Aug 2013     Mar 2014
       CXI 9.1   CxiDs2-0|Cspad-0 Mar 2014     Aug 2014
   Sacla.MPCCD        Sacla.MPCCD None         None
       XPP 7.1   XppGon-0|Cspad-0 Jan 2013     Aug 2013
  XPP 7.marccd  XppGon-0|marccd-0 Jan 2013     Aug 2013
       XPP 8.1   XppGon-0|Cspad-0 Aug 2013     Mar 2014
  XPP 8.marccd  XppGon-0|marccd-0 Aug 2013     Mar 2014
       XPP 9.1   XppGon-0|Cspad-0 Mar 2014     Aug 2014
```

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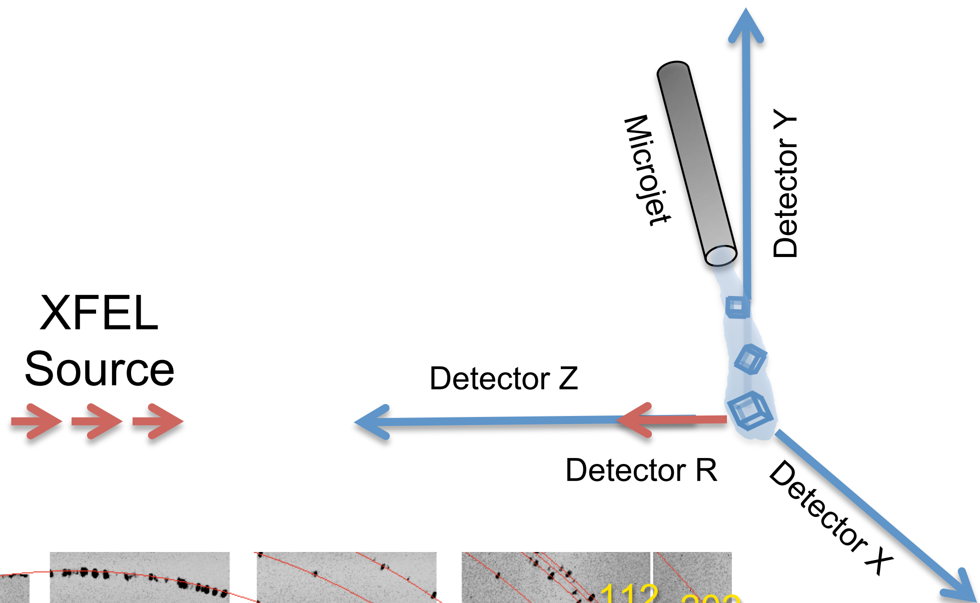
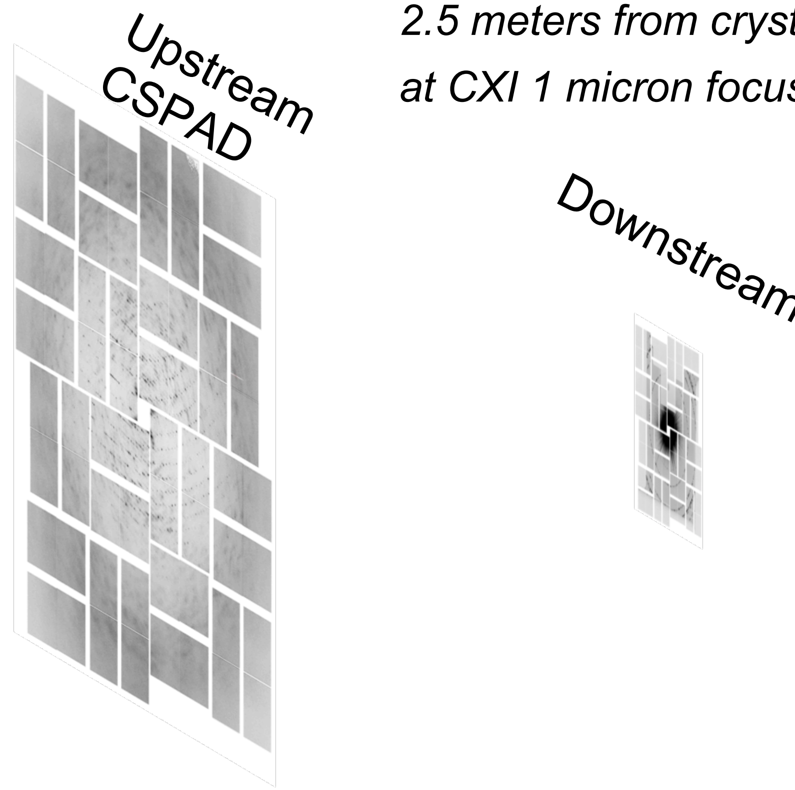
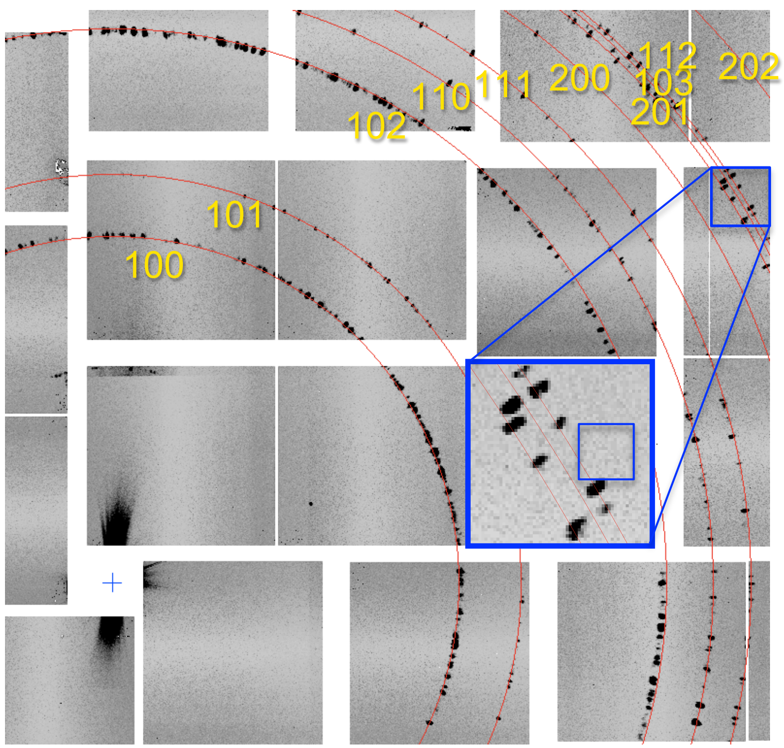
Downstream detector





2.5 meters from crystal
at CXI 1 micron focus



Indexed the lowest-resolution thermolysin powder rings

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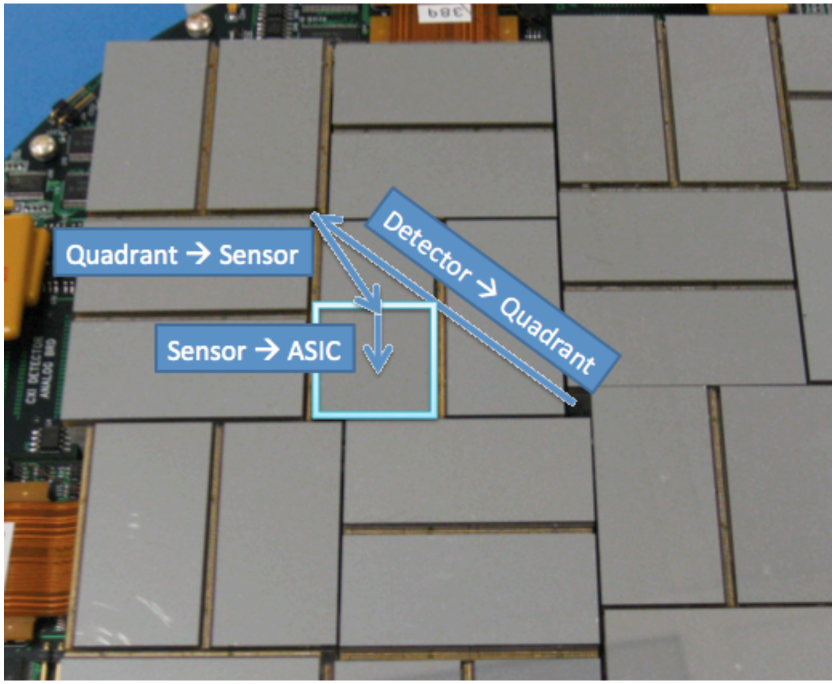
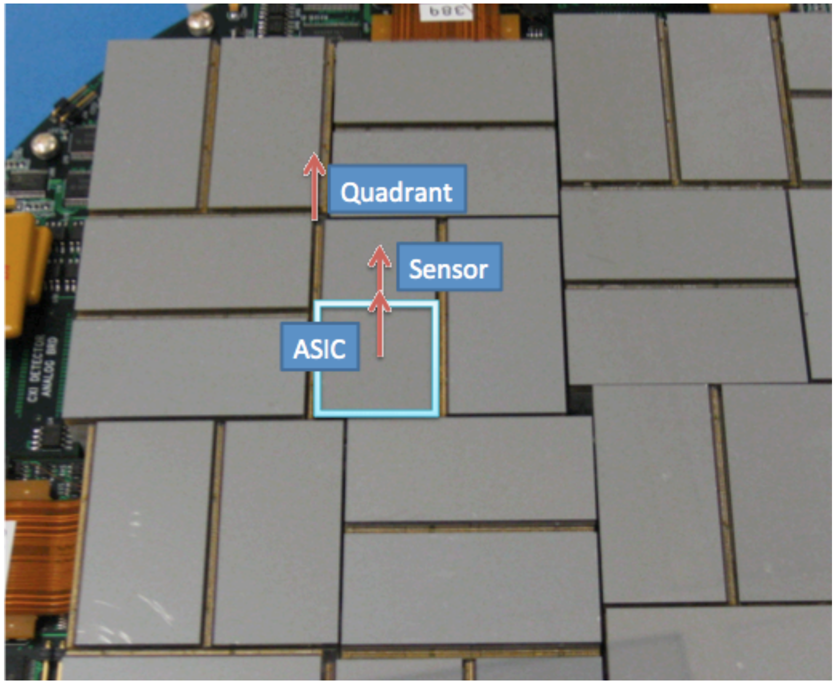
We are converging on a data format to capture the hierarchical design

- ImageCIF/ CBF: international standard file format
- Formal language for describing the experiment (and the data)
- Herbert Bernstein – Dowling College; critical collaborator
- DIALS software package will handle this standard format
- Will use HDF5 containers



Hierarchical organization:

Detector -> Quadrant -> Sensor -> ASIC

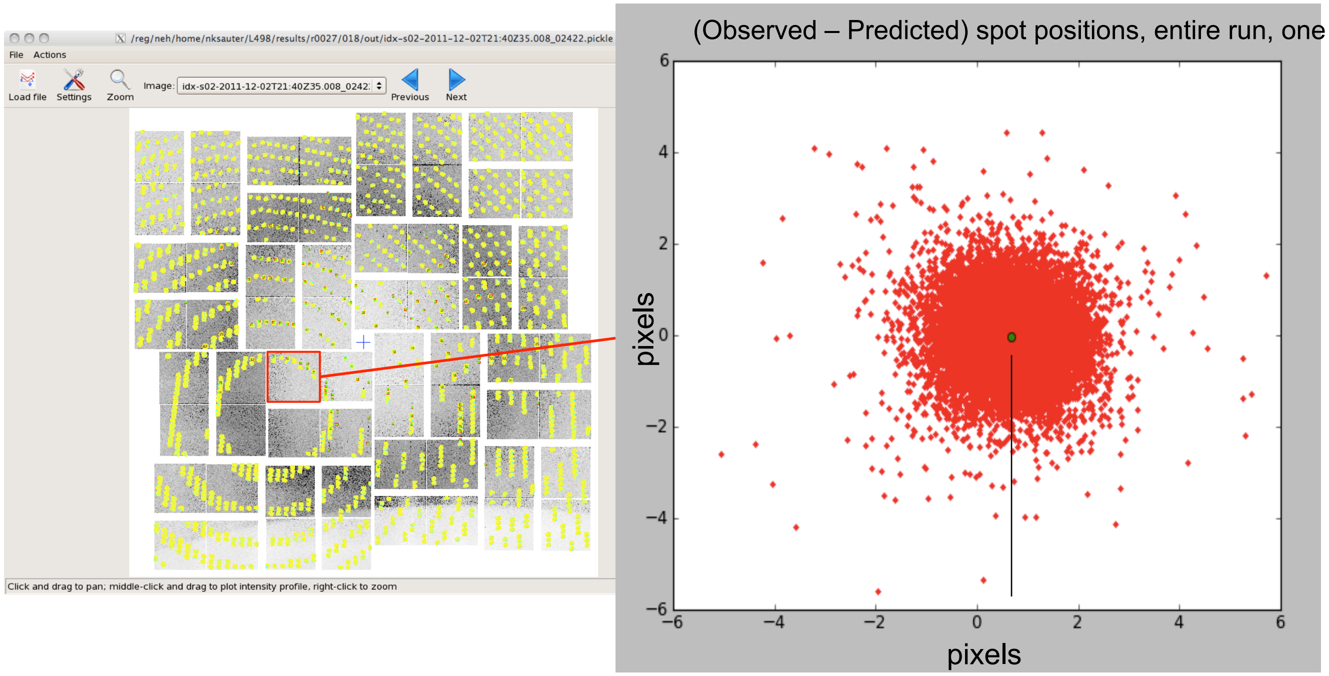


Each level:

Relative 3D translation and 3D rotation, as well as readout fast & slow axes

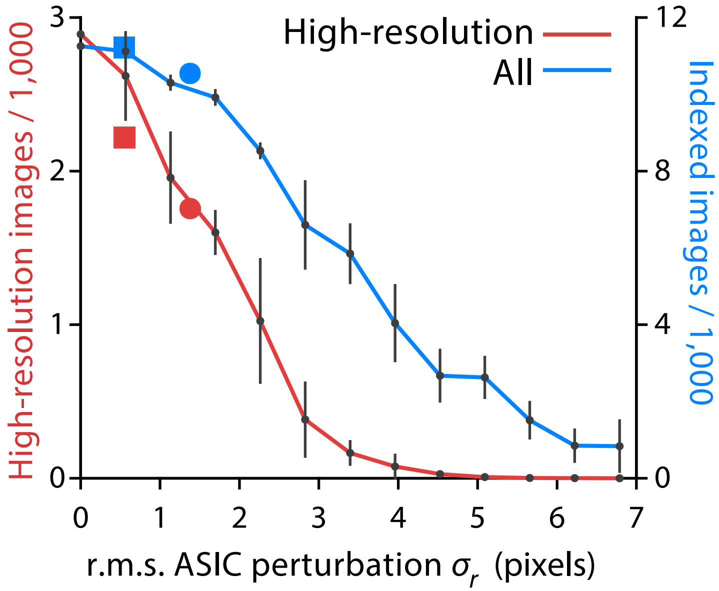
Brewster AS et. al (2014). "XFEL Detectors and ImageCIF." Comp. Crystallogr. Newsl. **5**:19–24

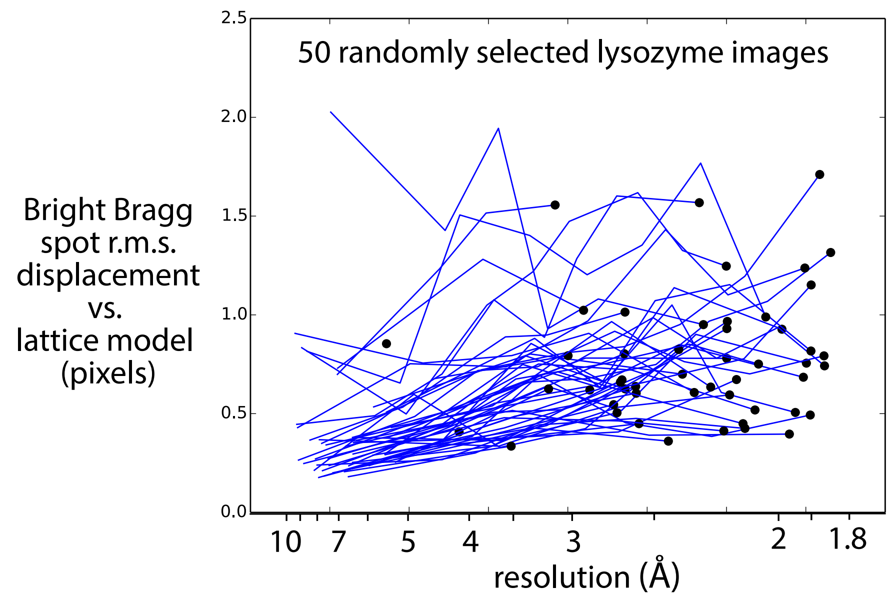
cspad.metrology tool: whole-pixel and sub-pixel corrections



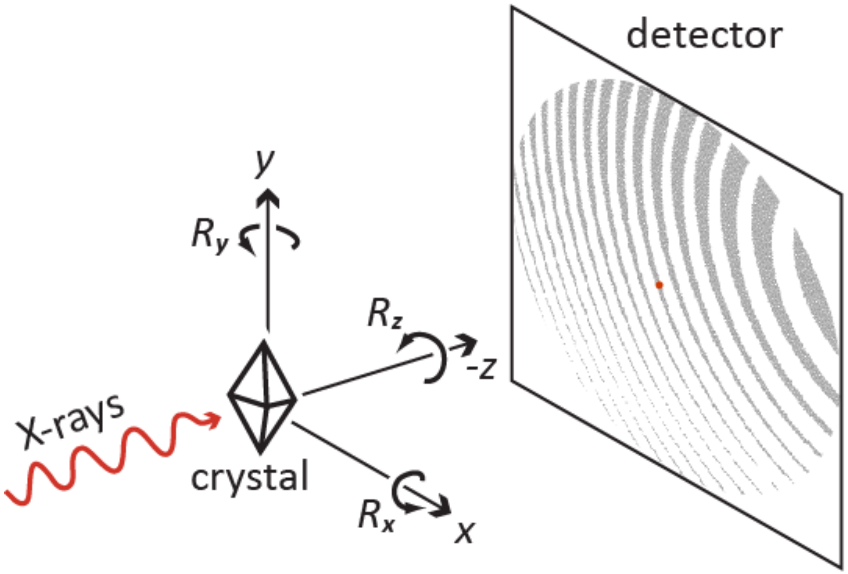
	Overall RMSD, all Bragg spots	Average Tile Displacement
As-given with manual quad placement `distl {quad_translations= }`	2.15 pixels	1.35 pixels
Nearest-pixel tile placement `distl {tile_translations= }`	1.86 pixels	0.40 pixels
Sub-pixel tile translations `integration {subpixel_joint_model{translations}}`	1.39 pixels	0.23 pixels
Rotations too `integration {subpixel_joint_model{rotations}}`	0.65 pixels	Near zero

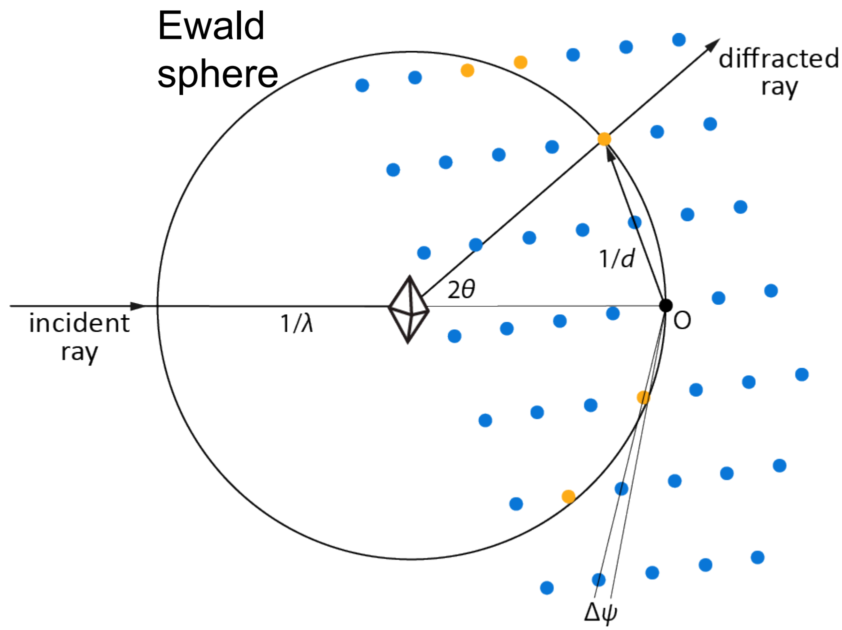
Varying the resolution cutoff for each image





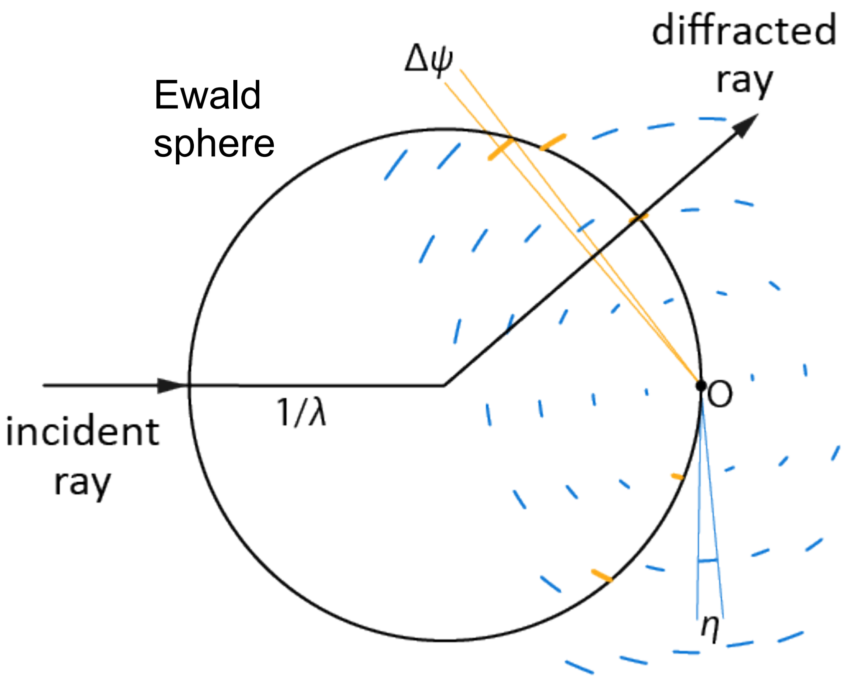
It is difficult to get crystal orientation from a still shot



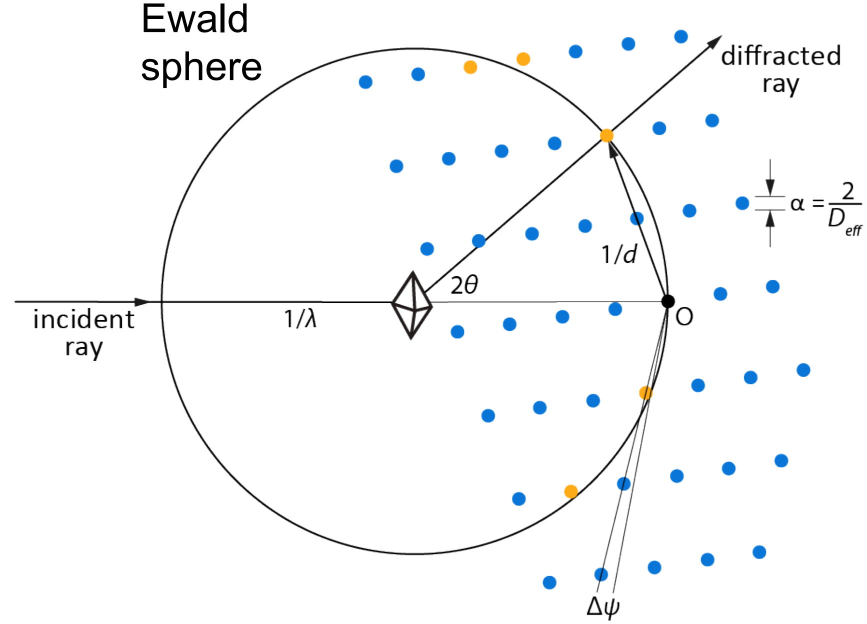


$$f = \sum_{\substack{spotfinder \\ spots}} \left(\mathbf{r}_{obs} - \mathbf{r}_{calc}\right)^2 + \left(\Delta\psi\left[rotx, roty\right]\right)^2$$

Crystal disorder is a combination of mosaicity and block size



Effective mosaicity η



Effective size D_{eff} of the coherently scattering mosaic block

Best-parameter fitting in cctbx.xfel; algorithm defined in phil file:
integration.mosaic.refinement_target=ML

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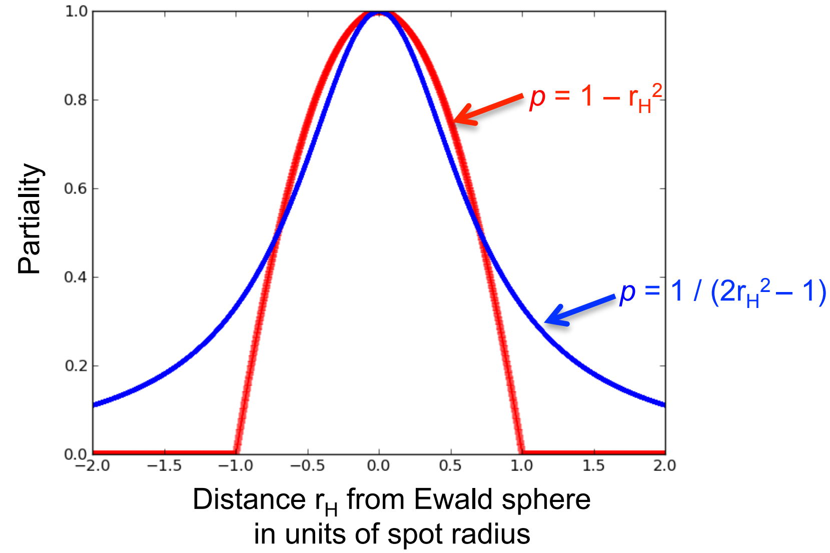
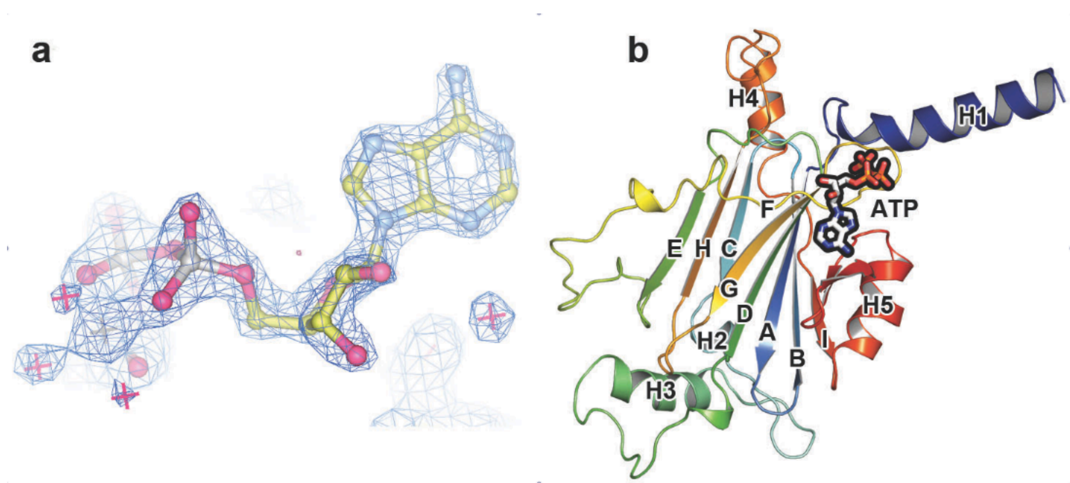
Rough mathematical expression for spot partiality





Cypovirus polyhedron crystal/ David Stuart group

- Molecular replacement, 10% sequence identity

Δψ angle offers a correction for partiality, supported by intensity statistics

N(Z) test

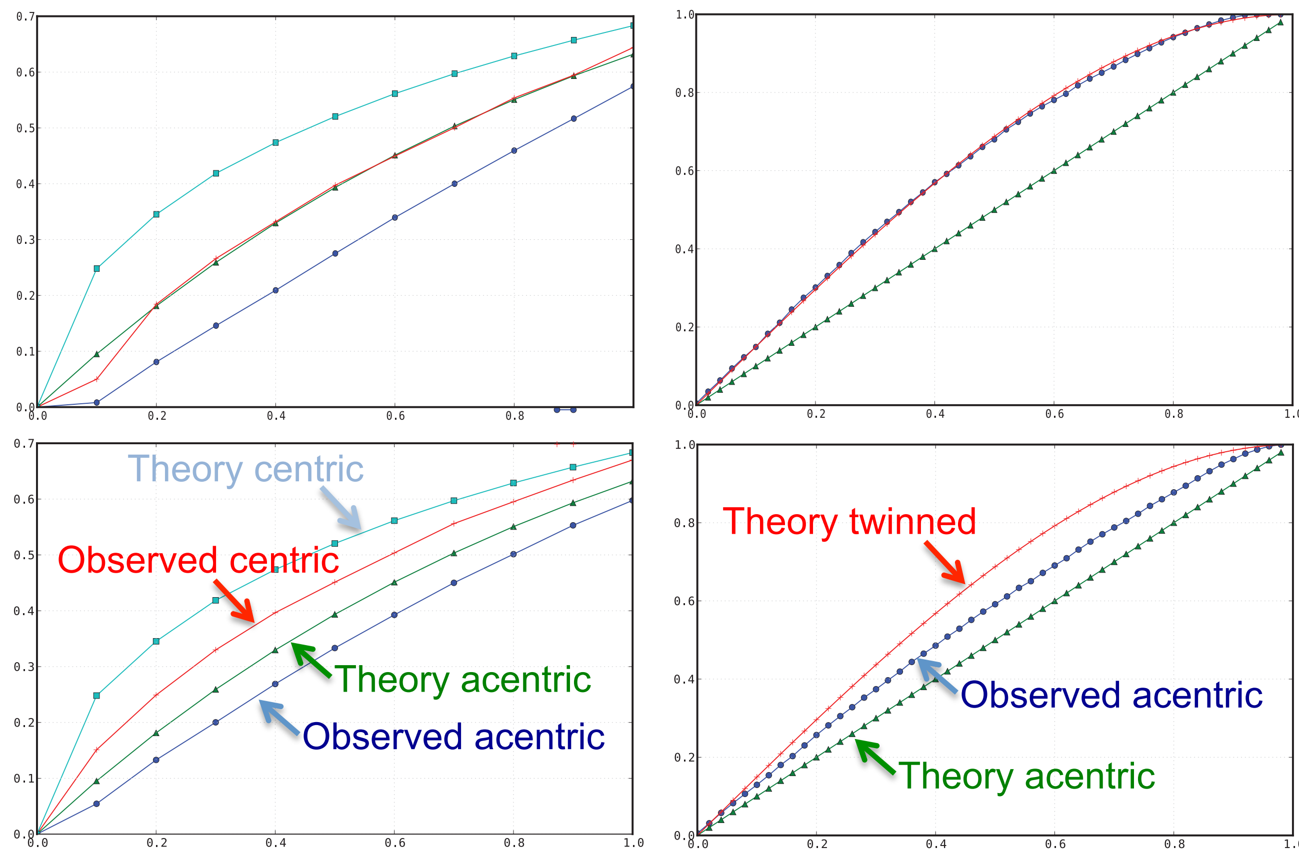
L test

Initial scaling & merging:

$\langle I^2 \rangle / \langle I \rangle^2 = 1.5$

Rough partiality correction:

$\langle I^2 \rangle / \langle I \rangle^2 = 1.7$ (theoretical 2)



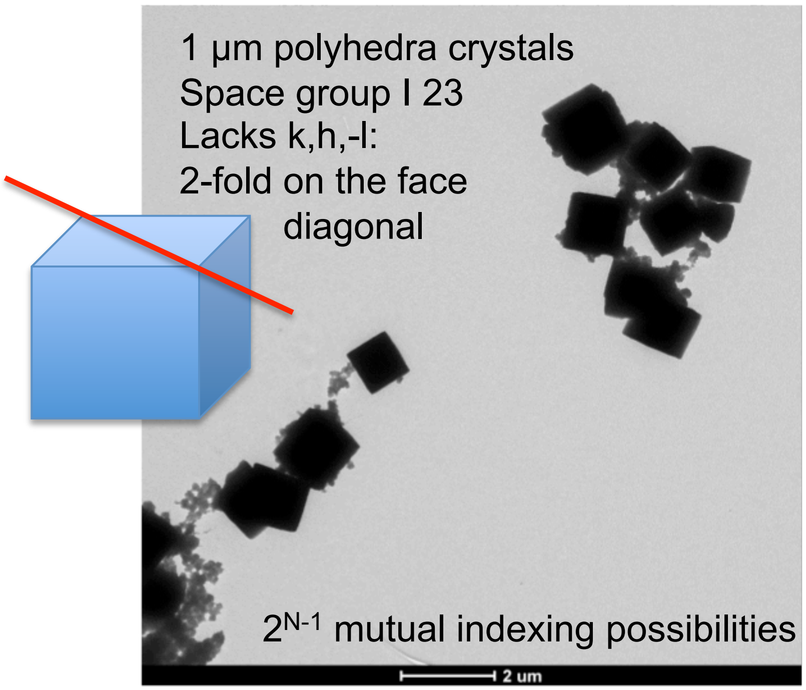
$Z = I / \langle I \rangle$

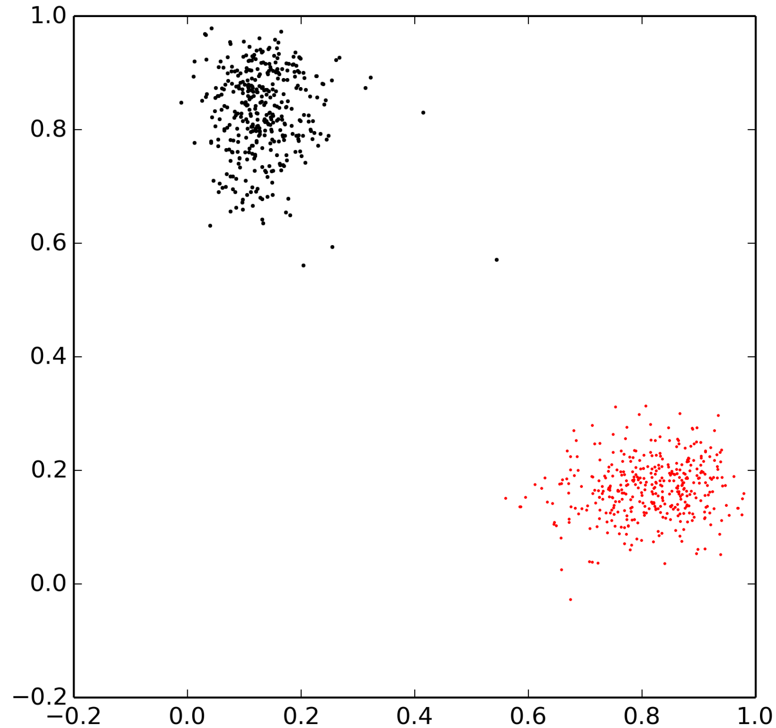
$|L| = |\ (I_1 - I_2) / (I_1 + I_2)\ |$

Thermolysin data

Resolving an indexing ambiguity

- 28% of crystal structures have space groups where the lattice symmetry is higher than the space group symmetry





- Brehm & Diederichs (2014) Acta D70: 101
- Break the ambiguity by pairwise comparison of image-to-image intensity correlation coefficient
- 768 lattices sorted into two piles

cxi.brehm_diederichs tool is documented at
http://cci.lbl.gov/xfel/index.php/Resolving_an_Indexing_Ambiguity

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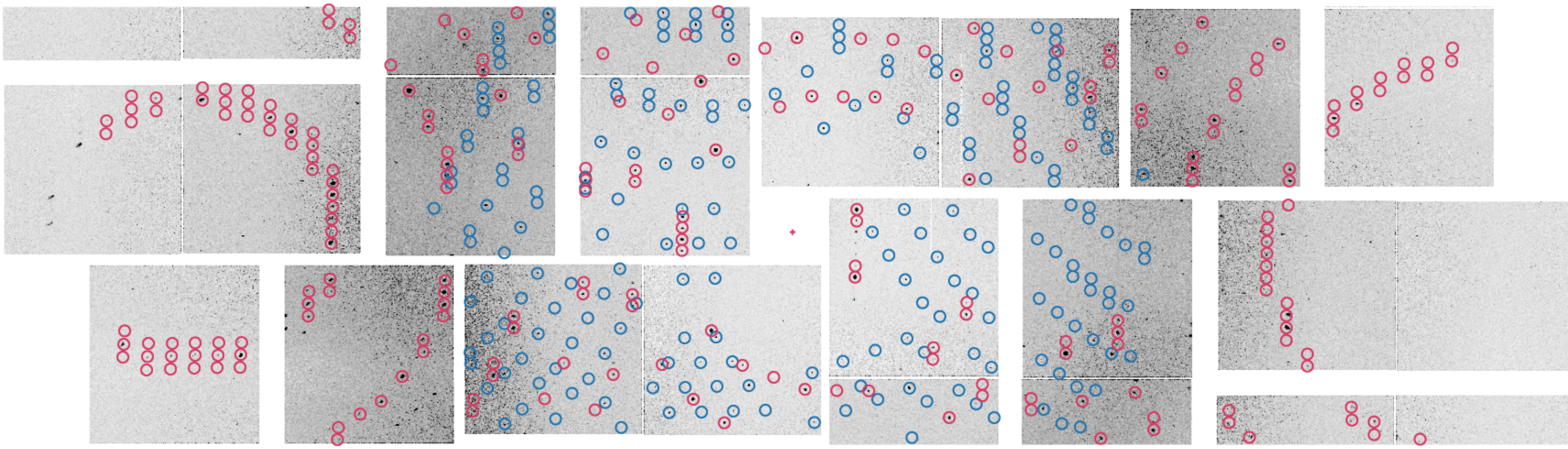
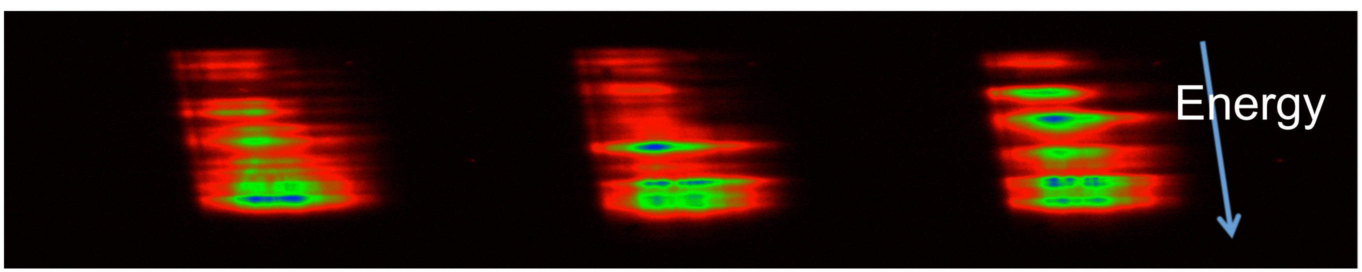
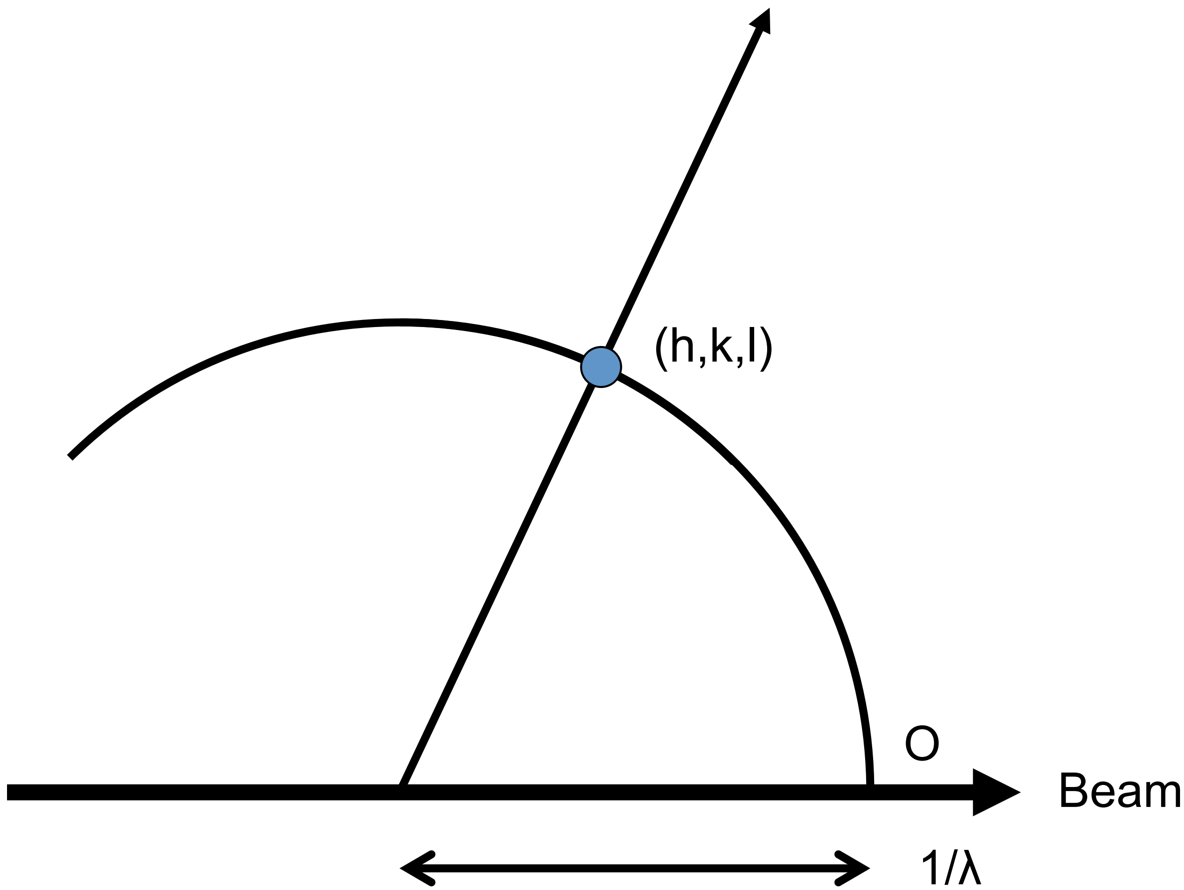
Challenges: Radial streaking





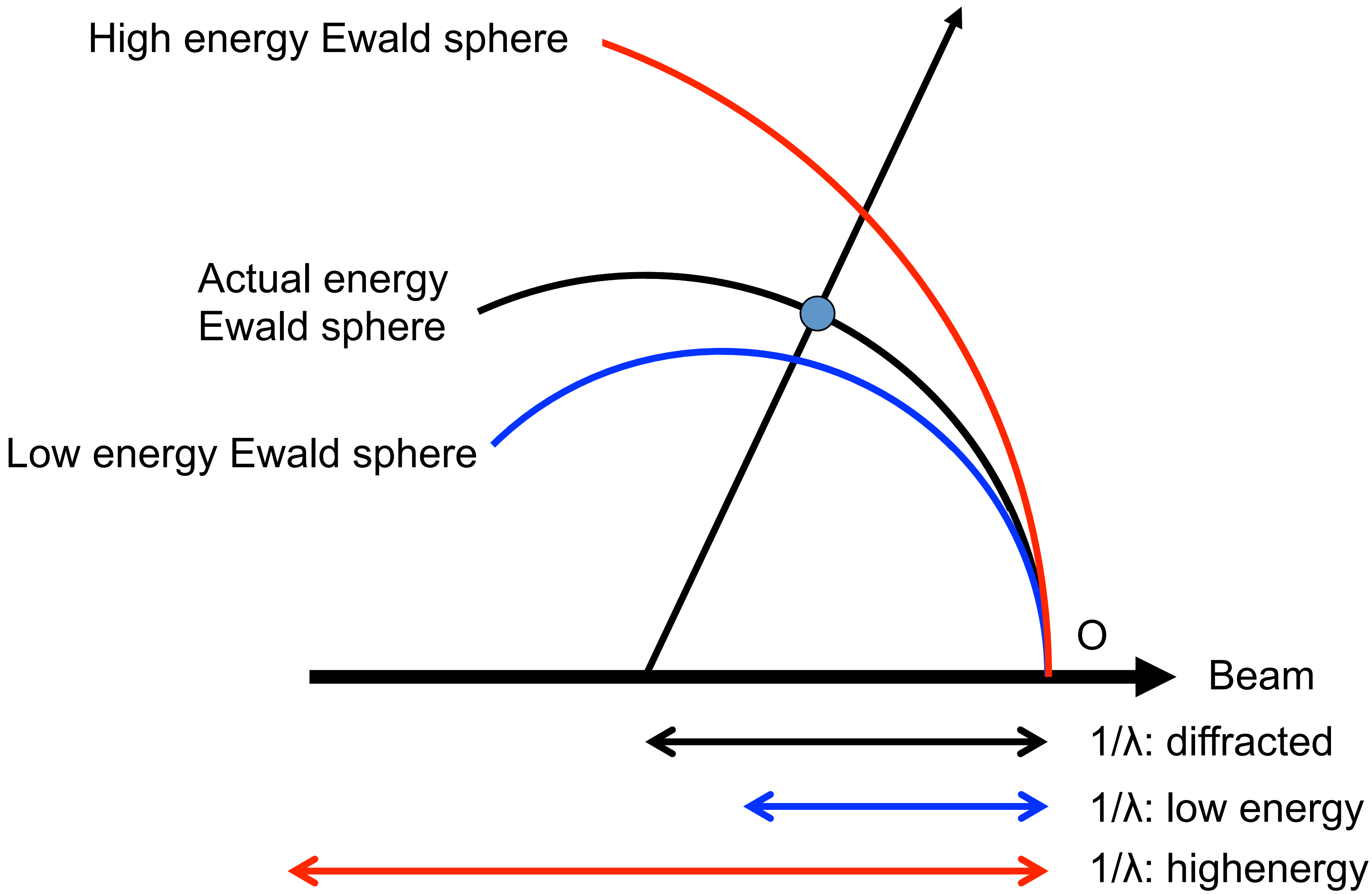
Shot-to-shot measurement of the X-ray incident pulse

Ewald sphere

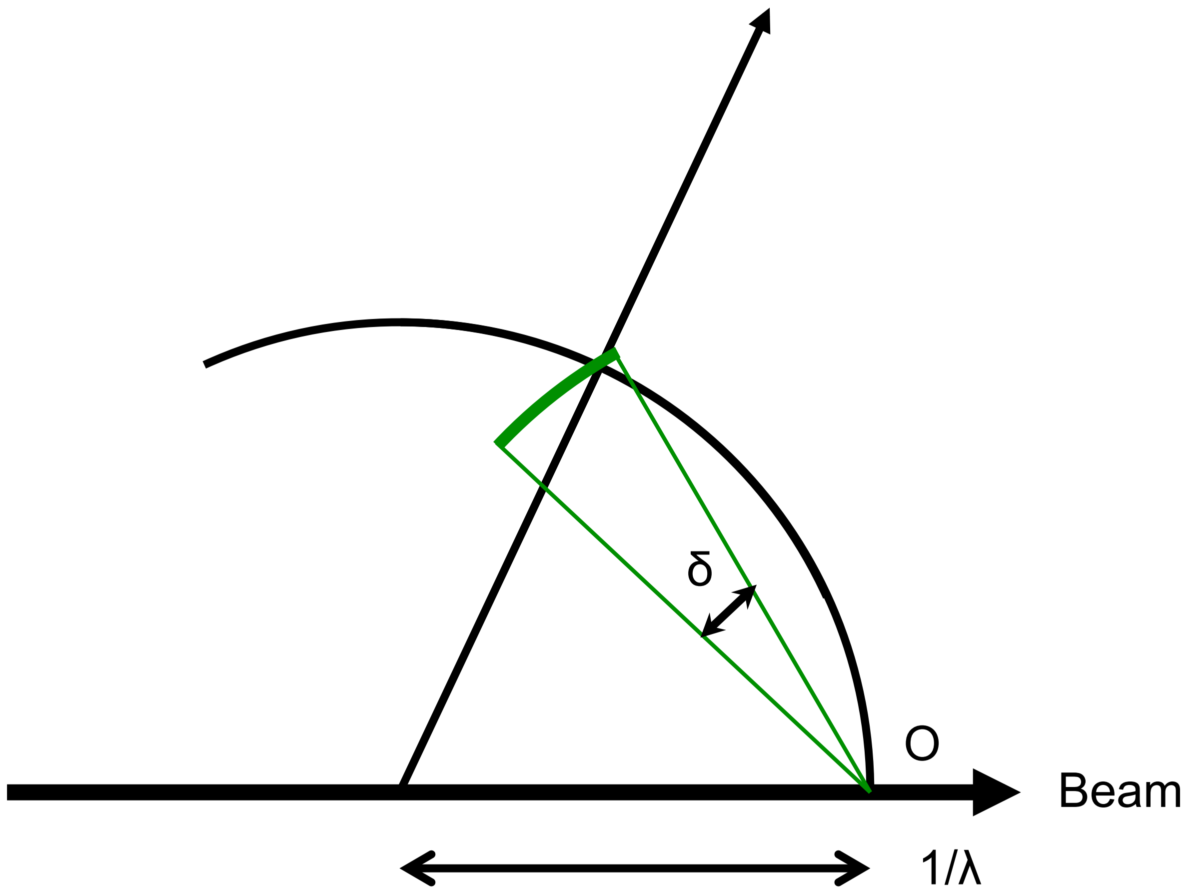


Slide sequence: Aaron Brewster

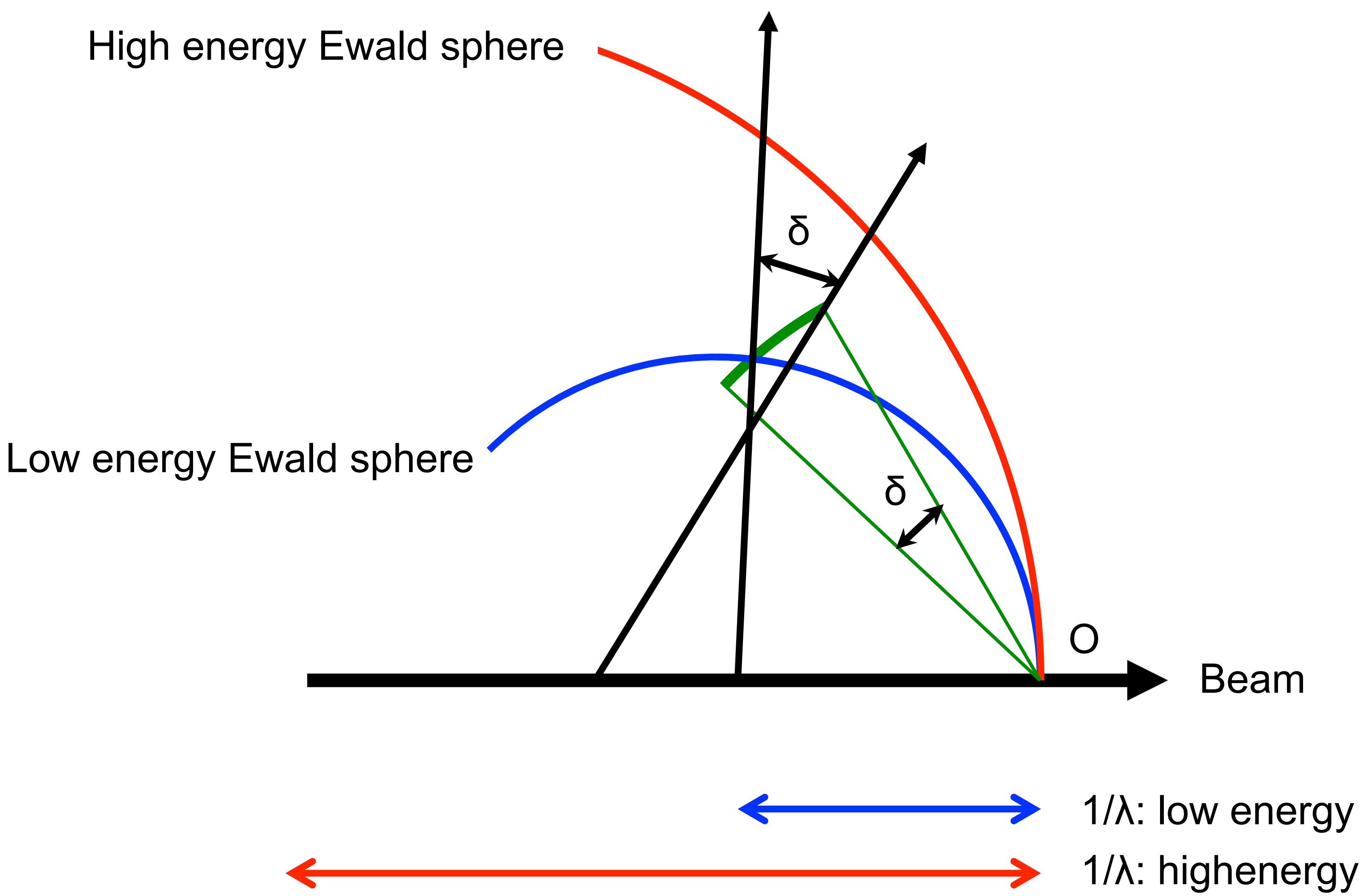
Ewald sphere paradigm with high/low bandpass limits



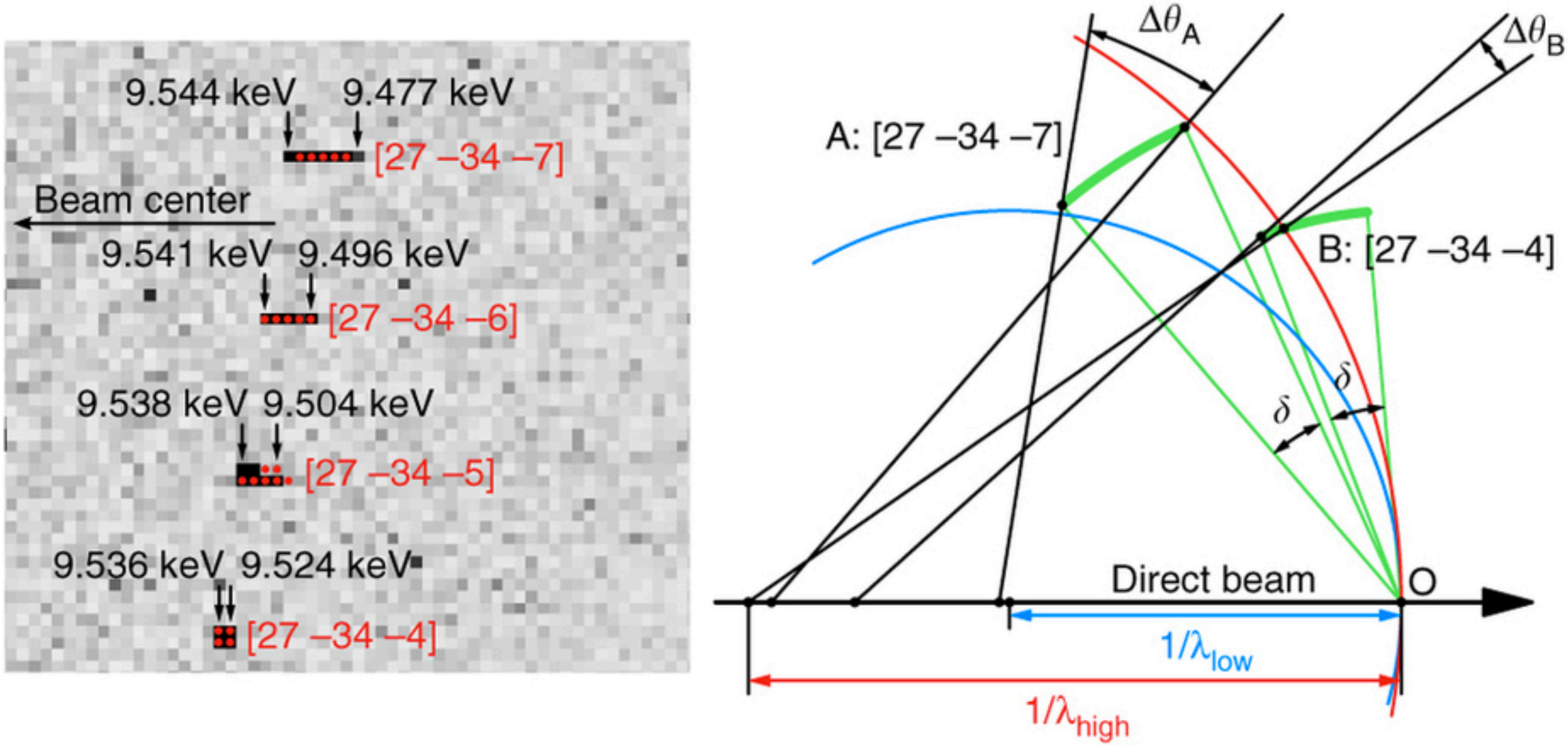
Ewald sphere with mosaicity arclets



Full model: bandpass + mosaicity

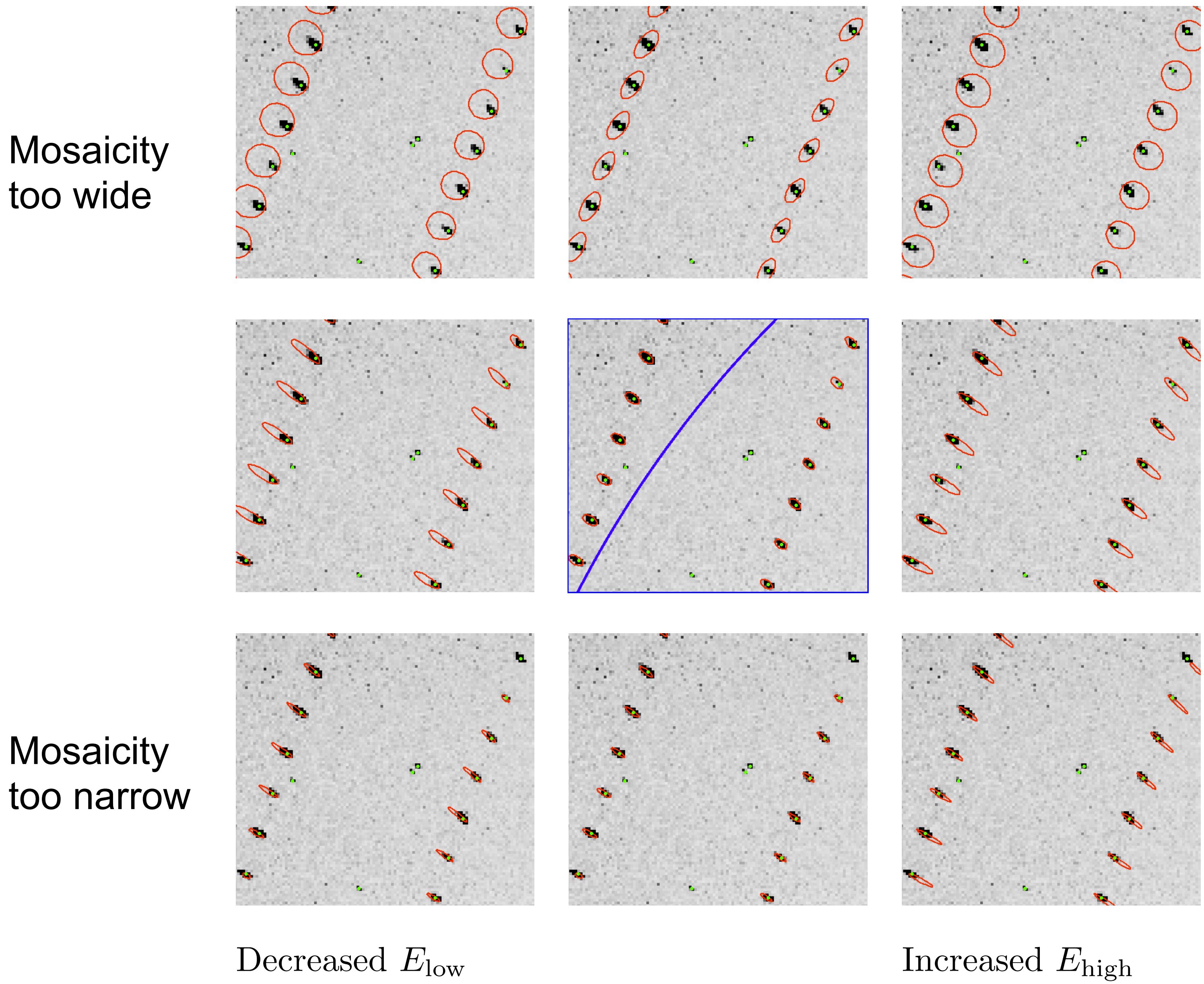


Radial dispersion: modeling each pixel

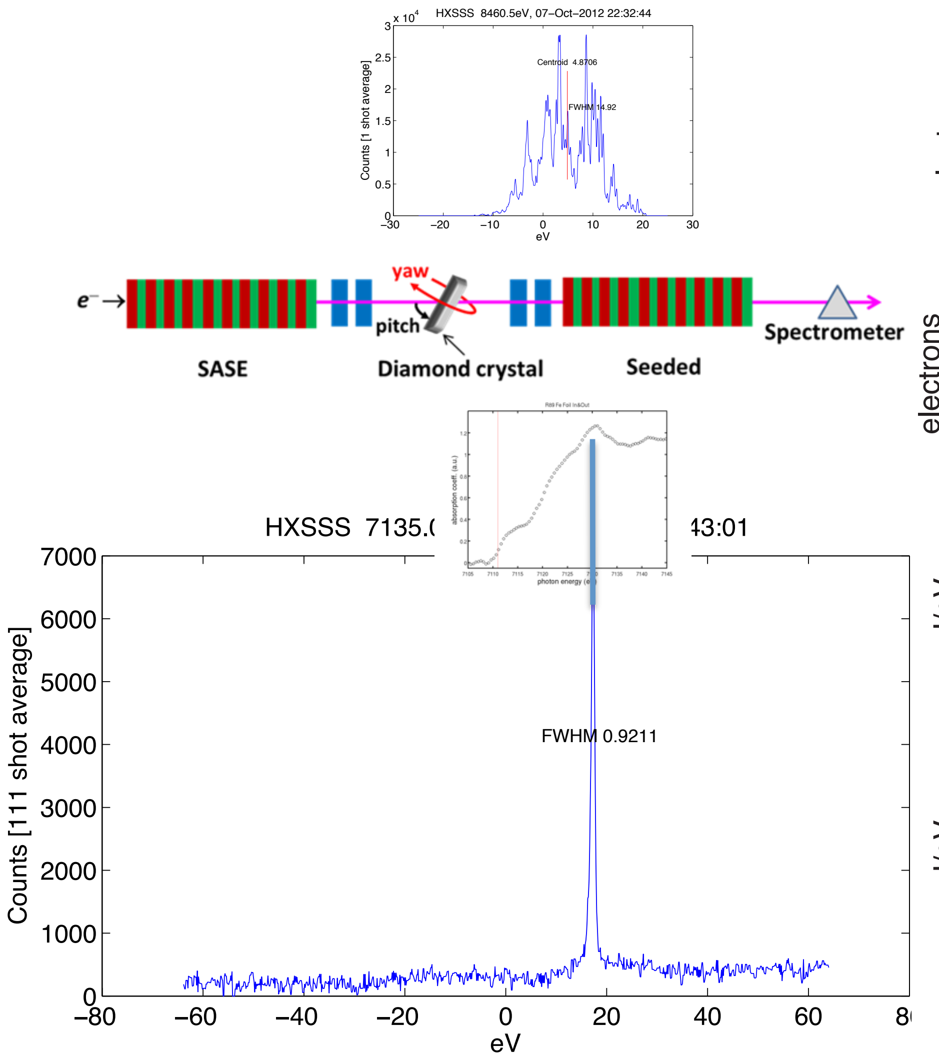


Hattne J et. al. (2014): *Nature Methods* **11**, 545-548.

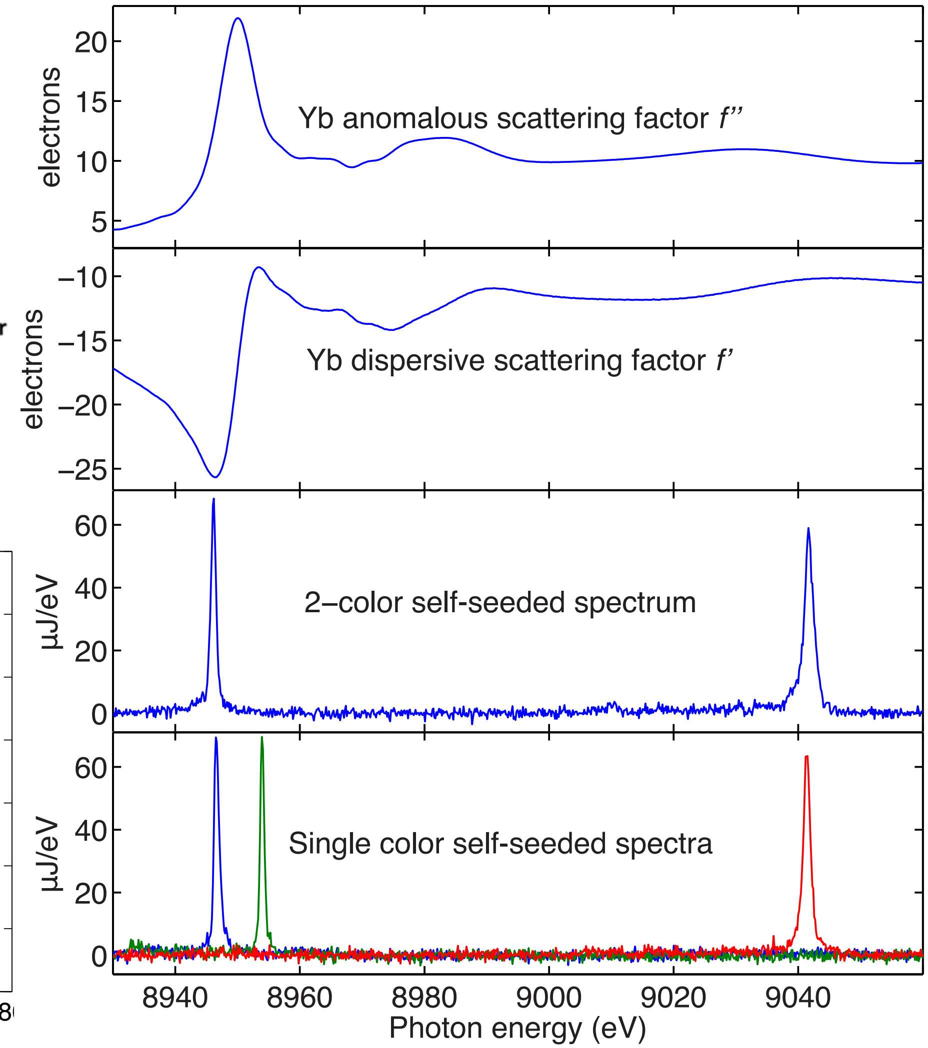
Optimal combination of dispersion + mosaicity to model spots



Single and two-color self seeded XFEL for SAD/MAD *de novo* phasing



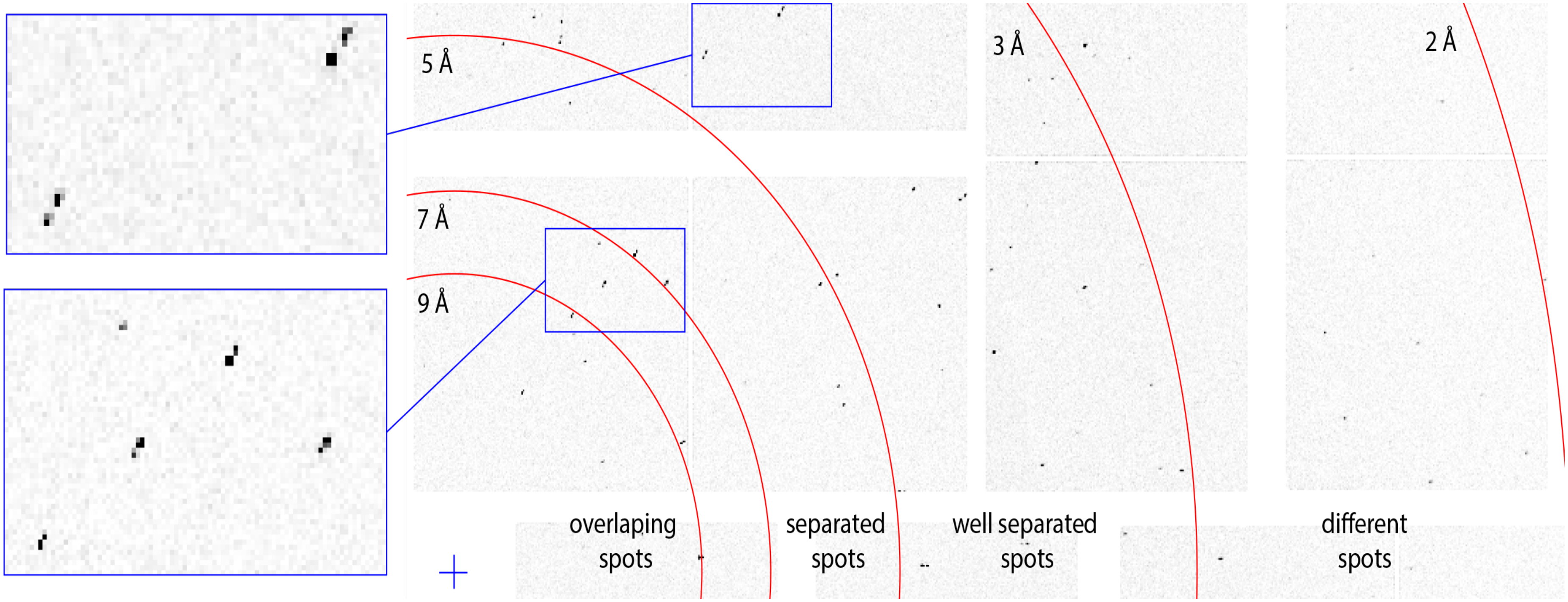
Optimized self seeded XFEL for SAD phasing

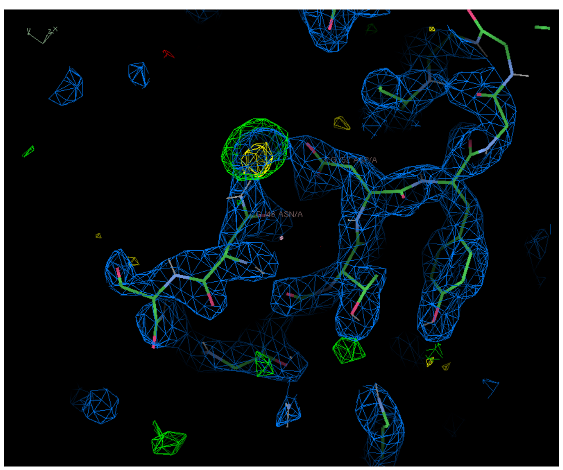


Two color self seeded XFEL with DE=95 eV
For Yb MAD phasing

Soichi Wakatsuki, Bill Weis, Axel Brunger (Stanford)

Two-color spectrum causes doubled Bragg spots





June 2014 preliminary results

Work in progress:

- Muhamed Amin
- Tara Michels-Clark
- Monarin Uervirojnangkoorn
- Ulf Lundstrom

So how good are my data?

Sources of systematic error:

- Variable crystal volume hit by beam
- Variable flux
- Detector metrology
- Inability to fully constrain the orientation model of still shots
- Varying crystal quality & internal disorder
- Stochastic SASE spectrum
- Non-isomorphism (Oliver Zeldin)

Measures of success:

- Bright spots r.m.s. displacement lattice model vs. observation
- Intensity statistics
- Wilson B factor
- Rfactors
- Peak height in the anomalous Fourier map

Acknowledgements

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Computational Methods
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Ray Sierra

LCLS
Spectroscopy
Uwe Bergmann

SSRL
Goniometry Methods
Mike Soltis
Ana Gonzalez
Ashley Deacon
Aina Cohen
Yingssu Tsai
Scott McPhillips

UCLA
BT toxin; amyloid
David Eisenberg
Duilio Cascio
Michael Sawaya
Jose Rodriguez
Luki Goldschmidt

BNL
Sample Delivery
Allen Orville
Christian Roessler

UCLA
BT toxin; amyloid
David Eisenberg
Duilio Cascio
Michael Sawaya
Jose Rodriguez
Luki Goldschmidt

Dowling College
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Herbert Bernstein

DIALS Development
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Richard Gildea
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Luis Fuentes-Montero

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Gregory Bell

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Polyhedra crystals
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Gwyndaf Evans
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Mona Uervirojnangkoorn
Oliver Zeldin

Phasing Methods
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Bill Weis

MPI Heidelberg
Data Processing
Ilme Schlichting
Karol Nass

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