Long Wavelength Phasing at SLS

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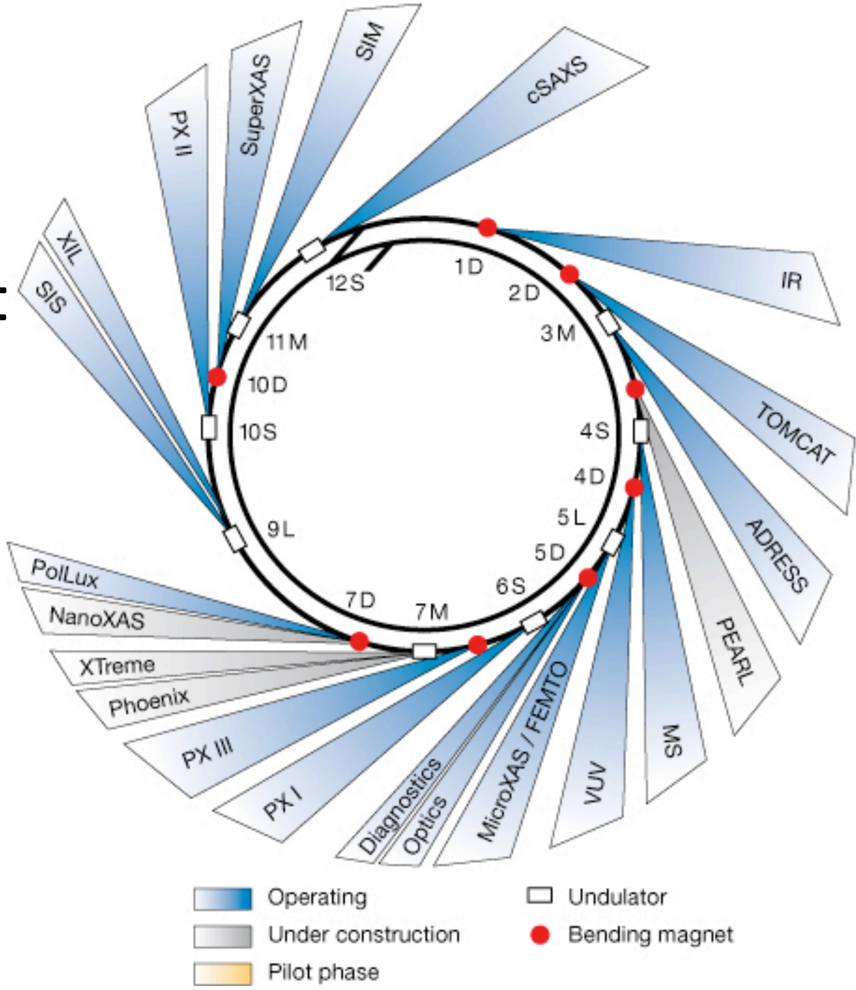
Outline

- Swiss Light Source and PX beamlines
- X06DA: a Beamline for Phasing Experiment
- Better Data for Phasing
- Phosphor-SAD Phasing

Swiss Light Source

- 2.4 GeV third generation synchrotron
- Top-up mode - 400 mA (± 1 mA)
- Low-emittance - $\varepsilon_x = 5.5$ nmrad
- 18 beamlines



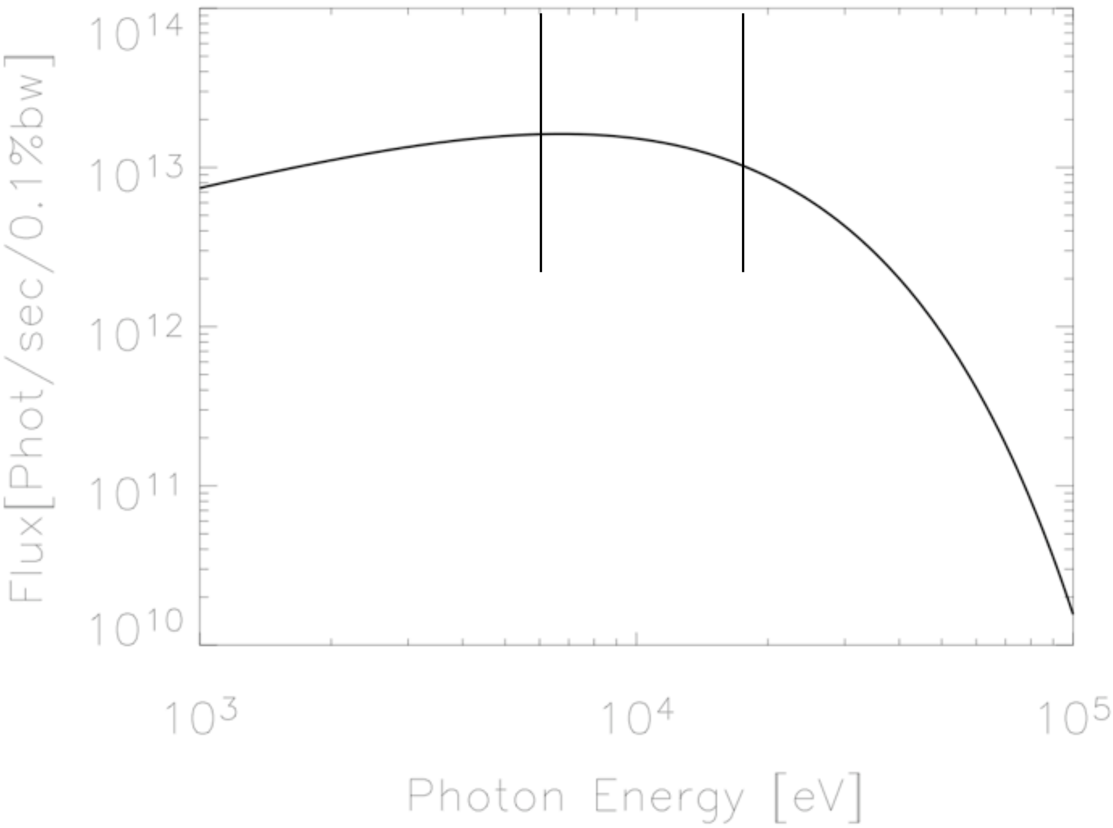
Protein Crystallography Beamlines

PX beamline	X06SA	X10SA	X06DA
Source	U19	U19	2.9T Superbend
Energy range (keV)	6.0 – 17.5	5.5 – 20.0	6.0 – 17.5
X-ray Optics	Dynamic focusing with adaptive optics		Achromatic fixed focusing
Focused beam size (µm) Divergence (mrad)	HR: 85 × 10 MD2: **25 × 5** ~ 0.4 × 0.06	50 × 10 0.6 × 0.1	90 × 45 2.0 × 0.5
Flux at 12.4 keV (photons/second)	2×10^{12}	2×10^{12}	5×10^{11}
Detector	**PILATUS 6M**	**PILATUS 6M**	**PILATUS 2M**
Sample changer	IRELEC CATS	CATS	CATS

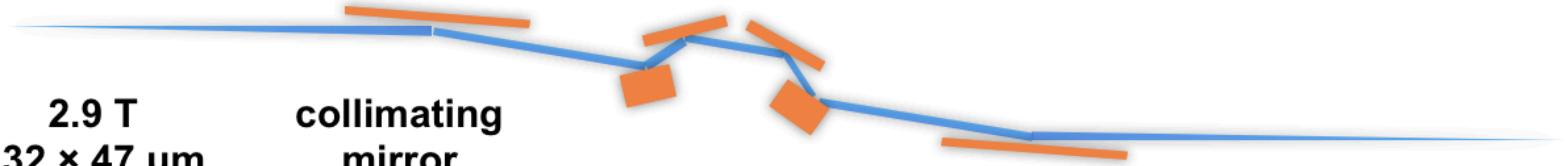
X06DA: A Beamline for Phasing

- Bright super-bending magnet source
- True fixed-exit monochromator
- Achromatic focusing
- PILATUS 2M FAST (Nov. 2011)
- Multi-axis goniometer (Jan. 2012)
- *In situ* diffraction screening with SBS plate
- Integrated crystallization facility

SuperBend 2.9T: Bright and Stable



Stable and Rapidly Tunable Optics



source

7 m

14 m

21 m

2.9 T
132 × 47 µm

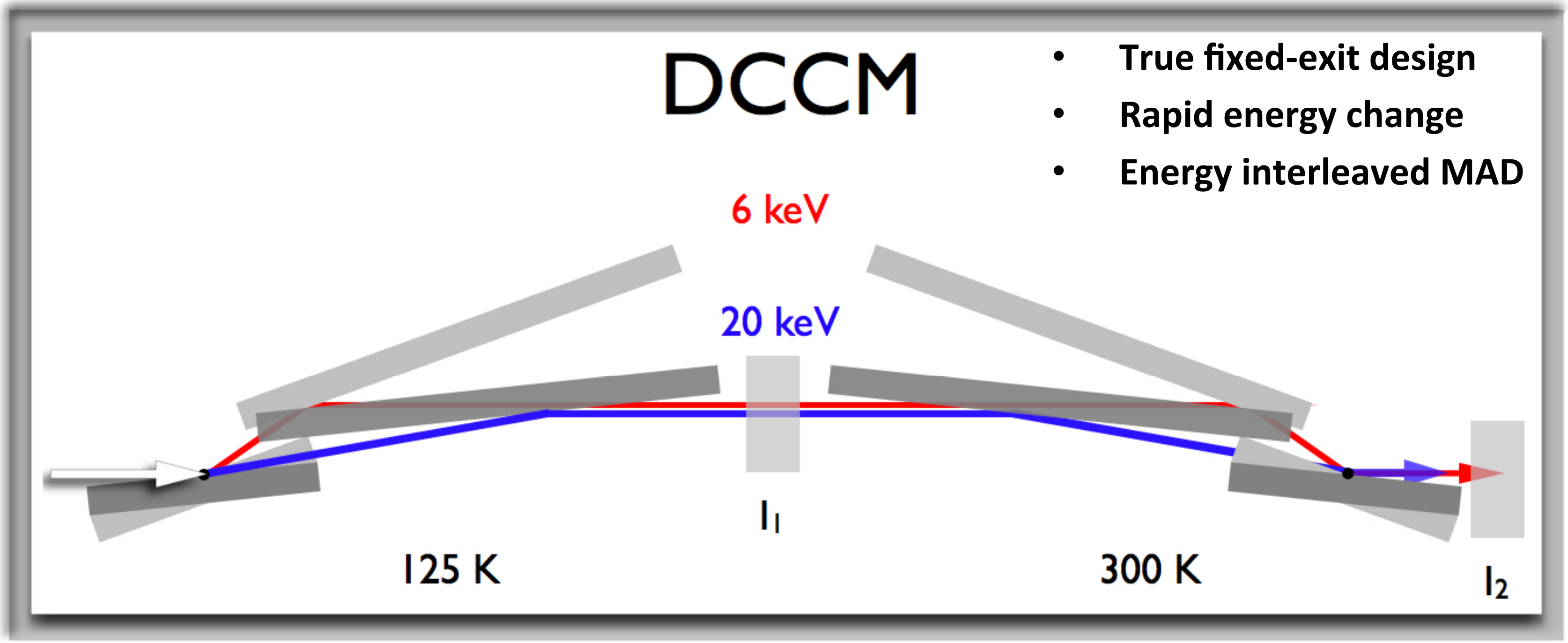
collimating
mirror

double channel-cut
monochromator

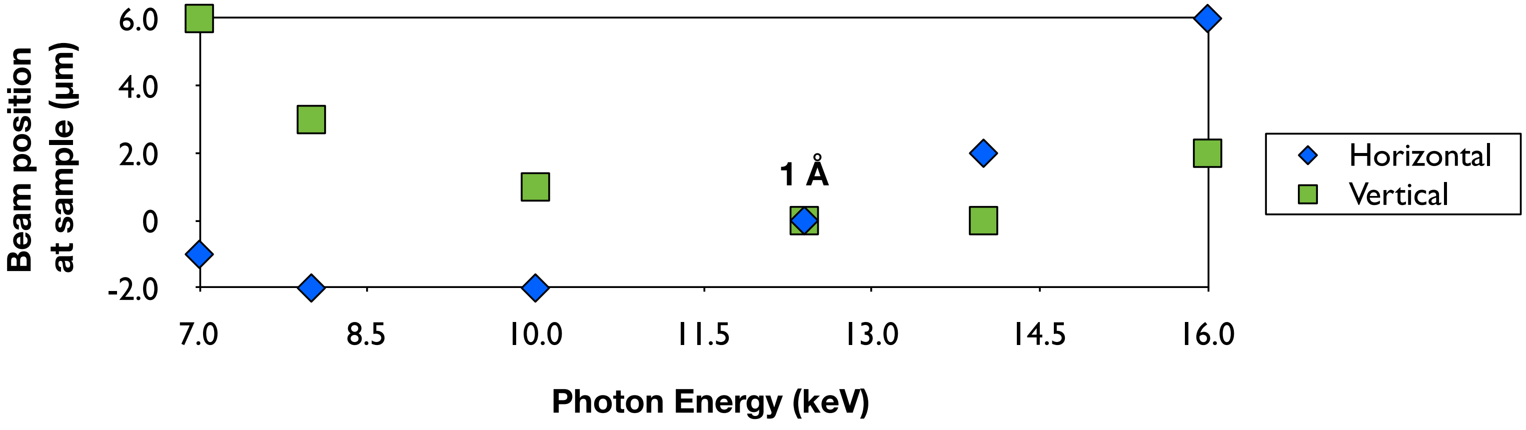
toroidal
focusing
mirror

demag
V 1:1
H 2:1

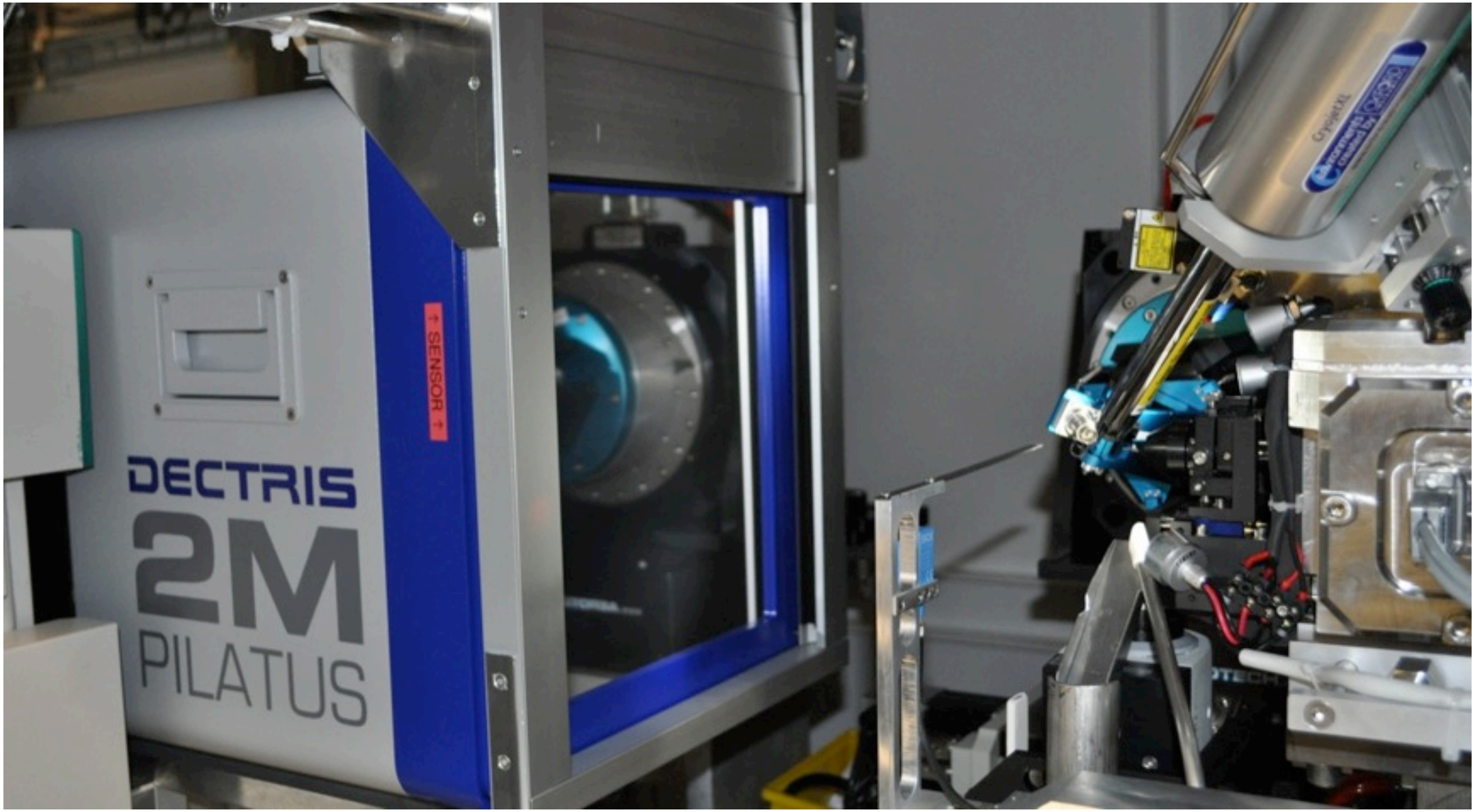
Double Channel-Cut Monochromator



- True fixed-exit design
- Rapid energy change
- Energy interleaved MAD



X06DA End-Station



PILATUS 2M FAST (60 Hz)
Excellent Data

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SUBSET OF INTENSITY DATA WITH SIGNAL/NOISE >= -3.0 AS FUNCTION OF RESOLUTION
RESOLUTION     NUMBER OF REFLECTIONS    COMPLETENESS R-FACTOR  R-FACTOR COMPARED I/SIGMA   R-meas  Rmrgd-F  Anomal  SigAno   Nano
  LIMIT     OBSERVED  UNIQUE  POSSIBLE     OF DATA   observed  expected                                      Corr

    5.01        3702     380       382       99.5%       1.1%      1.2%     3701  170.73     1.2%     0.4%    68%   1.699     275
    3.55        5798     646       646      100.0%       1.0%      1.2%     5798  164.48     1.1%     0.4%    35%   1.111     535
    2.89        8225     834       834      100.0%       1.3%      1.4%     8225  149.63     1.3%     0.5%    32%   1.163     739
    2.51        9896     966       966      100.0%       1.6%      1.6%     9896  124.92     1.7%     0.7%    29%   1.120     870
    2.24       11029    1100      1100      100.0%       1.9%      1.9%    11029  102.49     2.0%     1.0%    17%   1.068     977
    2.05       10859    1187      1187      100.0%       2.4%      2.2%    10859   82.11     2.5%     1.3%    11%   1.022    1072
    1.90       13102    1326      1326      100.0%       3.3%      3.2%    13102   61.08     3.5%     2.1%     7%   0.941    1231
    1.77       14043    1382      1382      100.0%       4.5%      4.5%    14043   45.70     4.7%     2.8%     7%   0.862    1285
    1.67       15441    1497      1497      100.0%       6.4%      6.6%    15441   32.86     6.7%     3.9%     5%   0.840    1405
    1.59       15253    1572      1572      100.0%       8.7%      8.7%    15253   24.43     9.2%     5.7%     3%   0.830    1412
    1.51       15304    1636      1636      100.0%      12.6%     12.6%    15304   17.27    13.3%     8.9%     1%   0.796    1525
    1.45       16779    1722      1722      100.0%      16.5%     16.9%    16779   13.17    17.4%    11.2%     2%   0.790    1625
    1.39       17288    1799      1801       99.9%      22.9%     23.8%    17288    9.49    24.2%    15.5%     1%   0.775    1704
    1.34       16702    1852      1853       99.9%      31.3%     32.8%    16701    6.70    33.2%    22.2%    -3%   0.738    1691
    1.29       12555    1932      1933       99.9%      37.7%     39.8%    12551    4.58    41.0%    34.7%    -1%   0.740    1402
    1.25        8302    1954      1960       99.7%      45.5%     48.7%     8222    2.88    51.9%    51.4%    -4%   0.699     949
    1.22        5796    2010      2072       97.0%      53.2%     58.5%     5436    1.86    63.9%    73.8%    -1%   0.696     540
    1.18        3505    1755      2108       83.3%      69.6%     75.5%     2863    1.14    89.6%   118.7%    -1%   0.612     134
    1.15        1558    1124      2181       51.5%      68.2%     76.4%      801    0.87    93.1%   138.2%   -12%   0.549      14
    1.12         338     303      2211       13.7%      71.3%     73.1%       70    0.71   100.9%   148.4%     0%   0.000       0
   total      205475   26977     30369       88.8%       2.3%      2.4%   203362   35.60     2.4%     7.0%     3%   0.871   19385
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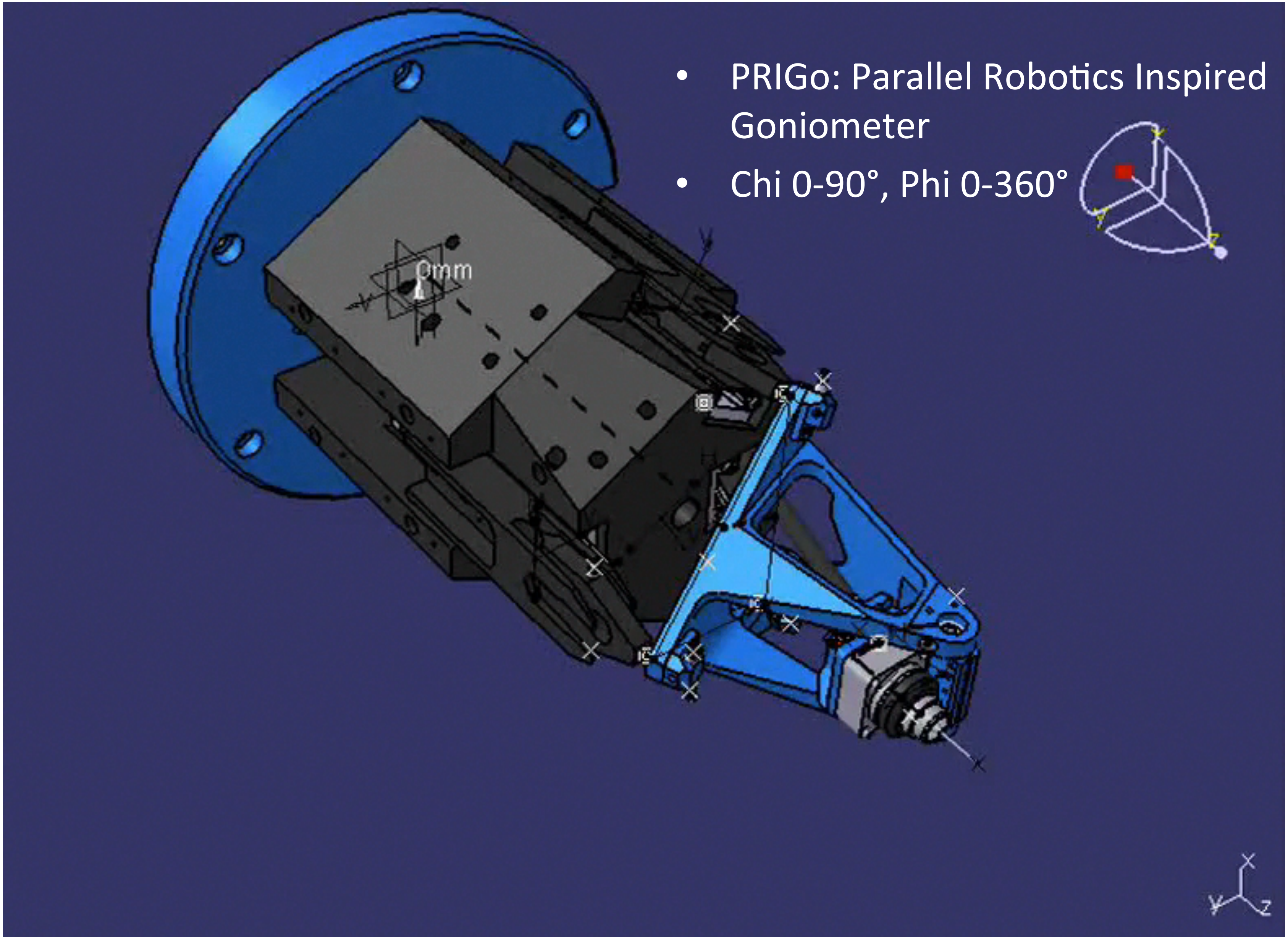
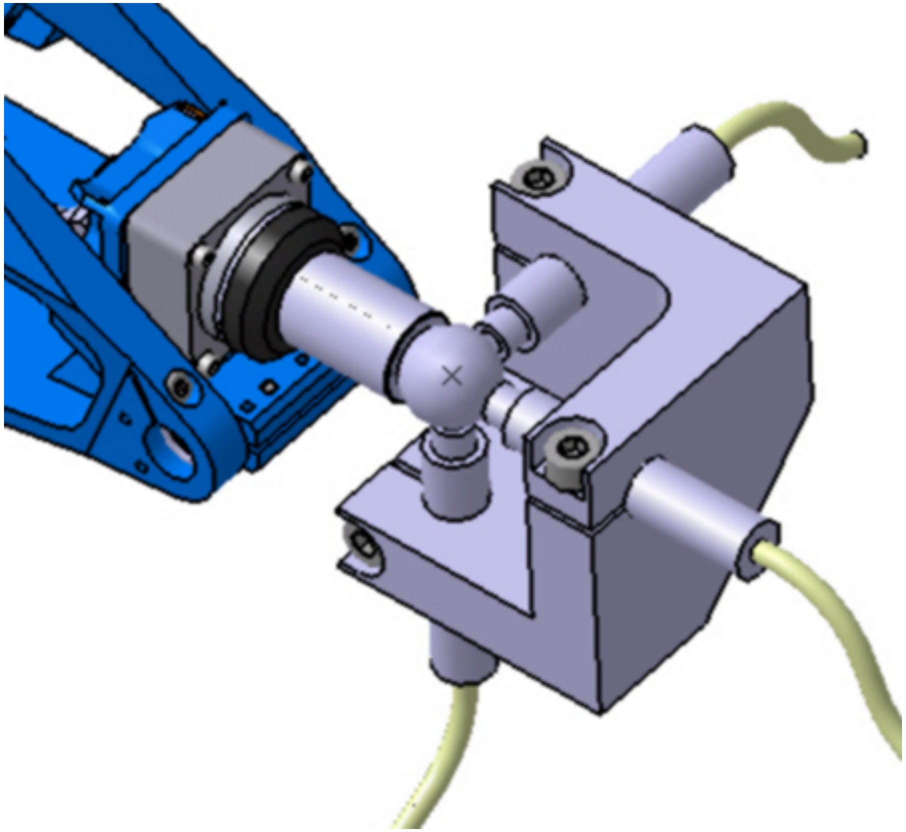
- Insulin
- 10 Hz fine φ-slicing - 0.1 deg/0.1 sec
- 900 images, 90 degree data

Multi-Axis Goniometer (PRIGo)

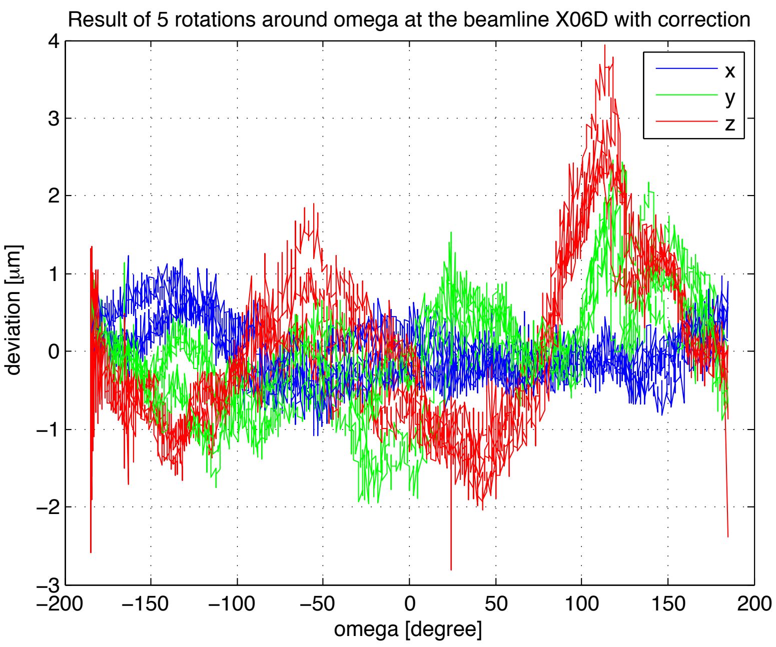


- PRIGo: Parallel Robotics Inspired Goniometer
- Chi 0-90°, Phi 0-360°

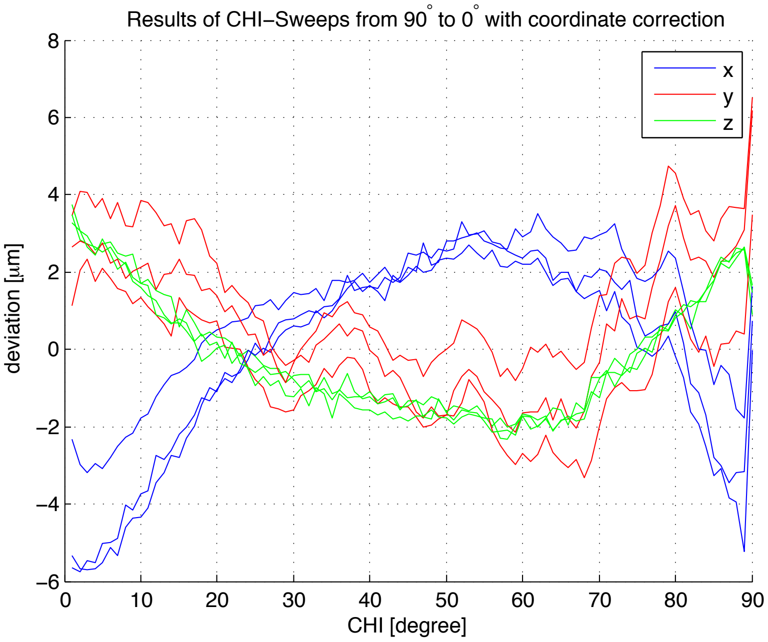
Sphere of Confusion Measurement



PRIGo: Sphere of Confusion < 5 µm



Omega

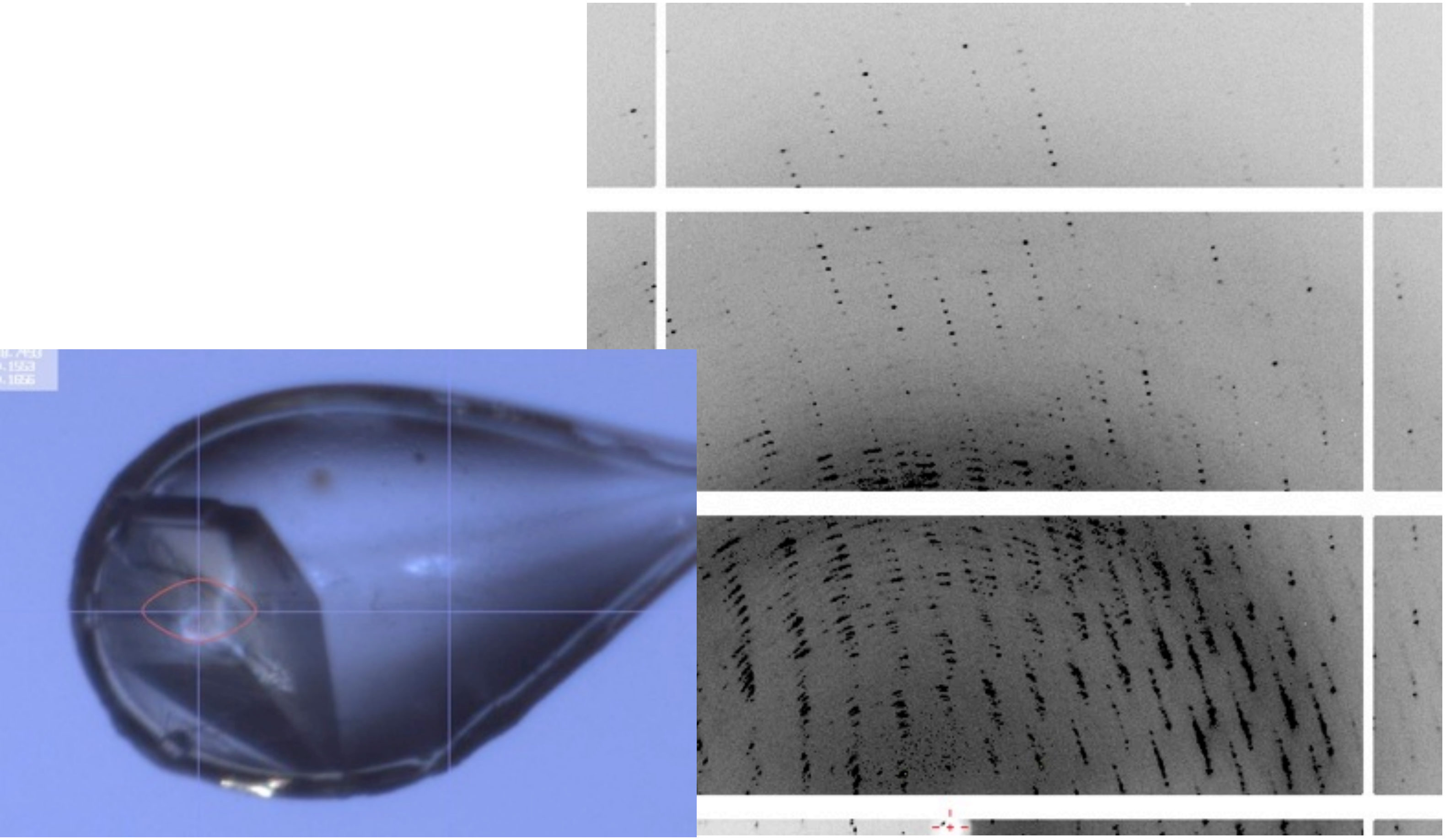


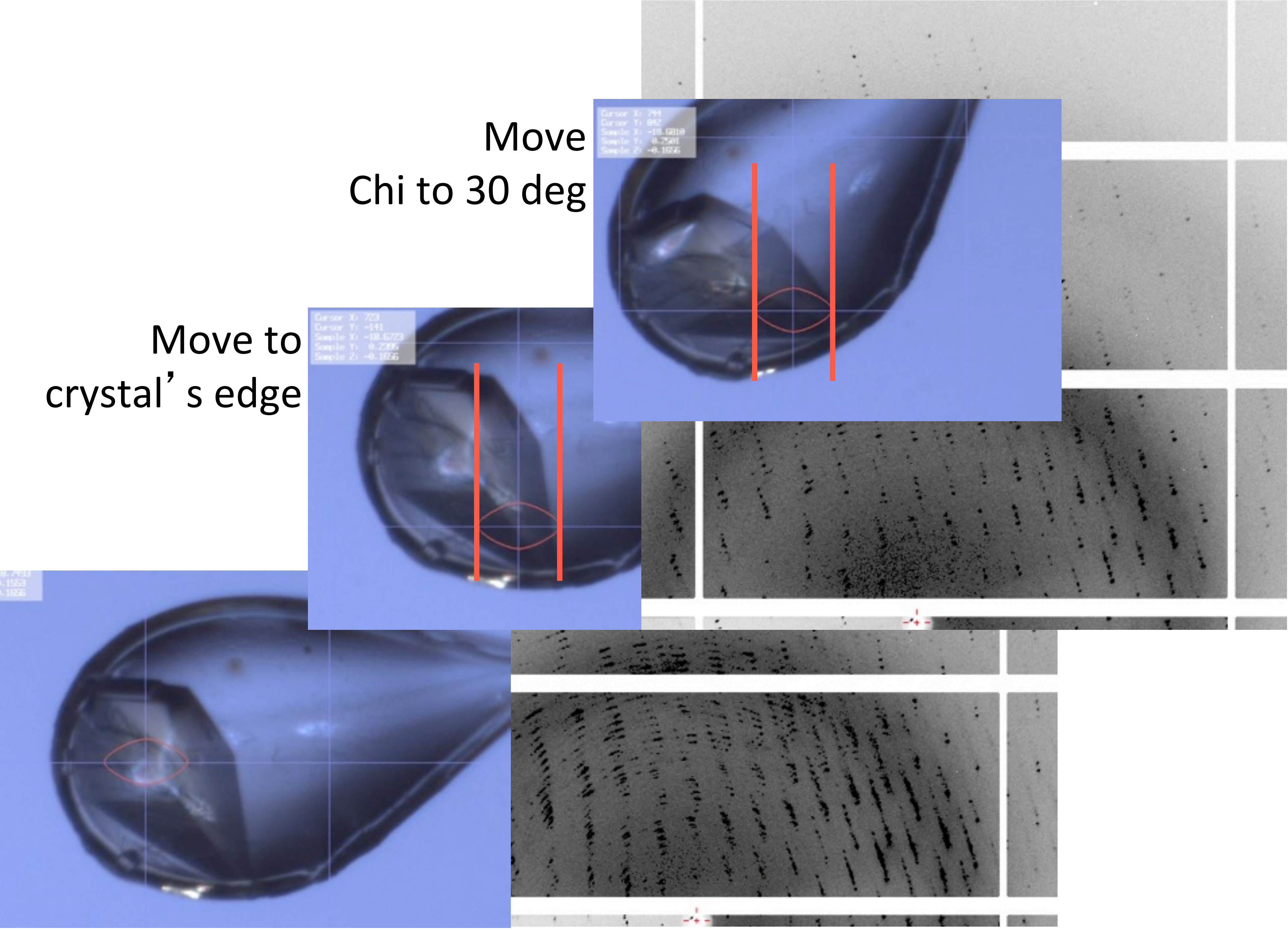
Chi

PRIGo User Application

- Select best part of crystal
- Align long-cell axis to avoid overlap
- Align even-fold rotation axis to record Bijvoet pairs on same image
- Multi-pass data collection with different crystal orientation for true redundancy and better absorption correction

PRIGo: Select Best Part of Crystal

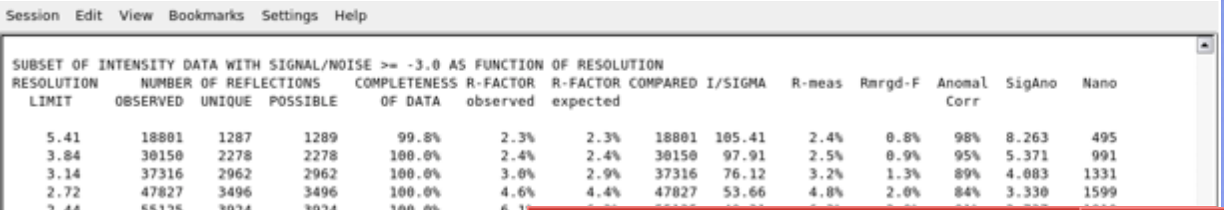




Move
Chi to 30 deg

Move to
crystal’s edge

720 deg data collection with Chi = 30 deg



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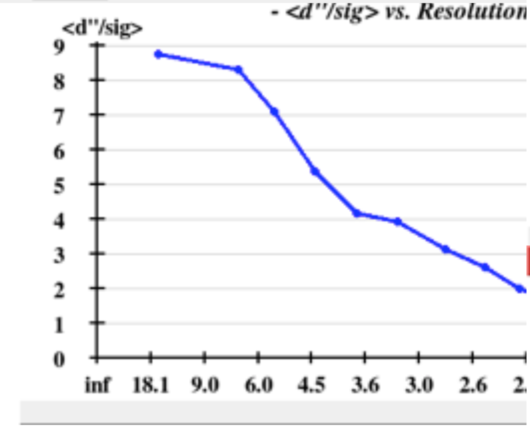
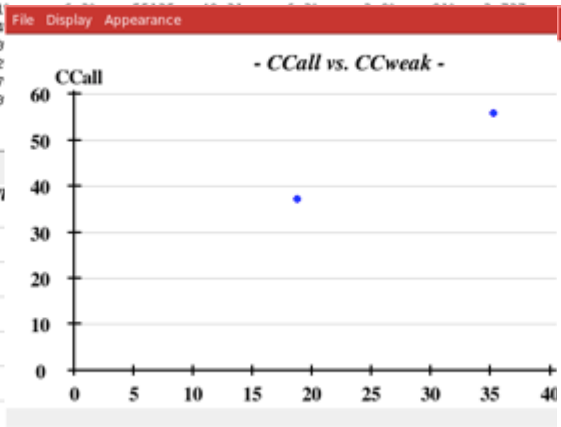
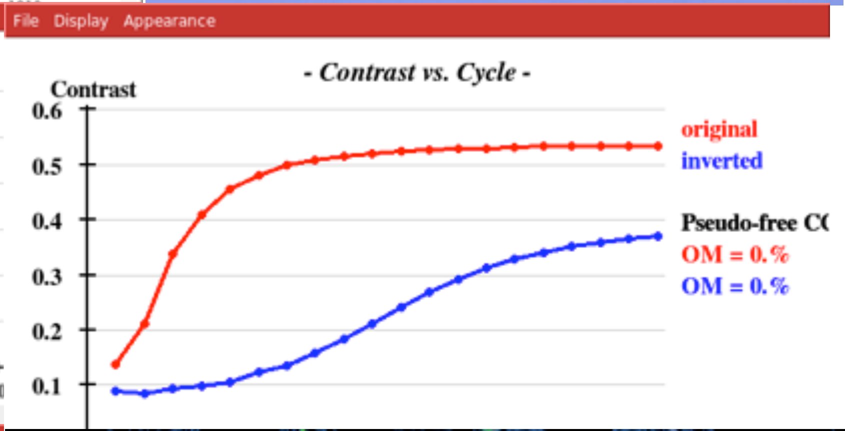
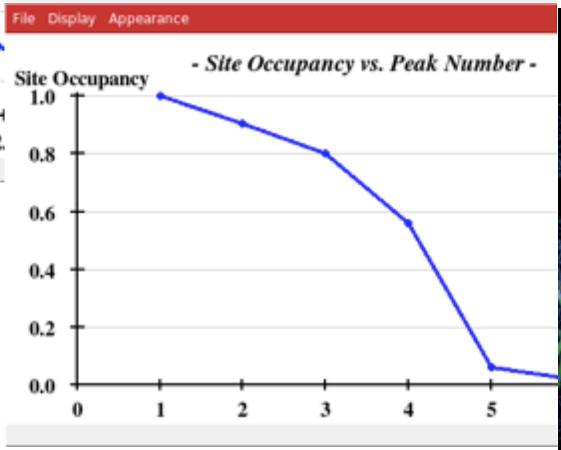
SUBSET OF INTENSITY DATA WITH SIGNAL/NOISE >= -3.0 AS FUNCTION OF RESOLUTION
RESOLUTION     NUMBER OF REFLECTIONS    COMPLETENESS R-FACTOR  R-FACTOR COMPARED I/SIGMA   R-meas  Rmrgd-F  Anomal  SigAno   Nano
  LIMIT     OBSERVED  UNIQUE  POSSIBLE     OF DATA   observed  expected                                     Corr

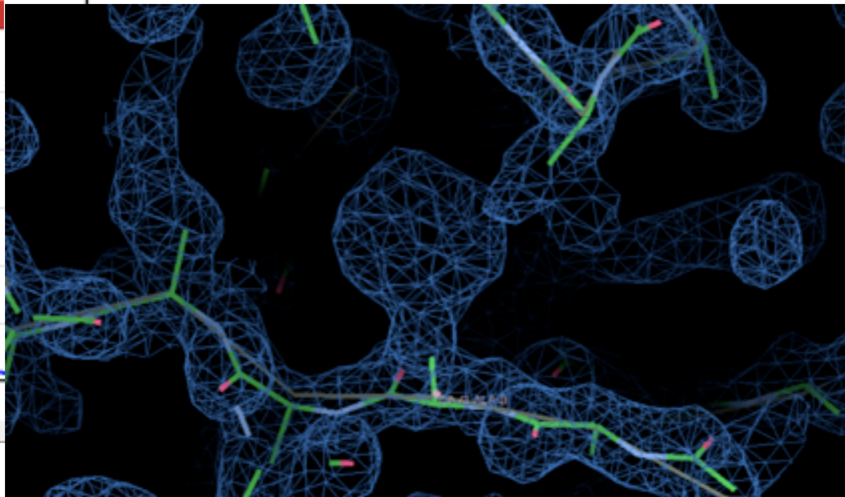
    5.41      18801    1287      1289       99.8%       2.3%      2.3%    18801  105.41     2.4%     0.8%    98%  8.263    495
    3.84      30150    2278      2278      100.0%       2.4%      2.4%    30150   97.91     2.5%     0.9%    95%  5.371    991
    3.14      37316    2962      2962      100.0%       3.0%      2.9%    37316   76.12     3.2%     1.3%    89%  4.083   1331
    2.72      47827    3496      3496      100.0%       4.6%      4.4%    47827   53.66     4.8%     2.0%    84%  3.330   1599
    2.44      55125    3924      3924      100.0%       6.1
    2.22      59559    4367      4367      100.0%       8.4
    2.06      57249    4768      4768      100.0%      11.0
    1.93      65351    5078      5078      100.0%      18.2
    1.82      69377    5294      5406       97.9%      27.7
   total     440755   33454     33568       99.7%       5.0
```



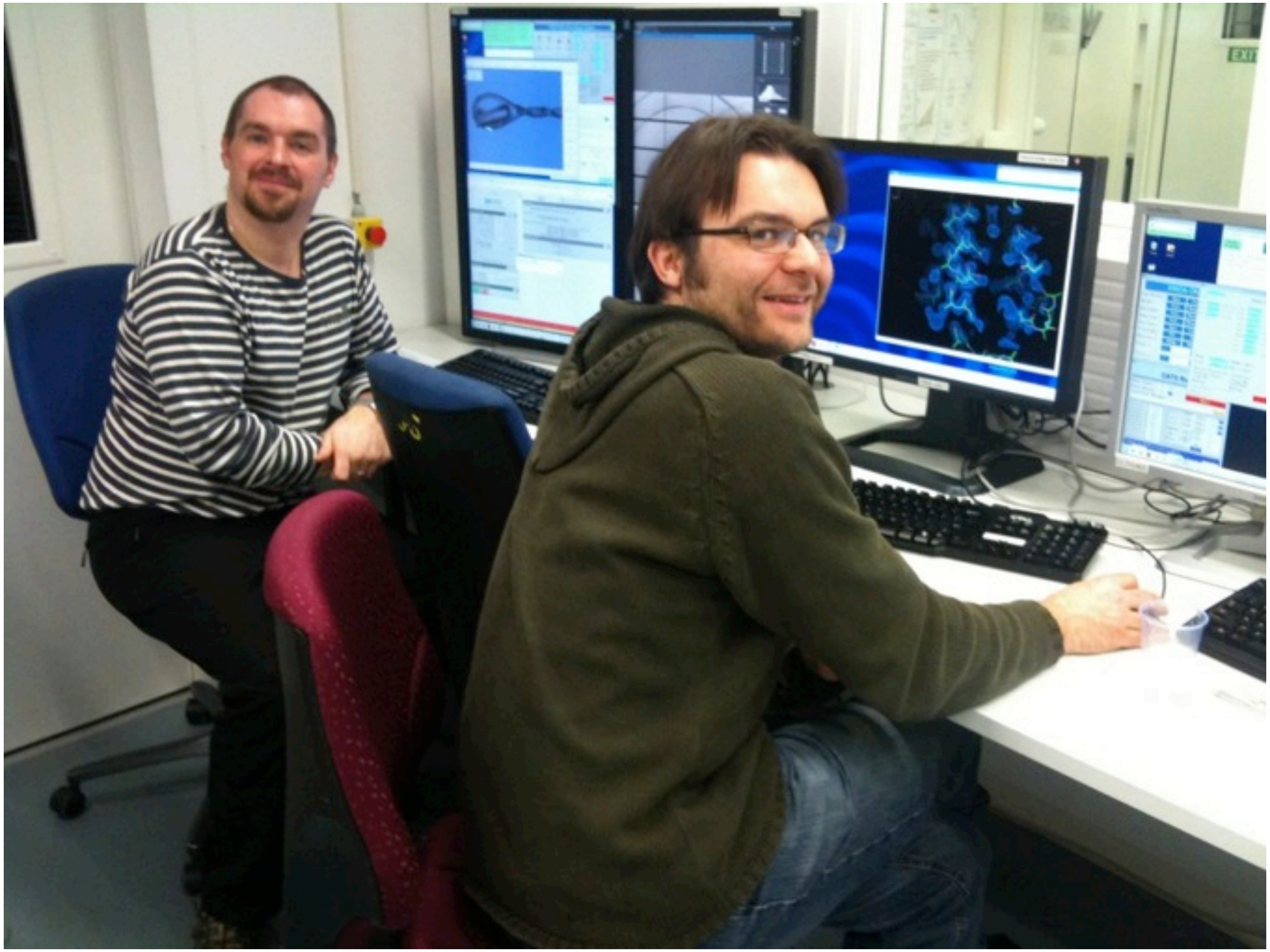






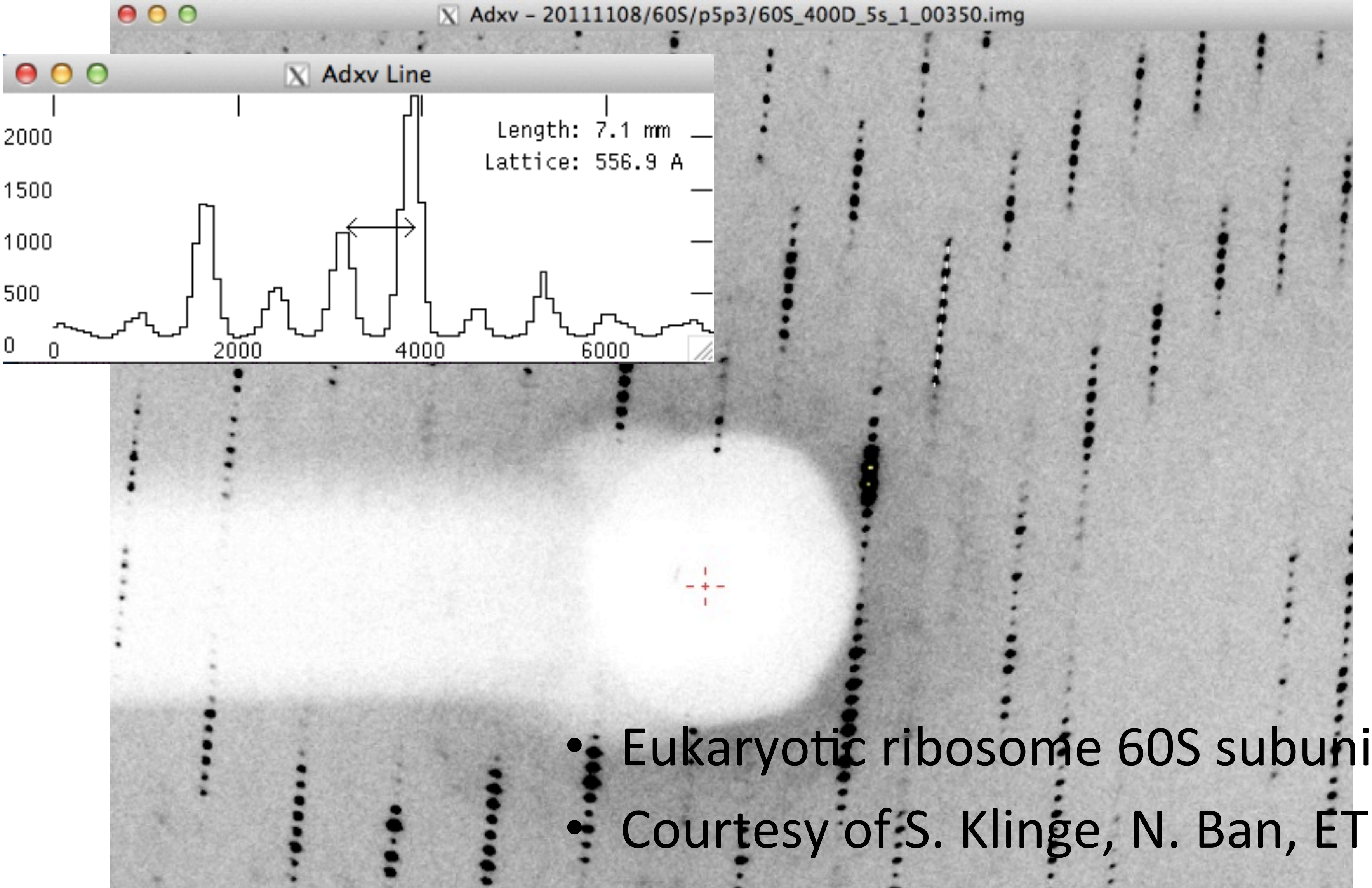


Jan. 27th 2012



Courtesy of E. Lorentzen, J. Basquin, MPG

PRIGo: Align Long-Cell Axis



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Length: 7.1 mm
Lattice: 556.9 A
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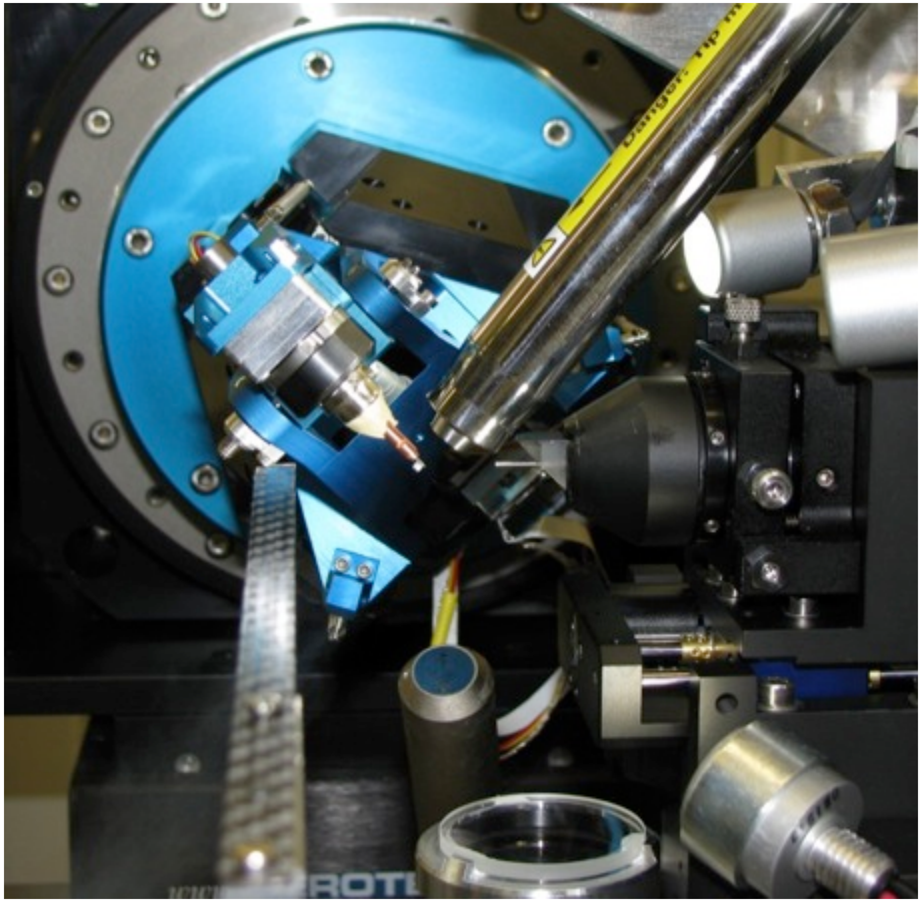
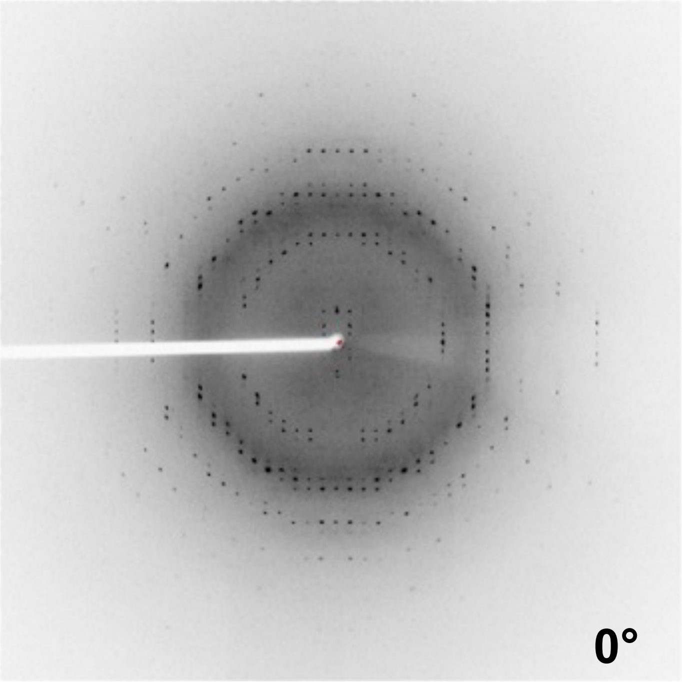
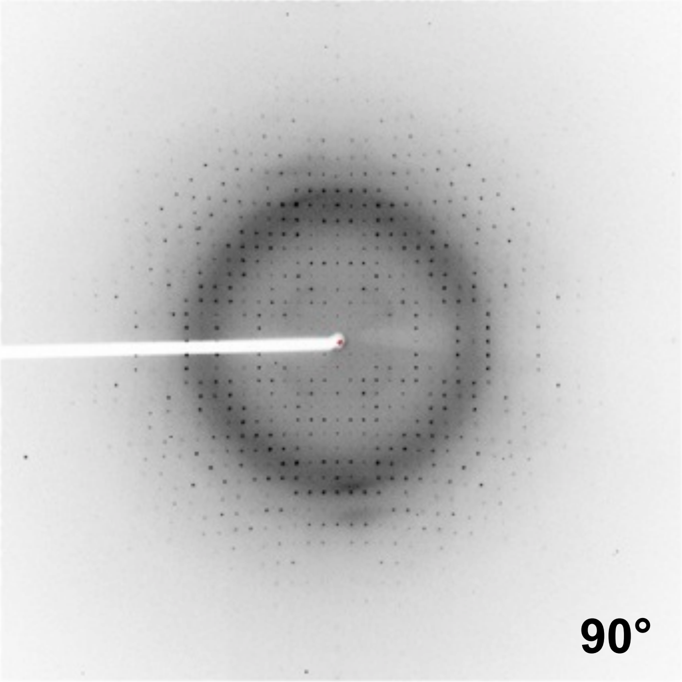
- Eukaryotic ribosome 60S subunit
- Courtesy of S. Klinge, N. Ban, ETH

PRIGo: Bijvoet Pairs on Same Image

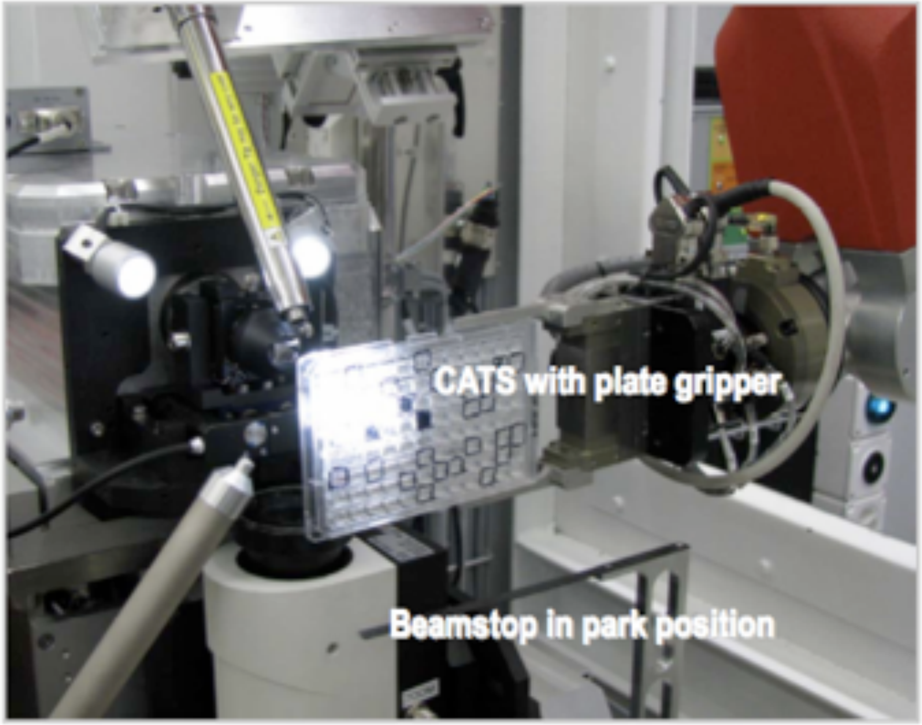


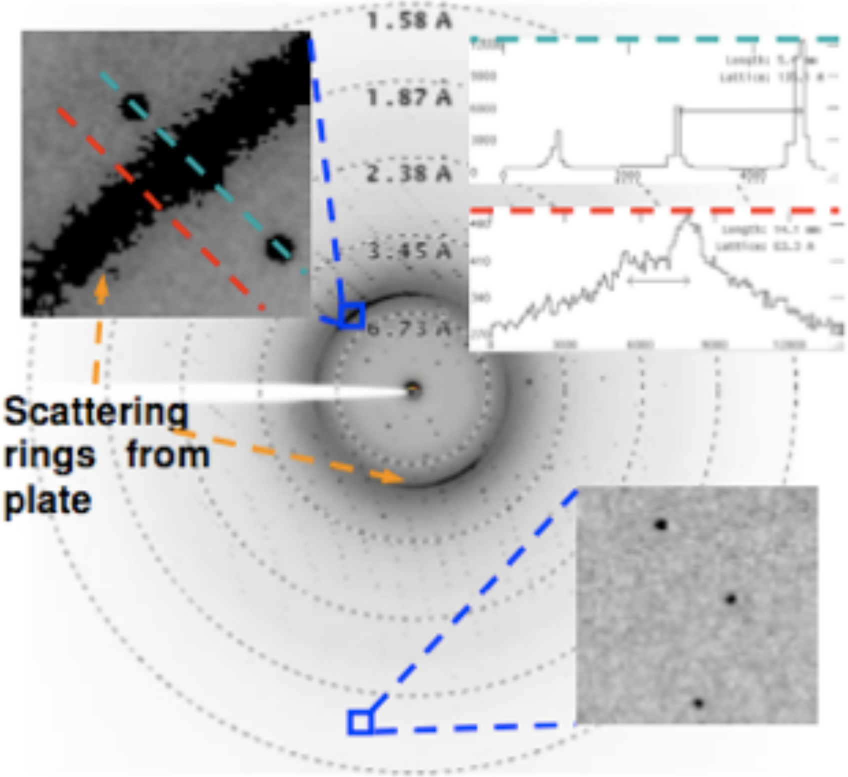




$P4_12_12$
47.41 47.41 70.44
Crystal oriented along a axis

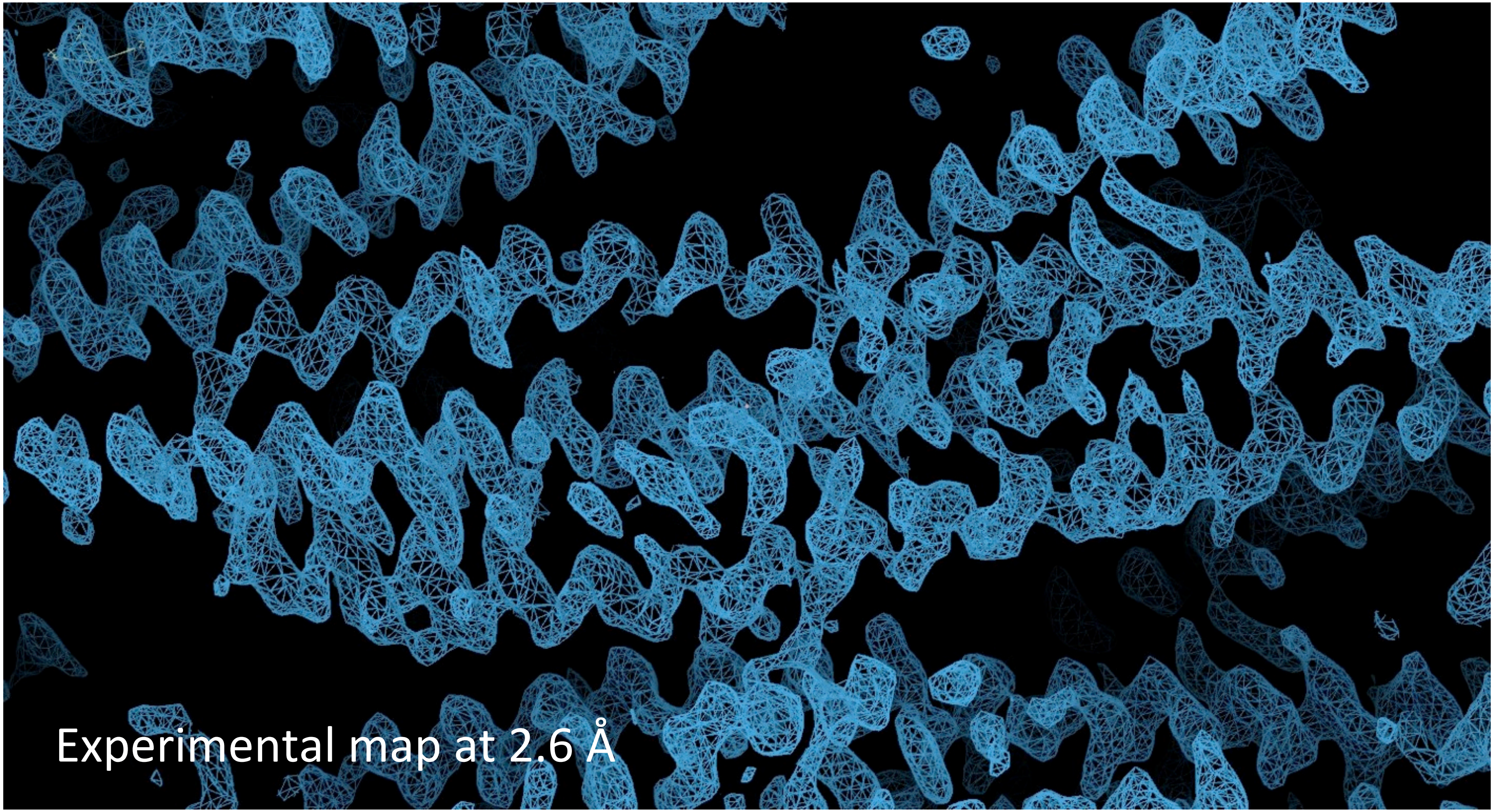
In situ Diffraction Screening





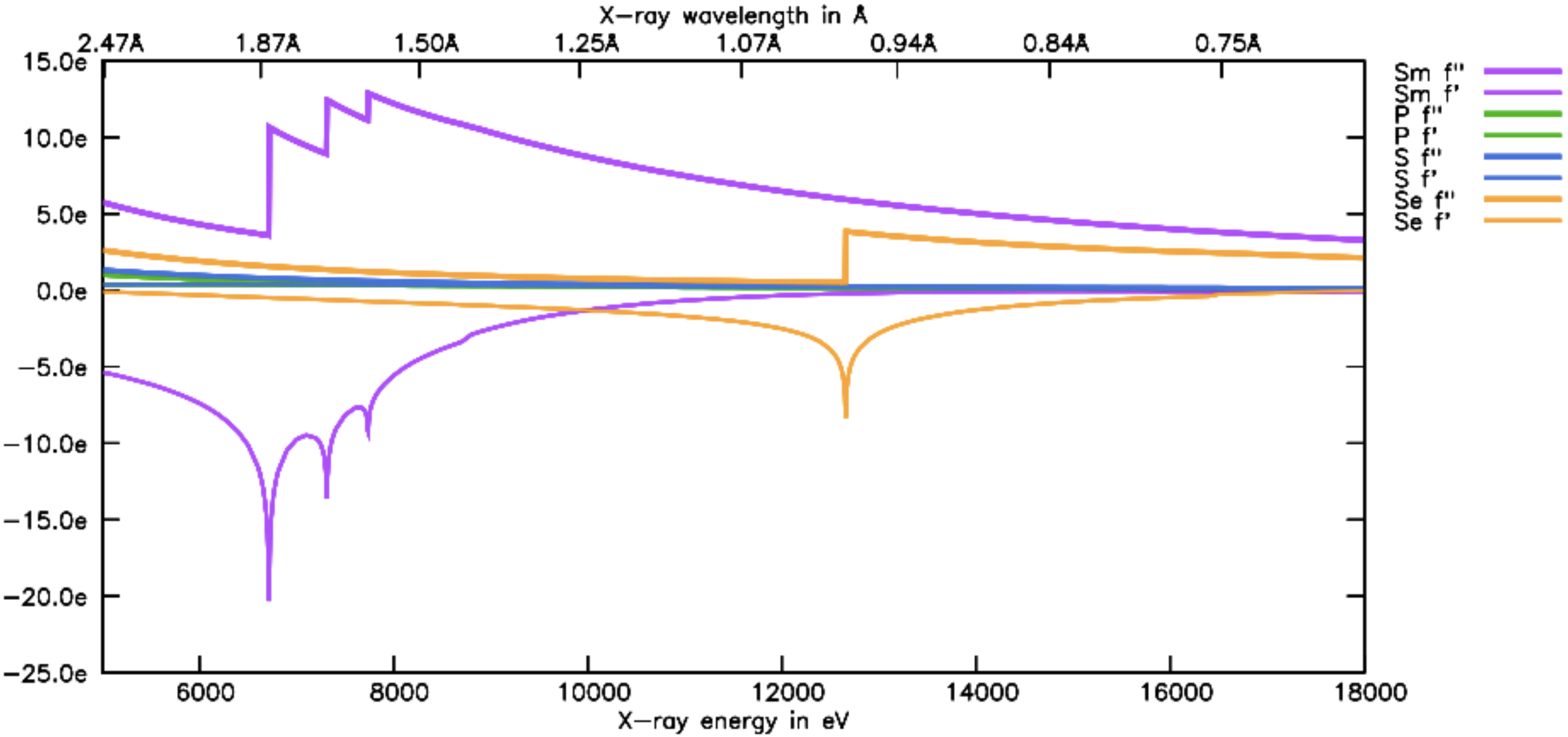
- In routine user operation
- 2 min switch time
- Same centering and data collection interface

Easy Long-Wavelength Case
Sm^{3+} L_{III}-edge, 1.846 Å


Experimental map at 2.6 Å

Courtesy of N. Stefan, S. Streikov, Belgium

Challenge in S-SAD and P-SAD Phasing



- There is really no much anomalous signal!
- $<\Delta F^{\pm}>/<F> \approx$ 1-2 % for S-SAD at 1.6-2.0 Å
- $<\Delta F^{\pm}>/<F> \approx$ 2-3 % for P-SAD at 1.6-2.0 Å

Signal/Noise

$$\langle \Delta F^{\pm} \rangle / \langle \sigma(F) \rangle$$

$$\langle \Delta I^{\pm} \rangle / \langle \sigma(I) \rangle$$

$$I/\sigma(I)$$

Improve Signal to Noise

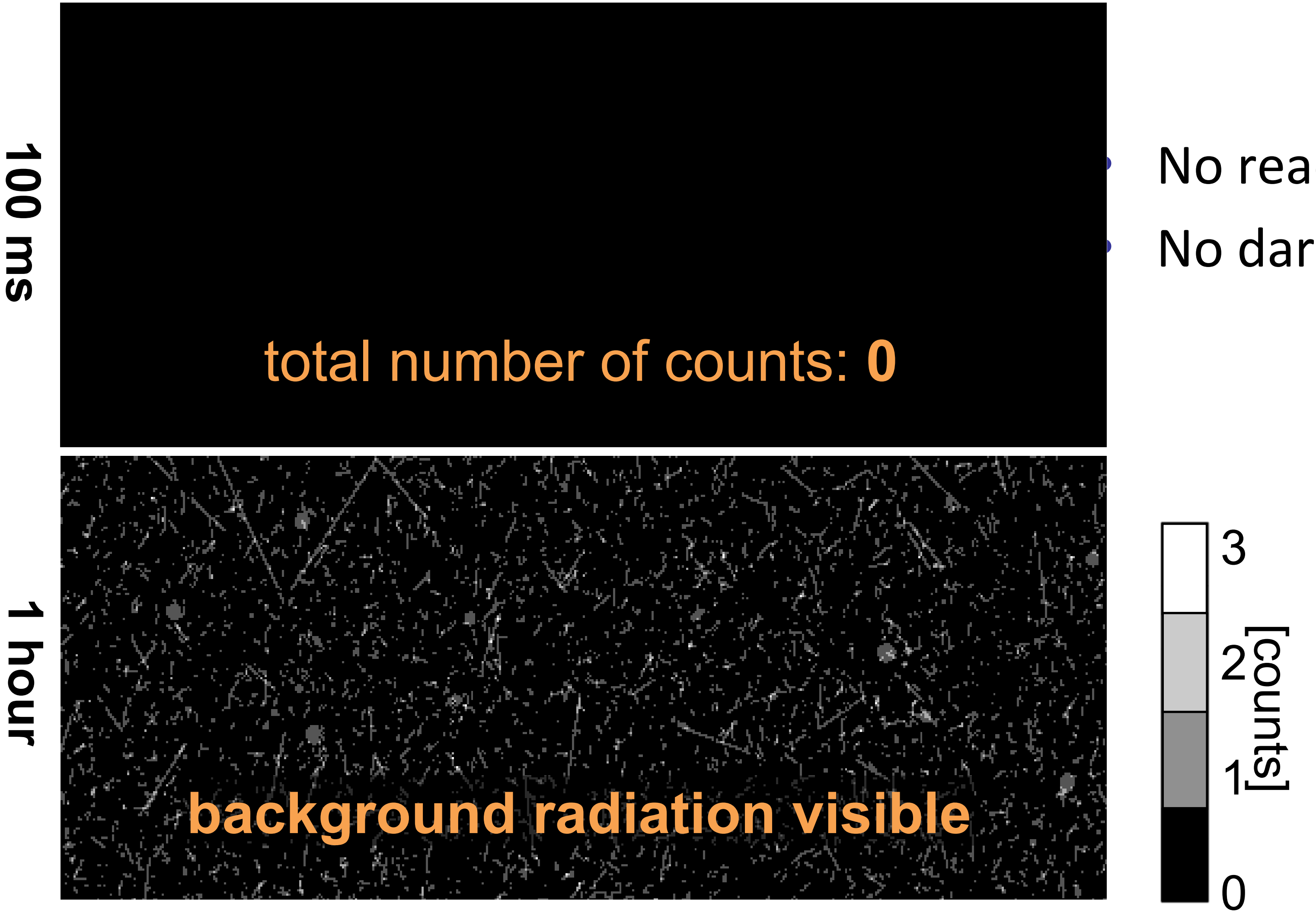
- Fine φ-slicing with PILATUS detector
- Dose fractionation with PILATUS detector (Multiple data sets (MDS) strategy by B.C. Wang)
- Multi-pass data sets with different crystal orientation with PRIGo
- Sample mounting is as important as beamline instrumentation

Features of PILATUS Detector

- Point spread function of 1 pixel
- No dark current
- No readout noise
- Fast readout (2.3 ms) => shutter-free continuous rotation
- High frame rates (25 Hz and 60Hz)
- High dynamic range of 20 bits

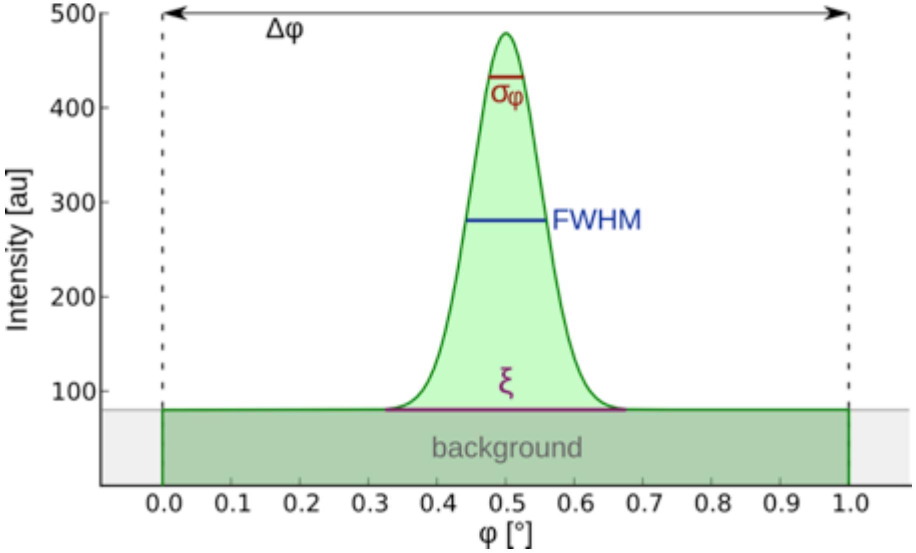
Hybrid pixel: noise free detection

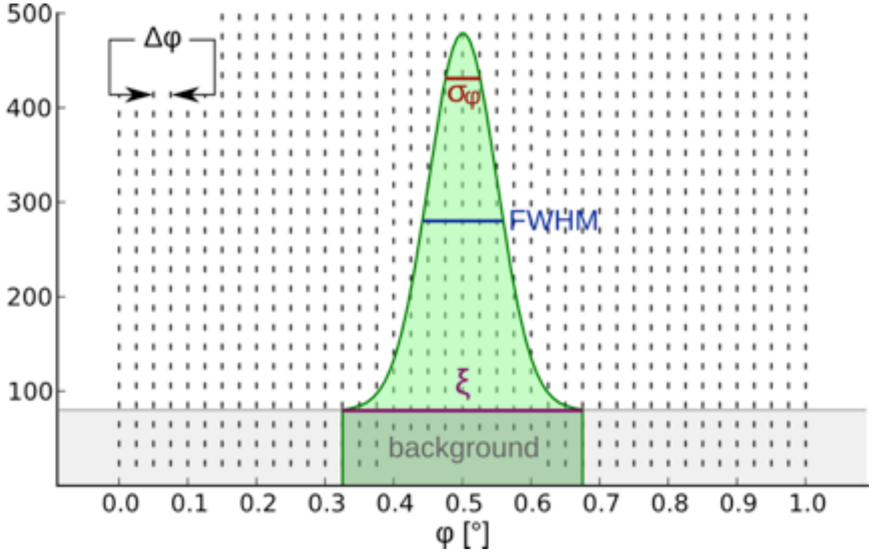
- No readout-noise
- No dark-current.



100K detector

Fine φ-slicing and background





Wide φ-slicing

- Large Δφ (Δφ > ξ)
- Large overlap of reflections and background along φ
- Few images

Fine φ-slicing

- Small Δφ (Δφ << ξ)
- Minimal overlap of reflections and background along φ
- Many images

Fine φ-slicing and Readout Noise

Detector with readout noise (CCD)

minimize readout noise ← compromise → minimize background

more images and smaller Δφ →

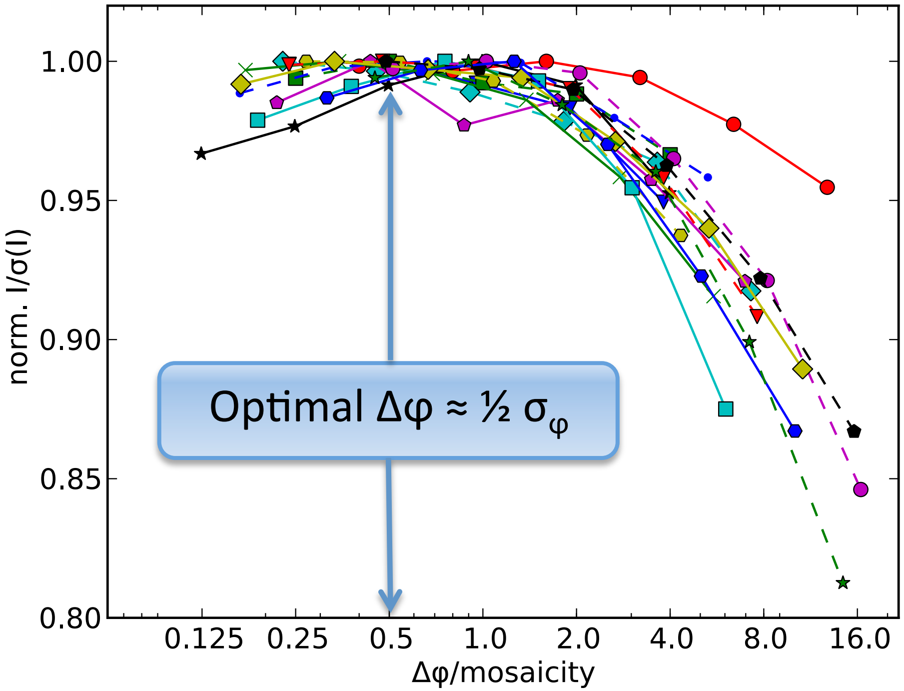
Detector without readout noise (PILATUS)

take full advantage of fine φ-slicing

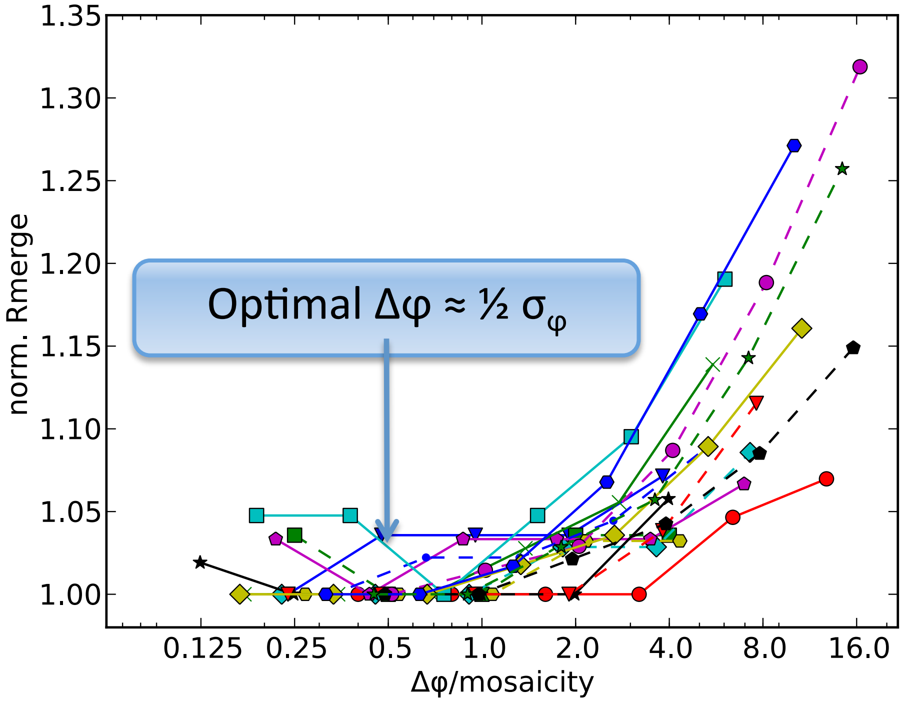
minimize background

more images and smaller Δφ →

Overall statistics: I/σ(I) and R_{merge}



Optimal Δφ ≈ ½ σ_φ



Optimal Δφ ≈ ½ σ_φ

- Test crystals: insulin, thaumatin, lysozyme, thermolysin
- 16 series of data sets with increasing Δϕ, each with 5 to 7 data sets
- 94 data sets in total

M. Mueller, *et al*, *Acta Cryst.* (2012) **D68**, 42

Dose Fractionation

Single data set approach:

Counting statistics

$$\sigma^2_{total} = G[I_s + I_{bg} + (m/n)I_{bg}] + m(K/A)^2 I_s^2$$

Systematic instrument error

Multiple Data set (MDS) approach:

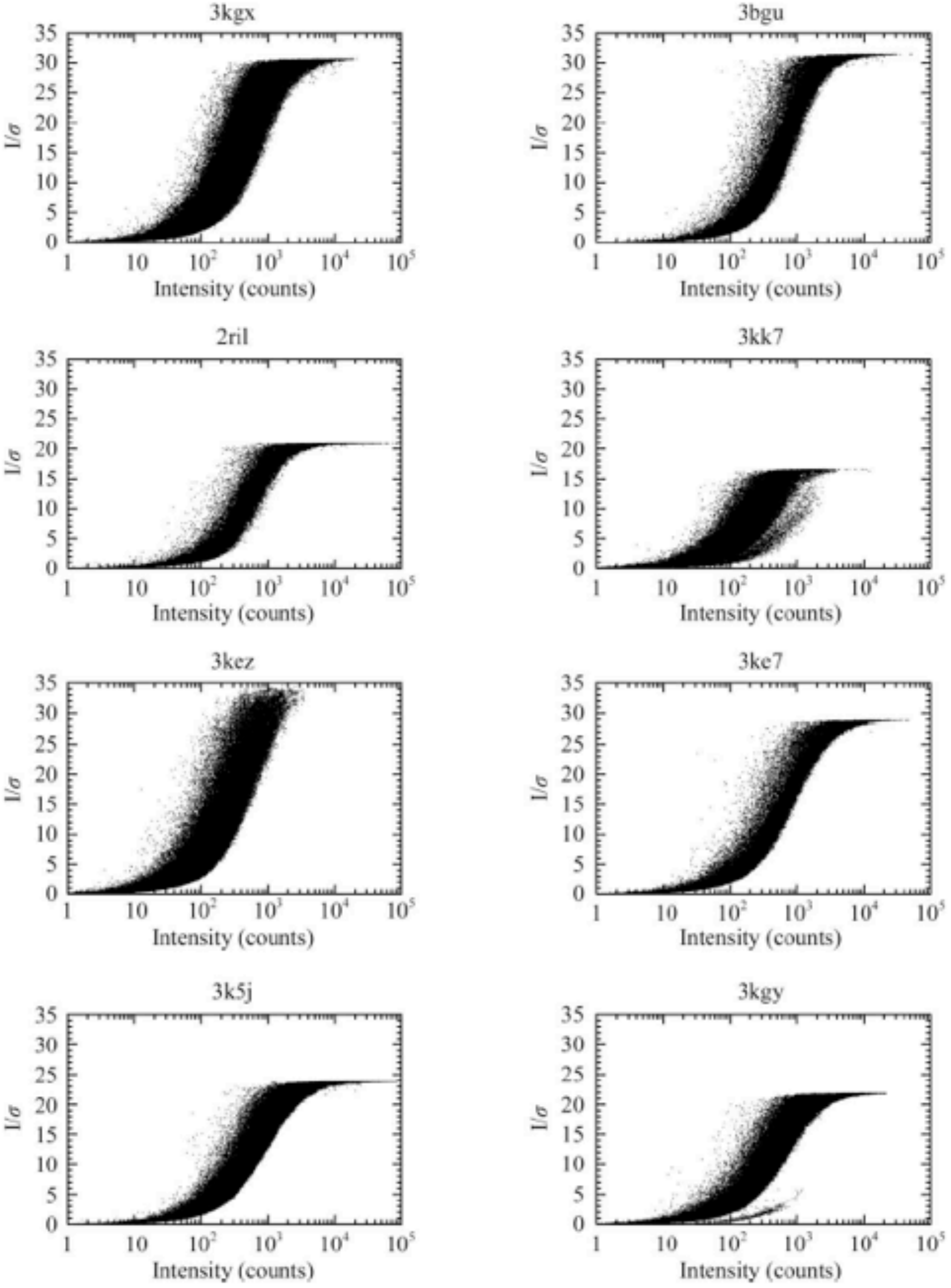
$$\sigma^2_{total} = G[I_s + I_{bg} + (m/n)I_{bg}] + \frac{m(K/A)^2 I_s^2}{N}$$

This illustrates the theoretical advantage of applying the MDS approach over the traditional approach, which is summarized in the statement below:

For a fixed X-ray dose, collecting Multiple Data Sets with short exposures can produce better data than collecting a single set of data using the long exposures.

B.C Wang

Z.J. Lui, et al, Acta Cryst. (1011) A67, 544



A better data-collection strategy might have been ***to increase the multiplicity while maintaing the total dose to avoid the inflation of the systematic error*** term which limits the I/σ of individual observations. This would have resulted in higher I/σ values after averaging

K. Diederichs, *Acta Cryst.* (2010), **D66**, 733

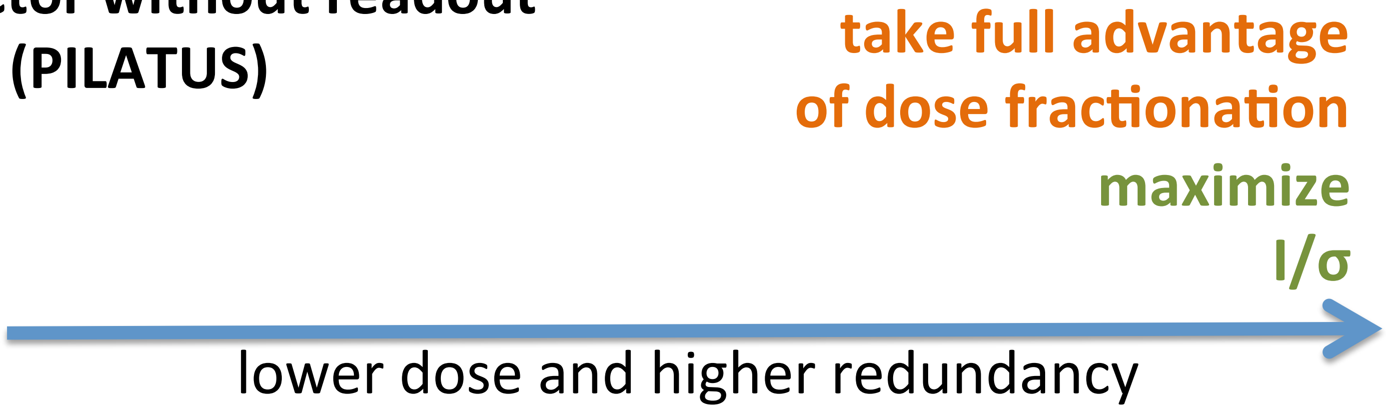
Dose Fractionation with PILATUS

Detector with readout noise (CCD)


minimize readout noise ← compromise → maximize I/σ

lower dose and higher redundancy

Detector without readout noise (PILATUS)

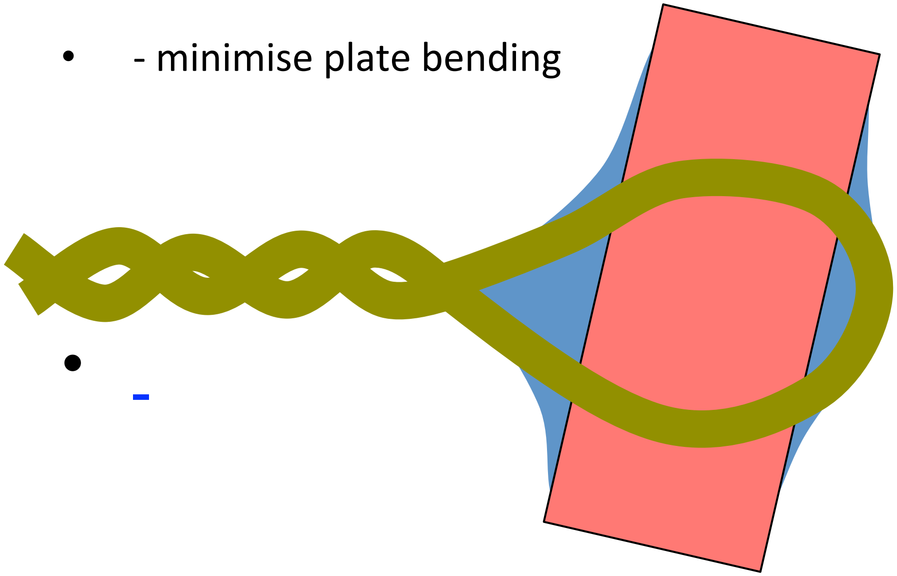

take full advantage of dose fractionation

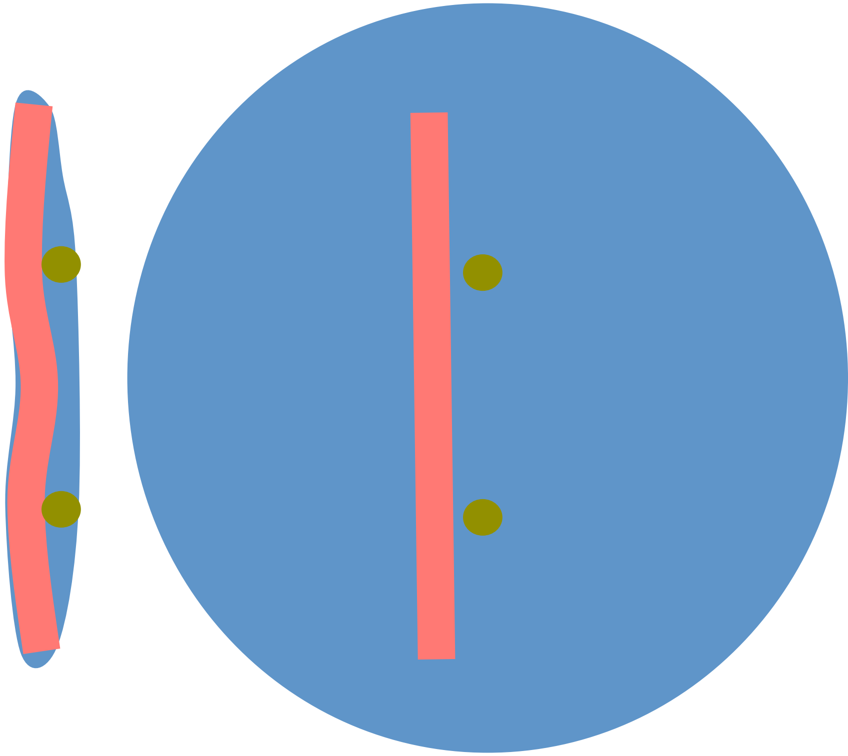
maximize I/σ

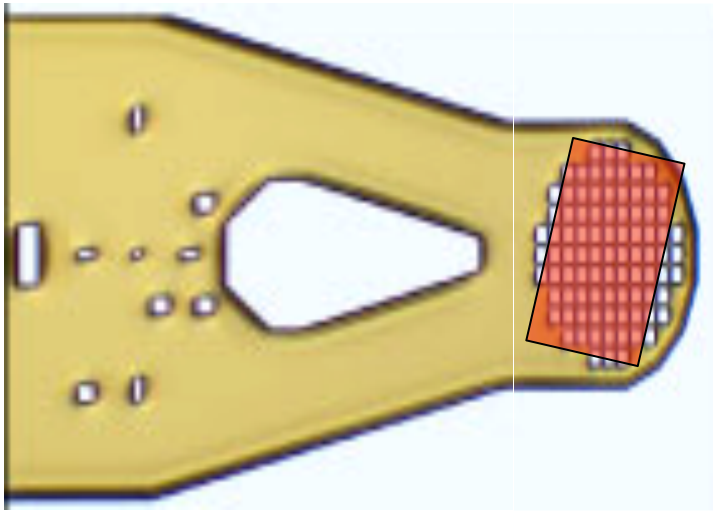
lower dose and higher redundancy

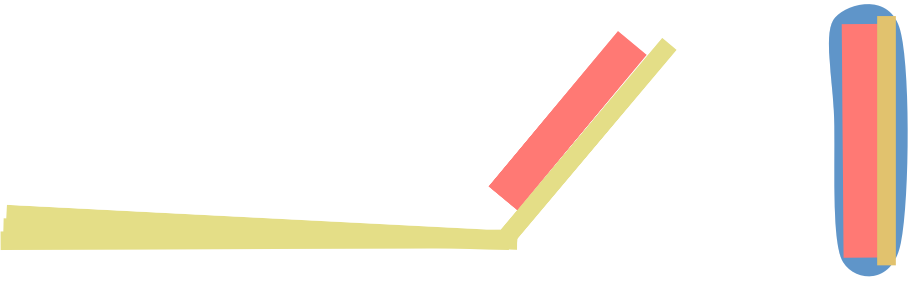
Better sample mounting

- - minimise plate bending
- -



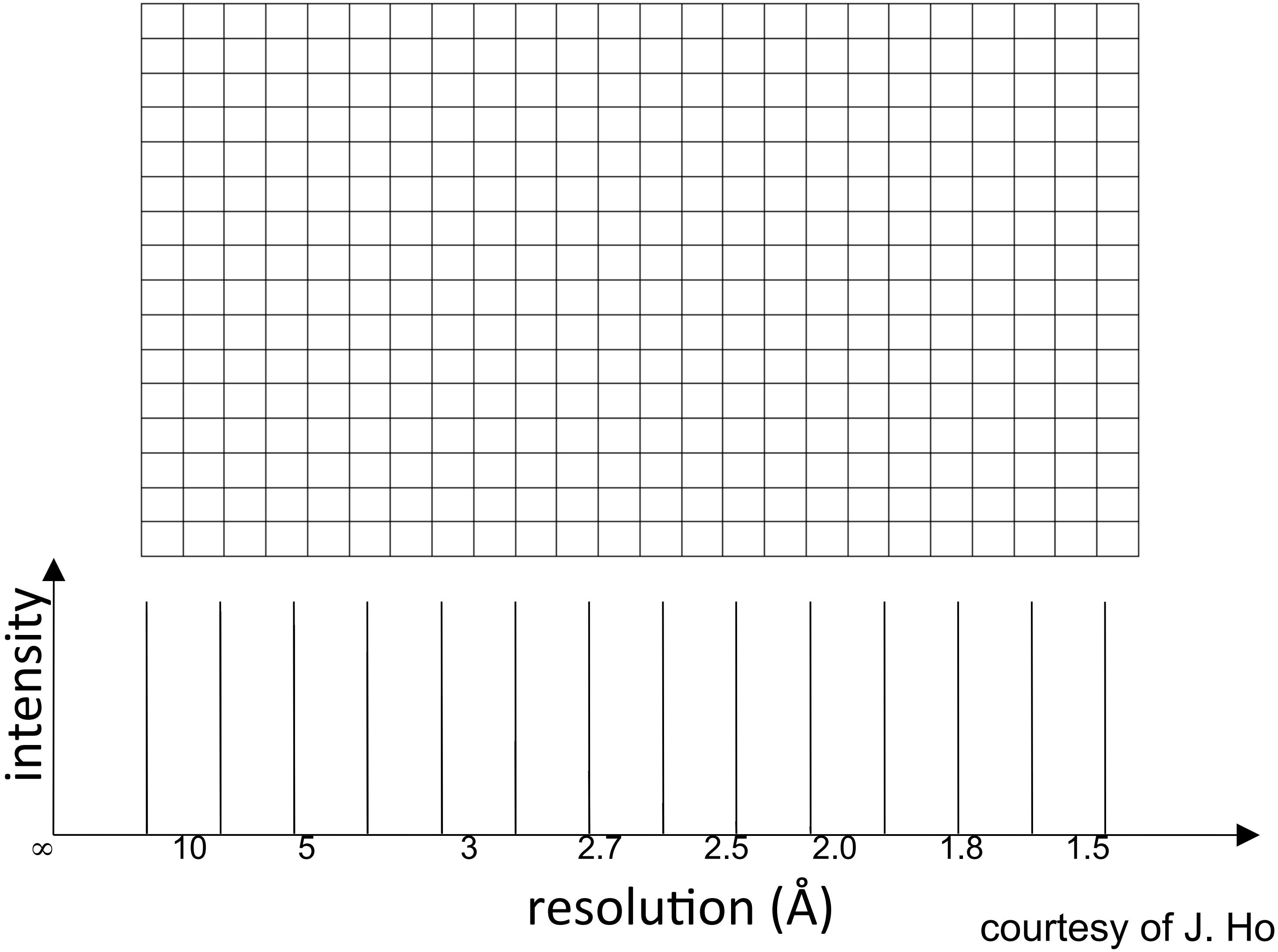






courtesy of J. Holton, ALS

Stress and Strain



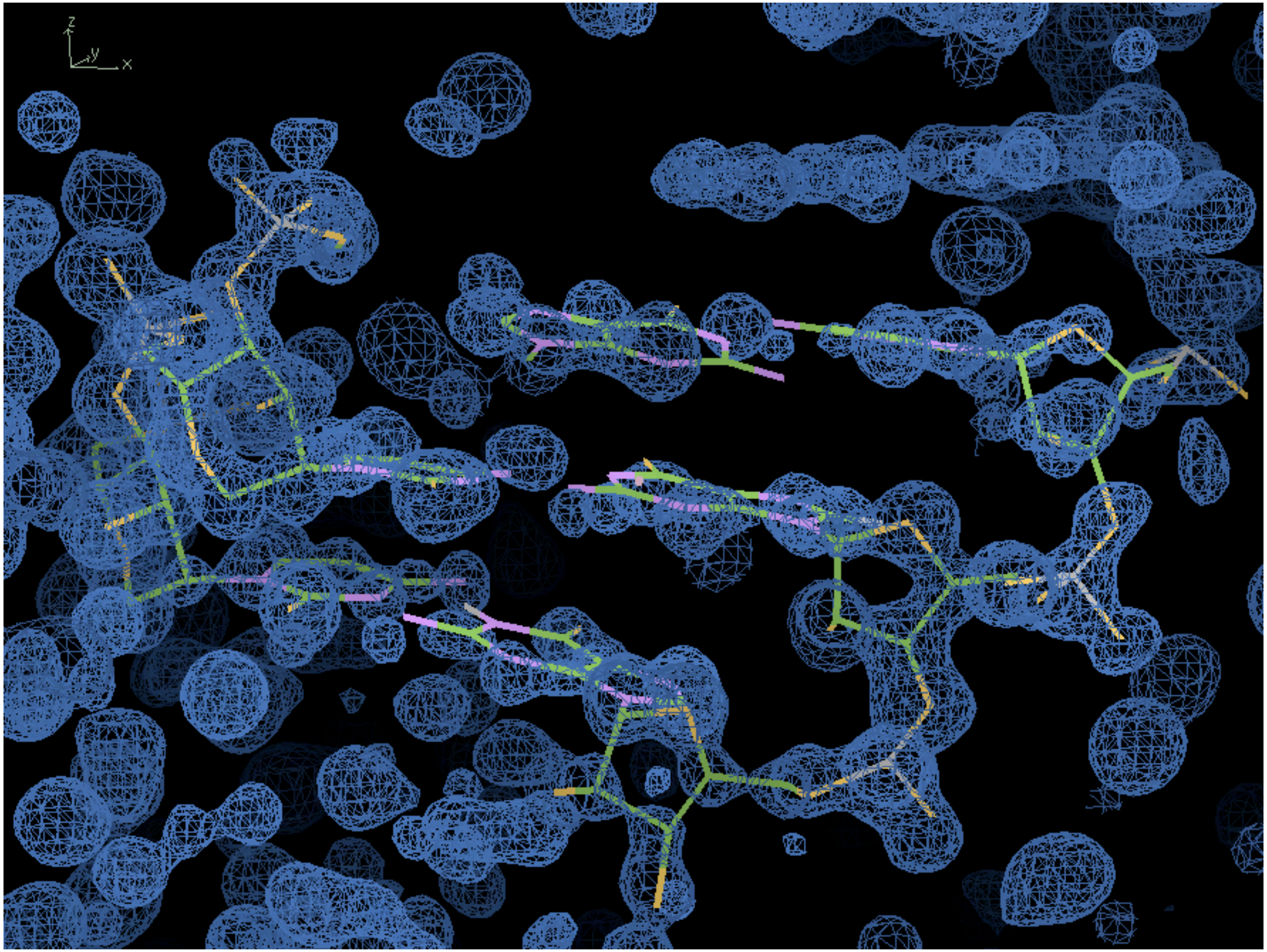
courtesy of J. Holton, ALS

Two Examples of P-SAD

Hybrid RNA/ANA

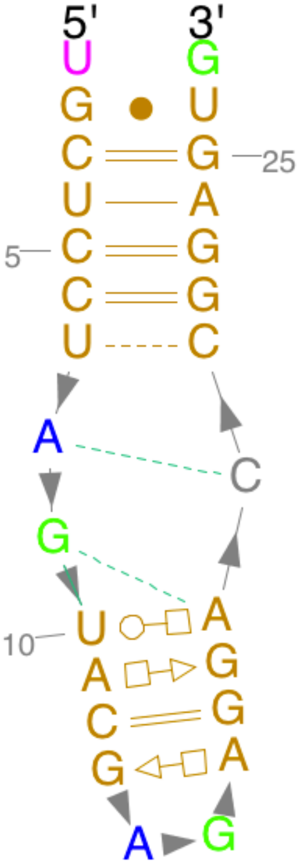
- Hybrid decamer with one RNA strand and ANA (altritol nucleic acid)
- Unit cell 34.2 × 34.2 × 170.1 Å^3 contains 1.5 hybrid decamer (15 base pairs ⇒ 30 phosphors) in asymmetric unit.
- *Sub-cell 34.2 × 34.2 × 34.0 (170/5) Å^3 contains only 3 base pairs ⇒ 6 phosphors in asymmetric unit.*
- SHELXD with P-SAD sub-cell data set
- SHELXE with additional high-resolution data set (1.14 Å), and -e0.914 to extrapolate to 0.914 Å resolution

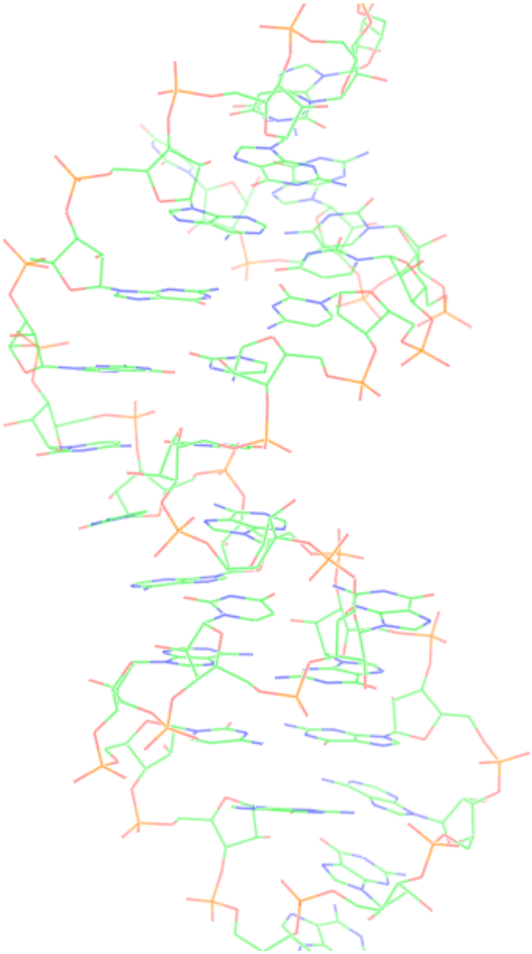
Hybrid RNA/ANA



Courtesy of M. Ovaere, L. van Meervelt, Belgium

Sarcin/Ricin Loop (SRL)

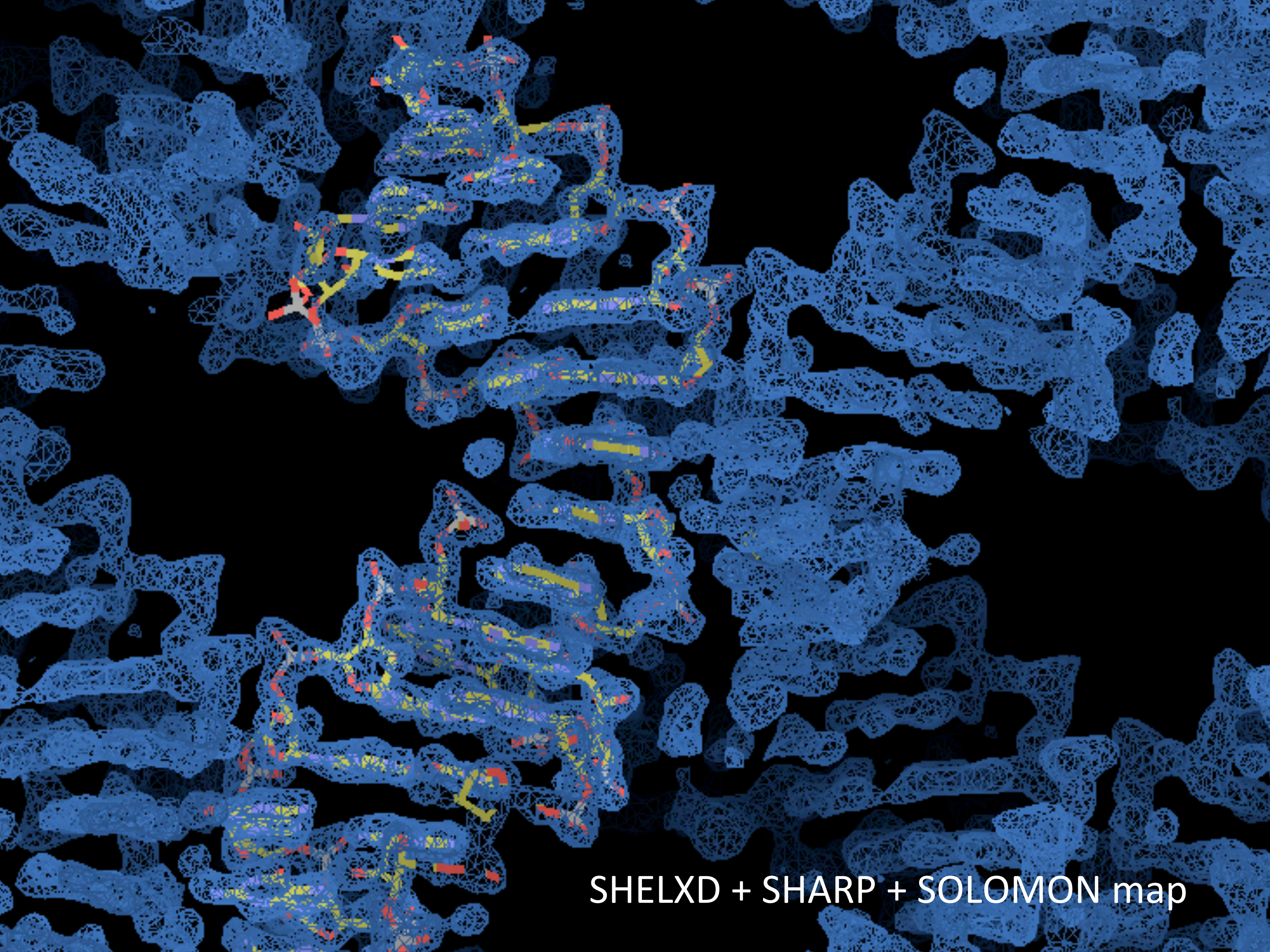




Sarcin/Ricin Loop (SRL), 26 P-site

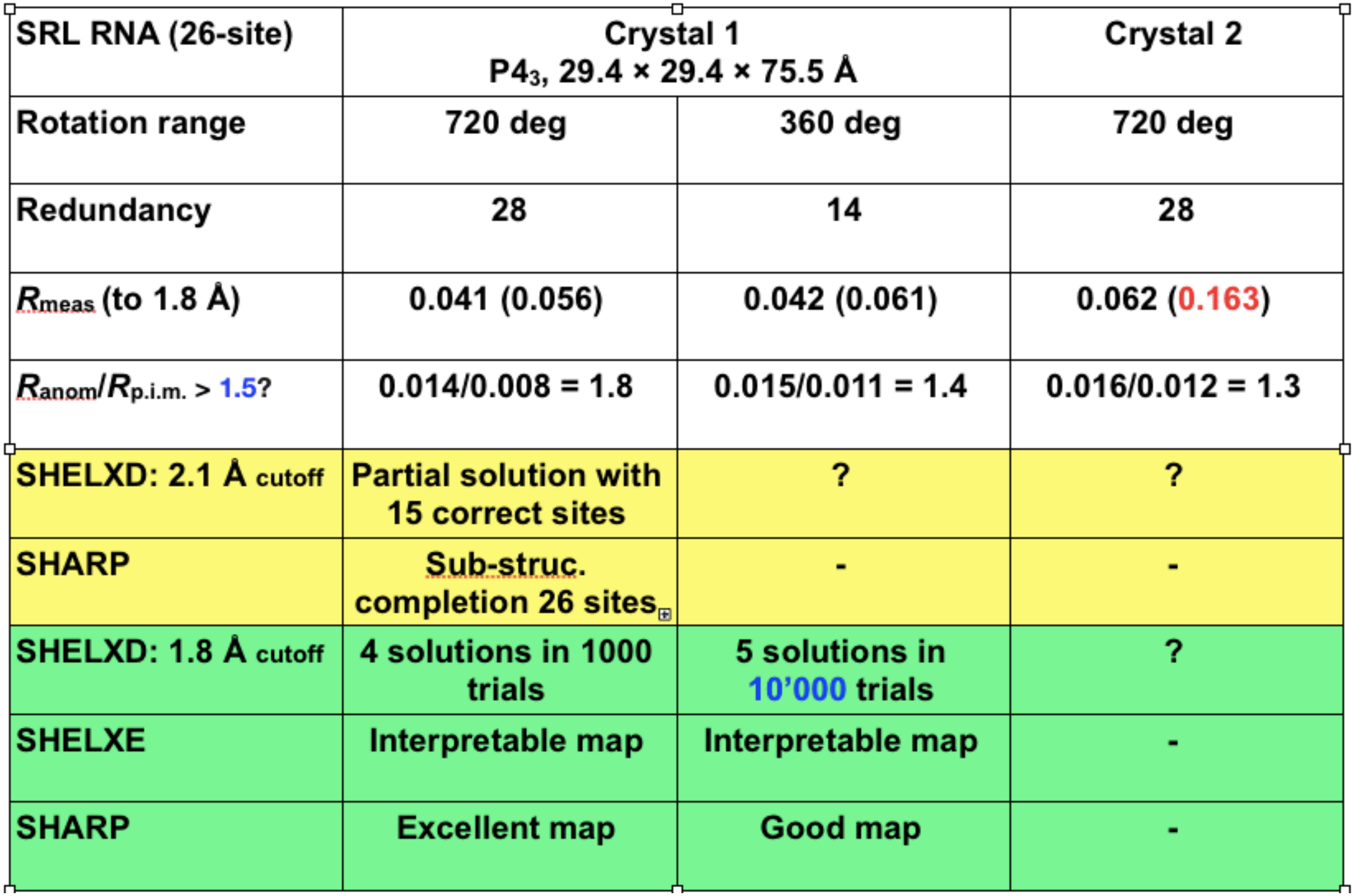
SRL RNA Data

Estimated $\Delta F^{\pm}/F$	**~ 2%**
Wavelength (Å)	1.6
Space group	$P4_3$
Unit cell (Å)	29.59 x 29.59 x 76.09
Resolution (Å)	1.8 (1.9-1.8) → 1.4
Oscillation range (°)	720
Completeness (%)	99.5 (97.3)
Redundancy	27.7 (23.4)
I / sigma	83.8 (52.2)
R_{meas}	0.041 (0.056)
R_{anom} / $R_{p.i.m.}$	0.014/0.008 = 1.75



SHELXD + SHARP + SOLOMON map

Sub-structure Determination

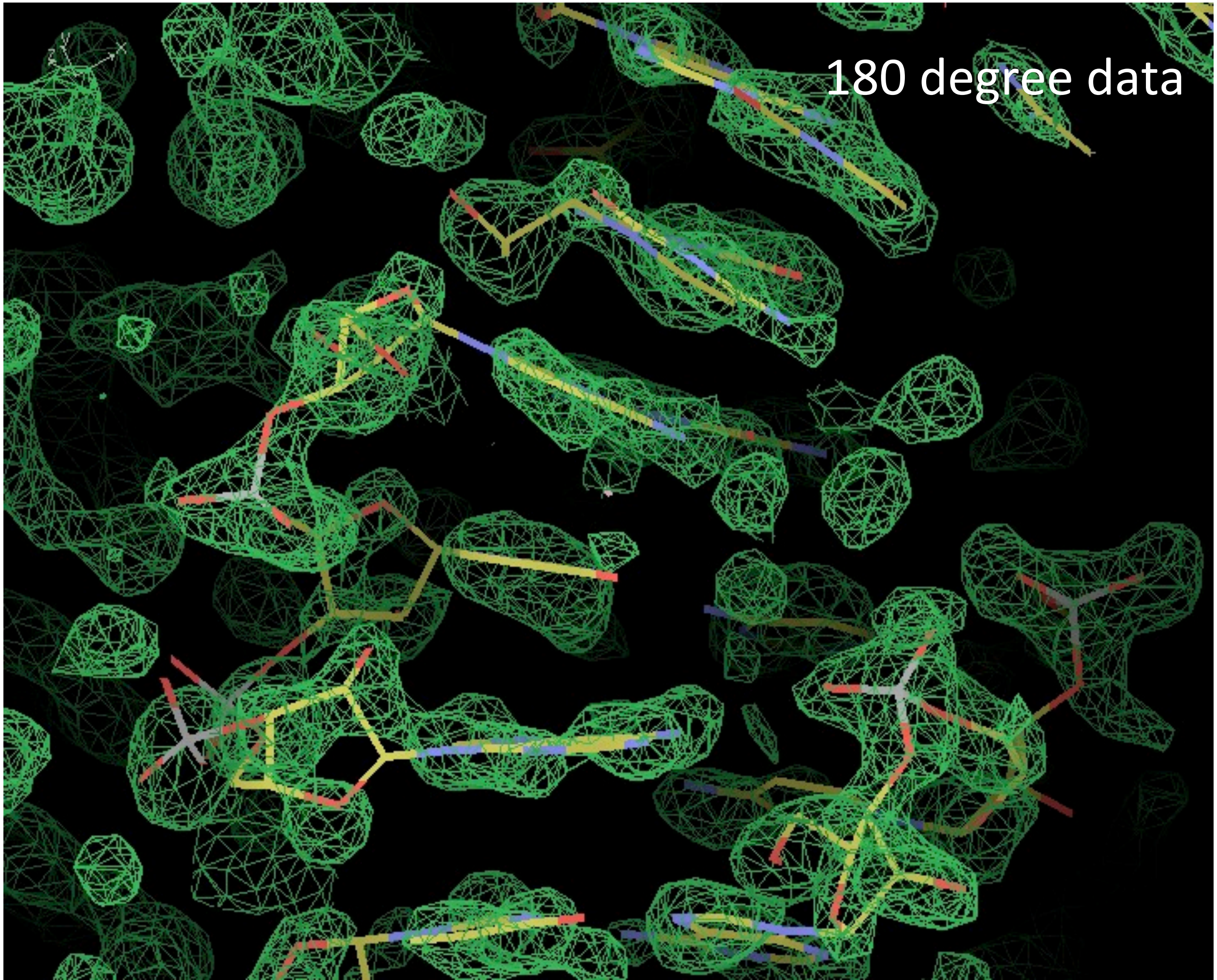


SRL RNA (26-site)	Crystal 1 $P4_3$, 29.4 × 29.4 × 75.5 Å		Crystal 2
Rotation range	720 deg	360 deg	720 deg
Redundancy	28	14	28
R_{meas} (to 1.8 Å)	0.041 (0.056)	0.042 (0.061)	0.062 (0.163)
$R_{anom}/R_{p.i.m.} > 1.5$?	0.014/0.008 = 1.8	0.015/0.011 = 1.4	0.016/0.012 = 1.3
SHELXD: 2.1 Å cutoff	Partial solution with 15 correct sites	?	?
SHARP	Sub-struc. completion 26 sites	-	-
SHELXD: 1.8 Å cutoff	4 solutions in 1000 trials	5 solutions in 10'000 trials	?
SHELXE	Interpretable map	Interpretable map	-
SHARP	Excellent map	Good map	-

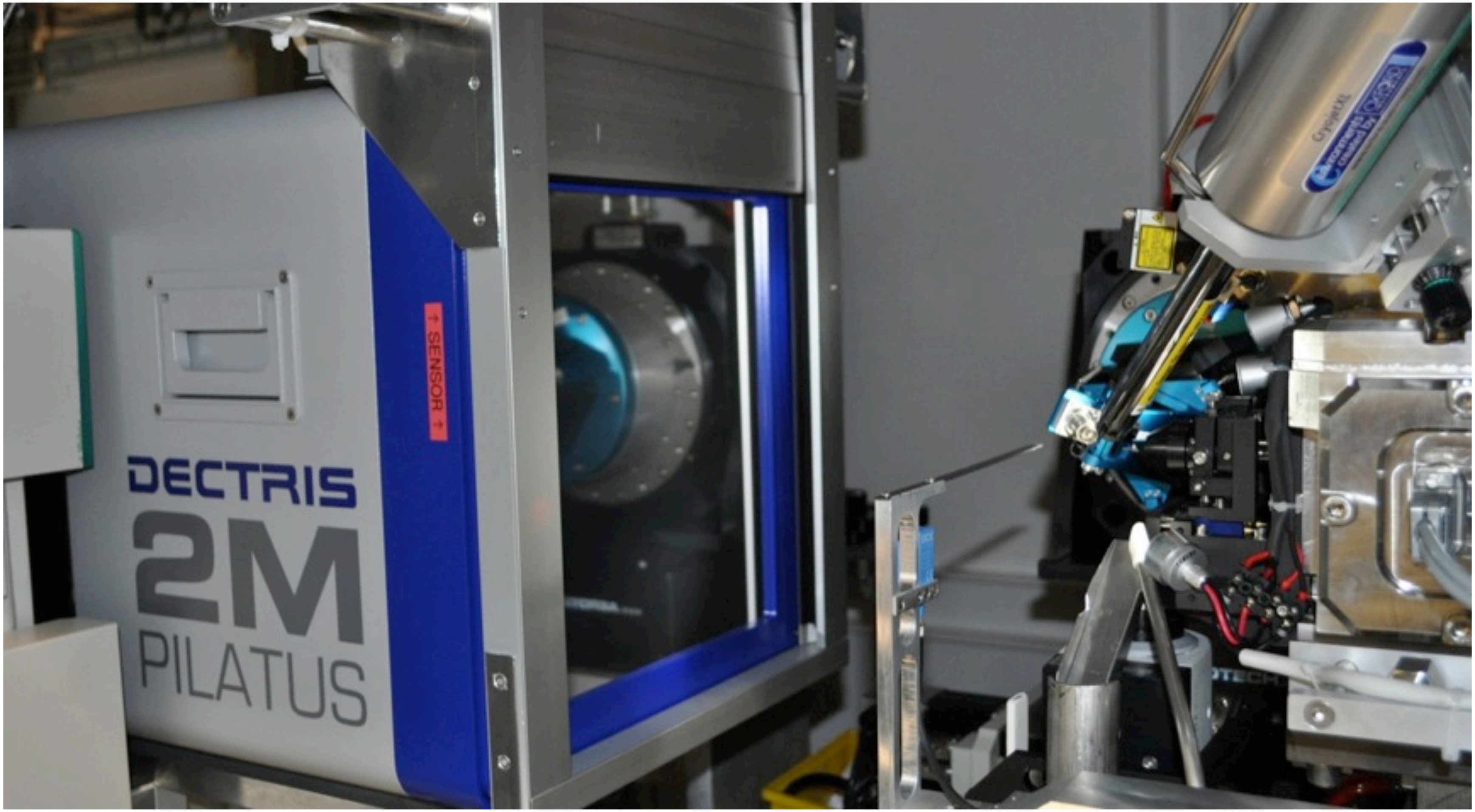
Phasing with Known P-Sites

SRL RNA (26-site)	Crystal 1			Crystal 2
Oscillation	720 deg	360 deg	180 deg	720 deg
Redundancy	28	14	7	28
R_{meas} (to 1.8 Å)	0.041 (0.056)	0.042 (0.061)	0.043 (0.059)	0.062 (0.163)
$R_{anom}/R_{p.i.m.}$	0.014/0.008= 1.8	0.015/0.011= 1.4	0.021/0.016= 1.3 0.014/0.016= 0.9	0.016/0.012=1.3
Sub-structure	26 correct sites			
PHASER+DM	Excellent map	Excellent map	Interpretable map	Excellent map
Map_CC	0.82	0.81	0.70	0.80

Phasing with Known P-Sites



X06DA for Phasing



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our users !!!

Thank You for Your Attention!