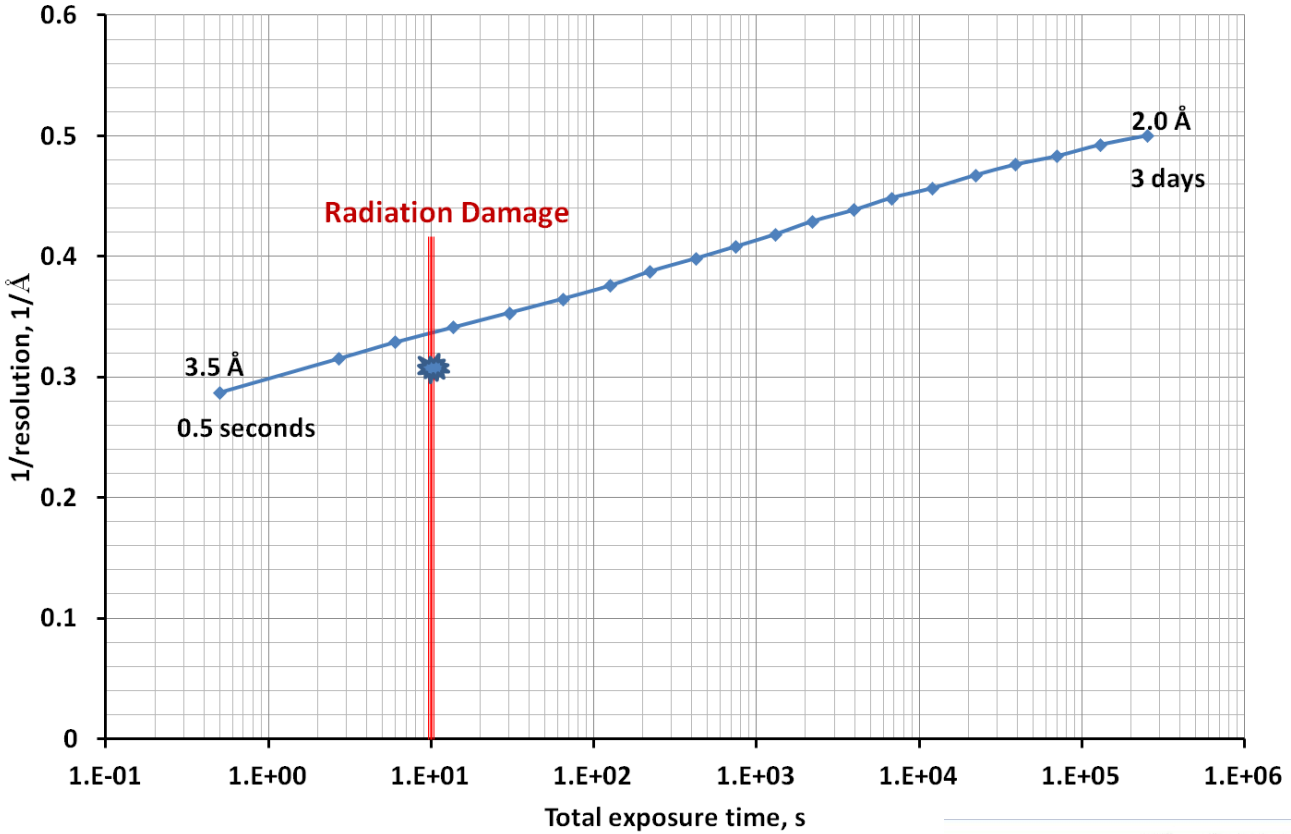
A Light for Science


European Synchrotron Radiation Facility

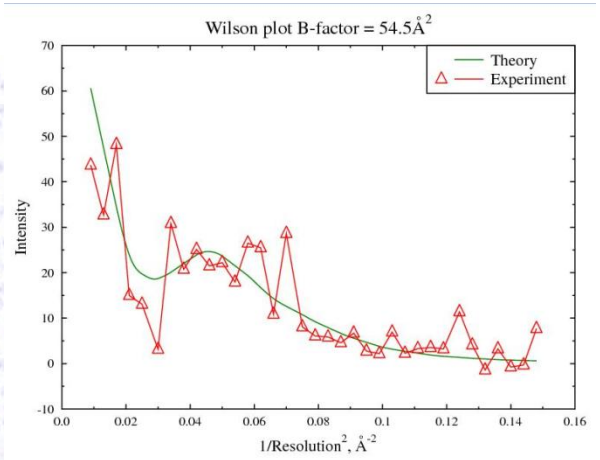
# 3-d Data Collection

# Resolution vs. Total_exposure

***BEST estimations,No radiation damage***

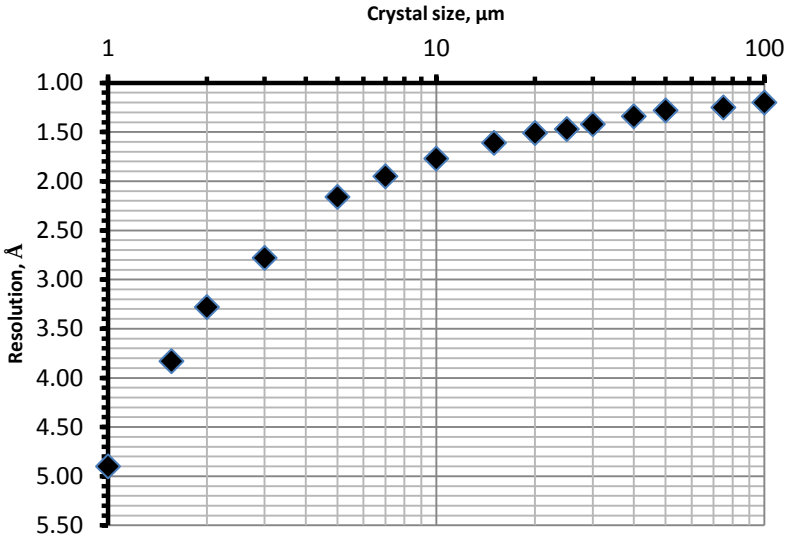



**Macrhodopsin**
**ID23-1,**
**Aperture 20**
**Flux =4.7e+11 [photons/s]**
**Dose rate =0.5 Mgy/s**

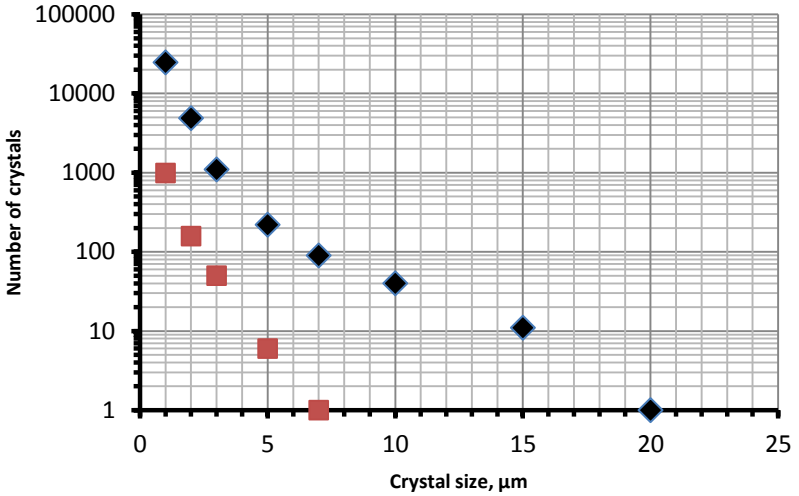



# Micro-crystallography

- Thermolysin, Space Group $P6_122$; B-factor=11.5 Å²



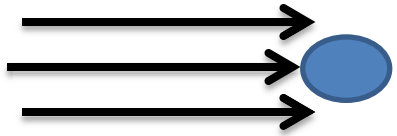

**Complete data set resolution *vs.* crystal size**




**Number of cryocooled crystals of a given size required to achieve dataset resolutions of 1.5 Å (black) and 2.0 Å (blue).**

- For a crystal 1x1x1 $\mu m^3$ in dimensions partial data sets *from about 1000 crystals* would be needed to achieve a final data set resolution of $d_{min}$ = 2.0 Å .

- Case 1:

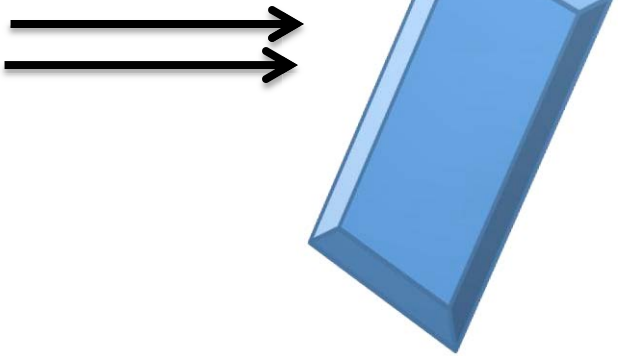


One-dimension DC

- Case 2:

Two-dimension DC

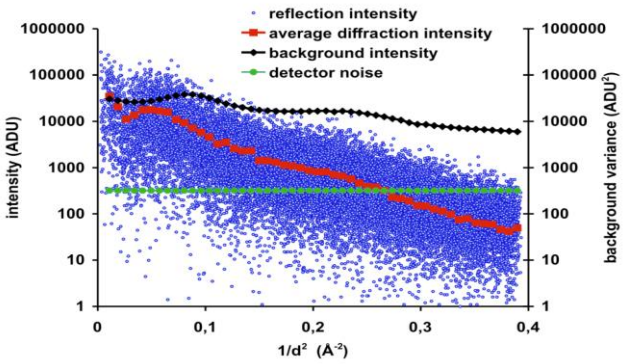
- Case 3:



Three-dimension DC

# *Case 1: One-dimension DC*

**Model for diffraction intensity *vs.* reciprocal space coordinate**



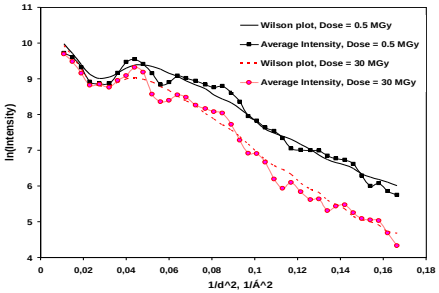

$$\hat{\mathrm{J}}(\mathbf{h}) = \frac{1}{s} \cdot \hat{\mathrm{J}}_u(h) \cdot \mathrm{Exp}(-\mathbf{h} \cdot \mathbf{B} \cdot \mathbf{h}^T)$$

$$\hat{\sigma}_J(h,\varphi) = \frac{1}{2N} \sum_{i=1}^{N} \int_o^{\infty} \sqrt{\mathrm{k}_{0i} + \mathrm{k}_{1i} J + \mathrm{k}_2 J^2} \left( \mathrm{p}(J \mid \hat{\mathrm{J}}(\mathbf{h}_{i1})) + \mathrm{p}(J \mid \hat{\mathrm{J}}(\mathbf{h}_{i2})) \right) dJ$$

## Radiation-damage model

Resolution-dependent intensity decay:

$$\hat{J}(\mathbf{h}, D) = \hat{J}(\mathbf{h}, D=0) scale(D) \exp(-B(D) h^2 / 2)$$

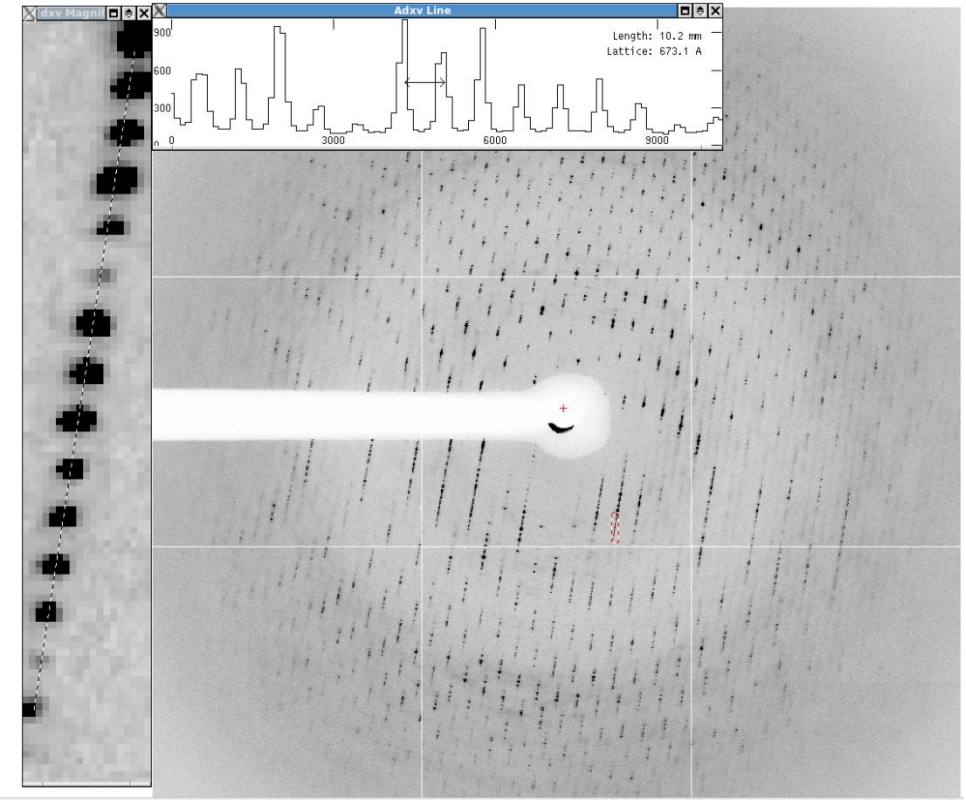


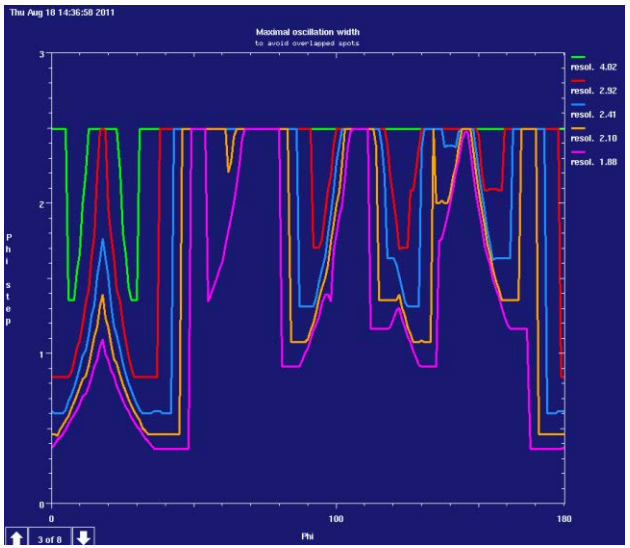

Radiation-induced non-isomorphism:

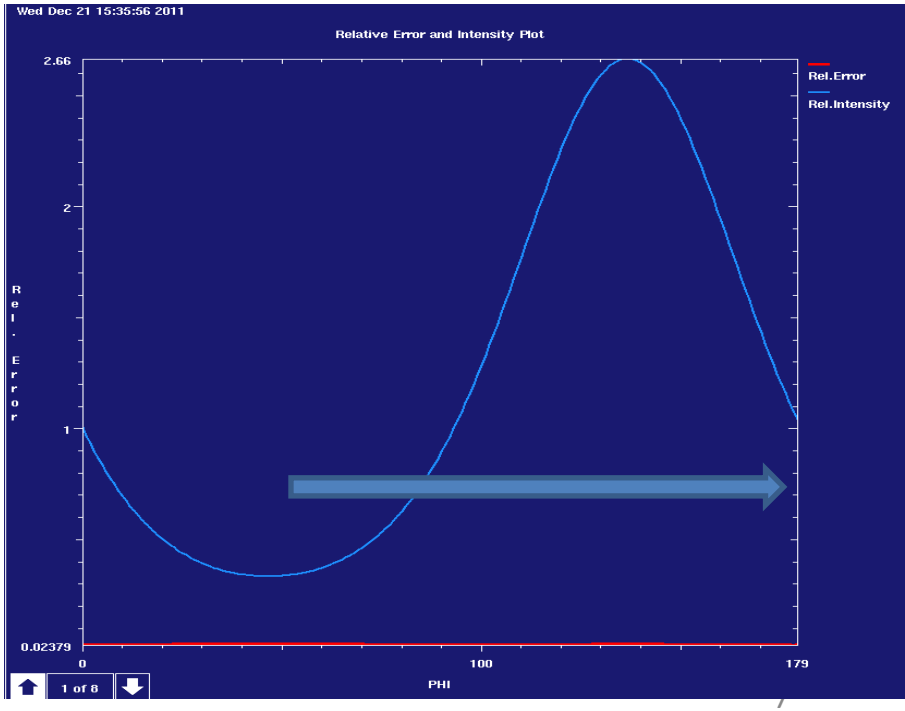
$$\sigma_a \approx e^{-\alpha D s^2}$$

$$R_{1I} = \left\langle \left| \frac{I_{D=0}}{\langle I_{D=0} \rangle} - \frac{I_D}{\langle I_D \rangle} \right| \right\rangle \approx (1 - \sigma_a^2)^{1/2}$$

```
Crystal
 Space Group                : P 4
 Cell                       : 141.55 141.55 671.01  90.00  90.00  90.00
 Mosaicity                  : 0.17 degree
```




```
Scaling
 Relative scale          :   31.44
 Overall B-factor        :  82.17 Angstrom^2
 B-factor eigenvalues    :  51.11 118.04 118.04 Angstrom^2
 Scaling error           :   3% at the resolution limit
```

# *Case 2: Two-dimension DC*

(a) micrometre-sized crystals

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Serial crystallography on *in vivo* grown microcrystals using synchrotron radiation

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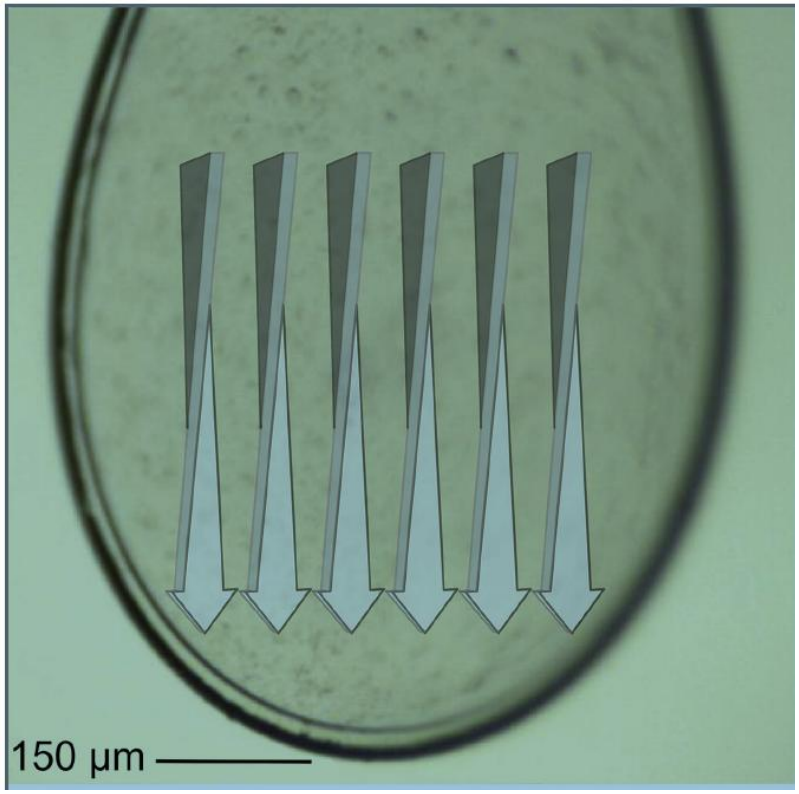
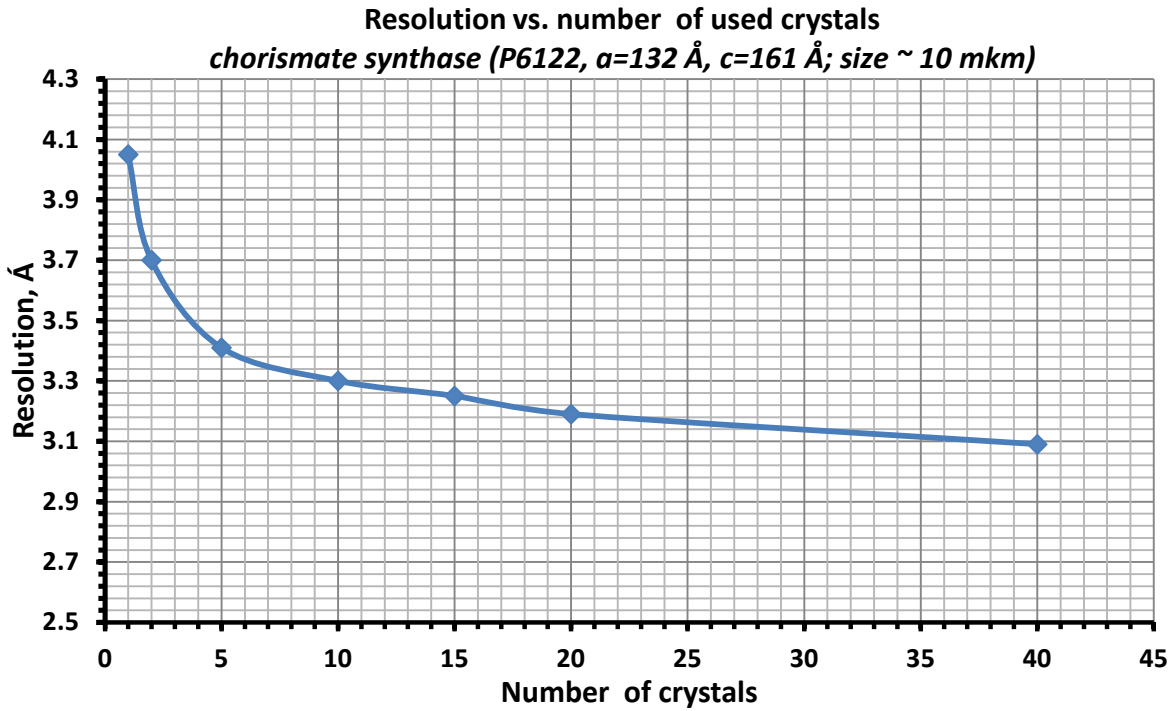
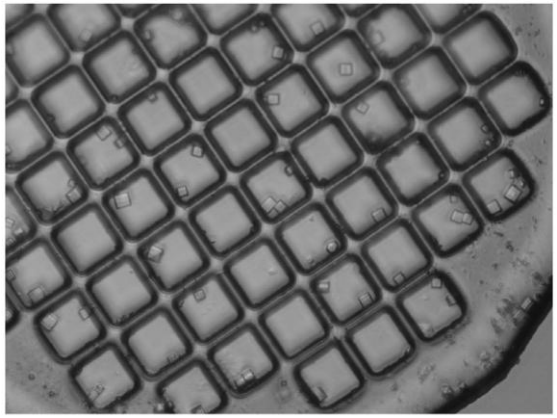
Accepted 16 December 2013

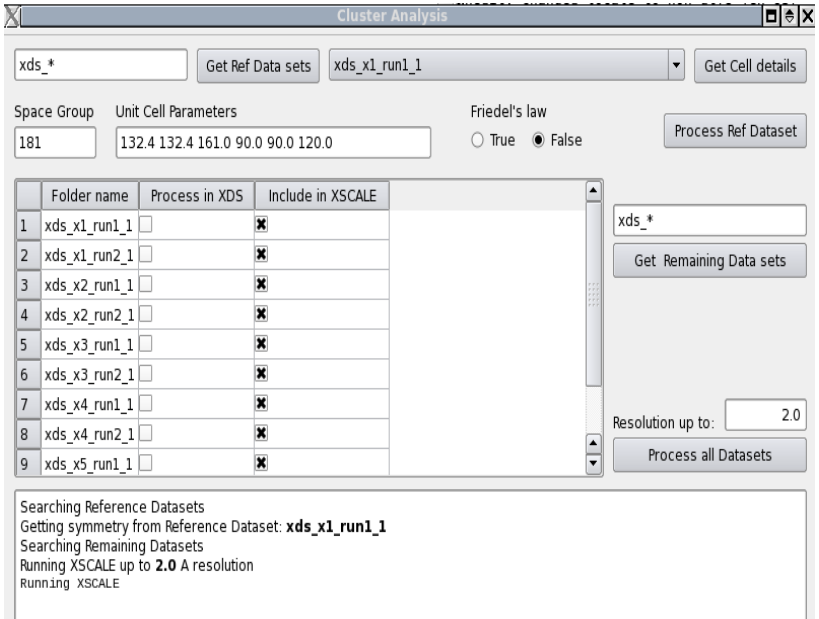
(b) sub-10 mu m crystals
Can not be characterized

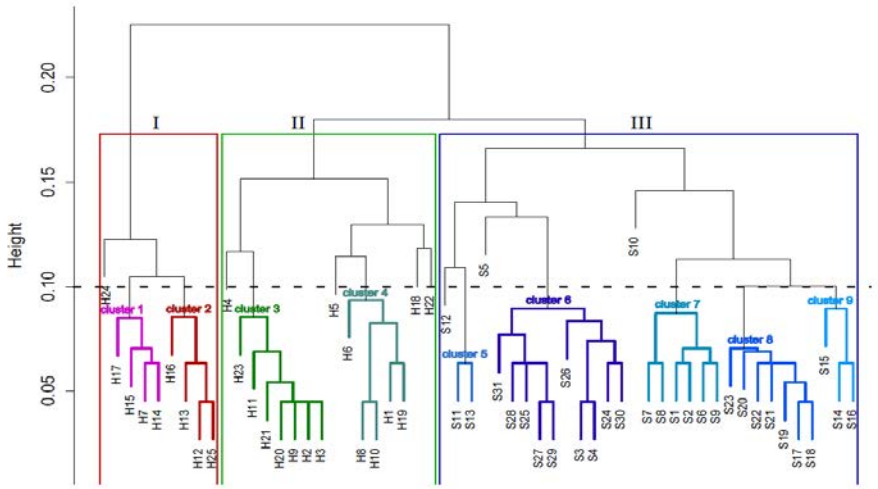


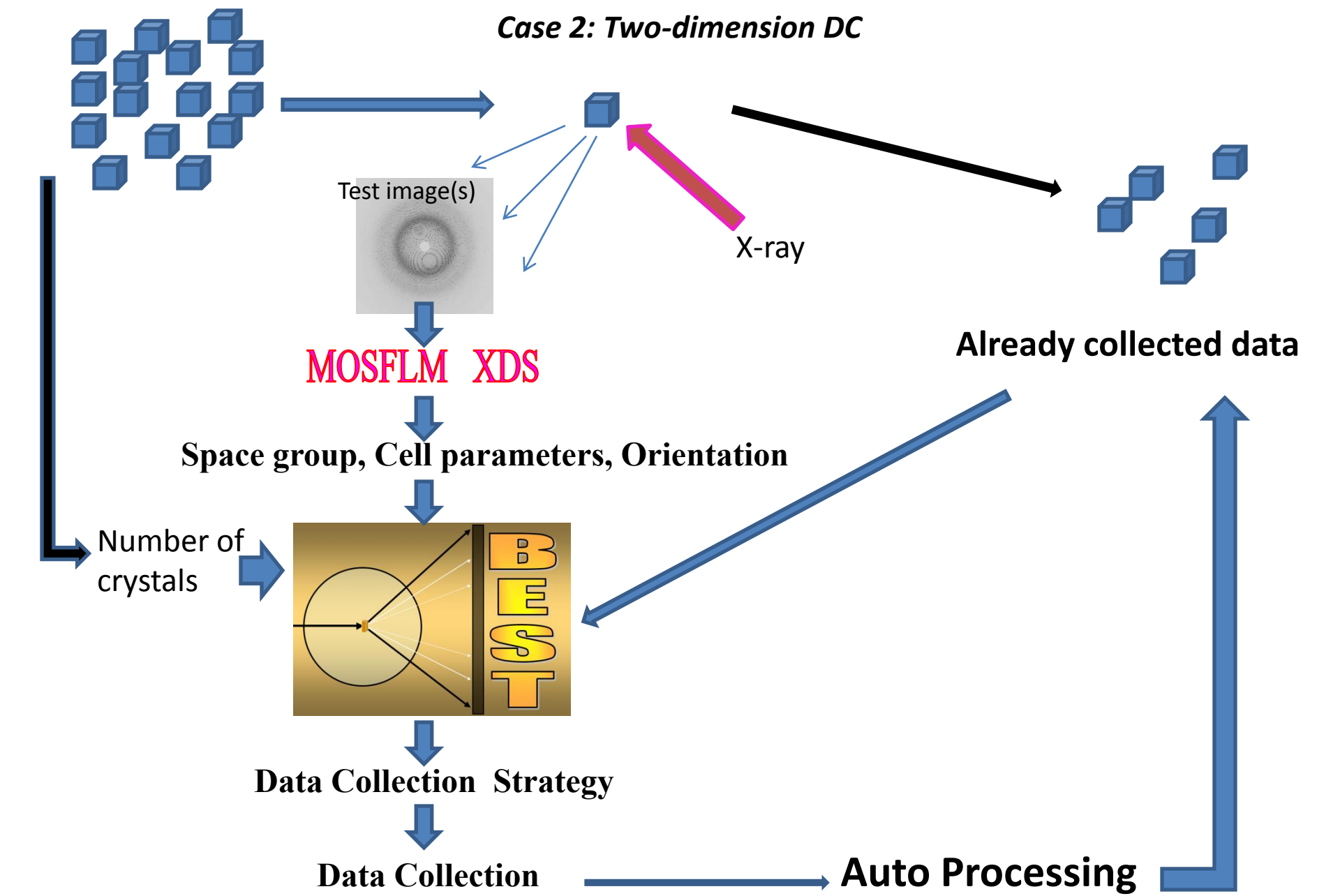
# *Case 2: Two-dimension DC* (b) sub-10 mu m crystals Crystals Can be characterized



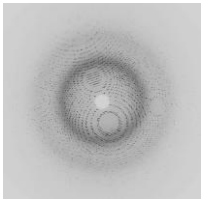


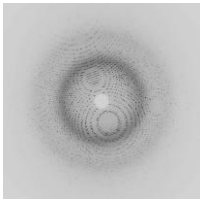

Case 2: Two-dimension DC
Test image(s)
X-ray
Already collected data
MOSFLM XDS
Space group, Cell parameters, Orientation
Number of crystals
BEST
Data Collection Strategy
Data Collection
Auto Processing

# Data collection strategy accounting radiation damage

Beamline Flux
Crystal contents



Initial Images



MOSFLM XDS DENZO

Space group, Cell parameters, Orientation, Mosaicity

$I[(h,k,l), T_{exposure})], I_{background}$

RADDOSE

Absorbed dose rate

Detector parameters
Beamline parameters and limitations

BEST 4.1

Crystal
Shape, size, position

**Geometry**

Optimal starting spindle angle and scan range

Maximum rotation angle without spot overlap

Optimal Multiplicity

**Statistics calculation**

Reconstruction of average intensity vs. resolution

Statistics modeling based on Wilson distribution

Radiation damage modeling

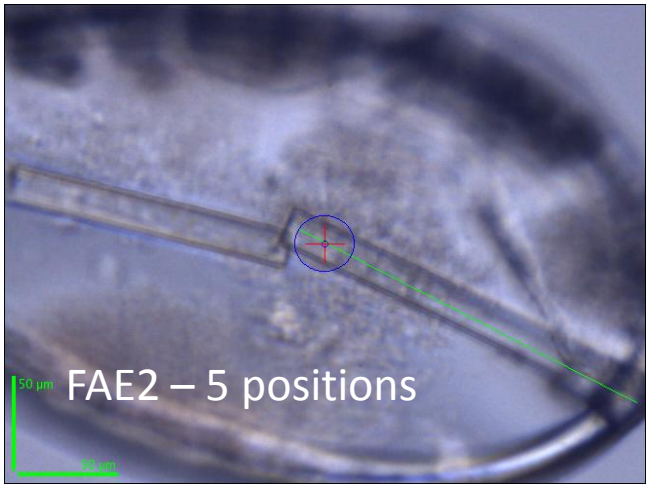
**Optimal plan(s) of data collection**
**Statistics**
**B-factor**

FAE crystals

ID23-1

E=12.75Kev, I=35 mA, Aperture=0.03 mm

Flux=1.5x$10^{11}$ Photon/sec




Multi-positions data collection

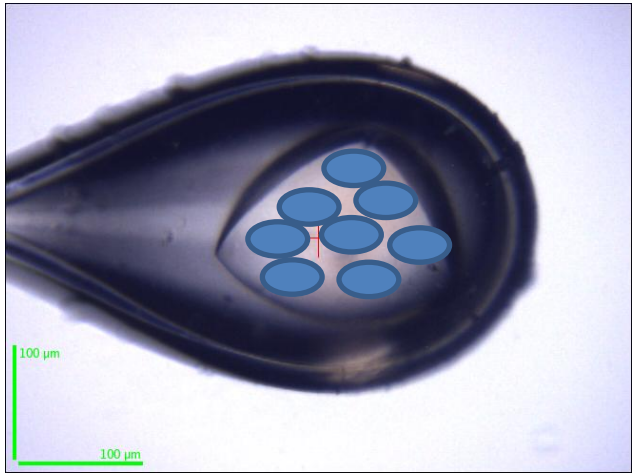
Resolution limit is set by the radiation damage

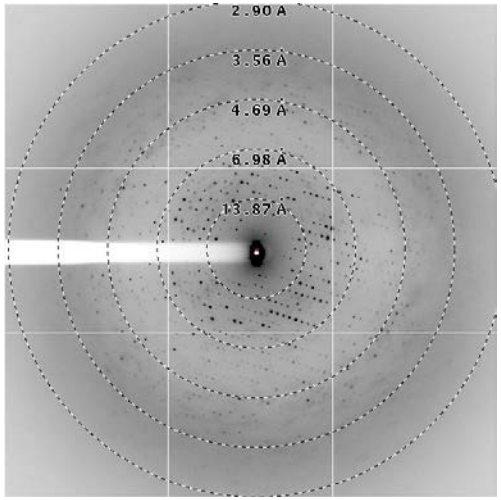
Resolution limit =1.73 Angstrom  Transmission =  100.0%  Distance = 244.6mm

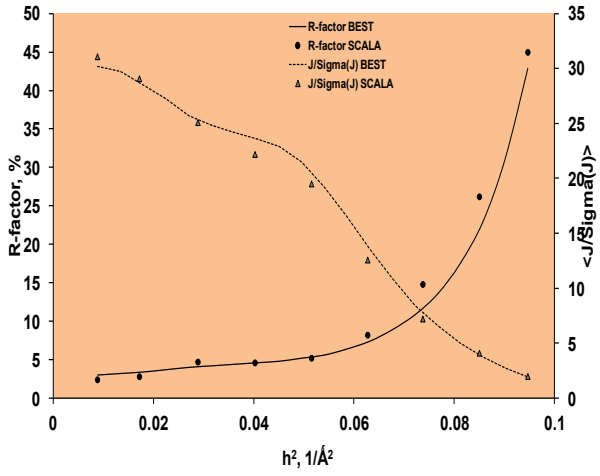
| sub-Wedge | Phi start degree | Rot. width degree | Exposure /image s | N.of ima-ges | Over-lap | sWedge width degree | Exposure /sWedge s | Exposure total s | Dose /sWedge MGy | Dose total MGy | Comple-teness % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Wedge number = 1 Crystal position = 1 | | | | | | | | | | | |
| 1 | 0.00 | 0.25 | 1.338 | 80 | No | 20.00 | 107.0 | 107.0 | 4.067 | 4.067 | 51.9 |
| Wedge number = 2 Crystal position = 2 | | | | | | | | | | | |
| 1 | 20.00 | 0.25 | 1.338 | 80 | No | 20.00 | 107.0 | 107.0 | 4.067 | 4.067 | 85.6 |
| Wedge number = 3 Crystal position = 3 | | | | | | | | | | | |
| 1 | 40.00 | 0.25 | 1.338 | 80 | No | 20.00 | 107.0 | 107.0 | 4.067 | 4.067 | 75.2 |
| Wedge number = 4 Crystal position = 4 | | | | | | | | | | | |
| 1 | 60.00 | 0.25 | 1.338 | 80 | No | 20.00 | 107.0 | 107.0 | 4.067 | 4.067 | 88.1 |

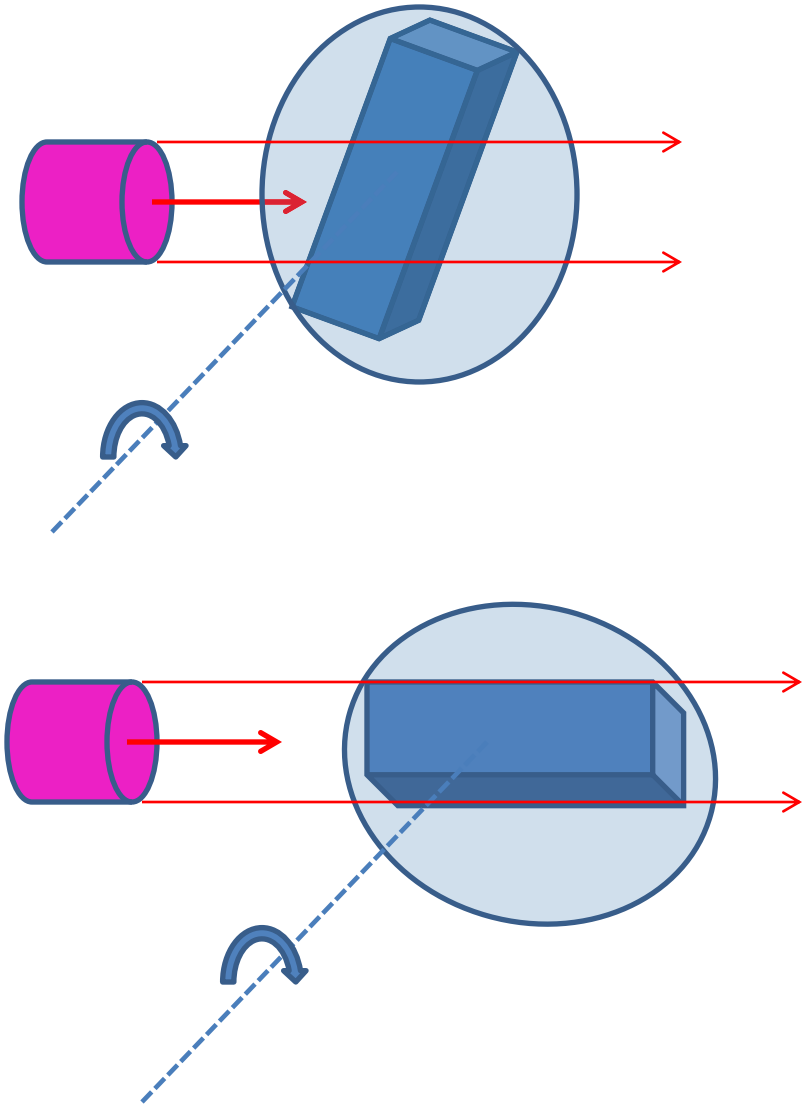
Phi_start - Phi_finish : 0.00 - 80.00
Total rotation range : 80.00 degree
Total N.of images : 320
Overall Completeness : 98.6%
Redundancy : 3.18
R-factor (outer shell) : 5.6% ( 36.8%)
I/Sigma (outer shell) : 22.9 ( 3.3)
Total Exposure time : 428.1 sec (0.119 hour)
Total Data Collection time : 1228.1 sec (0.341 hour)

**The 70 kDa membrane protein FtsH from Aquifex aeolicus  I222, a = 137.9, b = 162.1, c = 170**

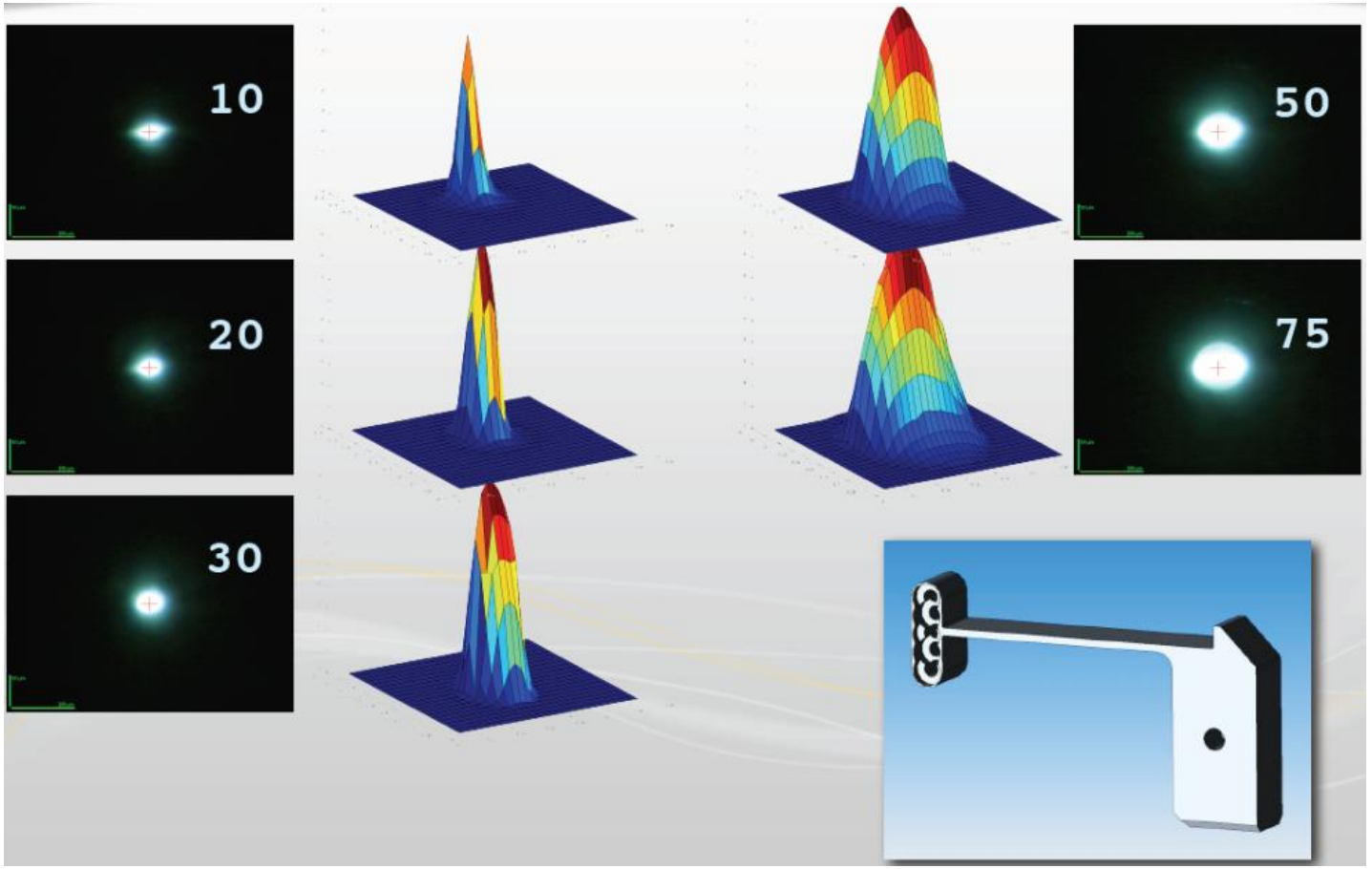




$$\hat{J}(\mathbf{h}, D, \Omega) = \hat{J}(\mathbf{h}, D = 0)\, scale(D, \Omega) \exp(-\mathbf{h} \cdot \mathbf{B}(D, \Omega) \cdot \mathbf{h}^T / 2)$$




**Flux**

**σx σy**

**Aperture**

**Slit sizes**

# Diffraction sample Modeling

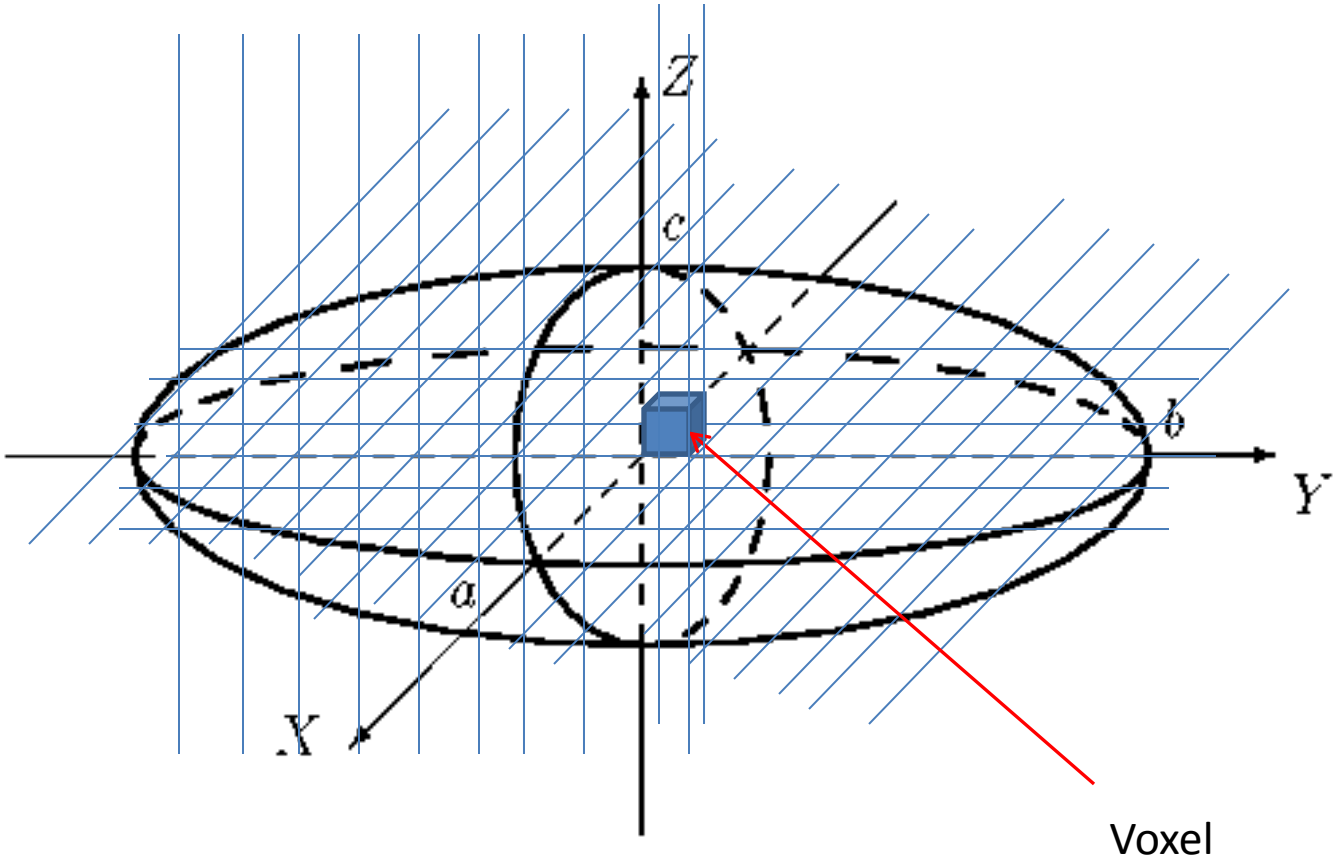
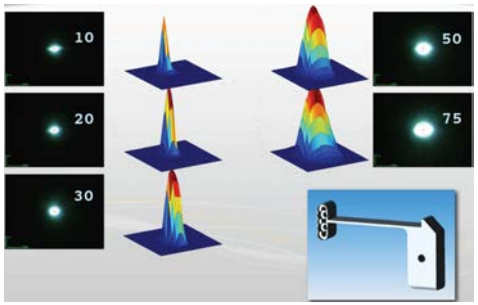



Рис. 1

Voxel

*Volumetric Picture Element*

$$Scale(\Omega) = Scale(voxel) \times NumberVoxel(\Omega)$$



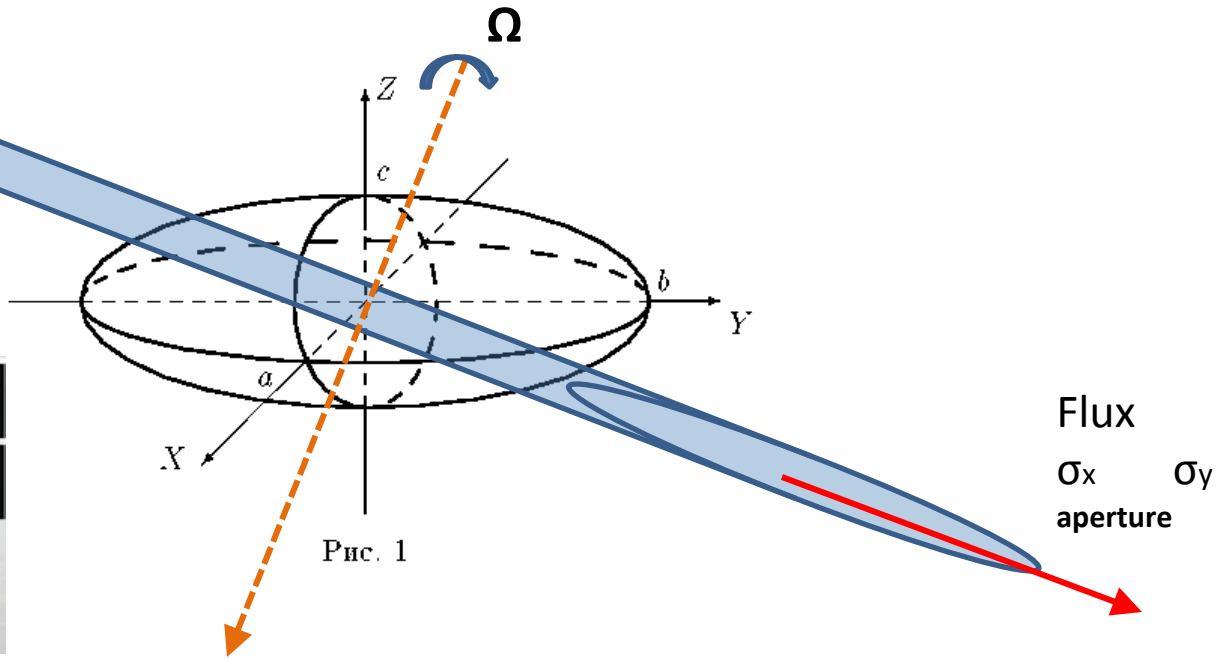
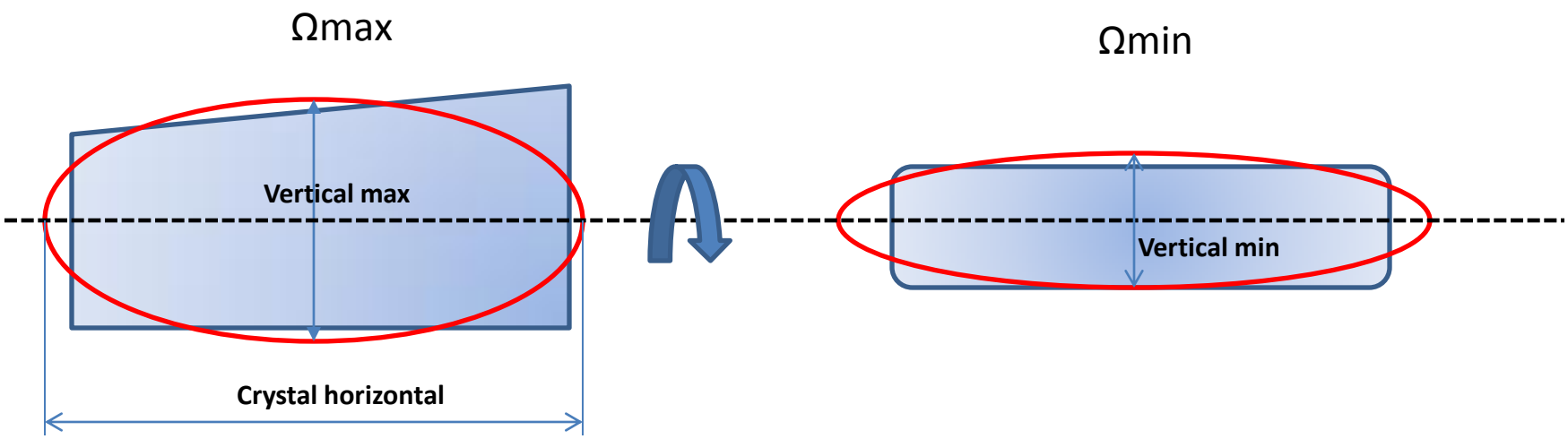




Рис. 1

$$\hat{J}(\mathbf{h}, D) = \hat{J}_o(h) \sum_{voxel} \sum_{x,y} I_{x,y}(beam) \times scale(voxel, D_{voxel}) \exp(-\mathbf{h} \cdot \mathbf{B}(D_{voxel}) \cdot \mathbf{h}^T / 2)$$

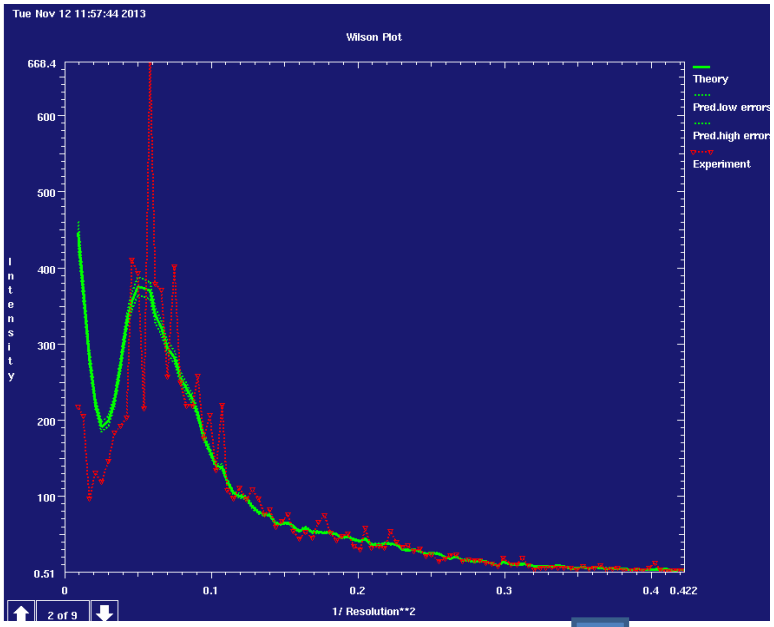


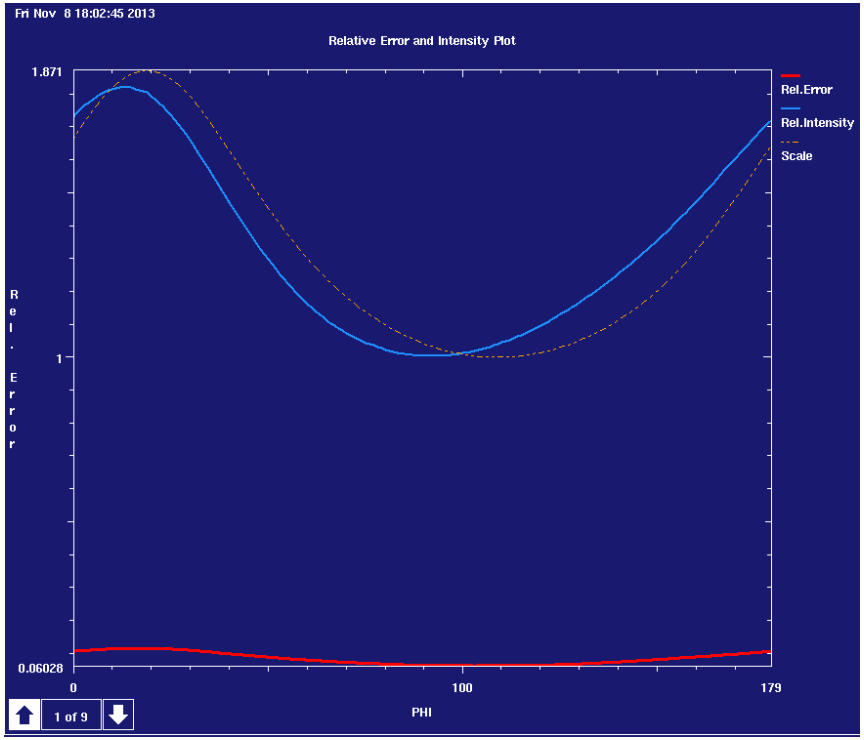

beam_crystal.dat - /mntdirect/_users/apopov/BEST4.1/

File Edit Search Preferences Shell Macro Windows Help

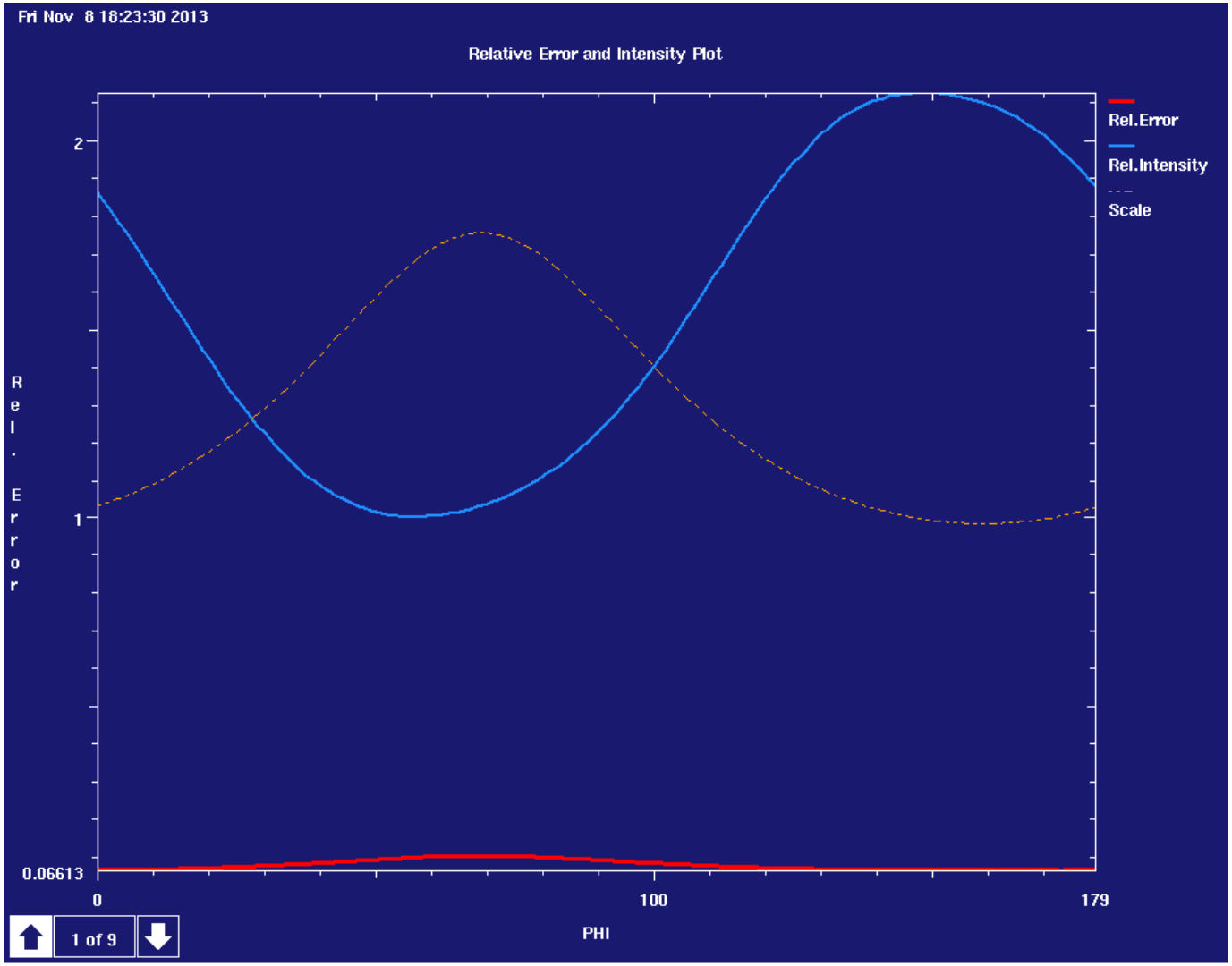
```
! - this is a comments
! all sizes in mm
horizontal_size 0.045
vertical_size 0.035
aperture_size 0.030
!defalt: no aperture
!horizontal_slit 0.1000
!vertical_slit 0.1000
beam_shift 0.01
!vertical shift relative to the rotation axis
!beam_flux 2.0e12
flux_2_dose 2.0e-5
!
crystal_vert_max 0.170
crystal_vert_min 0.090
crystal_hor 0.100
omega_min 20
!description of crystal shape and position- a,c,b
end
```

# First step - scaling



# First step - scaling



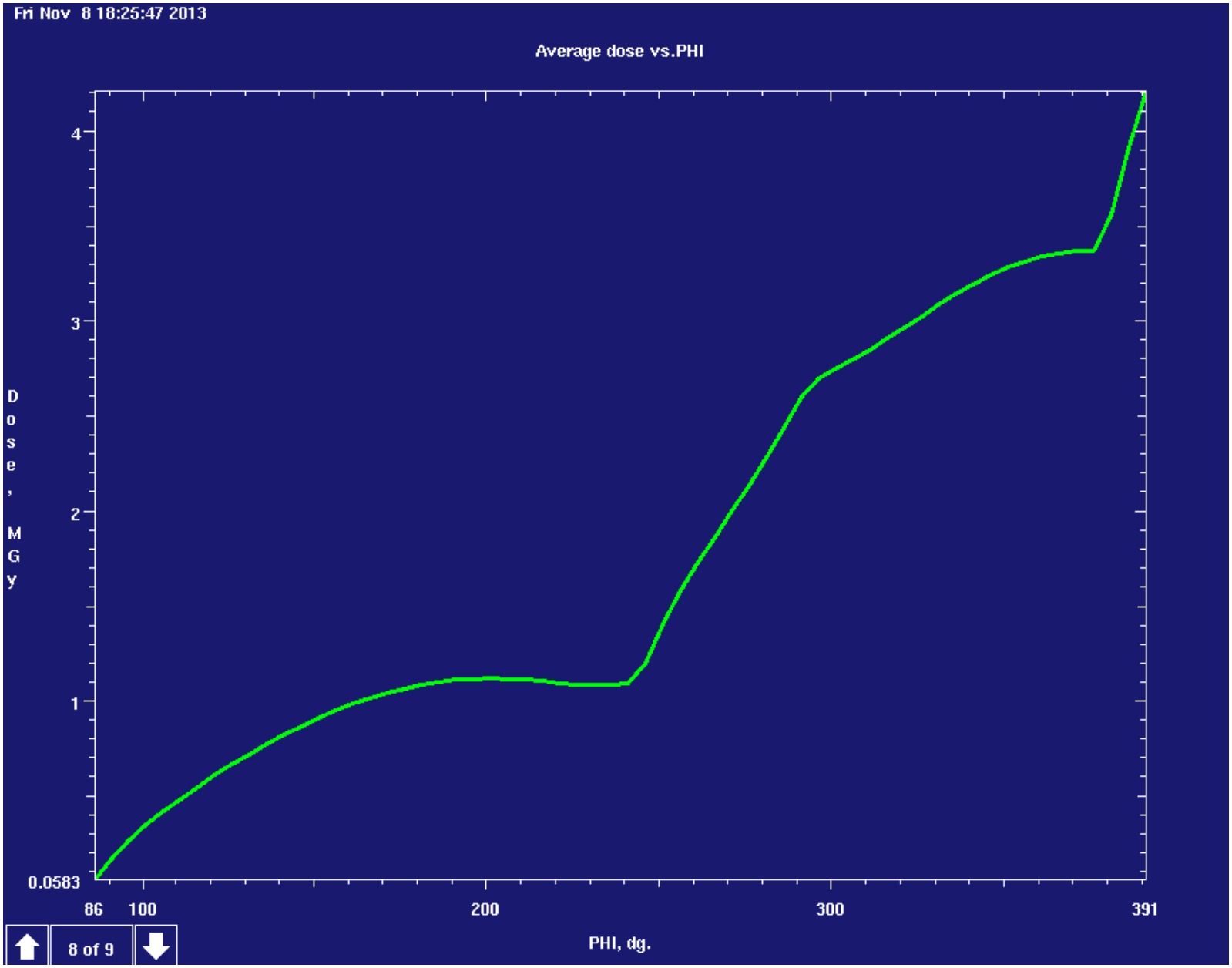
Main Wedge

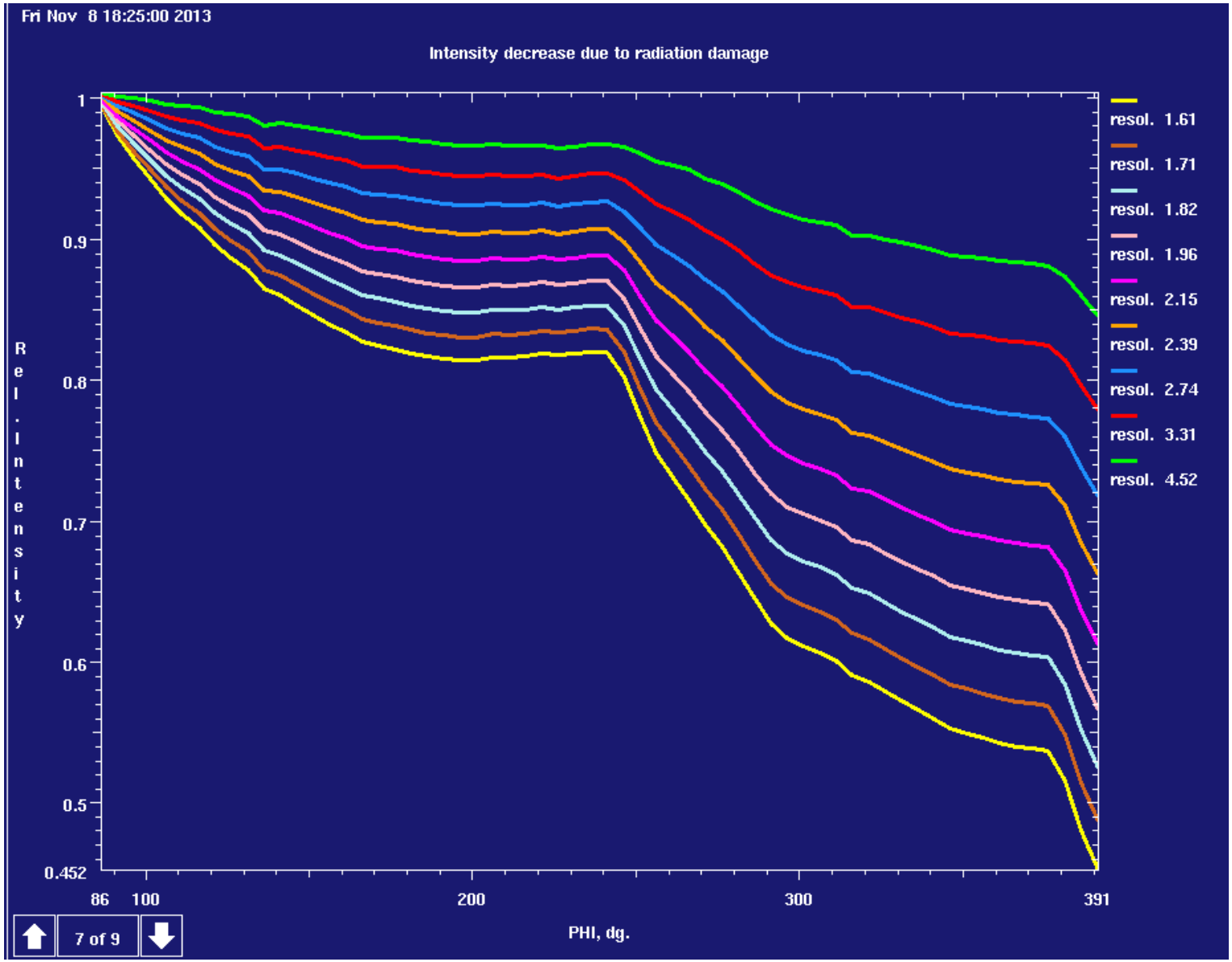
Resolution limit is set by the radiation damage

Resolution limit =1.61 Angstrom  Transmission =  100.0%  Distance = 306.2mm

| sub-Wedge | Phi start degree | Rot. width degree | Exposure /image s | N.of images | Over-lap | sWedge width degree | Exposure /sWedge s | Exposure total s | Dose /sWedge MGy | Dose total MGy | Completeness % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 81.00 | 0.15 | 0.122 | 1067 | No | 160.05 | 130.5 | 130.5 | 3.581 | 3.581 | 99.9 |
| 2 | 241.05 | 0.15 | 0.358 | 333 | No | 49.95 | 119.3 | 249.9 | 3.274 | 6.855 | 100.0 |
| 3 | 291.00 | 0.15 | 0.223 | 567 | No | 85.05 | 126.2 | 376.1 | 3.463 | 10.319 | 100.0 |
| 4 | 376.05 | 0.15 | 0.647 | 100 | No | 15.00 | 64.7 | 440.8 | 1.774 | 12.093 | 100.0 |

Phi_start - Phi_finish : 81.00 - 391.05
Total rotation range : 310.05 degree
Total N.of images : 2067
Overall Completeness : 100.0%
Redundancy : 12.69
R-factor (outer shell) : 10.5% (  89.7%)
I/Sigma (outer shell) : 30.7 (  3.2)
Total Exposure time : 440.8 sec (0.122 hour)
Total Data Collection time : 449.0 sec (0.125 hour)


Fri Nov 8 18:25:47 2013
Average dose vs.PHI
Dose, MGy
4
3
2
1
0.0583
86
100
200
300
391
PHI, dg.
8 of 9


Fri Nov 8 18:25:00 2013
Intensity decrease due to radiation damage
Rel. Intensity
1
0.9
0.8
0.7
0.6
0.5
0.452
86
100
200
300
391
PHI, dg.
resol. 1.61
resol. 1.71
resol. 1.82
resol. 1.96
resol. 2.15
resol. 2.39
resol. 2.74
resol. 3.31
resol. 4.52
7 of 9

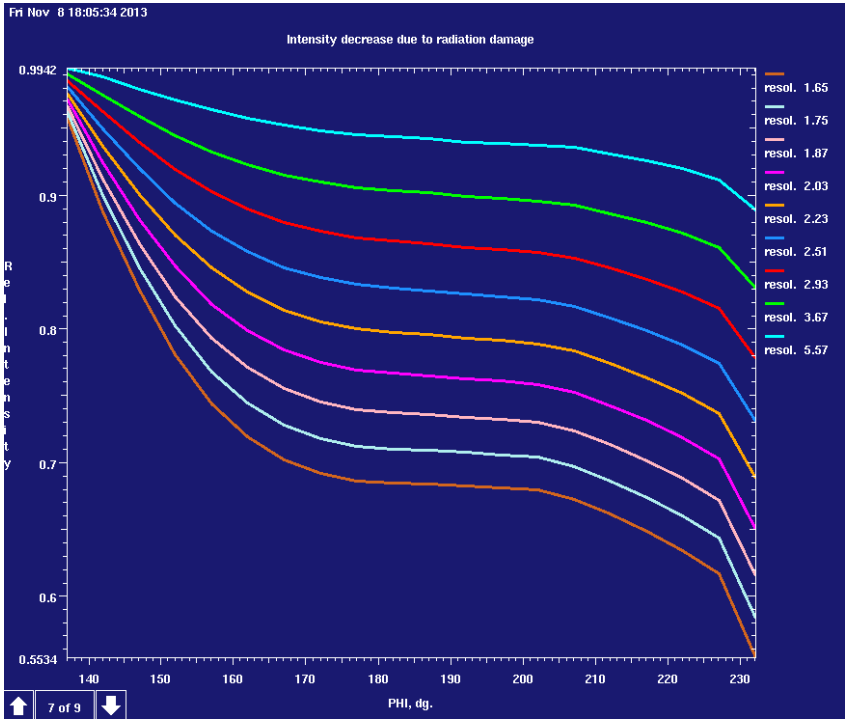
Main Wedge

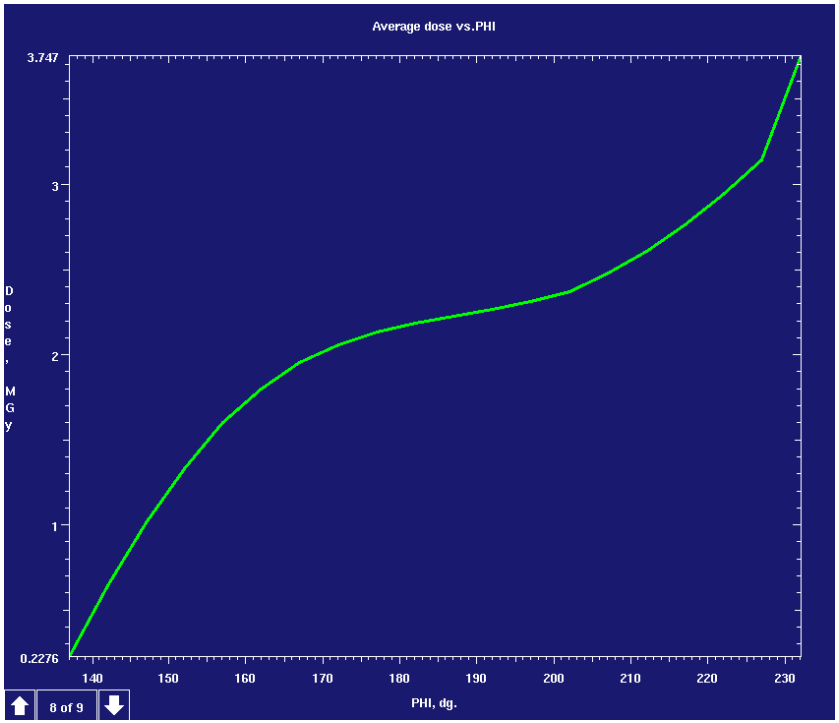
Resolution limit is set by the radiation damage

Resolution limit =1.65 Angstrom  Transmission =  100.0%  Distance = 315.4mm

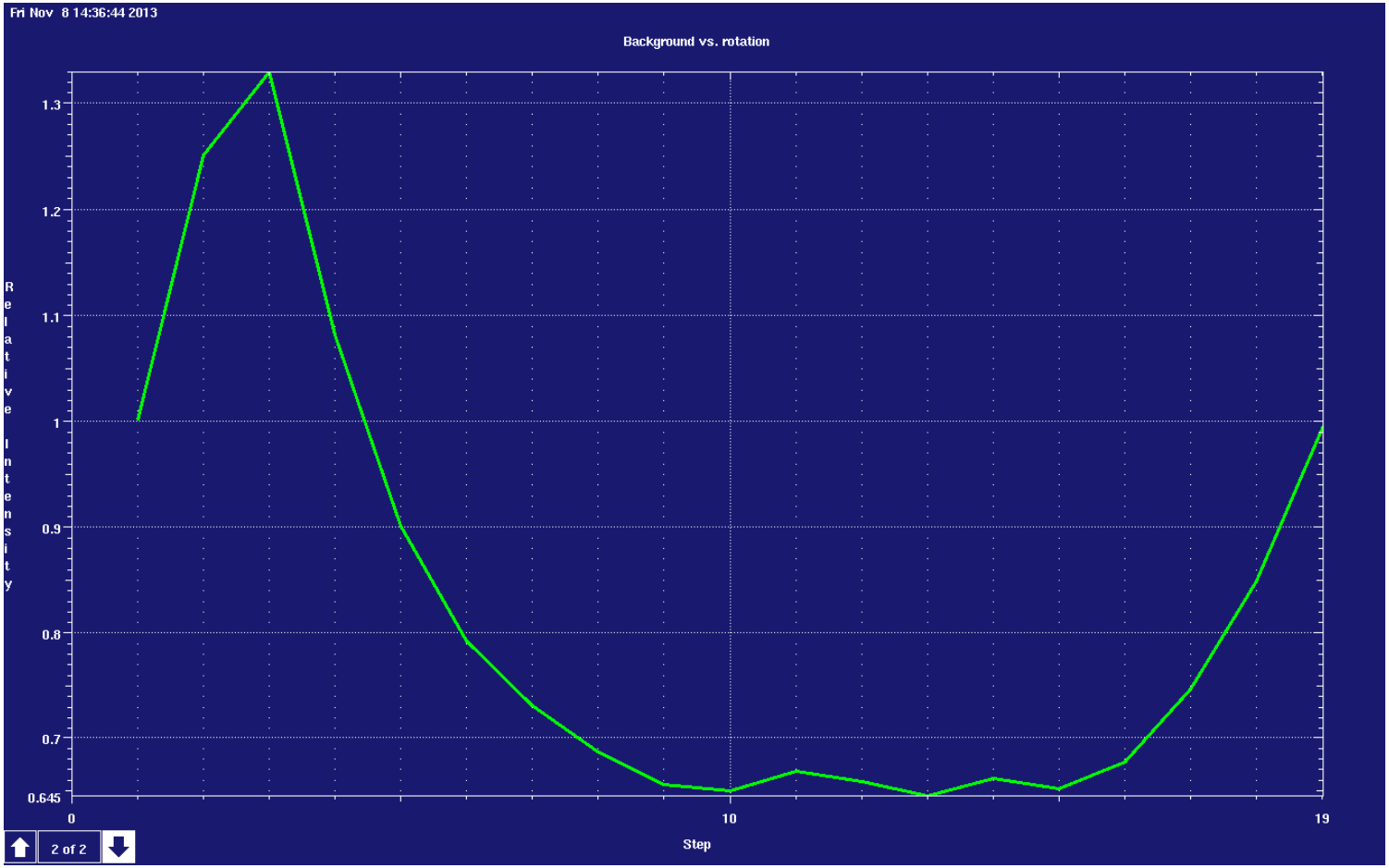
| sub-Wedge | Phi start degree | Rot. width degree | Exposure /image s | N.of images | Overlap | sWedge width degree | Exposure /sWedge s | Exposure total s | Dose /sWedge MGy | Dose total MGy | Completeness % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 132.00 | 0.15 | 0.478 | 634 | No | 95.10 | 303.1 | 303.1 | 8.315 | 8.315 | 97.0 |
| 2 | 227.10 | 0.15 | 1.328 | 33 | No | 4.95 | 43.8 | 346.9 | 1.202 | 9.518 | 99.0 |

Phi_start - Phi_finish : 132.00 - 232.05
Total rotation range : 100.05 degree
Total N.of images : 667
Overall Completeness : 99.0%
Redundancy : 4.13
R-factor (outer shell) : 6.3% ( 48.1%)
I/Sigma (outer shell) : 27.2 ( 2.9)
Total Exposure time : 346.9 sec (0.096 hour)
Total Data Collection time : 349.6 sec (0.097 hour)

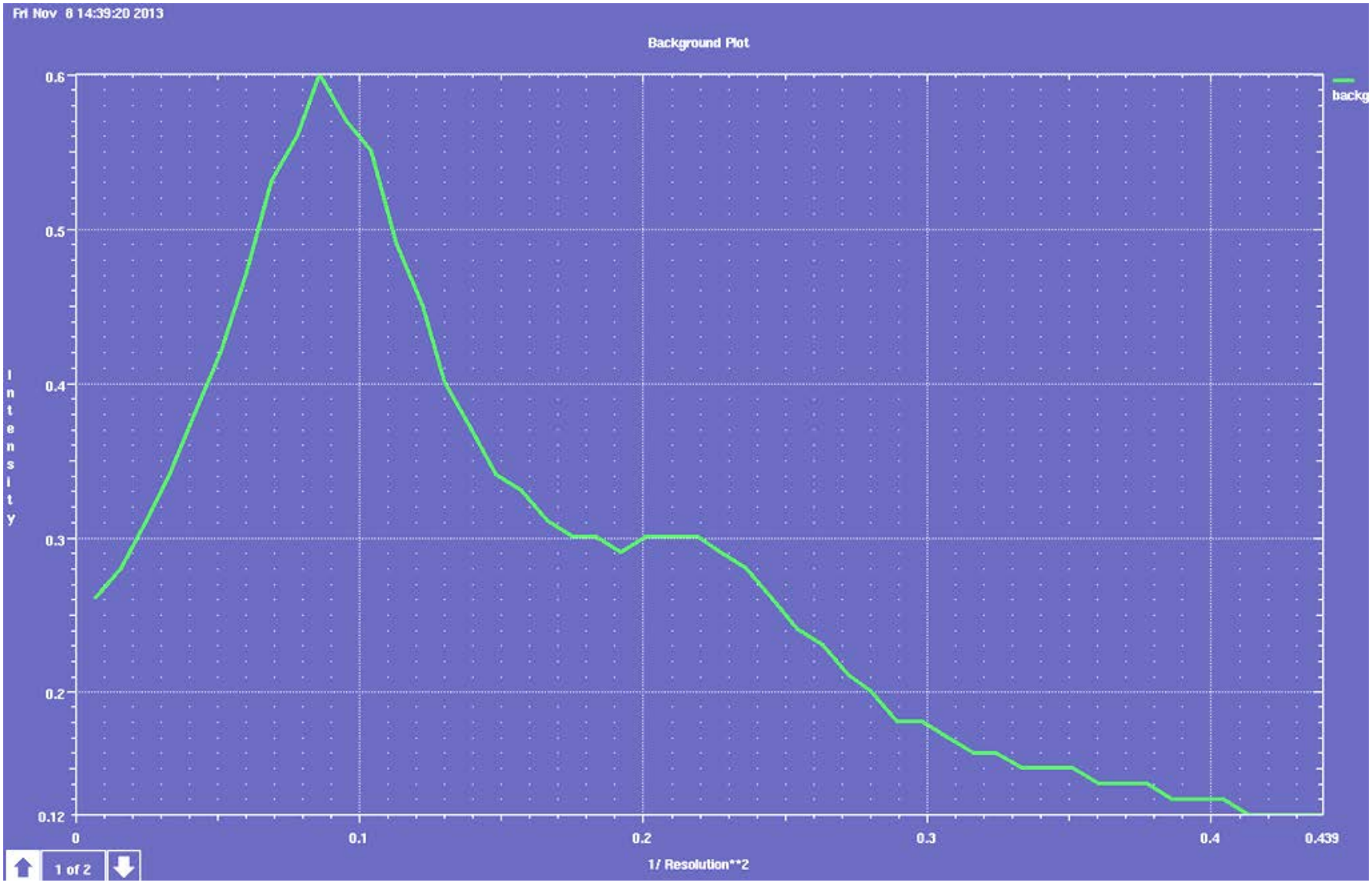


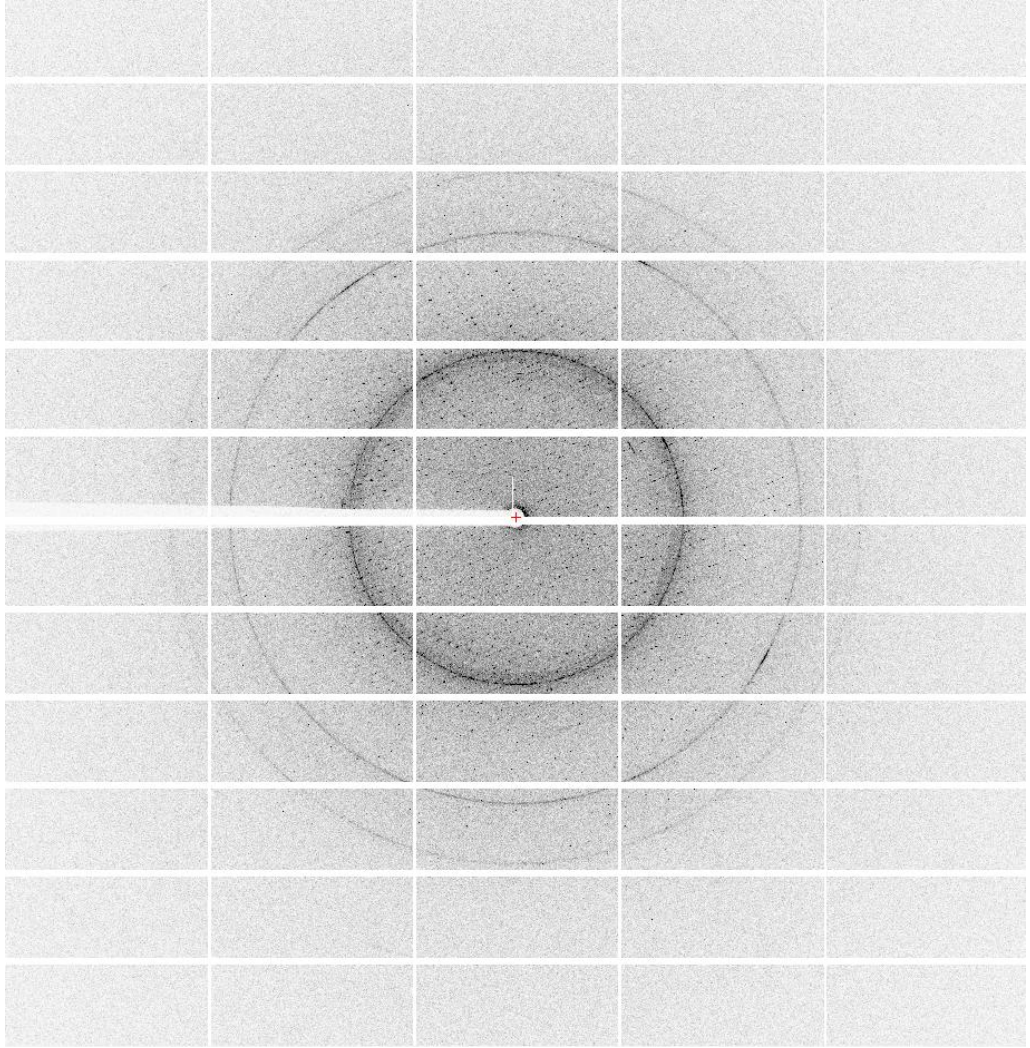



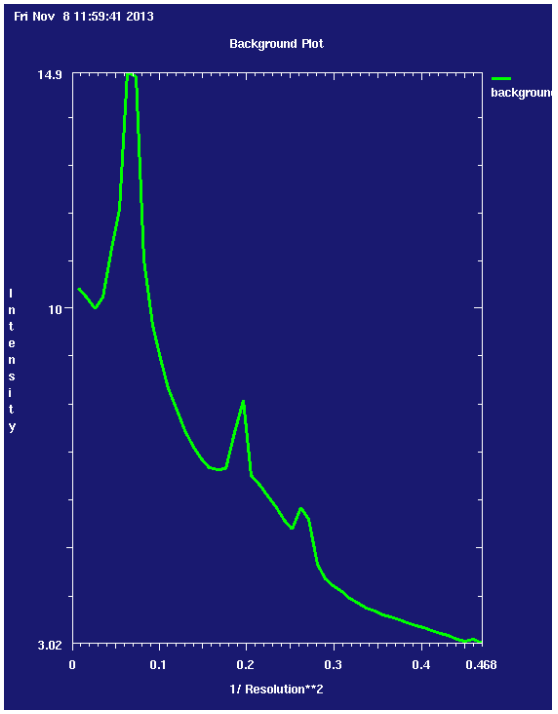
# Background vs. Crystal position



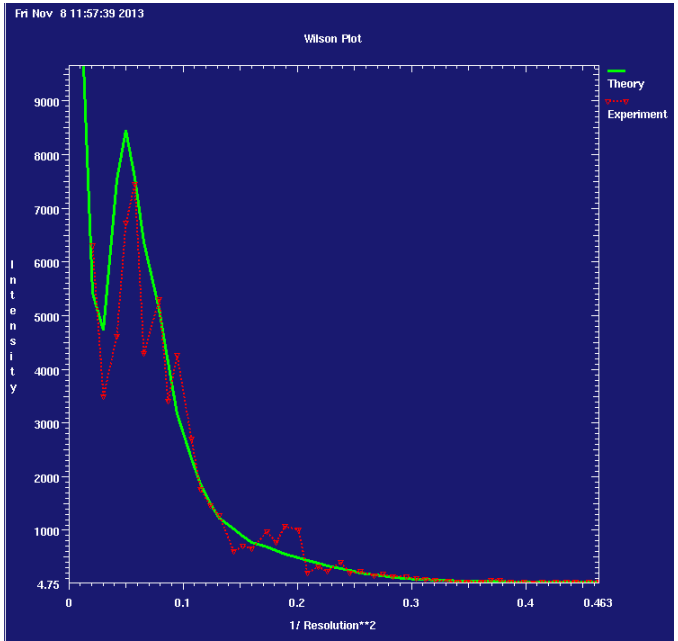
# Background vs. Crystal position


Fri Nov 8 14:39:20 2013
Background Plot
backg
Intensity
0.6
0.5
0.4
0.3
0.2
0.12
0
0.1
0.2
0.3
0.4
0.439
1/ Resolution**2
1 of 2

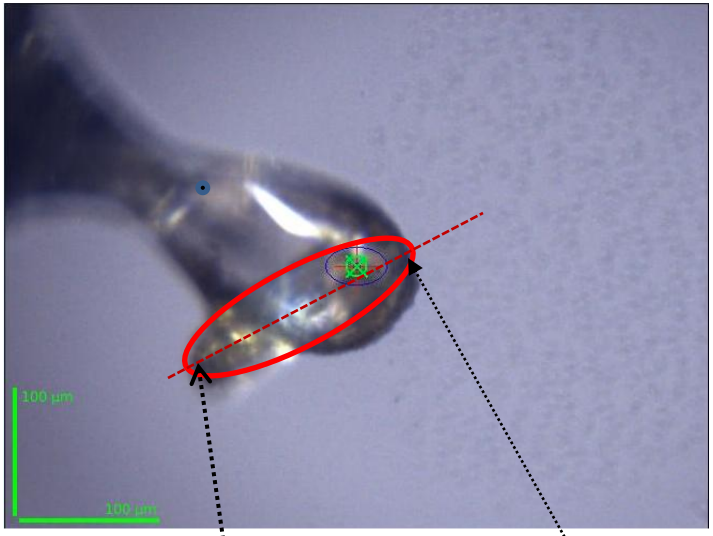


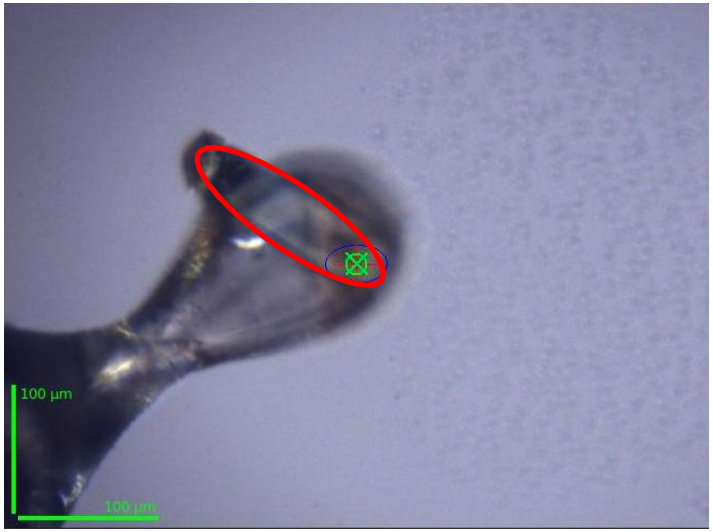



```
image number =           1
Relative scale       :    9.08
Overall B-factor     :  23.74 Angstrom^2
Resolution used      :    1.7 Angstrom
Correlation =    84.6 %
Rfactor =    17.7 %
```



# How to define a form and the position of a crystal?

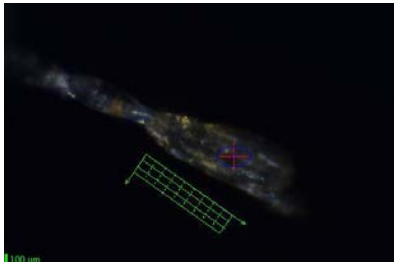


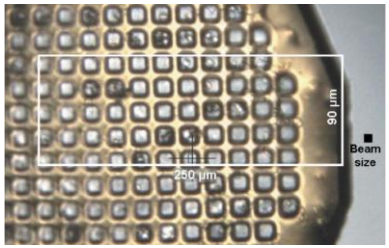




Point 1

Point 2

# crystal detection and characterization






computer programs

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**Automated diffraction image analysis and spot searching for high-throughput crystal screening**

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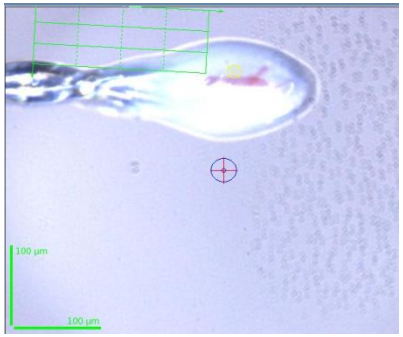
LABELIT
MOSFLM

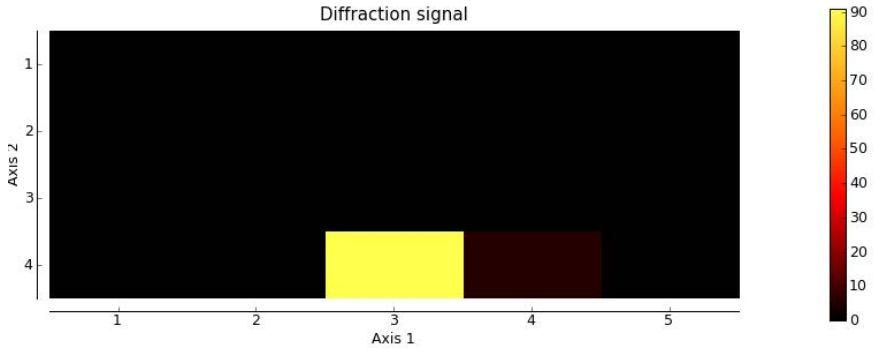
**Best position**

| Grid index Y | Grid index Z | Image file | sampx | sampy | phiy | Total integrated intensity | Bravais lattice |
|---|---|---|---|---|---|---|---|
| 10 | 2 | mesh2d-opid291_1_0020.cbf | -0.089 | 0.005 | -0.545 | 1.3e+07 | P3 |

**All positions**

| Grid index Y | Grid index Z | Image file | sampx | sampy | phiy | Total integrated intensity | Bravais lattice |
|---|---|---|---|---|---|---|---|
| 1 | 1 | mesh2d-opid291_1_0001.cbf | -0.043 | -0.041 | -0.345 | 1.61e+06 | _ |
| 2 | 1 | mesh2d-opid291_1_0002.cbf | -0.046 | -0.038 | -0.367 | 6.17e+06 | _ |
| 3 | 1 | mesh2d-opid291_1_0003.cbf | -0.049 | -0.035 | -0.390 | 9.35e+06 | _ |
| 4 | 1 | mesh2d-opid291_1_0004.cbf | -0.053 | -0.031 | -0.413 | 1.08e+07 | P3 |
| 5 | 1 | mesh2d-opid291_1_0005.cbf | -0.056 | -0.028 | -0.436 | 1.14e+07 | _ |
| 6 | 1 | mesh2d-opid291_1_0006.cbf | -0.059 | -0.025 | -0.458 | 9e+06 | _ |
| 7 | 1 | mesh2d-opid291_1_0007.cbf | -0.062 | -0.022 | -0.481 | 6.49e+06 | _ |
| 8 | 1 | mesh2d-opid291_1_0008.cbf | -0.065 | -0.019 | -0.504 | 3.04e+06 | _ |




## Best position

The sample has automatically been moved to the best position.
In order to move the sample to an other position please copy/paste the commands from the right column into SPEC EXP.

| Axis 1 | Axis 2 | Image file | Signal 1 | Signal 2 | Bravais lattice | SPEC command for moving sample to position |
|---|---|---|---|---|---|---|
| 3 | 4 | mesh-x_1_0018.cbf | 90.9 | 75846 | P1 | mv sampx 0.280; mv sampy -0.382; mv phiy 22.465 |

Signal 1: Criteria that uses intensities over background vs resolution. Popov 2014, to be published.
Signal 2: Labelit distl spotfinder total integrated intensity.

## All positions

| Axis 1 | Axis 2 | Image file | Signal 1 | Signal 2 | Bravais lattice | SPEC command for moving sample to position |
|---|---|---|---|---|---|---|
| 3 | 4 | mesh-x_1_0018.cbf | 90.9 | 75846 | P1 | mv sampx 0.280; mv sampy -0.382; mv phiy 22.465 |
| 4 | 4 | mesh-x_1_0019.cbf | 5.2 | 8975 | _ | mv sampx 0.278; mv sampy -0.381; mv phiy 22.515 |
| 1 | 1 | mesh-x_1_0001.cbf | 0 | 165 | _ | mv sampx 0.351; mv sampy -0.415; mv phiy 22.370 |
| 2 | 1 | mesh-x_1_0002.cbf | 0 | 368 | _ | mv sampx 0.348; mv sampy -0.414; mv phiy 22.419 |
| 3 | 1 | mesh-x_1_0003.cbf | 0 | 87 | _ | mv sampx 0.346; mv sampy -0.413; mv phiy 22.469 |
| 4 | 1 | mesh-x_1_0004.cbf | 0 | 199 | _ | mv sampx 0.344; mv sampy -0.411; mv phiy 22.519 |
| 5 | 1 | mesh-x_1_0005.cbf | 0 | 811 | _ | mv sampx 0.341; mv sampy -0.410; mv phiy 22.569 |
| 1 | 2 | mesh-x_1_0006.cbf | 0 | 274 | _ | mv sampx 0.329; mv sampy -0.405; mv phiy 22.368 |
| 3 | 2 | mesh-x_1_0008.cbf | 0 | 365 | _ | mv sampx 0.324; mv sampy -0.402; mv phiy 22.468 |
| 4 | 2 | mesh-x_1_0009.cbf | 0 | 514 | _ | mv sampx 0.322; mv sampy -0.401; mv phiy 22.518 |
| 5 | 2 | mesh-x_1_0010.cbf | 0 | 281 | _ | mv sampx 0.319; mv sampy -0.400; mv phiy 22.567 |
| 1 | 3 | mesh-x_1_0011.cbf | 0 | 825 | _ | mv sampx 0.307; mv sampy -0.394; mv phiy 22.367 |
| 2 | 3 | mesh-x_1_0012.cbf | 0 | 690 | _ | mv sampx 0.305; mv sampy -0.393; mv phiy 22.417 |
| 3 | 3 | mesh-x_1_0013.cbf | 0 | 1251 | _ | mv sampx 0.302; mv sampy -0.392; mv phiy 22.467 |
| 4 | 3 | mesh-x_1_0014.cbf | 0 | 4402 | _ | mv sampx 0.300; mv sampy -0.391; mv phiy 22.516 |
| 5 | 3 | mesh-x_1_0015.cbf | 0 | 76 | _ | mv sampx 0.297; mv sampy -0.390; mv phiy 22.566 |
| 1 | 4 | mesh-x_1_0016.cbf | 0 | 553 | _ | mv sampx 0.285; mv sampy -0.384; mv phiy 22.366 |
| 2 | 4 | mesh-x_1_0017.cbf | 0 | 539 | _ | mv sampx 0.283; mv sampy -0.383; mv phiy 22.416 |
| 5 | 4 | mesh-x_1_0020.cbf | 0 | 83 | _ | mv sampx 0.276; mv sampy -0.380; mv phiy 22.565 |
| 2 | 2 | mesh-x_1_0007.cbf | -0.005 | 6889 | _ | mv sampx 0.327; mv sampy -0.403; mv phiy 22.418 |

| Number | Scaler | B-factor | Resolution | Correlation | R-factor |
|---|---|---|---|---|---|
| 1 | 7.896 | 310.8 | 5.3 | 12.4 | 74.9 |
| 2 | 43.779 | 124.7 | 4.1 | 39 | 43.6 |
| 3 | 74.18 | 53.1 | 3 | 71.2 | 20.4 |
| 4 | 89.461 | 43.6 | 2.9 | 76.2 | 20 |
| 5 | 108.249 | 37.1 | 2.9 | 78.1 | 21.9 |
| 6 | 255 | 38.5 | 3.1 | 77.9 | 22.6 |
| 7 | 916.497 | 46.1 | 5.3 | 60.5 | 49.7 |
| 8 | 2075.394 | 67.8 | 5.3 | 10.7 | 87.6 |
| 9 | 0 | 0 | 0 | 0 | 0 |
| 10 | 0 | 0 | 0 | 0 | 0 |
| 11 | 424.232 | 43.5 | 4.8 | 62.5 | 36.2 |
| 12 | 31.177 | 34.8 | 2.4 | 80.5 | 17.4 |
| 13 | 14.481 | 28.3 | 2 | 84 | 15.4 |
| 14 | 8.728 | 31.3 | 1.9 | 81.7 | 17.9 |
| 15 | 5.714 | 37.1 | 2 | 78.7 | 18.5 |
| 16 | 5.048 | 38.9 | 2.1 | 77.2 | 20.3 |
| 17 | 6.151 | 34 | 1.9 | 79.7 | 17.4 |
| 18 | 11.242 | 29.3 | 1.9 | 83.7 | 15.1 |
| 19 | 15.979 | 38.7 | 2.1 | 76.6 | 21.5 |
| 20 | 51.573 | 39.3 | 2.9 | 76 | 22.7 |
| 21 | 0 | 0 | 0 | 0 | 0 |
| 22 | 203.738 | 49.6 | 3.1 | 71.4 | 27.2 |
| 23 | 37.04 | 47.9 | 2.9 | 73.1 | 21.5 |
| 24 | 13.783 | 49.1 | 2.4 | 71 | 24.4 |
| 25 | 14.12 | 30.1 | 2.4 | 82.5 | 16.6 |
| 26 | 7.71 | 34.2 | 2 | 79.3 | 19.3 |
| 27 | 5.854 | 35.4 | 1.9 | 80 | 17.1 |
| 28 | 5.696 | 37.9 | 2 | 79.1 | 16.9 |
| 29 | 6.887 | 35.6 | 1.9 | 79.5 | 16.3 |
| 30 | 9.08 | 23.7 | 1.7 | 84.6 | 17.7 |
| 31 | 0 | 0 | 0 | 0 | 0 |
| 32 | 0 | 0 | 0 | 0 | 0 |
| 33 | 0 | 0 | 0 | 0 | 0 |
| 34 | 0 | 0 | 0 | 0 | 0 |
| 35 | 219.289 | 42.8 | 3.1 | 74.7 | 26.8 |
| 36 | 166.848 | -9.9 | 2.4 | 37.6 | 62.2 |
| 37 | 49.944 | 12.6 | 2.4 | 85.5 | 24.5 |
| 38 | 17.325 | 40.3 | 2.4 | 74.9 | 24.2 |
| 39 | 11.697 | 49.7 | 2.4 | 70.9 | 24.9 |
| 40 | 15.37 | 36.1 | 2.1 | 77.9 | 20.8 |