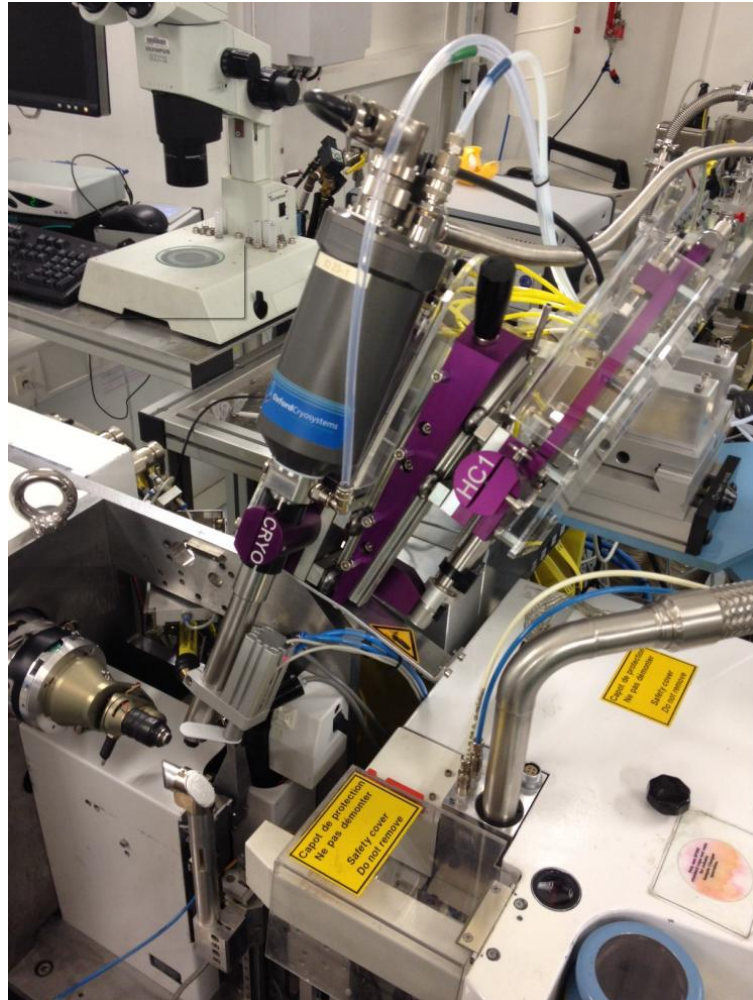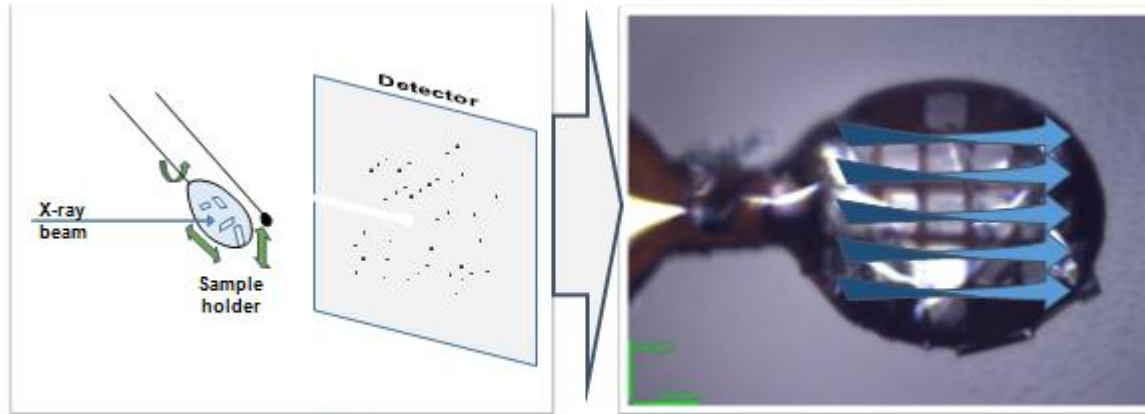# ID23-1 news

Rapid Exchanger (ReX) for automated exchange between HC1 humidifier nozzle and cryo-stream nozzle. Including mechanical support system with alignment axes

Application:provides an easy to use dehydration setup

New software developments

X-ray Mesh Scans micro X-ray beam, high-precision diffractometry , shutterless data acquisition

Experimental setup for X-ray crystallography

Translation with slight rotation is the principle of Mesh Scan data collection
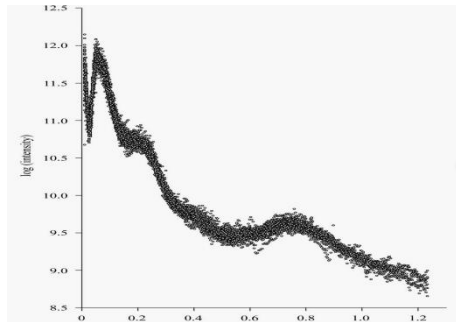
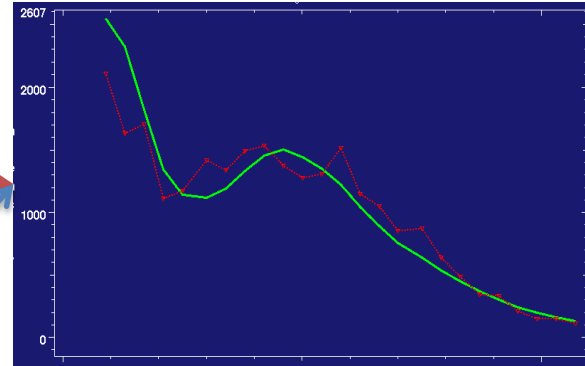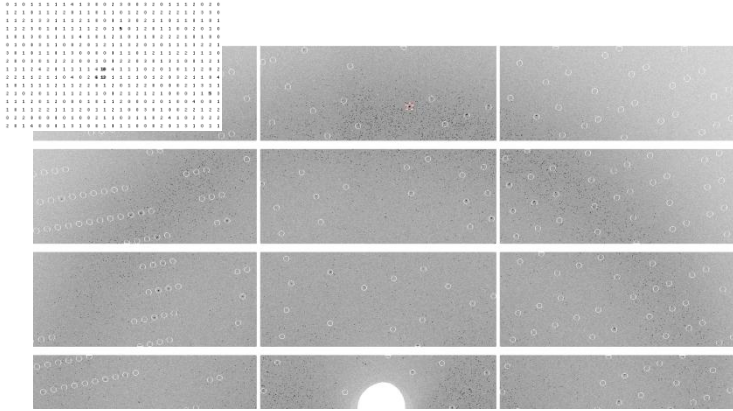The Complex Analysis of the X-ray Mesh Scan
DOZOR

Crystals Mapping – positions, size, diffraction quality

BEST – Data Collection Strategy accounting radiation damage and crystal size

Data Collection and Processing

Evaluating diffraction signal with DOZOR

score =

total scattered intensity × radial shape similarity

- Use Wilson plot as a prior
- Use all pixels, not just the local maxima

```
Program dozor /A.Popov & G.Bourenkov/
Version 1.3.6 // 02.02.2016
Copyright 2014 by Alexander Popov and Gleb Bourenkov
N    |      SPOTS     |        Powder Wilson            |    Main   Spot   Visible
image | num.of INTaver Res.|  Scale B-fac. Res. Corr. R-factor|   Score  Score  Resolution
-------------------------------------------------------------------------------
   1 |   44     4.  3.4|  154.25  26.5  2.9  72.0   26.7 |   1.426   1.74    2.73
   2 |   46    39.  3.6|   21.35  28.0  2.9  61.2   25.7 |   9.097  12.40    2.86
   3 |   40    76.  3.4|    5.88  44.6  2.9  69.1   28.2 |  22.485  29.69    2.73
   4 |   51    44.  3.4|   18.10  24.2  2.8  64.0   24.8 |  12.837  16.96    2.69
   5 |   46    32.  3.3|   22.98  30.4  2.9  71.6   24.9 |   9.561  13.63    2.62
   6 |   11     5.  6.4|  142.22  18.1  2.9  62.8   29.6 |   0.235   0.13    5.09
   7 |   43     4.  3.4|  232.69  16.7  2.8  62.9   31.0 |   1.258   1.97    2.69
   8 |   30    78.  3.4|   19.31  13.1  2.9  56.1   31.3 |  15.888  30.38    2.69
   9 |   33    83.  3.2|    9.34  28.5  2.9  52.3   25.0 |  20.799  40.67    2.59
  10 |   31    66.  3.4|    8.86  39.8  3.0  62.3   24.7 |  16.076  24.23    2.69
```
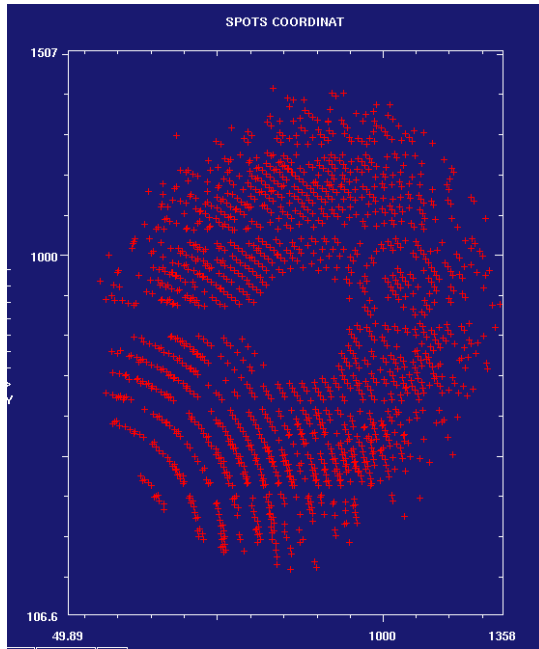
DOZOR output

```
Program dozor /A.Popov & G.Bourenkov/
Version 1.3.6 //  02.02.2016
Copyright 2014 by Alexander Popov and Gleb Bourenkov
```

| N image	SPOTS			Powder Wilson					Main	Spot	Visible
	num.of	INTaver	Res.	Scale	B-fac.	Res.	Corr.	R-factor	Score	Score	Resolution
1	44	4.	3.4	154.25	26.5	2.9	72.0	26.7	1.426	1.74	2.73
2	46	39.	3.6	21.35	28.0	2.9	61.2	25.7	9.097	12.40	2.86
3	40	76.	3.4	5.88	44.6	2.8	69.1	28.2	22.485	29.69	2.73
4	51	44.	3.4	18.10	24.2	2.8	64.0	24.8	12.837	16.96	2.69
5	46	32.	3.3	22.98	30.4	2.9	71.6	24.9	9.561	13.63	2.62
6	11	5.	6.4	142.22	18.1	2.9	62.8	29.6	0.235	0.13	5.09
7	43	4.	3.4	232.69	16.7	2.8	62.9	31.0	1.258	1.97	2.69
8	30	78.	3.4	19.31	13.1	2.9	56.1	31.3	15.888	30.38	2.69
9	33	83.	3.2	9.34	28.5	2.9	52.3	25.0	20.799	40.67	2.59
10	31	66.	3.4	8.86	39.8	3.0	62.3	24.7	16.076	24.23	2.69

Spots list
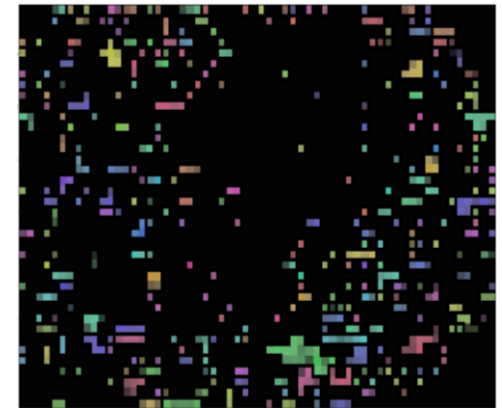
Crystals Mapping

detects single crystals in the crystal mess as well as crystal overlapping
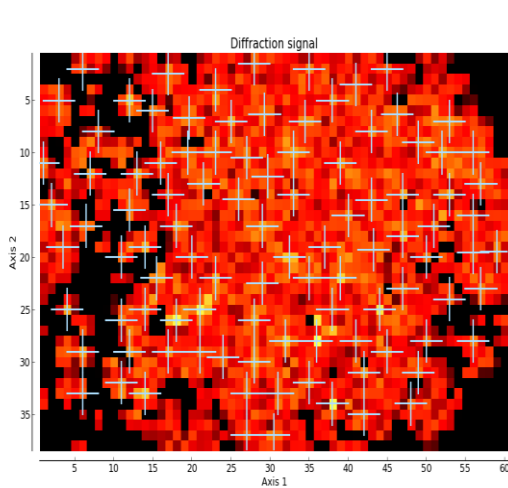
Initial diffraction map, showing regions with better protein crystal diffraction quality
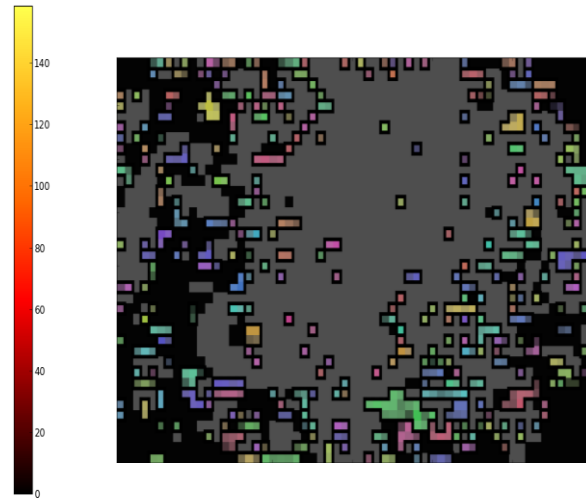
Crystal map after Dozor analysis (different colors correspond to different homogeneities; gray regions correspond to pattern overlapping)

Crystal map omitting regions of overlapping

Thaumatin ~50 µm crystals measured on ESRF ID23_1, beamsize 20µm, oscillation 0.017°

Crystal mapping
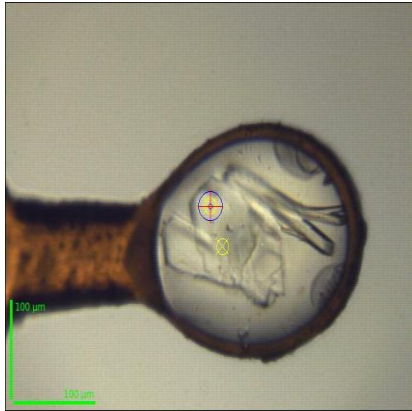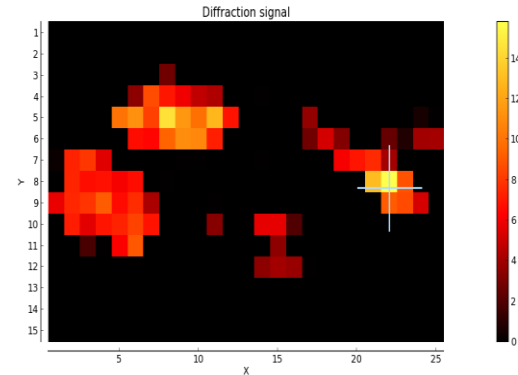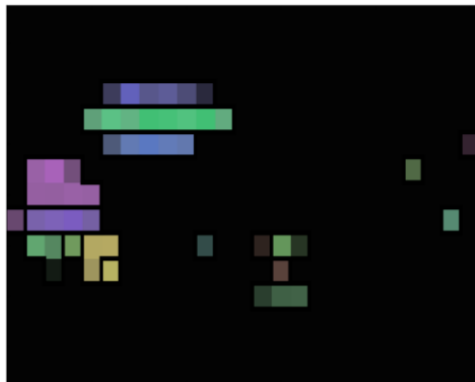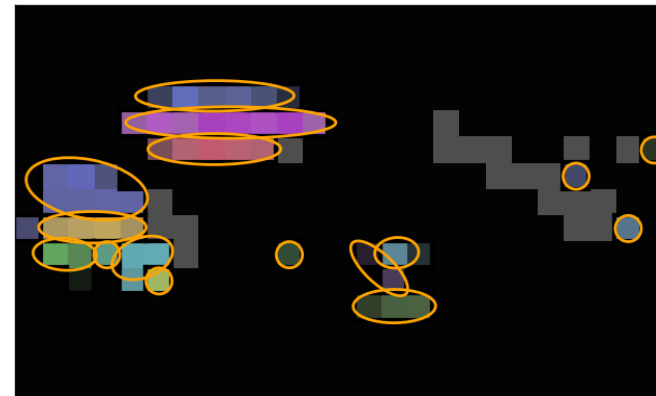## determine regions of crystal homogeneity and size

Photo snapshot of the sample

Initial diffraction map, showing regions with better protein crystal diffraction quality
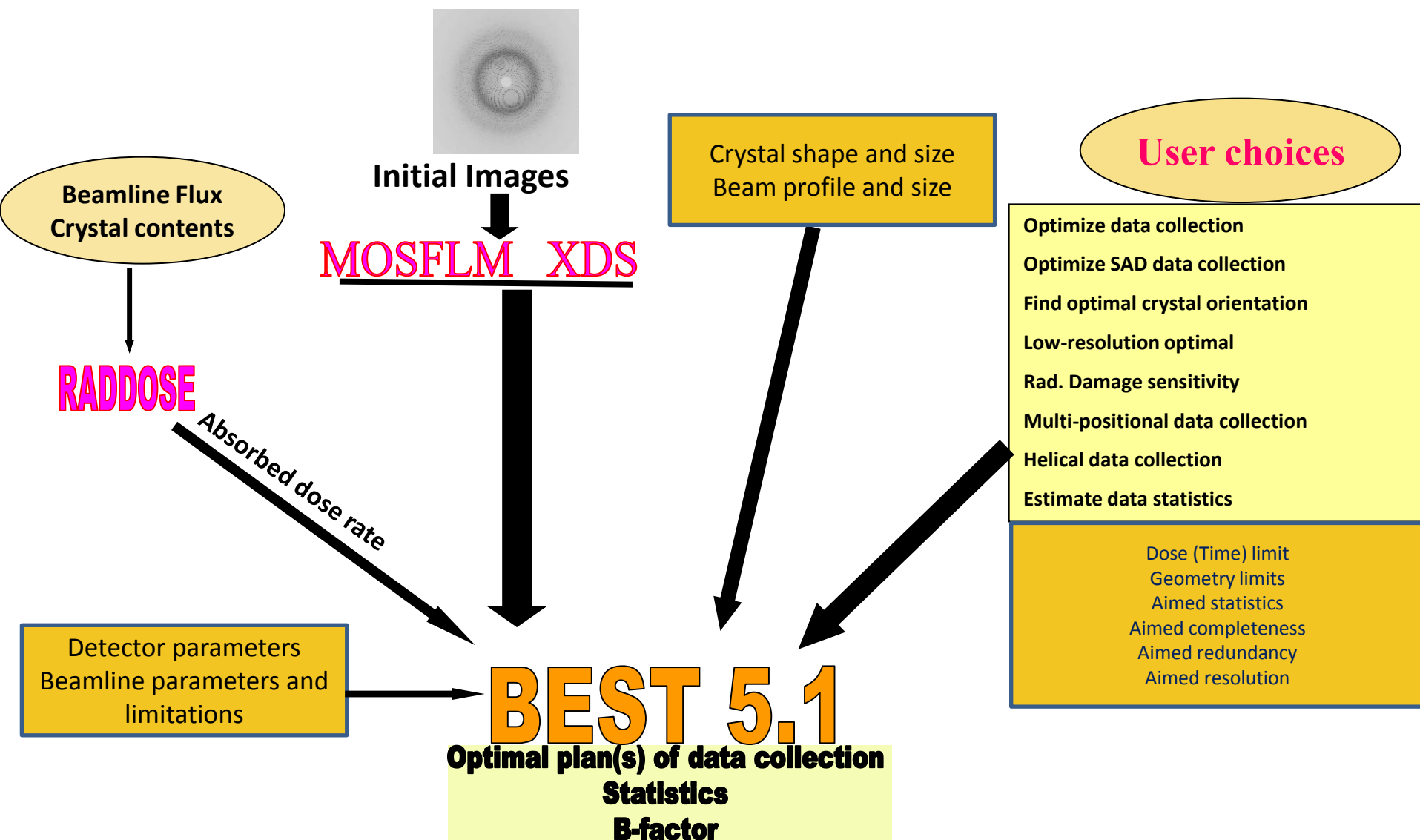

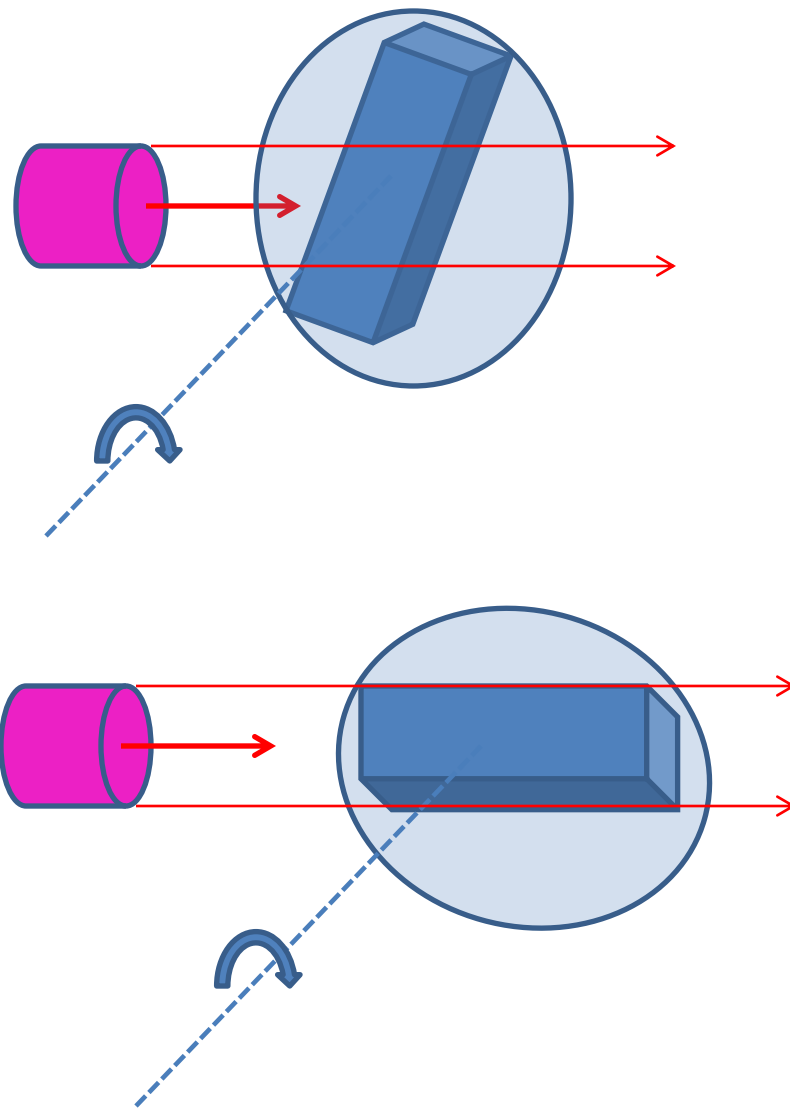Crystal map omitting regions of overlapping

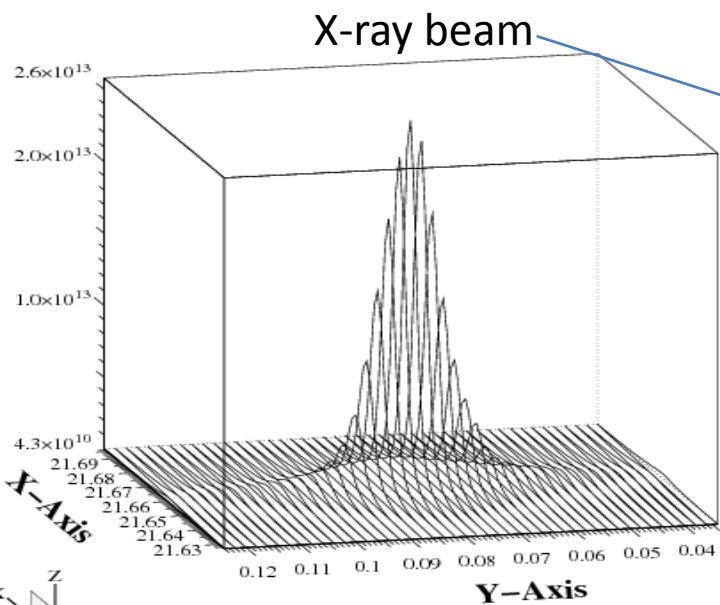fit crystal shapes, corresponding to regions of crystal integrity.

(measured on ESRF ID23_1, beamsize 10μm, oscillation 0.1°)

Initial Images

Beamline Flux
Crystal contents

MOSFLM   XDS

Crystal shape and size
Beam profile and size

User choices

Optimize data collection

Optimize SAD data collection

Find optimal crystal orientation

Low-resolution optimal

Rad. Damage sensitivity

Multi-positional data collection

Helical data collection

Estimate data statistics

RADDOSE

Absorbed dose rate

Detector parameters
Beamline parameters and
limitations

Dose (Time) limit
Geometry limits
Aimed statistics
Aimed completeness
Aimed redundancy
Aimed resolution

BEST 5.1

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

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

Vertical max   0.15 mm

Vertical min 0.03 mm

Crystal horizontal 0.05 mm

X-ray beam

```
!    TRYPSIN TEST
! all sizes in mm
! beam size
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 0.0
!vertical shift relative to the rotation axis
!
crystal_vert_max 0.150
crystal_vert_min 0.03
crystal_hor 0.050
omega_min 0
!description of crystal shape and position- a,c,b
end
```

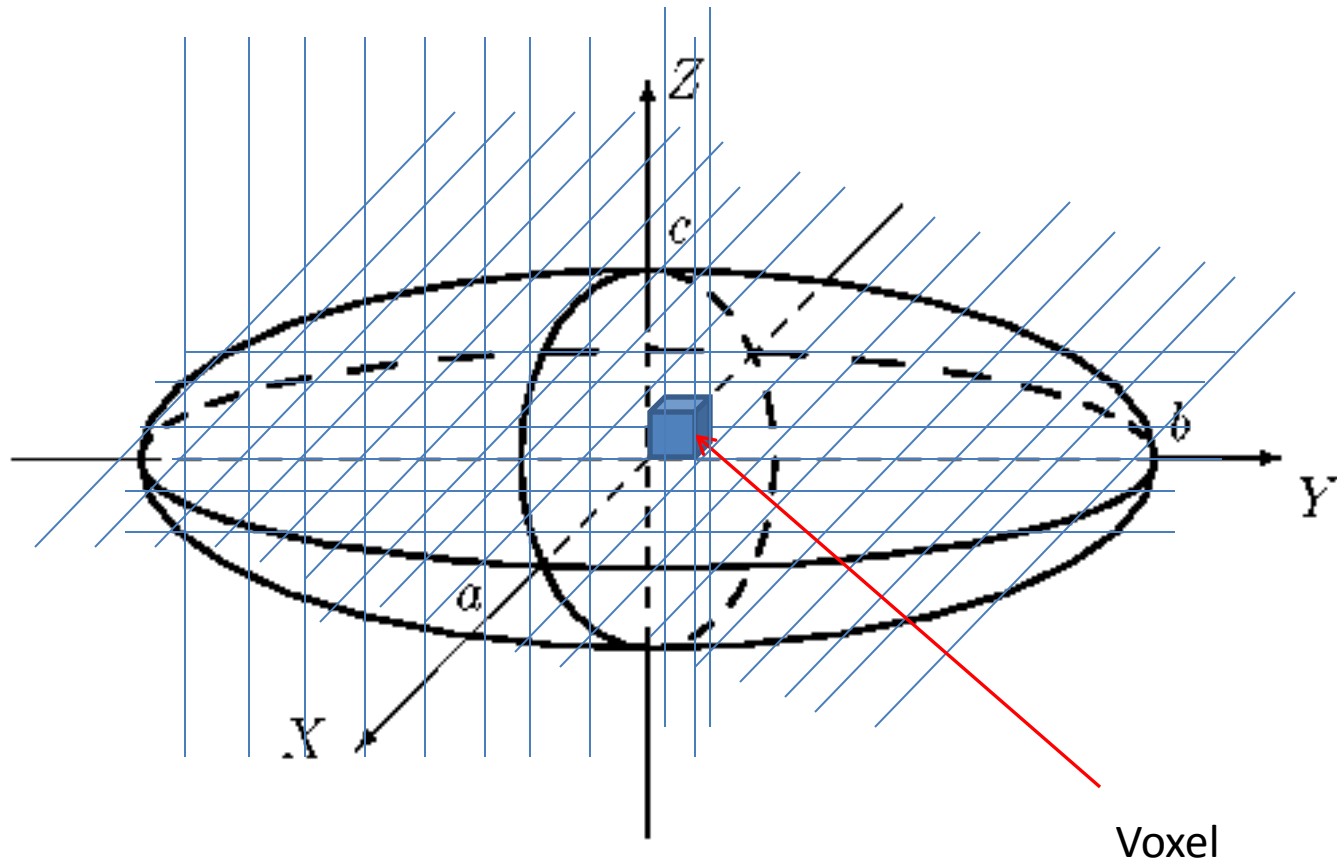beam_crystal.dat - /mntdirect/_users/apopov/BEST4.1/T3D/

File   Edit   Search   Preferences   Shell   Macro   Windows

Diffraction sample Modeling
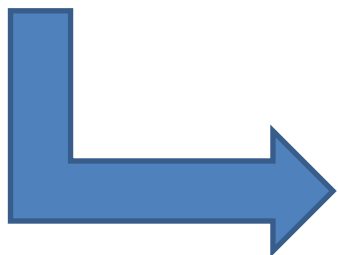
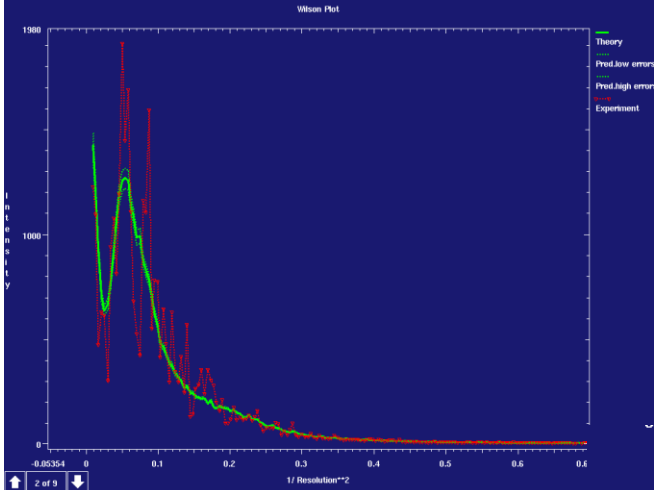

Рис. 1

Voxel
Volumetric Picture Element

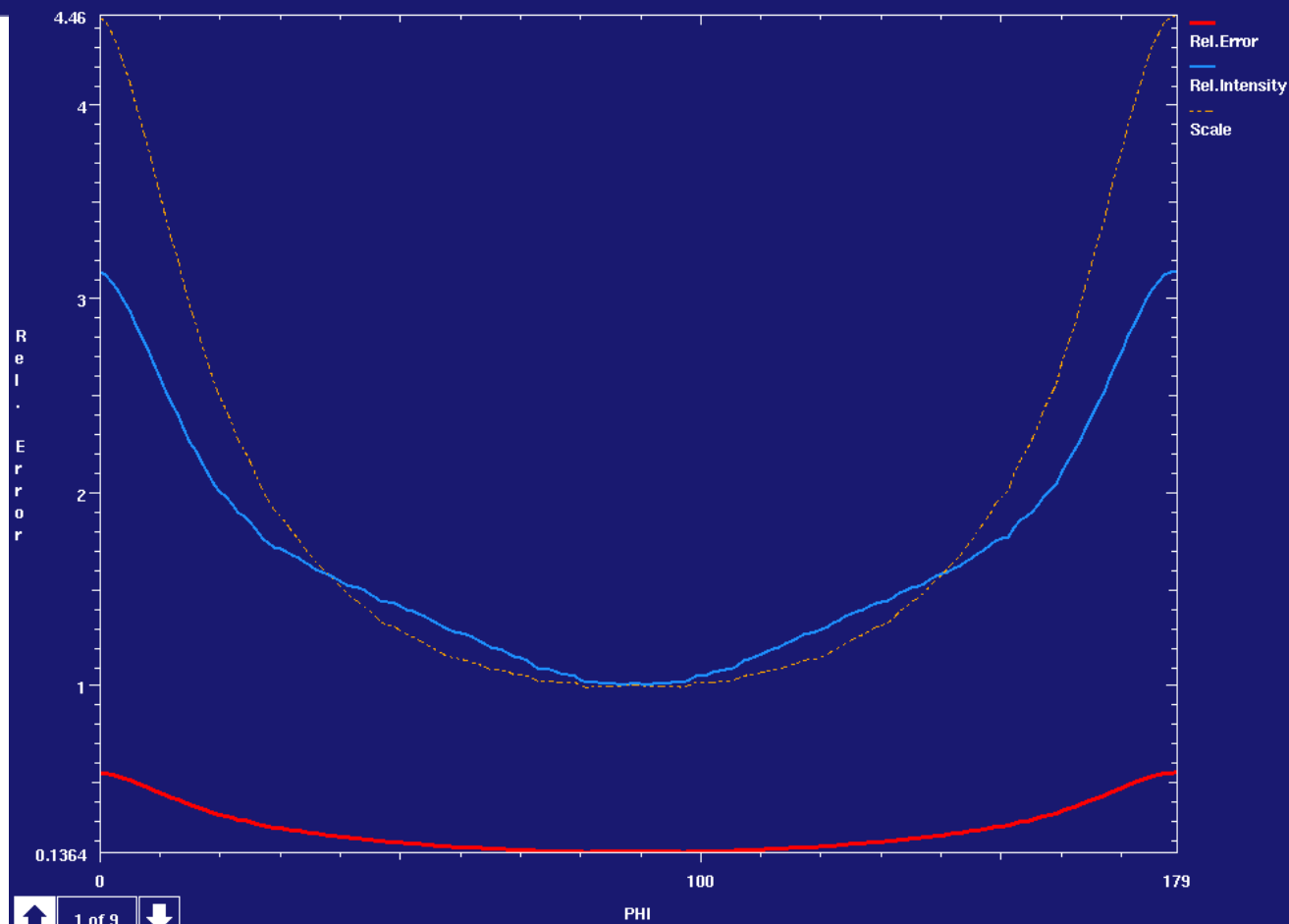# First step - scaling


```
Scaling
  Relative scale          :    77.02
  Overall B-factor        :    12.63 Angstrom^2
  B-factor eigenvalues    :     9.84   12.45   18.01 Angstrom^2
  Scaling error           :    13% at the resolution limit
```
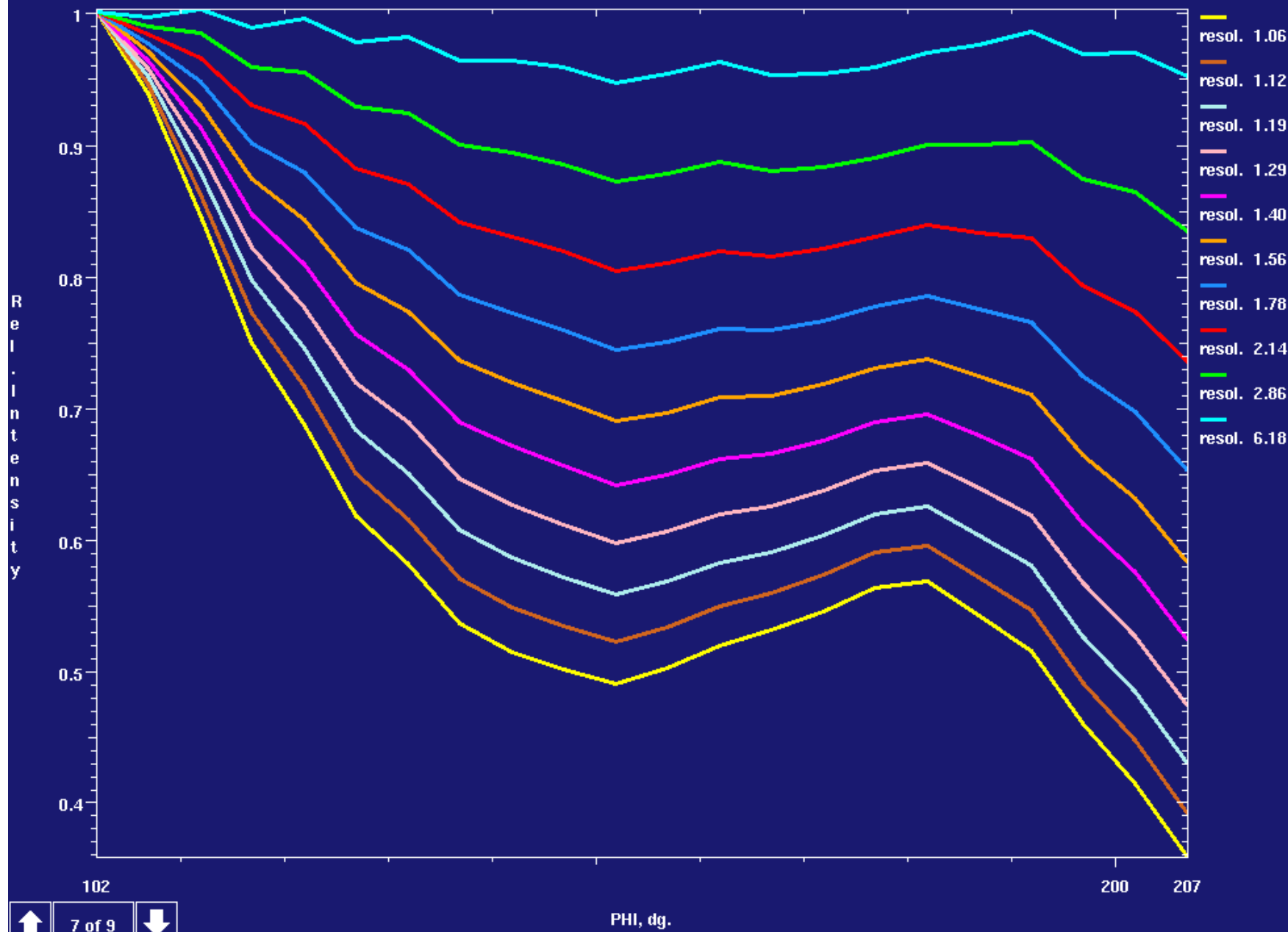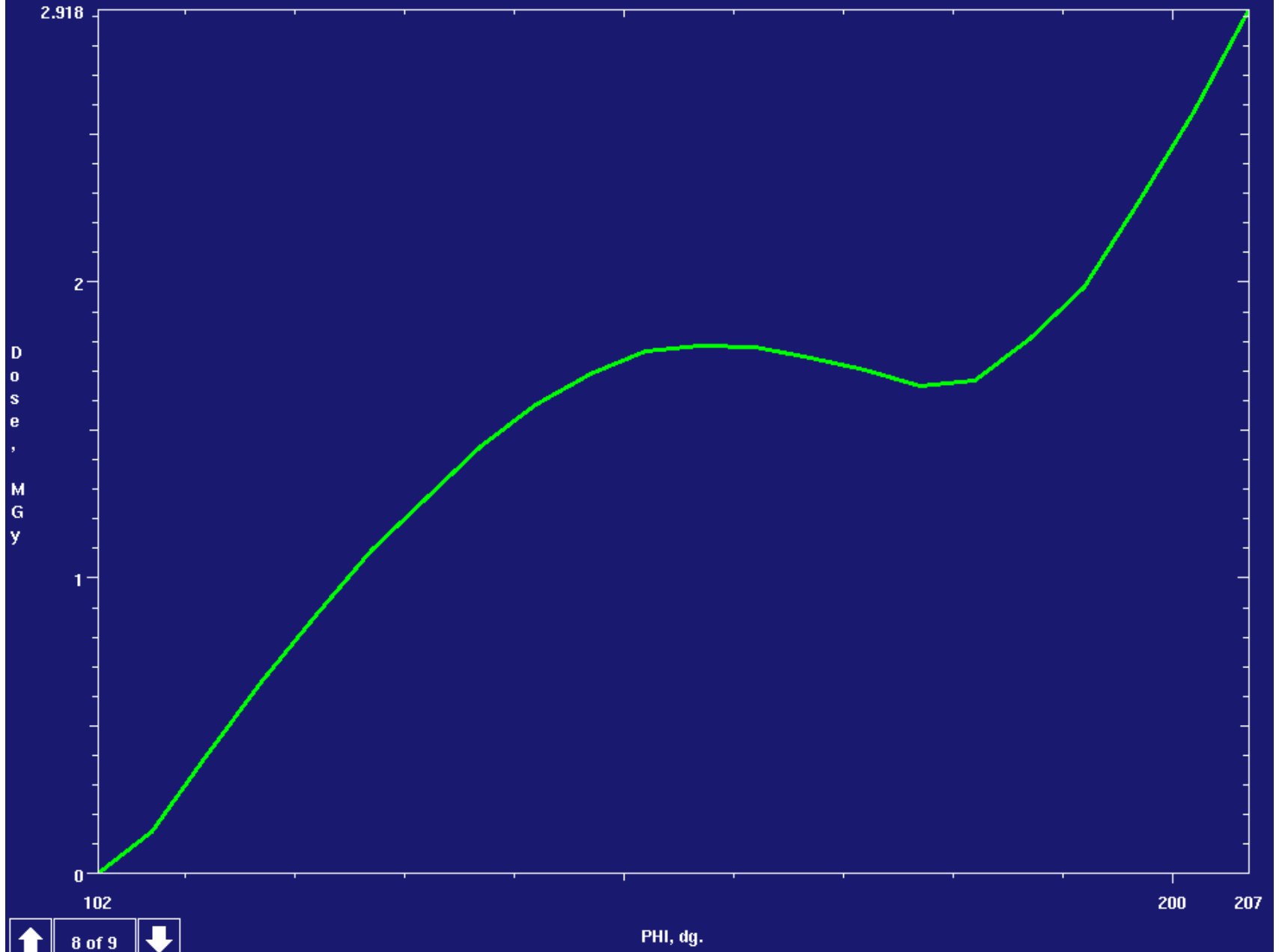
Intensity decrease due to radiation damage

PHI, dg.

resol. 1.06
resol. 1.12
resol. 1.19
resol. 1.29
resol. 1.40
resol. 1.56
resol. 1.78
resol. 2.14
resol. 2.86
resol. 6.18

Average dose vs.PHI

Dose vs. sample