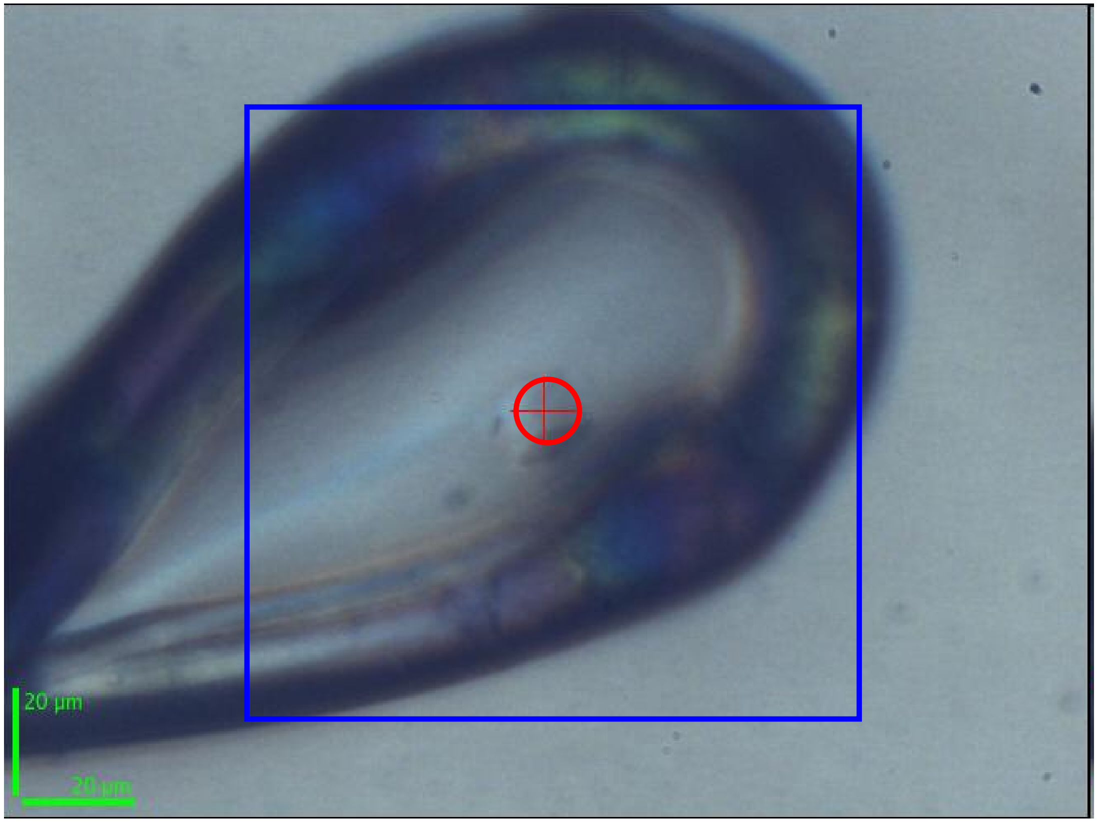
Microfocusing – Recent development for a better use of a microbeam

David FLOT (ESRF)

Microbeams improve signal to noise by minimising solvent diffraction.

(Beam size; 100X100 µm vs 10 µm Ø)



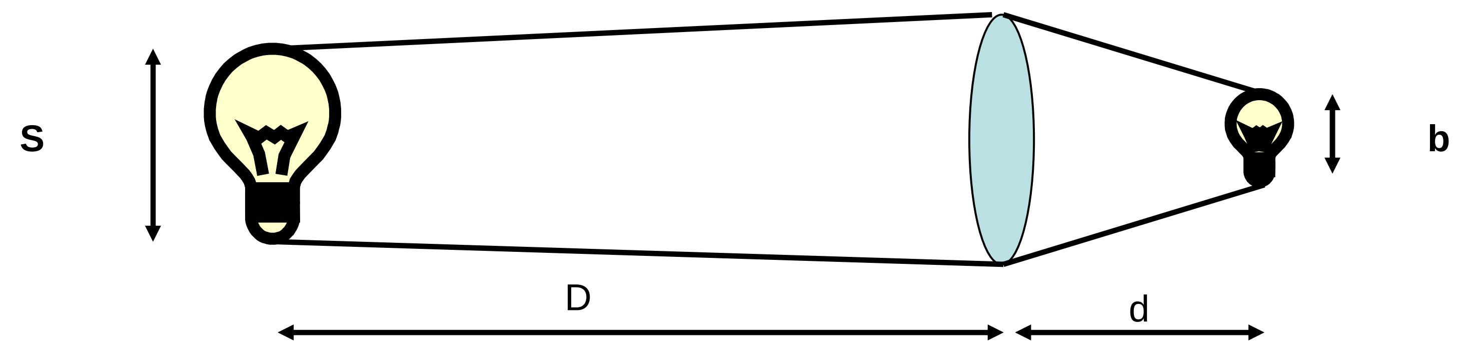
- $\mathbf{I\ (HKL)} \sim \mathbf{I_{beam}}\ (\mathbf{V_{crystal}\ /\ V_{cell}})$ x (some factors) x $|F(HKL)|^2$

- If the crystal size is small, if the unit cell is large, we need to increase dramatically the number of incoming photons.

- ➔ we need a small and intense X-ray beam …

How to achieve a small and intense beam?

How to achieve a small and intense beam?



b = S x (d / D)

D = source to focusing element distance, d = focusing element to focus point (sample) distance

S = source size, b = beam size

- **reduce S** (property of the electron beam …),
- **increase the "source to focusing element distance"** (more sensitive to the angular variation of the incoming beam),
- **reduce the "focusing element to focus point (sample) distance "** (increase of the divergence)

The quality of the focusing device has a contribution to the beam shape.

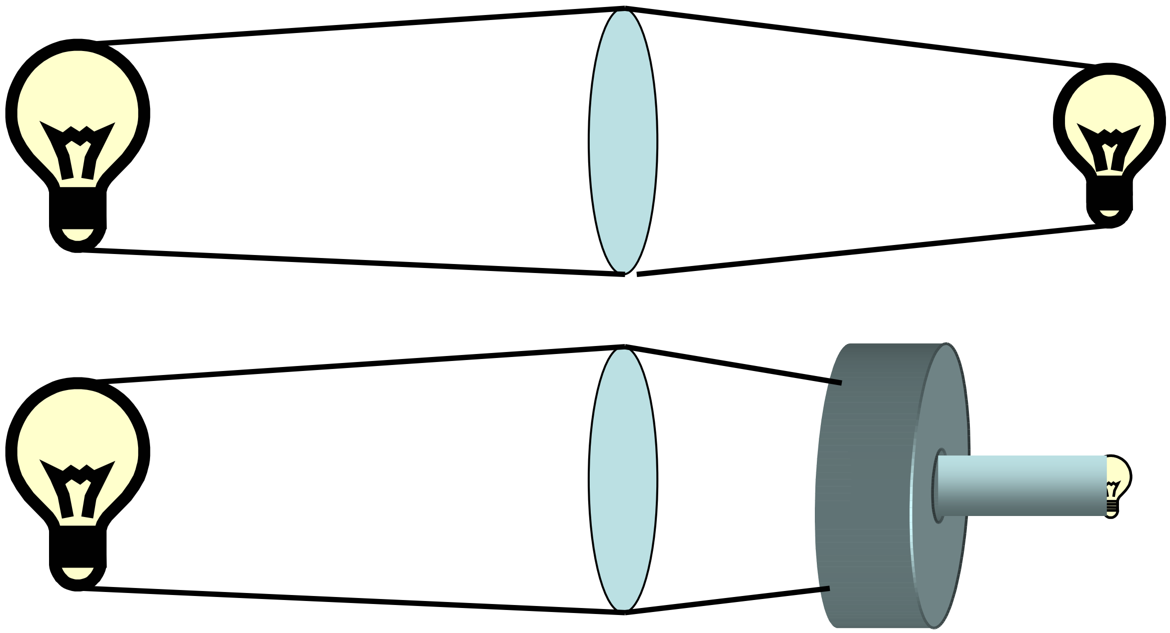
If we used a mirror, it is the mirror surface (“roughness”) and the mirror shape (“slope error”) which have a contribution to the beam shape.

2 approaches (at the ESRF):

- a moderately focussed beam (~50 µm Ø) + beam defining aperture

- 2 smaller flat mirrors mechanically bended.

1st approach : ID29 (MD2).

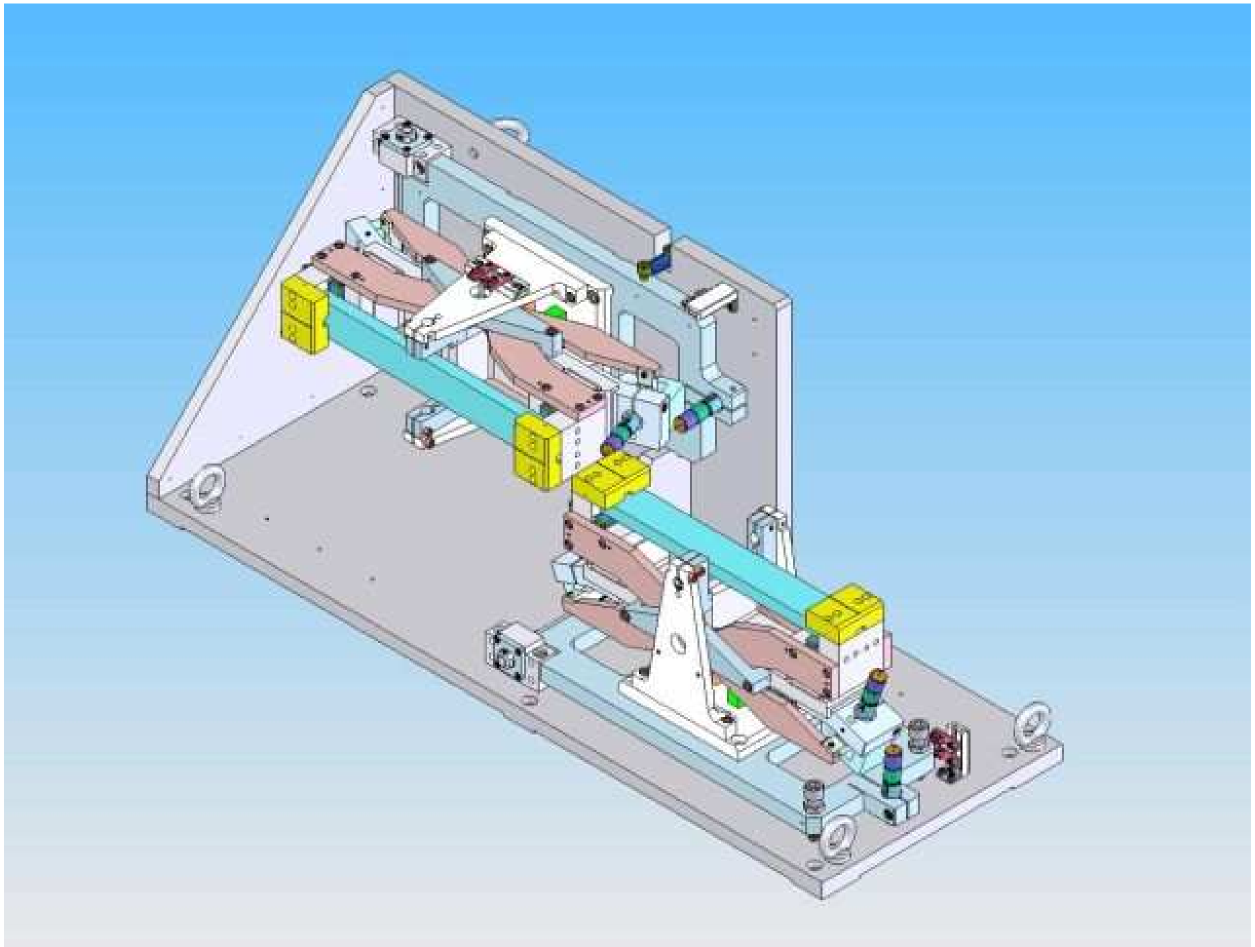


-Full beam

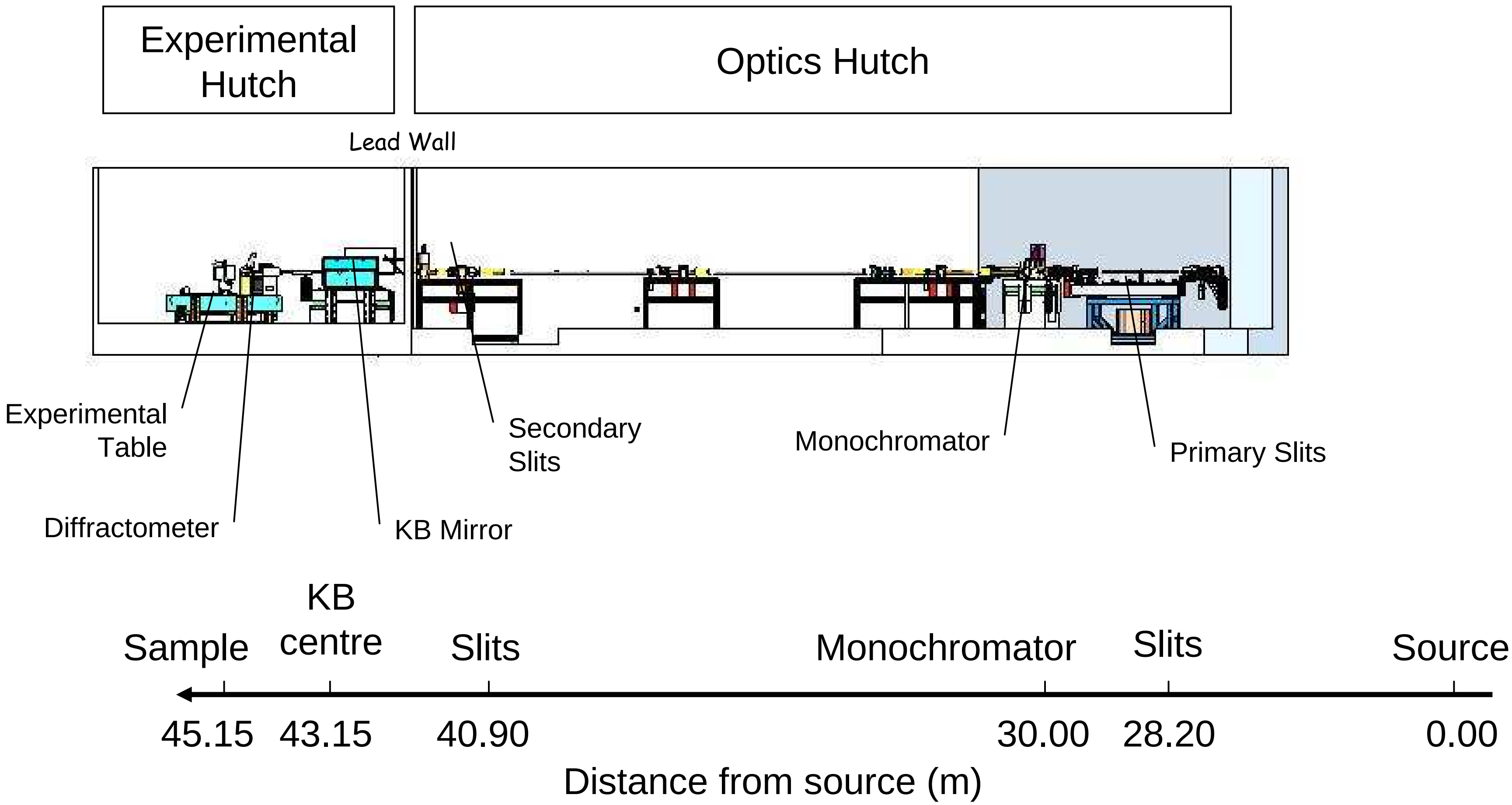
- 50 µm Ø beam defining aperture

-15 µm Ø beam defining aperture

2nd approach : ID23-2 (KB system).

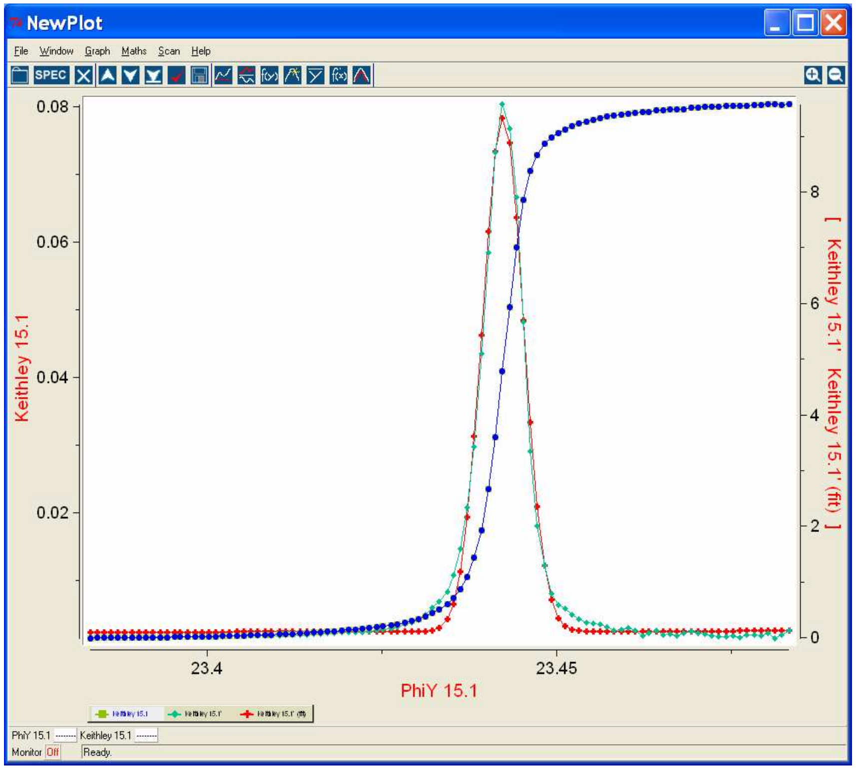


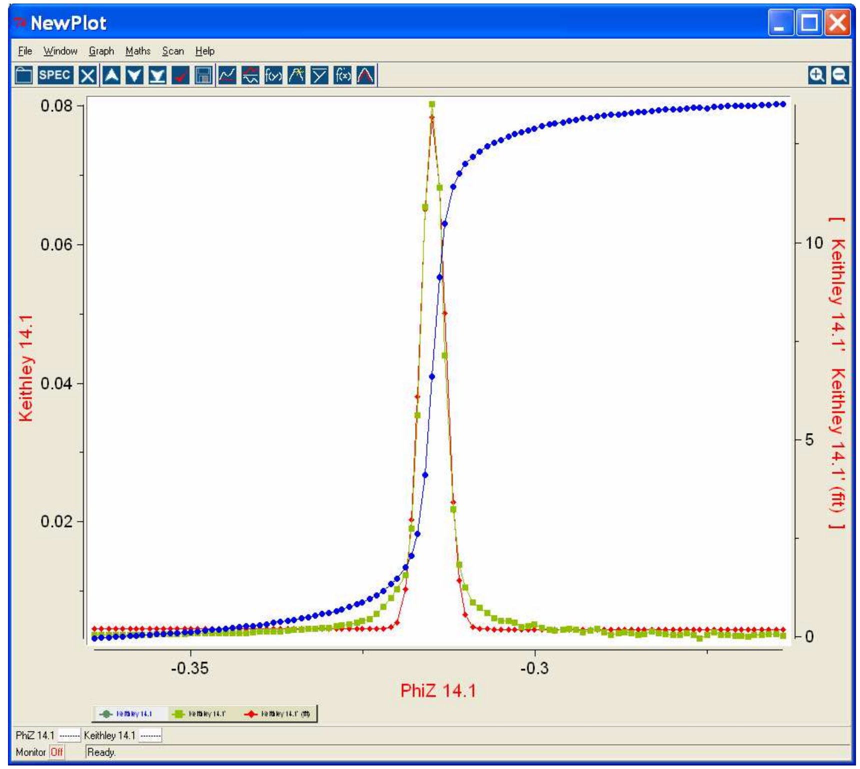
ID23-2



February 2010

Horizontal beamsize: ~ 7 μm (FWHM)
Vertical Beamsize: < 5 μm (FWHM)



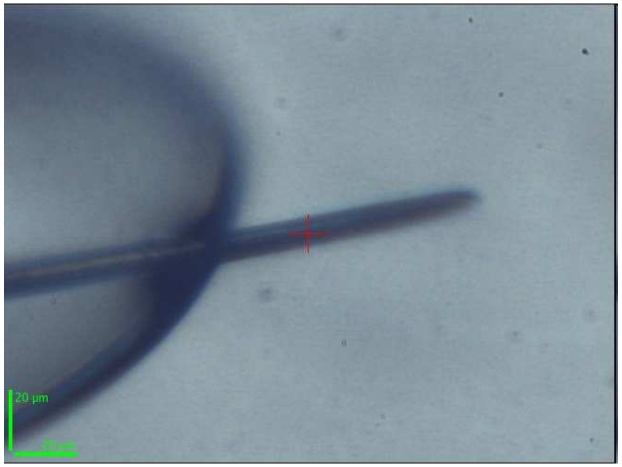


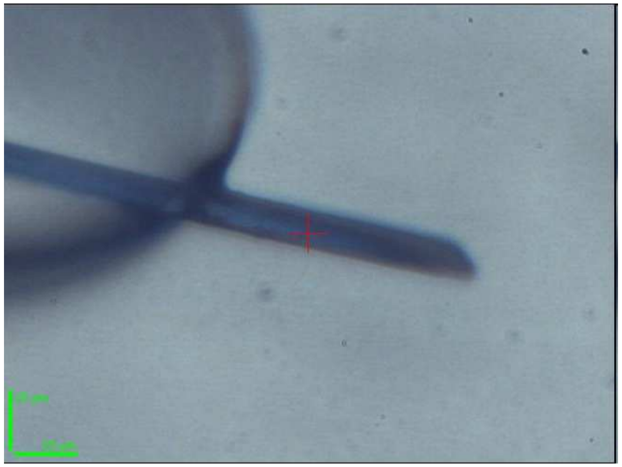
Measurement : absorption scan with a W wire (200 μm diameter), 1 μm step

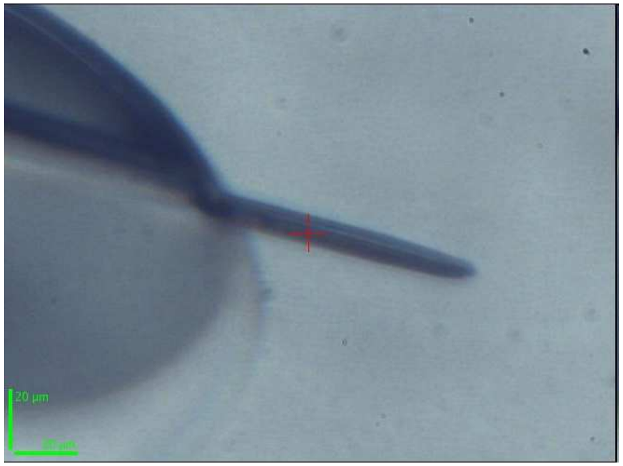
Crystal centring

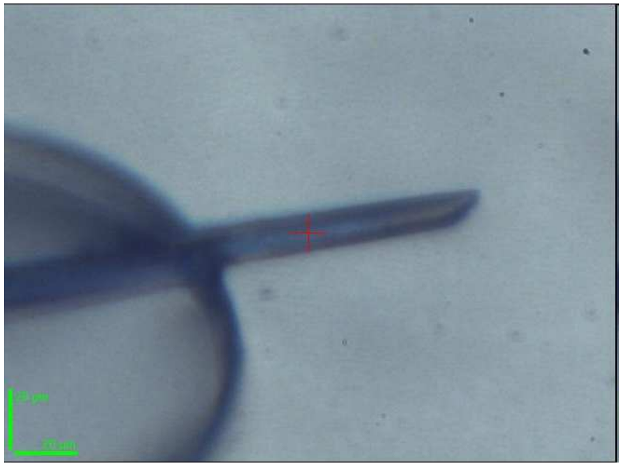
- Very important (but not always necessary…),
- aim: the crystal should rotate around itself (and not around the beam…),
- Problem: we need to see the crystal (importance of the sample preparation),
- Tactic: align the loop “edge on”, move back by 90° and start the “3 click procedure”:
  - First click: face on (good view),
  - Second click: middle of the loop, on the same vertical line,
  - third click: face on again, on the same vertical line.

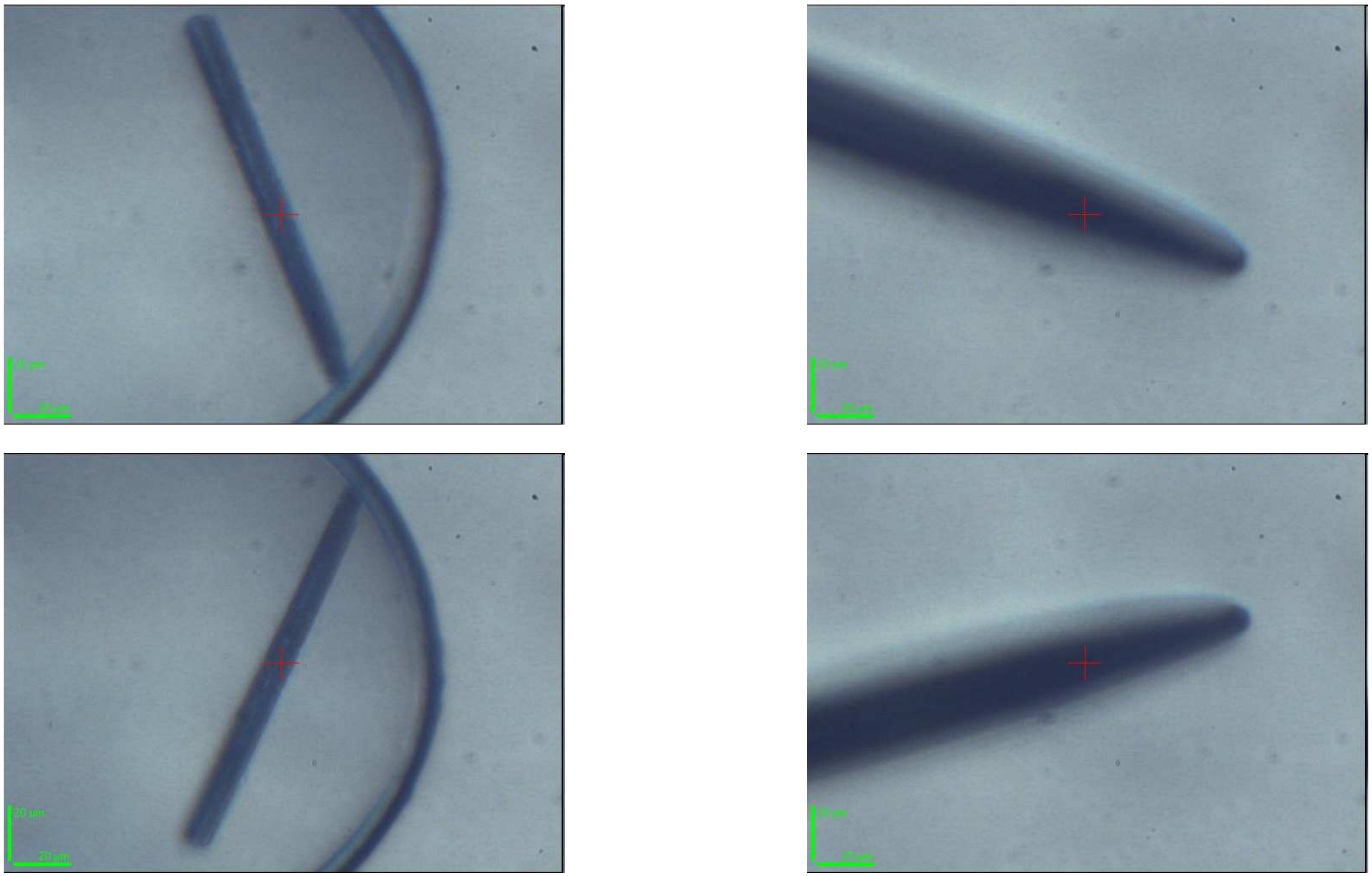
Easy case

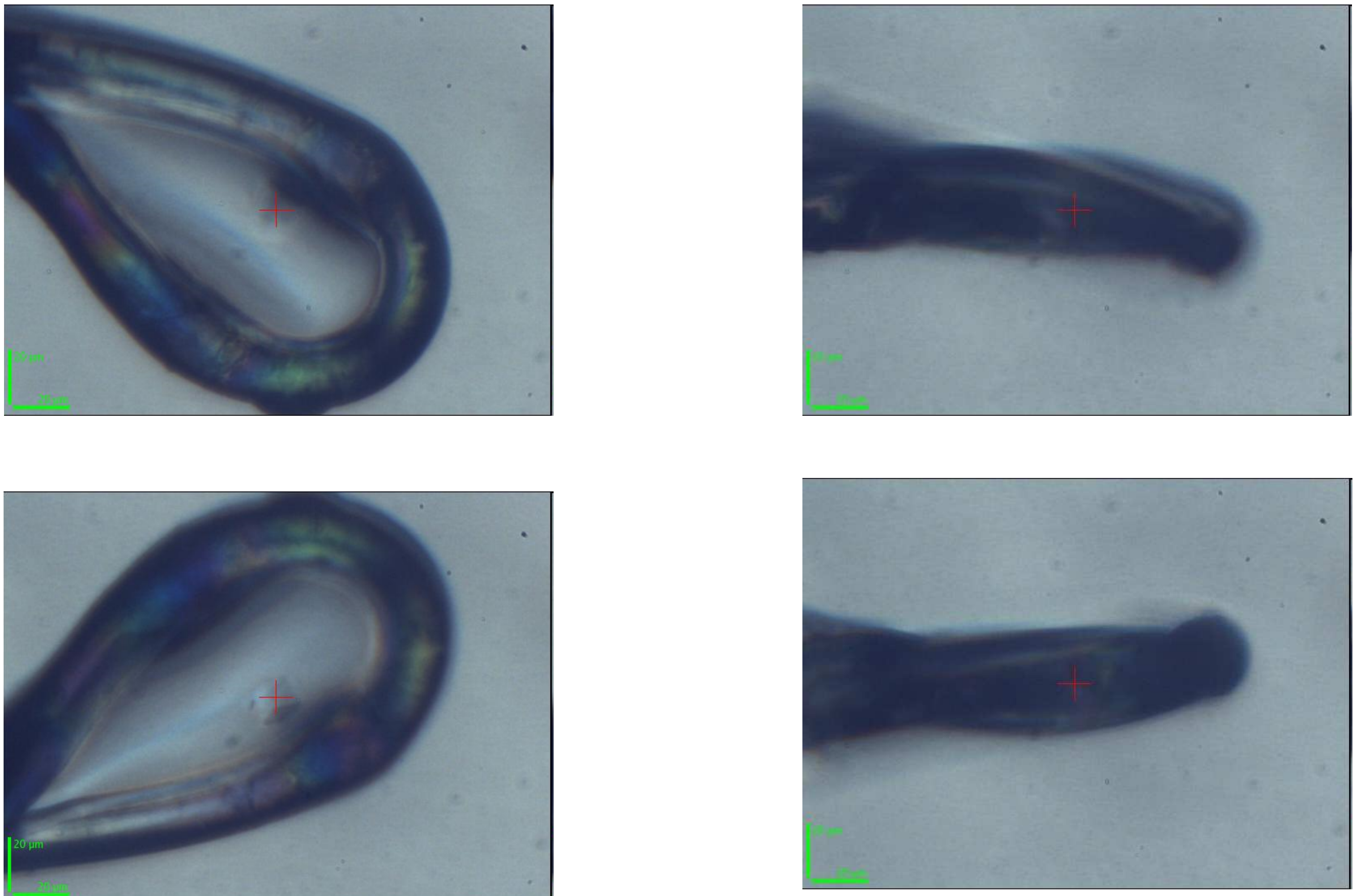


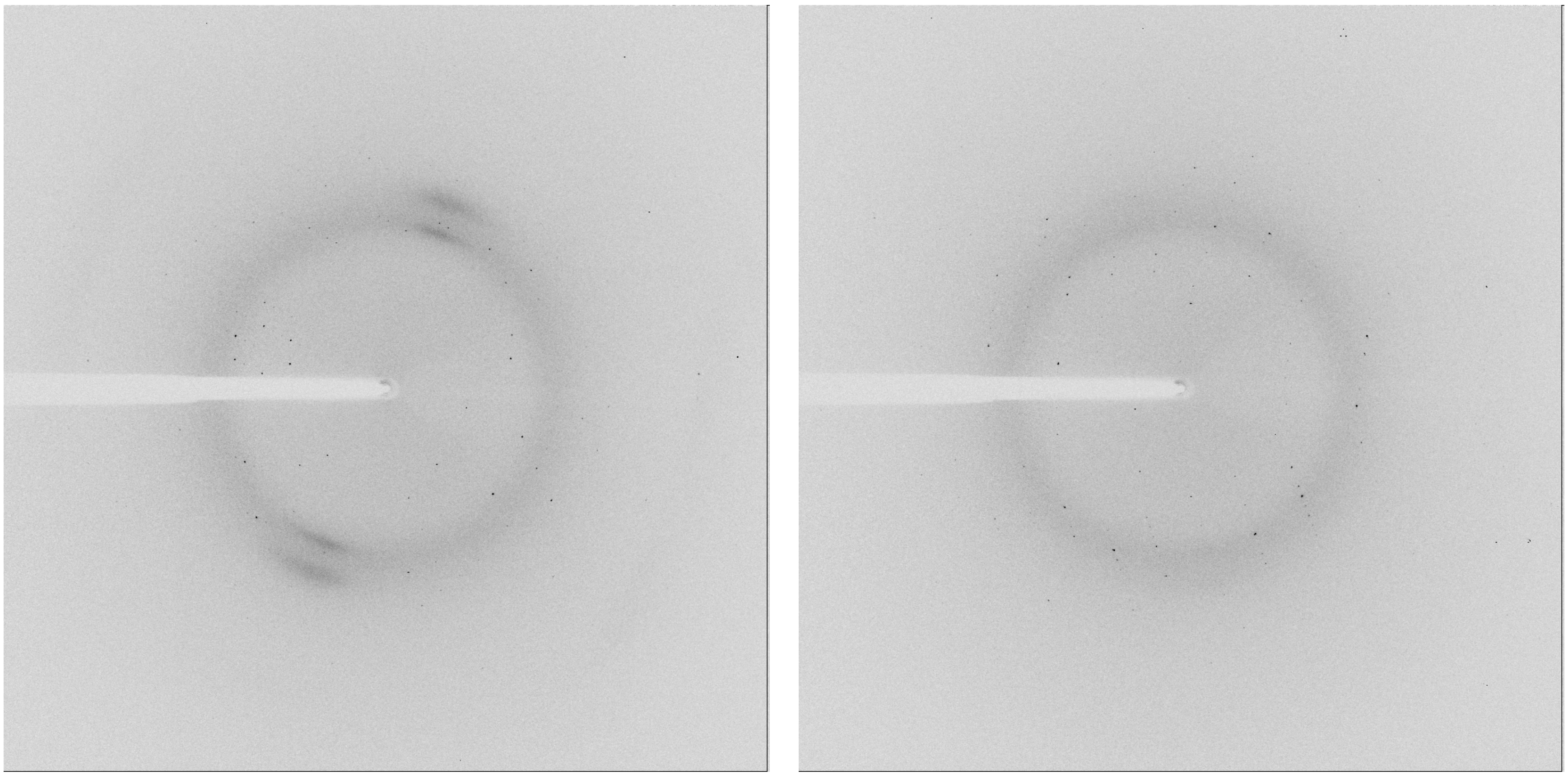




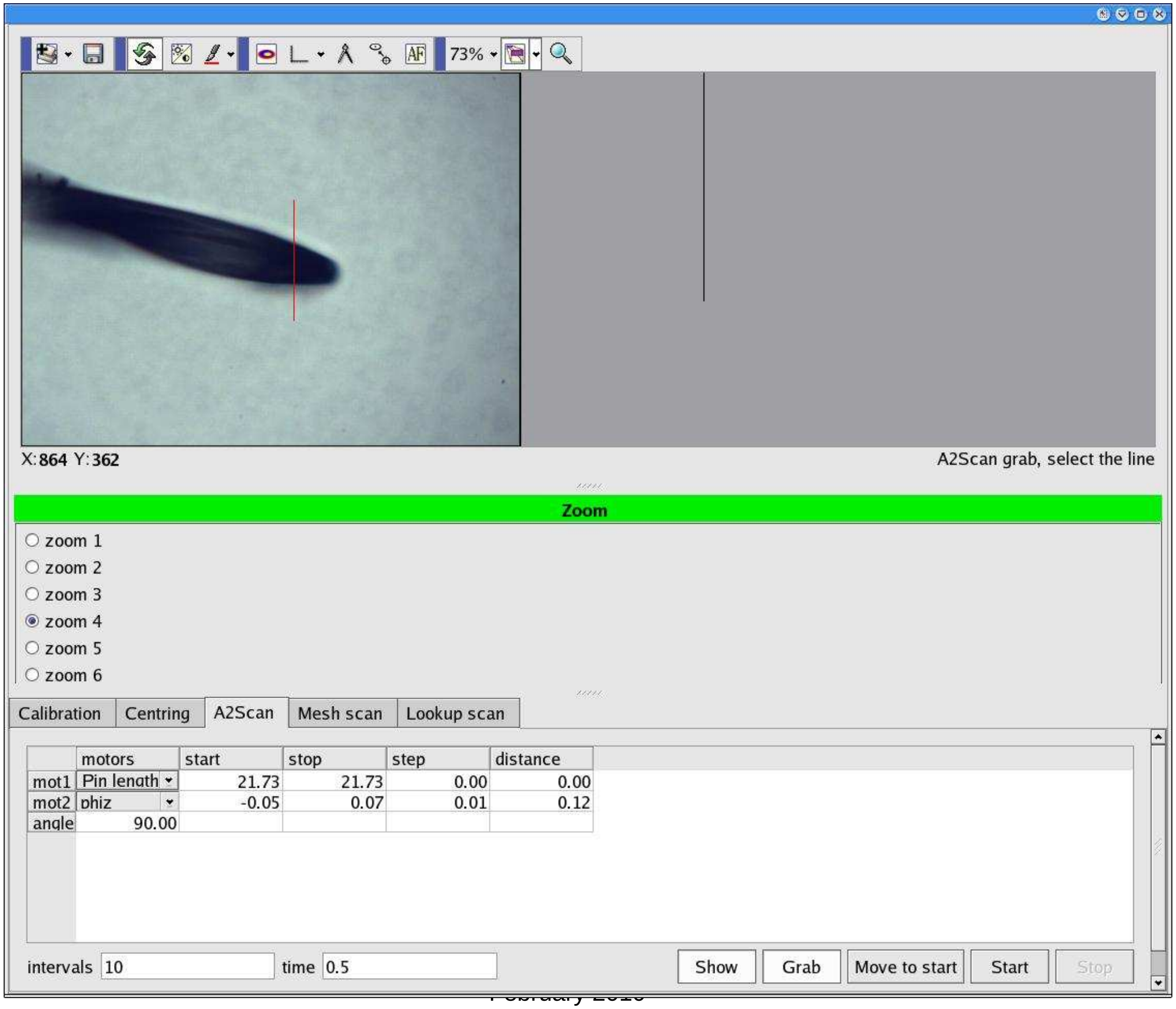





20 µm
20 µm

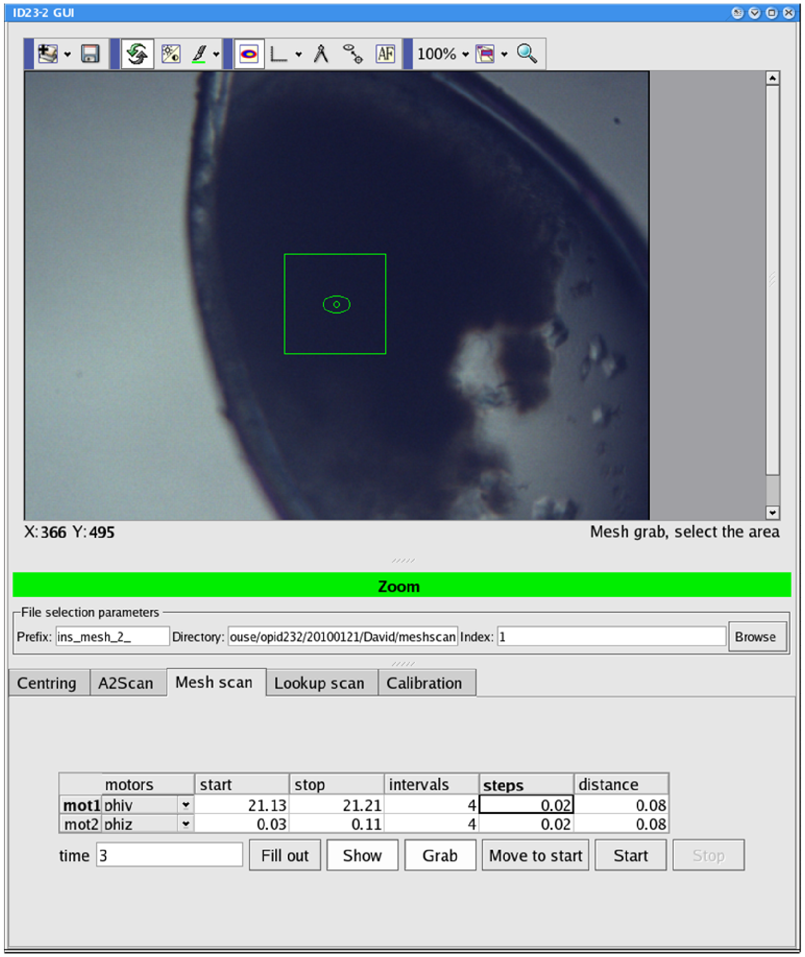


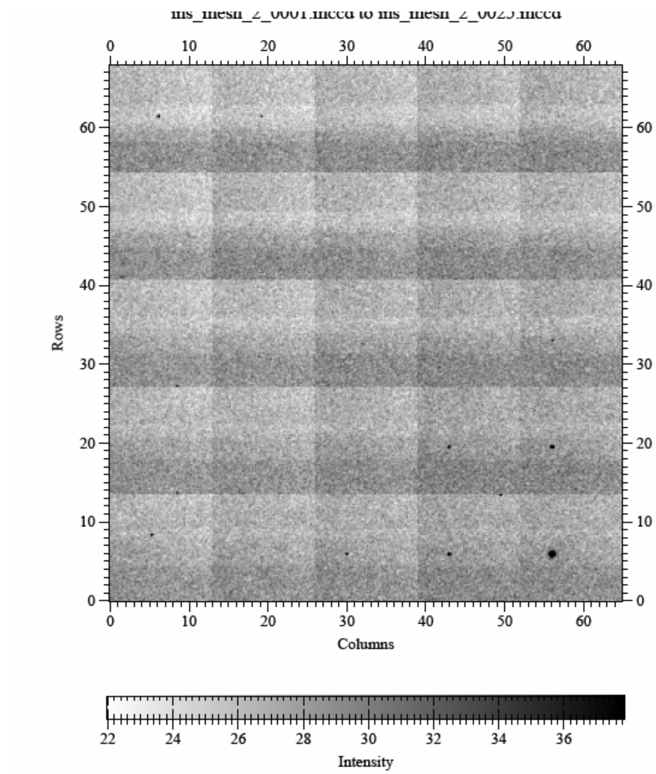
Line scan



73%
X: 864 Y: 362
A2Scan grab, select the line
Zoom
zoom 1
zoom 2
zoom 3
zoom 4
zoom 5
zoom 6
Calibration
Centring
A2Scan
Mesh scan
Lookup scan
motors
start
stop
step
distance
mot1 Pin length 21.73 21.73 0.00 0.00
mot2 phiz -0.05 0.07 0.01 0.12
angle 90.00
intervals 10
time 0.5
Show
Grab
Move to start
Start
Stop

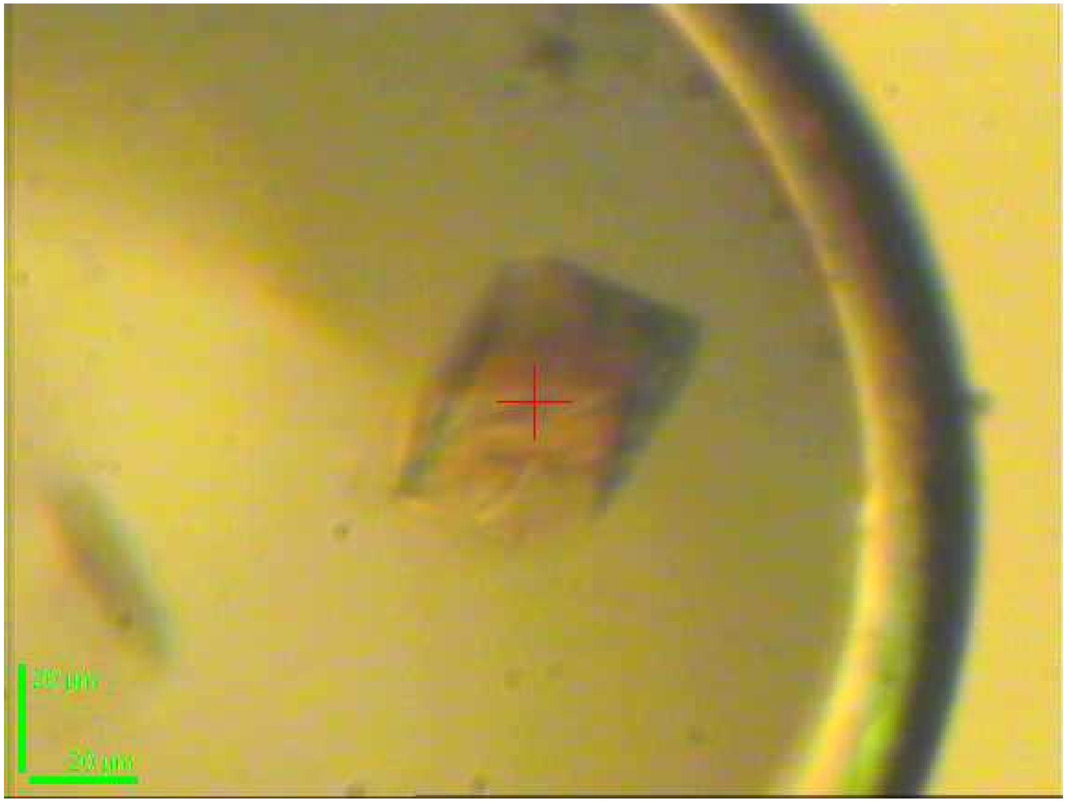
Mesh scan for crystal search





5 x 5 , 80 by 80 µm² grid

1 exemple : the use of the spatial resolution provided by a small beam (Carlo Petosa, IBS-Grenoble)



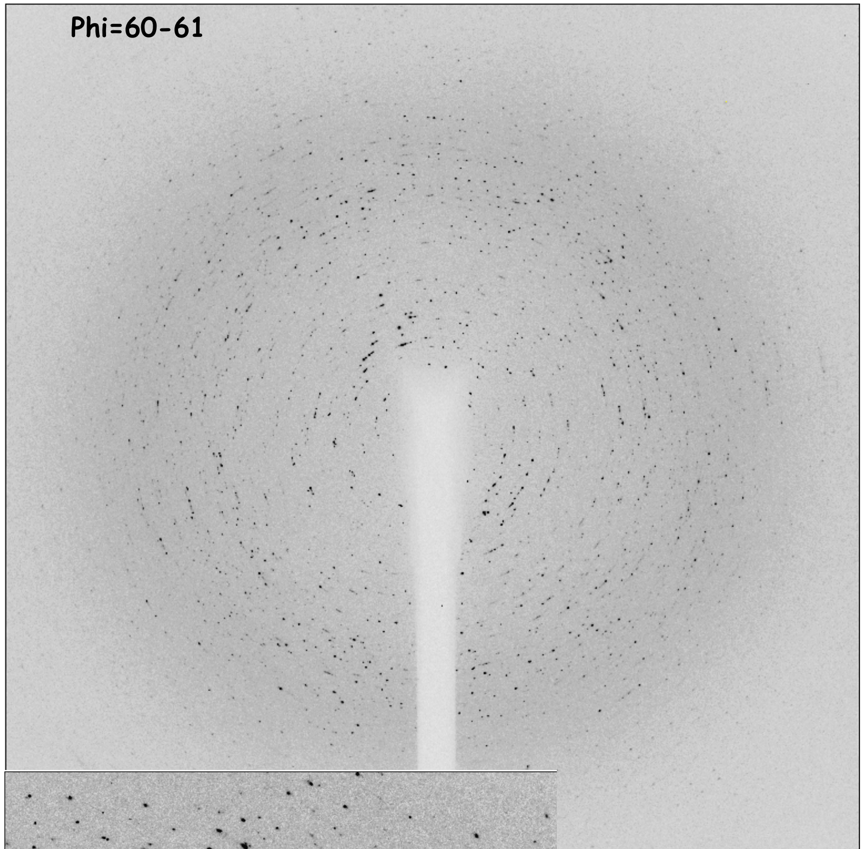
Sample: domain BD1 (res.17-136) + 20-mer peptide

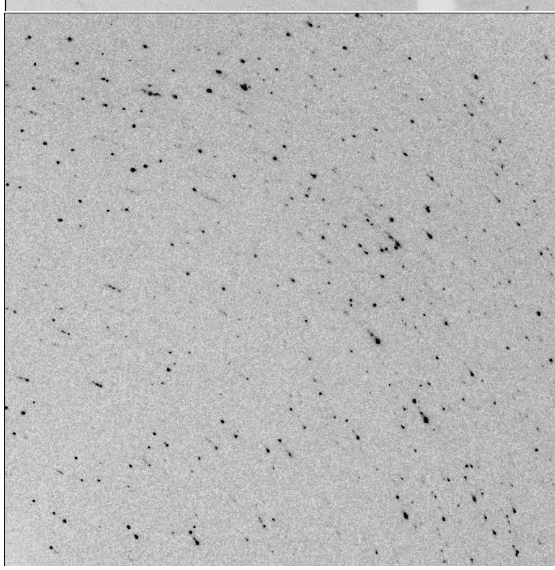
Crystals grown from robot screen.

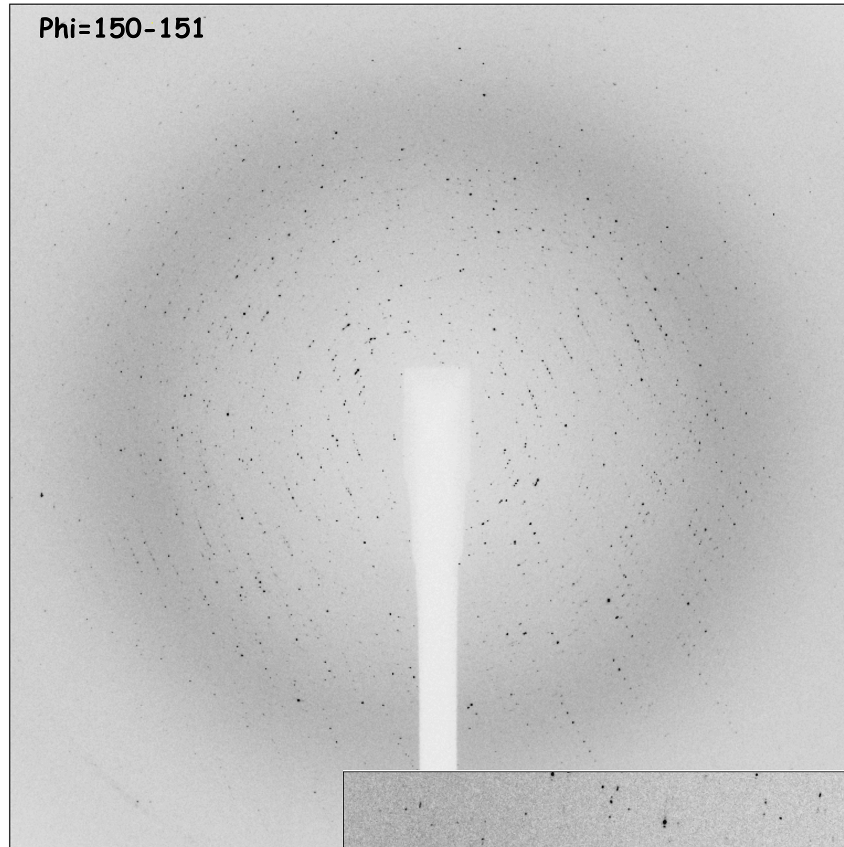
Crystal (~30x30 μm²) cryoprotected in 30% glycerol

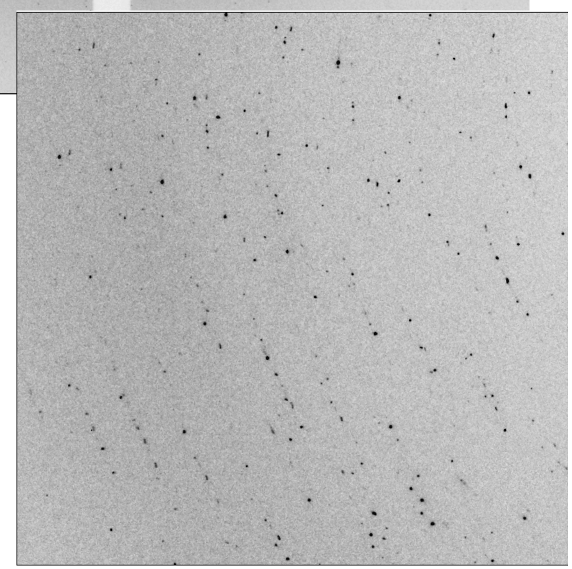
Data Collection: 2/3 bunch 160 mA

Images obtained by shooting centre of xtal







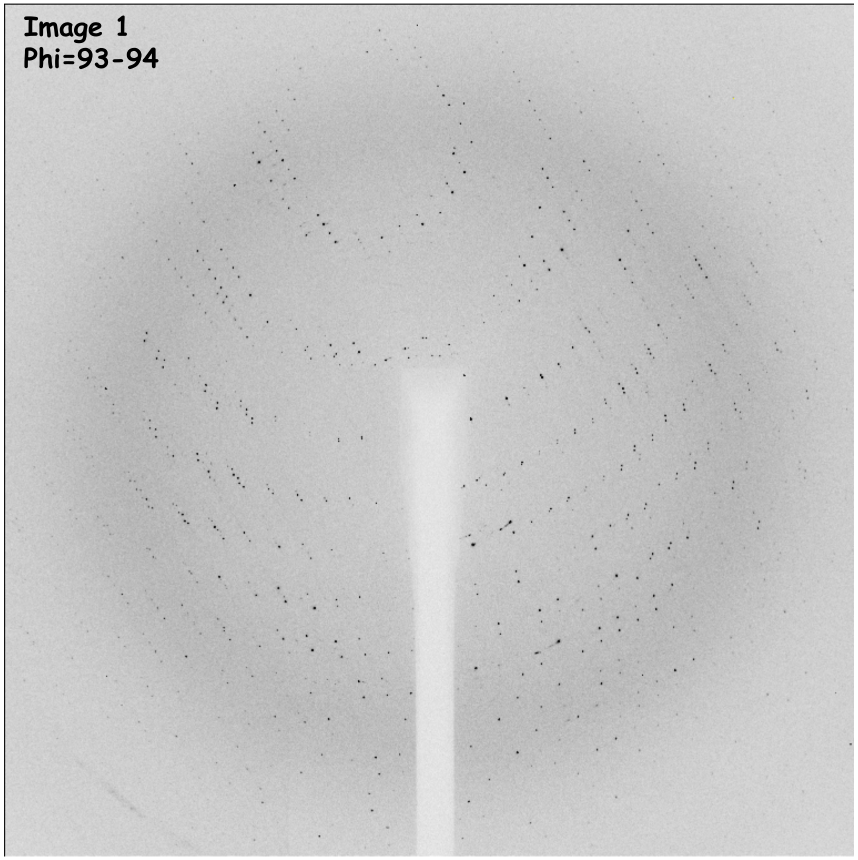


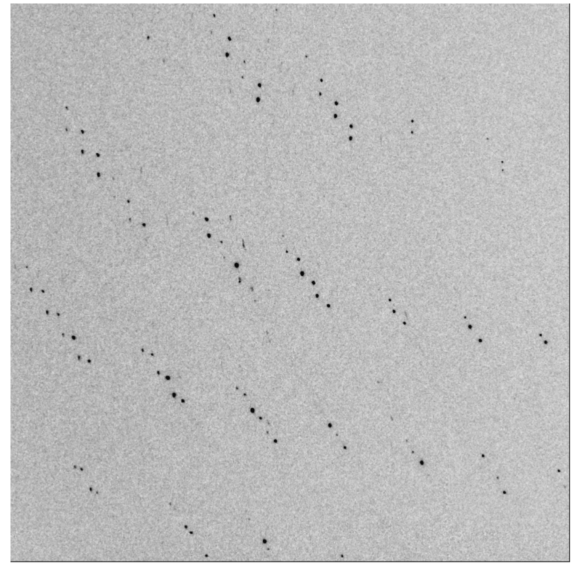
too many spots!

Exposure: 4 sec/image
10% transmission

David Flot, ESRF MX School 2010, 9th of February 2010

Images obtained by shooting left tip of crystal (volume 1)



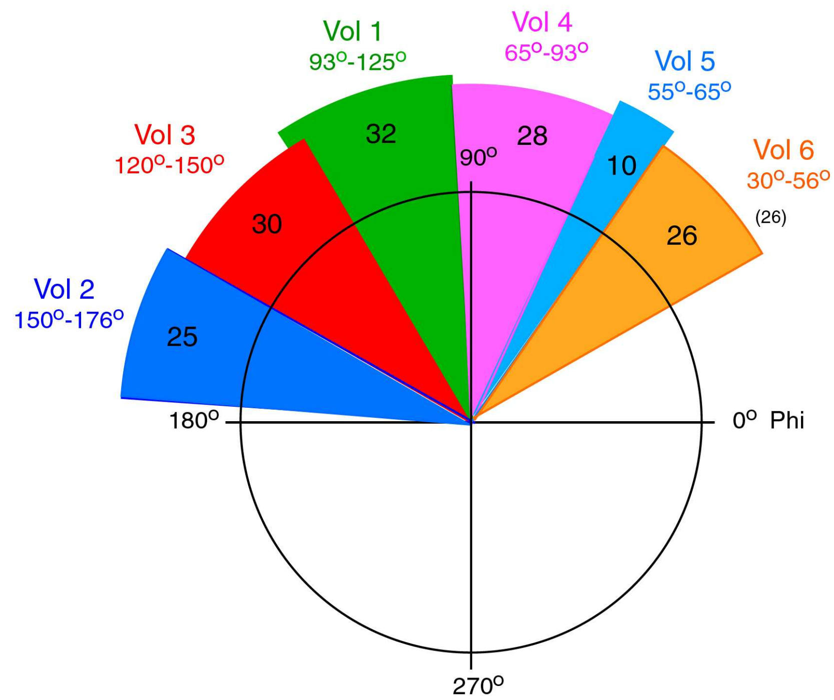


Nice-looking spots visible to 2.3

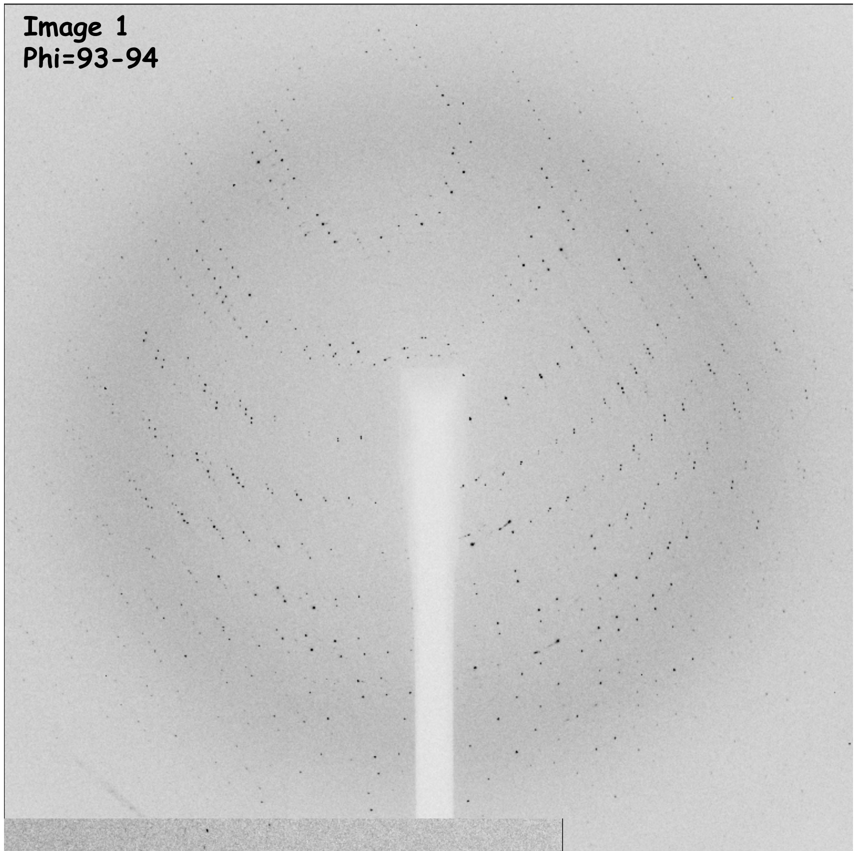
One major (and one minor) lattice visible

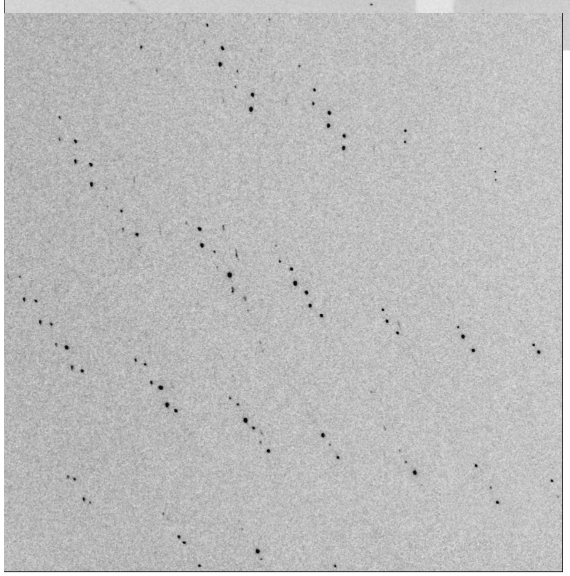
Strategy:

Shoot 6 different corners/edges of crystal
Collect 10-30 images per volume
Find combination of images giving best data

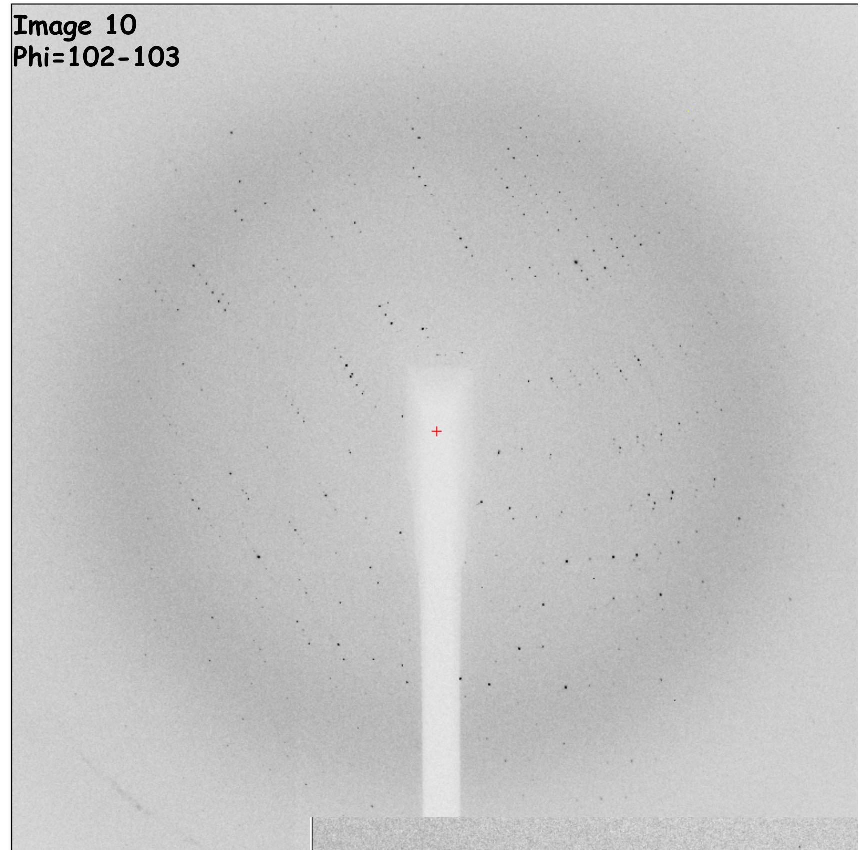


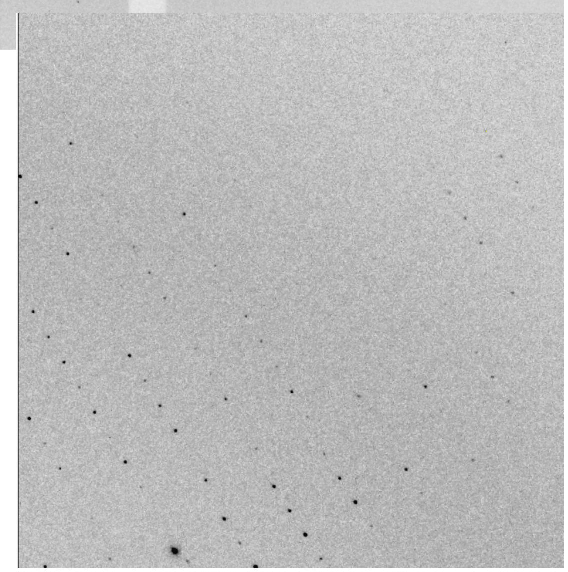
Radiation Damage





spots visible to 2.3





spots visible to 2.6

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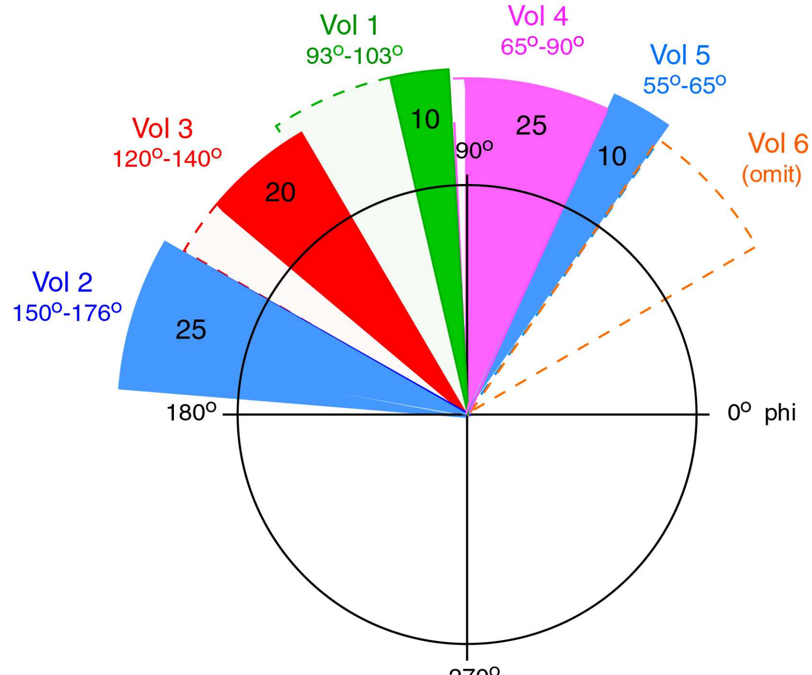
Best 90 Images

Completeness: 96.7%

Rfactor : 14.3%

I/Sig (2.4-2.5A): 2.8



XSCALE statistics

RESOLUTION LIMIT	NUMBER OF REFLECTIONS OBSERVED	UNIQUE	POSSIBLE	COMPLETENESS OF DATA	R-FACTOR observed	R-FACTOR expected	COMPARED	I/SIGMA	R-meas	Rmrgd-F	Mult
20.00	46	20	29	69.0%	11.8%	8.9%	46	5.14	14.2%	25.3	2.300
12.00	231	83	93	89.2%	6.0%	7.2%	231	13.16	7.2%	4.5	2.783
8.00	697	245	259	94.6%	7.1%	7.4%	697	14.39	8.4%	5.2	2.845
6.00	1403	456	479	95.2%	9.1%	8.9%	1403	11.89	10.7%	7.1	3.077
5.00	1818	560	579	96.7%	10.2%	9.5%	1818	11.70	12.0%	7.7	3.246
4.50	1604	504	515	97.9%	9.6%	9.2%	1604	12.44	11.3%	7.2	3.183
4.00	2586	770	787	97.8%	9.1%	9.2%	2586	12.53	10.7%	6.9	3.358
3.50	4321	1279	1290	99.1%	10.5%	10.5%	4321	10.50	12.4%	9.4	3.378
3.20	4138	1196	1205	99.3%	13.1%	13.7%	4138	8.18	15.5%	12.9	3.460
3.00	3785	1089	1096	99.4%	17.7%	17.9%	3785	6.49	21.0%	18.6	3.476
2.80	4831	1379	1385	99.6%	21.5%	22.2%	4831	5.39	25.5%	23.9	3.503
2.60	6430	1851	1860	99.5%	29.3%	29.8%	6430	4.19	34.8%	34.1	3.474
2.50	4065	1163	1175	99.0%	37.7%	37.2%	4065	3.48	44.9%	48.6	3.495
2.40	3477	1266	1363	92.9%	41.2%	40.5%	3477	2.82	49.7%	51.4	2.746
2.37	439	281	444	63.3%	41.2%	41.2%	439	2.31	51.5%	51.0	1.562
total	**39871**	**12142**	**12559**	**96.7%**	**14.3%**	**14.3%**	**39871**	**7.11**	**16.9%**	**18.4**	**3.284**

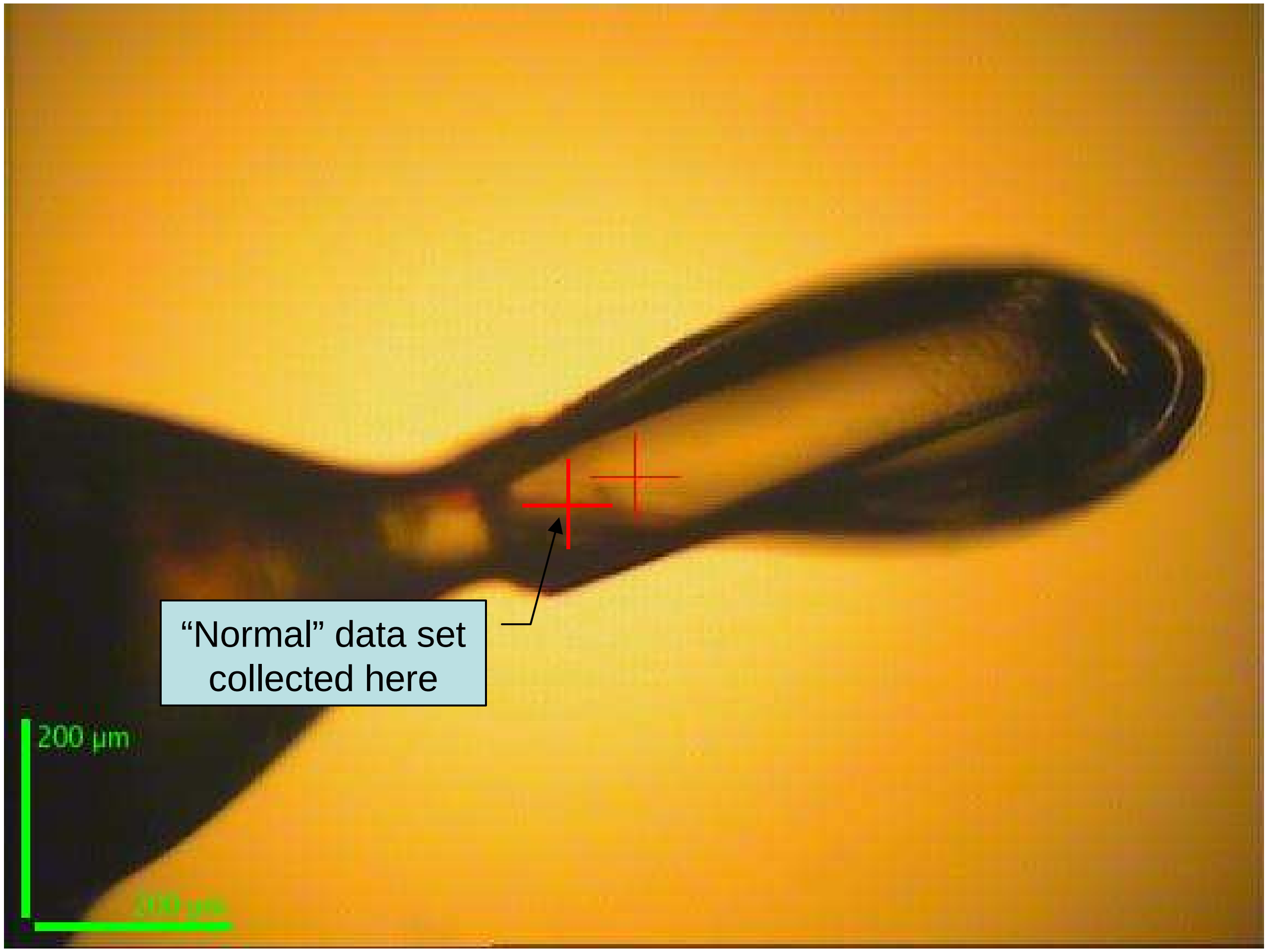
- Crystal quality not the same along the crystal,
- A beam smaller than the crystal allows to choose the best place and / or to collect several small datasets,
- “hot beam”: the beam damage is important (no more that 30s total exposure time per point) (*but difficult to predict if the cross section between the crystal and the beam change*).

Small beam / large crystal

- **One crystal**, **multiple datasets** : available through mxCuBE on all the stations.

- **One crystal**, **one dataset**, **data collection between two centred positions**: available on request (will be implemented on a GUI soon).

FAE crystal



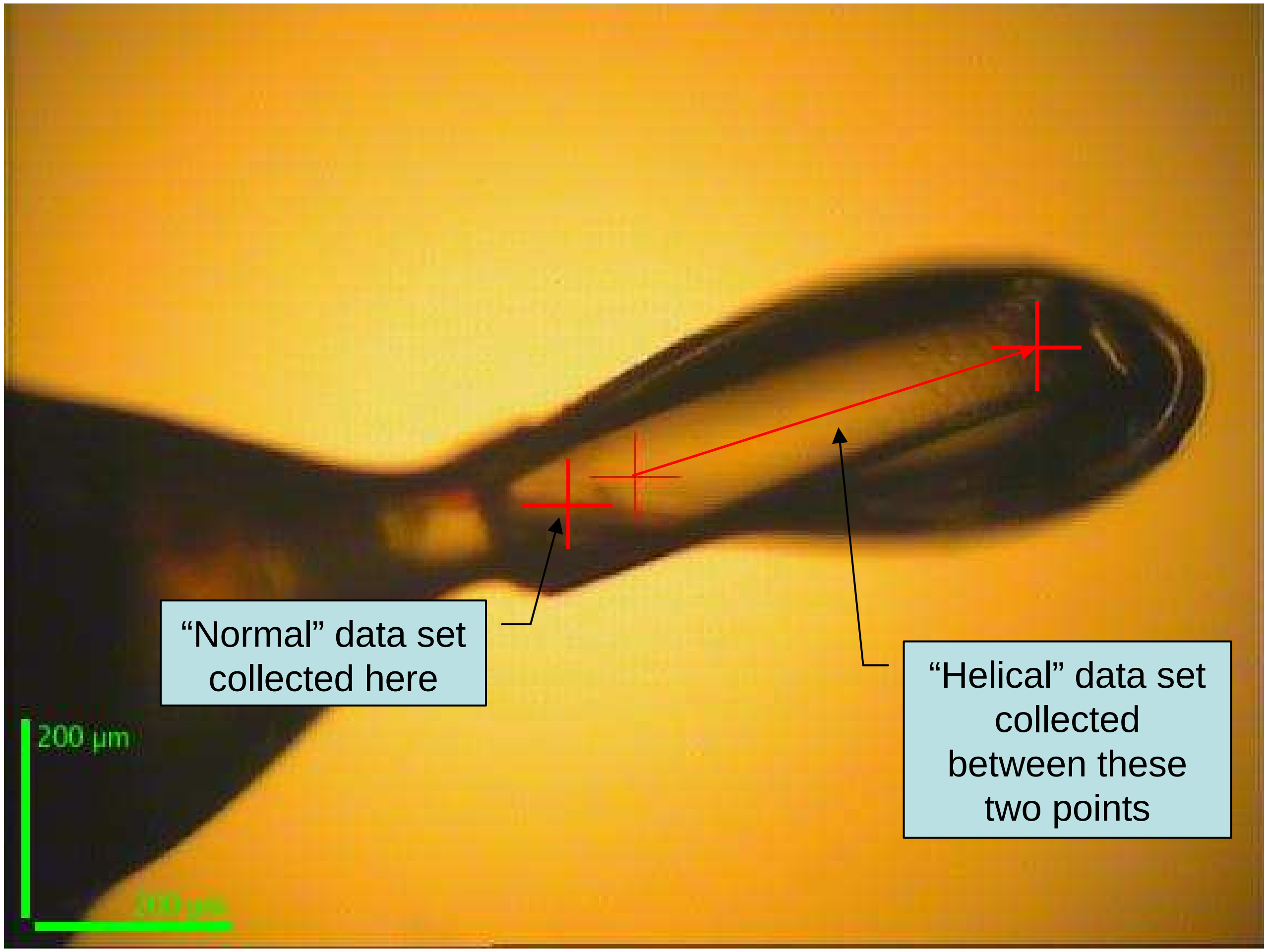
Statistics fron XDS (Correct step)

SUBSET OF INTENSITY DATA WITH SIGNAL/NOISE >= -3.0 AS FUNCTION OF RESOLUTION

RESOLUTION LIMIT	NUMBER OF REFLECTIONS OBSERVED	UNIQUE	POSSIBLE	COMPLETENESS OF DATA	R-FACTOR observed	R-FACTOR expected	COMPARED	I/SIGMA	R-meas	Rmrgd-F	Anomal Corr	SigAno	Nano
5.07	36929	6490	6526	99.4%	2.9%	3.5%	36928	43.82	3.2%	1.6%	90%	3.133	2853
3.61	67454	11654	11654	100.0%	3.1%	3.6%	67454	42.72	3.4%	1.7%	78%	2.172	5425
2.95	87304	15019	15019	100.0%	3.7%	4.1%	87304	36.28	4.1%	2.2%	73%	2.067	7113
2.56	103798	17801	17802	100.0%	4.9%	5.1%	103798	28.66	5.3%	3.1%	69%	1.926	8502
2.29	117267	20173	20173	100.0%	6.1%	6.3%	117267	24.02	6.7%	4.1%	59%	1.637	9687
2.09	128809	22249	22249	100.0%	7.7%	7.7%	128809	19.93	8.5%	5.4%	49%	1.418	10722
1.94	139178	24169	24169	100.0%	10.8%	10.7%	139178	15.15	11.9%	7.8%	38%	1.245	11689
1.81	149092	26055	26055	100.0%	16.0%	16.0%	149092	10.73	17.6%	11.8%	26%	1.075	12622
1.71	106265	25577	27669	92.4%	23.2%	23.4%	103797	6.12	26.3%	22.6%	14%	0.927	10900
total	936096	169187	171316	98.8%	5.6%	5.9%	933627	21.09	6.1%	5.6%	53%	1.523	79513

First data set: normal data collection , 270 degrees, **1 s**, 1 degree per image

FAE crystal



First data set: normal data collection , 270 degrees, **1 s** 1 degree per image
Second dateset: "helical" data collection, **1.5 s** exposure time per image

Statistics fron XDS (Correct step)

SUBSET OF INTENSITY DATA WITH SIGNAL/NOISE >= -3.0 AS FUNCTION OF RESOLUTION

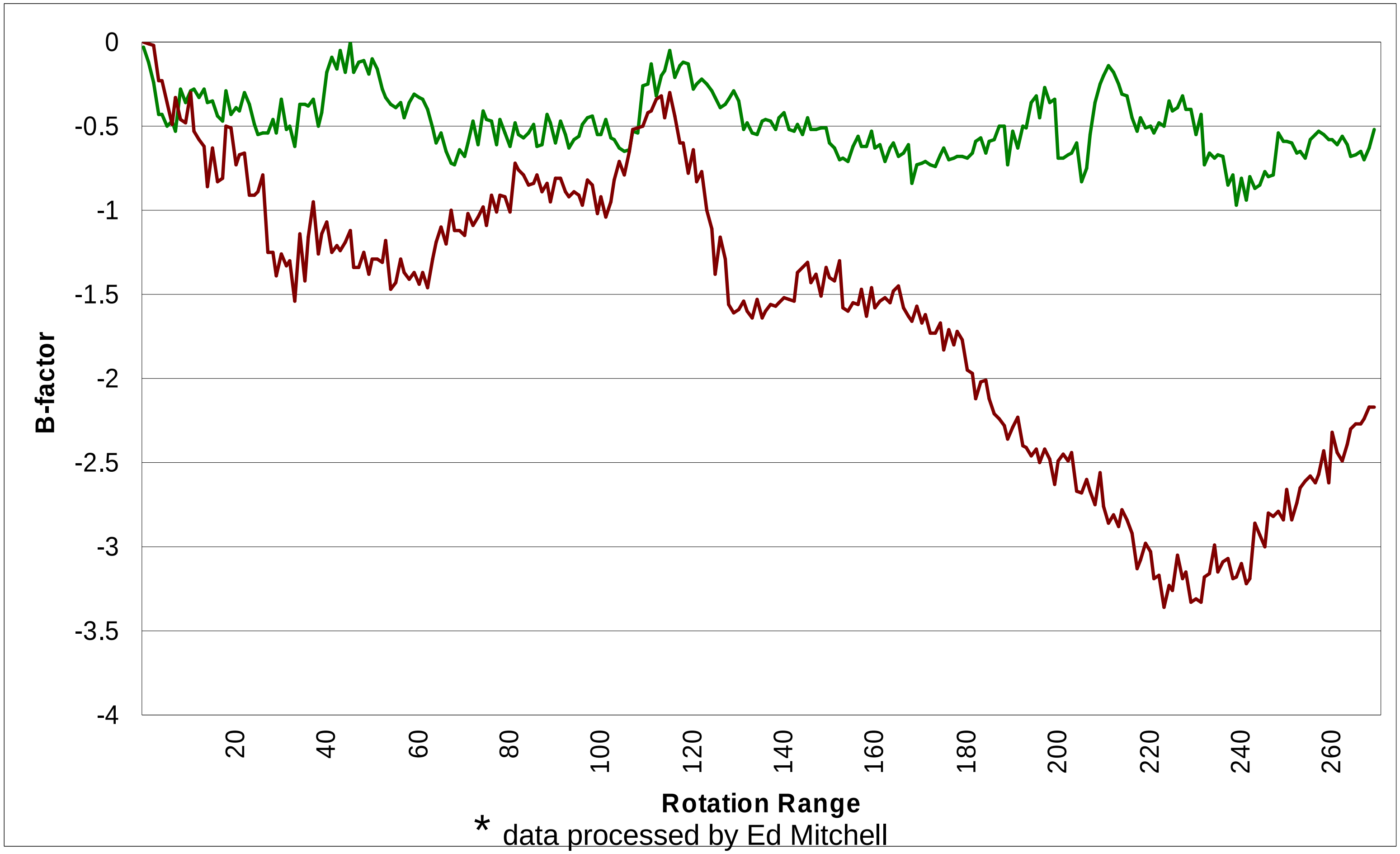
RESOLUTION LIMIT	NUMBER OF REFLECTIONS OBSERVED	UNIQUE	POSSIBLE	COMPLETENESS OF DATA	R-FACTOR observed	R-FACTOR expected	COMPARED	I/SIGMA	R-meas	Rmrgd-F	Anomal Corr	SigAno	Nano
5.07	36929	6490	6526	99.4%	2.9%	3.5%	36928	43.82	3.2%	1.6%	90%	3.133	2853
3.61	67454	11654	11654	100.0%	3.1%	3.6%	67454	42.72	3.4%	1.7%	78%	2.172	5425
2.95	87304	15019	15019	100.0%	3.7%	4.1%	87304	36.28	4.1%	2.2%	73%	2.067	7113
2.56	103798	17801	17802	100.0%	4.9%	5.1%	103798	28.66	5.3%	3.1%	69%	1.926	8502
2.29	117267	20173	20173	100.0%	6.1%	6.3%	117267	24.02	6.7%	4.1%	59%	1.637	9687
2.09	128809	22249	22249	100.0%	7.7%	7.7%	128809	19.93	8.5%	5.4%	49%	1.418	10722
1.94	139178	24169	24169	100.0%	10.8%	10.7%	139178	15.15	11.9%	7.8%	38%	1.245	11689
1.81	149092	26055	26055	100.0%	16.0%	16.0%	149092	10.73	17.6%	11.8%	26%	1.075	12622
1.71	106265	25577	27669	92.4%	23.2%	23.4%	103797	6.12	26.3%	22.6%	14%	0.927	10900
total	936096	169187	171316	98.8%	5.6%	5.9%	933627	21.09	6.1%	5.6%	53%	1.523	79513

SUBSET OF INTENSITY DATA WITH SIGNAL/NOISE >= -3.0 AS FUNCTION OF RESOLUTION

RESOLUTION LIMIT	NUMBER OF REFLECTIONS OBSERVED	UNIQUE	POSSIBLE	COMPLETENESS OF DATA	R-FACTOR observed	R-FACTOR expected	COMPARED	I/SIGMA	R-meas	Rmrgd-F	Anomal Corr	SigAno	Nano
5.09	48730	6401	6439	99.4%	2.0%	2.5%	48727	74.62	2.2%	0.9%	97%	5.649	2814
3.61	88981	11607	11607	100.0%	2.2%	2.6%	88981	72.69	2.4%	1.0%	93%	3.836	5403
2.95	115505	15025	15025	100.0%	2.6%	2.8%	115505	63.40	2.8%	1.2%	91%	3.700	7114
2.56	137168	17774	17774	100.0%	3.1%	3.3%	137168	52.18	3.4%	1.6%	89%	3.498	8489
2.29	155443	20164	20164	100.0%	3.7%	3.8%	155443	45.10	4.0%	2.0%	84%	2.963	9680
2.09	171089	22225	22225	100.0%	4.5%	4.5%	171089	38.57	4.8%	2.5%	79%	2.534	10710
1.93	185579	24196	24196	100.0%	5.8%	5.9%	185579	30.71	6.3%	3.4%	72%	2.139	11703
1.81	198774	26041	26041	100.0%	7.9%	8.0%	198774	23.18	8.5%	4.7%	63%	1.762	12616
1.71	141547	25989	27679	93.9%	10.6%	11.0%	139734	13.94	11.7%	8.6%	47%	1.334	11683
total	1242816	169422	171150	99.0%	3.5%	3.7%	1241000	39.41	3.7%	2.6%	79%	2.634	80212

First data set: normal data collection , 270 degrees, 1 s 1 degree per image
Seconda dateset: helical data collection, 1.5 s exposure time per image

B factor from mosflm/scala *



But…

- Crystal centring…
- Crystal homogeneity…
- Cell parameters…

Good practices with a small beam

1. Use a loop which matches the crystal size,
2. Minimise the amount of cryoprotectant, use one which is not too viscous,
3. Come to the station with a diffraction plan,
4. Do a quick centring, move the crystal to the beam, collect one diffraction pattern,
5. Centre the crystal properly if the diffraction quality is good enough,
6. Be care full with **radiation damage** when collecting (no more that 30s total exposure time per point) (*but difficult to predict if the cross section between the crystal and the beam change*),
7. Check the data quality when collecting,
8. When collecting small datasets, use “overlap” between the datasets.

Summary

- Possible to collect data on “**desesperate” subjects** (balance between more time in the wet lab or more time in the beam line / computer room),
- Small crystals can be **less** affected by **twinning,**
- **Special shapes like needle** like crystals can profit from a small beam,
- **Micro-volumes** of larger crystals can be **better ordered** than the **macrocrystal** (due to twinning, high mosaicity, etc…).

Thank You
for your attention!