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)
• \( I(\text{HKL}) \sim I_{\text{beam}} \left( \frac{V_{\text{crystal}}}{V_{\text{cell}}} \right) \times \text{(some factors)} \times |F(\text{HKL})|^2 \)

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

• \( \Rightarrow \) 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?

\[ b = S \times \left( \frac{d}{D} \right) \]

\( D = \) 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 ("slo... 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
2\textsuperscript{nd} approach : ID23-2 (KB system).
ID23-2

Distance from source (m)

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<th>Sample centre</th>
<th>Slits</th>
<th>Monochromator</th>
<th>Slits</th>
<th>Source</th>
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Monochromator Slits
KB centre
Secondary Slits
Lead Wall
Experimental Hutch
Optics Hutch
Experimental Table
Diffractometer
KB Mirror
Primary Slits

February 2010
Horizontal beamsize: $\sim 7 \mu m$ (FWHM)
Vertical Beamsize: $< 5 \mu m$ (FWHM)

Measurement: absorption scan with a W wire (200 $\mu m$ diameter), 1 $\mu m$ 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
Line scan
Mesh scan for crystal search

5 x 5, 80 by 80 µm² grid

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

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

Image 1
Phi=93-94

Image 10
Phi=102-103

spots visible to 2.3

spots visible to 2.6

David Flot, ESRF MX School 2010, 9th of February 2010
Best 90 Images

Completeness: 96.7%
R-factor: 14.3%
I/Sig (2.4-2.5Å): 2.8

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<th>NUMBER OF REFLECTIONS</th>
<th>XSSCALE statistics</th>
<th>COMPLETENESS</th>
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David Flot, ESRF MX School 2010, 9th of February 2010
• 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).
“Normal” data set collected here

FAE crystal
Statistics from XDS (Correct step)

| Resolution Limit | Observed | Unique | Possible | Completeness | R-Factor Observed | R-Factor Compared | I/|SIGMA | R-meas | R-merge | Anomalous | SigW | SigH | 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%    | 73%    | 2.172  | 5425  |
| 2.95             | 87304   | 15019  | 15019    | 100.0%       | 3.7%              | 4.1%              | 87304       | 38.28 | 4.1%   | 2.2%    | 73%    | 2.067  | 7133  |
| 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  | 9637  |
| 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%       | 10.3%             | 10.7%             | 139178      | 15.15 | 11.9%  | 7.8%    | 39%    | 1.245  | 11699 |
| 1.81             | 149092  | 25955  | 26055    | 100.0%       | 16.0%             | 16.0%             | 149092      | 10.73 | 17.6%  | 11.8%   | 25%    | 1.075  | 12622 |
| 1.71             | 16265   | 25577  | 25669    | 92.4%        | 23.2%             | 23.4%             | 103797      | 6.12  | 26.3%  | 22.6%   | 14%    | 0.927  | 10900 |
| Total            | 936096  | 169187 | 171316   | 98.9%        | 5.5%              | 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
"Normal" data set collected here

"Helical" data set collected between these two points

First data set: normal data collection, 270 degrees, 1 s 1 degree per image
Second dataset: "helical" data collection, 1.5 s exposure time per image
Statistics from XDS (Correct step)

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<tr>
<th>Resolution Limit</th>
<th>Number of Observations</th>
<th>Number of Possible Observations</th>
<th>Completeness of Data (%)</th>
<th>R-Factor</th>
<th>R-Factor Compared to I/\sigma</th>
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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

David Flot, ESRF MX School 2010, 9th of February 2010
B factor from mosflm/scala *

Rotation Range

* data processed by Ed Mitchell

David Flot, ESRF MX School 2010, 9th of February 2010
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 careful with radiation damage when collecting (no more than 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 “desperate” 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!