High-brightness X-ray sources for structural biology

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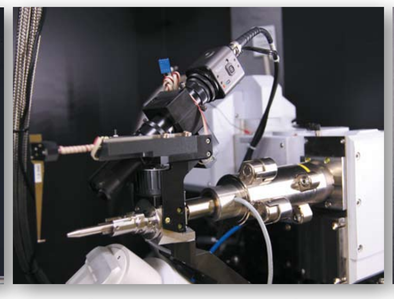
Modern microfocus X-ray sources define the state-of-the-art for protein crystallography and small angle scattering in the home lab. These sources have anode spot sizes of 100 μm or smaller. To preserve their brilliance, these sources are combined with high-quality Montel multilayer optics as beam shaping devices that image the source spot onto the position of the sample, magnifying the beam to a suitable size. Depending on the shape of the substrate, multilayer optics deliver a parallel or focused monochromatic beam. Below, we present results of three different microfocus X-ray sources: a sealed tube source, a rotating anode generator, and a liquid metal jet X-ray source. The power density detemines the brightness of an X-ray source and ranges from several kW/mm² for the sealed tube source to a few tens of kW/mm² for the rotating anode source, and to several hundreds of kW/mm² for the liquid metal jet X-ray source.

Microfocus sealed tube IμS (Incoatec, Cu anode, 50 μm focal spot) with QUAZAR multilayer optics.



Microfocus rotating anode X-ray generator HB-TXS (Bruker AXS, Cu anode, 100 μm focal spot, 2.5 kW) with HELIOS MX optic.

Liquid metal jet X-ray source METALJET (Excillum, 20 μm focal spot, 200 W) with a dedicated HELIOS MX optic for Ga radiation.

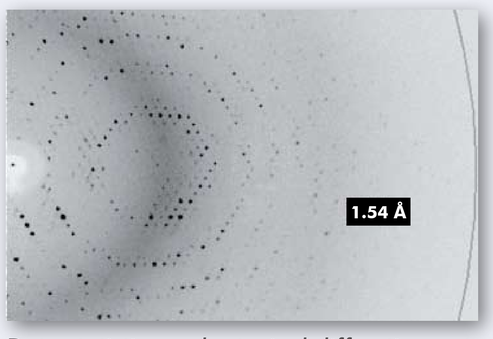
Incoatec Microfocus Source IμS

The IμS is a low-maintenance, highly brilliant sealed tube X-ray source. When combined with a dedicated multilayer optic, such as the QUAZAR (FWHM = 0.25 mm, 5.1 mrad) or the QUAZAR MX optics (FWHM = 0.10 mm, 7.6 mrad), the IμS delivers a flux density of more than 2 x 10^{10} photons/(s mm²) in a 2D focused beam. The latest version of the IμS, the new IμS $^{\text{High Brilliance}}$, has an improved X-ray optical design that leads to a flux density of more than 3 x 10^{10} photons/(s mm²). Therefore, the IμS delivers intensities comparable to older microfocus rotating anode generators which are suitable for structural analysis on protein crystals and poorly diffracting small molecule samples. This makes the IμS ideal for screening and structure determination of most protein crystals, including SAD phasing experiments.

SAD Phasing

Sample: Glucose Isomerase
a = 93.88 Å, b = 99.68 Å, c = 102.90 Å; I222;
T = 100 K; 388 amino acids

crystal size	0.24x0.24x0.15 mm³	<redundancy>	14.4 (4.5)
exposure time	40 s / 0.5°	<completeness>	98.0 % (89.8 %)
total time	~ 43 h	R_{int}	0.0652 (0.4191)
resolution	35-1.50Å (1.60-1.50Å)	$R_{p.i.m.}$	0.0149 (0.1959)
$< I/\sigma >$	21.8 (3.5)	R1(I > 2σ(I) ; all)	0.1587; 0.2047
anom. signal limit	2.7 Å (Ca^{2+}, 9 S)		

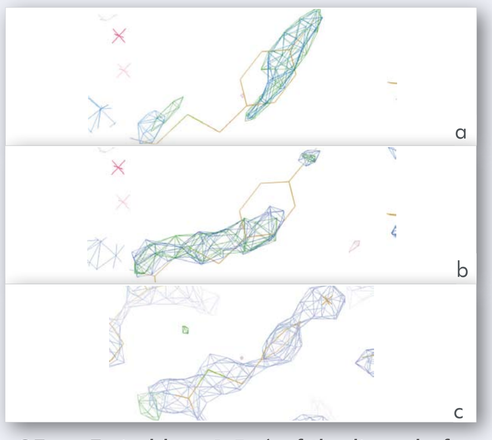


Data statistics and a typical diffraction pattern of glucose isomerase, measured with a Bruker AXS diffractometer equipped with the IμS (QUAZAR optics). The Ca^{2+} site has been found in the initial phasing with SHELXD.

Fragment Screening

Sample: Twinned crystal of an enzyme in complex with a small molecule fragment

crystal size	0.45x0.07x0.04 mm³	<completeness>	96.9 %
exposure time	15 s / 0.5°	$< I/\sigma >$	9.1
total time	3.5 h	R_{int}	0.0820
resolution	41–2.59Å	R_1	0.1331



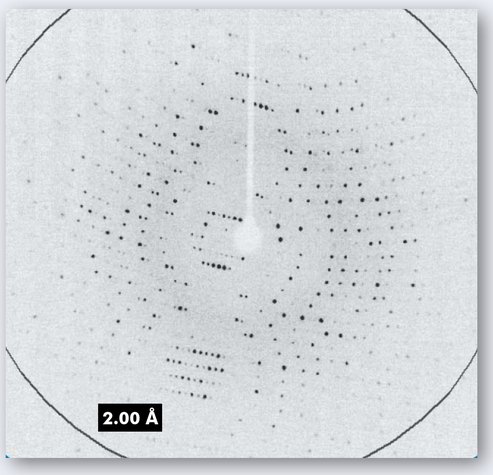
Data statistics and electron density map (Fo-Fc in green, 3 σ; 2Fo-mFc in blue, 1.5 σ) of the ligand after rigid body refinement with 60 % completeness (a, after 1 h), 80 % completeness (b, after 1.3 h) and 96 % data completeness (c, after 3.5 h and SHELXL refinement). Significant electron density in the active site was already visible after 1 h indicating the presence of the fragment. The complete data set was measured within 3.5 h and has a quality suitable for a twin refinement of the structure including the inhibitor. (The samples were provided by Dr. A. Heine and Prof. G. Klebe, Philipps University of Marburg.)

Microfocus Rotating Anodes

Modern microfocus rotating anode generators (μRAG's), such as the High-Brilliance Turbo X-ray Source (HB-TXS), allow significant higher power loads than microfocus sealed tube sources. In combination with high performance multilayer optics, this type of μRAG delivers a flux density that is about an order of magnitude higher compared to microfocus sealed tubes, resulting in intensities that are close to those obtained from second generation synchrotron beam lines. This enables diffraction studies on very small and poorly diffracting protein crystals and complete data collections with significantly shorter exposure times, e.g. for high-throughput studies.

30 sec Lysozyme Data Set

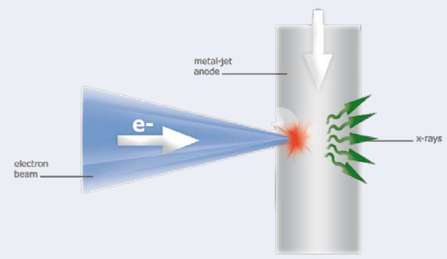
crystal size	0.17 mm diameter
exposure time	0.5 s/°
total time	30 s
resolution	22 – 2.00 Å (2.10 – 2.00)
$< I/\sigma >$	15.7 (4.2)
<redundancy>	3.68 (1.7)
<completeness>	97.6 % (85.1 %)
R_{int}	0.0634 (0.1789)



Data statistics and a typical diffraction pattern of a 30 sec lysozyme data set, measured with a Bruker AXS X8 PROTEUM system equipped with Helios MX optics.

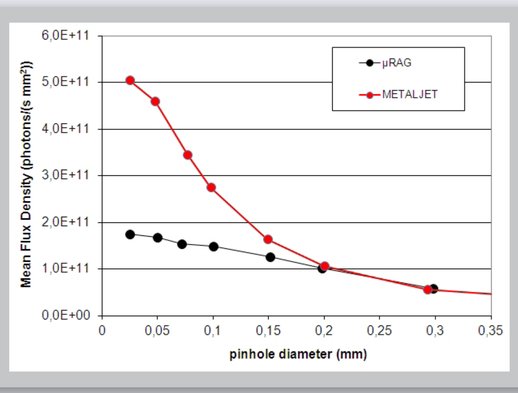
Liquid Metal Jet X-ray Source – The METALJET

The maximum power load that can be applied to solid metal anodes is limited by the thermal properties of the anode material and by the heat dissipation mechanism. This sets a hard limit for future improvements of X-ray sources based on solid metal targets. In Excillum's liquid metal jet X-ray source, however, X-rays are generated by an electron beam that is focused on a high-speed jet of a molten Gallium alloy. Such a high-speed liquid metal-jet target allows for power loads of several hundreds of kW/mm² in a spot size of < 20 μm. In order to preserve the extreme brightness of this source, a dedicated synchrotron-class optic of highest quality was designed for Ga-K_α radiation (9.25 keV). With this optics, the METALJET source is the brightest home-lab X-ray source, delivering unprecedented intensities of > 4 x 10^{11} photons/(s mm²) for a focused beam (FWHM = 0.07 mm). With a dedicated parallel beam Montel optics, a flux of more than 3 x 10^9 photons/s has been observed, giving a superb performance for small angle scattering experiments.



Working principle of the METALJET X-ray source.

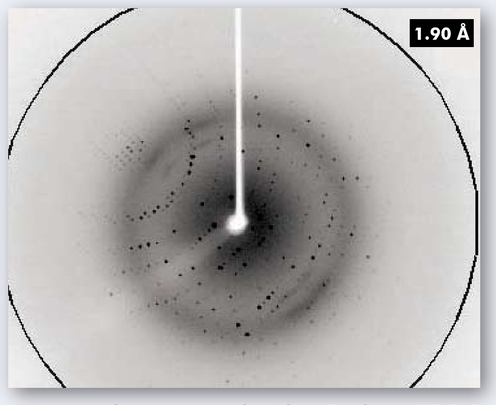
Protein Crystallography



Flux density measurement of the METALJET equipped with a dedicated HELIOS MX optic for protein crystallagraphy. The comparison with a microfocus rotating anode shows that the METALJET is the X-ray source of choice for small and poorly diffracting samples with a size of 0.1 mm or smaller.

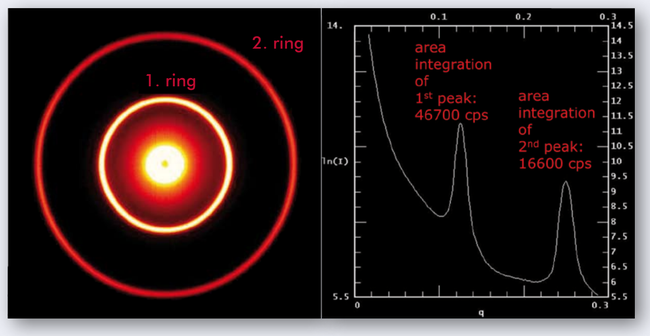
Sample: HIV Protease Complex
a = 46.38 Å, b = 57.86 Å, c = 84.87 Å; $P2_12_12$;
T = 100 K; 99 amino acids

crystal size	0.16 x 0.10 x 0.02 mm³
exposure time	30 s/0.5°
total time	2.5 h
resolution	34 – 1.90 Å (2.00 – 1.90)
$< I/\sigma >$	14.5 (3.6)
<redundancy>	5.4 (5.4)
<completeness>	99.9 % (100 %)
R_{int}	0.0855 (0.4331)

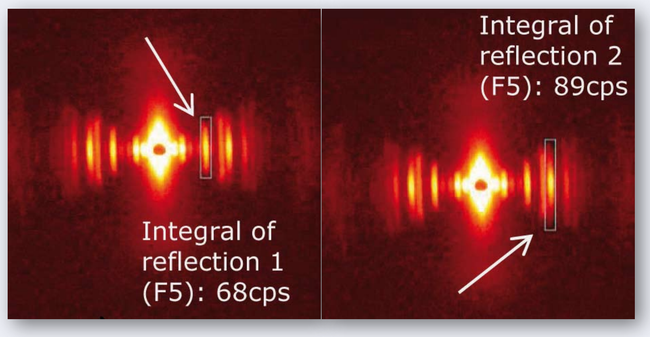


Data statistics and typical diffraction patterns of two small protein crystals, measured with a Bruker AXS D8 VENTURE equipped with the METALJET X-ray source.

Small Angle Scattering



SAXS scattering plot and radial integration of a Ag Behenate sample, measured with a Bruker AXS NANOSTAR equipped with the METALJET X-ray source.



SAXS scattering plot of a very thin fiber from a rat tail tendon, measured with a Bruker AXS NANOSTAR equipped with the METALJET X-ray source.