The MANACA beamline at Sirius, structural biology at 4th generation

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MANACA (**MA**cromolecular micro and **NA**no Serial **C**rystA**l**lography) is the first macromolecular crystallography beamline at Sirius, optimised for high flux, micro-beam size and small beam divergence (0.44 mrad). The focus is optimized to 10 (h) x 7 (v) µm² at sample position, but the beam size can be adjusted from 10 (h) x 7 (v) µm² to 100 (h) x 80 (v) µm² allowing match the beam to the crystal size. Additionally, the beam can also be cut to achieve smaller sizes (e.g. 5 (h) x 5 (v) µm²). The photon flux at sample will be ~$3x10^{12}$ ph/s/100 mA at 12.5 keV and the energy range from 5 to 20 keV. The experimental station has a mini-kappa [1] goniometer that allow the optimal alignment of crystals with long cell axes. Setups for serial crystallography data collection and analyses, as well as automation procedures, are under development.

The great beam characteristics (high brilliance; low emittance) provided by Sirius [2], the high stability and precision of the optics and experimental station will allow the diffraction of challenging samples such as viruses (and other big unit cell crystals), membrane proteins and complexes, which commonly yield small crystals. The energy range and beamline setup will allow native SAD phasing, reducing the necessity of additional experiments to solve new structures. The experiment control will be done using a user-friendly graphical interface (MXCuBE) [3], and automatic data processing (from data reduction to initial modelling) will be available.

Several strategies are being implemented in order to reduce the radiation damage, from beamline/data collection controls to data collection modes (e.g. multi-crystal/serial data collection). Our end station setup have an on-line intensity/position monitor and a photodiode beamstop that will allow monitoring the incident beam flux and size and the sample absorption in real time. The beam information will be used to generate data collection strategies and to estimate the dose absorbed by the crystal. We have also several attenuators that provide a fine control of beam transmission over the most of energy range. Last but not the least, multi-crystal and serial data collection setups and strategies are being developed to reduce even more the dose and allow room-temperature data collection.

Beamline layout

MANACA beamline uses two X-ray mirrors as focusing optics. In the optical hutch, the first optical element of the beamline is a horizontal Double-Crystal Monochromator (DCM) equipped with two pairs of crystals Si 111 and 311, followed by the vertical focusing mirror (side-bounce sagittal cylindrical mirror). The horizontal focusing mirror (tangential elliptical mirror) is located in the experimental hutch. This system will provide a beam cross-section of about 10 (h) x 7 (v) µm² (FWHM). This optical configuration allows for varying the beam size at sample by slightly changing the mirrors incidence angle (figure 1), a great feature for match beam and crystal size, and is optimised for high flux (figure 2).

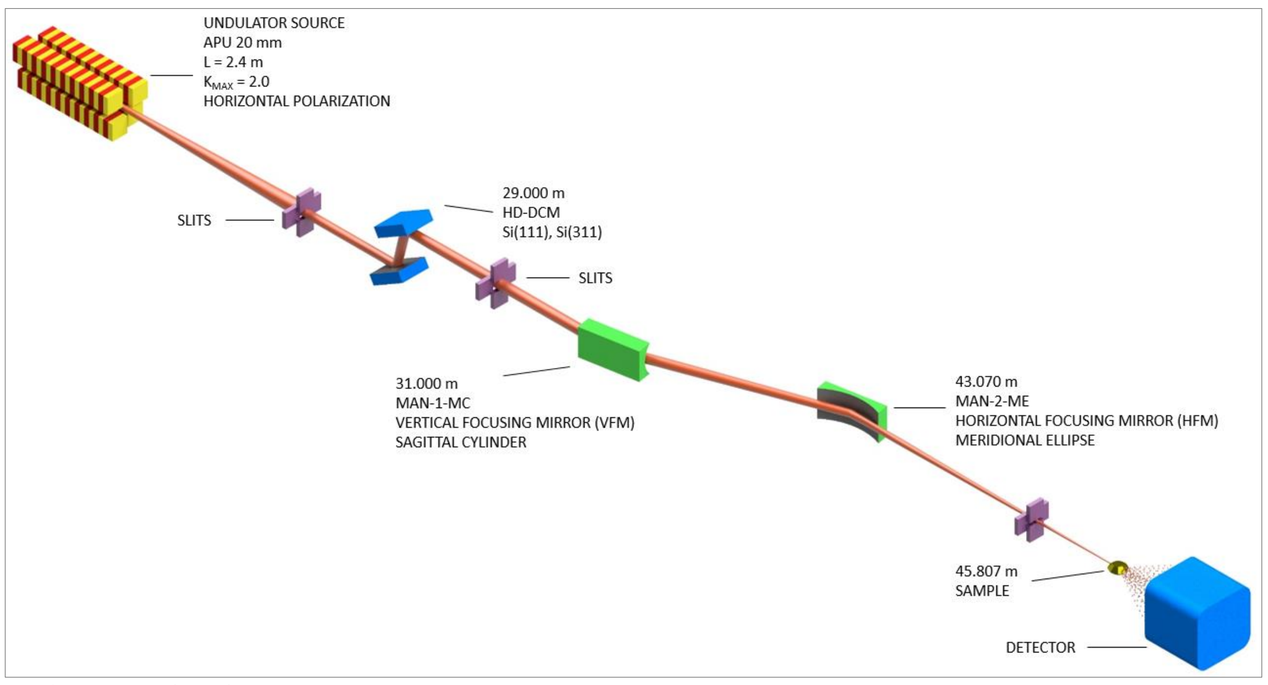

***Figure 1**. Beamline schematics.*

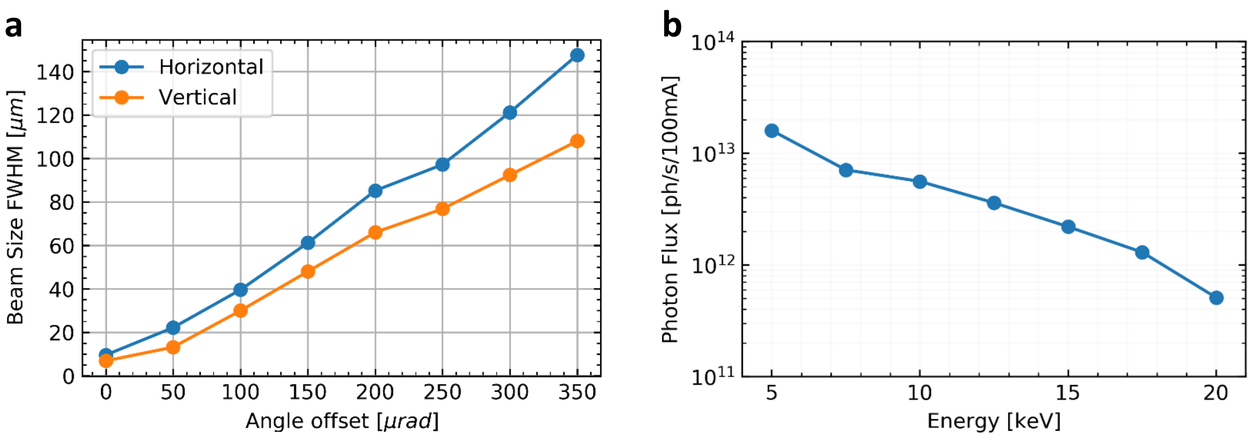

***Figure 2**. Beam size and flux at sample position. (a) Variable beam size at sample by changing both mirrors incidence angle by a small offset. (b) Photon flux (for I=100mA) at the sample.*

Current status and perspectives

MANACA has started the scientific commissioning on July in parallel to installation finalization and technical commissioning activities. We have adopted an emergency commissioning plan to delivery the basic instrumentation to perform data collection of SARS-CoV-2 related samples. Thus, so far, the beamline has operated on a fixed-energy regime (9 keV) with manual crystal mounting, single-axis goniometry and adjustable beam size from about 15 (h) x 17 (v) µm² (figure 4b) to 100 (h) x 80 (v) µm² (FWHM). Even with these limitations, the beamline has been showed good data quality (figure 4b and table 1).

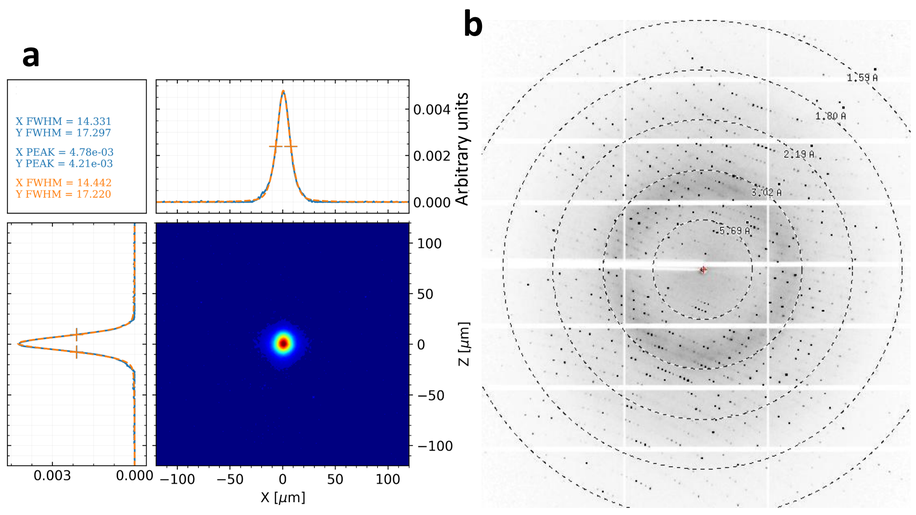

***Figure 4**. Beam size and protein diffraction. (a) Despite the undergoing commissioning we have already reached a beam size of 15 (h) x 17 (v) µm², very close to the nominal size of 10 (h) x 7 (v) µm². (b) Diffraction pattern from the first hen egg-white lysozyme (HEWL) crystal diffracted at MANACA, showing good data processing statistics (table 1).*

Table 1. Data collection statistics.

	HEWL
Wavelength (Å)	1.36697
Ring current (mA)	14
Beam size (µm)	~100 x 90
Exposure time (s)	0.1
Transmission (frac.)	0.75
Oscillation/image (°)	0.1
Total oscillation (°)	360
Images	3600
Resolution range	35.29 - 1.48 (1.53 - 1.48)
Space group	P 4_3 2_1 2
Unit cell (Å, °) (a=b, c, α=β=γ=90°)	78.90 36.81
Total reflections	416141 (7202)
Unique reflections	19160 (1336)
Multiplicity	21.7 (5.4)
Completeness (%)	96.13 (68.51)
Mean I/sigma(I)	77.16 (24.07)
Wilson B-factor	10.70
R-meas	0.0378 (0.0495)
CC1/2	1 (0.997)

Statistics for the highest-resolution shell are shown in parentheses.

Moreover, amazing scientific results have been obtained in the first month of scientific commissioning. Two structures solved at MANACA have been already deposited on PDB (SARS-CoV-2 3CL protease; PDBID: 7JR3 and 7JR4).

In the next months we expected to have access to the full energy range (5 – 20 keV), allowing SAD/MAD experiments, start the installation and commissioning of the automatic sample changer at the beamline and improve the focus size (reach the nominal focus size of 10 (h) x 7 (v) µm²). The beamline developments will focus on the improvement of classical rotation methods as well as serial methods, native phasing and new sample delivery methods (jet and fixed- target). The area detector will also be replaced for a faster and smaller pixel size detector (PiMEGA; in house development), which will allow faster and new experiments (serial and time resolved crystallography).

A second experimental hutch (nano-beam hutch) is in design phase and will be dedicated to non-goniometer-based sample delivery methods (serial crystallography), yet serial and room temperature methods will be implemented already on the current micro-beam hutch.

The MANACA micro-beam station has been designed to cover most of the classical and new macromolecular crystallography data collection methods and deal with a 4th generation X-ray beam. We expected, after conclude the commissioning, to use all this instrumentation (mirrors, BPMs, photodiodes, slits, fast detector, etc.) not only to delivery a high quality beam, but also to match beam and crystal size and provide an on-line dose measurement in order to control and reduce the radiation damage.

Beam conditioning elements and detector

The diffractometer base is equipped with two sets of horizontal-vertical slits (AT-C30, JJ X-Ray) each one followed by a diamond quadrant-based X-ray beam position monitor (A4 diamond XBPM, Cividec), a horizontal air-bearing goniometer and a photodiode beamstopper (Sentinel, MiTeGen). These slits can be used to cut-down the monochromatic beam just before the sample position (330 and 747 mm up-stream sample position). The beam can also be attenuated by a set of 12 metal foils (ABS-300, ADC) (figure 3). The detector (Pilatus 2M) is mounted on a separate base and can be positioned from 86 to 1000 mm far from the sample.

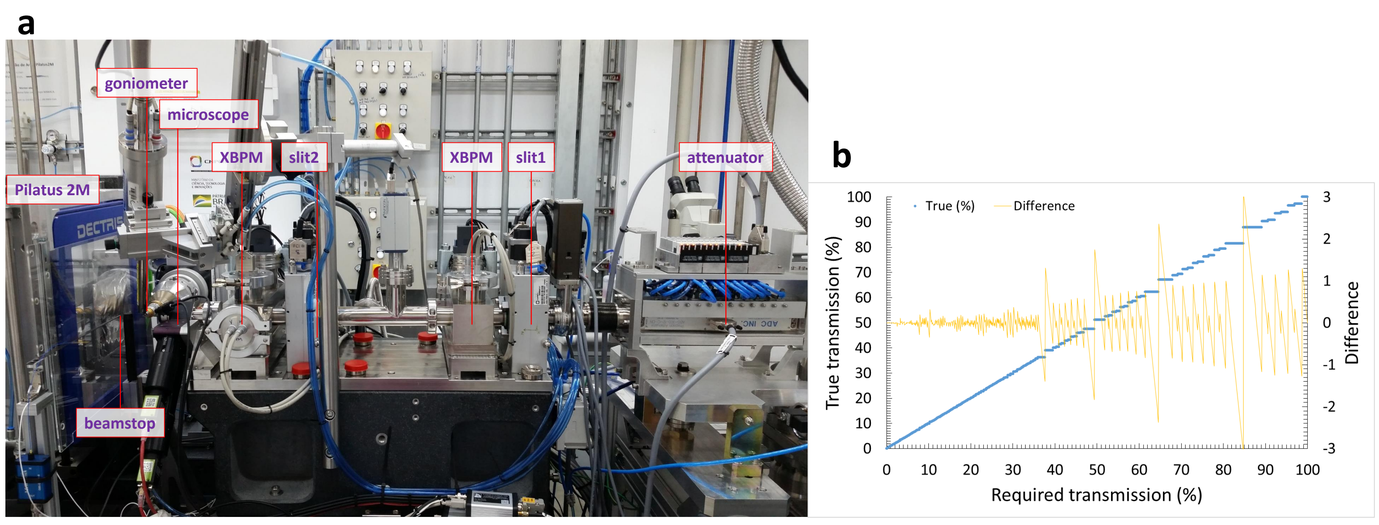

***Figure 3**. Beam conditioning elements and transmission control at 12.7 keV. (a) The end station is equipped with attenuators, two sets of slits, X-ray beam position monitors (BPM) and a photodiode beamstop. (b) The attenuator system can provide a continuous range of transmission and a precision of at least 0.5 from 0% to 35% of transmission. The range from 35% to 100% have just a few gaps in the range and a mean precision better than 1.*

Difference: Required transmission required - True Transmission.

References

[1] Brockhauser, S., Ravelli, R. B. G. & McCarthy, A. A. The use of a mini-κ goniometer head in macromolecular crystallography diffraction experiments. *Acta Cryst D* **69**, 1241–1251 (2013).

[2] Liu, L., Milas, N., Mukai, A. H. C., Resende, X. R. & Sá, F. H. de. The Sirius project. *J Synchrotron Rad* **21**, 904–911 (2014).

[3] Oscarsson, M. *et al.* MXCuBE2: the dawn of MXCuBE Collaboration. *J Synchrotron Rad* **26**, 393–405 (2019).

Sirius and MANACA beamline

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