Macromolecular Crystallography (MX)

MAX IV review report

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2023-11-15

Table of contents

1 General introduction

This report describes the MAX IV facilities for Macromolecular Crystallography (MX) experiments. MAX IV has two MX beamlines: BioMAX and MicroMAX. It also hosts a fragment-based drug discovery facility, FragMAX.

BioMAX was the first beamline at MAX IV to open to users and started operations in 2017. The beamline was designed to be very versatile, with a large energy and beam size range; catering to the needs of the user community, while also harnessing the special characteristics of 4th generation beamlines to attract new users interested in challenging projects involving microcrystals, large unit cells, and Serial Synchrotron Crystallography (SSX). The beamline design prioritized simplicity and relied on commercially available, custom solutions for robustness and easy-to-implement automation. BioMAX underwent a beamline review at the end of 2019 (see attached document). Both the review committee and the BioMAX users pointed out the need for remote access and increased experiment automation, capabilities for fluorescence scans and experimental phasing, and the continuing development of SSX experiments, mainly to build up a user community and serve as a sandbox for MicroMAX. We have made significant progress since then , with the implementation of many new beamline features now available to users: full remote access for cryo experiments with developments in automated data collection at room temperature; automated, X-ray based crystal centering procedures; fully unattended data collection for simple experiments using well-behaved samples, achieving a maximum throughput of 3-4 minutes per sample; XRF and energy scans and semiautomatic optimized phasing experiments; and alternative methods for sample delivery suited to SSX data collection that can be used or further developed at MicroMAX. BioMAX has a stable and satisfied user community and has achieved a good scientific output.

MicroMAX was conceived as a more ambitious project, both in terms of the beamline optics and experimental applications. While it has a similar energy range to BioMAX, the minimum beam size will be 1 x 1 μm^2 when the focusing mirrors are added in 2025 (compared with the 20 x 5 μm^2 BioMAX focused beam). The focusing using X-ray lenses gives high flexibility to rapidly change the beam size at the sample and will provide a focused beam size of around 10 x 5 μm^2. MicroMAX has two monochromators: a double crystal Si(111) monochromator like at BioMAX, as well as a Multi-Layer Monochromator (MLM) that can achieve a flux about 50 times higher than what is available at BioMAX. To be able to cope with the increased diffraction intensity, MicroMAX will be equipped with two detectors: a Jungfrau integrating detector, with single photon accuracy for low pixel values, and photon-counting EIGER detector. It will also be equipped with a tunable laser source for pump and probe experiments and a chopper for complex sample exposure regimes and X-ray pulses down to 10 µs. MicroMAX will be primarily dedicated to SSX, microfocus, and time-resolved experiments on the scale of microseconds and will also serve as a training center to promote the advantages of these techniques and their use within the MX community. At the same time, it will also be well-suited for automated oscillation experiments at cryo-temperatures. Unfortunately, MicroMAX was particularly affected by the Covid-19 pandemic and the following component shortages and delivery delays, with user operation delayed by about one year.

Besides sharing some instrumentation and a degree of overlap in experimental capabilities, BioMAX and MicroMAX also share the same low level beamline control software and computing facilities common to all MAX IV beamlines. They also share sample preparation laboratories, MXCuBE and experiment control, ISPyB for sample handling, and data stewardship. This arrangement means that both beamlines can benefit from developments tested and lessons learned in one of them.

FragMAX started as an offshoot of the scientific activity at BioMAX. Given the growing importance of fragment-based drug research for academic and industrial users, FragMAX has become an independent activity with its own goals and metrics, while remaining formally attached to BioMAX and being well integrated in the MX group. FragMAX uses the same data collection procedures and software used at BioMAX and has been a testbed for the development of unattended data collection. At the same time, FragMAX has developed its own protocols for crystallization and fragment binding –a service heretofore non-existent at MAX IV– using its own pipelines for data analysis, MR phasing, structure refinement, and

ligand location. FragMAX also uses a unique interface, FragMAXapp, developed in-house with the collaboration of the MX group at BESSY.

We have determined that the best way to achieve an optimal synergy between the two beamlines was to unify efforts and operate our facilities jointly. All MX group staff contribute to the development to either beamline according to their level of expertise. We are also planning to implement joint beamline proposals so that we can place our users on the most appropriate beamlines depending on the particular sample properties or specific experiment. This is useful not only for the users, but also for us, since it is easier to achieve complementarity between the beamlines with a joint user community. It can also be an advantage for promoting the use of MicroMAX at the difficult starting stage, when it will be competing with already established facilities like ID29 at ESRF.

In the future, developments at BioMAX will focus on implemented fully autonomous experiments and developing facilities for data collection at room temperature. Increased automation will be crucial to supporting FragMAX operations and creating an opportunity to expand the industrial user base at MAX IV. For FragMAX, an important goal is to increase the throughput at the sample preparation stage. At MicroMAX, we expect to achieve a basic scope to support high-throughput crystallography data collection along with serial and time-resolved experiments. Further development of data processing pipelines to monitor data collection on the fly, merge data from different samples and obtain phases and electron density maps shortly after data collection are underway.

There are risks to the plan related to lack of resources in the context of the difficult current financial situation of MAX IV. Aging instrumentation at BioMAX is becoming a concern. We also depend on external resources for developing our user interfaces, MXCuBE and ISPyB. While the MXCuBE collaboration is very productive and seems to be on a robust path for the near to medium term, the future of the ISPyB collaboration is more uncertain.

1.1 Charge questions

MAX IV asks the review committee to evaluate the material presented in the written report and the oral presentations and discussions and address the following charge questions:

A. Technical realization of the beamlines:

1. Do the beamlines provide adequate capabilities, and do the beamline teams address areas of necessary improvement?
2. Do the beamlines offer unique capabilities to the user community?
3. How do the beamlines compare to leading beamlines in their field worldwide?

B. User communities, science program, and impact:

1. Are the beamlines attracting and supporting the relevant user communities?
2. Is there sufficient focus between the different activities?
3. Do the capabilities meet the needs of the relevant scientific communities (as laid out in the original science case)?
4. Are the staff research and development projects of appropriate quality and in line with the current and future direction of the science program at the beamline?
5. Are the user and in-house science programs productive and making a sufficient impact in their science fields?
6. Are the beamlines missing opportunities regarding user communities, science programs, or research directions?
7. Do the beamlines / MAX IV employ an adequate outreach and training program?

C. Beamline operation:

1. Is the user support at the beamlines of high quality and allowing for a productive user program with high impact?
2. Are the beamlines or the facility missing out on opportunities for further improving user productivity?
3. Are there sufficient support labs and related facilities available to enable high-quality research at the beamlines?
4. Is the facility setting the right priorities in providing high-quality supporting infrastructure, services, and procedures?

D. Future directions:

1. Are the proposed plans and roadmap for the MX facilities adequate, or do you see changes that could increase the impact?
2. With the user community and national and international developments in mind, are the right priorities set out for these developments?
3. What resources are needed to make FragMAX successful? Are the proposed developments well chosen?
4. Do the beamlines / MAX IV have an adequate funding strategy and making use of funding opportunities?
5. Are there additional opportunities (funding, development, science directions) that the beamline or the facility should take into account?
6. Are the (envisioned) operation and science programs at the beamline well-adjusted?
7. In terms of types of experiments, are the overlap and specialization of BioMAX and MicroMAX adequate?
8. What types of sample delivery methods should MicroMAX prioritize? What types of serial and time-resolved experiments should MicroMAX concentrate on?
9. Is the joint operation of BioMAX and MicroMAX with centralized user management, scheduling and user support the best option? Are there any unforeseen risks in that strategy?

2 MX User facilities at MAX IV

2.1 BioMAX

2.1.1 Beamline description

The BioMAX beamline has been designed to have high reliability and stability while allowing certain flexibility for the delivered beam parameters. The photon beam energy is adjustable between 5 – 25 keV, while the transverse beam size (horizontal x vertical) at the sample position can vary from about 100 x 100 μm^2 down to 20 x 5 μm^2 FWHM for normal operation. Some main beamline parameters of BioMAX are presented in Table 2.1.

Insertion device	In-vacuum undulator, 18 mm period, 111 periods
Monochromator	Si (111) horizontal double crystal monochromator
Focusing optics	Kirkpatrick-Baez mirror pair
Energy / wavelength range	5 – 25 keV / 0.5 – 2.5 Å

Focused beam size	20 x 5 μm^2 (FWHM, horizontal x vertical)
Focused beam divergence	0.14 mrad (horizontal) x 0.11 mrad (vertical)
Flux at sample	10^{13} photons/second at 1 Å and 400 mA ring current

Table 2.1 BioMAX parameters

The layout of the BioMAX beamline is illustrated in Figure 2.1. This section gives a brief description of the beamline and focuses on the modifications and upgrades of various components performed in the recent years. For further details see Ursby *et al.* (2020).

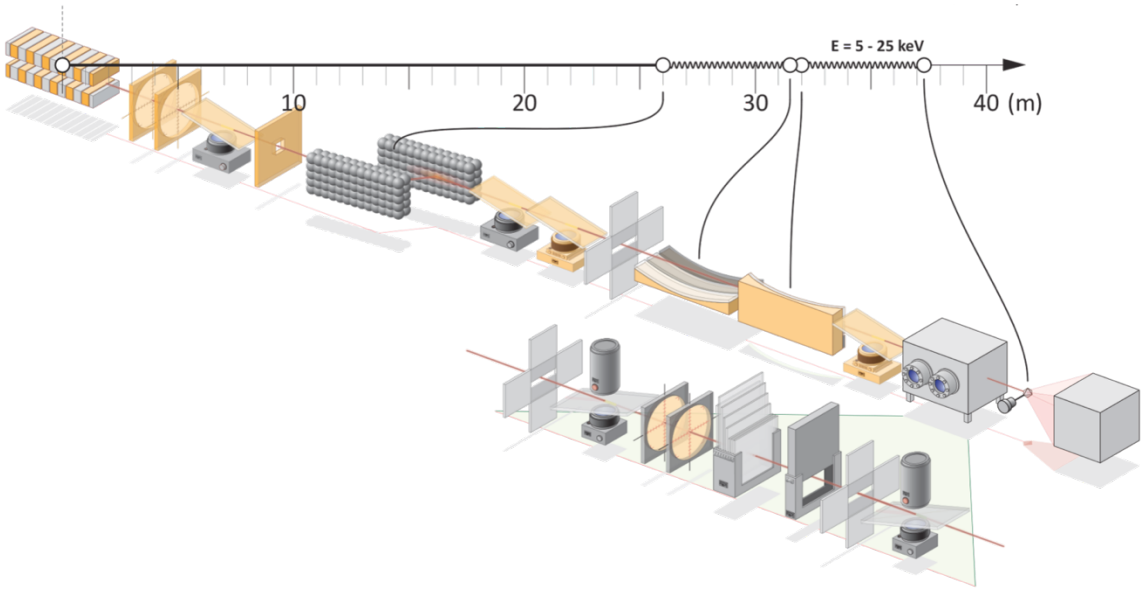


Figure 2.1 Schematic showing the BioMAX components: undulator, two front-end beam position monitors, front-end fluorescent screen, optics hutch fixed mask, double-crystal monochromator, fluorescent screen, NanoBPM, slits, vertical focusing mirror, horizontal focusing mirror, NanoBPM, beam conditioning unit (BCU), sample and detector. The components in the BCU chamber are shown in the enlargement and includes slits, diagnostics, two diamond beam position monitors, attenuators, shutter, slits and diagnostics.

2.1.1.1 Undulator

The X-ray source of BioMAX is an in-vacuum room temperature undulator based on the design by Yamamoto *et al*. (1992). It is able to produce a high-brilliance X-ray beam and allows tapering to generate wiggler-like radiation (Tarawneh, *et al*., 2019).

The motion control of the undulator was upgraded in 2021. The upgrade consisted of replacing the old encoders on the undulator motors and adding two more to ensure that all four motors controlling the undulator gap are equipped with an encoder. This upgrade has improved the undulator gap control and has facilitated the fast step and continuous scanning described in Section 5.3.

2.1.1.2 Optics

Monochromator

BioMAX uses a Si(111) horizontal double crystal monochromator (FMB Oxford, UK) that is able to select the X-ray photon energy with a relative bandwidth of $2x10^{-4}$. In 2021, we observed an elevated pressure in the

monochromator vacuum chamber, which was eventually found to be caused by a leak in the liquid nitrogen cooling circuit. This was addressed with a number of interventions to exchange faulty components over several accelerator shutdown periods. Despite the poor vacuum level, we could continue beamline operation by careful adjustment of the cooling settings in the monochromator and increasing the vacuum alarm level, so this problem did not result in downtime for the users.

We have extended the energy range over which automated energy changes can be performed. It is now possible to automatically change the beam energy between 6 keV and 21.5 keV, thanks to the use of two feedback loops that continuously correct the beam position at the monochromator exit and in the experimental hutch, respectively. For higher energies (up to 24 keV) some manual beam realignment by beamline staff may be required. Some test measurements were performed at BioMAX for machine studies using the 15th harmonic of the undulator at a photon beam energy of around 27 keV, but operation at such high energies is hindered by the low sensitivity of the nanoBPM (FMB Oxford) beam diagnostic that is used in the monochromator feedback loop. Since the current energy range allows collecting data to 0.66 Å resolution, sufficient for ultrahigh-resolution and small molecule work, we are not considering extending the energy range for user experiments in the foreseeable future.

Focusing mirrors

The BioMAX beam is focused to the sample position using Kirkpatrick–Baez (KB) mirror geometry (Kirkpatrick *et al*., 1948). The surface curvature of the horizontally (HFM) and vertically (VFM) focusing mirrors (Winlight X, France) can be changed to achieve the desired focusing at the sample position. We offer three beam focusing modes for general user operation: 100 x 100 μm^2, 50 x 50 μm^2, and 20 x 5 μm^2. The majority of operations use the 50 x 50 μm^2 focus mode because that most closely matches the typical size of crystals users bring to the beamline. For larger crystals, the defocused 100 x 100 μm^2 beam size is preferable, and the focused 20 x 5 μm^2 mode is suitable for work with microcrystals. Users can easily change between these three modes from the MXCuBE experimental control interface. The final beam size on the sample is determined by a MD3 diffractometer circular aperture (see Section 2.1.1.5).

In early 2020 the lead screw of the focusing motor on VFM was damaged, which prevented control of the vertical beam focus. The screw was replaced in February 2020, which resulted in almost one month of downtime, between repairs and vacuum recovery. A second screw was purchased to keep as a spare. A preventive maintenance procedure, to ensure regular movement of the benders through the entire range, was established with the vendor to prevent similar problems in future. Another issue occurred with VFM mirror focusing mechanics in 2022, which caused irreproducibility in the beam size. This did not affect most users, since the beam size is ultimately determined by apertures in the diffractometer, but we had to check and refocus the beam manually for experiments requiring fully focused beam. Eventually this problem was resolved by the vendor through a replacement of mechanical components.

We are considering replacing the KB mirror pair with CRL lenses. Potential advantages are the elimination of future problems with the focusing arising from the bending mechanism as well as the possibility to optimize the performance of the beamline for sample imaging experiments, for which the beamline is currently sub-optimally suited due to the structure observed in the defocused beam, shown in Figure 2.2. On the other hand, we have not found evidence that the beam structure impacts diffraction data quality. We will re-evaluate this option based on our experience using the CRL system at MicroMAX and future needs regarding beam size at BioMAX.

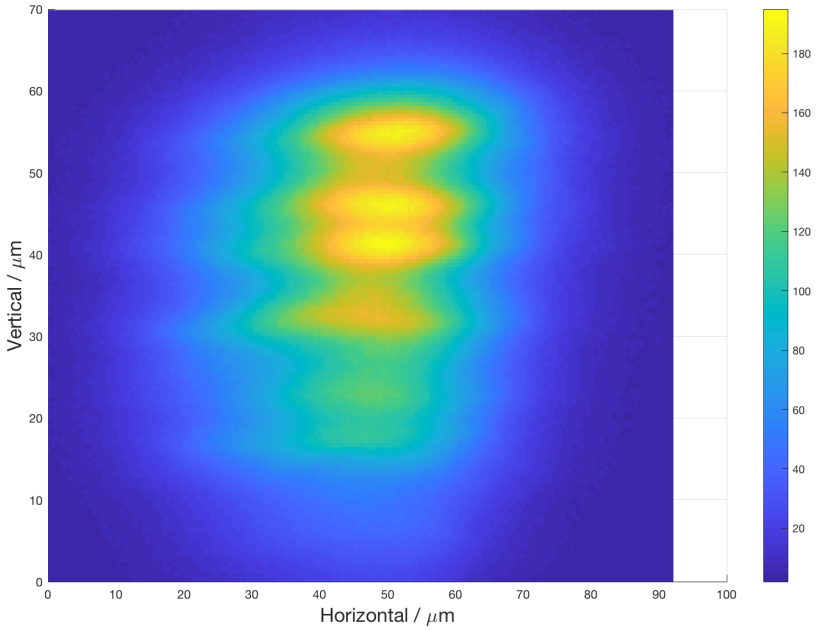


Figure 2.2 Profile of the defocused beam at BioMAX. The fringes in the vertical direction are an image of the structure of the KB mirror.

2.1.1.3 Beam conditioning unit

The first component in the BioMAX experimental hutch is the beam conditioning unit (BCU) (Cianci *et al.*, 2017) dedicated for beam manipulations and diagnostics. The BCU operates in vacuum and is connected to the vacuum system of the beamline. In 2021 a vacuum valve with a diamond window was installed just upstream of the BCU vessel. This upgrade isolates the BCU from the rest of the windowless vacuum system of the beamline and accelerator and reduces the time required to pump down the BCU after venting from several days to several hours.

Among other components in BCU, there are two X-ray beam position monitors (XBPMs) (CIVIDEC Instrumentation GmbH, Austria) that are used in the feedback loop that holds the beam position at the sample, a fast shutter (CEDRAT Technologies, France) and a set of three filter wheels for the adjustment of beam transmission. In the past years the piezo positioners (Attocube systems AG, Germany) used for the vertical alignment of the XBPMs have failed a number of times. This has disrupted user operation; failure of these components does not only prevent automated beamline alignment and changes of energy, but they can also fully block the X-ray beam under certain circumstances. Through multiple contacts with the supplier, we discovered that these positioners were unable to support the weight of the XBPMs. They were subsequently replaced by their “high-load” version in 2021. The new positioners are better suited for the weight of the XBPMs and have performed reliably.

An additional piezo positioner was added to the fast shutter to enable horizontal adjustment, in addition to the already available vertical adjustment. This allows the shutter to follow any possible beam alignment changes in horizontal direction and increases the acceptable pass-through aperture of the beam. The configuration of the filter wheels was upgraded in 2022 to improve the positioning of the filters and achieve a better alignment with respect to the beam.

2.1.1.4 Sample changer

BioMAX is equipped with an ISARA robotic sample changer (IRELEC, Fance), which is used for mounting and exchanging samples on the diffractometer. It consists of a Stäubli TX-60 six-axis industrial robot arm, the control unit, and a large sample dewar that can accommodate up to 29 standard universal pucks

(UniPucks), for a total of 464 samples. Since its installation and commissioning the sample changer system has been continuously improved and upgraded to make it more robust and reliable.

One of the most significant upgrades to the sample changer is the modification of the double gripper: by adding a small cut between the jaws of the gripper it was possible to increase the tolerances for the length of SPINE-like sample supports. This eliminated many instances where samples with longer support pins (>22 mm) were tipped on the goniometer head by the sample changer during the mounting/unmounting process, which often caused a collision between the sample changer and the diffractometer. Since the upgrade, we have had fewer problems with longer than normal pins; in combination with automated software detection of long pins based on the difference between the goniometer head at transfer and center positions, we have all but eliminated this source of collisions.

In 2022 the sample changer's control system firmware was upgraded, which eliminated the occasional loss of communication with external clients during operation and disruption of the auto-filling of the sample dewar.

The ISARA system was upgraded in 2022 in order to mount *in-situ* crystallization plates (see Section 2.1.2.4) The ISARA "plate hotel" is located behind the cryogenic dewar and can accommodate up to four crystallization plates that can be exchanged by the robotic arm onto a special plate gripper attached to the MD3 diffractometer (Figure 2.3).

Figure 2.3: Sample changer mounting a crystallization plate on the diffractometer.

In 2023 a new single gripper tool was commissioned for the sample changer to mount samples at room temperature. The "warm" single gripper has a unique design to grab sample bases without damaging the fragile glass capillaries attached to them. Three new UniPuck positions were installed outside the dewar to accommodate 48 room-temperature samples. The new gripper and the puck bases ("hot tools") are shown in Figure 2.4. More details about the single gripper operation are presented in Section 5.2

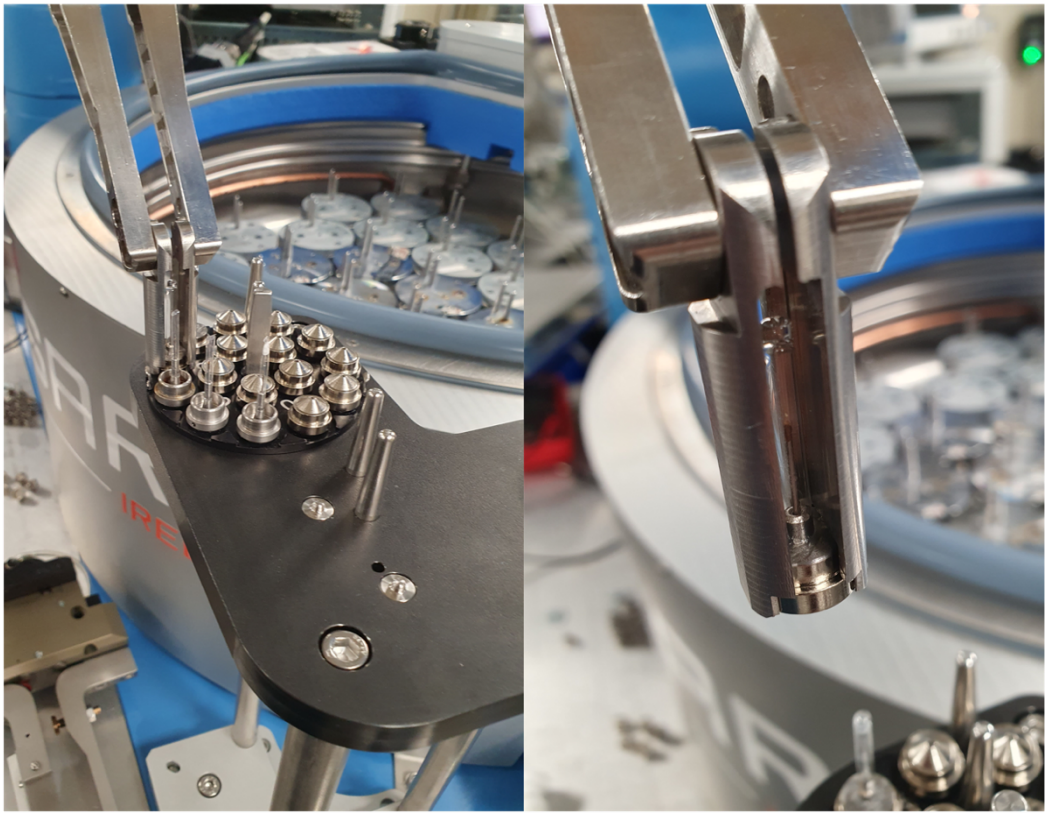


Figure 2.4: ISARA hot tool with single gripper. Note how the gripper picks up the sample at the base, leaving plenty of space to avoid damaging the attached capillary.

In order to improve the liquid nitrogen autofilling regulation of the ISARA dewar, a new phase separator was installed at BioMAX in 2023. The new device controls the liquid nitrogen regulation independently from the main control unit of ISARA, so that autofilling does not stop in case of failure of the unit. Moreover, the electronics controller of the phase separator allows adding more functionality to the sample changer system that would not be compatible otherwise with the older generation electronics controller of ISARA. The phase separator installation by the ISARA dewar is shown in Figure 2.5.

Figure 2.5: Phase separator (highlighted in red) installed by the ISARA dewar.

Occasional miscommunication between robot and diffractometer during sample transfer has been the main detriment to sample changing reliability resulting in sample loss. To avoid this, we have recently added a safety timeout mechanism, which returns the sample to the dewar if the sample changer does not receive the signal that the diffractometer is ready for sample exchange within a set time.

2.1.1.5 Diffractometer

Goniometry is performed by a microdiffractomer MD3-down (Arinax, France), which has a fast rotation axis (up to 800 deg per second) and can perform 60 Hz raster scans. For the majority of experiments, we use the "standard configuration": a mini-kappa goniometer head, penta-aperture (with 100, 50, 20, 10 and 5 µm diameter apertures), scatter-cleaning capillary tube, scintillator screen for beam visualization, and beamstop. We use other goniometer heads, e.g. the plate adapter and HVE adapter, to support *in-situ* and HVE SSX experiments, respectively. The Oxford Cryojet is supported by a Rapid nozzle EXchanger (REX) which also holds the HCLab humidity controller nozzle.

In the last few years, we have carried out a number of improvements to the MD3 system:

- Re-orientation of the REX. In the original setup (Figure 2.6 A), the HCLab nozzle was mounted further downstream of the sample position; this created a large shadow area on the detector at small sample to detector distances, which prevented collection of a complete data set at high resolution (above 1.8 Å at a beam energy of 12.7 keV). To avoid the shadow, we rotated the REX by 90 degrees so that both the cryo and HCLab nozzles are on the upstream side of the sample position (Figure 2.6 B). With this modification, no shadow from either the REX or the nozzles is observed on the diffraction images.

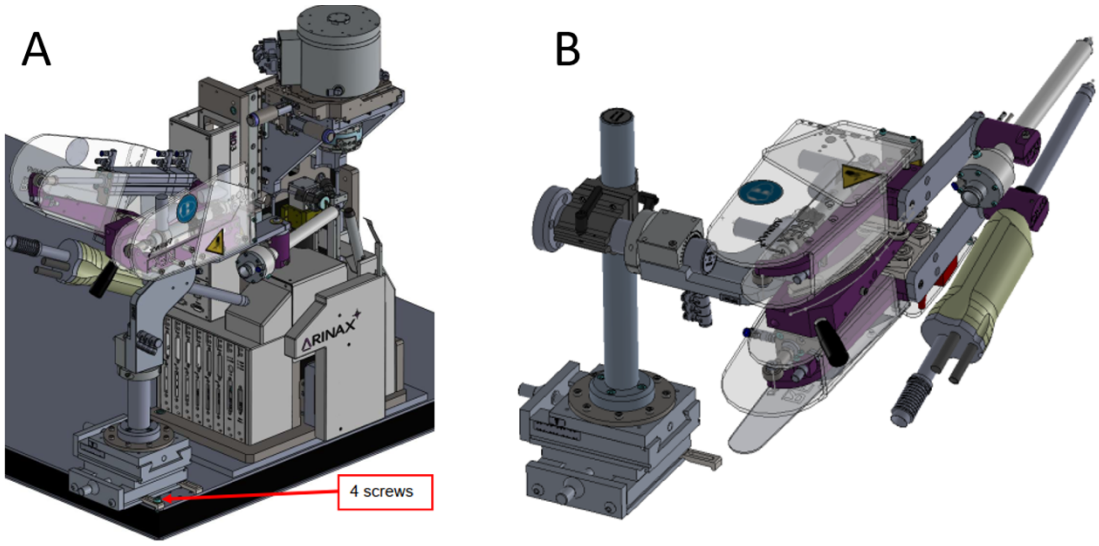


Figure 2.6 REX original (A) and new (B) orientation after modification.

- Commissioning of the plate adapter to support *in-situ* data collection. Currently, users can collect data from three types of crystallization plates (Section 2.1.2.4). While commissioning was slowed down by issues mainly related to collision potential with other MD3 instrumentation and communication between the sample changer and MD3, these were solved by 2022. However, the standard aperture and scatter-cleaning capillary ensemble used in the standard configuration does not fit in the reduced space between the beam exit and the plate, and using only the penta-aperture results in a higher background scatter. Using a larger beamstop reduces the total background, but, in addition, we want to obtain a "canon aperture" (combining an aperture and a cleaning capillary) to improve the data quality; this project is ongoing.
- Comparison of the scintillator crystals Bismuth Germanate (BGO) and Cadmium Tungstate (CWO). With the high photon flux at BioMAX, the original BGO crystal suffered radiation damage, which severely affected the fluorescence image of the beam– a critical component to automated beam alignment. In collaboration with Arinax, we compared BGO and CWO performance and radiation damage at different energies, and ultimately switched to a CWO crystal, which proved to be more robust. Recently we discovered the radiation damage was inflicted only on the surface coating on top of the BGO crystal while the crystal itself was not affected by the radiation.
- (Auto-) calibration of mini-kappa. To make better use of the mini-kappa geometry and provide user collection strategies with different kappa orientations, we have recently started a collaboration with Global Phasing and the MX-group in SOLEIL.

MD3 operation had been smooth and reliable for the first few years of beamline operation. However, when we further explored advanced functionalities and tried to improve the automation of the beamline, we were slowed down by many issues, such as 1) timeouts reading instrument settings; 2) equipment stopping during a configuration change, for example during preparation to mount a new sample or to start data collection; 3) random failure to trigger the detector and fast shutter during raster and helical scans; 4) failure of automatic loop centering due to bad video images; and 5) inconsistent motor positions after motor homing. A substantial part of beamline development has been dedicated to solving or mitigating the impact of these.

Furthermore, we have started to experience MD3 parts failure at an unusually high rate: between 2020 and October 2023 we have had nine major motor failures due to electronics or mechanical wear-out. To proactively prevent down-time, we have requested a replacement of all the original motors that have not

been exchanged yet, together with maintenance and troubleshooting plans. In the longer term, we want to upgrade the MD3, probably in conjunction with the sample changer, which is also by now an obsolete model. This would provide the chance to change the geometry to a MD3-up and facilitate operation of the sample changer. Such a major upgrade will require special funding efforts and cannot realistically be undertaken until MicroMAX is fully functional.

2.1.1.6 Area detector

The area detector in operation is an EIGER 16M (Dectris, Switzerland) hybrid pixel detector with 75 µm pixel size and a 450 µm thick silicon sensor. As described in the earlier BioMAX review report in 2019 (see attached document), there were many problems in the first few years of operation, but it has become more stable and reliable with time.

In October 2021 we installed flow sensors to monitor both the circulation of the external water coming to the chiller and the internal cooling liquid going to the detector head. The external water flow was adjusted to make sure it was within the working range requested by the chiller. Since then, the chiller operation has become smoother and we have hardly observed any failures related to detector temperature.

In January 2022 during the winter shutdown, we upgraded the EIGER SIMPLON firmware from 1.6.6 to 1.8.0 with help from Dectris. The upgrade included the installation of a graphics card driver, the upgrade of the ALBULA data visualization software, and the adoption of the new MX Gold standard, which uses the McStas coordinate system (Bernstein *et al*., 2020). This upgrade has been a game changer for detector operation. After the upgrade, the only major problem we have experienced is the failure of one module. Furthermore, it ended the need for daily reboots of the data acquisition unit to prevent the stream interface from crashing.

The current EIGER 16M was installed and commissioned in 2016. As we no longer have a maintenance contract with Dectris and there is no other detector with similar characteristics at MAX IV, any failure could potentially lead to months of downtime. Meanwhile, it has been shown that EIGER CdTe detectors have excellent data quality at high X-ray energies (Storm *et al*., 2021) but also with tender X-rays (communication with MX staff at ESRF and PETRA-III beamlines). Given the energy range (6-24 keV) at BioMAX, we have chosen a CdTe EIGER2 XE 16M for the detector upgrade. In addition to the improved sensitivity of CdTe sensors at higher energies, EIGER2 XE boasts a higher maximum frame rate (560 Hz vs 133 Hz for the current EIGER 16M). The preliminary plan is to receive and install the EIGER2 in January 2024, during the winter shutdown. The current detector will become a spare that could be used at BioMAX, at MicroMAX, and even at other MAX IV beamlines.

2.1.2 Experiment options

BioMAX was intended to be a very versatile beamline, capable of handling most conventional crystallography techniques, challenging cases involving the study or large molecules and complexes, as well as cutting edge experiments based on Serial Synchrotron Crystallography (SSX) and time resolved experiments. Even as advances in CryoEM and the prediction of protein structures made the initial scientific case somewhat obsolete as the experiment facilities were being developed, we have striven to fulfill the initial specifications for the beamline, while at the same time, expand in the new directions guided by shifting demand by the community. This section summarizes the main experimental facilities developed in the last years and in the present.

2.1.2.1 High throughput experiments and unattended data collection

The most common experiment setup requested by BioMAX users is single axis data collection at cryogenic temperatures using the “standard” MD3 configuration (Section 2.1.1.5). The level of automation implemented for these experiments makes remote control straightforward, and since the introduction of remote access in 2020, most data collection in this mode is done fully remotely (see Section 2.6.2).

By default, data are collected using a 0.1° oscillation angle and 11 ms exposure per image. Since typical X-ray transmission values range between 10 and 30 %, the use of the new EIGER2 detector (Section 2.1.1.6) will allow for a shorter exposure time with full beam transmission.

The upgrades to the sample changer described in Section 2.1.1.4 have helped improve the reliability of the experiment and reduced the down time caused by sample changer issues. Many of the most common issues experienced by users tend to be caused by glitches in communications between MXCuBE and other servers or problems with the MD3 diffractometer.

The introduction of an automated algorithm for X-ray diffraction-based sample centering has had a large impact in user operations and data quality, since it makes it easier to center small and thin samples accurately. The user can choose between performing the manual "three-click centering" procedure, which is still the preferred option with bulky and visible crystals, or setting up an area to be scanned by the X-rays and using the automated X-ray centering tool described in Section 2.3.1.1.

The development of an automated procedure for X-ray centering allowed us to fully automate data collection, following the scheme illustrated in Figure 2.7. This unattended data collection differs from manual data collection in the introduction of a "diffraction plan" –the data collection strategy parameters that will be applied to all the samples. The current setup is highly suitable for FragMAX experiments, which have been using it routinely for the last year with excellent results (see Section 2.4.1.4). Our most frequent industrial user, AstraZeneca, has also made partial use of unattended data collection during most of their beamtime sessions. In the future, users will be able to enter different diffraction plans for different samples; we are also collaborating with Global Phasing to be able to calculate a data collection strategy based on test images, instead of relying on user input. This will extend use of unattended data collection to most user experiments, including complex data collection involving optimization of data completeness by using the mini-kappa axis and anomalous pair collection to obtain experimental phase information (Section 2.1.2.3).

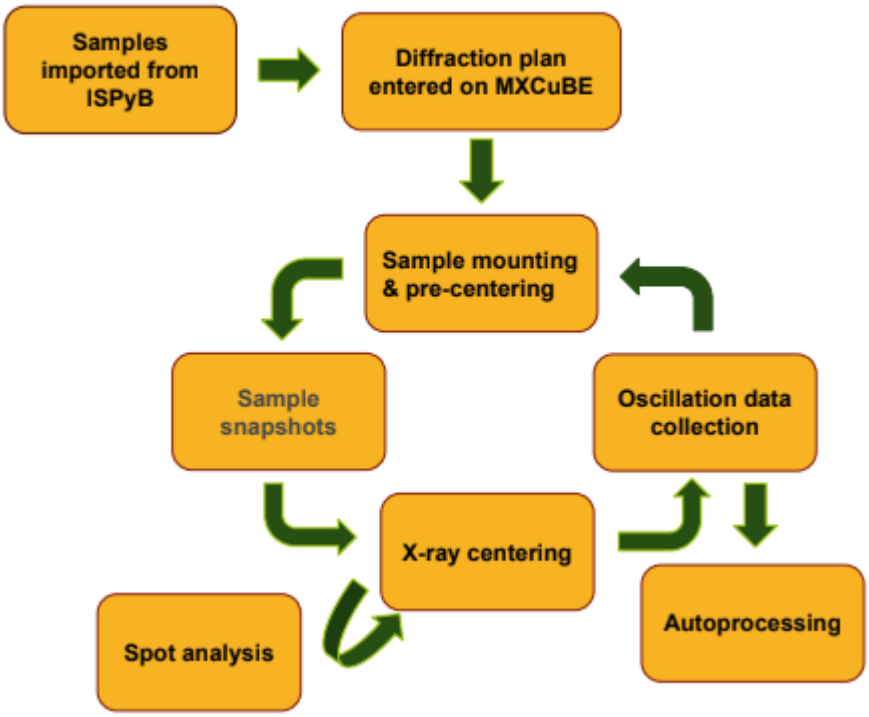


Figure 2.7 Scheme of the unattended data collection currently implemented at BioMAX. Collection of sample snapshots is optional. Except for the automated X-ray centering, the procedure relies on routines previously available in MXCuBE.

2.1.2.2 Serial Synchrotron Crystallography (SSX) experiments

Serial crystallography experiments have been carried out at BioMAX with both flow-based and fixed-target sample delivery systems. The development of SSX facilities has been a high priority at BioMAX since the beginning of operations. We have tested different systems that will continue to be developed at MicroMAX. We are also working on on-the-fly analysis of individual frames in SSX data which will provide user feedback about the hit rate and data statistics as explained in Section 2.3.3.2.

Fixed target SSX

Although fixed-target approaches facilitate SSX data collection from cryocooled samples, the relatively small dose (of the order of kGy) absorbed by the sample during a single diffraction pattern recording, along with the availability of the HClab humidity control system and sealed sample holders, makes it possible to perform data collection at room temperature and obtain a more comprehensive picture of protein dynamics. The usual setup consists in mounting microcrystals on a chip or similar support and performing rastering over the entire area without predefining the sample location. Figure 2.8 shows different sample holders, mostly based on a SPINE pin base and thus fully compatible with the BioMAX MD3 goniometer head. Although fixed target data collection at room temperature cannot currently be done remotely, we are exploring the potential use of the hot tools (Section 2.1.2.5) for automated sample exchange of chips of suitable dimensions.

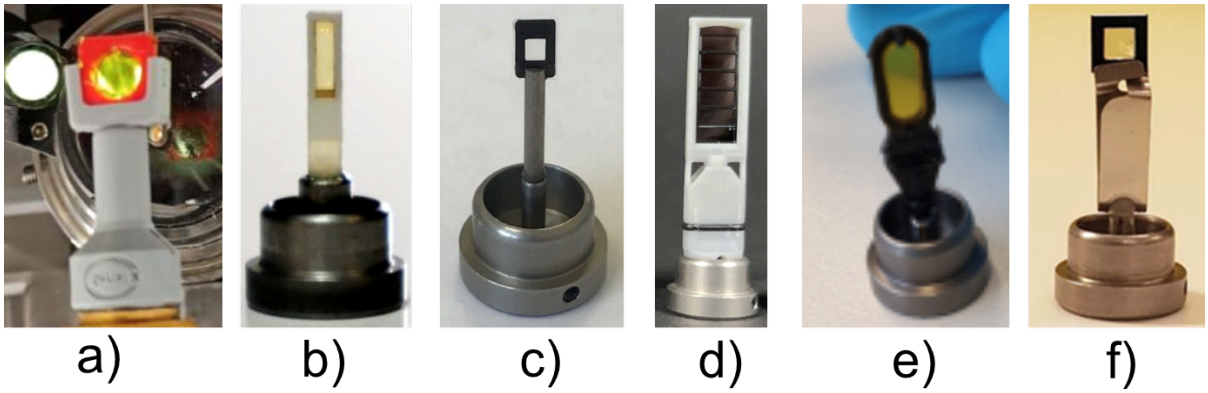


Figure 2.8 High-degree rotation fixed-targets. a) Serial-X, b) MiTeGen (Ilava et al., 2021), c) SwissMX (Karpic et al., 2020), d) Suna (Lieske et al., 2019), e) XtalTool (Feiler et al., 2019), f) Silson (Coquelle et al., 2015)

After a long delay due to the Covid-19 pandemic, we performed a test of the Roadrunner instrument developed at DESY, Hamburg (Roedig *et al*., 2017) in 2023. While the data obtained was of high quality (see Section 5.1) extensive work needs to be done before the instrument can be well integrated in the beamline environment for user work. We envision that this instrument could be used in the MicroMAX second endstation (EH2) at a later stage.

Flow-based SSX

The standard instrument for injector-based SSX is a high-viscosity extrusion (HVE) injector, shown in Figure 2.9. with a volume of 130 µl and a flow rate of 1 µl per minute that can use different viscous matrices (Shilova *et al*. 2020). This instrument was used to collect the time resolved data in the Jungfrau test experiment described in Section 5.4.

We have collaborated with Richard Neutze´s group at Gothenburg University in the development of the Serial-X flow cell device, consisting of an X-ray transparent glass capillary mounted on a goniometer-compatible 3D-printed support, connected to a syringe pump via lightweight tubing (Ghosh *et al.,* 2023). A third device, currently undergoing testing is the AdaptoCell, a microfluidic sample environment also in use at two other MAX IV beamlines: Balder and CoSAXS. This device is very suitable to perform time-resolved

experiments such as *in-situ* mixing. These two instruments, shown in Figure 2.9, d) and e), can be made available to general users on a collaboration basis.

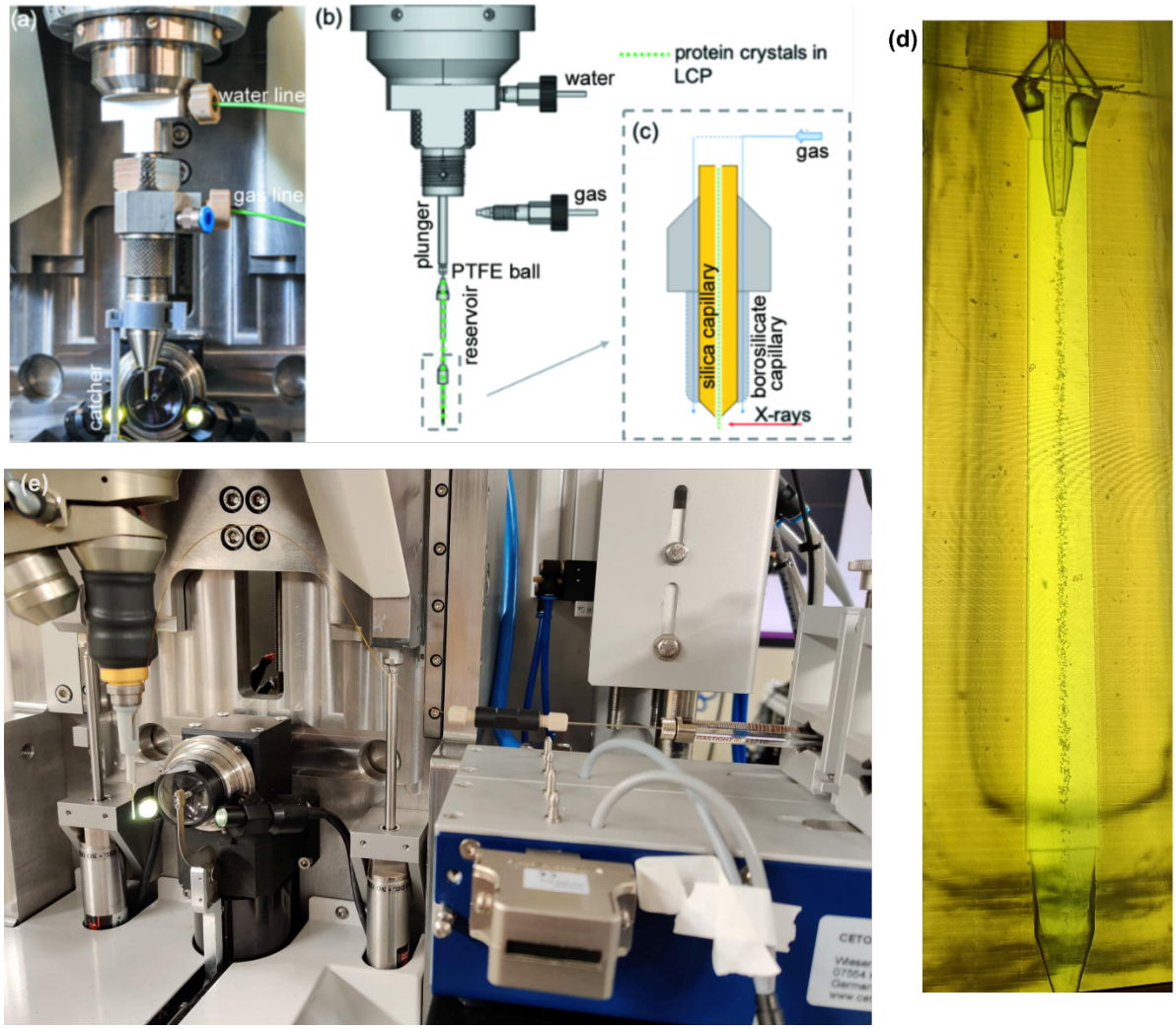


Figure 2.9 a,b,c) HVE setup at BioMAX d) AdaptoCell chip, e) Serial-X flow-cell setup

2.1.2.3 Experimental phasing capabilities

While solving the phases of macromolecular crystal structures is now done primarily with molecular replacement, *de novo* phasing with anomalous scattering is still used by some of our users (see, for example, Hackney *et al*., 2023). Single (SAD) or multiple wavelength (MAD) anomalous phasing can be necessary in cases where either no high-quality search model exists, or the data cannot be phased by molecular replacement. In addition, anomalous scattering can be valuable for location and identification of heavy atoms in the structure, among other purposes (Ma *et al*., 2023). Therefore, we have decided to develop and maintain the special beamline capabilities required for proper data collection for SAD/MAD and make their use automated, available remotely, and, in general, as simple as possible for users.

Fully automated changes of energy (Section 2.1.1.2) are a critical requirement. Optimized SAD/MAD data collection also requires measurement of the X-ray fluorescence spectra around the element absorption peak of interest. The fast energy scans developed at BioMAX (see Section 5.3) enable fast and reliable determination of peak and inflection energies. Users need only select the element of interest from a menu in MXCuBE, and the program automatically handles the energy scan and calculation of peak, inflection points, and remote energies using CHOOCH (Evans & Pettifer, 2001). The output energies from CHOOCH

are parsed back into MXCuBE, and when setting up a data collection, users can select one of the calculated energies from a drop-down menu.

Because minimizing dose, and therefore radiation damage, on a sample can be critical to successful phasing, users can also benefit from estimated doses for a data collection shown on the Data Collection panel. Advanced data collection protocols for SAD/MAD phasing are also available for users (Finke *et al*., 2016). Collecting anomalous or dispersive differences as close together as possible in time can help mitigate the effects of radiation damage (Gonzalez, 2007). To achieve this, we have implemented interleaved data collection in MXCuBE: data are collected in small angle wedges at either two crystal orientations separated by 180° degrees (the ”inverse beam” protocol, useful mainly for SAD data collection); or at different energies (for MAD data collection).

Because of the HDF5 format we use for writing out the diffraction data, interleaved data collection generates separate data sets for each wedge. In order to simplify both automated and manual data processing, we have developed a routine that takes sequential wedges of HDF5 data and repacks them to generate a single, full HDF5 dataset that users can take home and can also be handled by the routine automated analysis on the computing cluster (see Section 2.3.3.1).

Future improvements planned for experimental phasing include the incorporation of data collection strategies making use of the mini-kappa goniometer. This work will be done in collaboration with Global Phasing.

2.1.2.4 *In-situ* plate data collection

Collecting data from crystals *in situ* in the crystallization plate is useful to assess crystal growing conditions and obtain data at room temperature while avoiding damage to crystals during mounting and cryocooling. We have implemented *in-situ* screening and data collection capabilities using the crystallization plate adaptor for the Arinax MD3 diffractometer. The adaptor supports several different types of plates, but we are currently focusing on CrystalDirect (Cipriani, 2021), In situ-1 (Mitegen) and CrystalQuick-X (Le Maire, 2011).

Plate navigation and data collection capabilities in single/multi-position or fast mesh scans have been integrated into MxCuBE (Figure 2.10). On average it takes 5 minutes per drop for screening, depending on the sample. The plate adaptor can be rotated a total of 30 degrees and a complete dataset can be typically collected from 2-20 crystals. In the future, we are planning to merge partial datasets automatically by using the Autoproc pipeline; this will facilitate adoption of this method for fragment-based drug discovery experiments. We have also successfully tested collecting SSX data from crystals *in situ*, by carrying out a mesh scan of the crystallization drops. The results of the initial experiments are described in Section 5.1.

We plan to incorporate plate exchange with the sample changer (see Section 2.1.1.4) in MXCuBE, and add support for crystallization plate handling in ISPyB.

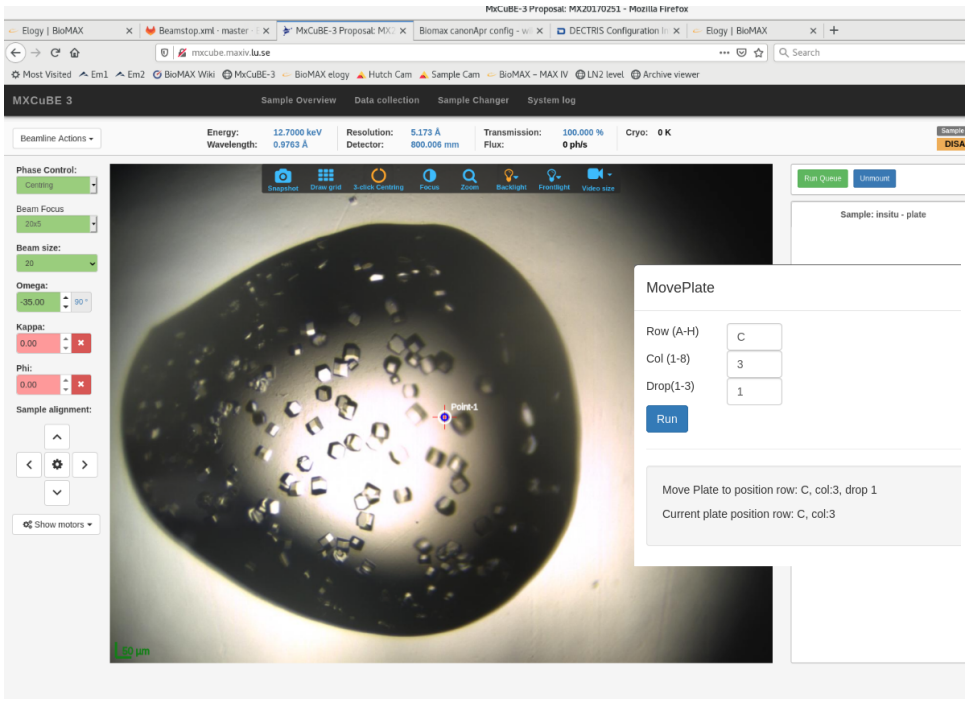


Figure 2.10 MxCuBE screenshot of in-situ plate setup.

Table 2.2 shows results of *in-situ* test data collections from Thaumatin and Triose phosphate isomerase single crystals.

	Thaumatin	**TIM**
Number of crystals	6	25
Resolution range	46.38-1.812 (1.876-1.812)	39.09-1.502 (1.556-1.502)
Space group	P 41 21 2	C 1 2 1
Unit cell	58.63,58.63,51.62 (90,90,90)	99.43,52.66,58.89 (90,118.072,90)
Total reflections	209877	368866
Unique reflections	24504 (2118)	42811 (4235)
Completeness (%)	98.09 (86.60)	99.68 (98.49)
Mean I/sigma(I)	7.83	9.0
Wilson B-factor	25.91	25.25
R-merge	0.168	0.217
R-meas	0.179	0.229
CC1/2	99.6	99.3

Reflections used in refinement	24473 (2113)	42802 (4234)
Reflections used for R-free	1204 (89)	2112 (233)
R-work	0.1653 (0.3075)	0.1454 (0.3062)
R-free	0.1874 (0.3379)	0.1659 (0.3250)

Table 2.2: Summary of data refinement statistics for test cases. Values in parentheses represent the high-resolution shell.

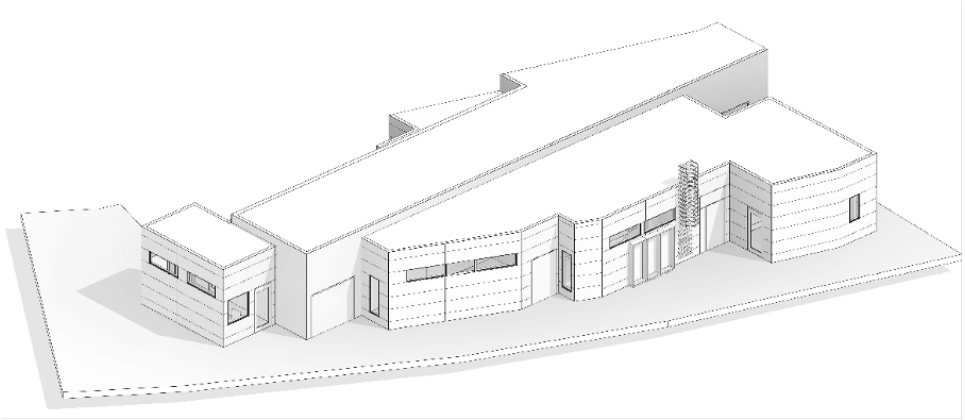
2.1.2.5 Single crystal data collection at room temperature in SPINE sample holders

BioMAX users wishing to carry out data collection at room temperature have two options:

1. Using the HClab humidity controller (Arinax, France) to stabilize the crystal at room temperature (Bowler *et al*., 2015). The advantage of this system is that it is possible to change the hydration state of this crystal in a controlled fashion and even cryocool the crystal thanks to the REX pneumatic stage, which can quickly swap between the HClab humidifier and the cryo-nozzle (see Section 2.1.1.5). The disadvantage of this approach is that it requires retrieving the crystals from the crystallization plate and mounting them one by one on the diffractometer. This system is not fully integrated in MXCuBE and for the foreseeable future will remain available as an on-site option.
2. Mounting crystals in glass capillaries or MiTeGen plastic sleeves. This is the more commonly used option, since users can prepare several samples in advance and, in the case of glass capillaries, which remain airtight, ship them. The recent introduction of the “hot tools” described in Section 2.1.1.4 to use the sample changer at room temperature can be very helpful to users interested in this experimental approach, potentially allowing for fully remote data collection. The results of the initial tests are given in Section 5.2.

Both approaches for single data collection at room temperature benefit by the dose estimate implemented in MXCuBE, with a specific maximum dose target for room temperature data collection. Currently the hot tools are only available to users on a collaborative basis, since the sample exchange can only be done using the sample changer server, which is not directly available to users. However, an industrial user (Sprint Bioscience) has already expressed interest in making use of the system and two groups in Lund University has used it for data collection on protein crystals and small molecule data collection (Li *et al*., 2023) respectively. One of our current priorities is to provide sample management for room temperature samples in ISPyB and integration within MXCuBE.

2.2 MicroMAX



2.2.1 Introduction

The MicroMAX beamline is funded by the Novo Nordisk Foundation. The funding covers the construction of the beamline and operation until 2031.

Initially (from 2004) there were two MX beamlines proposed for the future MAX IV facility, a high throughput beamline and a microfocus beamline, that have evolved to the BioMAX and MicroMAX beamlines. In the successful application for MicroMAX, the beamline was proposed to provide "structures from macromolecules that cannot be crystallized to sufficient size or quality, room temperature structures, and time resolved structures down to the micro- or millisecond timescale." The end station was to cover both automated rotational crystallography including remote operation, and serial crystallography using different sample delivery systems with the possibility for time-resolved experiments.

With the developments of CryoEM and structure prediction methods, the user demand for MX beamlines has changed, for example with studies of large complexes having moved to CryoEM. Even though large complexes were one of the intended use cases of the microfocus beam, this has not changed the capabilities that MicroMAX needs to offer.

We believe that MicroMAX should aim to provide world-leading capabilities in serial and time-resolved crystallography. It should also provide remotely –operated, automated rotational crystallography, albeit not in the same capacity as is planned for BioMAX (see also Section 2.2.6). MicroMAX will need to cover both serial crystallography and rotational crystallography to be able to adapt to user demands, while at the same time determining the limits of the scope of the beamline, given finite available resources.

A leading idea in the design of MicroMAX has been to build a beamline that is flexible both in terms of X-ray beam and experiment setup. The X-ray optics system will provide a beam with very high flux and small focus but that can also be easily tuned to fit the experiment. The energy is tunable in the range 5-25 keV, but the mirrors, required for the smallest beam size, will have an upper energy limit of 20 keV. The energy range of the multilayer monochromator that provides the highest flux is limited to 10-13 keV. The goal is that the X-ray beam properties will not set any limitations, that it can provide what is needed for the experiment.

The experiment setup will also be flexible providing both high-throughput data collection with automation, as well as serial and time-resolved crystallography. Initially the first experiment hutch will be used for all types of experiments, but a second hutch can later be taken in operation to make it easier to accommodate different types of experiments.

Construction of MicroMAX started in 2018 with the aim to start user operation in 2022. The start of operation has been delayed, mainly by delays in optics deliveries (including late orders due to the procurement being contested in court) and unforeseen issues with the installation and setup of optics components (see Section 2.2.2.3). The insertion device delivery was late but was not a limiting factor. There

have also been very long delivery times for electronic components, delaying, for example, installation of the equipment protection system and the personal safety system. These delays have been related to the pandemic and the global shortage of components and materials. The beamline is now close to ready for users, and which we plan to gradually ramp up both in terms of users and functionality (see Section 2.2.3).

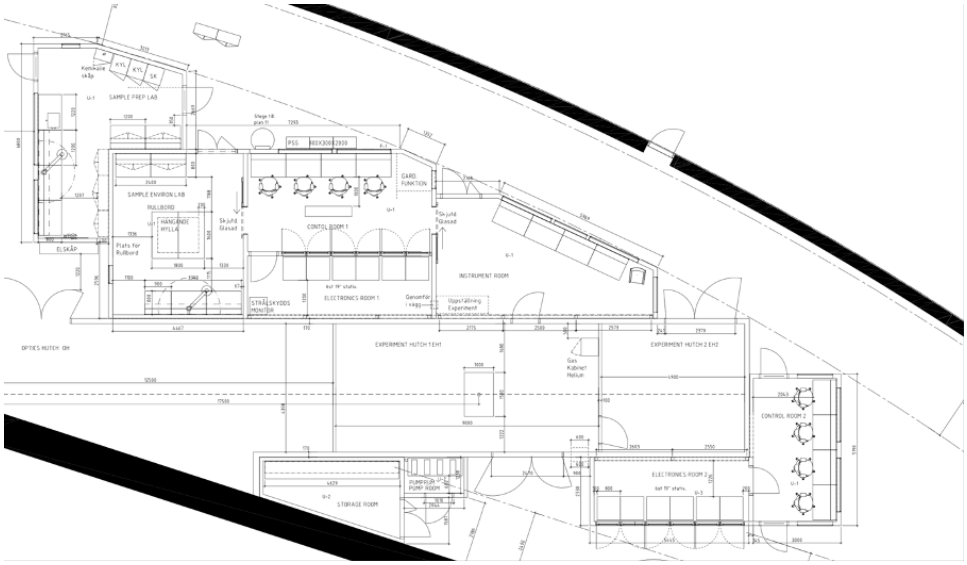


Figure 2.11 Overview of the MicroMAX beamline physical layout with radiation hutches, labs, and control rooms.

2.2.2 X-ray system

The MicroMAX X-ray system has been designed to take advantage of the 4th generation, 3 GeV storage ring with the aim of achieving high flux, small focus, stable beam, and wide energy range, while still maintaining the flexibility to easily vary flux, beam size, and photon energy. This section describes the function of the design shown in Figure 2.12.

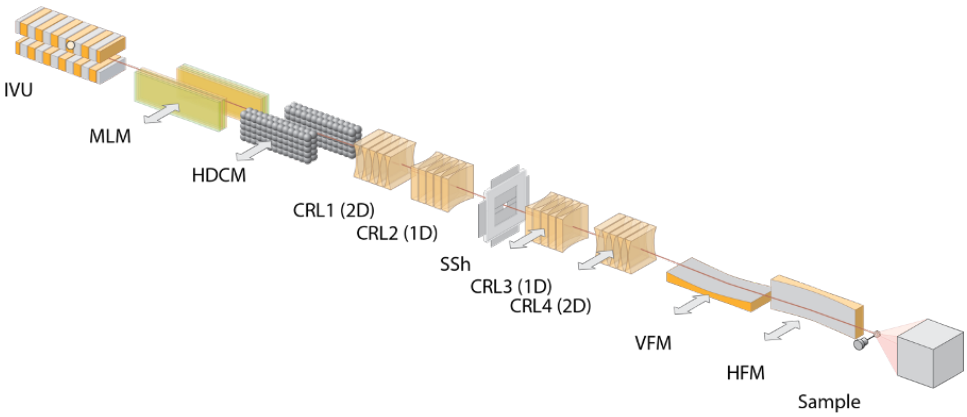


Figure 2.12 Overview of the X-ray system with the undulator and optical components to the sample, including in-vacuum undulator (IVU), multi-layer monochromator (MLM), HDCM (horizontally deflecting double crystal monochromator), compound refractive lenses (CRL) with either lenses refracting in only the horizontal direction (1D) or cylindrical refracting in both horizontal and vertical direction (2D), secondary source, horizontal (SSh), vertical focusing mirror (VFM) and horizontal focusing mirror (HFM).

2.2.2.1 Insertion device

MicroMAX is equipped with an in-vacuum, room-temperature, permanent-magnet undulator (Hitachi Metals, Japan). The undulator has a magnetic period of 18 mm, a minimum gap of 4.2 mm, Kmax=1.98 and a magnetic lattice length of 2.8 m (156 periods) that, like the one at BioMAX, can be tapered to widen the undulator peaks.

2.2.2.2 Monochromators

MicroMAX has two monochromators (FMB Oxford, UK), a double crystal monochromator (DCM) and a multilayer monochromator (MLM), with energy ranges of 5-25 keV and 10-13 keV, respectively. A DCM equipped with Si(111) crystals gives a bandwidth of around 2 x 10^{-4} and is typical for MX beamlines. At MicroMAX, the DCM is horizontally deflecting with a flux of around 10^{13} photons/s. It will be used for rotational crystallography and for other types of experiments requiring narrow bandwidth at the cost of photons. The MLM gives a wider bandwidth, with two different coatings providing bandwidths of 0.4% and 1%, and will deliver a flux of more than 10^{14} photons/s. By design, switching between the two monochromators is simple and fast, on the order of a minute (some thermal stabilization might be required but this has not yet been experimentally verified).

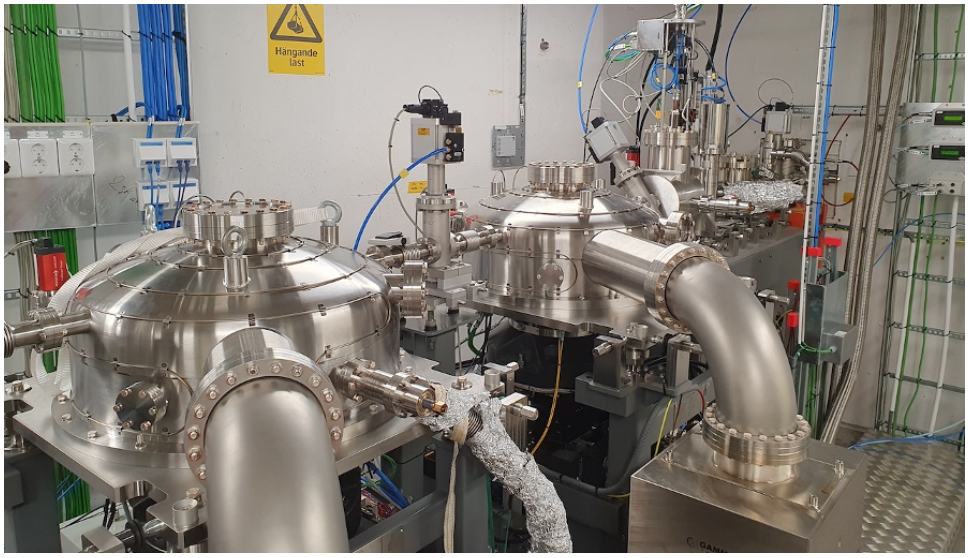


Figure 2.13 Photo of the monochromators (multi-layer monochromator on the right followed by the crystal monochromator on the left).

Beam focusing will be done using either X-ray lenses (Compound Refractive Lenses, CRLs) (Axilon, Germany) only or a combination of CRLs and mirrors. The CRL-only mode covers the energy range 5-25 keV, while the mirrors can operate from 5-20 keV. The two CRL units in the optics hutch collimate the beam, which is then focused using the two CRL units in the experiment hutch. The CRL-only mode focuses down to around 10 x 5 μm^2 (horizontal x vertical). To reach the minimal focused beam size of around 1 μm, focusing mirrors are required. We have chosen a KB mirror pair with an inhouse mechanical system and fixed curvature mirrors for this purpose. In the mirror configuration, the CRLs in the optics hutch focus the beam horizontally onto a secondary source. The beam is then refocused by a KB mirror pair onto the sample. In addition to smaller possible beam sizes, the mirror mode will also deliver a somewhat higher flux. The beamline will start with the CRL-only mode, while the focusing mirrors are planned to be installed in 2025. Focusing with the CRLs is quite flexible, as it is fast and easy to switch between different CRL configurations once they have been experimentally defined, of the order of tens of seconds determined by the speed of the longitudinal translations of the CRLs. There are many possible configurations, for example combining the mirrors with the CRLs to achieve a larger beam size without having to move out the mirrors (as it can be expected to take longer time to change between CRLs-only and mirror modes).

Figure 2.14 Photo of two of the CRL units.

The micro-focusing CRLs give the possibility to easily and rapidly change the beam size at the sample and is expected to give a more homogeneous defocused beam than the mirrors. The lenses are also less sensitive to vibrations than the mirrors. The chromatic nature of the lenses requires adjustments when the energy is changed, and it can be a challenge to cover the full energy range smoothly. Another challenge is that horizontally we require a large demagnification and therefore a relatively large number of lenses.

The switch between CRLs and KB mirrors will lead to a change of direction of the beam and a translation of the beam at the sample position of around 7 mm vertically and 5 mm horizontally.

At the time of writing this report, the actual performance of the optics has not been measured. The beamline will start operating with the CRLs-only mode at a fixed energy. Either monochromator can be used but the DCM will be used for the initial rotational crystallography data collection.

Beam for the future second experimental hutch could be delivered using existing CRL focusing, but this would give a horizontal beam size of around 50 μm. Alternatively, additional focusing in EH2 would have to be added for smaller focus.

Higher harmonics are suppressed by different effects. Calculations and simulations have shown that the contamination of harmonics is not a problem but, in some configurations, it might be necessary to detune the second crystal of the DCM (at energies below 8 keV and when using the unfocused beam).

Energy	**SSh open, KB**	**1 μm, KB**	**SSh open, CRL**	**1 μm, CRL**
5 keV (hDCM)	$2.8 \cdot 10^{13}$	$7.8 \cdot 10^{12}$	$6.3 \cdot 10^{12}$	$1.2 \cdot 10^{12}$
11 keV (hDCM)	$5.3 \cdot 10^{13}$	$1.5 \cdot 10^{13}$	$2.0 \cdot 10^{13}$	$3.4 \cdot 10^{12}$
18 keV (hDCM)	$1.7 \cdot 10^{13}$	$4.9 \cdot 10^{12}$	$6.0 \cdot 10^{12}$	$8.8 \cdot 10^{11}$
11 keV (MLM)	$2.6 \cdot 10^{15}$	$7.3 \cdot 10^{14}$	$8.0 \cdot 10^{14}$	$1.3 \cdot 10^{14}$

Table 2.3. Calculated flux at the sample at three different energies (corresponding to closed gap for 3rd, 7th and 11th undulator harmonics). In case of 11 keV / 7th harmonic the flux is also given using the MLM instead of the hDCM. The different configurations are with the secondary source (SSh) either open or cutting the beam so that the beam at the sample is 1 x 1 mm FWHM (1 x 1.6 mm in the case of MLM, CRL) and with either the KB mirrors or the CRLs for final focusing. Flux in photons/s. Simulations using xrt (REF) not taking into account the effect of heat load or mirror errors. Without any filters or windows included in the simulations.

2.2.2.3 Status of the X-ray system

Both monochromators have been aligned and the beam focused on the sample position. We have encountered many issues with the X-ray system during installation, including vacuum and stability issues with both monochromators, mechanical problems with the X-ray lens focusing systems, and misalignment of a fixed aperture. As of writing, most of these problems have been solved, but the performance still needs to be verified, including detailed commissioning of all components of the X-ray system.

2.2.3 EH1 Endstation

Experiment hutch 1 (EH1) hosts the first and primary MicroMAX endstation, centered around an MD3-up diffractometer (Arinax, France) on an in-house motion system, an ISARA2 sample changer (IRELEC, France), a diffraction detector table for two detectors (in-house design) currently housing an EIGER2 X 9M detector (Dectris, Switzerland), and a Cryostream 1000 open-flow sample cryo cooler (Oxford Cryosystems, UK).

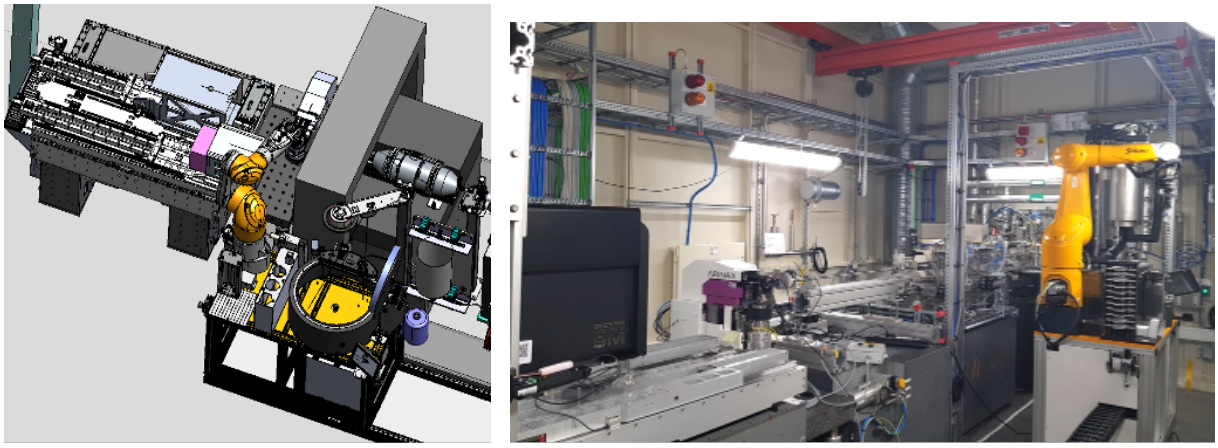


Figure 2.15 CAD drawing and photo of the EH1 experiment setup.

2.2.3.1 Diffractometer

The MD3-up is an integrated diffractometer system featuring an air-bearing, vertically-aligned omega axis, on-axis imaging, and beam shaping tools. Five exchangeable goniometer heads are currently available: a SPINE single-axis, a SPINE mini-kappa, a holder for crystallization plates, one for SSX fixed-target chips, and an empty head. Commissioning to date has primarily been done with the SPINE-based goniometer heads. The empty goniometer head is intended for less standardized experiment setups, like injectors used for SSX. The SSX fixed-target goniometer head has gone through site-acceptance tests without beam and is awaiting full beamline integration. The B-ZOOM on-axis viewing system, with user-variable magnification and a motorized zoom, was adapted with a monochrome camera for the high-magnification optical path for increased performance in high-speed diagnostics of the SSX goniometer head. The in-house sample table design used to position the MD3 was designed to accommodate operation with a one-micron X-ray beam, and to re-position the MD3 between the beam directionality coming from CRL or KB-mirror-based focusing.

2.2.3.2 Sample changer

The ISARA2 sample changer system is based around a 6-axis robot arm from Stäubli. The LN2 dewar layout is equivalent to the ISARA at BioMAX, holding up to 29 UniPucks under cryogenic conditions with automatic refilling. Four different tools are available for the arm to use: SPINE double gripper, laser tool (for calibrations), a crystallization plate gripper and an empty tool (for custom equipment fitting).

The table housing the ISARA2 system has one area for room temperature environment, currently housing a crystallization plate storage and one warm/ambient UniPuck position. This room temperature storage is fitted on a breadboard, with the intention to be easily exchangeable and later be able to accommodate features like a humidity/temperature-controlled sample storage for sample holders of interest.

A translation system allows the ISARA2 to be moved further upstream of the sample position to better accommodate experiments and experiment access in cases where the sample changer is not needed.

2.2.3.3 Area detectors

The first diffraction detector of the beamline is a Dectris EIGER2 X CdTe 9M. The detector has a pixel size of 75x75 μm^2 and consists of 18 individual detector modules. Capabilities include two independent photon energy thresholds, a deadtime of 100 ns, and a maximum continuous frame rate of at least 230 Hz in 16-bit mode. A region of interest mode allows the detector to operate with a subset of 8 modules and increase the 16-bit frame rate to 560 Hz. The detector surface is protected by a commercial detector cover from ADS Engineering, and fitted with a photodiode for flux measurements and beamstop positioning. The 750 µm cadmium telluride sensor layer allows a high quantum efficiency also above 20 keV, with a specified energy range of the detector of 8 to 100 keV. At MicroMAX, operating in the high energy range, and possibly benefitting from photoelectron escape from small crystals, will be possible when using the double crystal monochromator combined with CRL focusing, while the multilayer monochromator and KB-mirror system are designed for lower energies. Site acceptance tests have been performed and a first protein diffraction dataset has been collected. The detector output is generated and collected using the EIGER2 Filewriter and Stream interfaces, similar to the EIGER at BioMAX.

The EIGER2 will occupy one of the two detector positions of the in-house designed detector table. This will provide a sample-to-detector distance of up to 1 000 mm, with the ability to offset the detector horizontally and vertically. A fully motorized switchover enables fast switching between the two detectors. The detector table has radial rails and flexures to accommodate the change in beam directionality between the CRL- and KB-mirror-based focusing.

A Jungfrau 9M from PSI will be on loan during 2024 and installed on the second detector position in EH1. This integrating detector will be able to cater to time-resolved experiments, in particular in the sub-ms time scale. We plan to replace it later with a dedicated Jungfrau 4M detector.

2.2.3.4 Sample cryocooling

For cryogenic operation two Cryostream 1000 systems are available. One standard system (6 mm diameter cold stream and 80 – 400 K temperature range) and one Plus system with a wide bore (9 mm diameter cold stream and 80 – 500 K temperature range). The system is mounted in a temporary geometry and motion system (in-house design) for translations needed for sample changer access. Crystal annealing is possible based on controlling the cold gas flow. The wide bore nozzle will be tested on small-format, fixed-target SSX sample supports under cryogenic conditions.

2.2.3.5 Coming developments in EH1

Future engineering and integration projects for EH1 are described below.

Gantry

The design and installation of the gantry over the sample setup, upstream of the MD3, is planned for 2024. This will be used for SSX injector mounting with improved geometry, load capabilities, and ease of user access. The sample cryo nozzle, and any putative humidity control nozzle, would be moved here for improved geometry and for motion capabilities to adjust for the sample position changing between CRL or KB-mirror final focusing.

Timing system

A central timing system is being developed with a first version planned for early 2024. This will act as a main experiment clock for the synchronization of key components like X-ray chopper, shutter, diffraction detectors, nanosecond laser, diffractometer/sample delivery systems, and X-ray beam diagnostics.

Nanosecond laser integration

The EKSPLA nanosecond laser placed in EH2 (see below) will be integrated into EH1 via optical fiber guiding and with a dedicated sample-proximal optical breadboard. The EH1 part of the setup will include beam diagnostics, post-fiber focusing and guiding to the sample position. Longer term goals include integrating the laser guiding into the on-axis viewing system.

BCU-installation

The beam conditioning unit (BCU, see Section 2.2.5.3) is planned to be installed in summer 2024 and will include many critical features for routine operations and for making efficient use of the higher flux from the multilayer monochromator. This includes X-ray beam position monitors, an attenuation system, and a high-frequency X-ray chopper with a diagnostics system.

KB-mirror operation

When the KB-mirror system is installed (planned 2025) all components downstream will need to be able to switch between the different trajectories of the beam, with and without mirrors. The BCU, sample table, gantry equipment, sample changer trajectories, and the detector table all must be repositioned, and switching between the two modes must be as smooth as possible.

2.2.4 EH2: Laser & spectroscopy lab

The lasers at the MicroMAX will either be used for triggering structural changes in samples, which are then probed by X-rays, or for optical spectroscopy measurements. An offline spectroscopy setup in EH2 will allow users to run preparatory measurements that do not require X-ray exposures, whereas a later online spectroscopy setup will serve as rapid feedback for the crystallographic measurements.

2.2.4.1 Laser1, nanosecond tunable laser

A class 4 nanosecond tunable laser system from Ekspla's NT230 series is already installed at MicroMAX (see Figure 2.16). This laser will be used as the pump source for both pump-probe crystallographic and spectroscopic measurements of light-sensitive samples. Laser 1 has five output ports. Among these 5 output ports, 2 of them are coupled to optical fiber, and the remaining are free-space output. This laser is placed in EH2 and the beam will be delivered for the planned pump-probe crystallographic measurements in EH1 through an optical fiber. The technical specification of Laser 1 includes the following: Wavelength tunability: 210 – 2600 nm. Pulse width: 3 ns. Repetition rate: 100 Hz. Maximum pulse energy: 10 mJ.

2.2.4.2 Laser 2, nanosecond white light laser

A class 3B white laser from NKT Photonics SuperK COMPACT series will be purchased and used exclusively in optical spectroscopy as a probe source. Laser 2 will also be placed in EH2 and the beam will be delivered through free space optics. Laser 2 will provide the following: Spectral coverage: 450-2400 nm. Pulse width: < 2 ns. Repetition rate: 1-20 kHz. Total power: > 110 mW.

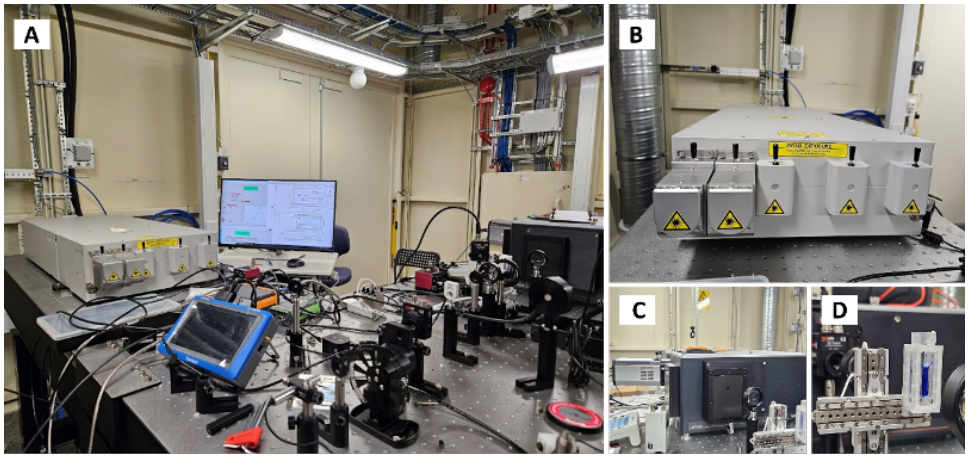


Figure 2.16 Present-day view of MicroMAX EH2. A) Off-line spectroscopy set-up. B) Ekspla nanosecond tunable laser. C) Andor spectrograph and CCD. D) SmarAct translation stage with a test sample in the cuvette.

2.2.4.3 Optical spectroscopy

An offline transient absorption spectroscopy lab is in development in EH2. Once this setup is fully built, it can resolve the spectra in a similar time scale as the diffraction data will be collected. The setup will be capable of measuring the spectra in most of the sample delivery methods used for the crystallographic measurements.

In the current configuration, the offline spectroscopy laboratory uses Laser 1 as the pump source and will use Laser 2 as the probe source. A combination of a spectrograph and CCD from Andor (see Figure 2.16 C) will serve as the detector. A PandABox FPGA will be employed to electronically control the synchronization and timing of the laser and detector. A LabVIEW program will be used to control all the instruments and data acquisition. It is possible to modify the current configuration of the offline spectroscopy so that it can additionally measure fluorescence/Raman spectra from the samples.

The development of an online spectroscopy setup is planned to be implemented in the next 3-4 years. This setup will be capable of measuring the absorption/fluorescence/Raman spectra simultaneously with the crystallographic measurements.

2.2.5 Inhouse developments (sample table, detector table, BCU)

2.2.5.1 Sample table

The sample table includes a granite surface with six degrees of freedom, intended to support and align experimental setups. It consists of two parts: a large block of support granite, mostly upstream of the diffractometer, that will later support the KB mirrors and the BCU; and a movable granite table. Table dimensions are 900 x 500 mm^2 and can hold up to 200 kg. The table granite is shown as transparent in Figure 2.17 in order to visualize the placement of the six linear actuators (legs). On the accessible surfaces, an arrangement of M5 anchoring points is available for securing the setups. In standard configuration, it supports the MD3-up goniometer.

Each leg has a displacement range of ±10 mm, with precision of 1 µm and two sets of linear encoders: one for the motion system (IcePAP), and the other for the PLC. Wiring of the legs to the PLC has a two-fold purpose: monitoring of experimental setup critical points for machine protection; and relaying the position changes to the ISARA2 sample changer for “on-the-fly” sample exchange coordinate adjustment.

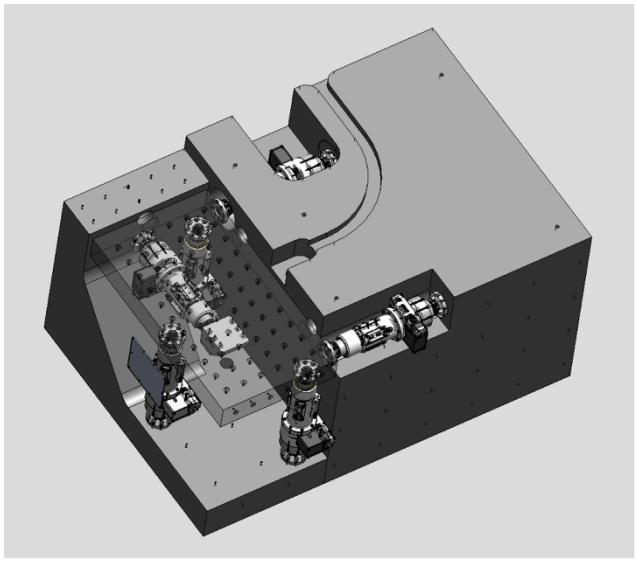


Figure 2.17 Sample table

Additionally, the KB mirrors and BCU will be housed on top of the upstream part (right) of the sample table support granite.

2.2.5.2 Detector table

In EH1, MicroMAX will have two detectors (inboard and outboard), which will be housed on the detector table. The table was designed with 5 axes of adjustment (x, y, z, pitch and yaw) to complement the specifics of our X-ray system. Each axis movement is monitored via absolute encoders. Figure 2.18 shows the arrangement of the axes.

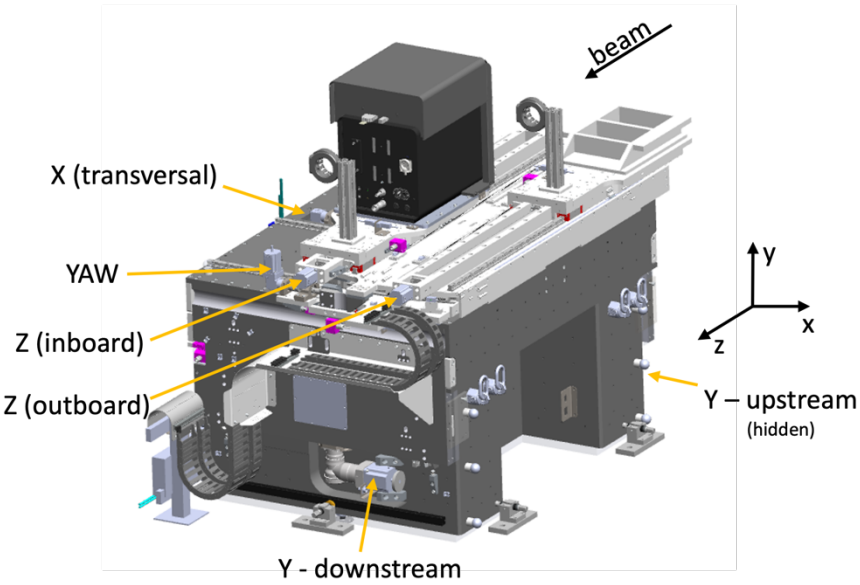


Figure 2.18 Detectors are mounted on a set of parallel rails. Each detector can be independently moved along the beam direction (z-axis) for 940 mm with precision of 0.1 mm. Rails can be rotated around the y-axis producing the YAW movement with respect to the beam with 0.1 µrad precision. This assembly is mounted on top of the table and can be moved perpendicularly to the beam (along x-axis, 10 µm precision), enabling simple swap between detectors. Finally, independent Y-axis adjustments on upstream and downstream sides of the table enable the pitch movement (1 µrad precision) with respect to the beam.

2.2.5.3 Beam conditioning unit (BCU)

The role of the BCU is to facilitate manipulation and characterization of the beam just prior to the sample. The BCU is placed on a set of linear actuators for aligning the chamber with the beam. The movement range is sufficient to cover the beam positions and orientations with respect to the CRL or KB mirrors.

Inside the chamber, all components, except the chopper, are mounted on a rail system. Additionally, each component is mounted on one or two fine resolution (100 nm) positioning stages for high precision alignment.

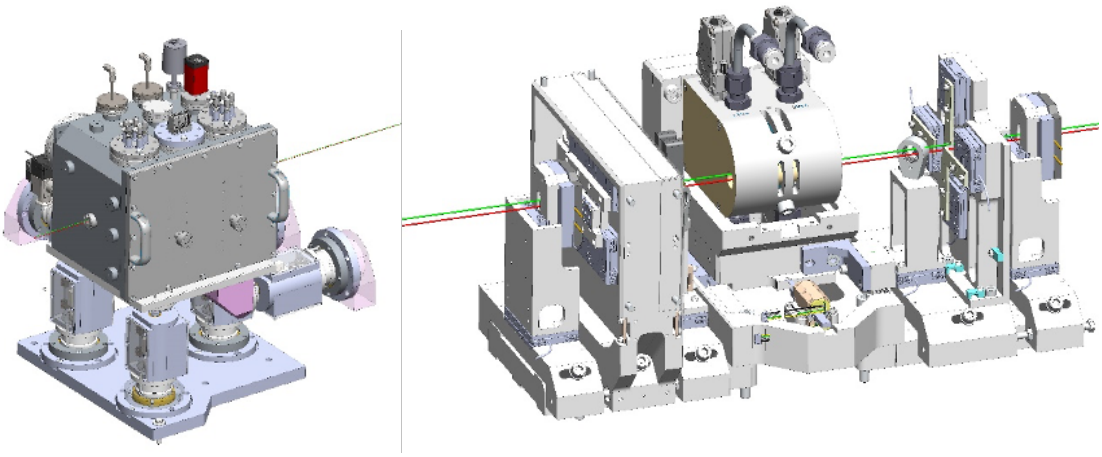


Figure 2.19: left - BCU with the linear actuators and right – arrangement of components inside the BCU. From right to left: XBPM1, Slits, Alignment mirror, Chopper, Shutter, Scintillator screen, Attenuator and XBPM2

Both XBPMs (Cividec B9) will be the same we have installed in the OH & EH1 optics. Slits are made with four independently moving tungsten blades, forming a rectangular opening. The magnetic-bearing chopper is from Celeroton. (Figure 2.20). It is positioned in the middle of the BCU. Its electronics are water-cooled. The rotating disc has six different cut-out profiles allowing for varying beam on/off ratios – from 0.8 % to 70 %.

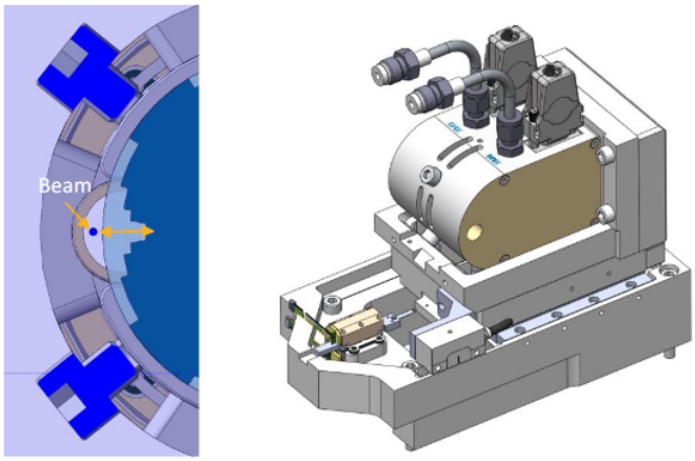


Figure 2.20: Cross-section view through the chopper (left). Chopper is mounted horizontally (right) due to its weight and stability requirements. Moving the chopper transversally to the beam, we can choose the desired cutout profile of the disc or the fully unobstructed beam.

As the chopper disc is magnetically levitating, the issue of cooling was resolved by designing the BCU to operate with a low pressure He atmosphere (<50 mbar). Inlet and outlet ports for pumping and venting of the chamber are not shown in the images above.

2.2.6 Science, experiments and roadmap

With MicroMAX commencing user operations, the coming years will come to need to include both commissioning activities and user operation. This transition further increases the need to prioritize and structure coming functional upgrades, as more staffing resources will be occupied with user operation.

To plan functional upgrades, a set of experimental milestones are currently being defined in terms of X-ray delivery requirements, equipment and integration needs, as well as what type of user program and

outreach would be needed. The milestone experiments will be outlined for the coming year with higher level of detail, and aim for a targeted but gradual deployment of new beamline capabilities. To highlight larger technological developments and larger extensions in possible science cases, certain milestone experiments will also be labelled as flagship experiments.

The scientific strategy is based around offering a set of standard experiment configurations (like rotation data collection on SPINE-mounts, or SSX injector experiment with nanosecond laser activation) to act as drivers for building the user community. In addition to such offered experiment setups, user proposals and custom requests can extend the targeted experiment types but likely with less extensive beamline integration (such as complex MXCuBE adaptations or custom ISPyB fields).

The user program will start with rotation data collection as a well-known quality metric to optimize beamline performance. A 5-micron beam in monochromatic mode will be the standard configuration, to welcome samples that benefit from microfocus beam, and offload BioMAX from such experiments. After reaching a remote-friendly (but not fully autonomous) operation state for rotation data, emphasis will be placed on SSX developments and time-resolved experiments. Initial work for SSX will target low-cost and user-accessible sample delivery methods such as SPINE-like fixed-target experiments and capillary injectors. Such setups can be sample-usage efficient and allow new, as well as experienced user groups, to establish suitable sample batches for either low-dose data collections or act as a stepping stone towards more complex time-resolved experiments. Sample feasibility beamtime will be used to lower the administrative burden for new user groups that could benefit from SSX experiments and want to find suitable sample preparation conditions.

A first flagship experiment will combine high-viscosity extrusion (HVE) sample delivery with laser-triggering and X-ray chopper timing control, to showcase the improved temporal resolution possible with the multilayer monochromator and storage cell mode of the Jungfrau detector. In addition to studies of light-sensitive samples or caged-compound release, this experimental setup can be used for temperature-jump experiments to generalize the types of sample systems and dynamics that can be probed.

Later developments will extend fixed-target SSX into more complex experiments and with increased experiment automation. This will include making use of large-format sample supports and high-performance scanning stages for fixed-target supports, to collect complete SSX data sets rapidly and with minimal sample consumption. The later flagship experiments include offering mixing experiments, like for enzyme reaction path studies, acting as a complement to laser-triggering and making on-(X-ray)-axis pump-probe experiments possible. With the KB-mirror system becoming available, the beamline will reach its maximum flux density and allow for shorter time scales and smaller samples to be studied.

Extending fixed-target-based SSX into more complex data collections like on-chip-mixing with hit-and-return time series, is seen as scientifically very interesting and as a highly sample-efficient way of doing time-resolved SSX experiments. It however requires extensive hardware developments and compete for in-house resources for motion control developments, at a time when several in-house design projects have already become delayed. It is therefore not targeted as heavily as flow-based sample delivery methods for the first years of operation. This could come to be revised based on user requests and/or possible collaboration-based developments.

The different milestone experiment goals will, in addition to experiment setup improvements, also be coupled to developments of EH2 (used as laser and spectroscopy lab in initial operation) and the beamline laboratories, for extended sample characterization and preparation, such as offline sample delivery optimization and spectroscopy for establishing in-crystal kinetics. The longer (4 year) goals include adapting EH2 for X-ray experiments and this will depend on the user requests and science/operational cases that develop during the initial years of EH1 operation.

#	Milestone	Timing	Goal	Motivation / Science case
M1	**Friendly user rotation data collection**	2023 Q4	First external user experiment: SPINE rotation data collection using MD3 diffractometer	Commence MicroMAX user operation
M2	**Routine remote rotation data collection**	2024 Q1	Remote users operating MicroMAX at fixed-energy and fixed-focus for cryogenic data collection, assisted by the ISARA2, MD3, Cryostream, EIGER2 and data auto-processing	Extend number of MX samples that can be collected on at MAX IV and provide a permanent configuration targeting the use of a 5 µm beam for MX.
M3	**Fixed-target SSX with SPINE-like sample supports**	2024 Q1	Collect SSX data from SPINE-like fixed-target mounts, with live image analysis Sample changer assisted and variable temperature [cryogenic and non-cryogenic]	Start of SSX user program in a low barrier-of-entry mode for sample screening and steady-state data collection
M4	**First injector experiment**	2024 Q2	Simple injector experiment on MD3 with basic MXCuBE integration User-provided setup if performing pump-probe	Offer flow-based SSX sample delivery to both cater to high-viscosity samples (e.g. LCP) as well as a steppingstone towards pump-probe injector experiments.
M5	**Extended SSX early user program**	2024 Q4	Open up for sample feasibility checks for users [fixed-target and flow-cell] – proposal-less screening beamtime Collect MLM data for such an experiment and easily switch between the 3 available photon energy bandwidths and variable attenuation	Simplified access route and beamtime scheduling to screen SSX samples. Make it easy to test data quality impact of different photon energy bandwidths, to optimize SSX experiments
M6 (F)	**High data-rate injector pump-probe experiment**	2025 Q1	HVE and JUNGFRAU used for chopper pump-probe experiment with nanosecond laser, using pink beam	Showcase the time-resolution of MicroMAX and provide a standard experiment setup for injector microsecond data collections.
M7	**Rotation data collection improved feature set**	2025 Q3	Offer rotation data collection across full DCM energy range, from MXCuBE Variable beam focus (CRLs) from 5 to ~ 200 micron, from MXCuBE < 5 micron beam with KB-mirrors proof-of-principle experiment	More variety to user adjustable beamline configurations to cater to topics like anomalous data collections or crystals with large size variation. Smallest focus to collect data on challenging sample systems with small crystals
M8	**Fixed-target SSX – new capabilities**	2025 Q3	Automatic collection of fixed-target samples (ISARA2 assisted) Collect data from crystallization plates Collect steady-state datasets with MD3 SSX head synchronized to X-ray chopper	Make fixed-target SSX of small-format (SPINE-like) sample supports available as a mail-in service, that can operate in a semi- to fully automatic mode. *In situ* SSX from crystallization plate data collection and complete SSX data collection from single large-format sample supports
M9 (F)	**Mixing experiments user operation**	2025 Q4	Offer ms-time-scale mixing experiments as routine user experiment To be evaluated if in-house driven or collaboration-based	Offer standard experiment setup for experiments where laser activation is not feasible or the most practical approach. Showcase with structural enzymology example
M10	**SSX automation**	2026 Q2	ISARA2 upgrade for improved room-temperature sample handling (incubator + large-format chips) Flowcell experiment setup with multiple inlets for mail-in/remote injector SSX in use	Automation improving throughput and sample screening abilities, to further lower barrier of entry for users that could benefit from SSX data and trivialize data collection
M11 (F)	**Large-format fixed-target supports with < 5 µm pump-probe experiments**	2026 Q3	SSX goniometer head used for HARE experiment assisted by ISARA2 sample exchange and on-axis reaction initiation KB-mirrors and MLM Evaluate feasibility of environment control around sample support	Allow hit-and-return data collection strategies with fixed-target sample supports to maximize data amount per prepared crystal. Able to handle more challenging samples and TR-SSX experiments due to KB-focusing and on-axis excitation.
M12	**First X-ray experiment in EH2**	2027 Q2	Proof-of-principle experiment in EH2 to showcase beam delivery and data acquisition capabilities	Offload complex experiment setups to EH2
M13 (F)	**Custom sample environment in EH2**	2027 Q4	User experiment that could benefit from the increased experiment setup space Integration with central timing system and MXCuBE	EH2 entering user operation – initial experiment depending on current user and science cases

Table 2.4. Proposed list of milestones for experiments / functionality. "F" in the first column refers to flagship experiments that constitutes major upgrades of the functionality of the beamline.

2.3 Beamline control and data analysis software

BioMAX and MicroMAX are intended to share software at different control levels so that it takes fewer resources to maintain and deploy new developments on both beamlines. For low level control, Tango (https://www.tango-controls.org/) and Sardana (https://sardana-controls.org/) are used, and they are supported by the MAX IV software group. On a higher level we continue to use MXCuBE (Mueller *et al.*, 2017; Oscarsson *et al.*, 2019), EDNA (Incardona *et al.*, 2009) and ISPyB (Delageniere *et al.*, 2011), which are jointly and continuously being developed via the MXCuBE/ISPyB collaboration. Local adaptations and new features have been constantly added to fit into our local hardware and software environment as well as to meet the requirements of user experiments.

Users interact with the beamline software as follows:

1. After the users generate a beamtime session in DUO and complete the required information they can set up the sample shipment in ISPyB and upload the sample information, which will be imported to MXCuBE during their beamtime (Figure 2.21).
2. Users control the beamline and collect data via MXCuBE, which sends the collection information to the ISPyB database and triggers different processing pipelines via EDNA, according to the experiment type (e.g., SSX or oscillation data collection)
3. Processing results are saved in ISPyB and may be sent back to MXCuBE where required by certain experiments. Users can view their collection and processing results in ISPyB both during and after their beamtime.

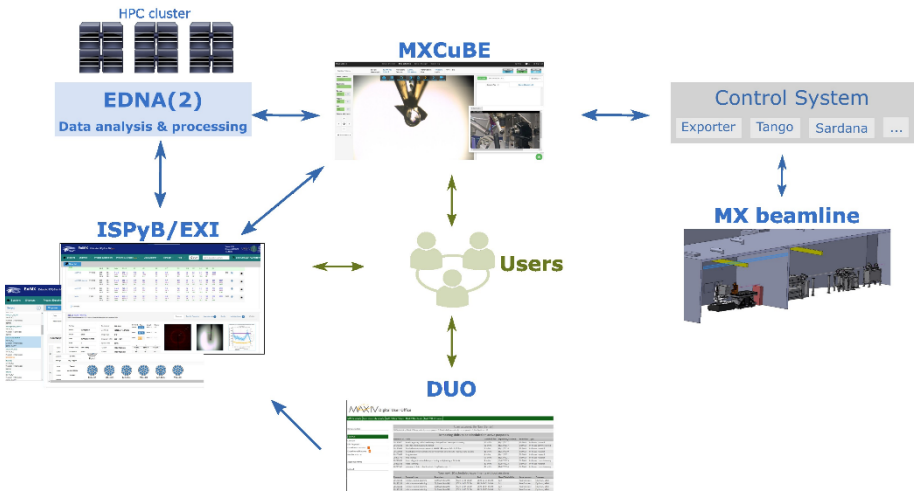


Figure 2.21 Overview of control and analysis software at BioMAX and MicroMAX. Arrows in green show the interactions between users and software, while arrows in blue show the communication between different software.

2.3.1 MXCuBE

MXCuBE stands for Macromolecular Xtallography Customized Beamline Environment, and it is the main beamline control software for users to carry out their experiments. Local users can use low level control interfaces only in special circumstances, like non-routine experiments or during tests of a new instrument, while remote users only have access to MXCuBE for experiment control.

The MXCuBE project is supported by most synchrotron facilities in Europe as well as several facilities on other continents (https://mxcube.github.io/mxcube/). The web version, MXCuBE3, was initially developed by MAX IV and ESRF, and first deployed at the BioMAX beamline at the beginning of its user operation in 2017 (Mueller *et al.*, 2017).

2.3.1.1 MXCuBE3 at BioMAX

After years of development, many new features and collection experiments have been implemented in the MXCuBE3 at BioMAX, including but not limited to the following.

- To minimize radiation damage and help users to plan the dose budget effectively, an interactive dose estimation (Figure 2.22) is displayed in the collection setup interface. The dose calculation is inspired by the crystal lifetime calculator at https://bl831.als.lbl.gov/xtallife.html (Holton, 2009). Using the given collection parameters, it gives an instant estimation of the dose as well as the maximum number of images that can be collected within a dose limit that can be selected depending on the experiment, or entered by the user. The flux at the sample position is measured on a calibrated photodiode before each data collection. The flux values before and after the data collection are written to ISPyB along with other beamline information.

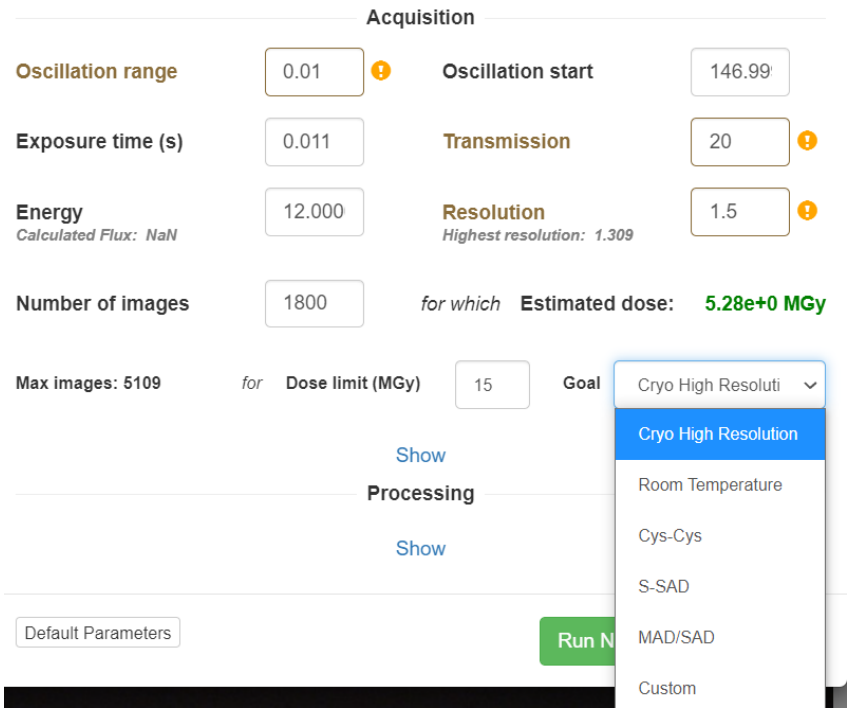


Figure 2.22 X-ray dose estimation in the acquisition configuration interface.

- Automatic X-ray centering. After auto loop centering, users can choose to center their crystal with X-rays. After users define the scanning area, the software does a mesh scan of the area, searches for spots in the images, locates and centers on the beam the best diffracting region in the scanned area, rotates the crystal by 90 degrees, performs a line scan to locate the sample at the new orientation, and finally centers the crystal (Figure 2.23 A). With the on-the-fly analysis by Dozor (Melnikov *et al*., 2018), the crystal can be identified immediately after the scan. Currently, a typical full centering procedure (with a mesh of 30 x 30 cells) takes one minute, and the running time can be reduced by parallelization and optimization of the collection procedure. The X-ray centering is very reliable (Figure 2.23 B) and is very useful to locate crystals, especially those that are difficult to identify optically.

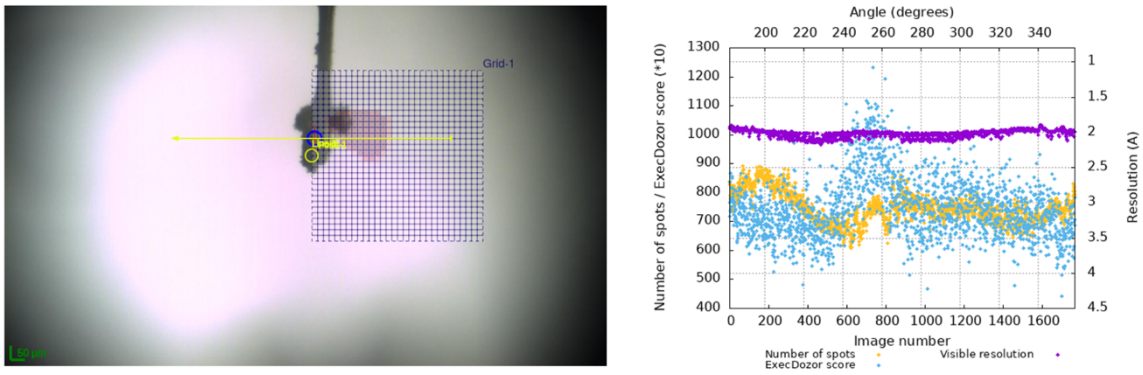


Figure 2.23 A) automatic X-ray centering. B) Plot of the image analysis results of a rotation dataset collected on the position determined from automatic X-ray centering.

- To provide a good starting point for manual or X-ray centering, we developed a simple fast, and robust auto loop centering that simply looks for the tip of the sample holder. It succeeds even when the sample holder is not clearly visible in the first image for different reasons, as shown in *Figure 2.24.* By default, the centering procedure checks five different orientations, and it takes around 11s in total.

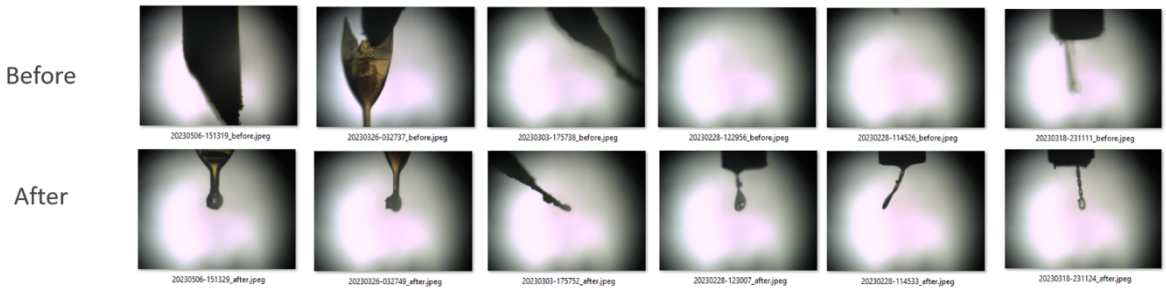


Figure 2.24 in-house developed simple, robust loop centering.

- With an accurate and automated way to center the crystal in the X-ray beam, we developed the first version of unattended data collection, which allows users to select a group of samples and set up the collection parameters ("diffraction plan") in MXCuBE that will be then applied for data collection for all selected samples (see Section 2.1.2.1). In order to extend unattended data collection to use different diffraction plans for different samples, we are developing ISPyB and MXCuBE so that users can include the diffraction plans in the sample list uploaded to ISPyB, which will be imported and used by MXCuBE. However, this plan still assumes that a) the users already have some experience working with the samples and b) the samples have consistent diffraction properties. To further provide users with an expert system in cases where those assumptions do not apply, we are collaborating with Global Phasing Ltd. to integrate their workflow of automatic sample characterization and strategy calculation, which would allow fully automated experiments from a wider range of projects and also be beneficial for non-automated data collection.
- MXCuBE was developed to support various experiments as described in Section 2.1.2, including:
    - Interleaved MAD data collection and inverse-beam collection
    - Improved support for injector-based experiments, including time-resolved SSX and automatic generation of input files for CrystFEL
    - *In-situ* collection from crystallization plates.
- We have introduced a series of "beamline actions", or short scripts to perform useful functions by interacting with beamline instruments which are not directly accessible to the users. For example, there is a beamline action that allows the users, with a click of a button, to open the beamline after an electron beam dump in the storage ring– a complex procedure that involves opening valves and

beam stoppers in the correct order, closing the undulator gap and resetting some motors. Other beamline actions include MD3 aperture alignment, flux calculation, and sample annealing. Beamline actions have also proven very useful for implementing workarounds or recovery procedures in cases of temporary failure of an instrument at the beamline (for example, resetting stuck motors in the MD3 microdiffractometer), which would have had a more serious impact on user operation otherwise.

- To speed up experiment changeovers and help diagnose problems at the beamline, an MXCuBE based script was developed that automatically prepares the beamline for user operation. The script checks several major devices, resets beamline servers, aligns the beam, measures the flux, takes air scattering images, measures the direct beam position, and cleans up open processes on the remote desktop server. All the preparation steps are logged, and the results are sent to staff by email with a short summary (Figure *2.25)*

20230530-130704_pre_...
26 KB

6 attachments (1 MB) Download all

!!!!!!!!!!!!!WARNINGS!!!!!!!!!!!!!!!
- Warning! Cryo nozzle is parked, please make sure it's not in manual position

++++++++++++++LOG++++++++++++++++
- Starting beam delivery...
- Checking the status of devices (md3, isara)...
- Aligning beam...
- Checking beamstop alignment...
- Taking air scattering image...
- Measuring direct beam position...
- Preparing open hutch...
- Checking software and data folder...
- Checking biomax remote server, logging out users and killing instances...
- Checking mxcube branches
++++++++++++++REPORT++++++++++++++++
Summary of prepare operation:
- energy 12.700000 keV
- focus beam size 50 micron
- Estimated flux at BCU is 8.76846702583e+12 ph/s (100% transmission)- md3 phase change is OK
- beam is aligned to the center, see the attached image
- aperture 50um is aligned to the center, see the attached image
- aperture 20um is aligned to the center, see the attached image
- aperture 10um is aligned to the center, see the attached image
- flux at sample position with 50 micro aperture and 100 percent transmission is 8.40E+12 ph/s
- measured direct beam @146mm is x,y: 2094.5316375, 2120.46820942

Find folder /mxn/groups/biomax on server biomax-remote

Find folder /data/visitors/biomax on server biomax-remote

Find folder /data/visitors/biomax on server localhost

- Have killed all albula instances on the biomax remote server.
- Have killed all xsession instances on the biomax remote server.
MXCuBE branches look good:
  * repo configuration : master
  * repo mxcube3 : biomax
  * repo hardwareObjects : 2.2
- please see the attachment for the aligned beam, air scattering and log file

Figure 2.25 Example of the email sent by the procedure that prepares the beamline for user operation.

2.3.1.2 MXCuBE-web at MicroMAX and MXCuBE upgrade

The current version of MXCuBE running at BioMAX is based on Python 2.7, which is no longer supported. Furthermore, it has diverged significantly from the master version of MXCuBE (https://github.com/mxcube) since the spring of 2019. Therefore, when we started MXCuBE at MicroMAX, the latest version of MXCuBE-web from Github was used and adapted for MicroMAX and the MAX IV infrastructure.

The new version supports the MD3-up diffractometer, the ISARA2 sample changer, the EIGER2 X CdTe 9M, and other beamline controls. The complete feature set for routine rotation data collection is present, and further testing as well as interfacing to external systems (for example, EDNA2 and ISPyB) are ongoing. The next step is to support basic SSX experiments, like injector-based data collection and basic raster scan of fixed-target chips. In the long run, MXCuBE will be adapted to various advanced SSX experiments.

Meanwhile, we need to upgrade the MXCuBE at BioMAX to the same version, so joint development can be resumed and we can take advantage of other improvements carried out by our international collaborators. As MicroMAX has similar low-level control software, scientific software environment, and the same MAX IV infrastructure, we will start the MicroMAX MXCuBE-web as the initial point for the upgrade and add missing features that are available in the current MXCuBE3 at BioMAX. The plan is to have the new version ready for testing during the coming winter shutdown and deploy it in February 2024.

2.3.2 ISPyB

ISPyB (Information System for Protein Crystallography Beamlines) is a Laboratory Information Management System (LIMS) combining sample tracking and experiment reporting at synchrotron beamlines (Delageniere *et al.*, 2021). Like the MXCuBE project, it is also supported by the major synchrotron facilities in Europe (https://ispyb.github.io/ISPyB/).

The ESRF has been the main driving force of the development of ISPyB. At MAX IV, ISPyB was adapted to work at the MX beamlines, particularly for sample shipment, sample management, and importing information from the DUO user portal. In 2020, the EXI interface to ISPyB was deployed at MAX IV. EXI provided faster display, a more intuitive user interface, and simplification of user processes. We have been using EXI with the ISPyB back-end since then.

Other user facilities have their own LIMS system for managing protein, crystallization, and crystal information, such as Icebear (Daniel, *et al.* 2021), which was developed by the Biocenter Oulu and now also used at several other facilities. We have collaborated with the Icebear team and developed some APIs so that they can create sample shipments automatically and can redirect their users to the collection entry in MAX IV ISPyB from their system. This work has been merged into the main ISPyB code, so that Icebear users have access to the same features when measuring data at other synchrotron facilities that use ISPyB.

Recently, we have begun implementing a new web interface project py-ispyb-ui, a React-based web interface for ISPyB. The React-based interface is advantageous because more software developers, including the software team at MAX IV, are familiar with it. The test py-ispyb-ui is already connected to the ISPyB backend and many of the functions are already in place (Figure 2.26). However, to be able to offer our users the new interface, we need to add the missing features that are present in the current EXI interface and that are required by our environment. In addition, we need to implement sample information management for the new systems we have implemented for data collection at room temperature, and, most critically, adapting sample diffraction plans for unattended data collection (2.3.1.1).

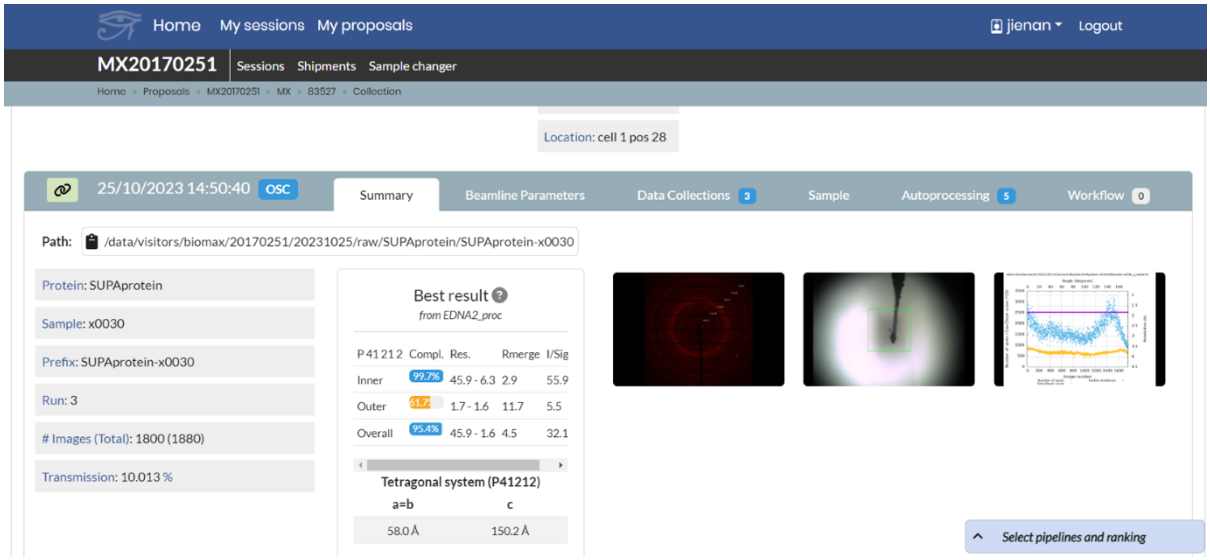


Figure 2.26 Py-ispyb-ui displaying data collection summary of a recently measured sample at BioMAX.

The current py-ispyb-ui presentation of data collection, processing results, and sample and data management, is not suitable for SSX experiments. Therefore, we have developed new interfaces and features for SSX in py-ispyb-ui. However, the "SSX py-ispyb-ui" interacts with the database with a different back-end. At MAX IV we are planning to pursue the development of a Python-based back-end (py-ispyb). The planned architecture is depicted in Figure 2.27.

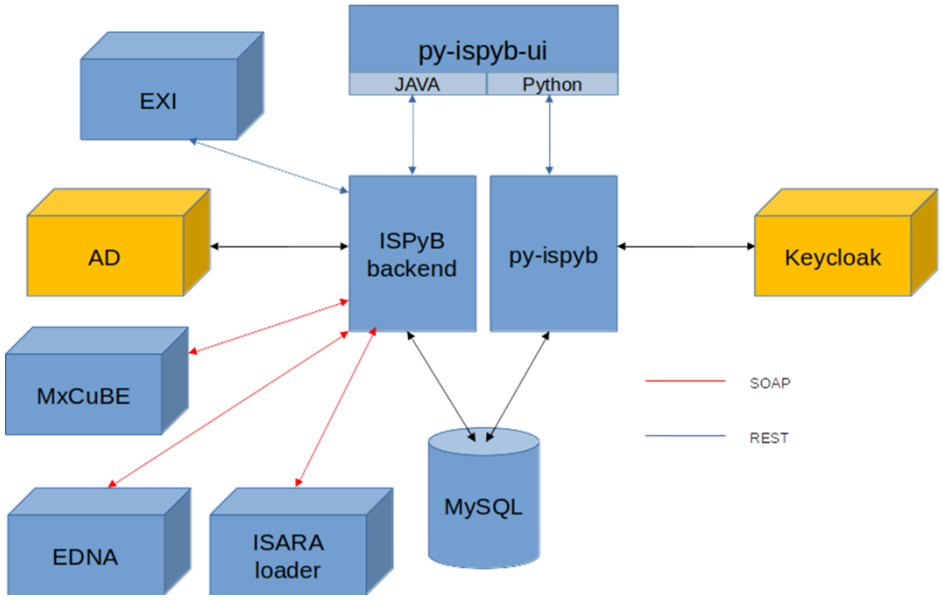


Figure 2.27 Architecture of the ISPyB server and its interaction with other systems.

2.3.3 Data analysis and processing

Given the highly automated data collection as well as the high data rates of EIGER2 and Jungfrau detectors, which are in the range of 100-2200 Hz for routine operation, automatic data analysis and processing are critical and different processing pipelines are required for rotational and serial data collections.

There is also a need for providing manual off-line processing facilities for the users, in case the automated data processing software does not provide optimal results or the users do not have the necessary computing capability or experience handling the MAX IV data format. For manual data processing, we use

the PReSTO platform, a project for the installation and adaptation of structural biology software for use in HPC environments (https://www.nsc.liu.se/support/presto/).

2.3.3.1 Automatic pipelines for rotation data

For rotation datasets, fast_dp (Winter & McAuley, 2011), EDNAProc, autoPROC (Vonrhein *et al.*, 2011) and Xia2/Dials (Winter, 2010) have been used for the data reduction routines at BioMAX. To provide users faster feedback of the data quality, we run a peak search of images using Dozor (Melnikov *et al.*, 2018). The Dozor score, number of spots, and resolution limit are plotted for each image (Figure 2.28). This tool is very useful to evaluate the centering quality (Fig. XB) and can also indicate radiation damage when cross-checked with other factors. Due to the history of instability with the EIGER 16M at BioMAX (Section 2.1.1.6), the peak search is carried out using the HDF5 images stored on disk. It takes less than one minute to analyze a dataset of 1800 images. In the near future, we plan to use the images from the zeromq stream interface, which will give users immediate feedback right after the data collection. This will become part of the MX_Suite software as described later.

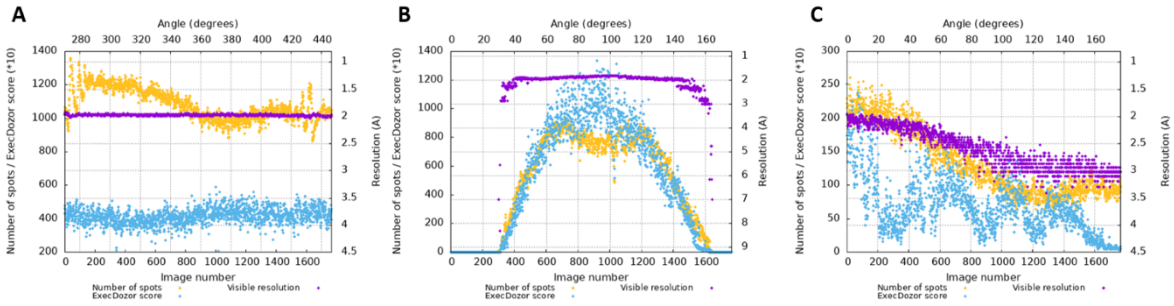


Fig. 2.28 Examples of plots of Dozor peak search. A) a good dataset. B) partially centered crystal. C) radiation damage

We have adapted fast_ep (https://github.com/DiamondLightSource/fast_ep), for automated experimental phasing. At the end of the reduction pipeline, if a significant anomalous signal is identified, fast_ep will be triggered. In addition, we are developing in-house MR pipelines. Users will soon have the option to upload a PDB file as a search model for automated MR. In addition, we have developed a workflow for running MR without the need for the user to supply a search model. Given an input sequence or UniProt ID, an EDNA2 Task (see below) runs Alphafold (Jumper *et al*., 2021) to generate search model(s). The best models are prepared for MR using model preparation and analysis tools from Phenix (Liebschner *et al*., 2019) then runs Dimple (CCP4) (Agirre *et al*., 2023) for MR analysis.

All processing pipelines are executed and managed in EDNA, which interacts with both MXCuBE and ISPyB. The original EDNA is a framework for plugin-based applications that are applied to online data analysis and was initially developed for different X-ray experiments at synchrotrons (Incardona *et al*., 2009). Today the MX part is still active and has become part of the MXCuBE collaboration. In 2019, ESRF initiated the EDNA2 project (https://github.com/olofsvensson/edna2), which is a complete rewrite of EDNA based on Python 3. Instead of plugins, EDNA2 handles individual processes as “Tasks.” Multiple Tasks can be run concurrently, and each Task can launch and monitor subtasks of their own. Task data input and output is handled by the JSON file type, as opposed to the less human-readable XML file type of EDNA-MX. In addition, EDNA2 has built-in support for submission of jobs to the Slurm job management system, which is used on MAX IV’s computing cluster. Tasks with compute-intensive processes can therefore be managed easily within EDNA2.

Recently we have set up EDNA2 and adapted it to our processing pipeline needs and the MAX IV computing infrastructure. We also developed a new inhouse EDNA2proc pipeline, which replaced the previous EDNAproc and is currently used for fast data processing. In addition, a wraparound Task, MAXIVAutoProcessingTask, runs and monitors each task (Figure 2.29). EDNA2proc and fast_dp are run first, giving users feedback in less than five minutes. The output from the fast pipelines, if they are successful,

are used as starting parameters for autoPROC and xia2/DIALS, which are subsequently run. Because these latter two pipelines are more focused on optimizing data quality over processing time, they can take anywhere from 15 min- 1 hr to finish. Thus, the "fast" pipelines can serve to guide users during their beamtimes, while using the output from the "comprehensive" pipelines for analysis at home.

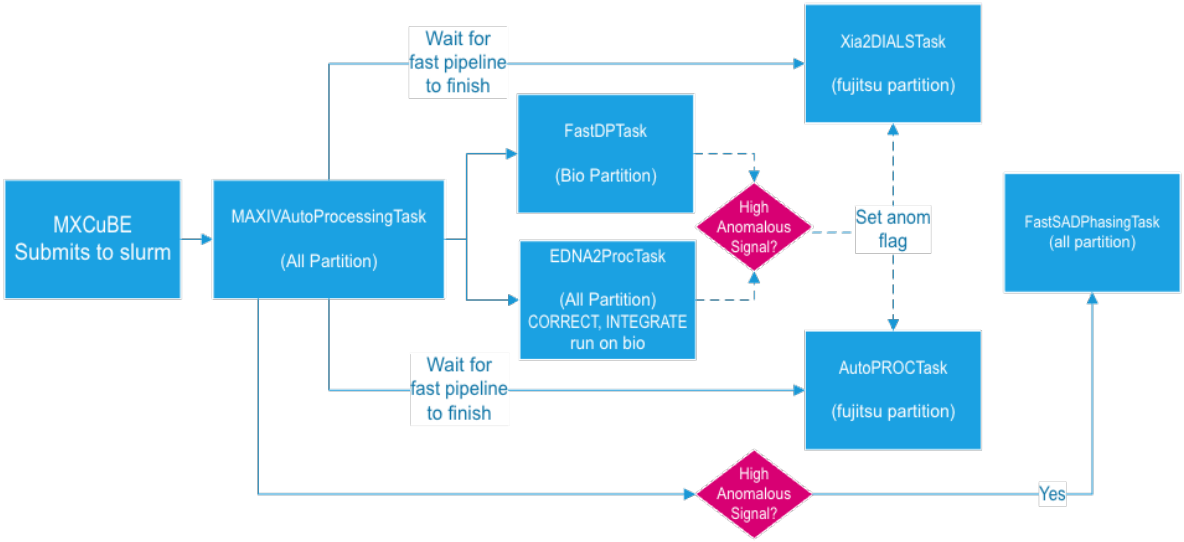


Figure 2.29 Flowchart of automated data processing pipelines for standard rotation data collection.

2.3.3.2 Real-time data analysis and automatic processing for SSX

For SSX experiments, it is crucial to get on-the-fly feedback so that users can use the information for further sample preparation and adjust the experimental setup. To meet the needs of different types of SSX collection, we are developing a real-time data analysis package, MX_Suite (Figure 2.30).

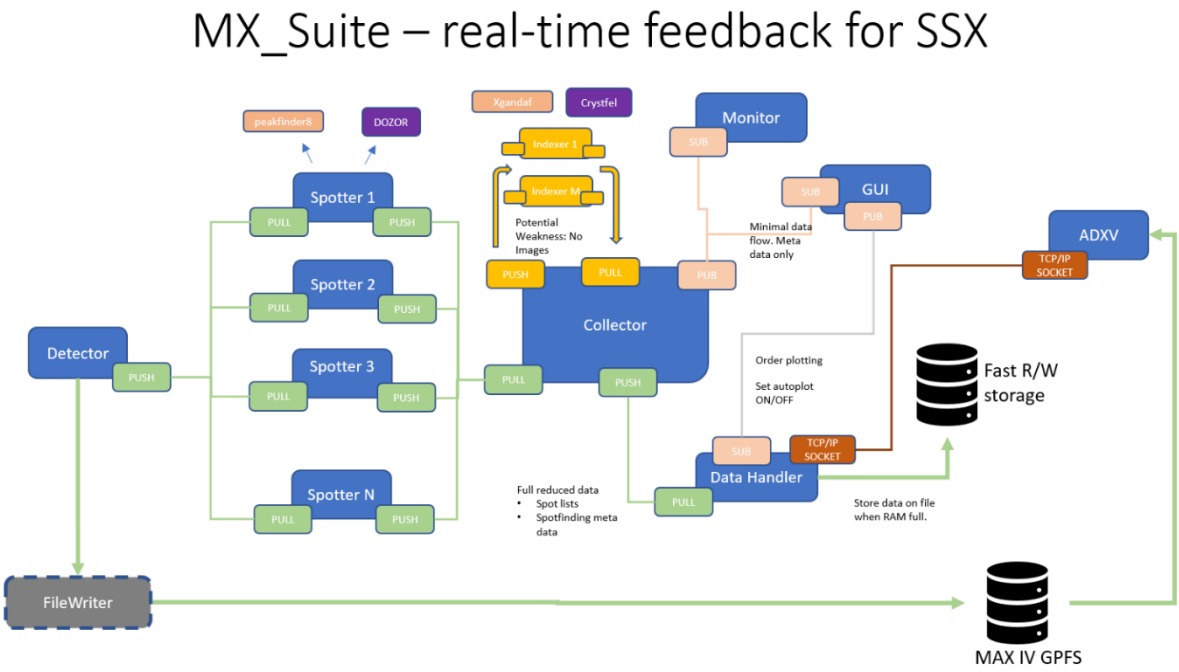


Figure 2.30 Flowchart of MX_Suite for real-time feedback.

MX_Suite will be kept running as a service, which has multiple parallel jobs on the cluster nodes. Before each data collection starts, all the jobs should be updated with the new meta information. Once the

detector is triggered, the peak search (spot finding) jobs receive images directly from the detector via the stream interface and analyzes them using a user- or system-defined spot finding program. The results, including spot list from all parallel jobs, will be collected by one collector job, which then forwards them to the indexing jobs for optional further analysis. The collector publishes the results in batches, which can be received by the user monitor, data handler, and the internal monitor service. The current focus is mainly on peak search and indexing, so for the user monitor interface we started with the GUI of Interceptor (https://github.com/ssrl-px/interceptor) and adapted it to our needs. We will integrate it with ADXV (https://www.scripps.edu/tainer/arvai/adxv.html), so that when user clicks a point on the interceptor GUI, the ADXV application will show the corresponding diffraction image, identified peaks and indexed peaks if applicable (Figure 2.31).

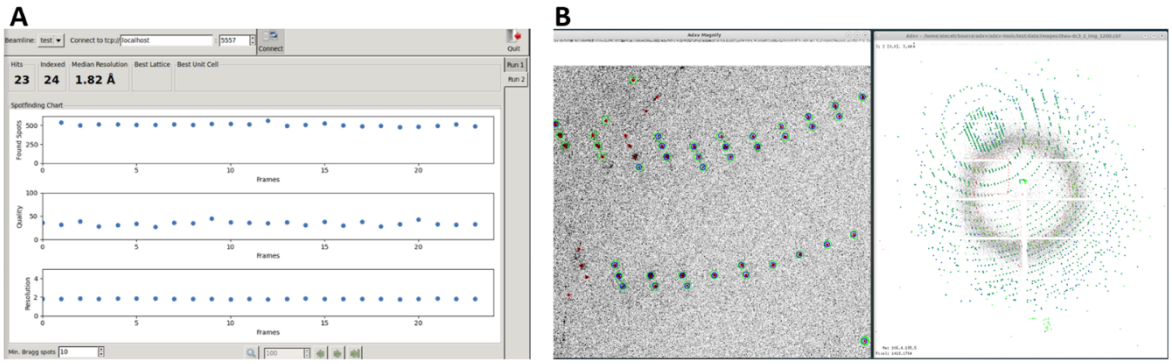


Figure 2.31 (A) modified Intereptor GUI, which is used as user monitor for MX_Suite. (B) ADXV view which shows diffraction image and multiple sets of spots.

MX_Suite can also be used for rotational experiments. Currently we are running two different Dozor based workflows for normal operation, one for X-ray centering using streamed images and one for data quality evaluation using HDF5 images. With some modification, MX_Suite should be able to replace both and provide faster feedback as discussed earlier.

We would like to set up or develop an automatic data reduction pipeline that can take the images from the hit-finding step and carry out indexing, integration, evaluation and merging. There are several software packages, including CrystFEL, nXDS (Kabsch, 2014), and cctbx.xfel (Sauter *et al.*, 2013), that have tools to serve such a function. From our tests and experience, it is most likely that we will develop or use CrystFEL-based processing pipelines.

2.3.3.3 Off-line data processing

MX users can use the MAX IV centralized computing resources for processing their data manually. In practice, this happens only rarely during data collection, given the high throughput and the fast availability of the autoprocessing results. However, the users can connect to an “offline” HPC (see Section 2.6.3) to reprocess the data.

To facilitate the access to the MX data processing software and hide the complexity of the HPC environment to users, we use PReSTO, a software stack for integrated structural biology available for the National Academic Infrastructure for Supercomputing in Sweden (NAISS) and the local MAX IV computer clusters. PReSTO is a collaboration between NSC, MAX IV, Linköping University, and Karolinska Institutet. Currently, the software packages XDS, CCP4, PHENIX, PyMOL, autoPROC/BUSTER/SHARP, ChimeraX, hkl2map, O, CrystFEL, and Alphafold are available through PReSTO. Many programs supported by PReSTO have built-in Slurm support, which can automatically submit jobs to the HPC queue. Jobs can also be submitted interactively. The PReSTO website has tutorials available for supported programs and we have also created MAX IV specific documentation on our web site.

2.4 FragMAX facility

2.4.1 Facility description

2.4.1.1 Introduction

The high throughput achievable at BioMAX has led to an extraordinary improvement in productivity and has enabled establishment of the FragMAX facility. The FragMAX facility supports structure-based drug and chemical tool compound discovery at MAX IV Laboratory (Lima *et al*., 2020). It was designed as a platform for crystal-based fragment screening, but the underlying workflows are applicable to all medium- to large-scale protein-ligand studies. The platform is comprised of four primary elements: (i) a medium-throughput crystal preparation facility, (ii) a collection of different fragment libraries, (iii) automated diffraction data collection at the BioMAX beamline, and (iv) novel tools for large-scale data processing. In 2019, the FragMAX platform began providing services to external users and has since established an international user program that is accessible to academic and industrial research organizations. Below is an overview of the different components of the facility and how they can be accessed.

2.4.1.2 Crystal preparation lab

FragMAX has a crystal preparation facility that is co-located with the Lund Protein Production Platform (LP3) at Lund University's biology department. The laboratory is approximately 2 kilometers away from MAX IV but is easily accessible by tram. The primary benefits of this arrangement are the shared use of instruments for protein crystallization and the strengthening of ties between FragMAX and researchers at Lund University. FragMAX has access to a Mosquito crystallization robot (sptlabtech) and a Minstrel crystal plate hotel and imaging system (Rigaku) through LP3. The IceBear web interface (Daniel *et al*., 2021) provides access to images of crystals. In addition, LP3 is equipped with a Dragonfly liquid dispenser for crystal screen optimization and a wide range of standard laboratory equipment for protein analysis and biophysical characterization.

At the core of the FragMAX lab is a custom-built project management application. The user interface of the program is based on a Jupyter notebook and guides users and staff through the workflow. In addition, it stores all experiment-related meta-data, from protein batch to PDB deposition, in an SQLite database and prepares machine instructions and reads their output into the database. This ensures that all experiments are recorded in a standardized fashion, eliminates the need for manual record keeping, and ensures robust logistics by eliminating the use of ad hoc conventions. Each project has its own SQLite database file, which is stored on the MAX IV file system.

FragMAX can support a variety of crystal preparation workflows if necessary. To date, however, all screening campaigns have been conducted by crystal soaking. The facility provides an automated crystal soaking process and a crystal harvester stage for semi-automated mounting (Figure 2.32). Typically, users send protein, crystallization solutions, and, if necessary, other ingredients (e.g. seeds, co-factors) to FragMAX. LP3 staff will set up crystallization trials according to user instructions. Crystal plates will be stored in a plate hotel, which will periodically image them. The staff will evaluate crystallization experiments and select droplets containing suitable crystals using an in-built crystal viewer within the project management software. The soaking process involves three steps: (i) preparation of a compound plate by mixing fragment stock solutions (see Section 2.4.1.3) in DMSO with a certain amount of stabilizing solution (usually crystallization solution), (ii) preparation of a soak plate with a liquid handling robot (Opentrons, USA) that contains fragment solutions in the same well positions where suitable crystals were identified, and (iii) rapid crystal soaking with a stationary 96 channel pipette (Integra MINI96, Switzerland). After a certain incubation period (usually overnight), crystals are mounted with the aid of a Crystal Shifter (OLT, UK). The crystal shifter is a motorized XY stage that guides the user through the experiment and prevents crystal droplets from evaporating. Moreover, FragMAX provides a large selection of pucks, pins, and storage dewars. The workflow allows for the two-day preparation of up to 500 crystals. Importantly, users and staff are only required to be actively engaged during crystal mounting, whereas the soaking

process largely runs in the background and frees up time for other tasks. In addition, the project management software ensures that all information is stored in a database and standardized file system, so that at the end of each day, the user can upload crystal information directly to EXI for subsequent data collection.

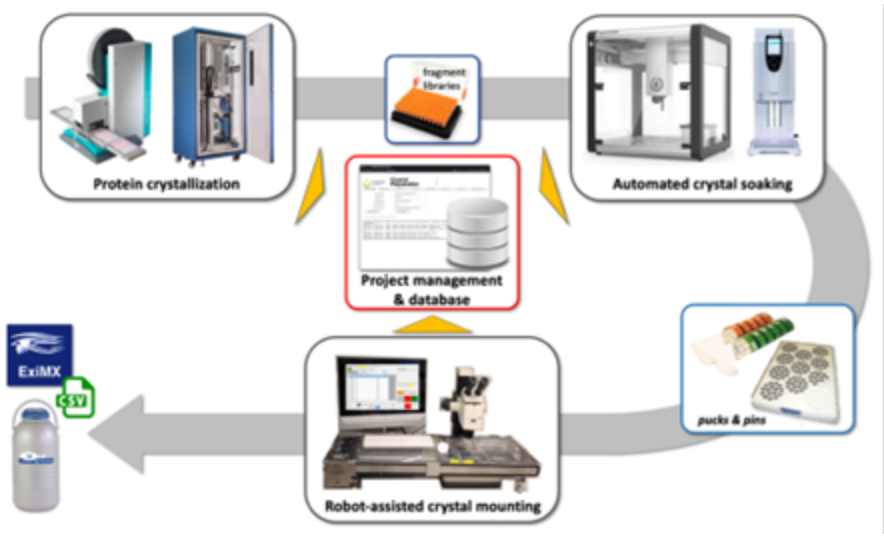


Figure 2.32 Schematic of the FragMAX crystal preparation workflow.

2.4.1.3 Fragment libraries

FragMAX provides several fragment libraries that are freely accessible to academic users. FragMAX has created a fragment library known as FragMAXlib. The 172-compound library was developed with input from scientists at Astra Zeneca and SARomics Biostructures AB. Each compound was bought as powder, dissolved in DMSO at a nominal concentration of 1M, and distributed between two 96-well plates. The library has been tested against more than 20 targets and has hit rates between 1 % and 10 %, comparable to those of more established libraries. FragMAXlib is the starting point for most screening campaigns. FragMAX also offers the EU-OPENSCREEN fragment library (968 compounds), the Astex MiniFrags library (80 compounds), and the Enamine DSI-poised library (860 compounds). All library plates are distributed in 96-well plates, with 1 mL of fragment solution in DMSO in each well. These plates are intended for a single use and are stored at -80 °C. Almost all screening campaigns conducted to date were done with fragments dissolved in DMSO. In cases where the crystal system was incompatible with DMSO, the DMSO was evaporated, and the fragment was dissolved in a suitable solvent/buffer.

2.4.1.4 Unattended data collection at BioMAX

Until the end of 2022, all data collection was performed manually at BioMAX, which became a bottleneck as the repetitive nature of the experiment put great stress on users and staff. FragMAX adopted the protocols for unattended data collection (see Section 2.1.2.1) as soon as they became available and now uses this mode exclusively. Interestingly, industrial user groups enthusiastically embraced this mode of action, whereas academic user groups sometimes require persuasion. Prior to launching unattended mode, a few manual datasets are collected to determine data collection parameters that yield the same results as previously reported by users while avoiding detectable global radiation damage. The same parameters (the “diffraction plan”) are then used throughout the collection of data without human intervention. After the collection is complete, FragMAX personnel examines the datasets in EXI and manually recollects those that were not properly centered. However, the failure rate is less than 1 % and, judging by our experience, the X-ray based centering used for unattended data collection provides superior results to manual “three-click" centering, even by experienced users, and particularly when working with small (< 50 µm) crystals and icy samples. The introduction of unattended data collection in 2023 was a game-changer, bringing FragMAX closer to becoming a truly internationally competitive facility.

2.4.1.5 Data analysis tools

Crystallographic fragment screening generates a large amount of raw diffraction data, but with typical hit rates ranging from 1 % to 10 %, the majority of soaked crystals have no fragments bound. Therefore, it is essential to rapidly process and analyze the data so that users can concentrate on the subset of interesting structures. Integration of X-ray diffraction data requires the most CPU power during data analysis. FragMAX thus relies heavily on the automated data processing system implemented by BioMAX.

Currently, FragMAX offers two options for rapid batch processing (Figure 2.33). Through the FragMAXapp web application, users can interactively perform data processing, initial refinement, and generation of ligand restraints (Lima *et* al., 2021). Additionally, they can use FragMAXapp as an interactive launching pad for the PanDDA algorithm (Pearce *et al*., 2017). This method is useful for smaller screening campaigns, but more difficult for larger experiments involving hundreds or thousands of data collections. As a result, we have developed a command line-based toolbox that enables exhaustive calculations for very large campaigns. In addition, these tools store a comprehensive set of meta-data in the respective FragMAX project database, enabling simple and reliable reporting. The latter option, which has been developed through discussions and feedback with our industrial users, produces structure results very quickly. Both tools make use of the MAX IV high-performance computing environment. Importantly, not only are all data stored in a relational database, but all files generated throughout the experiment are also stored in a structured, human-readable format, allowing reconstruction of the experiment long after the screening campaign has concluded.

Figure 2.33 Automated data analysis options at FragMAX. The platform offers a command line processing toolbox which is usually launched by FragMAX staff, as well as an interactive web application (FragMAXapp) that can be run by users.

2.4.1.6 Additional software tools for model building and refinement

FragMAX actively supports the data-to-map process, but final model building and refinement must be performed by the users, either remotely at MAX IV via the ThinLinc client (see Section 2.6.2) or by transferring the processed data to their home laboratory. We provide two plugins for coot via the code-sharing platform GitHub that enable accelerated building of models and refinement on all operating systems. The first plugin is used to review and model bound ligands identified by the PanDDA algorithm (Pearce *et al*.), while the second plugin enables ligand model building and refinement in standard crystallographic maps. These tools are accessible to all interested scientists and have received a consistent number of downloads over the years.

2.4.1.7 PDB deposition support

FragMAX offers users assistance with the submission of structures to the Protein Data Bank (PDB), if desired. FragMAXapp facilitates the entry of project-specific information, while the SQLite data source stores all other relevant information required for PDB deposition. FragMAX personnel will generate a file suitable for uploading to the RCSB PDB group deposition interface if users only provide their final structures. This enabled users to deposit 269 fragment-bound structures in a single session in 2022 (Barthel *et al*., 2022).

2.4.1.8 Access modes

FragMAX users may use the MAX IV user program (Section 3.1) to submit standard proposals biannually or fast access proposals, subjected to the same review procedure as other proposals. Through the iNEXT Discovery program, which runs until August 2024, European users can access FragMAX at any time throughout the year. FragMAX is accessible to industrial users through the MAX IV Industrial Relations Office. Potential users are encouraged to contact the FragMAX project manager prior to submitting an application in order to discuss the technical requirements for a successful screening campaign and to understand the requirements of a specific crystal system. The crystal system is critical to the success of a screening campaign; in fact, insufficient quantities of well-diffracting crystals delay approximately one-third of projects.

2.4.2 Collaborations

FragMAX collaborates with Manfred Weiss's group from HZB at BESSY II to develop FragMAXapp. FragMAXapp is deployed at MAX IV and HZB, and since 2021, the teams from MAX IV and HZB have met at least every other month to discuss bugs and program features.

Jens Carlsson from Uppsala University and Tobias Krojer from FragMAX submitted a joint project application to the PRISMAS PhD program. A PhD student was successfully recruited, and he began his position in September 2023 at Uppsala University. The PhD student will develop new methods for computational fragment elaboration and test and refine his algorithms using data collected at MAX IV. Together with Pål Stenmark of Stockholm University, Tobias has submitted a second project to the PRIMAS program. Here, the objective is to find a Ph.D. candidate who will use fragment-based lead discovery to develop chemical tool compounds for a novel cancer target protein.

As part of the iNEXT Discovery project, FragMAX collaborates with other European fragment screening centers and the European Protein Data Bank (PDBe) to develop a new data exchange format to describe the entirety of a crystallographic fragment screen. The collaboration proceeds through regular meetings and hackathons. A prototype of the data file has been developed so far and the screening centers are working towards implementing it in their workflows.

2.4.3 External funding

Swedish Research Council (grant No. 2018-06454, 2019 – 2022, 8.5 MSEK)

Uwe Mueller and Gustavo Lima from MAX IV, along with applicants from Lund University, Astra Zeneca, and SARomics Biostructures, submitted a successful "Grant for accessibility to infrastructure" application to the Swedish Research Council. The application titled "FragMAX - a facility for high throughput fragment screening in drug development by X-ray crystallography" served as the foundation for FragMAX by funding the acquisition of necessary equipment, fragment libraries, and personnel to implement the facility.

iNEXT Discovery (project number 871037, 2020– 2024, 196 685 Euro)

iNEXT-Discovery is a project funded by the European Commission's Horizon 2020 program. In addition to providing transnational access to infrastructures and methods, it also finances scientific research initiatives between access providers. MAX IV has agreed to provide 632 access hours, of which 440 are for FragMAX and 192 are for serial crystallography experiments. In addition, nine months of FTE funding for a researcher to implement data management and exchange protocols are included.

Vinnova (2019-02567_Vinnova, 2019 – 2020, 392 kSEK)

Lionel Trésaugues (Sprint Biosciences AB) and Gustavo Lima received a project grant from Vinnova entitled "Fragment-screening by X-ray crystallography at MAX IV on a human oncology-related protein". The objective of the project was for Sprint Bioscience, with the guidance and support of the FragMAX team at BioMAX, to acquire the expertise necessary to use X-ray screening by crystallography as a fragment-screening method and to identify new starting points for an oncology-related protein.

Carl Tesdorpfs stiftelse (2021, 66 kSEK)

We received a grant from the Carl Tesdorpfs stiftelse to fund the acquisition of an Opentrons OT-2 liquid handling robot. The machine is now in routine operation.

Crafoordska stiftelsen (2023 – 2024, 300 kSEK)

The Crafoordska stiftelsen awarded us a grant for the project "Structural analysis of PHIP, a new target against Glioblastoma from the dark proteome." The project will lay the groundwork for the structural analysis of an understudied protein implicated in human disease and also result in the development of novel FragMAX workflows.

2.4.4 Development of the platform

2.4.4.1 History and staff development

The Swedish Research Council (VR) provided funding for the development of the FragMAX facility. Uwe Müller, the former group manager of the MAX IV MX team, led the initiative. Gustavo Lima, a former postdoc in the group, assisted him. As co-applicants, they were accompanied by Wolfgang Knecht (LP3, LU), Derek Logan (SARomics Biostructures AB), and Tove Sjogren (AstraZeneca). The grant began in January 2019 and funded the purchase of a Crystal Shifter (OLT), sample holders, and pucks, as well as the construction of the FragMAXlib fragment library. In addition, the grant made it possible to hire Elmir Jagudin as software developer for FragMAXapp and Vladimir Talibov as postdoctoral fellow for workflow development and fragment library assembly. Uwe Müller and Gustavo Lima left MAX IV in 2020, while Vladimir Talibov left in February 2021. Tobias Krojer was hired in 2020 as the new FragMAX project manager and began in January of 2021. In May of 2021, Sandesh Kanchugal joined him as a postdoctoral fellow. Sandesh left MAX IV in November 2022, and the remaining funds for personal in the VR grant were used to fund fifty percent of the salaries of Maria Gourdon (until October 2023) and Celeste Sele (until December 2023) from LP3.

2.4.4.2 Development of sample throughput

Since 2019, FragMAX has increased its overall sample turnover significantly (Figure 2.34). However, 2021 witnessed a decline in activity due to the Covid-19 pandemic, the temporary closure of MAX IV to on-site users, and a complete staff turnover. Since then, however, the facility's throughput has returned to pre-pandemic levels, and in 2023, both project and sample throughput have begun to increase. Given the screening campaigns scheduled for November and December 2023, it is reasonable to assume that FragMAX will reach more than 4000 datasets in 2023, which is twice as many as in previous years and demonstrates the efficacy of current workflows, as 2023 was also the year with the smallest number of staff. The objective for 2024 is to increase the number of datasets to at least 5000, and ideally 8000, depending on the future staffing development.

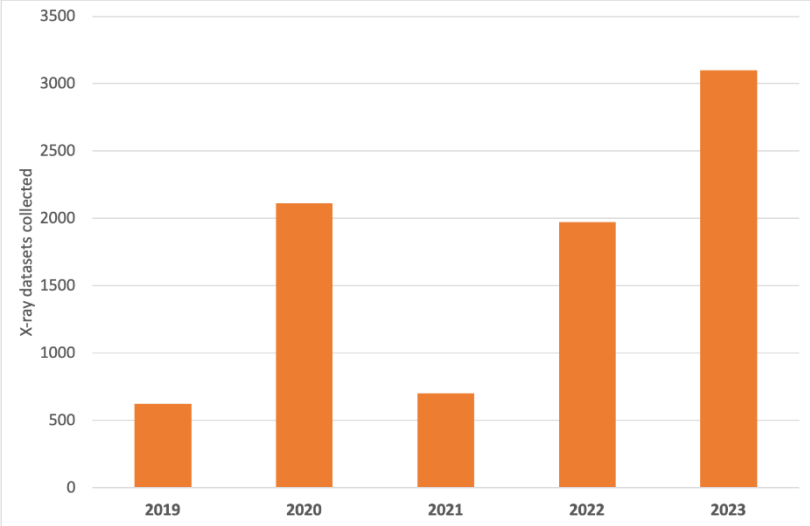


Figure 2.34 Development of the number of crystals collected at FragMAX from 2019 to 2023. In 2023, more than 3000 datasets have been collected until October 2023 and approximately another 1000 are planned until the end of the year.

2.4.4.3 Development of screening campaigns

The increased number of screening campaigns (Figure 2.35) mirrors the increased number of data collections. Initially dominated by MAX IV internal projects, FragMAX now focuses almost exclusively on academic and industrial user campaigns. The first industrial user project was completed in 2020, albeit as part of an academic collaboration. FragMAX and the MAX IV Industrial Relations Office initiated the development of a fee-per-service model in 2021, which has since attracted several national and international biotech companies.

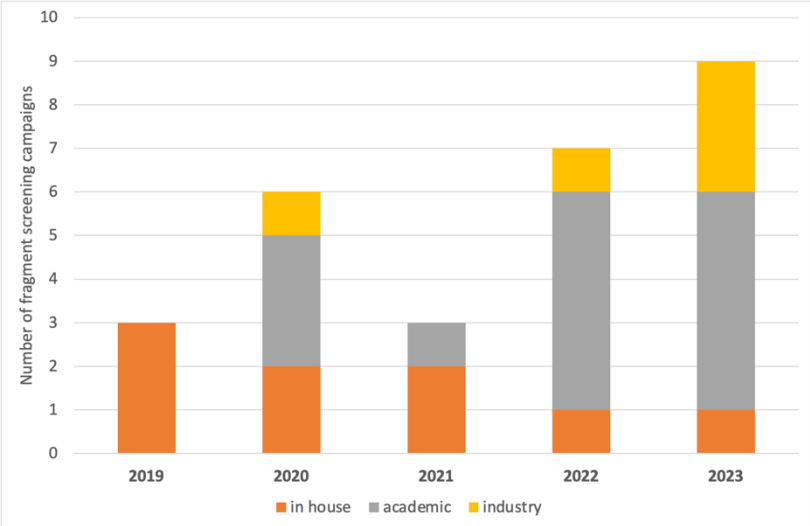


Figure 2.35 Development of screening campaigns at FragMAX from 2010 to 2023.

2.4.4.4 Geographical origina of FragMAX user groups

The majority of FragMAX user groups come from Sweden and neighboring Scandinavian countries Denmark and Norway (Figure 2.36). The next largest group are users from continental Europe, particularly from Poland. But FragMAX has also seen interest from groups in the United States and Brazil.

Figure 2.36 Geographical origin of FragMAX user groups. The numbers in parenthesis indicate the number of groups from a given country. The number includes groups from industry and academia.

2.5 Supporting laboratory facilities

The BioMAX and MicroMAX beamlines users and staff have access to three sample preparation labs: one at each beamline and the MAX IV Biolab (common to all life science beamlines at MAX IV). A laboratory is also available at MicroMAX for preparation of complex sample delivery environments. All laboratories are located within 20 m of each other. Complex or bulky equipment is shared by the users of either beamline, regardless of physical location.

2.5.1 Beamline laboratories

BioMAX sample preparation laboratory

The sample preparation laboratory at BioMAX (see Figure xx) is used by staff and users mainly to handle samples at room temperature or cryo-cool samples prior to the beamtime. We have two high end Leica M80 stereo microscopy stations with video screens and 20 °C and 5 °C crystal storage cabinets. The laboratory is equipped with opaque blinders and color filters for light-sensitive experiments.

MicroMAX sample preparation laboratory

Incubators and biochemical laboratory equipment will be housed in this lab, together with microscopy solutions. Initial adaptations for handling light-sensitive samples have been performed. A video microscope for (in particular) surface characterizations, and a humidity chamber for loading fixed-target sample supports are available.

MicroMAX sample environment laboratory

The sample environment laboratory offers space for electronics work and offline characterization of sample delivery setups. Cooling infrastructure for electronics racks is available if needed.

2.5.2 MAX IV Biological lab

The MAX IV Biological Lab serves as the central facility for life science-related synchrotron experiments conducted across multiple beamlines. In addition to the sample preparation rooms connected to specific beamlines, the Biolab offers comprehensive instrument access and support.

Currently, the Biolab provides 8 bookable benches for users and a range of services including solution creation, protein concentration, centrifugation, dialysis, gel electrophoresis, incubators set at various temperatures, etc. The lab also offers freeze drying facility, UV-Vis spectroscopy for different volumes,

dynamic light scattering (DLS), analytical-scale protein purification through High-Performance Liquid Chromatography (HPLC), microscopy services, and secure storage for chemicals and samples at temperatures of 4 °C, -20 °C, -80 °C, and –152 °C. Additionally, the Biolab features an anaerobic chamber with a microscope and UV-Vis setup and various consumables.

Continuous development and enhancement of the Biolab are undertaken in response to user requirements and evolving scientific needs.

2.6 Computing infrastructure

IT resources for beamline control and computing at MAX IV are centrally organized with emphasis on sharing and reuse of resources when appropriate. This will allow software and IT support groups to manage systems for all beamlines in a similar way and, to a large extent, use the same building blocks everywhere. The purpose of having different beamlines, however, is to focus on some unique experimental technique, so there is also a lot of dedicated IT equipment to support those unique capabilities. In addition, there is an HPC resource available to all beamlines during data collection with a total of 2560 cores and 48 GPUs out which 784 cores are reserved for the BioMAX beamline. In the future, some computing resources will be dedicated to MicroMAX as well.

2.6.1 Data storage and retrieval

The experiment control system is running in a clustered virtual machine environment to provide a highly available infrastructure with inbuilt redundancy. The data acquisition chain is centered around a cluster of servers that receive the data from the detector system, apply any necessary corrections and write the data to permanent storage. This DAQ cluster includes a high bandwidth network to the detector, a dedicated storage network for writing the data to disk, and a third network for streaming the data to the HPC system for on-the-fly data analysis.

Data storage is based on a clustered system with a dedicated low latency network for all data movement. The system uses different performance tiers that are configured to match the stages in the data life cycle. At the time of collection, that data is stored on the fast tier and available to the autoprocessing routines described in Section 2.3.3. It is later migrated to a medium tier, perhaps to be re-analyzed manually or retrieved by the users. It will eventually be migrated to a slower tape tier, where files still can be listed. If the file content is requested, the file will automatically be restored to a disk tier. In addition, all data, with the exception of proprietary data, will be backed up to tape. We maintain a SciCat data catalogue of all the archived data sets and their data identification number. The current data policy states that the data will be kept for at least seven years.

A dedicated resource providing high bandwidth for external data transfer is available to the user. The standard tools provided are Globus Connect and sftp, but custom tools for transfer to object storage are also being deployed. Archived data can be made public at any time by the users and downloaded upon request via the SciCat interface.

2.6.2 Remote access

Remote access to MAX IV is done via VPN and login is using 2-factor authentication. The remote user is then given appropriate privileges based on the active proposal. At BioMAX the remote user can run their experiment after login to a virtual desktop via the ThinLinc client. Remote access is always granted by default to all the members of the proposal during scheduled beamtime, so on-site experiments can be “hybrid” and benefit from the help of remote collaborators.

The remote desktop has all the software and tools for controlling the beamline and analyzing the data and is set up to look identical to the desktop at the beamline workstations, so that on-site users do not need be retrained when they collect data remotely, and vice-versa. We plan to replicate the current BioMAX setup for MicroMAX.

A computer cluster for post-analysis is also available remotely via a virtual desktop. Unlike the beamline remote desktop, which is only available to the users during their beamtime, the so called “offline cluster” can be accessed by all users with a proposal at any time.

2.7 Competitive analysis

The MAX IV MX beamlines can compete with all beamlines worldwide in terms of X-ray beam characteristics, beamline instrumentation, and experiment control for a wide range of macromolecular crystallography experiments. Compared to larger facilities, the MX program cannot always implement the same amount of functionality in terms of experiment control and analysis, but with the high level of competence of the staff, we can be highly competitive.

2.7.1 BioMAX

Most synchrotrons have at least one macromolecular crystallography beamline in their portfolio comparable to BioMAX in characteristics and experimental purpose. It is expected that BioMAX shares its user community with ID30A-1 / MASSIF-1 at ESRF, I03 & I04 at Diamond and P13 at PETRA III and these beamlines have been compared to BioMAX in terms of beam properties and scientific output (Table 2.5). The beam properties, including flux on sample, beam size, energy range are all comparable or somewhat better at BioMAX. The annual publication rate at BioMAX is quite comparable with MASSIF-1 at ESRF and P13 at PETRA III but the Diamond beamlines are more productive (> 100 annually published articles compared to BioMAX 40 publications in 2021). BioMAX has a relatively large proportion of proprietary users that do not publish.

	BioMAX (MAXIV)	**MASSIF-1 (ESRF)**	**I03 (Diamond)**	**I04 (Diamond)**	**P13 (PETRA III)**
Operation start	2017	2018	2007	2007	2013
Number of publications	109	216	1802	1733	320
Flux [ph/s]	2×10^{13}	5×10^{12}	1.7×10^{12}	1×10^{12}	5×10^{12}
Spot size [microns] – focused beam	20 x 5	10 x 10	90 x 20	10 x 5	30 x 20
Energy-range [keV]	5 - 25	12.65	5.2 - 21.0	6 - 18	4 – 17.5
Detector	EIGER 16M	PILATUS3 2M	EIGER2 XE 16M	EIGER2 XE 16M	EIGER 16M

Table 2.5 Comparison of beam properties and scientific output between BioMAX and some other similar beamlines

We estimate that BioMAX has several strengths that make it attractive to users, like the remote capabilities, the FragMAX platform, the use of MXCuBE, and the large range of functionality. At the same time, there are some areas of growth:

- Not being able to offer fully autonomous data collection brings down the amount of used beamtime, particularly during the night and weekends. It also limits somewhat the demand of proprietary beamtime, with some users at present preferring DLS or the ESRF for projects amenable to full automation. The unattended data collection described in Section 2.1.2.1 is a first step towards improving the situation.
- Swedish users (the largest user community at MAX IV, see Section 3.1.1) do not get any travel or sample shipment support from MAX IV, which makes it cheaper for them to use other European

facilities. The procedure to handle sample shipments, described in Section 3.3.3 can also be burdensome to beamline staff. Solutions are being explored to improve this issue.

- A general challenge, common to all mature MX-dedicated beamlines, is how to adapt to rapidly evolving user demands (related to developments in Cryo-EM and use of structure prediction using AI methods.

2.7.2 MicroMAX

With its emphasis on serial crystallography MicroMAX is similar to ID29 at ESRF, I24 at Diamond, T-REXX at PETRA III (2nd endstation of the P14 beamline), 14 ID at APS, MX3 at the Australian Synchrotron (see Table 2.6). ID29 is more specialized, not capable of single crystal rotational crystallography. P14 offers serial time-resolved crystallography in a second hutch as MicroMAX could do in the future, allowing preparation work to take place while the first hutch is used for other data collections.

MicroMAX strengths are expected to be flexibility both in terms of X-ray beam and experimental setup, short pulses with the chopper, high flux with the multilayer monochromator, flexibility with photon-counting (CdTe) and integrating (Si) detectors.

	MicroMAX (MAX IV)	**ID29 (ESRF)**	**I24 (Diamond)**	**P14 (PETRA III)**	**14 ID (APS)**	**MX3 (ANSTO)**
Operation start	tbc	2022	2008	2012	2000	tbc
Functionality	Single xtal SSX / T-R	SSX / T-R	Single xtal SSX / T-R	Single xtal SSX / T-R (2 EH)	Single xtal SSX / T-R	Single xtal SSX / T-R
Flux [ph/s]	>1e^{14}	>5e^{15}	3e^{12}	2e^{13}	7e^{13}	>1e^{13}
Spot size [microns] – focused beam	10 x 5 (later 1 x 1)	0.5 x 0.5	7 x 6	2 x 6	20 x 20	2 x 2
Energy-range [keV]	5-25	10-25 + 35	6.4-20	7-20	7-15	10-15

Table 2.6 Summary of some similar beamlines.

3 Beamline operation

3.1 Beamtime access schemes and review process

MAX IV issues calls for beamtime proposals twice per year, with deadlines in mid-March and mid-September for the following Autumn or Spring beamtime period respectively. The beamline team checks beamtime proposals for technical feasibility; afterwards, the Program Advisory Committee (PAC) for structural biology assesses the proposals on scientific merit. The PAC advises MAX IV management on beamtime allocation. Their recommendations are always followed in practice. Beamtime is allocated in units of 4-hour shifts. Users can apply for beamtime through multiple proposal schemes, described further below.

Starting at the Fall 2023 call, the User Office implemented the possibility to submit joint standard and Block Allocation Grant (BAG) proposals for BioMAX and MicroMAX. The rationale for this is that even within a single project, there may be samples or experiments that may benefit from the particular properties or facilities available at one of the beamlines (for example, pink beam at MicroMAX or experimental phasing at BioMAX); at the same time, the existing overlap between the capacities of the two beamlines allows for scheduling flexibility and a better chance for users to receive make-up time in case of disruptions to the beamtime. So far, one BAG and one standard proposal have requested time at both beamlines. The low number is not surprising since most BAG proposals are not up for renewal until the Fall 2024 call; thus, we expect to see more joint proposals in 2024.

Standard proposals

Standard proposals comprise single research projects, submitted by typically one principal investigator (PI) or by a small collaboration of PIs for a limited number of beamtime shifts. Normal proposals are valid for one beamtime period, when accepted.

Block allocation grant (BAG) proposals

BAG proposals consolidate a larger number of projects and PIs from one research environment, like an institution, a city-based selection, or a country wide-area BAGs arrange the use of their shifts amongst themselves; since 2020, BAG proposals are valid for two years (four beamtime periods) with a mid-term report and review by the PAC. This has removed the focus for the BAGs from a basically repetitive proposal review process to more of a performance review.

Fast access proposals

Fast access proposals were introduced in 2020 to open up BioMAX beamtime for Covid-19 related and medically relevant research outside the call periods, but the scope was soon expanded to accept any project in immediate need of beamtime or to characterize samples prior to an application for a normal proposal (“feasibility experiment”). Under this scheme users can continuously submit proposals, which get reviewed by one member of the PAC and receive a limited number of shifts, typically within one month. Since the fast access proposals were formalized, BioMAX has received 6 proposals (of which 2 of them are FragMAX proposals) and awarded 14 shifts. At MicroMAX, rapid access proposals are currently accepted for sample feasibility experiments. The User Office has announced the temporary suspension of the fast access program from November 2023 due to staff shortages. Because fast access only takes a small fraction of the beamtime and yet the existence of this access mode is important to MX users, we plan on continuing accepting these proposals at BioMAX as internal proposals.

Expert commissioning proposals

Two expert commissioning proposals were submitted by users to help test and develop the functionality of MicroMAX in advanced serial crystallography techniques and time-resolved experiments. These proposals are no longer accepted for the current or future calls. However, external collaborators can be invited to participate in in-house proposals for new developments at any beamline; an example is the collaboration with researchers from PSI to test the performance of Jungfrau 4M detector for time-resolved experiments at BioMAX, described in Section 5.4.

iNEXT-Discovery

The iNEXT-Discovery program is an initiative funded by the European Commission Horizon-2020 framework from February 2020 until August 2024 to enable access to structural biology research infrastructures for “translational” structural biology projects with applications in biomedical, biotechnological, biomaterial, food or environmental research. Initially, the program was due to end in December 2023, but received an extension until August 2024 due to Covid-19 related delays. iNEXT Discovery is a transnational access program, therefore, all European and non-Swedish user groups use it to apply for access at FragMAX (see

Section 2.4.1.8). MAX IV agreed to provide 440 access hours for FragMAX and 192 access hours for serial crystallography experiments. We are due to complete the number of access hours for FragMAX by the end of 2023 and will request to transfer the remaining 192 access hours for fragment screening projects.

Industrial proposals

Over the last years, BioMAX has typically dedicated between 10 and 15 % of the available shifts for industrial use. The industrial user base consists of both pharmaceutical companies directly as well as commercial analytical research organizations serving the pharmaceutical industry with protein crystallography related services (mediator companies). In the Fall 2023 period, we are giving about 20 % of the beamtime to proprietary research, and we expect to dedicate an even higher percentage in the future, because of the interest of industry in the FragMAX platform (see Section 2.4.4.3) and the development of unattended data collection, which will likely result in increasing demand from industrial users.

Training and education proposals

MAX IV aims to have about 12 shifts of beamtime per user period dedicated to training and education. Initially, the call for training and education proposals happened twice per year, at the same time as the call for Standard proposals. In 2023 the call for proposals was discontinued, pending review of the program implementation by the user office, but the users can still apply for training and education beamtime through the beamline staff.

3.1.1 Statistics

In the following graphs we summarize user statistics since 2020 (the first year of operation following the previous BioMAX review) until June 2023. The statistics about beamline usage in Figure 3.1 are given as a percentage of beamtime available to users, not counting accelerator shutdown periods, time dedicated to accelerator studies or beamline commissioning time; the total down time has varied somewhat from year to year, as shown in Figure 3.2; long R3 accelerator shutdowns for maintenance are scheduled over the summer (over two months) and winter (about one month). Usually there are 2-3 weeks per beamtime period dedicated to machine studies in addition, as well as all Mondays during the beamtime period. As of 2023, with all beamlines in the first phase of construction finished, beamline commissioning time has been reduced from every Tuesday to only one Tuesday per month, and the total time available to users has increased.

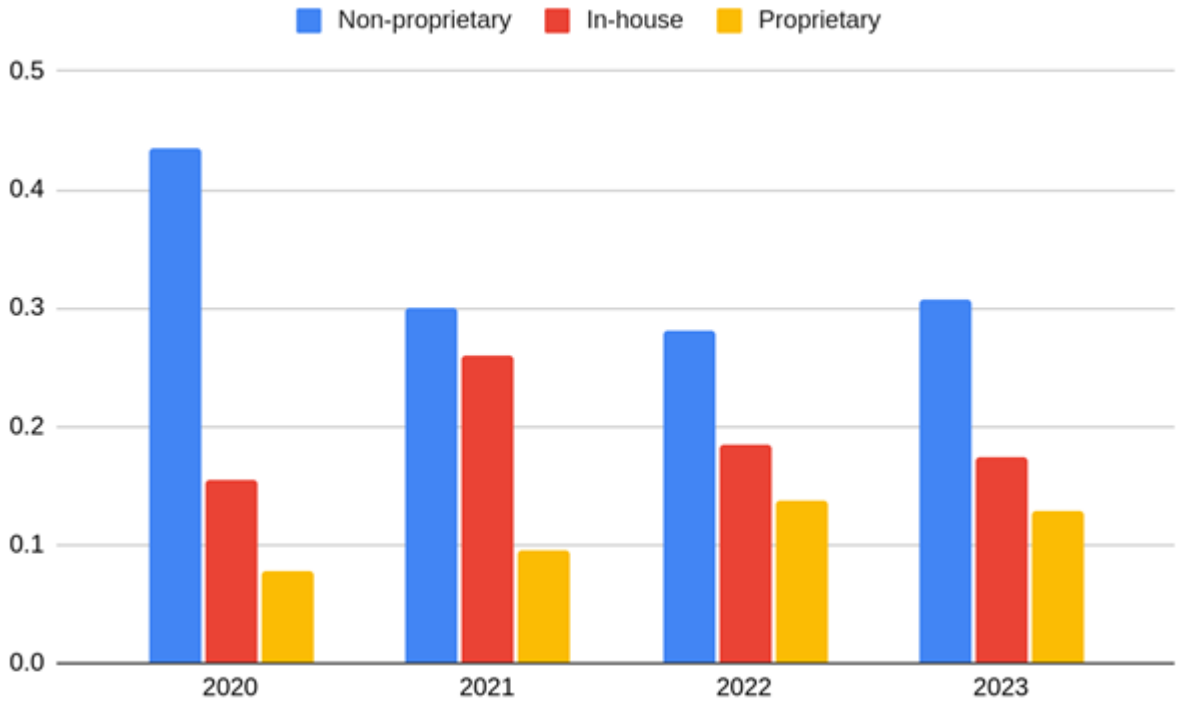


Figure 3.1. Beamtime usage in relation to the available shifts. For 2023, the numbers apply to the first half of the year. The statistics include FragMAX proposals.

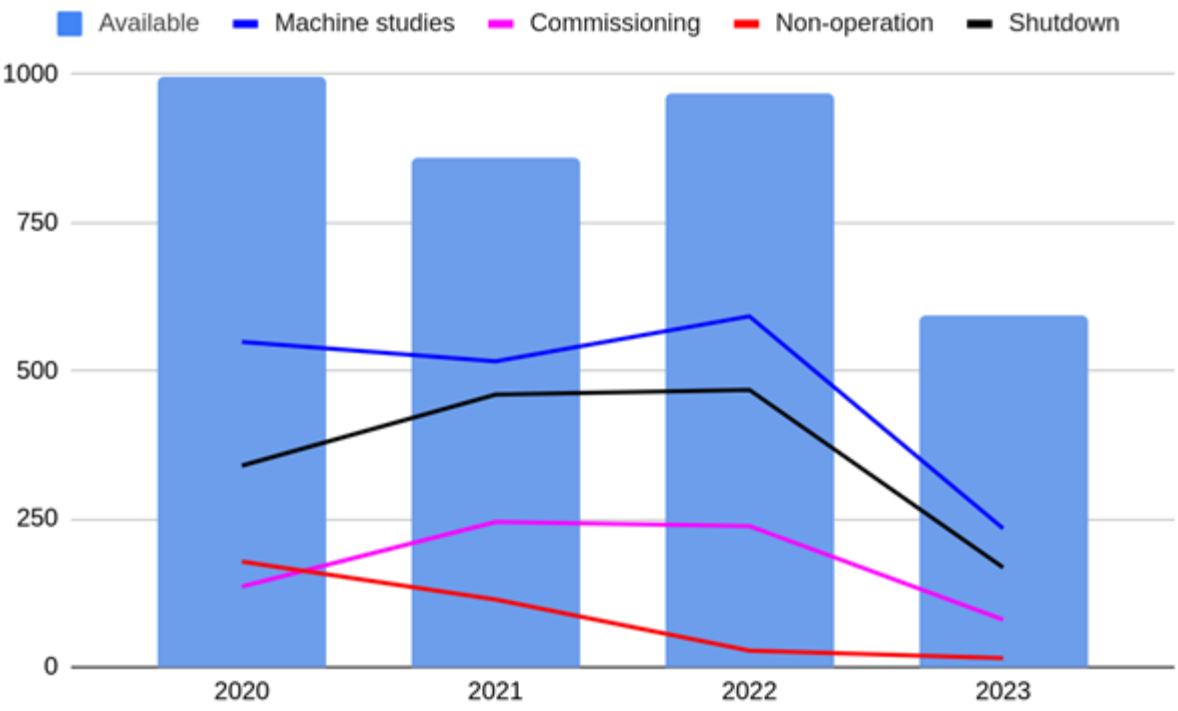


Figure 3.2. Number of shifts available to users, compared to shifts dedicated to machine studies, beamline commissioning and down time (accelerator shutdown or other reasons for non-operation). The high number of non-operation shifts in 2020 and 2021 is due to the repair of the KB mirrors and pandemic-related stand-downs. For 2023, only shifts until June 30th are counted.

2020 has been thus far the year where we had most shifts used by external users. We attribute this uptick to the ESRF upgrade and other sources restricting user operation because of the Covid-19 pandemic. Thanks to the very timely implementation of remote access and the decision by MAX IV to continue remote user operations, we were able to provide beamtime at BioMAX despite the travel restrictions in place. Non-proprietary usage flattened out over the next two years, although we expect to see an increase for over one year starting in the 2023 Fall period, due to the shutdown of SLS and APS.

There has also been an increase in proprietary beamtime. As mentioned in Section 3.1, we expect this trend to continue. Achieving the objective of fully automated data collection to attract more industrial users is a very important part in our plans for further development of the MX program at MAX IV and of the BioMAX beamline in particular. See Section 6.

Regarding geographical location of the users, the majority of proposals come from Sweden (42 %, 52 proposals in total), Denmark (18 %), and Germany (8 %). These three countries, together with other Baltic and Nordic countries account for a large majority of the MAX IV MX users (87 %). Figure 3.3 shows a heat map of the recent proposals by country based on the affiliation of PI. Usage from other countries is usually more related to punctual restrictions in access to other sources (for example, two USA proposals are currently big users of MAX IV because of the APS upgrade).

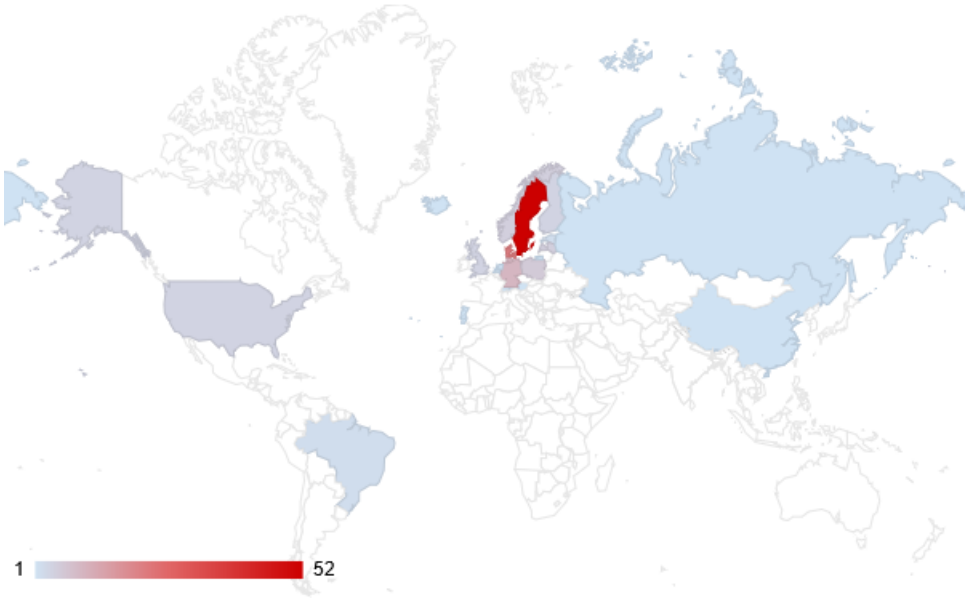


Figure 3.3 Heat map of proposals by country since 2020.

3.1.2 User feedback

MAX IV users are encouraged to submit feedback on their experience at MAX IV after their experiment. Typically, about 40 responses per year are submitted. Figure 3.4 lists the average scores for different aspect divided in three areas: Facilities, Beamline, Support and Overall success. The maximum score is 5.

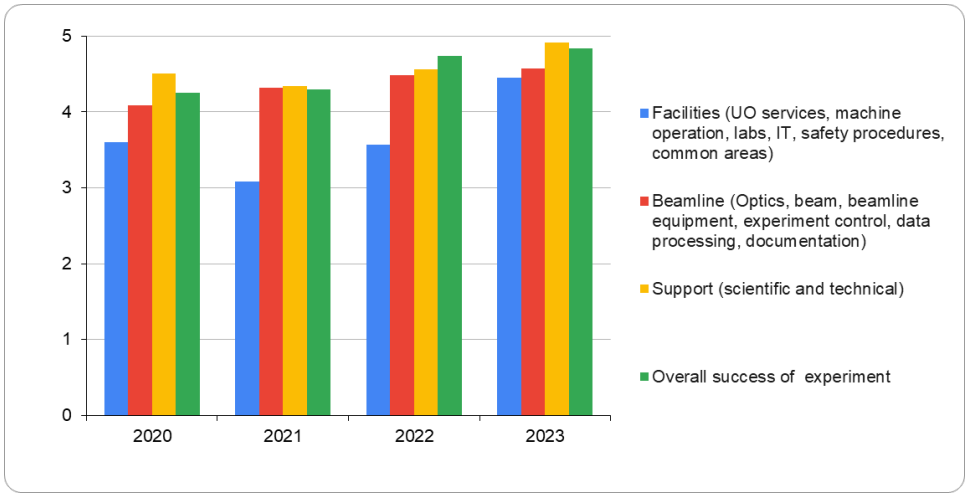


Figure 3.4 User feedback summary since 2020

Although the data from 2023 are not complete yet, since only the responses from the first half of the year are included, there is an upwards trend, particularly regarding the beamline performance and beamline facilities and the overall success of the experiment. Staff support is almost always the highest regarded aspect of the experiment, while facilities is the worst; scrutiny of the data suggests that some remote users, who do not use the laboratory facilities or the common areas, tend to score these with 0. Omitting the evaluation for the facilities only available for on-site users, raises the overall facility average score above 4.

Until 2021 we got negative comments about the lack of grid scans or, after they were implemented in 2021, difficulties using them. The semiautomated X-ray centering introduced in 2023 was, unsurprisingly, well received. Software instability or slowness and problems with the remote connection were also commented upon. Staff, on the other hand, were almost always mentioned in a positive way.

3.1.3 Publications

Figure 3.5 lists the publications per year in the MX group. The immense majority are from experiments carried out at BioMAX; work done at the MAX-lab beamlines is still being published and credited to BioMAX, although the number of these publications typically does not exceed one per year.

As of now, there are no publications for MicroMAX. The impact of the new beamline in the scientific output of the MX groups will depend on whether the new capabilities offered by MicroMAX results in a higher number of users or proposals or if it is exploited mainly by the current user base at BioMAX.

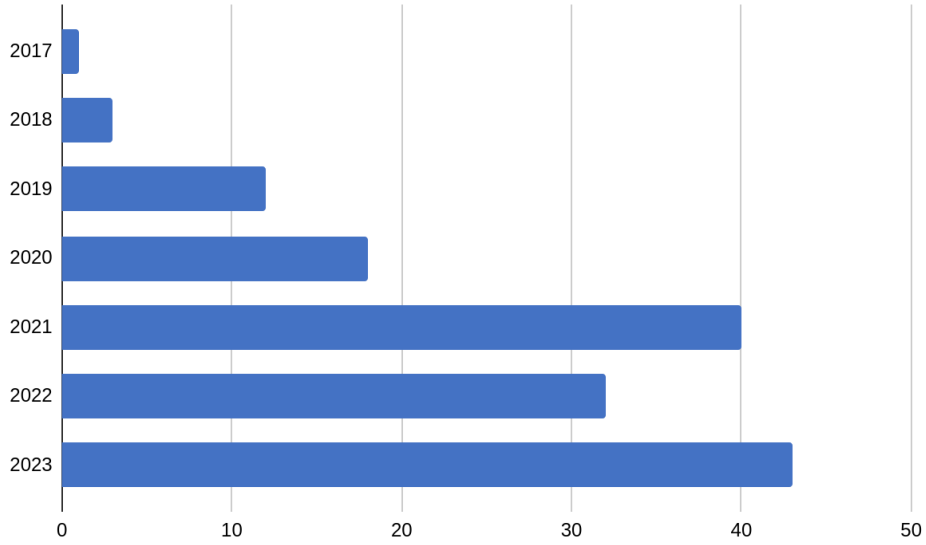


Figure 3.5 Publications per year. For 2023, publications are current as of October 19th. The fully up to date list can be consulted at https://publications.maxiv.lu.se/BioMAX

3.2 MX program

The MX program was started by MAX IV in 2023 as a pilot program to test a new way to more efficiently handle beamline projects, by grouping them in program areas based on similar experimental techniques and managing them jointly. The different programs would be able to decide internally on priorities for development and shared resources. While supporting computing, software and engineering groups remain centralized, staff in these groups are allocated to work in a reduced number of programs, thus contributing to build a solid expertise in the specific systems needed for different scientific areas and foster a stronger collaboration between developers, support staff and beamline staff.

The MX group was selected as the best one to test this approach because of the overlap between BioMAX and MicroMAX (using the same experimental techniques, the same control software and similar instrumentation) and the already existing close-knit collaboration within the group, with MicroMAX staff contributing to the development and support of BioMAX since the beginning of operation and BioMAX staff participating also in the planning and commissioning of MicroMAX experimental facilities.

The MX program is now permanently implemented. Project management within the MX program is carried out in a dynamic way, with representatives from the MX staff, the Project Office and the Life Science director meeting biweekly to update on progress, identify bottlenecks, and reallocate priorities, if needed.

3.2.1 Staffing

BioMAX has three permanent beamline scientists, one of whom is funded to manage the FragMAX project; one permanent instrument scientist, and a postdoc (3-year). It has also a two-year beamline scientist, which is a permanent position but it is unclear if it will be renewed under the present budget constraints. MicroMAX has two beamline scientists, one computational scientist, and one instrument scientist. Additionally, one beamline scientist and one postdoc are about to be recruited to MicroMAX. There is one PhD student in the group who is graduating in November 2023. Both beamlines share a research engineer / technician. In the practice, though, we operate as a team, with MicroMAX staff involved in operation and maintenance of BioMAX and vice-versa.

One of the beamline scientists takes the role of beamline manager (BLM) for each beamline. The BLM position carries tasks like beamtime scheduling, as well as budget responsibility, and a general steering of the course of the beamline concerning developments and upgrades, aided by the rest of the team. Usually, at MAX IV, beamlines sharing a common technical scope have a group manager (GM) who acts as a link

between the beamlines and upper management, is responsible for supervision of the staff in the group, coordination of the beamline budgets and has an important say in the scientific development of the MX program. In the MX group this role has always been fulfilled by one of the BLMs.

Compared to the beamline scientists, the instrument scientists in the MX group are more centered on instrument maintenance and development rather than methods development in the area of X-ray crystallography and scientific support of the users. In the practice, we encourage all the members of staff to have a working knowledge of the beamline optics, instruments and the scientific applications. Most staff members participate in user support and we try to achieve a good balance between the areas of expertise of all the members of the group, so everyone can contribute to scientific and technical developments and smooth operation of the user program.

The current MX group members are:

BioMAX:

- Swati Aggarwal, Postdoc
- Monika Bjelcic, PhD student
- Aaron Finke, Beamline Scientist
- Tobias Krojer, Beamline Scientist, FragMAX responsible
- Jie Nan, Beamline Scientist
- Ana Gonzalez, Beamline Scientist, current BioMAX BLM
- Ishkhan Gorgisyan, Instrument Scientist

MicroMAX:

- Oskar Aurelius, Beamline Scientist
- Cecilia Casadei, Computer scientist
- Manoop Chenchiliyan, Research Engineer / Technician
- Mirko Milas, Instrument Scientist
- Thomas Ursby, Beamline Scientist, current MX GM and MicroMAX BLM

The MAX IV Controls and IT group (KITS) designates two members of their group to be a beamline contact for software and instrumentation related issues. The MX beamline contacts are:

- Mikel Eguirain (BioMAX software)
- Julio Lidón-Simón (BioMAX instrumentation)
- Elmir Jagudin (MicroMAX software)
- Siwen An (MicroMAX instrumentation)

MicroMAX is presently also funding:

- Staffan Bengtsson (Mechanical Design Engineer)
- Alberto Nardella (Software)

3.2.2 External funding

The funding for the construction of BioMAX was awarded by the Knut and Alice Wallenberg Foundation and for the construction and operation of MicroMAX by the Novo Nordisk Foundation. In addition to the external funding for FragMAX as described in section 2.4.3 the following are the major grants that have been received by the MX facilities:

European Commission Horizon 2020 EUCALL (grant no 654220, 2015-2018, 277 kEuro)

The project "The European Cluster of Advanced Laser Light sources (EUCALL)" covered a 2-year scientist position, equipment and travel cost for developing standardized sample holders for high-repetition experiments.

Swedish Research Council (RÅC grant no 2017-06734, 2018-2022, 3.44 MSEK)

MAX IV together with Gisela Brändén and Richard Neutze from University of Gothenburg, Lars Redecke from University of Lübeck, Matthias Wilmanns from EMBL-Hamburg, Alke Meents from DESY and Adrian Mancuso from European XFEL received funding for the project "Expanding the domain of serial crystallography: membrane protein & in cellulo crystallization" that covered the cost for one PhD student at MAX IV. The project aimed at developing serial crystallography by streamlining efficient delivery of microcrystals for diffraction studies, developing advanced approaches for the growth of microcrystals and training the user community to take advantage of these new methods.

Swedish Foundation for Strategic Research (2019-2022)

MAX IV together with collaborators Uppsala University, Halmstad University, Chalmers, University of Gothenburg, RISE and Lund University received funding for the project AdaptoCell. The project aimed at developing a microfluidic flow-cell platform (AdaptoCell) for MAX IV Laboratory users, to be integrated onto the X-ray beamlines and adapted to each specific X-ray method. One part has covered costs for developing a device for serial crystallography at MicroMAX.

Swedish Research Council (RÅC grant no 2021-05981, 2022-2025, 2.93 MSEK)

MAX IV together with Gisela Brändén from University of Gothenburg, Alke Meents from DESY, Matthias Rarey from University of Hamburg, Katerina Dörner from European XFEL and AstraZeneca received funding for the project "X-ray based drug design platform" to cover the cost for one 2-year postdoc, consumables and travel. The project aims at developing new methods to improve X-ray screening for drug discovery.

3.3 User operation processes at BioMAX

3.3.1 Beamtime scheduling

Once the PAC review decision has been communicated to the user, the beamtime can be scheduled. Staff uses the daytime shifts on Mondays, Tuesdays and Wednesday mainly for weekly maintenance, tests, upgrades, user training and staff beamtime (both research and development). Occasionally we schedule challenging experiments requiring extra staff support on those days.

Currently, between 8 and 10 shifts every week are prebooked for proprietary beamtime. Proprietary beamtime scheduling is handled by the Industry Relations Office at MAX IV. General users can book the 22-24 shifts available every week on a first-come-first served basis using a Doodle poll. iNEXT beamtime is also booked by the FragMAX staff via the Doodle poll.

General user startup times during weekdays are 09:00 and 17:00. During weekends and holidays, the usual starting time is 9:00 am. Remote access, together with developments in automated procedures to condition the beam and reset the beamline software, allow supporting four-hour (single shift) beamline slots; we have started this practice routinely for proprietary beamtime, with starting times at 9:00, 13:00 and 17:00. Academic proposals may also be scheduled for single shifts at short notice (3 weeks or less in advance), but for advanced bookings, a minimum of 8 hours is currently enforced for a more efficient use of the beamline.

3.3.2 Preparing the beamline for experiments

Any scientist in the MX group can support the users at BioMAX on a regular basis. This will also be true for the user support at MicroMAX.

About one hour before the start of the first user group of the day, support staff does a thorough check of the beamline and makes sure that the beam is delivered and well aligned and that all the instrumentation and software are working properly. This task is facilitated by the use of automated scripts. The summary of the beamline preparation script (see Section 2.3.1) is logged and can also be e-mailed to the support staff. Staff also removes the previous user samples from the sample dewar, if necessary, and loads the samples

for all the day's user groups, as long as the samples are present at the beamline. Sample handling is described in more detail in Section 3.3.3.

For user groups starting back-to-back, less preparation is required. A simplified beamline reset script that checks that the last sample collected is dismounted, does a quick beam alignment and server reset can be run by staff or by the finishing user group from MXCuBE. Support staff gets an e-mail notification when users run the "beamtime end" script.

Once the beamline is ready, the scheduled user group can start. Remote users typically get a phone call from staff indicating that they can log in to the beamline remote server and start the experiment. Support staff can share ("shadow") the remote desktop with users, which is useful for helping less experienced users, or use Zoom. Local users collecting data on-site receive the required annual on-site safety training if it has expired, and, depending on the level of experience, an introduction to the beamline setup, and instructions to run the experiment.

In addition to the on-site user support, MX staff provides a call-service up to 11:00 pm on weekdays and 8:00 pm on weekends and holidays. MAX IV has recently hired floor coordinators that can assist users 24/7. Since the Fall 2023, the floor coordinators are the initial contact for users, contacting the MX call-service if required.

3.3.3 Sample logistics

Due to the typical MX beamline throughput, and the prevalence of remote experiments, handling the users' samples plays an important part of the BioMAX user support. It is worth noting that MAX IV has no involvement in user sample shipments and, despite the high volume of samples received for MX experiments, there is no dedicated staff assisting the MX group with issues related to shipment (customs clearing issues, shipping documentation, dewar handling on-site, etc.). This usually creates a non-negligible amount of work for support staff.

The following steps are done in preparation of a user experiment:

1. Sample safety evaluation by MAX IV chemical safety.

2. Shipment setup in ISPyB. This is mandatory for cryo-cooled samples to be mounted with the beamline sample changer.

3. Sample dewar shipment and arrival by delivery company. Local users, regardless of whether they conduct the experiment remotely or on site, are encouraged to deliver their samples to goods receiving the day before their experiment so that they can be loaded before the first user shift in the day. If the users bring the samples themselves, the samples are loaded during their beamtime.

4. Sample transport to the beamline, LN2 top-up by MAX IV logistics

5. Samples are loaded into the ISARA dewar. On-site users can load and unload their own samples if they wish, but often staff takes care of this task too.

6. After unloading the samples, the sample dewar is handed out to the courier company by MAX IV logistics. If the dewar is missing shipping paperwork or the users have more beamtime in the near future, the dewar is stored in a designated area, and topped up periodically (Figure 3.6) until the user arranges the return shipment.

Non-cryocooled samples are currently most often transported by users, since they are not supported remotely yet, but they can also be shipped. We encourage the users to use ISPyB, although sample annotation in this case is not implemented yet.

Figure 3.6 Topping up of user's dewars stored at MAX IV

3.4 Community outreach

Training of the MX user community at MAX IV is important to ensure a smooth operation of the MX beamlines in the context of a turn-key experiment setup and a large proportion of remote users. To this end, we periodically organize one-day workshops, usually twice per beamtime period. The training is supplemented with extensive documentation of all the experimental procedures available on the MAX IV web site. The MX group is also engaged in educational and dissemination activities addressed to young scientists, educators and the general public with the aim of increasing public interest in our activities and develop future user communities. BioMAX regularly hosts and co-organizes workshops and tutorials on macromolecular crystallography and structural biology techniques and accepts Training and Education proposals.

3.4.1 User training

We have been offering users training on the BioMAX beamline operation since 2019. At the beginning, users traveled to MAX IV to attend the workshop, but in 2020, after the start of travel and meeting restrictions during the pandemic, we started organizing remote workshops instead. Since remote access has been the norm for most users since, we have not gone back to on-site workshops, although we will most likely provide on-site training for MicroMAX at the beginning of operations. During the training the user gets an overview on use of ISPyB for sample and shipment management, an introduction to MXCuBE, remote operations, and the IT-environment for data processing and data storage/backup at MAX IV. We encourage new users to attend this training, but infrequent users and old users who want to get up to date with the latest developments of the beamline facilities are also welcome.

3.4.2 Web documentation

Detailed and complete user documentation is needed in order to improve users' experience, reduce the workload for staff, and supplement user training. Over the last years, we have developed extensive user documentation for BioMAX on the public MAX IV site (https://www.maxiv.lu.se/beamlines-accelerators/beamlines/biomax/user-information/) that describes in detail all the procedures pertaining to the experiment: shipping samples, setting up remote access, using MXCuBE for data collection, manual data processing and data transfer. Besides written instructions, we have also videos of important procedures, like experiment preparation in EXI and connecting to the remote desktop, among others (see Figure 3.7). FragMAX has its own pages (https://www.maxiv.lu.se/beamlines-accelerators/science-initiatives/fragmax-biomax-fragment-screening-platform/ describing the project for potential users and providing a practical guide to evaluate sample suitability for fragment screening. The MicroMAX webpage will be developed as soon as user experiments start.

We plan to create a common portal for both beamlines and FragMAX, to emphasize the links within the MX group and also increase the visibility of MicroMAX among the current (mainly remote) BioMAX user community. From a practical point of view, a common site will also give us an opportunity to consolidate documentation for procedures that are common to all the facilities.

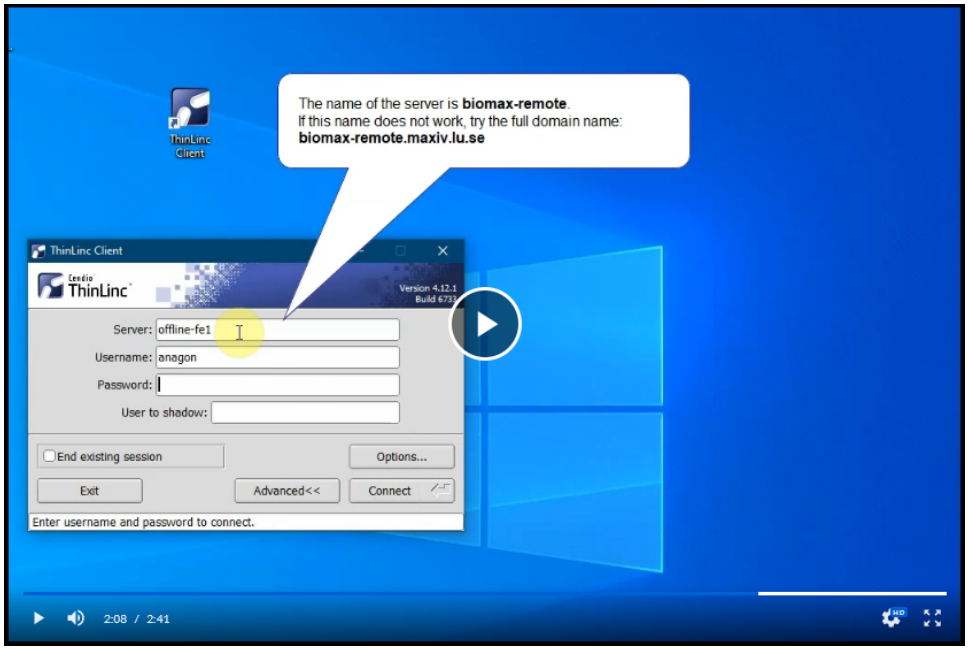


Figure 3.7 Video included in the Web documentation showing how to connect remotely to BioMAX

3.4.3 MAX IV-hosted courses and workshops

On June 6-10, 2022, the MX group organized the MicroMAX Masterclass Summer School on data collection and structure determination in macromolecular crystallography, a five-day event at LINXS and MAX IV, combining lectures by experts in the field and practical tutorials on standard and SSX data collection and data analysis, phasing, and model building. See https://indico.maxiv.lu.se/event/5039/overview for a description of the event. The course attended by 24 international students and was highly rated by participants and tutors.

A RÅC workshop on sample delivery for Serial Crystallography took place in May 22-23 2023. The workshop comprised lectures, demonstrations and discussions with the participants. There were five participants, with preference given to PhD students, postdocs, and participants in the InCellSX RÅC (Röntgen Ångström Cluster) collaboration between the University of Gothenburg and MAX IV Laboratory in Sweden, and the University of Lübeck, EMBL Hamburg, DESY and the European XFEL in Germany to “expand membrane protein & in cellulo crystallization”. The program can be found at https://indico.maxiv.lu.se/event/5199/program.

Both the Masterclass Summer School and the RÅC Workshop were funded by the Novo Nordisk Foundation as part of the MicroMAX Education and Training Initiative.

FragMAX co-organized a symposium and workshop on structure-based drug discovery at the LINXS Institute of Advanced Neutron and X-ray Science in Lund. The event took place from September 18-22, 2023, and was part of the ongoing LINXS theme on Integrated Pharmacology and Drug Discovery (IPDD). The event was structured into three parts. It started with a one-day symposium on structure-based drug discovery, followed by two days of lectures on methods and strategies in fragment-based lead discovery. Finally, a selected group of early career scientists completed a two-day workshop which included one day of hands-on data collection and analysis at BioMAX, followed by computational chemistry work at the Kemicentrum of Lund University. The symposium and lectures attracted more than 70 on-site and online participants and 11 students from diverse scientific backgrounds took part in the workshop.

3.4.4 Training & Education proposals

BioMAX hosts some tutorials which are part of courses in crystallography organized by different universities. The Lund University course KEMM15 and the Gothenburg University KEM360 Structure and dynamics of biomolecules course have had a class on data collection at MAX IV for several years. The students travel to MAX IV for the course and the MX staff is heavily involved in teaching and organizing the tutorials. In 2022, the University of Copenhagen also started applying for training and education time for Master/PhD courses. We also host annually the KFKN10 Biophysical Chemistry course, but it does not involve actual use of the beamlines.

In June 2023, The University of Oulu (Finland) organized an Instruct-ERIC course on macromolecular X-ray, electron, and neutron crystallography and the practical data collection tutorials took place remotely at MAX IV. MX staff traveled to Oulu to teach and lead the data collection tutorials. Assisting a remote data collection from the users' location was a very positive experience, and something we have plans to reproduce in future workshops or even regular user training.

4 User highlights

4.1 Research to Rescue: How MAX IV Drives Nerve Agent Antidote Advancements

Fredrik Ekström, FOI, CBRN Defence and Security, Umeå, Sweden

This presentation delves into our research aimed at developing antidotes to combat the devastating effects of nerve agent intoxication, a concern in the context of warfare, terrorism, and targeted assassinations. We initiate the presentation by revisiting the well-established knowledge of nerve agents' covalent binding to the vital enzyme acetylcholinesterase (AChE) and the use of antidotes to restore normal nervous system function. Our approach combines diffusion-trap cryocrystallography and density functional theory (DFT) calculations, leading to new insights into the mechanism of nerve agent antidotes. These findings provide a foundation for improving antidotes' effectiveness while shedding light on the complex workings of enzymatic reactions.

Expanding our research horizons, we shift our focus to the enzyme Choline O-acetyltransferase (ChAT), a key player in neurotransmitter production and a potential drug target. Through the investigation of prototypical arylvinylpyridinium-based compounds, previously considered inhibitors, we reveal that these compounds are actually substrates in a coenzyme A-dependent hydrothiolation reaction, resulting in the *in-situ* formation of active inhibitors. This unique mechanism creates an adduct deeply embedded within ChAT's active site tunnel, with significant interactions in a hydrophobic pocket adjacent to the choline binding site. This discovery opens the door to the development of more potent and bioactive ChAT inhibitors, potentially reshaping pharmacological possibilities.

In addition, we introduce an exciting discovery of a unique molecular switch that responds to ionic strength and regulatory phosphorylations. This switch influences the structure and dynamics of ChAT, with broader implications for understanding and manipulating the functions of the entire choline/carnitine enzyme family in various diseases.

To conclude, we discuss the pivotal role of X-ray synchrotron radiation in fragment-based screening. This cutting-edge technique offers distinctive opportunities for discovering compounds that target ChAT and other challenging drug targets.

4.2 Crystallography of biomass-degrading enzymes: towards better protection and utilization of renewable resources

Leila Lo Leggio, University of Copenhagen, Copenhagen, Denmark

The world faces major resource challenges which necessitate identification of renewable alternatives to the oil-based industrial feedstock, at the same time as agriculture is under pressure due to climate change. Conversion of polysaccharide/aromatic rich plant cell wall waste materials can make a contribution to overcoming these challenges. In my group we study the structure and function of biomass degrading enzymes and their interaction with biomass components – often themselves very poorly characterized at the structural level. Synchrotron crystallography at MAX IV continues to be a major technique we use to guide fundamental understanding of the functioning of these enzymes and in turn their use. GEs (Glucuronyl esterases) are thought to decouple polysaccharides from lignin while LPMOs (Lytic Polysaccharide Monooxygenases) are copper enzymes which through their flat binding sites are able to attack crystalline substrates, thus they both counteract biomass recalcitrance to enzyme degradation.

I will show briefly:

- high resolution structures of GEs providing the basis of theoretical calculations helping us understand reaction mechanism with model and realistic substrates
- high resolution structures of LPMOs challenging the limit of coordinate precision
- how crystallographic studies can uniquely characterize interactions with complex glycans and hint at novel specificities

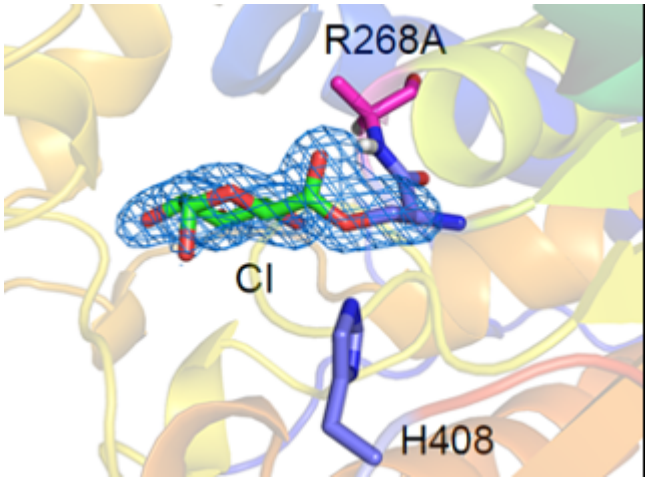


Figure 4.1 Covalent intermediate in the R268A mutant of a glucuronyl esterase (from Zong et al Nature communications 13,1449 (2022)

4.3 Synchrotron studies for drug discovery: an industrial perspective

Tove Sjögren, Discovery Sciences AstraZeneca R&D, Mölndal, Sweden

The structural chemistry laboratory at AstraZeneca Gothenburg recently celebrated its 25th anniversary. Over the years more than 100 drug discovery projects, mainly in the areas of cardiovascular, metabolic and respiratory disease have been successfully supported with 3D structure information and biophysical data. Access to protein crystallography beamlines has always been central to our work, and in the past 6 years we have relied entirely on synchrotron data. I will discuss how data impact on our projects, illustrated by examples. In addition to the tactical support of the current drug discovery portfolio, we are exploring developments in serial crystallography through academic collaborations. Finally I will provide a perspective on our need for synchrotron radiation and a strong relationship with synchrotrons going forward.

5 In-house research

5.1 Sample delivery methods for SSX

The success of methods in crystallization, sample delivery, data collection, and data processing at XFELs led to the development of serial crystallography at synchrotron sources (SSX) for collecting high-resolution diffraction data at room temperature. Interest in utilizing SSX has increased, as data collection is available at ambient temperature, while preventing or minimizing radiation-induced damage. These methods yield more biologically pertinent structural insights compared to conventional X-ray crystallography conducted at cryogenic temperatures with a single crystal (Diederichs & Wang, 2017).

The SSX methods available at BioMAX can be grouped into two categories: flow-based and fixed-target techniques. In flow-based SSX, sample is constantly replenished, which requires more sample than fixed-target approaches but requires less handling of fragile crystals and is amenable to membrane protein crystals suspended in lipidic cubic phase (LCP). Fixed-target SSX needs less sample due to higher hit rates, but greater handling of crystals is required, which can damage them (Zhao *et al*., 2019).

Under the fixed-target approach, we further distinguish between high-degree rotation (with sample supports that can rotate up to 90° during the data collection), and low-degree rotation options (with supports that can rotate up to 20° during the data collection). In the high-degree rotation category, there are many chip-based solutions compatible with the BioMAX MD3 standard goniometer head (see Section 2.1.2.2), Among these, the Serial-X chips were developed in collaboration with the University of Gothenburg. For our work on oxygen-sensitive samples, we developed a dedicated chip and introduced a new protocol for collecting MX data at room temperature under anaerobic conditions. It utilizes silicon-nitride chips in a "sandwich" setup, with custom 3D-printed tools and a MX sample holder. The effectiveness of this method was demonstrated by analyzing deoxy and met-hemoglobin samples at various beamlines, including X-ray macromolecular crystallography at BioMAX and X-ray absorption spectroscopy at Balder (Bjelčić *et al*., 2023).

In the low-degree rotation category, SSX data collection is also possible from crystallization plates, as we demonstrated by collecting data from hemoglobin and lysozyme by scanning crystallization drops during commissioning of the plate adapter (Section 2.1.2.4). This was followed by a successful data collection SSX data on light-sensitive crystals of the photosensory module of the *P. aeruginosa* phytochrome. This work was done in collaboration with the Westenhoff group at Uppsala University.

in April 2023 we carried out an experiment with the Roadrunner II (Roedig *et al*. 2017) at BioMAX. The Roadrunner II was equipped with a humidity chamber which provided good control of the sample environment. This was an intensive, highly collaborative experiment, in which nearly all MX-group staff were involved in some way, and included assistance from Alke Meents's group at DESY. We collected data from test crystals of Hen Egg White Lysozyme, soluble Epoxide Hydrolase (sHE), and Cytochrome *c* Oxidase (C*c*O). The resulting electron density maps from these experiments are shown in Figure 5.1.

For flow-based SSX, besides the standard HVE option (Shilova *et al*., 2020) we have developed the Serial-X flow cell (Ghosh *et al*., 2023) in collaboration with the University of Gothenburg. This device is also in use at other facilities. The Serial-X flow cell offers more flexibility and easier sample handling during data collection compared to HVE, but has slightly higher background because of use of a glass capillary. Both Serial-X and HVE are used for collection of LCP-based samples and are not suited for solution-based data collection. We bridged that gap with the development of the AdaptoCell microfluidic device, which is in its final testing phase.

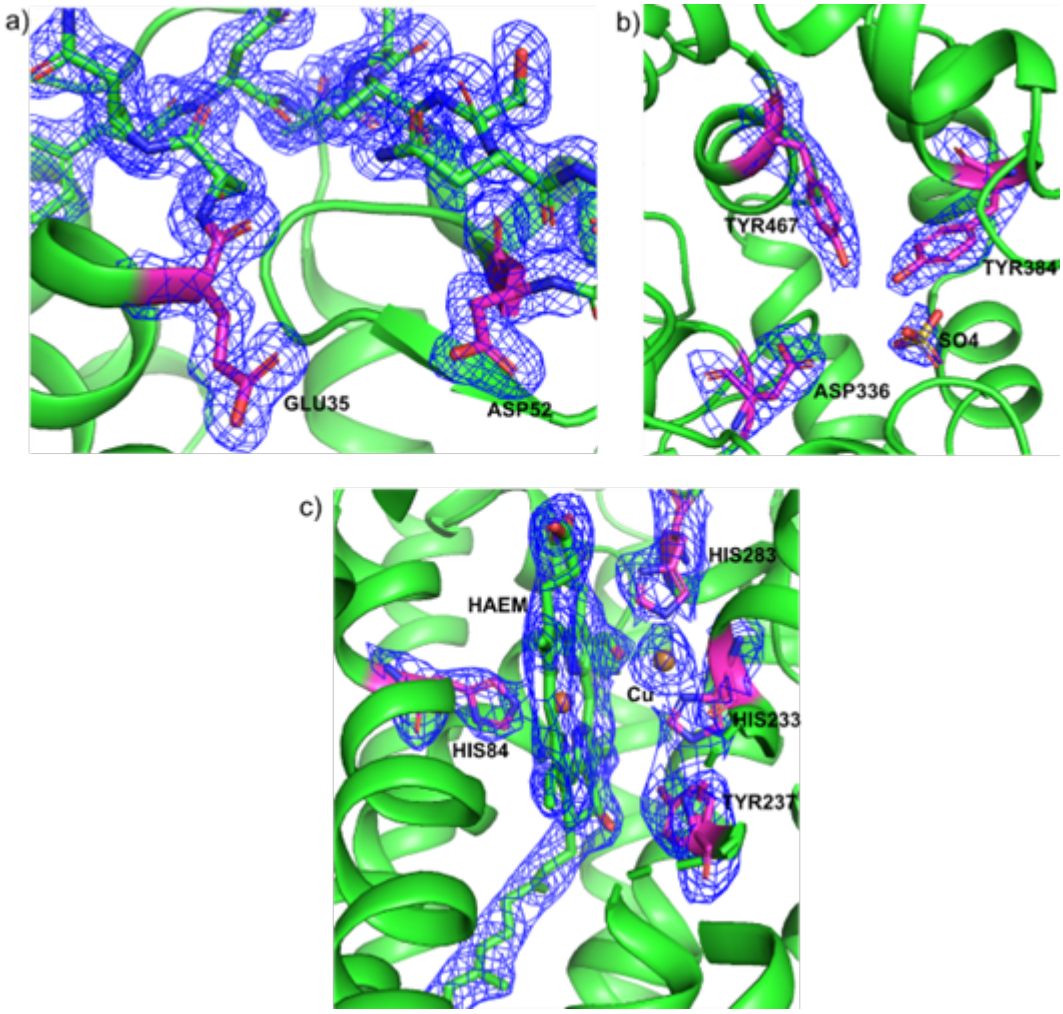


Figure 5.1. Roadrunner results. View of the final 2mFo–DFc electron density map: a) Lysozyme active site, b) sHE active site, c) CcO haem a3 active site. Density is contoured at 1.1σ.

5.2 Development of facilities for data collection at room temperature

Data collection at room temperature (RT) can result in a better understanding of protein dynamics relevant to function. The development of automated approaches for sample handling and exchange at RT is important to make these experiments easier and more convenient to the BioMAX user community. With Europe's most advanced and high-power neutron spallation source being constructed in Lund, the development of a portable crystal mounting setup to prevent crystal dehydration opens the door to collecting both neutron and X-ray data on the same crystal (Figure 5.2). The approach we have adopted consists in mounting the crystals on SPINE-like pins covered with a glass capillary or MiTeGen sleeve filled with reservoir solution. Vacuum grease or epoxy can be used to seal the capillary on the magnetic base to avoid the risk of dehydration. The samples are placed on UniPuck bases placed on the new hot tool puck positions described in Section 2.1.1.4. The crystals can then be mounted on the goniometer by using the warm Sample Changer gripper. Currently, we are using the Sample Changer server interface for mounting and unmounting samples, until this function is made available in MXCuBE. Using such automated system offers the possibility of remote data collection at room temperature in near future.

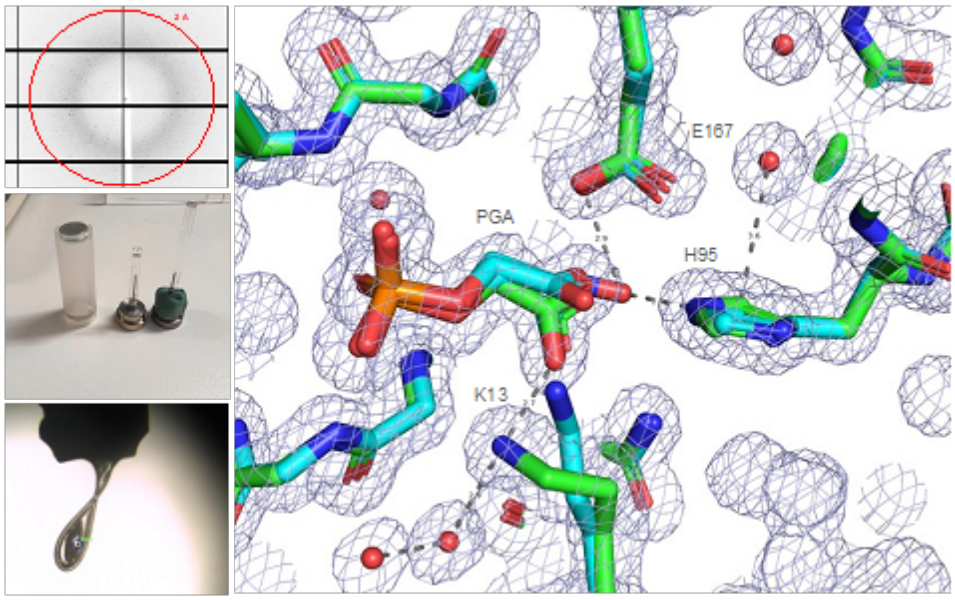


Figure 5.2. Sample mount for RT data collection of Triose phosphate isomerase (TIM) from Leishmania mexicana. The crystals of TIM variant E97Q diffracted X-rays at a resolution of 1.8 Å at room temperature. Active site conserved residues of room temperature structure superimposed with cryo-structure (PDB entry:2VXN) is shown in right image. Alternate conformations for the inhibitor (PGA) and Lysine 13 was observed in RT structure. 2mFo-DFc electron density map is contoured at 1.0σ level.

Due to geometrical restrictions of the gripper and the BioMAX diffractometer, the maximum sample mount-capillary ensemble is 25 mm. This length allows placing the sample in cryo-vials and shipping them with minimum risk of damage in CombiPucks. We have already tested this shipment method by packing the CombiPuck in a styrofoam box; the samples survived a trip between France and Sweden. In future, we are also planning to design chips for in-situ and serial data collection as per the configuration of the gripper.

In situ data collection capabilities have been implemented using the crystallization plate adaptor for the MD3 Arinax diffractometer to facilitate more efficient crystallization screening and avoid the time-consuming process of crystal harvesting, as described in Section 2.1.2.4. The most commonly used in-situ plates have been configured in MD3. The results from single data collection from test crystals were good (Figure 5.3), and we implemented in situ screening a standard facility for general users in 2023. Currently we are working with Lund protein production platform to configure CrystalDirect and Mitegen in-situ plates in Mosquito LCP robot for automated plate setup.

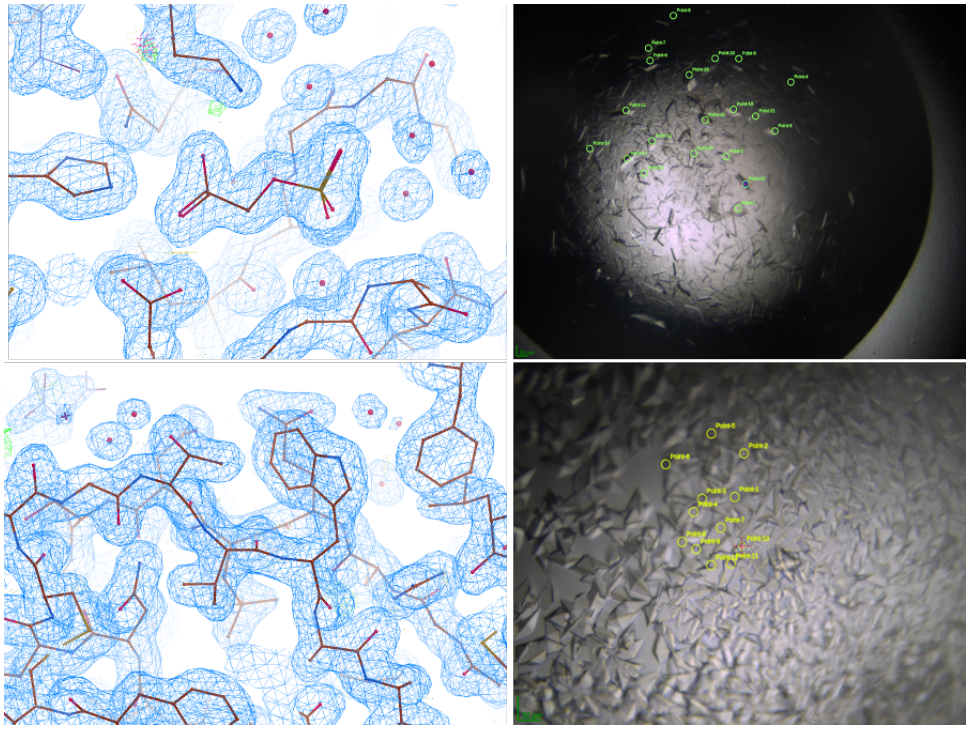


Figure 5.3. 2mFo–DFc electron density map of TIM active site (Top) and Thaumatin (bottom) collected and merged covering 30° of oscillation width using 0.1° per frame and 0.1 s exposure per frame.

5.3 Fast energy scans

At MX beamlines, single-wavelength anomalous dispersion (SAD) and multi-wavelength anomalous dispersion (MAD) techniques (Guss *et al*., 1988) are commonly used for obtaining experimental phases. These methods require performing an energy scan over the absorption edges of anomalous scatterers that can be present in the crystals. Such scans allow the identification of the energies at which the real and imaginary components of the anomalous scattering factor f' and f'' reach local extrema. This information can be used for obtaining experimental phases, as well as for identifying heavy atoms in anomalous difference maps.

Fully automated energy scans at BioMAX were first implemented as conventional step scans. In this operation mode, the sample would only be exposed to the X-ray beam for data acquisition once all the motors involved in the energy change had finished moving for each energy step. These step scans took about 2–3 minutes, not counting the duration of the scan preparation and recovery phases which amounted to a total of 5-6 minutes on average. The overall duration of the step scan was well in excess of the typical time (18–36 s) required to collect a full rotation data set at the beamline. This served as a motivation to develop a continuous (flying) energy scan procedure at BioMAX which improves the overall scanning routine and reduces its duration (Gorgisyan *et al*., 2023).

The continuous energy scan covers 100 eV energy range and carries out a synchronous motion of the BioMAX undulator gap and the Bragg angle of the monochromator while simultaneously measuring the fluorescence signal from the sample at 100 Hz with 1 eV steps. The duration of the scan itself is now only 1 s which is two orders of magnitude shorter than the step scan. The overall duration of the scanning routine, including the preparation and recovery phases is about 90 s on average, which is a factor of 4 faster compared to the step scan.

During the scan the fluorescence is measure by XR-100SDD detector (Amptek, USA), and the signal from this detector is read out using Xspress 3 Mini unit (Quantum Detectors, UK). BioMAX utilizes a PandABox (Zhang *et al*., 2017) for triggering of the Xspress 3 Mini and the fast X-ray shutter, as well as the overall timing and synchronization control of the motors involved in the scanning routine. Due to the synchronous

motion of the undulator gap and the Bragg angle, the beam intensity and alignment remain unchanged throughout the 100 eV energy range.

Figure 5.4 shows a comparison of a continuous and a step scan over a 100 eV range around Br K absorption edge at 13473.7 eV. One can see from the figure that the two scans look similar, and the data analysis following the scan procedure provides the same results for both of them.

The continuous energy scan routine was developed during 2021-2022 and was put in production at the end of 2022 to replace the step scan routine. It has been operating reliably since.

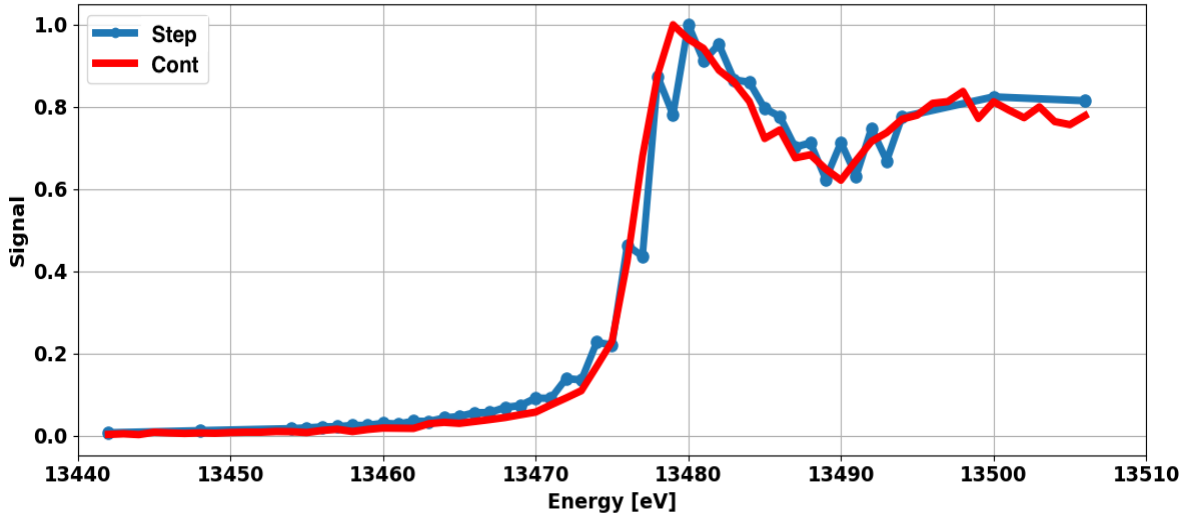


Figure 5.4 Continuous and step scans around Br K edge.

5.4 Time-resolved SSX using a Jungfrau detector

When MicroMAX operates with its multilayer monochromator, it will have a polychromatic beam (or "pink" beam) of 1-2 % bandwidth with over 10^{14} photons/s that can be focused into a 1 × 1 µm^2 spot. For an MX experiment, 10^9 ph/pixel/s can be expected on the detector, which is far beyond the EIGER2 maximum count rate of 10^7 ph/pixel/s. In time-resolved studies, to explore the dynamics down to millisecond time frame, a high framerate detector with kilohertz refresh rates is required. Therefore, MicroMAX has chosen to use a Jungfrau detector.

The Jungfrau detector is a novel integrating pixel detector which was developed at the PSI for high performance photon science applications (Mozzanica *et al.*, 2014). It showed not only excellent performance for XFEL experiments (Nass *et al.*, 2020), but also promising results for synchrotron-based MX (Leonarski *et al.*, 2018). However, the Jungfrau detector is not a commercial product, and it could be more demanding in terms of computing infrastructure, operation, and maintenance. To evaluate its performance in serial crystallography and to get hands-on experience on the operation, we collaborated with the several groups at the PSI and carried out several injector-based SSX experiments including time-resolved studies using the Jungfrau4M at the BioMAX beamline.

With the help from PSI group, we were able to integrate the Jungfrau 4M into the MAX IV infrastructure. The data-acquisition system Jungfraujoch (Leonarski *et al*., 2023) was installed on an IBM IC922 server (Jungfraujoch server), which received the raw readout from Jungfrau detector, converted it to photon counts, compressed the data and published to the downstream system. The compressed data was received by the filewriter (Fujitsu) server and written to the GPFS storage.

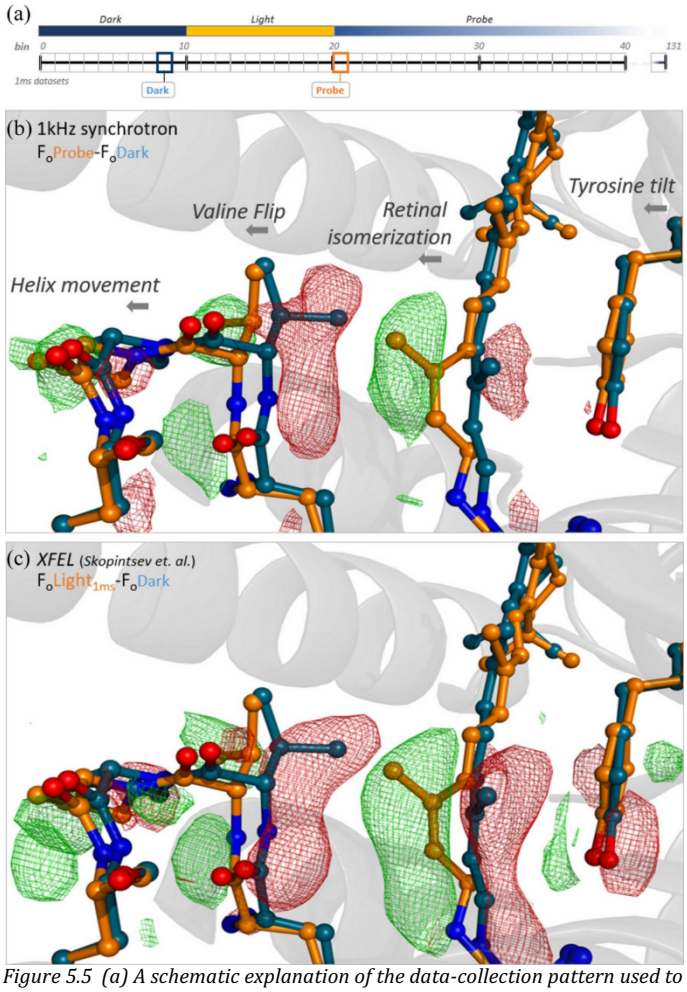


Figure 5.5 (a) A schematic explanation of the data-collection pattern used to record the millisecond datasets. (b) A difference map of Fo from bin #21 (probe, 1 ms after illumination) minus Fo of bin #8 (dark). (c) A difference map of 1 ms Fo (PDB ID 6TK2) minus dark Fo (PDB ID 6TK6) recorded at SwissFEL (data from Skopintsev et al., 2020). All the maps are shown at ±3σ and resolution was cut at 2.38 Å.Skopintsev et al., 2020). All the maps are shown at ±3σ and resolution was cut at 2.38 Å.

In this study, SSX data of lysozyme samples were collected at different frame rates up to 2 kHz and the data quality acquired at kilohertz was comparable to that obtained at 100 Hz. From a 5 s collection at 2 kHz, 2.05 Å resolution structure of lysozyme could be obtained. Next, we continued with time-resolved experiment and used a pump-probe laser setup to study a light-driven sodium pump (KR2). A time range of 130 ms was chosen, including 10 ms dark state, 10 ms excitation by laser and 110 ms probe period (Figure 5.5 a), and data were collected at 1 kHz for around 10 hours. The data from the 10–11 ms revealed retinal isomerization, the valine flip and the movement of the α-helix (Figure 5.5 b), which are the characteristic features occurring in the millisecond time range of the KR2 photocycle, as observed previously (Skopintsev *et al*., 2020). Comparing to the data measured at SwissFEL (Figure 5.5. c), most of the features are observed in both data sets, despite the electron density map from the BioMAX data showing less defined densities owing to limitations in dose and radiation damage when collecting synchrotron data at room temperature. This work showed the high quality SSX data at kilohertz frame rates by the Jungfrau detector, but also demonstrated the possibility to push the time resolution for synchrotron-based time-resolved experiments to the low millisecond regime. An article describing this experiment was recently published in IUCrJ. (Leonarski *et al.*, 2023).

The estimated delivery time for the MicroMAX Jungfrau 4M is in 2025. We have started a new collaboration with PSI on operating the PSI JUNGFRAU 9M detector and associated Jungfraujoch data acquisition system at the MicroMAX beamline during the Swiss Light Source dark time.

6 Roadmap

The goals of the MX facilities over the next years will be to maintain and develop the present strengths to be able to meet the demands of the user community. We foresee developments of room-temperature, serial, and time-resolved crystallography to widen and expand the capacity and scope of FragMAX and to develop experiment control and data analysis to cover the needs for the different types of data collections including un-attended data collection and serial crystallography.

To maximize the impact of these developments it will be important to provide training to the user community, for example to make serial and time-resolved crystallography accessible to a wider user community and to train new PhD students making MX the method of choice for those cases when it is the most suitable technique.

6.1 Short-term planning (1-2 years)

- Further develop the reliability and functionality of unattended data collection at BioMAX (Section 2.1.2.1)
- Establish the ongoing developments of room-temperature data collections as standard methods for users at BioMAX (Sections 2.1.2.4 and 2.1.2.5).
- Further development of data analysis pipelines for single crystal data collections and implement available methods to collect optimal data (Global Phasing).
- Reach the full basic scope of MicroMAX with user operation of serial, time-resolved and high throughput crystallography (including experiment control and automated data analysis). This also includes acquisition of an integrating detector as well as user operation of the beam conditioning unit, the mirror focusing system, and the laser and spectroscopy lab.
- Implement automatic data processing for serial crystallography with rapid feedback during data collection, including online monitoring of for example hit rates and automatic data reduction pipelines.
- Consolidate, document and standardize existing FragMAX workflows and software to support preparation of up to 10,000 crystals per year and develop and commission a semi-automatic crystal mounting device.
- Extend the academic and industrial FragMAX user base with the aim to run 10 – 15 screening campaigns or structure-based drug discovery projects per year.
- Build a strong user community for BioMAX, FragMAX and MicroMAX with a strong community outreach program (see Section 3.4).

Key performance indicators:

- Number of publications (BioMAX: an average of 40 per year; MicroMAX > 5 per year)
- Number of industrial users (> 5 per year) and number of delivered proprietary beamtime hours (> 400 h per year)
- Number of academic and industrial fragment screening campaigns (> 10 per year)
- Number of experiments exploiting the unique capabilities of MicroMAX (> 5 per year) and number of publications on the results of these experiments (> 2 per year)

6.2 Mid-term planning (3-4 years)

- Study and prepare for an upgrade of BioMAX: diffractometer and sample changer upgrade (see Section 2.1.1.5), replacement of focusing mirrors with CRLs (see Section 2.1.1.2).
- Implement online spectroscopy at MicroMAX and further develop sample delivery and time-resolved methods at MicroMAX.
- Develop and implement a new working and access model with other Swedish infrastructures that establishes FragMAX as a gateway environment for structure-driven chemical biology and drug development projects.

- Extend the existing collaboration with the Lund Protein Production Platform (LP3) to enable routine clone-to-structure support for non-structural biologists.
- Establish a workflow with, for example, National Bioinformatics Infrastructure Sweden (NBIS) for streamlined computational hit-to-lead development.

Key performance indicators:

- Number of publications (BioMAX: average of 40 per year; MicroMAX: > 20 per year)
- Number of industrial users (> 5) and number of delivered proprietary beamtime hours (> 400 h per year).
- Number of academic and industrial fragment screening campaigns (> 10 per year)
- Number of experiments (> 10 per year) and publications (> 5 per year) based on the unique performance of MicroMAX

6.3 Long-term planning (> 4 years)

- Upgrade the experiment setup (including diffractometer and sample changer) and beam focusing at BioMAX
- Bring MicroMAX EH2 into operation
- Extension of FragMAX into a general access point for all areas of structure-driven small molecule development

7 References

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