Article

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EXtra-Xwiz: A Tool to Streamline Serial Femtosecond Crystallography Workflows at European XFEL

Oleksii Turkot [1,†], Fabio Dall'Antonia [1,†], Richard J. Bean [1], Juncheng E [1], Hans Fangohr [1,3], Danilo E. Ferreira de Lima [1], Sravya Kantamneni [1], Henry J. Kirkwood [1,4], Faisal H. M. Koua [1], Adrian P. Mancuso [1,2,5], Diogo V. M. Melo [1], Adam Round [1], Michael Schuh [1], Egor Sobolev [1], Raphaël de Wijn [1], James J. Wrigley [1] and Luca Gelisio [1,†,*]

[1] European XFEL GmbH, Holzkoppel 4, 22869 Schenefeld, Germany
[2] La Trobe Institute for Molecular Science, 3086 Victoria, Australia
[3] Current affiliation: Max Planck Institute for the Structure and Dynamics of Matter, 22761 Hamburg, Germany
[4] Current affiliation: PlantTech Research Institute, 3110 Tauranga, New Zealand
[5] Current affiliation: Diamond Light Source, Harwell Science and Innovation Campus, OX11 0DE Didcot, United Kingdom
* Correspondence: luca.gelisio@xfel.eu; Tel.: +49-40-8998-6761
† These authors contributed equally to this work.

Abstract: X-ray free electron lasers deliver photon pulses that are bright enough to observe diffraction from extremely small crystals, at a time scale outrunning their destruction. As crystals are continuously replaced, this technique is termed serial femtosecond crystallography (SFX). Due to its high pulse repetition rate, the European XFEL enables the collection of rich and extensive data sets, suited to study various scientific problems including ultrafast processes. The enormous data rate, data complexity, and the nature of the pixelized multi-modular area detectors at European XFEL pose severe challenges to users. To streamline the analysis of SFX data, we developed the semi-automated pipeline *EXtra-Xwiz* around the established *CrystFEL* program suite, processing diffraction patterns on detector frames into structure factors. Here we present *EXtra-Xwiz*, and introduce its architecture and use by means of a tutorial. Future plans for its development and expansion are also discussed.

Keywords: serial femtosecond crystallography; SFX; *EXtra-Xwiz*; pipelin; *CrystFEL*

1. Introduction

The structural arrangement of atoms in matter and the nature of their chemical bonds can be deciphered by employing X-ray crystallography. This experimental technique has been pivotal in structural biology (see, e.g., [1–3] and references therein) and contributes to date to approximately 85% of the structures released in the Protein Data Bank (calculated using data from [4,5]). X-ray crystallography requires samples to be present in the form of crystals: periodic repetitions of a unique unit cell which results in Bragg peaks upon exposure to X-rays. These encode part of the information to reconstruct the electron density of the sample. In a simplified description, assuming that the kinematic approximation holds, the overall intensity of Bragg peaks increases with (i) the squared number of unit cells in the crystal and its degree of perfection, and (ii) the number of photons interacting with the sample [1]. The size and quality of crystals can only be controlled to a limited extent, also due to the need to maintain realistic near-physiological conditions when biological compounds are investigated. The key to elucidate increasingly complex macromolecules, from e.g., Hemoglobin [7] to the Ribosome [8], instrumental to advance structural biology, has thus been the exploitation of more and more advanced

[1] For an extensive description the reader is referred to, e.g., [6]

photon sources. The photon flux generated from modern sources, which are either based on storage rings or X-ray free electron lasers (XFELs), has enabled the collection of diffraction data allowing up to Ångstrom resolution from smaller and smaller crystals, down to sub-micrometer size at XFELs as a result of their exceptional brilliance. The interaction of crystals with intense X-ray beams results in several physical processes that can lead to permanent radiation damage (see, e.g., [9]). Classical crystallography experiments consist of rotation scans exposing repeatedly the same region of the crystal. Consequently, it accumulates damage during data acquisition. To mitigate this, cryogenic conditions can be employed so as to hamper the processes of radical formation from photolectrons [10]. Another strategy consists in collecting data from fresh regions of the sample, either of the same crystal or different ones, in a serial fashion (see, e.g., [11]). The latter paradigm is adopted at XFELs as a single X-ray pulse typically deposits enough energy to completely destroy the sample [12]. However, the temporal duration of pulses is short enough – of the order of several femtoseconds – that signals from almost undamaged crystals can be detected, as the timescale of the ion movement is much longer [2]. At XFELs, crystals are continuously replaced typically using liquid jets or movable fixed-target stages and intercept the X-ray beam with a certain probability (the so-called hit rate). This technique is termed serial femtosecond crystallography (SFX) [14–18]. Furthermore, owing to the femtosecond duration of their pulses, XFELs are exceptional tools to perform time-resolved investigations at resolutions not achievable by photon sources based on storage rings [13,19–23].

In parallel to the development of X-ray sources and instrumentation, the computer hardware and software to process data from raw diffraction images to the final structural model has evolved in several aspects, including the development of novel crystallographic methods, the usage of parallel processing computational methods, and the design of graphical interfaces to facilitate and automate the data processing. The processing of X-ray crystallography data commences with the reduction of raw detector frames to a unique set of structure factors [24]. This includes finding Bragg peaks, indexing them, integrating pixel intensities in three-dimensions, and averaging the symmetry-equivalent reflection observations with proper scaling. These steps have been implemented in popular software packages such as XDS [25], Mosflm [26], or *DIALS* [27]. Owing to the nature of data collection, serial crystallography requires different algorithms and approaches, and includes a hit-finding step for identifying detector frames containing the signature of diffraction, which is a prerequisite for indexing the reciprocal lattice [17]. Also in this case, dedicated software suites have been developed in the last decade. Notably, *DIALS* has been extended to process serial crystallography data [28], and the *CrystFEL* suite [29] has been developed. Subsequent data analysis, from structure factors to the final atomic model, requires software for crystallographic phasing, model building from derived electron density and its refinement and validation [3].

The entire crystallography data processing pipeline consists of the sequential execution of several tasks, often performed by different software programs. As the input and output data formats, as well as the user experience, might differ greatly across tools, several software pipelines have been developed with the aim of abstracting complexity and increasing analysis throughput and automation level [30–33]. In fact, X-ray crystallography beamlines at storage rings are exceptional examples of sophisticated ecosystems including state-of-the-art robotics, information systems and processing tools, allowing for a comprehensive automation [30,34,35]. Such simplification empowers inexperienced users to focus on scientific questions. It should be pointed out that the need for expert knowledge persists in demanding cases, e.g., when the diffraction signal is particularly weak, or extensive parameter optimization is required.

Several challenges are intrinsic to serial crystallography at XFELs, such as pulse-to-pulse jitter of the X-ray beam in space, wavelength, and energy, which are typically reflected in the amount of

[2] For a comprehensive introduction to structure determination using XFELs the reader is referred to [13]

[3] For a detailed explanation, the reader is referred to, e.g., [30]

diagnostics necessary to interpret the outcome of the experiment. Additionally, the rate and amount of data collected to solve the scientific problem under investigation as well as the often-complicated nature of custom-built detectors further complicate processing and interpretation [15,17,36]. For example, the European XFEL (EuXFEL) [37,38] generates up to 27,000 X-ray pulses per second. A fraction of these is collected by multi-modular pixelized area detectors, such as the Adaptive-Gain Integrating Pixel Detector (AGIPD, up to 3,520 frames or 14 GiB per second) [39], the Large Pixel Detector (LPD, up to 5,120 frames or 10 GiB per second) [40], and the JUNGFRAU detector (up to 160 frames or 1.9 GiB per second) [41], which are synchronized with X-ray pulses. Due to technical reasons, the data acquisition system stores each detector module separately. In particular, predefined sequences of data from each module are stored in different HDF5 [4] files in the EuXFEL data format (EXDF), which might be a significant barrier for several users, and currently cannot be used directly by popular software like *CrystFEL*. Additionally, the sheer volume of the data to be analysed makes workflows practically unfeasible unless distributed computing on high-performance computing (HPC) clusters is employed, whose usage is an additional burden to scientists. Finally, photon sources like EuXFEL enable investigation of ultrafast processes, which are often performed utilizing some form of excitation of the sample (the so-called pump) to then probe the induced molecular dynamics with the XFEL beam. The cost of this is tedious bookkeeping of the data frame subsets, given the pump-probe patterns and verification of correct time sampling, and the same applies in general to diagnostic means.

With the aim of abstracting as much complexity as possible so as to allow scientists to focus on their biological question, we developed *EXtra-Xwiz* [43]. This allows for a high degree of automation of data analysis workflows through its integration with other services provided at EuXFEL. In this paper, we introduce *EXtra-Xwiz* and discuss its current status and future goals. In Section 2 we describe the *EXtra-Xwiz* design and architecture, followed by a step-by-step tutorial with an example of processing SFX data in Section 3. Finally, we give an outlook on planned extensions to the pipeline in Section 4.

2. Design and implementation of the EXtra-Xwiz pipeline

EXtra-Xwiz consists of an SFX workflow-managing tool bundled with some auxiliary programs. In the simplest scenario, a workflow is run as a linear pipeline that will (i) prepare input data, (ii) distribute the input frames into subsets that can be processed in parallel, (iii) perform merging and scaling of structure factor intensity observations, and (iv) create crystallographic figures of merit (FOMs) [44].

To accomplish this, *EXtra-Xwiz* mostly utilizes programs from the *CrystFEL* suite. These include:

- *indexamajig* – main command-line program for indexing and integrating diffraction patterns;
- *cell_explorer* – a tool for displaying and determining unit cell parameters of the crystalline sample;
- *partialator* – for scaling, merging, and post-refining reflections data;
- *check_hkl* – for calculating FOMs based on the full set of merged reflections, such as completeness, average signal strengths, and redundancy;
- *compare_hkl* – for calculating FOMs based on the merged reflections split into two sets, such as R-factors and correlation coefficients.

The full list of the *CrystFEL* programs can be found in the dedicated publication [45] and documentation [46]. *CrystFEL* is freely available at [46].

EXtra-Xwiz is released as an open source project at [47].

2.1. Structure of the EXtra-Xwiz pipeline

A schematic representation of the pipeline is shown in Figure 1. *EXtra-Xwiz* requires a configuration file with parameters for each step, organized into sections.

[4] Hierarchical Data Format v.5 [42]

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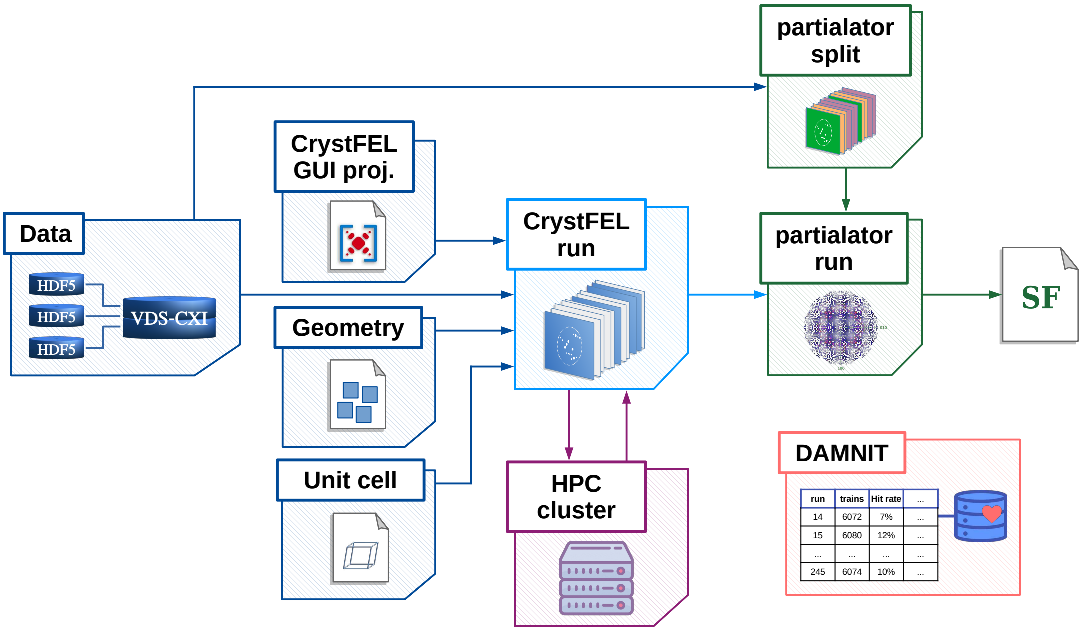

run	trains	Hit rate	...
14	6072	7%	...
15	6080	12%	...
...	...	...	...
245	6074	10%	...

Figure 1. Schematic of *EXtra-Xwiz*. The input expected by *EXtra-Xwiz* consists of (i) experimental data, which is represented as a virtual data set (“Data” block), (ii) a detector geometry description (“Geometry” block), and (iii) optionally a unit cell file (“Unit cell” block). Reduction and processing of the data is performed by *CrystFEL* (“CrystFEL run” block), and input parameters can be either specified in the configuration file or imported from the *CrystFEL* graphical user interface project file (“CrystFEL GUI proj.” block). The former is executed on the High-Performance Computing (HPC) cluster Maxwell (“HPC cluster” block). Afterwards the output reflections are post-processed by the *partialator* program from the *CrystFEL* suite (“partialator run” block), which produces a unique set of structure factors (“SF” file). Reflections can be split by *EXtra-Xwiz* into custom subsets (“partialator split” block) using conditions derived from the input data (see text for details). Automatic execution of *EXtra-Xwiz* and harvesting of metadata can be obtained exploiting the *DAMNIT* tool (“DAMNIT” block). Adapted from [43].

The first step of *EXtra-Xwiz* pipeline is to provide the data in a format suitable for processing by *CrystFEL*. *CrystFEL* can read diffraction data stored in Crystallographic Binary Format (CBF) or Hierarchical Data Format v.5 (HDF5) [48]. For the latter, various standards are supported, including NeXus [49,50] and the native format of the Coherent X-ray Imaging Data Bank (CXIDB) [51].

Due to technical requirements, data from each module of X-ray detectors at EuXFEL is saved to separate files. As this is not compatible with *CrystFEL*, the library *EXtra-data* [52] is used to generate a single HDF5 file with a "virtual" layout (virtual dataset file, VDS) containing references to the relevant original EXDF data. This processing step, as illustrated in Figure 2, is represented as a block "Data" in Figure 1 and corresponds to the section "[data]" in the configuration file.

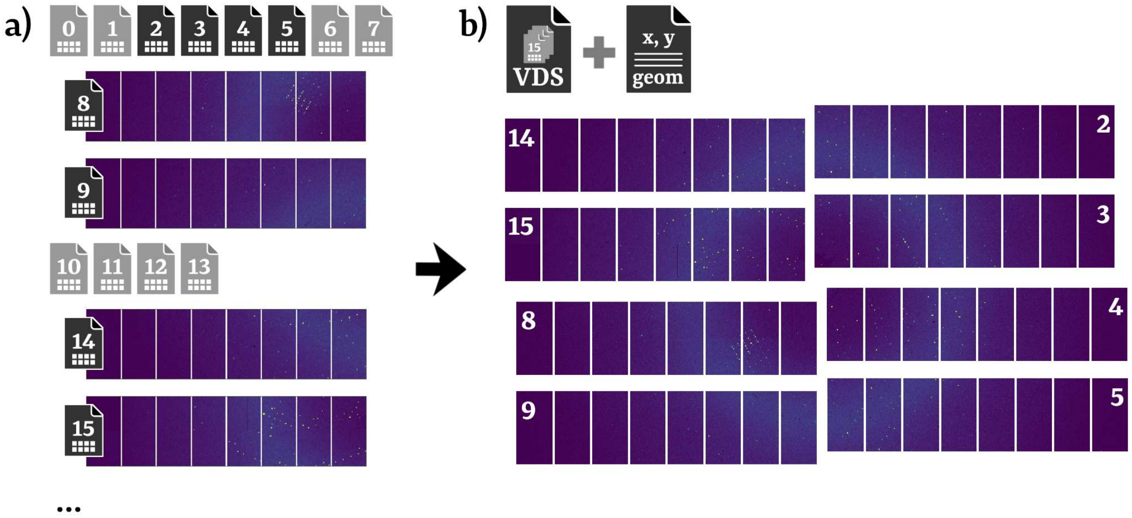

Figure 2. Schematic representation of the virtual dataset file (VDS). (a) Data from each module of EuXFEL X-ray detectors is stored as a different file. (b) Data from all modules assembled as a single array in a VDS file. A geometry description ("geom") can then be used to spatially arrange modules in the laboratory frame. In this example, only the eight inner modules are displayed, corresponding to the black file icons in (a).

To cope with variable spatial configurations of detector modules, *CrystFEL* uses a generalized detector representation – the geometry file [29]. This describes the position of each detector pixel in the laboratory coordinate system, as well as the pixel size and the X-ray beam energy. It also contains information on the internal HDF5 paths of the image dataset and any bad pixels mask in the input data file. The geometry file, represented with the block "Geometry" in Figure 1, is specified in the section "[geom]" of the *EXtra-Xwiz* configuration file.

If prior information on the unit cell is known, it can be used to filter auto-indexing results that agree to the expected reference. Such reference unit cell parameters (a, b, c, α, β, γ) can be specified in the unit cell file schematically represented with a block "Unit cell" in Figure 1. It can be provided to *EXtra-Xwiz* in the "[unit_cell]" section of the configuration file. Some of the indexing methods can derive the crystal symmetry even without prior information on the lattice. In this case, *EXtra-Xwiz* can be run without specifying the unit cell file, as illustrated in Figure 3. After the indexing step, the *cell_explorer* graphical user interface (GUI) will be launched to display the histograms of resulting lattice parameters. This allows users to determine the cell parameters with a fit and to save them into a unit cell file which will be expected by *EXtra-Xwiz* to continue processing the data as shown in Figure 1.

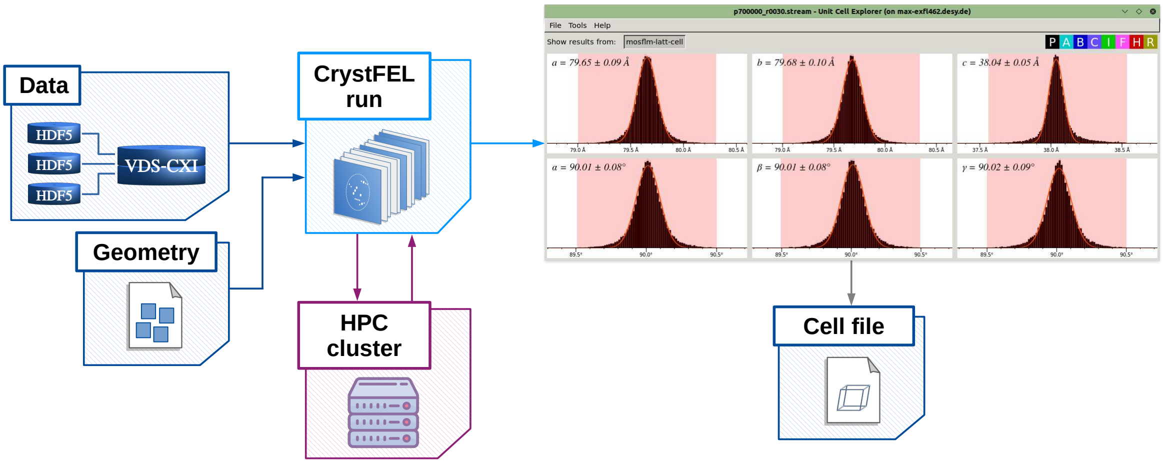

Figure 3. Schematic of the *EXtra-Xwiz* workflow used to determine the unit cell in case no initial unit cell file is available. The blocks are the same as defined in Figure 1, except for the *cell_explorer* graphical user interface displayed in the top right corner.

Main processing and reduction of the SFX data in *EXtra-Xwiz* is performed with the *indexamajig* tool from *CrystFEL*. It is used for finding the location of Bragg peak candidates in each detector image, indexing the patterns, and integrating peak intensities. For each image frame *CrystFEL* writes information on identified Bragg peak candidates, lattice parameters found by the auto-indexing algorithm, and integrated intensities into a so-called *stream* file in a form of a plain text. This processing step is represented with a "CrystFEL run" block in Figure 1. Parameters for *indexamajig* should be specified in the "[indexamajig_run]" section of the *EXtra-Xwiz* configuration file.

Identifying optimal parameters for the Bragg peaks search is crucial for successful indexing. If too many true peaks are missed or too many false peaks (for example, from the detector background or misbehaving detector pixels) are included, the indexing algorithm may fail completely or identify wrong unit-cell parameters and crystal orientation. The sample and data taking conditions may differ significantly during the experiment and therefore it is a good practice to regularly tune parameters. The graphical user interface of the *CrystFEL* suite offers a convenient tool for performing such a preparative step based on visual inspection of a few of image frames. A session of the *CrystFEL* GUI can be stored into a project file and *EXtra-Xwiz* provides a command-line tool *xwiz-import-project* for importing parameters from such file into the *EXtra-Xwiz* configuration file. This procedure is represented by the "CrystFEL GUI proj." block in Figure 1.

The most time-consuming step of processing SFX data is indexing. Due to the independent nature of the SFX frames, portions of collected data sets can be processed separately, which allows for parallelization. *EXtra-Xwiz* automatically distributes *indexamajig* jobs on the local HPC cluster Maxwell operated with the *Slurm* [53] batch-queue system. The results are then concatenated into a single *stream* file. This is represented by the "HPC cluster" block in Figure 1. In the configuration file the name of the *Slurm* partition as well as the number and expected duration of distributed tasks have to be specified under the "[slurm]" section.

After indexing, reflection intensities from the whole data set are merged for each symmetrically unique reflection. This task is accomplished by the *CrystFEL partialator* program, which can determine scaling factors for individual measurements, correct for partiality, and post-refine the model parameters for each crystal [54]. This process is usually repeated in an iterative procedure to bring the individual measurements into agreement [48,54]. The *partialator* program operates on the content of the *stream* file and therefore does not require the initial SFX data. This data analysis step corresponds to the "partialator run" block in Figure 1 and "[merging]" section of the *EXtra-Xwiz* configuration file. Merged reflections are stored in the *hkl* file format as plain text, which can be imported into most structure-solution packages. Output from *partialator* is used by *EXtra-Xwiz* to calculate figures of merit with *check_hkl* and *compare_hkl*. The completeness of unique reflections and signal-to-noise ratio (SNR) $I/\sigma(I)$ are estimated with *check_hkl* from a single list of reflections merged from all available data. To calculate the correlation coefficients $CC_{1/2}$ and CC^* as well as R-factor R_{split}, the reflection observations for each unique Miller index are randomly split before merging by *partialator* into two halves. Both half-data sets are merged separately and their correlation computed with *compare_hkl*. Values of the listed FOMs for all data and outer reflections shell are stored by *EXtra-Xwiz* into a table in a summary file.

Certain types of SFX experiments are performed varying some external parameter, which results in data subsets having different characteristics. These include time-resolved SFX experiments, in which sample states excited for example by visible light (*pump on*) are probed by X-rays as a function of time. Interpretation of such data usually relies on the difference between the subsets. It is therefore crucial to ensure that all other experimental conditions, e.g., the X-ray beam fluence, as well as data processing are kept as close as possible between the sets. To satisfy this, *pump on* frames are often collected interleaved with the *pump off* frames within the same train of X-ray pulses. In this case, *partialator* can be used to split reflections after scaling and post-refinement and prior to merging. This option requires a text file with a "data set identifier" (e.g., the pump status) for each frame in the input data. *EXtra-Xwiz* produces such files either using the frame pattern information specified by a user, or automatically utilizing information from a diode which records the signal from the pump laser. This procedure is represented with the "partialator split" block in Figure 1. Parameters for the splitting have to be specified in an optional "[partialator_split]" section of the *EXtra-Xwiz* configuration file. Merged reflections *hkl* files as well as FOMs are produced for each subset as well as for the overall data set.

The execution of *EXtra-Xwiz* can be automatically triggered as soon as experimental data become available by exploiting its integration with the software *DAMNIT* [55]. Furthermore, *DAMNIT* provides a graphical interface to *EXtra-Xwiz* results.

3. Data processing with EXtra-Xwiz by example

This section describes an example of processing SFX data from hen egg-white lysozyme (HEWL) microcrystals collected at the SPB/SFX instrument using the AGIPD detector (run 30, proposal 700000). Only basic knowledge of the Unix commands and environment are expected from the reader. Lines starting with a "$" indicate commands which should be executed in a Unix shell. For readers who are not users of the European XFEL, the Virtual Infrastructure for Scientific Analysis (VISA) [56] service can be used and additional instructions are provided in Section 3.2.

Access to the Maxwell cluster is exclusive to EuXFEL users and detailed instructions on how to connect to it can be found in the EuXFEL Data Analysis user documentation [57]. In general, a user with an active account can connect to one of the interactive cluster nodes:

```
$ ssh <user name>@max-exfl-display.desy.de
```

To start using *EXtra-Xwiz* a dedicated module has to be loaded at the cluster with:

```
$ module load exfel EXtra-xwiz/crystals2023
```

As mentioned in Section 2.1, *EXtra-Xwiz* requires a configuration file in TOML format [58] for its operation [5]. It should be named "xwiz_conf.toml" and a template of such file can be generated by starting the pipeline for the first time in an empty folder with the following command:

```
$ xwiz-workflow
```

The configuration file contains parameters for each of the pipeline processing steps organized into sections such as "[data]", "[geom]", "[unit_cell]", "[indexamajig_run]", and "[merging]". A copy of the

[5] Detailed description of all available configuration options is available at the *EXtra-Xwiz* documentation [59]

configuration file used in this example along with all other files necessary for the pipeline execution can be downloaded from [47].

Data to be processed by the pipeline can be specified with just a proposal number and a list of runs in the "[data]" section of the configuration file:

```
[data]
proposal = 700000
runs = [30]
```

It is possible to select a subset of frames from each run with an optional `frames_range` parameter in the same section, for example:

```
frames_range = {start = 0, end = 200000, step = 1}
```

This parameter has values organized into a dictionary similar to the Python range object but inclusive for the end value with `end = -1` representing the last frame of the run.

For the purpose of reproducibility of the SFX analysis, *EXtra-Xwiz* supports a list of different versions of the *CrystFEL* suite which can be selected in the "[crystfel]" section:

```
[crystfel]
version = '0.10.2'
```

Currently, recent major *CrystFEL* versions are available, as well as a "maxwell_dev" option which corresponds to the constantly updated installation of the latest *CrystFEL* version.

Geometry and unit cell parameters files should be provided to the pipeline in the "[geom]" and "[unit_cell]" sections, respectively:

```
[geom]
file_path = "agipd_p700000_r0030.geom"
[unit_cell]
file_path = "hewl.cell"
```

A representative detector frame is shown in Figure 2(b). There detector modules are positioned in the laboratory frame layout with the use of *EXtra-geom* library [60].

In case the unit cell of the sample is not known prior to the analysis, it can be generated by setting the option `file_path = "none"` as described in section 2.1. During the *EXtra-Xwiz* session, after the indexing step, an interactive *cell_explorer* session will start. At this point, the user is expected to determine the cell parameters from the histograms of indexing results (as explained in [48]), and save them into a unit cell file which will be requested by *EXtra-Xwiz*. This procedure is illustrated in Figure 3.

Parameters for Bragg peak identification and indexing with *indexamajig* program have to be specified in the "[indexamajig_run]" configuration block:

```
[indexamajig_run]
resolution = 4.0
peak_method = "peakfinder8"
peak_threshold = 800
peak_snr = 5
index_method = "mosflm"
integration_radii = "2,3,5"
...
min_peaks = 10
extra_options = "--no-non-hits-in-stream"
```

Documentation regarding all *indexamajig* options can be found at [46]. In the current state of *EXtra-Xwiz* not all of these options are covered by the default configuration file parameters, and if any of such options are required for data processing they can be specified in the string for `extra_options` parameter. Data processing with *indexamajig* is the most time-consuming step of the whole pipeline but the computations are usually performed in parallel on multiple nodes of the Maxwell cluster. Cluster partition, number of nodes to use in parallel and maximum expected duration of the individual jobs should be specified under "[slurm]" section of the configuration file:

```
[slurm]
partition = "upex"
n_nodes_all = 20
duration_all = "10:00:00"
```

For testing the pipeline on a small subset of data (e.g., a hundred frames), without exploiting the *Slurm* jobs scheduler, it is advised to select the "local" partition. In this case *EXtra-Xwiz* will run *indexamajig* on the same node the pipeline is running.

Reflection intensities obtained from the Bragg peaks indexing are merged and post-refined with the *partialator* tool and required parameters have to be specified under the "[merging]" block of *EXtra-Xwiz* configuration:

```
[merging]
point_group = "422"
scaling_model = "unity"
scaling_iterations = 1
max_adu = 100000
```

Point groups corresponding to the symmetry groups of crystallized samples can be identified with the table in *CrystFEL* documentation [61].

In case of the time-resolved SFX experiments *pump on* (sample illuminated with the "pump" laser) and *pump off* (sample in the non-excited state) frames are processed in the same manner and separated only on the merging step of *partialator*. As already mentioned in section 2.1, for such separation *partialator* requires an additional input file labelling accordingly each frame of the input data. *EXtra-Xwiz* can generate such file according to parameters specified in the "[partialator_split]" block. Let us assume that the machine delivers only at 1/8th of the 4.5 MHz maximum repetition rate and the detector is configured to record only these pulses. The sample is illuminated by infrared light every third delivered pulse (i.e., the 24th assuming 4.5 MHz operation), resulting in the following labels, "pump_on pump_off pump_off pump_on ...". The latter can be set in the configuration as:

```
[partialator_split]
execute = true
mode = "by_pulse_id"
[partialator_split.manual_datasets]
  pump_on = {start=0, end=-1, step=24}
  pump_off = [{start=8, step=24}, {start=16, step=24}]
```

Any user-defined set of labels can be specified with a corresponding list of inclusive range-like dictionary objects or pulse id values. Usually, in time-resolved experiments a diode is used to record data relative to the state of the pump laser. *EXtra-Xwiz* can utilize signal from this diode and automatically generate labels accordingly if the `mode` parameter is set to either "on_off" or "on_off_numbered":

```
[partialator_split]
execute = true
mode = "on_off_numbered"
```

```
xray_signal = ["SPB_LAS_SYS/ADC/UTC1-1:channel_0.output", "data.rawData"]
laser_signal = ["SPB_LAS_SYS/ADC/UTC1-1:channel_1.output", "data.rawData"]
```

The difference between the "on_off_numbered" and the "on_off" mode is that in the former case consequent events of the same kind (e.g., pump off) are identified by an increasing number. For the example given above, the "on_off_numbered" labels are "on_1 off_1 off_2 on_1, ...". Paths to the diode data specified for `xray_signal` and `laser_signal` are provided by beamline scientists. As data used in this tutorial does not originate from the pump-probe experiment, the splitting into datasets does not make sense and should be avoided by either setting `execute = false` or simply removing the "[partialator_split]" block from the configuration file.

After all the configuration parameters have been set the *EXtra-Xwiz* pipeline can be executed in an automatic mode with:

```
$ xwiz-workflow -a
```

Without the "-a" ("–automatic") optional argument the pipeline will verify each configuration parameter with a user in the interactive procedure. When the *EXtra-Xwiz* operation finishes, it will generate a summary file containing information on the processed data statistics and FOMs, for example:

```
Step #    d_lim    source       N(crystals)     N(frames)     Indexing rate [%]
  1         1.6    indexamajig      46899       639616            7.3
...
Crystallographic FOMs:
                        overall   outer shell
Completeness              100.0         100.0
Signal-over-noise         4.224          0.99
CC_1/2                   0.8974       0.03244
CC*                      0.9726        0.2507
R_split                   27.28          80.6
```

These results are provided only to demonstrate the capabilities of the pipeline and could be improved, for example by tuning selected parameters and processing more runs of the collected data. The resulting *CrystFEL stream* file can be found in the same folder and the output *hkl* file with full FOMs tables in the "partialator" folder.

3.1. Automatic scan over EXtra-Xwiz configuration parameters

Sometimes it is required to run the *EXtra-Xwiz* pipeline modifying one or multiple configuration parameters over a list of values, for example to assess the sensitivity of a given parameter. For such use case an *xwiz-scan-parameters* tool have been developed. Similar to *xwiz-workflow*, it requires a configuration file, and a template is generated on the first use of the tool in an empty folder:

```
$ xwiz-scan-parameters
```

The configuration file "xwiz_scan_conf.toml" for parameters scan tool consists of four sections: "[settings]", "[xwiz]", "[scan]", and "[output]". Main parameter of the "[settings]" block is `xwiz_config` which determines the path to the initial *EXtra-Xwiz* configuration file.

The "[scan]" section can contain any number of sub-sections. Each sub-section will be treated as a next level of a nested loop, therefore number of iterations in each scan are multiplied. Names of the parameters within each scan sub-section represent full names of the parameters in the *EXtra-Xwiz* configuration files and their values should contain either a list or an inclusive range-like dictionary of values to scan over. If multiple parameters are listed within one scan they have to contain the same number of values which will be modified simultaneously on each step of the scan, for example:

```
[scan.SNR]
'indexamajig_run.peak_snr' = {start = 3, end = 7, step = 2}
'indexamajig_run.peak_threshold' = [1000, 800, 700]
```

Here a scan with 3 iterations is defined: first an SNR value of 3 will be used with a threshold of 1000, next an SNR of 5 will be set with a threshold of 800, and finally an SNR of 7 with a threshold of 700.

In the "[output]" section, the file names to store the tables with the results can be specified. When the configuration file is ready, the parameters scan can be started by running the *xwiz-scan-parameters* in the same folder. It will run *EXtra-Xwiz* sequentially over all scan iterations and collect data processing statistics and overall values of FOMs for each scan step into a table similar to this one:

```
                          index_rate(%)  ...  cc_half  cc_star  r_split
peak_snr  peak_threshold
       3            1000          0.080  ...    0.051    0.313   105.40
       5             800          7.332  ...    0.894    0.972    27.44
       7             700          7.219  ...    0.919    0.979    25.81
```

3.2. Running EXtra-Xwiz tutorial at VISA

VISA is a platform for cloud-based data analysis, developed by the Institute Laue-Languevin and deployed at several European photon and neutron facilities [56]. Its documentation can be found at [62].

In order to perform this tutorial using VISA, an instance containing an *EXtra-Xwiz* installation can be generated and used in a web browser:

1. navigate to https://visa.xfel.eu;
2. click "Create a new instance";
3. click "Search for experiments" and select the proposal "p700000 - SFX on Hen egg-white lysozyme, AGIPD detector";
4. click on "EXtra-Xwiz_Crystals2023" environment;
5. choose the virtual hardware;
6. create the instance.

There is no need to load any additional modules and the tutorial described above applies except for the distribution of computations on the HPC cluster, which is not accessible from VISA. Because of that, the following should be used:

```
[slurm]
partition = "local"
[indexamajig_run]
n_cores = 1
...
```

Be careful, this would result in a very slow processing of the input data. Therefore, instead of running *EXtra-Xwiz* on the entire run, a pre-selection of "good frames" can be used by specifying in the "[data]" section:

```
[data]
...
frames_list_file = "indexed_p700000_r0030.lst"
```

Please note that the "indexed_p700000_r0030.lst" file has been produced specifically for this VISA example and this option is never used in the actual processing of the experimental data.

4. Discussion and outlook

A challenge that requires expert knowledge and/or iterative processing steps lies in the optimization of parameters, such as minimum signal-to-noise ratio, peak finder thresholds, etcetera. Technically, as these parameters are passed to the *CrystFEL* programs through a configuration file, iterative runs require a re-editing of the configuration, which can quickly become tedious. Currently, *EXtra-Xwiz* offers two ways to simplify this: first, the software includes a grid search, as described in Section 3.1, that can scan over some parameter space. This requires some estimate of reasonable parameter ranges, and enough time to compute the necessary grid nodes. Second, peak-finding parameter optimization can be done manually, and with visual feedback, using the *CrystFEL* GUI. Results of a GUI session can be stored to a project file to be used by *EXtra-Xwiz* for batch processing of one or more entire runs. Both of these approaches have their limitations, in particular related to the interpretation of their results by inexperienced users. Alternatively to brute-force searches, optimization methods, for example based on artificial intelligence, can be employed. In particular, we developed a method based on Bayesian optimization that optimizes *EXtra-Xwiz* parameters by maximizing the indexing rate, and reduces the need for expertise in the interpretation of results [63]. This solution is being deployed at EuXFEL and integrated into *EXtra-Xwiz*. The same approach can also be used to tune the detector geometry representation in laboratory space. Indeed, the overall SFX workflow can greatly benefit from automating this task. At the moment, this is largely done manually. The operator is typically using graphical software for the visual centering and quadrant fitting of powder diffraction rings, followed by iterations of *indexamajig* and *geoptimizer* [64] for maximizing the yield of indexable frames.

As a more long-term outlook, we strive to give users an end-to-end experience, that is, a pipeline covering all the steps from detector images to an atomic structure built into the reconstructed electron density. For this purpose, downstream modules handling the program execution related to the tasks of crystallographic phasing, model building and (preliminary but automated) structure refinement need to be included to the *EXtra-Xwiz* framework. Furthermore, owing to the modular nature of *EXtra-Xwiz*, in the future, software different from *CrystFEL* (e.g., *DIALS*), might be considered. It should be pointed out that challenging problems, in particular with respect to de novo structures, will require user experience for decisions along the way, for instance which phasing method to apply, or which search model to use for molecular replacement. This means that the design of the workflows have to be well thought, allowing user intervention where required and good balance of parameters with reasonable defaults versus expert input, boiling down to a useful semi-automatic approach. For this, an improved user interface is being designed.

5. Conclusions

EXtra-Xwiz has been designed to support scientists performing SFX at the European XFEL. It handles tasks such as data preparation, discrimination of data based on pump status, and parallel processing which often pose a significant barrier to inexperienced users.

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Abbreviations

The following abbreviations are used in this manuscript:

SFX	Serial femtosecond crystallography
XFEL	X-Ray Free-Electron Laser
EuXFEL	European XFEL facility
EXDF	EuXFEL Data Format
AGIPD	Adaptive-Gain Integrating Pixel Detector
LPD	Large Pixel Detector
HPC	High-Performance Computing
HDF5	Hierarchical Data Format v.5
CBF	Crystallographic Binary Format
CXIDB	Coherent X-ray Imaging Data Bank
GUI	Graphical User Interface
VDS	Virtual Dataset File
FOM	Figure Of Merit
SNR	Signal-to-Noise Ratio
HEWL	Hen Egg-White Lysozyme
VISA	Virtual Infrastructure for Scientific Analysis

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