

Serial Femtosecond Crystallography – Supplementary Information

Thomas R.M. Barends¹, Benjamin Stauch², Vadim Cherezov², Ilme Schlichting^{1†}

¹ Department for Biological Mechanisms, Max Planck Institute for Medical Research, Heidelberg, Germany

² Department of Chemistry, The Bridge Institute, University of Southern California, Los Angeles, CA, USA

†email: ilme.schlichting@mpimf-heidelberg.mpg.de

Synchrotron, location	Beam-line	Sample devices	Detector/ Frame rate	Analysis support /pipeline	Comments
APS, Chicago, USA	SBC/19ID	ALEX Holder, Chips, Fixed Target	PILATUS 6M / 100 Hz	Kanzus/Globus Flows Pipeline	Time resolved serial available in collaboration with BioCARs at the APS
	BioCARs	Chips DoT HVE inj. Microdrop	Rayonix 340HS (10 Hz), epix10k (1 kHz)	Precognition (Laue data processing; https://biocars.uchicago.edu/facilities/software/precognition-documentation)	Time-resolved Laue crystallography; ns and ps lasers
	GM/CA /23ID	chips, HVE inj., in-situ plates	Pilatus3-6M, Eiger-16M	crystfel, cctbx_xfel	Contact beamline staff regarding types of in-situ plates
CHESS, Cornell, USA	ID7B2	Chips	EIGER2 16M (130 Hz)	Semi-automatic procedure based on XDS and Python scripts	Collaboration with MiTeGen provides a variety of chips, as well as a humidity-controlled box for loading chips. Users are welcome to bring their own sample-handling devices.
DIAMOND, Harwell, UK	I24	Chips HVE inj.*	PILATUS 6M (100 Hz) EIGER CdTe 9M (230 Hz)	Auto hit-finding/ hit-rate monitoring (DIALS). Semi-automated indexing and integration (DIALS) Other packages will can also be used/will work.	Primarily silicon chips but any fixed target/chipless chip. PORTO laser (https://lightcon.com/product/orpheus-opa/) and high power LEDs for photoexcitation. Drop on chip mixing. Anaerobic fixed targets available. Acoustic chip loading. Fixed target travel ranges (default setup) 50mm.
ESRF, Grenoble, France	ID29	Chips HVE inj. microfluidic	JUNGFRAU 4M up to 2kHz (typical 925 Hz)	Under development (NanoPeakCell, CrystFEL)	SSX fast translating horizontal range up to 5 cm Nanosecond laser 400-2000nm + 355 nm TR UV-vis absorption spectroscopy at icOS Dedicated sample preparation laboratory Possible to adapt user developed sample devices
MAX IV, Lund, Sweden	BIOMAX	Chips HVE injection	EIGER16M 100Hz (500Hz @4M)	(py)Dozor for spots-finding, Cctbx/crystFEL via PReSTO	For time-resolved experiment, so far we have mostly provided trigger device(s) and control, while users bring their own laser system. Pipelines to provide users feedback of hit-rate, indexing-rate etc are under development.

					Chip size (travel range of scanning stage) 2.5 x 2.5 mm ²
	Micro MAX	- Chips - HVE & capillary injection	-EIGER2 X CdTe 9M (230 Hz @ 9M, 550 Hz @ 4M) - JUNGFRUA Si 4M (2.2 kHz), from 2023	(py)Dozor (spots-finding) and CrystFEL (indexing) online pipeline under development PReSTO software stack (https://www.nsc.liu.se/support/presto/) and HPC resource for offline use	- Wavelength-tunable nanosecond laser - X-ray chopper down to 10 μs pulses and variable photon energy bandwidth (0.02, 0.3 & 1.0%) - Support for chips up to ~ 30x30 mm ² - Offline injector setup and laboratory space for light-sensitive samples.
NLSL-II , Brookhaven Natl. lab. USA	FMX	Chips HVE inj.*	EIGER X 16M (133 Hz)/ 4M (750 Hz)	PyMDA CrystFEL	Oxford Chip ¹ Droplet ejector (under construction)
PETRA III , Hamburg, Germany	P11	DoT and Chips	EIGER 2 16M (130 Hz)	nXDS and CrystFEL	Time resolved experiments are carried out with the tape drive in combination with a mixing device. A chopper wheel is available (up to 500 Hz) Automated data processing with CrystFEL is under development
	P14.1 P14.2 (T-REXX)	HVE inj.* Chips	EIGER 2 16M CdTe (1120 Hz) EIGER 4M (750 Hz)	CrystFEL/DIMPLE CrystFEL/DIMPLE	532, 630, 660 nm cw; 355, 532 nm pulsed 532, 630, 660 nm cw 355, 532 nm pulsed HARE* ² LAMA* ³
SLS , Villigen, Switzerland	PXI/P XII VESP A	Chips HVE inj.* Single shot chips (2023)	EIGER 16M (130 Hz) JUNGFRUA 4M (2 kHz)	ADP / XDS for small rotation wedges. Single shot analysis pipeline in preparation	Tunable ns laser (https://ekspla.com/product/nt230-series-nanosecond-tunable-dpss-lasers), acoustic levitator
SPring-8 , Sayo, Japan	BL32X U	Chips	Eiger X 9M (238 Hz)	• Auto hit-finding using SHIKA (backend Cheetah) • Semi-automated indexing and integration (CrystFEL)	Automated data collection using SSX-ROX down to sub-micron crystals
	BL45X U	Chips	PILATUS3 X 6M (100 Hz)		Automated data collection using SSX-ROX
	BL41X U	Chips, HVE inj. ⁴ (being installed)	Eiger X 16M (133 Hz) Eiger2 X CdTe 4M (500 Hz)		• Automated data collection using SSX-ROX • Tunable ns laser NT230-30Hz (Ekspla) • Fixed target stage with 50 mm stroke (under installation)
SSRL , Stanford, CA, USA	12-1	Chips/fix ed target, injectors (liquid or viscous media)	EIGER 16M 2-XE (500 Hz)	Real-time SX data analysis pipeline	SX-Rot (90°/sec), pink beam multilayer in commissioning, sister beam line to LCLS-MFX standard goniometer setup at MFX, fully remote access fixed-target collection at controlled humidity, controlled temperature or cryogenic conditions
	12-2	Chips/fix ed-target, Injectors (liquid and viscous media)	EIGER 16 M (100 Hz)	Real-time SX data analysis pipeline	

Supplementary Table 1: Synchrotron sources with serial synchrotron crystallography capability.

* In collaboration with facility/ instrument scientists

1. Ebrahim, A. *et al.* Dose-resolved serial synchrotron and XFEL structures of radiation-sensitive metalloproteins. *IUCrJ* **6**, 543-551 (2019).
2. Schulz, E.C. *et al.* The hit-and-return system enables efficient time-resolved serial synchrotron crystallography. *Nat Methods* **15**, 901-904 (2018).
3. Mehrabi, P. *et al.* Liquid application method for time-resolved analyses by serial synchrotron crystallography. *Nat. Methods* **16**, 979-982 (2019).
4. Shimazu, Y. *et al.* High-viscosity sample-injection device for serial femtosecond crystallography at atmospheric pressure. *J Appl Crystallogr* **52**, 1280-1288 (2019).