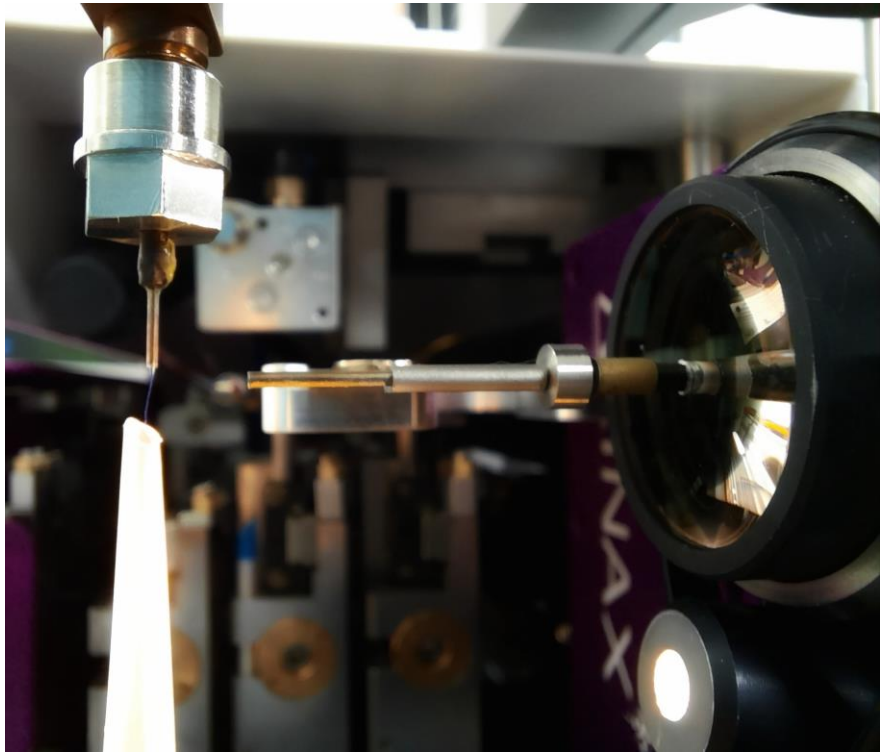
# LEAPS MEETS LIFE SCIENCES CONFERENCE



## New opportunities for time-resolved serial crystallography at the new ID29 from the ESRF extremely brilliant source

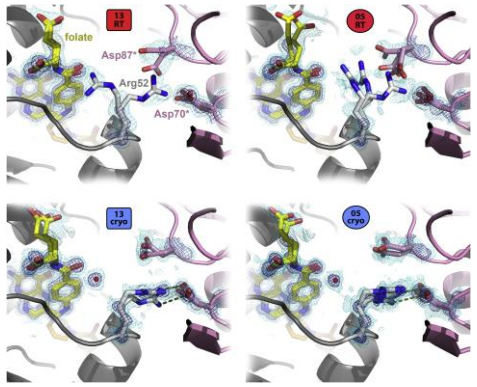
**Julien ORLANS (ESRF ID29)**

# ROOM-TEMPERATURE DATA COLLECTION

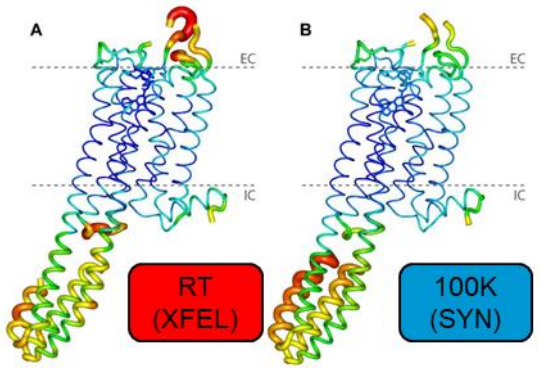
Cryo-structures do not display the same range of conformations as the room-temperature structures:

- Might hide **functional conformations**
- Might prevent **binding of substrates or inhibitors**
- Inhibit **thermal motion** closer to “native” conditions

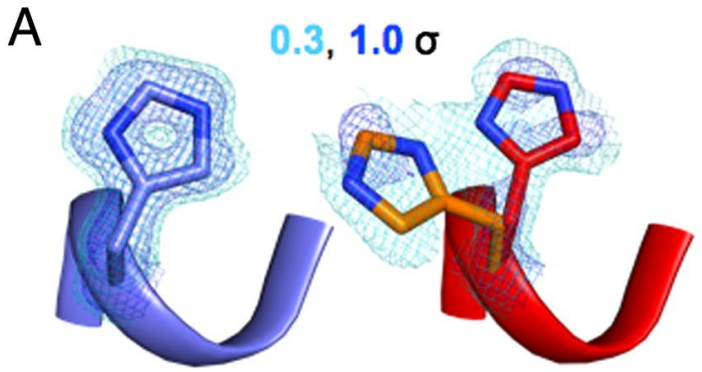
Room-temperature temperature crystal structures reveal physiologically relevant conformations “hidden” at 100 K.

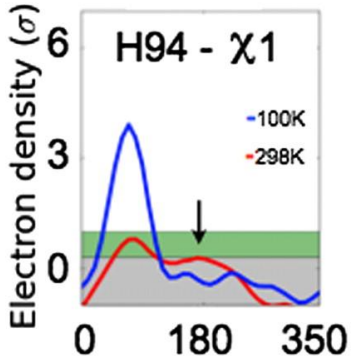



Keedy *et al.*, Structure (2014)

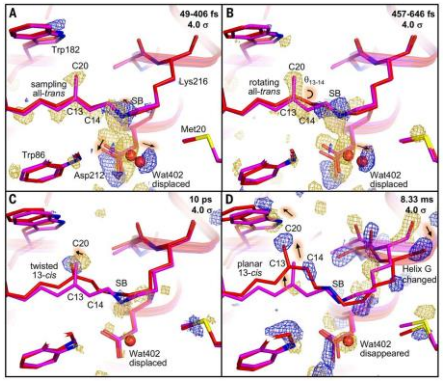



Liu *et al.*, Science (2013)

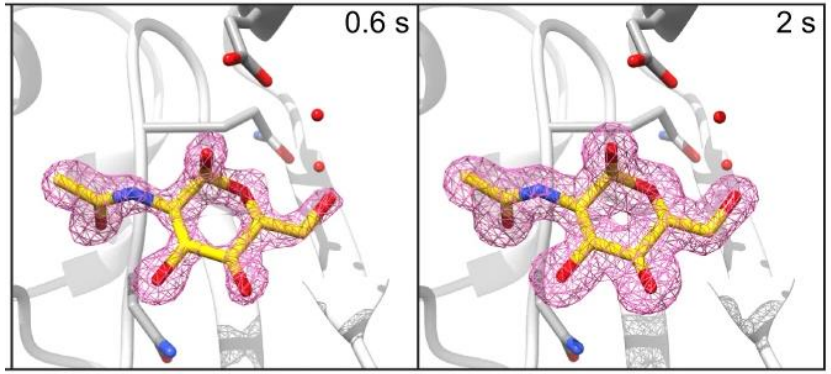



Fraser *et al.*, PNAS (2011)



Nogly *et al.*, Science (2018)



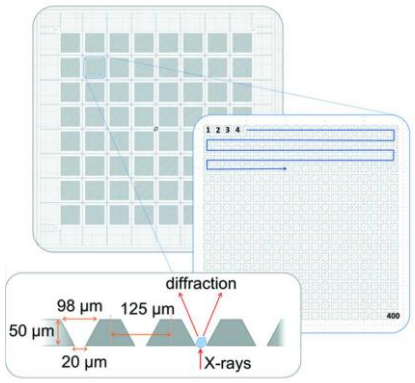

Butryn *et al.*, Nat. Comm. (2021)

Because of radiation damage, **serial crystallography** is the most valuable route to obtaining room-temperature structures.
**(1 crystal = 1 diffraction pattern)**

# SERIAL CRYSTALLOGRAPHY

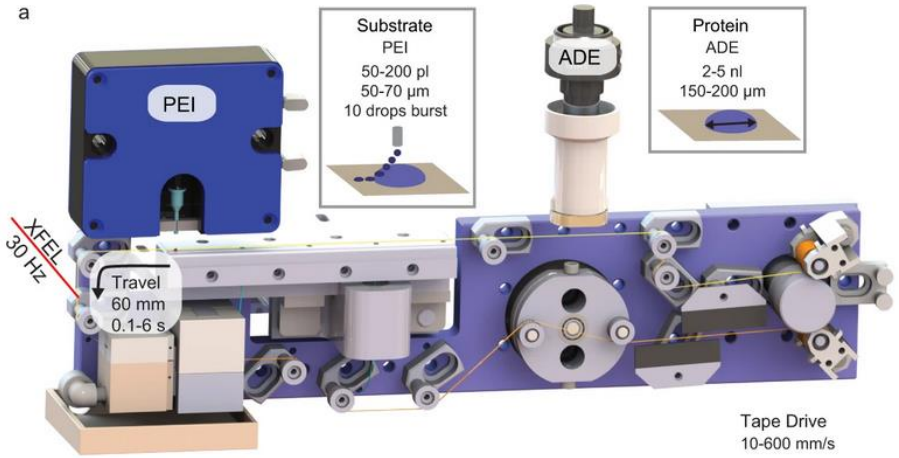
## There are 4 family of sample delivery methods:

### Fixed-Target



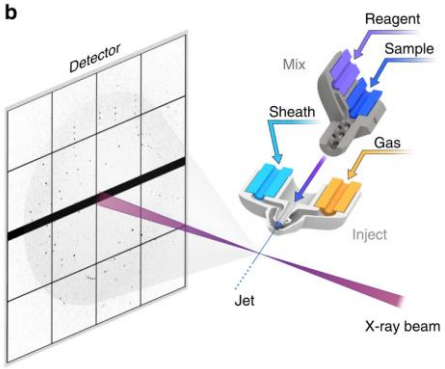

Ebrahim *et al.*, IUCrJ (2019)

### Hybrid (Tape-Drive)

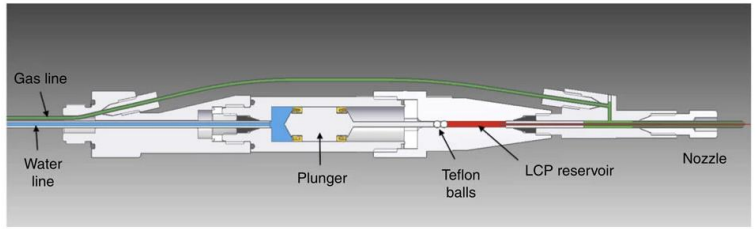



Butryn *et al.*, Nat. Comm. (2021)

### Liquid and Viscous Injectors

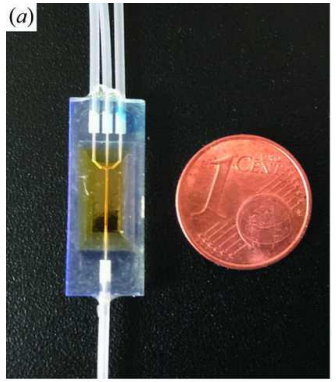



Knoška *et al.*, Nat. Comm. (2020)




Weierstall *et al.*, Nat. Comm. (2014)

### Microfluidics



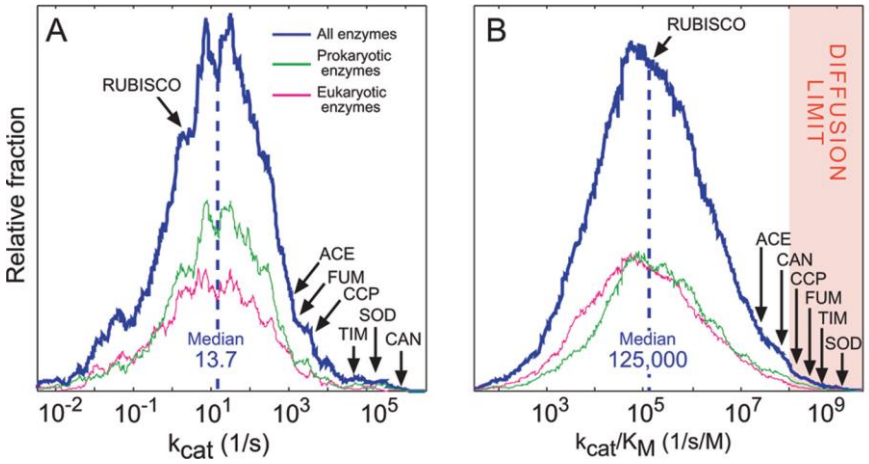

Monteiro *et al.*, IUCrJ (2019)

# ENZYME TIME-SCALE

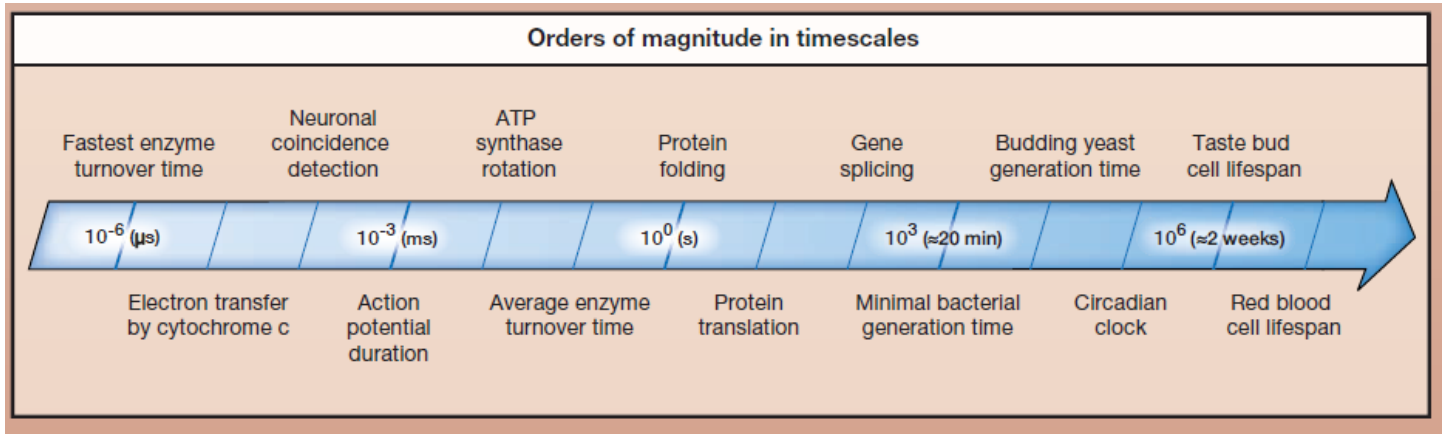
**The median turnover time for catalysis in solution is about 70 ms**

**More than 60% of enzymatic reactions exhibit a kcat value between 1 and 100 $s^{-1}$**

**Enzymes catalyzing reactions related to secondary metabolism are typically 30-fold slower than those of central metabolism**




Bar-Even *et al.*, Biochemistry (2011)




Shamir *et al.*, Cell (2016)

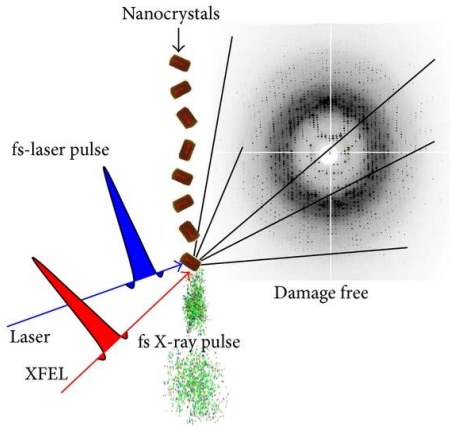
**To observe molecular mechanism *in-crystallo*, the reaction can be activated by:**

- **Photoactivation**
- **Mixing**

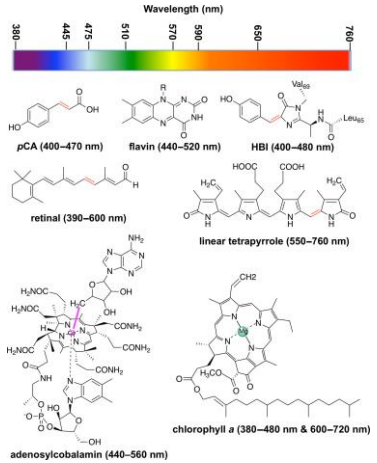
# PROTEIN DYNAMICS IN CRYSTALS

## Pump & probe

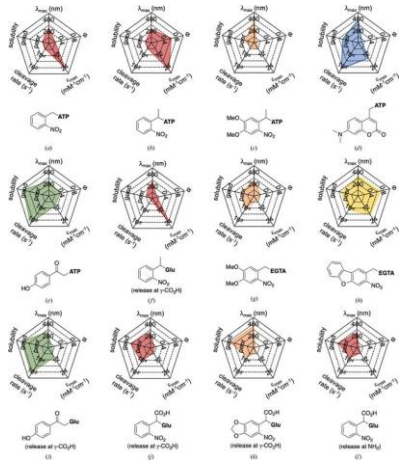
- Photoactivatable proteins (UV-vis)
- Photoactivatable ligands (UV-vis)
  - Cleavable cage compounds
  - Activatable cofactors
- Temperature jumps (IR)




Schmidt, Advances in Condensed Matter Physics (2013)



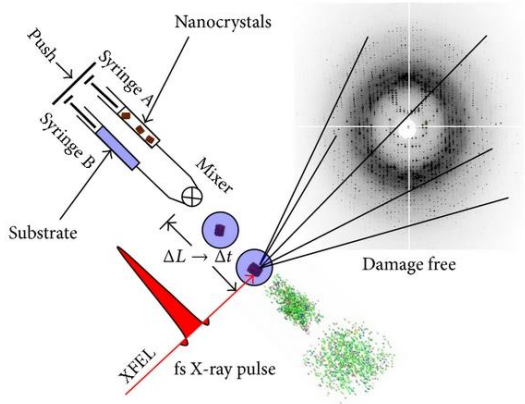

Poddar *et al.*, FEBS (2021)



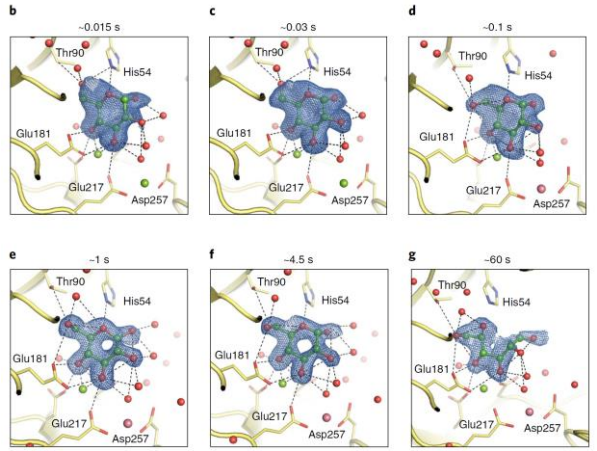
Monteiro *et al.*, Acta D. (2021)

## Ligand and buffer mixing

- Drug binding
- Enzymatic reaction
- pH jump



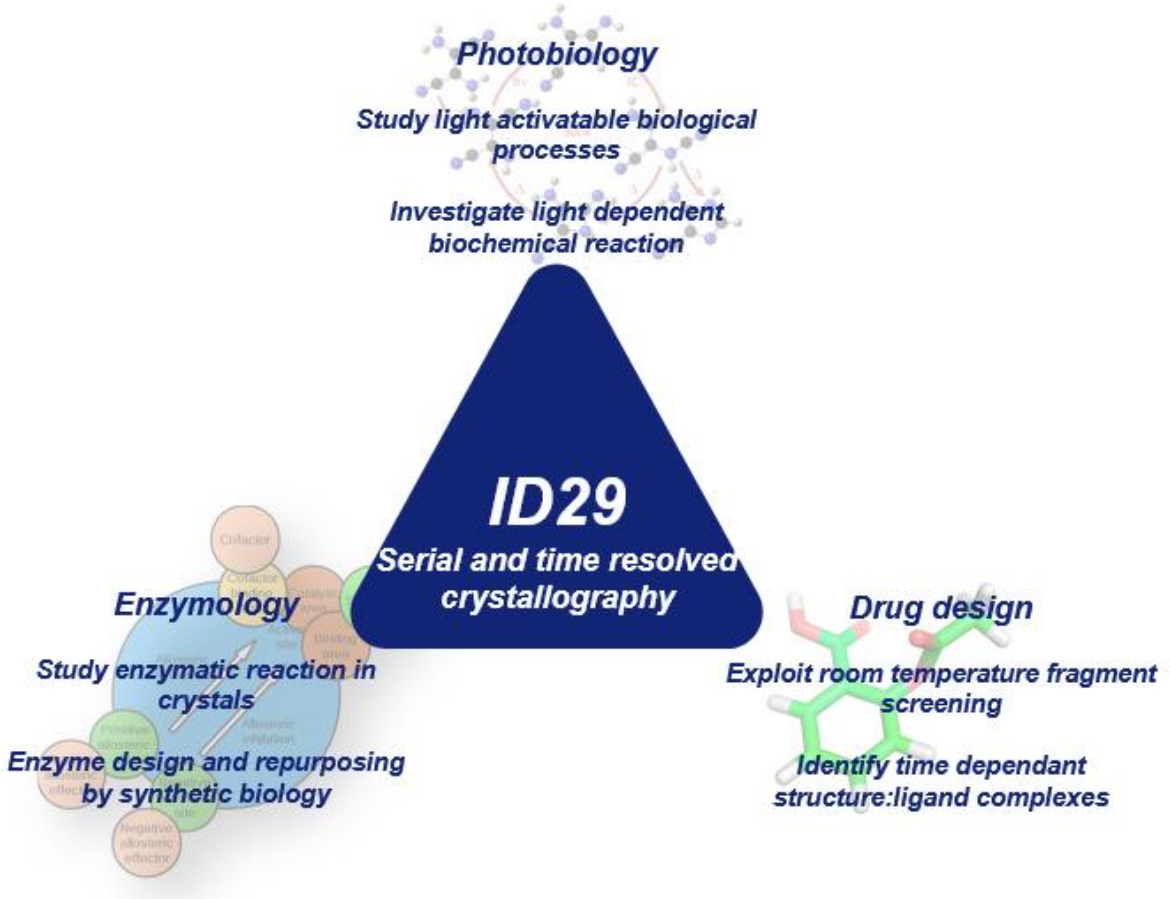

Schmidt, Advances in Condensed Matter Physics (2013)



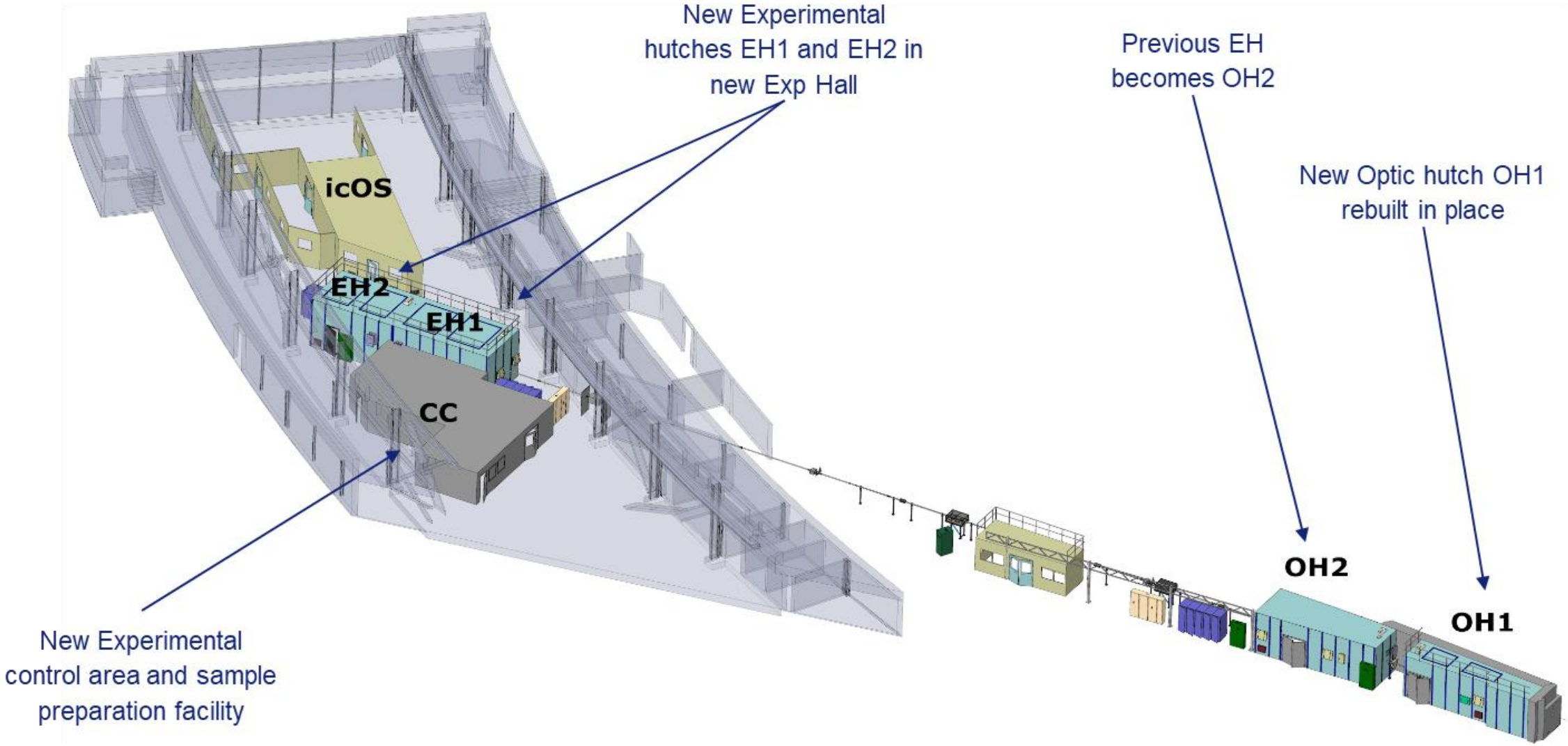

Mehrabi *et al.*, Nat. Meth. (2019)

# ID29 MISSION

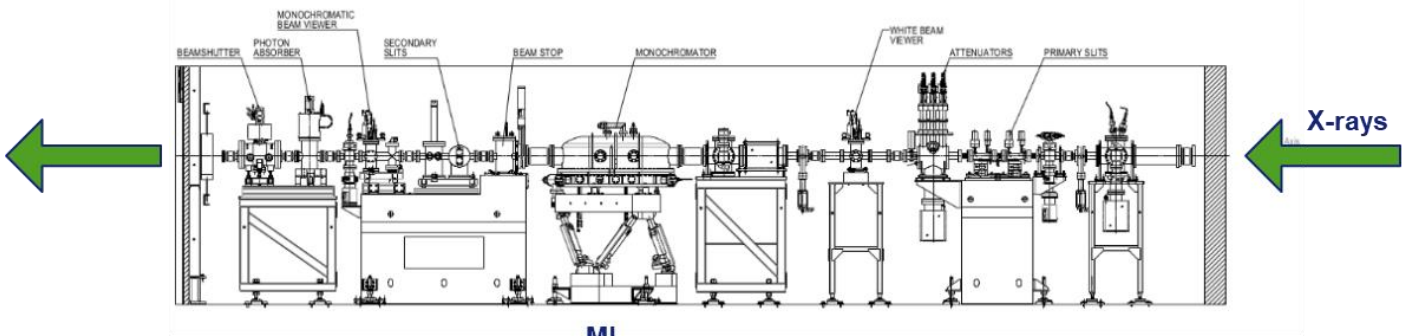
- Room temperature serial crystallography experiment
- Extremely high flux with exposure time in µs range and high repetition rate
- Tunable over a large energy range
- Accurate control timing system to trigger events
- Adapt different sample environments and crystal delivery systems
- Optimize sample consumption
- Equipped with sample preparation laboratory and data analysis area



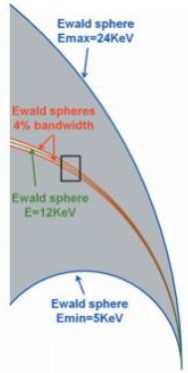
# BEAMLINE LAYOUT



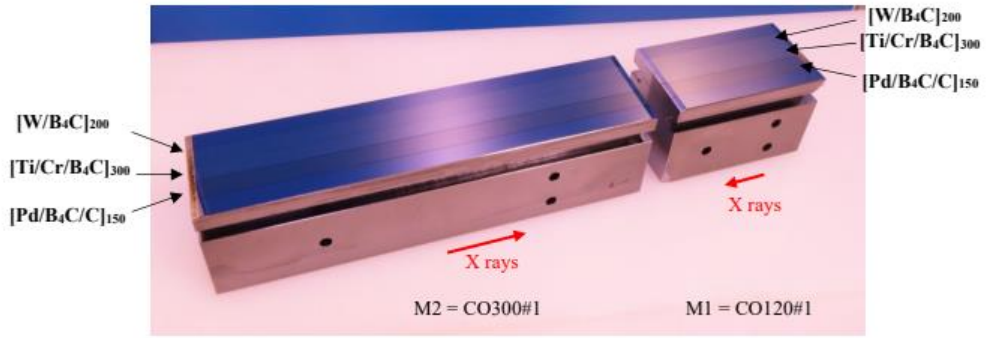
# OPTIC HUTCH 1 (OH1)



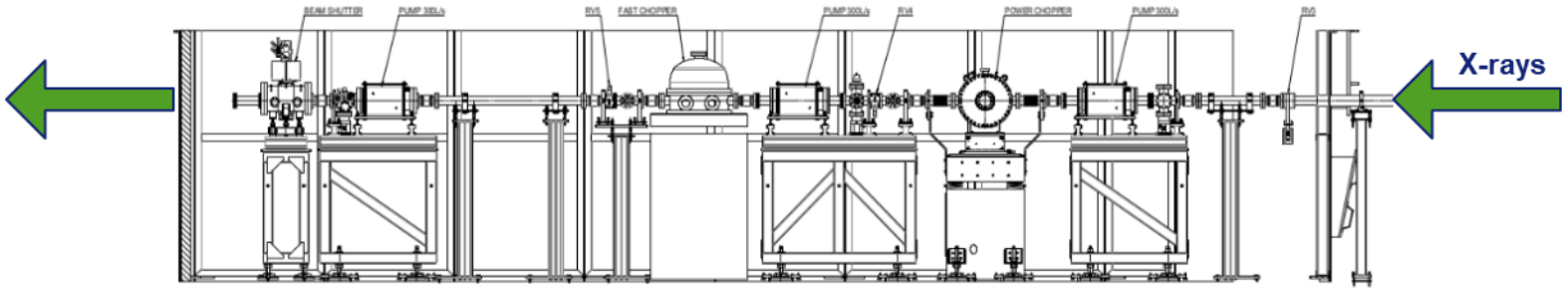

**ML**
**Monochromator**




- A multilayer monochromator system was designed to increase bandwidth
- Adjust bandwidth by changing stripe
- The multilayer monochromator permits to deliver higher flux in larger bandwidth
- Three stripes are present to cover whole energy range 10-20 keV (+35 keV) with 0.4% and 1% bandwidth

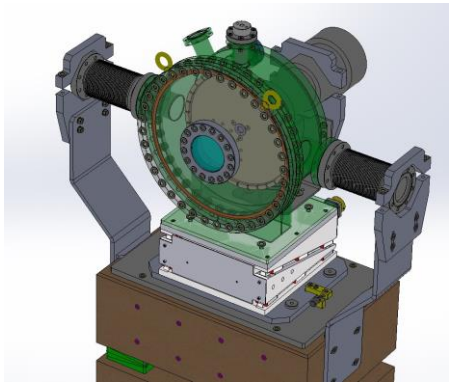


# OPTIC HUTCH 2 (OH2)

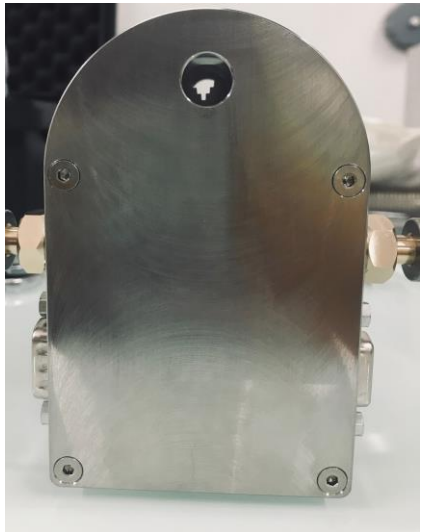



## Fast chopper

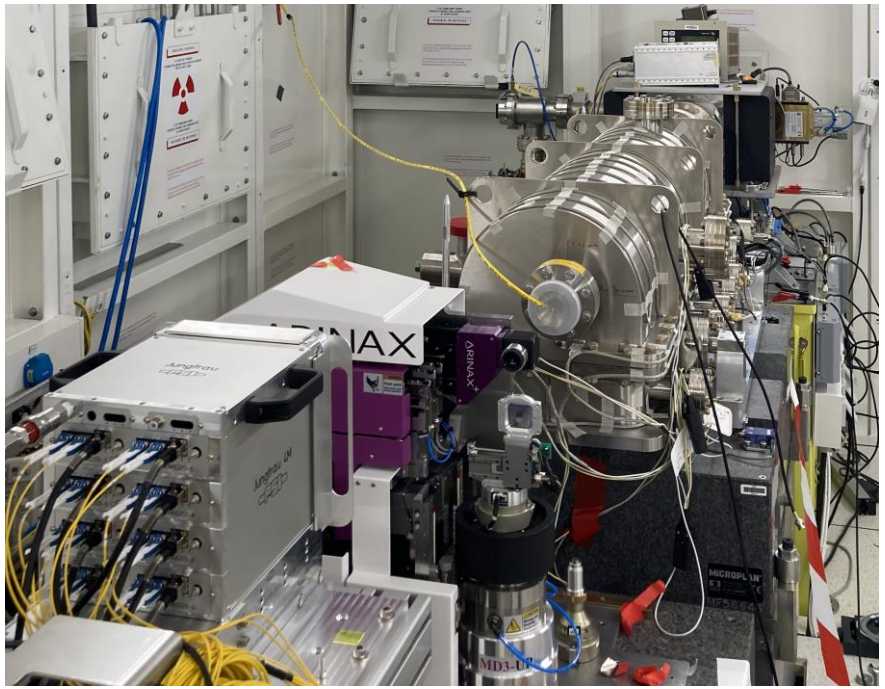
## Heat load chopper

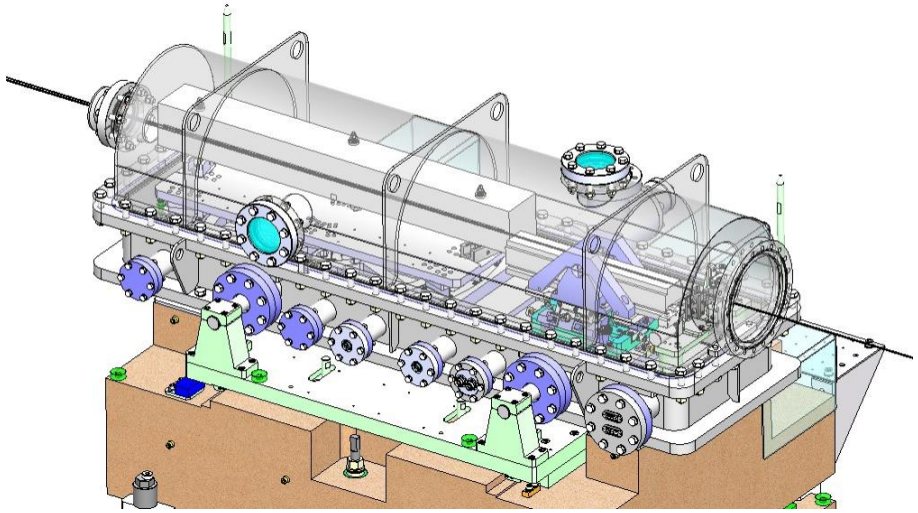


- A double chopper system removes heat load and defines short exposure times
- Heat load chopper generates a 90 µs pulse at different frequencies: 925, 462.5, 231.25 & 115.625 Hz
- Fast chopper generates pulses of 10, 20 & 30 µs

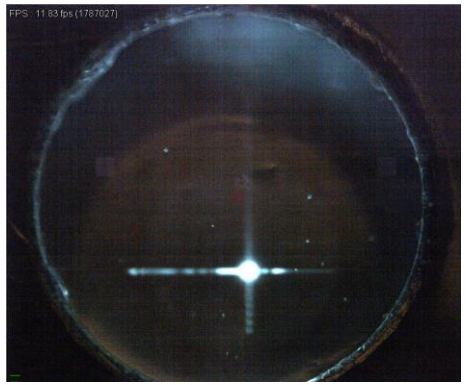


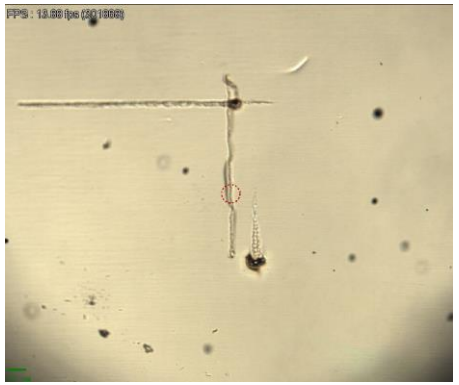
# EXPERIMENTAL HUTCH



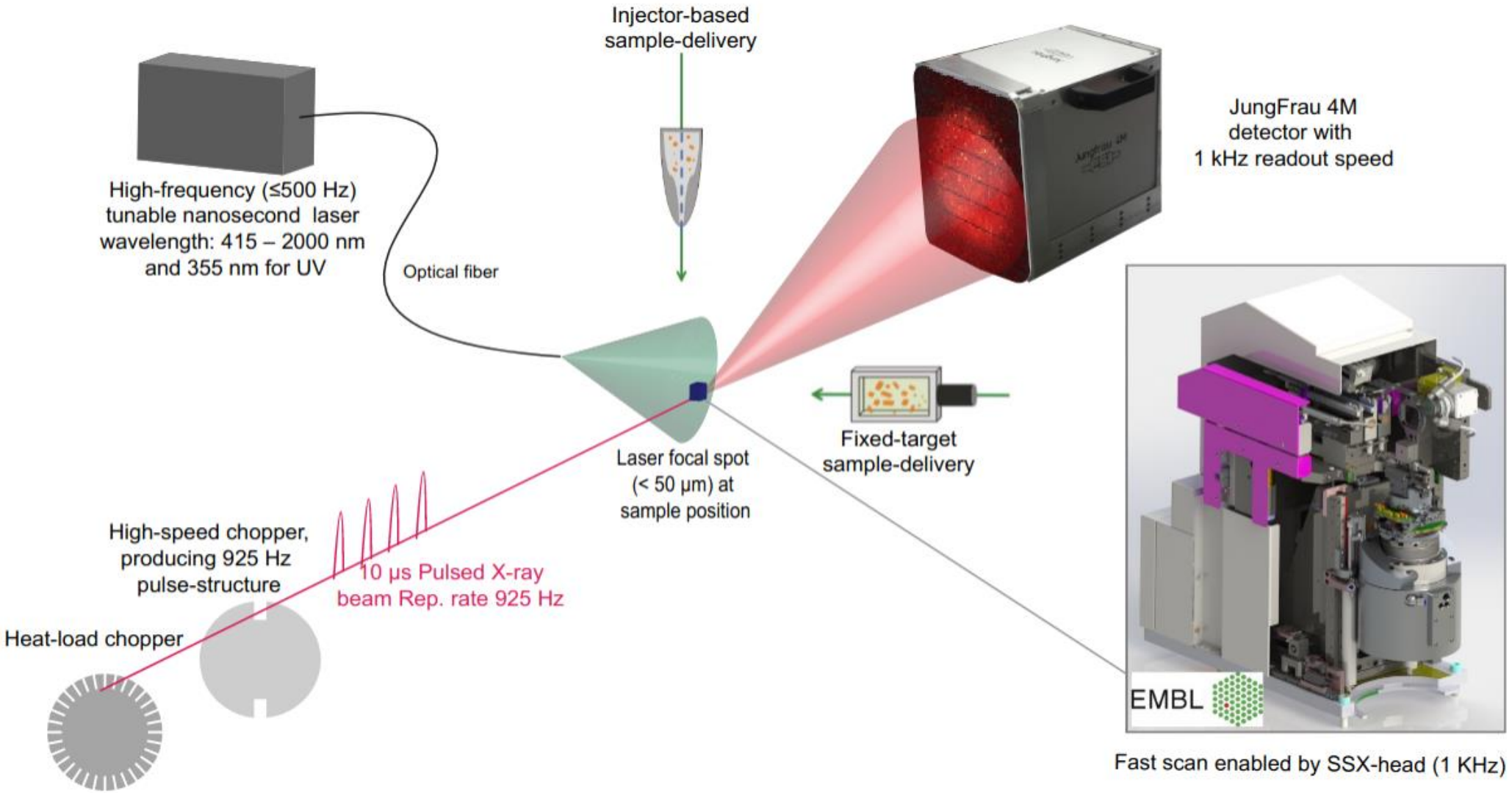


- Sample at 107 m from source
- Elliptical KB mirrors to focus the beam
- Measured beamsize ~ 2 x 4 µm (VxH)
- Beam resizing by tuning incident angle
- Flux at the sample position of ~ $10^{15}$ ph/sec

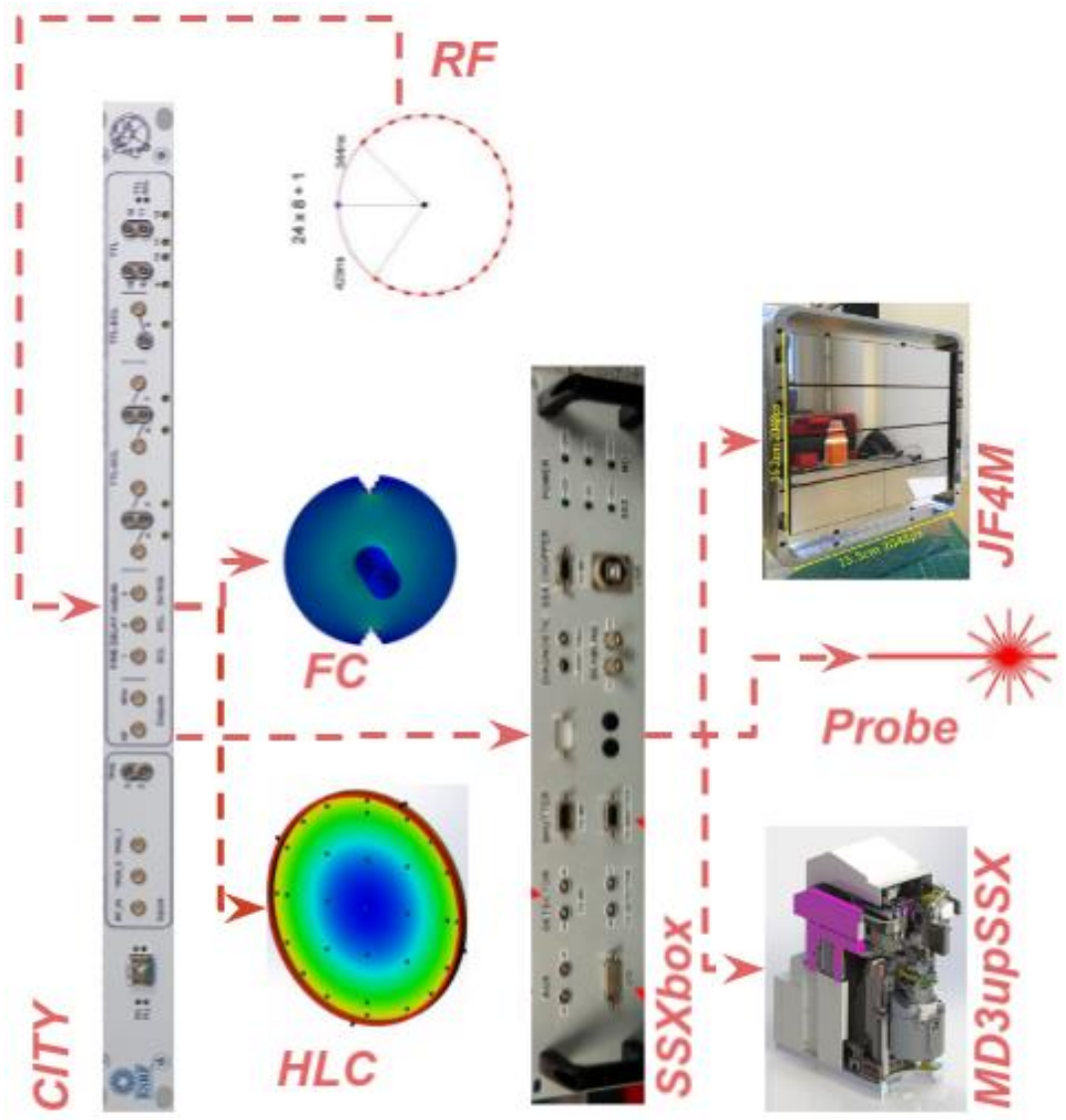




# EXPERIMENTAL SET-UP

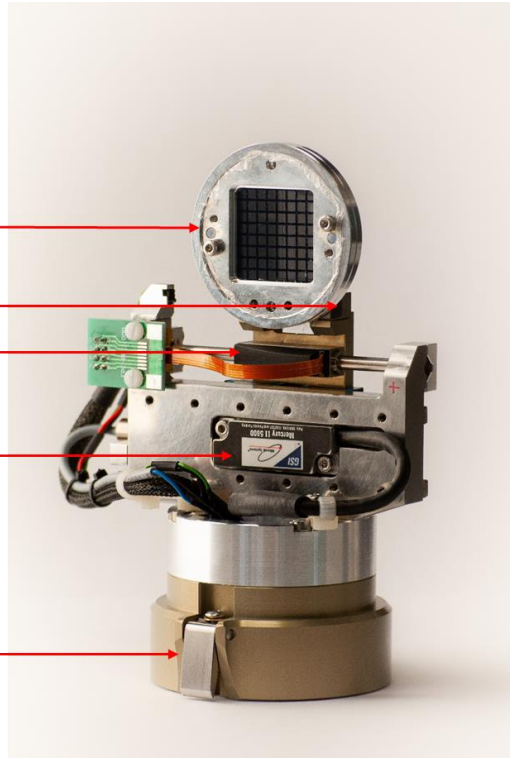


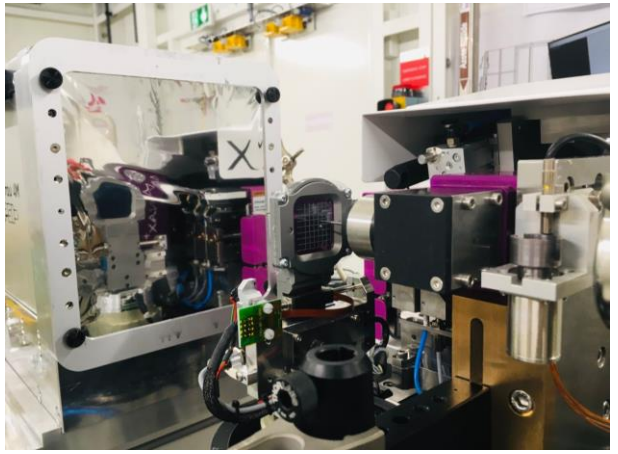
# EXPERIMENTAL SET-UP

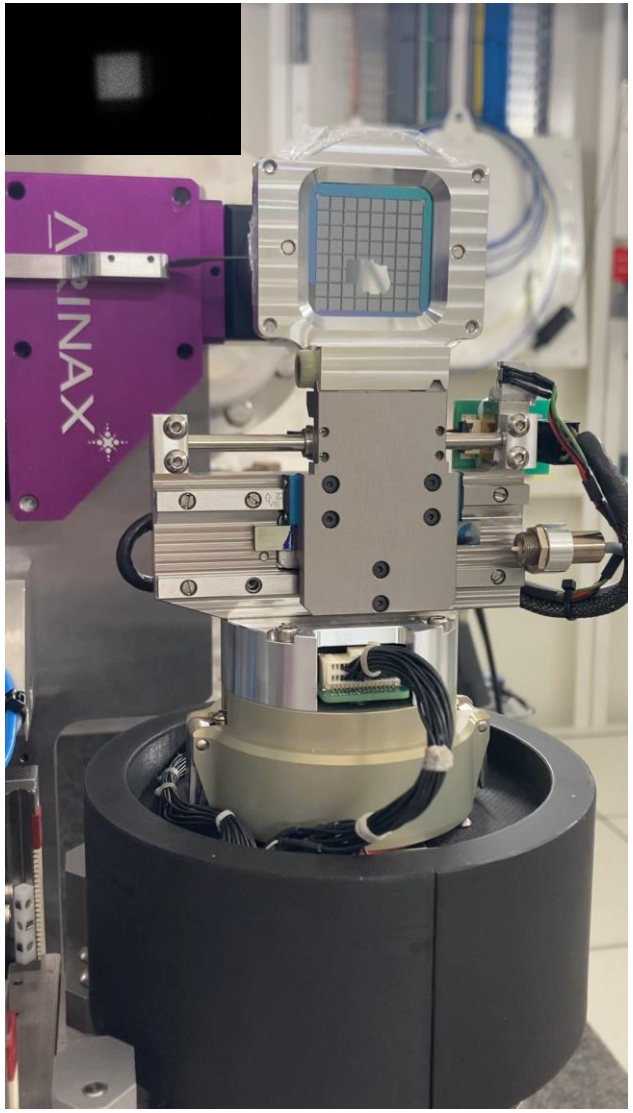



- A new developed timing system synchronises every step of the acquisition with the radiofrequency of the storage ring
- CITY and SSXbox are the two hearts of the system
- Heatload and Fast chopper are synchronised
- SSXbox propagate the clock to the data acquisition devices, MD3upSSX, X-ray detector and additional triggerings
- X-ray pulses of tunable length from 10 to 90 microseconds
- Synchronous triggering of external probes

# FIXED-TARGET

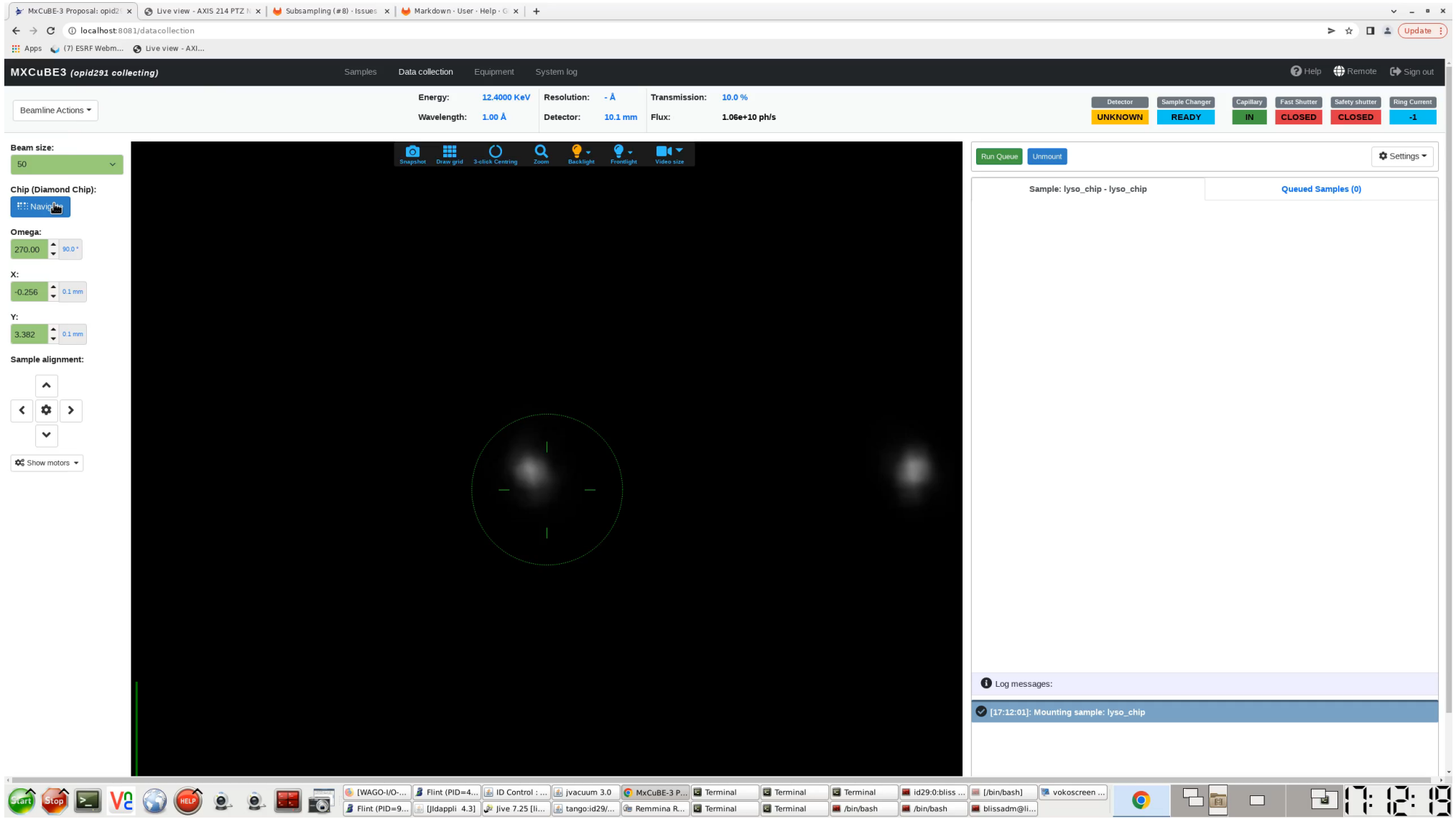


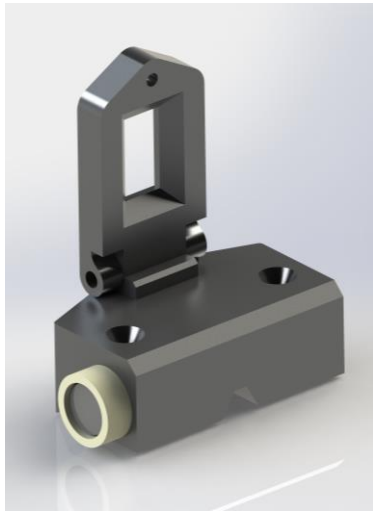


- **In Fixed-Target, crystals are captured in apertures of a silicon chip**
- **Scan speed: up to 125 mm/s**
- **Desired precision at meeting point < 1µm**
- **Run synchronously with the chopper system**

# FIXED-TARGET

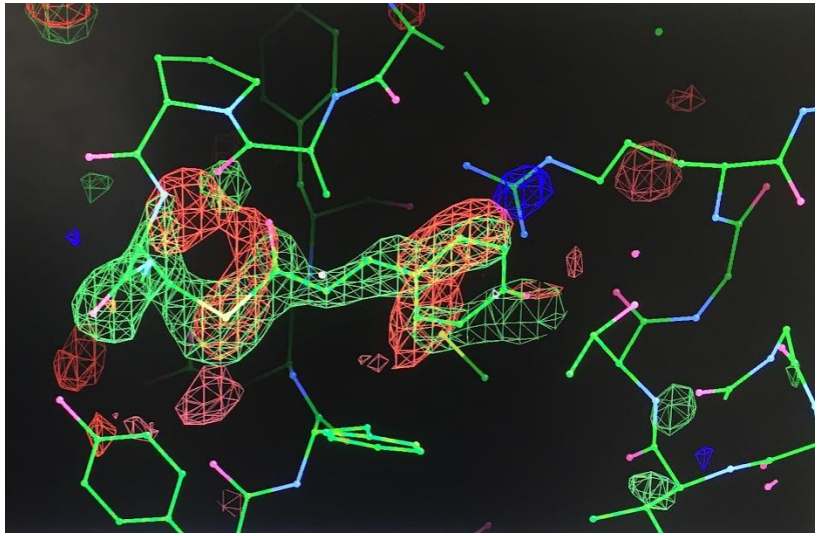


# CHIPLESS CHIP

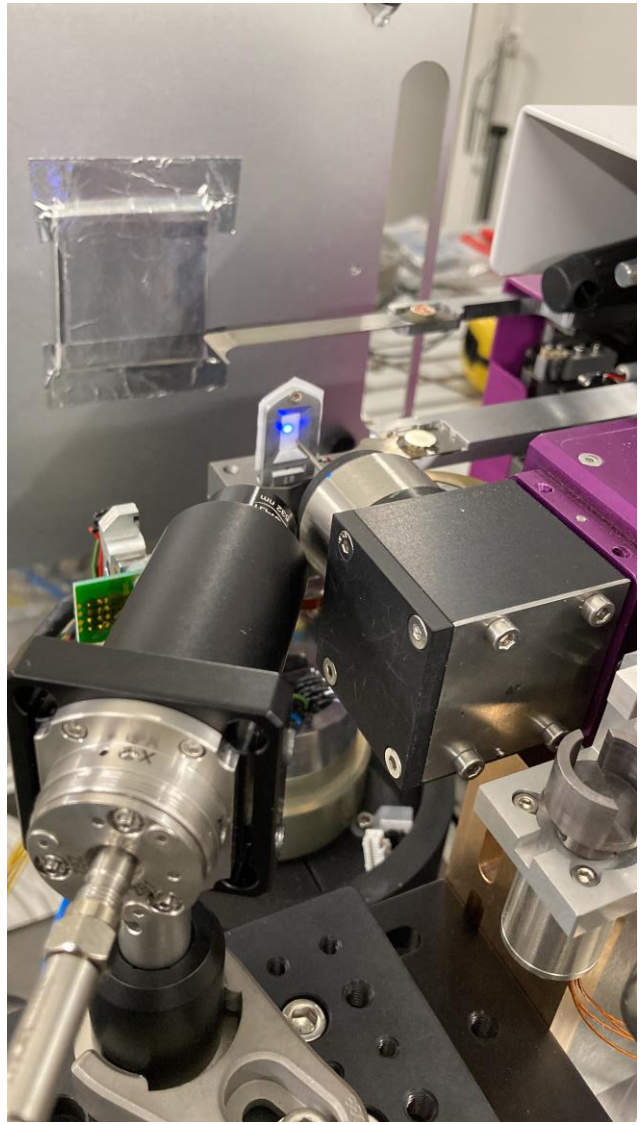


MPI-SOSchip

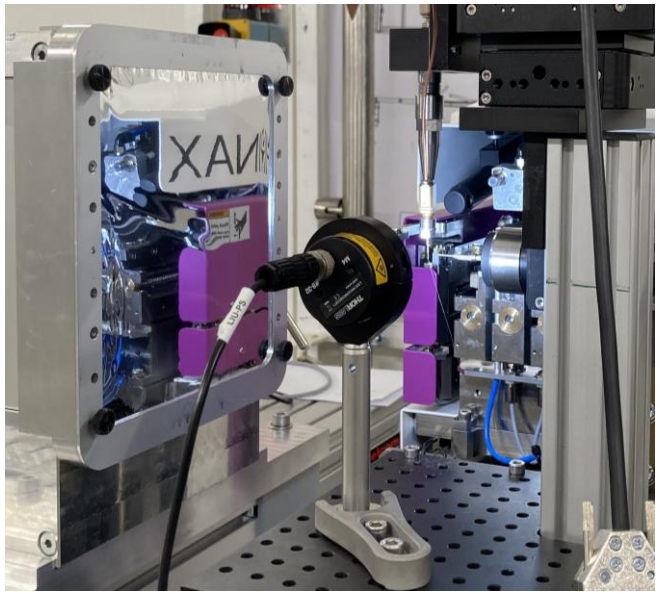
- Test the sample diffraction with only 3 µL
- Can collect a complete dataset depending on crystal density
- 8 min to collect 80k images
- Steady-state studies



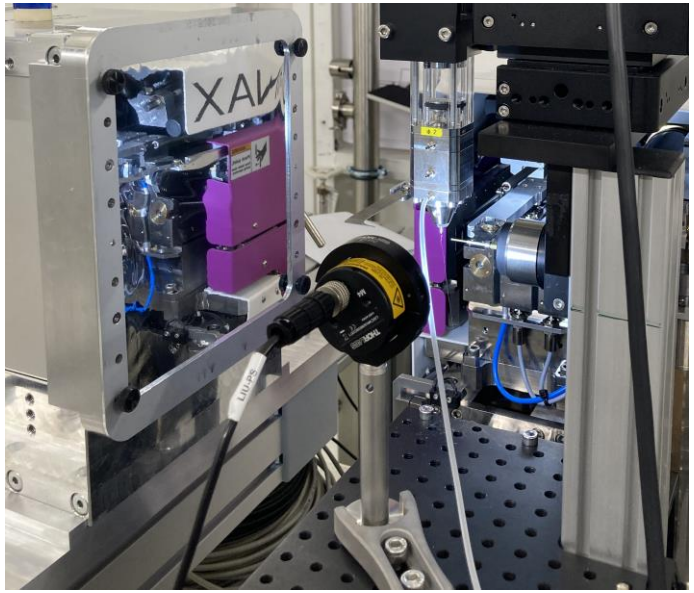
Schmidt group



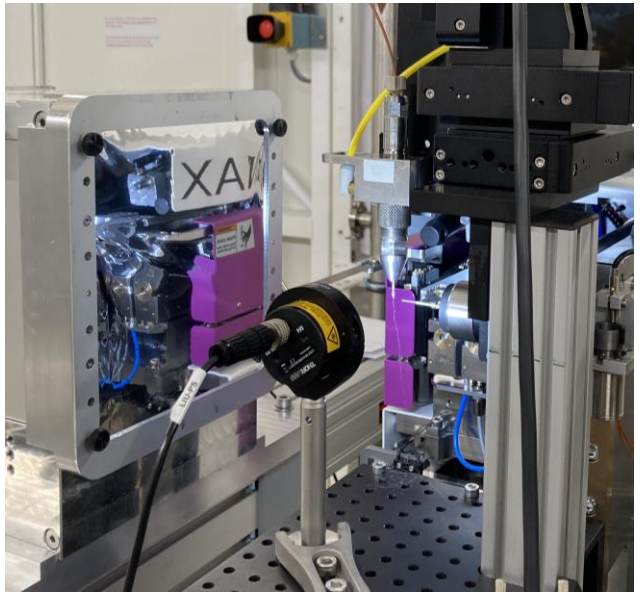
# HIGH-VISCOSITY INJECTOR



ASU injector



SACLA injector



MPI injector

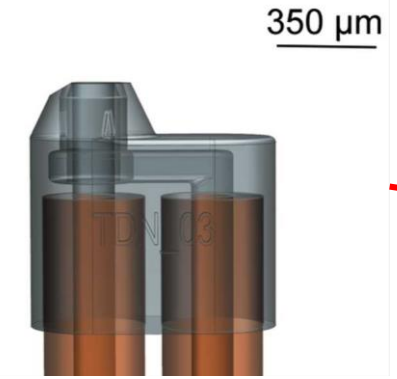
**Microcrystals are embeded in viscous media:**

- **LCP**
- **HEC (Hydrogel-based)**
- **Grease (Oil-based)**

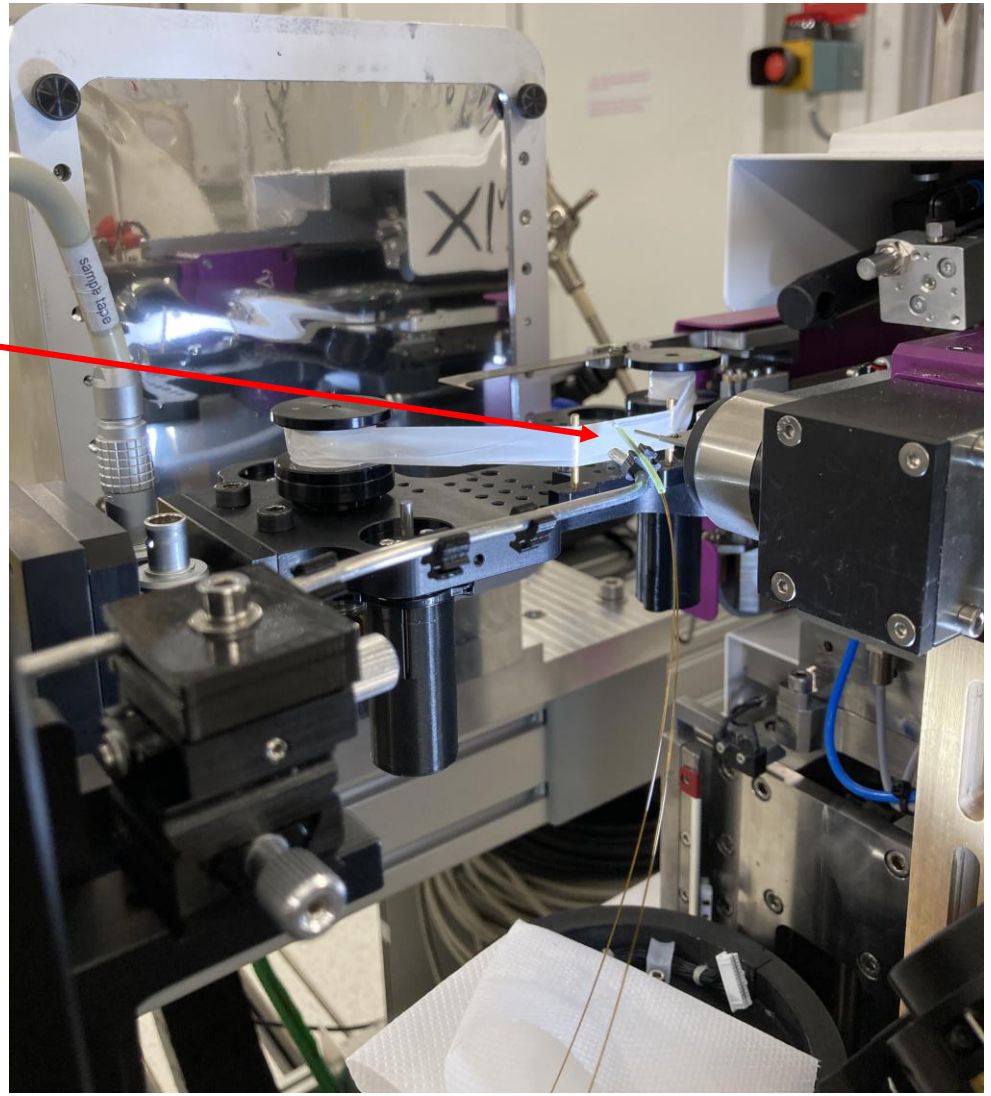
# TAPE-DRIVE

Lübeck University

**3D-printed nozzle from CFEL**



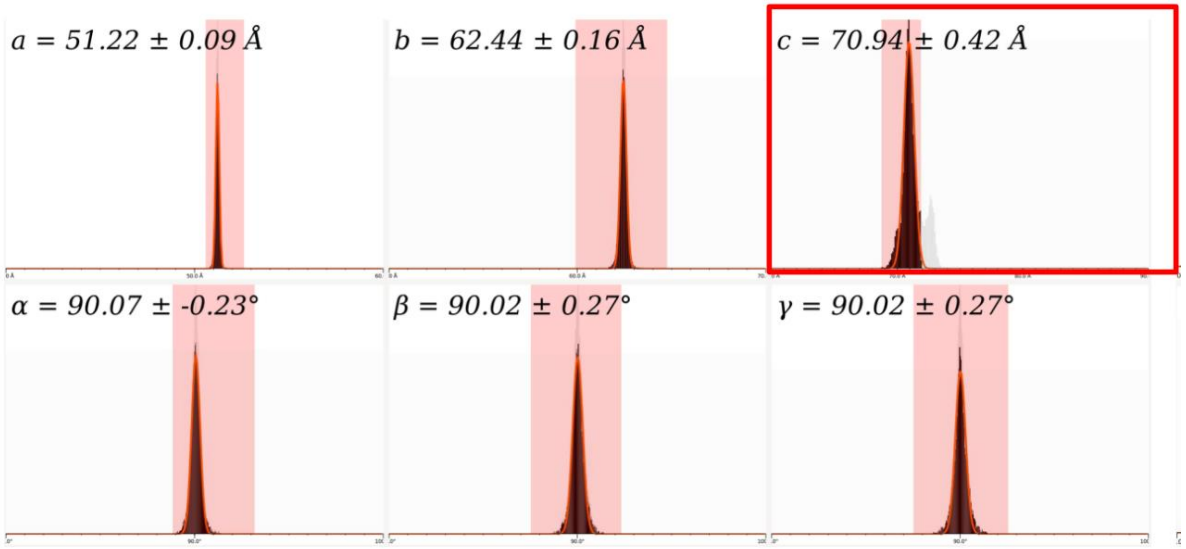

Henkel *et al.*, IUCrJ (2023)



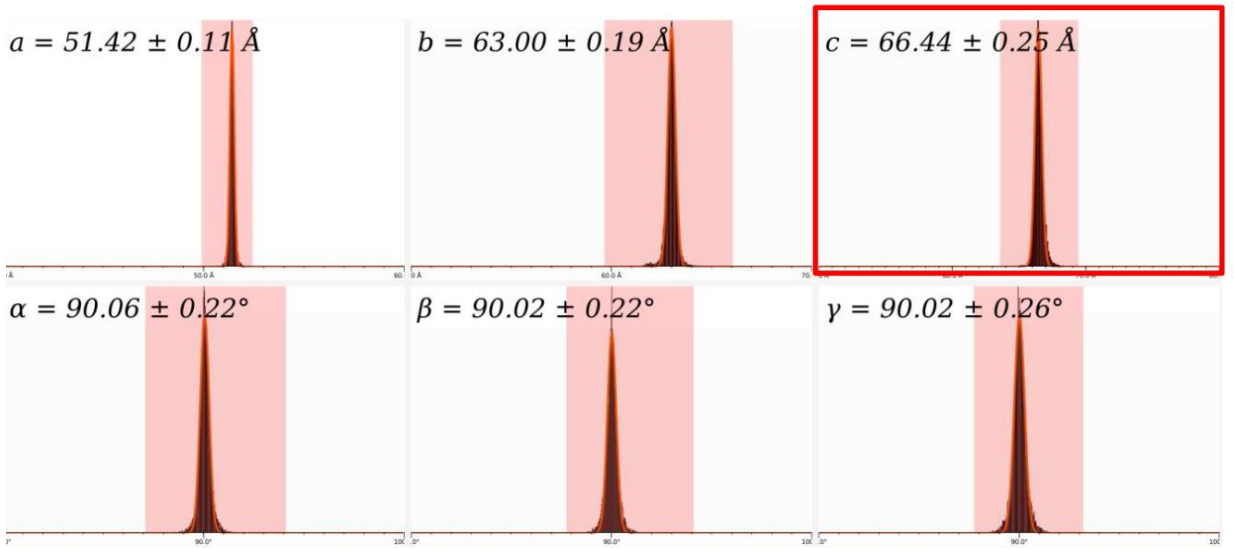

- Time-delay from 0.5 s to several minutes
- Enzyme catalysis
- Ligand/inhibitor binding
- pH jump

# TAPE-DRIVE

## T-Cer pH-jump showed a shrinkage on the c axis

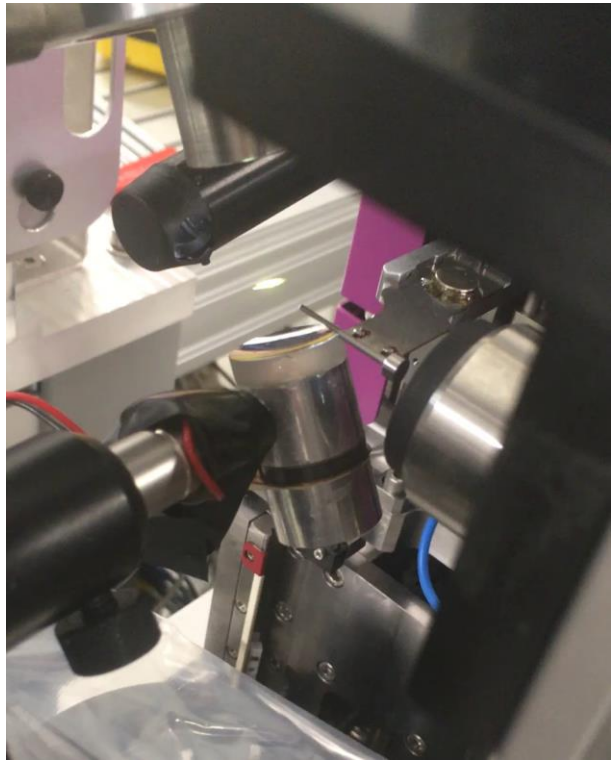



Resting state

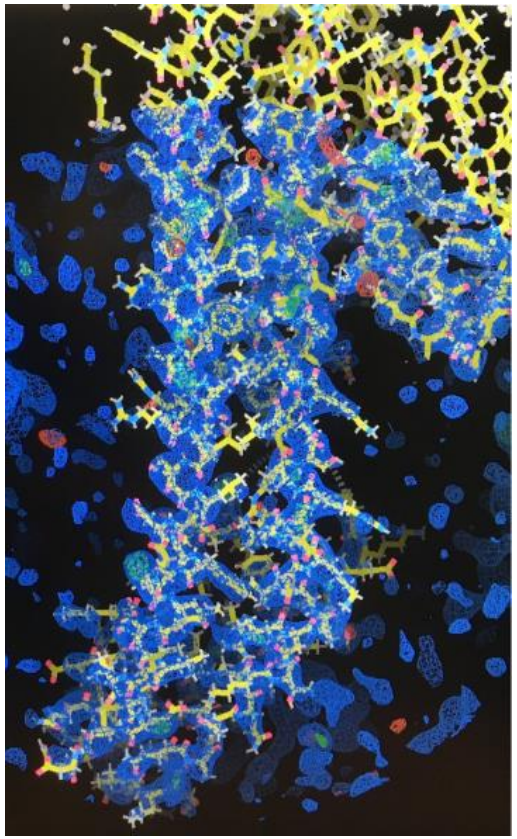



1min after mixing

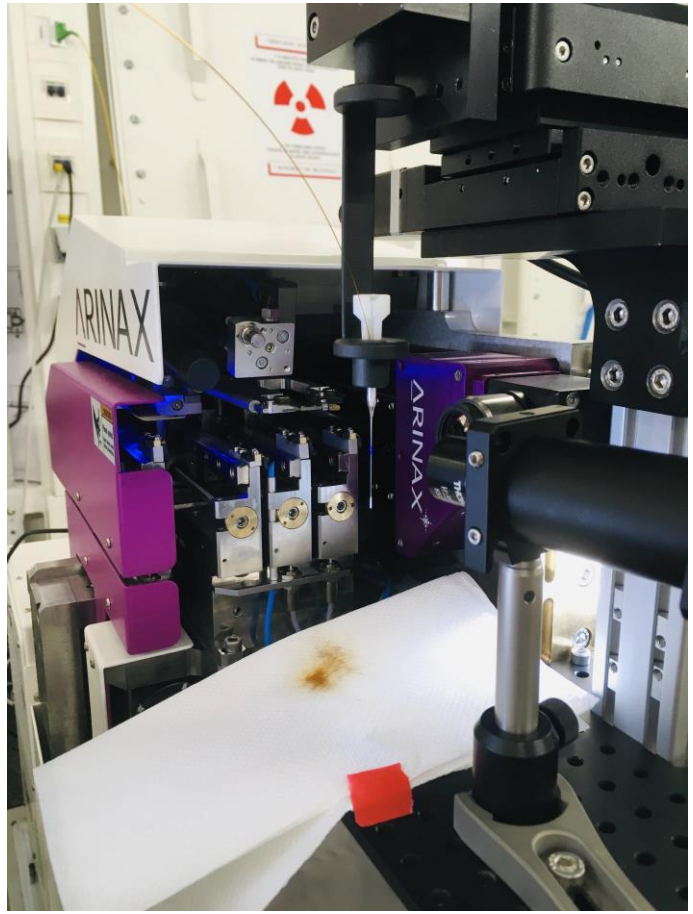
# OTHER SAMPLE DELIVERY METHODS



**PSI Acoustic Levitator**

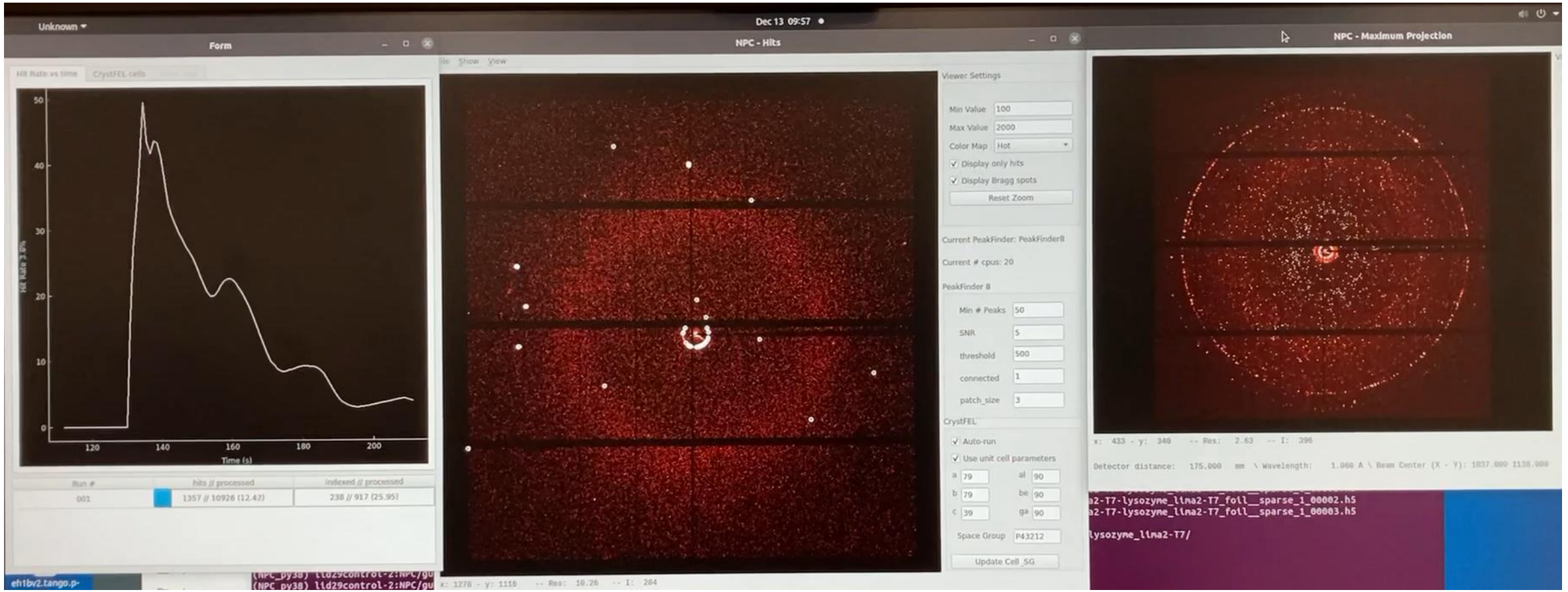


**First membrane protein collected on ID29**

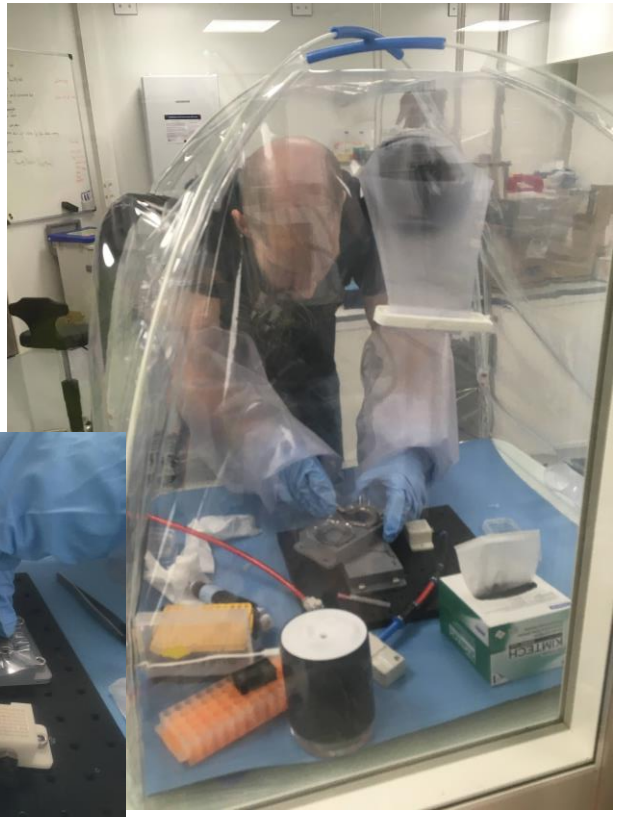


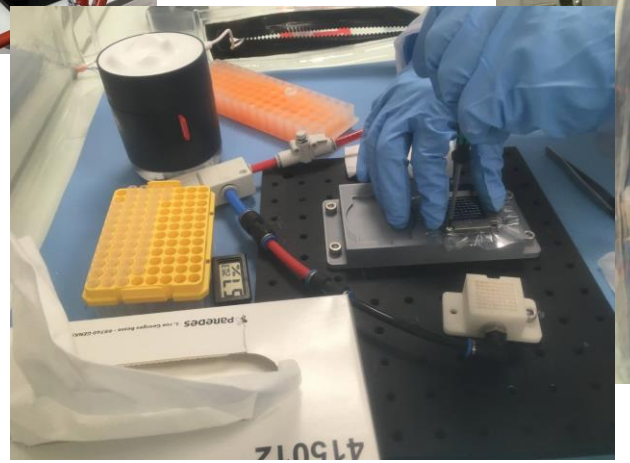

**SerialX Gothenburg University**

# NPLIVE – NEAR REAL TIME FEEDBACK ON PROCESSING WITH GPU

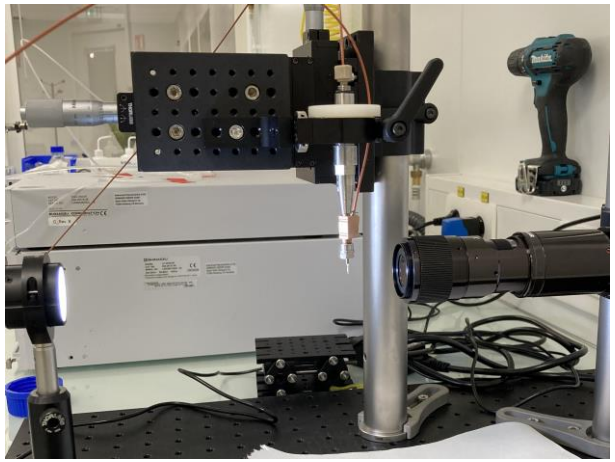


# SAMPLE PREPARATION LABORATORY

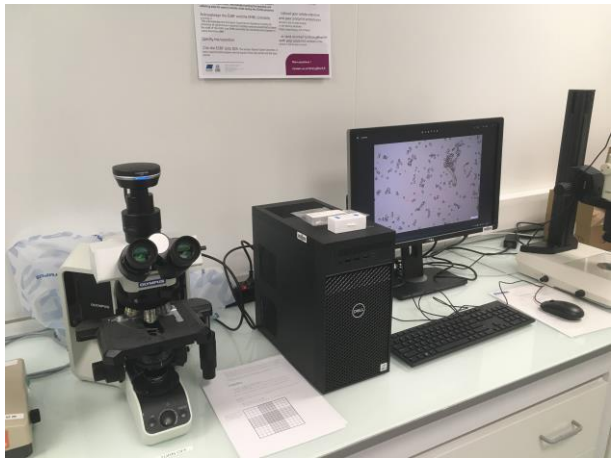




Humidity Tent

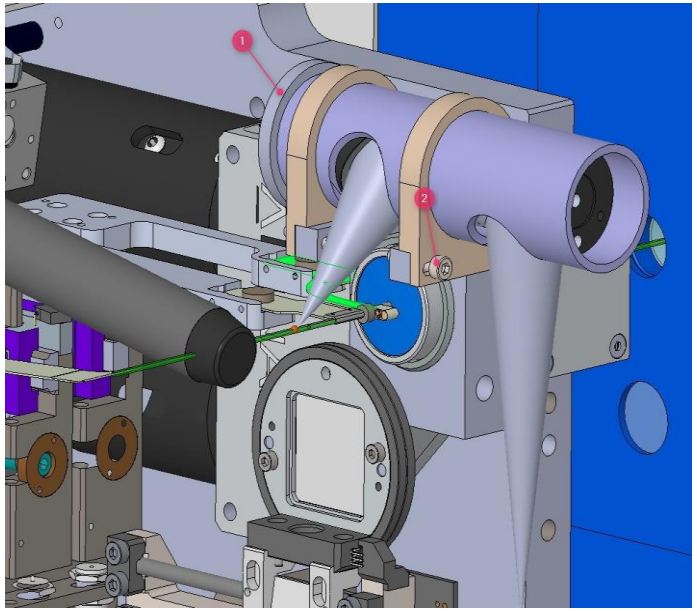


HVE Offline Bench



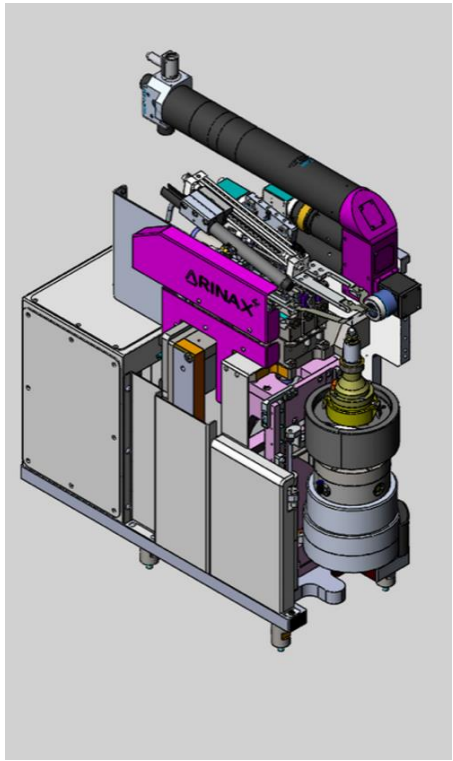
# NANOSECOND LASER INTEGRATION

- 400 - 2000 nm laser (+355 nm) at 500 hz, 10 mJ max pulse energy
- New development injection through OAV
- Synchronised with experiment clock

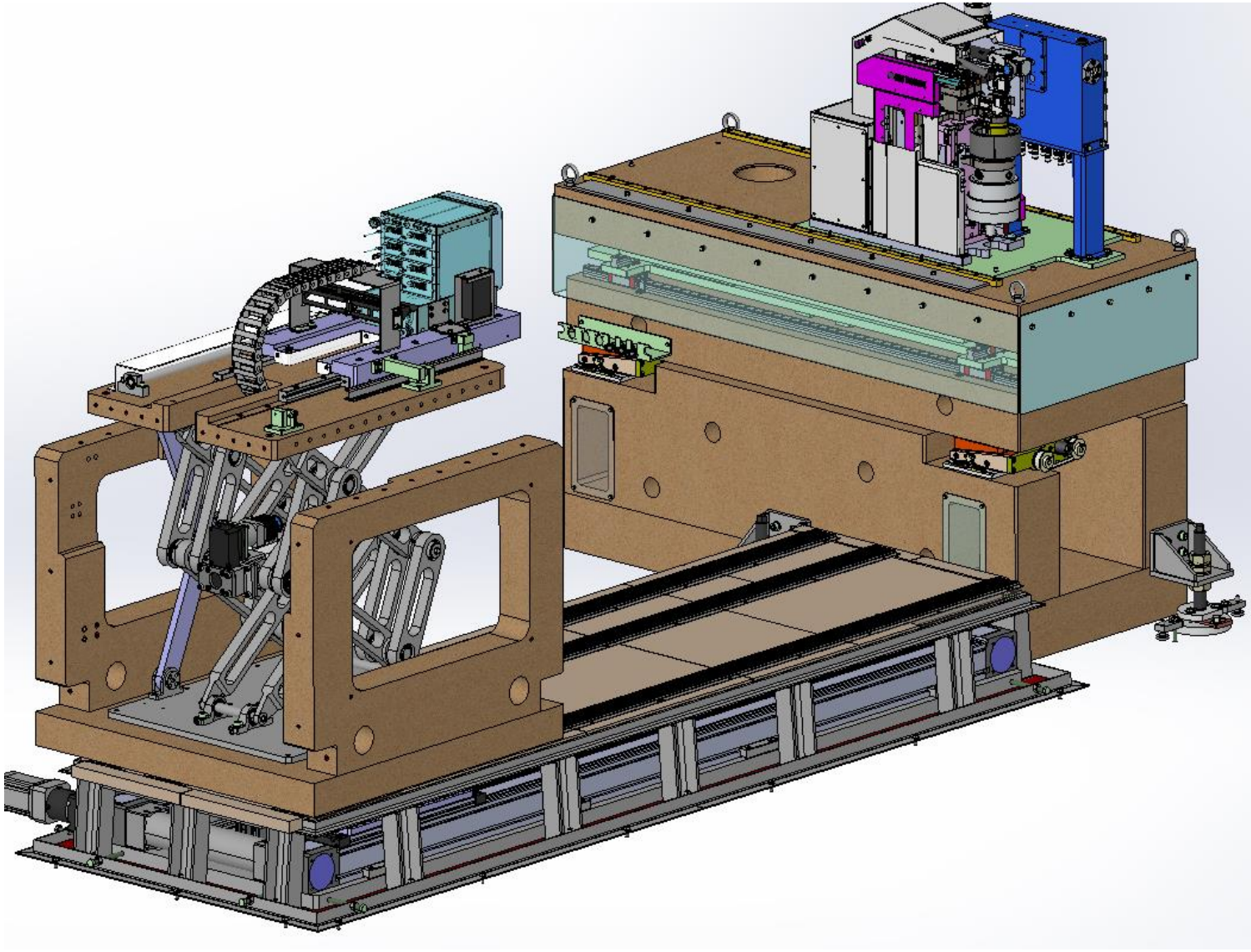


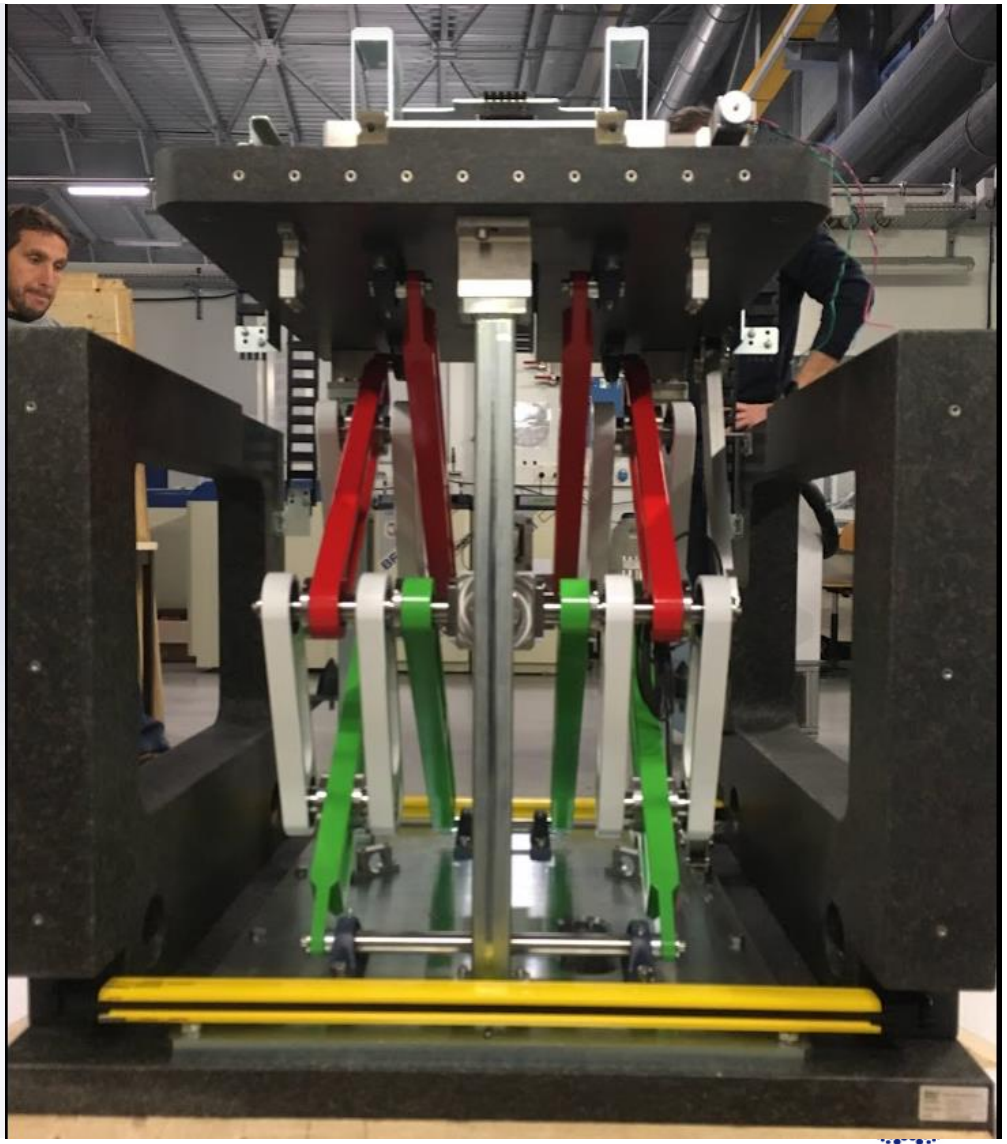
Laser specification:

- wavelength range (400-2000 nm)
- pulse energy (~10–20 mJ)
- pulse duration (~5 ns)
- externally triggered
- repetition rate ~500 Hz minimum
- and portable with optical fibre



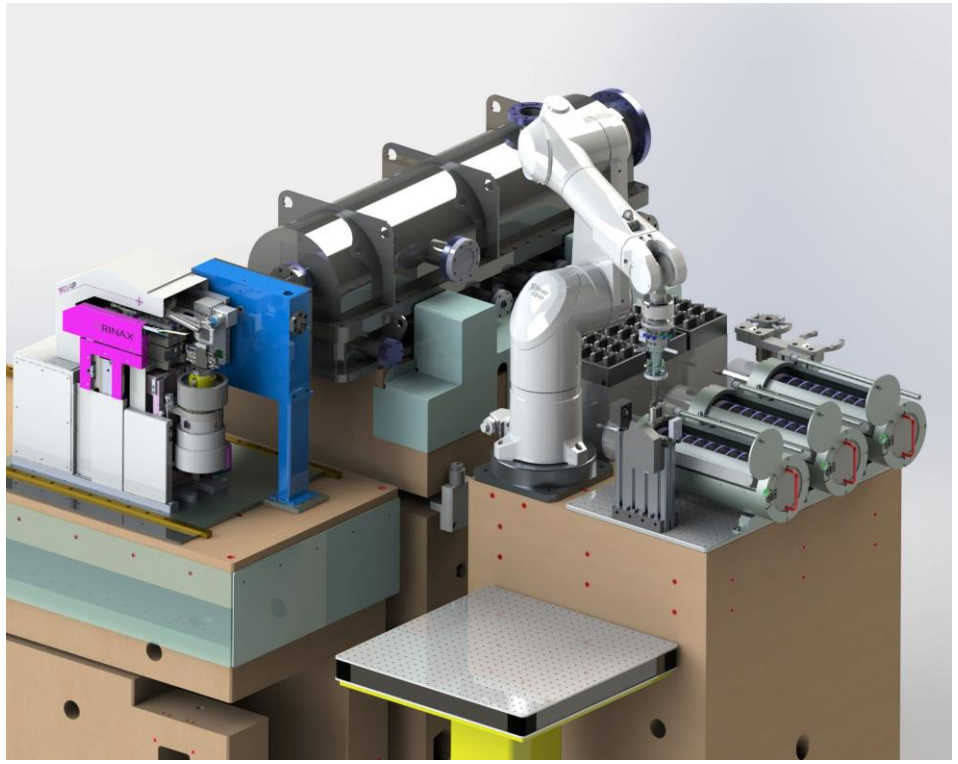
# DETECTOR TABLE

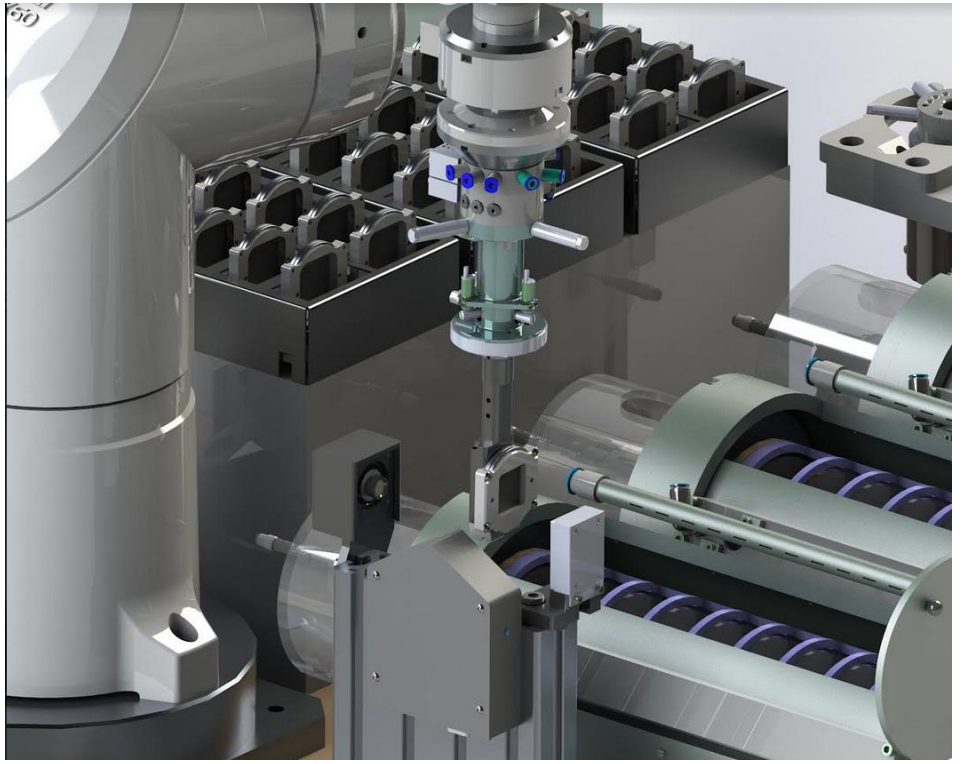




# FLEX SAMPLE-CHANGER FOR FIXED-TARGET

- **Brand new FLEX gripper to mount fixed target chips**
- **Dedicated humidity controlled hotel for storing Si-chips**
- **Standardization in sample mounting**



# ID29 IN NUMBERS

**Photon flux: ~ $10^{15}$ ph/sec**

**Pulse length: 90, 30, 20 & 10 µs**

**Repetition rate: 925, 462.5, 231.25 & 115.625 Hz**

**Energy: 10-20 kEv (+35 kEv)**

**Beam size: ~ 2 x 4 µm (VxH)**

**11 BAG proposals world wide (Operation from Sept, 2022)**

**More than 600Tb collected in 6 months**

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**Shibom Basu**
**Samuel Rose**

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**Users**