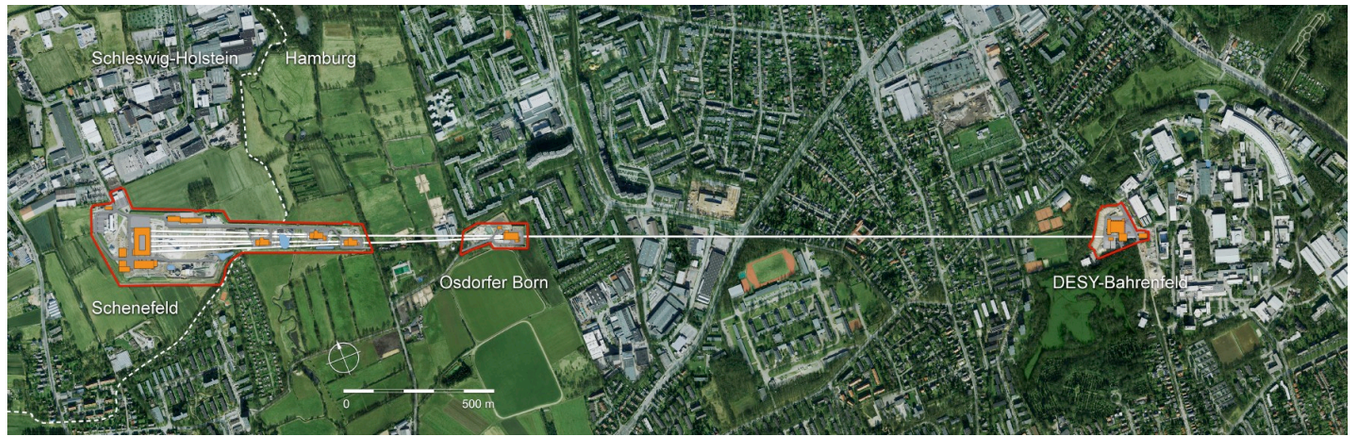
Serial crystallography at Diamond and XFELs

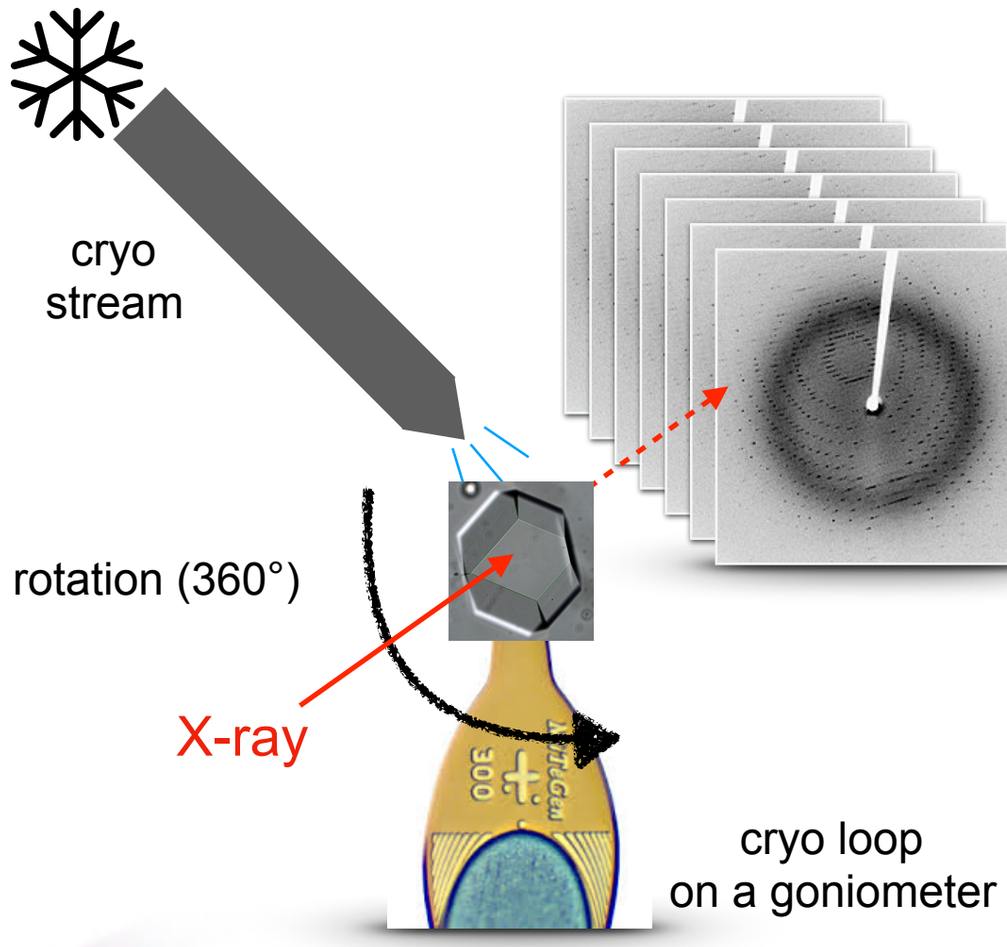


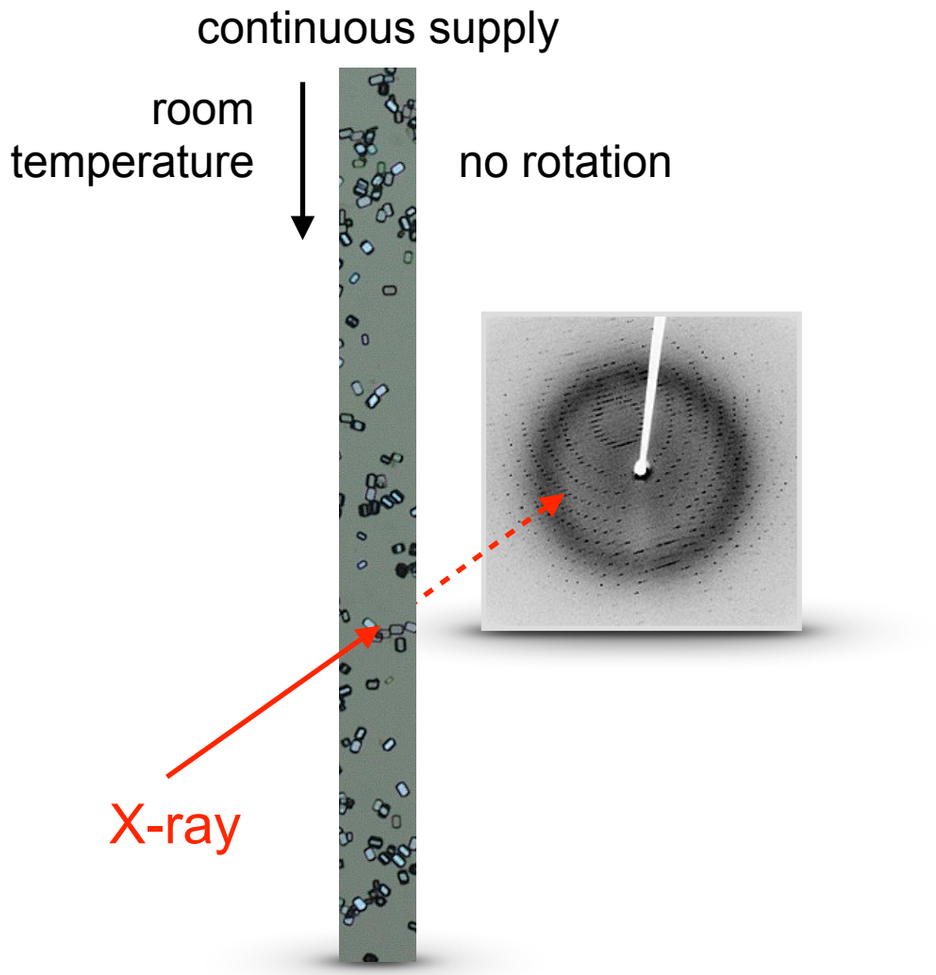


Agata Butryn, PDRA
XFEL-Hub at DLS

diamond

Standard vs serial crystallography





single crystal rotation:

dataset = 1000s images from 1 crystal

serial methods:

dataset = 1000s images from 1000s crystals

diamond

Why serial crystallography?

Perfect for:

- small crystals
- sensitive samples (radiation, cryo)
- RT experiments (is cryo relevant?)
- dynamic experiments

Why serial crystallography?



protein-substrate complex
ground state

?



protein-product complex
final lit state

diamond

Why serial crystallography?



protein-substrate complex
ground state



protein-product complex
final lit state

reaction intermediate



Why serial crystallography?



protein-substrate complex
ground state



protein-product complex
final lit state

reaction intermediate



reaction intermediate



reaction intermediate

diamond

Why serial crystallography?



protein-substrate complex
ground state



protein-product complex
final lit state

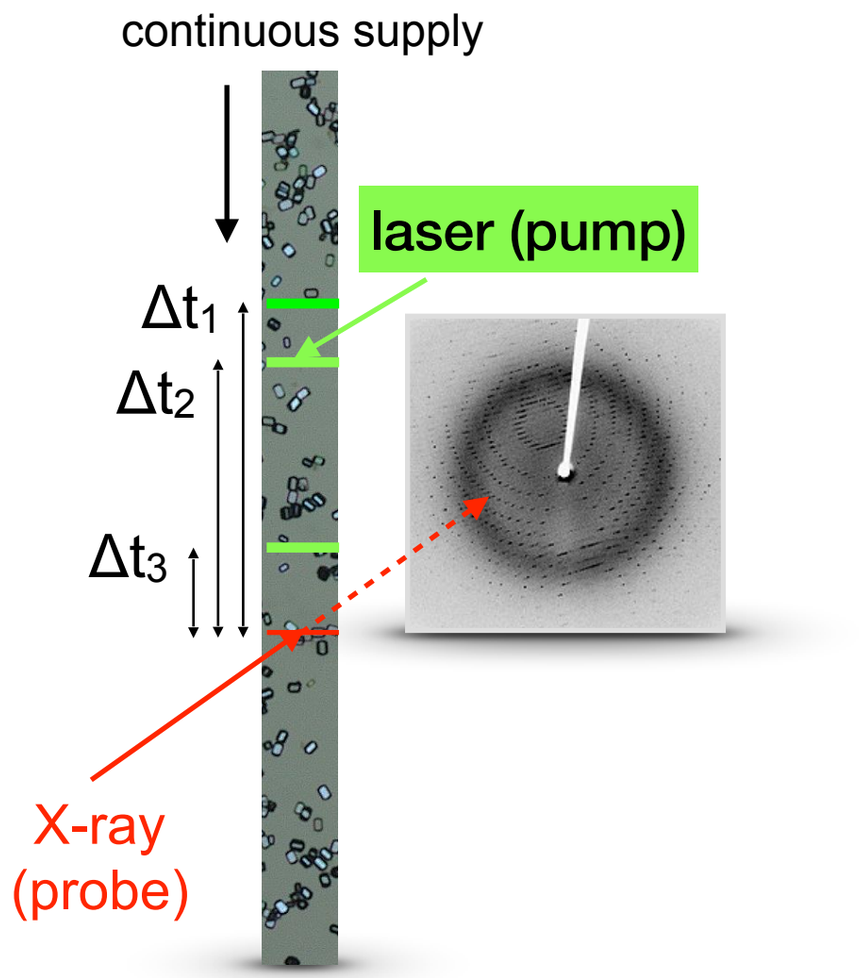


diamond

Why serial crystallography?

Perfect for:

- small crystals
- sensitive samples (radiation, cryo)
- RT experiments (is cryo relevant?)
- dynamic experiments



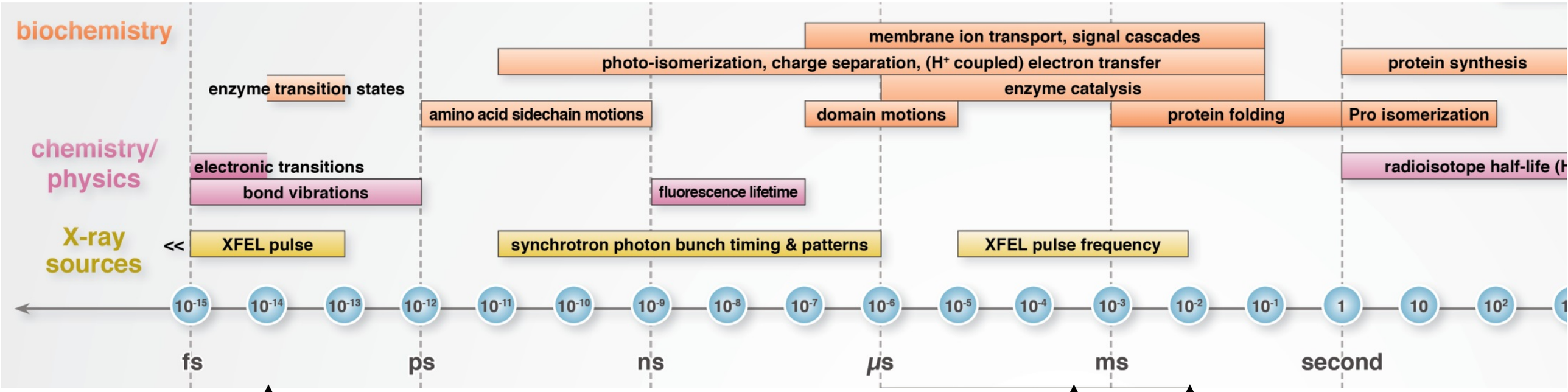
pump-probe:

t_0: 1000s images = dataset 1
Δt_1: 1000s images = dataset 2
Δt_2: 1000s images = dataset 3
Δt_3: 1000s images = dataset 4
(…)

diamond

What is conceivable at synchrotron/XFEL?

What is conceivable at SR/XFEL?

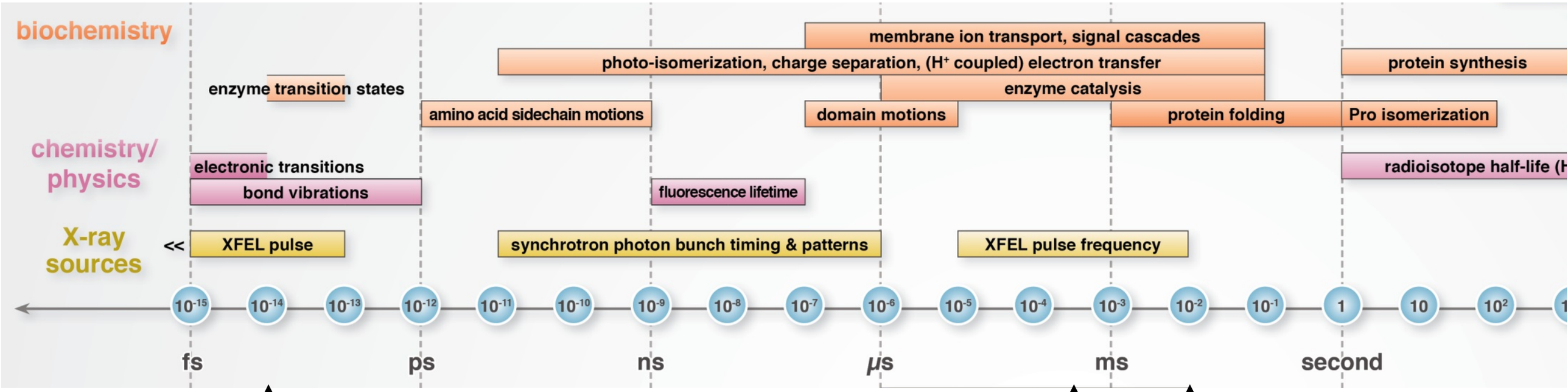


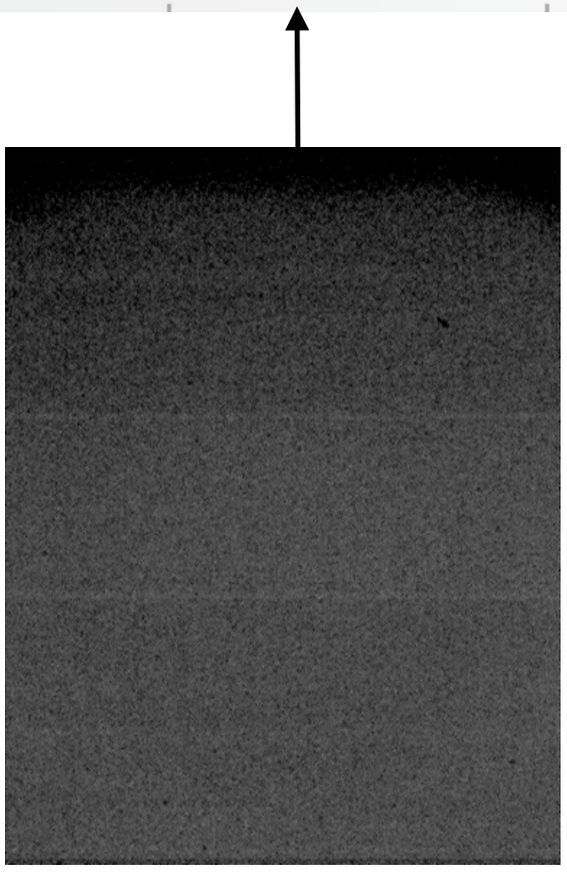
XFEL:
- higher time resolution
- smaller crystals
- single particles

SR

Enzyme catalytic turnover
~ 1 µs – seconds
Average enzyme turnover ≈ **60 ms**

What is conceivable at SR/XFEL?





SR

Enzyme catalytic turnover
~ 1 μs – seconds
Average enzyme turnover ≈ **60 ms**

Synchrotrons available:

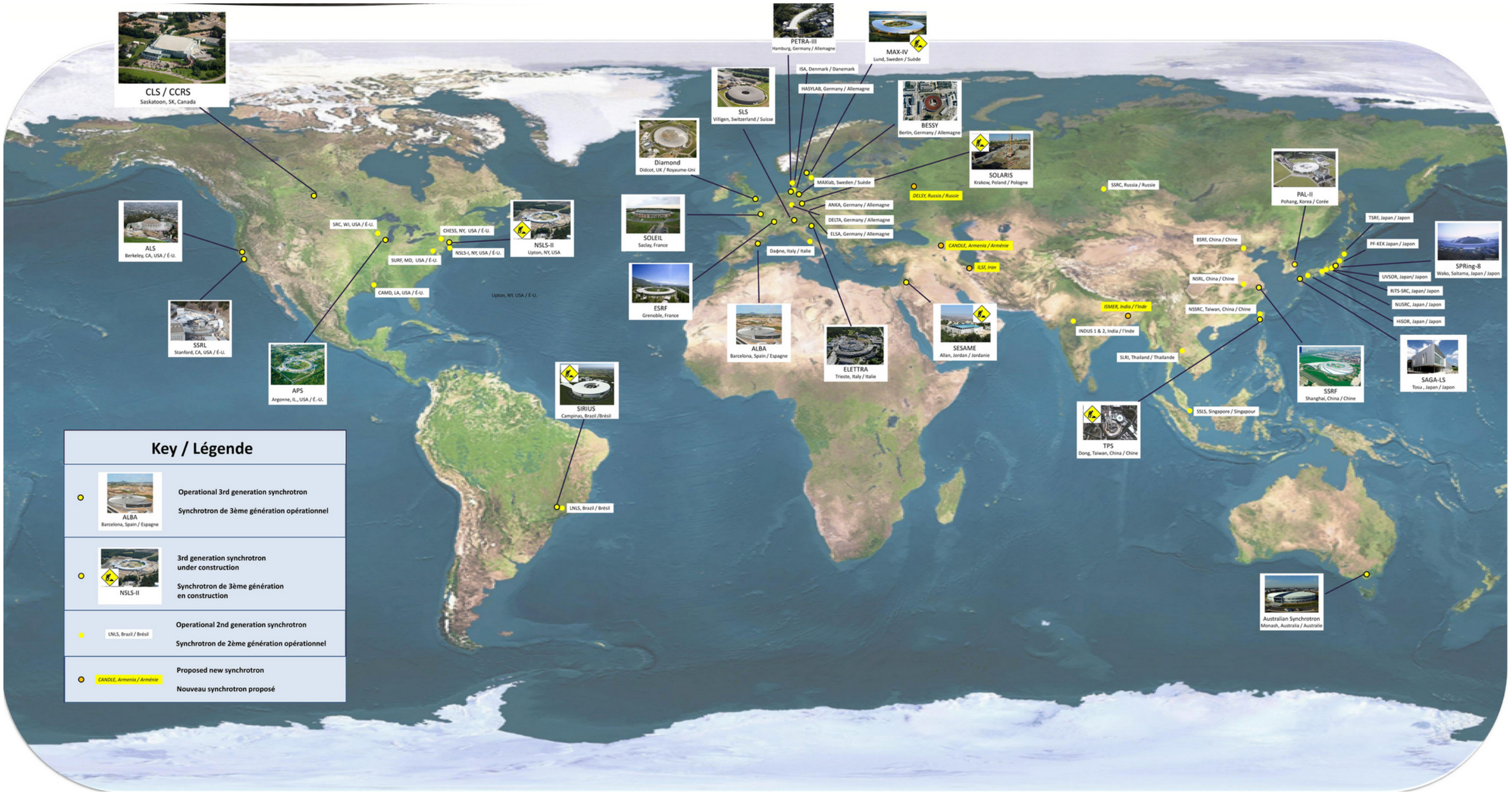
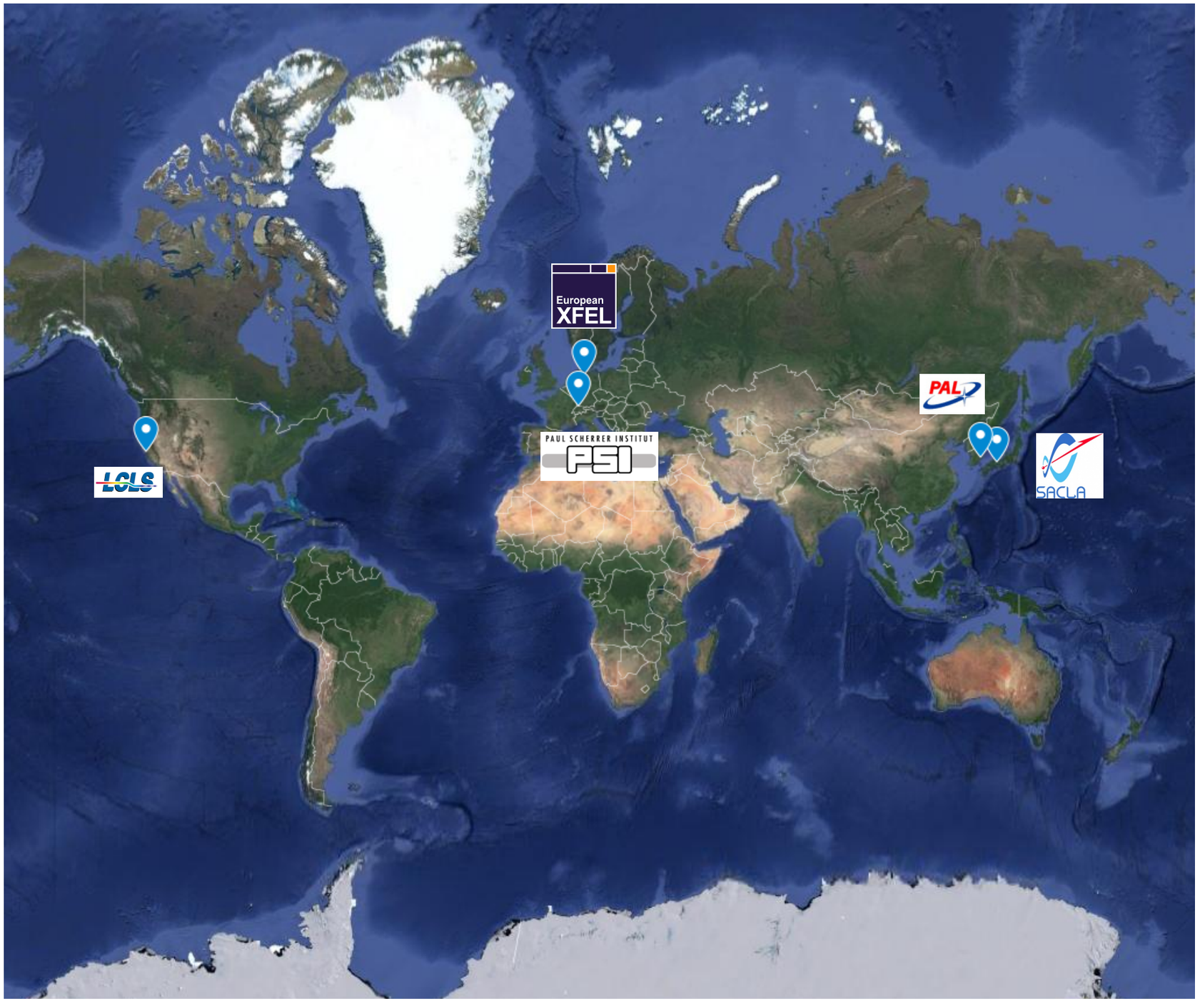


Image from Canadian Light Source

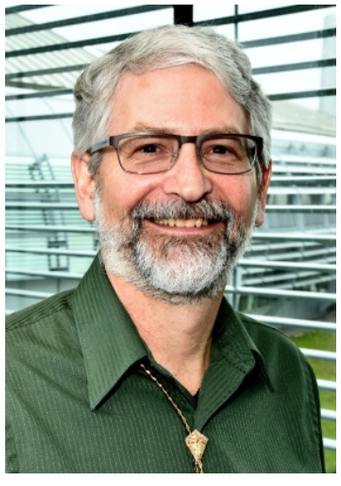
XFELs available:

XFELs in operation:

- **LCLS** (USA)
- **SACLA** (Japan)
- **PAL XFEL** (South Korea)
- **SwissFEL** (Switzerland)
- **European XFEL** (Germany)



The XFEL-Hub at Diamond



Allen Orville
PI



Pierre Aller
Beamline scientist



Agata Butryn
PDRA

Science & Technology Facilities Council

wellcome trust

BBSRC bioscience for the future

MRC Medical Research Council

Support of serial structural biology experiments (SR/XFEL) via:

- sample preparation,
- sample testing (BAG beamtime),
- hardware for sample delivery,
- data collection,
- data processing,
- training,
- travel assistance for UK XFEL users

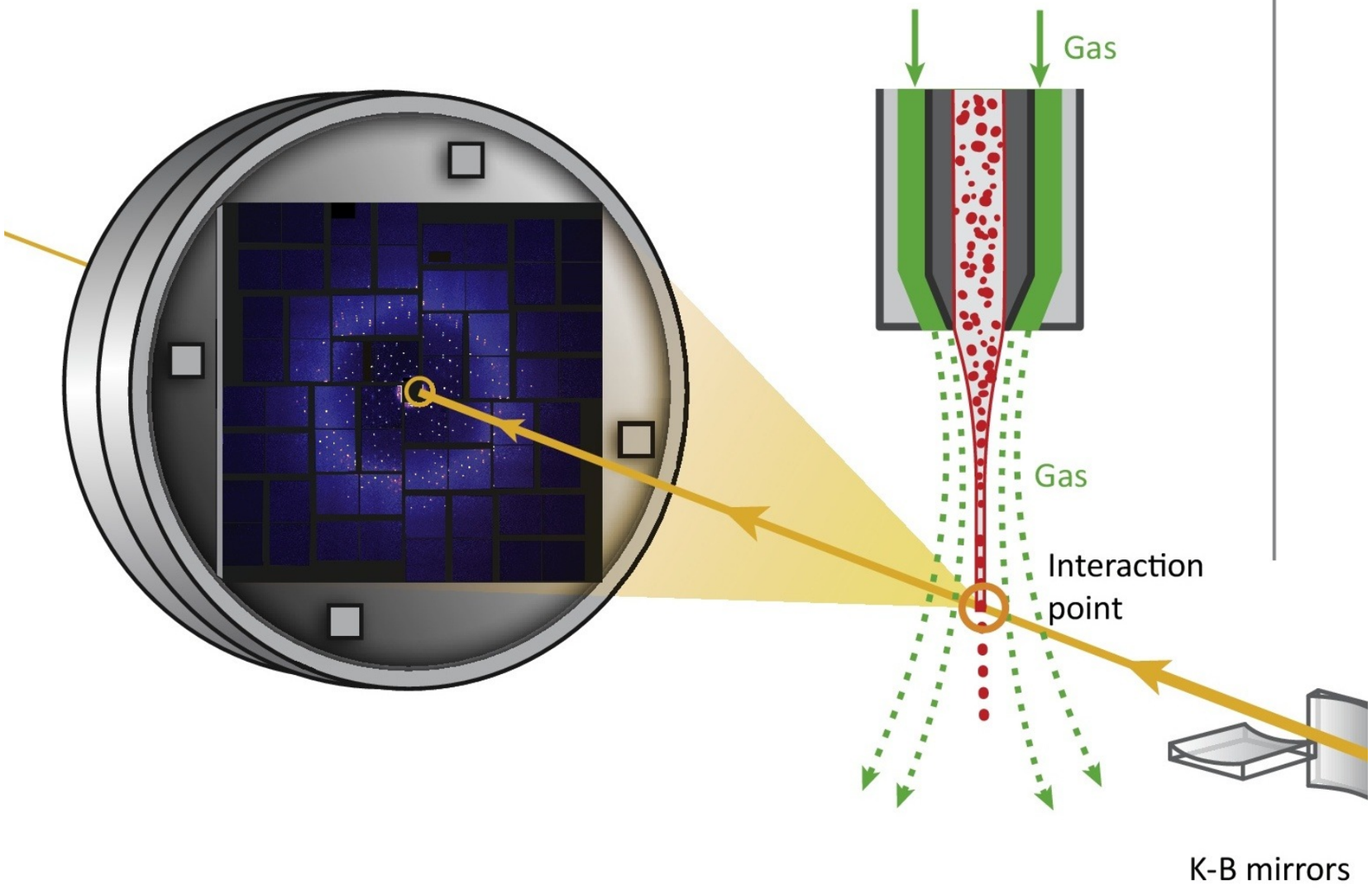
Transfer of methods between XFEL, synchrotron, and cryo-EM

diamond

Sample delivery

Liquid jet

Gas Dynamic Virtual Nozzle (GDVN)



- ~10 uL/min
- ~10 m/s
- 10-100 mg protein

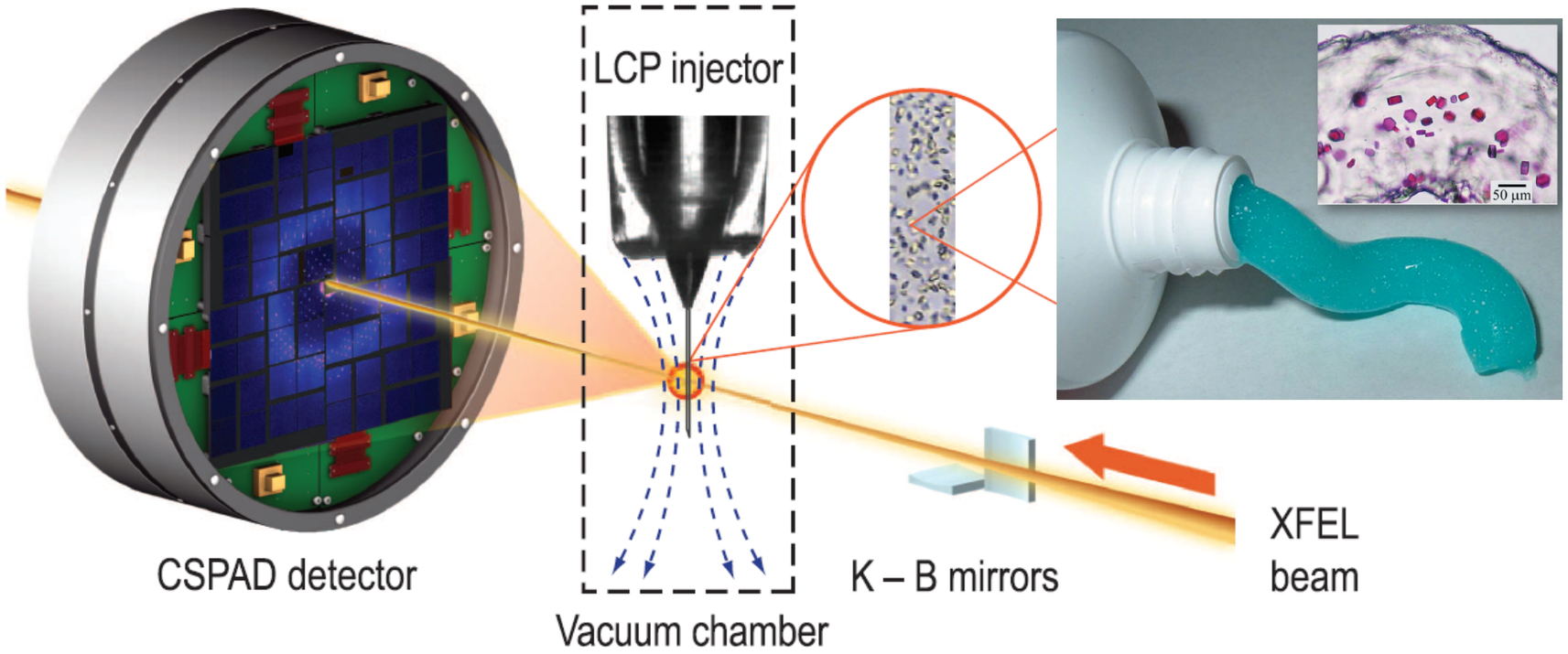


50 fs @ 30 Hz = 1 min every ~300 years…

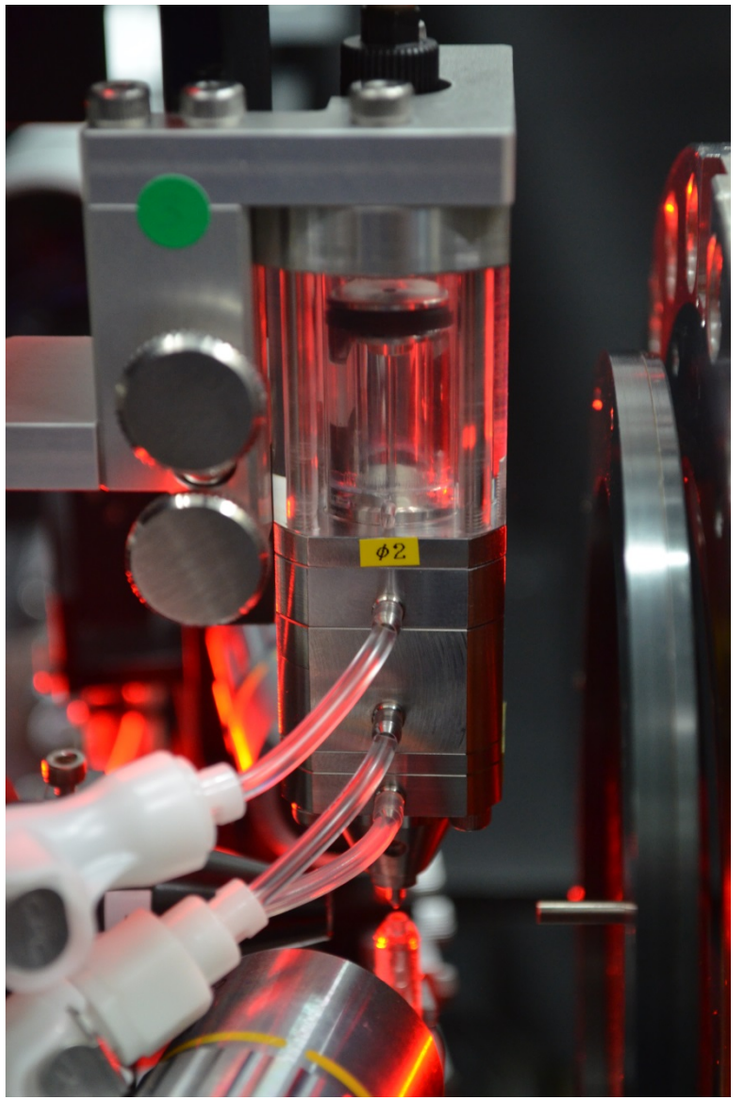
microcrystals
10^{7}-10^{11}/mL

Johansson et al., Nat Meth (2012)
Johansson et al., Trends Biochem Sci (2014)

High-viscosity (LCP) extruder



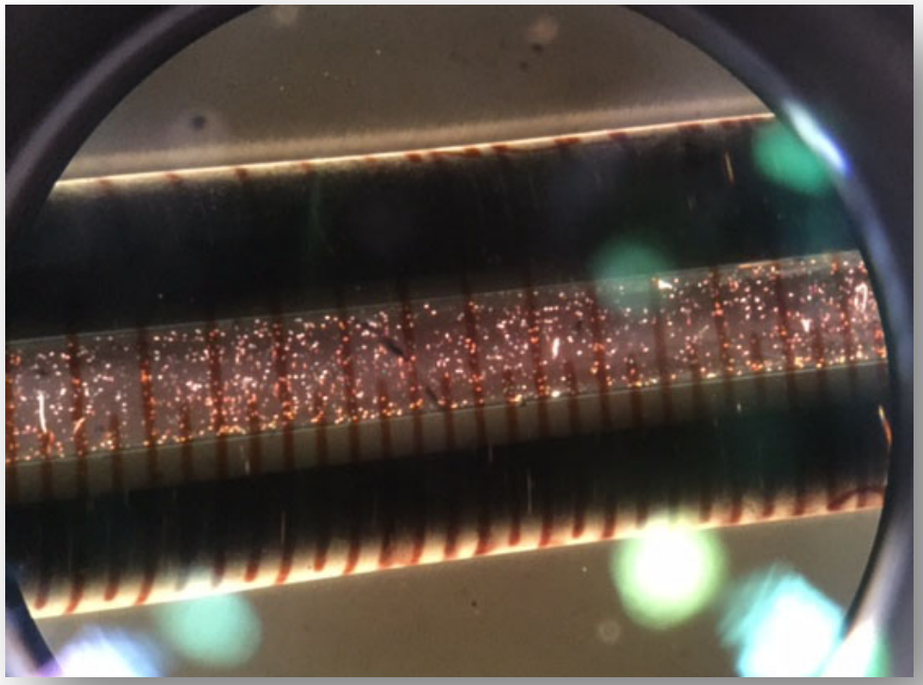
SACLA extruder



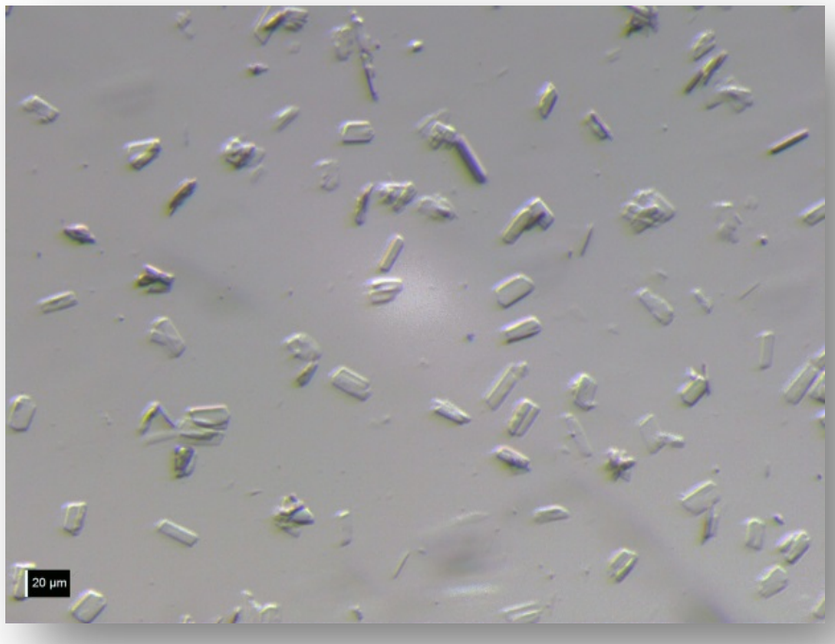
- 3-300 nL/min
- 100 uL of microcrystals

Liu et al., Science (2013)

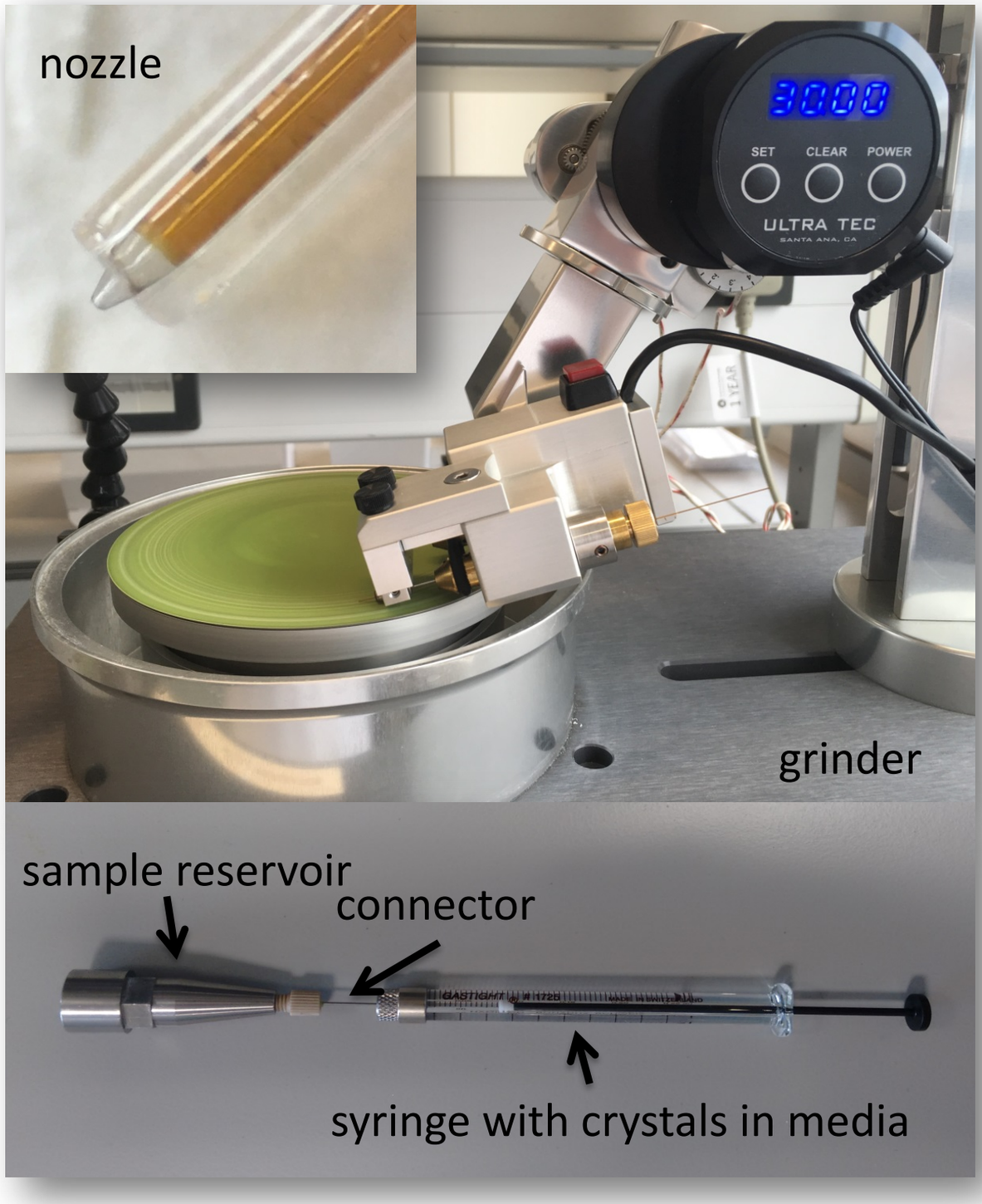
LCP/viscous media extruder



membrane protein
crystals in LCP



soluble protein crystals
in viscous media



LCP-SSX at DLS I24

gas line

reservoir (20µl)

nozzle

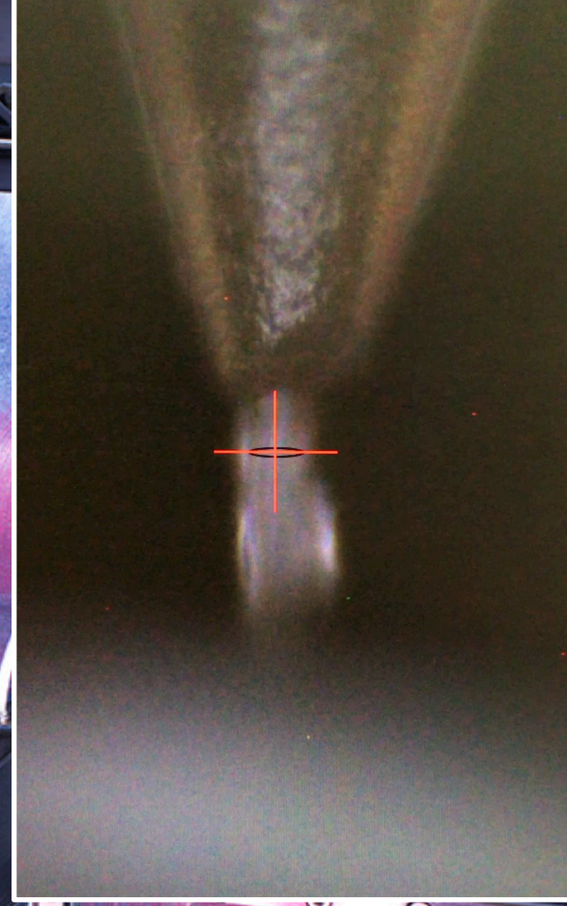
scatter guard

horizontal goniometer

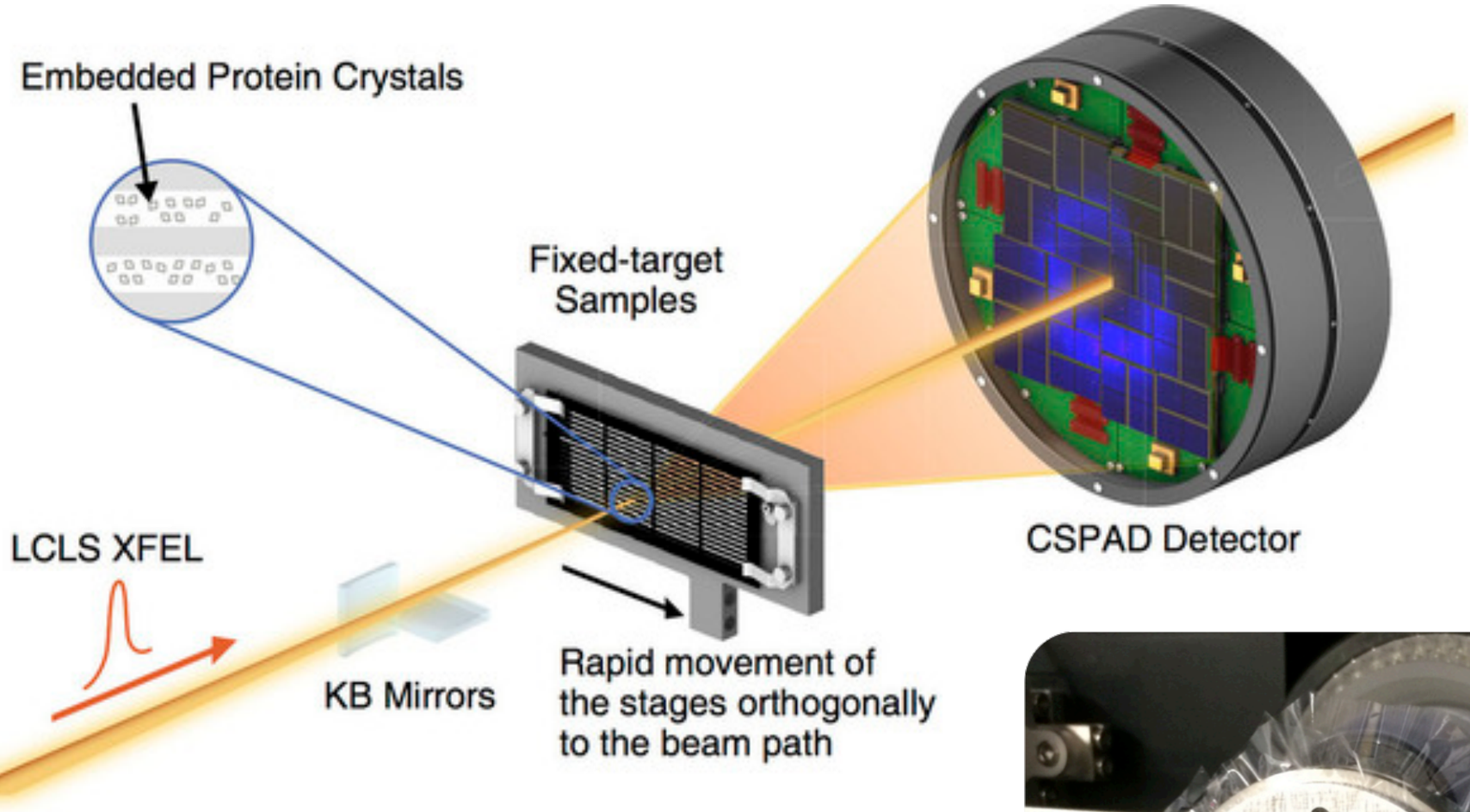
catcher

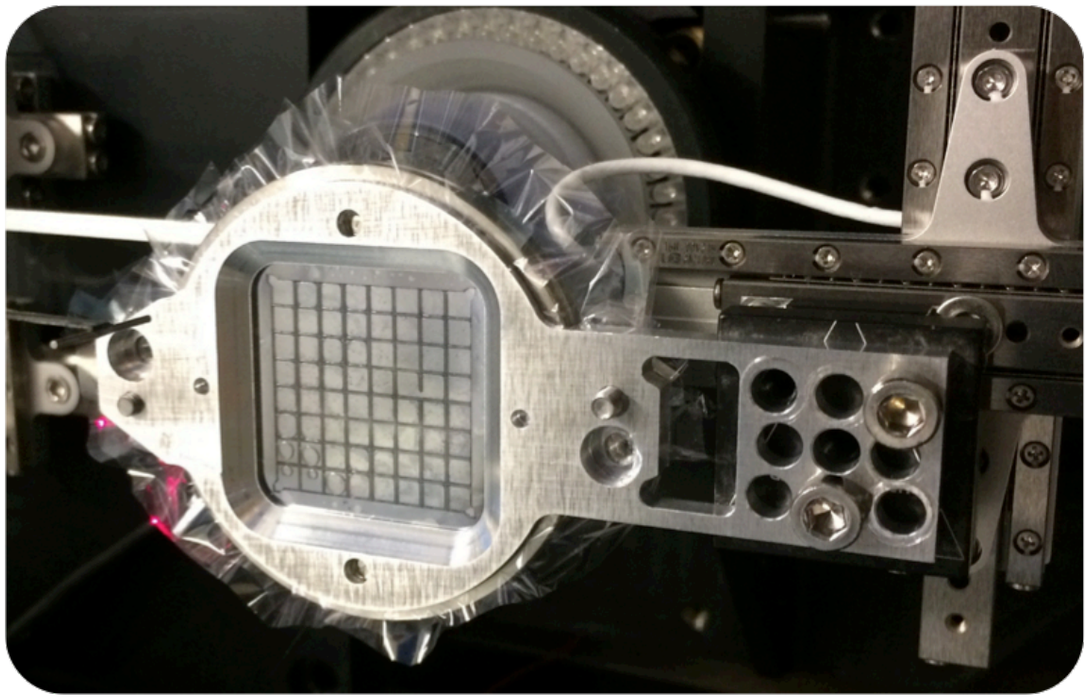
beamstop

vertical goniometer



Fixed-target approaches

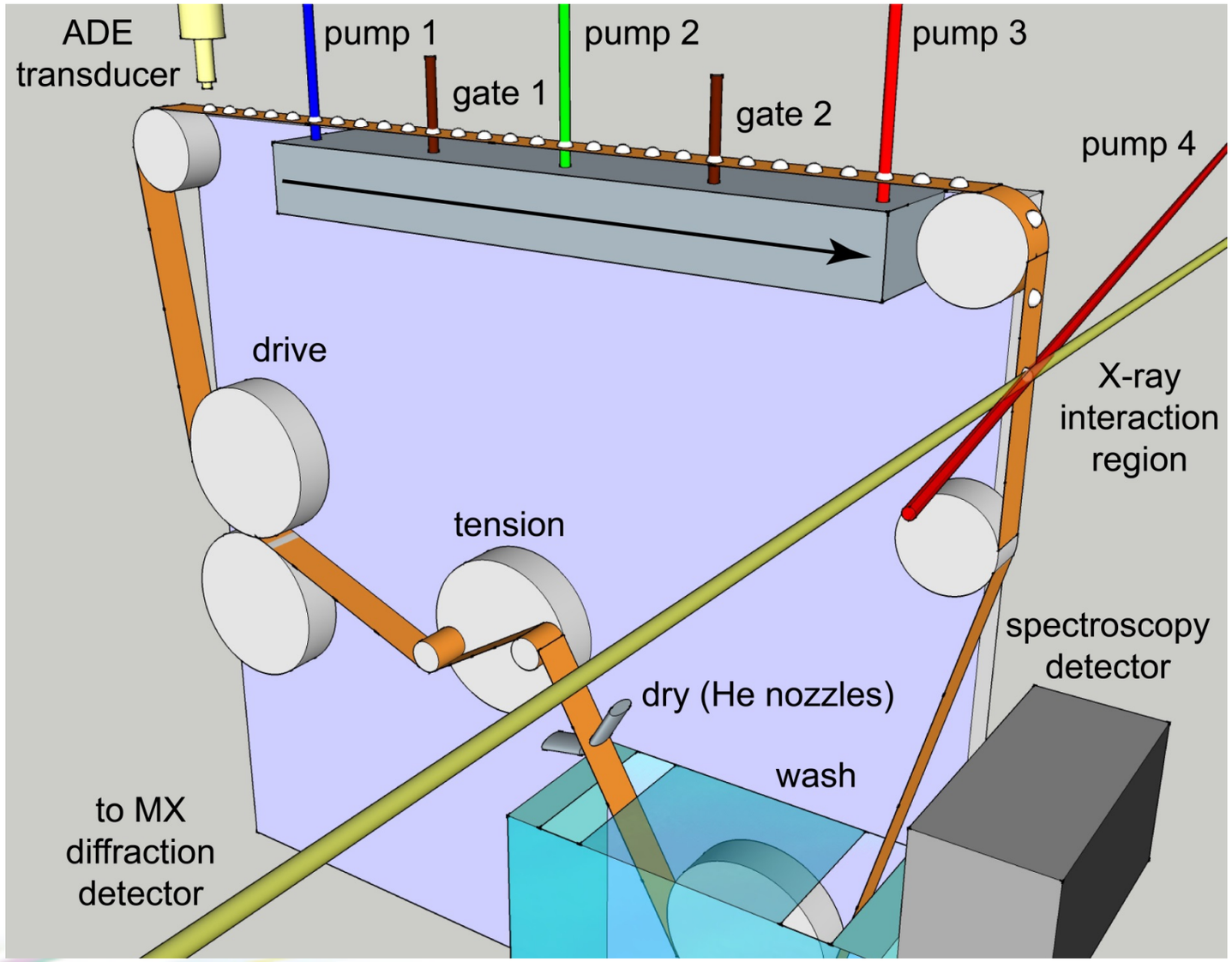


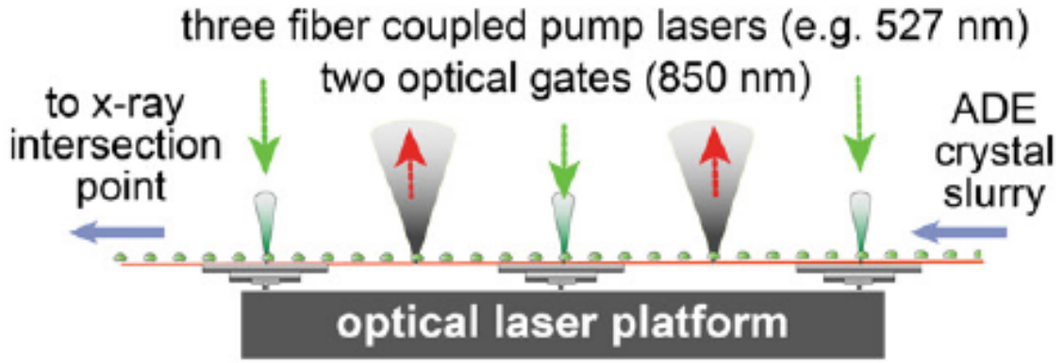


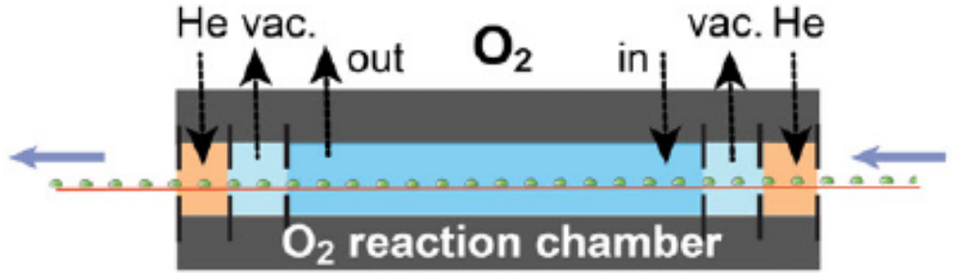
- scarce samples, “easy” to load
- several chip & well sizes
- hit ratio of ~100%
- room temp & cryo
- pump-probe, mixing

Hunter et al., Sci Rep (2014), https://www.diamond.ac.uk/Instruments/Mx/I24/

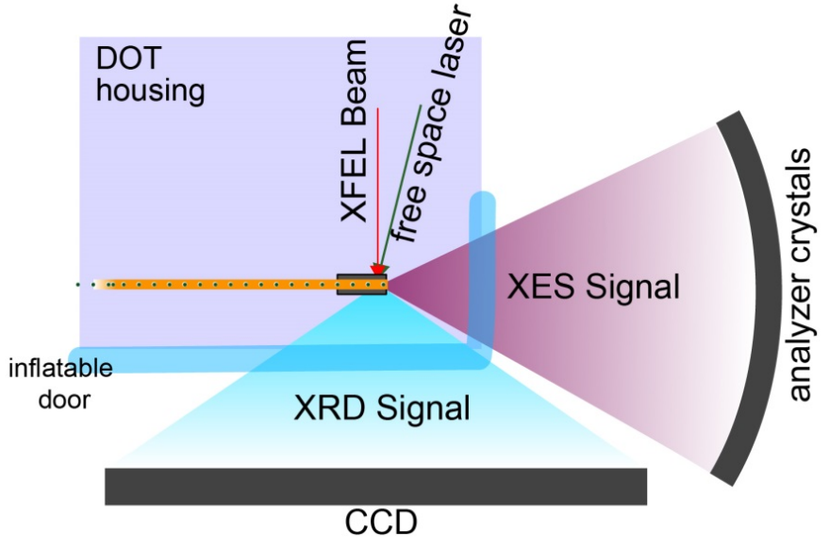
On-demand acoustic ejection with conveyor belt







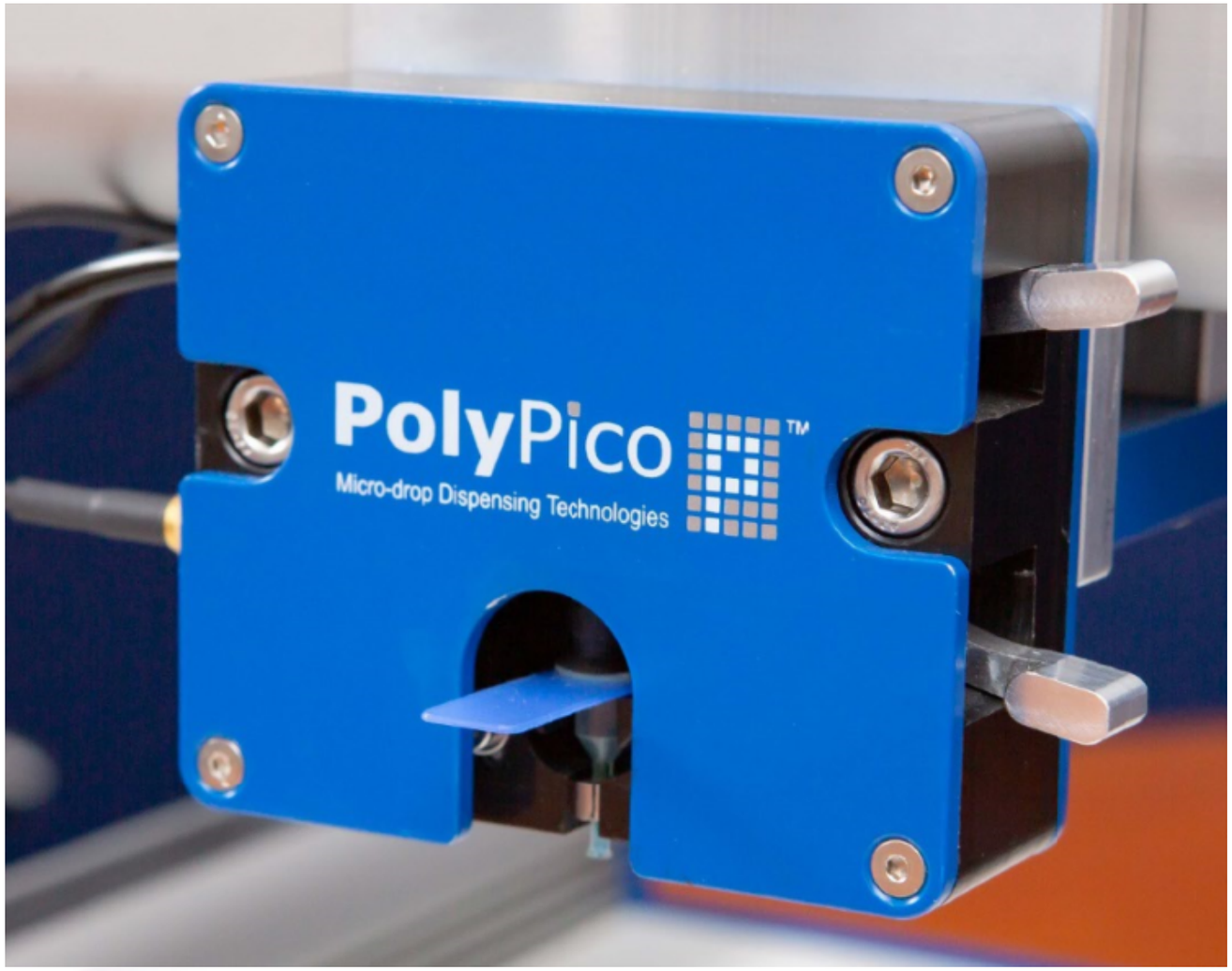
Fuller et al.,
Nat Meth. (2017)

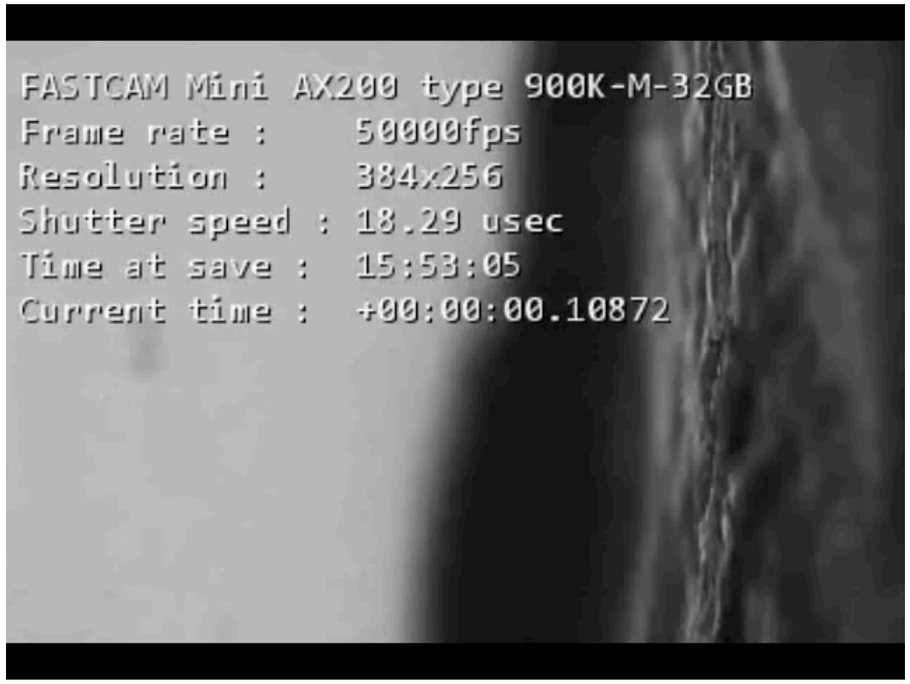


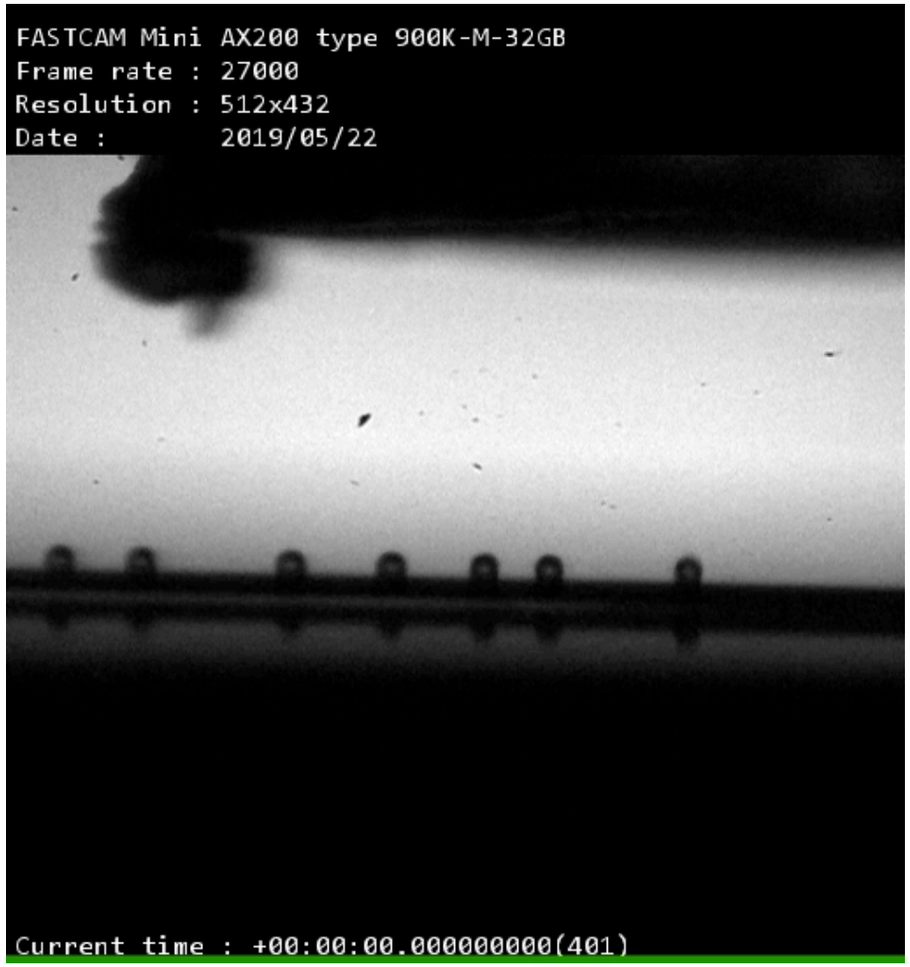
diamond

On-demand acoustic ejector at LCLS

PolyPico acoustic ejector





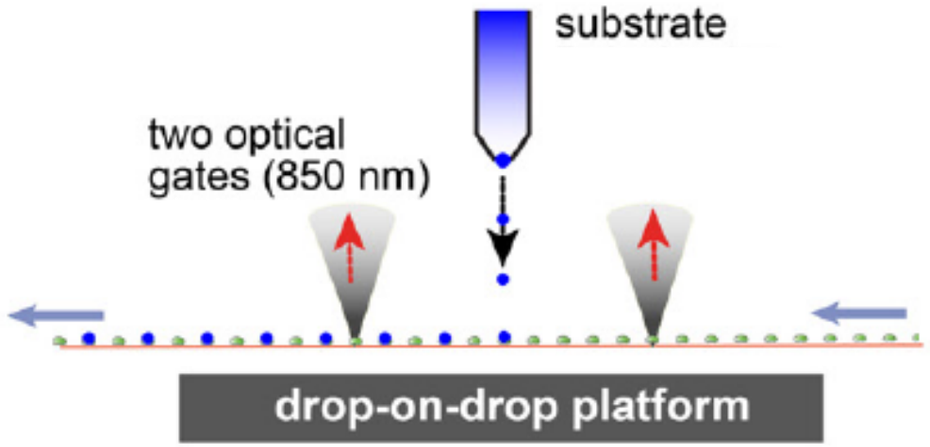


PolyPico sample delivery

- 30-100 pL droplets
- up to 38 kHz
- disposable cartridge
- cartridge dead volume 7 µL

diamond

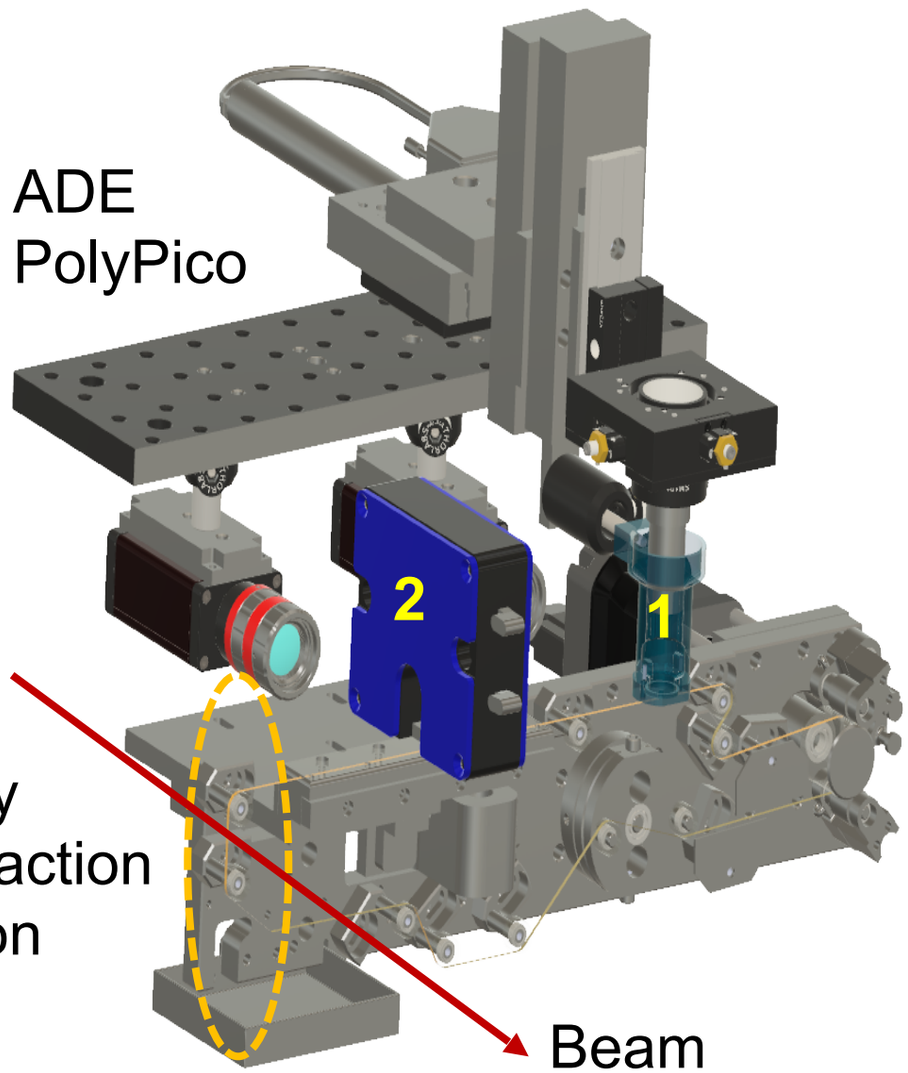
SACLA XFEL 9-12 July 2019

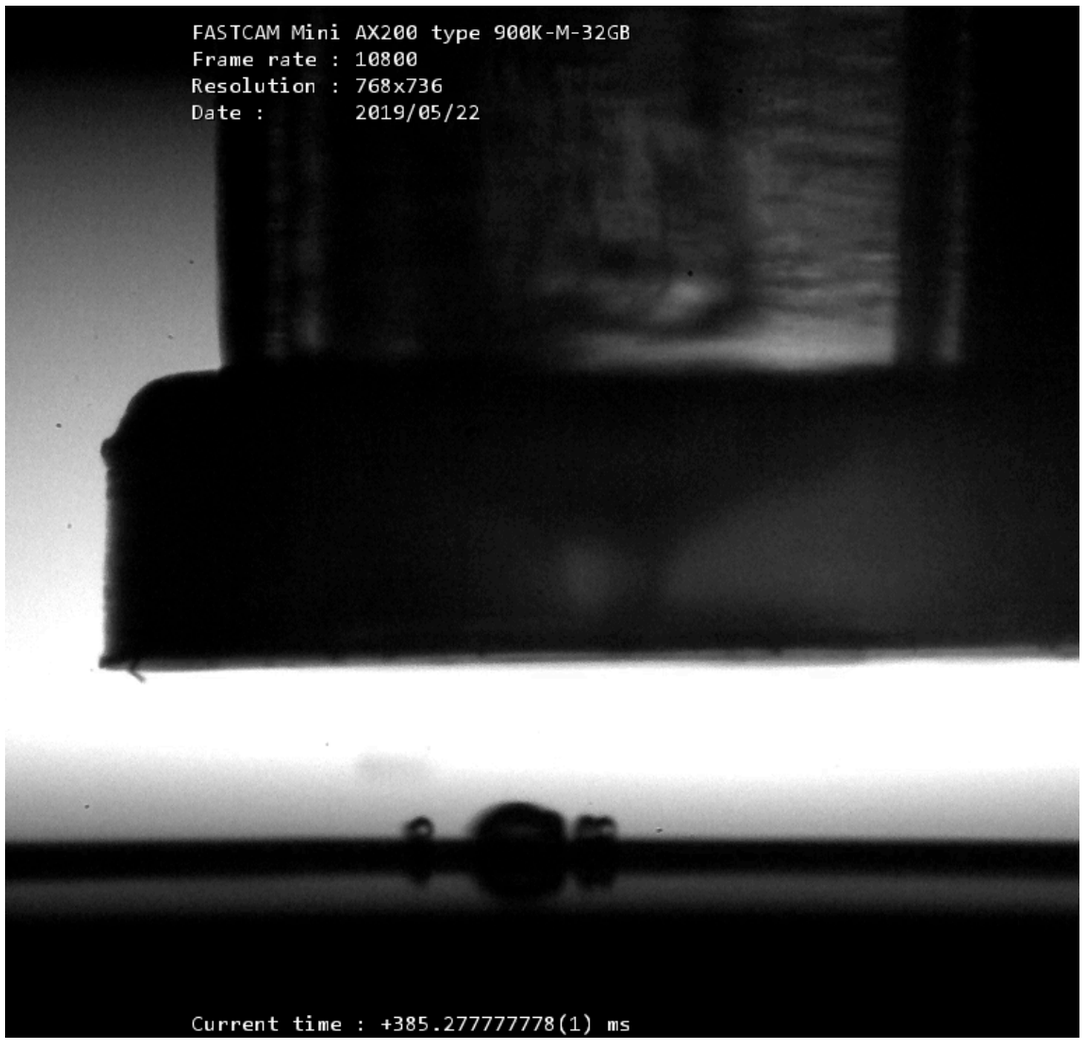


“Deciphering the structural basis of antimicrobial resistance with non-equilibrium, tr-SFX studies of beta-lactamase turnover” (Orville et al.)

- Belt speed: 15 mm/s to 600 mm/s (100 ms - 4s)
- ADE: 1 nL-2.5 nL @ 30 Hz
- PolyPico > 30 pL @ up to 38 kHz in bursts

1) ADE
2) PolyPico





SACLA XFEL 9-12 July 2019

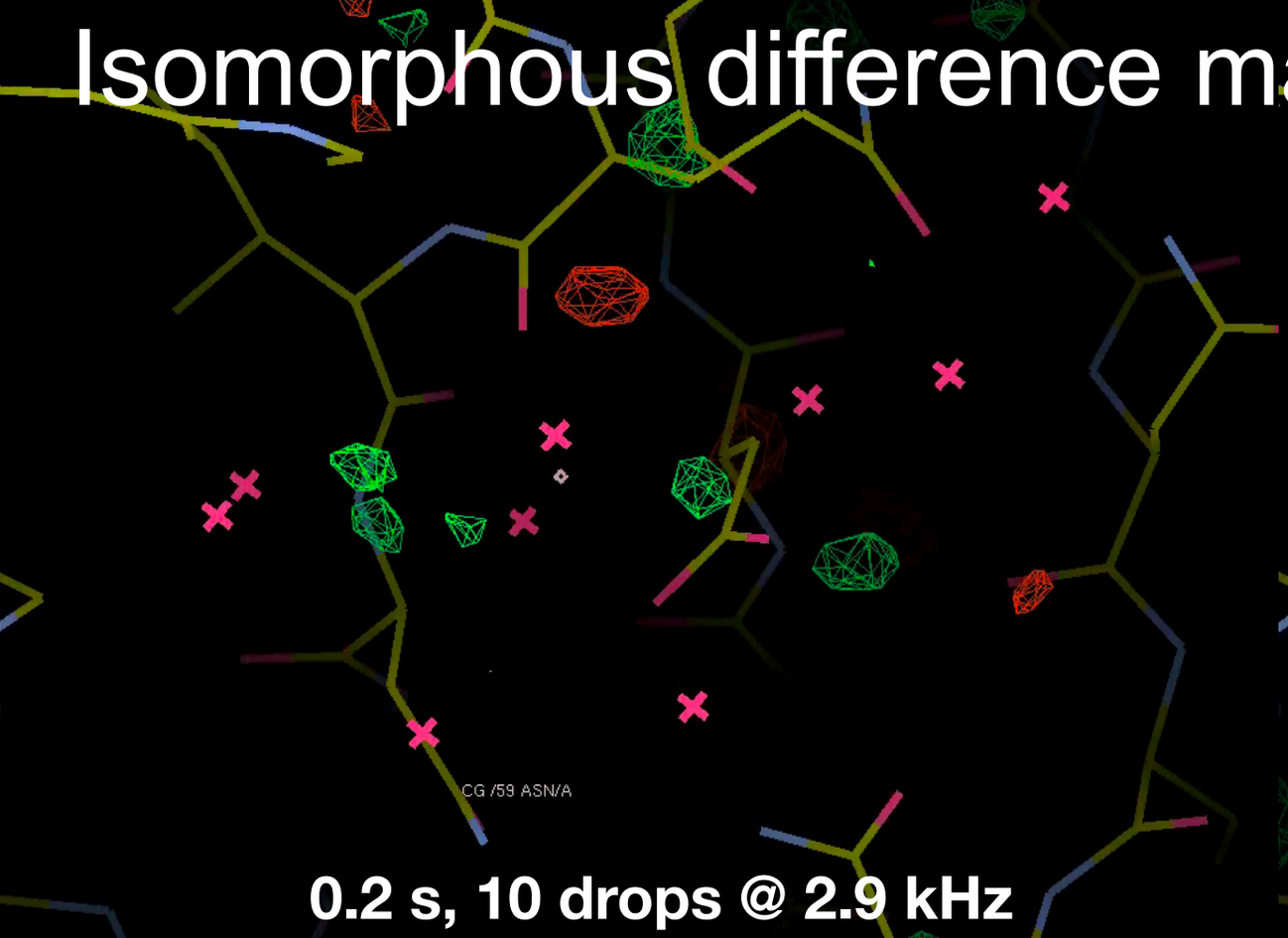
PolyPico Head

PolyPico Camera

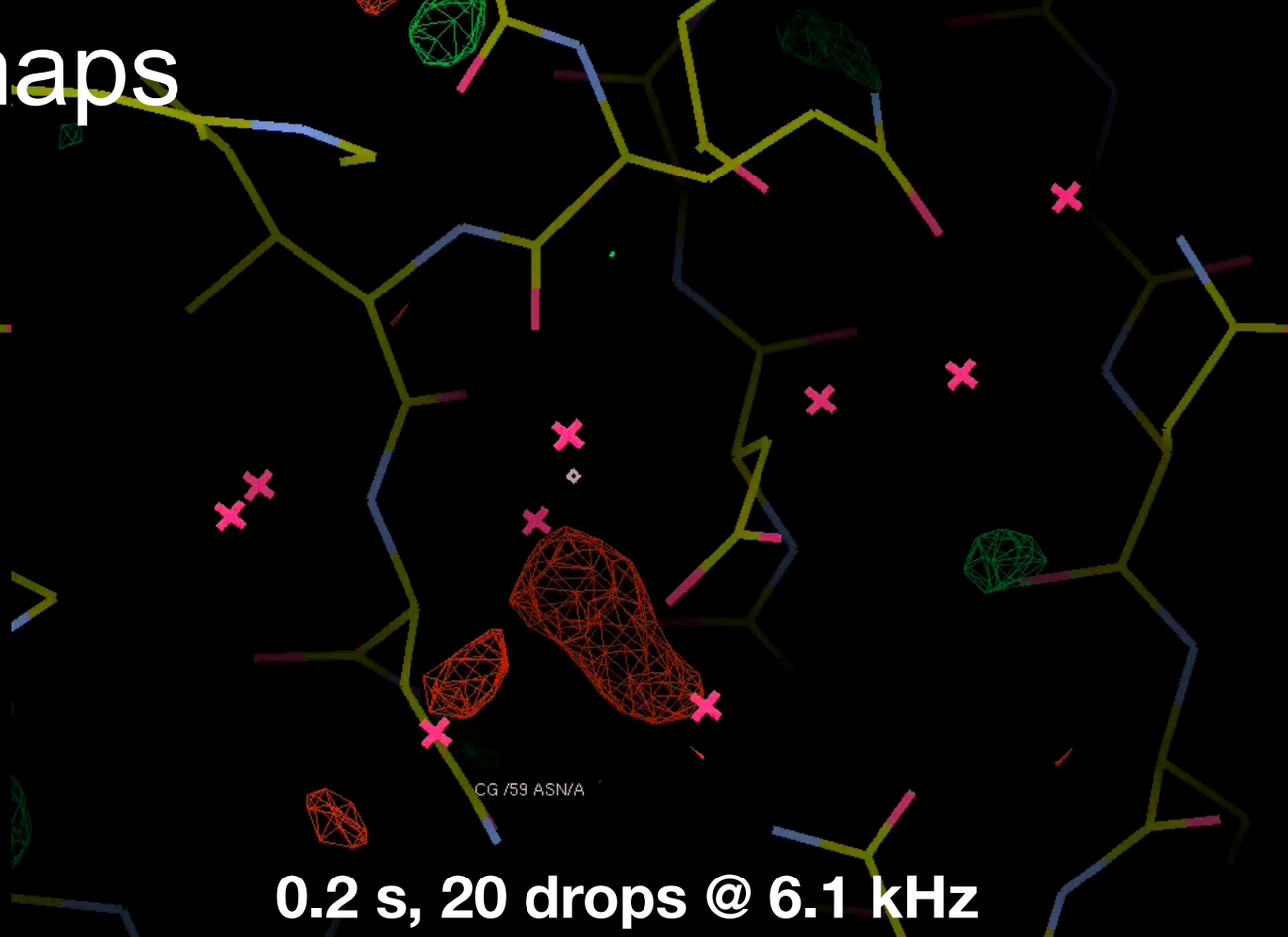
Transducer

Tape direction

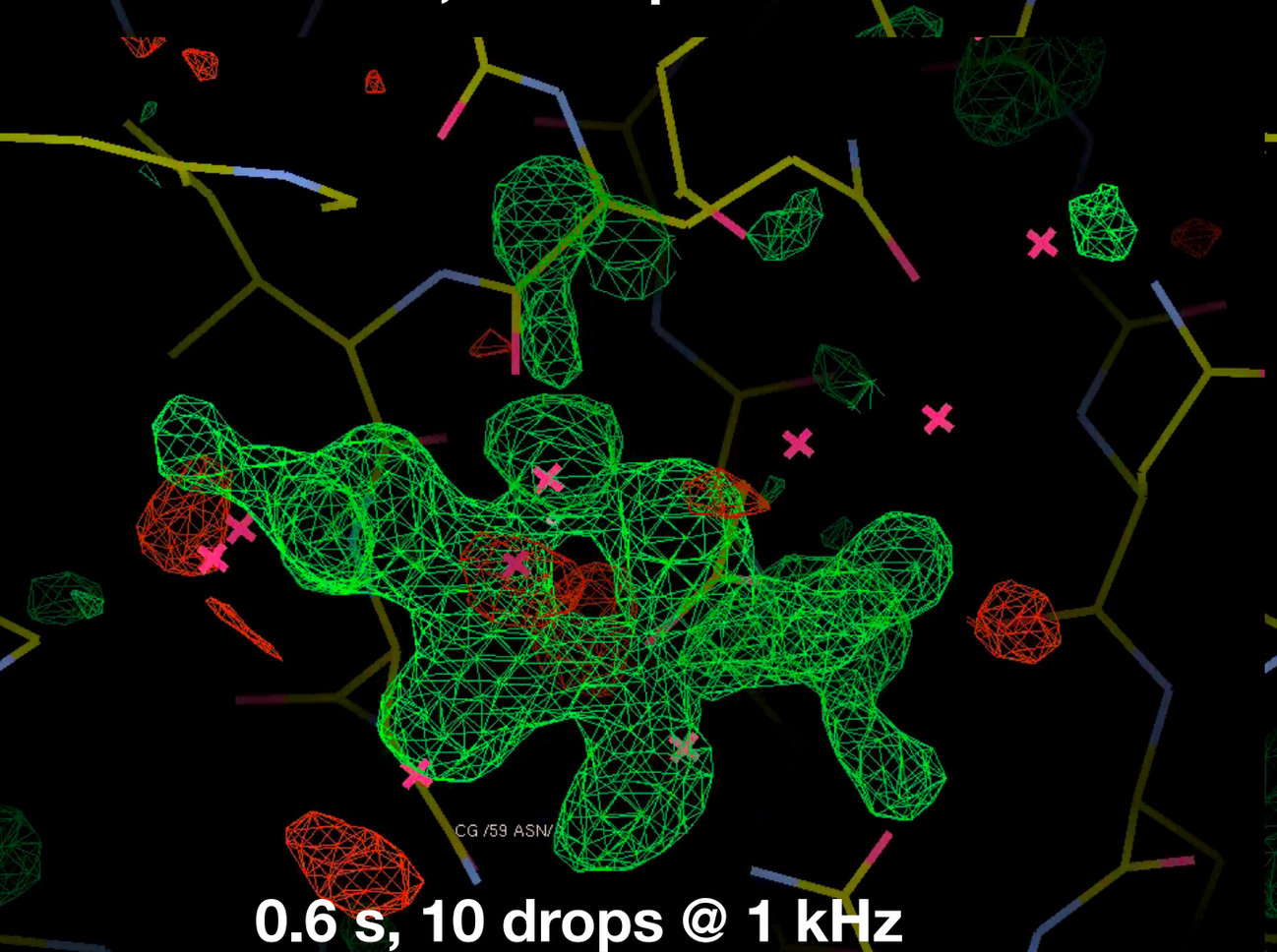
Isomorphous difference maps



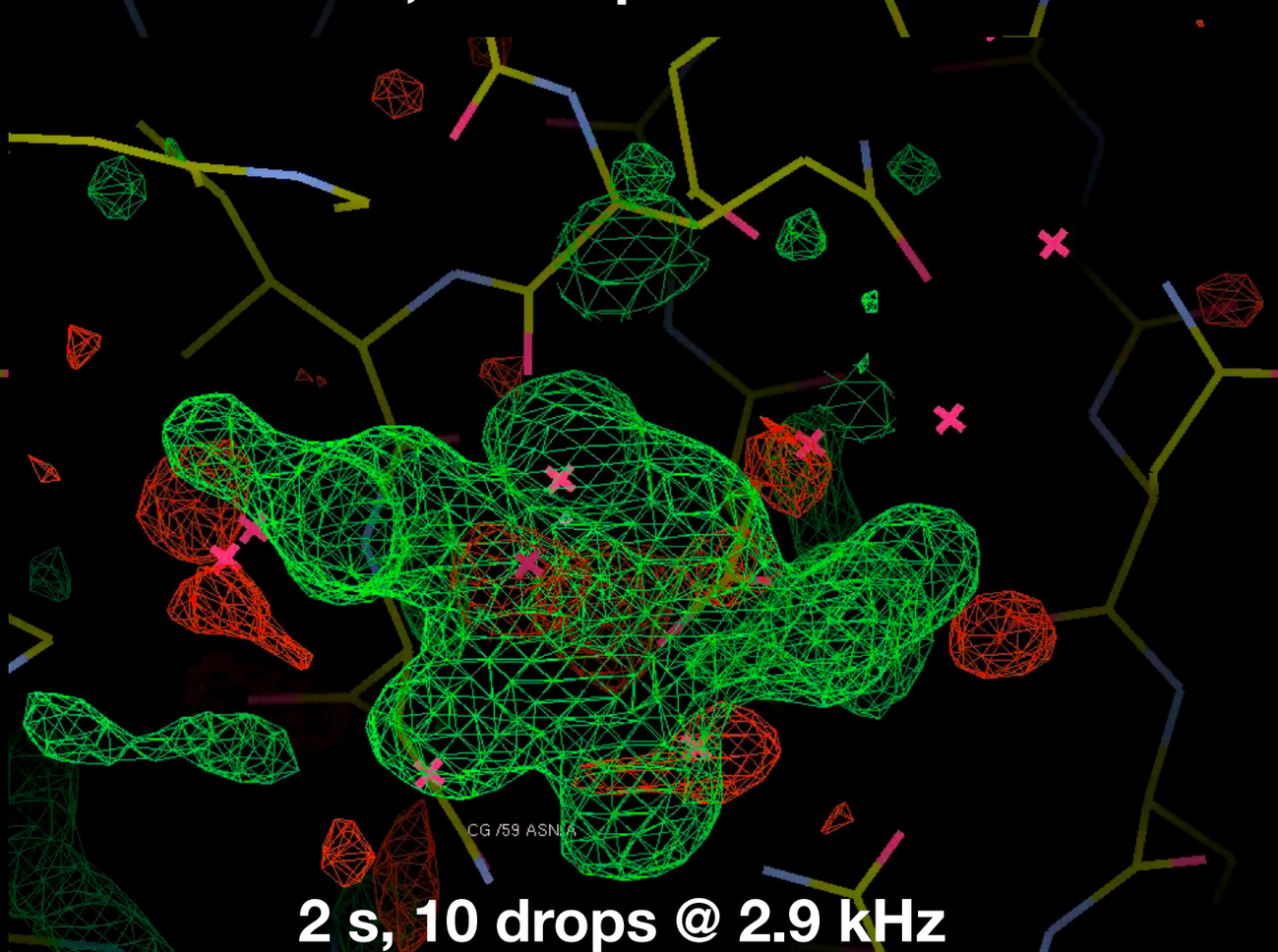
0.2 s, 10 drops @ 2.9 kHz



0.2 s, 20 drops @ 6.1 kHz

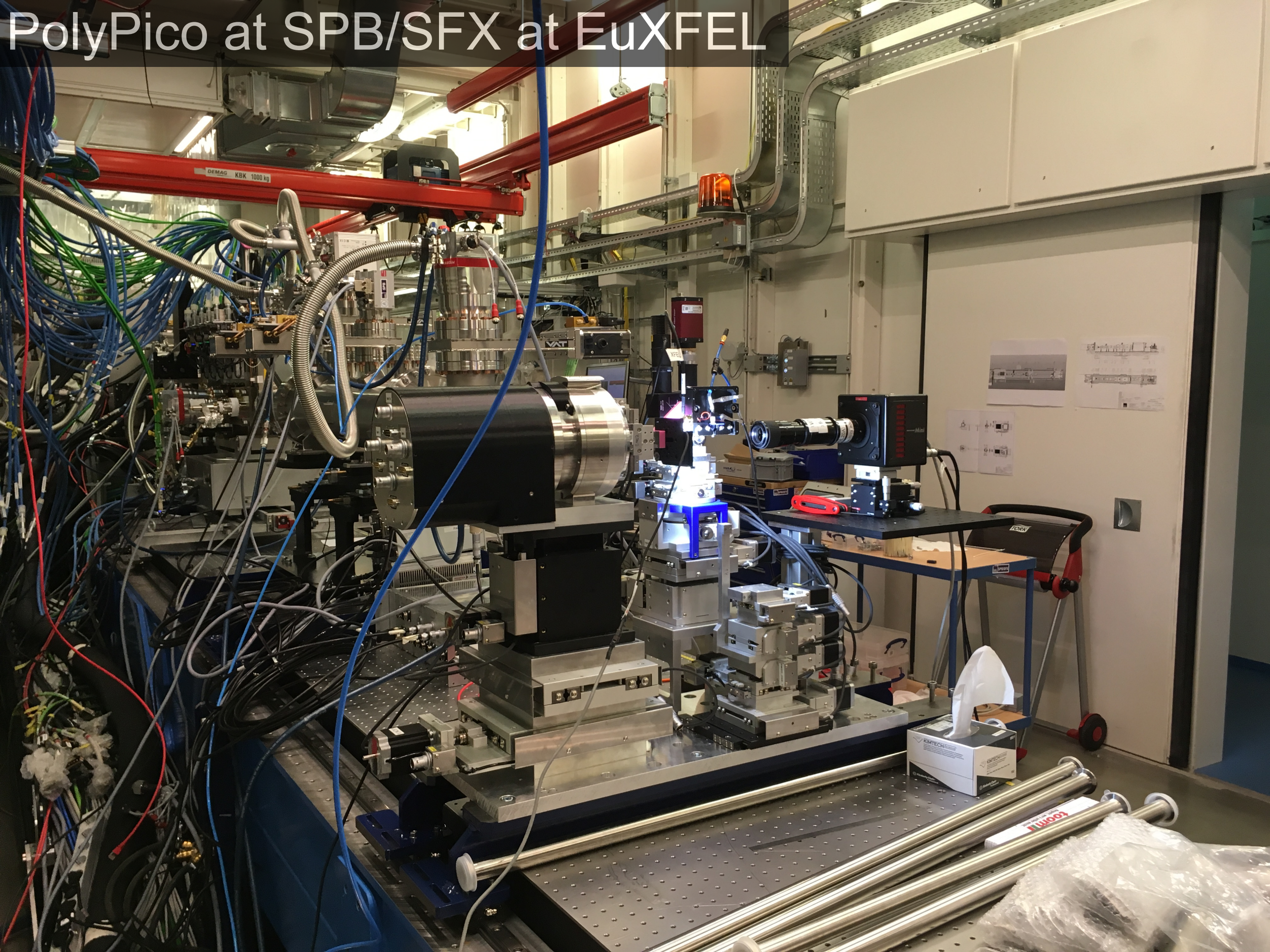


0.6 s, 10 drops @ 1 kHz

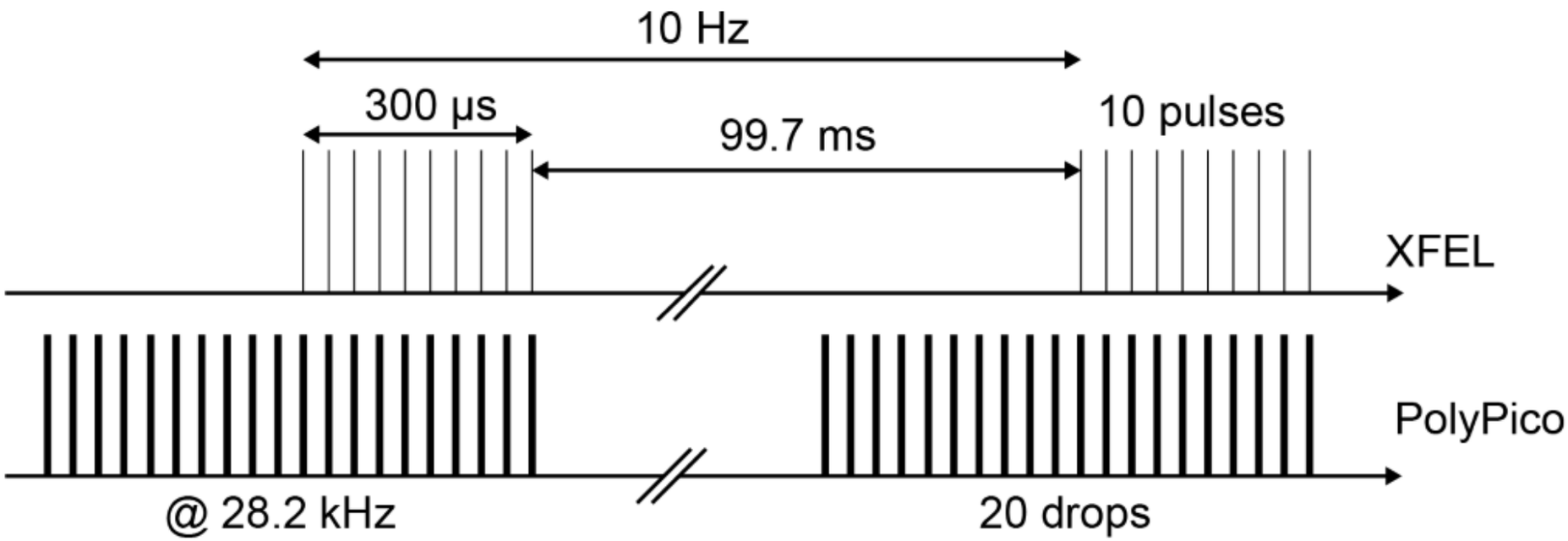


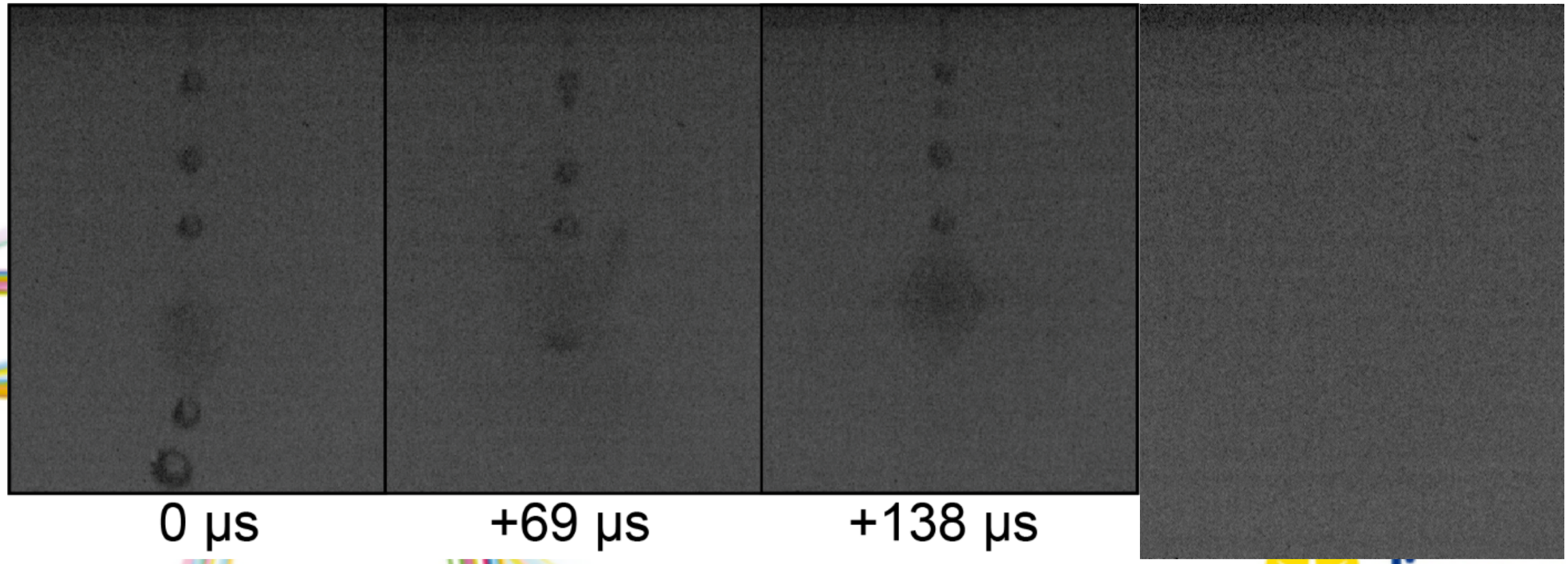
2 s, 10 drops @ 2.9 kHz

PolyPico at SPB/SFX at EuXFEL



IRDa commissioning SPB/SFX @ EuXFEL





diamond

Diamond Light Source

XFEL-hub:
Allen Orville
Pierre Aller
Julien Orlans

I24:
Robin Owen
(Martin Appleby)
Danny Axford
(John Beale)
(Brad Davy)
Ali Ebrahim
(Darren Sherrell)
Selina Storm

VMXi:
Juan Sanchez-Weatherby

DIALS:
Nick Devenish

Oxford University

Christopher Schofield
Jürgen Brem
Ian Clifton
Jos Kamps
Pauline Lang
Anka Lucic
Mike McDonough
Patrick Rabe

EuXFEL

Adrian Mancuso
Adam Round
Robin Schubert
Joachim Schultz

PolyPico

Gabriel Leen

Berkeley National Lab

Junko Yano
Vittal Yachandra
Ruchira Chatterjee
Franklin D. Fuller
(Sheraz Gul)
In-Sik Kim
Jan Kern
(Louise Lassalle)
(Ernest Pastor)
Philipp Simon

Nicholas K. Sauter
Aaron Brewster
Asmit Bhowmick
Kyle Sutherlin
(Iris Young)