What can you do at I24?

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Outline

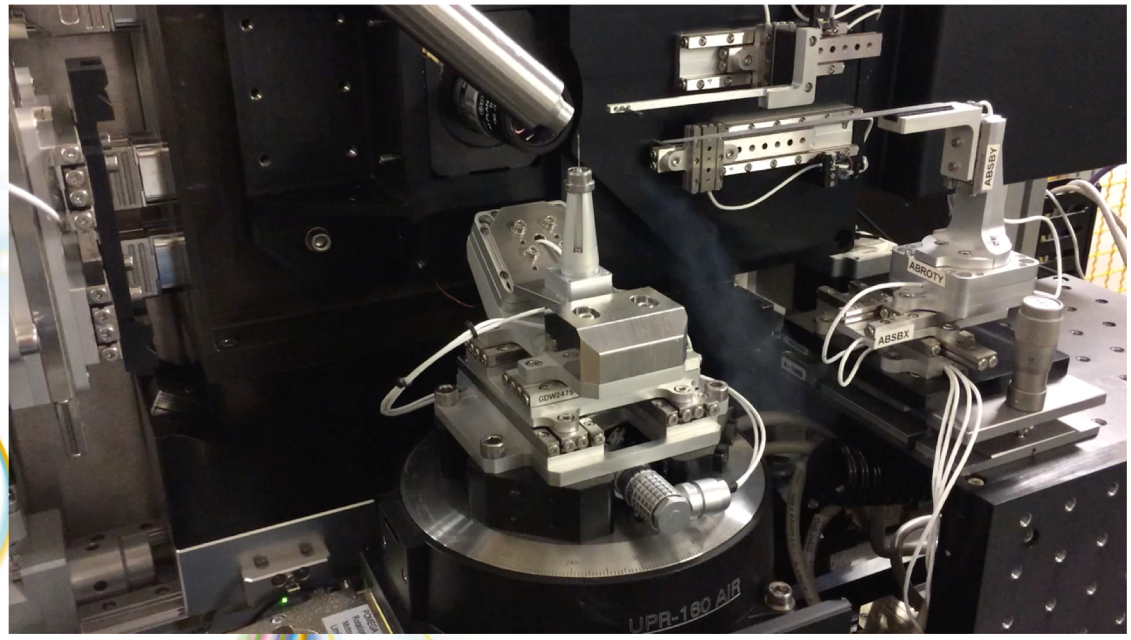
- What can I24 do?
  - standard data collection
  - *in situ* data collection
  - benefits of multiple crystal data collection
- Serial crystallography at I24
  - sample delivery systems
    - data processing
      - X-ray induced structural changes
- Future possibilities at I24
  - time-resolved and rotational serial crystallography
  - new CdTe Eiger



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Standard data collection at I24

- I24 is a microfocus beamline with beam sizes of 7x7 um to 50x50 um
- the flux is the same for all beamsizes → 4x10^{12} ph/s at our standard energy of 12.8 keV
- vertical goniometer as a standard, kappa goniometer on request



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In situ data collection at I24 - plates

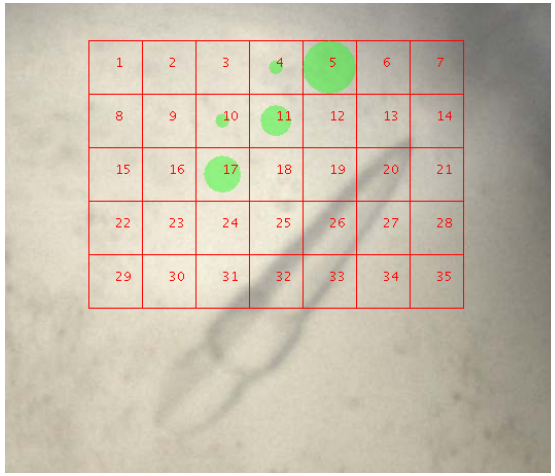
- change between pin mode and tray mode takes about 2 minutes – easy to switch back and forth
- automated plate exchange possible
- rotation range ± 30º, room temperature only



In situ data collection at I24 - plates

- most 96 well plates can be accommodated
  - also custom plates with enough notice
  - preferred plate type: Greiner Crystal Quick X or Mitegen *in-situ*-1

- highly recommend not using plates with curved bottoms
  - greatly complicates centring as apparent position of crystals becomes offset.
  - size and direction of offset dependent on location within well



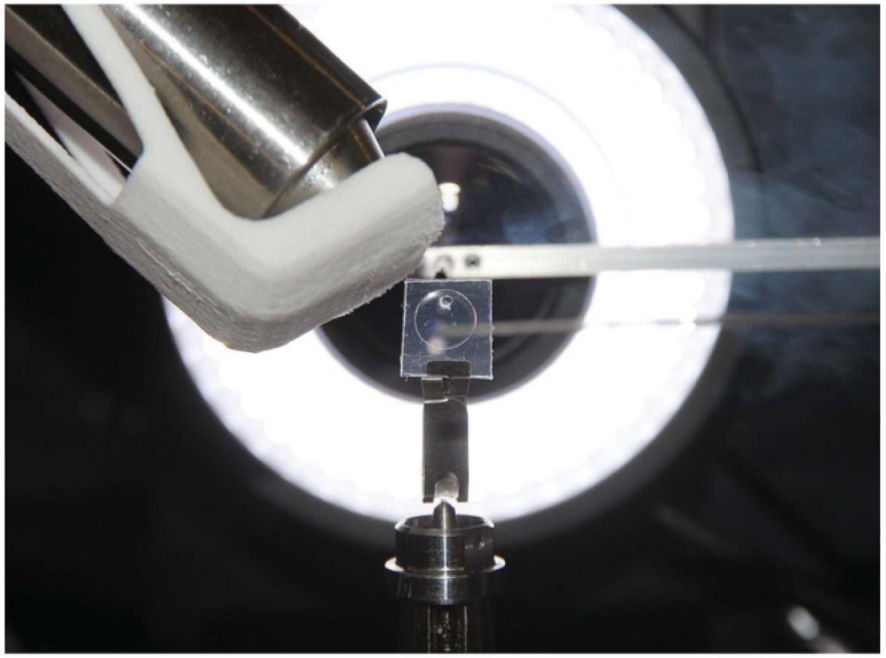
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In situ data collection at I24 – thin film

- to overcome the high background, samples can also be crystallized or mounted in thin film

- in thin film (mylar) or thin polymer-film sandwiches, data collection at cryo-temperature is possible

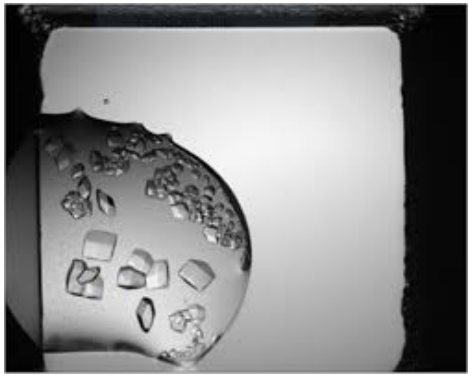
Axford et al., Acta Cryst. (2016). F72, 313–319



Why using multiple crystals?

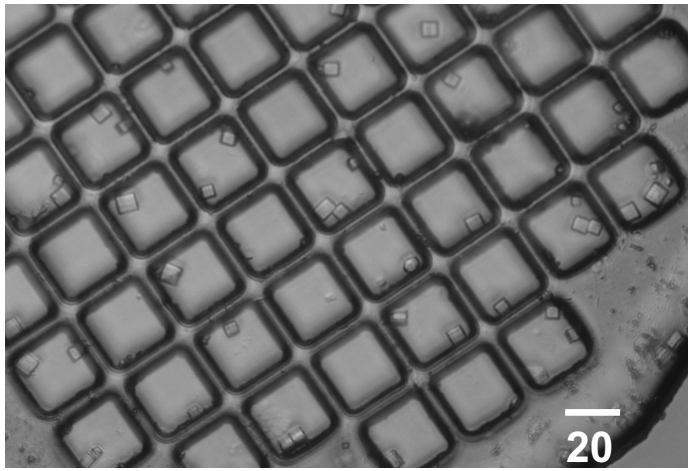
- radiation damage
  - room-temperature measurements
  - very rarely complete data sets from small crystals
  - damage-free structures

- crystals are too fragile or too small to be fished



Experimental requirements

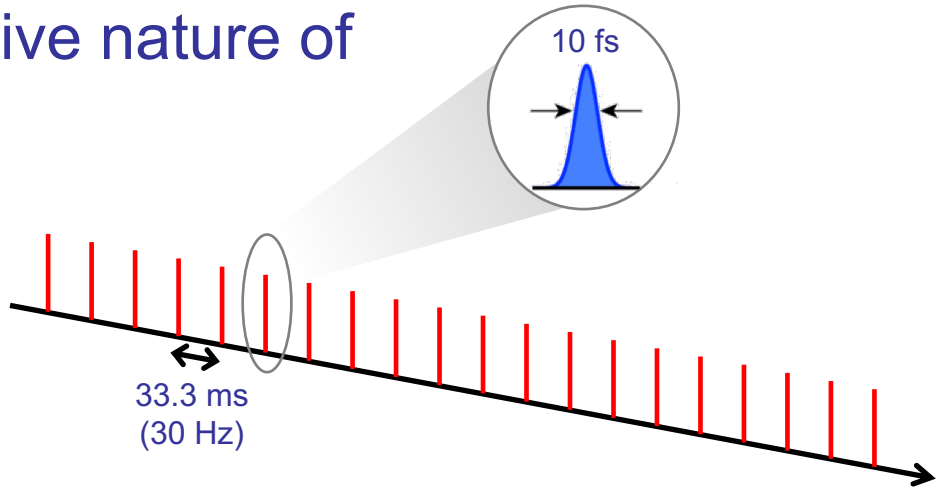
- the basics really matter:
  - good crystal mounting (very little surrounding liquid)
  - isomorphous crystals necessary
  - matching the beam to the crystal to increase signal to noise
  - adapting the rotation range to the sample holder

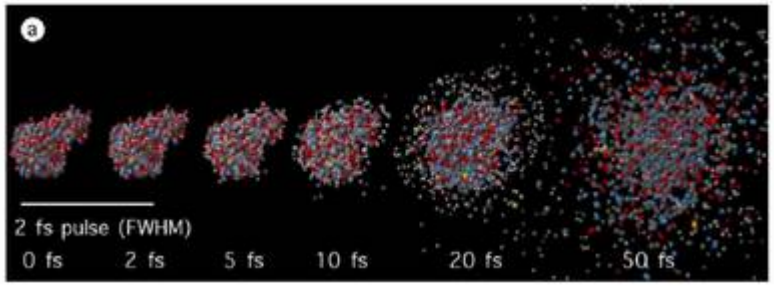


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The move to serial crystallography

- development of serial approaches has been driven by the destructive nature of XFEL
- a new sample must be presented to each pulse
- many pulses per second





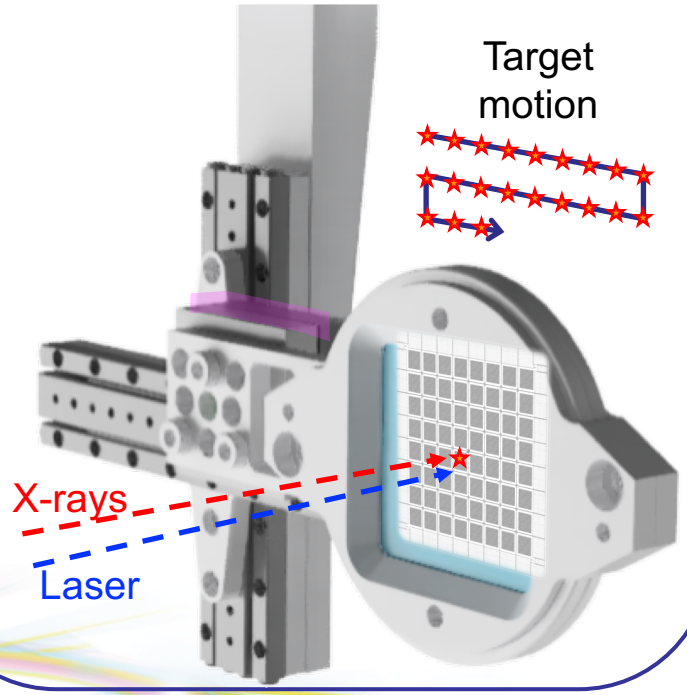
Neutze et al, Nature 406, 752 (2000)

- different methods of sample delivery
- synchrotrons for testing

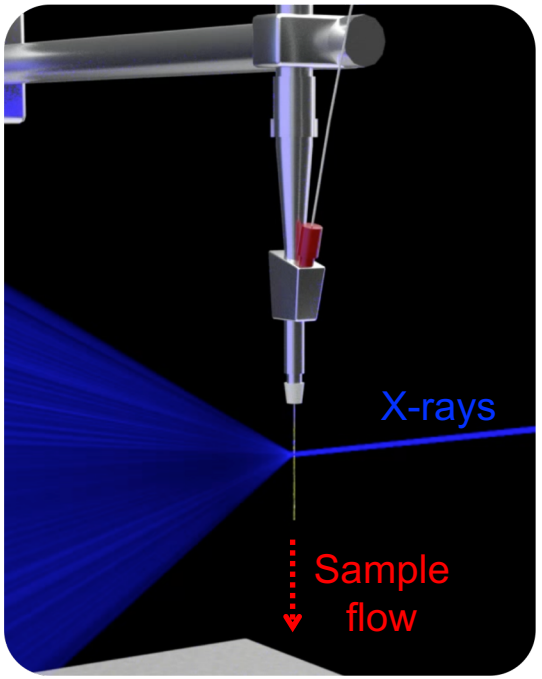
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Modes of sample delivery at I24

Fixed targets



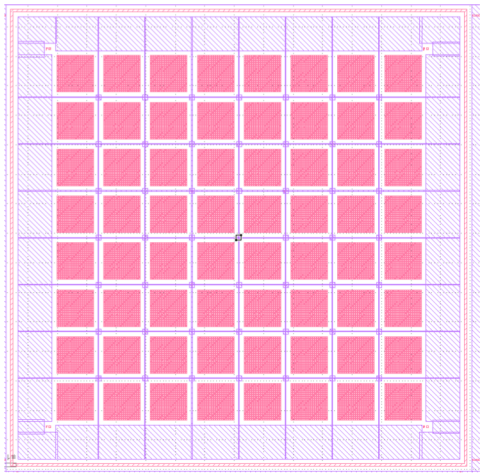
LCP extruder

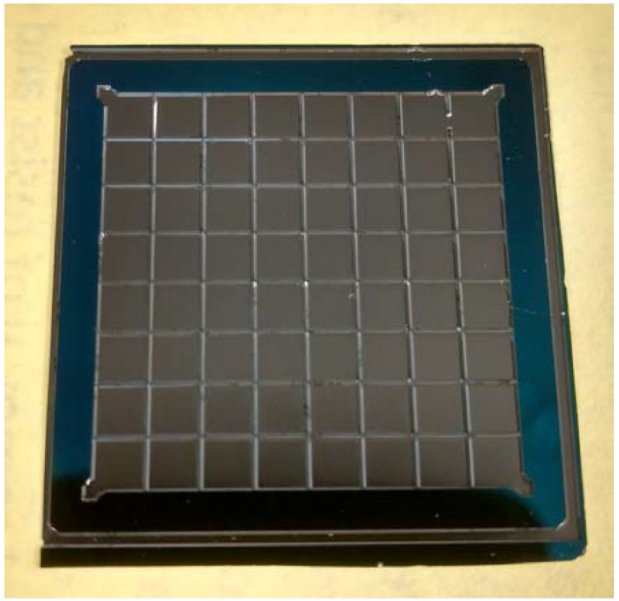


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‘New’ chips

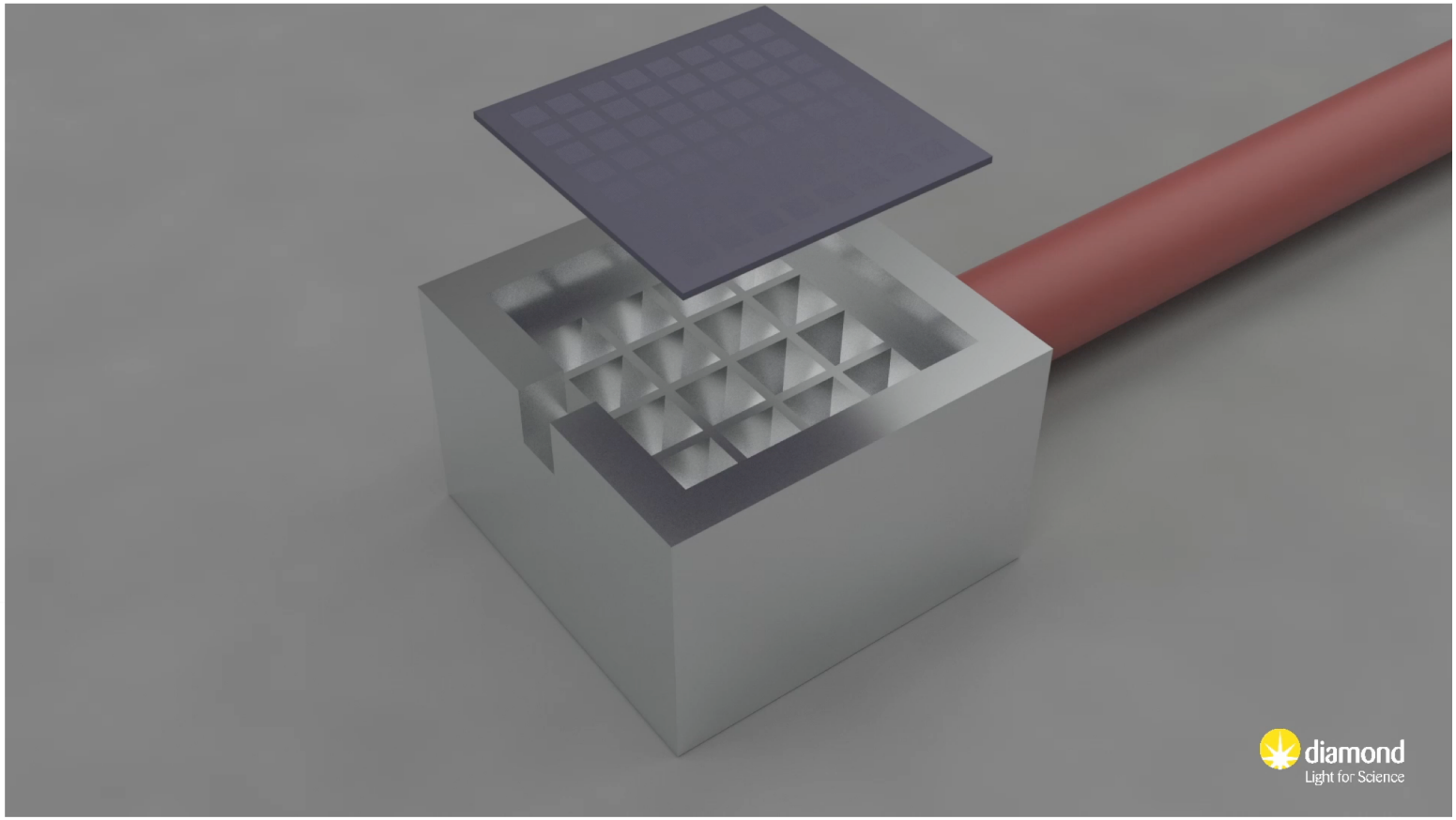
- developed by I24 and the Southampton Nanofabrication Centre
  - 20×20 apertures per city block; 8×8 city blocks
  - chip capacity = 25,600
  - 125 µm repeat distance, variable aperture size
  - chip approx. 30×30 mm^2
  - robust design
  - accurate: extremely well spatially defined





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Fixed targets: getting crystals onto chips

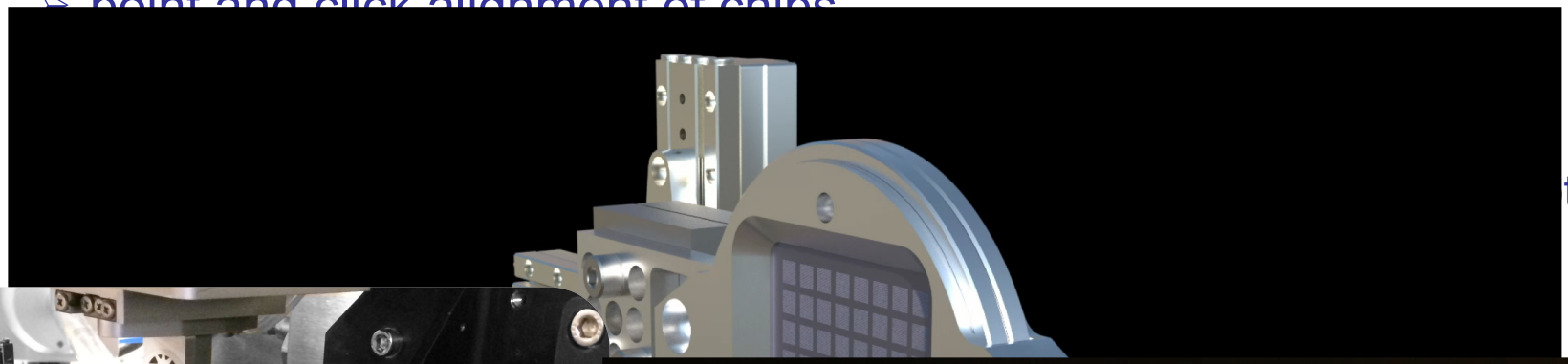


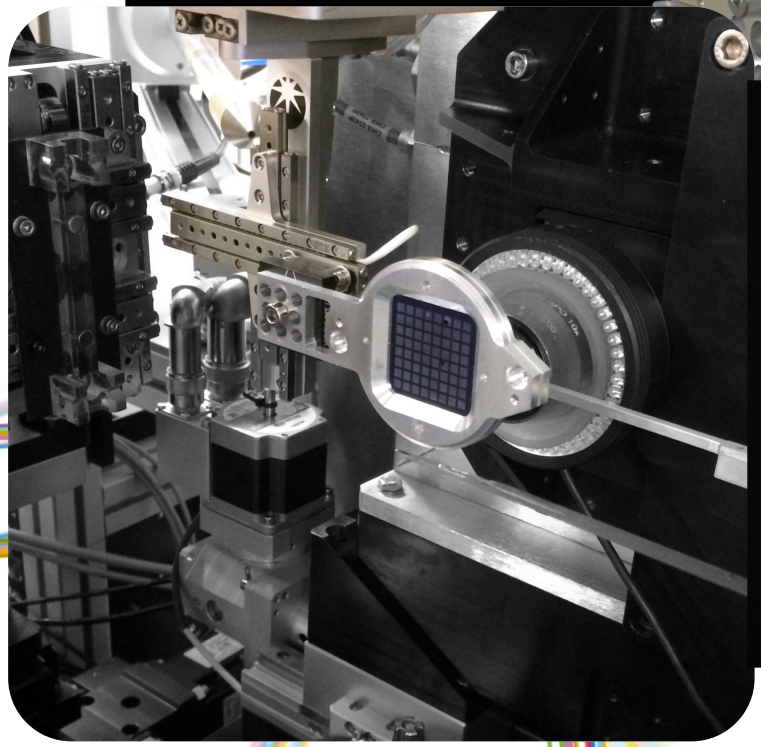
https://www.diamond.ac.uk/Instruments/Mx/I24/I24-serial.html

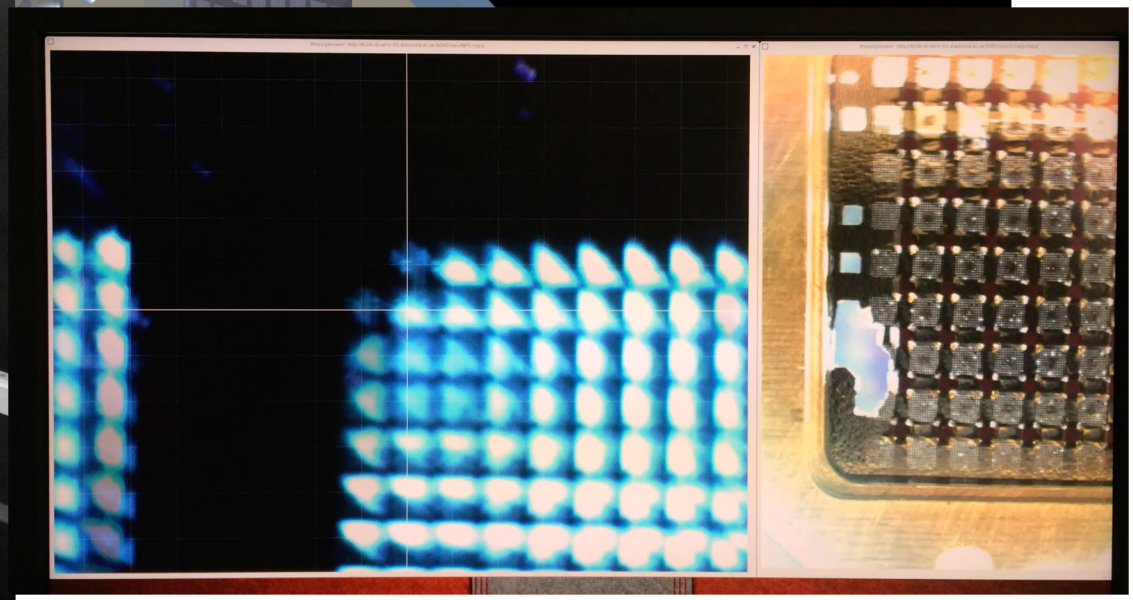
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In practice: fixed targets at I24

- fixed target data collection:
  - point and click alignment of chips



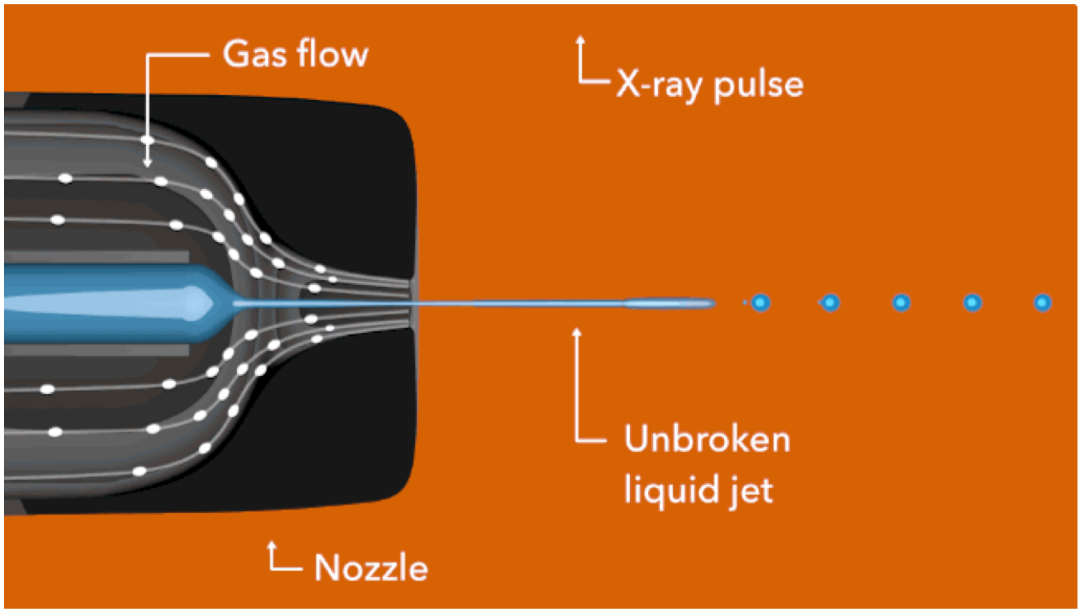




animations https://www.diamond.ac.uk/Instruments/Mx/I24/I24-serial.html

Liquid jets

Gas dynamic virtual nozzle (GDVN) liquid jet

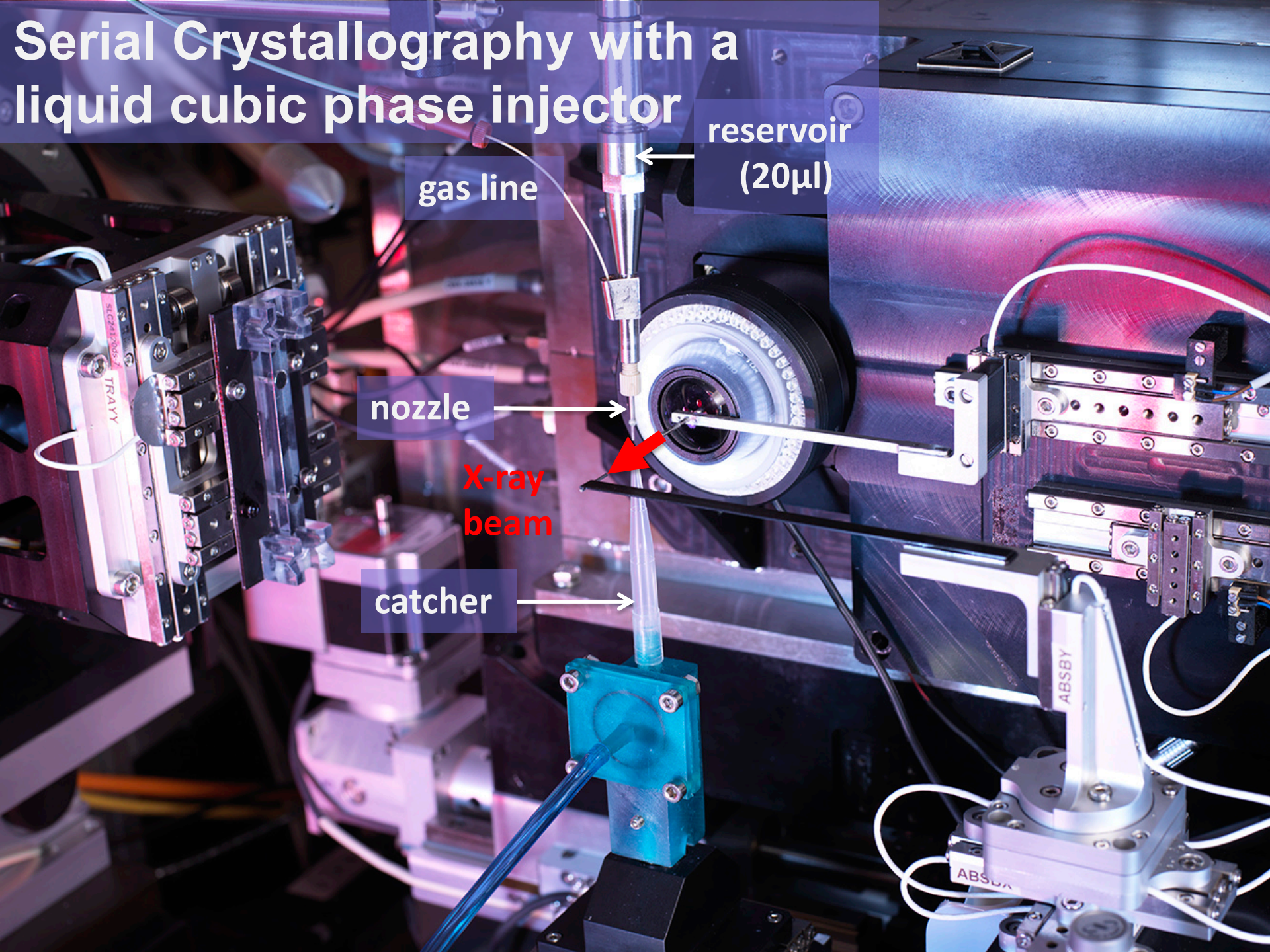


- air flow focuses liquid jet
- most common injector
- ~5 μm diameter stream reduces background noise
- prone to clogging
- large numbers of crystals do not see X-rays

Lipidic cubic phase injectors

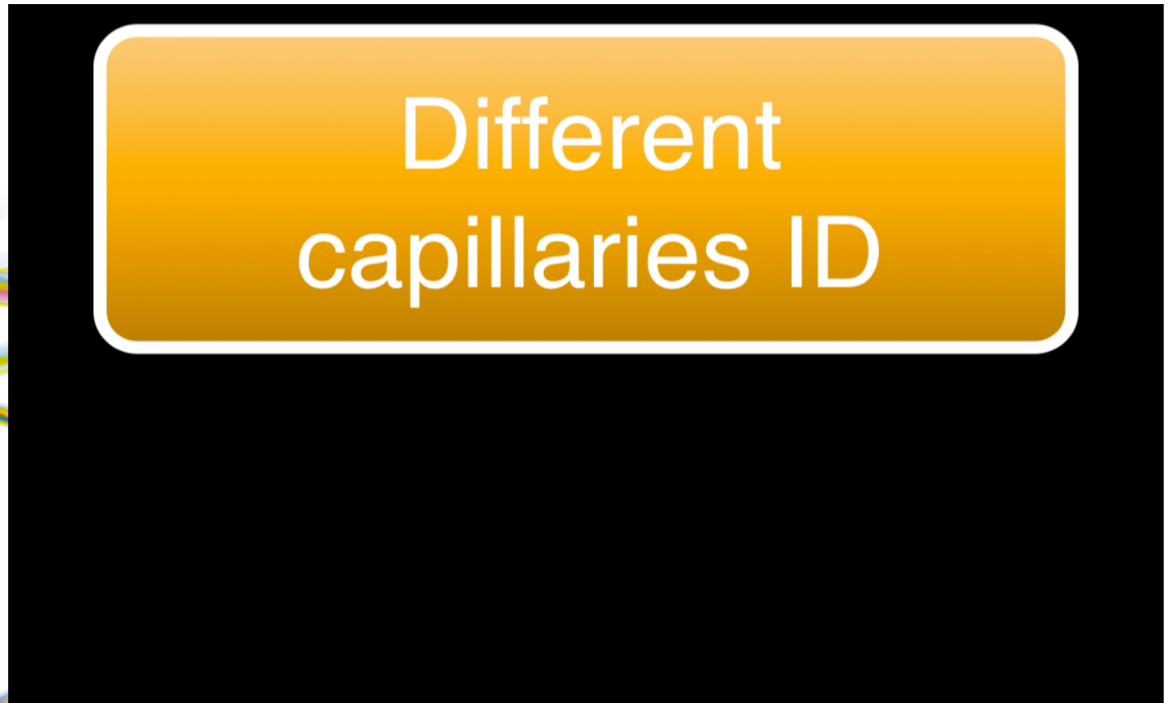
- similar principle. Makes use of extremely high viscosity liquid
- extruded at 2-3 mm s^{-1}: more efficient sample use

Orange animations
https://www6.slac.stanford.edu/news/2015-02-02-5-ways-put-tiny-targets-front-x-ray-laser.aspx


Serial Crystallography with a liquid cubic phase injector
reservoir (20µl)
gas line
nozzle
X-ray beam
catcher

Capillaries, flow rate, and sample consumption

- 100, 50 and 20 µm diameter capillaries used
- mostly ran using 50 µm capillary: 40 µL syringe lasts 3hrs
- flow rate rather variable (compare below), working to characterise optimal setup and parameters



In practice: I24 data collection…

Measure data from chip(s)

↓

dials.stills_process (data processing)

↓

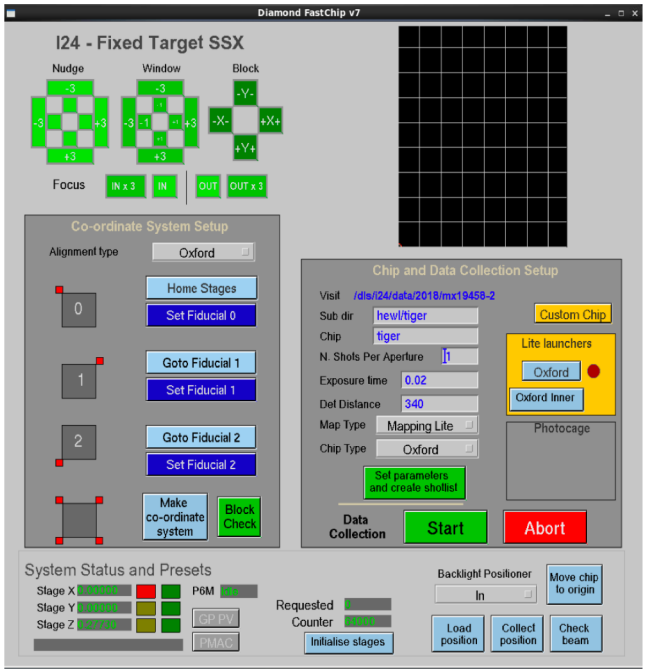
Prime (scaling & merging)

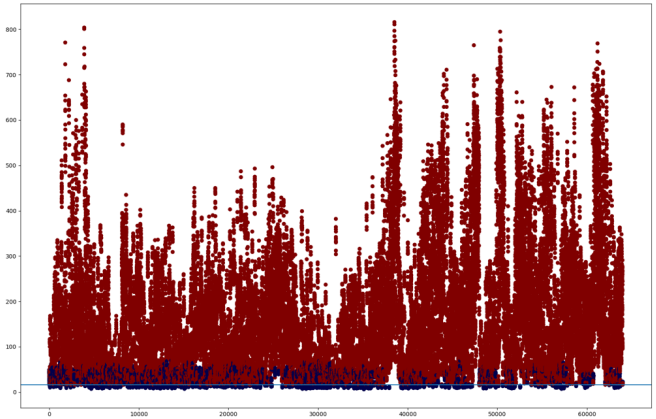
↓

Examine maps (CCP4,Phenix)

↓

Good enough data?



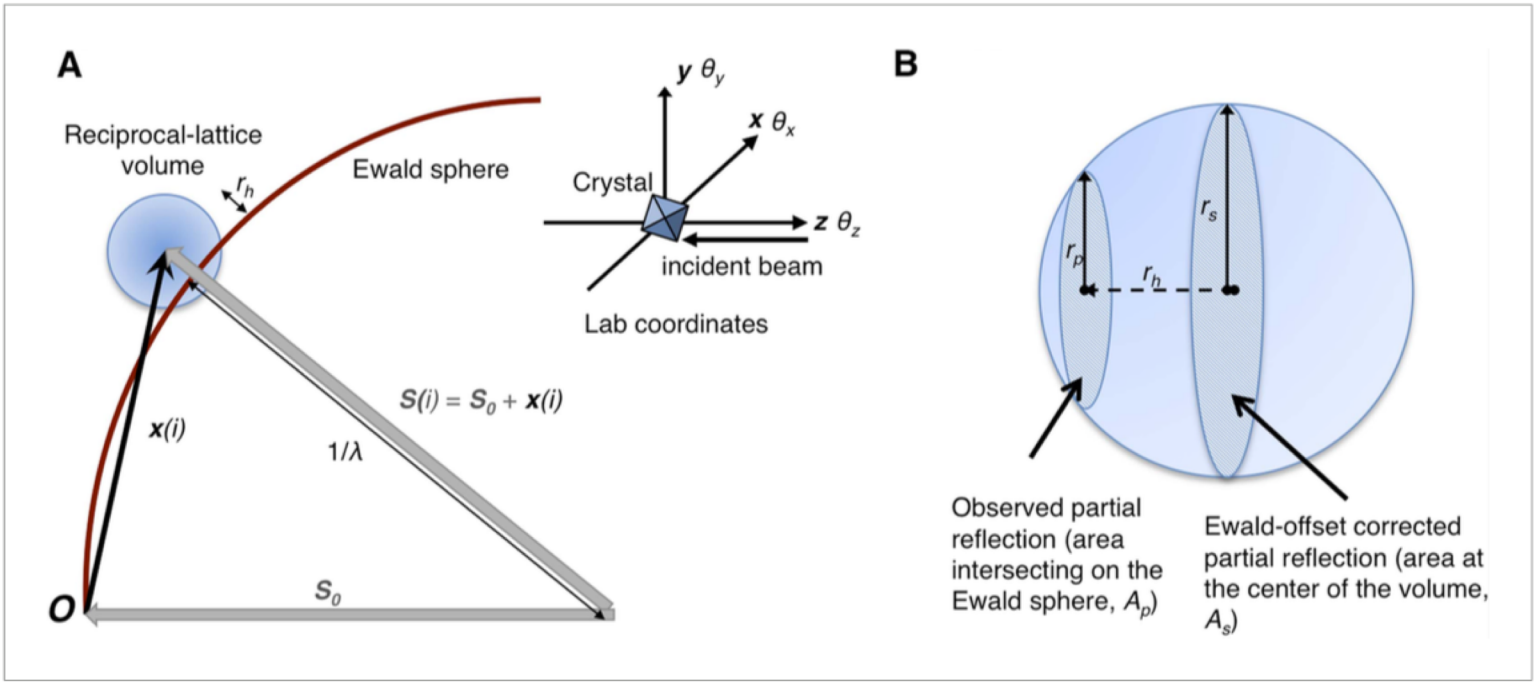


- simple GUI based data collection
- real time hit rate monitoring (above)

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… and analysis of ‘still’ data

- only partially recorded reflections from randomly oriented crystals



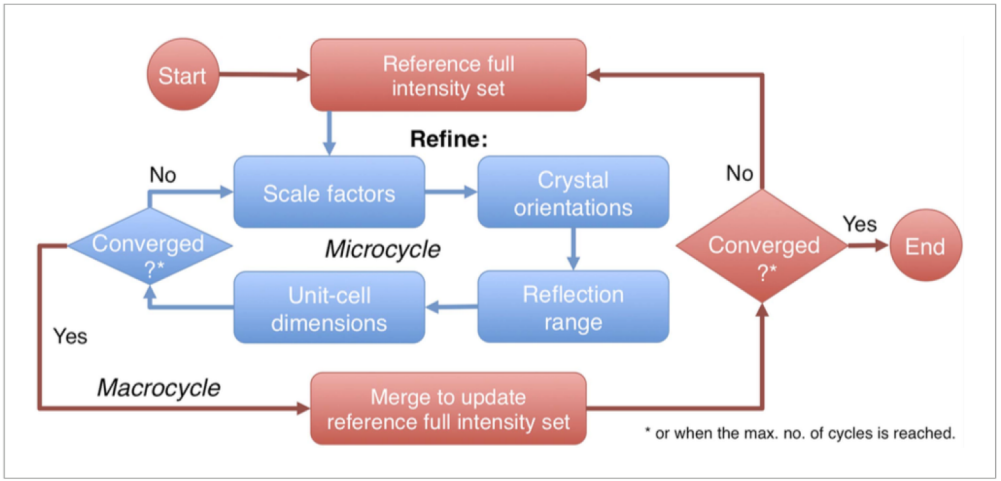
Uervirojnangkoorn et al. eLife: e05421 (2015)

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Reconstructing the reflection intensities

- option 1: use the ‘Monte-Carlo method’ in which thousands of partial intensity observations of a given reflection are summed and normalized by the number of observations (CrystFEL)

- option 2: process the images one by one (dials.stills) and apply extensive post-refinement (prime = post-refinement and merging) -> less images required

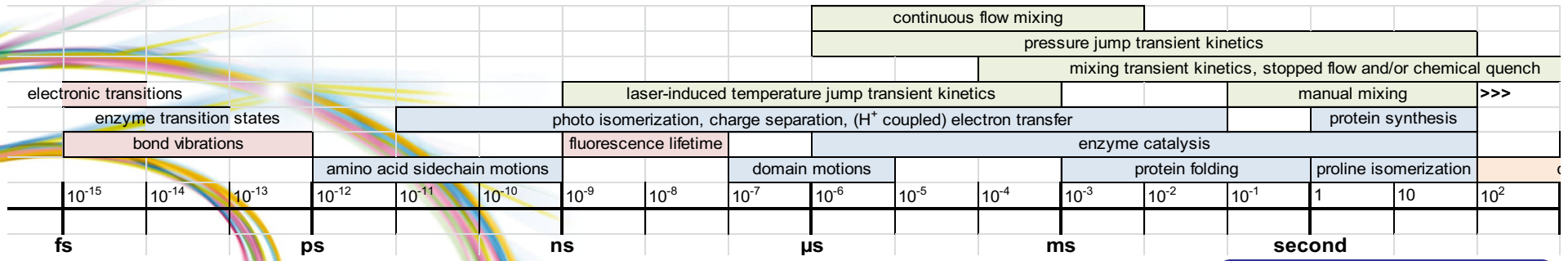


Uervirojnangkoorn et al. eLife: e05421 (2015)

Future possibilities - moving into the time domain

- serial approaches really open the door to time resolved experiments on non-reversible reactions at synchrotrons

- can't reach fastest timescales – this is really where XFEL and SR complement each other - but a lot of interesting changes are in microsecond+ region
  - How do we minimise the contribution of radiation damage?
  - What about non light triggered/non reversible reactions?

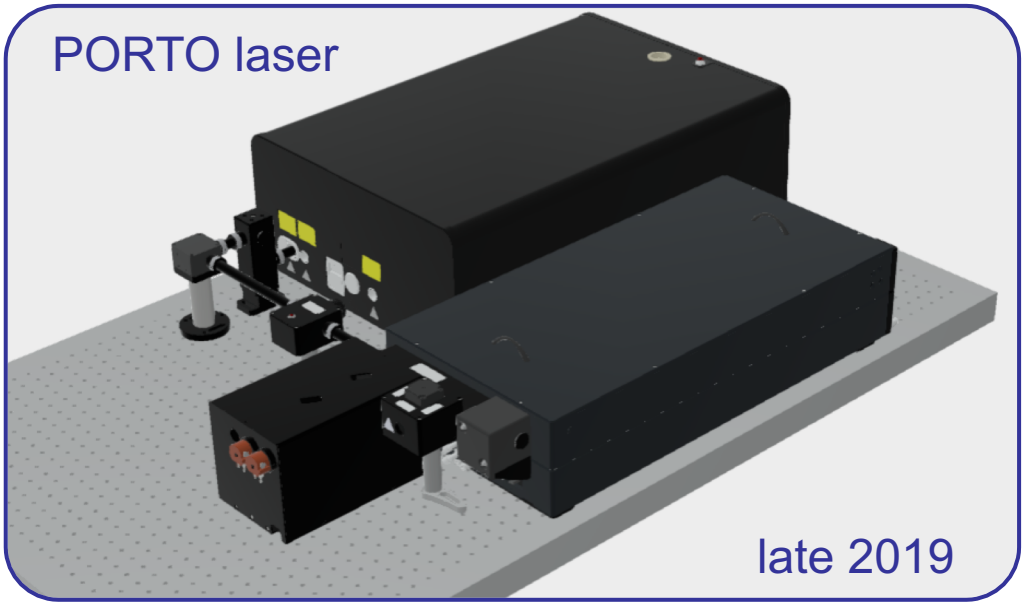


kinetic-crystallography, freeze-trapping

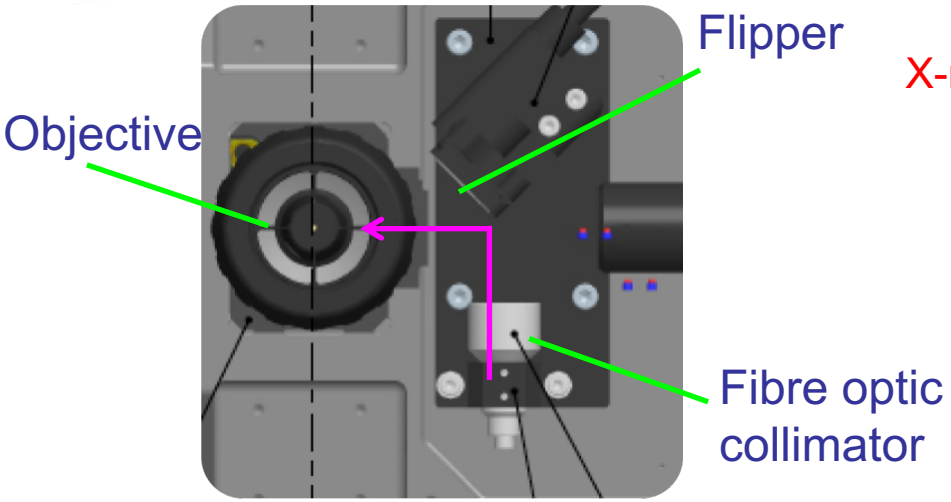
??? ⟵

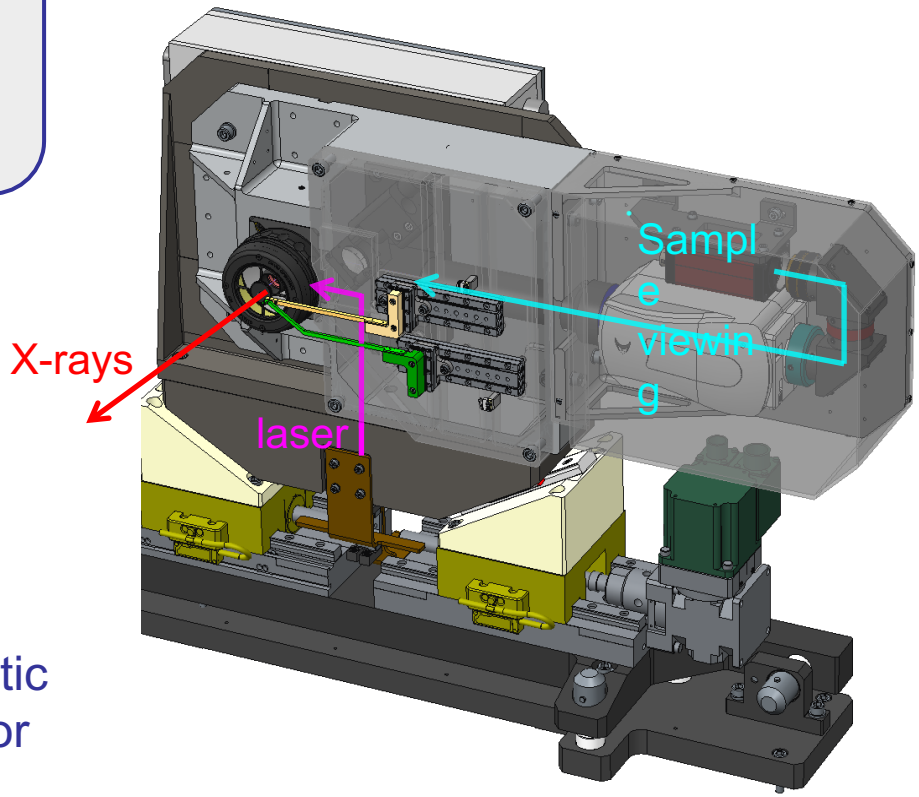
Serial at I24

Future possibilities: photoexcitation

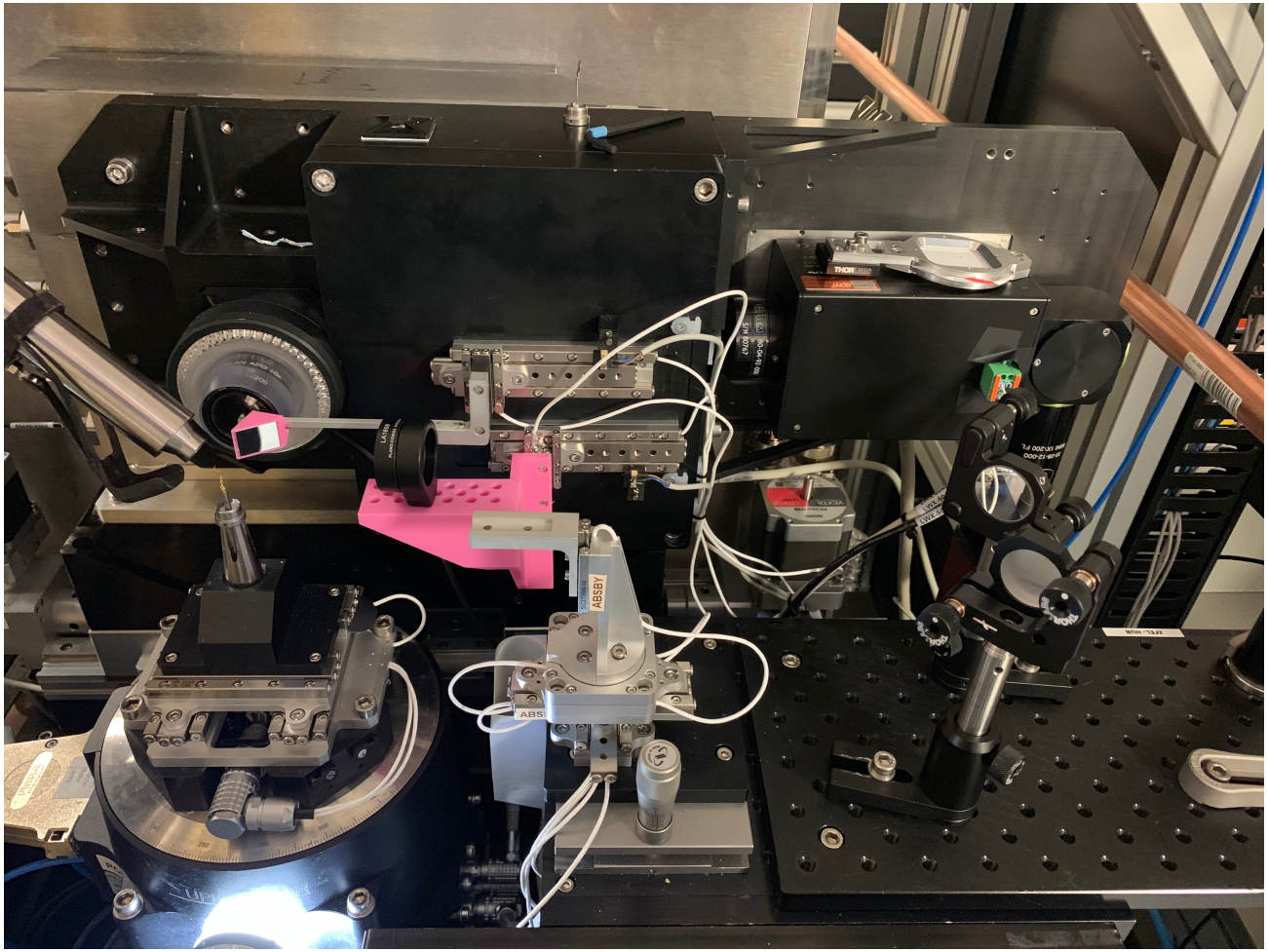


- portable Pharos laser system for Diamond
  - tuneable 210 nm - 2500 nm
  - pulse <250 fs (can be broadened)



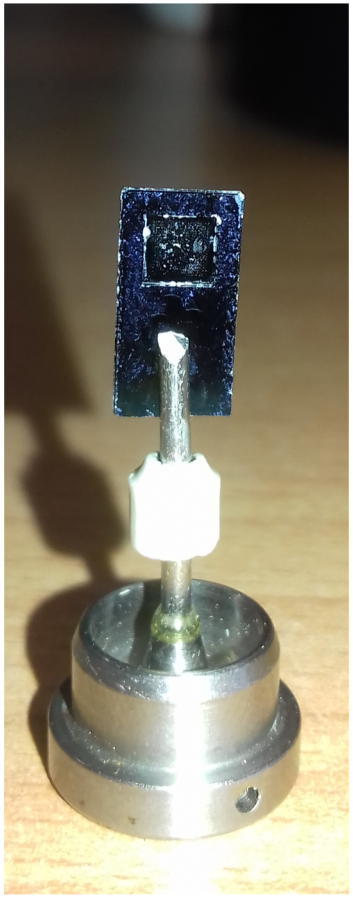


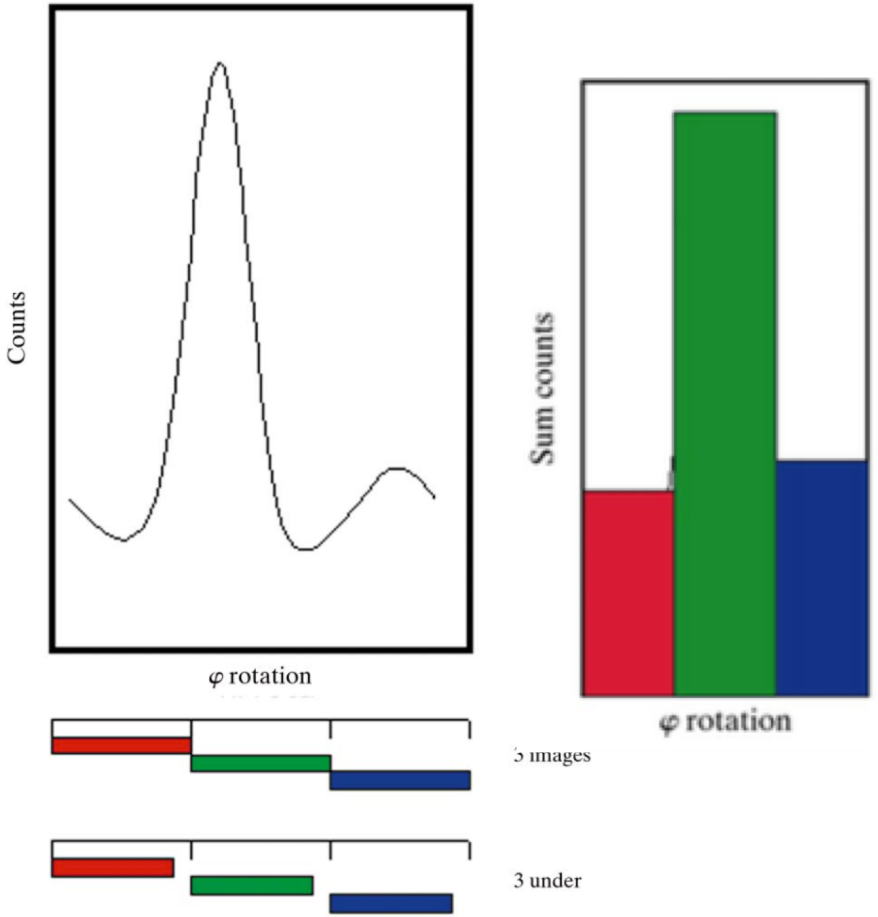
Future possibilities: photoexcitation



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Future possibilities: rotational serial crystallography





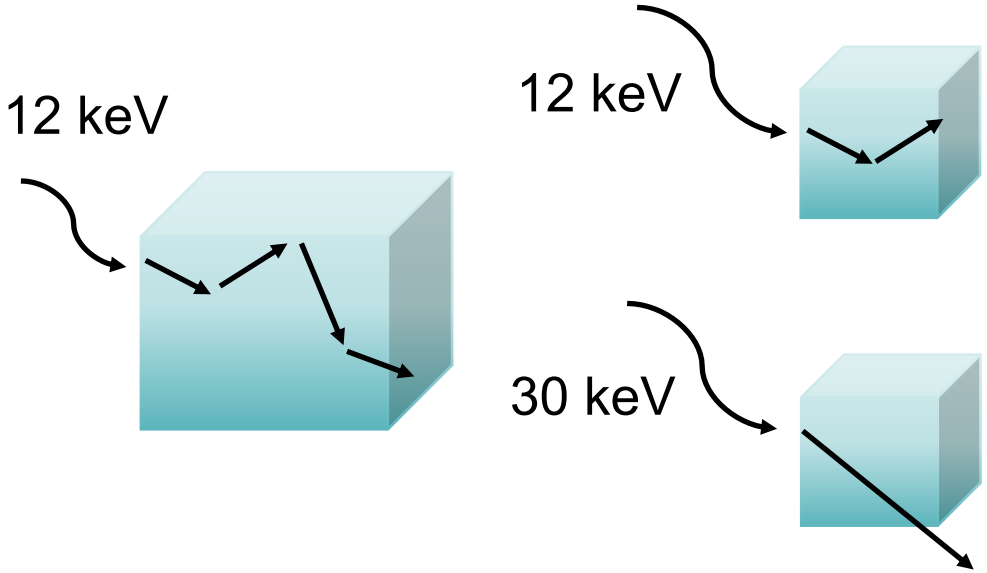
Pflugrath, Acta Cryst, D55, 1718-1725 (1999)

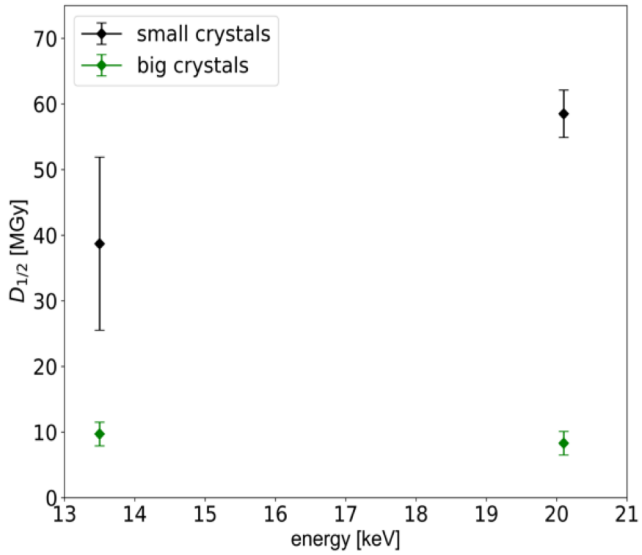
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Future possibilities: CdTe Eiger

- can run at up to 238 Hz, 3 us readout time
- optimised for high energies
- enables us to exploit photoelectron escape







Storm et al., IUCrJ (2020)

Acknowledgements

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Thank you for your attention!



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