Serial Femtosecond Crystallography on tiny 3D crystals

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Introduction

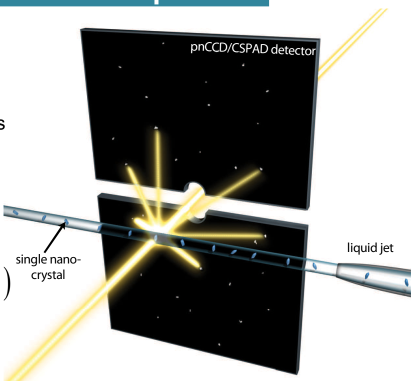
The elucidation of structures of macromolecules is an important step in the quest of understanding the chemical mechanisms underlying biological function. X-ray crystallography is a mature method that is only limited by the quality of the crystals investigated and by radiation damage. Intense, femtosecond X-ray pulses provided by X-ray free-electron lasers promise to break the nexus between radiation damage and crystal size, thereby allowing structure determination using nano- and microcrystals. Recent serial femtosecond crystallography (SFX) experiments at the LCLS have shown the feasibility of this approach[1]. A continuous liquid microjet was used to inject randomly oriented crystals into the FEL beam[2,3]. The diffraction patterns were collected in vacuum at the repetition rate of the FEL in the CAMP[4] or CXI instruments[5] using pnCCD or CSPAD detectors, respectively.

Due to the mismatch between continuous sample flow and stroboscopic data collection, sample consumption is huge. FEL-triggered drop-on-demand approaches have been proposed and are being explored[3]. For very precious samples, other possibilities need to be explored which include preparation on fixed targets and cryo-stages, which are ideally integrated into dedicated endstations with appropriate detectors. Since the crystals intersect the FEL beam very fleetingly, only thin slices through the rocking curve are recorded, requiring many measurements of the reflections to allow a Monte-Carlo like integration of the beam profiles[6,7]. A pink or Laue beam has not only more flux than a monochromatic beam but is also more efficient in sampling reciprocal space. A shot-to-shot analysis of the spectrum would be highly desirable, for example to allow accurate profile fitting including coherent diffraction features as would be the availability of a divergent beam that can be matched to the sample size.

Typical Experimental Setup

Data collection

Detectors:
pnCCD[4]: four 512 x 1024 panels
75 µm pixel size
CSPAD: 1520 x 1520 pixels
110 µm pixel size



Interaction volume:

$$d_{\text{beam}} \times d_{\text{jet}} \times \min\left(d_{\text{beam}}, d_{\text{jet}}\right)$$

Typically 1 x 1 x 5 µm³

LCLS pulse parameters:
λ =2-1.3 Å, 6-9.4 keV
5-40 fs pulse duration (e-beam)
120 Hz
~ 3 mJ/pulse
$10^{11} – 10^{12}$ photons/pulse

Gas focused liquid jet[2]:
~ 5 µm diam., flow rate ~10 µl/min, ~10 m/s
Hit rate = concentration x interaction volume
Effective hit rate smaller (multiple hits, hit finding issues, indexing efficiency)

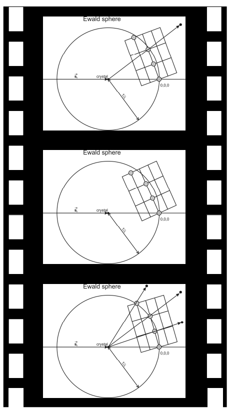
Liquid jet sample consumption

$$\text{sample consumption} = \frac{\text{patterns needed} \times \text{flow rate}}{\text{hit rate}_{\text{eff}} \times \text{rep rate}}$$ ➔Typically > 1ml of 10^9 ml^{-1}

$$\text{sample efficiency} = \frac{\text{interaction volume} \times \text{rep rate}}{\text{flow rate}}$$ ➔Typically 10^{-6}

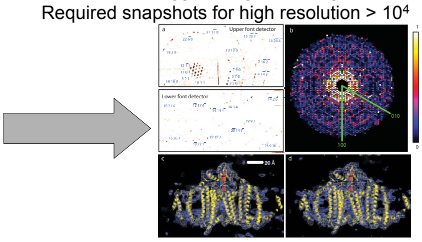
➔Alternative sample delivery schemes needed!

Data collection and analysis



Problems:
-Different sizes of crystals
-Random orientations
-Thin slices through Ewald sphere
-Shape-transform effects

➔Monte-Carlo-type integration is possible[6]:
Required snapshots for high resolution > 10^4

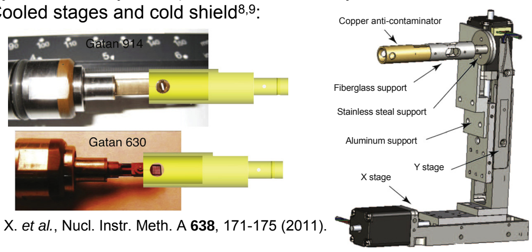


Chapman, H. N. *et al.*, *Nature* **470**, 73-77 (2011).

Reducing Sample Consumption

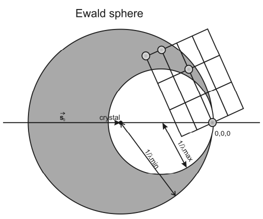
Fixed cryo targets

-Crystals on wafer in random orientations
-Synchronized fly scan (translation ~ 10 x crystal/beam size between pulses)
-Cooled stages and cold shield[8,9]:



Huang, X. *et al.*, Nucl. Instr. Meth. A **638**, 171-175 (2011).

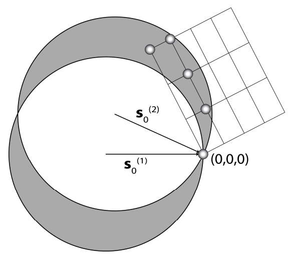
Pink-Laue SFX



-1-3% bandwidth
-Better data collection efficiency
-Better SNR
➔reduced sampling requirements
➔Need to know spectrum for each shot

Split beam

-Cover 0.1° to 1°
-Better data collection efficiency
-Better SNR
➔reduced sampling requirements
➔Need to know intensity for each beam



Suggested Beam Parameters

Parameter		Unit	Requirement	Motivation / Remarks
Beam parameters				
Energy		keV	6 - 13 keV	SFX, MAD-SFX
	stability		0.1%	energy measured/shot
Bandwidth		%	0.1% ,1-3%	conventional SFX, pink-Laue SFX
	stability		< ± 10% bw	record spectrum for each shot
Beam position	stability		< 1 µm	to keep alignment
Beam size		µm	adjustable 0.2 - 10/20	adjustable to size of nano- / microcrystal batch
Photons per pulse		#ph	»10^{10}	maximum fluence
	stability		---	not an issue but record for each shot
Pulse length (e-beam?)		fs	< 20	diffract-then-destroy
	stability			record for each shot
Pulse arrival time	stability	fs		N/A
Beam parameter changes during experiment				
Energy	range/step	eV	6 - 13 keV	adjust cross-section/resolution for each sample
	rate	eV/min	200	minimize downtime
Beam size (microfocus only)	range/step	µm	0.2 - 10/20	Steps of 1 µm, adjust for each sample
	rate	µm/min	2	10 minutes for sample change
Pulse length	range/step	fs	---	
	rate		---	
Beam geometry				
Beam slope	max. tolerable	µrad	100	not noticeable shift on detector
Working distance	min. required	mm	50	max. diffraction angle ~ 60°
Other				
Transverse coherence			full	Required for coherent diffraction at extended object

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