



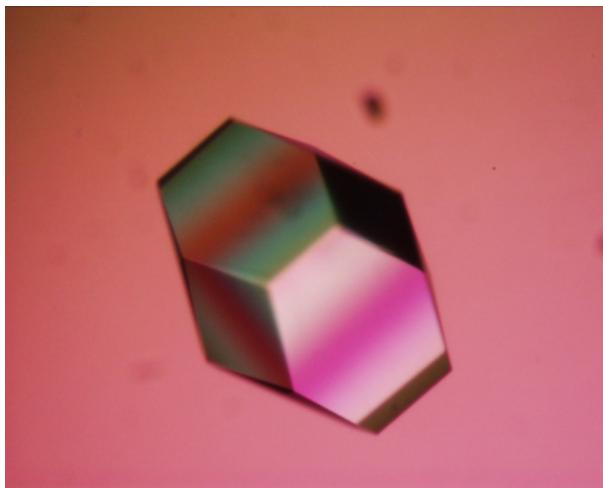
Kay Diederichs

# Serial Crystallography at the Synchrotron

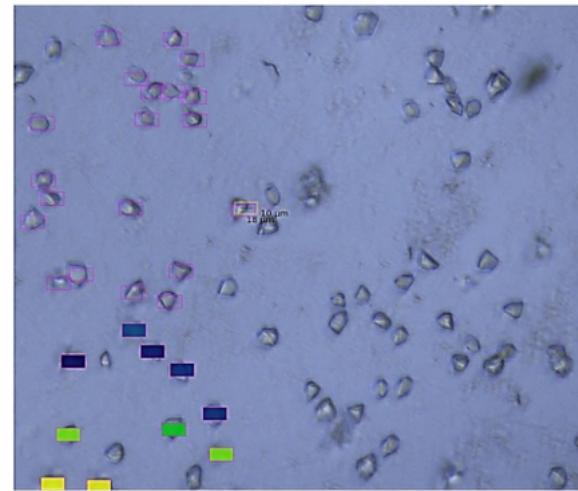


# Conventional vs. Serial Crystallography

**CX**



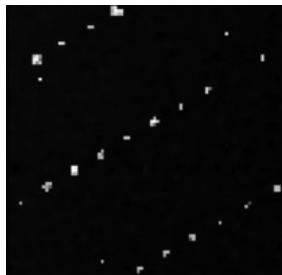
**SX**



- One or a few crystals
- $\sim 50 \times 50 \times 50 \mu\text{m}^3$
- Complete data set
- Cryogenic temperature
- Rotation data processing

- 10 – 10000 crystals
- $\sim 5 \times 5 \times 5 \mu\text{m}^3$
- Small wedges or stills
- Cryogenic and room temperature
- Data sets selection

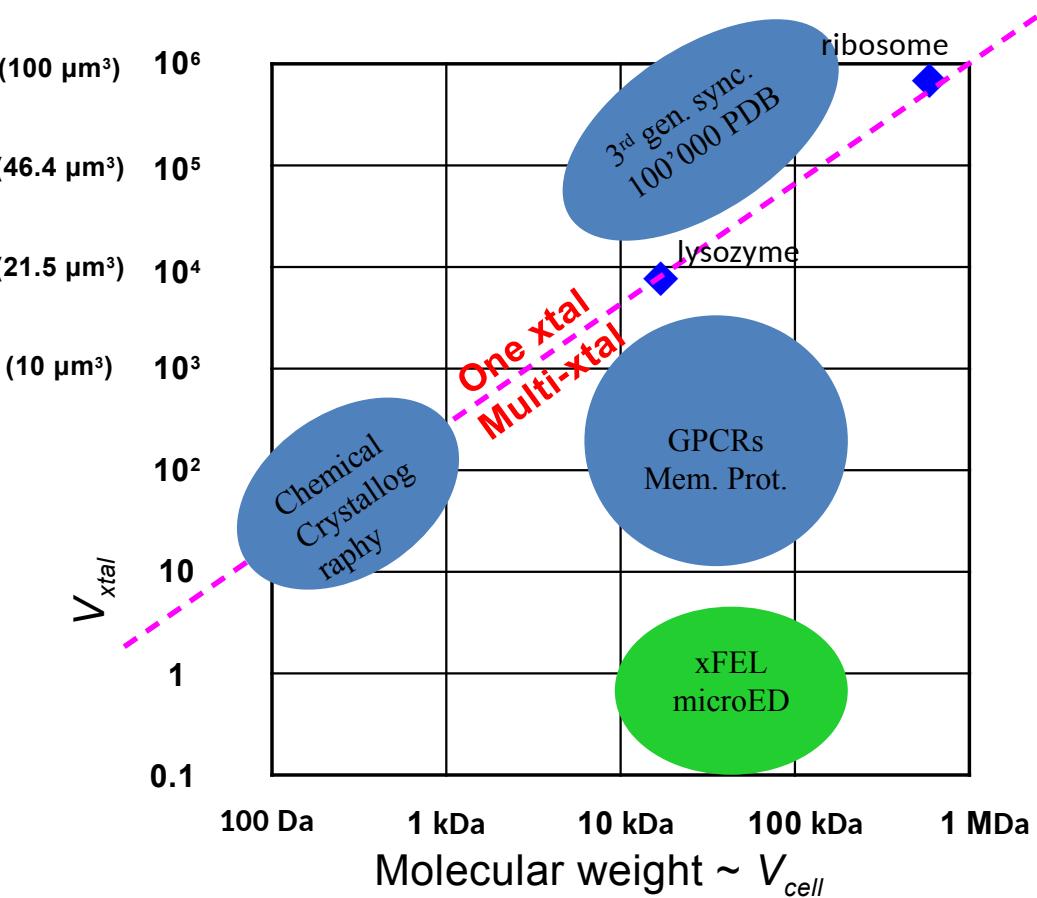
# Why Serial Crystallography?



$$I_{hkl} \propto I_0 \frac{V_{xtal}}{V_{cell}}$$

## Signal-to-noise ratio

- Diffraction volume
- Radiation damage
- Background scattering



2016 PDB  
10'000 total  
672 membrane proteins

# Challenges in SX

## Challenge

Micro-crystal, weak diffraction

Crystal delivery

Crystal screening and data collection

Data analysis

## Method

Variable focusing of X-rays to match crystal size and reduce background scattering

LCP injector and *in situ*

Fast and automated grid scan (rastering) and serial data collection

**Selection of data sets (how?)**

# Micro-focusing, variable beam size, EIGER detector

One micron beam



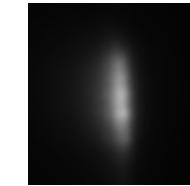
$2 \times 1 \mu\text{m}^2$



$10 \times 2 \mu\text{m}^2$



$10 \times 12 \mu\text{m}^2$



$2 \times 12 \mu\text{m}^2$

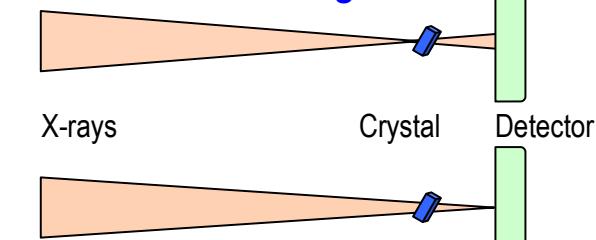
$2 \times 10^{11} \text{ p/s}$

$1 \times 10^{12} \text{ p/s}$

$2 \times 10^{12} \text{ p/s}$

$2 \times 10^{11} \text{ p/s}$

Low divergence



X-ray focus on sample vs. on detector

Source ( $\mu\text{m}$ ,  
 $\mu\text{rad}$ )  
H:  $200 \times 140$   
V:  $23 \times 25$

Beamline X06SA at SLS

Primary  
focusing

Movable  
secondary  
source

Sample  
Secondary  
focusing



EIGER 16M

Standard micro-focusing



$5 \times 5 \mu\text{m}^2$



$10 \times 5 \mu\text{m}^2$



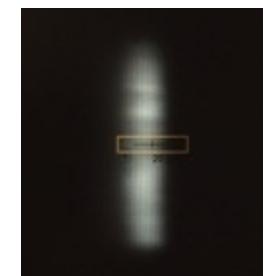
$20 \times 5 \mu\text{m}^2$



$40 \times 10 \mu\text{m}^2$



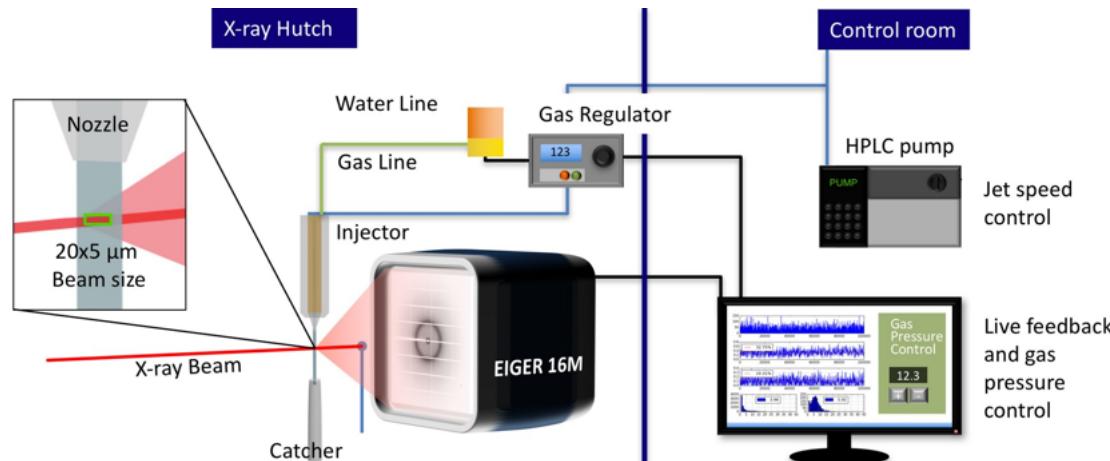
$40 \times 60 \mu\text{m}^2$



$10 \times 60 \mu\text{m}^2$

# Sample delivery: LCP injector at synchrotron

## Experimental Setup



## Features

Optimal hit rate  
versus signal-to-noise

Multiple patterns per crystal with  
high frame rate

Simple setup

Setup at beamline X06SA  
with an Arizona injector

- + Container free
- + Low X-ray background scattering
- + Continuous operation
- + Room temp. structure (pump-probe)

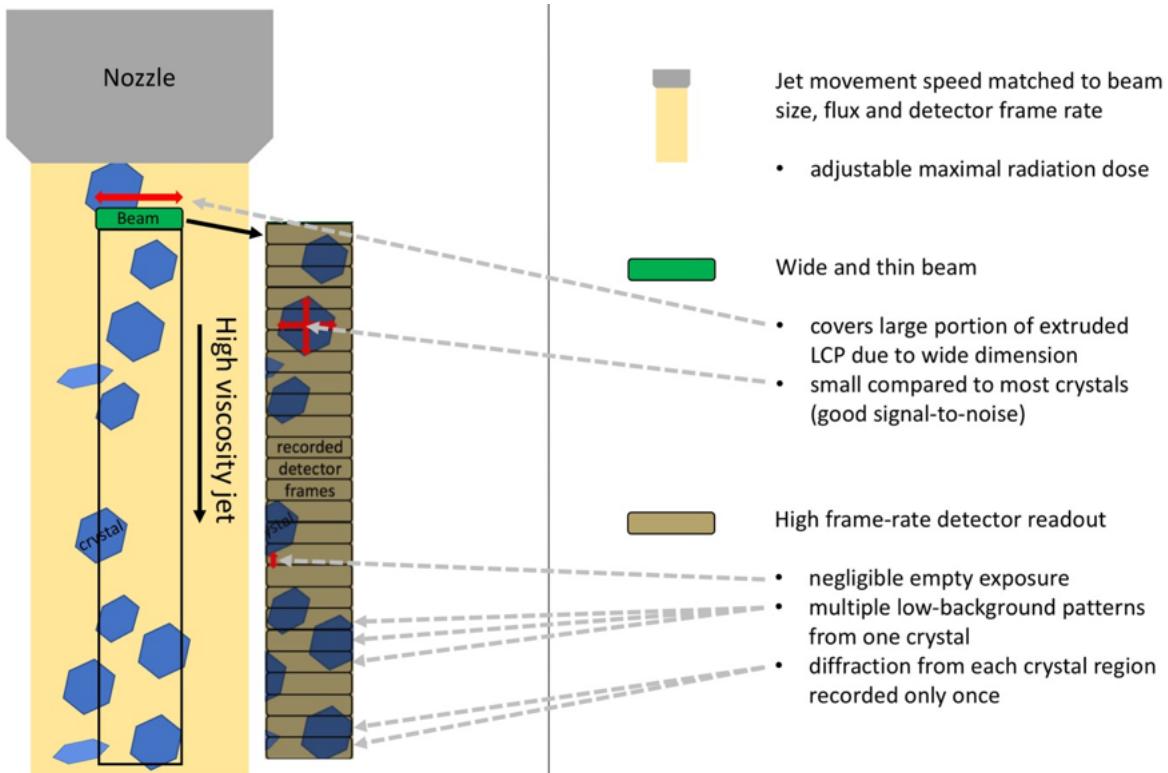
- Limited control of crystal flow
- Radiation damage at RT (100 kGy)
- Still diffraction images
- Data analysis

S. Botha, *et al.* *Acta Cryst. D* **71**, 387 (2015)

P. Nogly, *et al.* *IUCrJ*, **2**, 168 (2015)

T. Weinert and J. Standfuss

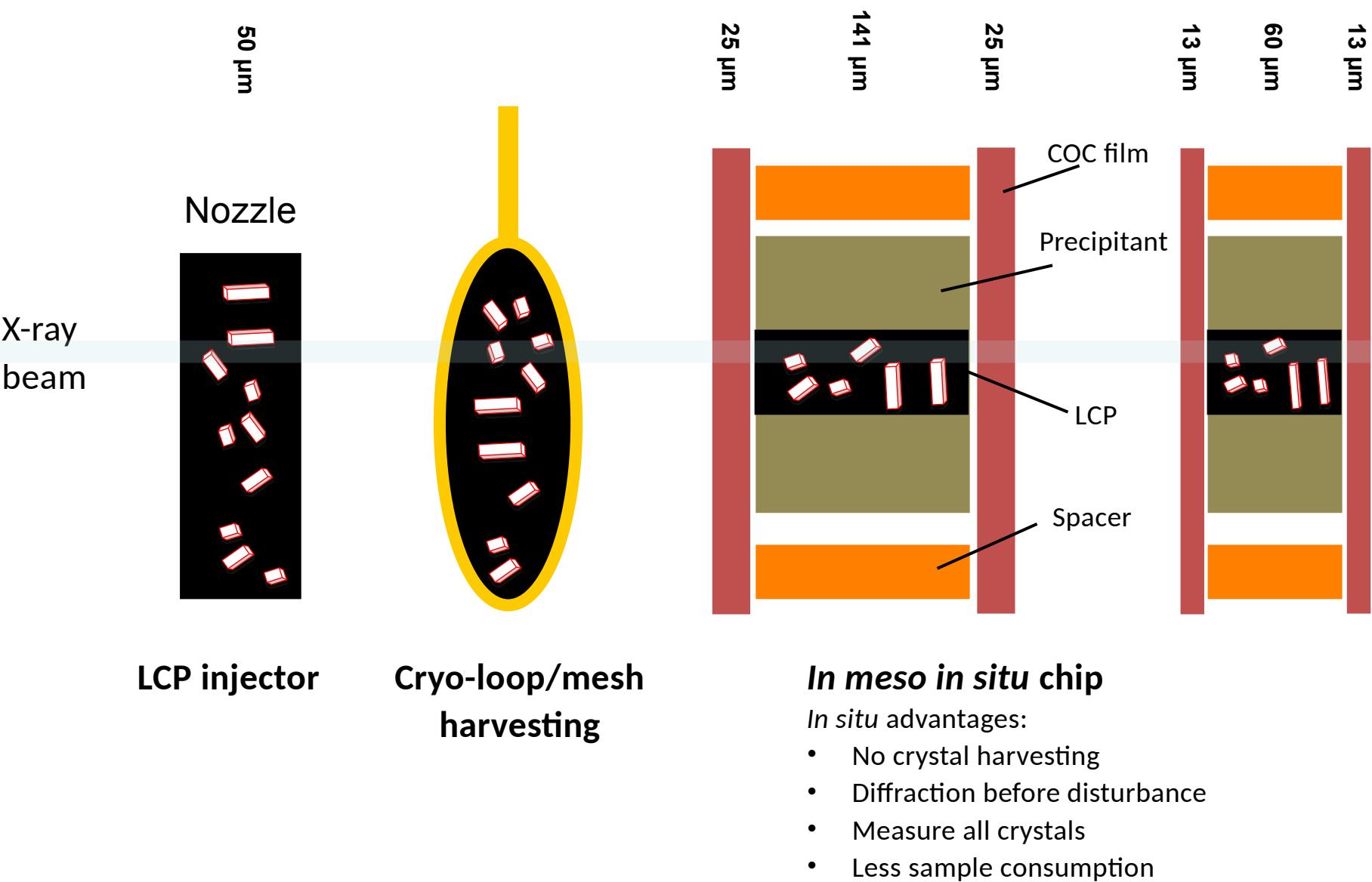
# Injector method: Crystal scanning SX



Optimize signal to noise in SX

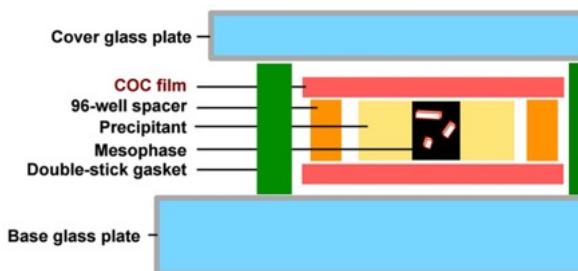
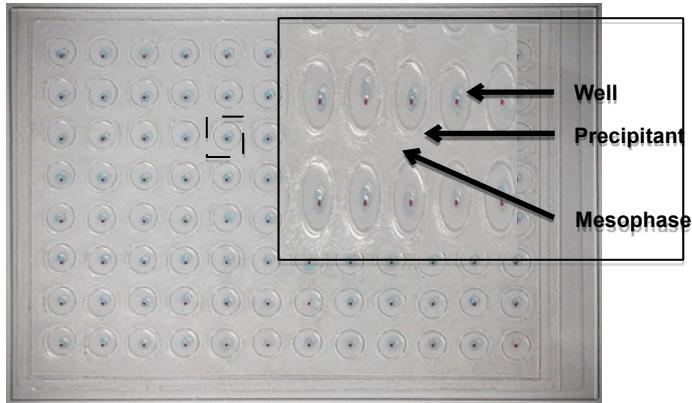
Tubulin
$P2_1$
74.0, 91.4, 83.6 Å, 96.8°
$15 \times 10 \times 10 \mu\text{m}$
1 Å, $7 \times 10^{11} \text{ ph/s}$
$10 \times 5 \mu\text{m}$ beam
30 kGy / crystal
50 Hz
1,388,078 images
CrystFEL analysis
66.271 indexed (4.8%)
36.1 – 2.13 Å
100% complete
$CC_{1/2}$ 0.99 (0.19)
$R_{\text{free}}$ 23.4%

# From injector to fixed-target, and *in situ*



# *In meso in situ* method: Crystallization

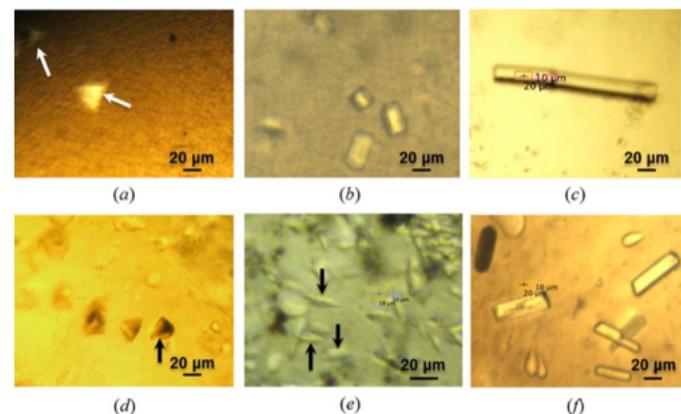
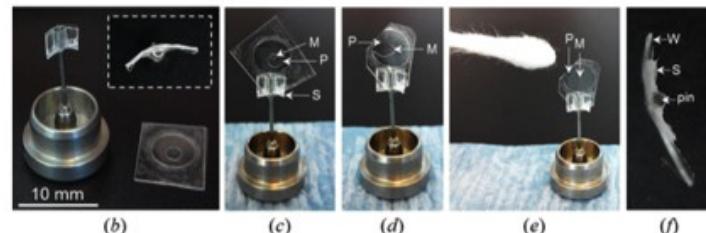
Membrane protein crystallization  
in lipid cubic phase (*in meso*)



IMISX plate: double sandwich plate with  
Cyclic Olefin Copolymer film (COC) and glass

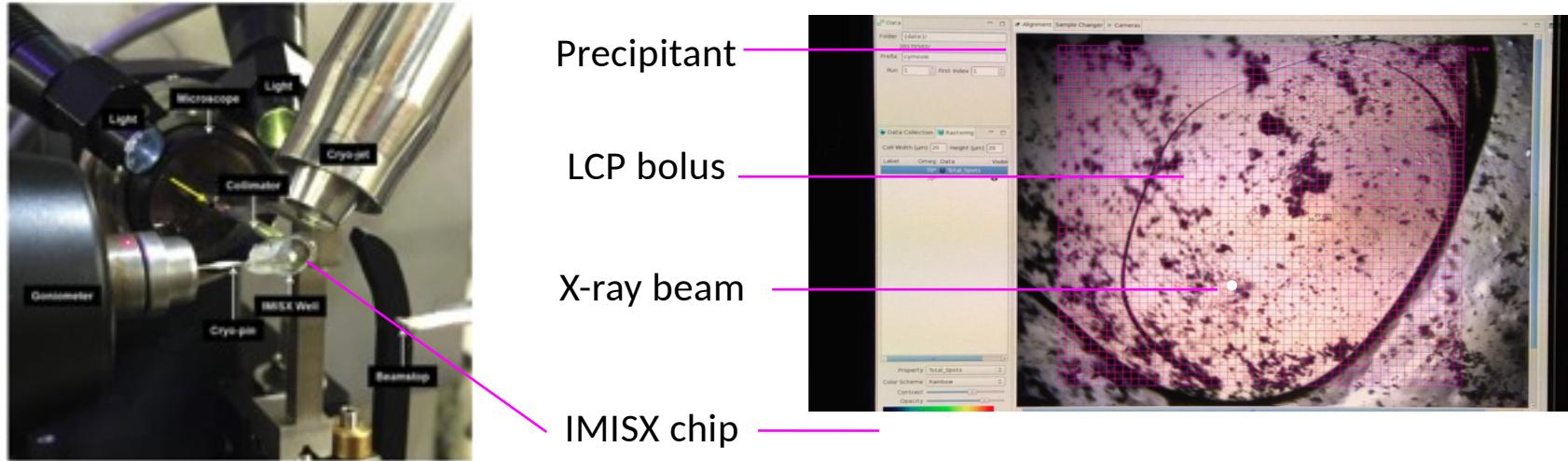
IMISX method, C.-Y. Huang, *et al.* *Acta Cryst.*  
**D71**, 1238 (2015); **D72**, 93 (2016)

IMISX chip mounting  
Cryo-freezing



Crystals viewed through an on-axis  
microscope at MX beamline

# *In meso in situ* method: Data collection automation at cryo-temperature

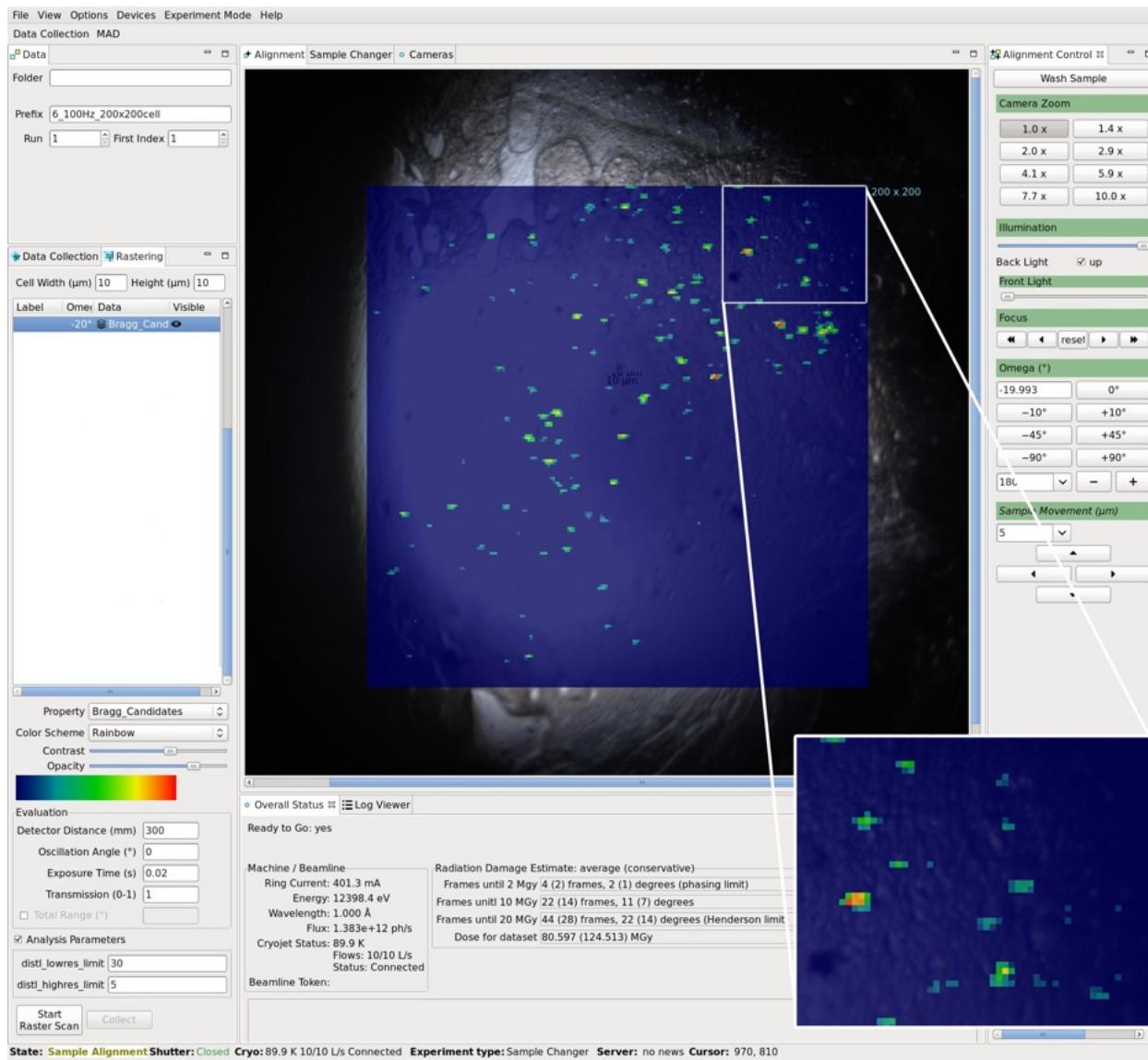


## The cryogenic advantages (IMISXcryo)

- Tolerable X-ray life-dose (50× more vs. RT)
- Prepare crystals in advance of beamtime
- Simplify crystal storage and transportation
- Compatible with sample changers
- Data collection automation

- 
- IMISX chip, LCP bolus of 1×1 mm
  - 20×20 μm beam, EIGER 16M 100Hz
  - Fast grid scan – 50×49 cell in one min.
  - Collect small wedge (5 – 20°) data at each crystal hit
  - > 100 crystals per hour

# In meso in situ method: Application



**Membrane Protein X**

**P2<sub>2</sub>2<sub>1</sub>2**

**78.1, 116.2, 73.2 Å**

**20 × 15 × 10 μm**

**200 × 200 cells grid scan**

**10 × 10 μm beam**

**30 MGy / crystal**

**10° data / crystal**

**155 crystals**

**One hour beamtime**

**XDS, XSCALE analysis**

**150 processed**

**60 merged**

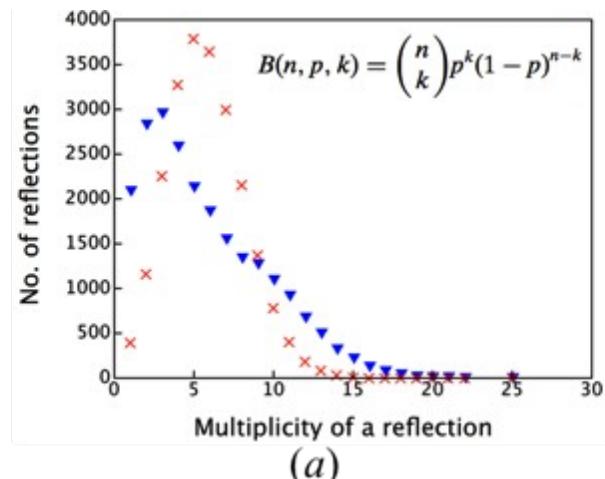
**40 – 3.0 Å**

**100% complete**

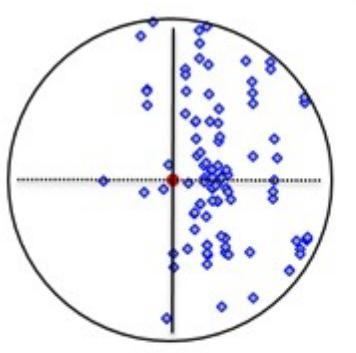
**CC<sub>1/2</sub> 0.99 (0.51)**

# Data analysis I: completeness and multiplicity

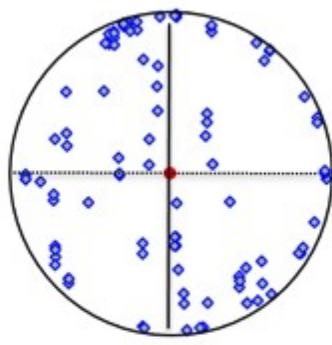
Preferential crystal orientations as shown in the skewed multiplicity distribution (a Binomial)



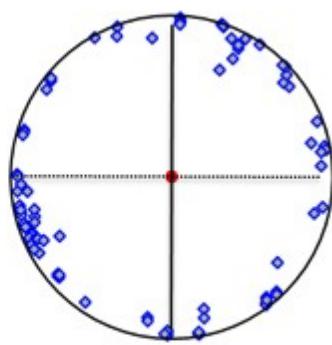
(a)



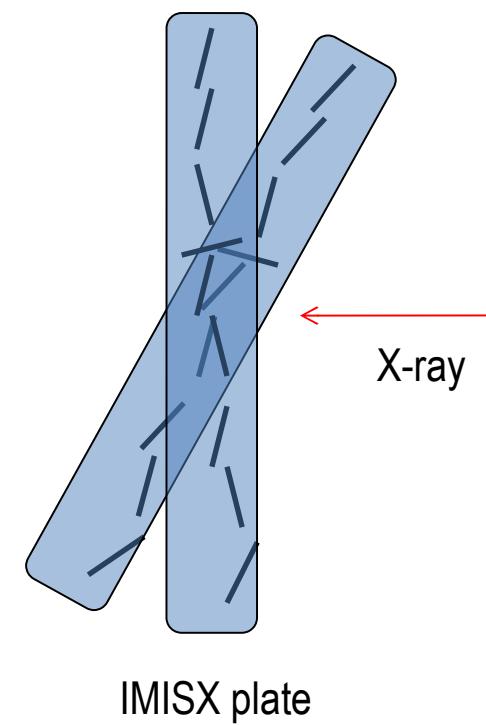
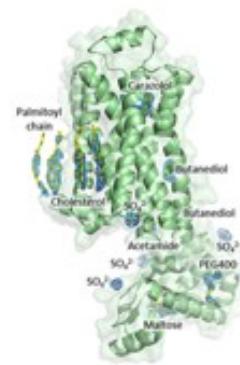
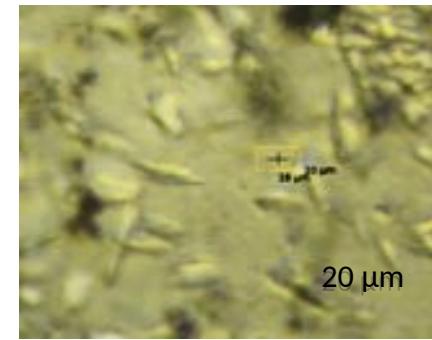
(b)



(c)



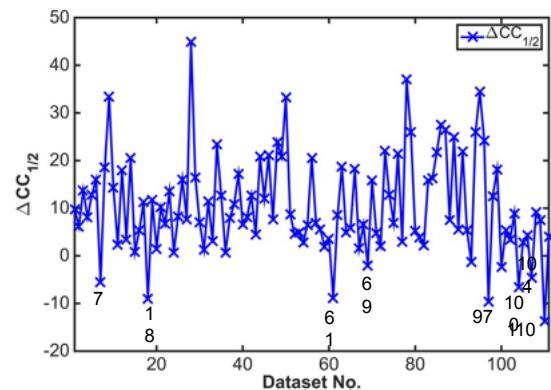
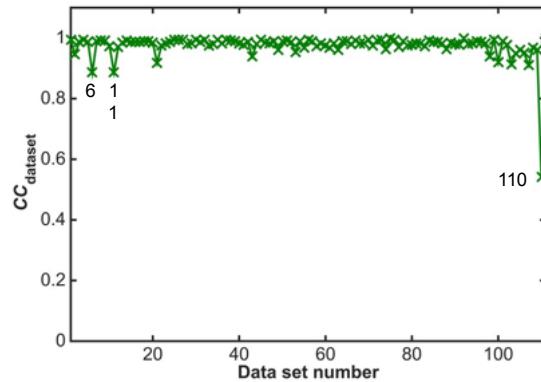
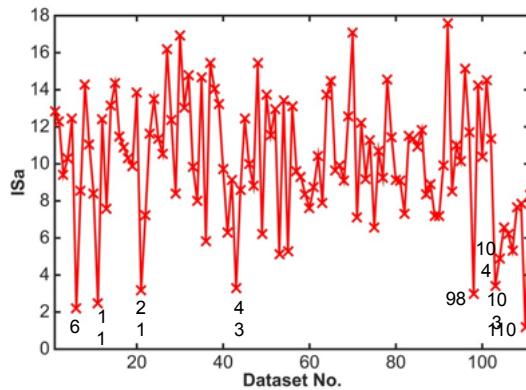
(d)



Projection of the unit-cell *a*, *b*, and *c* axis along the X-ray beam direction (red dot)  
Unit cell: 108.0 Å, 170.6 Å, 40.4 Å, 90°, 106.3°, 90°

# Data analysis II: data sets selection

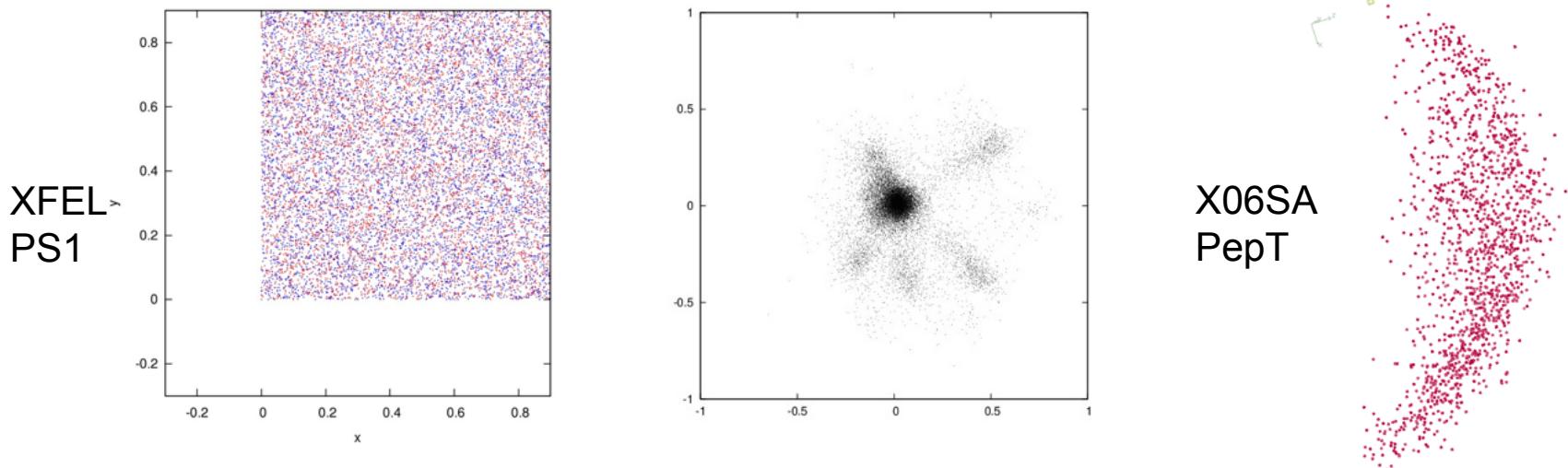
- Index and integrate each data set individually with XDS
- $I_{hkl,1}, I_{hkl,2}, I_{hkl,3}, \dots I_{hkl,n}$  – common unique intensities of each data set
- $ISa$  –  $\langle I/\sigma(I) \rangle$  of each individual data set in the merged intensities
- $CC_{dataset}$  – Intensity correlation coefficients and their clustering
- $\Delta CC_{1/2}$  – Improvement of the overall data quality



C.-Y. Huang, et al. *Acta Cryst. D72*, 93 (2016)  
G. Assmann, et al. *J. Appl. Cryst. 49*, 1021 (2016)

# Data analysis III: the meaning of isomorphism

- **Heterogeneity** of data sets: differences in composition, conformation, RH, ...
- a new theory that represents each data set as a vector in low-dimensional space, based on pairwise correlation coefficients (Diederichs 2017)
- length of vector: signal-to-noise ratio; angles between vectors: systematic differences → clustering in angle space
- can be used for selection, but also for analysing spread of conformations
- *may be used for any type of noisy data where systematic effects exist*

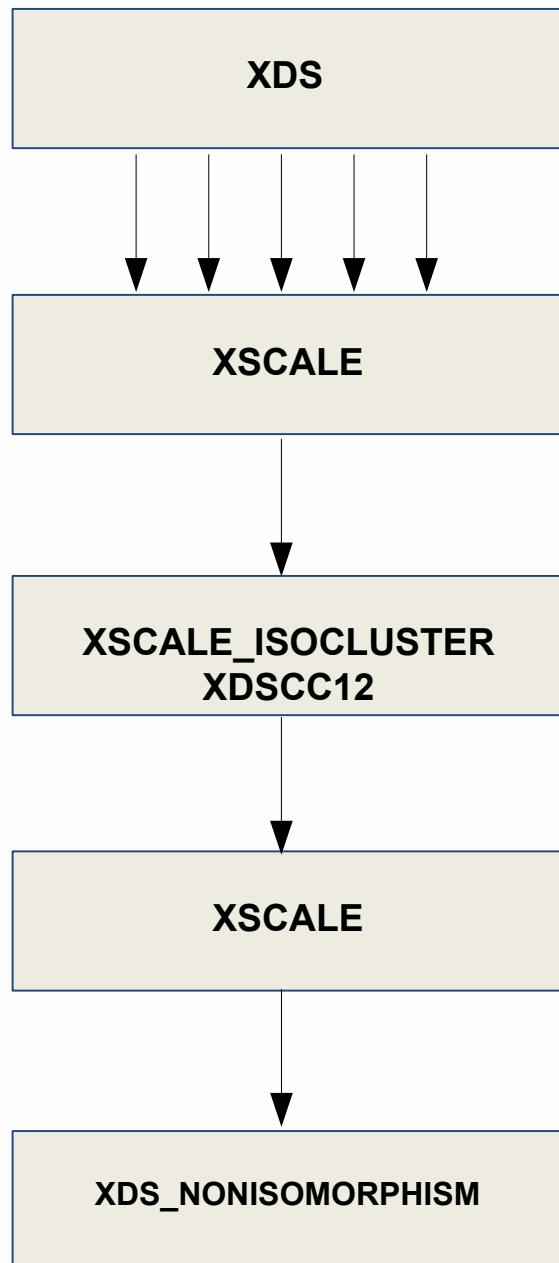


W. Brehm, K. Diederichs. Breaking the indexing ambiguity in serial crystallography (2014) *Acta Cryst. D70*, 101

K. Diederichs. Dissecting random and systematic differences between noisy composite data sets (2017) *Acta Cryst. D73*, 286

output:

explanation:



XSCALE.HKL

first time: use all datasets  
(MINIMUM\_I/SIGMA=0 or 1)

XSCALE.#.INP

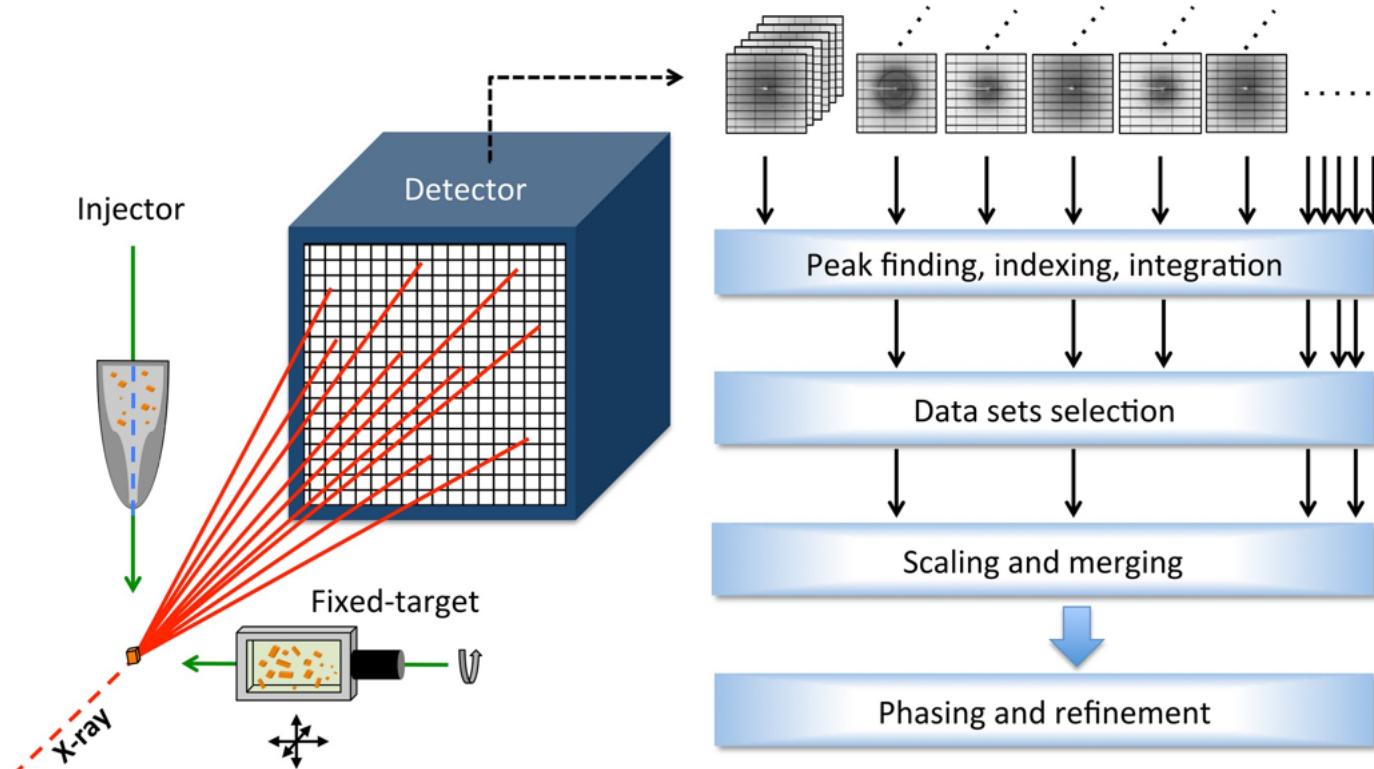
separate into clusters & rank

XSCALE.#.HKL

specify cluster to work with: (e.g.)  
ln -s XSCALE.1.INP XSCALE.INP  
possibly iterate with  
XSCALE\_ISOCLUSTER

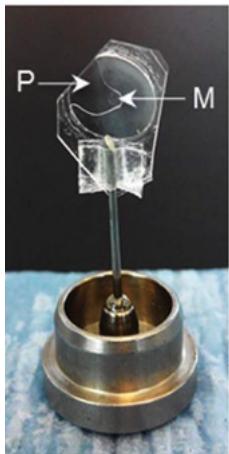
Use equation 2 of Diederichs (2017) to  
quantify coordinate differences between  
datasets

# Summary: Serial Synchrotron Crystallography (SSX)

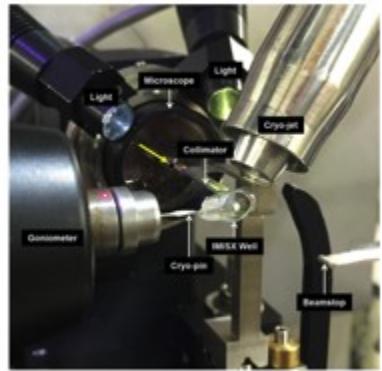


- SSX has emerged as an attractive method in macromolecular micro-crystallography at synchrotron beamlines
- Continuous delivery of crystals (high-throughput)
- Collect data from all crystals systematically instead of from a random selection
- “Overcome” radiation damage by supplying fresh crystals
- Selection of and merging within high-isomorphism data sets / clusters
- Promises to see not just one conformation – new software (`xscale_isocluster`)

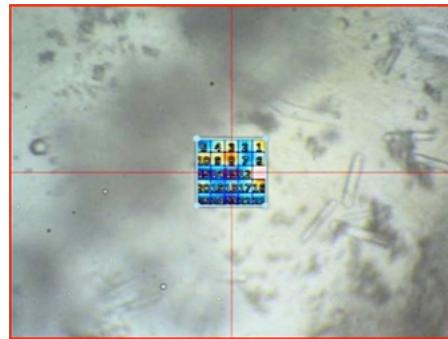
# IMISXcryo is available at beamlines near you



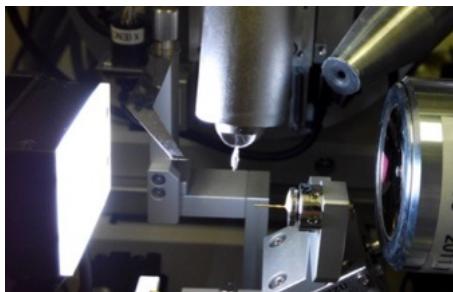
**IMISXcryo**



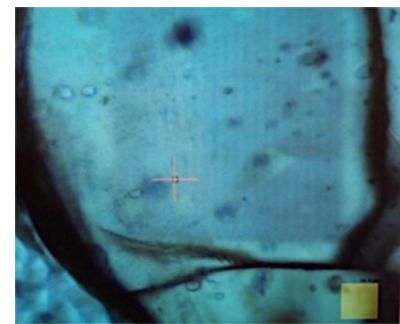
**X06SA, SLS, Switzerland**



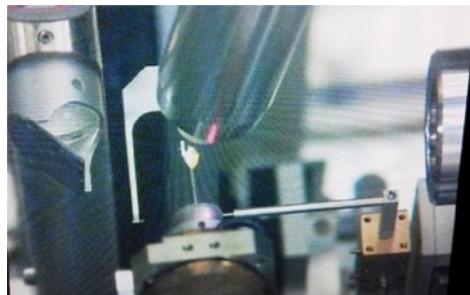
**ID-D-23, APS, US**



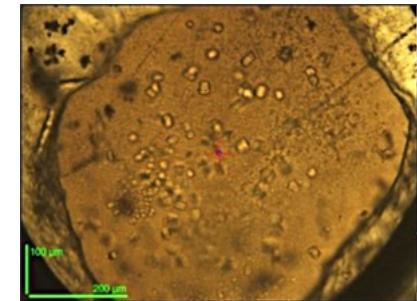
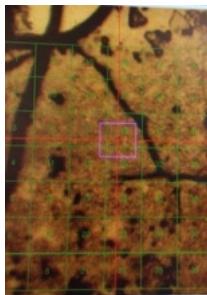
**BL32XU, SPring-8, Japan**



**I24, DLS, UK**



**BL-1A, KEK, Japan**



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