First experiments – SFX & operation schemes

Jaehyun Park & Ki-Hyun Nam
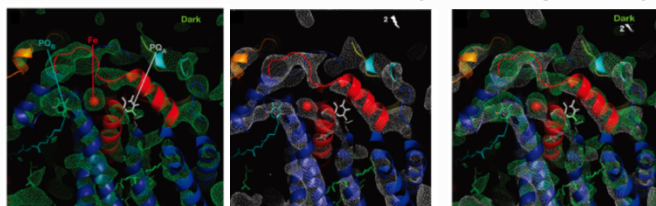
Nano Crystallography & Imaging (NCI) station
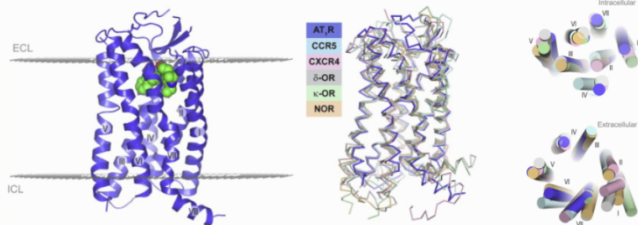
Transmission Mode
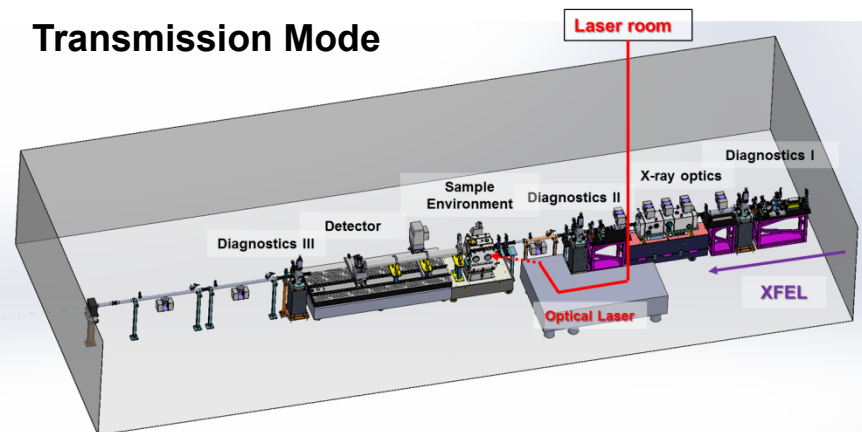
NCI Experiments:

Serial femtosecond crystallography (SFX)

Time-resolved SFX

Coherent diffraction imaging (CDI)

Single-pulse nano-particle imaging

Single-pulse bio-imaging

Ultrafast time-resolved imaging

Serial Femtosecond Crystallography
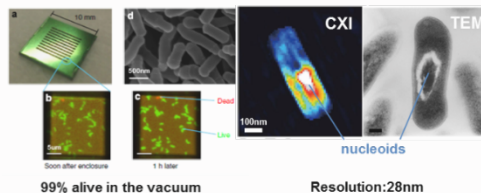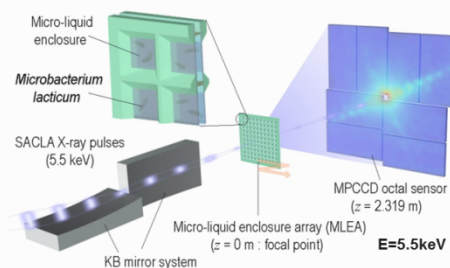

Structure of the PSII

Kupitz et al. Nature. (2014)

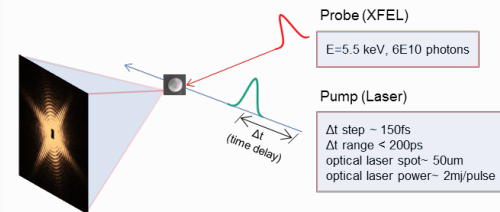**Structure of the Angiotensin Receptor**

Zhang et al. Cell (2015)

Single-pulse Bio-imaging

Living cell (lacticum) Imaging

Takashi. et al., Nature comm. (2014)

Time-resolved Imaging

Probe (XFEL)
E=5.5 keV, 6E10 photons

Pump (Laser)
Δt step ~ 150fs
Δt range < 200ps
optical laser spot~ 50um
optical laser power~ 2mj/pulse

Melting process of Au nanocrystal
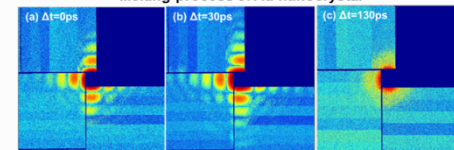
Single shot diffraction pattern of 150nm Au particle (square shape) at the time delay of (a)Δt=0ps (b)Δt=30ps (c)Δt=130ps. Dramatic change of diffraction pattern between Δt=30ps and 130ps indicates that laser-induced melting occurred during this interval.

Dr. Song et al., Postech Physics

NCI Layout

Experimental station spec

Energy Range	2-20 keV 5-12 keV (Optimized)
Beam Size	1500 μm (Unfocused beam) @12.4 keV 2 μm (KB mirror) @12.4 keV
Sample Environment	High Vacuum (~ 10^{-7} Torr) He ambience Fixed target , Liquid and LCP injectors
Detector	1M MPCCD (50 x 50 um^2 pixels size) MX225-HS (Rayonix)

XFEL
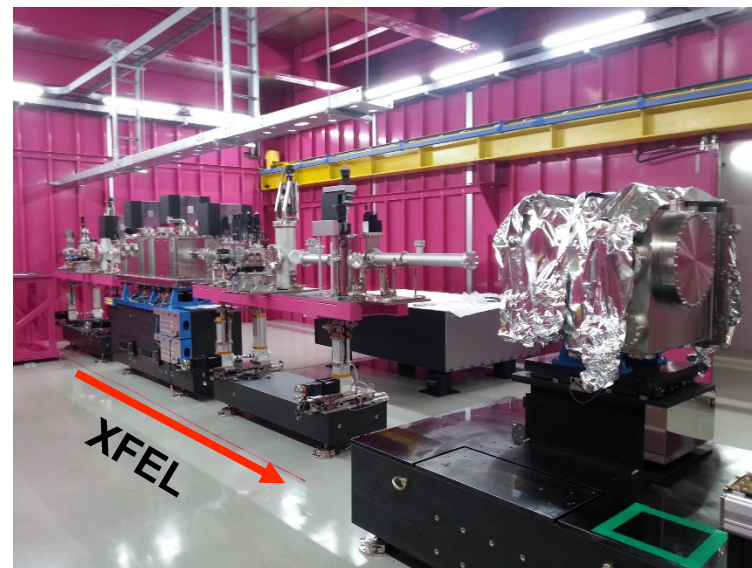
Diagnostics III **Detector stage** **Sample** **Diagnostics II** **Focusing optics** **Diagnostics I**

Pop-In

1M MPCCD

MX225-HS

Sample Chamber

Pop-In QBPM Slit

KB mirror

Pop-In QBPM Slit

Ref. Laser Attenuator

Optical Laser

XFEL

Distance (m)   148   142   138
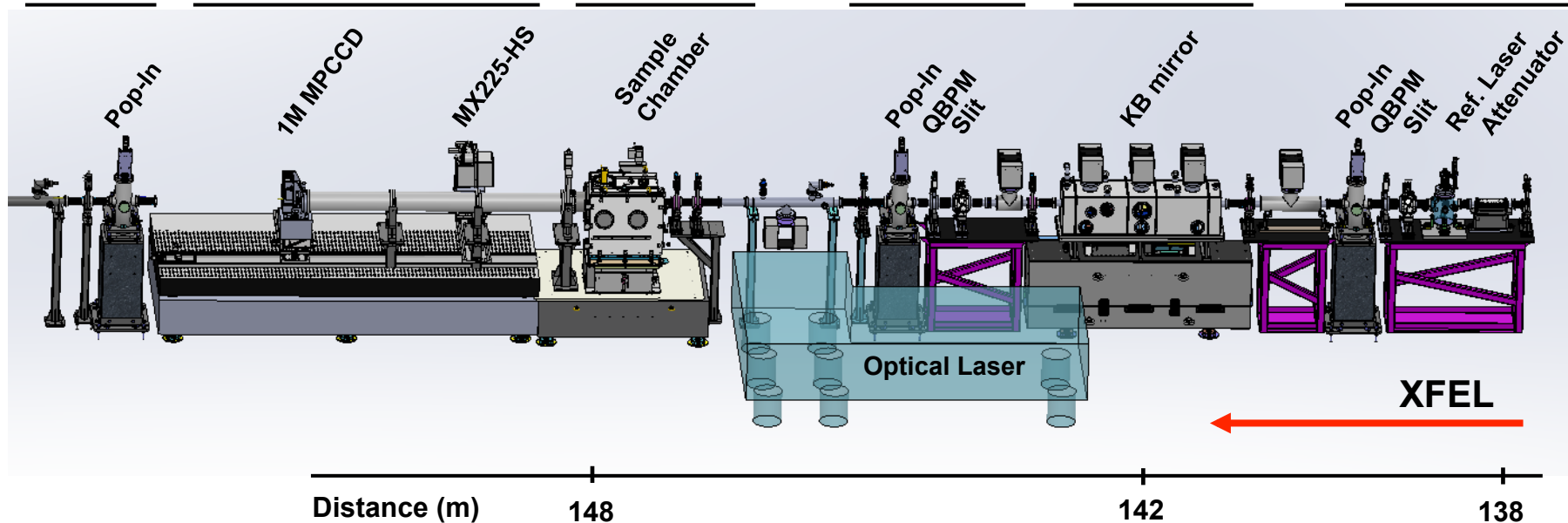
X-ray optics: Kirkpatrick-Baez Mirror
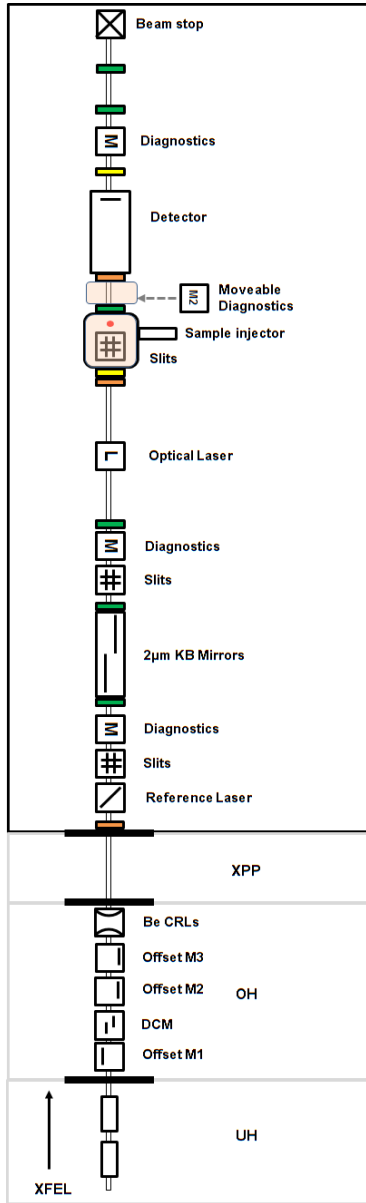
> **Purpose**
  - Increases photon flux at interaction point

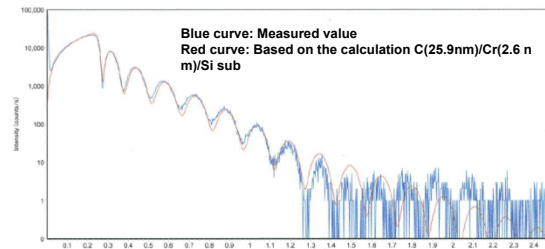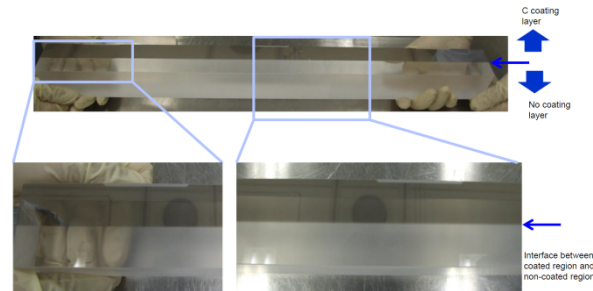> **Requirements**
  - Beam size: 2 μm
  - Focal length: 5.68 m (center of mirrors)
  - Reflectivity: >99% (at 5 ~ 12. 4 keV)
  - Mirror length: accept 4 σ or more
  - Vacuum environment: $<10^{-8}$ Torr

> **Production**
  - Mirror substrate: JTEC (Japan)
  - Mirror coating: NTT-AT (Japan)
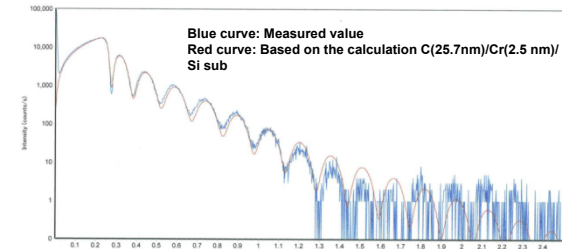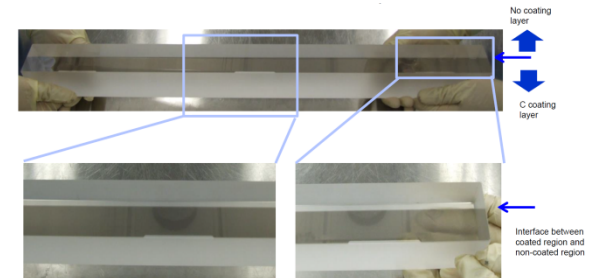
> **Mirror coating**

> **Substrate inspection**

2-1. Specifications and measurements

			Unit	Specification	Measurement	Judgment
1	Substrate size	Length	mm	600 ±0.5	599.99	OK
		Width	mm	50 ±0.2	49.99	OK
		Thickness	mm	50 ±0.2	50.15	OK
2	Material		—	Quartz	—	—
3	Shape		—	Tangential ellipse	—	—
4	Useful area		mm	>580	588.20	OK
			mm	>20	22.00	OK
5	Tangential Slope error		μrad rms	<0.1	0.096	OK
6	Sagittal Slope error		μrad rms	<2.0	0.171	OK
7	Roughness (50X)		nm rms	<0.3	0.175	OK

Table2-1: First Mirror (VFM)

			Unit	Specification	Measurement	Judgment
1	Substrate size	Length	mm	600 ±0.5	600.00	OK
		Width	mm	50 ±0.2	50.00	OK
		Thickness	mm	50 ±0.2	50.09	OK
2	Material		—	Quartz	—	—
3	Shape		—	Tangential ellipse	—	—
4	Useful area		mm	>580	580.50	OK
			mm	>20	20.50	OK
5	Tangential Slope error		μrad rms	<0.1	0.092	OK
6	Sagittal Slope error		μrad rms	<2.0	0.278	OK
7	Roughness (50X)		nm rms	<0.3	0.168	OK

Table2-2: Second Mirror (HFM)

Blue curve: Measured value
Red curve: Based on the calculation C(25.9nm)/Cr(2.6 nm)/Si sub

VFM

Blue curve: Measured value
Red curve: Based on the calculation C(25.7nm)/Cr(2.5 nm)/Si sub

HFM

X-ray optics: Mirror manipulator

Beam stop
Diagnostics
Detector
Moveable Diagnostics
M2
Sample injector
Slits
Optical Laser
Diagnostics
Slits
2µm KB Mirrors
Diagnostics
Slits
Reference Laser
XPP
Be CRLs
Offset M3
Offset M2
DCM
Offset M1
OH
UH
XFEL
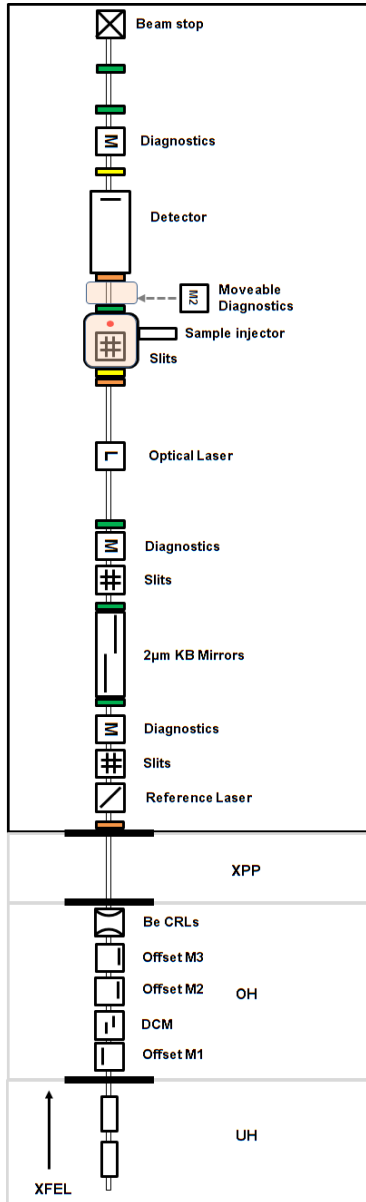
➢ **Purpose**
- Alignment of two KB focusing mirrors

➢ **Requirements**
- Mirror acceptance: 600 -1000 mm mirror
- Resolution: $\leqq$ 0.05 (beam rms = 1.4 µm)
- Stability: $\leqq$ 0.07 / 8 hours (arcsec)
- Vacuum: <10^{-9} Torr (in operation: <10^{-6} Torr)

➢ **Production**
- Manipulator design: PAL staff (Hyo Yoon Kim)
- Mirror manipulator: Vactron (Korea)

➢ **Manipulator inspection**

Motion	Parameter	Specification	Measurement	Judgment
Horizontal (X-axis)	Range (mm)	± 10	± 10	OK
	E_Resolution (µm)	$\leqq$ 0.5	0.05	OK
	M_Resolution (µm)	$\leqq$ 0.05	0.01	OK
	Precision (µm)	$\leqq$ 2.0	0.7	OK
	Stability (µm)	$\leqq$ 3.0 / 8 hours	1.9	OK
Vertical (Y-axis)	Range (mm)	± 10	+10,-15	OK
	E_Resolution (µm)	$\leqq$ 0.2	0.2	OK
	M_Resolution (µm)	$\leqq$ 0.1	0.08	OK
	Precision (µm)	$\leqq$ 1.0	0.3	OK
	Stability (µm)	$\leqq$ 2.0 / 8 hours	1.6	OK
Roll[1] (gonio axis)	Range (Degree)	± 1.5	± 1.5	OK
	E_Resolution (arcsec)	$\leqq$ 0.4	0.093	OK
	M_Resolution (arcsec)	$\leqq$ 0.1	0.014	OK
	Precision (arcsec)	$\leqq$ 2.0	1.23	OK
	Stability (arcsec)	$\leqq$ 1.0 / 8 hours	0.2	OK
Pitch[1] (gonio axis)	Range (Degree)	± 1.5 (linearly =±8.86 mm)	± 1.5 (linearly =±8.86 mm)	OK
	E_Resolution (arcsec)	$\leqq$ 0.05 (beam 변위 = 10 µm)	0.028 (beam 변위 = 7 µm)	OK
	M_Resolution (arcsec)	$\leqq$ 0.02 (beam 변위 = 4 µm)	0.0006 (beam 변위 =1.4 µm)	OK
	Precision (arcsec)	$\leqq$ 0.2 (beam 변위 = 20 µm)	0.17	OK
	Stability (arcsec)	$\leqq$ 0.07 / 8 hours	0.076	OK
Base Alignment	Driver	Manual		OK
	Range	Z direction, ± 25 mm (with roll, pitch)	± 25 mm	OK
		X, Y direction, ± 15 mm	± 15 mm	OK
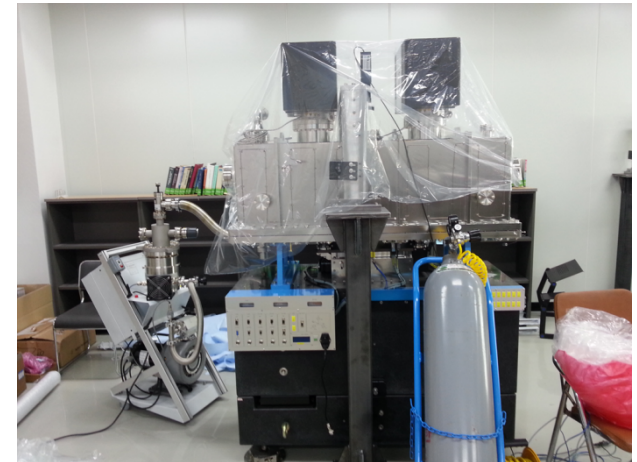
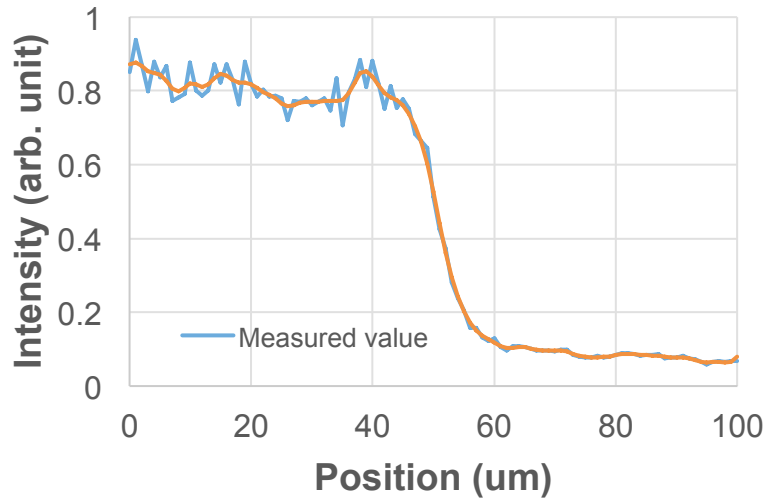

Motion & stability test at PAL-XFEL

Mirror manipulator assemble at PAL-XFEL

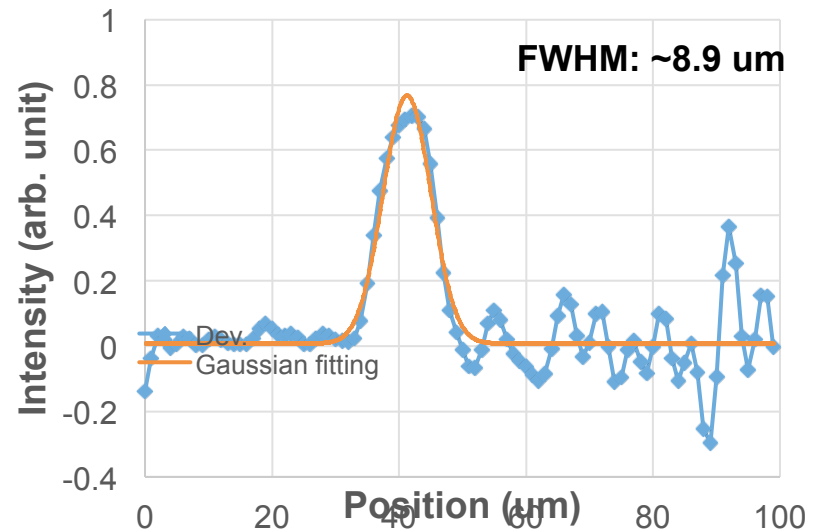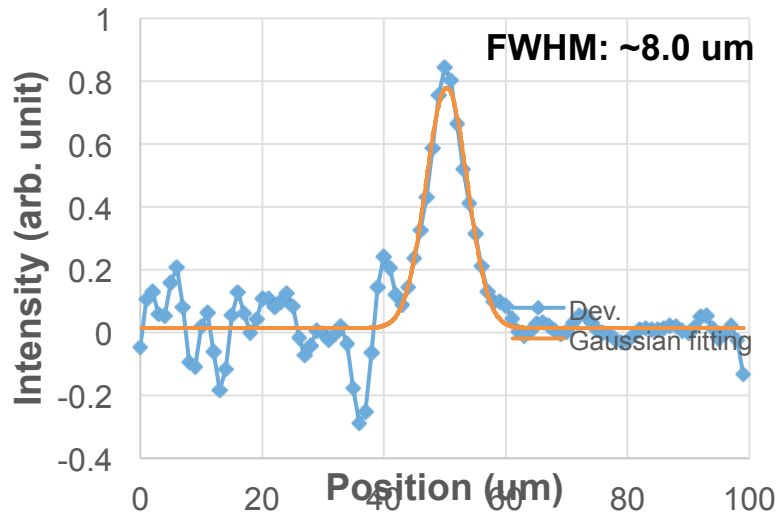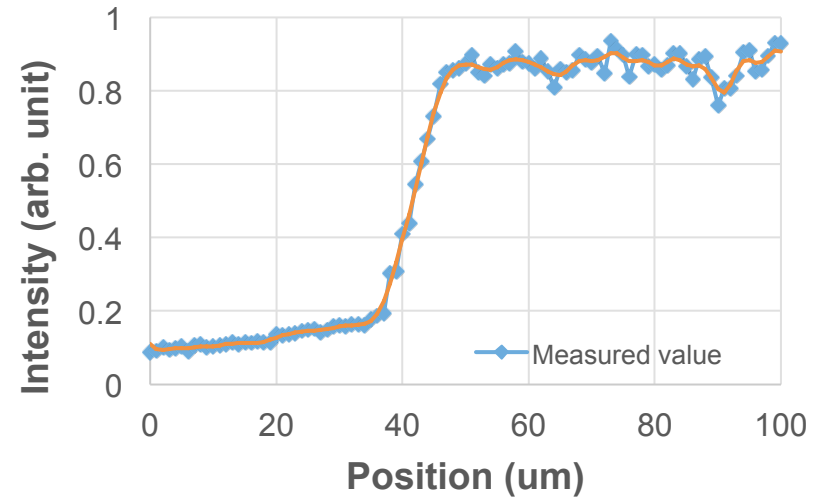# K-B mirror focusing @ NCI (6.1 keV)

Vertical direction

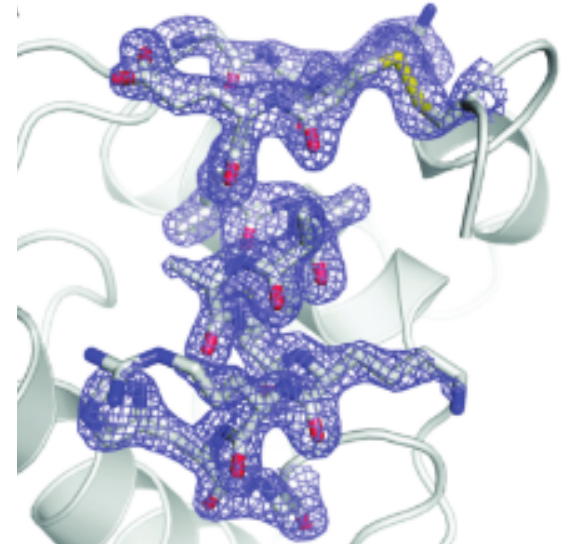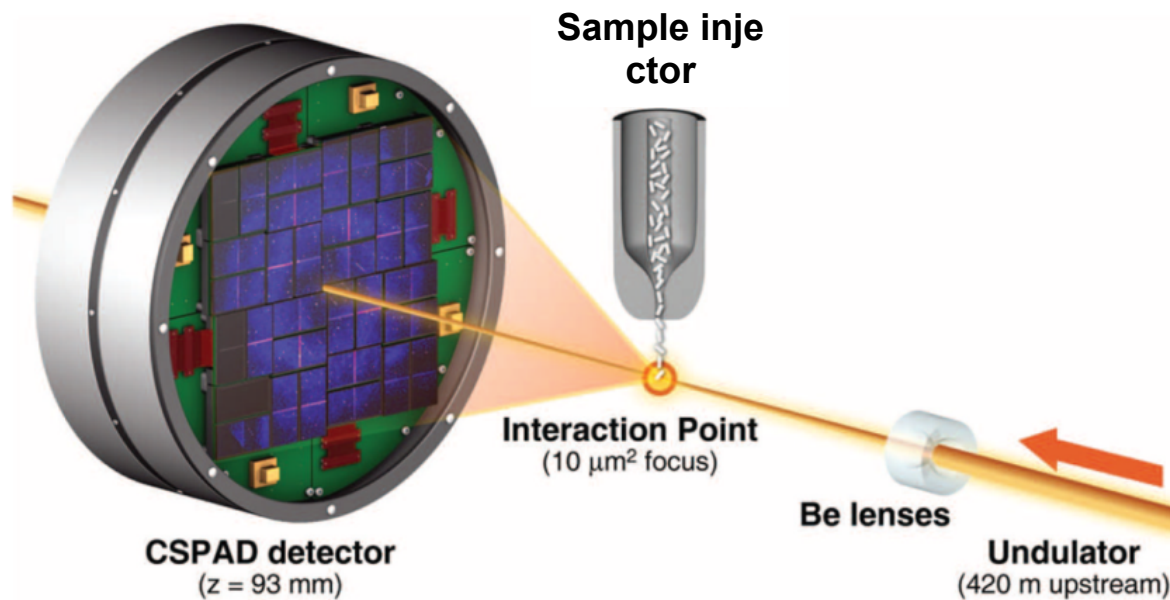## Horizontal direction

FWHM: ~8.0 um

FWHM: ~8.9 um

Serial femtosecond crystallography

PAL

➢ High resolution Protein Structure Determination

Sample injector

Interaction Point
(10 μm² focus)

Be lenses

Undulator
(420 m upstream)

CSPAD detector
(z = 93 mm)

Experimental geometry for SFX

Boutet et al. Science (2012)

- All the nanocrystals posses the same crystal symmetry, a series of nanocrystals could be illuminated by FEL X-ray pulses and the diffraction patterns recorded.

- The variations in alignment of the crystal axes from sample to sample can be determined from the indexing of the Bragg peaks in the diffraction patterns.

SFX experiments strategy

Sample preparation
- Growing crystal
- Crystal quantity
- Buffer condition
- Size control

Crystal analysis
- Real protein crystal?
- Proper size?
- Density control

Experiment
- Buffer test
- Liquid jet injector
- LCP injector
- Dedicated chamber

Users side

Structure analysis
- CrystFEL / cctbx.xfel
- Structure refinement

Data treatment
- Hit rate, Completeness
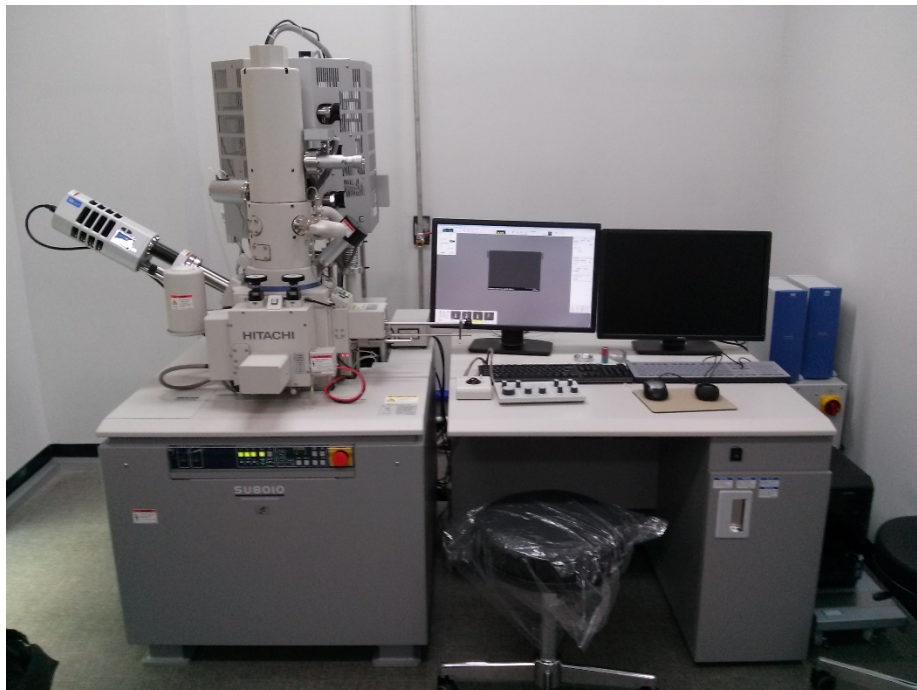- Background calibration
- Peak finding

Data acquisition
- Large area detector: high resolution data
- Distance control: adapt for sample features

HX beamline sample preparation room

Room166

SEM
Nano particle characterization

Hitachi Su8010

- Cold FE gun
- Semi-in-lens type
- High resolution image (1.0nm@15kV)
- Deceleration function (1.3nm@1.0kV)
- Dual SE detector (Lower/Upper)
- EDS (Energy Dispersive Spectrometer)
- 3-axis goniometer stage
- Ion sputter with Pt target
- Consumable and spare parts

Room276

Long term bio sample preparation

Sample preparation

Sample analysis instruments

SONICC - sample observation
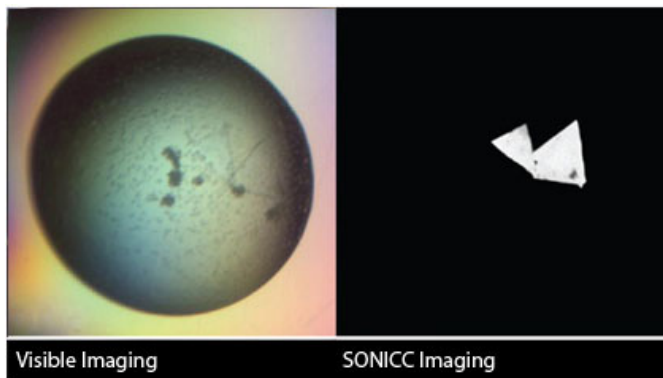**(Second Order Non-linear Imaging of Chiral Crystals)**

DLS - size determination
(Dynamic Light Scattering)

Room161-1

FORMULATRIX
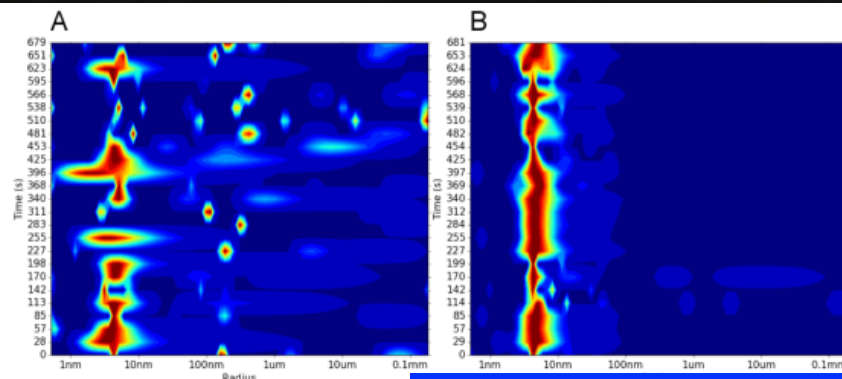Crystallization - Liquid Handling - dPCR

SpectroSize™300 *for Sizing of Nano-particles like Proteins*

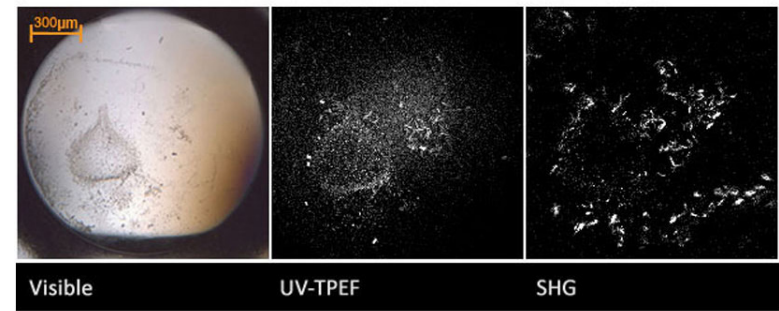Visible Imaging | SONICC Imaging

Crystal analysis

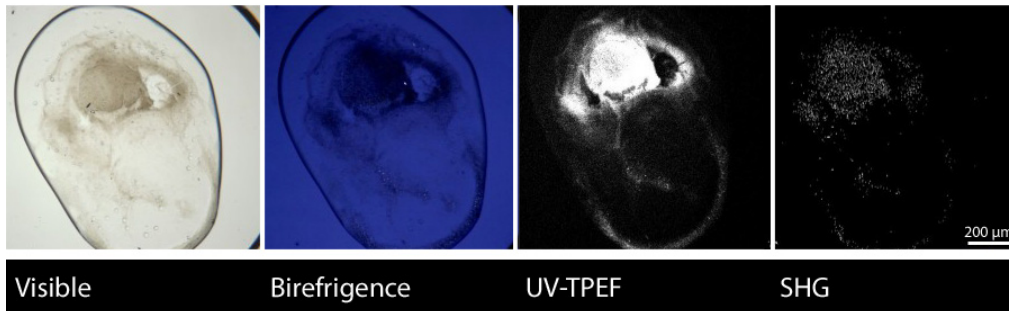# Protein crystal detection - SONNIC

visible   UV-TPEF   SHG

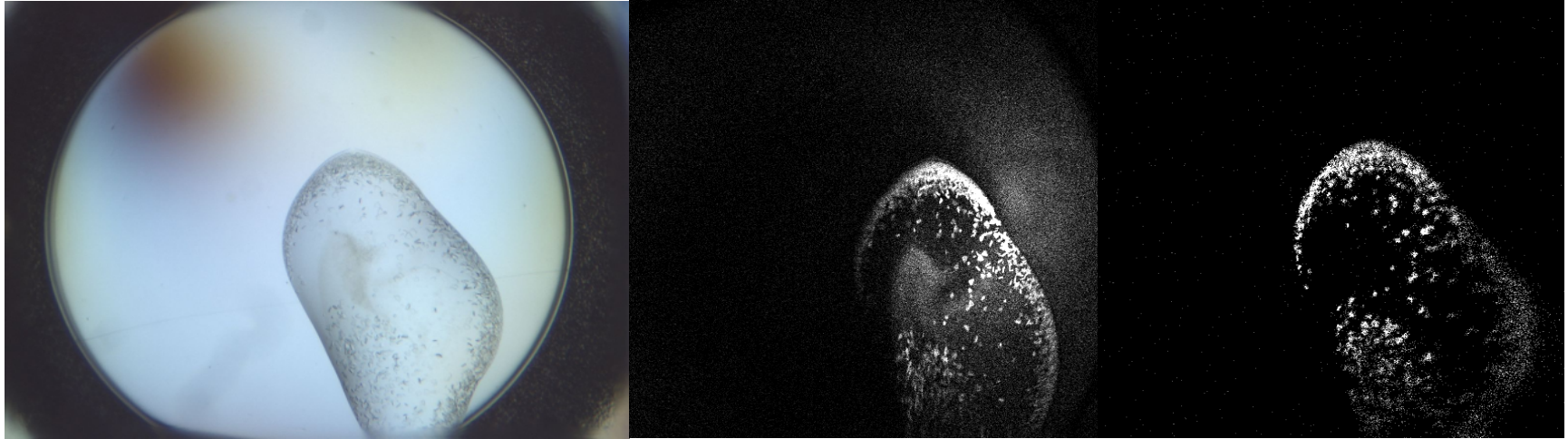Visible   Birefrigence   UV-TPEF   SHG
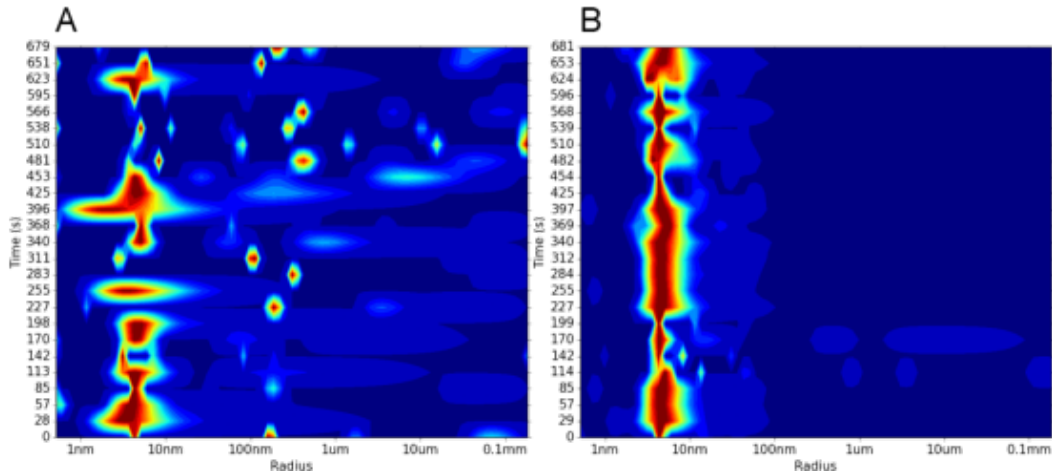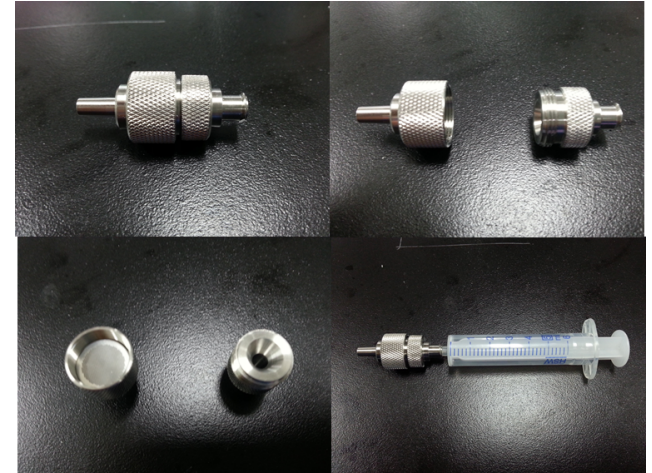
Visible   UV-TPEF   SHG

The combination of visible, UV-TPEF and SHG imaging make for a powerful tool for protein crystal imaging

Crystal analysis

Sample size characterization - DLS

In situ DLS in linbro plates. Radius distribution plot of a membrane protein detergent complex before gelfiltration (A) and after gelfiltration (B).
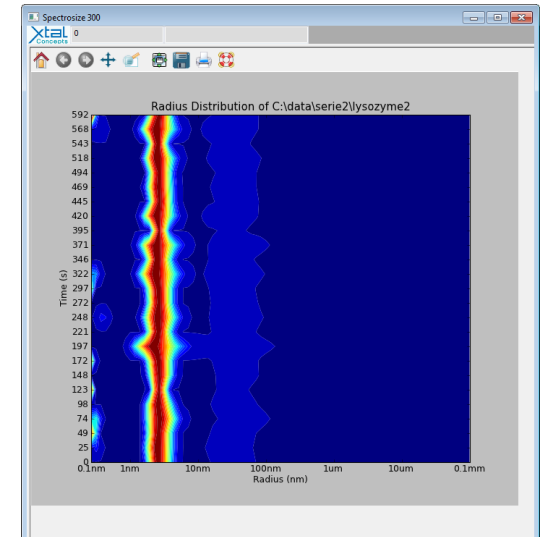
SpectroSize™300 *for Sizing of Nano-particles like Proteins*

The minimum required sample volume is ~8 µl.♪

### SpectroSize™300 TECHNICAL DATA

|	
Laserdiode	*T*emperature stabilized single-mode Laser diode ✓ Wavelength: 660 nm ✓ Optical power: 100 mW ☐ Wavelength: ☐ 785 nm ☐ 532 nm ☐ 405 nm
Particle size	*M*easurement range ✓ Minimum: 1 nm ✓ Maximum: approx. 6 µm
Sensitivity	*S*ample concentration with standard laser (660 nm) ✓ Minimum 0.1 mg/ml of lysozyme ✓ Maximum > 100 mg/ml

Crystal analysis

Sample injectors for SFX
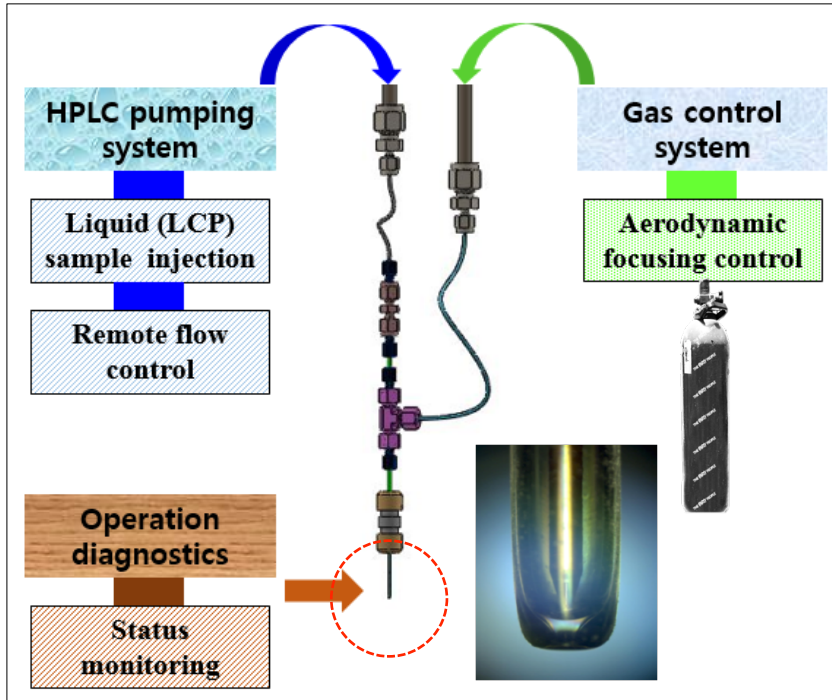
Liquid sample injector operation scheme
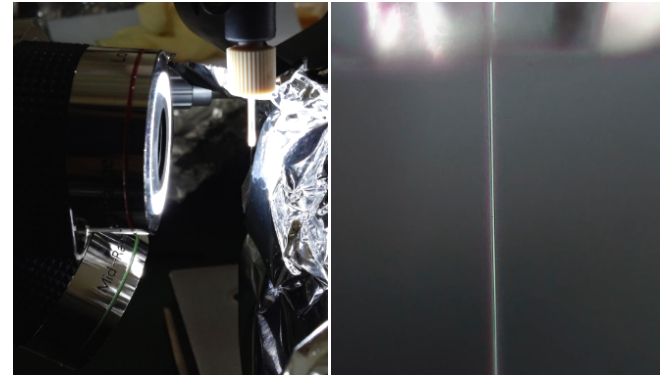
Liquid jet injector
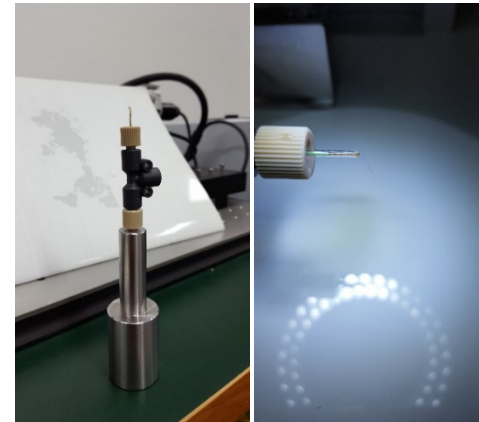
LCP injector

➤ **Liquid jet injector:**
- **Gas dynamic virtual nozzle (GDVN)**
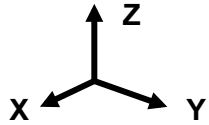- **Soluble protein crystals and nano particles**
- **Flow rate: 20 ~ 30 ul/min**
- **Diameter control: 3 ~ 30 um**

➤ **LCP injector:**
- **Low sample consumption**
- **Membrane proteins & micro/nano crystals**
- **Operation time: 7 hours with 94 nl/min**
- **Flow rate: 90 ~ 200 nl/min**
- **Diameter: 50, 75, 100 um**
- **Reservoir volume: 40 ul**

Experiment

SFX dedicated chamber at PAL-XFEL

Multi ports for injectors

Injector position manipulation feedthrough

Stage	Travel	Resolution
X	50 mm	10 nm
Y	50 mm	50 nm
Z	200 mm	50 nm

XFEL

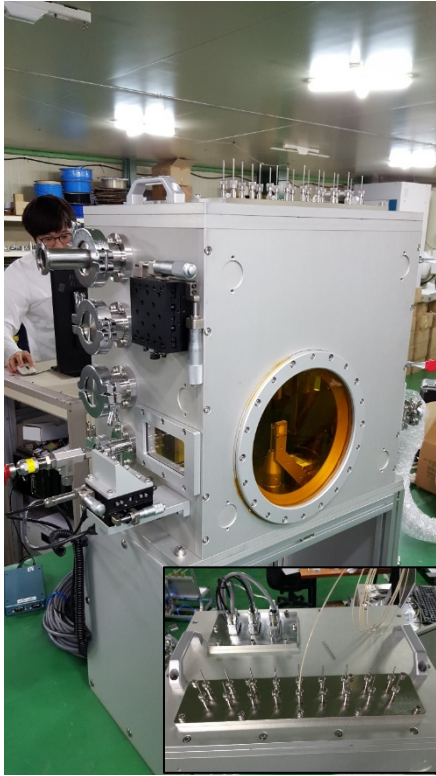**Pump laser input port**

Diffraction signal port

405

437

835

SFX dedicated chamber at PAL-XFEL

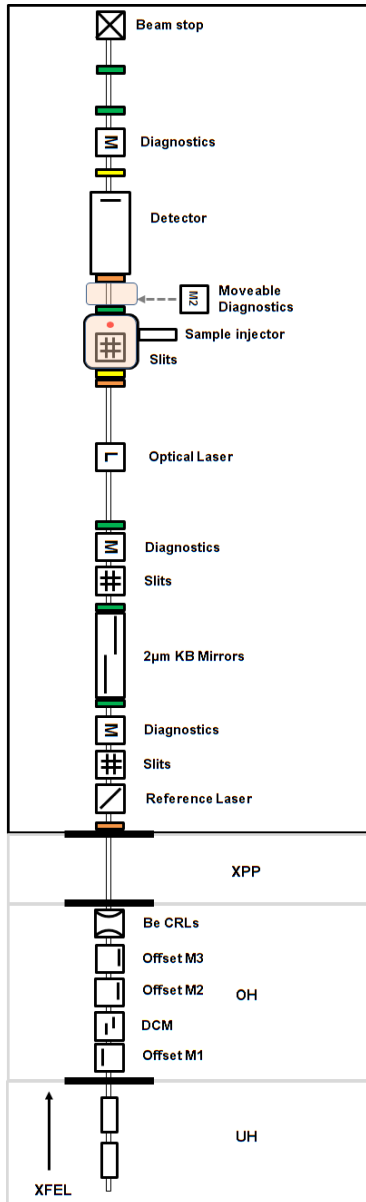> **Sample delivery :**
- Soluble samples (protein crystals, nano particles in solution)
- Samples in lipidic cubic phase (LCP)
- Carrier matrix with nano crystals
- Compatible with Liquid jet injectors (GDVN) & LCP injectors

> **Chamber operation :**
- In **helium** atmosphere
- **Multi port** injection
- Oxygen concentration sensor
- Injector diagnostics ports
- Pumping laser ports

Experiment

Detectors

Left diagram (beamline, top to bottom):
- Beam stop
- Diagnostics
- Detector
- Moveable Diagnostics — M2
- Sample injector
- Slits
- Optical Laser
- Diagnostics
- Slits
- 2µm KB Mirrors
- Diagnostics
- Slits
- Reference Laser
- XPP
- Be CRLs
- Offset M3
- Offset M2 — OH
- DCM
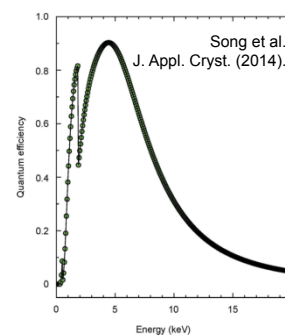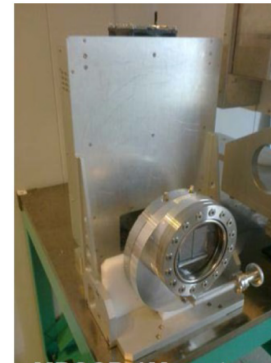- Offset M1
- UH
- XFEL

➢ **Purpose**
- Measure the 2D coherent diffraction patterns

➢ **Requirements specification**
- Energy range: 2-20 keV
- Quantum efficiency over at least 0.8
- Random noise of <0.18
- Detector size of > 20 cm
- Pixel size of < 30 - 50µm
- Dynamic range of > 10^4
- Frame rate of 60 fps
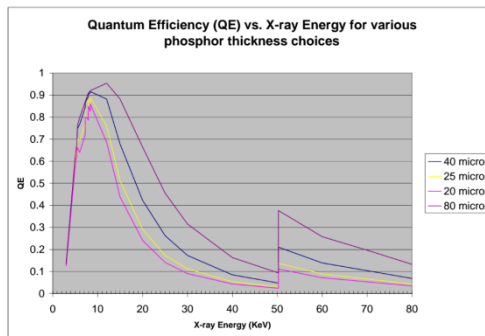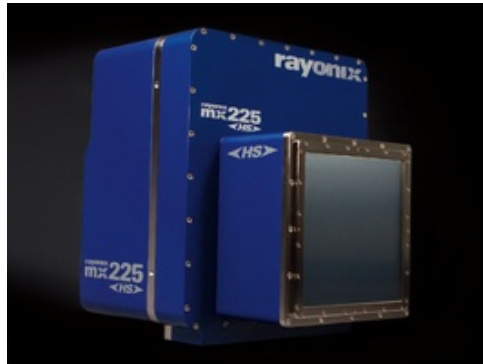
MX225-HS

On-chip Binning	
2 X 2	
Readout Speed	10 frames/second
Pixel Size (µm)	78µm
Frame Size	2880 x 2880 pixels
3 X 3	
Readout Speed	20 frames/second
Pixel Size (µm)	117µm
Frame Size	1920 x 1920 pixels
4 X 4	
Readout Speed	40 frames/second
Pixel Size (µm)	156µm
Frame Size	1440 x 1440 pixels

Quantum Efficiency (QE) vs. X-ray Energy for various phosphor thickness choices
- 40 micron
- 25 micron
- 20 micron
- 80 micron

MX225-HS

Song et al. J. Appl. Cryst. (2014).

MPCCD

Company/Institute	PSI	Basic Module
Type	JUNGFRAU 0.1 Hybrid-pixel (Integrating-Dynamic gain switching(3-fold))	75 µm x 75 µm 512 x 1024 (0.5M) 62.5kSFr.
Energy range	>3keV	
ADC	14bit	
Dynamic Range	>1·10^4@12keV	
Noise	<150 e⁻	
Frame Rate (Hz)	2kHz	
Pixel Size (µm)	75x75	
Active area [mm²]	40x80	
System	(module)	
Status	Prototype	

JUNGFRAU (2018-2020)

Data acquisition

Detectors for SFX
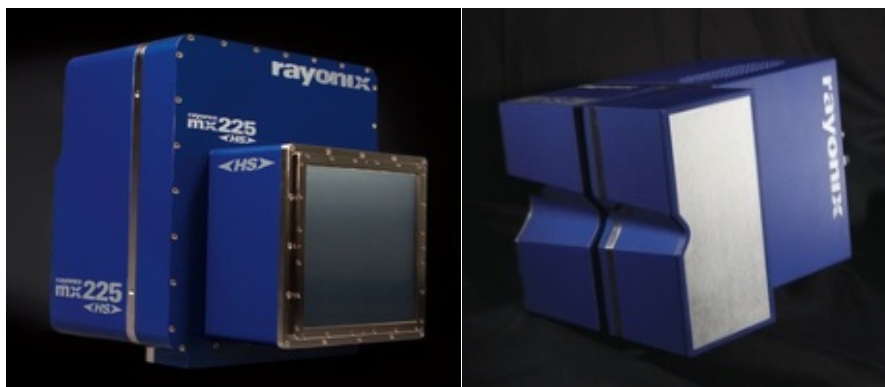
Energy (keV)	λ (Å)	Crystal to detector distance (mm)					
		75	100	125	150	175	200
12.4	1.00	1.22	1.58	1.4	1.58	1.77	1.97
11	1.13	1.19	1.38	1.57	1.78	2	2.22
10	1.24	1.31	1.51	1.73	1.96	2.2	2.45
9	1.38	1.46	1.68	1.92	2.18	2.44	2.72
8	1.55	1.64	1.89	2.16	2.45	2.75	3.06
7	1.77	1.88	2.16	2.47	2.8	3.14	3.49
6	2.07	2.19	2.52	2.88	3.27	3.67	4.08

Recordable structure resolution (Å)

Available resolution with MX225-HS

|	MX255-HS	LX255-HS
Pixel Size	39 μm × 39 μm	44 μm × 44 μm
Pixel Number	5760 × 5760	1920 × 5760
Active Area	225 mm × 225 mm	85 mm × 225 mm
Operating Temperature	-80°C	
Quantum Efficiency	<80% (Detected Quantum Effy.) @ 8-12 keV	
Noise	High Speed mode: 8 e−/ pixel Low Noise mode: 4 e− / pixel	
Dynamic Range	~ 10^4 photons/ 2x2 pixels with 12 keV X-ray	
Frame Rate	2.5 Hz (1x1) / 10 Hz (2x2: standard) / 40 Hz (4x4)	
PSF	100 μm FWHM with 40 μm thick. phosphor	

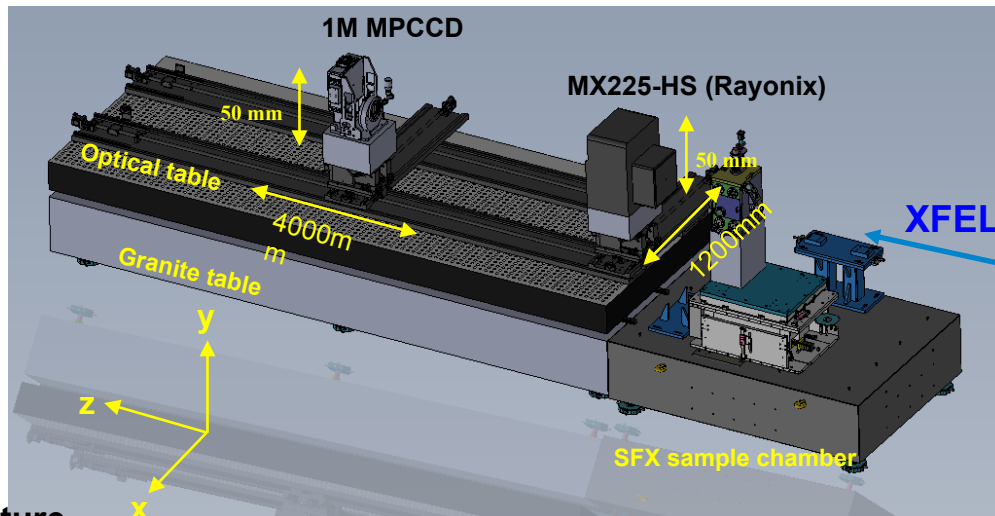**Data acquisition**

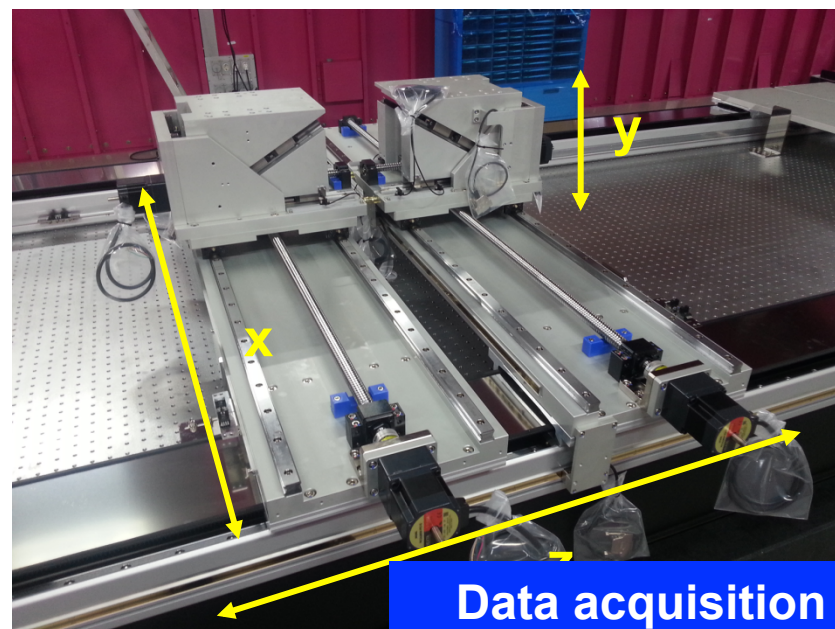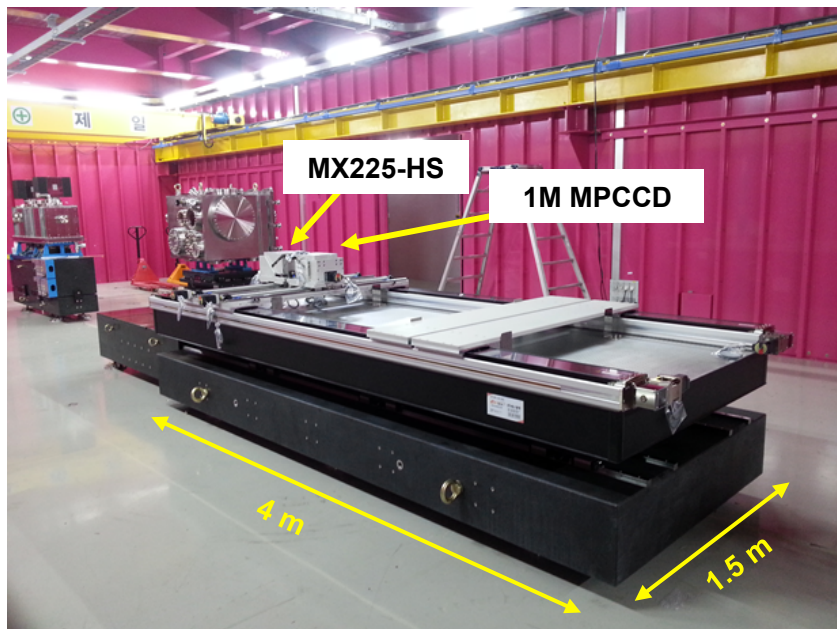# Detector stages

1) Detectors

MX225-HS (Rayonix)
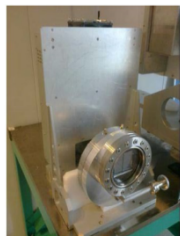
1M MPCCD

2) Detector distance manipulation
- Optimizing preferred resolution and peak separation depending on the crystal structure features


1M MPCCD

MX225-HS (Rayonix)

50 mm

Optical table

50 mm

4000mm

Granite table

1200mm

XFEL

SFX sample chamber

y

z

x

MX225-HS   1M MPCCD

MX225-HS
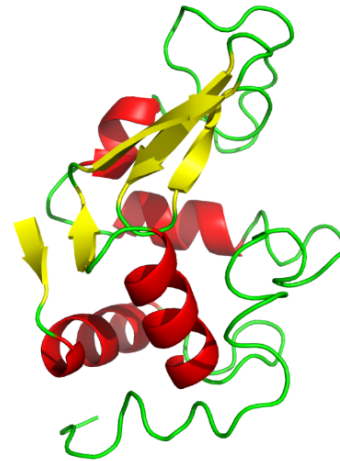
1M MPCCD

4 m

1.5 m

y

x

Data acquisition

Demo experiment

- Sample: Lysozyme microcrystals
- Purpose:
  - Instrument test (injector, sample chamber, detector…)
  - Alignment of the key features (sample-to-detector distance…)
  - Data processing
- Sample delivery method: Liquid jet sample injector, LCP injector
- Sample environment: He ambience
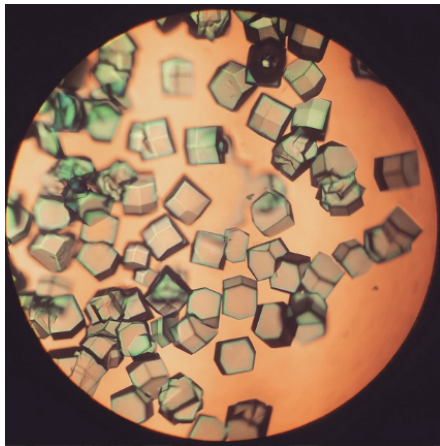
Lysozyme crystals

**Crystal structure of L
ysozyme (at PLS-II)**

Summary

SFX: High resolution structure analysis for protein crystals in perfectly hydrated state @ room temperature

- **NCI: serial femtosecond crystallography & coherent diffraction imaging**
- **SFX: sample injectors (liquid jet & LCP) + dedicated chamber**
- **Liquid jet injector delivery: micro/nano sized crystal w/ buffer solutions**
    - **multi port injection : diverse sample loading w/o nozzle change**
    - **easy handling and maintenance**

- **LCP injector delivery: membrane proteins & nano/micro crystals in carrier matrix**
    - **low sample consumption**
- **Dedicated chamber: compatible w/ liquid jet & LCP injectors operated in He gas environment**
- **Detector stage: convenient adjustment of sample-to-detector distance optimizing resolution and peak separation**
- **Demo experiment: Lysozyme microcrystals**