Construction and utilization of Korea-4GSR's life science and imaging beamline

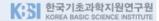
- 1. ID21 BioPharma-BioSAXS Beamline
- 2. ID22 Bio Nano Crystallography (BioNX) Beamline
- 3. BM10 High Energy Microscopy (HEM) Beamline

2025.06.27

Mi-Jeong Kwak (ID22), Yong Sung Park (BM10), Ji-hun Kim (ID21)
Yeon-Gil Kim, Jae-Hong Lim, Kyeong-Sik Jin (PLS-II)
Yong-Seok Heo, Dong In Yu, Sang-ho Lee (KOSUA)
Michel Fodje, Marco Stampanoni, Byeong-du Lee (MAC)







Overview of the Bio and imaging beamlines

BL	Design keyword	Source	Energy (keV) (mainly)	Flux (ph/s)	Sample	Beam size at sample	Results	Applications
ID21 BioPharma -BioSAXS	q-vector optimizing layout, High- throughput, Convenience	IVU24	5~20 (optimized: 8~25) (12)	~ 2 × 10 ¹³	Biomacromolecules <u>in solution</u>	~100×100 µm²	SAXS profile Model 1. 2. 3. Molecular resolution 4. Validation Current Research in Struc. Biol. (2020)	High-throughput Experiment SEC-SAXS Protein-based therapeutics Development of drug carrier using Lipid
ID22 BioNX	Micro beam, High flux, Rapid beam resizing, Stable, High- throughput	IVU20	8~25 (12.4, 20)	~5 x 10 ¹³	Biomacromolecules <u>as crystallized</u>	2x2~ 40x40 μm² @ 12.4 keV 1x1~5x5 μm² @ 20 keV	Diffraction Density map Model 2. Atomic resolution https://www.biospectator.com/news/view/15766	Microcrystallography Room temperature crystallography Drug discovery Quick screening of initial crystal
BM10 HEM	High energy, Large beam, X-ray Phase contrast imaging	Bending Magnet (2T)	5~100 (E _c :21.2)	~10¹³	Biological tissue, Battery, Developmental prototype, Electronic components, Cultural heritage etc.	1.9 x 1.1 ~200x28 mm² @ 100 m	CT image XPCI Post-processed 3D Image 1. 2. 3. 4.	Soft tissue imaging Operando or in situ CT Defect inspection Non-destructive diagnosis



Proc. SPIE 5535, Developments in X-Ray Tomography IV (2004)

https://www.esrf.fr/home/news/general/contentnews/general/a-unique-atlas-of-the-human-heartfrom-cells-to-the-full-organ.html



ID21 BioPharmaBioSAXS Beamline

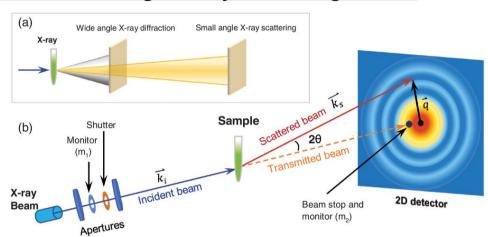
Priority support for industries

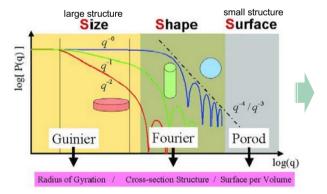






Solution Small Angle X-ray Scattering (SAXS)



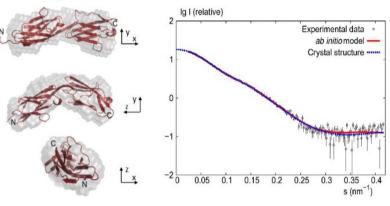


1. Size of particle

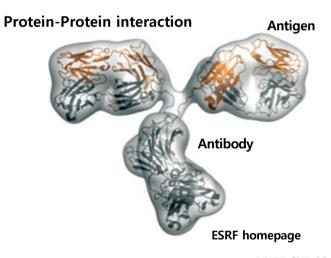
 $I(q) \sim \exp(-q^2R_g^2/3)$ (R_g : Radius of Gyration)

- 2. Form of particle $I(q) \sim q^{-1} \sim 2$
- 3. Surface structure $I(q) \sim q^{-4}$

Protein structure characterization



https://biorontech.com/biosaxs







Design Summary

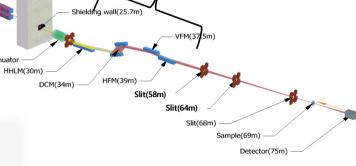
1. q-vector optimizing layout

2. High-throughput

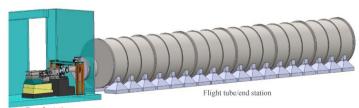


IVU24(0m)

Main
Optics
components → Placed close to the source



Automatic Sample exchanger robot



Vacuum flight tube

3. Convenience





ID21 BioPharma-BioSAXS beamline comments from MAC

\Box HHLM

• Instead of having three strips (Si, Rh, Pt) as originally planned, consider whether using only a Rh coating might suffice.

☐ Focusing Optics

 APS uses a CRL (Compound Refractive Lens) for convenience in focusing. Is there any plan to use CRLs at the 4GSR BioPharma-BioSAXS beamline?

☐ H-DCM vs V-DCM

 At APS-U, there were issues with vertical DCMs (V-DCMs) due to beam vibration and drift. Consequently, H-DCMs are now more commonly used, so it would be good to review this aspect.

☐ 3 slit system

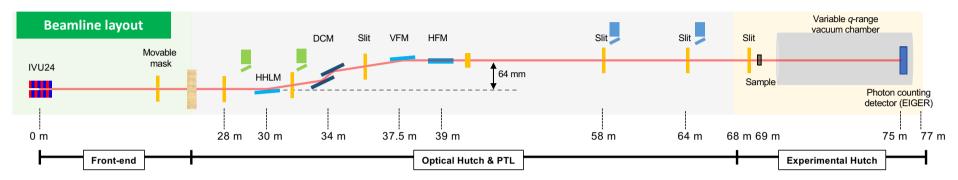
- Currently, the beam-defining slit is placed behind the KB mirror.
 Consider whether placing the slit between the DCM and KB mirror or leaving it behind the KB mirror is more advantageous.
- Depending on the final decision, calculate the size of the beam stopper at the detector.

☐ Focal point location

• In APS-U, the beam is focused at the detector. In the current design, the beam is focused on the sample. This could potentially damage the sample, and might also result in a very large beam size at the detector. Therefore, the selection of the focal point is crucial. Ideally, the beam spot on the detector should be smaller or at least comparable to the detector's pixel size, but it does not have to be strictly smaller.



Overview of BioPharma-BioSAXS Beamline



Specification

Beamline	ID21 BioPharma-BioSAXS	
Light source	In Vacuum Undulator 24 (3m)	
Energy range [keV] (mainly)	5 ~ 20 (optimized: 8 ~ 25) (12.4)	
Energy resolution (ΔΕ/Ε)	< 2 x 10 ⁻⁴	
Techniques	Solution SAXS	
Beam size at sample (μ m ²) H × V, FWHM	~ 100μm × 100μm	
Beam flux (ph/s)	~ 2 × 10 ¹³	

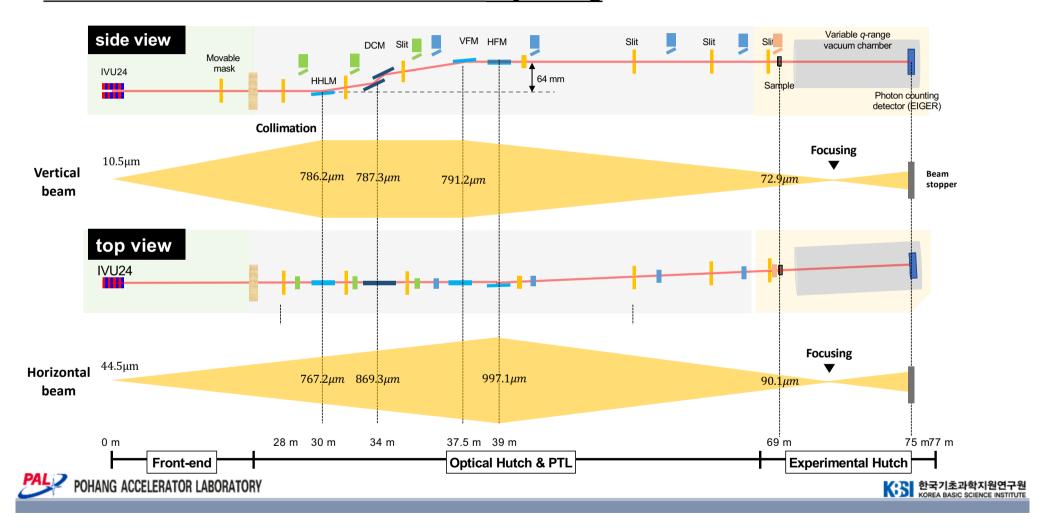
Beamline characteristics

- As an industry-prioritized beamline, an <u>automated sample exchange</u> <u>robot</u> is implemented to enable high-throughput experiments.
- A large vacuum chamber including the detector allows rapid adjustment of the sample-to-detector distance from 0.5 to 6 meters, enabling efficient analysis of samples with various sizes.
- The system is designed to <u>perform experiments under a wide range of conditions</u>—such as pH, temperature, and denaturant concentration for studies on the 3D structure and stability of biomolecular proteins.

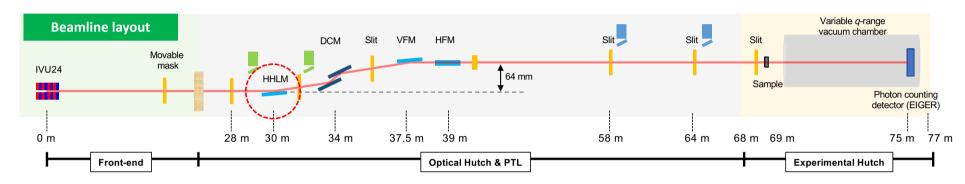


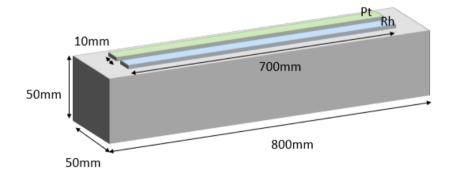


Overview of BioPharma-BioSAXS Beamline-Ray-tracing



1. M1 mirror – High Heat Load Mirror (HHLM)





Purpose of M1

(Heat load management) Reducing the heat of the white beam.

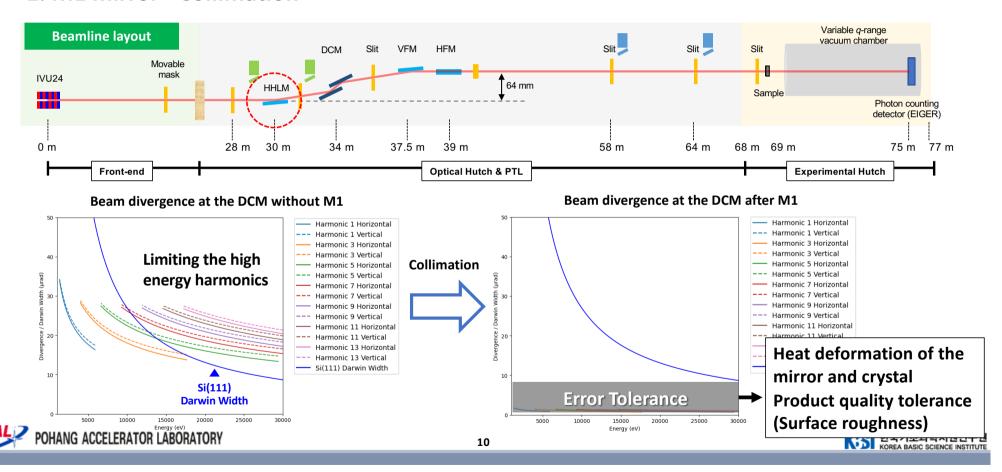
The optical components can generally be positioned further upstream, which is advantageous for securing a smaller source size.

(Collimation) By reducing the beam divergence to be smaller than the Darwin width, the flux can be preserved and energy resolution can be enhanced.

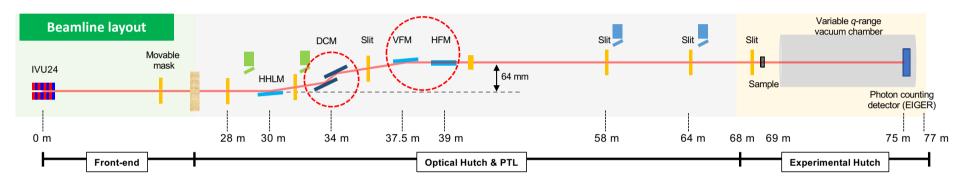




1. M1 mirror - Collimation

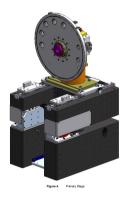


2. Double Crystal Monochromator (DCM) and 3. Focusing mirror



2. Vertical-DCM

Specification	1 st Crystal	2 nd Crystal	
Shape	Plane		
footprint	0.86 x 4.76 mm ²	0.86 x 4.76 mm ²	
Size	60(L) x 25(W) x 50(H) mm ³	150(L) x 25(W) x 30(H) mm ³	
Substrate	Si<111>		

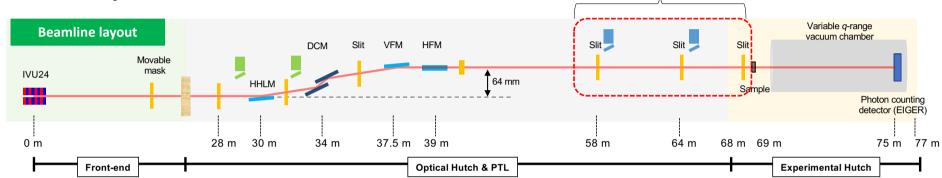


${\bf 3} \ . \ {\bf Focusing} \ {\bf mirror}$

Specification	Horizontal focusing	Vertical focusing	
Shape	Parabolic	Elliptical	
footprint(FWHM)	0.94 x 303.5 mm ² 0.74 x 377.3 r		
Size	50 x 500 x 50 mm ³		
Substrate	Si		
Coating material	Rh(50nm), Pt(50nm)		
Incident angle	2.6mrad		
p, q parameter	p: inf q: 34.125m	p: 39m q: 32m	

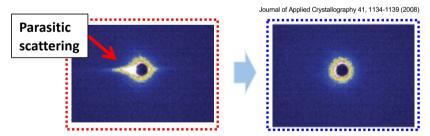


4. 3 Slit system



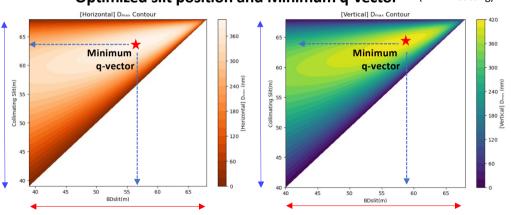
Purpose of 3 slit system

- The parasitic scattering removal
- Enhancing the q-vector



Optimized slit position and Minimum q-vector (FWHM cutting)

3 slit system

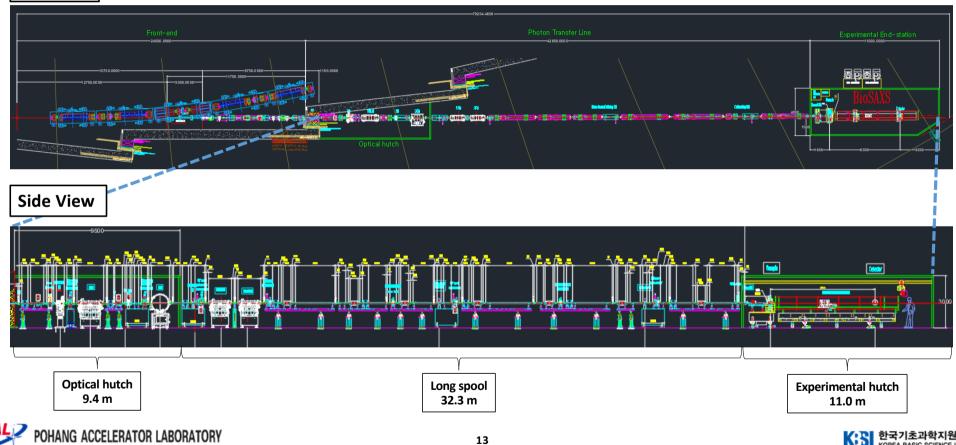






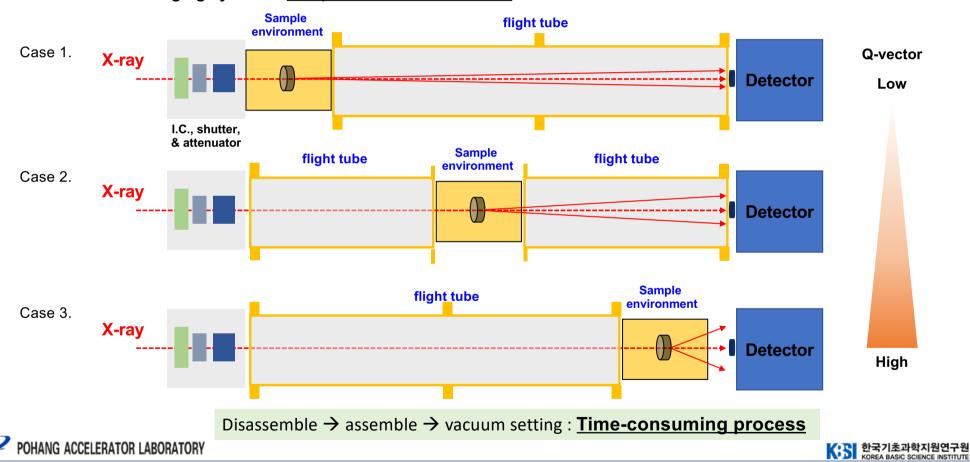
Design drawing

Top View



End-station

♦ Classical SDD changing system – Sample move, Detector fixed



End-station

◆Rapid sample to detector distance (SDD) changing system – Sample fixed, Detector move

X-ray SDD can be changed inside the flight-tube (Detector is movable along the beam flow direction)

Variable q-range vacuum flight tube

Time reduction for sample to detector distance (SDD) change

SDD: 0.5 ~ 6 m

- By fixing the sample environment position, it is easy to install robot system and build applied experiment tools
- Improvement of data quality owing to minimizing air scattering (background)

~ 0.33 electrons /Å³ (water) ~ 0.43 electrons /Å³ (biomolecules)

Vacuum flight tube examples



Example; 12 ID-B, AP-



Example: 13A BioSAXS, TPS



Example; SWING, Soleil





End-station

♦Sample changer robot

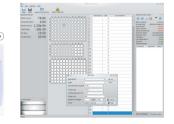
ARINAX BioSAXS



- Sample Exposure Unit (SEU)Sample Changer Unit (SCU)
- Control Software
- Control Electronics Rack
- · Fluid Management Unit (FMU)

High-throughput experiments: ~ 50 samples / 1 hour

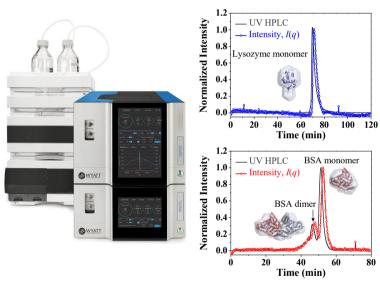
Performances				
Solution transfer volume	5 to 200 uL			
Typical cycle time - loading/unloading - cleaning (wash, rinse, dry)	50 s 15 s / 15 s 20 s			
Controls				
Sample exposure modes	Static Flow (0.05 to 20 uL/s)			
Temperature	Exposure (2 ~ 60 °C) Storage (4 ~ 40 °C)			
Dimension (W x H x D)				
Main unit (robot)	550 x 800 x 1100 mm ³			



♦ Size-exclusion chromatography (SEC)-SAXS

· Continuous-flow in-line sample purification

In-line purification of solution



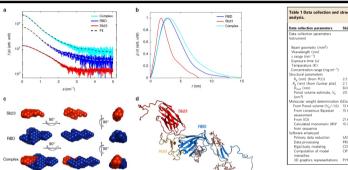
- · Agilent infinity 1260 system, RI, UV, MALS, & DLS
- Separation and Analysis of polydisperse species or mixture





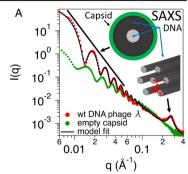
Beamline applications

Conformational Ensembles of Flexible Protein

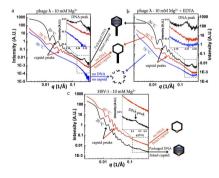


Nature Communications 11, 5588 (2020)

DNA & Capsid structure







Journal of Virology 89, 9288-9298 (2015)

Industrial application ••

Therapeutic Antibody



ex) Tracking structure of antibody molecules in solution

Company: Boehringer

Facility: ESRF

Sample: Antibody molecule in solution

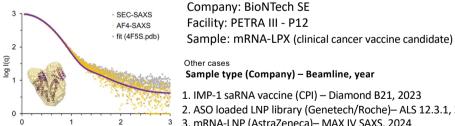
Other cases

Antibody type (Company) - Beamline, year

- 1. IgG1 mAb (Genetech) SSRL BL4-2, 2024
- 2. Pembrolizumab (Merch & Co.) Diamond B21, 2024
- 3. TrYbe (UCB Pharma) ESRF BM29, 2023
- 4. IgG1 mAb2 (Eli Lilly x UT Austin) CHESS ID7A, 2023

mRNA-LNP

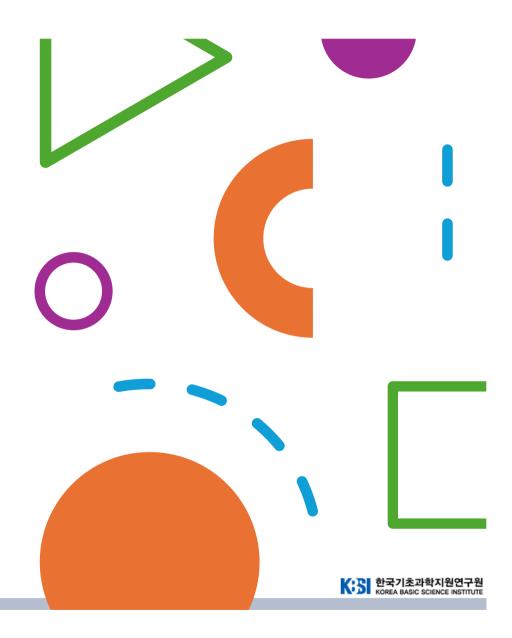
Quantitative size-resolved characterization of mRNA nanoparticles



Sample type (Company) - Beamline, year

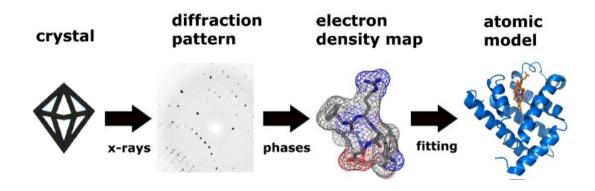
- 1. IMP-1 saRNA vaccine (CPI) Diamond B21, 2023
- 2. ASO loaded LNP library (Genetech/Roche) ALS 12.3.1, 2023
- 3. mRNA-LNP (AstraZeneca)- MAX IV SAXS, 2024
- 4. Moderna bivalent vaccine (Moderna) NSLS-II I22, 2024

ID22 Bio Nano Crystallography (BioNX) Beamline

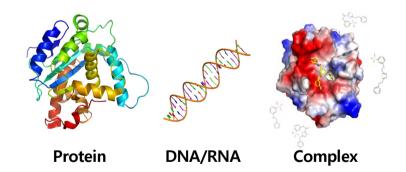




Macromolecular crystallography (MX)



The atomic resolution structure of the biomacromolecules



Users



Academia
Protein structure
and function

Industry
Structure based drug
discovery (SBDD)

Design summary

Size of Protein crystals



- 1. Micro-focusing beam
- 2. High flux

CRL defocusing

3. Rapid beam resizing



CRL Sample

R

Defocused Bigger beam size

Compound refractive lens (CRL)

4. Stable



CRL-only focusing mode

5. High-throughput



Sample exchange robot system

Smallest beam size

ID22 BioNX beamline comments from MAC, PLS-II, etc.

Optics

1. Compound refractive lens (CRL)

- ✓ Simplify the step of the beam size adjustment
- ✓ CRL-only focusing have high stability, it would be suitable for the high-throughput experiment

2. Diagnostics

- ✓ A diagnostic device used for DCM feedback should be placed as far from the DCM as possible to ensure high resolution.
- ✓ For beam stability, it is essential to use a diagnostic system with high resolution.

3. Detector

✓ It is advisable to make the purchase at the final stage, as new technology may emerge.

Data acquisition

- ✓ The data frame rate can be configured higher than the originally targeted 30 Hz it is expected to be set at 100 Hz or above.
- ✓ It is essential to secure sufficient facility computing infrastructure and local computing resources. This will be planned in conjunction with the data center strategy, and the local computing resources will be purchased at the final stage to ensure high specifications.

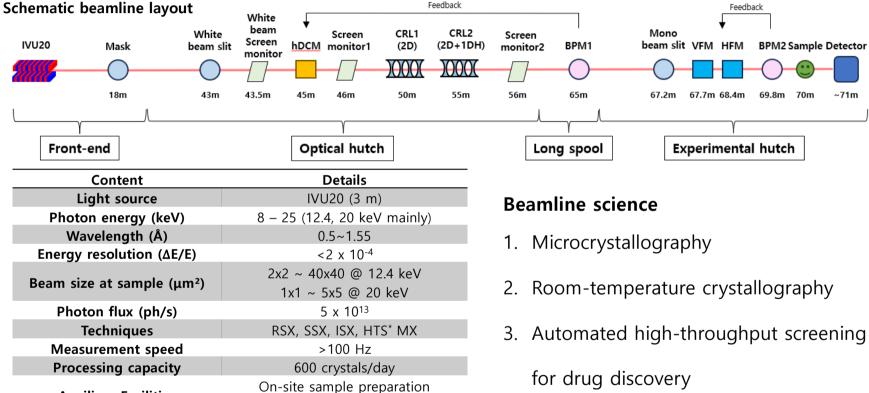
Beamline science

✓ It is necessary to consider the prioritization of beamline science objectives.





Overview of BioNX beamline



laboratory

- 2. Room-temperature crystallography
- 3. Automated high-throughput screening for drug discovery



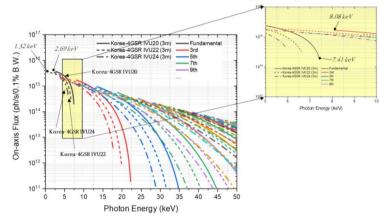
Auxiliary Facilities



Feedback

^{*)} RSX: Rotating single crystallography, SSX: Serial synchrotron crystallography, ISX: In-situ crystallography, HTS: High throughput screening

❖ Undulator: In Vacuum Undulator 20 (IVU20)

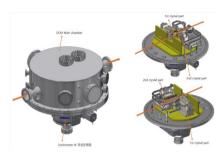


IVU Type	IVU20	IVU22	IVU24
On-Axis Flux	D+	90% of IVU20's	80% of IVU20's
On-Axis Flux	Best	between 10-30 keV	between 10-25 keV
Coherent Flux	Dt	80% of IVU20's	60% of IVU20's
Conerent Flux	Best	between 10-30 keV	beween 10-30 keV
Spectral Continuity	Missing/compromised between 7.41 keV and 8.08 keV	Perfect at gap size <5.58 mm	Perfect at gap size <6.46 mm
Heat Load on			
Beamline by	Best: 12 kW at the	15 kW at the minimum	17 kW at the
Off-Axis	minimum gap size	gap size	minimum gap size
Radiation			

Ha, et al., JKPS (2024)

- ✓ Low heat load
- ✓ High flux
- ✓ Missing 7.41 keV and 8.08 keV (limited the usage of this range) (Cobalt (7.7 keV) energy scan range)

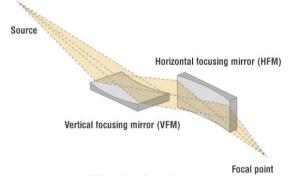
Optics Features

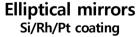


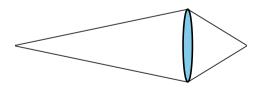
Horizontal DCM Si(111) crystal



Compound refractive lens (CRL)
Diamond lens (large aperture)







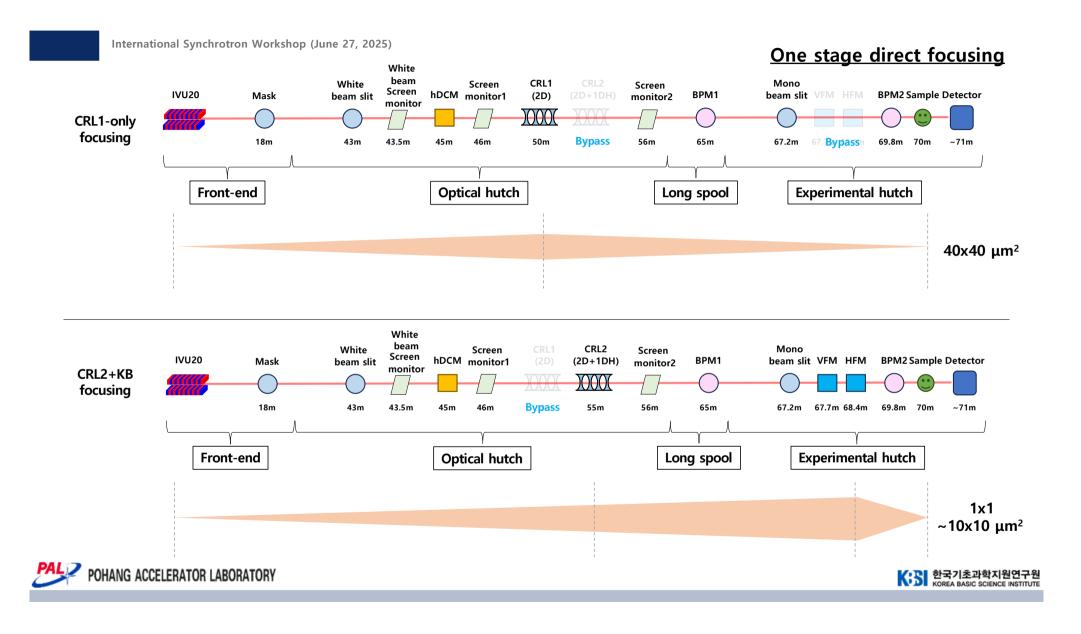
One stage direct focusing

Full beam

Simplifying optics
Improved manageability







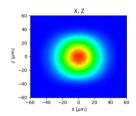
Two focusing modes

1) CRL1-only focusing mode

- High Beam position stability
 - → Suitable for continuous and long-term experiments
- For rotating single crystallography
- For high-throughput screening
- 24 h experiment, automation, remote experiment
- 12.4 keV fixed, Beam size fixed focusing (40x40 micron)

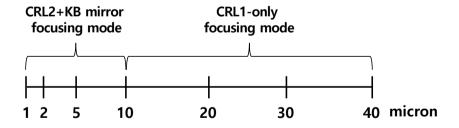
CRL spec

2D diamond CRL, ROC 1 mm, Aperture 1.5 mm, 7 ea



Beam size: 40x40 um² (FWHM) Flux: 3.0x10¹³ ph/s

* 10~40 micron: diffractometer aperture

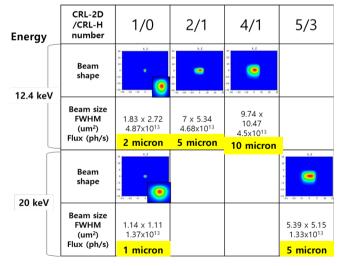


2) CRL2+KB focusing mode

- Beam position stability could be low due to the vibration of the KB mirror → Suitable for short-term and on-site experiment
- For micro-focusing (<10 micron)
- For serial crystallography, in-situ crystallography
- 12.4 keV or 20 keV, Beam size 1~10 micron

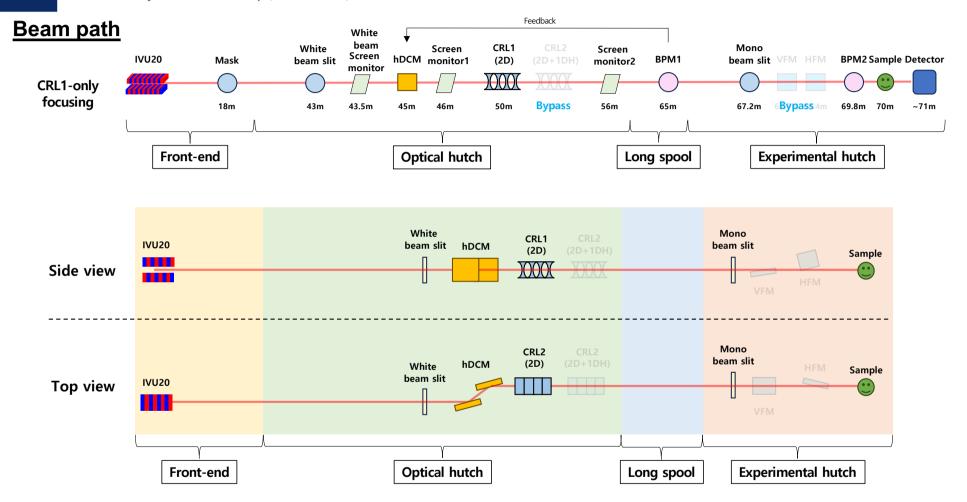
CRL spec

2D diamond CRL, ROC 6 mm, Aperture 2 mm, 5 ea 1D diamond CRL, ROC 2.1 mm, Aperture 2 mm, 3 ea



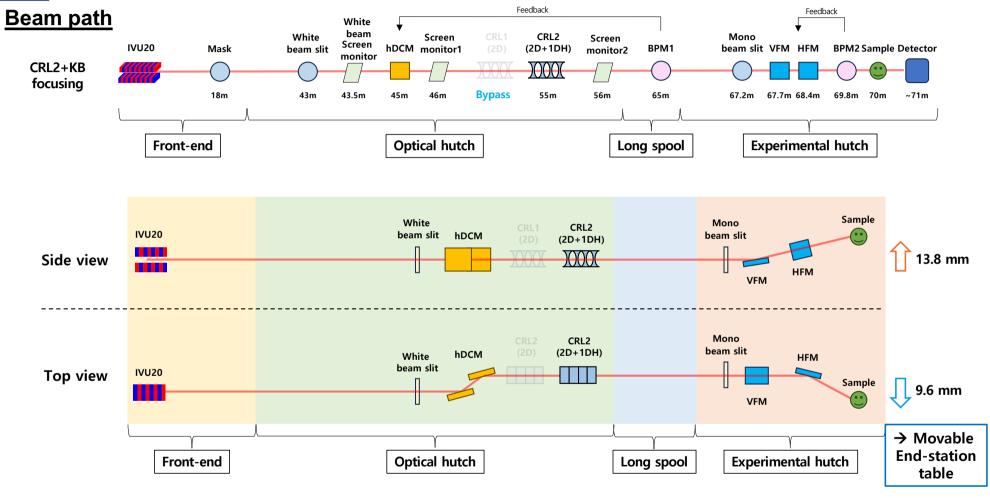
3 beam sizes 2~10 micron ~5x10¹³ ph/s

2 beam sizes 1~5 micron 1.3x10¹³ ph/s





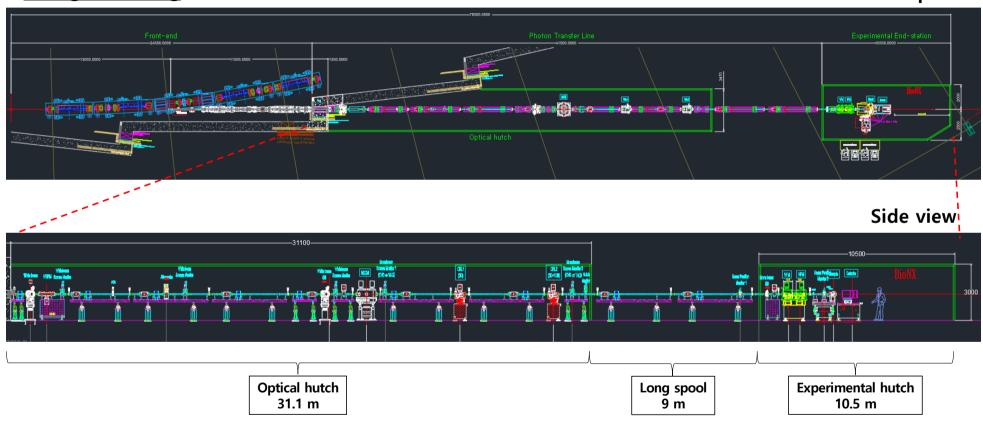






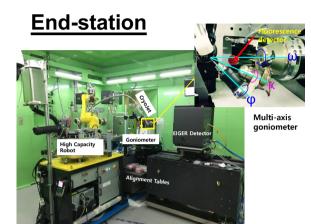


<u>Design drawing</u> Top view









High precision diffractometer



- Sphere of confusion: 100 nm
- Raster scan at 15 mm/s
- Rotation speed: 720 deg/s
- Easy to change various goniometer heads

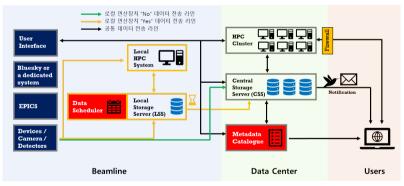
DAQ

Data acquisition/management

Web-based User Interface (WUI) Remote access



Computing infrastructure



Local HPC system and data center

Silicon drift X-ray detector

Layout

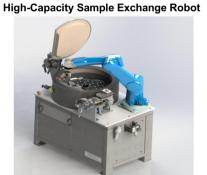


- X-ray fluorescence
- Element scan



EIGER2 XE 16M

- Frame rate: 560 Hz (16 bit), 700 Hz (8 bit)
- Active area: 311 x 328 mm2
- Energy range: 6-40 keV



592 sample/storage chamber ~600 sample/day



Sample preparation laboratory



~ fragment library on ECHO plates (ex. 1200 fragments)



~ Crystallization Plates (ex. 1200 crystal drops)

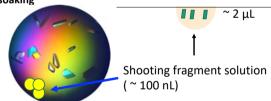




Precise, Accurate, and Efficient Liquid transfer using sound



- ~ 30 sec for 100 fragments (one crystallization plate)



Crystallization robot Crystal imaging system

- Facility for sample preparation of FBDD experiment
- Support drug discovery experiment





Floor plan of sample preparation laboratory

실명벤치 (개수대 불포함)

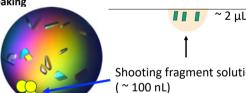
실험벤치 (개수대 불포함)

Cold room

내벽A_1F_BASIC_230731



- ~ Soaking fragments into crystal drops on plate
- ~ singleton soaking

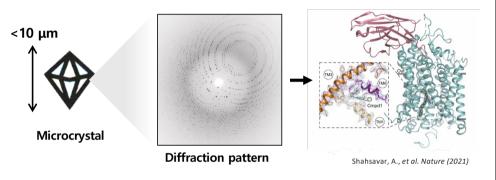


한국기초과학지원연구원 KOREA BASIC SCIENCE INSTITUTE

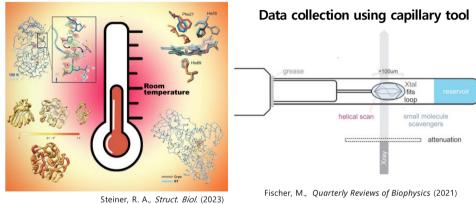
내벽A_1F_BA

Beamline applications

High-quality data from microcrystal

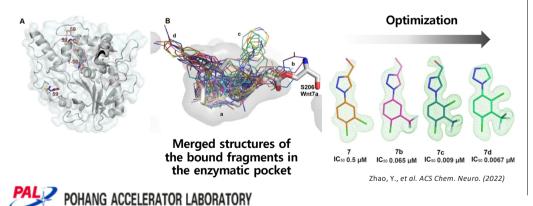


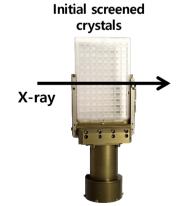
Room-temperature crystallography



Quick screening of initial crystal

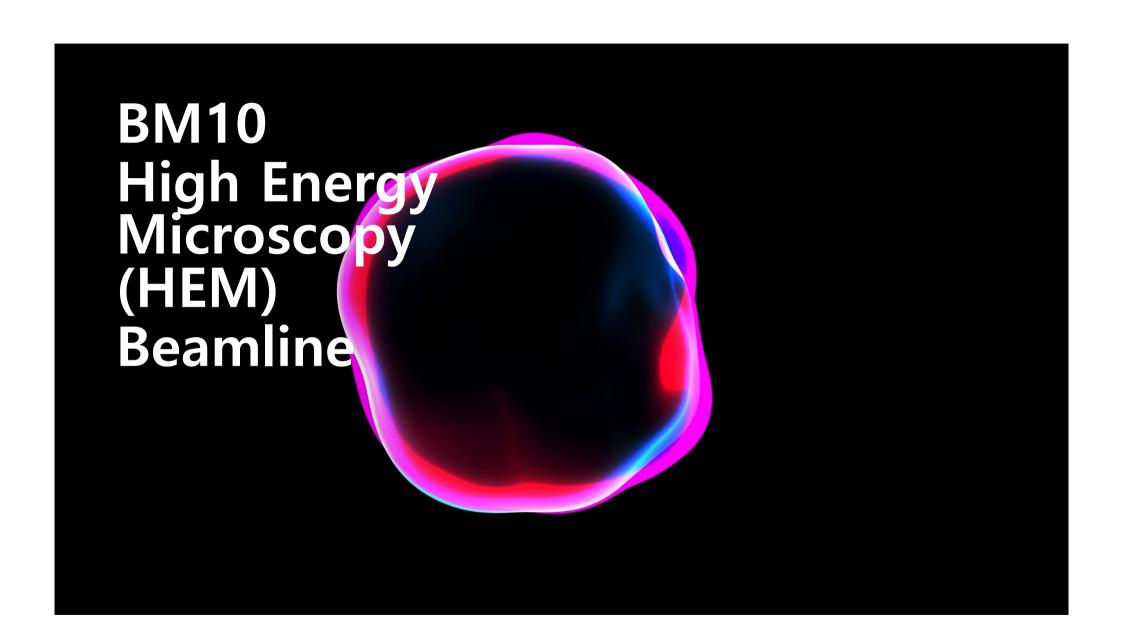
Drug discovery





Diffraction or not?



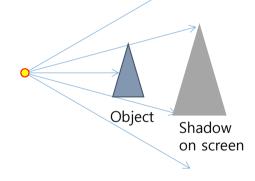


Projection imaging

Conventional projection image

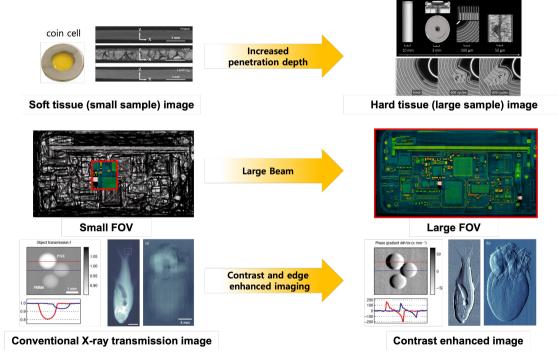






Synchrotron source

- · High resolution imaging
- High photon flux
 - Fast scan
 - ➤ In situ/operando imaging studies







Design summary

1. High energy beam

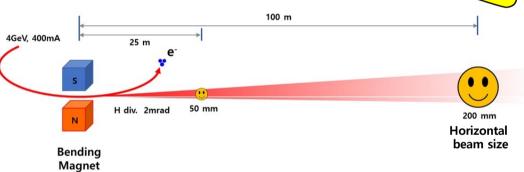
Light source: Bending magnet Electron bunch

X-ray

100 m

2. Large beam

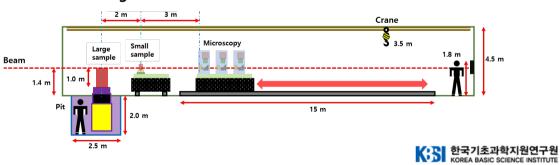
Long beamline 121 m



End-station design

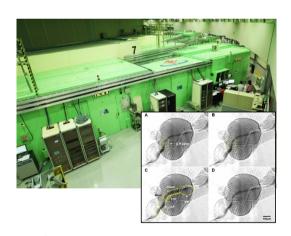
3. Phase contrast effect

SDD: 5~20 m



Beamline Design concept

- Full field imaging
- Hard sample > 100 keV
- Large sample 200 mm beam size (Bending magnet or Wiggler and Long beamline)
- Fast scan
 - ✓ e.g. 20 hours/scan conventionally -> 10 minutes/scan using synchrotron radiation
- In situ/operando imaging studies



High Energy Microscopy beamline





PLS-II X-ray imaging beamlines

Commercial X-ray imaging systems





BM10 HEM beamline Comments

Source

- Was the possibility of using a 3-pole wiggler as a source investigated? (MAX-IV Dr. Aymeric Robert)
- In low-emittance storage rings, the use of a wiggler can produce a spectrum similar to that of an undulator, with discrete harmonic peaks rather than a broad bending magnet spectrum. Furthermore, the photon beam profile tends to exhibit a donut shape rather than a Gaussian distribution.
- Although the flux from a 4th-generation storage ring (4GSR) bending magnet is generally lower than that of a wiggler, it is still possible to meet the beamline requirements (~100 keV) by utilizing a 2 T bending magnet source.
- At the sample position, the photon flux achievable with this setup is comparable to that of the ESRF-EBS BM18 beamline, which employs a 3-pole wiggler, particularly in the key energy range of 40–100 keV.

End-station

- ➤ In designing the beamlines, it is important to utilize state-of-the-art technologies, rather than those available today, for key components such as X-ray optics and detectors. International technical reviews will be recommended for this purpose. (IAC)
- We have already recruited International Machine Advisor Committee (IMAC) for each beamline, and as of January 2025, most beamlines have undergone reviews of their key components (optics, end-stations, detectors, etc.), with corresponding reports completed.

Data acquisition

- > Is it possible to improve the time resolution of the detector? (KOSUA)
- It is possible to achieve higher time resolution by replacing the detector with one that offers faster readout speed. However, the primary objective is to obtain high-quality images.

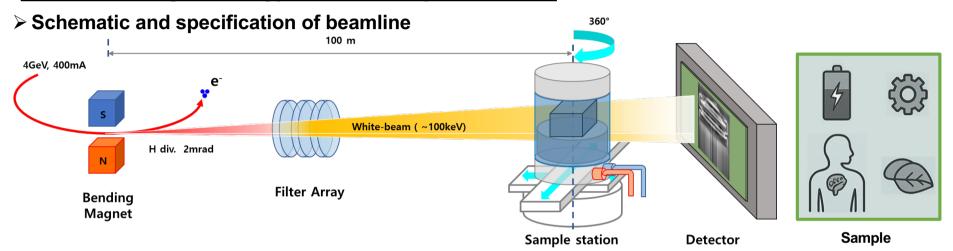
Application

- ➤ Is bio-medical research feasible? (KOSUA)
 - Yes, it is feasible. The experimental hutch is designed with ample space to accommodate a wide range of experiments beyond animal studies.





Overview High Energy Microscopy Beamline



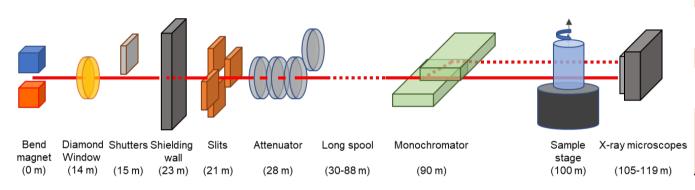
> Beamline Science

- High-energy beam : Energy & Materials applications
- Large beam size : Industrial applications
- Phase contrast effect : Bio-medical imaging

Light Source	Bending Magnet (2T)		
Photon energy	5 ~ 100 keV (E _c :21.2 keV)		
Beam size (FWHM)	200 mm x 28 mm @ 100 m		
SDD	< 20 m		
Spatial resolution	> 0.5 um		
Technique	Projection imaging		
Sample space	a few m (100 kg)		

Layout of the HEM beamline

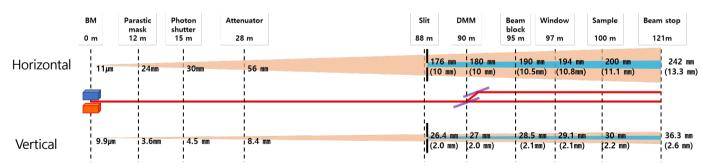
> Beamline Layout



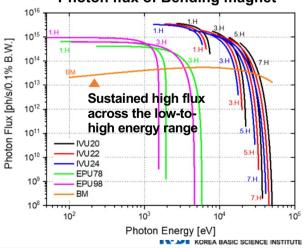
> Source

Source Type	Bend Magnet (BM)	
Magnetic Field	2 T	
Magnetic Radius	6.67 m	
Total Power	1.36 kW	
Critical Energy	21 keV	
Electron source size (σ_x, σ_y)	4.6 μm, 4.4 μm	
Electron source divergence (σ'_x, σ'_y)	12 µrad, 1.3 µrad	
Photon source size in FWHM (Horizontal, Vertical)	11 μm, 9.9 μm	
Photon source divergence in FWHM (Horizontal, Vertical)	2 mrad (433 W) 0.3 mrad @ 10 keV	

> Raytracing

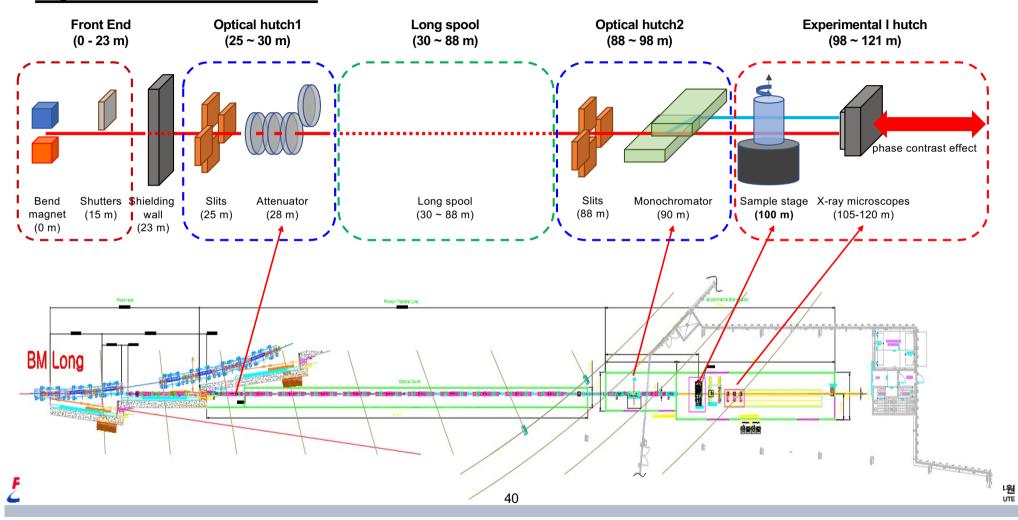


Photon flux of Bending magnet

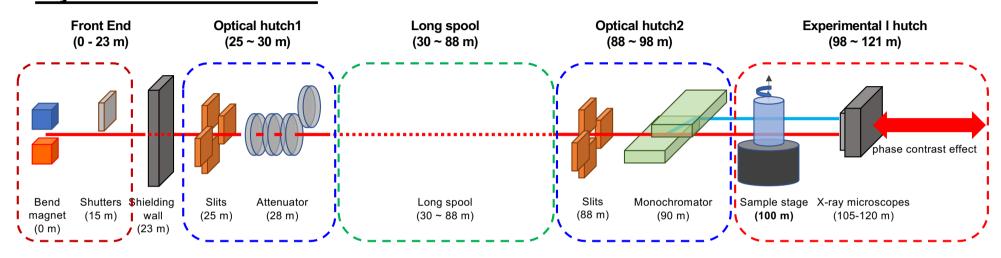


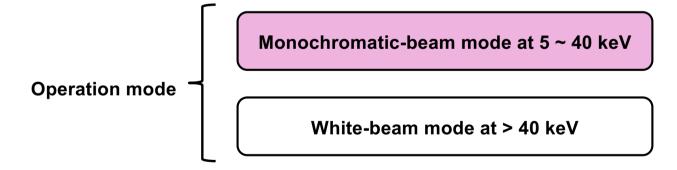
PAL POHANG ACCELERATOR LABORATORY

Layout of the HEM beamline



Layout of the HEM beamline

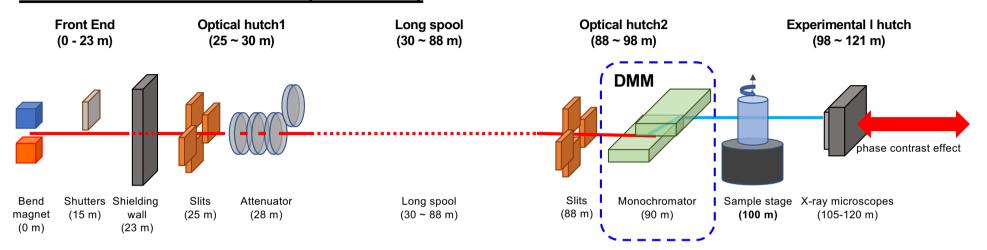








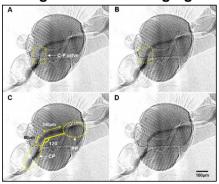
Monochromatic-beam mode (5 ~ 40 keV)



Double Multilayer Monochromator (DMM) Specifications

Properties	DMM (2strips)			
Properties	Ru/C (5~20keV)	W/B4C (20~40keV)		
Mirror size	55 mm (W) x 310 mm (L) x 50 mm (H)			
Optical footprint	10 mm (W) x 258 mm (L) 10 mm (W) x 258			
Coating strip number	2 ea			
Coating strip size	24 mm (W) X 304 (L) mm	24 mm (W) X 304 (L) mm		
Beam size	10 mm × 2.0 mm @ 20 keV	10 mm × 2.0 mm @ 40 keV		
Coating material	Ru/C	W/B4C		
Period Thickness	4 nm	2 nm		
Number of Periods	100	200		
Substrate material	Si			
Mirror surface shape	Plane			
Reflection direction	vertical			

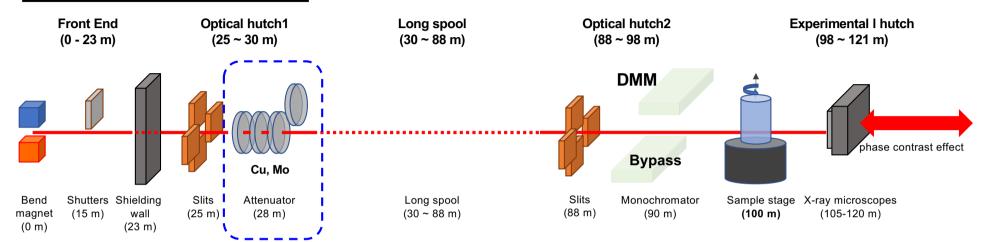
Sub-micron High resolution imaging



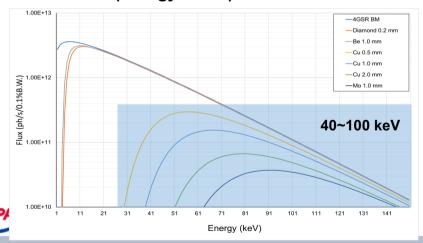




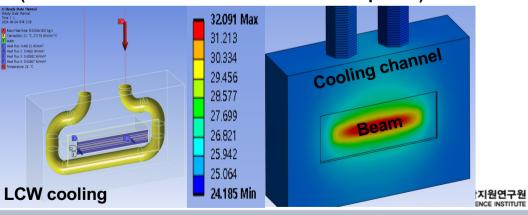
White-beam mode (40 keV ~)

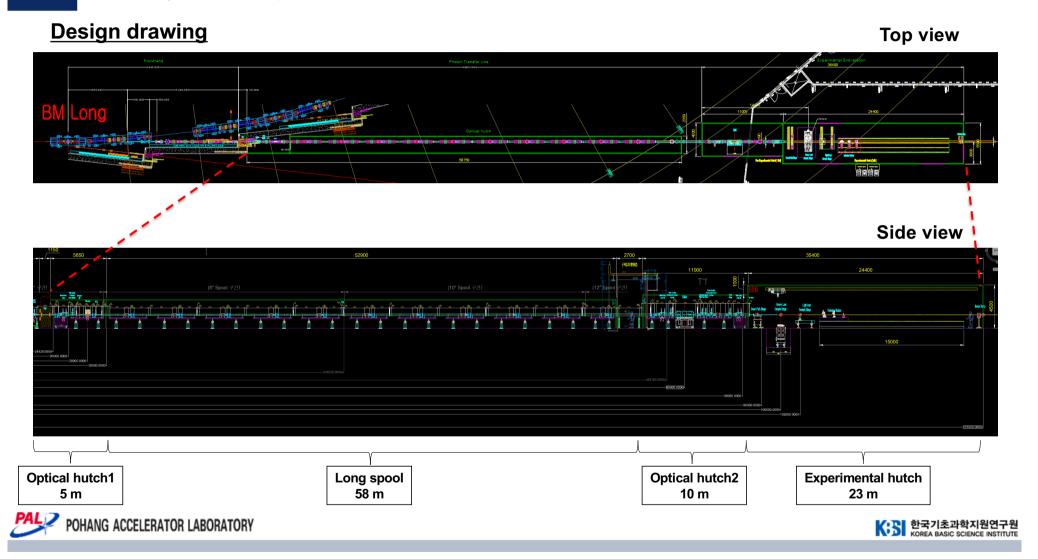


> Attenuator (Energy filters)

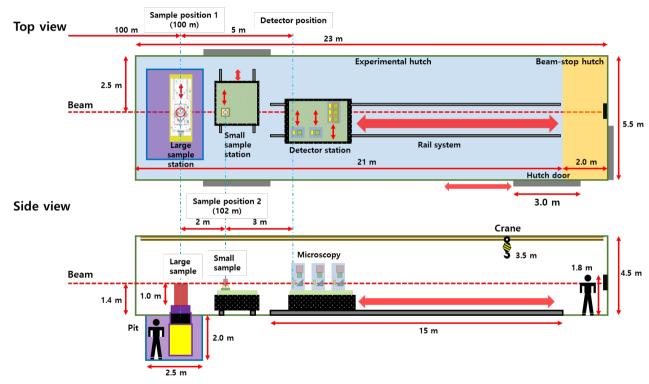


> Thermal analysis for attenuator (The white beam attenuator is under development)

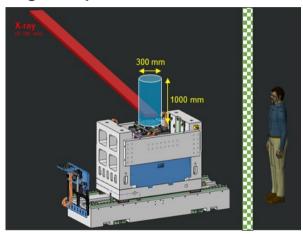




End-station



≻Large sample station



➤ Large sample stage Specifications

Data	Dimension	
Sample weight	100 kg	
Sample dimensions	H=1.0 m, D=0.3 m	
Rotation stage	RT500S	
Angular accuracy	< 2 arcsec	
Max. speed	60 rpm	

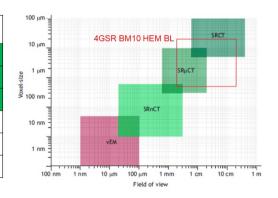




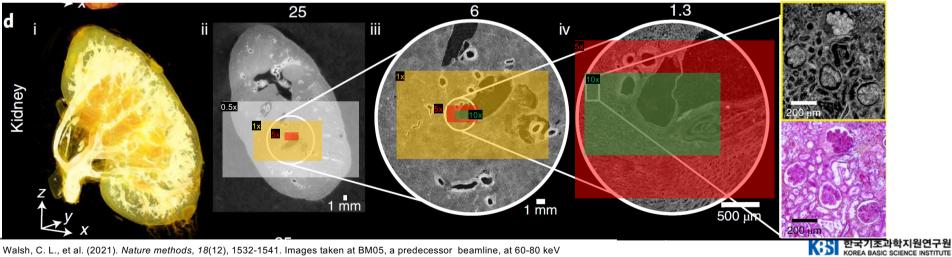
Multiple scale CT scan (Establishing a strategy of the collection of Large FOV image and Mass Data Process)

➤ Image specification (4GSR BM10)

X-ray microscope	Array detector	Single detector			
Field of View	200 x 25 mm ²	38 x 21 mm ²	19 x 11 mm ²	3.8 x 2.1 mm ²	1.9 x 1.1 mm ²
Magnification	0.25x	0.5x	1x	5x	10x
Pixel size	20 µm	9.2 µm	4.6 µm	0.92 μm	0.46 μm
Pixel number	26 million	9 million			
Data size	52 MB/image	18 MB/image			
3D data size	205 GB/set	25 GB/set			



Multiscale Phase-Contrast Tomography (ESRF-EBS BM18)





Beamline applications

