Macromolecular SAXS

Size • Shape • Flexibility • Assemblies • Solution State

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Photo left:
Dr. Y. Shimura (founder of Rigaku) and Professor A. Guinier. This photo was taken in 1959 at the front gate of Rigaku Science & Research Laboratory in Tokyo. Shimura and Guinier became friends when Rigaku undertook the project to publish the Japanese version of Guinier's famous X-ray book, "The Theory and Technique of X-ray Crystallography."

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OUR PASSION

The determination of the first protein structure (myoglobin) in 1957 served as a watershed event for structural biology. Crystallography and NMR continue to provide accurate, high resolution structural information that can be used in understanding the function of biological molecules, but an additional technique (SAXS) is now becoming widely used as a complementary tool for the structural biologist. As larger and more complex molecular systems are being studied, SAXS provides the ability to determine the size and shape of macromolecules in solution. Moreover, SAXS can be useful for providing information about the molecular arrangements of assemblies, when combined with complementary biophysical techniques. Rigaku Oxford Diffraction is proud to play an integral part in developing and providing the tools that help advance the science of structural biology.

ROD BioSAXS-2000^{nano}_brochure_en_Ver1_2018.6.07

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Solution scattering from biological molecules



Rigaku
oxford diffraction

Rigaku's BioSAXS-2000^{nano} system

DESIGNED FOR MAXIMUM PERFORMANCE

Rigaku's BioSAXS-2000^{nano} instrument is a small angle X-ray scattering (SAXS) system designed to measure SAXS data on both isotropic and anisotropic samples. The system features beam conditioning components such as Rigaku's precision-milled and polished Kratky block along with Rigaku's OptiSAXS optic, a double-bounce confocal optic with a large capture angle to deliver a spectrally pure beam with high flux. Because the focal point of the OptiSAXS optic remains at the detector, there are no requirements for data corrections caused by instrumental smearing. The system also features Rigaku's HyPix-3000 hybrid photon counting (HPC) detector with small pixel size, high sensitivity and essentially no noise.



Rigaku HyPix-3000



BioSAXS-2000^{nano} system with Automated Sample Changer (ASC)

The versatile BioSAXS-2000^{nano} can be installed on a variety of X-ray sources, including the open port of a Rigaku rotating anode. The new design has the known features of the proven BioSAXS-2000[1] with the addition of hot-swappable stages that enable measurements on anisotropic samples, as well as wide angle X-ray scattering (WAXS) and grazing incidence (GISAXS). The currently installed stage is automatically detected by hardware and software so there is no downtime associated with software or hardware reconfiguration –two simple mouse clicks in the software and a different type of measurement can begin.

BioSAXS-2000^{nano} system specifications

X-ray generator	MicroMax™-007 HF	FR-X
Camera length	~500 mm	
Sample volume	20 – 30 µL	
Beam size	Variable width x 1500 µm (at sample), 530 µm (avg. at detector)	
Cu $K\alpha$ flux at sample	3.4 x 10^9 ph/s	8.4 x 10^9 ph/s
Incident beam optics	OptiSAXS	
Collimation	2D Kratky (X stage and tilt, motorized)	
Sample stage	3 capillaries (X,Y stage, motorized) with a flow cell option	
Beam stop	PIN diode detector (motorized)	
q range	0.006 – 0.65 Å^{-1} (up to 3.6 Å^{-1} with variable distance)	
Size	238 mm (width), 1392 mm (depth), 546 mm (height), 150 kg	
Detector	HyPix-3000 HPC detector Active area: 77.5 mm x 30.5 mm Pixel size: 100 µm	

DESIGNED FOR EASE OF USE

The BioSAXS-2000^{nano} system, the BioSAXS AUTO components and the SAXSLab control software are each designed for maximum ease of use. Updates to the vacuum enclosure include a large access door that allows easy access to internal components for quick stage swaps and a one step easy door lock. Special parking hardware inside the vacuum chamber means that you do not have to uninstall the sample changer flow cell when swapping components to perform materials science experiments. Other ease-of-use design features include:

- Automated system alignment of collimation hardware and beam stop.
- Automated sample alignment for multiple samples.
- Software integrated help videos and quick start guides.
- Remote assistance and troubleshooting from applications and service personnel.
- Automated processing software to provide automatic checks for radiation damage and assessment of the useful experimental data range and q_{max} determination.

KEY FEATURES

- 2D modern Kratky system with OptiSAXS confocal optic.
- Automated alignment of collimation system, sample positions and beam stop.
- Photodiode beam stop with real time intensity measurement.
- Rigaku's HyPix-3000 HPC detector for high-sensitivity, low-noise measurement.
- Automated sample loading and data analysis for aqueous macromolecular samples.
- Optional stages for studying materials and anisotropic samples.

Unique benefits of the BioSAXS-2000^{nano}

- Easily installed on the open port of an existing Rigaku generator.
- 2D Kratky design means higher flux on the sample without the need for data corrections due to smearing.
- OptiSAXS optic offers a large X-ray capture angle to deliver outstanding flux performance and faster SAXS experiments.
- Rigaku's HyPix-3000 HPC detector delivers highest sensitivity and lowest noise, making it ideal for measuring the weak scattering signal from biological solutions.
- The BioSAXS AUTO package adds automated sample loading, data collection and analysis to make SAXS experiments faster to perform and easier to evaluate for both novices and experts.
- New "nano" options expand the measuring capabilities of the instrument for measuring WAXS data, as well as measuring data for anisotropic samples.

[1] Licai Jiang, Rigaku Innovative Technology US patent: 8,094,780., Japan patent: 5,214,442. Europe: patent pending

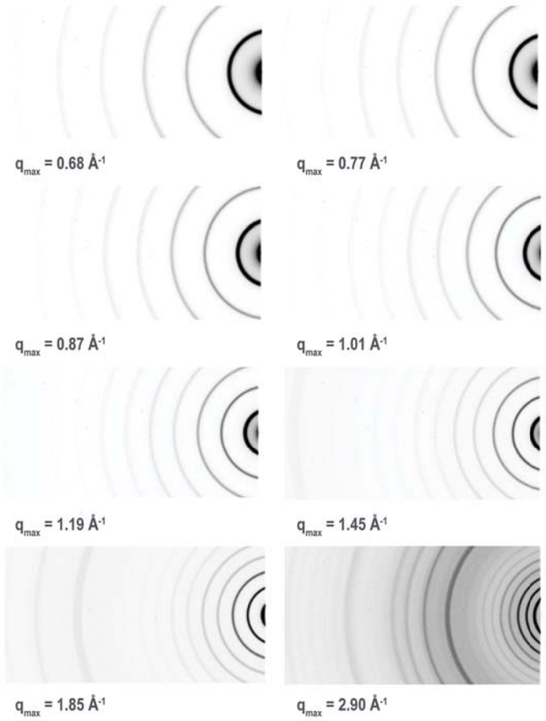
BioSAXS-2000^{nano} – A single platform to support a broad range of samples

VARIABLE q STAGE

For experiments that require measurement at higher scattering angle the optional variable q stage provides that capability. In this case, the sample stage is mounted on a motorized Z-stage that allows translation along the beam to decrease sample-to-detector distance and effectively increase the maximum measurement angle. Moreover, users can still use the optional measurement stages.

The q range is continuously selectable from the SAXSLab software and features the following specifications:

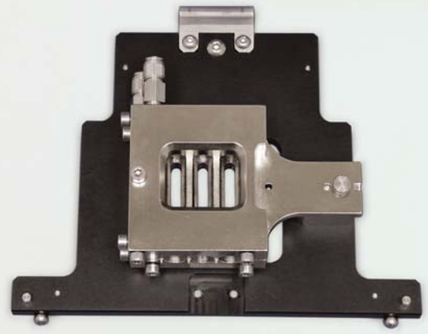
q range coverage		
SAXS	0.004 Å^{-1}	0.6 Å^{-1}
WAXS	0.03 Å^{-1}	3.6 Å^{-1}



Example images collected at varying sample to detector distances.

CAPILLARY STAGE

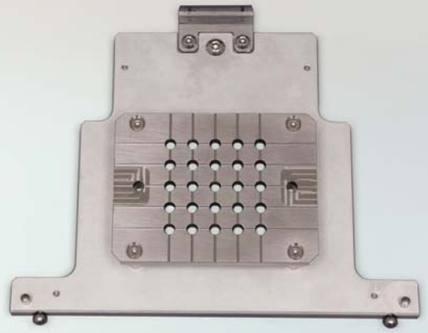
All BioSAXS-2000^{nano} systems come standard with a capillary stage. The capillary stage accommodates one calibration standard and up to 3 static, capillary cells. If the ASC is used then one of these positions is occupied by a flow cell. The stage is motorized along X and Y to position solution samples and calibration standards in the beam.



Capillary stage

POWDER STAGE

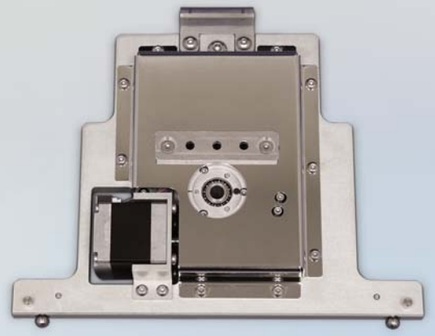
The optional powder stage holds an array of up to 27 experimental powder samples or calibration standards. As with the capillary stage, samples and calibration standards are positioned by motorized X and Y movement.



Powder stage

ROTATION STAGE

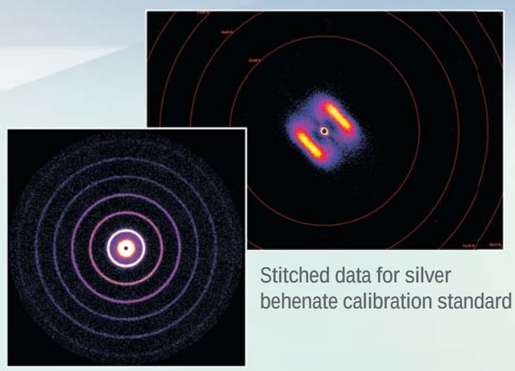
The optional rotation stage allows 360° data collection of anisotropic scattering samples. Simply collect at the desired number of rotation angles and the software will stitch the images together seamlessly. Example stitched images are shown below.



Rotation stage



Stitched data for stretched LDE polymer



Stitched data for silver behenate calibration standard

ADDITIONAL STAGES AND CONFIGURATIONS

The BioSAXS-2000^{nano} includes options for additional stages and configurations. Contact Rigaku with your application needs for more information.

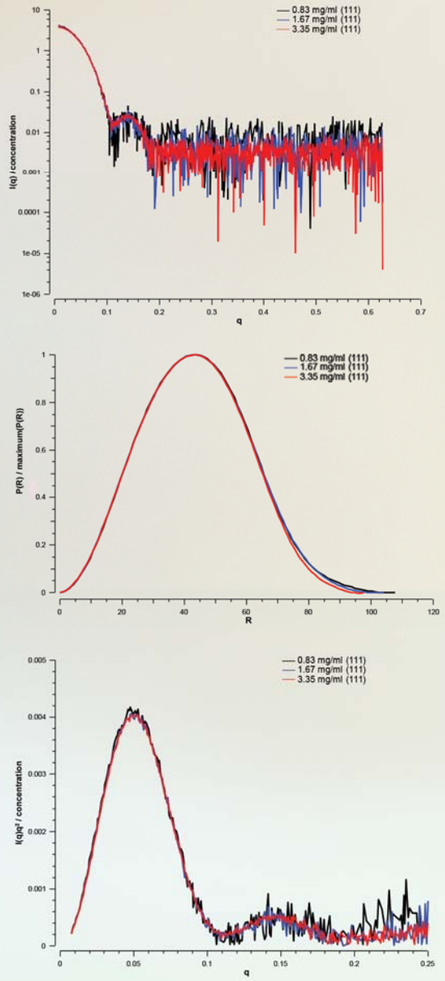
Experimental example

GLUCOSE ISOMERASE

The BioSAXS-2000^{nano} system with AUTO package and HyPix-3000 HPC was used to automatically collect and analyze SAXS data for a concentration series of glucose isomerase (GI), a tetramer with molecular weight of 172 kDa and published Rg of 32.5 Å. SAXS data were collected for GI solutions (0.83 mg/mL, 1.67 mg/mL and 3.35 mg/mL) and buffer (10 mM Tris pH 7.5, 200 mM NaCl, 1 mM $MgCl_2$) for 15 minutes with images acquired every 5 minutes.

The SAXS data were radially averaged, converted to 1D plots of I vs. q, buffer subtracted and analyzed using the Automatic Analysis Pipeline (AAP) in the Rigaku SAXSLab software package. A separate human serum albumin (HSA) standard was also measured for molecular weight (MW) estimates. Scattering, Kratky and P(r) plots are shown below and structural parameters are reported in the table.

All plots overlay and structural parameters are consistent across the concentration series. Moreover, the experimental scattering overlays well with corresponding calculated SAXS profiles. These results illustrate the effectiveness of the BioSAXS system and automated tools.



GI SAXS profiles (a), P(r) plots (b), Kratky plots (c)

Conc.	Guinier I(0)/c	Guinier R_g AutoR_g[1]	Real Space R_g autoGNOM[1]	D_{max}	MW (by HSA)	MW (by Porod volume)	q_{max} shanum[1]
0.83 mg/mL	4.3	33.7 (1.0) Å	33.4 Å	108 Å	175 kDa	163 kDa	0.63 Å^{-1}
1.67 mg/mL	4.2	33.3 (0.5) Å	33.3 Å	104 Å	170 kDa	161 kDa	0.63 Å^{-1}
3.35 mg/mL	4.1	32.8 (1.2) Å	32.8 Å	98 Å	166 kDa	148 kDa	0.63 Å^{-1}

BioSAXS AUTO - Automation

AUTOMATED SAMPLE CHANGER

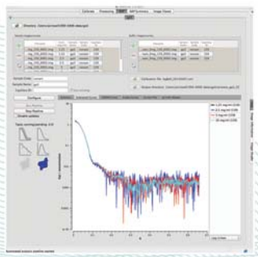
For those labs that desire unattended sample mounting and data collection, the BioSAXS-2000^{nano} includes an optional flow cell and ASC. Together, these components add automated sample loading, washing and data collection capabilities to the system. The ASC supports samples supplied in 96-well plates and 0.2 mL PCR tube arrays. The ASC seamlessly integrates with the BioSAXS-2000^{nano} system and SAXSLab software to allow easy setup of unattended or overnight experiments so that you can focus more time where it counts, on SAXS data analysis and publication of results. Features of the BioSAXS-2000^{nano} ASC include:

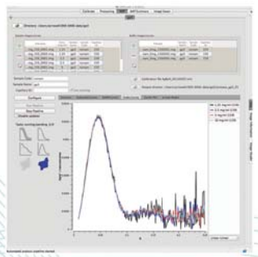
- Flow cell for unattended sample loading from samples in 96-well plates and PCR tubes.
- Foil-sealed samples to ensure that your samples won't evaporate prior to data collection.
- Multiple cleaning solutions.
- Temperature controlled sample storage, including option for separate storage versus data collection temperatures.

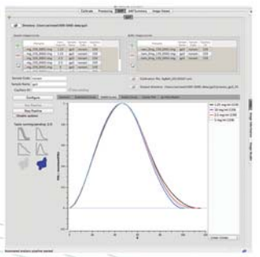


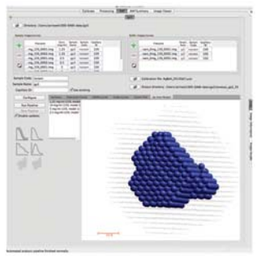
Automated Sample Changer











AUTOMATED DATA COLLECTION AND ANALYSIS

The BioSAXS-2000^{nano} system is controlled by the Rigaku SAXSLab software, a single package that integrates data collection, data processing and data analysis. Rigaku offers an optional AAP that utilizes the industry standard ATSAS package to provide the following automatic analyses for each sample:

- Automatic sample evaluation and aggregation identification.
- Automatic profile averaging and buffer subtraction.
- Automatic Guinier plot generation and calculation of R_g and I(0).
- Automatic Kratky plot generation.
- Automatic Porod volume and MW calculation.
- Automatic P(r) calculation with report of real space R_g and D_{max}.
- Automatic estimation of qmax from useful Shannon channels.
- Automatic MW calculation by the Volume of Correlation, Vc.
- Automatic envelope calculation, averaging and analysis.
- Automatic shape classification.
- PDF report generator and easy results review for previous AAP runs.

The AAP displays results for all concentrations of a sample with a comprehensive table of calculated structural parameters along with warnings to the user in cases of poor sample quality, aggregation or other problems. With the AAP, users get a gauge of sample quality as soon as data collection is complete.

[1] Petoukhov, M.V. et al., (2012). J. Appl. Cryst. 45, 342-350.