Solution scattering experiments at SSRL BL4-2

Autosampler, SEC-SAXS and others

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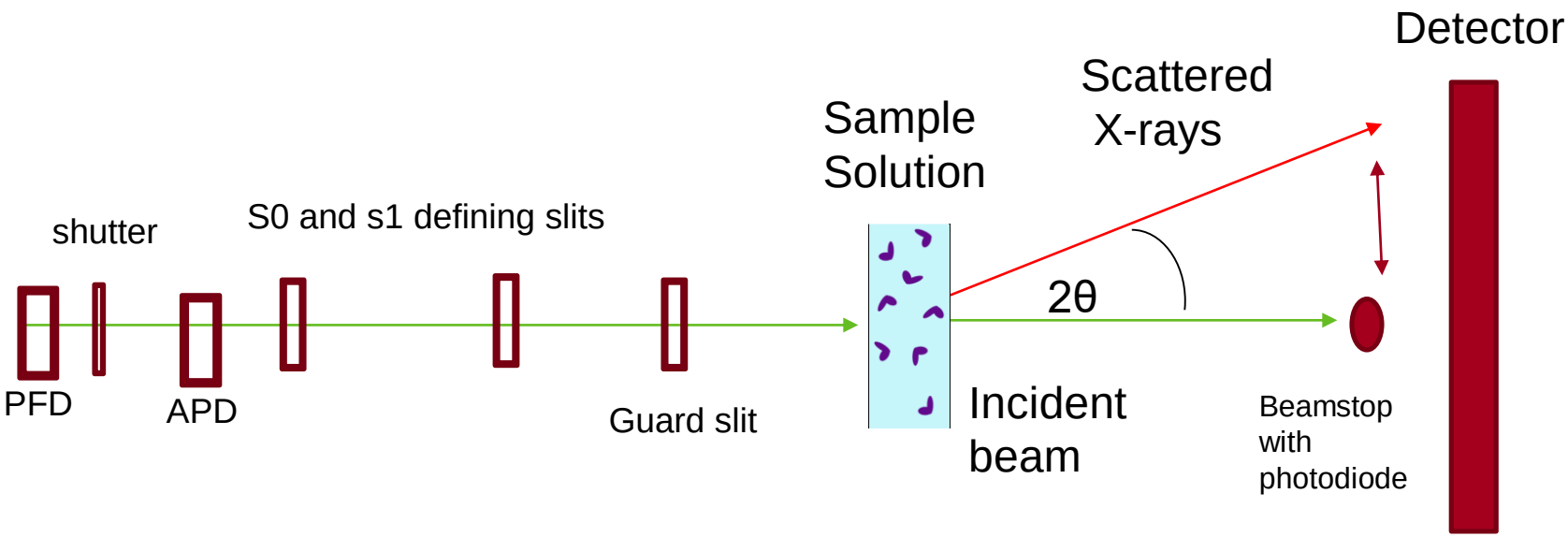
BioSAXS workshop 2019

U.S. DEPARTMENT OF ENERGY
Office of Science

SLAC NATIONAL ACCELERATOR LABORATORY

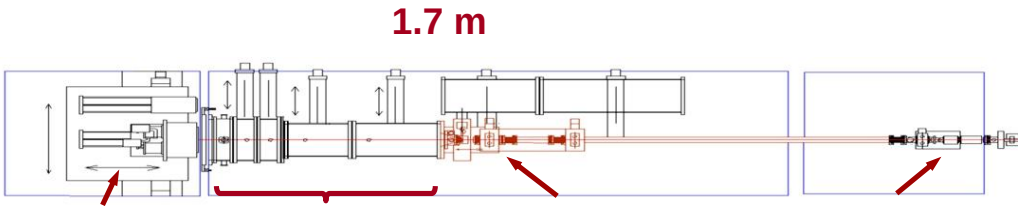
Beamline layout



The intensity at q is measured, where q is the angular dependence defined as:

$$q = 4\pi \sin \theta / \lambda$$

Beamline layout

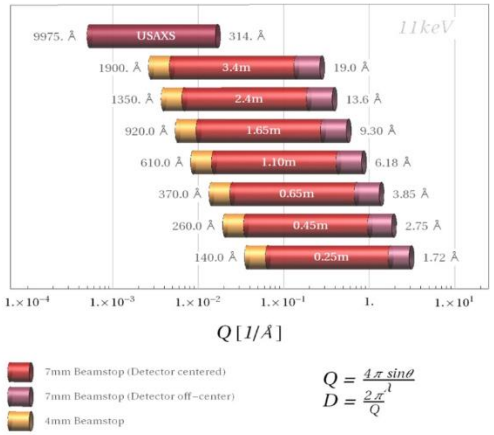


motorized detector stage

adjustable flight path

movable guard slit assembly and sample stage

shutter, filter and pitch feedback



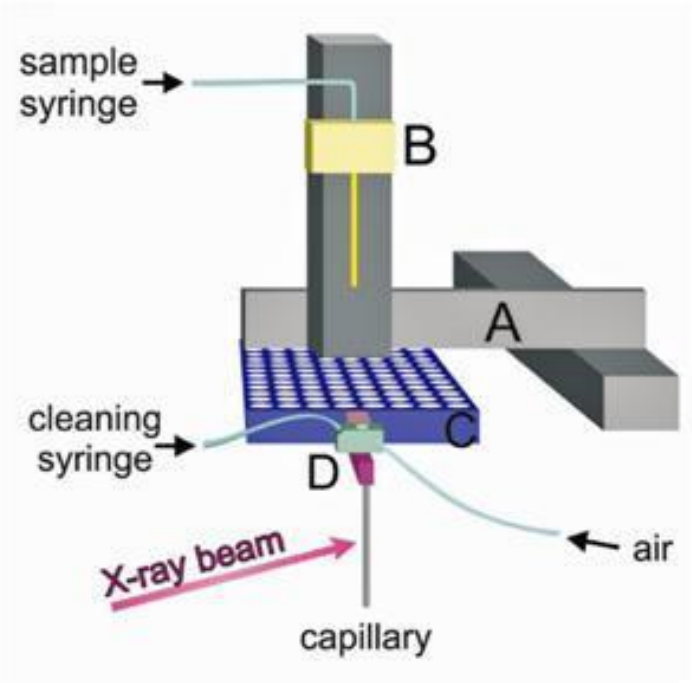
Solution SAXS setup

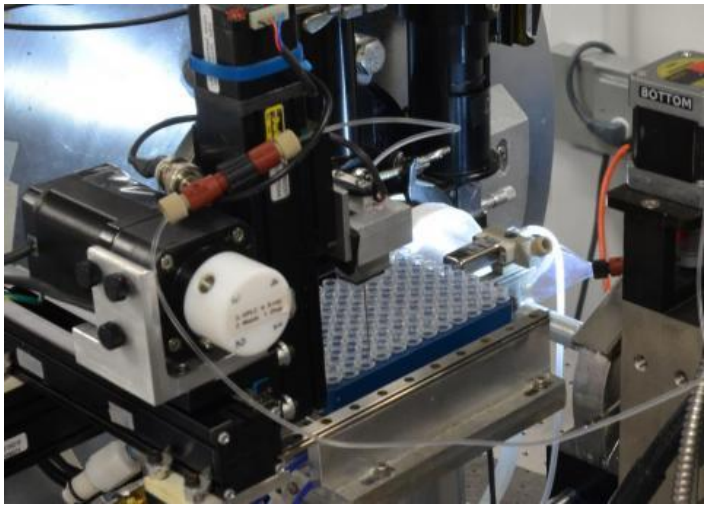
- Autosampler
- SEC-SAXS
- Time-resolved experiments (Stopped-flow mixer, laser (in preparation))
- Manual cell
- Static sample capillaries

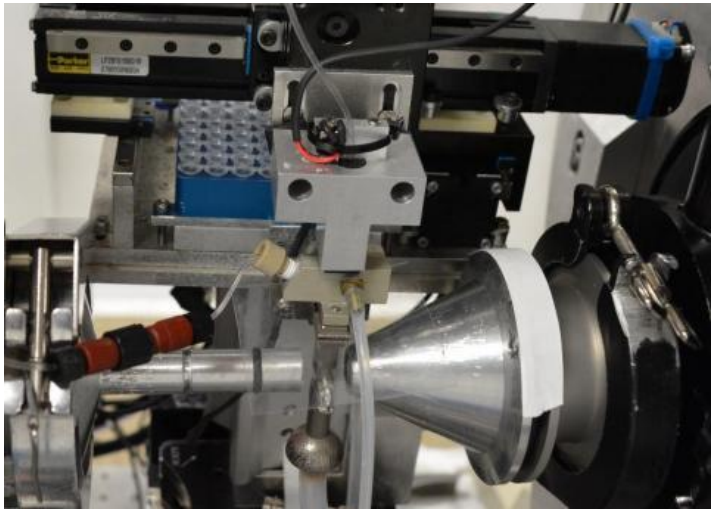
Autosampler

Fully automatic sample delivery device for solution scattering experiments

Dual dispenser

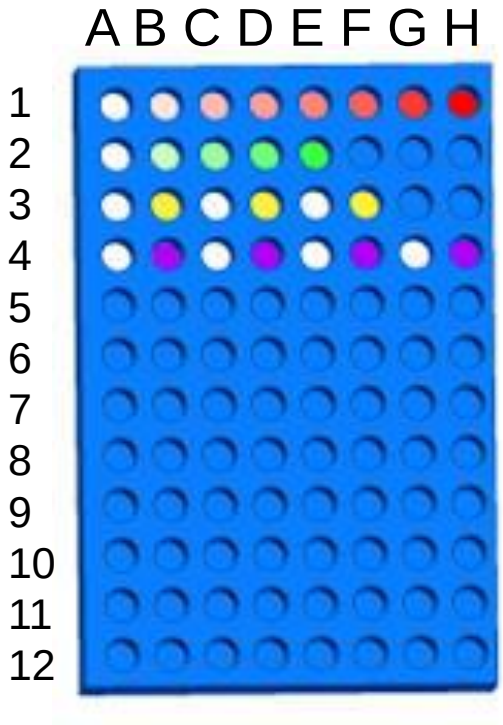


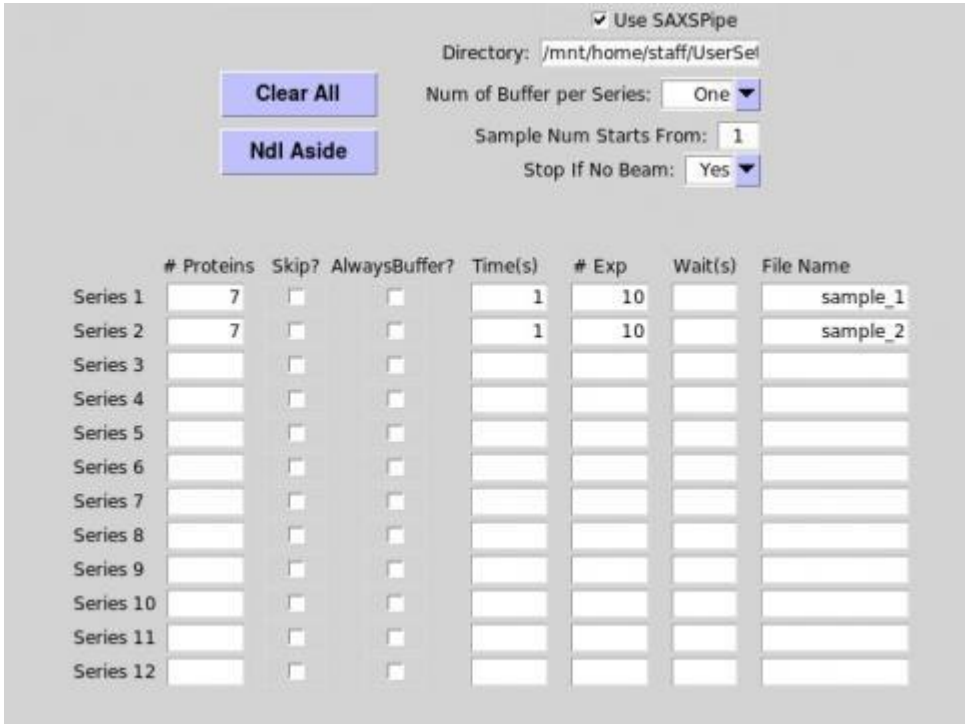




Autosampler

It is based on a standard 96-well micro plate format and fully integrated into the BluIce control system of the beamline.



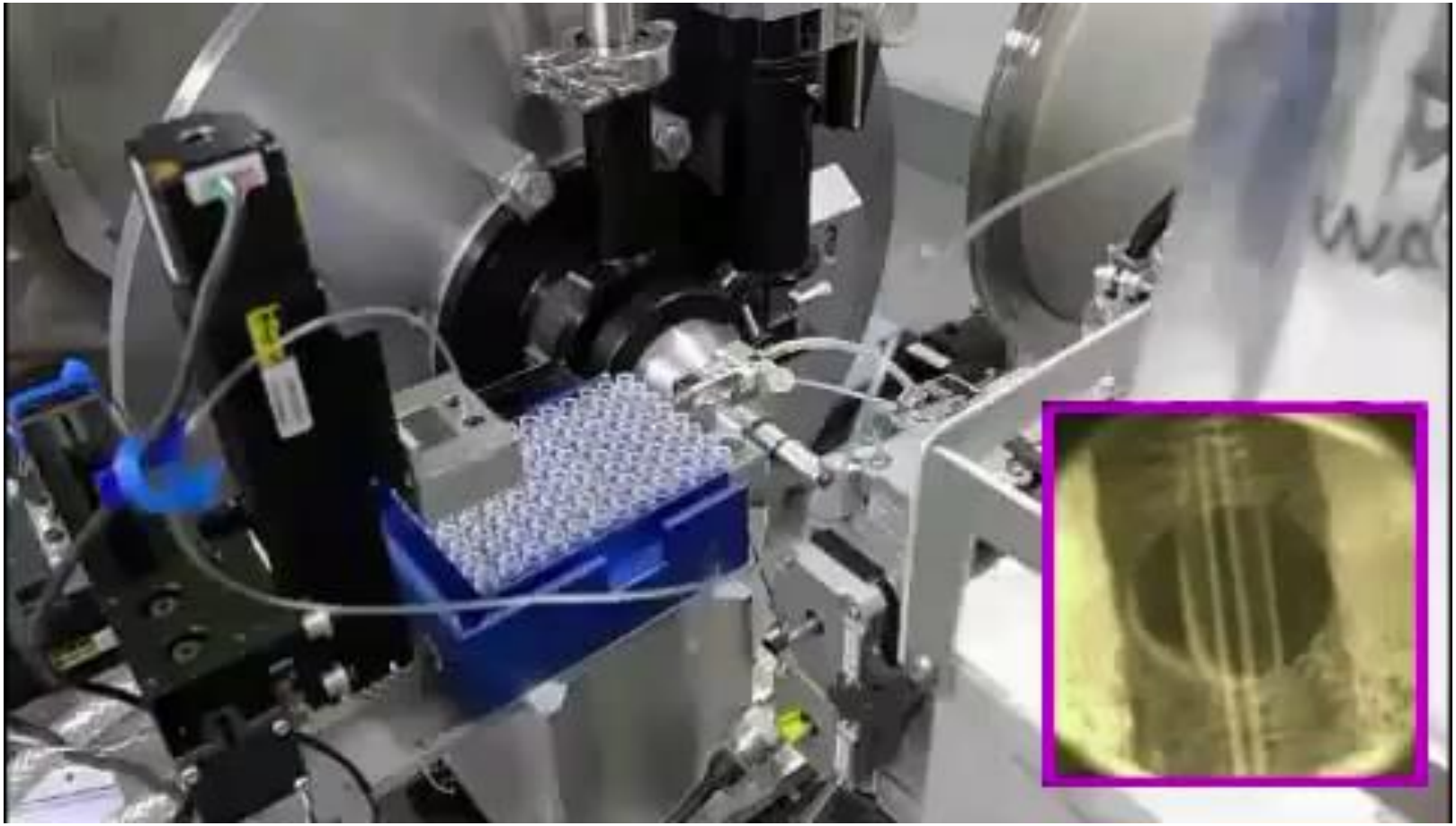


Naming: sample1_01A_B001_0_001.tif, sample1_01B_S002_0_001.tif …

Autosampler

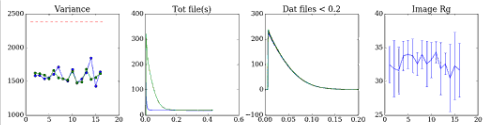
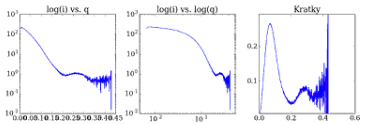
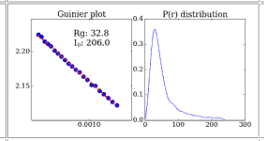
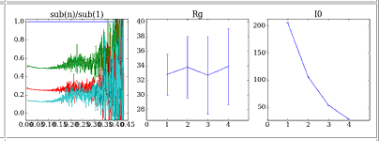
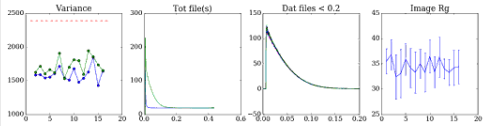
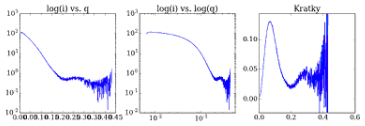
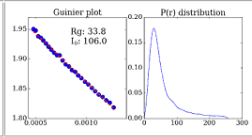
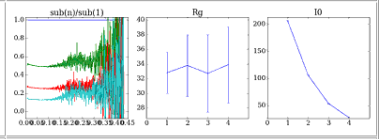
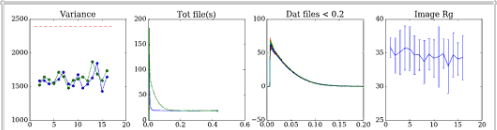
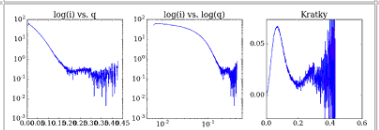
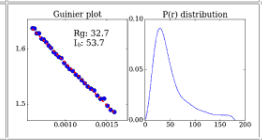
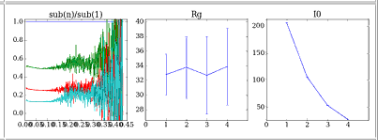
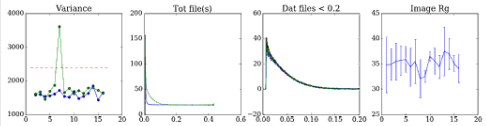
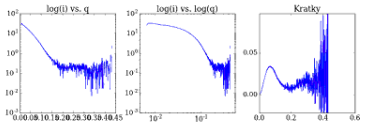
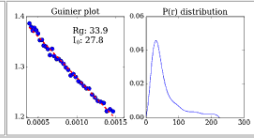
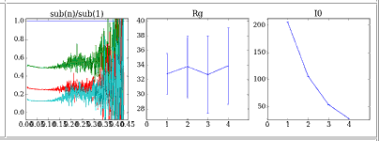
- Usually 30ul sample/buffer volume per injection
- Minimizing radiation damage by oscillating sample during data collection
- Fast data collection (3-5min/set, 96 samples in 5-8 hours)
  (Depending on data collection and washing procedure)
- Thorough cleaning of capillary, needle and tube (with user customizable cleaning procedure)
- 96-well plate compatible sample rack with temperature control
- Uses VMR 0.2ml 8x PCR strip (catalog number: 20170-004)
- 1.5mm quartz capillary cell
- Sample recovery after data collection (optional)

Autosampler



Saxspipe

Automatic on-line data analysis

Results	Time	Sub (or tot) file	Buffer (reject) Sample (reject)	Guinier rg i0	Datgnom realspace rg	Datgnom dmax	Image graphs	Data graphs	Data analysis graphs	Row analysis
Check data - real and reciporocal Rgs differ for more than 5%	13 Jul 2018 16:30:46	o1_02B_S060-o1_02A_B059	16 (0) 16 (0)	32.8 ± 2.8 206.0 ± 0.52 53 to 54 (22 total)	48.23	239.59				
Check data - real and reciporocal Rgs differ for more than 5%	13 Jul 2018 16:35:37	o1_02C_S061-o1_02A_B059	16 (0) 16 (0)	33.8 ± 4.2 106.0 ± 0.39 29 to 55 (27 total)	51.75	256.30				
Check data - real and reciporocal Rgs differ for more than 5%	13 Jul 2018 16:40:29	o1_02D_S062-o1_02A_B059	16 (0) 16 (0)	32.7 ± 5.3 53.7 ± 0.19 34 to 63 (30 total)	43.70	179.41				
Check data - real and reciporocal Rgs differ for more than 5%	13 Jul 2018 16:45:20	o1_02E_S063-o1_02A_B059	16 (0) 16 (1)	33.9 ± 5.2 27.8 ± 0.15 25 to 60 (36 total)	50.40	224.19				

This information is meant for a quick data check only. Please analyse your data carefully by hand! SasTool Version 0.9.7.6, ATSAS 2.7.2 (r7883)

Saxspipe

Automatic on-line data analysis

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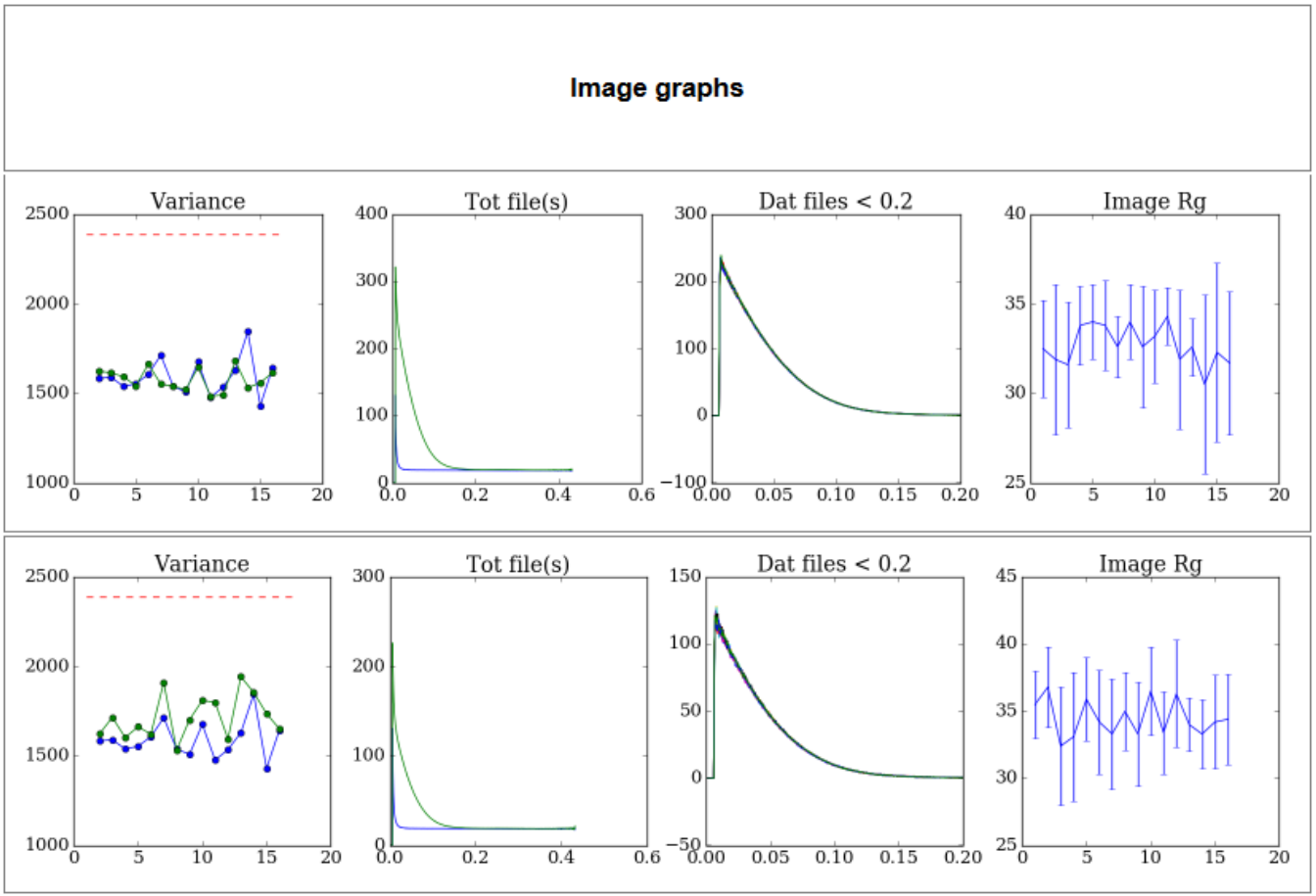
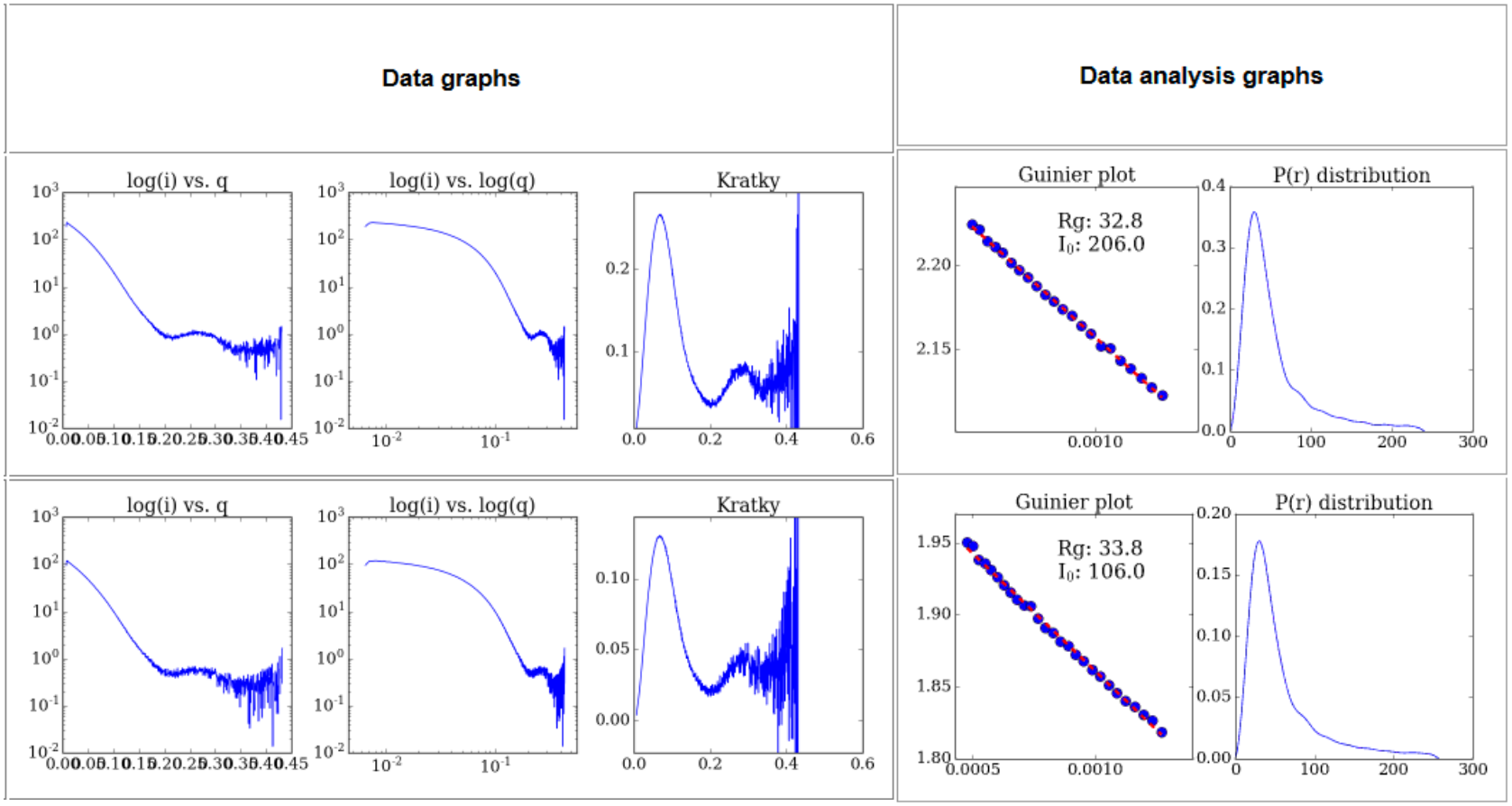
Saxspipe

Automatic on-line data analysis



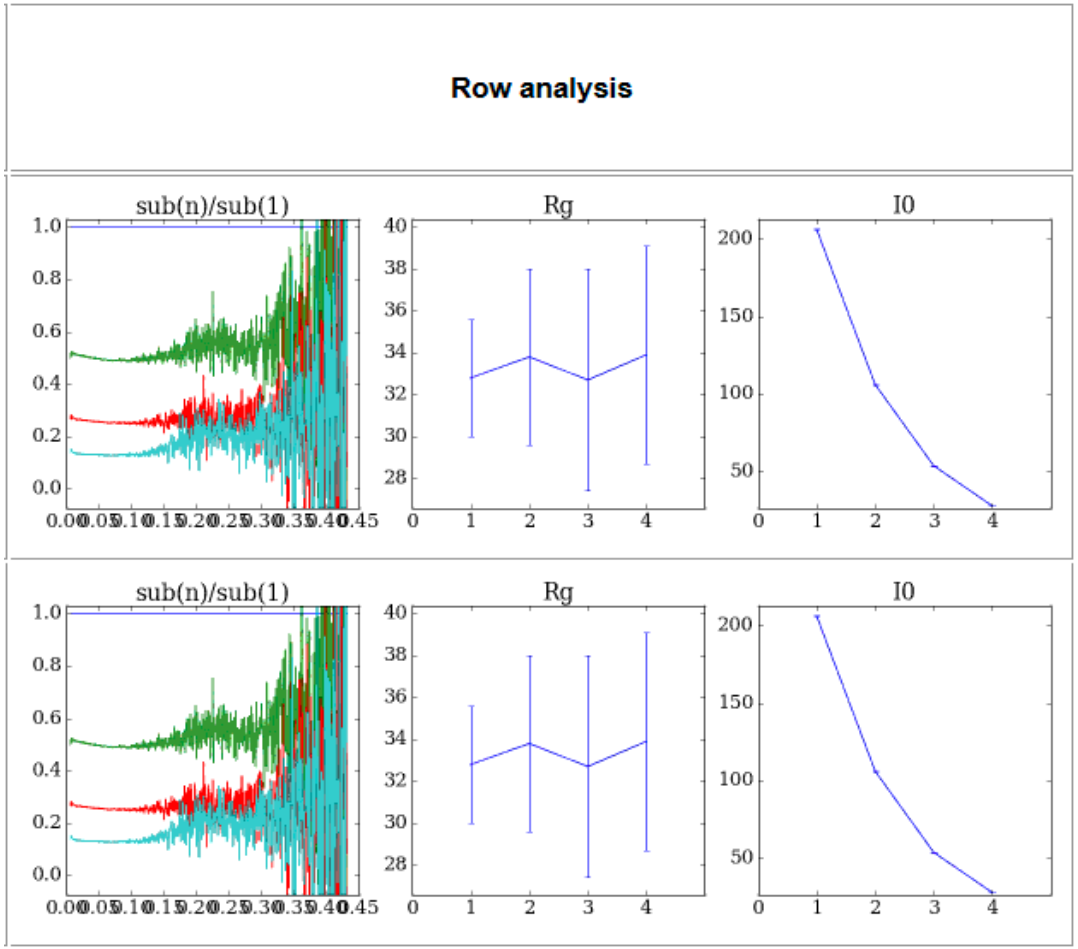
Saxspipe

Automatic on-line data analysis



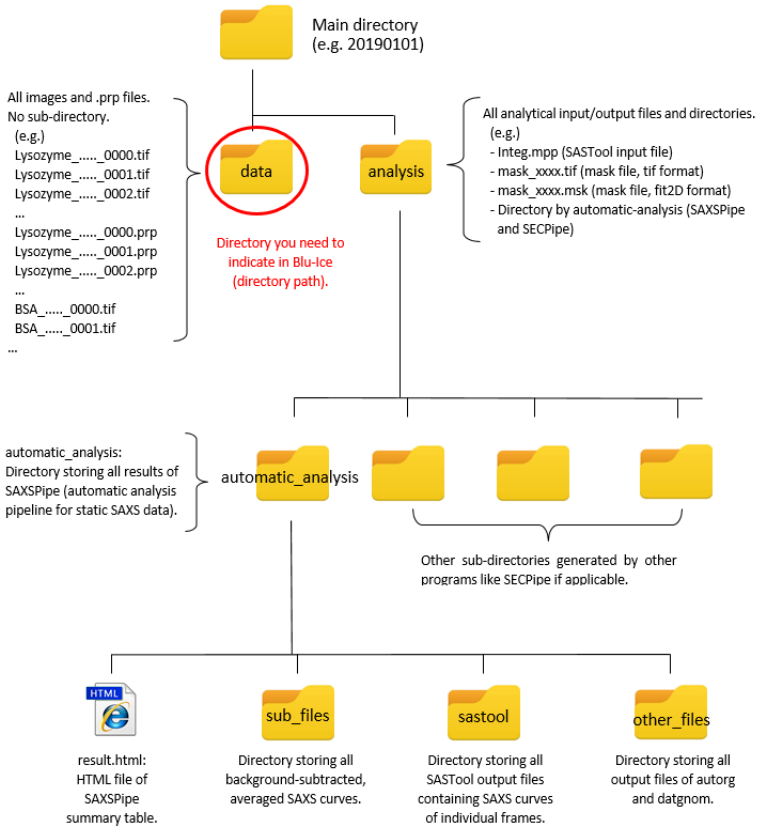
Saxspipe

Automatic on-line data analysis



Saxspipe

Can be used from the command line to re-analyze the data:
$ saxspipe2.py <sample_number> [buffer_number]

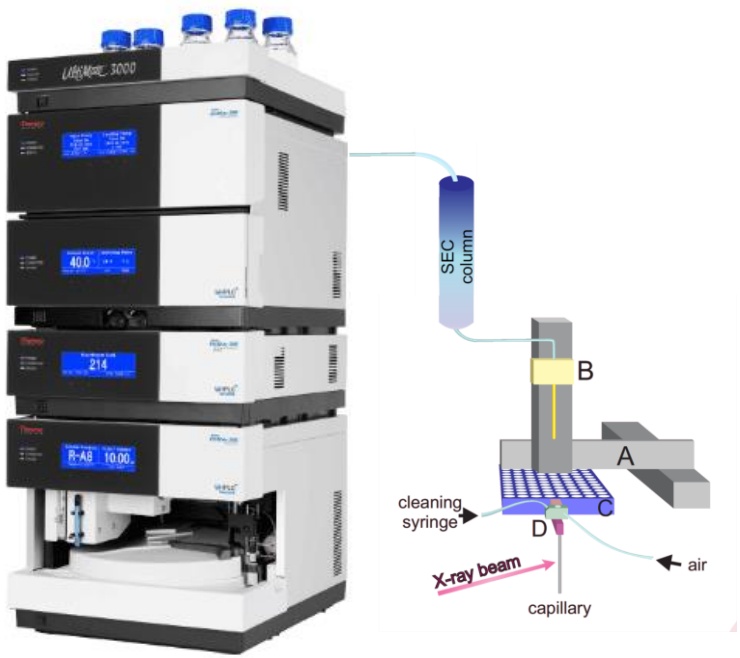


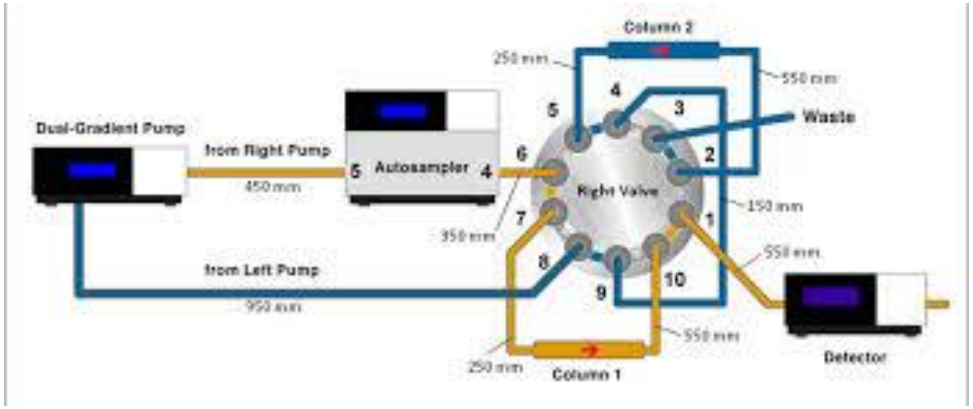
SEC-SAXS

SEC-SAXS is a powerful tool to overcome and/or mitigate problems resulting from non-monodisperse samples.

UltiMate 3000 UHPLC (Thermo Scientific)

- 2 pumps (6 solutions) w/ degasser
- 4 UV-vis channels (up to 200 Hz)
- Auto-sample changer
- Tandem analyses for HPLC high throughput
- Refractive index detector
- Software: Chromeleon

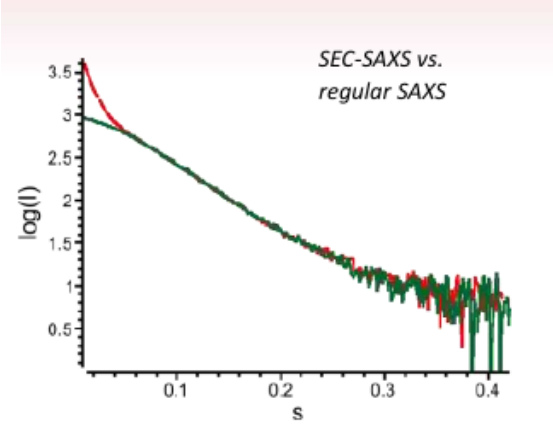




SEC-SAXS

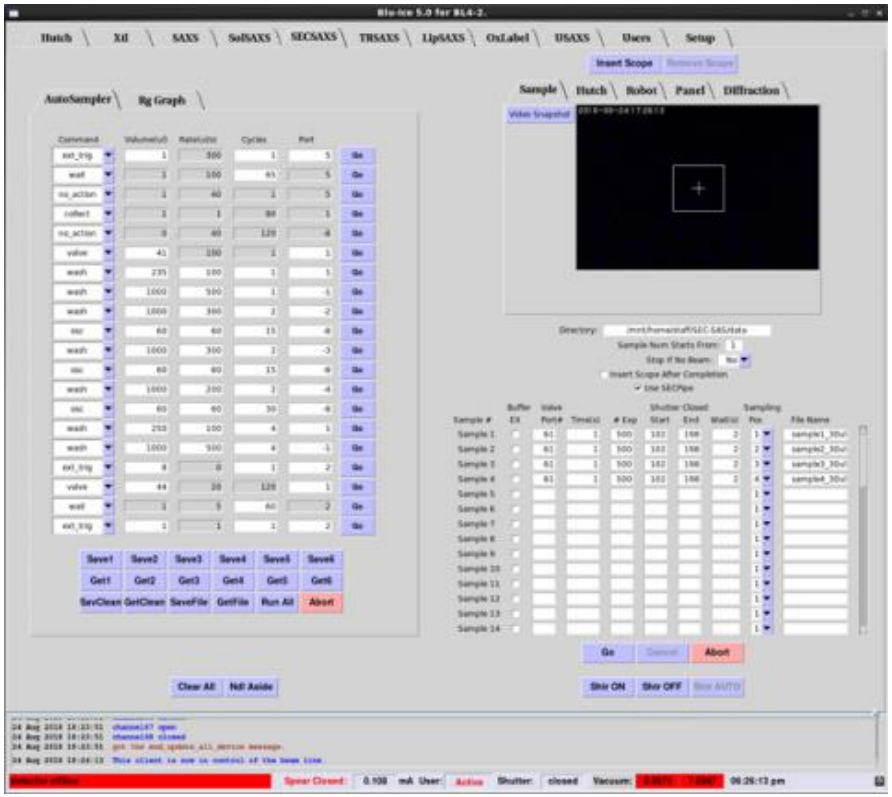
- Small column (2.4ml) is used to minimize dilution.
- Elution profile would be different from that with large column.
- Just to remove aggregates from monodisperse & monomodal sample (single peak), 100ul injection generally works great.
  - Dilution factor at X-ray position: ~60-70%
  - Good for low concentration sample.
- Available columns:
  - Superdex 200 Increase PC 3.2/300
  - Superdex 75 PC Increase 3.2/300
  - Superose 6 Increase PC 3.2/30

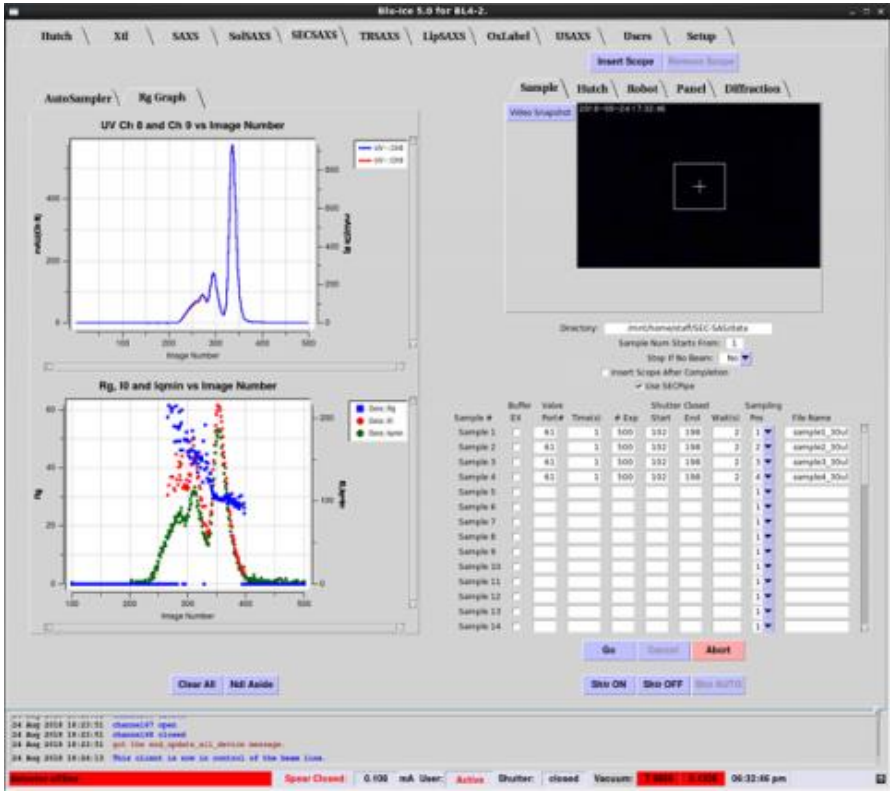
  or bring your own column



SEC-SAXS

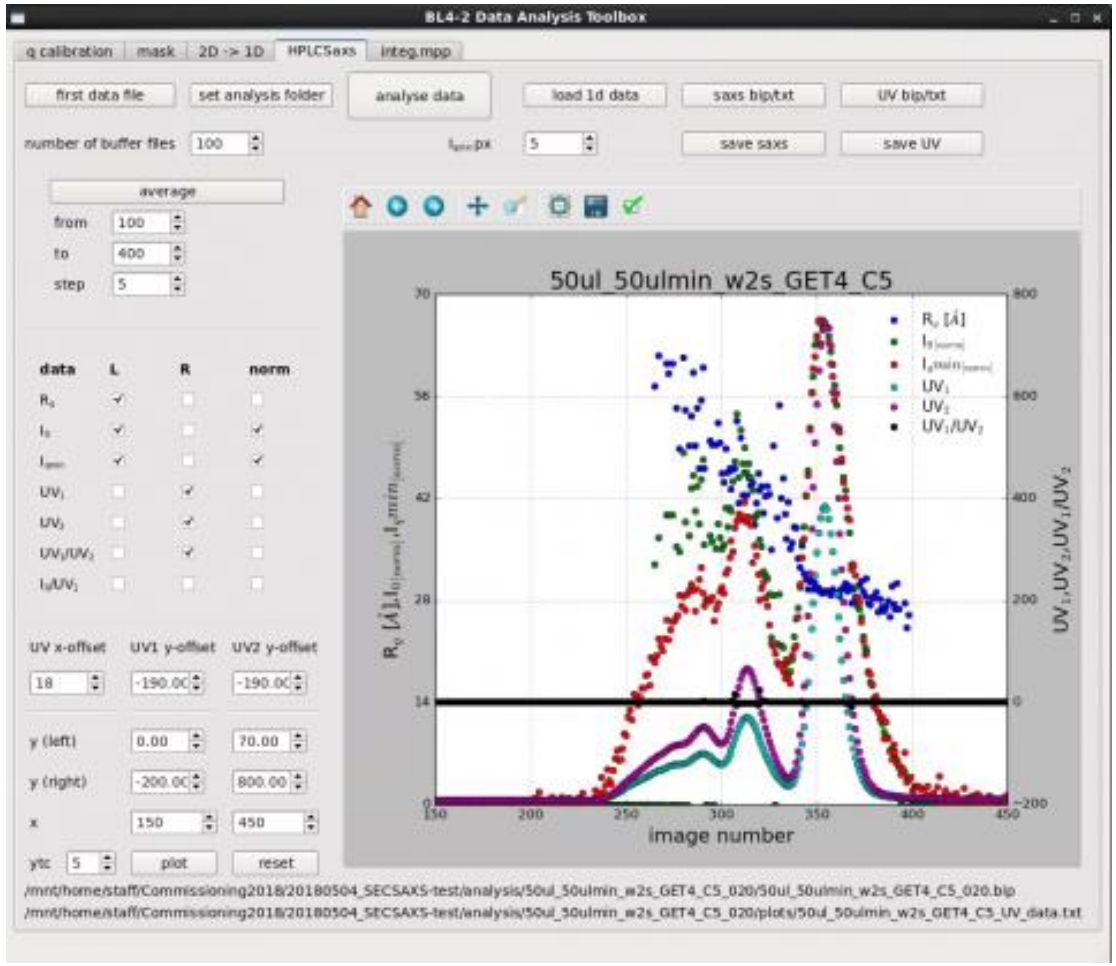
- Blu-Ice control for the experiment and on-line analysis.





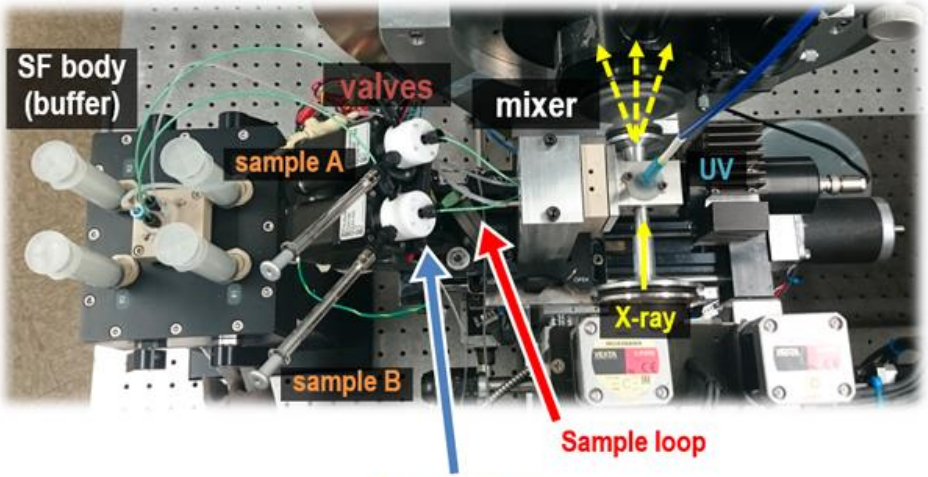
bl42-dat

- Post-analysis/averaging of the data.



Stopped-flow mixer

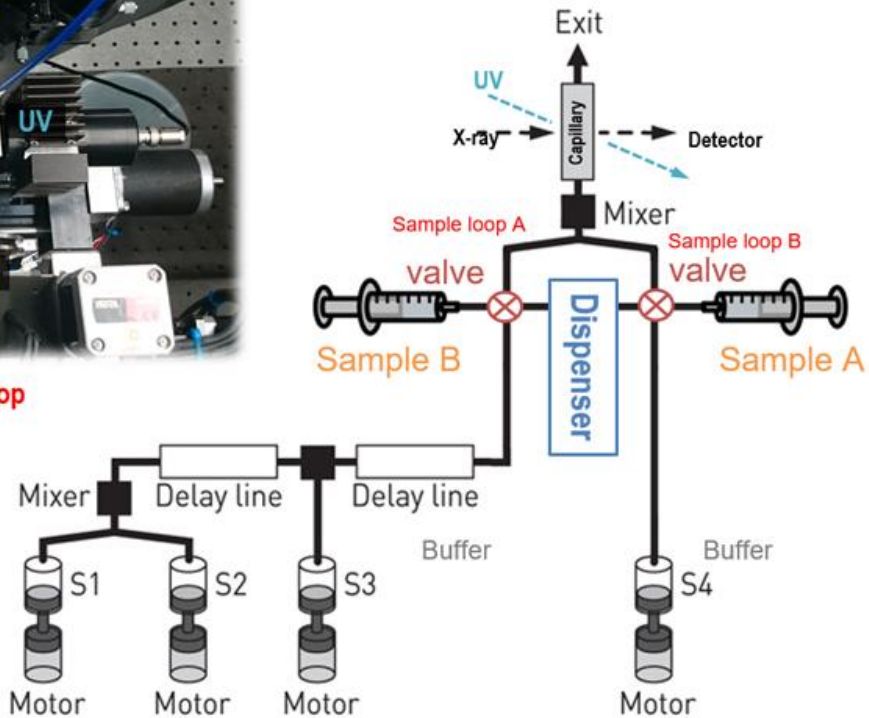
Time-resolved solution SAXS (TR-SAXS) is a powerful experimental tool to investigate conformational changes of macromolecular systems and allows to monitor biological processes under near physiological conditions.



Sample loop

Dispenser

Washing solutions
Water
Detergent
EtOH
Bleach
Buffer

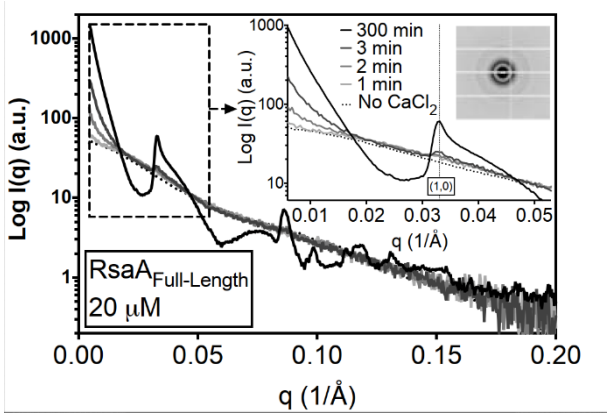


SFM400 w/ switching valves

Stopped-flow mixer

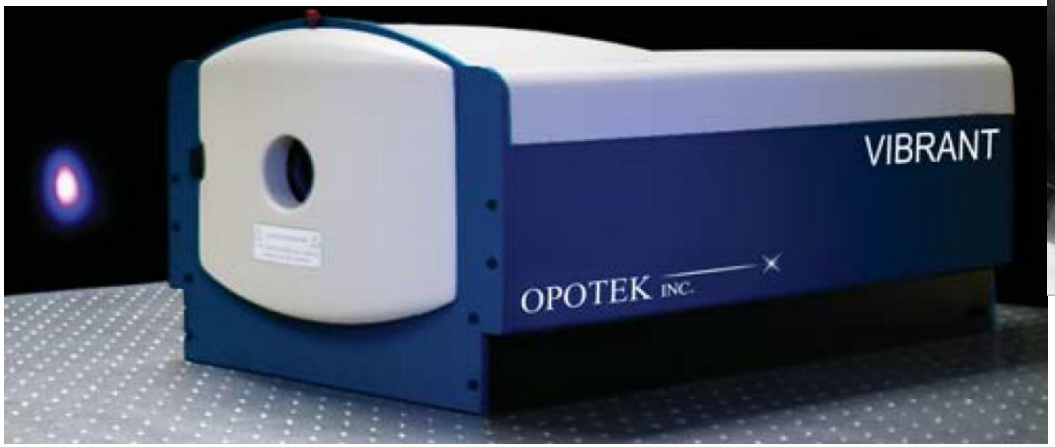
Main features

- Customized Bio-Logic 4 syringe stopped-flow mixer (SFM400)
- Semi-automatic data collection and analysis with user friendly software
- 30-200ul per data set without any dead volume (standard: 100ul)
- 2 msec time resolution achievable (limited by detector frame rate)
- Customizable and powerful cleaning of capillary after every single shot
- Computer-controlled electric rotary valves
- Each module is temperature controllable
- Monitor and record mixing signals and timings by oscilloscope
- High flux beam by Mo/B4C multilayer is available for short exposure
- Specialized Blu-Ice TR-SAXS tab for TR-SAXS data collection
- UV port (under development)



Photoreaction and T-jump

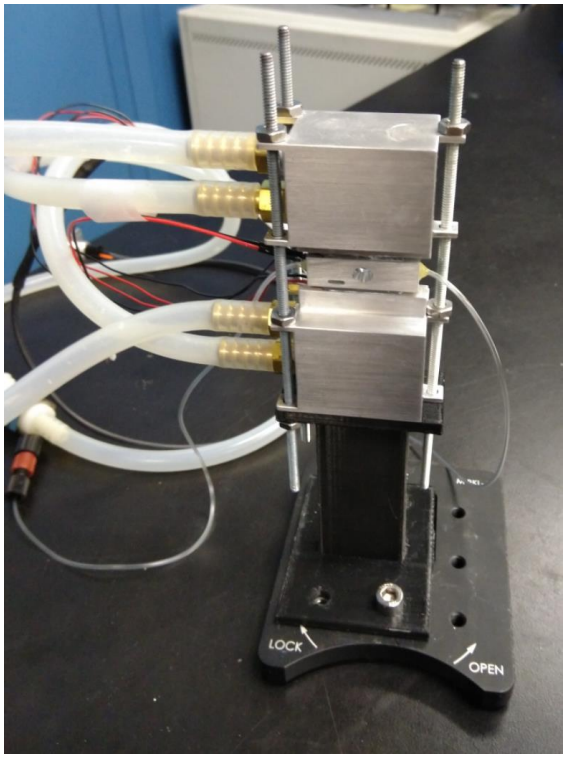
- Opotek Vibrant 355HE tunable laser
- Wavelength range: 410 nm – 2400 nm
- Peak energy 45 mJ, 5ns pulse duration
- Max. repetition rate 10 Hz
- Full computer controll



Manual cells

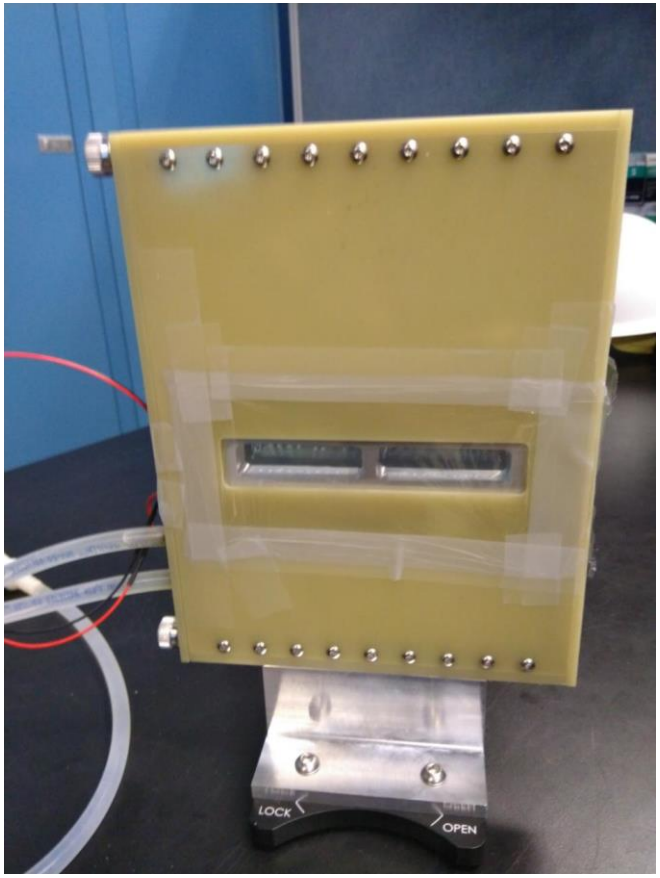
Temperature controlled capillary cells with manual loading

Peltier modules
0-100°C



Water chiller
5-80°C

Multy-capillary holder

Temperature controlled holder for 20 capillaries

Peltier modules, 0-100°C