



| The European Synchrotron

BM29 REFURBISHMENT

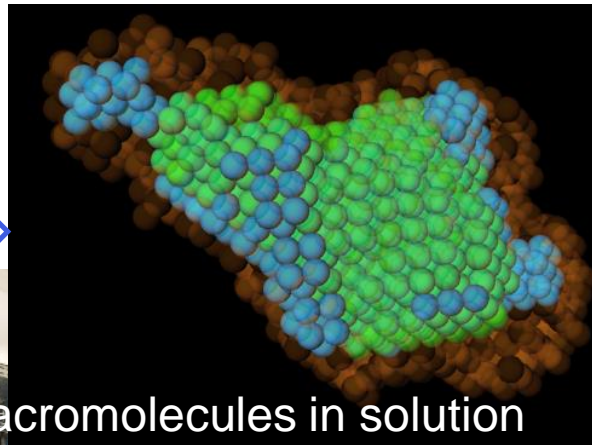
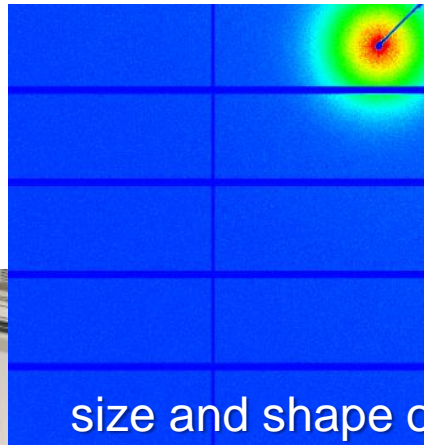


*Petra Pernot
Structural Biology Group
ESRF
8th February 2021
BAG Meeting*


BM29 post EBS changes:

- source = 2 PW
 - detector = Pilatus3 2M in vac
 - sample changer = Arinax
 - exposure unit(s)
 - software updates:
 - SPEC to BLISS,
 - BsxCUBE3,
 - EDNA to DAHU
 - user 'covid' mode = mail_IN
- Remote access inappropriate to experiments on macro-molecules in solution

BM29 NEW SOURCE

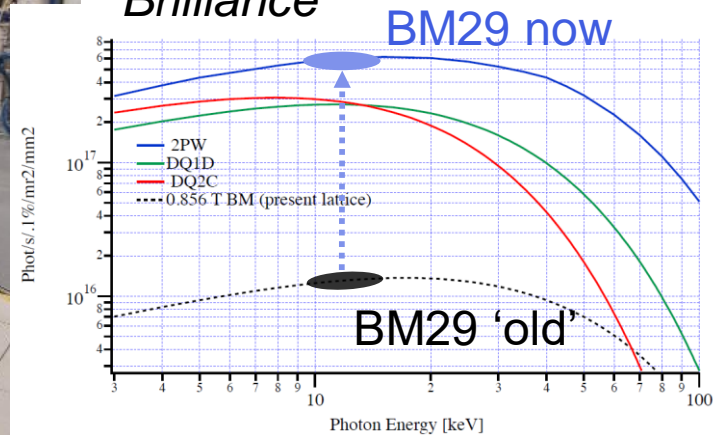


size and shape of macromolecules in solution

- new source = 2 pole wiggler
 - similar spectrum, twice flux
 - sample-to-source distance increased by 3m
 - much smaller source size(s)
- 
- brilliance higher by factor 50
 - smaller beam on sample

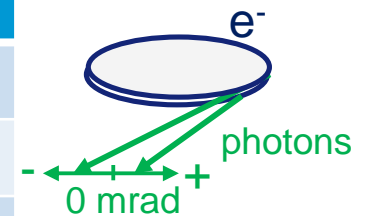


Brilliance



Horizontal transverse position of the photon beam axis at the Front End

Source	Lattice	H position at FE [mm]
0 mrad reference	present	0
0.856 T BM @ -9 mrad	present	-204.29
0 mrad reference	new	2.419
2PW conf. A @ -7.05 mrad	new	-180.97 ($\Delta_{\text{POS}} = + 23$)
2PW conf. B @ -8.65 mrad	new	-221.79 ($\Delta_{\text{POS}} = -17.5$)

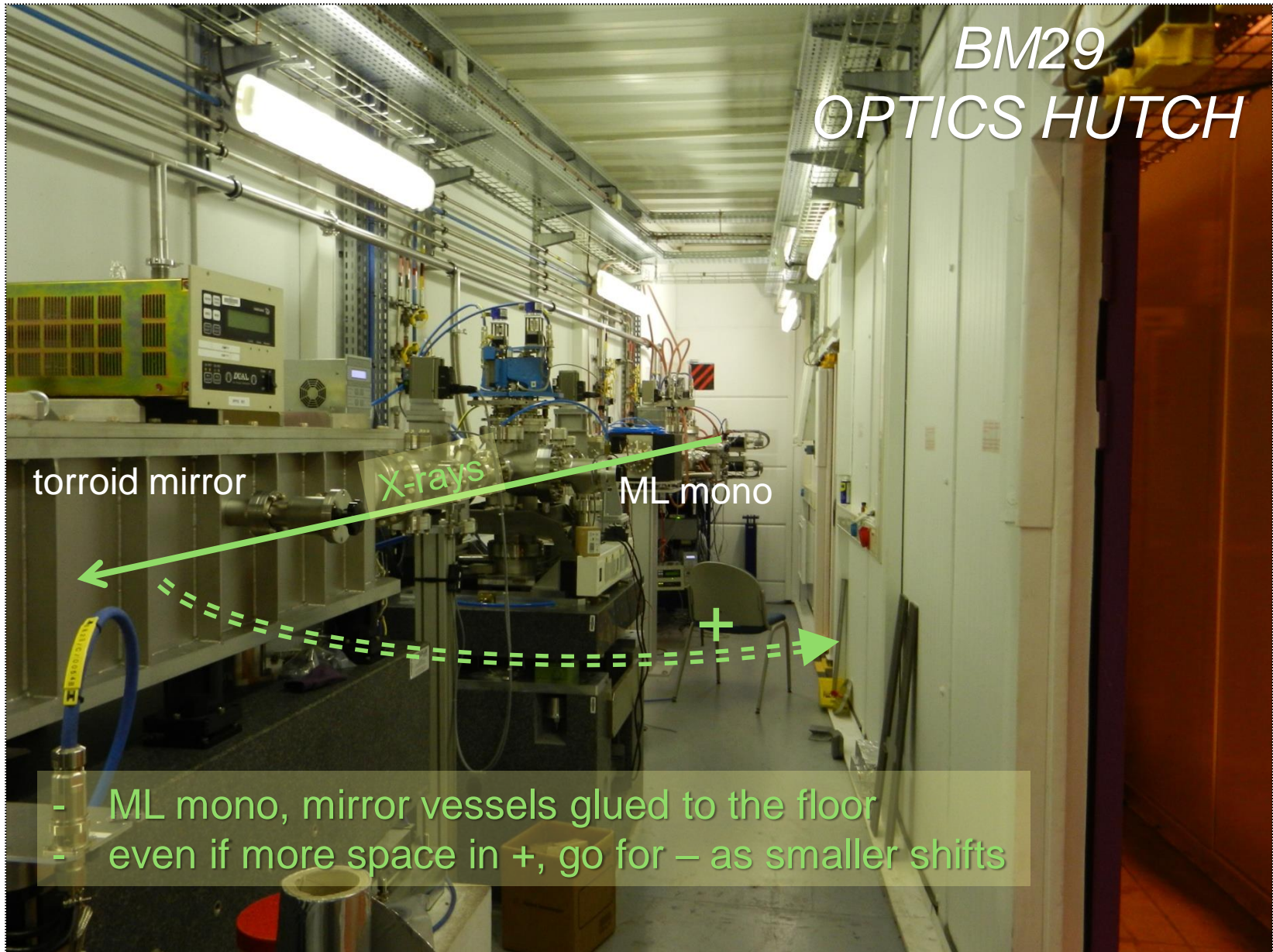


$$\Delta_{\text{MRAD}} = + 1.95$$

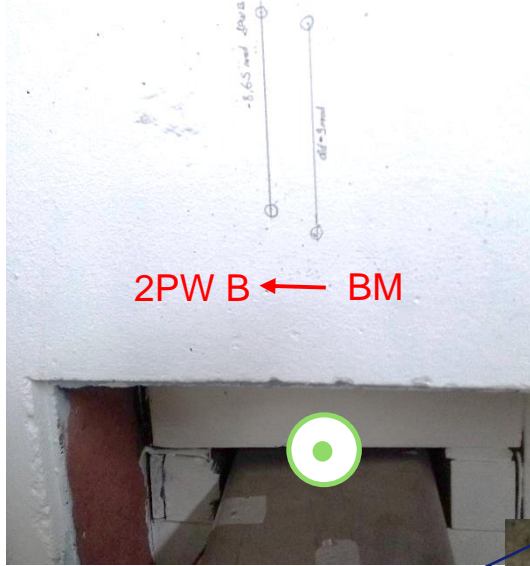
$$\Delta_{\text{MRAD}} = + 0.35$$

BM29 beam shift	FE	Mono	Mirror	Sample	Detector
$D_{\text{source-to-element}}$ [m]	25.51	31	35	45	48
2PW conf. A [mm]	+23	+33	+41	+60	+66
2PW conf. B [mm]	-17.5	-15.5	-14	-10.5	-8.5

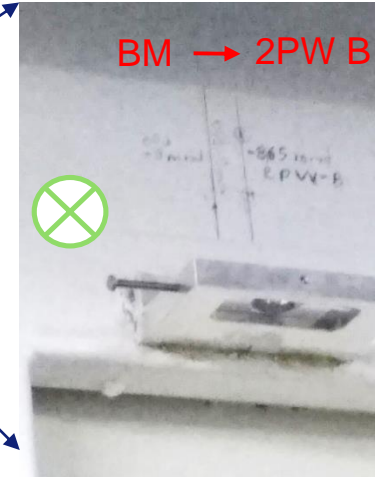
Distance of 2PW central point to FE: 25.51 m (was 22.54 m)



OH - FE -19.7 mm

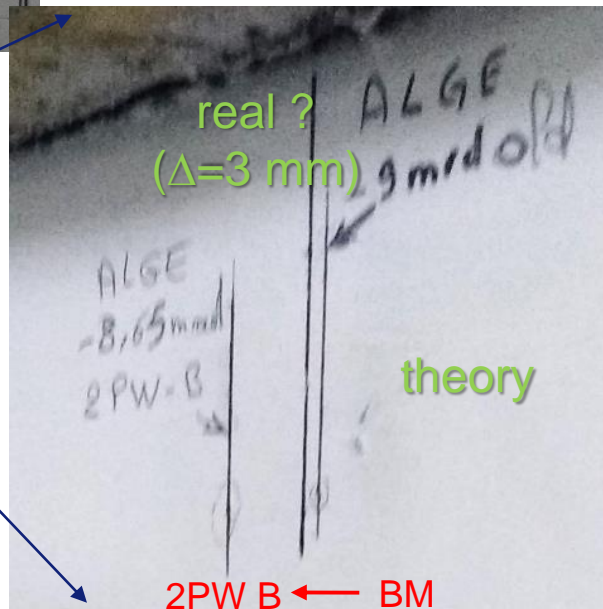
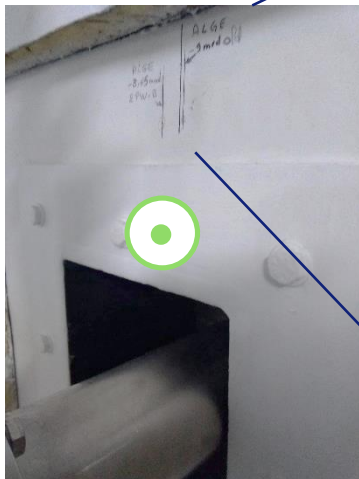


OH - EH wall



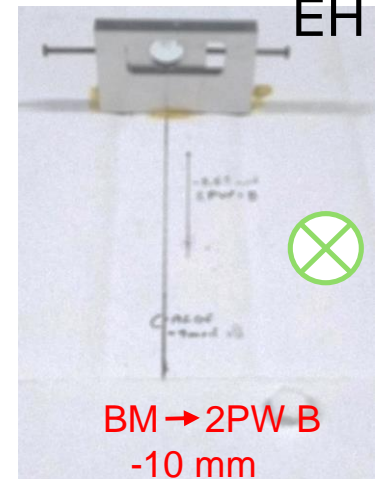
Juliette Aubin,
Daniel Schirr-Bonnans

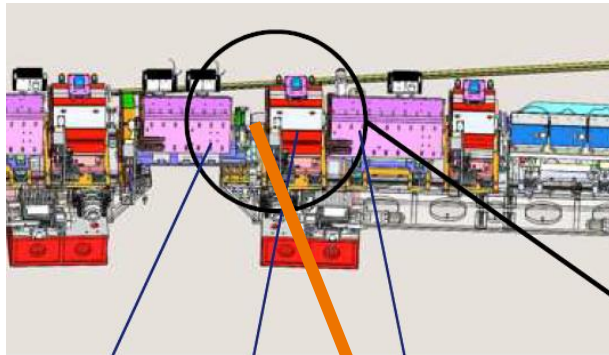
EH entry



-14 mm

EH end



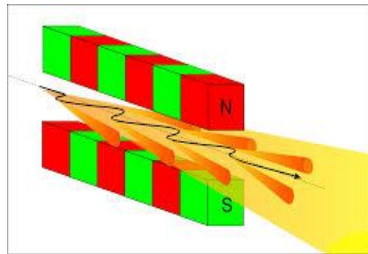


DQ2C
0.39 T

High gradient
quadrupole
90 T/m

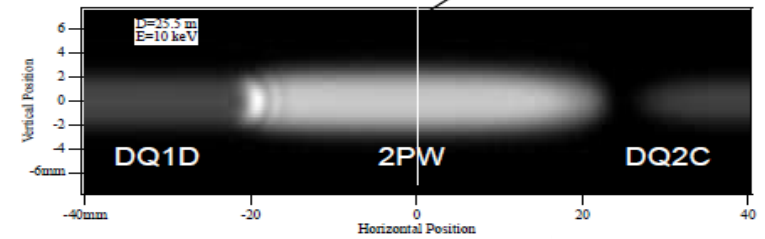
DQ1C
0.57 T

2 Pole Wiggler

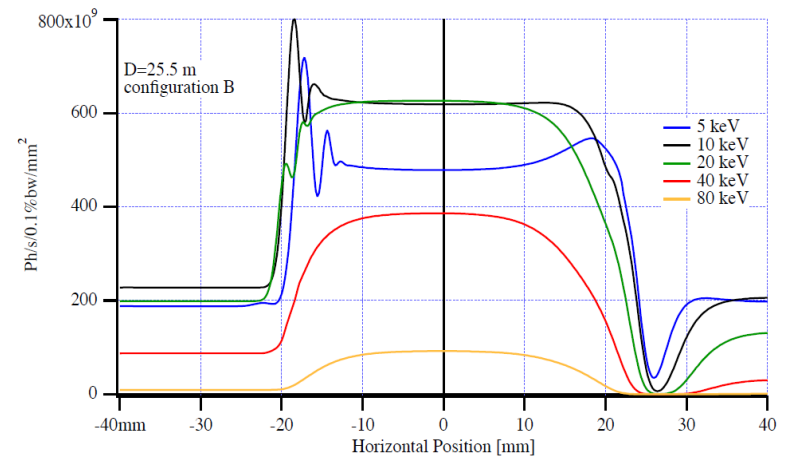


Configuration B:

- 2PW and DQ1D beams superimposed
- 2PW x-ray fan axis @ -8.7 mrad



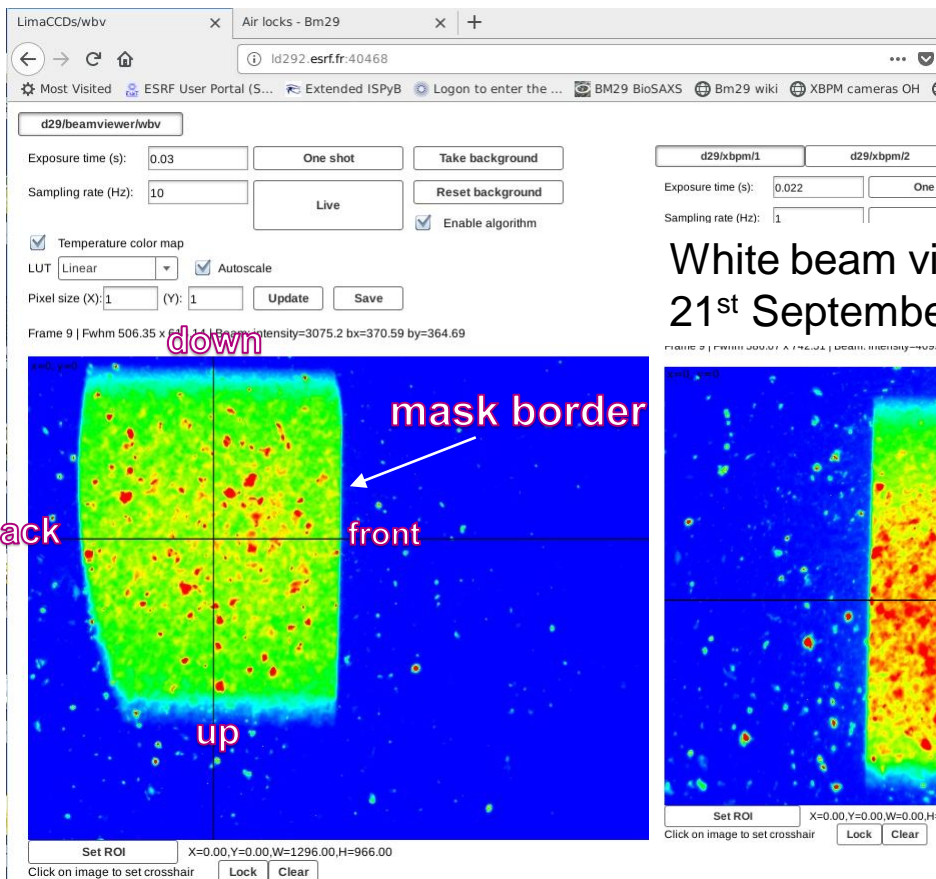
PHOTON FLUX DENSITY OF 2PW configuration B AT FRONT END



1ST EBS PHOTONS AT BM29, I = 9.3MA ON 11 MARCH 2020

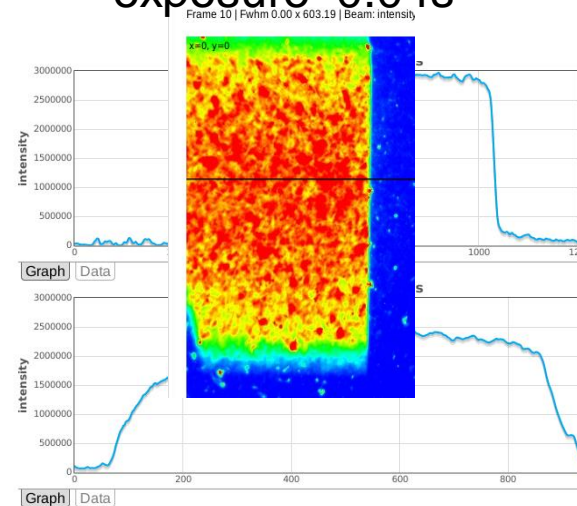
2PW_B installed in the EBS ring 10th March 2020 morning: safety key given on **11 March 2020** at 8.50h (take out at 17.01h)

White beam viewer (polycrystalline diamond), PS: ~4 mm x ~4 mm



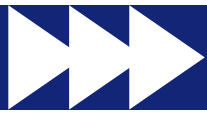
White beam viewer : recorded 21st September 2018, I ~ 40mA

2 PW exposure 0.04s



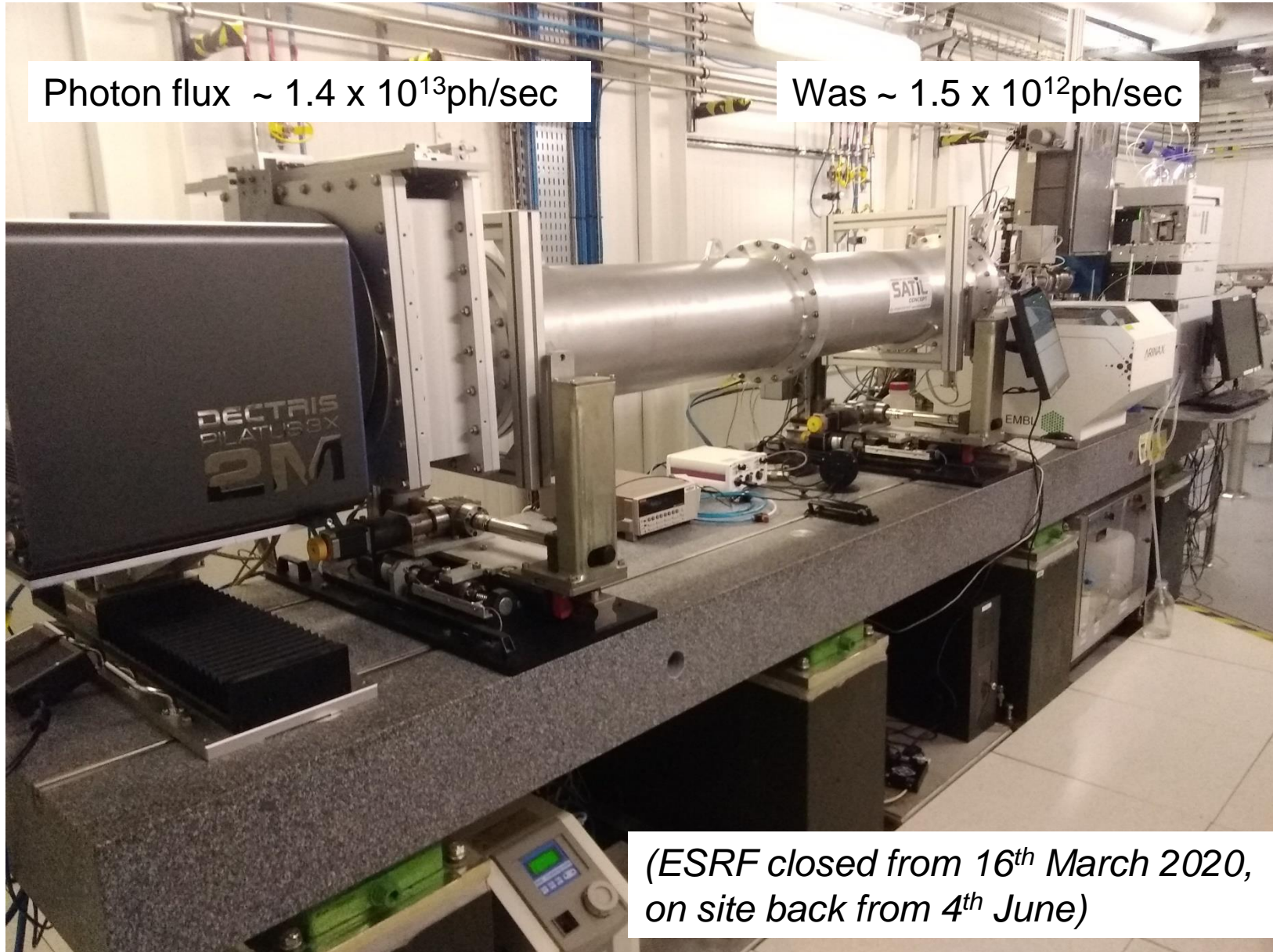
➔ 2 x BM flux

Next beam allowed on 30th June 2020 ...



Photon flux $\sim 1.4 \times 10^{13}$ ph/sec

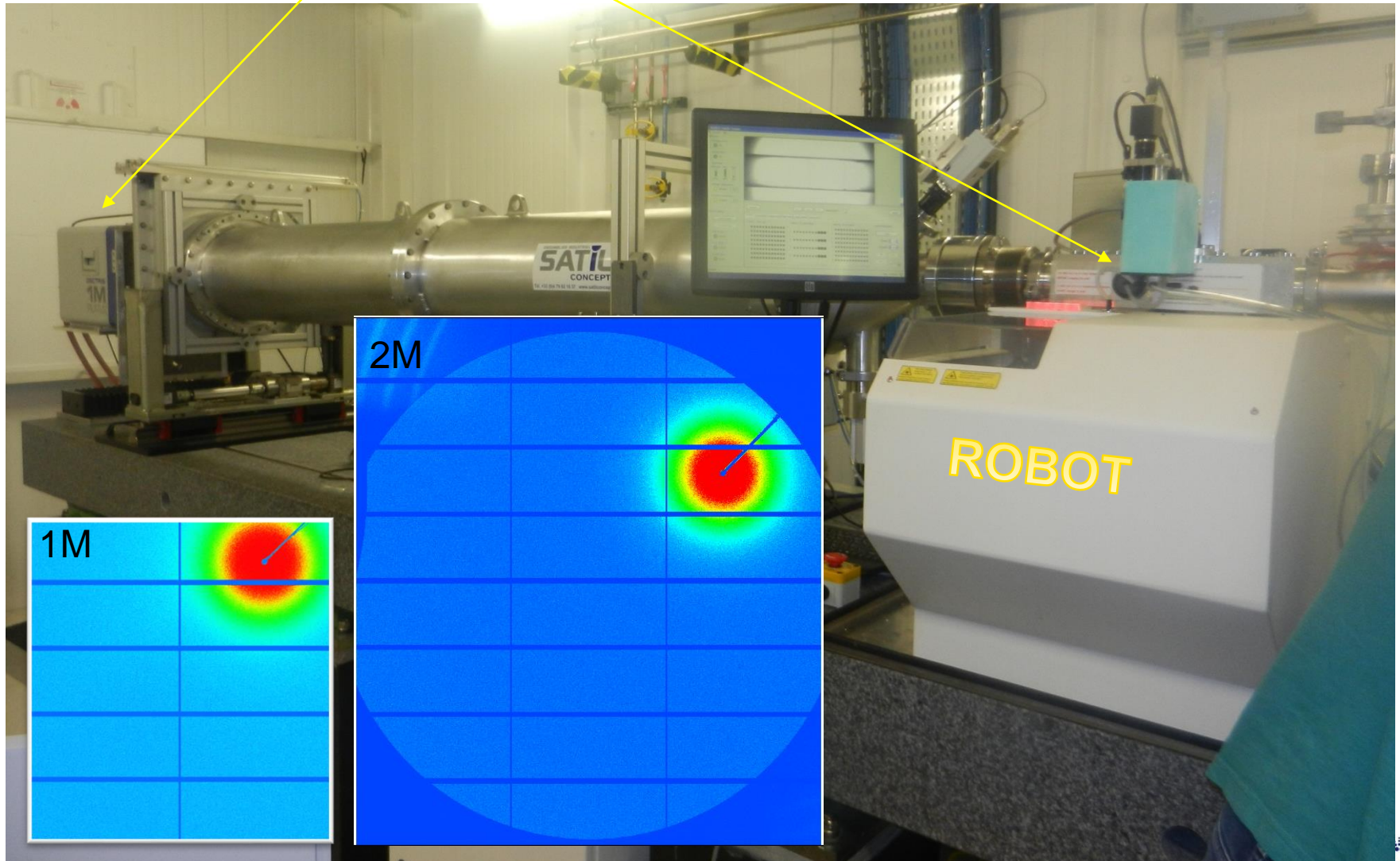
Was $\sim 1.5 \times 10^{12}$ ph/sec

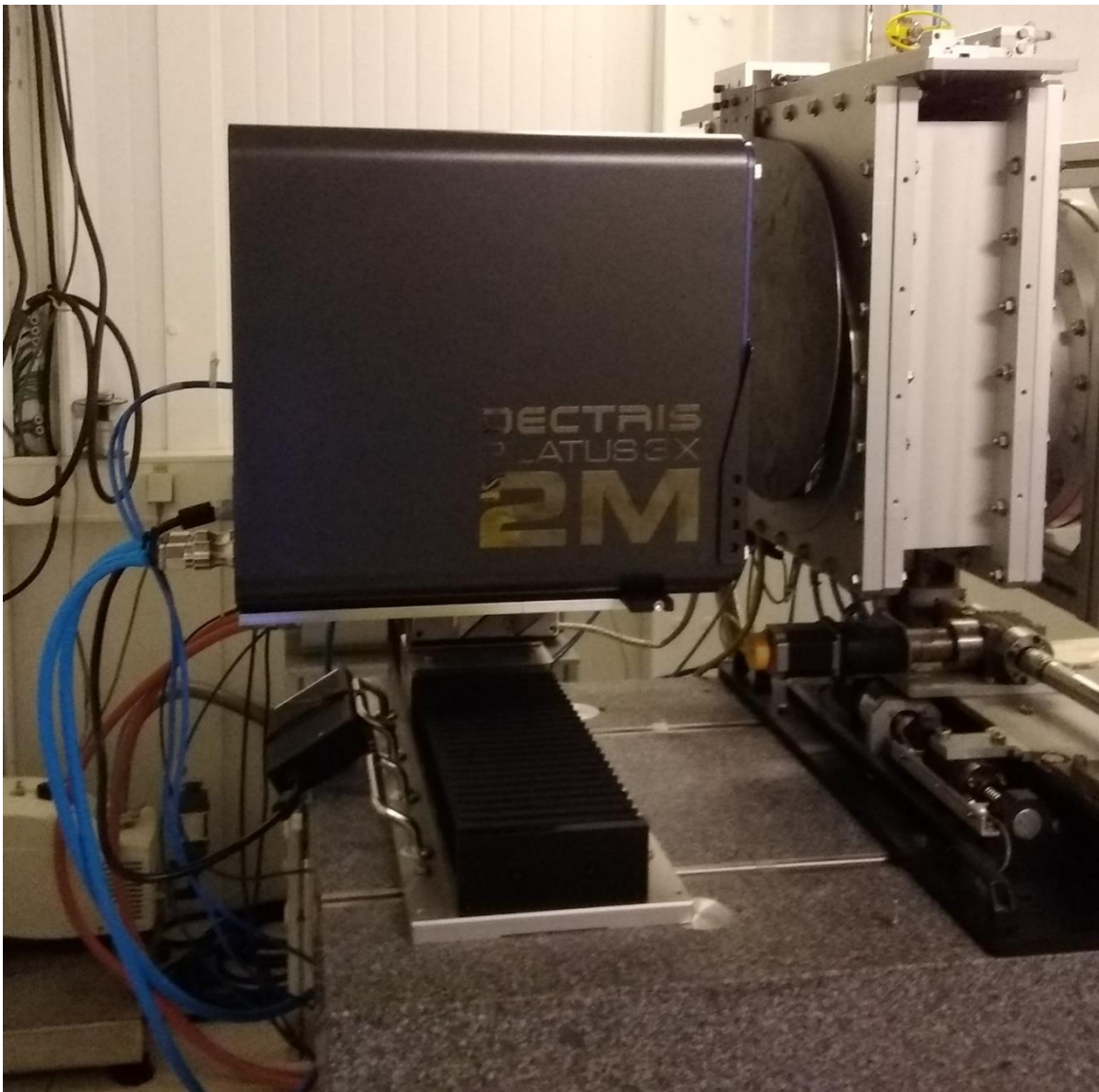


(ESRF closed from 16th March 2020, on site back from 4th June)

ACTIONS TO TAKE ADVANTAGE FROM EBS

- new bigger in-vacuum **detector**: better s/n ratio with less radiation damage and background;
- new sample changer and **exposure unit(s)**: adapted to new beam characteristics

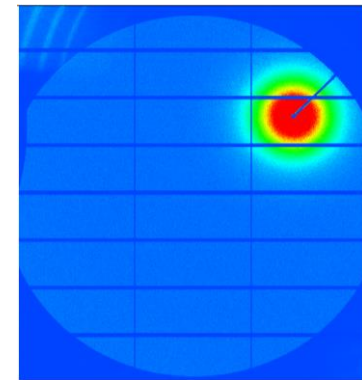




PILATUS3 2M

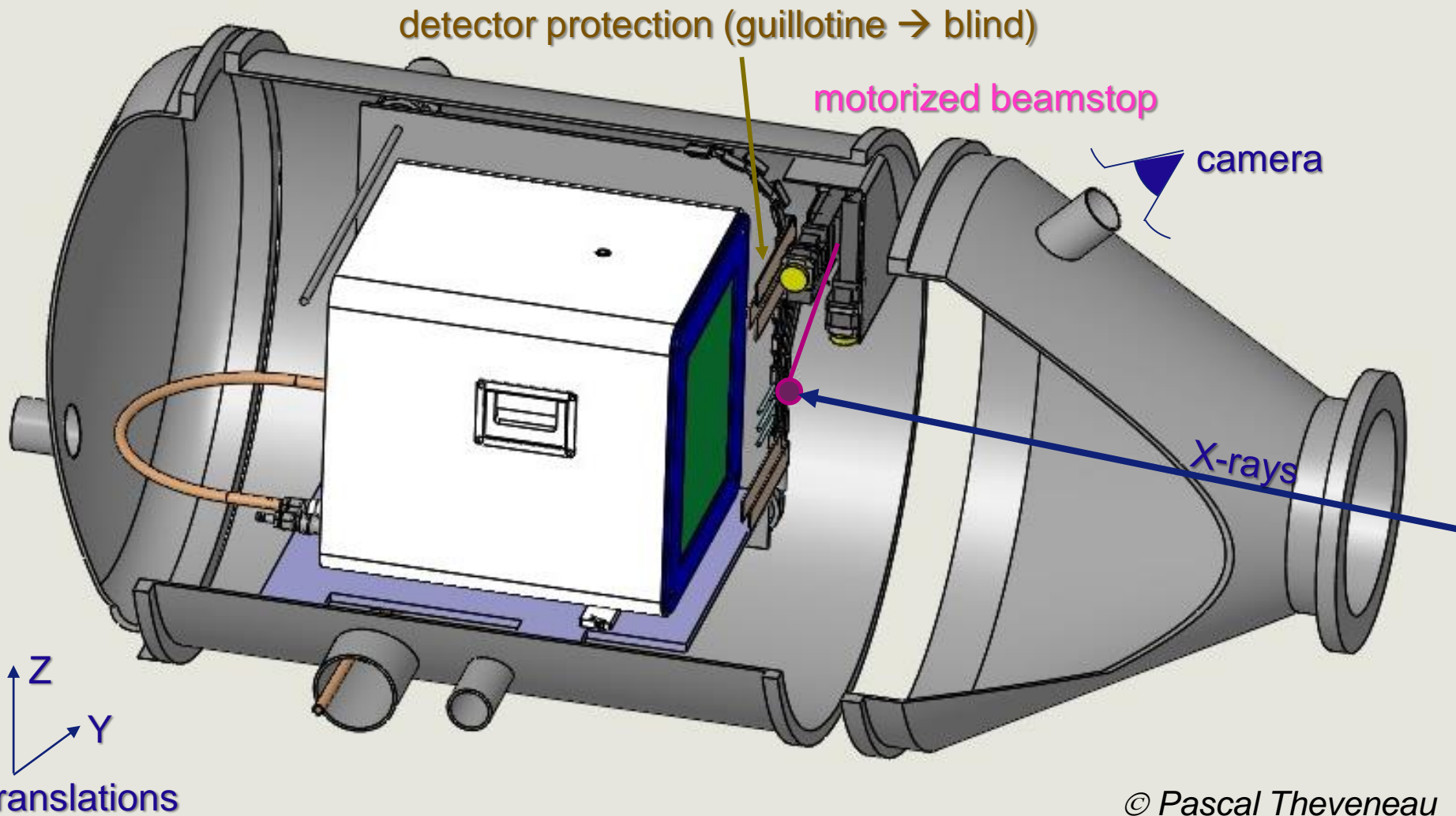
in vacuum compatible

- delivered Dec 2019
- sensitive area 253.7 x 288 mm²
- frame rate = 250 Hz
- up-to-date software
- better S/N: less parasitic scattering, no kapton window, air



To be hold within a new flight tube: project delayed

BM29 BIOSAXS DETECTOR IN VACUUM



Vacuum vessel: $\phi \sim 80\text{cm}$

NEW SAMPLE CHANGER ROBOT

- sample transfer: 5 to 200 μL ;
- cycle time (load-clean) $\sim 50\text{s}$
- less height: more place for sample environment

Samples stored in 96 well plates or PCR tubes



Thermo-regulation
- storage: 4-40°C,
- exposure cell: 4-60°C

SAMPLE CHANGER EXPOSURE UNIT

- capillary pods



capillaries with different ϕ ,
shapes and materials

circular

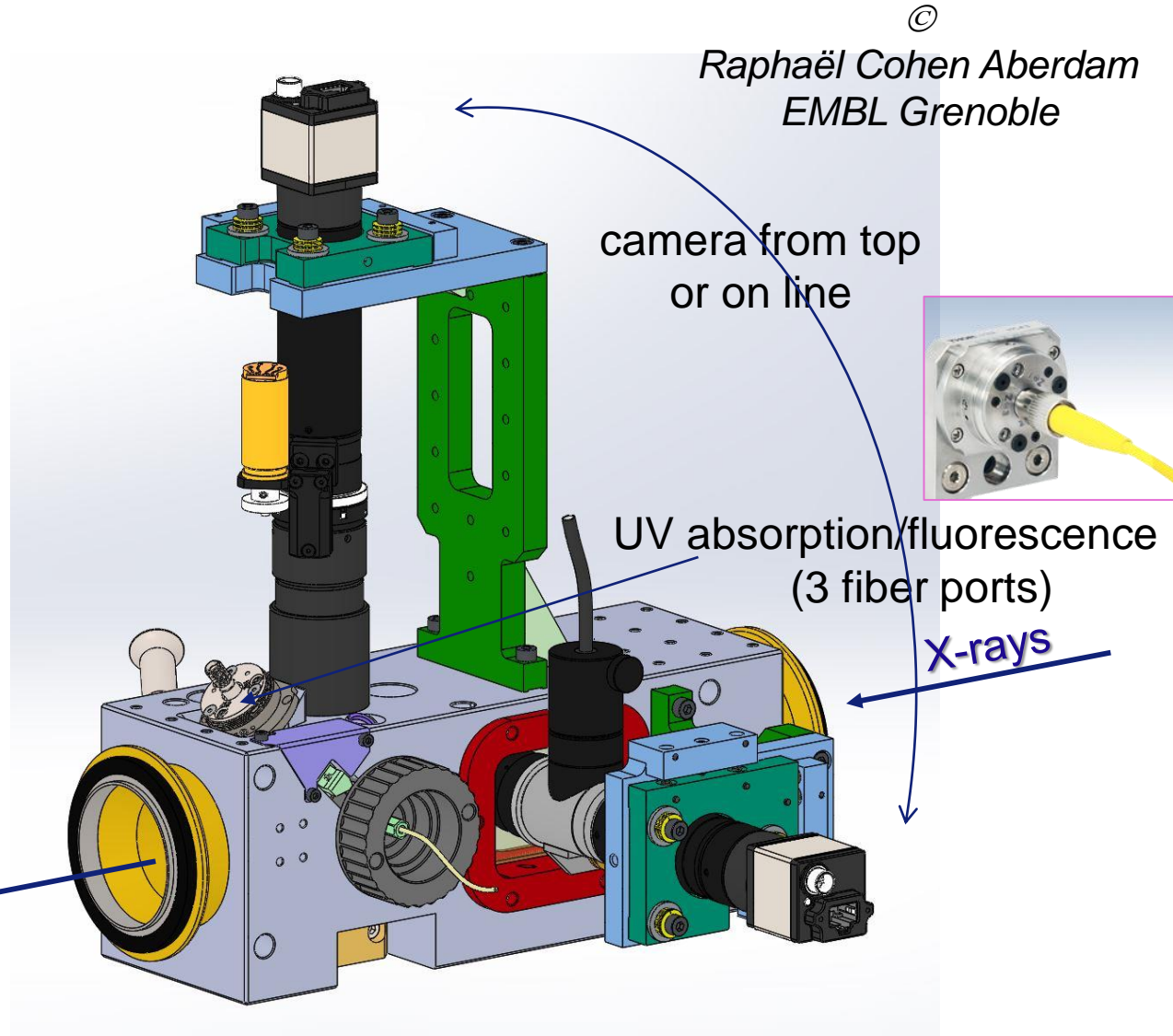
rectangular

\varnothing 0,5 mm

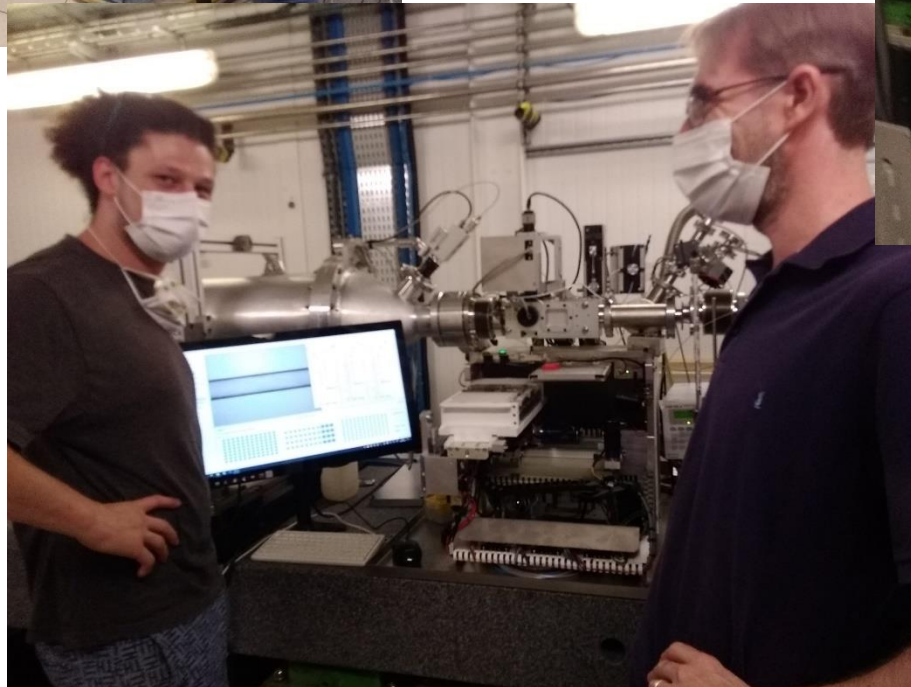
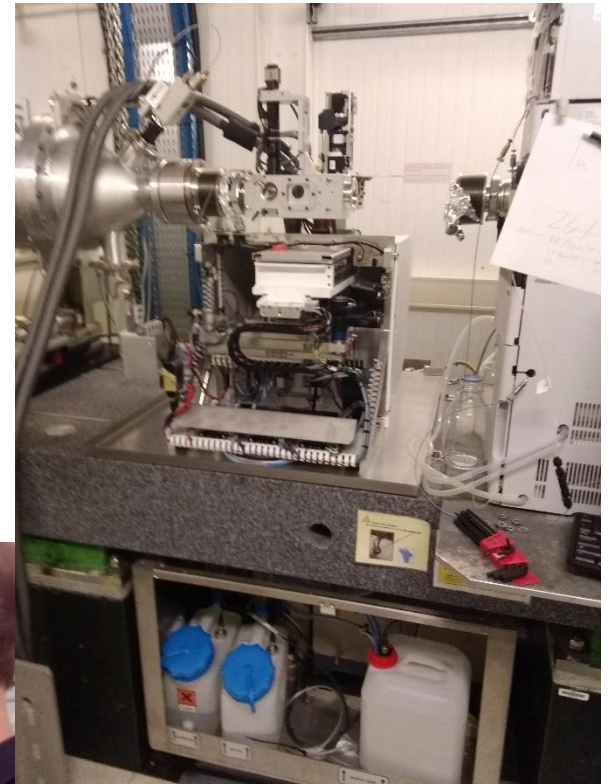
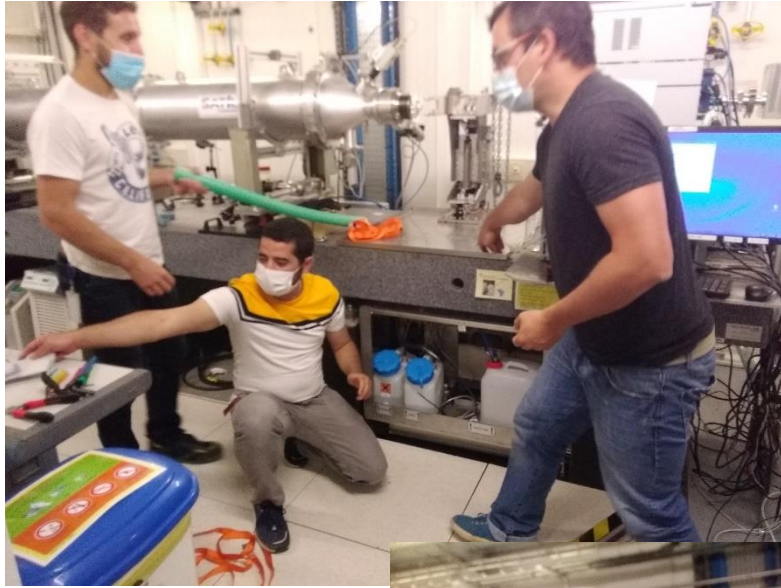
0,8 x 0,4 mm²

Robot and SEU

- optimized for smaller capillaries: 1 mm standard
- more precise syringe for loading used



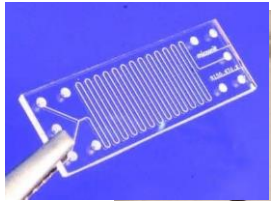
SAMPLE CHANGER AND ITS EXPOSURE UNIT INSTALLATION



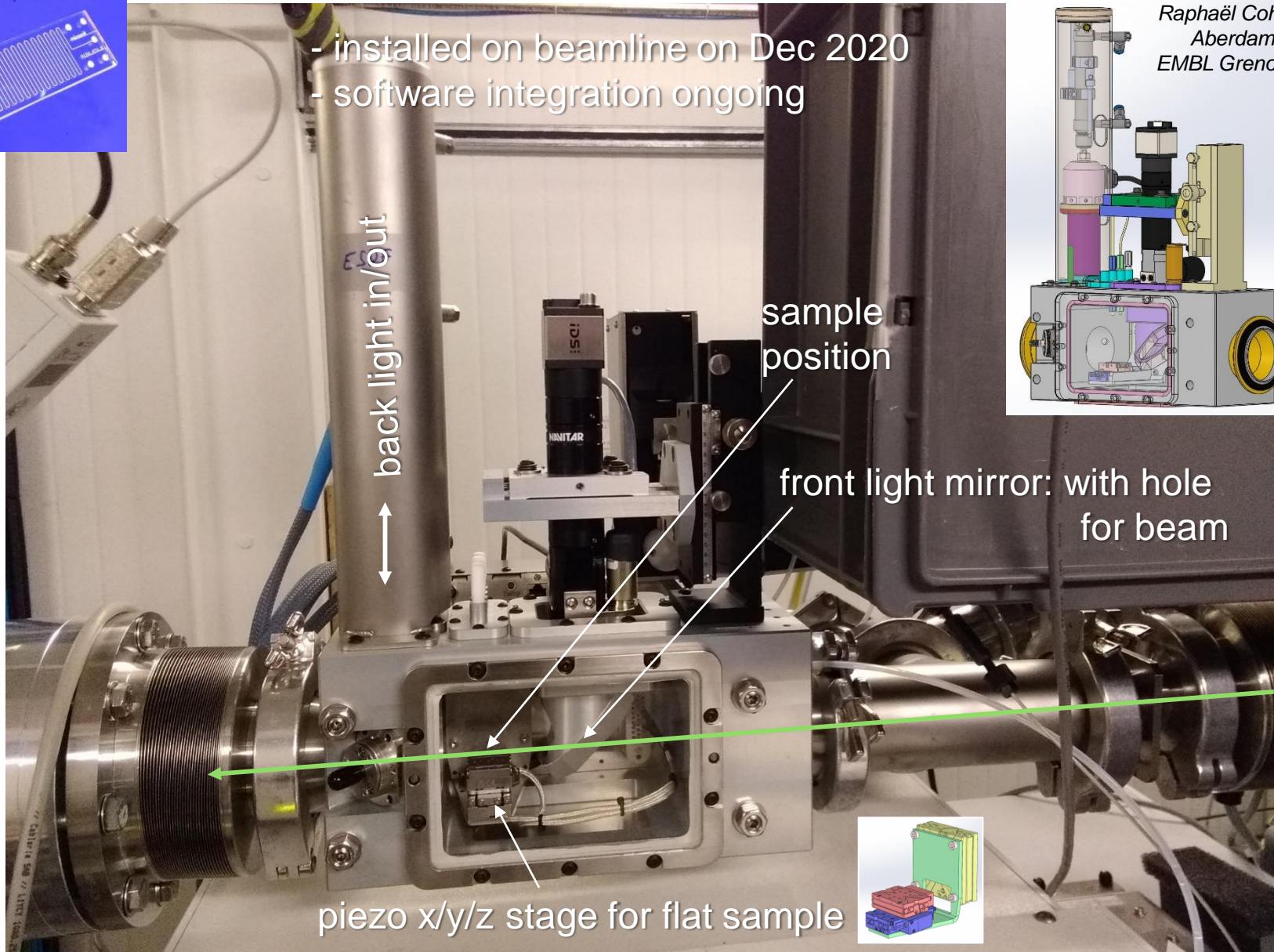
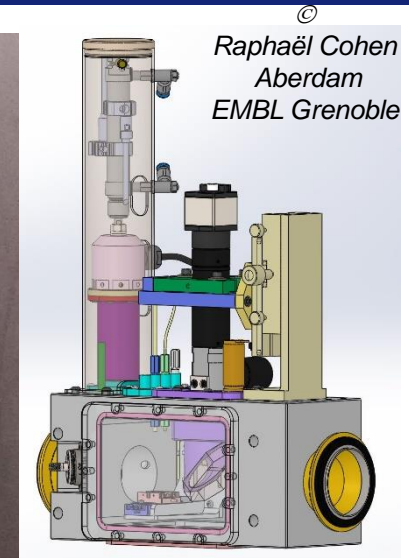
SAMPLE CHANGER AND ITS EXPOSURE UNIT



SCANNING EXPOSURE UNIT



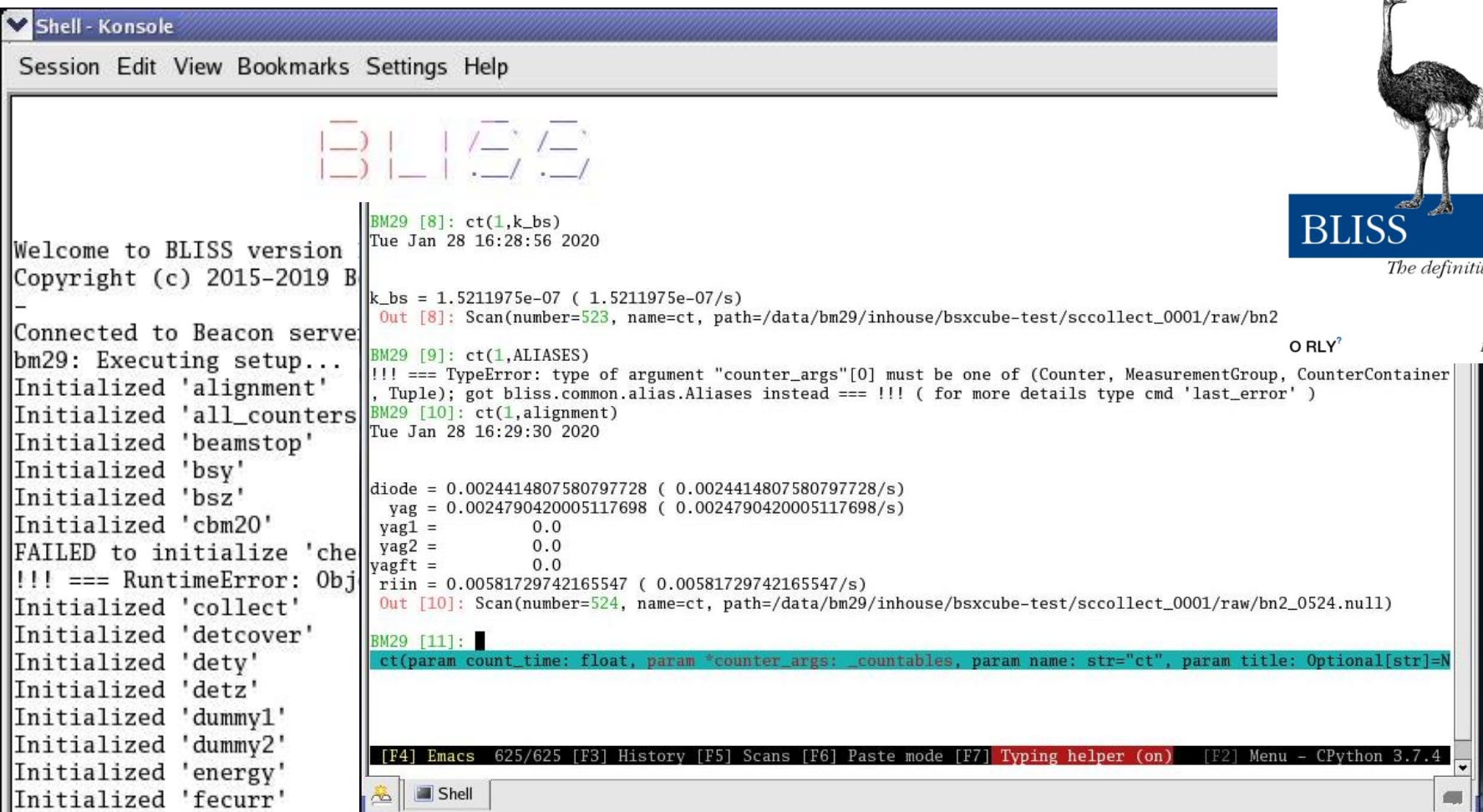
- installed on beamline on Dec 2020
- software integration ongoing



BeamLine Instrumentation Support Software

BM29: Antonia Beteva

- ESRF in-house development, Python based
- installation, configuration and commissioning ongoing from 2019
- done



```
Shell - Konsole
Session Edit View Bookmarks Settings Help

Welcome to BLISS version
Copyright (c) 2015-2019 B
-
Connected to Beacon server
bm29: Executing setup...
Initialized 'alignment'
Initialized 'all_counters'
Initialized 'beamstop'
Initialized 'bsy'
Initialized 'bsz'
Initialized 'cbm20'
FAILED to initialize 'che
!!! === RuntimeError: Obj
Initialized 'collect'
Initialized 'detcover'
Initialized 'dety'
Initialized 'detz'
Initialized 'dummy1'
Initialized 'dummy2'
Initialized 'energy'
Initialized 'fcurr'

BM29 [8]: ct(1,k_bs)
Tue Jan 28 16:28:56 2020
k_bs = 1.5211975e-07 ( 1.5211975e-07/s)
Out [8]: Scan(number=523, name=ct, path=/data/bm29/inhouse/bsxcube-test/sccollect_0001/raw/bn2

BM29 [9]: ct(1,ALIASSES)
!!! === TypeError: type of argument "counter_args"[0] must be one of (Counter, MeasurementGroup, CounterContainer
, Tuple); got bliss.common.alias.Aliases instead === !!! ( for more details type cmd 'last_error' )
BM29 [10]: ct(1,alignment)
Tue Jan 28 16:29:30 2020
diode = 0.0024414807580797728 ( 0.0024414807580797728/s)
yag = 0.0024790420005117698 ( 0.0024790420005117698/s)
yag1 = 0.0
yag2 = 0.0
yagft = 0.0
riin = 0.00581729742165547 ( 0.00581729742165547/s)
Out [10]: Scan(number=524, name=ct, path=/data/bm29/inhouse/bsxcube-test/sccollect_0001/raw/bn2_0524.null)

BM29 [11]:
ct(param count_time: float, param *counter_args: _countables, param name: str="ct", param title: Optional[str]=N

[F4] Emacs 625/625 [F3] History [F5] Scans [F6] Paste mode [F7] Typing helper (on) [F2] Menu - CPython 3.7.4
```



DATA COLECTION GUI: BSXCUBE3

Technology stack as for MXCuBE3

Backend: Python 3.7, Flask

Frontend: ES6 (JavaScript)React 16, Bootstrap 4

HTML web interface and includes an intuitive user interface with a logical flow

Markus Oskarsson

Jean-Baptiste Florial (EMBL)

Acquisition Acquisition result Beamline Setup System log

The image displays two screenshots of the BSXCuBE 3 web interface. The left screenshot shows the 'Acquisition' view, which includes a navigation bar with 'Acquisition', 'Acquisition result', 'Beamline Setup', and 'System log'. Below the navigation bar, there are three status indicators: Energy (0.000 keV), Transmission (0.00 %), and Ring Current (0). A 'Data path' field shows a sample name and protein acronym. The main content area features a welcome message 'Welcome blissadm!' and a prompt 'To get started, select experiment below'. Three large buttons are visible: 'Sample changer', 'HPLC', and 'Scan'. The right screenshot shows the 'Beamline setup' view, which includes the same navigation bar and status indicators. The main content area is divided into several control panels: 'Minihutch Slits', 'EH Slits 1', 'EH Slits 2', 'Beamstop', and 'Sample'. Each panel contains 'Position' and 'Aperture' controls, represented by directional arrows and a central red 'X' button.

BSXCUBE3 SAMPLE CHANGER DATA ACQUISITION

BSXCuBE 3 | multivisor | Extended ISPyB

localhost:3000/acquisition/sc

BSXCuBE 3 | Acquisition result | Beamline Setup | System log

Front End: Wait for permission | Safety shutter: Fault state | Fast shutter: Closed | Energy: 12.500 keV | Transmission: 0.00 %

Data path: [icon]

Queue: 1

Sample Changer | Experiment Name: pb_fle_pankaj | Load Parameters | Save

Sample: [] Buffer: []

Plate 1: 96 Deep Well Plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	[]	[]	[]	[]	[]	[]	[]	[]	[]	[]	[]	[]
B	[]	[]	[]	[]	[]	[]	[]	[]	[]	[]	[]	[]
C	[]	[]	[]	[]	[]	[]	[]	[]	[]	[]	[]	[]
D	[]	[]	[]	[]	[]	[]	[]	[]	[]	[]	[]	[]
E	[]	[]	[]	[]	[]	[]	[]	[]	[]	[]	[]	[]
F	[]	[]	[]	[]	[]	[]	[]	[]	[]	[]	[]	[]
G	[]	[]	[]	[]	[]	[]	[]	[]	[]	[]	[]	[]
H	[]	[]	[]	[]	[]	[]	[]	[]	[]	[]	[]	[]

Plate 2: 4 x (8 + 3) Block

	1	2	3	4	5	6	7	8	9	10	11
A	[●]	[●]	[●]	[●]	[●]	[●]	[]	[]	[●]	[●]	[]
B	[]	[]	[]	[]	[]	[]	[]	[]	[]	[]	[]
C	[]	[]	[]	[]	[]	[]	[]	[]	[]	[]	[]
D	[]	[]	[]	[]	[]	[]	[]	[]	[]	[]	[]

Plate 3: 96 well Plate

	1	2	3	4	5	6	7	8	9
A	[]	[]	[]	[]	[]	[]	[]	[]	[]
B	[]	[]	[]	[]	[]	[]	[]	[]	[]
C	[]	[]	[]	[]	[]	[]	[]	[]	[]
D	[]	[]	[]	[]	[]	[]	[]	[]	[]
E	[]	[]	[]	[]	[]	[]	[]	[]	[]
F	[]	[]	[]	[]	[]	[]	[]	[]	[]
G	[]	[]	[]	[]	[]	[]	[]	[]	[]
H	[]	[]	[]	[]	[]	[]	[]	[]	[]

Clear Table | Clear Selected Row | Optimisation | Expand Parameters: [] | Parameters: []

	Name	Buffer	Plate	Row	Column	C (mg/mL)	Flow	Extra Flow t(s)	Volume (µl)
[]		--Choose--	1	A	1	1	[]	5	50
[]	1	pbcc4a	2	A	1	5.6	[]	10	50
[]	2	pbcc4b	2	A	2	2.8	[]	10	50
[]	3	pbcc4c	2	A	3	1.4	[]	10	50
[]	4	FleR1	2	A	4	10	[]	10	50
[]	5	FleR2	2	A	5	5	[]	10	50
[]	6	FleR3	2	A	6	2	[]	10	50

< To Buffer Table

Clear Queue []

PROCESSED 1

PB_FLE_PANKAJ_SC

- 1: PB20CC4B
- 2: PBCC4A
- 3: PB20CC4B
- 4: PB20CC4B
- 5: PBCC4B
- 6: PB20CC4B
- 7: PB20CC4B
- 8: PBCC4C
- 9: PB20CC4B
- 10: TRIS
- 11: FLER1
- 12: TRIS
- 13: TRIS
- 14: FLER2
- 15: TRIS
- 16: TRIS
- 17: FLER3
- 18: TRIS

Start Queue

a few seconds ago 17:07
Actor added to the queue (scollect, 5bdfce5a-b794-4751-b32c-76c8bd13fae5)

6 hours ago 10:53
Queue completed, pausing

Run now or Add to Queue

BSXCUBE3 HPLC DATA ACQUISITION

BSXCuBE 3 | Acquisition result | Beamline Setup | System log | mx415 | Help | Signout

Front End: Wait for permission
Safety shutter: Fault state
Fast shutter: Closed
Energy: 12,500 keV
Transmission: 0.00%

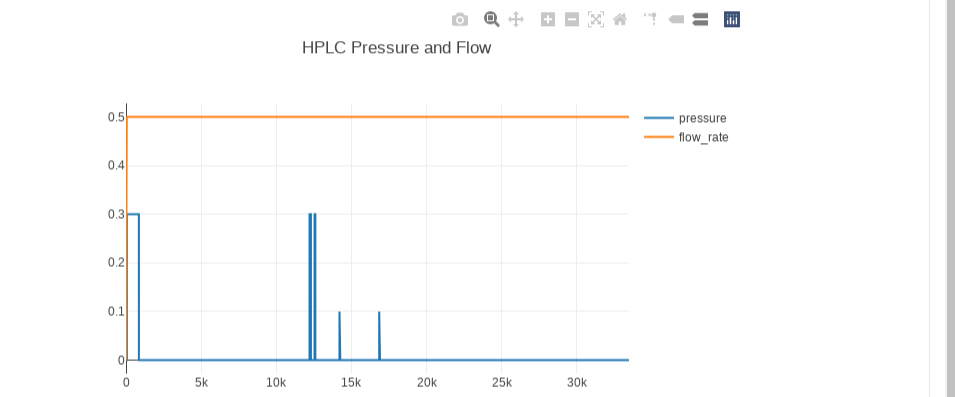
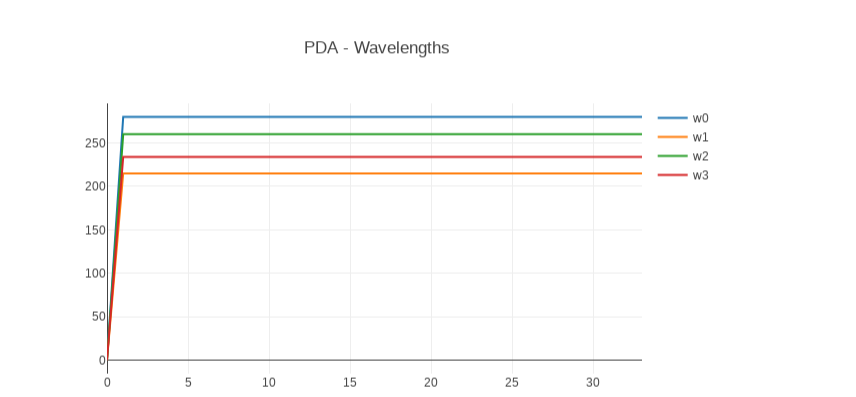
Data path:

Ring Current: 0
CBM20: Controlled
PDA: Controlled

Queue: 1

HPLC | Experiment Name: myhplc | Load Parameters | Save | Equilibrate | Sample Collect | HPLC Control

Disconnect to PDA | Disconnect to CBM | Start Injection | Stop Injection



HPLC Control

Flow Rate (mL/min) : 0.5 Min Pressure (MPa) : 0.1 Max Pressure (MPa) : 13.7	Port : A Pump (OFF) : Start Pump Purge (Stopped) : Start Purge	Auto Sample : ON Auto Sample Temp : (20) : 20 Vial Number * : 1	Lamp : ON Purge RI : OFF Auto Zero RI : OFF
---	---	---	---

BSXCUBE3 HPLC DATA ACQUISITION

BSXCuBE 3 | multivisor | Extended ISPyB | Acquisition result | Beamline Setup | System log | mx415 | Help | Signout

Front End: Wait for permission | Safety shutter: Fault state | Fast shutter: Closed | Energy: 12.500 keV | Transmission: 0.00 %

Ring Current: 0 | CBM20: Controlled | PDA: Controlled

Data path: | Queue: 1

HPLC | Experiment Name: myhplc | Load Parameters | Save | Equilibrate | Sample Collect | HPLC Control

Auto Sample: Set OFF | Plate: HPLC Well Plate | Selected Vial Number: 1

Auto Sample Temp: (20) 20 ✓

Sample Parameters:

Sample Name: sample_z | Vial Number: 1 | Injection Volume (µl): 50

Number of Frame: 240 (Recommended Value: 240.0) | Column Elution T (min): 4 (Recommended Value: 4.0) | Exposure Time (s): 1

λ1 (nm): 260 | λ2 (nm): 280 | λ3 (nm): 350

λ4 (nm): 450 | Exposure Temp °C: 20 | Flow Rate (mL/min): 1

Comment: c

Add to Sample Table

Clear Table | Clear Selected Row | Optimisation | Expand Parameters: | Parameters: | Number of HPLC Sample's: 1

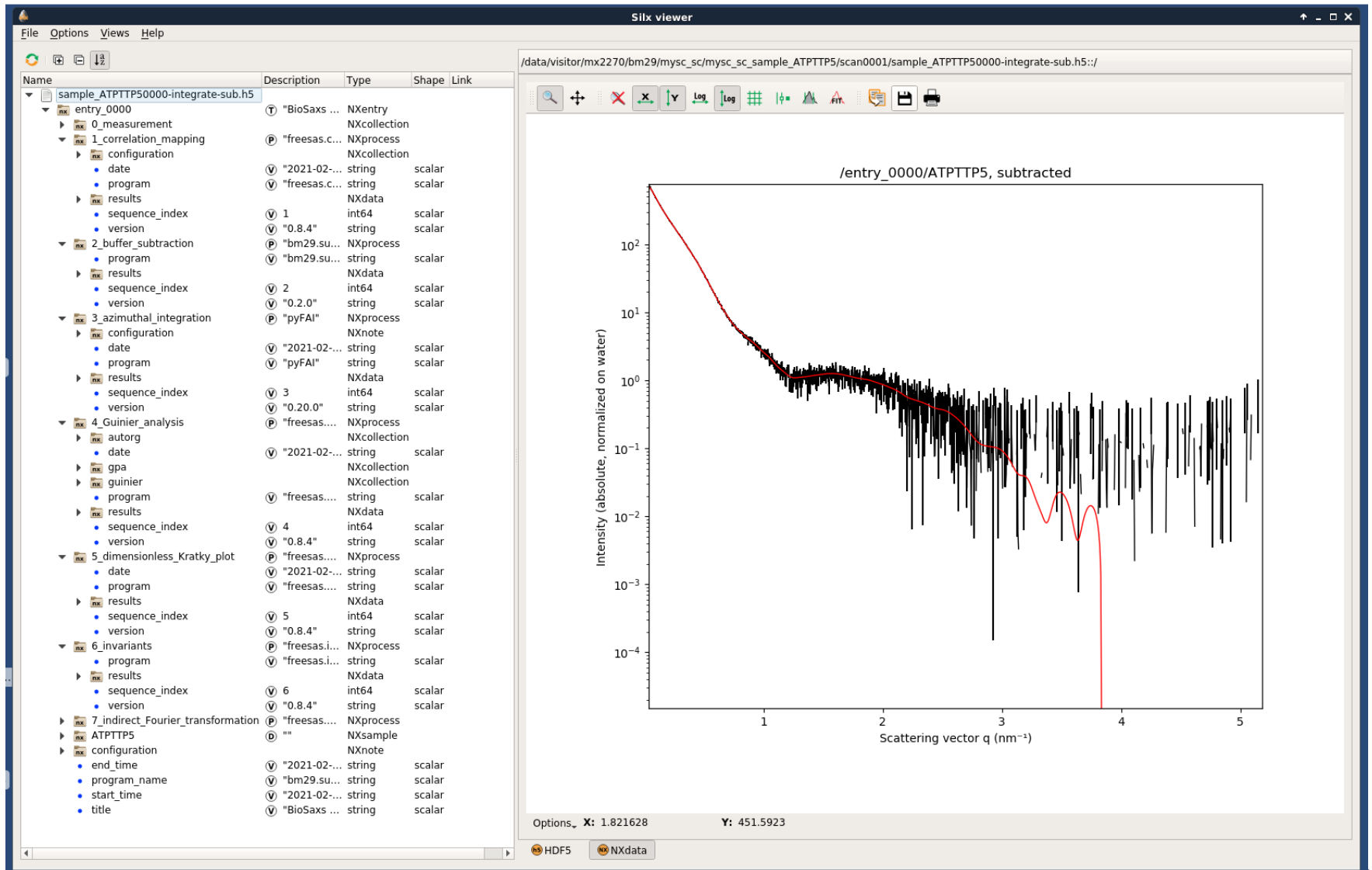
Sample Name	Flow R. (mL/min)	Vial Number	Injection Volume (µl)	λ1 (nm)	λ2 (nm)	λ3 (nm)	λ4 (nm)	Concentration	Exposure Temp °C	Exposure Time (s)	Number of Frames	Column Elution (min)	Comment	
z	1	1	50	260	280	350	450	1	20	2	240	4	c	
1	z	1	1	50	260	280	350	450	1	20	2	240	4	c

Back: To Equilibrate

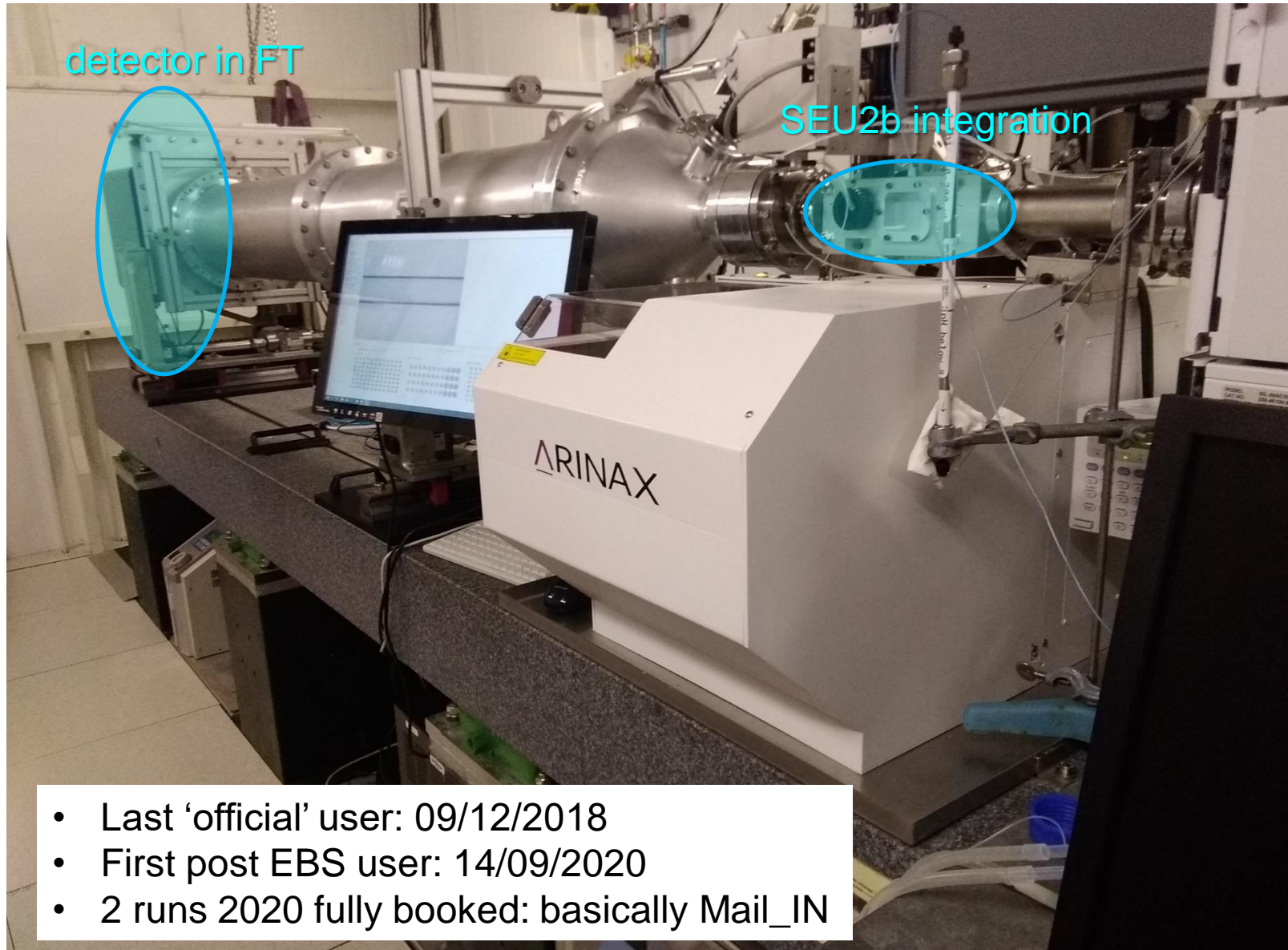
SC Initial Cleaning: | Wait for Beam: | Run Now | Add to Queue

opensource data reduction pipeline, FreeSAS

Jerome Kiefer



CURRENT STATUS OF BIOSAXS BM29 REFURBISHMENT – NEARLY THERE



- Last 'official' user: 09/12/2018
- First post EBS user: 14/09/2020
- 2 runs 2020 fully booked: basically Mail_IN

Remote access inappropriate to experiments on macromolecules in solution:

- sample preparation just prior to measurement often necessary,
- robot can not be loaded for hours of data collection,
- hplc set-up manualy

1 external user allowed per session to help LC (travelling abroad difficult)

3 users per session up to 3 times per run and beamline (1/3 this run filled)

We try to limit pure mail_IN sample quantity to what is humanly possible to process (setting up the beamline, sample prep, hplc column equilibrium, running experiment and watching errors) in 10h: ~ 100 samples in batch mode or 10 in sec-saxs (in same buffer) and combinations.

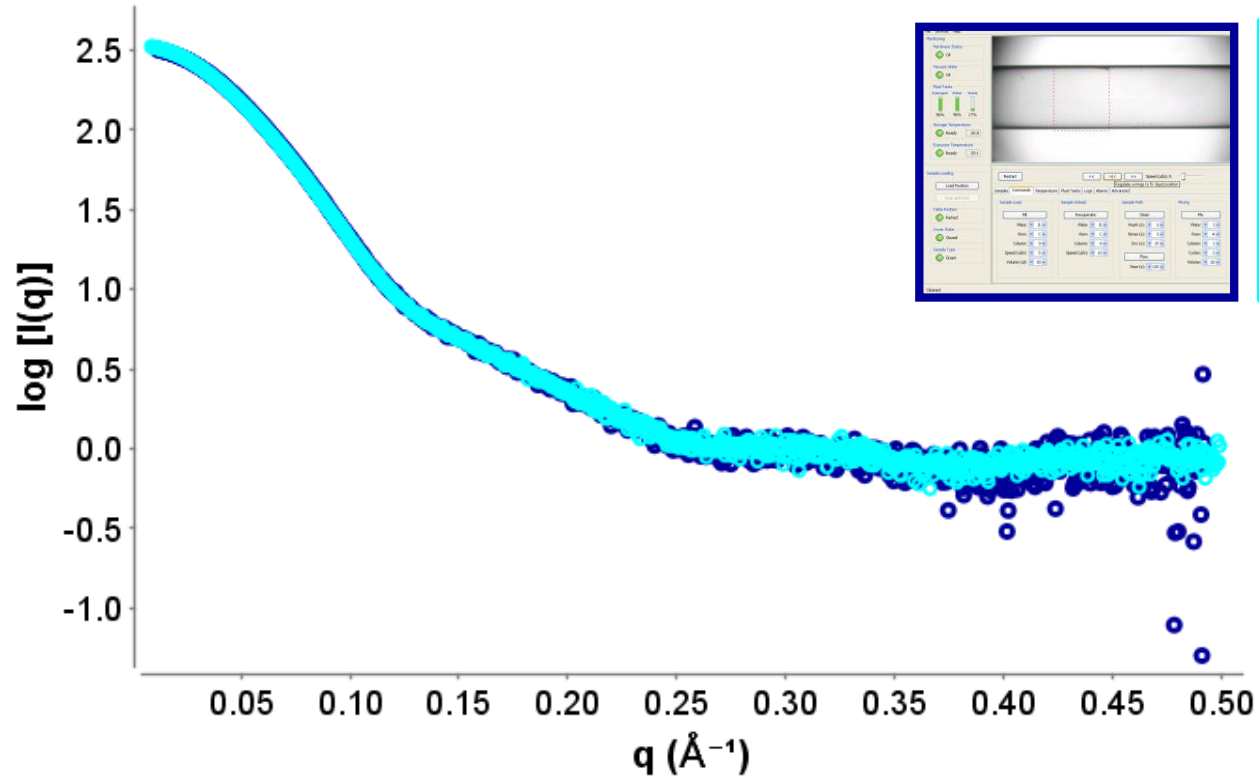
Experiments available :- dilution series (3 dilutions) / varying temperature from 4 – 50 °C / change in energy/ change in attenuation

Columns for SEC-SAXS provided

Samples, buffers and instruction for sample prep and experiment should be filled in Delivery form and send to LC before the experiment date.

Details in BM29 Mail_In Protocol.pdf (send to all BAG/Rolling responsables)

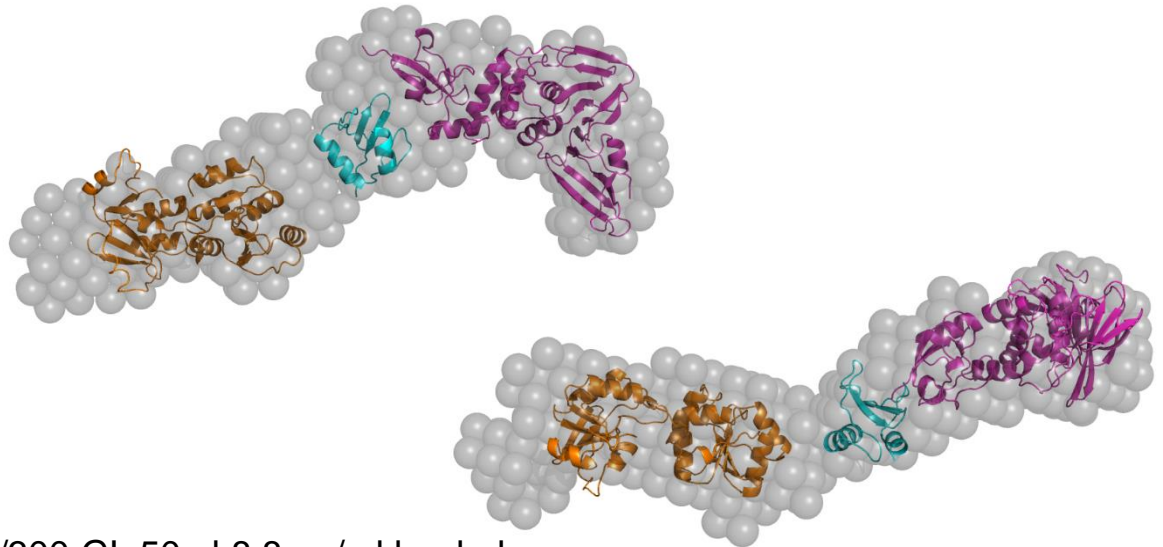
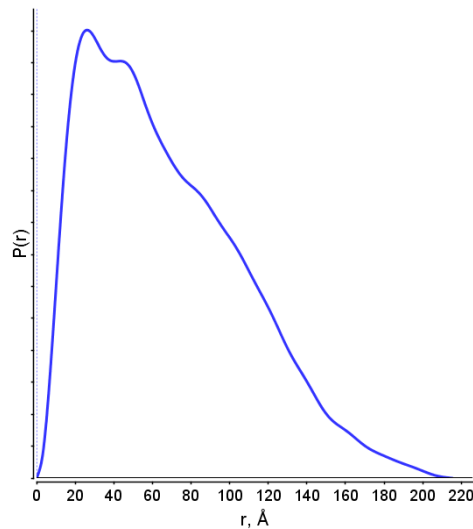
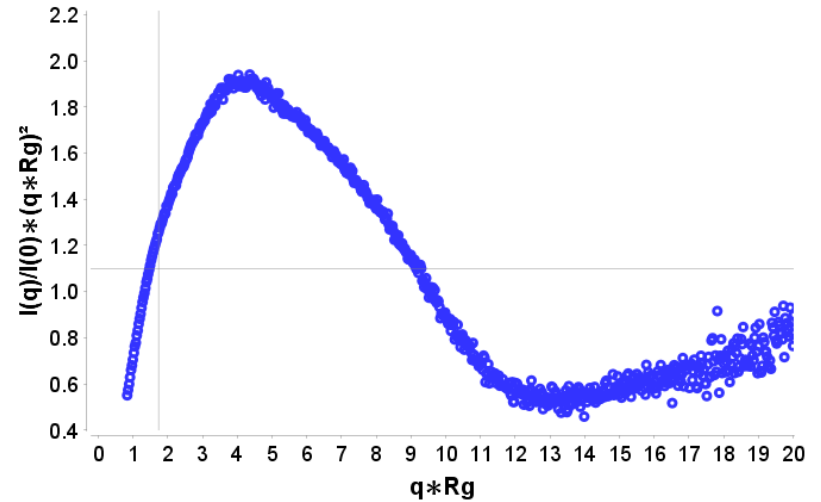
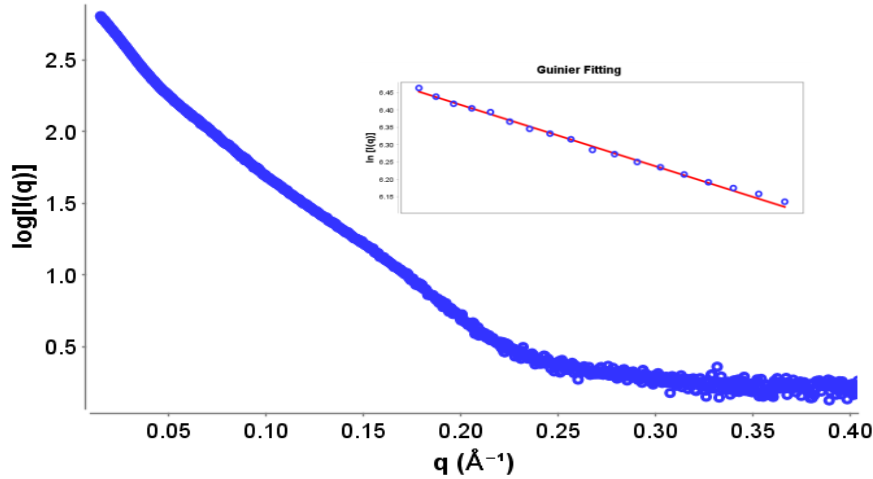
BSA Pre and Post upgrade using Robot



- 10 frames at 1 second 50% Post EBS (1 mm capillary)
- 10 frames at 1 second 100% Pre EBS (1.8 mm capillary)

Buffer 50 mM Hepes pH 7.5

User Data – Post EBS SEC-SAXS

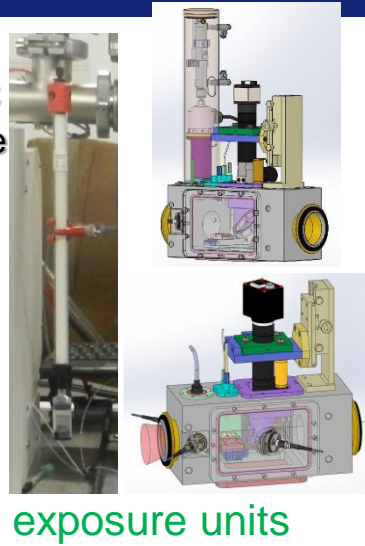
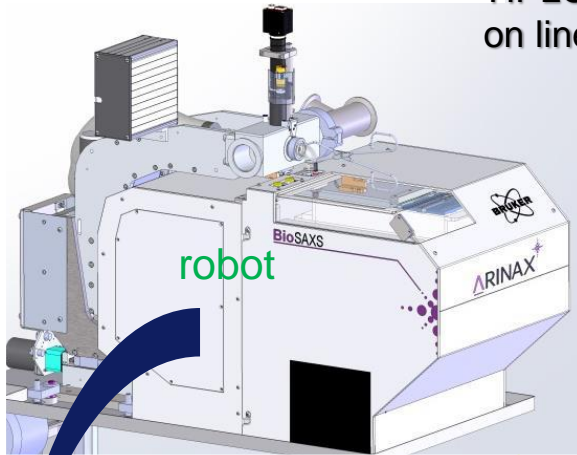


SEC-SAXS using Superdex200 10/300 GL 50 μ l 8.8mg/ml loaded.

AFTER EBS BIOSAXS BEAMLINE OUTLOOK

SAMPLE DELIVERY

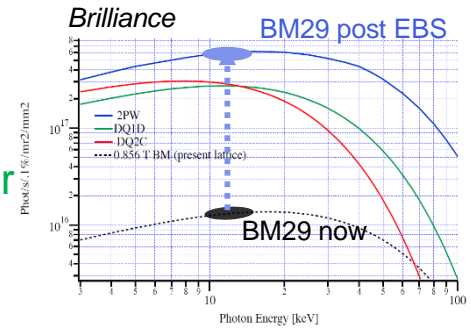
HPLC
on line



exposure units

SOURCE

BM → 2 pole
wiggler



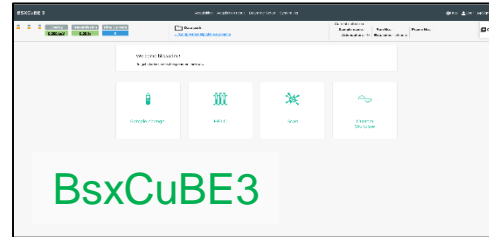
DETECTION

1M → 2M in
vacuum



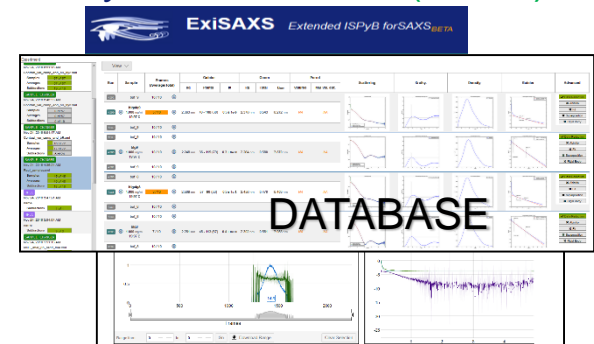
SOFTWARE

SPEC → BLISS
data collection GUI



including workflows

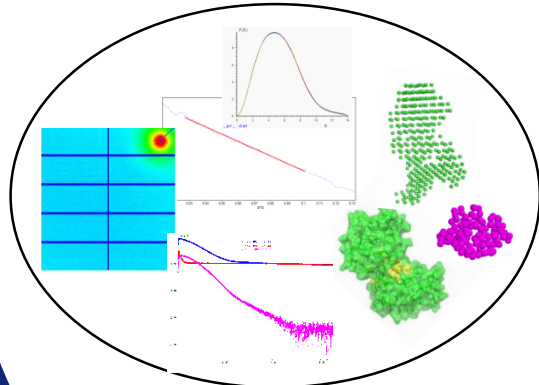
ISPyB: EXI → EXI2 (React)



DATABASE

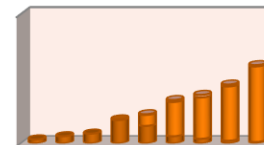
sample tracking and data flow

EDNA → DAHU



data processing and analysis pipeline

rise of BioSAXS long may it continue



AKNOWLEDGMENT

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ESRF BIOSAXS PUBLICATIONS

As on January 2021

$$\Sigma_{\text{TOTAL BIOSAXS ESRF}} = 738$$

