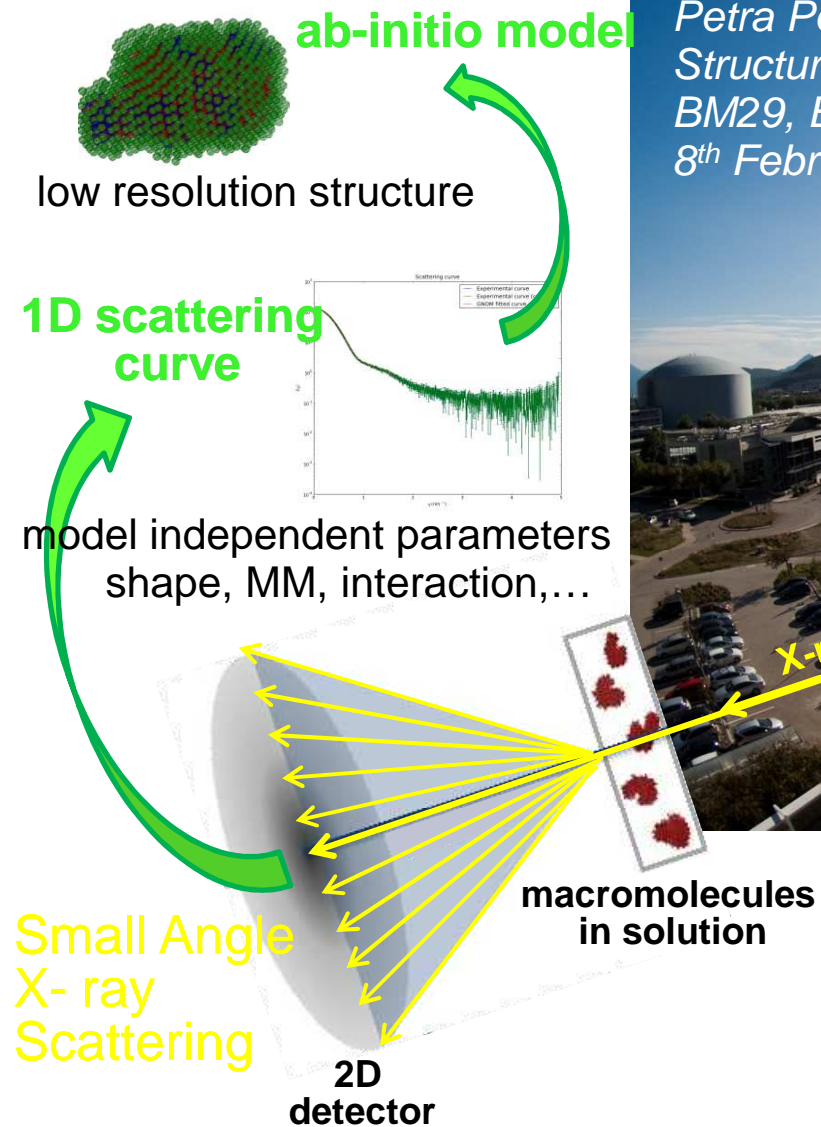




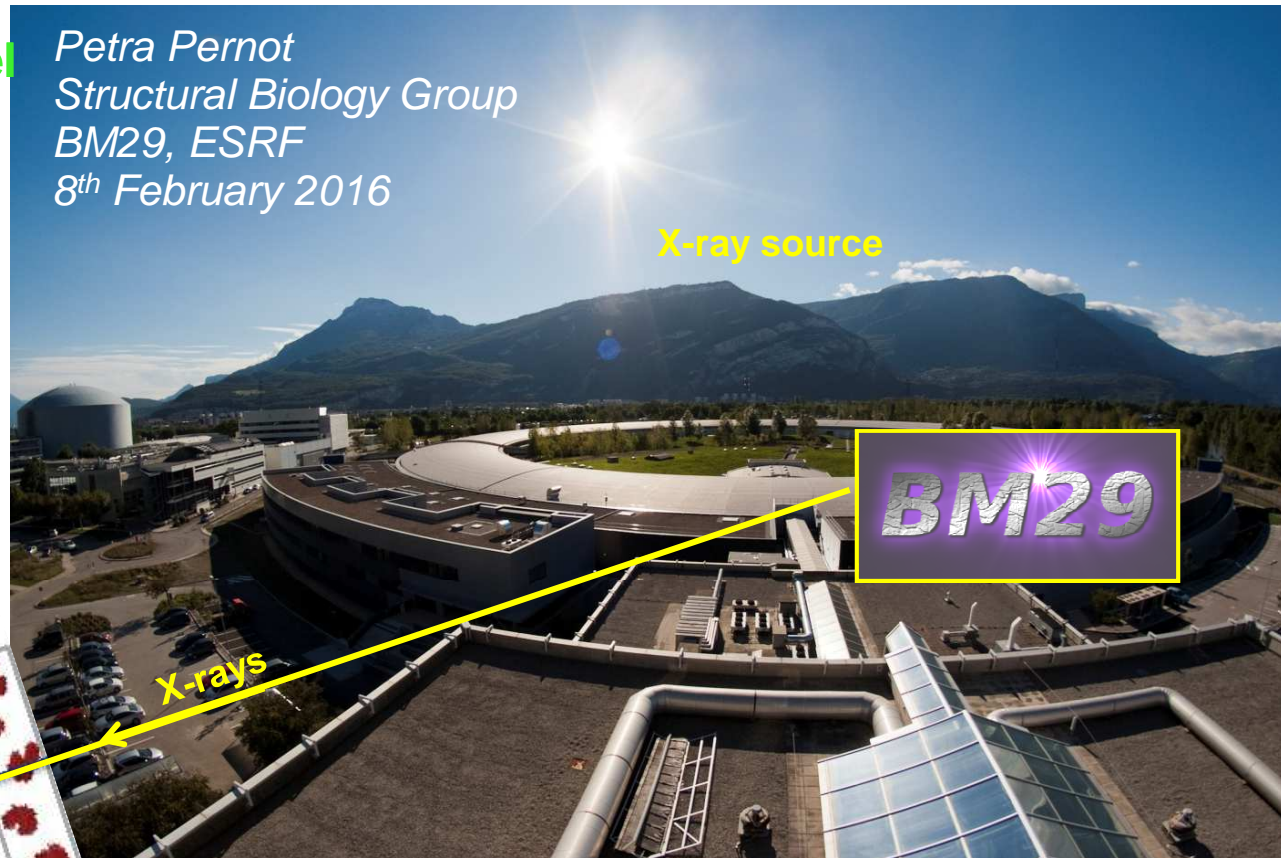
ESRF

| The European Synchrotron

BM29 IN GLANCE = BIOSAXS POWER



Petra Pernot
Structural Biology Group
BM29, ESRF
8th February 2016



OUTLINE

- ESRF BioSAXS beamline(s) key features and stats
- automated data collection and analysis
- microfluidics: crystallisation in situ
- complementarity with SANS: joint access
- news

BM29 BIOSAXS KEY FEATURES

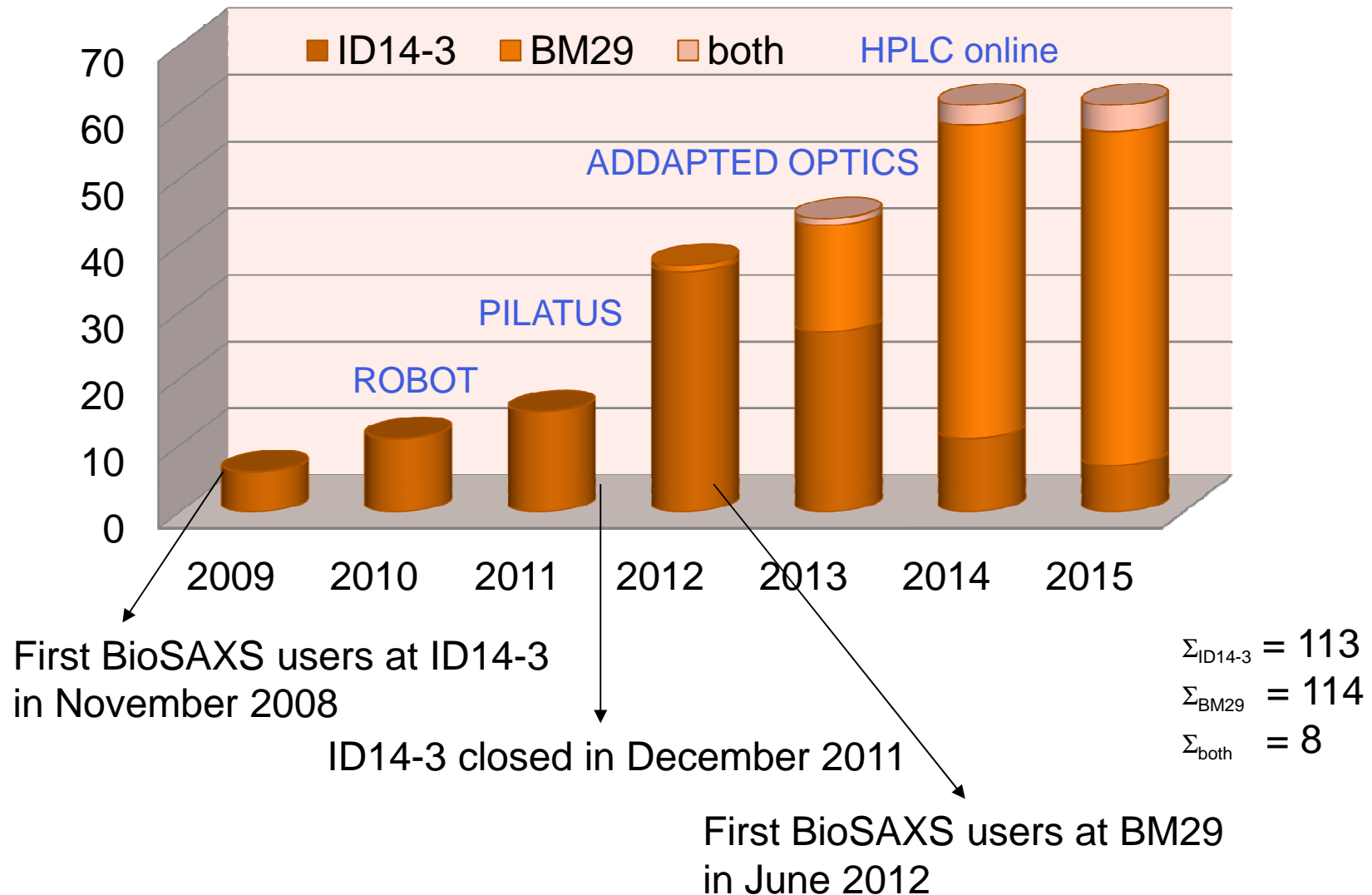
- beamline is dedicated exclusively to small-angle scattering experiments of biological macromolecules in solution: technique for shape, assembly & architecture to complement & aid MX, NMR, EM,... and SANS (joint SAX/NS proposals);
- provides information on every sample from moderate volumes (~ 15 μ l) & concentrations (1-10 mg/ml);
- allows rapid data collection & broad range of conditions (pH, temperature, substrates, co-factors, etc.) with automated data analysis and data tracking;
- high throughput facility “easy” to use in
 - a) **sample changer** mode 10” (cycle loading-exposure-cleaning ~ 1.5 ’)
 - b) **HPLC on-line** mode 30’ (column elution time);
- can test/suggest functionally important assemblies & conformation in solution vs. MX & provide overall envelopes for fitting high resolution domains.

Downsides - information is spherically averaged, resolutions restricted (worse than 12Å) & models can be underdetermined

ESRF BIOSAXS PUBLIS

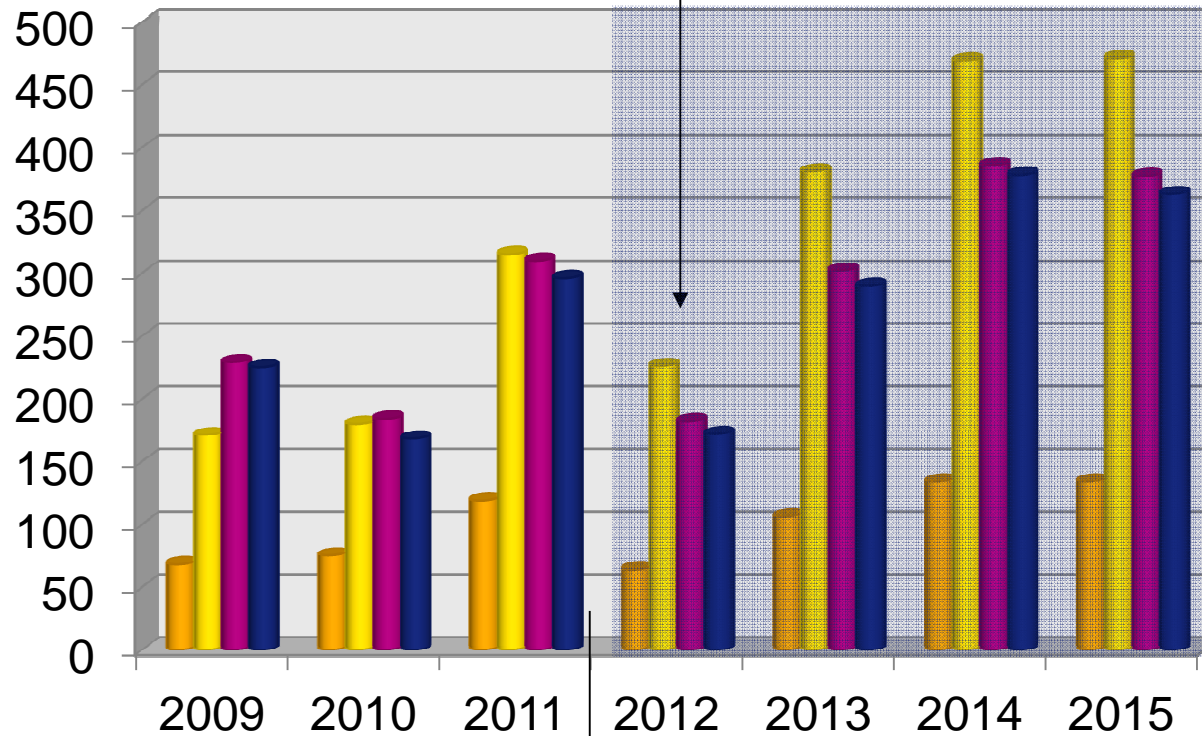
As on 7th January 2016

$$\Sigma_{\text{TOTAL}} = 235$$



ESRF BIOSAXS EXPERIMENTAL SESSIONS, USERS, SHIFTS

First BioSAXS users at BM29
in June 2012



■ N of exp. sessions

$$\Sigma_{2015} = 134$$

■ User counts

$$\Sigma_{2015} = 471$$

■ Scheduled shifts

$$\Sigma_{2015} = 377$$

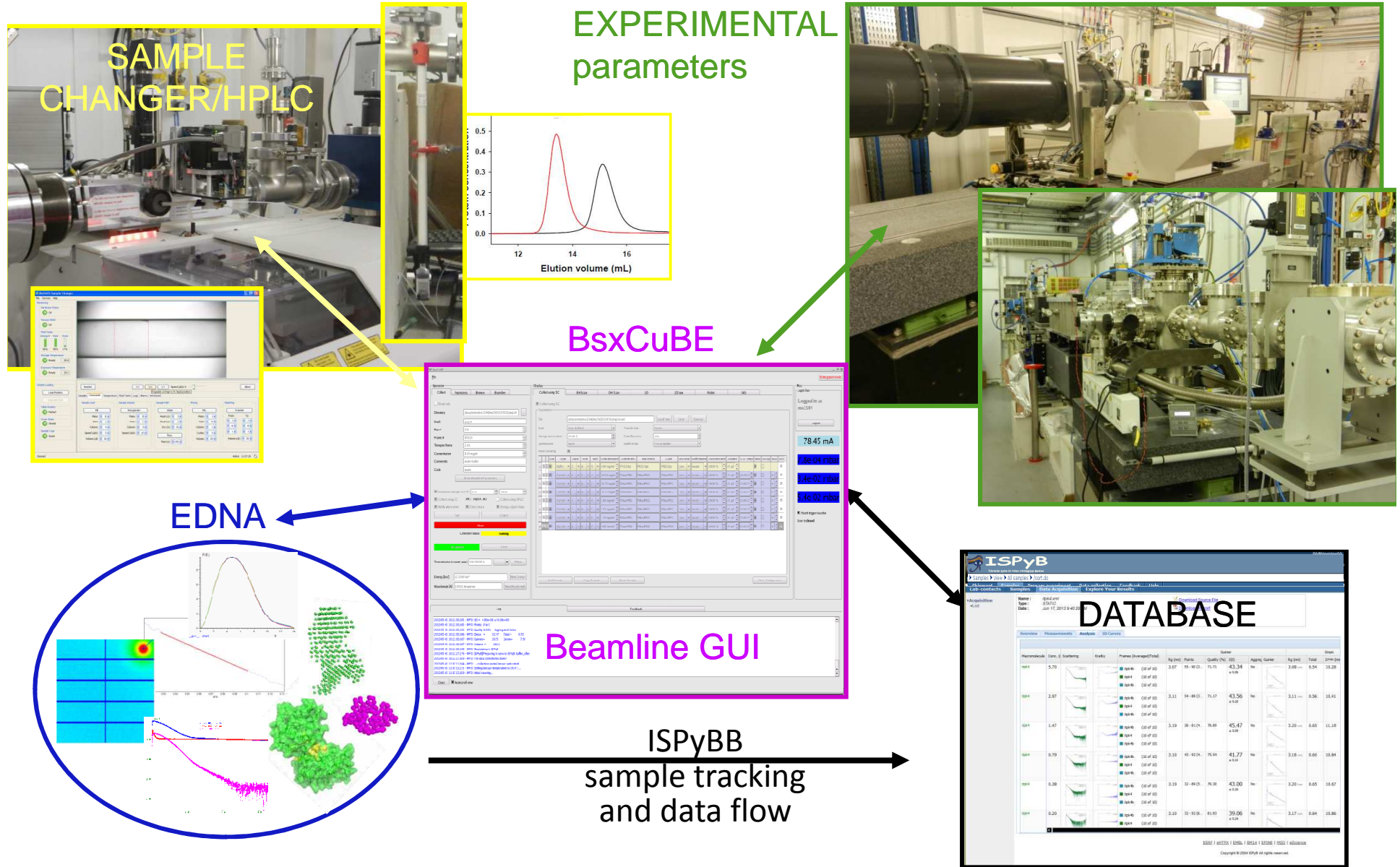
■ Done shifts

$$\Sigma_{2015} = 363$$

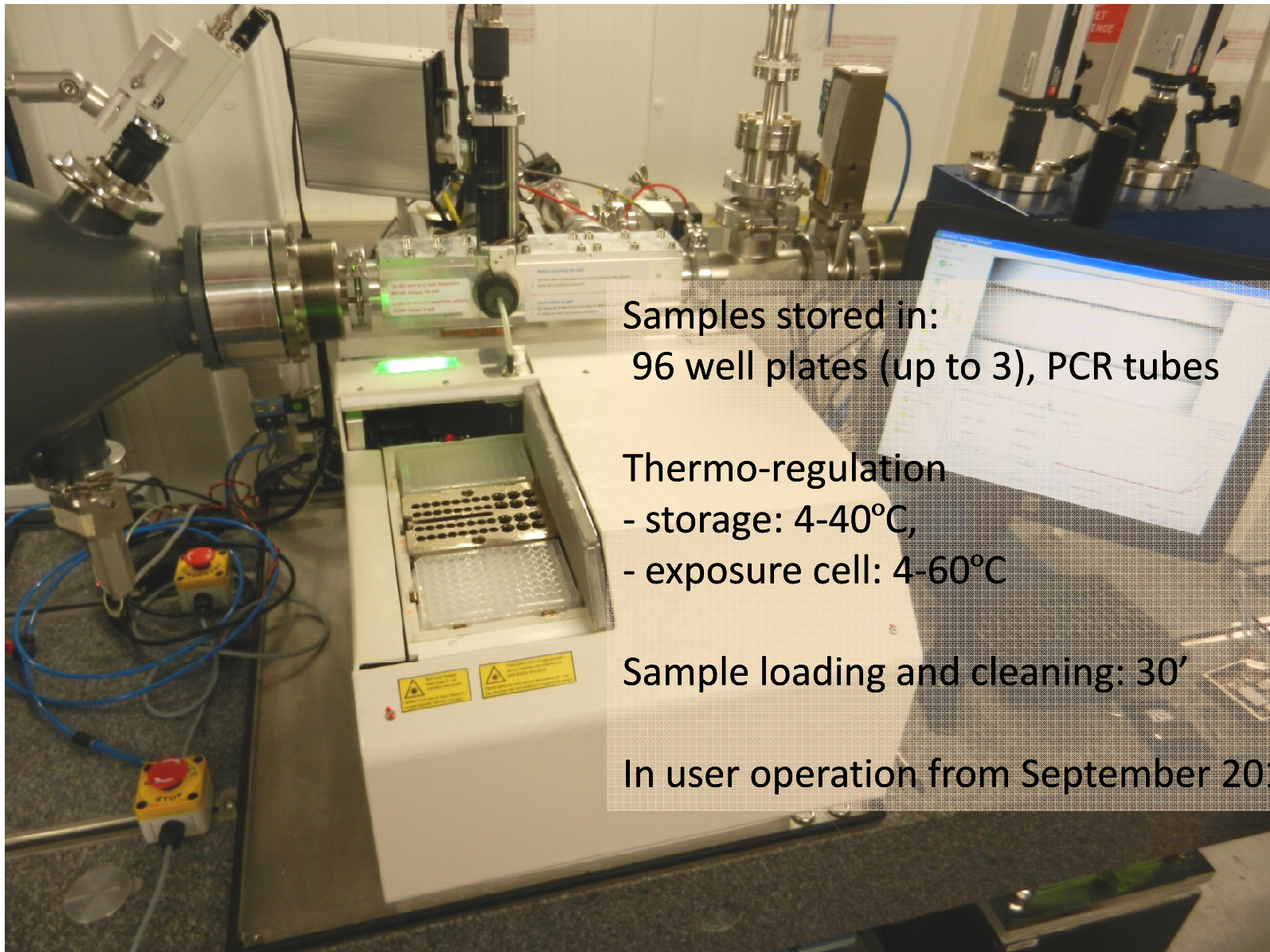
ID14-3 closed in December 2011

	ID14-3	BM29	Σ total
Σ N°Exp.	262	438	700
Σ Users	666	1547	2213
Σ sched. shifts	723	1243	1970
Σ done shifts	689	1203	1892

BM29 OUTLOOK – AUTOMATISATION



BM29 OUTLOOK – SAMPLE CHANGER



Samples stored in:
96 well plates (up to 3), PCR tubes

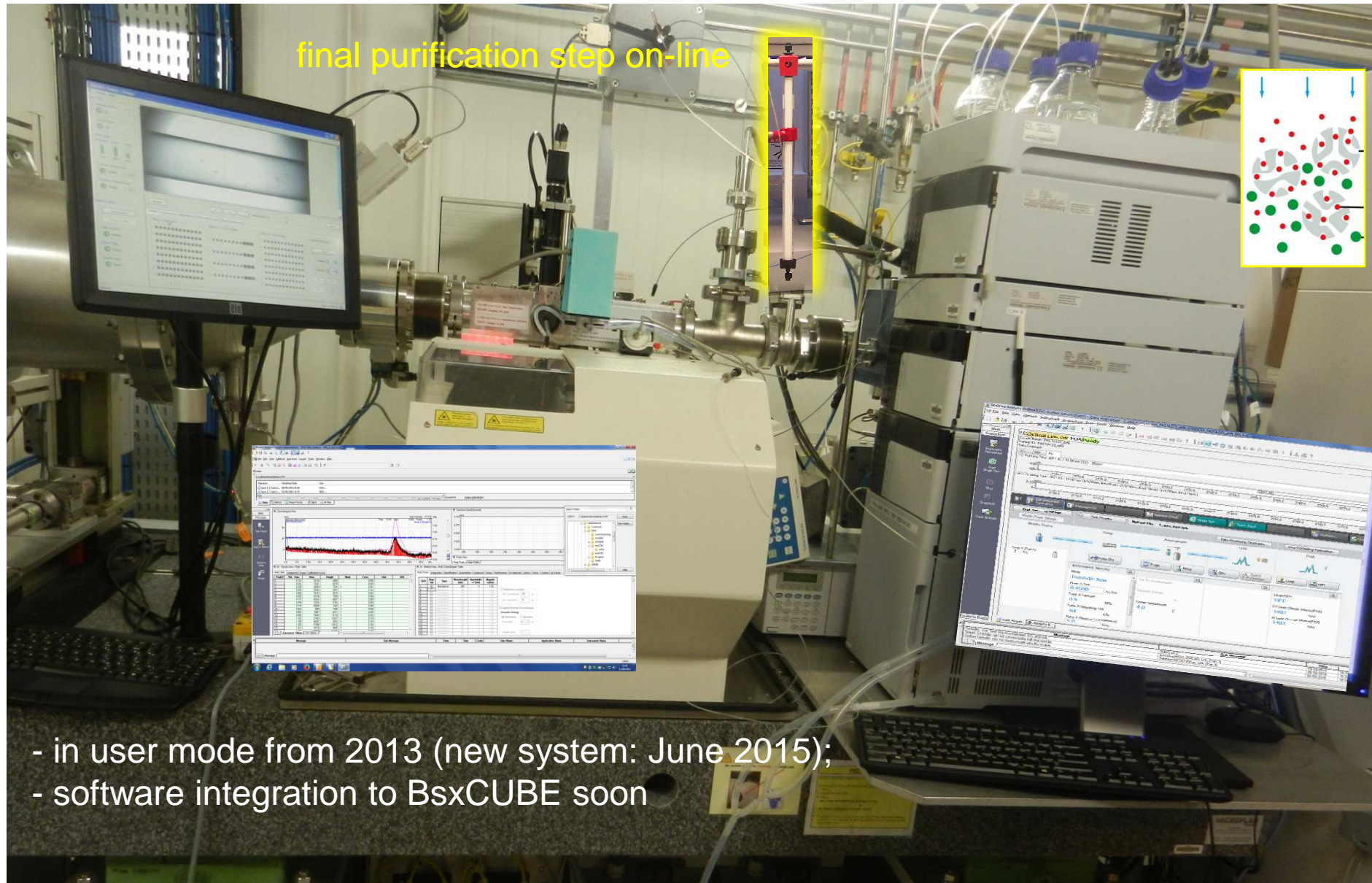
Thermo-regulation

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

Sample loading and cleaning: 30'

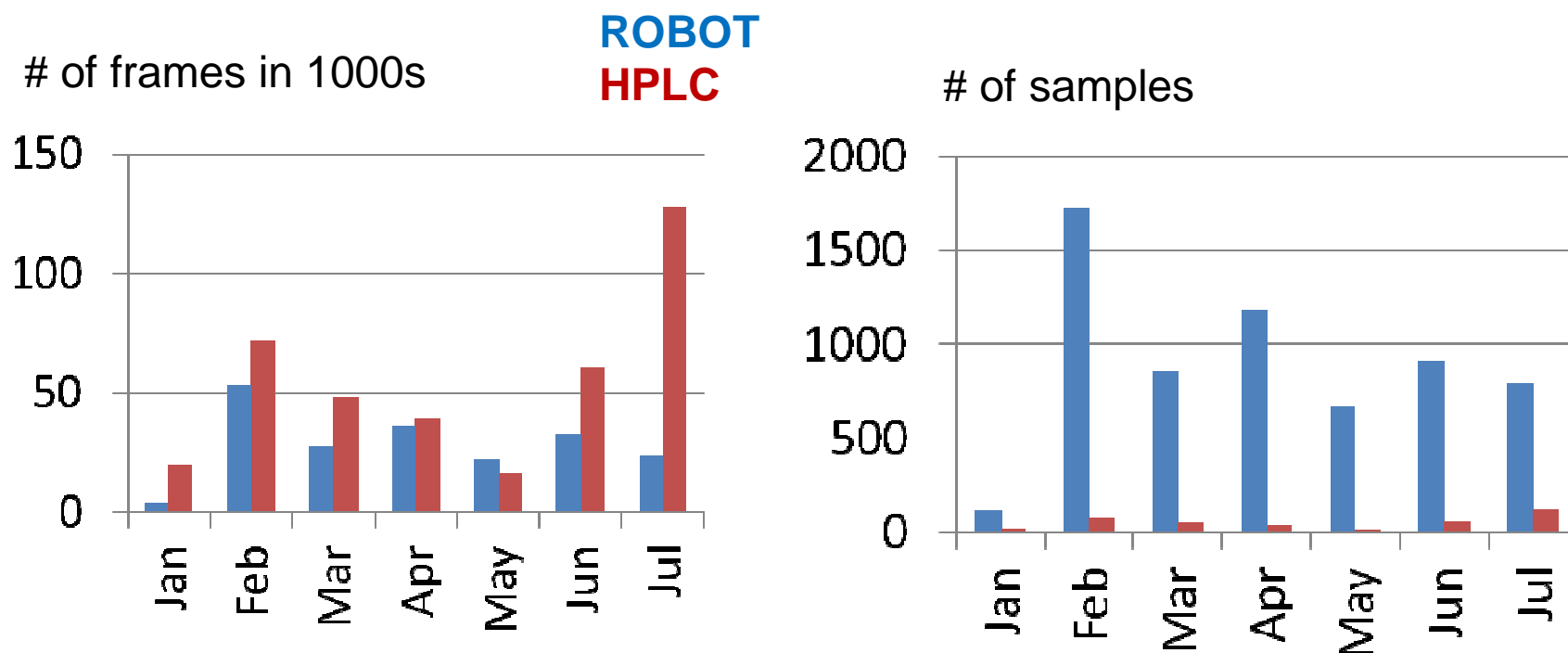
In user operation from September 2010

BM29 OUTLOOK – HPLC SYSTEM



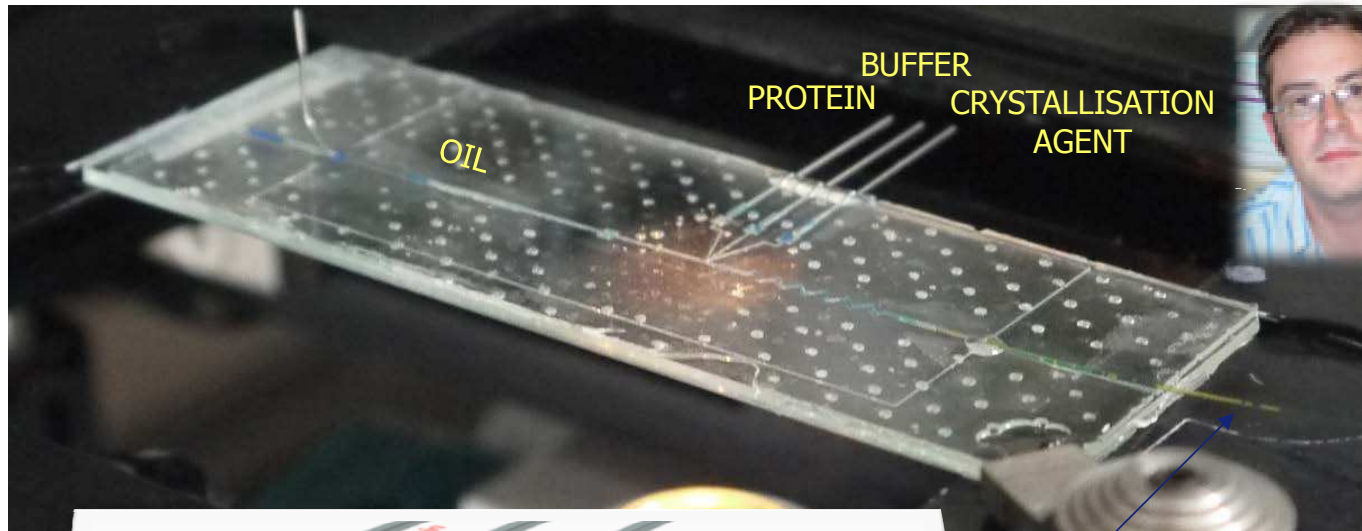
HPLC x ROBOT USE

Liquid chromatography in situ with SAXS × concentration series using robot

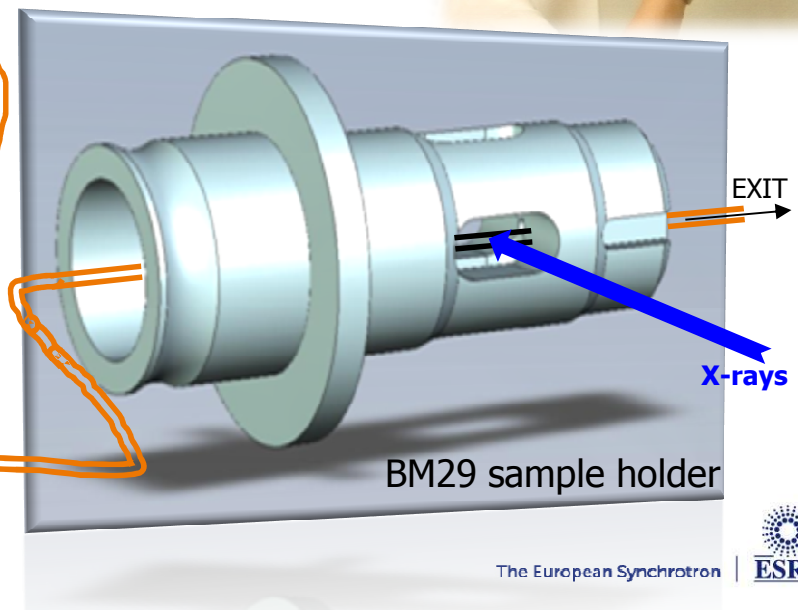
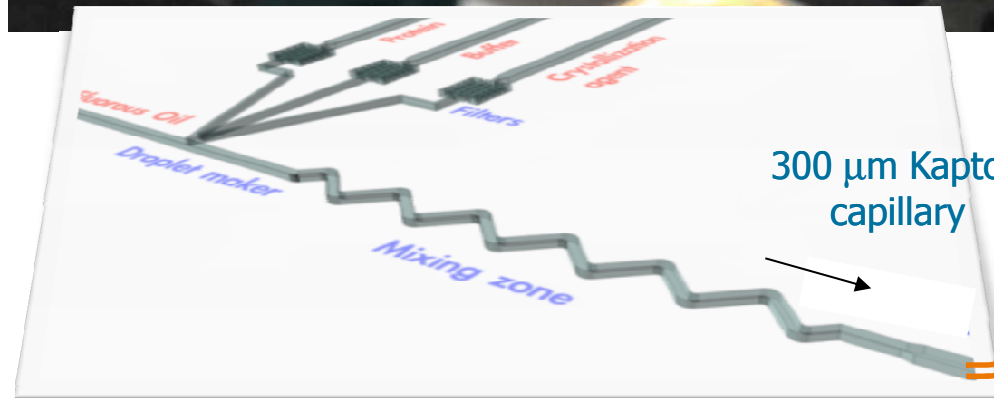


Characteristic example generated using ISPyB data base for first half of year 2014. Most user groups combine robot and HPLC experiments within one exp. session.

MICROFLUIDICS INTEGRATION AT BM29



Collaboration within LTP
Sébastien Teychené
Laboratoire de Génie
Chimique, Toulouse
and
Françoise Bonneté
Institut des Biomolécules
Max-Mousseron, Avignon

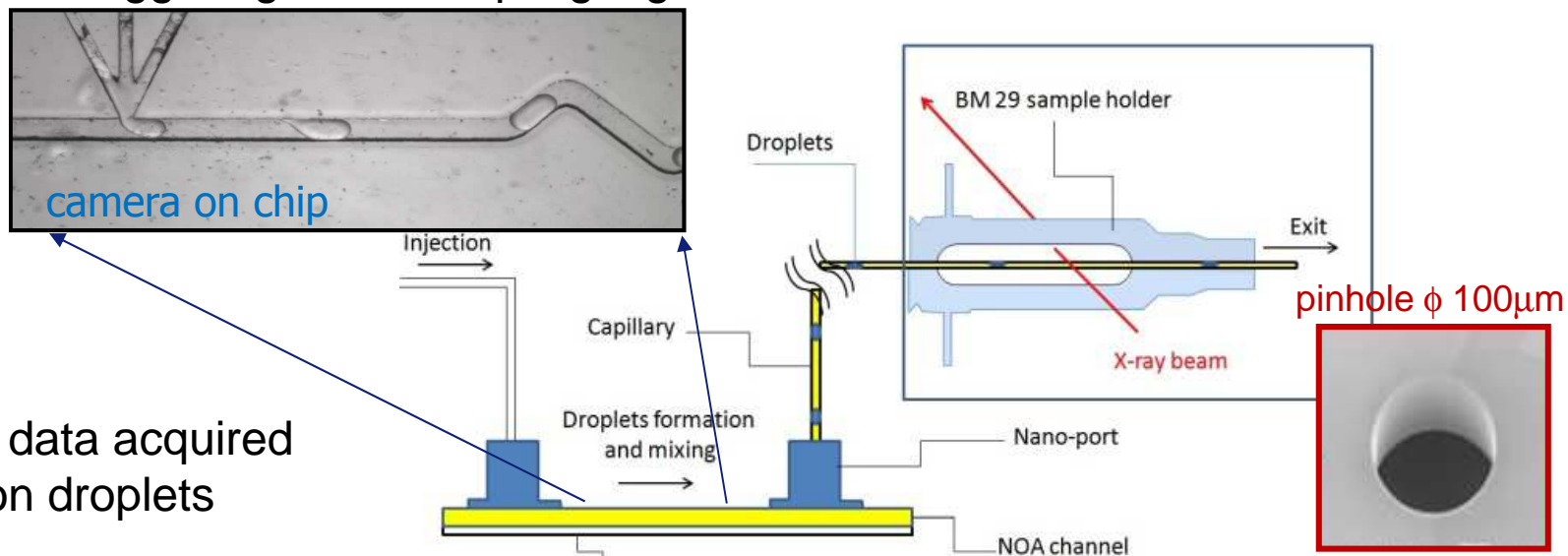


“Coupling digital microfluidics and Small-Angle X-ray scattering to study the whole crystallization process of proteins in solution”

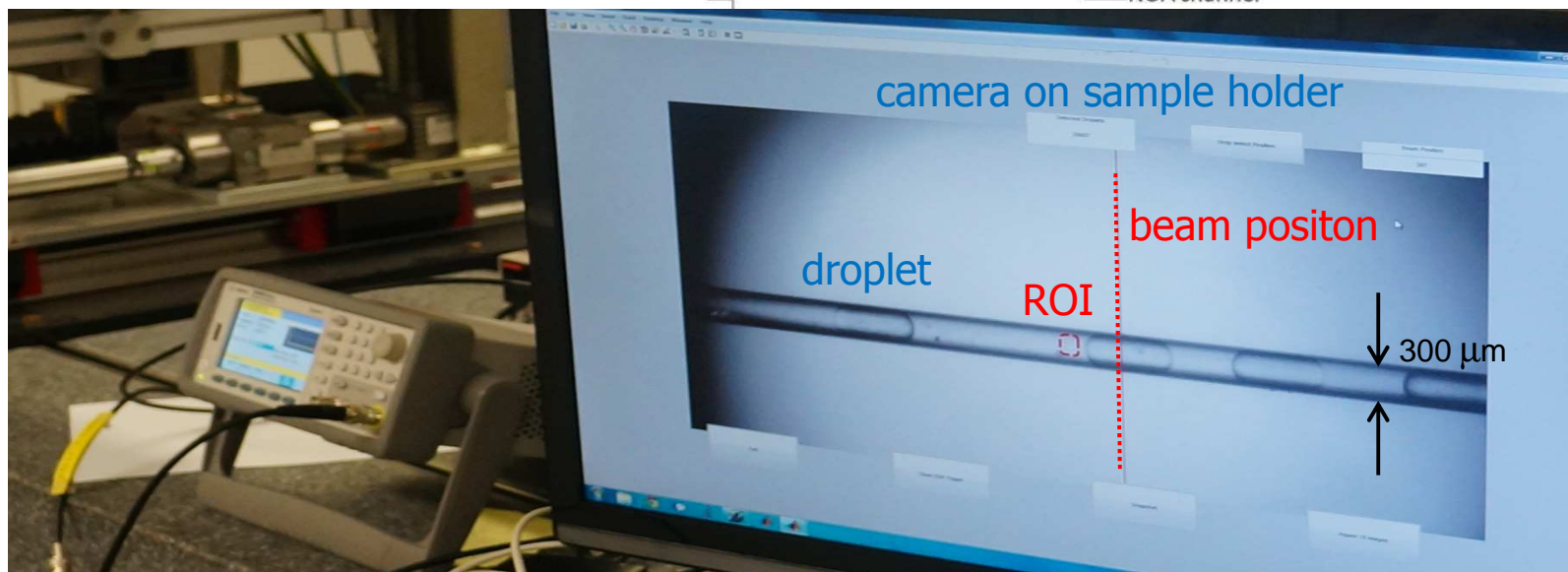
N.V. Pham, D. Radajewski, P. Guillet, A. Round, M. Brennich, P. Pernot, B. Biscans, F. Bonneté and S. Teychené, *Crystal growth and design* (2016)

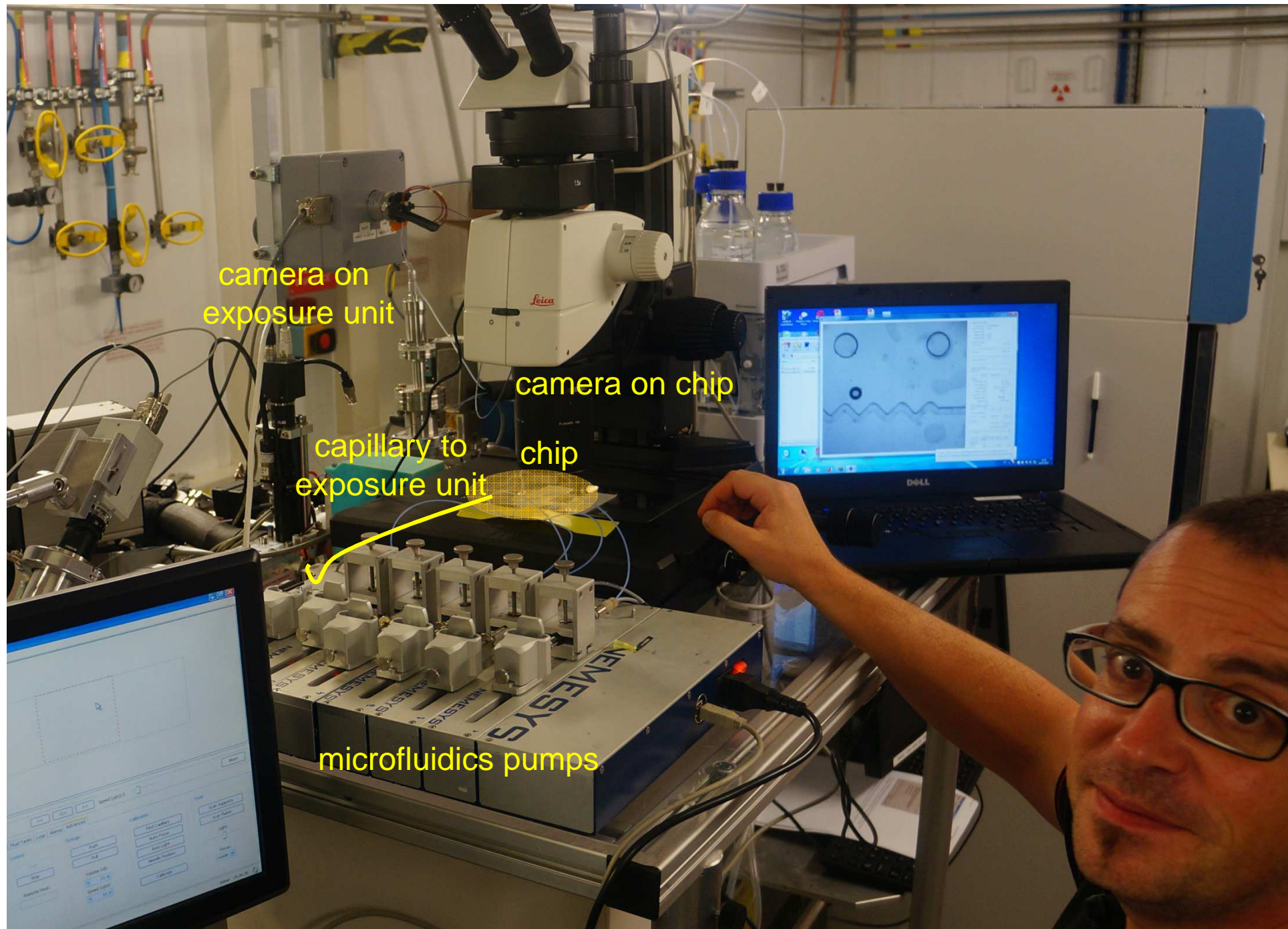
COUPLING DIGITAL MICROFLUIDICS AND SAXS AT BM29

External triggering when coupling digital microfluidics and SAXS:



X-ray data acquired only on droplets





camera on exposure unit

camera on chip

capillary to chip

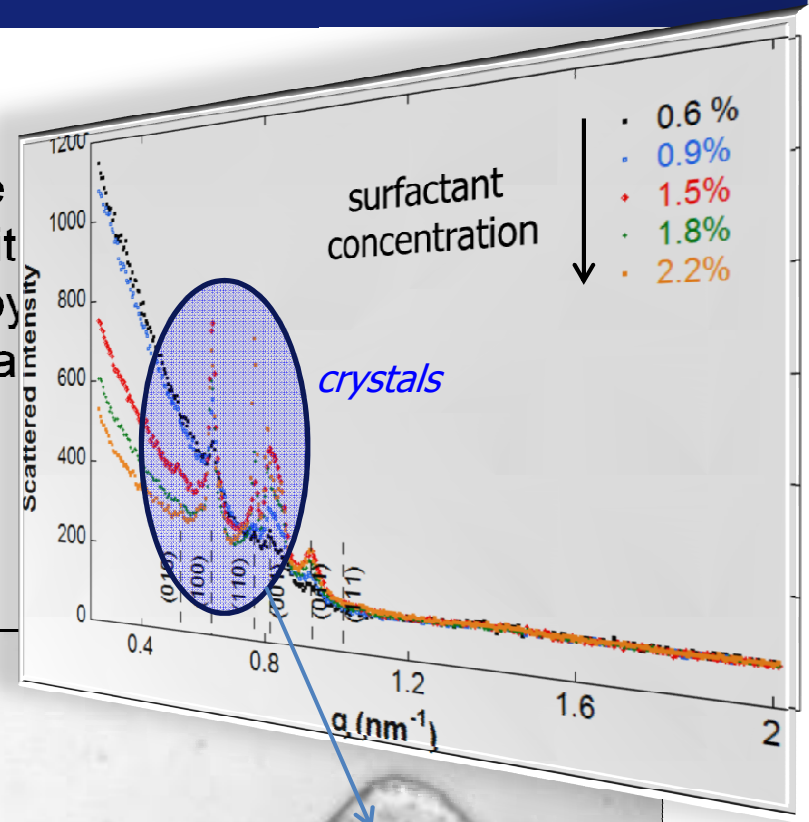
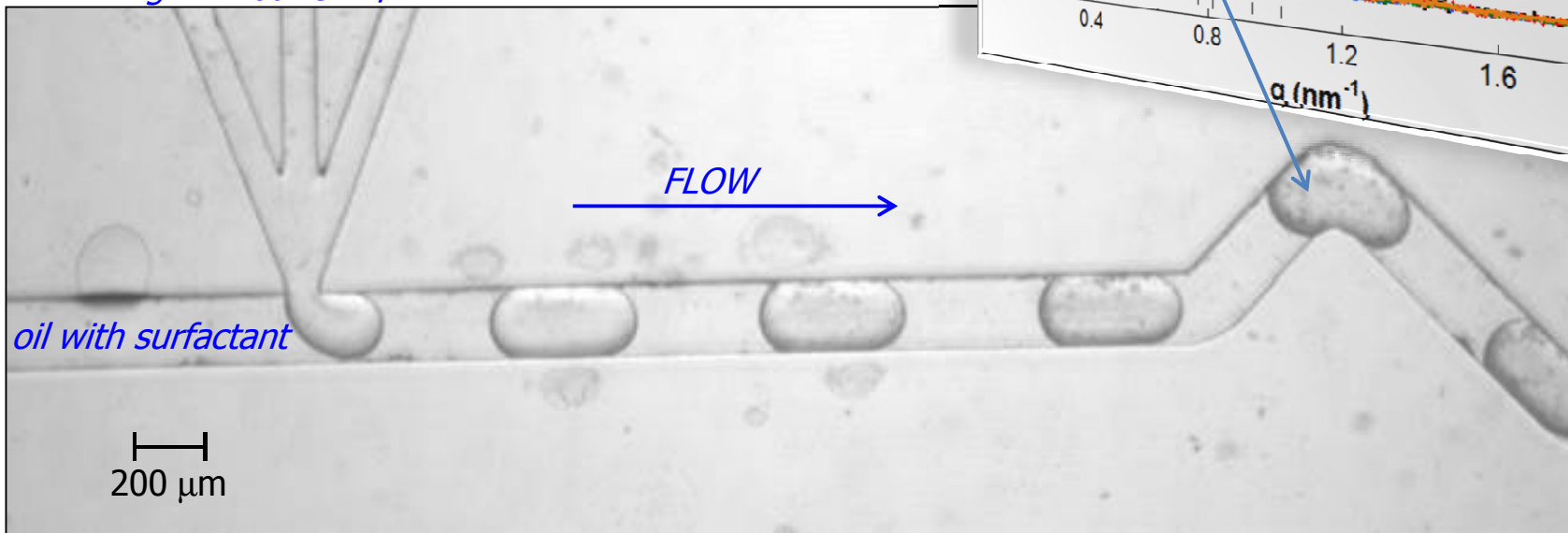
microfluidics pumps

CRYSTALLISATION IN SITU

Each droplet acts as a microreactor in which the pH, ligands, and additives) can be fine-tuned, with As the conditions can be adjusted dynamically by range of conditions can be investigated quickly a

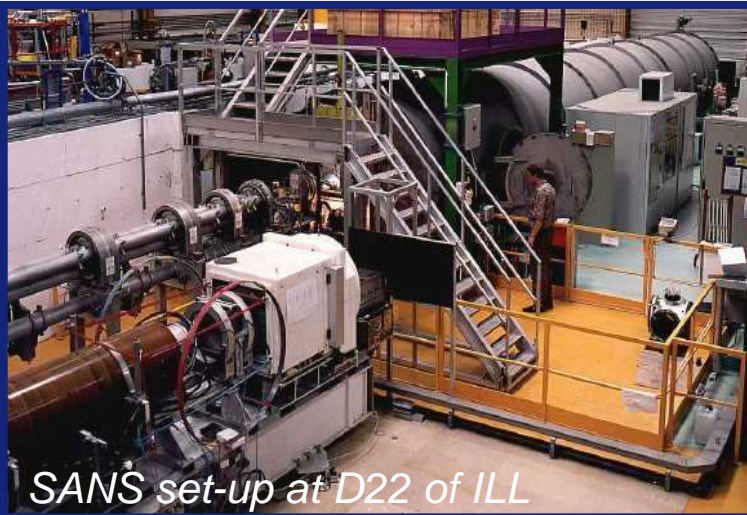
Glucose isomerase + surfactant

crystallisation
agent buffer protein



It can works for you too...

SAX/NS COMPLEMENTARITY



Neutron small angle scattering provides additional information for macromolecular complexes that are made of several types of molecules such as proteins, nucleic acids or lipids. **Contrast variation** experiments obtained by exchanging the solvent for deuterated or partially deuterated solvent enhances the signal from one component.

	SAXS	SANS
volume	small < 50 μ l	larger ~ 300 μ l
concentration	> 0.1 mg/ml	> 1 mg/ml
measuring time	short ~ s	longer ~ m÷h
radiation damage	yes	no
contrast variation	no	yes
sensitive to salts, denaturants	yes	no

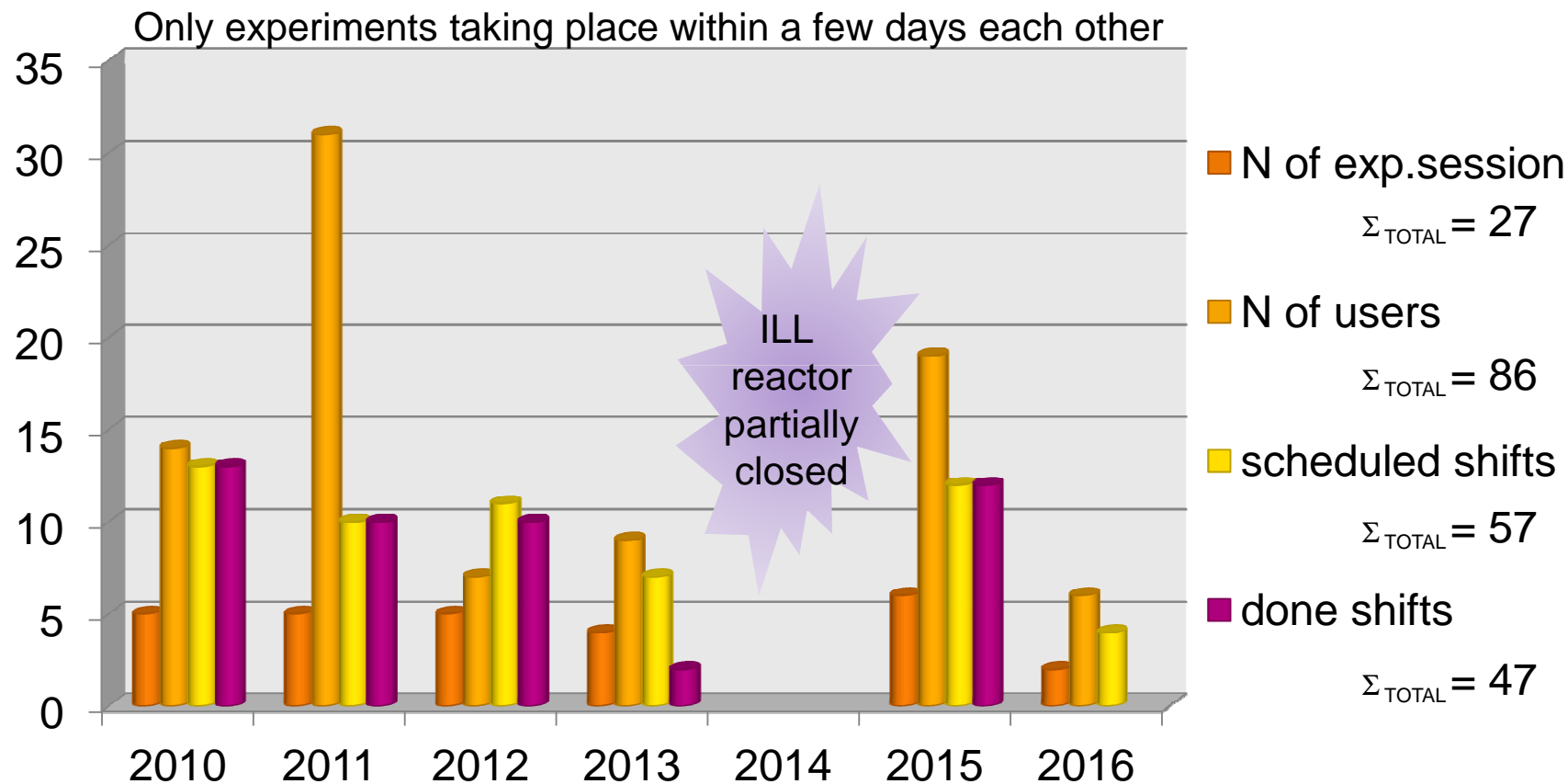


Joint access possible – ESRF and ILL SAS experiments during one trip to Grenoble.

SAX/NS BAG coming soon: when SAX/NS proposal accepted (and scheduled) by the ILL, possibility to ask time from this BAG for BM29 beamtime without rolling proposal.

JOINT SAX/NS

- This proposal is directly linked to an ILL proposal for SANS measurements on the same sample(s), submitted during the last ILL proposal round and for which time at the ILL has already been awarded Yes No If yes, ILL Proposal Number:



in A-form D₂O

Sample environment for this experiment session

Samples will contain

Deuterium If checked, specify the volume per ml

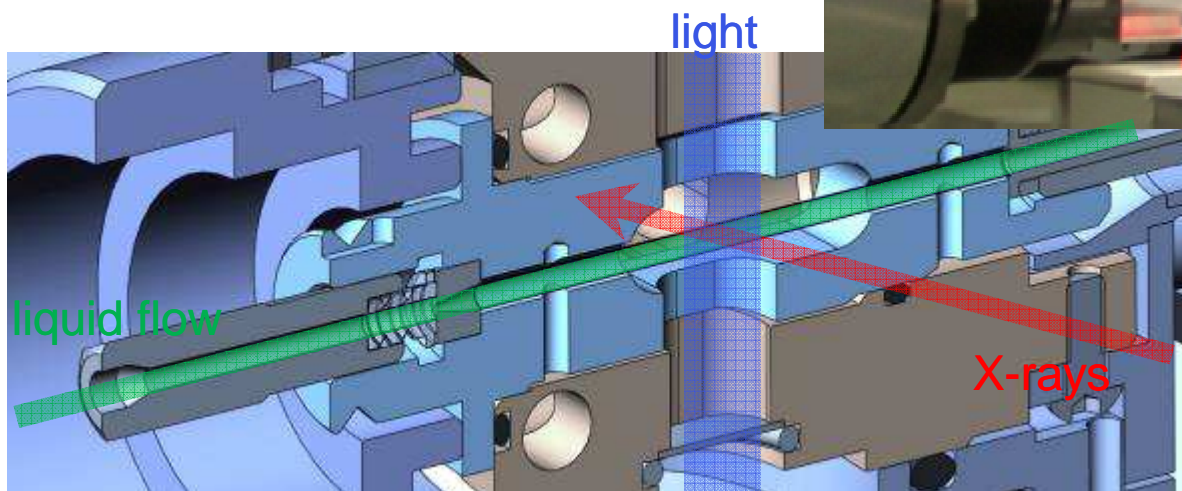
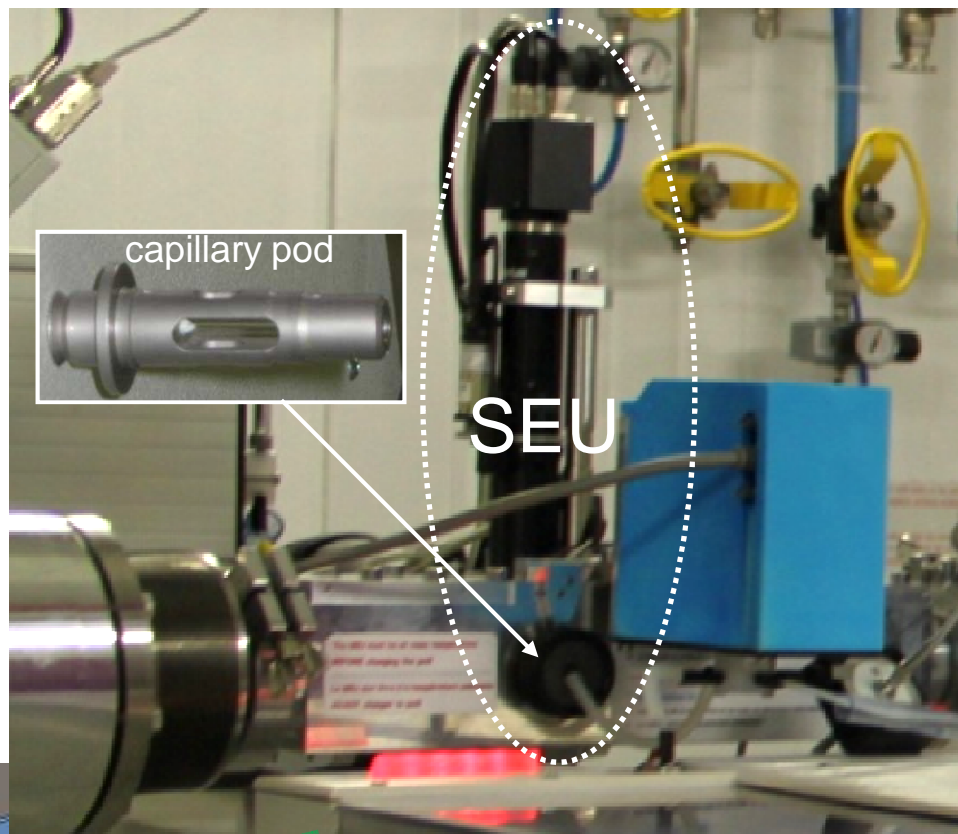
BM29 NEWS: SAMPLE THERMALISATION TIME

The screenshot displays the 'wbm29scbio - Remote Desktop Connection' window. On the left, a 'Monitoring' sidebar shows various system statuses: Hardware Status (OK), Vacuum State (OK), Fluid Tanks (Detergent, Water, Waste), Storage (61%), and Exposure (61%). Below this, 'Sample Loading' includes buttons for 'Load Position' and 'Scan and Park'. Further down, 'Table Position' (Parked), 'Cover State' (Closed), and 'Sample Type' (Green) are listed. The main area features a large video feed of the sample stage, overlaid with a red dashed box. At the bottom, a 'Restart' button and a tabbed interface are visible. The 'Temperature' tab is active, showing 'Storage Temperature' (Setpoint: 20.0, Actual: 20.2) and 'Exposure Temperature' (Setpoint: 20.0, Actual: 20.0). A 'Sample Thermalisation' dialog box is open, showing 'Set Wait Time (s): 0' and 'Current Wait Time (s): 0'. A purple circle highlights the 'Sample Thermalisation' status indicator in the sidebar, which shows a radio button next to 'Standby'. Another purple circle highlights the 'Sample Thermalisation' dialog box.

REDESIGN SAMPLE EXPOSURE UNIT

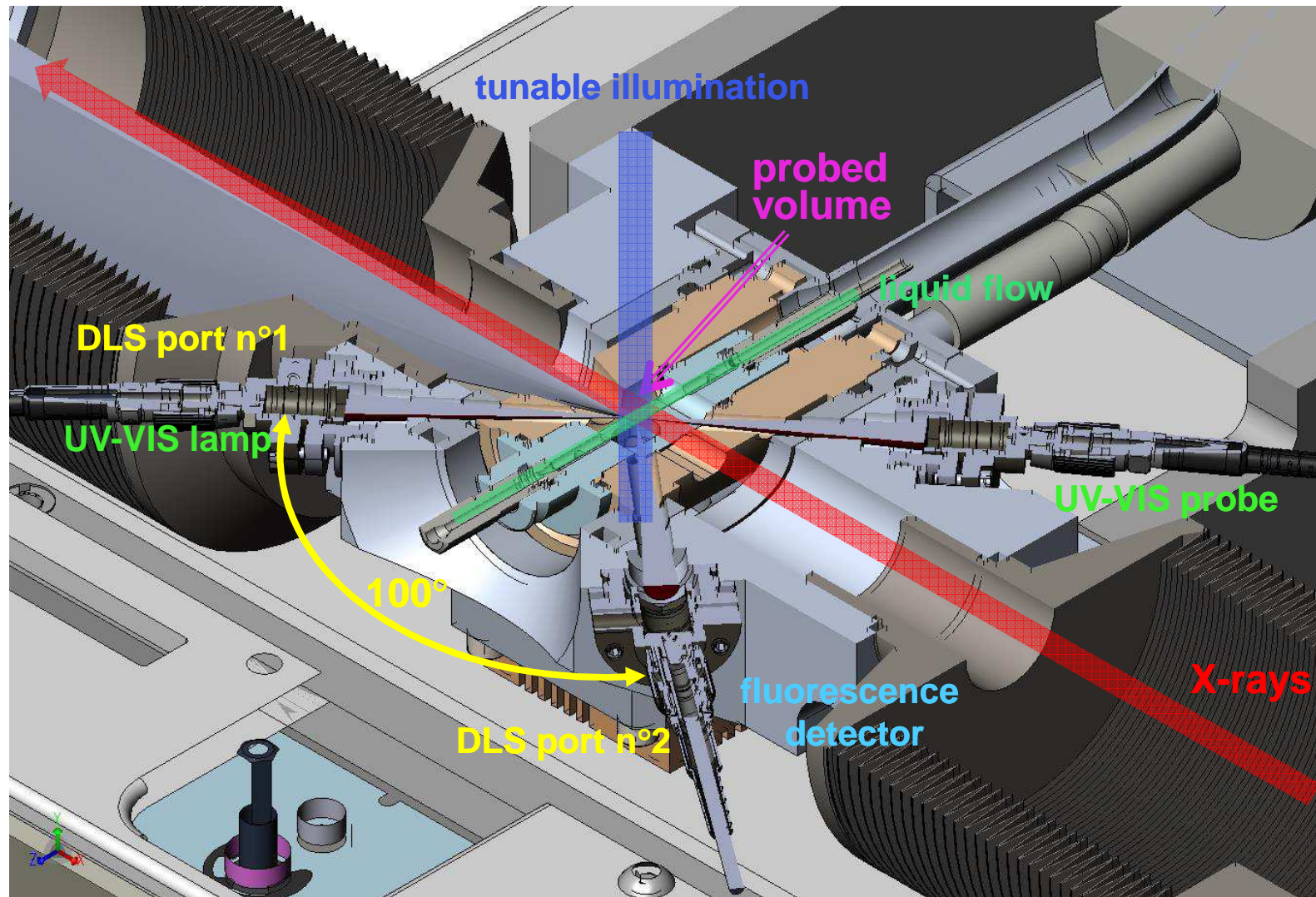
BM29 multiprobe exposure unit :

- multicolor light source photo-activated/sensitive proteins
- on-line UV/Vis and fluorescent detector at 90°
- (moderate) Wide Angle Scattering



© Frank Felisaz ,
Florent Cipriani, EMBL

REDESIGN SAMPLE EXPOSURE UNIT

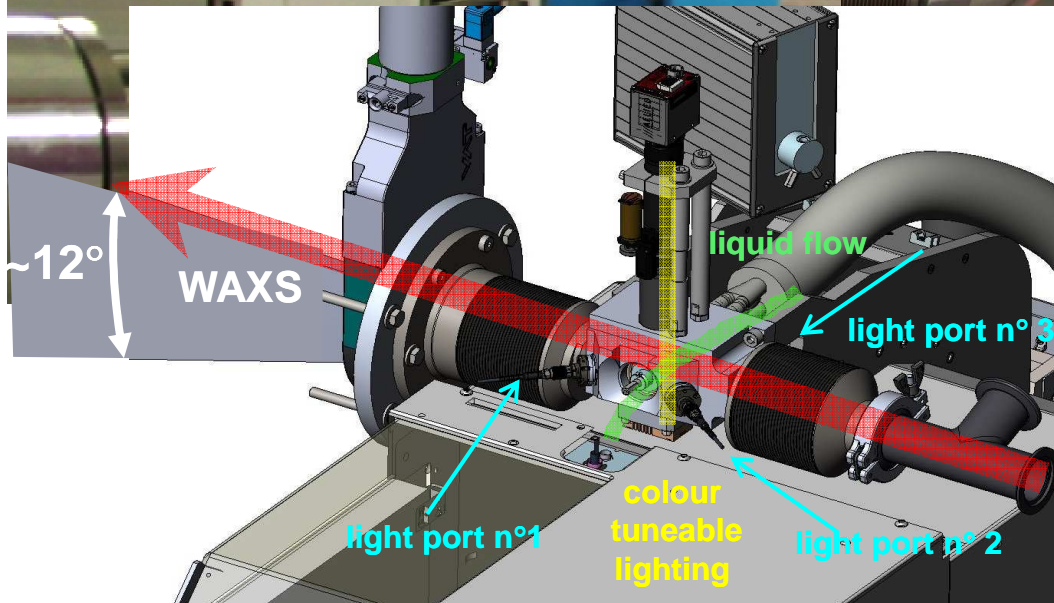


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Florent Cipriani, EMBL



NEW SEU: MULTIPROBE EXPOSURE UNIT

- multiprobe exposure unit: **multicolor light source**, on-line **DLS**, **UV/Vis** and **fluorescence** detector,



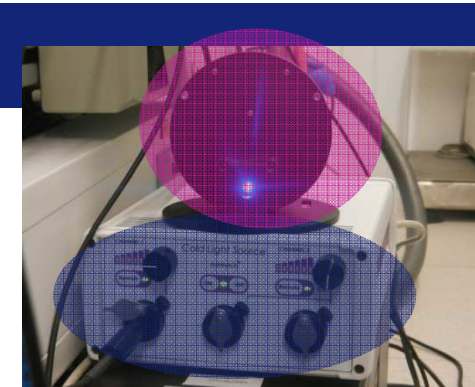
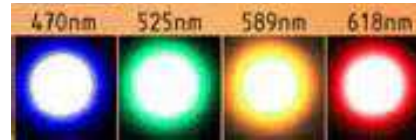
X-rays

Installed and commissioned
end of January 2016, in use

© Frank Felisaz ,
Florent Cipriani, EMBL

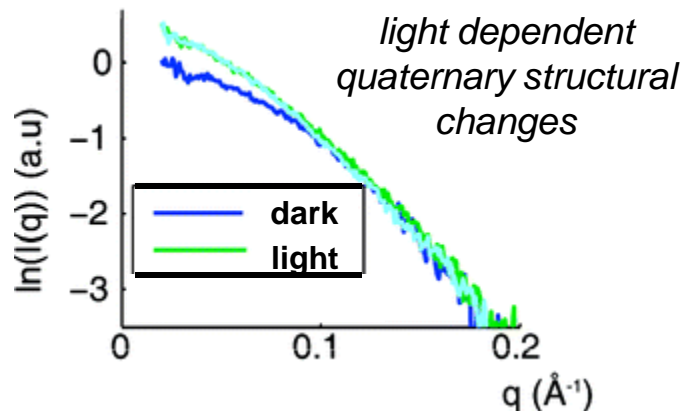
LIGHTINGS WITH TUNABLE WAVELENGTH

- cold light source + filter box + optical fibers
- photo-activated/sensitive proteins

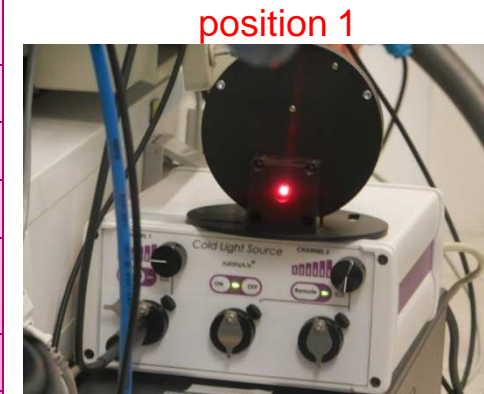


Example

Signal perception in LOV (Light, Oxygen, Voltage) domains is achieved via a blue-light ($\lambda_{440-485\text{nm}}$) absorption by non-covalently bound flavin chromophore ('light' state). This triggers the formation of a transient adduct between the flavin C4a atom and the S atom of a strictly conserved cysteine residue in the LOV domain. Once illumination has ceased, this covalent bond is broken: decay in seconds to hours depending on LOV protein.



Pos	Wavelength	Nick name
1	610 nm	red
2	325-385 nm	UV
3	495 nm	yellow
4	315-445 nm, 715-1095 nm	violet
5	275-375 nm	UV _{low}
6	BG7	blue



On-line UV/Vis spectrophotometer would help to verify a complete signalling state population before each experiment.

SEE YOU AT BM29 BIOSAXS BEAMLINE

Thank you for attention

BM29

