From automated sample handling to robot based goniometer: few steps towards a fully robotized pipeline for protein crystallography

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IBS/Synchrotron Group (Grenoble, France)

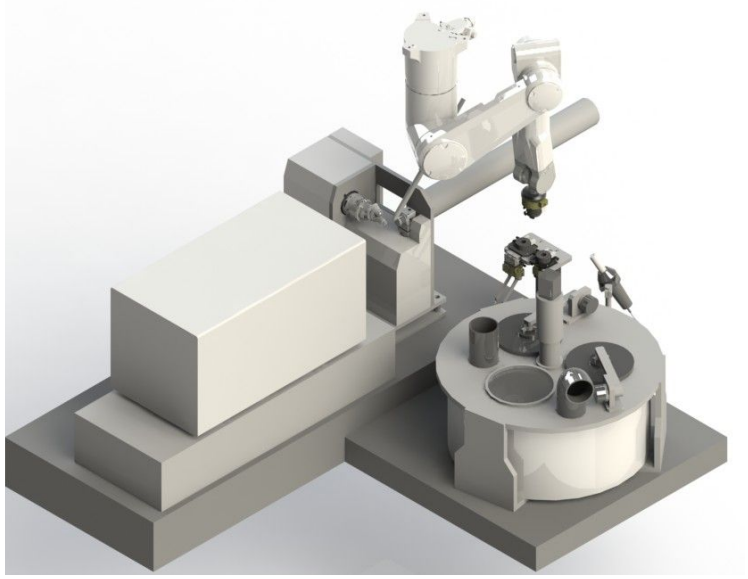
PSDI meeting, November 2013

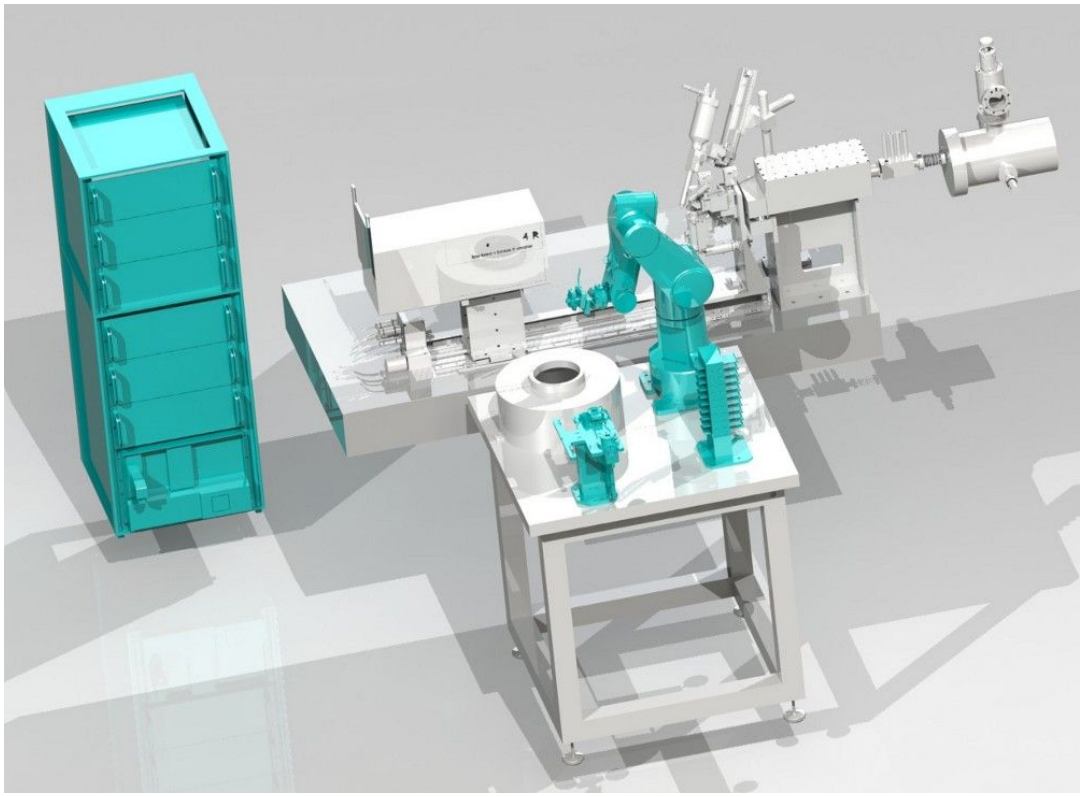
Systems commercialized by NatX-ray *(visit NatX-ray booth, room 3)*

G-Rob for beamlines

Usually including

- G-Rob functionalities
- (Sample environment)
- Supporting frame



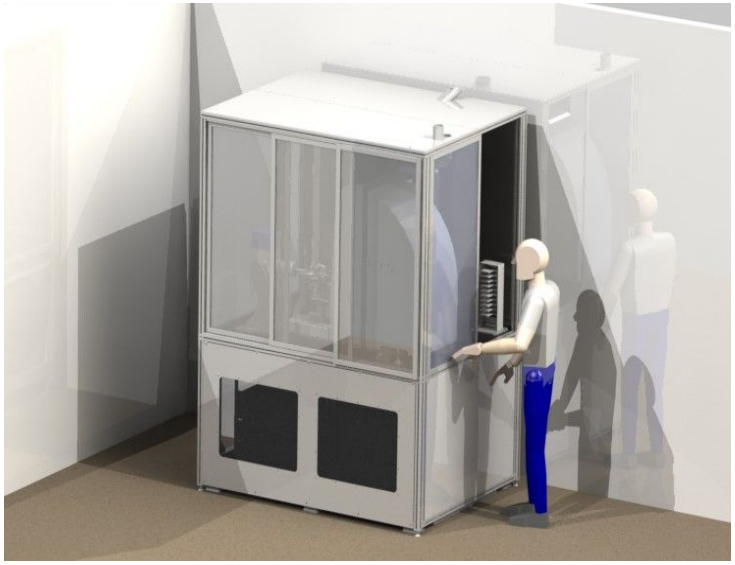


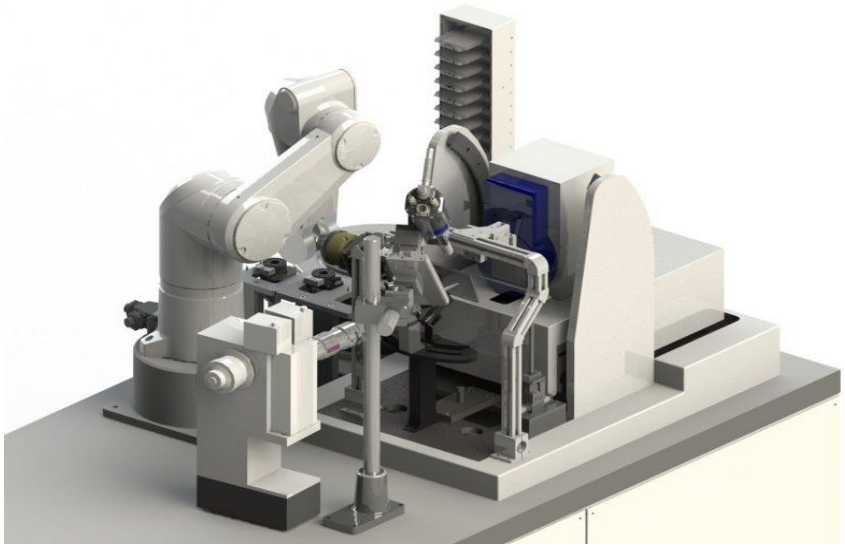
In operation at LNLS (Brazil), ESRF (France), NSLS (USA)

G-Rob lab systems

All-in-One laboratory solution, with

- G-Rob functionalities
- X-ray source
- Detector
- Sample environment
- Table and X-ray shielding





Available as

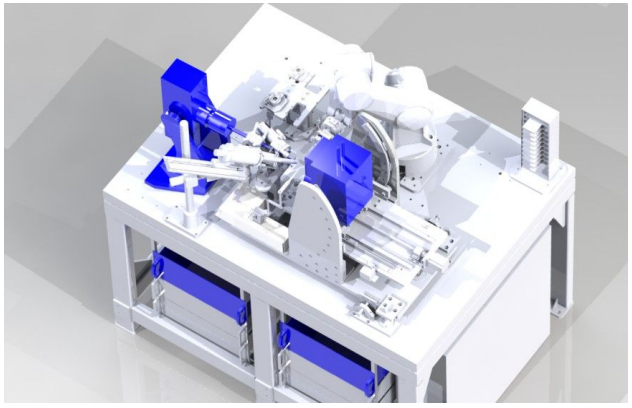
- A complete system
- An upgrade for existing lab diffraction systems

In operation at EPFL (Switzerland), CBS (France)

G-Rob configurations

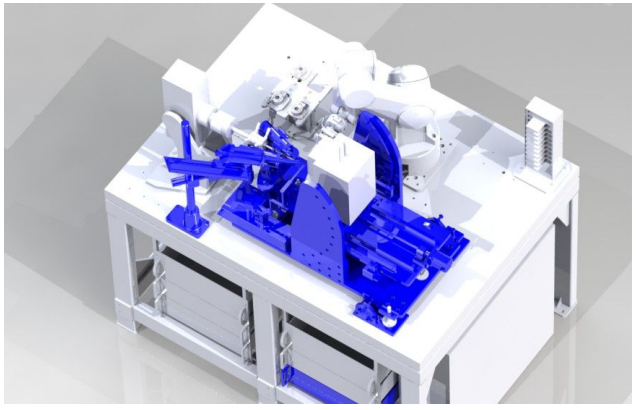
A large variety of X-ray sources

Sealed tube, rotating anode



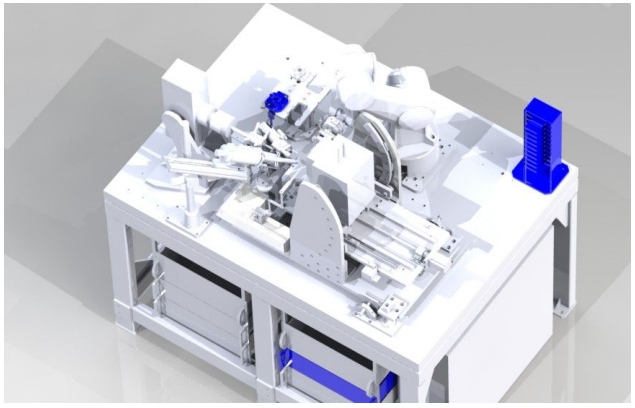
A large variety of detectors

IP, CCD, Solid-State detectors

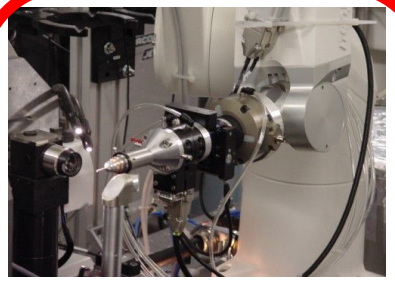


A complete sample environment

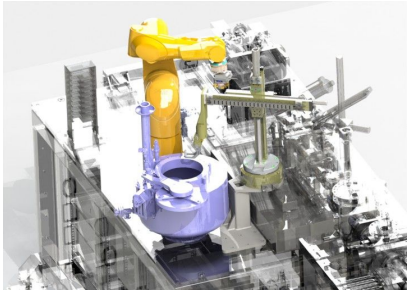
- Sample microscope with motorized zoom
- Motorized detector translation
- Motorized 2-Theta detector rotation
- Motorized beam stop
- Cryo-cane translation



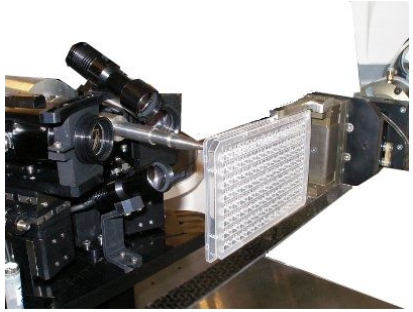
A choice of G-Rob functionalities



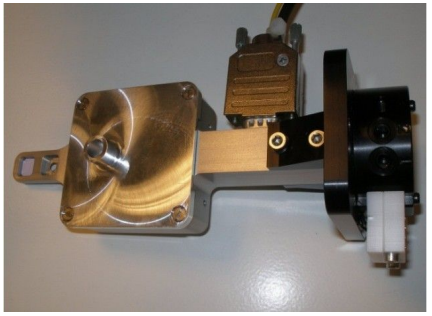
Frozen crystals, capillaries, powder



Cryo-sample transfer

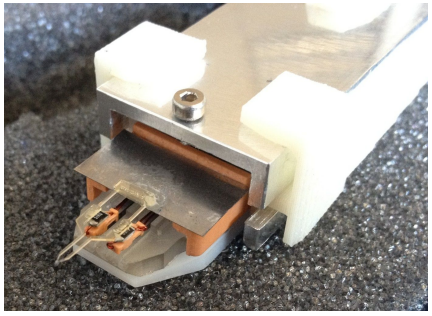


Plates, microchips (*in situ* screening & datacoll.)



Beam monitoring, quick-realign

G-Rob 1D/1D+



Sample harvesting

a goniometer for single sample

- frozen crystal, capillary

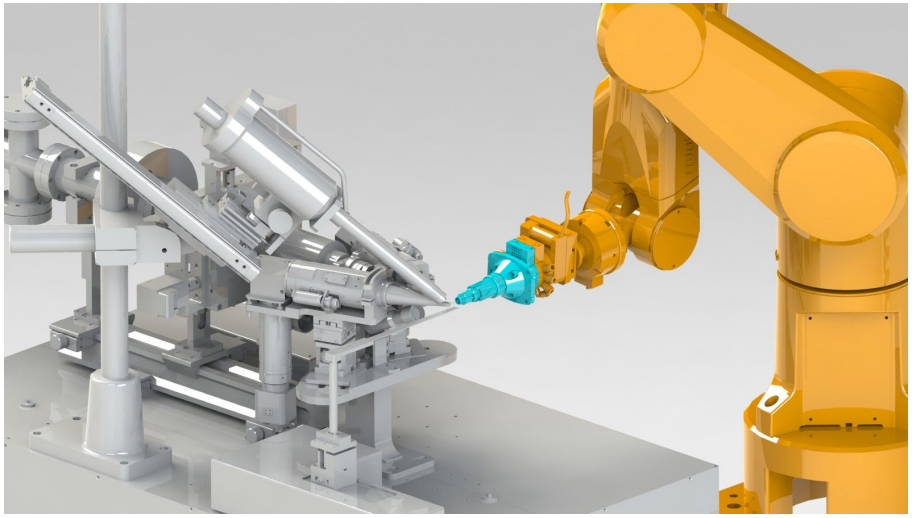
Applications

- classical data collection
- shutter-less data collection
- Phi data collection
- powder diffraction

G-Rob function: 1D/1D+

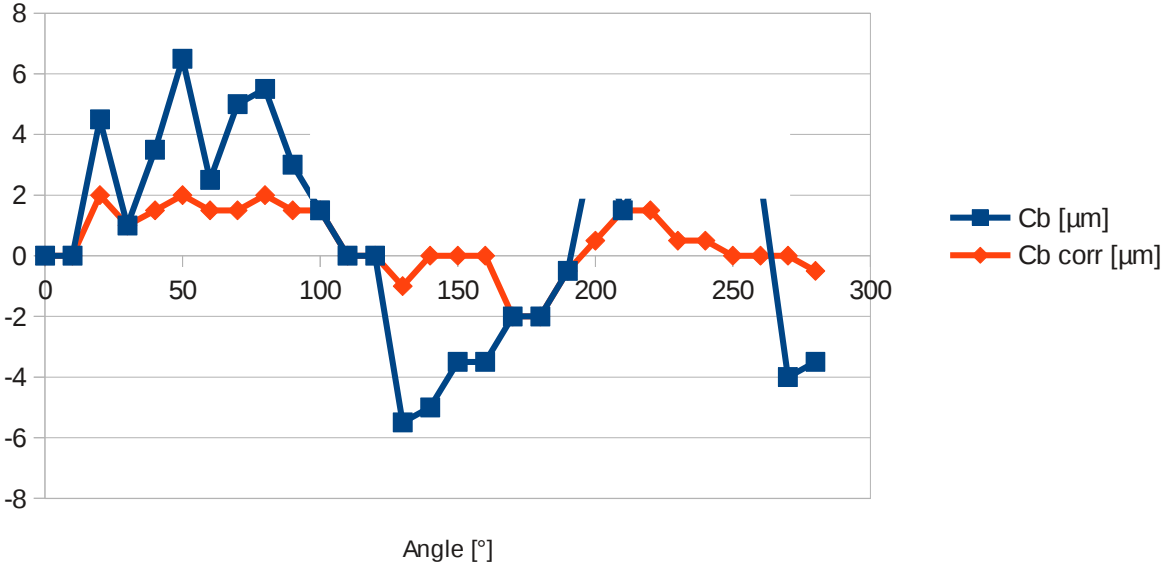
- Goniometer capability
- Validated with beam down to 90 µm
- Exposure time as short as

  0.1s for 1° oscillation



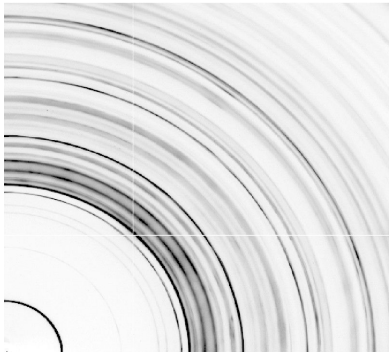
1D → 1D+

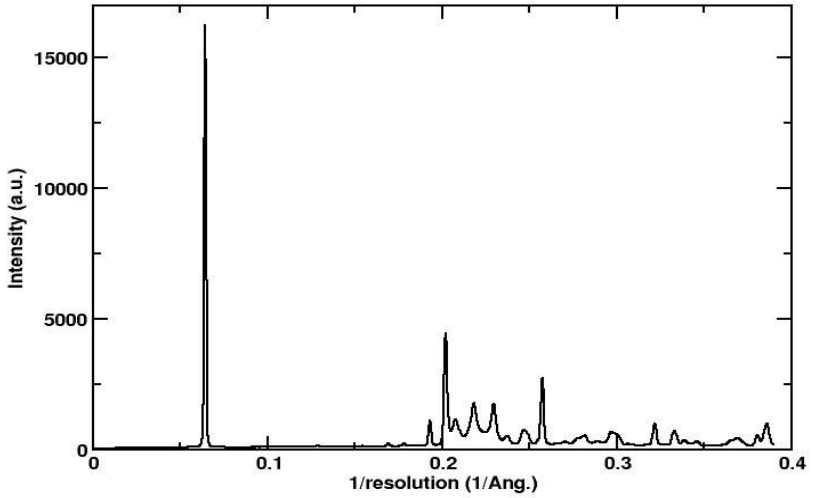
- Improved goniometer capability
- A 6 µm radius sphere of confusion for small beams



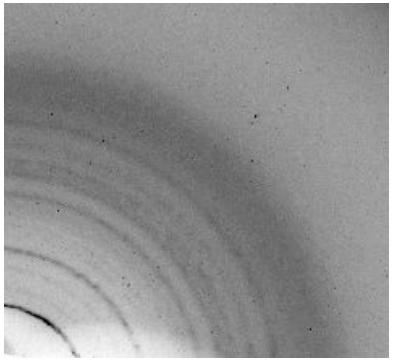
G-Rob function: Powder diffraction

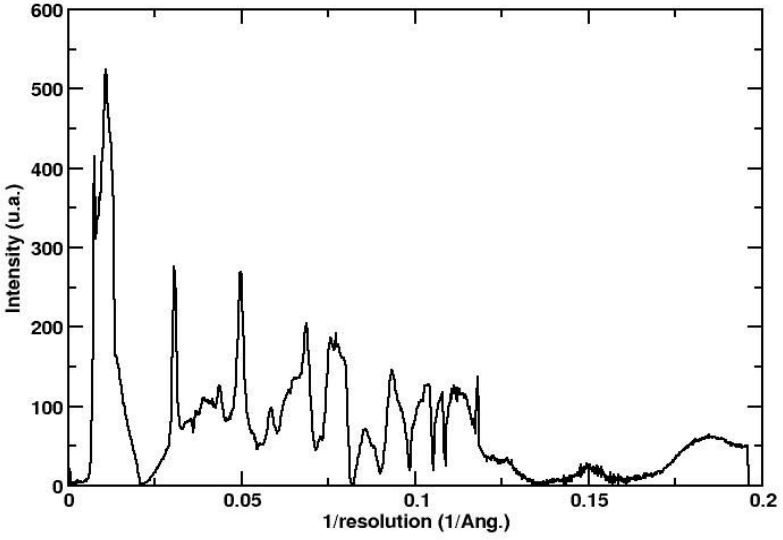
Small molecule powder



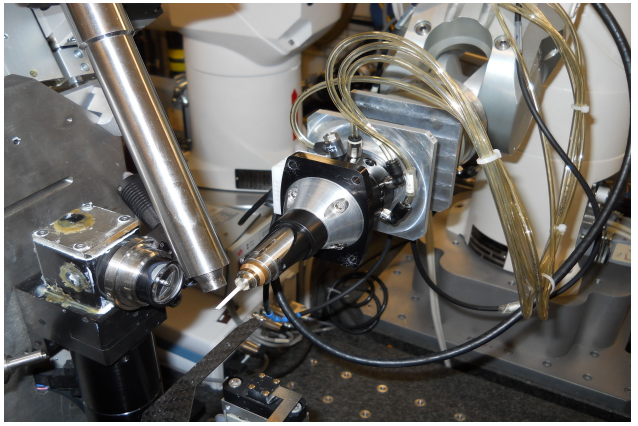


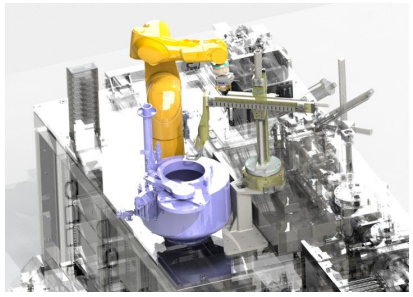
Protein powder



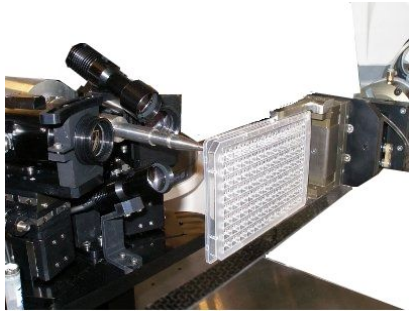


2-4 rotation / sec
Continuous translation

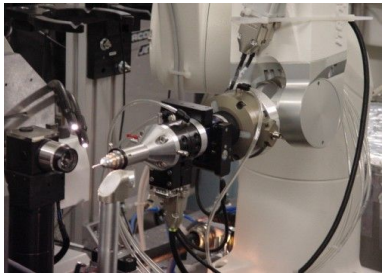




Cryo-sample transfer

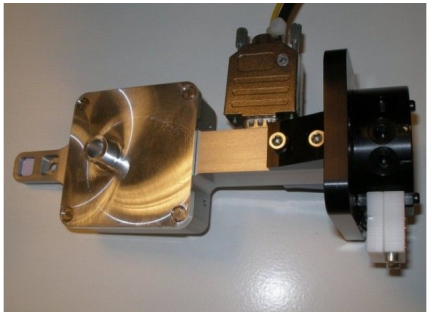


Plates, microchips
(*in situ* screening & datacoll.)

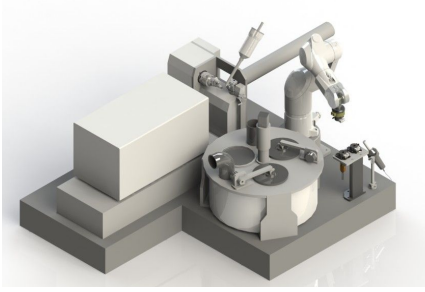


Frozen crystals,
capillaries, powder

G-Rob 1DT



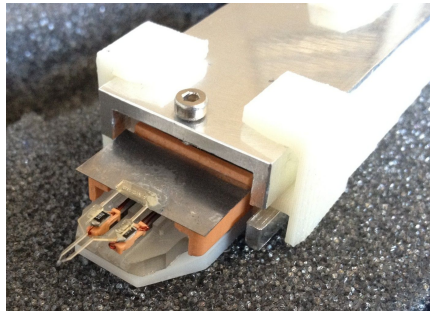
Beam monitoring,
quick-realign



Sample changer

Cryo-frozen samples transfer

- Automated transfer of frozen samples
- 90 to 240 samples storage Dewar
- SPINE standard format



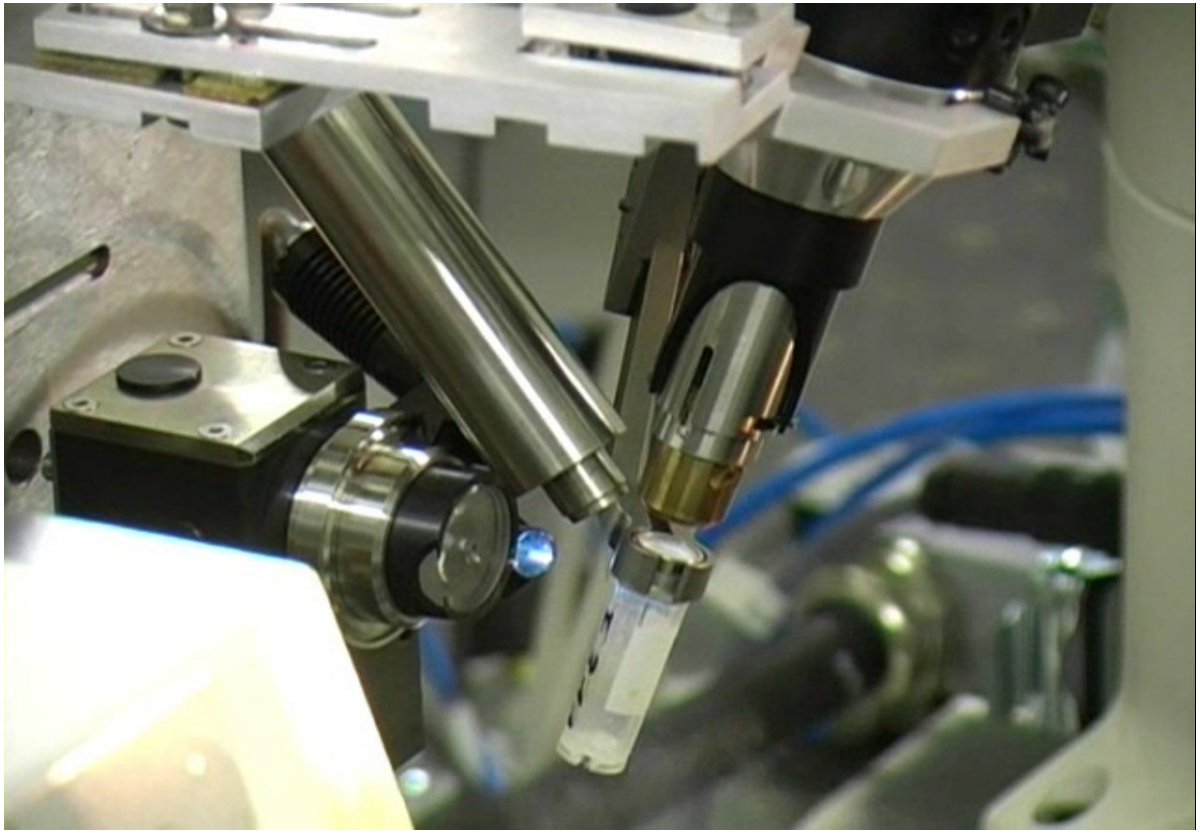
Sample harvesting

Applications

- high throughput screening of frozen crystals
- remote controlled experiments

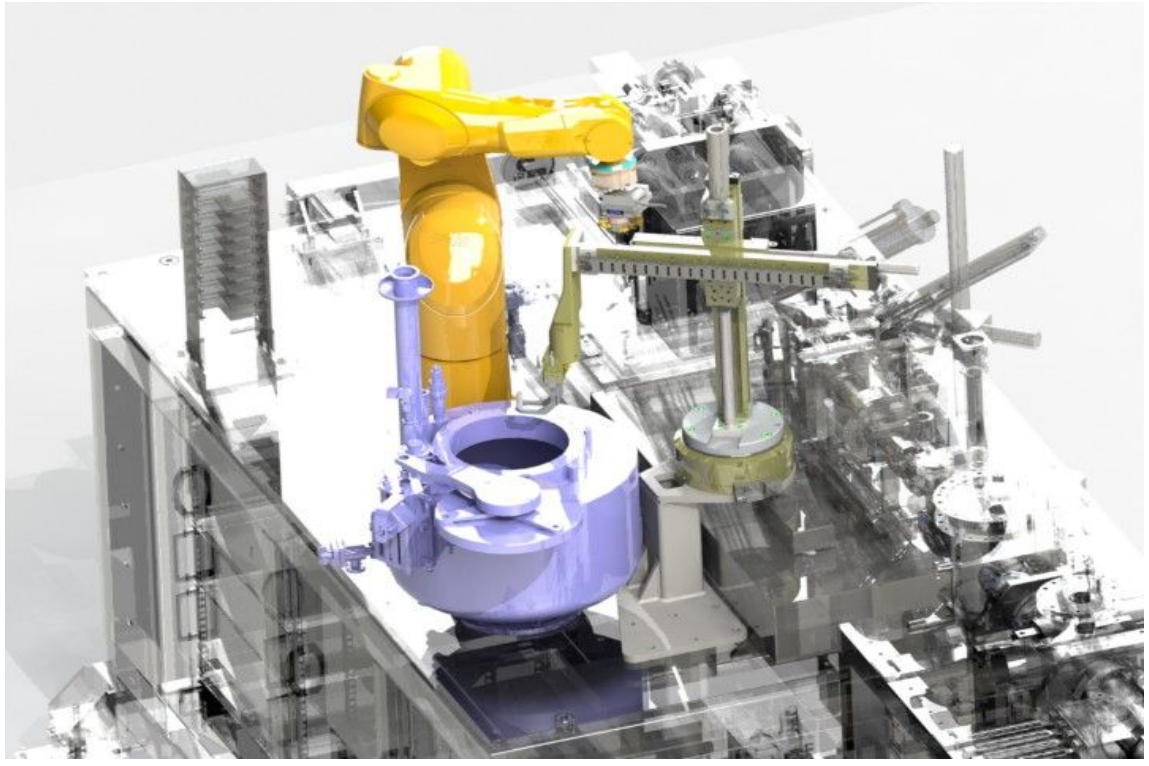
G-Rob 1DT for in-house systems

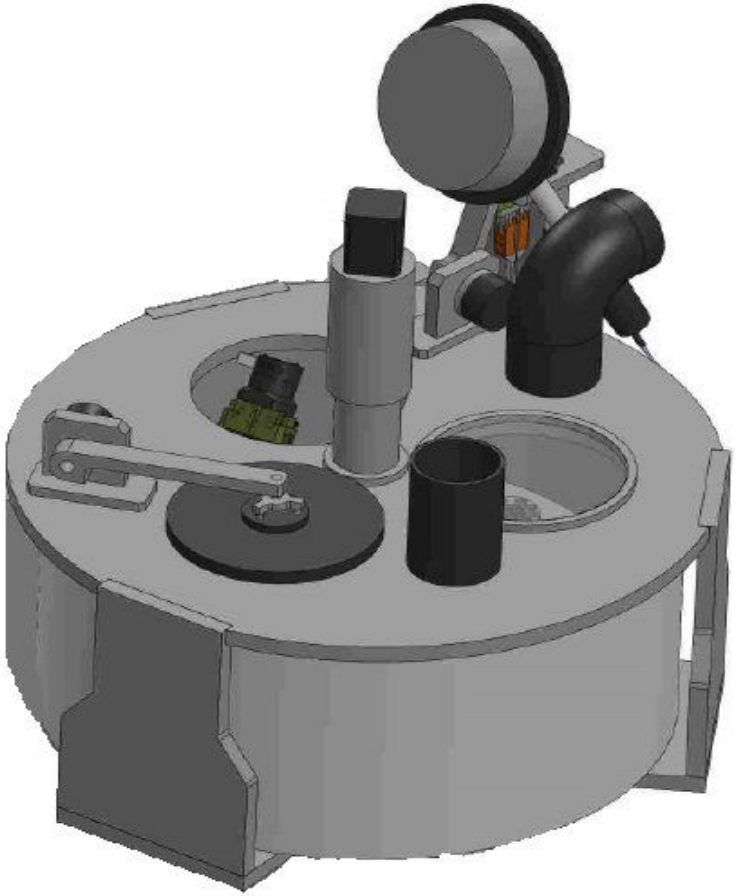
- Rapid sample changer cycle time
- Compatible with SPINE standard
- Storage Dewar for 90 samples

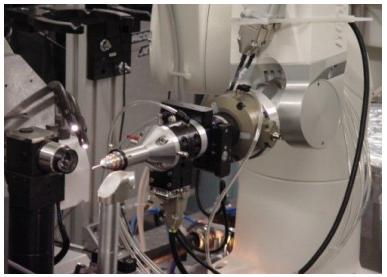


G-Rob 1DT for beamlines

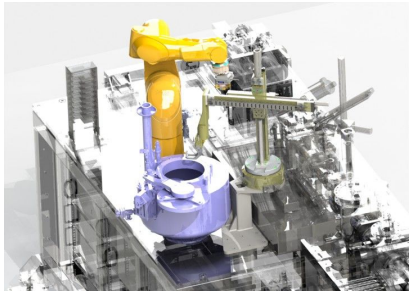
- Very fast: cycle time < 40 sec
- Compatible with SPINE standard
- Storage Dewar for 90 up to 240 samples



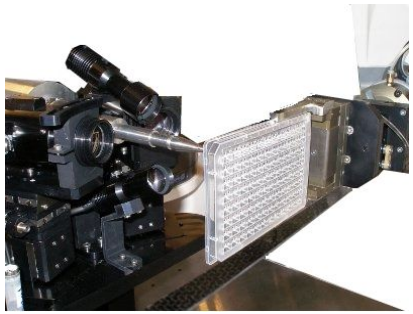




Frozen crystals, capillaries, powder



Cryo-sample transfer



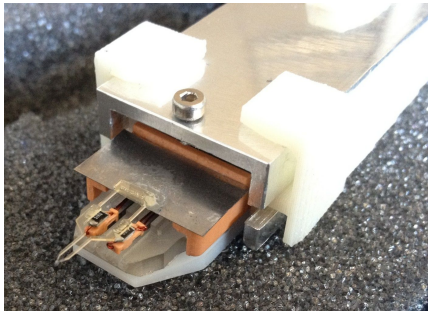
Plates, microchips (*in situ* screening & datacoll.)

Beam monitoring, quick-realign

G-Rob 2D

in situ screening & data collection

- SBS micro-plates (sitting/hanging drops)
- SBS high density batch plates
- micro-chips
- high pressure cells



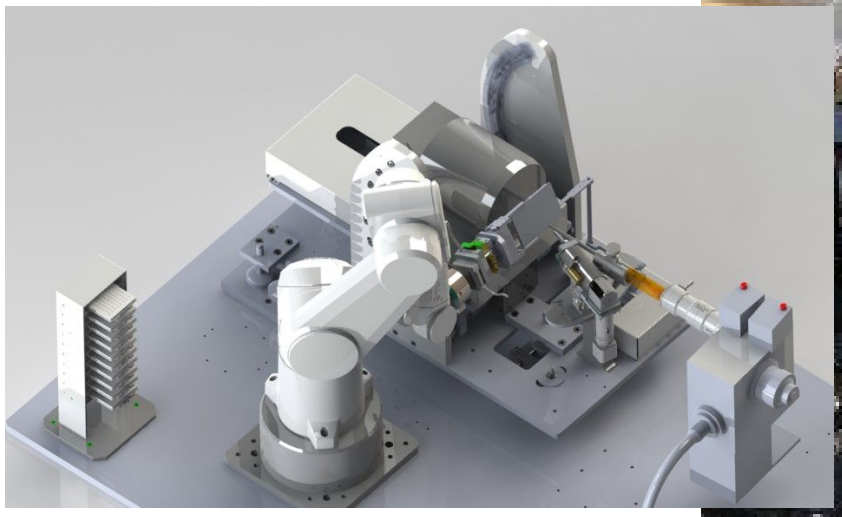
Sample harvesting

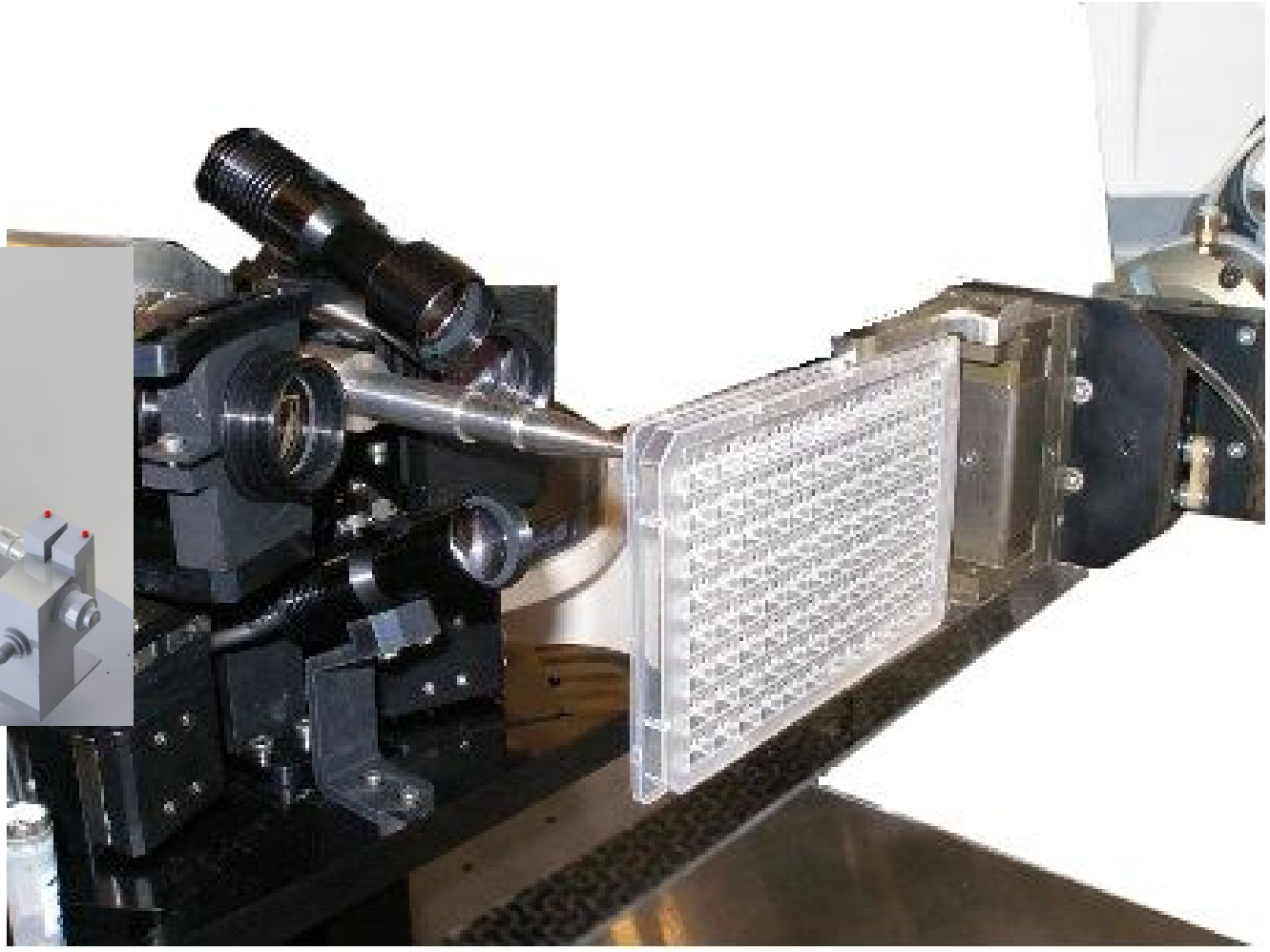
Applications

- rapid crystallization screening
- data collection at room temperature on series of crystals
- automated screening of compounds, fragments, heavy atoms

G-Rob function: 2D

- *In situ* screening for crystallization plates
- Up to 80 degree rotation range for *in situ* data collection





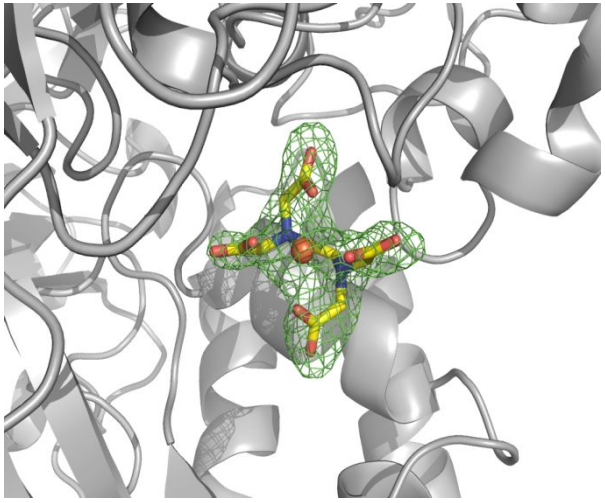
Crystal Listing

Position off all crystals on a plate can be recorded by single clicks.
Then data can be recorded in a row on these crystals, in a fully automated way.

Listing on:	G-Rob
Number of samples	79
<|X|> error (µm)	3
<|Y|> error (µm)	3
<|radius|> error (µm)	5
X standard deviation (µm)	2
X standard deviation (µm)	2
radius standard deviation (µm)	3

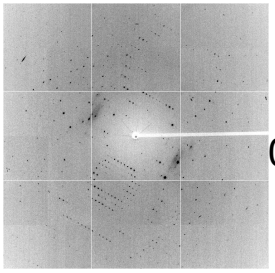
Experiments performed on the
in-house G-Rob system
of the EPFL crystallography platform
(Prof. S. Cole laboratory, Lausanne).

	Lysozyme	NikA-FeEDTA
Data collection		
Resolution (last shell) (Å)	2.10	2.45
Completeness (last shell) (%)	71.6 (75.0)	68.4 (71.4)
R_{sym}[a] (last shell) (%)	13.9 (38.5)	13.8 (41.6)
I/σ (last shell) (I)	5.61 (2.75)	4.45 (2.20)
Refinement		
R_{work}[b] (%)	18.82	17.39
R_{free}[c] (%)	23.11	25.07



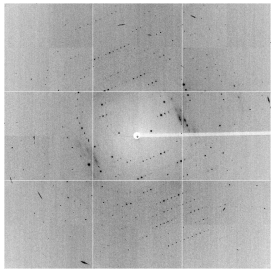
Fe(III)-EDTA binding site in NikA.
Omit Fourier electron density map
of Fe-EDTA contoured at 3 sigma

High pressure cell
(R. Kahn *et al*., IBS, Grenoble)

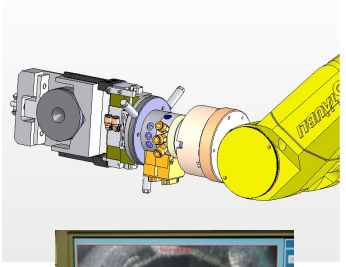


Z-DNA

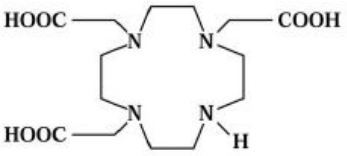
0 Mpa



540 MPa

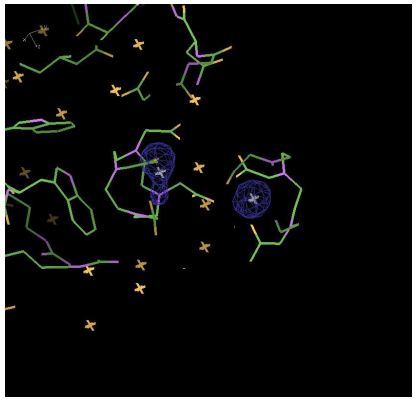


Anomalous data of Yb-DO3A / lysozyme complex solved "in the drop"



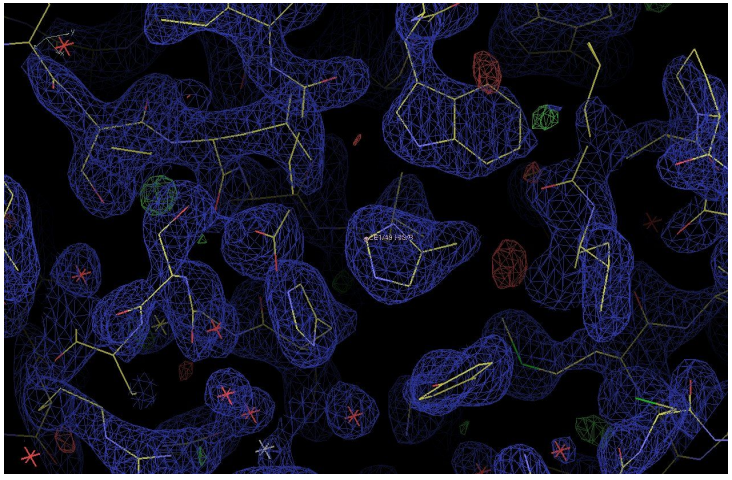
resolution: 1.8 Å
complet.: 90 %
(anomalous)

Anomalous diff. map (threshold: 6 σ)

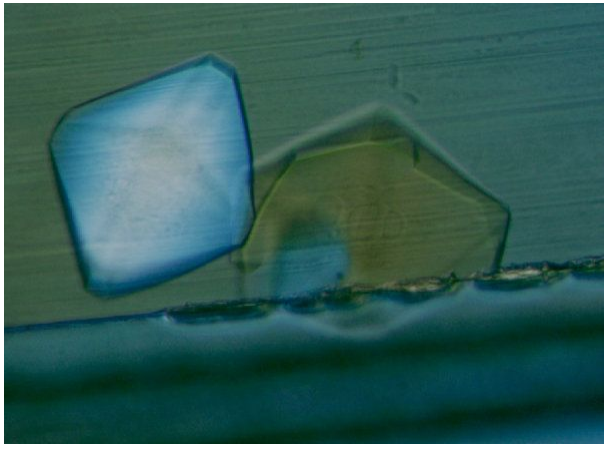


Structure of NDK from *A. Polyphaga* Mimivirus solved "in the drop"
(C. Abergel, L. Jacquamet, CNRS)

space group: p6(3)
a/b/c: 70.8/70.8/106.3
resolution: 2.3 Å
completness: 80 %, I/σ(I): 3.3
Rsym: 19.6 %, Rfree: 27.4 %



Extra-cellular domain of a membrane protein
(A. Haouz, Inst. Pasteur, Paris)

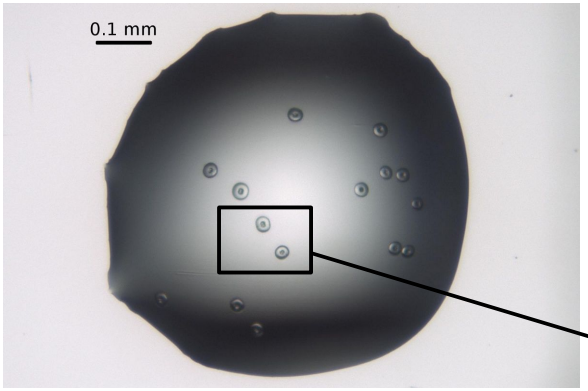


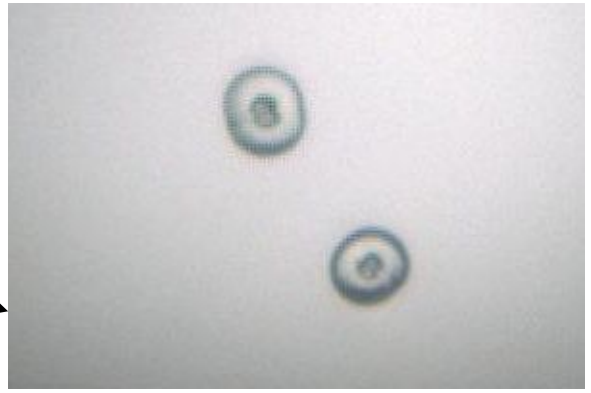
>300 um crystals
No diffraction when frozen

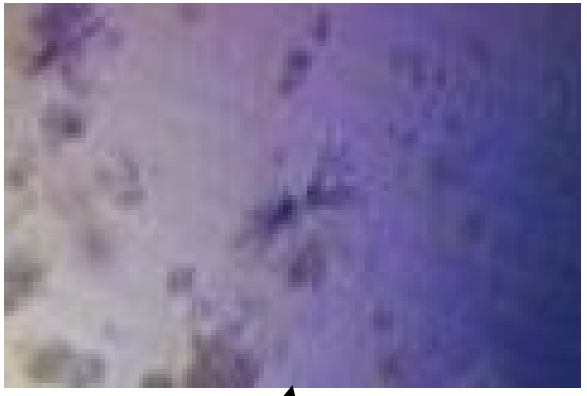
Beamline: FIP-BM30A
Plate: X-ray plate
Resolution: ~ 10 Å

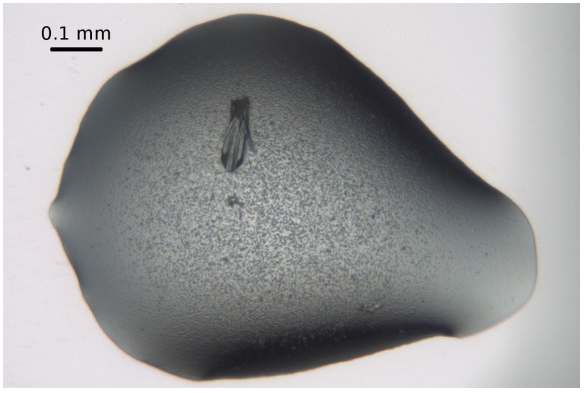
In situ screening

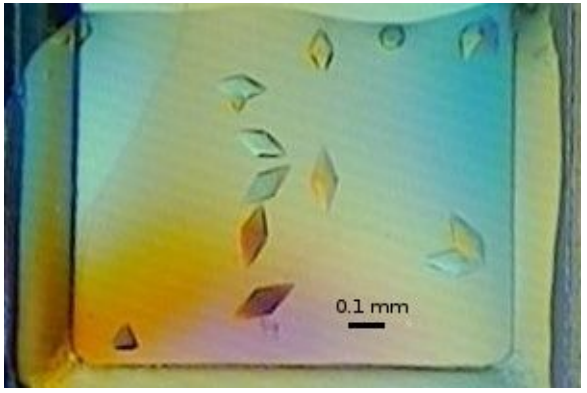
Samples recently proved to be diffracting protein crystals using G-Rob 2D ("*in situ*") screening

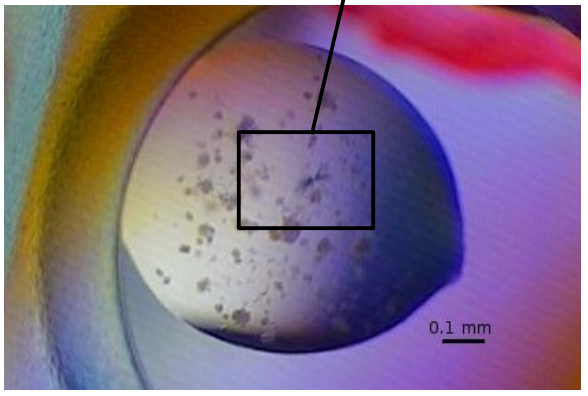








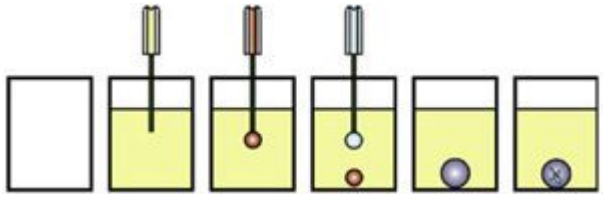




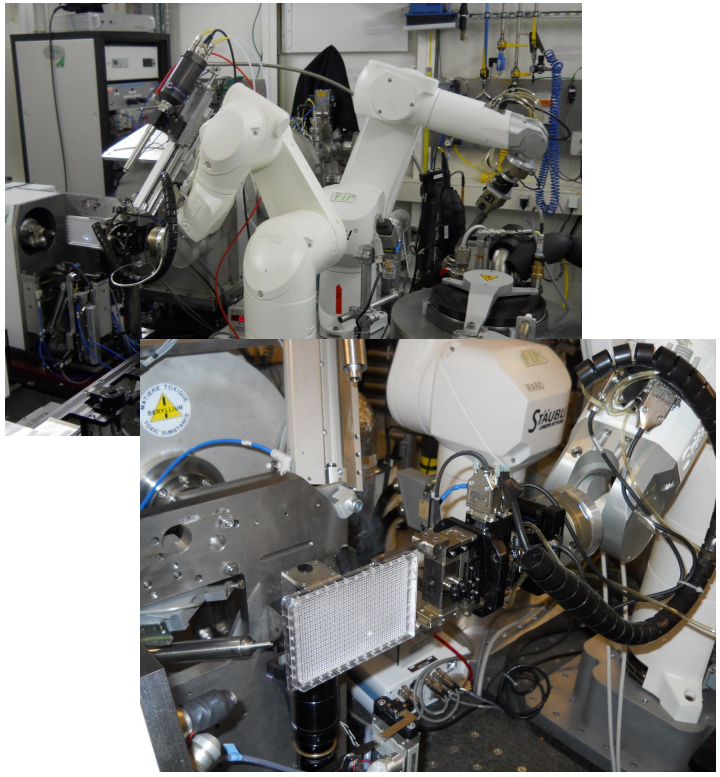
Adenovirus surface protein in complex with its receptor

In situ experiment performed on FIP-BM30A (5 Oct 2012)

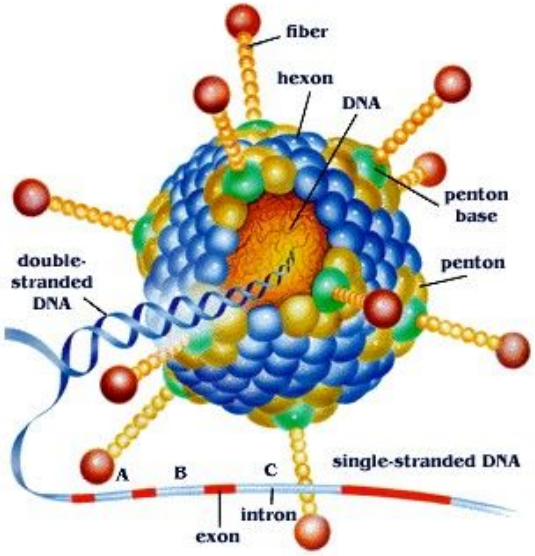
C. Zubieta, P. Fender (EMBL-Grenoble), A. Lieber (Washington Univ., Seattle)



Microbatch crystallization assay (HT platform, HWI Buffalo)

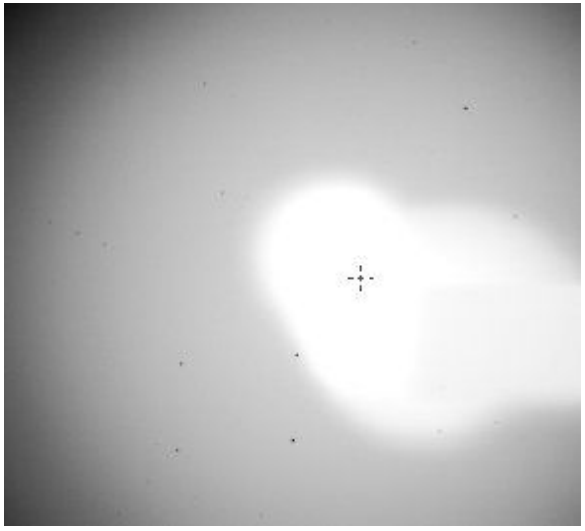


in situ screening in 1536-well plate with G-Rob on FIP-BM30A



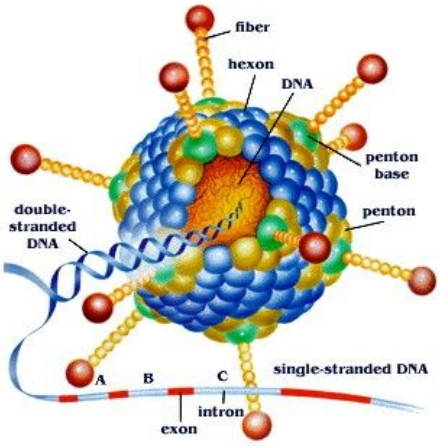
Aim of the experiment: study of the complex between the virus capside fiber and the human Desmoglein-2 receptor. This interaction leads to the opening of epithelial cells inter-cellular junctions, responsible for virus entry.

Diffraction at 6.3 Å on 30x10 µm² crystals

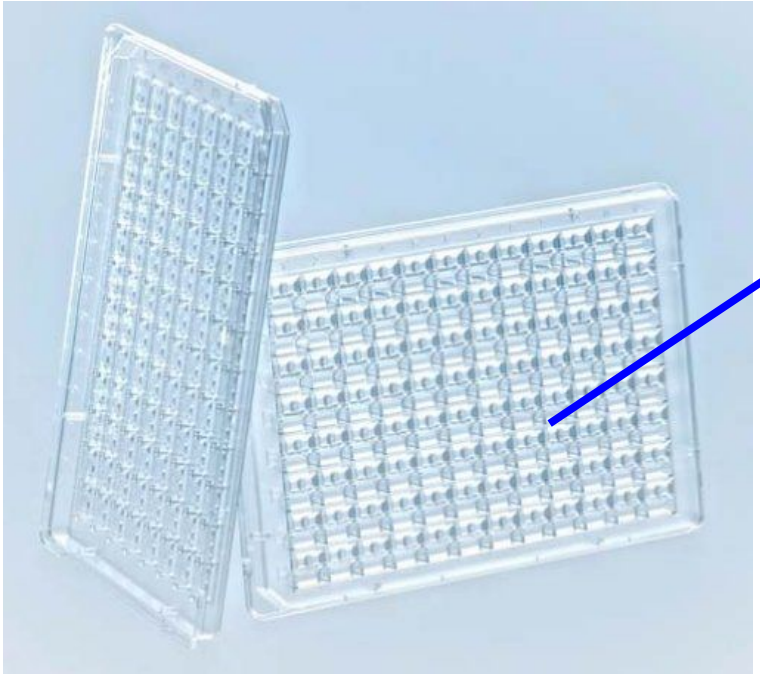


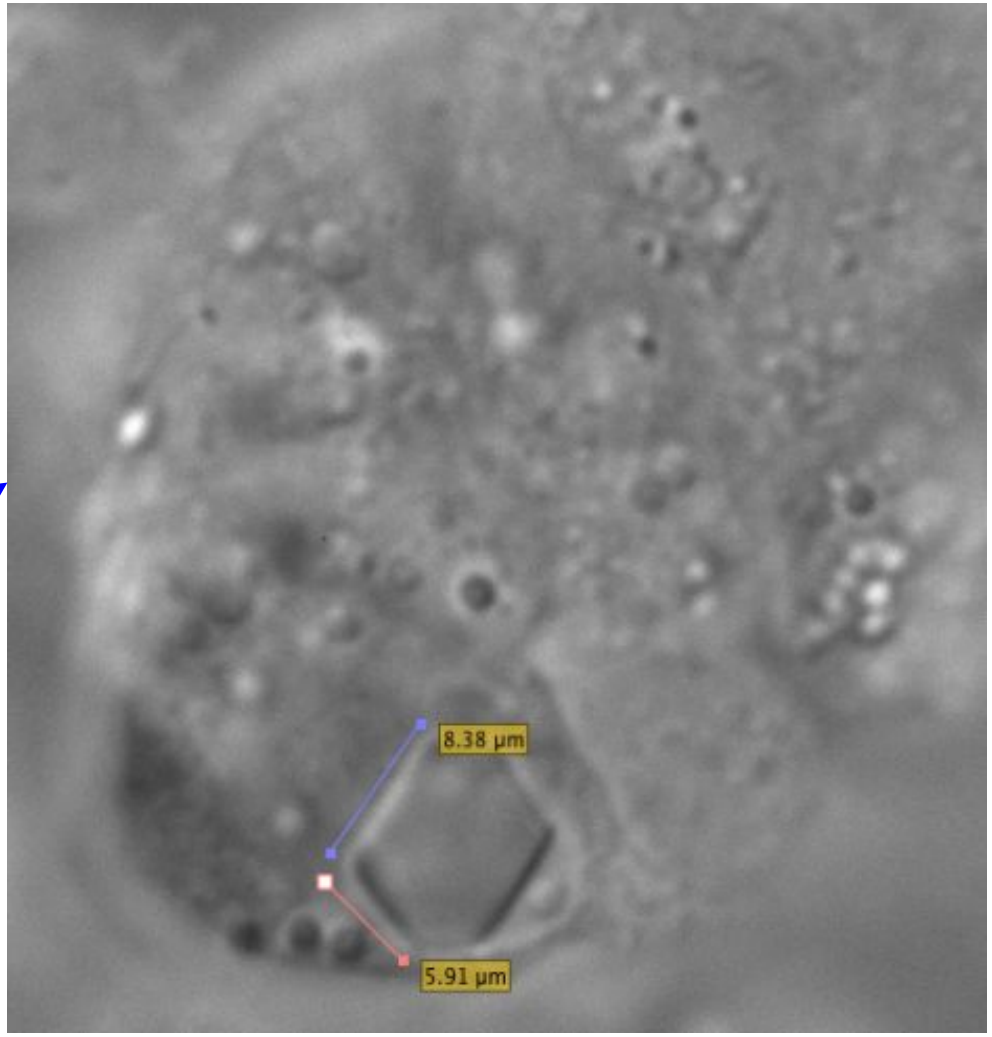
Intranuclear Adenovirus cristals

P. Fender *et al.*, EMBL



In situ analysis of crystals appeared in the insect cells used for expression.

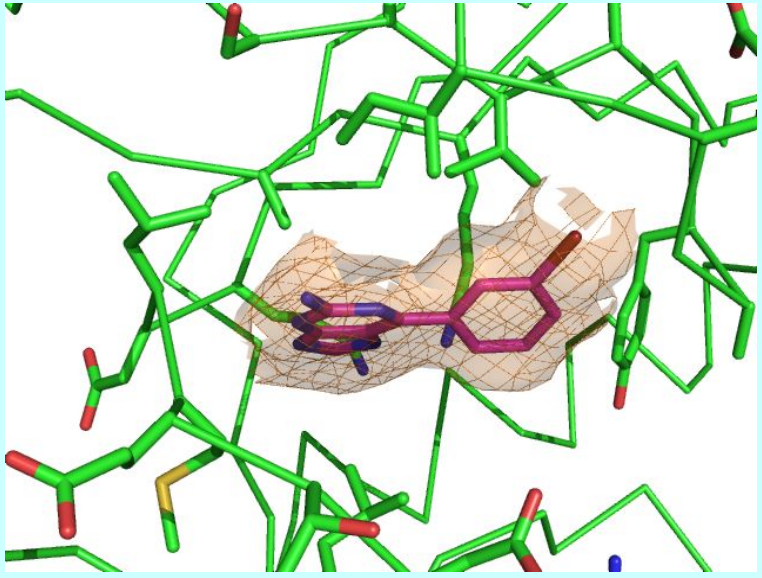




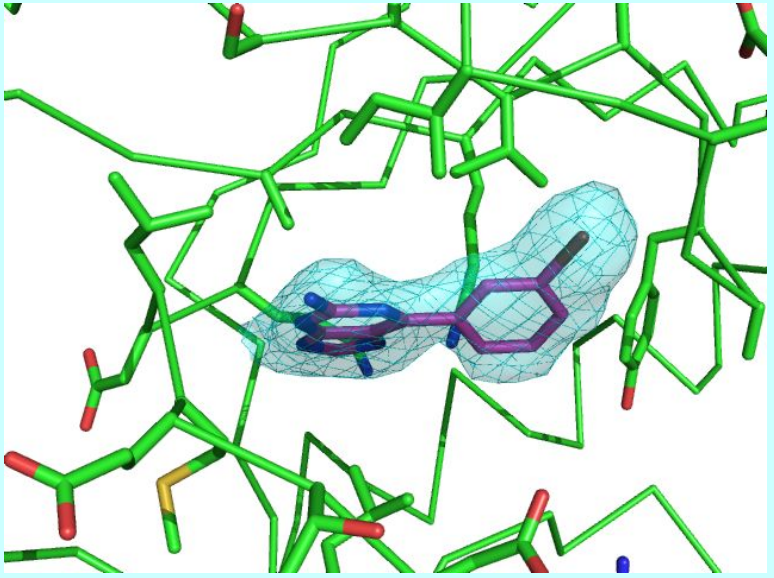
Test of ERK-2 in P21 with bromated ligand

Completness of 83%
by merging 3 dataset (50+50+41 frames)
Rsym ~5.4 at 2.15 Å.
Refinement against the structure of ERK-2, with no ligand.

ERK-2 (6PB)
Refinement: Refmac/Coot
Without ligand (R/Rfree ~ 20.4/25.5)



fo-fc (orange) 0.9 sigma



2fo-fc (blue) 0.9 sigma

Collab. G. Labesse, CNRS/CBS (Montpellier)

Interest of *in situ* for protein dynamic

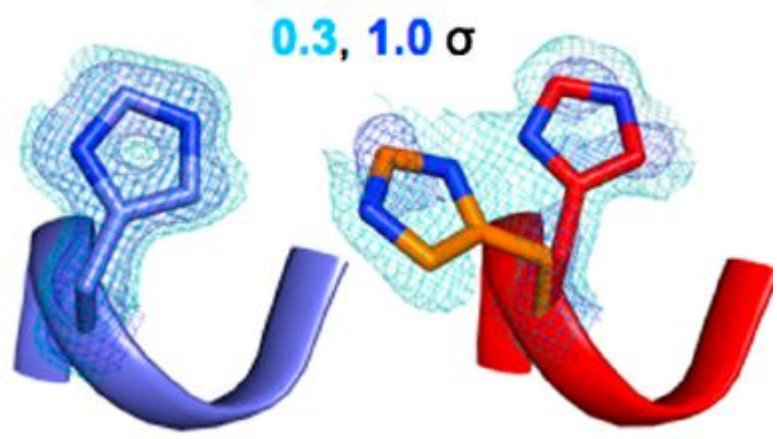
Flash cooling of protein crystals

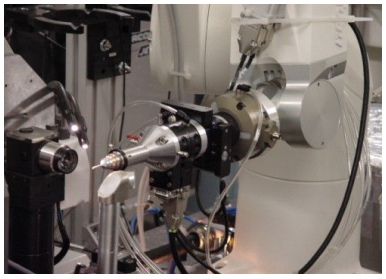
- biases structural collective motions in protein crystals;
- remodels the conformation of > 35% of side chains;
- eliminates packing defects necessary for functional motions;
- induces bias toward smaller, overpacked, and unrealistically unique models.

Instead, **room-temperature** X-ray crystallography experiments, such as the *in situ* experiments, helps in revealing

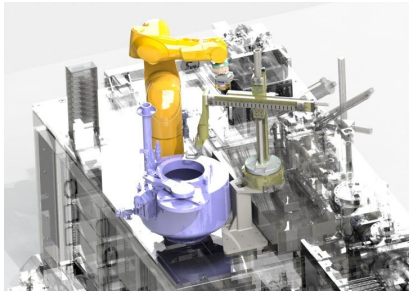
- motions crucial for catalysis,
- ligand binding,
- allosteric regulation.

In the signaling switch protein, H-Ras, an allosteric network consistent with fluctuations detected in solution by NMR was uncovered in the room-temperature, but not the cryogenic, electron-density maps (Fraser *et al.*, PNAS, 2011 (108), 16247-52).

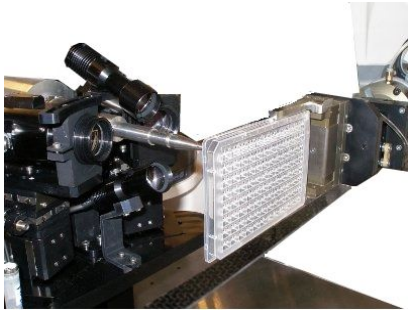




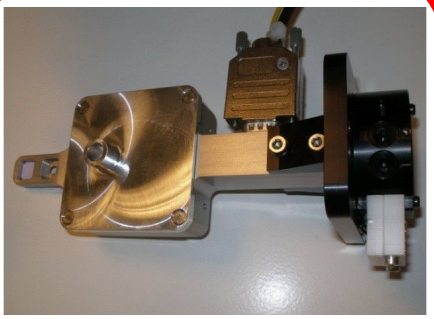
Frozen crystals, capillaries, powder



Cryo-sample transfer

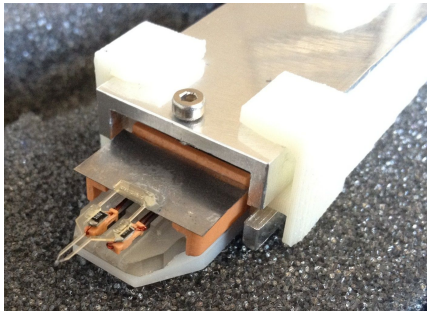


Plates, microchips (*in situ* screening & datacoll.)



Beam monitoring, quick-realign

G-Rob Monitoring



Sample harvesting

Beam monitoring

- fluorescent screen
- diode monitor

Application

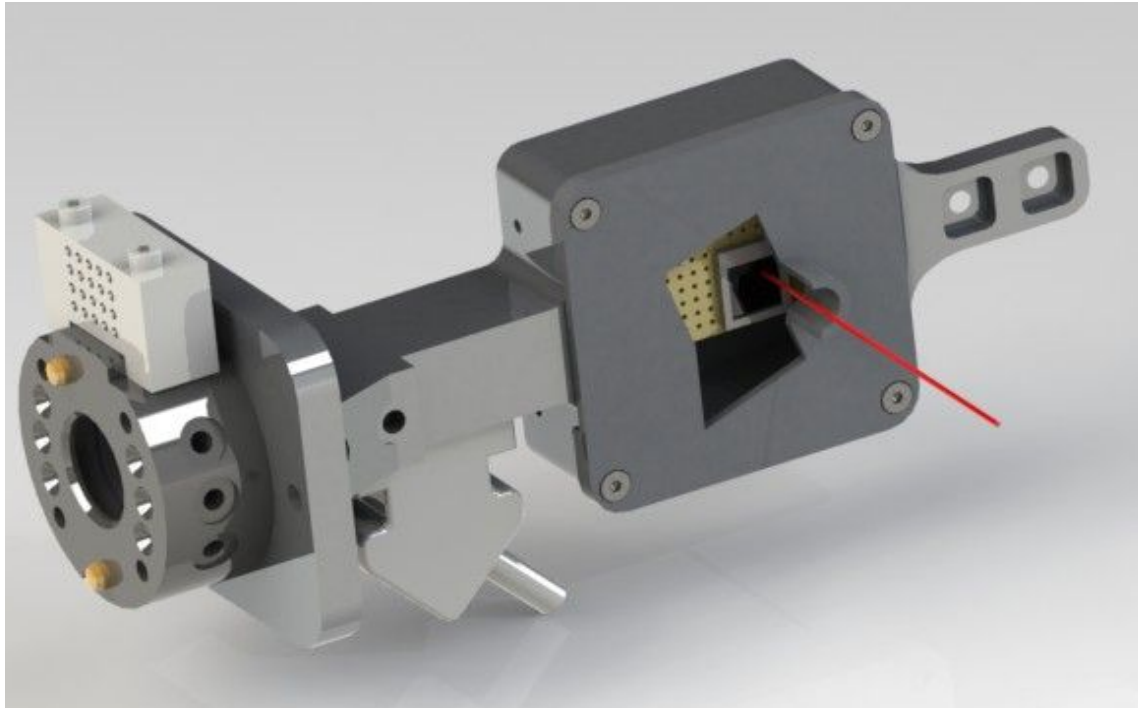
- Check the beam position
- Check the beam intensity
- automated beam optimization (~”Quick Realign”) by users

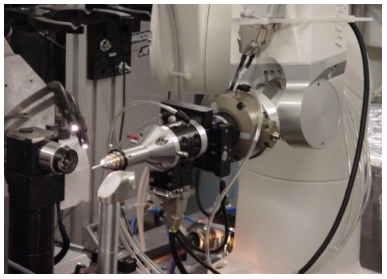
G-Rob function: Monitor

A robot tool for beam monitoring

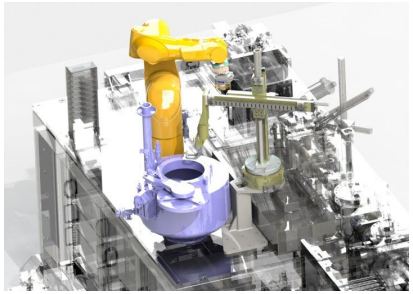
- A diode for intensity measurement
- A fluorescence screen for beam imaging

In association with motorized optics ➡ **automated beam alignment**

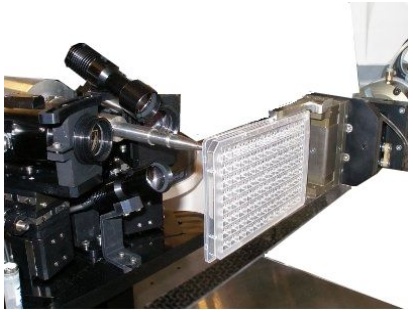




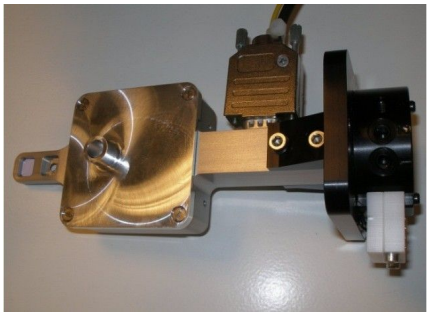
Frozen crystals, capillaries, powder



Cryo-sample transfer



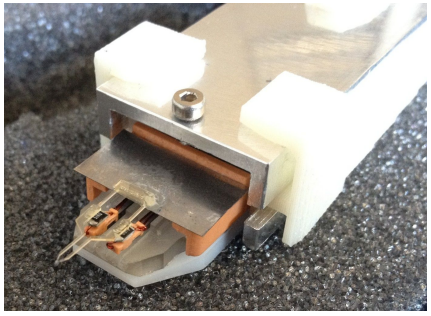
Plates, microchips (*in situ* screening & datacoll.)



Beam monitoring, quick-realign

G-Rob Harvesting

(in development)



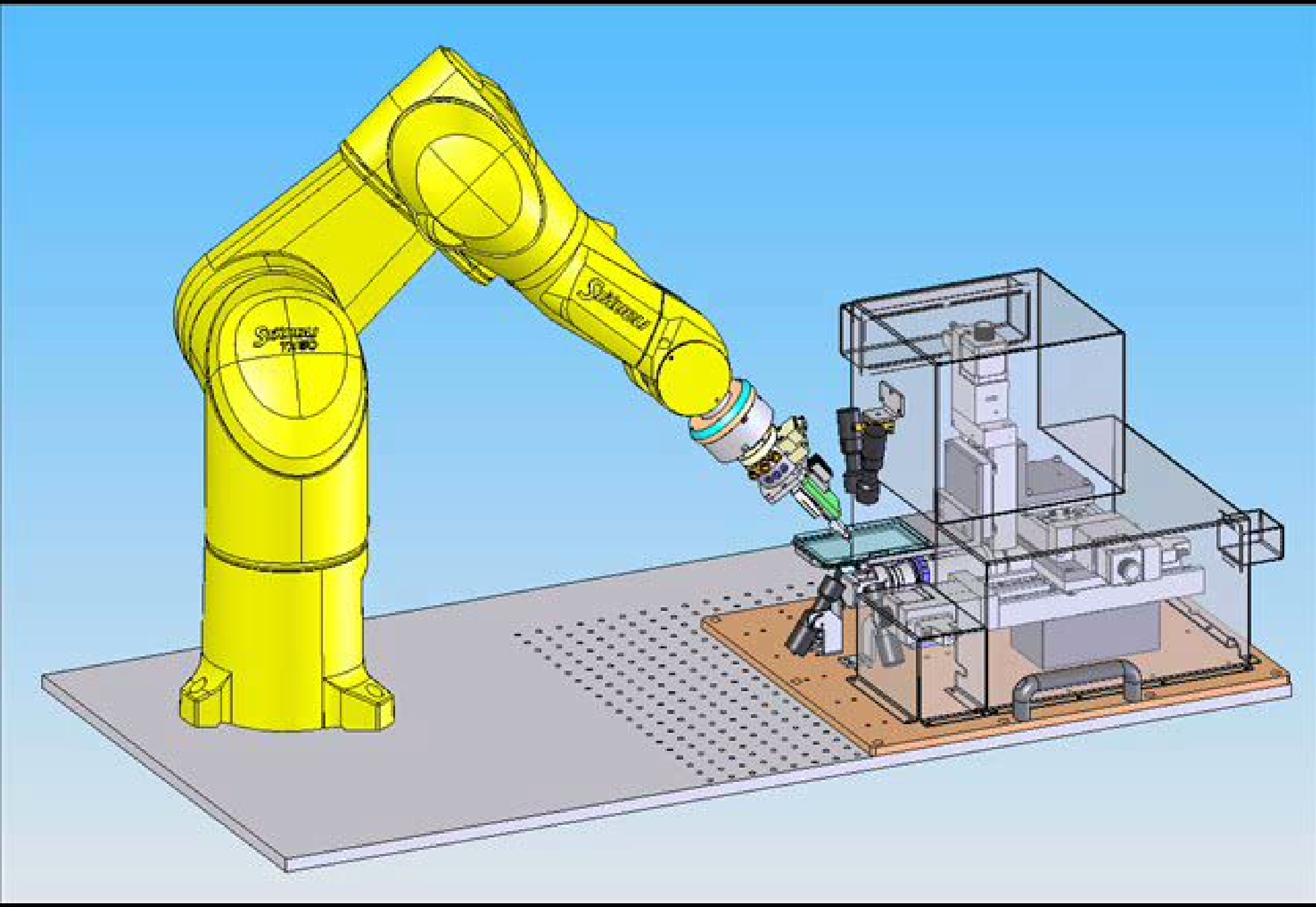
Sample harvesting

Sample Harvesting

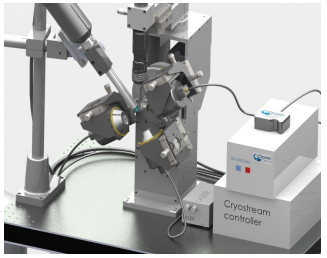
- Semi-automated harvesting
- Remote controlled micro-gripper

Applications

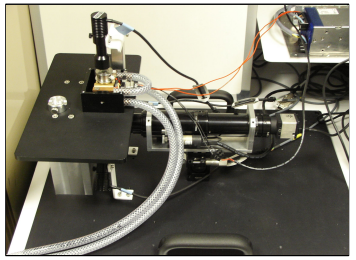
- Remote harvesting
- Crystal freezing
- Transfer to loops for storage



VisuBench & Crystal-Listing



Misc



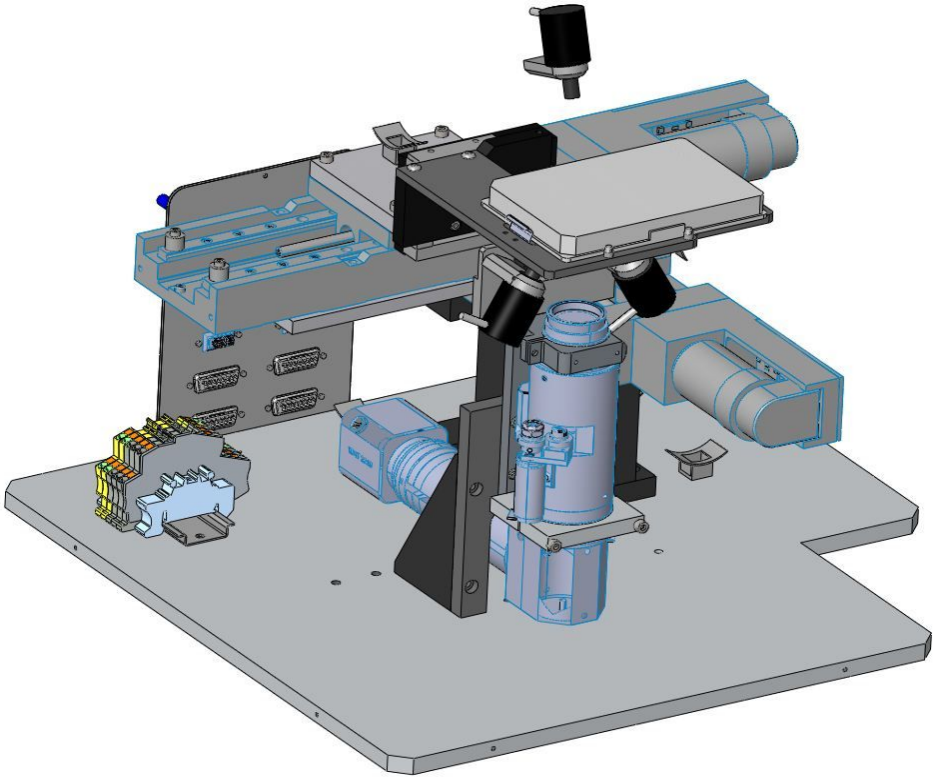
OptiCryst

The Visualization Bench

Beam monitoring
- Inverting microscope
- Crystal Listing

Applications
- Visual screening
- Crystal position recording
- On line: sample harvesting

Visualization bench

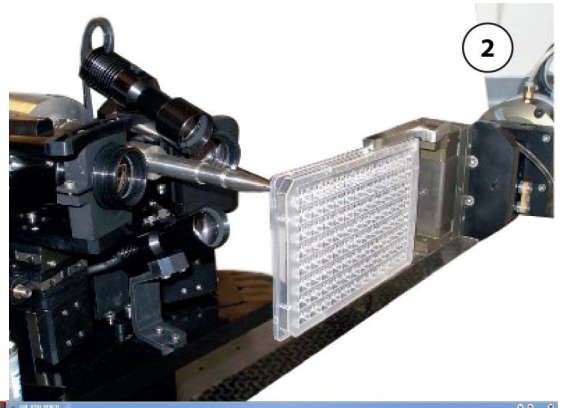


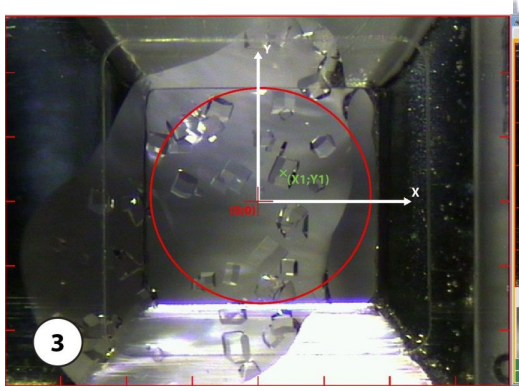
Fully motorized inverted microscope designed for the analysis of micro-plates.
Equipped with motorized zoom, front and back LEDs for sample lightening.
Controlled through a user friendly graphical interface.

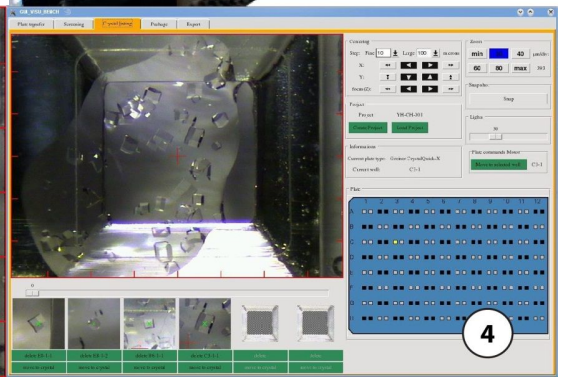
Crystal Listing

A dedicated microscope can be used off-line for
-Automated image recording
-Selection of crystals (one click)

→ Position of crystals recorded in a local coordinate reference
→ Data uploaded to the G-Rob database for automated screening

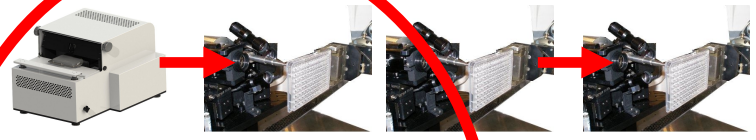






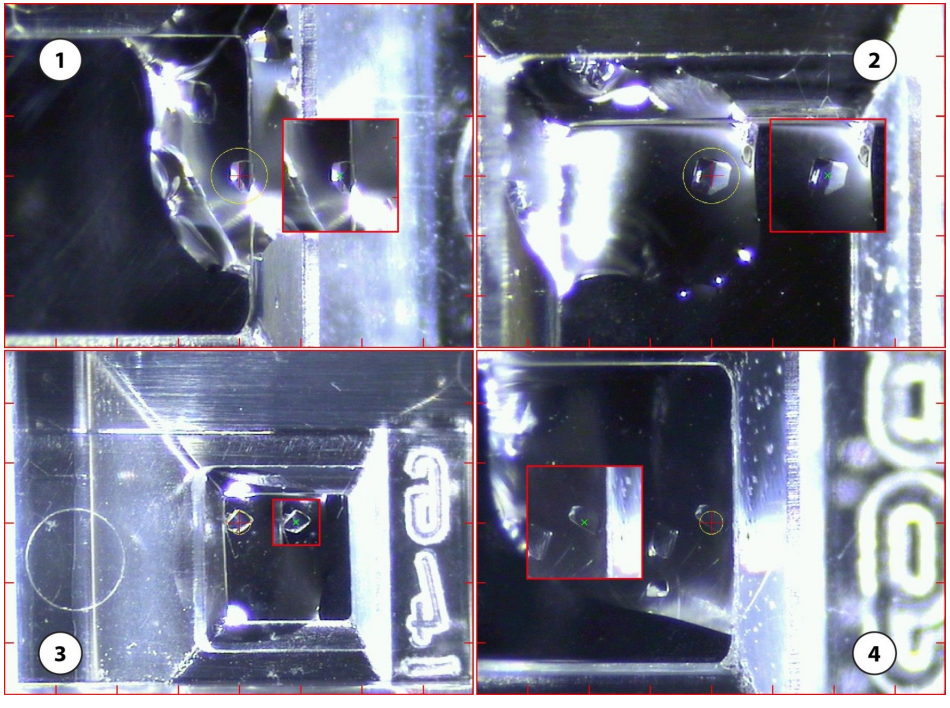
① Visualization Bench
② *In situ* X-ray diffraction with G-Rob
③ Automatically centered well with crystal coordinates in the local reference
④ Crystal Listing tab in Visualization Bench GUI

Crystal Listing

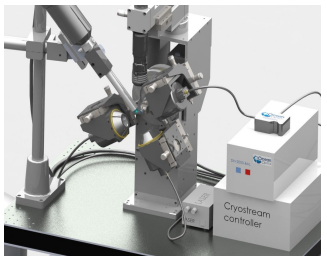


Listing on:	Visualisation bench	G-Rob
Number of samples	44	79
<|X|> error (µm)	33	3
<|Y|> error (µm)	21	3
<|radius|> error (µm)	41	5
X standard deviation (µm)	17	2
X standard deviation (µm)	14	2
radius standard deviation (µm)	17	3

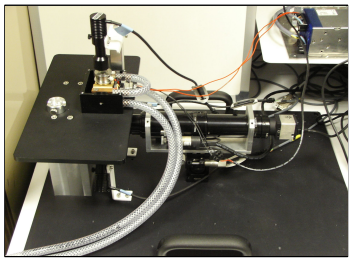
Experiments performed on the
in-house G-Rob system
of the the EPFL crystallography platform
(Prof. S. Cole laboratory, Lausanne).



VisuBench & Crystal-Listing



Misc



OptiCryst

Based on the Cryobench available at the ESRF on ID29:
D. Bourgeois, et al. (2002). J. Appl. Cryst. 35, 319-326.
Head of the Cryobench team at ESRF: A. Royant

MiSC: a MicroSpectroPhotometer

MiSC

- Goniometer + microscope + cryo-cane
- Reflective optics
- Spectrometer

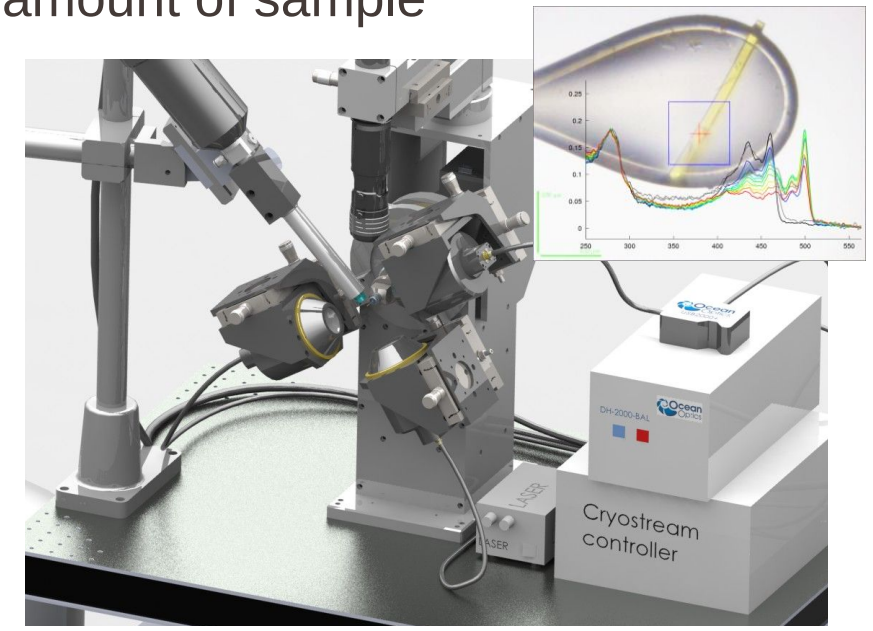
Applications

- Fluorescence measurements
- Absorption measurements
- Synchronized experiment

MiSC

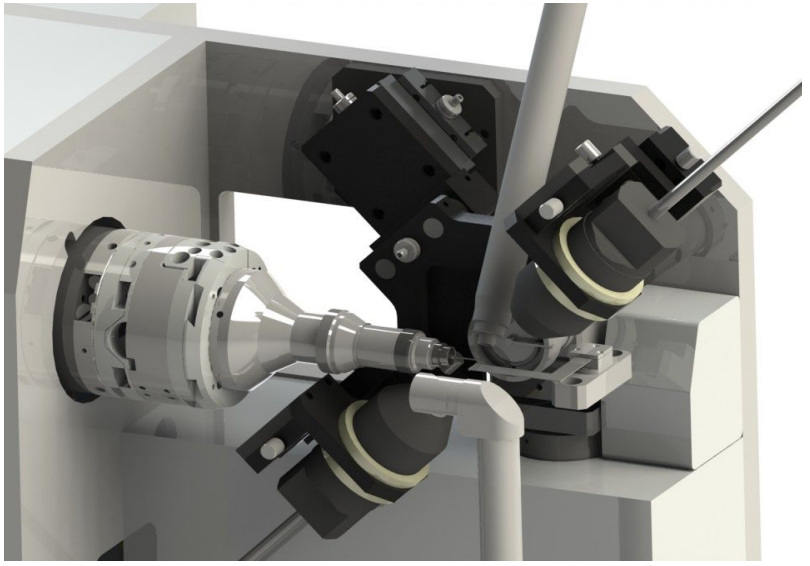
A Micro-Spectrophotometer for Crystals

- **UV/Visible absoption**, measured on the 200-1100 nm range
- **Fluorescence measurements** at 90° on micro-amount of sample
- **Samples can be:**
  - Crystal down to 10 µm
  - Nanoliters solutions
- **Spectrometer :** 200-1025 nm range



Based on the CryoBench (IBS & ESRF/ID29)

Stand alone version
As a G-Rob function
In line version for goniometers
Detector as close as 40 mm
Access for sample changer



Absorption source: 210-1700 nm UV-NIR deuterium/tungsten
Fluorescence source: 455 nm LED source or 473 nm SS laser
Sample holder: 2-axis gonio head (crystals), cuvette holder
Sample visualization: with video microscope
Polarizer, optical fibers

IBS/GSY & FIP-BM30A

X. Vernede
Y. Sallaz-Damaz

N. Larive

For testing the in-house G-Rob system: EPFL, Switzerland
For testing the synchrotron G-Rob system: FIP-BM30A at ESRF, France

In both case contact: **jean-luc.ferrer@ibs.fr**

R. Richaud

E. Girard
A. Royant

…and the crystallography platform at the EPFL: F. Pojer, S. Cole

Funding: CEA (TS Program), CEA/DSV, CNRS, IBS, Rhone-Alpes