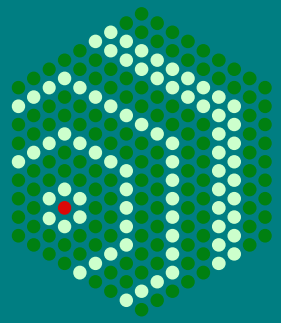
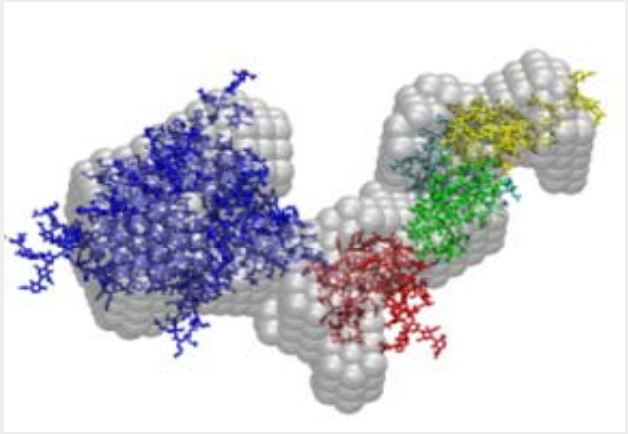
INSTRUMENTS for X-ray crystallography and solution scattering experiments

EMBL Grenoble outstation
Instrumentation Group
Diffraction Instrumentation Team, Florent Cipriani



EMBL Member States: Associate Member:

EMBL Basic research in **Molecular Biology**



- Study the **STRUCTURE** of biological macromolecules
  - ➔ Nature and position of the ATOMS
  - ➔ Understand the function of the macromolecules
    - ➔ Understand the mechanisms of life
      - ➔ Understand diseases
        - ➔ Design drugs
- **TOOLS** X-rays and Neutrons scattering

ESRF
→ X-Rays

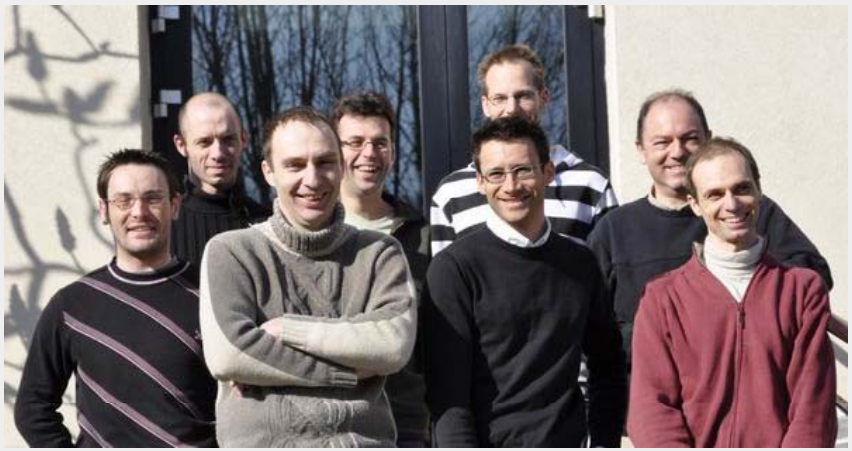
ILL
→ Neutrons

Diffraction Instrumentation Team

Mission: Develop Instruments and Methods for diffraction experiments

The TEAM

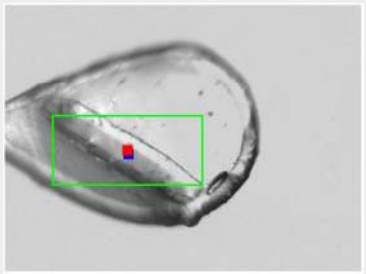
Franck ***Felisaz***
Jerome ***Halbwachs***
Raphael ***Moya***
Alexandre ***Gobbo***
Gergely ***PAPP***
Julien ***Huet***
Christophe ***Landret***
Silvia ***Russi***
Florent ***Cipriani***



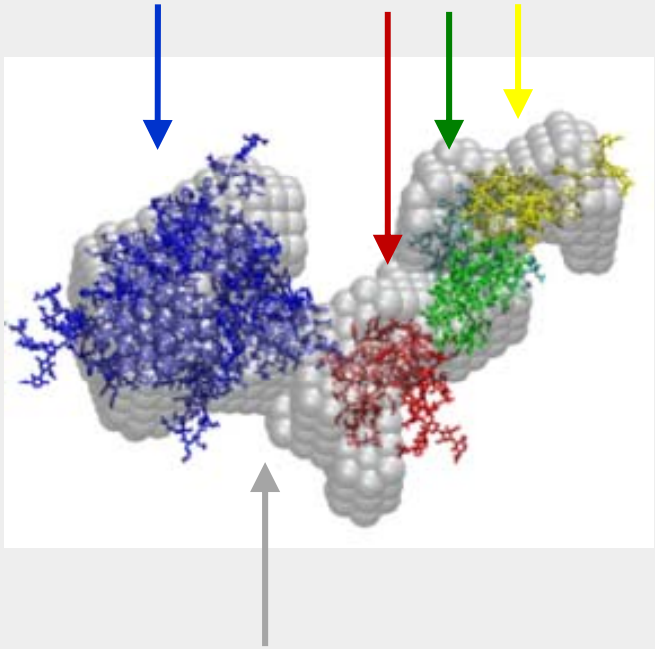
Structure determination

Two X-ray diffraction techniques

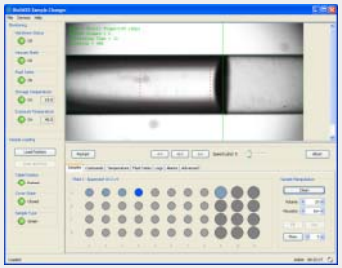
1 – Macromolecular crystallography



- Atomic resolution (0.8 Å -50 nm)
- Crystallised form
- Size of macromolecules is limited



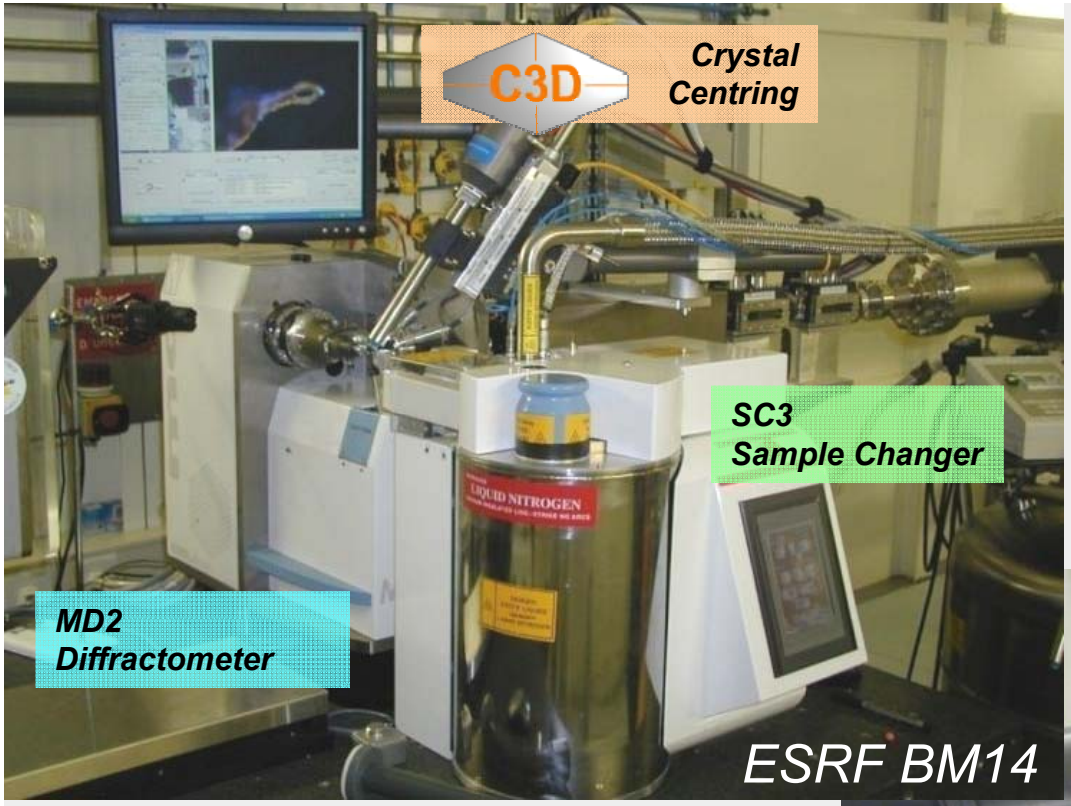
2 – Small angle scattering (SAXS)



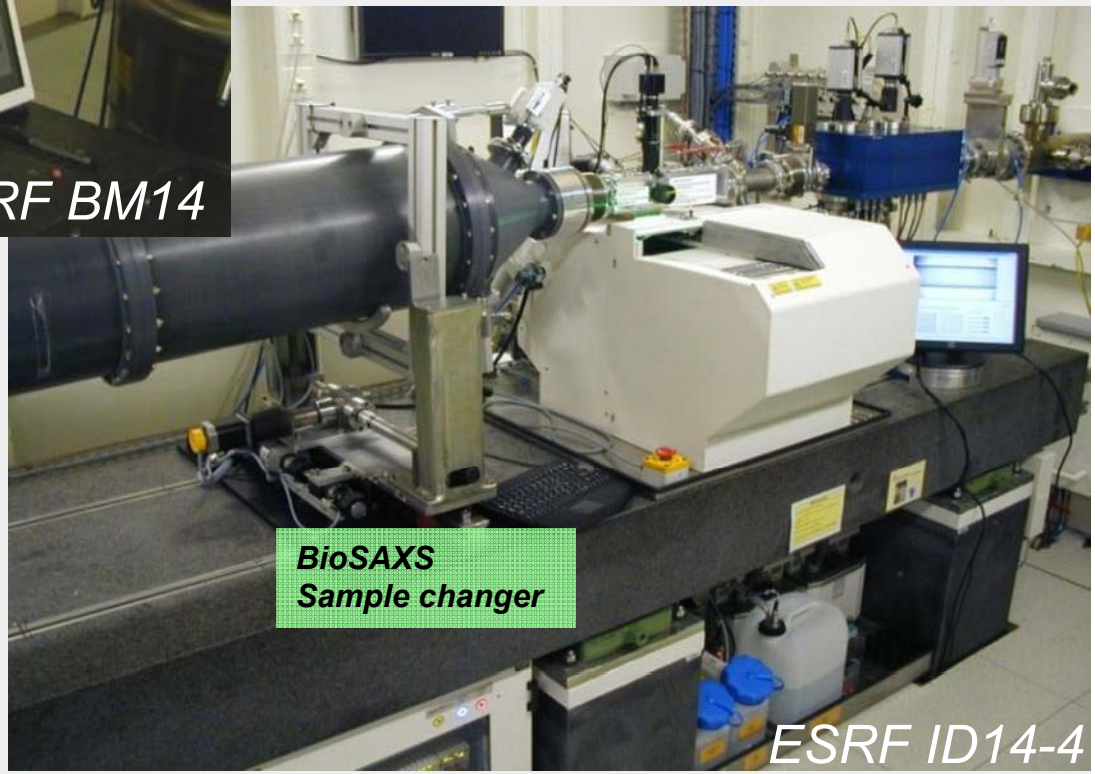
- Sample in solution
- Large macromolecules, assemblies (complexes)
- Kinetics
- Resolution is limited to 10 Å (Up to 500 nm)

Typical Instruments

◄ Crystallography



▼ Small Angle X-ray Scattering



MAATEL

BRUKER

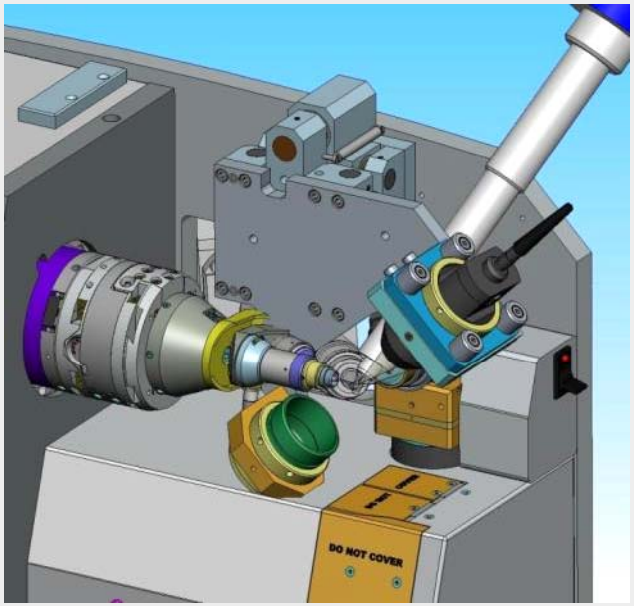
- 25 beamlines equipped in Europe, US, Canada, Australia, Asia

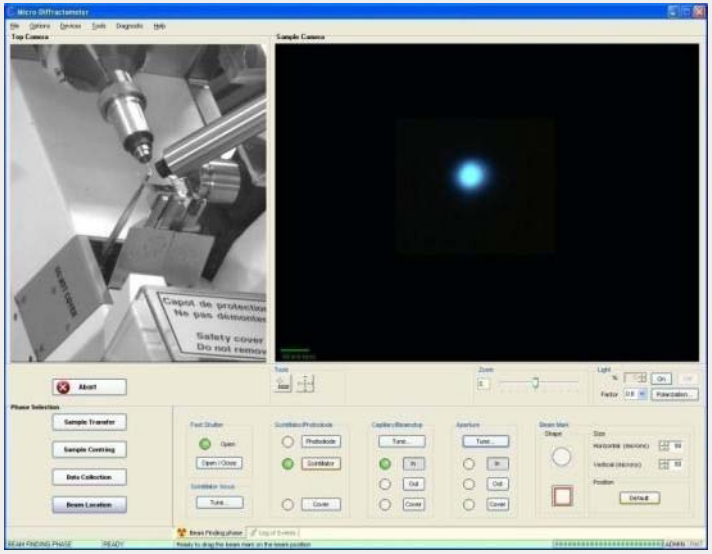
Diffraction Instrumentation TEAM

Our expertise: **System engineering**

Precision mechanics
Optics
Cryogenics

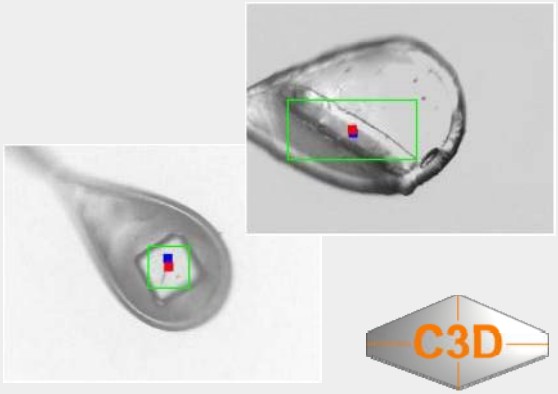
Analog/digital Electronics
Motion control





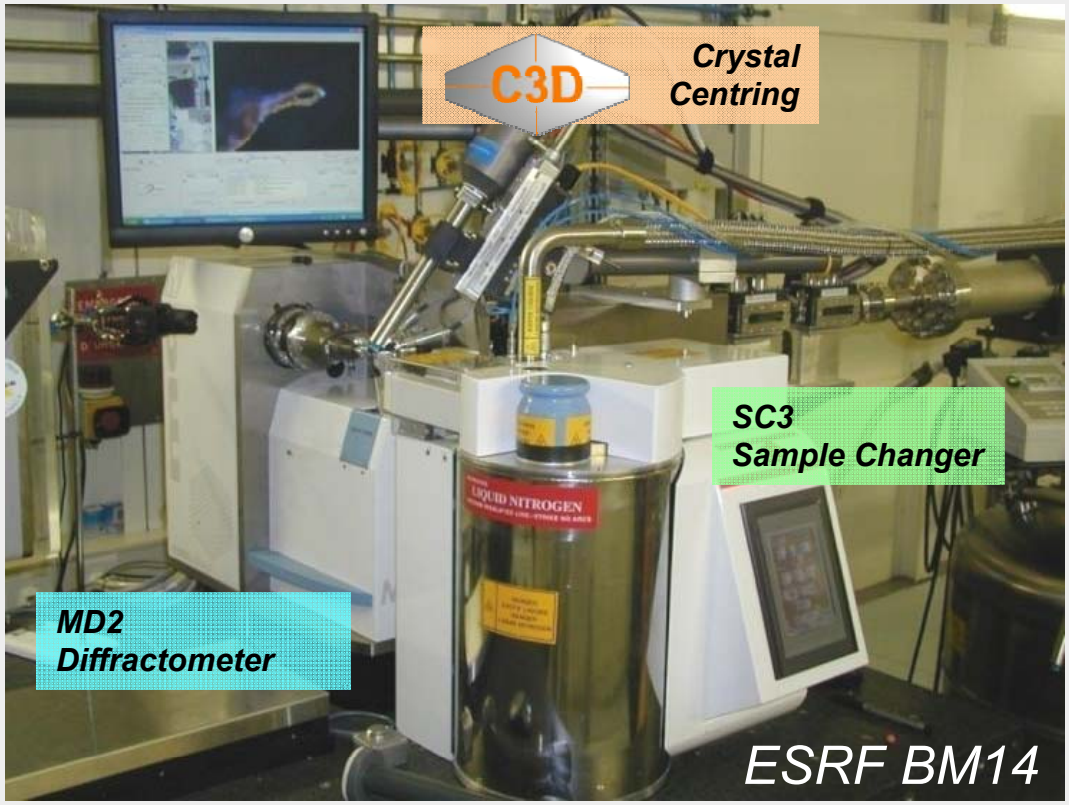
Software

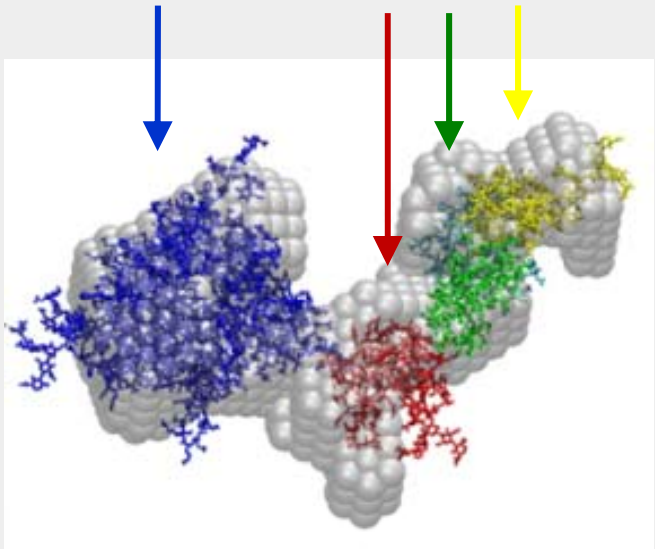
← Instrument control
Image computing →



Automatic crystal detection & alignment
Bernard Lavault

Instruments for Macromolecular Crystallography

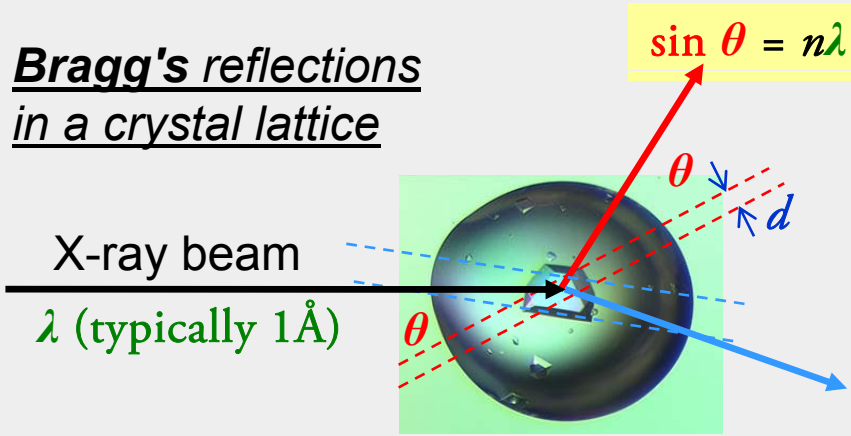


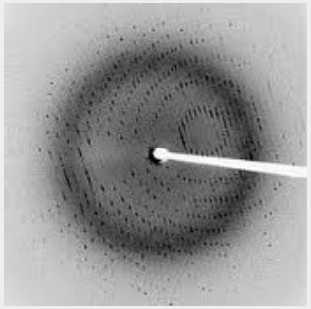


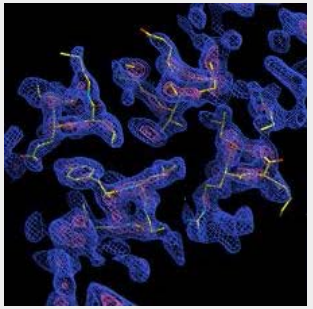
Macromolecular X-ray crystallography Reminder

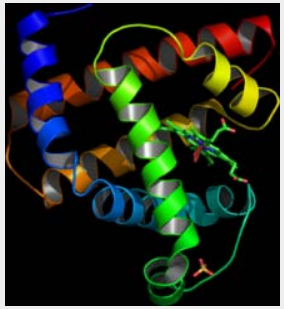
***Bragg's** reflections in a crystal lattice*

$\sin\theta = n\lambda / 2d$




Diffraction image

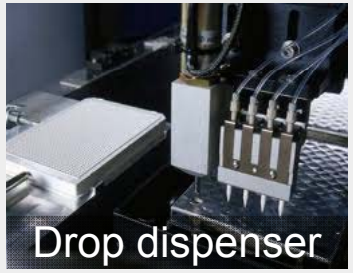

Electron density map


Structure

First crystallize the macromolecules ...

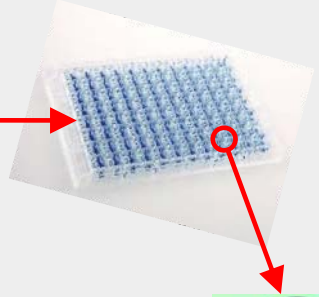

Protein

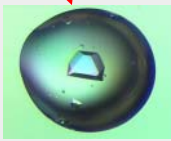
96 wells plate




crystallization solutions

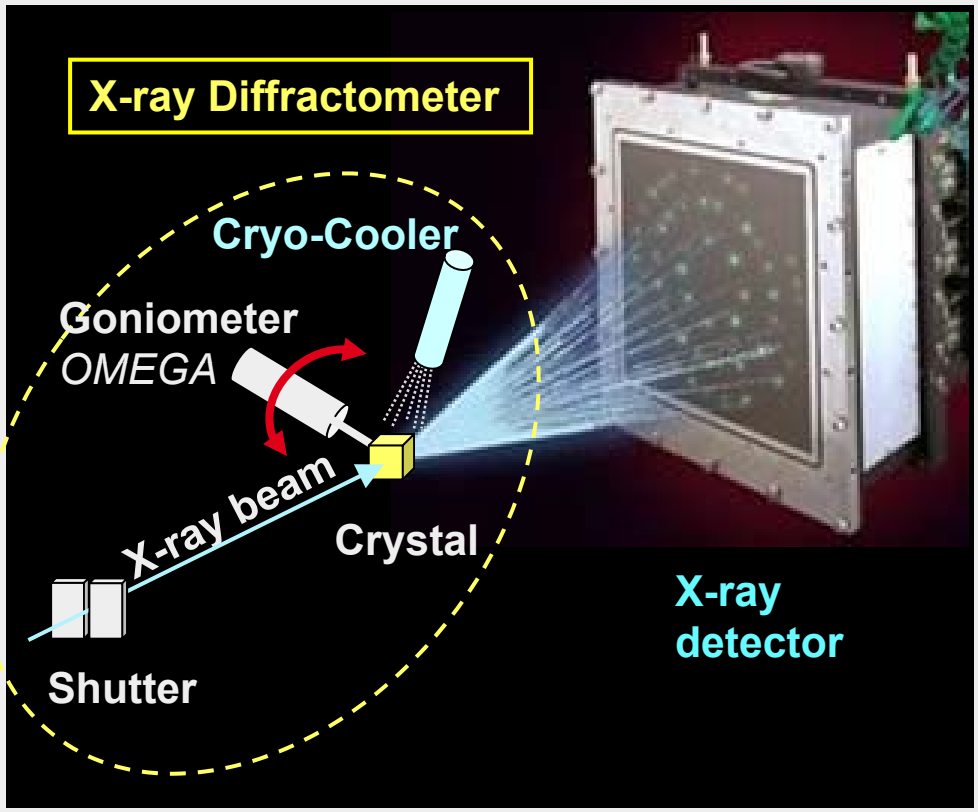

Crystallization farm



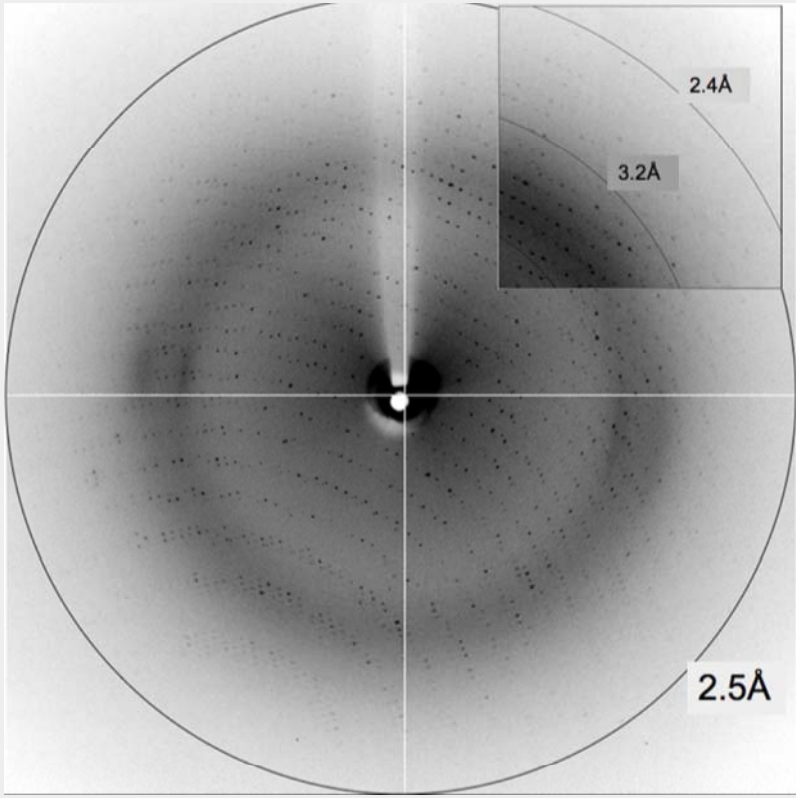


X-ray crystallography data collection **Reminder**

Typical **Experimental setup**



Crystals mounted in a “cryo-loop”



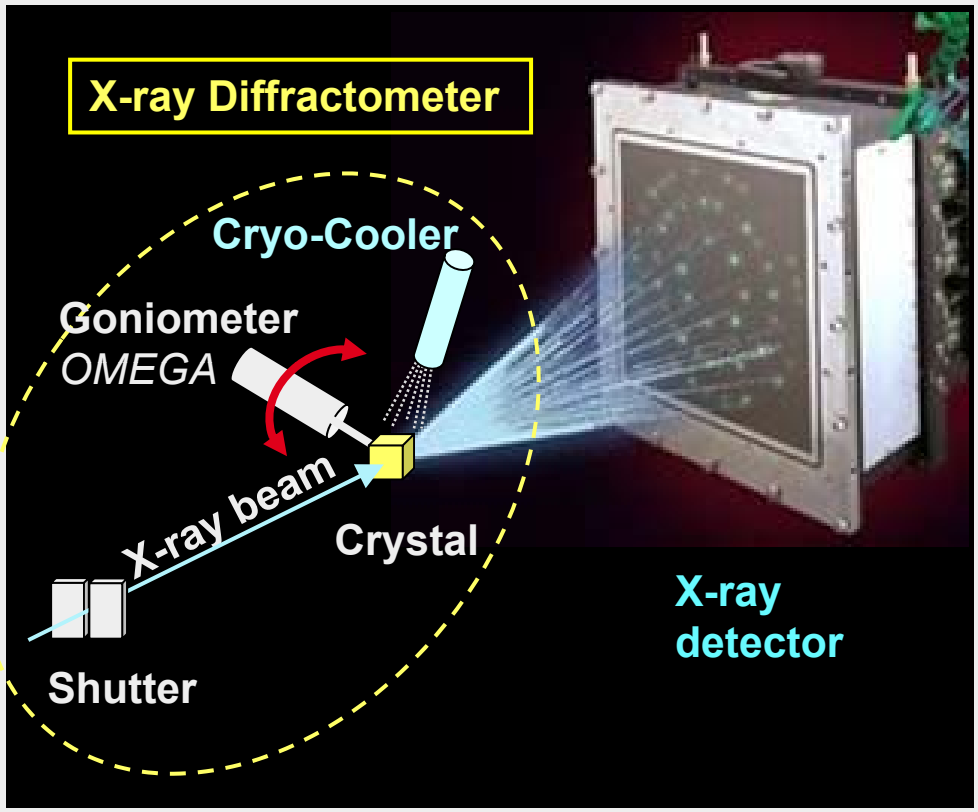
Collecting a diffraction data set

Several hundred images collected during ***angular Scans***

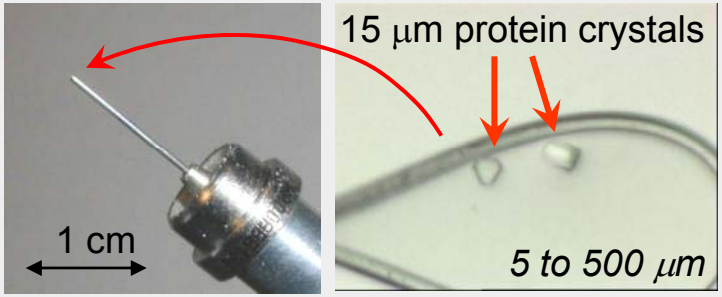
Typical ***scan****: 1 degree in 0.1 to 5 sec*

X-ray crystallography data collection **Reminder**

Typical **Experimental setup**



Crystals mounted in a “cryo-loop”



Data quality

- ***Alignment of the crystal with the beam***
- ***Precision of the scans***
- ***Signal to noise ratio***
- ***Radiation damage***

Collecting a diffraction data set

*Several hundred images collected during **angular Scans***

*Typical **scan**: 1 degree in 0.1 to 5 sec*

Our solution the MD2 diffractometer family

Diffractometer

Control electronics

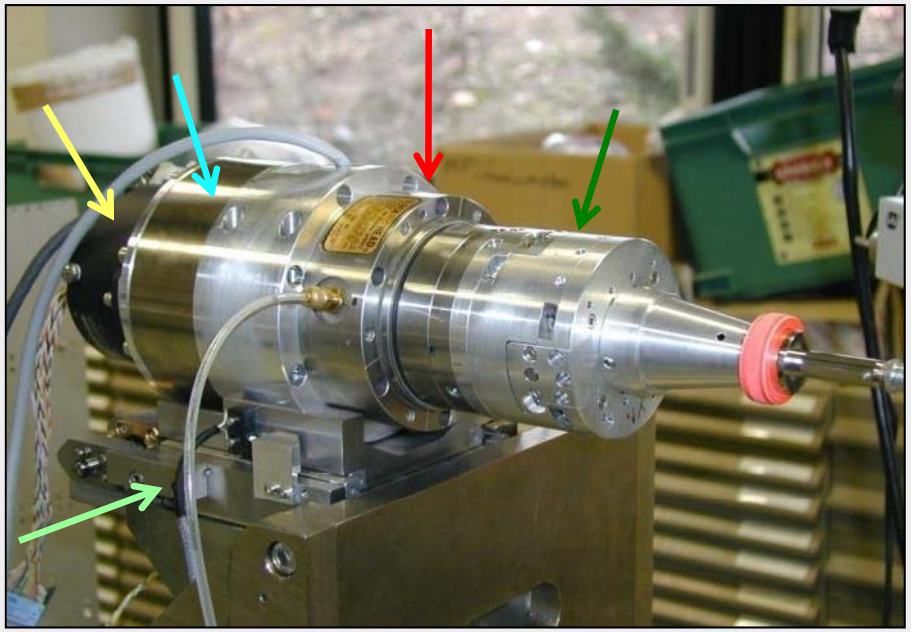
Control software
Windows
(.NET, C++, VB)

C3D Crystal Centring software

***Designed for optimal* Data Quality *and* Automation**

The MD2 Goniometer

High precision **Air bearing goniometer** with crystal **alignments** & **centring** table



- **Air bearing spindle** *a few nm error motion*
- **Torque motor** driven (no gearbox)
- **Direct encoding** 4.6M pulses/turn

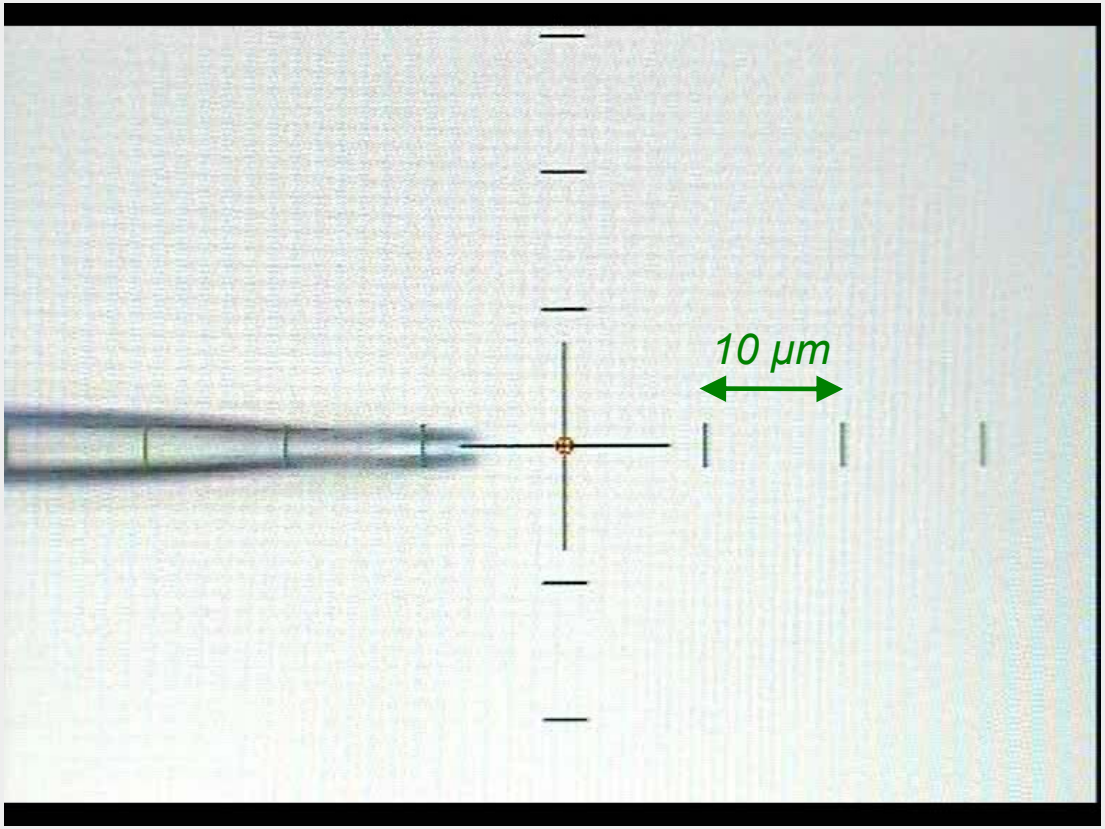
- **XY centring table** 0.2 µm resolution..
- **XYZ Alignment table** 0.2 µm resolution

- **PMAC motion control (Close loop PID)**

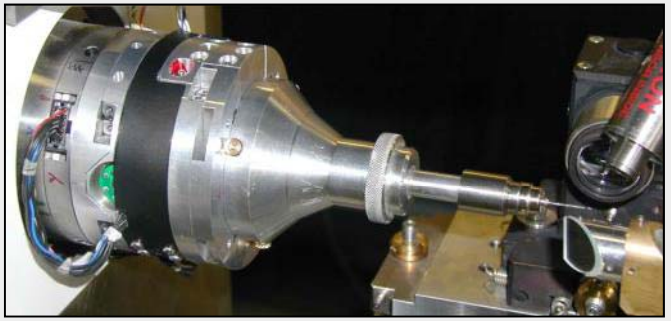
➔ Angular error <1 mDeg @ 20 Deg/s
➔ Shutter synchronisation error <<1 mDeg (scans)

MD2 Goniometer

Précision control



Observed sphere of confusion ≈ 1 µm



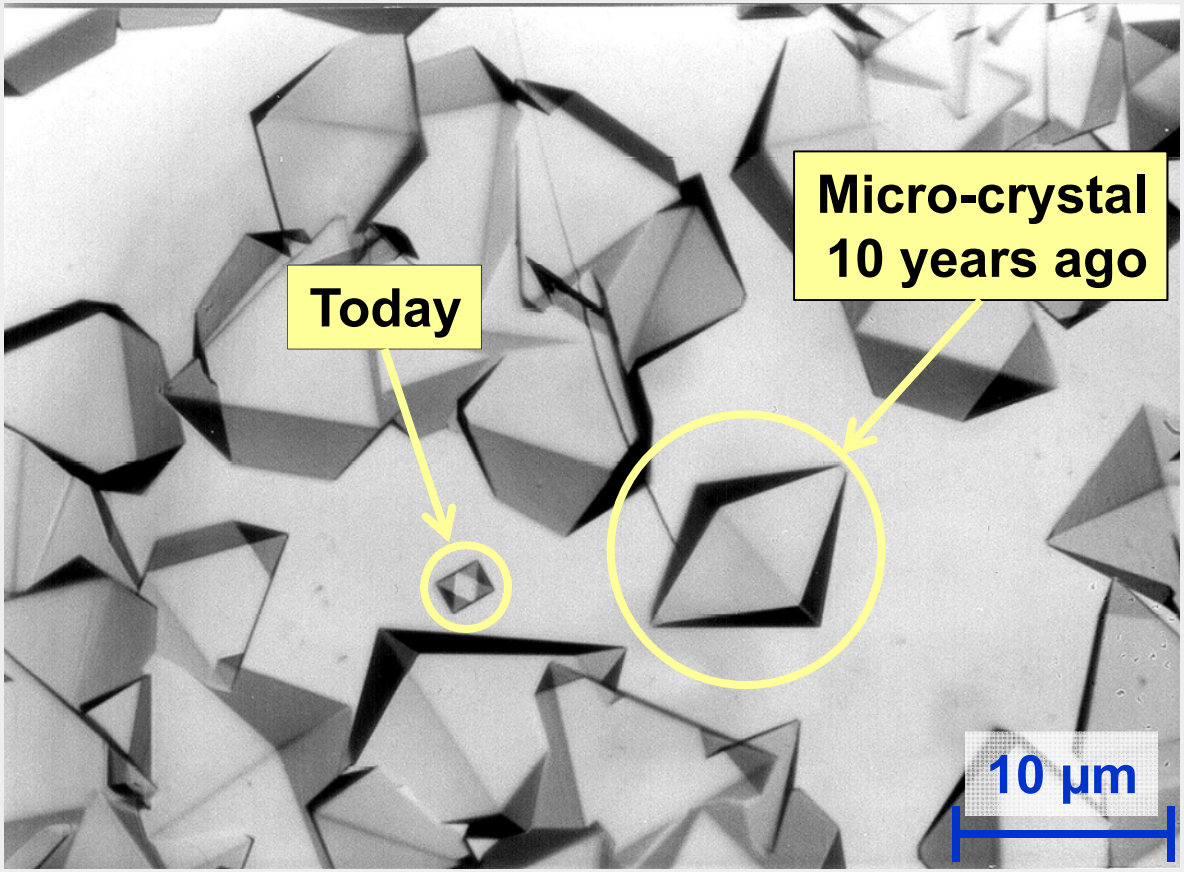
Scales

Green grid:10 µm
Red circle: 1 µm diameter
Screen resolution: 0.25 µm / pixel

Real time video
Needle with 1 µm hole at tip
Rotation 45 deg/sec

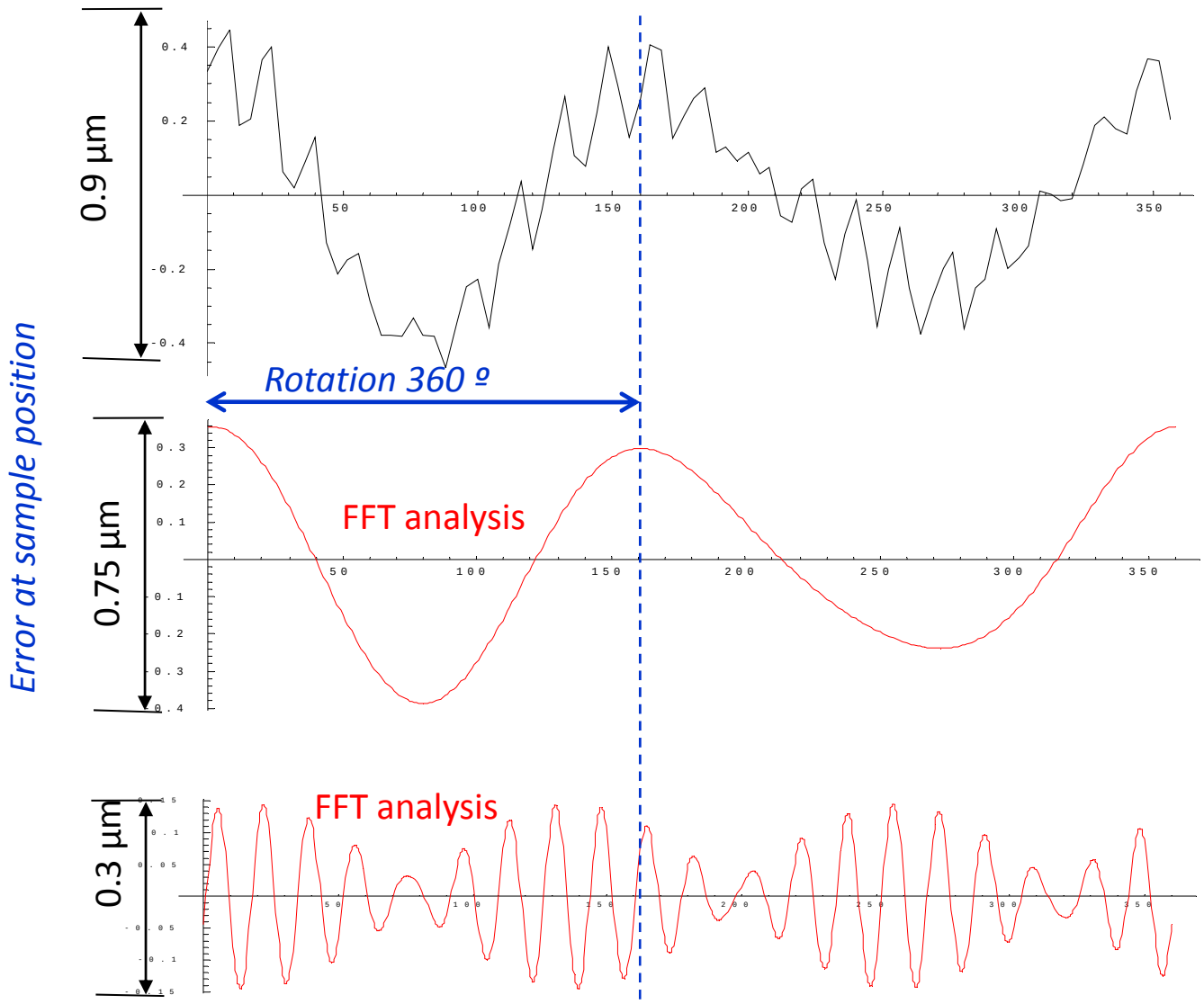
Beam like view

Processing micro-crystals



How to improve the precision of Goniometers?

Improving the goniometer precision



SOR diameter
- **0.9 µm pp**
- **0.25 µm r.m.s**

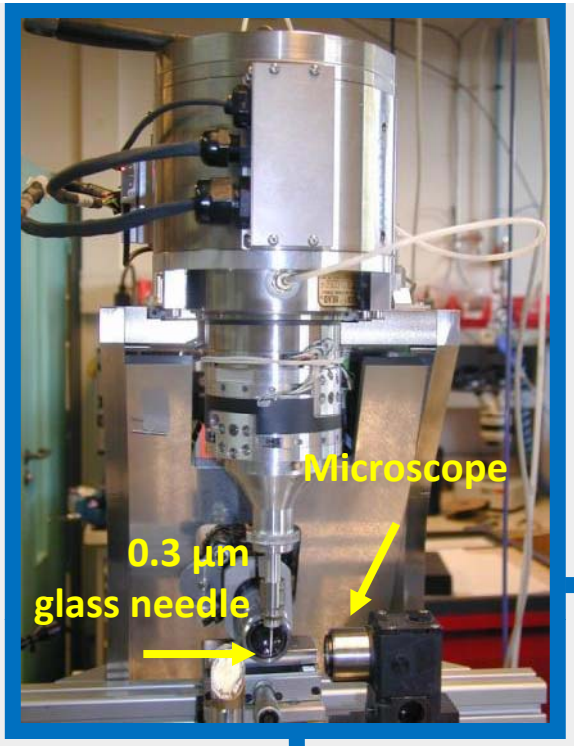
2nd+3rd harmonics

Gravity effect on Centering table

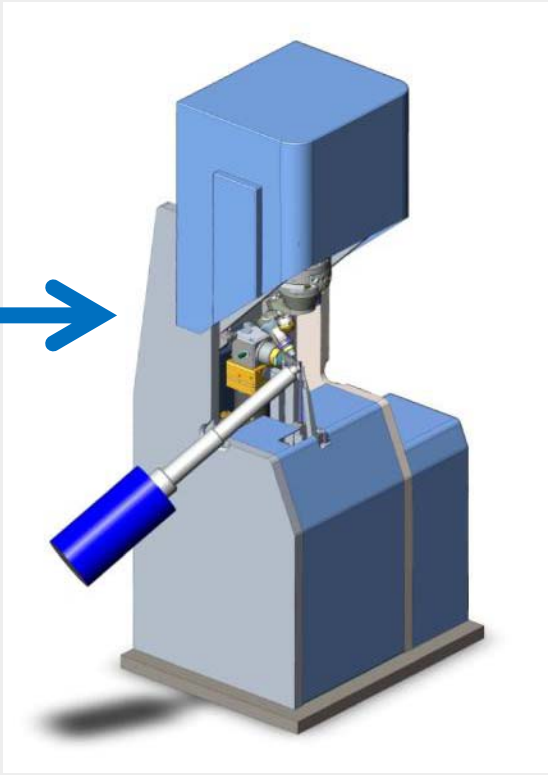
20th+23rd harmonics

Poles of the OMEGA motor

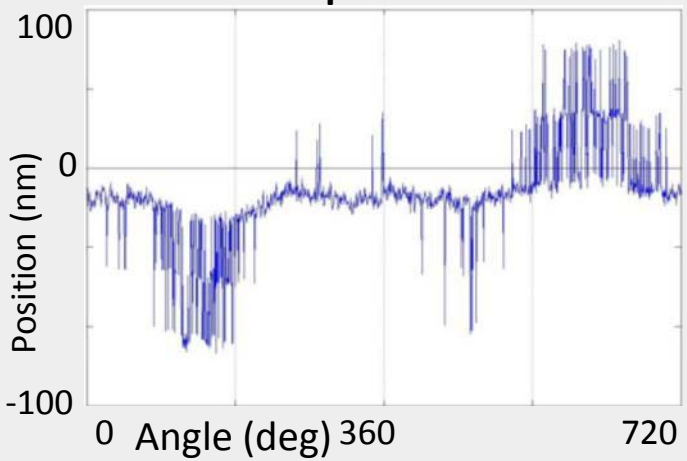
New Goniometer
Set in vertical orientation



- **SOC <0.5 *µm* with Kappa**
  Crystal down to **2-3 µm**



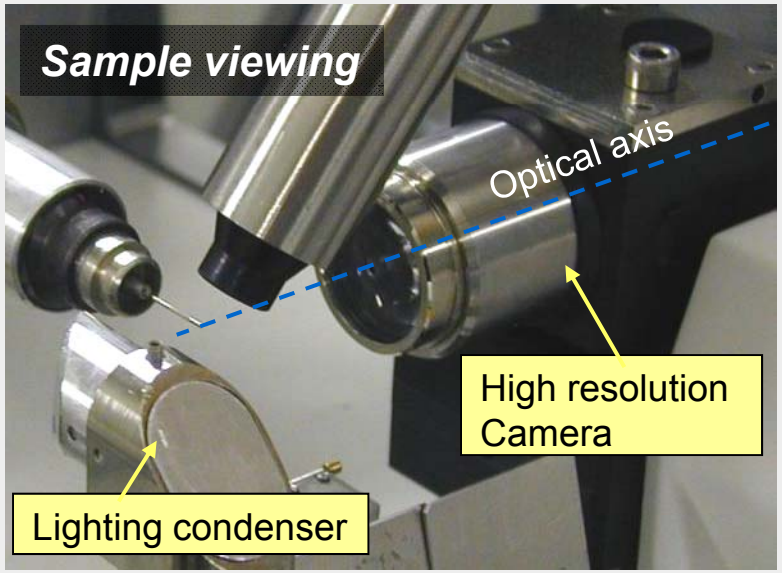
Needle position[2] 2 turns

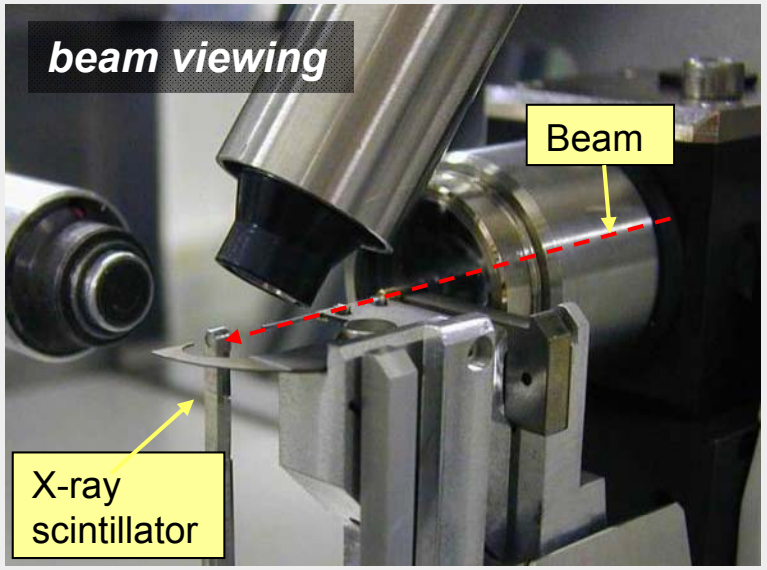


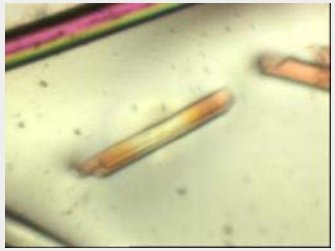
[2] Glass needle observed with a microscope (125 nm/pixel) + sub pixel interpolation + Image computing

The MD2 – On beam axis video-microscope

*High resolution **On Beam Axis** video-microscope* **Patented**







12µm needle

Real view of the collimated beam

No parallax error → Perfect alignment with the beam

*The **MD2** micro-diffractometer*
to get the best data from the crystals

BUT… not all the crystals are good…

Before automation

*An ordinary day on a beamline… **Screening** crystals*

Changing a Crystal:

…Opening the hutch

Unmounting the previous

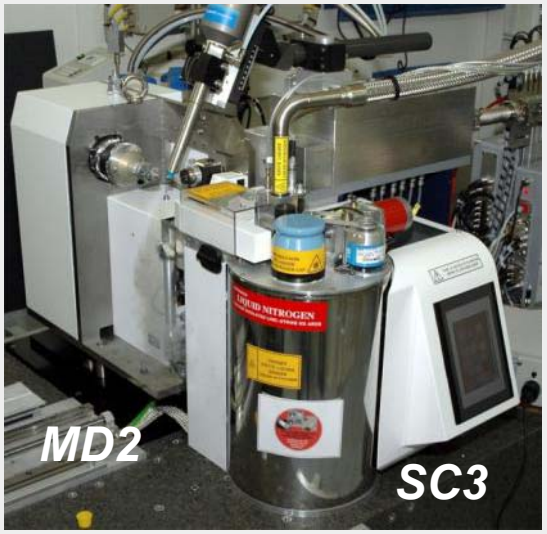
Mounting the new one

Aligning it

Closing the hutch

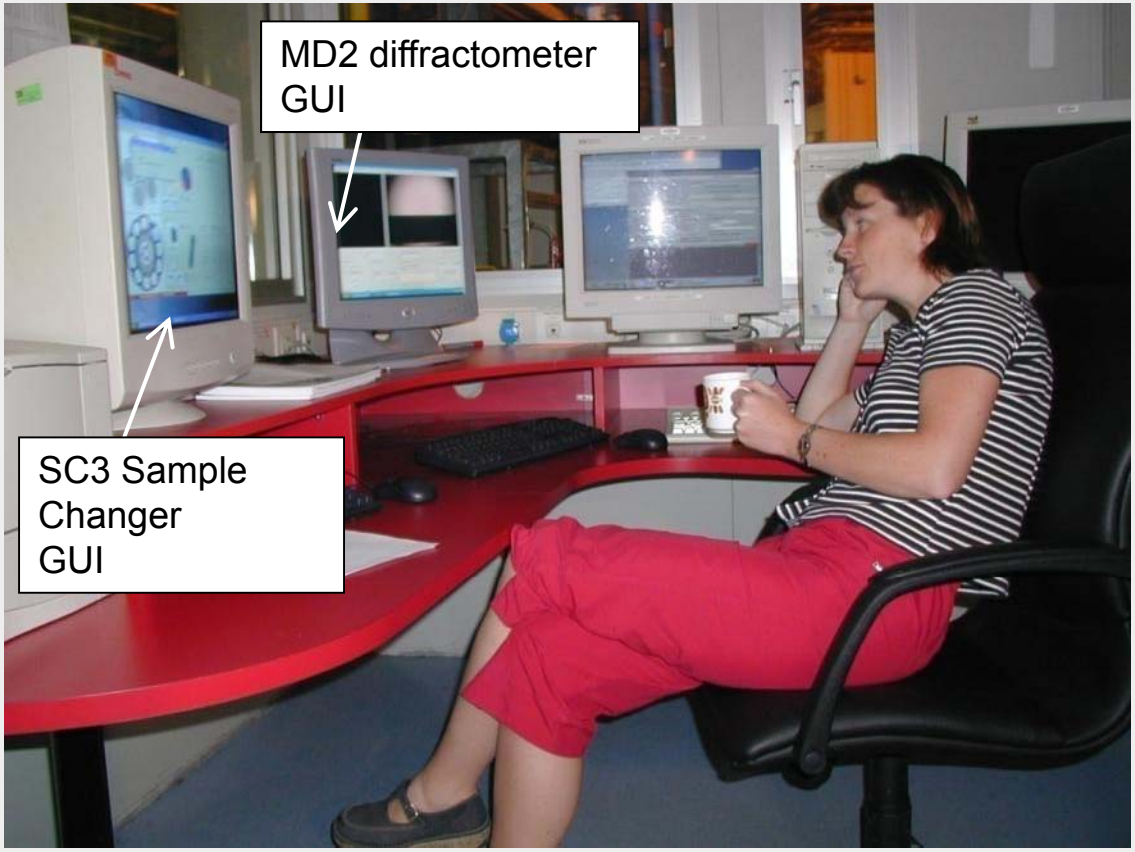
Starting data collection…

*An ordinary day on a beamline! …**Screening** crystals…*



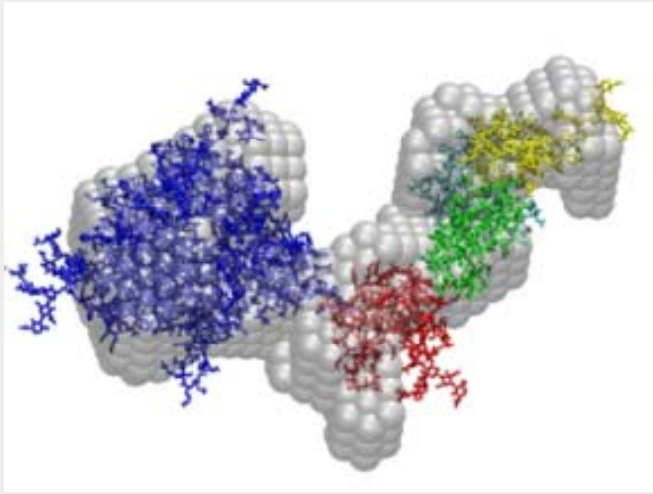
Automatic screening

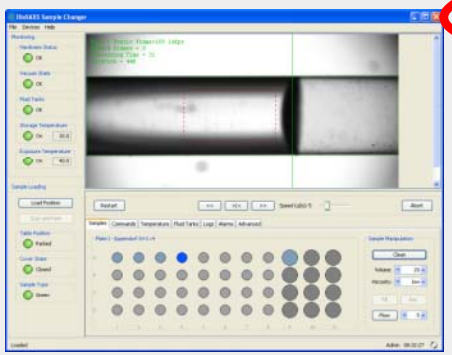
- **50 samples** in 2H30'



… And also thanks to C3D , the automatic crystal centring software developed by Bernard Lavault

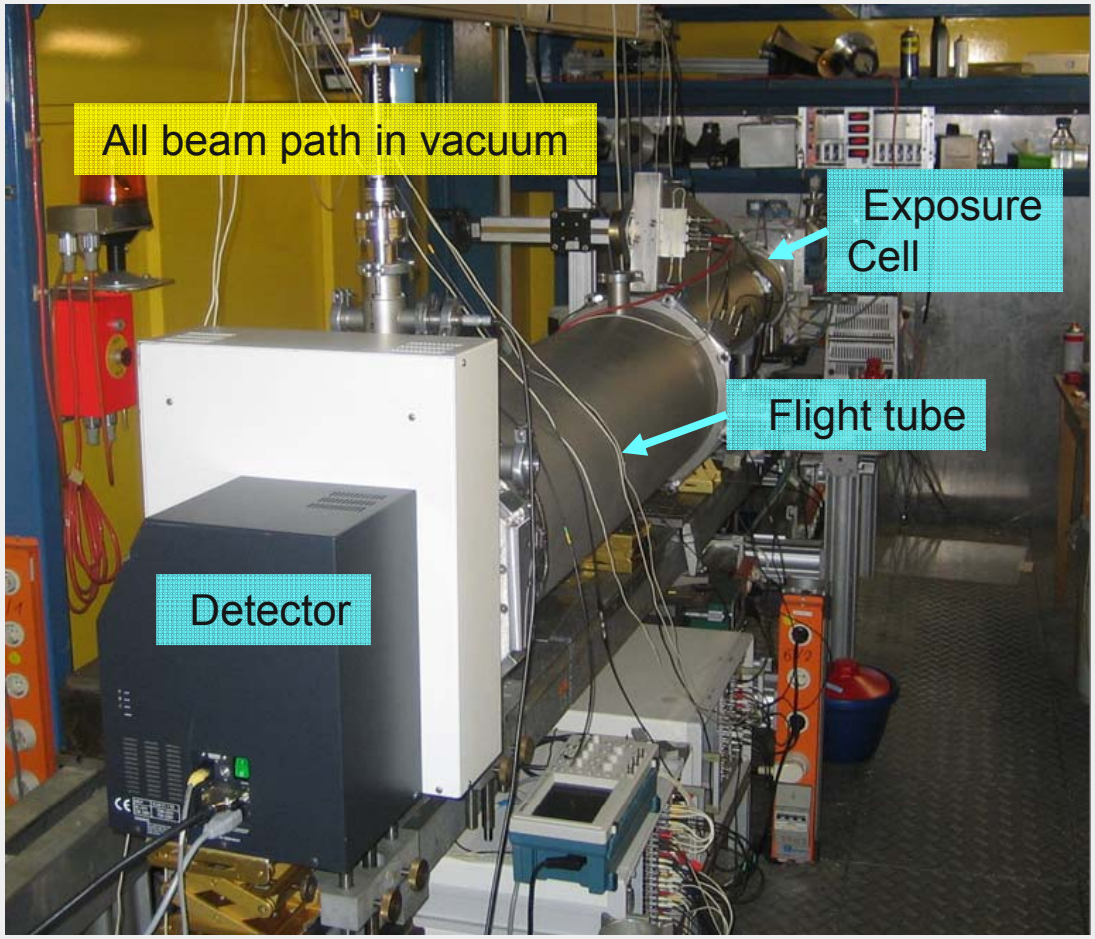
Instruments for Small Angle Scattering experiments



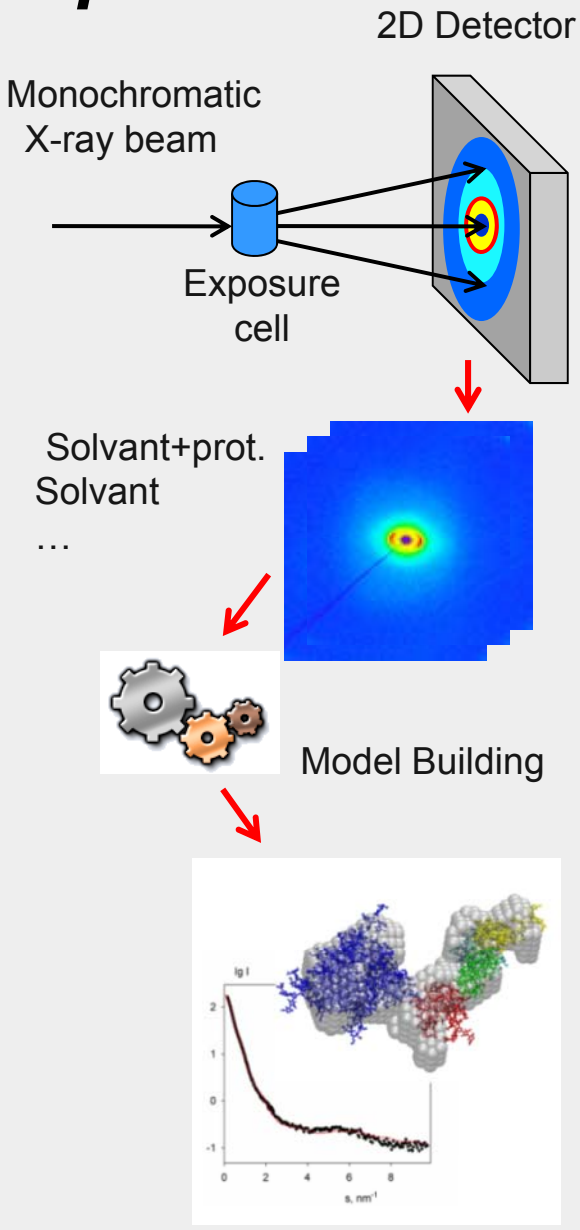


- Sample in solution
- Large macromolecules, assemblies (complexes)
- Kinetics
- Resolution is limited to 10 Å (Up to 500 nm)

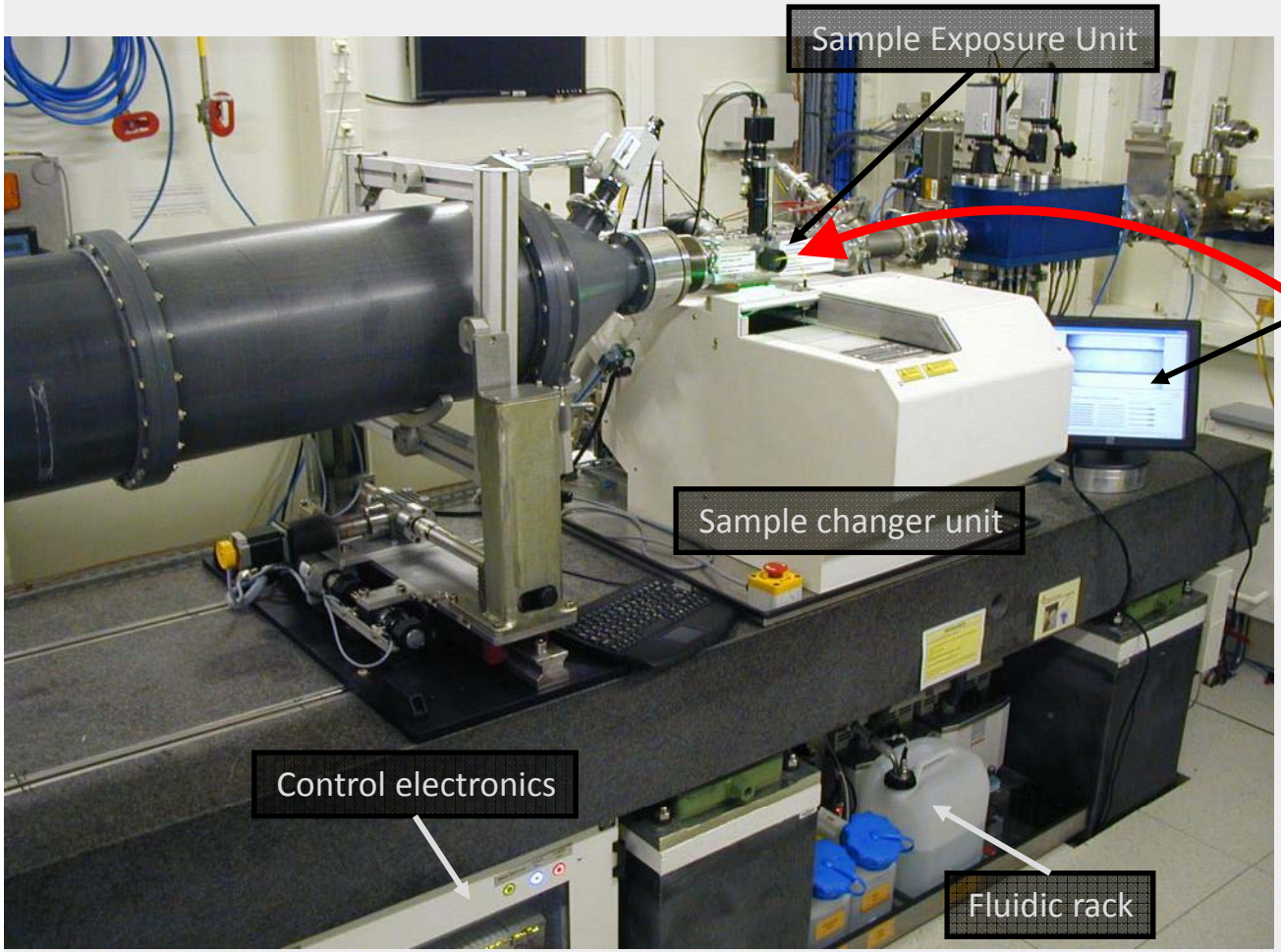
BioSAXS Typical experimental setup

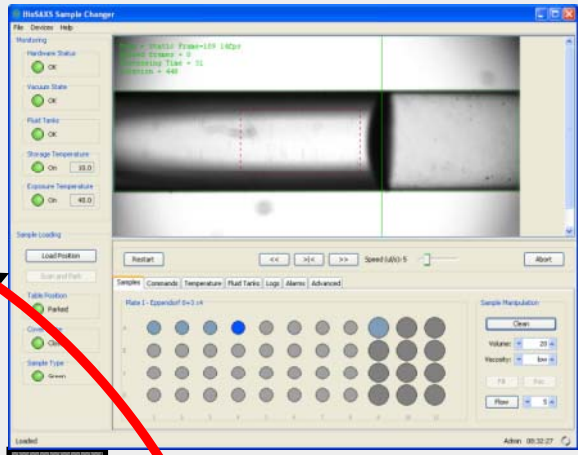


X33 beamline EMBL-HH/Doris *D. Svergun, M. Roessle*



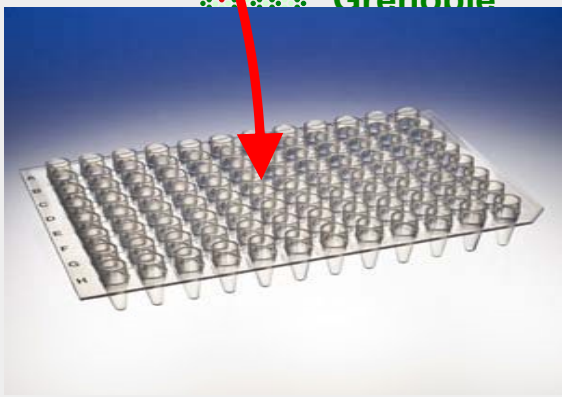
BioSAXS Sample Changer at ID 14-3



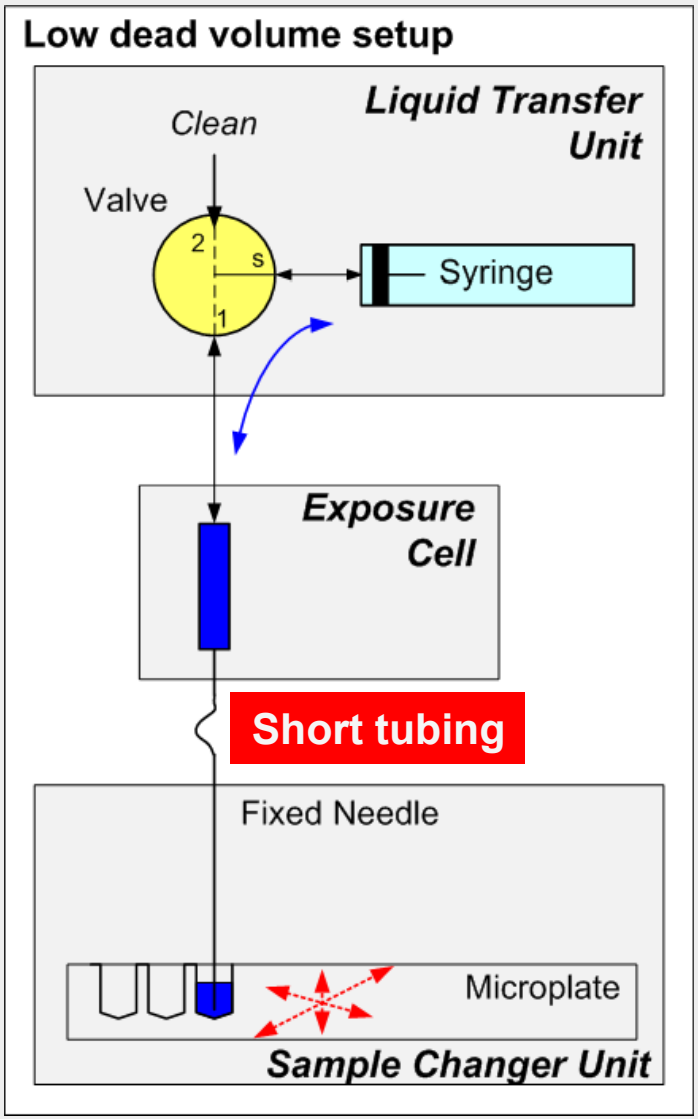


5-200µl

EMBL Grenoble



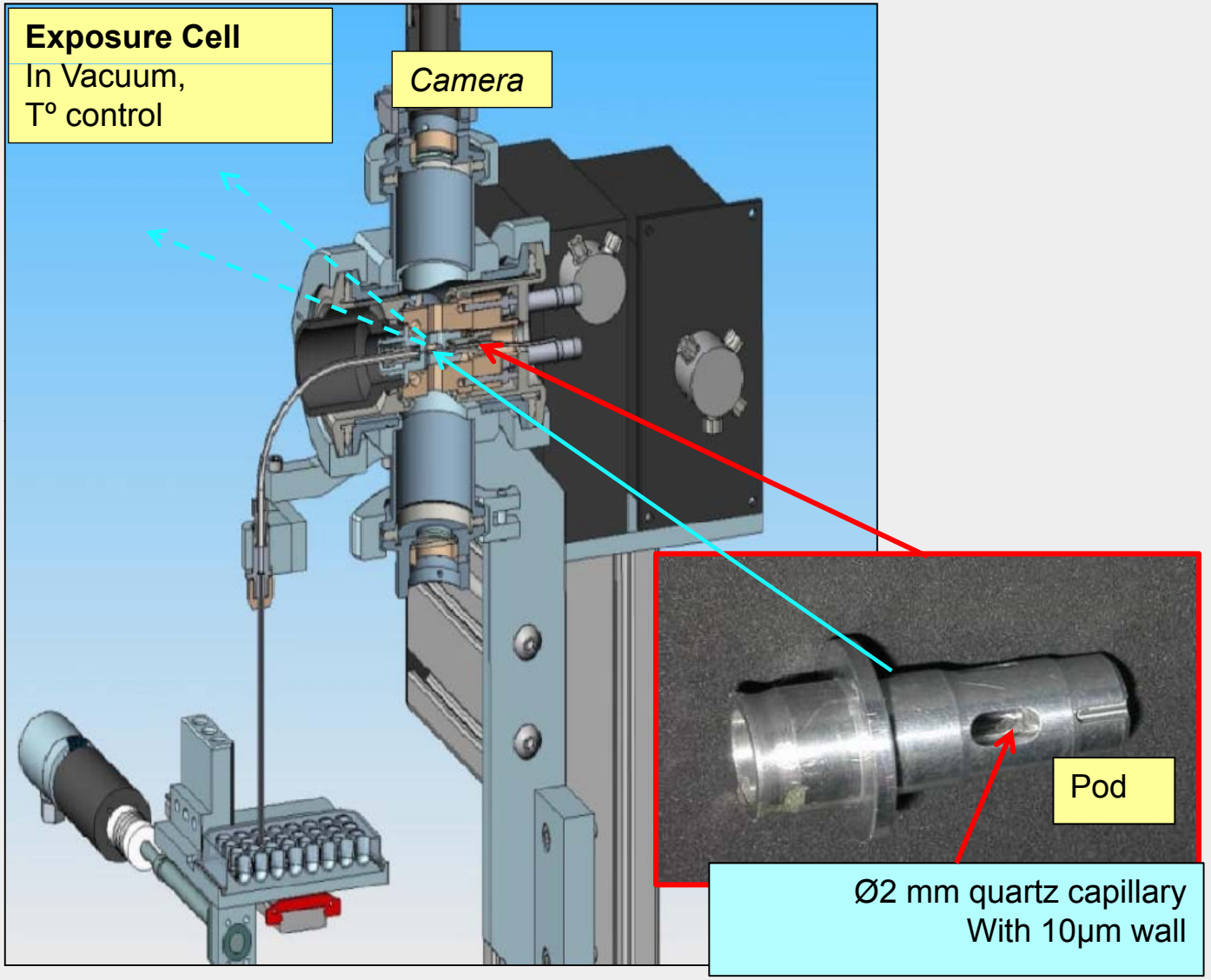
A design for low volumes & high speed



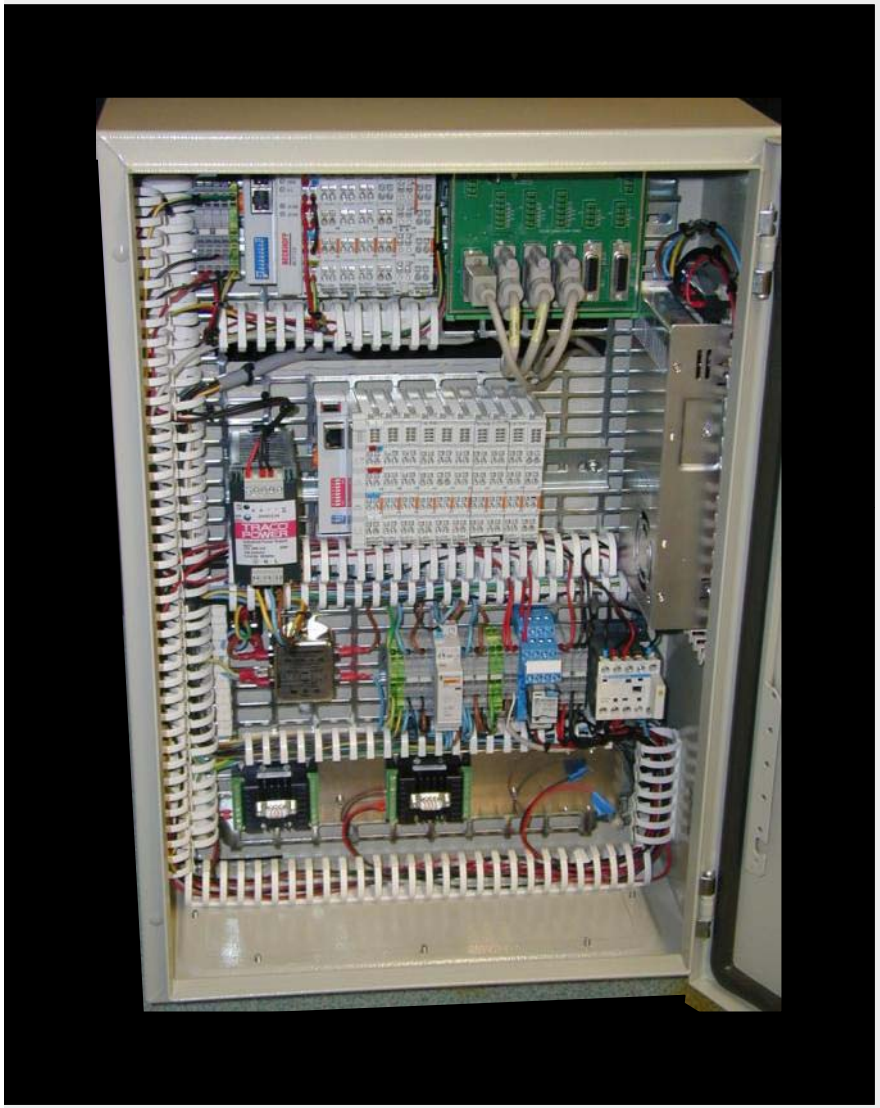
Challenging design

- Two separated units
- Pipetting needle is fixed
- Microplates are moved to the samples

BioSAXS Sample Changer – Sample Exposure Unit



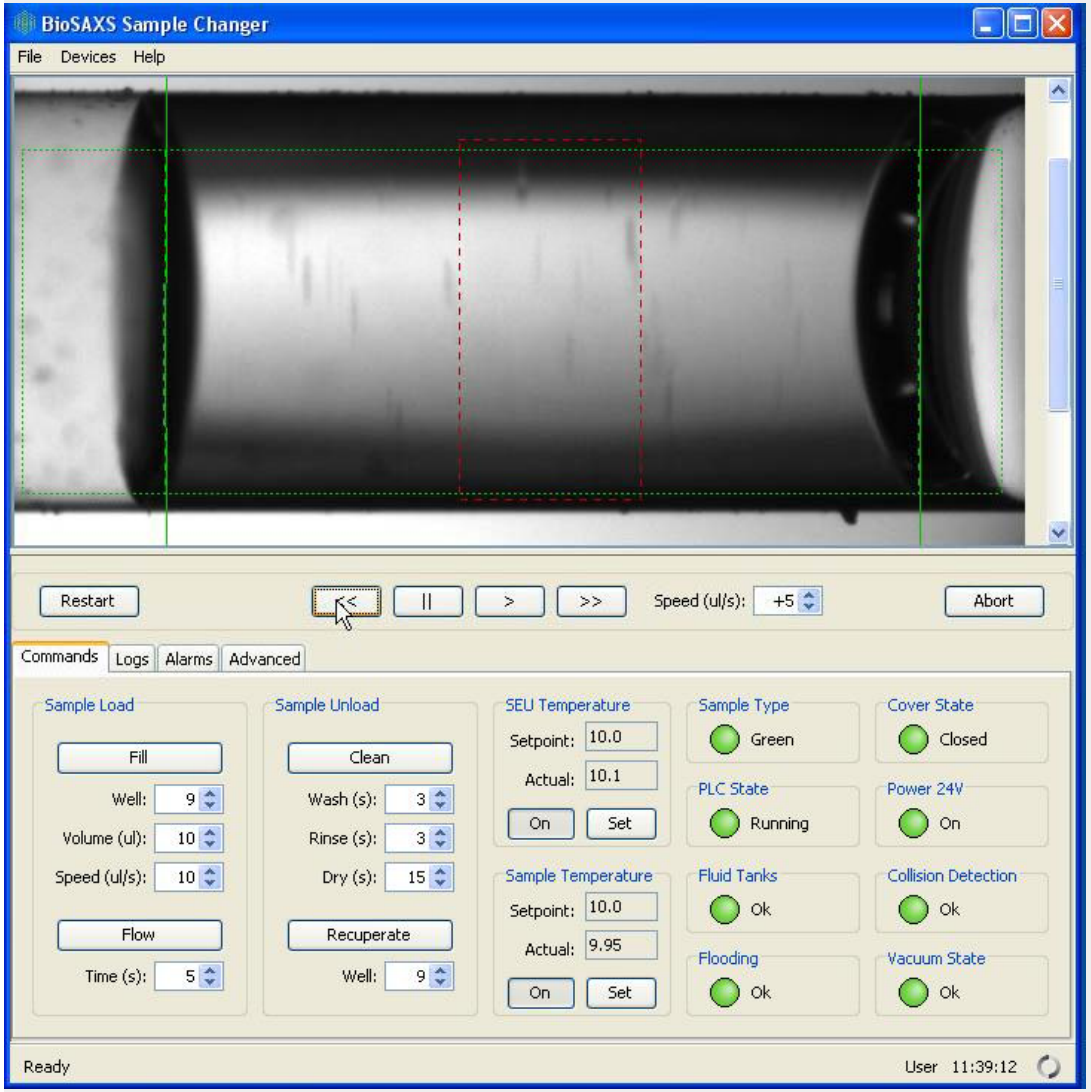
Control electronics



- Field bus electronics ***EtherCat*** *(Beckhoff)*
- **PLC and** et **motion control:** ***TwinCat*** real time layer in a **Windows PC**

EtherCAT

Control software



Design

- Core & GUI Written in **Java**
- Process scripted in **Phyton**
- Image computing **C++/Java**

- **Full remote control**
  - High and low level control Socket + Libraries
  - **Tango** device server (ESRF)
  - **Tine** device server (EMBL-HH)

Load Sample

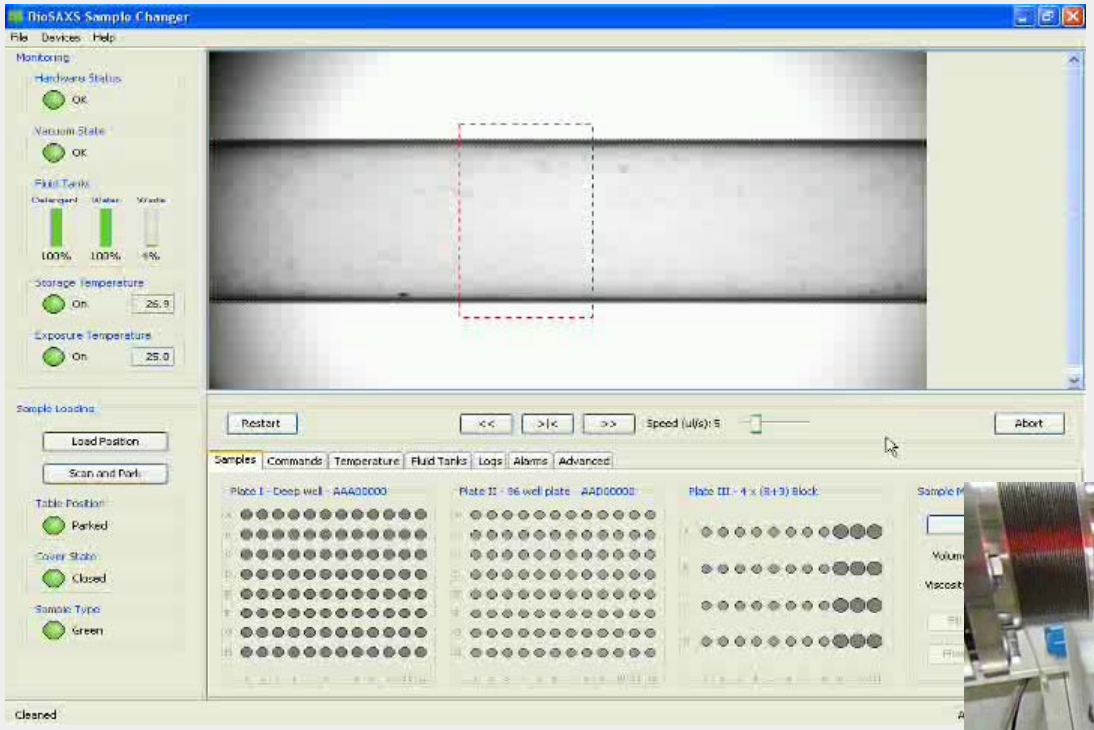
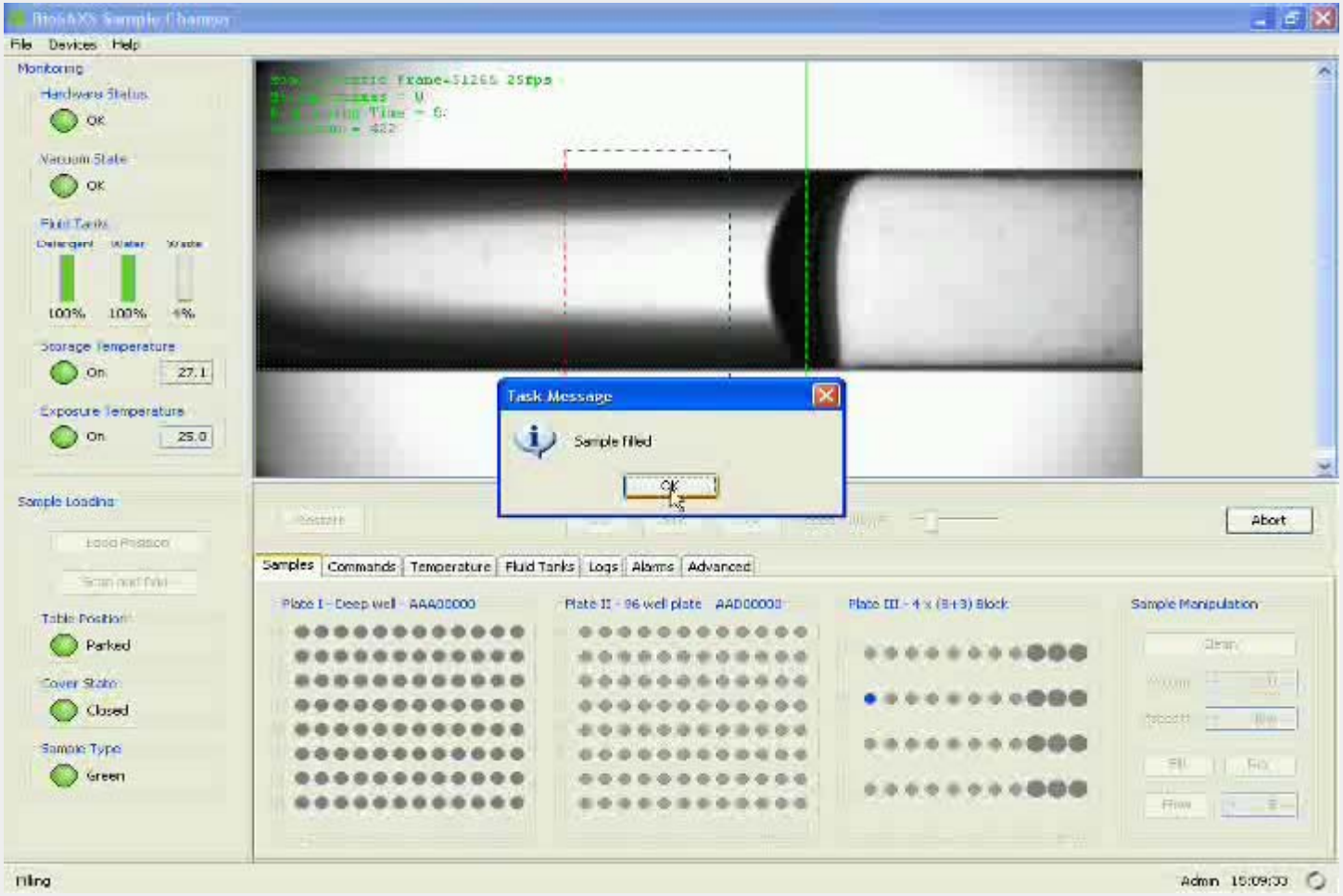


Table is moving in sample aspirated exchange

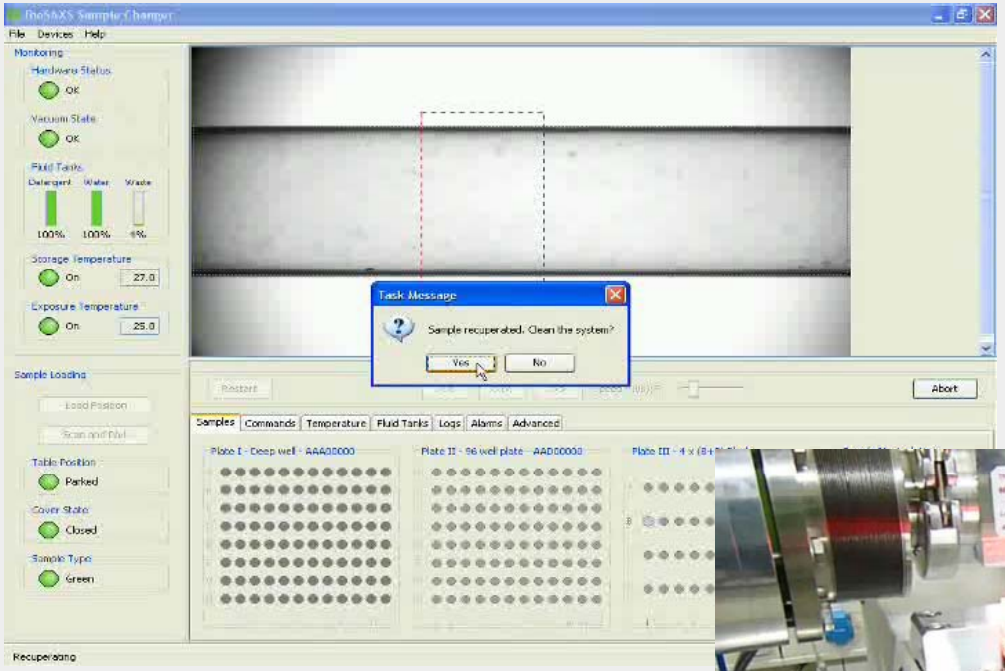
Sample arrives in field of view: positon controll takes over

Flow sample During exposure to X-rays



Optimal use of solution to reduce radiation damage

Clean Needle, tubing and Exposure Capillary



Washing with detergent
Washing with water
Drying
Parking the cleaning station

BioSAXS Sample Changers today

- **Two machines installed**

  ESRF ID14-3, routinely used

  EMBL@PETRA-III, commissioning

- **One machine under construction**

  for Diamond Light Source

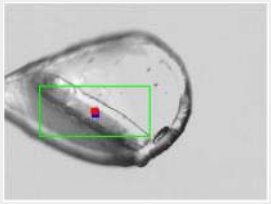
  (Maatel/Bruker)

Macromolecular Crystallography ***Crystal harvesting***

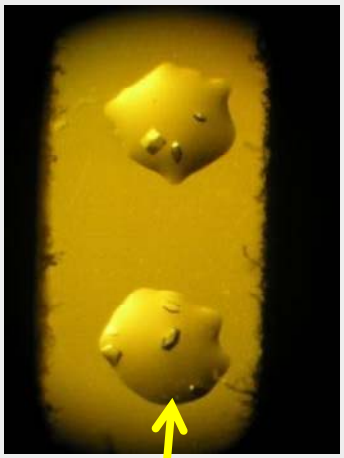
Crystallization is automated

MX beamlines are automated

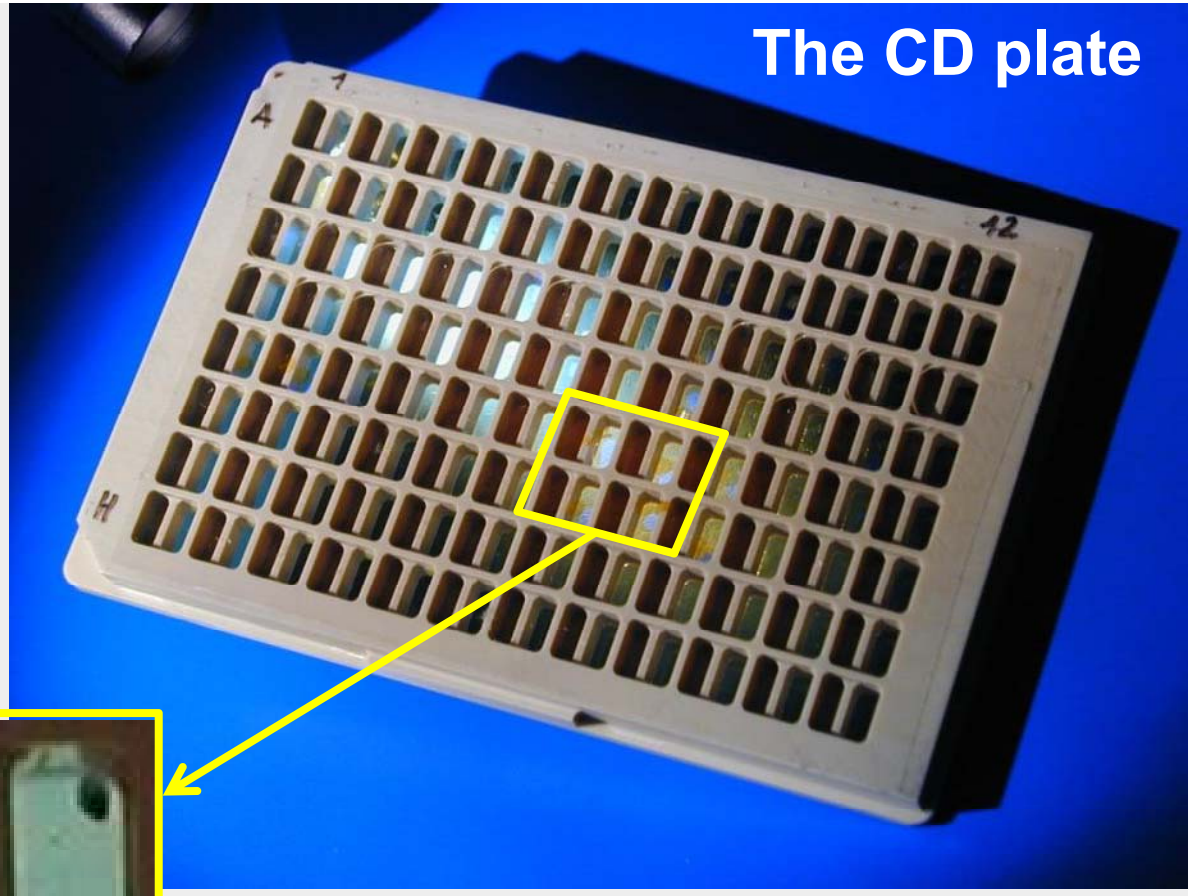
?

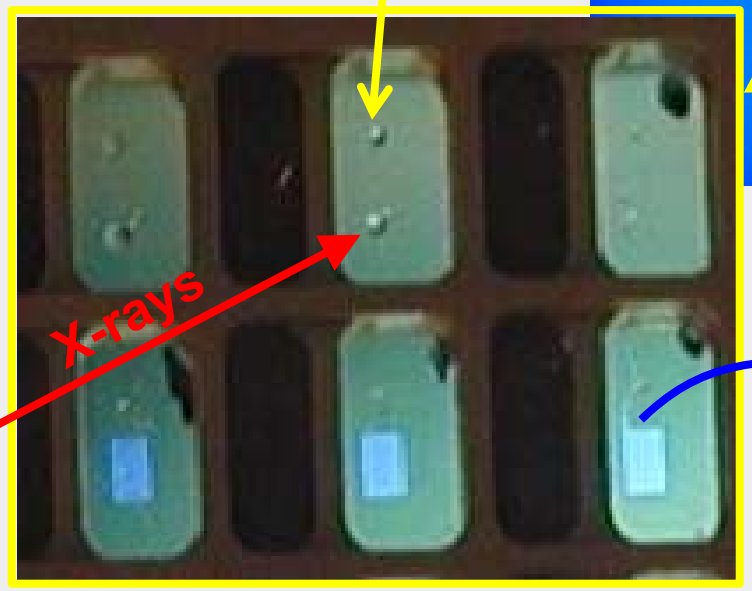


Crystal Direct

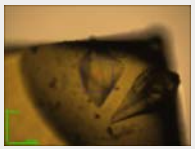


In-plate
crystal screening

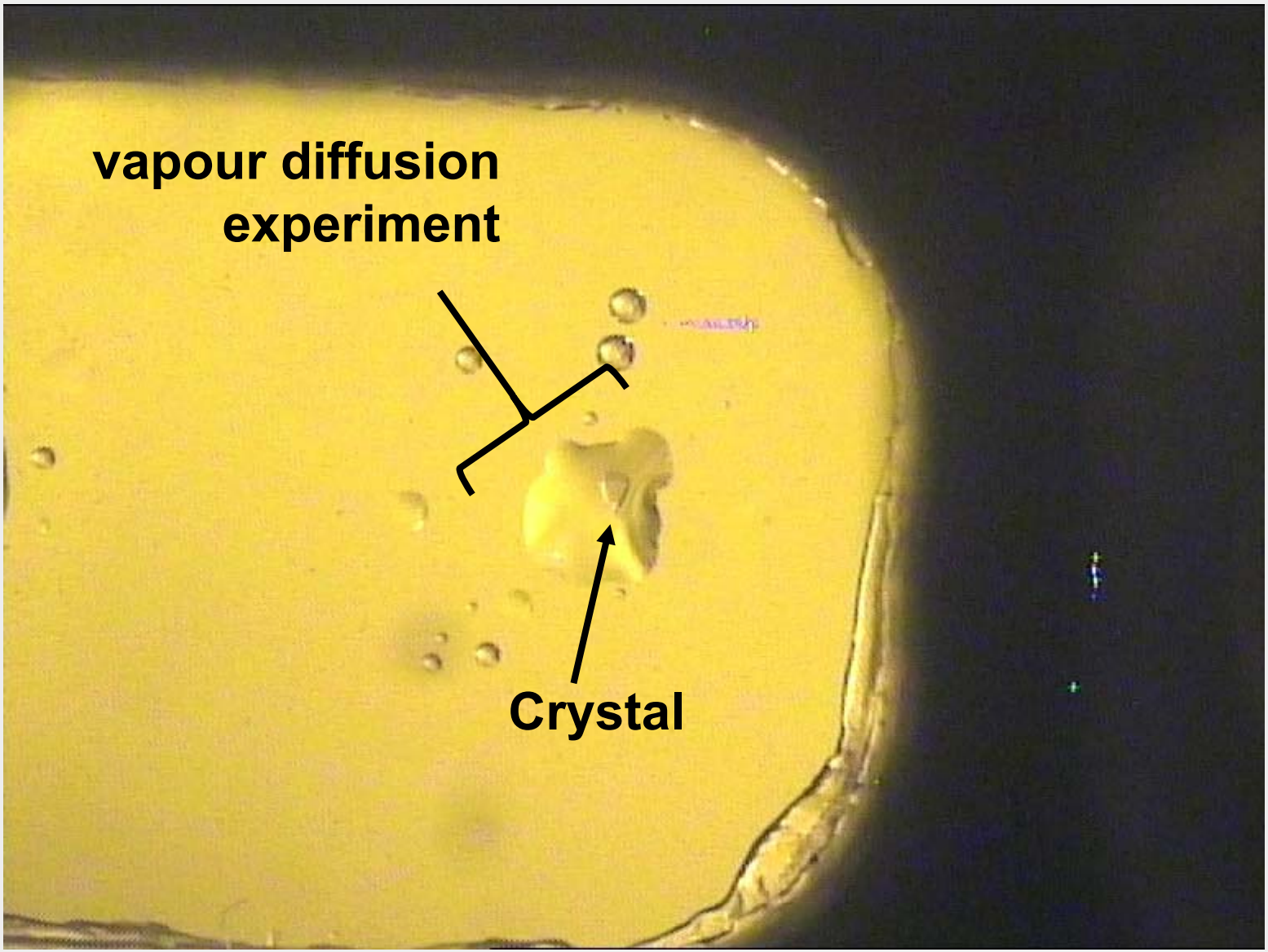




Harvesting by photo-ablation



Crystal Direct crystal harvesting (Photo ablation)

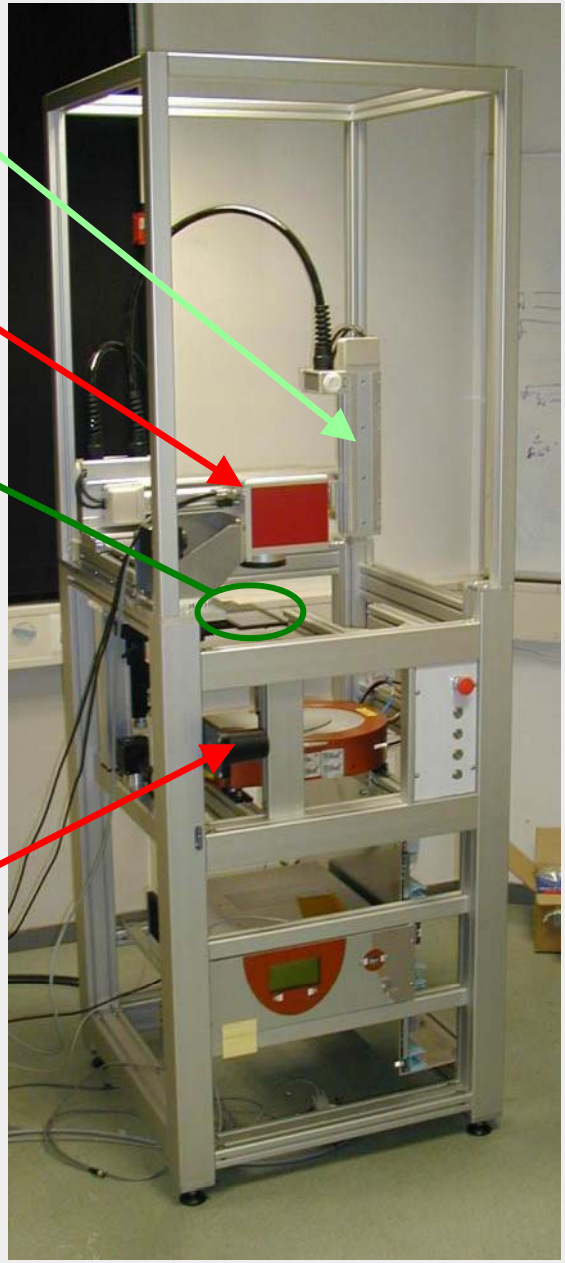


Cartesian XYZ robot

Scanning head

CD plate

fs laser



Crystal Direct
Harvester prototype

- Precision mechanics
- Optics (Laser, microscope)
- Robotics & Motion control
- Fluidics (gluing, pico-drops of cryo-protectant)
- Image computing (Laser Auto focus, alignment control)
- Low level automation (PLC)
- Process automation (Java/Phyton/C++)
- Communication (Device server, Databases)

Again a good mix
of physics, mechanics electronics and software!

Thank you for your attention!

Franck ***Felisaz***
Jerome ***Halbwachs***
Raphael ***Moya***
Alexandre ***Gobbo***
Gergely ***PAPP***
Julien ***Huet***
Christophe ***Landret***
Silvia ***Russi***
Florent ***Cipriani***