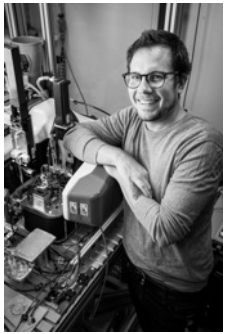
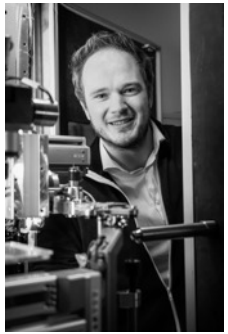
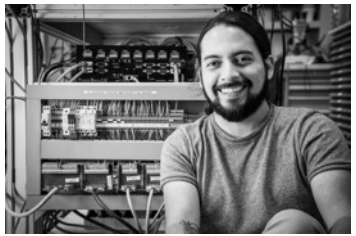
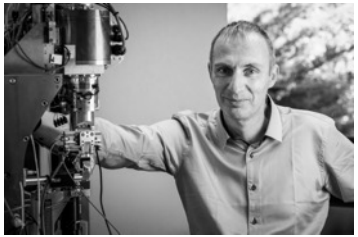
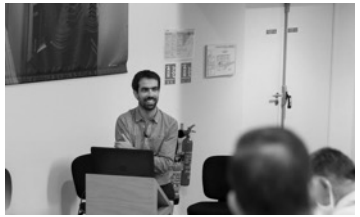
Instrumentation, Automation and Standards in Structural Biology

Gergely PAPP
Interim Head – EMBL Grenoble Instrumentation Team

EMBL

EMBL Grenoble Instrumentation Team - Focus

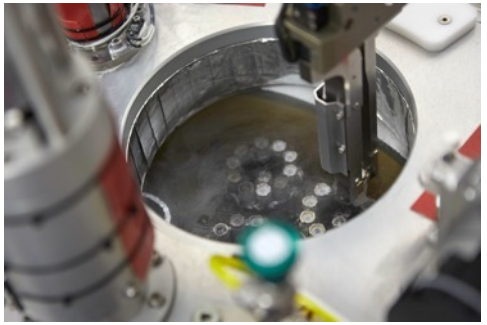

- Sample Environment of MX and SAXS beamlines
  - Diffractometers (20 years)
  - Automated sample changers (15 years)
  - Automated crystal harvesting CrystalDirect™ (10 years)
  - Sample holder standards & tracking solutions (15 years)
- Preparation of Cryo-EM sample grids (2017)

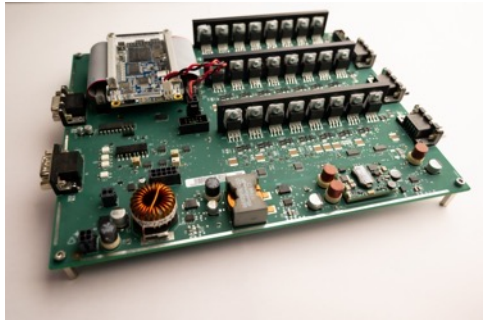
Our objectives

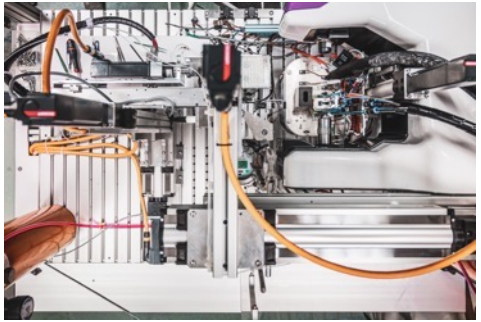
- Develop tools **from scientific demand** that are not available on the market
- Make them available for the entire scientific community by the bias of industrial partners
- Scientific collaborations are essential for our group:
  - EMBL-GR Crystallographers
  - ESRF Structural Biology Group
  - EMBL – Hamburg Crystallographers
  - EMBL – Heidelberg Cryo Electron microscopists
- As well as collaboration with industry
  - ARINAX
  - MiTeGen
  - MolecularDimensions
  - …

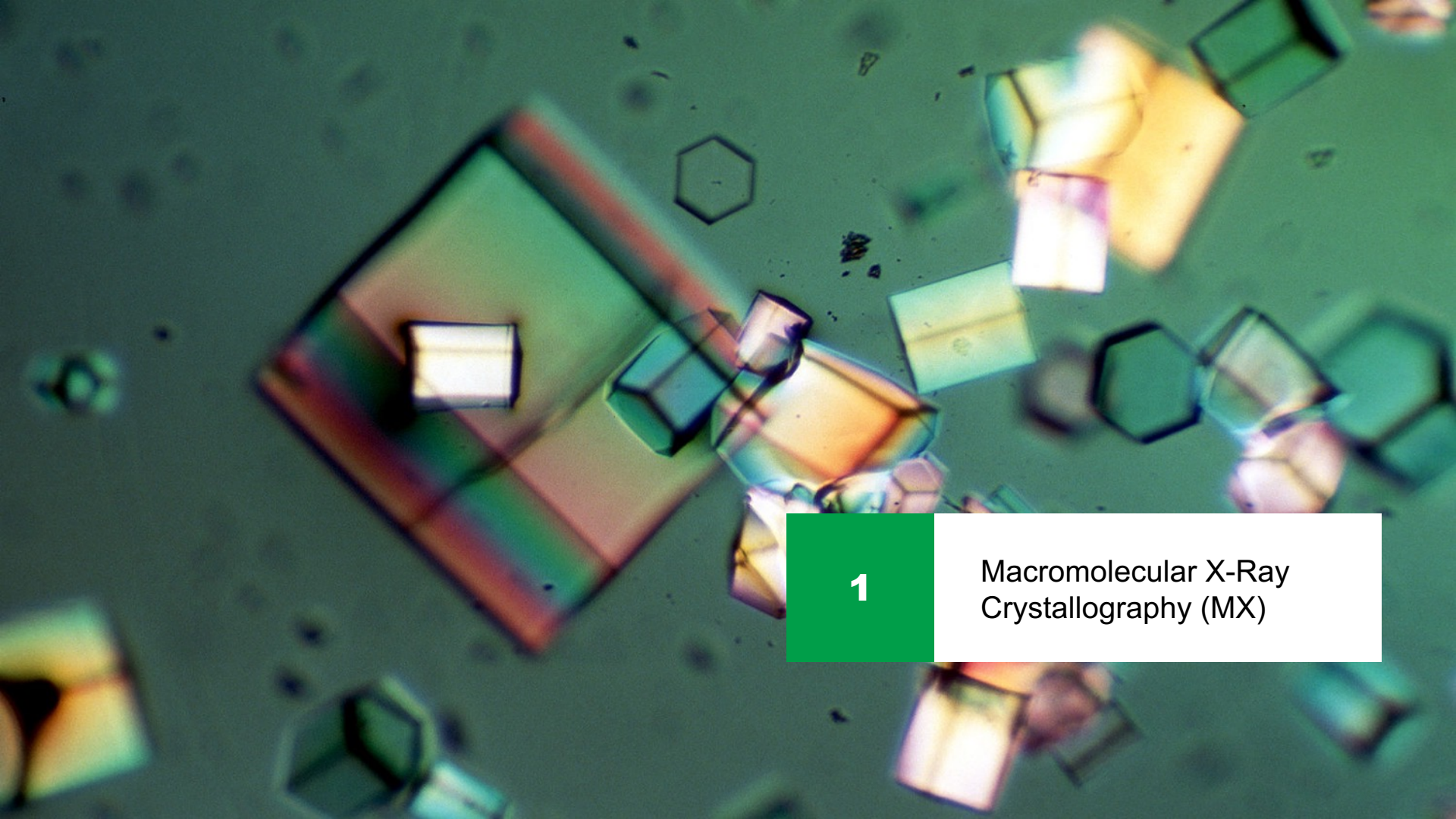
Expertise – System Engineering

- Precision mechanics
- Cryogenics
- Analog/Digital Electronics
- Motion control
- Software engineering



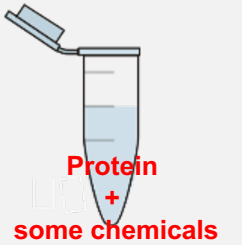


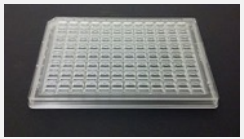




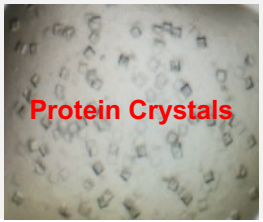
1 Macromolecular X-Ray Crystallography (MX)

MX in brief

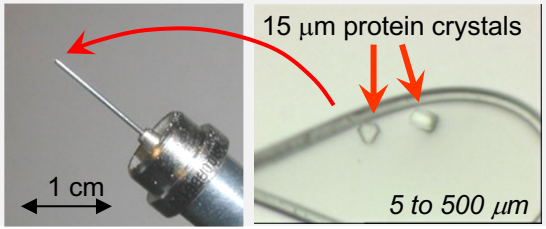




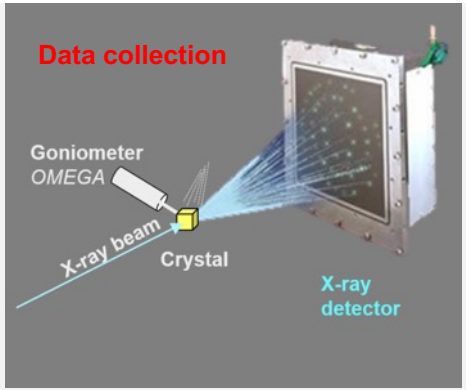
By chance



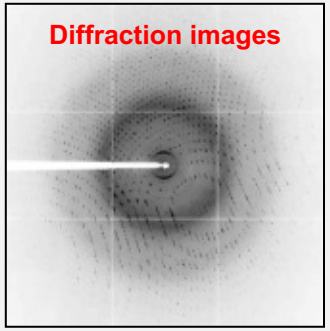
Crystal mounting



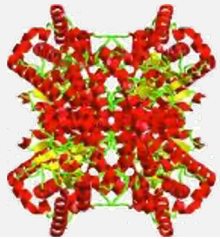
Sample handling



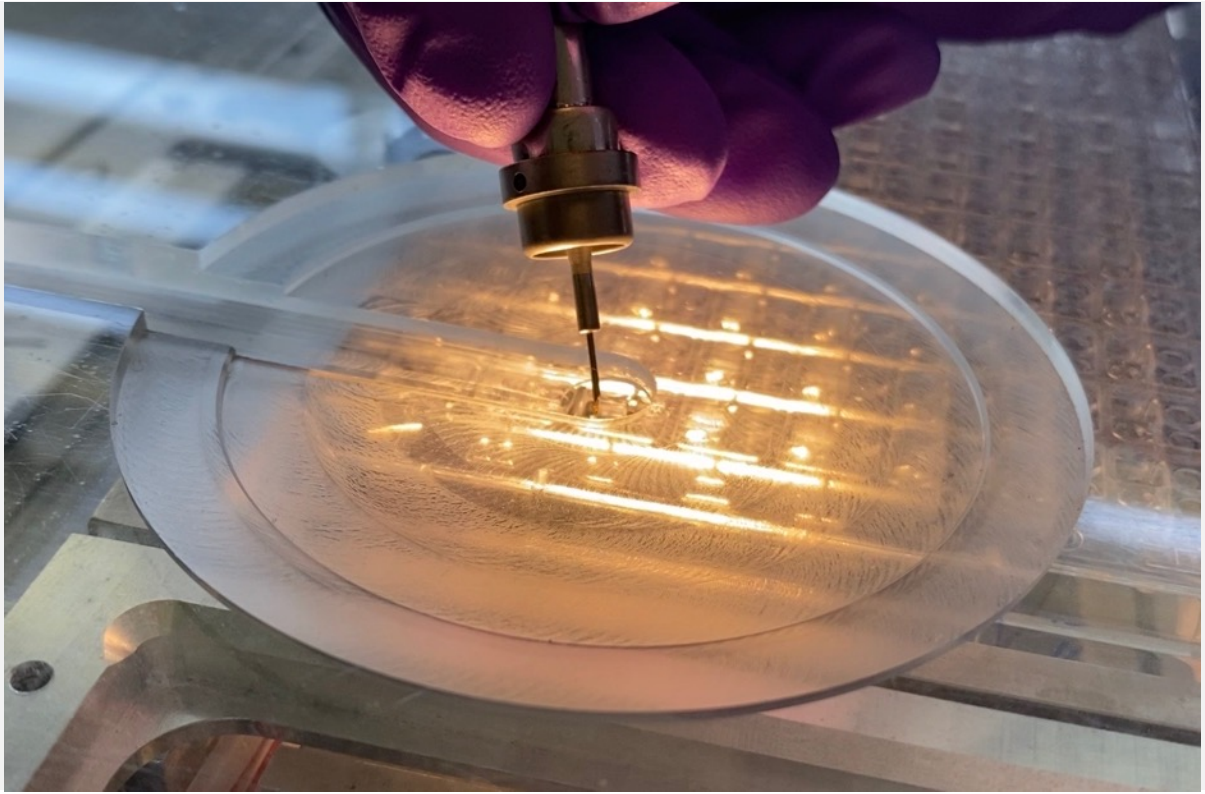
Hopefully



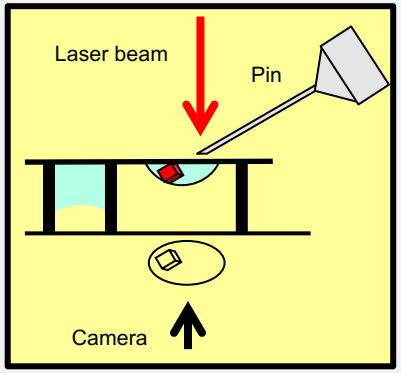
Structure



Crystal Harvesting – The traditional way

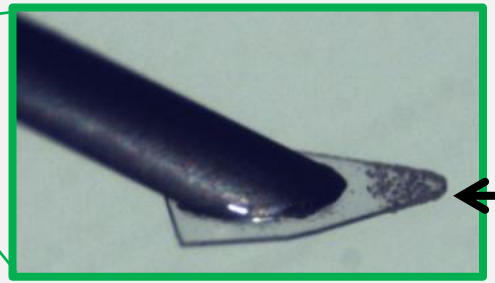


CrystalDirect: Harvesting by laser photoablation (2010)



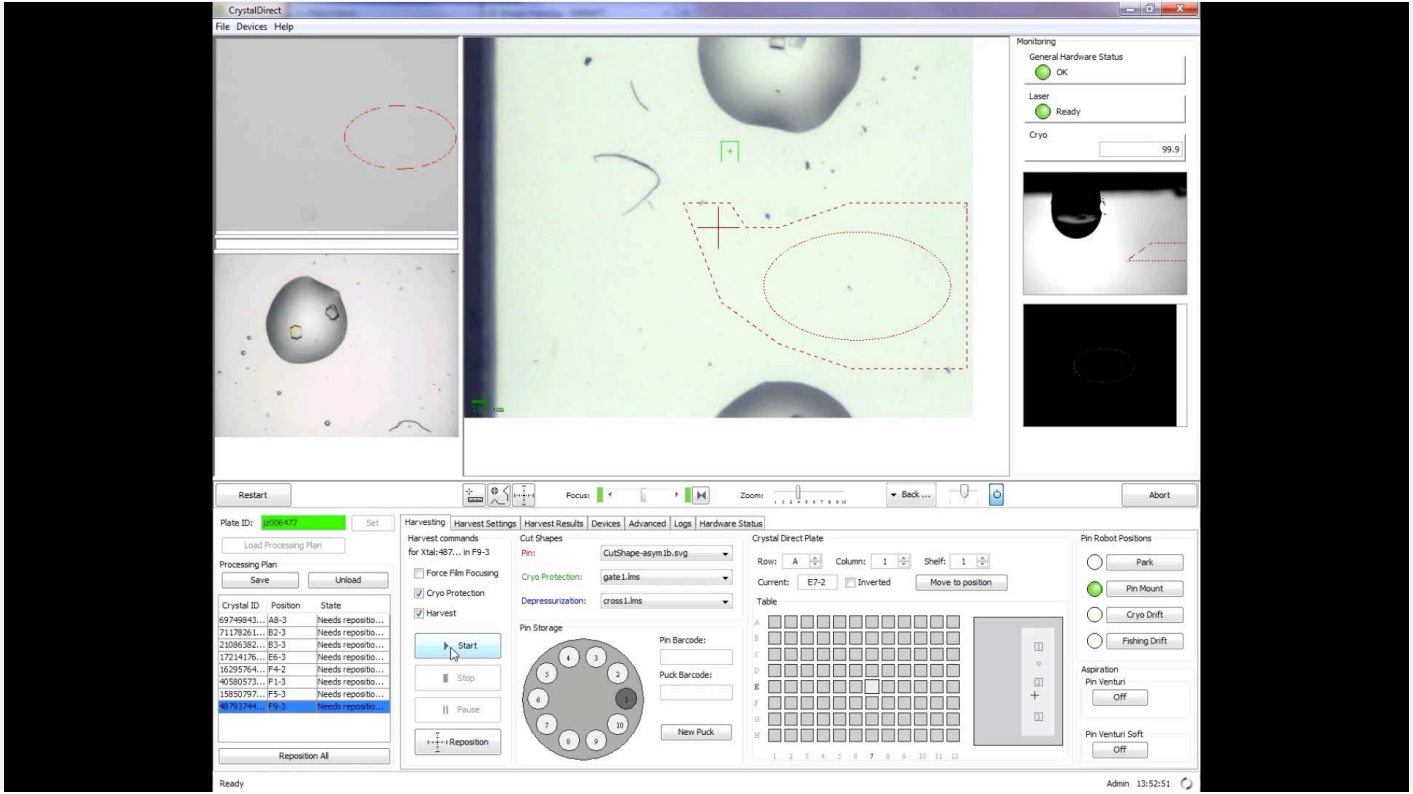
Film with crystal **cut** with a **femtosecond** laser beam

Automatically mounted on a **SPINE sample holder**



Crystal(s)

CrystalDirect : Harvesting video



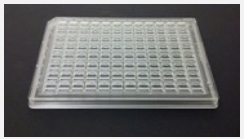
CrystalDirect machine family

CD1 Prototype – 2011
- Automated harvesting

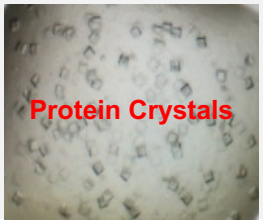
CD2 – 2016
- Pin can be oriented
- Coupled to a sample storing robot
- Commercial version available

CD3 – 2021
- Temperature regulation (4 – 20 degrees)
- Fast harvesting (< 1 min/crystal)

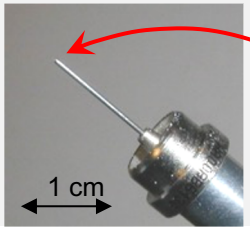
MX in brief

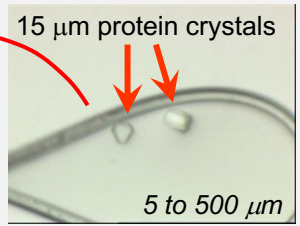


By chance

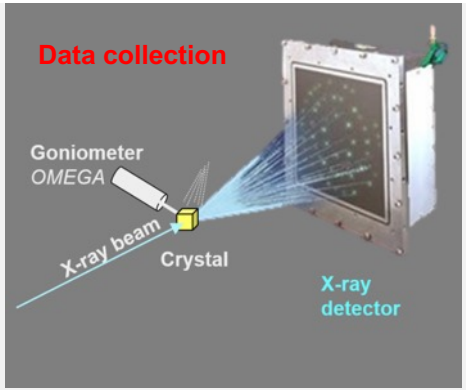


Crystal mounting

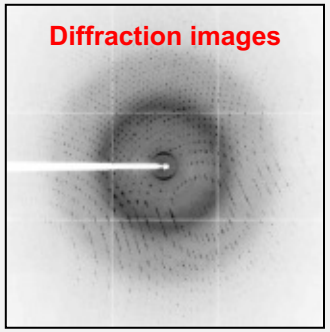




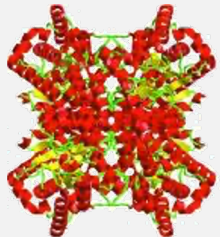
Sample handling



Hopefully

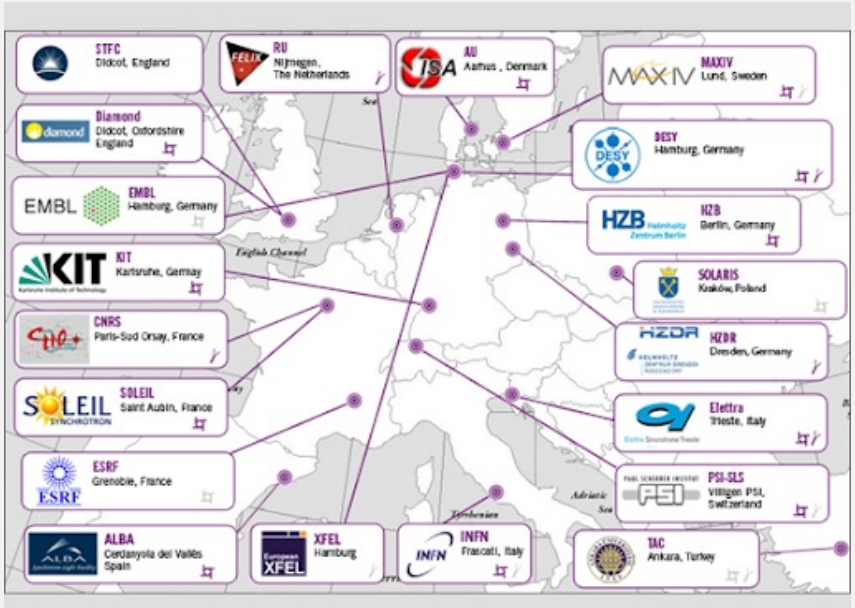


Structure



Sample holders

- Tens of synchrotron radiation facilities across europe
  - Samples can be collected at different facilities

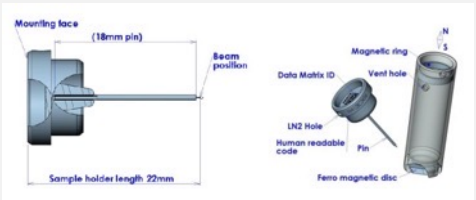


Need for

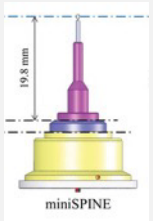
STANDARD (S)

Sample holder standards

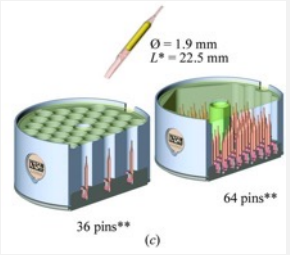
SPINE puck and pin standard – 2004
- Up to 10 samples in standard pucks
- *16 samples in unipucks*



miniSPINE sample holder and puck – 2016
- *Up 36 samples*

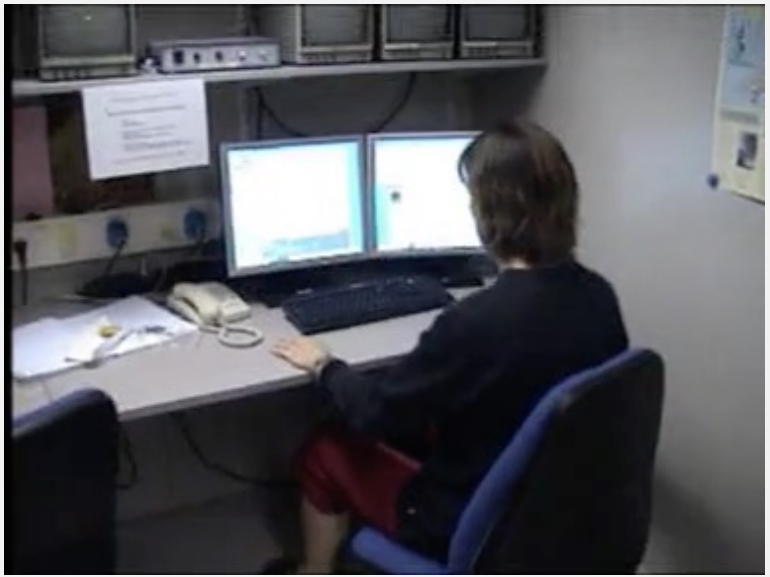


NewPin sample holder and puck – 2016
- High density & high repositioning precision
- Up to 64 samples in a puck

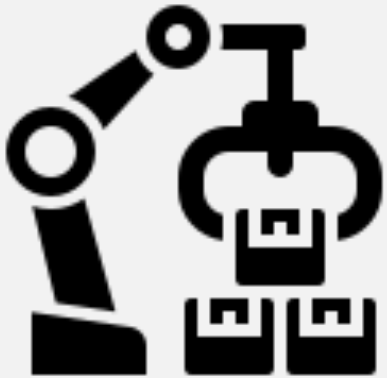


Sample handling on beamlines

- Synchrotrons compatibility
- How to handle samples on beamlines

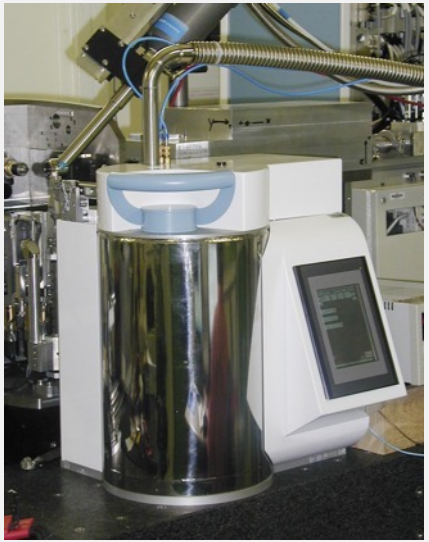


Automation needed



Sample changer robots

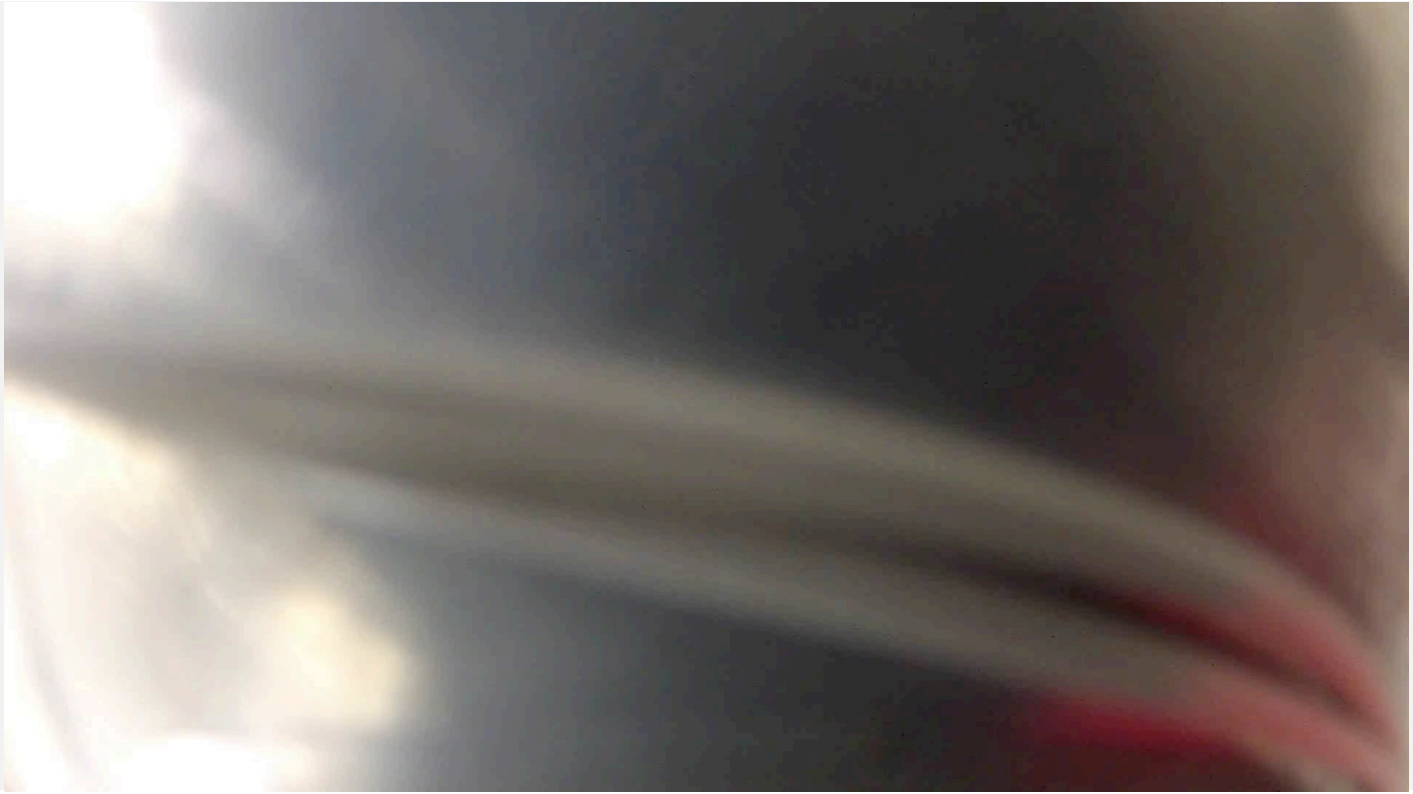
SC3 sample changer – 2006
- **EMBL / ESRF**
- Capacity: 50 samples



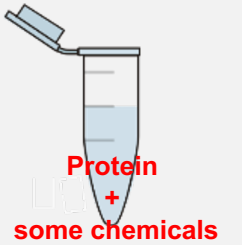
FlexED8 – 2013
- **EMBL**
- Capacity: 128 samples
- *Up to 288 with miniSPINEs*

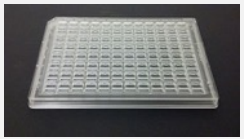
FlexHCD – 2016
- **EMBL / ESRF**
- Capacity: 312 samples
- *Up to 864 with miniSPINEs*

Sample loading video



MX in brief





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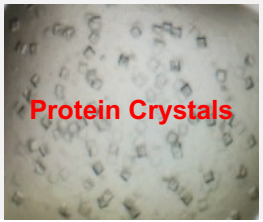
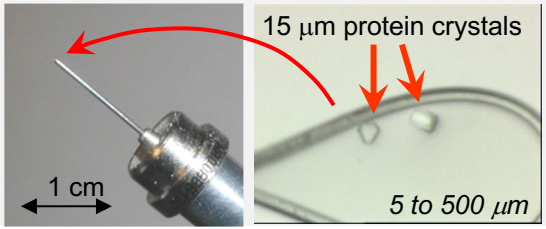
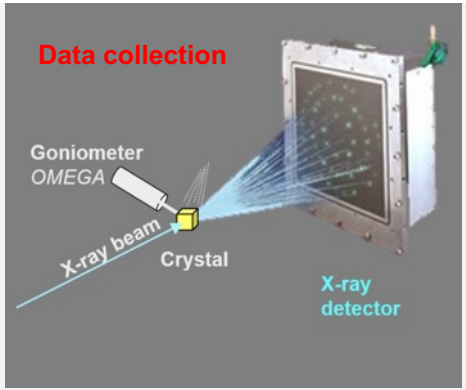


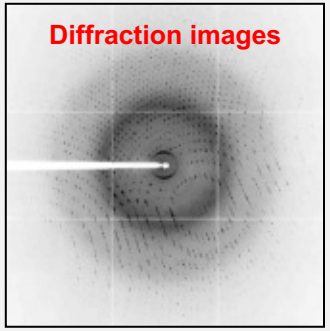
Crystal mounting



Sample handling



Hopefully



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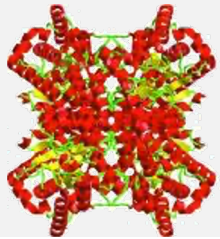
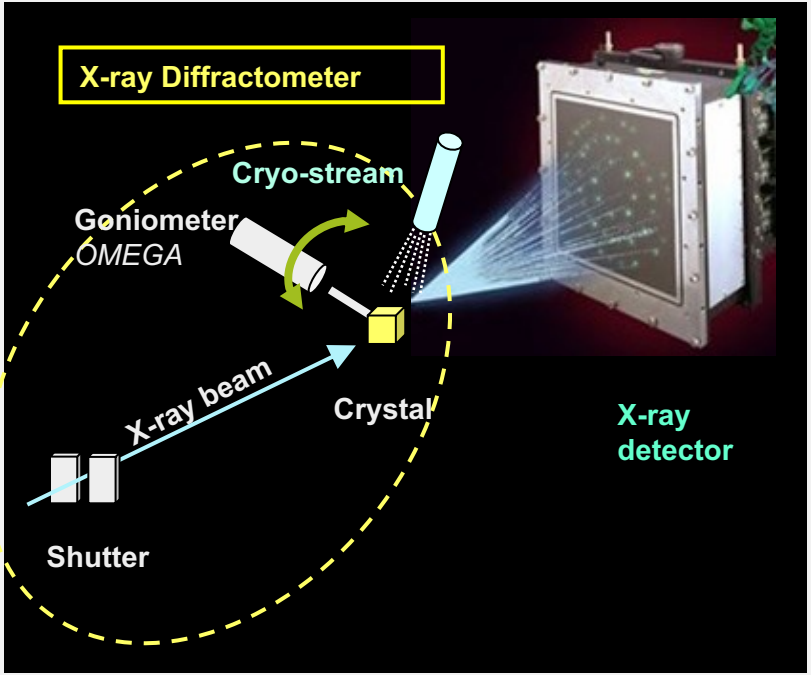
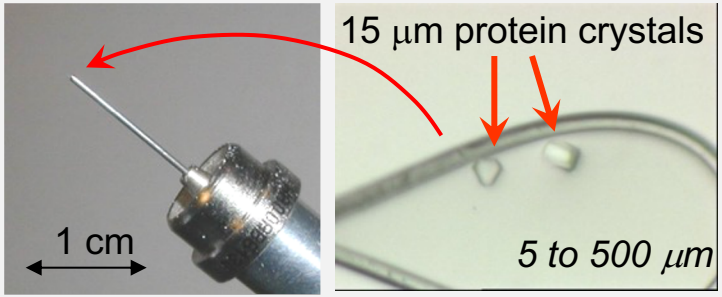


X-ray data collection at synchrotron

Typical experimental setup



Crystals mounted in a “cryo-loop”

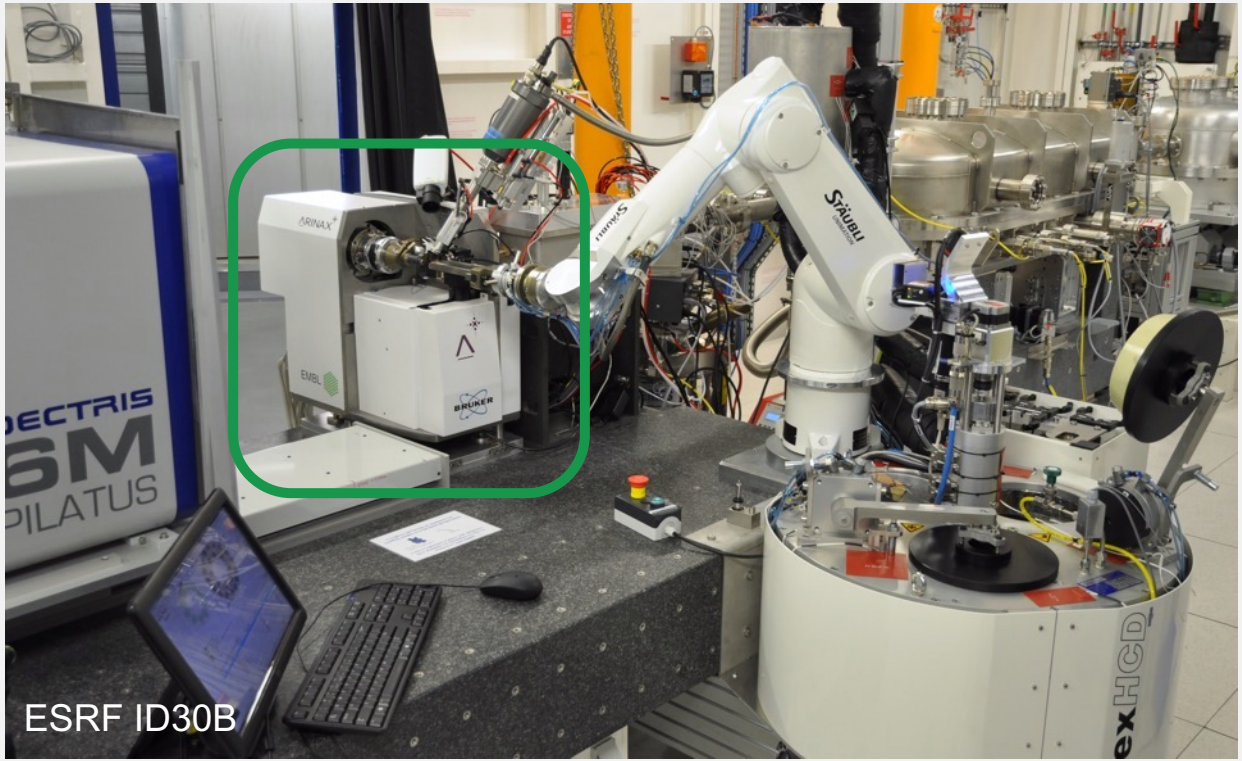


Collecting a data set

recording hundreds of diffraction images during ***angular Scans***

In minutes, seconds for the most intense beamlines

MX beamline: Diffractometers



MD goniometer family

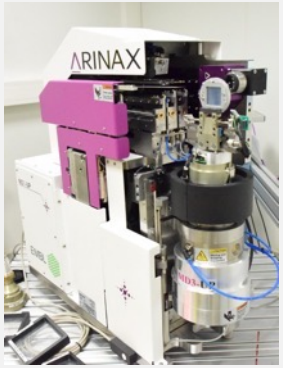
MD1 Prototype - 1998
EMBL GR / ESRF
Process 10 µm crystals

MD2 industrial version - 2002
EMBL GR

MD3 - 2012
EMBL GR / HH / Arinax
*Process routinely **1 µm** crystals*

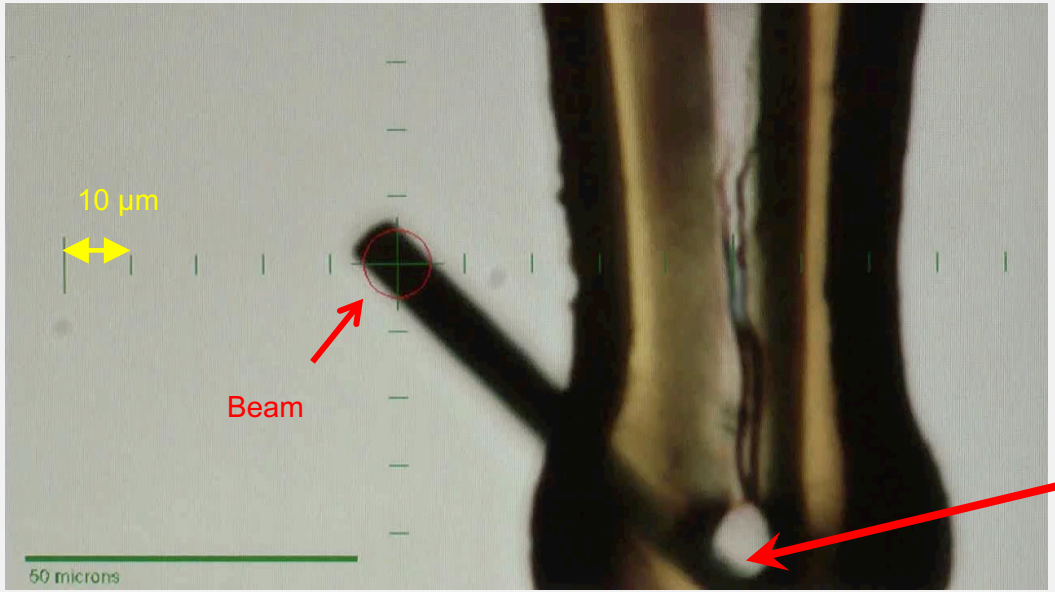
MD3up - 2016
EMBL GR/ Arinax
Easy sample changer access

MD3ssx - 2020
EMBL GR/ ESRF / Arinax
Serial data-collection on SSX chips



MD3 – High dynamic precision

- Video



Synchronous move

- OMEGA (rotation)
- Centring table (XY)
- Alignment table (YZ)

4D Scan along a 8 µm Ø needle - 180 deg.,10 sec

MD goniometer family

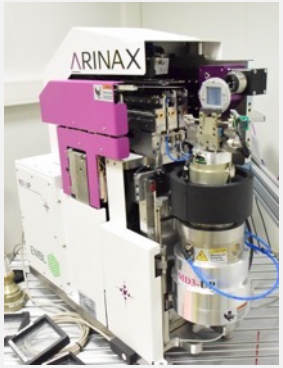
MD1 Prototype - 1998
EMBL GR / ESRF
Process 10 µm crystals

MD2 industrial version - 2002
EMBL GR

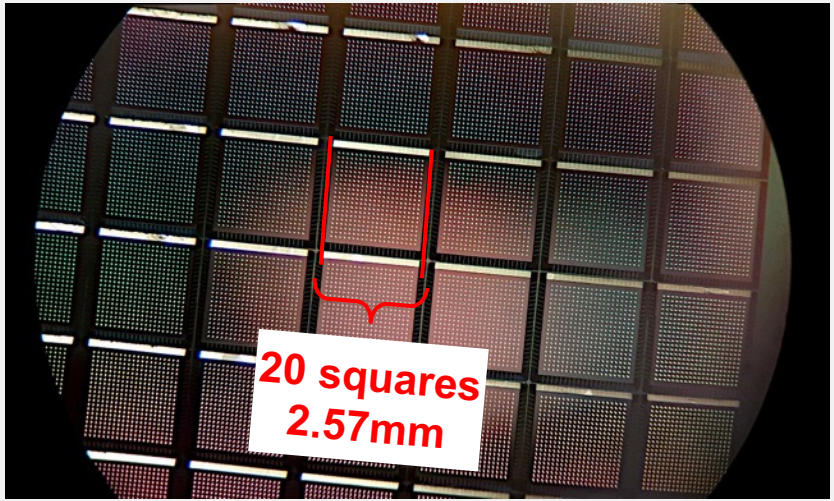
MD3 - 2012
EMBL GR / HH / Arinax
*Process routinely **1 µm** crystals*

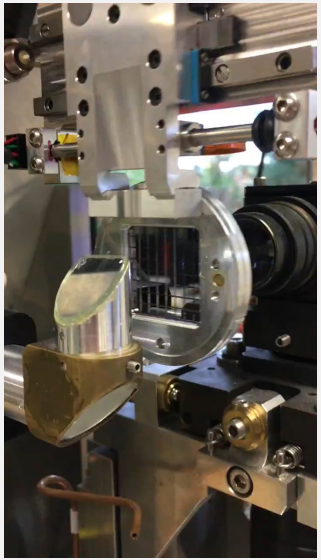
MD3up - 2016
EMBL GR / Arinax
*Process routinely **1 µm** crystals*

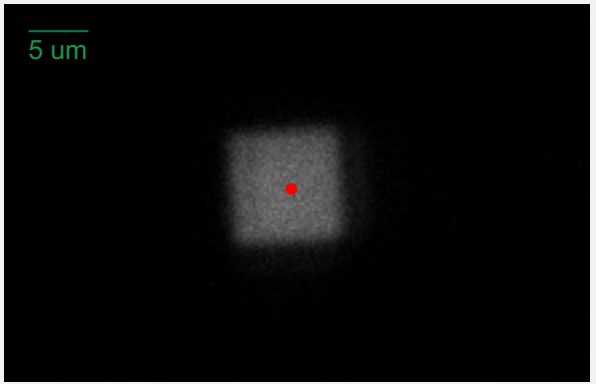
MD3ssx - 2020
EMBL GR / ESRF / Arinax
Serial data-collection
SSX chips



MD3 SSX – Chopper synchonized data collection on silicon chips



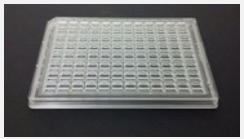




Captured at **20 mm/s**
Synchronized on **1kHz beam chopper**
Dynamic **positioning precision < ±1um**

MX in brief

Protein
+
some chemicals



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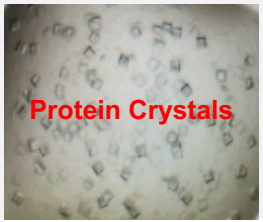
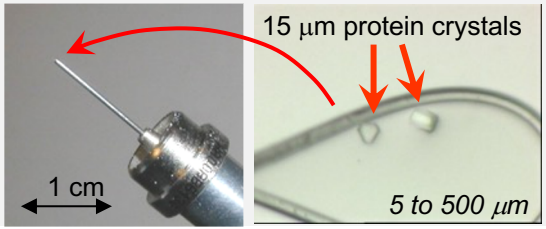
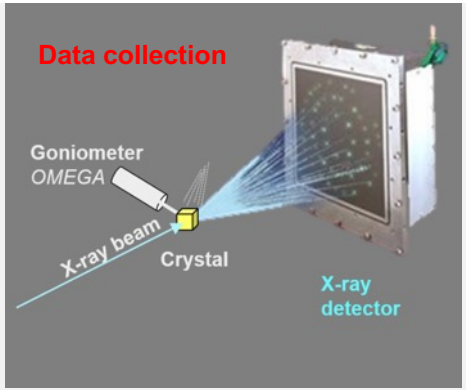


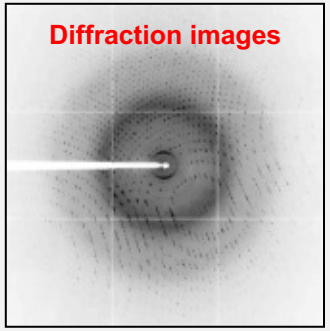
Crystal mounting



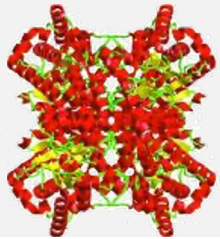
Sample handling



Hopefully

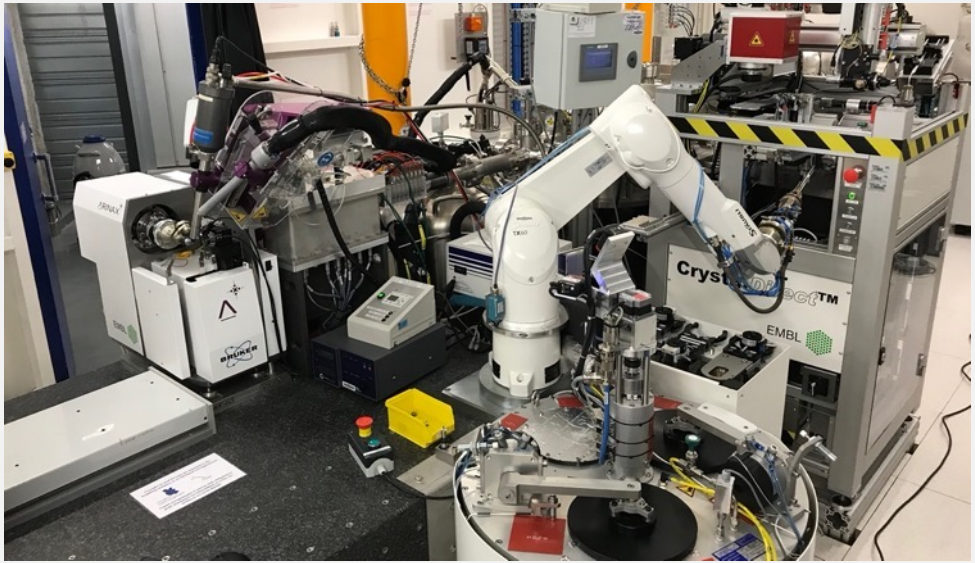


Structure

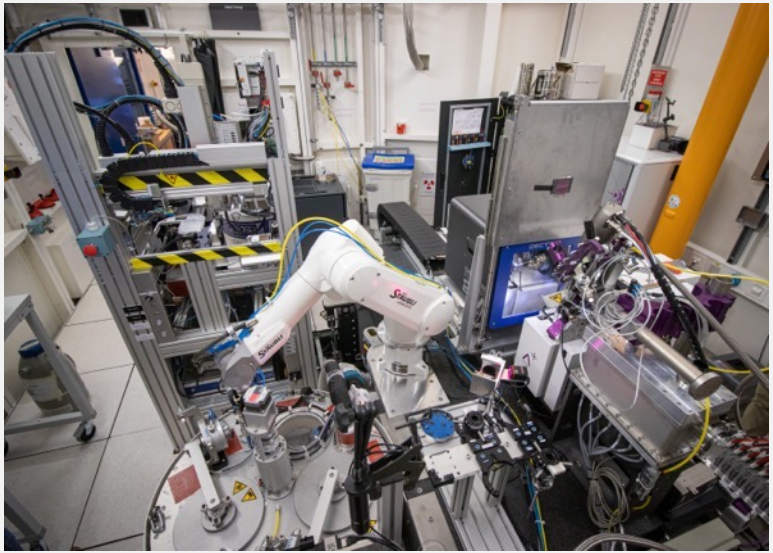


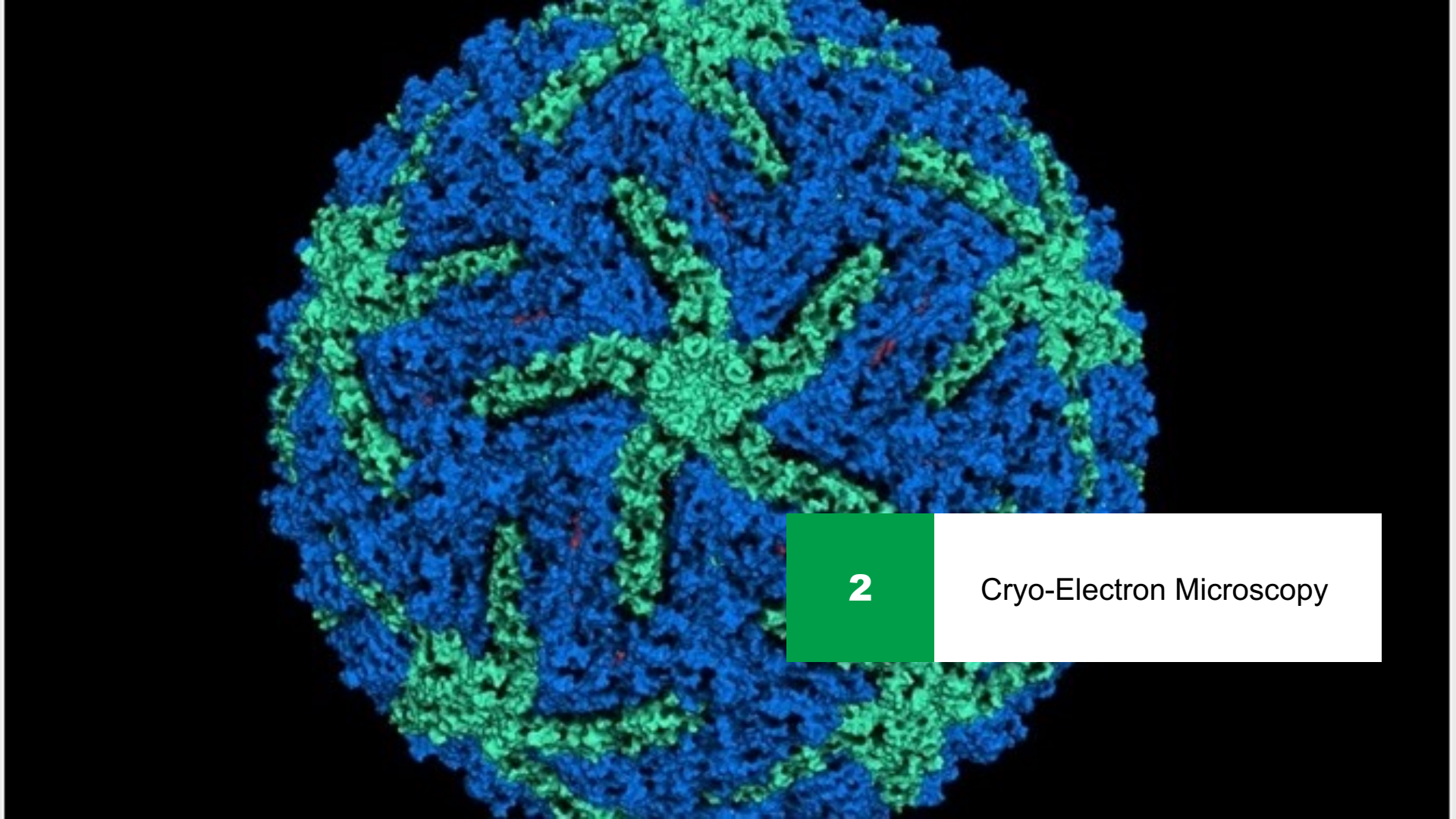
CrystalDirect to Beam
Fully automated Harvest & Collect pipeline

CD1 Prototype - 2017
Proof of principle at ID30B - 2017



CD2 – 2021 (ongoing)
EMBL / ESRF
Permanent installation on Massif1

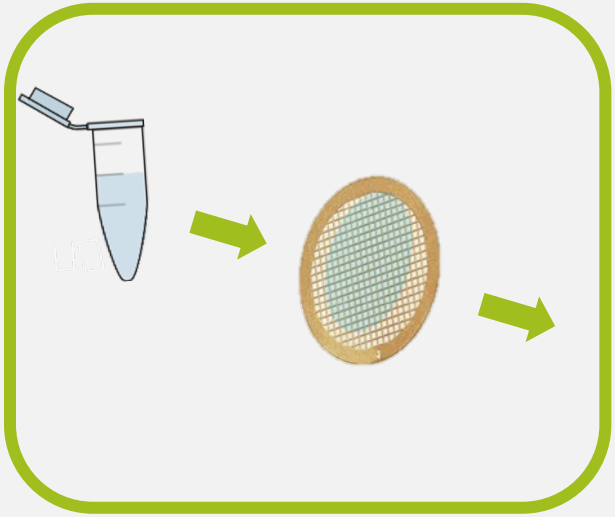




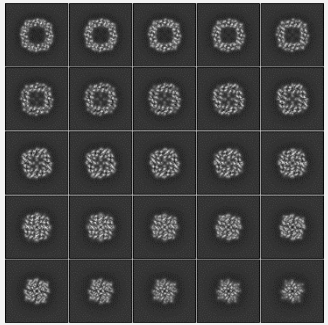
2

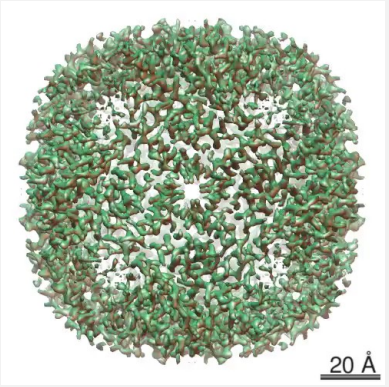
Cryo-Electron Microscopy

Cryo-EM in short

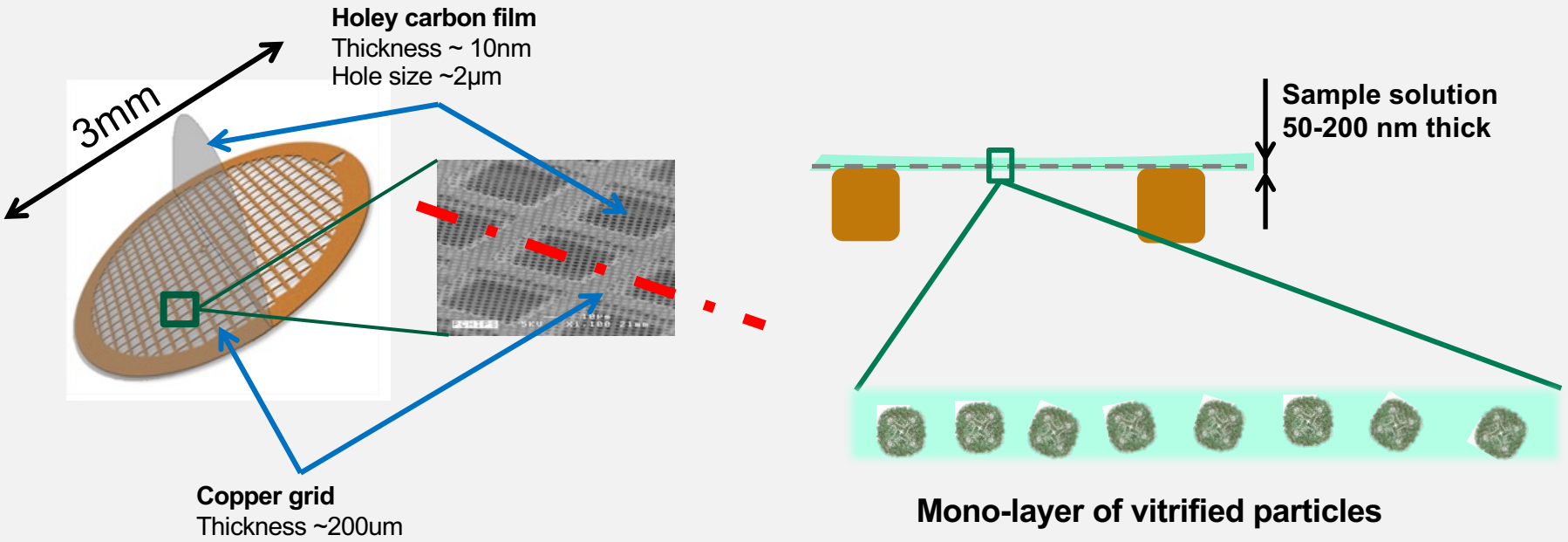


Sample preparation

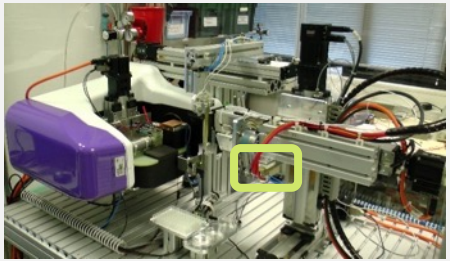


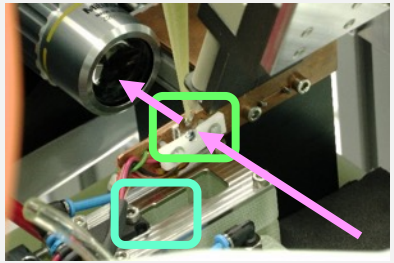


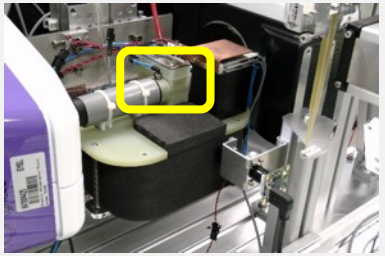
Cryo-EM : sample grid and optimal sample layout

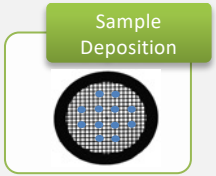


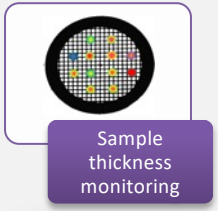
EasyGrid: automated Cryo-EM sample preparation

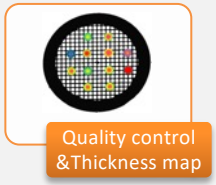


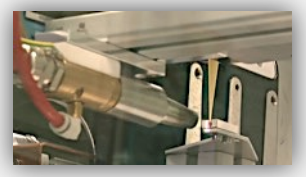






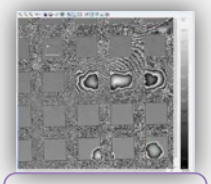




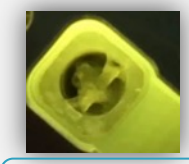


Plasma treatment

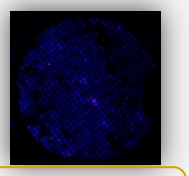
Picoliter drop dispenser



Interferometric method

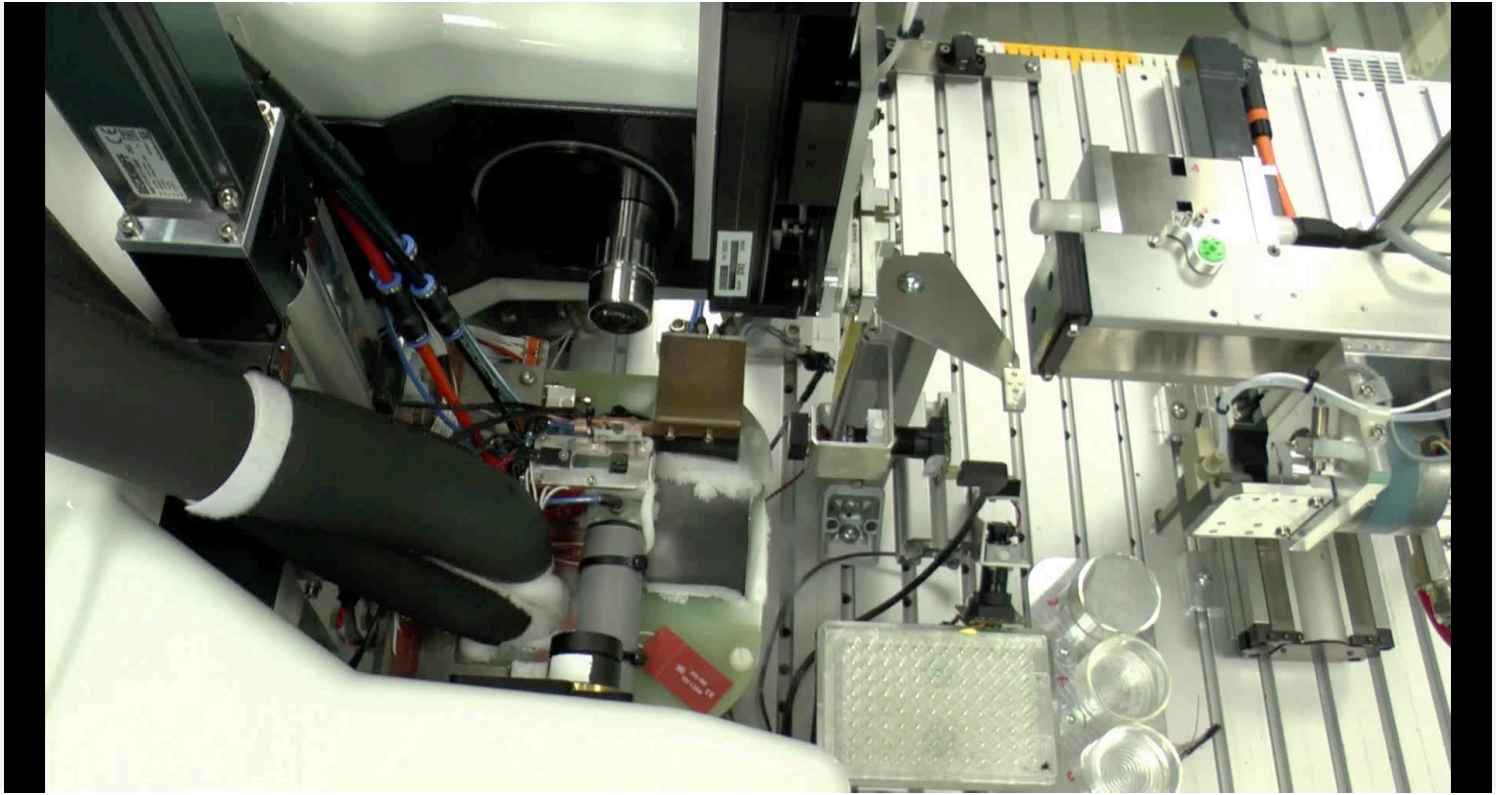


Liquid Propane -Ethane jet



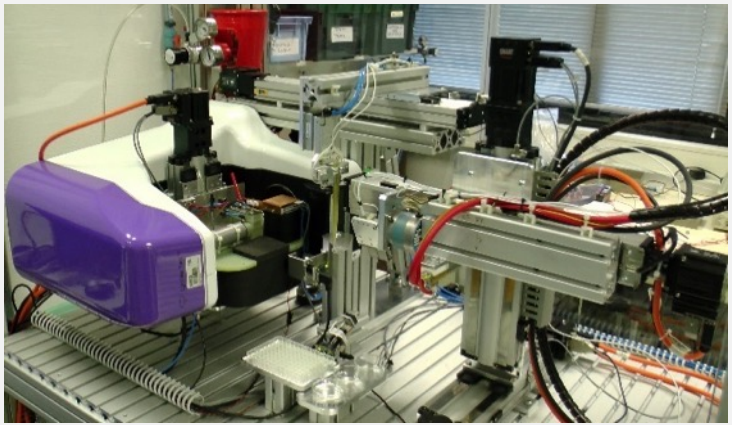
Interferometric method

EasyGrid video

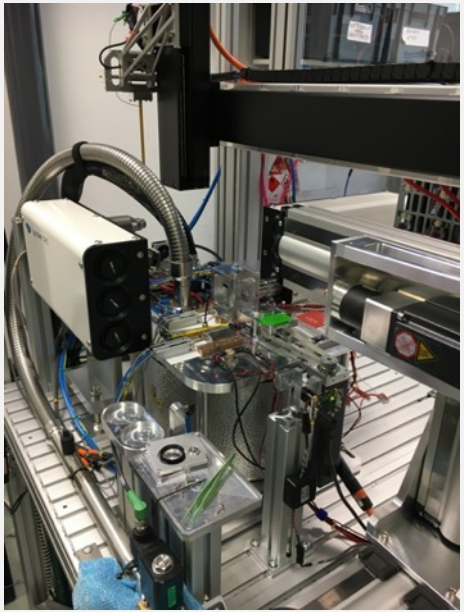


EasyGrid machine family

EG1 Prototype – 2018
- *Preparation of 1 grid*
- *Manual grid transfer to boxes*



EG2 – 2021 (ongoing)
- *Automated preparation and storing up to 40 grids*



3 Cryogenic Sample tracking

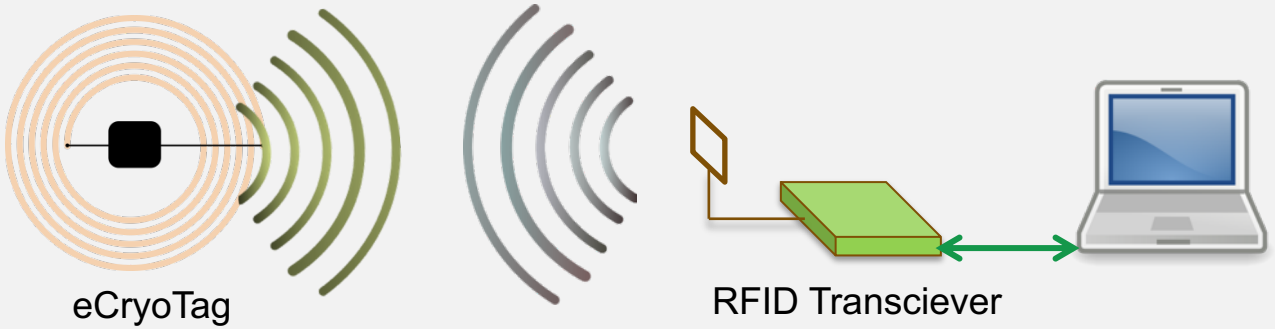
More and more samples at platforms

- Traditional tracking methods:
  - Datamatrix codes
    - Frost makes reading difficult
    - Damaged barcodes due to handling
  - Hand written codes
    - Difficult to read
    - Possible human errors
  - But
    - Samples represent months of work
    - Loss is hardly tolerated

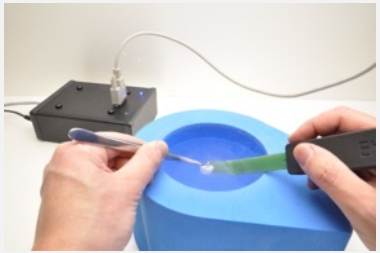
Need for reliable

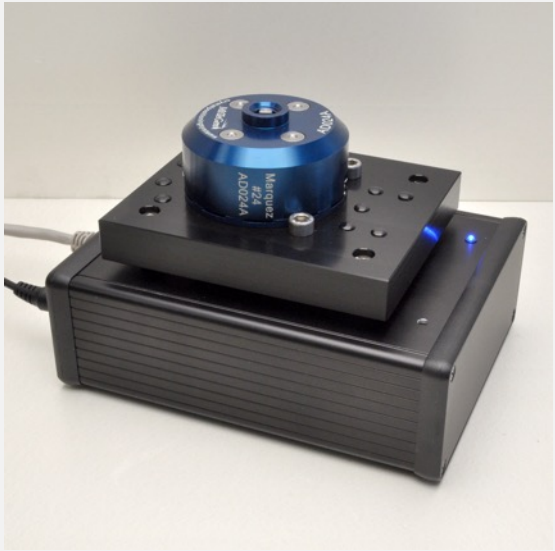
How to effectively track samples at cryogenic temperature?

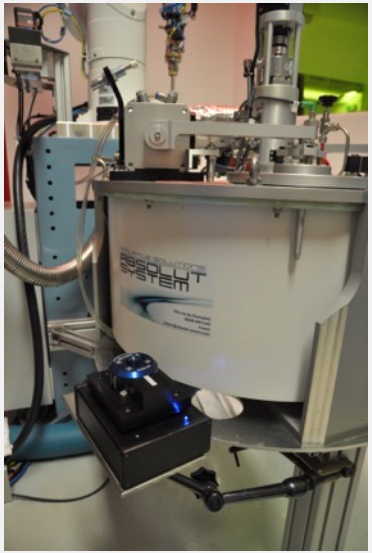
- **eCryoTag**
  - RFID (Radio Frequency Identification) technology
  - Contactless
  - Fast & reliable reading



A new standard for MX and Cryo-EM sample tracking



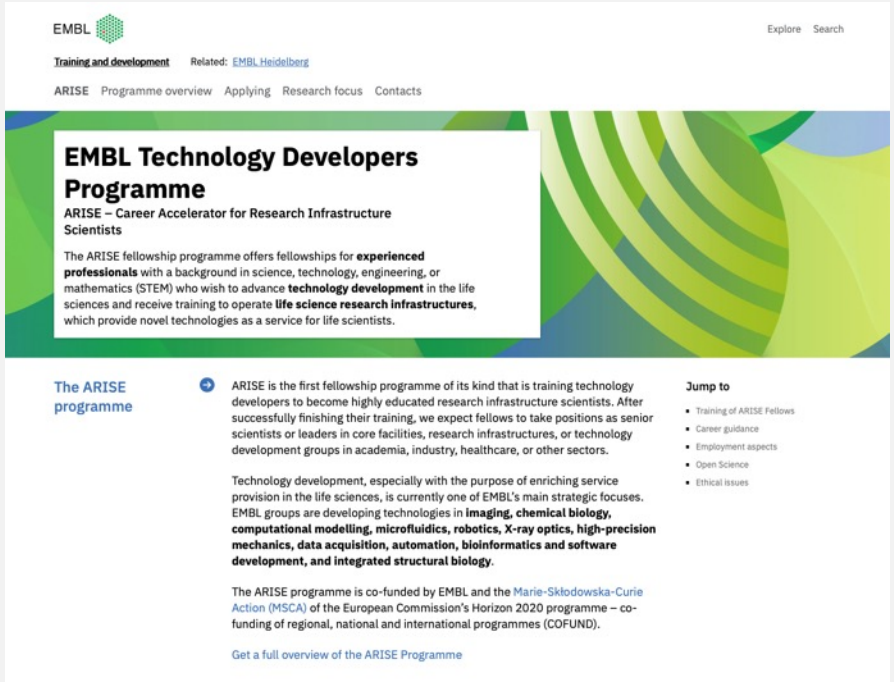




eCryoTag usecase at HTXlab crystal harvesting platform

ARISE Fellowship opportunities

https://www.embl.org/training/technology-developers-programme/arise/



2nd Call:
1st September – 31 October 2021

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