

Instrumentation, Automation and Standards in Structural Biology

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Interim Head – EMBL Grenoble Instrumentation Team



EMBL Grenoble Instrumentation Team - Focus



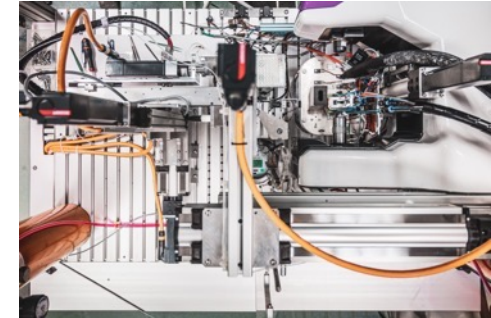
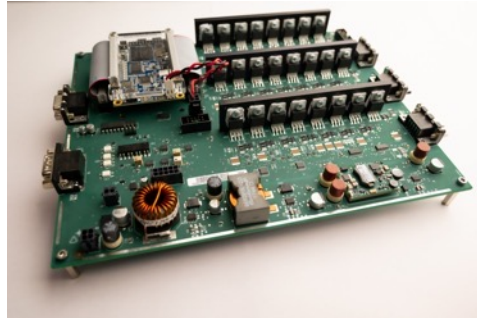
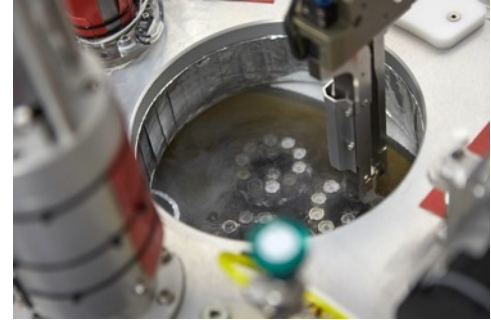
- Sample Environment of MX and SAXS beamlines
 - Diffractometers (20 years)
 - Automated sample changers (15 years)
 - Automated crystal harvesting CrystalDirect™ (10 years)
 - Sample holder standards & tracking solutions (15 years)
- Preparation of Cryo-EM sample grids (2017)

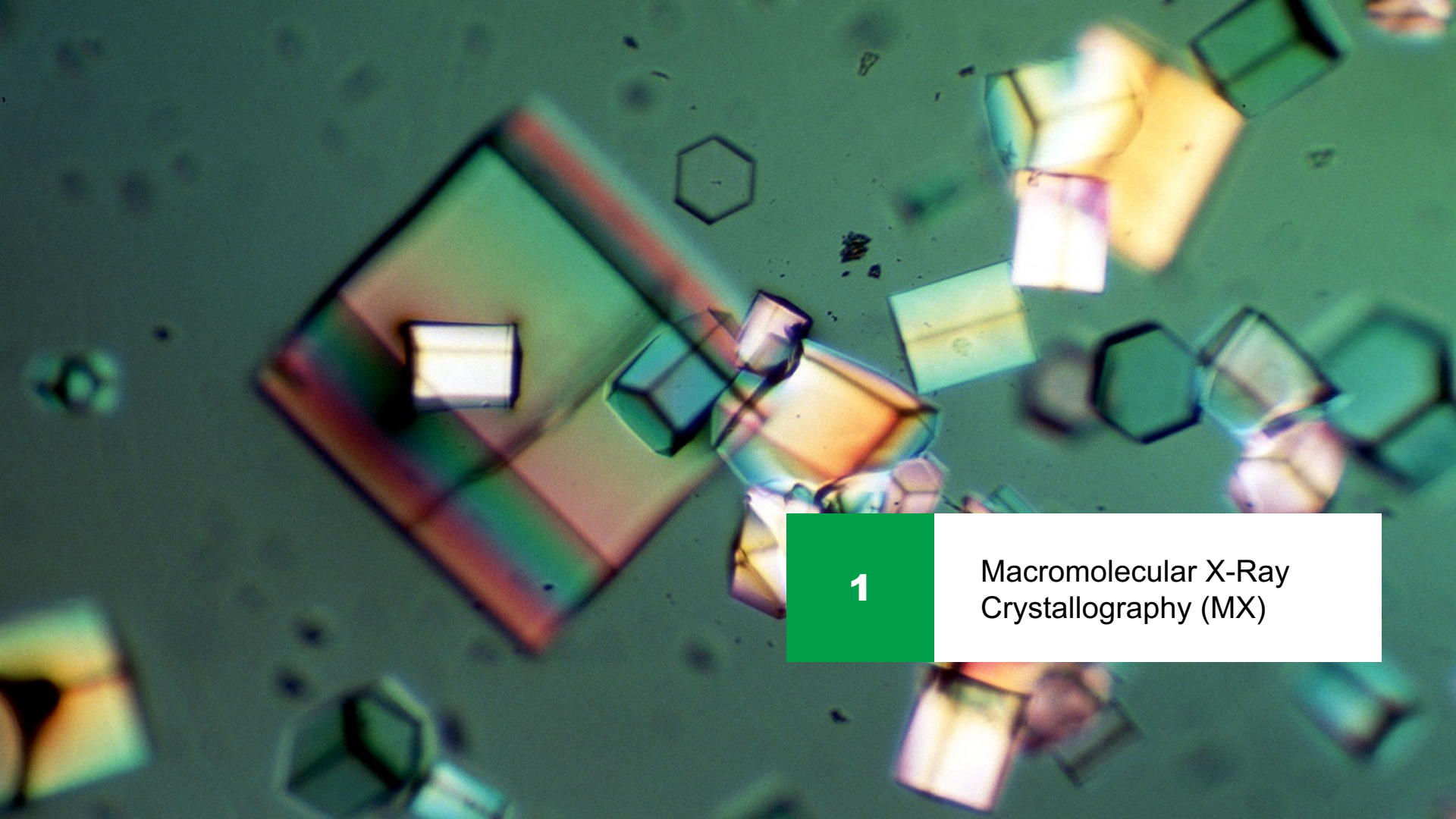
Our objectives

- 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

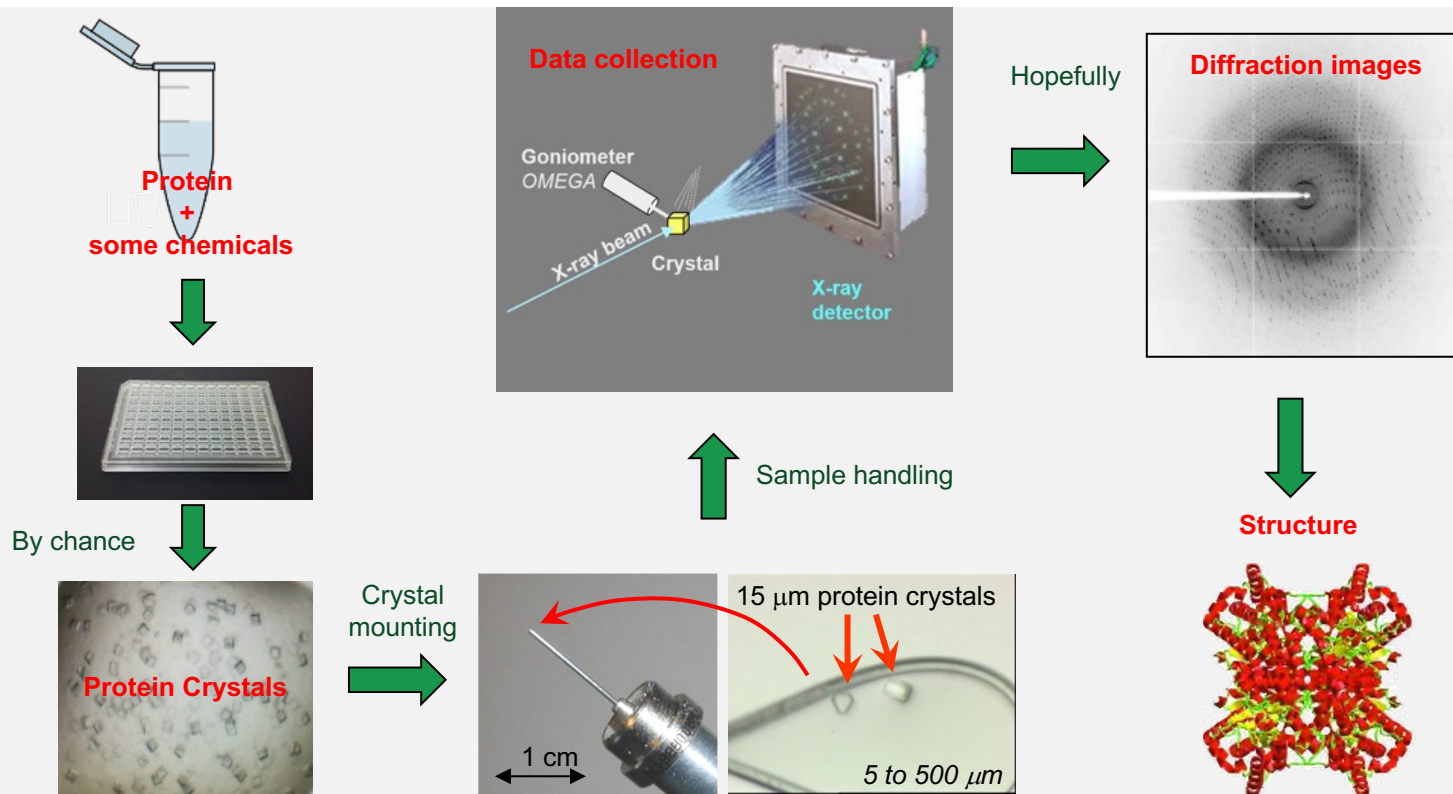




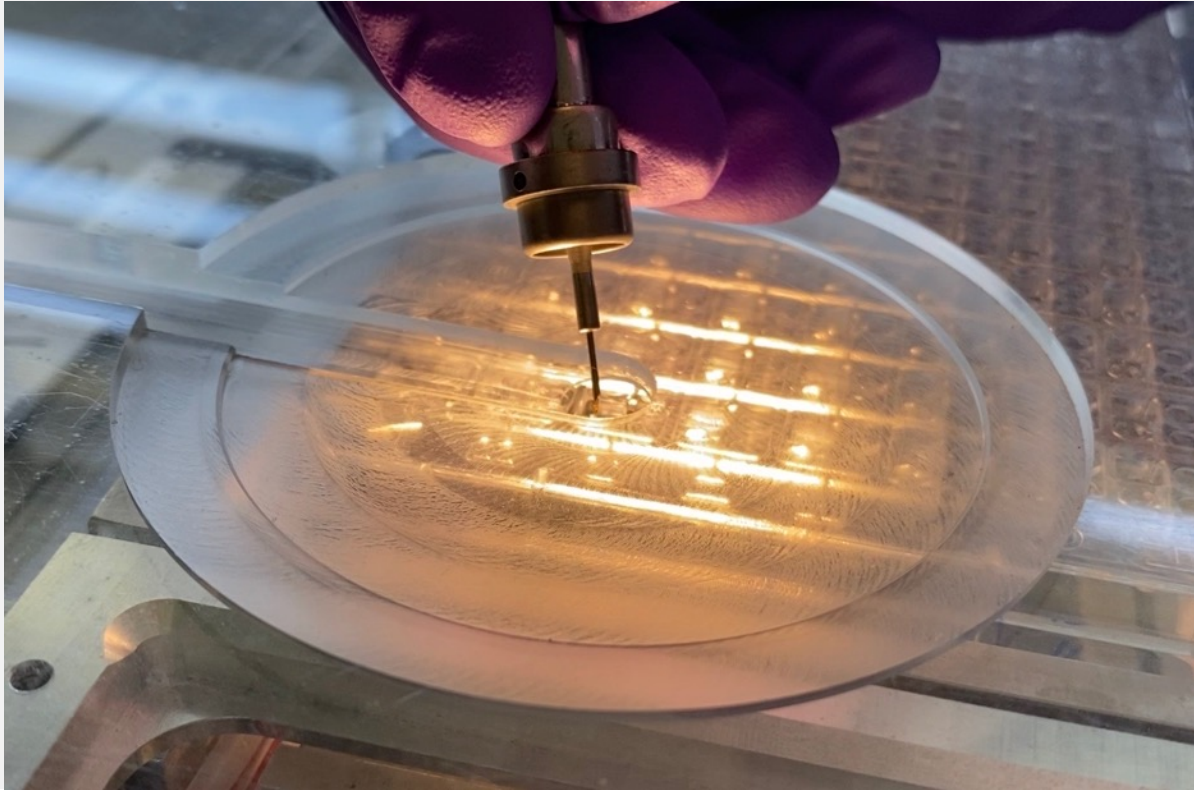
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Macromolecular X-Ray
Crystallography (MX)

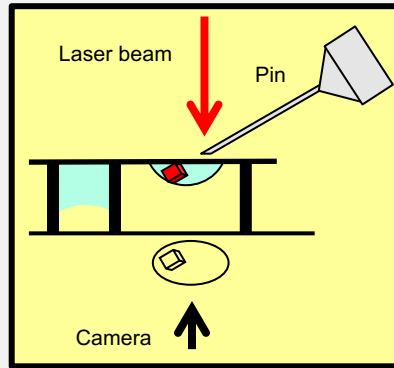
MX in brief



Crystal Harvesting – The traditional way

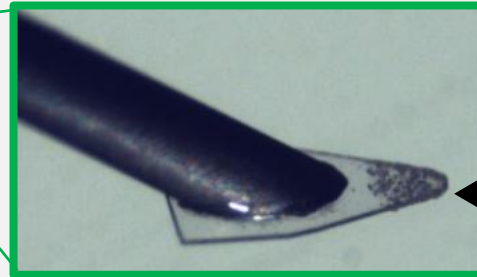
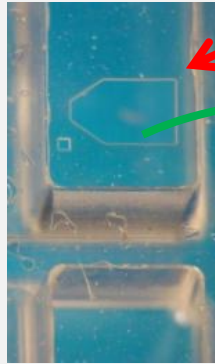


CrystalDirect: Harvesting by laser photoablation (2010)



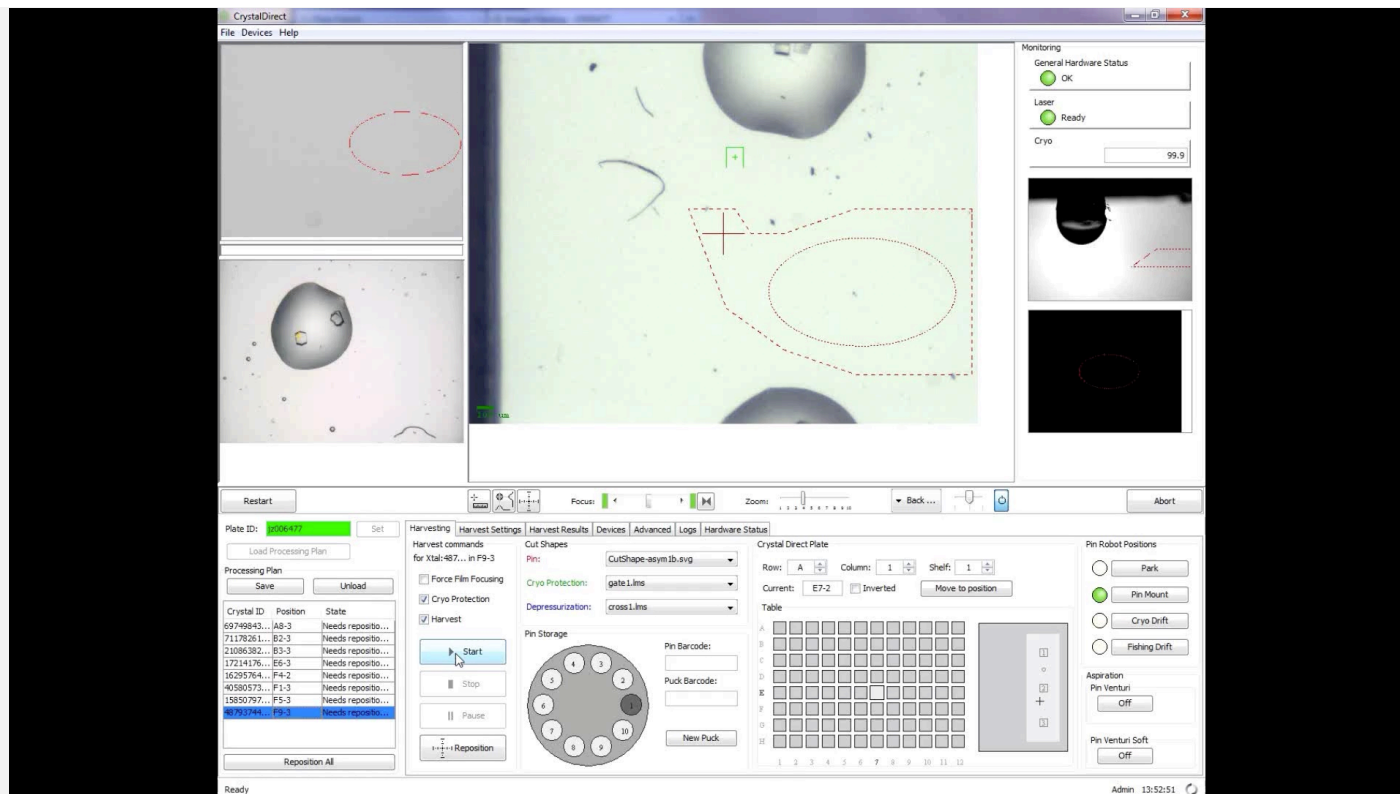
Film with crystal **cut** with a **femtosecond** laser beam

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

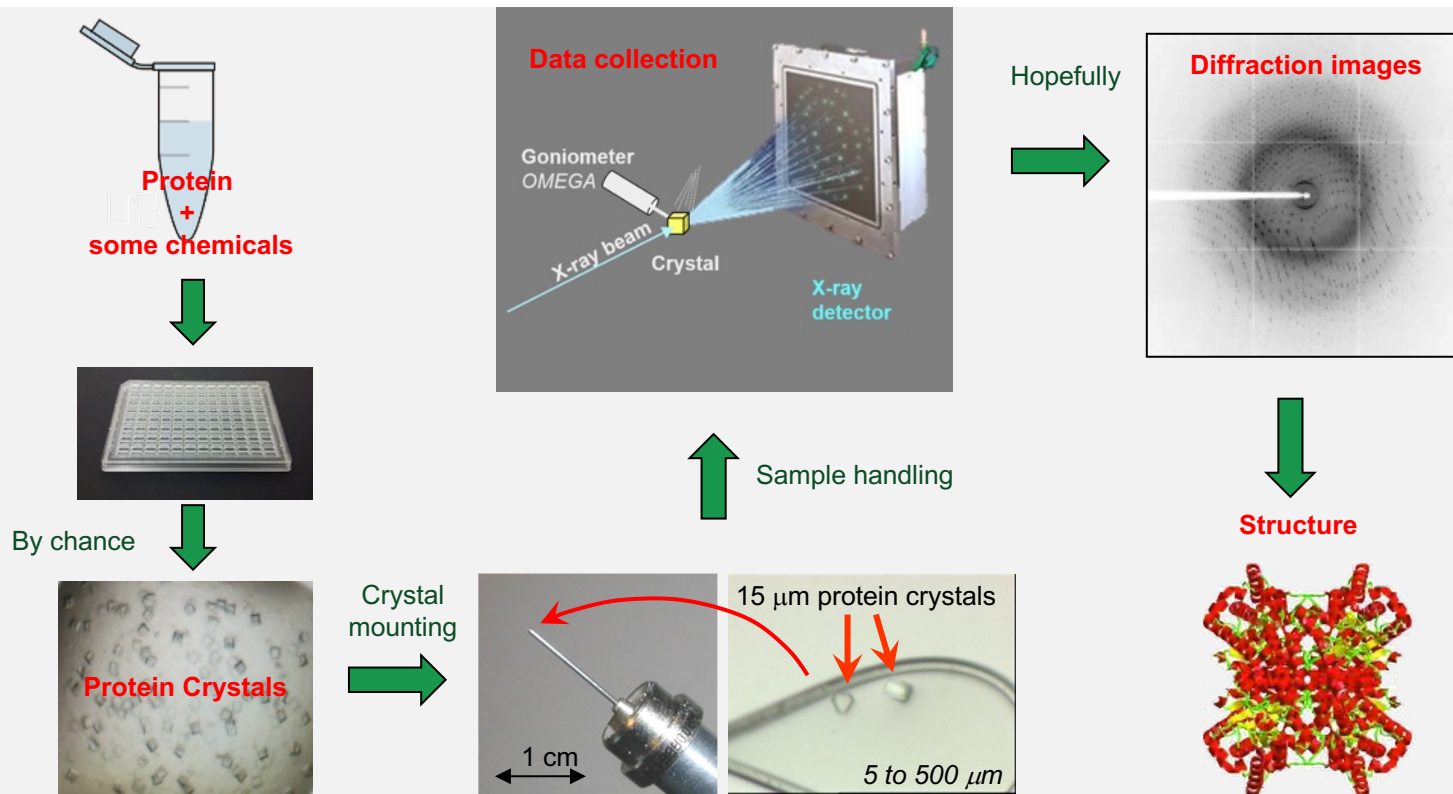


CD3 – 2021

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

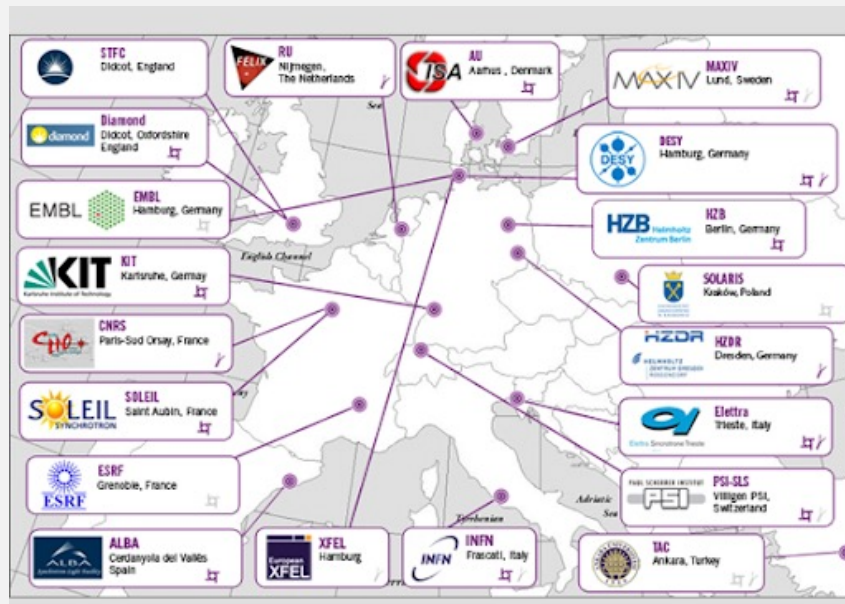


MX in brief



Sample holders

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



Need for

STANDARD (S)

Sample holder standards

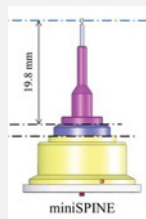
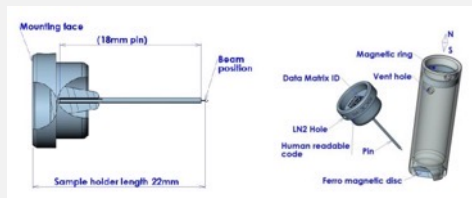
SPINE puck and pin standard – 2004

- Up to 10 samples in standard pucks
- 16 samples in unipucks



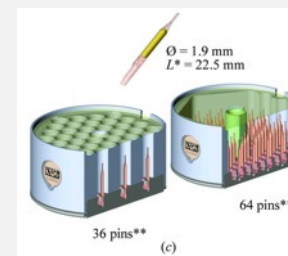
miniSPINE sample holder and puck – 2016

- Up to 36 samples



NewPin sample holder and puck – 2016

- High density & high repositioning precision
- Up to 64 samples in a puck

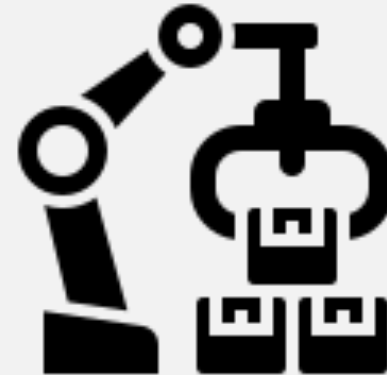


Sample handling on beamlines

- 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

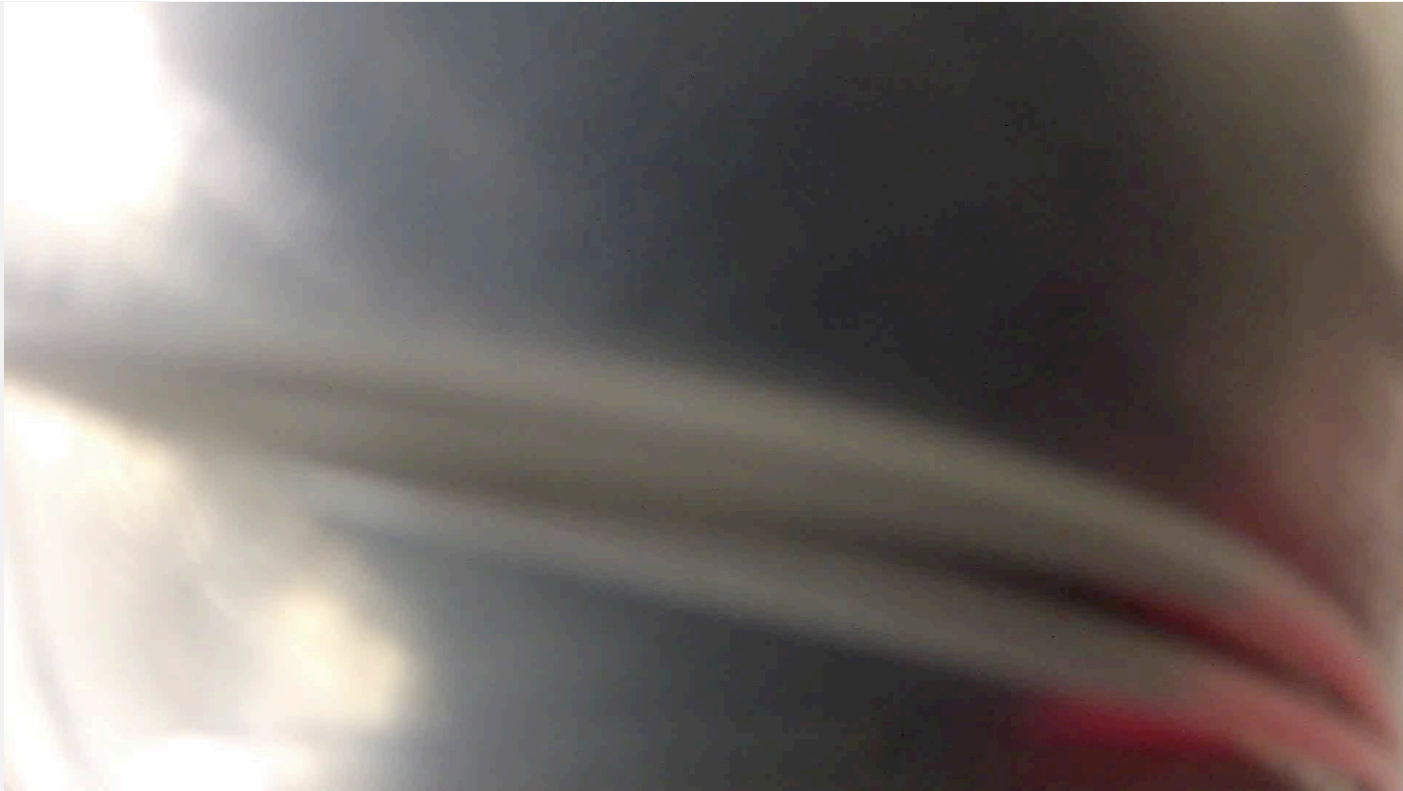


FlexHCD – 2016

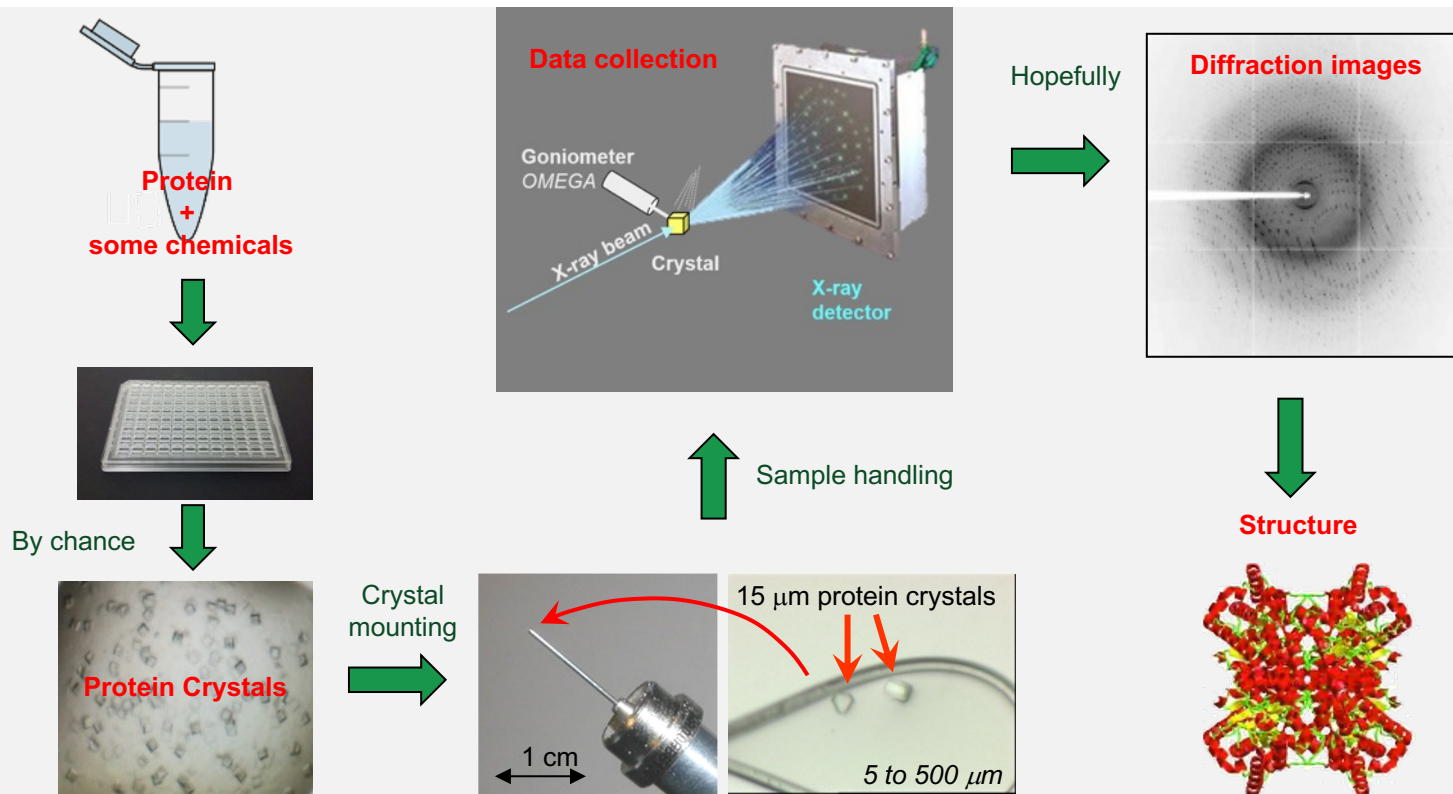
- EMBL / ESRF
- Capacity: 312 samples
- Up to 864 with miniSPINEs



Sample loading video

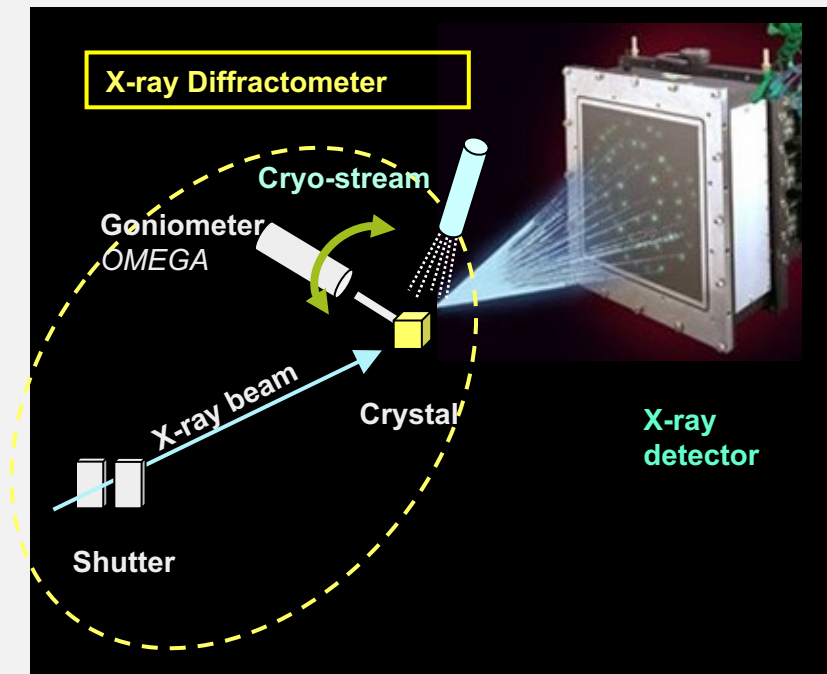


MX in brief

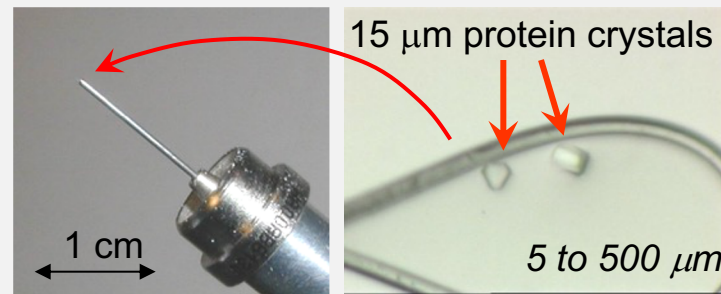


X-ray data collection at synchrotron

Typical experimental setup



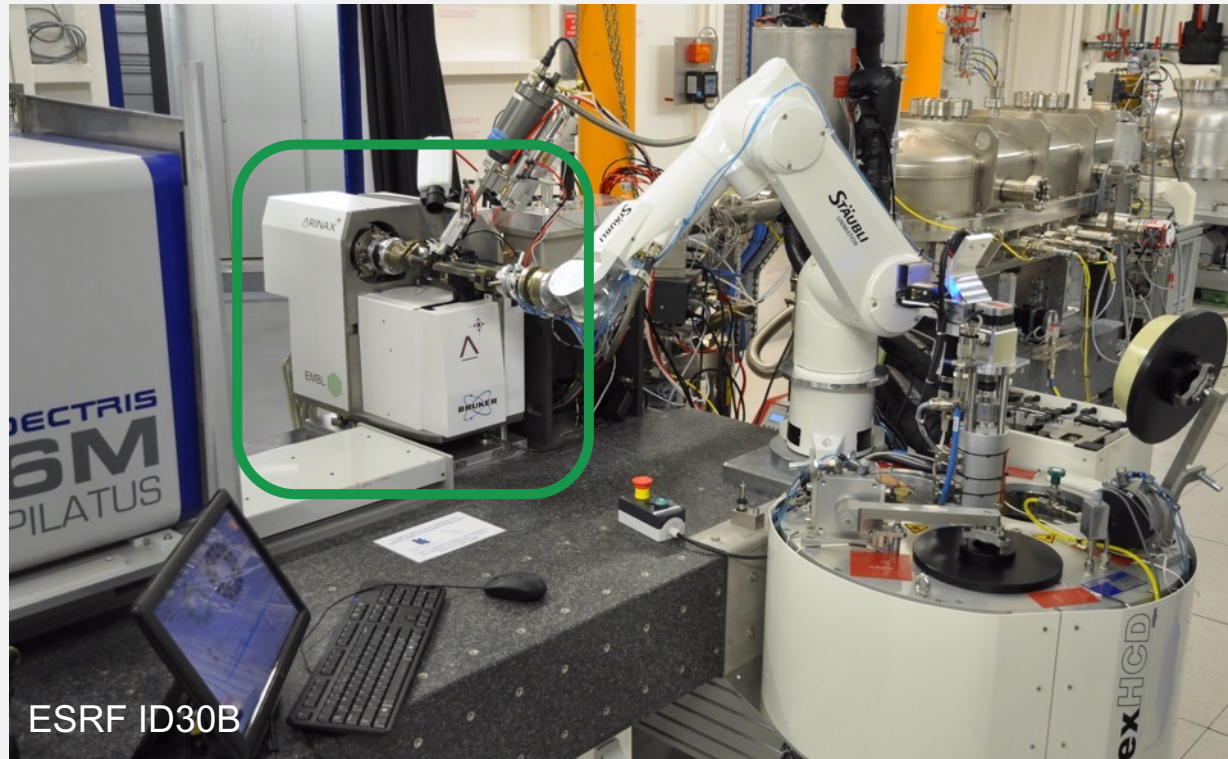
Crystals mounted in a “cryo-loop”



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 μ m crystals



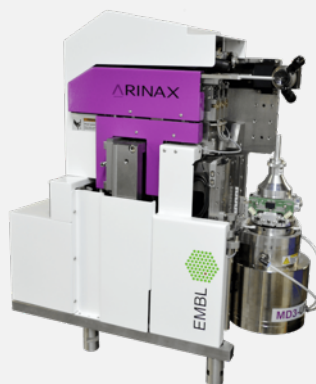
MD2 industrial version - 2002
EMBL GR



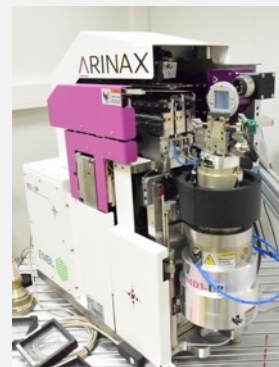
MD3 - 2012
EMBL GR / HH / Arinax
Process routinely 1 μ m crystals



MD3up - 2016
EMBL GR / Arinax
Easy sample changer access

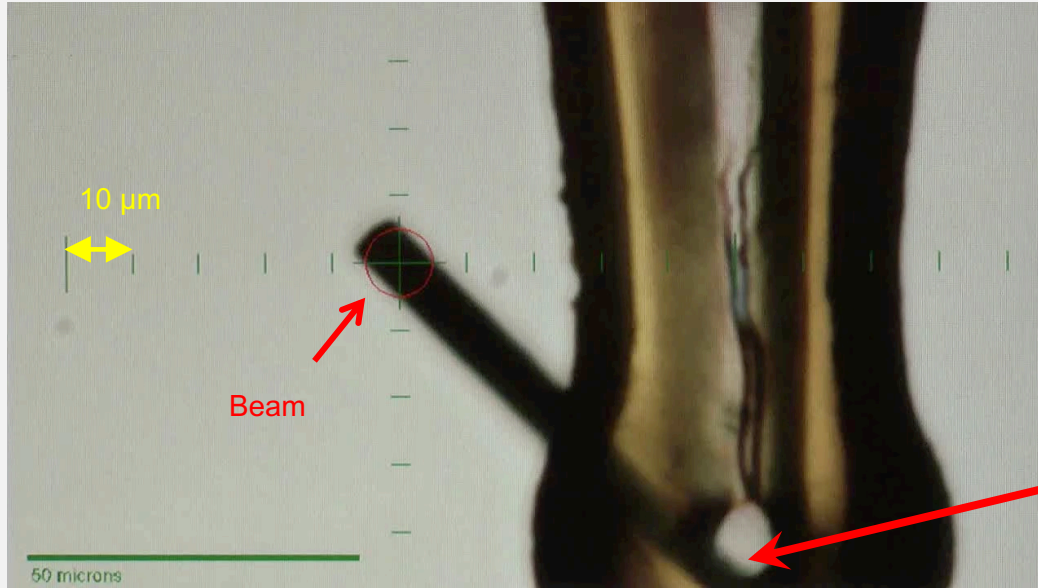


MD3ssx - 2020
EMBL GR / ESRF / Arinax
Serial data-collection on SSX chips



MD3 – High dynamic precision

- Video



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



Synchronous move

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

MD goniometer family

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



MD2 industrial version - 2002
EMBL GR



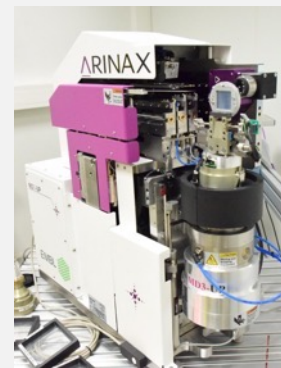
MD3 - 2012
EMBL GR / HH / Arinax
Process routinely 1 μm crystals



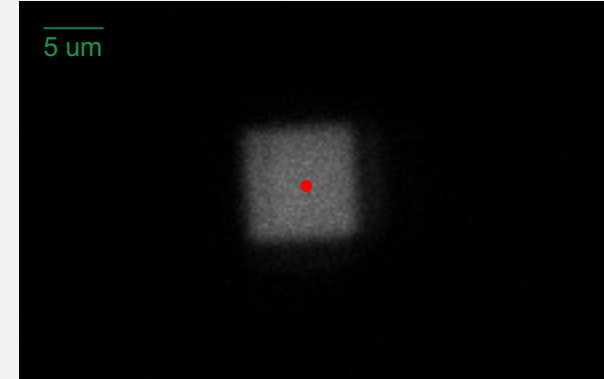
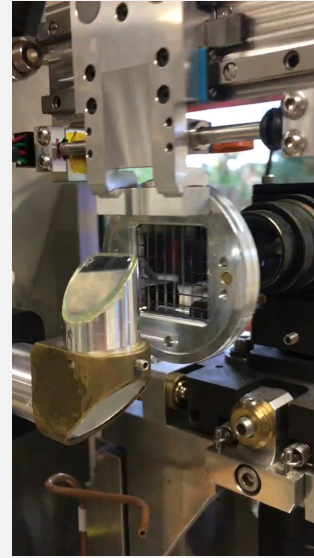
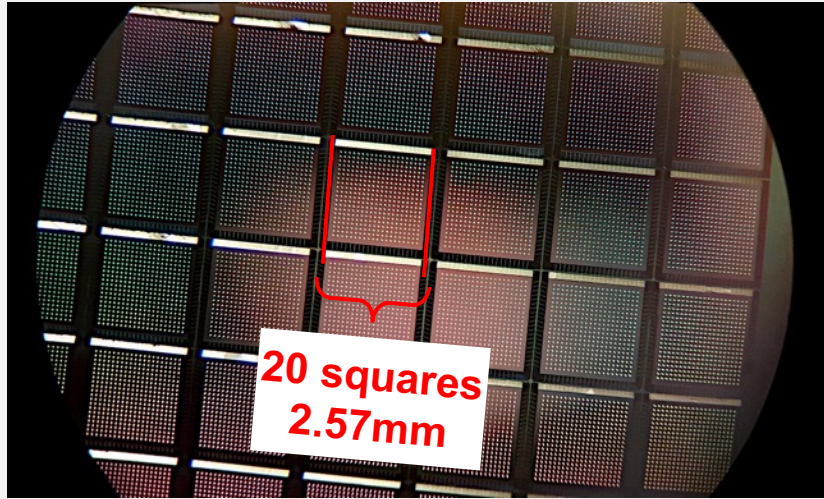
MD3up - 2016
EMBL GR / Arinax
Process routinely 1 μm crystals



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

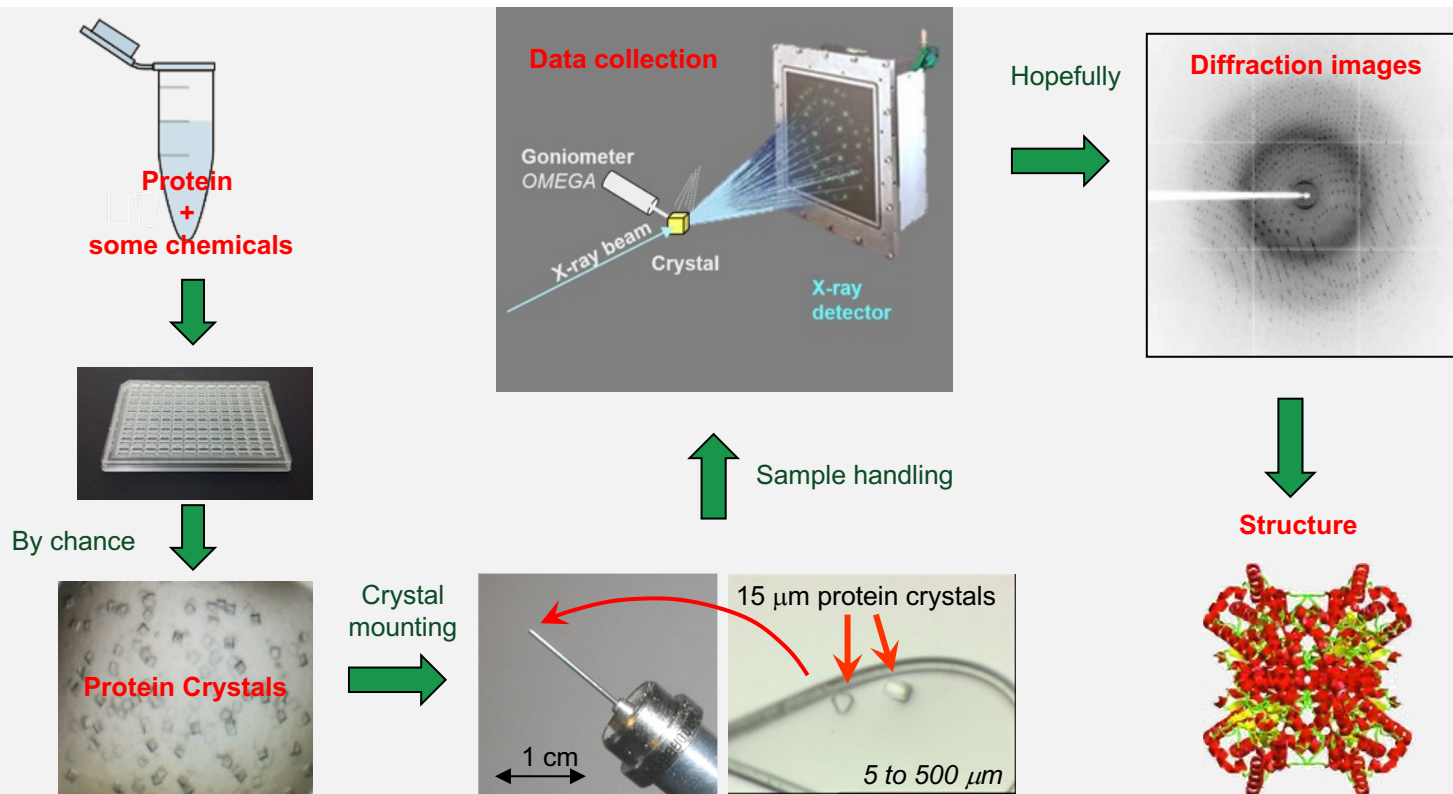


MD3 SSX – Chopper synchronized data collection on silicon chips



Captured at **20 mm/s**
Synchronized on **1kHz beam chopper**
Dynamic **positioning precision** $< \pm 1\mu\text{m}$

MX in brief

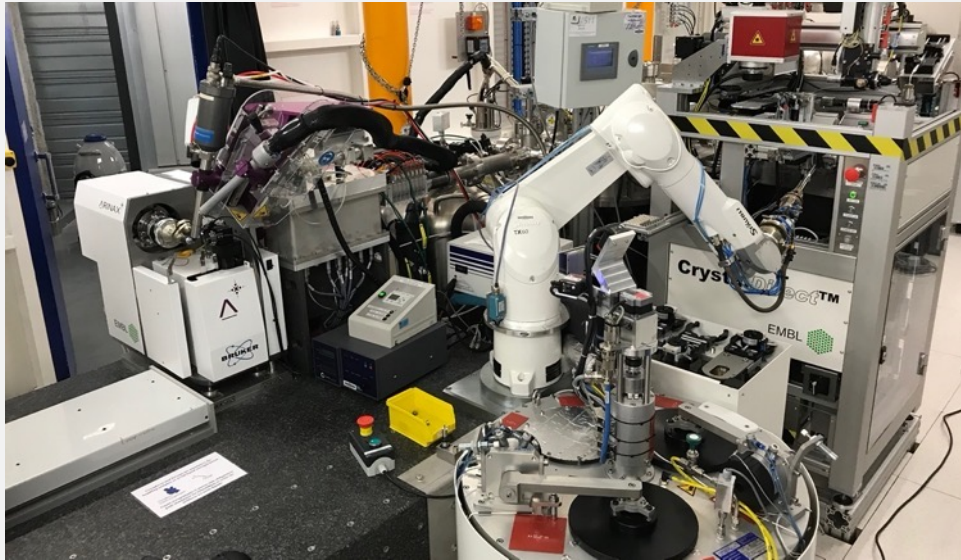


CrystalDirect to Beam

Fully automated Harvest & Collect pipeline

CD1 Prototype - 2017

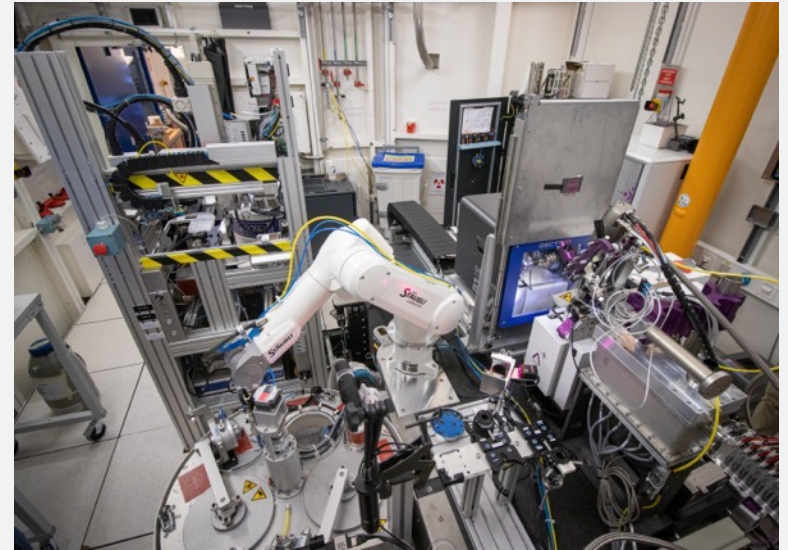
Proof of principle at ID30B - 2017

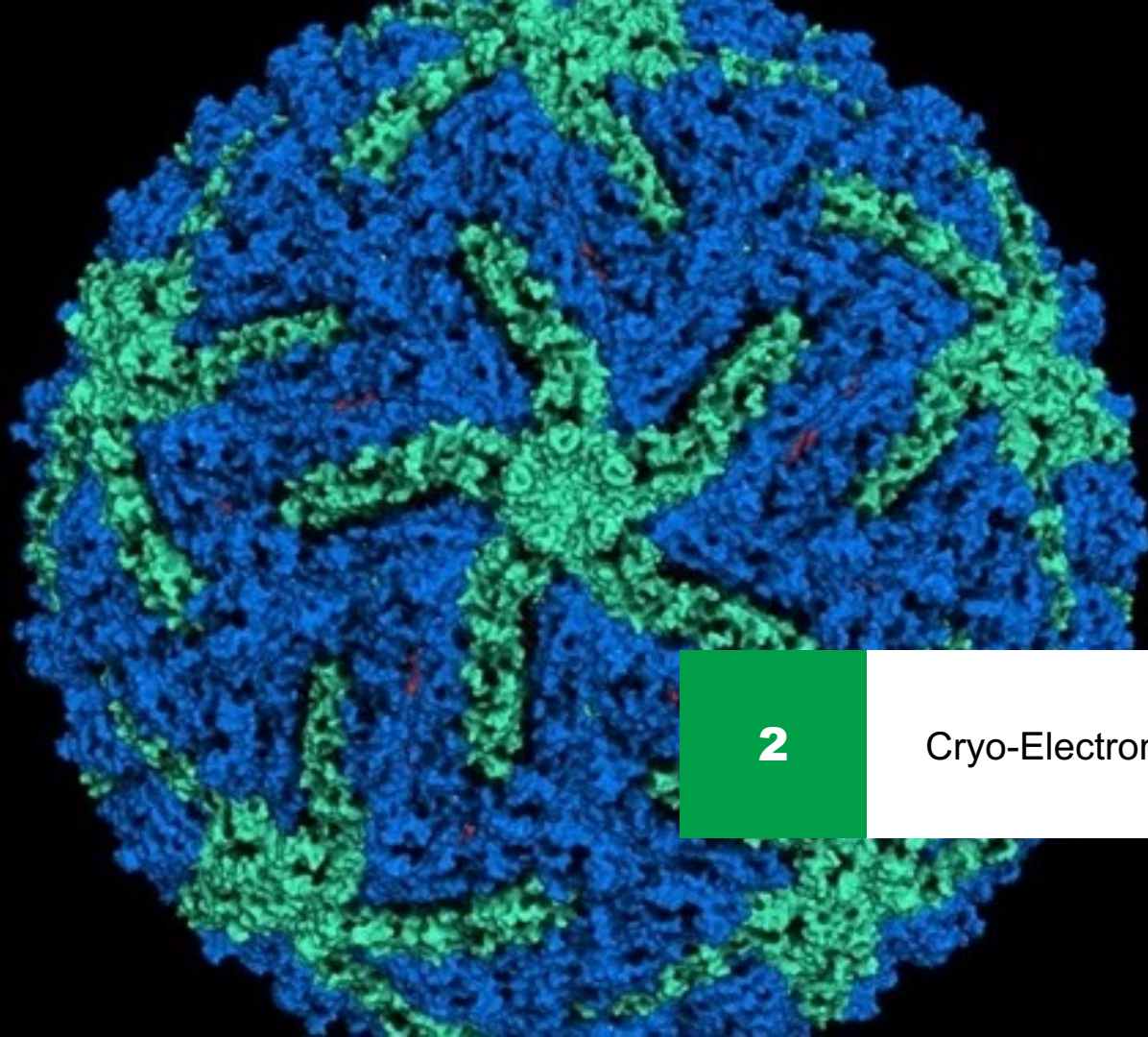


CD2 – 2021 (ongoing)

EMBL / ESRF

Permanent installation on Massif1

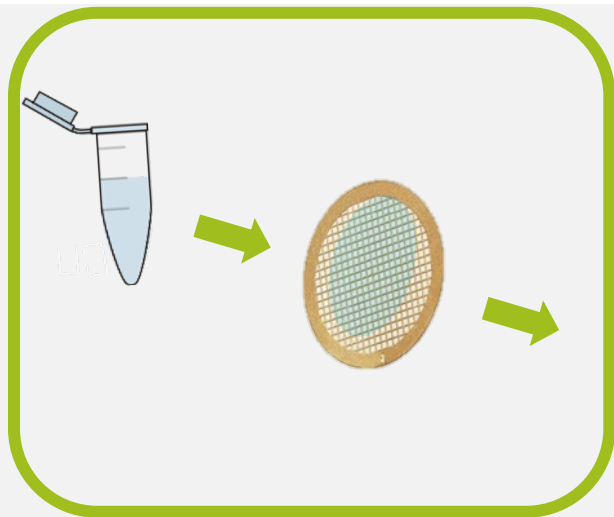




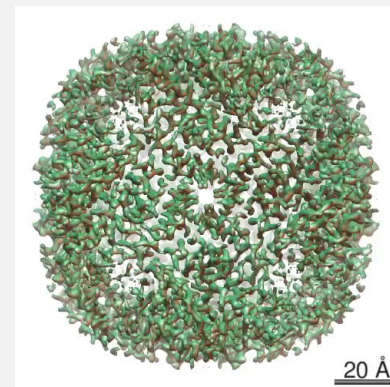
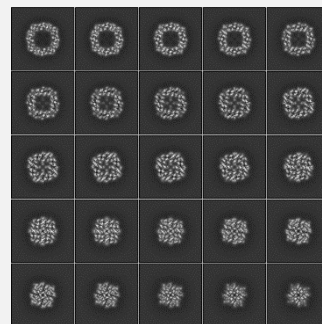
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Cryo-Electron Microscopy

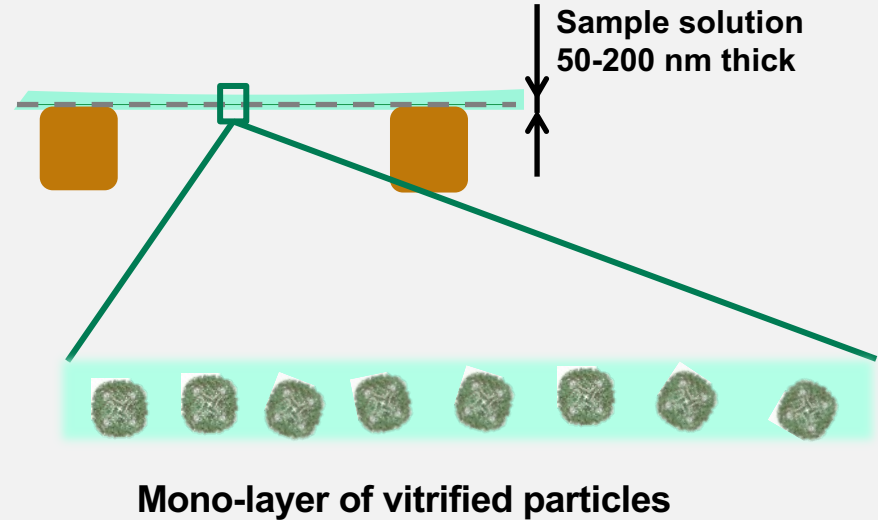
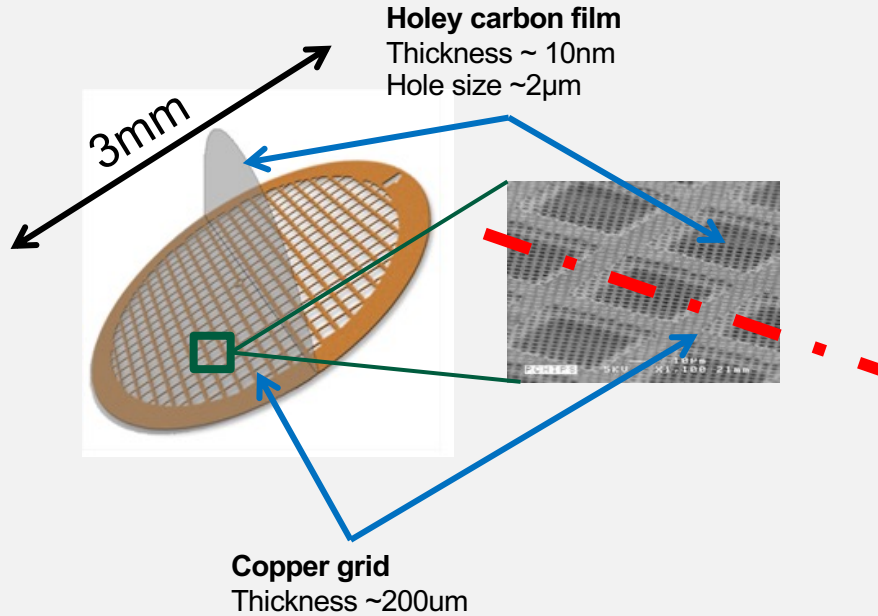
Cryo-EM in short



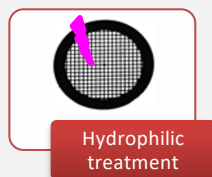
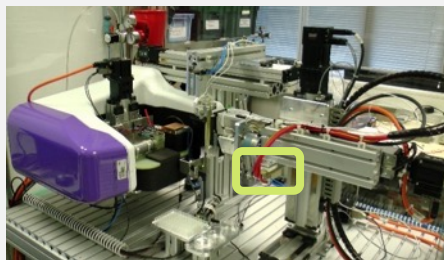
Sample preparation



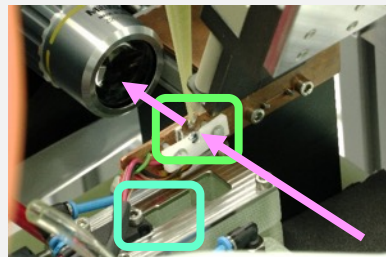
Cryo-EM : **sample grid** and **optimal sample layout**



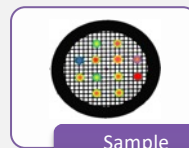
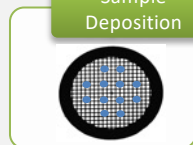
EasyGrid: automated Cryo-EM sample preparation



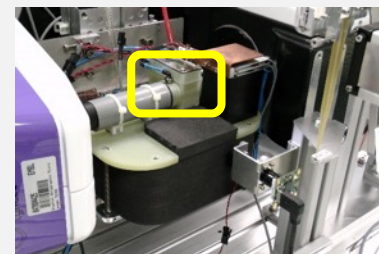
Hydrophilic treatment



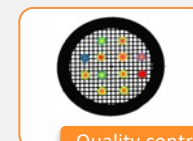
Sample Deposition



Sample thickness monitoring



Vitrification



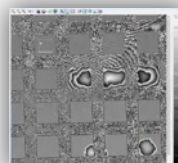
Quality control & Thickness map



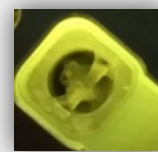
Plasma treatment



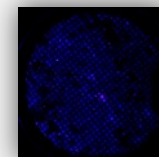
Picoliter drop dispenser



Interferometric method

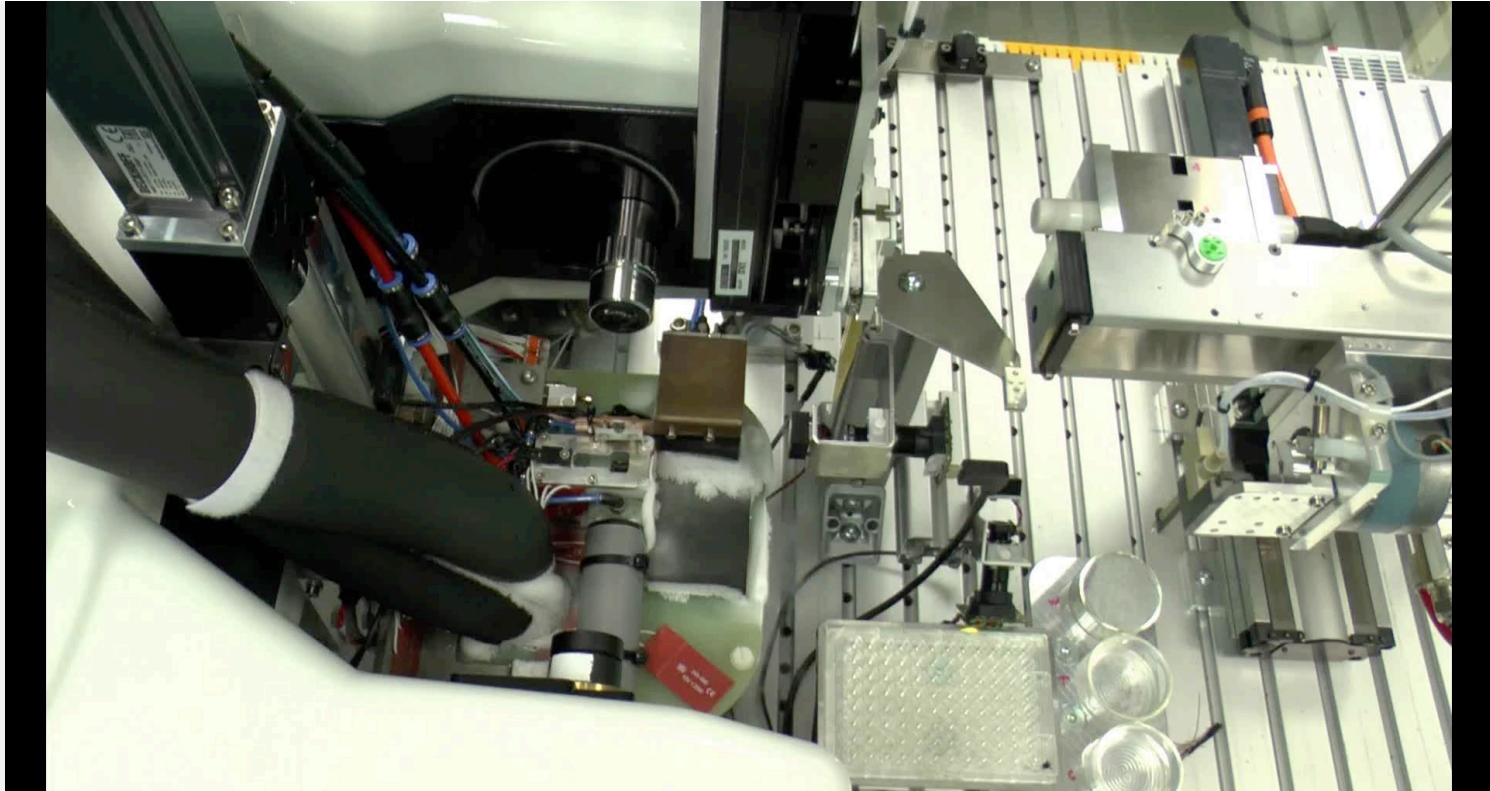


Liquid Propane-Ethane jet



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

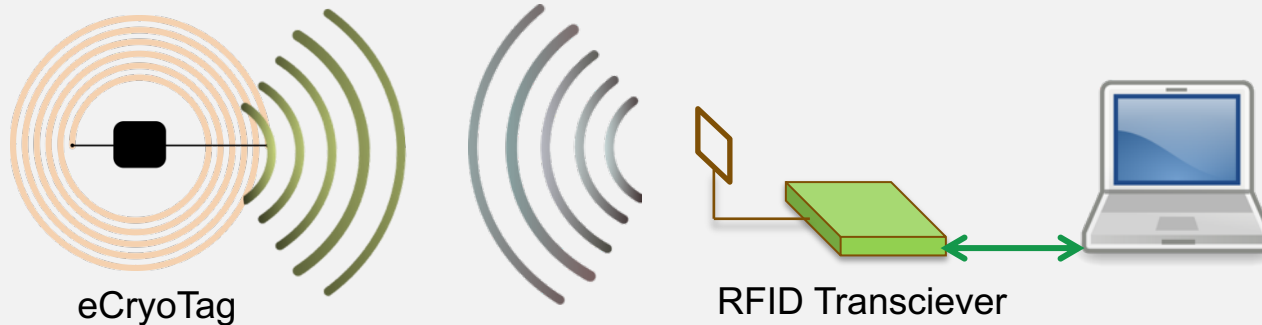
Need for reliable



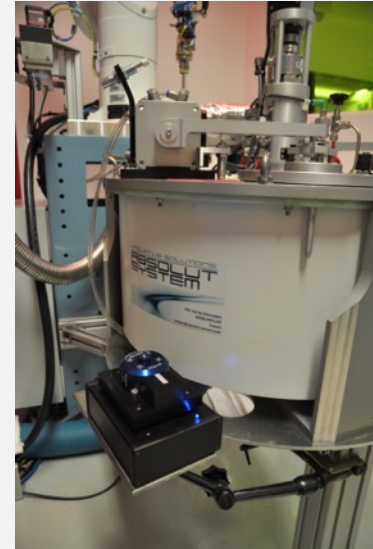
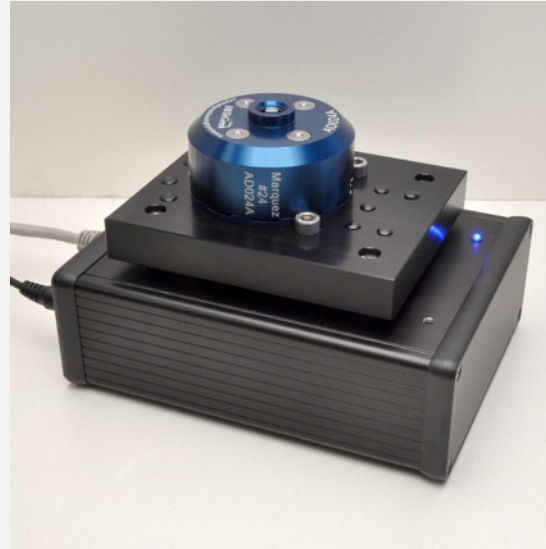
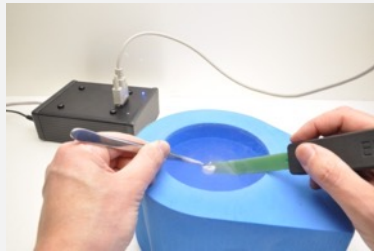
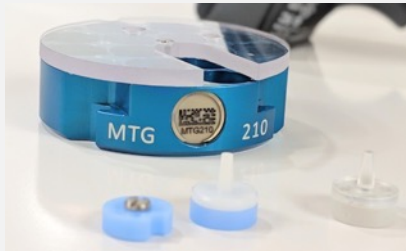
How to effectively track samples at cryogenic temperature?

- **eCryoTag**

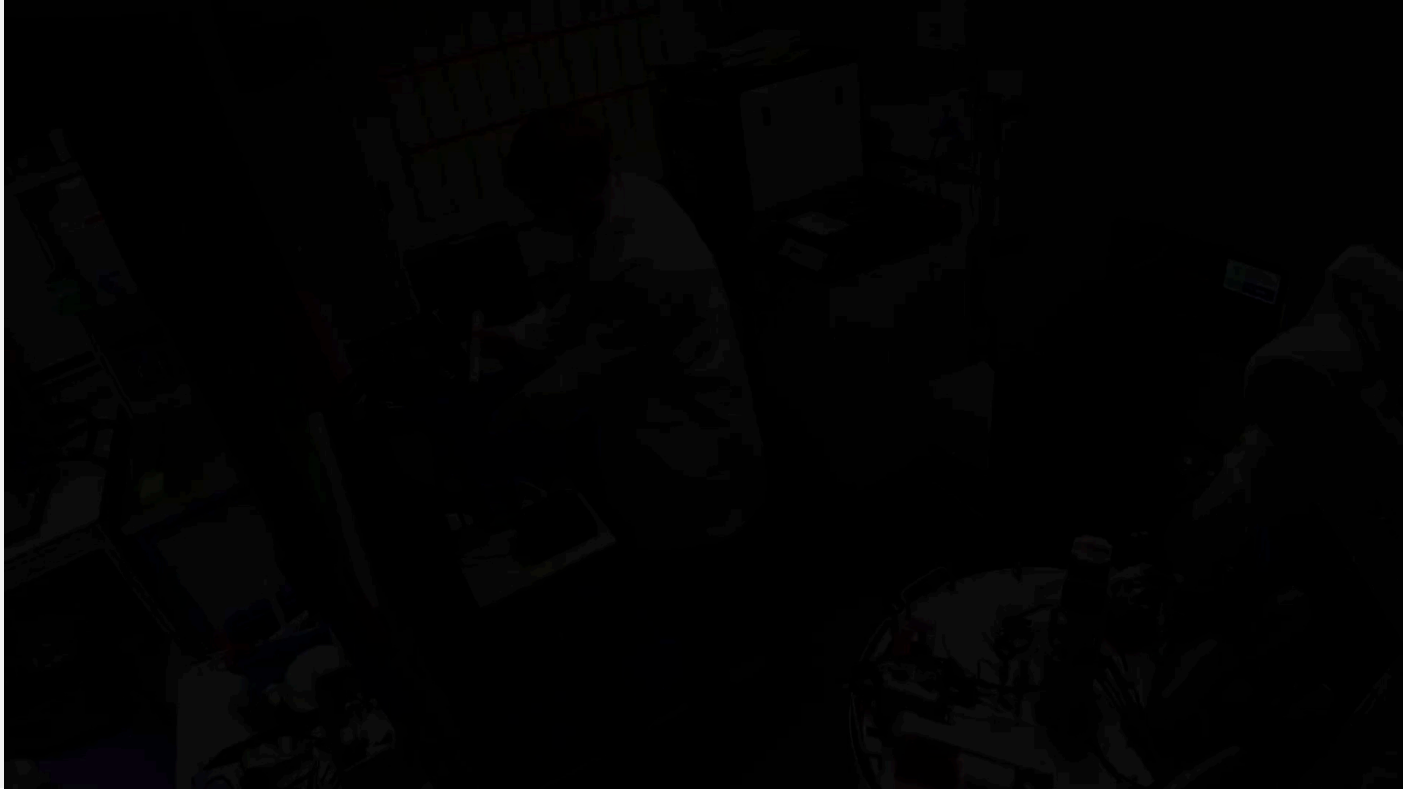
- RFID (Radio Frequency Identification) technology
- Contactless
- Fast & reliable reading



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/>

EMBL  Explore Search

Training and development Related: [EMBL Heidelberg](#)

ARISE Programme overview Applying Research focus Contacts

EMBL Technology Developers Programme

ARISE – Career Accelerator for Research Infrastructure Scientists

The ARISE fellowship programme offers fellowships for **experienced professionals** with a background in science, technology, engineering, or mathematics (STEM) who wish to advance **technology development** in the life sciences and receive training to operate **life science research infrastructures**, which provide novel technologies as a service for life scientists.

The ARISE programme

ARISE is the first fellowship programme of its kind that is training technology developers to become highly educated research infrastructure scientists. After successfully finishing their training, we expect fellows to take positions as senior scientists or leaders in core facilities, research infrastructures, or technology development groups in academia, industry, healthcare, or other sectors.

Technology development, especially with the purpose of enriching service provision in the life sciences, is currently one of EMBL's main strategic focuses. EMBL groups are developing technologies in **imaging, chemical biology, computational modelling, microfluidics, robotics, X-ray optics, high-precision mechanics, data acquisition, automation, bioinformatics and software development, and integrated structural biology.**

The ARISE programme is co-funded by EMBL and the [Marie-Sklodowska-Curie Action \(MSCA\)](#) of the European Commission's Horizon 2020 programme – co-funding of regional, national and international programmes (COFUND).

[Get a full overview of the ARISE Programme](#)

Jump to

- Training of ARISE Fellows
- Career guidance
- Employment aspects
- Open Science
- Ethical issues

2nd Call:
1st September – 31 October 2021

Acknowledgements

- EMBL - GR
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 - MiTeGen
 - Molecular Dimensions
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