Scientific instruments to answer biological questions at the EMBL

Andrew McCarthy (andrewmc@embl.fr)

Team leader, EMBL-Grenoble

EMBL

14/05/2019

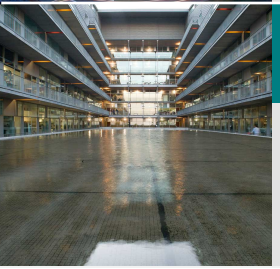
EMBL sites – over 1800 people and more than 80 nationalities

Hinxton
EMBL-EBI

Bioinformatics

Grenoble

Structural biology



Barcelona

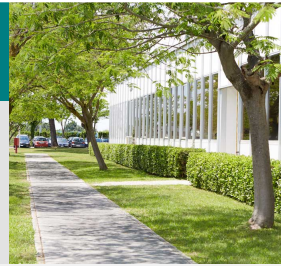
Tissue biology and disease modelling

Hamburg

Structural biology

Heidelberg

Life sciences

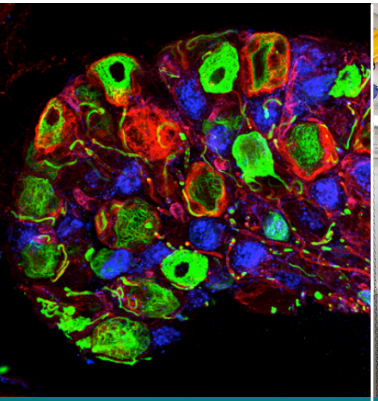


Rome

Epigenetics and neurobiology

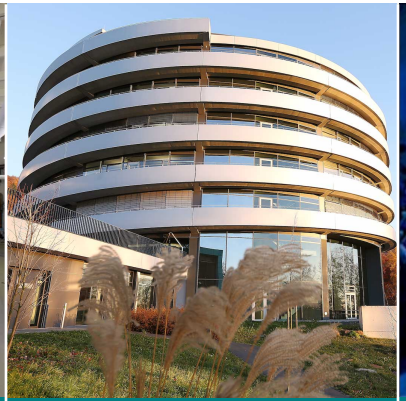
EMBL

EMBL's missions



Basic research

Services



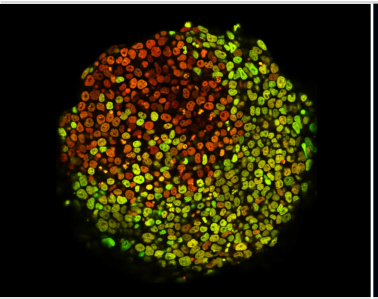
Advanced training

Technology development & transfer

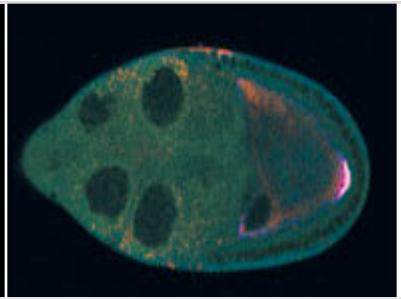
Integration of life science research

EMBL

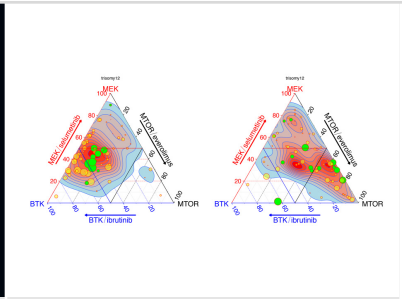
EMBL research units – over 80 independent research groups



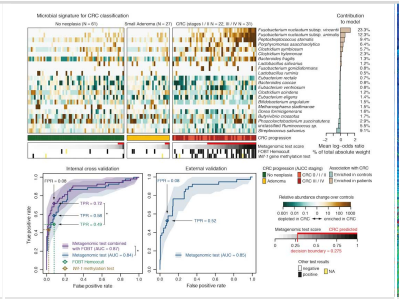
Cell biology and biophysics – Heidelberg



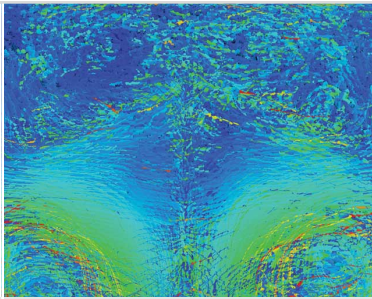
Developmental biology – Heidelberg



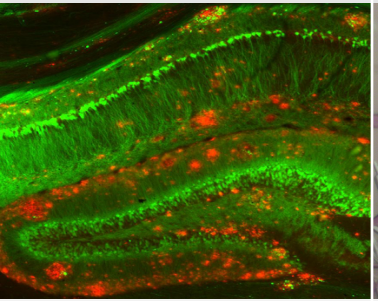
Genome biology – Heidelberg



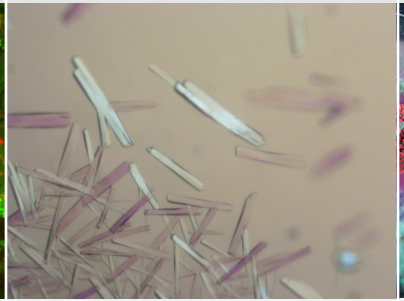
Structural and computational biology – Heidelberg



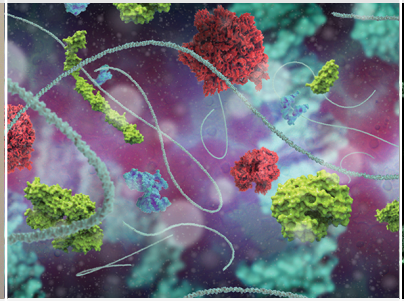
Directors' research – Heidelberg



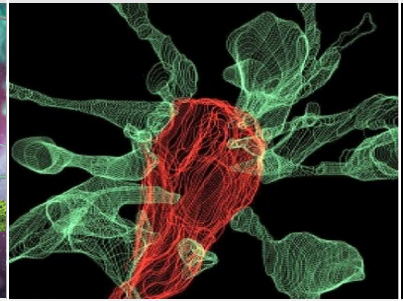
Structural biology – Hamburg



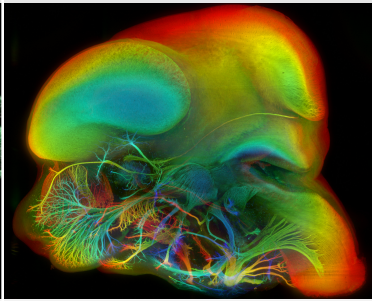
Structural biology – Grenoble



Bioinformatics – EMBL-EBI, Hinxton



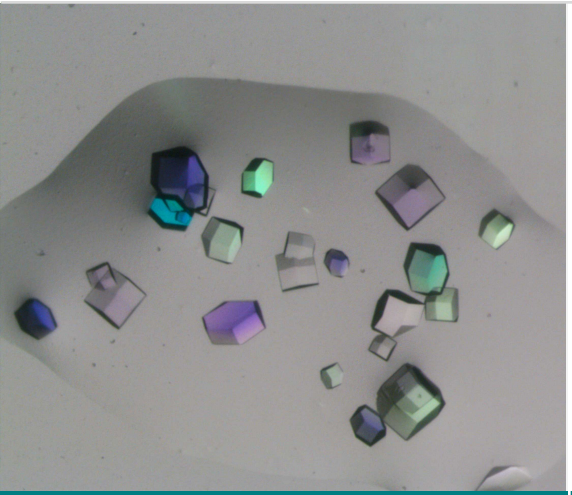
Epigenetics and neurobiology – Rome

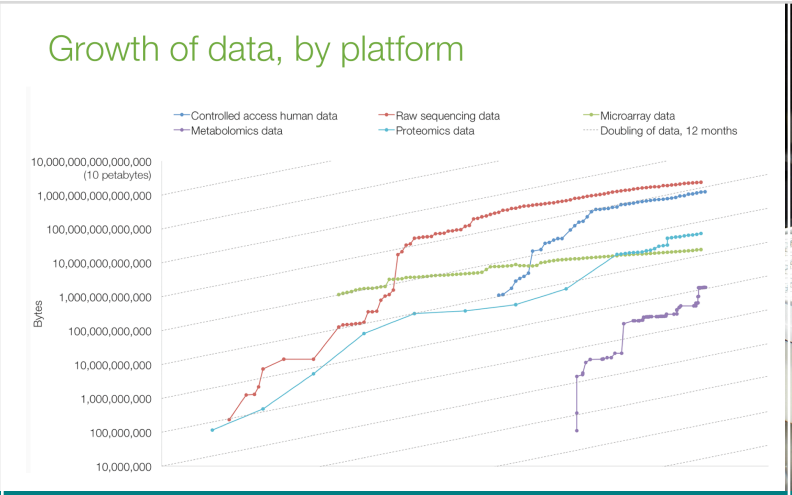


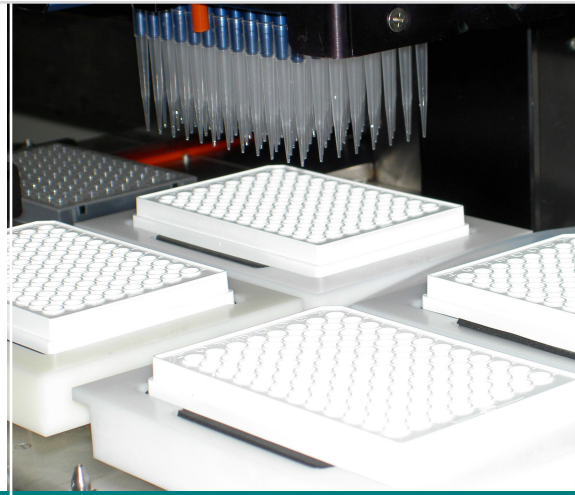
Tissue biology and disease modelling – Barcelona

EMBL

EMBL infrastructure and services







Structural biology services

> 3,000 user visits per year

Many users of complementary services

Bioinformatics services

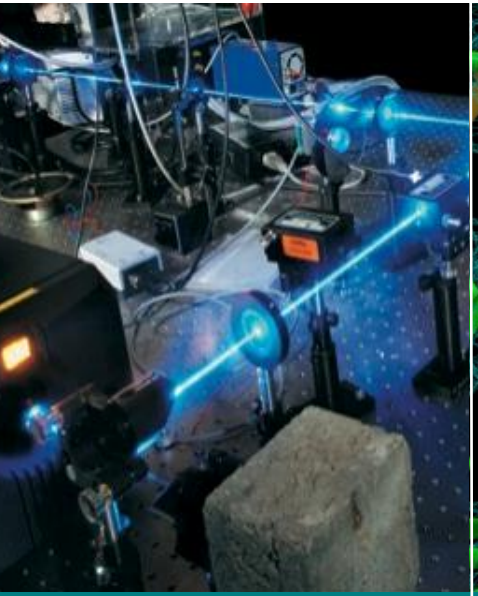
~ 38,000,000 web visits to EMBL-EBI databases per day

Core Facilities

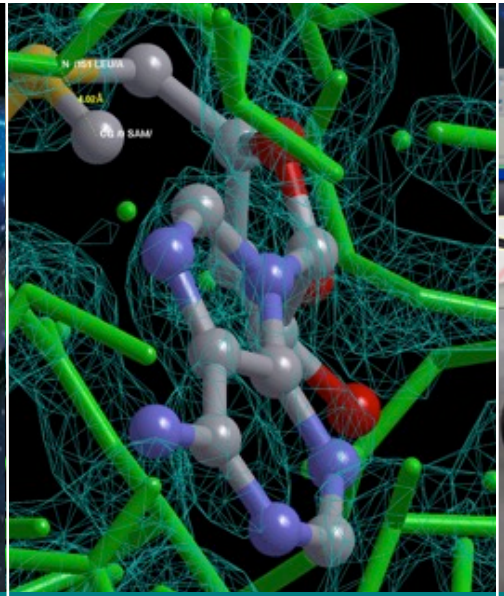
~1,200 internal and external users per year

EMBL

Technology development and transfer



Imaging technology



Software development

Synchrotron instrumentation

EMBL develops a broad spectrum of technology and instrumentation for life science research

EMBL makes its discoveries and inventions available to the scientific community and to society through EMBLEM

EMBL

Influenza polymerase mechanism (*Cusack group)* - EMBL-Grenoble

2

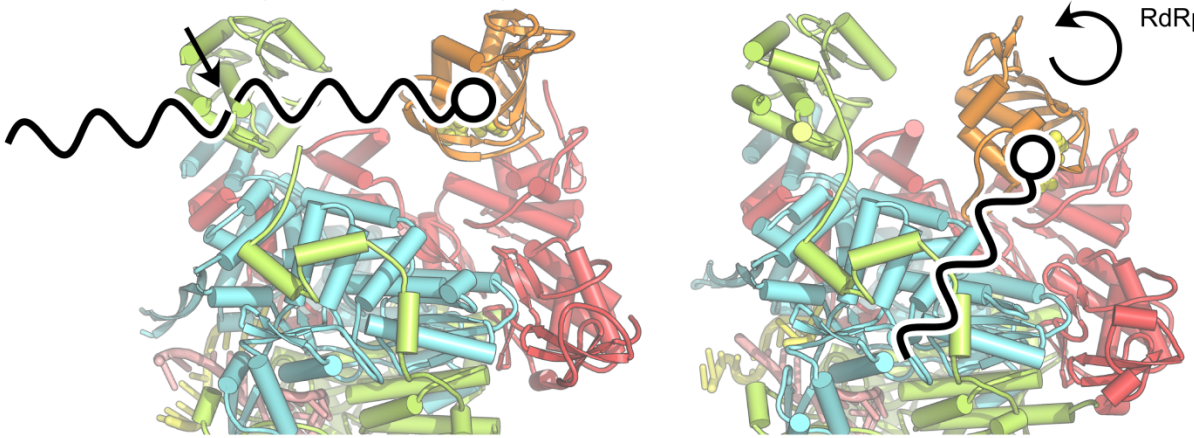
the endonuclease domain cuts mRNA 10-15 bases downstream of the cap

1

the cap binding domain binds host mRNA on its cap structure

3

the cap binding domain rotates by around 70° and thereby repositions the cleaved mRNA to the RdRp active site



4

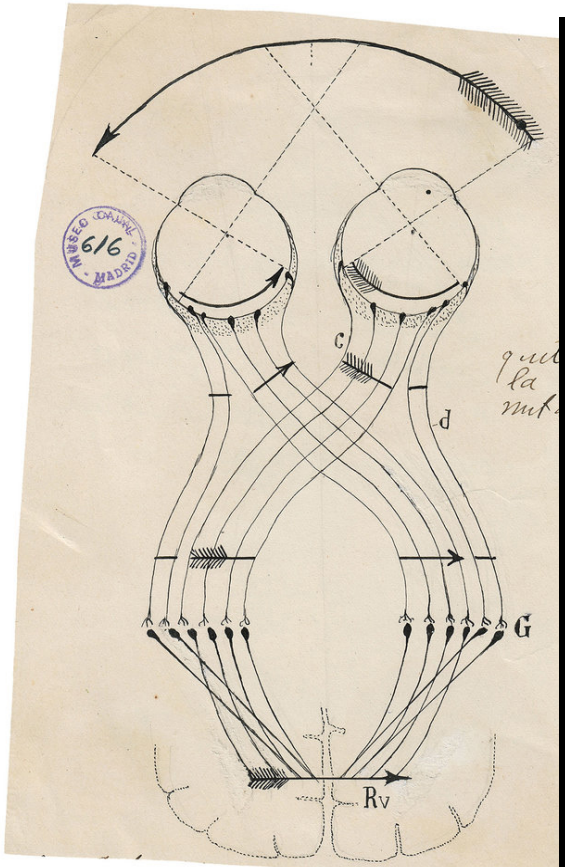
the cleaved host mRNA acts as primer for the transcription of viral RNA

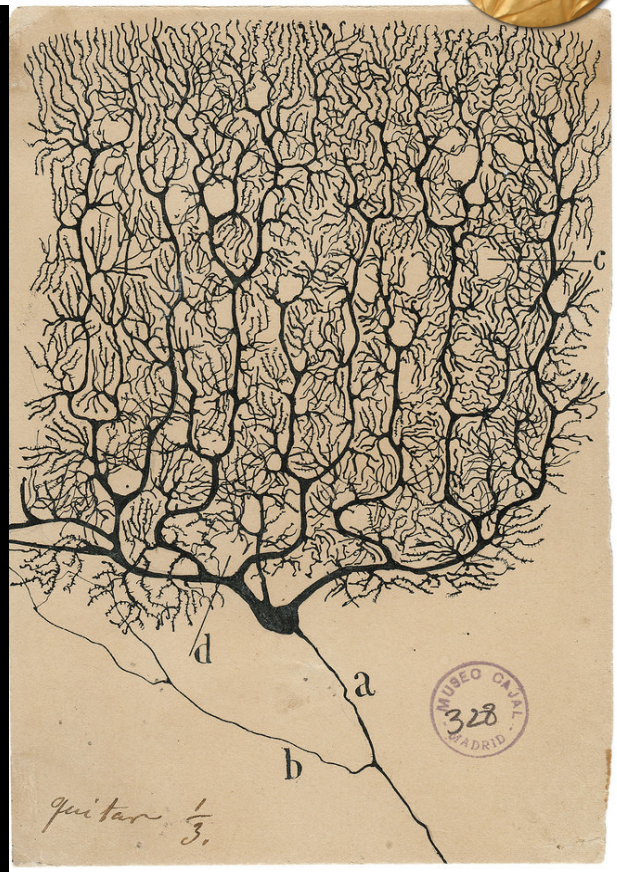
Pflug *et al*. (2014) *Nature*, **516**, 355–360.
Reich *et al*. (2014) *Nature*, **516**, 361–366.
Thierry *et al*. (2016) *Mol*. *Cell*, **61**, 125-137.
Lukarska *et al*. (2017) *Nature*, **541**, 117–121.
Omoto *et al*. (2018) *Sci. Rep.,* **8**, 9633. - Baloxavir marboxil

EMBL

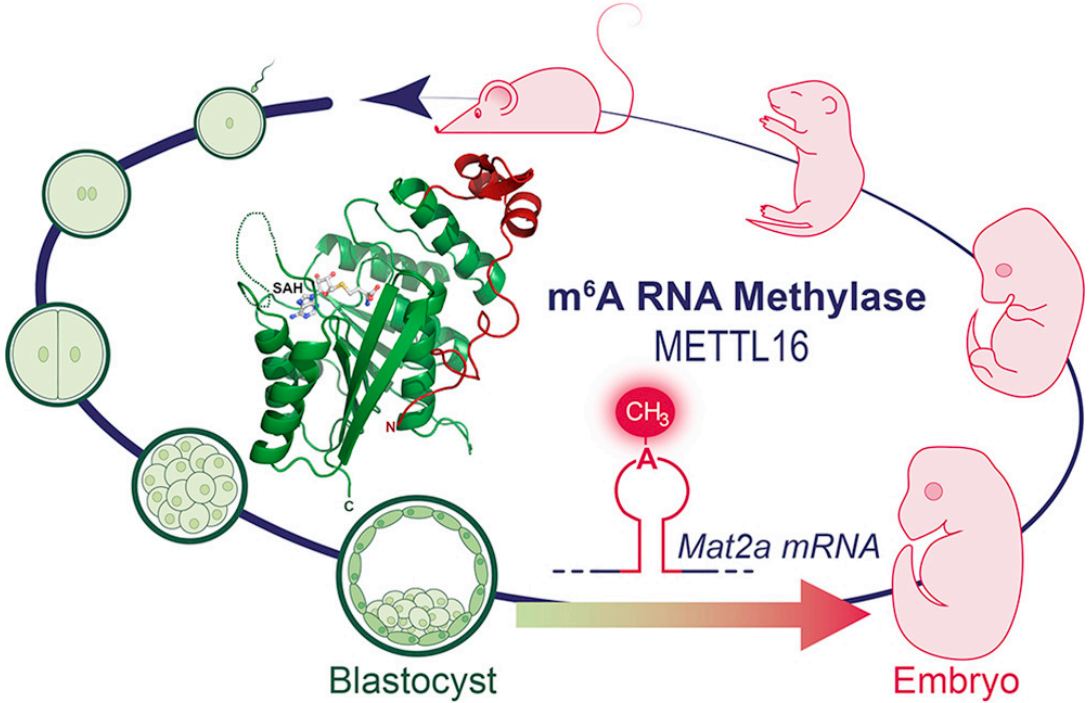
Ramón y Cajal (1852-1934)

Nobel prize in physiology or medicine (1906)
– *shared with Camillo Golgi*





METTL16 (*McCarthy team*) - EMBL-Grenoble outstation



Medel *et al*. (2018) *Mol*. *Cell*, **71**, 986-1000.

UNIVERSITÉ DE GENÈVE
FACULTÉ DES SCIENCES

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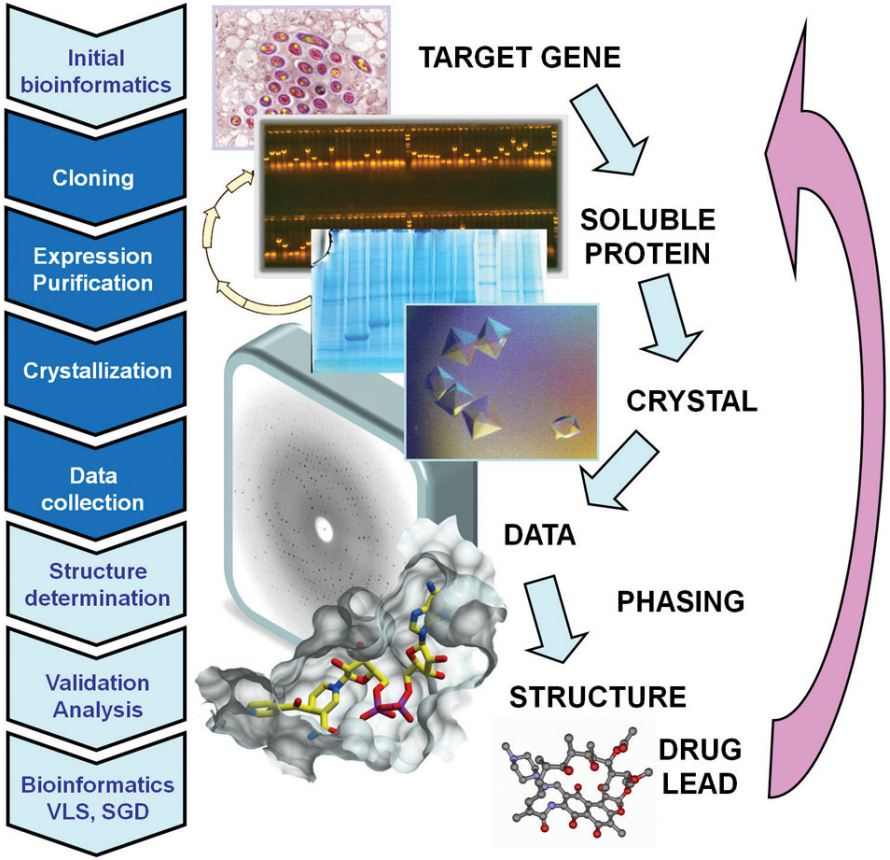
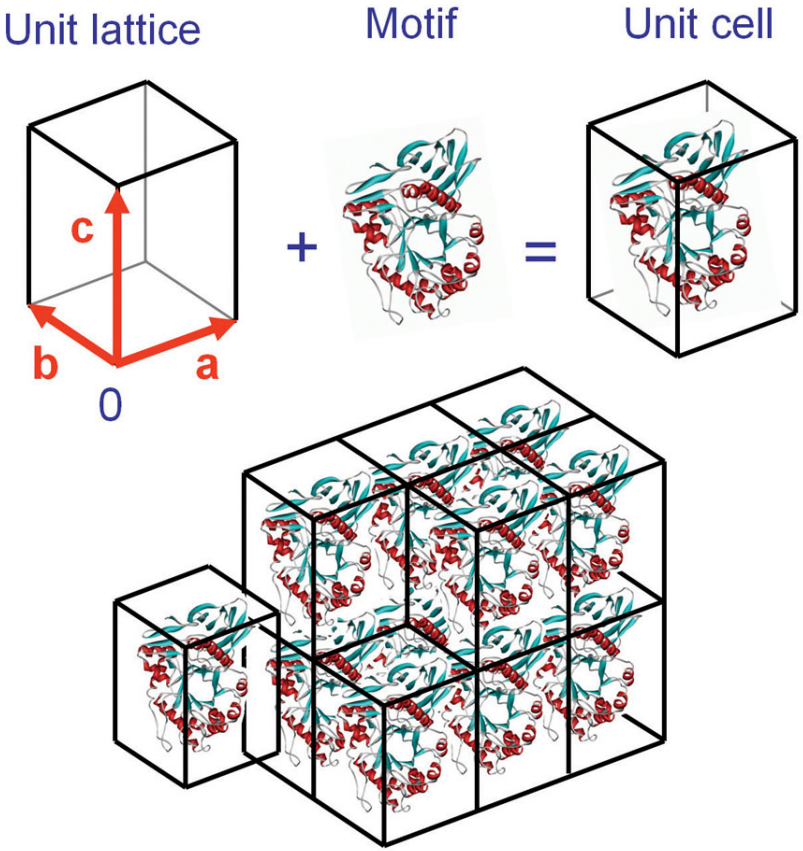
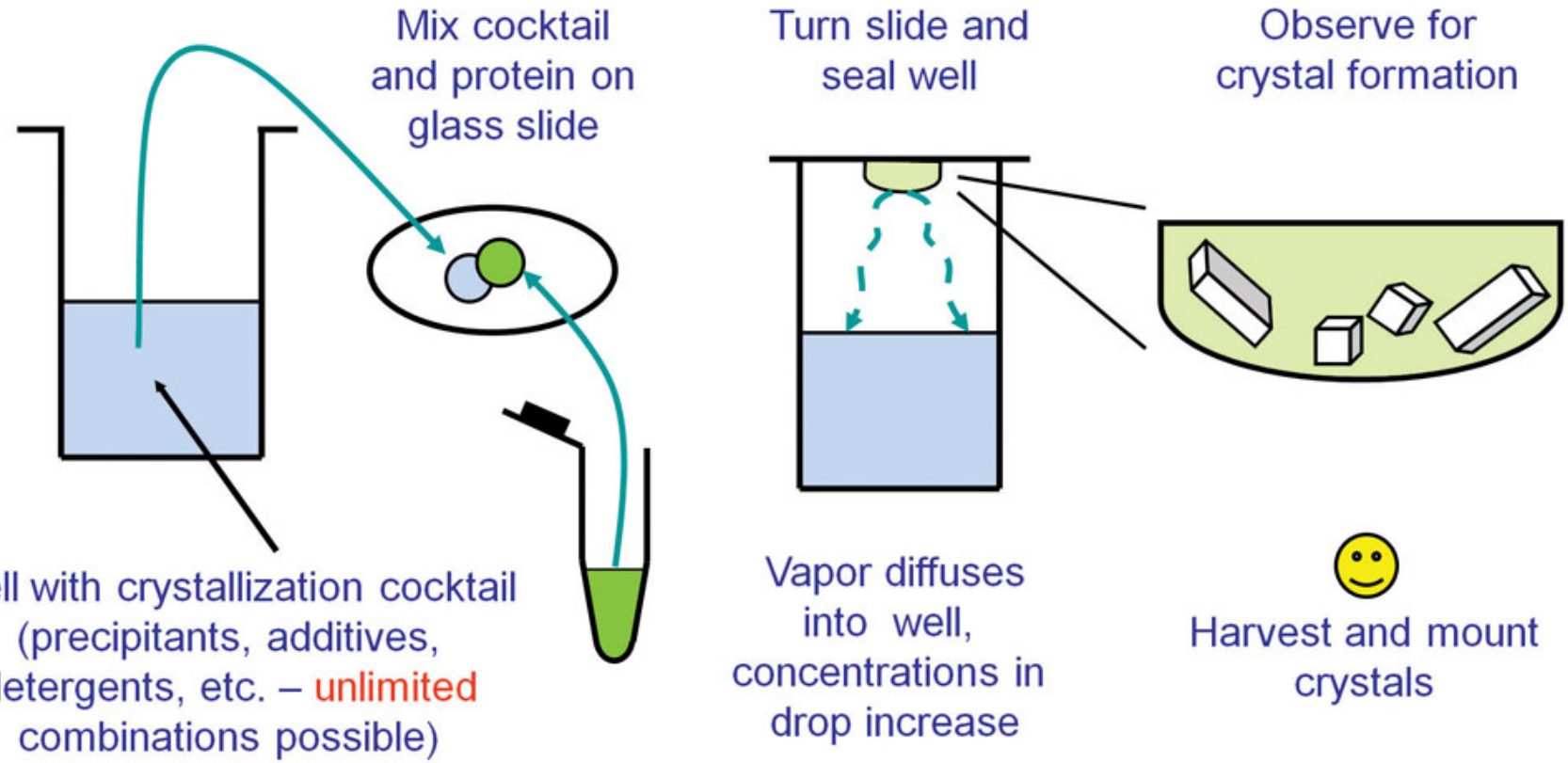
Structural biology techniques (MX)

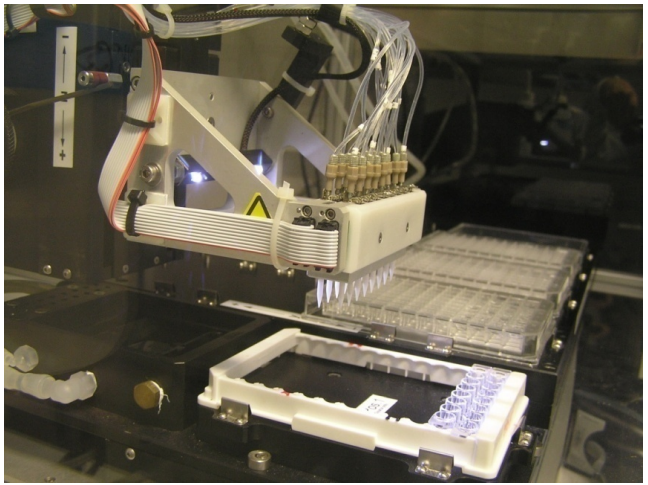


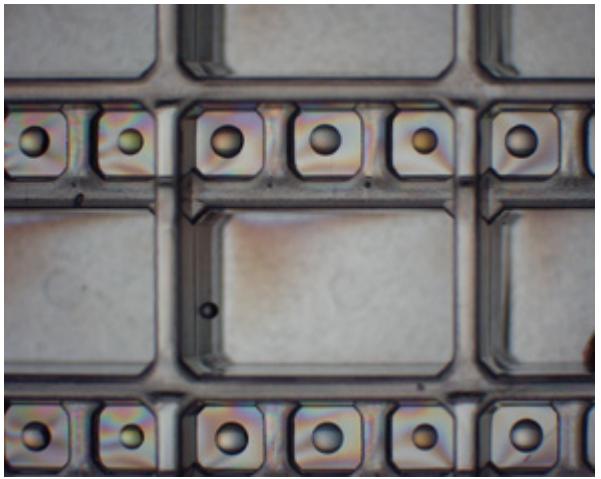


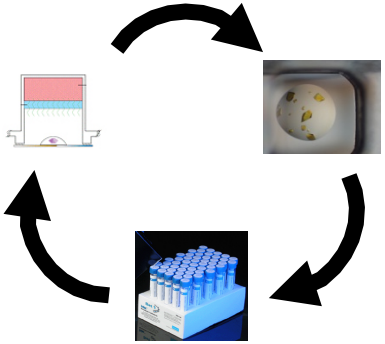
Protein crystallisation

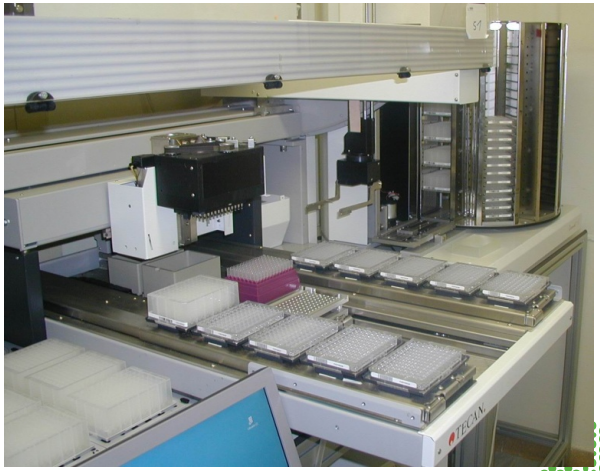


Automation of crystallisation



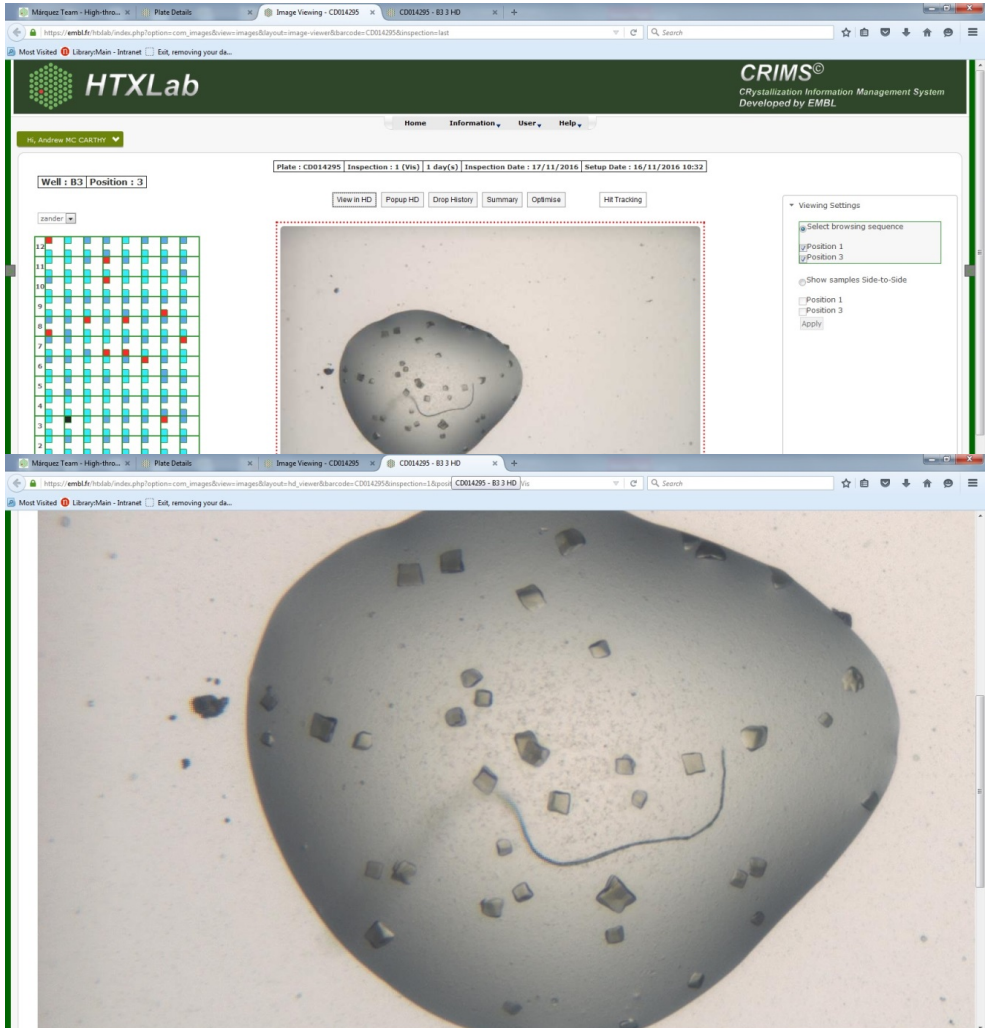




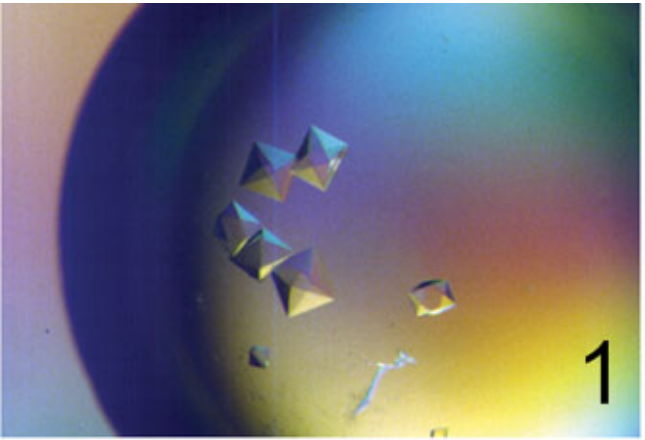


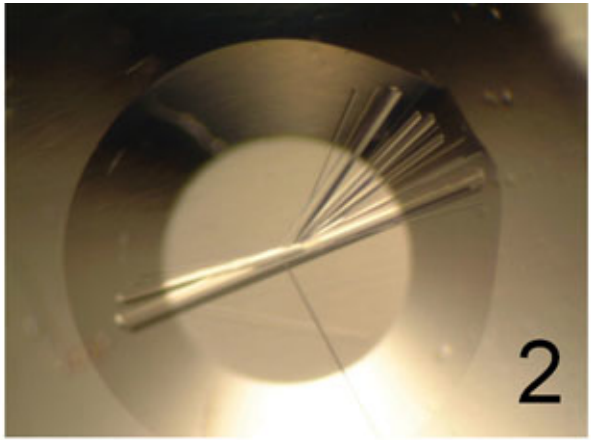
- ✓100 nl + 100 nl drops (50nl + 50nl)
- ✓3 samples per plate
- ✓6-9 samples in parallel
- ✓90 µl of sample for std. screening
- ✓Strict Quality Control

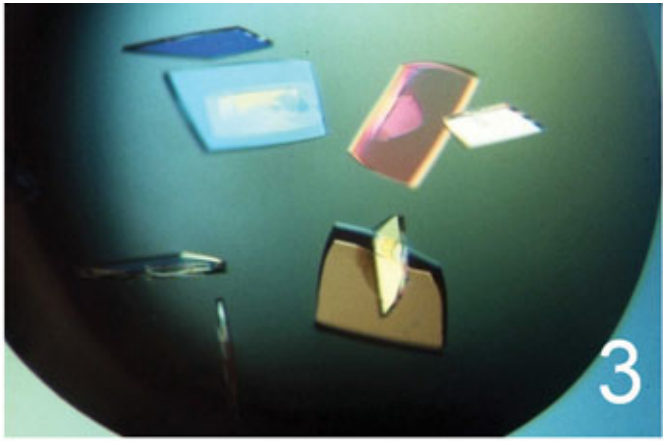
Automation of crystallisation

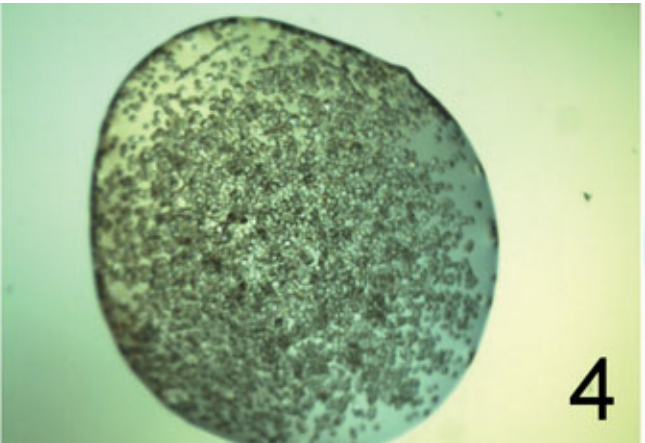


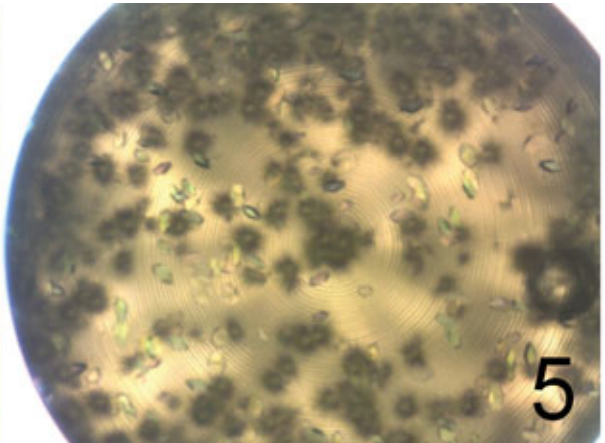
Protein crystals

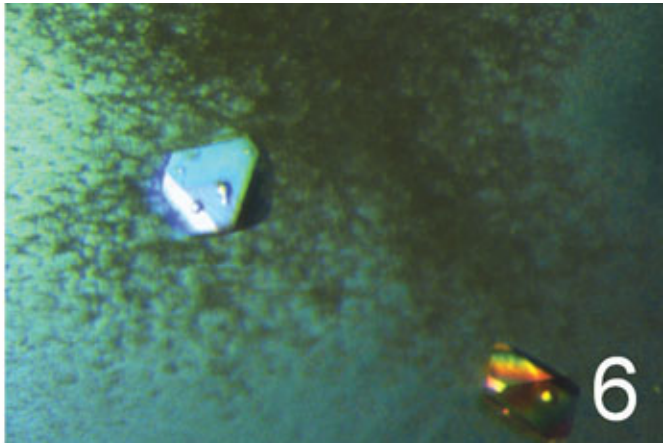












Darwin's formula

$$I(hkl) = I_{beam}\, r_e^2\, \frac{V_{xtal}}{V_{cell}}\, \frac{\lambda^3 L}{\omega V_{cell}}\, P\, A\, |F(hkl)|^2$$

I(hkl) - photons/spot (fully-recorded)

I_{beam} - incident (photons/s/m^2)

r_e - classical electron radius (2.818×10^{-15} m)

V_{xtal} - volume of crystal (in m^3)

V_{cell} - volume of unit cell (in m^3)

λ - x-ray wavelength (in meters!)

ω - rotation speed (radians/s)

L - Lorentz factor (speed/speed)

P - polarization factor

$(1+\cos^2(2\theta) - Pfac \cdot \cos(2\Phi)\sin^2(2\theta))/2$

A - absorption factor

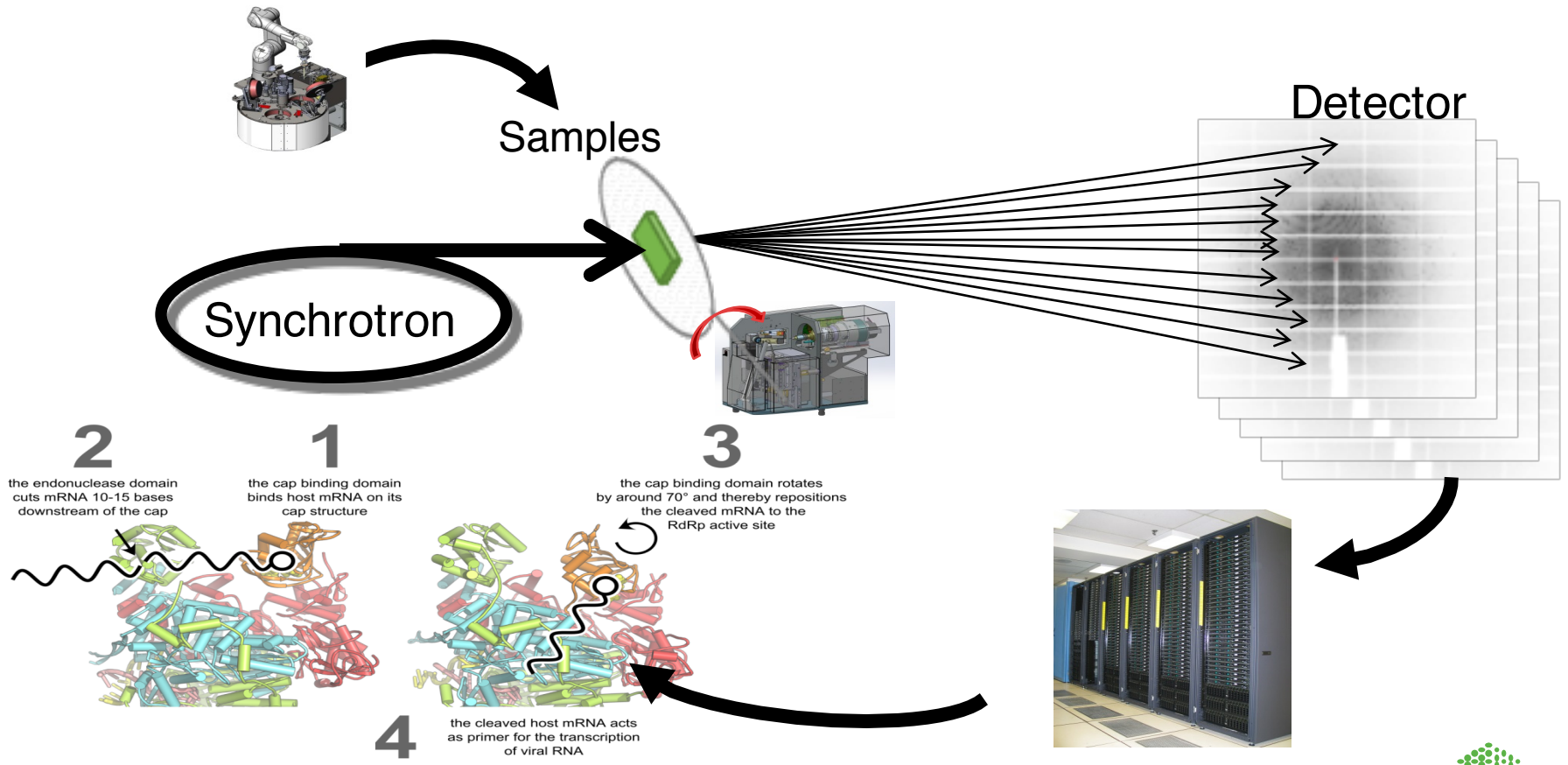
$\exp(-\mu_{xtal} \cdot l_{path})$

F(hkl) - structure amplitude (electrons)

C. G. Darwin (1914)

EMBL

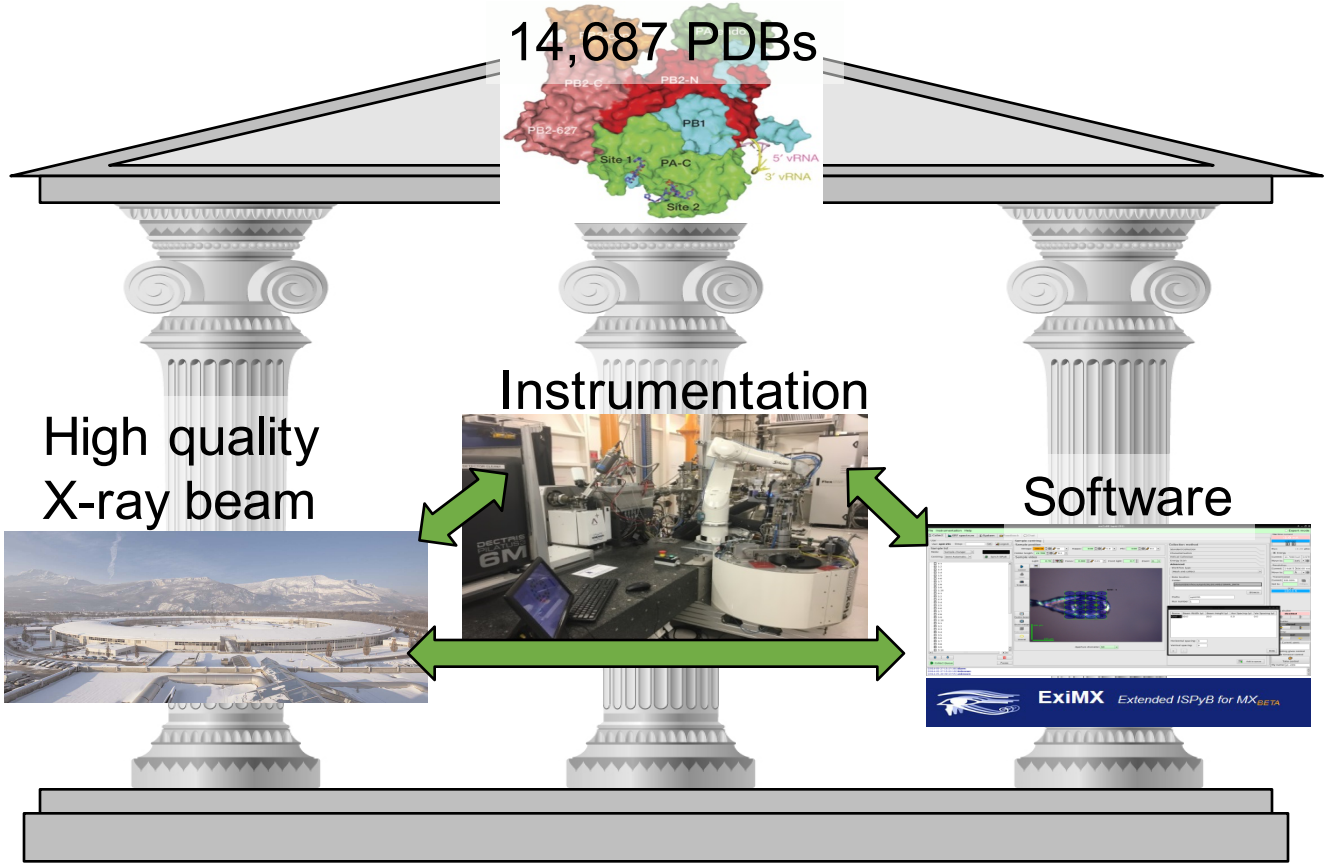
EMBL-Grenoble – EPN campus



Lukarska *et al*. (2017) *Nature*, **541**, 117-121.

EMBL

ESRF-EMBL Pillars of success



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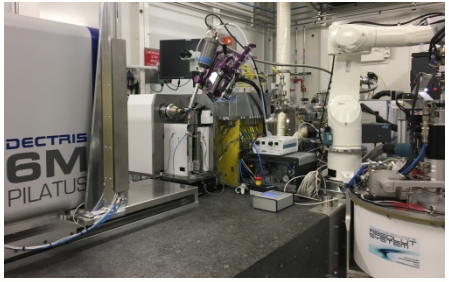
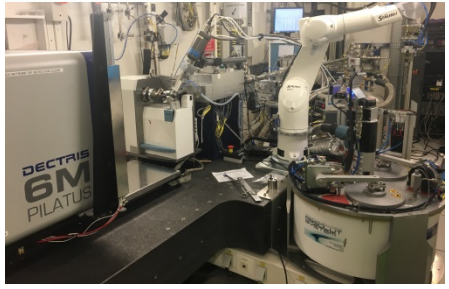
ESRF-EMBL Joint Structural Biology Group Beamlines (2018)

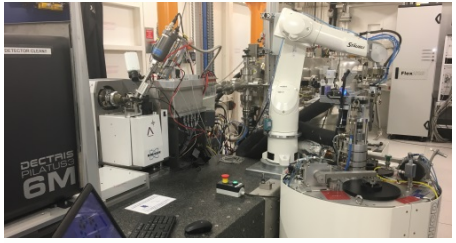
ID23-1 (tunable)



ID29 (tunable)



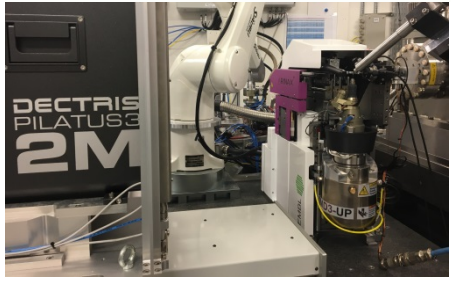
ID30B (tunable)



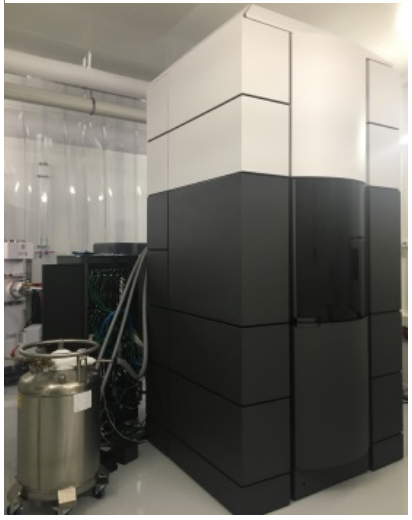
ESRF-EBS
Extremely Brilliant Source

12/2018 – 8/2020

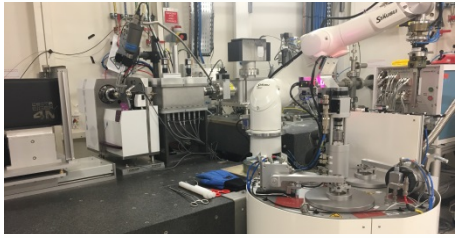
ID23-2 (μ focus)



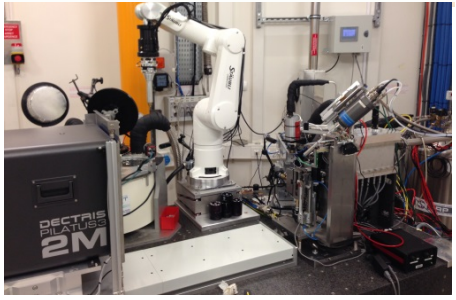
CM01



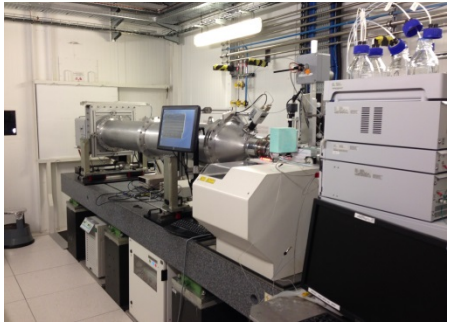
ID30-A3 (μ focus)



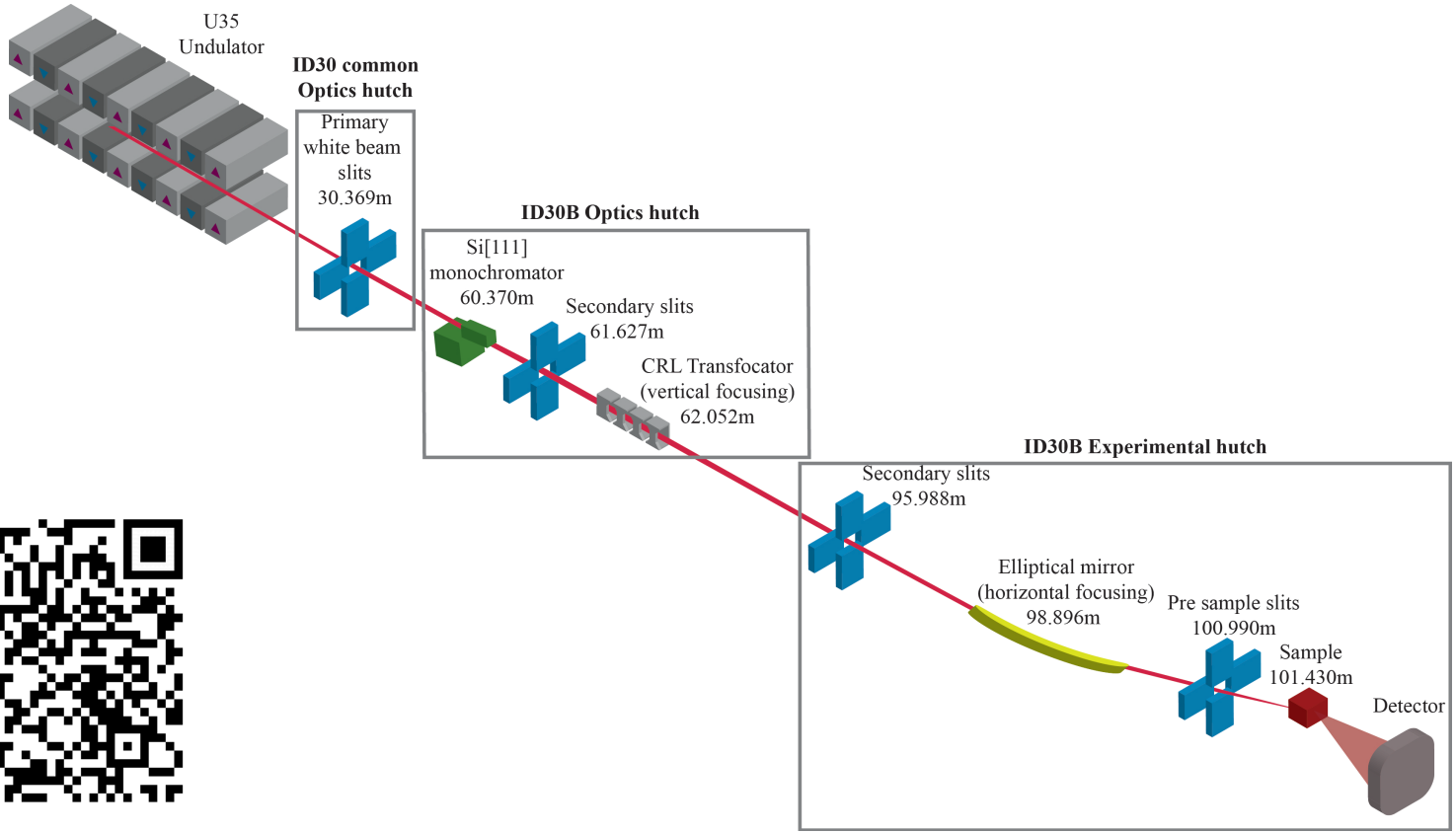
ID30-A1 (MASSIF)



BM29 (BioSAXS)



ID30B optical layout



McCarthy *et al.* (2018) J. Synchrotron Rad. **25**, 1249-1260

EMBL

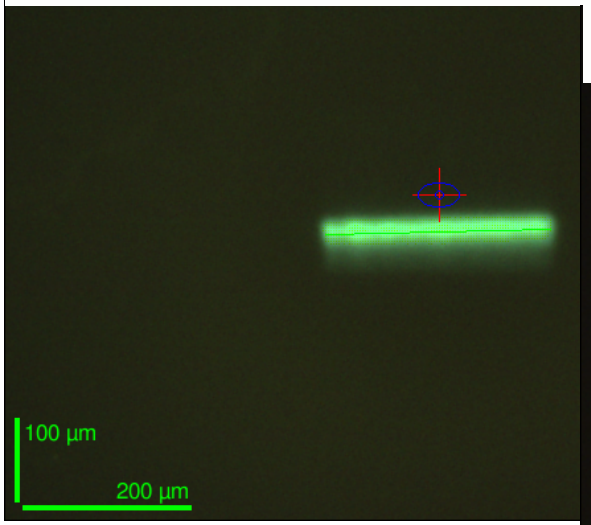
ID30B variable focus

Energy range: 6-20 keV

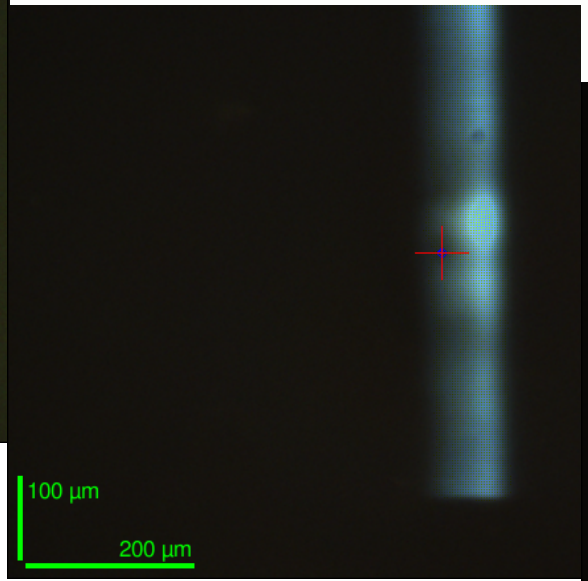
Flux ~ 5x10^{12} phs/sec/mm^2 at 12.7 keV

Beam size: <40μm^2 (apertures – 10, 20, 30, 50 and 75 μm^2)

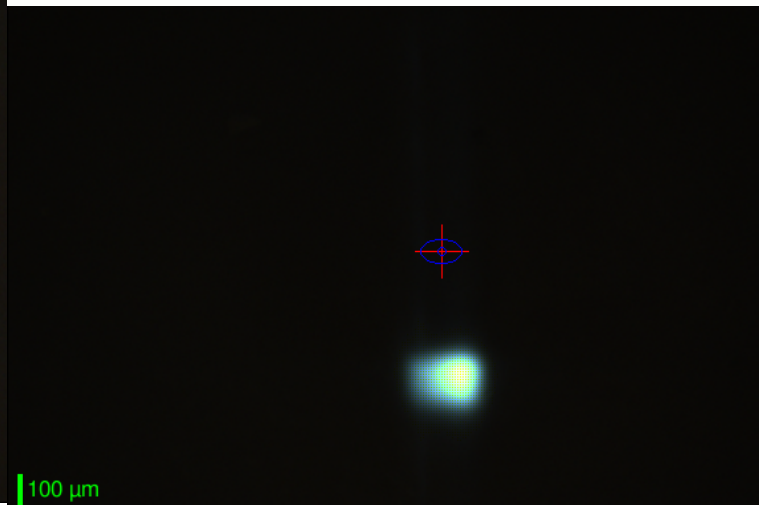
Vertical focusing, no horizontal focusing (mirror unbent)



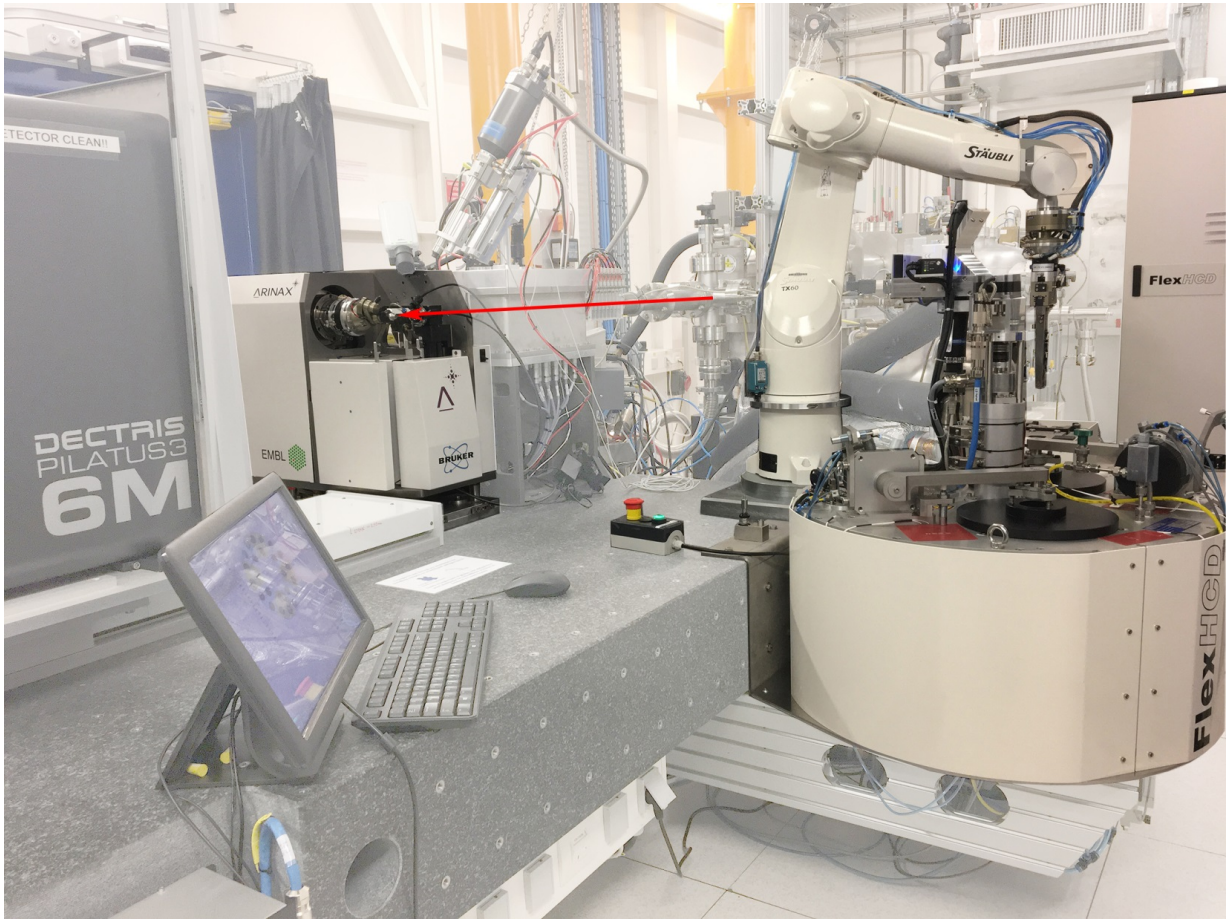
No vertical focusing – mirror bent



Focused beam

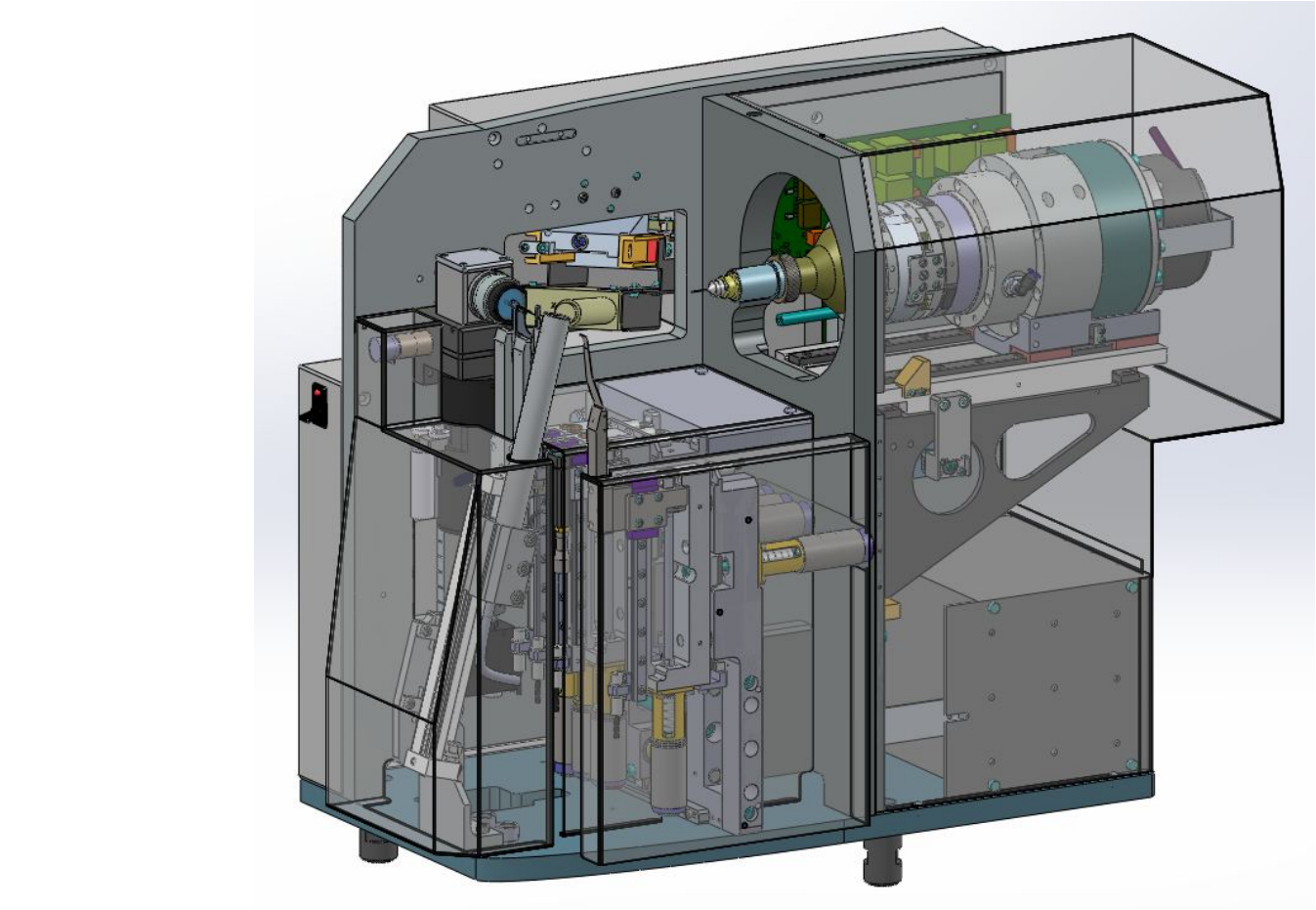


ID30B at the ESRF



EMBL

Microdiffractometer – MD2S

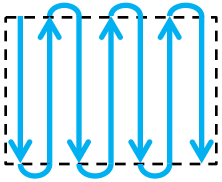


EMBL

MXCUBE

Prepare for data collection
(frame on hardware trigger)

Detector



Do a grid scan
Scan Finished

Full PMAC
with latch

Trigger

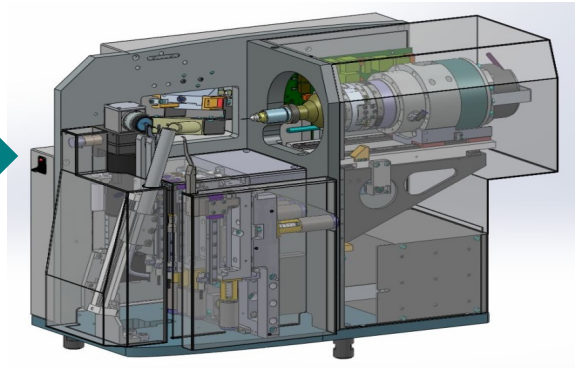
Frame readout
For precise coder latch

Latched
Position

Done

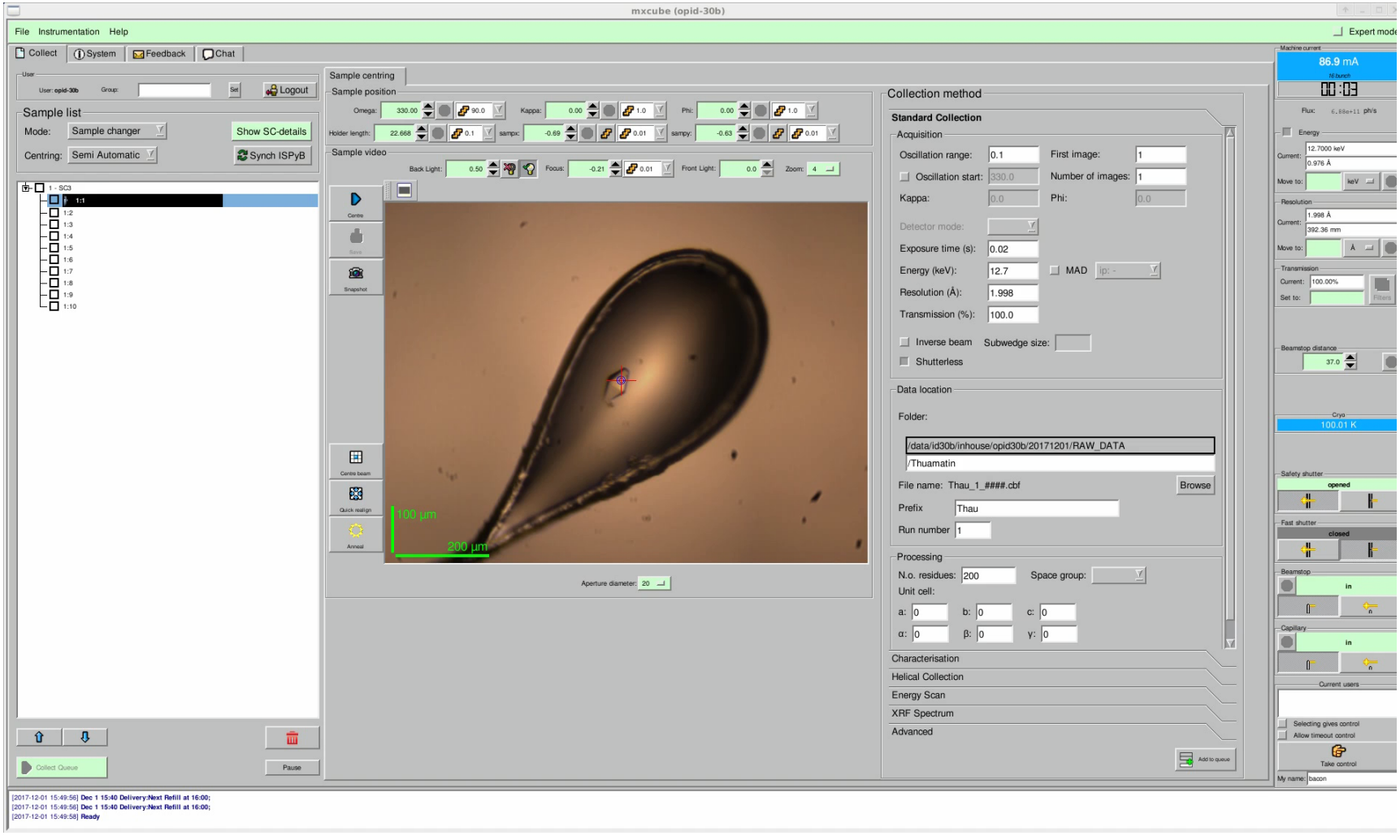
MD software

PMAC rack

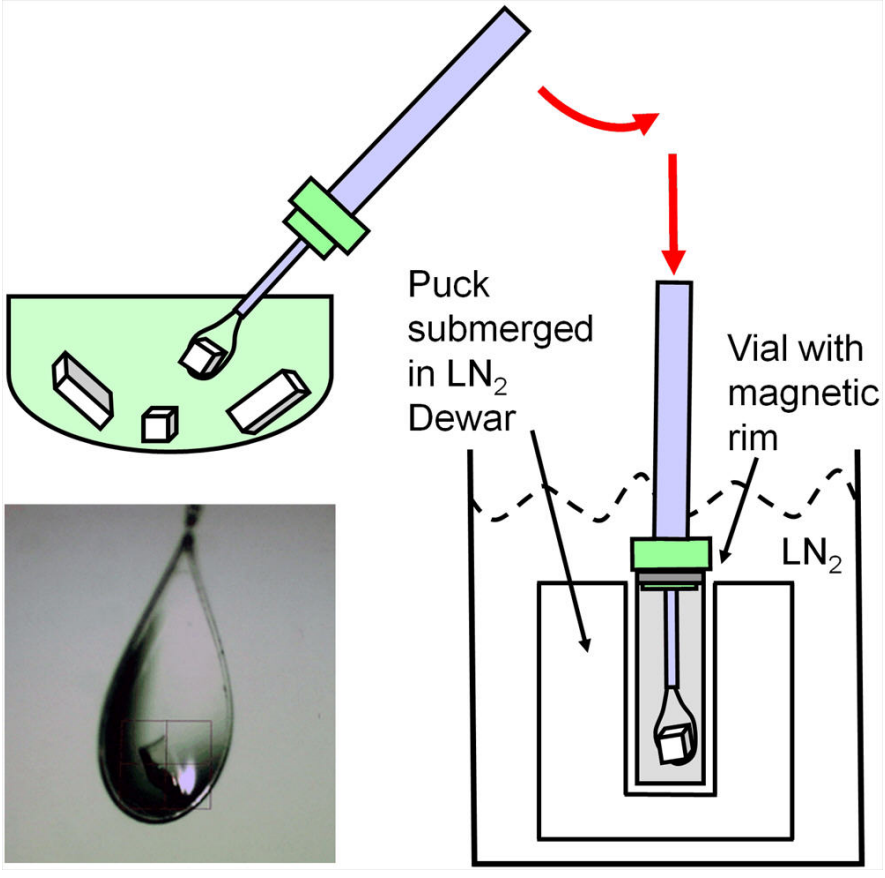


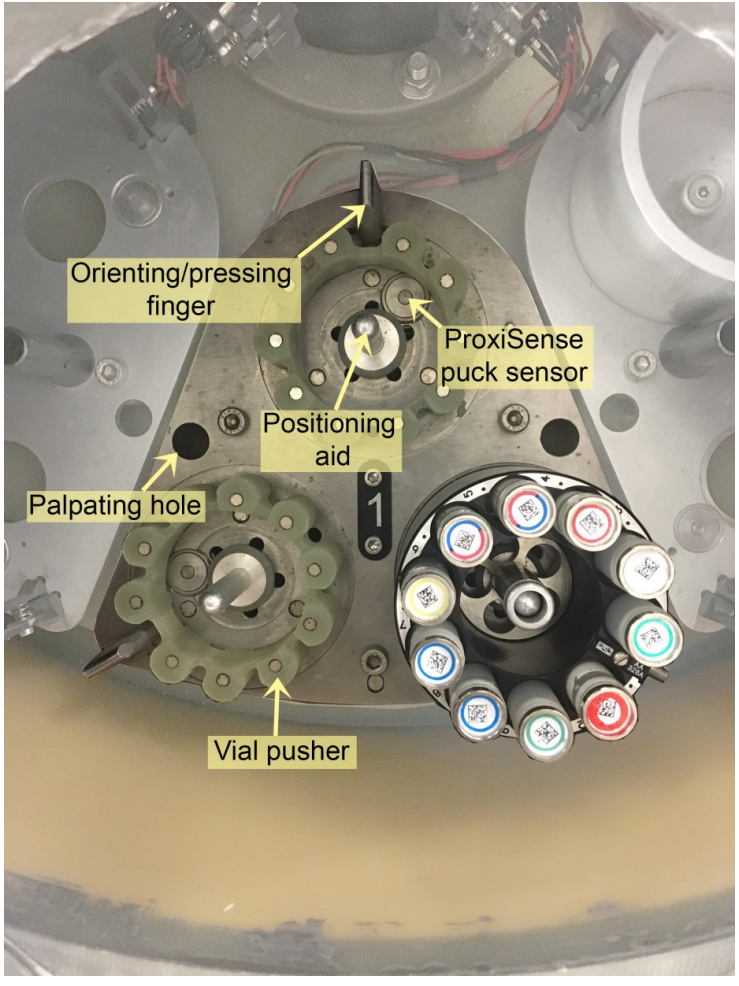
Diffractometer

EMBL


mxcube (opid-30b)
File Instrumentation Help
Expert mode
Collect System Feedback Chat
User
User: opid-30b Group: Set Logout
Sample list
Mode: Sample changer Show SC-details
Centring: Semi Automatic Synch ISPyB
1 - SC3
1:1
1:2
1:3
1:4
1:5
1:6
1:7
1:8
1:9
1:10
Collect Queue
Pause
Sample centring
Sample position
Omega: 330.00 90.0 Kappa: 0.00 1.0 Phi: 0.00 1.0
Holder length: 22.668 0.1 sampx: -0.69 0.01 sampy: -0.63 0.01
Sample video
Back Light: 0.50 Focus: -0.21 0.01 Front Light: 0.0 Zoom: 4
Centre
Save
Snapshot
Centre beam
Quick realign
Anneal
100 µm
200 µm
Aperture diameter: 20
Collection method
Standard Collection
Acquisition
Oscillation range: 0.1 First image: 1
Oscillation start: 330.0 Number of images: 1
Kappa: 0.0 Phi: 0.0
Detector mode:
Exposure time (s): 0.02
Energy (keV): 12.7 MAD ip: -
Resolution (Å): 1.998
Transmission (%): 100.0
Inverse beam Subwedge size:
Shutterless
Data location
Folder:
/data/id30b/inhouse/opid30b/20171201/RAW_DATA
/Thuamatin
File name: Thau_1_####.cbf Browse
Prefix Thau
Run number 1
Processing
N.o. residues: 200 Space group:
Unit cell:
a: 0 b: 0 c: 0
α: 0 β: 0 γ: 0
Characterisation
Helical Collection
Energy Scan
XRF Spectrum
Advanced
Add to queue
Machine current
86.9 mA
Flux:
Energy
Current: 12.7000 keV
0.976 Å
Move to:
Resolution
Current: 1.998 Å
392.36 mm
Transmission
Current: 100.00%
Set to:
Filters
Beamstop distance
37.0
Cryo
100.01 K
Safety shutter
opened
Fast shutter
closed
Beamstop
in
Capillary
in
Current users
Selecting gives control
Allow timeout control
Take control
My name: bacon
[2017-12-01 15:49:56] Dec 1 15:40 Delivery:Next Refill at 16:00;
[2017-12-01 15:49:56] Dec 1 15:40 Delivery:Next Refill at 16:00;
[2017-12-01 15:49:58] Ready

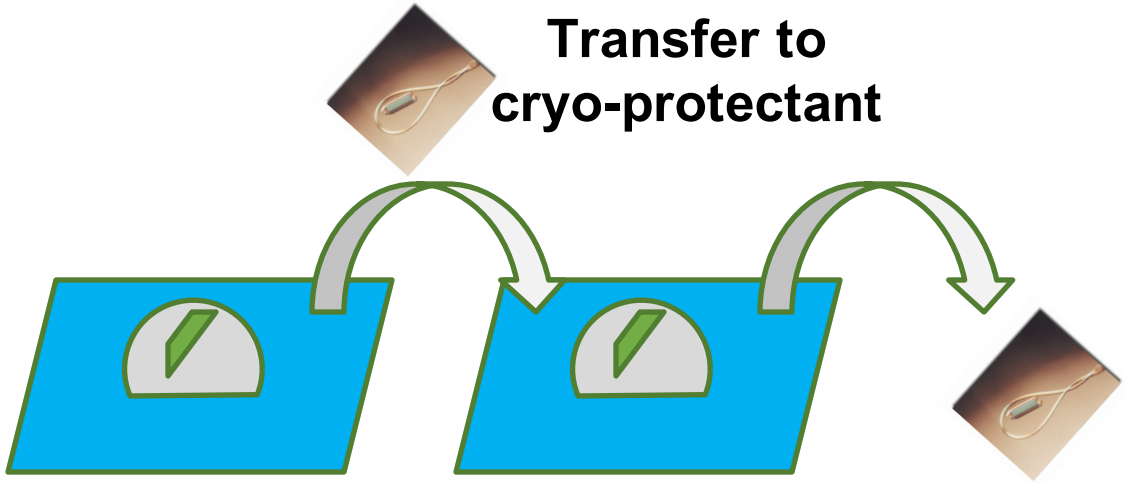
Crystal fishing and storage





Crystal fishing and storage

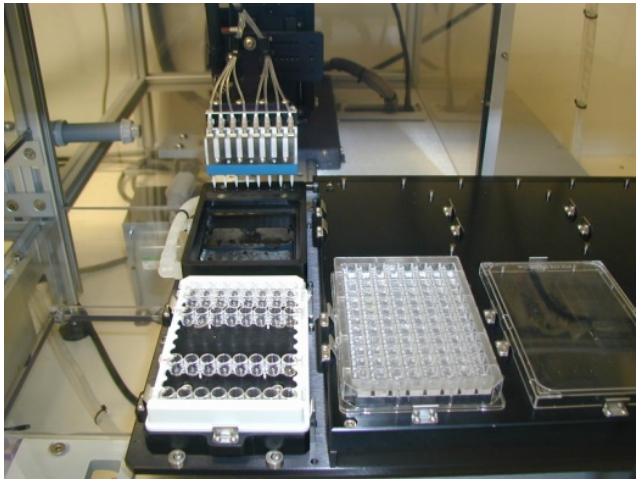
Cryo-protectants and/or ligands through serial transfer



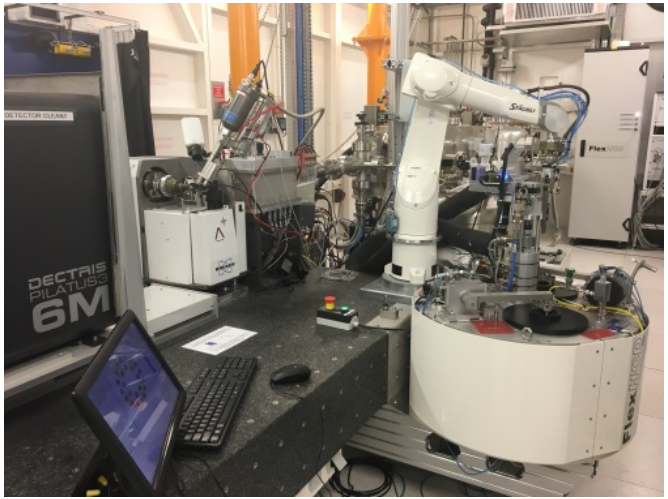
EMBL

Crystal Direct™: a new concept for Crystal harvesting

HTX Lab, J.A. Marquez



Instrumentation, F. Cipriani



Open Access HTX Facility

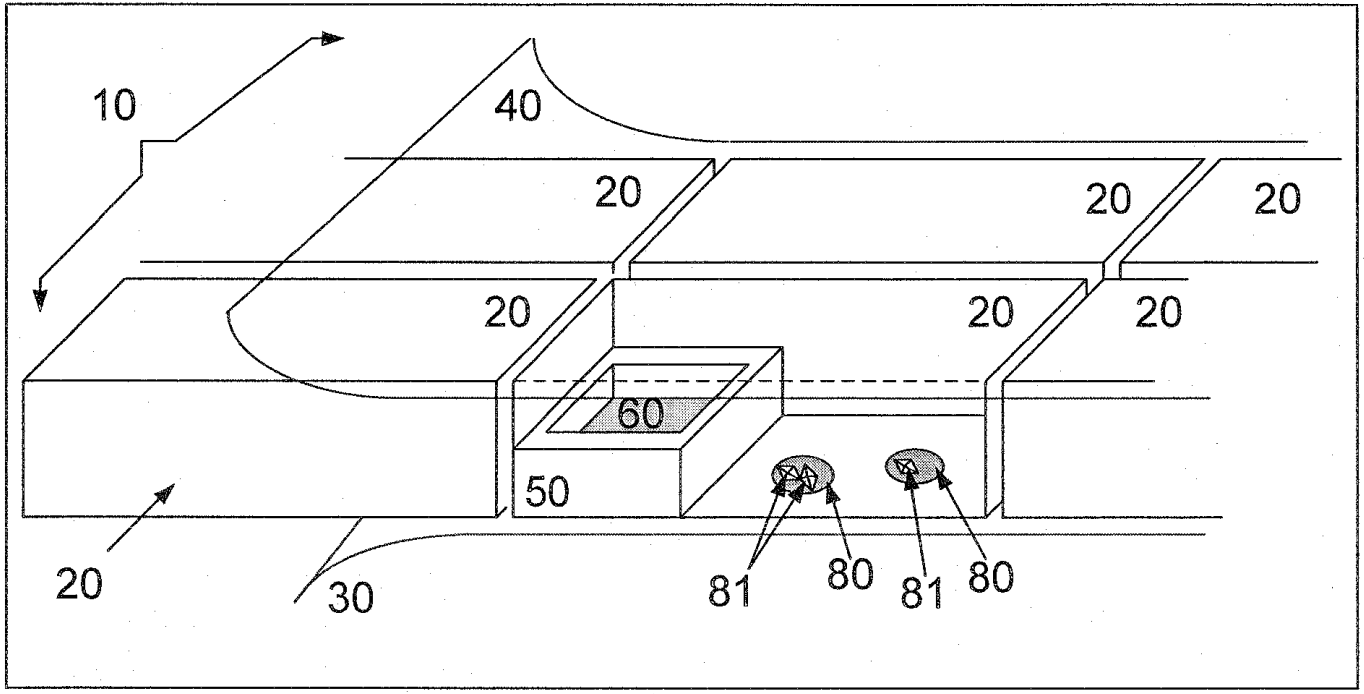
- Over 5000 registered users
- 1200 samples processed per year
- Over 3.9 million individual crystallization experiments performed

Synchrotron Instrumentation

- Instruments for MX and SAXS beamlines
- Beamline automation
- Sample holder standards
  - SPINE
  - NewPin

EMBL

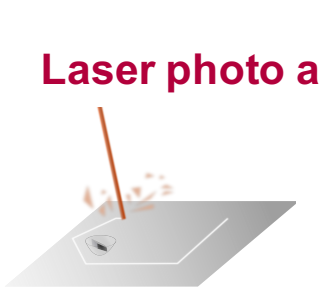
Crystal Direct™: a new concept for Crystal harvesting

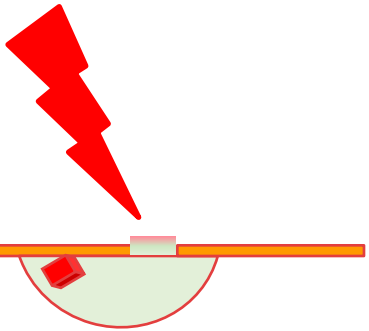


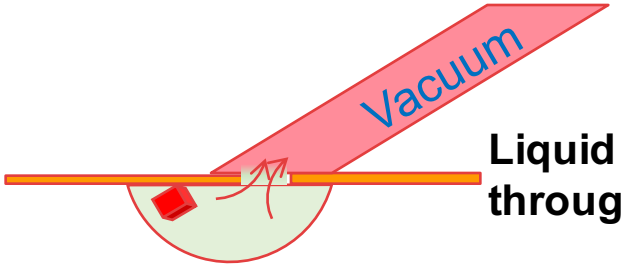
EMBL

Crystal Direct™: a new concept for Crystal harvesting

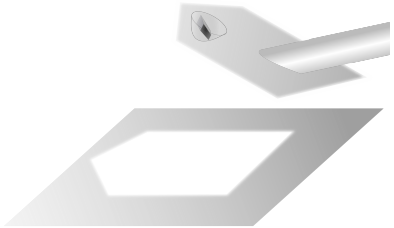
Laser photo ablation



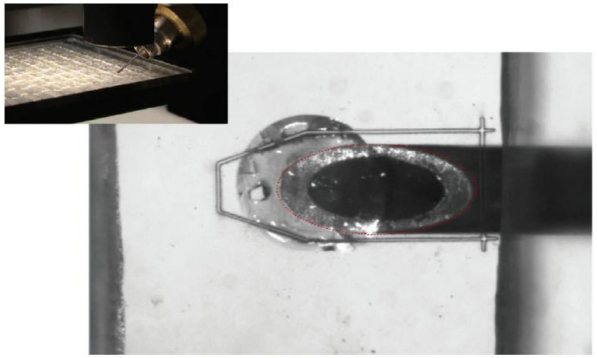




Liquid removal through aspiration



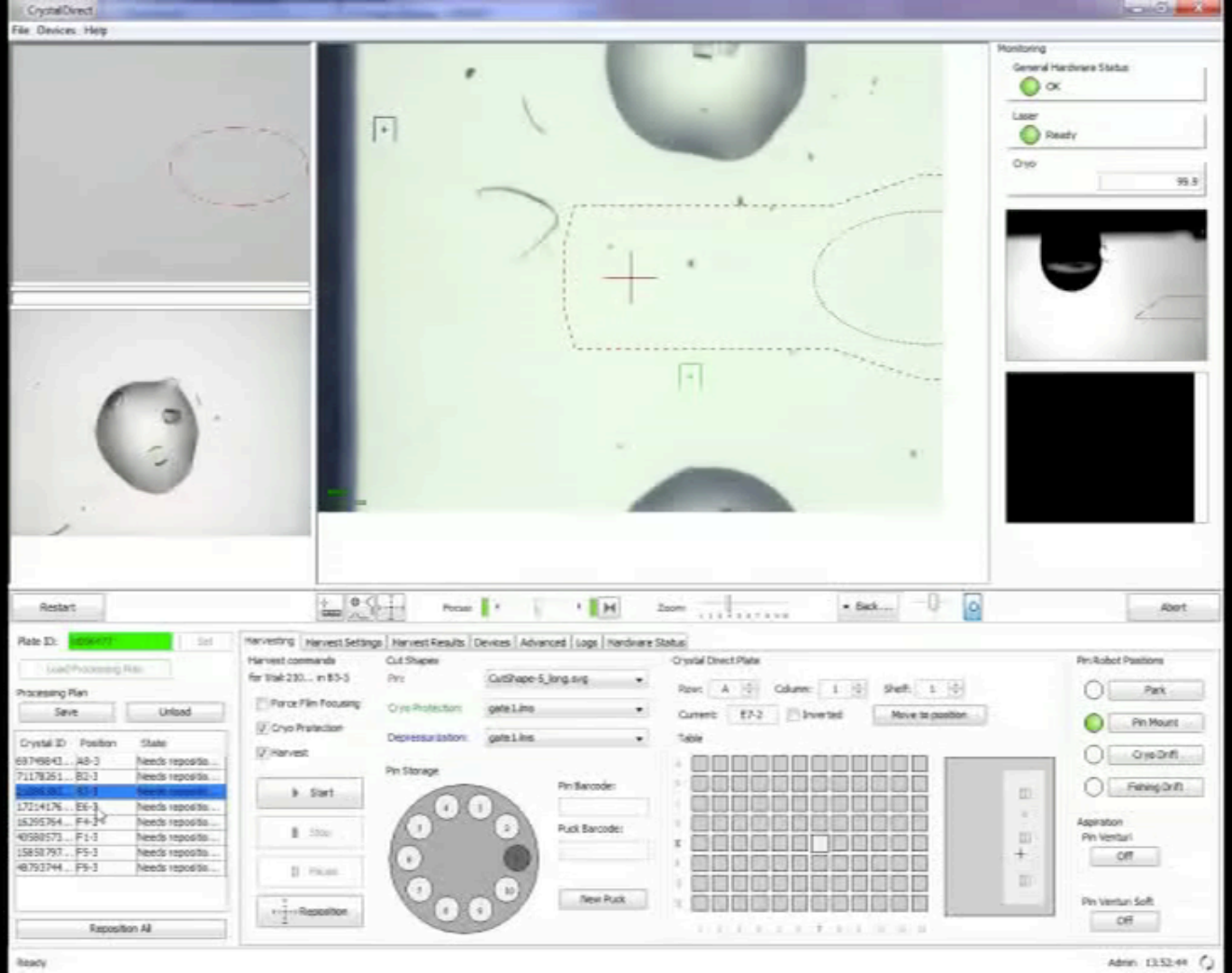
Reduce sample size
increased cooling rate
Reduce background scattering



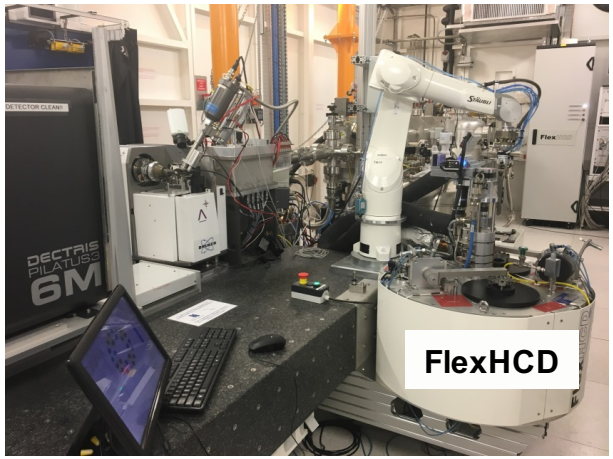
Harvesting crystals grown on a thin film by photo ablation

Zander et al. (2016) Acta Cryst. D**72**, 454-466

EMBL


CrystalDirect
File Devices Help
Monitoring
General Hardware Status
OK
Laser
Ready
Cryo
99.9
Restart
Focus
Zoom
Back...
Abort
Plate ID:
Processing Plan
Save
Unload
Crystal ID
Position
State
Reposition All
Harvesting
Harvest Settings
Harvest Results
Devices
Advanced
Logs
Hardware Status
Harvest commands
Cut Shapes
Pin:
CutShape-5_long.svg
Cryo Protection:
gate1.imo
Depressurization:
gate1.ins
Force Film Focusing
Cryo Protection
Harvest
Start
Stop
Pause
Reposition
Pin Storage
Pin Barcode:
Puck Barcode:
New Puck
Crystal Direct Plate
Row:
Column:
Shelf:
Current:
Inverted
Move to position
Table
Pin Robot Positions
Park
Pin Mount
Cryo Drift
Fishing Drift
Aspiration
Pin Venturi
Off
Pin Venturi Soft
Off
Ready
Admin

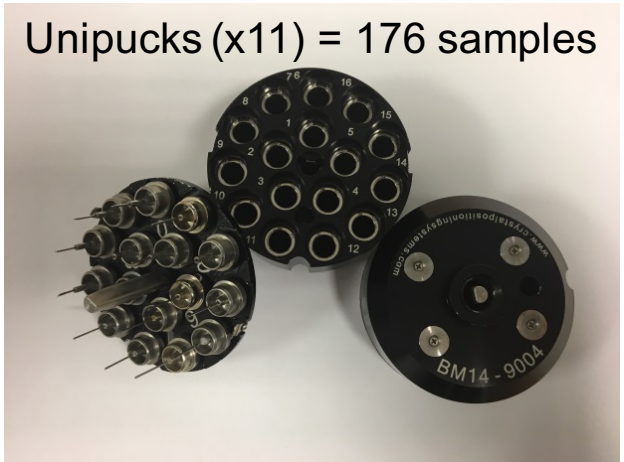
FlexHCD – A versatile sample changer



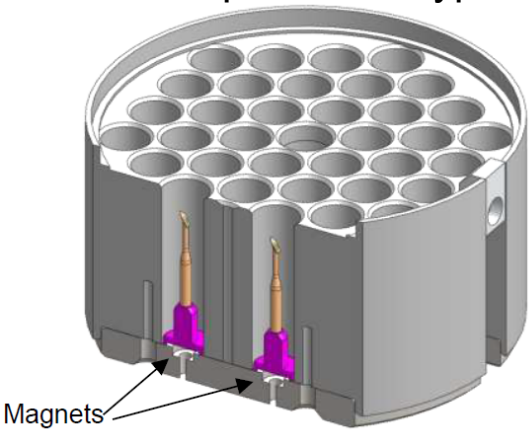
SPINE baskets (x12) = 120 samples

ESRF
SLS
BESSY
PETRAIII
ALBA

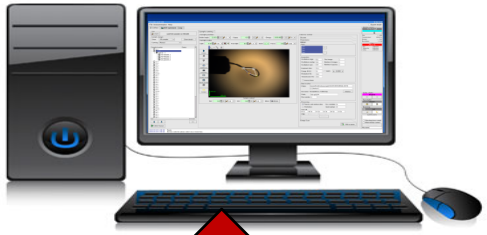
Unipucks (x11) = 176 samples



New sample holder types



MXCuBE



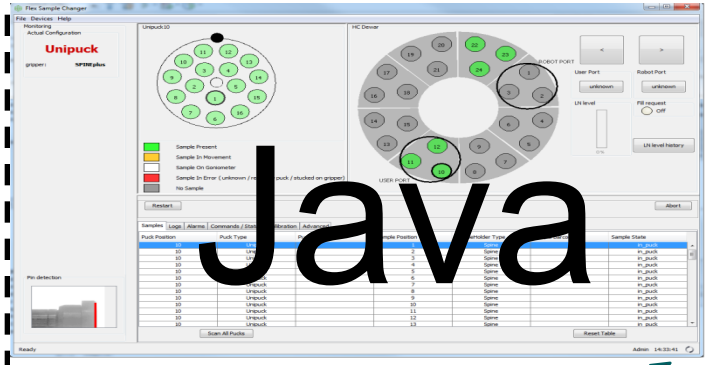
Tango (Exporter…)

VAL3 movement sequences:
- Load trajectory, Unload trajectory…

Local regulation loops (PLCs)
- Dewar temperature
- De-freezing station temperature
- LN level regulation

GenericStaubCom server

StaubCom protocol



PC windows (or Linux)

RS232

Ethernet

Ethernet

RS232

OneWire:
Gripper identification

DM reader

Camera + image processing:
Gripper calibration
Pin detection

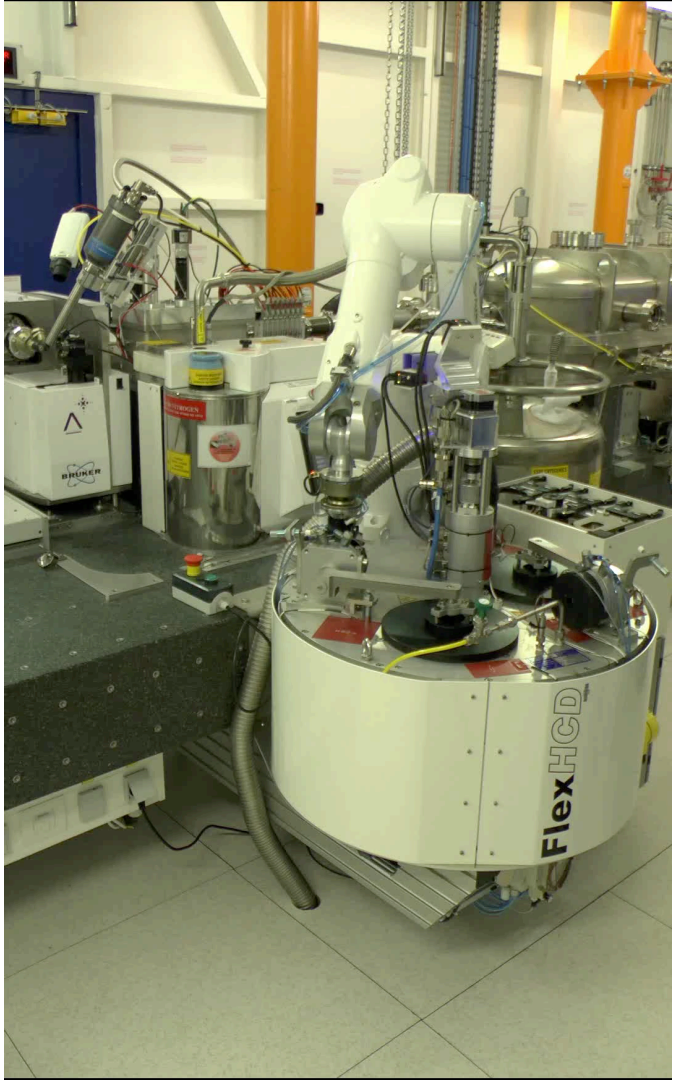
ProxiSense Card:
Puck presence & type detection

modbus

HCD:
- Plate rotation
- LN filling
- Open/Close ports

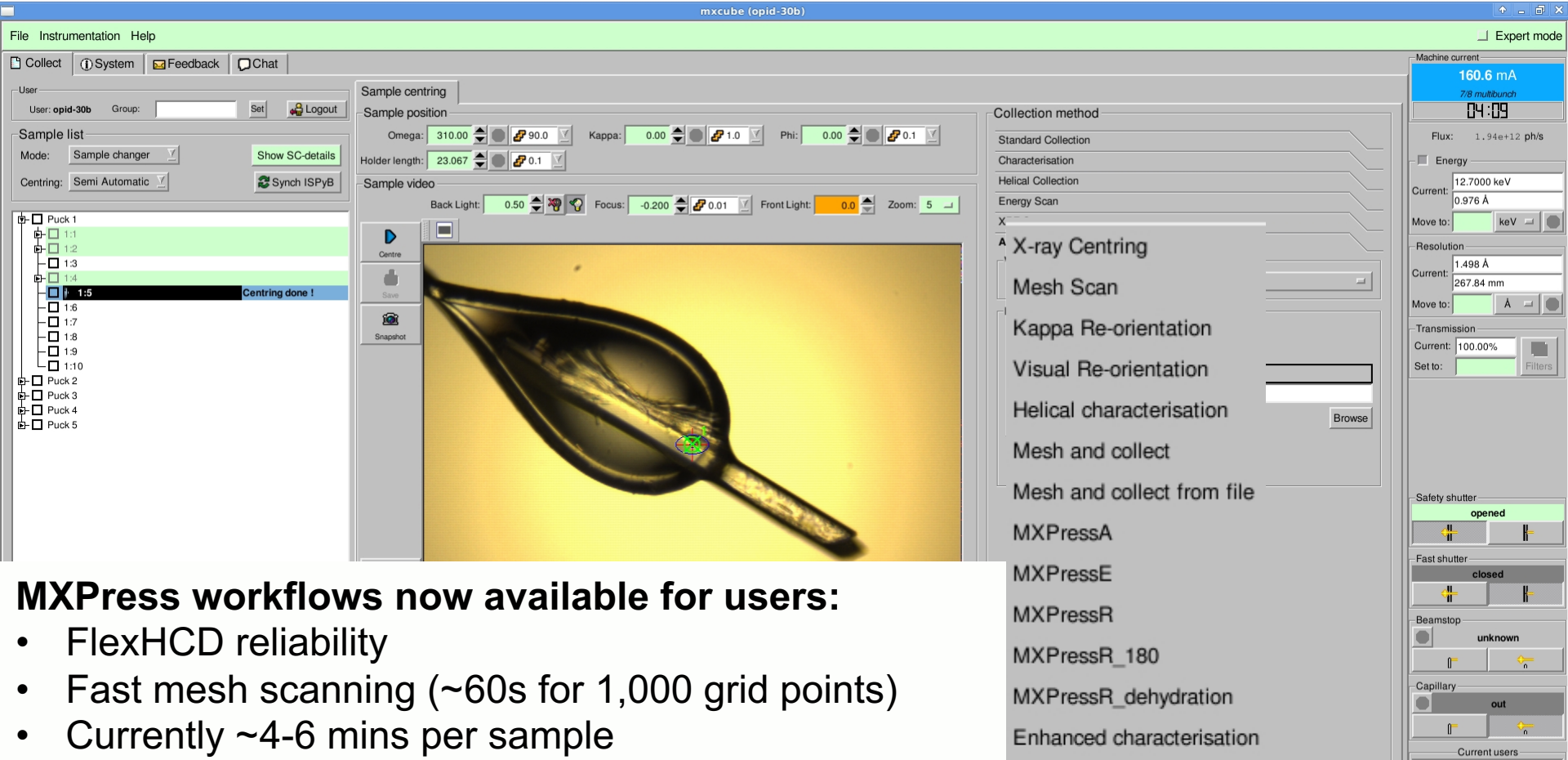
Local touch screen

EMBL



EMBL

Automation of MX experiments

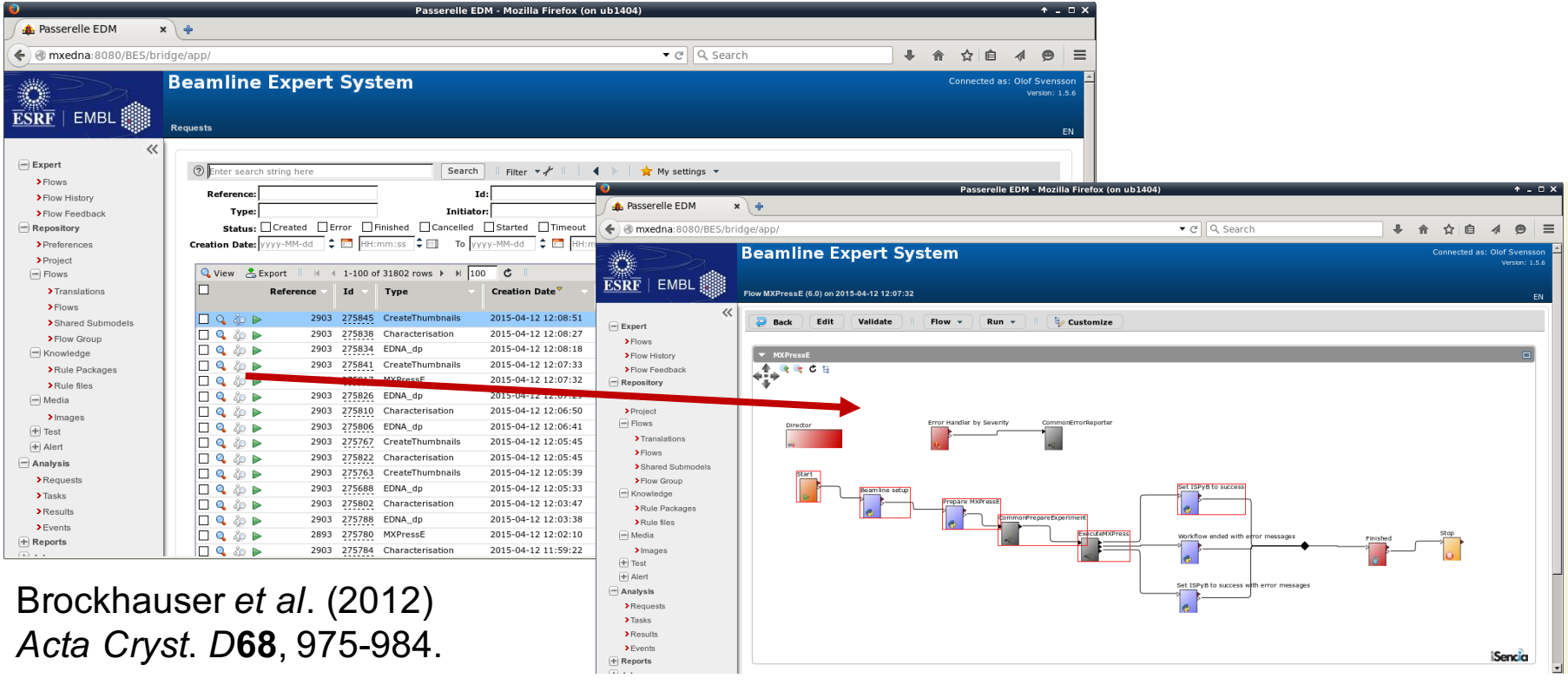


MXPress workflows now available for users:

- FlexHCD reliability
- Fast mesh scanning (~60s for 1,000 grid points)
- Currently ~4-6 mins per sample

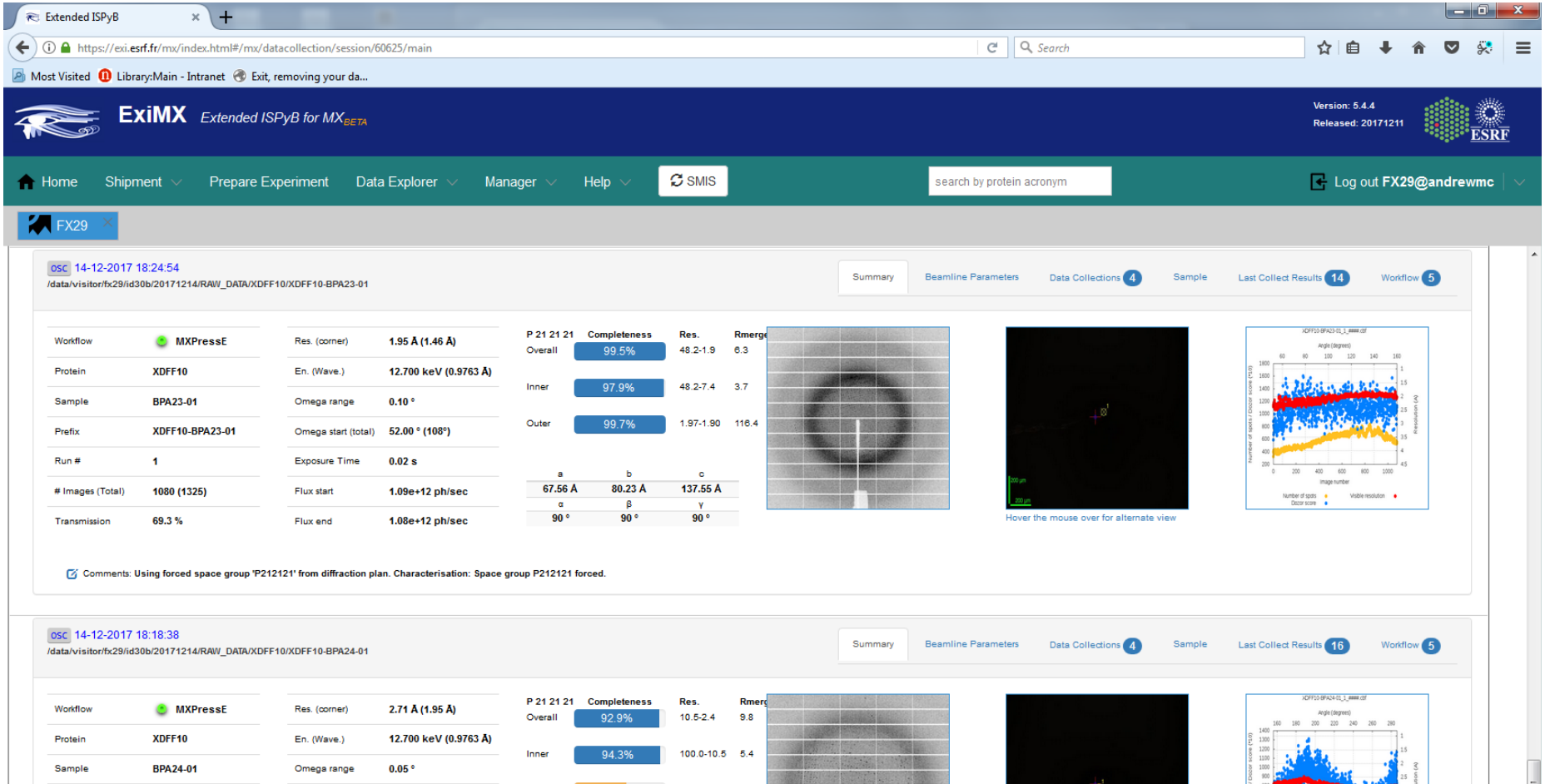
ESRF-EMBL Beamline Expert System

- Passerelle-EDM – A Web interface for workflow execution that logs all metadata stored in database

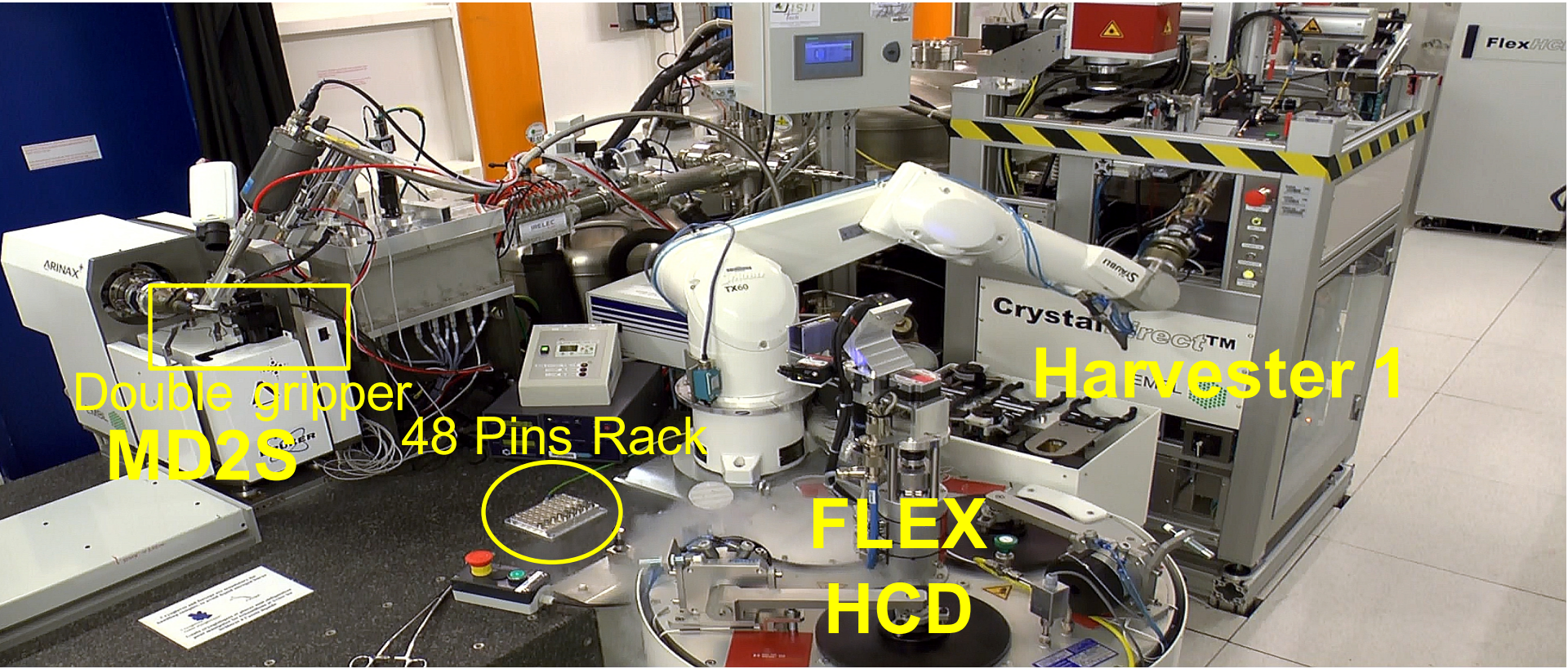


Brockhauser *et al*. (2012)
Acta Cryst. *D***68**, 975-984.

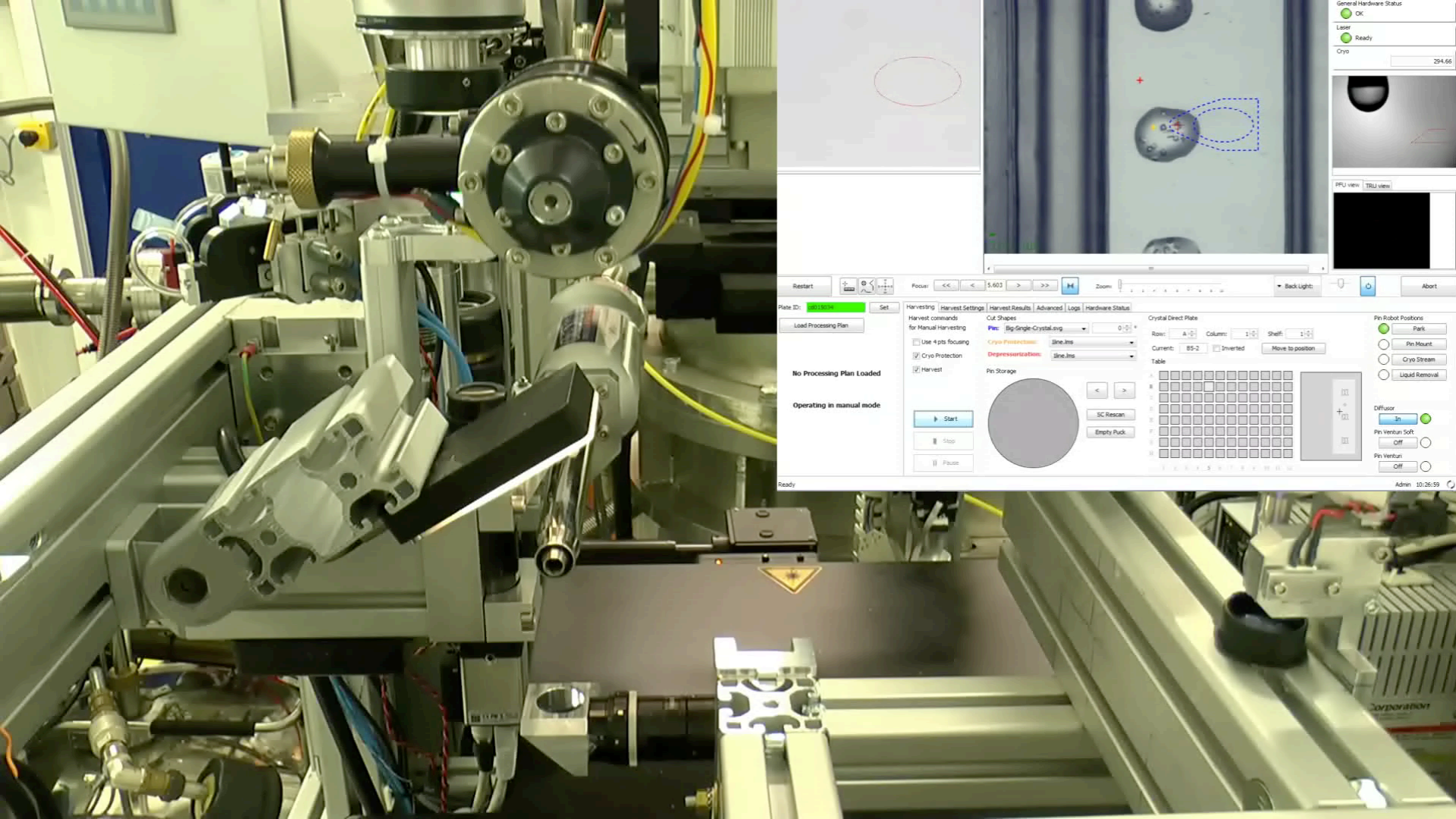
Results displayed in databases for display and use



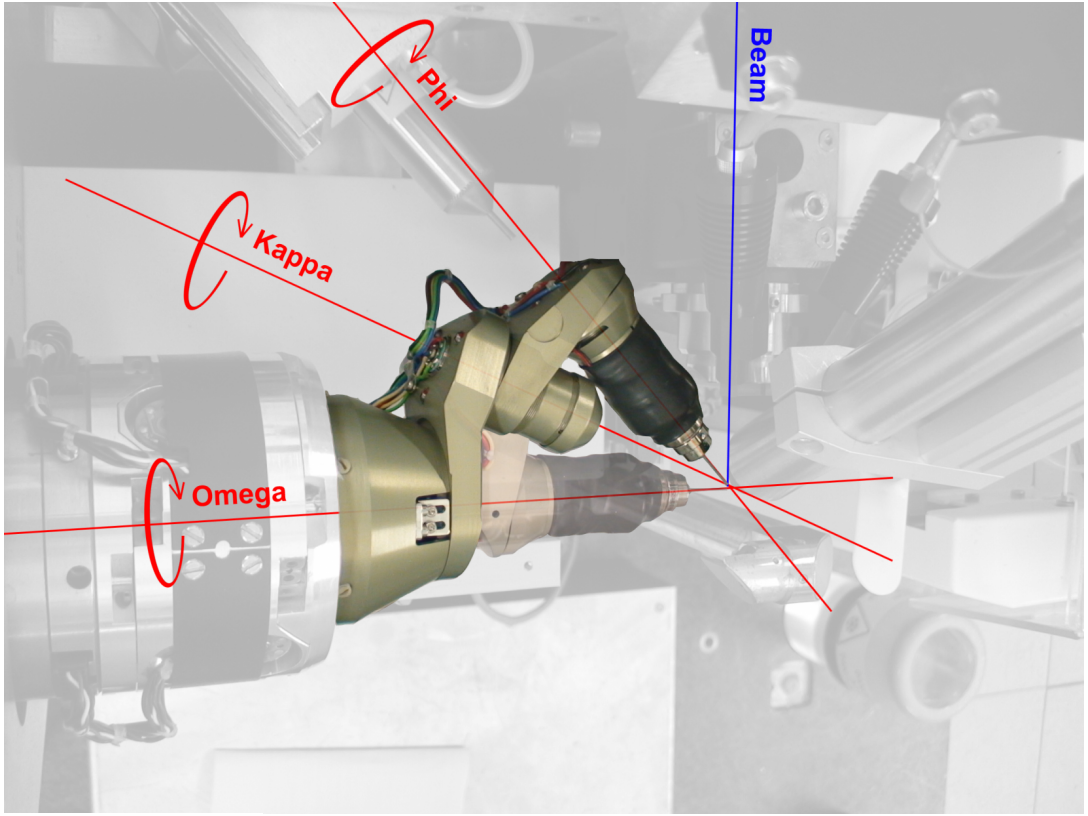
CrystalDirect-To-Beam on ID30B



EMBL


General Hardware Status
OK
Laser
Ready
Cryo
294.66
PPU view
TRU view
Restart
Focus:
5.603
Zoom:
Back Light:
Abort
Plate ID:
Set
Load Processing Plan
Harvesting
Harvest Settings
Harvest Results
Advanced
Logs
Hardware Status
Harvest commands for Manual Harvesting
Use 4 pts focusing
Cryo Protection
Harvest
No Processing Plan Loaded
Operating in manual mode
Start
Stop
Pause
Cut Shapes
Pin:
Big-Single-Crystal.svg
Cryo Protection:
3line.lms
Depressurization:
3line.lms
Pin Storage
SC Rescan
Empty Puck
Crystal Direct Plate
Row:
Column:
Shelf:
Current:
B5-2
Inverted
Move to position
Table
Pin Robot Positions
Park
Pin Mount
Cryo Stream
Liquid Removal
Diffusor
In
Pin Venturi Soft
Off
Pin Venturi
Off
Ready
Admin
10:26:59

ID30B Mini-Kappa goniometer head



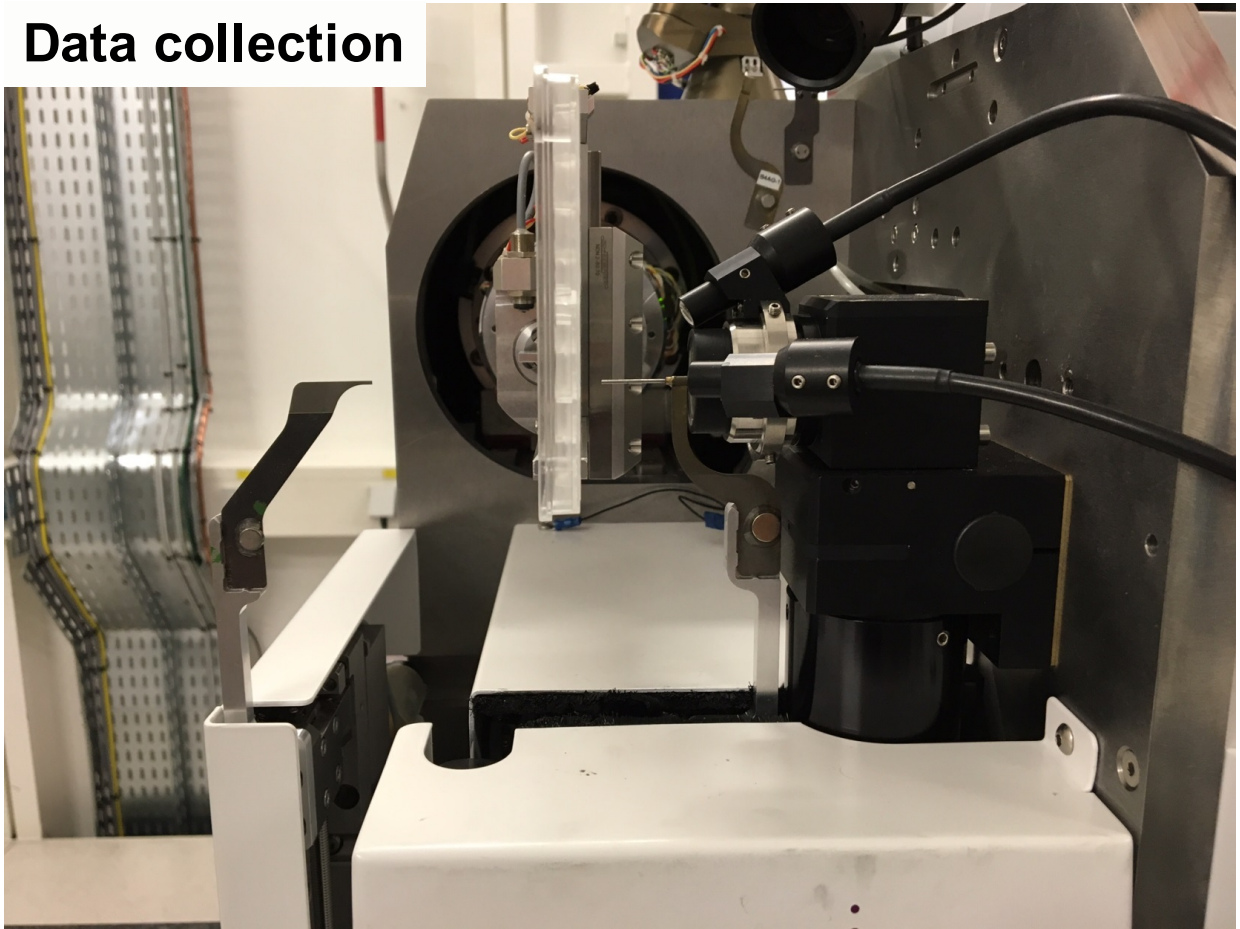
Acta Cryst D BIOLOGICAL CRYSTALLOGRAPHY

Brockhauser *et al.*

Volume 69 | **Part 7** | July 2013 | Pages 1241-1251

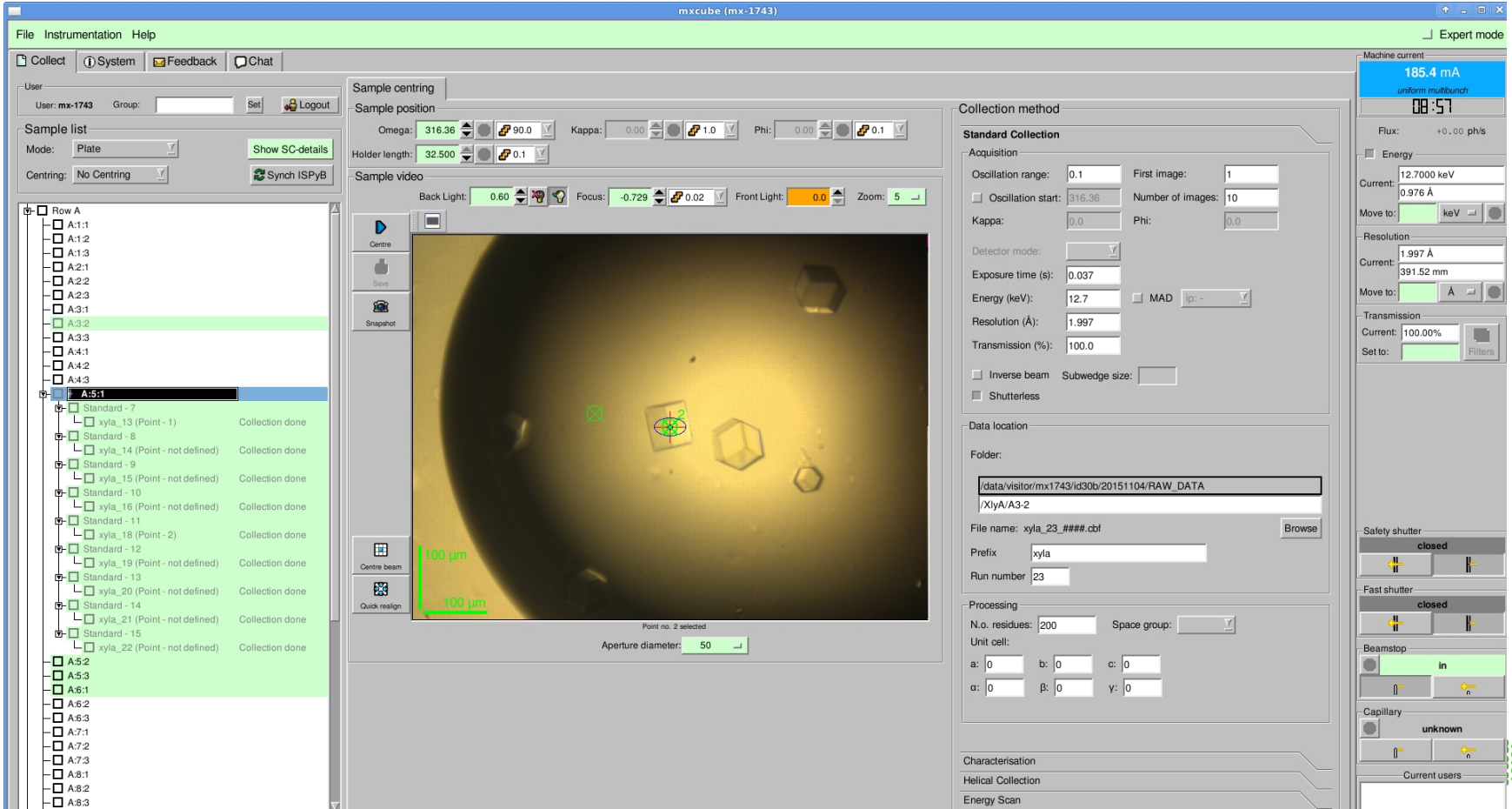
EMBL

ID30B MD2S in situ plate screening

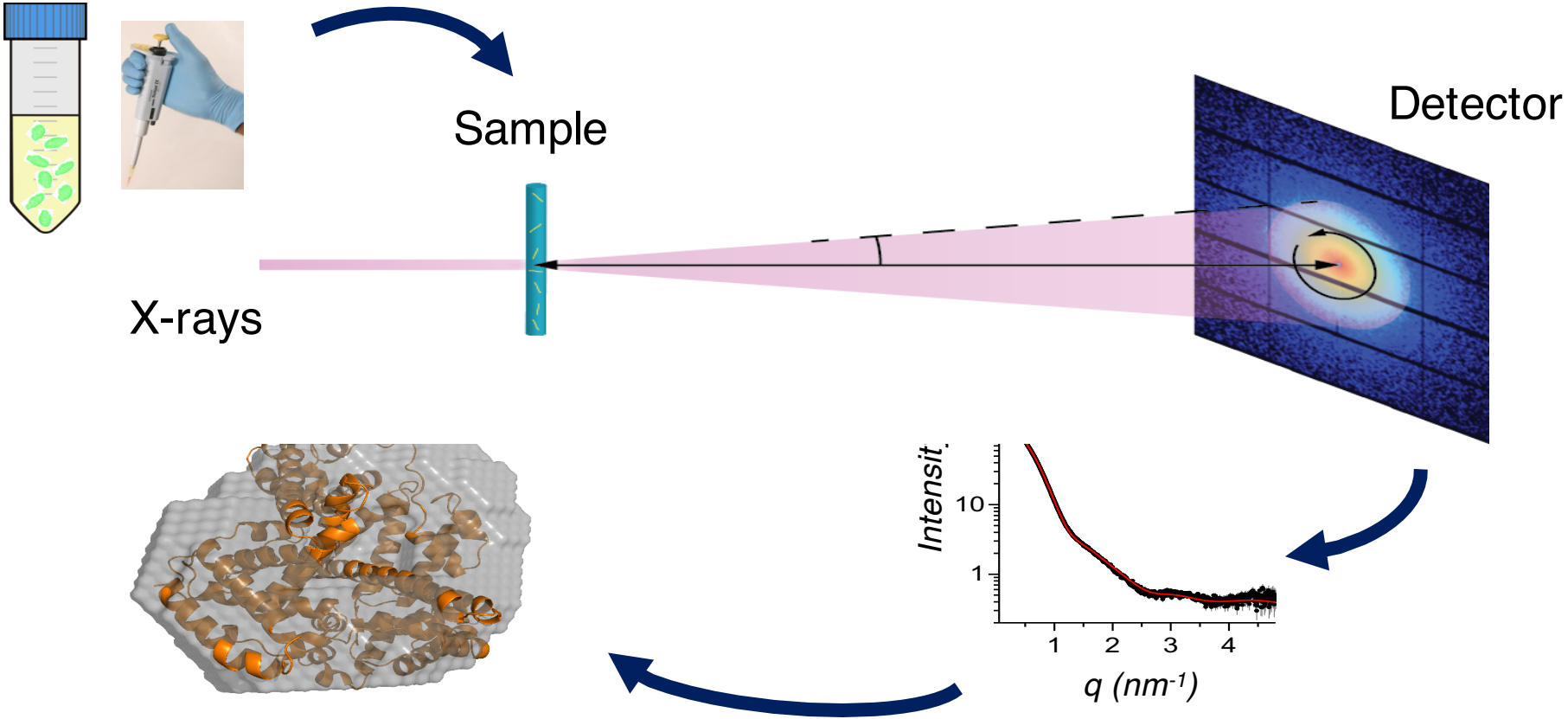


EMBL

ID30B MD2S in situ plate screening in MxCuBE

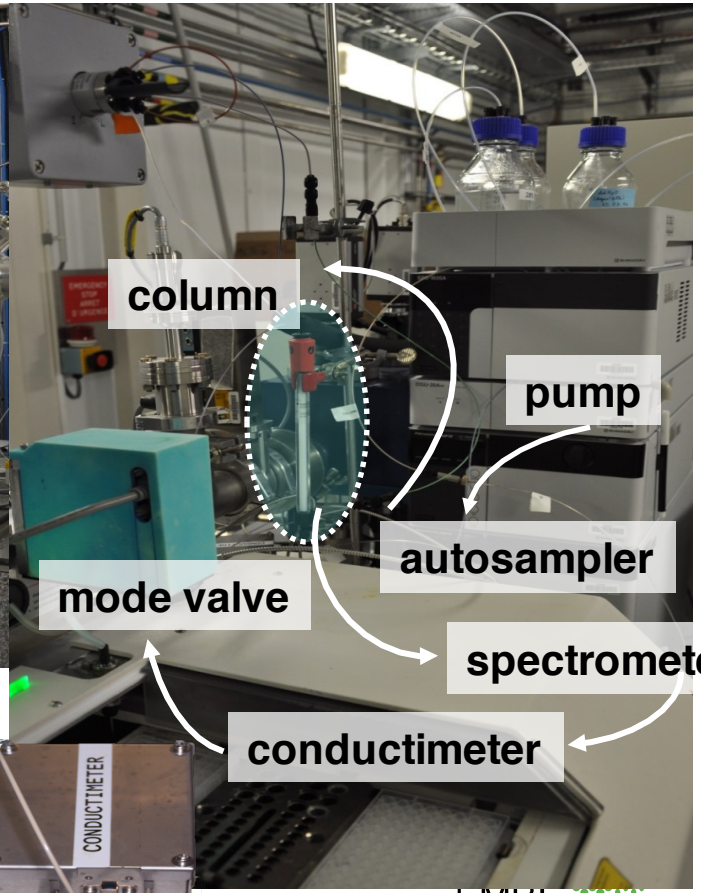


Biological small angle X-ray scattering (bioSAXS)



EMBL

BioSAXS on BM29



JOURNAL OF SYNCHROTRON RADIATION

Pernot *et al.*
Volume 20 | **Part 6** | July 2013 | Pages 660-664

BIOLOGICAL CRYSTALLOGRAPHY

Round *et al.*
Volume 71 | **Part 1** | January 2015 | Pages 65-75

EMBL

ESRF Extremely Brilliant Source upgrade

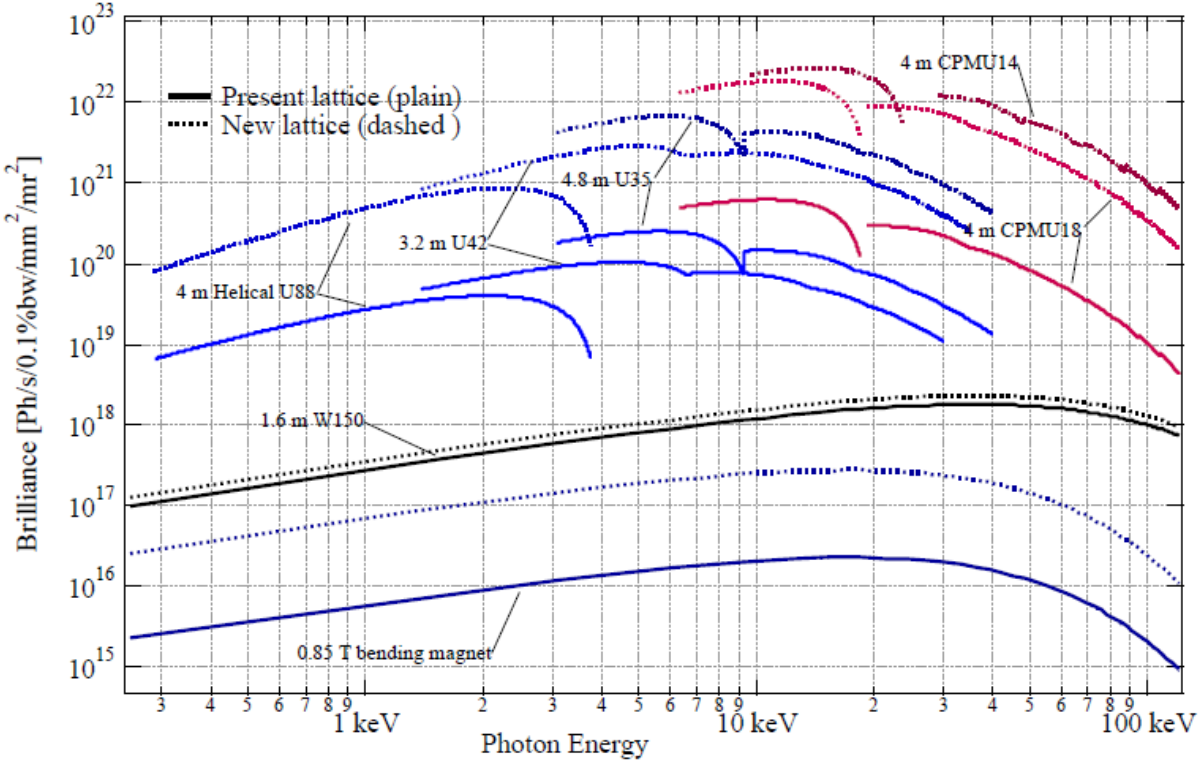


Figure 4: ESRF brilliance for the existing and new lattices

EMBL

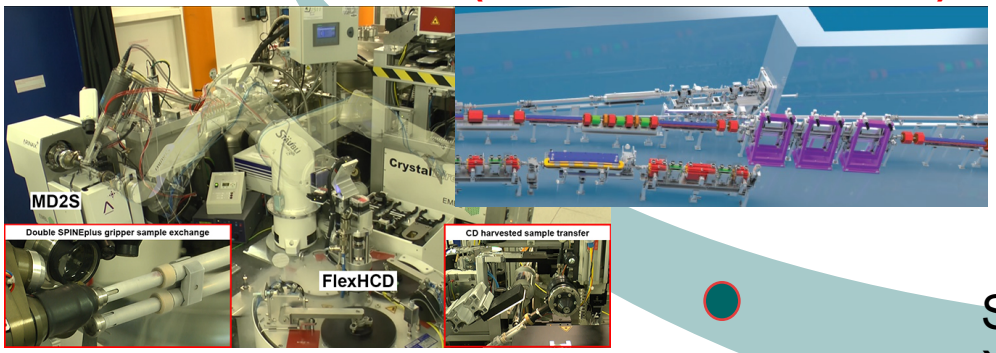
ESRF Extremely Brilliant Source upgrade

Medium term

(2019)

Supercharge the source

(Dec. 2018 - June 2020)



A brighter future

2020->

Smaller and brighter X-rays

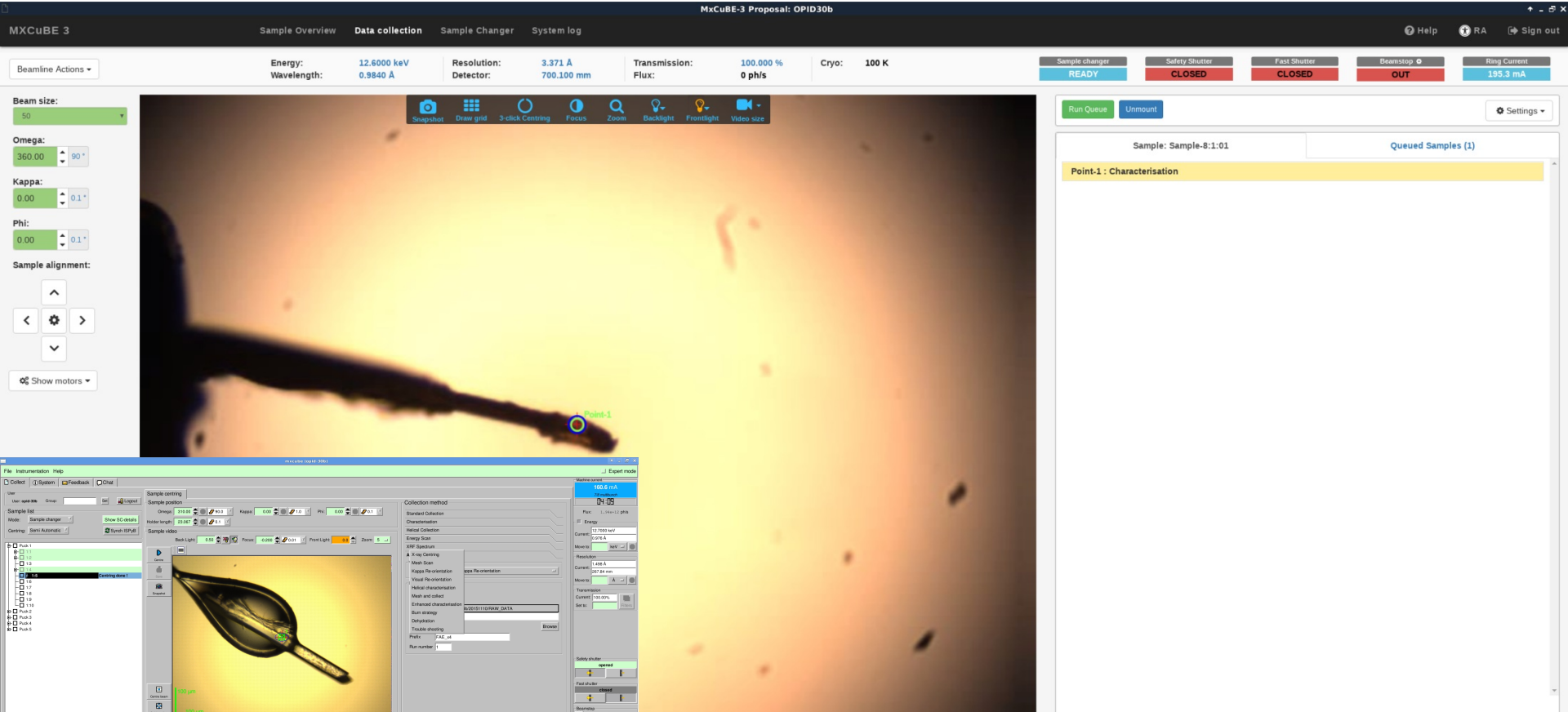
- **Beamline upgrades**
  BM29, MASSIF-1, ID29
- **Software developments**
  MxCuBE^3/BsxCuBE^3

New science:

- RT serial crystallography
- Time resolved (μs)
- Using coherence

EMBL

Software upgrade: MxCuBEv2 -> MxCuBE^3

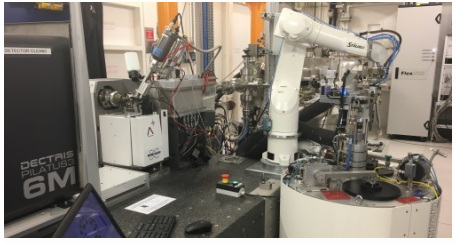


ESRF-EMBL Joint Structural Biology Group Beamlines

ID23-1 (tunable)

ID29 (tunable)

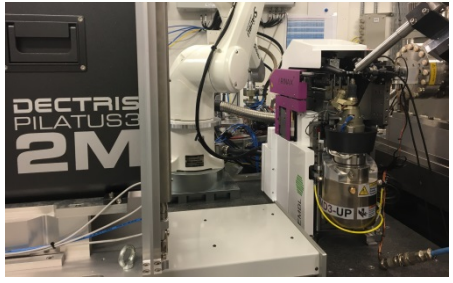
ID30B (tunable)



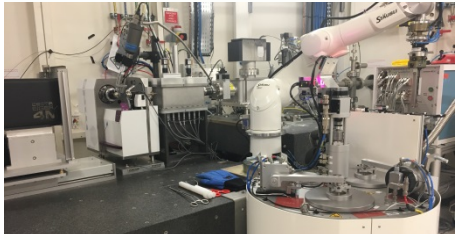
ESRF-EBS
Extremely Brilliant Source

12/2018 – 8/2020

ID23-2 (μ focus)



ID30-A3 (μ focus)

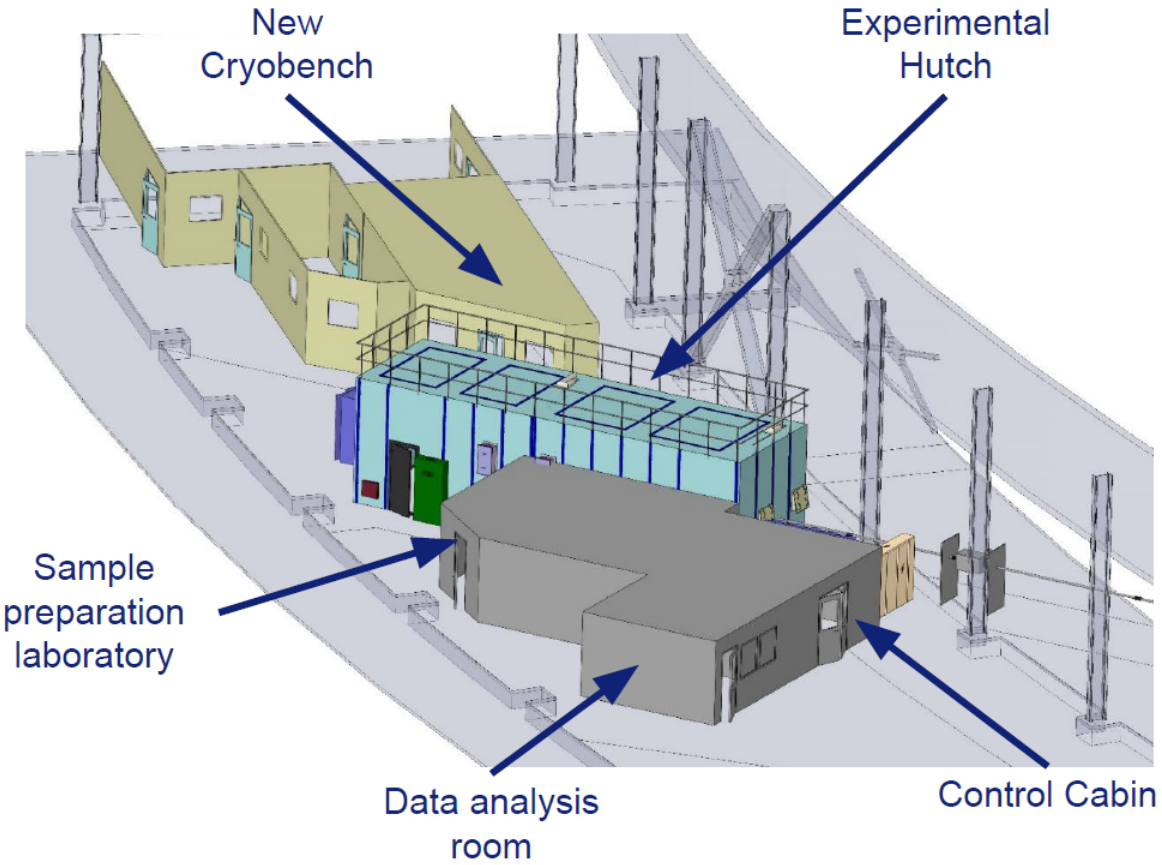


ID30-A1 (MASSIF)

BM29 (BioSAXS)

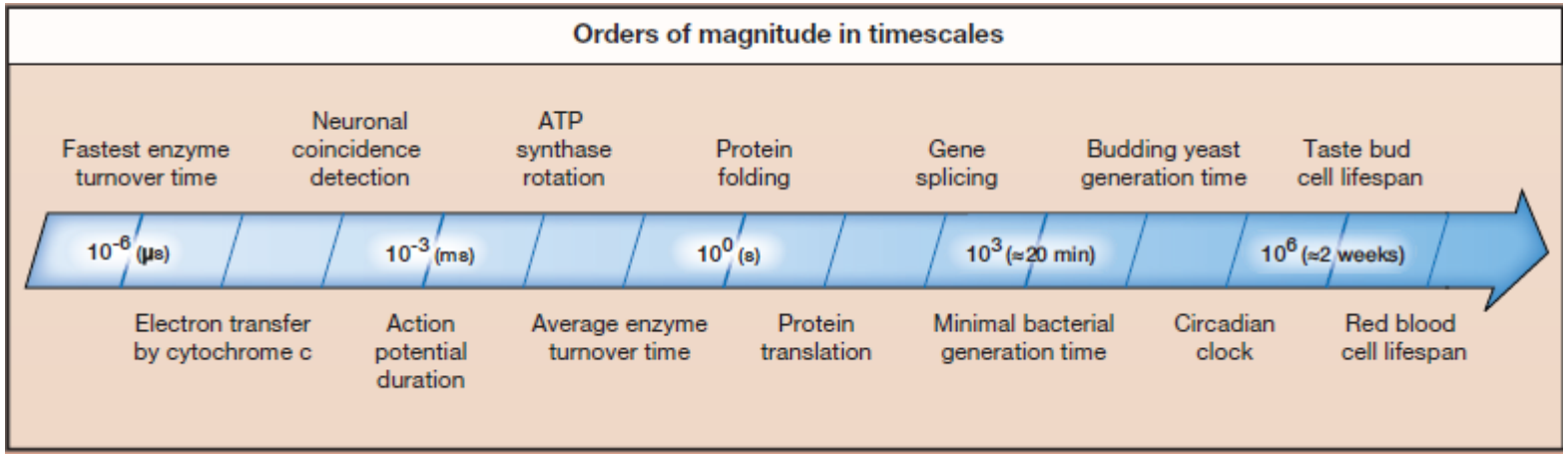
ID29 Experimental hutch (EH1/2) in the Chartreuse extension

- >5 x 10^{15} ph/s/mm^2
- Multilayer mono
- KB mirrors
- 0.5 μm^2
- 1 μs chopper
- 1 kHz detector speed



Daniele de Sanctis (ESRF)
Shibom Basu (EMBL-Grenoble)

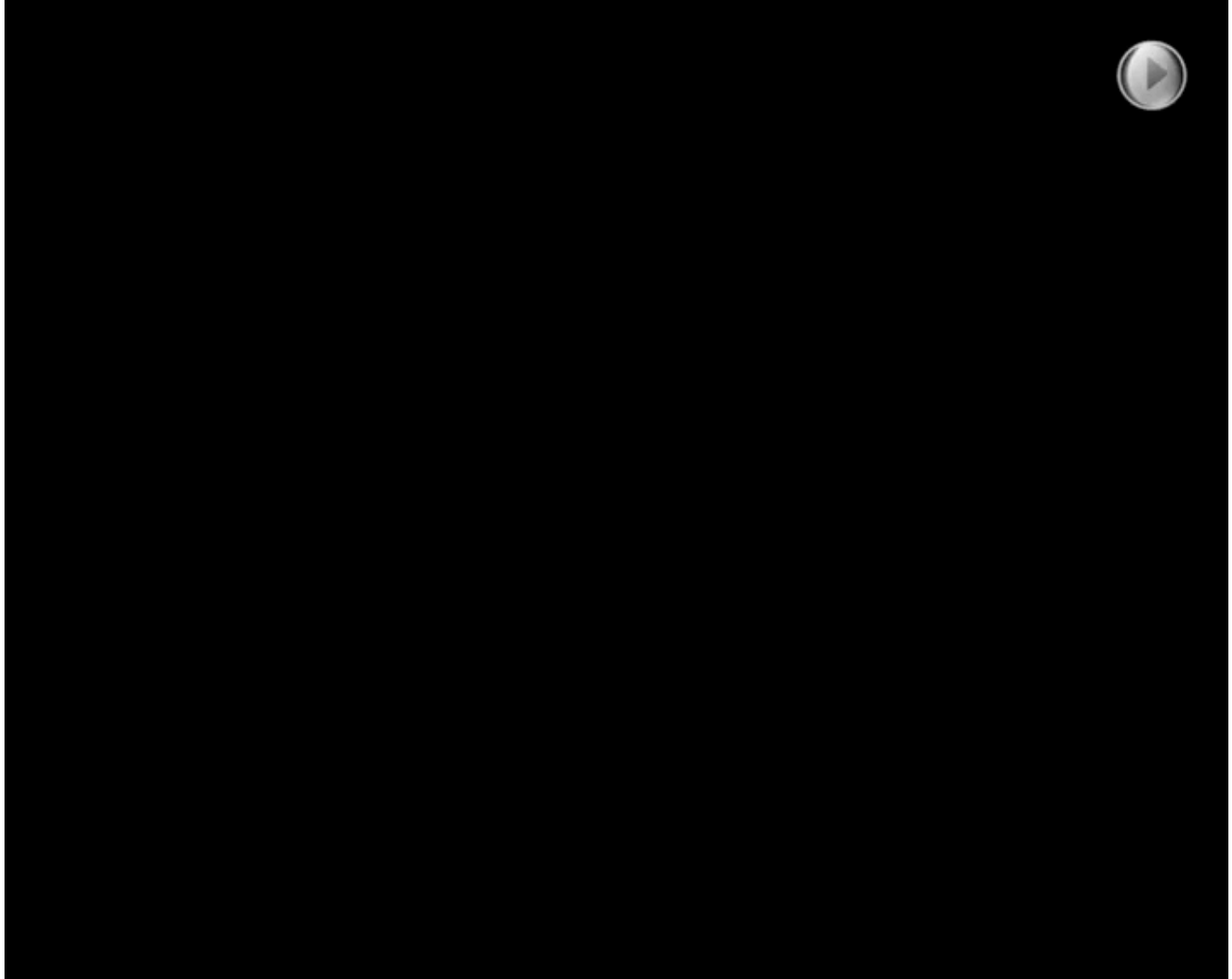
ID29 upgrade – Serial synchrotron crystallography (SSX) and high E



Shamir *et al*. (2016) *Cell* **164**, 1302.e1

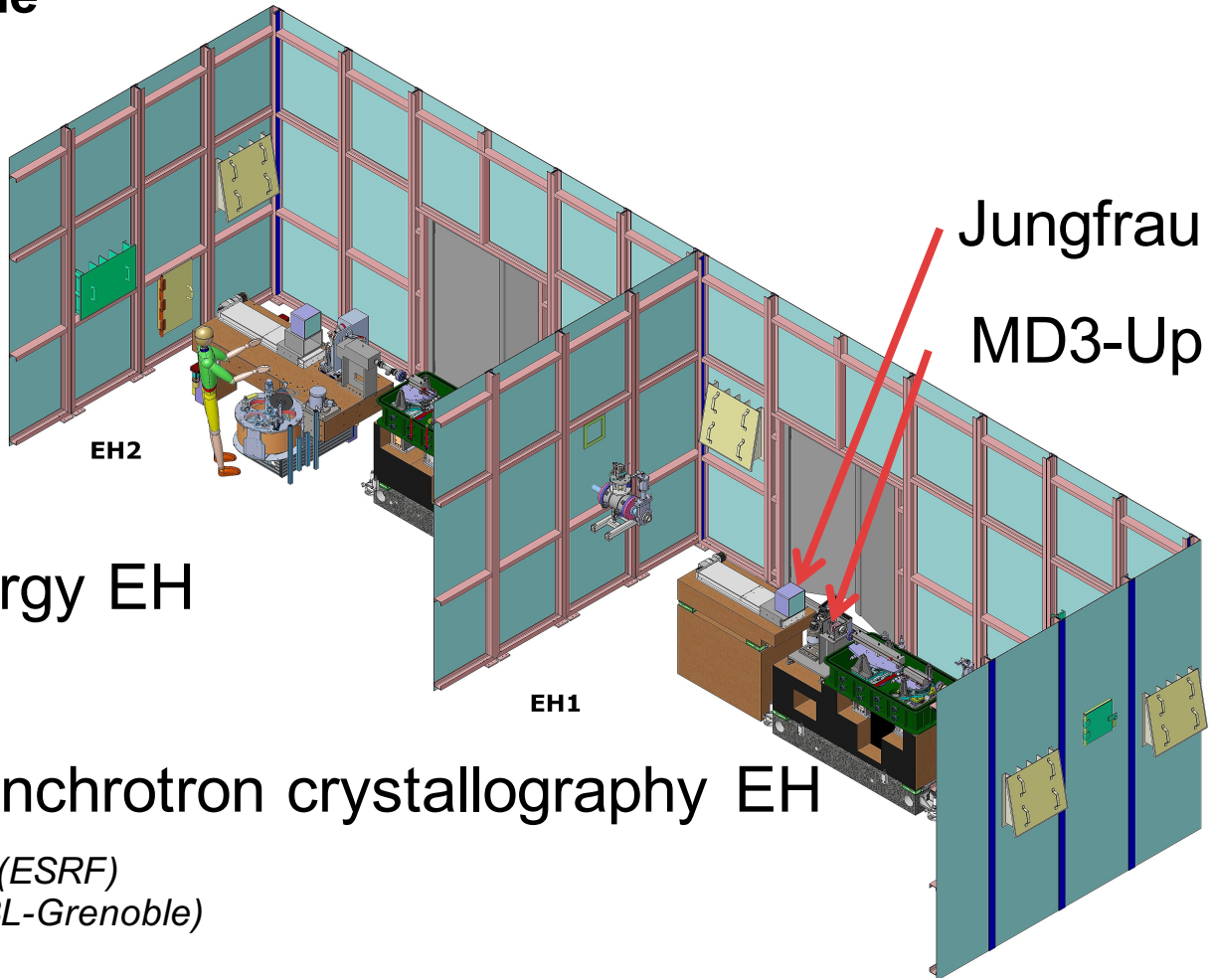
Daniele de Sanctis (ESRF)
Shibom Basu (EMBL-Grenoble)

EMBL



EMBL

ID29 Upgrade



Jungfrau (1 kHz)

MD3-Up

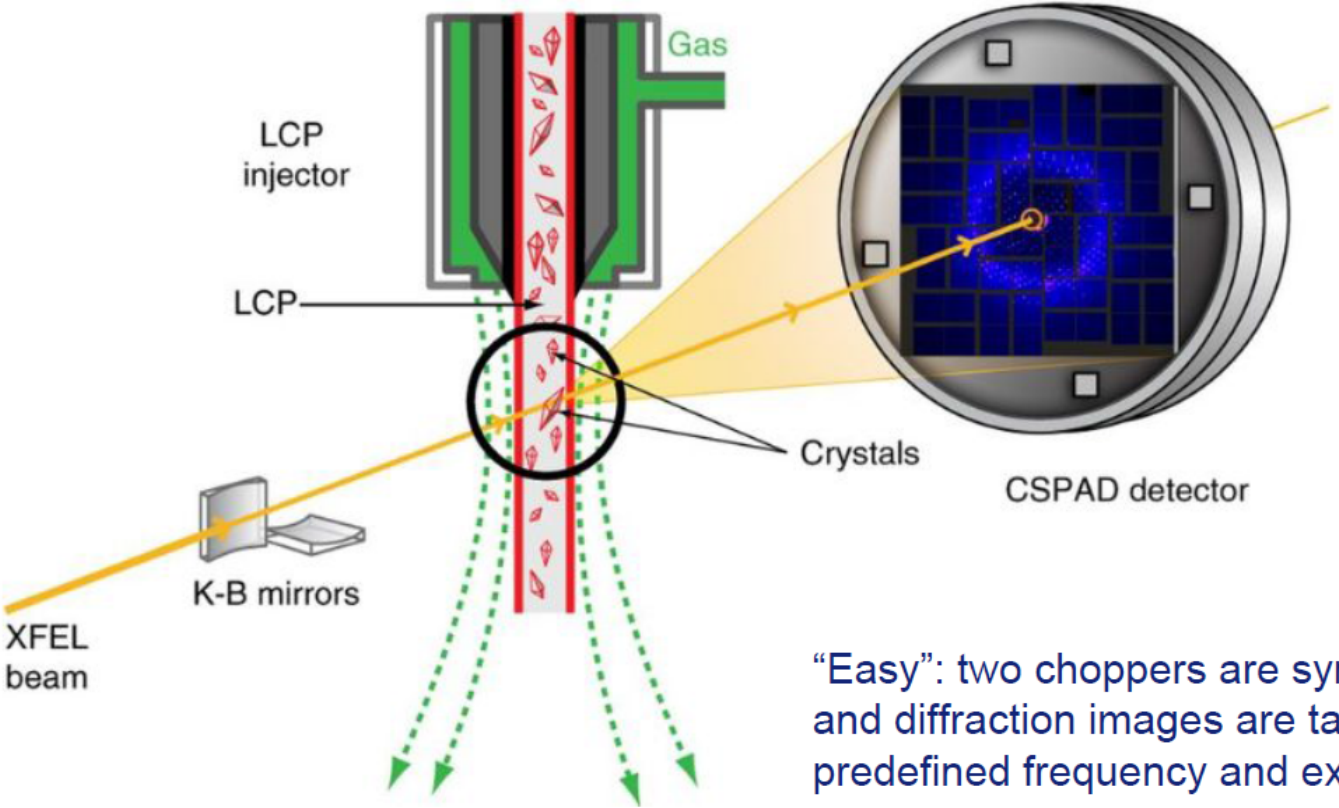
High energy EH

Serial synchrotron crystallography EH

Daniele de Sanctis (ESRF)
Shibom Basu (EMBL-Grenoble)

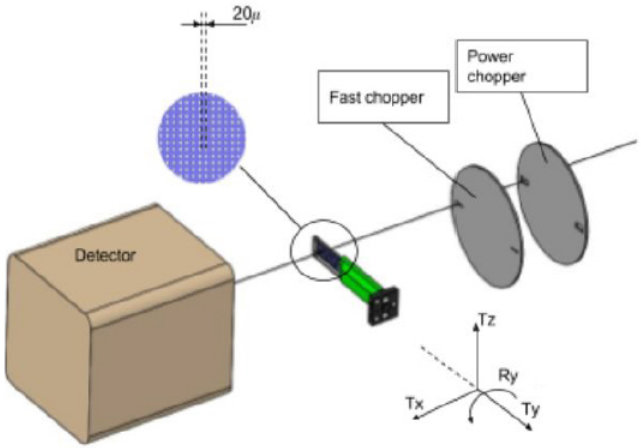
EMBL

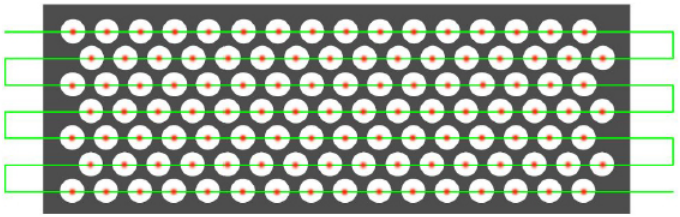
Jet experiments

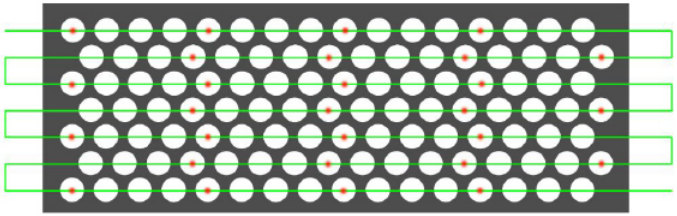


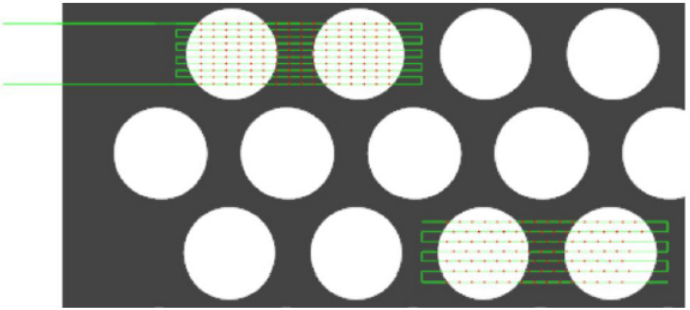
"Easy": two choppers are synchronized and diffraction images are taken at predefined frequency and exposure time

Fixed target support





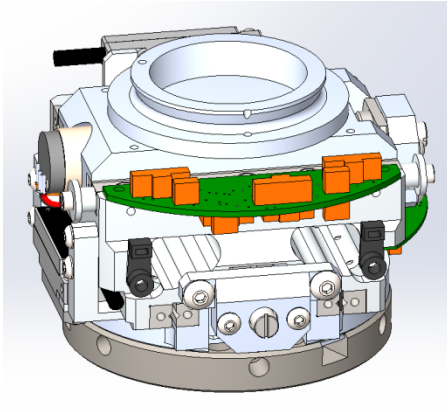




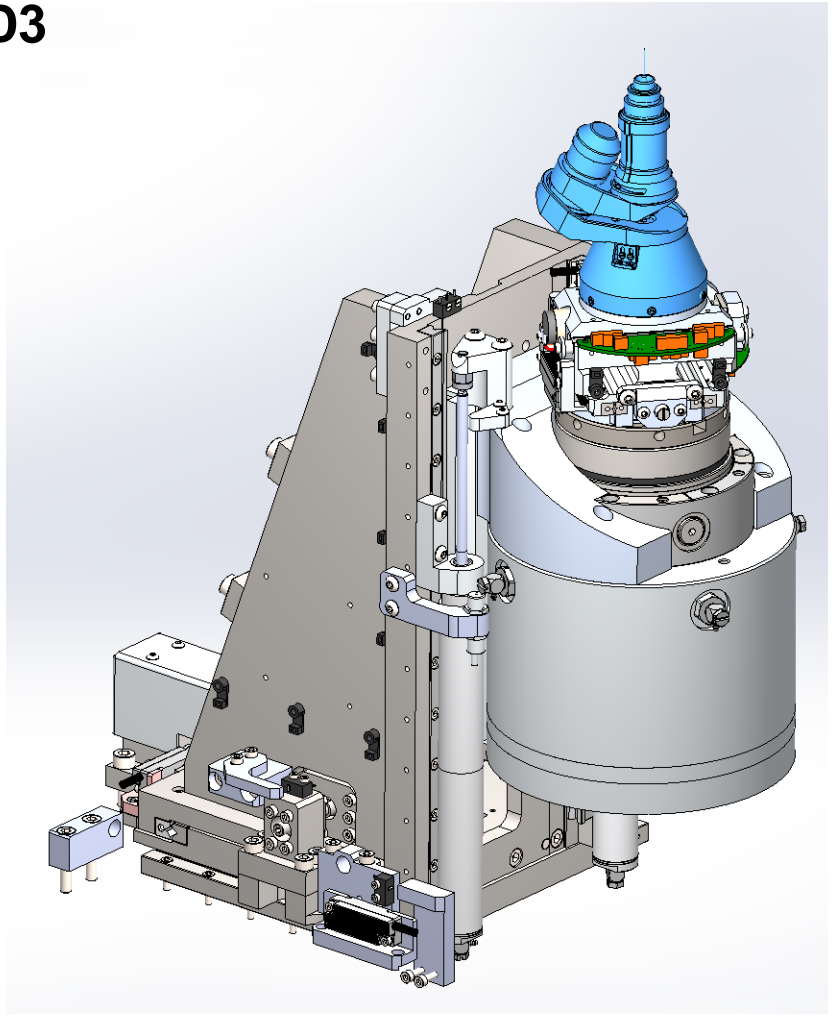
- Choppers
- **MD3-Up** – synchronous scans
- Lasers
- Jungfrau detector (3 kHz)

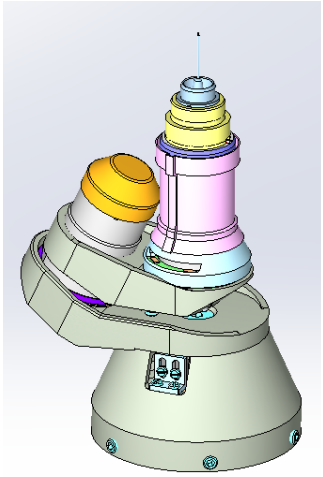
ID29 Upgrade – MD3

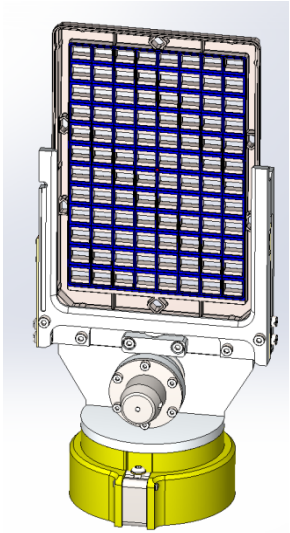
X : +/- 5mm 5mm/s
Y : +/- 5mm 5mm/s
Z : 118 mm 30mm/s
Encoders 5nm



XY table
+/- 5mm
Encoders 5nm

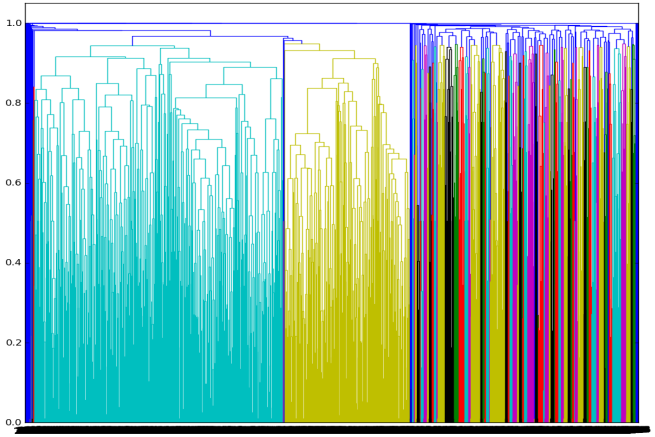




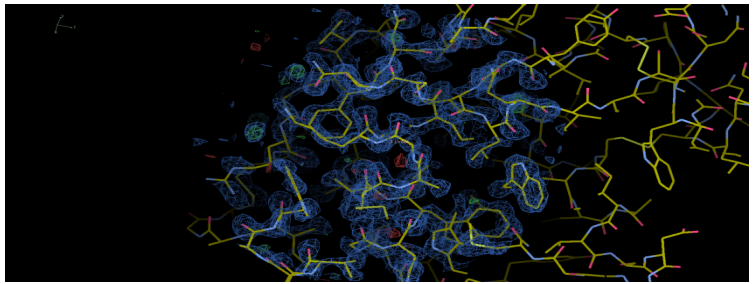


ID23-2: MD3-Up ESRF-EBS ready

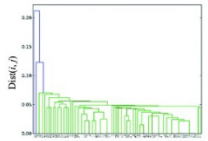
- Cyan cluster: 1351 datasets,



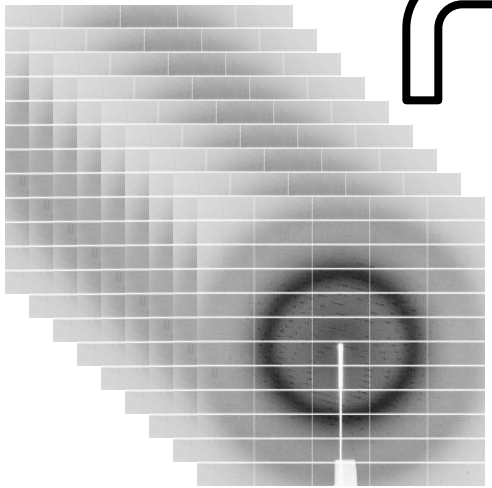
- Resolution (Å) = 20-1.3
- $R_{p.i.m.}$ (%) = 18.3
- Completeness (%) = 74.2

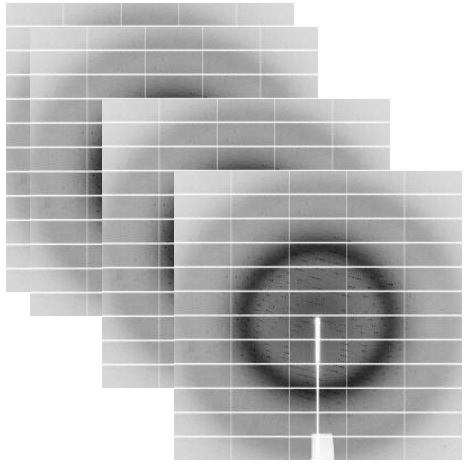


EMBL

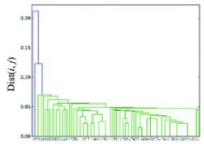


Cell 1

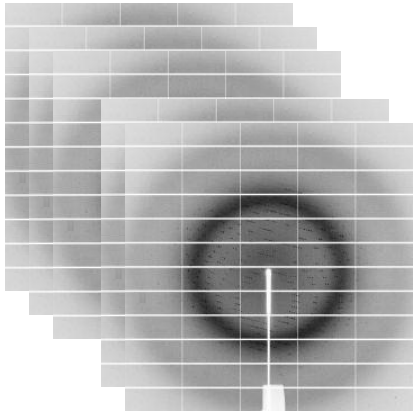




Apo structure



Cell 2



Ligand bound

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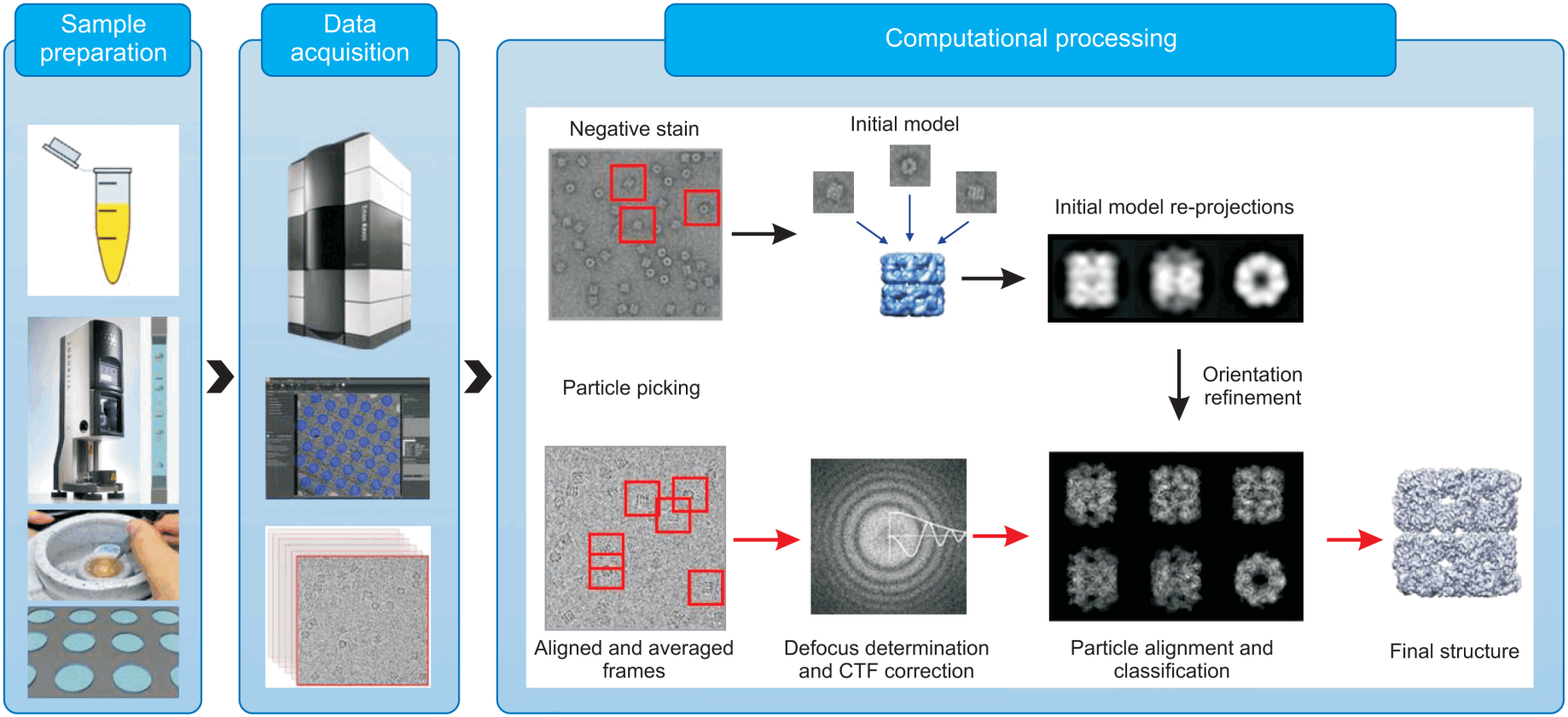
Structural biology techniques (Cryo-EM)

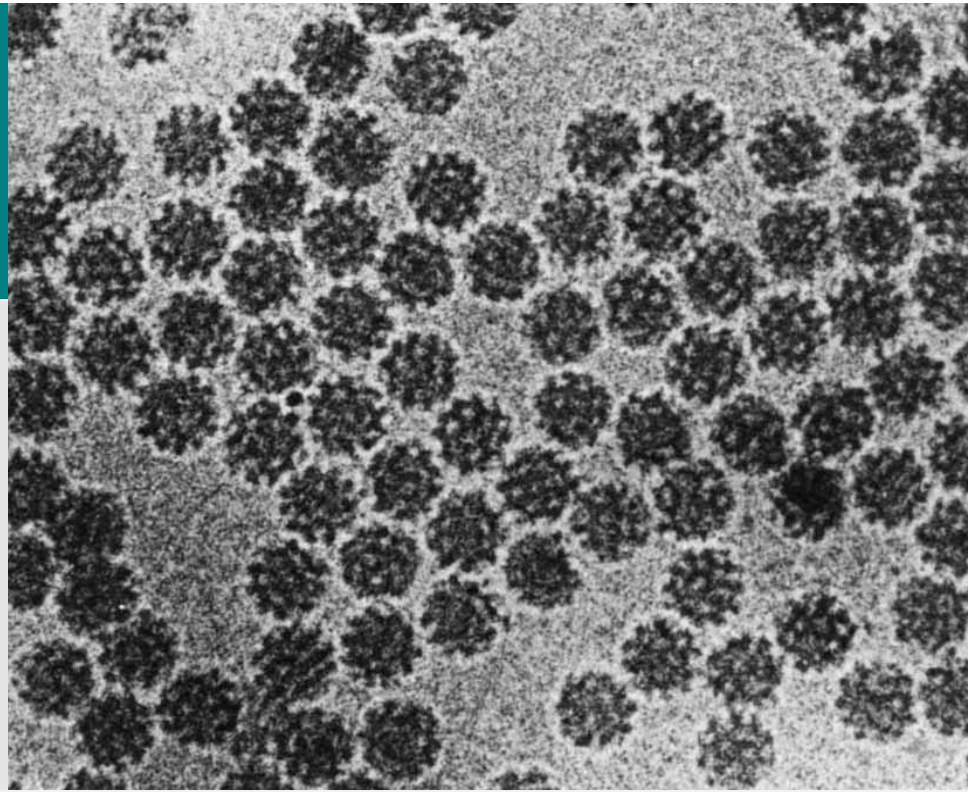


Long history of Cryo-EM developments at the EMBL

2017 Nobel Prize
CHEMISTRY

Jacques Dubochet

“for developing cryo-electron microscopy
for the high-resolution structure determination
of biomolecules in solution”



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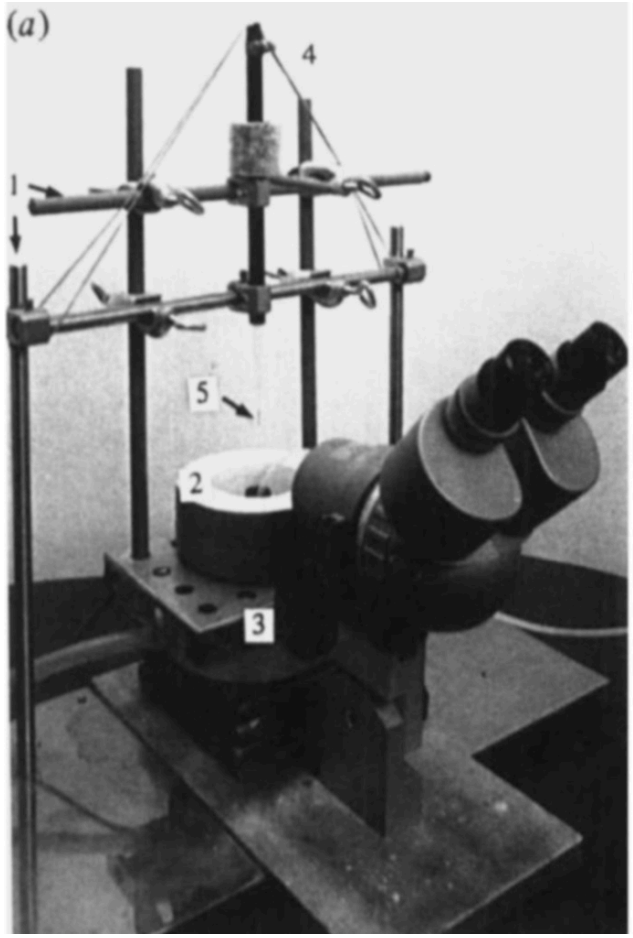
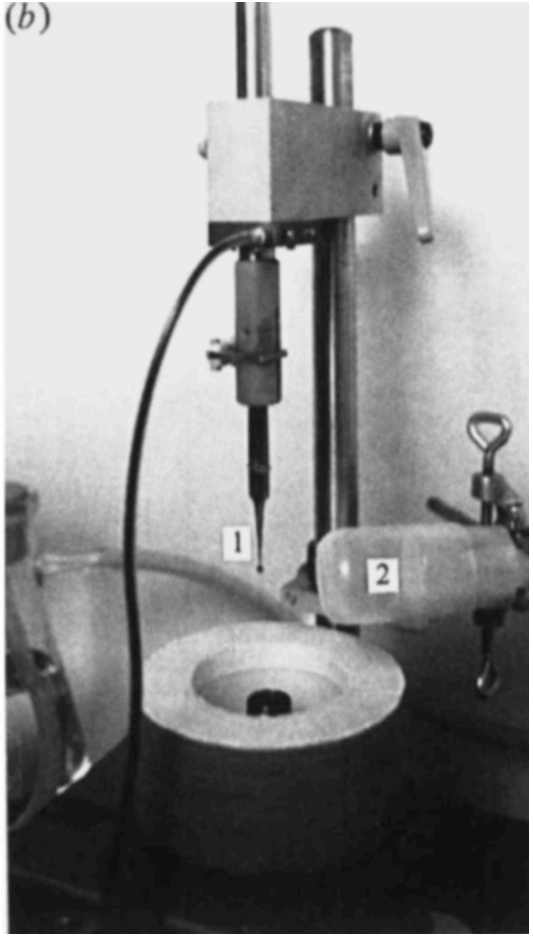
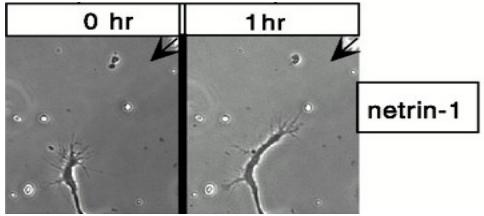
Cryo-EM freezing in the 1980s and now

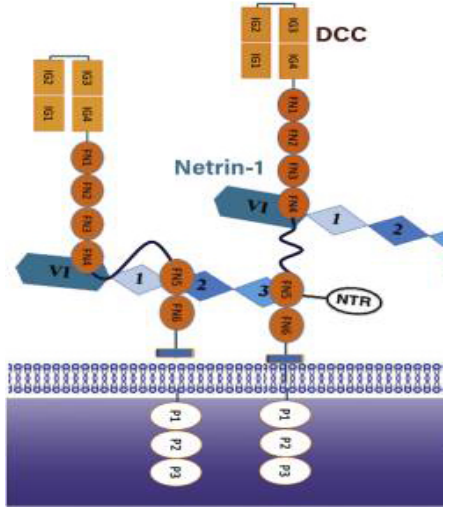




Vision – Retinal ganglions

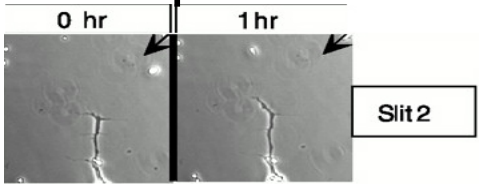
1. Attraction

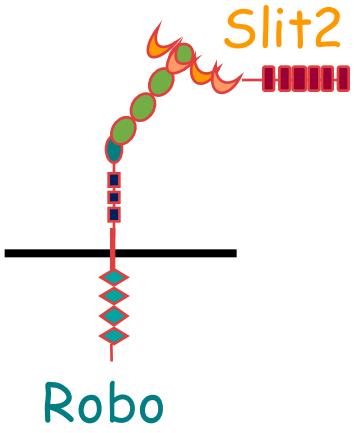


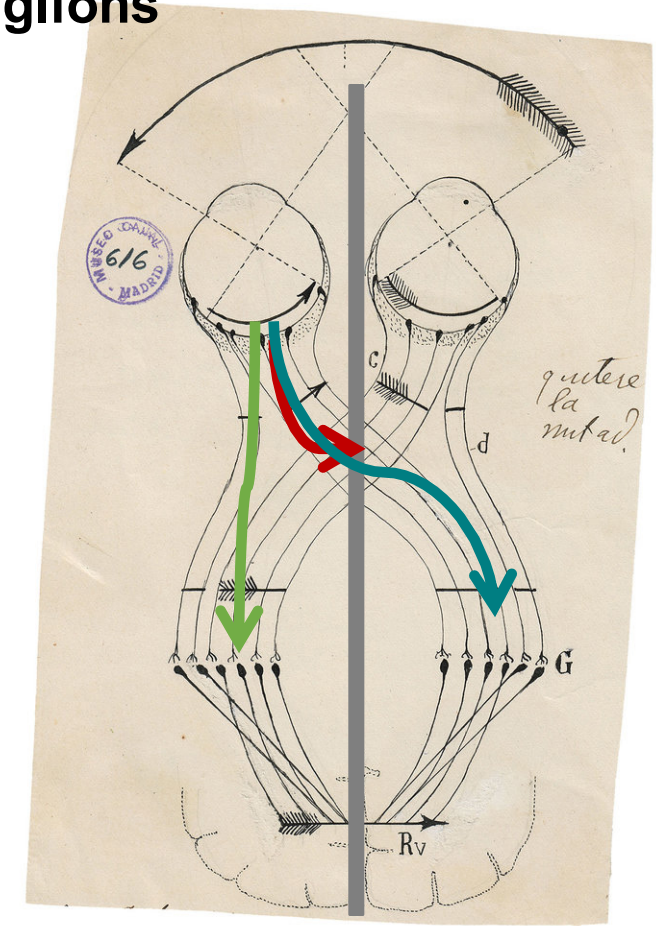


Liu *et al*., (2018)
Neuron **97**, 1261-1267

2. Repulsion



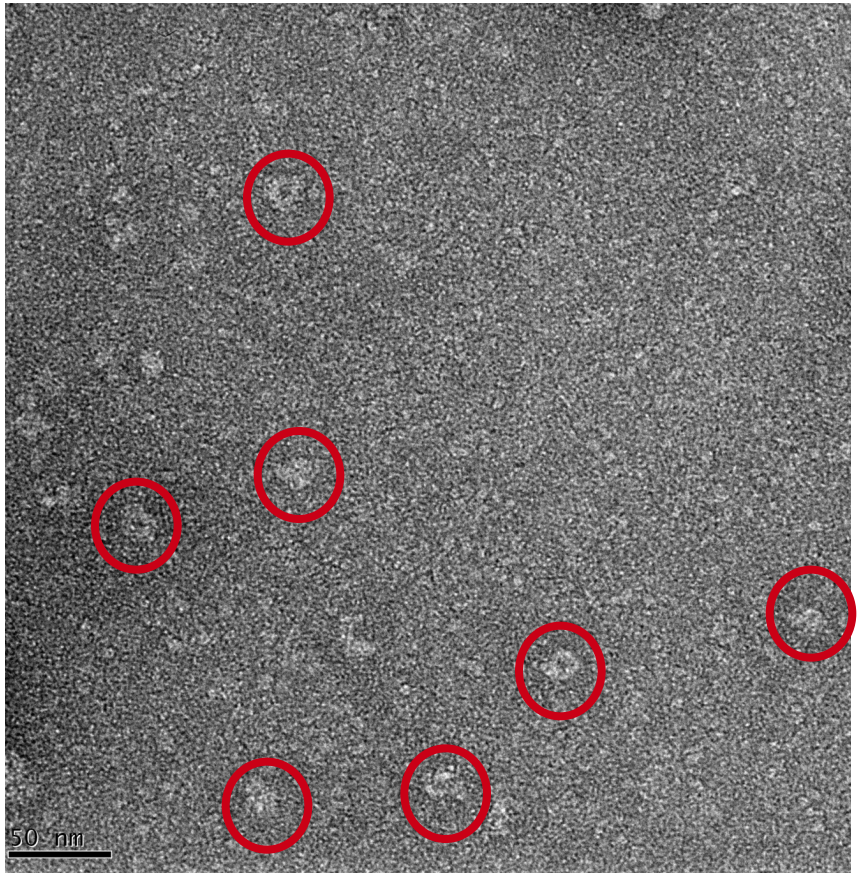


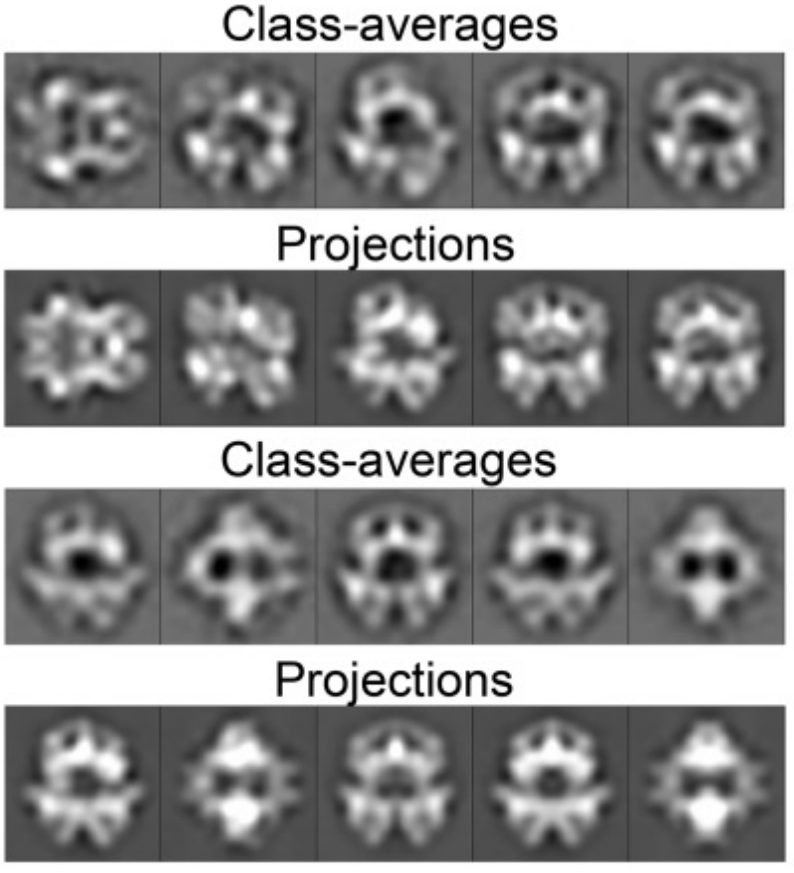


The midline

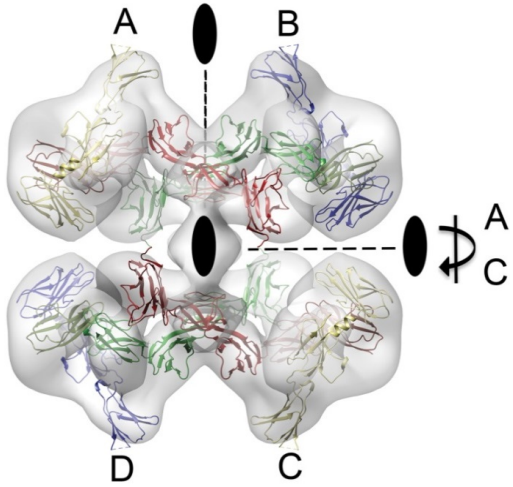
EMBL

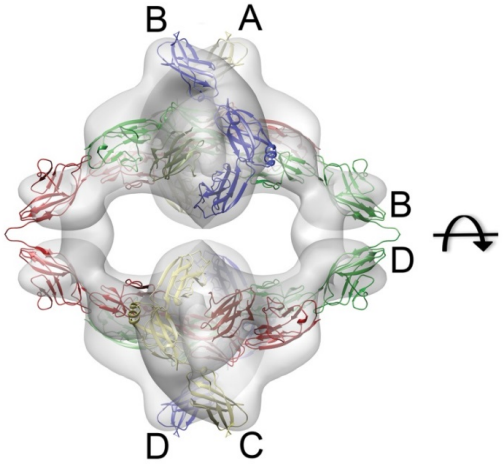
Negative stain 3D EM imaging of Robo1 - Aleksandrova *et al*. (2018) Structure **26**, 166-171

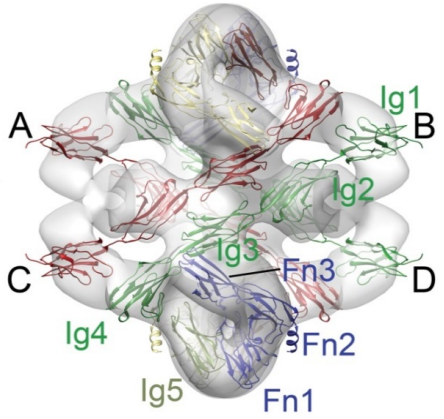


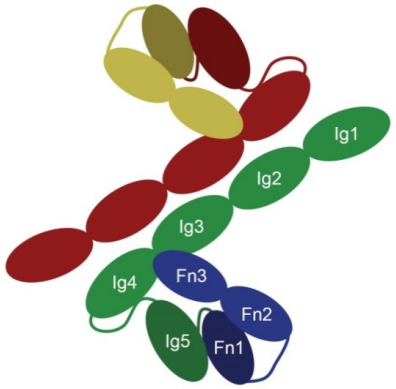


Robo1 forms tetramers – Cell surface autoinhibition

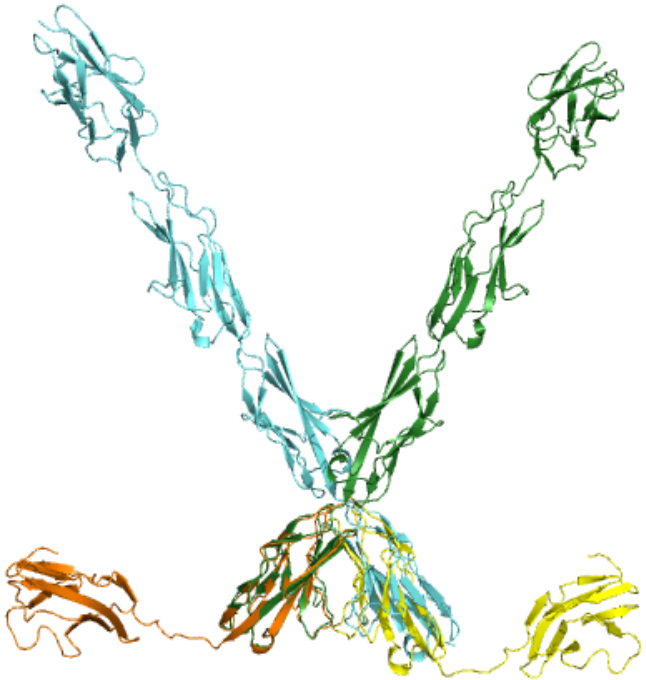




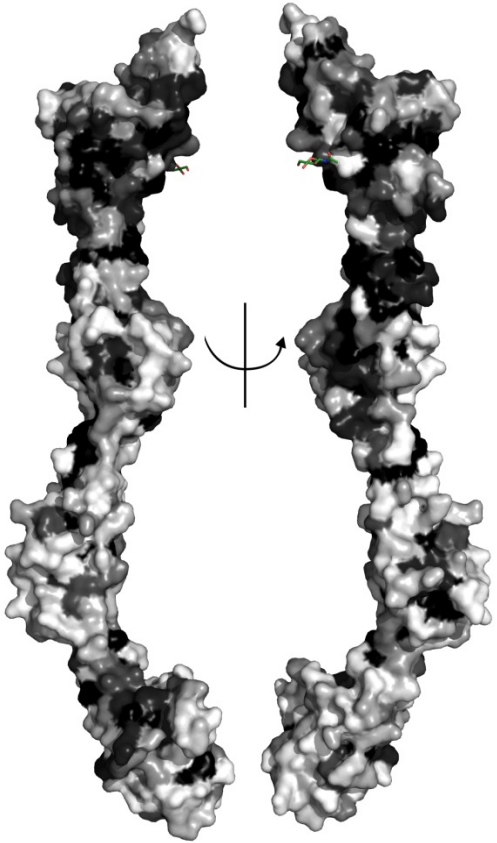




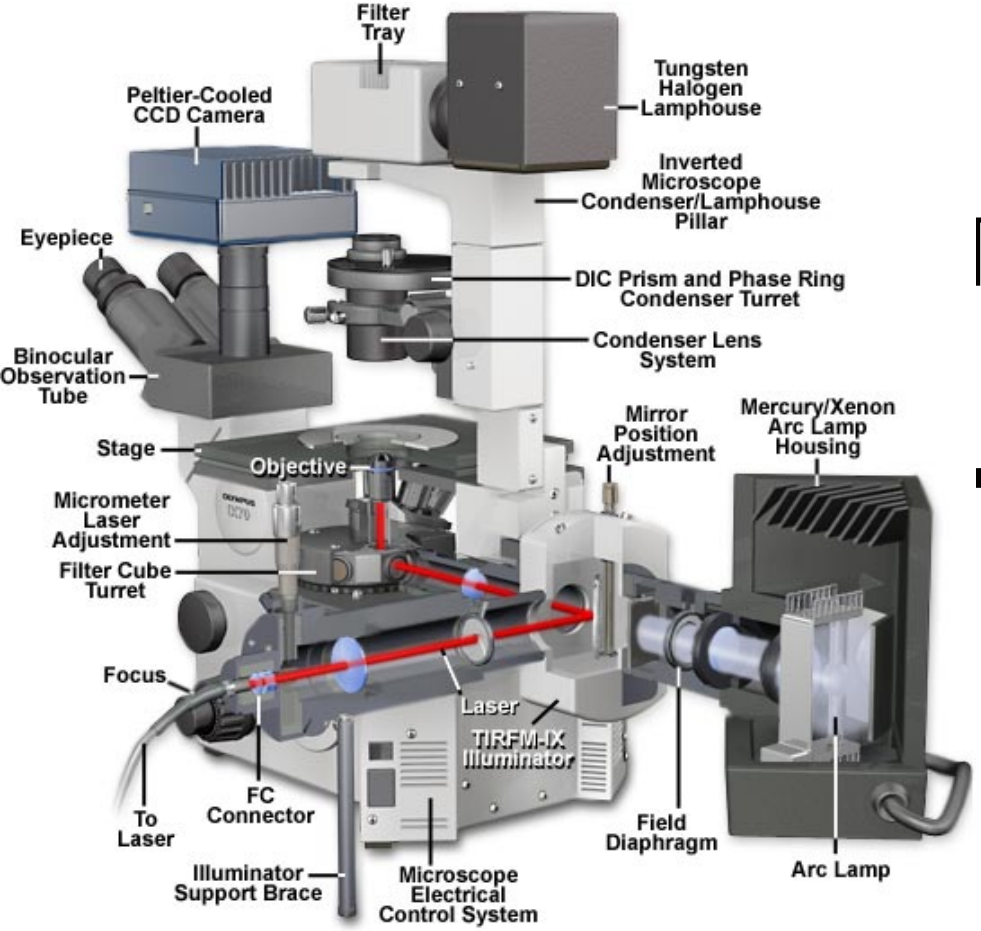
Robo1/2 activation involves dimerisation

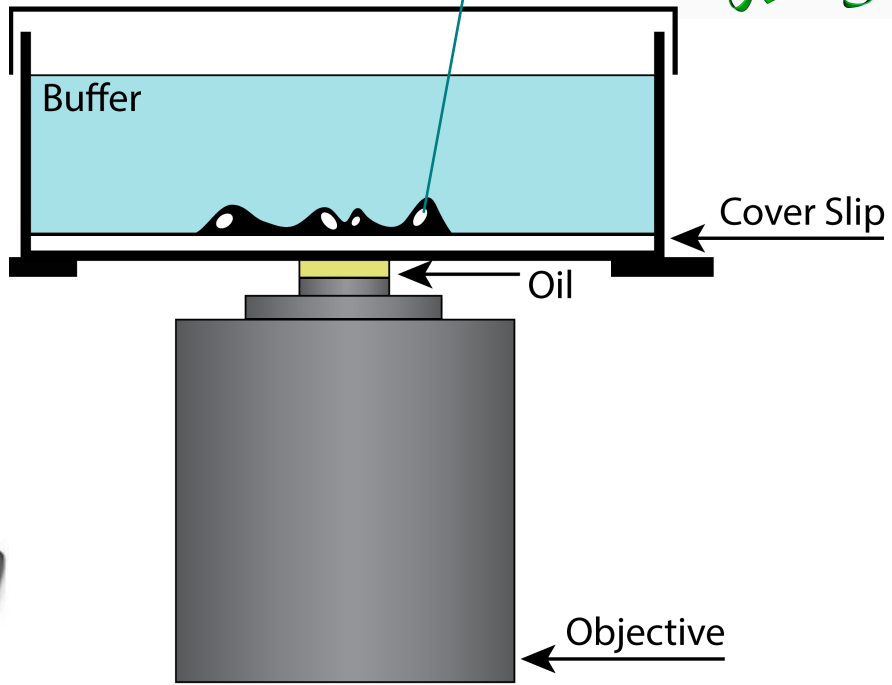


Barak *et al*. (2019) Cell **177**, 272-285

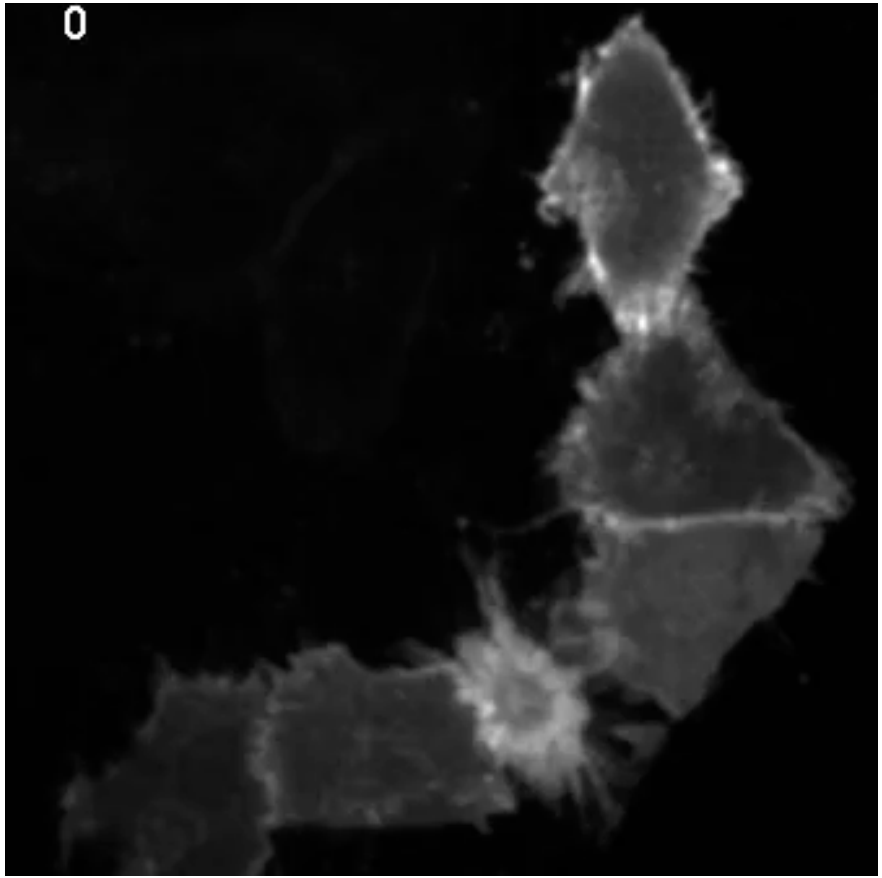


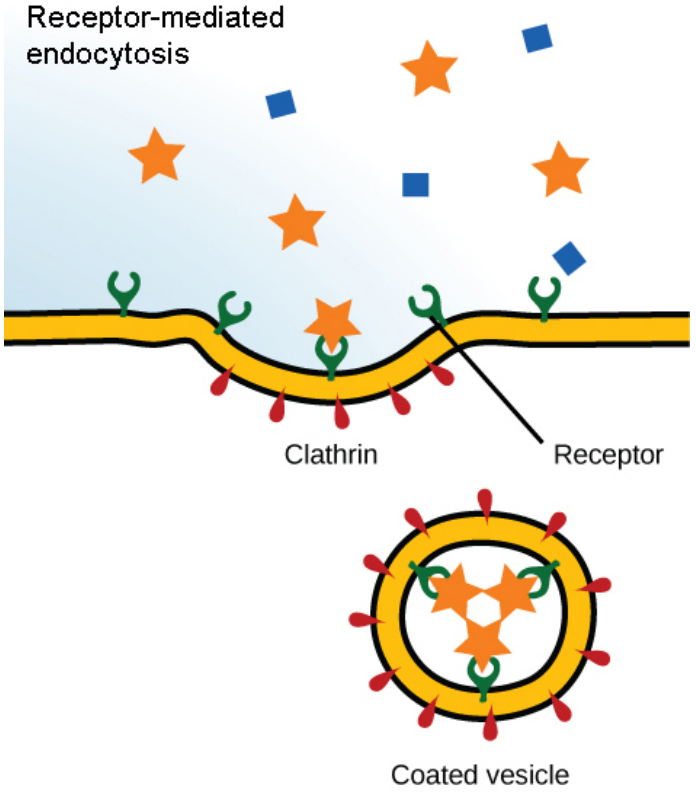
Live cell imaging





Robo1 undergoes endocytosis upon Slit binding





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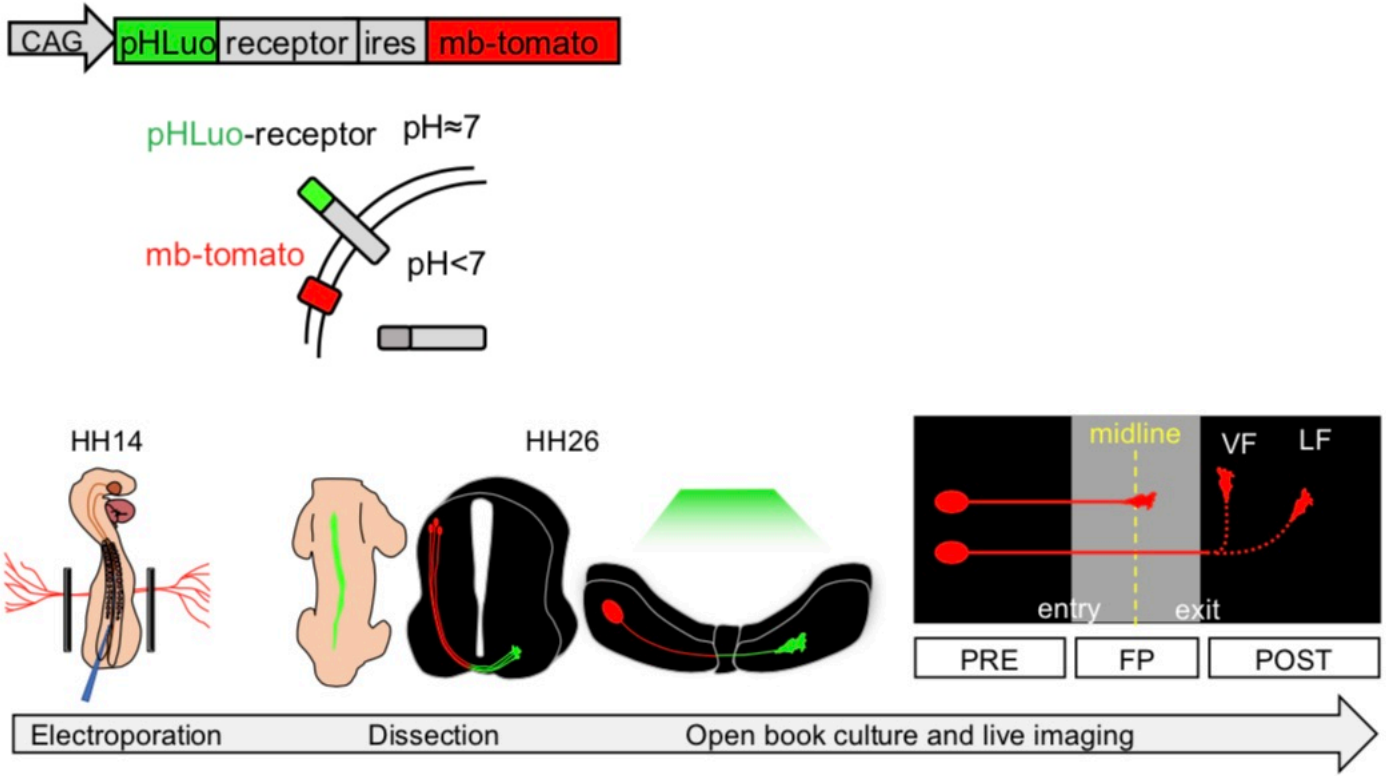
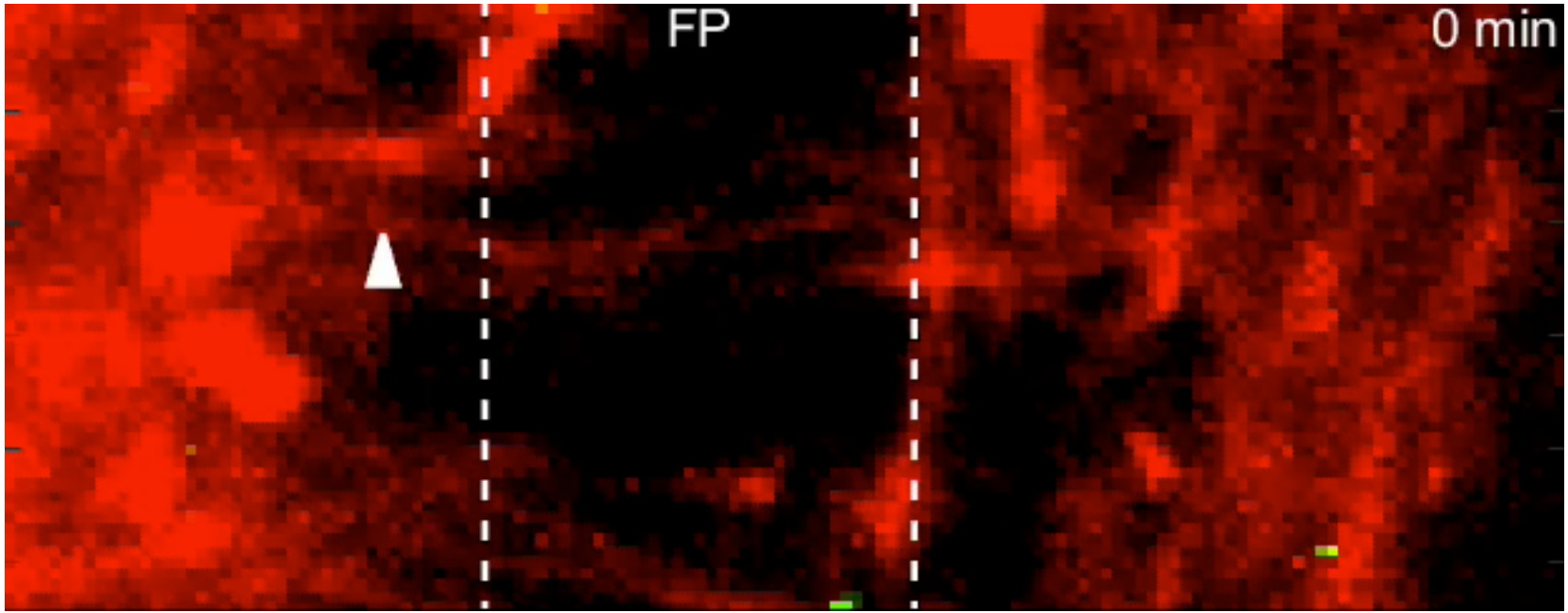
Robo1/2 spatial and temporal profiling



Pignata *et al*. (2019) BioRxiv

EMBL

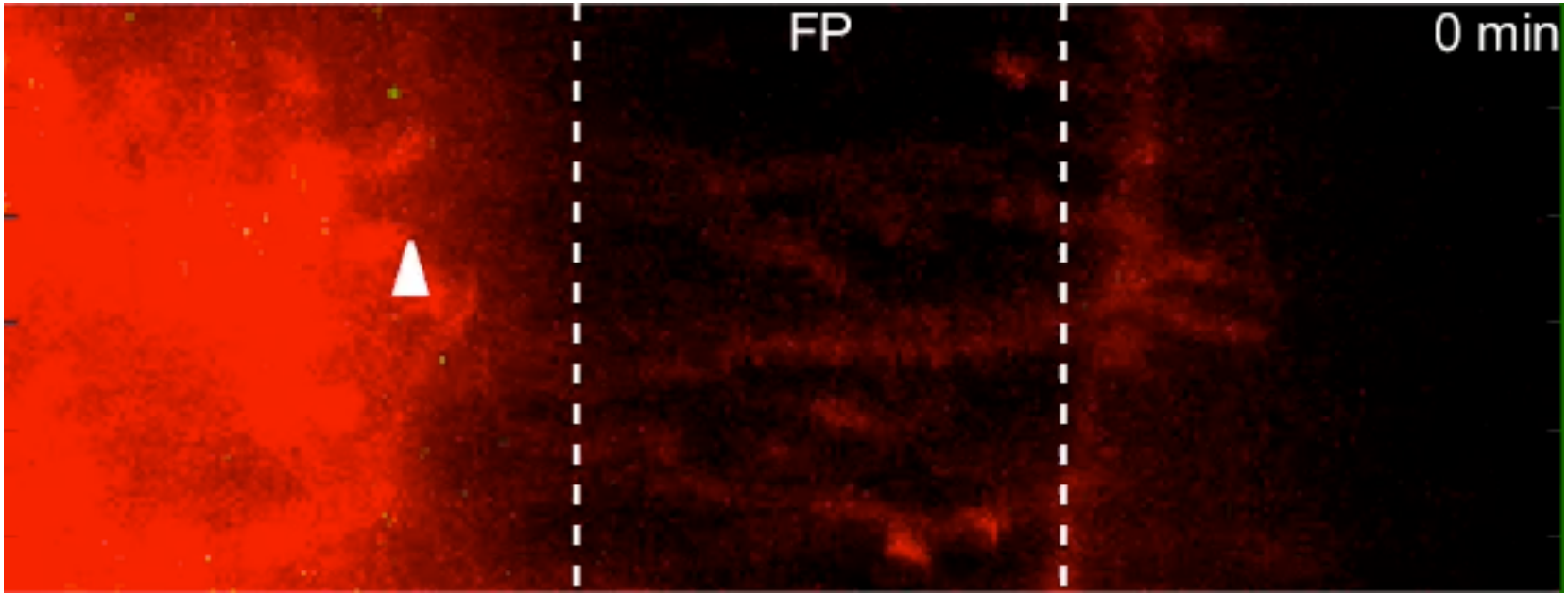
Robo1/2 spatial and temporal profiling



Pignata *et al*. (2019) BioRxiv

EMBL

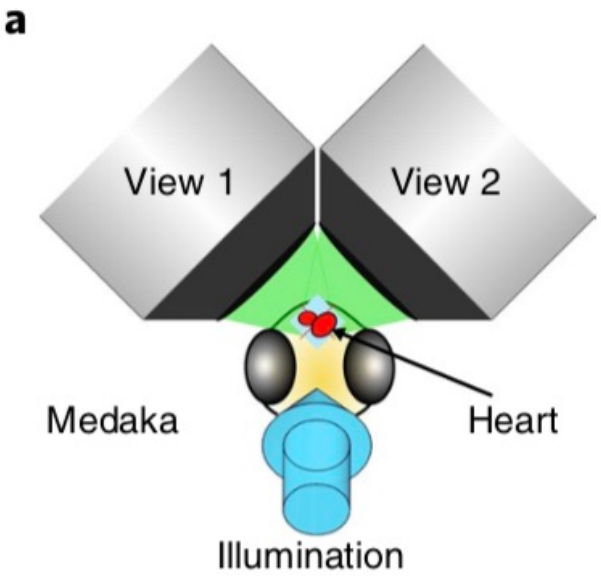
Robo1/2 spatial and temporal profiling

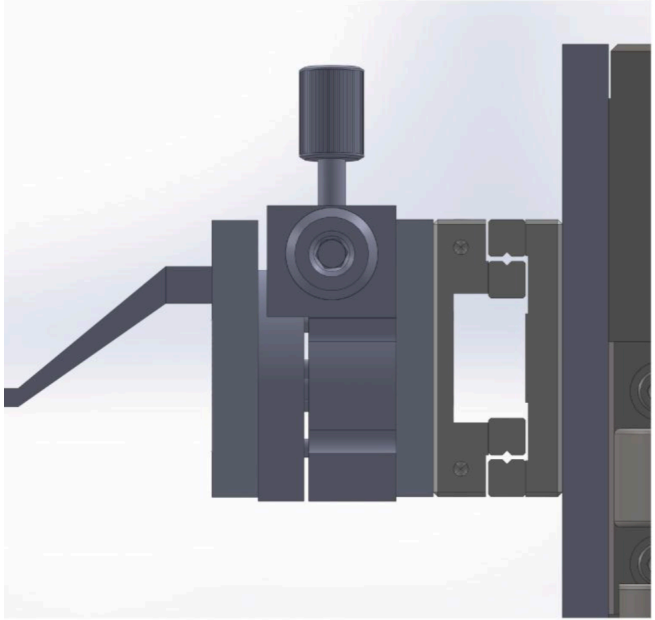


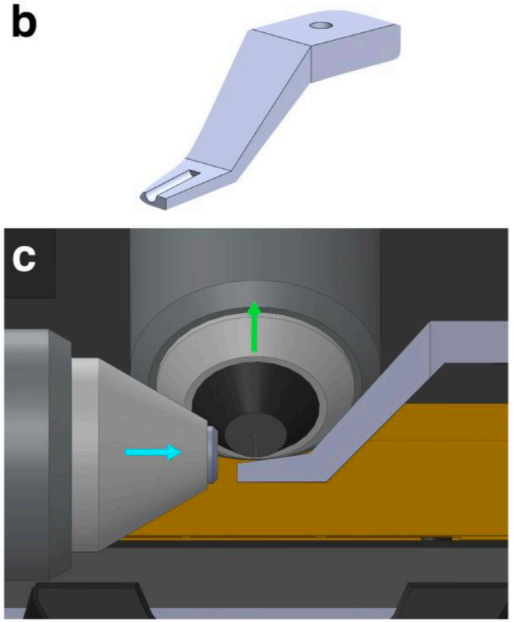
Pignata *et al*. (2019) BioRxiv

EMBL

Imaging fast biological processes in 3D







Wagner *et al*. (2019) Nat. Methods

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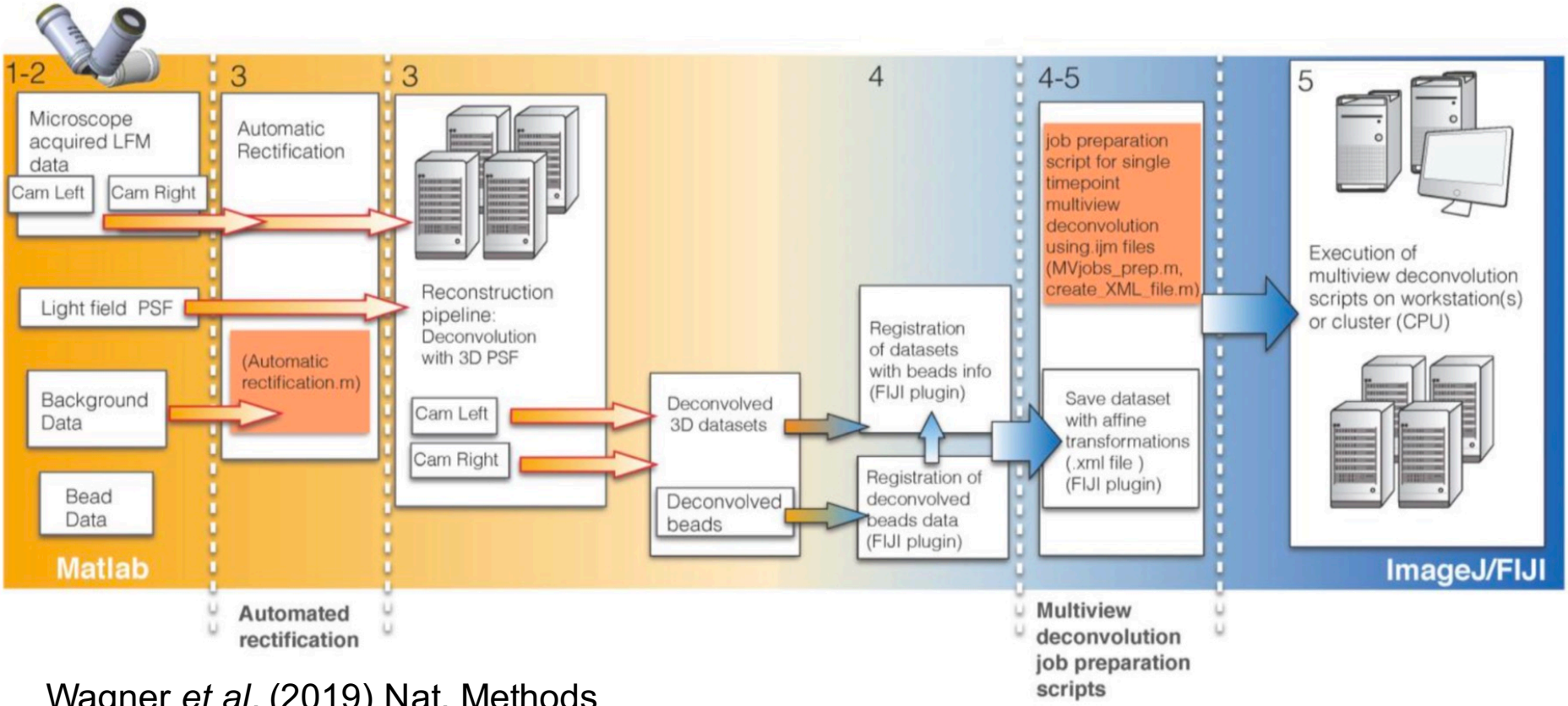
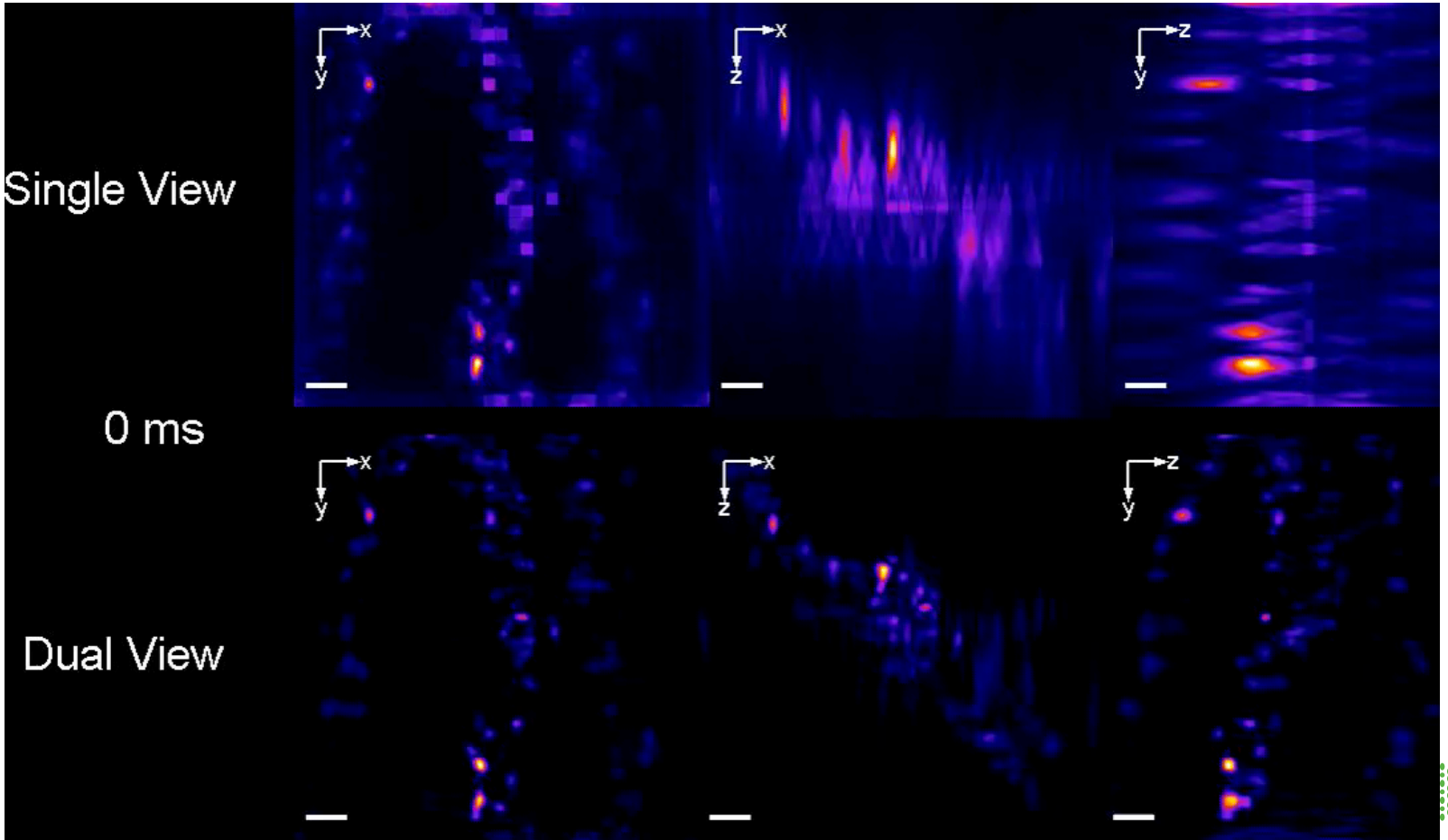
Imaging fast biological processes in 3D

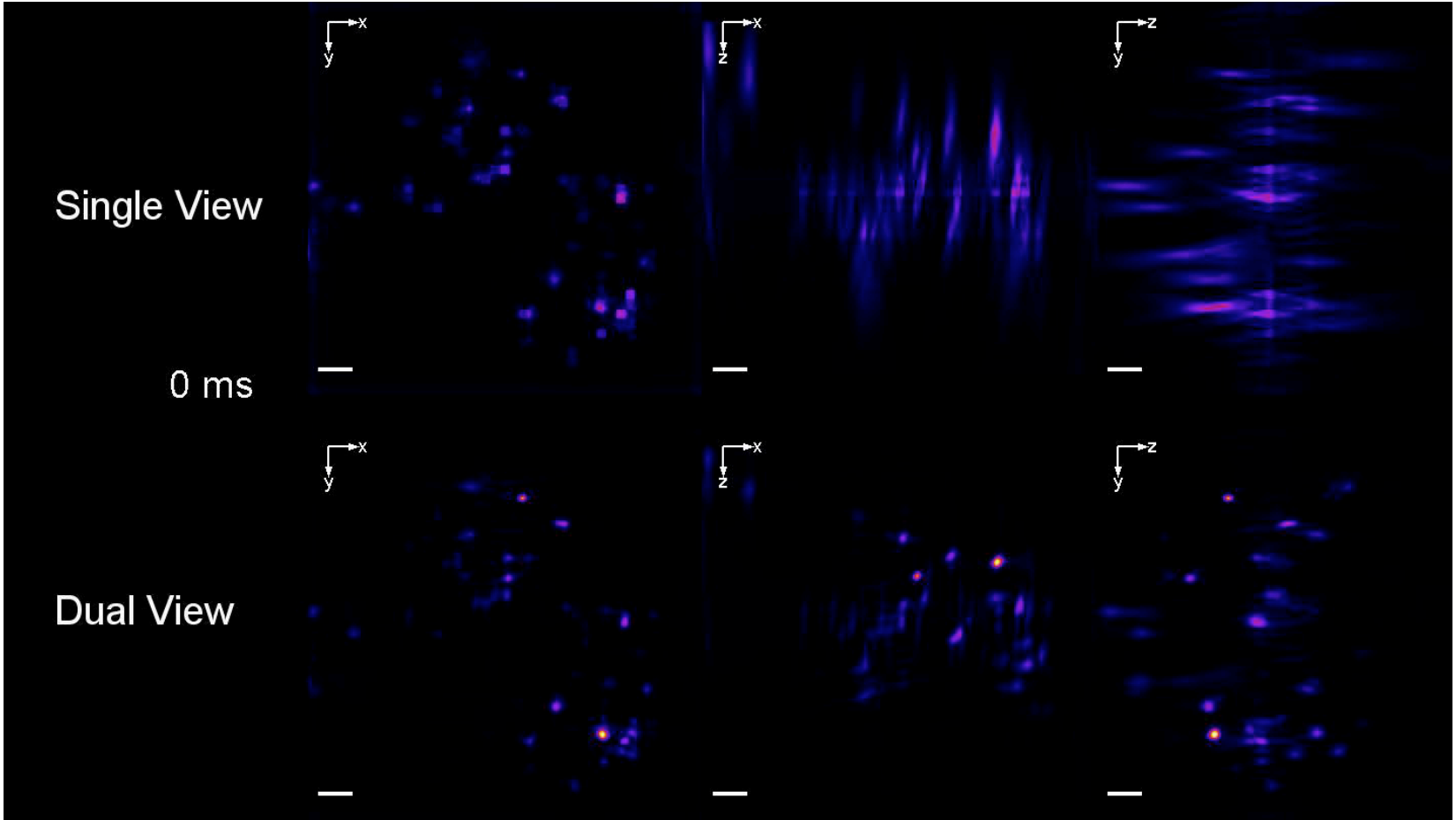


Wagner *et al*. (2019) Nat. Methods

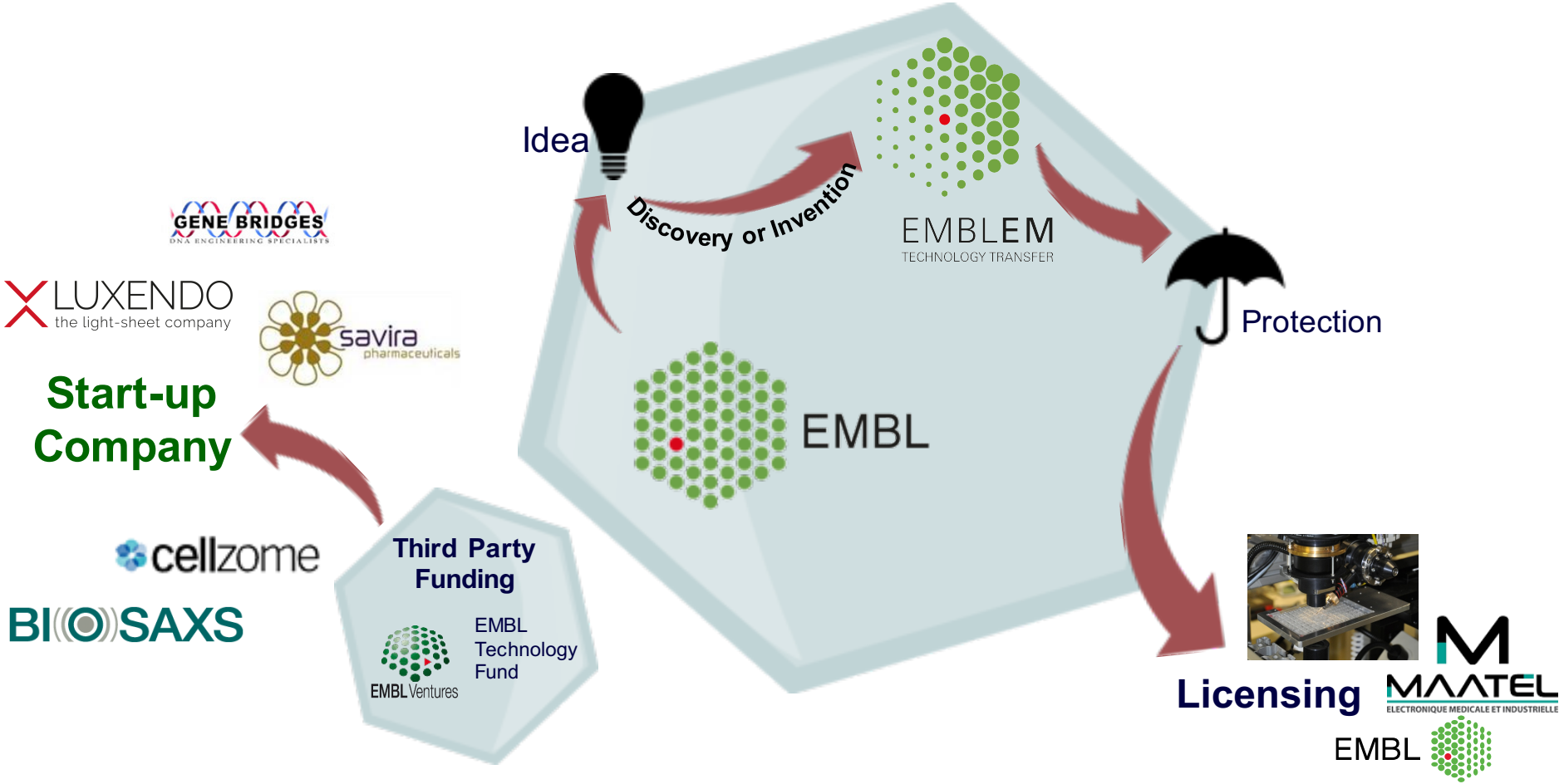
Single cell imaging of blood flow dynamics



Single cell imaging of blood flow in the heart



EMBL Technology & knowledge transfer: The players



ATTRACT

ACCELERATING DEVELOPMENT OF HIGH-PERFORMANCE DETECTOR AND IMAGING TECHNOLOGIES FOR SCIENCE AND MARKETS

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1. **ATTRACT** is a new, open, pan-EU initiative to accelerate the development of high-performance detector (sensor) and imaging technologies for both scientific and industrial use.

2. **It involves** European Research Infrastructures (ERIs), European Research institutes and Research and Technology Organisations (RTOs), Small and Medium Enterprises (SMEs), companies, universities and business and innovation specialists.

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Thank you

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Franck Felisaz
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