

Scientific instruments to answer biological questions at the EMBL

Andrew McCarthy (andrewmc@embl.fr)

Team leader, EMBL-Grenoble

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



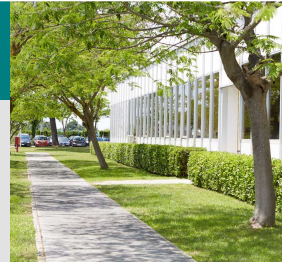
Heidelberg

Life sciences

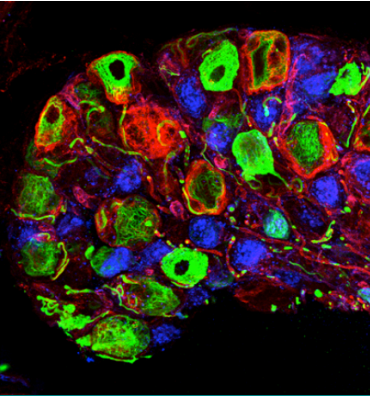


Rome

Epigenetics
and
neurobiology



EMBL's missions



Basic research



Services



Advanced
training

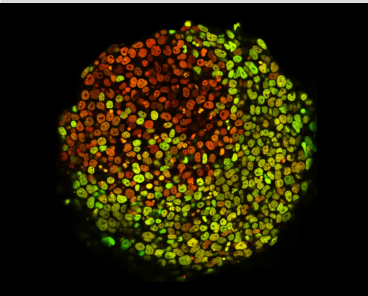


Technology
development
& transfer

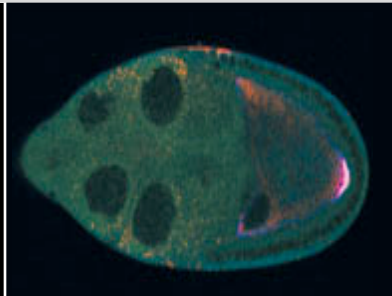


Integration
of life science
research

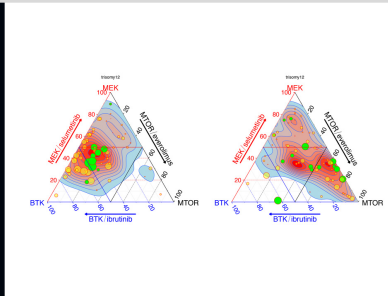
EMBL research units – over 80 independent research groups



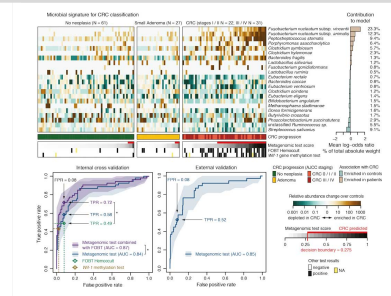
Cell biology and biophysics
– Heidelberg



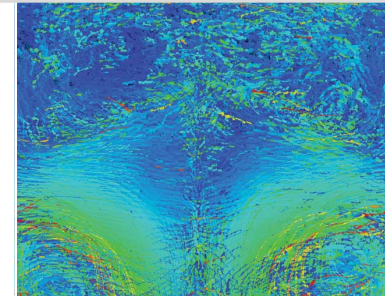
Developmental biology
– Heidelberg



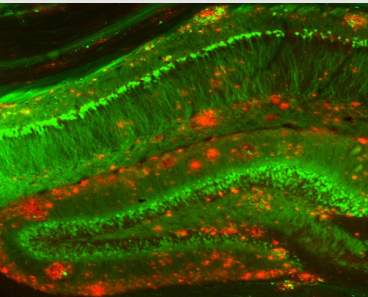
Genome biology
– Heidelberg



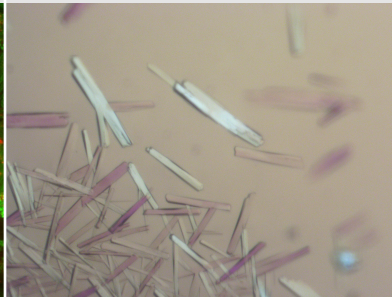
Structural and computational
biology – Heidelberg



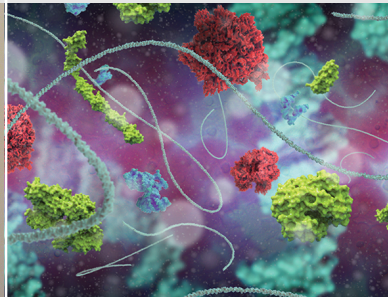
Directors' research
– Heidelberg



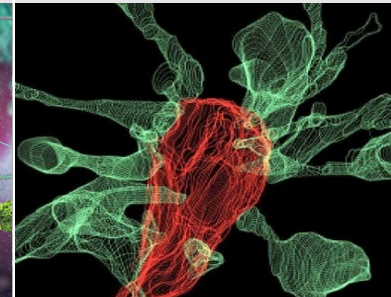
Structural biology
– Hamburg



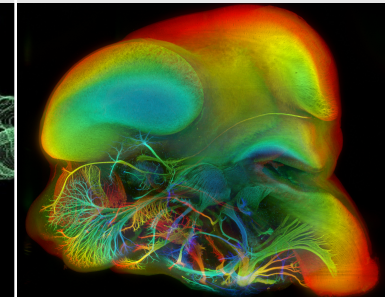
Structural biology
– Grenoble



Bioinformatics
– EMBL-EBI, Hinxton

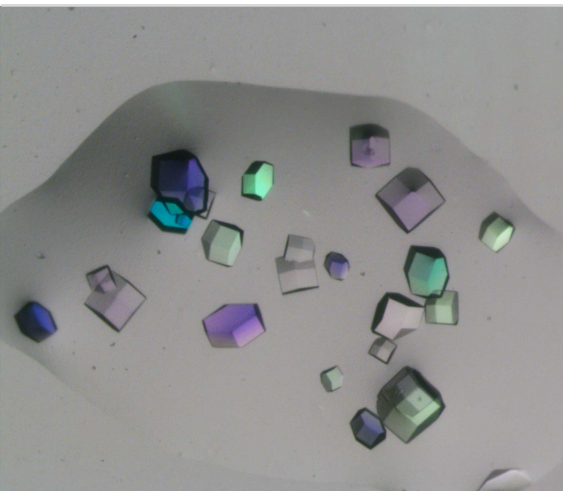


Epigenetics and neurobiology
– Rome



Tissue biology and disease
modelling – Barcelona

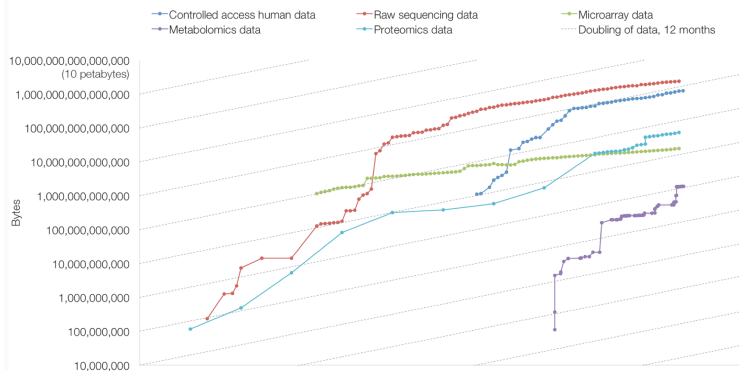
EMBL infrastructure and services



Structural biology services

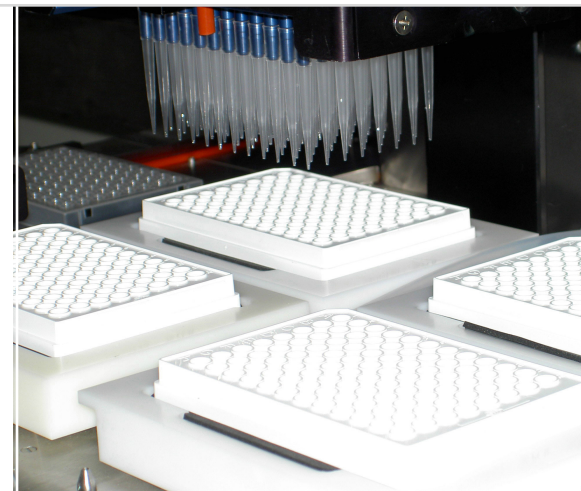
> 3,000 user visits per year
Many users of complementary services

Growth of data, by platform



Bioinformatics services

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



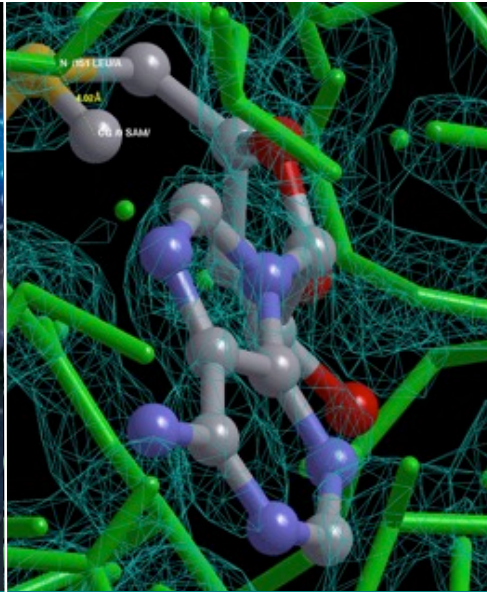
Core Facilities

~1,200 internal and external users per year

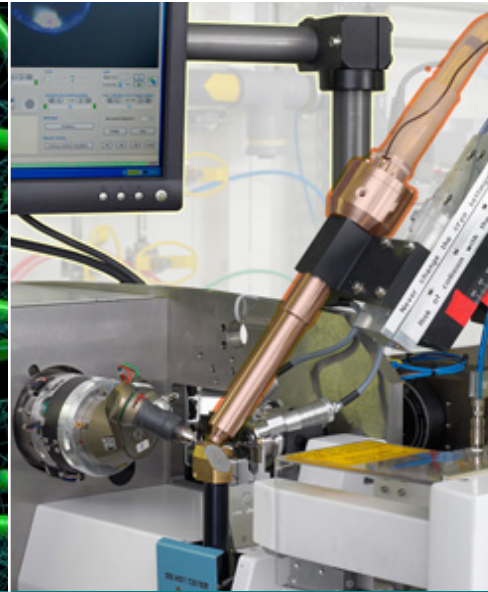
Technology development and transfer



Imaging
technology



Software
development



Synchrotron
instrumentation

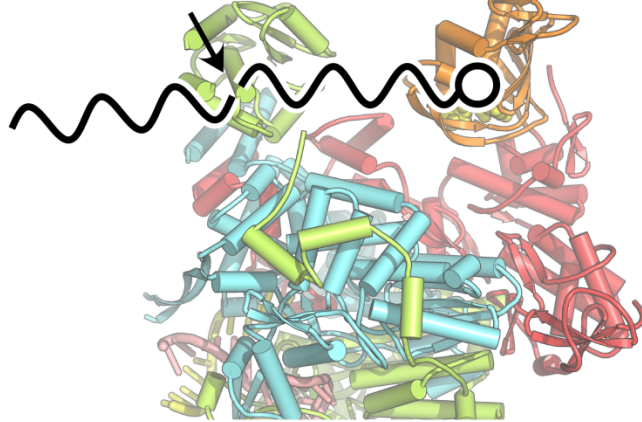
EMBL develops a broad spectrum of technology and instrumentation for life science research

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

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

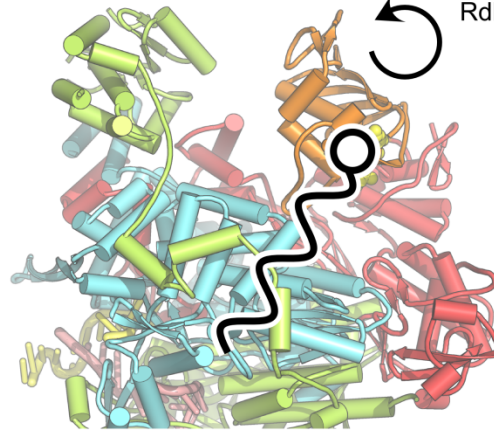
2

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



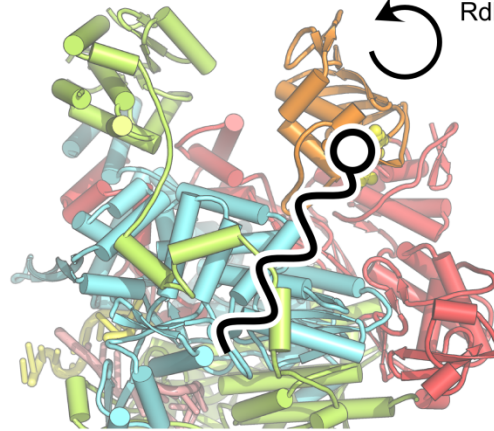
1

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



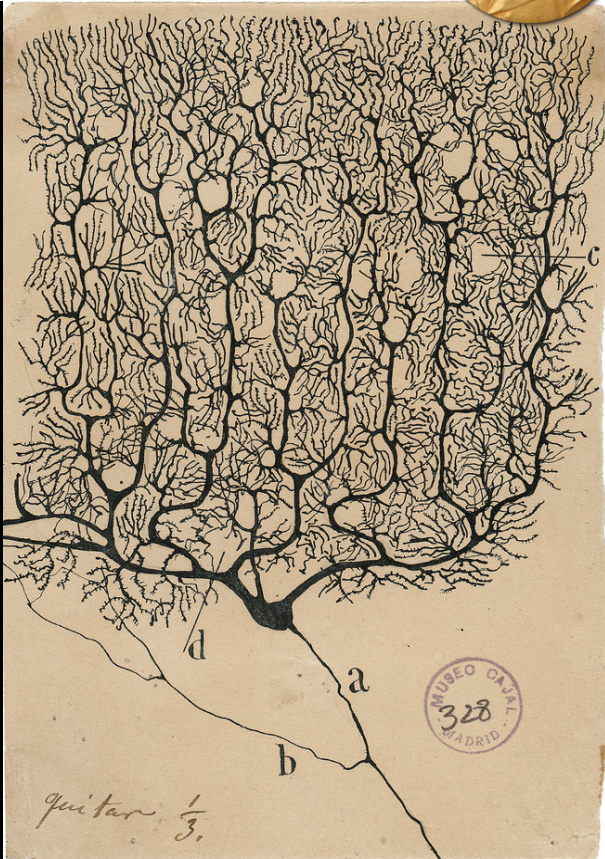
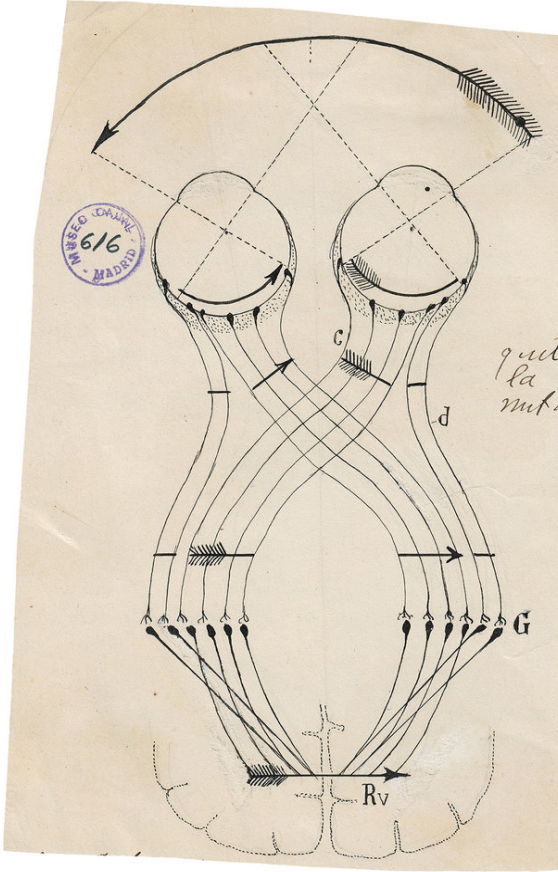
4

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

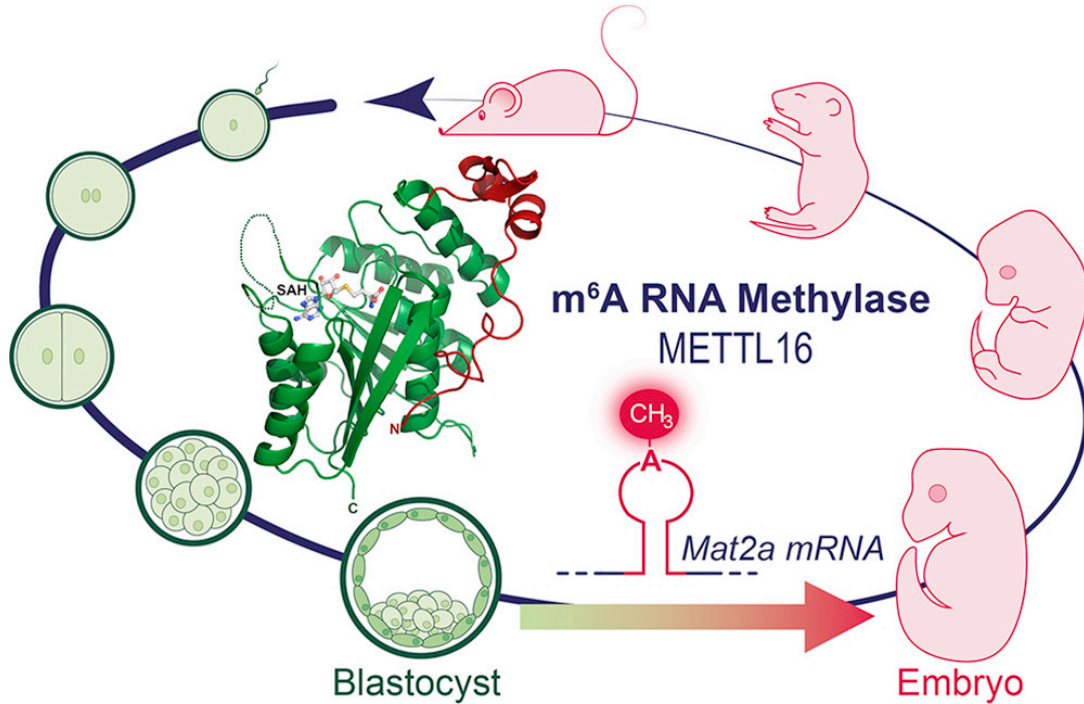
Pflug *et al.* (2014) *Nature*, **516**, 355–360.
Reich *et al.* (2014) *Nature*, **516**, 361–366.
Thierry *et al.* (2016) *Mol. Cell*, **61**, 125–137.
Lukarska *et al.* (2017) *Nature*, **541**, 117–121.
Omoto *et al.* (2018) *Sci. Rep.*, **8**, 9633. - Baloxavir marboxil

Nobel prize in physiology or medicine (1906)

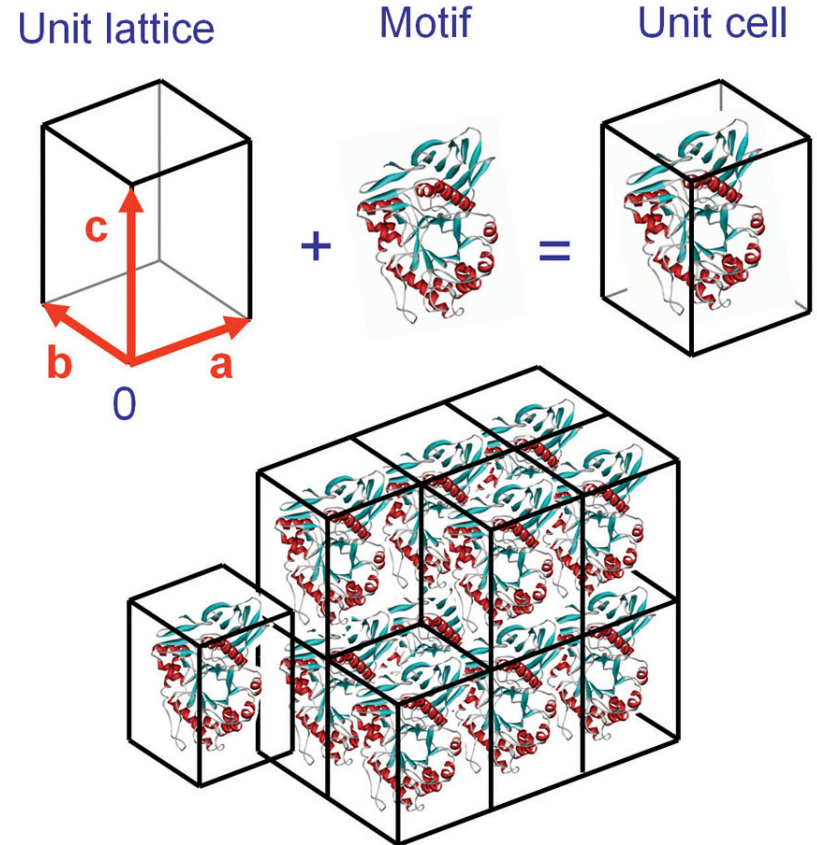
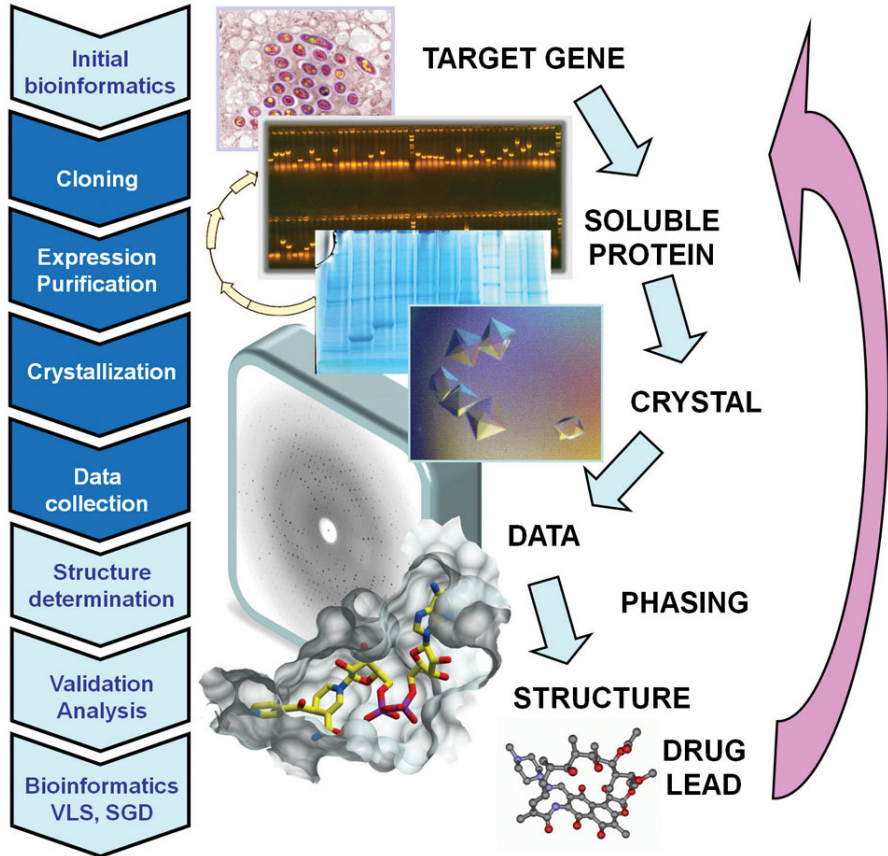
Ramón y Cajal (1852-1934) – *shared* with Camillo Golgi



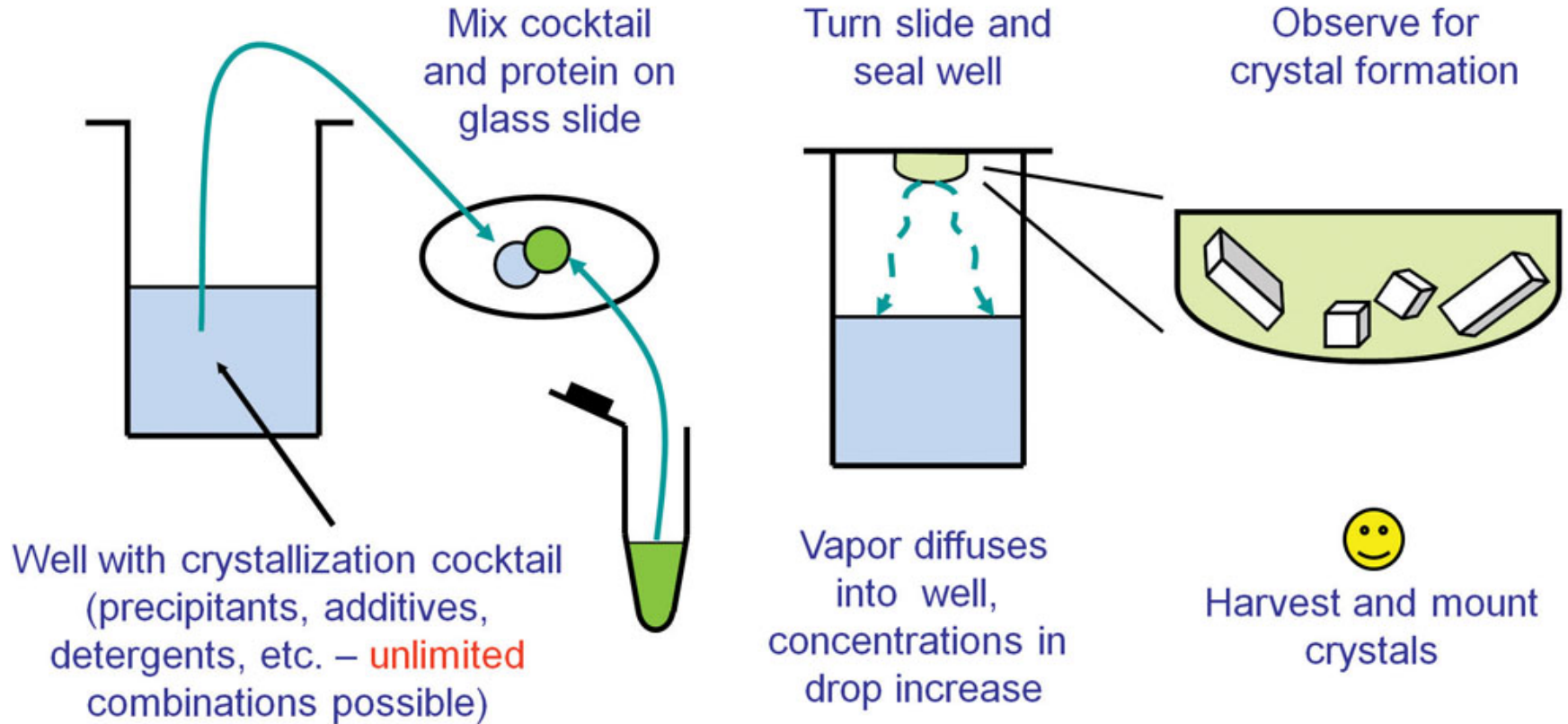
METTL16 (*McCarthy team*) - EMBL-Grenoble outstation



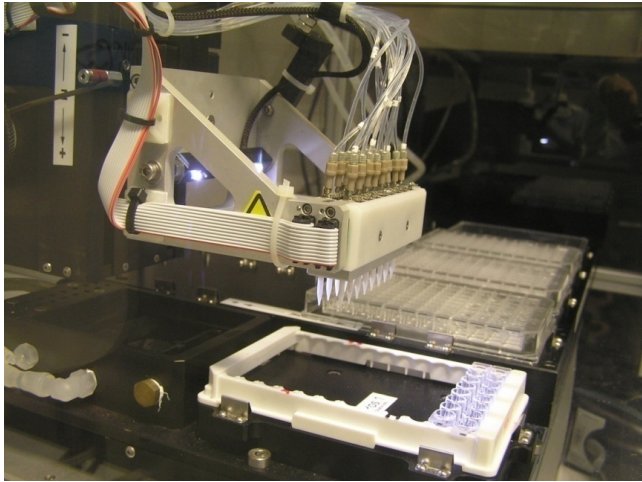
Structural biology techniques (MX)



Protein crystallisation



Automation of crystallisation



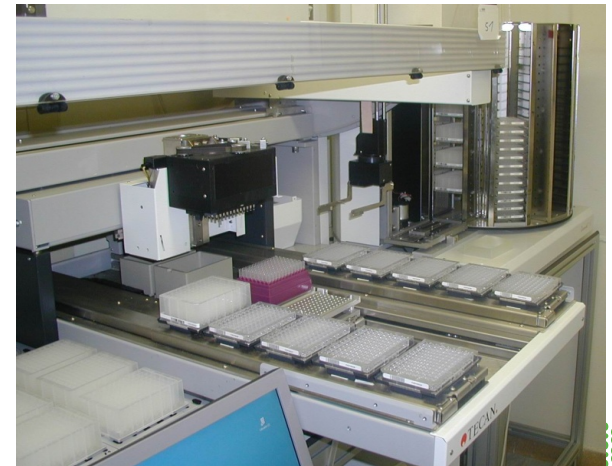
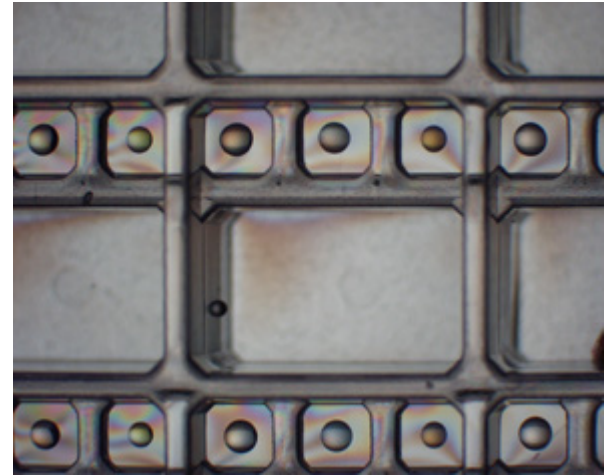
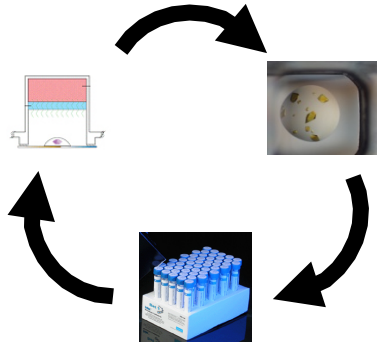
✓ 100 nl + 100 nl drops (50nl + 50nl)

✓ 3 samples per plate

✓ 6-9 samples in parallel

✓ 90 µl of sample for std. screening

✓ Strict Quality Control



Automation of crystallisation



Marquez Team - High-thro... x Plate Details x Image Viewing - CD014295 x CD014295 - B3 | HD x

https://embl.fr/htxlab/index.php?option=com_images&view=images&layout=viewer&barcode=CD014295&inspection=last

Most Visited Library/Main - Intranet Exit, removing your da...

HTXLab
Crystallization Information Management System
Developed by EMBL

Home Information User Help

Dr. Andrew MC CARTHY

Well : B3 | Position : 3

Plate : CD014295 | Inspection : 1 (Vis) | 1 day(s) | Inspection Date : 17/11/2016 | Setup Date : 16/11/2016 10:32

View in HD Popup HD Drop History Summary Optimize Hit Tracking

zorder

12
11
10
9
8
7
6
5
4
3
2

Viewing Settings

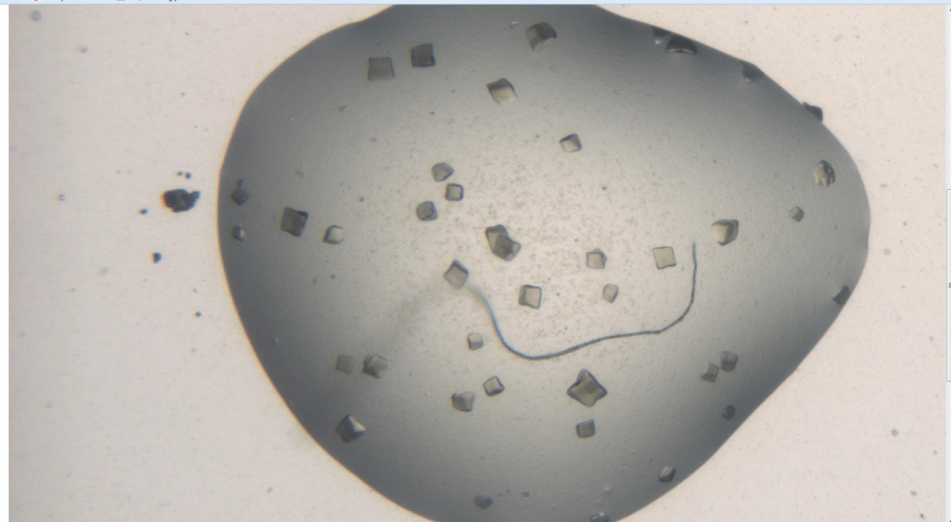
- Select browsing sequence
 - Position 1
 - Position 3
- Show samples Side-to-Side
 - Position 1
 - Position 3

Apply

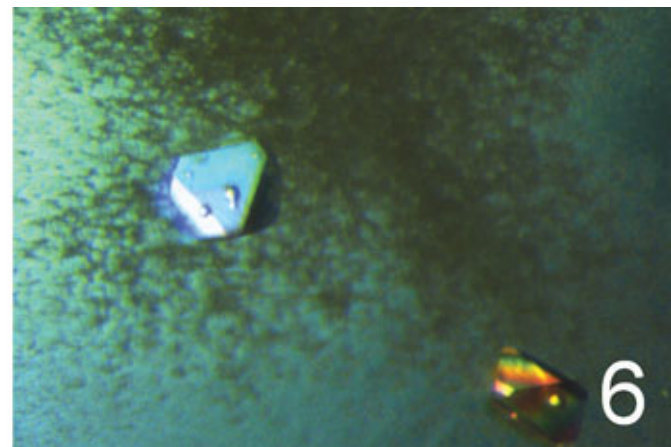
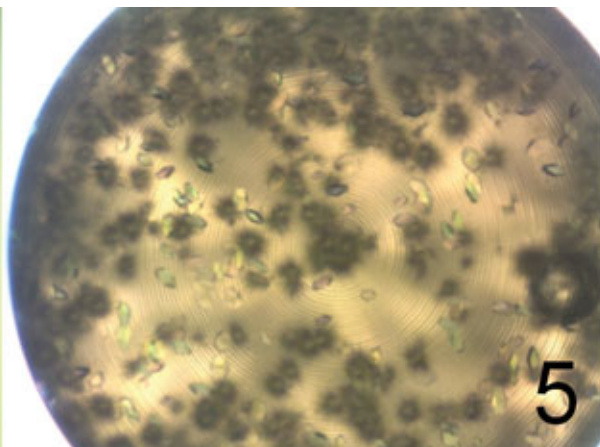
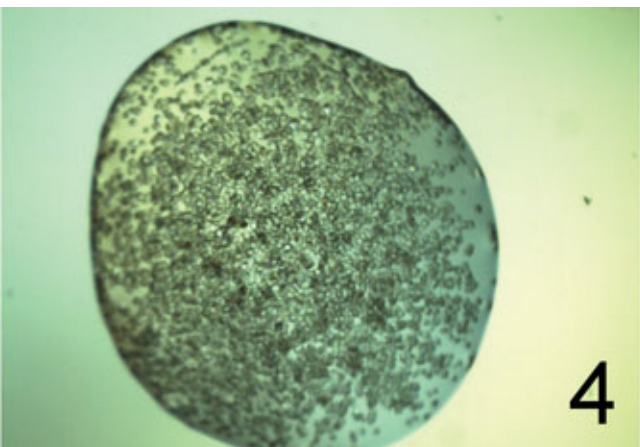
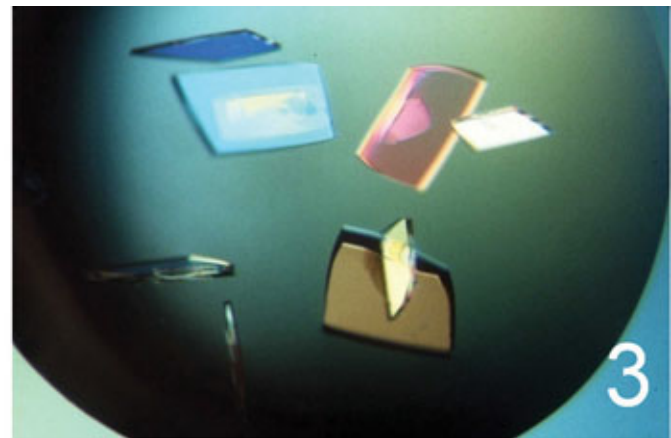
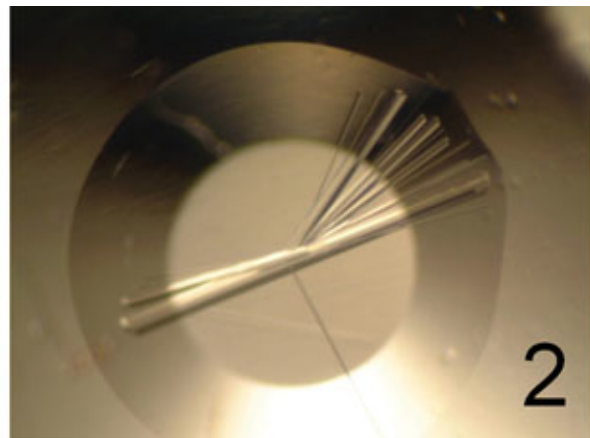
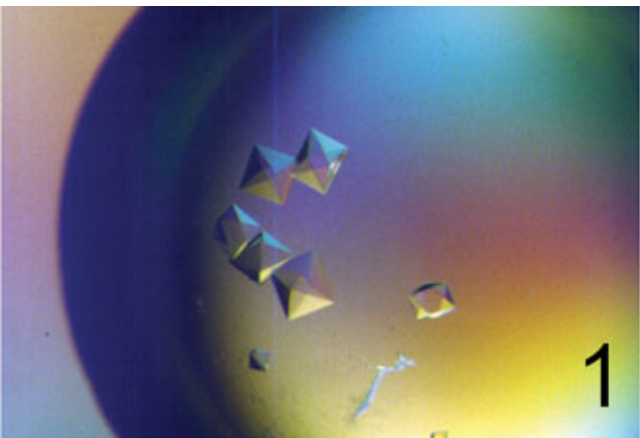
Marquez Team - High-thro... x Plate Details x Image Viewing - CD014295 x CD014295 - B3 | HD x

https://embl.fr/htxlab/index.php?option=com_images&view=images&layout=viewer&barcode=CD014295&inspection=1&popup=CD014295-B3 | HD | Vis

Most Visited Library/Main - Intranet Exit, removing your da...



Protein crystals



Darwin's formula

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

$I(\mathbf{hkl})$ - photons/spot (fully-recorded)

I_{beam} - incident (photons/s/m²)

r_e - classical electron radius
(2.818x10⁻¹⁵ m)

V_{xtal} - volume of crystal (in m³)

V_{cell} - volume of unit cell (in m³)

λ - x-ray wavelength (in meters!)

ω - rotation speed (radians/s)

L - Lorentz factor (speed/speed)

P - polarization factor

$$(1 + \cos^2(2\theta) - P_{\text{fac}} \cdot \cos(2\Phi) \sin^2(2\theta)) / 2$$

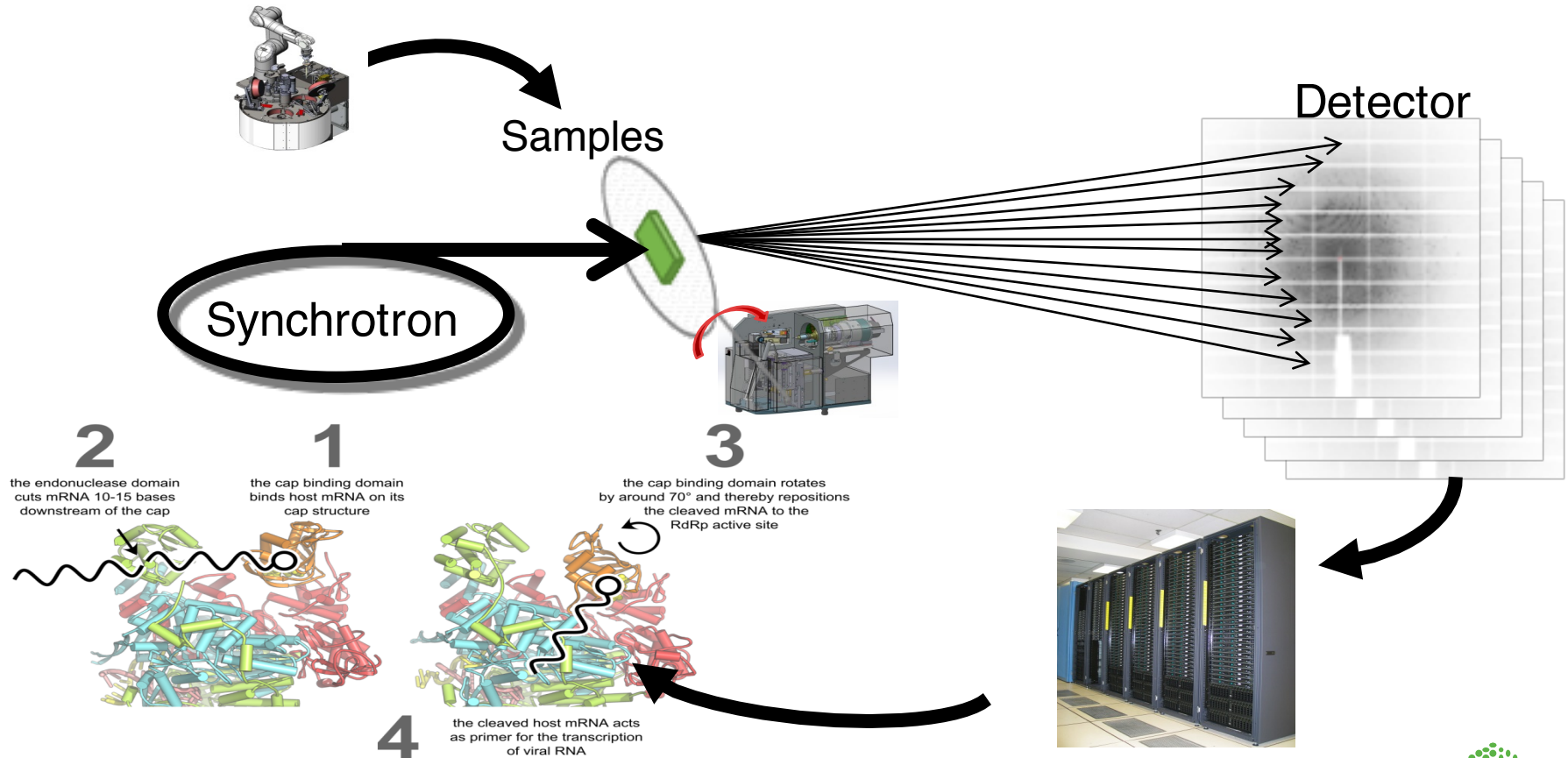
A - absorption factor

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

$F(\mathbf{hkl})$ - structure amplitude (electrons)

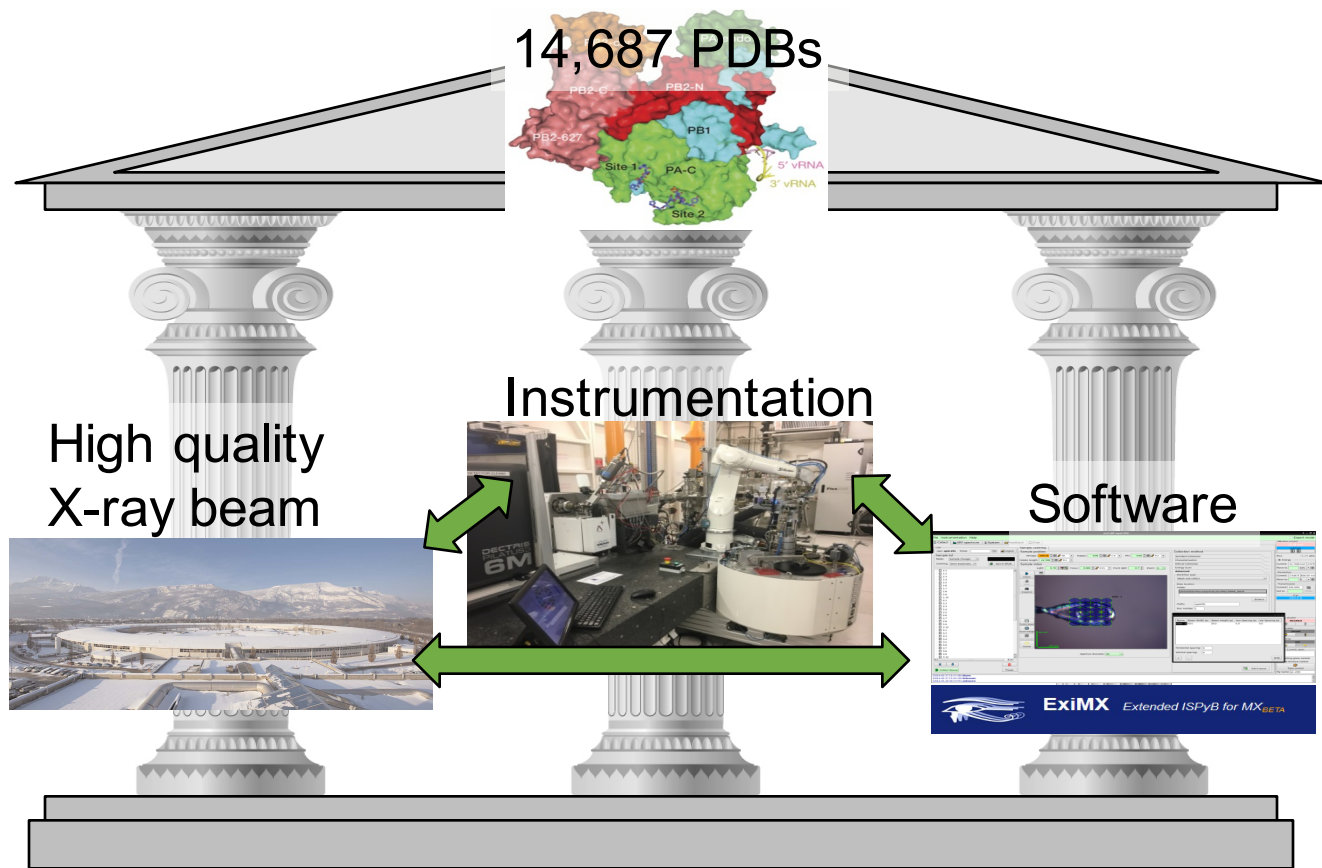
C. G. Darwin (1914)

EMBL-Grenoble – EPN campus



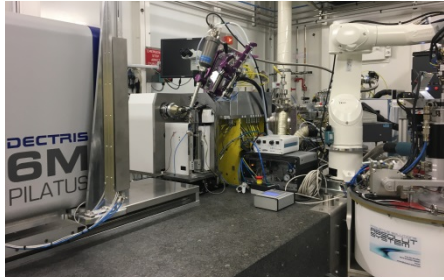
Lukarska *et al.* (2017) *Nature*, **541**, 117-121.

ESRF-EMBL Pillars of success

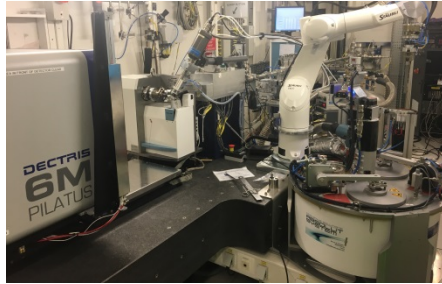


ESRF-EMBL Joint Structural Biology Group Beamlines (2018)

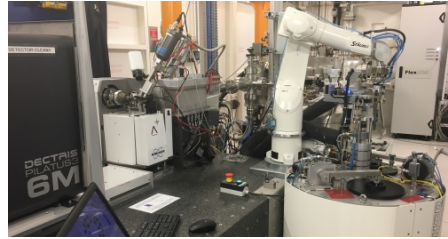
ID23-1 (tunable)



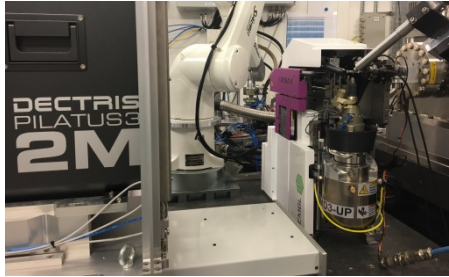
ID29 (tunable)



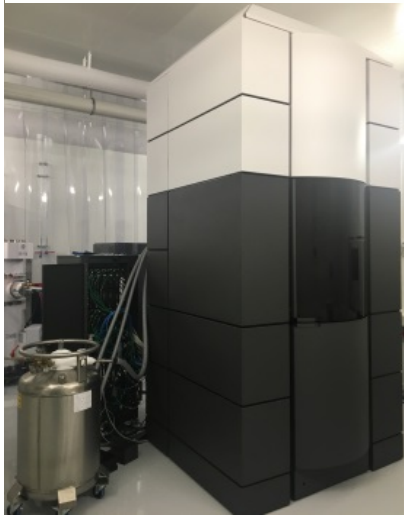
ID30B (tunable)



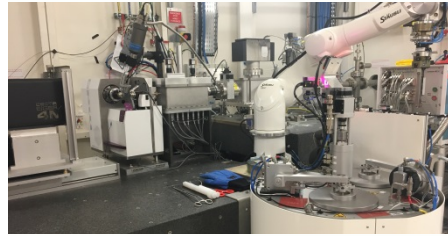
ID23-2 (μ focus)



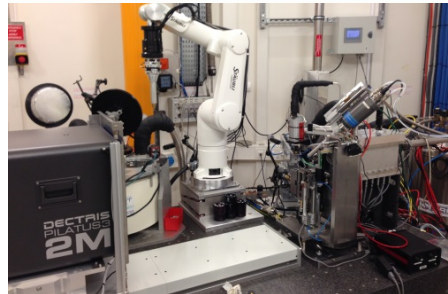
CM01



ID30-A3 (μ focus)



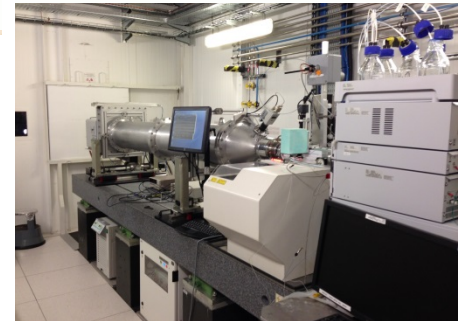
ID30-A1 (MASSIF)



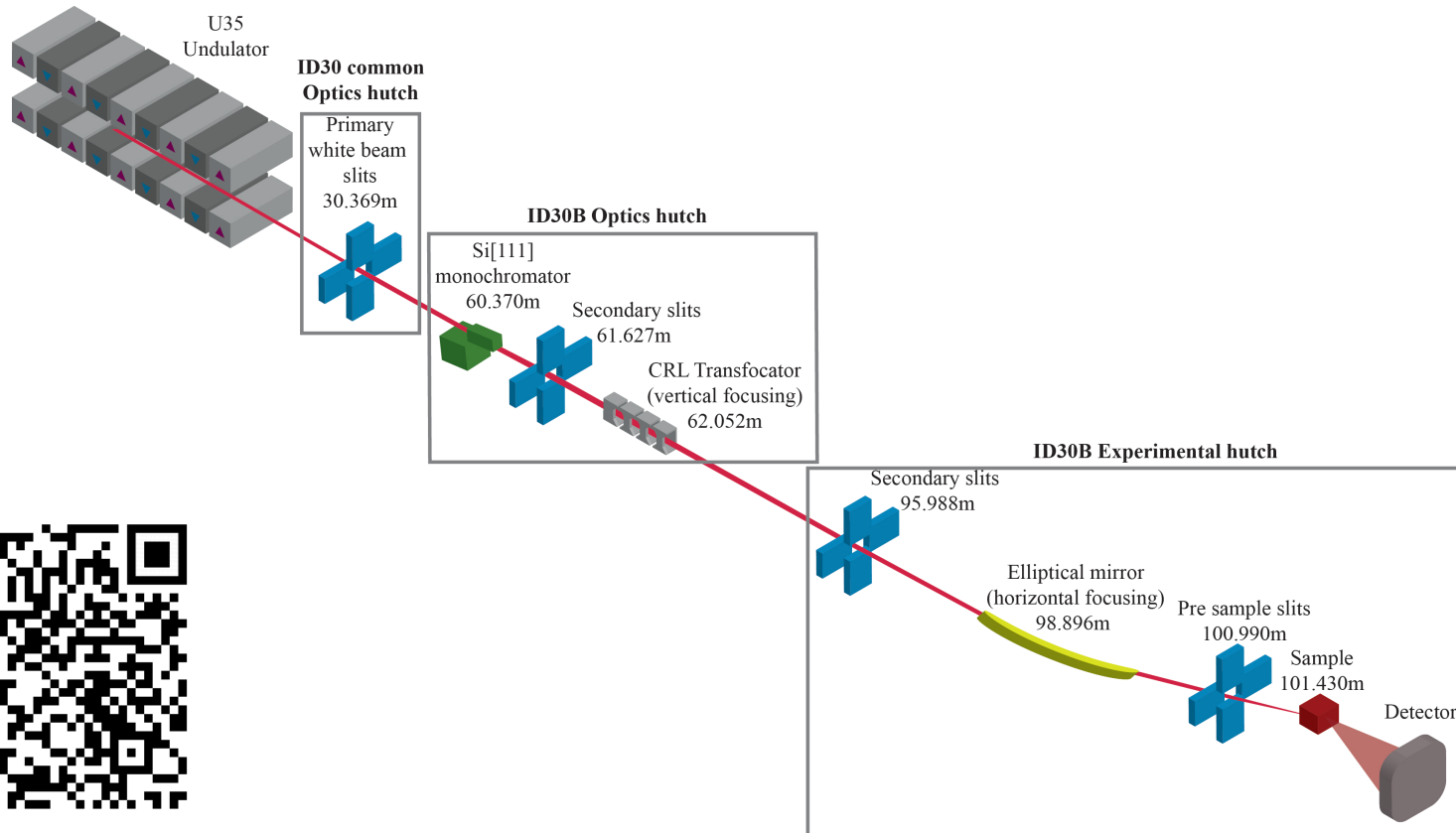
ESRF-EBS
Extremely Brilliant Source

12/2018 – 8/2020

BM29 (BioSAXS)



ID30B optical layout



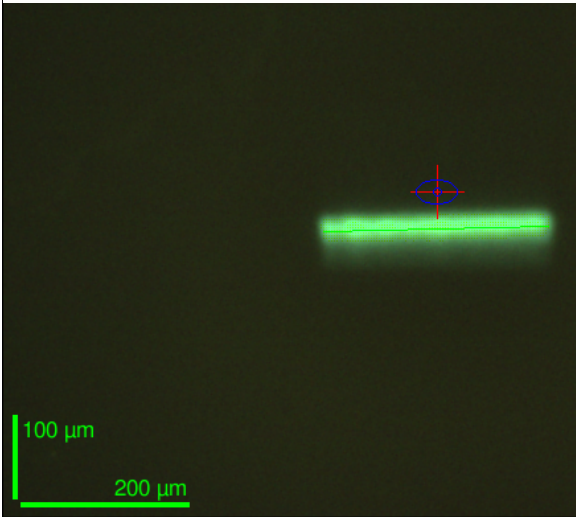
ID30B variable focus

Energy range: 6-20 keV

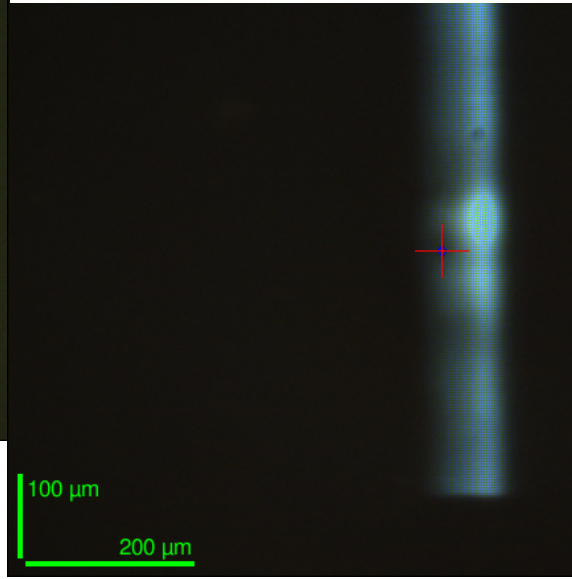
Flux $\sim 5 \times 10^{12}$ phs/sec/mm² at 12.7 keV

Beam size: $< 40 \mu\text{m}^2$ (apertures – 10, 20, 30, 50 and 75 μm^2)

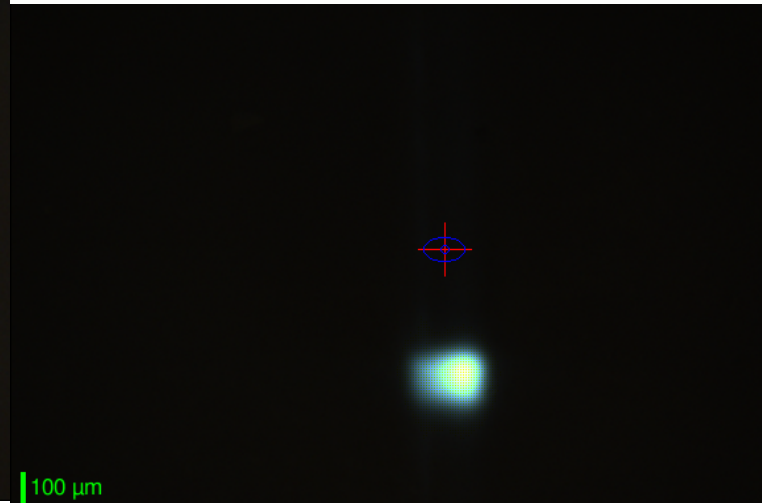
Vertical focusing, no horizontal focusing
(mirror unbent)



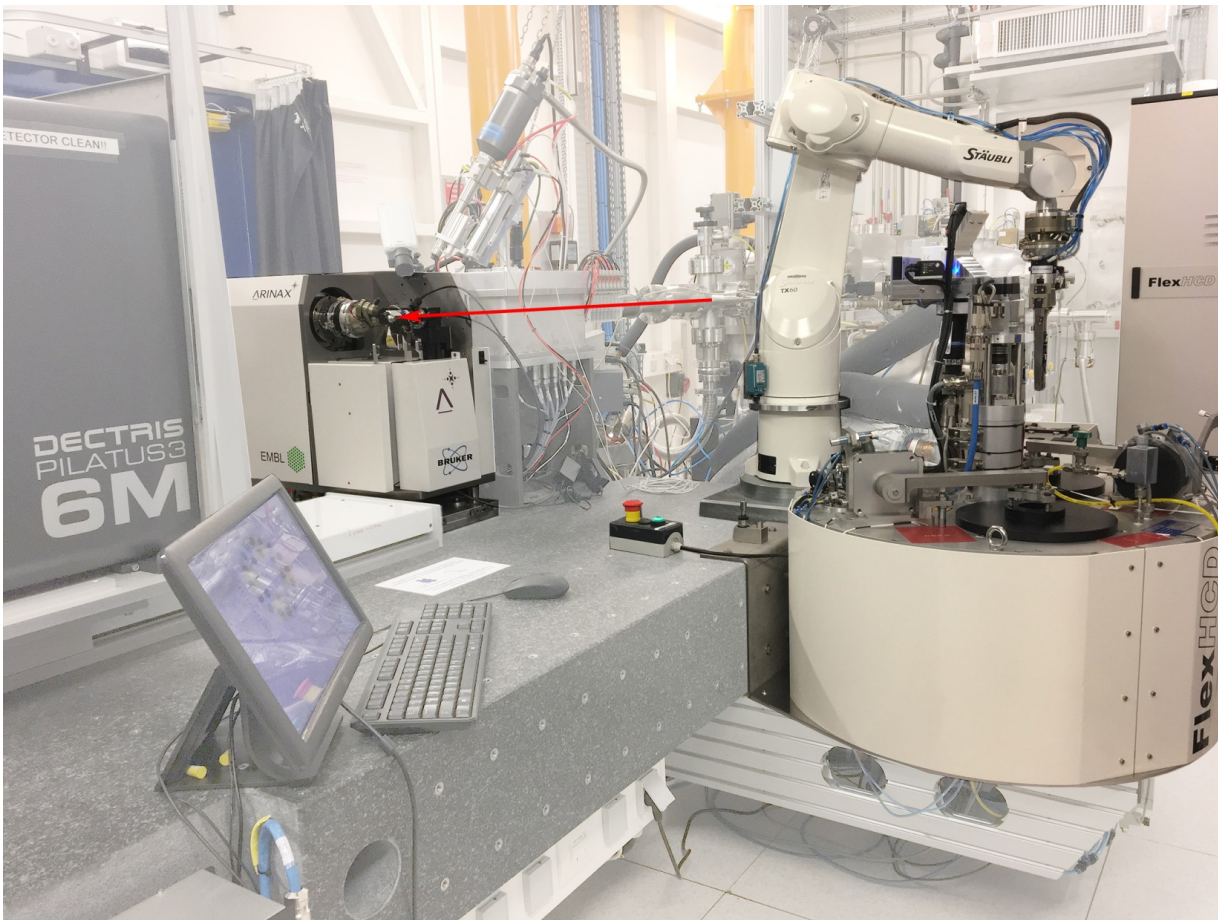
No vertical focusing – mirror bent



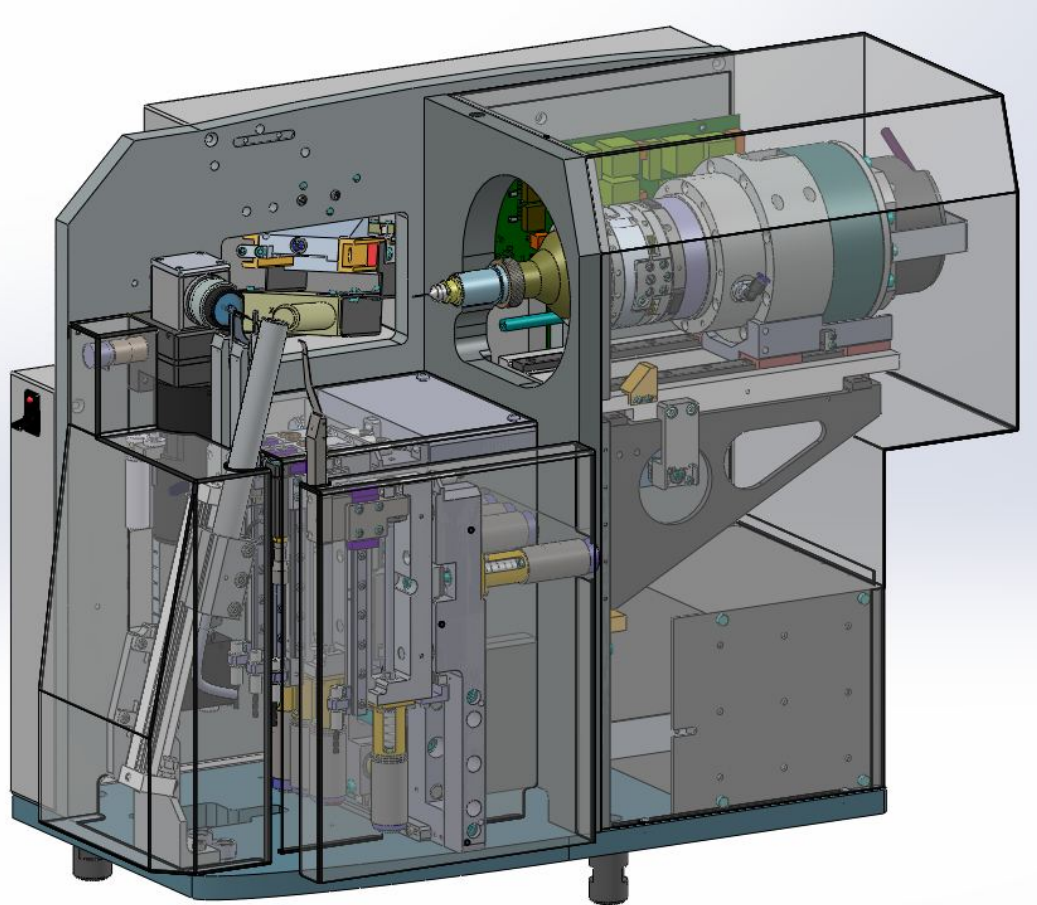
Focused beam

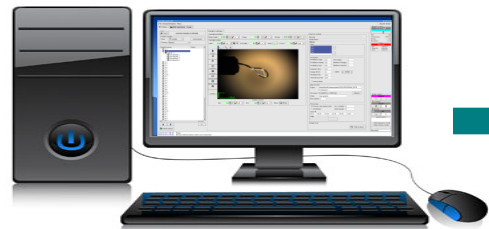


ID30B at the ESRF



Microdiffractometer – MD2S





Prepare for data collection
(frame on hardware trigger)

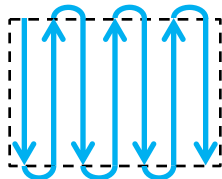


MXCUBE



Do a grid scan
Scan Finished

**Full PMAC
with latch**



Trigger

Detector

Frame readout
For precise coder latch



Latched
Position

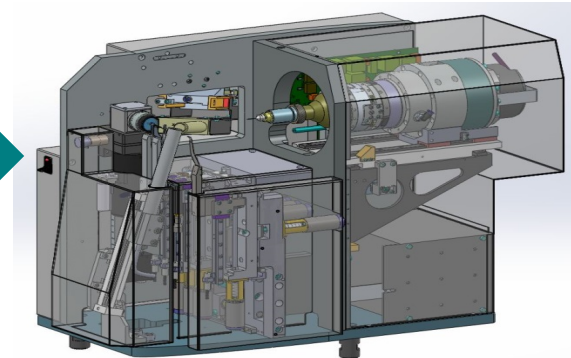


Done

MD software



PMAC rack



Diffractometer

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

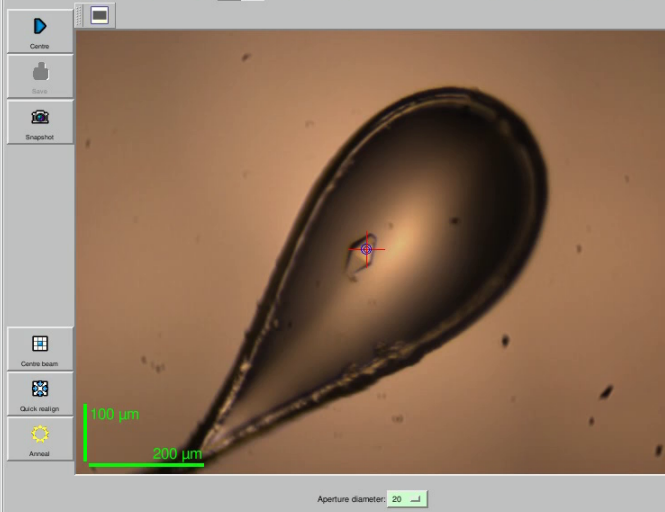
Sample centring

Sample position

Omega: 330.00 Kappa: 0.00 Phi: 0.00
 Holder length: 22.668 samp: -0.69 sampy: -0.63

Sample video

Back Light: 0.50 Focus: -0.21 Front Light: 0.0 Zoom: 4



- Centre
- Save
- Snapshot
- Centre beam
- Quick re-align
- Annul

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: [Y]
 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:

 File name: Thau_1_####.cbf Browse
 Prefix: Thau
 Run number: 1

Processing

N.o. residues: 200 Space group: [Y]
 Unit cell:
 a: 0 b: 0 c: 0
 α: 0 β: 0 γ: 0

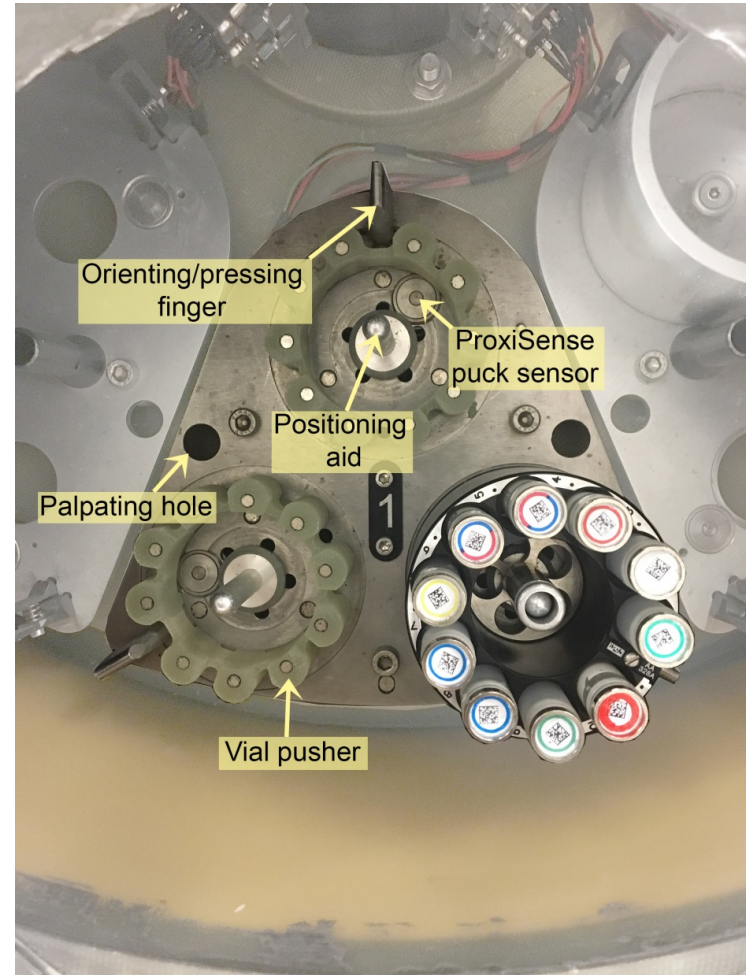
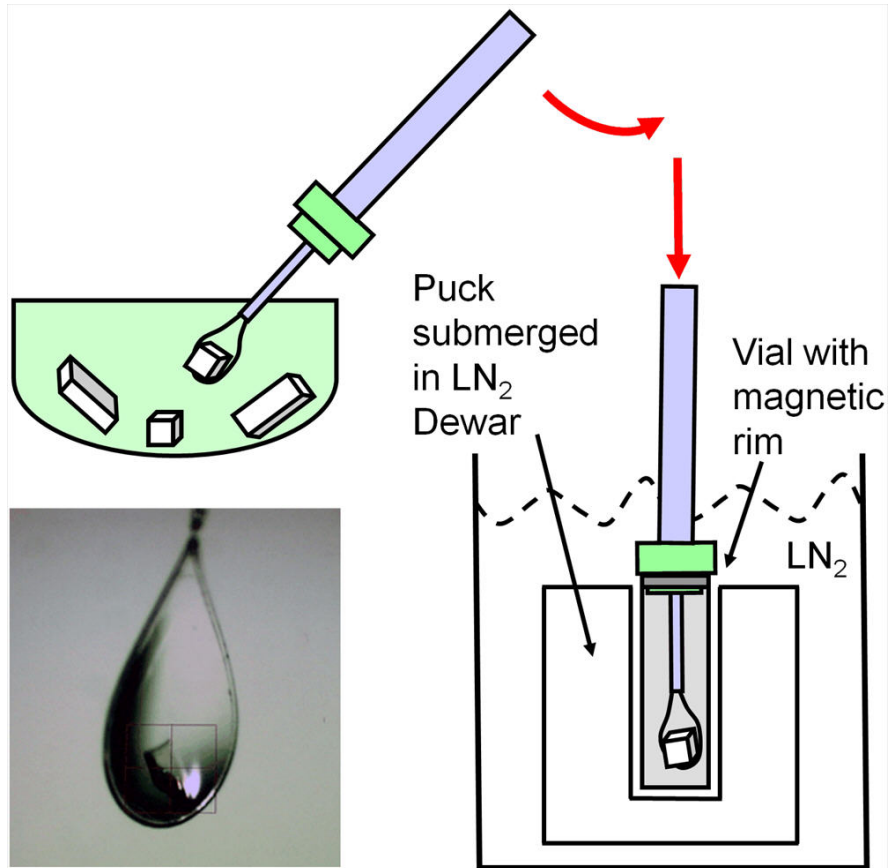
Characterisation

- Helical Collection
- Energy Scan
- XRF Spectrum
- Advanced

Add to queue

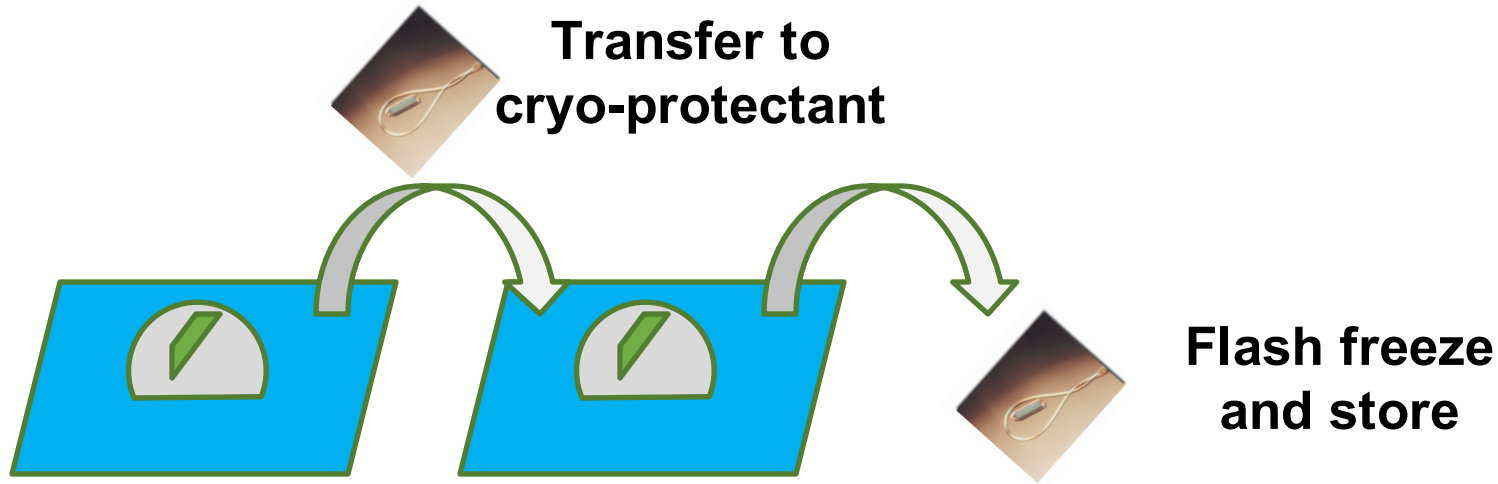
Machine current: 86.9 mA
 Flux: 6.88e+11 p/s
 Energy: []
 Current: 12.7000 keV, 0.976 A
 Move to: [] keV
 Resolution: 1.998 Å, 392.36 mm
 Current: [] A
 Move to: [] A
 Transmission: 100.00%
 Current: []
 Set to: []
 Beamstop distance: 37.0
 Cryo: 100.01 K
 Safety shutter: opened
 Fast shutter: closed
 Beamstop: in
 Capillary: in
 Current users: []
 Selecting gives control
 Allow timeout control
 Take control
 My name: bacon

Crystal fishing and storage



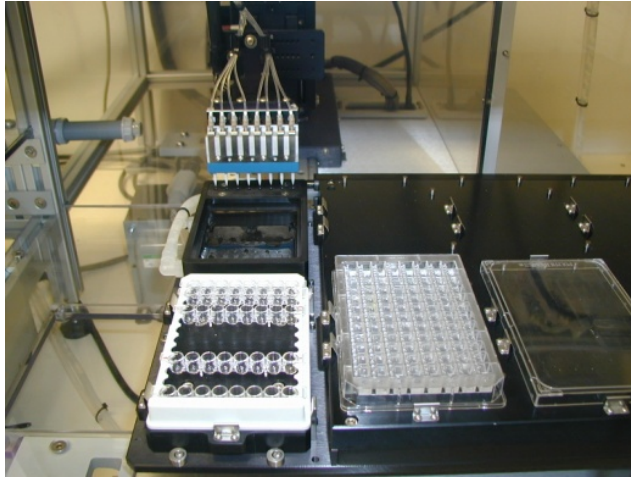
Crystal fishing and storage

Cryo-protectants and/or ligands through serial transfer

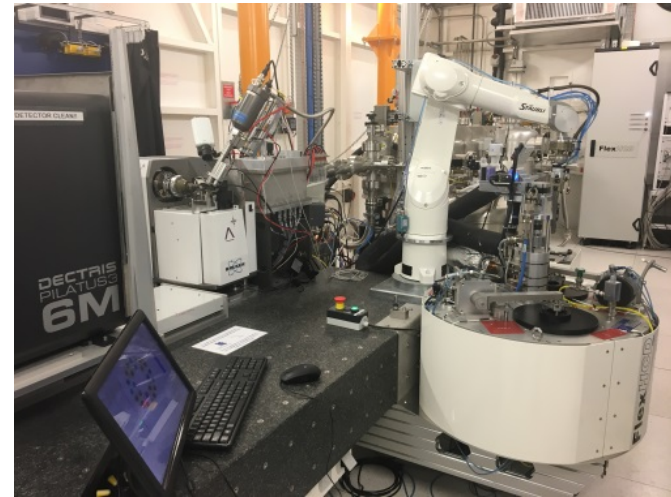


Crystal Direct™: a new concept for Crystal harvesting

HTX Lab, J.A. Marquez



Instrumentation, F. Cipriani



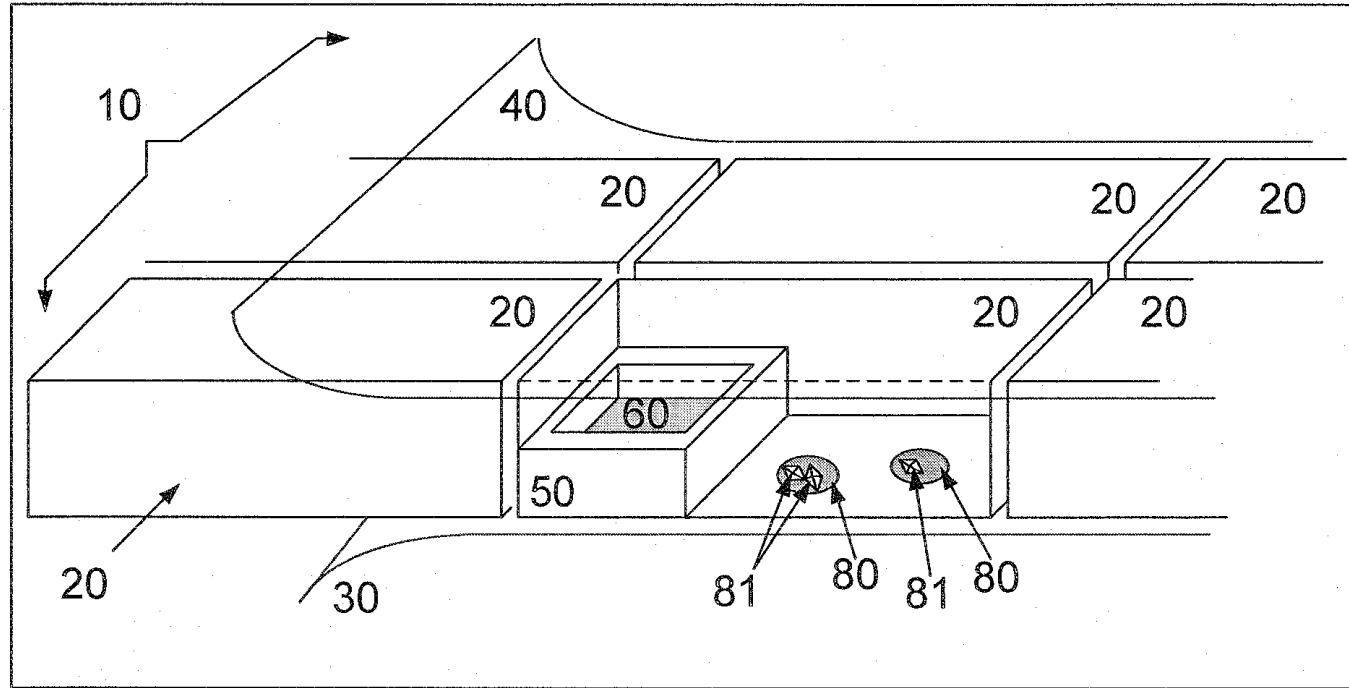
Open Access HTX Facility

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

Synchrotron Instrumentation

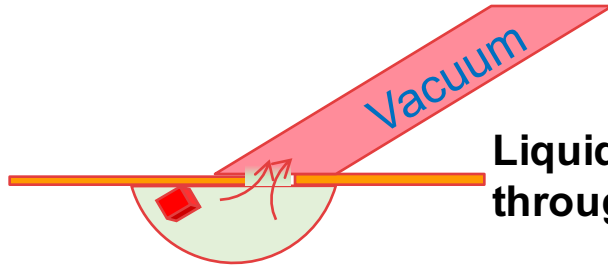
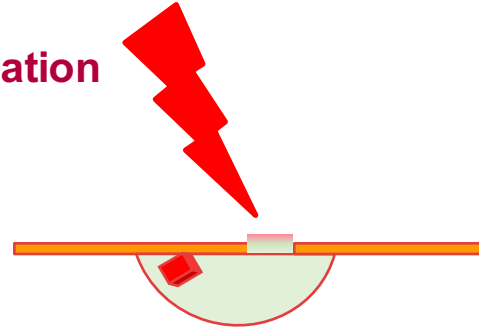
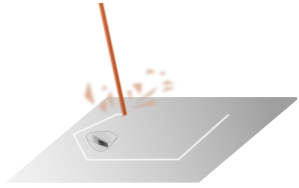
- Instruments for MX and SAXS beamlines
- Beamline automation
- Sample holder standards
 - SPINE
 - NewPin

Crystal Direct™: a new concept for Crystal harvesting

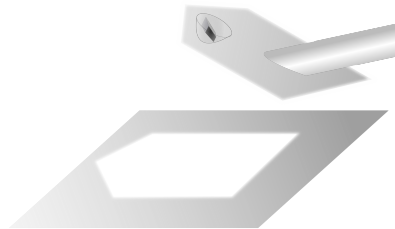


Crystal Direct™: a new concept for Crystal harvesting

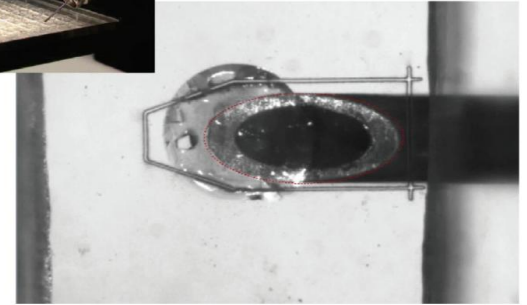
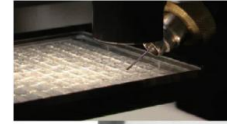
Laser photo ablation



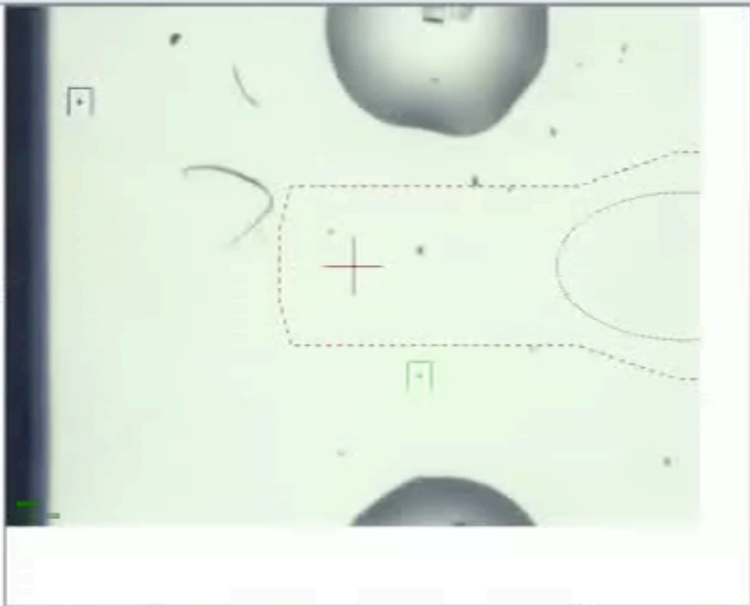
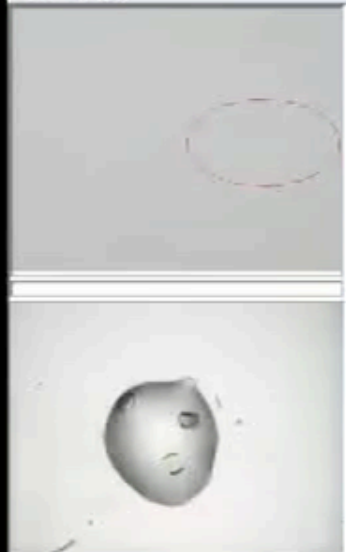
Liquid removal
through aspiration



Reduce sample size
increased cooling rate
Reduce background scattering



Harvesting crystals grown on a thin film by photo ablation



Monitoring

General Hardware Status: ● OK

Laser: ● Ready

Cryo: 99.9

Restart Pocus Zoom Back... Abort

Plate ID: 100001 Get

Load Processing Plate

Processing Plan

Save Unload

Crystal ID	Position	Status
69749643	A8-3	Needs reposition...
71178251	B2-3	Needs reposition...
10864301	B3-1	Needs reposition...
17214176	E6-3	Needs reposition...
16205764	F4-3	Needs reposition...
40989573	F1-3	Needs reposition...
15859797	F5-3	Needs reposition...
48753744	F9-3	Needs reposition...

Reposition All

Harvesting | Harvest Settings | Harvest Results | Devices | Advanced | Logs | Hardware Status

Harvest commands for 100001 in B3-3

Force Film Focusing

Cryo Protection gate Line

Depressurization gate Line

Harvest

Pin Storage

Pin Barcode:

Puck Barcode:

New Puck

Start Stop Pocus Reposition

Crystal Direct Plate

Row: A Column: 1 Shift: 1

Current: B7-2 Inverted Move to position...

Table

Pin-Robot Positions

Park

Pin Mount

Cryo-Drift

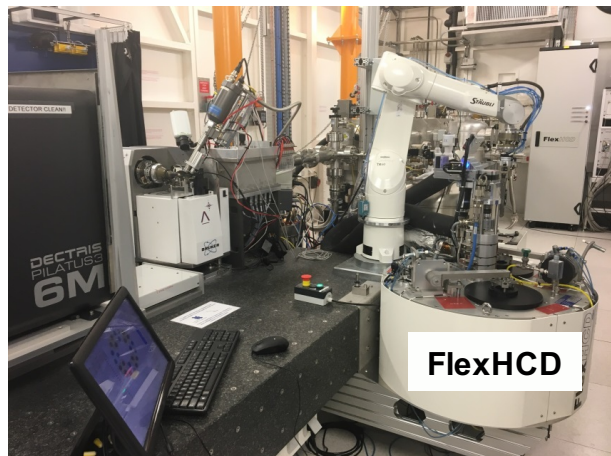
Fibreg-Drift

Aspiration

Pin Venturi OFF

Pin Venturi Soft OFF

FlexHCD – A versatile sample changer



SPINE baskets (x12) = 120 samples

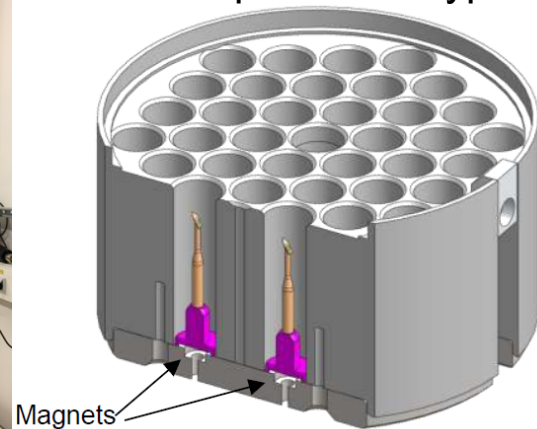


ESRF
SLS
BESSY
PETRAIII
ALBA

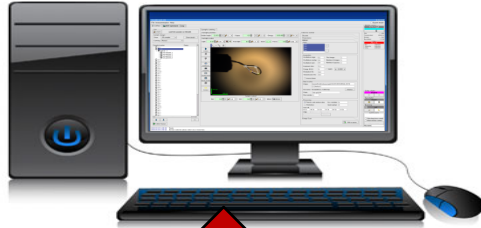
New sample holder types



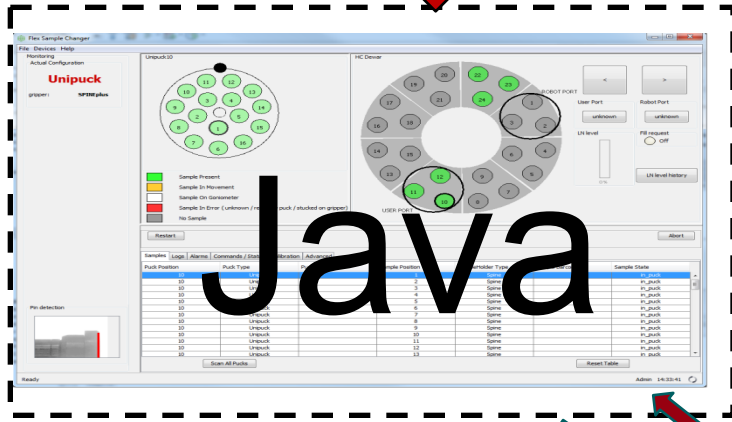
Unipucks (x11) = 176 samples



MXCuBE



Tango (Exporter...)



Java

PC windows (or Linux)



Local touch screen

StaubCom protocol



Generic StaubCom server



modbus



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

RS232

Ethernet

Ethernet

RS232



OneWire:
Gripper
identification

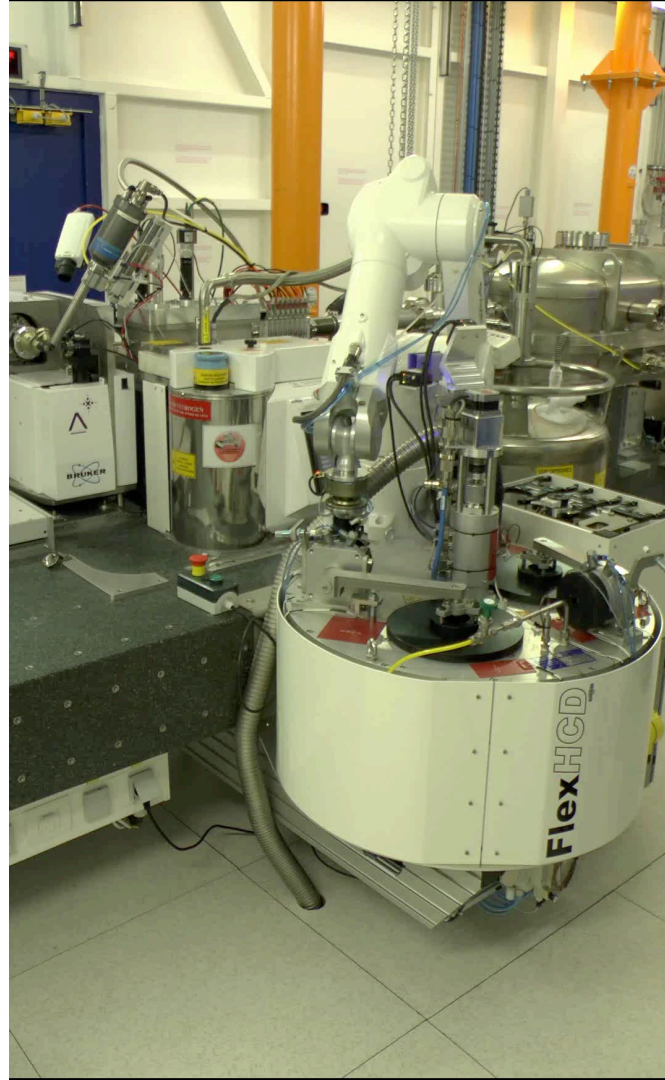
DM reader

Camera + image
processing:
Gripper calibration
Pin detection

ProxiSense Card:
Puck presence &
type detection

- VAL3 movement sequences:
- Load trajectory, Unload trajectory...
- Local regulation loops (PLCs)
- Dewar temperature
 - De-freezing station temperature
 - LN level regulation





Automation of MX experiments

The screenshot displays the mxcube (opid-30b) software interface. The top menu bar includes 'File', 'Instrumentation', and 'Help'. Below the menu bar, there are buttons for 'Collect', 'System', 'Feedback', and 'Chat'. The main interface is divided into several panels:

- User:** Shows 'User: opid-30b' and 'Group:'. There are 'Set' and 'Logout' buttons.
- Sample list:** A tree view showing 'Puck 1' through 'Puck 5'. Under 'Puck 1', items 1.1 through 1.10 are listed. Item 1.5 is highlighted with a blue bar and the text 'Centring done!'. There are buttons for 'Show SC-details' and 'Synch ISPyB'.
- Sample centring:** A panel with 'Sample position' controls: Omega (310.00), Kappa (0.00), Phi (0.00), Holder length (23.067), and Focus (-0.200). There are also 'Back Light' (0.50) and 'Front Light' (0.0) controls. A 'Zoom' control is set to 5. Below these are 'Centre', 'Save', and 'Snapshot' buttons.
- Sample video:** A large video window showing a close-up of a dark, elongated sample on a thin rod, with a green crosshair indicating the centring point.
- Collection method:** A menu with options: 'Standard Collection', 'Characterisation', 'Helical Collection', 'Energy Scan', 'X-ray Centring', 'Mesh Scan', 'Kappa Re-orientation', 'Visual Re-orientation', 'Helical characterisation', 'Mesh and collect', 'Mesh and collect from file', 'MXPressA', 'MXPressE', 'MXPressR', 'MXPressR_180', 'MXPressR_dehydration', and 'Enhanced characterisation'. A 'Browse' button is visible at the bottom of the menu.
- Machine current:** A blue box showing '160.6 mA' and '7/8 multibunch'. Below it is a digital clock showing '04:09'. Further down, 'Flux: 1.94e+12 ph/s' is displayed.
- Energy:** A section with 'Current: 12.7000 keV' and '0.976 A'. There is a 'Move to:' field set to 'keV'.
- Resolution:** A section with 'Current: 1.498 A' and '267.84 mm'. There is a 'Move to:' field set to 'Å'.
- Transmission:** A section with 'Current: 100.00%' and 'Set to:' field.
- Safety shutter:** A section with a green bar and the text 'opened'.
- Fast shutter:** A section with a grey bar and the text 'closed'.
- Beamstop:** A section with a grey bar and the text 'unknown'.
- Capillary:** A section with a grey bar and the text 'out'.
- Current users:** A section at the bottom right.

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

The screenshot displays the Passerelle EDM web interface in Mozilla Firefox. The main window shows a list of requests with columns for Reference, Id, Type, and Creation Date. A red arrow points from a row in the table to a workflow diagram in a separate window.

Reference	Id	Type	Creation Date
2903	275845	CreateThumbnails	2015-04-12 12:08:51
2903	275838	Characterisation	2015-04-12 12:08:27
2903	275834	EDNA_dp	2015-04-12 12:08:18
2903	275841	CreateThumbnails	2015-04-12 12:07:33
2903	275847	MXPressE	2015-04-12 12:07:32
2903	275826	EDNA_dp	2015-04-12 12:07:29
2903	275810	Characterisation	2015-04-12 12:06:50
2903	275806	EDNA_dp	2015-04-12 12:06:41
2903	275767	CreateThumbnails	2015-04-12 12:05:45
2903	275822	Characterisation	2015-04-12 12:05:45
2903	275763	CreateThumbnails	2015-04-12 12:05:39
2903	275688	EDNA_dp	2015-04-12 12:05:33
2903	275802	Characterisation	2015-04-12 12:03:47
2903	275788	EDNA_dp	2015-04-12 12:03:38
2893	275780	MXPressE	2015-04-12 12:02:10
2903	275784	Characterisation	2015-04-12 11:59:22


The workflow diagram shows a sequence of steps: Director, Error Handler by Severity, CommonErrorHandler, START, Beamline setup, Prepare MXPressE, CommonPrepareExperiment, Set ISPyB to success, Workflow ended with error messages, Finished, and STOP. A red arrow points from the 'MXPressE' row in the table to the 'Prepare MXPressE' step in the workflow.

Brockhauser *et al.* (2012)
Acta Cryst. D68, 975-984.

Results displayed in databases for display and use

Extended ISPyB <https://exi.esrf.fr/mx/index.html#/mx/datacollection/session/60625/main> Search

Most Visited Library:Main - Intranet Exit, removing your da...

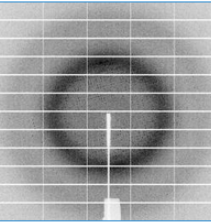

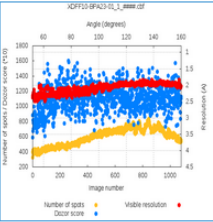
ExiMX Extended ISPyB for MX_{BETA} Version: 5.4.4 Released: 20171211 

Home Shipment Prepare Experiment Data Explorer Manager Help SMIS search by protein acronym Log out FX29@andrewmc

FX29

OSC 14-12-2017 18:24:54 /data/visitor/fx29/id30b/20171214/RAW_DATA/XDFF10/XDFF10-BPA23-01

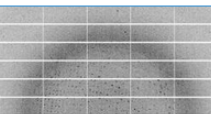
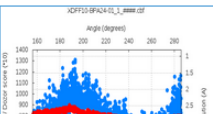
Summary Beamline Parameters Data Collections 4 Sample Last Collect Results 14 Workflow 5

Workflow	MXPressE	Res. (corner)	1.95 Å (1.46 Å)	P 21 21 21	Completeness	Res.	Rmerge												
Protein	XDFF10	En. (Wave.)	12.700 keV (0.9763 Å)	Overall	99.5%	48.2-1.9	6.3												
Sample	BPA23-01	Omega range	0.10 °	Inner	97.9%	48.2-7.4	3.7												
Prefix	XDFF10-BPA23-01	Omega start (total)	52.00 ° (108°)	Outer	99.7%	1.97-1.90	116.4												
Run #	1	Exposure Time	0.02 s	<table border="1"> <tr> <td>a</td> <td>b</td> <td>c</td> </tr> <tr> <td>67.56 Å</td> <td>80.23 Å</td> <td>137.55 Å</td> </tr> <tr> <td>α</td> <td>β</td> <td>γ</td> </tr> <tr> <td>90 °</td> <td>90 °</td> <td>90 °</td> </tr> </table>			a	b	c	67.56 Å	80.23 Å	137.55 Å	α	β	γ	90 °	90 °	90 °	
a	b	c																	
67.56 Å	80.23 Å	137.55 Å																	
α	β	γ																	
90 °	90 °	90 °																	
# Images (Total)	1080 (1325)	Flux start	1.09e+12 ph/sec																
Transmission	69.3 %	Flux end	1.08e+12 ph/sec																

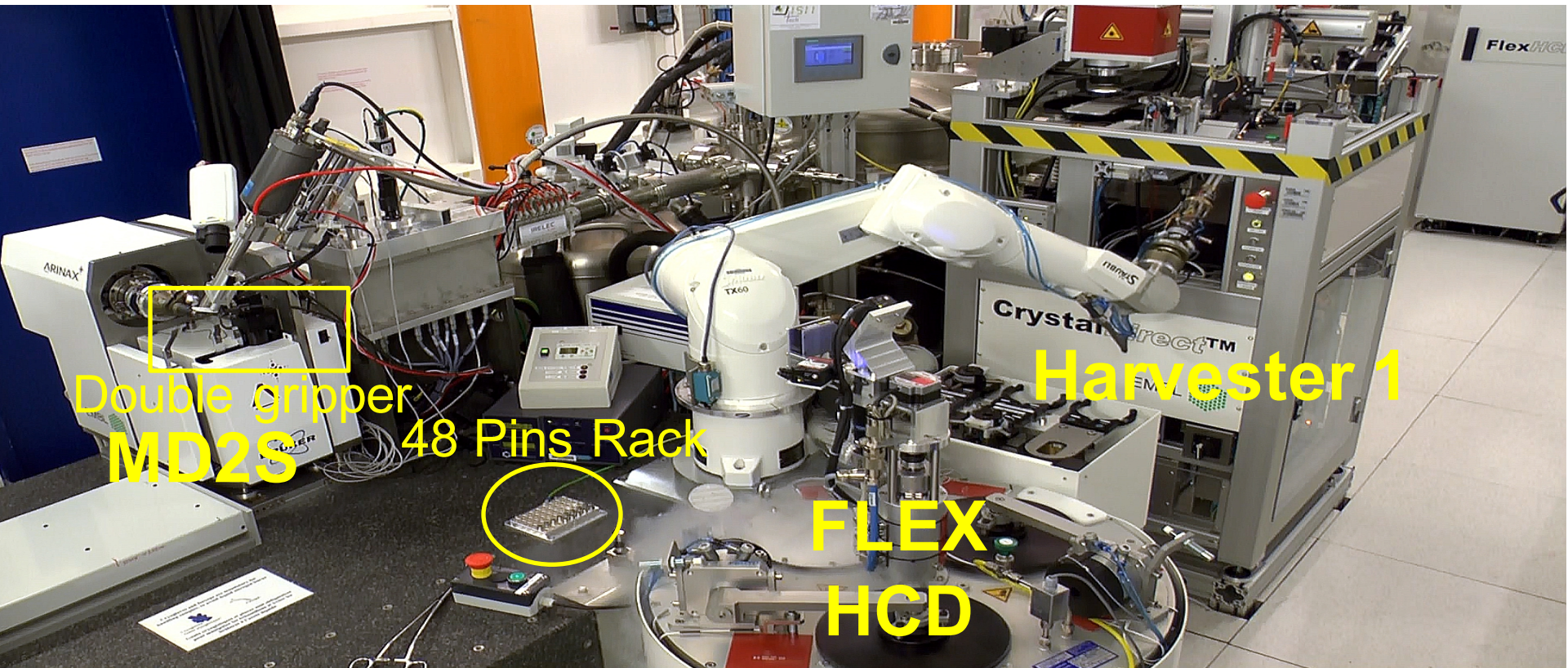
Comments: Using forced space group 'P212121' from diffraction plan. Characterisation: Space group P212121 forced.

OSC 14-12-2017 18:18:38 /data/visitor/fx29/id30b/20171214/RAW_DATA/XDFF10/XDFF10-BPA24-01

Summary Beamline Parameters Data Collections 4 Sample Last Collect Results 16 Workflow 5

Workflow	MXPressE	Res. (corner)	2.71 Å (1.95 Å)	P 21 21 21	Completeness	Res.	Rmerge	
Protein	XDFF10	En. (Wave.)	12.700 keV (0.9763 Å)	Overall	92.9%	10.5-2.4	9.8	
Sample	BPA24-01	Omega range	0.05 °	Inner	94.3%	100.0-10.5	5.4	
								

CrystalDirect-To-Beam on ID30B

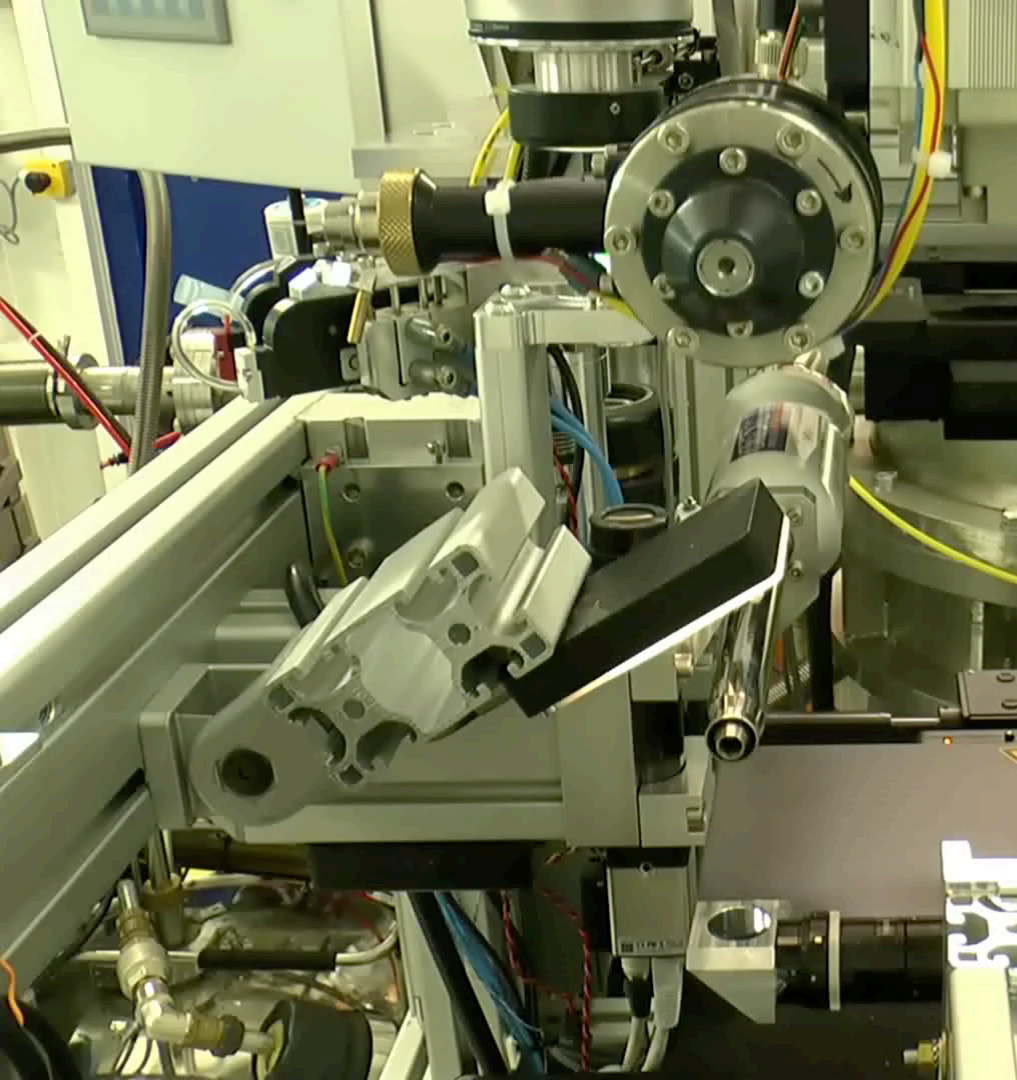


Double gripper
MD2S

48 Pins Rack

FLEX
HCD

Harvester 1



General Hardware Status
OK
Laser Ready
Cryo 294.66

Restart Focus: 5.603 Zoom Back Light Abort

Plate ID: **Big-Single-Crystal-orig** Set
Load Processing Plan

No Processing Plan Loaded
Operating in manual mode

Start Stop Pause

Harvesting Harvest Settings Harvest Results Advanced Logs Hardware Status
Harvest commands for Manual Harvesting
 Use 4 pts focusing
 Cryo Protection
 Harvest

Cut Shapes
File: **Big-Single-Crystal-orig**
Cryo Focus/Class: **line.ins**
Depressurization: **line.ins**

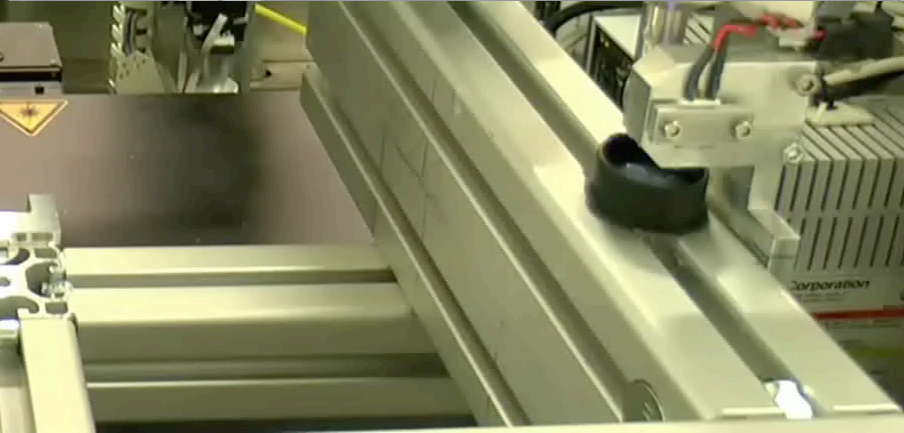
Crystal Direct Plate
Rows: **A-2** Columns: **1-2** Shelf: **1-2**
Current: **B5-2** Inverted Move to position

Pin Storage
SC Rescan Empty Puck

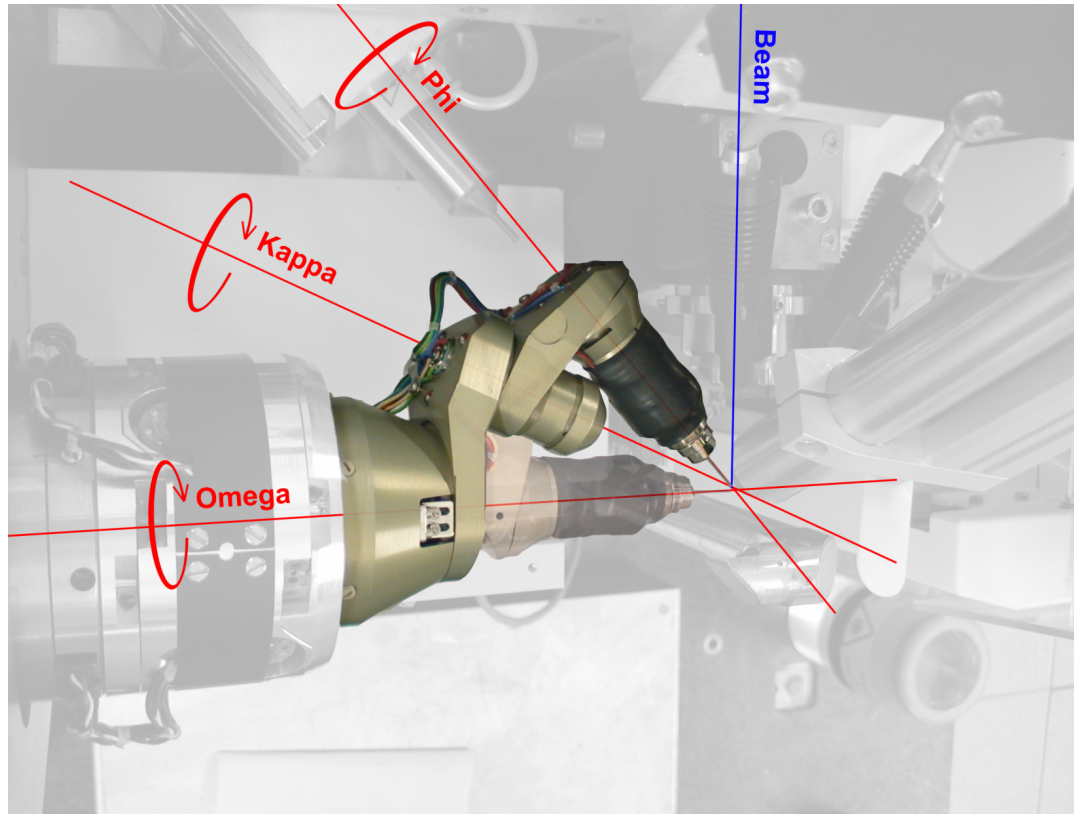
Pin Robot Positions
 Park
 Pin Mount
 Cryo Stream
 Liquid Removal

Diffuser On Off
Pin Venturi Soft Off On
Pin Venturi Off On

Ready Admin 10:26:59

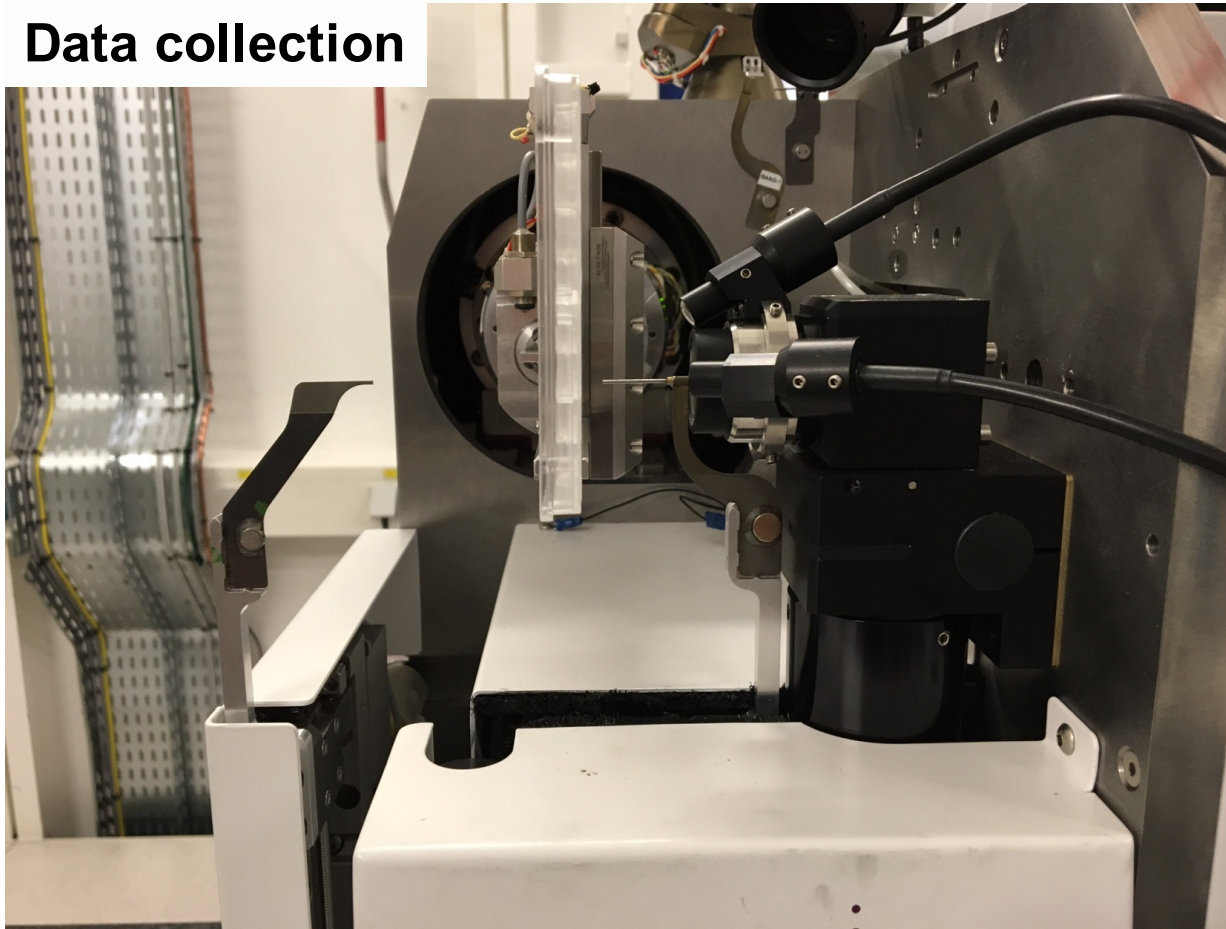


ID30B Mini-Kappa goniometer head



ID30B MD2S in situ plate screening

Data collection



ID30B MD2S in situ plate screening in MxCuBE

mxcube (mx-1743) Expert mode

File Instrumentation Help

Collect System Feedback Chat

User: mx-1743 Group: [] Set Logout

Sample list
Mode: Plate Show SC-details
Centring: No Centring Synch ISPyB

Sample centring
Sample position
Omega: 316.36 90.0 Kappa: 0.00 1.0 Phi: 0.00 0.1
Holder length: 32.500 0.1

Sample video
Back Light: 0.60 Focus: -0.729 0.02 Front Light: 0.0 Zoom: 5

Collection method
Standard Collection
Acquisition
Oscillation range: 0.1 First image: 1
Oscillation start: 316.36 Number of images: 10
Kappa: 0.0 Phi: 0.0
Detector mode:
Exposure time (s): 0.037
Energy (keV): 12.7 MAD ip: -
Resolution (Å): 1.997
Transmission (%): 100.0
Inverse beam Subwedge size:
Shutterless

Data location
Folder:
/data/visitor/mx1743/id30b/20151104/RAW_DATA
/XtyA/A3-2
File name: xyla_23_###.cbf Browse
Prefix: xyla
Run number: 23

Processing
N.o. residues: 200 Space group:
Unit cell:
a: 0 b: 0 c: 0
α: 0 β: 0 γ: 0

Machine current
185.4 mA
uniform multibunch
08:57
Flux: +0.00 ph/s
Energy
Current: 12.7000 keV
0.976 Å
Move to: keV
Resolution
Current: 1.997 Å
391.52 mm
Move to: Å
Transmission
Current: 100.00%
Set to: Filters

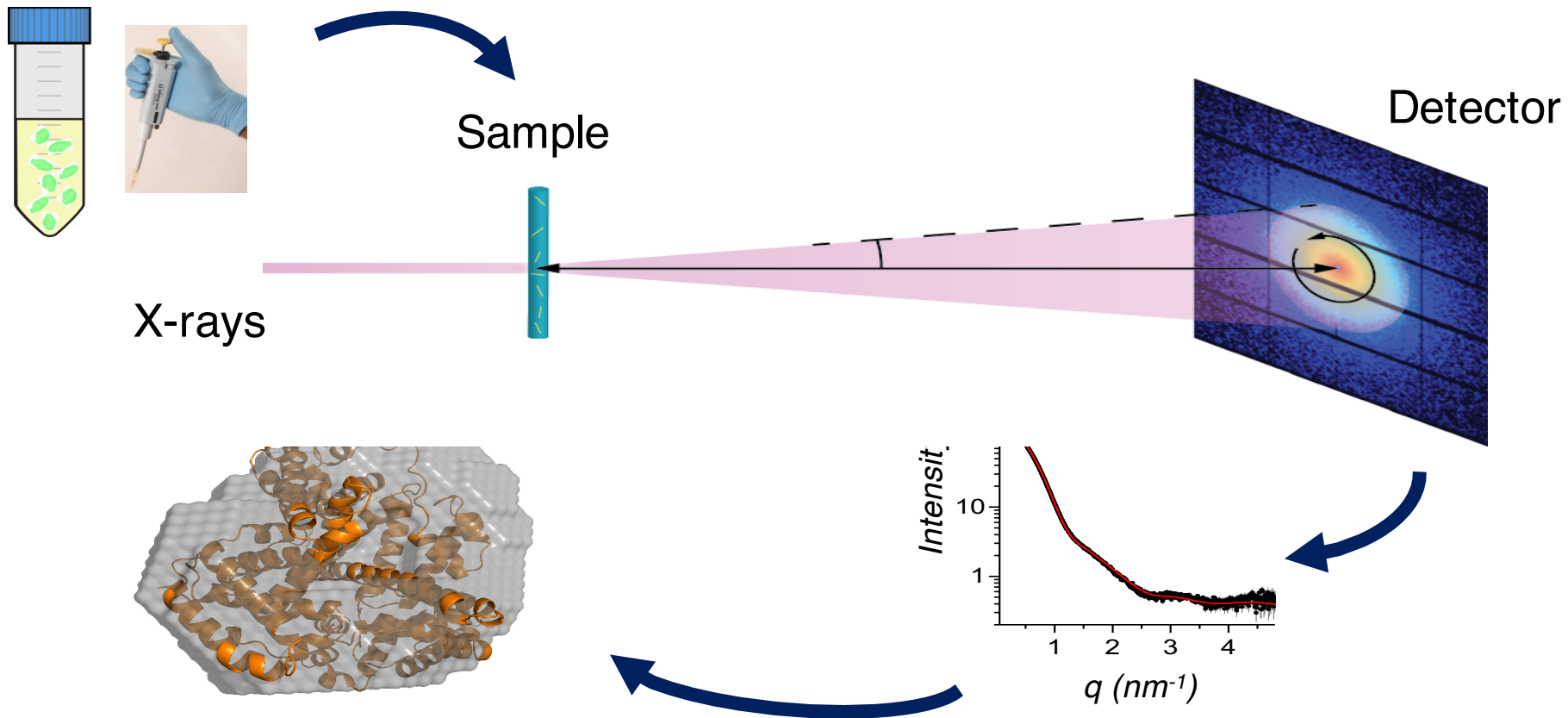
Safety shutter closed
Fast shutter closed
Beamstop in
Capillary unknown
Current users

Row A
A:1:1
A:1:2
A:1:3
A:2:1
A:2:2
A:2:3
A:3:1
A:3:2
A:3:3
A:4:1
A:4:2
A:4:3
A:5:1
Standard - 7
xyla_13 (Point - 1) Collection done
Standard - 8
xyla_14 (Point - not defined) Collection done
Standard - 9
xyla_15 (Point - not defined) Collection done
Standard - 10
xyla_16 (Point - not defined) Collection done
Standard - 11
xyla_18 (Point - 2) Collection done
Standard - 12
xyla_19 (Point - not defined) Collection done
Standard - 13
xyla_20 (Point - not defined) Collection done
Standard - 14
xyla_21 (Point - not defined) Collection done
Standard - 15
xyla_22 (Point - not defined) Collection done
A:5:2
A:5:3
A:6:1
A:6:2
A:6:3
A:7:1
A:7:2
A:7:3
A:8:1
A:8:2
A:8:3

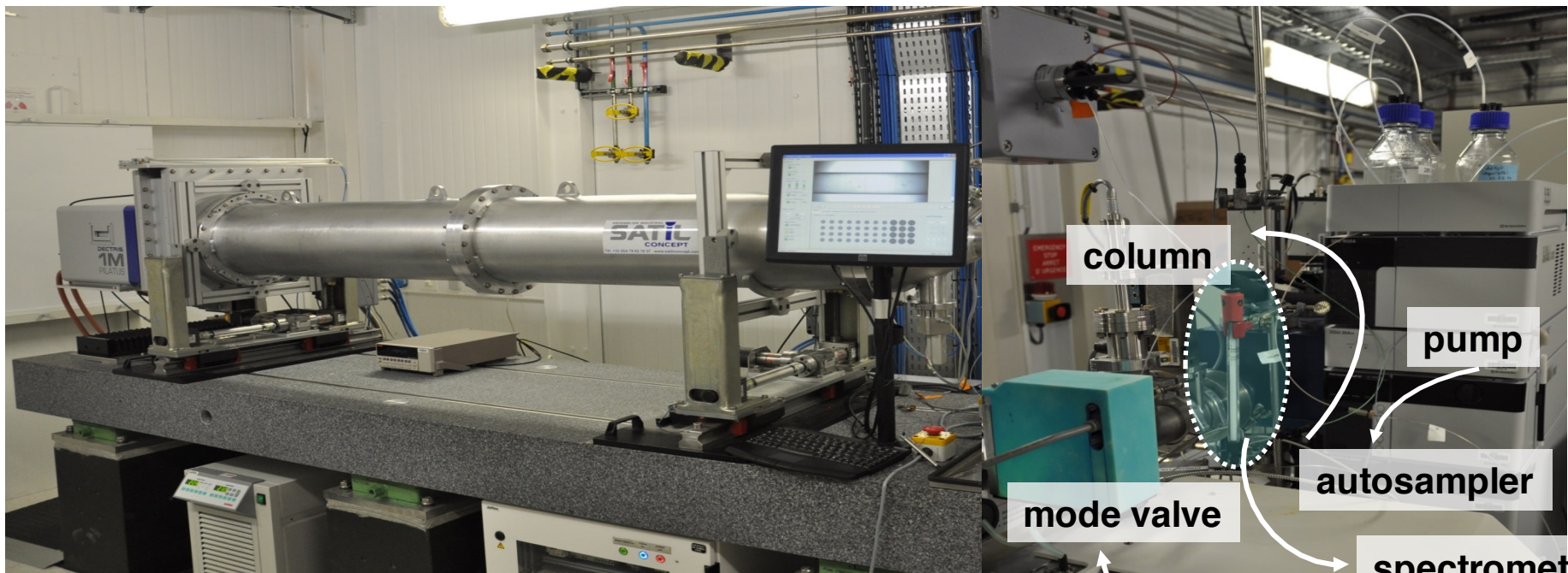
Centre
Save
Snapshot
Centre beam
Quick realign

100 μm
100 μm
Point no. 2 selected
Aperture diameter: 50

Biological small angle X-ray scattering (bioSAXS)



BioSAXS on BM29



Synchrotron
JSR

JOURNAL OF
SYNCHROTRON
RADIATION

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

Acta Cryst
D

BIOLOGICAL
CRYSTALLOGRAPHY

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

ESRF Extremely Brilliant Source upgrade

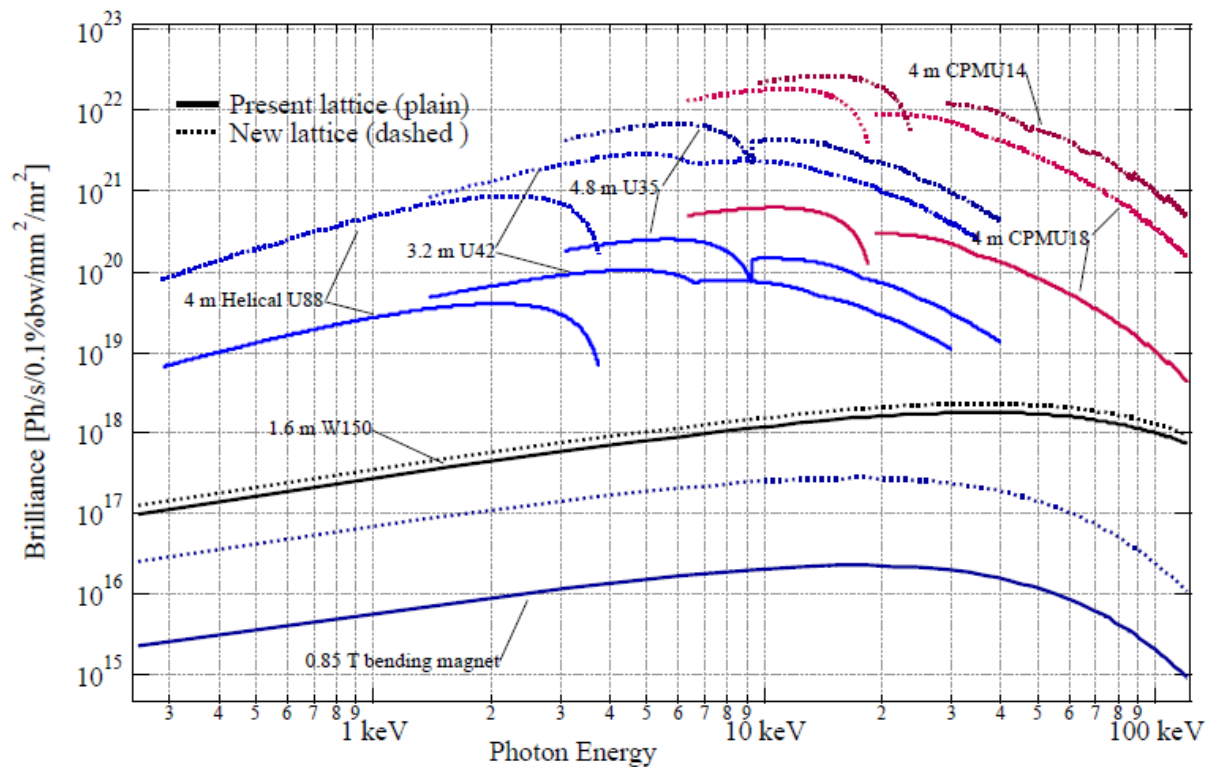


Figure 4: ESRF brilliance for the existing and new lattices

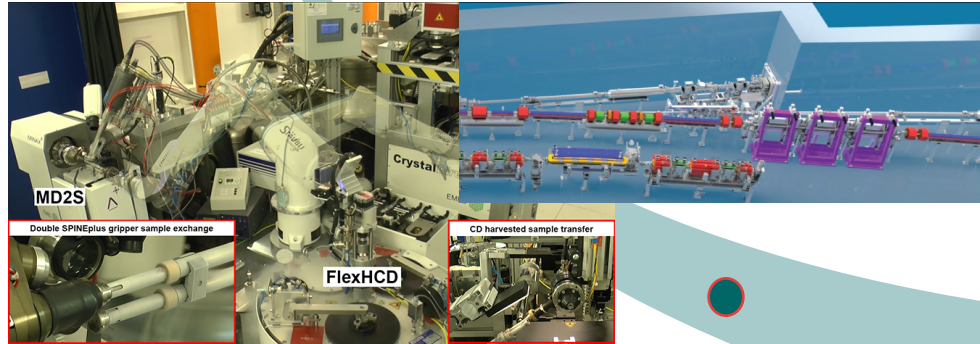
ESRF Extremely Brilliant Source upgrade

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**
MxCuBE³/BsxCuBE³

New science:

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

Software upgrade: MxCuBEv2 -> MxCuBE³

The screenshot displays the MxCuBE-3 software interface for the OPID30b proposal. The main window shows a microscope view of a sample with a green circle highlighting a region labeled "Point-1". The interface includes several control panels and status indicators.

Beamline Actions

Energy:	12.6000 keV	Resolution:	3.371 Å	Transmission:	100.000 %	Cryo:	100 K
Wavelength:	0.9840 Å	Detector:	700.100 mm	Flux:	0 ph/s		

Sample Overview

Sample changer:	Safety Shutter:	Fast Shutter:	Beamstop:	Ring Current:
READY	CLOSED	CLOSED	OUT	195.3 mA

Beam size: 50

Omega: 360.00 (90°)

Kappa: 0.00 (0.1°)

Phi: 0.00 (0.1°)

Sample alignment: Show motors

Run Queue / Unmount: Sample: Sample-8:1:01, Queued Samples (1), Point-1: Characterisation

Instrumentation Help / Expert mode

Sample control: Omega: 360.00, Kappa: 0.00, Phi: 0.00

Collection method: Standard Collection, Characterisation, Helical Collection, Energy Scan, XRF Spectroscopy, A-Scan Centring, Mesh Scan, Riggs Repetition, Visual Repetition, Helical characterisation, Near and distant, Extended characterisation, Burn strategy, Detectors, Tissue staining, Phly: PAZ_v4, Run number: 1

Parameters: Flux: 0.000 ph/s, Energy: 12.600 keV, Current: 0.370 A, Resolution: 3.371 Å, Current: 287.84 µA, Magnification: 100.00%, Beamstop: 100.00%

Shutter status: Safety shutter: OPEN, Fast shutter: OPEN, Beamstop: OPEN

ESRF-EMBL Joint Structural Biology Group Beamlines

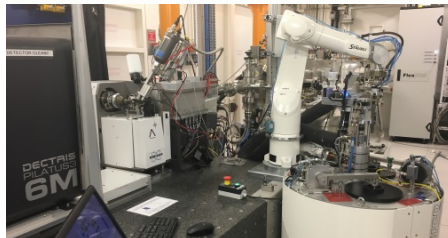
ID23-1 (tunable)



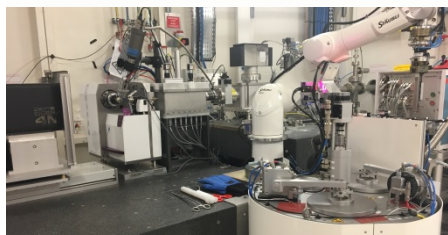
ID29 (tunable)



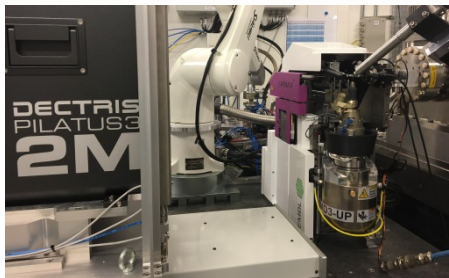
ID30B (tunable)



ID30-A3 (μ focus)



ID23-2 (μ focus)



ID30-A1 (MASSIF)



ESRF-EBS
Extremely Brilliant Source

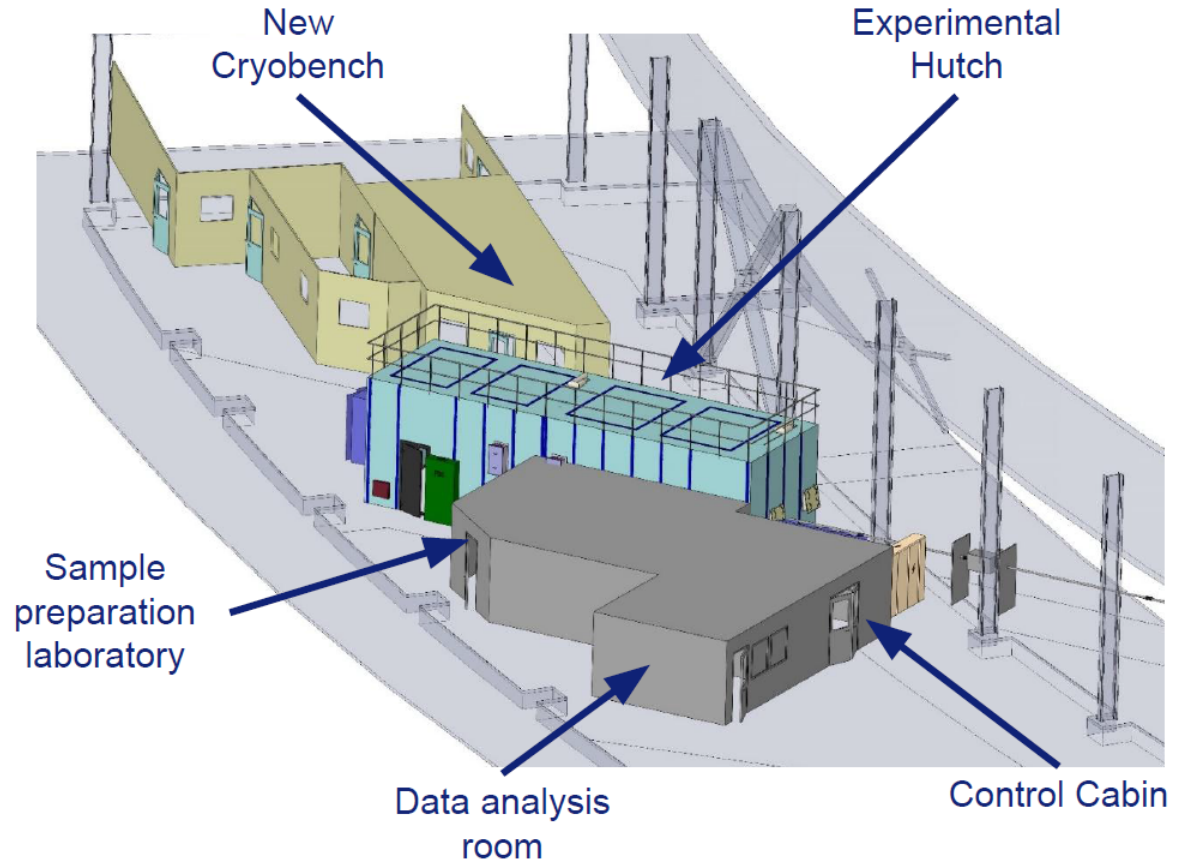
12/2018 – 8/2020

BM29 (BioSAXS)



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

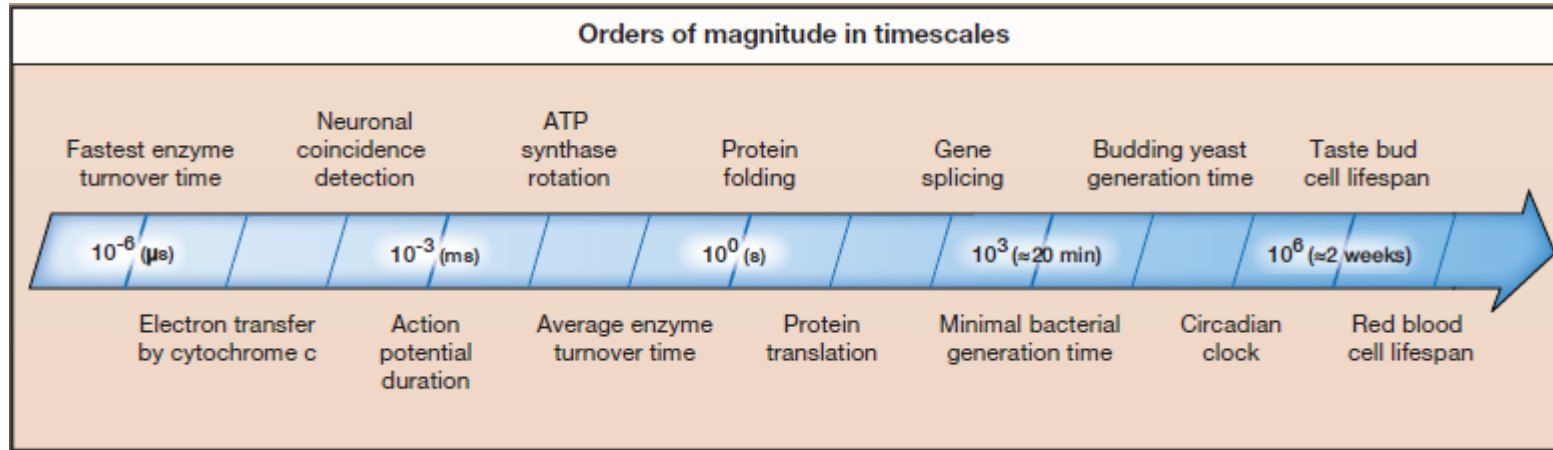
- $>5 \times 10^{15}$ ph/s/mm²
- Multilayer mono
- KB mirrors
- 0.5 μm^2
- 1 μs chopper
- 1 kHz detector speed



Daniele de Sanctis (ESRF)

Shibom Basu (EMBL-Grenoble)

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

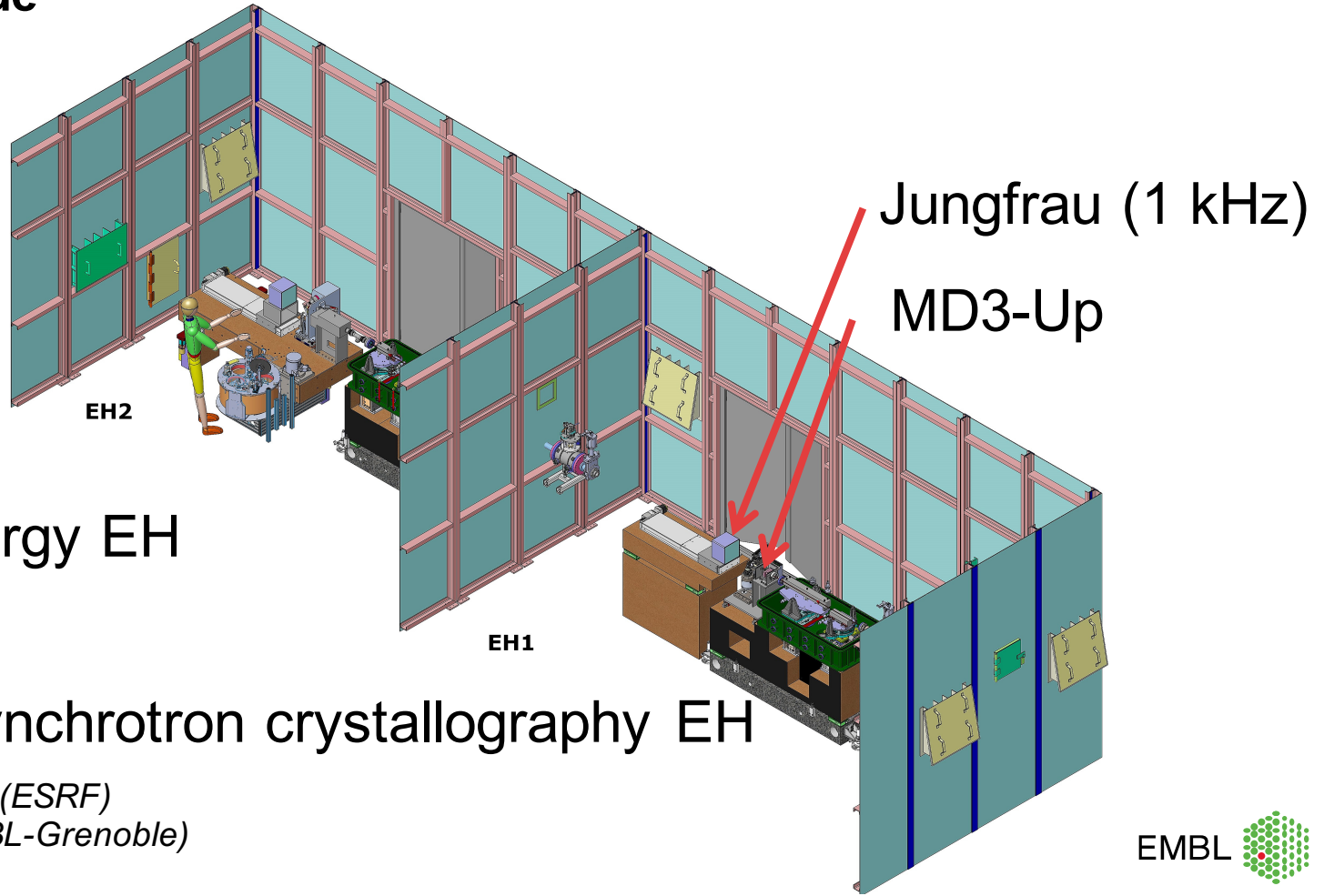


Shamir *et al.* (2016) *Cell* **164**, 1302.e1

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



ID29 Upgrade



Jungfrau (1 kHz)

MD3-Up

EH2

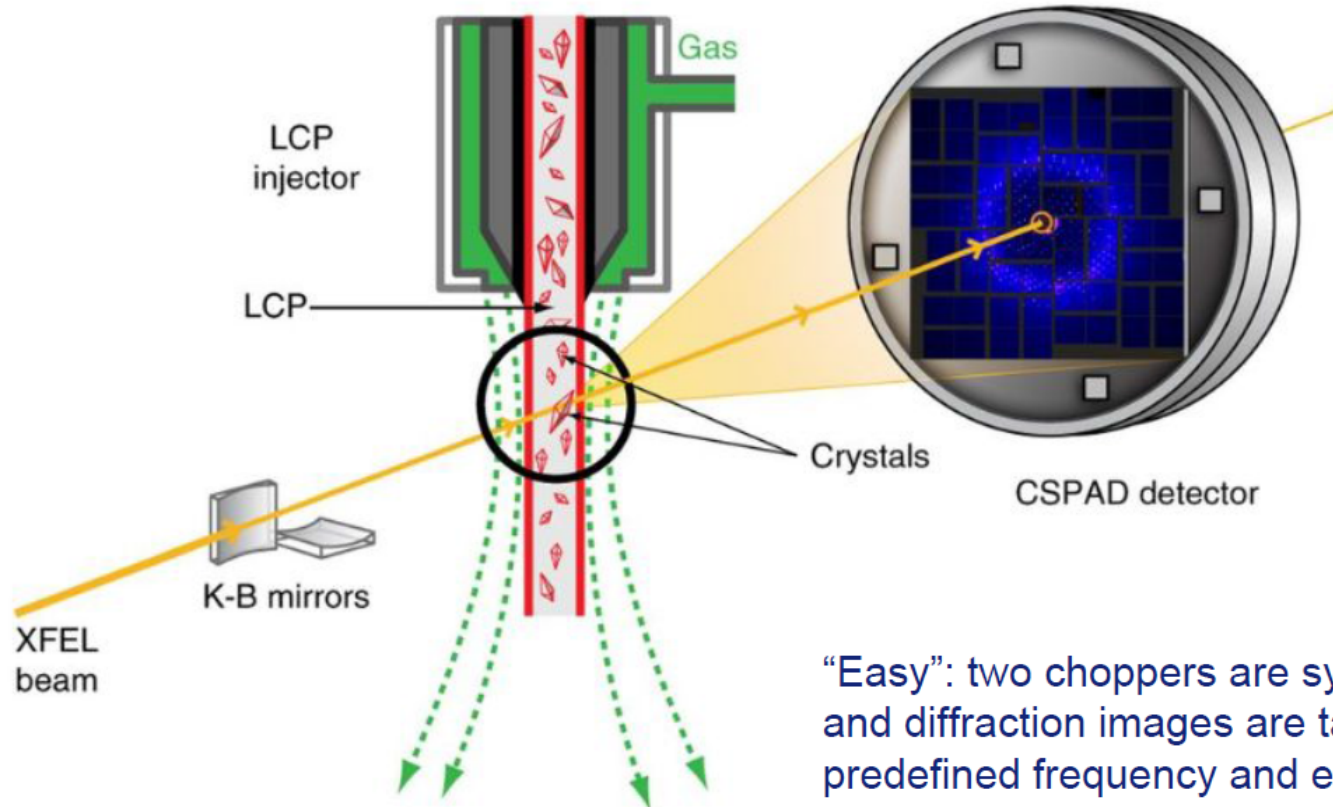
EH1

High energy EH

Serial synchrotron crystallography EH

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

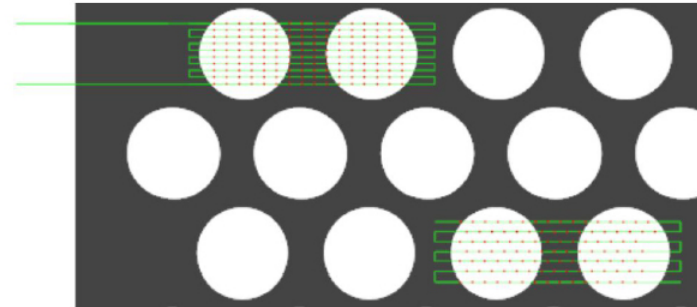
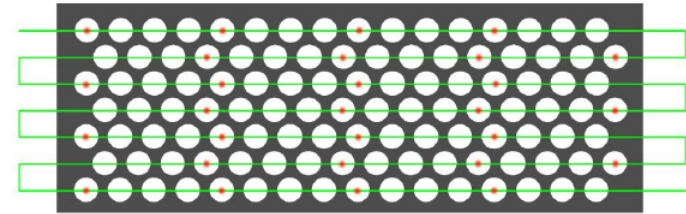
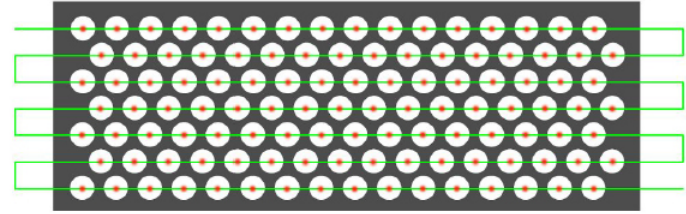
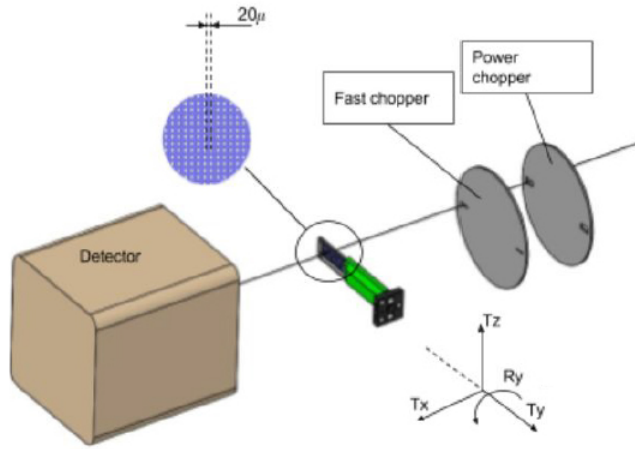
Jet experiments



“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)



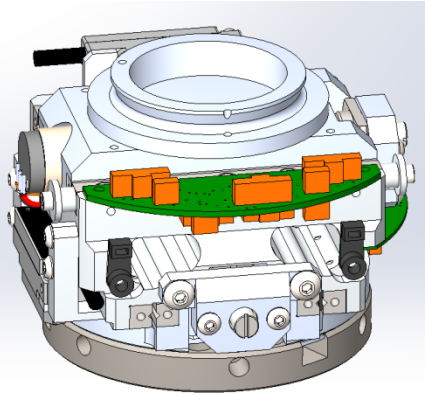
ID29 Upgrade – MD3

X : +/- 5mm 5mm/s

Y : +/- 5mm 5mm/s

Z : 118 mm 30mm/s

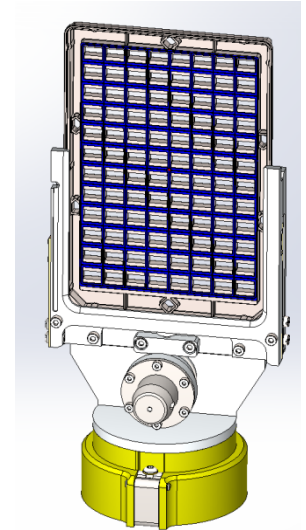
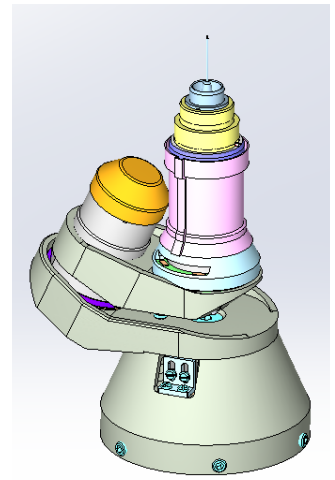
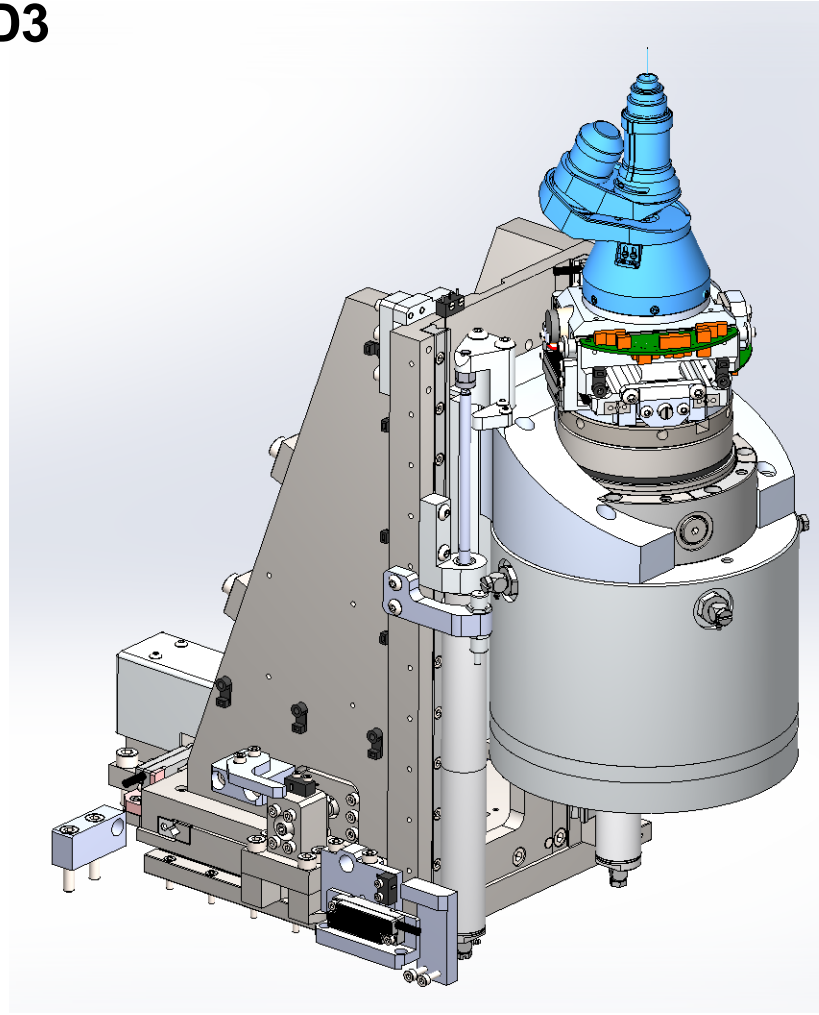
Encoders 5nm



XY table

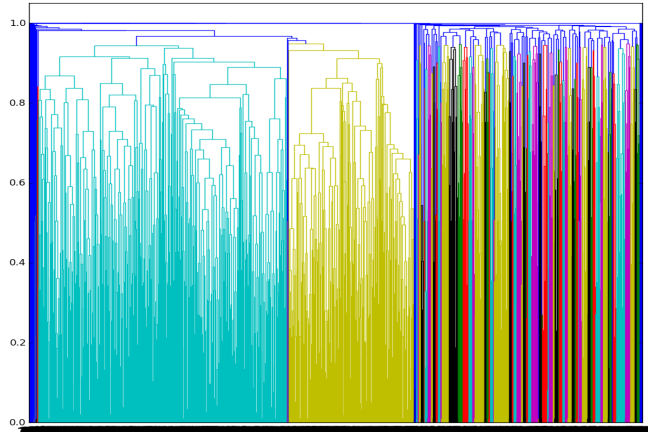
+/- 5mm

Encoders 5nm

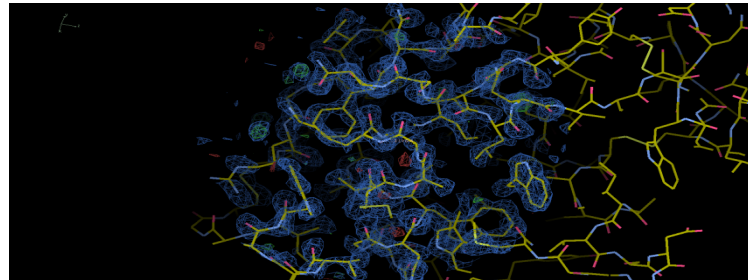


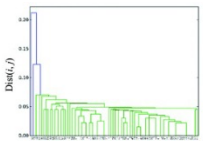
ID23-2: MD3-Up ESRF-EBS ready

- Cyan cluster: 1351 datasets,

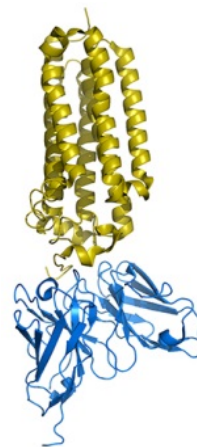
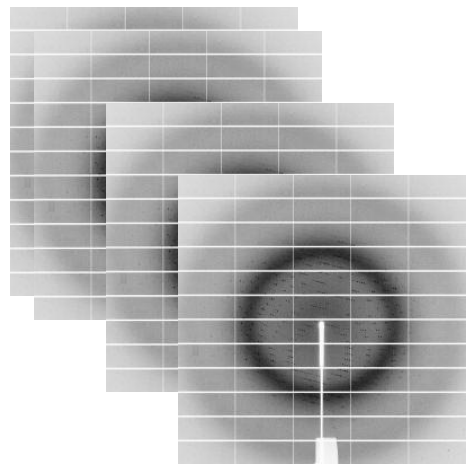
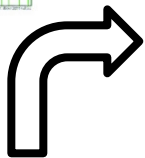


- Resolution (\AA) = 20-1.3
- $R_{p.i.m.}$ (%) = 18.3
- Completeness (%) = 74.2

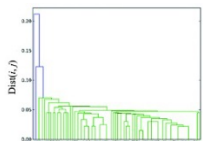




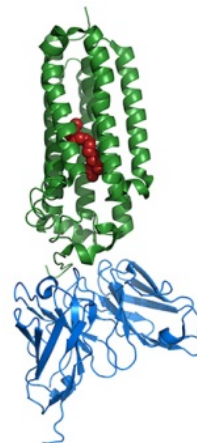
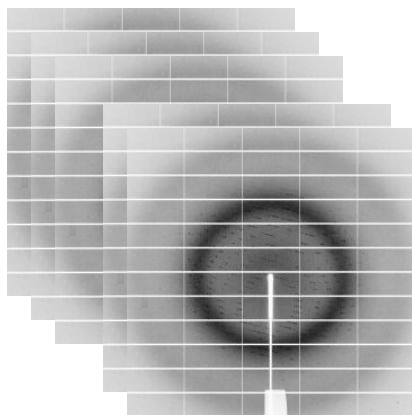
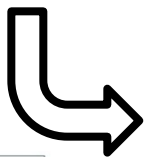
Cell 1



Apo structure

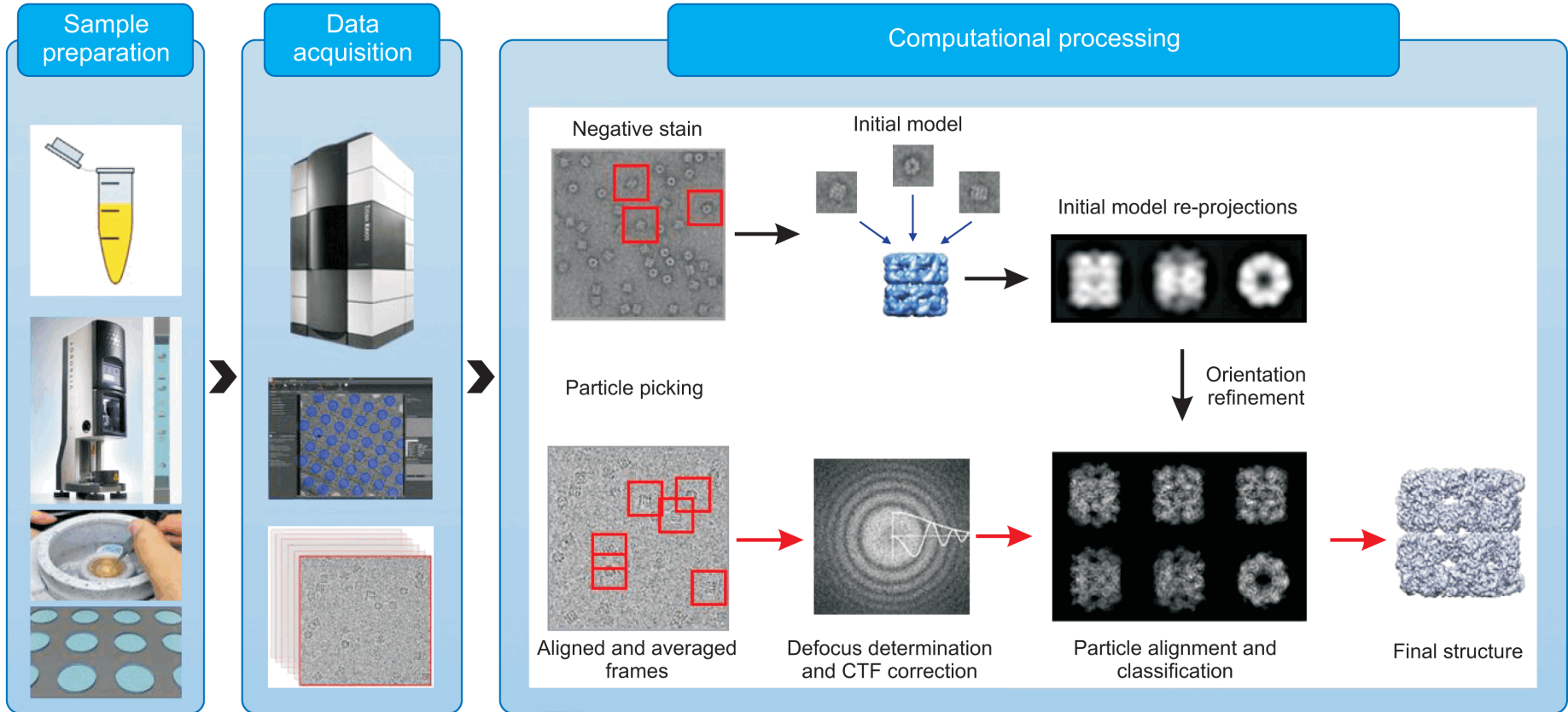


Cell 2



Ligand bound

Structural biology techniques (Cryo-EM)

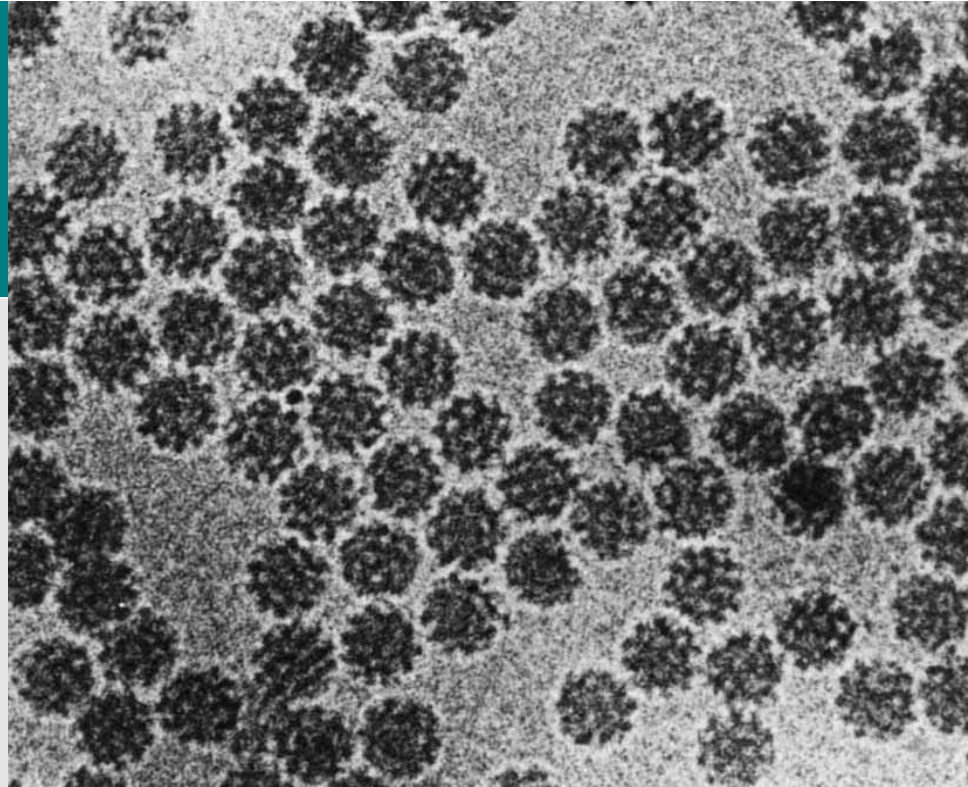


Long history of Cryo-EM developments at the EMBL

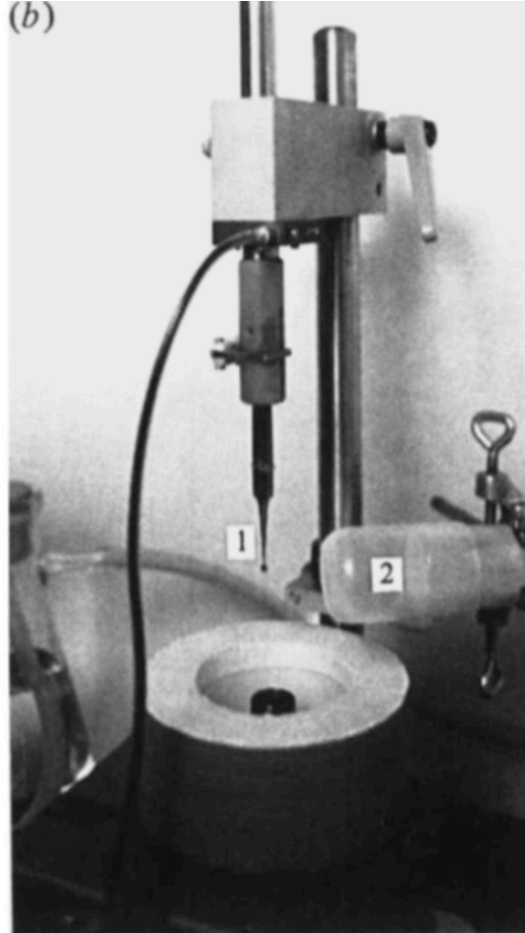
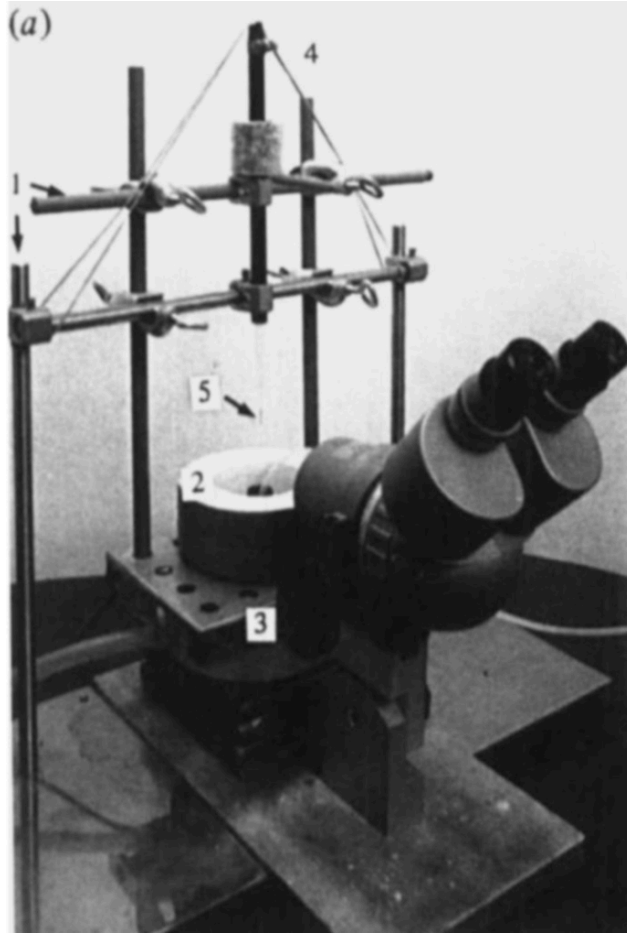
2017 Nobel Prize
CHEMISTRY

Jacques Dubochet

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

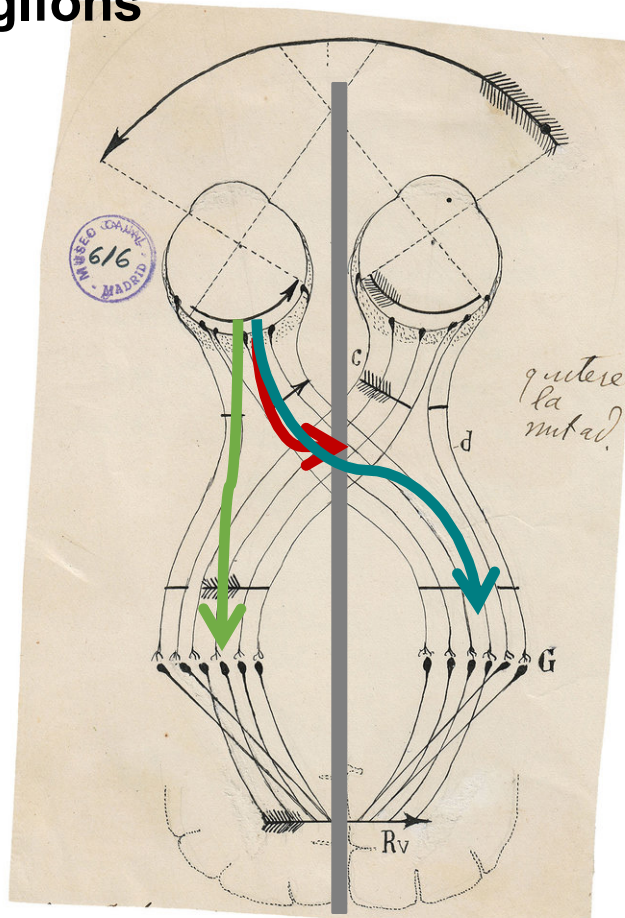
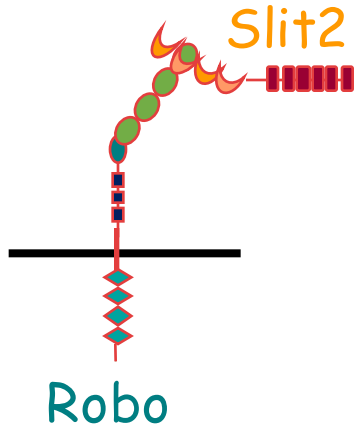
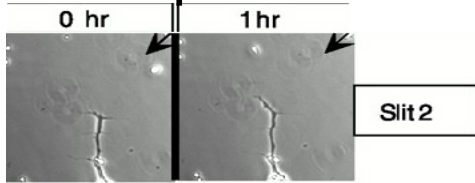


Cryo-EM freezing in the 1980s and now



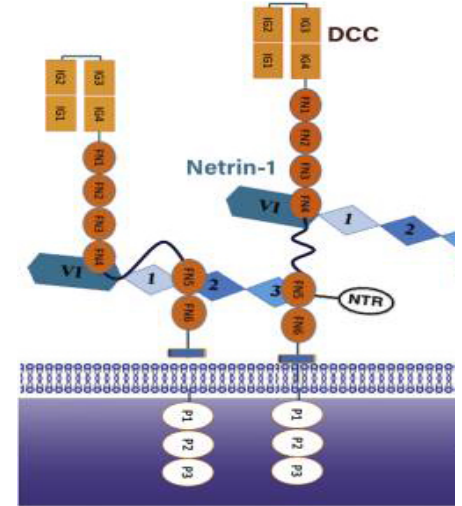
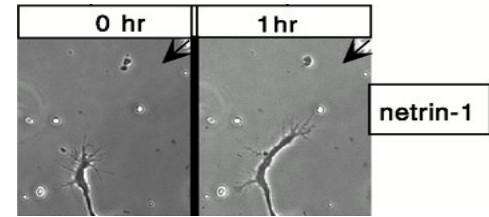
Vision – Retinal ganglions

2. Repulsion



The midline

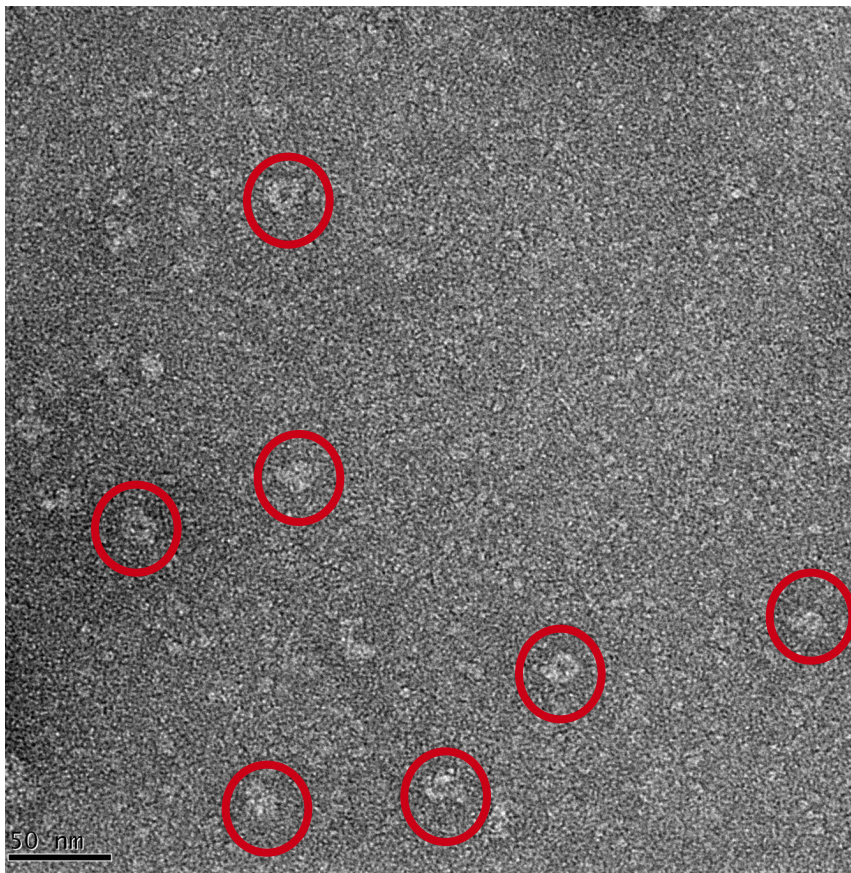
1. Attraction



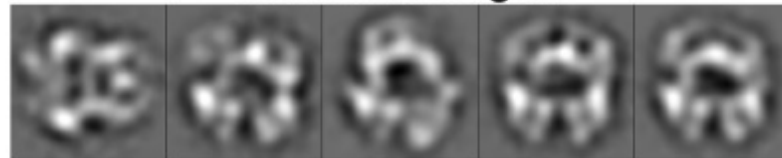
Liu *et al.*, (2018)
Neuron **97**, 1261-1267

Negative stain 3D EM imaging of Robo1 - Aleksandrova *et al.* (2018)

Structure **26**, 166-171



Class-averages



Projections



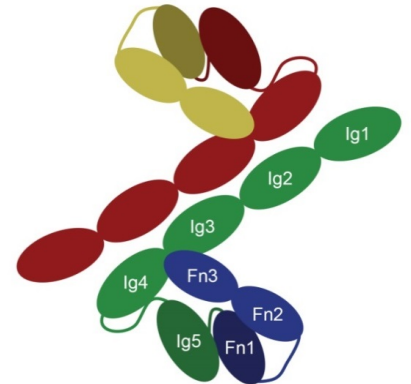
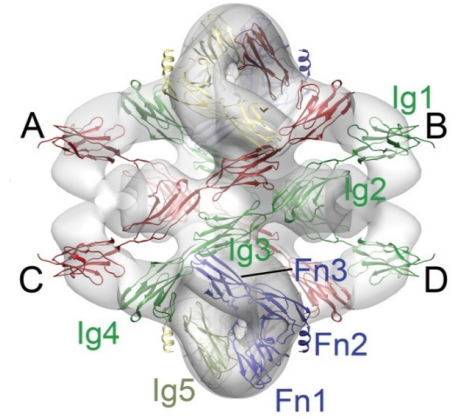
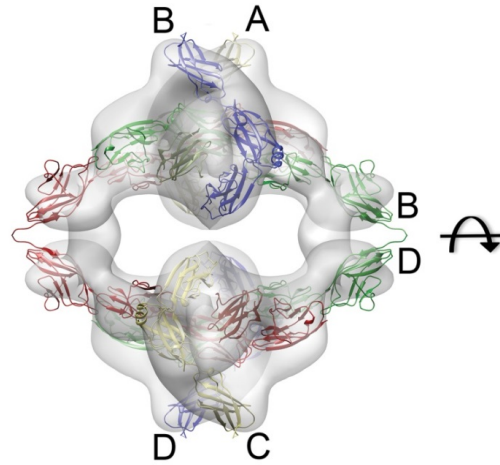
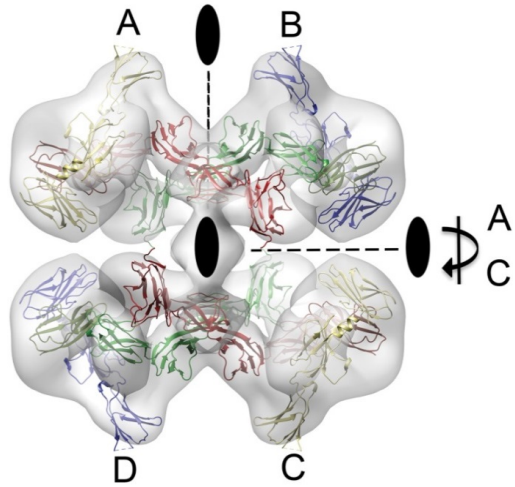
Class-averages



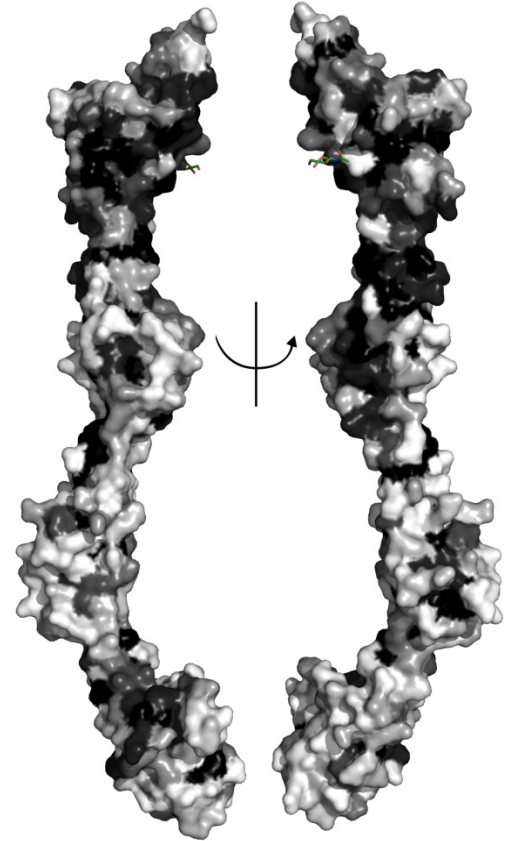
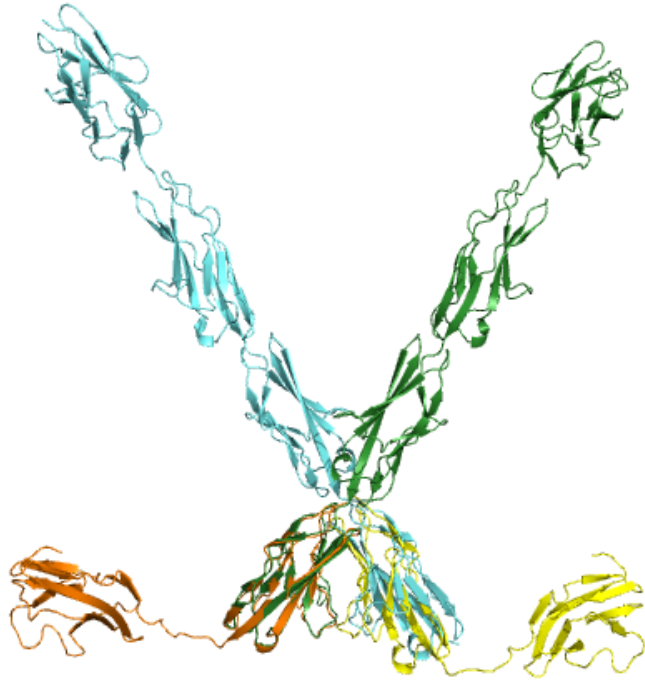
Projections



Robo1 forms tetramers – Cell surface autoinhibition

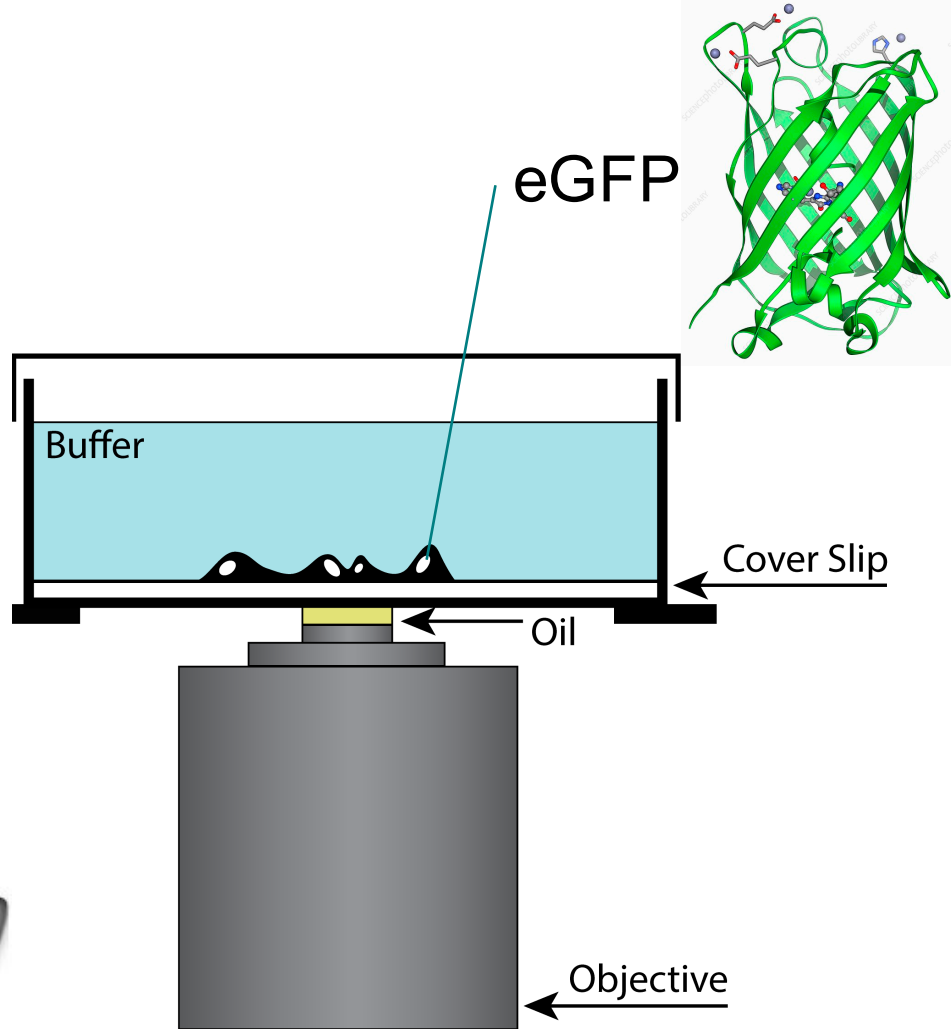
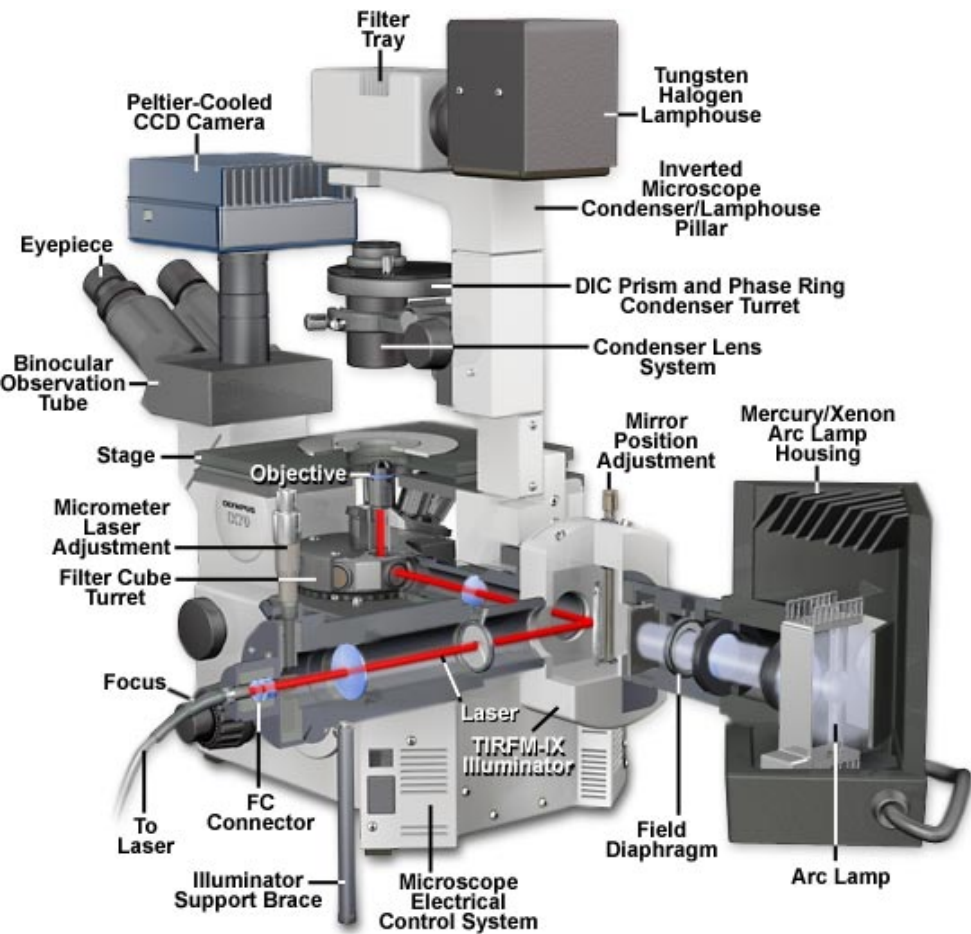


Robo1/2 activation involves dimerisation

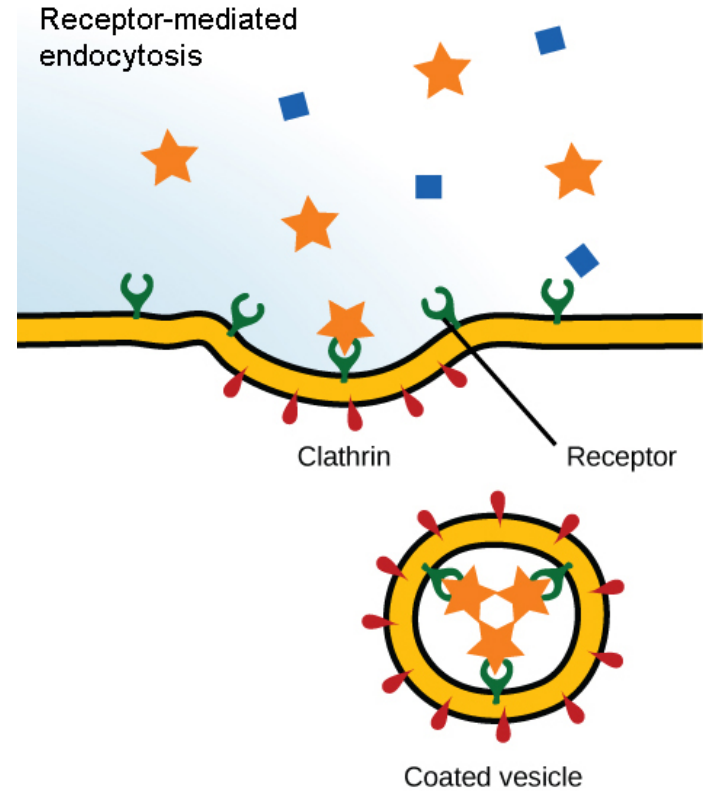
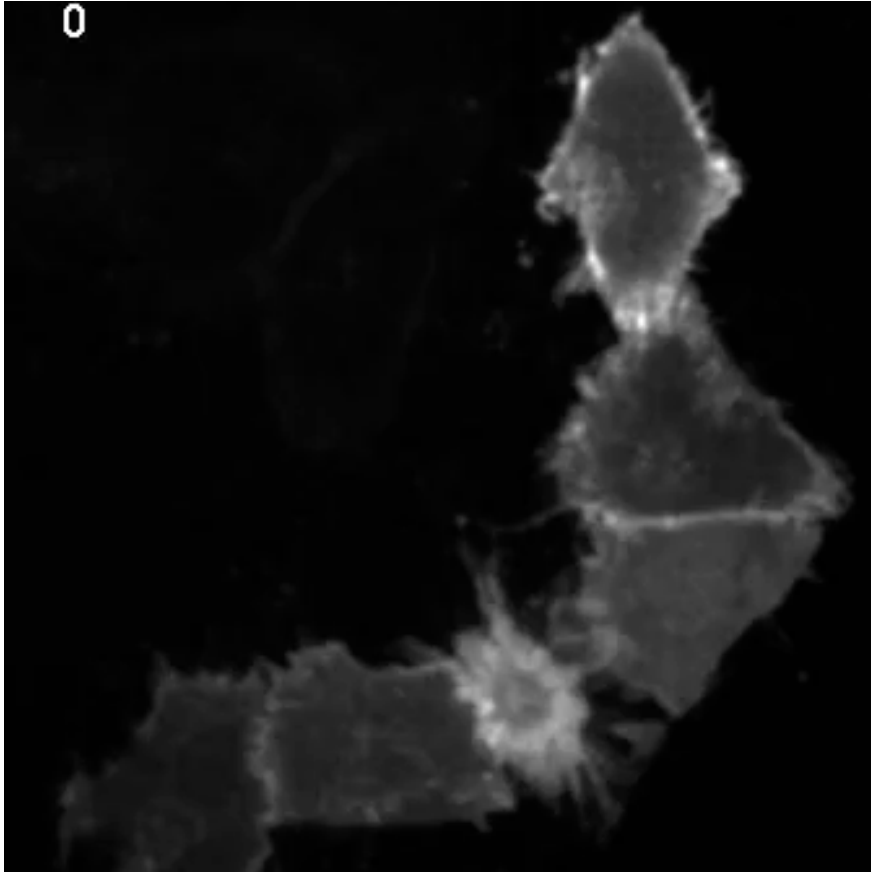


Barak *et al.* (2019) *Cell* **177**, 272-285

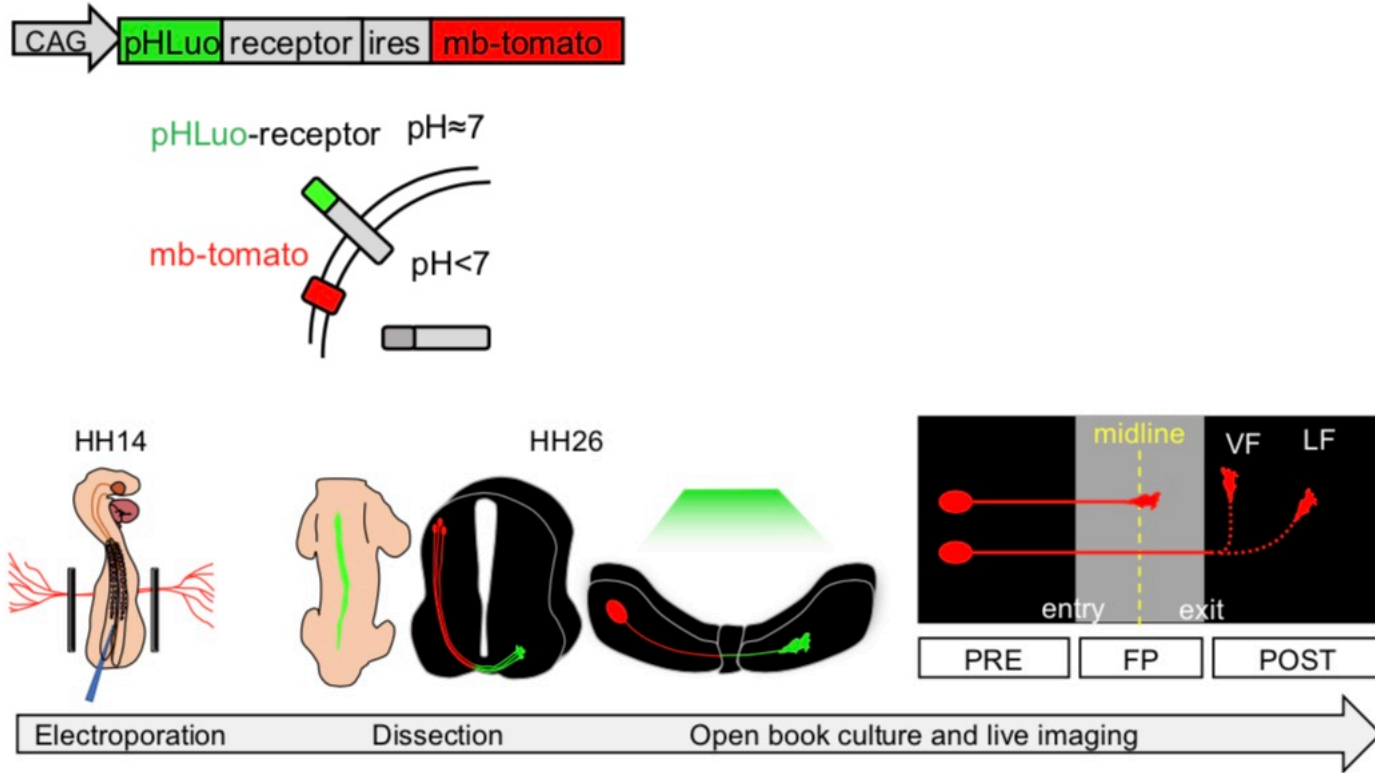
Live cell imaging



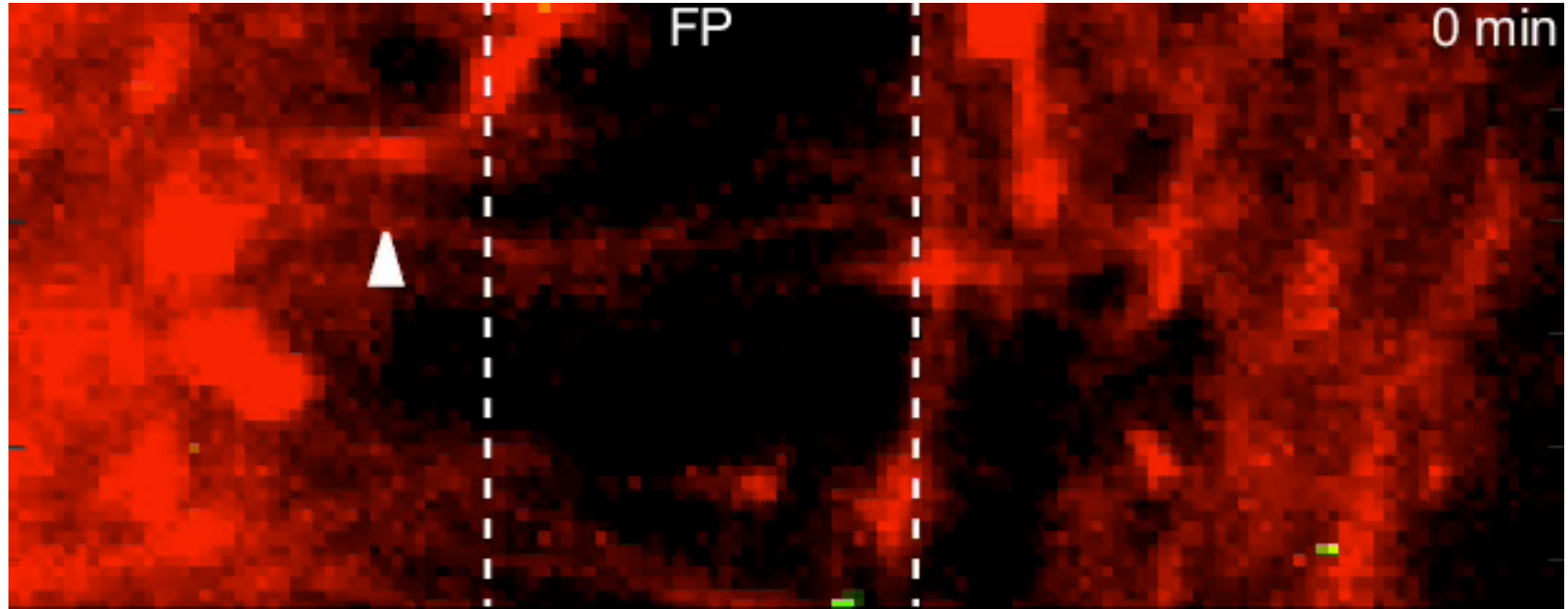
Robo1 undergoes endocytosis upon Slit binding



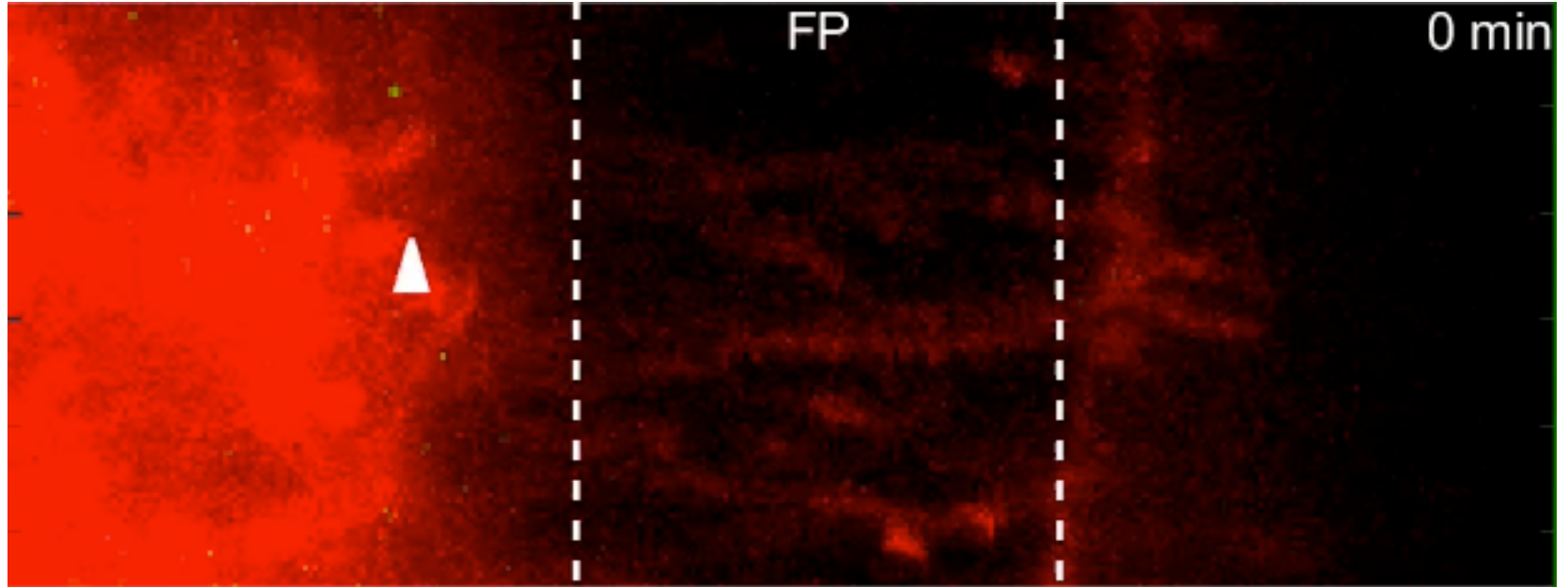
Robo1/2 spatial and temporal profiling



Robo1/2 spatial and temporal profiling

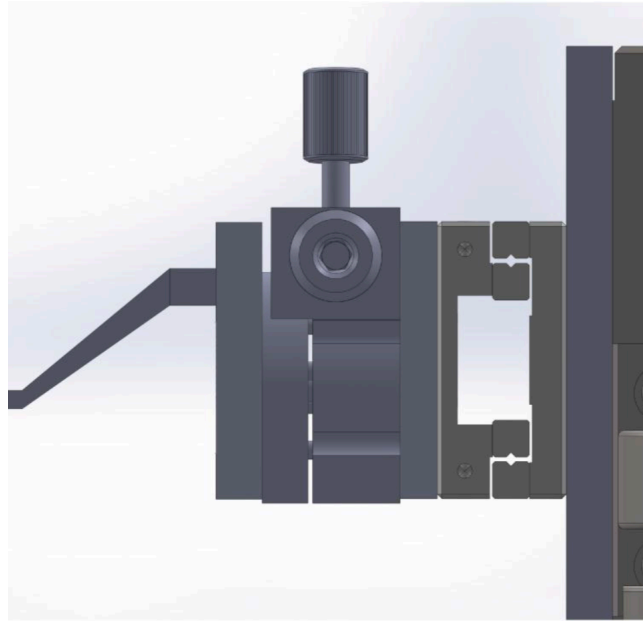
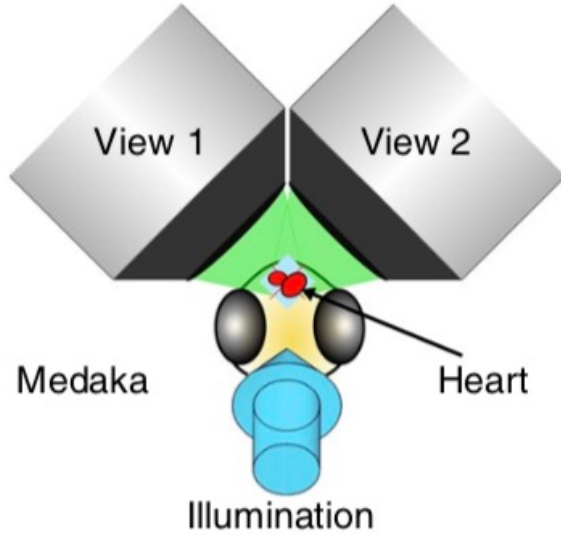


Robo1/2 spatial and temporal profiling

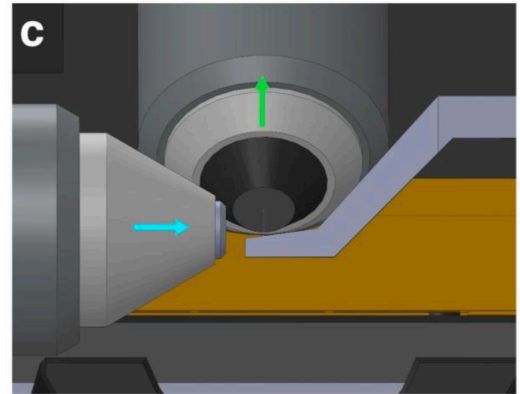
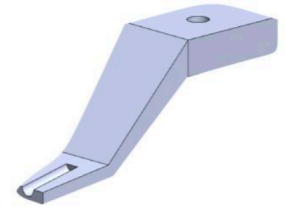


Imaging fast biological processes in 3D

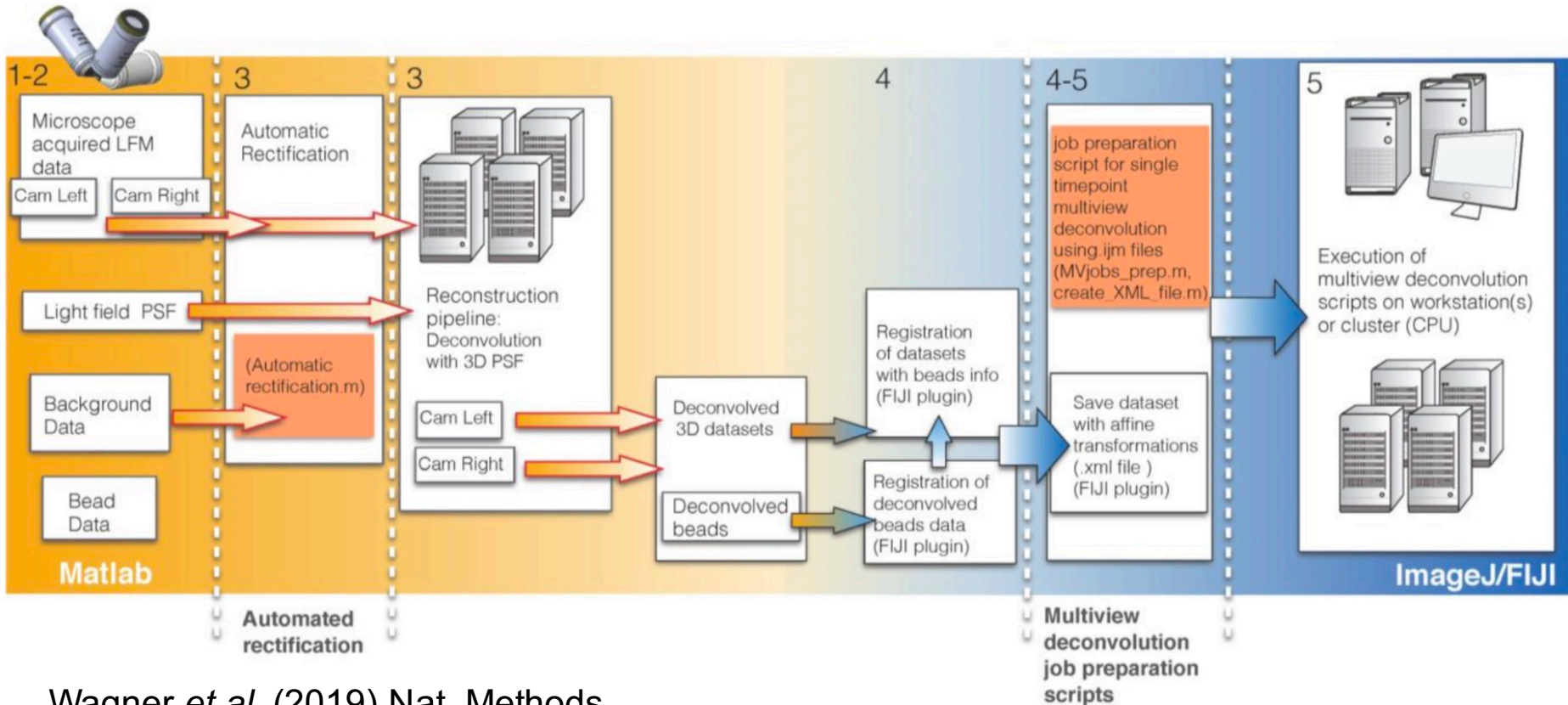
a



b

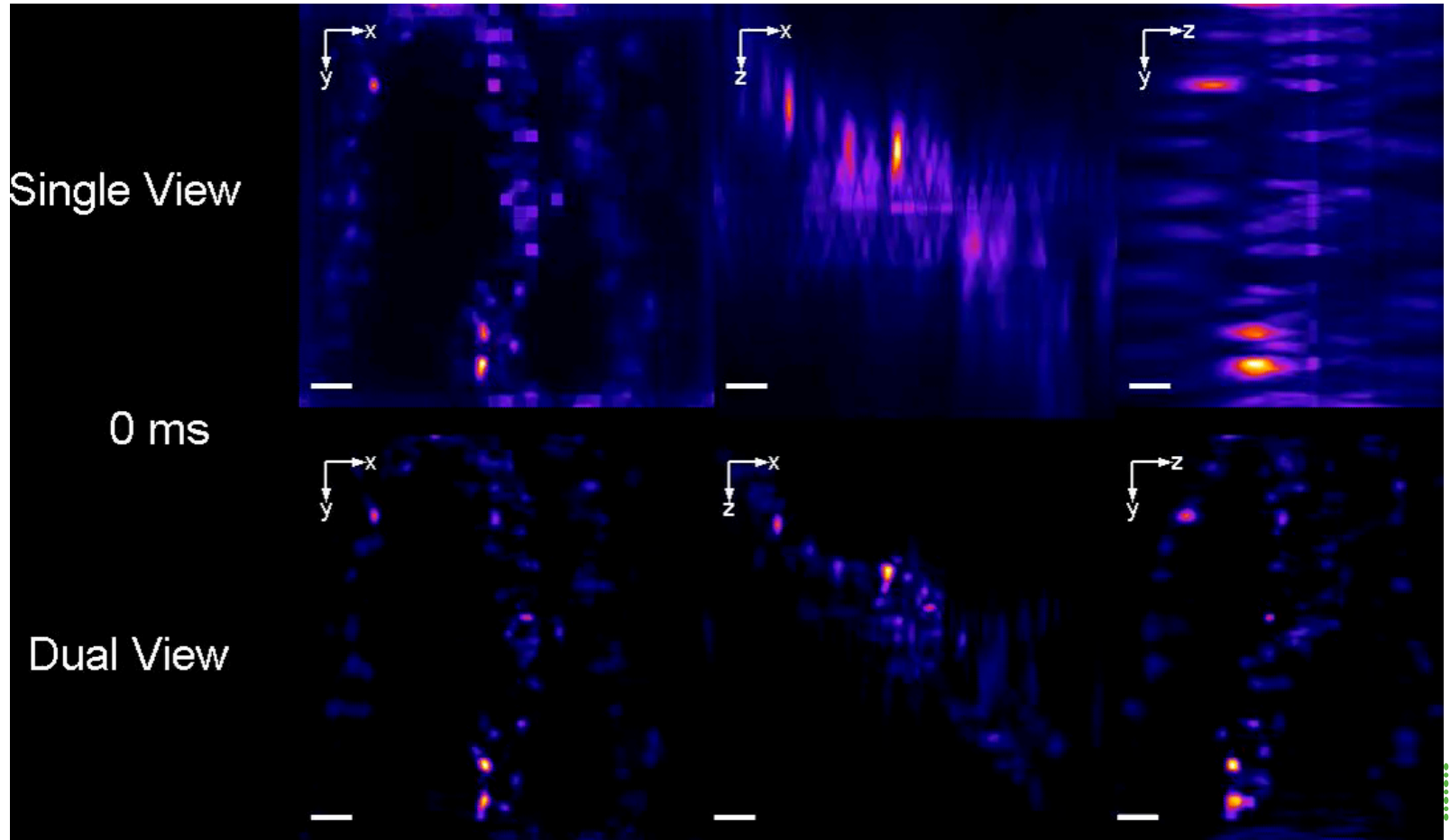


Imaging fast biological processes in 3D

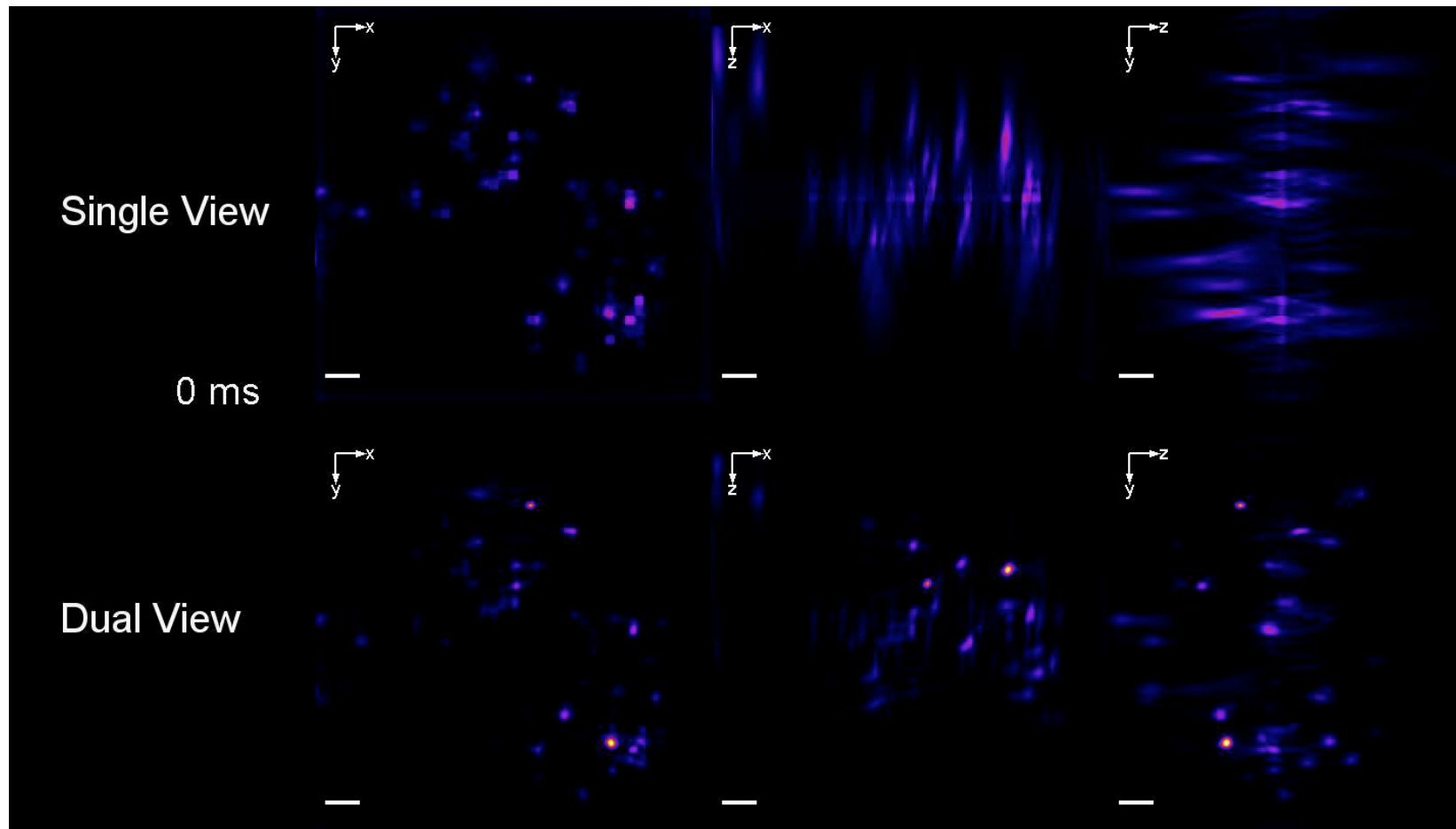


Wagner *et al.* (2019) Nat. Methods

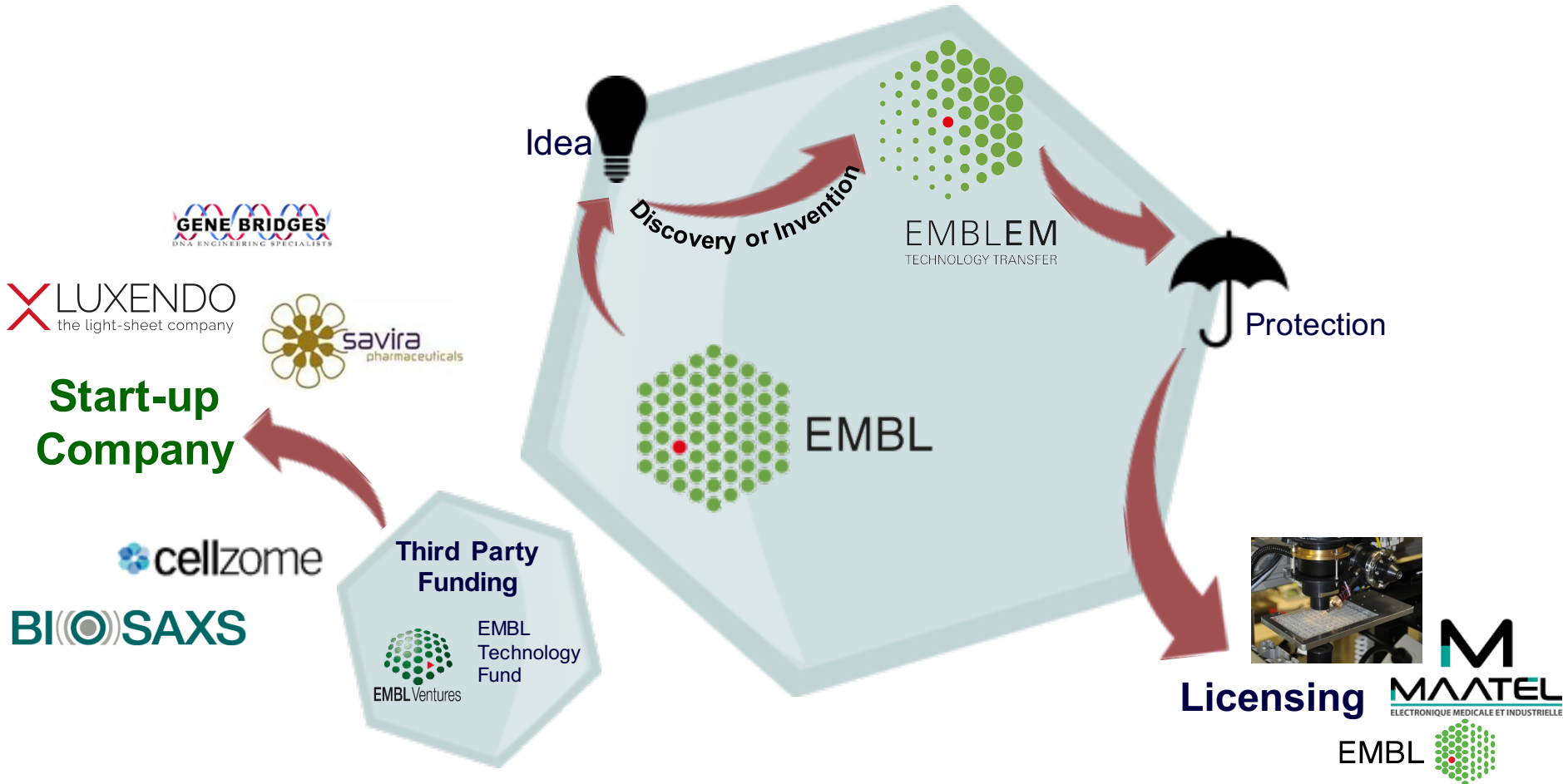
Single cell imaging of blood flow dynamics



Single cell imaging of blood flow in the heart



EMBL Technology & knowledge transfer: The players





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



WHAT IS ATTRACT?



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.

Thank you

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