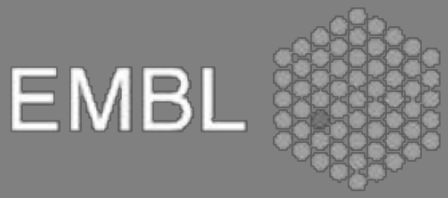
Aspects of Instrumentation at the European Molecular Biology Laboratory

Thomas R. Schneider, EMBL Hamburg

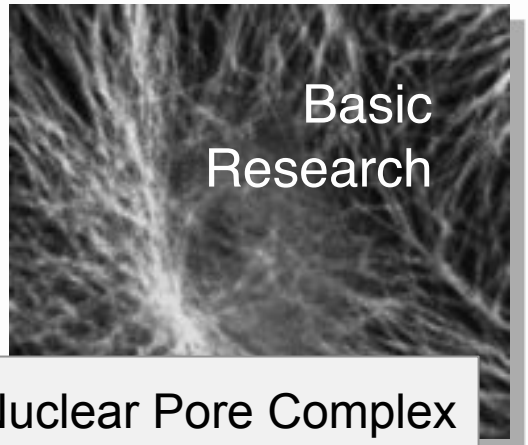
EIROforum School on Instrumentation
ESO (Garching, Germany) 15/06/2015



EMBL's Five Missions

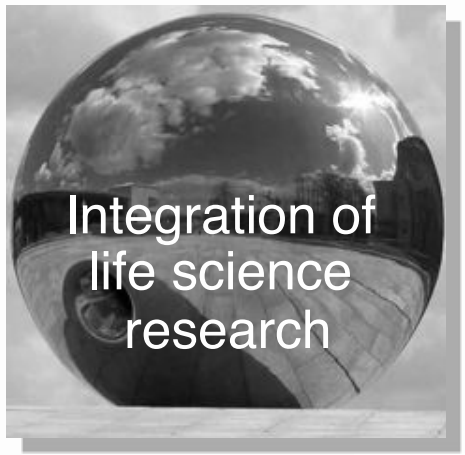
Core Facilities

Synchrotron beamlines



Nuclear Pore Complex

EMBL-EM



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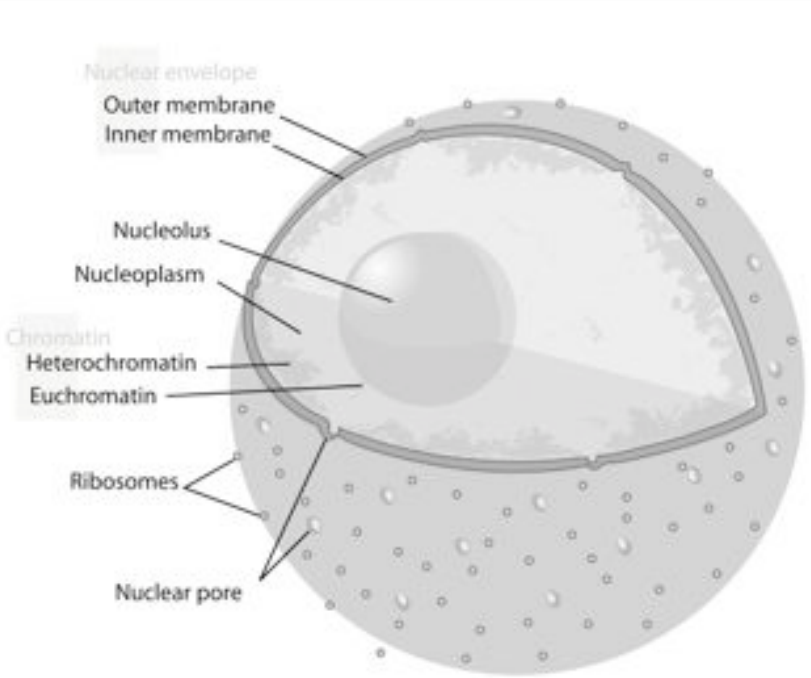
The Nuclear Pore Complex

- An example of multi-scale ‘hybrid’ structural biology

The Nuclear Pore Complex (‘NPC’)



Linear dimensions: 3 – 100 µm

https://en.wikipedia.org/?title=Nuclear_pore
http://book.bionumbers.org/how-big-is-a-human-cell/

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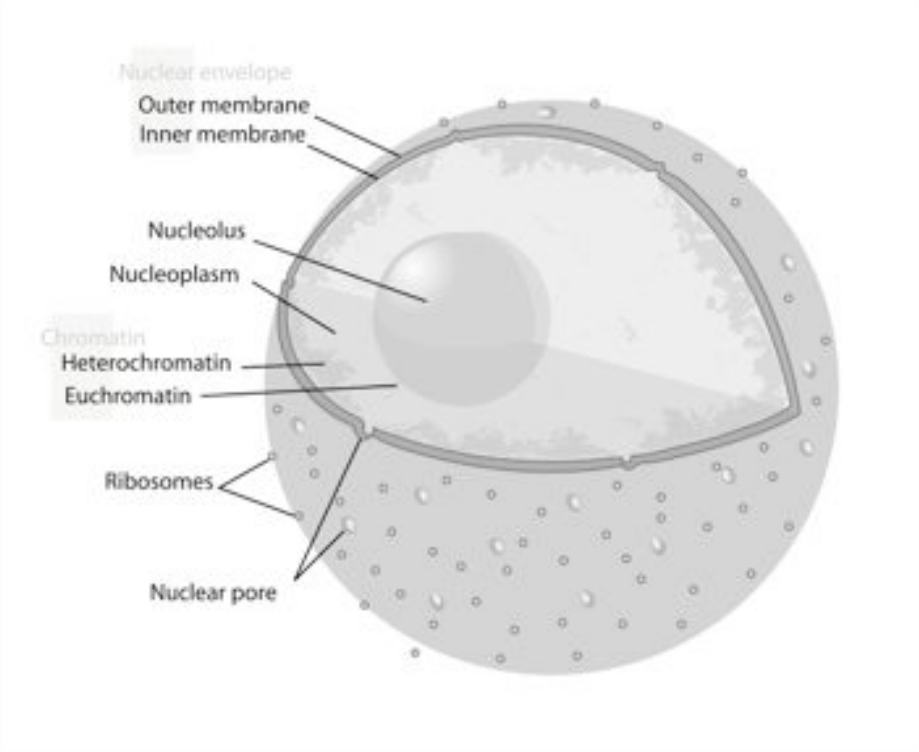
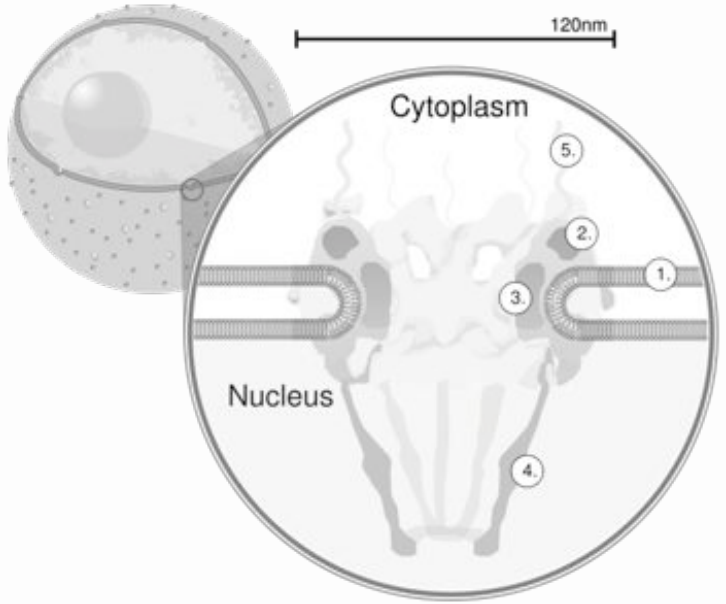
The Nuclear Pore Complex ('NPC')





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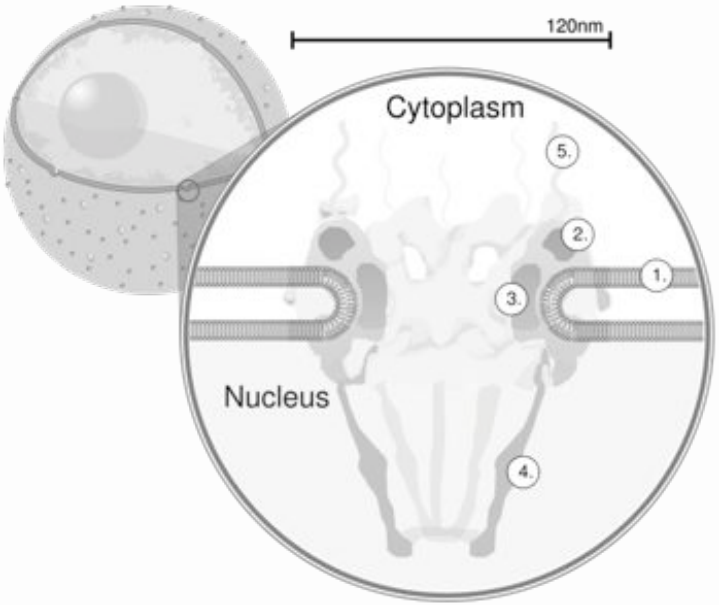
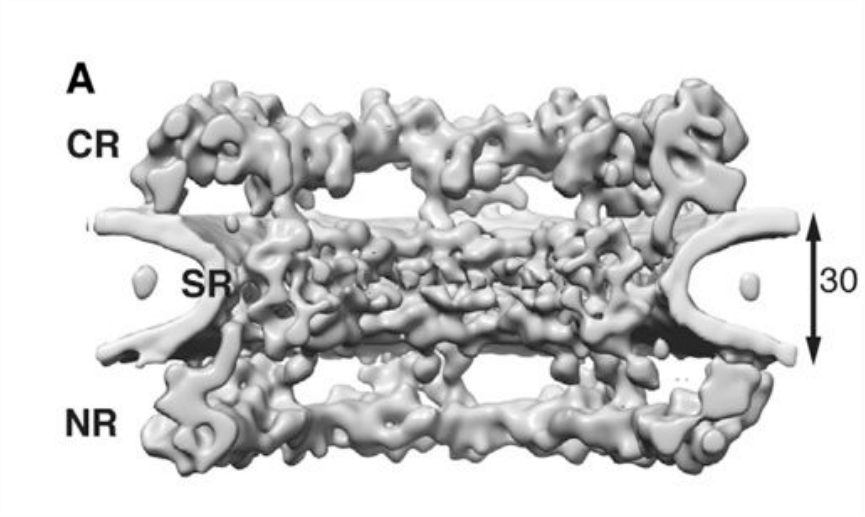
The Nuclear Pore Complex ('NPC')



~ 1000 protein molecules of 30 kinds

assemble / disassemble



NPC by Cryo Electron Tomography: 3.2 nm

Bui et al. (2013). Integrated Structural Analysis of the Human Nuclear Pore Complex Scaffold. Cell, 155:1233

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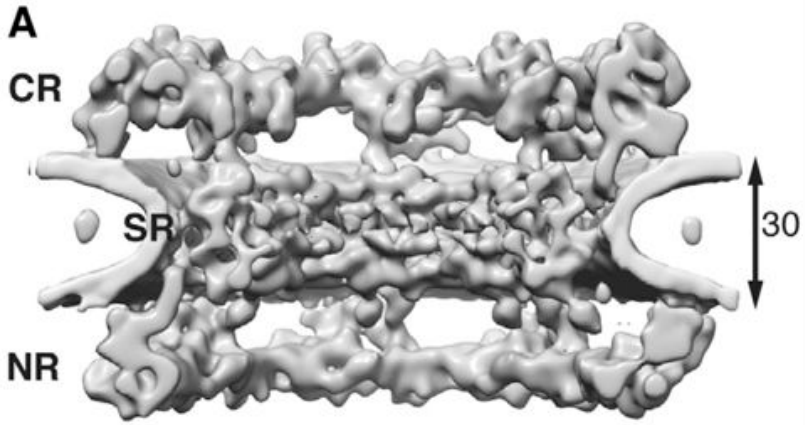
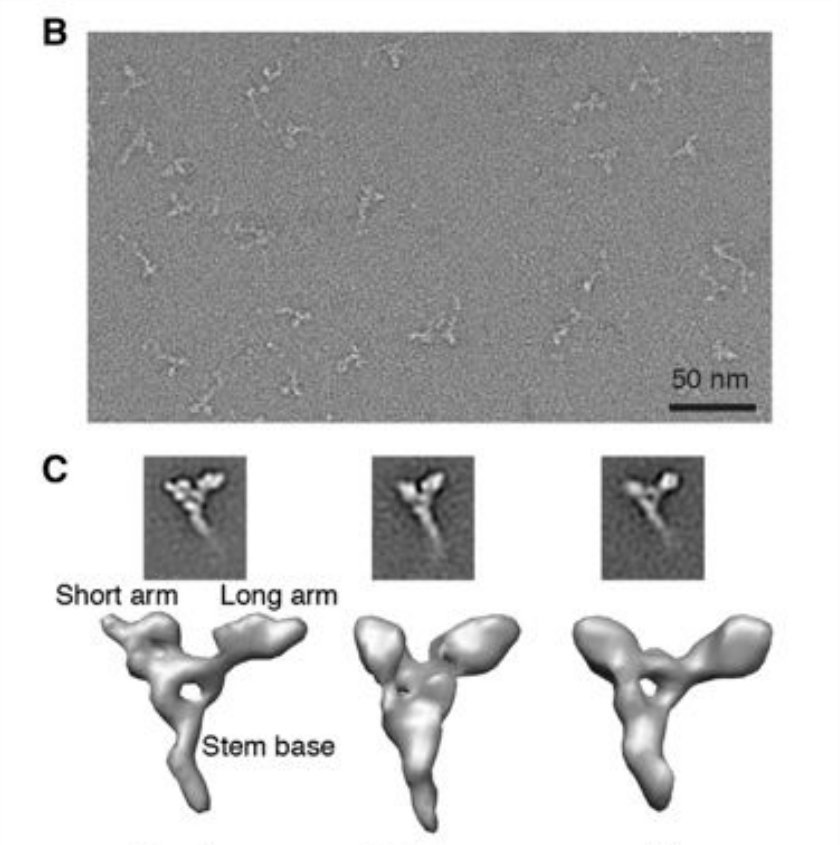
Bui et al



Composition

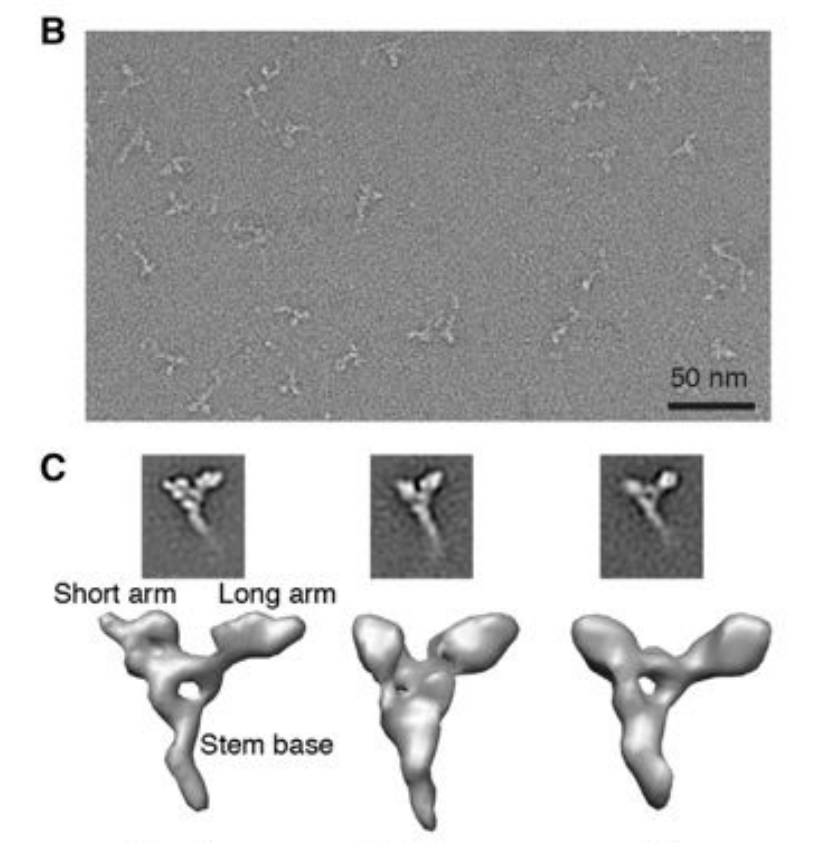
Numerous proteins: e.g. hNup107, Nup358, Nup214, Nup88, Aladin, hCG1, Nup153, Nup50



CNC by Single Particle Cryo Electron Microscopy: ~1 nm

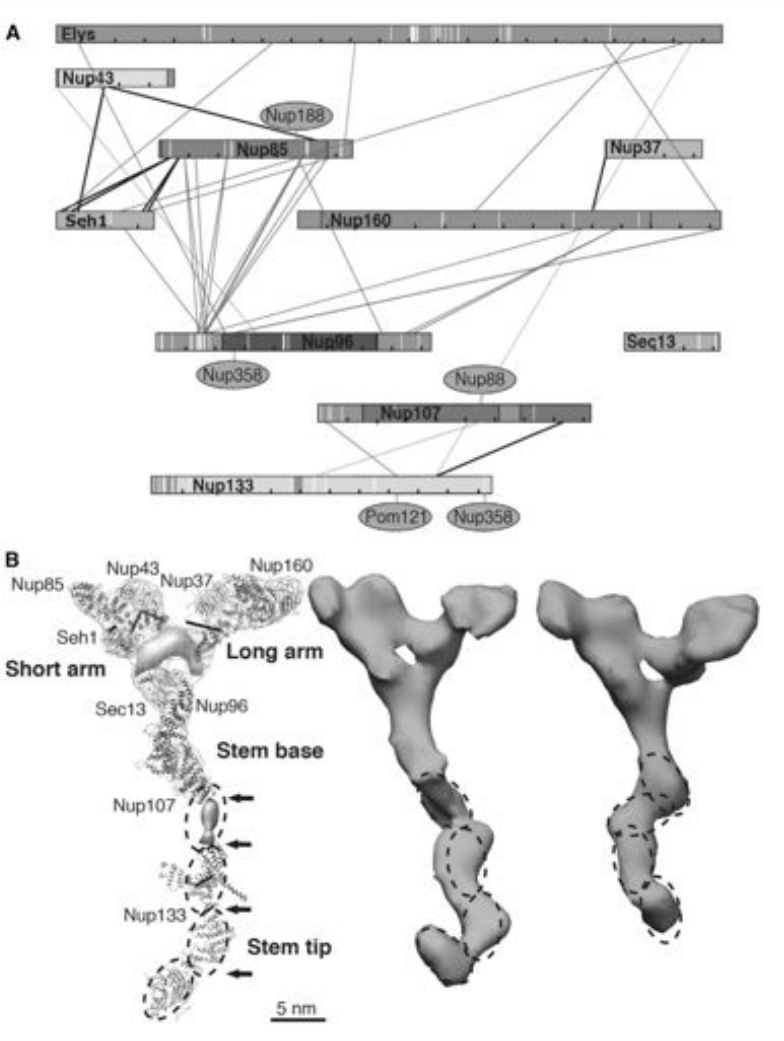
Bui et al. (2013). Integrated Structural Analysis of the Human Nuclear Pore Complex Scaffold. Cell, 155:1233

Nuclear Pore Complex



Nup107 Composition

Nup107 consists of 10 proteins



Spatial arrangement by cross-linking mass spectrometry

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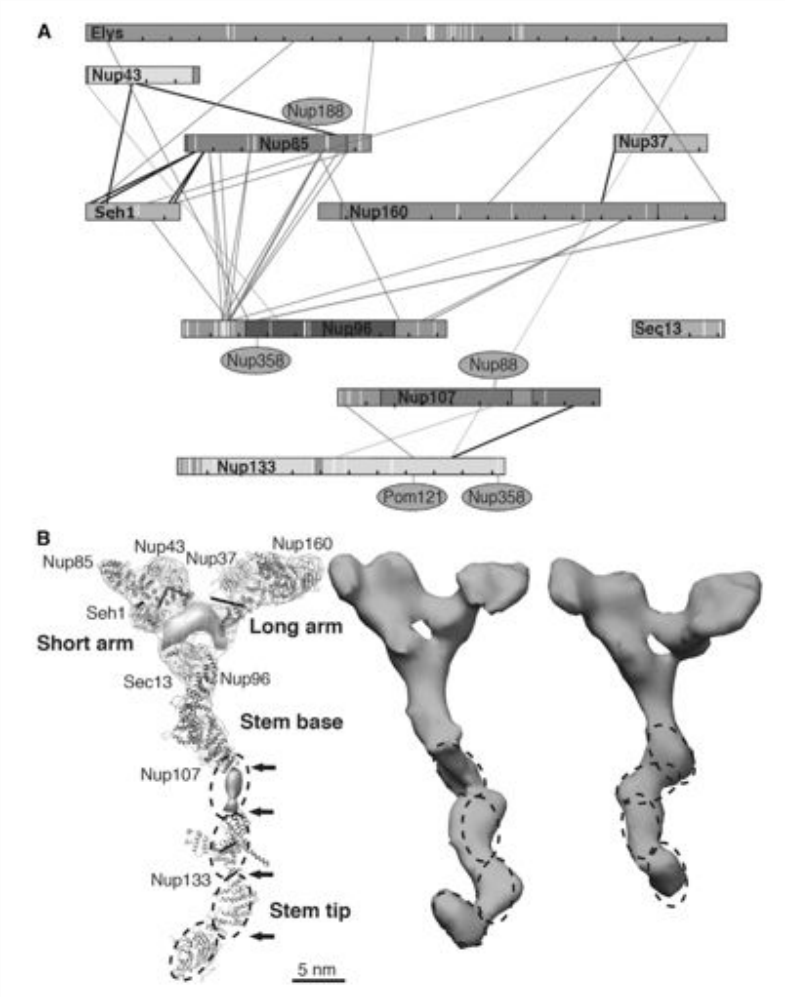
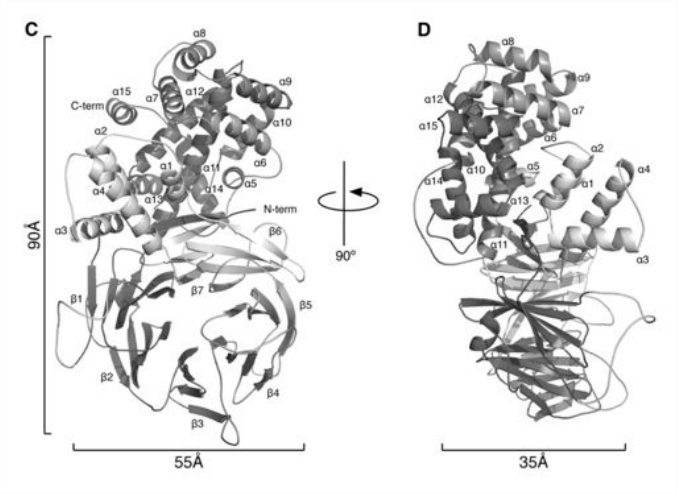
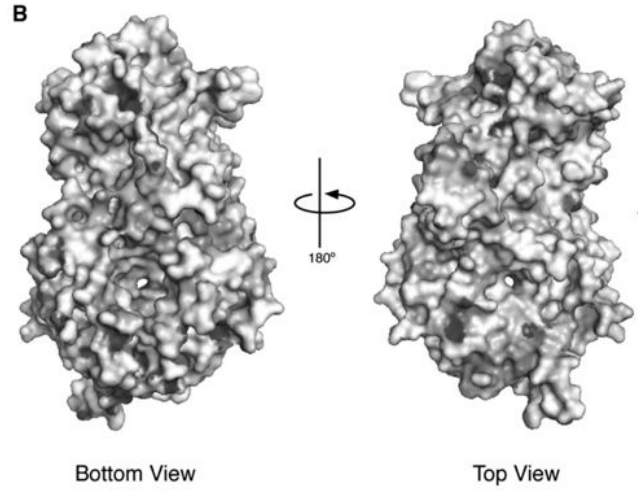
Nuclear Pore Complex



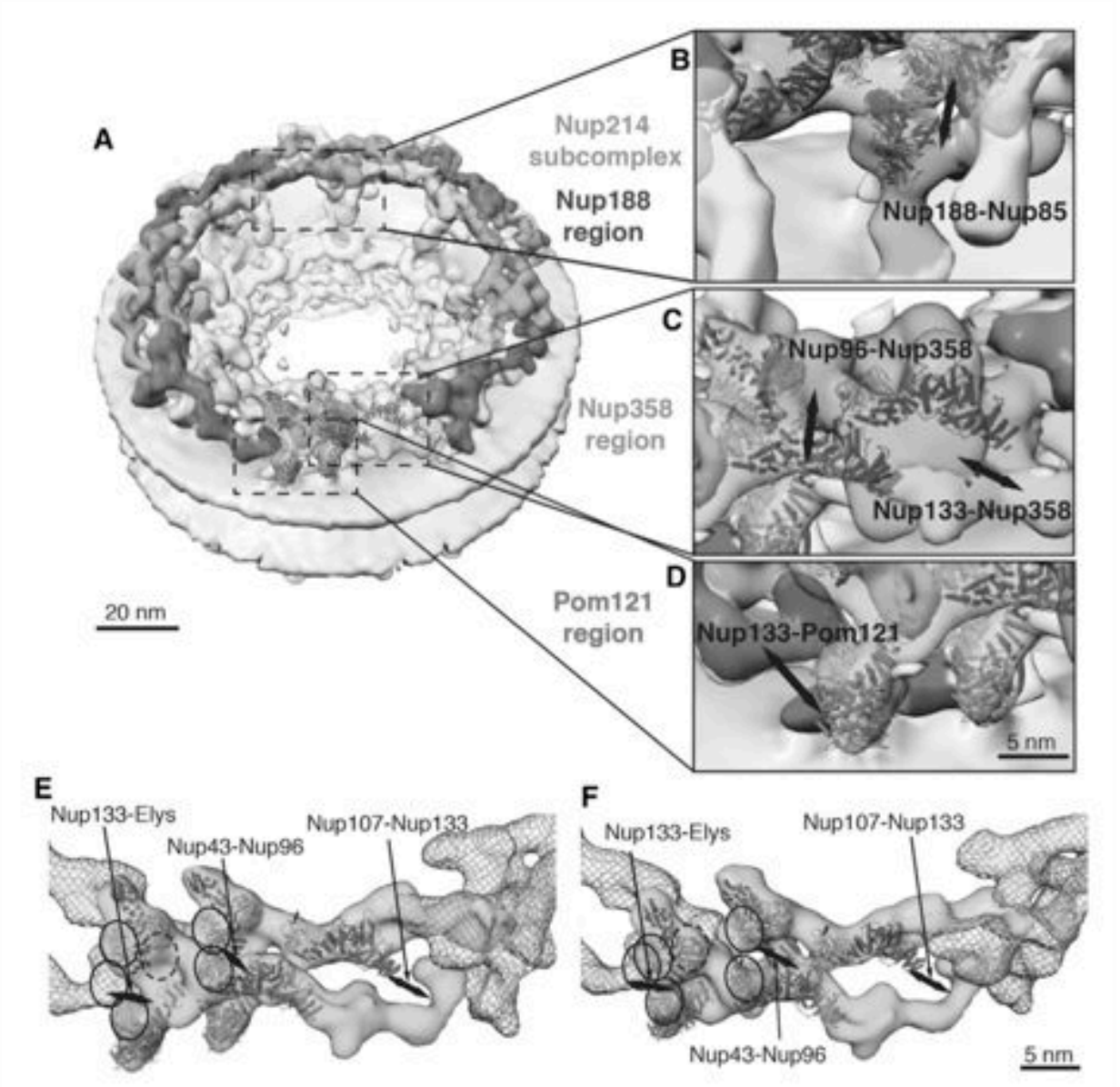




Nup120 by X-ray Crystallography: 3.0 Å

Leksa et al. (2009). The Structure of the Scaffold Nucleoporin Nup120 Reveals a New and Unexpected Domain Architecture. Structure, 17:1082

Putting things back together



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Reconstructing the NPC by Super Resolution Microscopy

Szymborska et al., 2013, Science 341:655
GSDIM = ground state depletion microscopy followed by individual molecule return

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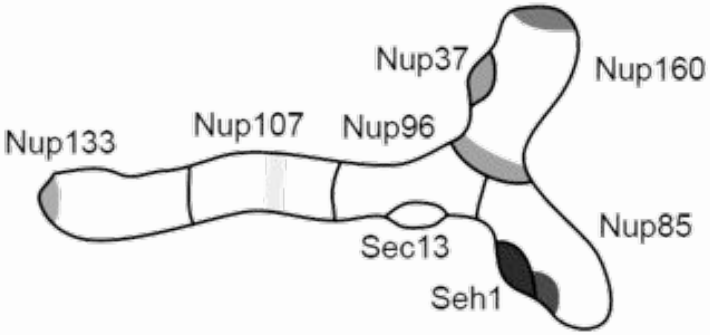
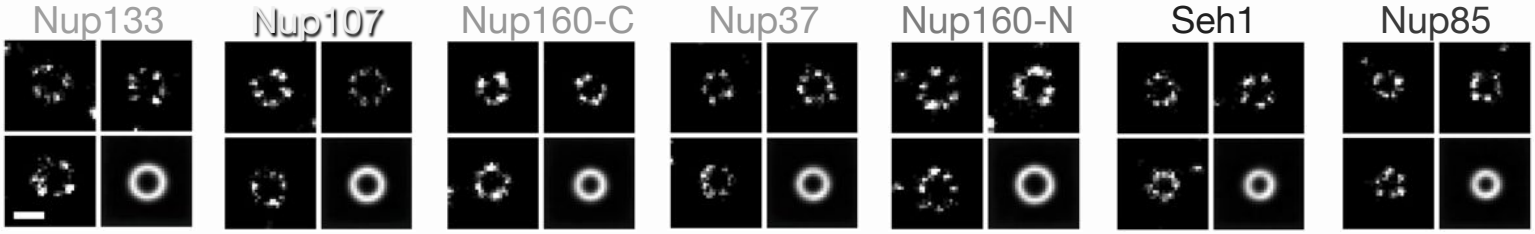
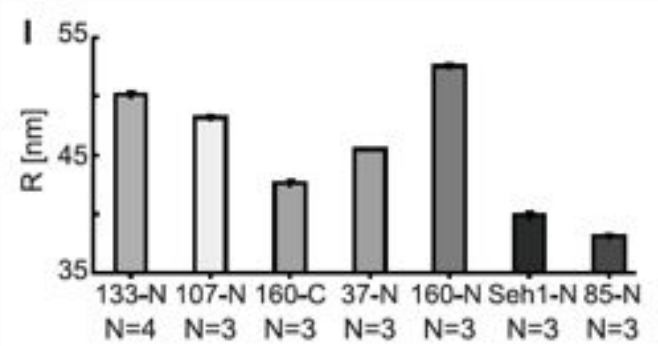
Mapping the radial orientation of the Y-shaped complex inside the NPC

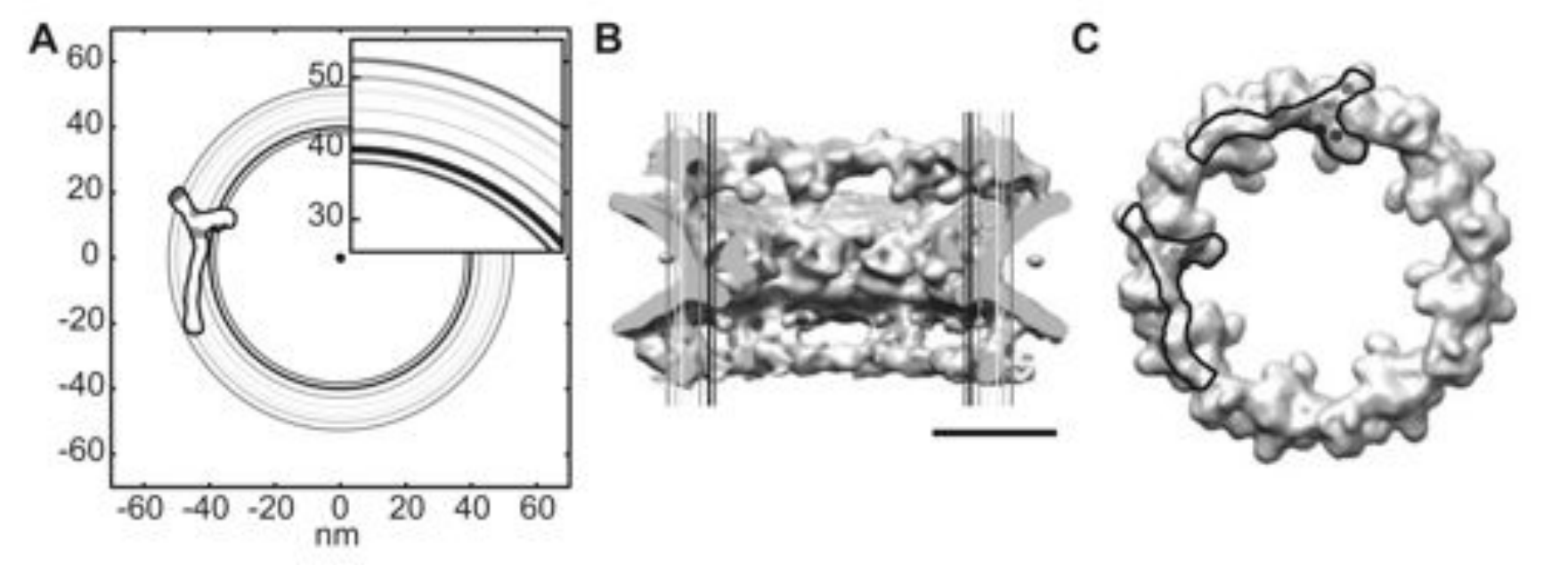






Szymborska et al., 2013, Science 341:655

Putting the Y-shaped complex



Szymborska et al., 2013, Science 341:655

4Pi-STORM 3D reconstruction of the NPC

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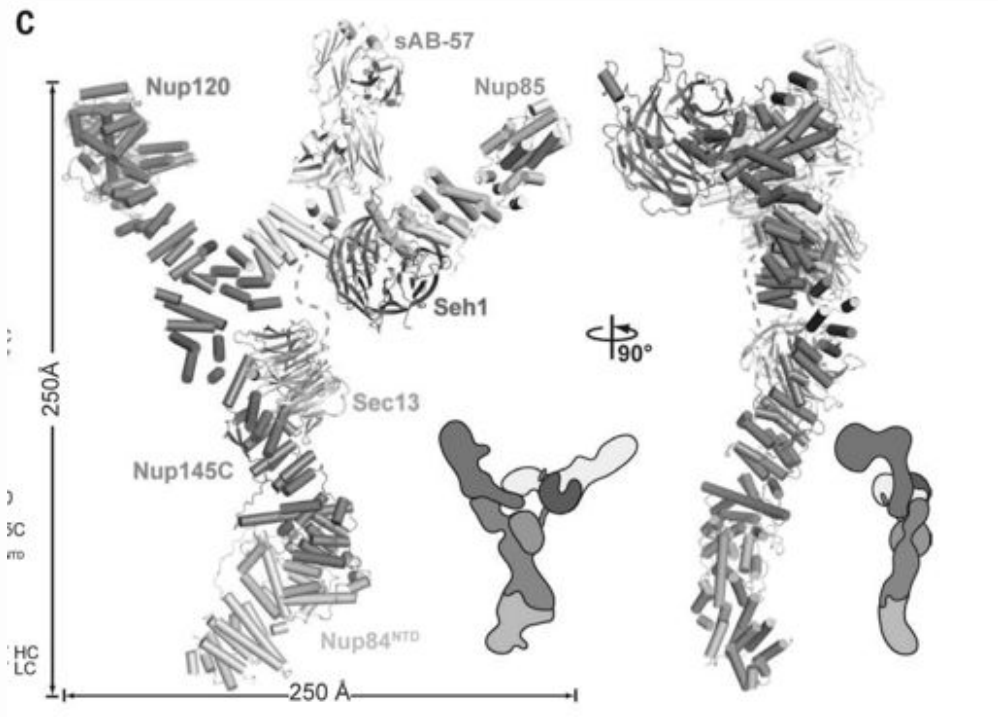
Crystallographic structure of the Y-shaped complex



CNC (Nup107) by X-ray Crystallography: 7.4 Å

Stuwe et al. (2015) Architecture of the nuclear pore complex coat. Science 347:1148

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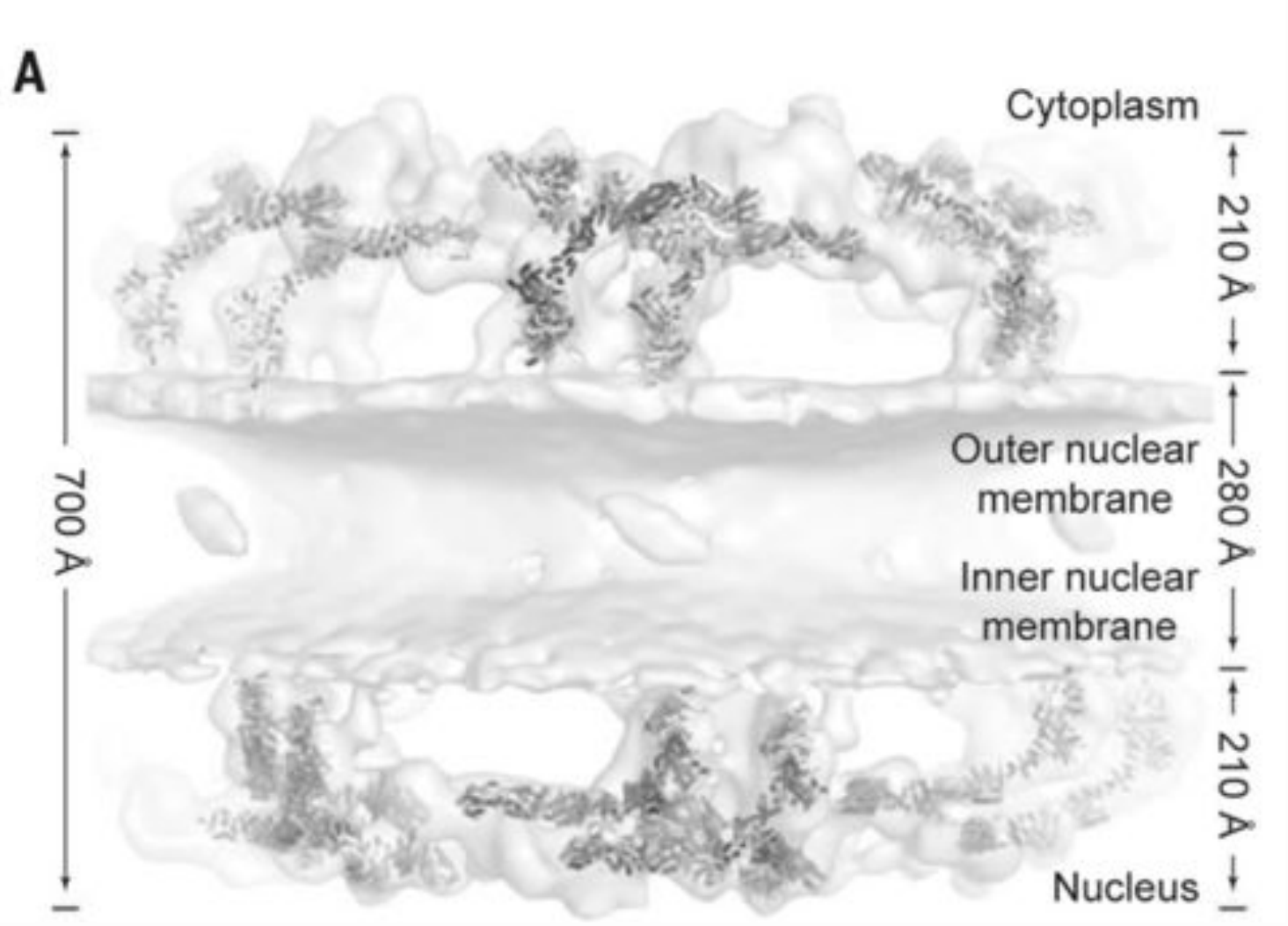
Putting things back together



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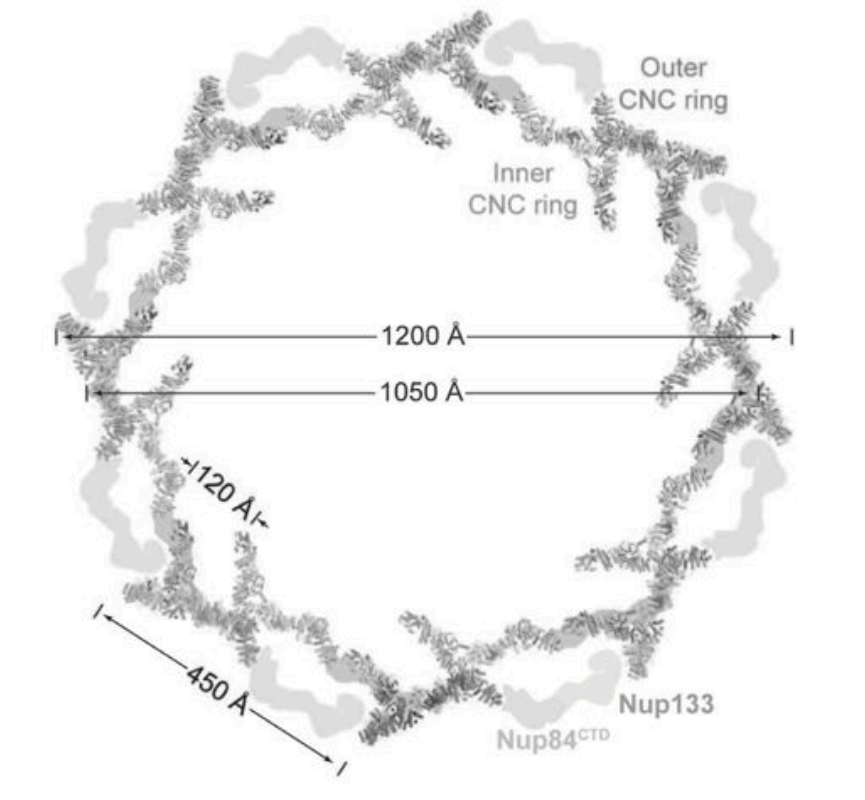
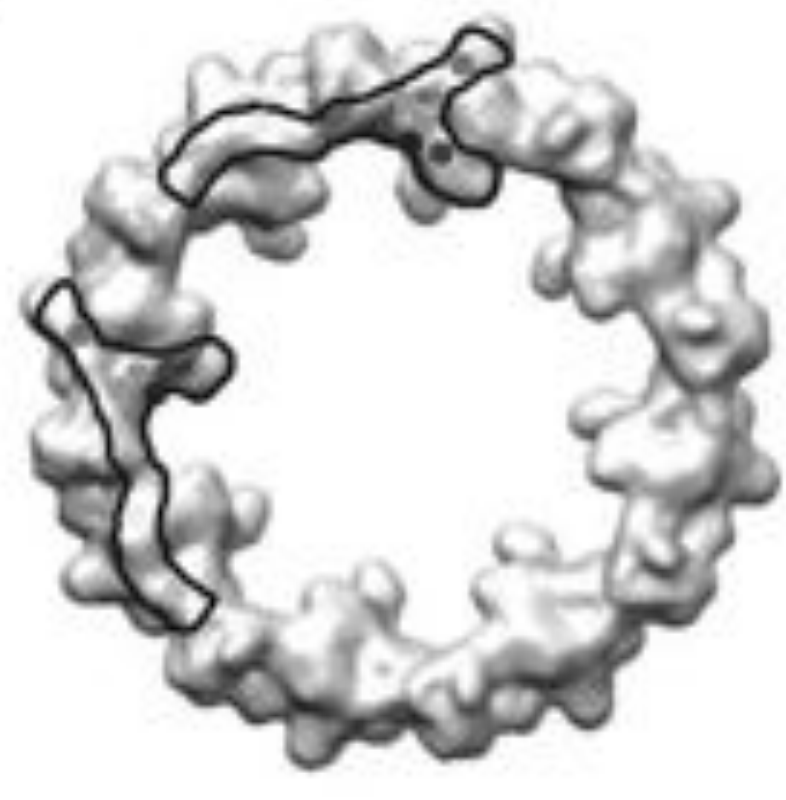
Consistency?



Stuwe et al. (2015)
Science 347:1148



Szymborska et al., (2013)
Science 341:655

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Core Facilities

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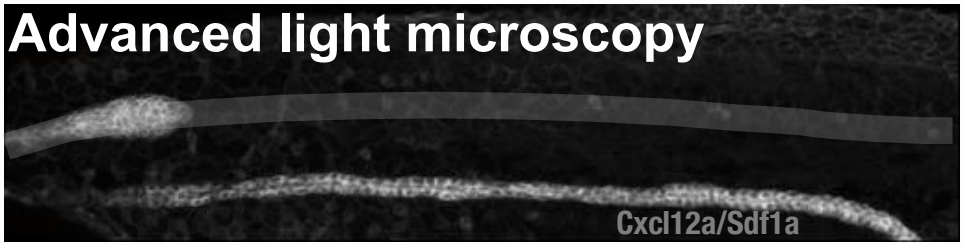
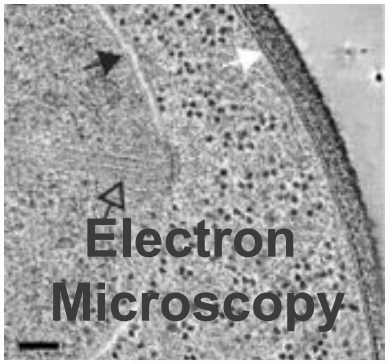
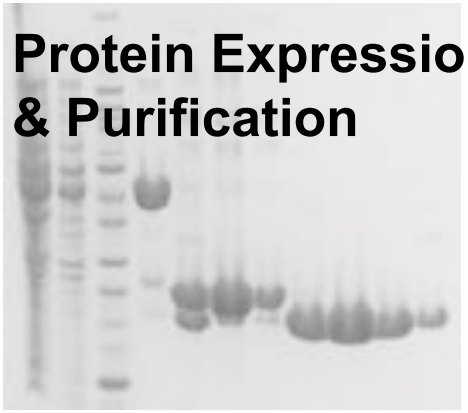
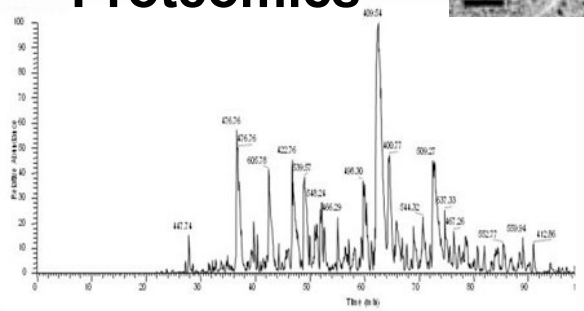
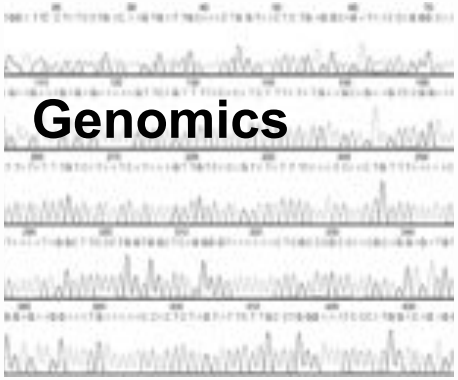
EMBL Scientific Core Facilities

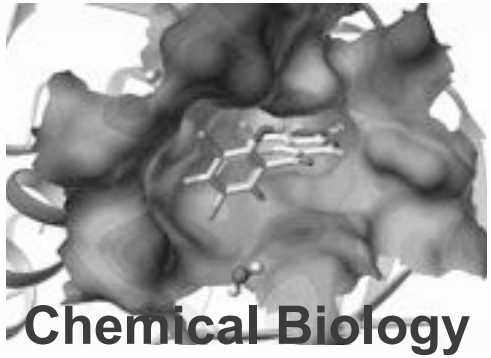












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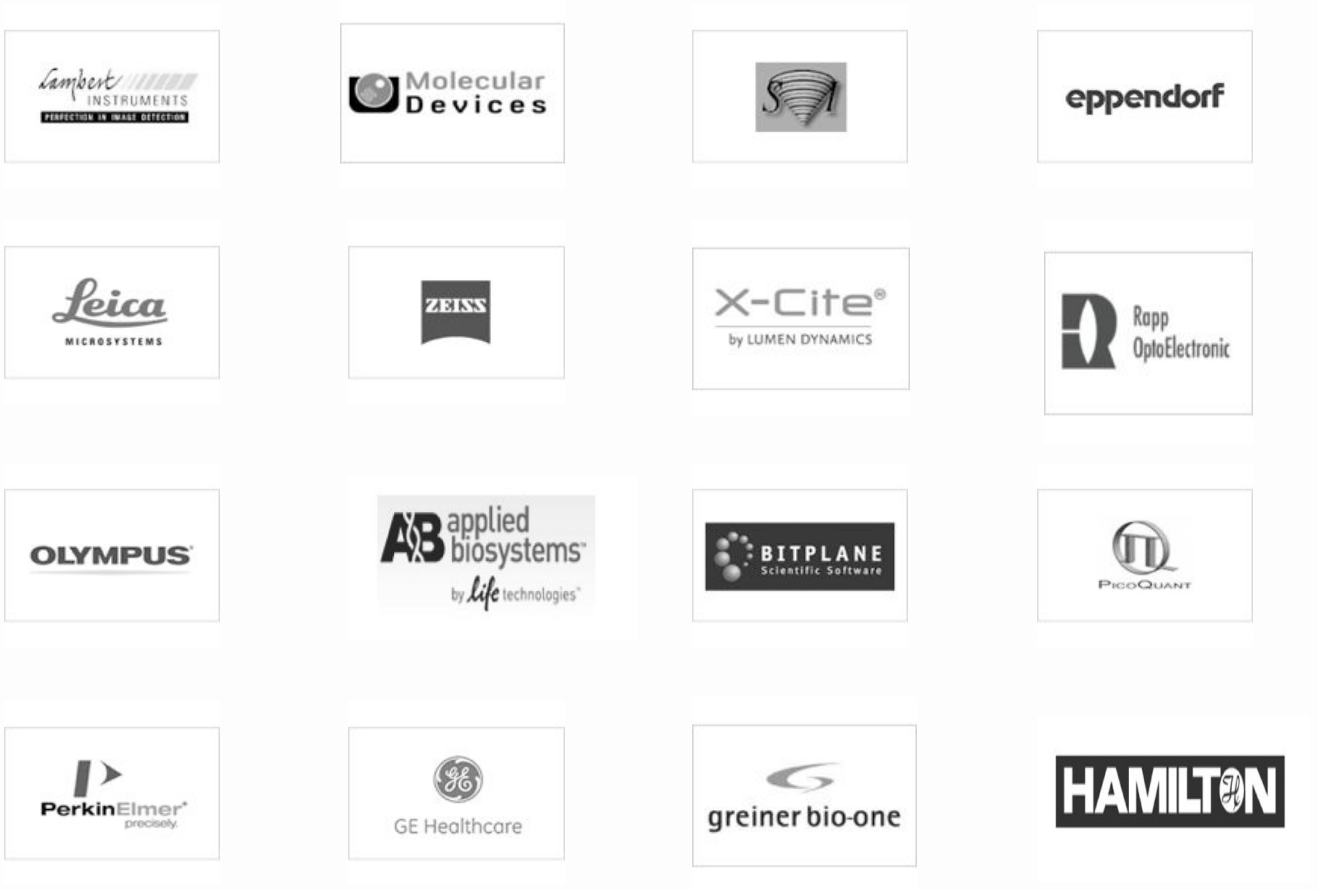
Current Industrial Partners of the ALMF



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HINXTON
HAMBURG
HEIDELBERG
GRENOBLE
MONTEROTONDO
EMBL Heidelberg
Germany
MAIN LABORATORY / GENERAL INFORMATION
EMBL Grenoble France
STRUCTURAL BIOLOGY
EMBL Hamburg
Germany
STRUCTURAL BIOLOGY
EMBL-EBI Hinxton
United Kingdom
EUROPEAN BIOINFORMATICS INSTITUTE
EMBL Monterotondo
Italy
MOUSE BIOLOGY

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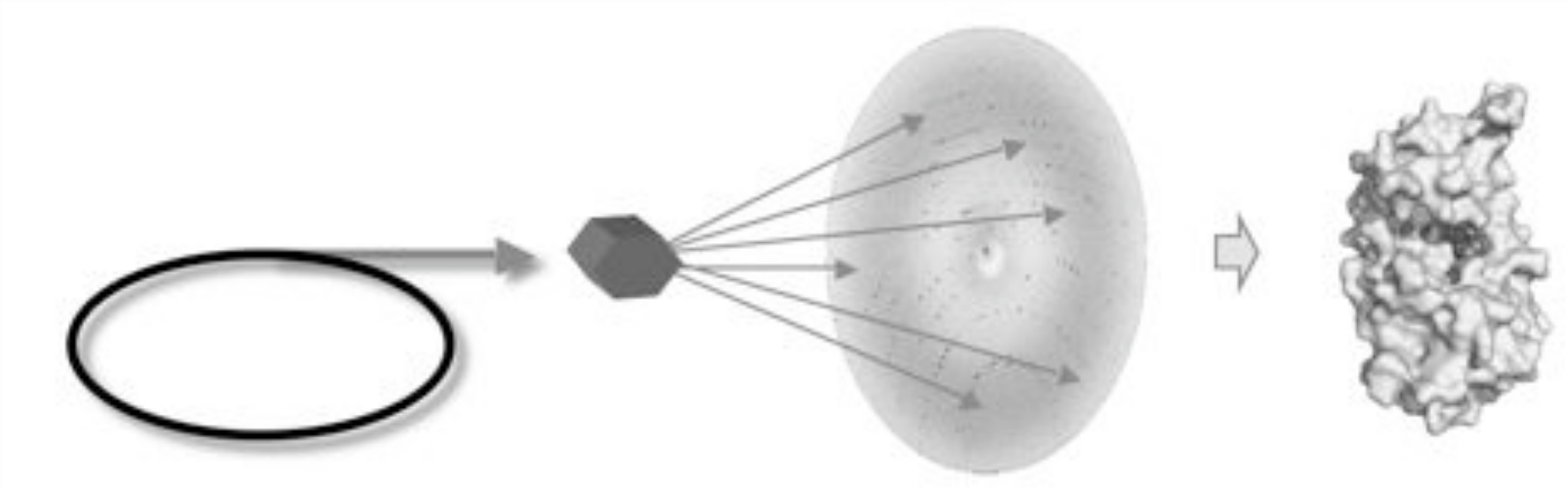
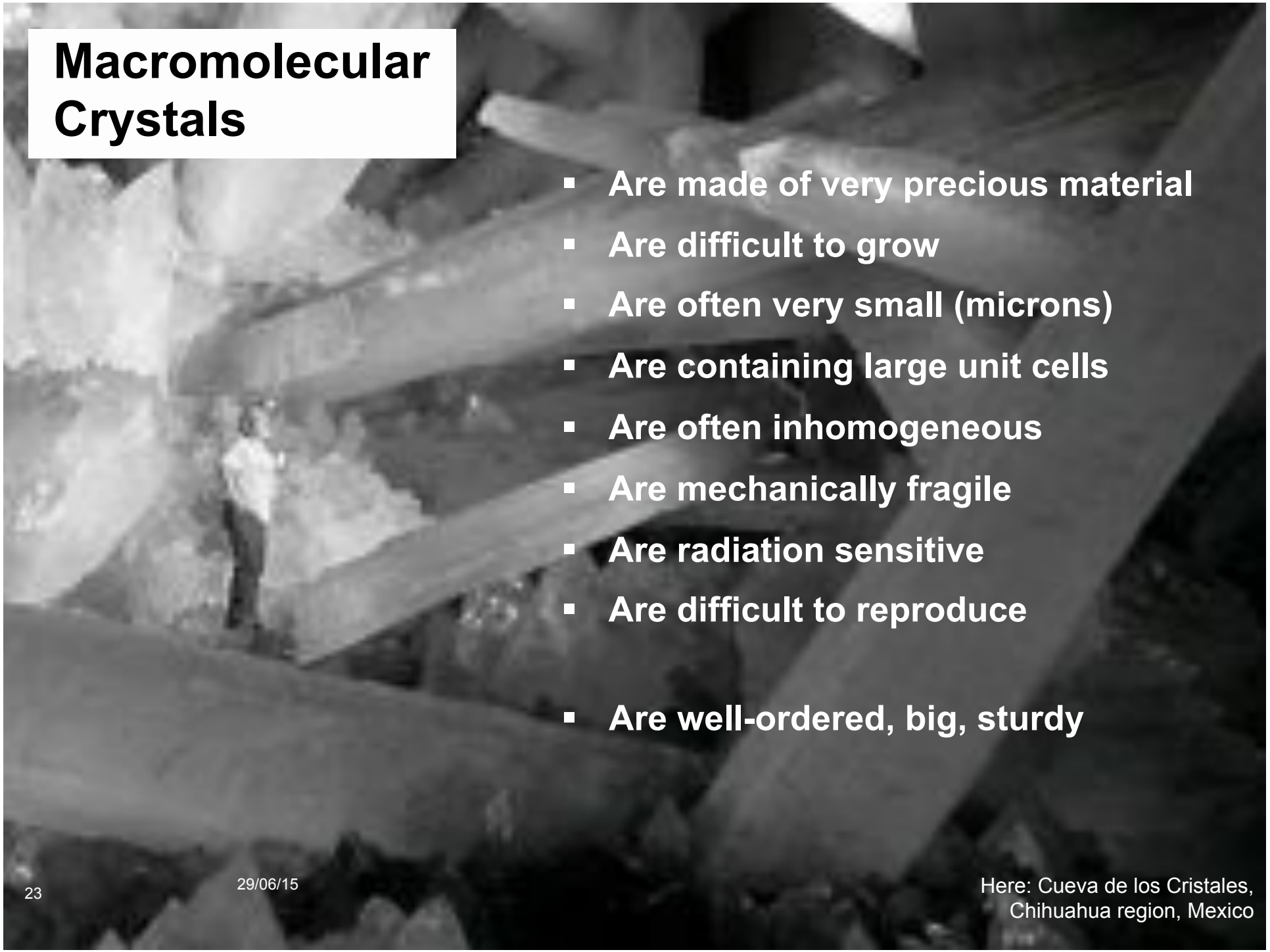


Macromolecular Crystals



- Are made of very precious material
- Are difficult to grow
- Are often very small (microns)
- Are containing large unit cells
- Are often inhomogeneous
- Are mechanically fragile
- Are radiation sensitive
- Are difficult to reproduce

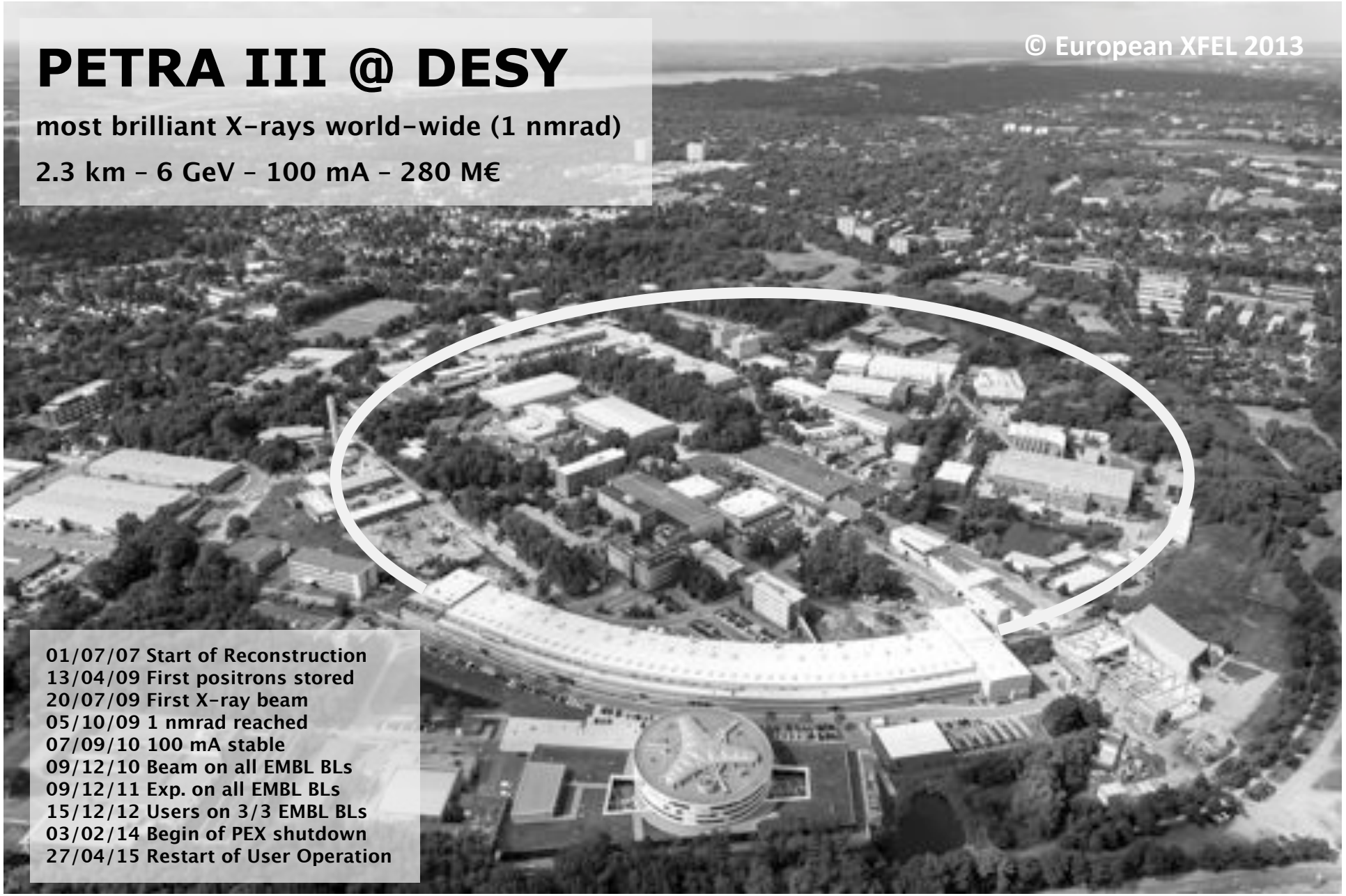
- Are well-ordered, big, sturdy

Here: Cueva de los Cristales, Chihuahua region, Mexico

PETRA III @ DESY

most brilliant X-rays world-wide (1 nmrad)

2.3 km – 6 GeV – 100 mA – 280 M€



01/07/07 Start of Reconstruction
13/04/09 First positrons stored
20/07/09 First X-ray beam
05/10/09 1 nmrad reached
07/09/10 100 mA stable
09/12/10 Beam on all EMBL BLs
09/12/11 Exp. on all EMBL BLs
15/12/12 Users on 3/3 EMBL BLs
03/02/14 Begin of PEX shutdown
27/04/15 Restart of User Operation

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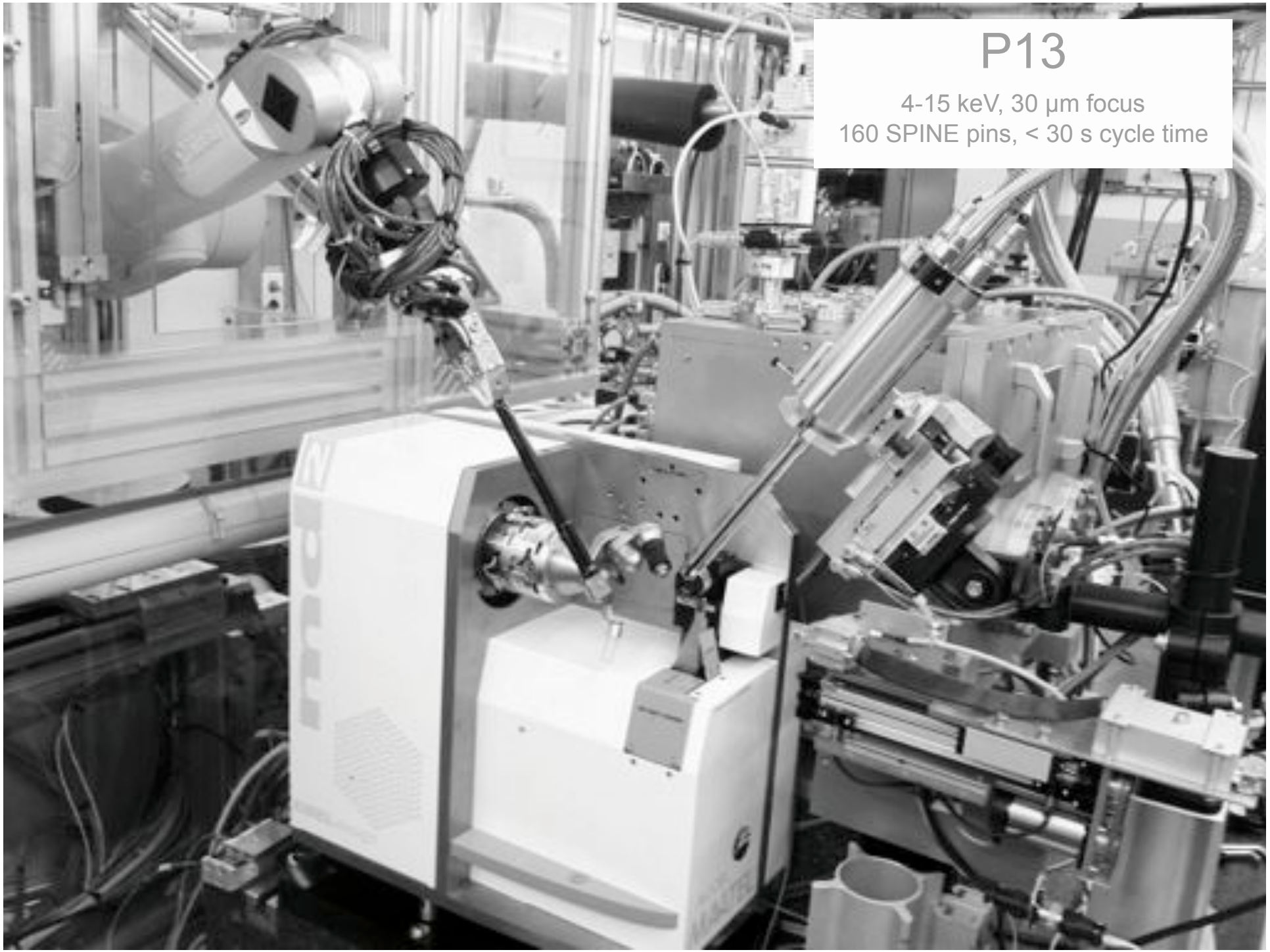
P13
4-15 keV, 30 µm focus
160 SPINE pins, < 30 s cycle time

P14

7-15 [35] keV, 4 x 6 [1x5] µm focus

100 MGy/s

- 5 x 10^{12} ph/s into 5 x 5 μm^2
- 500 ms at 100 K
- 10 ms at 300 K

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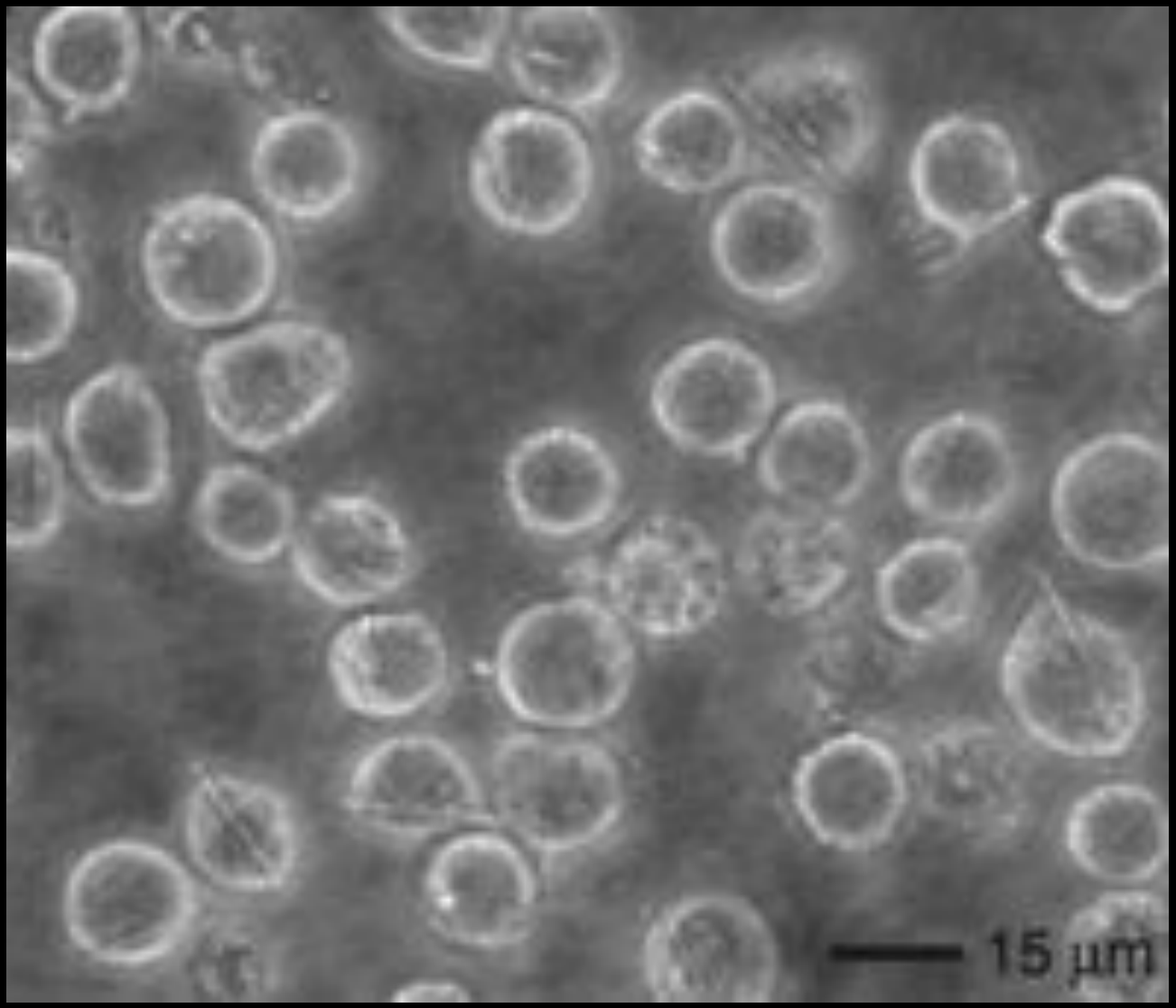
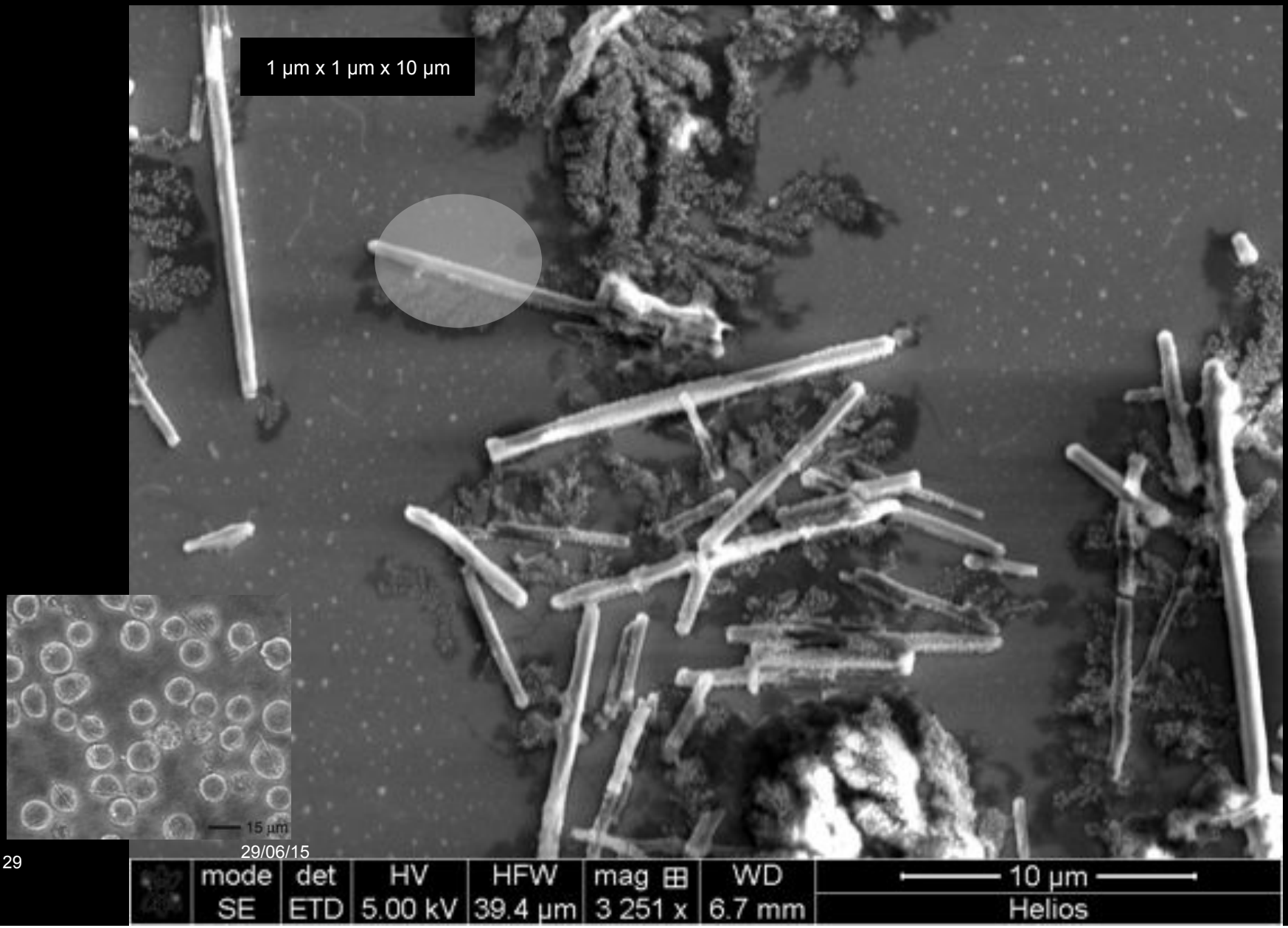
Cathepsin B



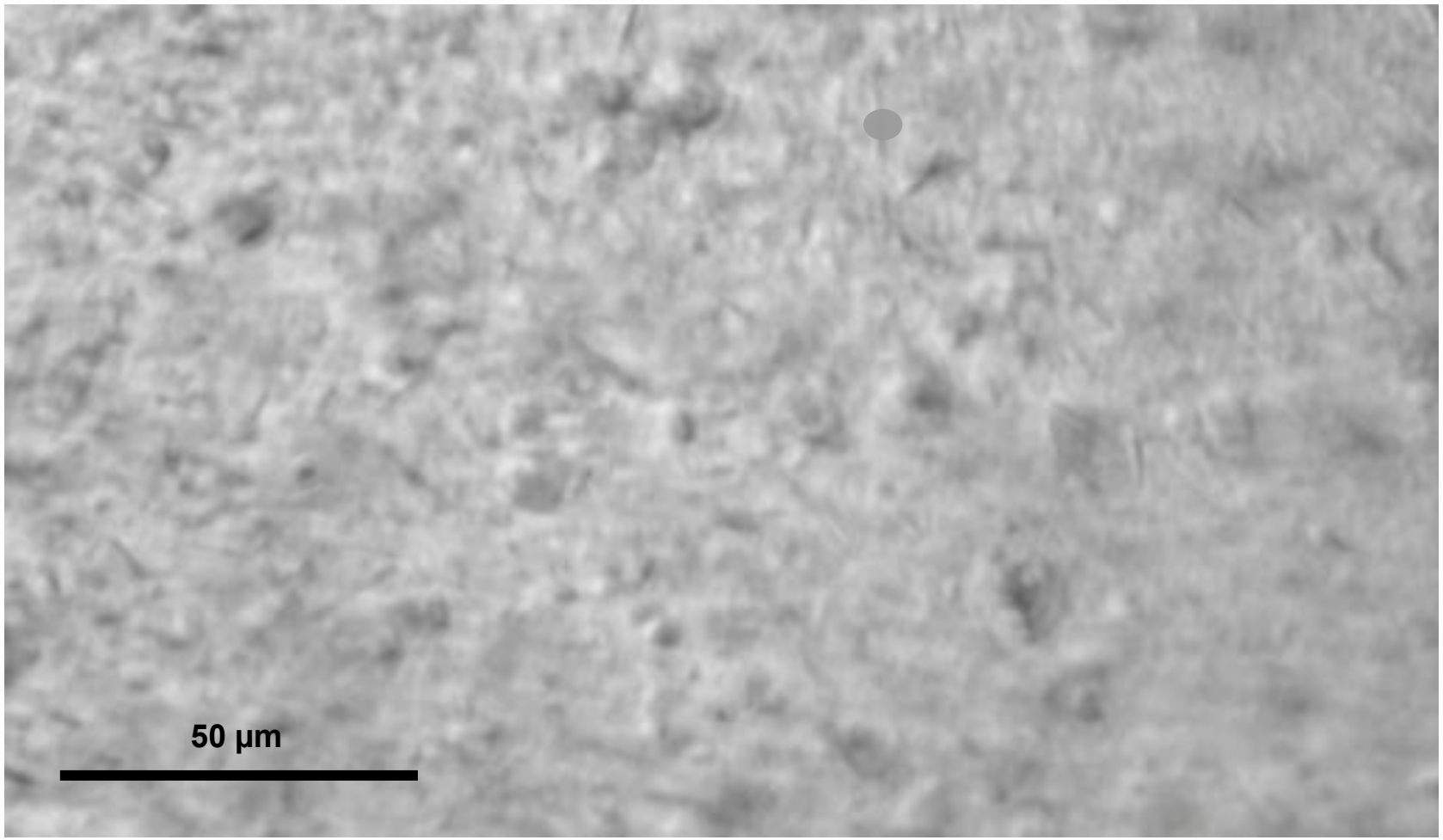
Cathepsin B

Redecke, L., ... Chapman, H. (2012): Natively Inhibited Trypanosoma brucei Cathepsin B Structure Determined by Using an X-ray Laser. Science 339:227 [4HWY:2.1 Å]



29/06/15

Cathepsin B – Suspension in a loop



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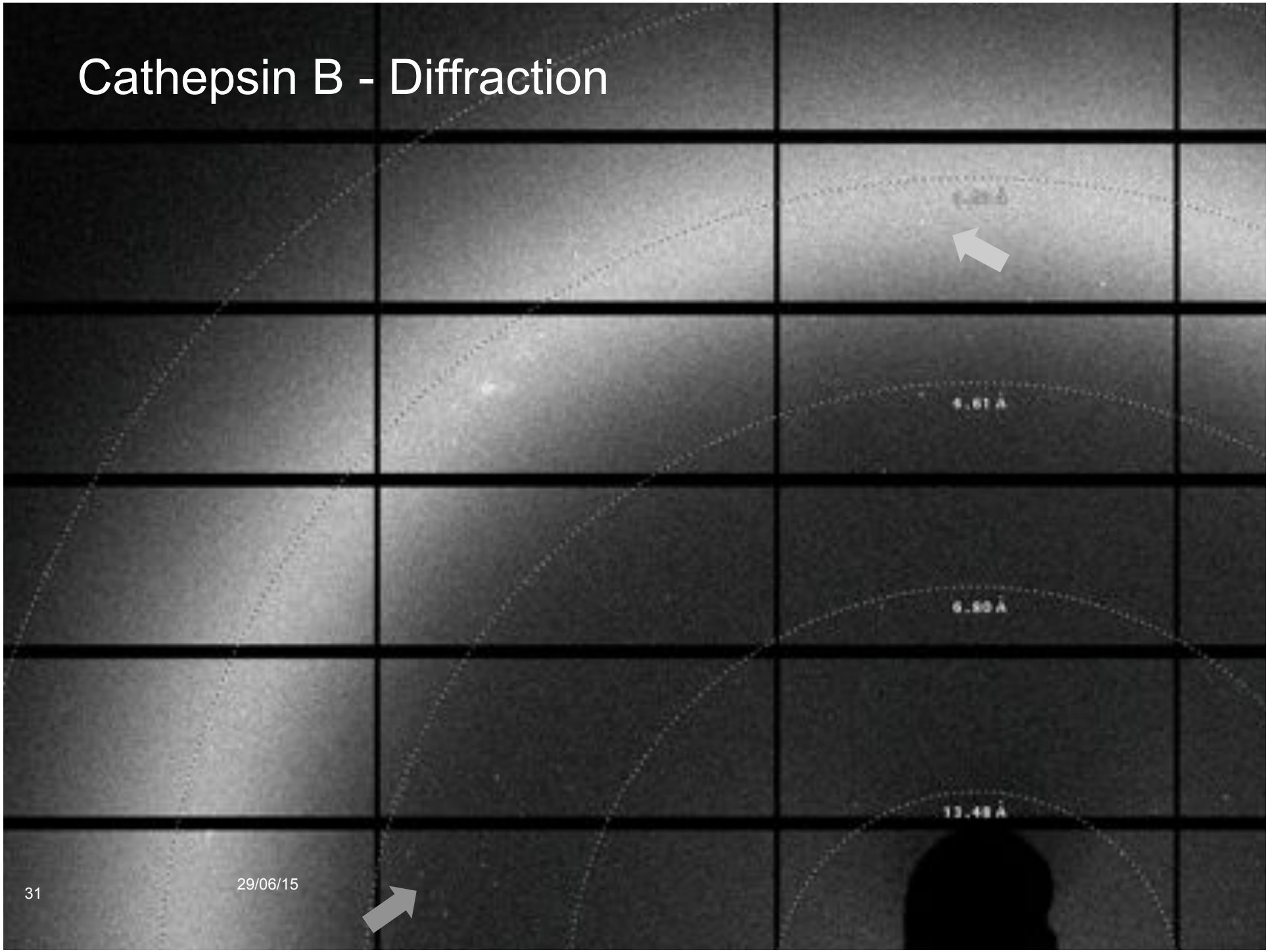
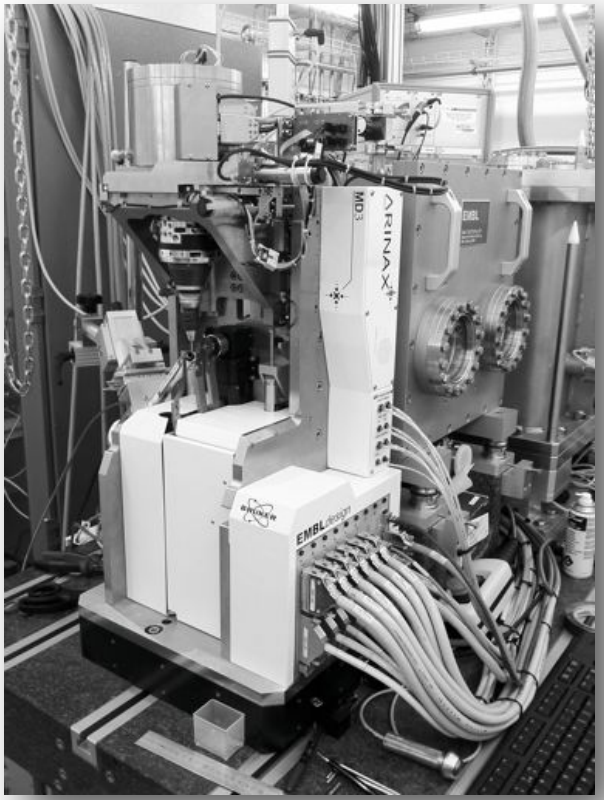
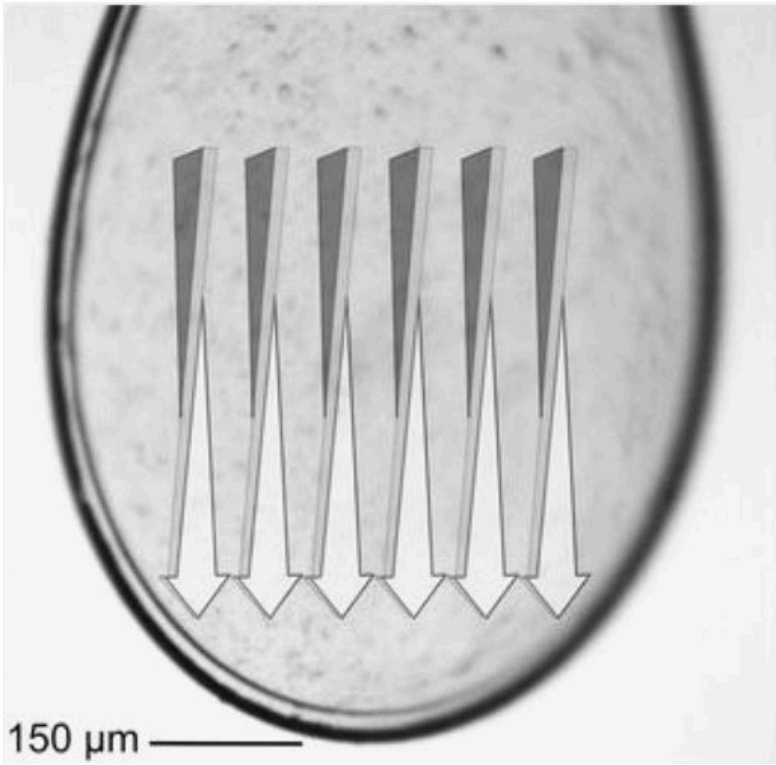
Cathepsin B - Diffraction



Serial Synchrotron Crystallography (2013)



Presentation of frozen suspension of micro-crystals to a micro-beam using an MD3 (EMBL-GR+HH)



Data collection using **continuous** highly-precise ‘serial helical scans’ as implemented on MD3.

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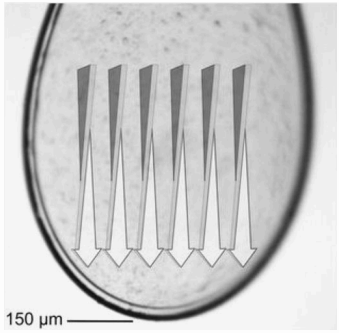
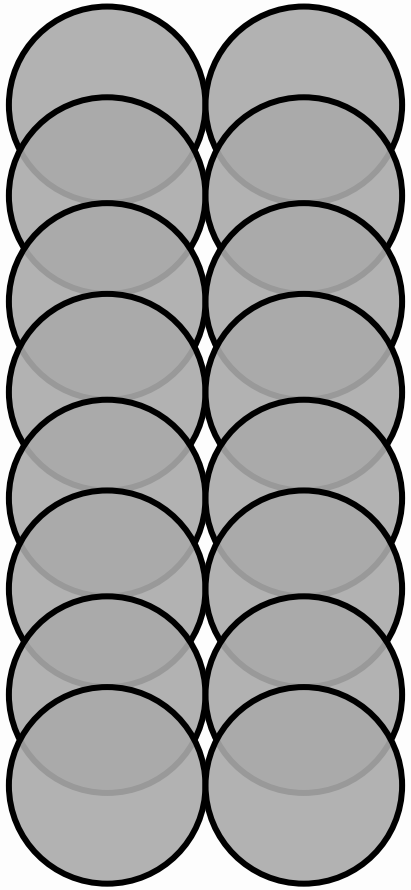
Exposure conditions (in 2013)

- 1.2 * 10^{12} ph/sec into 4 x 5 μm^2
  - < 1 s crystal lifetime
- 600 x 600 μm^2 ROI
  - 120 parallel scans 5 µm apart
  - Each scan 600 µm from -45º to +45º with 240 images
  - 2.5 µm per image, 0.375º per image
  - 50 – 60 MGy for each volume / crystal
- 28800 frames in 8 hours.
- Flux limited! (1 Hz)

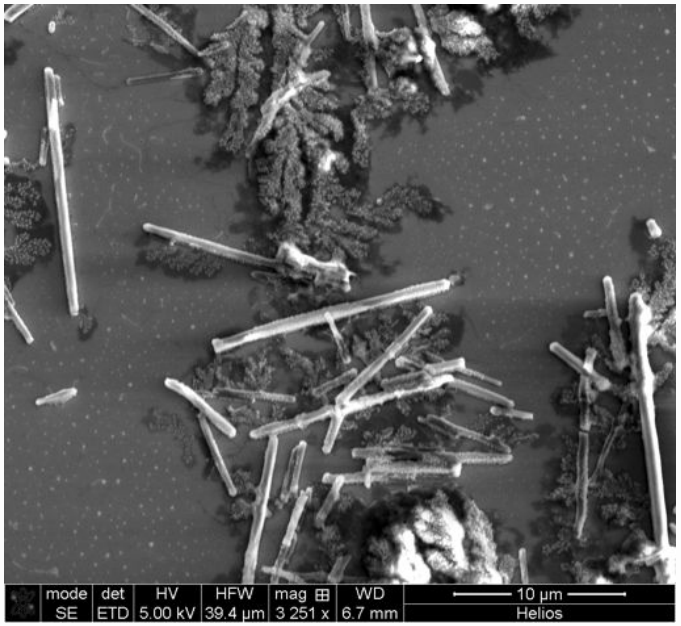




Extraction of data

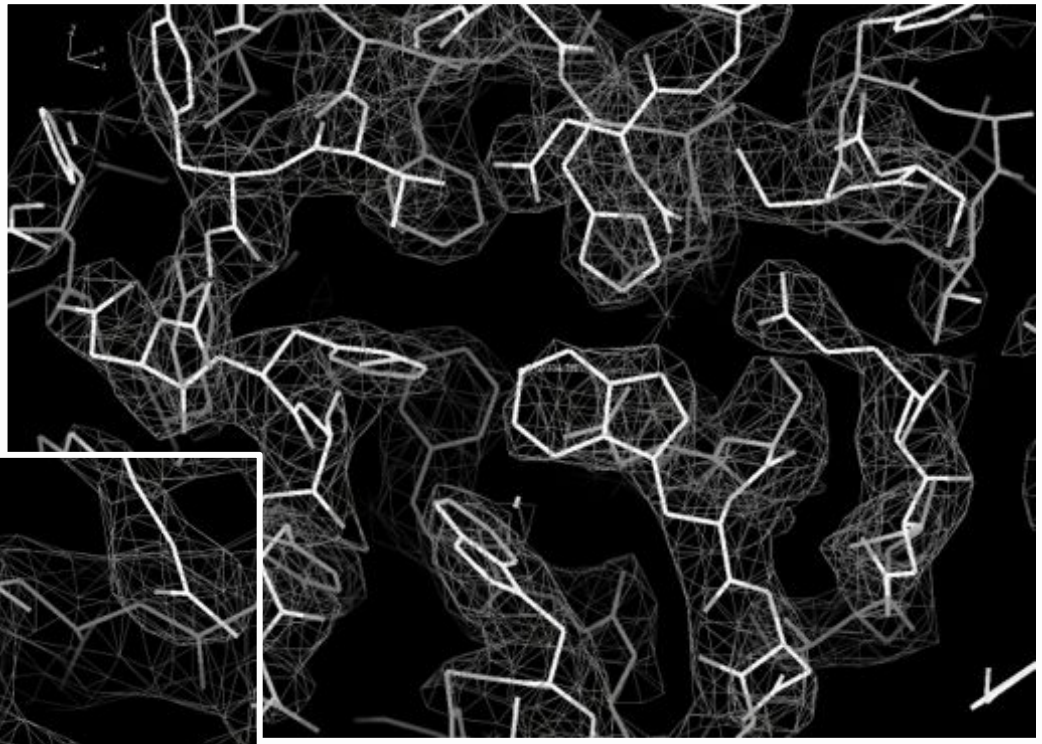
- 120 * 240 = 28800 frames = 172 GB
- ~2000 crystal hits identified by CrystFEL [White et al.]
- 500 clusters of adjacent hits along scan direction (i.e. the same crystal hit more than once).
- 150 series of frames integrated with XDS
- 97 partial data sets were merged with XSCALE

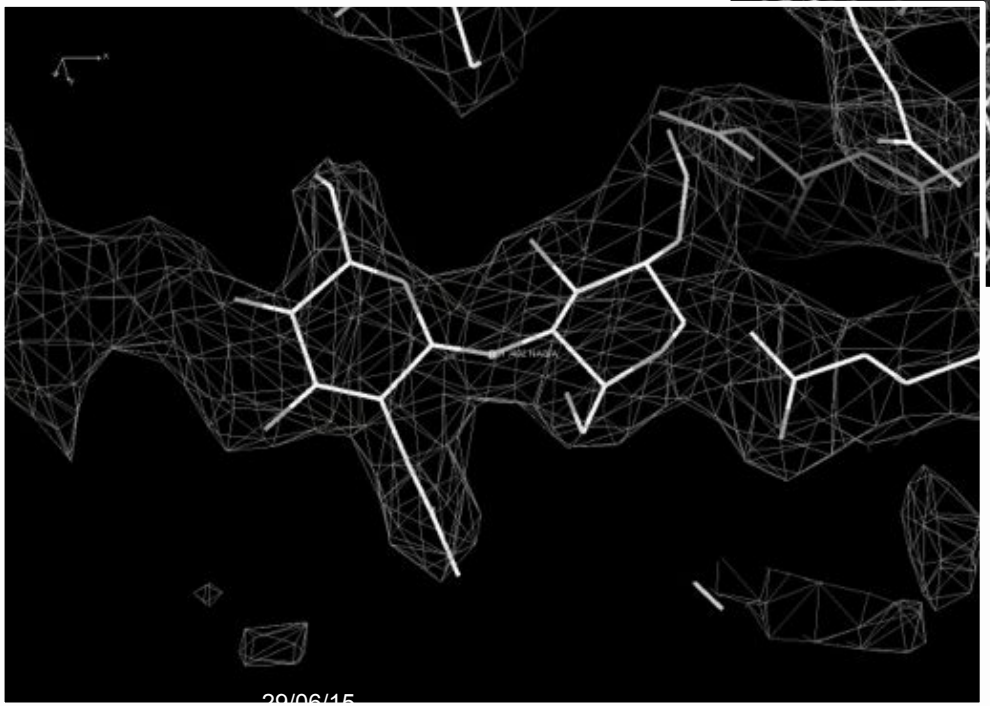
- ~ 3 weeks for processing



Cathepsin B – Refinement

- REFMAC5. 2442 atoms (jelly body) against 8394 reflections [87.4, 3.0] Å.
- $R_{work,free}$ = 22.9, 26.1





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Serial crystallography on *in vivo* grown microcrystals using synchrotron radiation

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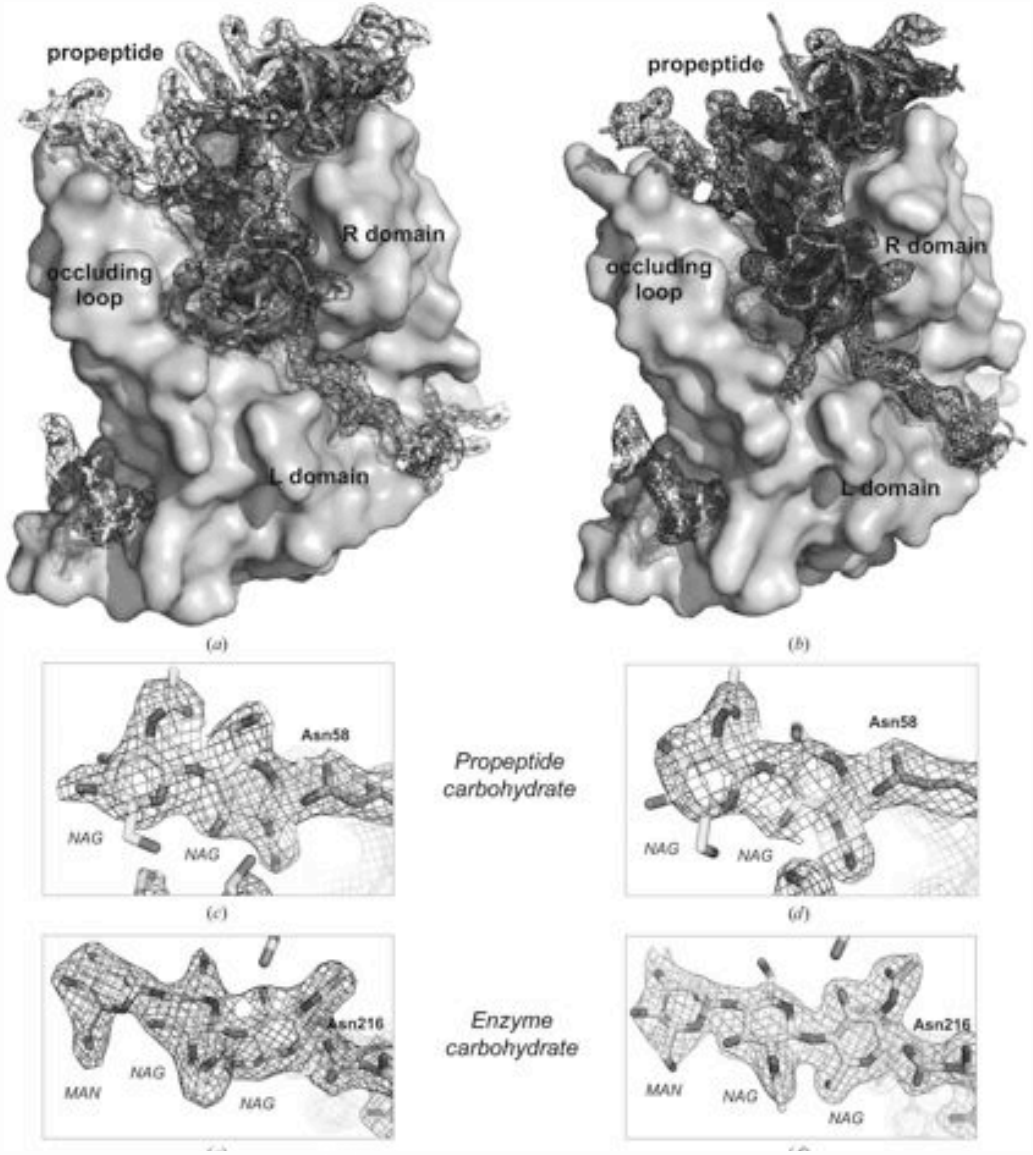
PETRA III XFEL



PETRA III	XFEL
Crystal Size	
10^7 unit cells [10 x less than smallest crystals used at synchrotrons before]	
Resolution	
3 Å	2.1 Å
Material used	
15 nl	10 ml
Result	
Models are identical within error	

Gati, Bourenkov, … Schneider, Redecke, Chapman (2014) IUCrJ 1:87

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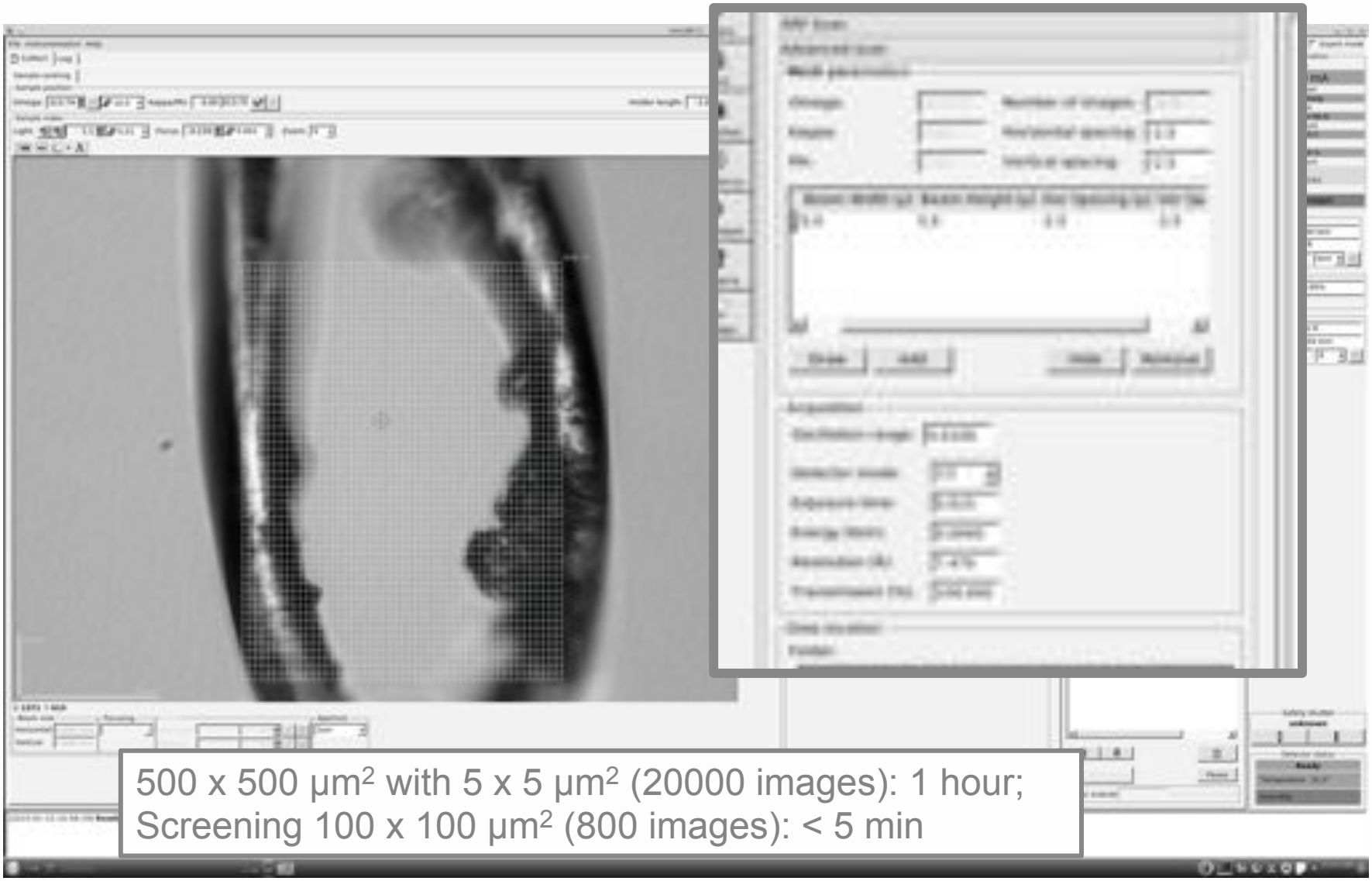
Implementation



500 x 500 µm^2 with 5 x 5 µm^2 (20000 images): 1 hour;
Screening 100 x 100 µm^2 (800 images): < 5 min

‘MxCuBE- collaboration with ESRF

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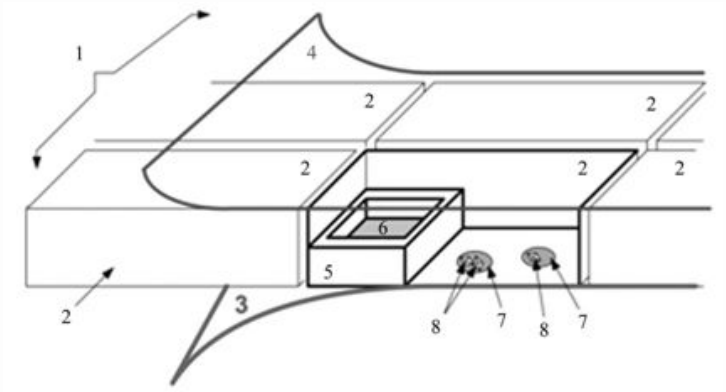
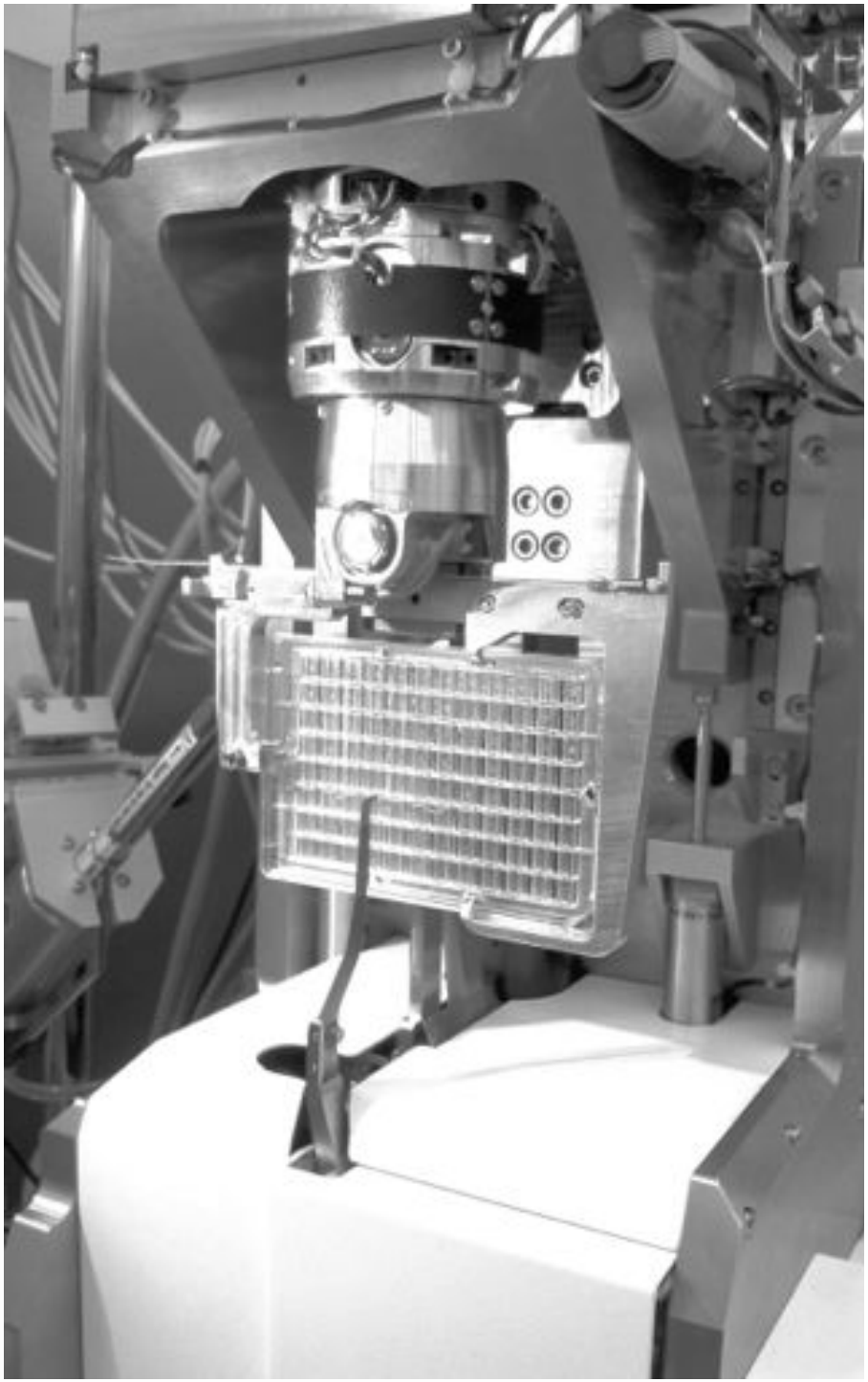
In Situ Experiments with CrystalDirect

- Mechanics and software are in place for data collection from CrystalDirect™ plates.
- Crystals grow on a thin polymer foil with negligible X-ray scatter
- Cryst. life-time in focused beam < 10 ms

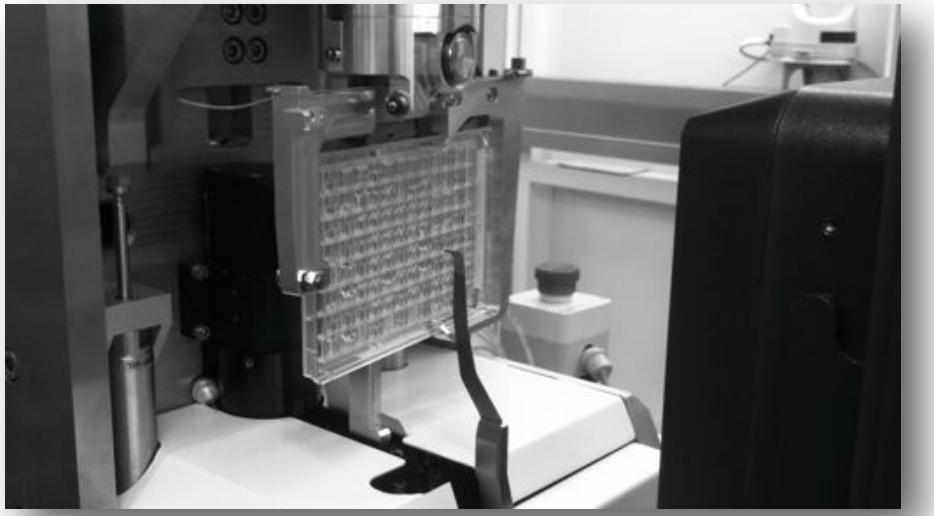


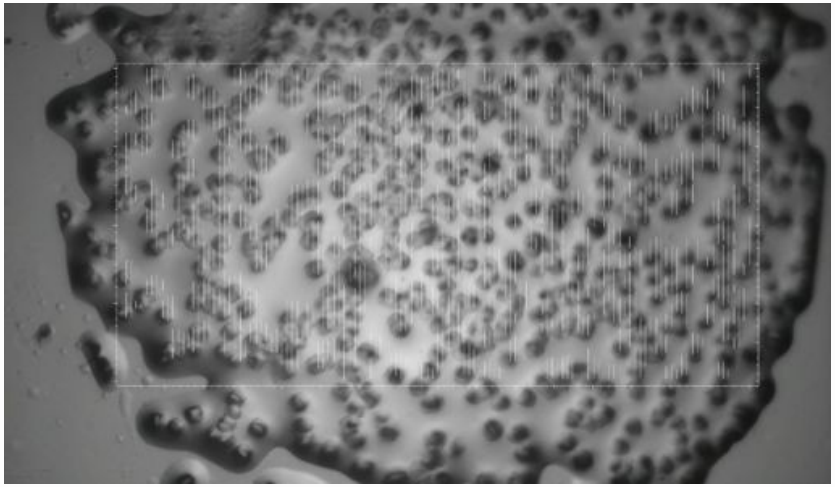
Cipriani, Marquez et al. (2012) 'CrystalDirect: a new method for automated crystal harvesting based on laser-induced photoablation of thin films.' Acta Cryst D68:1393.

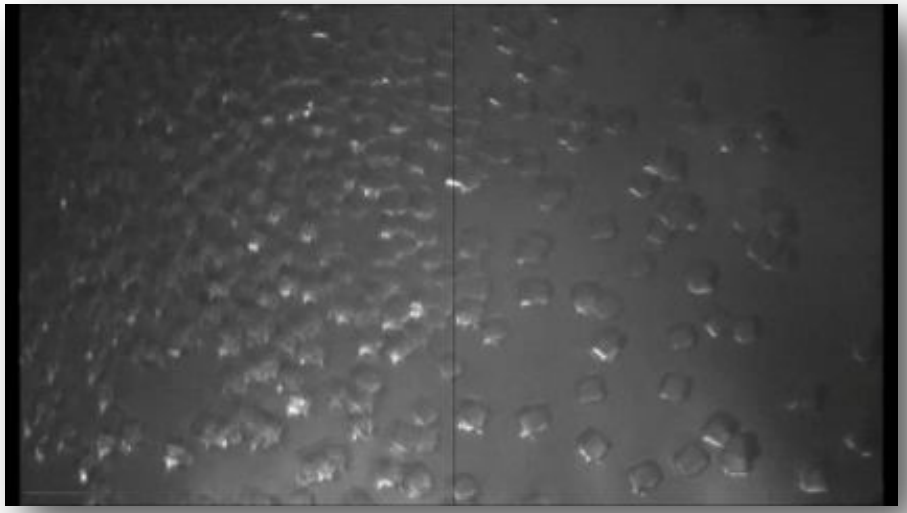


In situ data collection

- Continuous serial helical scans with a micro-beam on CrystalDirect plates



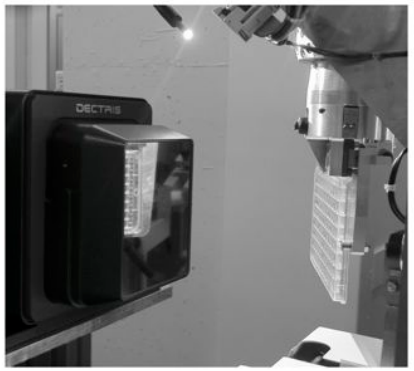




In situ serial data collection at room temperature

Detectors?

- PILATUS3 can run at 500 Hz.
- Time required for read-out is ca. 1 ms
- In continuous scan mode, the detector is ‘blind’ for 50% of the time



- EIGER 4M can run at 750 Hz
- Time required for read-out is ca. 3 µsec
- ‘blind’ for < 1% of the time

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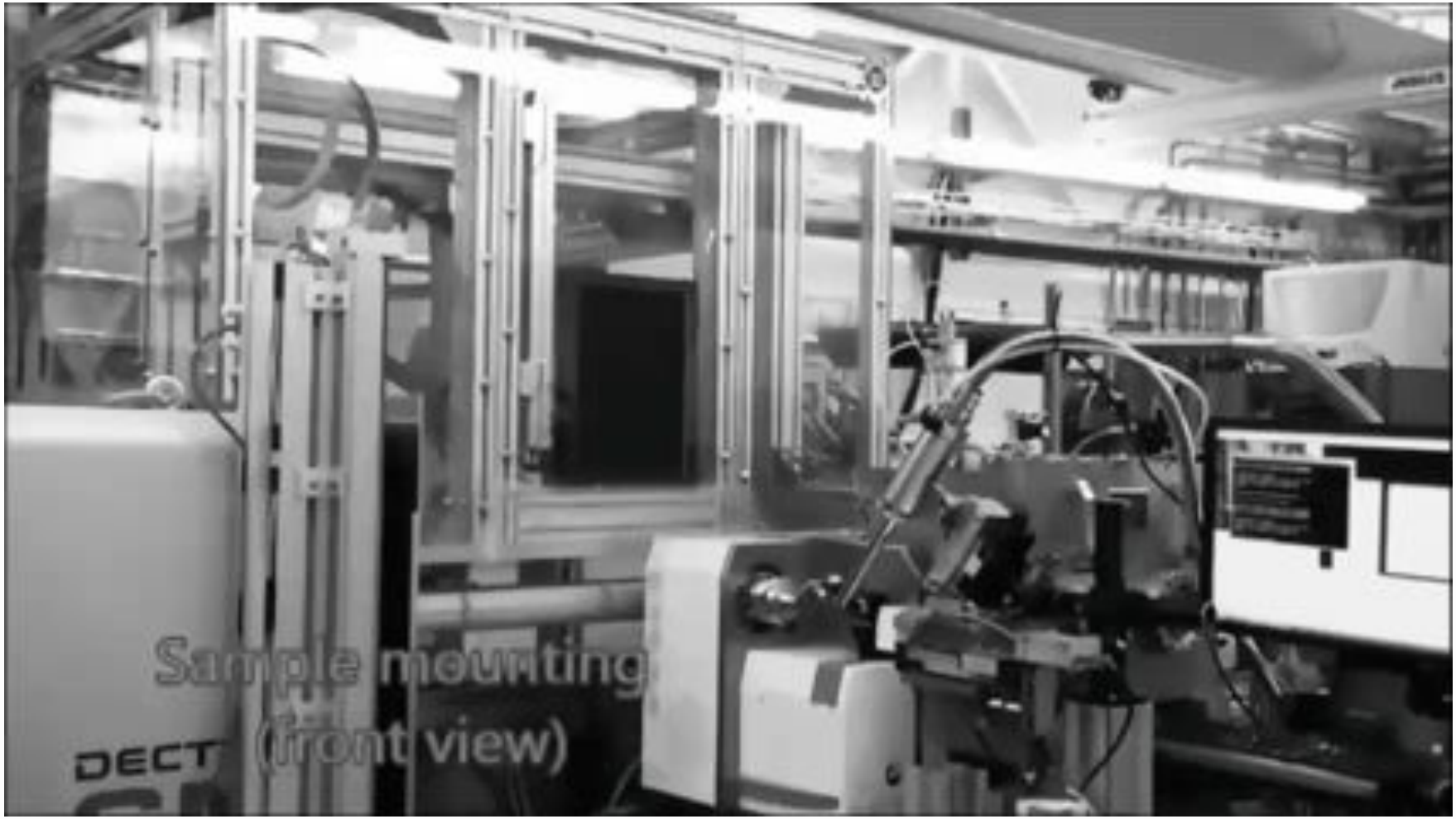
Robotics ...



No operator error / remote operation / no hot bodies

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Business ...

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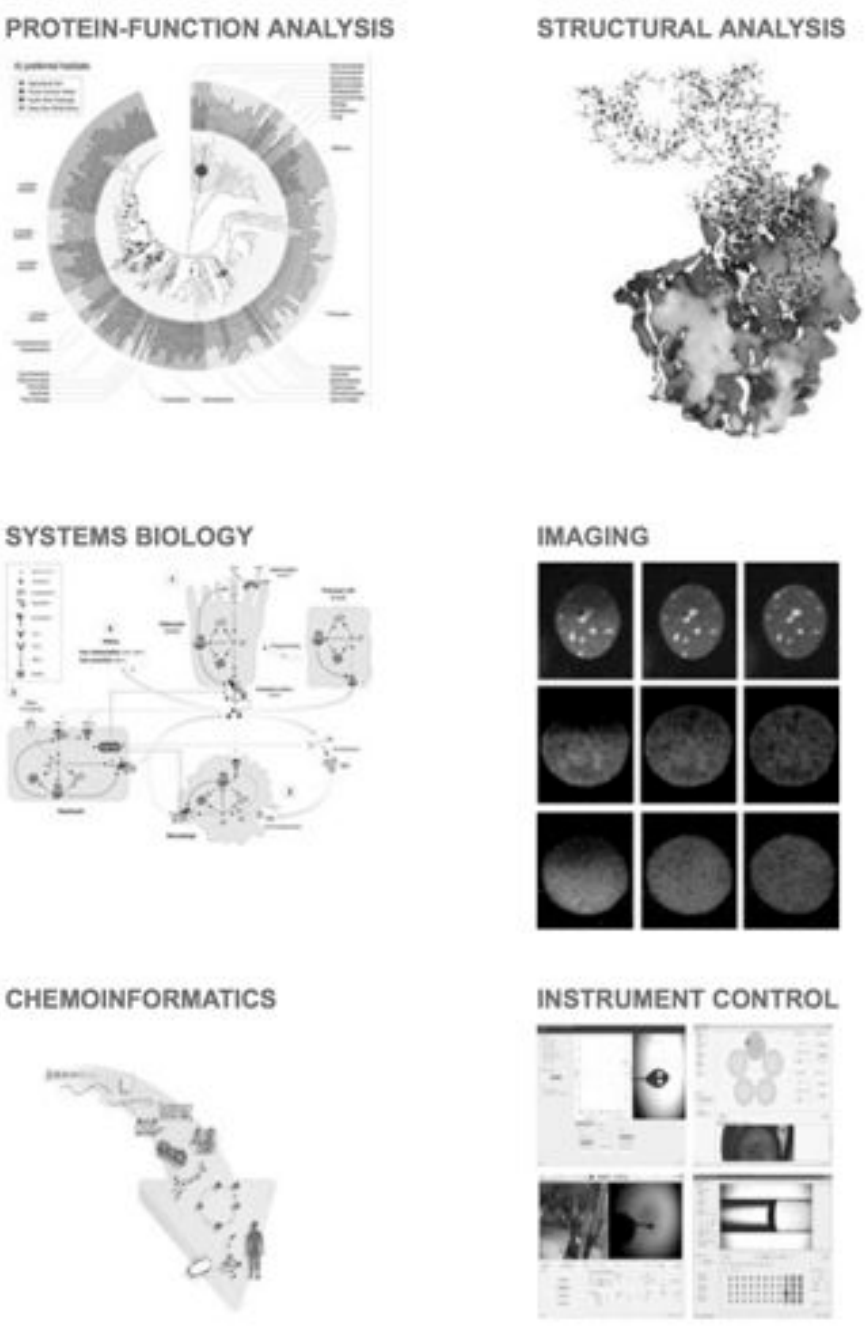
The goals of EMBLEM's technology transfer activities are to:

- promote the commercial use of research results and innovations;
- foster exchange between EMBL research and industry;
- return value to stakeholders including society at large;
- support EMBL spin-off companies working in the life sciences;
- increase the attractiveness of EMBL for top researchers by providing professional technology transfer service;
- create high-tech jobs.

Software downloads

- Licensing for different scenarios
  - Academic
  - For profit



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EMBL-EM: Portfolio companies

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GENE BRIDGES
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ELARA pharmaceuticals

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Summary

- Cutting edge instrumentation is crucial for pushing the boundaries of Structural Biology (object size and imaging resolution).
- Whatever probe is useful will be used (light, e-, n, ...).
- Technology development, technology provision (as service), technology dissemination (commercial) for research in the member states.
- Biologists, biochemists, chemists, physicists, computer scientists, engineers, lawyers, and others working together makes the difference.
- Transition from *experiment* to *measurement* needs to be made.
- Software becomes ever more important (download, web-services).
- Centralized user facilities / ‘infrastructures’ are a (the only possible?) way to deal with technical complexity and cost.