Microfocus Beams and Crystallography

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ESRF
ERL-NANOBIO

6 GeV synchrotron radiation source
4 nmRad emittance
18 European partners
36 public; 12 national beamlines

Microdiffraction techniques

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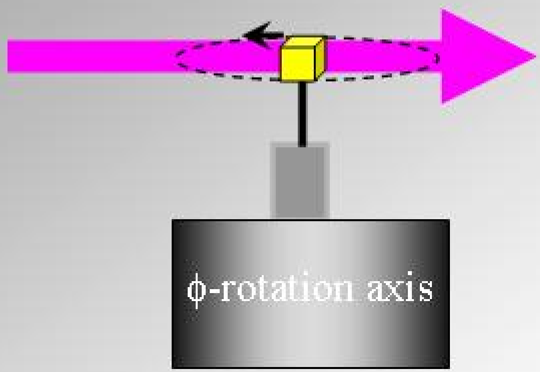
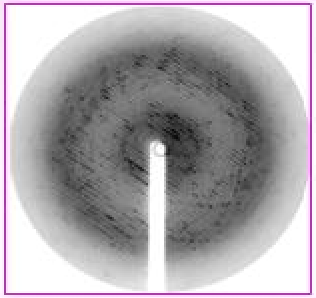
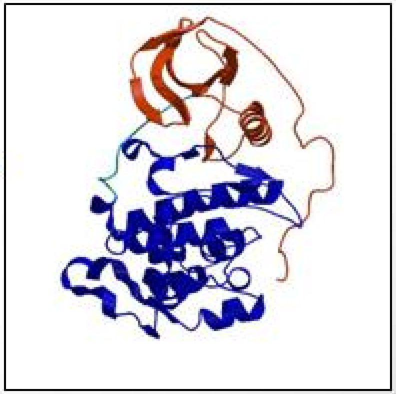
single crystal diffraction



ϕ-rotation

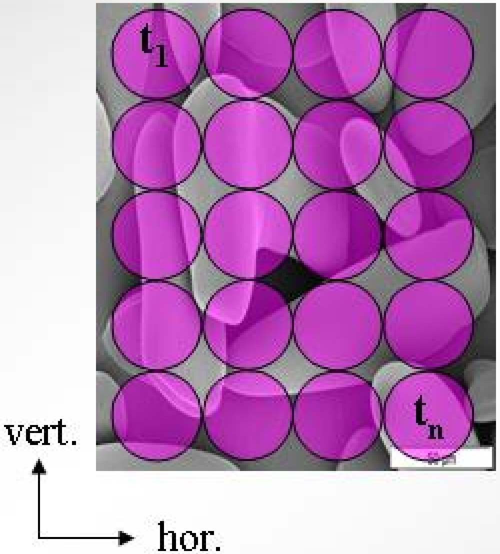


n detector-frames: $\phi_i \ldots \phi_n$



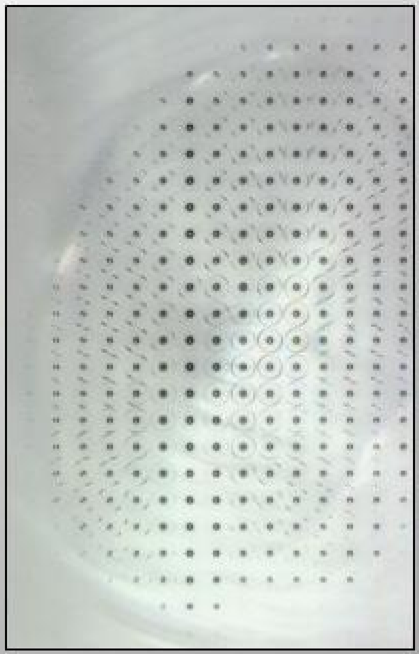
crystal structure

SAXS/WAXS microscopy



mesh-scan

n detector frames: $t_i \ldots t_n$



Irradiated single crystal volumes overview

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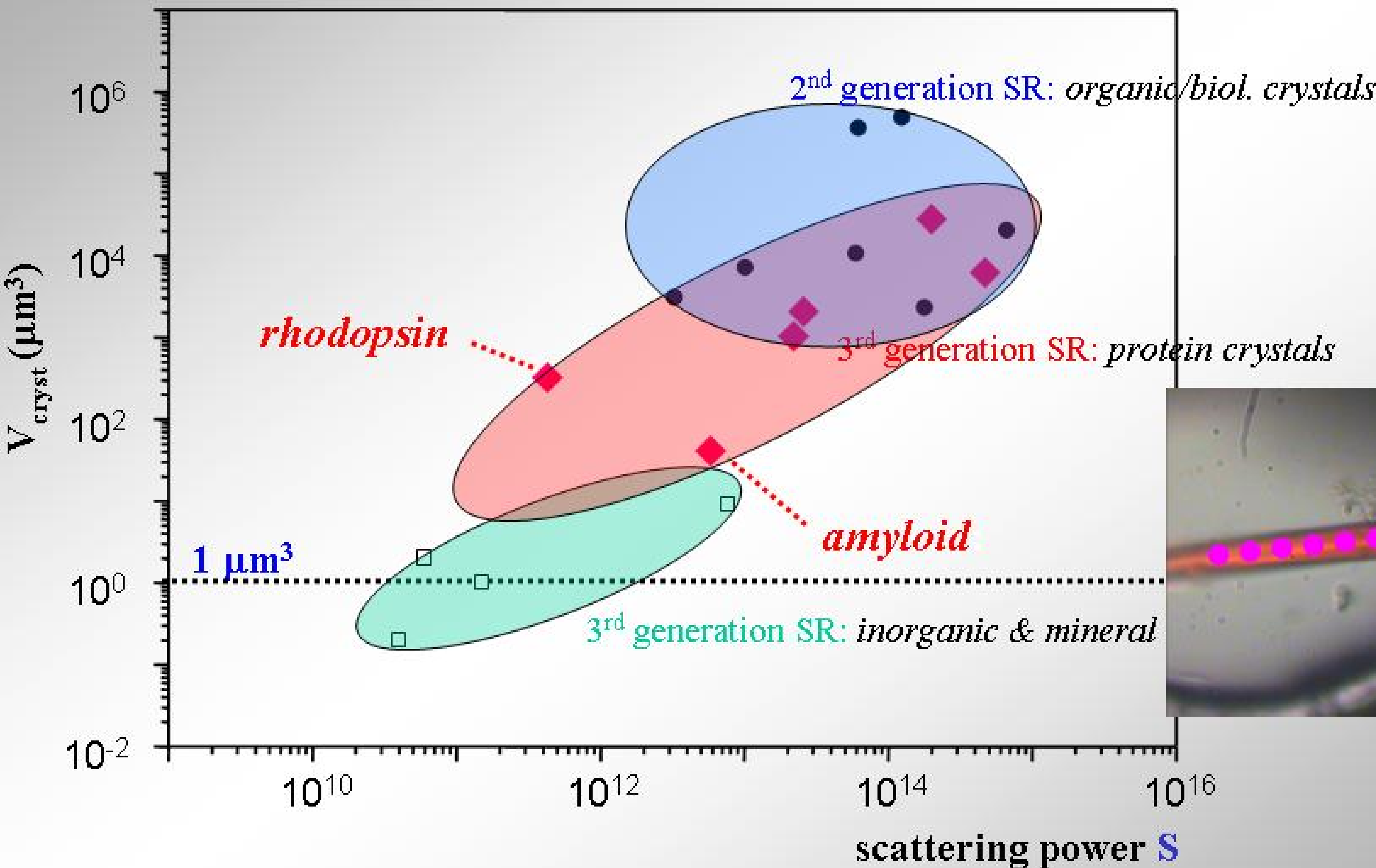
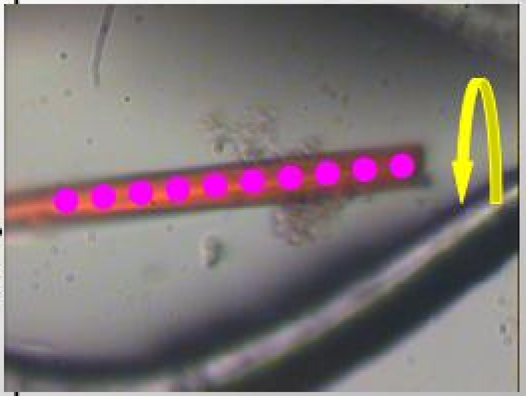
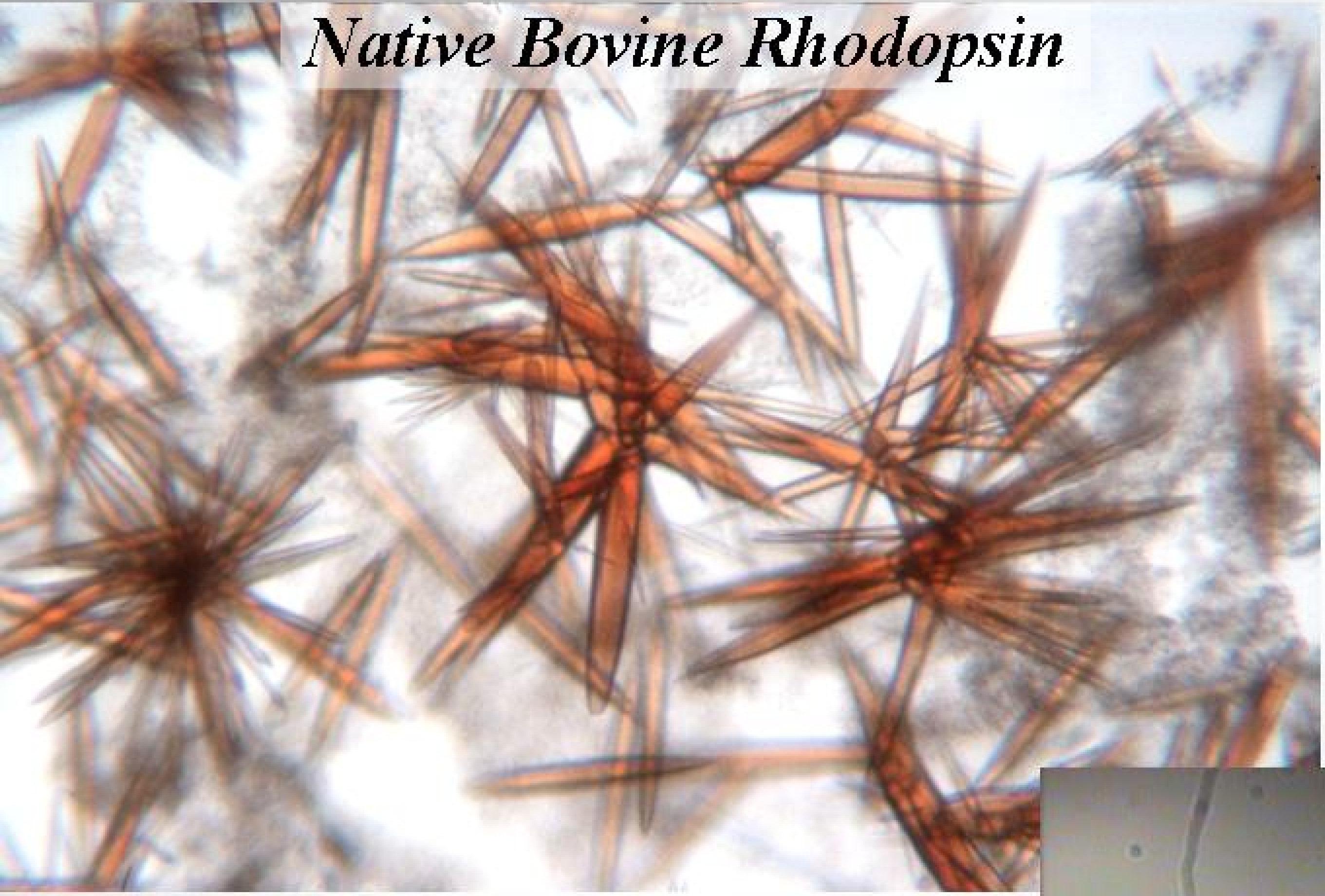




$$S = (F_{000}/V_{cell})^{2} * \lambda^{3} * V_{cryst}$$

Native Bovine Rhodopsin

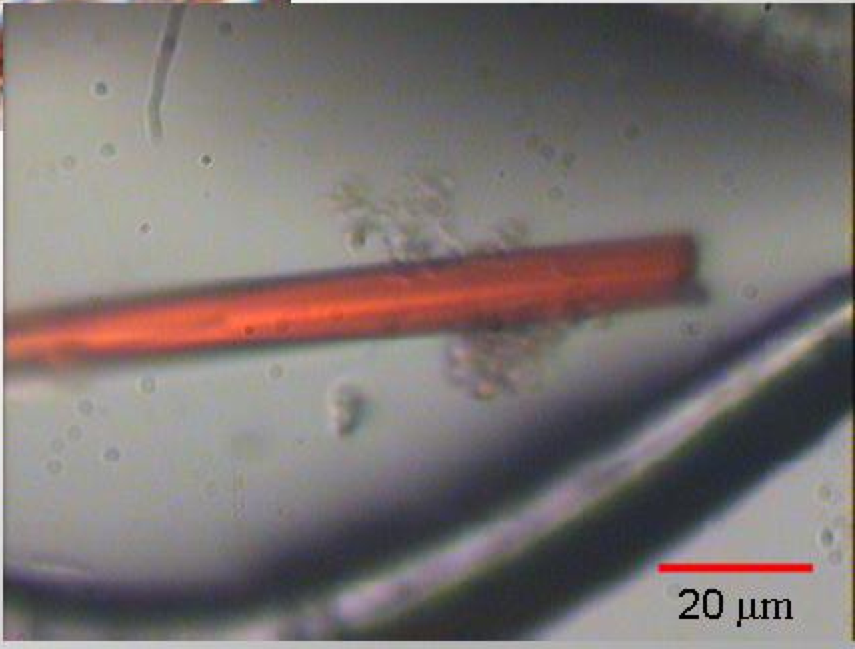


light sensitive protein

$P3_1$ crystal form

a=10.38, c=7.66 nm

0.265 nm resolution



Li et al., *J. Mol. Biol.* (2004) **343**, 1409

Electron micrograph and electron diffraction pattern

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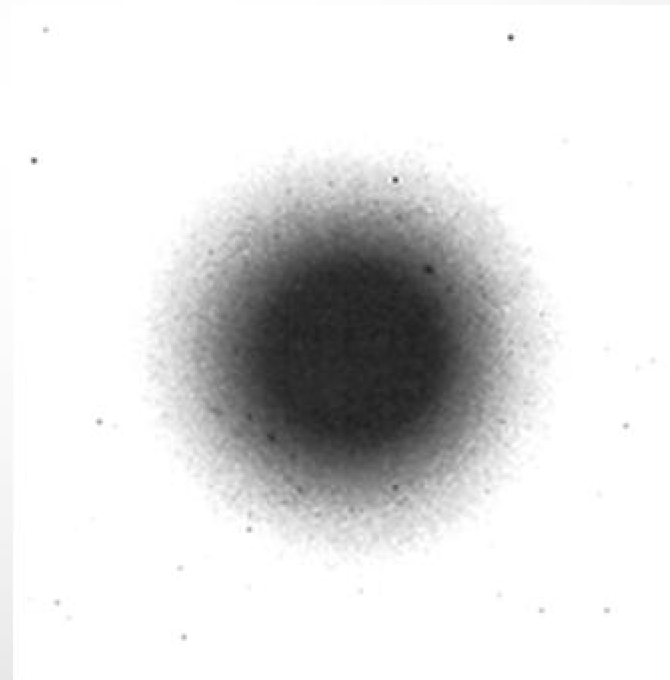

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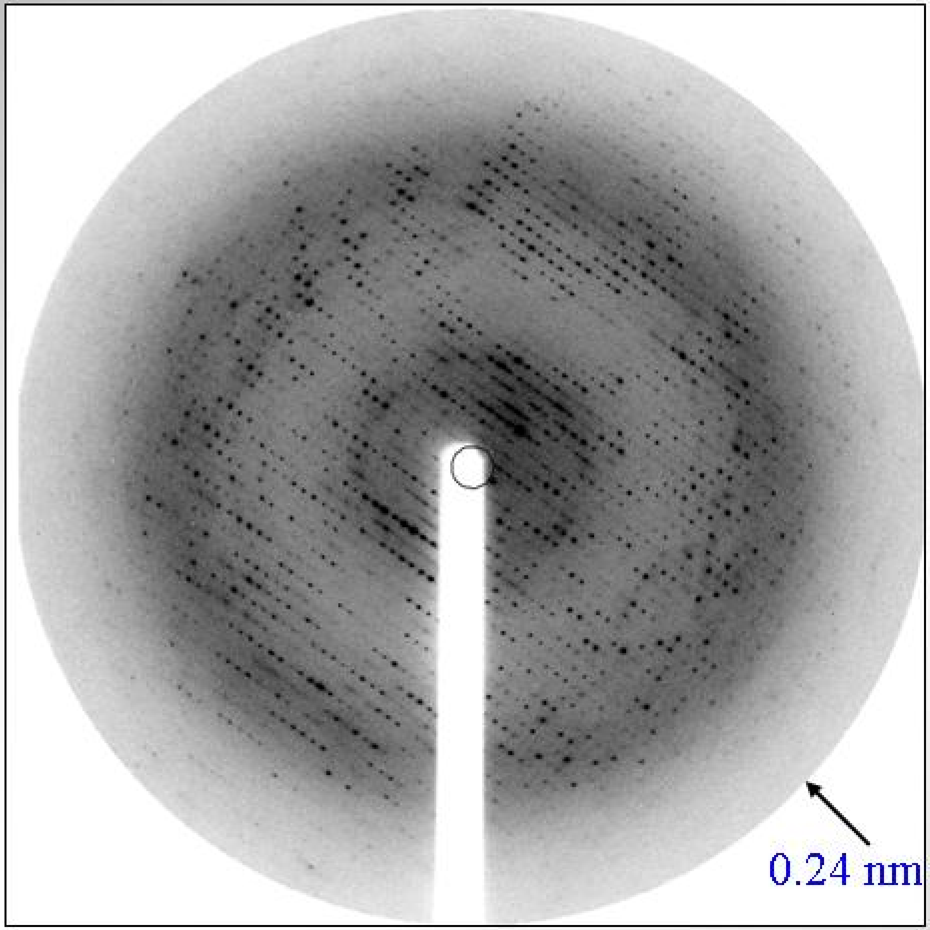

* 5 micron beam
* only about one rhodopsin crystal in 30 diffracts to 0.3 nm
* systematic search for optimum crystallization conditions required
* radiation damage severe, often only one shot/crystal

P3(1) crystal packing

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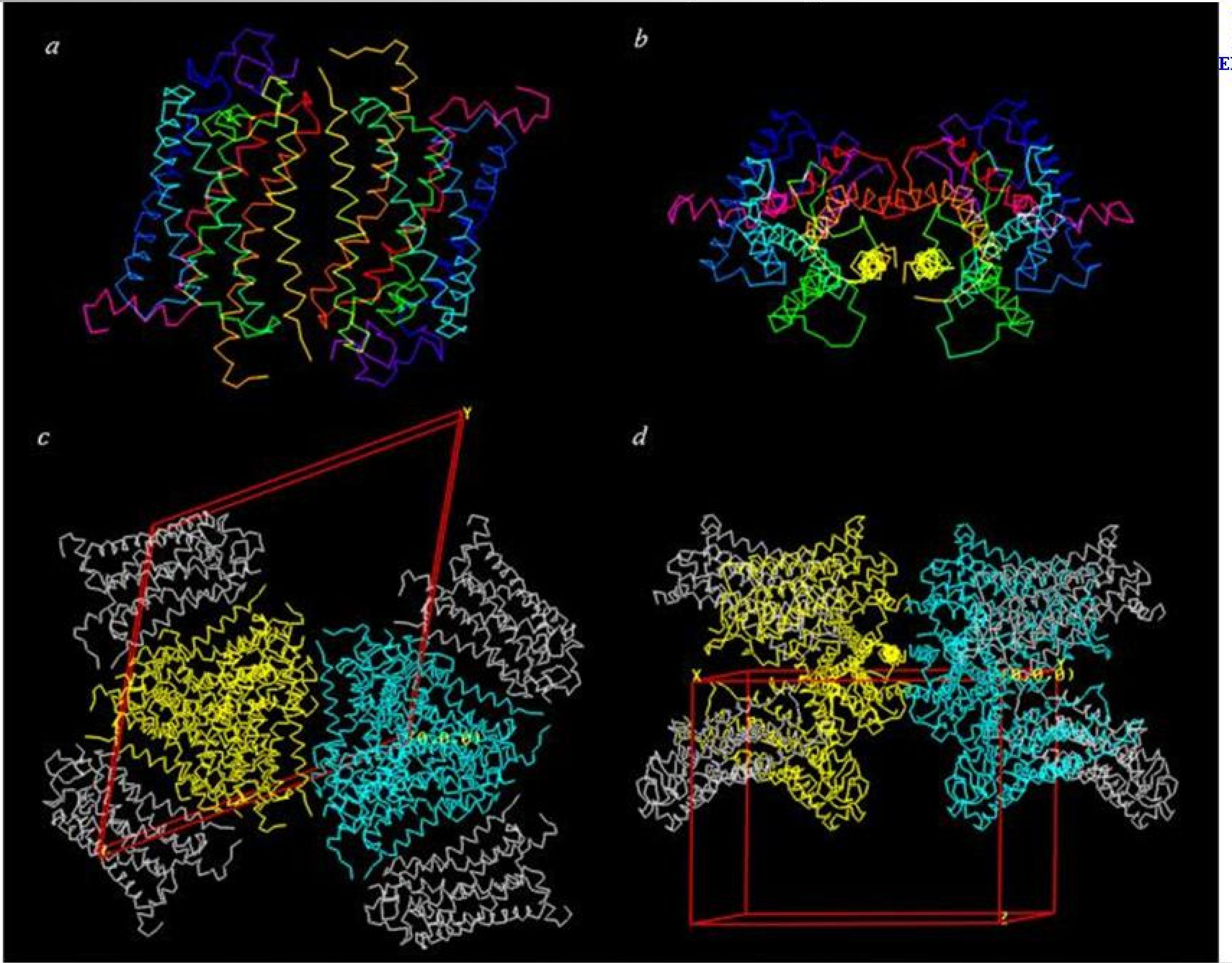

Amyloidic microfibrils from yeast protein

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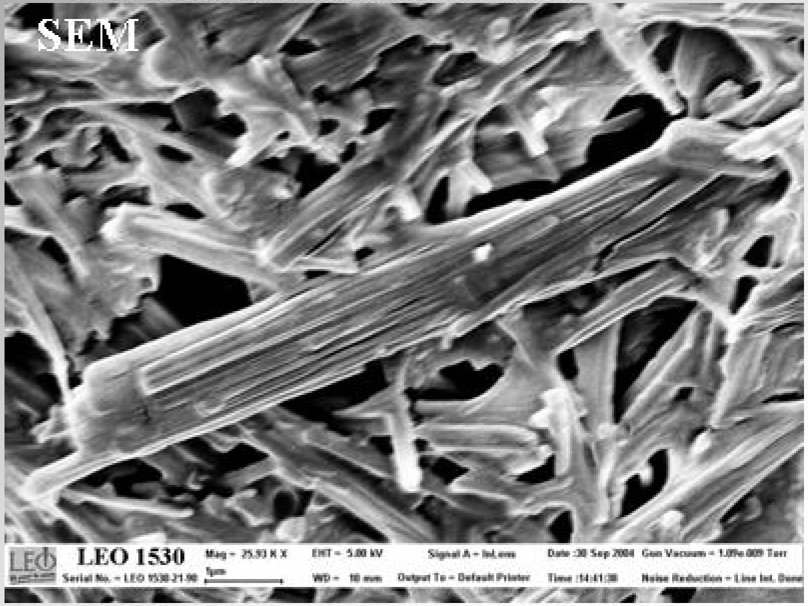
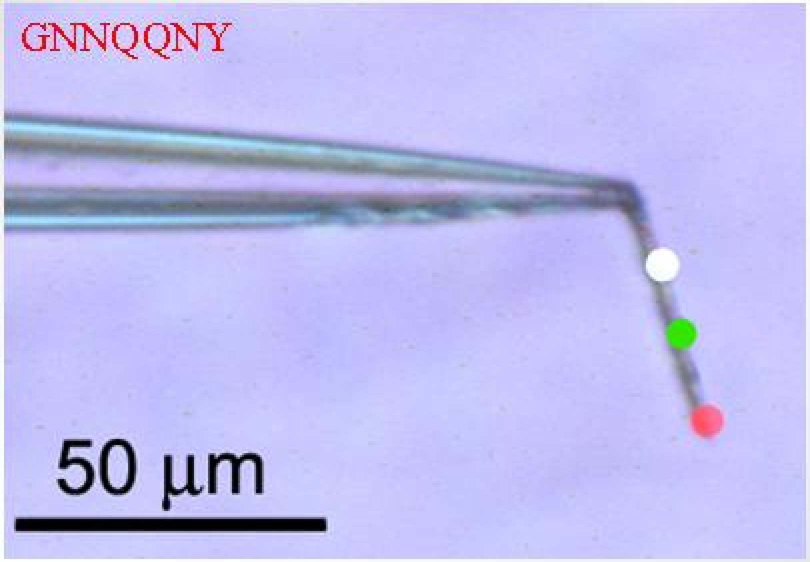
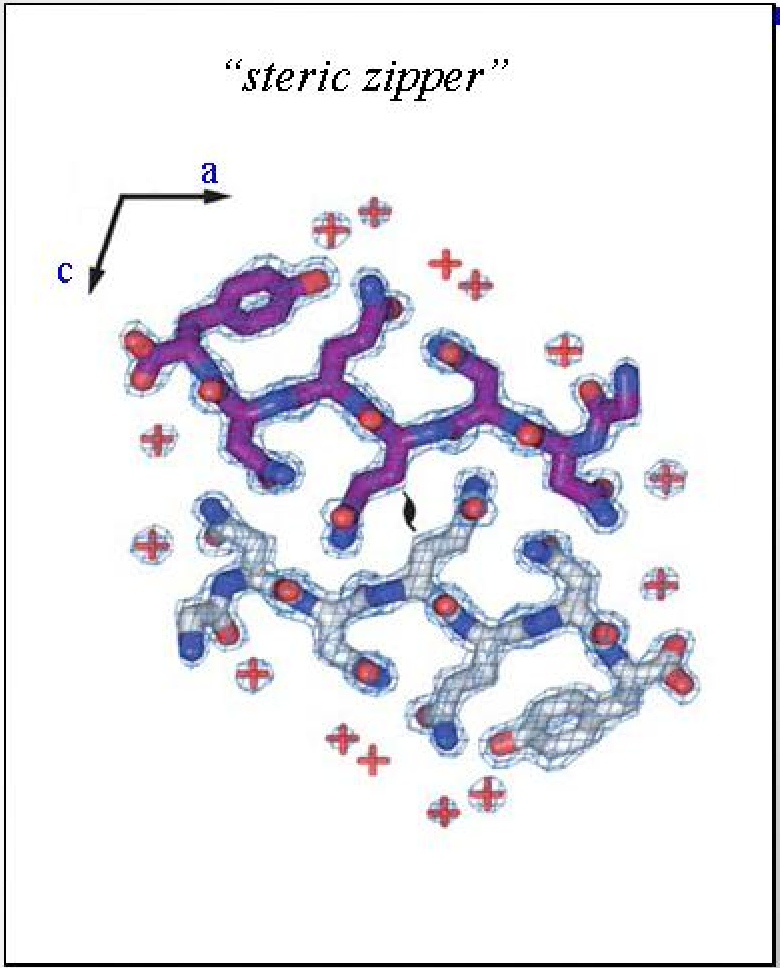
aminoacid peptidyl fragments







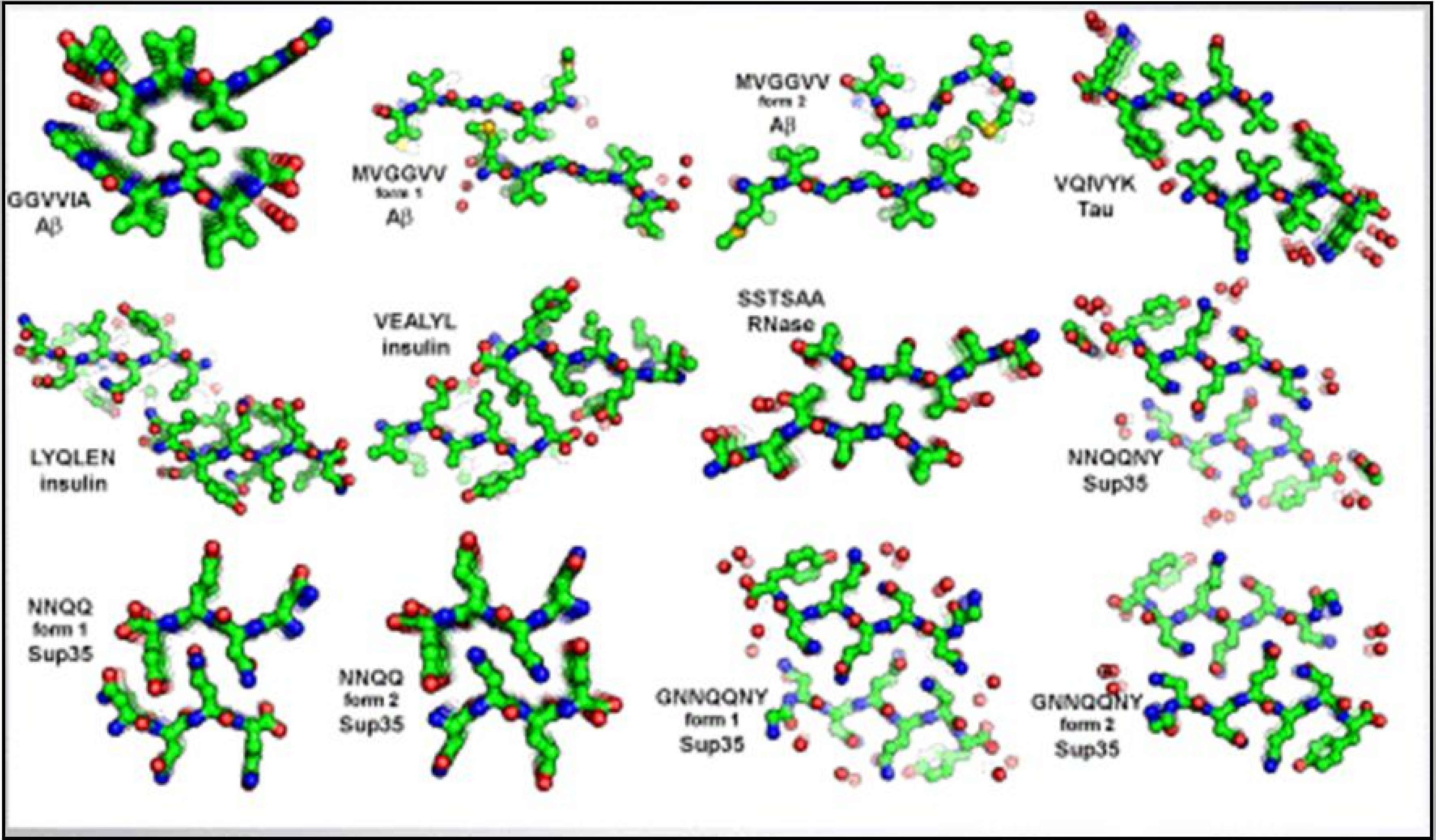
$P2_1$

a=2.194, b= 0.487, c=2.348 nm, β=107.08

0.18 nm resolution

Nelson et al., *Nature* (2005) **435**, 773

Overview on refined amyloid structures



Courtesy: M. Sawaya; Eisenbergs Lab-UCLA

Optics limitations

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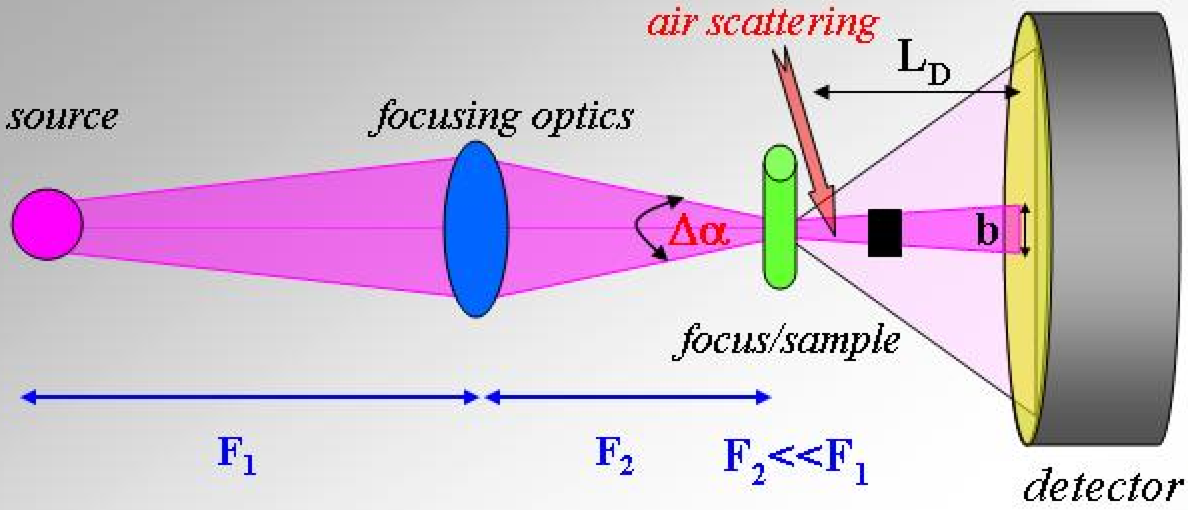

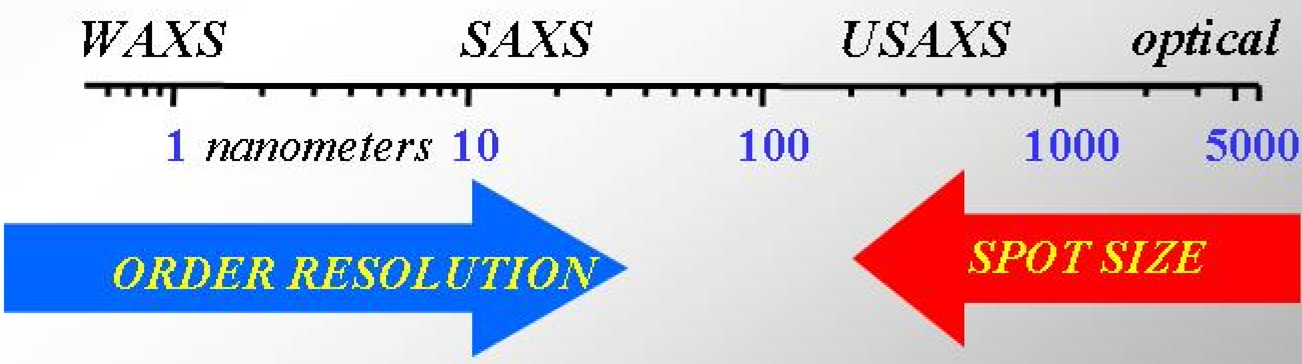

X-ray interference in single muscle fibers

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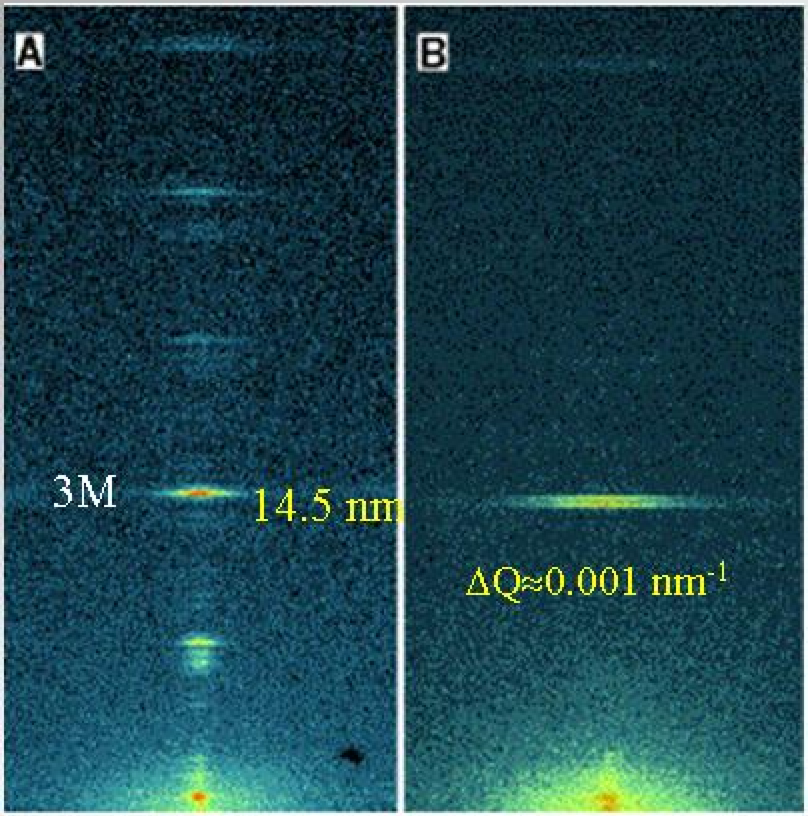
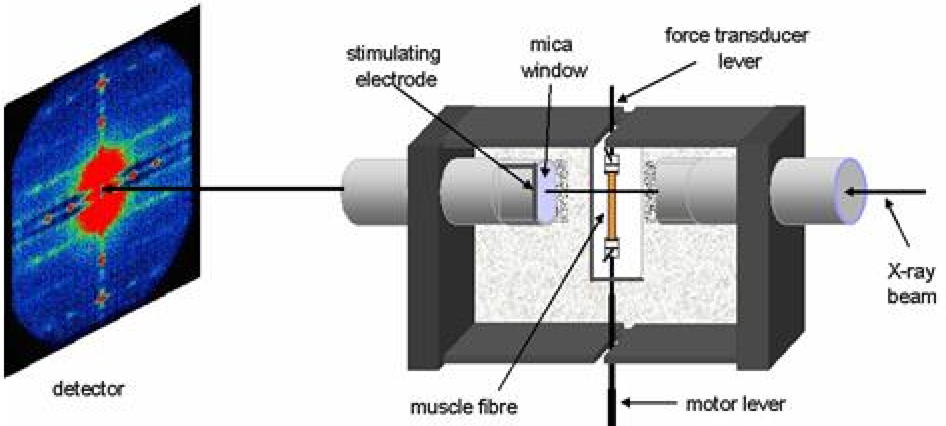
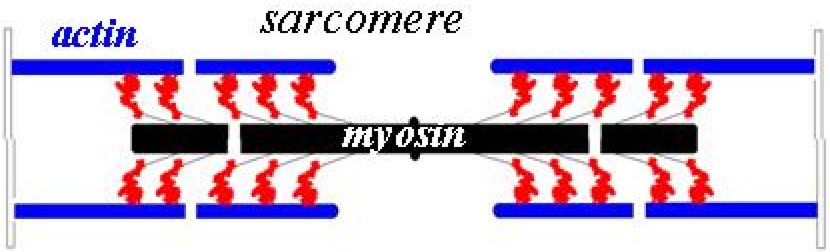




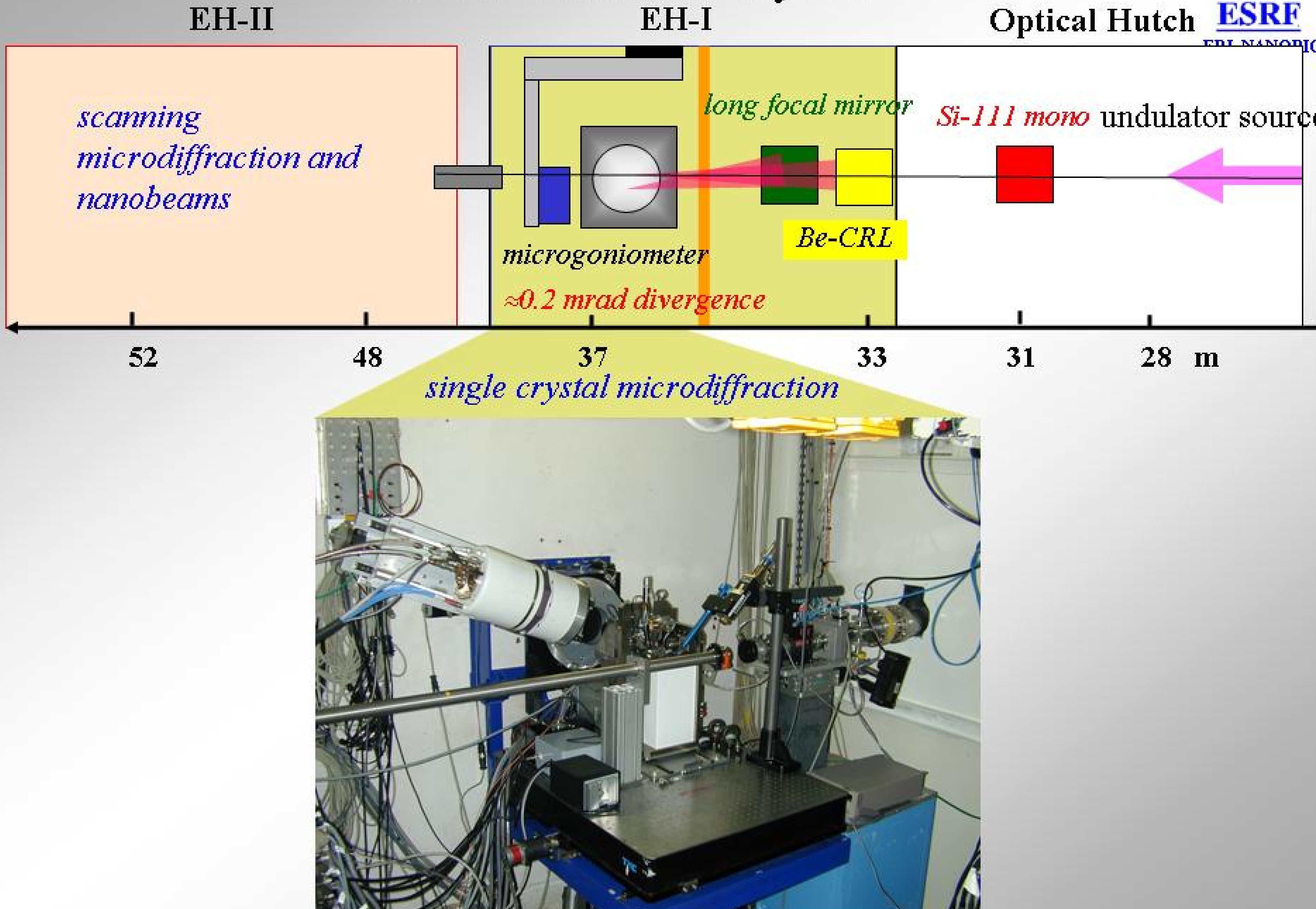
Piazzesi *et al.* *Nature* (2002) **415**, 659

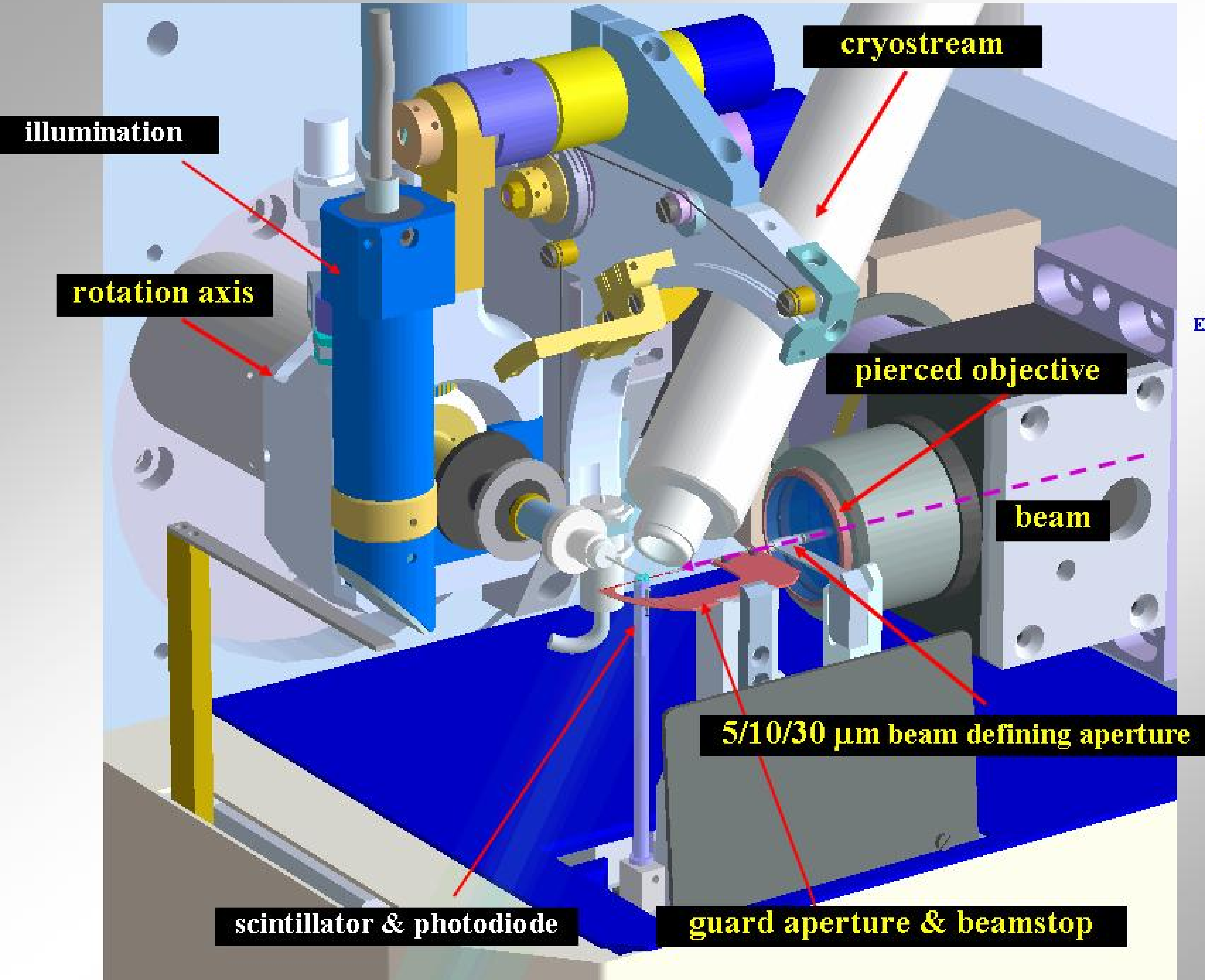


bipolar arrangement of *myosin heads* on either side of sarcomere leads to interference in 3M reflection

ID02: 10 μrad vertical divergence

ID13 beamline layout





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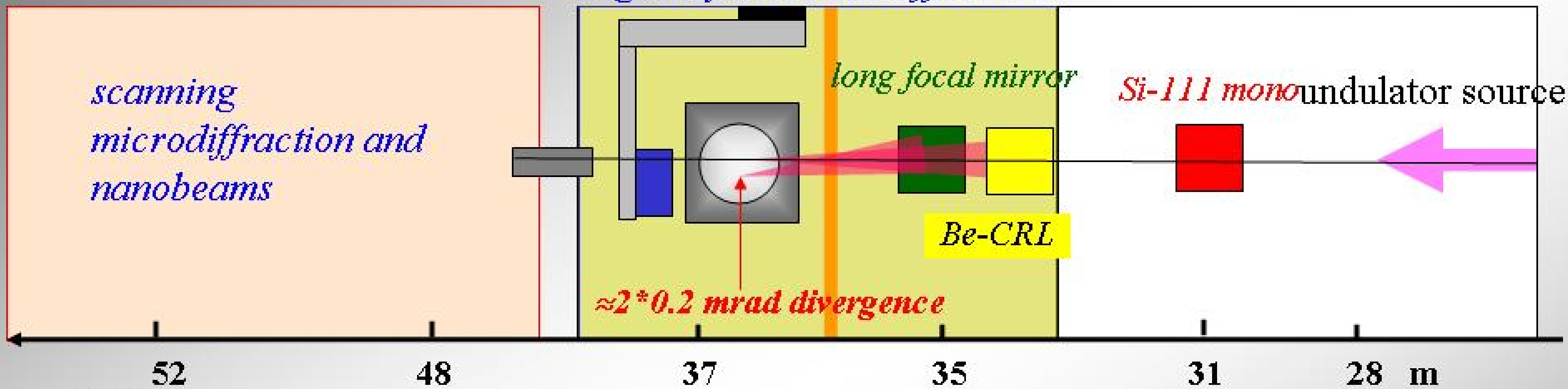
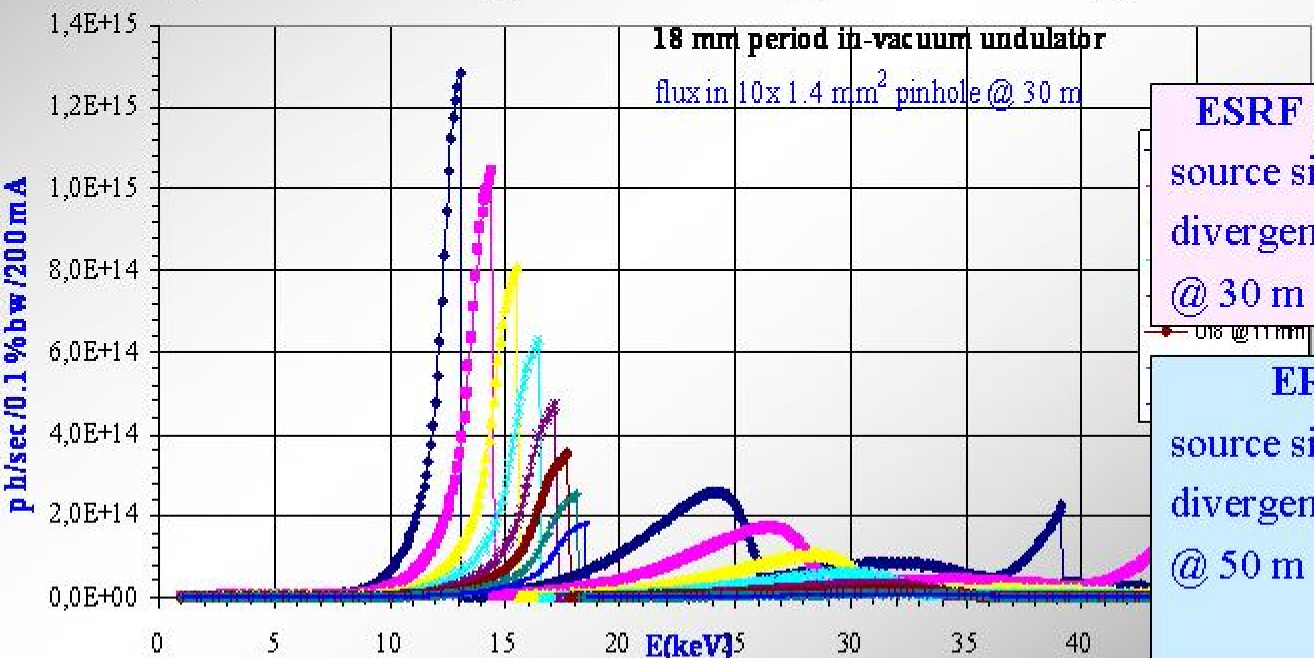
ESRF ID13 beamline: layout

EH-II EH-I Optical Hutch

single crystal microdiffraction





ESRF (13 keV) 1.2 m low-β undulator

source size	$134_h * 25_v$	μm^2
divergence	$0.2_h * 0.02_v$	$mrad^2$
@ 30 m	$6.2_h * 0.6_v$	mm^2

ERL (15 keV) 5 m undulator

source size	$12.2_h * 12.2_v$	μm^2
divergence	$0.014_h * 0.014_v$	$mrad^2$
@ 50 m	$0.5_h * 0.5_v$	mm^2

fwhm values!

Comparison of 5 μm beam optics

ESRF ERL NANOBIO

ESRF-ID13: 5 μm beam (13 keV/Si-111)
1:10 focus + collimator

divergence	≈ 200_h*200_v	μrad²
flux	≈ 10^{11}	ph/s

ESRF-ID13: 5 μm beam (13 keV/Si-111)
1:2.3 focus + collimator

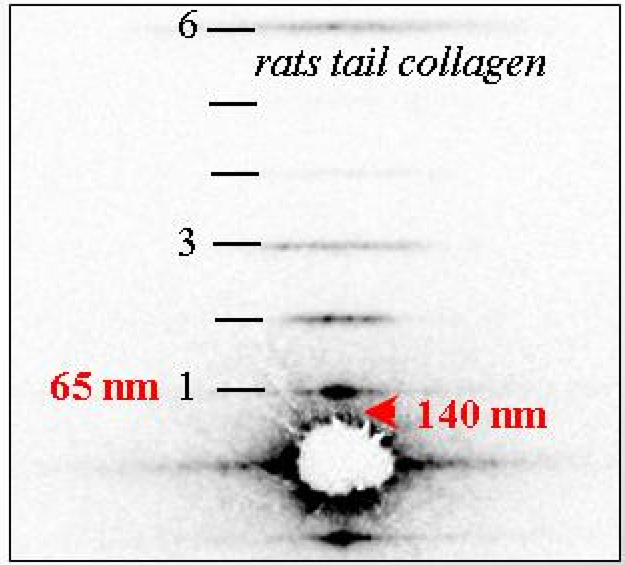
divergence	≈ 45	μrad²
flux	≈ $5*10^9$	ph/s

ESRF-ID02: 5 μm beam (13 keV/Si-111)
1:1 focus + collimator

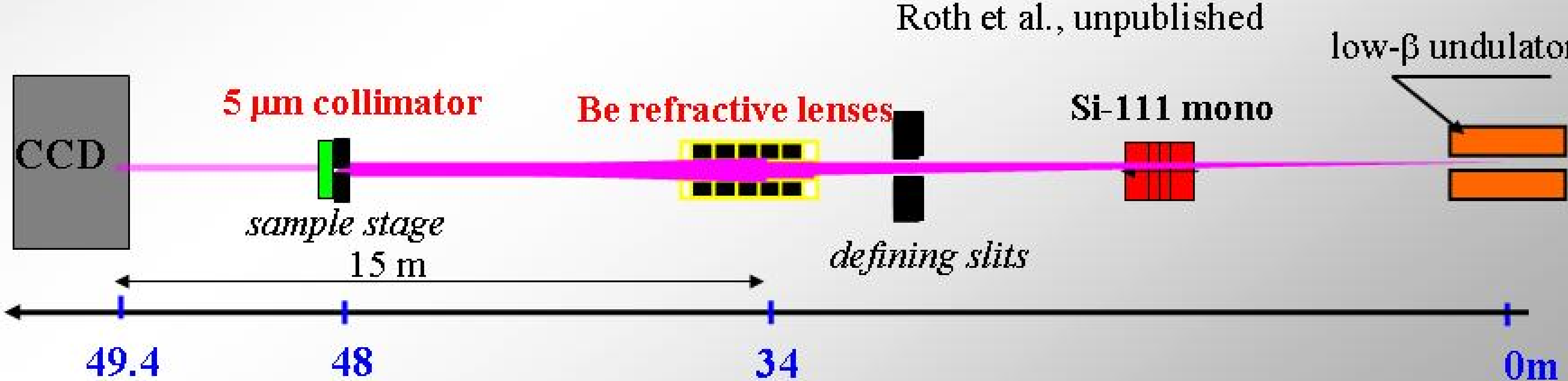
divergence	≈ 38_h*15_v	μrad²
flux	≈ $5*10^9$	ph/s

ERL 5 μm beam (15.1 keV/Si-220)
unfocused + collimator

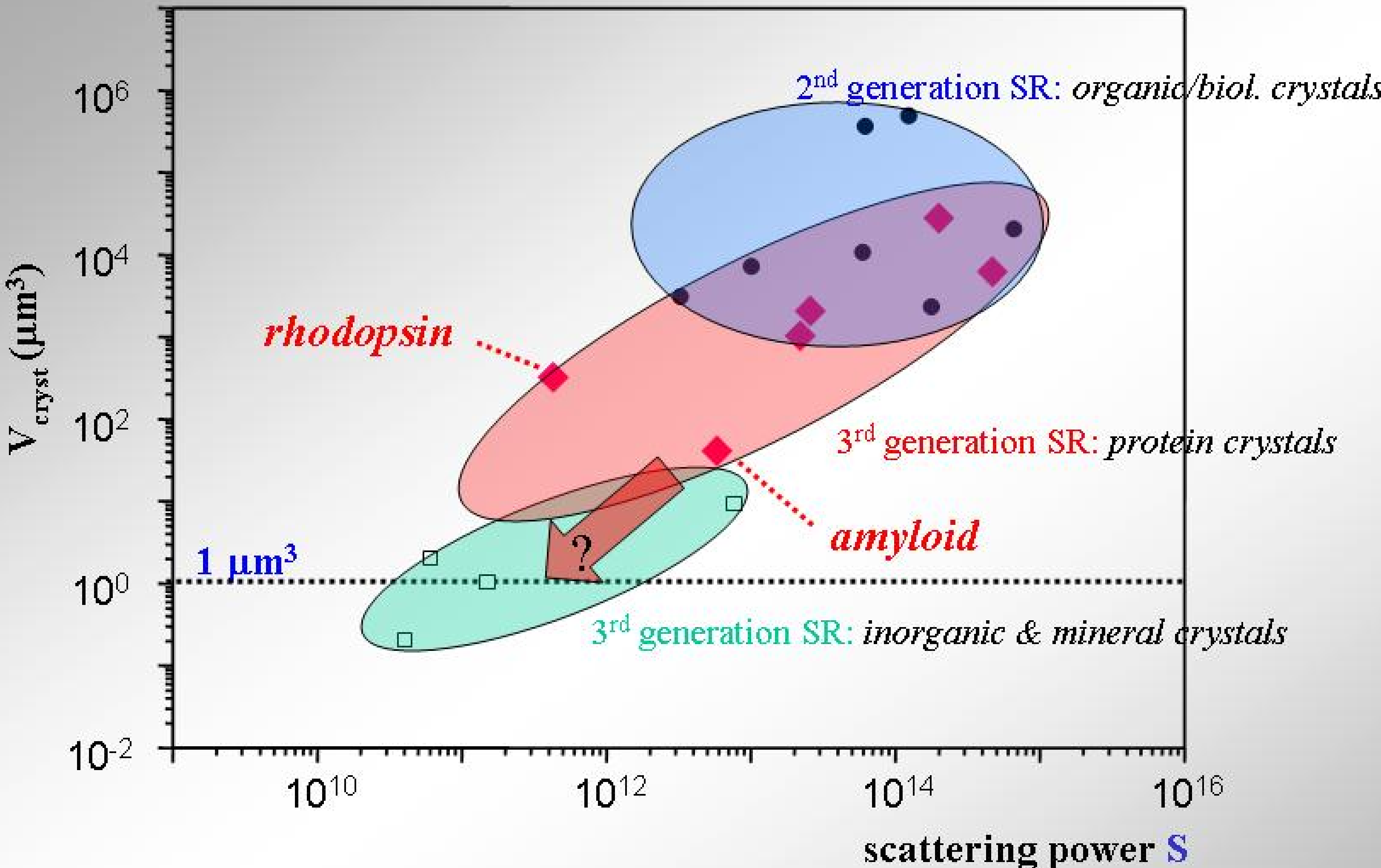
divergence	$17.6_h*14.1_v$	μrad²
flux	≈ $2.5*10^{12}$	ph/s



Roth et al., unpublished



Reduction of irradiated crystal volume



$$S = (F_{000}/V_{cell})^2 * \lambda^3 * V_{cryst}$$

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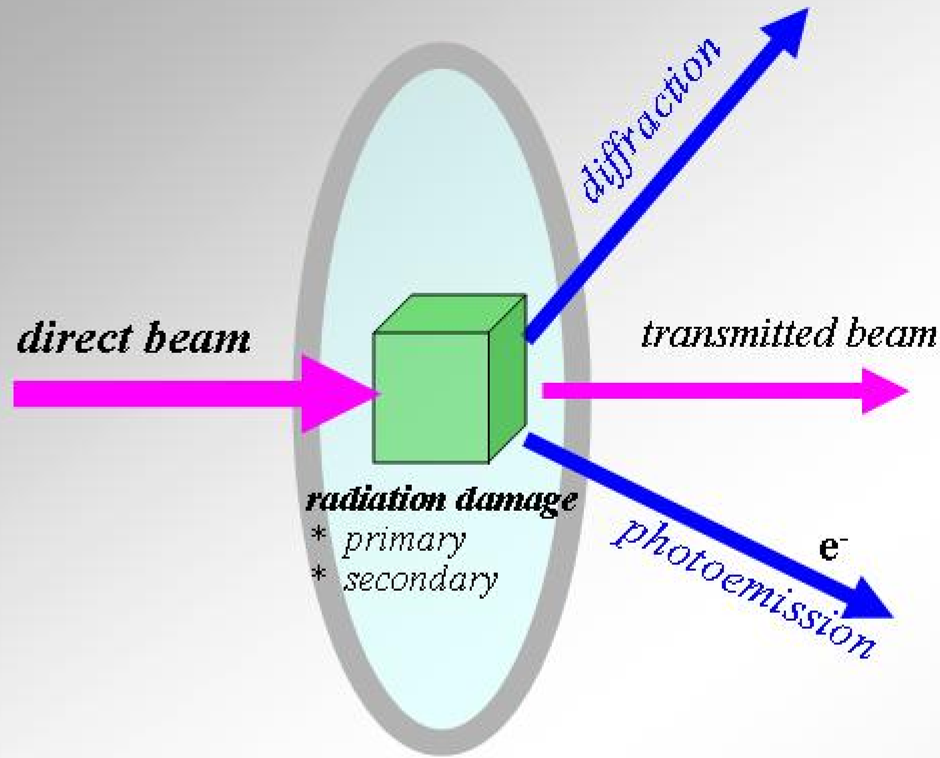
New approaches to be explored

- * *observation, manipulation and characterization of microocrystals*
- * *generation of micron- and submicron, low-divergent beams*
- * *sample environment and goniometer*
- * *detectors*



maximize diffraction, minimize radiation damage

* *sample dimensions should be small enough* (a few μm) so that photoelectron can escape

* *minimize sample support* and surrounding material

* *avoid Compton scattering* (E> 30 keV); 20 keV allows somewhat larger escape depth than 13 keV

Nave & Hill *JSR* (2005) **12**, 299

* *reduce measuring and frame transfer time*: optimize flux density and fast detector

Manipulation of subμm^3 inorganic crystals

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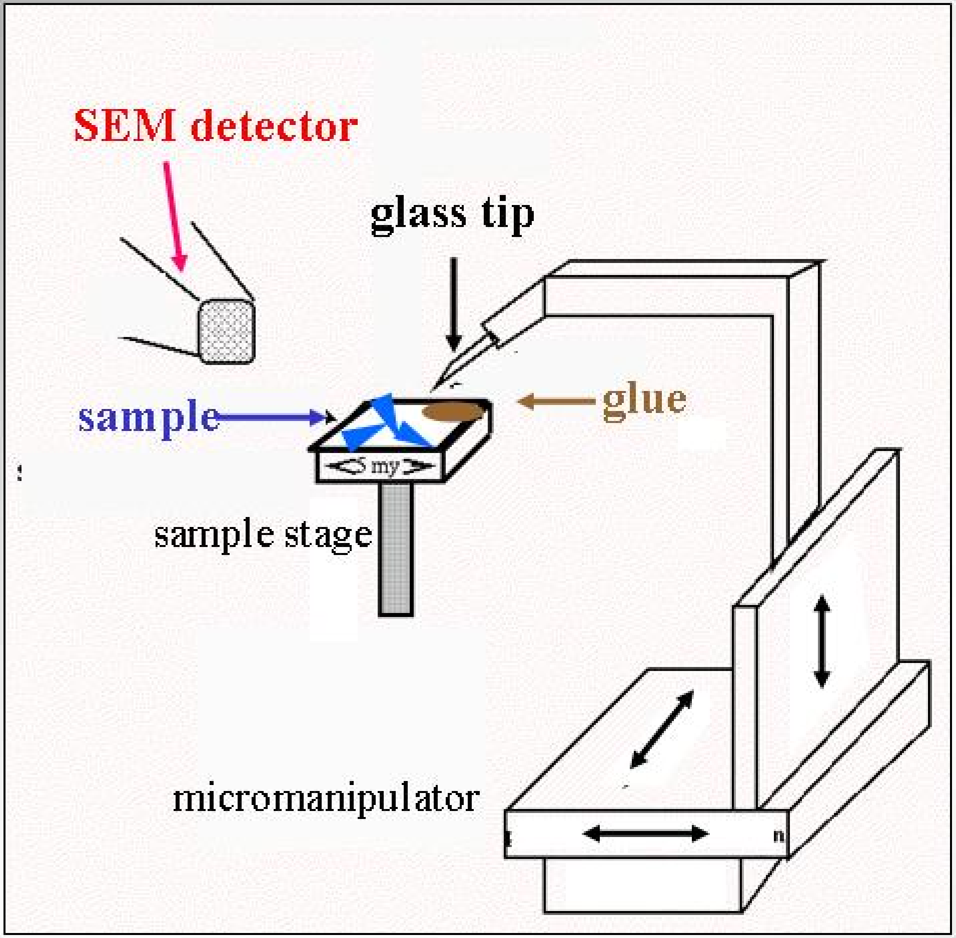

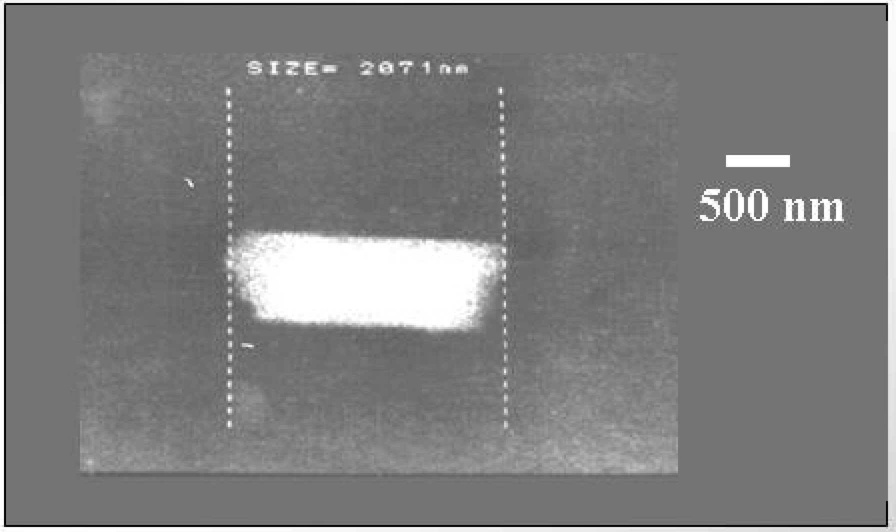

SEM picture of subμm3 Kaolinite crystal

Burghammer: PhD-thesis; University of Munich (1997)

Manipulation with optical tweezers

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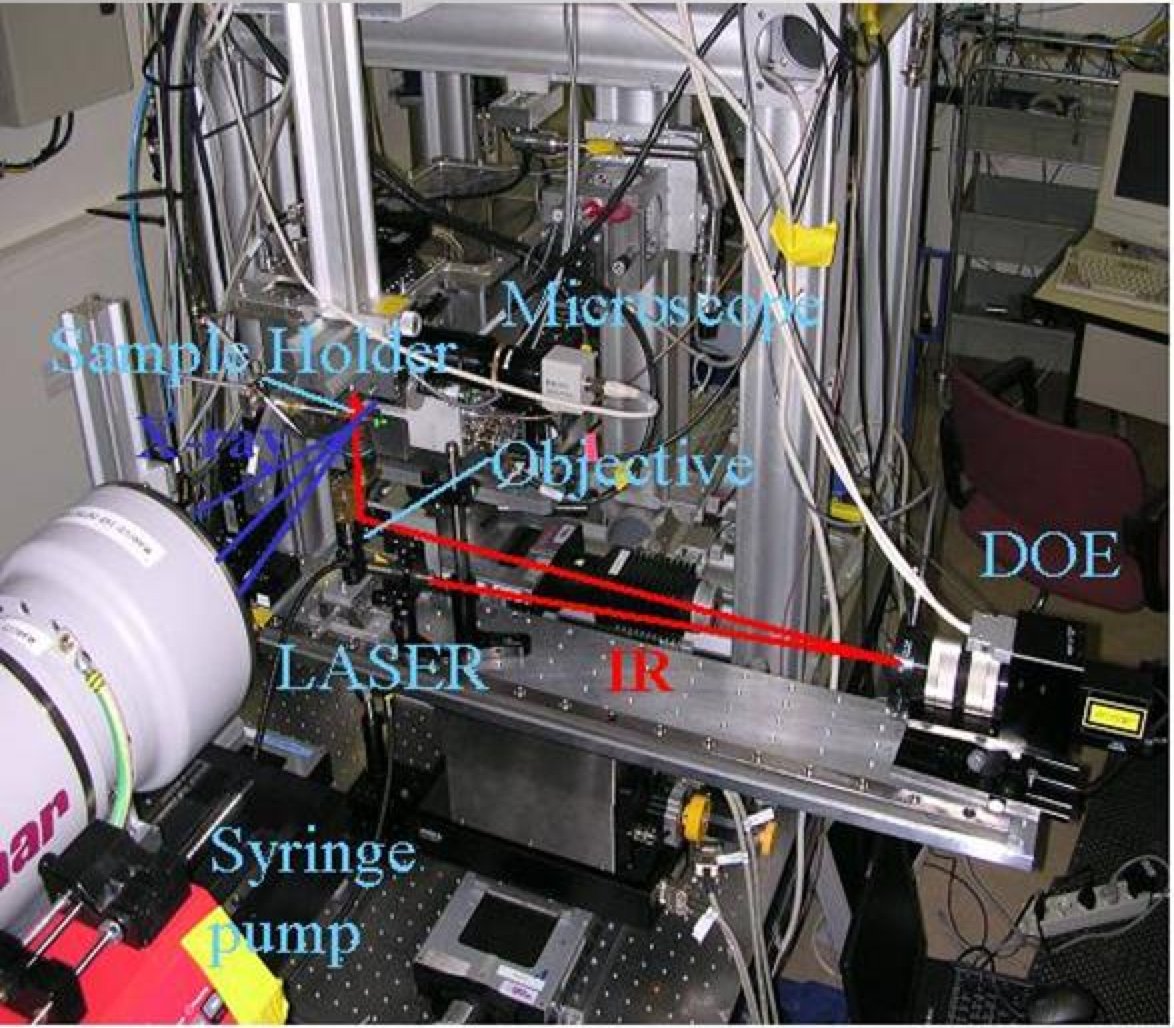


Amenitsch et al., SRI-2006 procedings

IBR	Amenitsch et al. Graz
advanced technology and nanoscience TASC national laboratory tecnologie avanzate e nanoscienza	Cojoc et al. Trieste

optical tweezer set-up at the ID13 beamline including capillary holder, syringe pump and top microscope.

Sample manipulation with optical tweezers

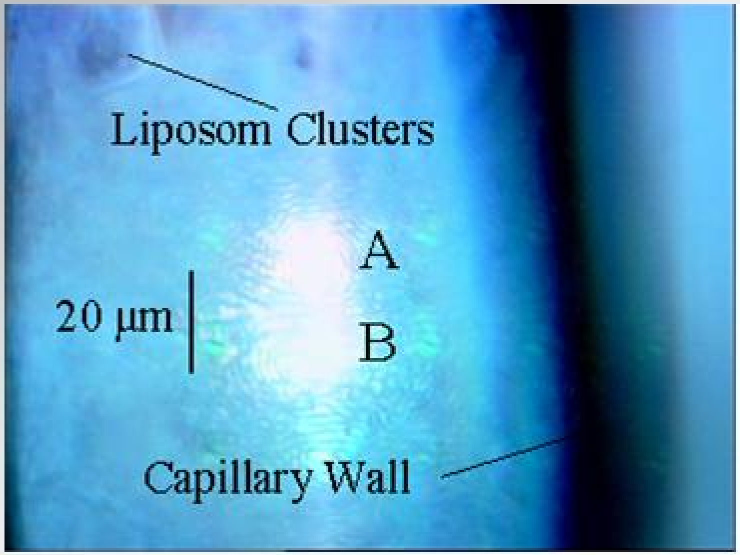
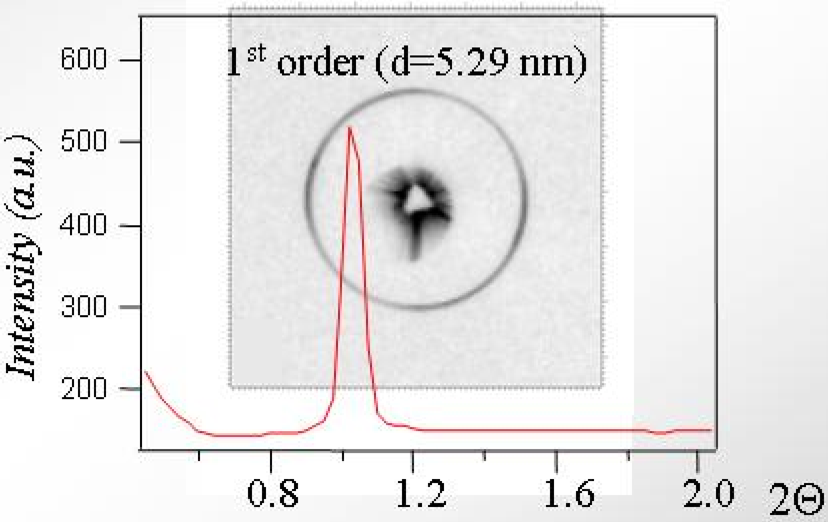


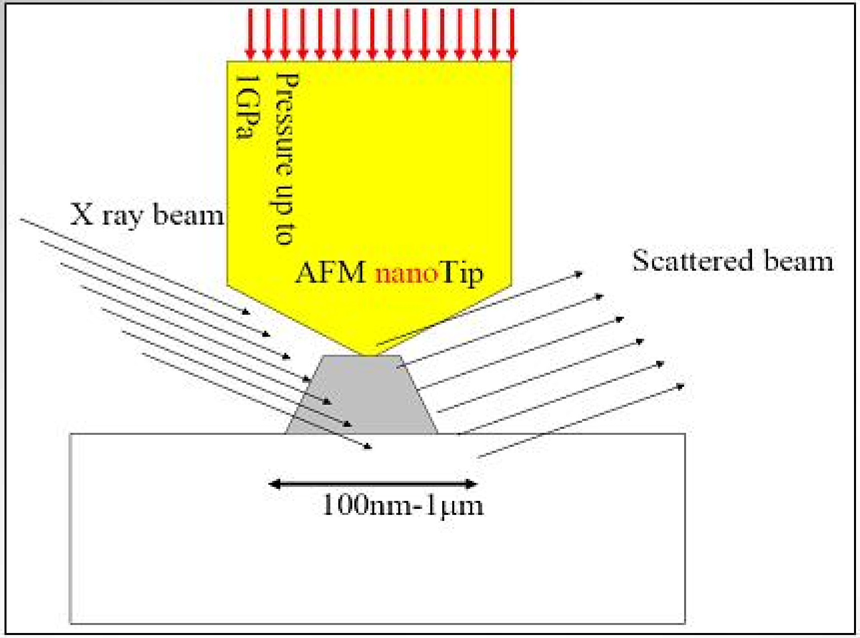
image of the ***two trapping spots*** *A, B in the capillary, under which two clusters of* ***POPE liposoms*** *have been trapped (not seen due to the intense IR light). A non trapped cluster is also indicated*

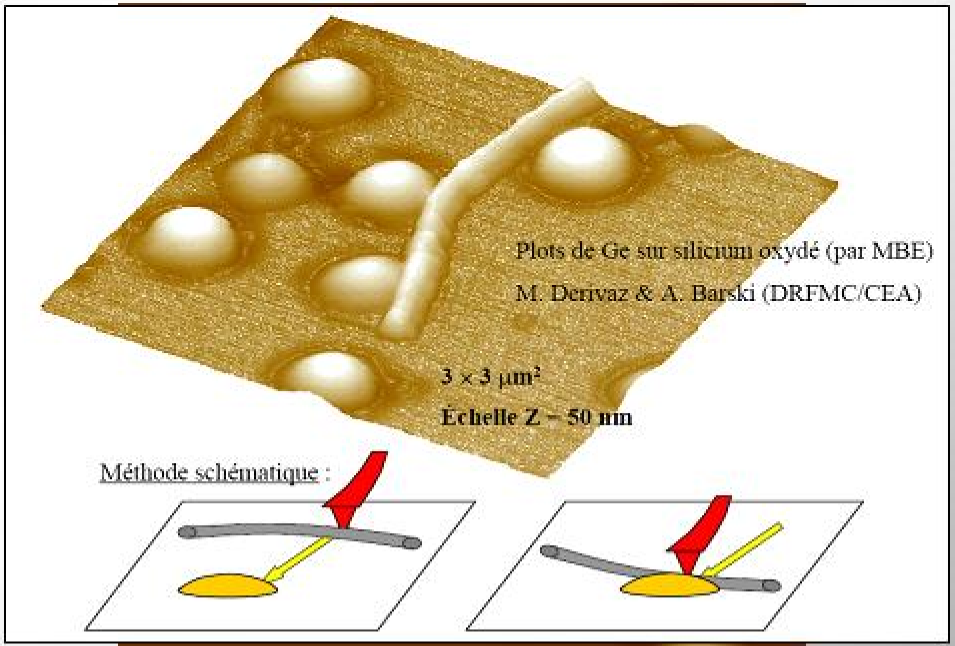


diffraction pattern obtained from a 10 µm large cluster of liposomes with an about ***1 µm beam****.*

Palmitoyl-Oleyl-Phosphatidylethanolamine

Manipulation of sub μm³ particles by AFM





EEC XTIP project: Comin (ESRF)...

ESRF ID13 beamline: focal spots

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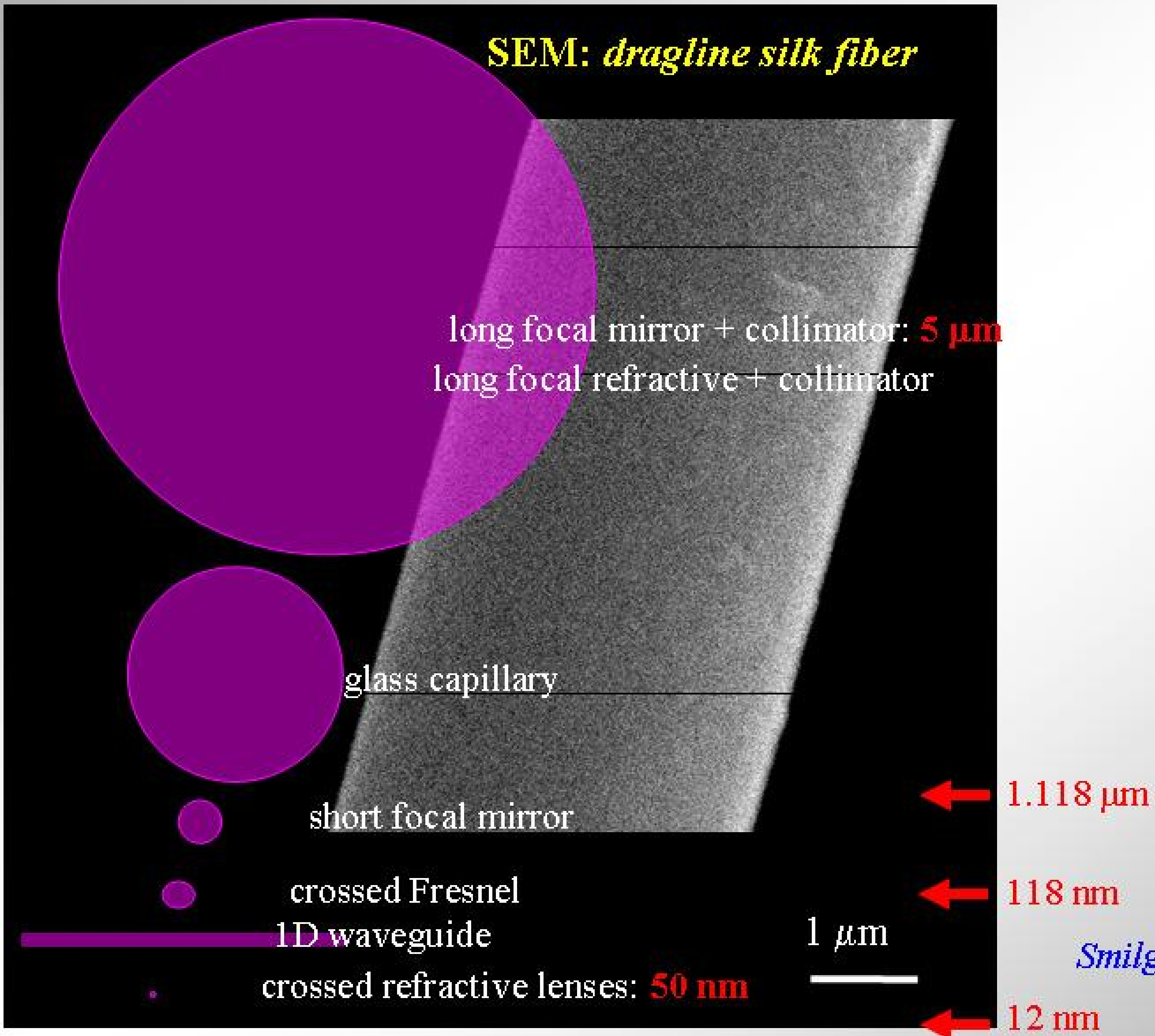
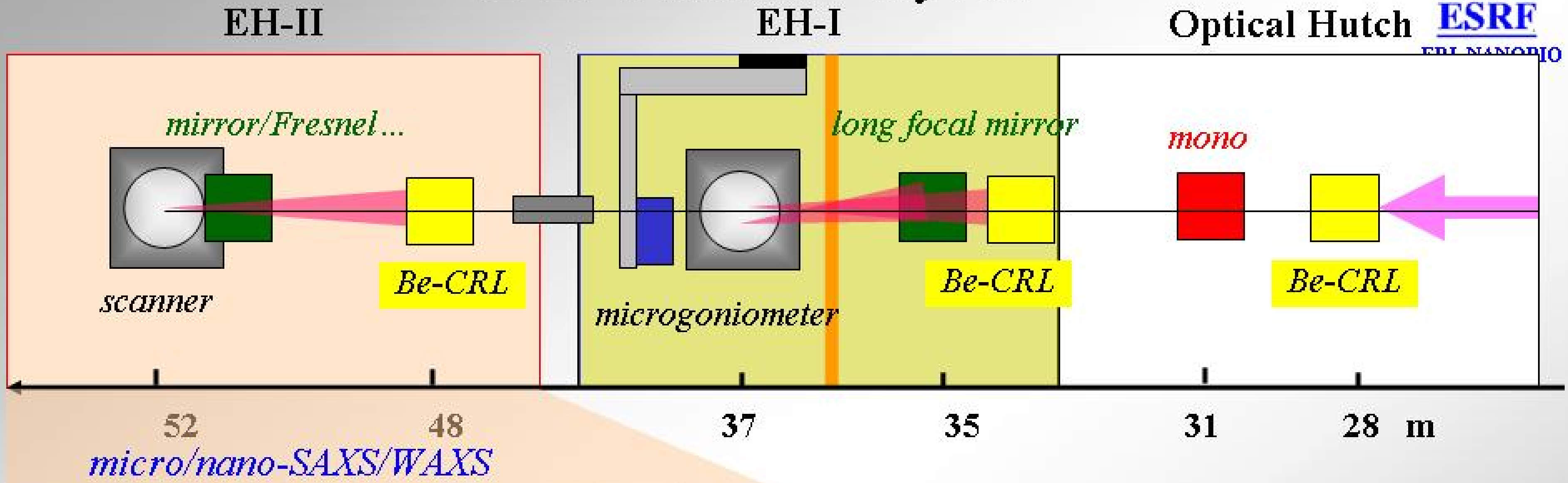
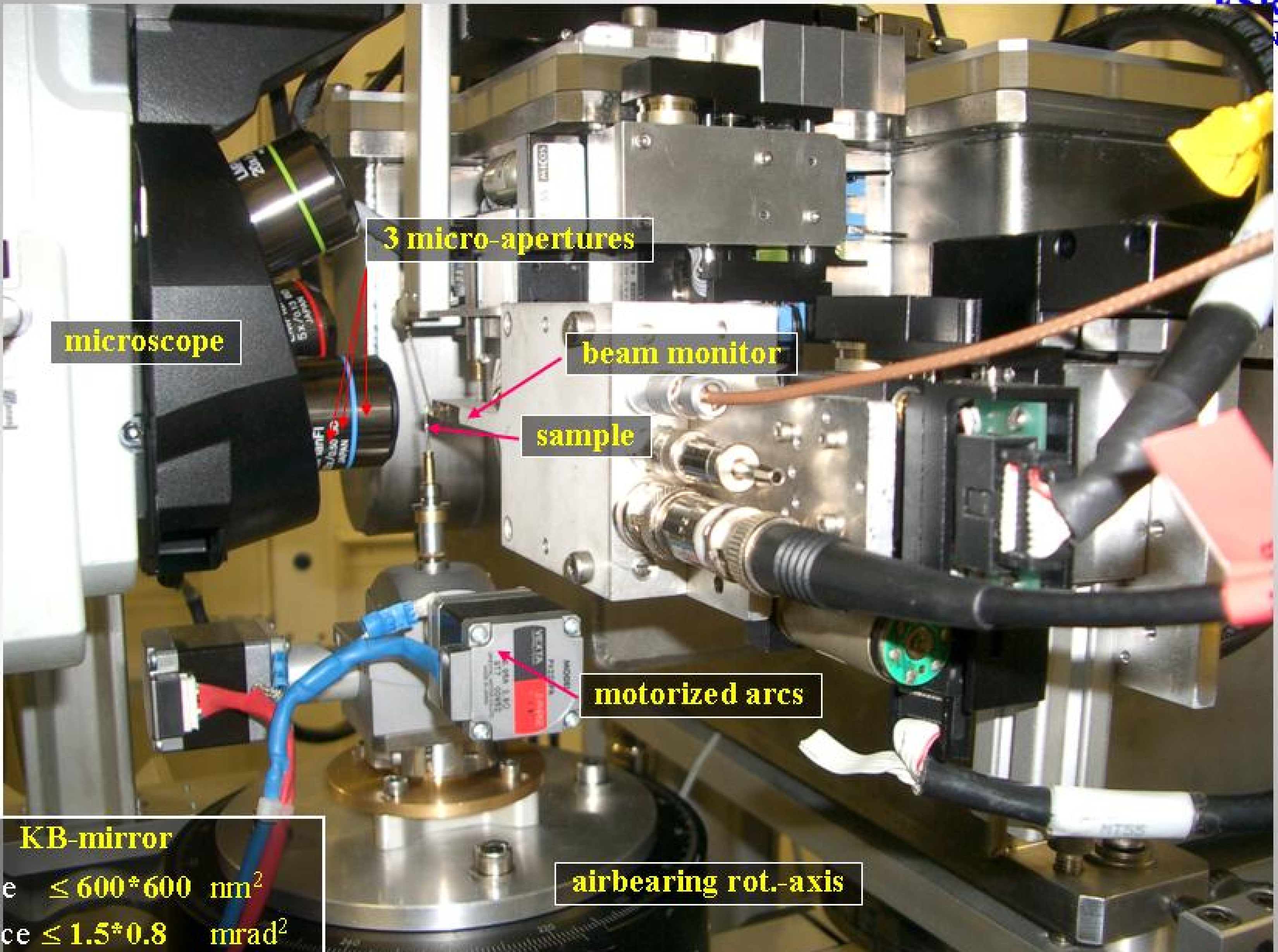


Smilgies Microbeam Scattering Report

ID13 beamline layout



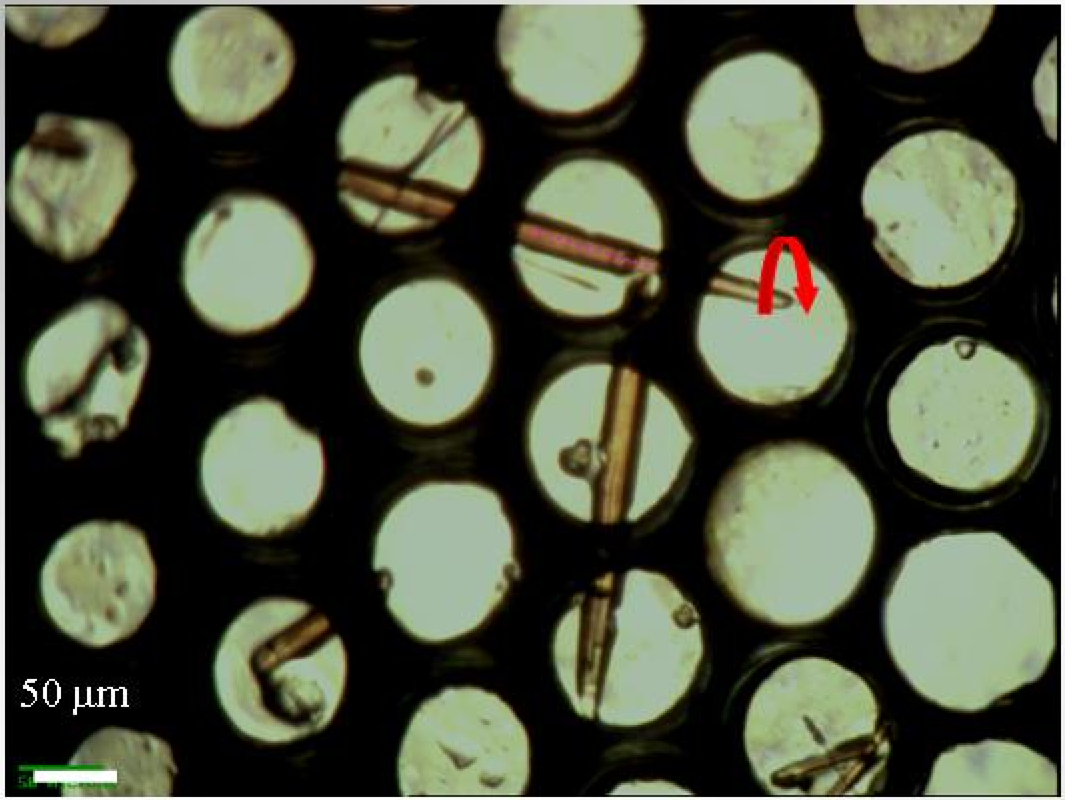
Scanning set-up and KB-mirror

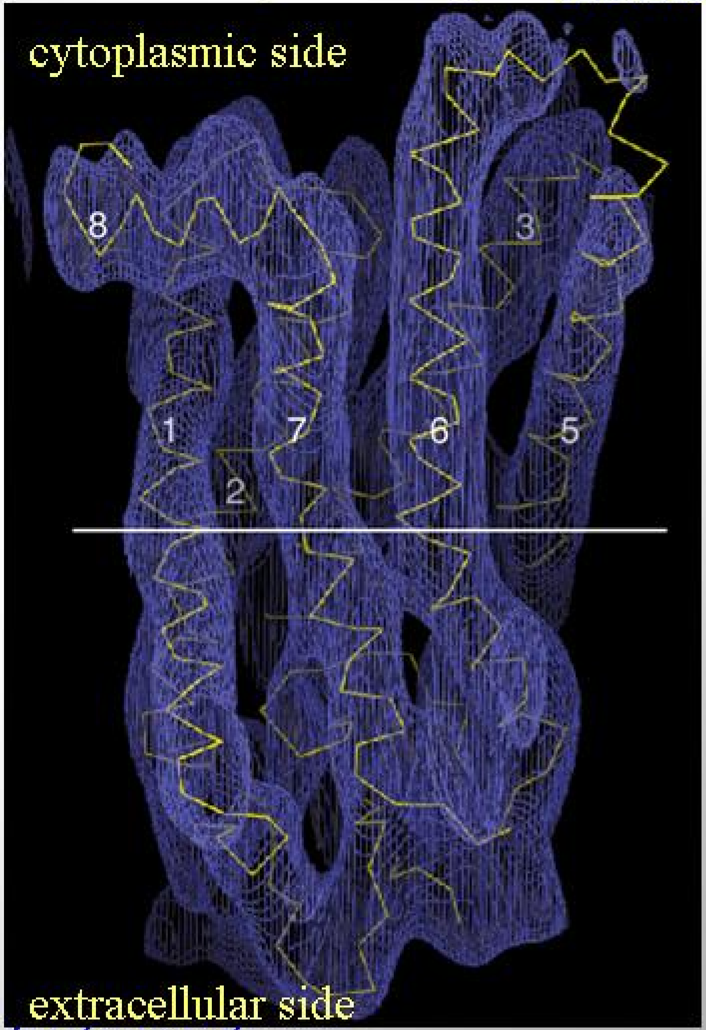


KB-mirror

beam size	≤ 600*600	nm^2
divergence	≤ 1.5*0.8	$mrad^2$
flux	≤ $8*10^{10}$	ph/s

structure of metarhodopsin I obtained from 98 randomly oriented tilted cryo-EM images





carbon grid mounting techniques: *avoid cryoprotectant background*

automate data collection: *scanning + rotation*

multiple single shot experiments: *randomly oriented crystals*

Ruprecht et al. *EMBO J.* (2004)

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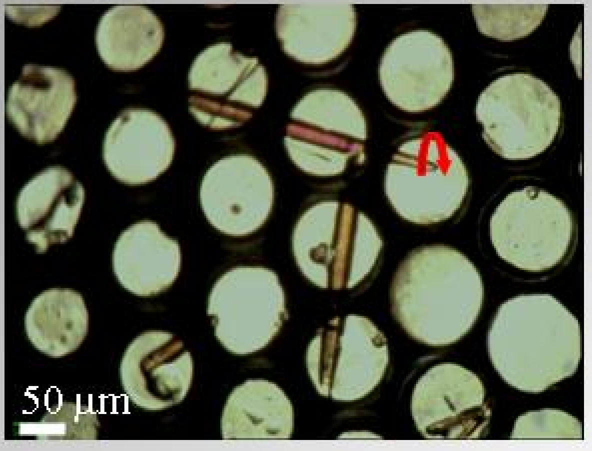

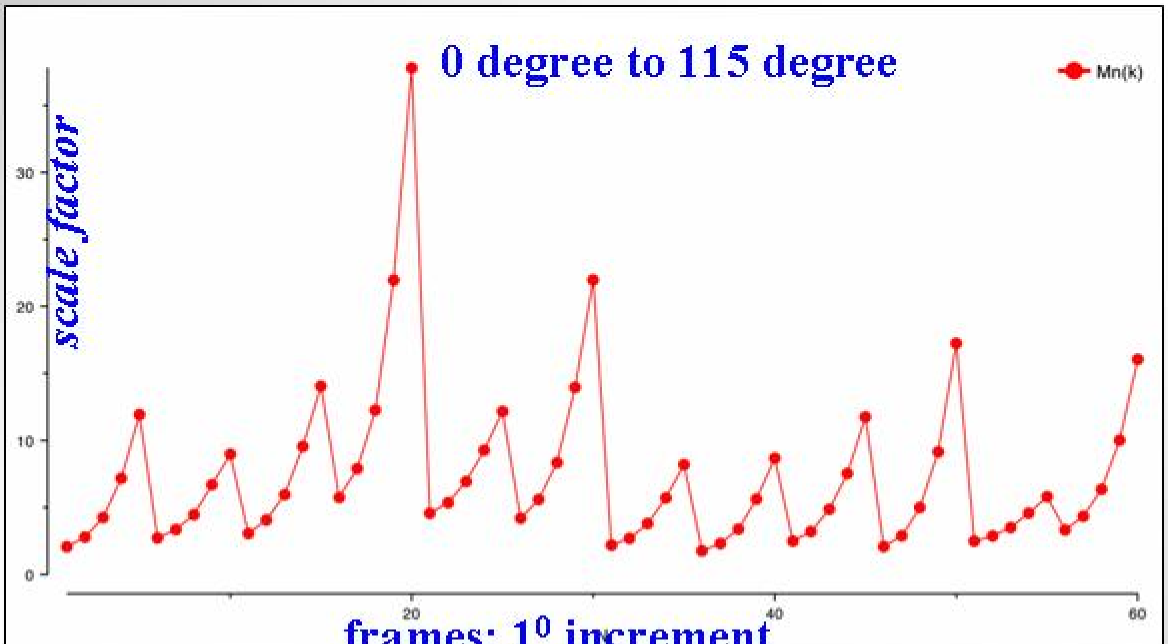

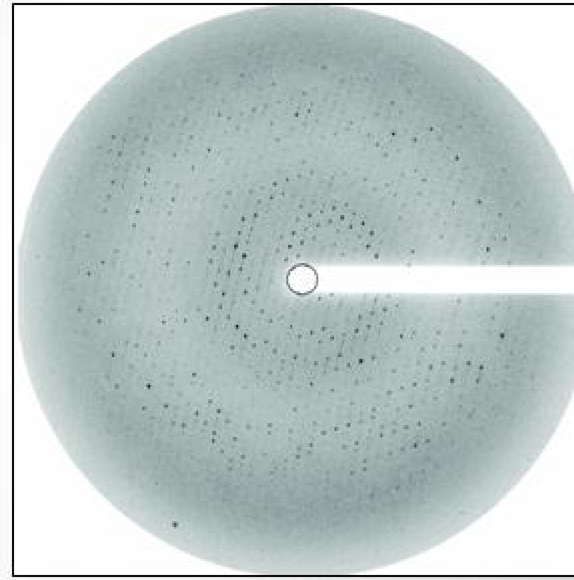

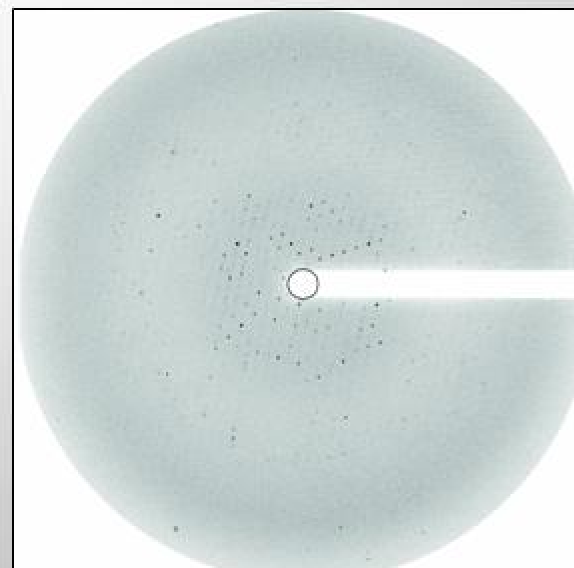

Coherence matched KB-Fresnel system

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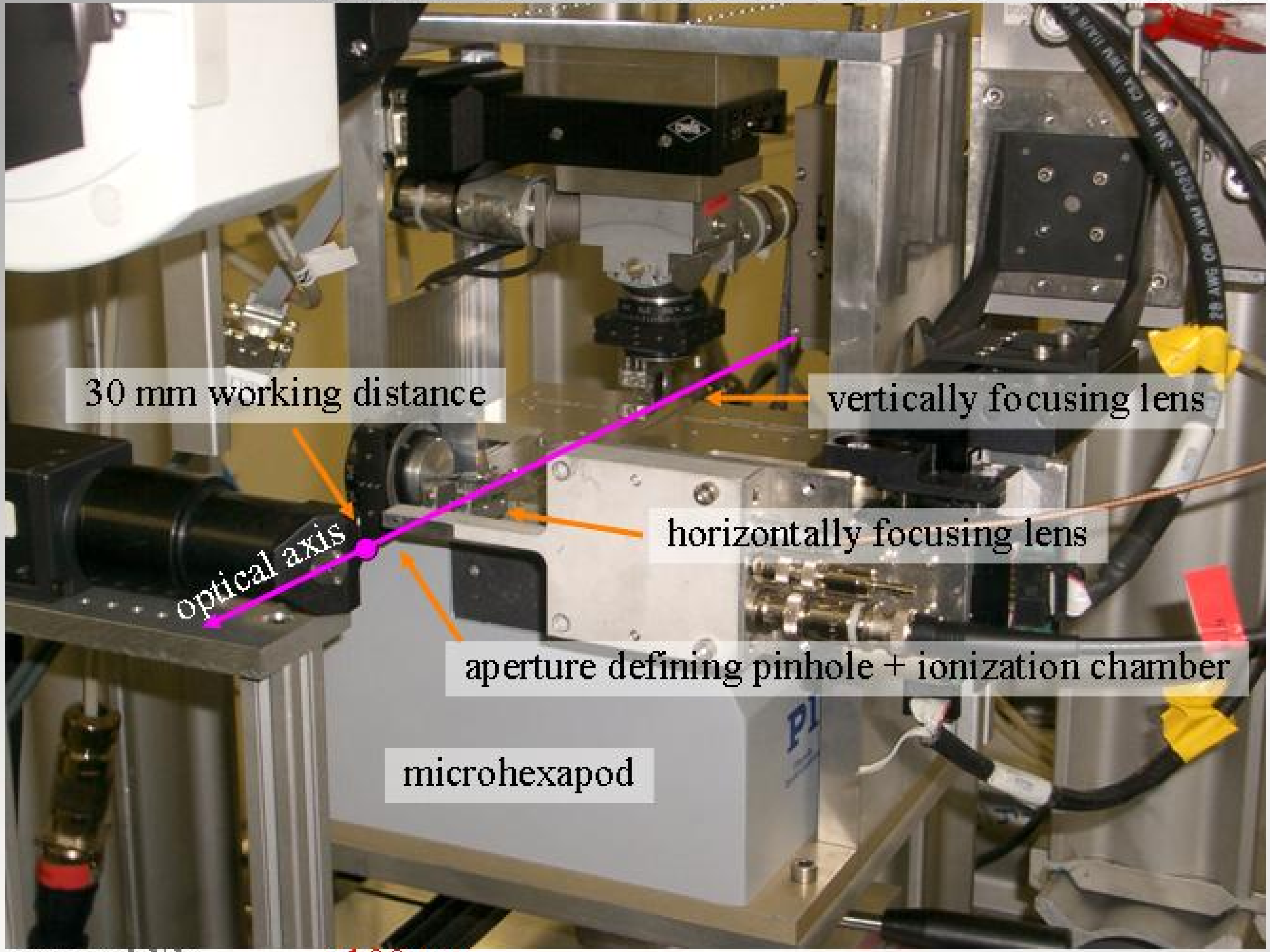
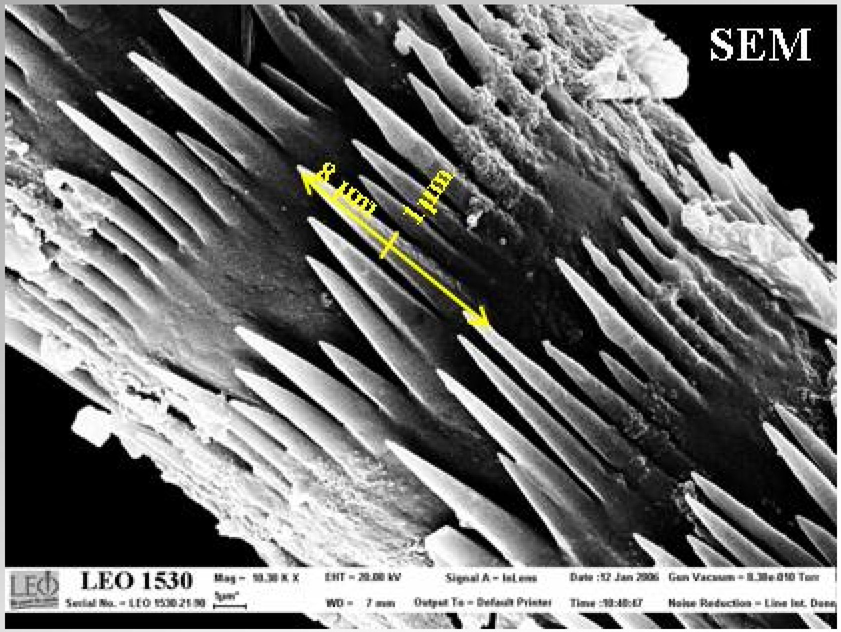
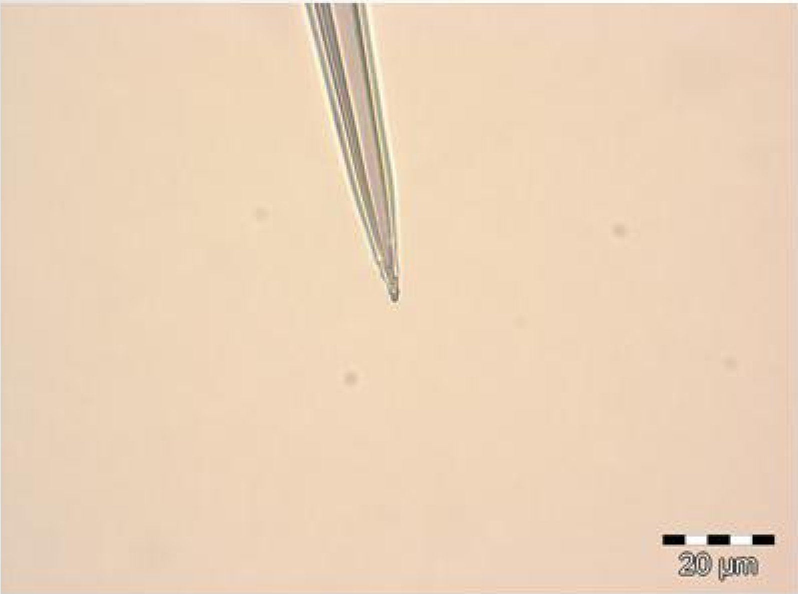


David et al., PSI

zone width:	≥100 nm	
lens diameter:	$200_h(140)^{*}$ 50_v (25) µm	Nöhammer et al., *APL* (2005) **86**, 163104
focal spot:	**300*300**	nm^2 routinely used; **140 nm** demonstrated
divergence:	**1**	mrad
Q_{min}	**≥0.1**	nm^{-1}
flux:	**$1*10^{10}$**	ph/s (Si-111; 12.7 keV)

Vaterite single crystal needles

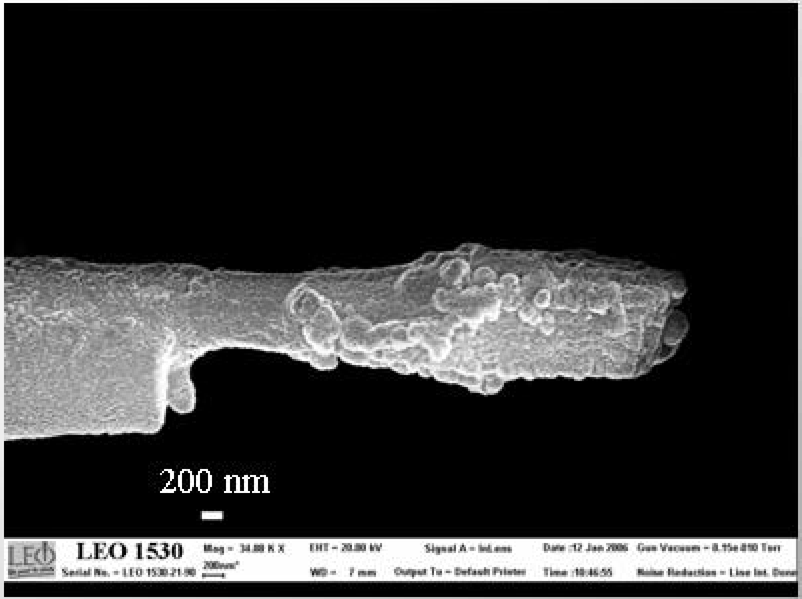




Vaterite ($CaCO_3$) single crystal needles from sea urchin

courtesy: Zolotoyabko & Pokroy

Israel Institute of Technology, Haifa



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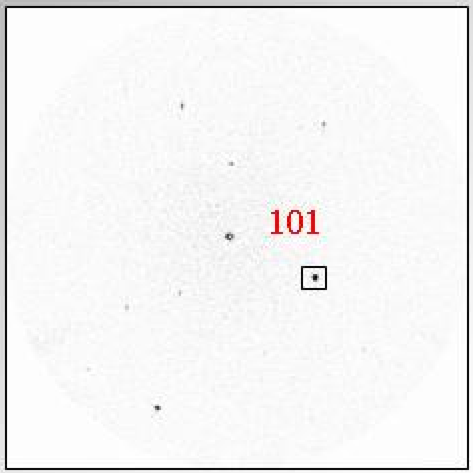
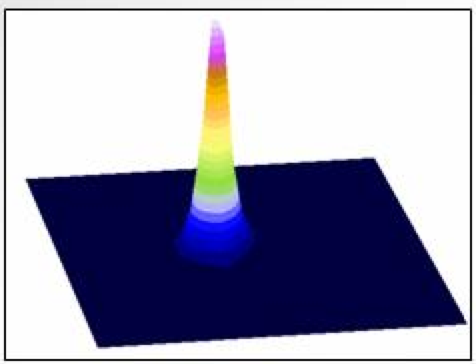
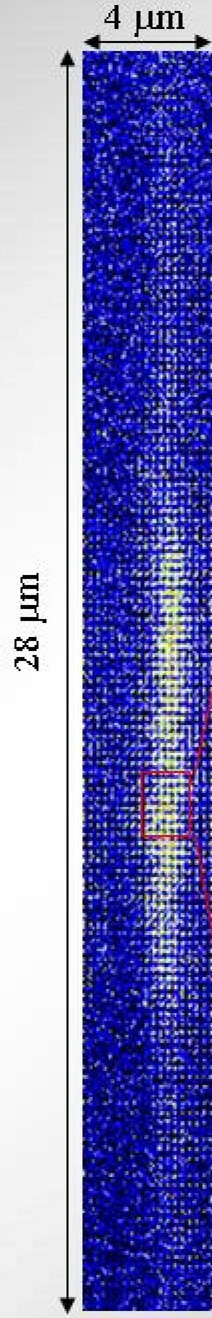
beam size: 300 nm

λ=0.098403 nm



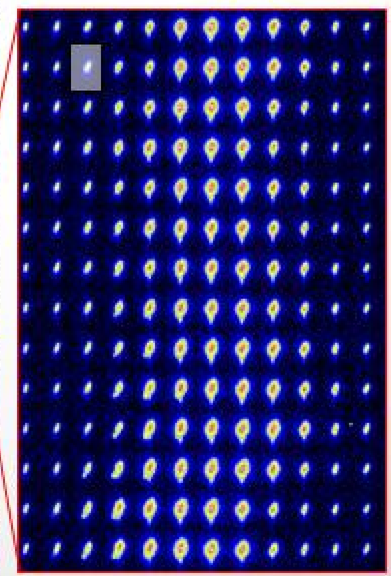


a=0.4133(1)nm, c=0.8476(2)nm; $P6_3/mmc$



composite image of 101 reflection from mesh-scan

200*200nm^2 "pixels"



single crystal data collection requires sub-100 nm eccentricity rotation spindle

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Summary

- ERL is unbeatable for the generation of intense and low divergent microbeams as compared to 3rd generation SR sources
- secondary radiation damage should be limited as far as possible by short readout times
- requires an integrated concept of fast framing, <50 μm pixel size pixel detectors
- new concepts of sample observation and manipulation have to be developed: look at neighbouring disciplines

ESRF
ERL-NANOSOFT

Acknowledgements

G. Schertler	MRC-Cambridge	*Rhodopsin, new sample environments*
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A. Madsen	Copenhagen Univ.	*amyloid fibres*
M. Roessle	EMBL-Hamburg	single cell diffraction
H. Amenitsch	Graz University	optical tweezers
D. Cojo	Elettra	optical tweezers
M. Burghammer	ESRF-ID13	*ID13 instrumentation*