Hard X-ray phase contrast microscopy and fluorescence mapping with KB optics

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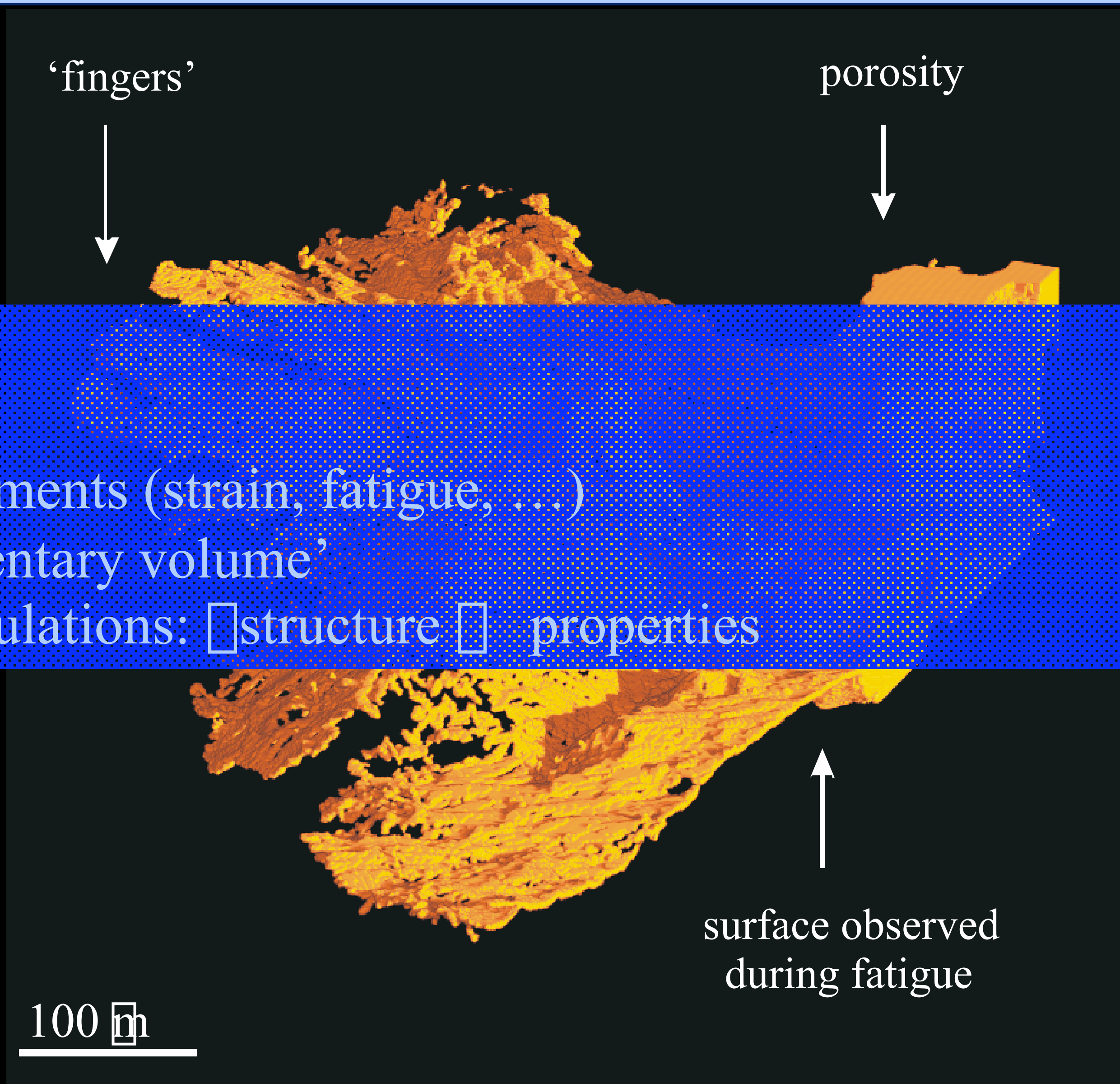
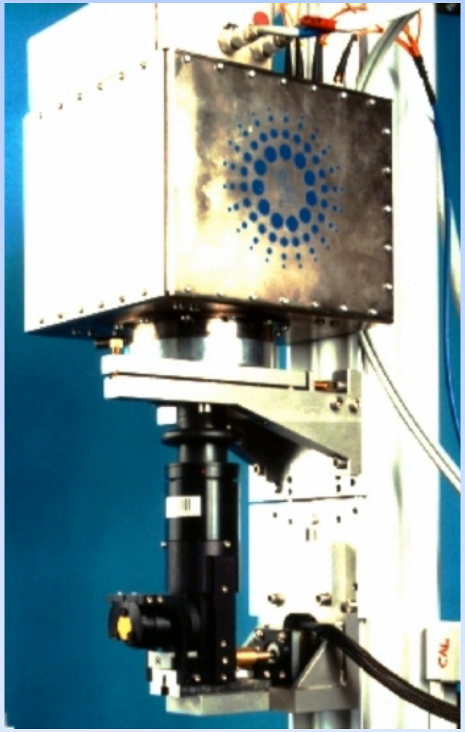
Motivation



- 3D microscopy
- non-destructive
  - in-situ experiments (strain, fatigue, ...)
- ‘representative elementary volume’
  - input for calculations: structure properties

Motivation



Improve the spatial resolution

resolution □ 0.5 μm

NA=0.95, 1 μm thick LAG:Eu, 11.5 keV

with scintillator based detectors

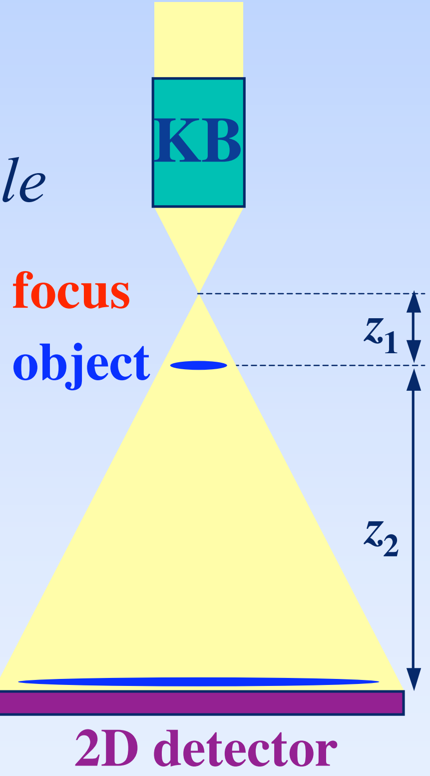
X-ray magnification using

- diffractive optics (FZP)
- refractive optics (CRL)
- *reflective optics (KB)*

Motivation

Gabor's Microscopic principle (Nature, 1948)

The object is illuminated by an electron beam brought to a fine focus… The object is a small distance behind (or in front) of the point focus, followed by a photographic plate at a large multiple of this distance…



Outline

KB optics

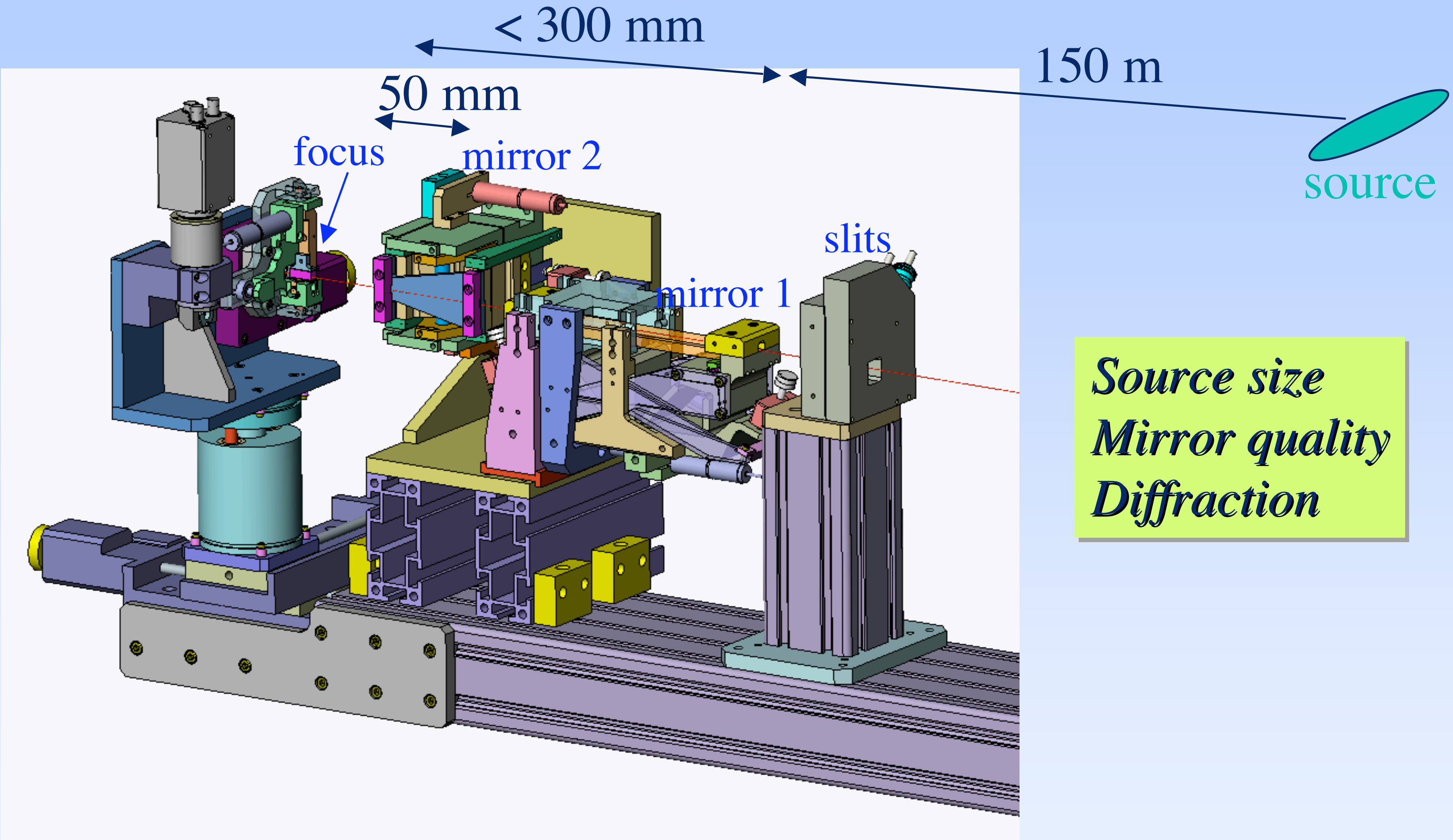
- Limits in KB focusing
- Measurement of the spot size

Applications

- Projection Microscopy
- Fluorescence imaging

Prospects

Kirkpatrick-Baez focusing



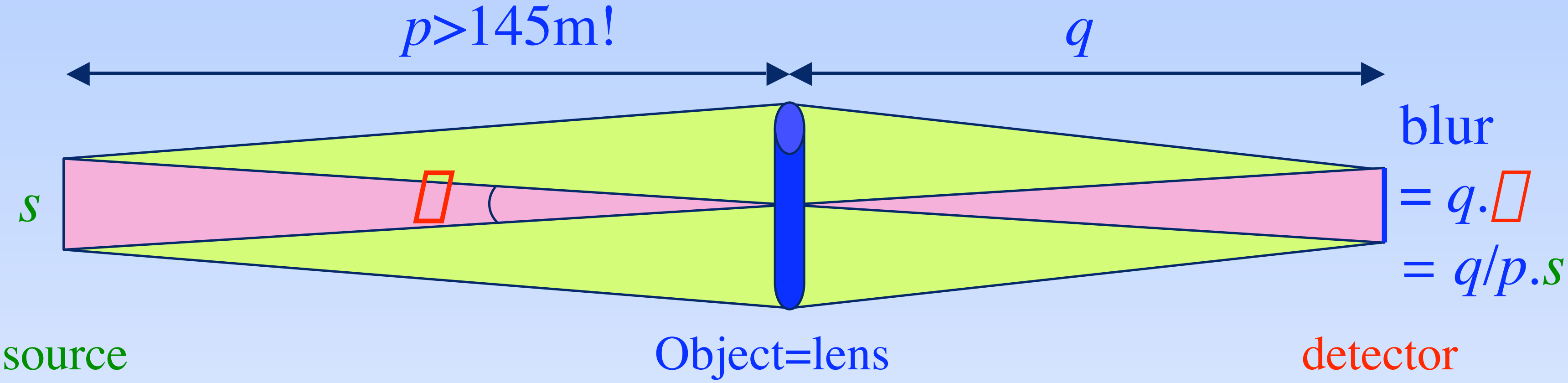
Source size
Mirror quality
Diffraction

O. Hignette, G. Rostaing

Limits in KB focusing

Source size

Focus = Demagnified image of the source



Same requirement as for Phase Contrast Imaging

Source ID19

125 □m(h) * 25 □m (v)
Low □ section!

Demagnification = p/q
Hor.: 1540
Ver.: 520

focus

87 nm * 45 nm

Limits in KB focusing

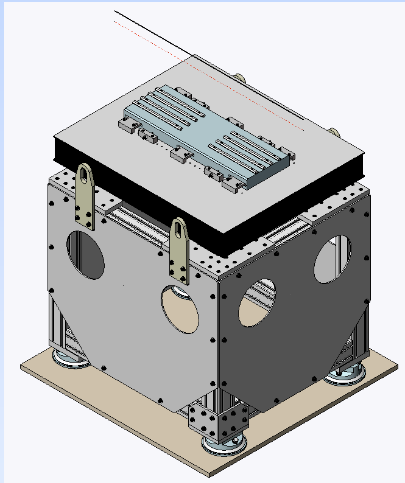
'Effective' Source size

Vibrations!

should be smaller than angular source size (0.15 μrad fwhm)

- *Complete* instrument on a single table

    light and *rigid* structure



KB-table
P. Bernard

- *No pre-monochromator* (cooling)

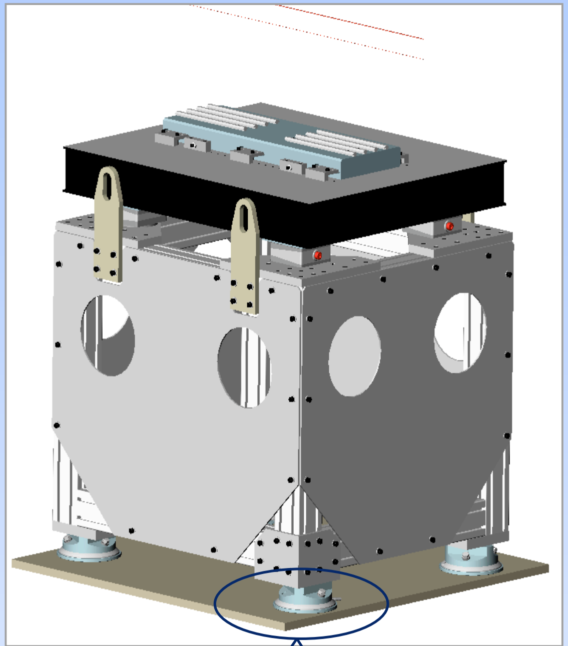
    Multilayer on first mirror as monochromator

    Enormous gain in flux

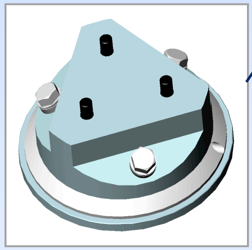
    Gain in vertical acceptance

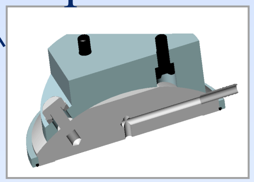
    $\Delta E/E \approx 10^{-2}$ (3d harmonic undulator)

Light frame KB table



Air pads





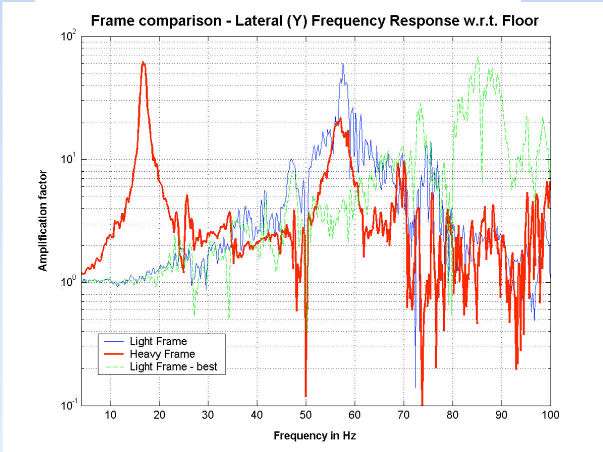
Natural frequency above floor excitation

$$f_{res} = \frac{1}{2\pi}\sqrt{\frac{k}{m}}$$

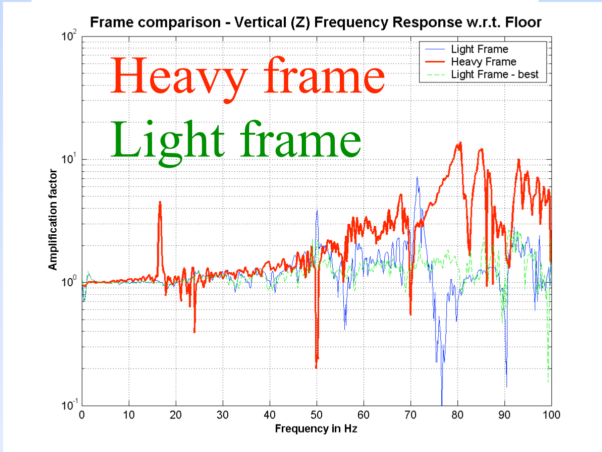
k → High stiffness

m → Light

Lateral amplification



Vertical amplification



		Vertical (Z) [μm]			Longitudinal (X)			Lateral (Y)			θy Rotation [μrad]
		Floor	Top plate	Rel. %	Floor	Top plate	Rel. %	Floor	Top plate	Rel. %	
Heavy Frame	dpp	0.76	0.83	9.7	0.94	1.2	27.8	0.46	1.97	326.7	0.25
	rms	0.12	0.13	5.5	0.09	0.12	44	0.07	0.29	291	0.04
Light Frame	dpp	0.67	0.69	2.5	0.31	0.33	7.7	0.49	0.53	7.6	0.09
	rms	0.11	0.11	1.5	0.05	0.05	3.1	0.07	0.07	4.9	0.01

θ_y

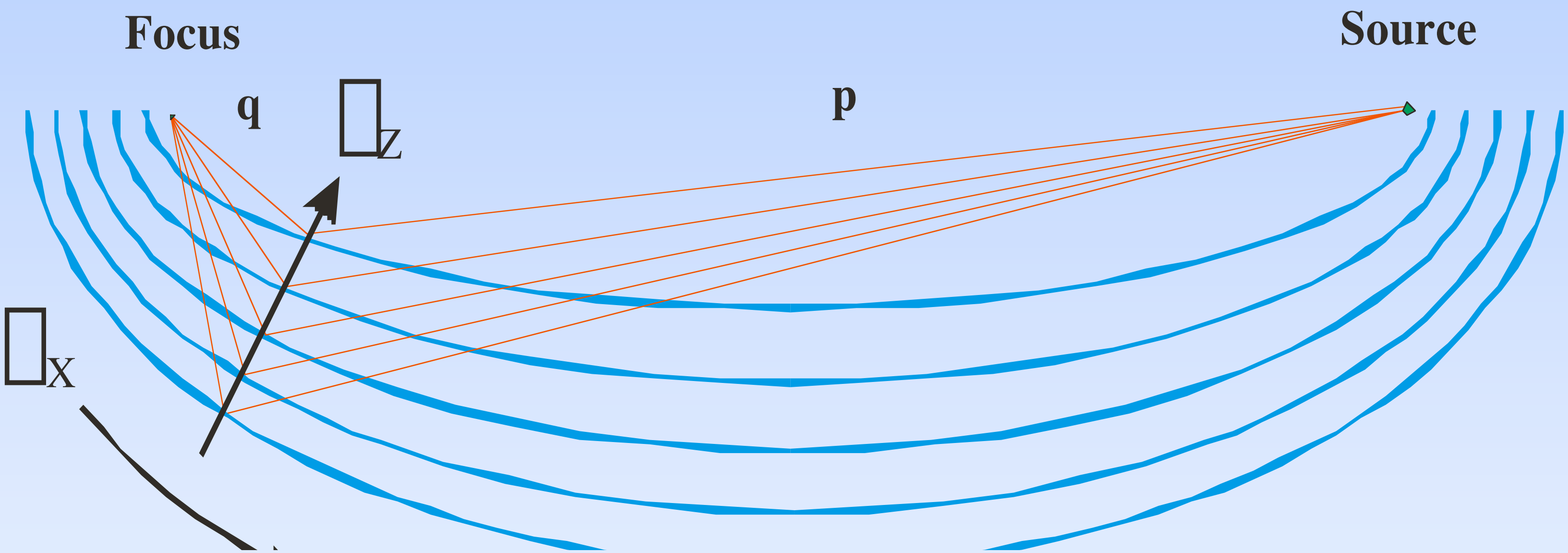
10 nrad rms

P. Bernard, M. Lesourd, L. Zhang

Multilayer coating

Incidence angle variation up to 50% ➔ laterally graded multilayer

Depth gradient negligible ($< 10^{-5}$)



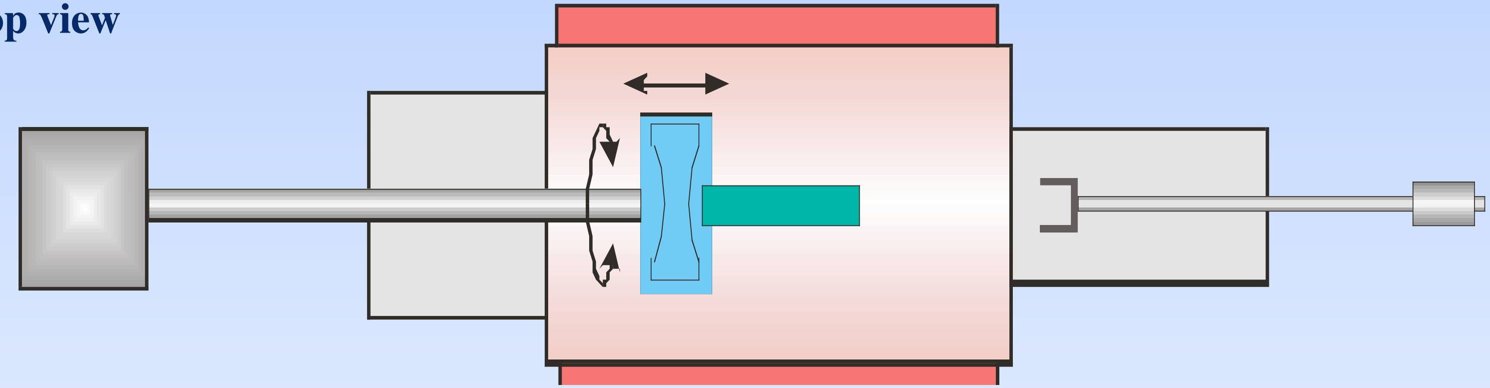
Ch. Morawe

Multilayer coating

ESRF deposition facility

- Distributed Electron Cyclotron Resonance (DECR) plasma sputtering
- 3 target positions, rotation and linear movement

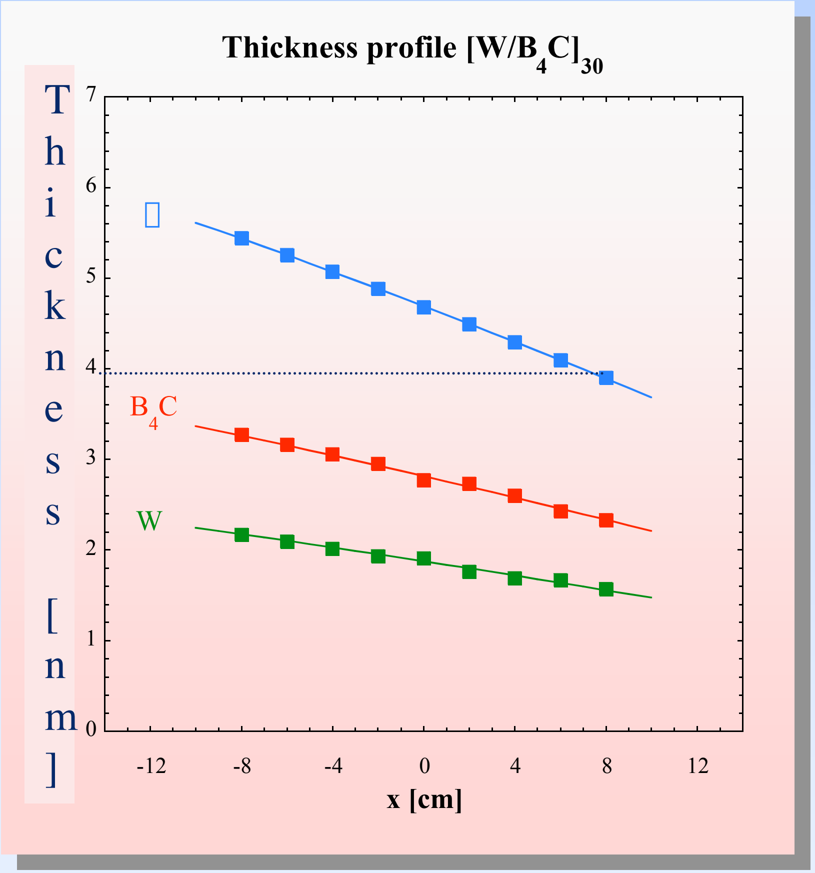
Top view

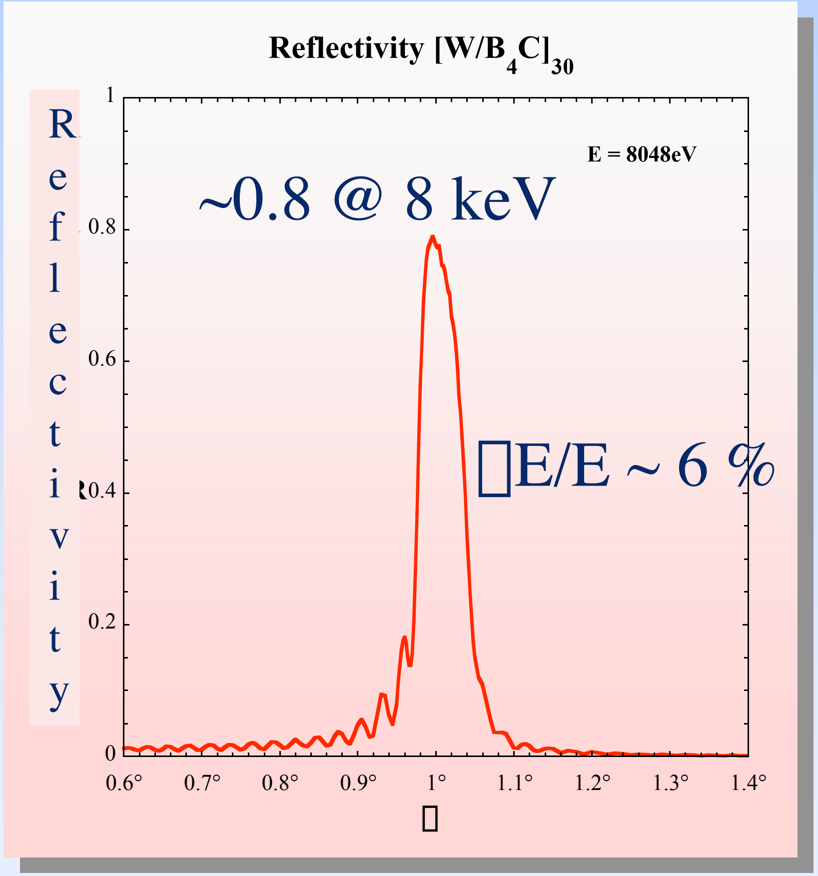


Base pressure	10^{-8} mbar
Ar pressure	1.3□10^{-3} mbar
Polarization voltage	U = -500...-2000 V (DC/RF)
Particle flux	R = 0.02…0.10 nm/s
Deposition temperature	T_{Depo}□ 100°C

Ch. Morawe

Multilayer coating





Ch. Morawe

Limits in KB focusing

Mirror quality

Elliptical shape

Incoherent view point

Slope errors □

Blur = 2.□.*q*

Coherent view point

Shape errors *h* □ Aberrations

□□ = - 4□/□ . *h* . sin□

Use small region of mirror

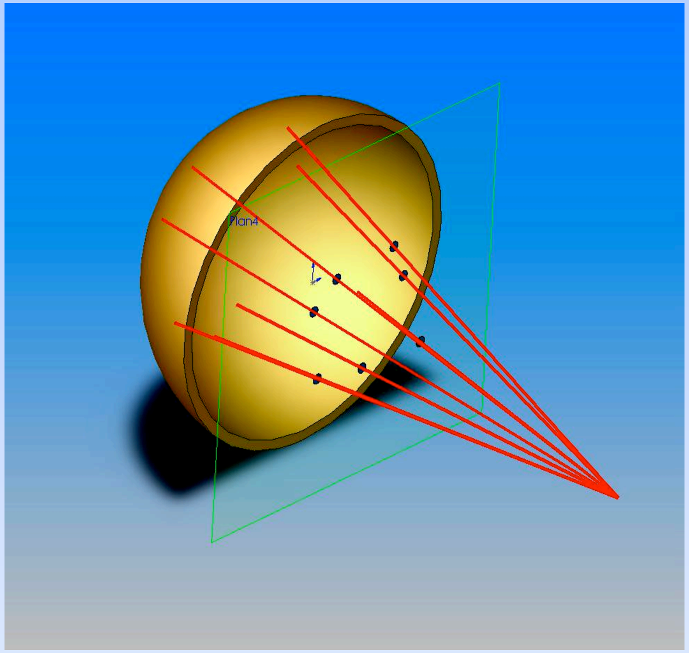
Mirrors manufactured by Wave Precision

Dynamical bending

2 actuators / mirror

automatic alignment procedure

Alignment procedure

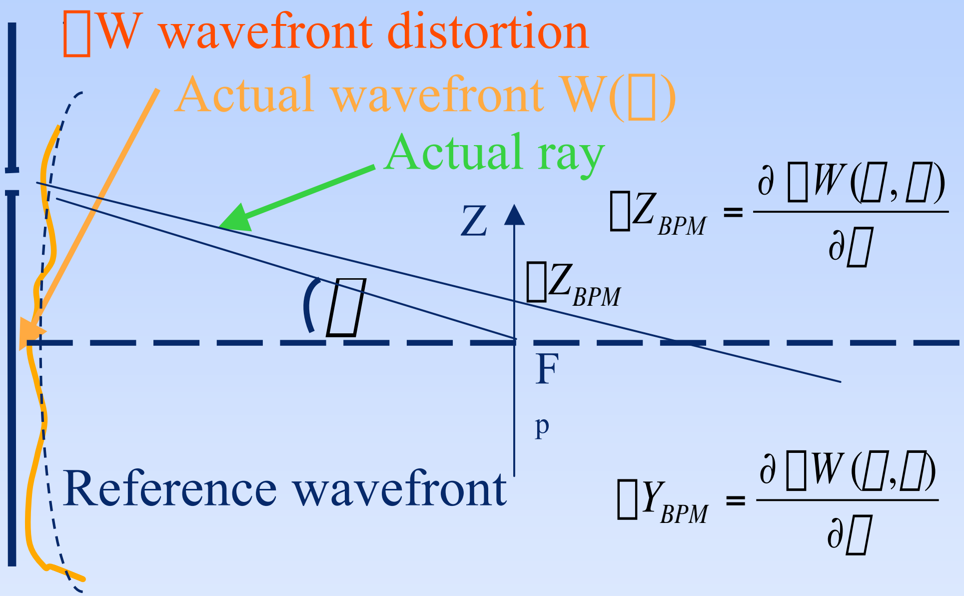


Wavefront W = equiphase surface

Normal rays defined by slits

BeamPositionMonitor = XCCD

ΔW = 2 *optical surface distortion * sin θ



Measuring ΔZ_{BPM}, ΔY_{BPM} with scanning slit and BPM

Integrating ➡ Optical surfaces information

O. Hignette

Alignment procedure

Linear optimization

interaction matrix scheme

- Measure the linear information Y (slope)
- Send a unit displacement on each actuator
- Store the resulting system differential metrology ΔY
- Build the interaction matrix H by grouping all ΔY
- Correction vector C to be sent to the actuators

$$C = \left(H^T H\right)^{-1} H^T Y$$

O. Hignette

Limits in KB focusing

Diffraction

Numerical aperture should be sufficiently large

'Blur'= $c\dfrac{\lambda}{w}q$ $\quad$ w = slit width

Use large region of the mirror

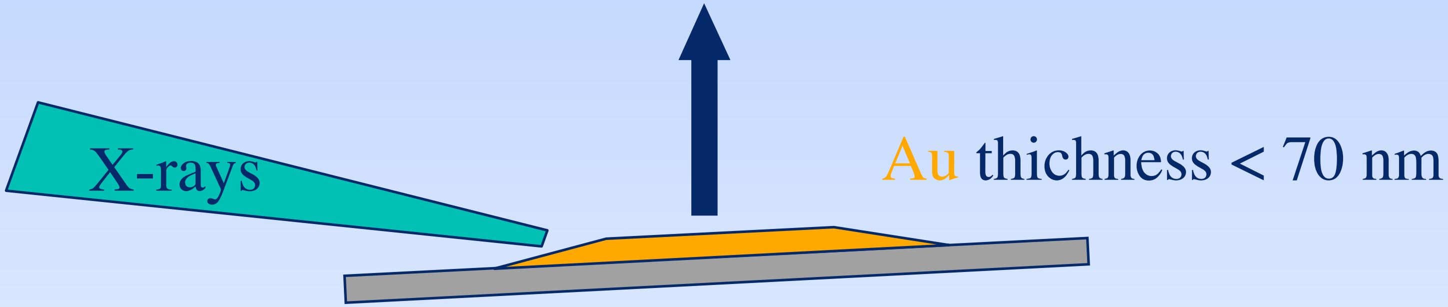
Focusing results at 20.5 keV

Measurement:

Direct line scan, no derivation

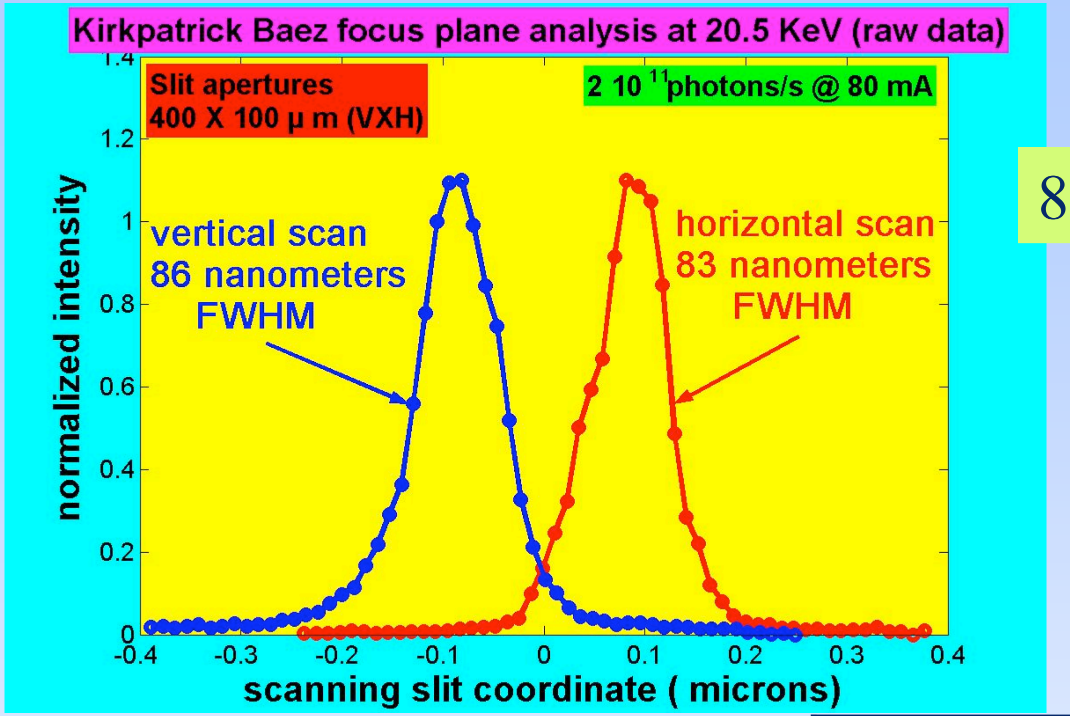
Fluorescence of Au slit

More direct and precise than an edge scan



O. Hignette, P. Cloetens

Focusing results at 20.5 keV



86 nm (v) x 83 nm (h)

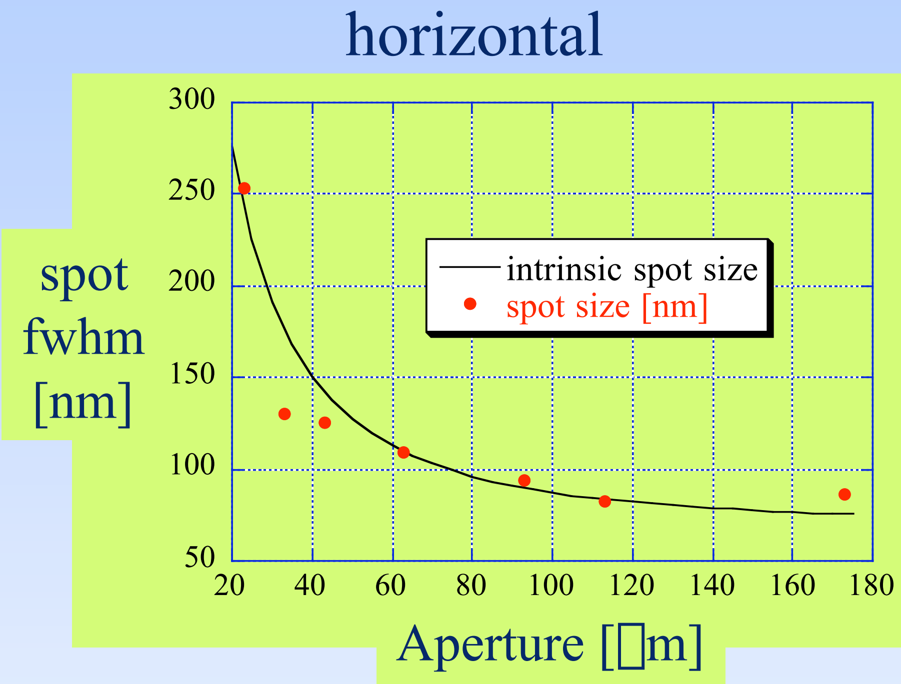
Aperture V*H (□m)	Spot fwhm V*H (nm)	Flux (□E/E=10^{-2}) ph/s @ 80 mA
200 x 50	118 x 109	5 10^{10}
400 x 100	86 x 83	2 10^{11}
600 x 160	116 x 90	4.5 10^{11}

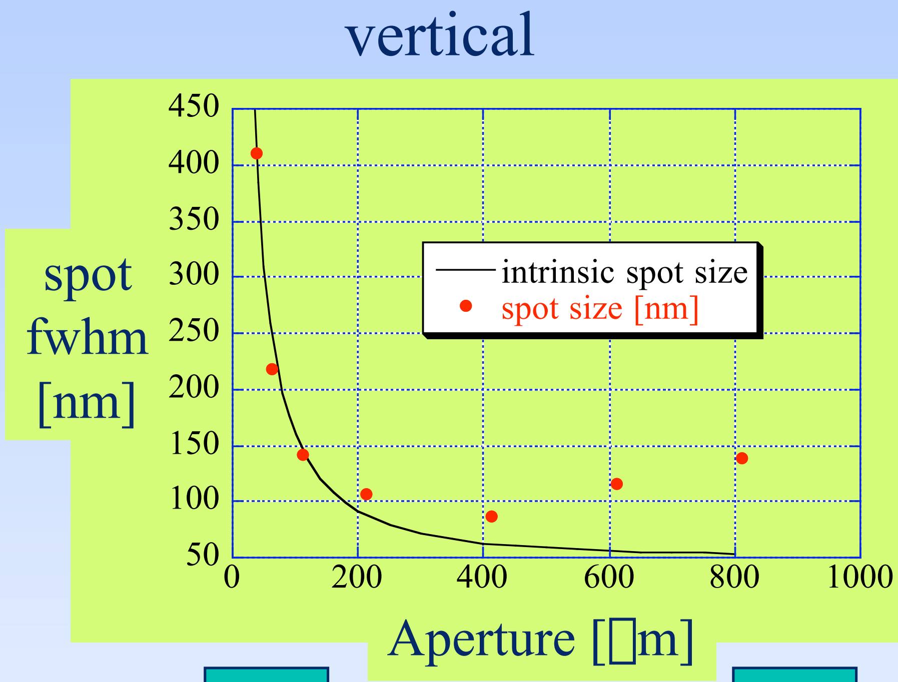
Gain > 3 10^{6} !!

O. Hignette, P. Cloetens

Focusing results at 20.5 keV

Effect of the aperture

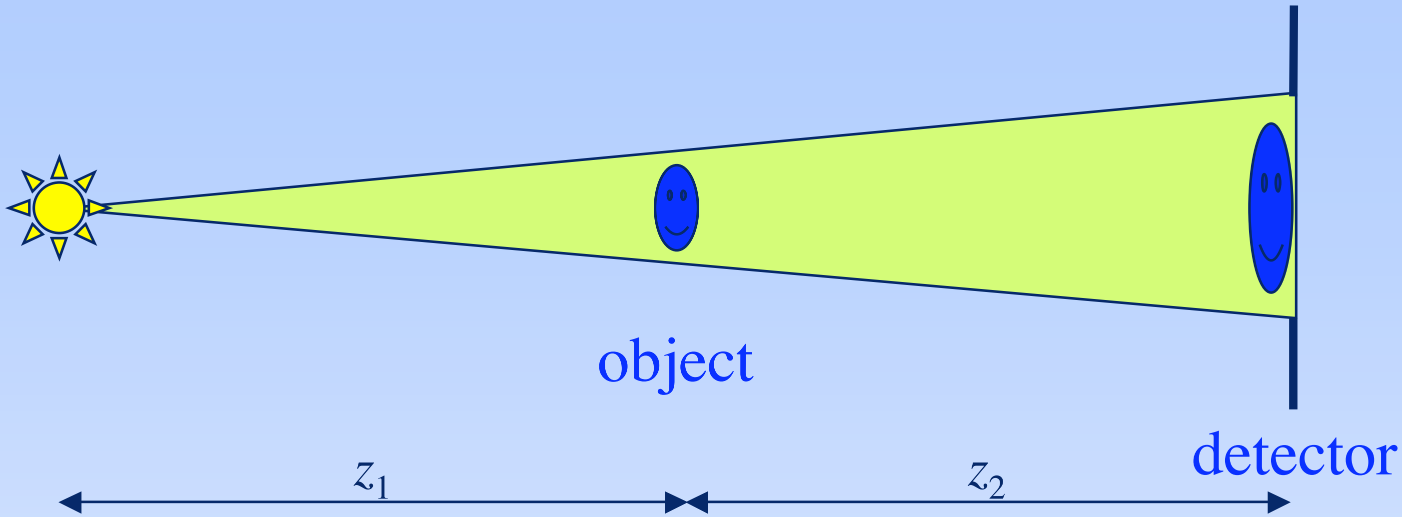




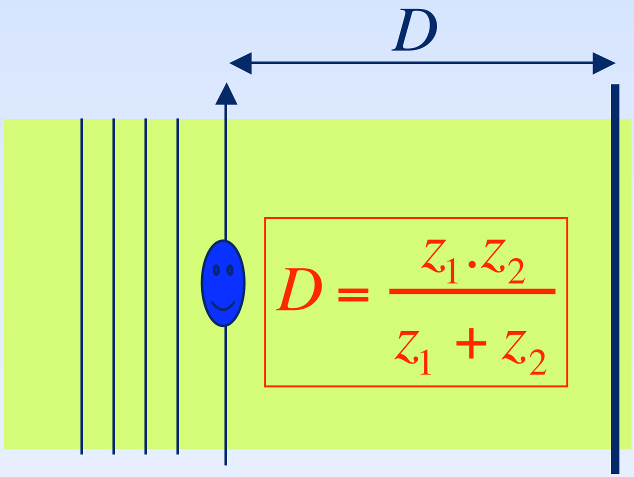
Diffraction limited

Increase tails

Applications: Projection Microscopy

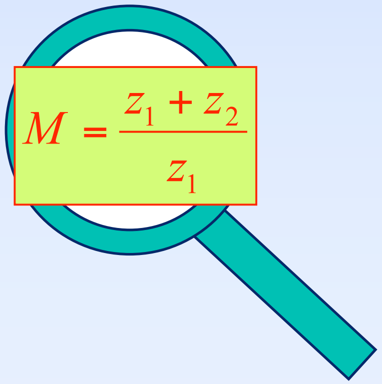


equivalent to



Plane wave illumination

+



Magnification

Limit cases

$z_1 >> z_2$

$D = z_2 \; ; M = 1$

$z_1 << z_2$

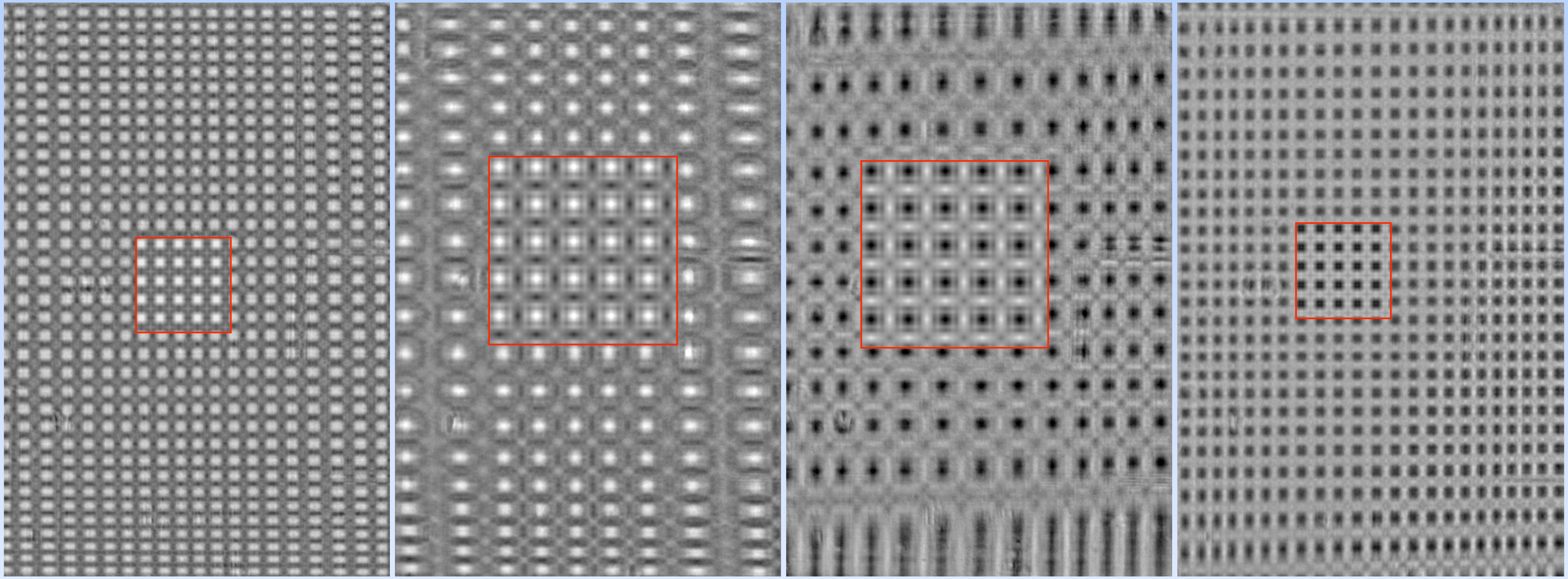
$D = z_1 \; ; M = z_2/z_1$

Projection Microscopy

2 μm pitch grating (Si)
C. David, PSI

10 μm

Mirrors fully illuminated
0.2 (H) x 1.1 (V) mm, E=20.5 keV

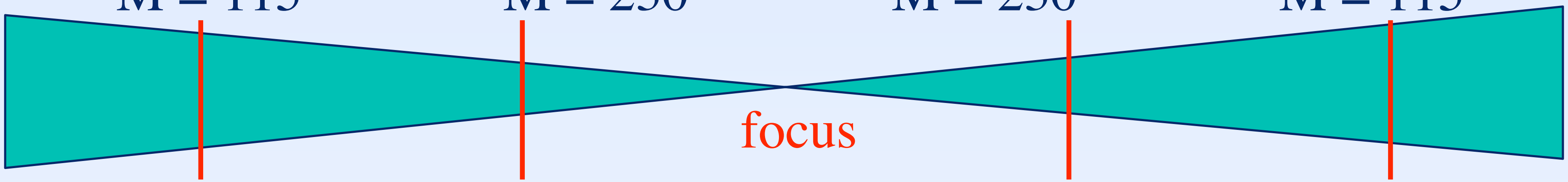


D = -20 mm
M = 115

D = -10 mm
M = 230

D = 10 mm
M = 230

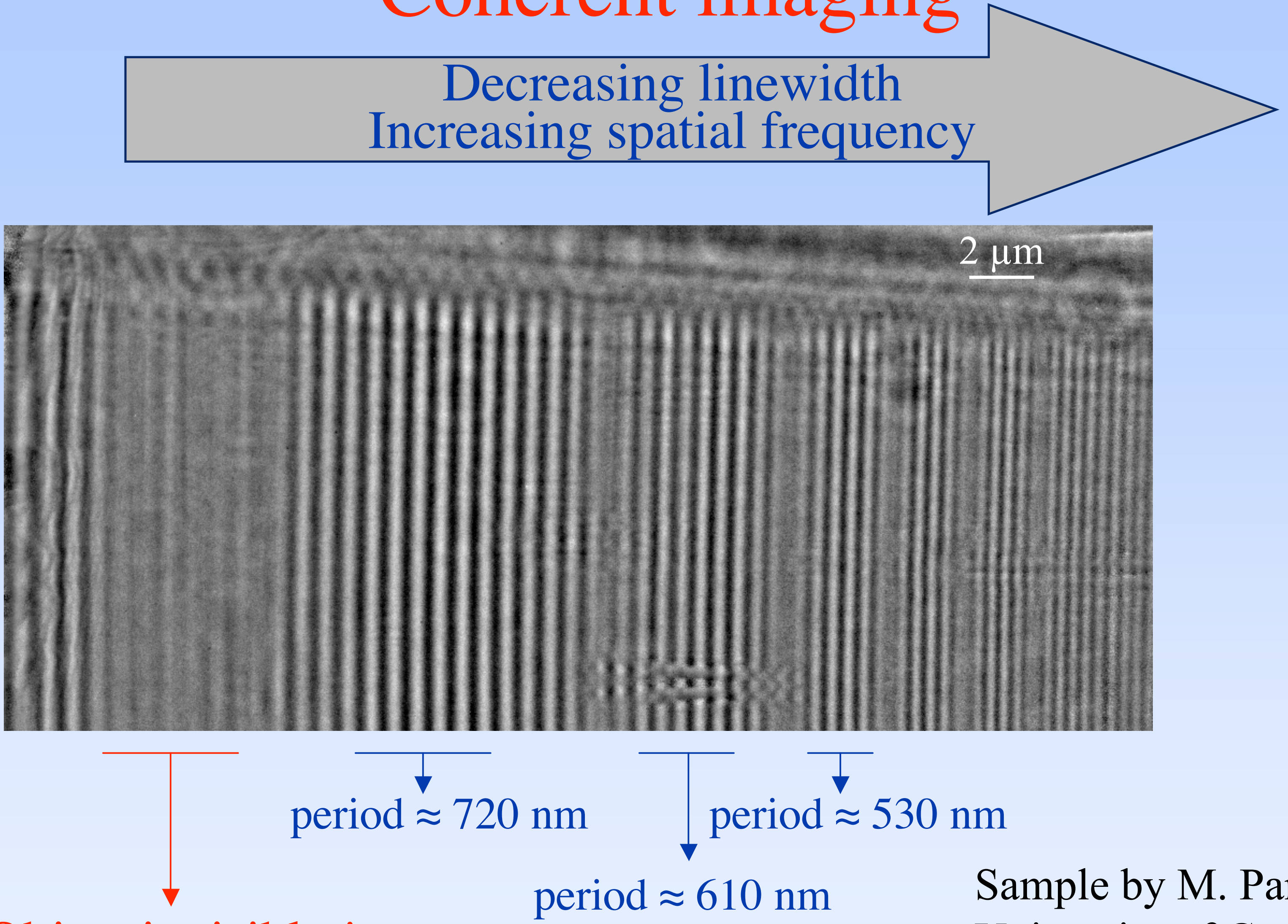
D = 20 mm
M = 115



Exposure time = 0.4 s ! (16-bunch, saturation CCD)

P. Cloetens, O. Hignette

Coherent imaging



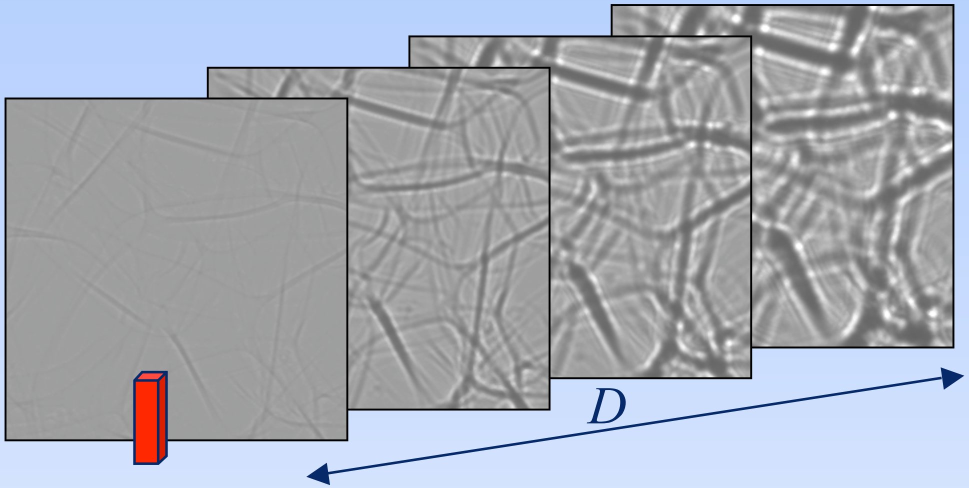
Sample by M. Panitz,
University of Goettingen

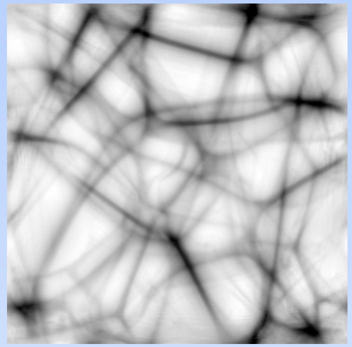
Contrast depends strongly on period or spatial frequency

Phase retrieval

Poster 6.106

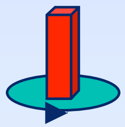
1) phase retrieval with images at different distances

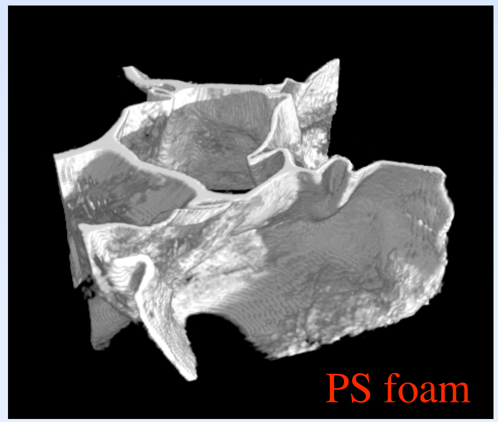




Phase map

2) tomography: repeated for □ 1000 angular positions



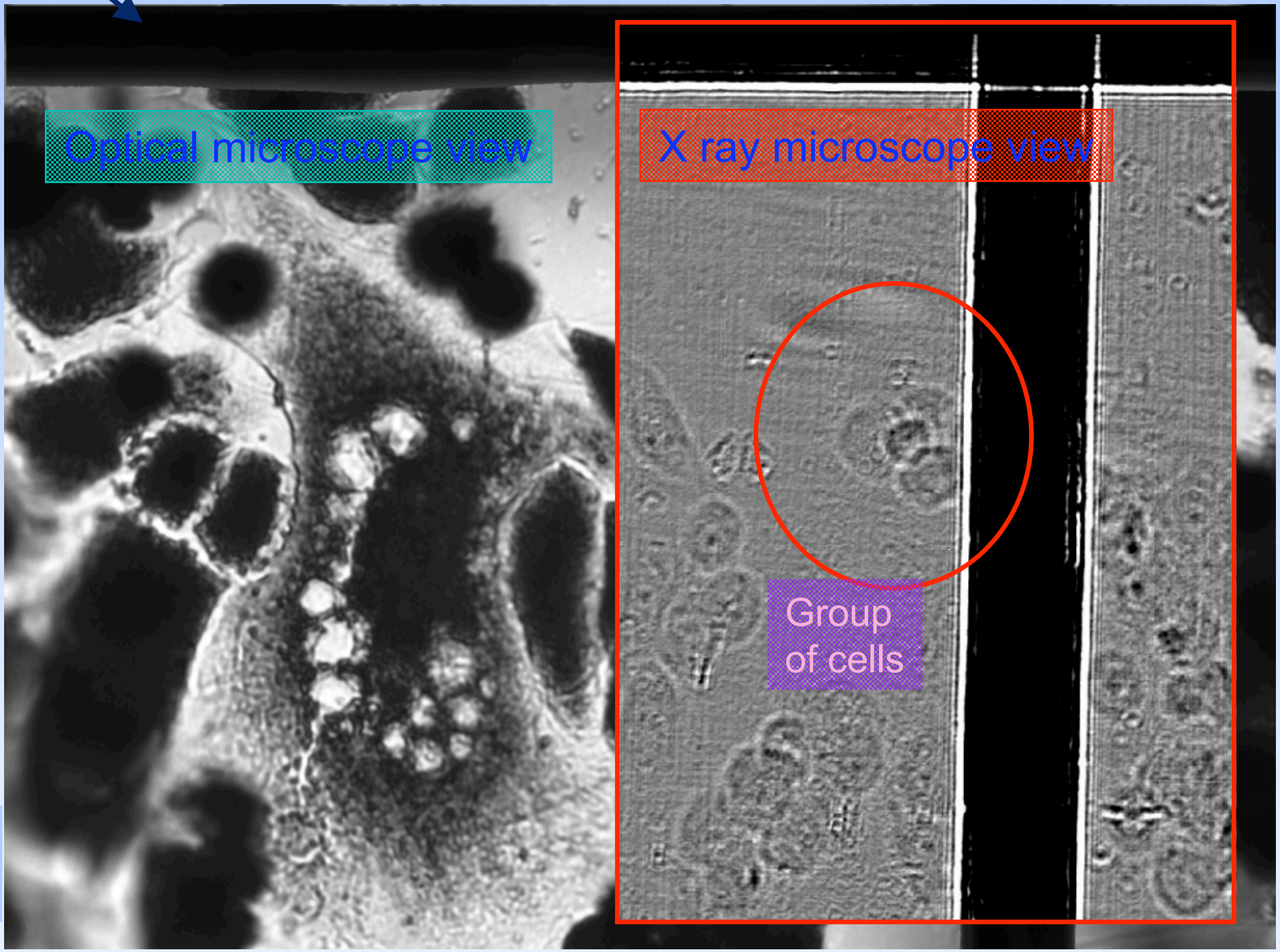


3D distribution of □ or the electron-density
improved resolution
straightforward interpretation
processing

P.Cloetens et al., Appl. Phys. Lett. 75, 2912 (1999)

Projection Microscopy

Cu fiber



25 μm

Cellular imaging

Cancerous cells
S. Bohic

Projection Microscopy

Cancerous cells
S. Bohic

Towards focus

10 μm

D = 50 mm
M = 45

D = 30 mm
M = 75

D = 10 mm
M = 230

E = 20.5 keV
Exposure time = 0.3 s ! (16-bunch)

No X-ray optics behind the sample → dose efficient

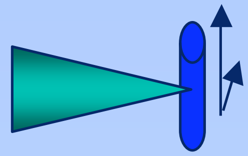
P. Cloetens, W. Ludwig

Applications: Fluorescence

Cancerous cells treated with anti-cancer drug (Cisplatin)

Step = 0.3 μm, 0.9 sec/pt

40 μm



K

Pt

E = 15 keV

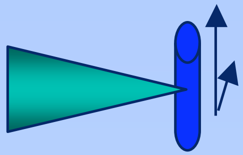
Phase Contrast

Fe

P. Cloetens,
W. Ludwig,
S. Bohic

Applications: Fluorescence

Cancerous cells (Coll. S. Bohic- ID22)



50 μm

K

Pt

Step = 0.5 μm
1 sec/pt

Fe

Traces of Pt-based drug can be imaged
New opens in elemental cellular chemistry

<u>Adapted length scale for</u>

- sub-cellular structures (cell organelles)
- unicellular organisms (bacteria, algae and fungi)

P. Cloetens, W. Ludwig, S. Bohic

Prospects

Nano-tomography

Starting in October

Next generation synchrotrons

Present source size limit with KB:

90 nm x 50 nm

(Source size)

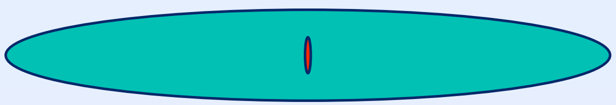
(Mirror quality: ion beam figuring)

Diffraction

ultimate focus: 20 nm (h) x 12 nm (v)

Much more efficient:

0.2 mm x 1.2 mm of a 20 mm x 3 mm beam



Conclusions

- Sub (90 nm)^2 focusing made 'easy' for hard X-rays
- High flux (5 10^{11}ph/s @ 200 mA)
  thanks to KB efficiency, multilayer optics
- Micro-structure: fast nano-radiography, nano-tomography
  +: fast and dose efficient full-field imaging
  good resolution (sub 100 nm in both directions)
  - : distorsions due to mirror quality
  +/-: defocused image = phase contrast image
- Fluorescence: probe for sub-micron element mapping (2D - 3D)

Acknowledgments

KB Optics

- Optics Group ESRF
  - O. Hignette, Ch. Morawe, A. Rommeveaux, Ph. Villermet
- Technical Services ESRF
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- Beamline contact
  - P. Bernard
- SciSoft ESRF
  - E. Chinchio
- Bliss ESRF
  - D. Fernandez

Experiments

- S. Zabler, K. Fezzaa (APS), W.K. Lee (APS)

Samples

- Ch. David PSI
- M. Panitz University of Goettingen