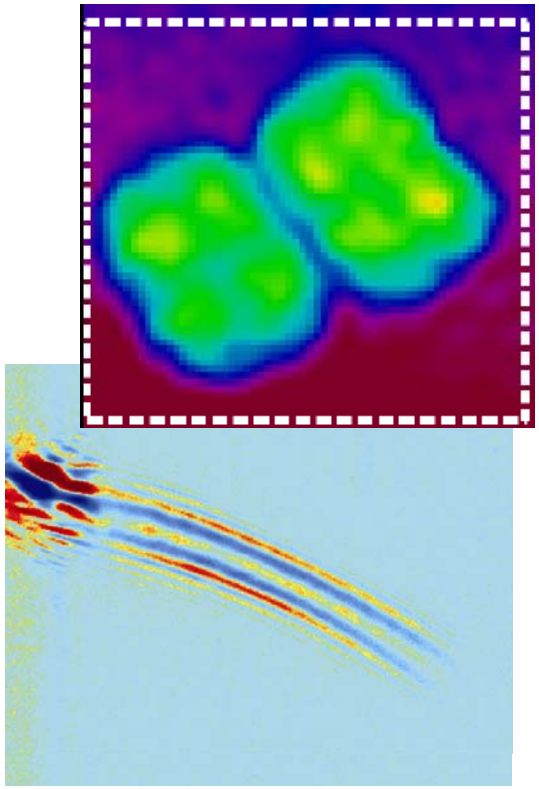
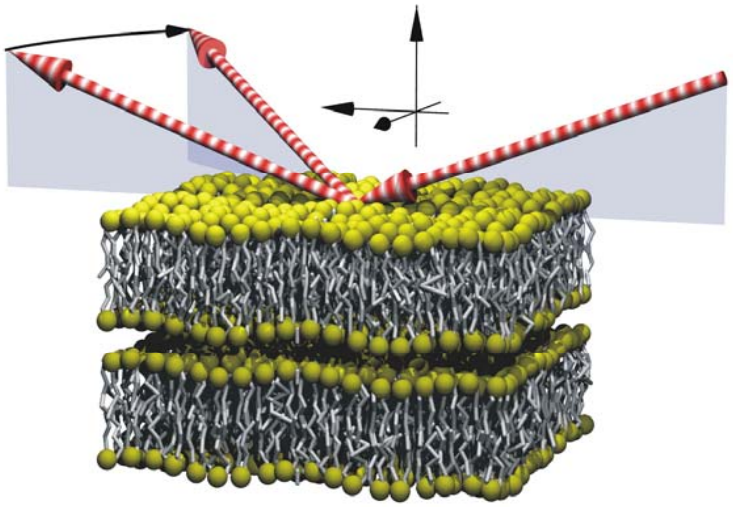
Biological Imaging and Dynamics of Biomolecular Assemblies at XFEL

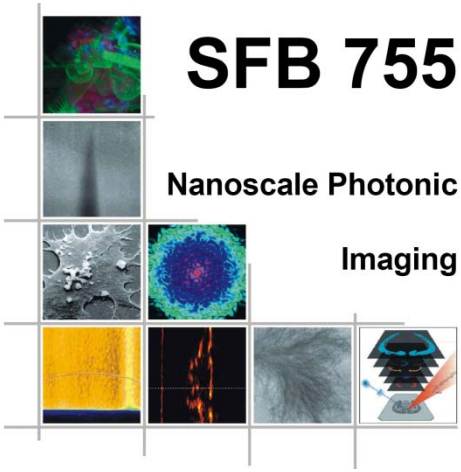




Andre Beerlink, Tim Salditt
Institut für Röntgenphysik,
Universität Göttingen

Sebastian Aeffner, Britta Weinhausen *membrane biophysics*
Klaus Giewekemeier Michael Mell *cells, coherent imaging*
Marius Priebe *microliquid jet*
Sebastian Kalbfleisch, Sven Krüger, Henrike Neubauer *holography*

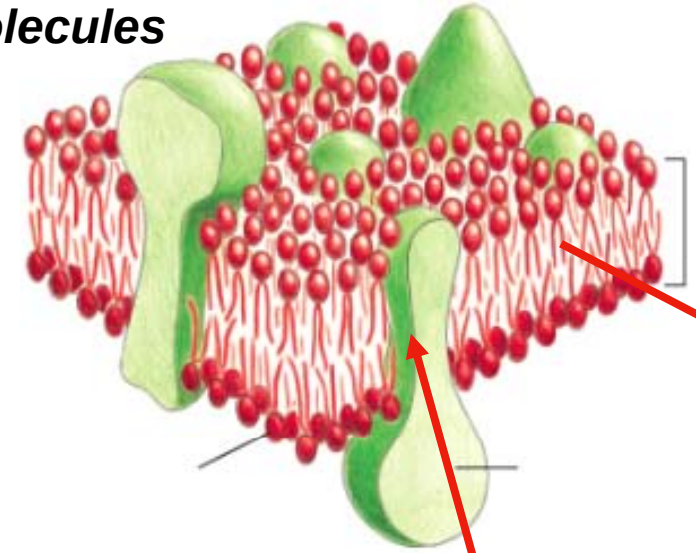
M. Rheinstädter, ILL Grenoble, McMaster Hamilton *bilayer dynamics*
P. Cloetens, R. Tucoulou , H. Metzger, E. Ziegler, L. Wiegert *ESRF*
Jochen Hub, Bert de Groet, MPI biophys. Chemie *MD simulation*

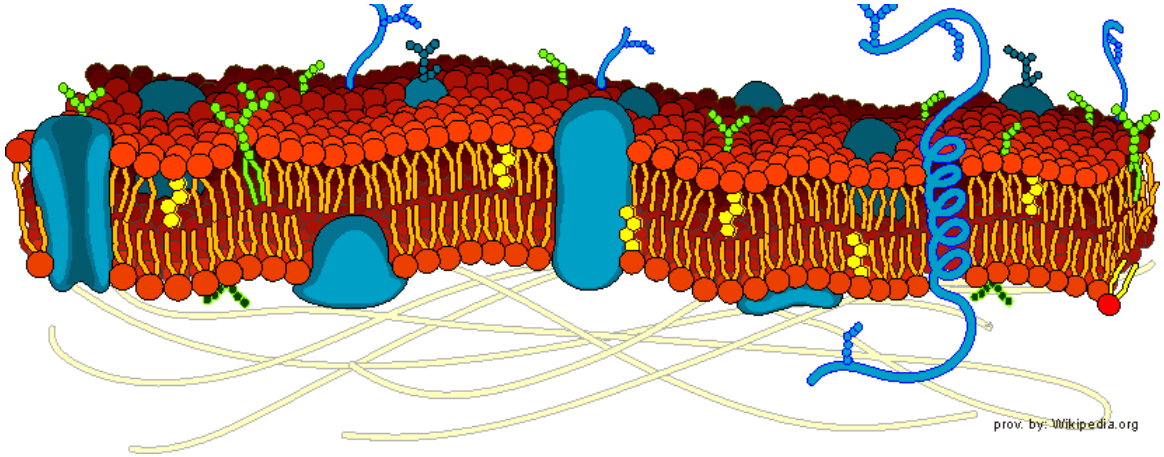


Support by **DFG, BMBF, SOFTCOMP, VI Helmholtz**

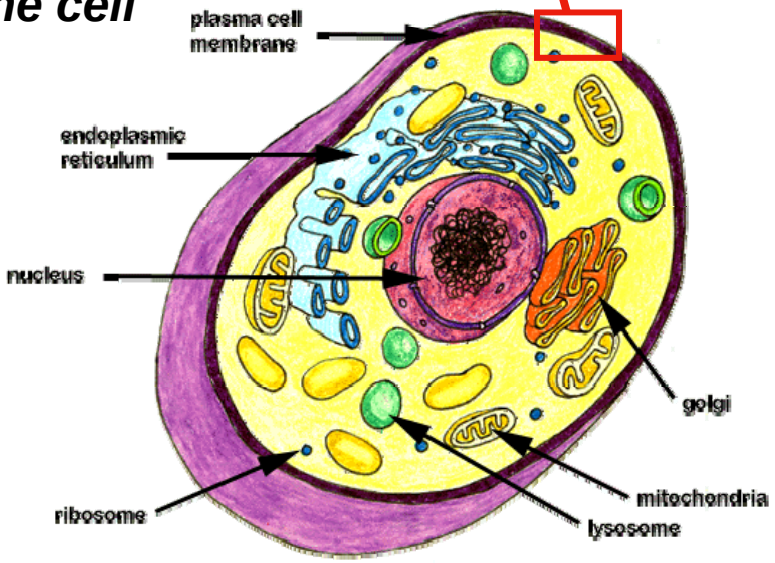
Structure of multi-component membranes by x-ray scattering and MD simulations

the membrane as the most important interface in biology: assemblies of biomolecules

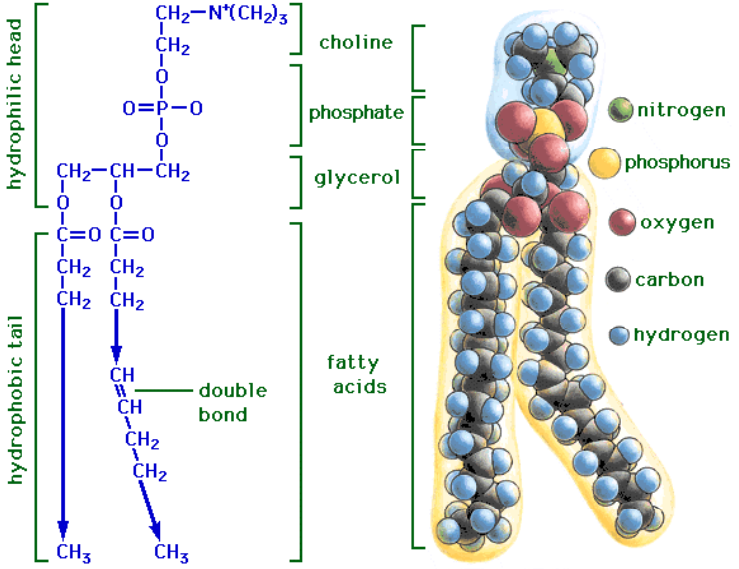




the cell



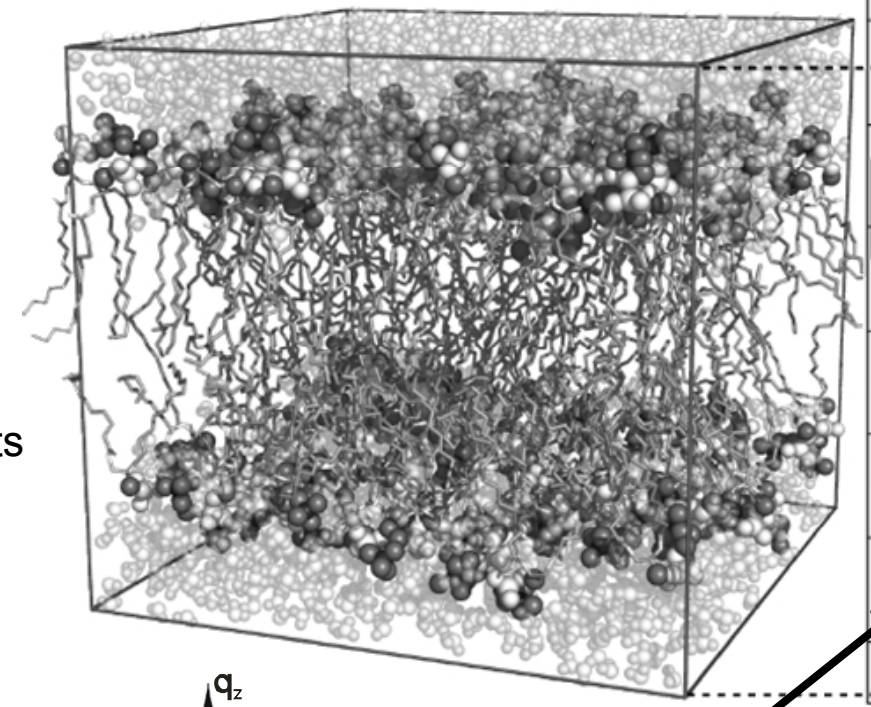
the molecules: <u>lipids</u>, cholesterol, proteins

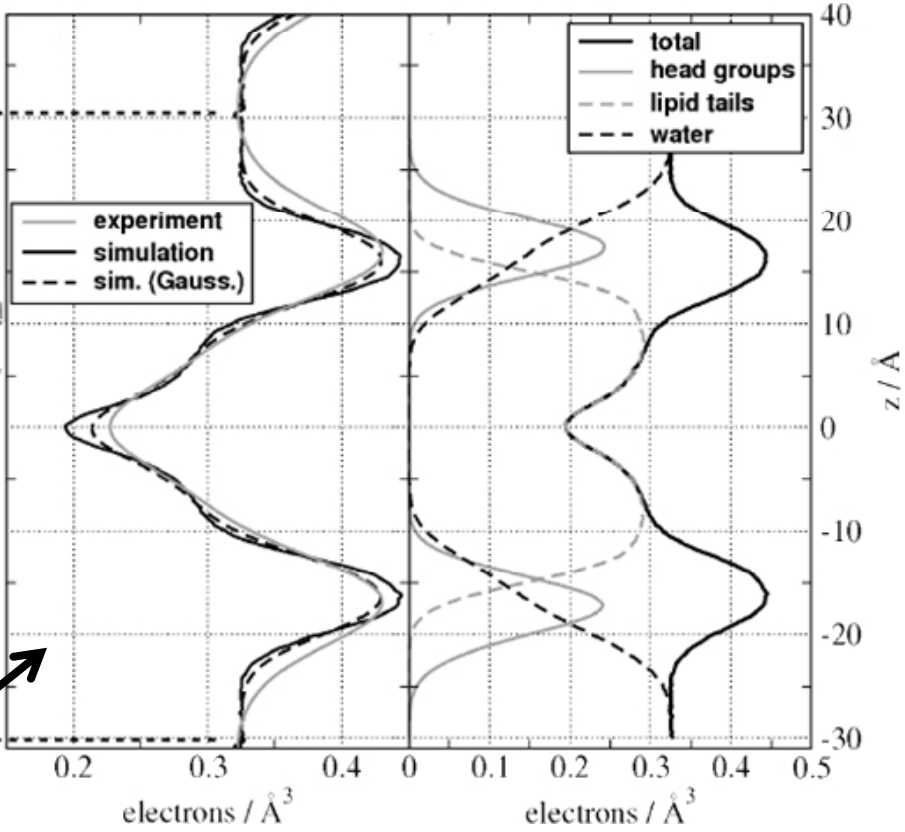


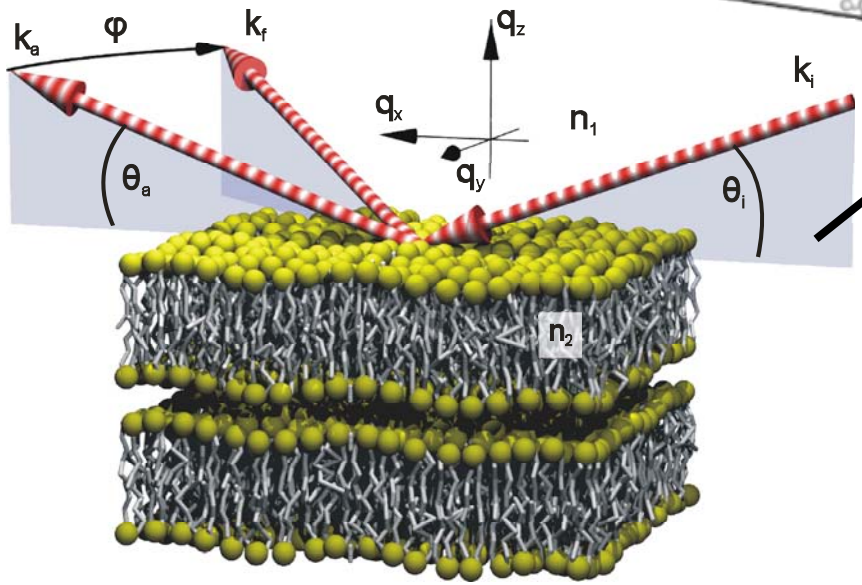
→ **multi-component model systems**

Structure of multi-component membranes by x-ray scattering and MD simulations

high consistency of experiments an MD simulations





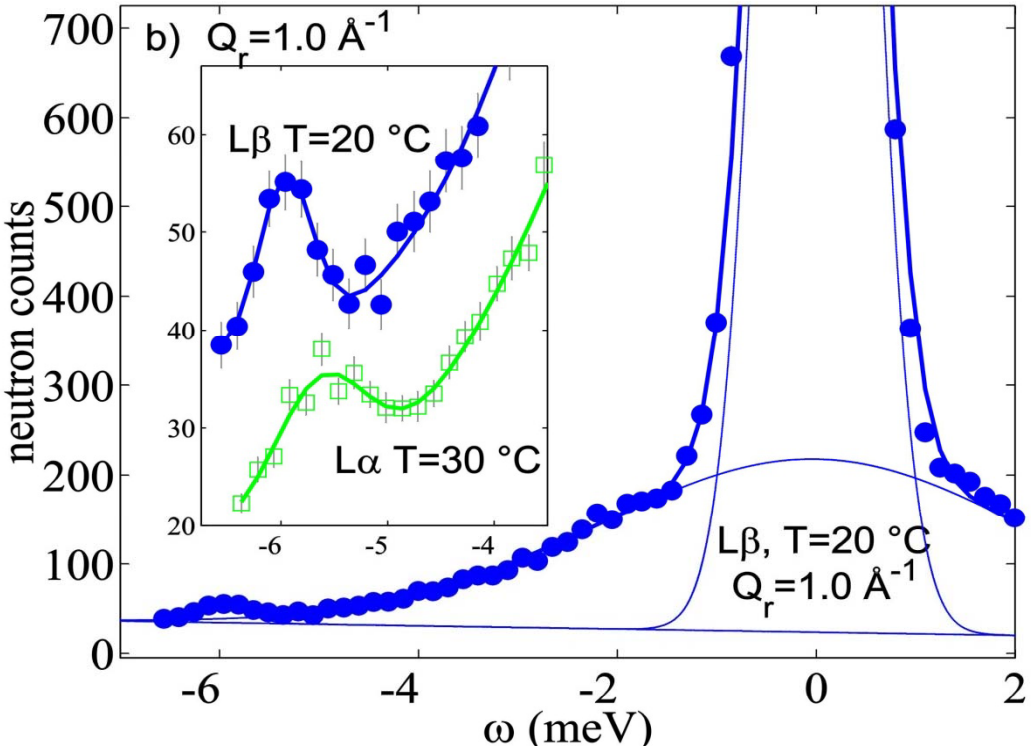


description of model membrane systems:

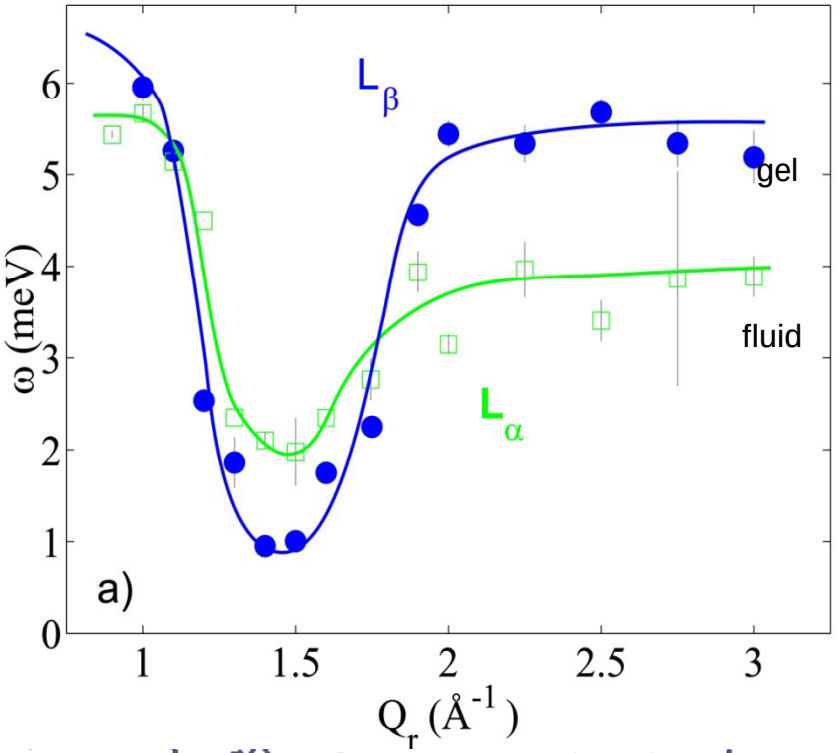
$$\rho_M(z) = \rho_{water} + \Delta_{\max} \sum_{n=1}^{N} f_n \nu_n \cos\left(\frac{2\pi n z}{d}\right)$$

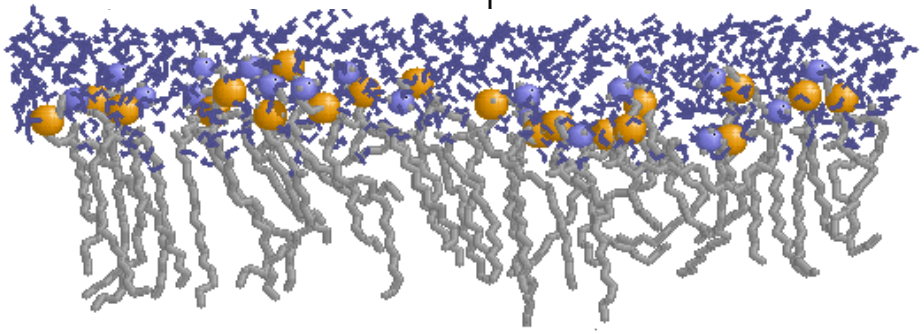
T. Salditt, J. Phys., Cond. Mat. 17 (2005)
T. Salditt, Current Opinion in Structural Biology 13, 467-478 (2003)
T. Salditt, M. Vogel, W. Fenzl, Phys. Rev. Lett. 90, 178101 (2003)

Inelastic neutron scattering with Three-Axes Spectrometer: chain dynamics on picosecond time scale



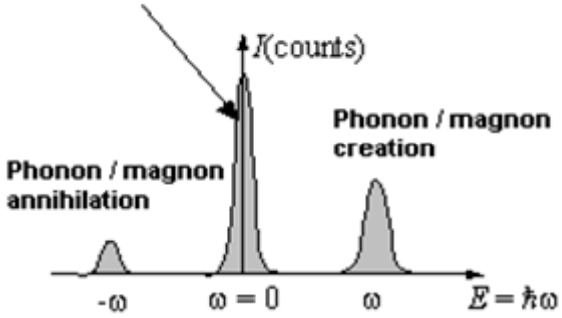
Dispersion relations in the gel- and the fluid phase of the DMPC model membrane

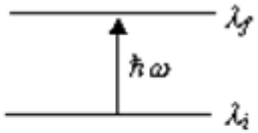




Quasi-elastic peak
(Zero energy transfer)

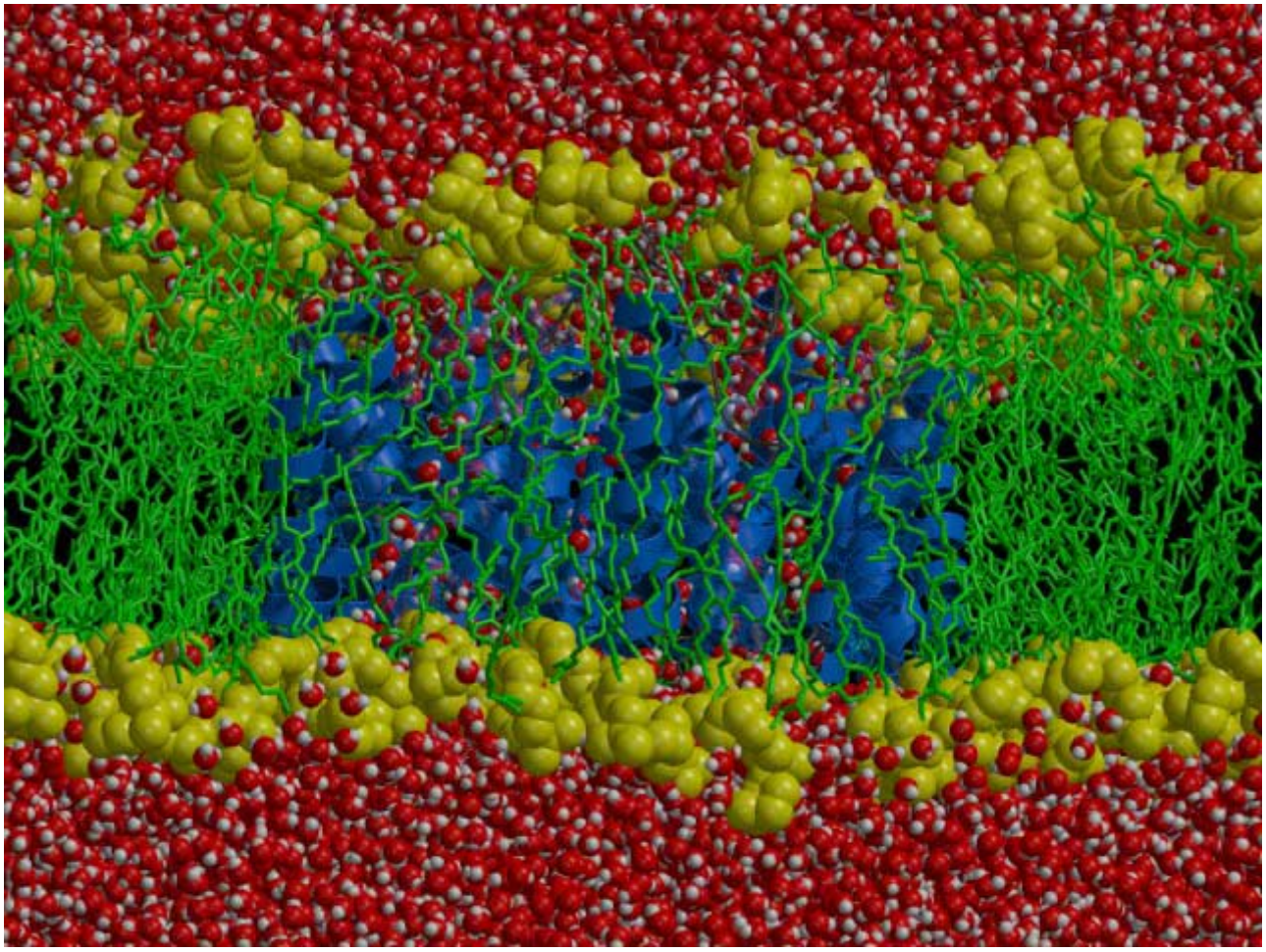
$$S(-\mathbf{Q},-\omega) = \exp\left(\frac{-\hbar\omega}{k_B T}\right) S(\mathbf{Q},\omega)$$





Rheinstädter et al., PRL (2004), (2006)
Salditt, Rheinstädter, Neutrons in Biology, Springer, (2006)

Dynamics of membranes (MD simulation)



Today:

static systems

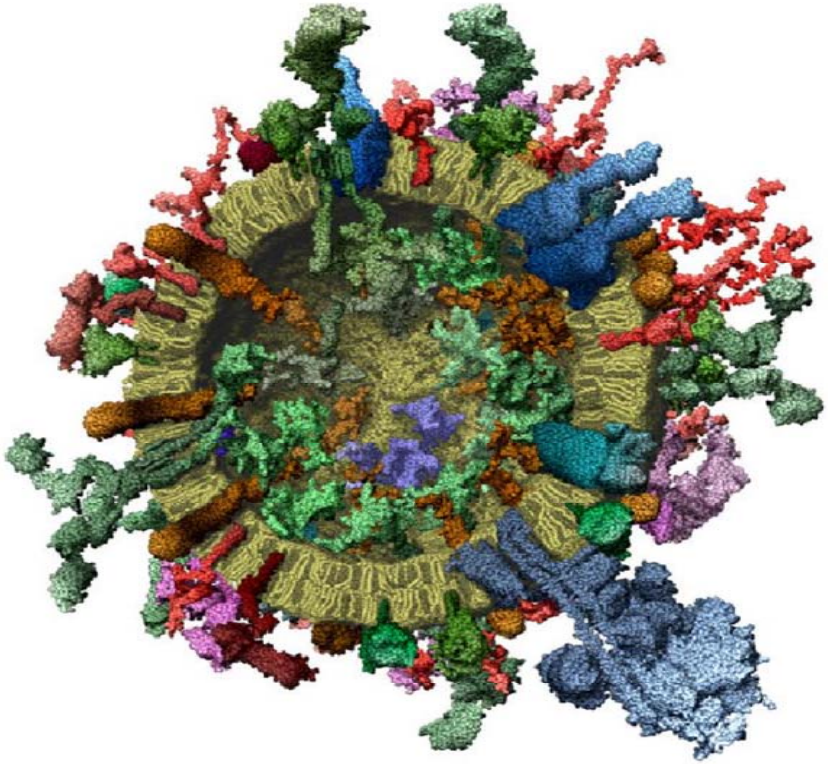
equilibrium dynamics by inelastic neutron scattering

Tomorrow at XFEL:

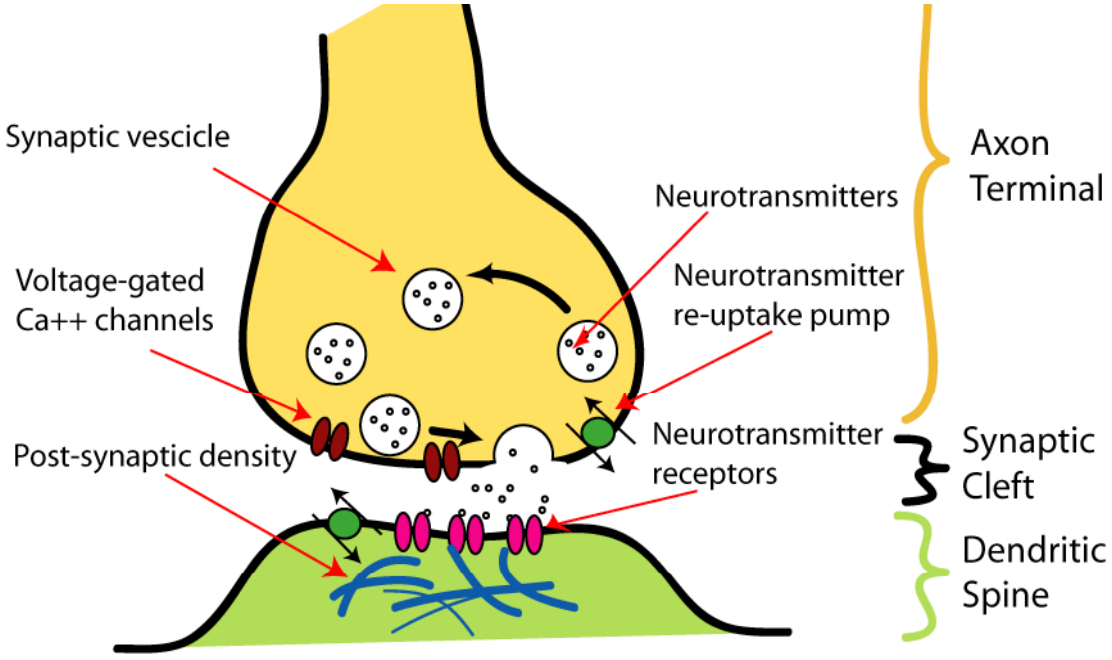
pump probe / out-of-equilibrium dynamics

pico-second time resolution

Membrane structure of synaptic vesicles by SAXS

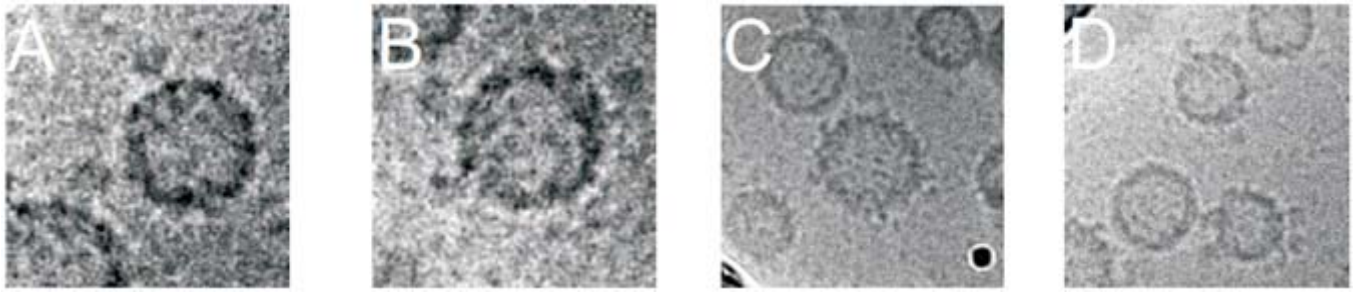


S. Takamori et al., Cell 127 (2006)



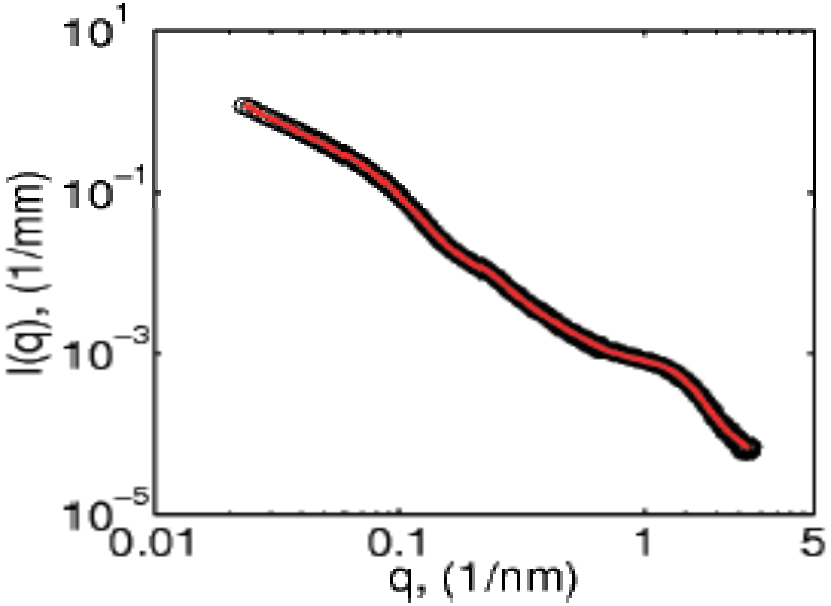
H. Lodish et al., Molecular Cell Biology (2000)
B. Alberts et al., Molecular Biology of the Cell (2002)

complemetary studies: Cryo-EM, Dynamic Light Scattering

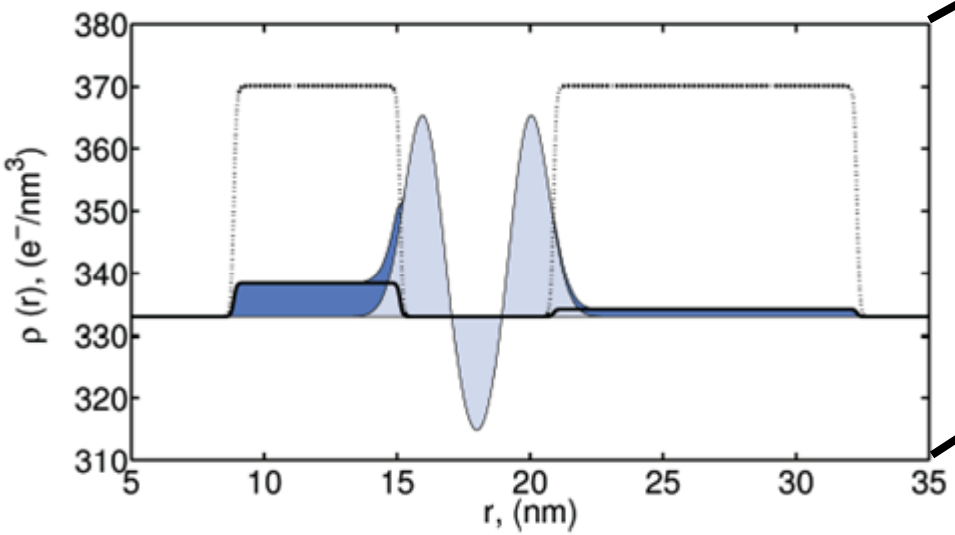


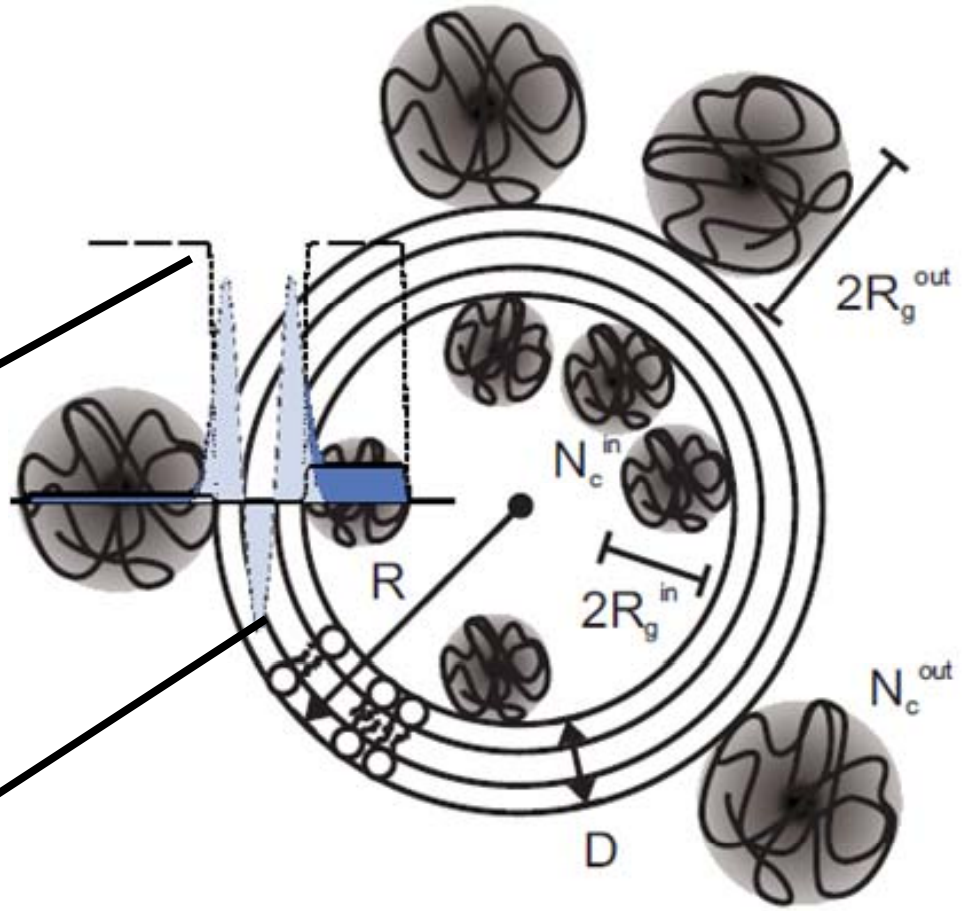
Cooperation Reinhard Jahn
and Helmut Grubmüller, MPI-bpc

Membrane structure of synaptic vesicles by SAXS

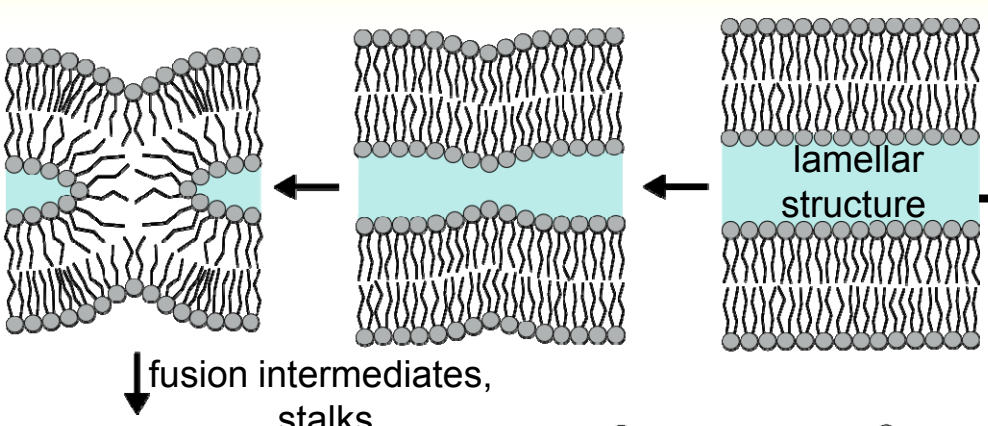


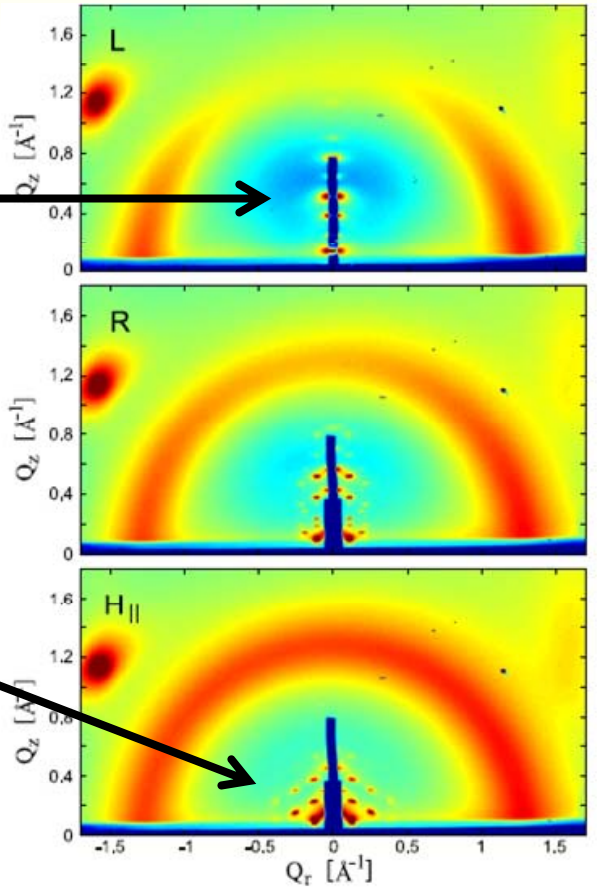
- electron density profile from large ensemble SAXS experiments
- increased complexity by high polydispersity





Formation of a stalks and pores during membrane fusion



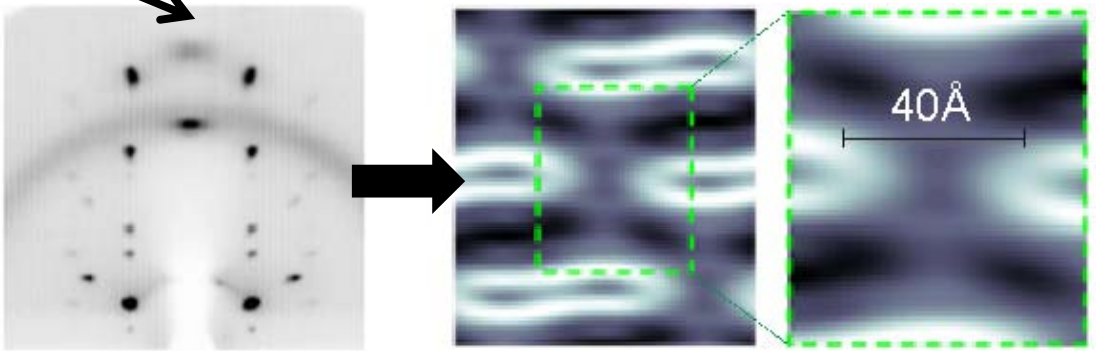


Today:

equilibrium intermediate structures by scattering on large ensembles

Tomorrow at XFEL:

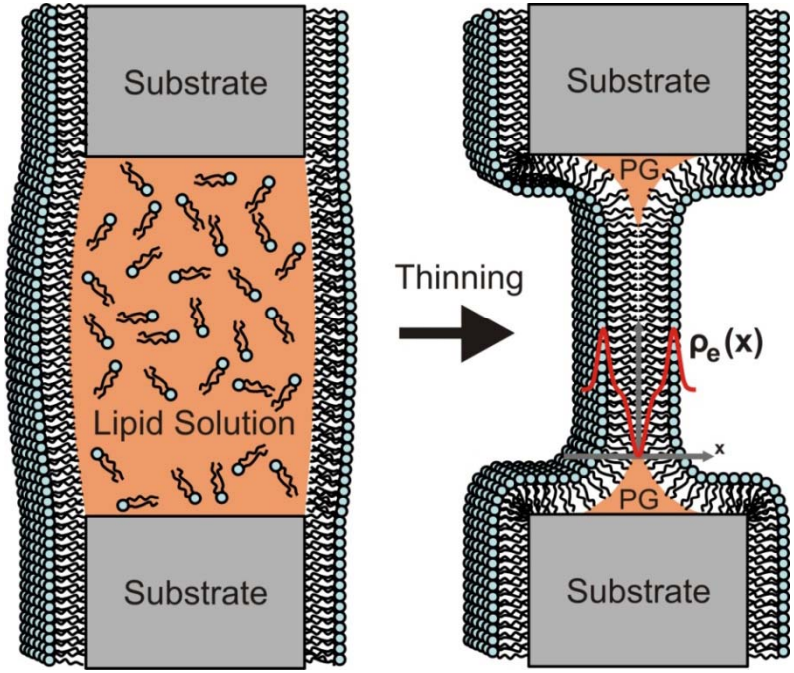
- *transient fusion pore by imaging*
  > 2D crystal in XFEL
- *spatial and temporal resolution of synaptic fusion event*



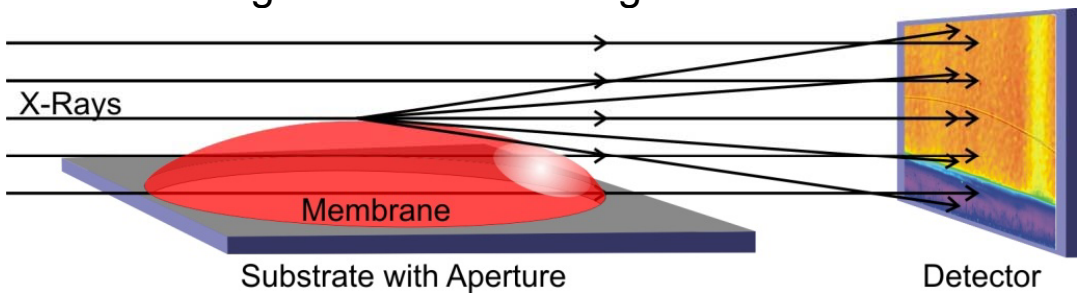
S. Aeffner et al, EPJE 2009

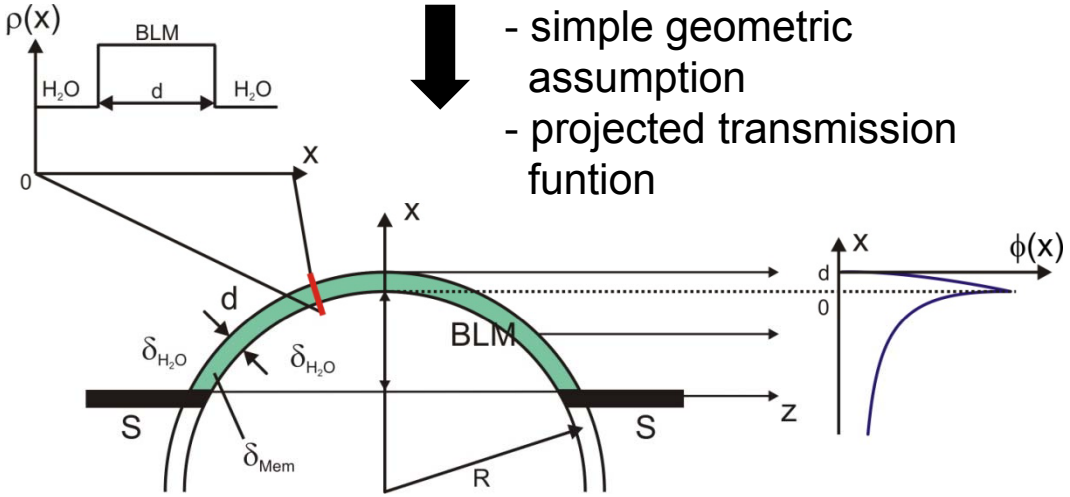
Hard x-ray phase contrast imaging of Black Lipid Membranes

freestanding model membrane system

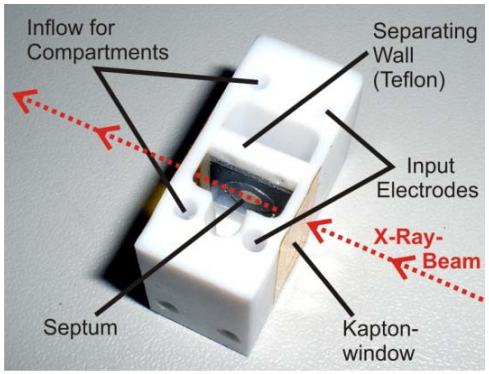


bulged membrane image formation





- simple geometric assumption
- projected transmission funtion

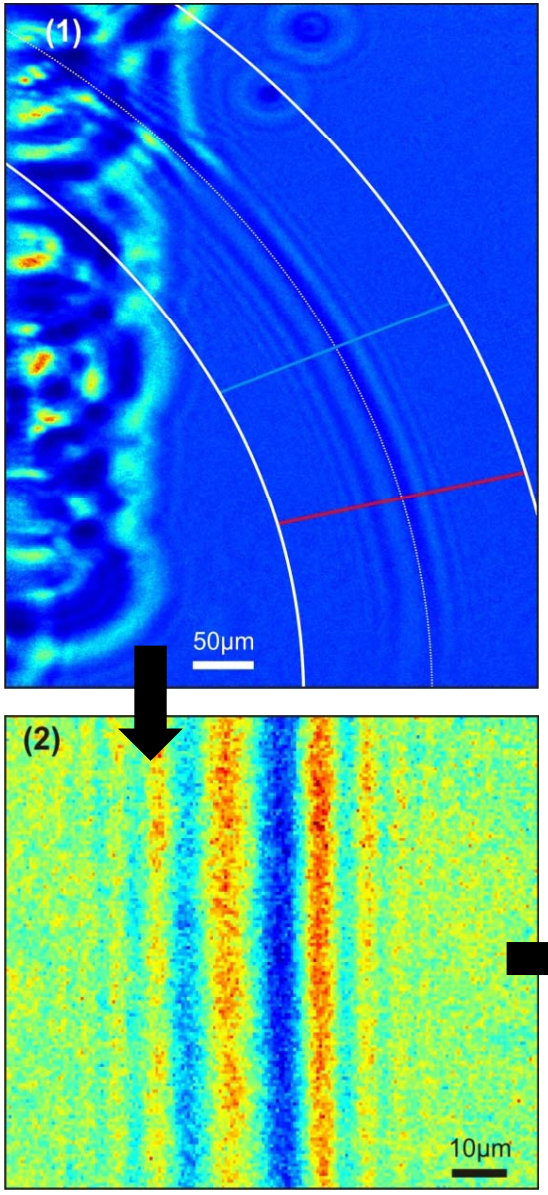


Studies of asymmetric conditions far away from equilibrium (i.e. fluctuations, electroporation, current flow, osmotic pressure)

A.Beerlink, E. Ziegler, T.H. Metzger, T. Salditt, Langmuir 24 (2008)
A. Beerlink, M. Mell, M. Tolkiehn, T. Salditt, APL, accepted (2009)

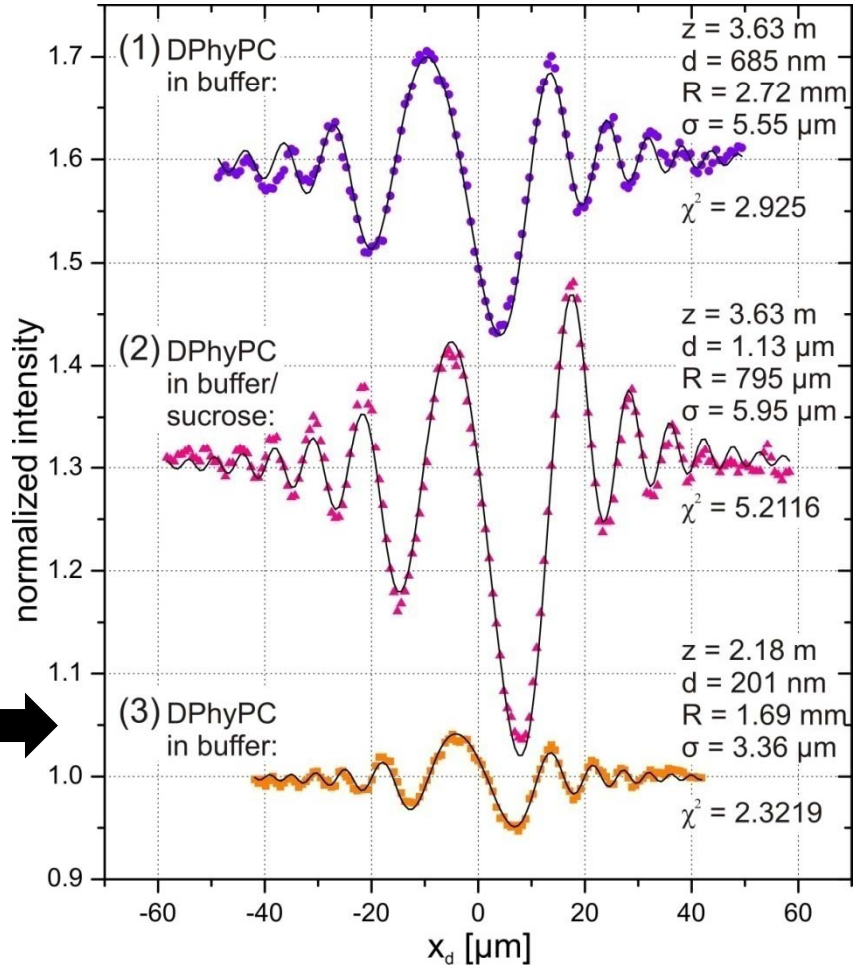
Hard x-ray phase contrast imaging of Black Lipid Membranes

phase contrast imaging

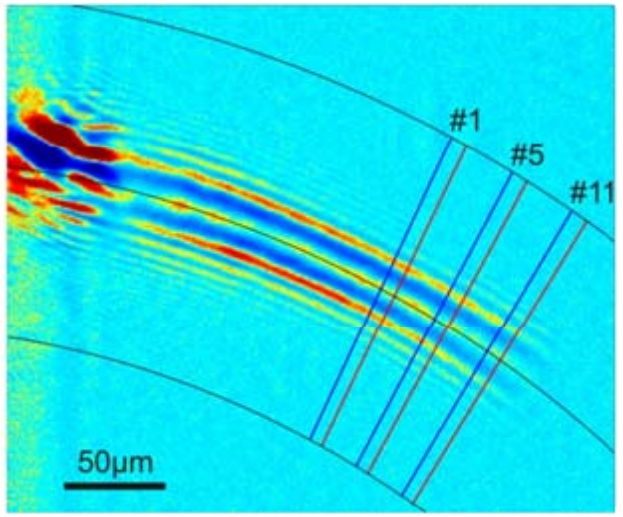


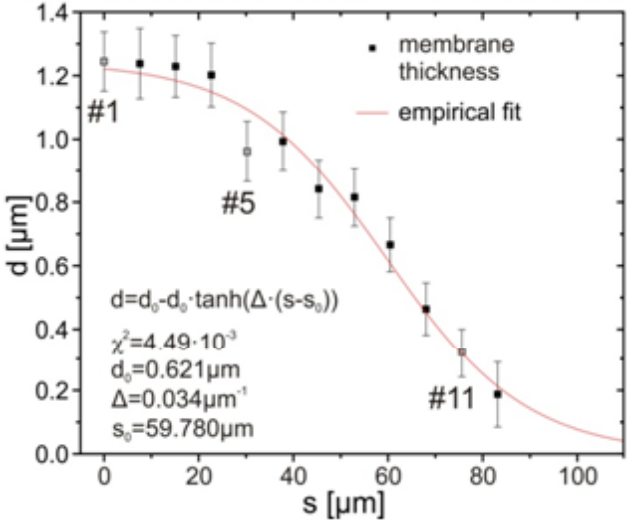
data fitting by slabwise propagation of projected transmission function in Fresnel approximation

$$E(x_d, z) = E_0 \sqrt{\frac{k}{2\pi z}} e^{-i\frac{\pi}{4}} \int_{-\infty}^{\infty} e^{i\varphi(x)} e^{\frac{ik(x-x_d)^2}{2z}} dx$$

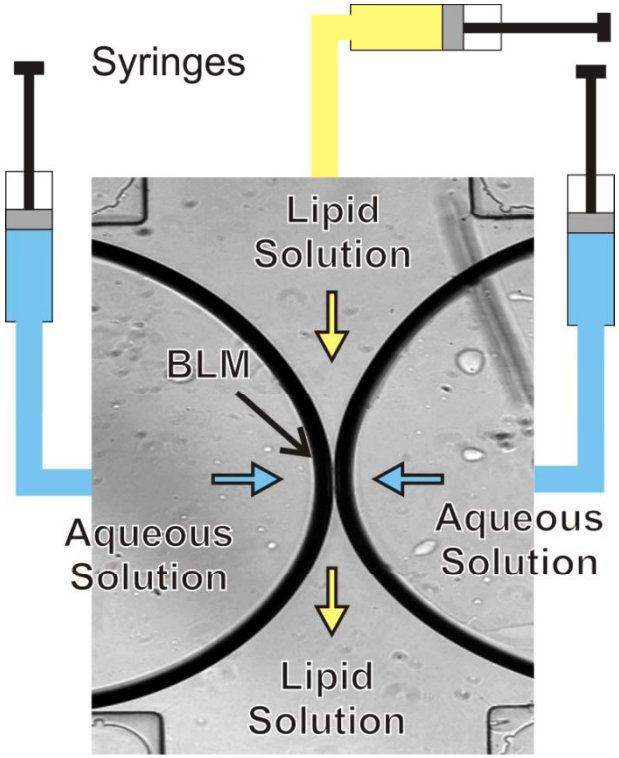


imaging of thinning process

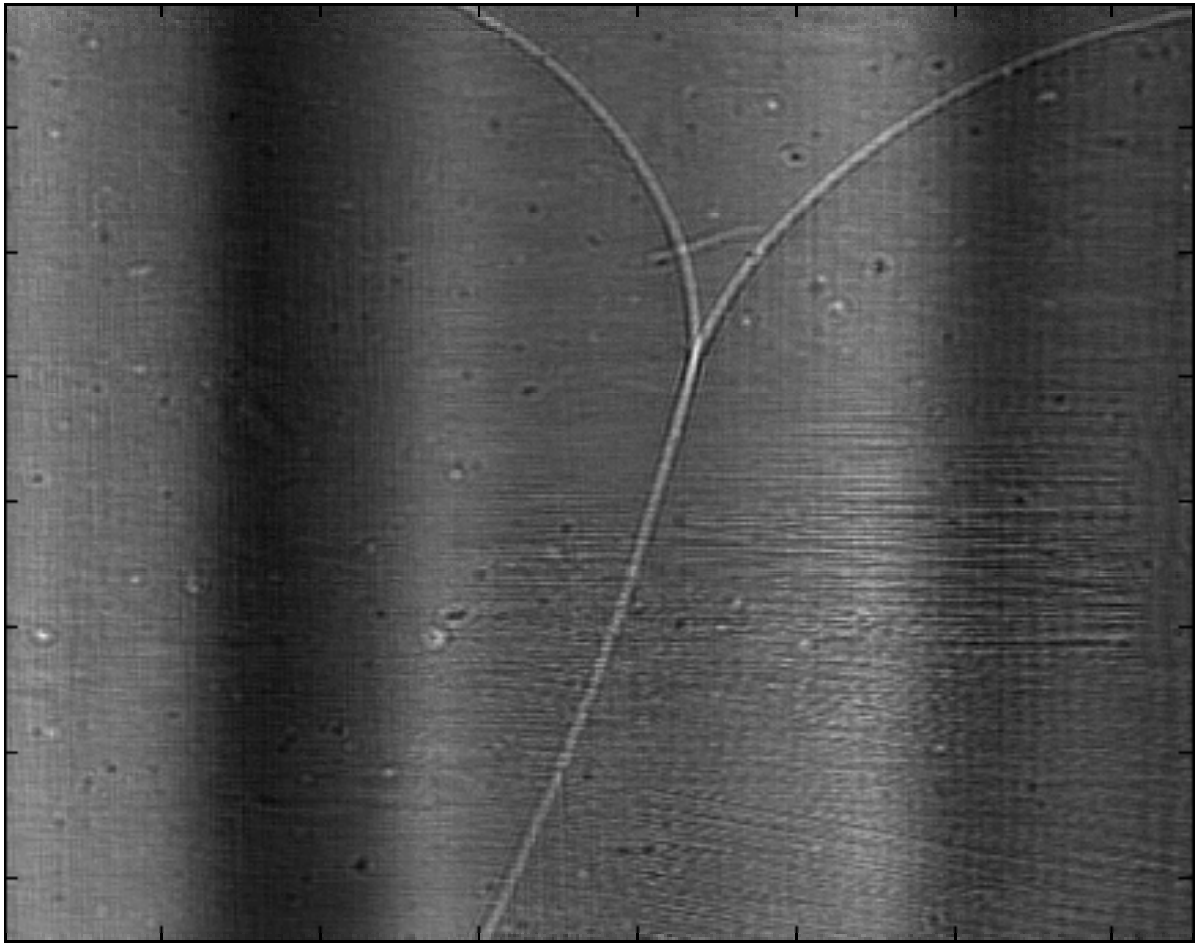




Recent results from BLM imaging in microfluidic devices (ID22NI)



fusion of two lipid monolayers to become a single bilayer (zipping effect)



Today:

- spatial resolution down to 200nm

- low time resolution

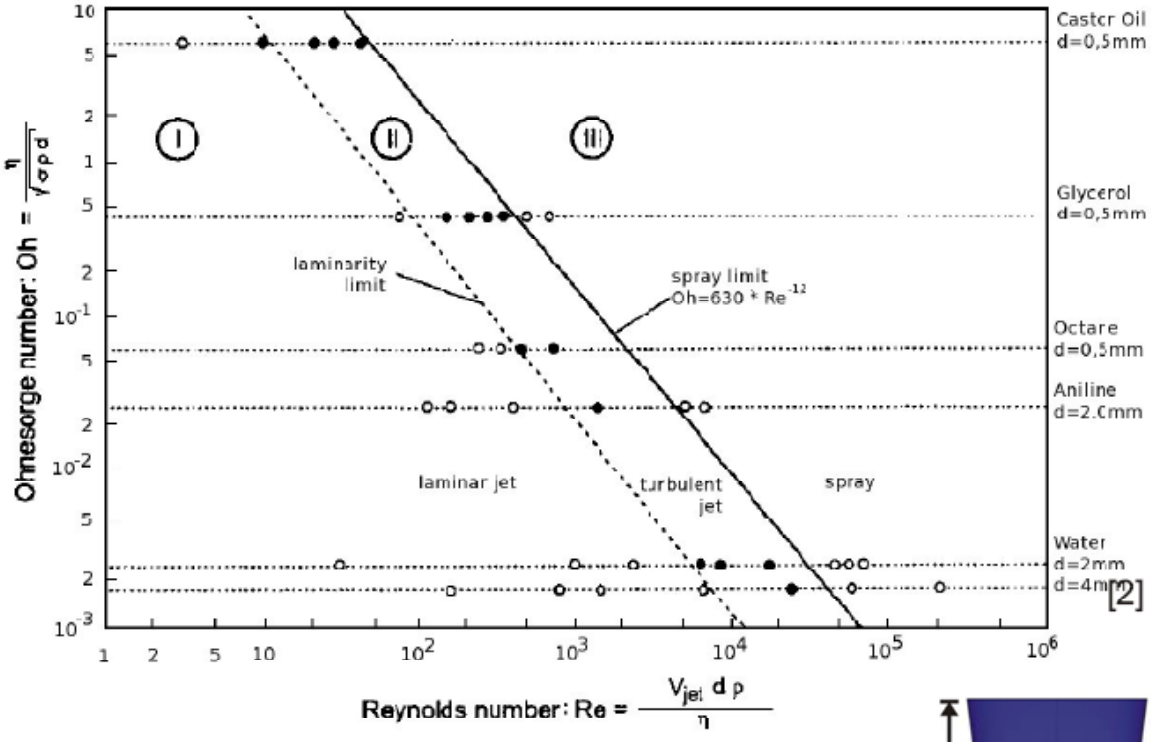
Tomorrow at XFEL:

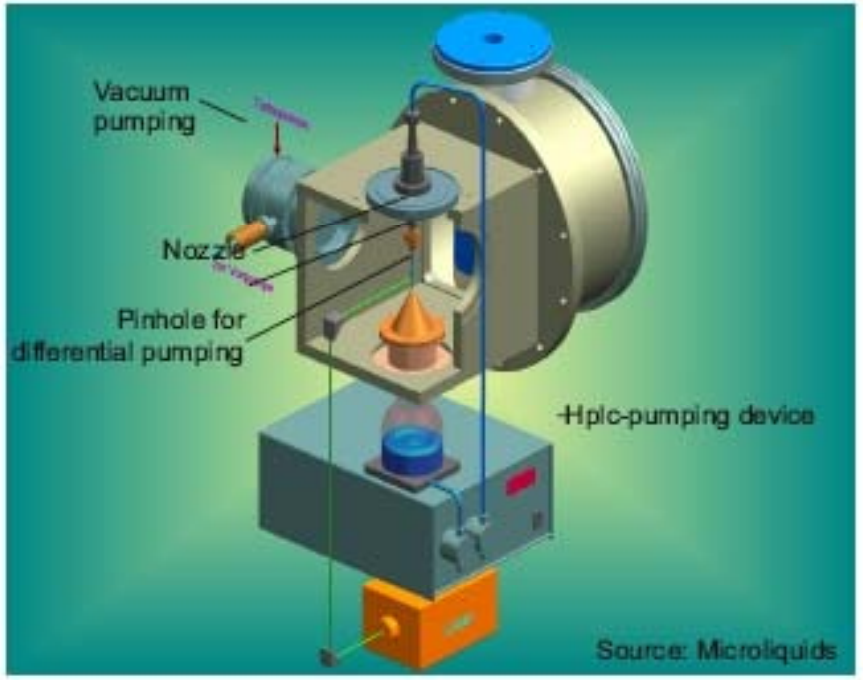
- short puls time resolution

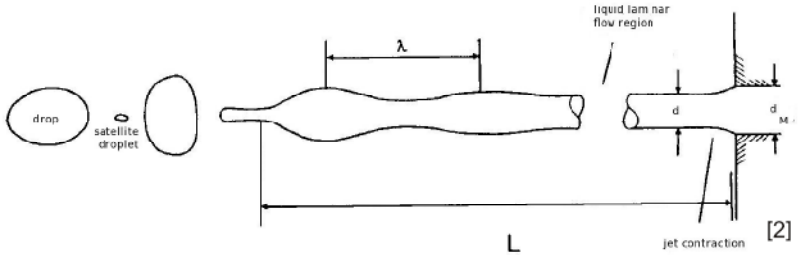
- coherence > increased phase contrast

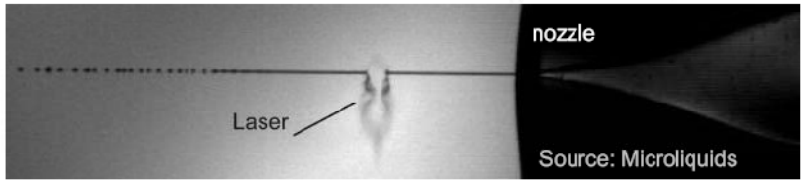
> high spatial resolution > resolution of single lipid bilayer (5nm)

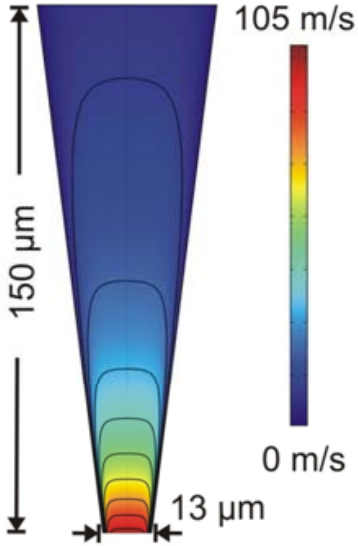
A vacuum compatible microfluidic jet





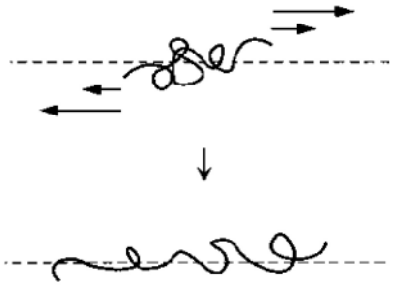




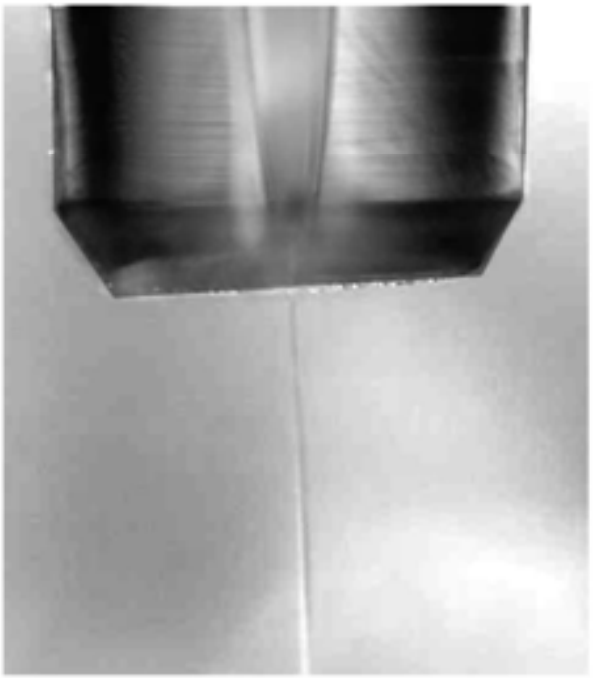


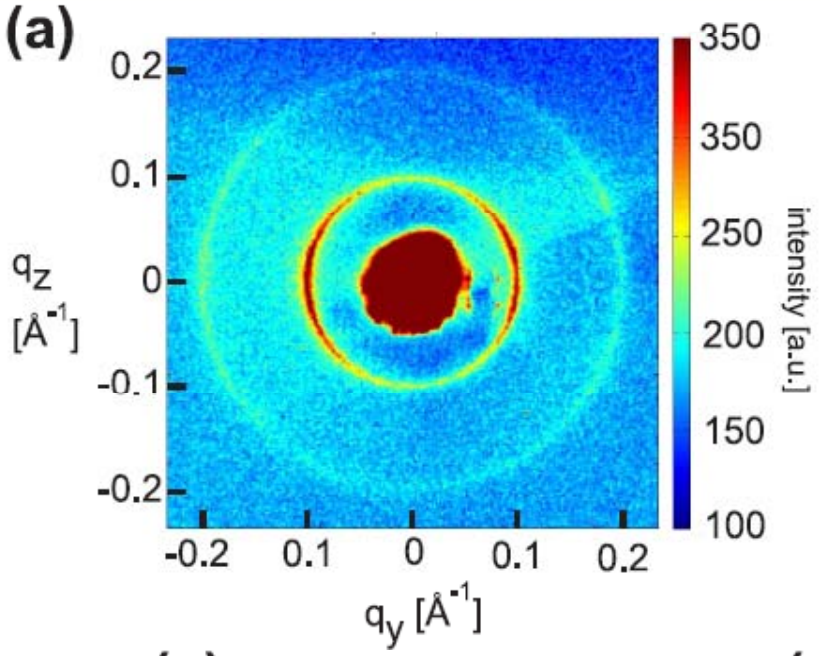
SAXS experiments with high shear rates: $10^7\ s^{-1}$

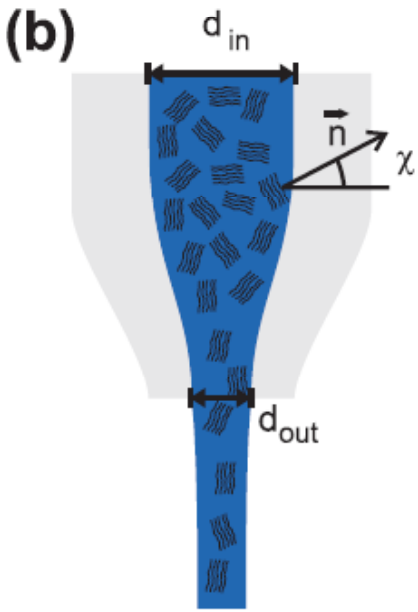
$$\dot{\gamma} = \left| \frac{dv_z}{dr} \right|$$



Microfluidic jet: hydrated biomolecules in XFEL beam





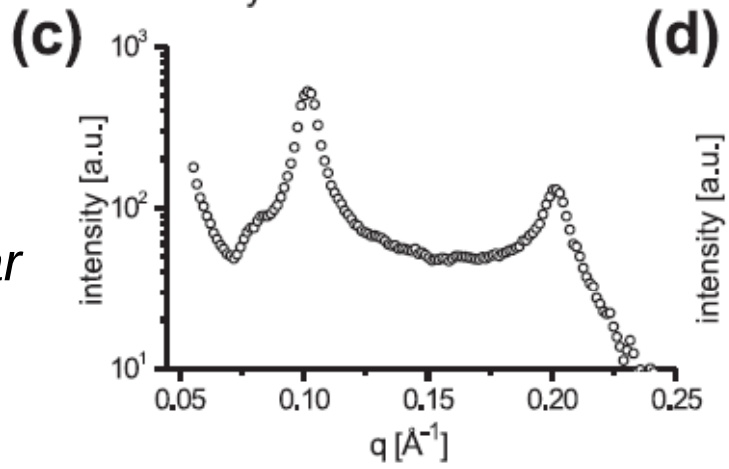


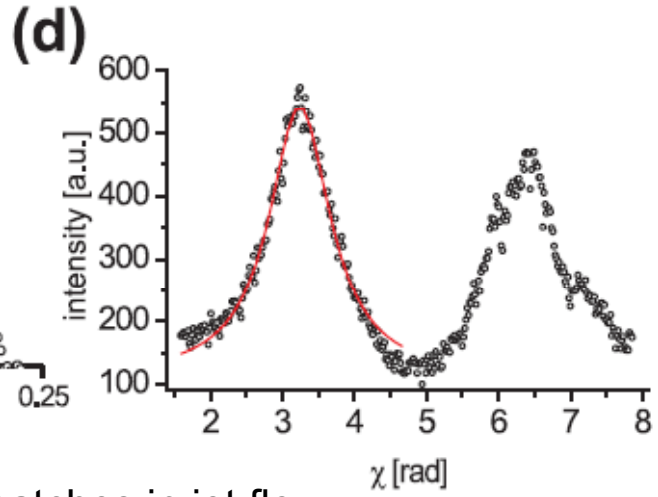
Today:

SAXS of hydrated biomolecular assemblies and ensembles

Tomorrow at XFEL:

single particle imaging / SAXS





- orientation of Lipid patches in jet flow
- determination of relaxation times.

Priebe et al. , submitted

X-ray Imaging of Cells:
present and (bright) future …

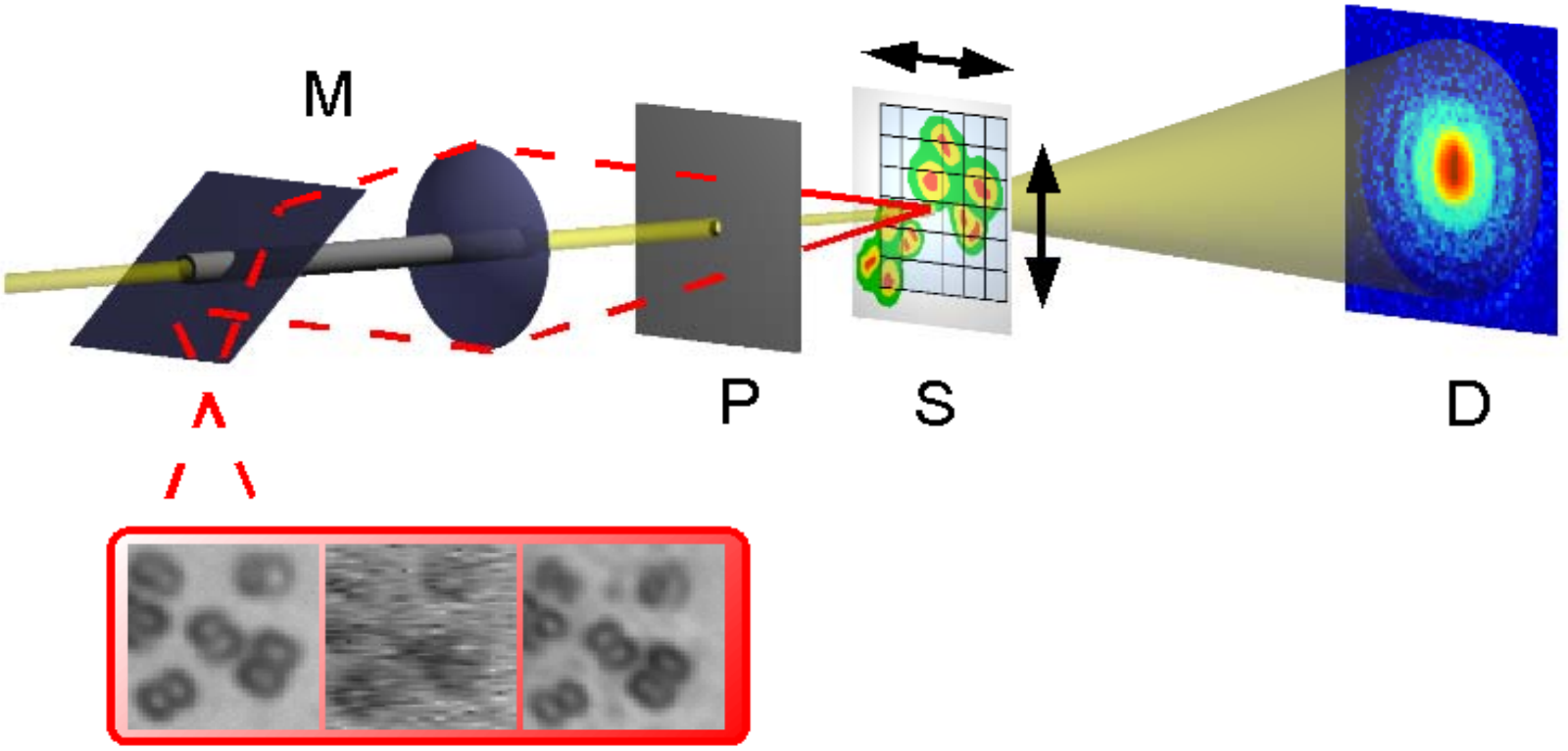
Experimental setup at cSAXS beamline (SLS)

51 x 51 scan positions, dwell time 1 s, 400 nm grid spacing, 6.2 keV

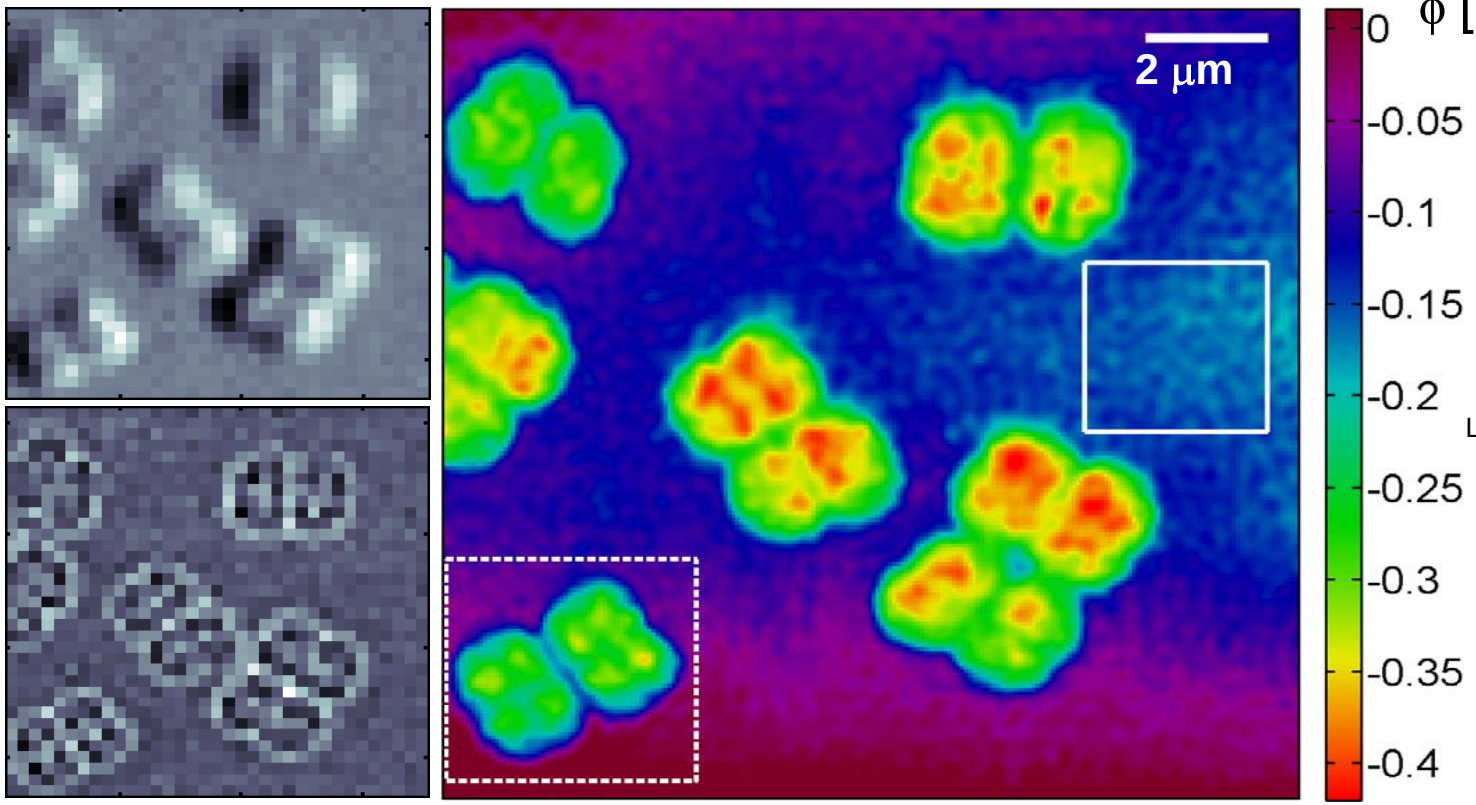
Average fluence $9 \cdot 10^5$ ph/μm^2, total dose ca. $2 \cdot 10^3$ Gy

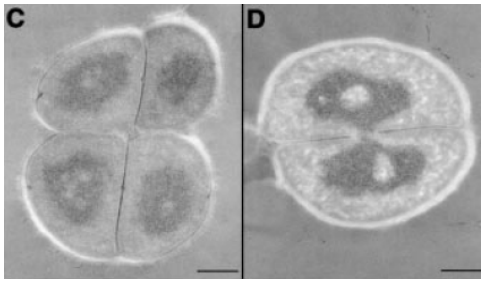
1.4 μm pinhole, defocus distance 1.4 mm

Pilatus detector at 7.22 m, on-axis optical microscope



Reconstructed phase



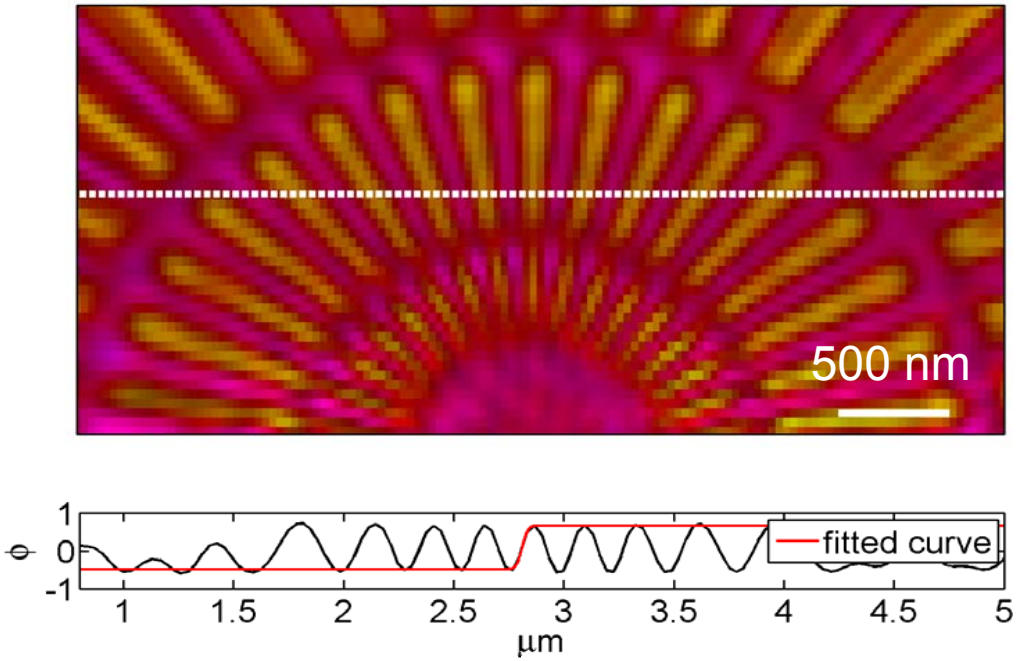


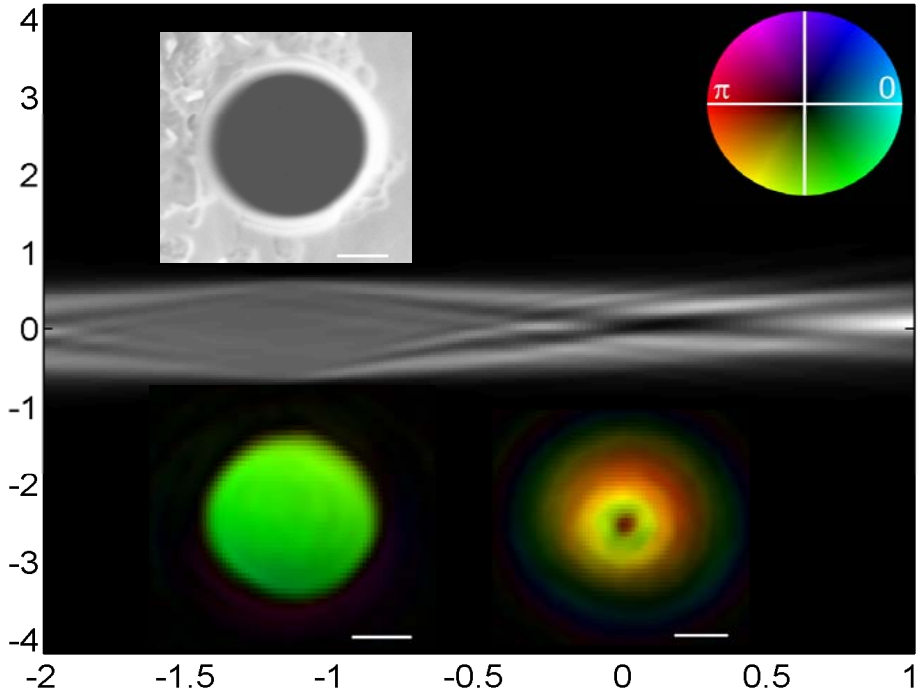
Levin-Zaidman S., Science **299** 254 (2003)

- Freeze-dried, unstained and unsliced cells
- Overall phase shift of single cell 0.25-0.3 rad (< 10% π), consistent with simulations
- 2500 iterations of SXDM algorithm, averaged over each 5th iterate, starting at 2000
- Half-period resolution 140

Giewekemeyer *et al.*, submitted

Object and probe reconstruction

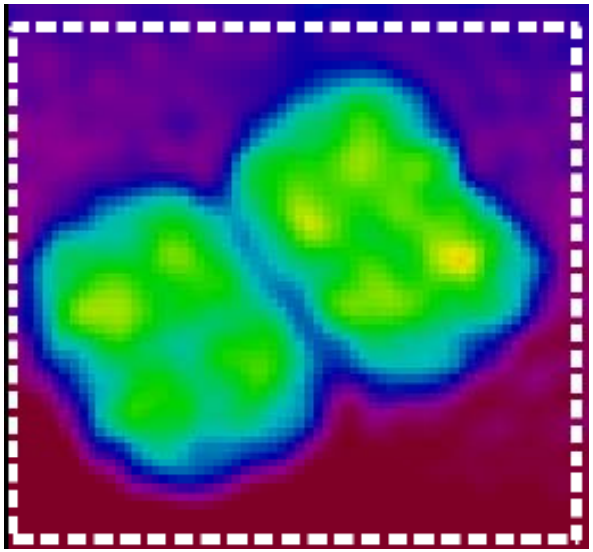




- 500 nm Ta test sample
- Resolution (FWHM) 50 nm, fluence $5.1 \cdot 10^6$ ph/μm^2
- Measured phase and amplitude change: 0.34π and 0.86 (expected: 0.34π and 0.88)

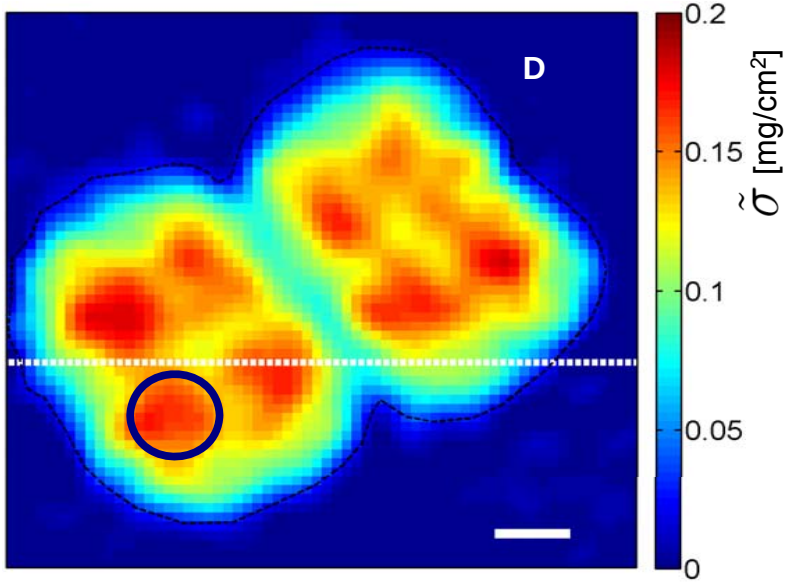
Quantitative mass density maps

$\phi(\mathbf{r})$



$$\tilde{\sigma}_m(\mathbf{r}) = -\left(\frac{2u}{\lambda r_0}\right) \cdot \phi(\mathbf{r})$$

$\tilde{\sigma}_m(\mathbf{r})$



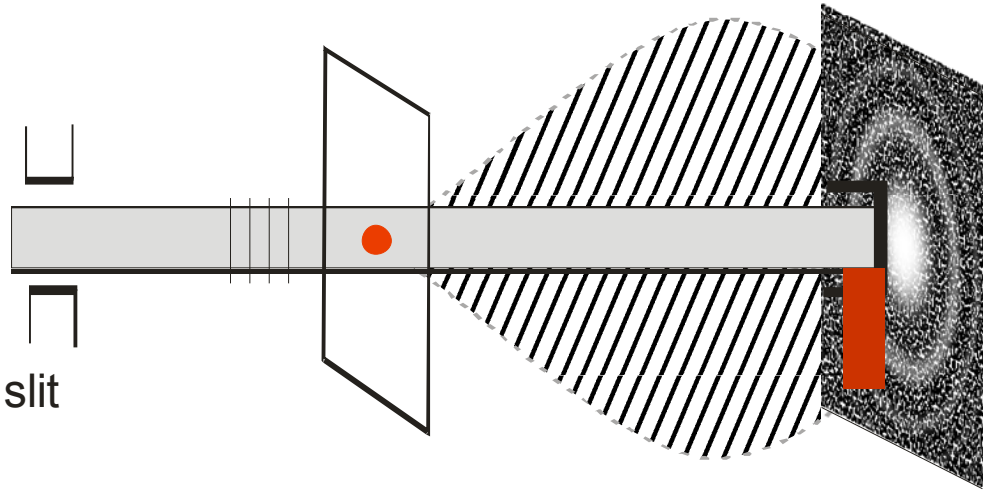
Absolute mass density $\sigma_m = \overline{A}/(2\overline{Z}) \cdot \tilde{\sigma}_m$

For biological material $\overline{A}/(2\overline{Z}) = 0.94$ ($H_{50}C_{30}N_9O_{10}S$, model protein)

Assume cell thickness between 1.5 and 2.5 μm → central density of 0.7-1.1 g/cm^3

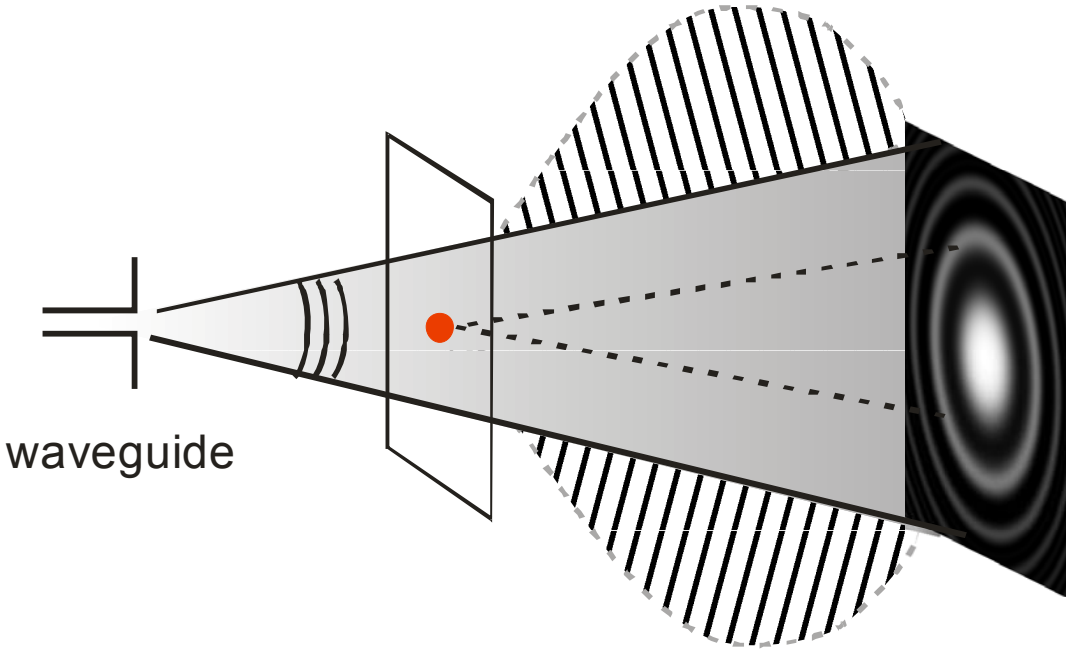
Experimental error of effective area mass density ca. 0.02 mg/cm^2

Design of the illumination function for coherent imaging



coherent diffraction (speckle):
iterative phase retrieval

beamstop: missing
low-q components

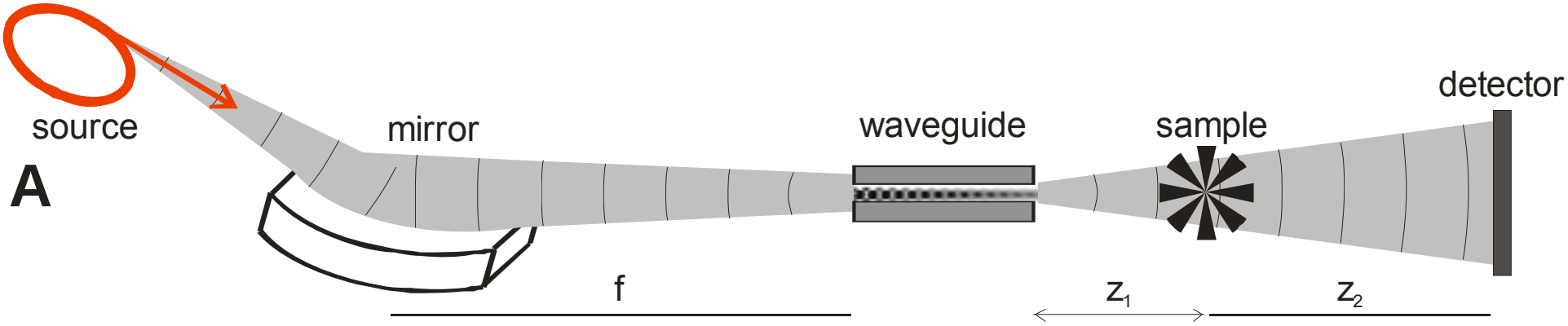


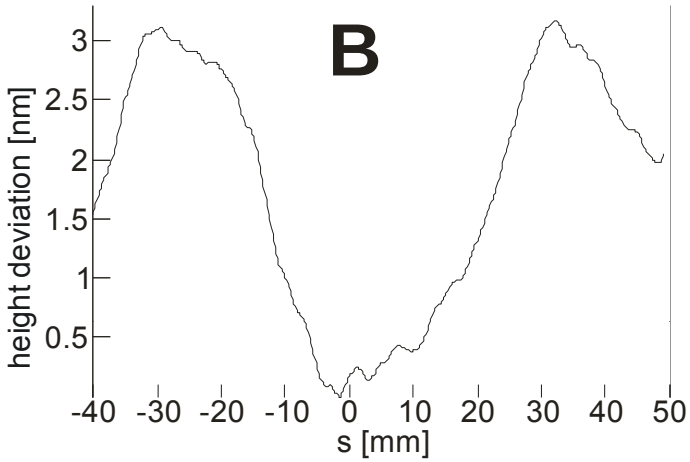
out-of-cone: coherent diffraction:
iterative phase retrieval

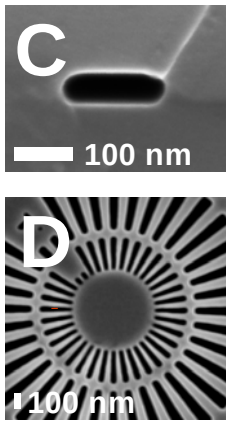
in-cone: hologram
backprojection

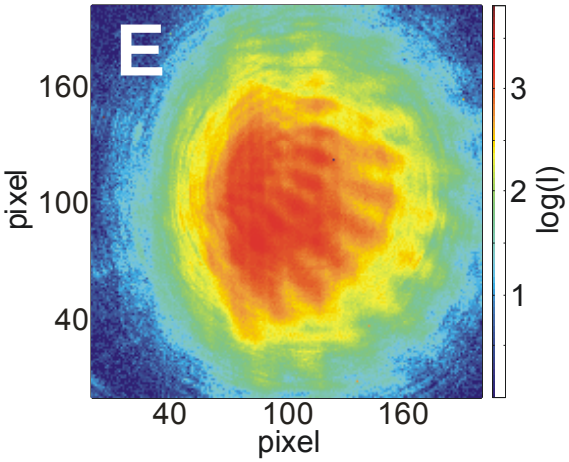
no beamstop

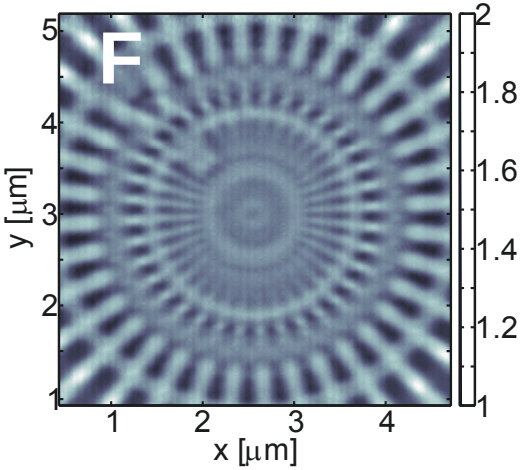
One-step holographic reconstruction (ID22NI)











S. Kalbfleisch *et al.*, unpublished

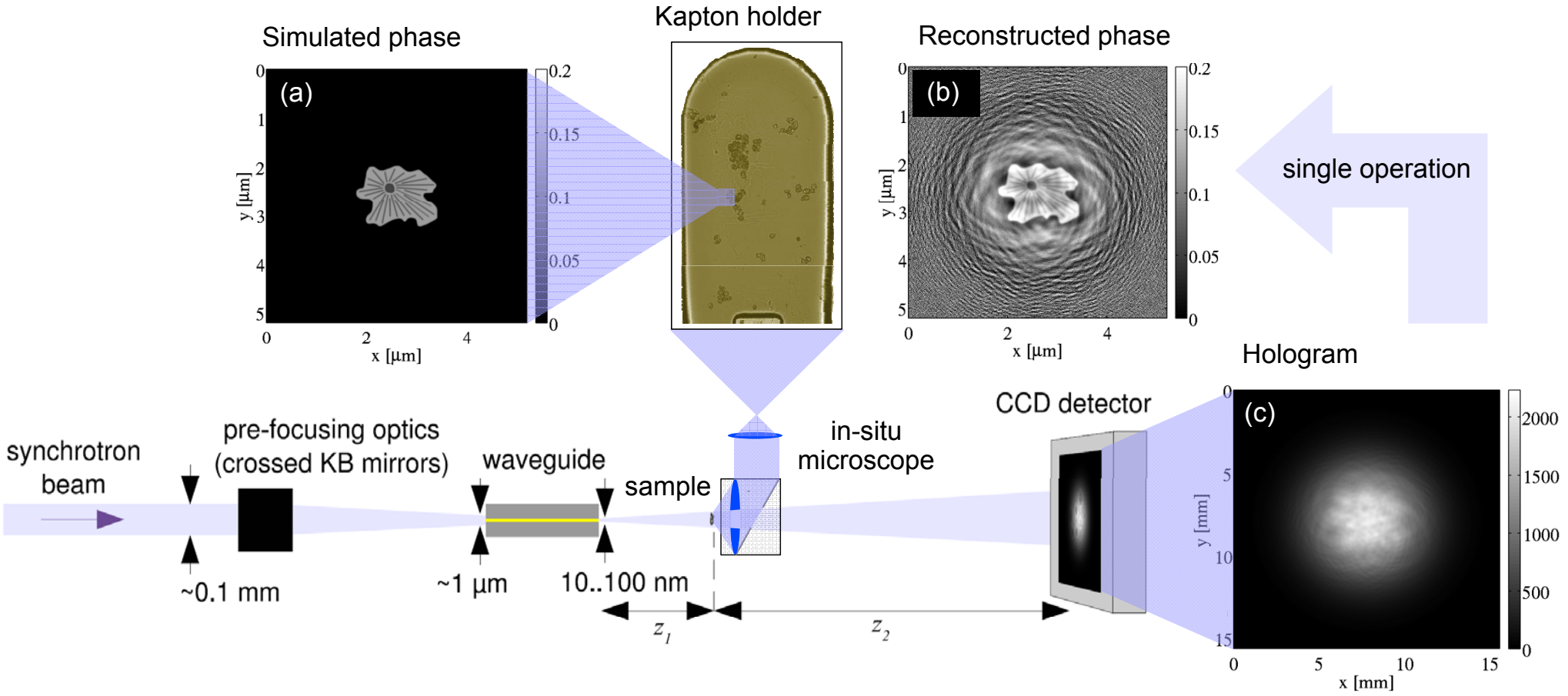
Holo-microscopy of biological cells (dedicated setup at Petra III / P10)

recent test experiment at ID22/ESRF

17.5 keV photon energy

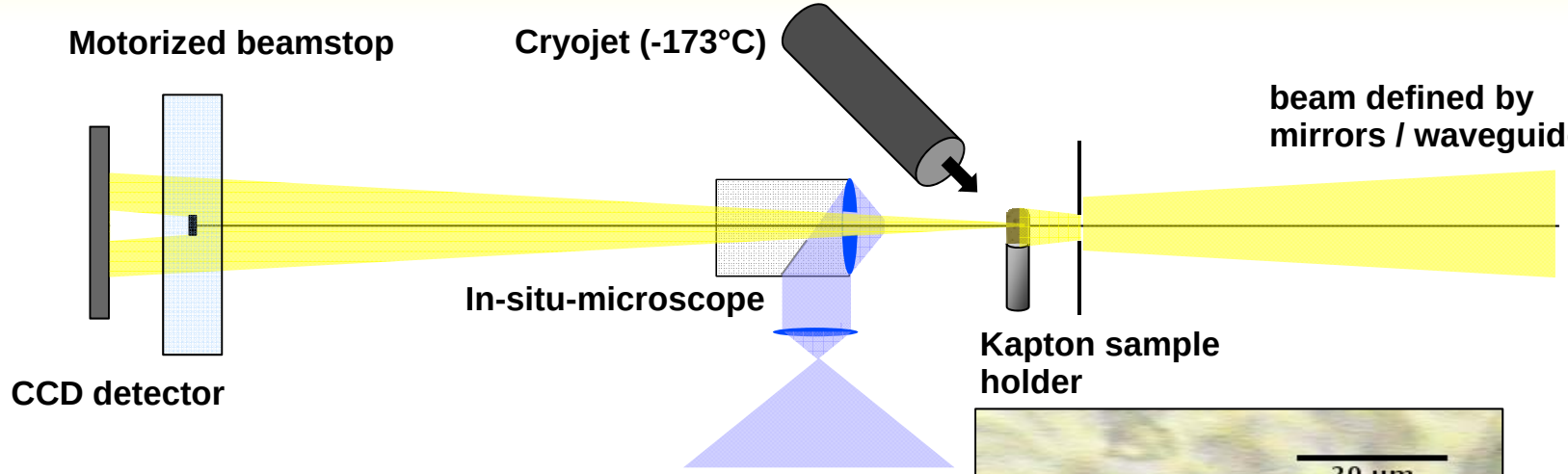
Maxipix single-photon counting detector (d_{pix} = 55 μm), z_2 = 3.1 m, z_1 = 1..10 mm

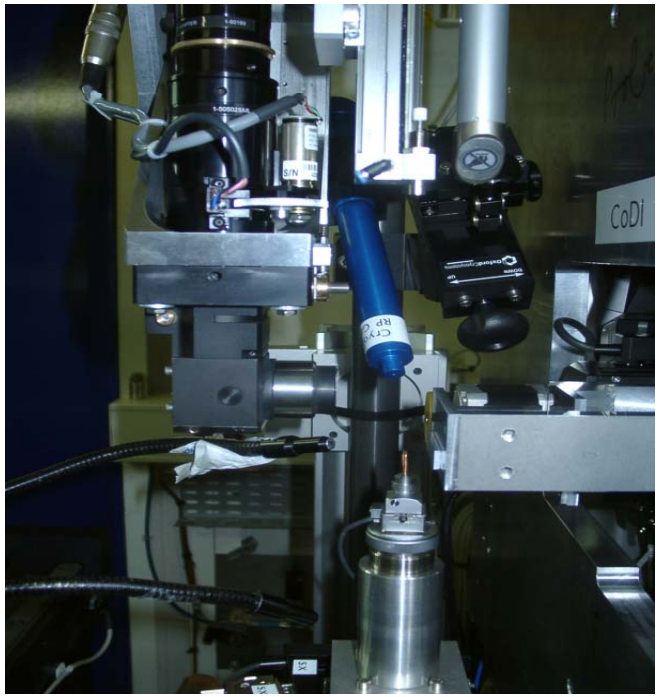
2d-channel and 2d-crossed waveguides

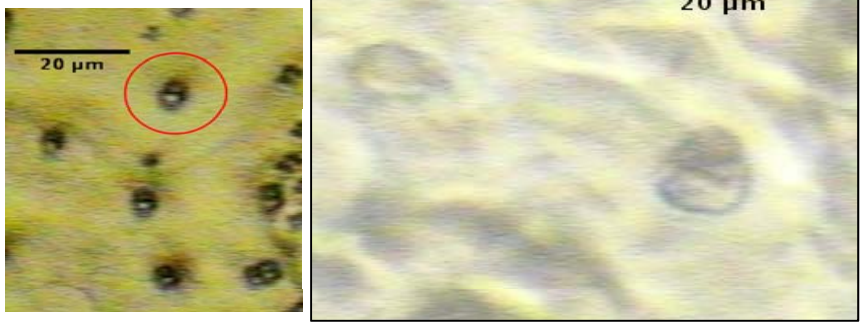


S. Kalbfleisch, K. Giewekemeyer, S. Krüger, H. Neubauer

Dedicated instrument at PETRA III coherence beamline

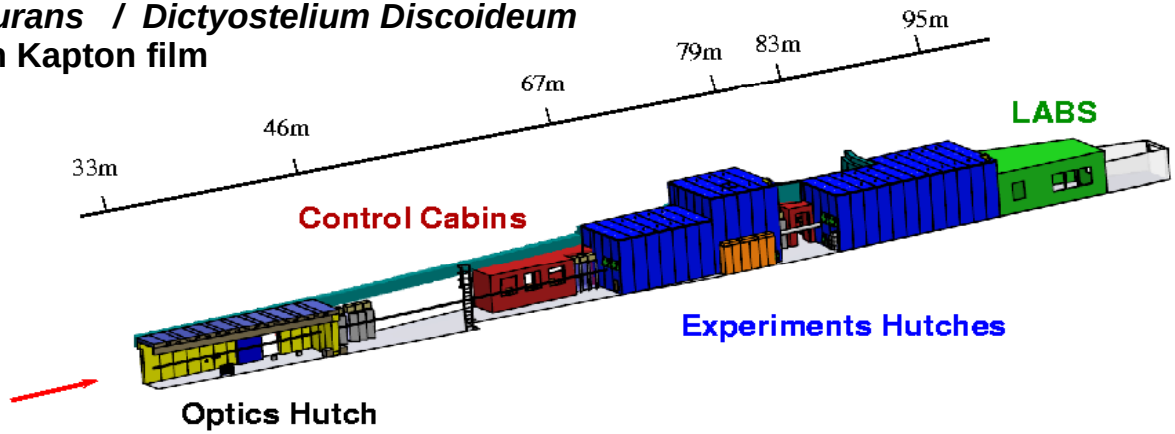




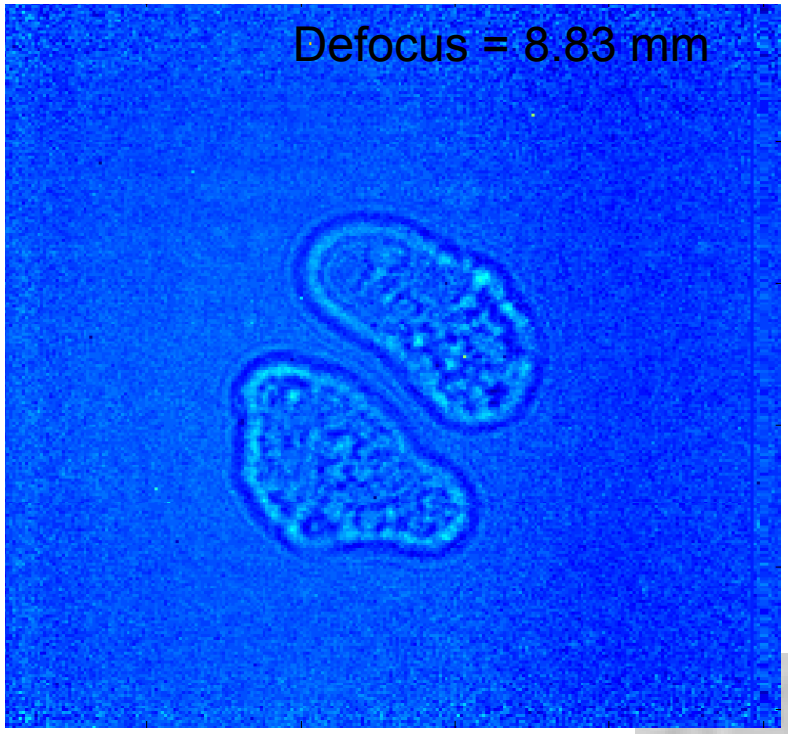


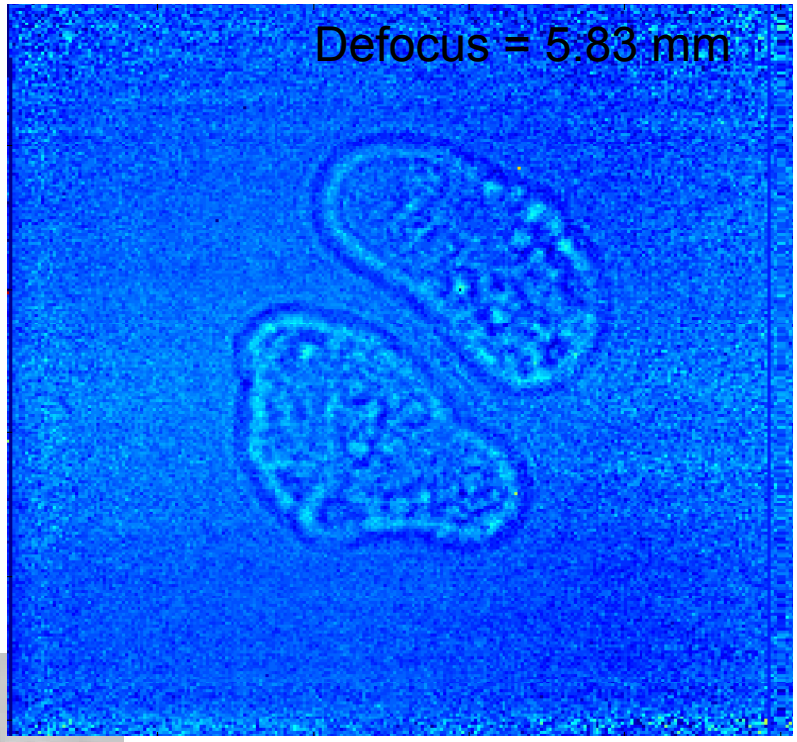
Freeze-dried (left) and frozen hydrated (right)
Radiodurans / *Dictyostelium Discoideum*
Cells on Kapton film

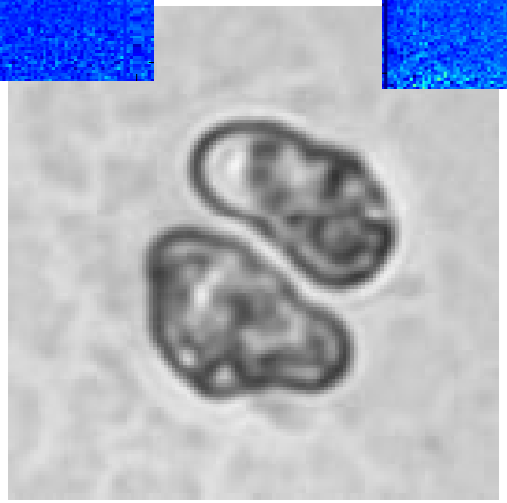
5m undulator,
29mm period,
low ß-section
energy range: 6-18keV
brilliance:
B= 4.5 10^{20} @ E=8keV
Commissioning
end-2009



Holographic imaging: Dictyostelium D. in a waveguide beam



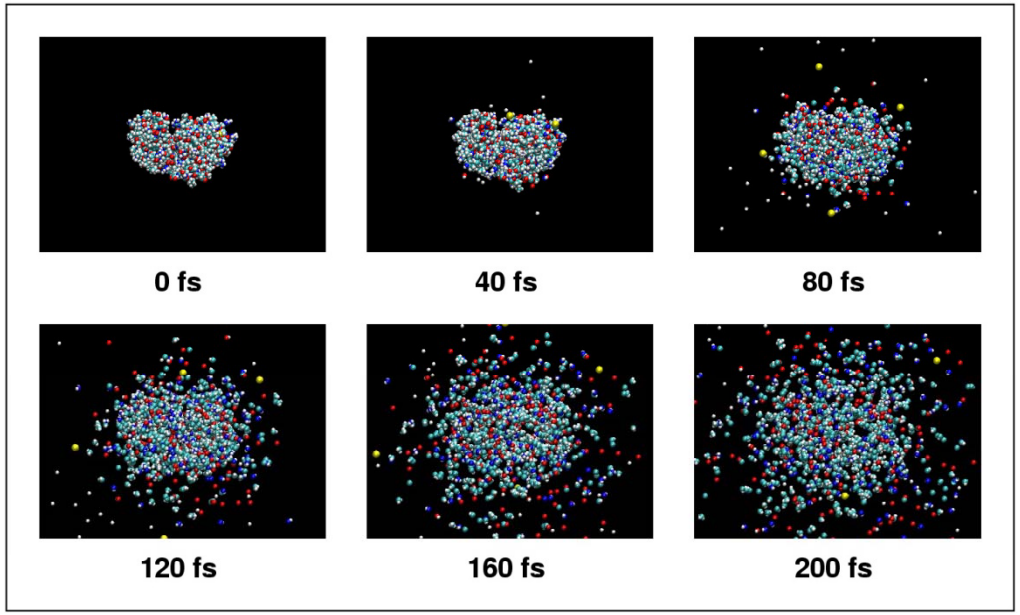




ID 22NI
35 x 35 nm crossed WG
17.5 keV

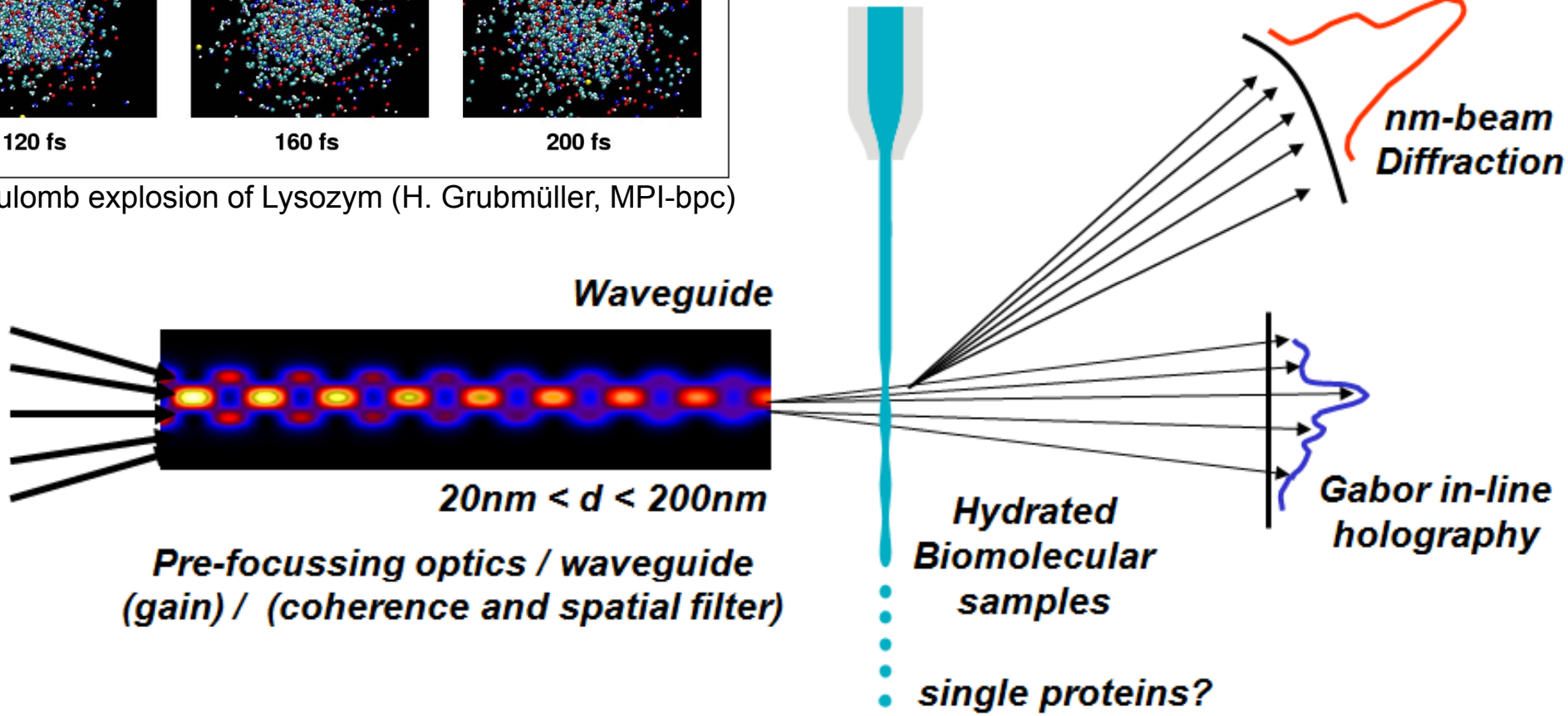
Giewekemeyer et al, unpublished
preliminary results

Goal: (Near-)atomic resoluton for hydrated biomolecules and cells in x-ray laser (XFEL) beam

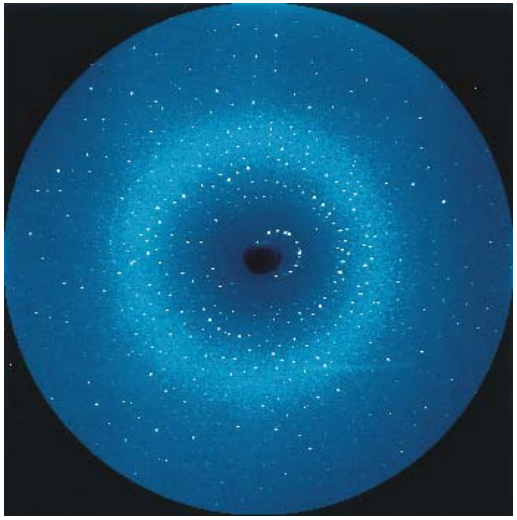


Coulomb explosion of Lysozym (H. Grubmüller, MPI-bpc)

Imaging Atomic Structure and Dynamics with Ultrafast X-ray Scattering
K. J. Gaffney, *et al.*
Science **316**, 1444 (2007);



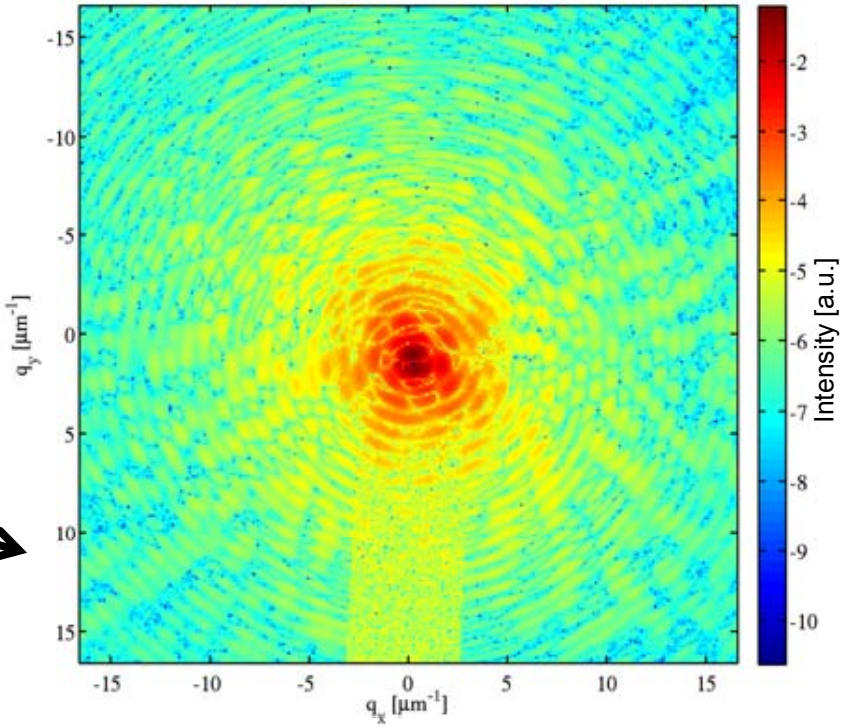
Contributions of the XFEL: from molecules to cells …



Protein crystallography
www.esrf.fr

high photon flux / ultrashort pulses:
- single shot / single particle experiments
- increased time resolution: pump – probe investigation of inner membrane dynamic s (membrane fusion, single bilayer formation)

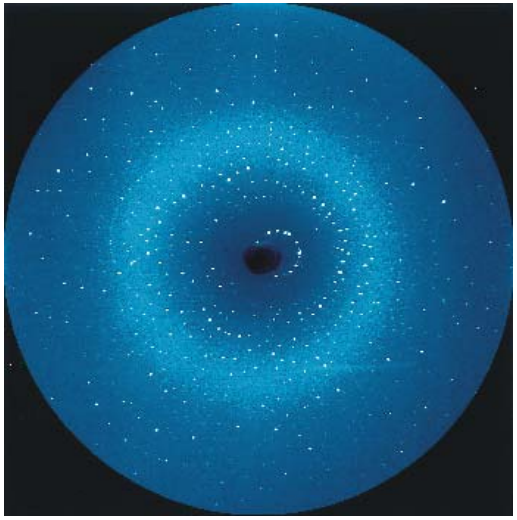
coherence:
- inline holography
  > single step reconstruction
- 3D – investigation of cells
- increased spatial resolution in phase contrast imaging especially weakly scattering objects (cells, single membranes)



Coherent diffraction pattern of a cell

microfluidics:
- single particle / cell sorting
- hydrated cell preparation
- renewable meterials
- high reproducability

Contributions of the XFEL: from molecules to cells …



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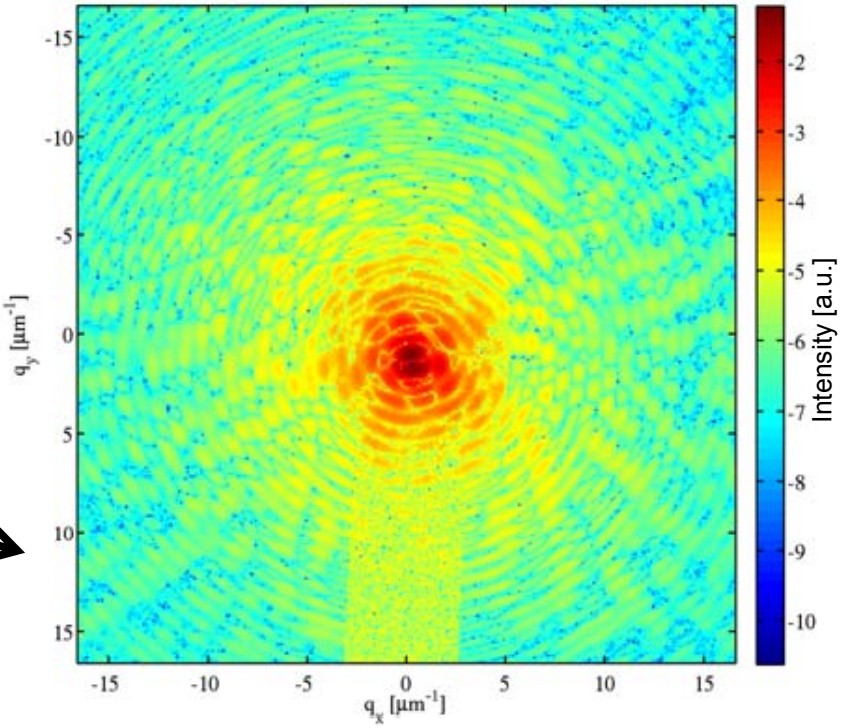


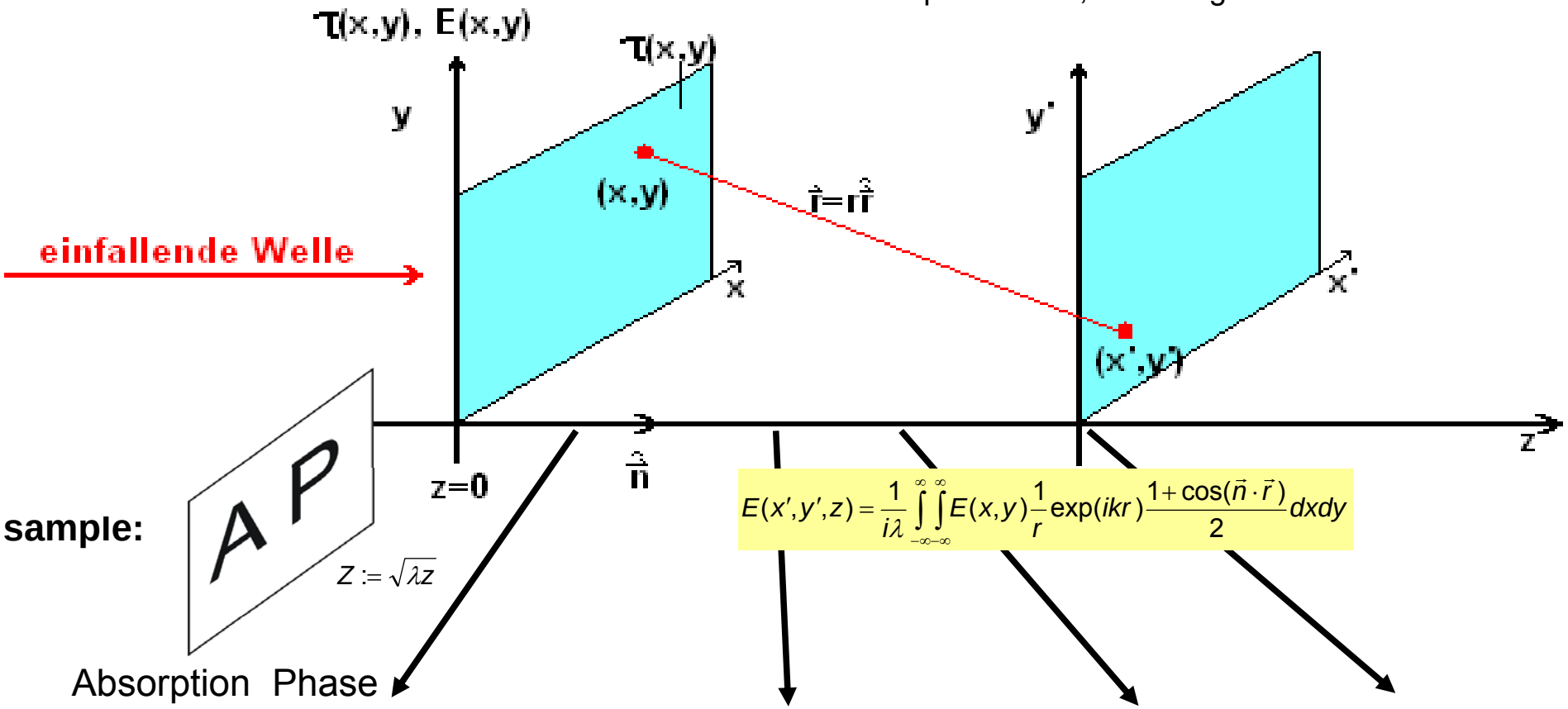
Coherent diffraction pattern of a cell

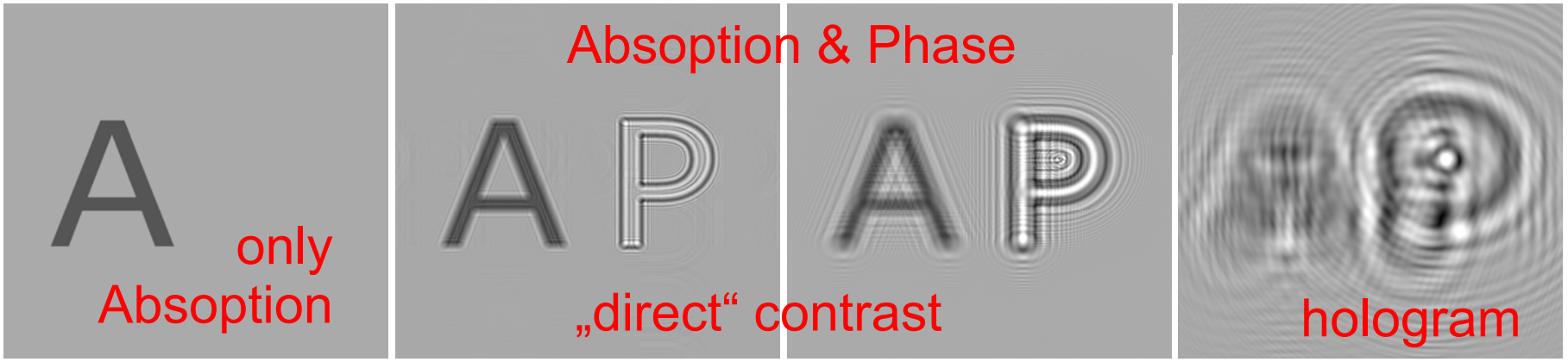
microfluidics:
- single particle / cell sorting
- hydrated cell preparation
- renewable meterials
- high reproducability

THANK YOU FOR YOUR ATTENTION!

Propagation imaging

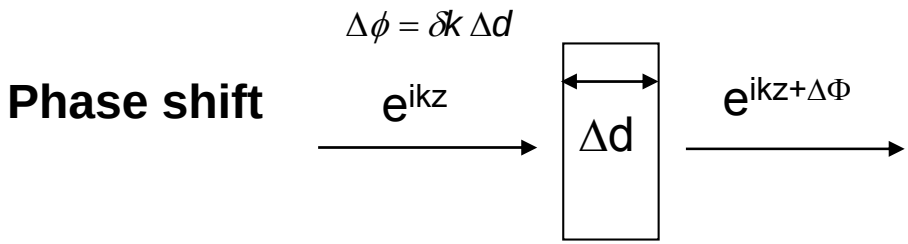
- Object plane z=0 with complex transmission $\tau(x,y)$
- illumination with plane wave, wavelength λ





coherent x-ray phase contrast imaging

$$n = 1 - \delta + i\beta$$



hologram recorded with the point source corresponds to a hologram recorded with a plane wave at an effective defocusing distance

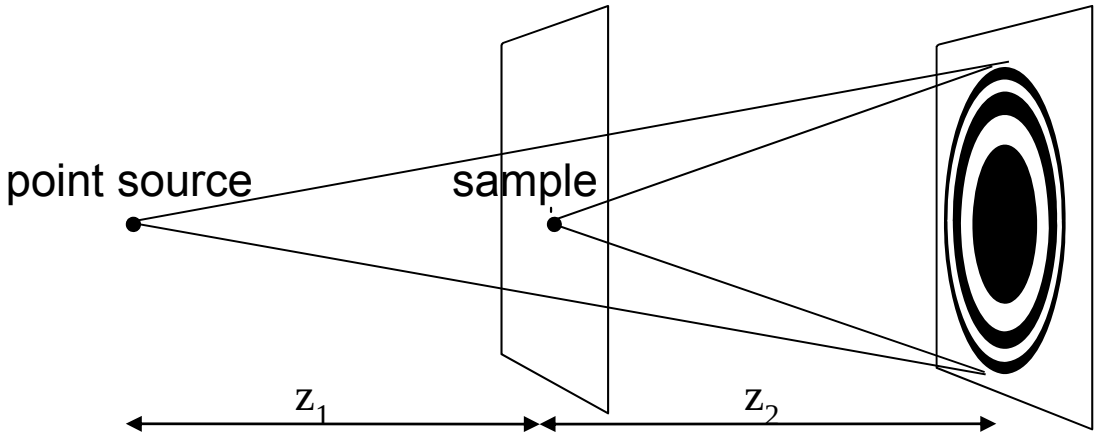
$$Z_{eff} = \frac{z_1 z_2}{z_1 + z_2}$$

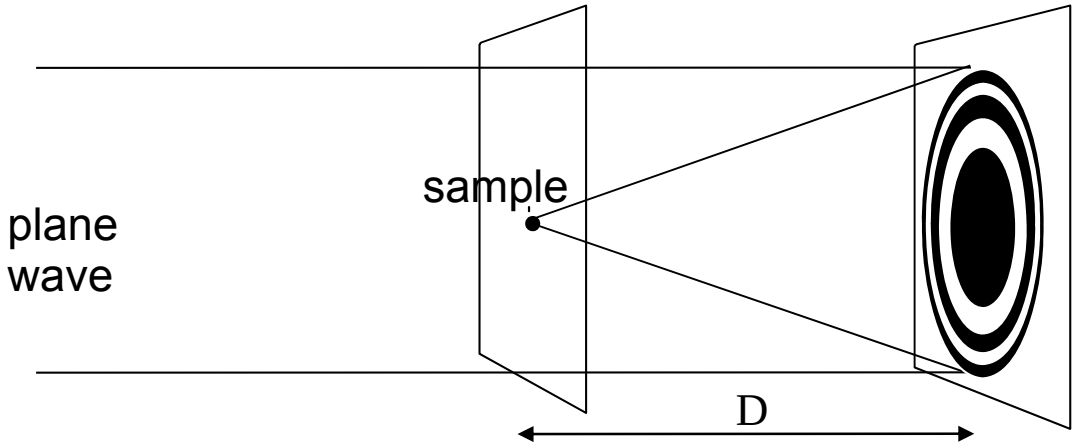
magnified by

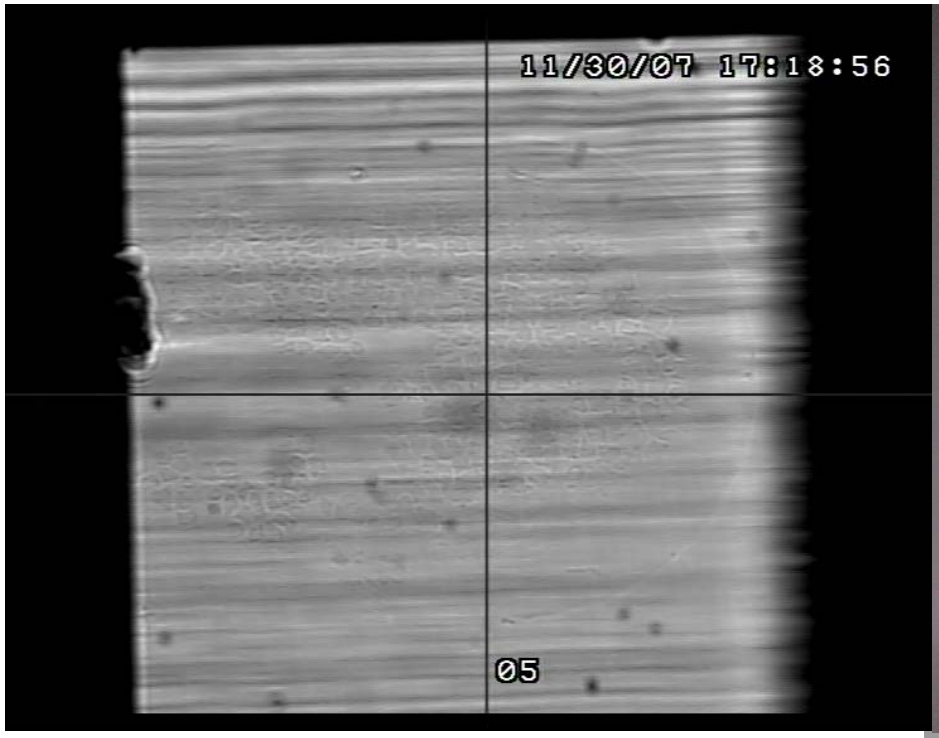
$$M = \frac{z_1 + z_2}{z_1}$$

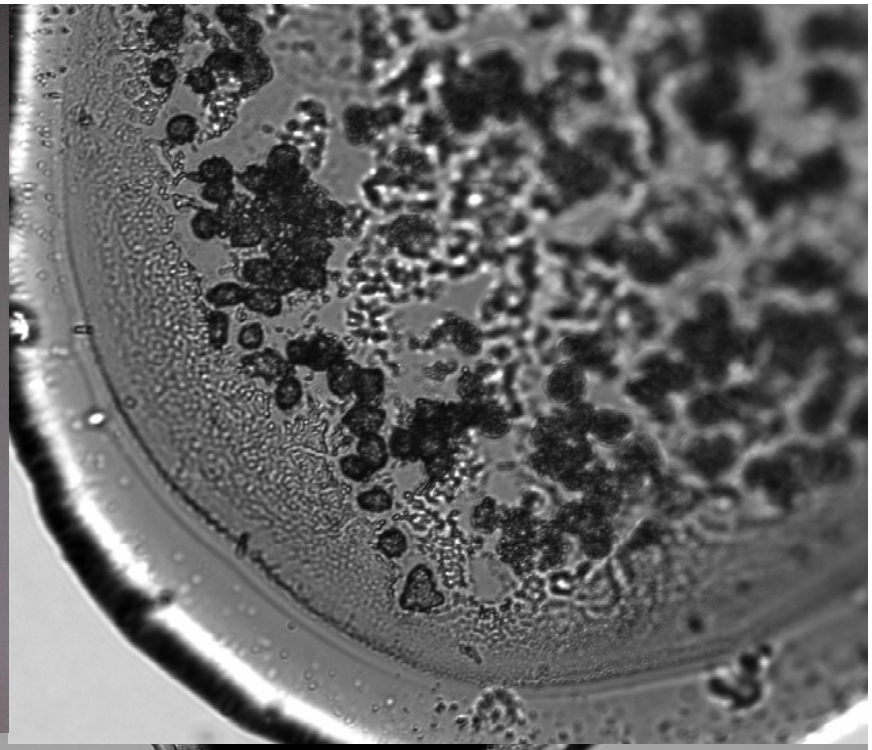
→magnification allows for a spatial resolution below detector pixel size!

→plane wave setup used for simulations and reconstruction

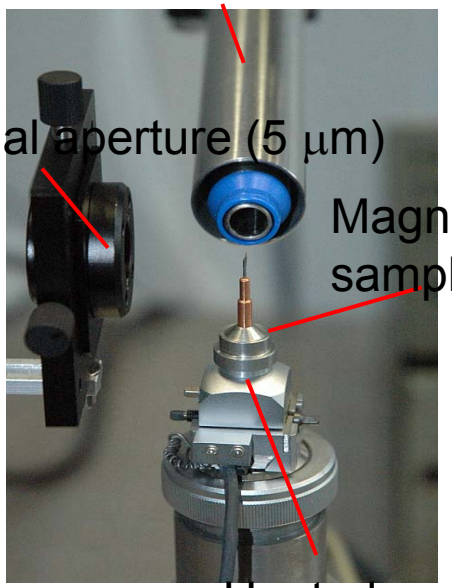






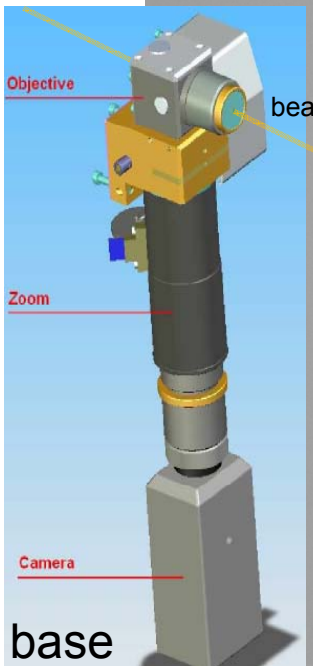


Cryo-Stream (-160°C)

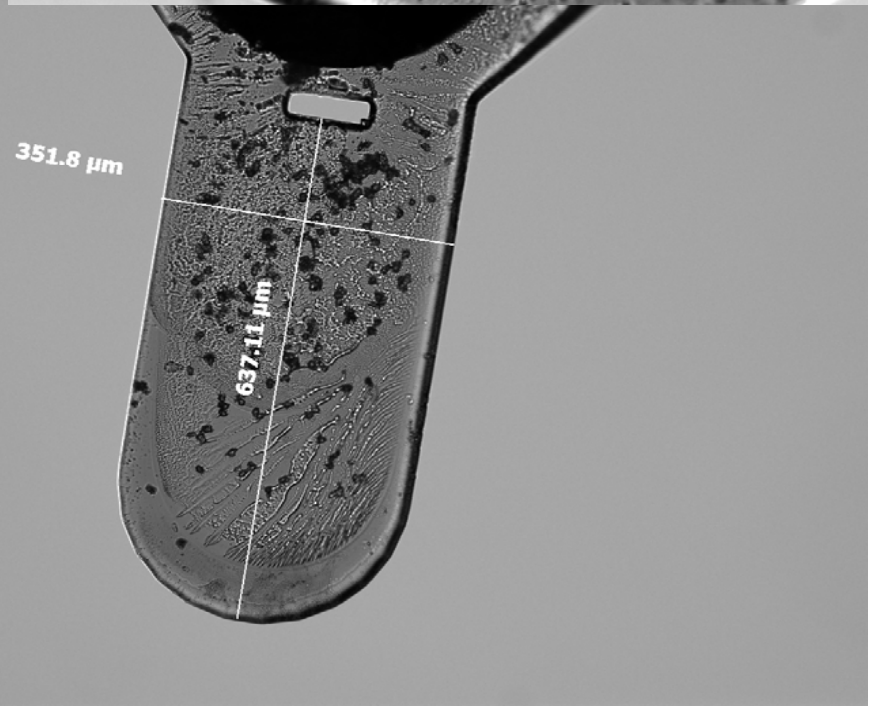


Final aperture (5 μm)

Magnetic
sample holder



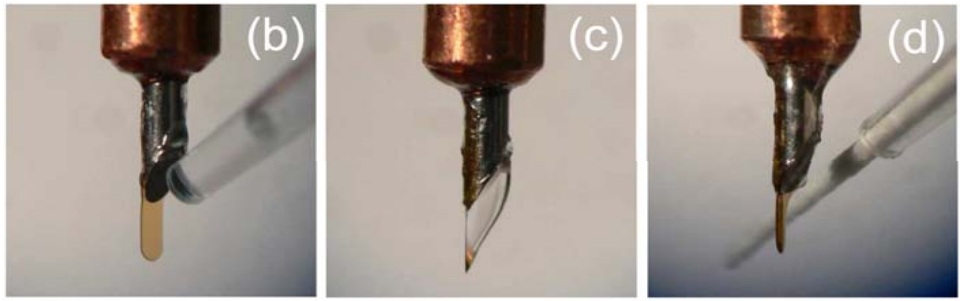
Heated goniometer base



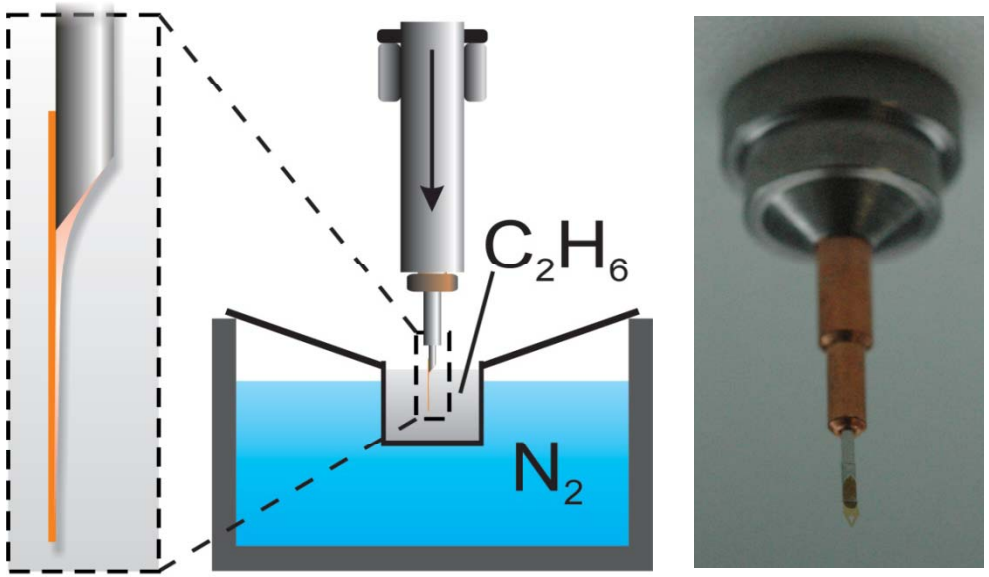
Sample preparation and environment for frozen hydrated cells

Home-built cryo-plunging device (K. Giewekemeyer)

Sample transfer (through stereo microscope)



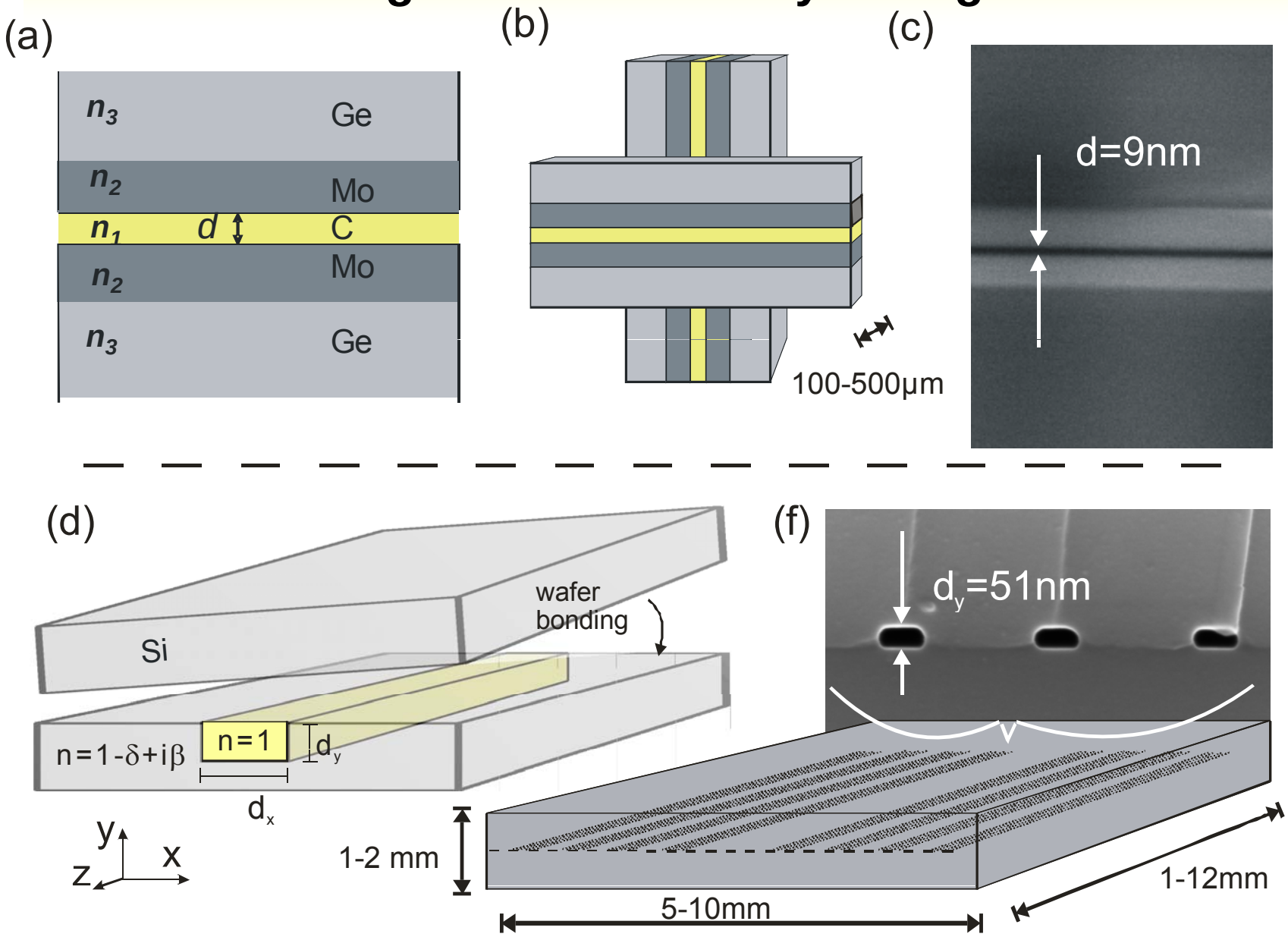
M. Hantke, IRP



Today:
Tedious cryo preparation protocol with potential risk

Tomorrow at XFEL:
Single shot imaging of hydrated cells

New generation of x-ray waveguides

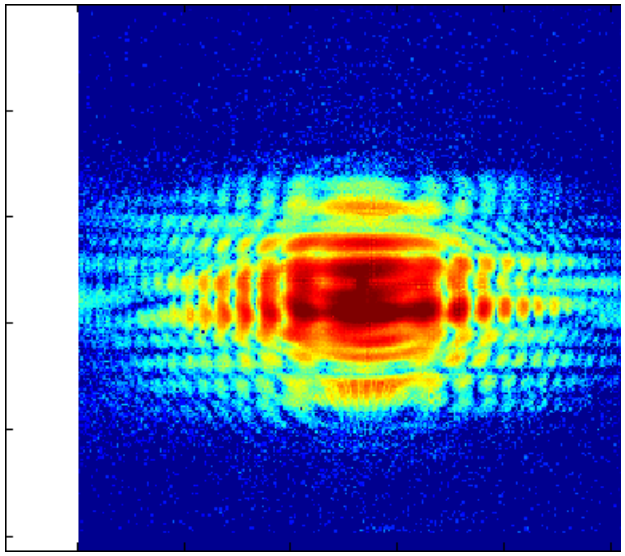


T. Salditt, S. Krüger, C. Baehtz, Phys. Rev. Lett. 2008
S. Krüger et al, unpublished

H. Neubauer, M. Kanbach, unpublished

Siemens-Star in WG beam

WG + Large defocus (holography)

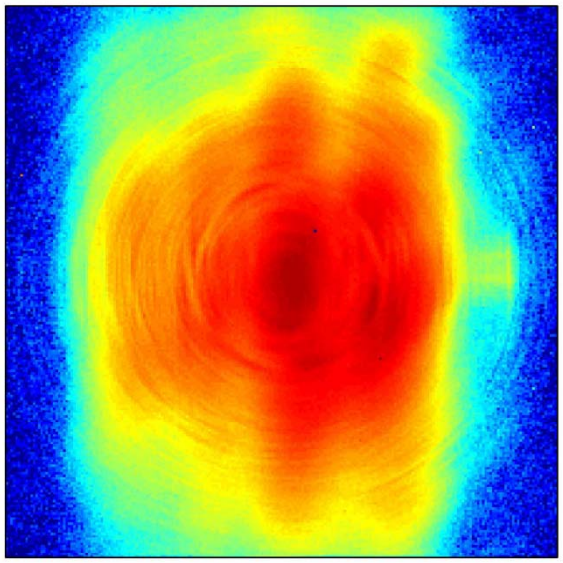


ID01 (ESRF), 8 keV, FZP focusing, MEDIPIX

WG: (< 200 x 200) nm^2 x 2 mm, Si-Air (bonded)

FOV(single projection): ~ 100 x 100 μm^2

WG + Small defocus

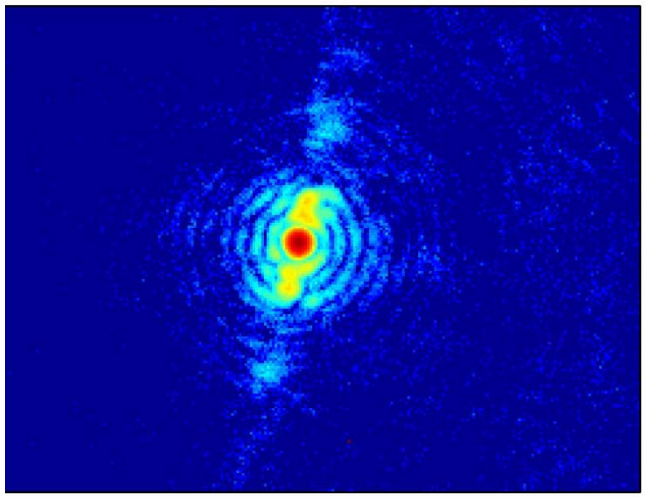


ID22NI (ESRF), 17.5 keV, KB focusing, MEDIPIX

WG: (30 x 100) nm^2 x 10 mm, Si-Air (bonded)

FOV: 3 x 3 μm, 16x16 positions

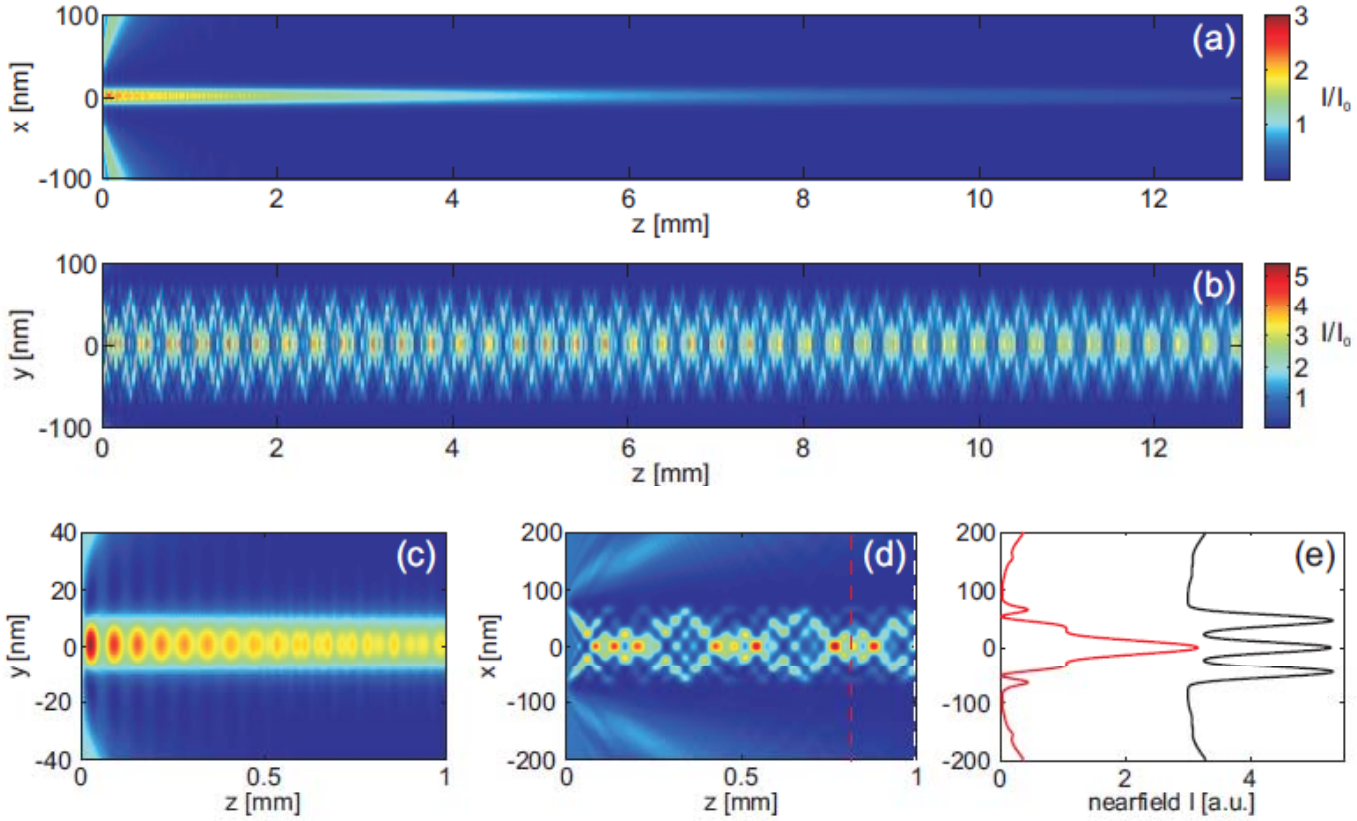
Pinhole + Small defocus (diffraction+holography)



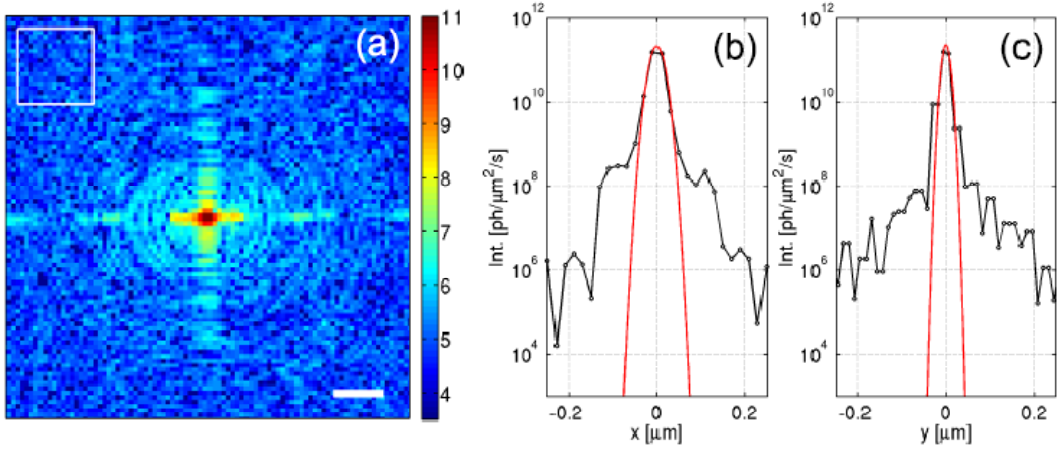
cSAXS (SLS), 6.2 keV, slight focusing, PILATUS

Pinhole: $\varnothing$ = 1.4 μm, 20 μm tungsten

FOV: 4.75 x 2.25 μm, 20x10 positions



Field simulations
140nm x 24 nm



Reconstruction
of near field
from experimental data