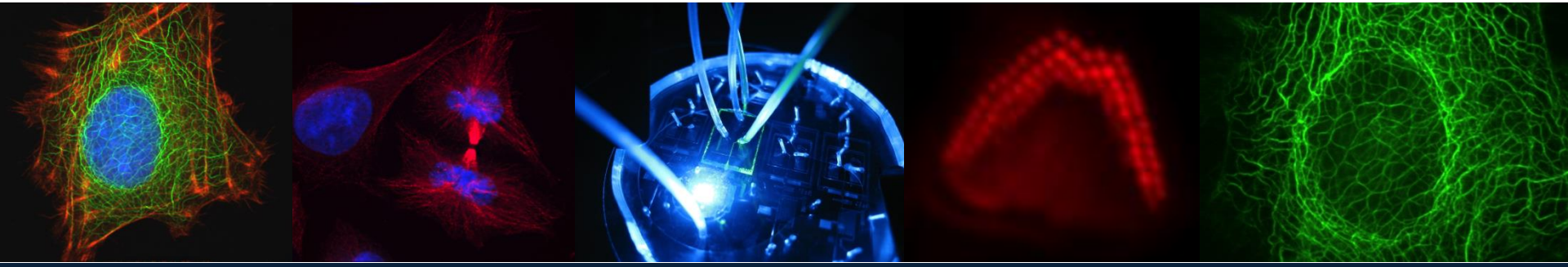


Ideas for microfluidics experiments at MID

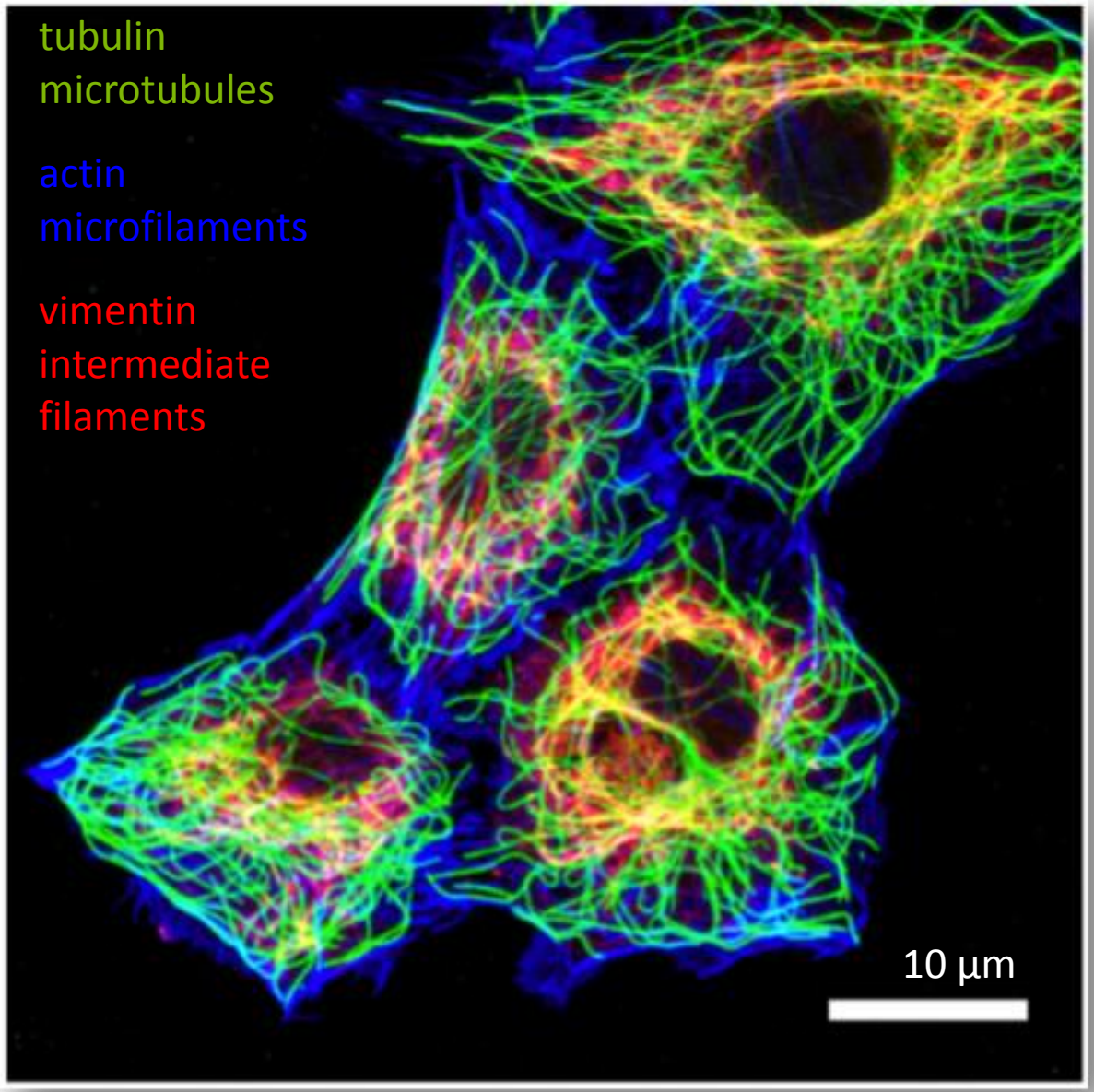
Sarah Köster, Tim Salditt

Institut für Röntgenphysik, Georg-August-Universität Göttingen

Early Science Workshop @ MID

27.01.2015

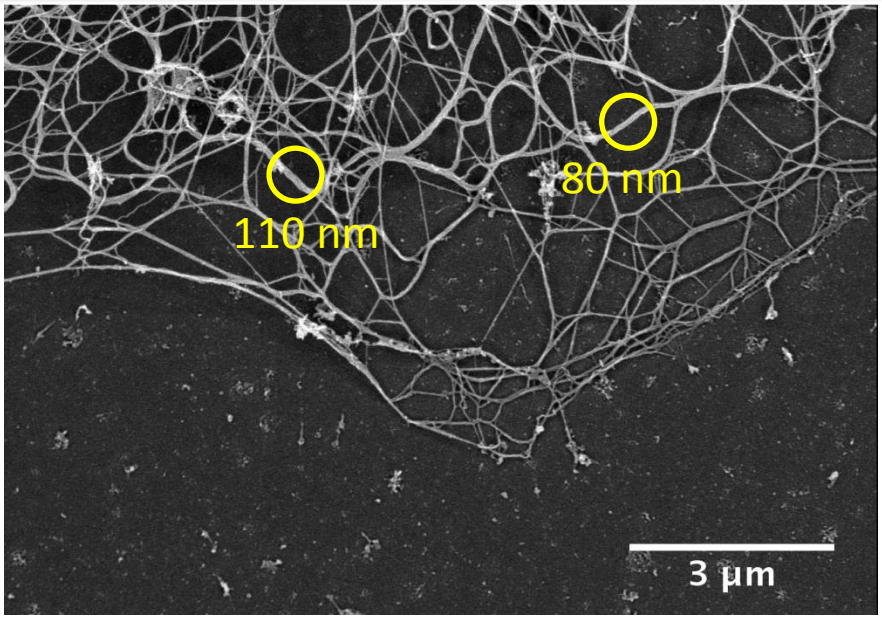
Cellular imaging – light microscopy



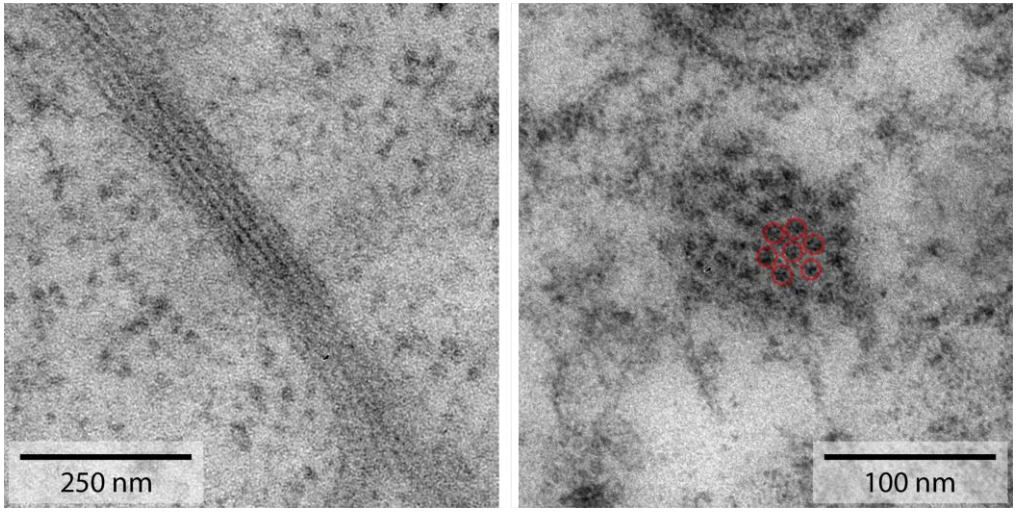
- live cell imaging
- molecule specific labeling
- extensive sample preparation
- small penetration power
- spatial resolution: ~200 nm (but: STED, STORM!!)

images: Rosmarie Sütterlin, Ueli Aebi (Biozentrum Basel)

Cellular imaging – electron microscopy



SEM image by P. Walther, M. Beil, Uni Ulm



TEM images by W. Möbius, MPI EM Göttingen

- static samples (dry, cryo…)
- imaging of surfaces/slices
- very extensive sample preparation
- spatial resolution: nm

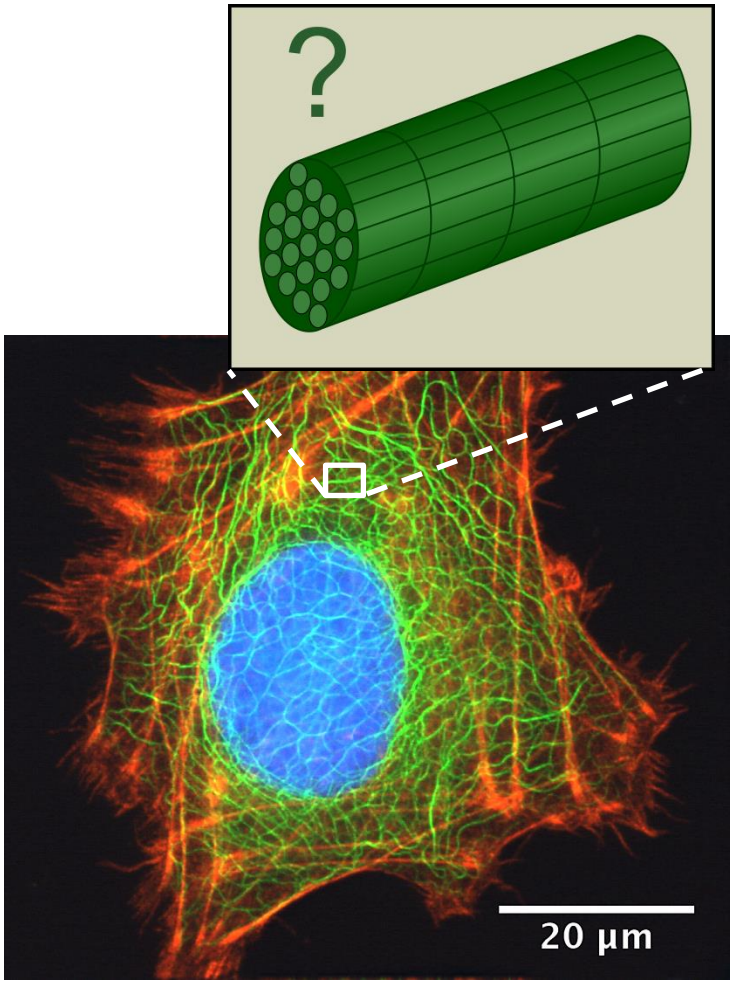
Cellular imaging – X-rays?!

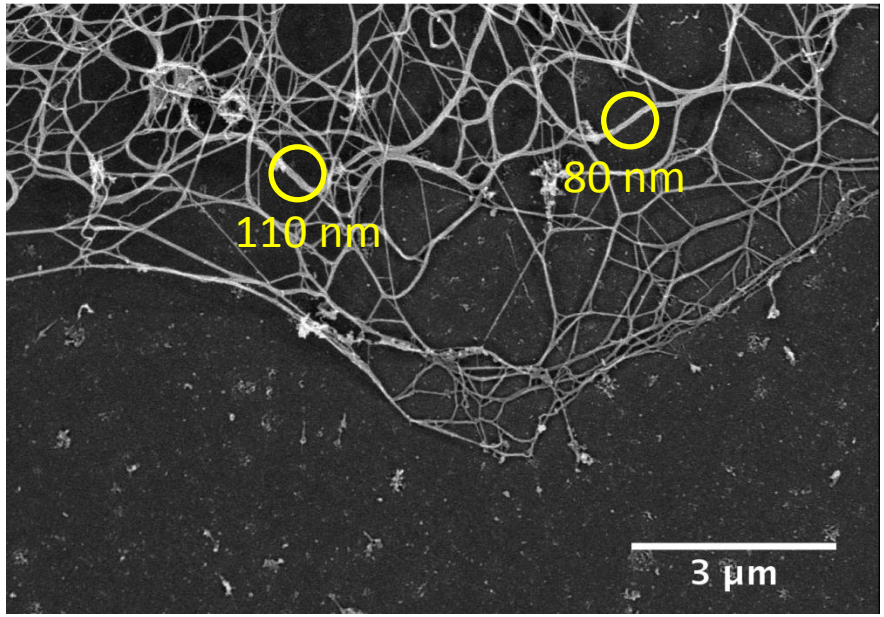
- Introduction
- **Imaging cells with X-rays: nano-diffraction, ptychography**
- Sample environments: microfluidics
- Experiments @ FLASH and SACLA &

  Ideas for microfluidics experiments @ MID

Networks in epithelial cells

keratin bundles

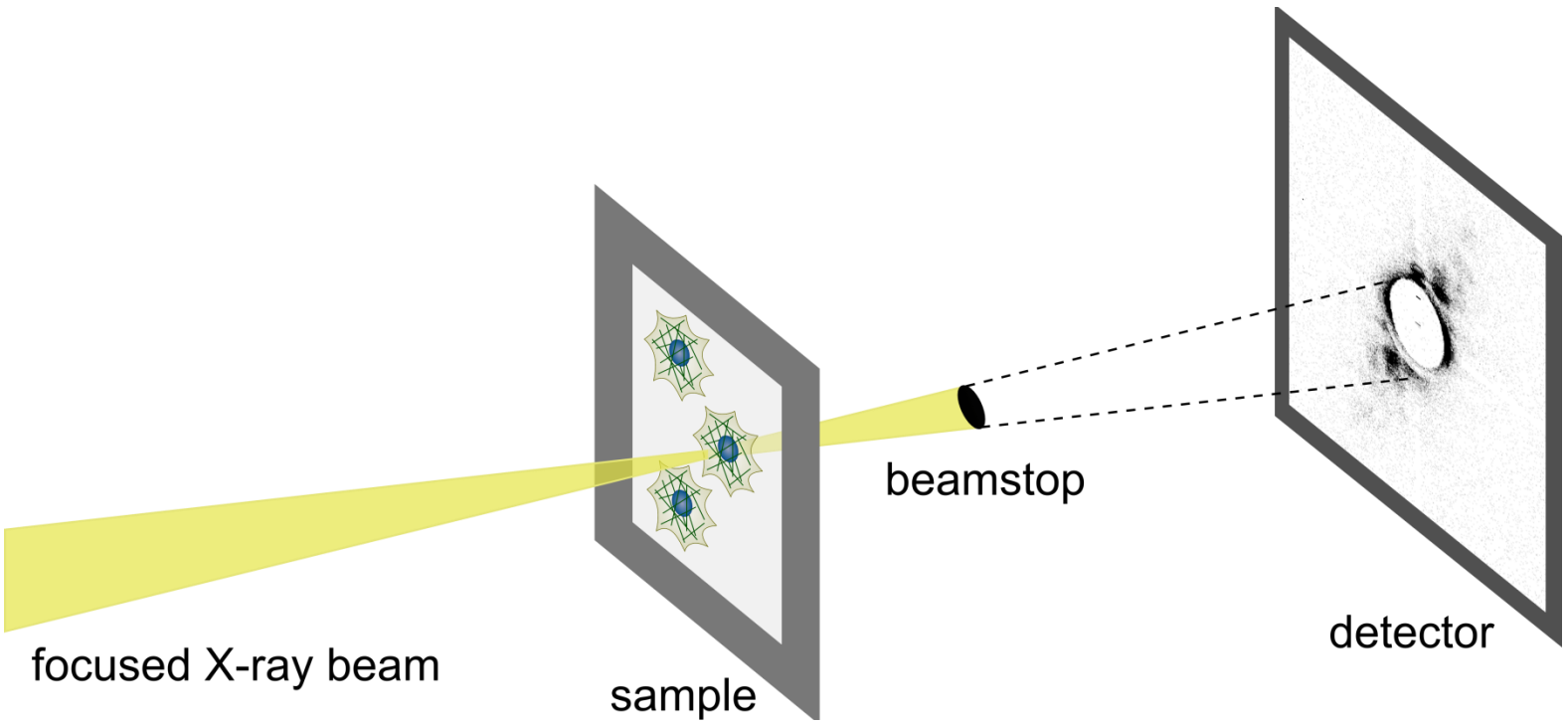




SEM image by P. Walther, M. Beil, Uni Ulm

- occur mainly in epithelial cells
- provide tensile strength
- single filament thickness ≈ 10 nm

Nano-diffraction setup



- small beam size on sample ≈ 150 × 150 nm²
- ***scattering***: high resolution in real space ***and*** in reciprocal space
- ***local, internal*** bundle structure and orientation
- ID 13/ESRF, P10/PETRAIII, cSAXS/SLS

Weinhausen et al, New. J. Phys. 2012, Weinhausen & Köster Lab Chip 2013

Strategy

① chemically fixed & plunge frozen & freeze-dried cells

- ➟ good electron density contrast
- ➟ can be prepared beforehand
- ➟ no biosafety-level necessary

② chemically fixed & hydrated (buffer) cells

- ➟ fewer preparation steps
- ➟ “test“: aqueous environment
- ➟ preparation beforehand
- ➟ no biosafety-level necessary

③ living cells (medium)

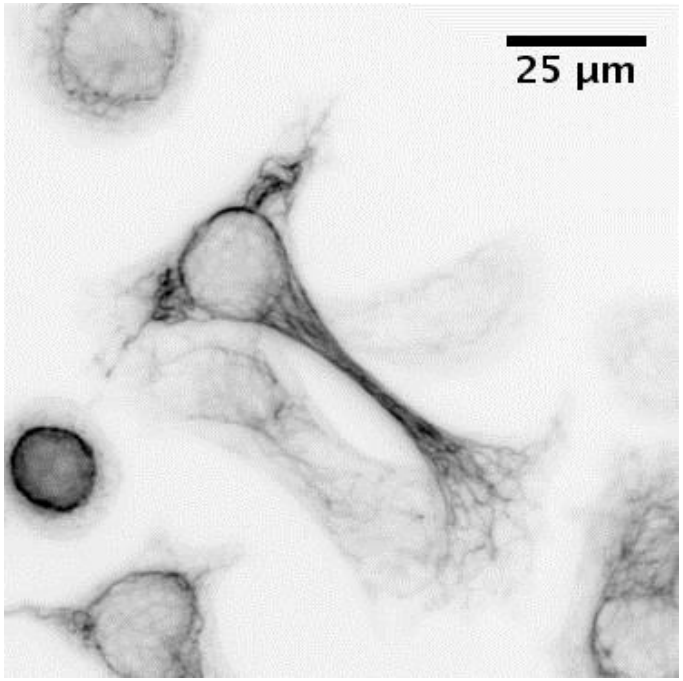
- ➟ dynamic measurements (microfluidics)
- ➟ “in situ”, “in operando”

X-ray dark field images

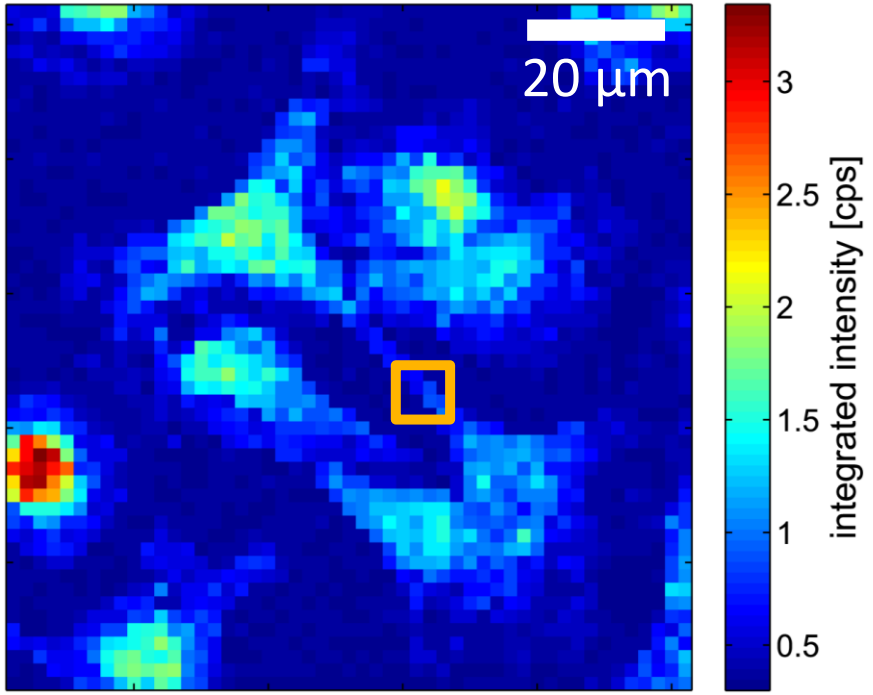
fixed, freeze-dried cells

ID13/ESRF

visible light fluorescence
(inverted gray scale)



X-ray dark field



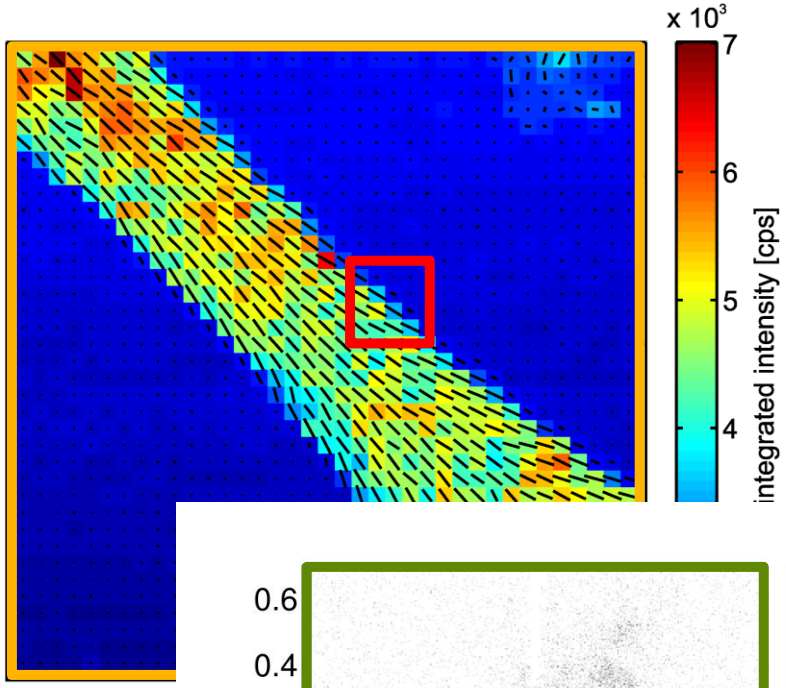
step size 2 µm

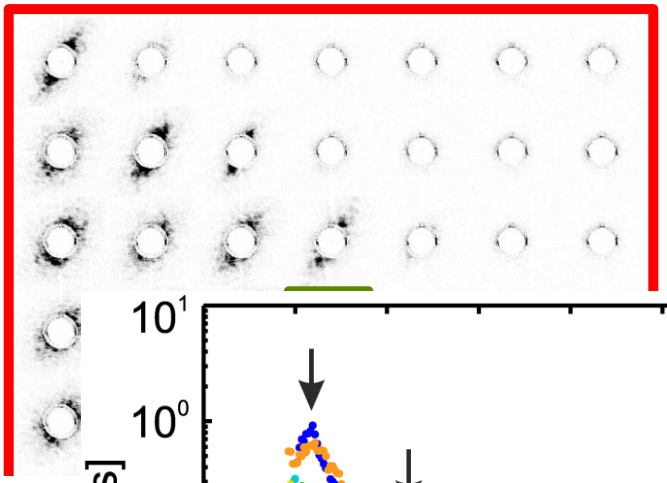
Weinhausen et al, New. J. Phys. 2012

Internal nanostructure

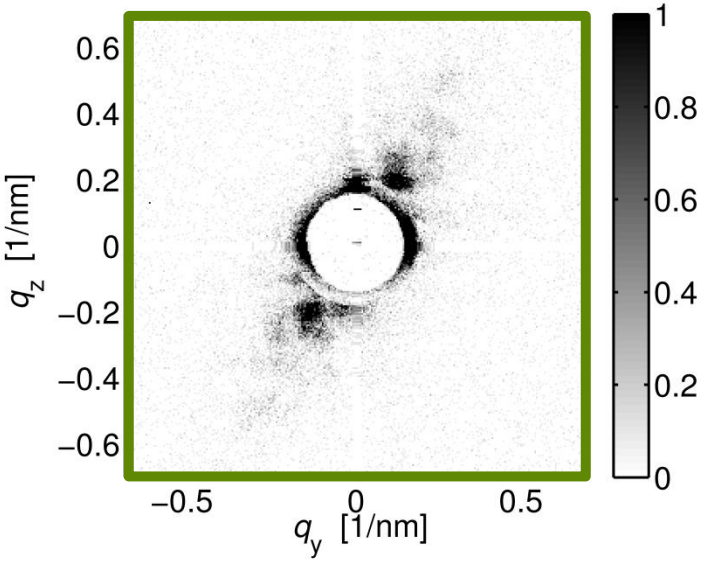
fixed, freeze-dried cells

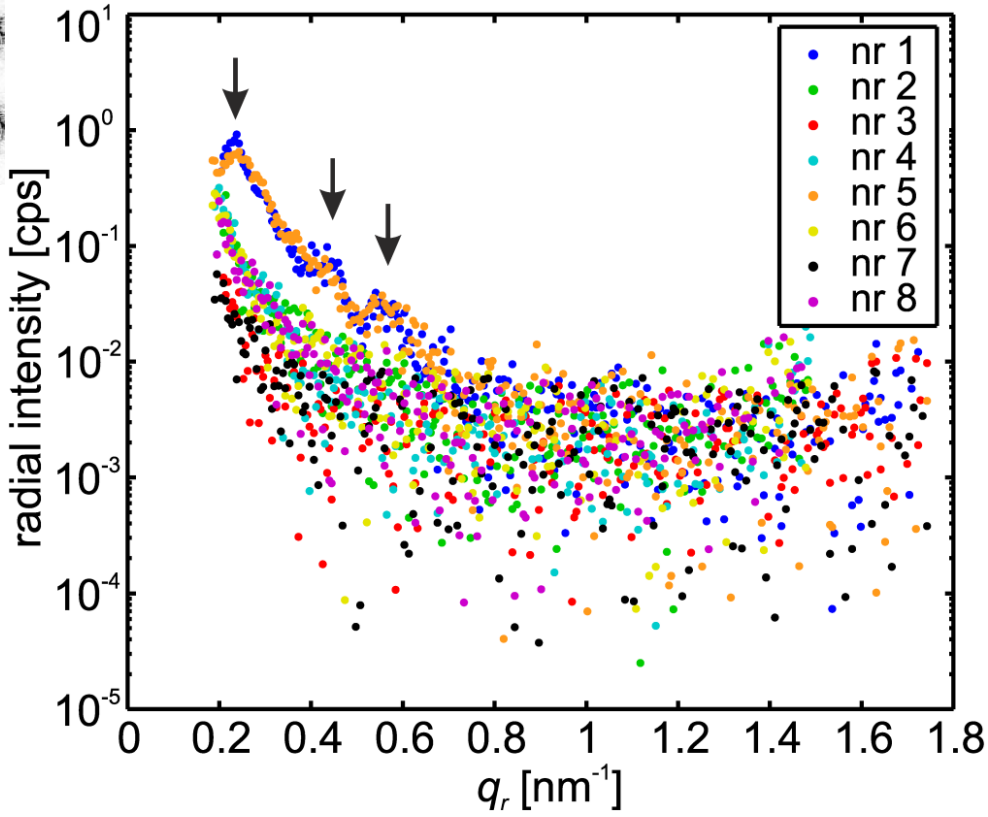
ID13/ESRF





step size



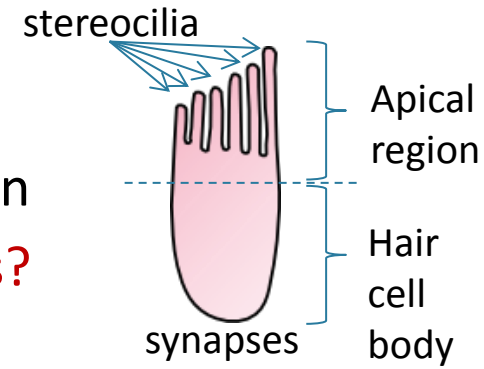


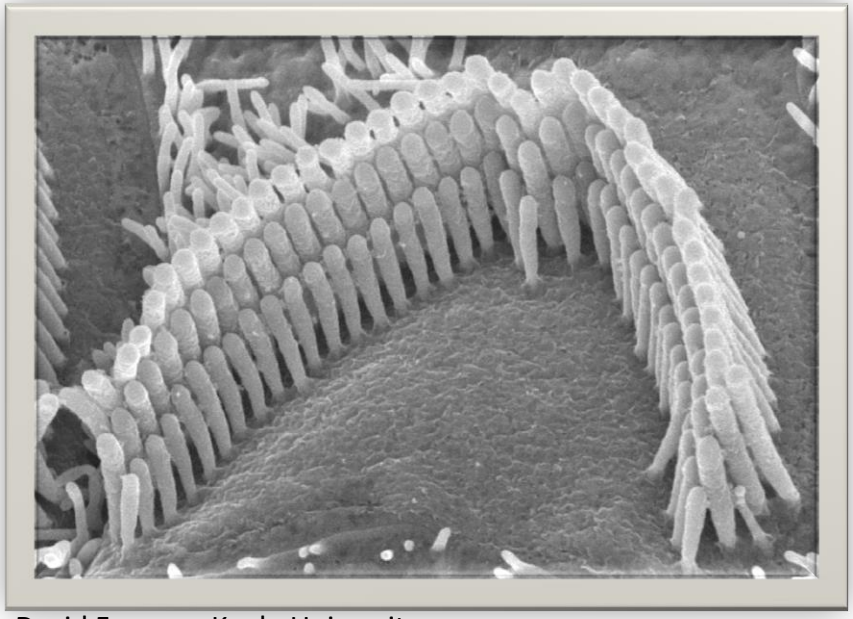
Weinhausen et al, New. J. Phys. 2012

Hair cell stereocilia

actin bundles

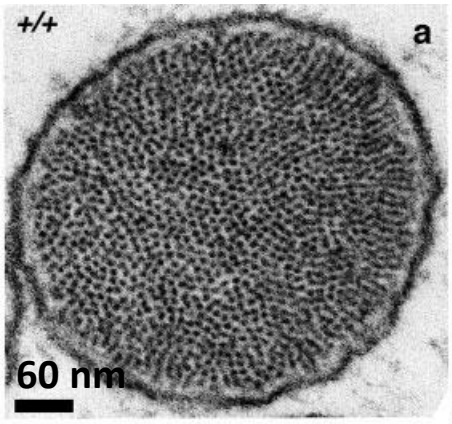
- sensors in the inner ear
- highly parallel bundles of actin
- arrangement of the filaments?

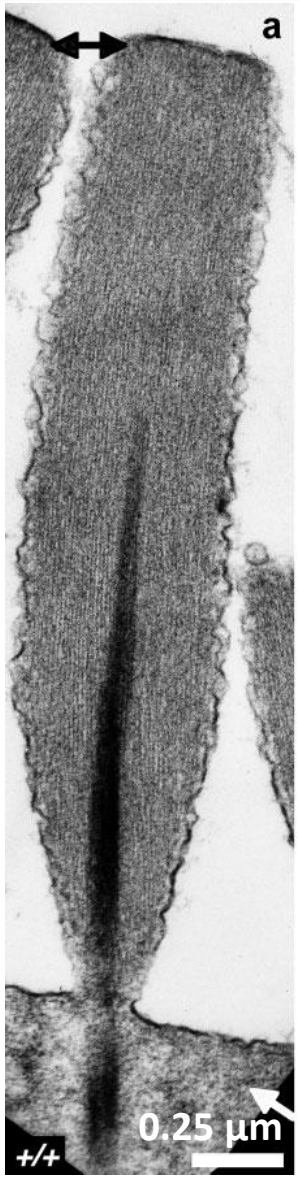




David Furness, Keele University

Mogensen et al., Cell Mot. Cytoskel. 2007

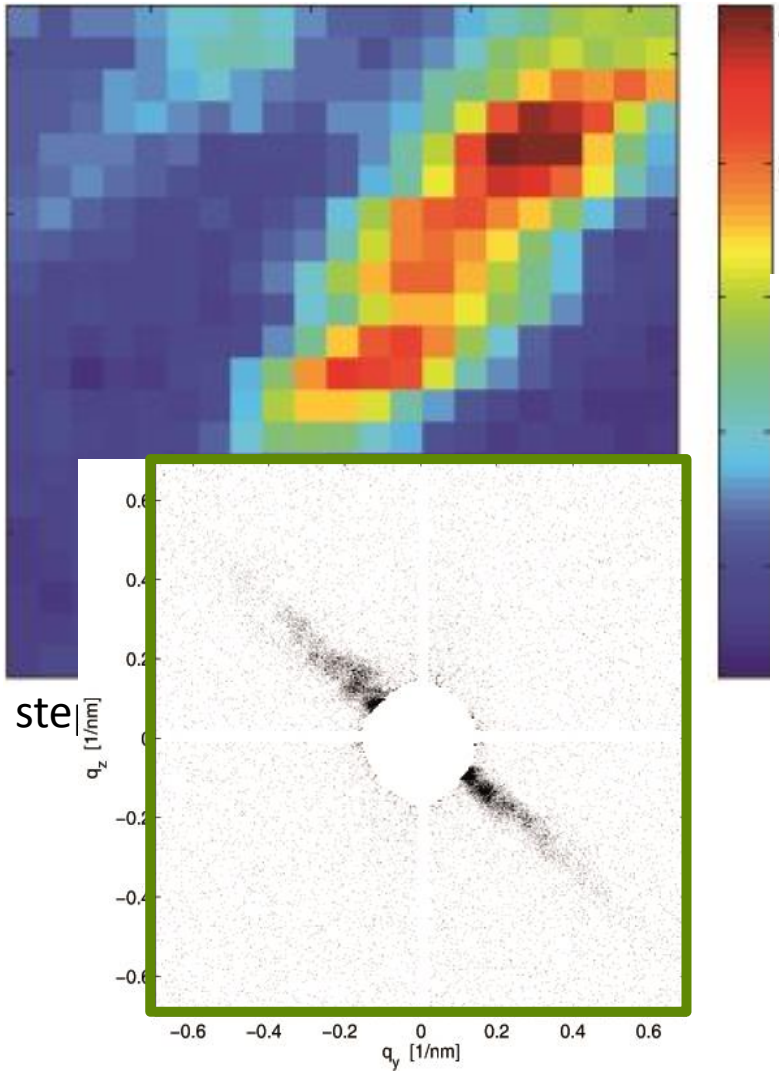


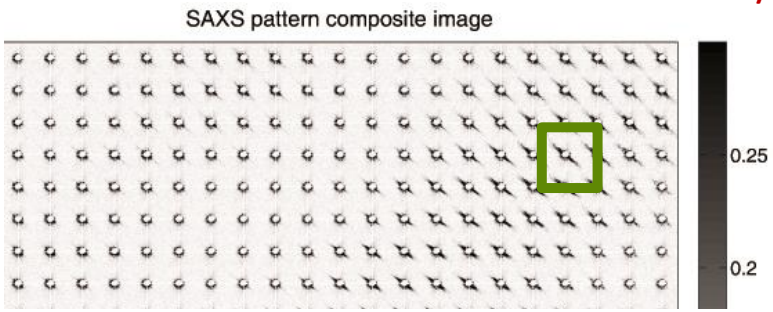


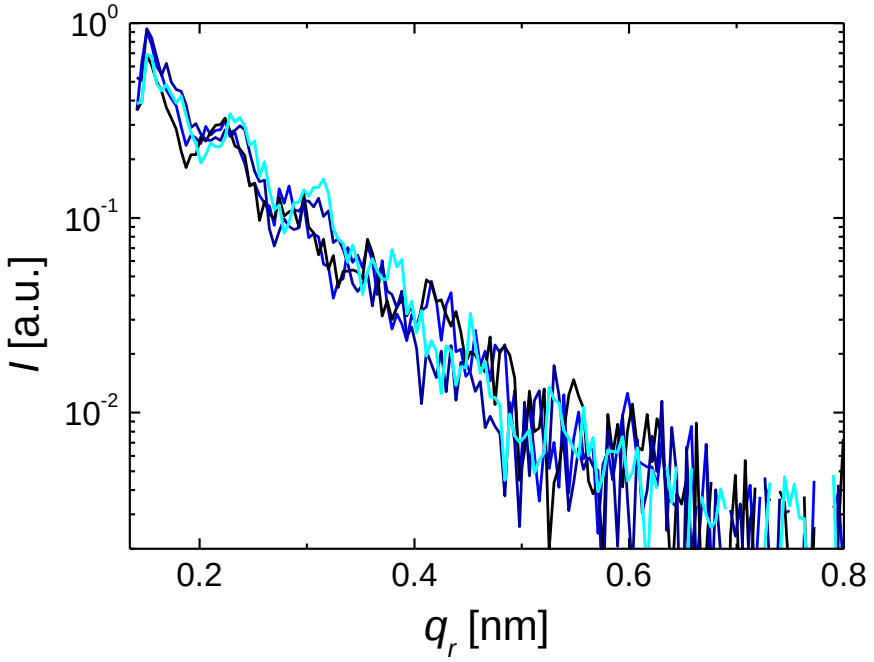
Internal nanostructure

actin; fixed, freeze-dried cells

ID13/ESRF

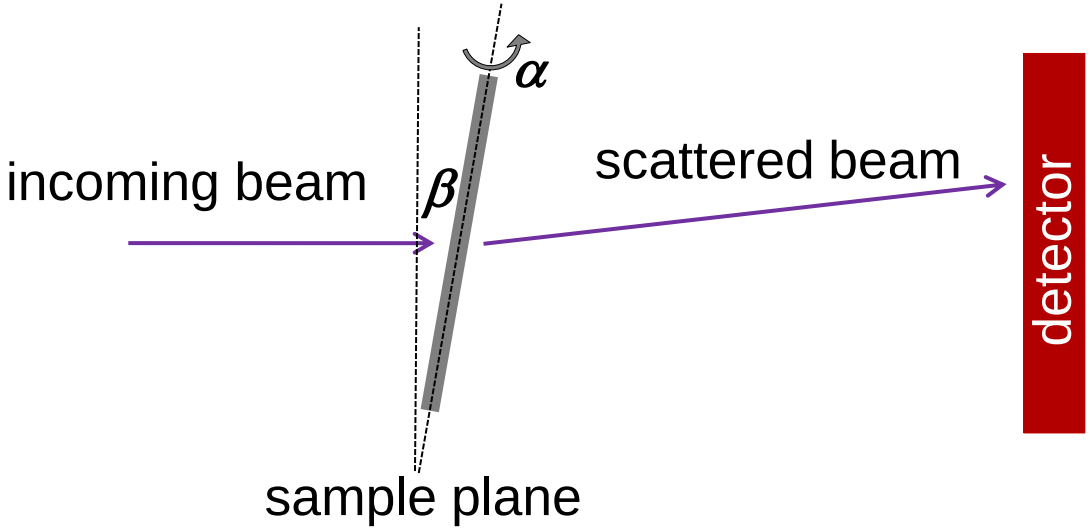


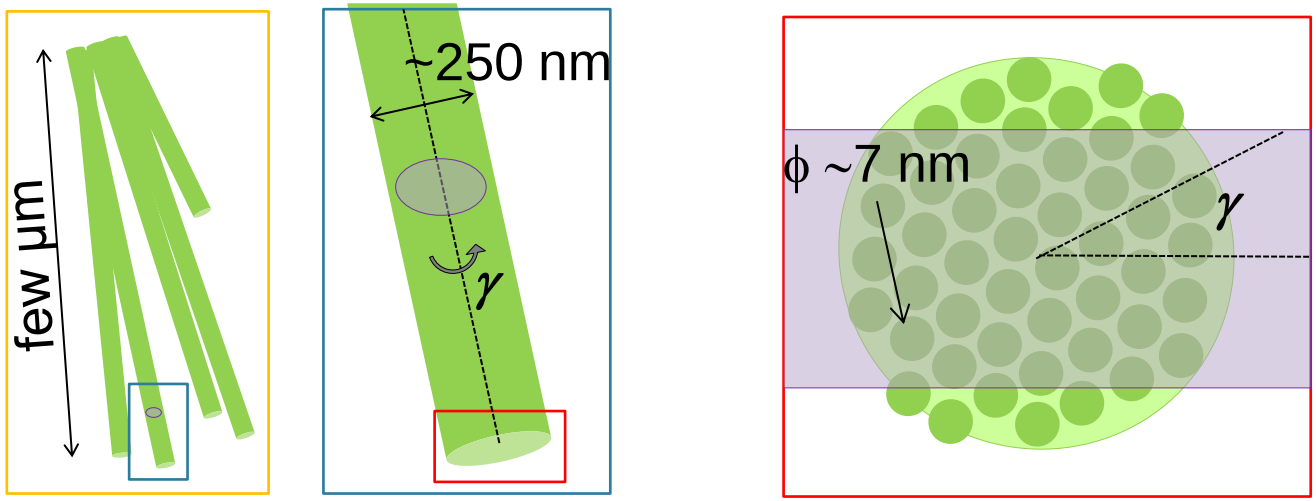




Piazza et al., ACSNano, 2014

Scattering geometry

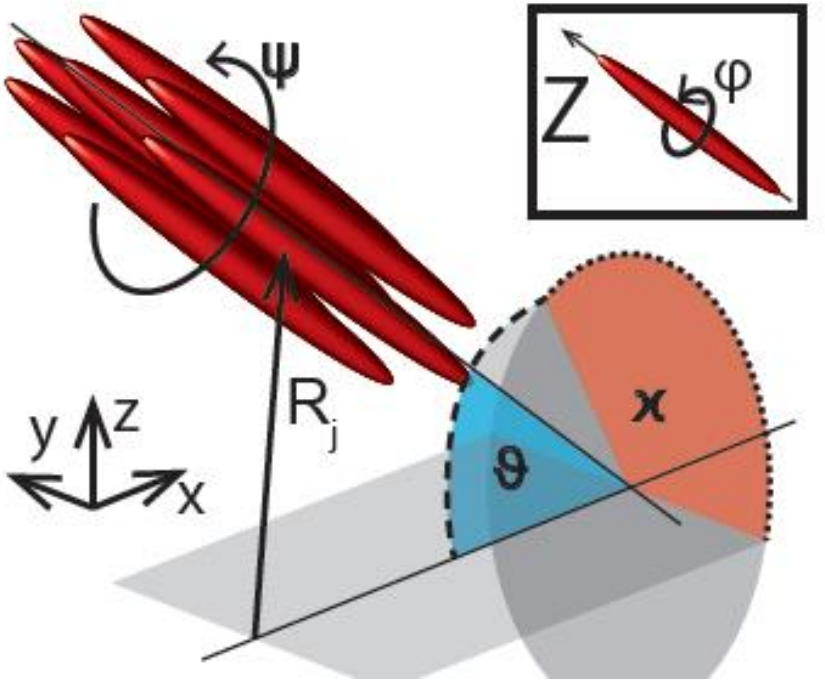


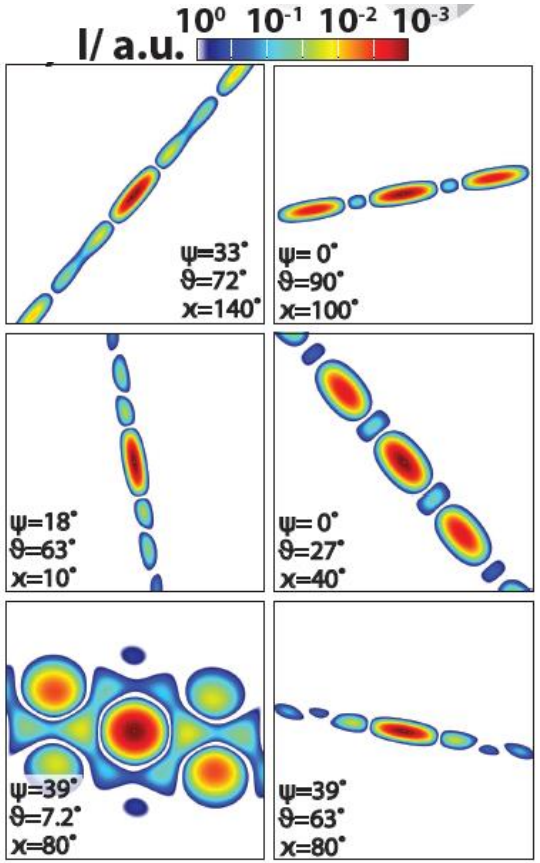


Piazza et al., ACSNano, 2014

Simulation

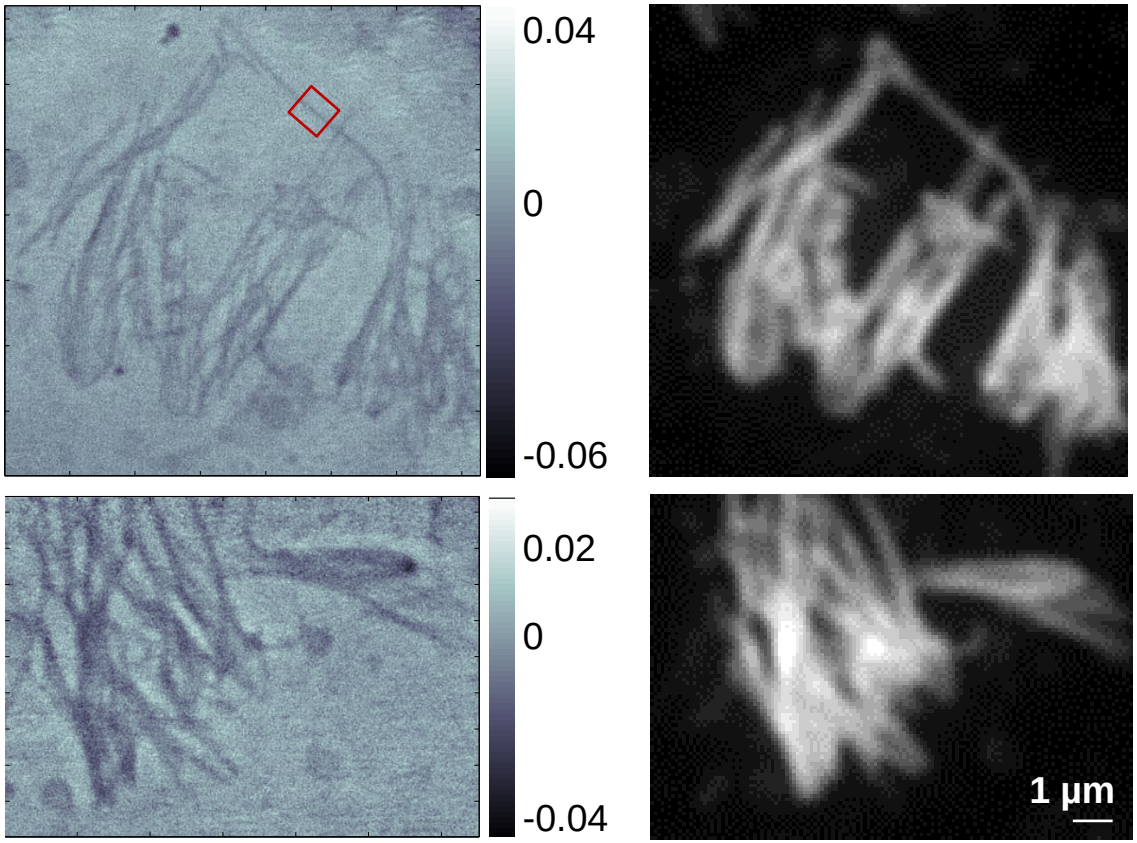
Tim Salditt, Göttingen

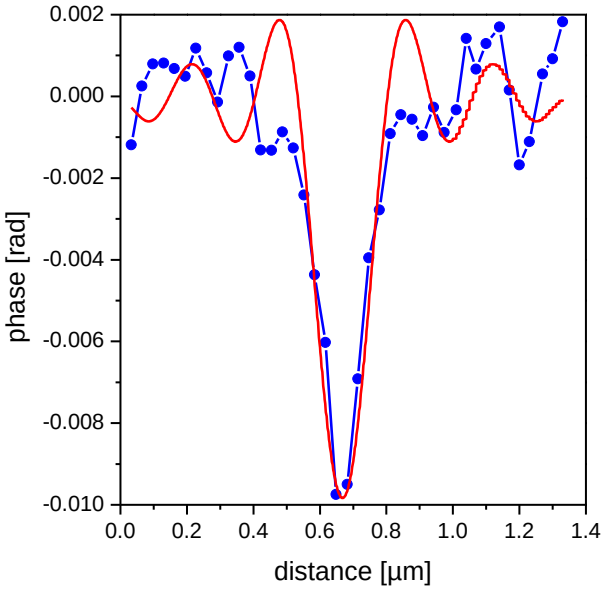




Priebe et al., Biophys. J., 2015

Ptychograpy





Piazza et al., ACSNano, 2014

Sample environments

- Introduction
- Imaging cells with X-rays: nano-diffraction, ptychography
- **Sample environments: microfluidics**
- Experiments @ FLASH and SACLA &

  Ideas for microfluidics experiments @ MID

A step forward: hydrated cells

measurements on hydrated cells

- native protein structure
- no artifacts from freezing/drying

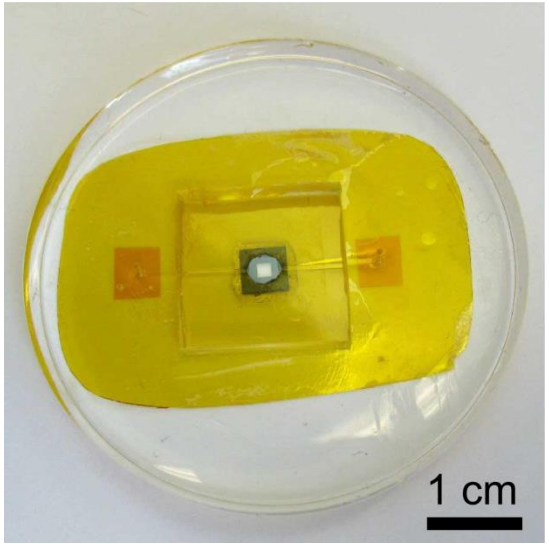
- lower electron density contrast
- absorption/scattering due to water layer
- increased radical production and mobility in water

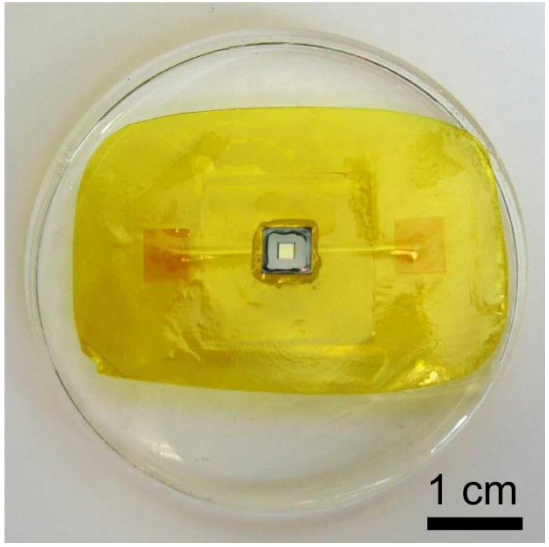
sample environment

- compatible with x-ray diffraction
- compatible with cell culture
- small/light enough to fit into the set-up

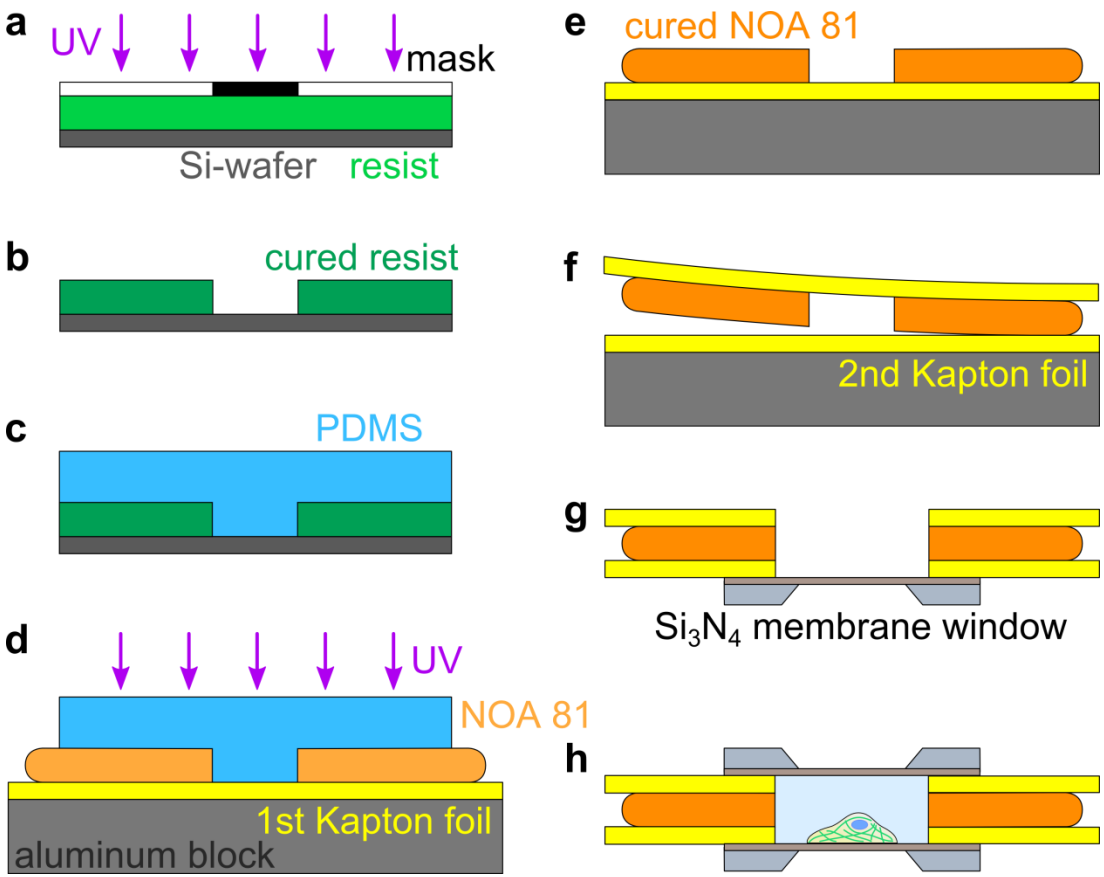
A microfluidic chamber for cells

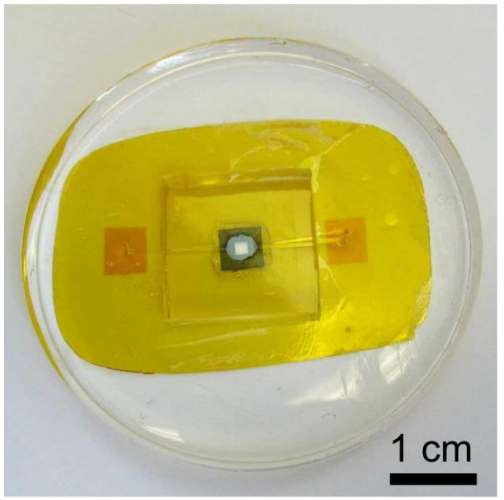
- fluid flow: nutrient supply and waste removal
- Si_3N_4 membrane windows as substrate and window material for X-rays

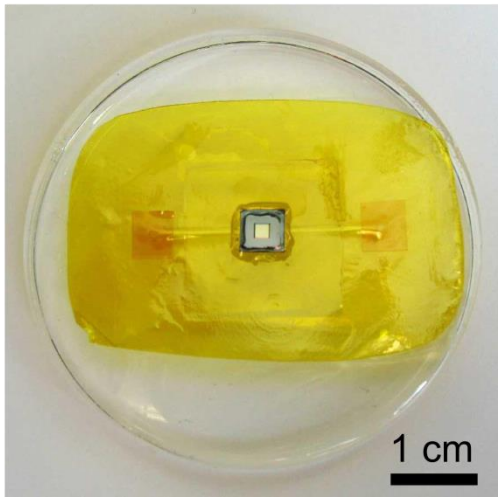




A microfluidic chamber for cells





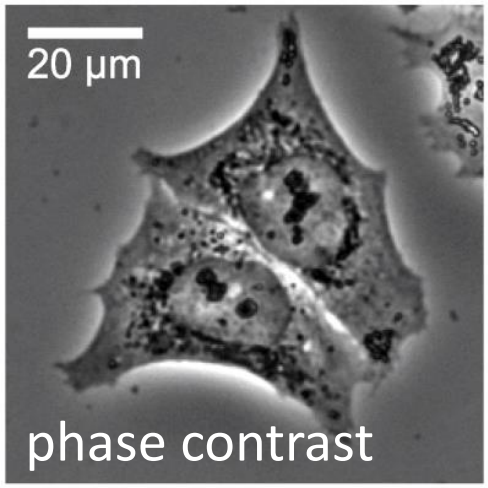


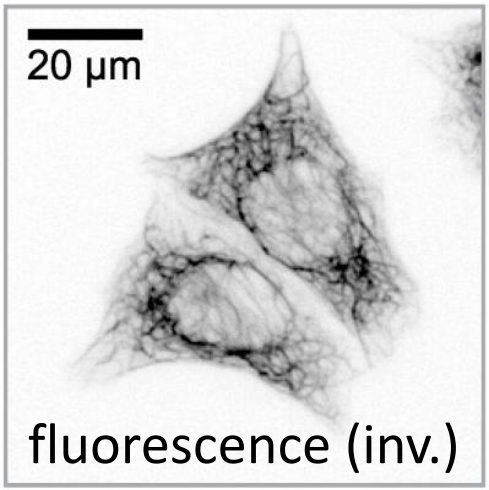
- channel size ≈500 × 150 µm²

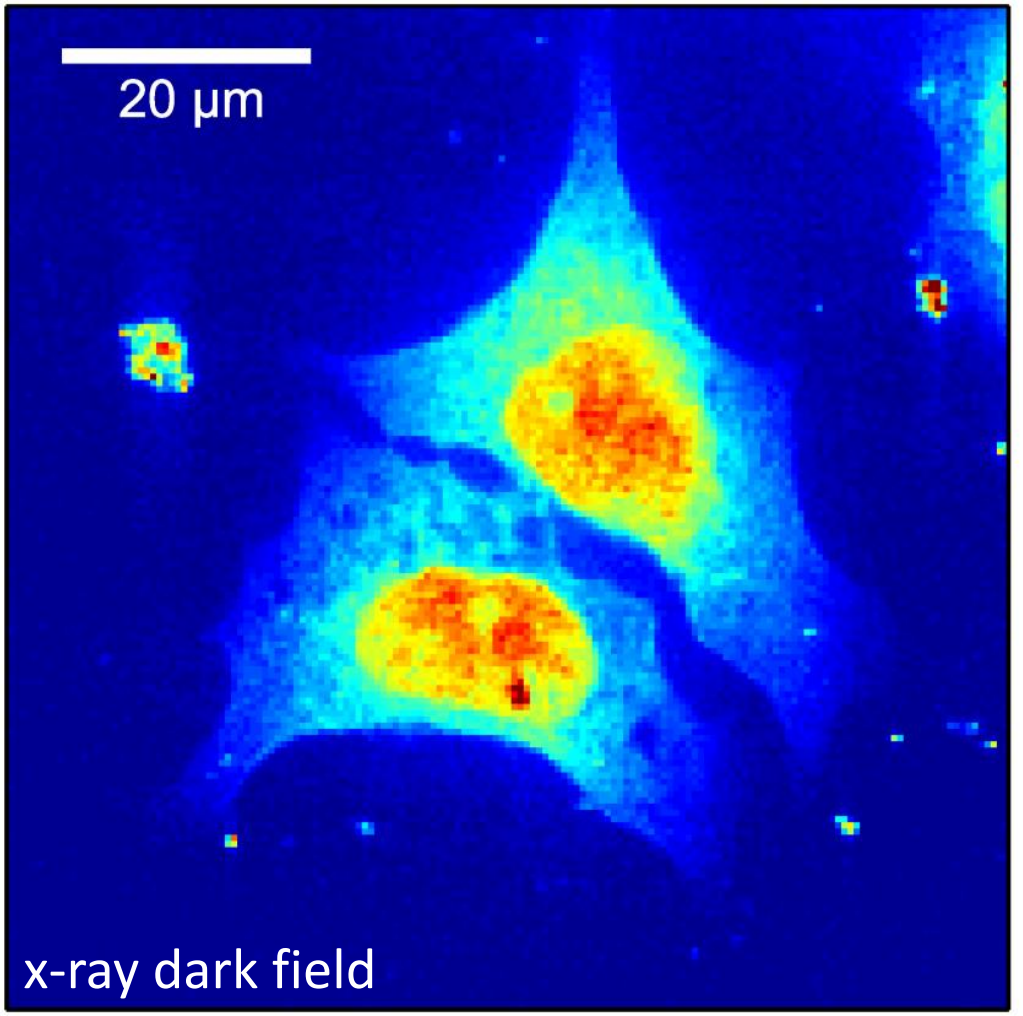
Fixed-hydrated cells

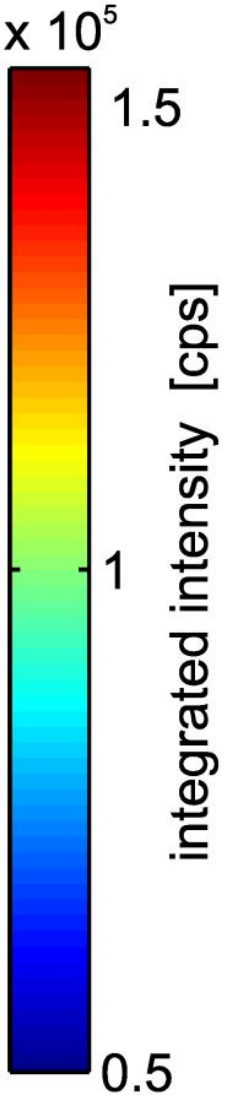
fixed, hydrated cells

P10/PETRA III







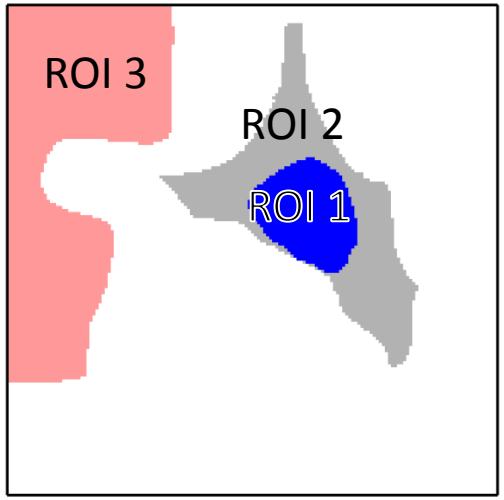


- good signal-to-noise ratio in dark-field image

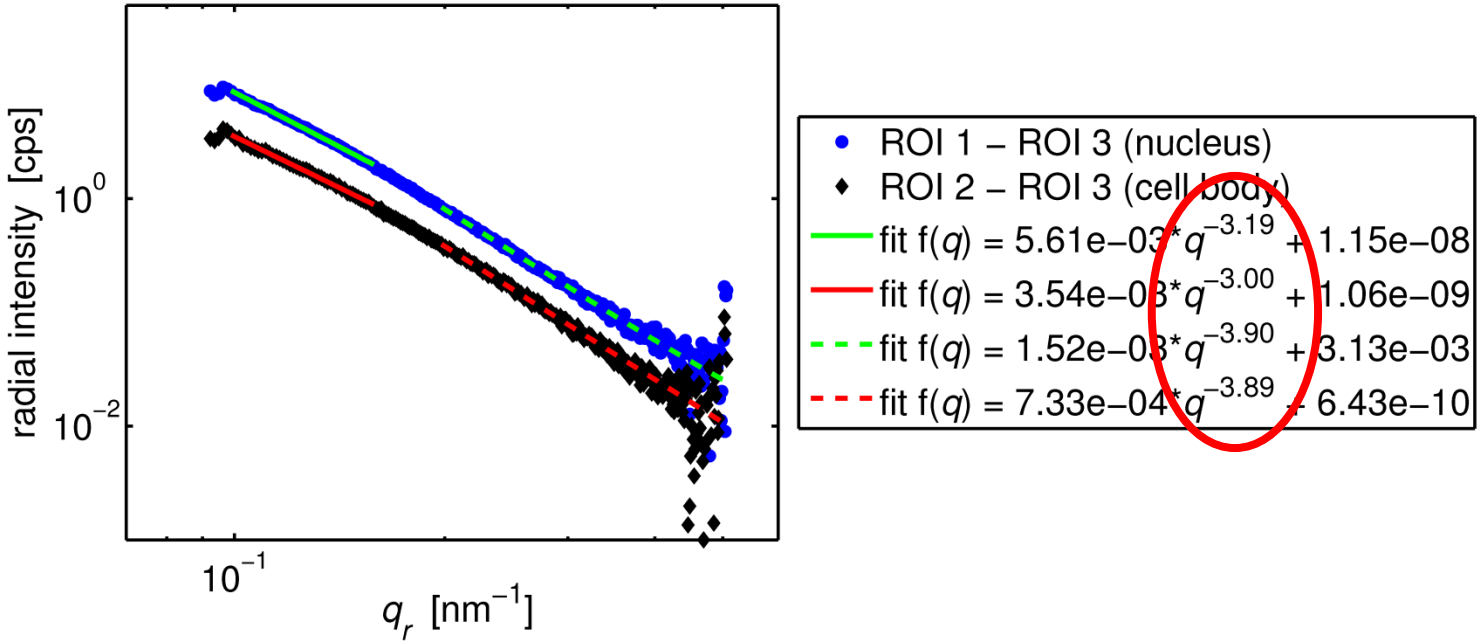
step size 250 nm

Weinhausen et al, PRL 2014

Radial intensity

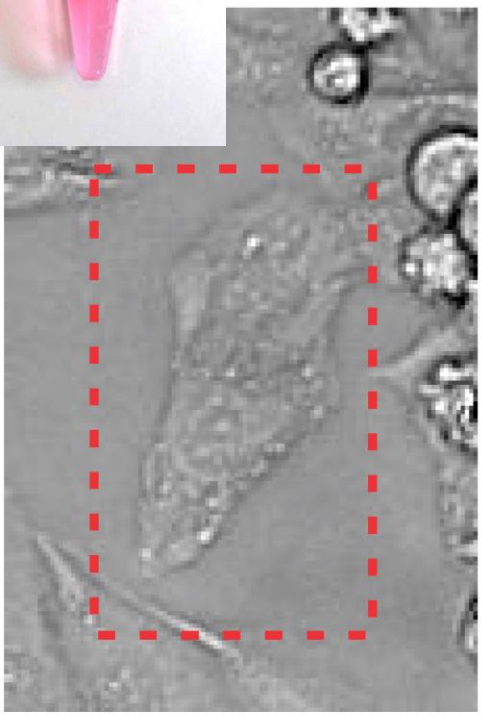


- different scan regions
- averaged scattering patterns
- azimuthal integration
- background subtraction
- power-law fit

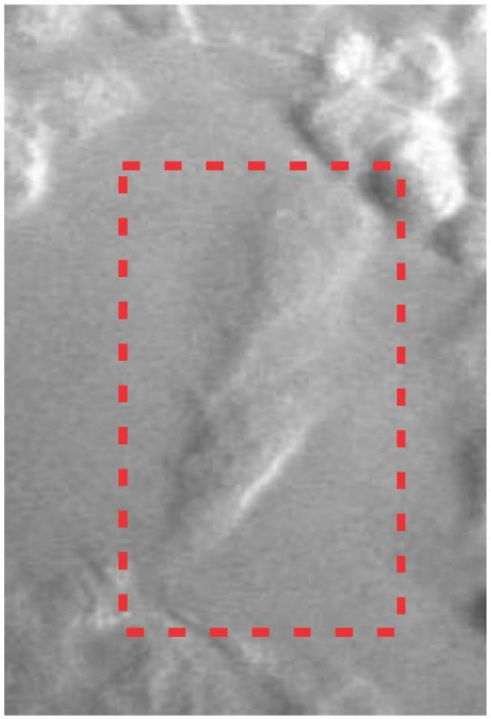


Initially living cells

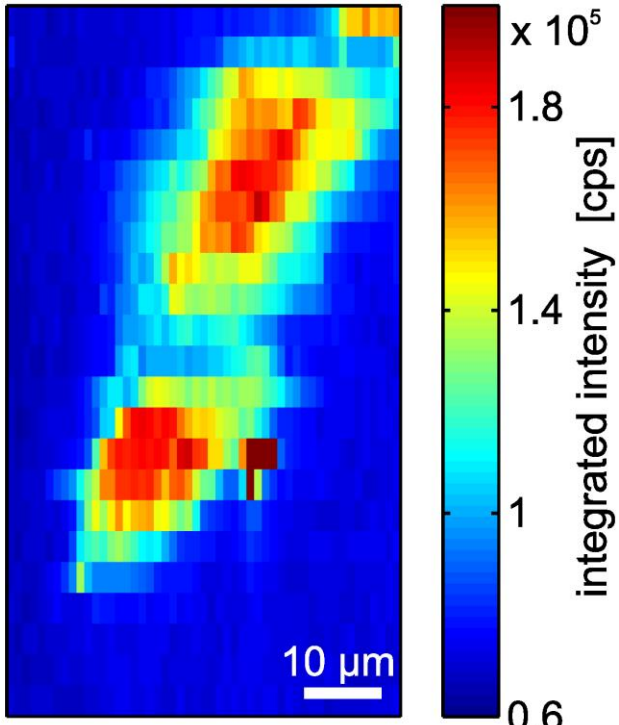
- transport in microtubes filled with medium
- storage in petri dishes incubator



lab microscope



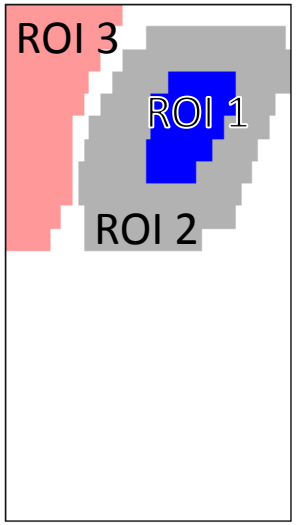
beamline microscope

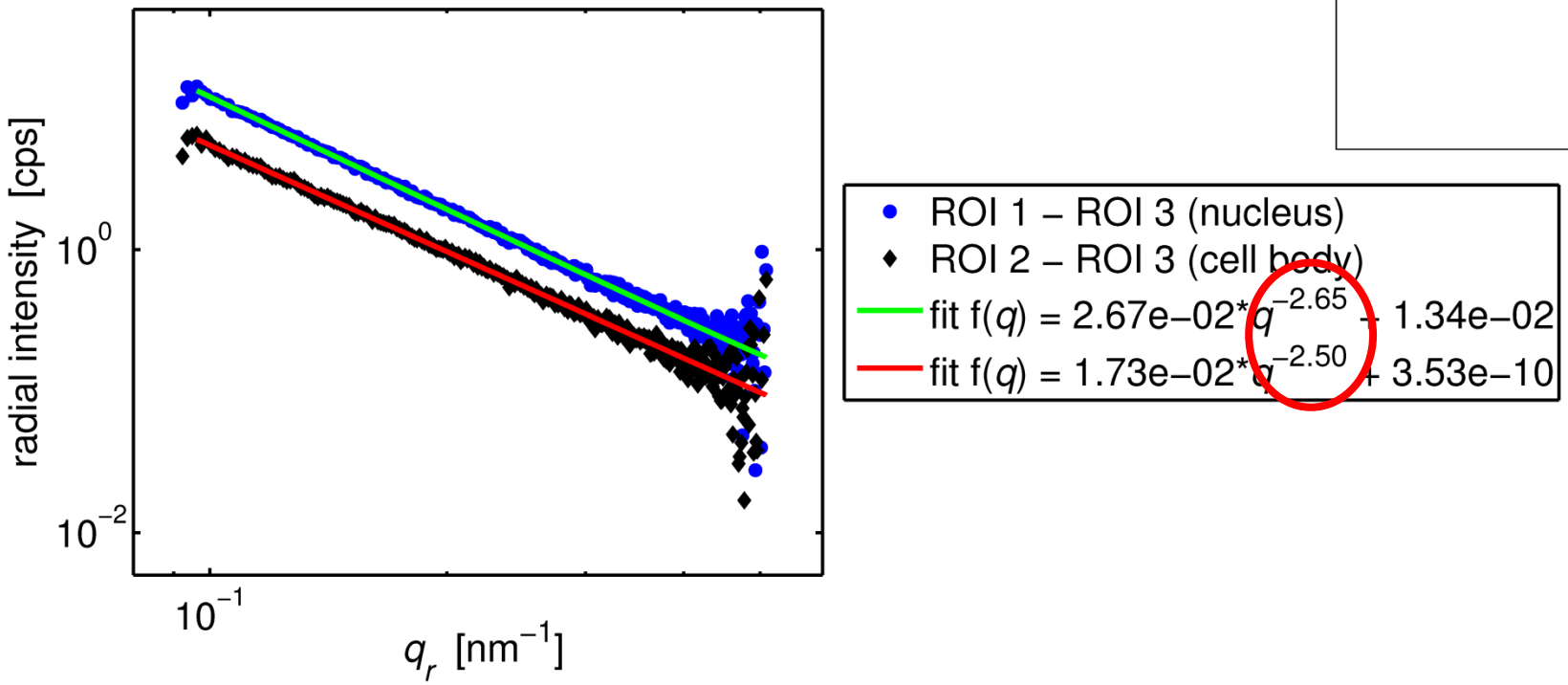


X-ray dark-field image

- asymmetric scans to skip damaged regions

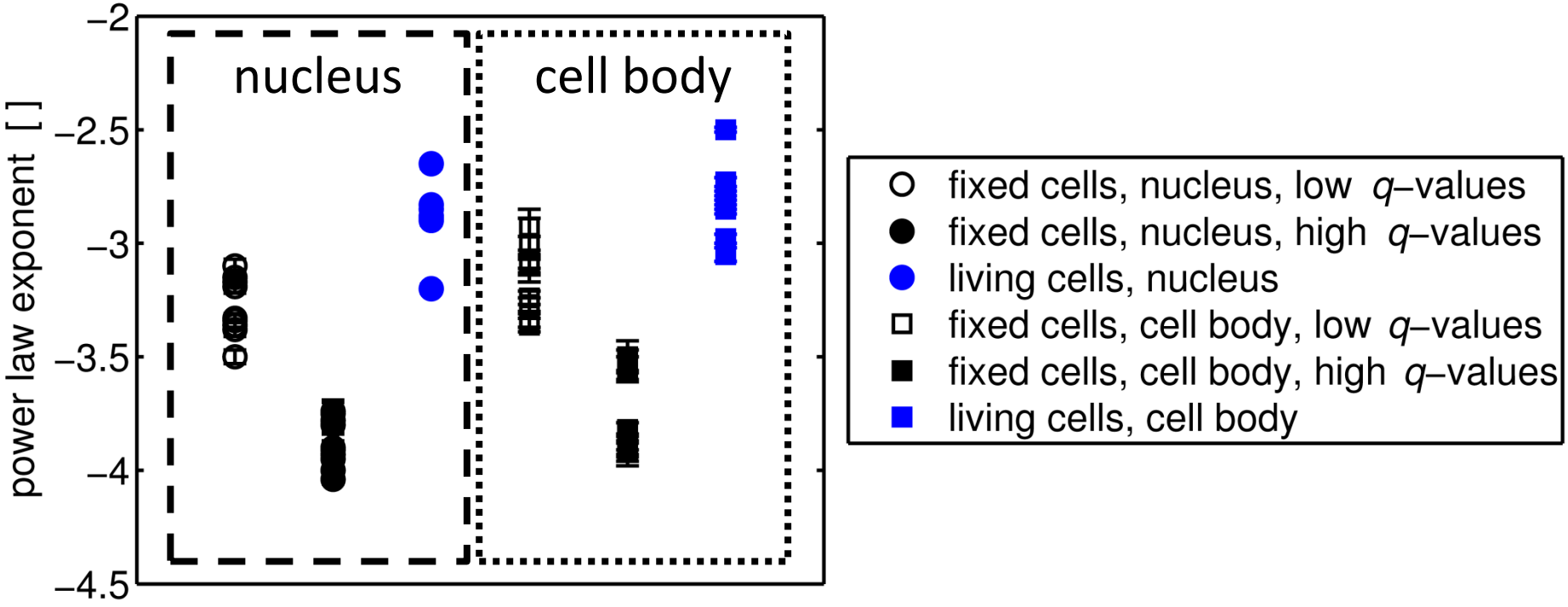
Radial intensity





Weinhausen et al, PRL 2014

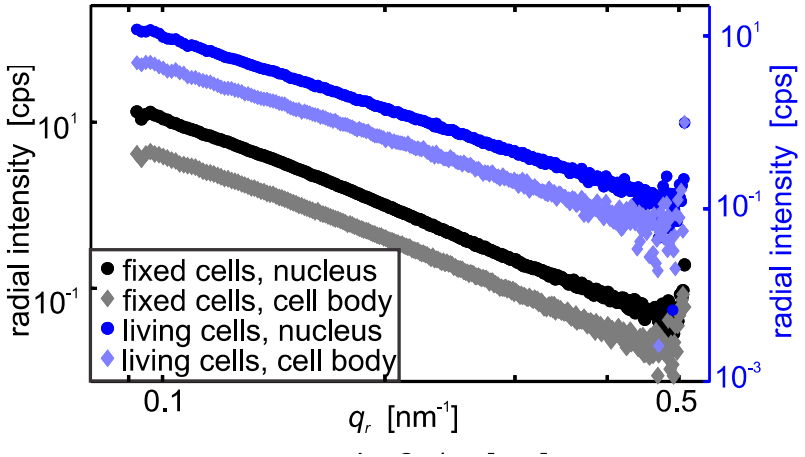
Power law exponents



- **different** (higher) power law exponents for living cells compared to fixed-hydrated cells

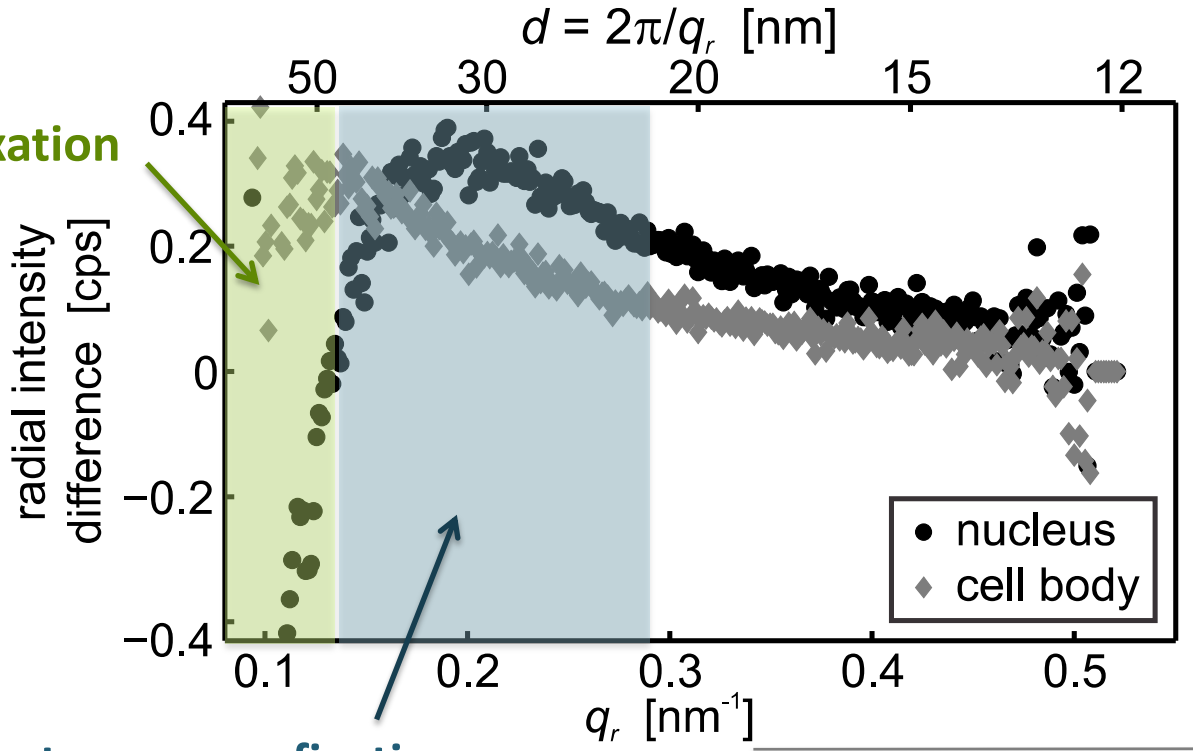
Weinhausen et al, PRL 2014

Structural changes



„living – fixed cells“

emerging structures upon fixation

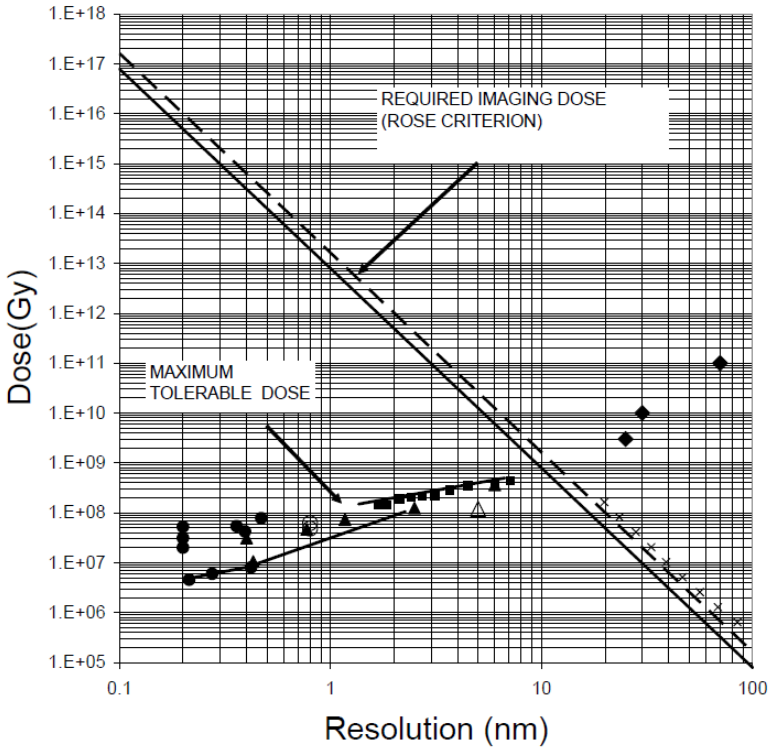


Weinhausen et al, PRL 2014

disapearing structures upon fixation

Conclusions

- X-rays provide a complementary approach for imaging cells (⇔ light microscopy, electron microscopy)
- different (contrast) methods are currently being developed and improved
- radiation damage remains a great challenge
- reciprocal space provides structural information at high resolution



Howells et al., 2009

Sample environments

- Introduction
- Imaging cells with X-rays: nano-diffraction, ptychography
- Sample environments: microfluidics
- **Experiments @ FLASH and SACLA &**

  Ideas for microfluidics experiments @ MID

Requirements @ MID

- scintillation-based detector (AGIPD) @ ~ 10 m from focus
- possibly second detector, “drilled-through”
- optical microscopy for sample alignment
- feed-through for connections to microfluidic chambers

Acknowledgements

Göttingen:
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Villigen: Ana Diaz, Andreas Menzel
Hamburg: Anders Madsen, Jörg Hallmann, Christian Schroer, Juliane Reinhardt
Dresden: Jens Patommel






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