Phase and index imaging for biologie

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MiFoBio 2021
Presqu'île de Giens - France, November 5-12, 2021

Intensity Microscopy

Dark-field
Oblique illumination
Rheinberg illumination
Hoffman modulation
Polarized microscopy
Phase contrast (Zernike)
Differential Phase Contrast (Nomarski)
Ultramicroscope (Siedentopf & Zsigmondy)

:-) Some control of the illumination
:-) A certain comprehension of the light specimen interaction phenomenon
:-(Inherently 2-D

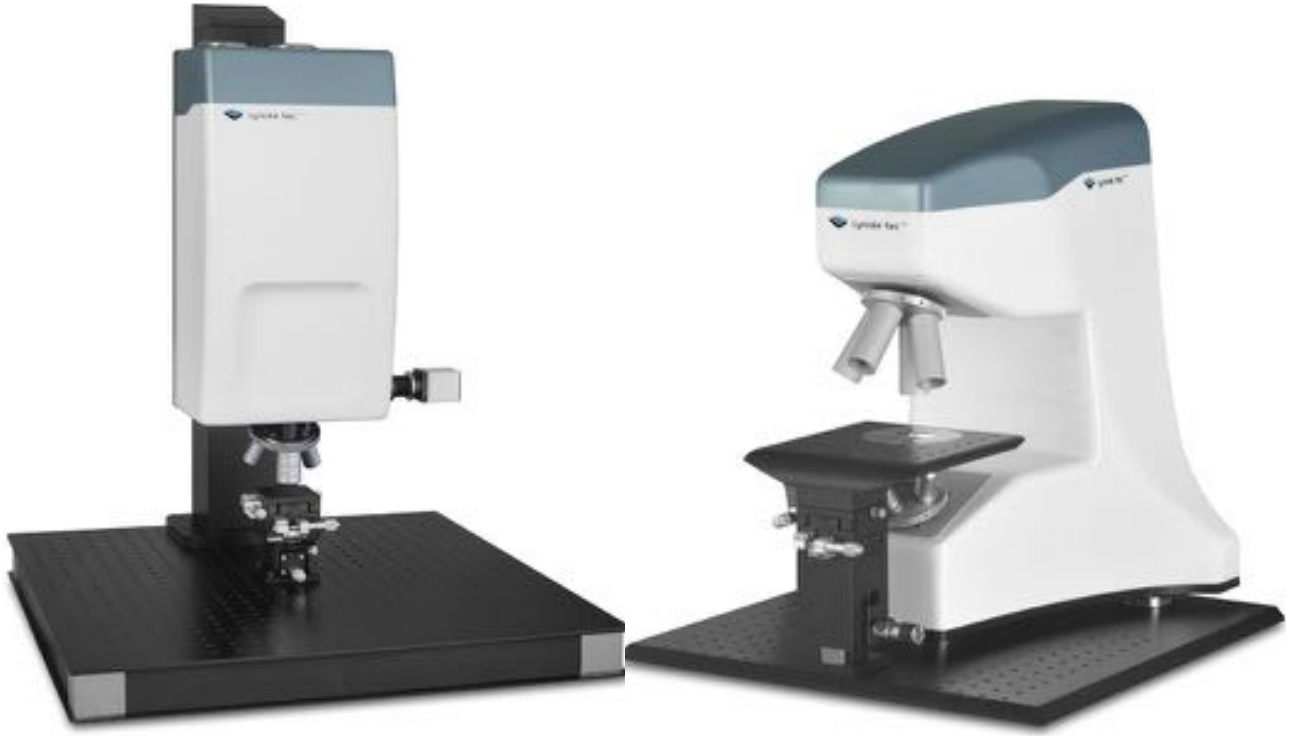
Phase Microscopy

:-(Usually, one records intensity only images

Gabor Holography
Phase Stepping Holography
Off-Axis Holography
Front Wave Analyser

=> One can (now easily) measure amplitude and phase of the diffracted field

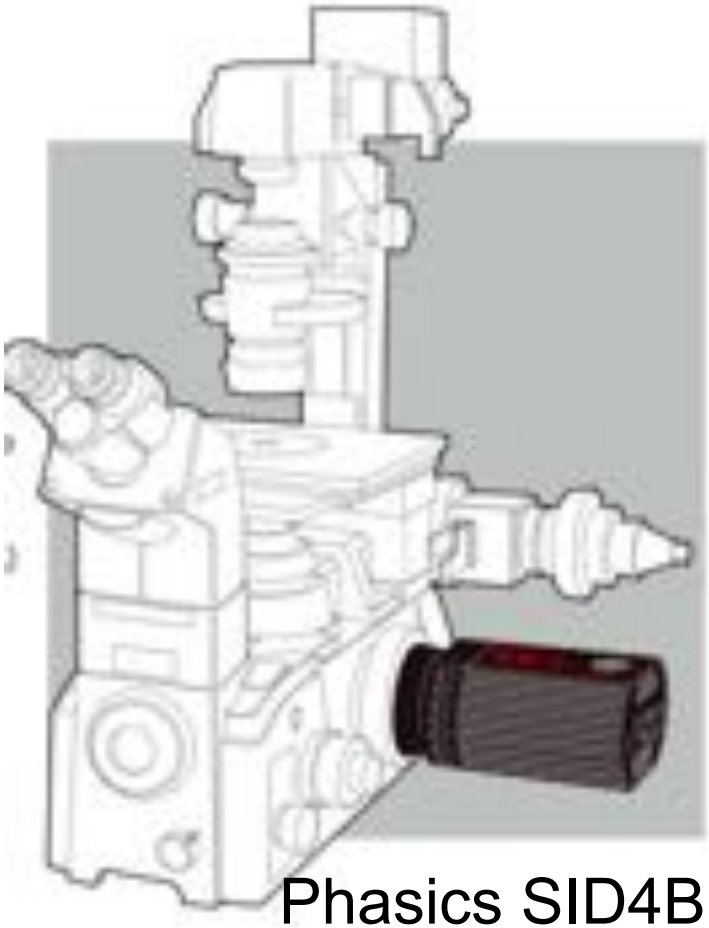
Commercial DHM



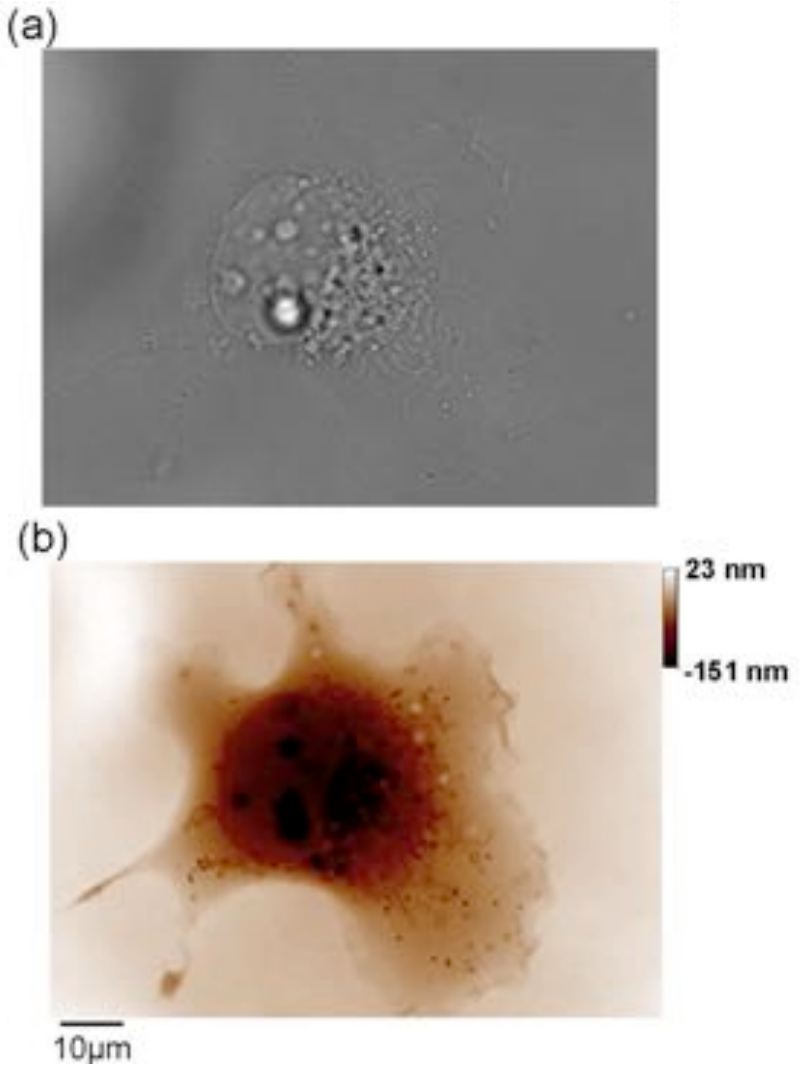
lyncée tec

Phase Holographic Imaging

Wavefront Analyser

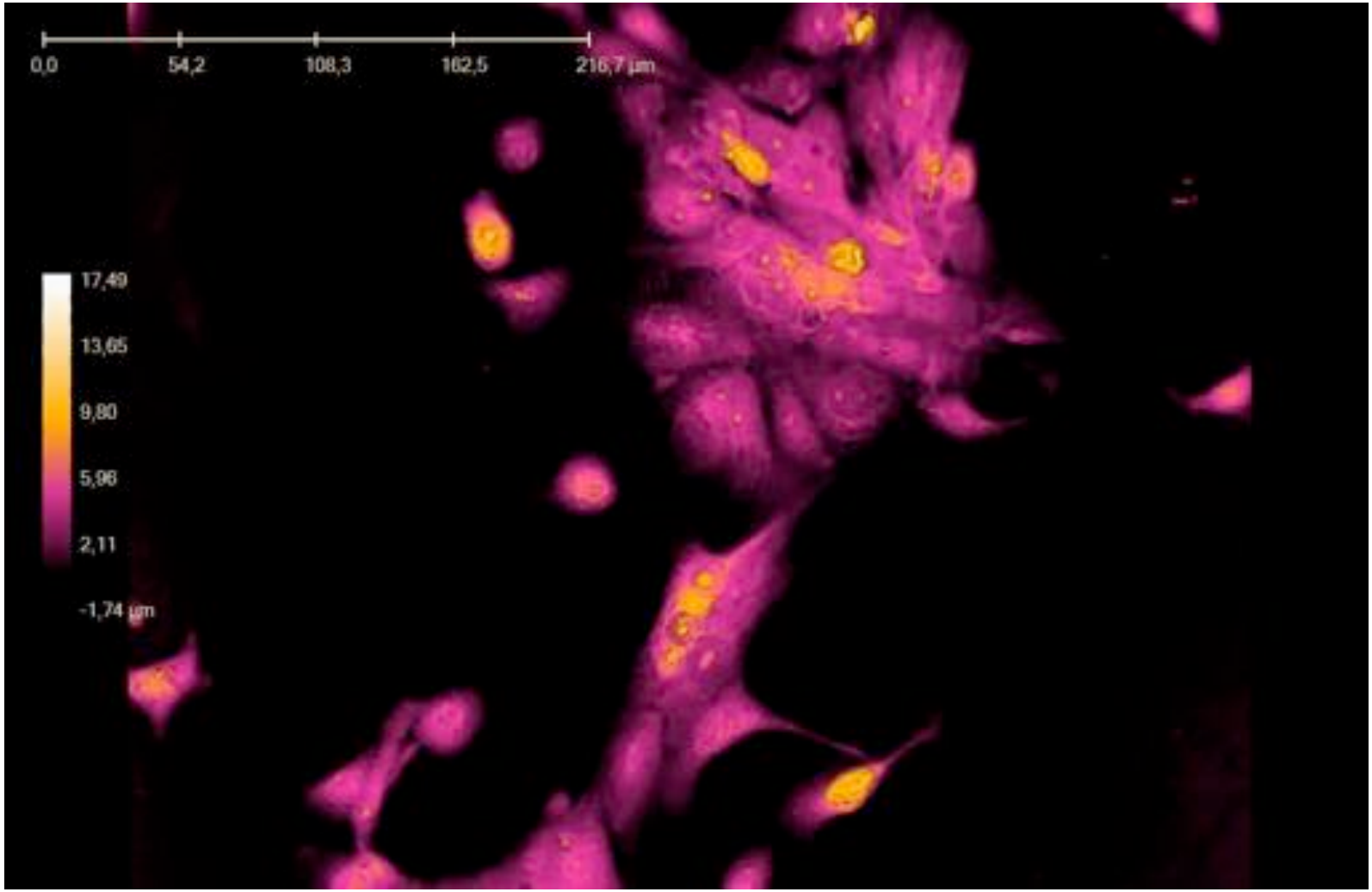


Phasics SID4Bio



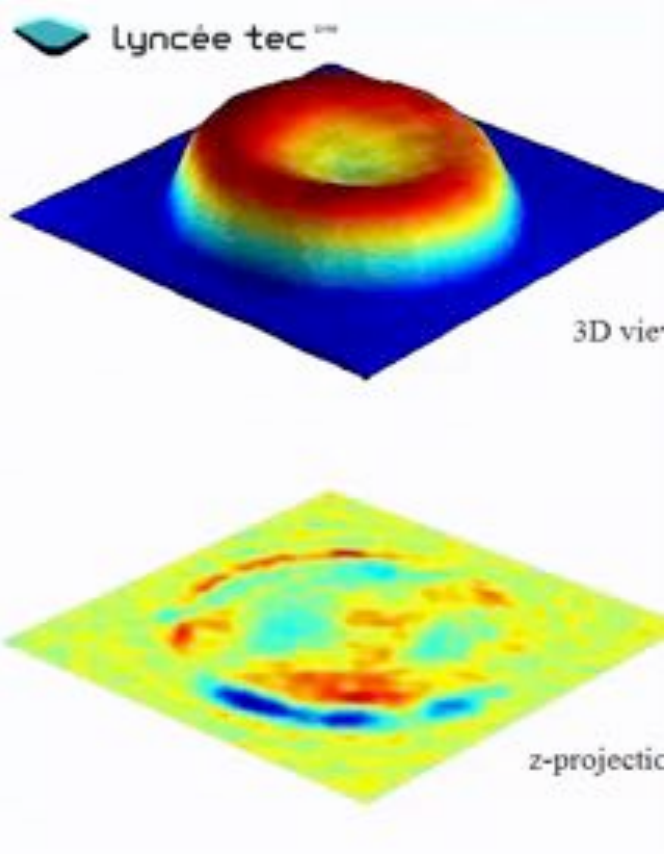
P. Bon, et al., Opt. Expr. **17**, p. 13080 (2009)

Suivi de cultures (DHM)



Living JIMT-1 cells, recording over 72 hours, 1 frame / 4 min
Birgit Janicke – Phase Holographic Imaging

Holographic Microscopy

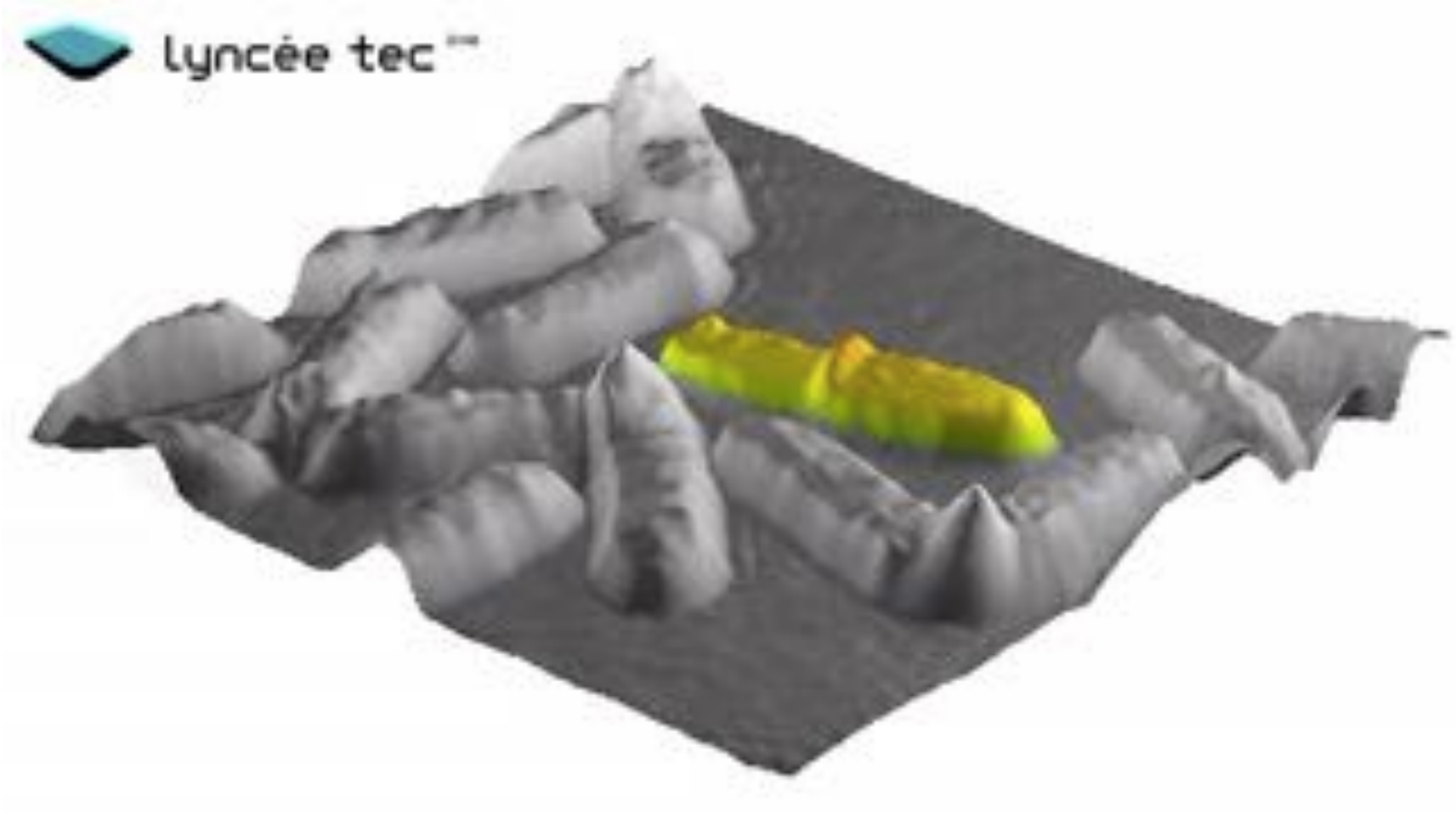


Measurement of the integral refractive index and dynamic cell morphometry of living cells with digital holographic microscopy

B. Rappaz, *et al.,*

Opt. Express 13 (23), 9361-9373 (2005)

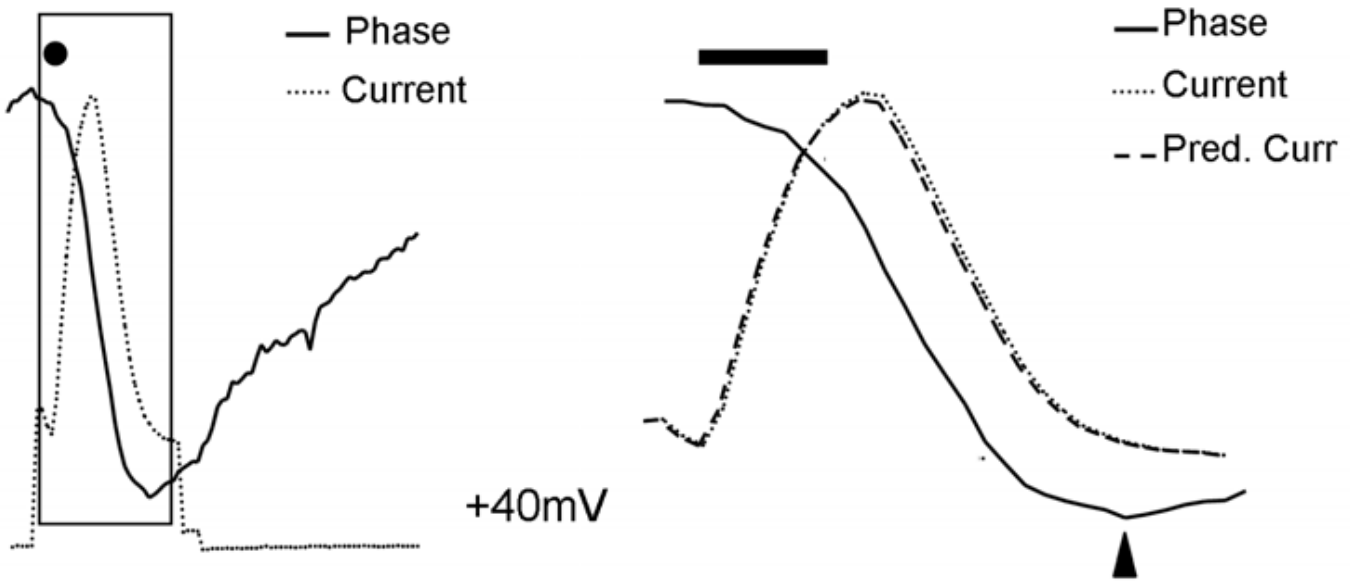
Cartographie de surface



Electrophysiologie optique (DHM)

$$I_{GABA}(t) = \frac{V_0}{\varepsilon^*_{GABA}} \frac{d}{dt} \left(\frac{\varphi_0}{\varphi(t)} \right)^{1/s-r}$$

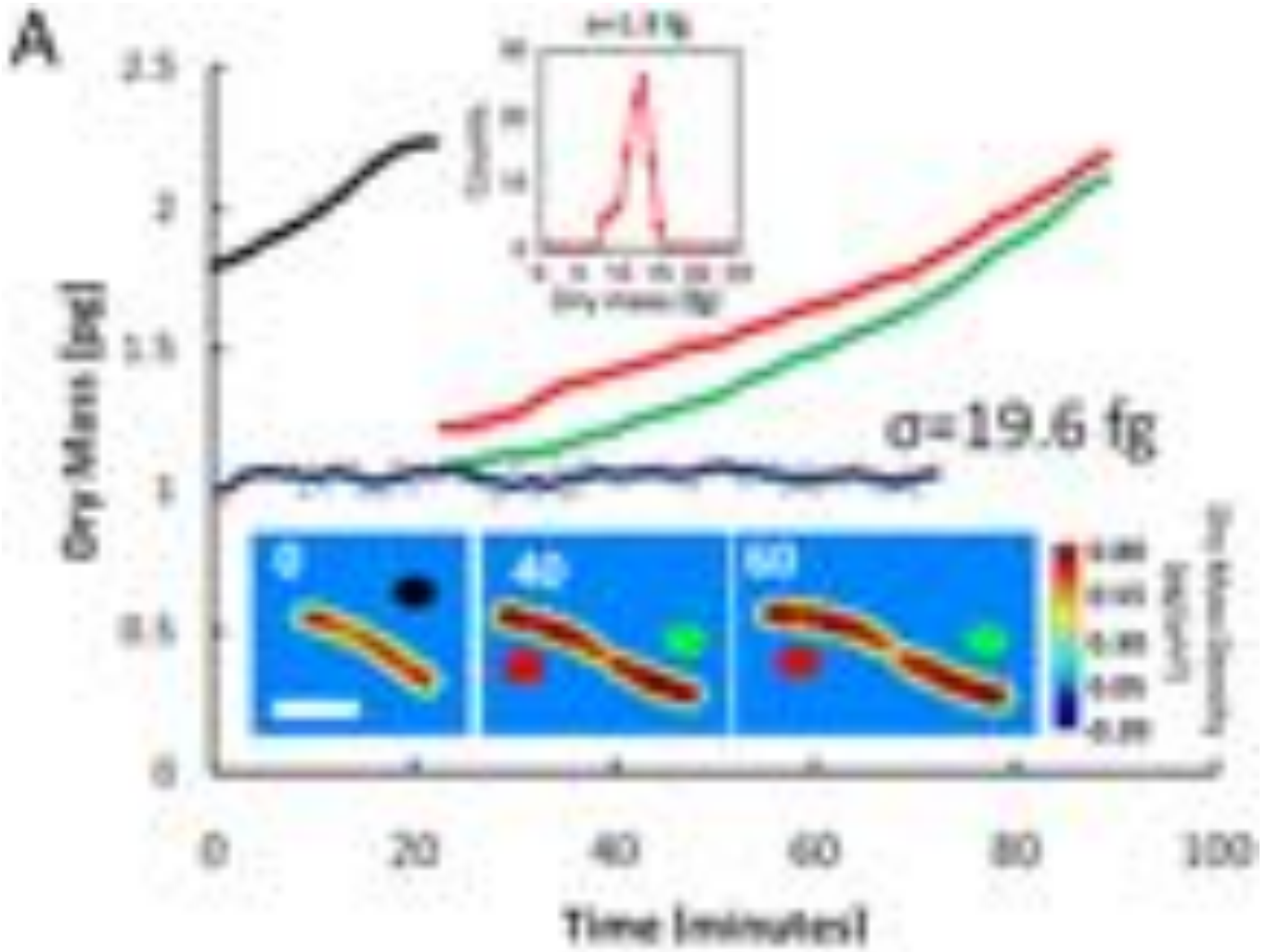
NaCl



"Simultaneous Optical Recording in Multiple Cells by Digital Holographic Microscopy of Chloride Current Associated to Activation of the Ligand-Gated Chloride Channel GABAA Receptor"

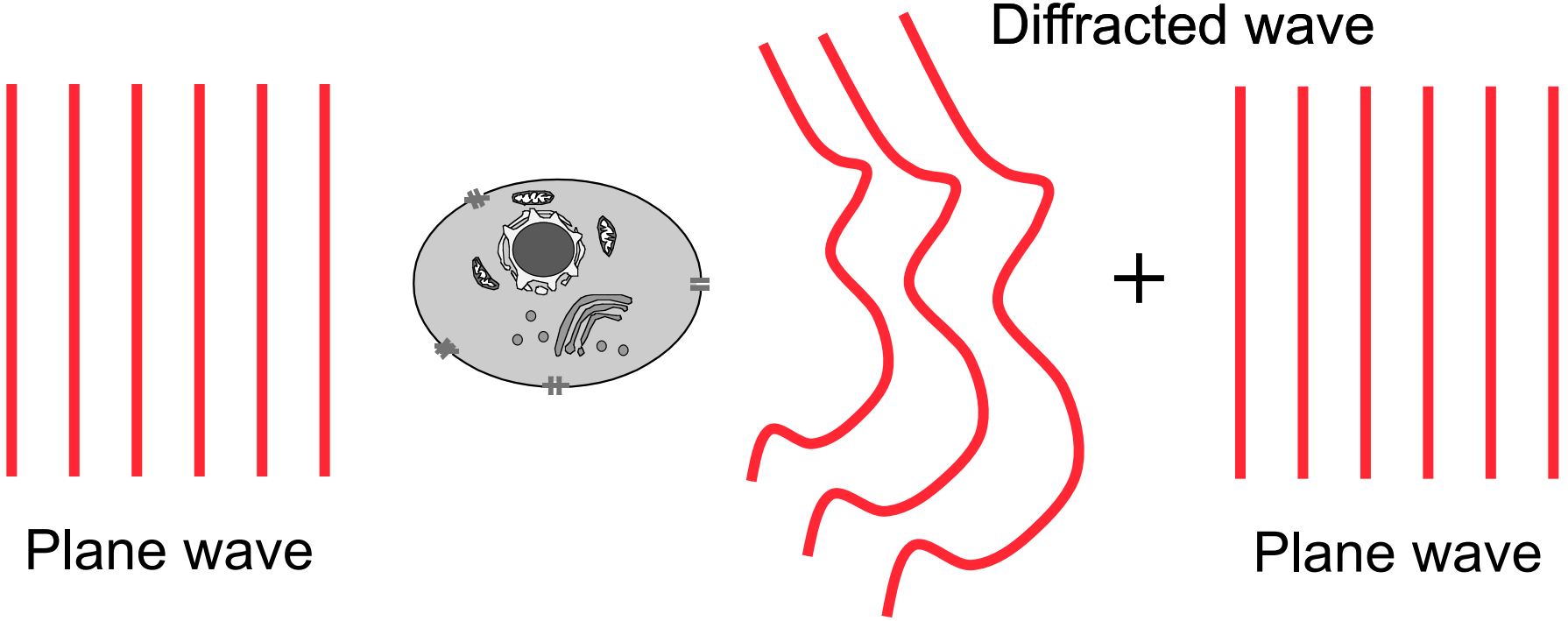
P. Jourdain, *et al.*, Plos One **7,** e51041(2012)

Mesure de masse sèche (SLIM)



“Optical measurement of cycle-dependent cell growth”
M. Mir, *et al.,* PNAS **108**, p. 13124 (2011)

Coherent Light Diffraction



Weakly diffracting/diffusing/absorbing object

1st Born approximation

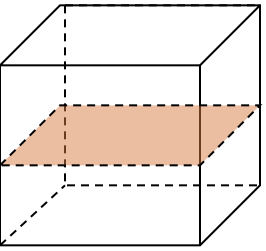
The diffracted wave is interpreted as a part of the 3-D Fourier 3D transform of <n>

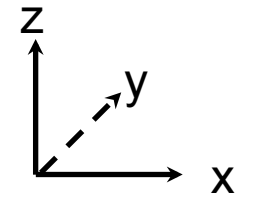
Semi-transparent object reconstruction from holographic data

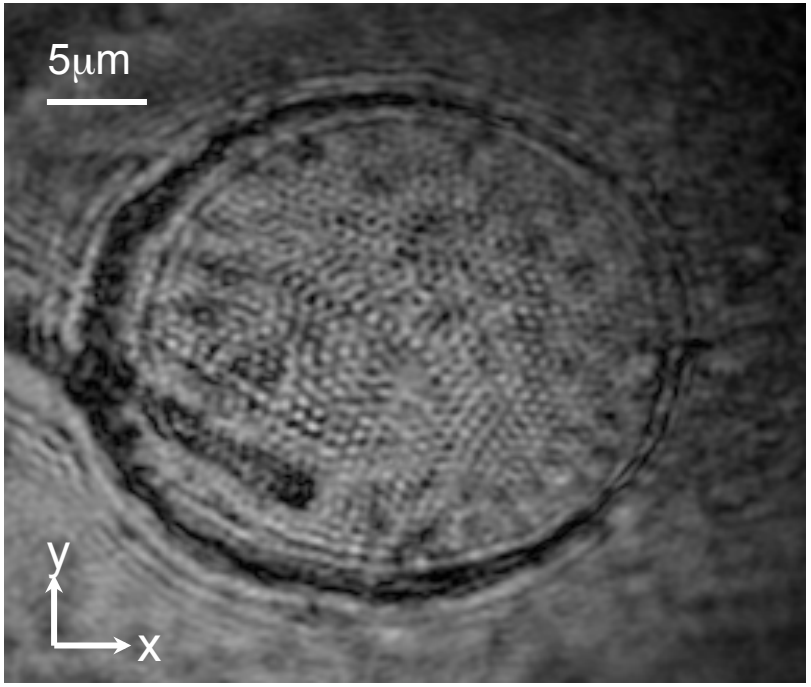
E. Wolf, Opt. Comm. **1**, p. 153 (1969)

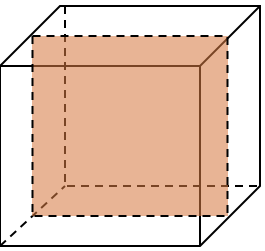
Holographic Miroscopy: Results

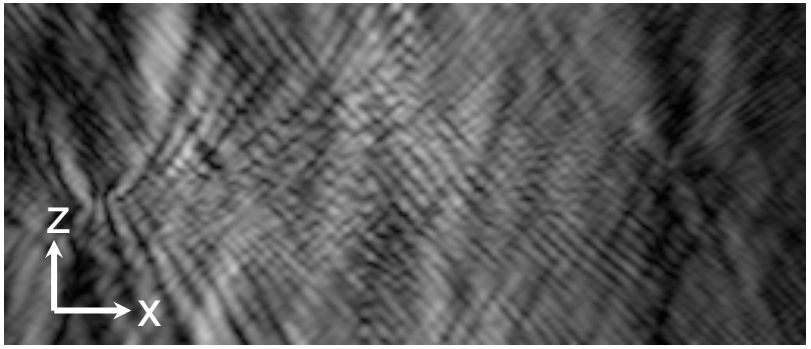
1 angle









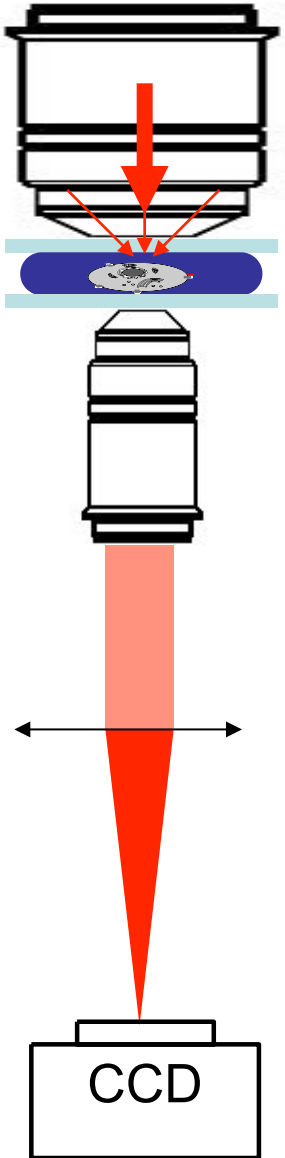


Limited 3-D resolution

Profilometry

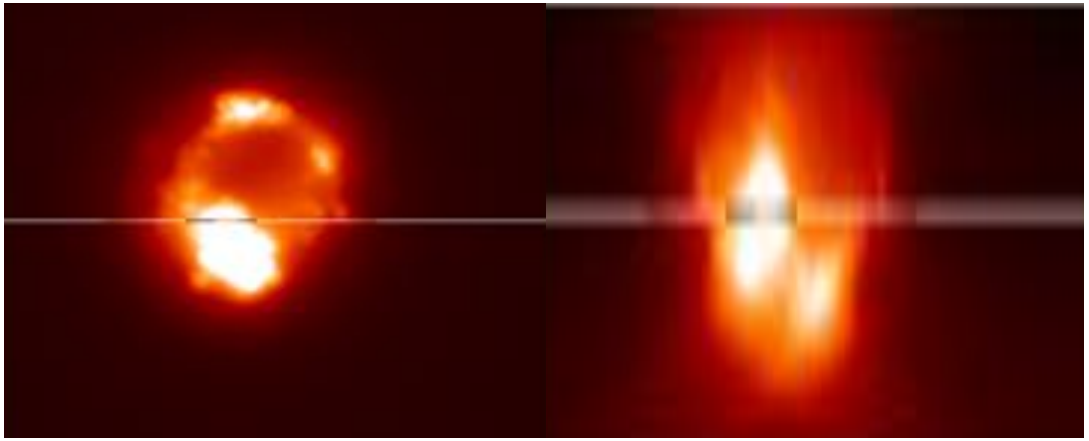
Integral measurements

Illumination Control: Consequences



A radical solution:

1 unique illumination direction!



Cellule CD34 - Image Georges Jung, Laboratoire d'Hématologie
Centre Hospitalier Régional Emile Muller - Mulhouse

$$R_{trans} = R_{fluo} = \frac{\lambda}{(NA_{cond} + NA_{obj})}$$

Limits of Label-free VS fluorescence

Sensitivity

→ Not a single molecule approach…

Resolution limit

→ Initial resolution = 50% of fluo. resolution

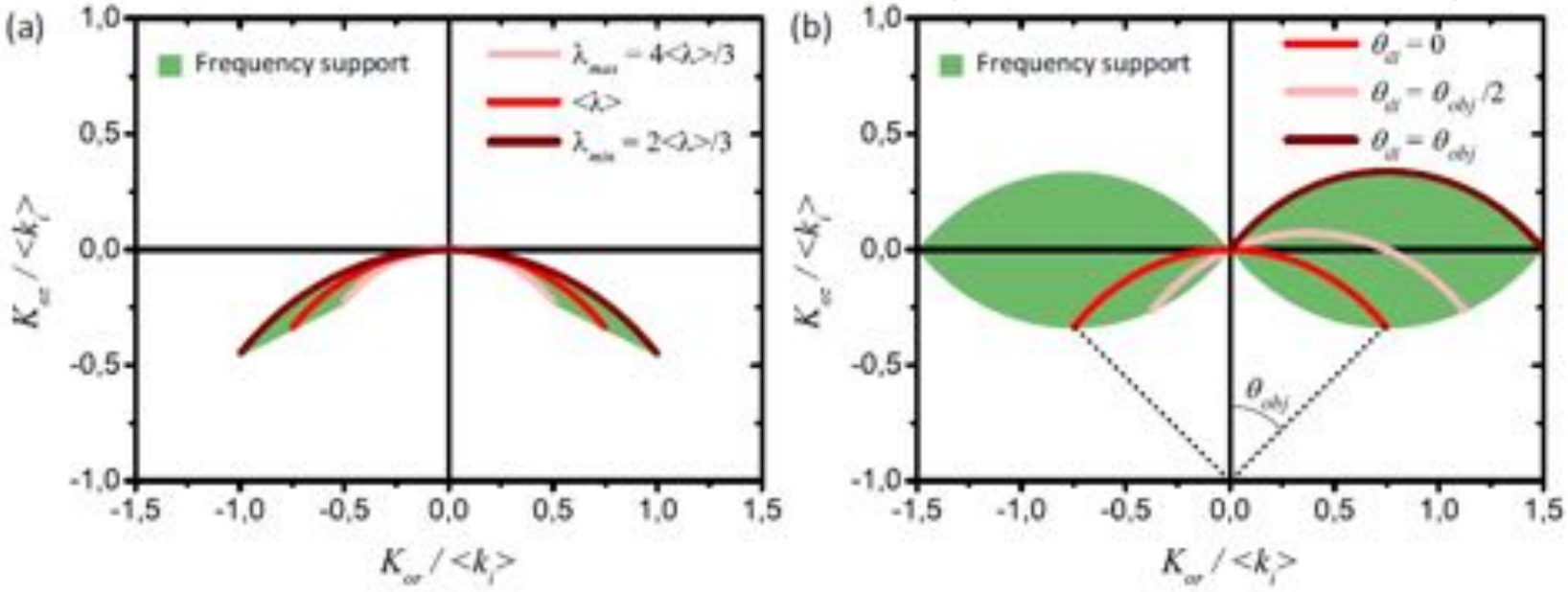
Selectivity/Specificity

→ How to identify and contrast a molecular type

Resolution limit

- Label-free = coherent process VS Fluo = incoherent process
  - → Phase imaging = usually interferometric → One illumination angle
  - → PSF phase imaging = $1{,}22\lambda/NA$ VS PSF fluo. = $1{,}22\lambda/2NA$

(Worst in the axial direction!!!!)



Resolution enhancement

- Aperture synthesis ➔ Multiplexing illumination angles

  Bertrand *et al.*, High-resolution tomographic diffractive microscopy of biological samples, **J. Biophotonics**, 3(7), 2010

- Incoherent illumination

  Parthasarathy *et al.*, Quantitative phase imaging using a partitioned detection aperture, **Opt. Express**, 37(19), 2012

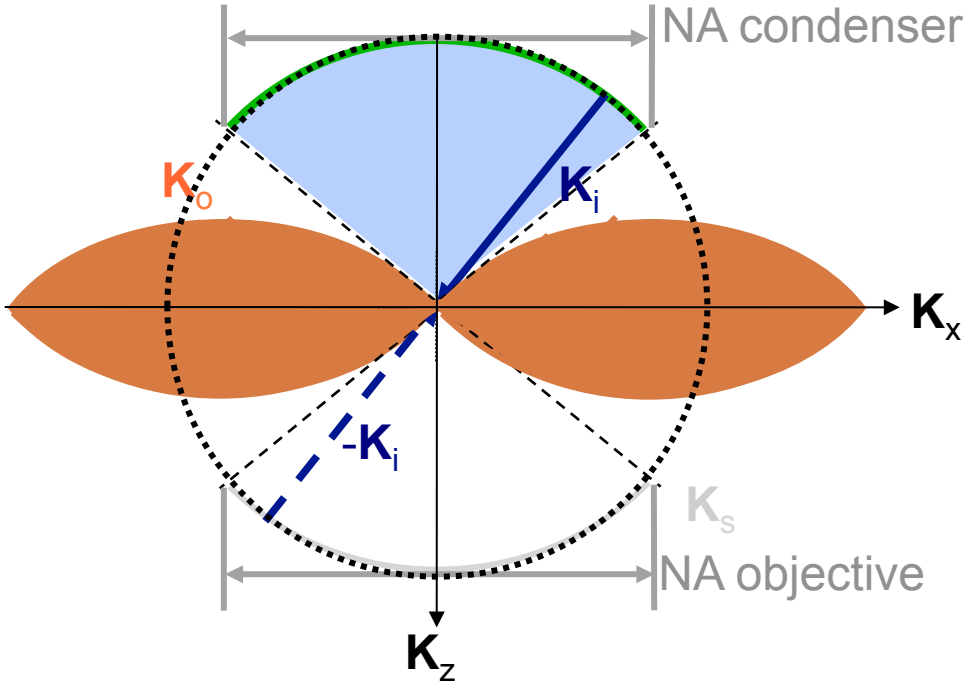
  Bon *et al.*, Enhanced 3D spatial resolution in quantitative phase microscopy using spatially incoherent illumination, **Opt. Express**, 22(7), 2014

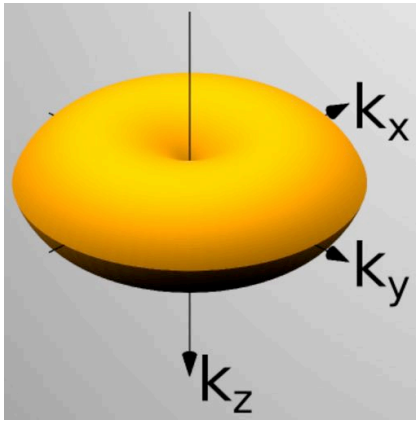
- Confocal illumination & structured illumination / structured refractive index modulation

  Chowdhury & Izzat, Structured illumination quantitative phase microscopy for enhanced resolution amplitude and phase imaging, **Biomed. Opt. Express**, 4(10), 2013

  Liu *et al.*, Quantitative phase-contrast confocal microscope, **Opt. Express**, 22(15), 2014

Tomography by Illumination Rotation



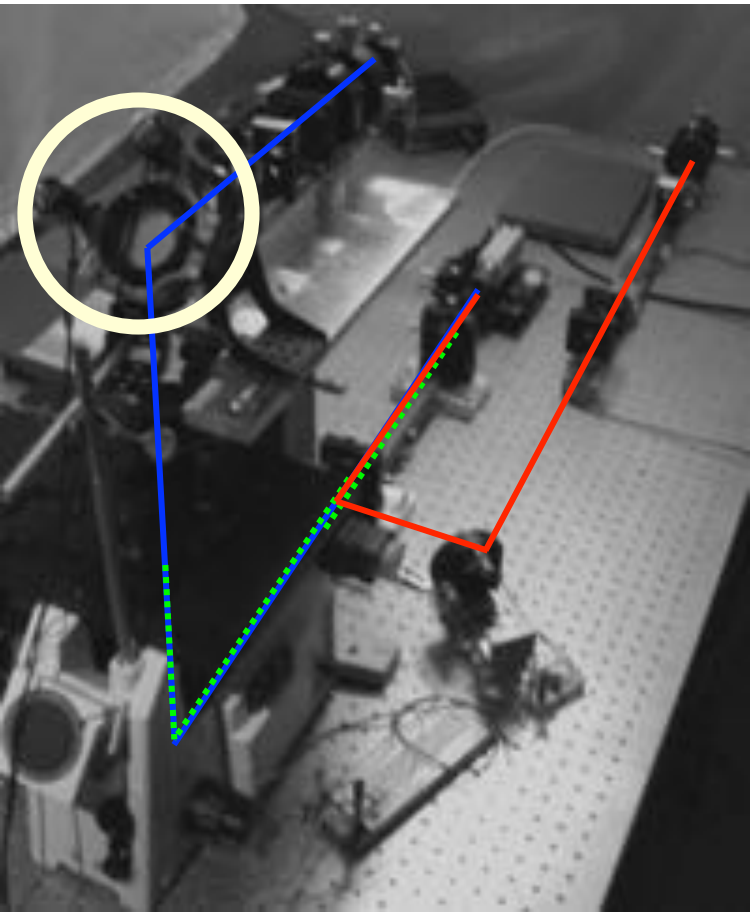


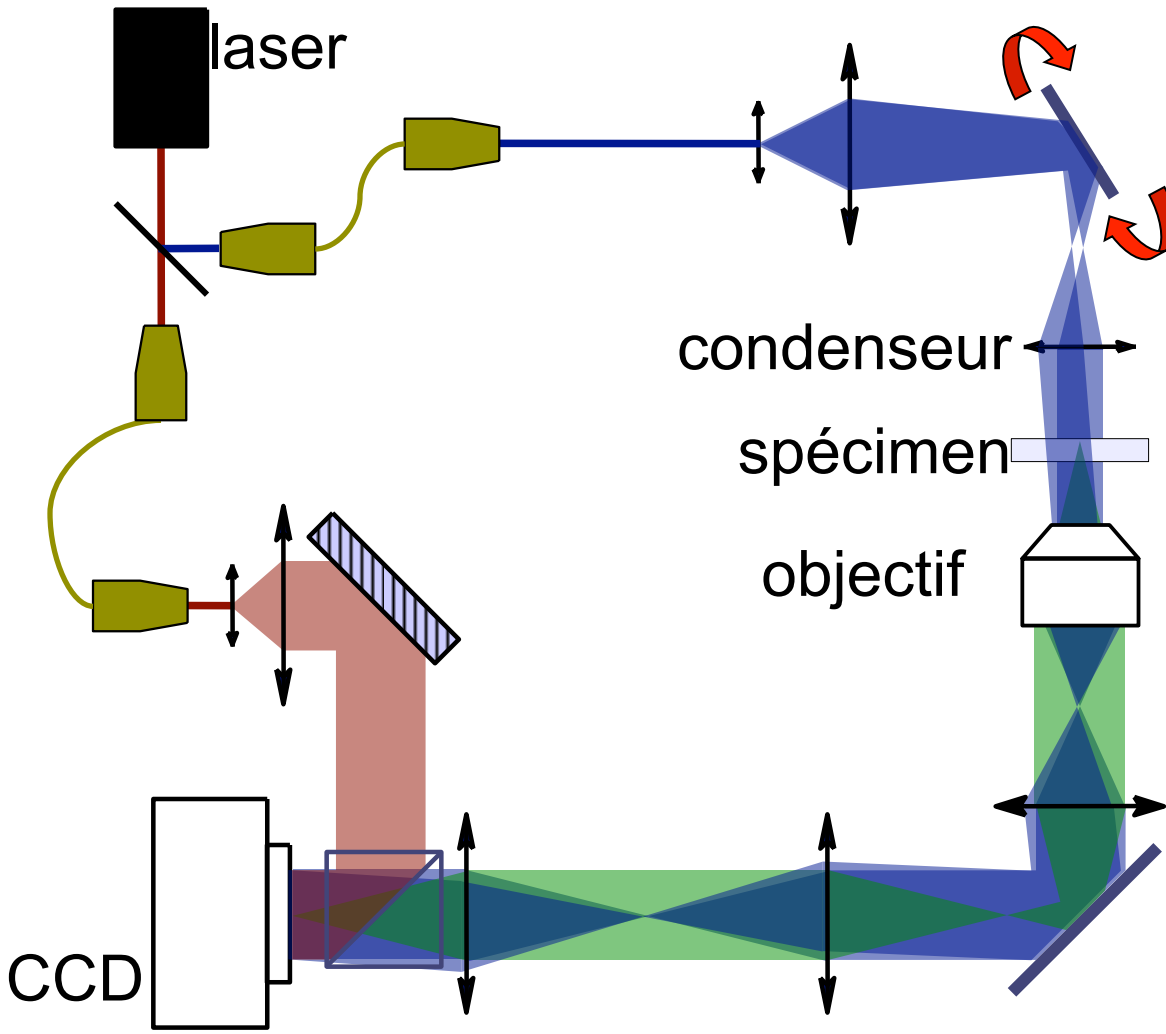
- Different angles of illumination ⇨ other object frequencies
- Large number of angles

⇨ Extended and filled frequency support

- **Objective numerical aperture ⇨ Limitation of the detection angle**
- **Condenser numerical aperture ⇨ Limitation of the illumination angle**

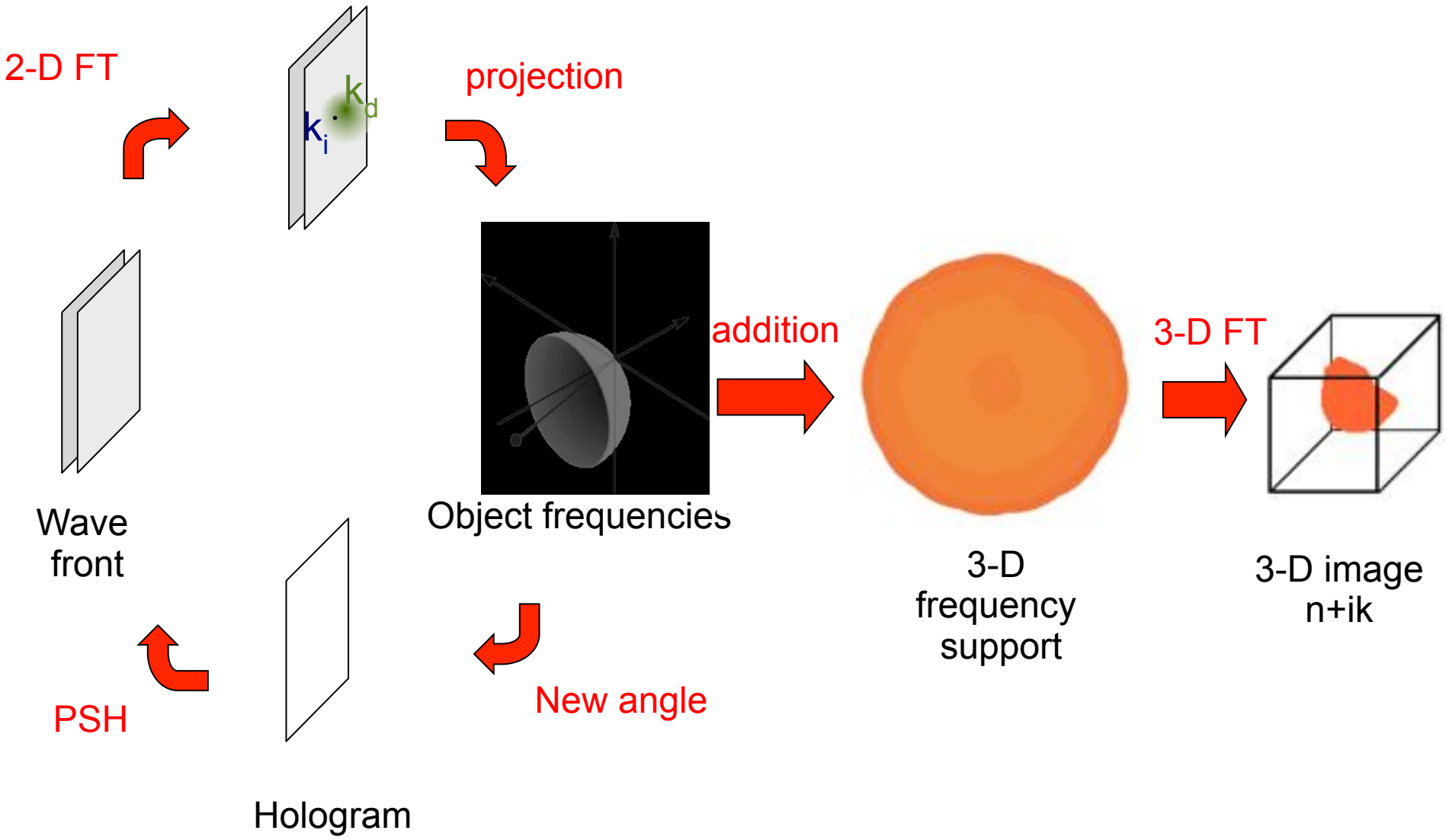
Tomographic Microscopy: Transmission





Holographic microscopy and diffractive microtomography of transparent samples, M. Debailleul, *et al.,* Meas. Sci. Technol. **19**, 074009 (2008)

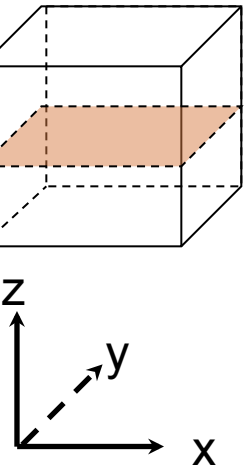
Object Reconstruction

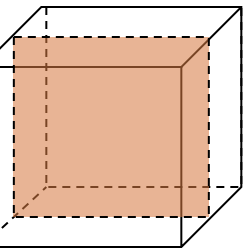


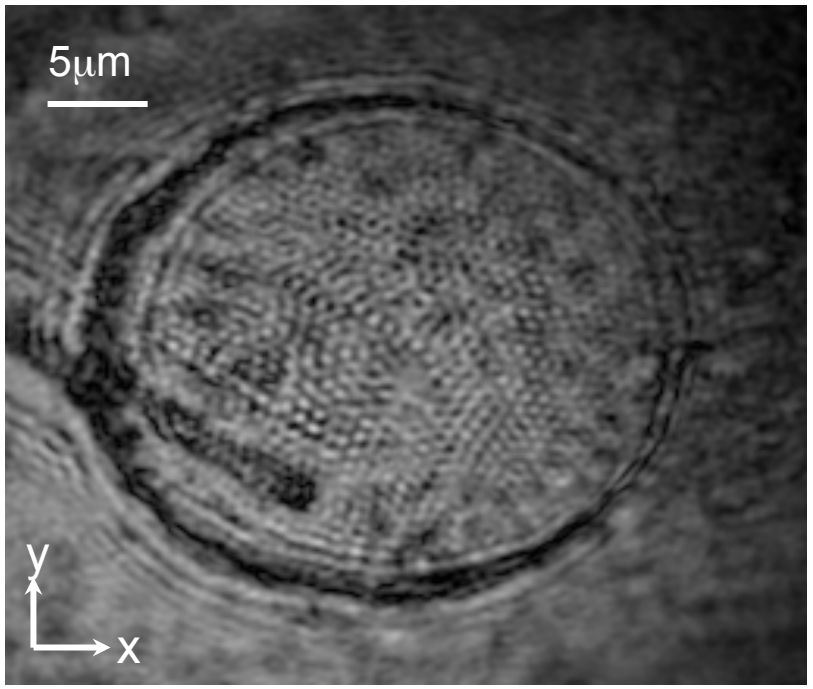
Holography / Tomography

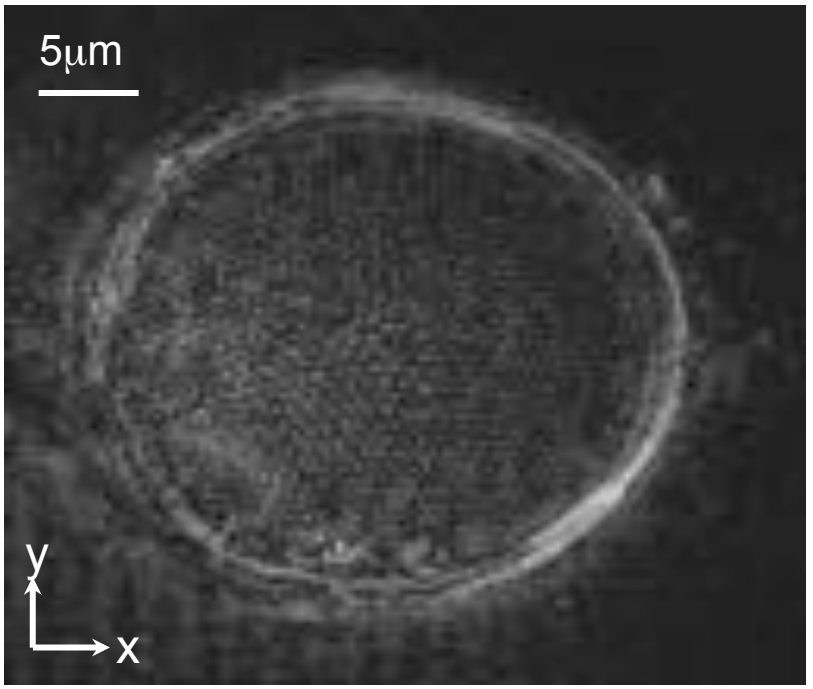
1 angle

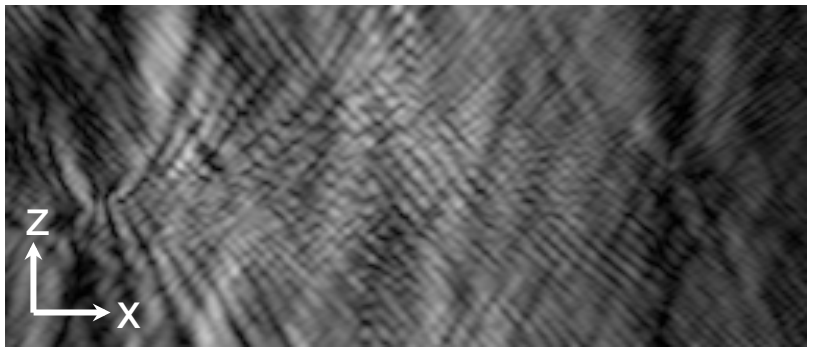
372 angles

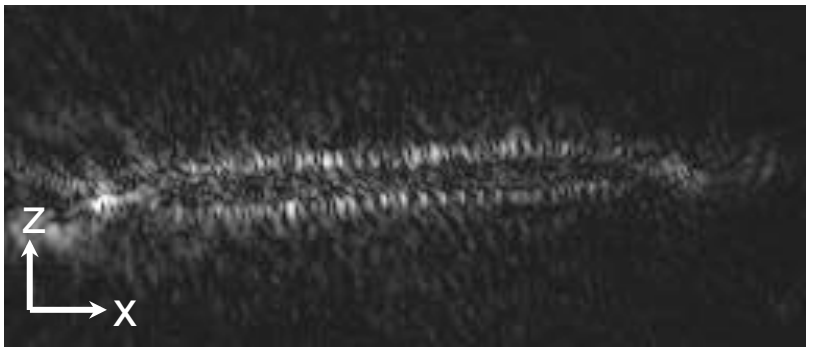




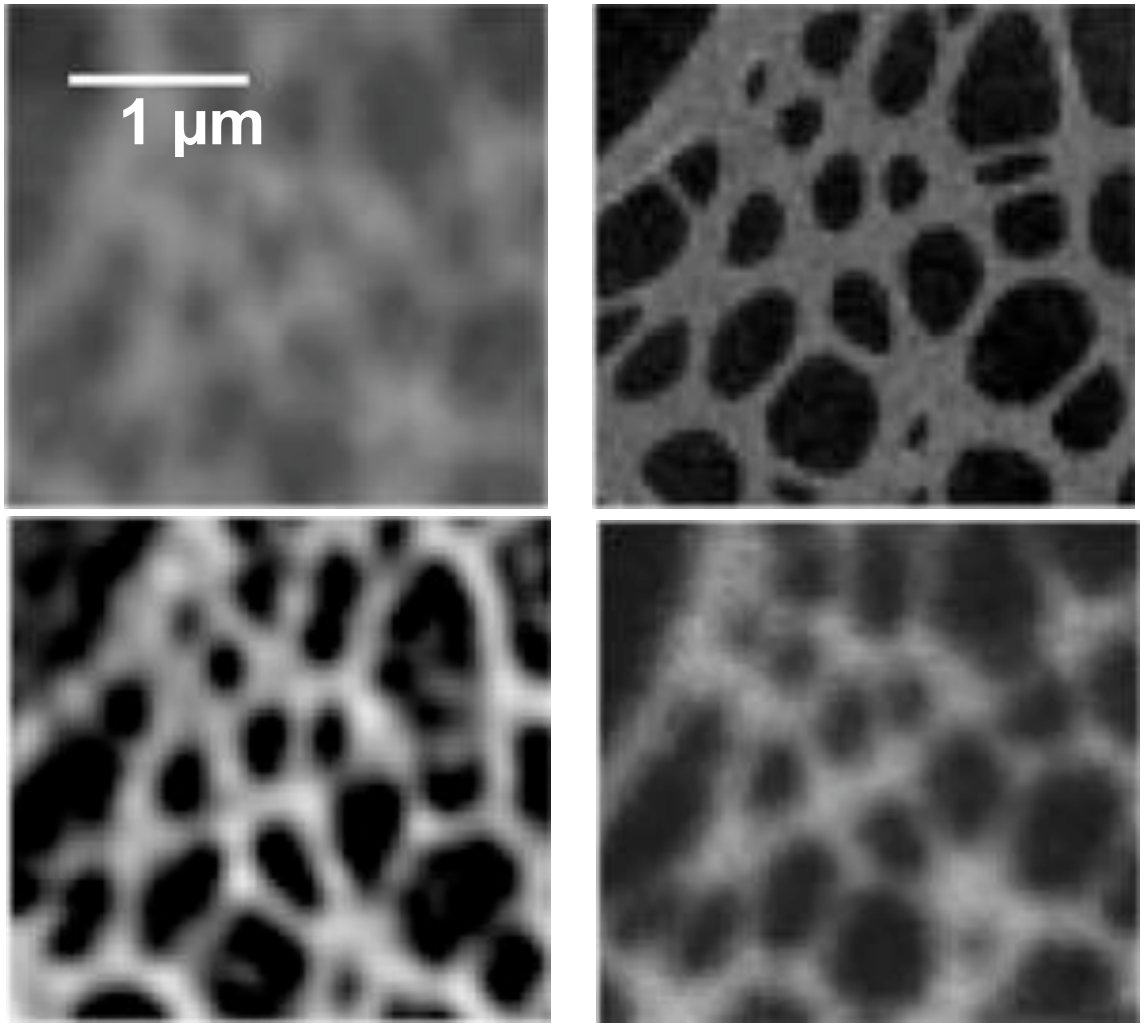








Carbon Mesh

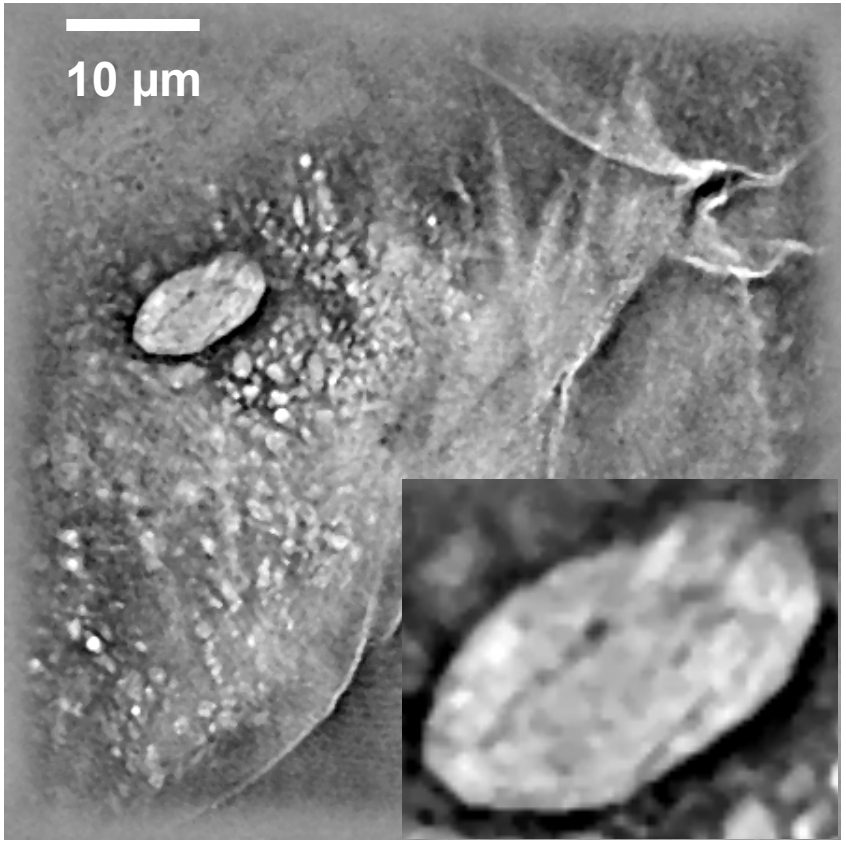


High-resolution three-dimensional tomographic diffractive microscopy of transparent inorganic and biological samples

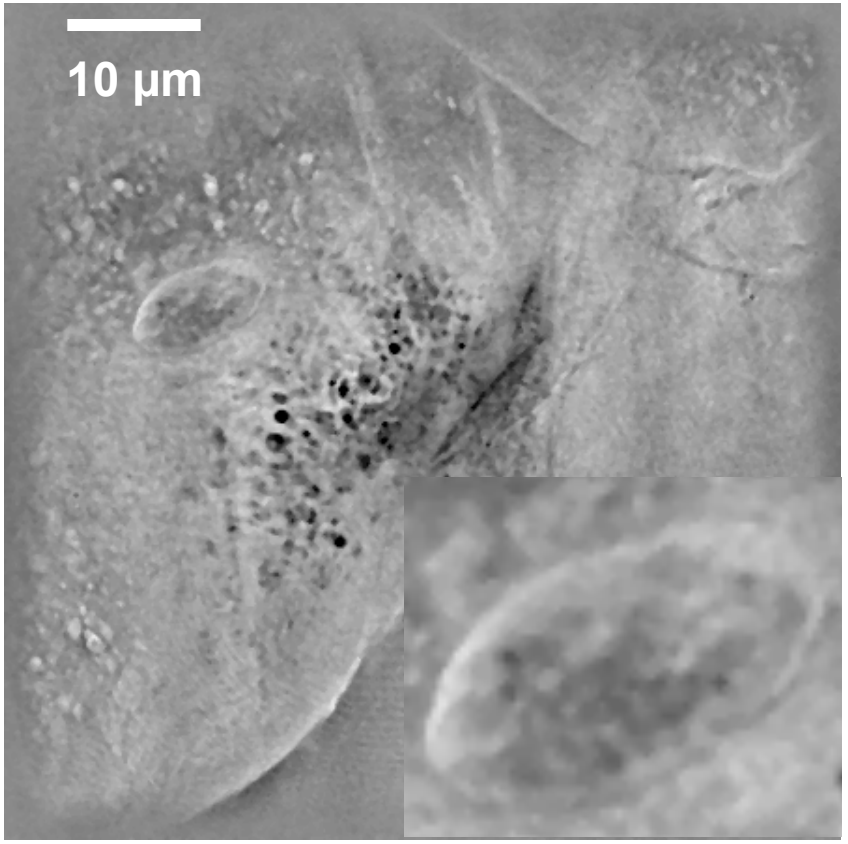
M. Debailleul, *et al.*, Opt. Lett. **34**, p. 79 (2009)

Tomography => Index of Refraction

Epithelial cells



Indice
Real part



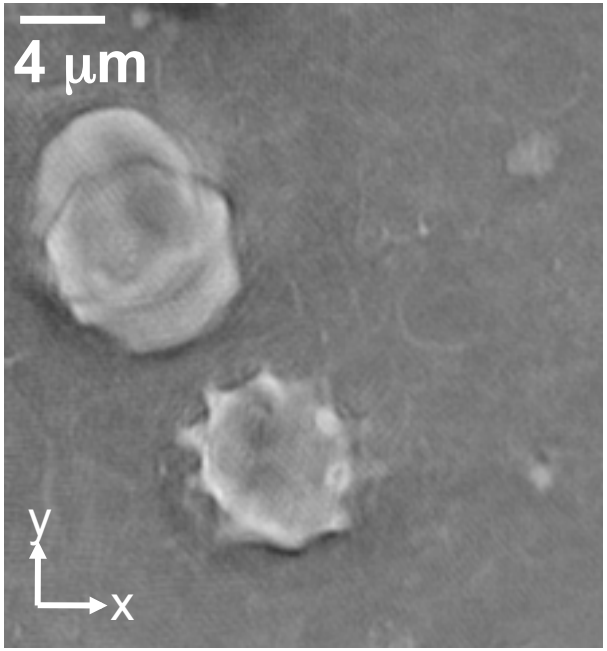
Indice
Imaginary part

High resolution tomographic diffractive microscopy of biological samples

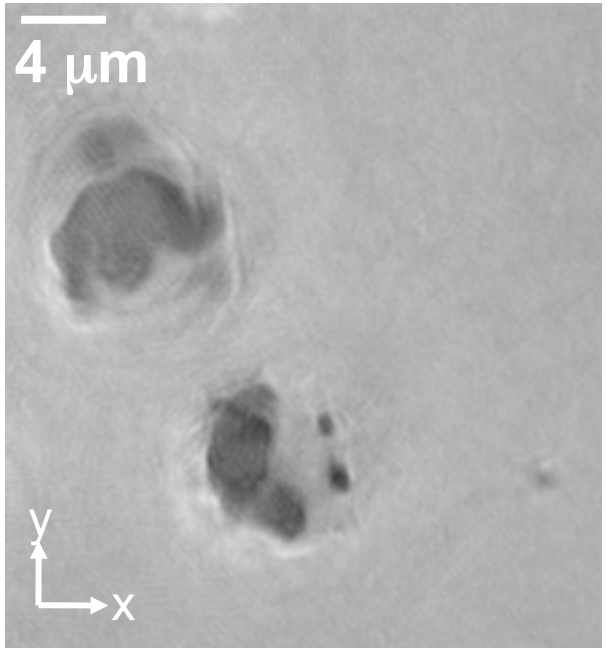
M. Sarmis, *et al.,* J. Biophotonics **3**, p. 462 (2010)

Tomography => Index of Refraction

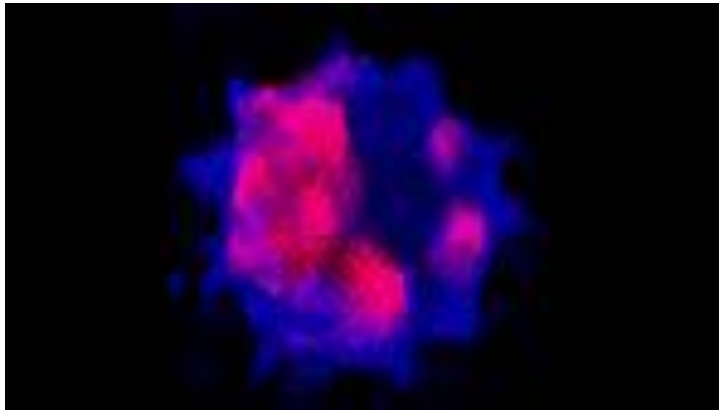
Granulocytes



Refraction

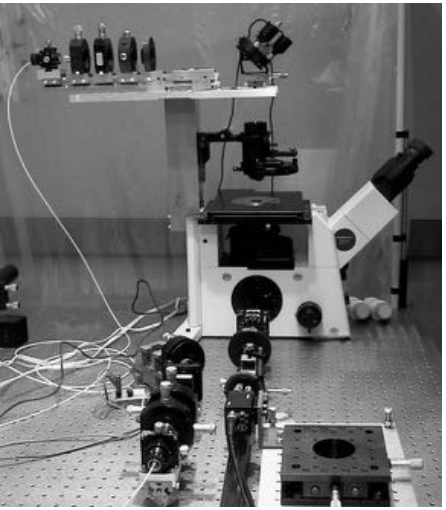


Absorption

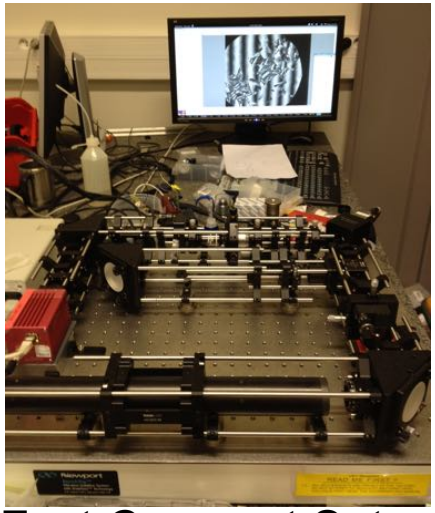


False colour rendering:
Red : absorption
Blue : refraction

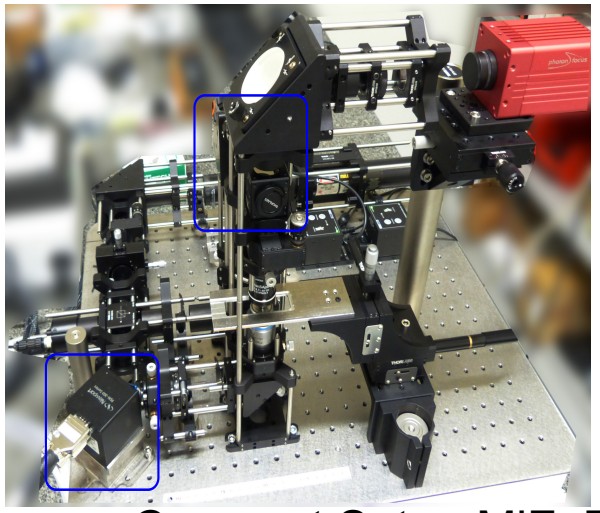
Solutions de labo

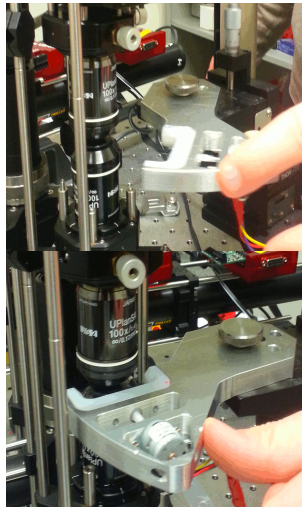


First prototype

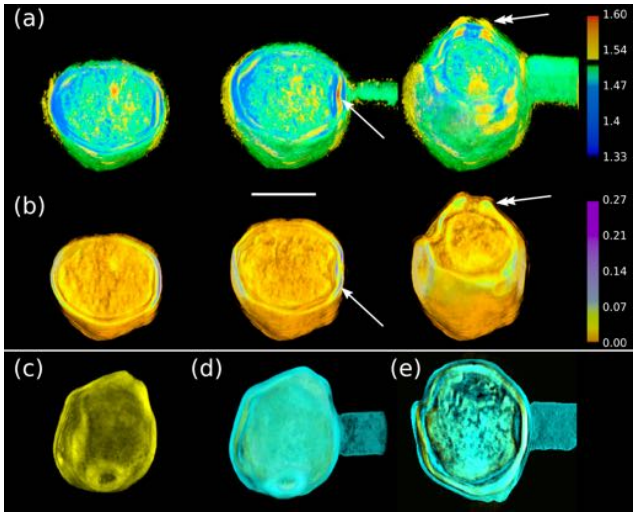


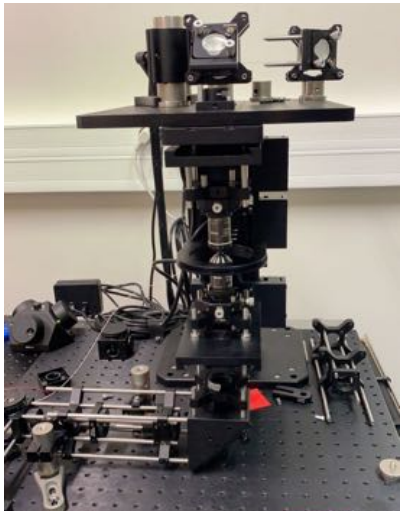
Fast Compact Setup
MiFoBio 2014-2016





Compact Setup MiFoBio 2012





New System

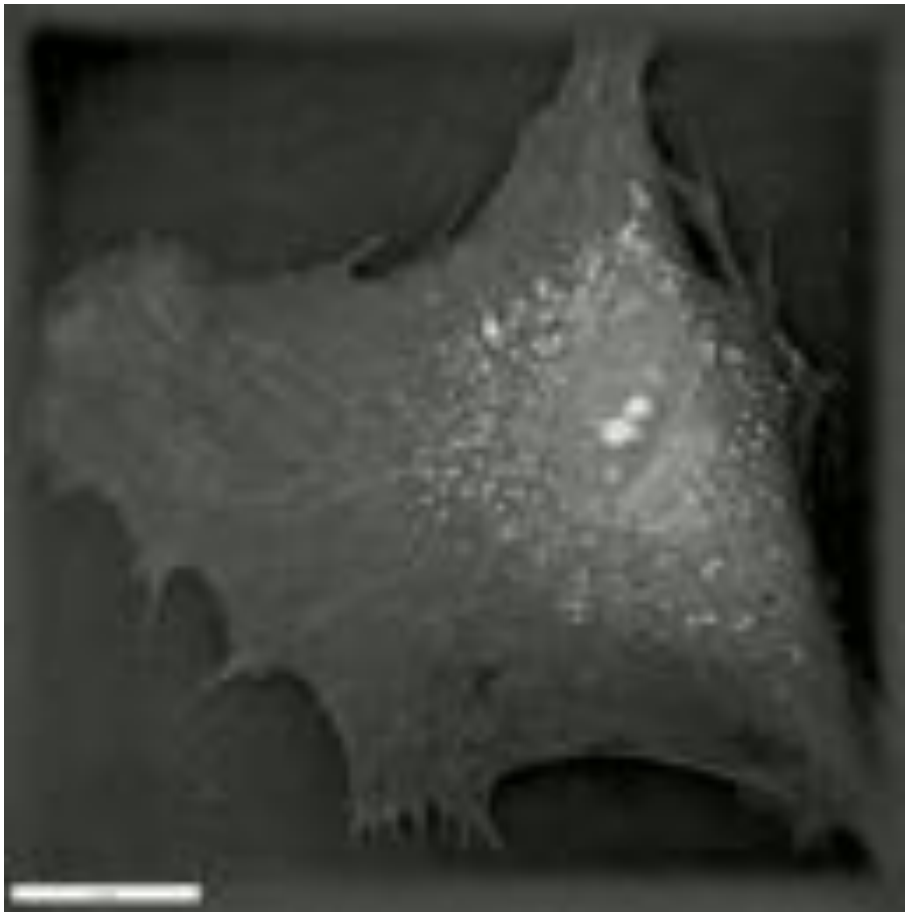
Commercially available!

NANOLIVE
Looking inside life

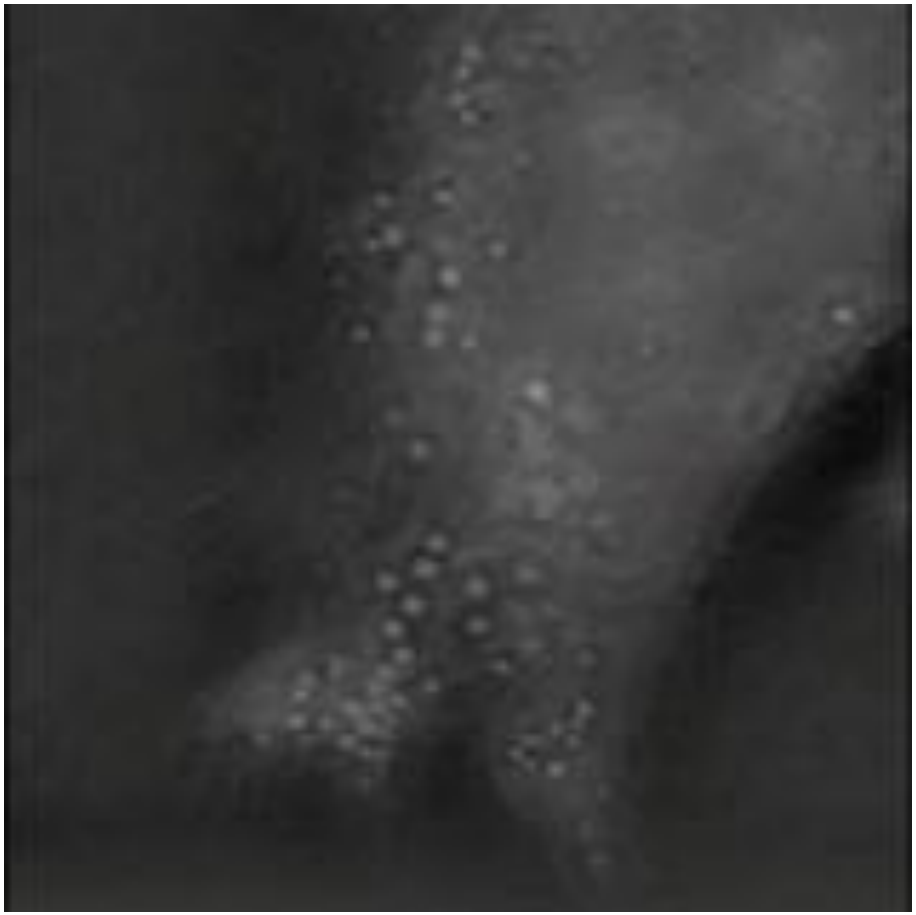
TOMOCUBE

See their website for interesting applications
Several active groups in the world (Korea, Poland, Taïwan, France, Germany, Italy…).

Applications http://nanolive.ch

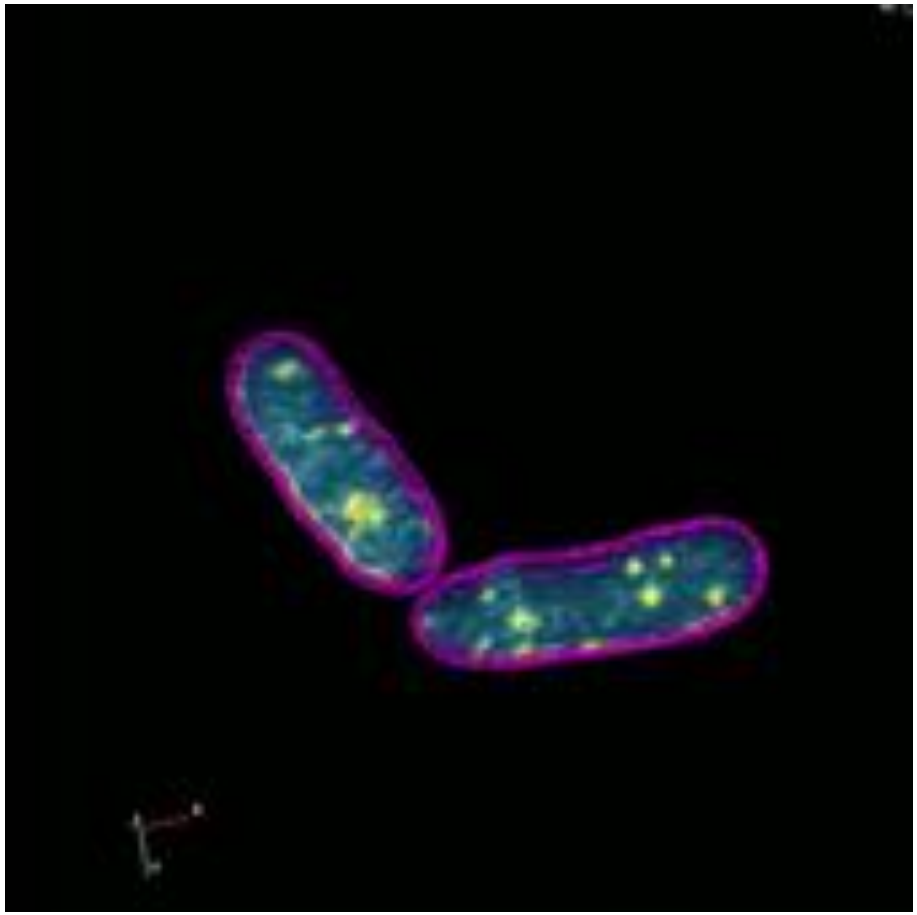


cellular morphological changes induced by drug treatment

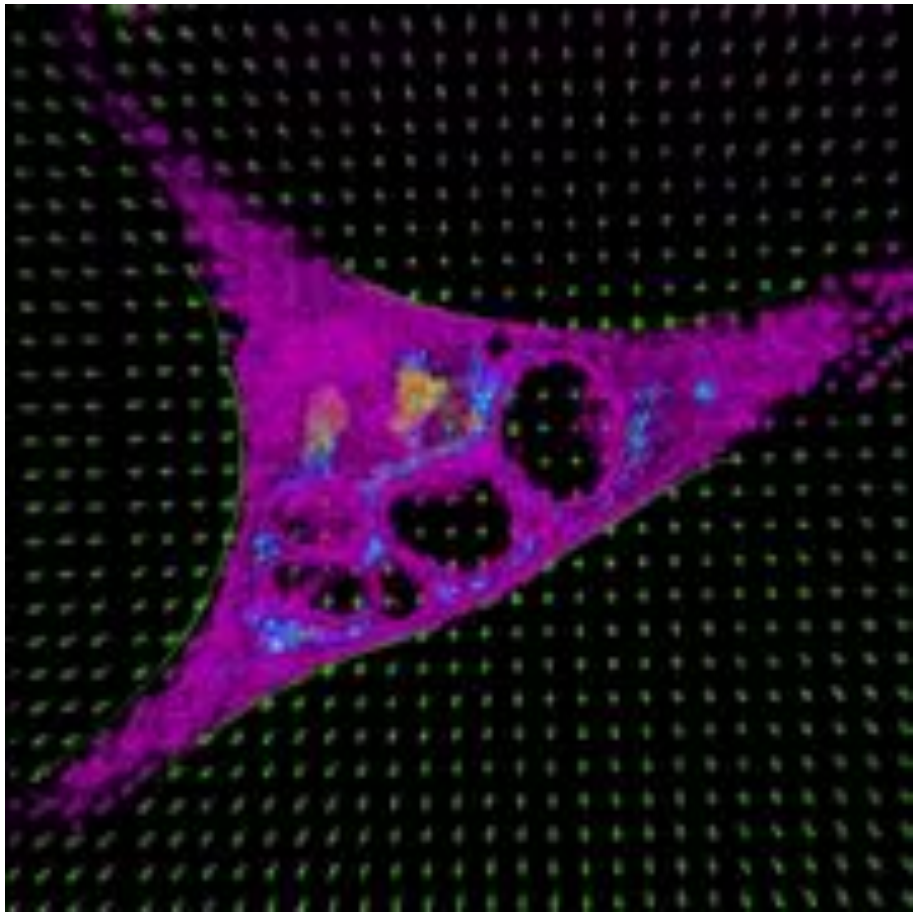


nanodiamonds internalization & 3D distribution in living cells

Applications http://nanolive.ch

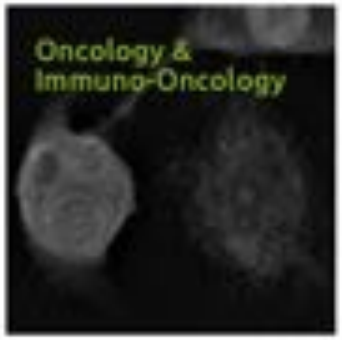


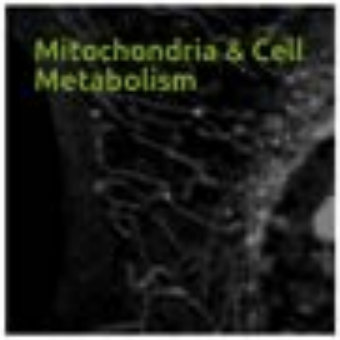
Fission yeast
(Schizosaccharomyces pombe)
during division

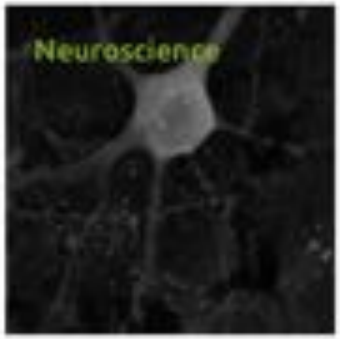


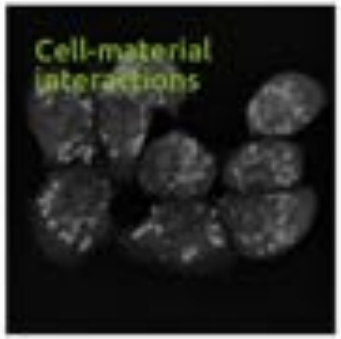
Fibroblast reticular cell seeded
on glass nanopillars

Applications http://nanolive.ch



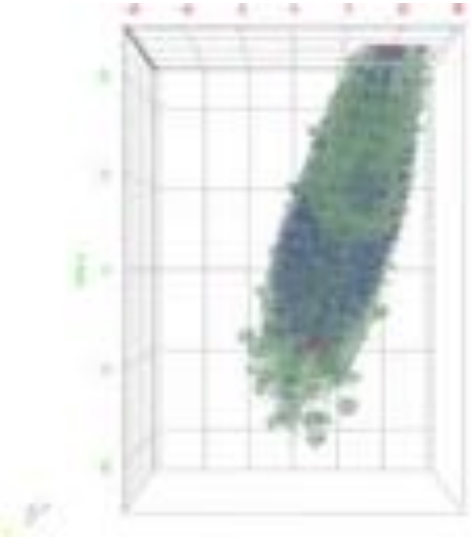


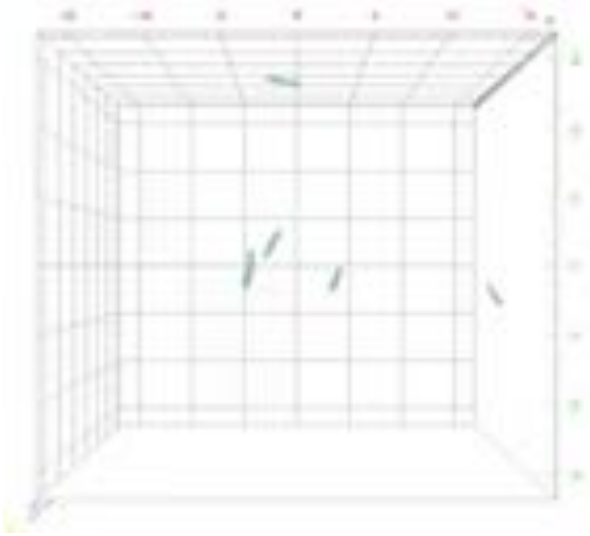




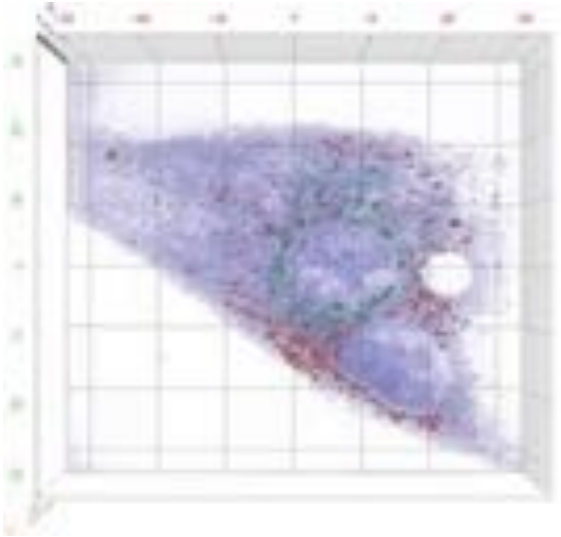
Applications http://www.tomocube.com

Cell apoptosis

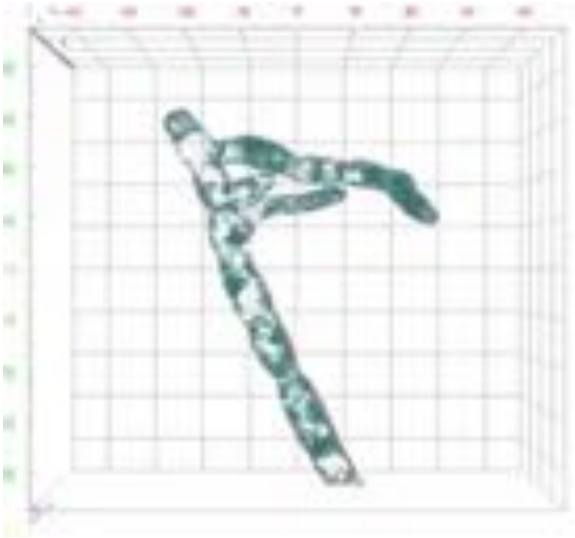




Bacterial growth



HeLa cell

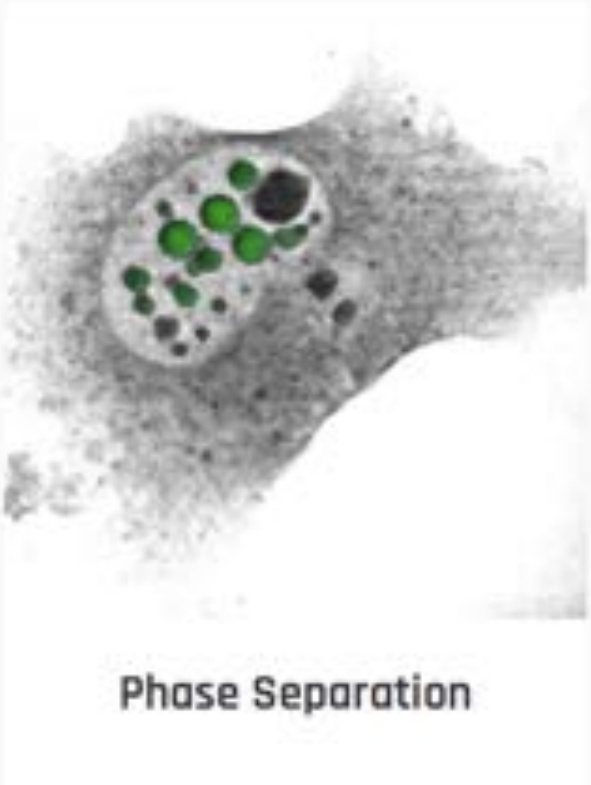


Microalgae

Applications http://www.tomocube.com

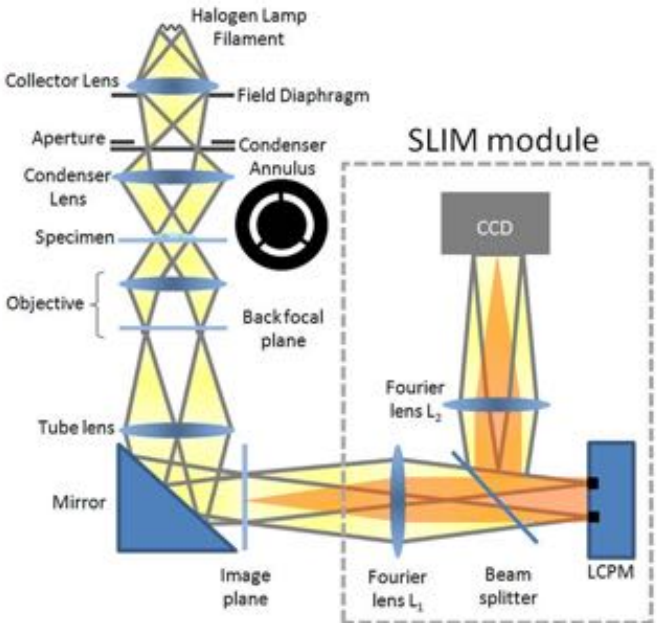
Lipid Quantification

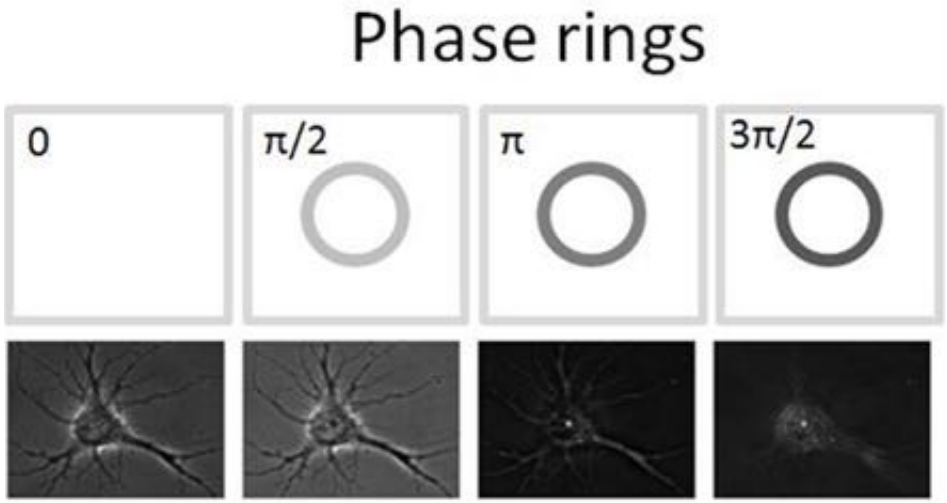
Bacteria Analysis

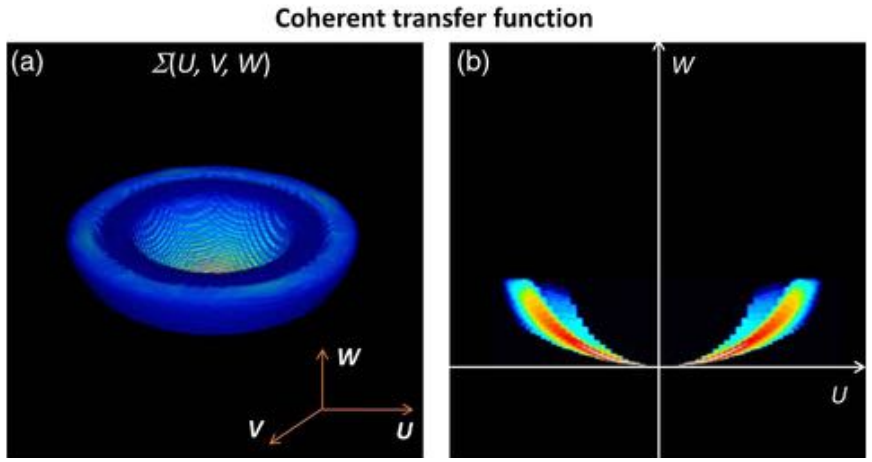


Phase Separation

White-light diffraction tomography



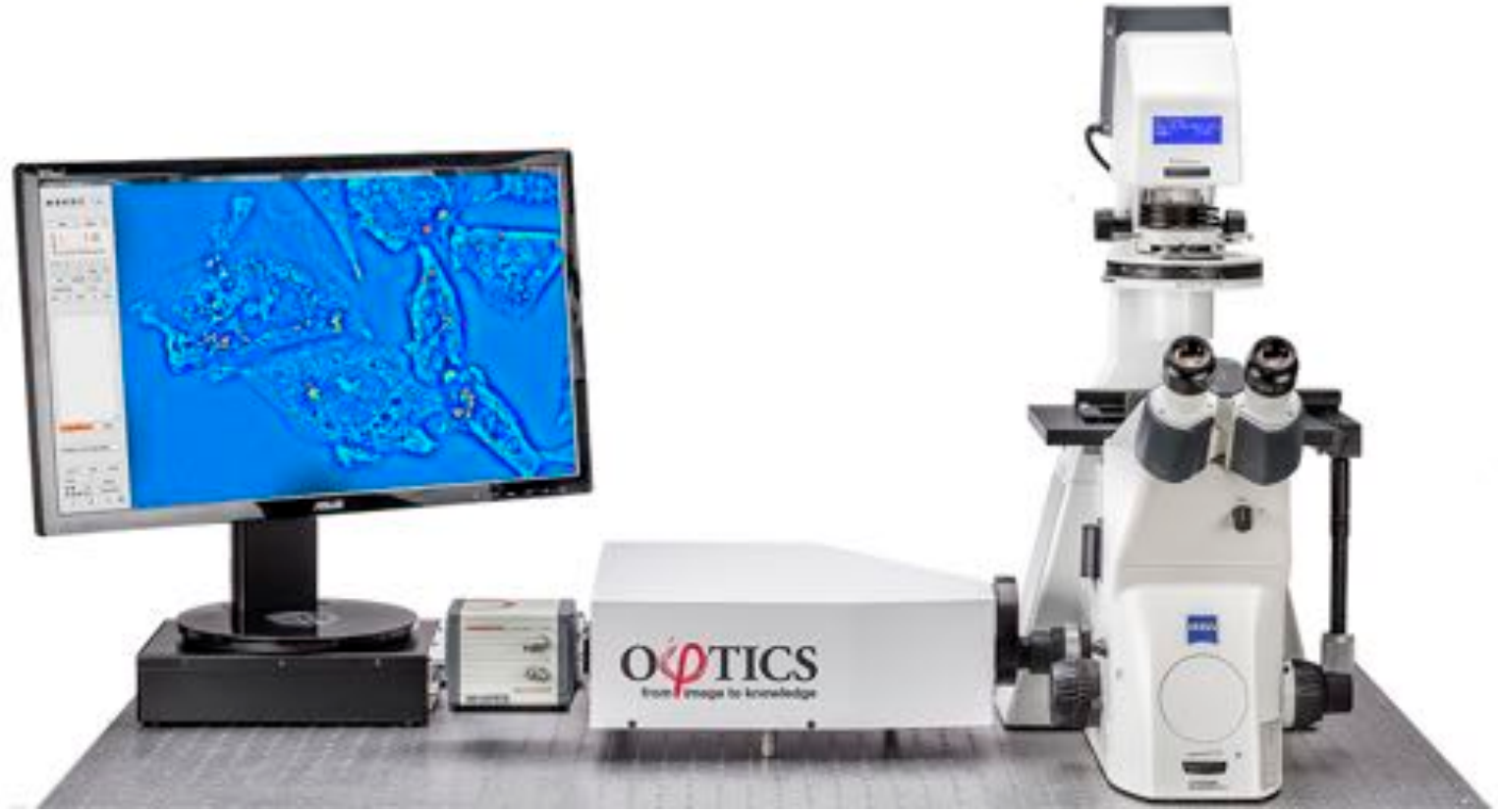




+ Z-scanning
+ data processing

White-light diffraction tomography of unlabelled live cells
T. Kim, *et al.* Nature Photonics **8**, p. 256 (2014)

Commercially available!

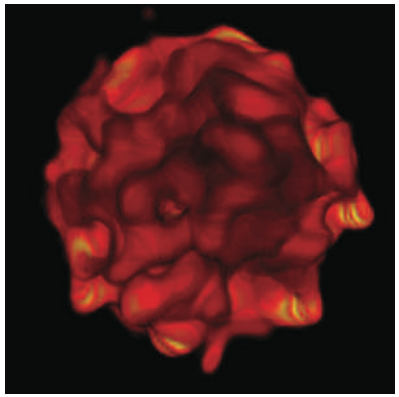


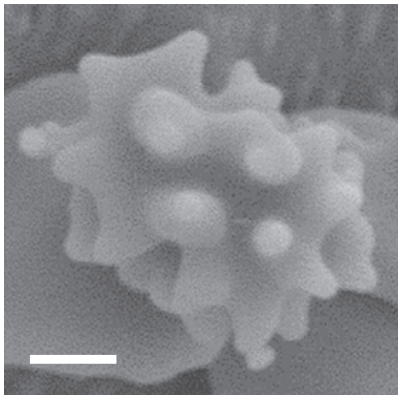
See their website for interesting applications

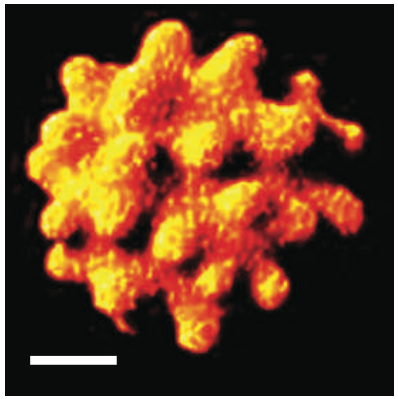
Applications http://phioptics.com

SEM

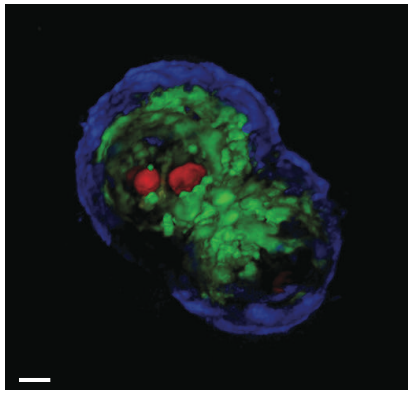
Confocal



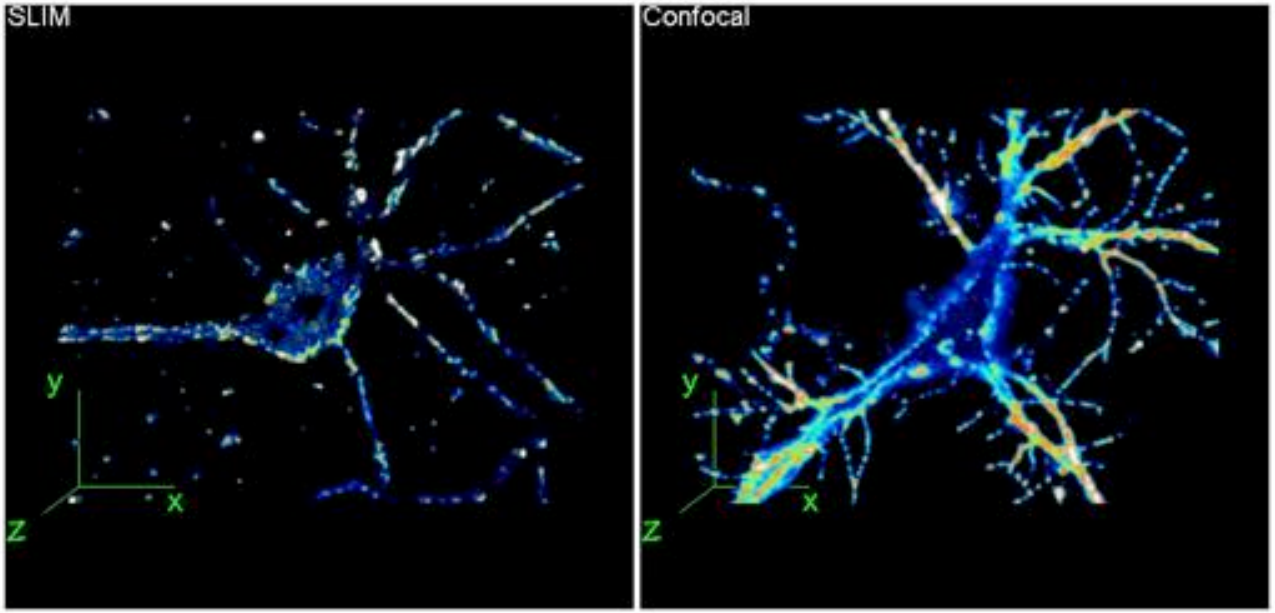




Spiculated RBC

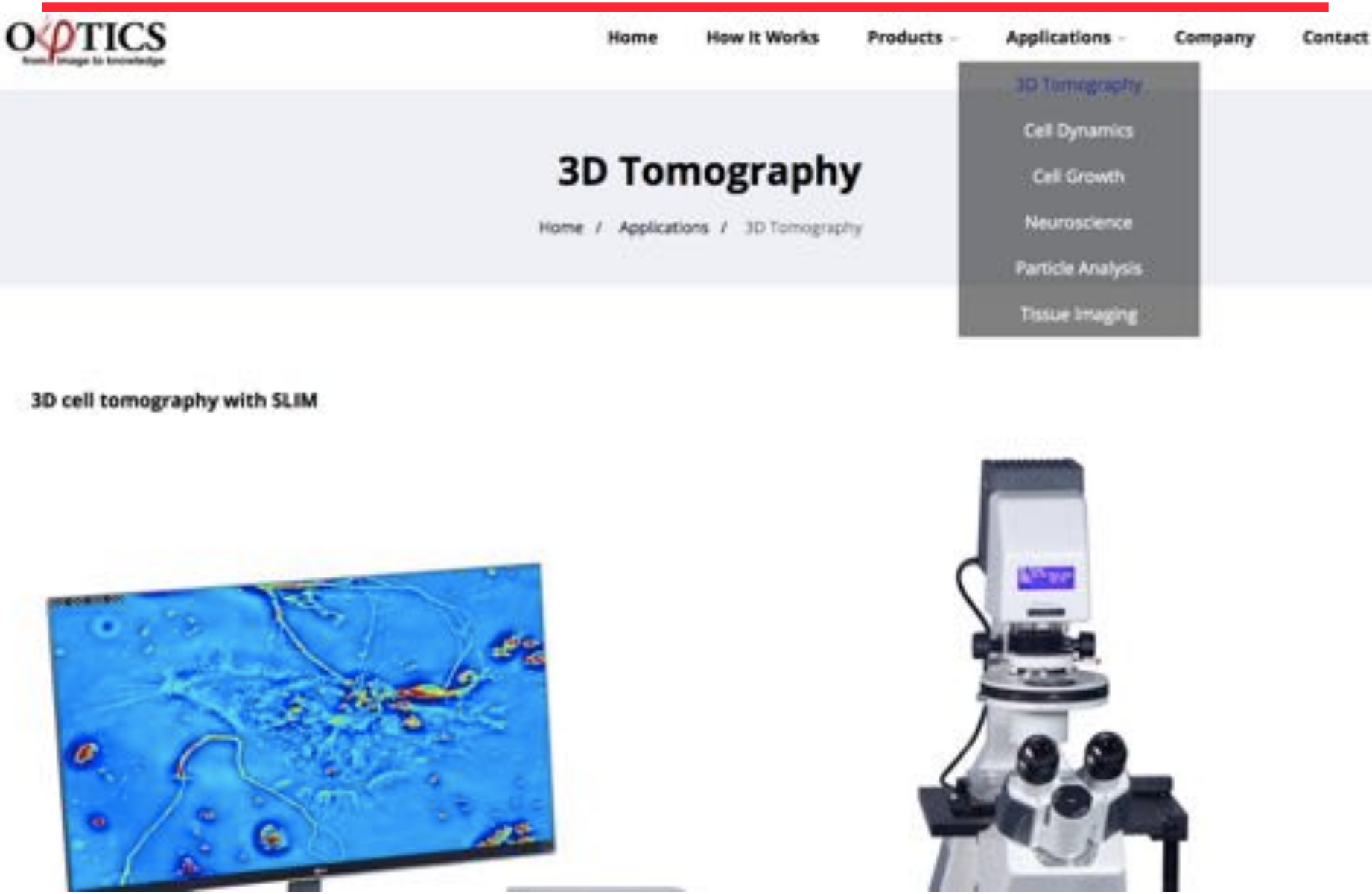


HT29 cell



Live neuron

Applications http://phioptics.com



Limits of Label-free VS fluorescence

Sensitivity

→ Not a single molecule approach…

Resolution limit

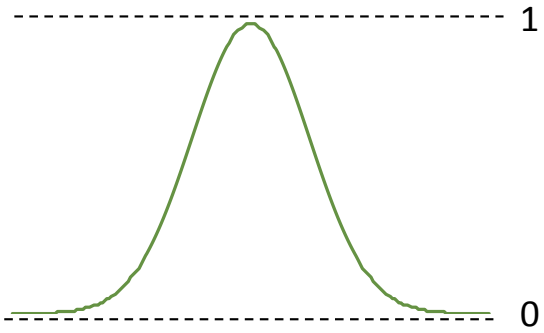
→ Initial resolution = 50% of fluo. resolution

Selectivity/Specificity

→ How to identify and contrast a molecular type

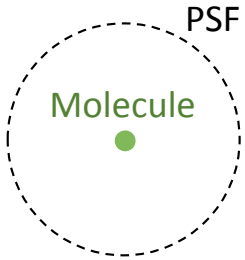
Sensitivity

Fluo



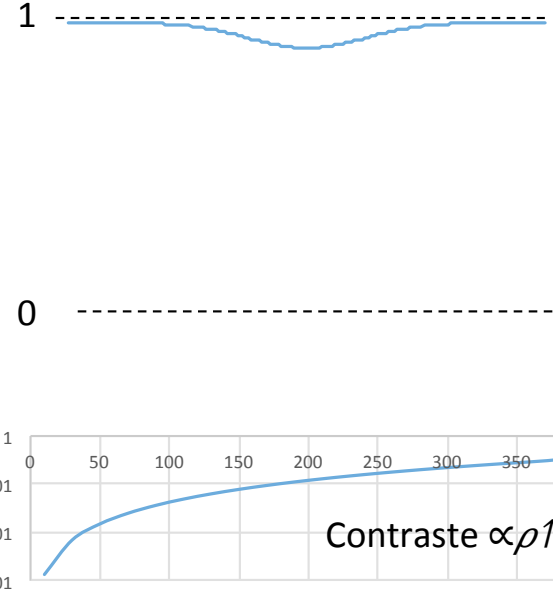
Contraste = 1

Signal ⇔ Brightness



ρ : molecule diam.

Label-free



Contraste $\propto \rho\hat{}3 \,/ PSF\hat{}3$

Signal ⇔ Refractive index variation

Sensitivity enhancement ?

- Signal = shot-noise limited ➔more photons!➔alternative sensor

  Hosseini *et al.*, Pushing phase and amplitude sensitivity limits in interferometric microscopy, **Optics Letters**, 41(7), 2016

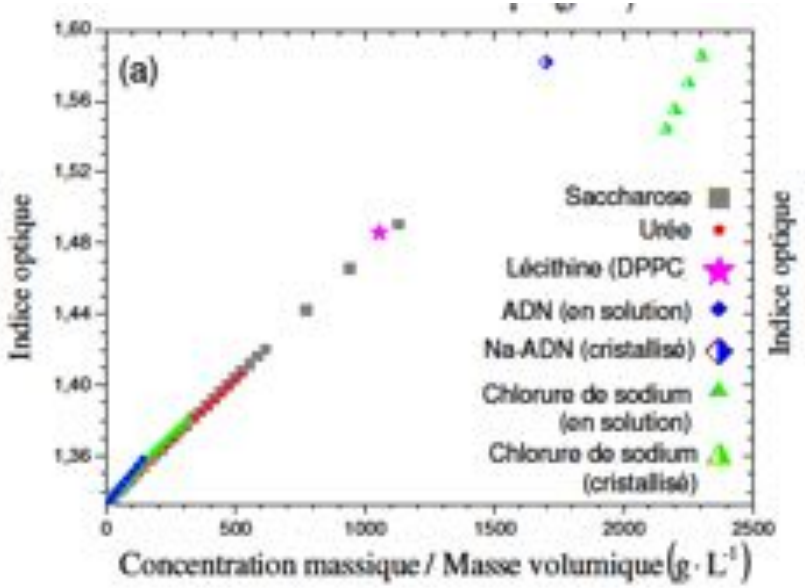
  Roose-Amsaleg *et al.*, Utilization of interferometric light microscopy for the rapid analysis of virus abundance in a river, **Research in microbiology**, 168(5), 2017

- Dark field

  Martinez-Marrades *et al.*, Stochastic 3D optical mapping by holographic localization of Brownian scatterers, **Opt. Express**, 22(23), 2014

Selectivity / sensitivity

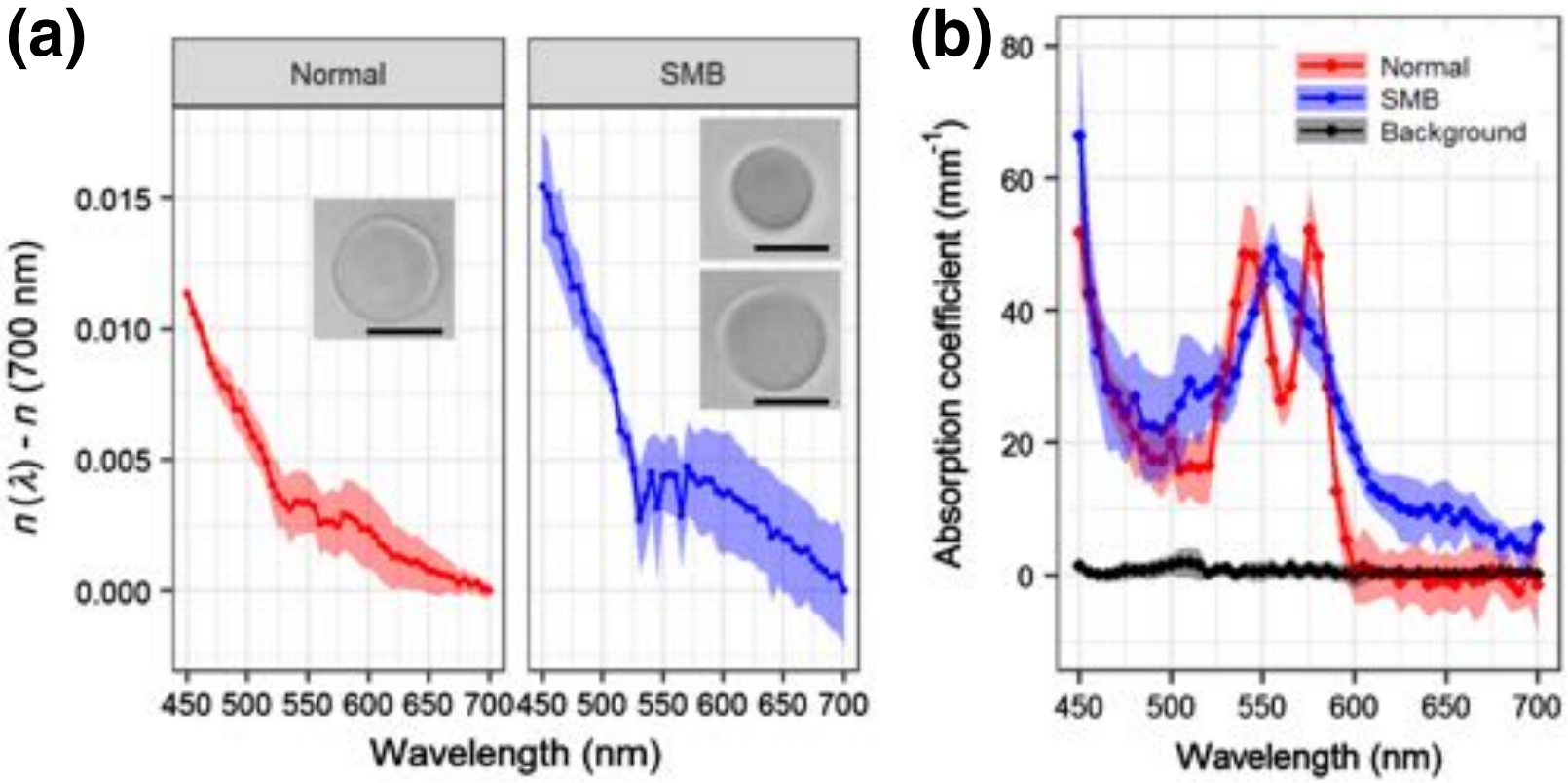
- Signal ⇔ Refractive index variation … not specific !



Selectivity / sensitivity enhancement

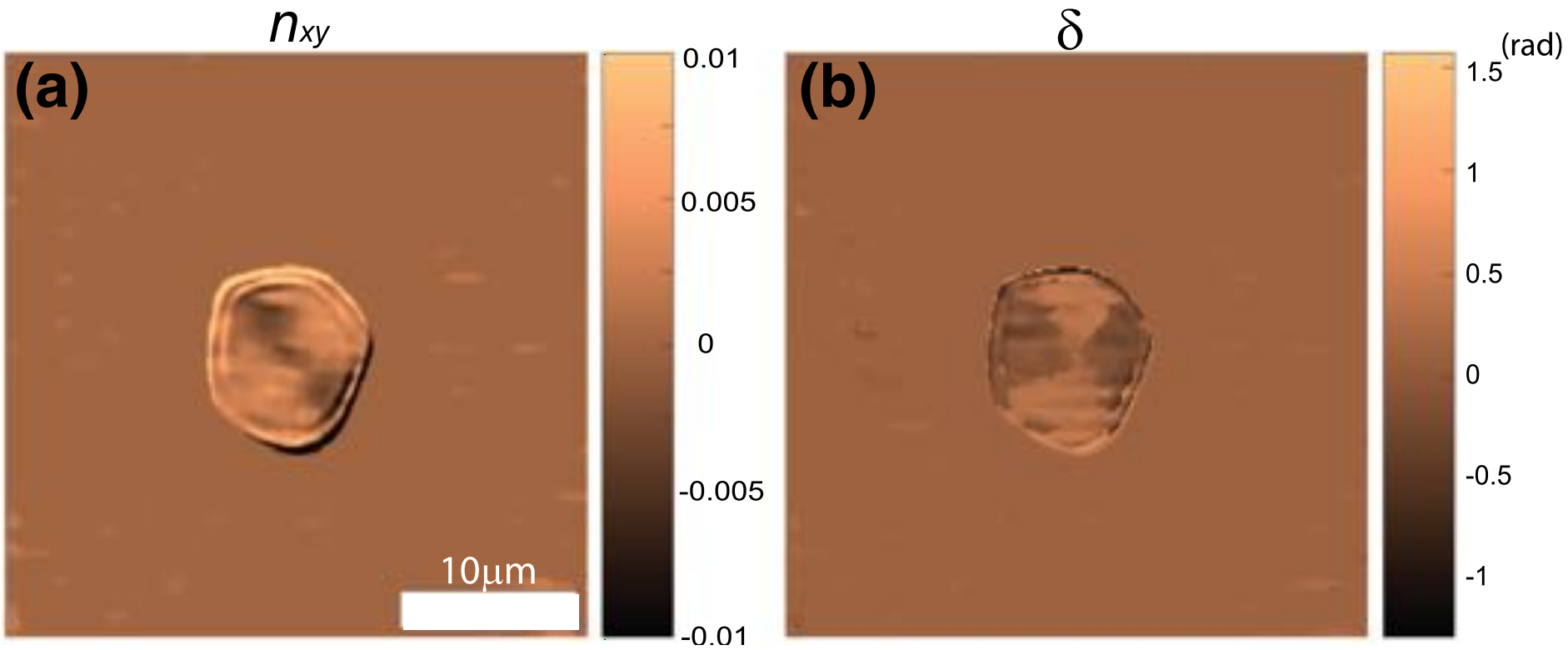
- Polarization → cytoskeleton, collagen…
- High refractive index / absorption / scattering probes (nano-diamond, Gold particle)
- Refractive index specific modulation

Spectroscopic Microtomography



Spectroscopic Microtomography in the Visible Wavelength Range
Y. Sung, Physical Review Applied **10**, 054041 (2018)

Polarized Microtomography



Polarization-sensitive optical diffraction tomography
A. Saba, et al., Optica **8**, 402 (2021)

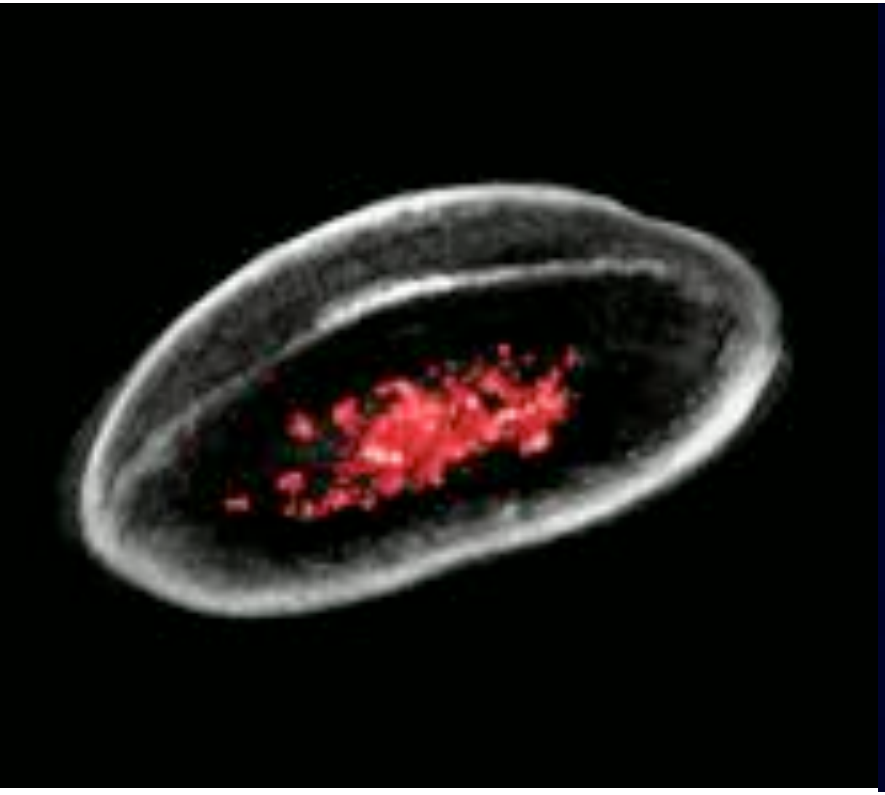
Polarized Microtomography

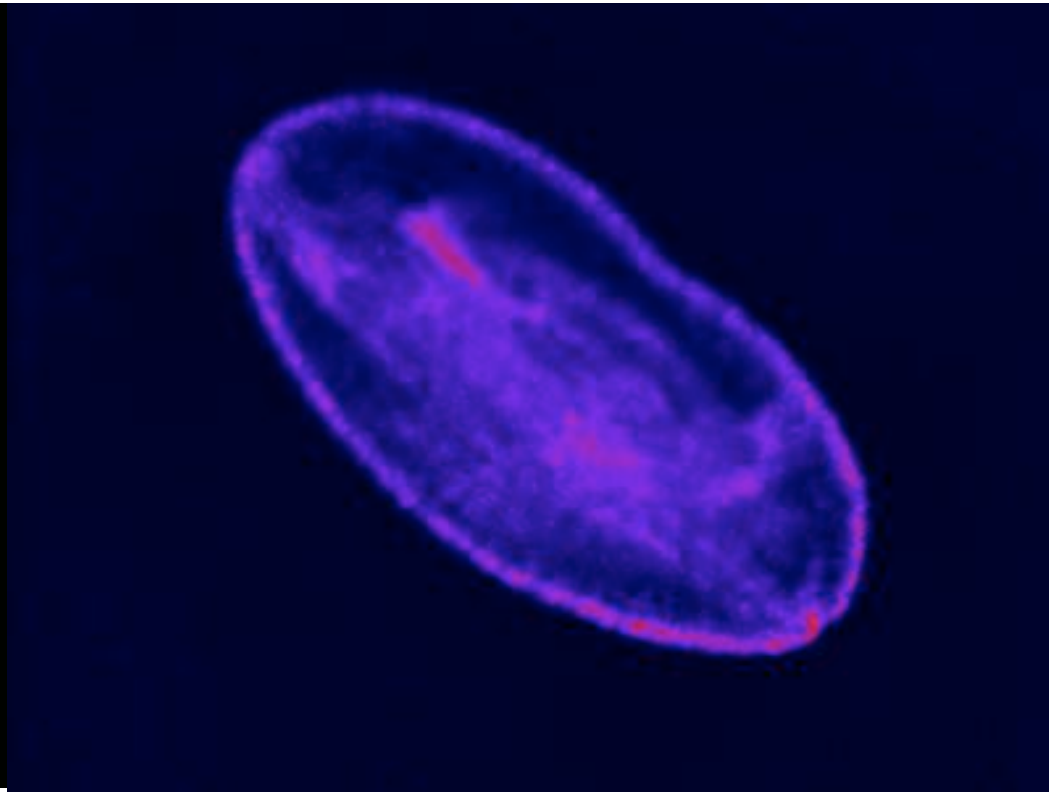
Polarization-sensitive optical diffraction tomography
A. Saba, et al., Optica **8**, 402 (2021)

What’s next ???

Tomography / Fluorescence Comparison

Snowdrop pollen





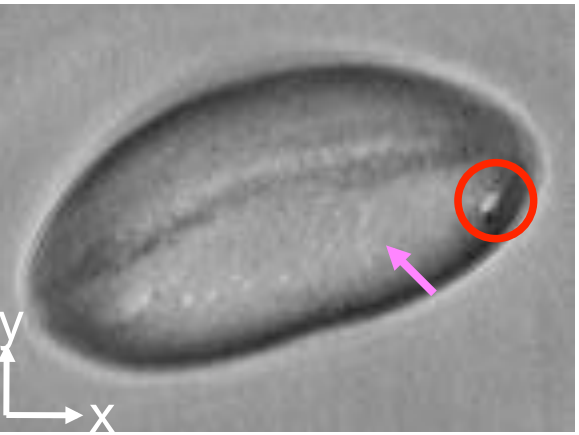
Tomography

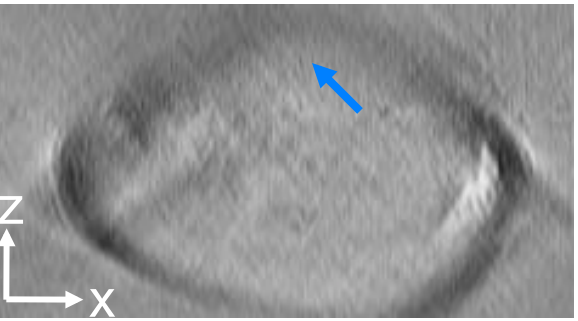
red : index n > index immersion medium

Fluorescence

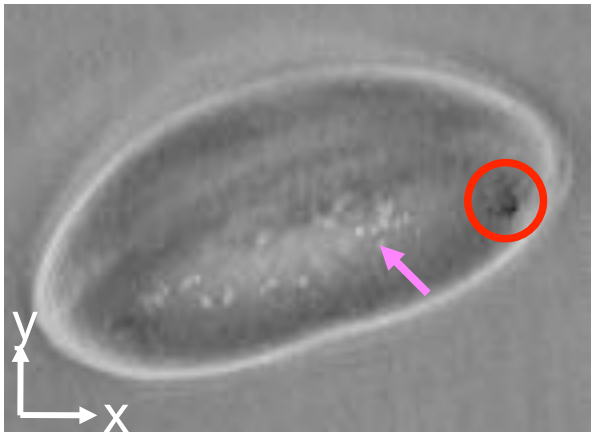
Non-Isotropic Resolution

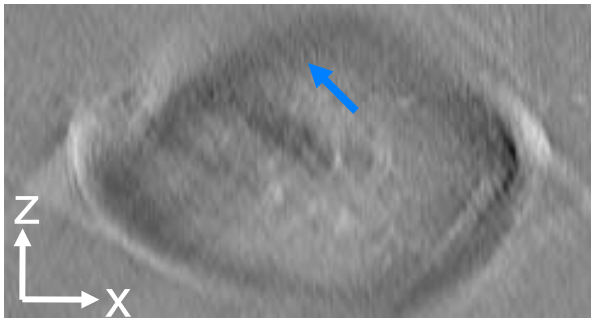
Absorption



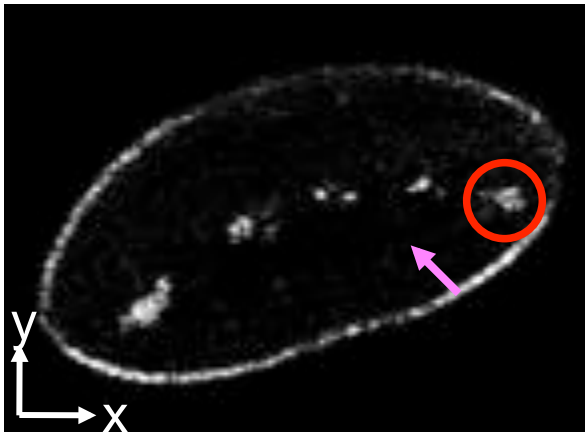


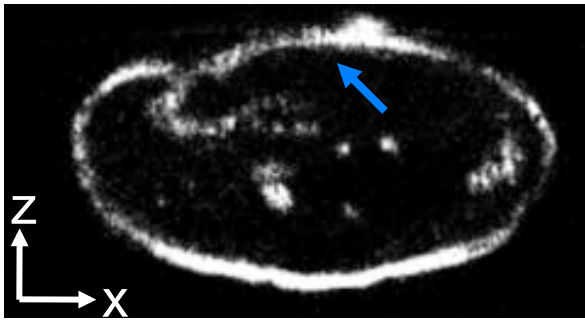
Index of refraction



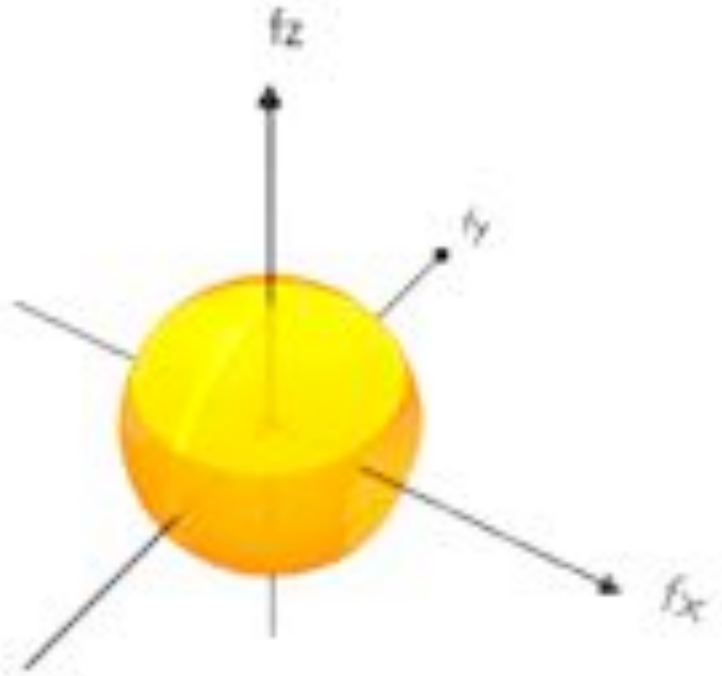


Autofluorescence





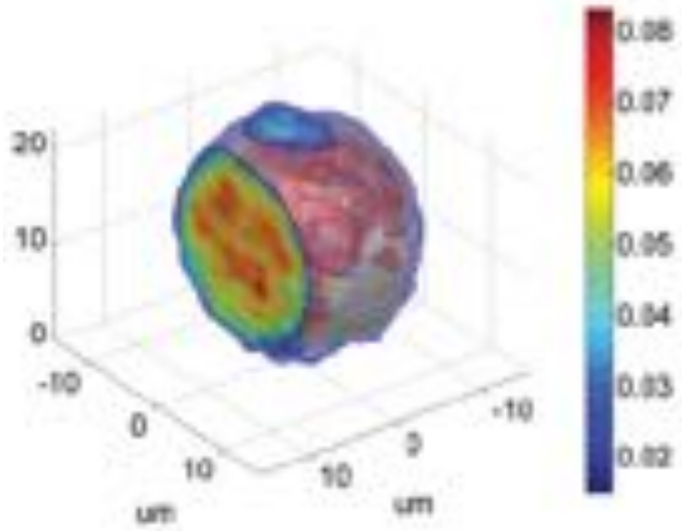
Tomography by Specimen Rotation



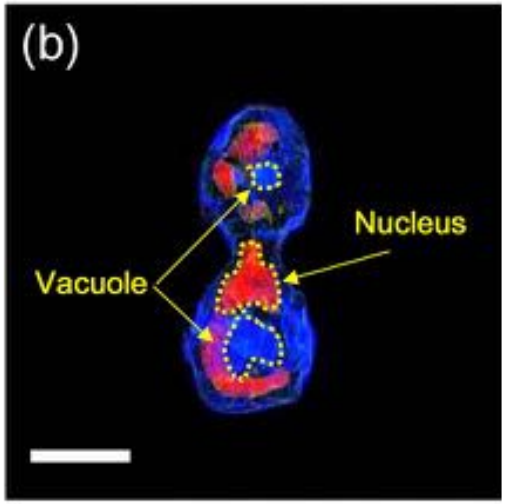
- Sample rotation may be difficult
- low NA => quasi-isotropic, but rather low resolution

Tomography by Specimen Rotation

Problems and Solutions in 3-D Analysis of Phase Biological Objects by Optical Diffraction Tomography
M. Kujawińska, *et al.,*
Int. J. Optomechatronics **8**, p. 357 (2014)

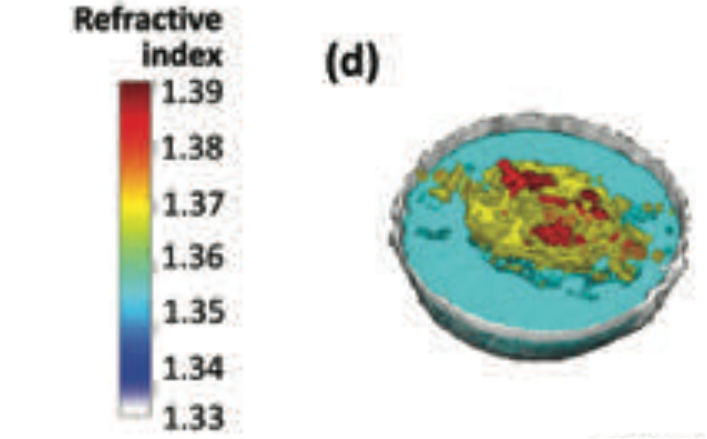


Tomographic phase microscopy with 180° rotation of live cells in suspension by holographic optical tweezers
M. Habaza, *et al.,* Opt. Lett. **40**, p. 1881 (2015)

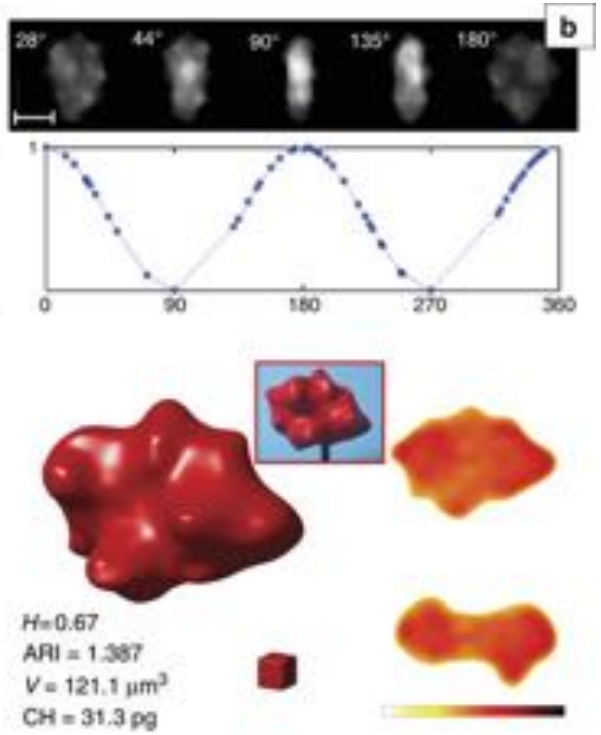


Tomography by Specimen Rotation

Rapid 3D Refractive-Index Imaging of Live Cells in Suspension without Labeling Using Dielectrophoretic Cell Rotation
M. Habaza, *et al.,*
Adv. Sci., paper 1600205 (2016)

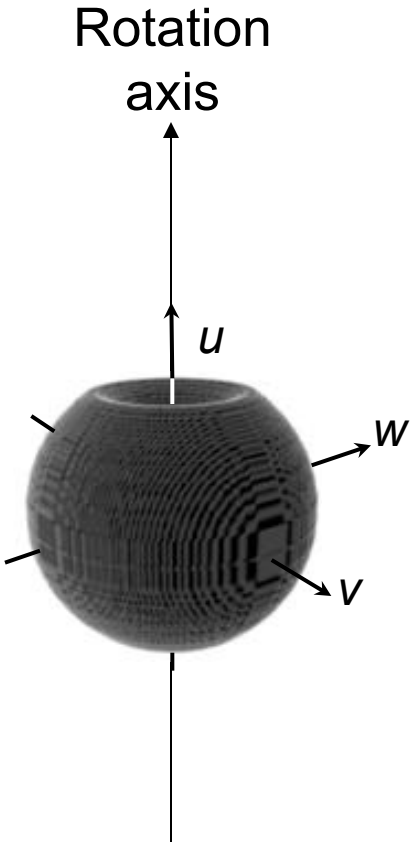


Tomographic flow cytometry by digital holography
F. Merola, *et al.,*
Light: Science & Applications **6**, paper e16241 (2017)



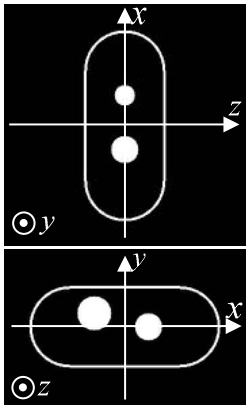
Missing Frequencies

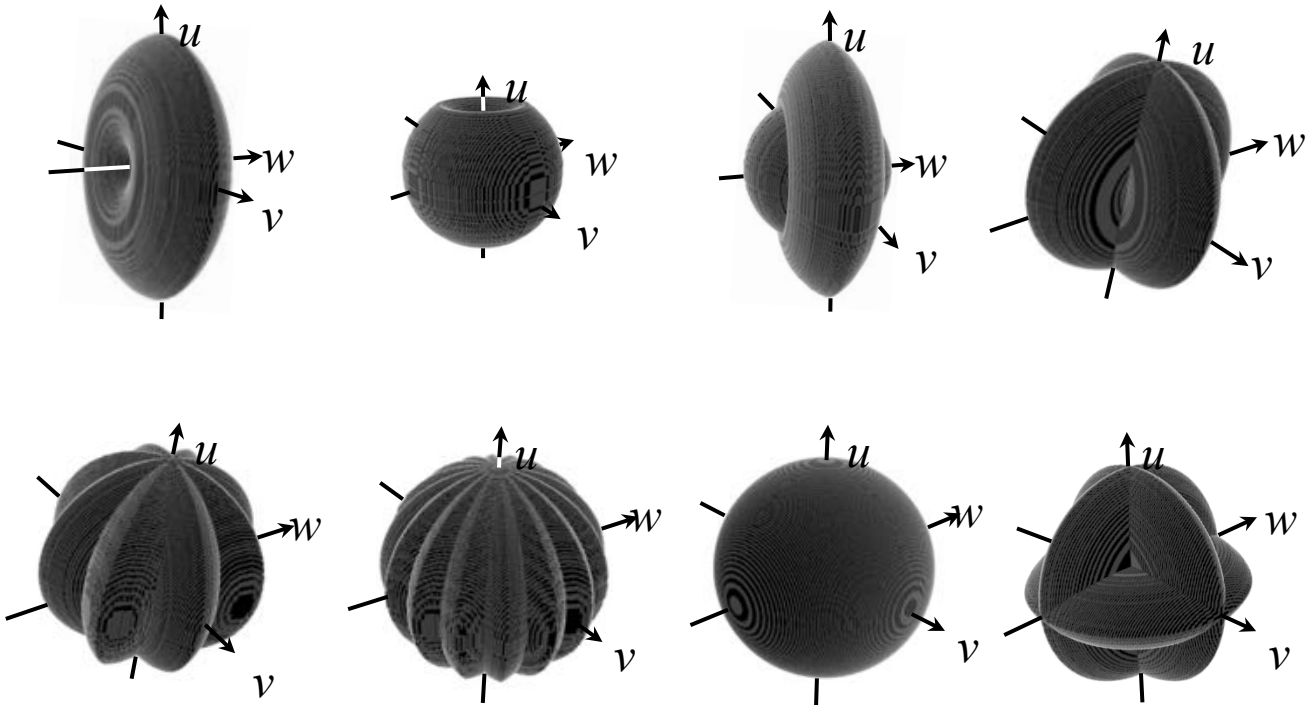
Recorded frequencies **Missing part** **"Missing apple core"**

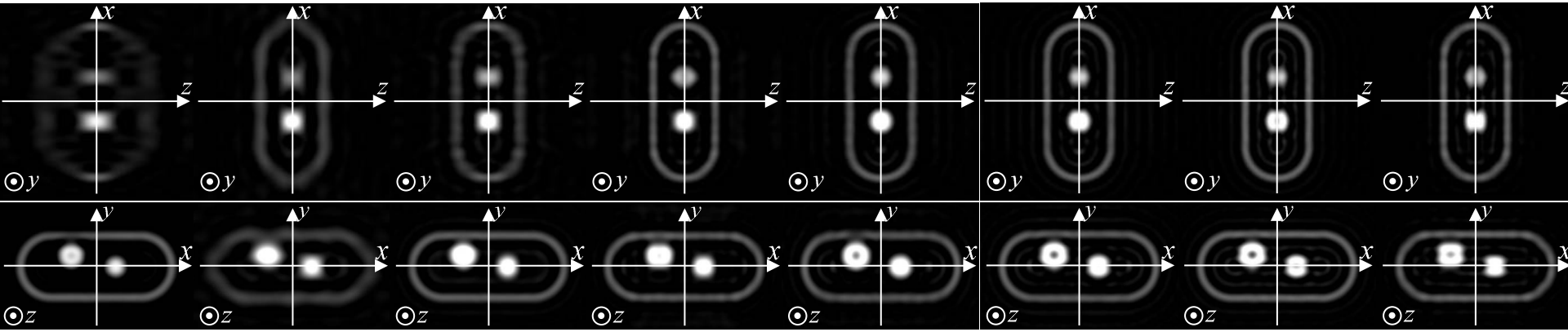


"Diffraction microtomography with sample rotation: influence of a missing apple core in the recorded frequency space"
S. Vertu, *et al.,* Centr. Eur. J. of Phys. **7**, p. 22 (2009)

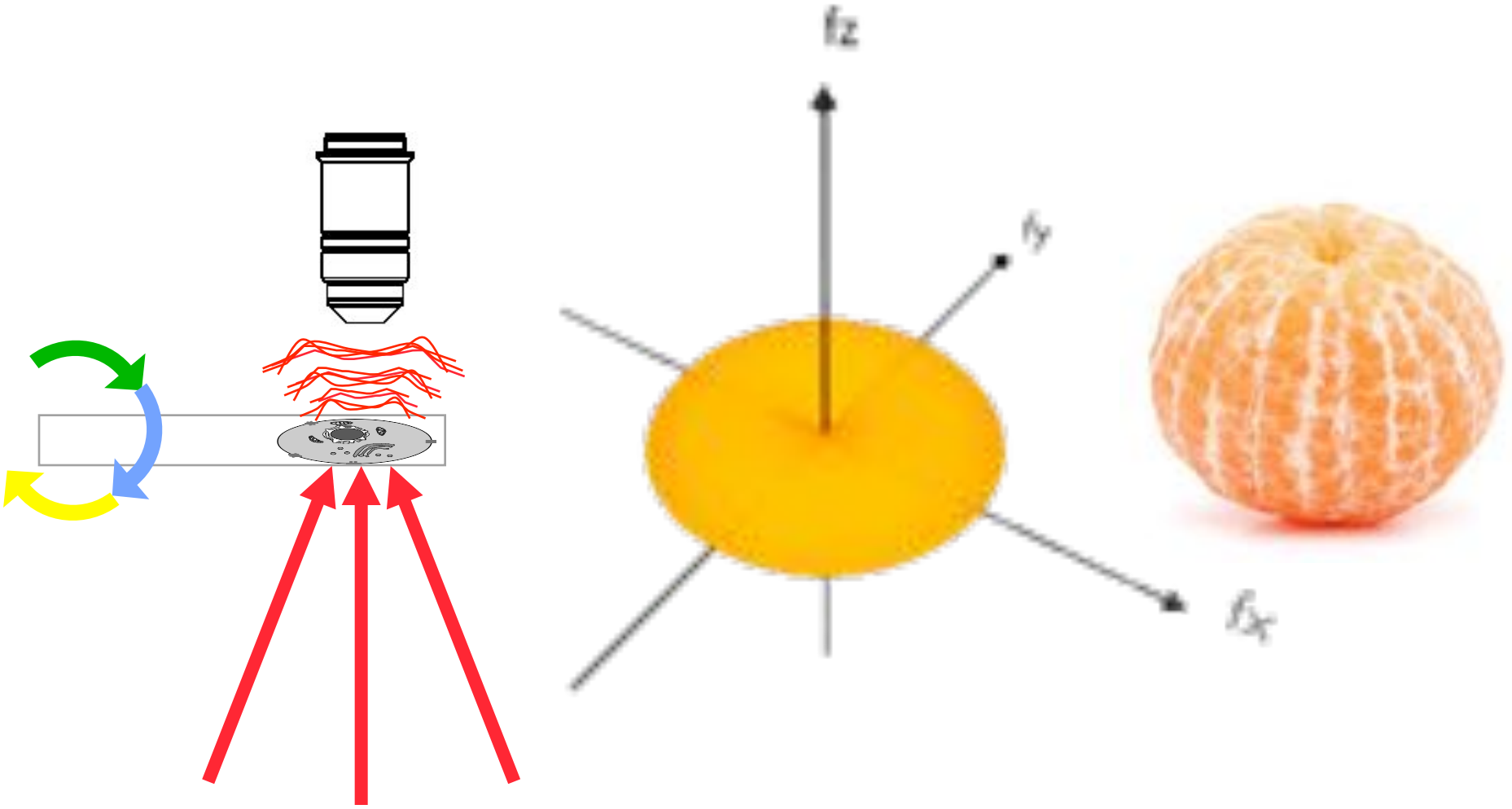
Multiview Tomography







Multiview Tomography

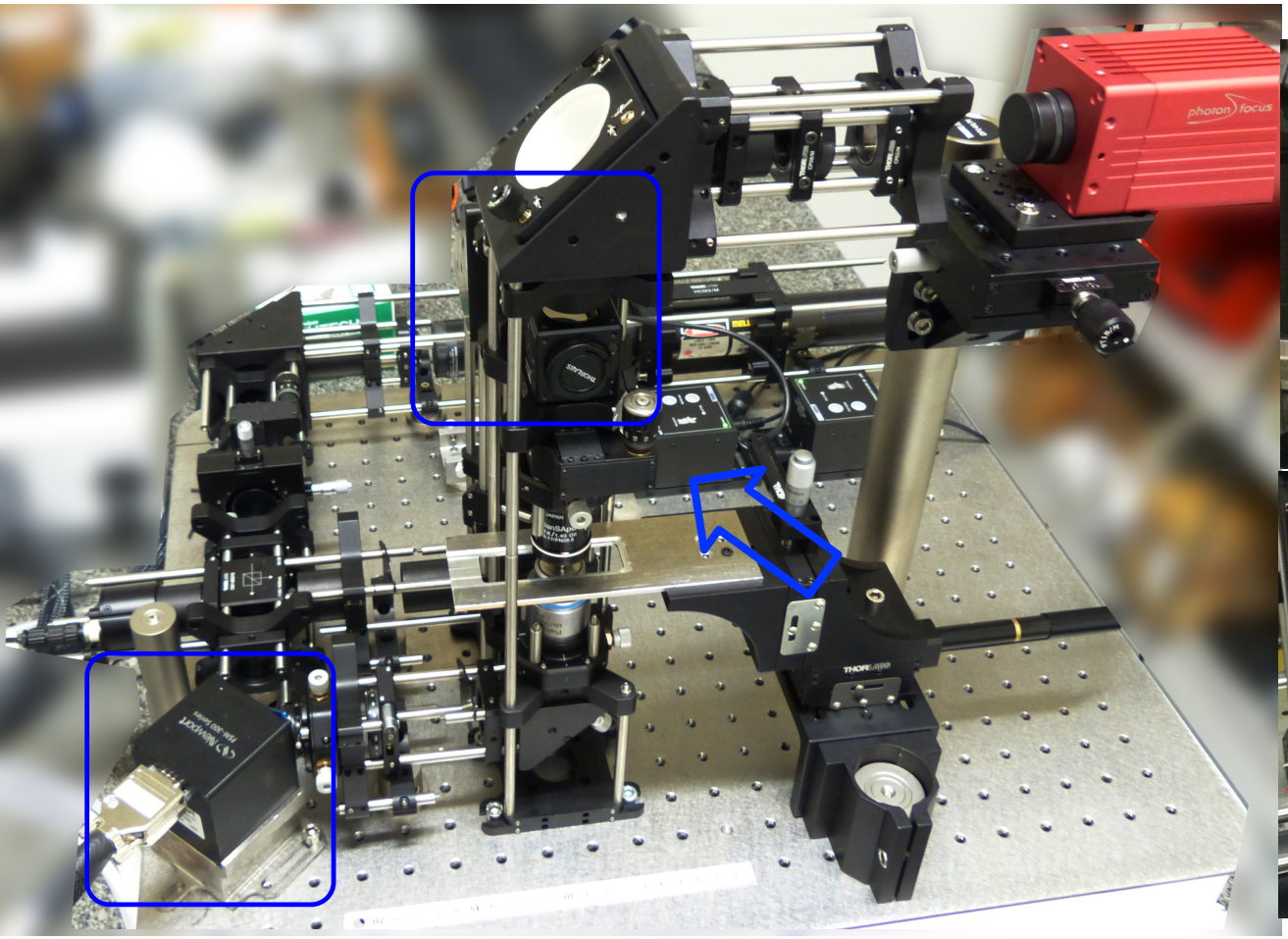


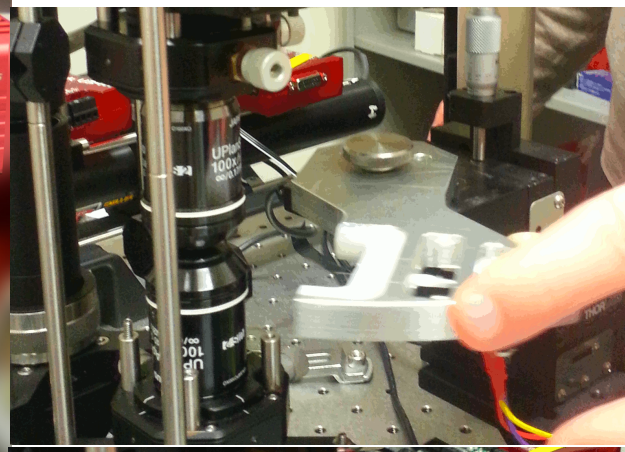
Improved and isotropic resolution in tomographic diffractive microscopy combining sample and illumination rotation

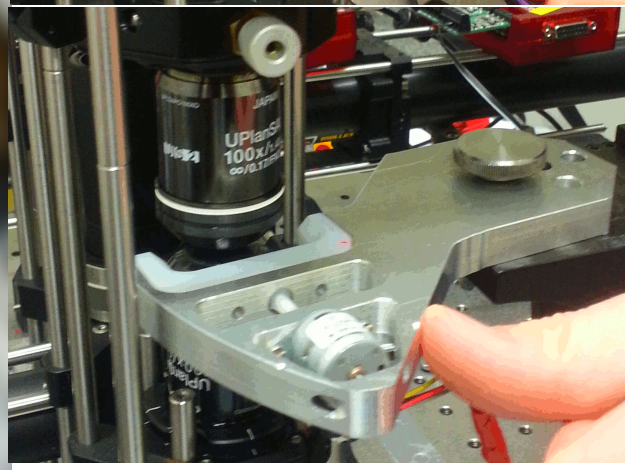
S. Vertu, *et al.*, Centr. Eur. J. of Phys. **9**, p. 969 (2011)

Towards High NA, IsoResolution

λ=633nm or 475nm, NA_{obj}=1.4, NA_{cond}=1.4

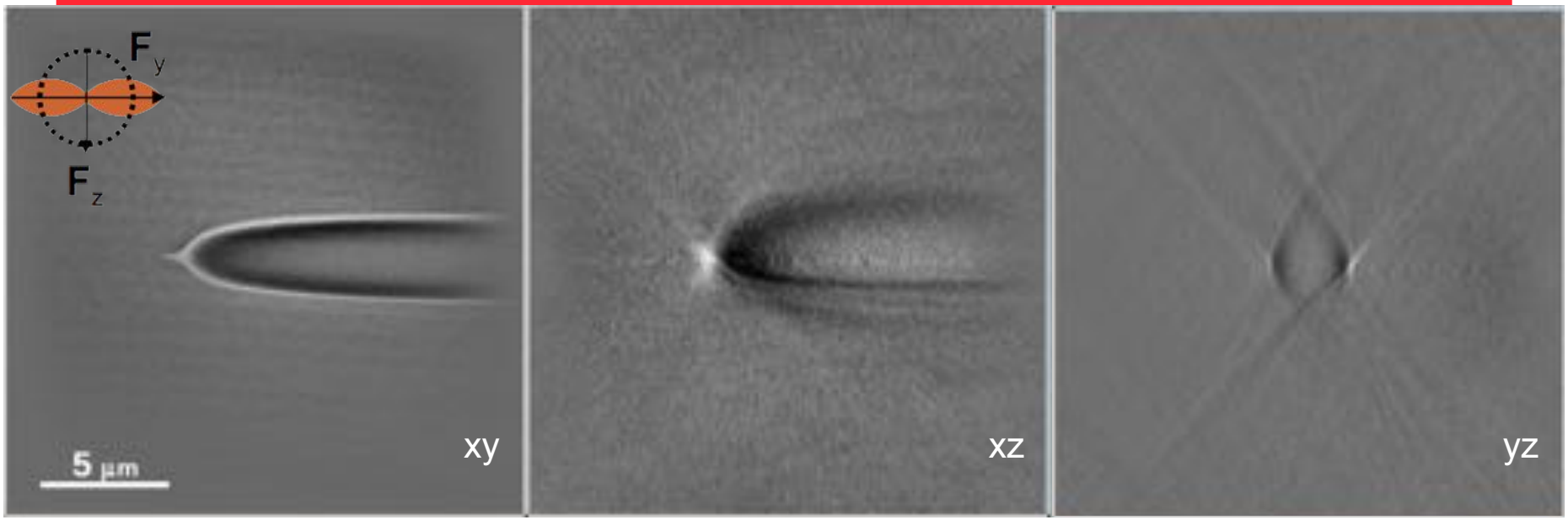


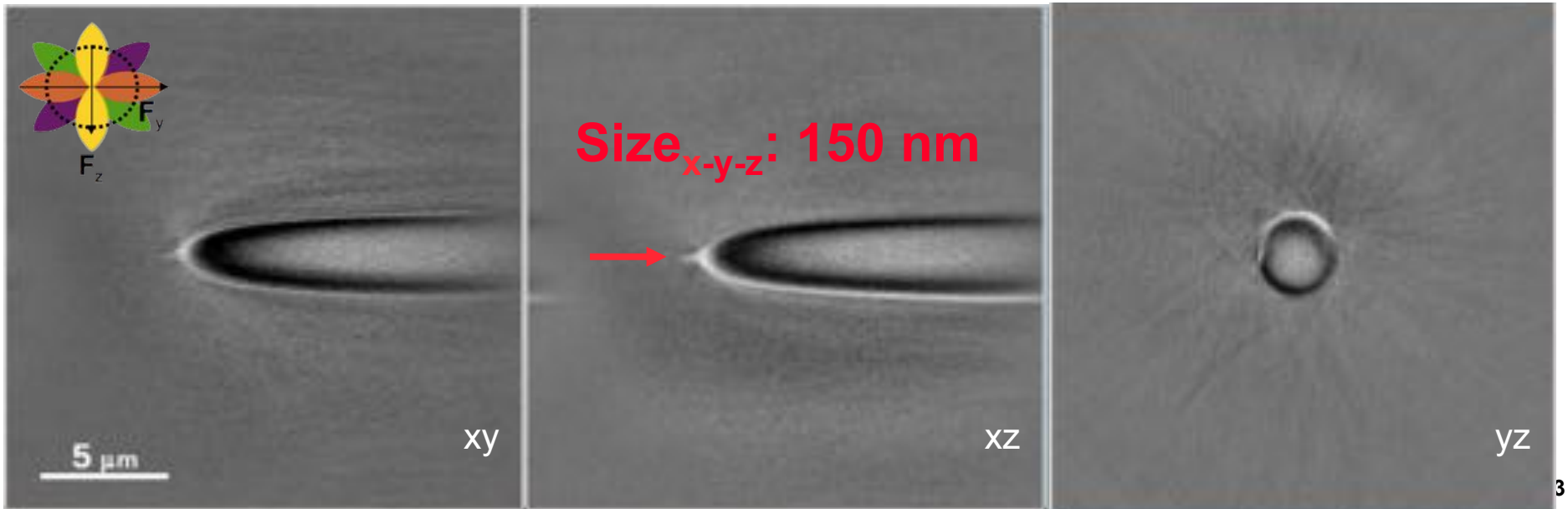




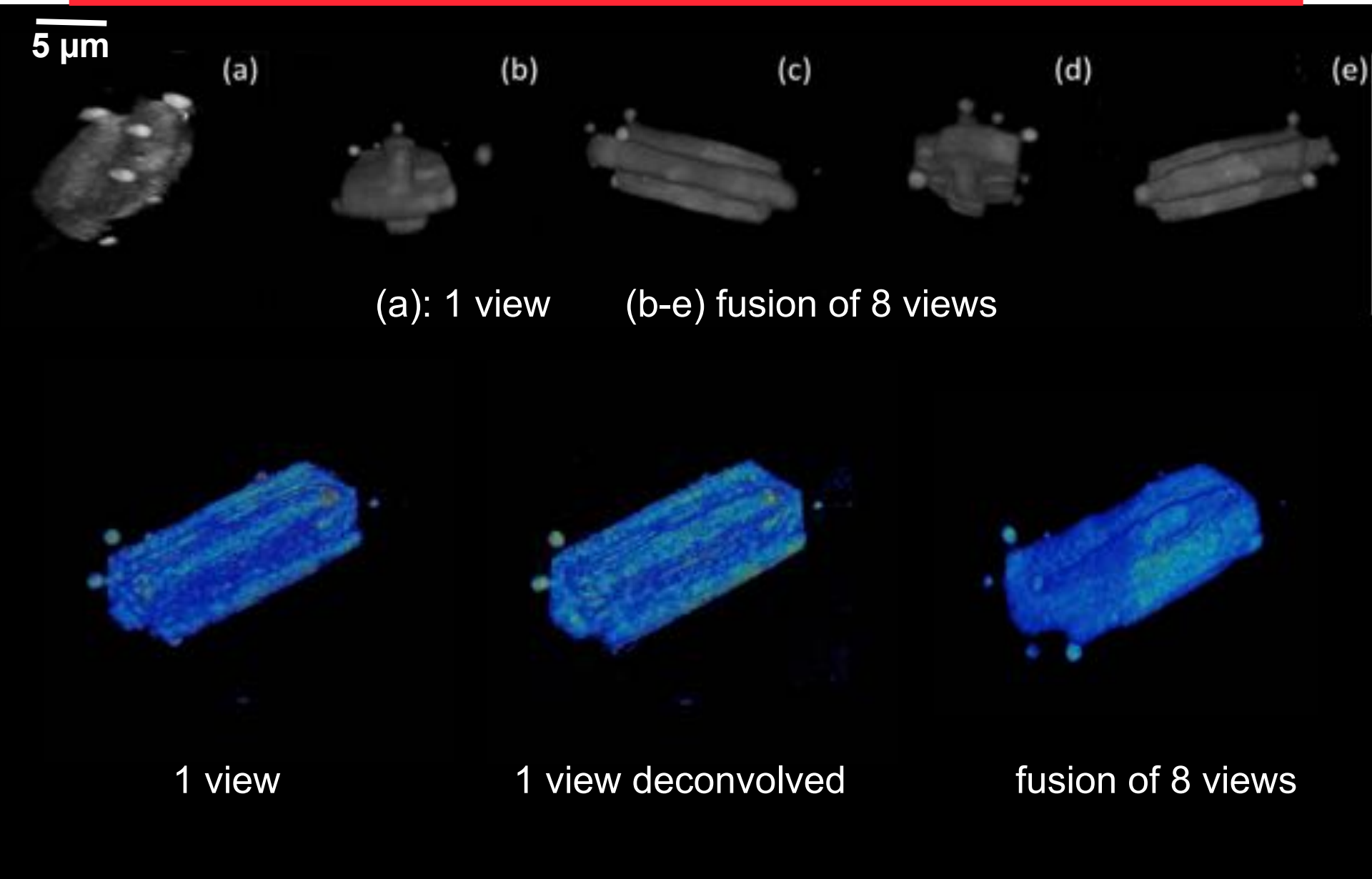
Fast acquisition (less than 10s for 1 object orientation / 400 illuminations)
Real-time reconstruction for each object orientation (1 volume of data each 3s)

Optical Fiber Tip (λ=475 nm $R_{predicted}$=95nm)

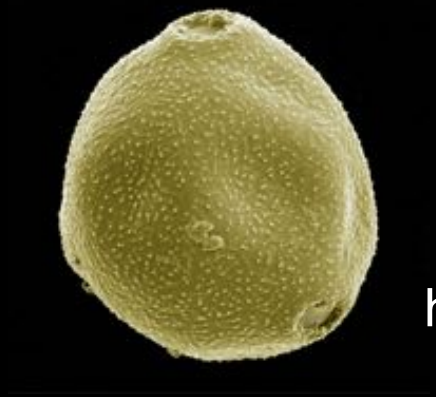




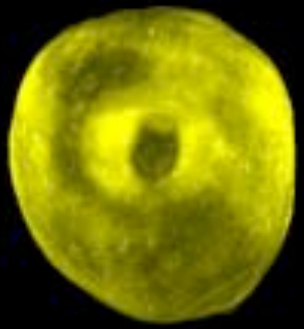
Zeolith microcrystal



Betula Pollen



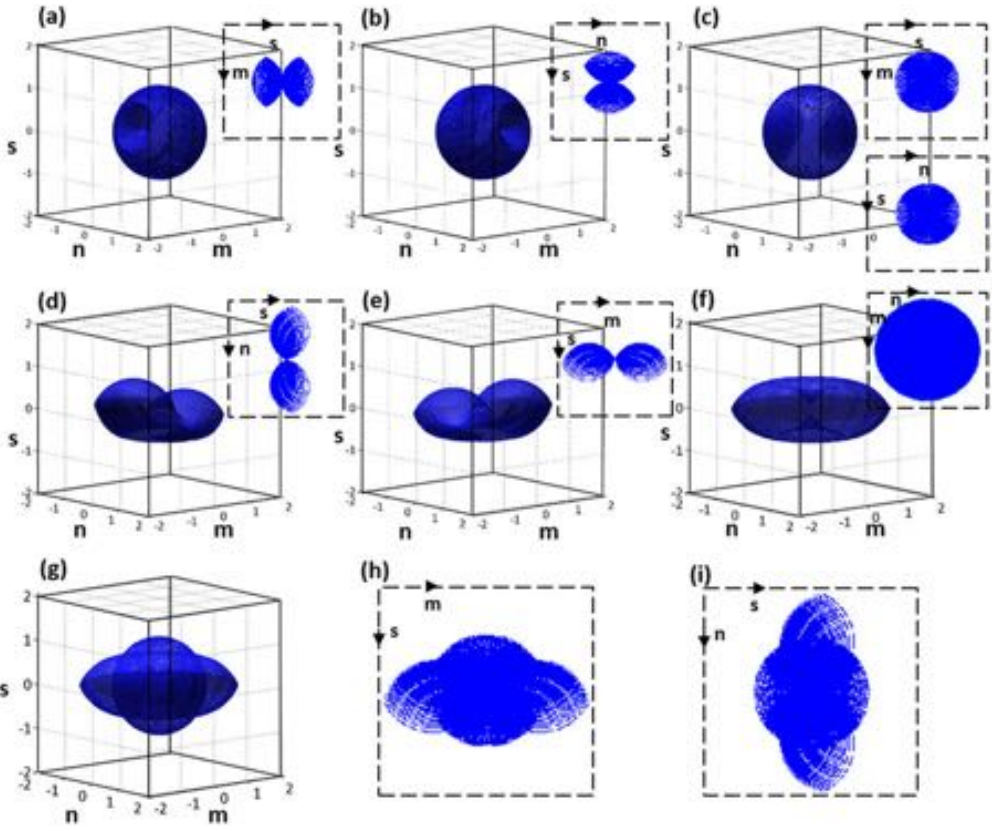
http://www.vcbio.science.ru.nl

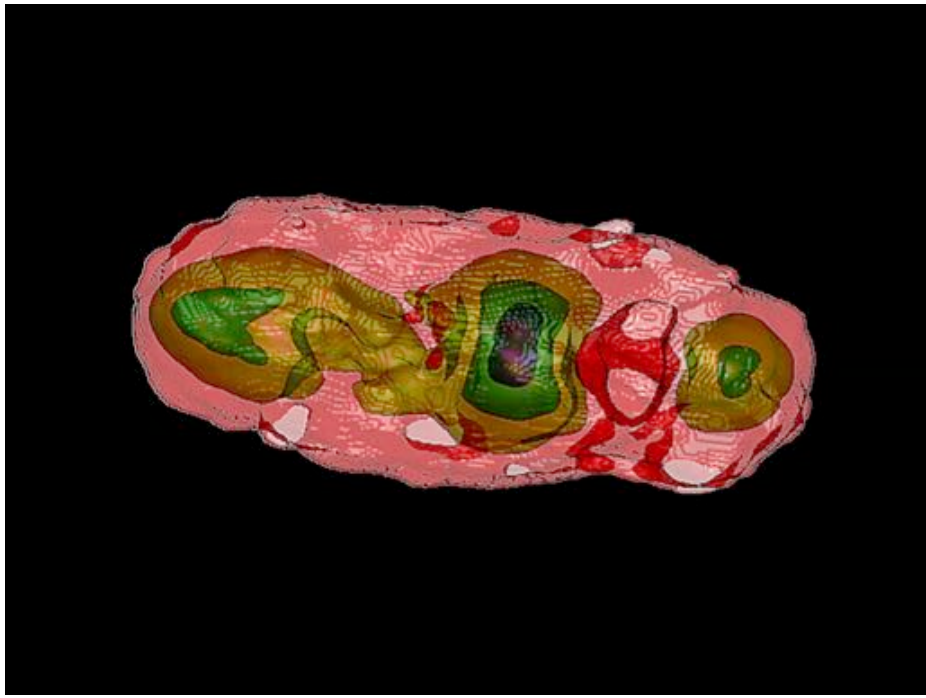


Betula Pollen

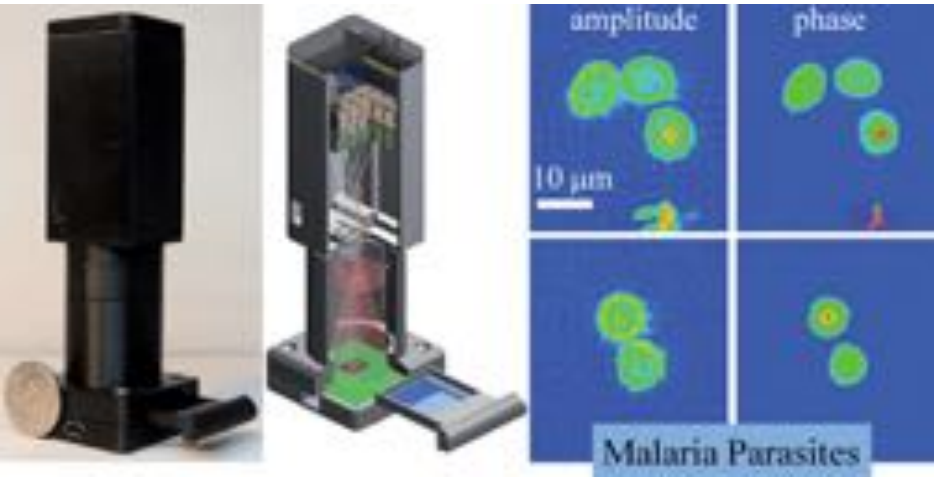
Integrated dual-tomography for refractive index analysis of free-floating single living cell with isotropic superresolution

B. Vinoth, *et al.,* Scientific Reports **8**, 5943 (2018)



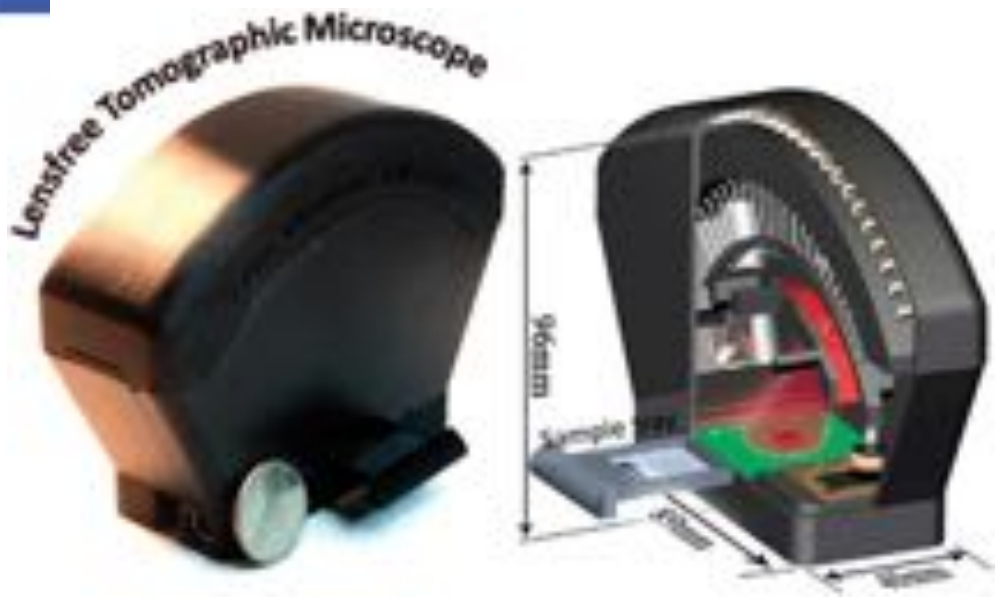


Low-cost microscopy/tomography

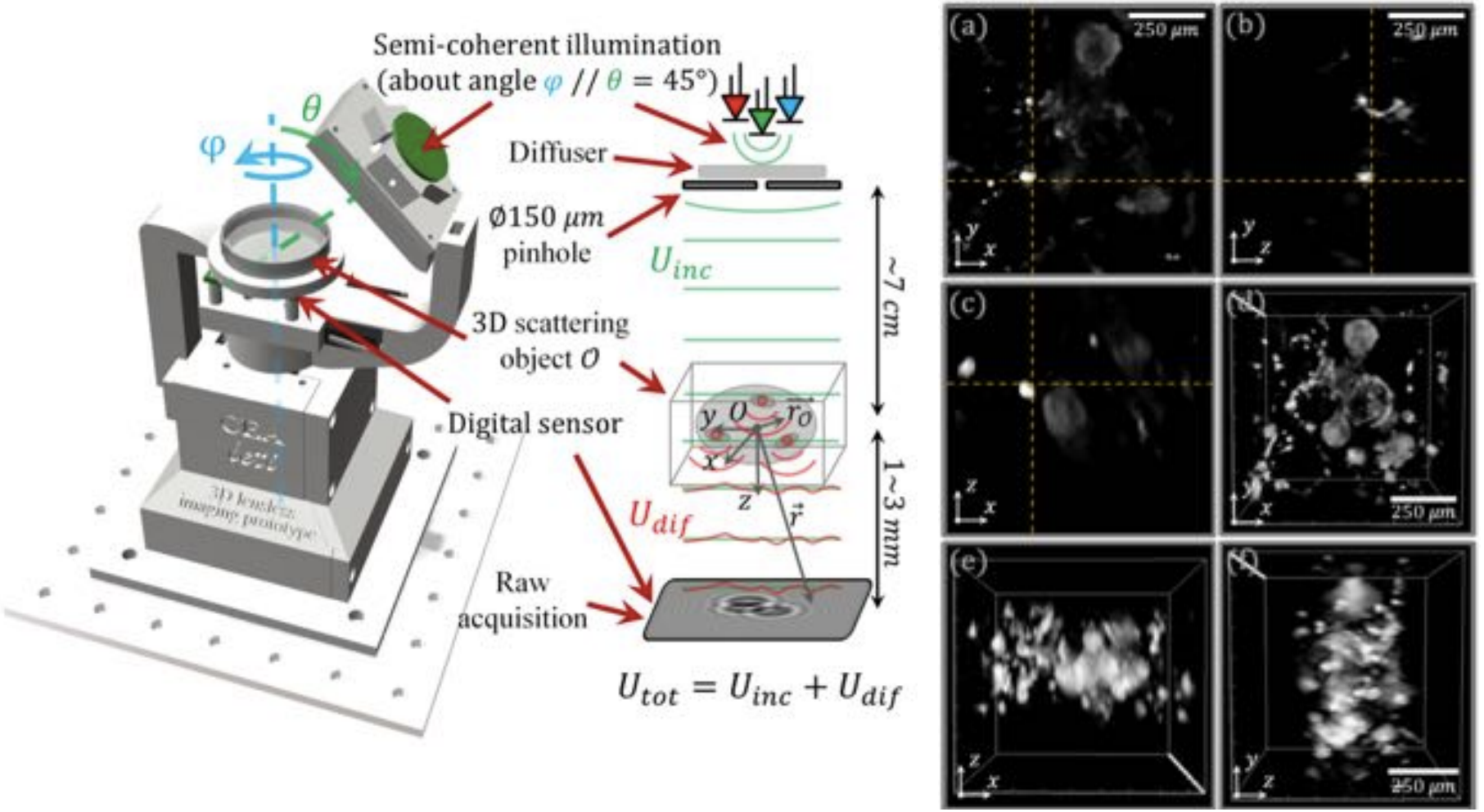


Aydogan Ozcan's group
UCLA

Lens-free optical tomographic microscope with a large imaging volume on a chip
S. O. Isikman, *et al.,*
PNAS 1015638108 (2011)



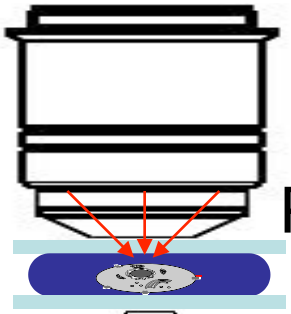
Low-cost microscopy/tomography



Comparative study of fully three-dimensional reconstruction algorithms for lens-free microscopy

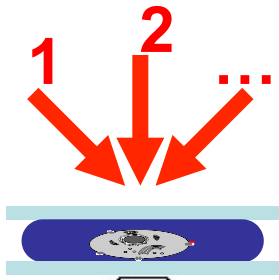
A. Berdeu, *et al.,* Appl. Opt. **56**, p. 3939 (2017)

Drawback : Speed



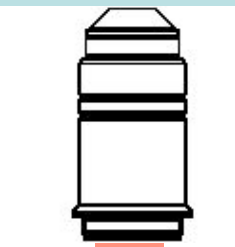
Intensity Microscopy:
Transmission,
Phase contrast, DIC…

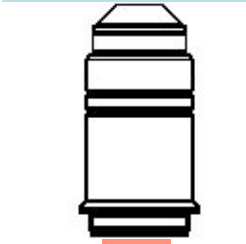
Diffractive
Tomographic
Microscopy



Incoherent
Parallel
⇒ Ultrafast ☺

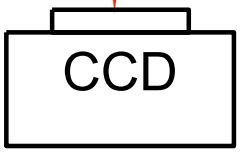
Coherent
Sequential
=> Slow ☹

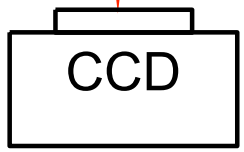




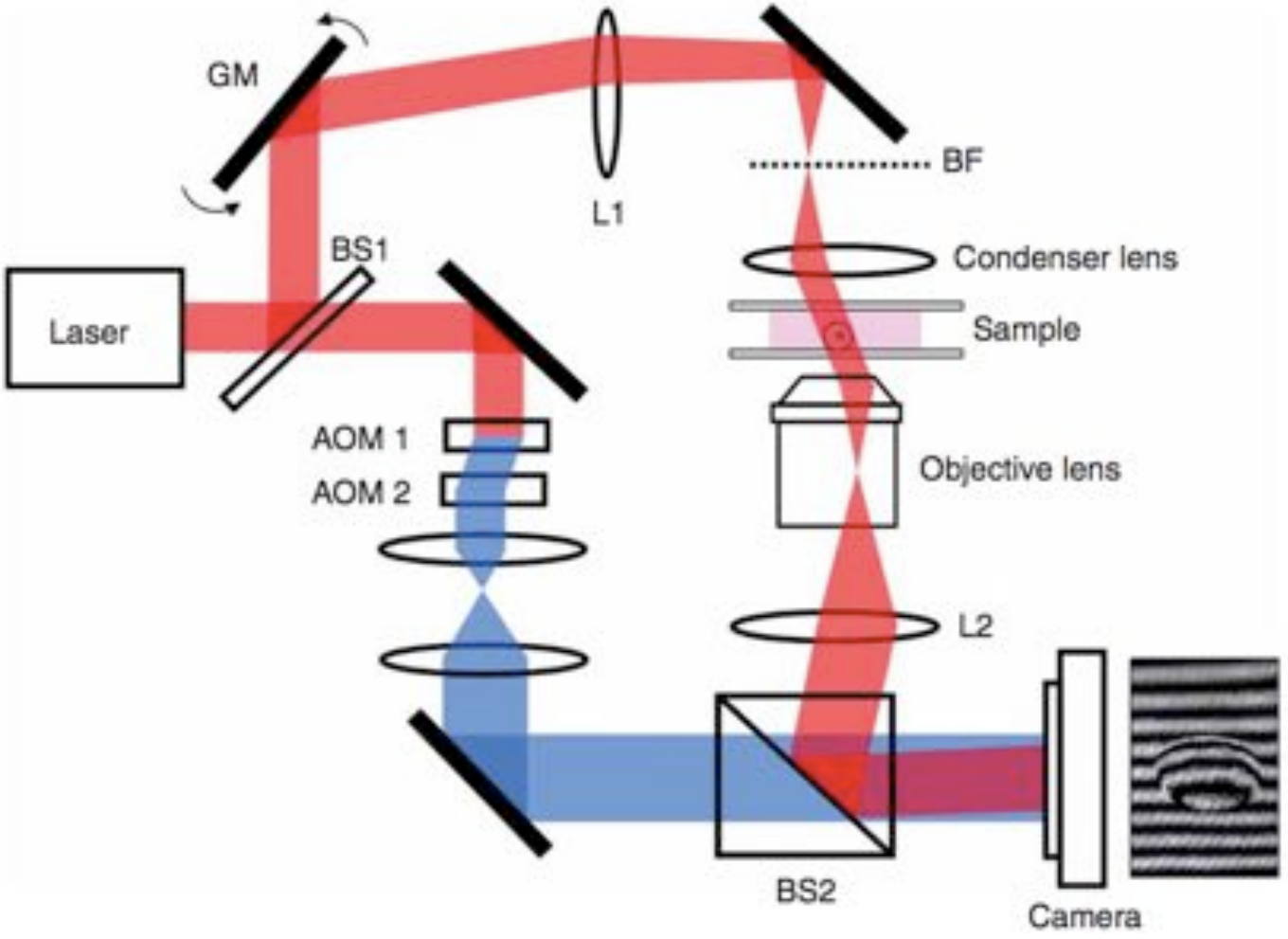
Not-quantitative
Low-resolution

Quantitative
High-resolution



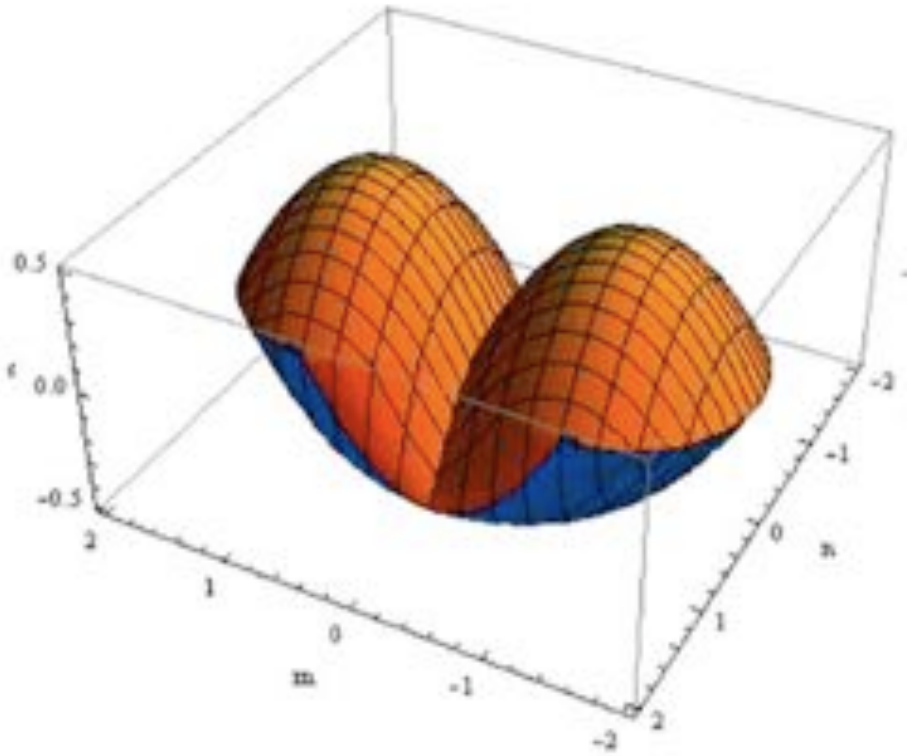


Fast 1-D Scanning



“Tomographic phase microscopy”
W. Choi, *et al.*, Nat. Meth. **4**, p. 717 (2007)

1-D Scanning

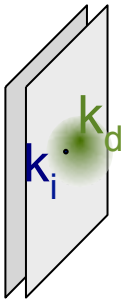


"The overall shape takes a form of what we might call a "peanut."

"Image formation in holographic tomography"
S. Shan Kou, and C. J. R. Sheppard, Opt. Lett. **33**, p. 2362 (2008)

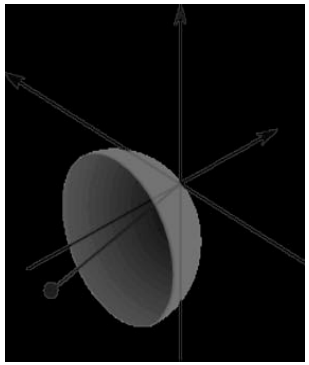
Acquisition Reconstruction Display

1.4 ms for 1 angle



Projection

GPU



Addition

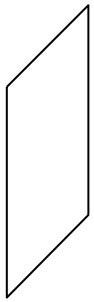
GPU

Off-axis

2-D FT

GPU

Object frequencies



New angle

CPU->Mirror

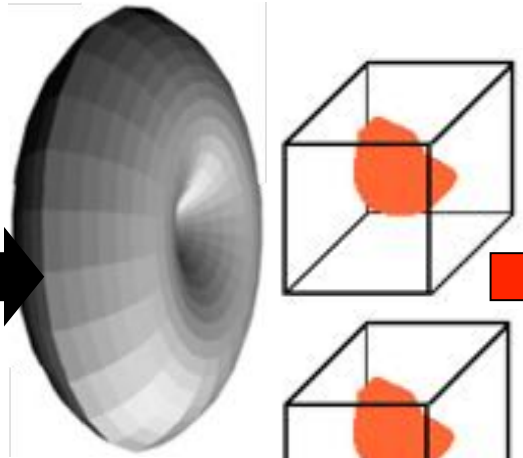
1 hologram

Camera->CPU

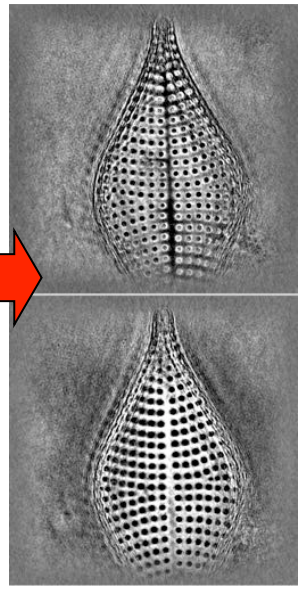
Data preparation
+ 3-D FT

GPU

240 ms

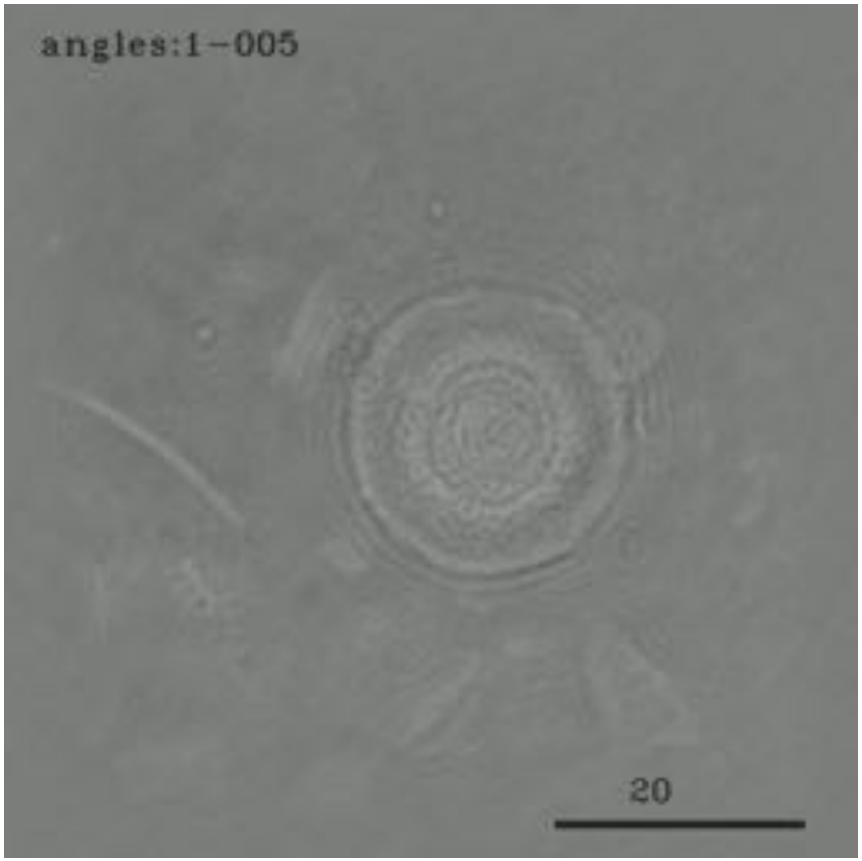


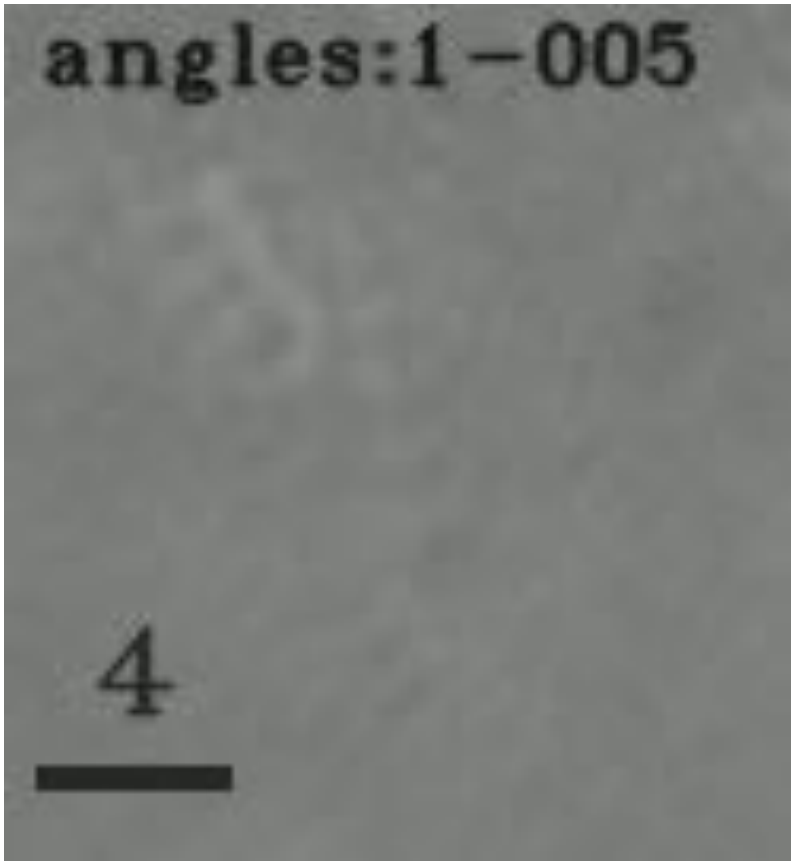
3-D
frequency
support



Display
(±1ms)

GPU Reconstruction





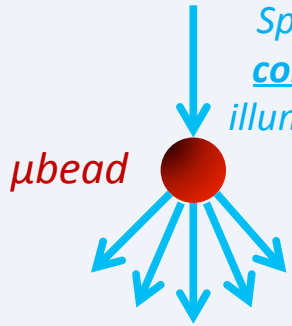
Nvidia Tesla C2075, Cuda, FFTW : 3.5 3D images/s

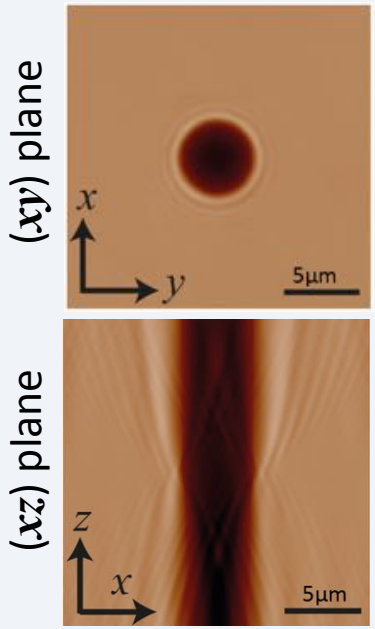
Tomographic diffractive microscopy: towards high-resolution 3-D real-time data acquisition, image reconstruction and display of unlabeled samples

J. Bailleul, *et al.*, Opt. Comm. **422**, p. 28 (2018)

Other possible approach

Conventional QPI

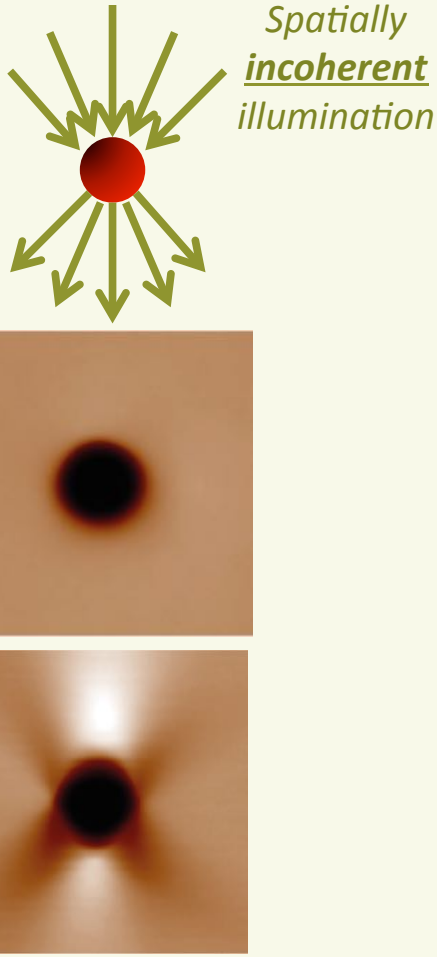




☹ Limited axial resolution

New approach

Bon *et al.*,
Optics Express
2014

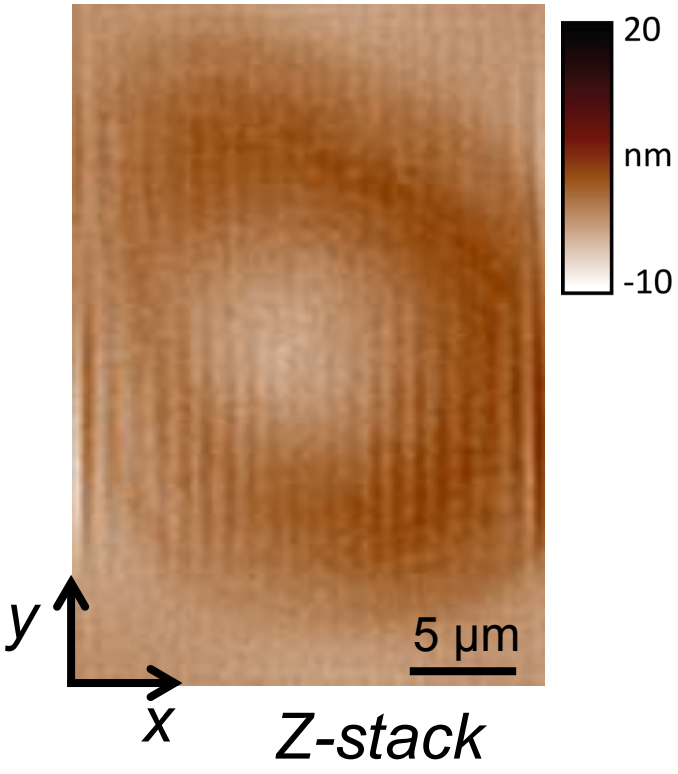


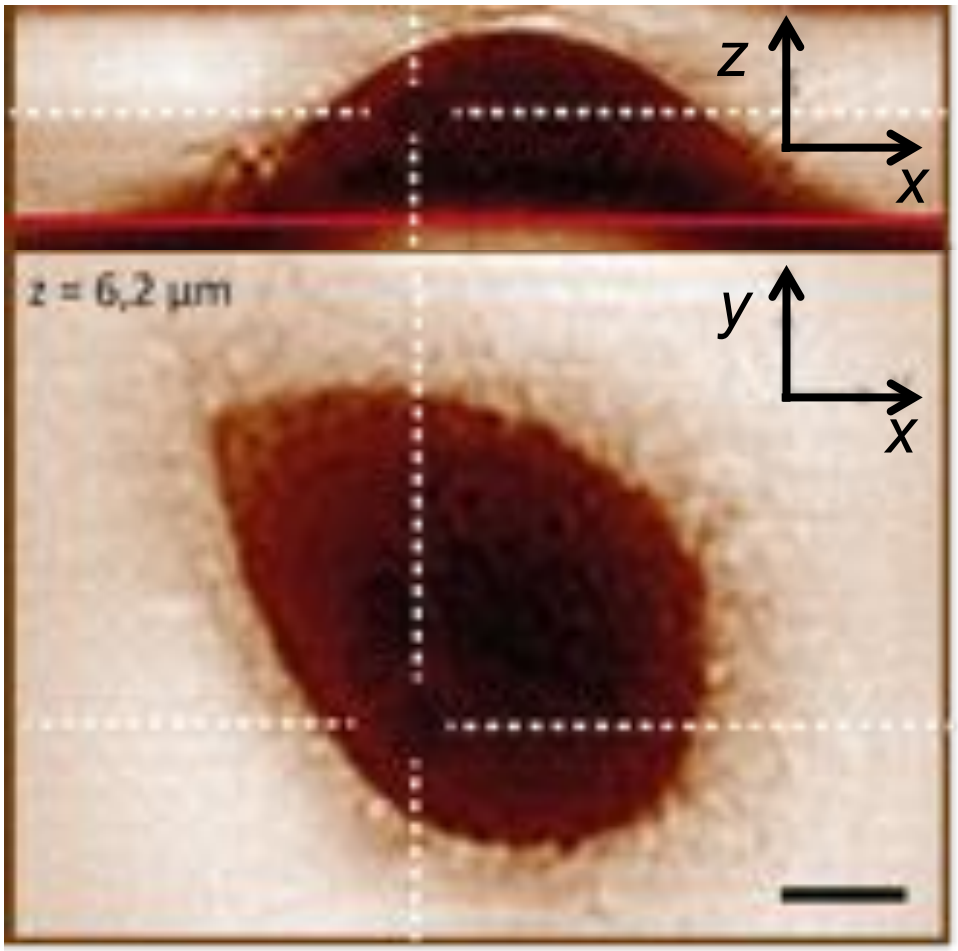
☺ 3D resolution ⇔ fluorescence

3D live cell imaging

Living COS-7 cell

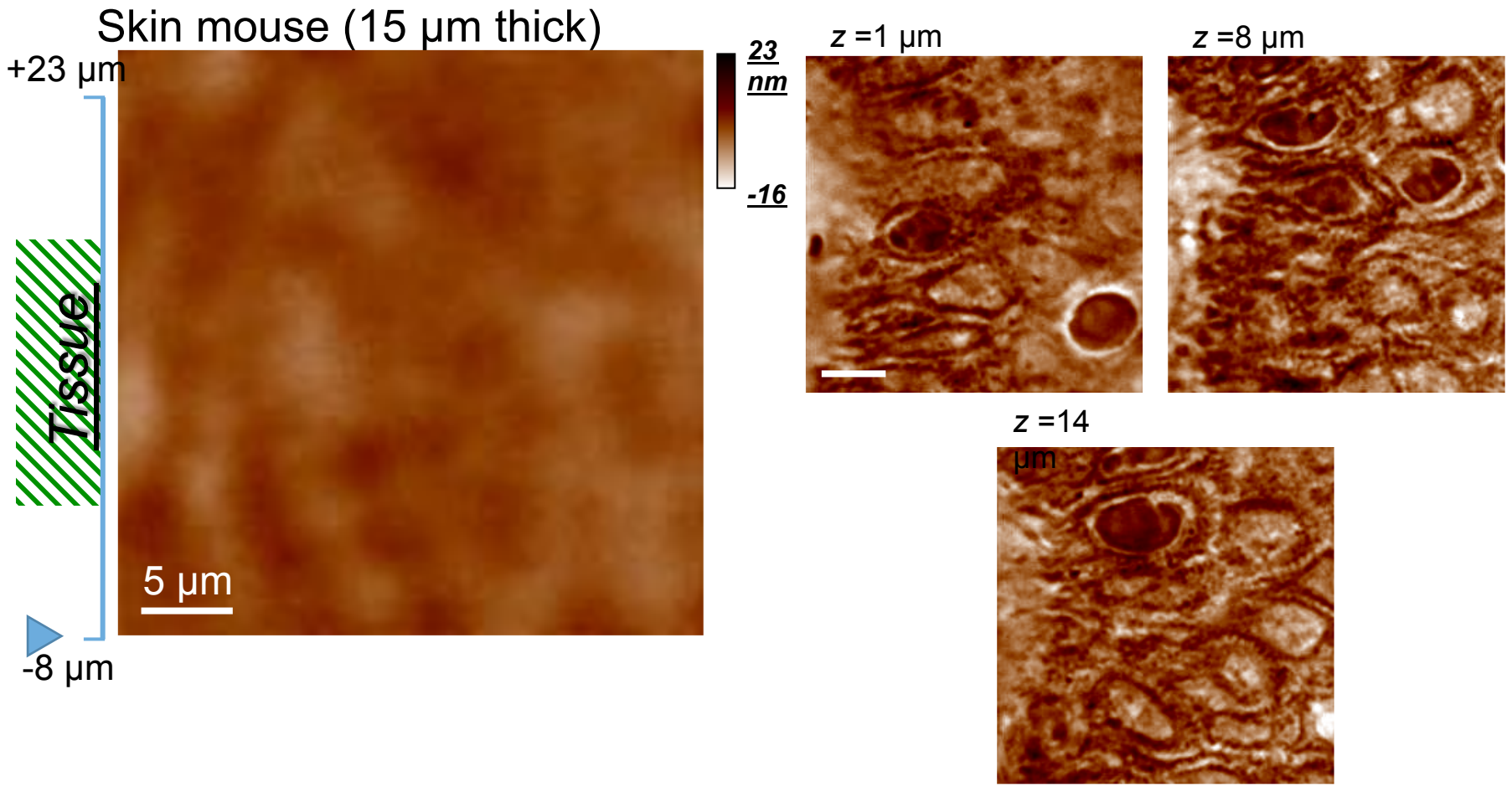
OPD with spatially Incoh. Illum.
+ 3D deconvolution





- ✓ ***3D shape of the cell***
- ✓ ***Fast acquisition, compatible with live imaging (just a z-stack!)***

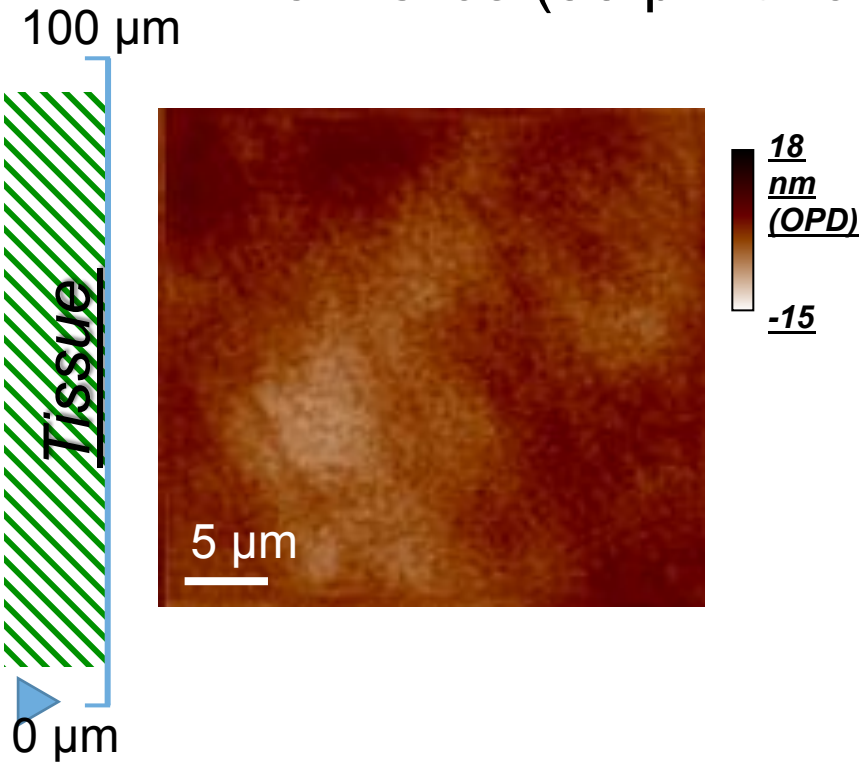
Fixed tissue imaging (1/2)

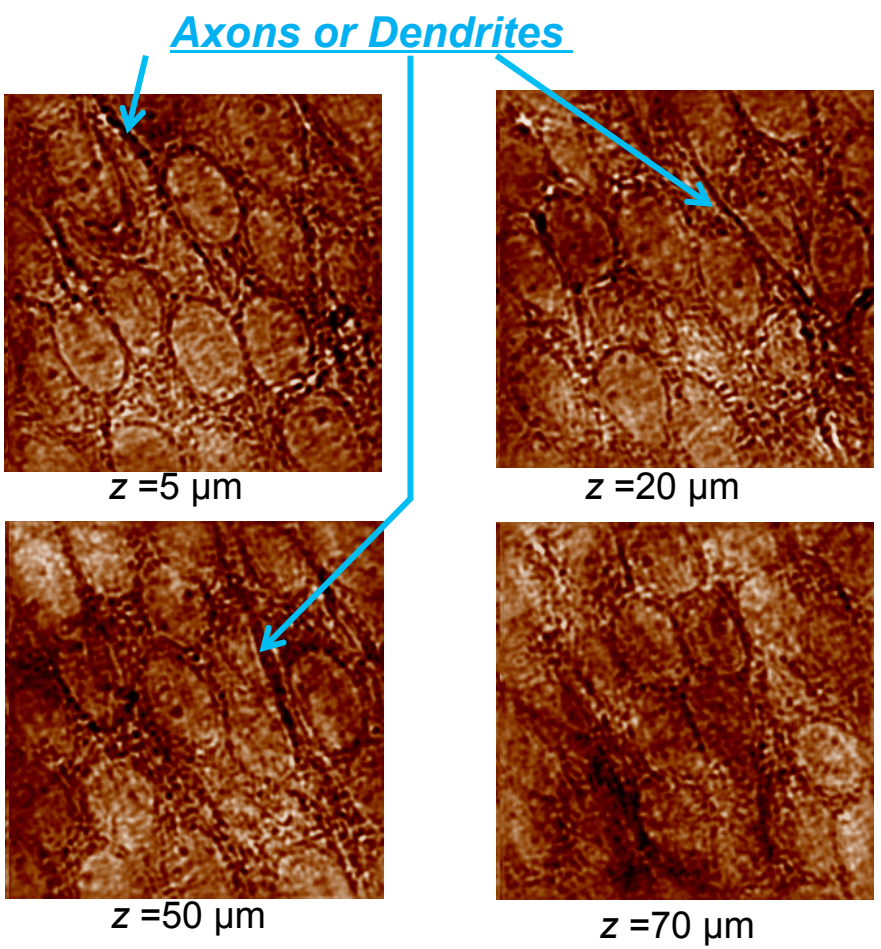


✓ ***Cell layer visualization without labeling***

Fixed tissue imaging (2/2)

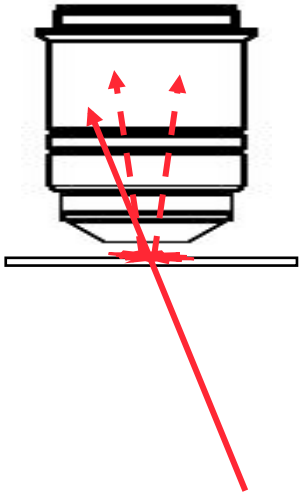
Brain slice (90 µm thick)



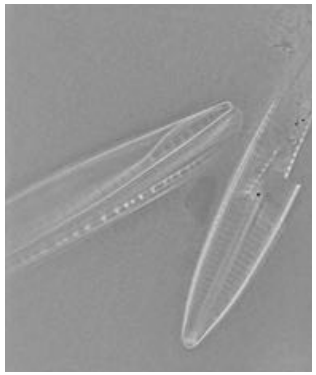


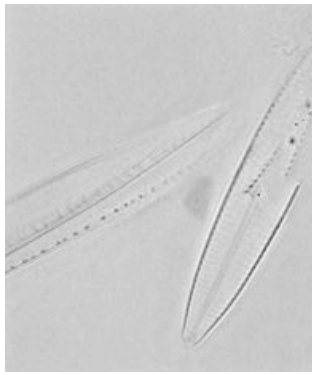
✓ ***Thin structures visible even after few tens of microns***

Multimode Imaging

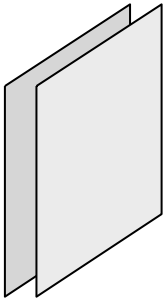


Synthetic reconstruction

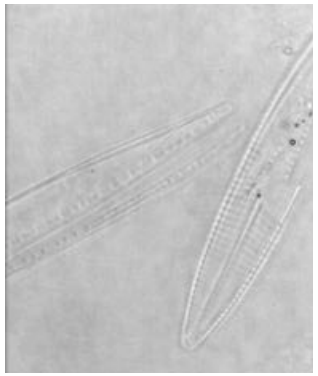




Refraction Microscopy

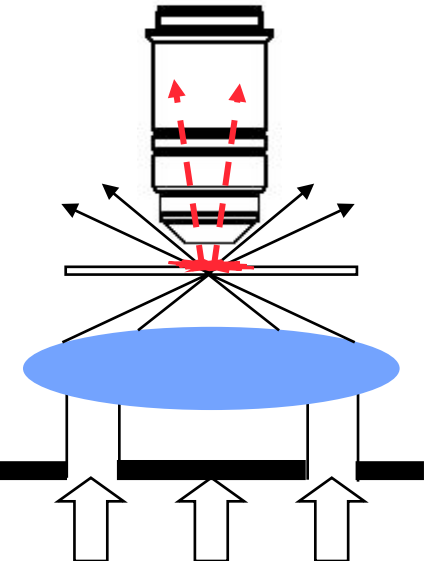


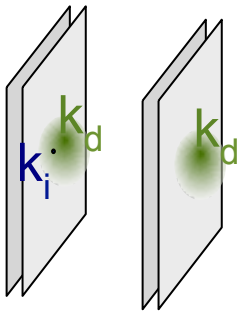
Compute Intensity (^2)



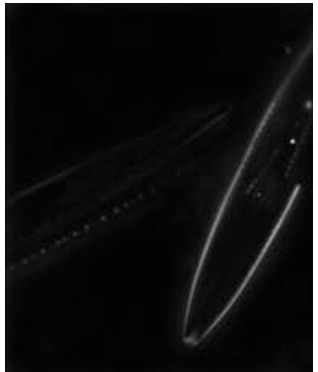
Wide-Field Microscopy

Wavefront





FT (^2)



High-NA Dark-Field Microscopy

Conclusion

Unprepared samples => use of a new kind of information <n>

High resolution imaging: λ/(3.5NA) lateral experimentally demonstrated

Challenges:
- RT acquisition/reconstruction/display
- polarimetric TDM
- “true” superresolution?