Tomographic diffractive microscopy: principles and applications

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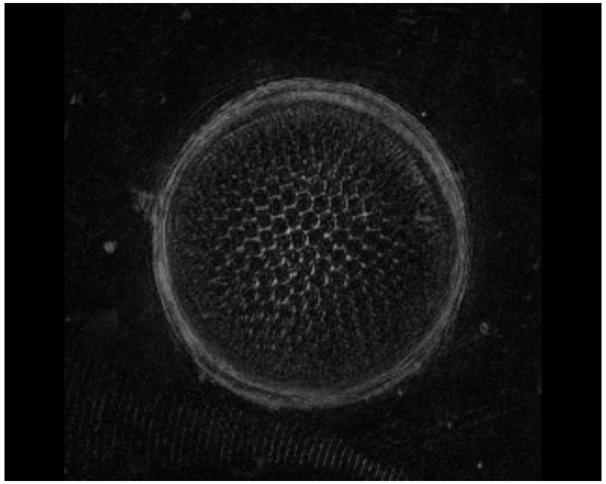


MiFoBio 2018
Seignosse - France, October 5-12, 2018

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

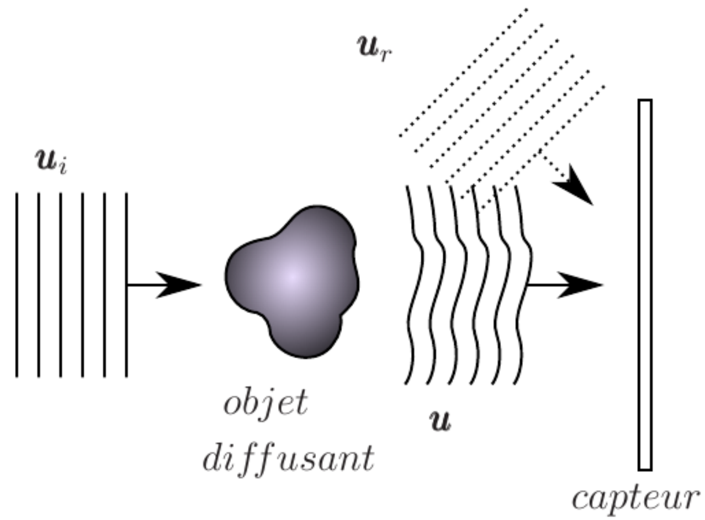
Phase Microscopy

:-(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

Principle of Holography



$$\mathcal{I}(x,y) = \underbrace{|\boldsymbol{u}(x,y)|^2 + |\boldsymbol{u}_r(x,y)|^2}_{\text{0-Order}} + \underbrace{\boldsymbol{u}(x,y)\boldsymbol{u}_r^*(x,y)}_{\text{Order 1}} + \underbrace{\boldsymbol{u}^*(x,y)\boldsymbol{u}_r(x,y)}_{\text{Order -1}}$$

0-Order Order 1 Order -1

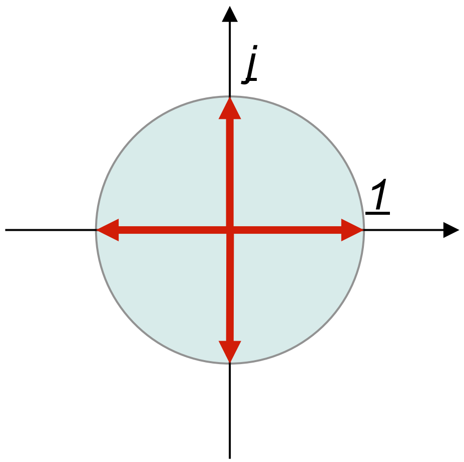
How to get the object wave (order 1) from the fringe pattern?

Nobel Prize 1971 Physics
Denis Gabor
""for his invention and development of the holographic method".".

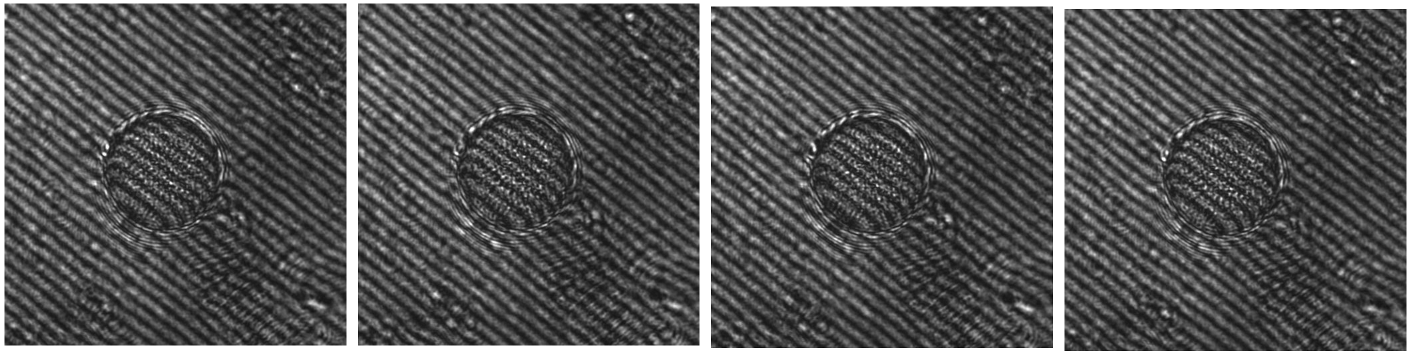
Phase Stepping Holography

4 holograms, with 4 different phases of the reference wave

$$\mathcal{I}_k(x,y) = |u(x,y)|^2 + |\boldsymbol{u}_r(x,y)|^2 + u(x,y)u_r^*(x,y)e^{-jk\pi/2} + u^*(x,y)\boldsymbol{u}_r(x,y)e^{jk\pi/2}$$



$$\mu = +u(x,y)u_r^*(x,y)$$



I0 *I1* *I2* *I3*

$$\begin{aligned}
\mathcal{I}_0 &= |\boldsymbol{u}(x,y)|^2 + |\boldsymbol{u}_r(x,y)|^2 + \mu + \mu^* \\
\mathcal{I}_1 &= |\boldsymbol{u}(x,y)|^2 + |\boldsymbol{u}_r(x,y)|^2 + -j\mu + j\mu^* \\
\mathcal{I}_2 &= |\boldsymbol{u}(x,y)|^2 + |\boldsymbol{u}_r(x,y)|^2 - \mu - \mu^* \\
\mathcal{I}_3 &= |\boldsymbol{u}(x,y)|^2 + |\boldsymbol{u}_r(x,y)|^2 + j\mu - j\mu^*
\end{aligned}$$

One obtains:

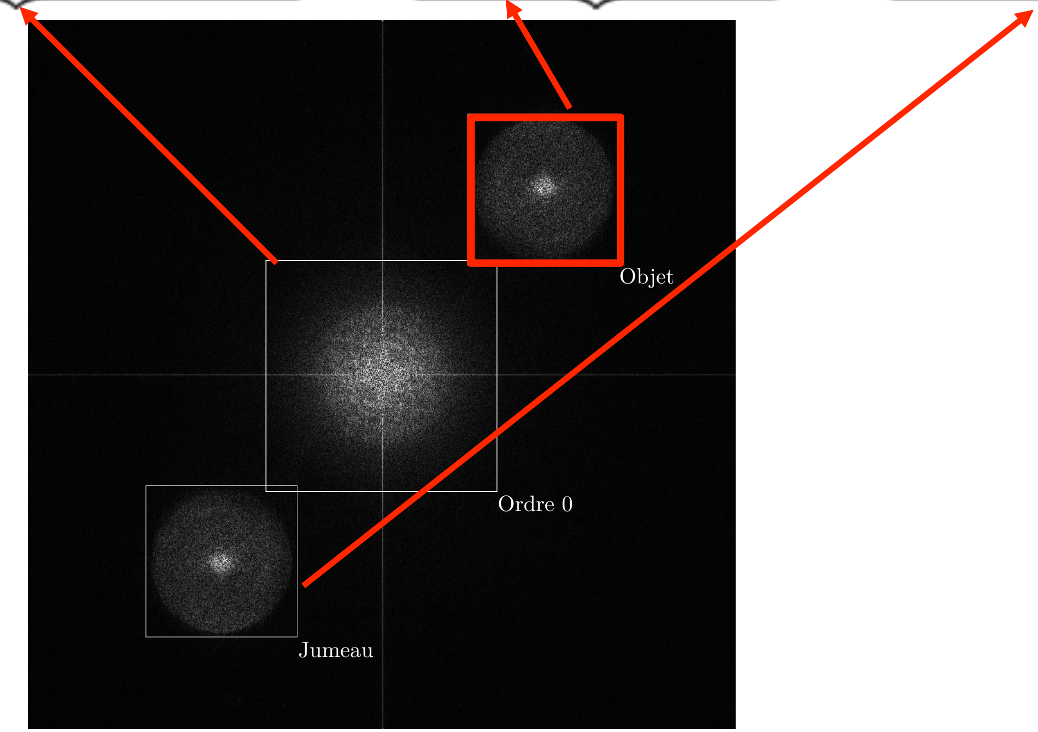
$$\begin{aligned}
\mathcal{I}_0 - \mathcal{I}_2 &= 4\Re(\mu) \\
\mathcal{I}_3 - \mathcal{I}_1 &= 4j\Im(\mu)
\end{aligned}
\longrightarrow
\boldsymbol{u} = \frac{(\mathcal{I}_0 - \mathcal{I}_2) + (\mathcal{I}_3 - \mathcal{I}_1)}{4\boldsymbol{u}_r}$$

Off-Axis Holography

The reference wave is angularly shifted => périodic modulation

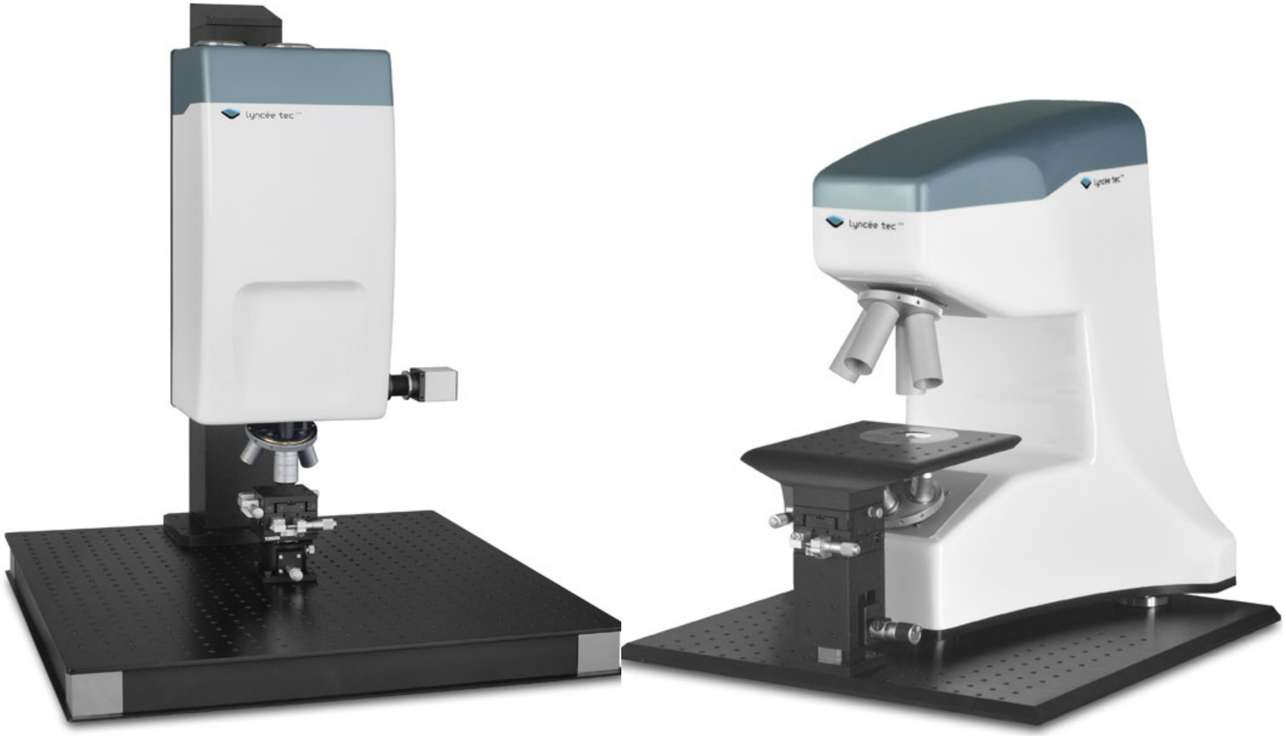
Fourier transform of the hologram splits the different orders:

$$\mathcal{I}(x,y) = \underbrace{|\boldsymbol{u}(x,y)|^2 + |\boldsymbol{u}_r(x,y)|^2} + \underbrace{\boldsymbol{u}(x,y)\boldsymbol{u}_r^*(x,y)} + \underbrace{\boldsymbol{u}^*(x,y)\boldsymbol{u}_r(x,y)}$$



Spatial filtering : selection of order 1

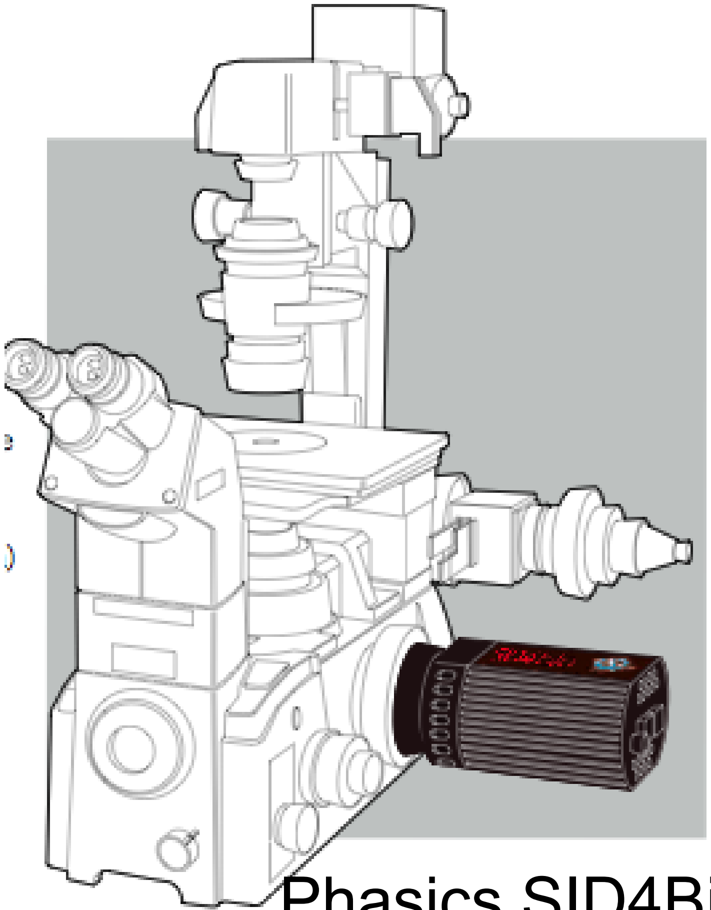
Commercial DHM

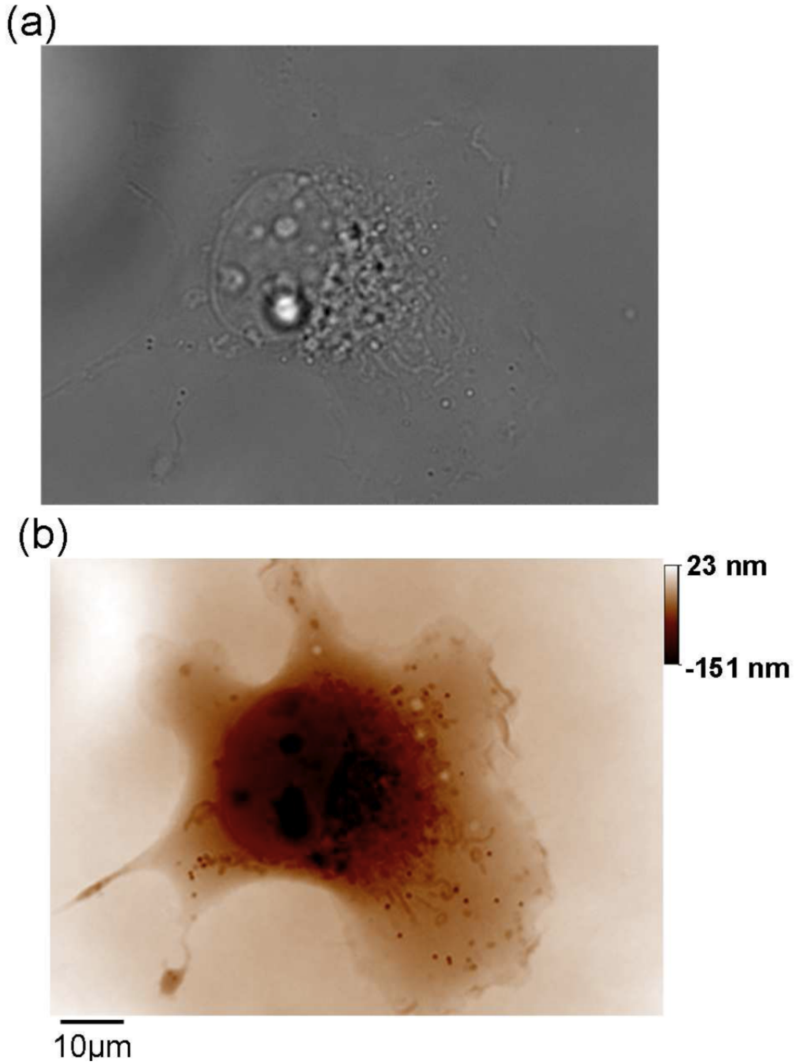


lyncée tec DHM

Phase Holographic Imaging

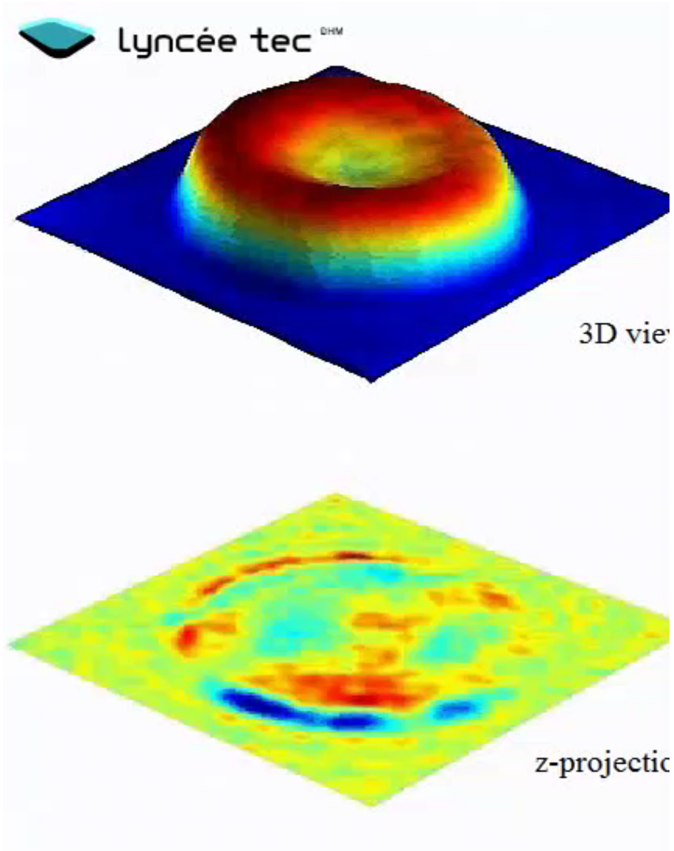
Wavefront Analyser





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

Holographic Microscopy

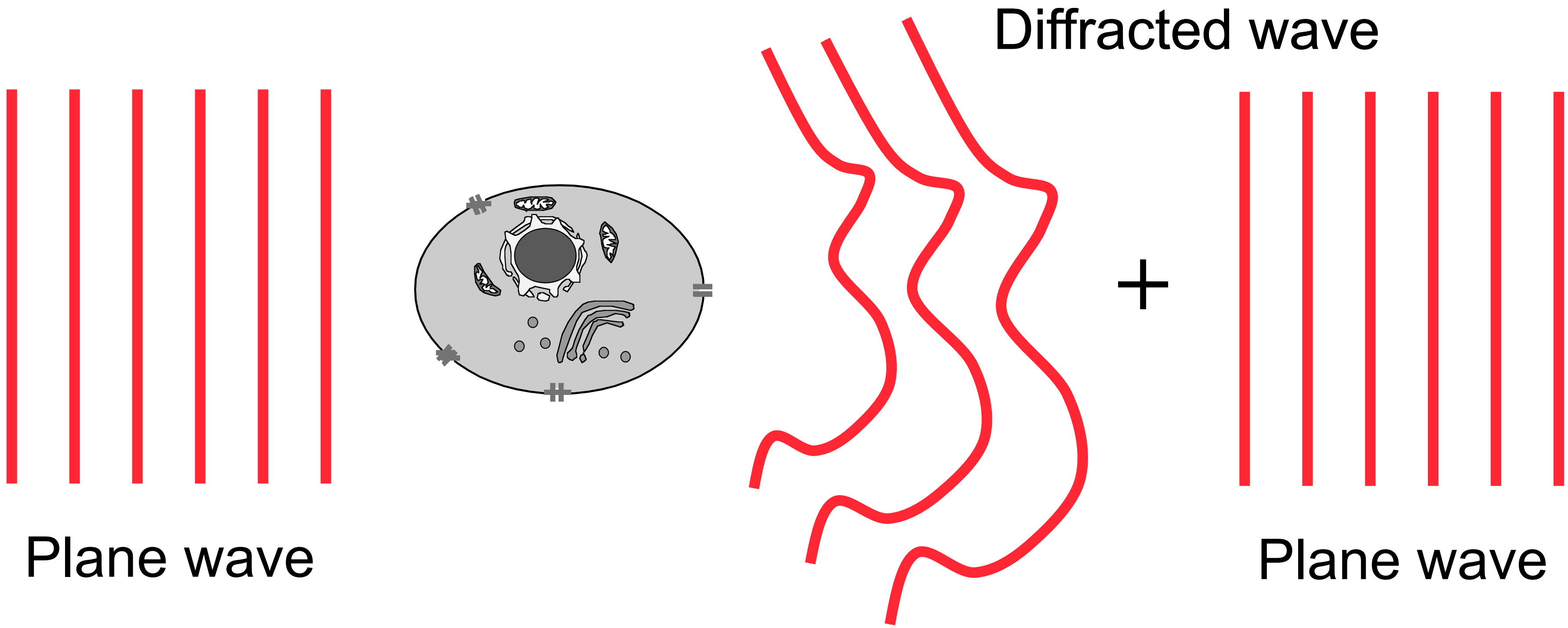


Measurement of the integral refractive index and dynamic cell morphometry of living cells with digital holographic microscopy

B. Rappaz, *et al.,*

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

Coherent Light Diffraction



Weakly diffracting/diffusing/absorbing object

1st Born approximation

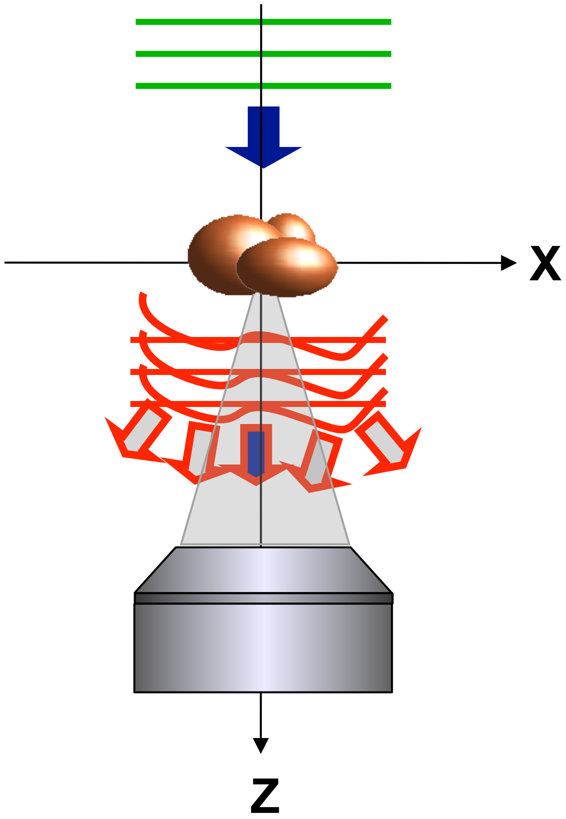
The diffracted wave is interpreted as a part of the 3-D Fourier 3D transform of <n>

Semi-transparent object reconstruction from holographic data

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

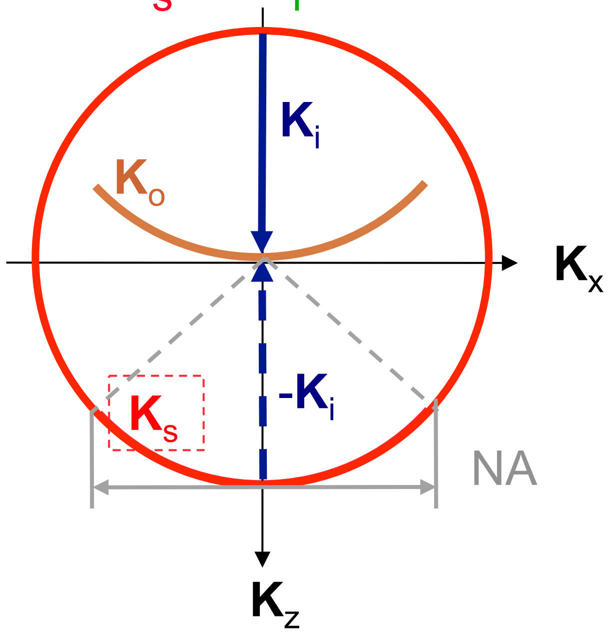
Image Space / Fourier space

Spatial domain



Frequency domain

$K_s = K_i = 2\pi/\lambda$

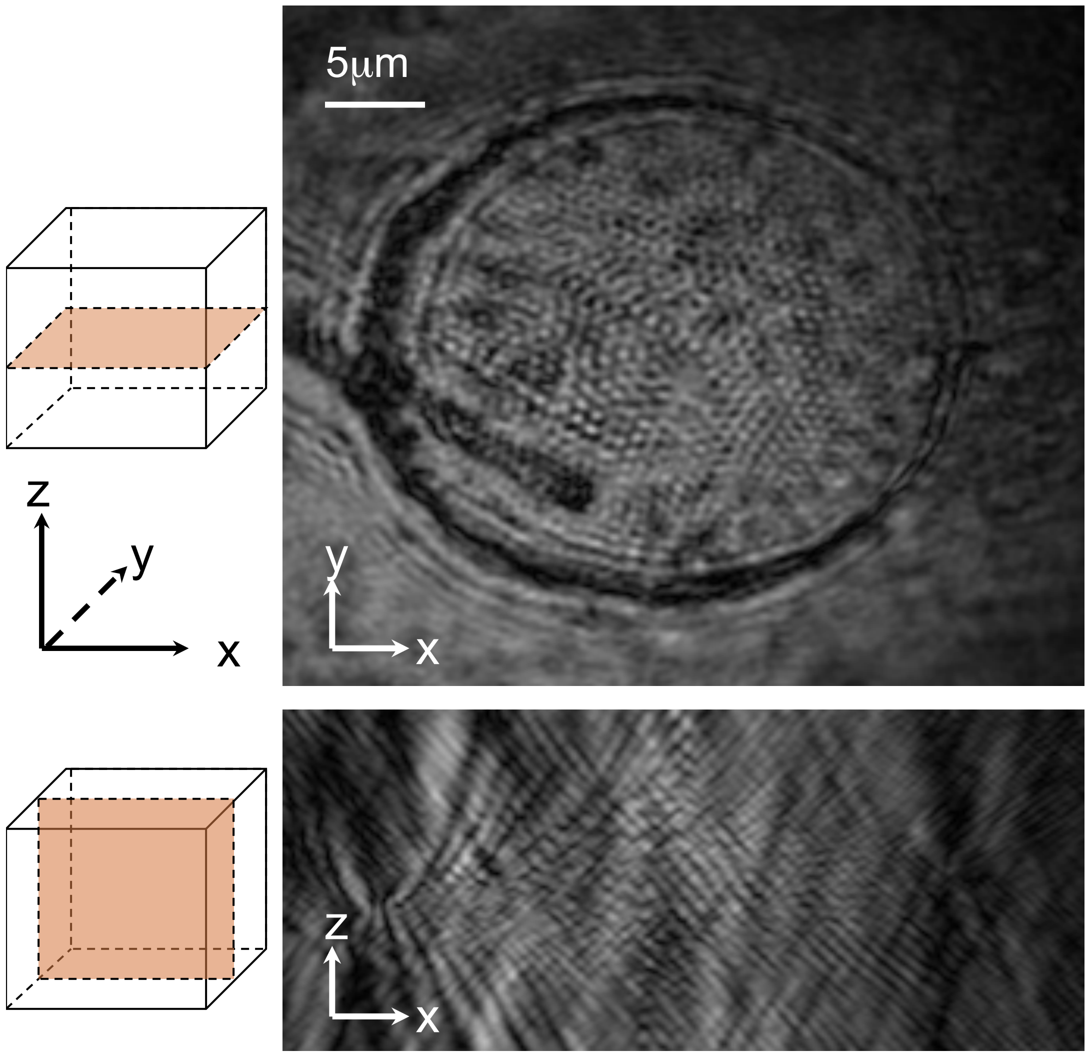


$\vec{K}_o = \vec{K}_s - \vec{K}_i$

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

Holographic Miroscopy: Results

1 angle

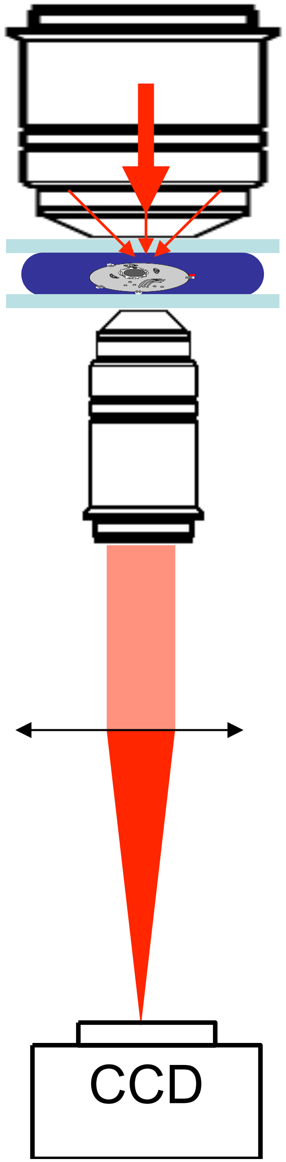


Limited 3-D resolution

Profilometry

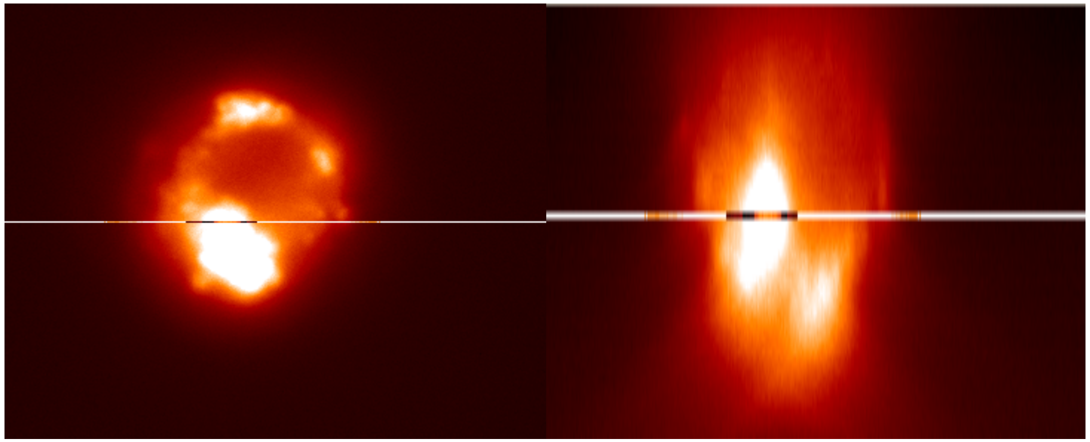
Integral measurements

Illumination Control: Consequences



A radical solution:

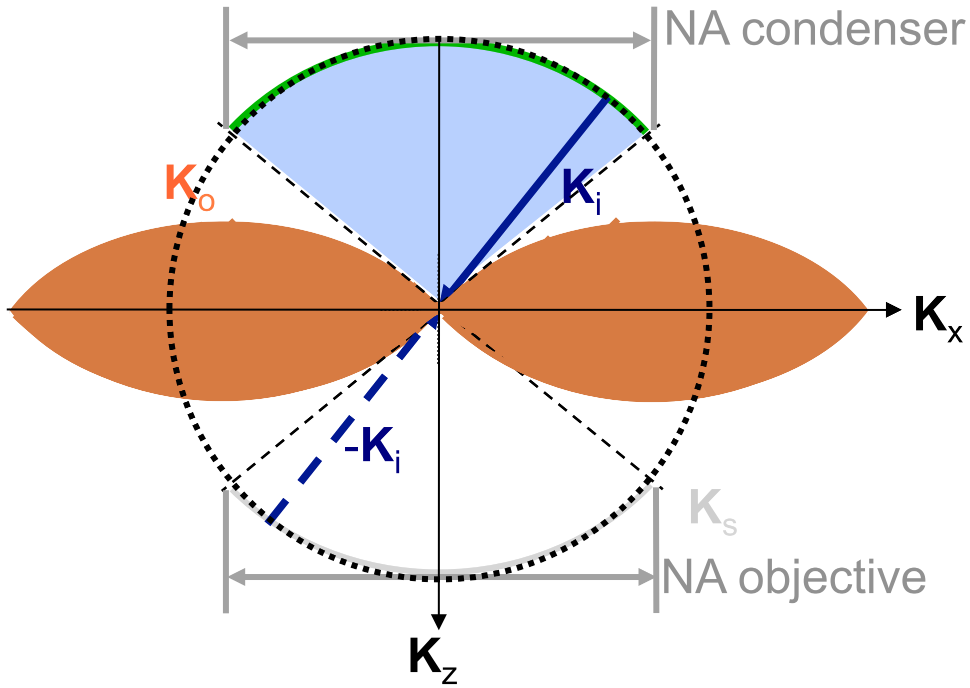
1 unique illumination direction!

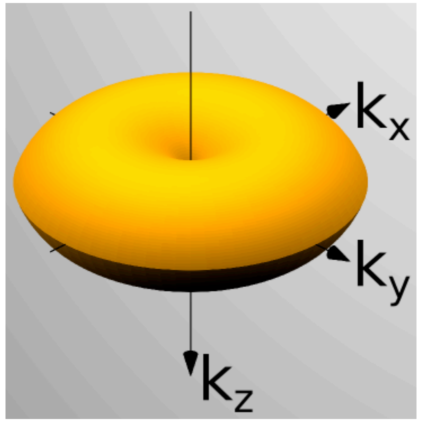


Cellule CD34 - Image Georges Jung, Laboratoire d'Hématologie
Centre Hospitalier Régional Emile Muller - Mulhouse

$$R_{trans} = R_{fluo} = \frac{\lambda}{(NA_{cond} + NA_{obj})} = \frac{\lambda}{2NA}$$

Tomography by Illumination Rotation



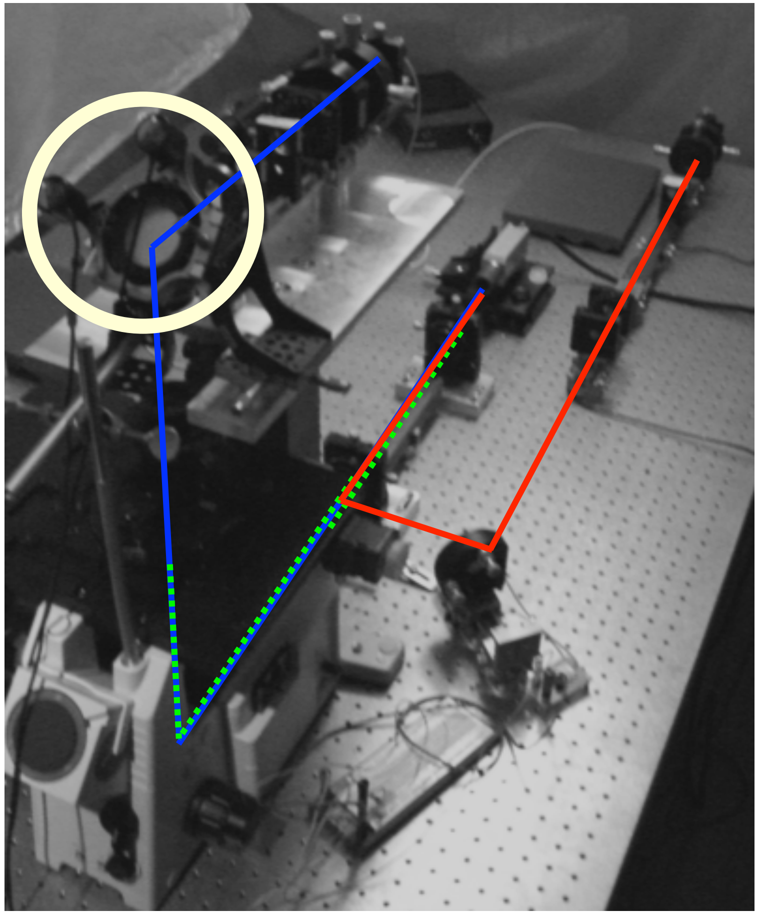


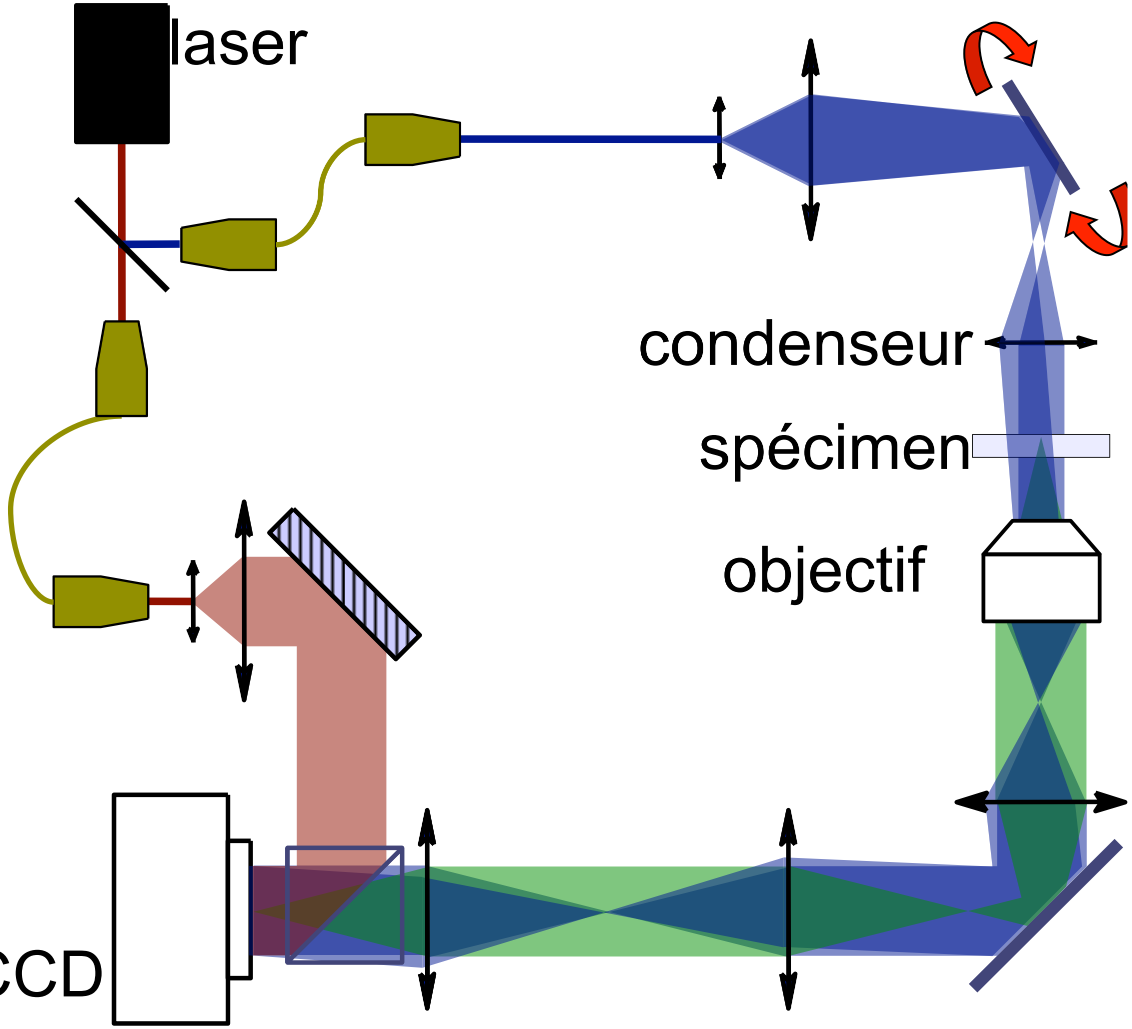
- Different angles of illumination ⇨ other object frequencies
- Large number of angles

⇨ Extended and filled frequency support

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

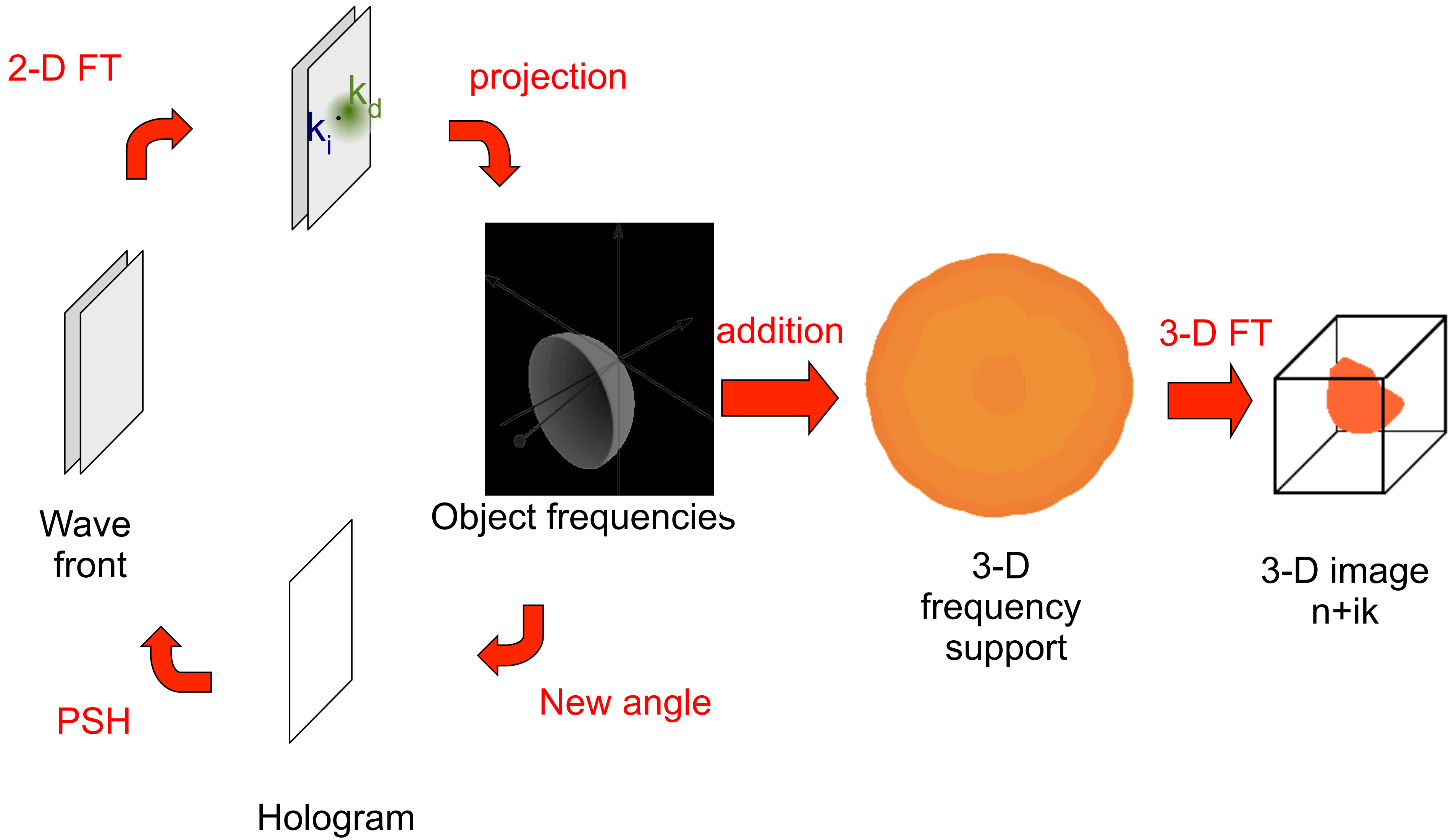
Tomographic Microscopy: Transmission



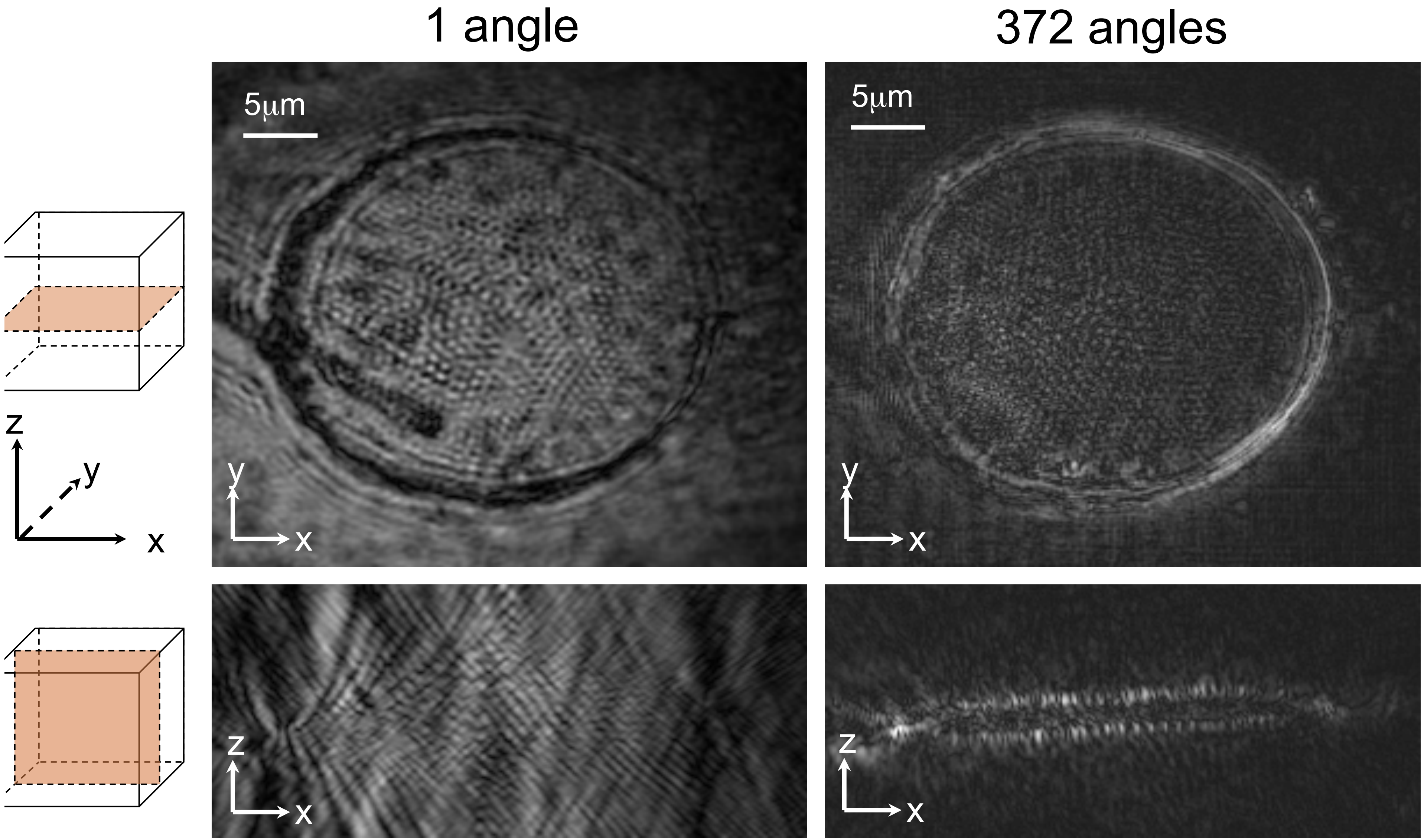


Holographic microscopy and diffractive microtomography of transparent samples, M. Debailleul, *et al.*, Meas. Sci. Technol. **19**, 074009 (2008)

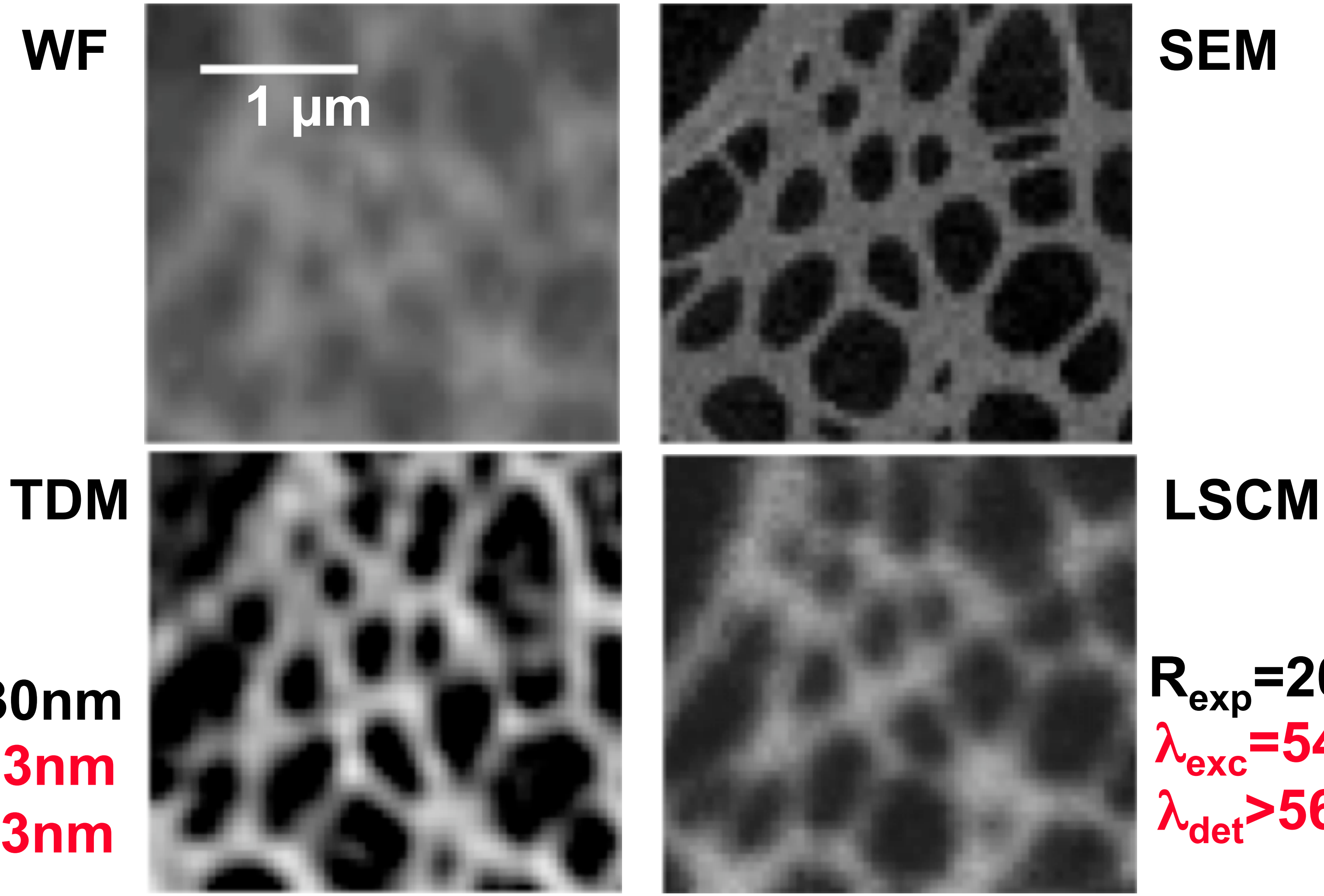
Object Reconstruction



Holography / Tomography



Carbon Mesh



R_{exp}=130nm
R_{the}=113nm
λ_{det}=633nm

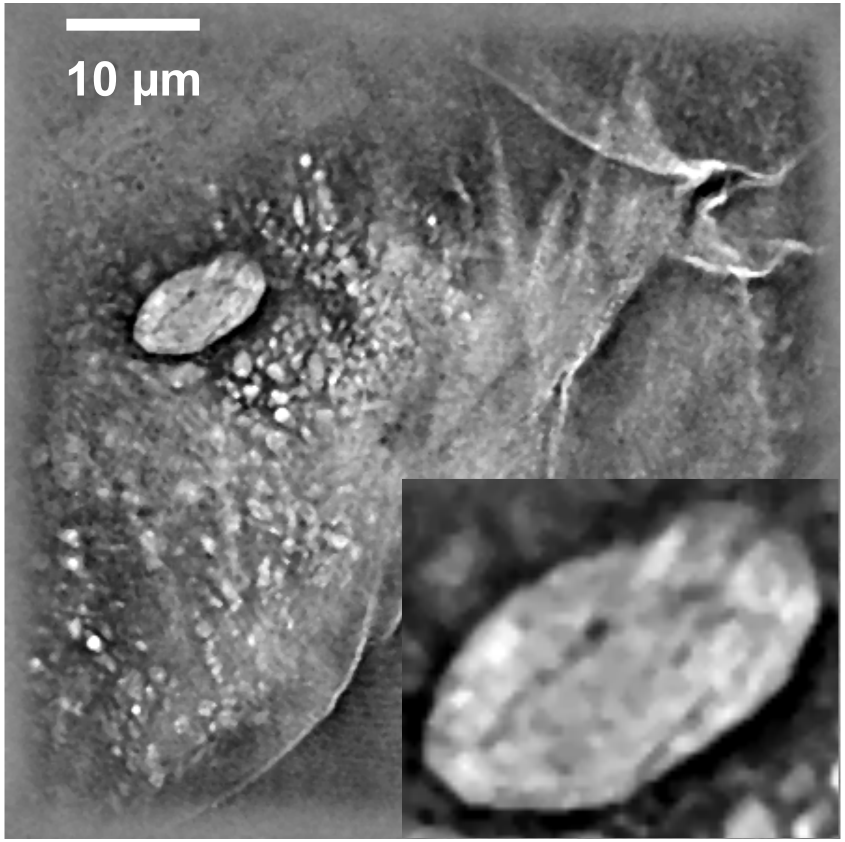
R_{exp}=200nm
λ_{exc}=543nm
λ_{det}>560nm

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

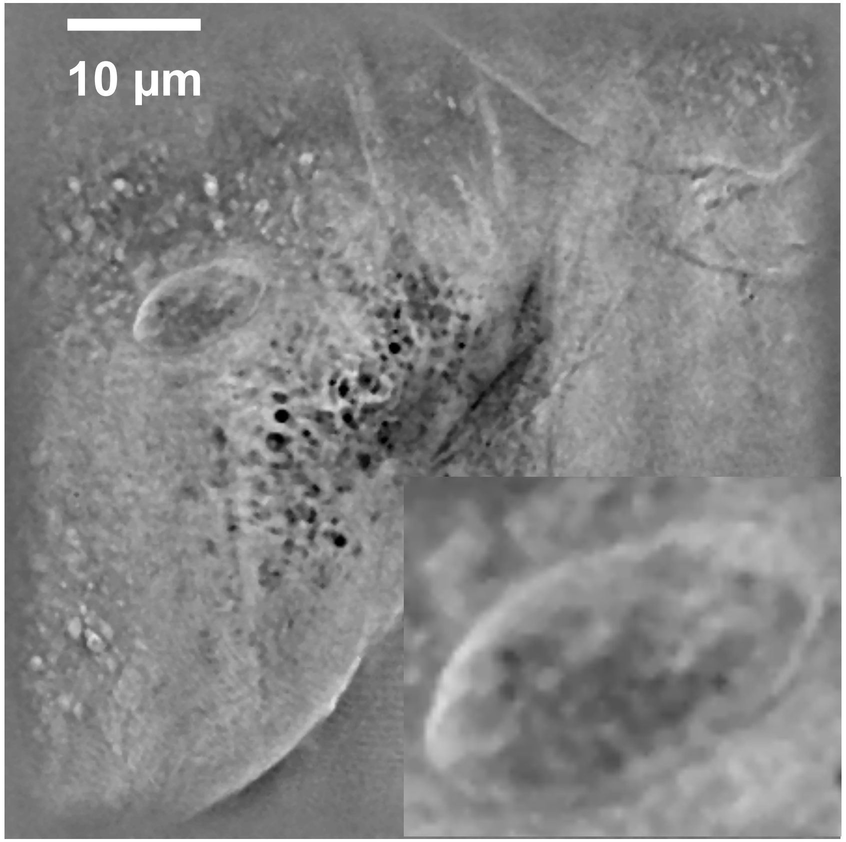
M. Debailleul, *et al.*, Opt. Lett. **34**, p. 79 (2009)

Tomography => Index of Refraction

Epithelial cells



Indice
Real part

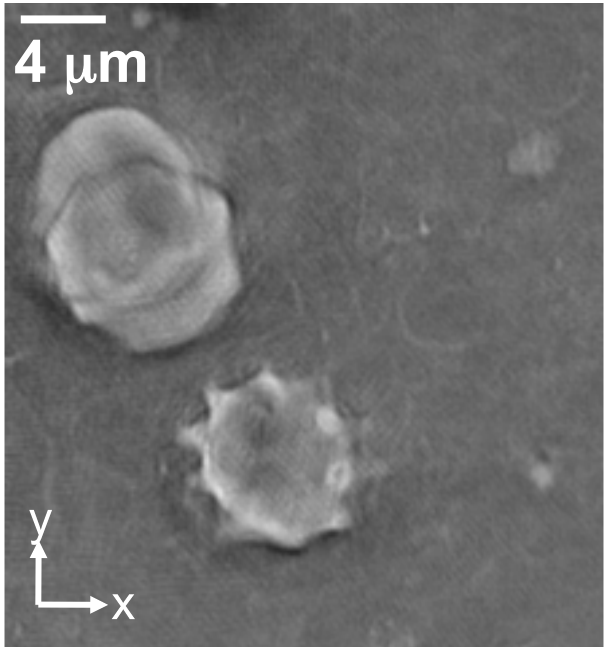


Indice
Imaginary part

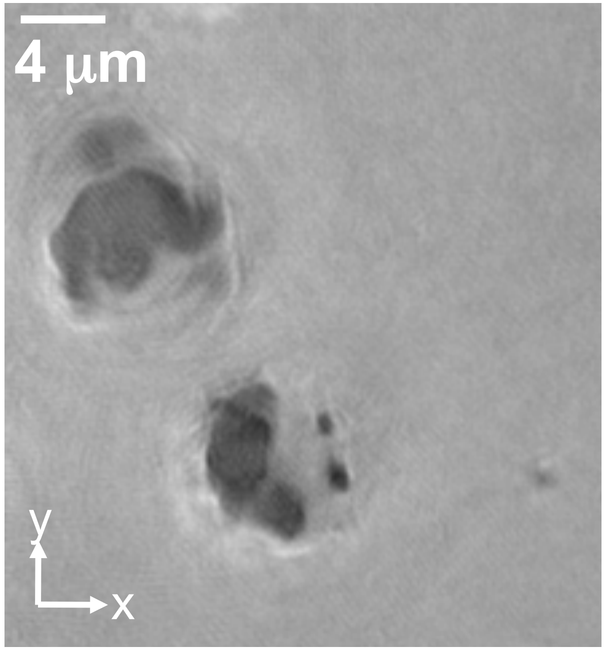
High resolution tomographic diffractive microscopy of biological samples
M. Sarmis, *et al.,* J. Biophotonics **3**, p. 462 (2010)

Tomography => Index of Refraction

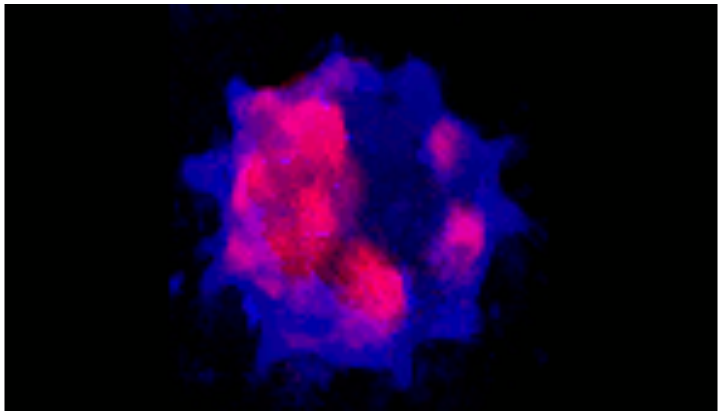
Granulocytes



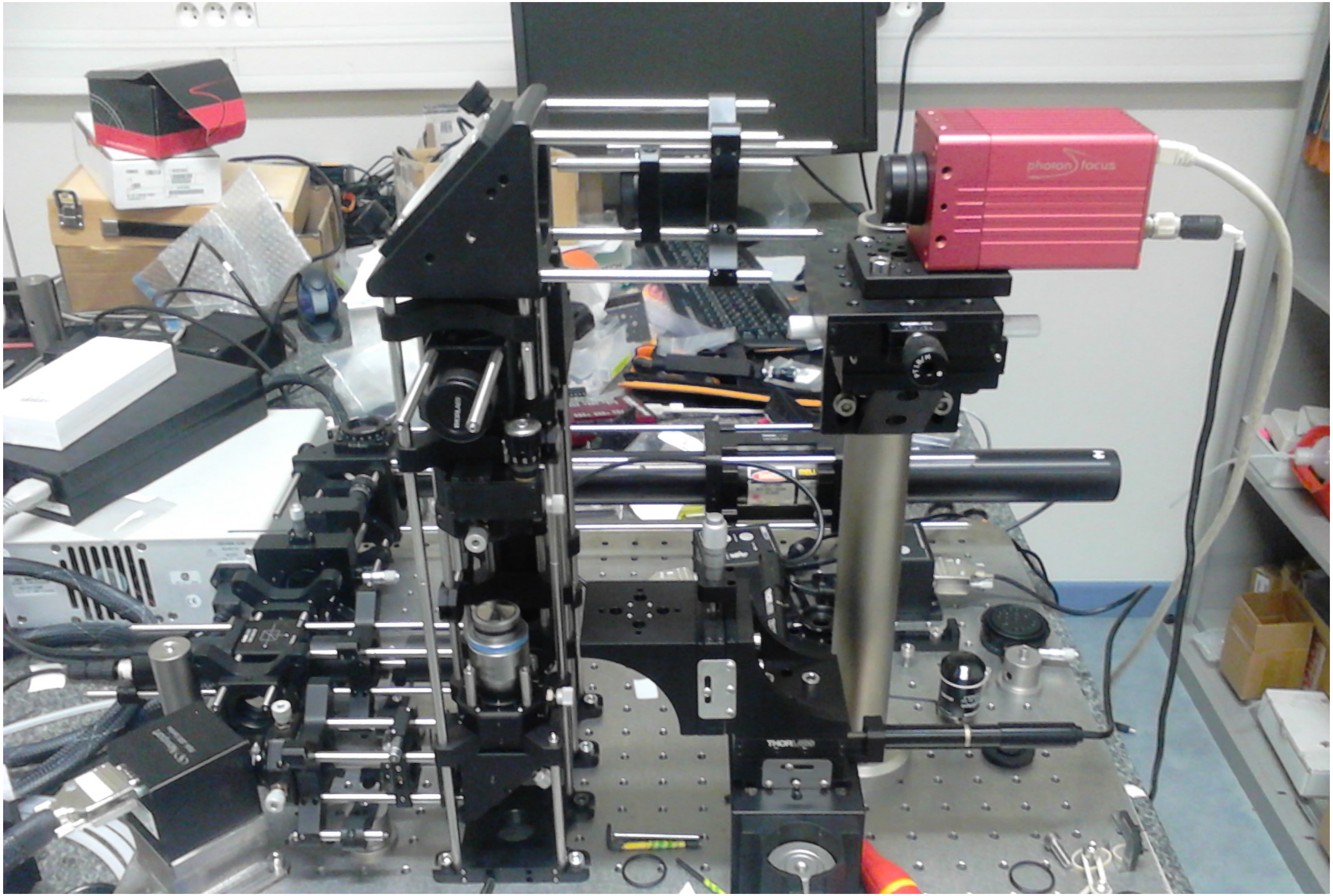
Refraction



Absorption



False colour rendering:
Red : absorption
Blue : refraction



Workshop MiFobio 2012 – 2014 – 2016

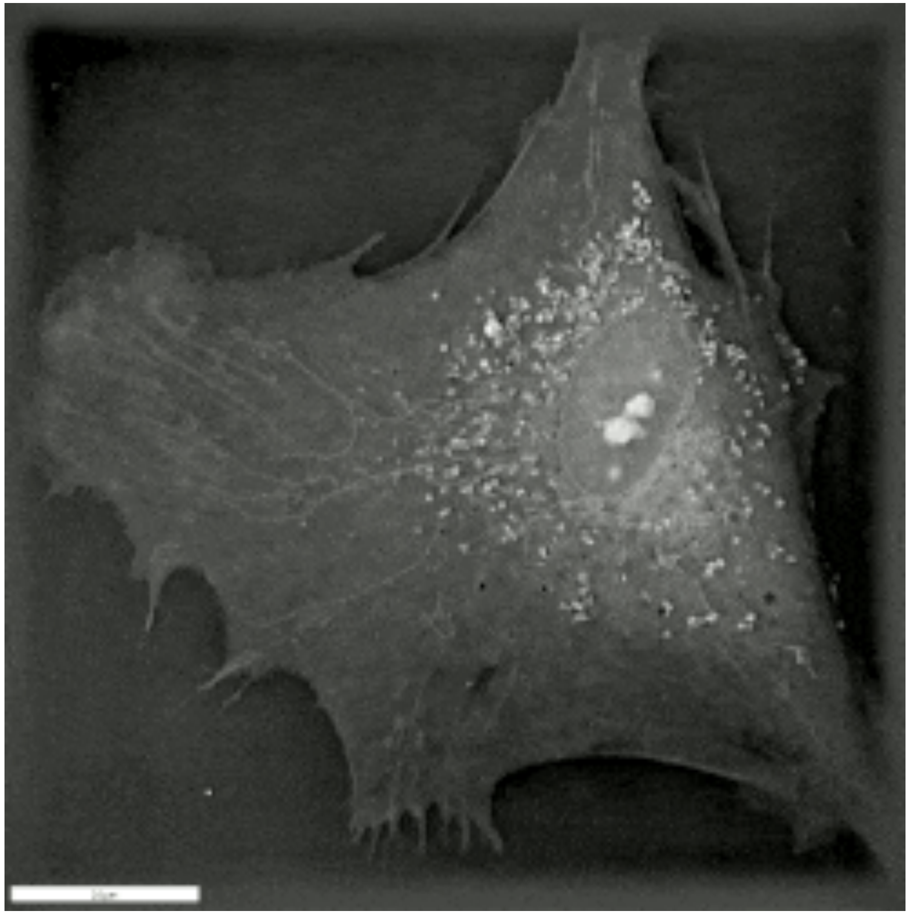
Commercially available!

NANOLIVE
Looking inside life

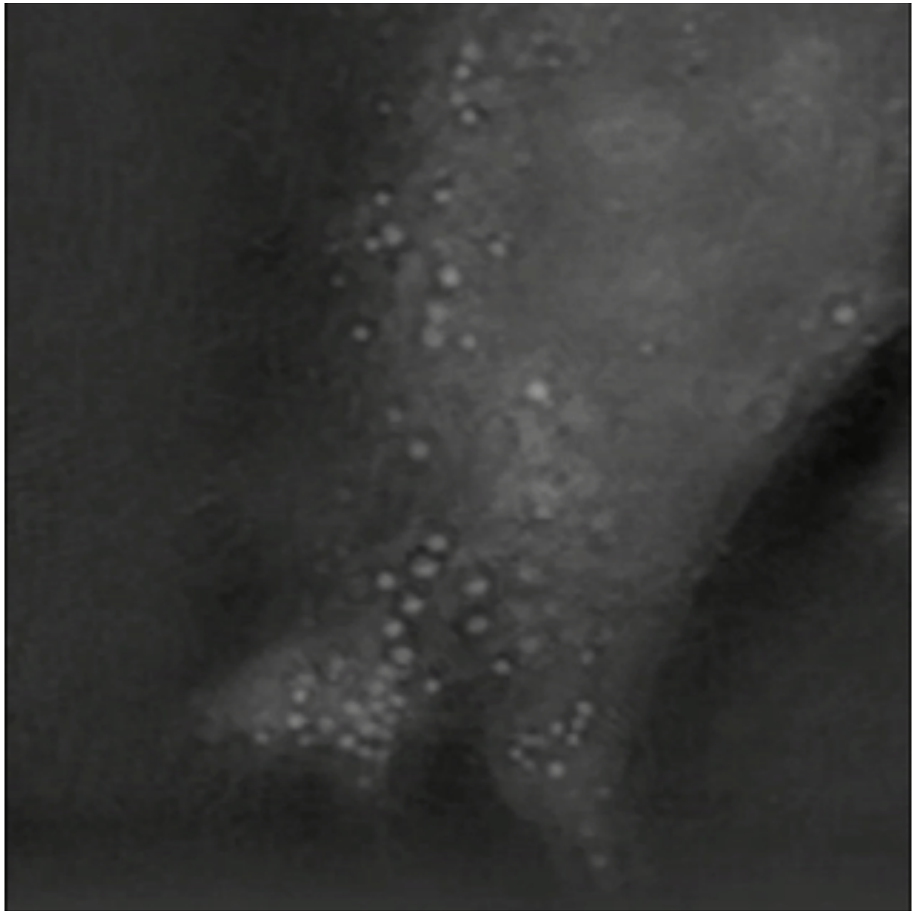
TOMOCUBE

See their website for interesting applications
Several active groups in the world (Korea, Poland, Taïwan, France, Germany, Italy…). Workshop MiFobio 2018

Applications http://nanolive.ch

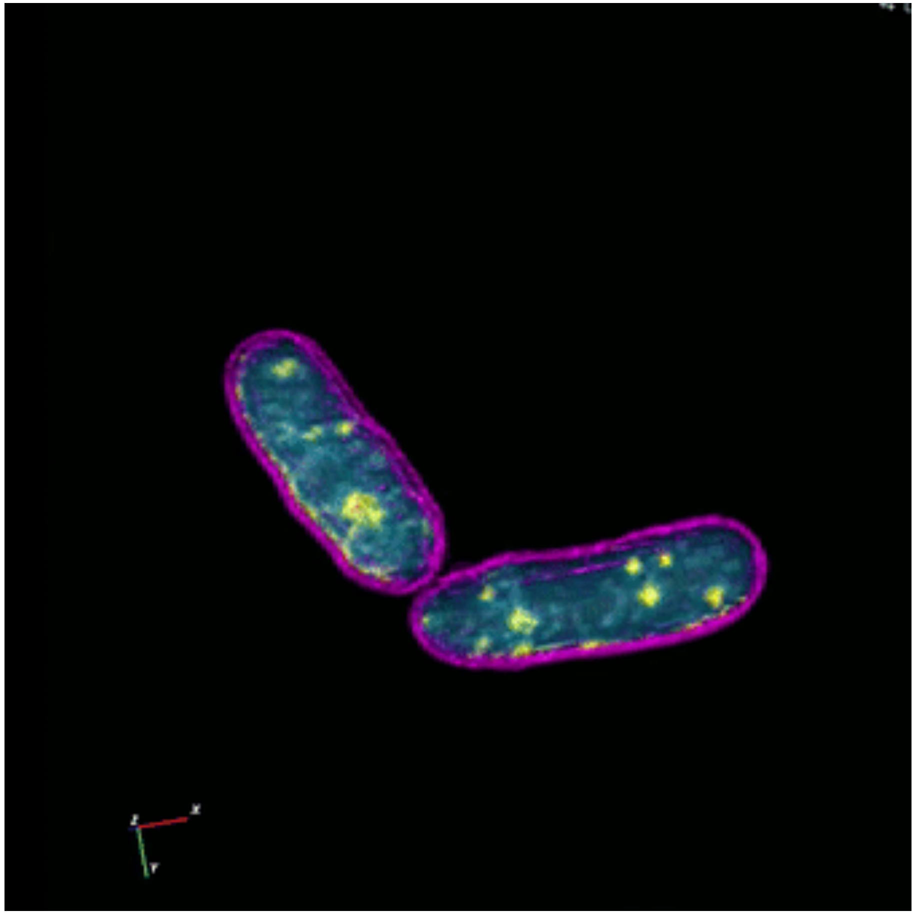


cellular morphological changes
induced by drug treatment

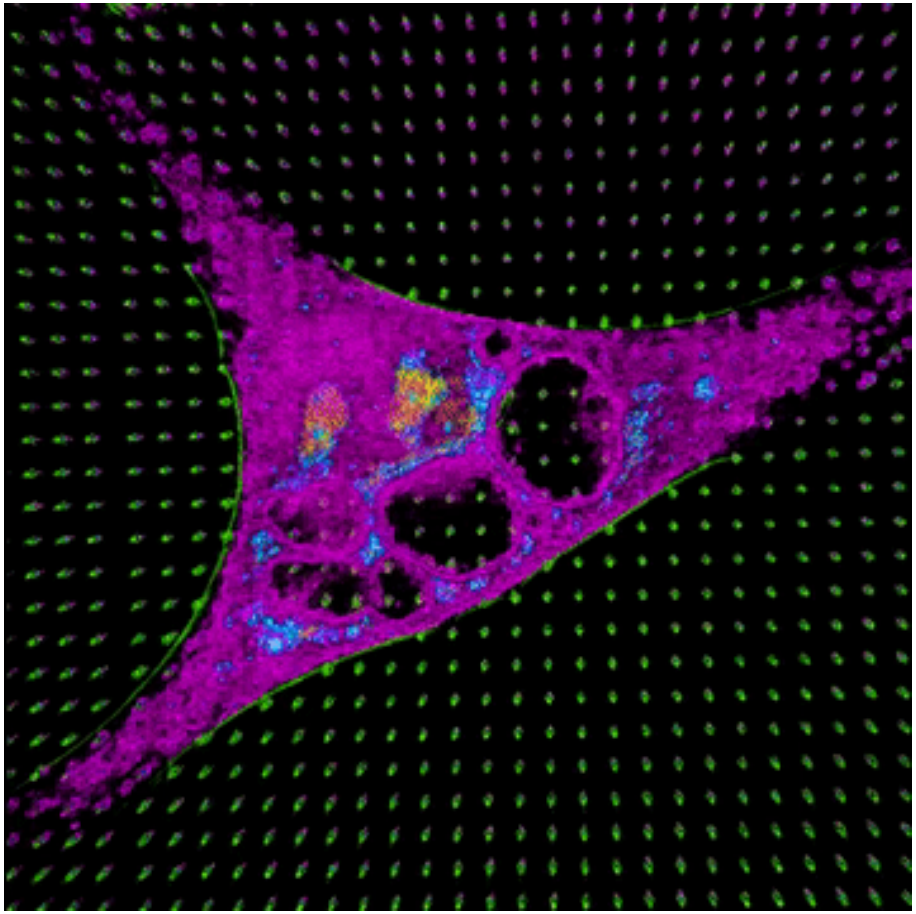


nanodiamonds internalization
& 3D distribution in living cells

Applications http://nanolive.ch



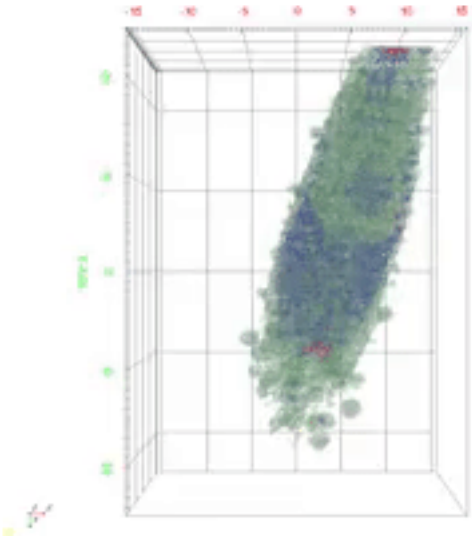
Fission yeast
(Schizosaccharomyces pombe)
during division

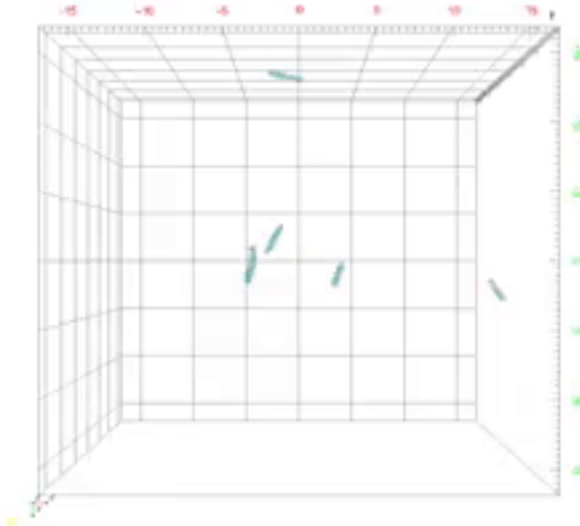


Fibroblast reticular cell seeded
on glass nanopillars

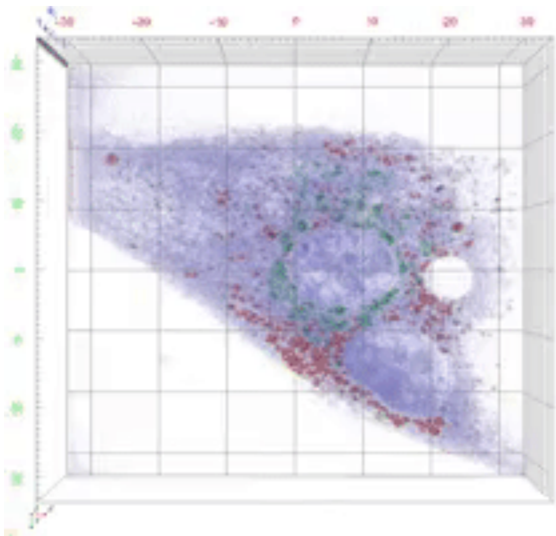
Applications http://www.tomocube.com

Cell apoptosis

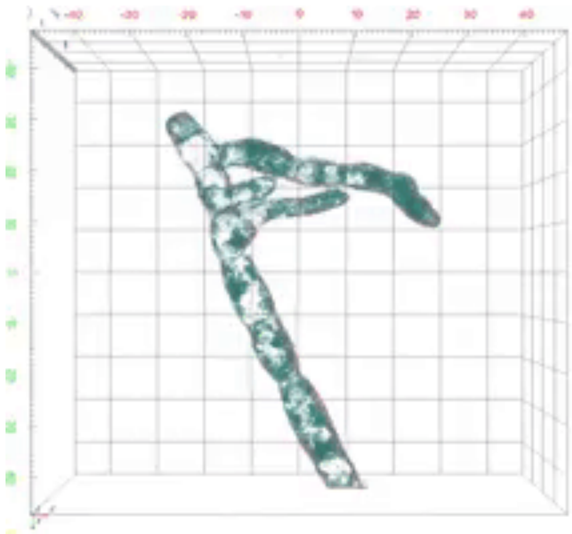




Bacterial growth

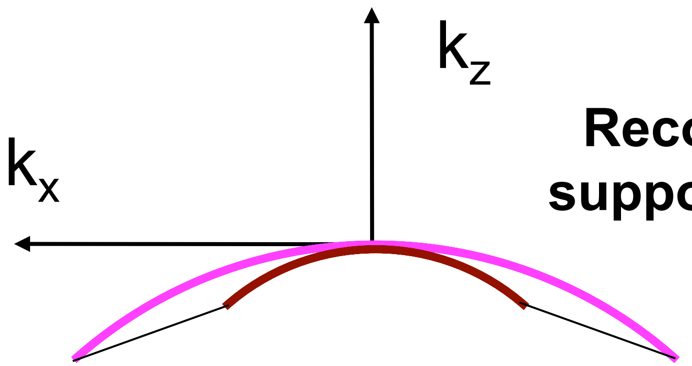


HeLa cell



Microalgae

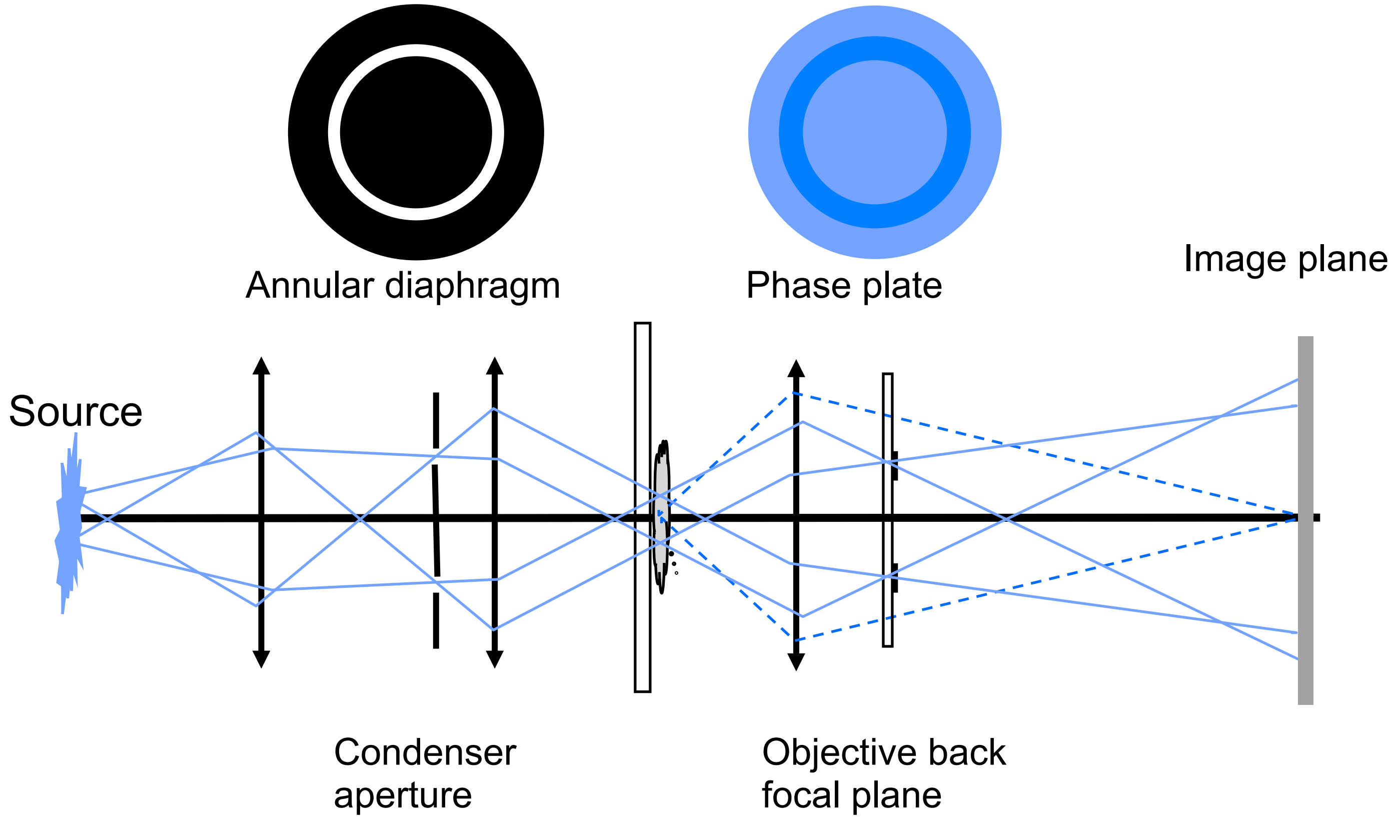
Tomography by Wavelength Variation

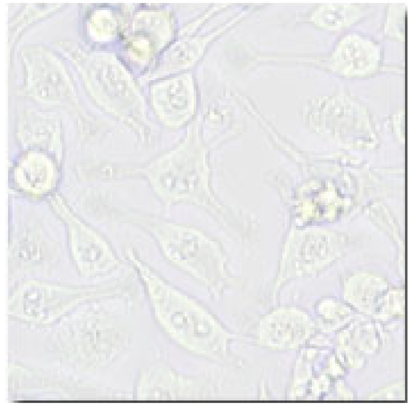


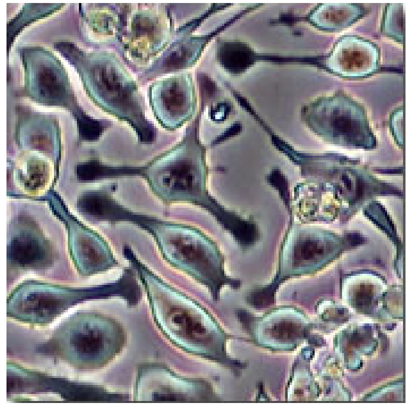
Recorded frequency support in transmission

- **No moving part**
- **Low gain in resolution**
- **Wide spectrum coherent sources ?**

Phase Contrast Microscopy



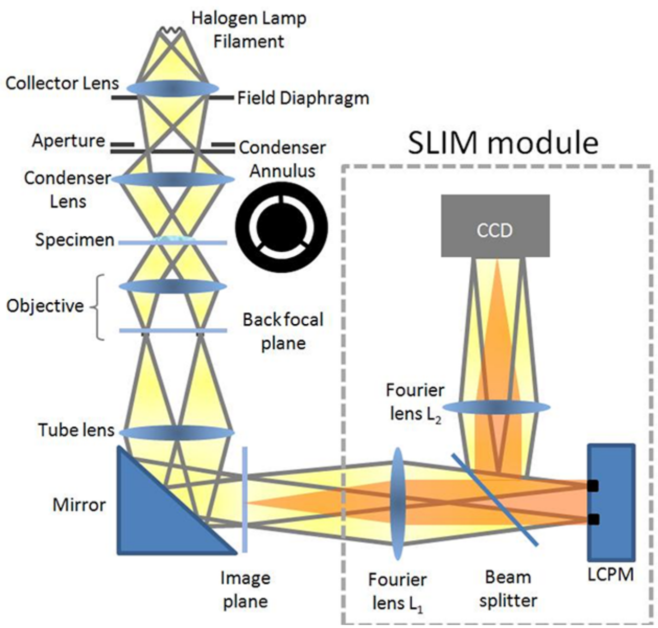




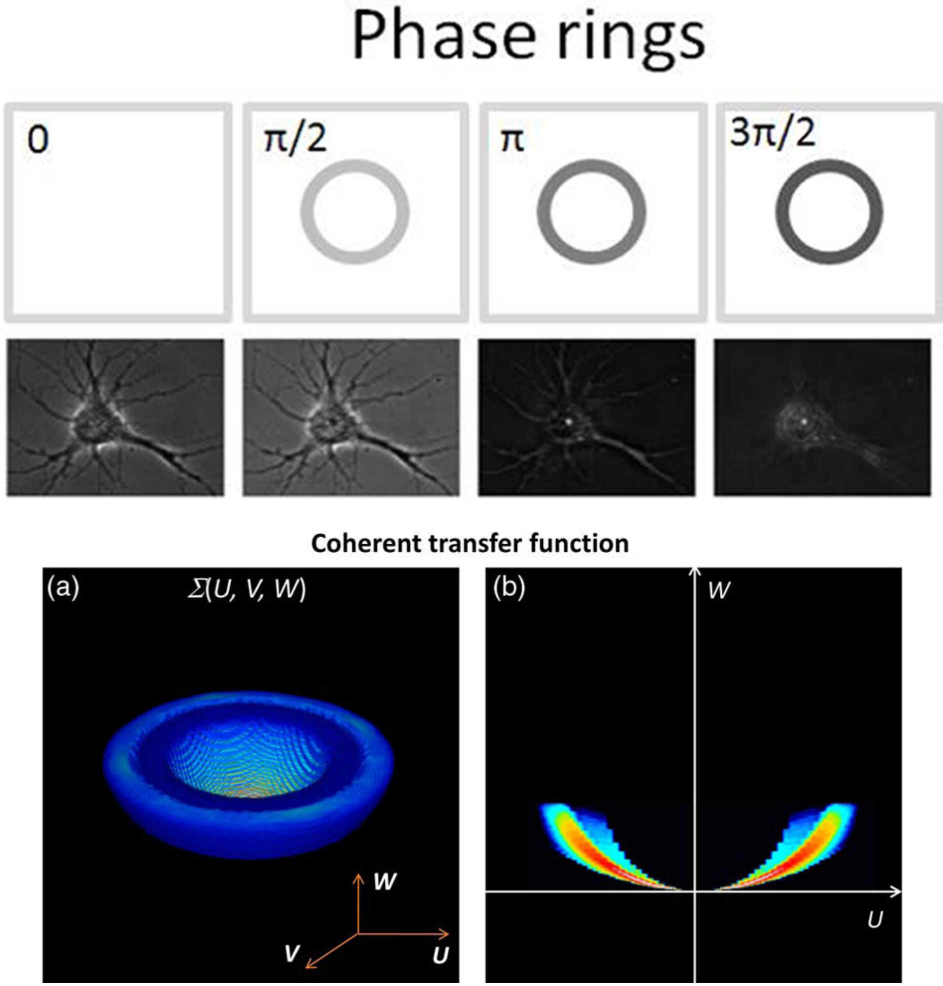
www.microscopyu.com

Nobel Prize 1953 Physics
Frits (Frederik) Zernike
"for his demonstration of the phase contrast method, especially for his invention of the phase contrast microscope".

White-light diffraction tomography

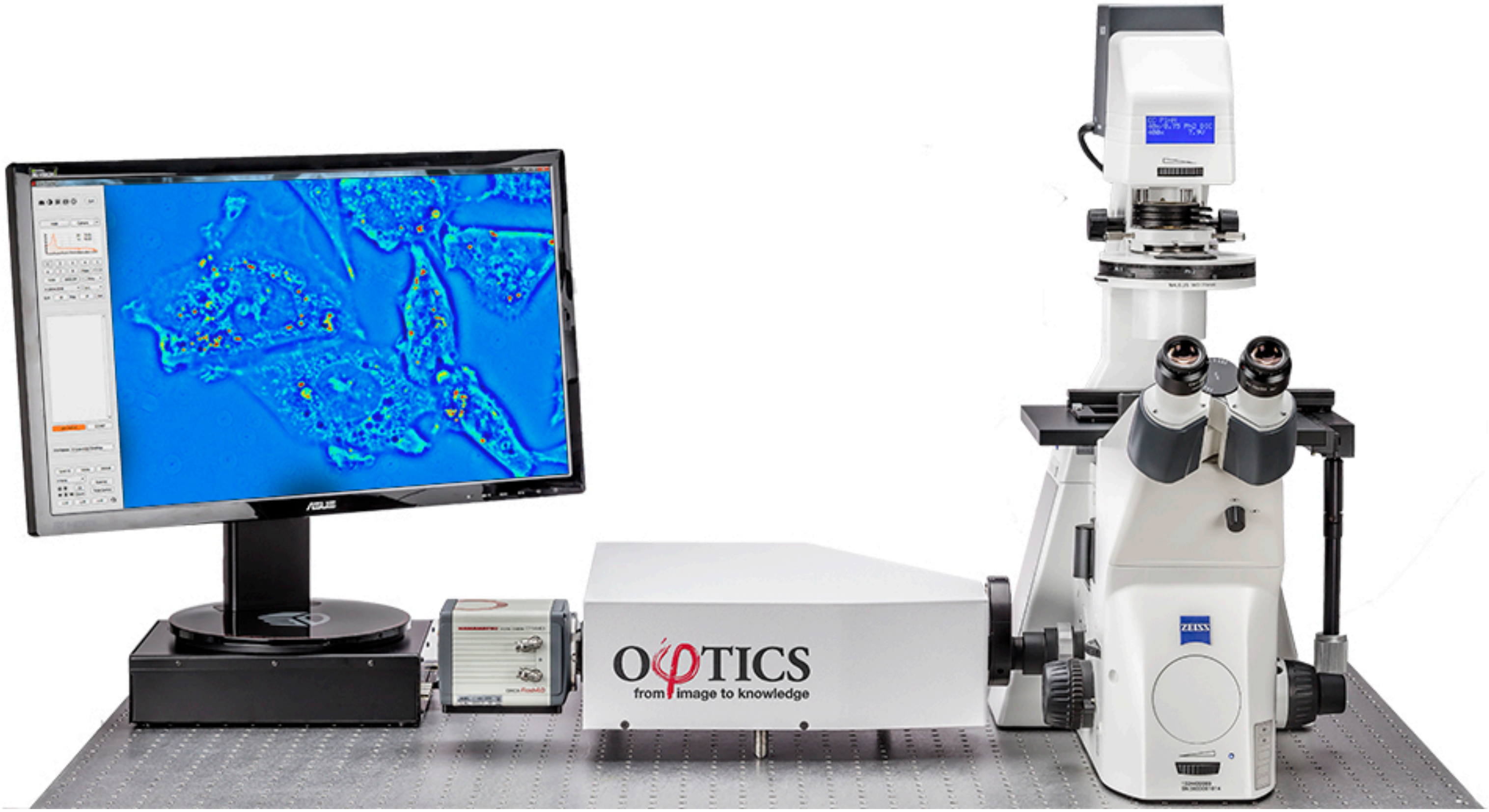


+ Z-scanning
+ data processing



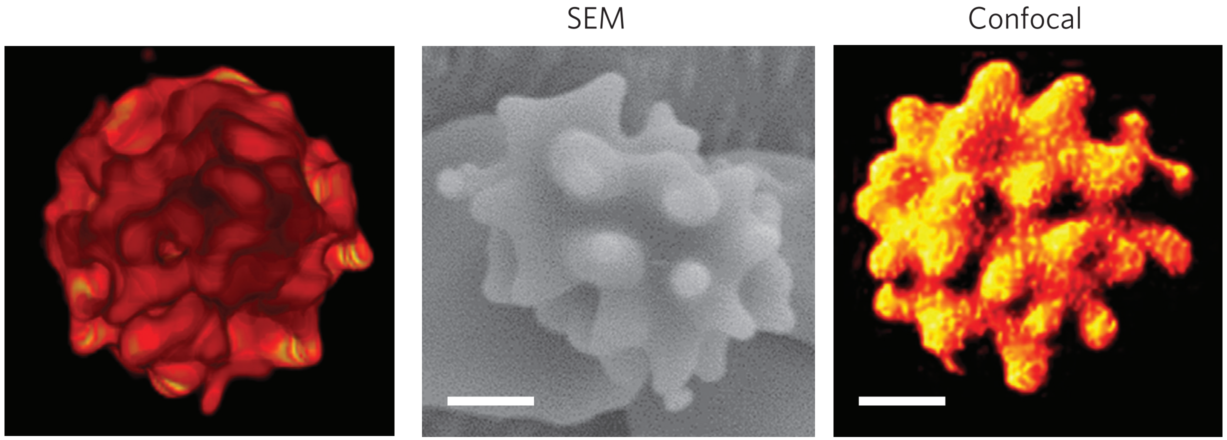
White-light diffraction tomography of unlabelled live cells
T. Kim, *et al.* Nature Photonics **8**, p. 256 (2014)

Commercially available!

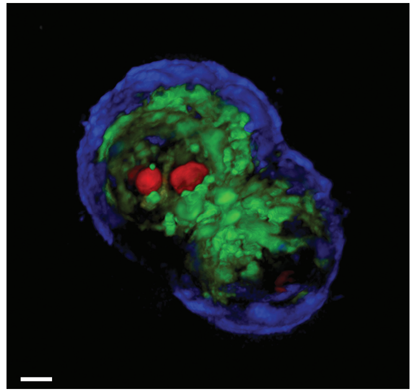


See their website for interesting applications

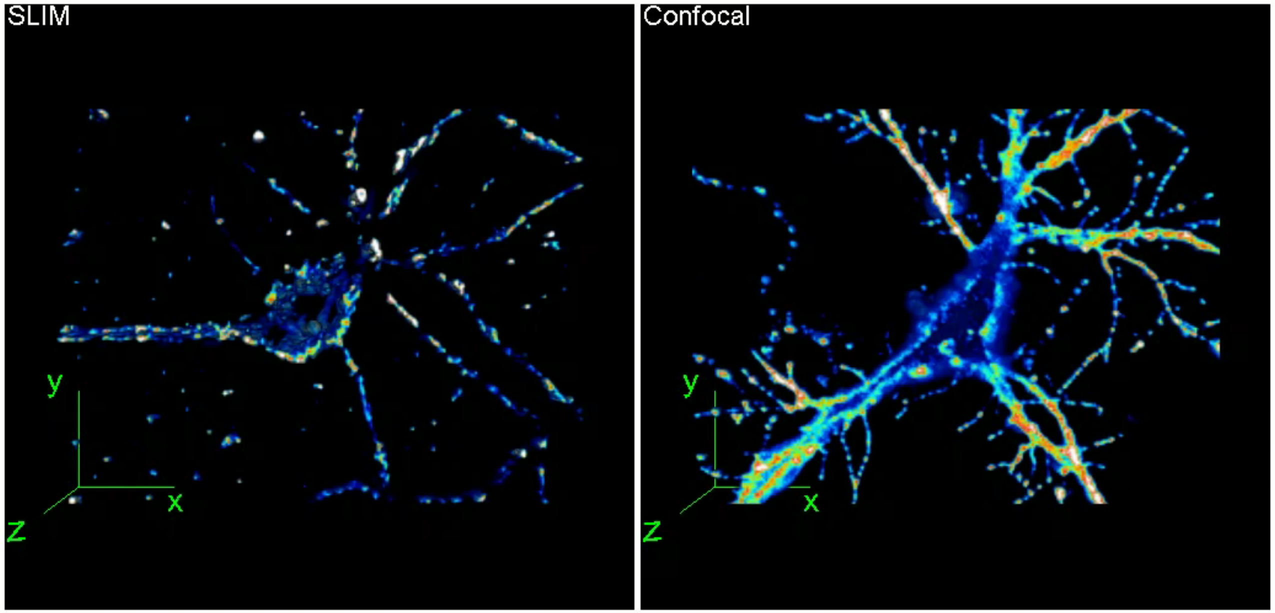
Applications http://phioptics.com



Spiculated RBC



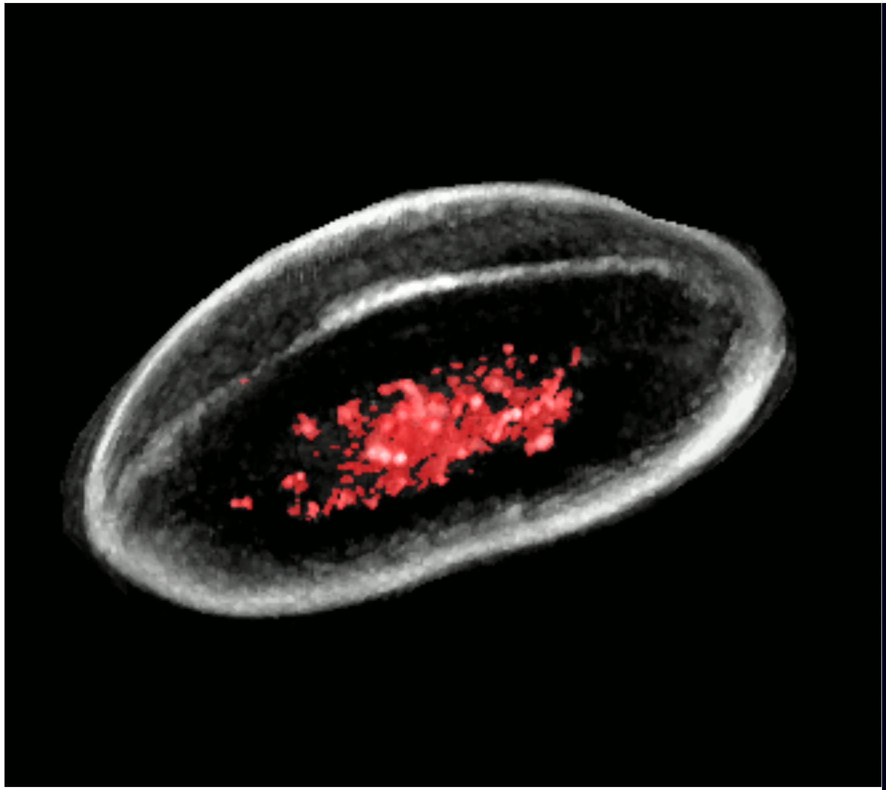
HT29 cell

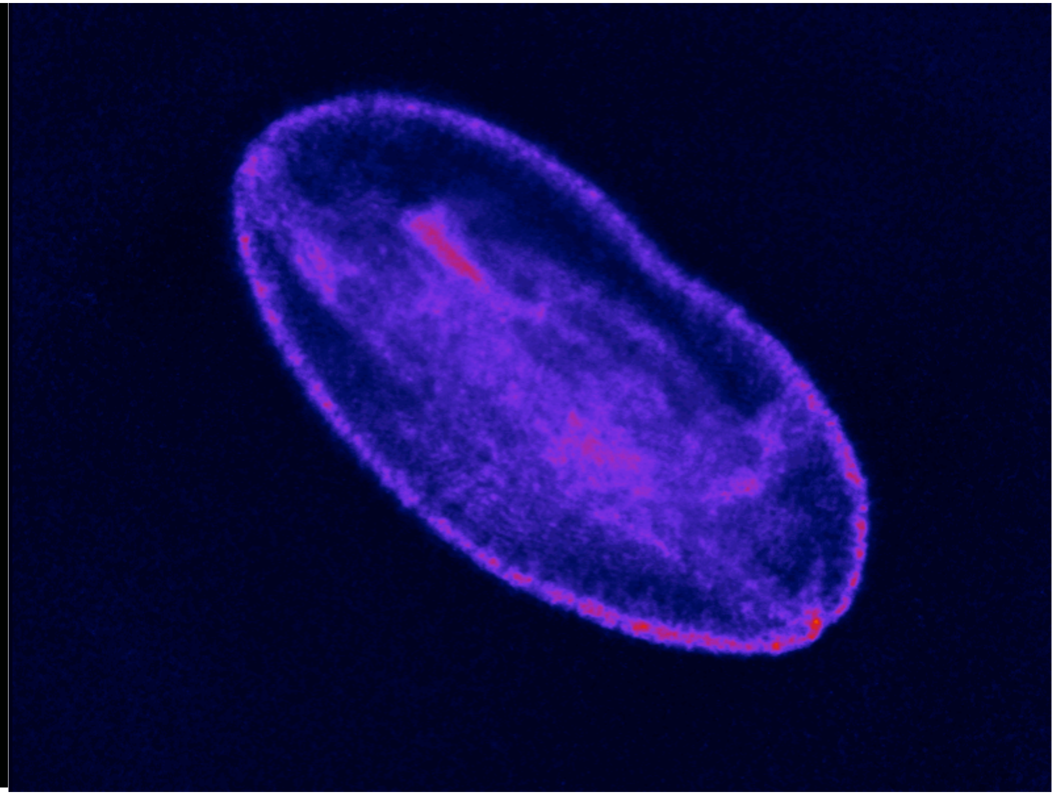


Live neuron

Tomography / Fluorescence Comparison

Snowdrop pollen



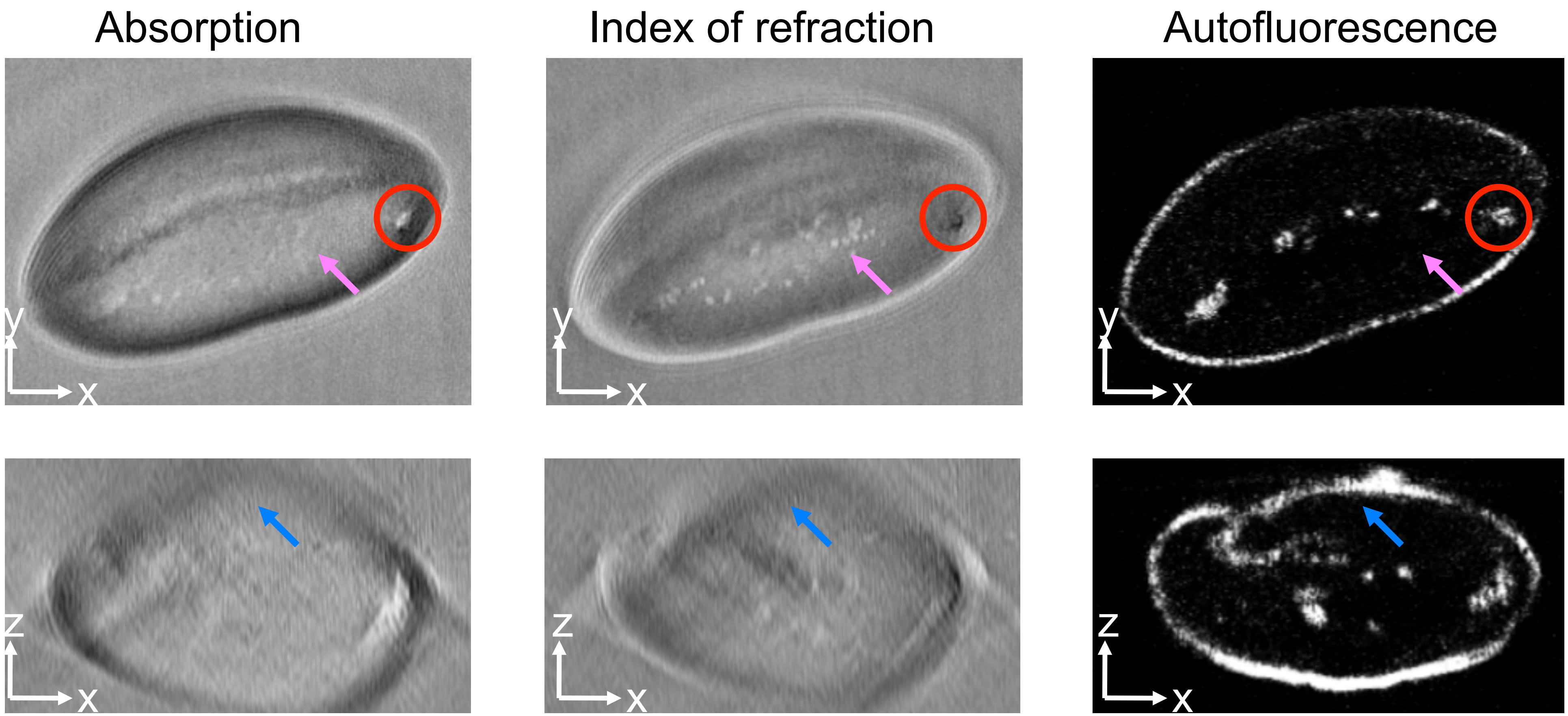


Tomography

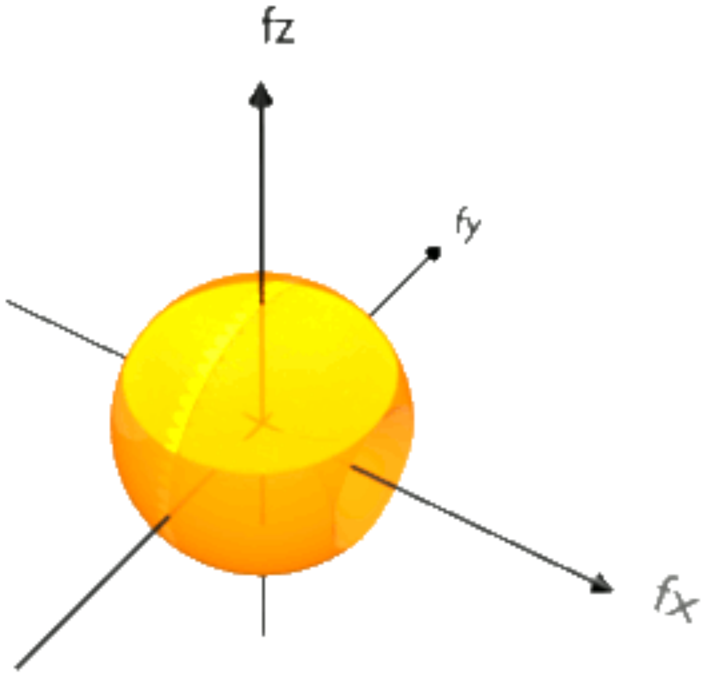
red : index n > index immersion medium

Fluorescence

Non-Isotropic Resolution



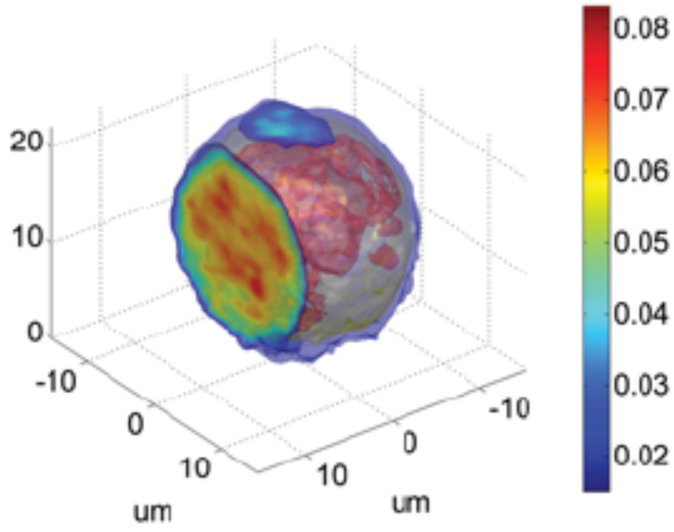
Tomography by Specimen Rotation



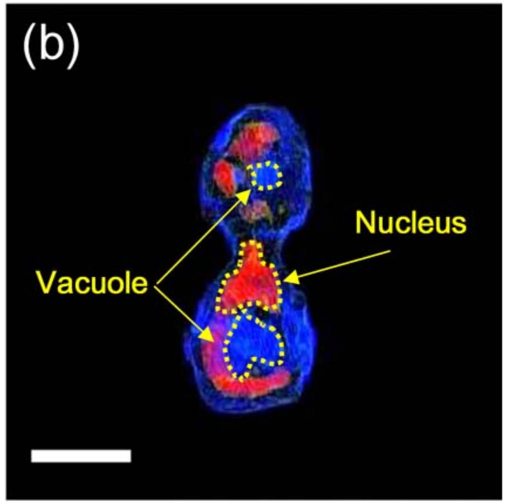
- Sample rotation may be difficult
- low NA => quasi-isotropic, but rather low resolution

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)

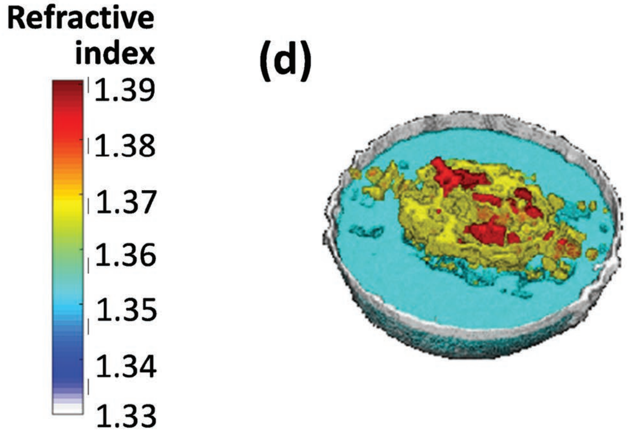


Tomographic phase microscopy with 180° rotation of live cells in suspension by holographic optical tweezers
M. Habaza, *et al.,* Opt. Lett. **40**, p. 1881 (2015)

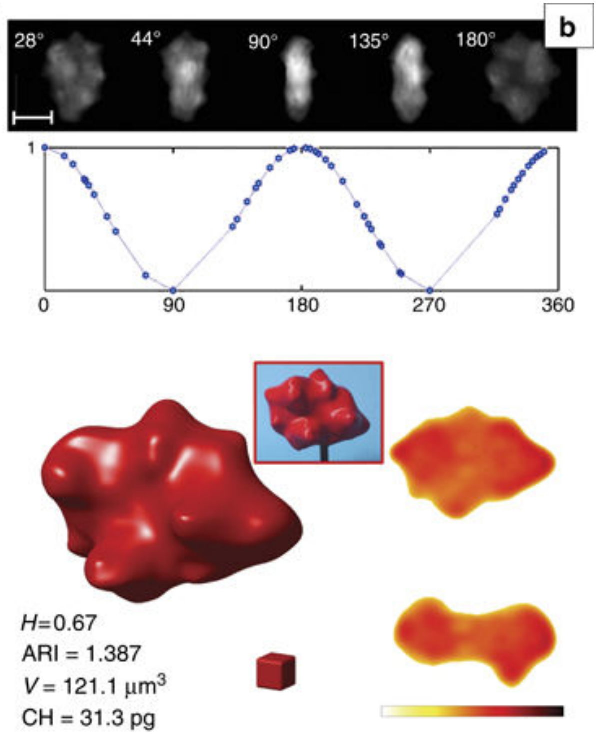


Tomography by Specimen Rotation

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)

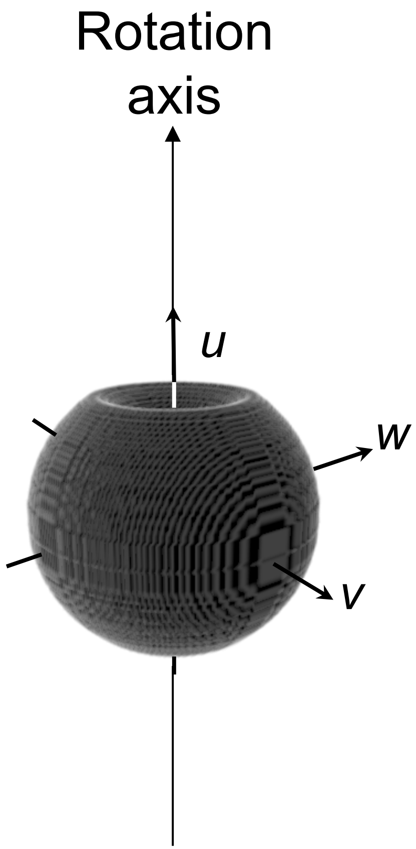


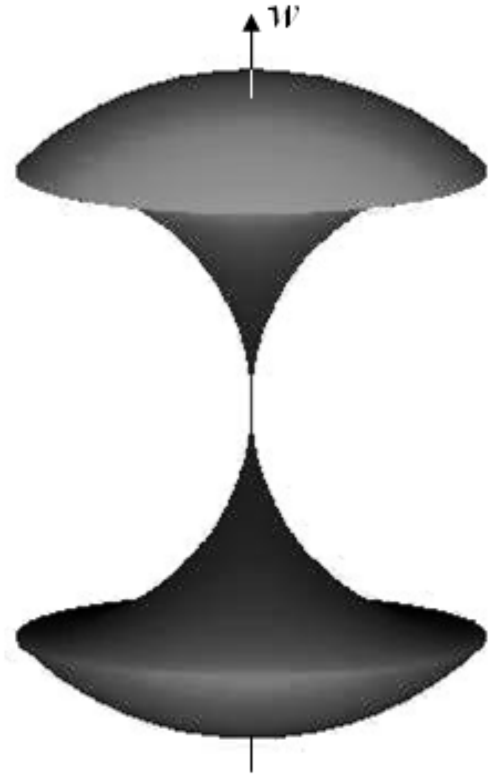
Tomographic flow cytometry by digital holography
F. Merola, *et al.*,
Light: Science & Applications **6**,
paper e16241 (2017)



Missing Frequencies

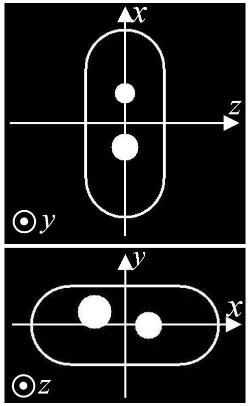
Recorded frequencies **Missing part** **"Missing apple core"**

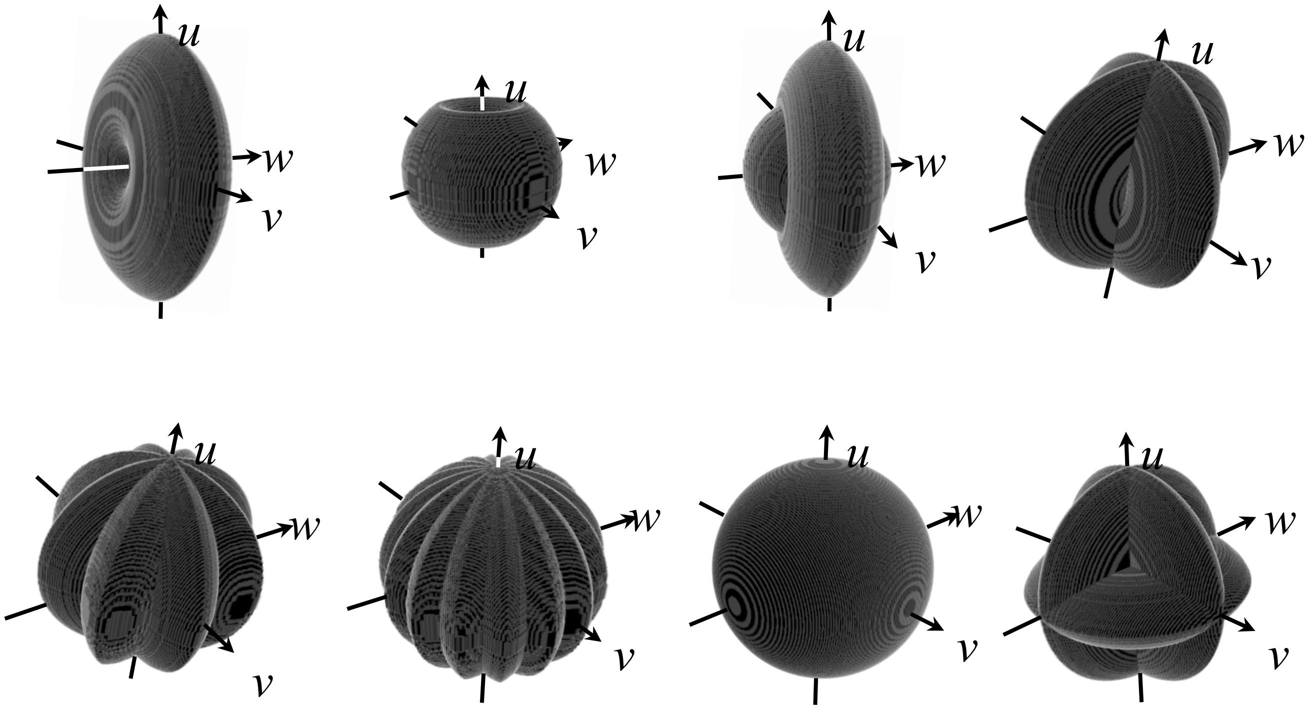


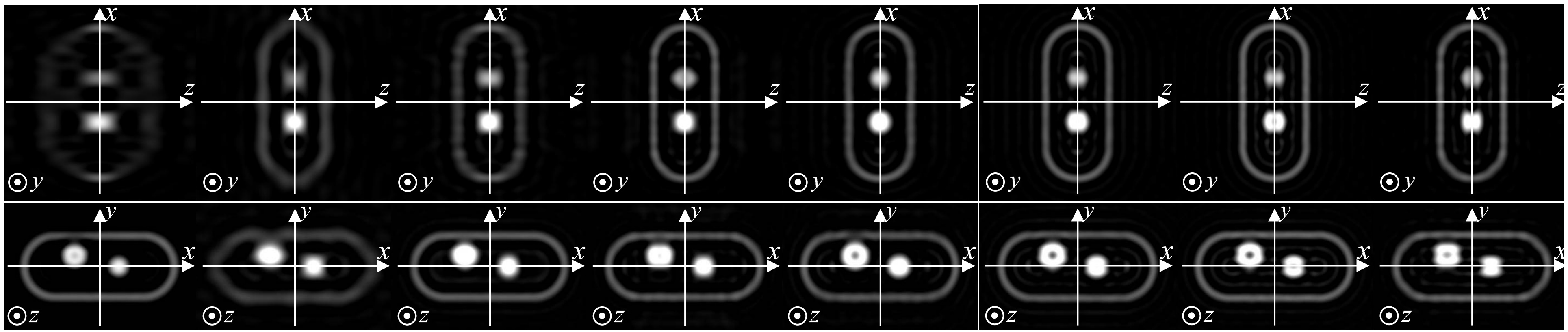


"Diffraction microtomography with sample rotation: influence of a missing apple core in the recorded frequency space"
S. Vertu, *et al.,* Centr. Eur. J. of Phys. **7**, p. 22 (2009)

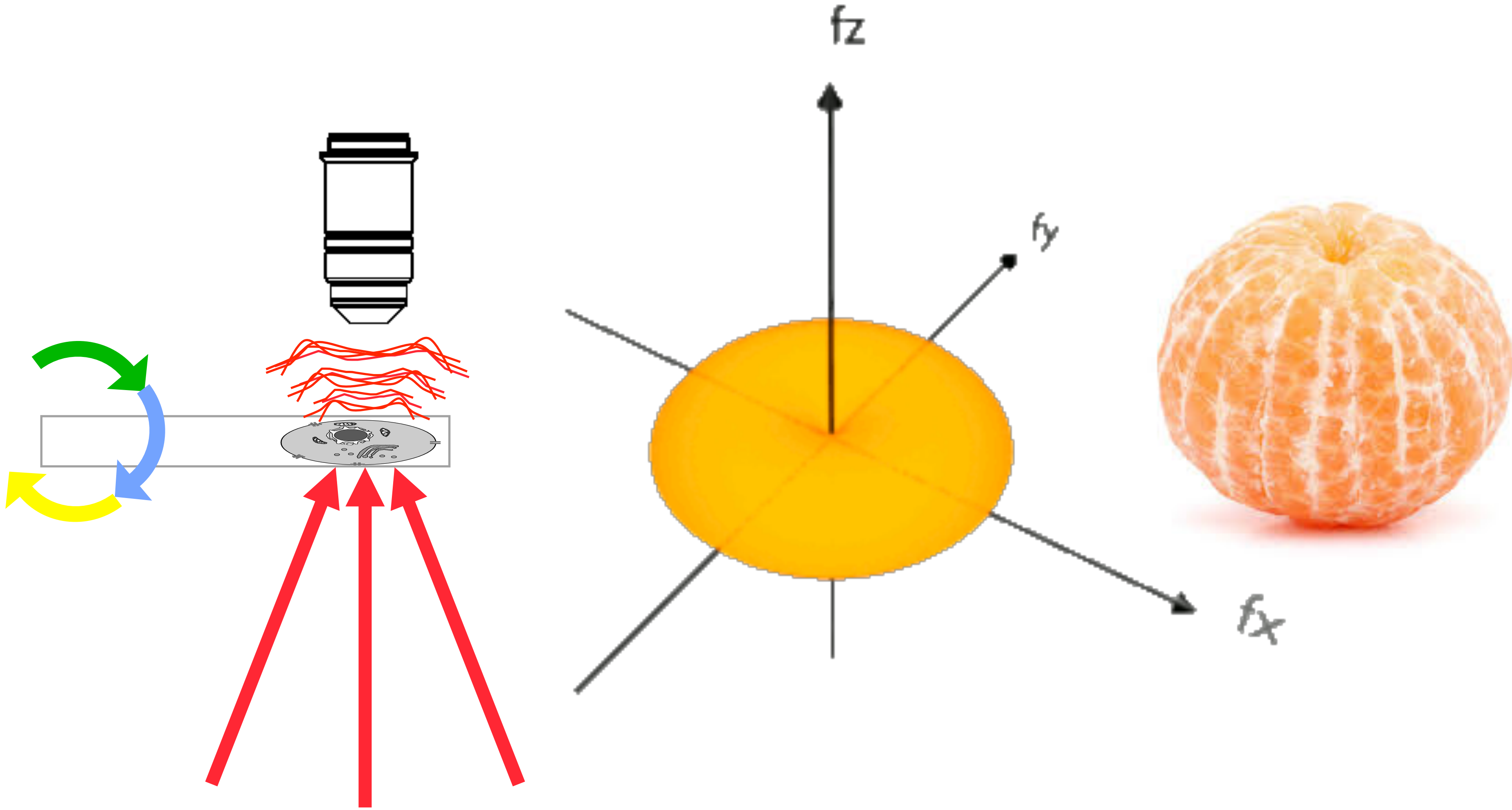
Multiview Tomography







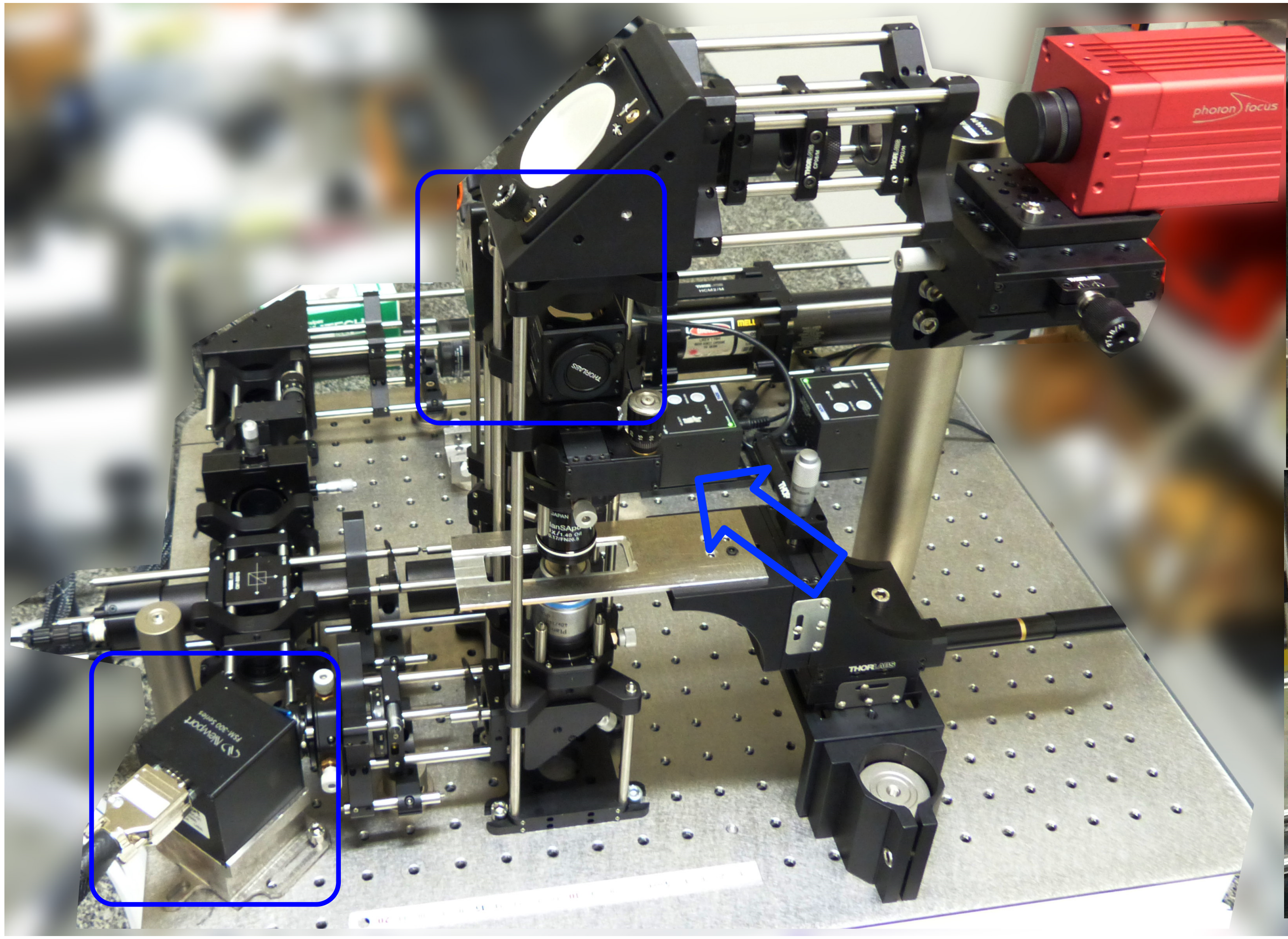
Multiview Tomography

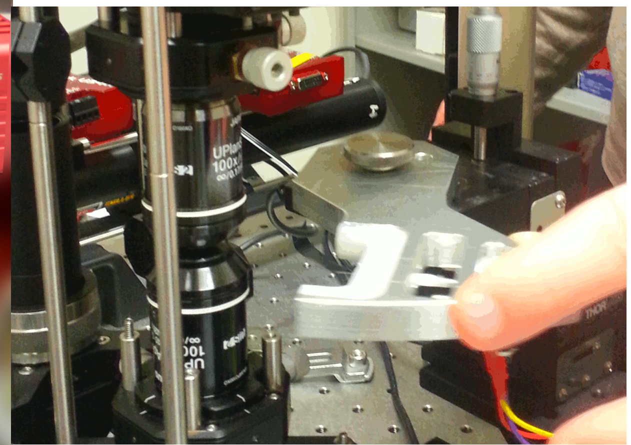


Improved and isotropic resolution in tomographic diffractive microscopy combining sample and illumination rotation
S. Vertu, *et al.*, Centr. Eur. J. of Phys. **9**, p. 969 (2011)

Towards High NA, IsoResolution

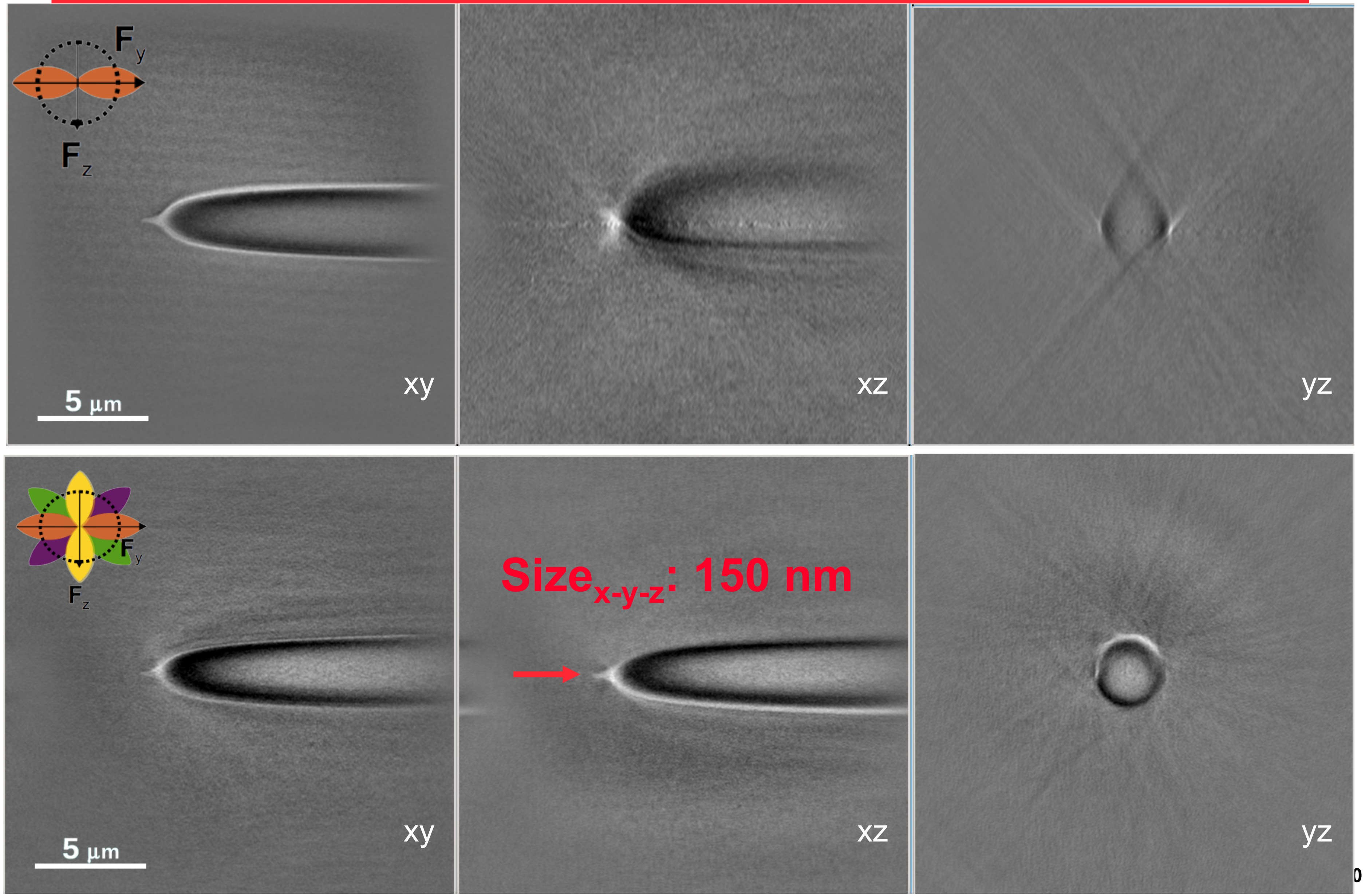
λ=633nm or 475nm, NA_{obj}=1.4, NA_{cond}=1.4



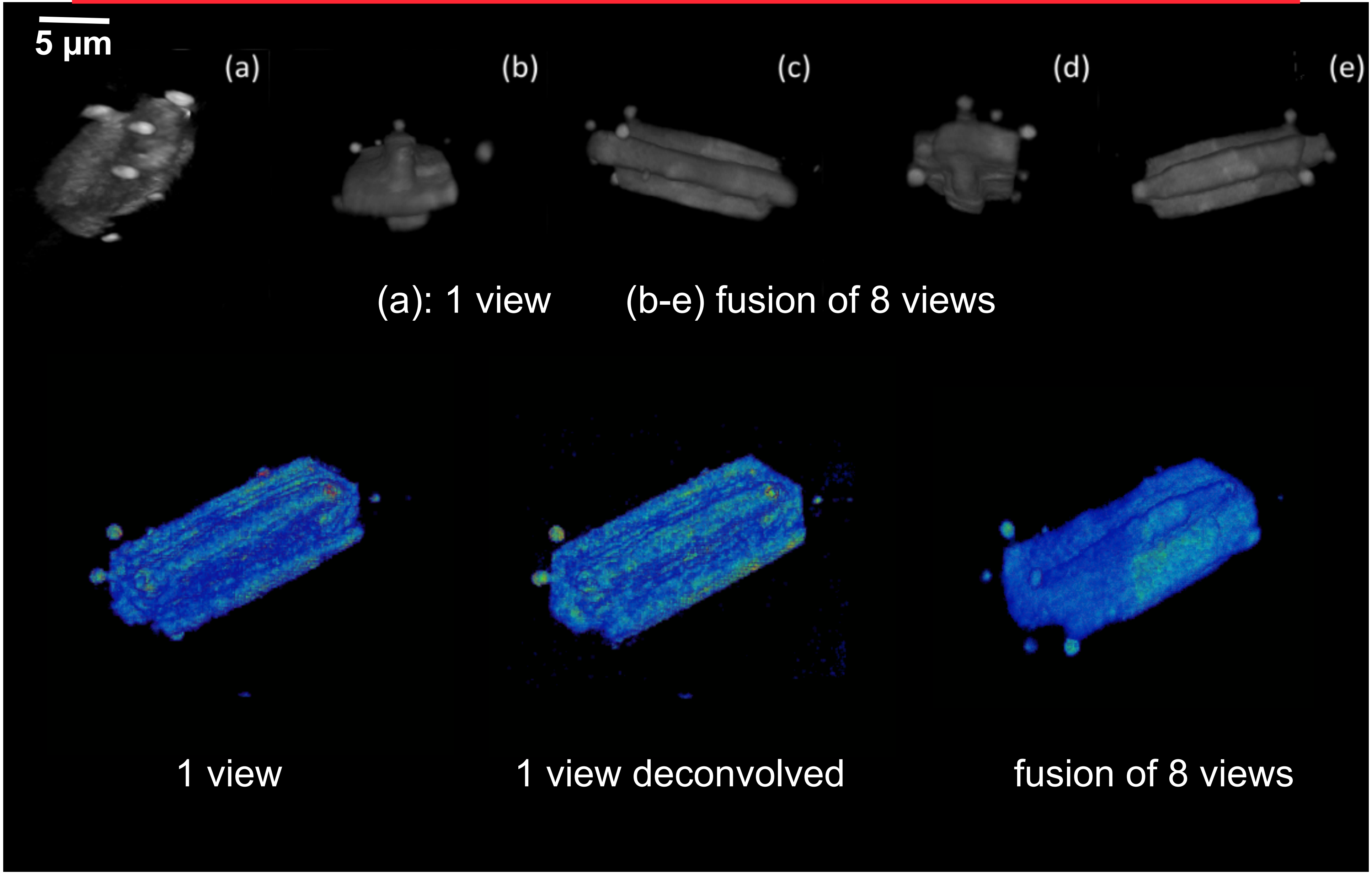


Fast acquisition (less than 10s for 1 object orientation / 400 illuminations)
Real-time reconstruction for each object orientation (1 volume of data each 3s)

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

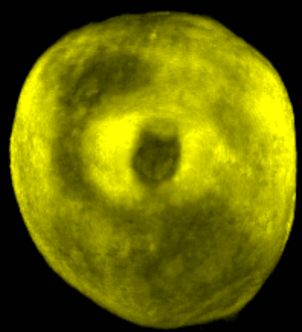


Zeolith microcrystal



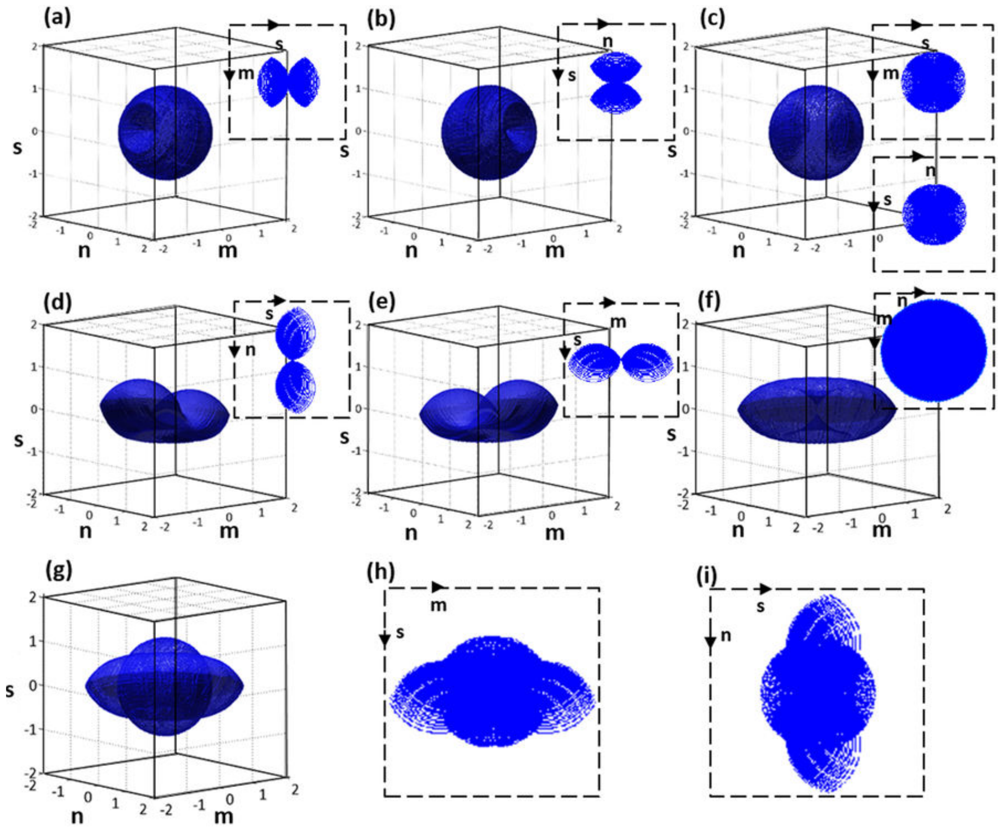
Betula Pollen

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

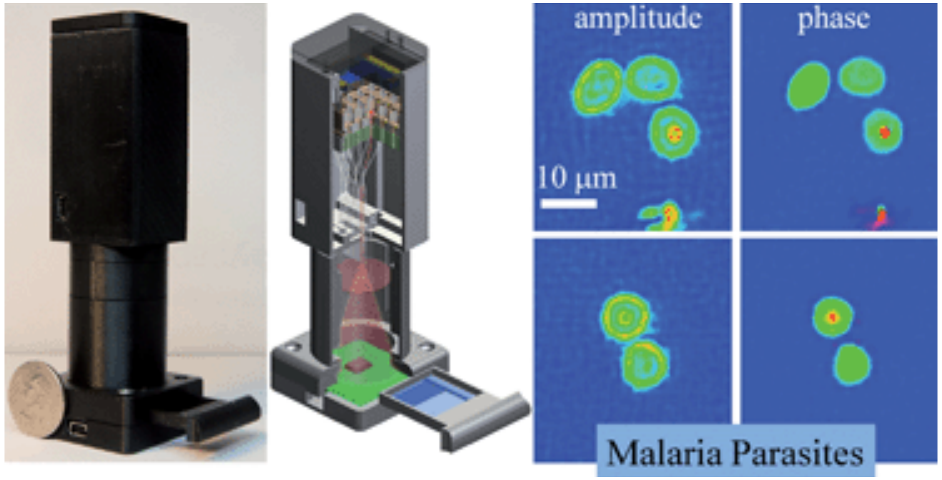


Betula Pollen

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)

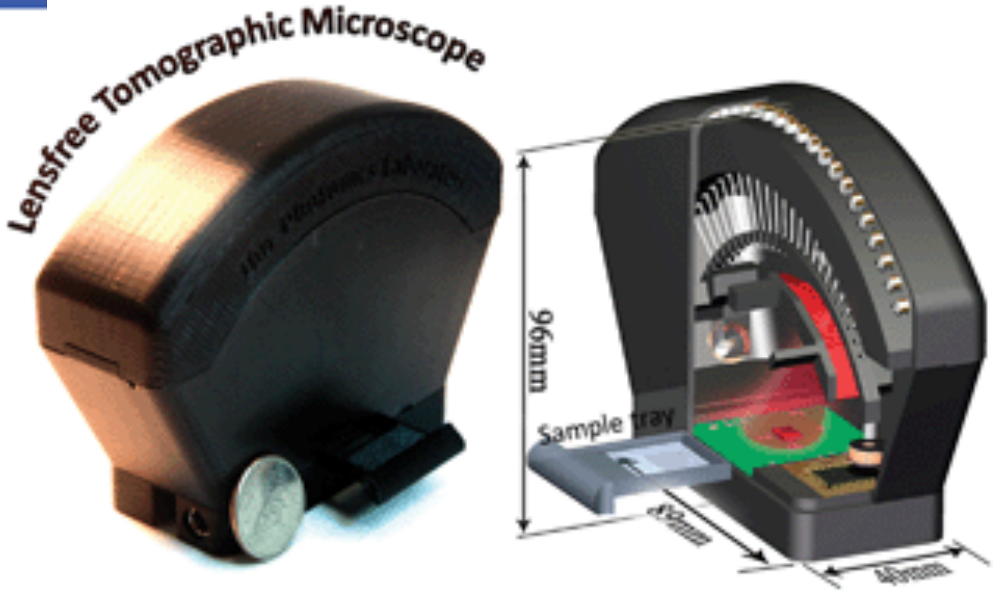


Low-cost microscopy/tomography

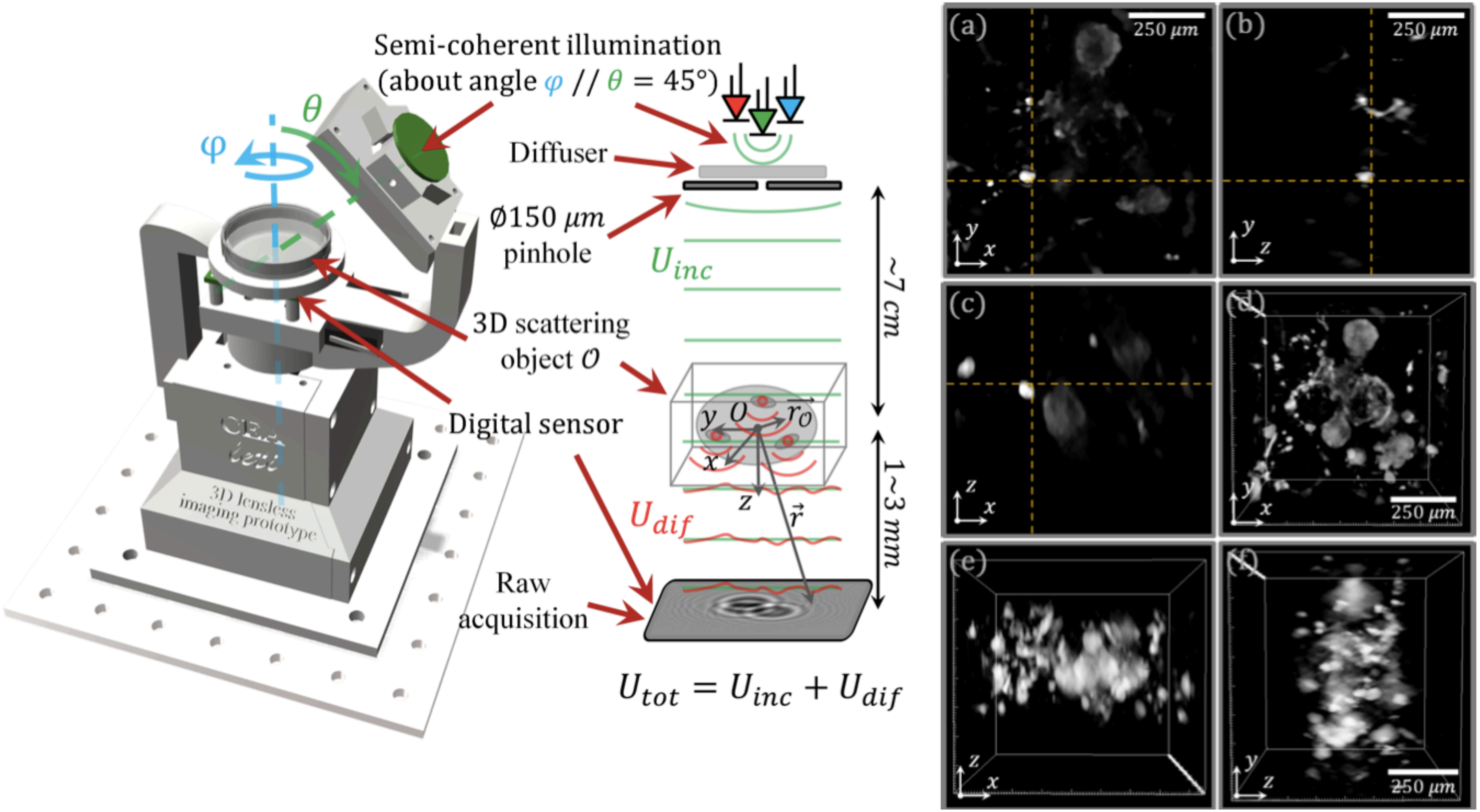


Aydogan Ozcan’s group
UCLA

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



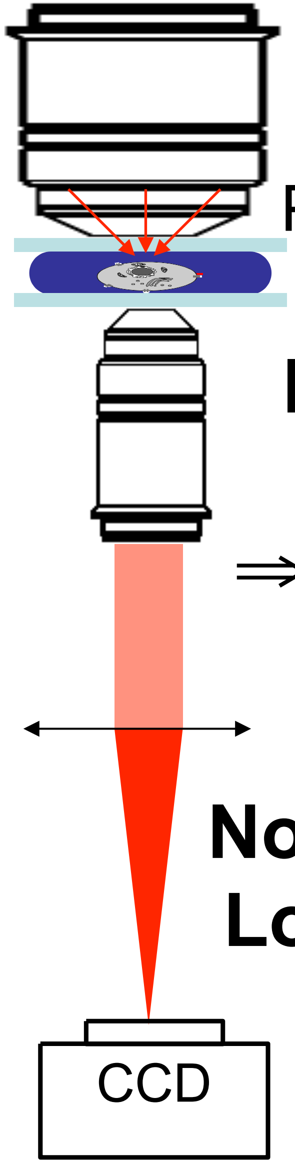
Low-cost microscopy/tomography



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…

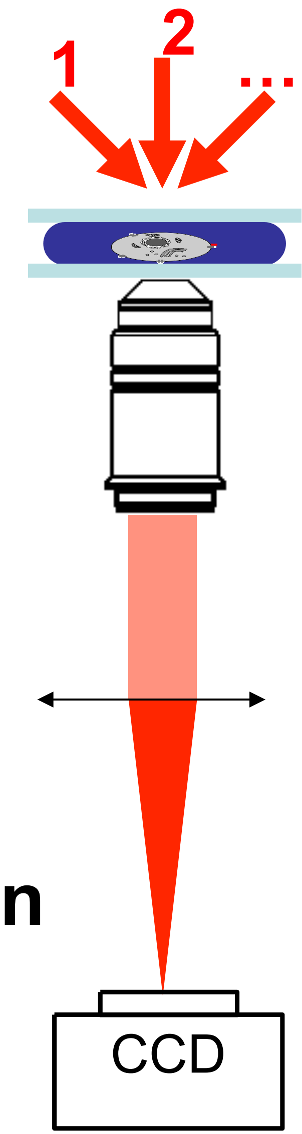
Incoherent
Parallel
⇒ Ultrafast ☺

Not-quantitative
Low-resolution

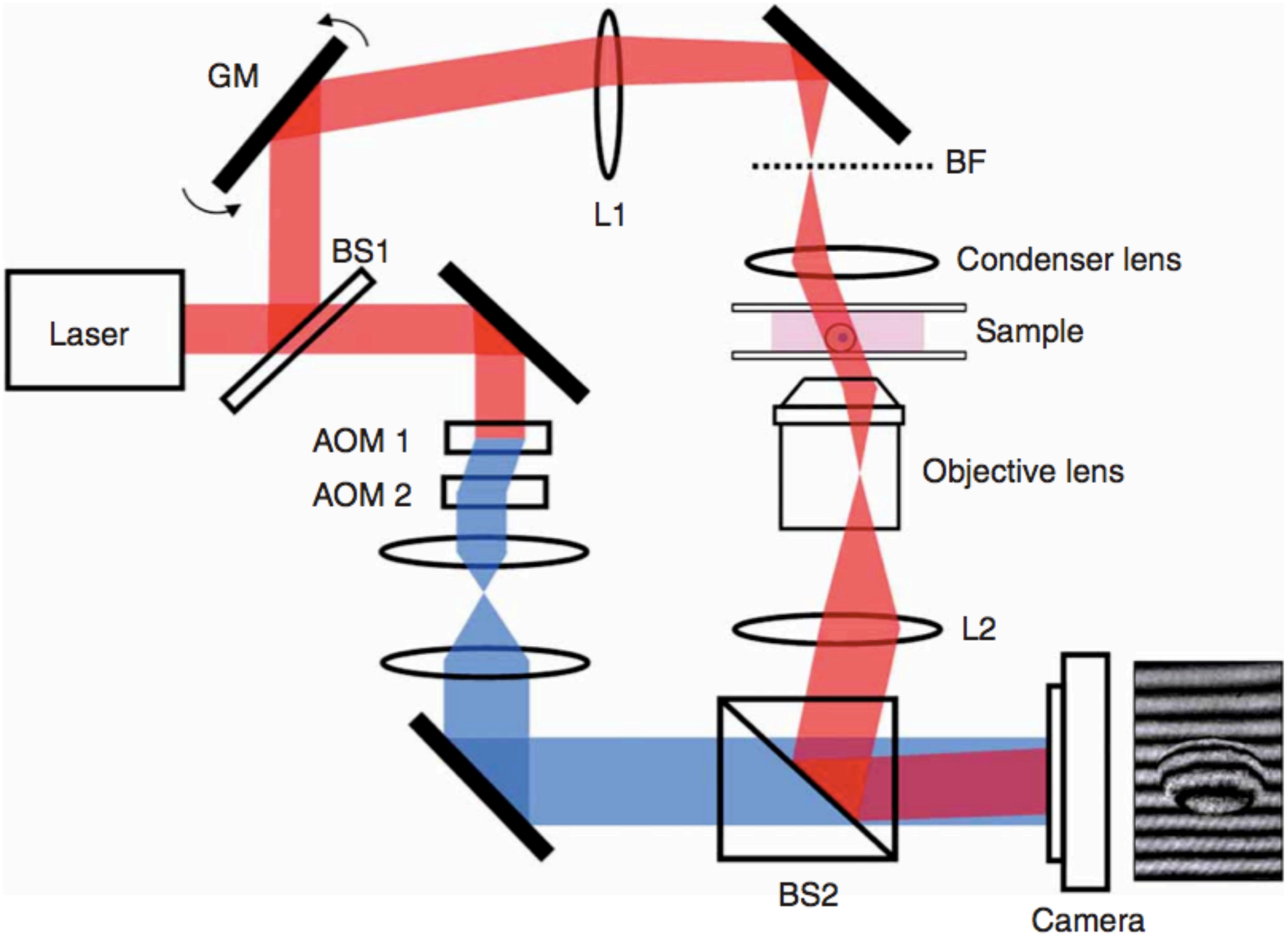
Diffractive
Tomographic
Microscopy

Coherent
Sequential
=> Slow ☹

Quantitative
High-resolution

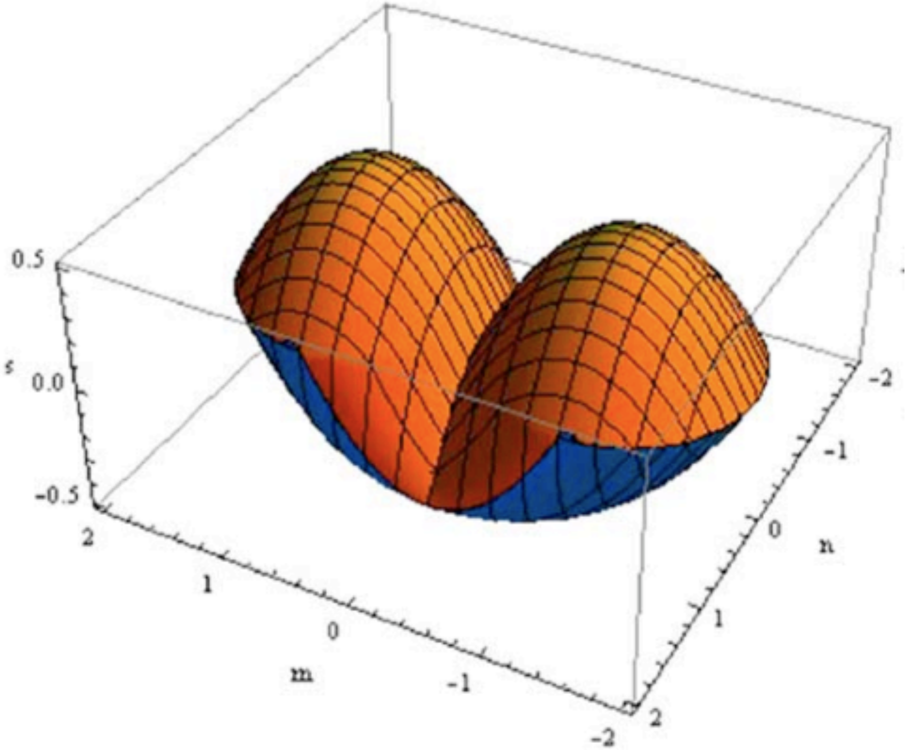


Fast 1-D Scanning



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

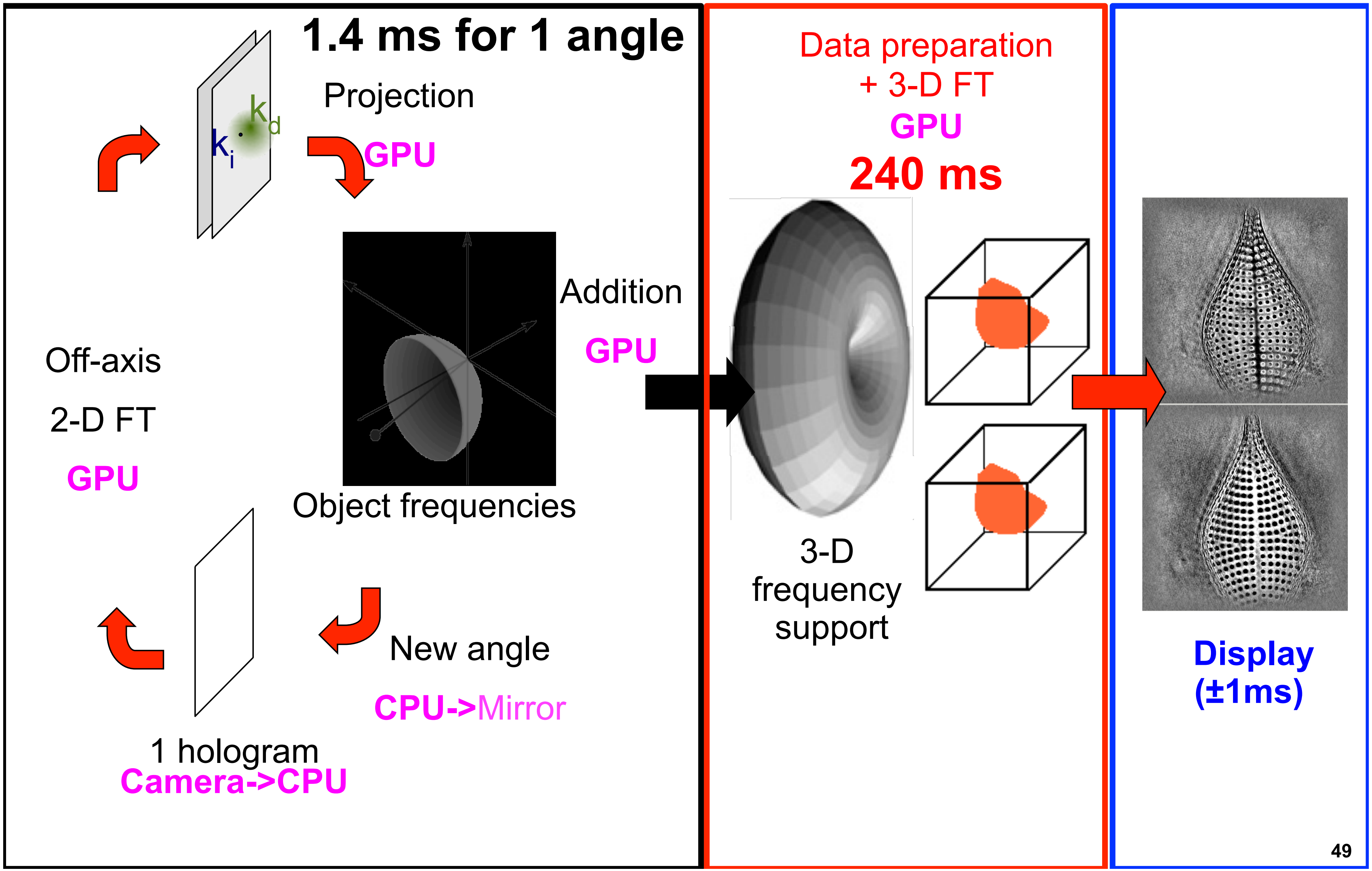
1-D Scanning



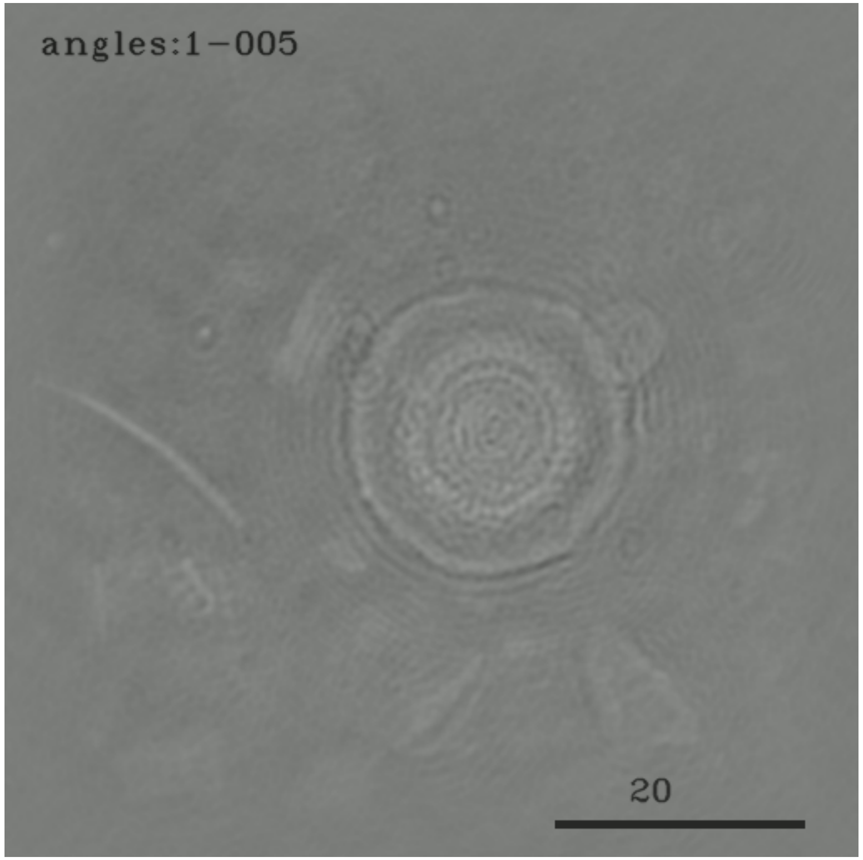
“The overall shape takes a form of what we might call a “peanut.”

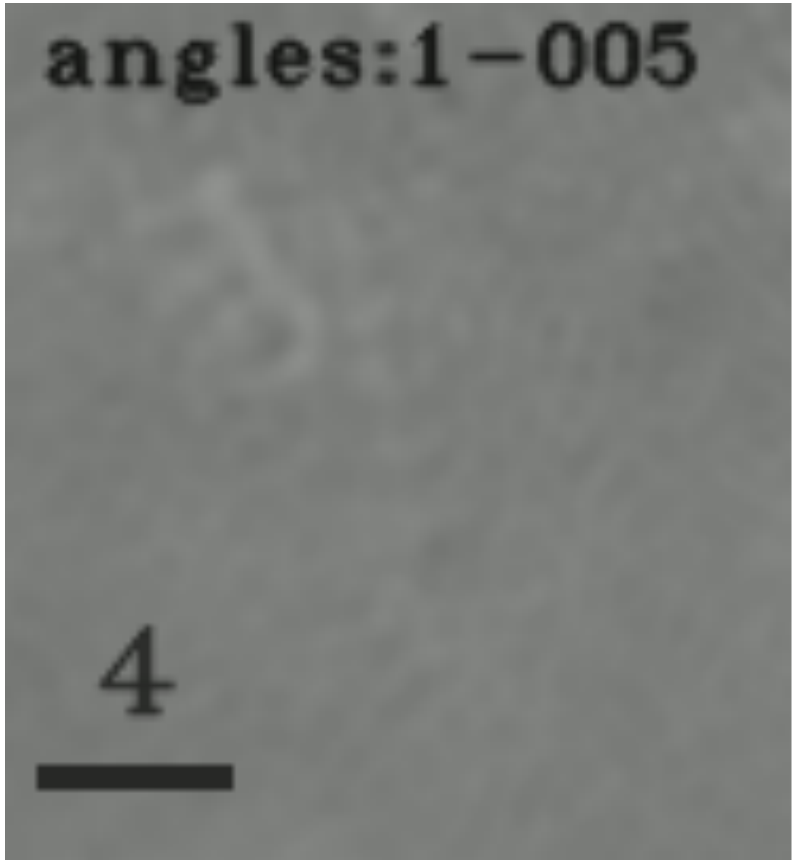
“Image formation in holographic tomography”
S. Shan Kou, and C. J. R. Sheppard, Opt. Lett. **33**, p. 2362 (2008)

Acquisition Reconstruction Display



GPU Reconstruction



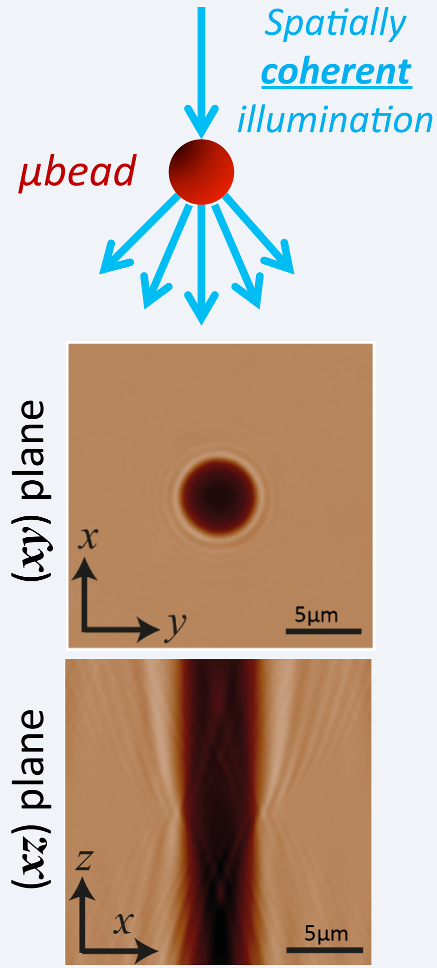


Nvidia Tesla C2075, Cuda, FFTW : 3.5 3D images/s

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

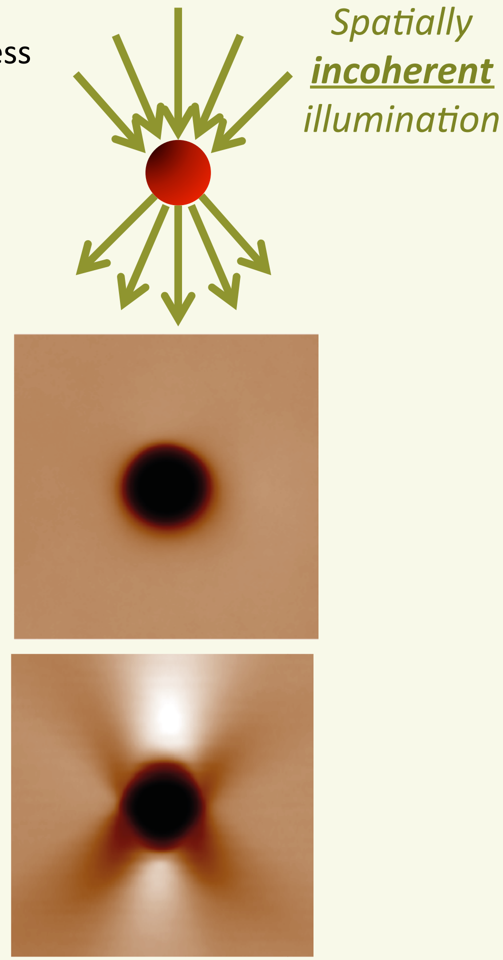
Conventional QPI



☹ Limited axial resolution

New approach

Bon *et al.*, Optics Express 2014

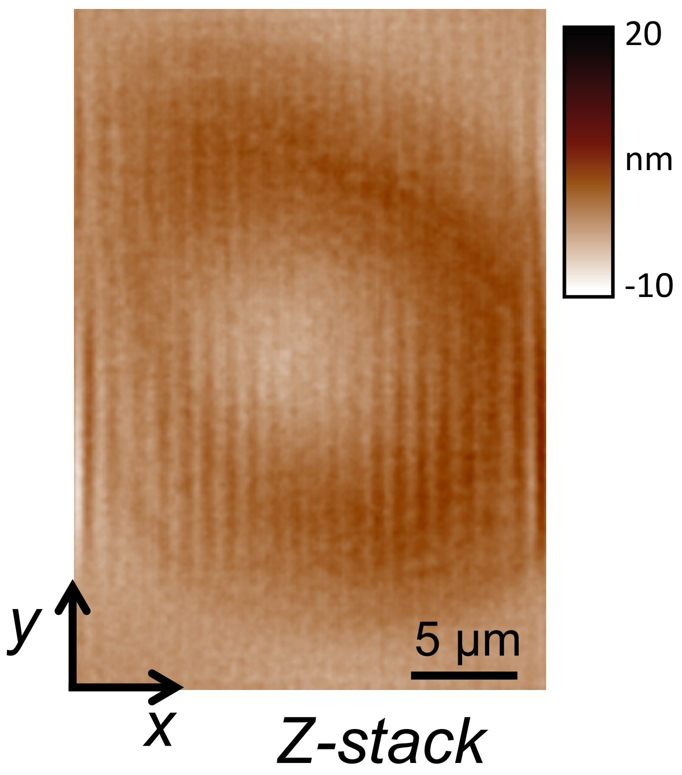


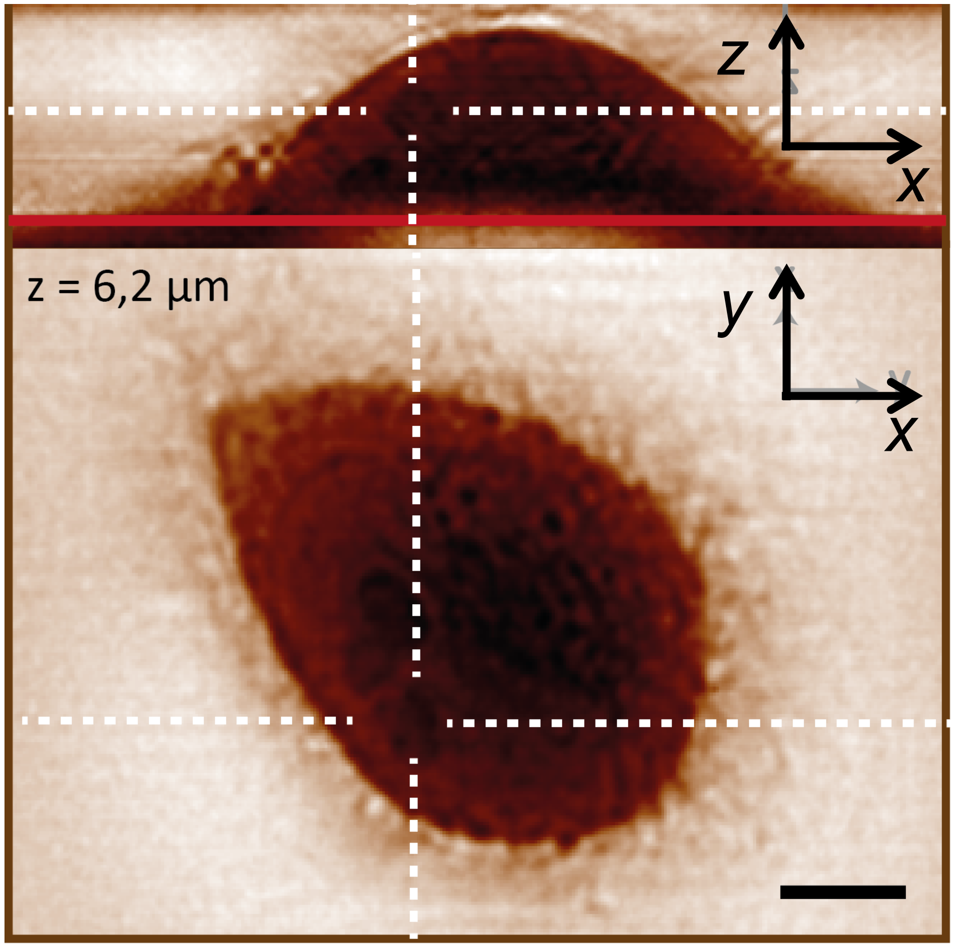
☺ 3D resolution ⇔ fluorescence

3D live cell imaging

Living COS-7 cell

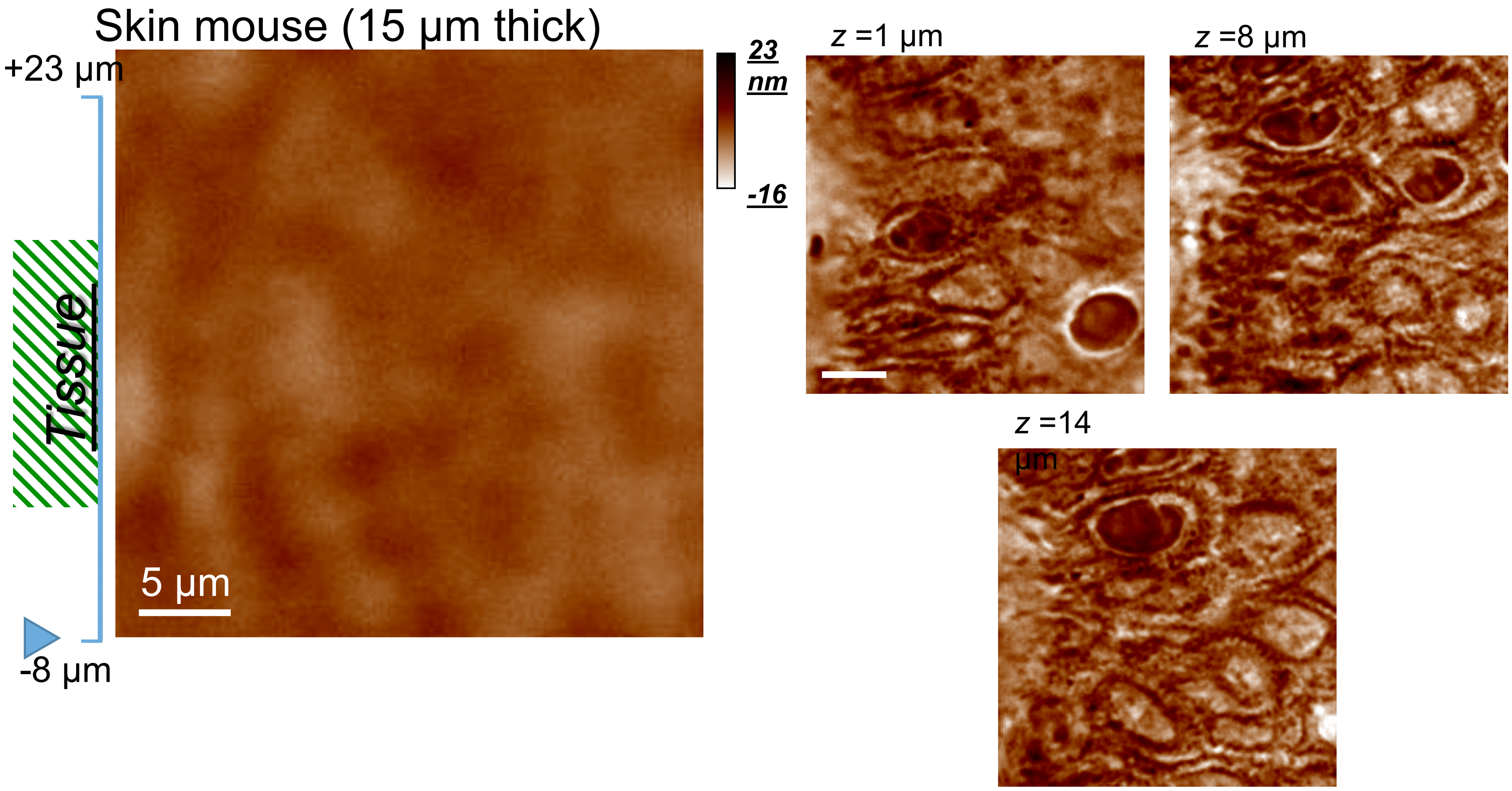
OPD with spatially Incoh. Illum.
+ 3D deconvolution





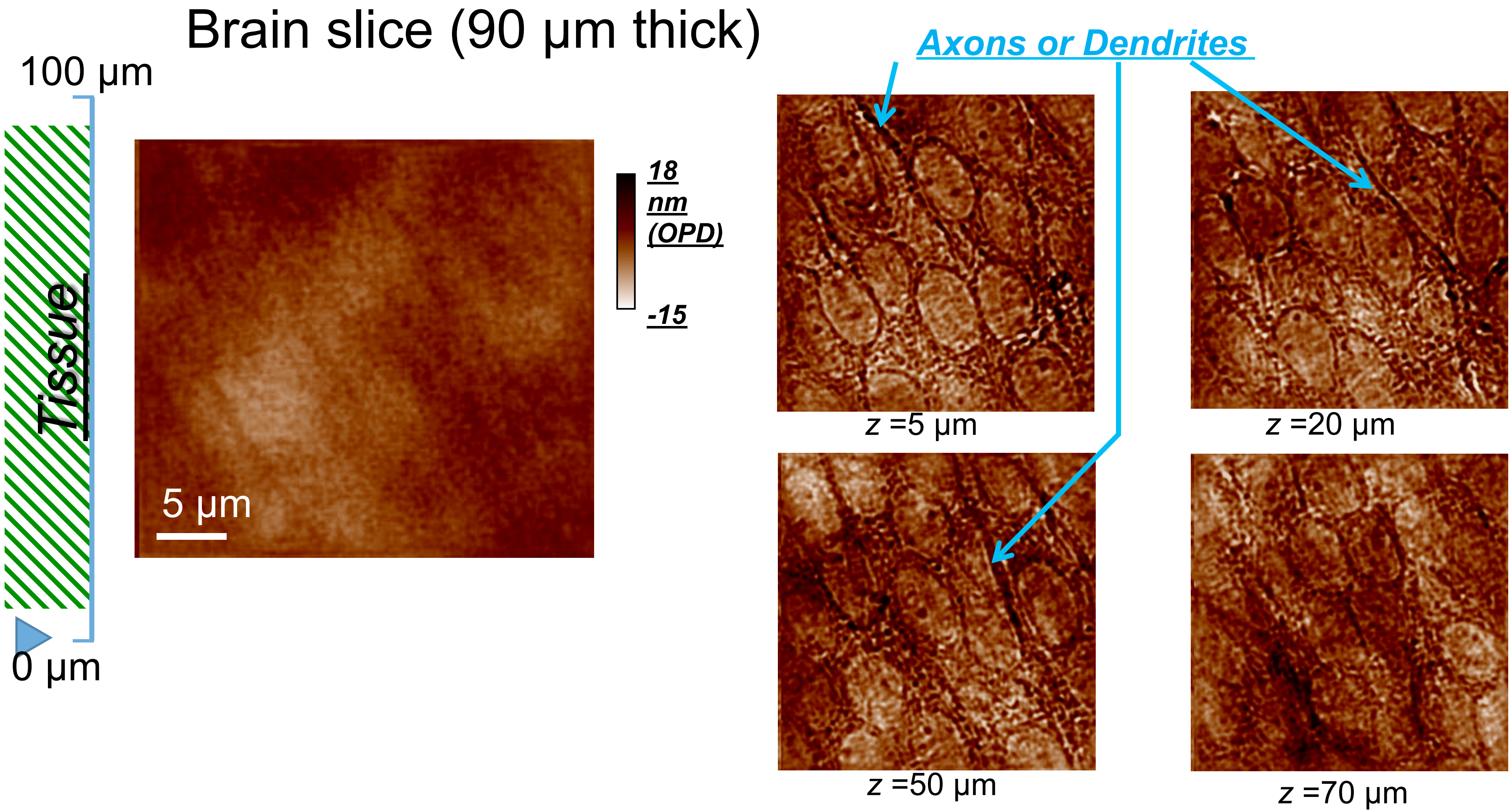
- ✓ ***3D shape of the cell***
- ✓ ***Fast acquisition, compatible with live imaging (just a z-stack!)***

Fixed tissue imaging (1/2)



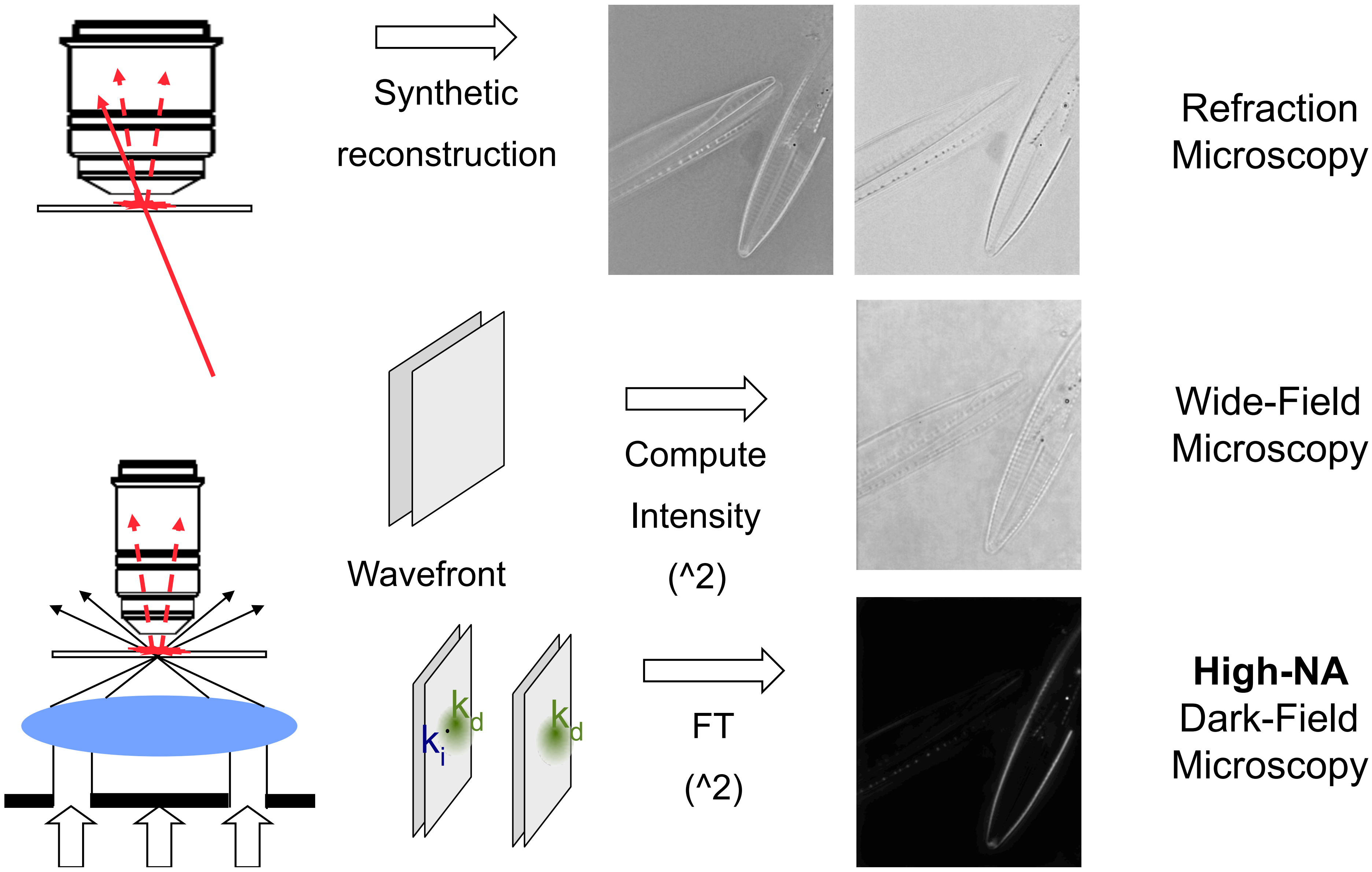
✓ ***Cell layer visualization without labeling***

Fixed tissue imaging (2/2)



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

Multimode Imaging



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?