OSA'S 95TH ANNUAL MEETING
FRONTIERS IN OPTICS 2011
LASER SCIENCE XXVII
APS/DLS 27TH ANNUAL MEETING

Postdeadline Session **A**
Reference - **PDPA2**

Deconvolved phase imaging for diffraction tomography

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ÉCOLE POLYTECHNIQUE FÉDÉRALE DE LAUSANNE

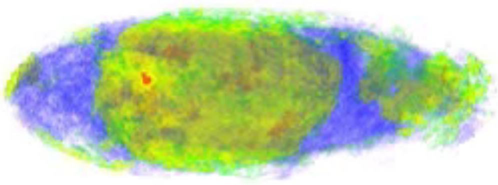
19. October 2011

New tomographic method:

Optical diffraction tomography for microscopy…

… a good tool for 3D imaging of biological cells.

- Transparent objects imaging in 3D thanks to phase information.
- Non invasive and label free.
- Based on digital holographic microscopy (DHM).
- Physical interaction between light and matter is considered.



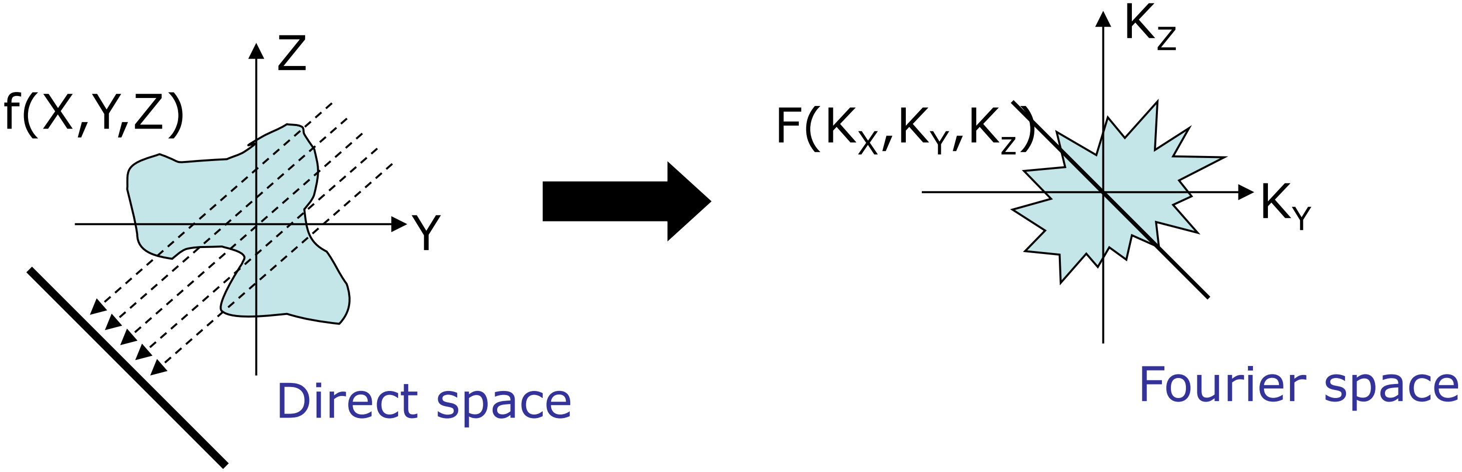
To help achieving high resolution:

3D complex deconvolution method is applied!

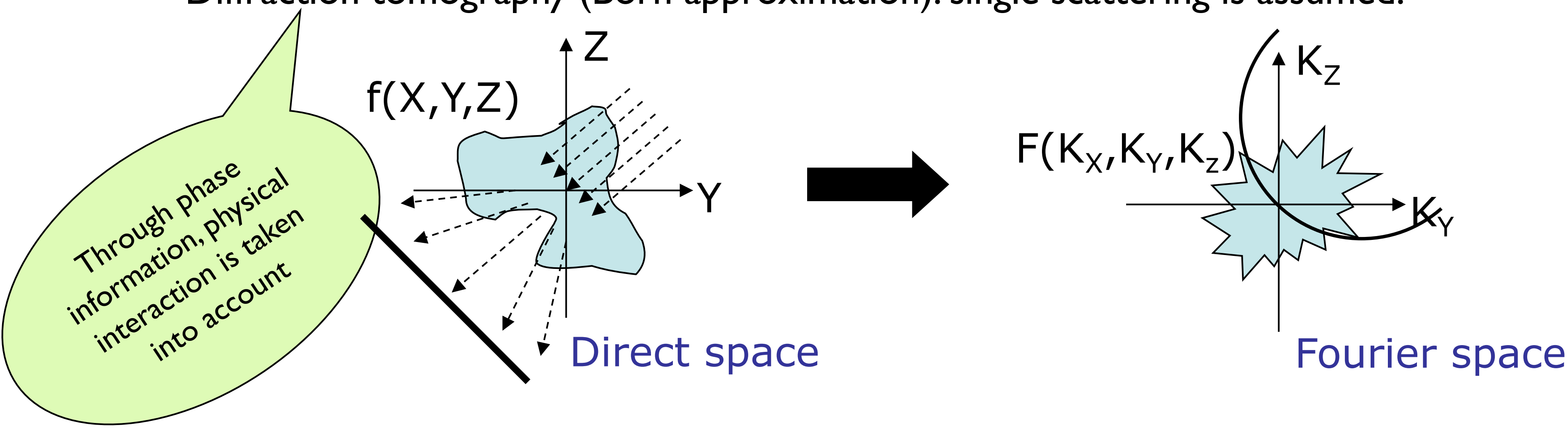
F. Charrière, et al., Living specimen tomography by digital holographic microscopy: morphometry of testate amoeba. Optics Express, 14(16):7005-7013, 2006.

Diffraction tomography

- Backprojection (Radon): light is considered travelling along straight rays.

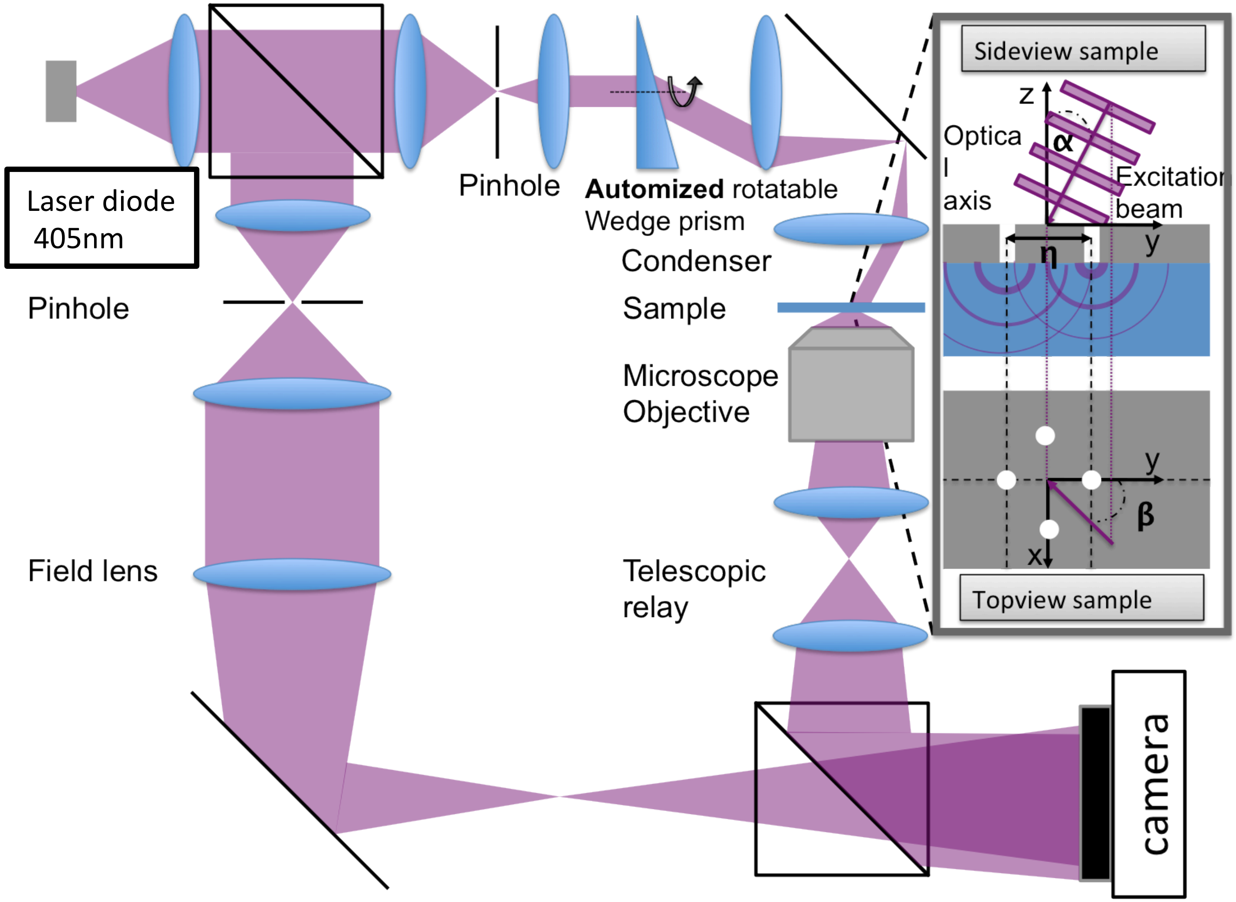


- Diffraction tomography (Born approximation): single scattering is assumed.



Devaney, "A filtered backpropagation algorithm for diffraction tomography," Ultrasonic Imaging 4(4), 336 –350 (1982).

Angular beam scanning holographic microscopy



Wavelength
405nm

Microscope objectives
NA_{MO}=1.4 (oil)

Condenser
NA_{Cond}=0.4 (air)

Wedge prism
scanning 1°

Relay magnification
Sampling=22nm

Cotte Y. et al., Journal of Biomedical Optics 16(10), 106007 (2011)

Fig. ▲ In DHM transmission configuration, a rotatable wedge prism is used to tune illumination conditions. The inset illustrates excitation beam's orientation in side and top perspective of the test target. The beam propagates in the direction of β (arrow: *k*-vector) and is inclined by angle α.

High-resolution imaging

To achieve higher spatial 3D resolution:

- higher **NA**
- shorter **wavelength**
- **steeper tomographic angles** (sample/illumination rotation)

Problem:

Extraction of scattered field From diffraction theory

Measured field | Incident field | scattered field

$$U(\vec{r}_1) = U^{(i)}(\vec{r}_1) + U^{(s)}(\vec{r}_1)$$

Object field

Coherent image formation

$$U(\vec{r}_2) = \iiint_{-\infty}^{\infty} o(\vec{r}_1) h(\vec{r}_2 - \vec{r}_1) \mathrm{d}x_1 \mathrm{d}y_1 \mathrm{d}z_1,$$

Object reconstruction in reciprocal space:

$$FT_{3D}[F] = \frac{f_z}{2\pi}\left(FT_{2D}\left[o^{(s)}\right]\right)e^{-i2\pi f_z z}$$

Object scattering potential

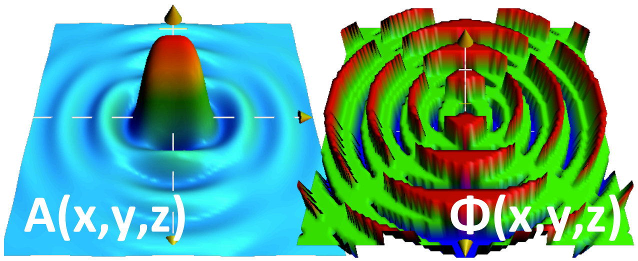
Scattered object field

Field convolved with APSF
=> We need scattered object field
=> Important for high-resolution!

Cotte Y. et al., Opt. Express 18, 19462-19478 (2010)

Coherent image inversion

complex image
$U(x_2,y_2)$



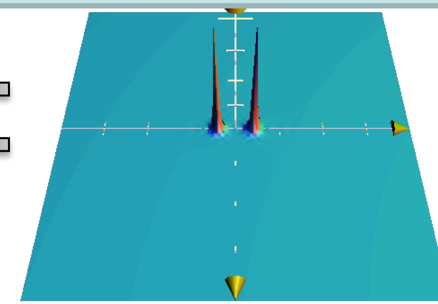
FFT

Inverse filtering
+ physical method: Inversion of imaging formation
- noise amplification
- phase loss

deconvolution theorem for coherent imaging

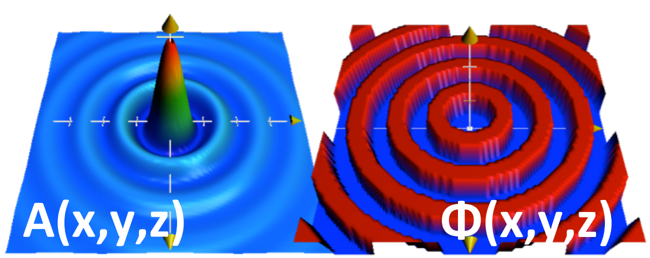
complex spectrum

$$o(\vec{r}_1) = \iiint_{-\infty}^{\infty} O(\vec{k}) exp[-\mathrm{i}2\pi(\vec{k}\cdot\vec{r}_1)]\mathrm{d}k_x\mathrm{d}k_y\mathrm{d}k_z = \mathcal{F}^{-1}\left\{\frac{G(\vec{k})}{c(\vec{k})}\right\}.$$



Coherent transfer function (CTF)

complex point spread function (APSF)
$h(x_2,y_2)$



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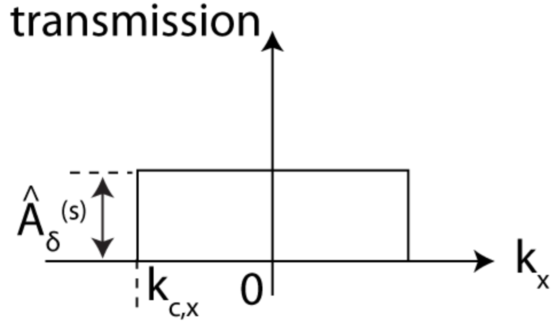
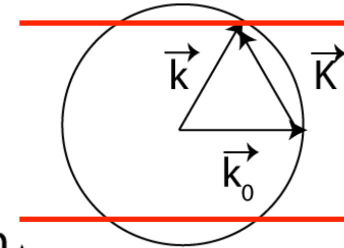
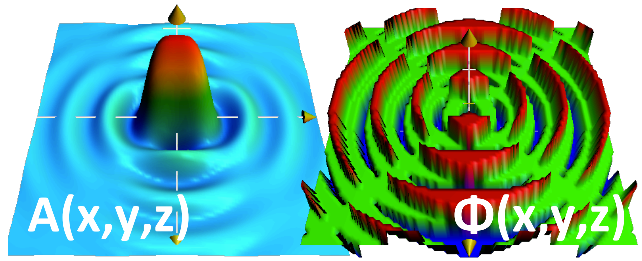


Problem: In 3D

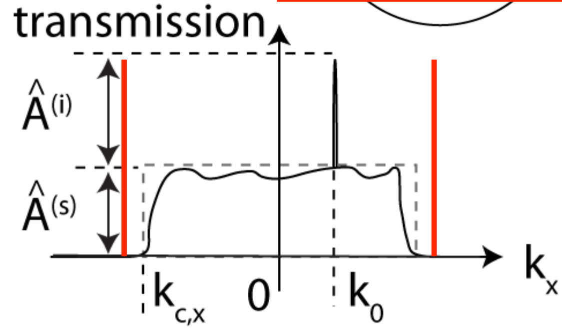


3D filtering

complex image
$U(x_2,y_2)$



FFT



deconvolution theorem for coherent imaging

complex spectrum

w\ low pass filter

$$o(\vec{r}_1) = \mathcal{F}^{-1}\left\{\frac{G(\vec{k})}{c(\vec{k})}\right\}.$$

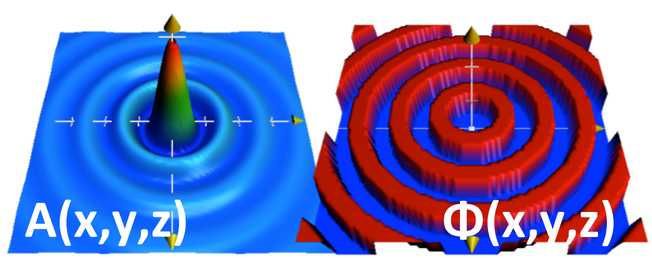
$$\tilde{c}(\vec{k}) = \begin{cases} c & \text{if } |c| > \tau \\ 1 \cdot \exp\left[\mathrm{i} \cdot \arg\left[c\right]\right] & \text{if } |c| \le \tau \end{cases} .$$

Complex regularization
+ noise suppression 3D
+ no phase loss

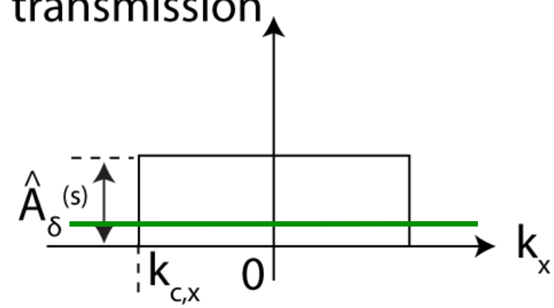
Coherent transfer function (CTF)

w\ complex regularization

complex point spread function (APSF)
$h(x_2,y_2)$

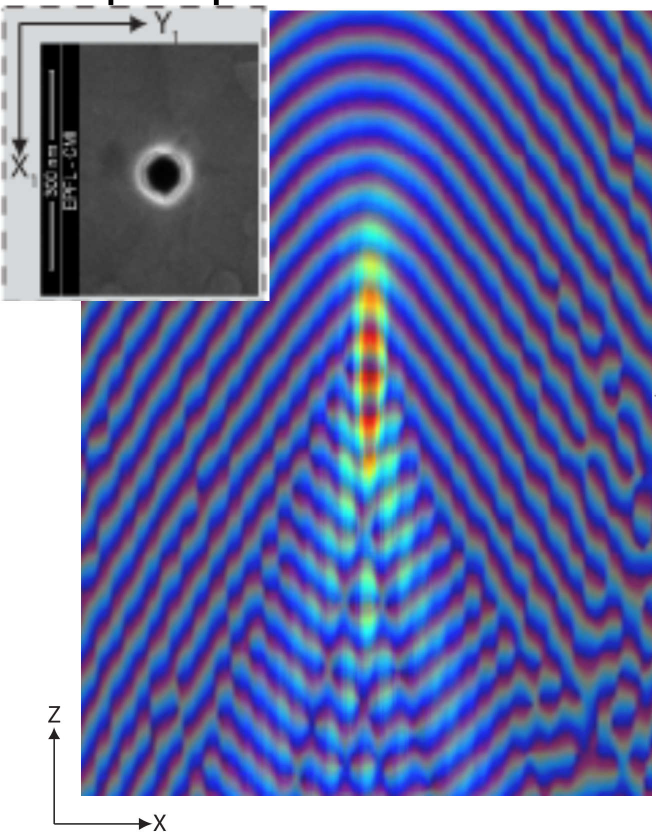


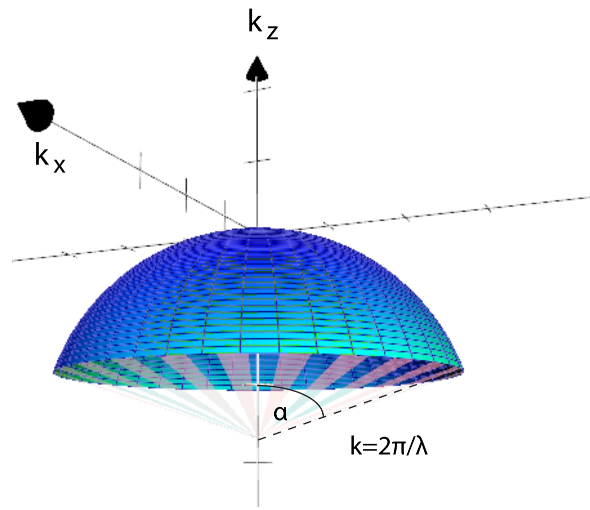
FFT



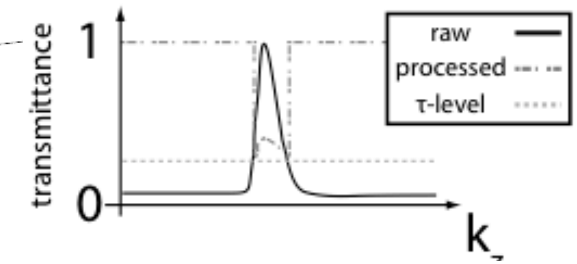
Experimental 3D Coherent transfer function

complex point source





Complex regularisation threshold



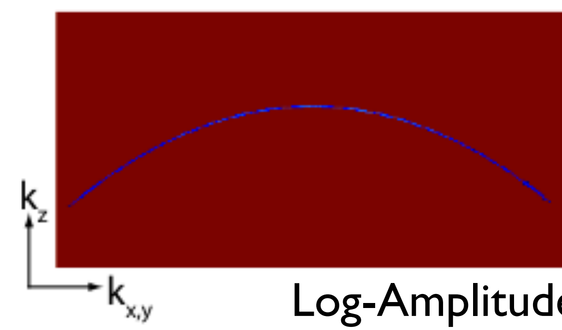


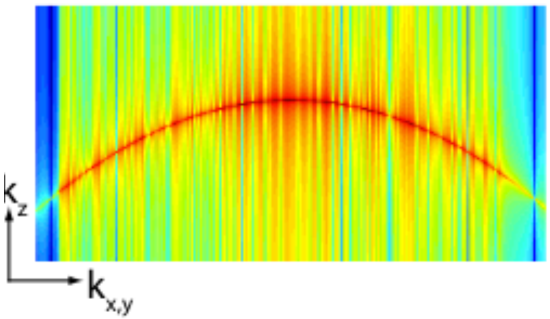
Fig. ▲ 3D Amplitude point spread function (APSF), phase (50%) and amplitude (50%)

Fig. ▶ 3D CTF [amplitude with a maximum subtended half-angle α = arcsin($NA/n_{immersion}$)].

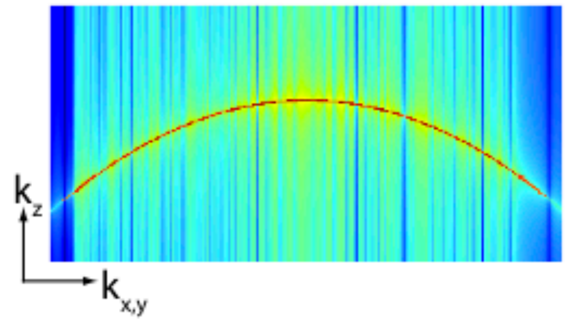
3D complex deconvolution applied to scanning diffraction tomography.

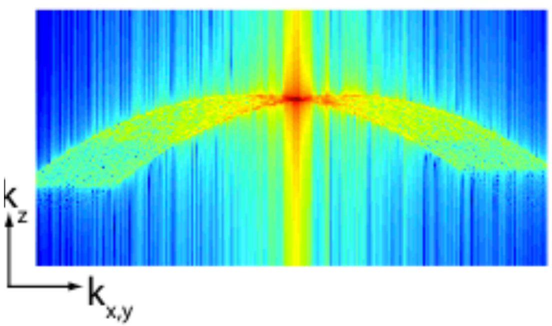
Raw spectrum

Complex deconvolved spectrum

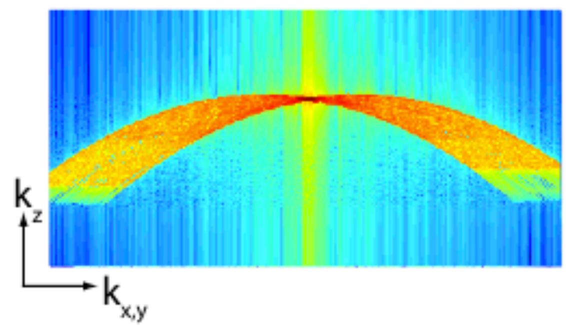


single acquisition





360° acquisition



Synthetic scattering potential

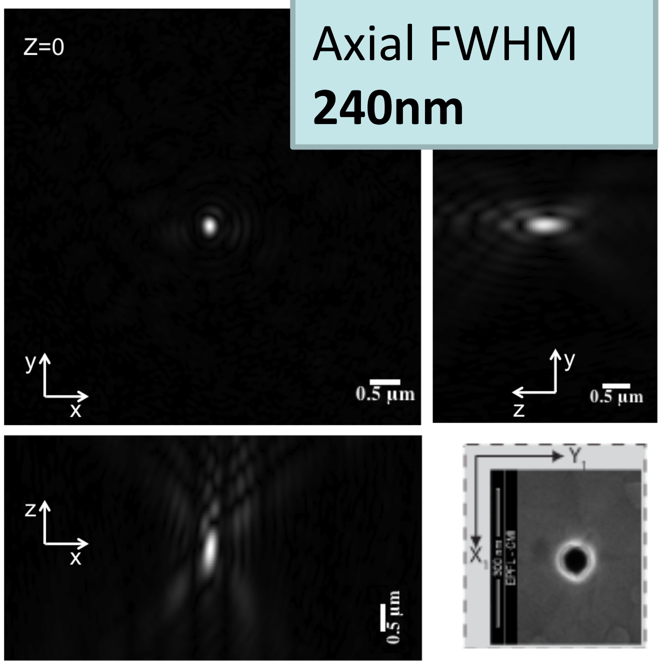
Complex deconvolved
Synthetic scattering potential

APSF of 3D complex deconvolved diffraction tomography

Synthetic

Lateral FWHM
180nm
Axial FWHM
240nm

(a)



Synthetic + Complex deconvolved

Lateral FWHM
110nm
Axial FWHM
180nm

(c)

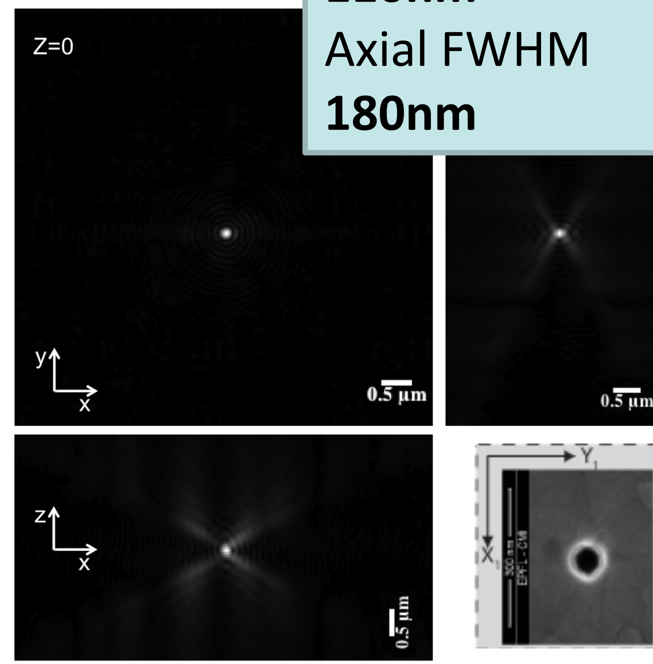


Fig. ▲ Characterization of angular scanning setup. Amplitude (a) and phase (b) show 3D APSF before, and (c)–(d) after 3D complex deconvolution

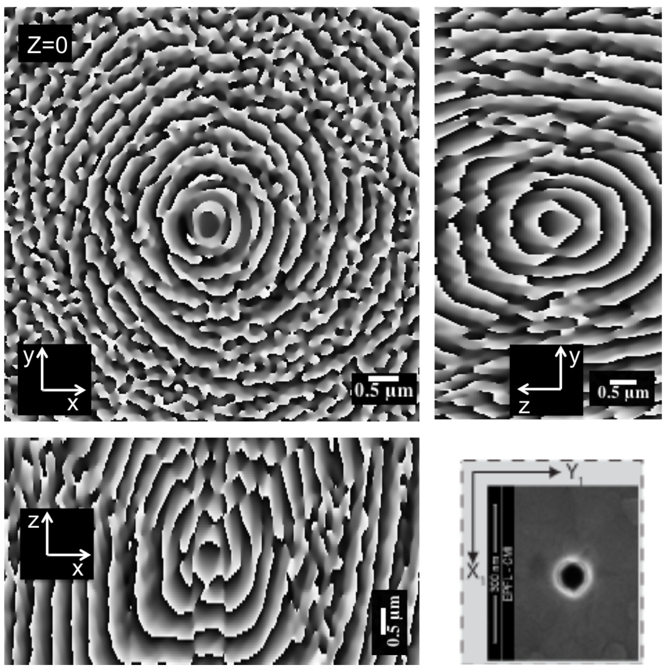
APSF of 3D complex deconvolved diffraction tomography

Synthetic

Synthetic
+ Complex deconvolved

(b)

(d)



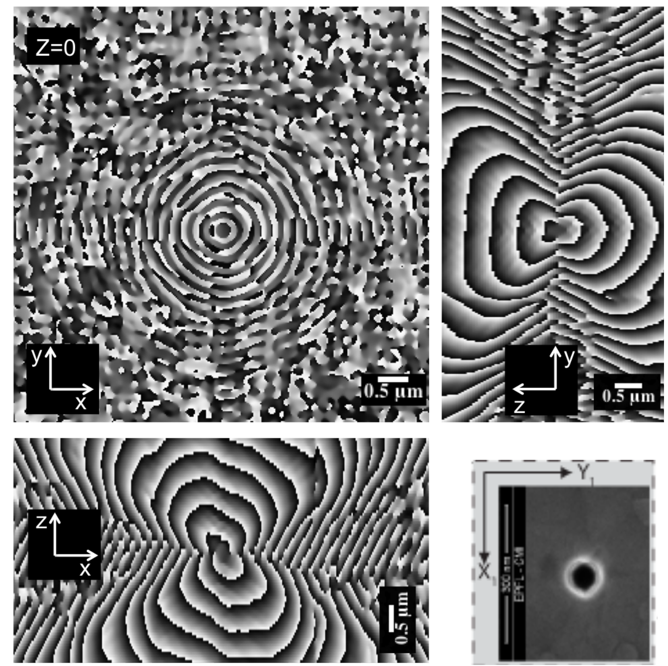


Fig. ▲ Characterization of angular scanning setup. Amplitude (a) and phase (b) show 3D APSF before, and (c)–(d) after 3D complex deconvolution

Experimental resolution assessment diffraction tomography

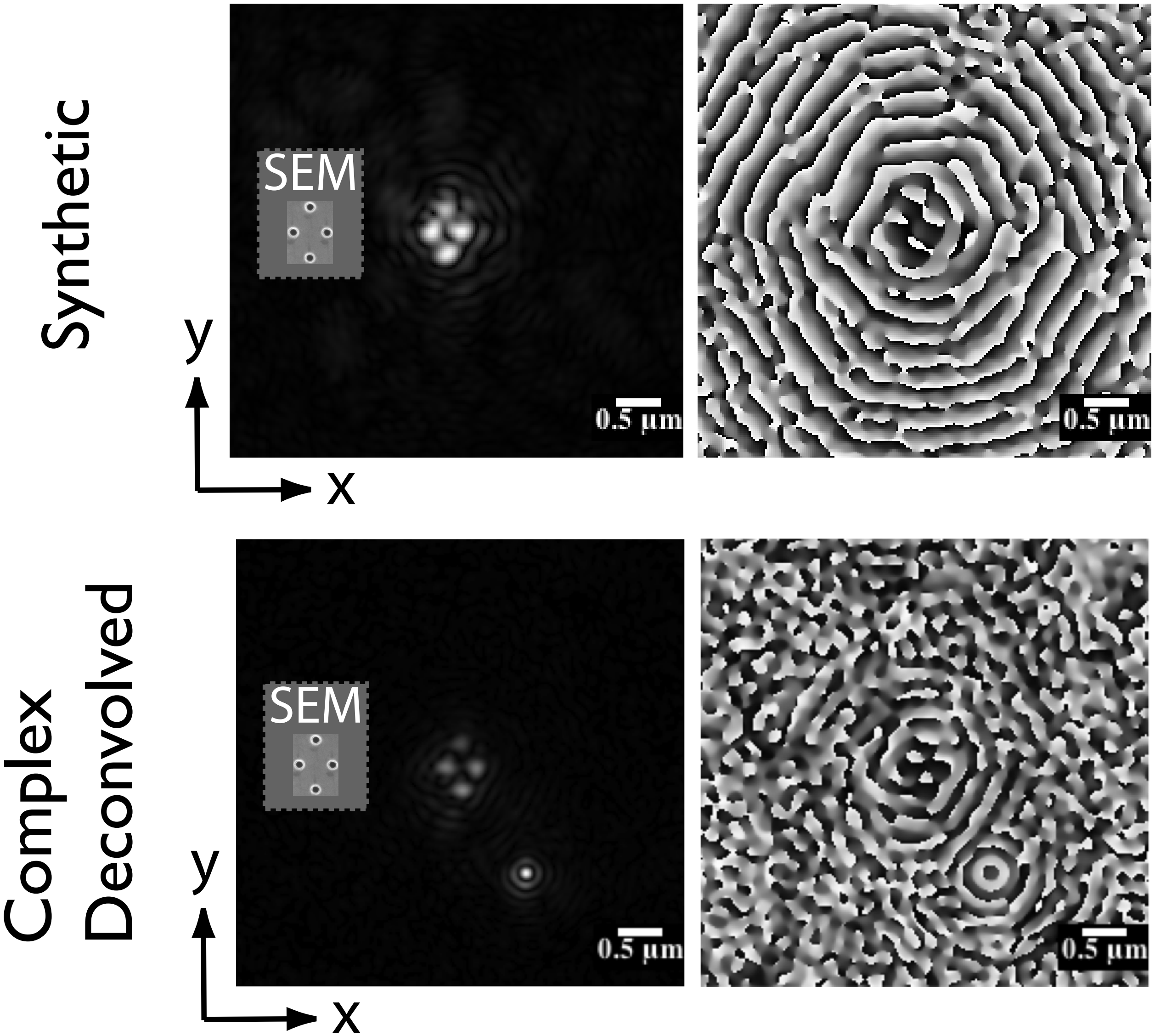
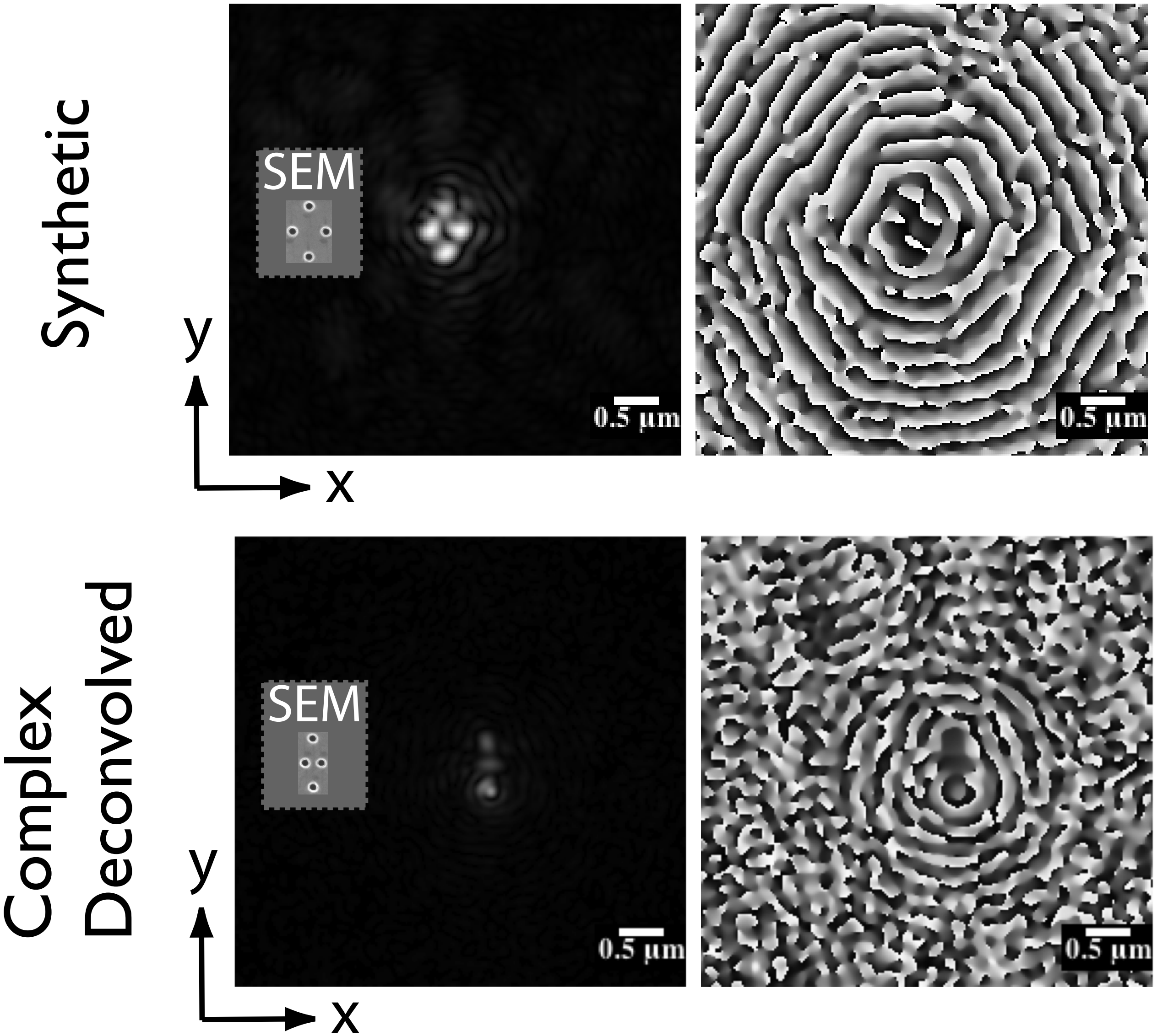
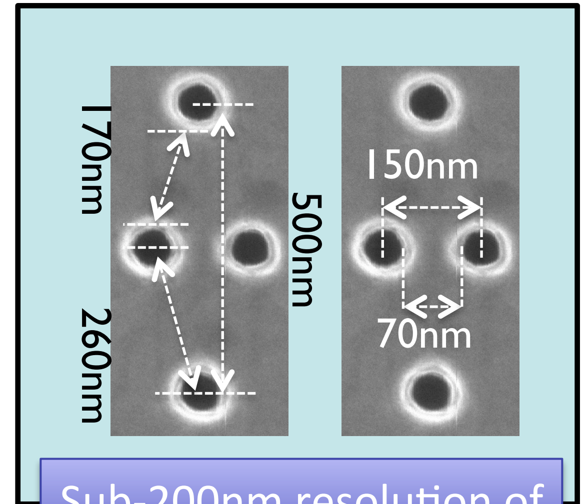


Fig. ◀
Four nano-holes (diameter=80nm) is depicted in amplitude (left) and phase (right) for η_v =500nm, η_h =300nm.

Experimental resolution assessment diffraction tomography





Sub-200nm resolution of about 150nm

Fig. ◀
Four nano-holes (diameter=80nm) is depicted in amplitude (left) and phase (right) for η_v =500nm, η_h =150nm in (b).

Resolution scale in neuronal axon phase imaging

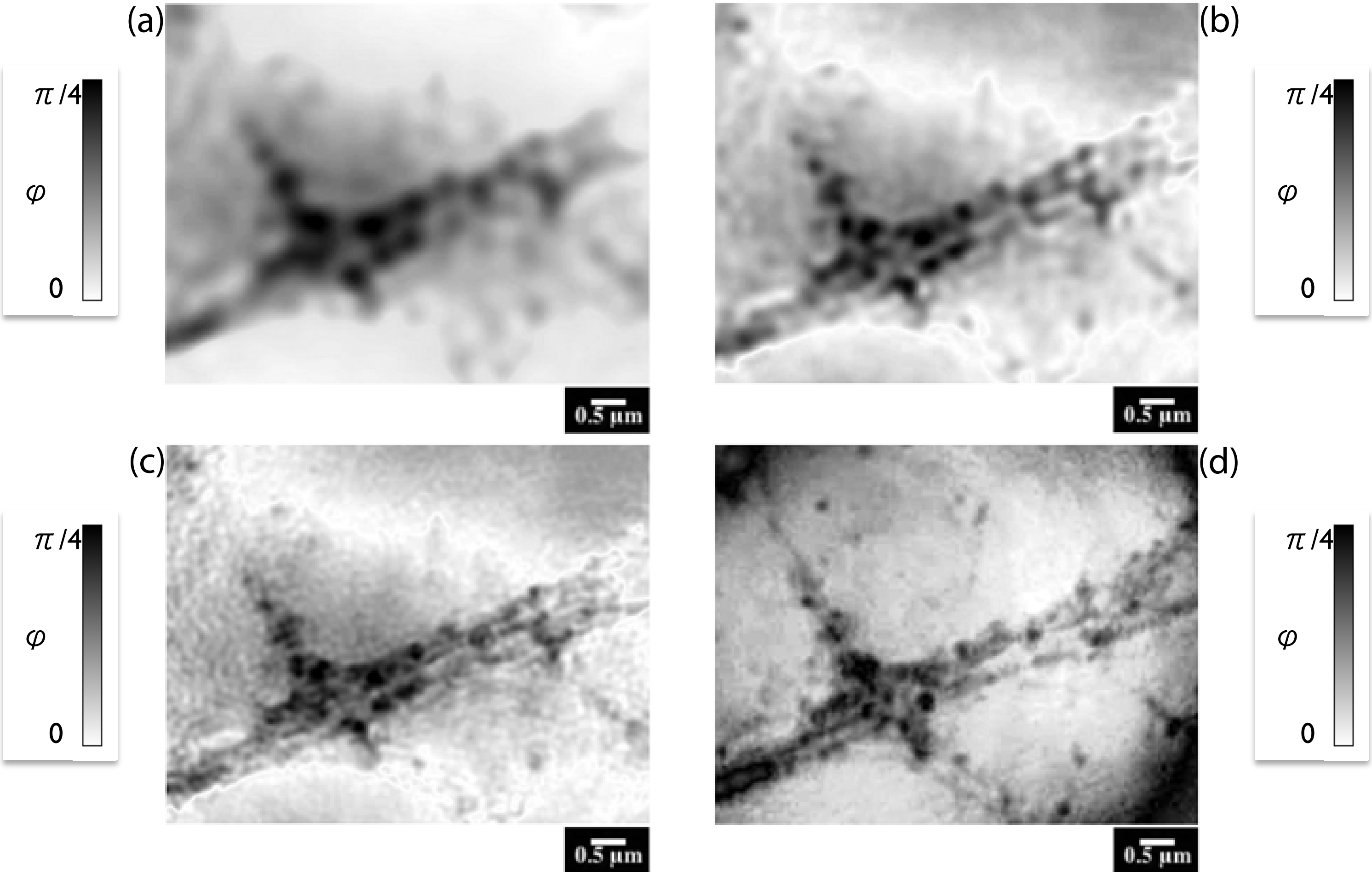
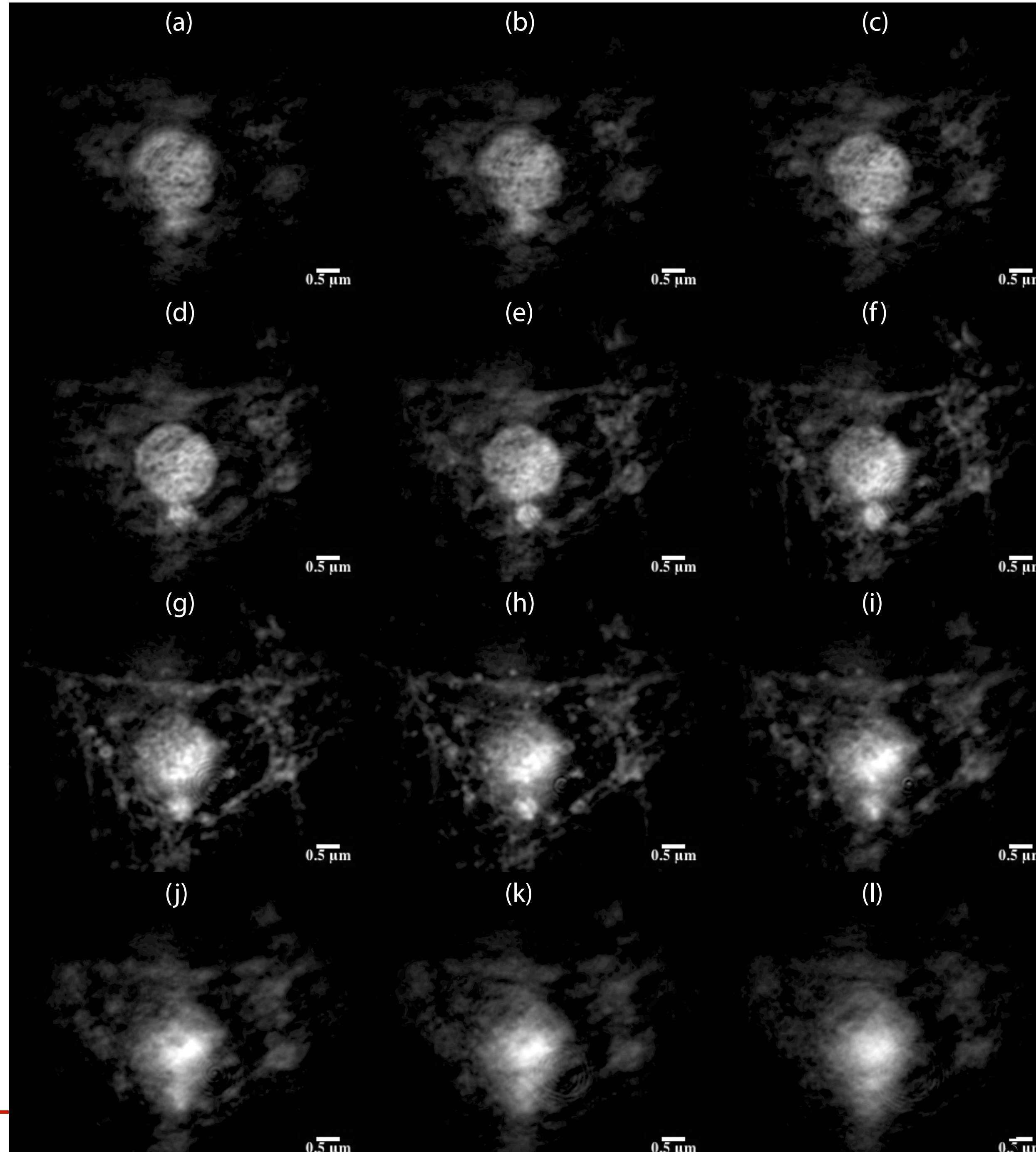


Fig. ▲ Fixed neurones. Phase images of a optical imaging system according to NA = 0.7 and λ =670nm in (a), of NA = 0.95 and λ =532nm in (b), and of NA = 1.4(oil) and λ =405nm (c) and processed (d).

Optical sectioning of refraction index

Fig. ▶
Complex deconvolved refraction tomogram of neuronal network. Images (a)–(l) show lateral slices through 3D-Re{n} (uncalibrated levels) with respective distances
Δz = 260nm.
The sections go from top-to-bottom and represent in total a depth of ≈2.4μm.



Deconvolved phase imaging for diffraction tomography

Theoretical Description

– Image formation and diffraction

Experimental methods

– DH tomography based on beam rotation
– 3D complex deconvolution

Main result:

A general scheme for **quantitative high resolution imaging** adaptable to all kind of acquisition methods

& QUESTIONS

V. ACKNOWLEDGEMENTS

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