

Vortex self-imaging experiments on aberration insensitive localization of microobjects



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Outline

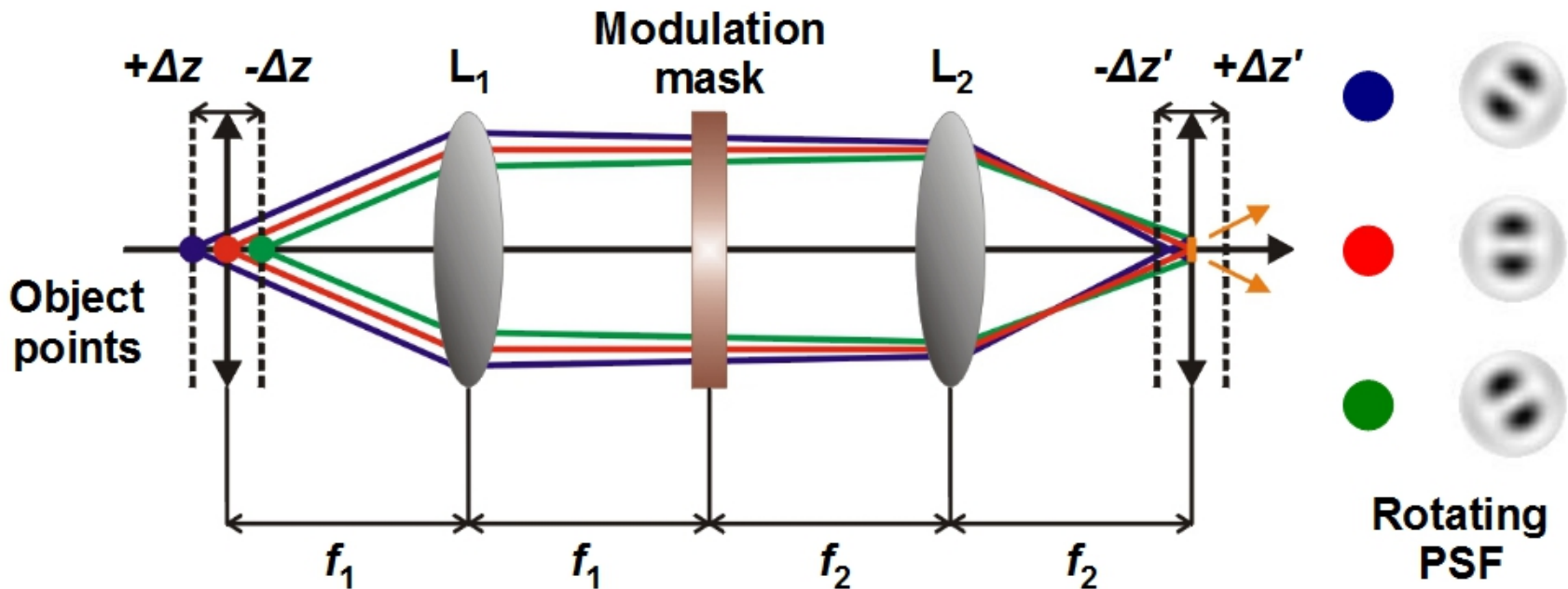
1. Introduction
2. Theoretical model
3. Experimental setup
4. Results
5. Conclusions

Rotating point spread function (PSF)

The rotating PSF has been incorporated into techniques of optical microscopy as a precise tool for three-dimensional localization and tracking of microparticles.

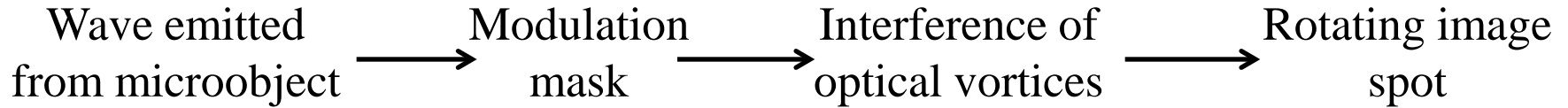
A. Greengard, Y.Y. Schechner, R. Piestun, *Depth from diffracted rotation*, *Opt. Lett.* 31, 181-183 (2006).

Principle of the rotating PSF



The axial position of each monitored point-like object is determined from angular orientation of a rotationally asymmetrical image spot, rotating under defocusing.

Methods of the rotating PSF implementation



1. Modulation mask composed of L-G modes

Double-helix PSF

S.R.P. Pavani, R. Piestun, High-efficiency rotating point spread function, Opt. Express 16, 3484-3489 (2008).

Corkscrew PSF

M.D. Lew, S.F. Lee, M. Badieirostami, W.E. Moerner, Corkscrew point spread function for far-field three-dimensional nanoscale localization of pointlike objects, Opt. Lett. 36, 202-204 (2011).

2. Sampled spiral phase mask

Azimuthal sampling

M. Baranek, Z. Bouchal, Rotating vortex imaging implemented by a quantized spiral phase modulation, J. Europ. Opt. Soc. Rap. Public 8, 13017 (2013).

Radial sampling

S. Prasad, Rotating point spread function via pupil-phase engineering, Opt. Lett. 38, 585-587 (2013).

Nobel prize in chemistry 2014

Super-resolution in optical microscopy

S.W. Hell, E. Betzig, W.E. Moerner

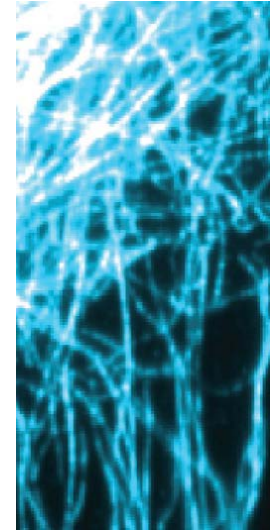
Fluorescence microscopy:

- SIM** - Structured Illumination Microscopy
- STED** - Stimulated Emission Depletion

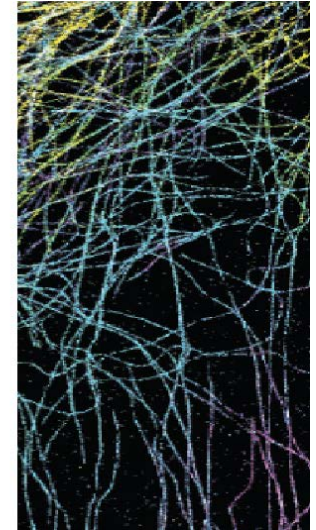
Fluorescence localization microscopy:

- STORM** - Stochastic Optical Reconstruction Microscopy
- PALM** - Photoactivated Localization Microscopy

Standard
imaging



3D
STORM



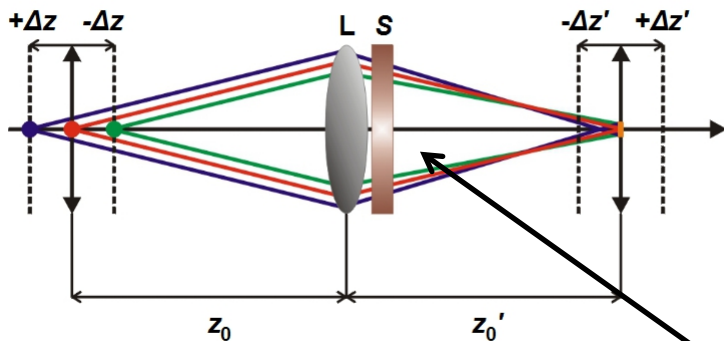
B. Huang, W. Wang, M. Bates, X. Zhuang, Three-dimensional super-resolution imaging by stochastic optical reconstruction microscopy, Science 319, 810-813 (2008).

S.R.P. Pavani, M.A. Thompson, J.S. Biteen, S.J. Lord, N. Liu, R.J. Twieg, R. Piestun, W.E. Moerner, Three-dimensional, single-molecule fluorescence imaging beyond the diffraction limit by using a double-helix point spread function, Proc. Nat. Acad. Sci. USA 106, 2995-2999 (2009).

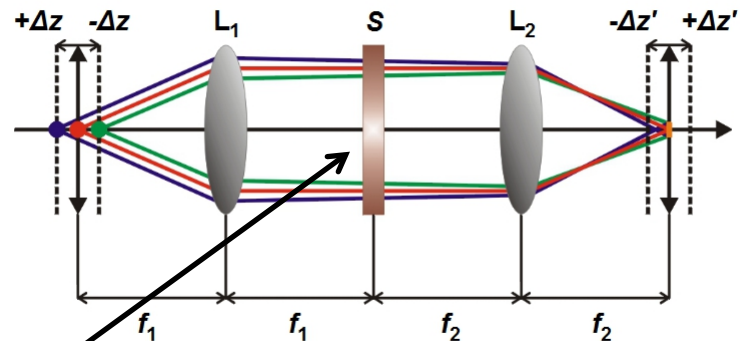
Rotating PSF => 3D super-resolution microscopy

Theoretical model

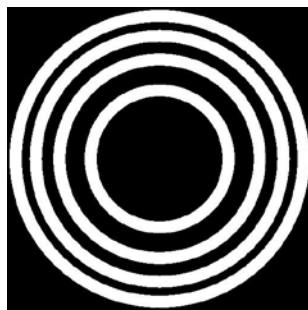
Modulation of complex amplitude



Modulation of spatial spectrum



Amplitude
transparency



Annular
spiral mask

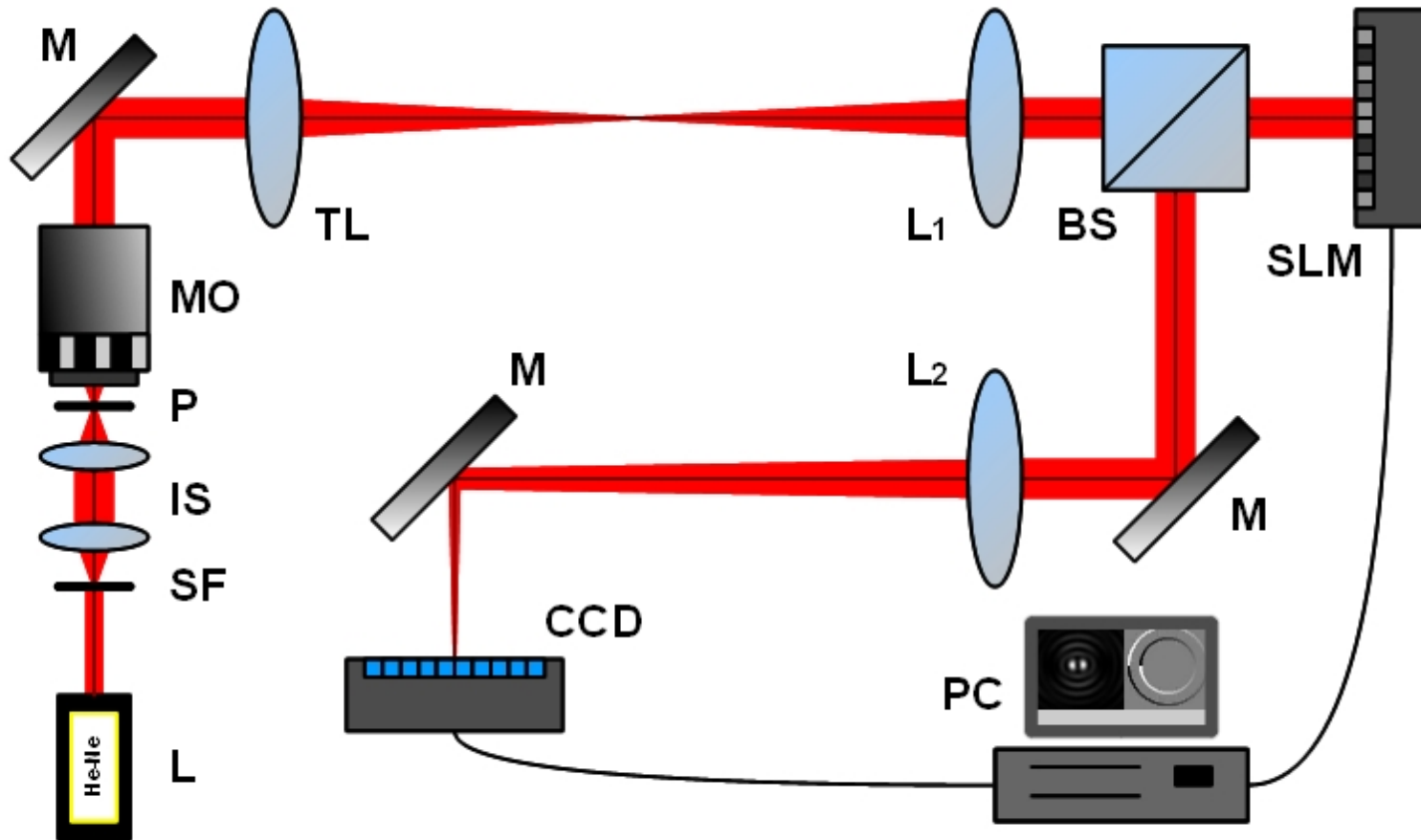


Phase
transparency

Narrow
rings

**Self-imaging of
nondiffracting vortices**

Experimental setup



L – He-Ne laser (20 mW, 632.8 nm); **SF** – spatial filter; **IS** – illumination system; **P** – pinhole ($d = 2 \mu\text{m}$); **MO** – microscope objective (Newport 20x, NA = 0.4, $f_o = 9 \text{ mm}$); **M** – mirrors; **TL** – tube lens ($f_t = 200 \text{ mm}$); **L₁**, **L₂** – lenses ($f_1 = 200 \text{ mm}$, $f_2 = 400 \text{ mm}$); **BS** – beam splitter; **SLM** – Hamamatsu (X10468, 600x800 pix); **CCD** – Olympus F-view II

Results

Shape invariant PSF rotation in extended axial range

Modulation mask

$$l_1 = -1$$

$$l_2 = 1$$



Numerical aperture:

$$A = 0.1$$

Wavelength:

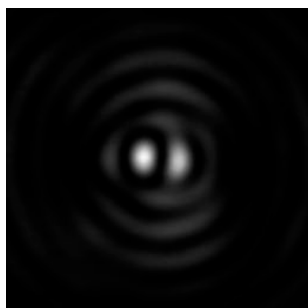
$$\lambda = 632.8 \text{ nm}$$

Depth of focus:

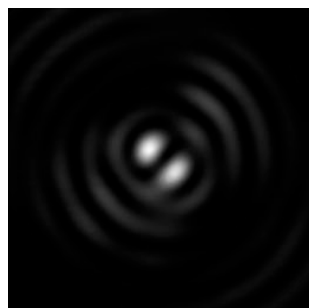
$$\Delta d \approx 32 \text{ } \mu\text{m}$$

Aberration free system:

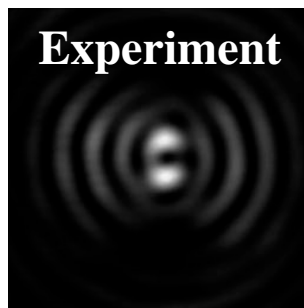
$$A_{040} = 0$$



$\Delta z = -120 \text{ } \mu\text{m}$



$\Delta z = -60 \text{ } \mu\text{m}$



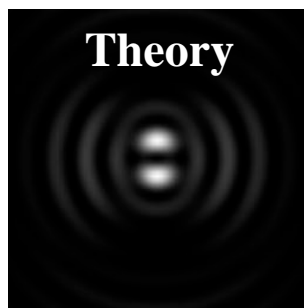
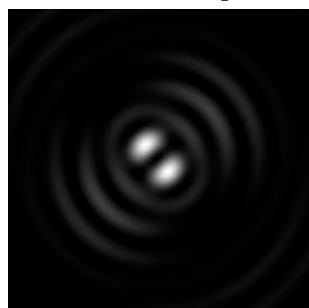
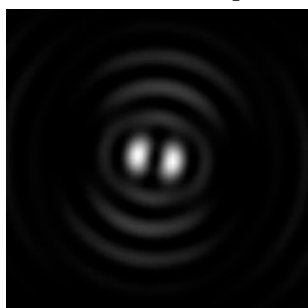
$\Delta z = 0 \text{ } \mu\text{m}$



$\Delta z = 60 \text{ } \mu\text{m}$



$\Delta z = 120 \text{ } \mu\text{m}$



M. Baranek, P. Bouchal, M. Siler, Z. Bouchal, Aberration resistant axial localization using a self-imaging of vortices, Opt. Express 23, 15316-15331 (2015).

Results

Aberration resistant PSF rotation

Modulation mask

$$l_1 = -1$$

$$l_2 = 1$$



Numerical aperture:

$$A = 0.1$$

Wavelength:

$$\lambda = 632.8 \text{ nm}$$

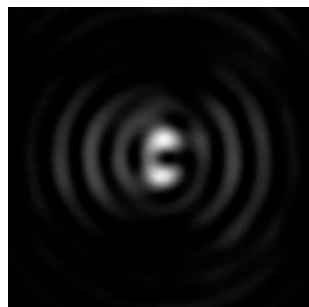
Depth of focus:

$$\Delta d \approx 32 \mu\text{m}$$

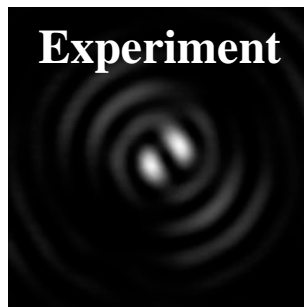
Spherical aberration: $A_{040} = 0.4 \lambda$



$\Delta z = -120 \mu\text{m}$



$\Delta z = -60 \mu\text{m}$



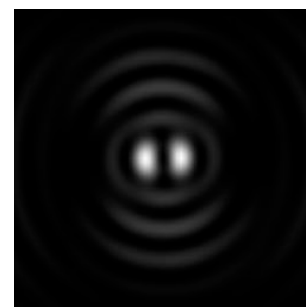
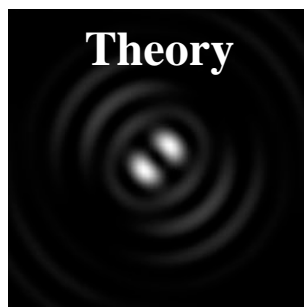
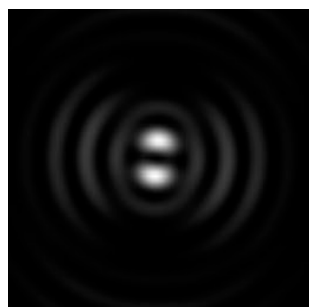
$\Delta z = 0 \mu\text{m}$



$\Delta z = 60 \mu\text{m}$



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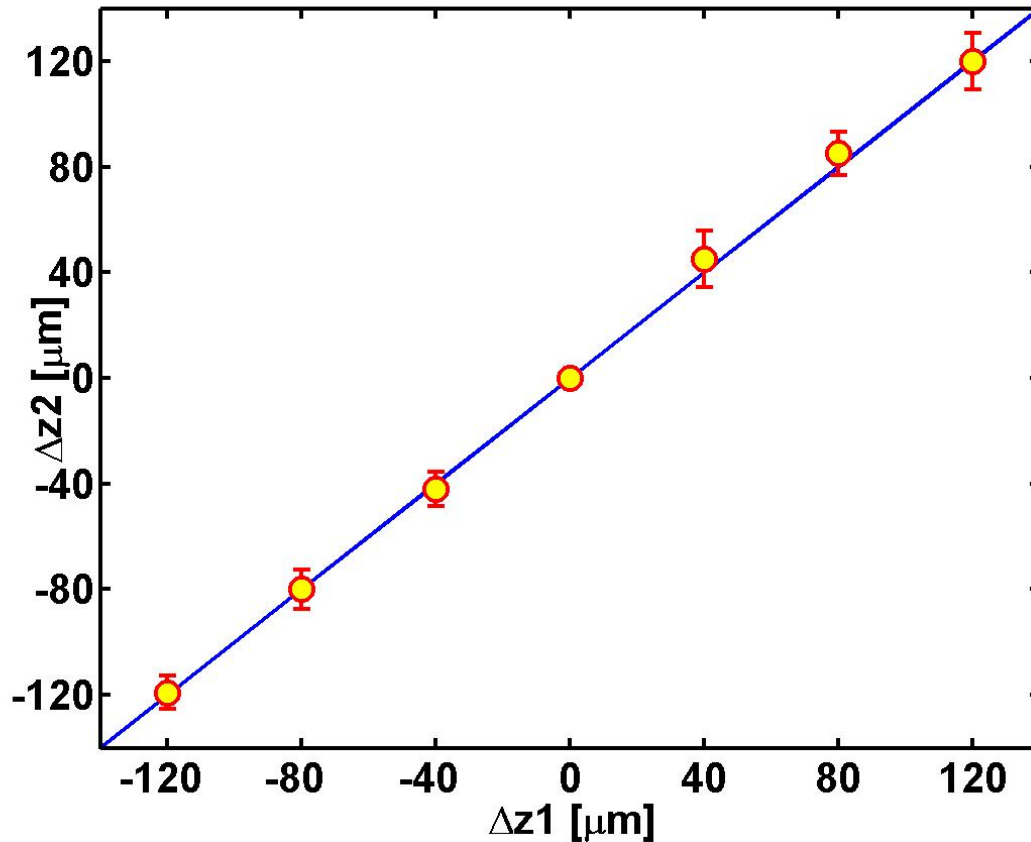
M. Baranek, P. Bouchal, M. Siler, Z. Bouchal, Aberration resistant axial localization using a self-imaging of vortices, Opt. Express 23, 15316-15331 (2015).

Results

Evaluation of the pinhole axial position

Δz_1 – pinhole axial position – set by precise microtranslation.

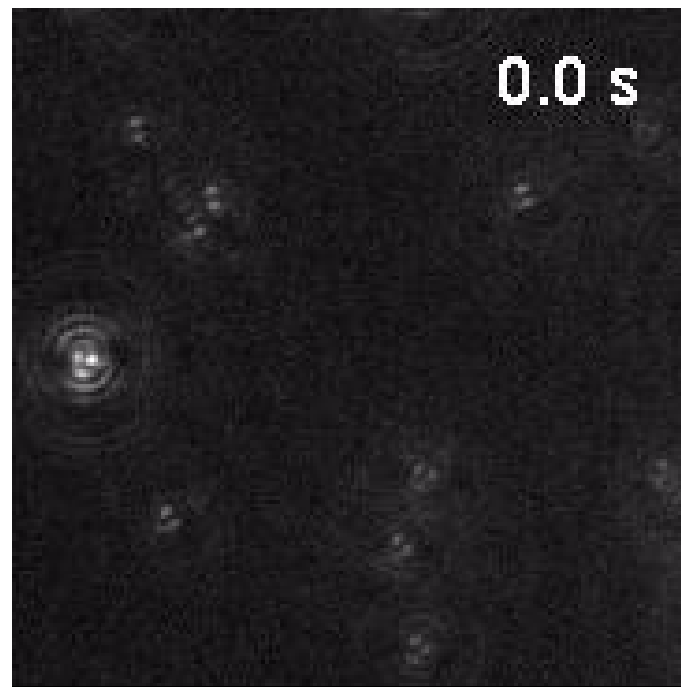
Δz_2 – pinhole axial position – obtained from rotated image.



Δz_1 [μm]	Δz_2 [μm]
-120 ± 1	-118.8 ± 6.1
-80 ± 1	-79.7 ± 7.4
-40 ± 1	-42.0 ± 6.5
40 ± 1	44.5 ± 10.7
80 ± 1	84.8 ± 8.0
120 ± 1	119.4 ± 10.7

Results

Defocusing rotation of microparticles



Vortex self-imaging applied to 3D localization of freely moving 1 μm polystyrene beads.

M. Baranek, P. Bouchal, M. Siler, Z. Bouchal, Aberration resistant axial localization using a self-imaging of vortices, Opt. Express 23, 15316-15331 (2015).

Conclusions

Summary

New technique for rotating PSF generation was presented.

The advantages of the method are:

- **resistance against aberrations,**
- **localization in a large axial range substantially exceeding the depth of field of the microscope objective used,**
- **shape and size invariance of the PSF during rotation,**
- **possibility to control the rotation sensitivity and the energy efficiency by SLM addressing.**

Outlook

Our future research is focused on application potential of designed rotating PSF.

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Thank You for Your attention