Incoherent Digital Holography with Axial Localisation by Rotating Point Spread Function (PSF)

Petr Bouchal,[1,2,*] Zdeněk Bouchal,[3] Radim Chmelík,[1,2]

[1] Institute of Physical Engineering, Faculty of Mechanical Engineering, Brno University of Technology, Technická 2, 616 69 Brno, Czech Republic.

[2] Central European Institute of Technology, Brno University of Technology, Technická 10, 616 00 Brno, Czech Republic.

[3] Department of Optics, Palacký University, 17. listopadu 1192/12, 771 46 Olomouc, Czech Republic.

***petr.bouchal@ceitec.vutbr.cz**

Technology Agency of the Czech Republic

CEITEC

Outline

- **3D imaging, localisation and tracking of particles**
  - methods and applications
  - techniques of axial localisation
- **Concept of PSF rotation in digital holography**
  - benefits of rotational holographic localisation
  - all-digital implementation of vortex beam holography
  - demonstration of depth estimation by diffraction-free vortex beams
- **Rotating PSF in Fresnel Incoherent Correlation Holography (FINCH)**
- **Experimental demonstrations in FINCH**
  - image rotation under numerical refocusing (5μm fluorescent beads)
  - tracking of 500nm polystyrene beads
  - 3D localisation of a single polystyrene bead
- **Conclusions**

Particle imaging, localisation and tracking

Applications: time-resolved imaging, single molecule tracking, microfluidics, particle image velocimetry.

Techniques of the depth estimation

❑ Optical microscopy

(*2D imaging – PSF engineering needed*)

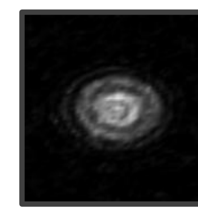
- ➢ evaluation of defocusing image spreading
- ➢ astigmatic imaging
- ➢ **rotating double helix PSF** ➡

- ○ instantaneous depth estimation (direct image detection) ✔
- ○ narrow range of localisation ✘

❑ Digital holographic microscopy (DHM)

(*Inherently 3D resolving technique*)

- ➢ reconstruction of axial intensity
- ➢ peak searching algorithms
- ➢ 3D deconvolution methods



- ○ extended range of localisation ✔
- ○ time-consuming successive reconstructions and iterative algorithms ✘

Digital holography with vortex localisation

Optical microscopy with double helix PSF

- based on filtration in Fourier domain
- optical implementation of 4-f system needed
- rotation evaluated by image intensity detection (**fast processing**, restrictions on PSF engineering)
- **high accuracy** – must be in compromise with localisation range $\Delta z \sim \lambda/NA^2$ x $L \sim \lambda/NA^2$

Digital holographic microscopy

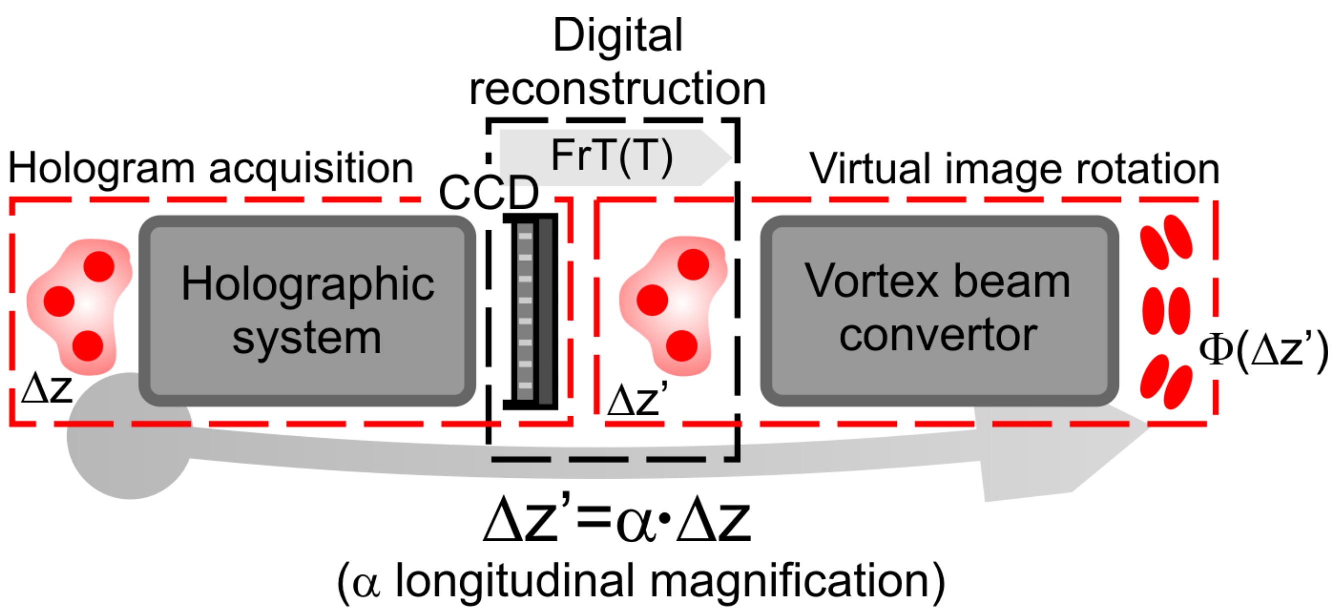
- **wide localisation range**
- lower accuracy
- time-consuming processing

New idea: DIGITAL VORTEX BEAM HOLOGRAPHY

- all-digital vortex implementation
- processing of a single in-line hologram without phase shifting
- 3D localisation from single plane hologram reconstruction
- fast processing enabling dynamic localisation and tracking of particles
- accuracy comparable with double helix localisation
- processing of electric field possible – an extreme variability in PSF control

Concept of PSF rotation in digital holography

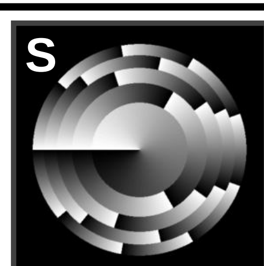
❑ Implementation of rotating PSF: hologram acquisition, standard reconstruction, spiral post-processing.



$$U' = \mathscr{F}^{-1}\{S \cdot \mathscr{F}\{FrT(T)\}\}$$

➢ Rotation occurs in intensity.

- ❖ Spiral modulation in Fresnel zones
- ❖ Interference of defocusing-invariant vortex beams
- ❖ Analogy with the self-imaging
- ❖ Limited rotation control

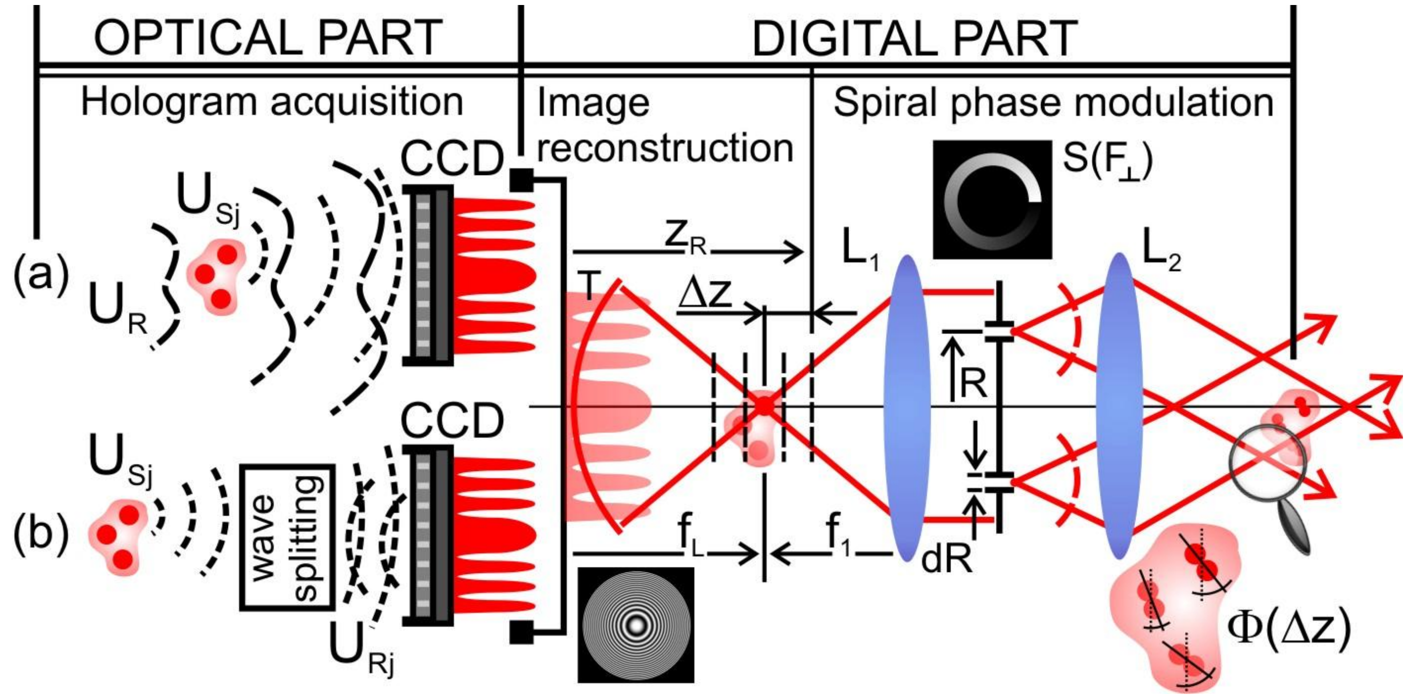


➢ Rotation occurs in electric field.

- ❖ Spiral modulation in single ring
- ❖ Rotating defocusing-invariant vortex beam
- ❖ Analogy with diffraction-free beam
- ❖ Full control of the rotation

Implementation of PSF rotation in digital holography

❑ Technique applicable to: (a) Classical holography, (b) Self correlation imaging.



❑ Processed image field:

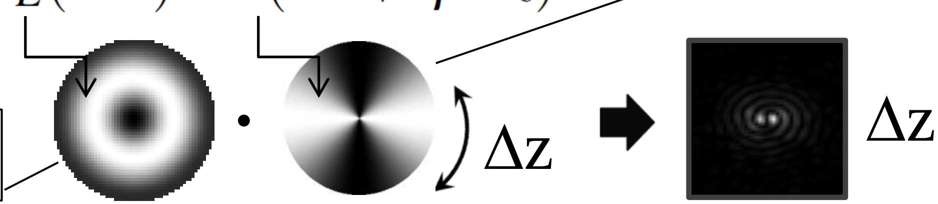
$$U' = \mathscr{F}^{-1}\{S\cdot\mathscr{F}\{FrT(T)\}\}$$

$$e_r \propto \Re(U')$$

❑ Rotating vortex image:

$$e_r \propto J_L(r'\alpha)\cdot\cos(i\Phi + i\beta\Delta z)$$

Defocusing-induced rotation

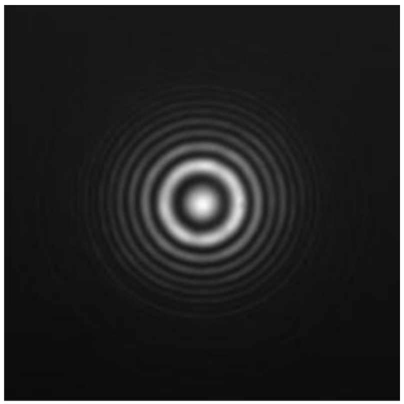


Defocusing-invariant shape

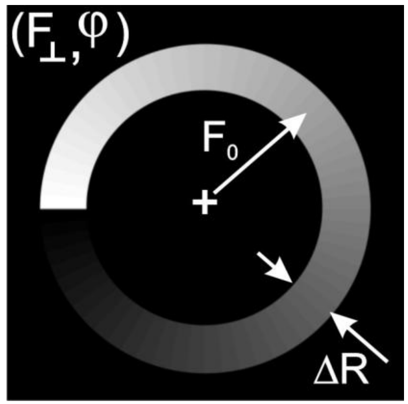
Bessel function (L>1) is azimuthally modulated, resulting in rotating PSF with multiple symmetry.

PSF scaling and control of rotation rate

Point hologram



Spiral phase filter



$$S(\mathbf{F}_\perp) = \delta\left(|\mathbf{F}_\perp| - F_0\right)\exp(il\varphi)$$

Single radial frequency

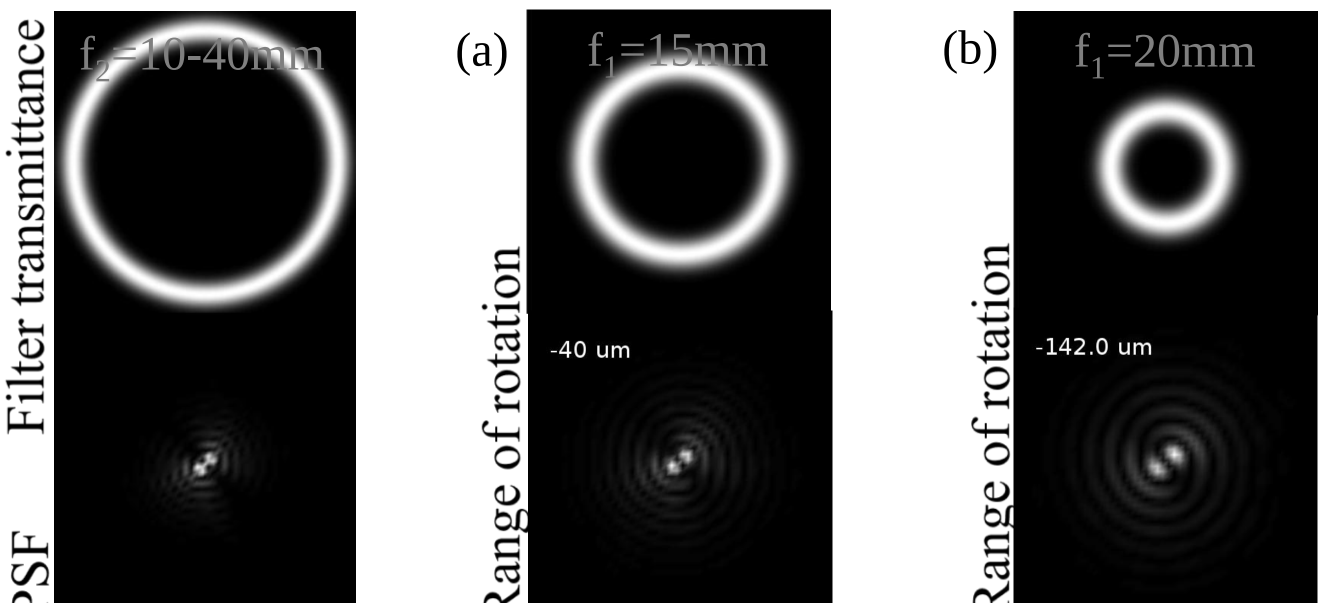
spiral phase

Parameters of the vortex image

$$e_r \propto J_L(r'\alpha)\cos(i\Phi + i\beta\Delta z), \text{ where}$$

$$\alpha = \frac{2\pi F_0}{\lambda f_2} \quad \text{and} \quad \beta = \frac{\pi F_0^2}{\lambda f_1^2}.$$

By α, β the PSF can be scaled and the rotation rate controlled.



Filter parameters can be adjusted in order to satisfy particular experimental conditions.

PSF size: $\Delta r' = \dfrac{r'_{L,1}\lambda f_2}{\pi F_0}$

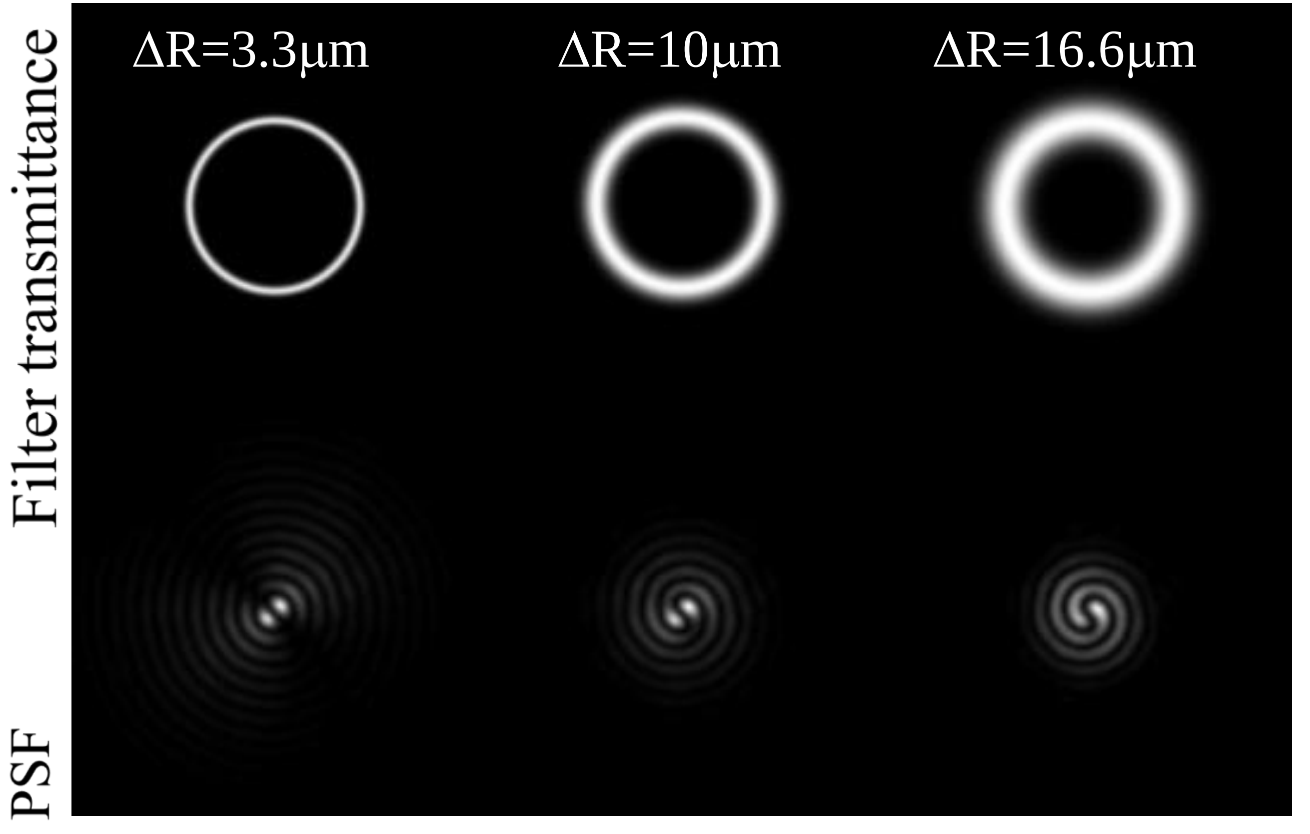
Rotation period: $\Delta Z = \dfrac{\lambda f_1^2}{F_0^2}$

PSF shaping and change of axial range

- Finite width and Gaussian apodization of the filter results in:

  - Image spot analogous to the Bessel-Gauss beam is created
  - Side lobes of the Bessel profile are attenuated
  - The axial range available for localisation is changed

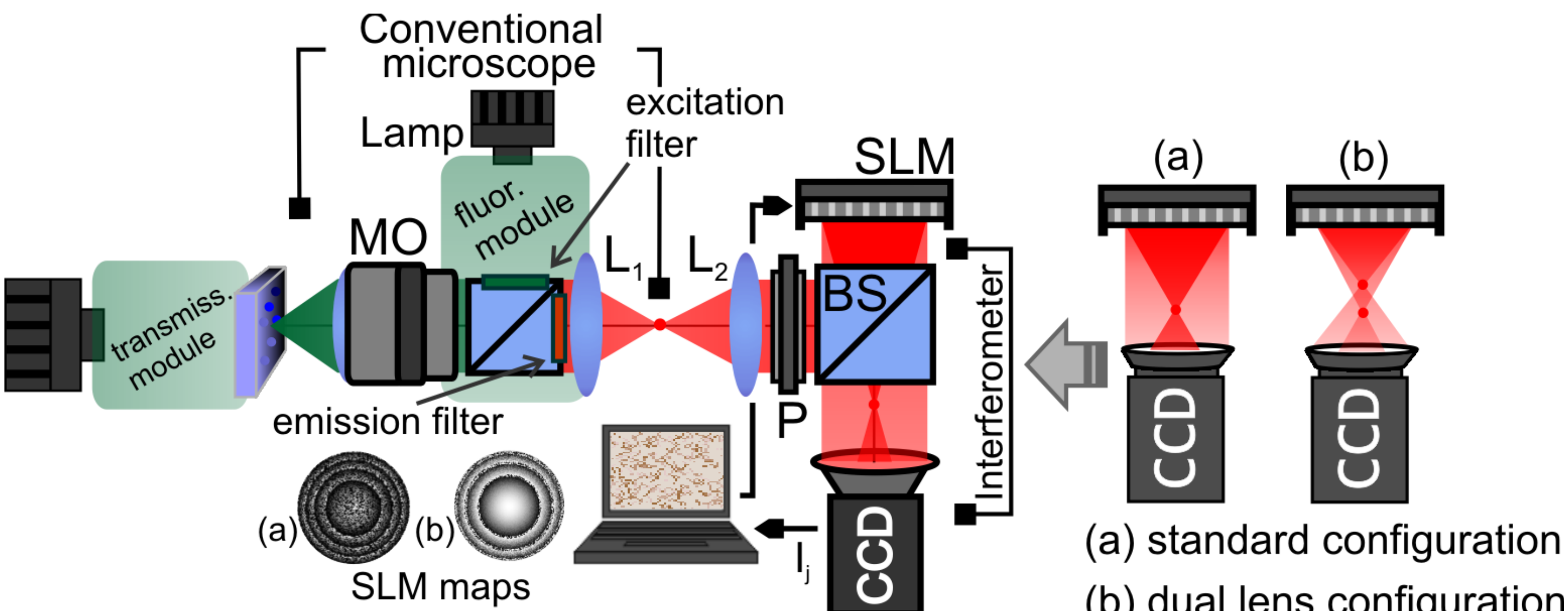
Gaussian apodization – FWHM ΔR



Fresnel incoherent correlation holography

- ❑ Experimental demonstrations were carried out in the system for the Fresnel Incoherent Correlation Holography (FINCH) [1,2].

- ➢ Conventional microscope connected to relay lenses L1, L2 [3] and a common-path interferometr.
- ➢ Wave splitting realized by a Spatial Light Modulator (SLM).
- ➢ Correlation records of incoherently illuminated 3D object captured at the CCD



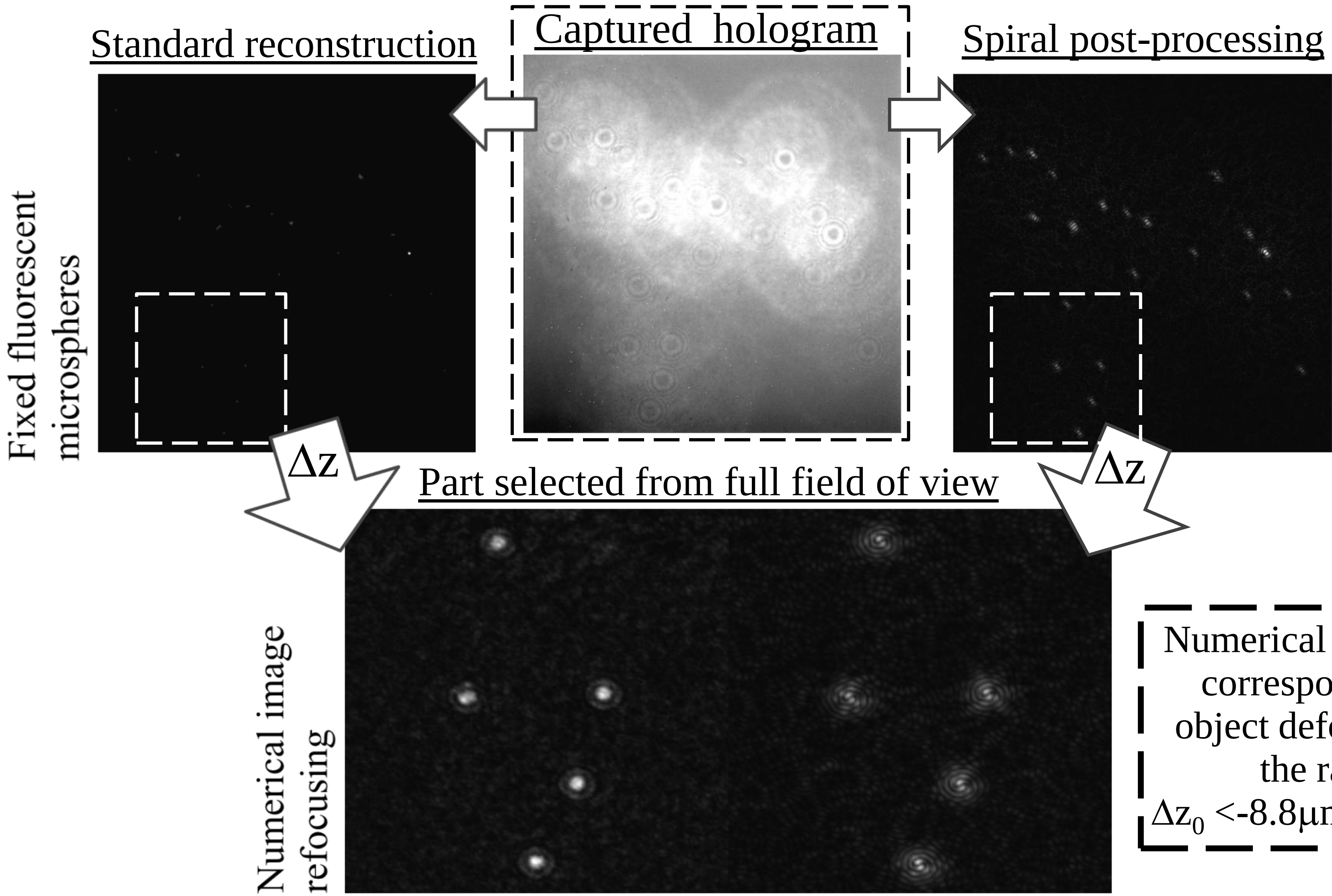
[1] J. Rosen and G. Brooker, "*Digital spatially incoherent Fresnel holography*," Opt. Lett. **32**, 912-914 (2007).

[2] B. Katz, J. Rosen, R. Kelner, and G. Brooker, "*Enhanced resolution and throughput of Fresnel incoherent correlation holography (FINCH) using dual diffractive lenses on a spatial light modulator (SLM)*," Opt. Express **20**, 9109-9121 (2012).

[3] P. Bouchal and Z. Bouchal, "*Wide-field common-path incoherent correlation microscopy with a perfect overlapping of interfering beams*," J. Europ. Opt. Soc. Rap. Public. **8**, 13011 (2013).

Experimental results

Sample: fluorescent focal check test, fixed microspheres-5μm in diameter, excit./emiss.-543nm/582nm.
Experimental configuration: microscope objective NA=0.55, standard FINCH (f=400mm).



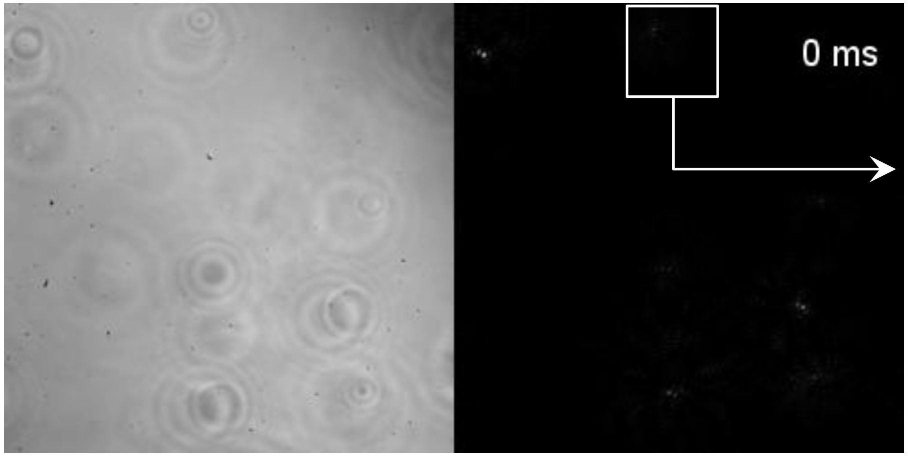
Numerical refocusing corresponding to object defocusing in the range Δz_0 <-8.8μm ; 8.8μm> .

Experimental results

Sample: suspension of polystyrene microspheres-0.5μm in diameter.
Experimental configuration: microscope objective NA=0.5, standard FINCH (f=400mm).

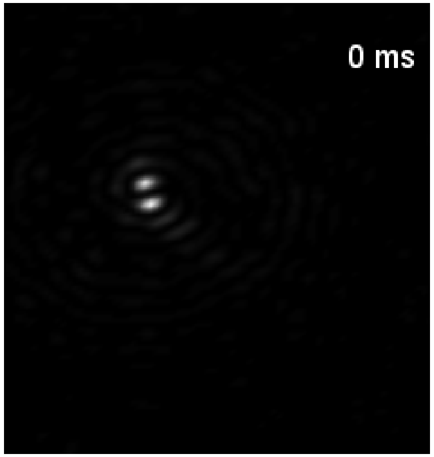
Hologram

Reconstruction with spiral post-proccessing

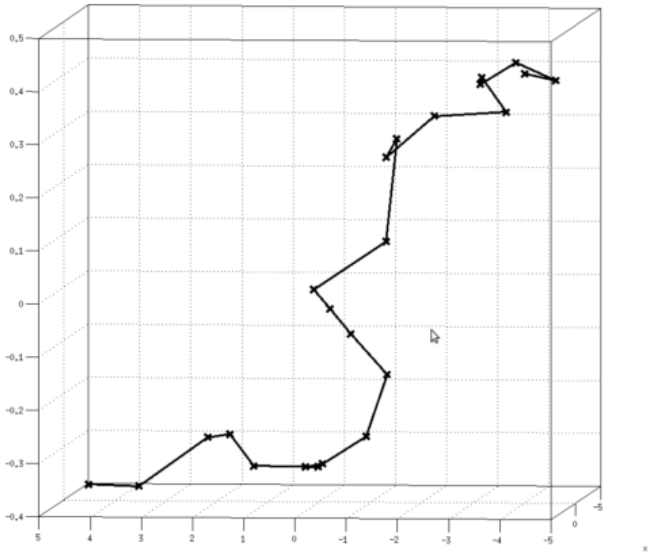


*Part selected from full field of view

Selected particle



3D trajectory



Range:
x <-5μm ; 4.7μm>
y <-2.8μm ; 4.6μm>
z <-6.6μm ; 7.6μm>

Experimental demonstration of dynamic, high resolution capabilities of proposed method.

Conclusions

❑ The benefits of the PSF rotation in inherently 3D resolving systems were discussed and demonstrated.

❑ The holographic axial localisation using the rotating diffraction-free vortex beams was examined.

❑ It was verified that just one reconstruction of a single in-line hologram is sufficient for 3D particle localisation.

❑ The proposed techniques of digital vortex holography were experimentally demonstrated by real-time localisation of fluorescent and polystyrene beads of sub-micrometer size.

The results presented were obtained in a close cooperation between the Brno University of Technology and the Palacký University Olomouc.

This work was supported by the project CEITEC - ”Central European Institute of Technology” No. CZ.1.05/1.1.00/02.0068 from European Regional Development Fund, the project No. TE01020229 of the Technology Agency of the Czech Republic and the IGA project of the Palacký University PrF 2014-14.

Thank you for attention.