Numerical Analysis of Tightly Focused Beams for Confocal Microscopy Illumination by Real Lens Systems

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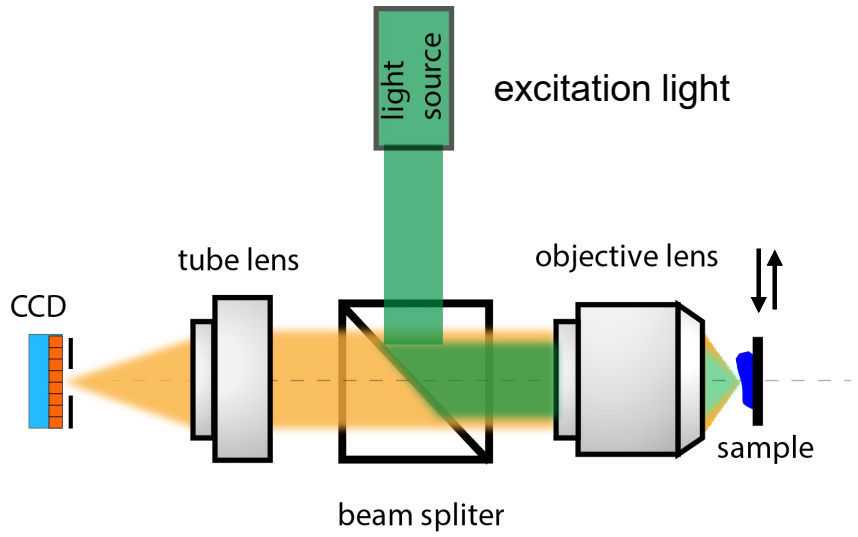
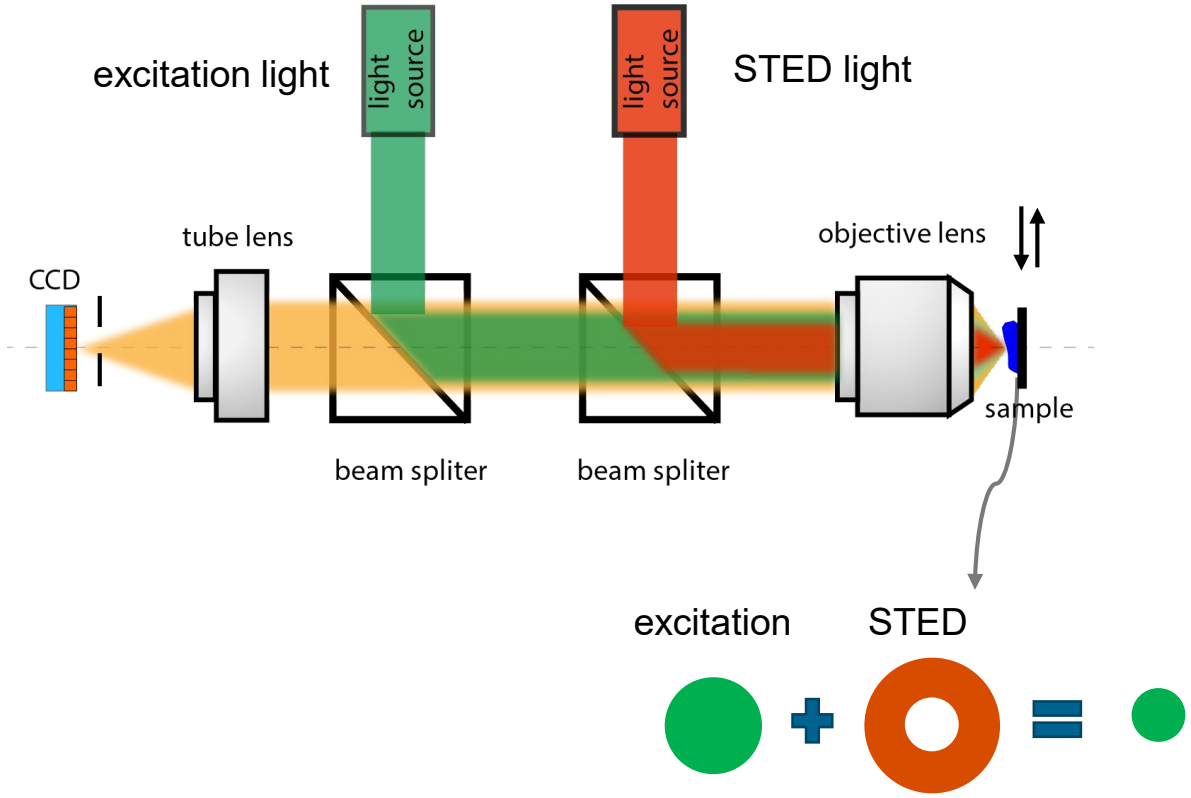
Background: Conventional Confocal Microscopy and STED

Conventional Confocal Microscopy

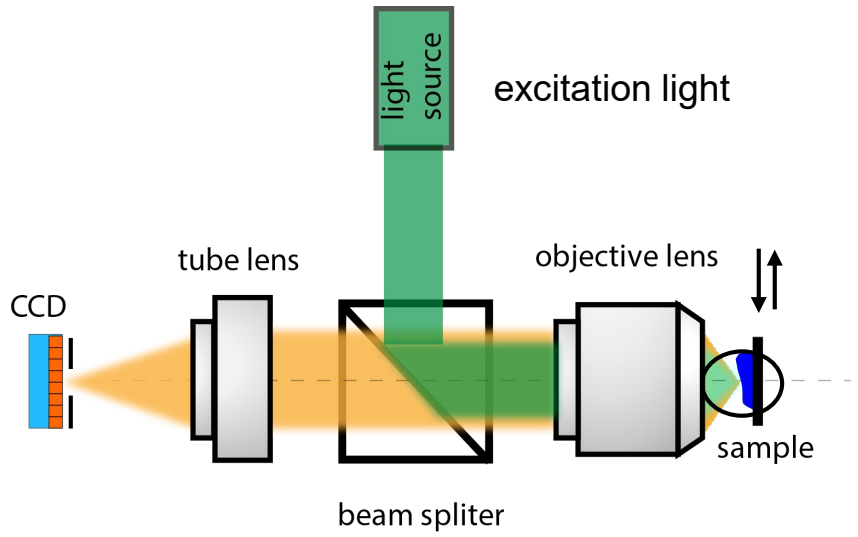


STimulated Emission Depletion (STED)

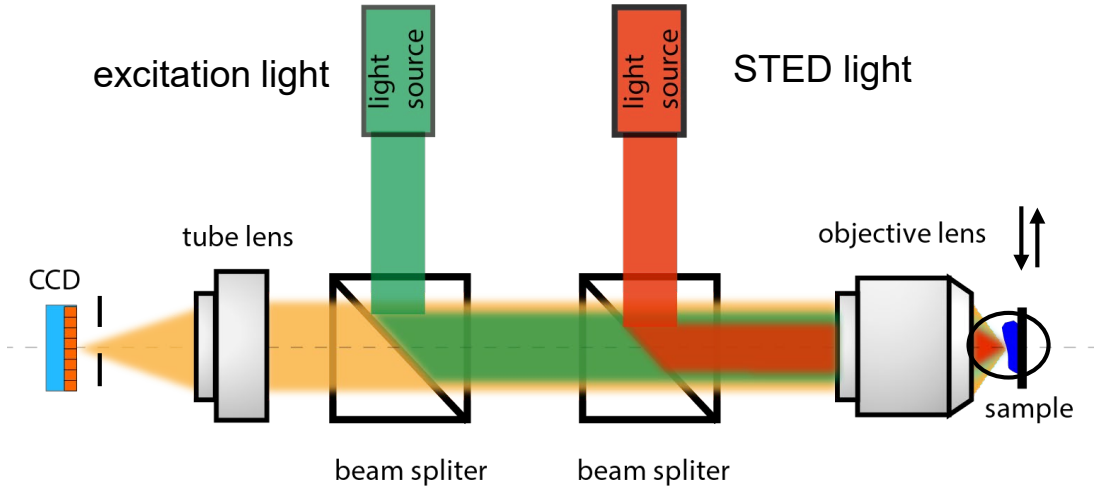


Motivation: Conventional Confocal Microscopy and STED

Conventional Confocal Microscopy



STimulated Emission Depletion (STED)



- Illumination spot is essential for analyzing the image quality.
- Ray tracing is very limited. The polarization, diffraction need to be included.
- Debye-Wolf integral is widely used, but very often limited to idealized aplanatic lens without aberration.

What is the illumination spot when a real lens is applied?

Definition of Quantities

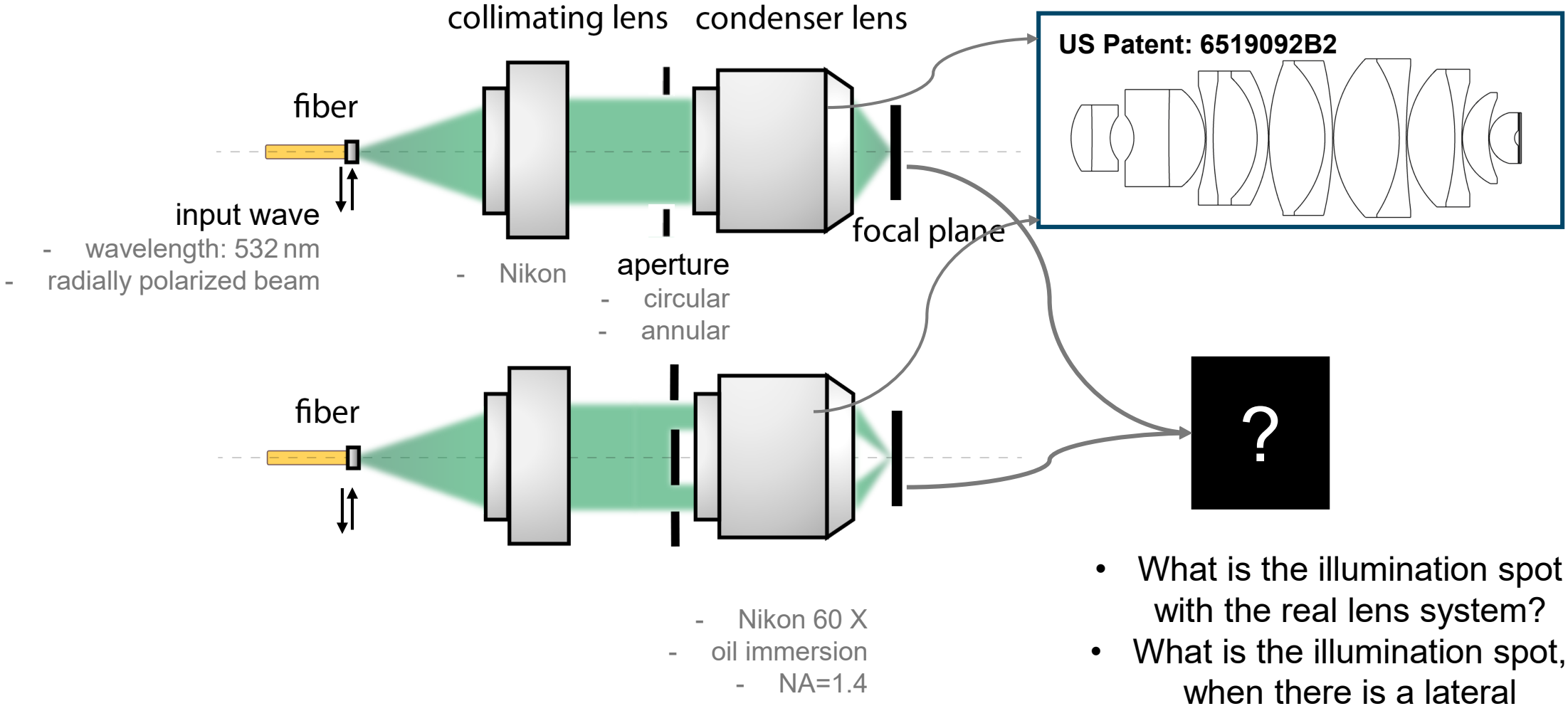
- Illumination spot is defined as the electric energy density:

$$w_e \propto \|\boldsymbol{E}\|^2 = |E_x|^2 + |E_y|^2 + |E_z|^2,$$

- Deviation:

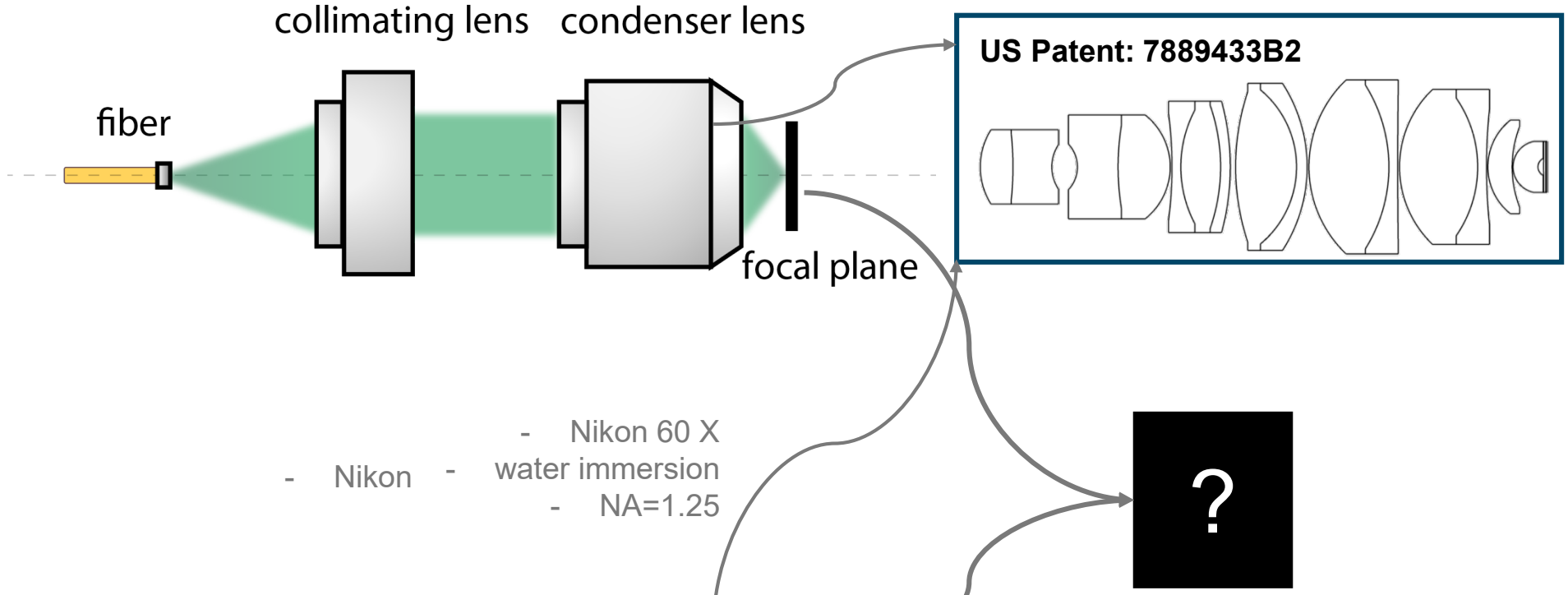
$$\sigma = \frac{\sum_{x,y} |f^{\mathrm{Eva}}(x,y) - f^{\mathrm{Ref}}(x,y)|^2}{\sum_{x,y} |f^{\mathrm{Ref}}(x,y)|^2}$$

Modeling Task 1: Conventional Confocal Microscopy



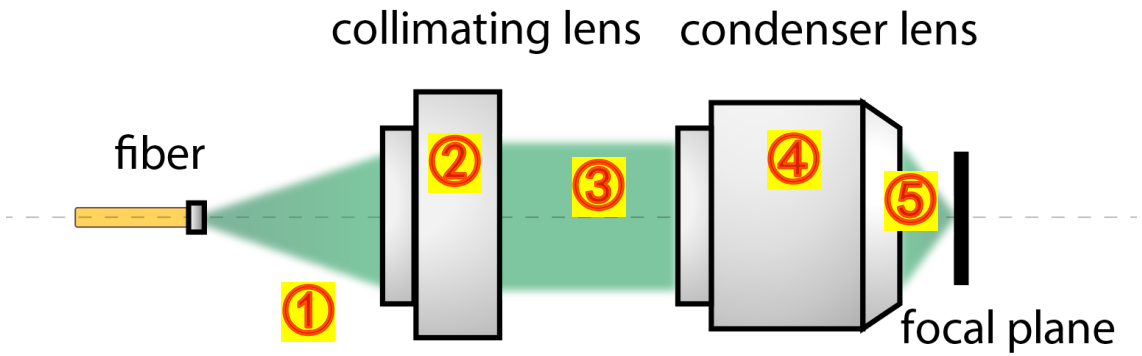
- What is the illumination spot with the real lens system?
- What is the illumination spot, when there is a lateral misalignment of the fiber?

Modeling Task 2: STED



- What is the illumination spot, considering the chromatic aberration?
- Is the axial shift of the source able to compensate it?

Fully Vectorial Modeling in the Framework of Field Tracing

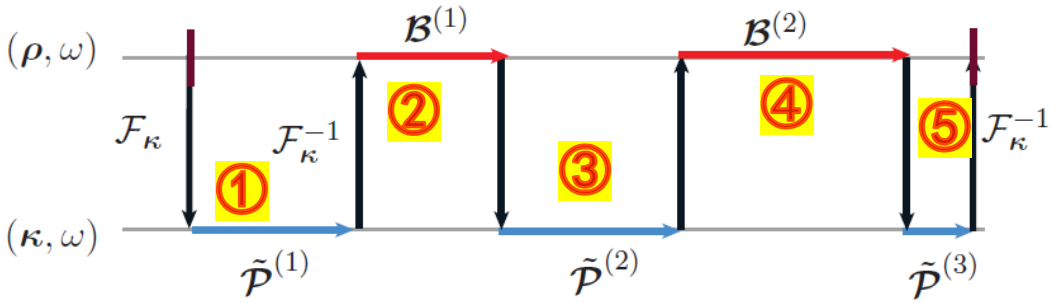


Space domain

$\boldsymbol{\rho} = (x, y)$

Fourier domain

$\boldsymbol{\kappa} = (k_x, k_y)$



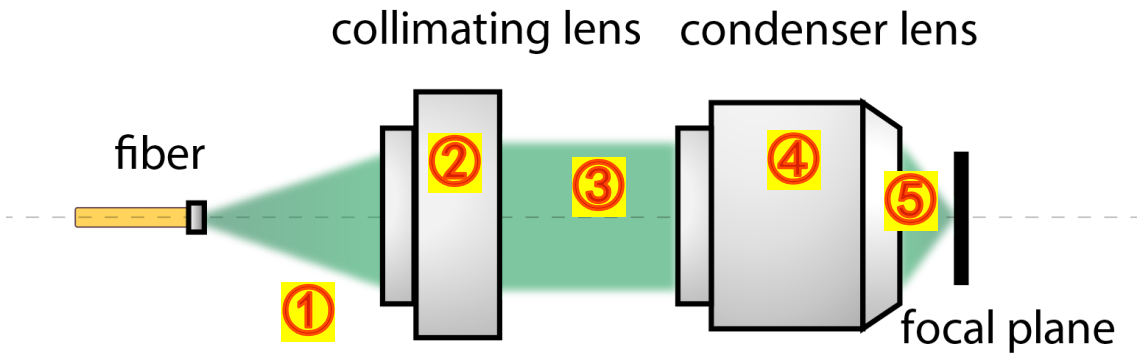
Free Space Propagation (FSP) | Local Plane Interface Approximation (LPIA)

① ③ ⑤ | ② ④

S. Zhang et al. Appl. Opt. (2016)

A. Pfeil et al., Appl. Opt. (2000)

Fully Vectorial Modeling in the Framework of Field Tracing



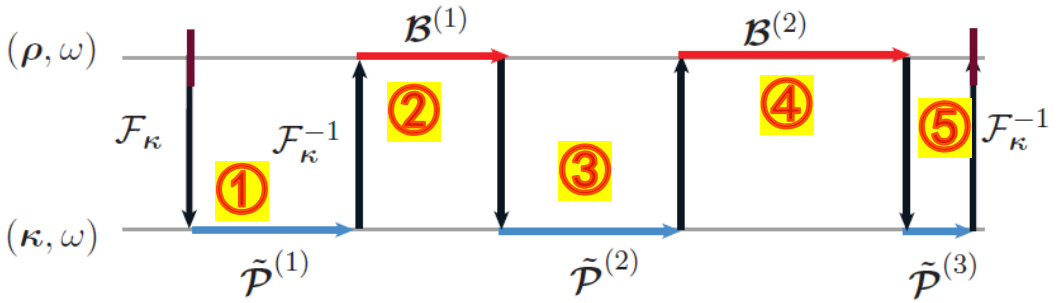
The excitation light follows the same logic, the only difference is the source

Space domain

$\boldsymbol{\rho} = (x, y)$

Fourier domain

$\boldsymbol{\kappa} = (k_x, k_y)$



Free Space Propagation (FSP) — Local Plane Interface Approximation (LPIA)

① ③ ⑤ — ② ④

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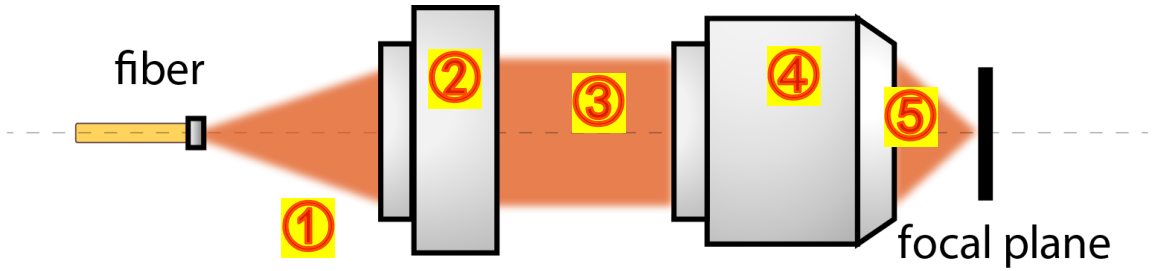
Fully Vectorial Modeling in the Framework of Field Tracing



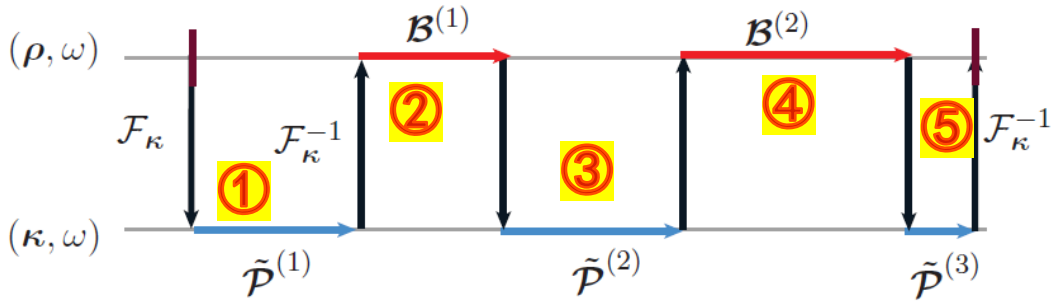
The STED light follows the same logic, the only difference is the source

Space domain

$\boldsymbol{\rho} = (x, y)$

Fourier domain

$\boldsymbol{\kappa} = (k_x, k_y)$



Free Space Propagation (FSP) — ① ③ ⑤

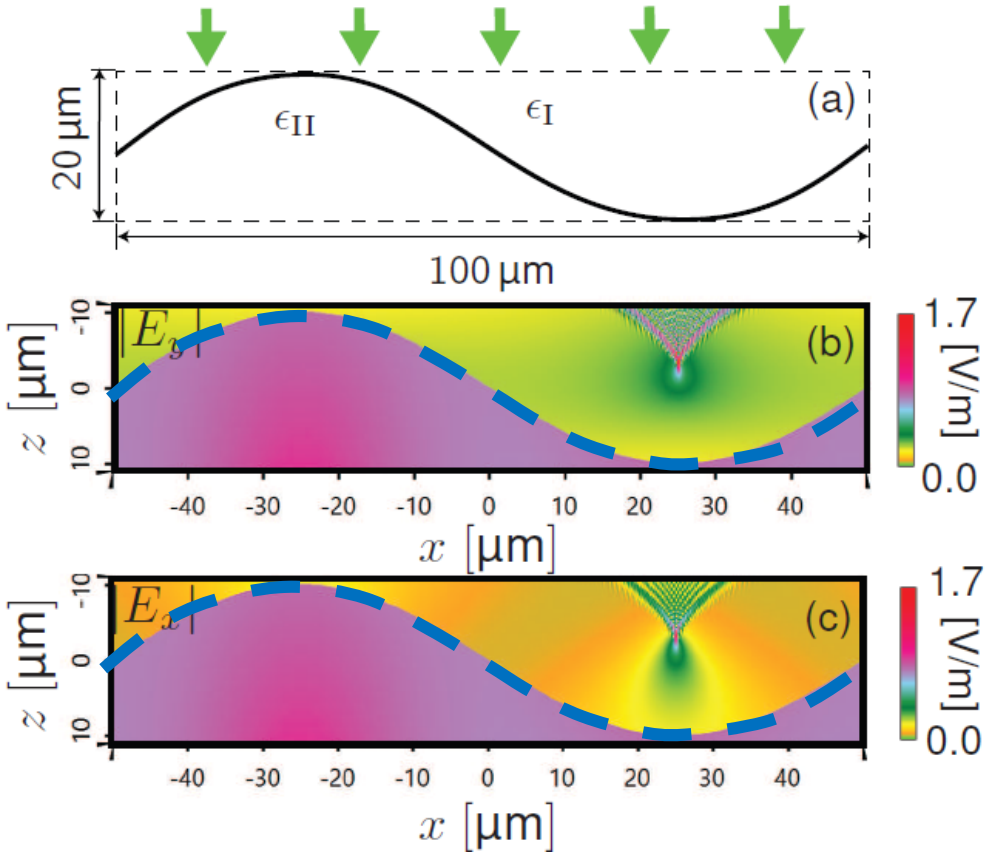
Local Plane Interface Approximation (LPIA) — ② ④

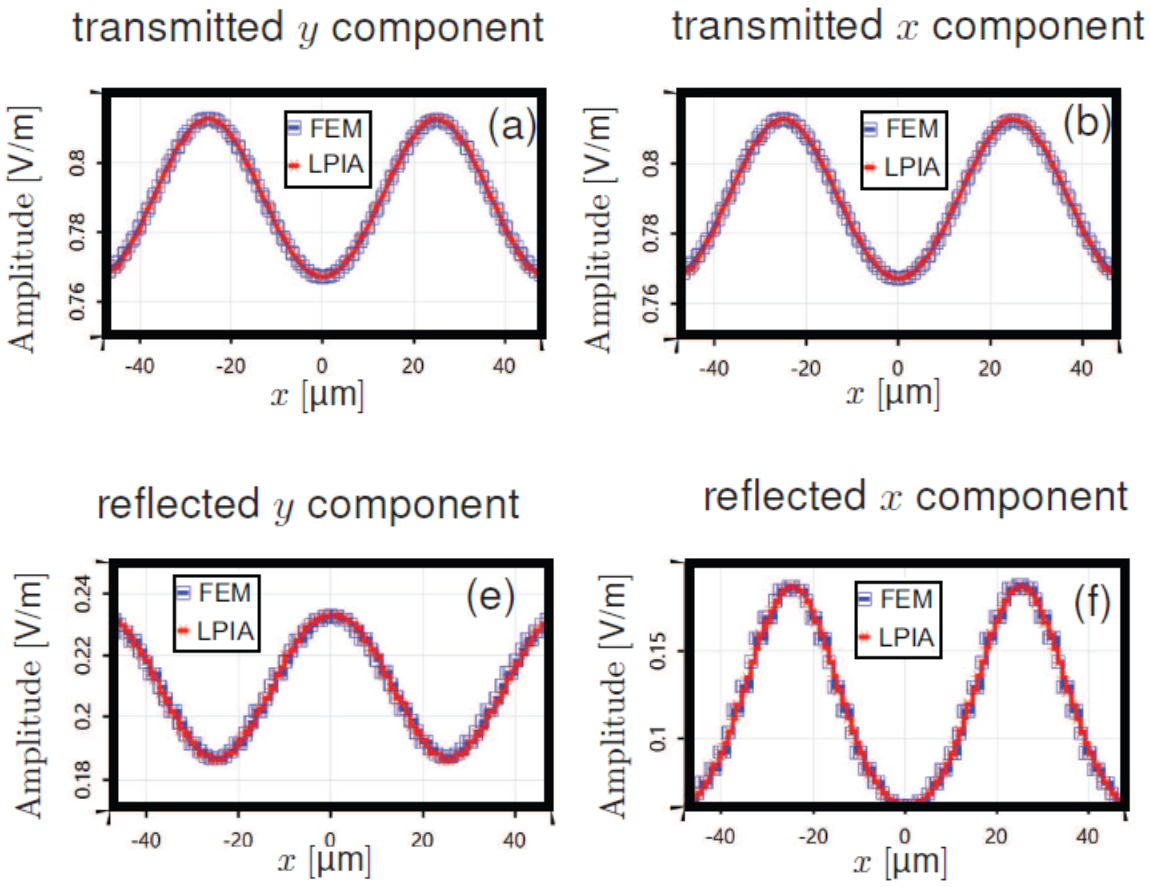
S. Zhang et al. Appl. Opt. (2016)

A. Pfeil et al., Appl. Opt. (2000)

Validation of LPIA

Results by FEM via JCMSuite





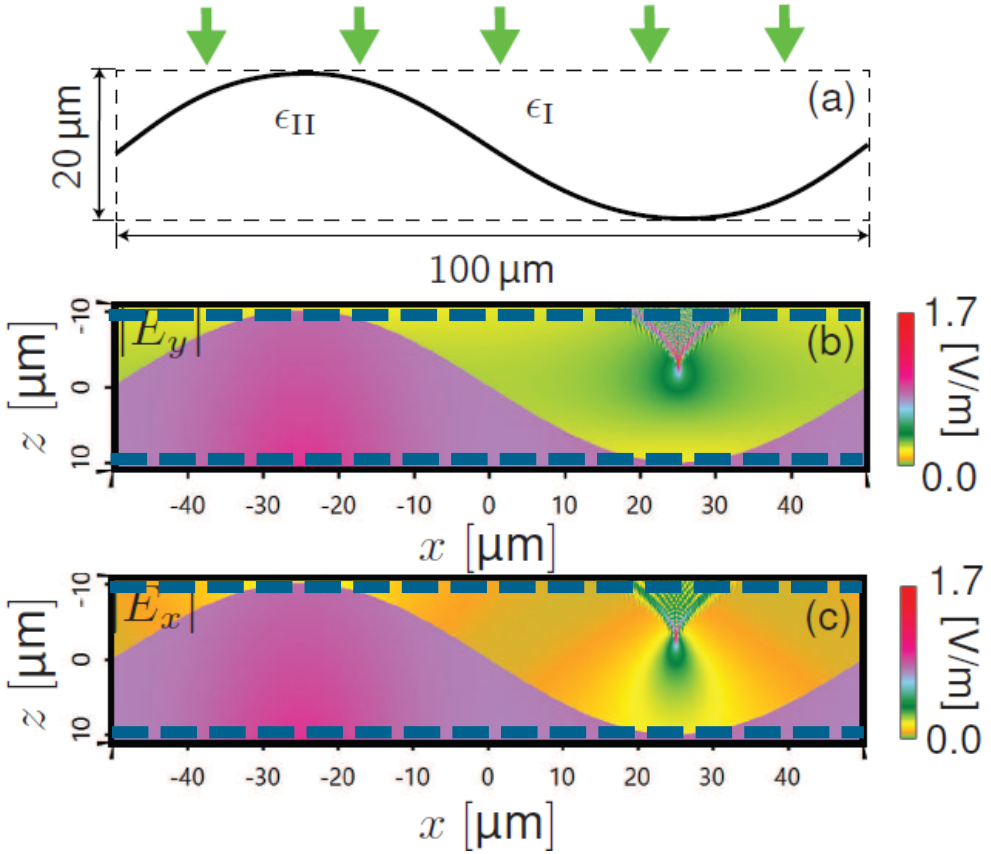
Time of LPIA: <1 s

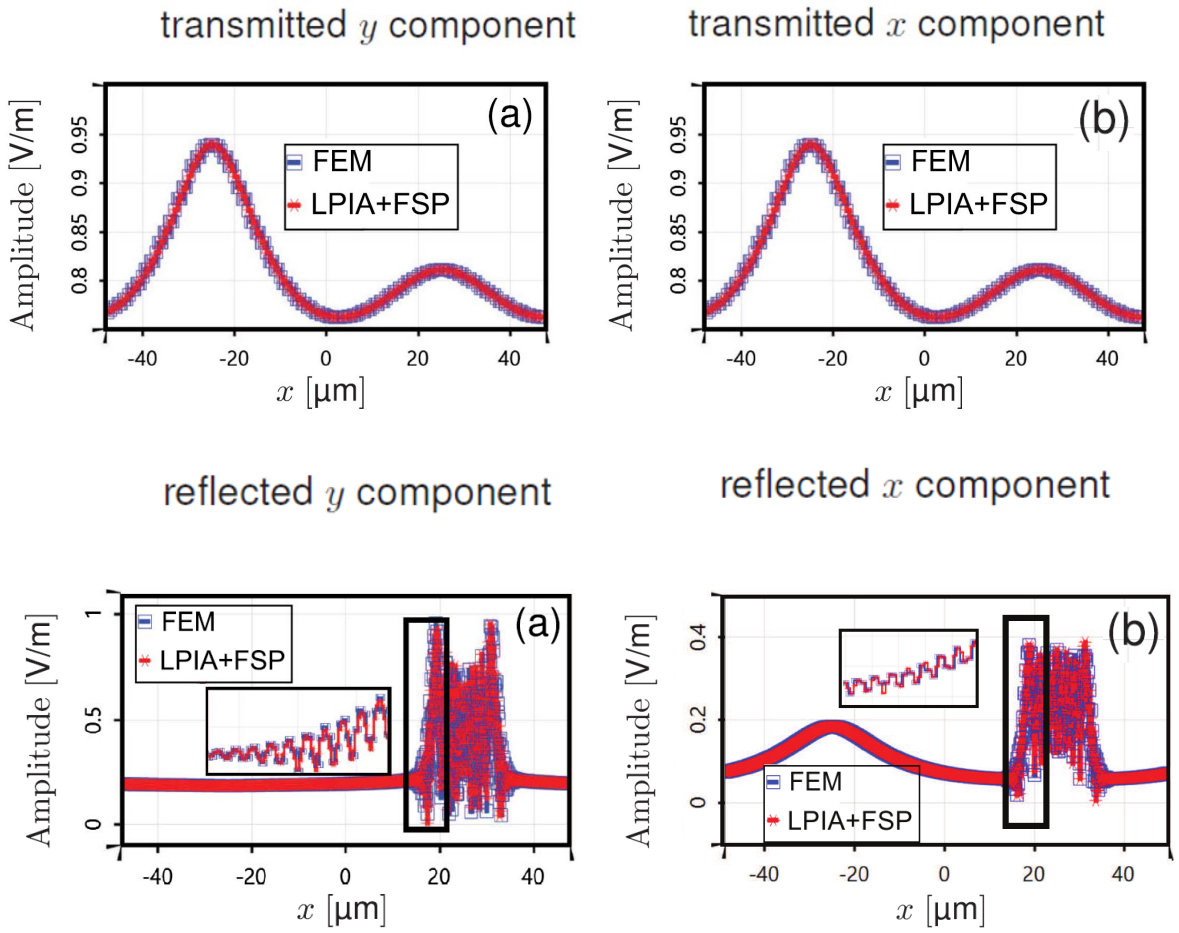
Time of FEM: ~20 mins

R. Shi, C. Hellmann, and F. Wyrowski, J. Opt. Soc. Am. A **36**, (2019).

Validation of LPIA

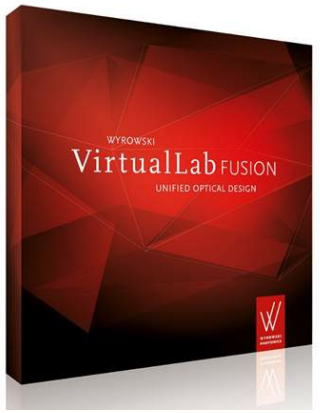
Results by FEM via JCMSuite





Time of LPIA+FSP: <1 s Time of FEM: ~20 mins

R. Shi, C. Hellmann, and F. Wyrowski, J. Opt. Soc. Am. A **36**, (2019).

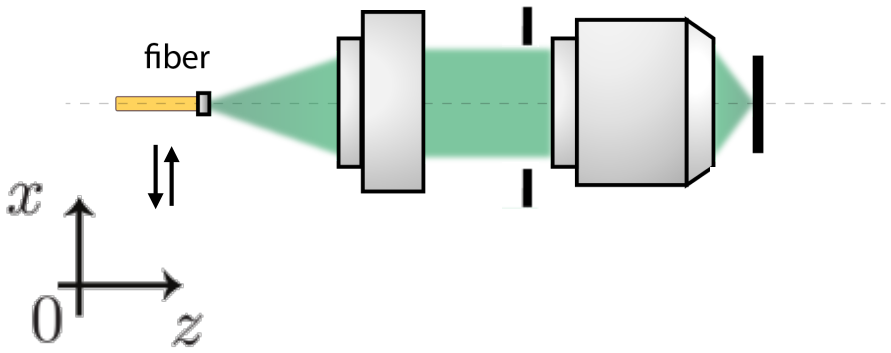


LIGHTTRANS

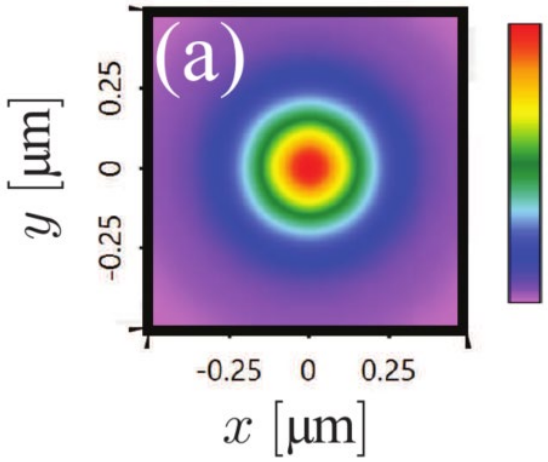
Simulation Results via VirtualLab Fusion

Conventional Confocal Microscopy

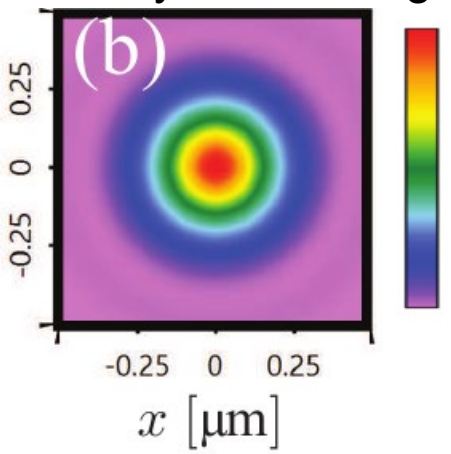
Focal Spot by Radially Polarized Beam



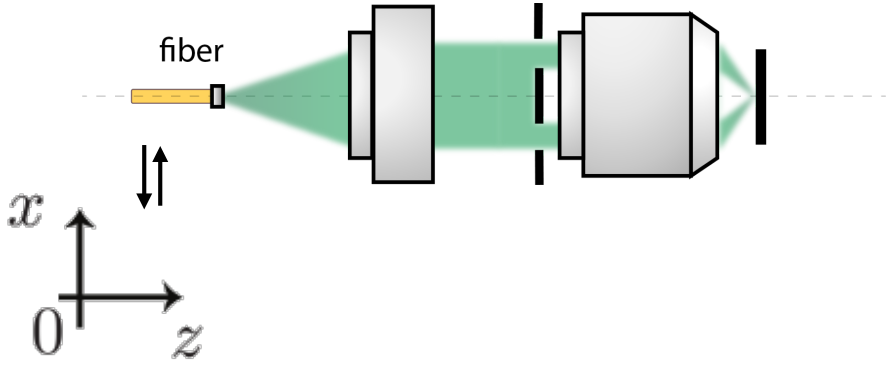
Real Lens System

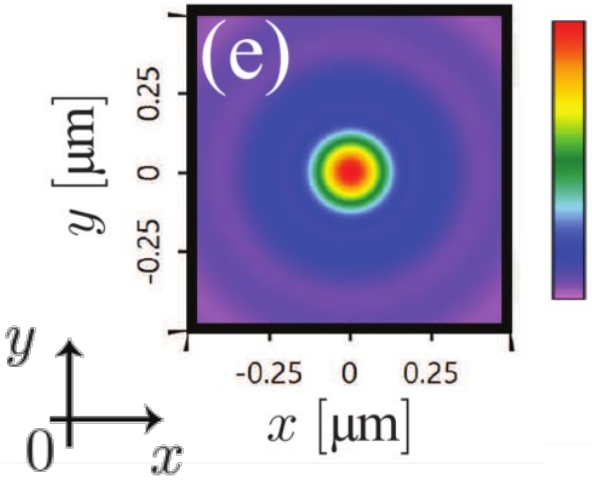


Idealized Aplanatic Lens
via Debye-Wolf integral

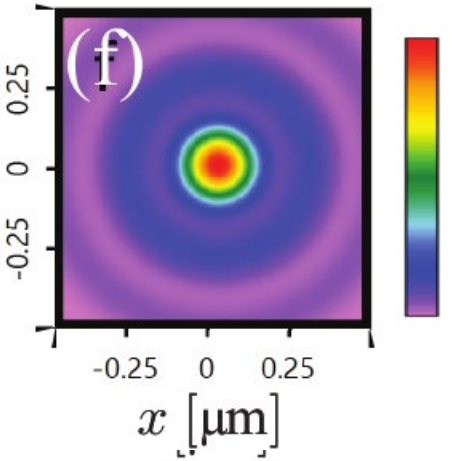


$\sigma < 1\%$



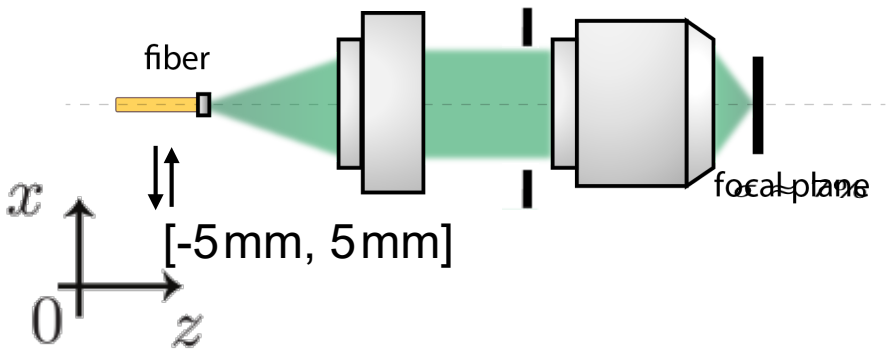


$\sigma < 1\%$

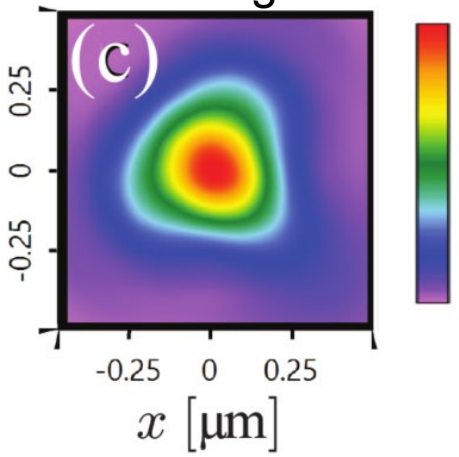


several seconds

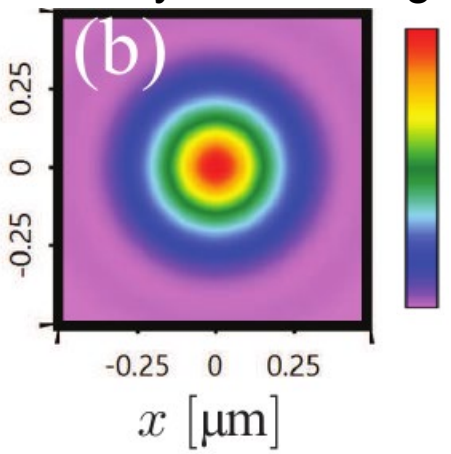
Distorted Focal Spot with Lateral Shift of Source



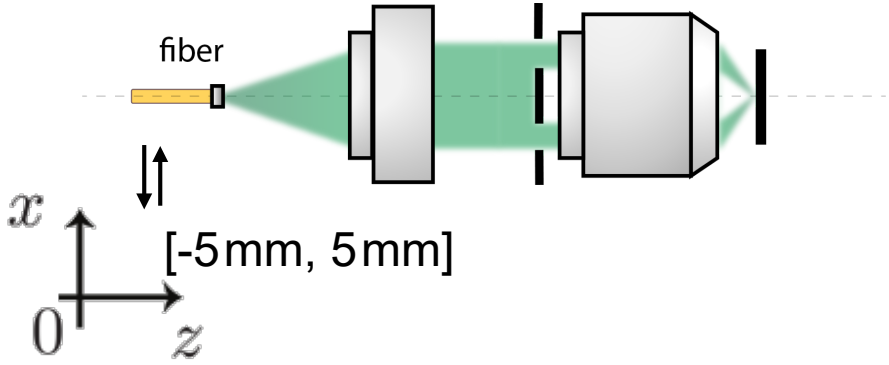
Real Lens System
with misalignment

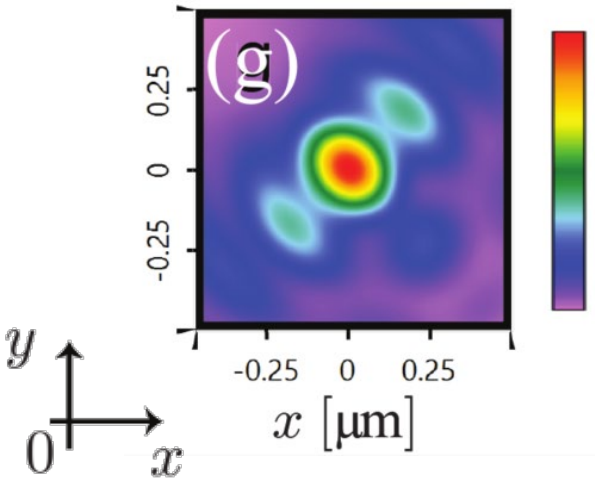


Idealized Aplanatic Lens
via Debye-Wolf integral

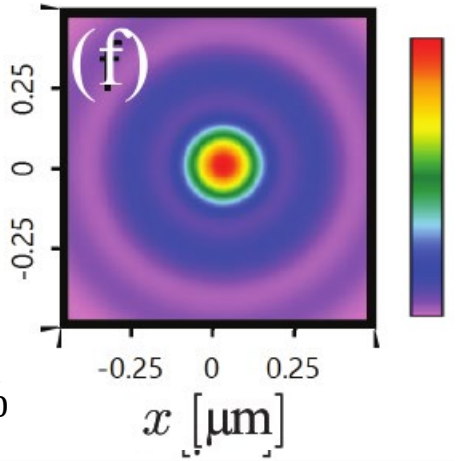


$\sigma \approx 7\%$



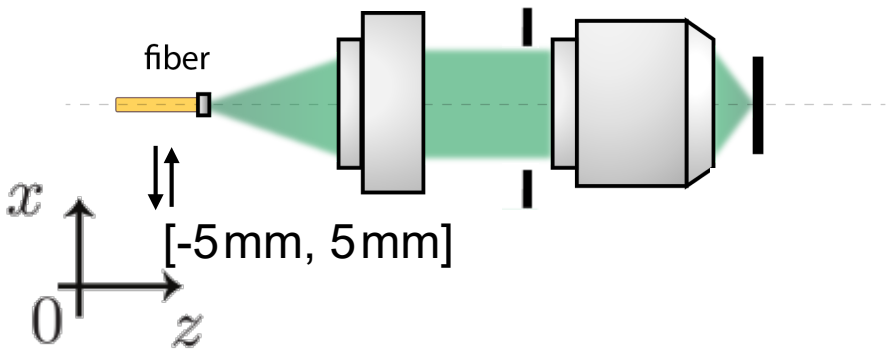


$\sigma \approx 16\%$

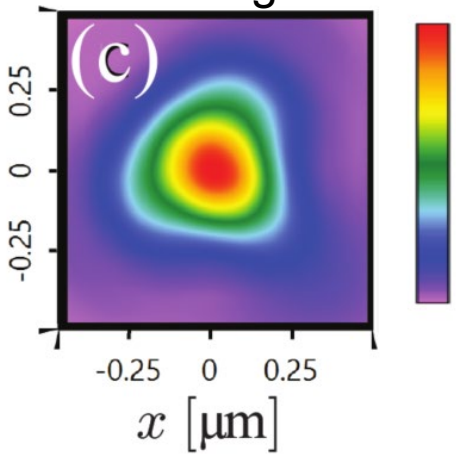


several seconds

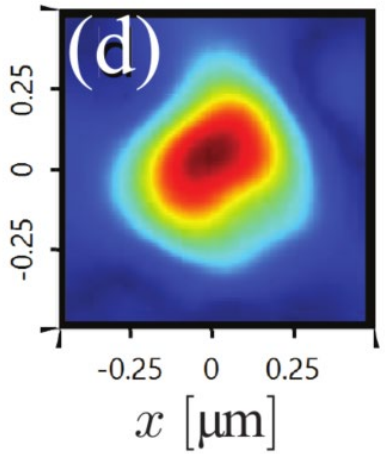
Distorted Focal Spot V.S. Experimental Results

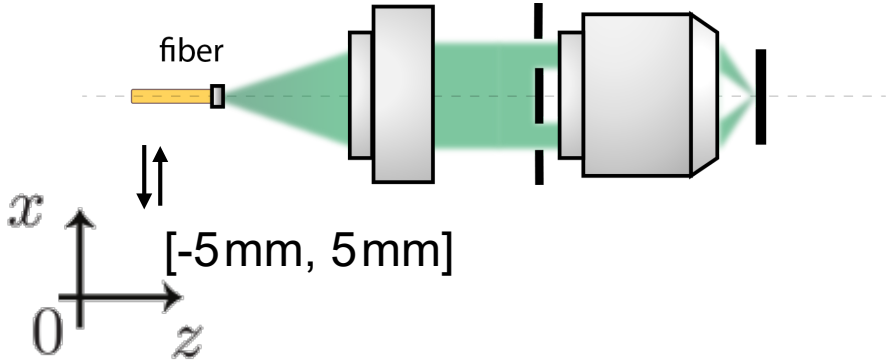


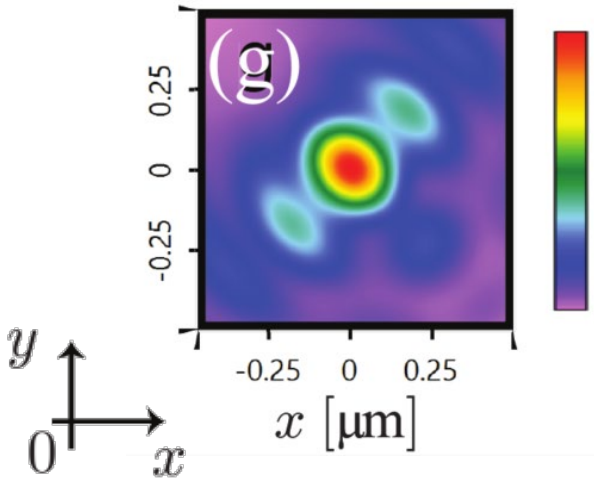
Real Lens System
with misalignment

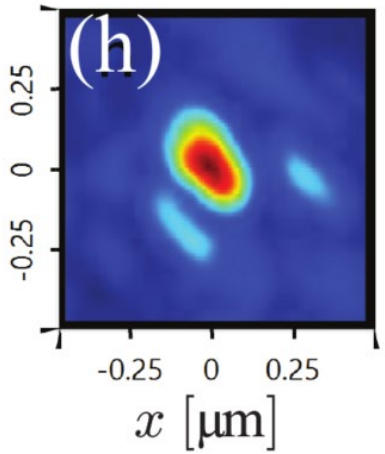


Experimental Results





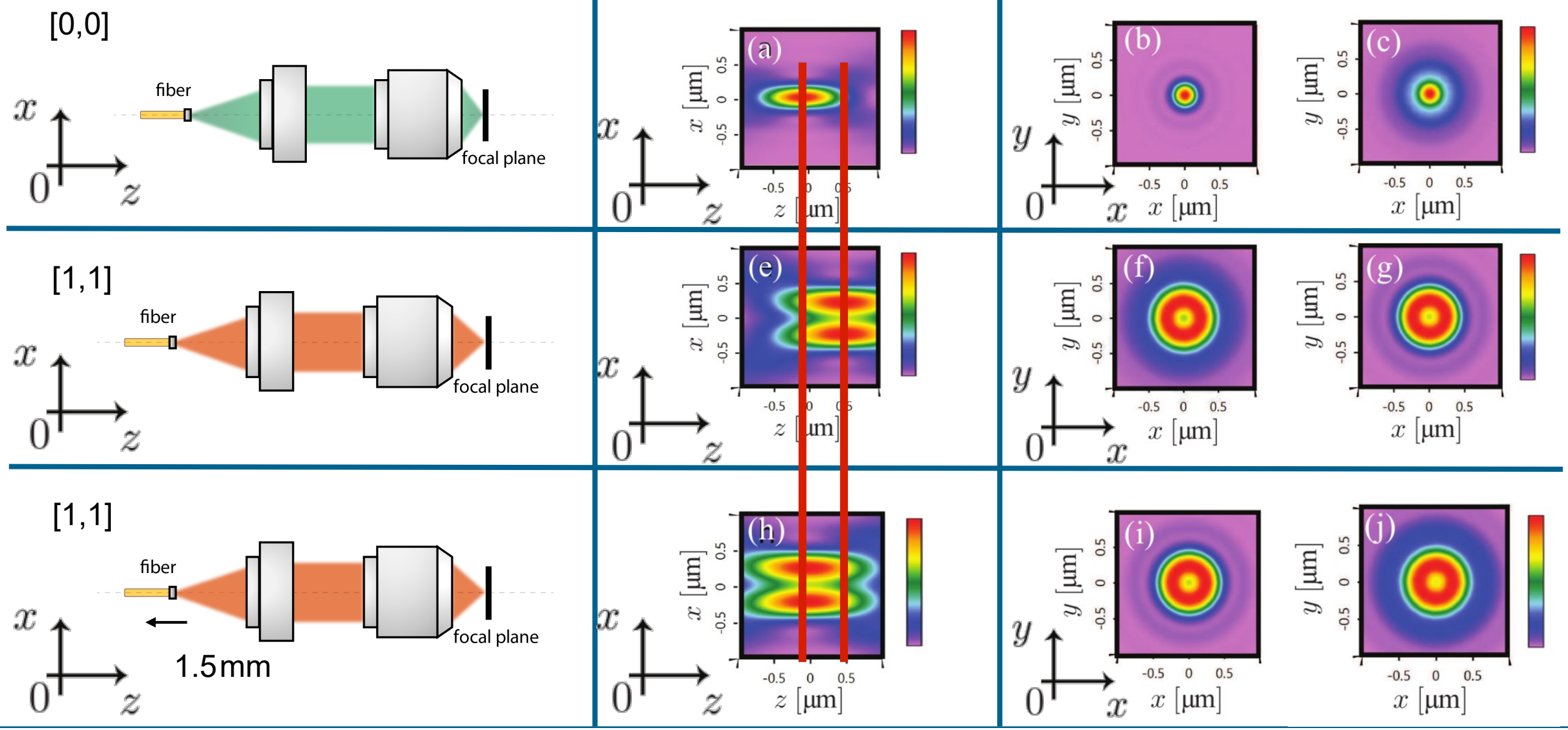




[Yang et al. (2013)]

STED

Focal Spot by Circularly Polarized Gaussian Laguerre Beam



Summary and Conclusion

- We do the fully vectorial physical-optics modeling of the complex illumination system for conventional confocal microscopy and STED.

- We analyze the influence of the focal spot with lateral misalignment of the source for the conventional confocal microscopy. And we find that it influences more in the case of annular aperture.

- We analyze the influence of the chromatic aberration for the STED. And we find that it can be compensated by the axial shift of the STED source.

Thanks!