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)

excitation light  STED light

excitation light
Motivation: Conventional Confocal Microscopy and STED

Conventional Confocal Microscopy

- 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.

STimulated Emission Depletion (STED)

What is the illumination spot when a real lens is applied?
Definition of Quantities

- Illumination spot is defined as the electric energy density:

\[ w_e \propto \|E\|^2 = |E_x|^2 + |E_y|^2 + |E_z|^2, \]

- Deviation:

\[ \sigma = \frac{\sum_{x,y} |f^{Eva}(x,y) - f^{Ref}(x,y)|^2}{\sum_{x,y} |f^{Ref}(x,y)|^2} \]
Modeling Task 1: Conventional Confocal Microscopy

- Input wave:
  - Wavelength: 532 nm
  - Radially polarized beam

- Collimated lens:
  - Nikon

- Condenser lens:
  - Aperture: circular, annular

- Object plane:
  - Nikon 60 X
  - Oil immersion
  - NA = 1.4

- 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?

- Input wave: Gaussian-Laguerre \([0,0]\)
  - Wavelength: 558 nm
  - Circularly polarized beam
  - [P. Török and P.R.T Monro, (2004)]

- Fiber

- Collimating lens

- Condenser lens

- Nikon
- Water immersion
- NA=1.25


- Input wave: Gaussian-Laguerre \([1,1]\)
  - Wavelength: 775 nm
  - Circularly polarized beam
  - [P. Török and P.R.T Monro, (2004)]

- Fiber

- Nikon 60 X

- 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

\[ \boldsymbol{\mathbf{\mu}} = (\kappa_x, \kappa_y) \]

\[ \boldsymbol{\mathbf{\eta}} = (x, y) \]

Space domain
\[ \boldsymbol{\mathbf{\rho}} = (x, y) \]

Fourier domain
\[ \boldsymbol{\mathbf{\kappa}} = (k_x, k_y) \]

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

Fully Vectorial Modeling in the Framework of Field Tracing

\[ \mathbf{\mu} = (k_x, k_y) \]

\[ \mathbf{\alpha} = (x, y) \]

Space domain
\( \rho = (x, y) \)

Fourier domain
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Free Space Propagation (FSP)
Local Plane Interface Approximation (LPIA)

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

Fully Vectorial Modeling in the Framework of Field Tracing

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

Space domain
\( \rho = (x, y) \)

Fourier domain
\( \kappa = (k_x, k_y) \)

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Validation of LPIA

Results by FEM via JCMSuite

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

Validation of LPIA

Results by FEM via JCMSuite

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

Simulation Results via VirtualLab Fusion
Conventional Confocal Microscopy
Focal Spot by Radially Polarized Beam

Real Lens System

Idealized Aplanatic Lens via Debye-Wolf integral

\( \sigma < 1\% \)

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

[0,0]

[1,1]

[1,1]

1.5mm
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!