Fast Physical-Optics Modeling of Two-Photon Fluorescence Microscopy with 3D Structured Illumination

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Background: Fluorescence Microscopy

Joblonski Diagram

excitation

emission

$h_v_1$

$h_v_2$
Motivation: Higher Resolution & Reducing Out-Of-Focus Light

<table>
<thead>
<tr>
<th>SIM</th>
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<tbody>
<tr>
<td>[Heintzmann et al., Proc. SPIE 1998]</td>
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<td>[Gustafsson, J. Microsc (2000)]</td>
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**ILLUMINATION**

- resolution: ~80 nm
- low power and high speed

**SAMPLE**

very good candidate for living cell

**Two-Photon Floreescence (TPF)**

with Temporal Focusing (TF)

[Zhu et al., Opt. Exp. (2005)]

only excites fluorescent sample near the focal region
Motivation: Higher Resolution & Reducing Out-Of-Focus Light

TF-TPF combined with 3D-SIM

- The interference pattern and the temporal focusing is calculated assuming an ideal system in literature.
- Is this assumption true? What is the influence from a real system?
- Ray tracing is not enough.
- Physical-optics modeling is required to include coherence, interference and diffraction from microstructure.

[Isobe et al., Jap. J. Appl. Phy. (2017)]
Definition of Quantities

- Interference pattern is the intensity which is defined as proportional to the time averaged energy density:

\[ I = \langle I(t) \rangle = \frac{1}{\Delta t} \int_I^{t+\Delta t} I(t) dt \]
\[ \propto \frac{1}{\Delta t} \int_I^{t+\Delta t} \|E(t)\|^2 dt \]

- Inhomogeneity:

\[ \sigma = \frac{I_{\text{max}} - I_{\text{min}}}{I_{\text{max}} + I_{\text{min}}} \]
Modeling Tasks:

What is the interference pattern near the focal region, influenced by the real system?

**Objective lens**
- Nikon 60 X water immersion
- NA=1.25
- effective NA~1.1

**US Patent: 7889433B2**

**Input wave**
- continuous laser: 780 nm
- linearly polarized in x direction

**Blazed grating**
- period: 42 um
- blazed angle: 12°

**Lens 1, 2 and tube lens**
- Thorlab achromatic
- AC254-200-B

What is the temporal focusing, influenced by the real system?

**Input wave**
- pulsed laser: 100 fs
- carrier wavelength: 780 nm
- linearly polarized in y direction
Fully Vectorial Modeling in the Framework of Field Tracing

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

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

Space frequency domain

Fourier domain

Free space propagation

Fourier Modal Method (FMM)

Local Plane Interface Approximation (LPIA)

Fully Vectorial Modeling in the Framework of Field Tracing

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

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

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

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

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

Free space propagation
Fourier Modal Method (FMM)
Local Plane Interface Approximation (LPIA)

Modeling of the temporal focusing follows the same logic.
Validation of LPIA

Results by FEM via JCMSuite

Time of LPIA: <1 s  Time of FEM: ~20 mins
Simulation Results via VirtualLab Fusion
Interference Pattern Near Focal Plane

- inhomogeneity is 0.
Interference Pattern Near Focal Plane

- Ideal system
- Real system with perfect alignment

- Inhomogeneity is 0.
- Inhomogeneity is ~0.

- Computational time is within seconds.
Interference Pattern Near Focal Plane

-理想系统
-实际系统与完美对齐
-实际系统与430μm横向位移

• 不均匀性是0.
• 不均匀性是~0.
• 不均匀性是~0.3
Temporal Distribution Near Focal Plane at Center Point

ideal system

\[ |E_y(z, t)| \]

\[ I \]

\[ \text{FWHM: } \sim 4.8 \mu\text{m} \]
Temporal Distribution Near Focal Plane at Center Point

Ideal system vs. real system with perfect alignment:

- Ideal system: FWHM: ~4.8 μm
- Real system: FWHM: ~5.4 μm

Computational time is within half a minute.
Temporal Distribution Near Focal Plane at Center Point

- Ideal system
- Real system with perfect alignment
- Real system with 1.5 mm lateral shift

\[ |E_y(z, t)| \quad I \quad |E_y(z, t)| \quad I \quad |E_y(z, t)| \quad I \]

- FWHM: \(~4.8 \, \mu m\)
- FWHM: \(~5.4 \, \mu m\)
- FWHM: \(~7.5 \, \mu m\)
Summary, Conclusion and Outlook

- We use the fully vectorial physical-optics modeling of the whole microscopy system with inclusion of the microstructure, e.g. blazed grating.
- The coherence, interference and aberration effects are directly included with a relatively fast modeling speed.

- For perfectly alignment, the lens is well-designed.
- For lateral shift of the objective lens, the inhomogeneity increases for interference pattern. The temporal focusing becomes even wider with excitation of more out-of-focus light.

- The combination of the interference pattern and temporal effects will be investigated in the future.
Thanks!