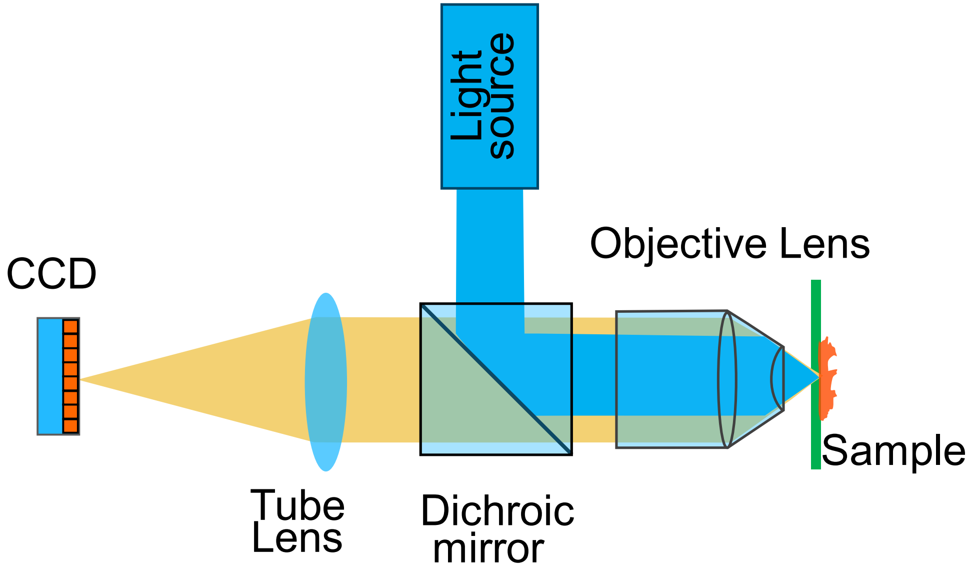
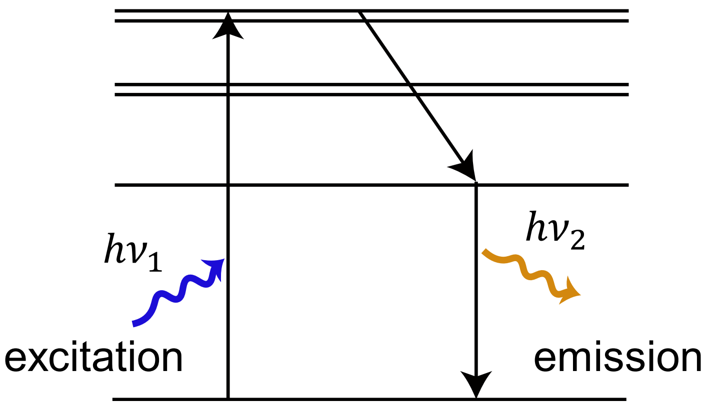
Fast Physical-Optics Modeling of Two-Photon Fluorescence Microscopy with 3D Structured Illumination

Rui Shi[1,2] , Site Zhang[2], Christian Hellmann[3], and Frank Wyrowski[1]
1 Applied Computational Optics Group, Friedrich Schiller University Jena, Jena, Germany,
2 LightTrans International UG, Jena, Germany,
3 Wyrowski Photonics GmbH, Jena, Germany,

Background: Fluorescence Microscopy



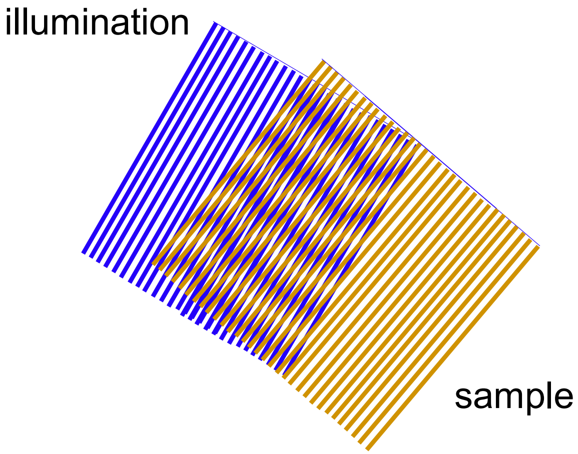
Joblonski Diagram



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

SIM

[Heintzmann et al.,**Proc. SPIE** 1998]
[Gustafsson, **J. Microsc**(2000)]



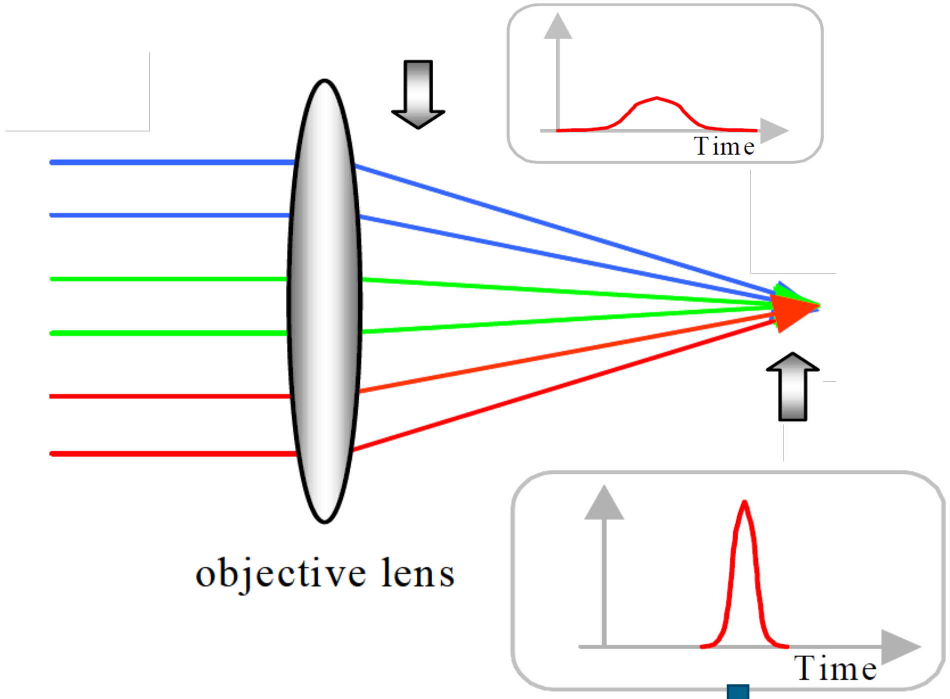
- resolution: ~80 nm
- low power and high speed

⬇

very good candidate for living cell

Two-Photon Florescence (TPF) with Temporal Focusing (TF)

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

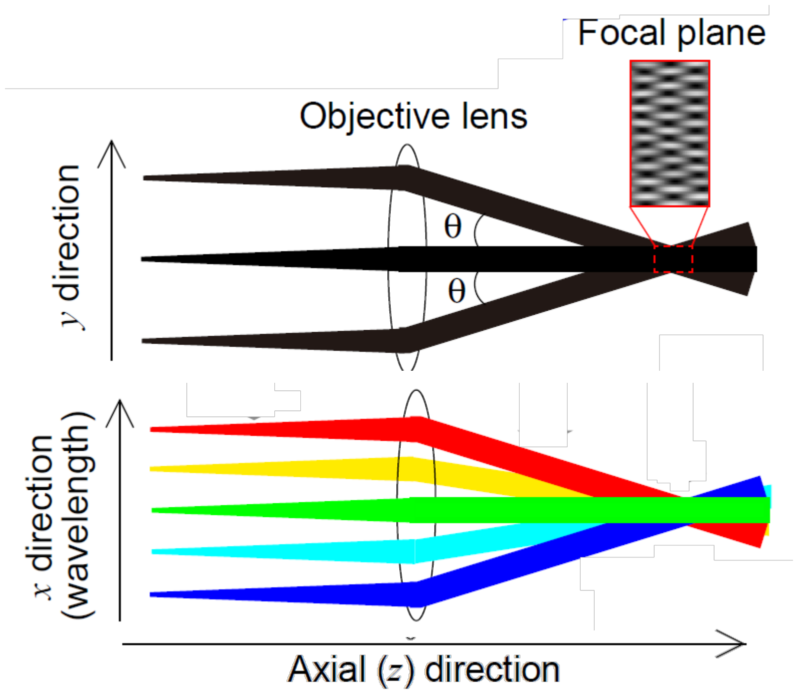


⬇

only excites fluorescent sample near the focal region

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

TF-TPF combined with 3D-SIM



[Isobe et al., **Jap. J. Appl. Phy.** (2017)]

- 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.

Definition of Quantities

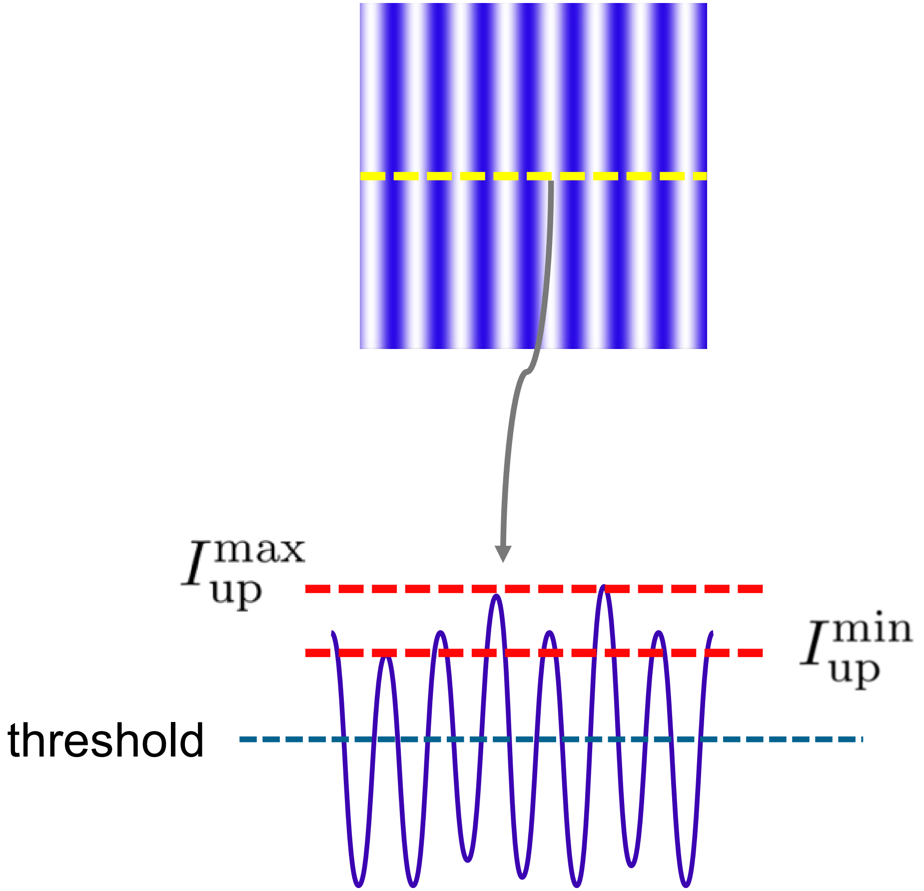
- Interference pattern is the intensity which is defined as proportional to the time averaged energy density:

$$I = < I(t) > = \frac{1}{\Delta t} \int_t^{t+\Delta t} I(t) dt$$

$$\propto \frac{1}{\Delta t} \int_t^{t+\Delta t} \|\boldsymbol{E}(t)\|^2 dt$$

- Inhomogeneity:

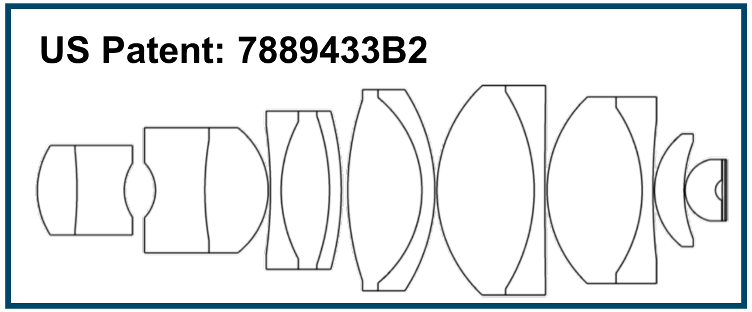
$$\sigma = \frac{I_{\text{up}}^{\text{max}} - I_{\text{up}}^{\text{min}}}{I_{\text{up}}^{\text{max}} + I_{\text{up}}^{\text{min}}}$$



Modeling Tasks:

objective lens

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



blazed grating

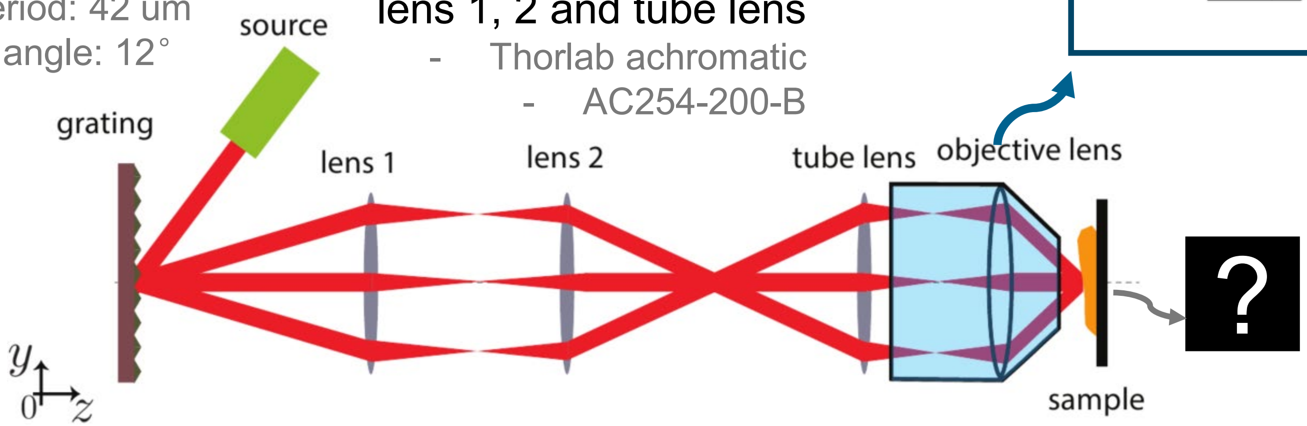
- period: 42 um
- blazed angle: 12°

lens 1, 2 and tube lens

- Thorlab achromatic
- AC254-200-B

input wave

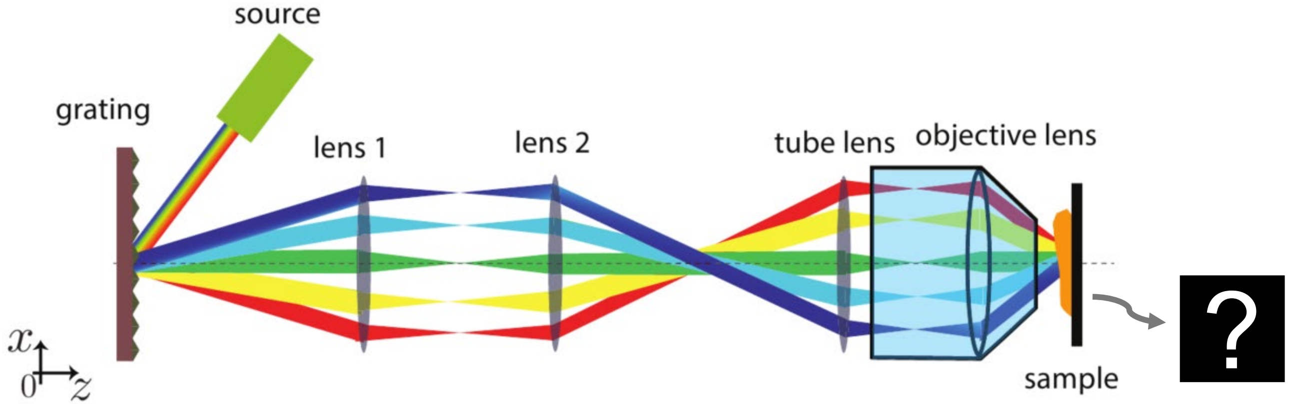
- continuous laser:
- wavelength: 780 nm
- linearly polarized in x direction



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

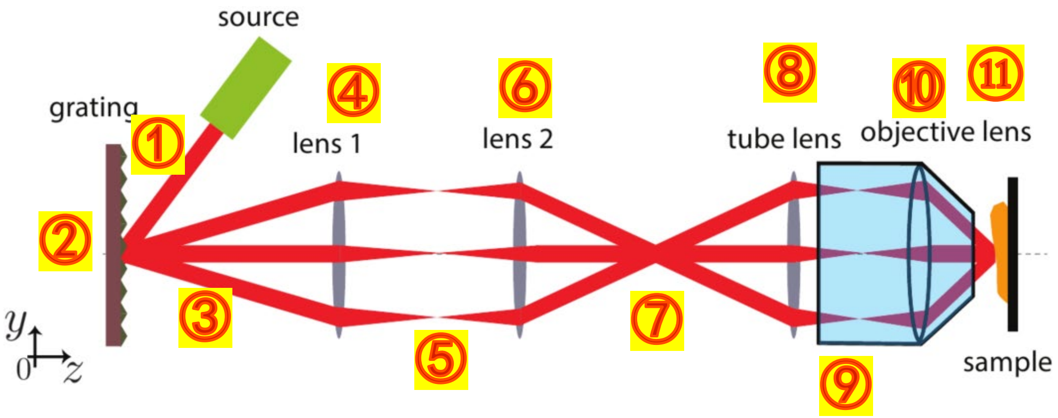
input wave

- pulsed laser: 100 fs
- carrier wavelength: 780 nm
- linearly polarized in y direction



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

Fully Vectorial Modeling in the Framework of Field Tracing

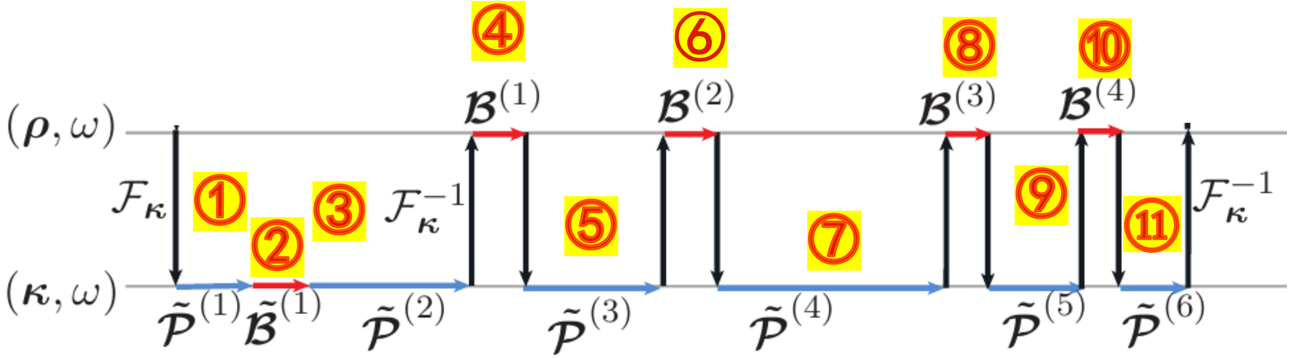


Space frequency domain

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

Fourier domain

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



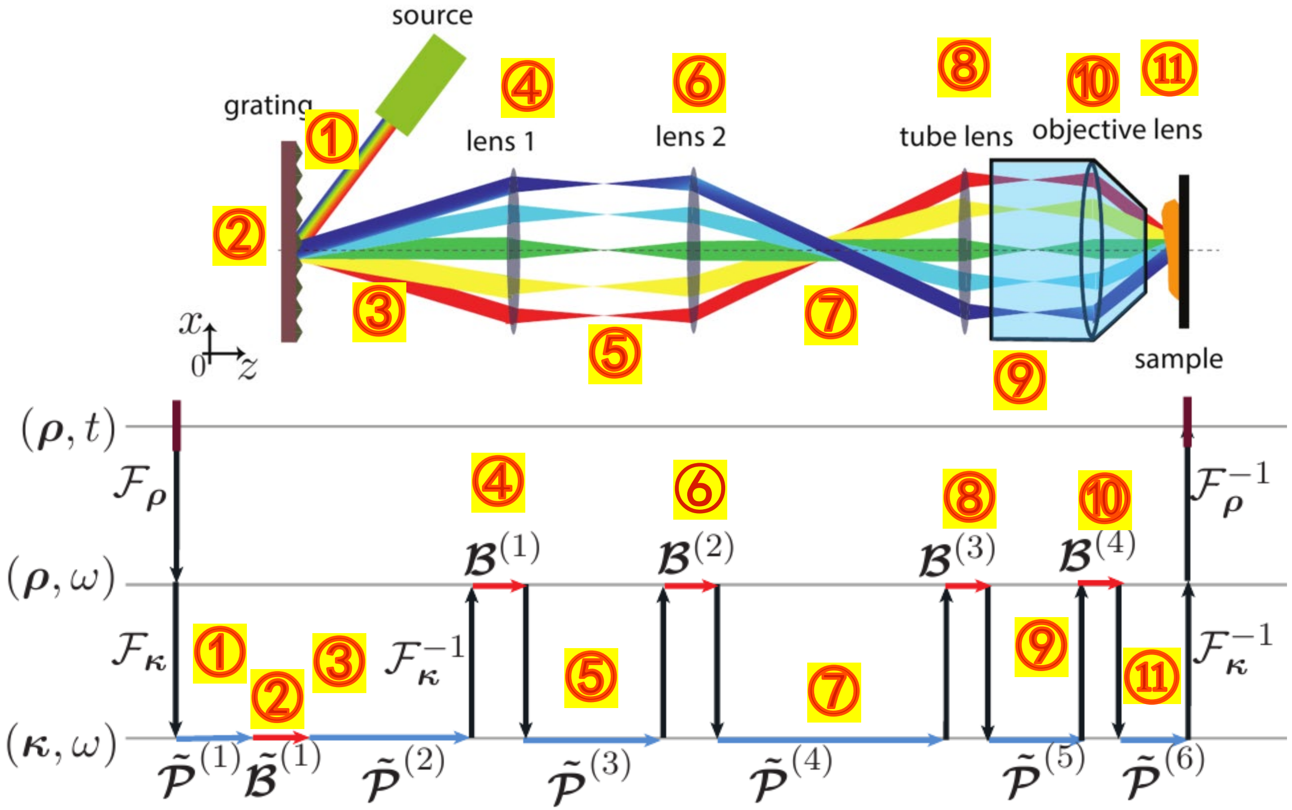
S. Zhang et al. Appl. Opt. (2016)

M. G. Moharam et al. Opt. Soc. Am. A (1995)

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

Free space propagation	Fourier Modal Method (FMM)	Local Plane Interface Approximation (LPIA)
① ③ ⑤ ⑦ ⑨ ⑪	②	④ ⑥ ⑧ ⑩

Fully Vectorial Modeling in the Framework of Field Tracing



Space time domain

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

Space frequency domain

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

Fourier domain

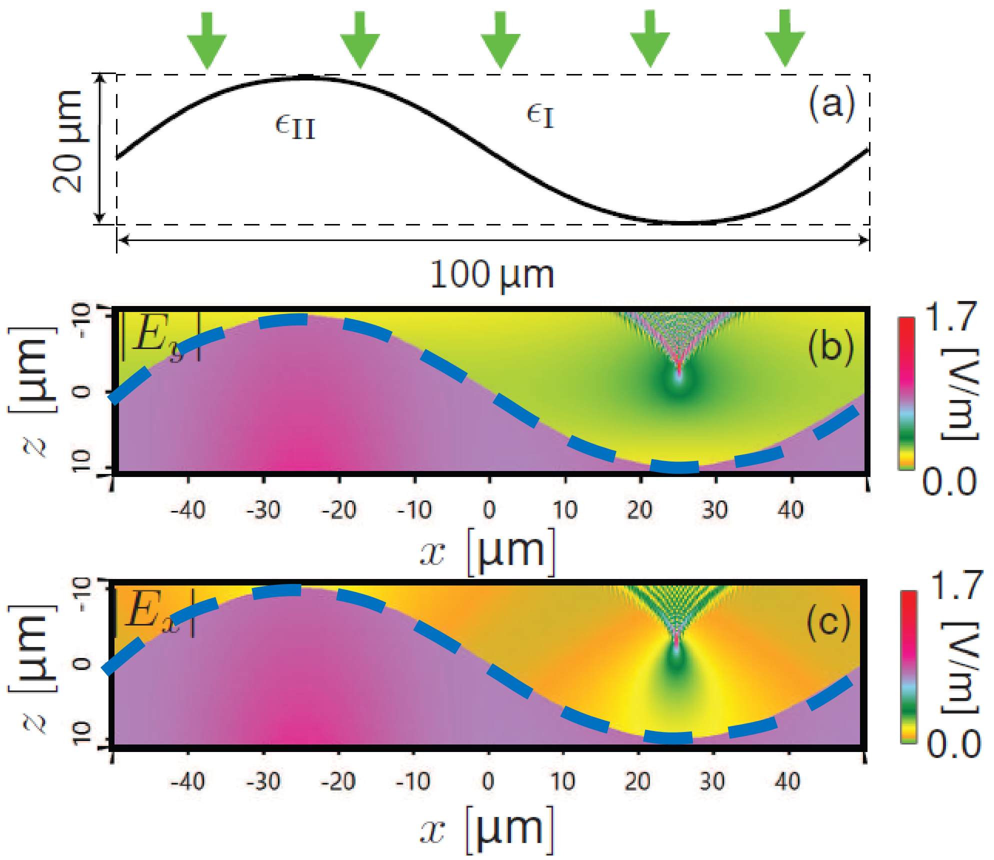
$\boldsymbol{\kappa} = (k_x, k_y)$

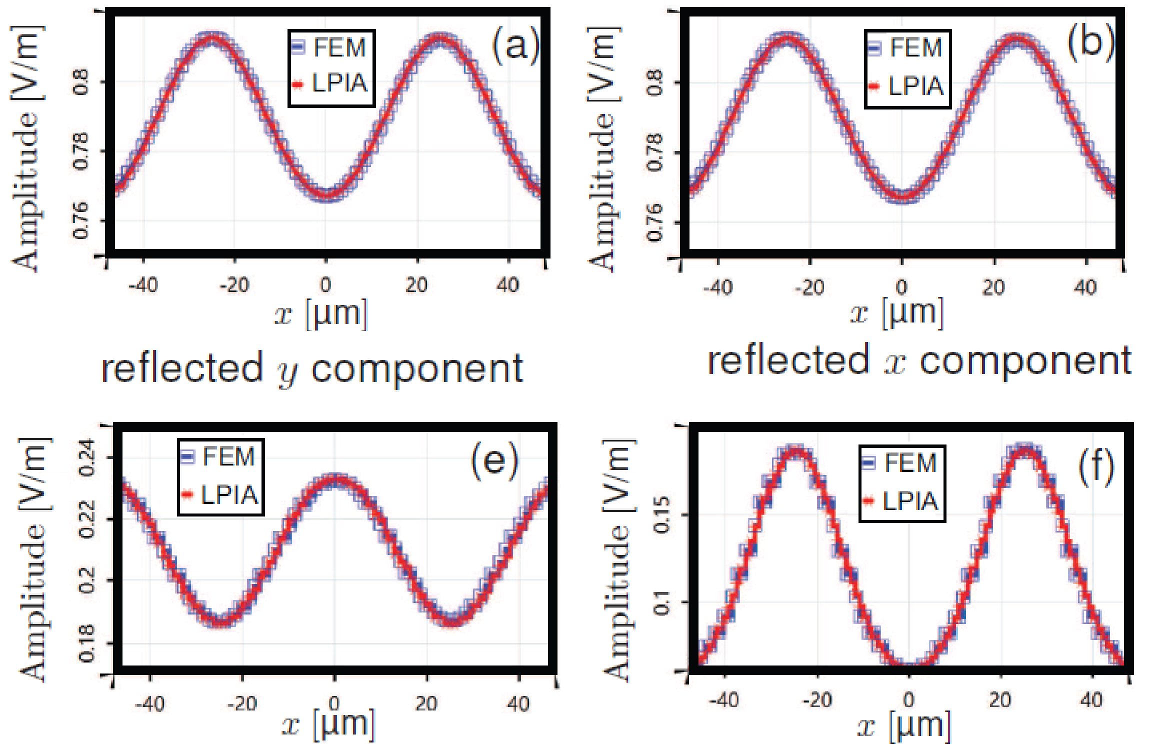
Modeling of the temporal focusing follows the same logic.

Free space propagation	Fourier Modal Method (FMM)	Local Plane Interface Approximation (LPIA)
① ③ ⑤ ⑦ ⑨ ⑪	②	④ ⑥ ⑧ ⑩

Validation of LPIA

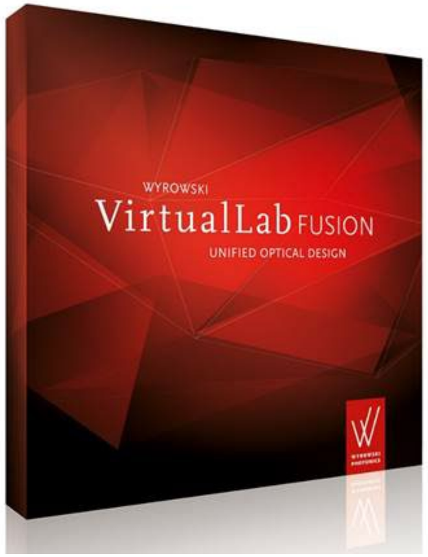
Results by FEM via JCMSuite





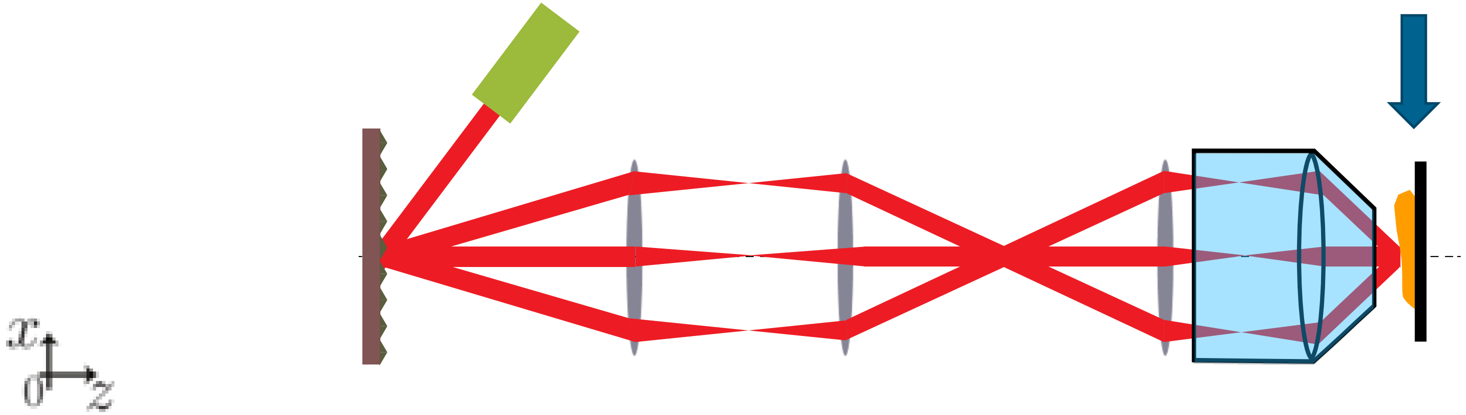
Time of LPIA: <1 s

Time of FEM: ~20 mins

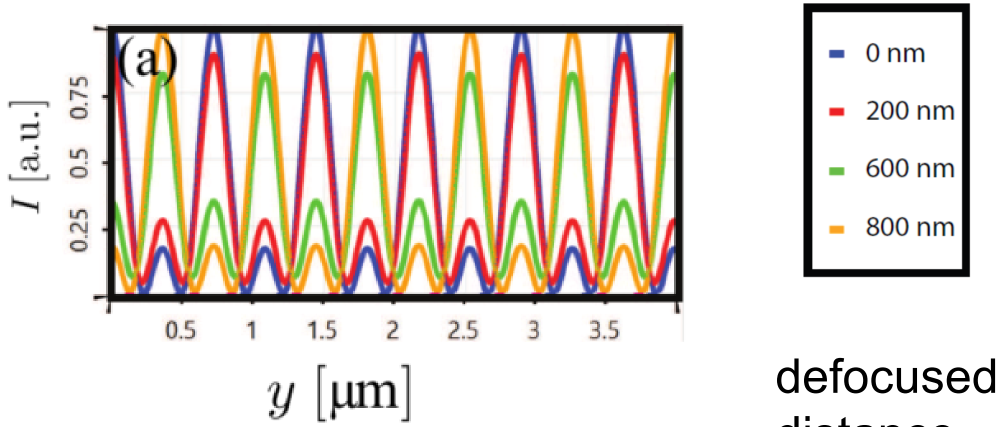


Simulation Results via VirtualLab Fusion

Interference Pattern Near Focal Plane



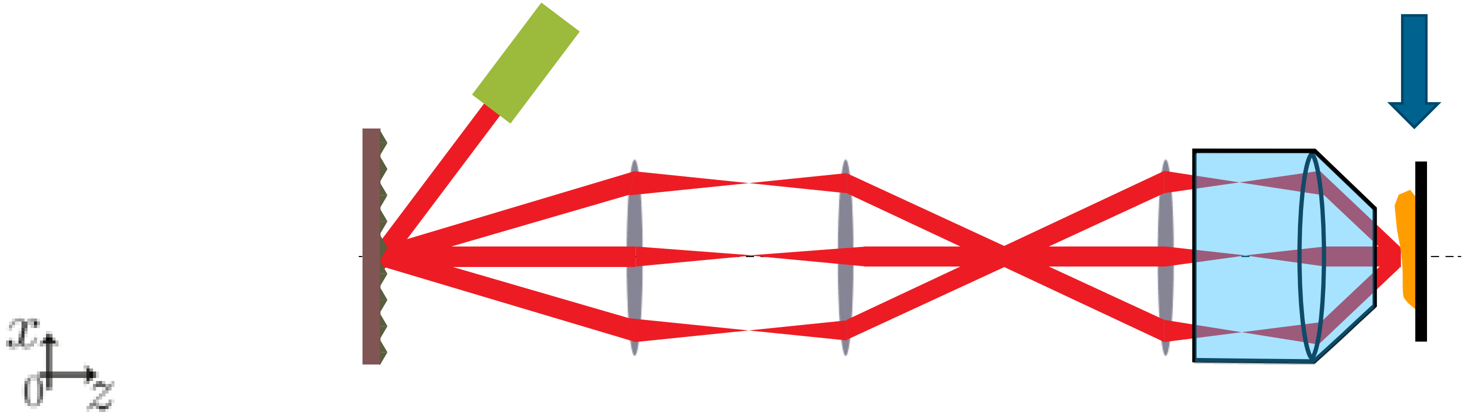
ideal system



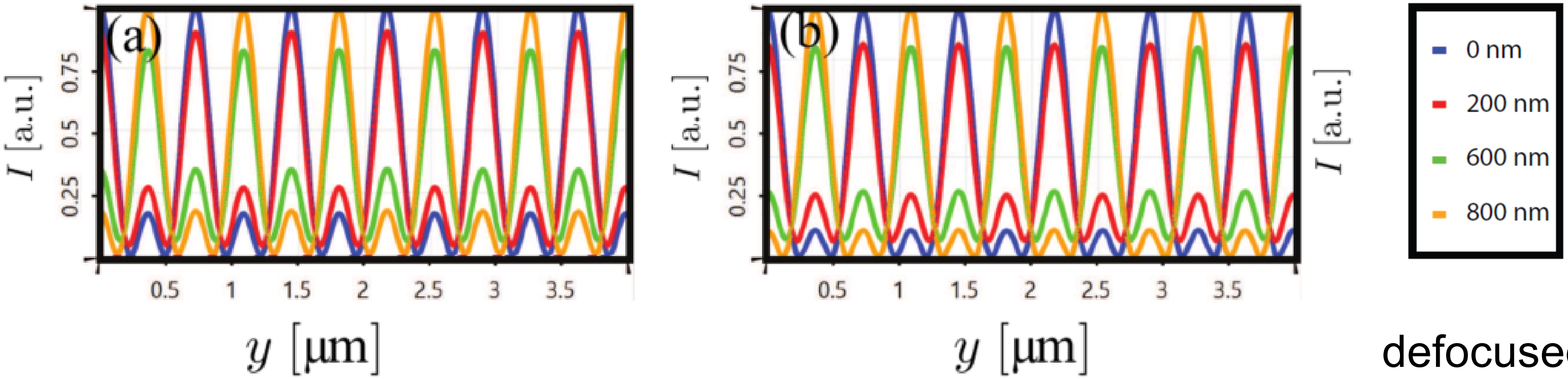
- inhomogeneity is 0.

defocused distance

Interference Pattern Near Focal Plane



ideal system — real system with perfect alignment

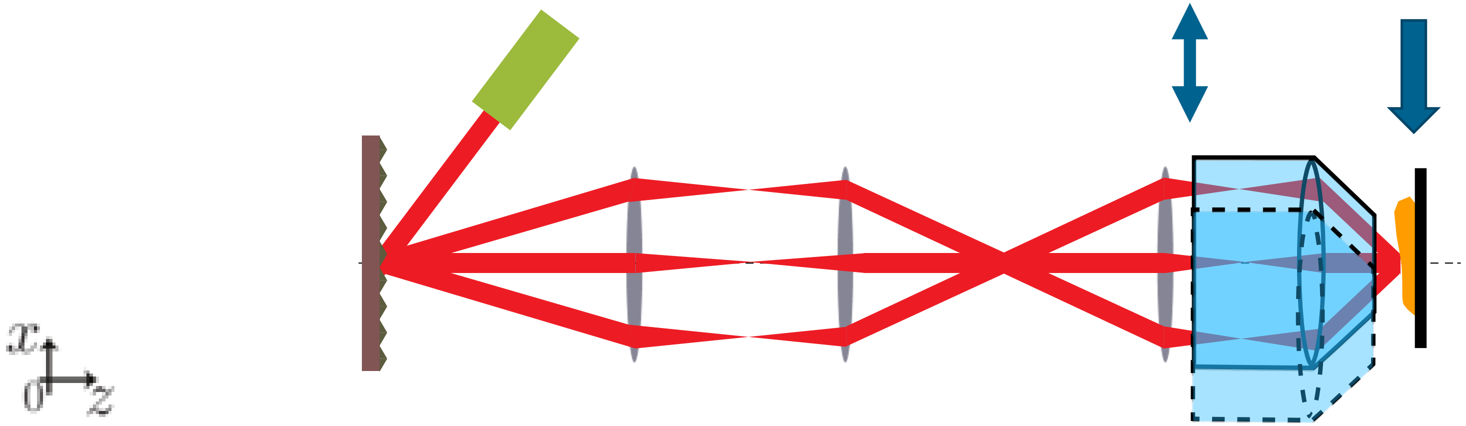


- inhomogeneity is 0.
- inhomogeneity is ~0.

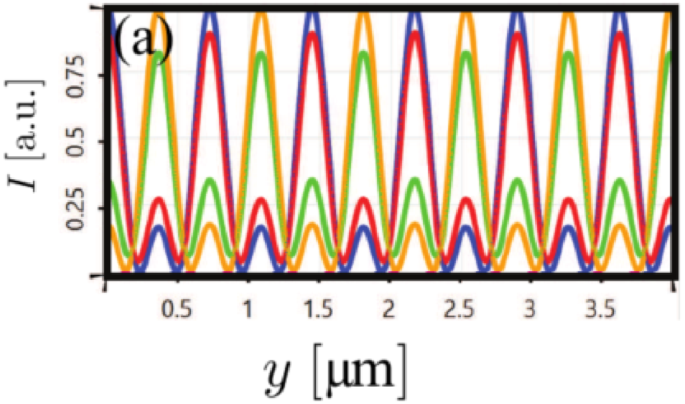
defocused distance

Computational time is within seconds.

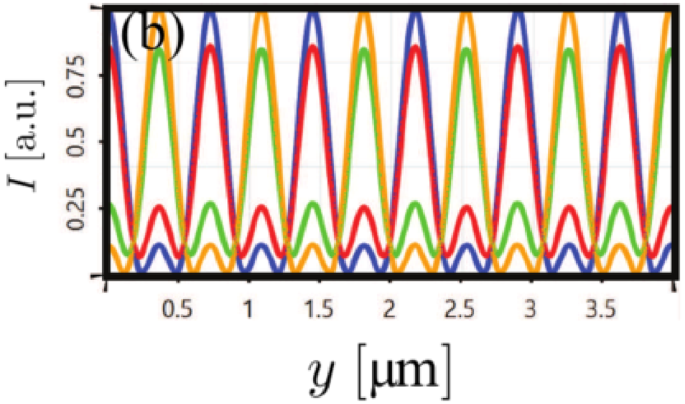
Interference Pattern Near Focal Plane



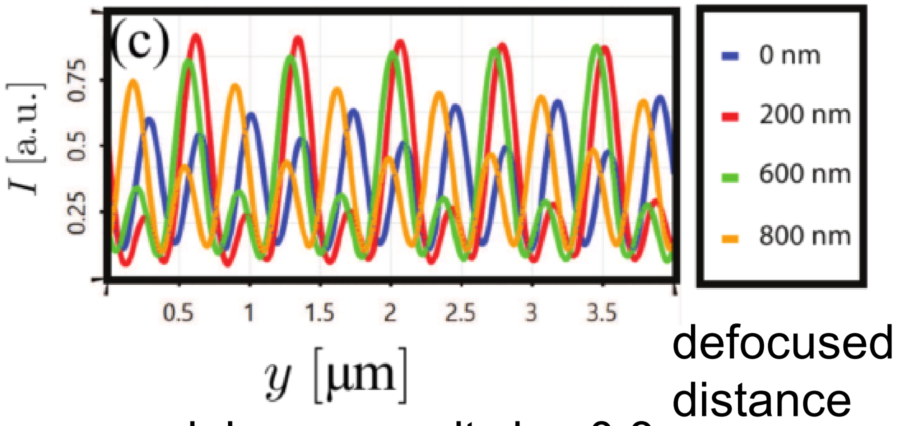
ideal system	real system with perfect alignment	real system with 430 μm lateral shift



- inhomogeneity is 0.

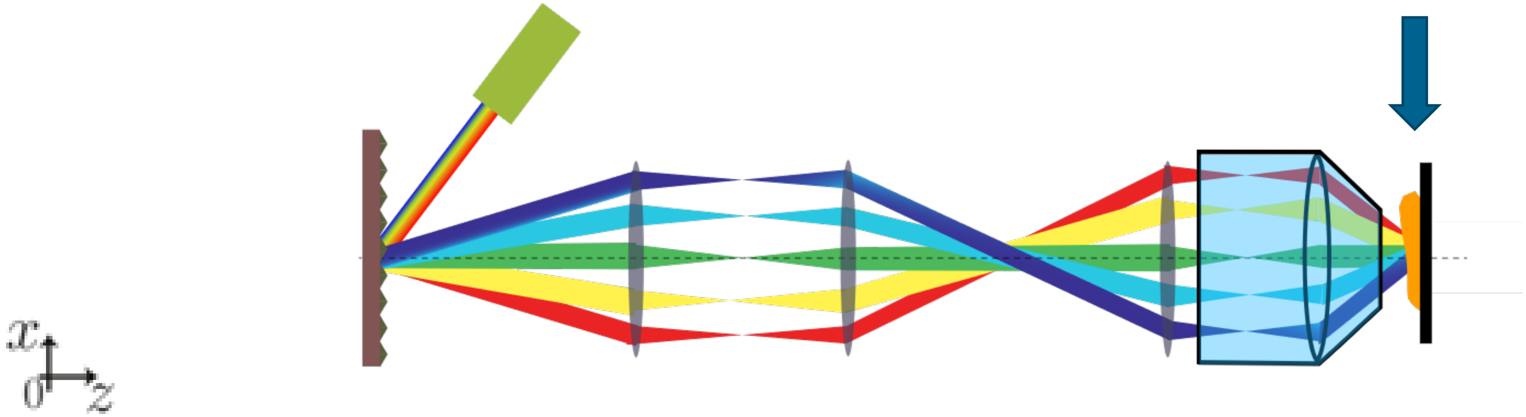


- inhomogeneity is ~0.

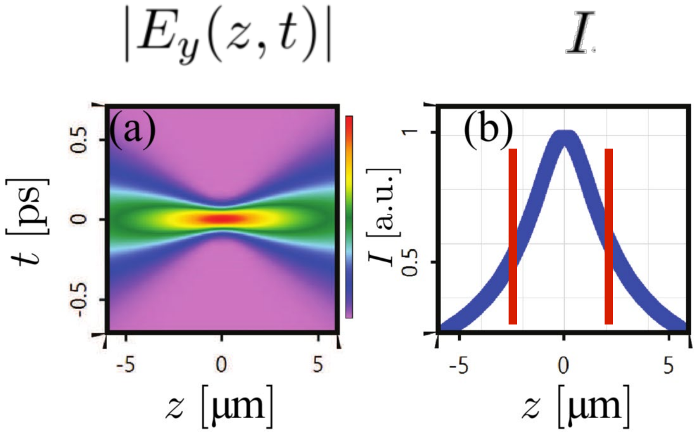


- inhomogeneity is ~0.3

Temporal Distribution Near Focal Plane at Center Point

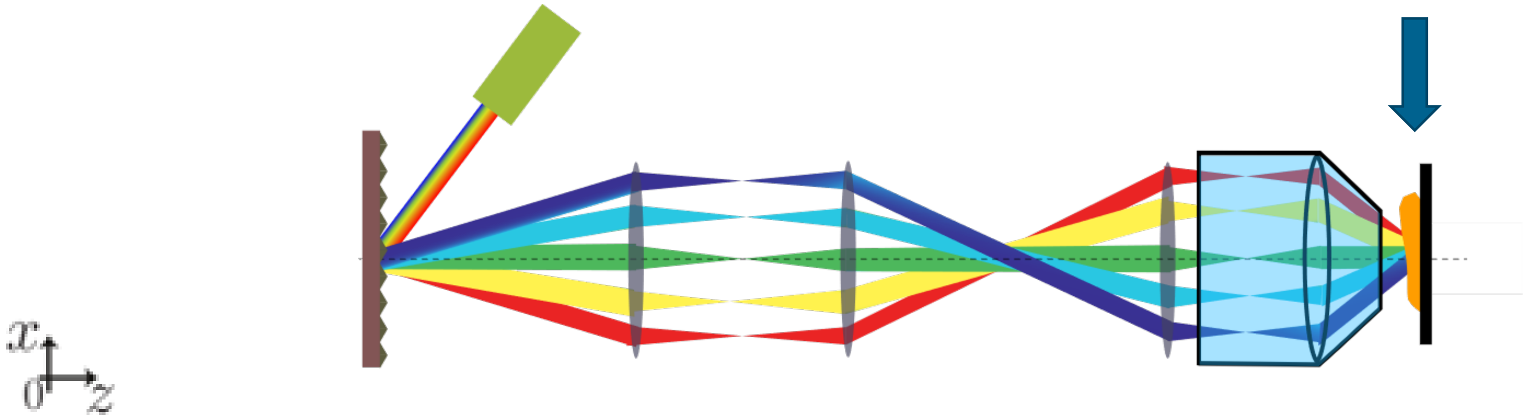


ideal system



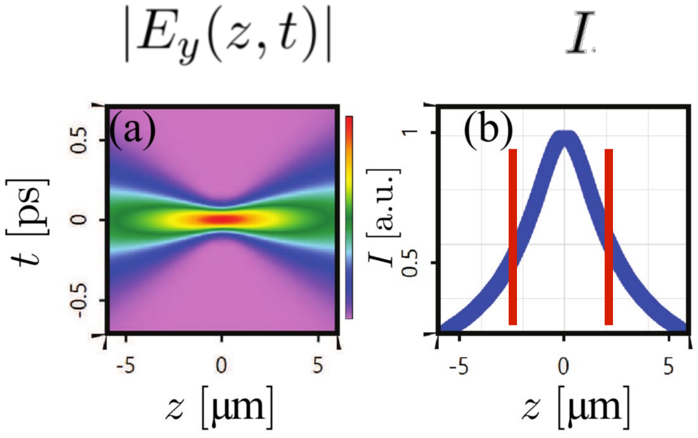
FWHM: ~4.8 µm

Temporal Distribution Near Focal Plane at Center Point

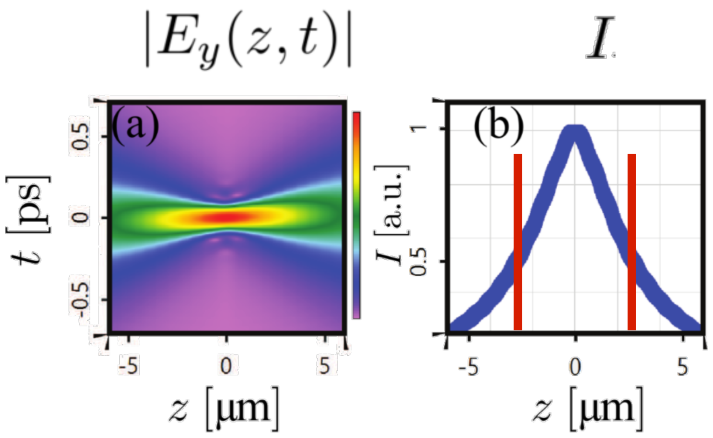


ideal system

real system with perfect alignment



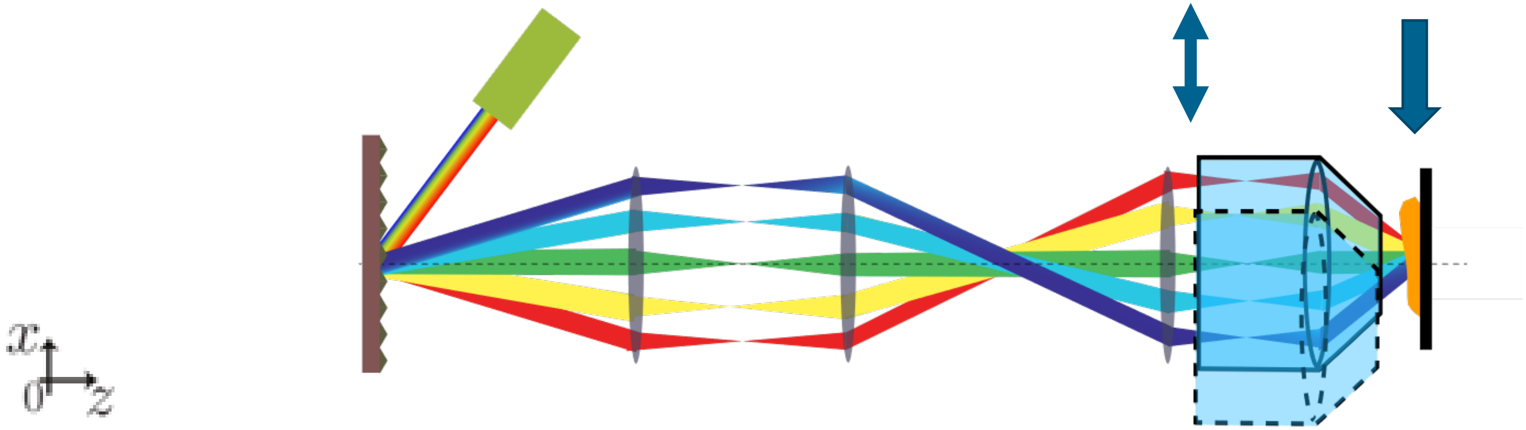
FWHM: ~4.8 µm



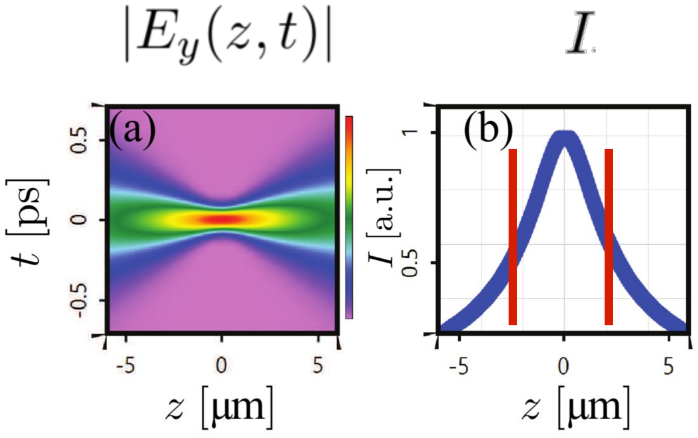
FWHM: ~5.4 µm

Computational time is within half a minute.

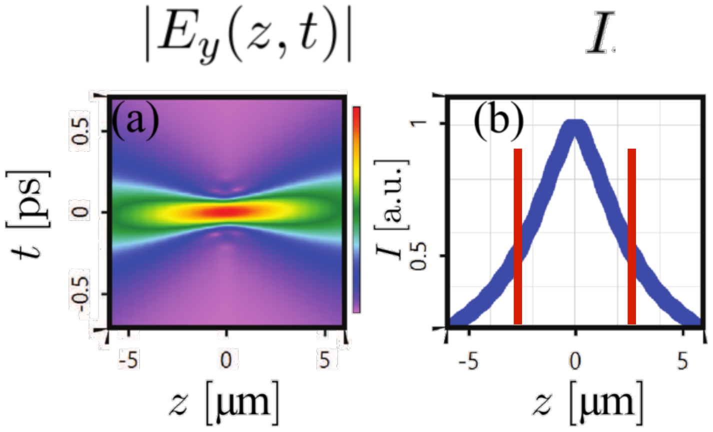
Temporal Distribution Near Focal Plane at Center Point



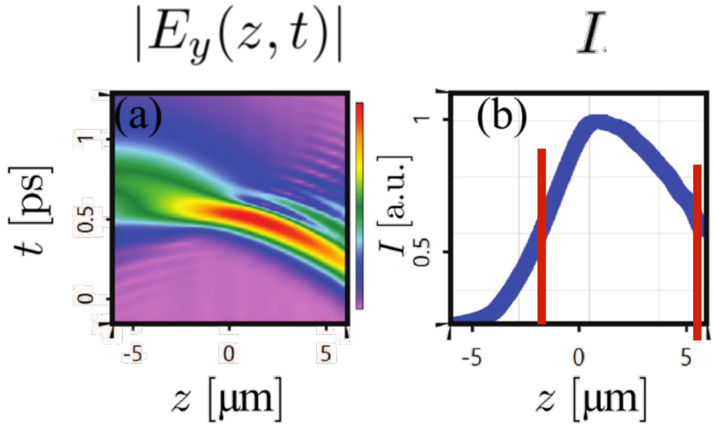
ideal system	real system with perfect alignment	real system with 1.5 mm lateral shift



FWHM: ~4.8 µm



FWHM: ~5.4 µm



FWHM: ~ 7.5 µm

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!