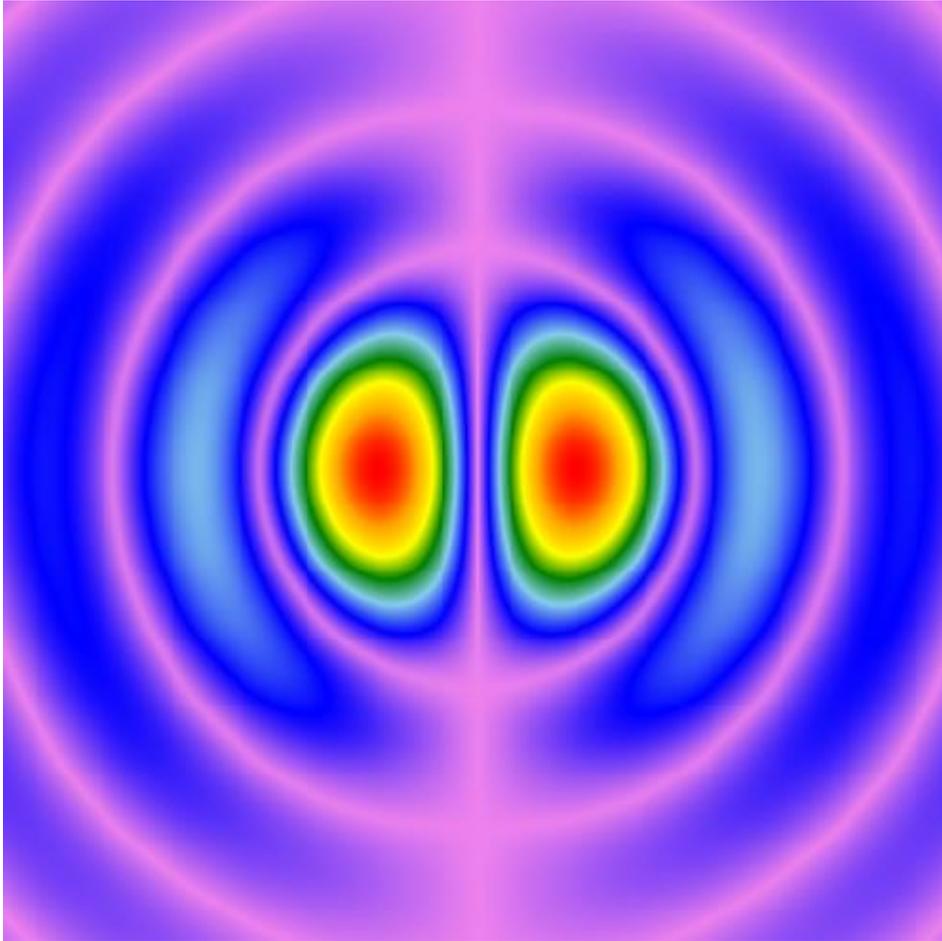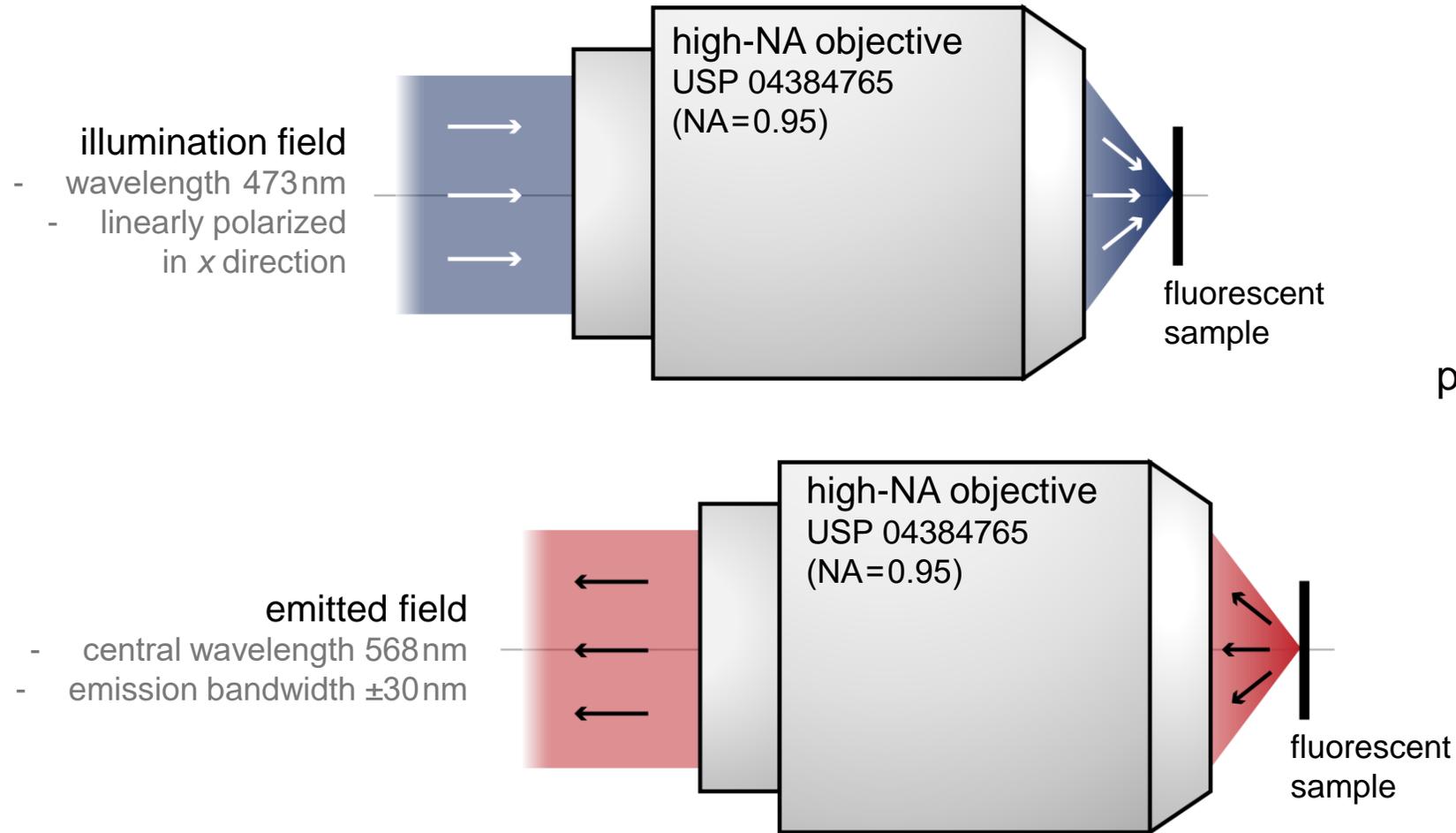# Chromatic Effect Analysis in Fluorescent Microscopy

Abstract

Chromatic aberration plays an important role in the reflection-type fluorescent microcopy, because the emitting wavelength and illumination wavelength are different. On the other hand, such microscopy systems often employ high-NA lenses as objectives. Therefore, the vectorial effect must be also taken into consideration for the performance analysis. In VirtualLab Fusion, chromatic effects of high-NA objective lens can be analyzed in a full vectorial manner. As an example, the performance of a patented objective lens is evaluated.

Modeling Task

illumination field
- wavelength 473 nm
  - linearly polarized in *x* direction

high-NA objective
USP 04384765
(NA=0.95)

fluorescent sample

emitted field
- central wavelength 568 nm
- emission bandwidth ±30 nm

high-NA objective
USP 04384765
(NA=0.95)

fluorescent sample

How to evaluate the performance of the high-NA objective lens for both illumination and emission wavelengths?

Performance at Illumination Wavelength 473 nm

plane wave
- wavelength 473 nm
- linearly polarized in x direction

high-NA objective
USP 04384765
(NA=0.95)
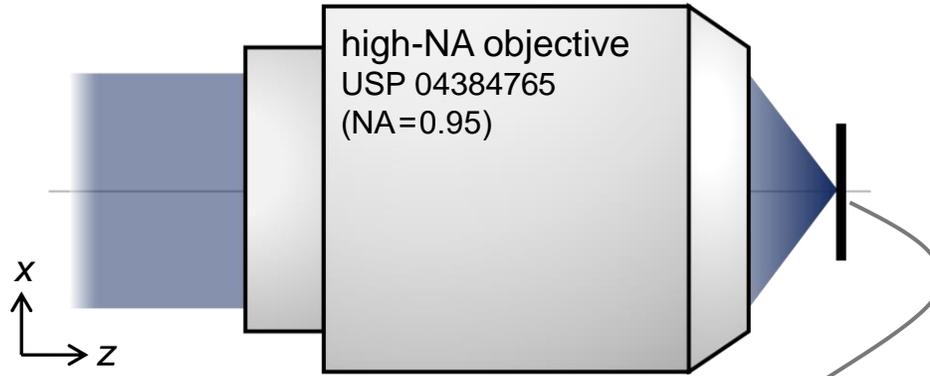
At 473 nm, far below the designed wavelength, the focal spot size is much larger than the predicted value from Debye-Wolf integral.

ideal situation calculated by Debye-Wolf integral

$|E_x|$

$|E_y|$

$|E_z|$

$\|\boldsymbol{E}\|^2$

$\|\boldsymbol{E}\|^2$

vs.

FWHM (x/y): 614/623 nm

FWHM (x/y): 346/236 nm
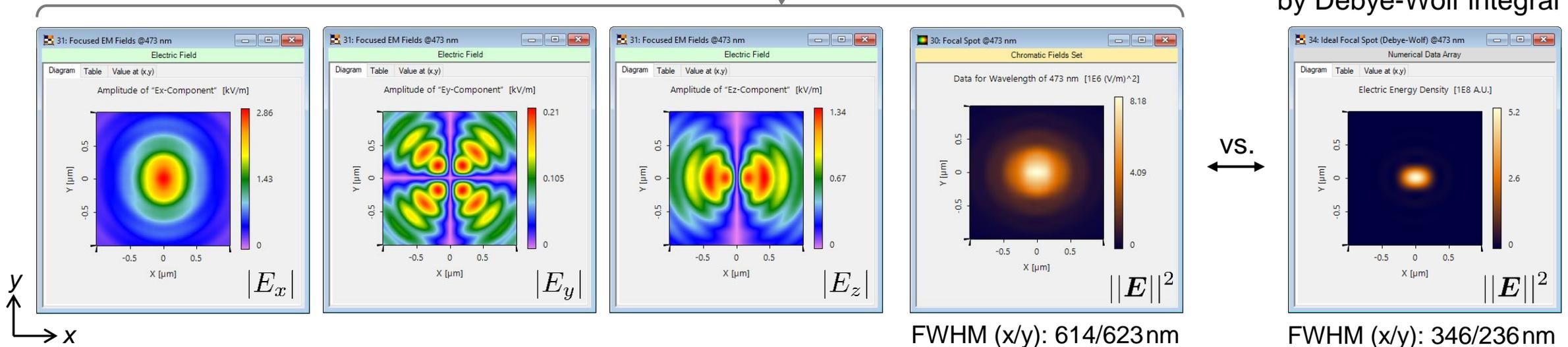
Performance at Emission Wavelength 568 nm

plane wave
- wavelength 568 nm
- linearly polarized in *x* direction

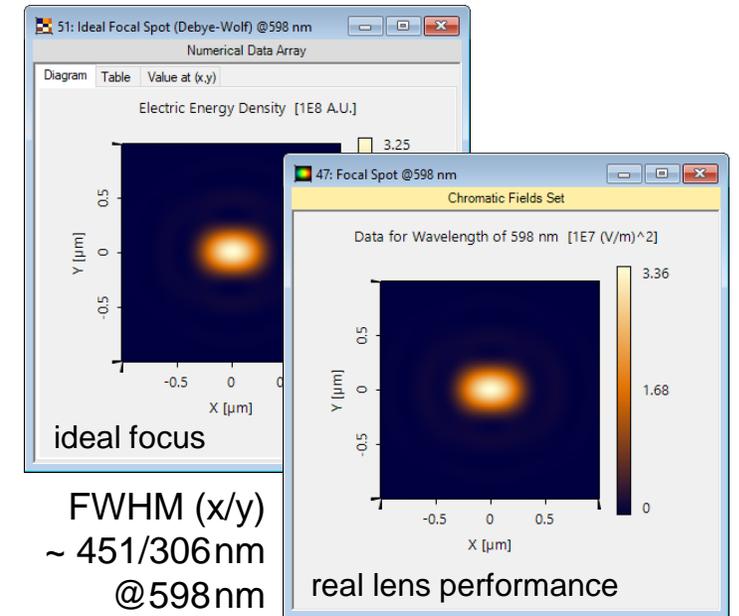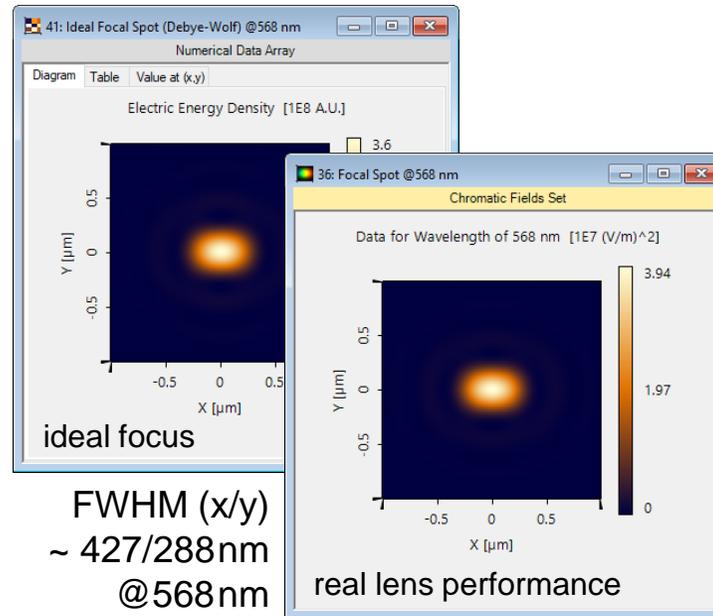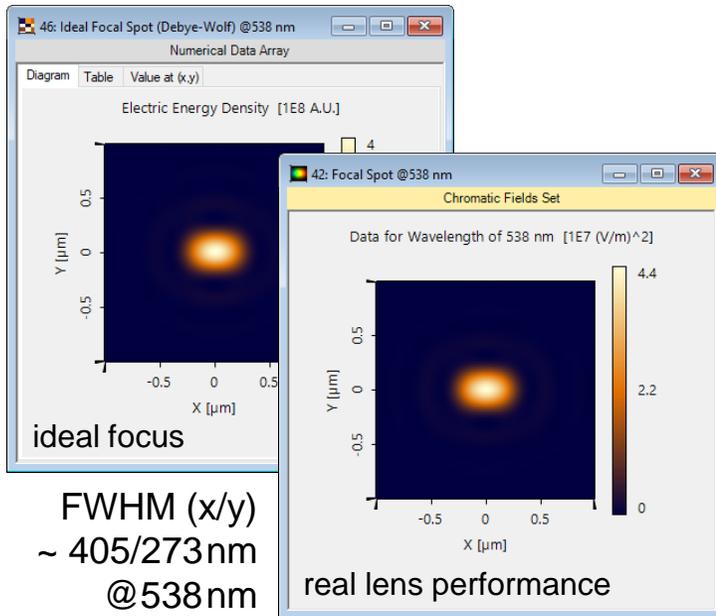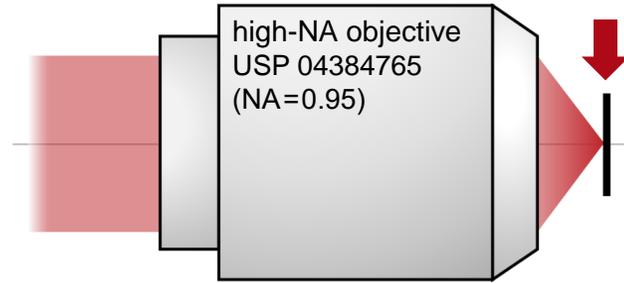high-NA objective
USP 04384765
(NA=0.95)
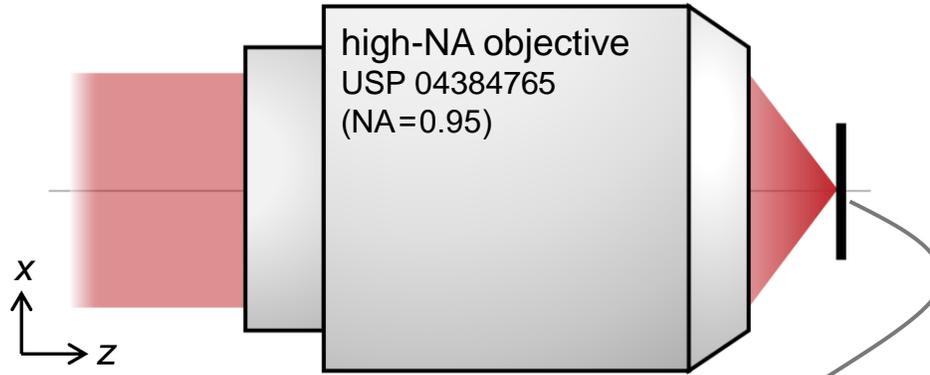
At 568 nm – the design wavelength, the focal spot size is comparable with the prediction of Debye-Wolf integral.

ideal situation calculated by Debye-Wolf integral

$|E_x|$ $|E_y|$ $|E_z|$ $||\boldsymbol{E}||^2$ vs. $||\boldsymbol{E}||^2$

FWHM (x/y): 427/288 nm   FWHM (x/y): 415/283 nm
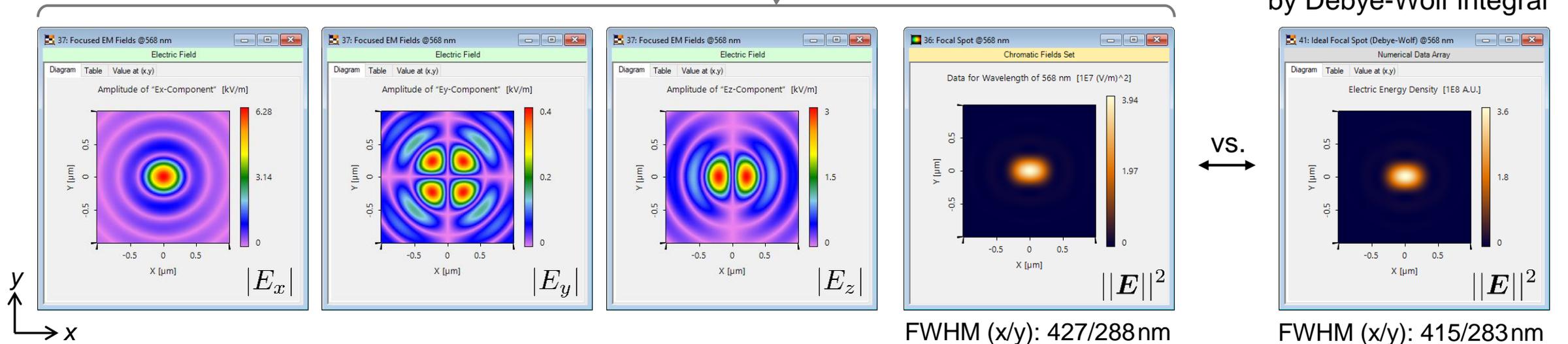
Performance around Emission Wavelength 568±30 nm

plane wave
- wavelength 568±30 nm
- linearly polarized
  in *x* direction

high-NA objective
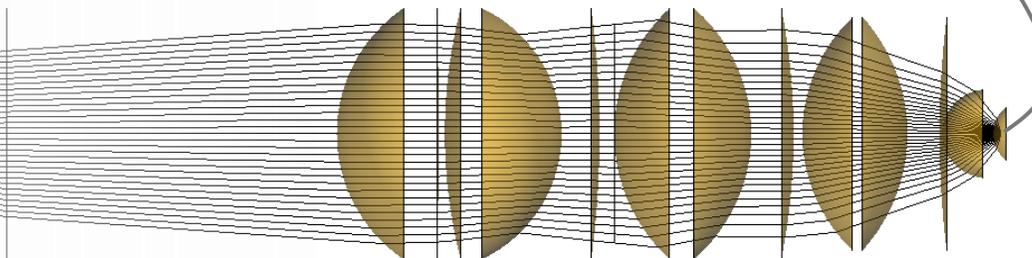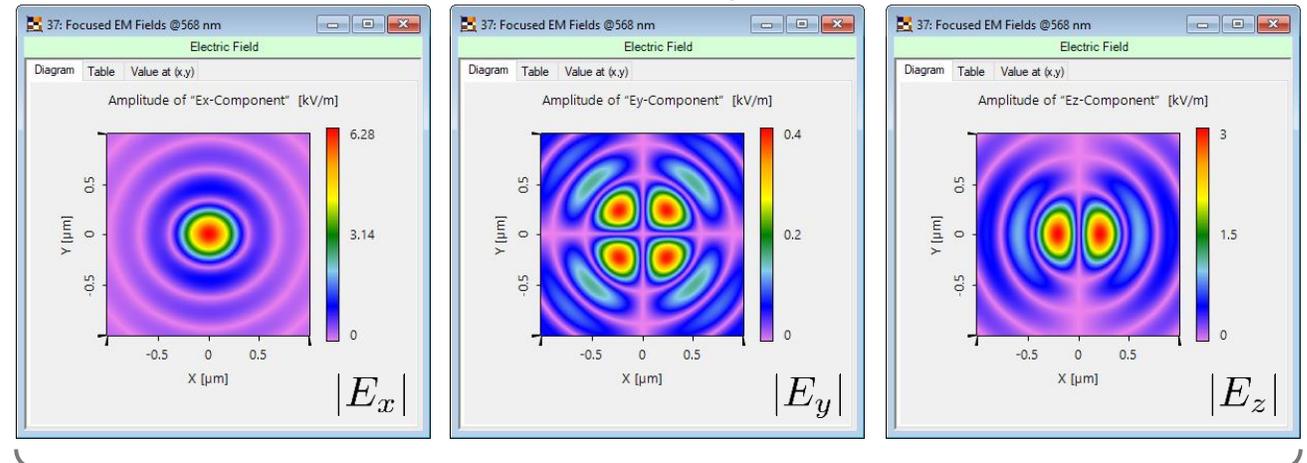USP 04384765
(NA=0.95)

ideal focus

FWHM (x/y)
~ 405/273 nm
@538 nm

real lens performance

ideal focus

FWHM (x/y)
~ 427/288 nm
@568 nm

real lens performance

ideal focus

FWHM (x/y)
~ 451/306 nm
@598 nm

real lens performance

configuration of complex lens system

calculation of the electromagnetic fields on the focal plane
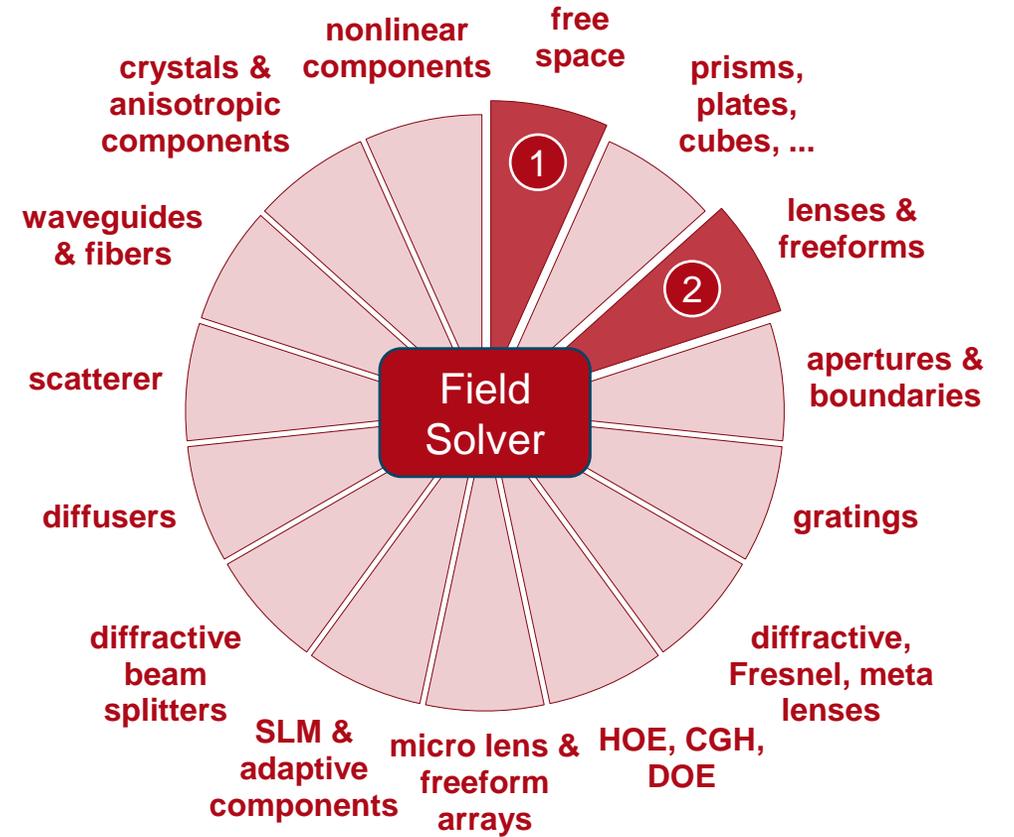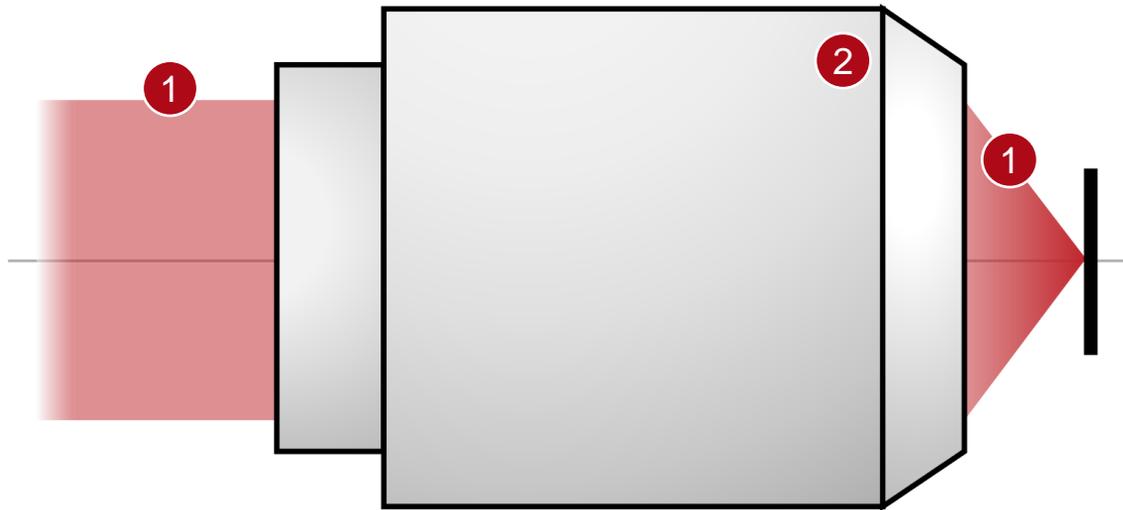

$|E_x|$ $|E_y|$ $|E_z|$

system analysis with ray tracing and visualization

Workflow in VirtualLab Fusion

- Import lens systems from Zemax OpticStudio®
  - Import Optical Systems from Zemax [Use Case]

- Analyze imaging performance of real lens system
  - Analyzing High-NA Objective Lens Focusing [Use Case]

- Use Debye-Wolf integral as a reference
  - Debey-Wolf Integral Calculator [Use Case]
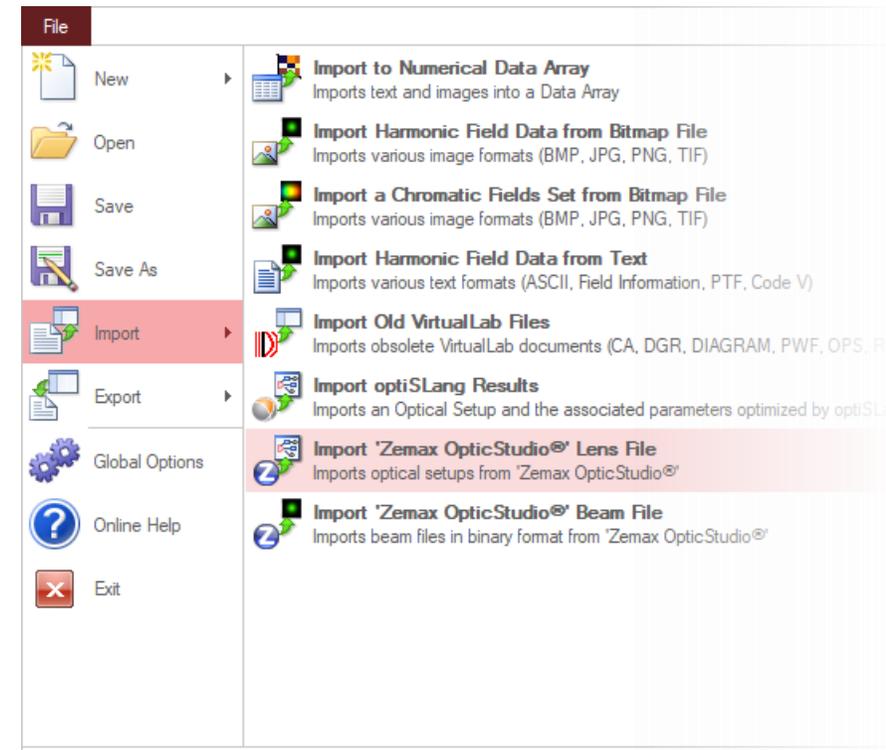
VirtualLab Fusion Technologies

Document Information

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version	1.2
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software version	2020.1 (Build 1.226)
category	Application Use Case
further reading	- Analyzing High-NA Objective Lens Focusing - Resolution Investigation for Microscope Objective Lenses by Rayleigh Criterion