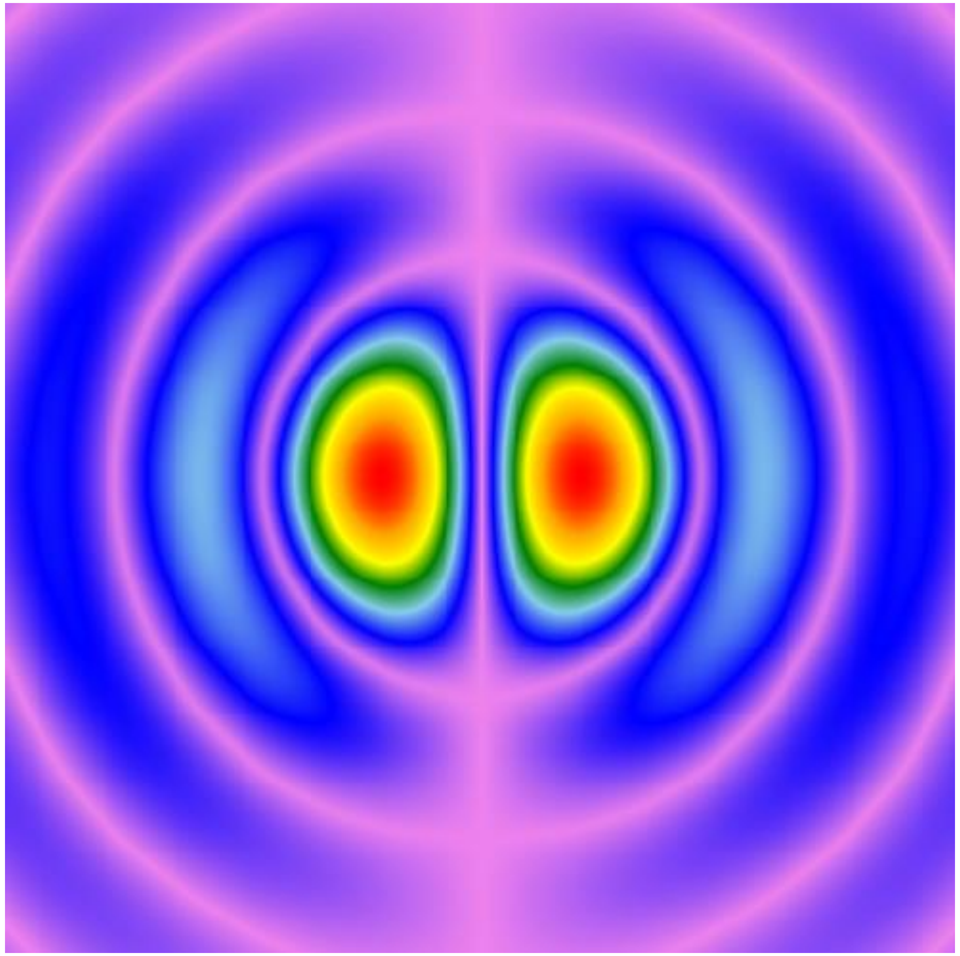
Chromatic Effect Analysis in Fluorescent Microscopy

Abstract

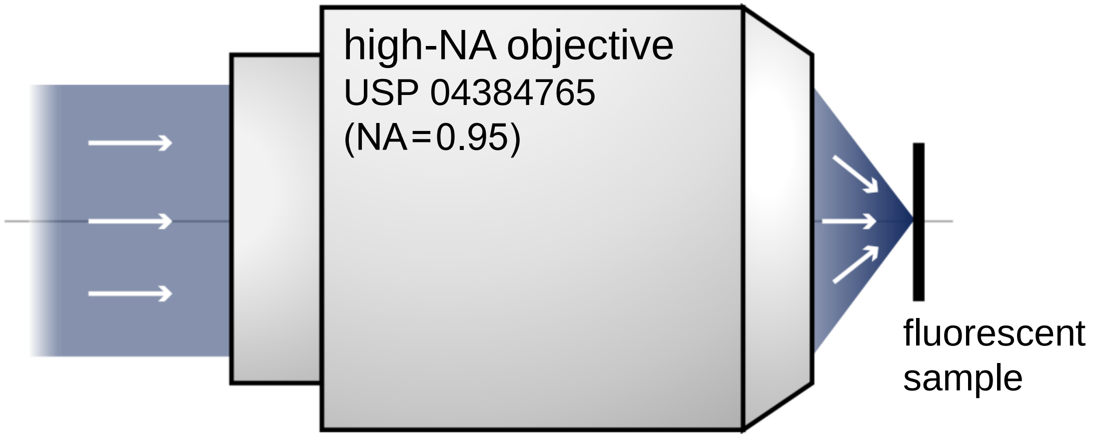


Chromatic aberration plays an important role in the reflection-type fluorescent microcopy, because the emitting wavelength and illumination wavelength are different. On the other hand, such microscopy systems often employ high-NA lenses as objectives. Therefore, the vectorial effect must be also taken 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

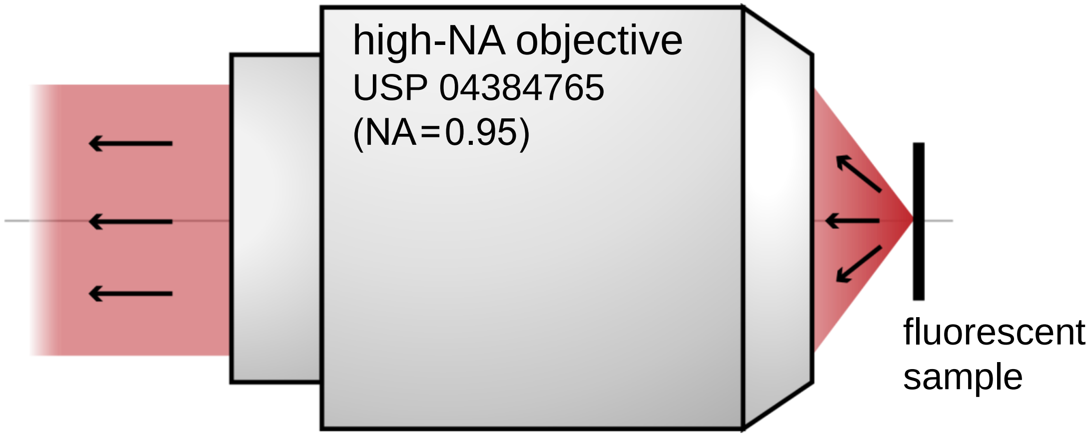
illumination field

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



emitted field

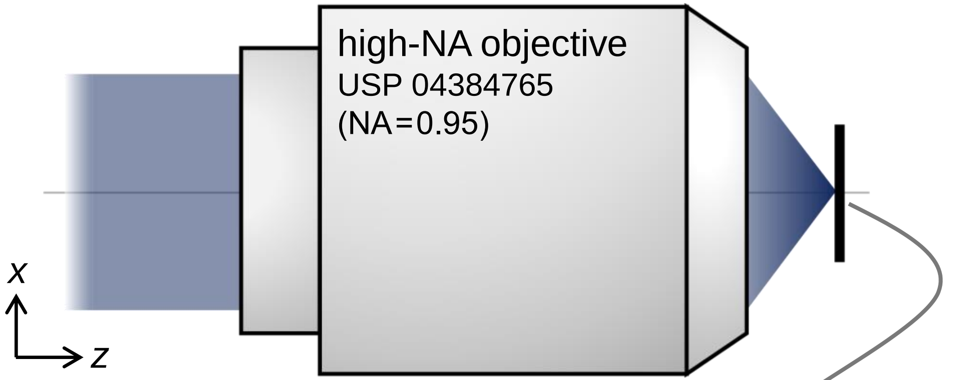
- central wavelength 568 nm
- emission bandwidth ±30 nm



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

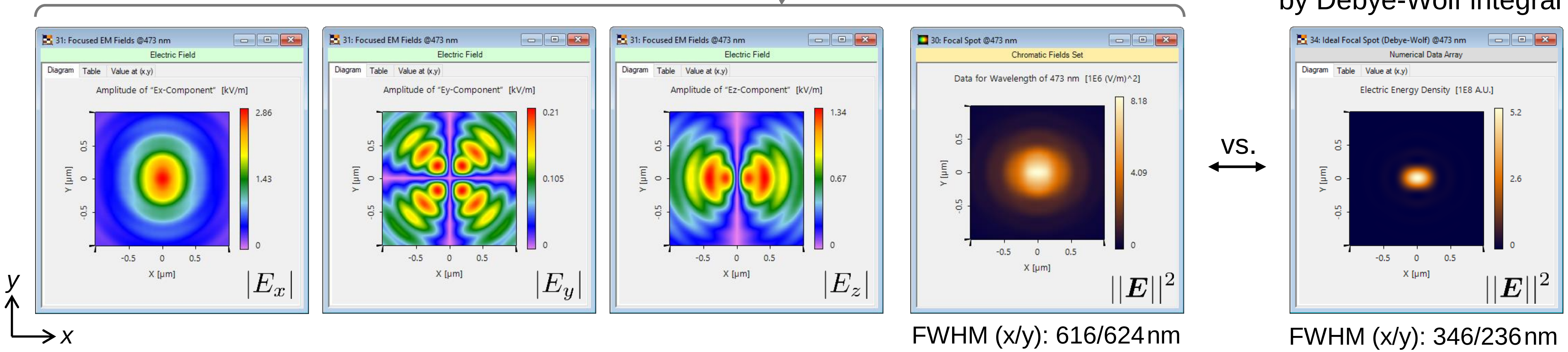
Performance at Illumination Wavelength 473 nm

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



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

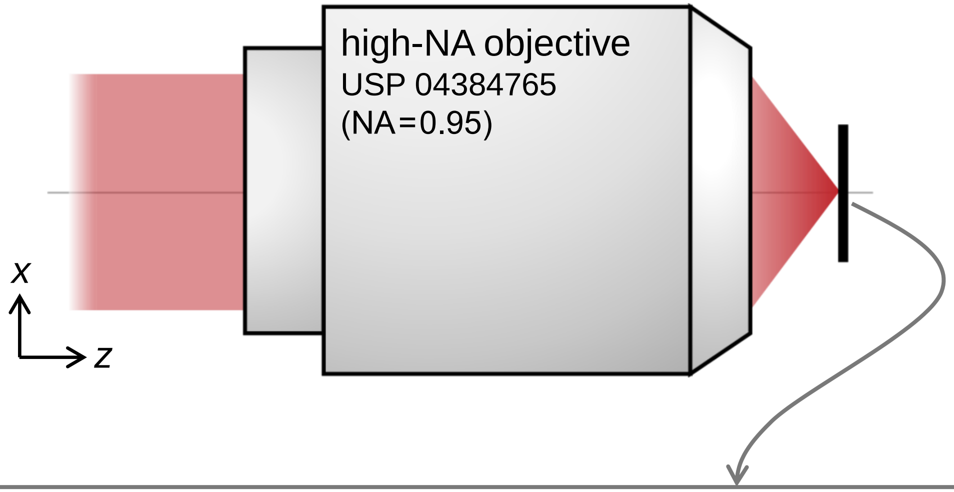


FWHM (x/y): 616/624 nm

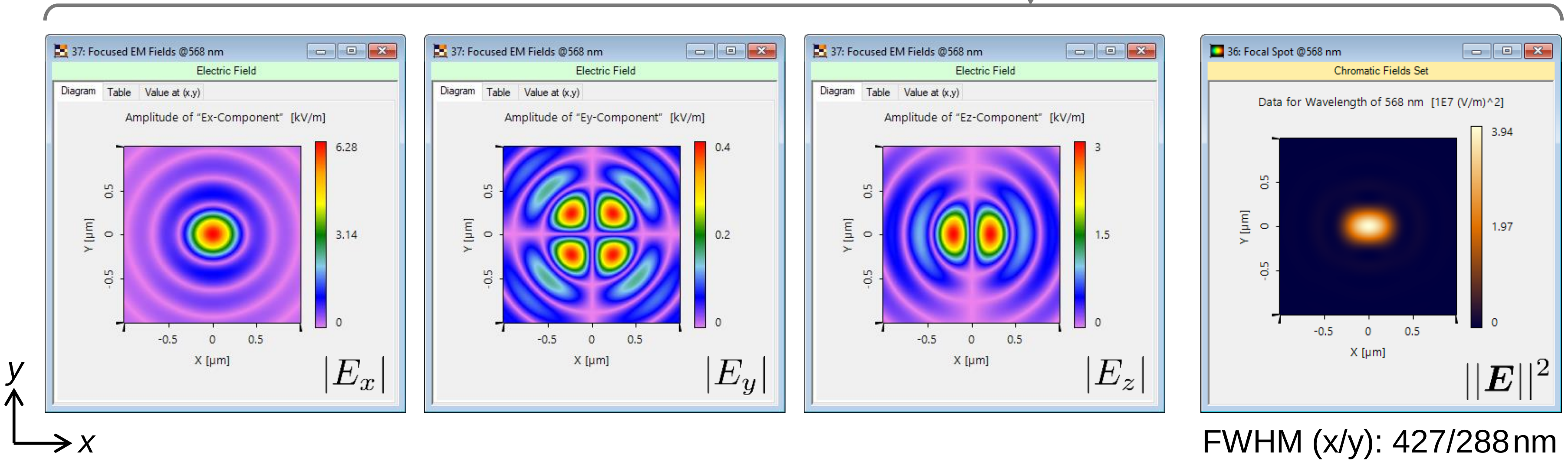
FWHM (x/y): 346/236 nm

Performance at Emission Wavelength 568 nm

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



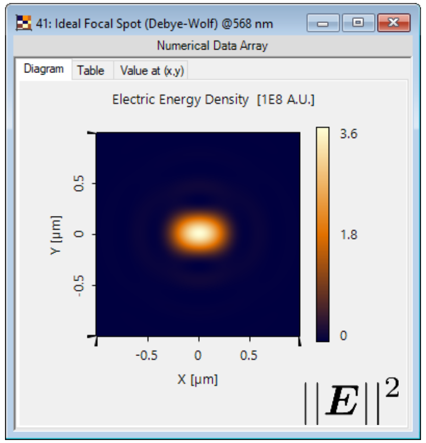
At 568 nm – the design wavelength, the focal spot size is comparable with the prediction of Debye-Wolf integral.



FWHM (x/y): 427/288 nm

vs.

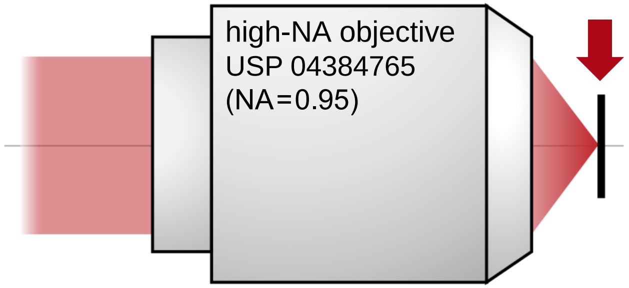
ideal situation calculated by Debye-Wolf integral

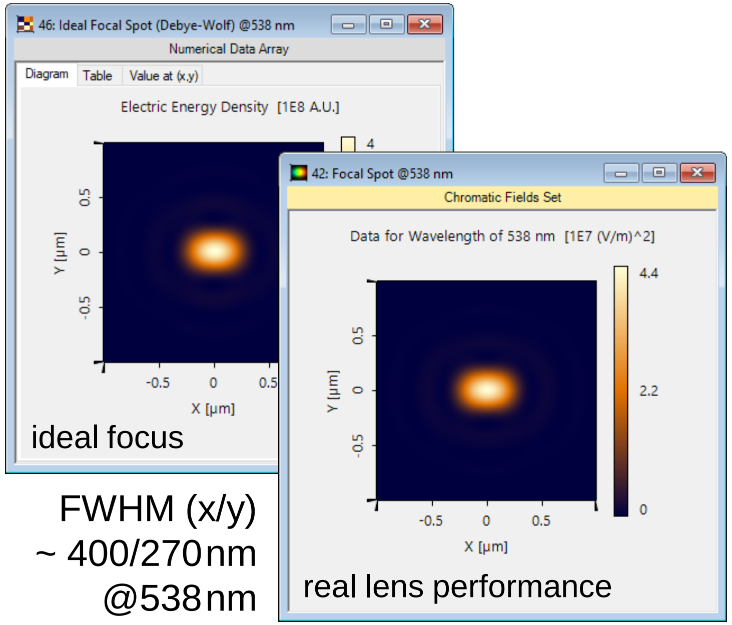


FWHM (x/y): 415/283 nm

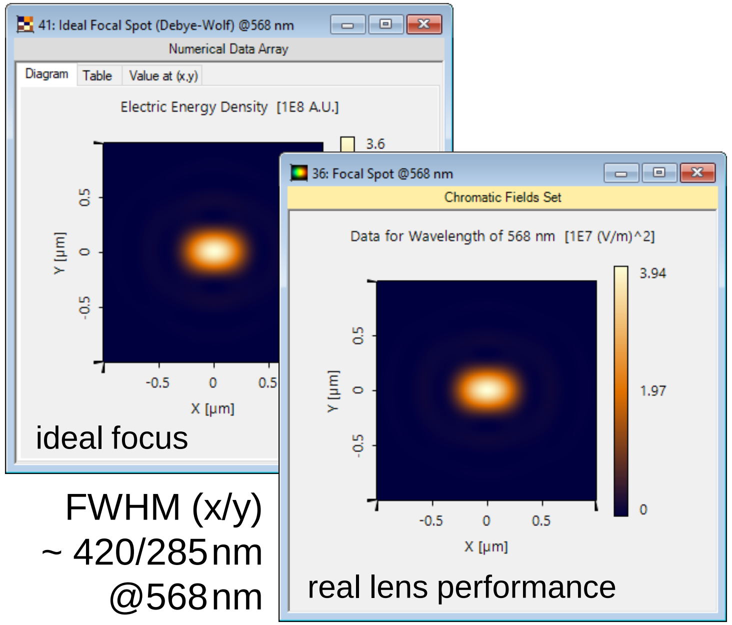
Performance around Emission Wavelength 568±30 nm

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

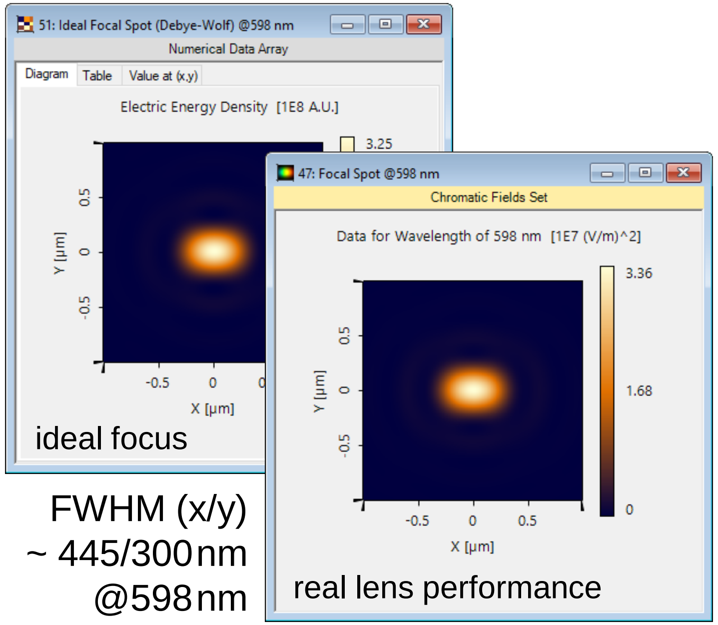




FWHM (x/y)
~ 400/270 nm
@538 nm



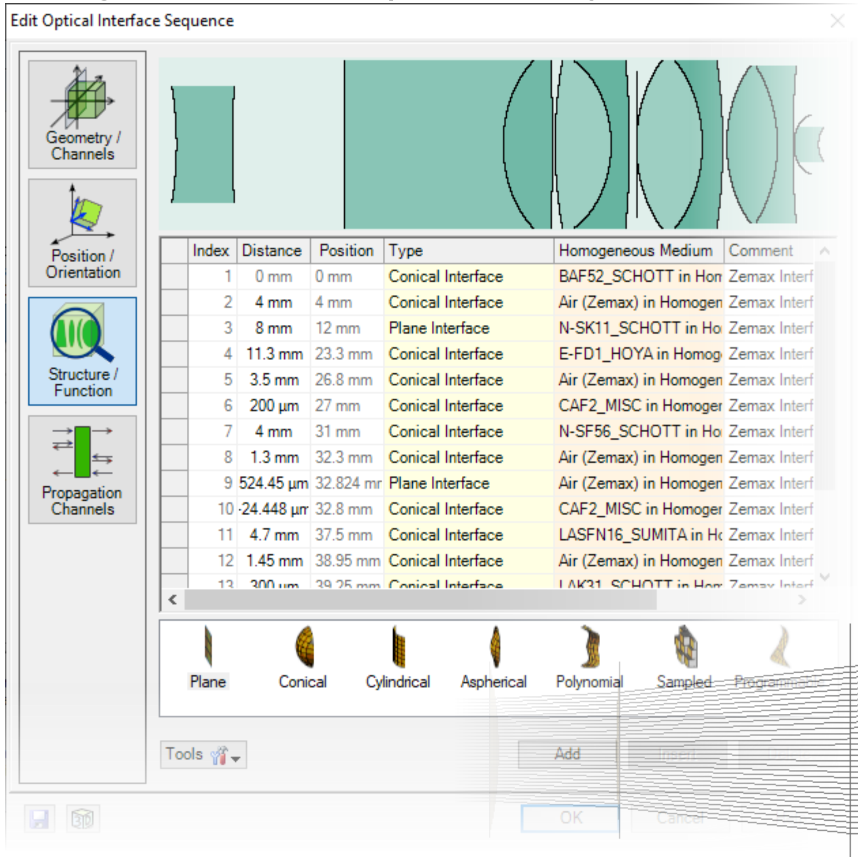
FWHM (x/y)
~ 420/285 nm
@568 nm



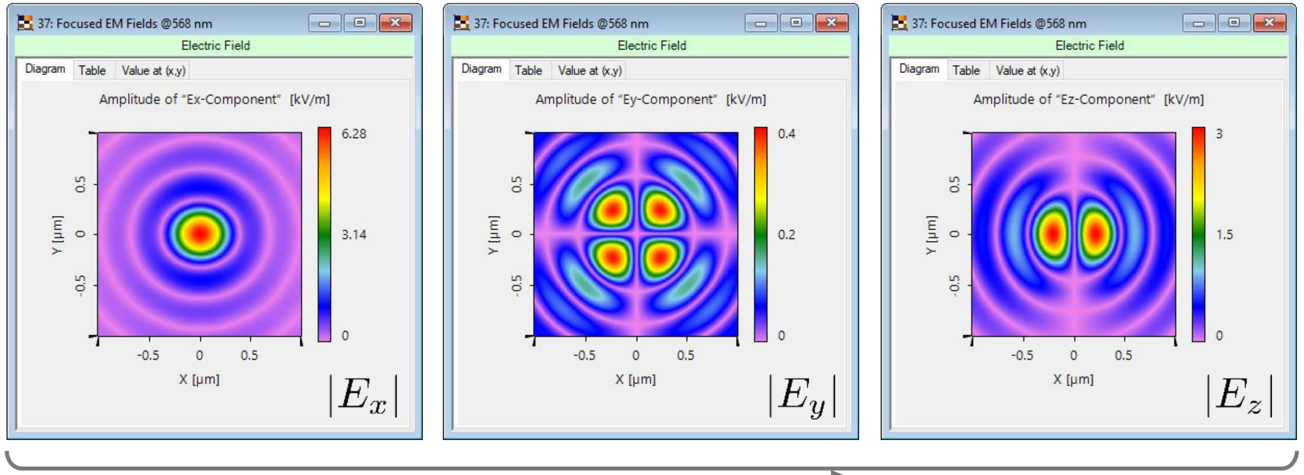
FWHM (x/y)
~ 445/300 nm
@598 nm

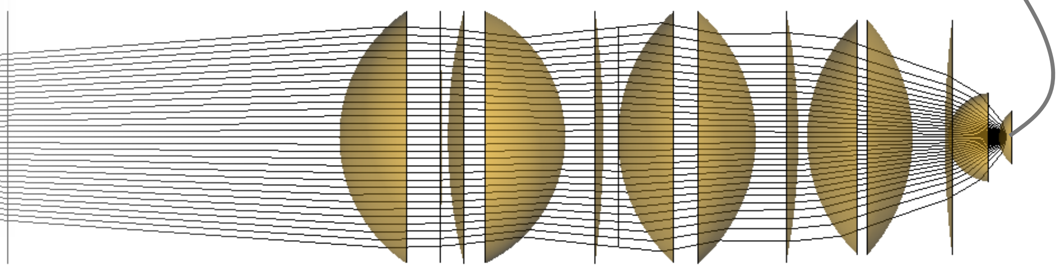
Peek into VirtualLab Fusion

configuration of complex lens system



calculation of the electromagnetic fields on the focal plane

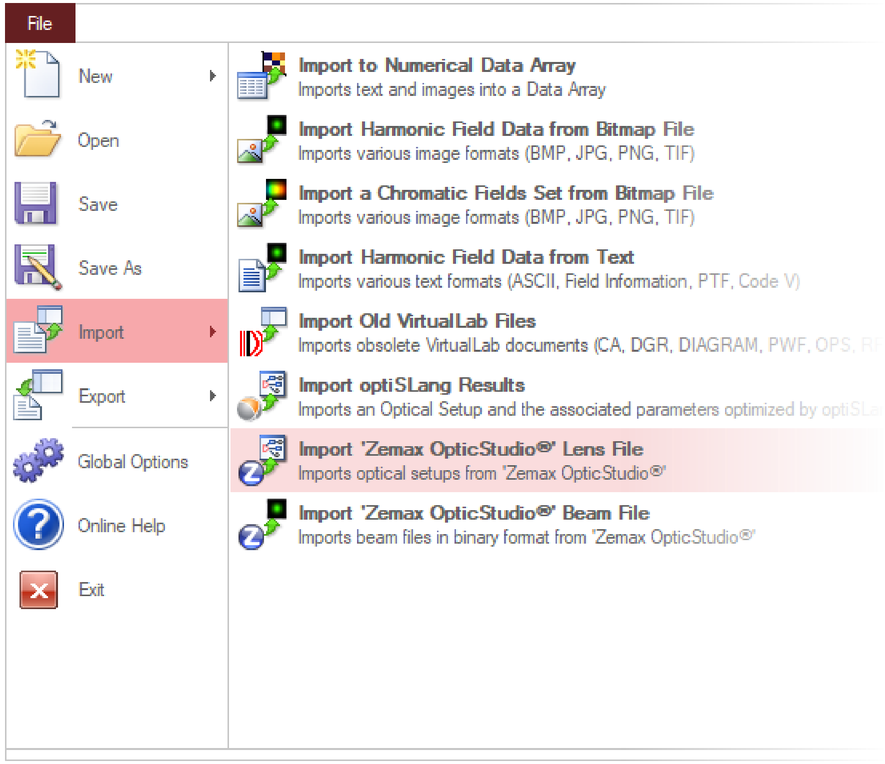




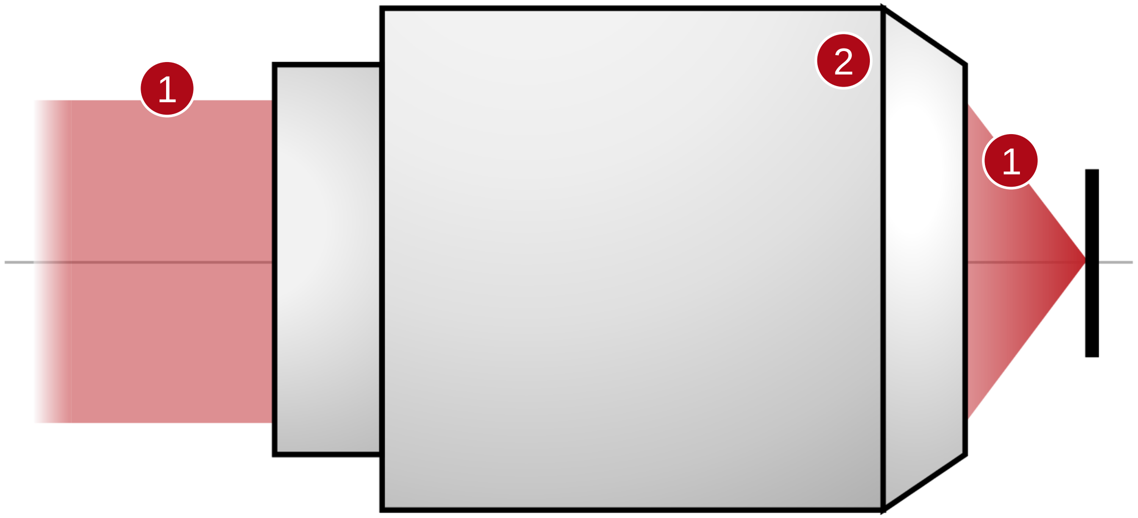
system analysis with ray tracing and visualization

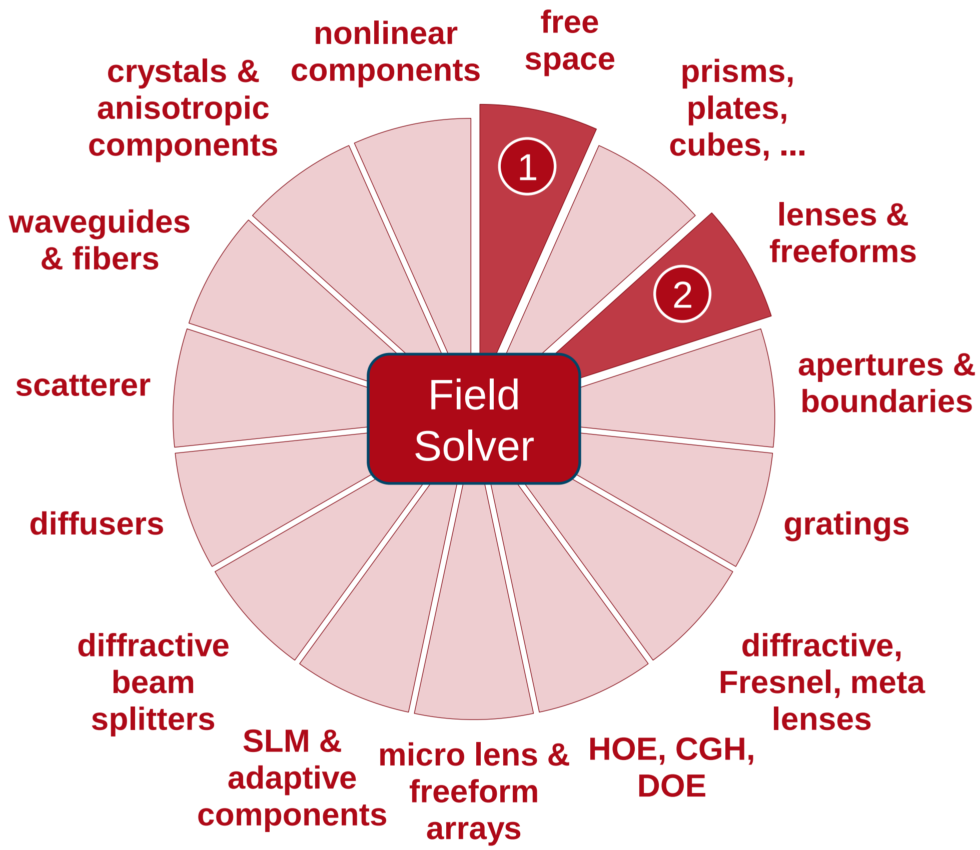
Workflow in VirtualLab Fusion

- 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

title	Chromatic Effect Analysis in Fluorescent Microscopy
document code	MIC.0004
version	1.0
toolbox(es)	Starter Toolbox
VL version used for simulations	VirtualLab Fusion 2019 Summer Release (7.6.1.18)
category	Application Use Case
further reading	- Analyzing High-NA Objective Lens Focusing - Resolution Investigation for Microscope Objective Lenses by Rayleigh Criterion