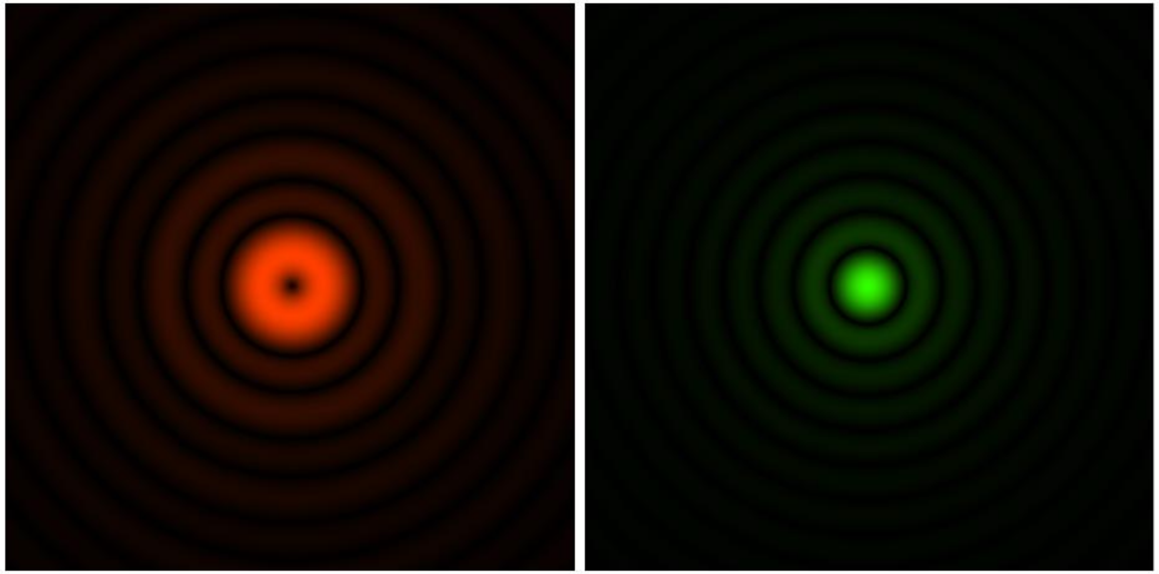
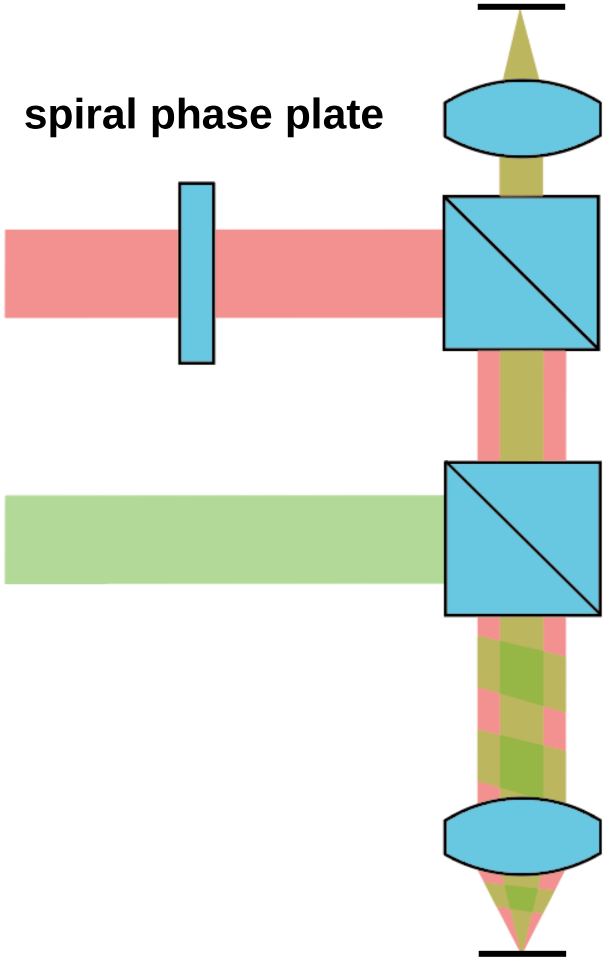
Principle of Stimulated Emission Depletion (STED) Microscopy

Abstract

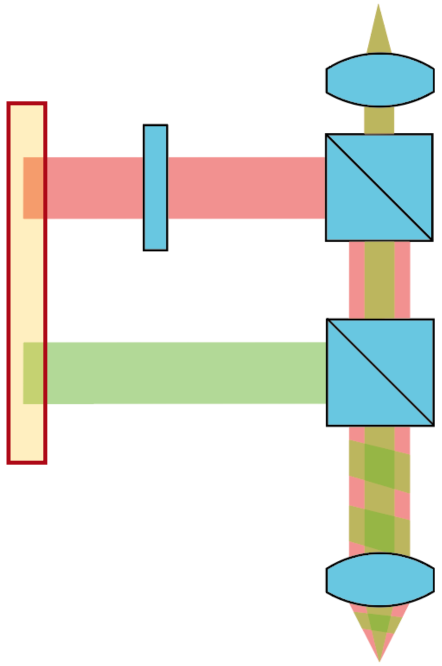


Stimulated Emission Depletion (STED) Microscopy describes a commonly used technique to achieve super resolution in biological applications. In this method two laser beams – one normal, one transformed into a donut-mode – are superimposed onto a fluorescent specimen. By using excitation and depletion of the fluorescent processes and exploiting the resulting saturation effects, the back reflected light exhibits a much higher resolution compared to usual microscopy techniques (e.g., widefield microscopy). In this document the basic setup of such a device is presented. For modeling the saturation effect, an equivalent aperture is applied in the focal region.

Task Description

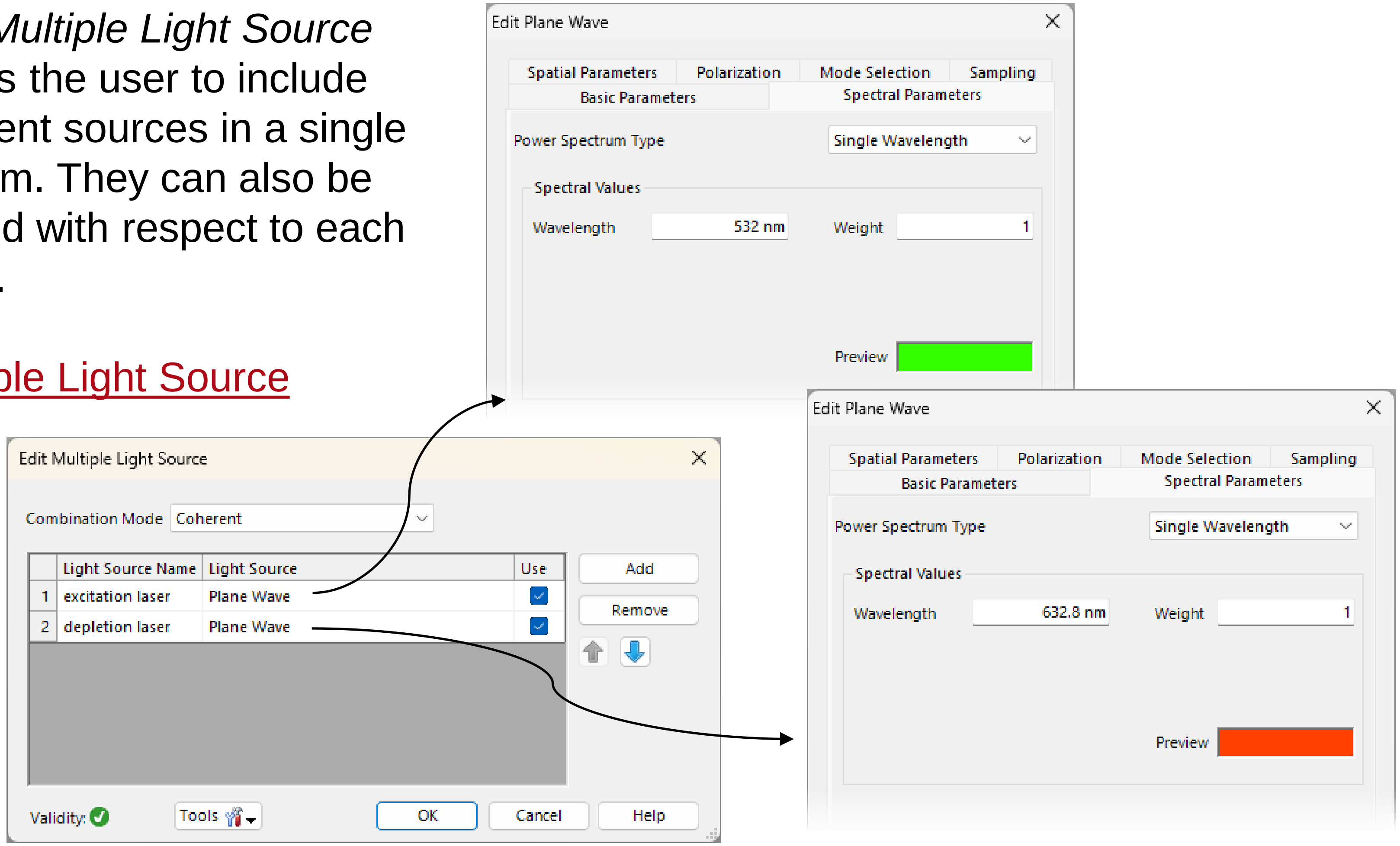


Multiple Light Source

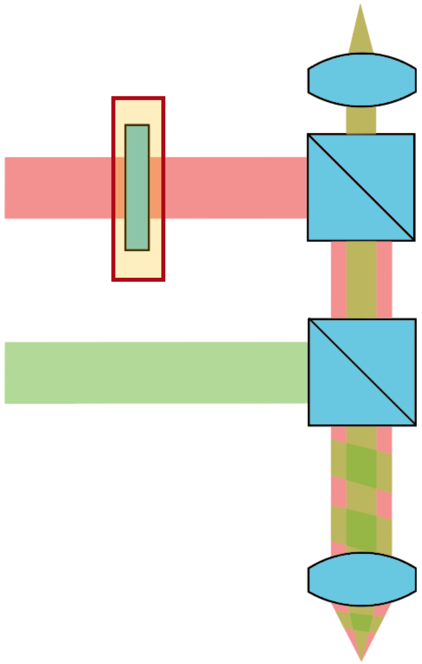


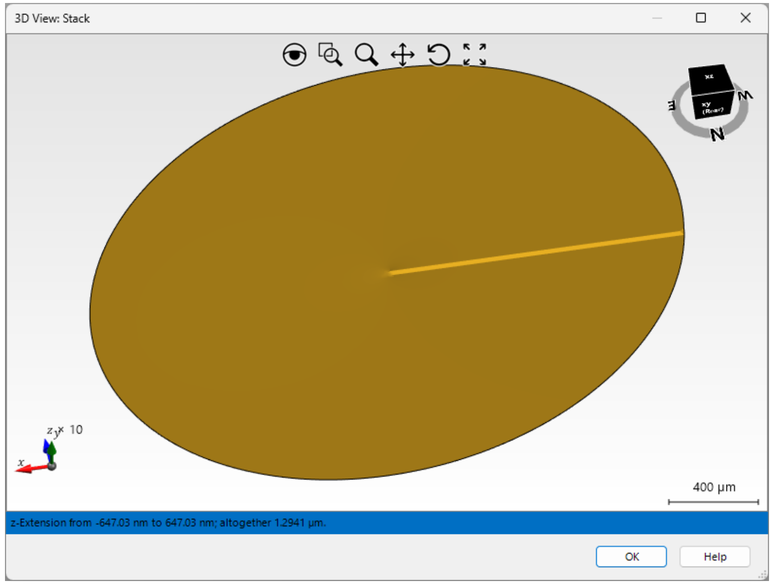
The *Multiple Light Source* allows the user to include different sources in a single system. They can also be shifted with respect to each other.

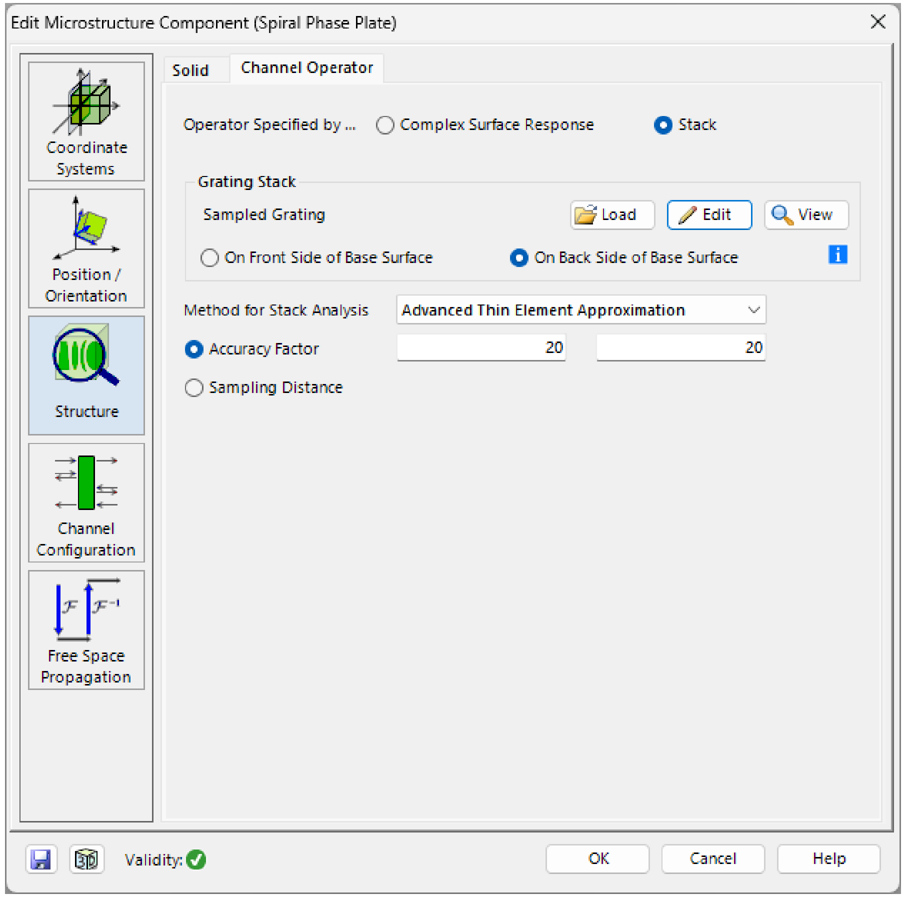
Multiple Light Source



Spiral Phase Plate

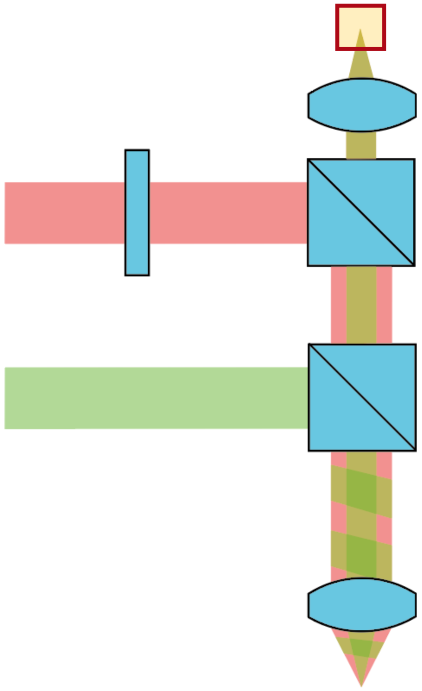






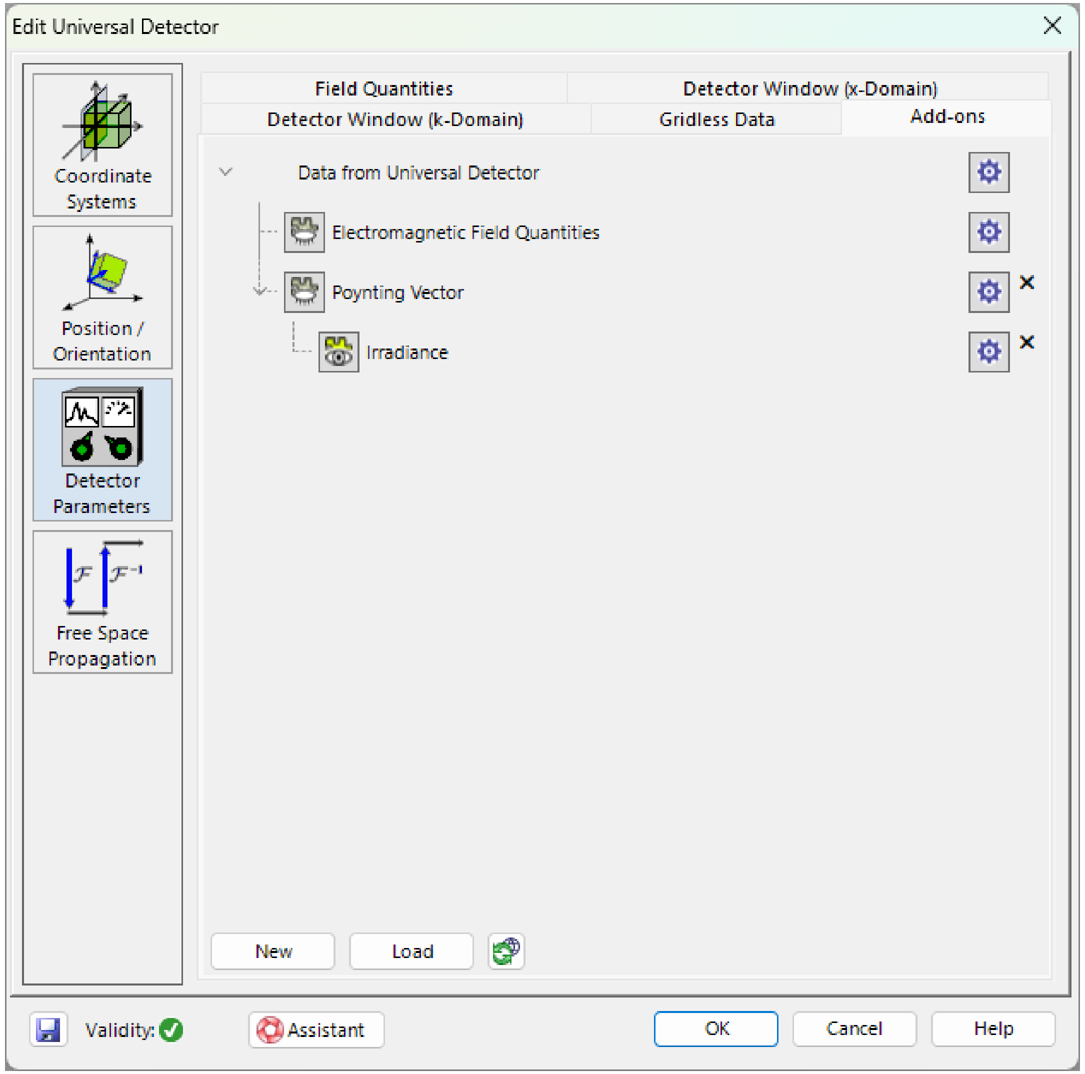
The *Microstructure Component* models diffractive structures using advanced TEA (Thin Element Approximation). In our example the spiral phase plate is given as a custom *Programmable Surface*. This surface can be included in a *Stack* and then loaded into the *Microstructure Component*.

Detector Addons

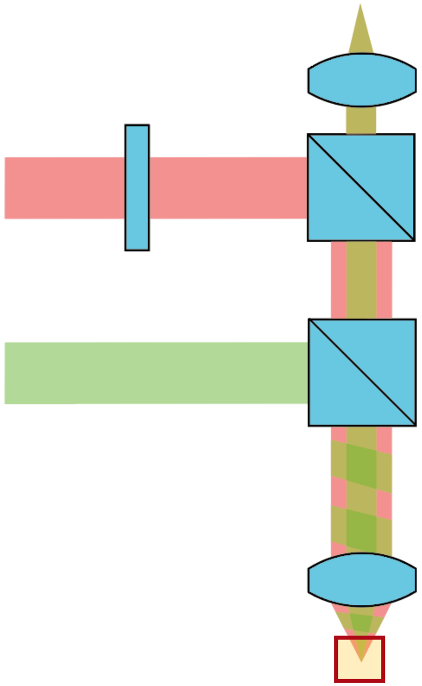


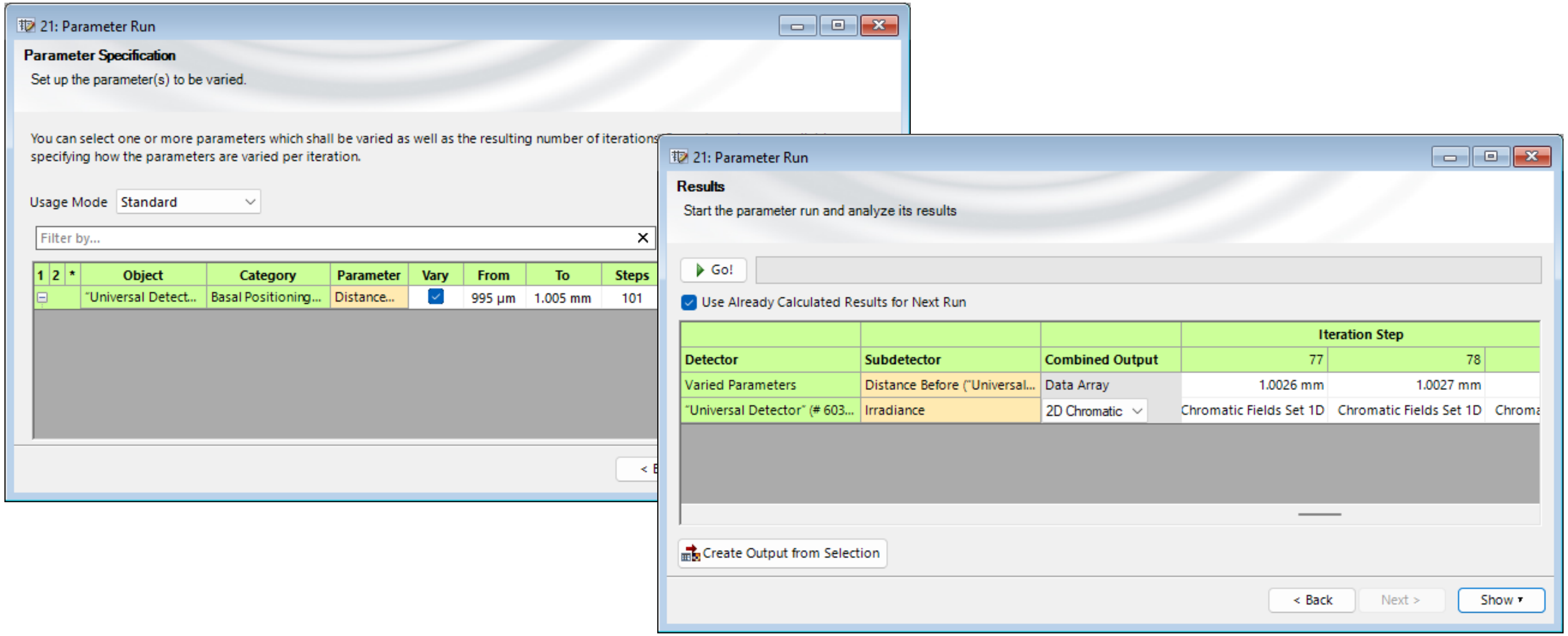
The *Universal Detector* enables the evaluation of the impinging field and the calculation of various physical quantities through so-called *Add-Ons*. In this example, the *Irradiance* is calculated. For more information, see:

Universal Detector



Parameter Run

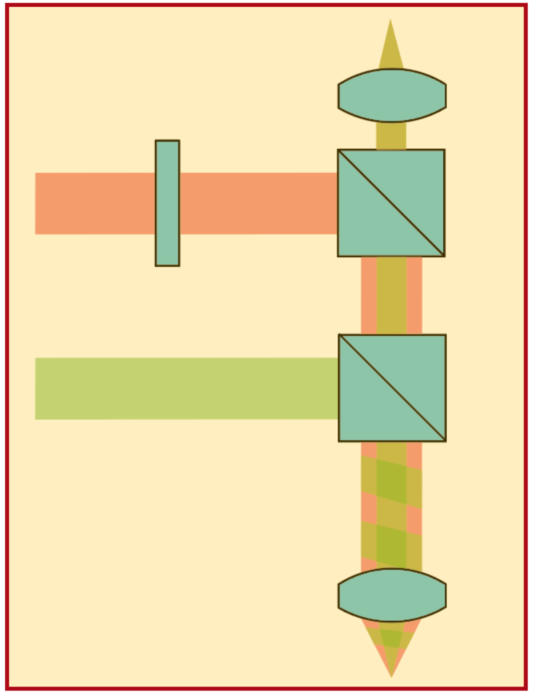


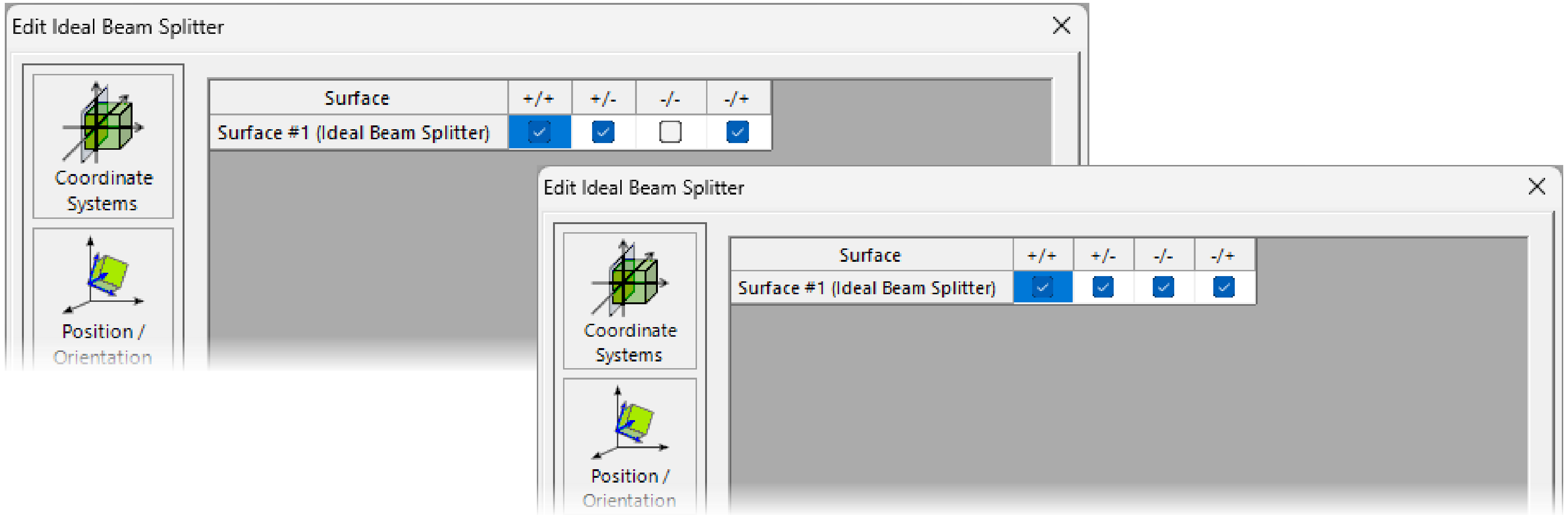


To achieve a z-scan of the focal region a *Parameter Run* can be performed. With this tool the user can easily vary an individual parameter or a set of parameters of the entire optical system. For more information, see:

Usage of the Parameter Run Document

Non-Sequential Modeling

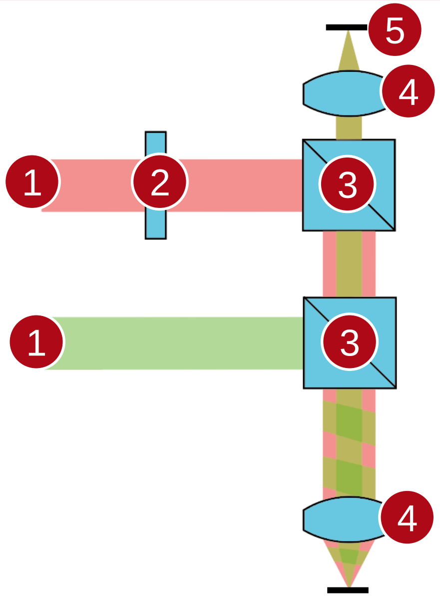




With the channel configuration mode toggle set to *Manual Configuration*, the user can specify, for each surface in the system, which channels to open for the simulation. When the simulation is run, a preliminary analysis of the active light paths will be performed (by the so-called *Light Path Finder*). The field will then be traced along these light paths by the engine to the detectors present in the system.

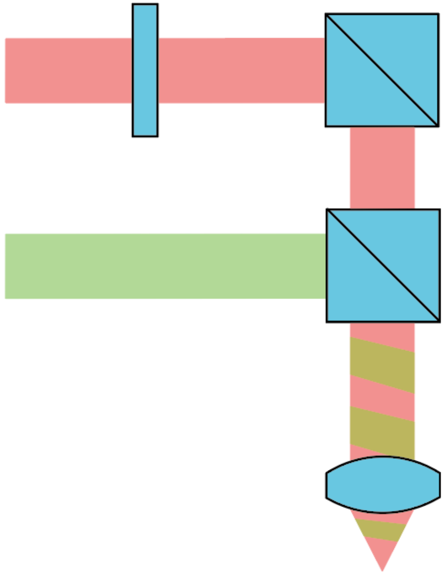
Channel Setting for Non-Sequential Tracing

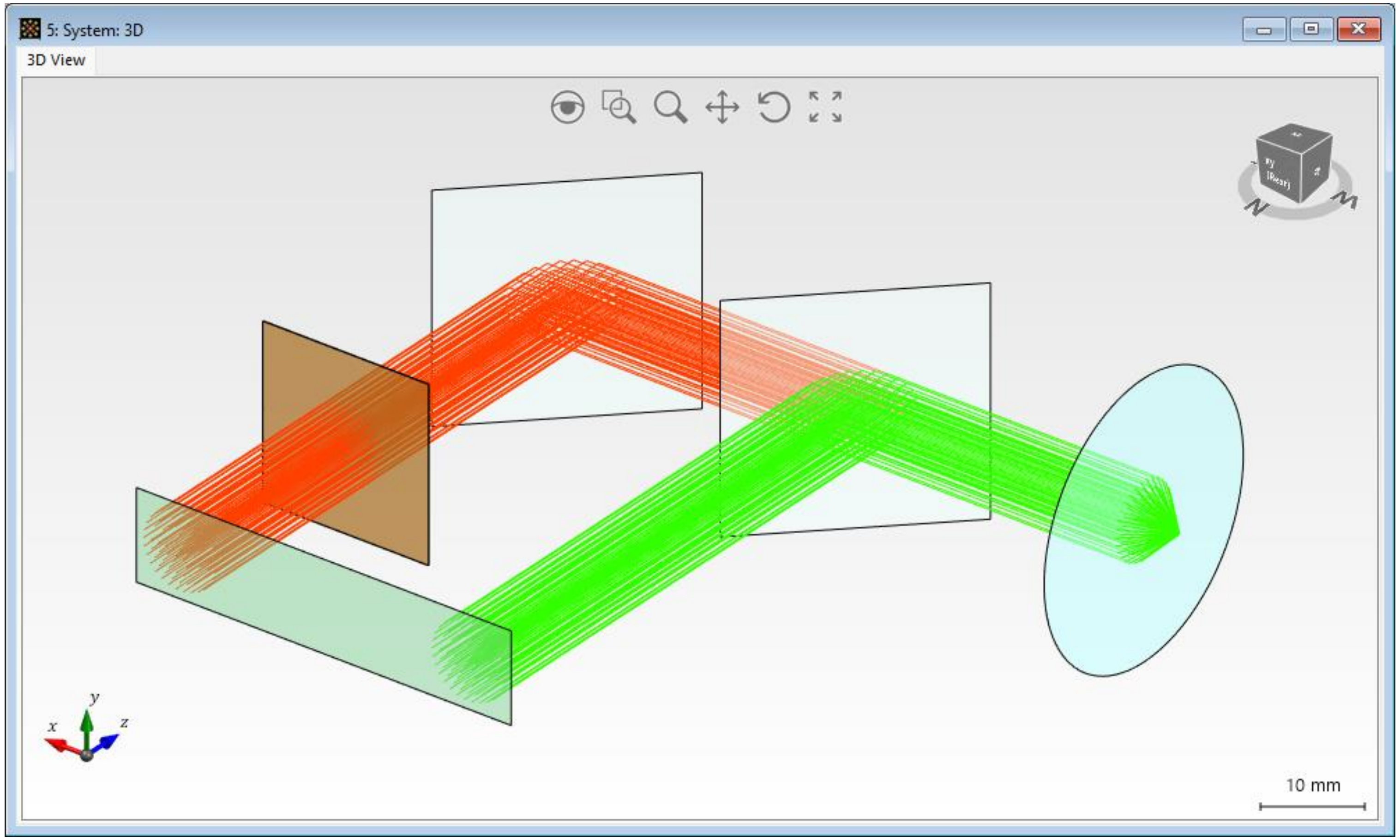
Summary – Components...



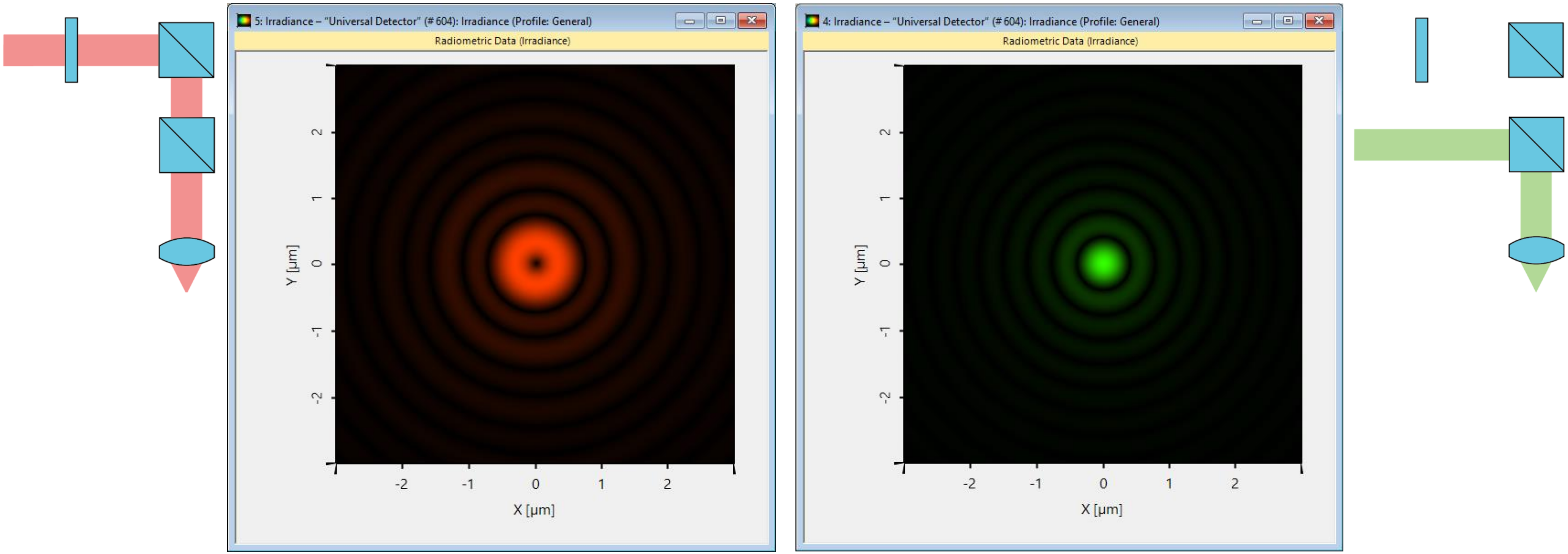
… of Optical System	… in VirtualLab Fusion	Model/Solver/Detected Value
1. source	*Multiple Light Source*	incoherent spatial source modes
2. phase plate	*Microstructure Component*	Advanced Thin Element Approximation
3. beam splitter	*Ideal Beam Splitter*	transmission function
4. lens	*Ideal Lens*	transmission function
5. detector	*Universal Detector*	irradiance

System Impressions



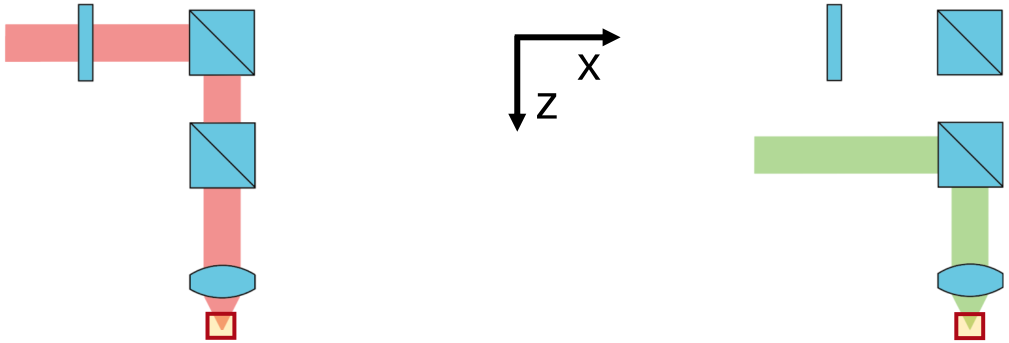


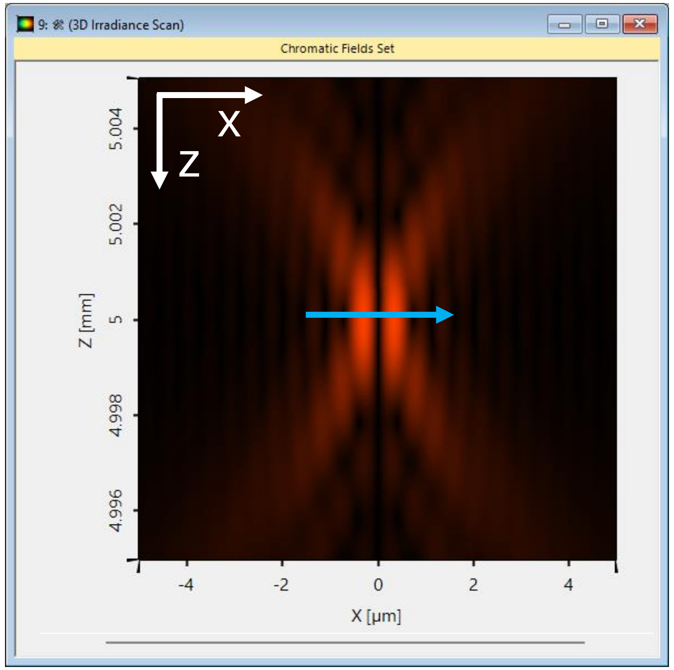
Excitation & Depletion Laser

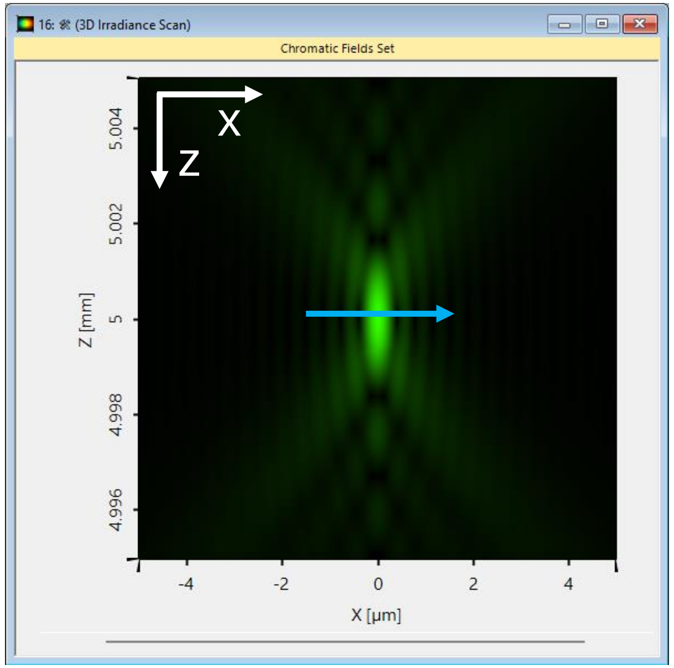


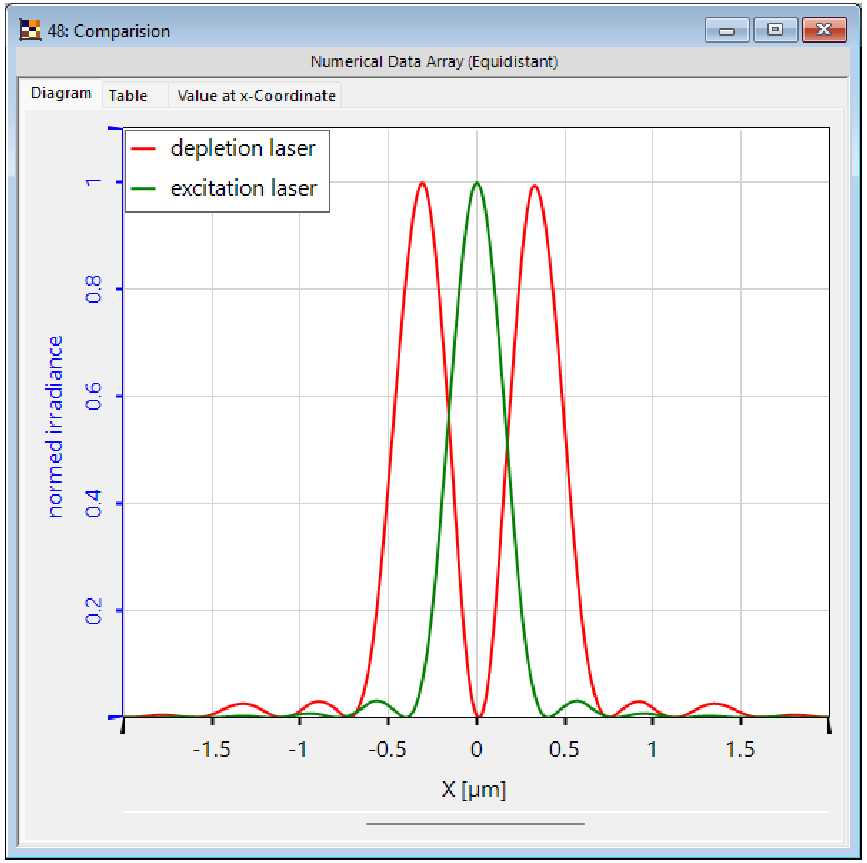
The propagation of light into the focal region reveals that light from the depletion laser generates a donut-shaped spot, wherein the central hole is smaller than the focal spot of the excitation laser. As the two beams compete in the fluorescence process on the target, this leads to an effective smaller beam size of the signal laser.

3D STED Profile





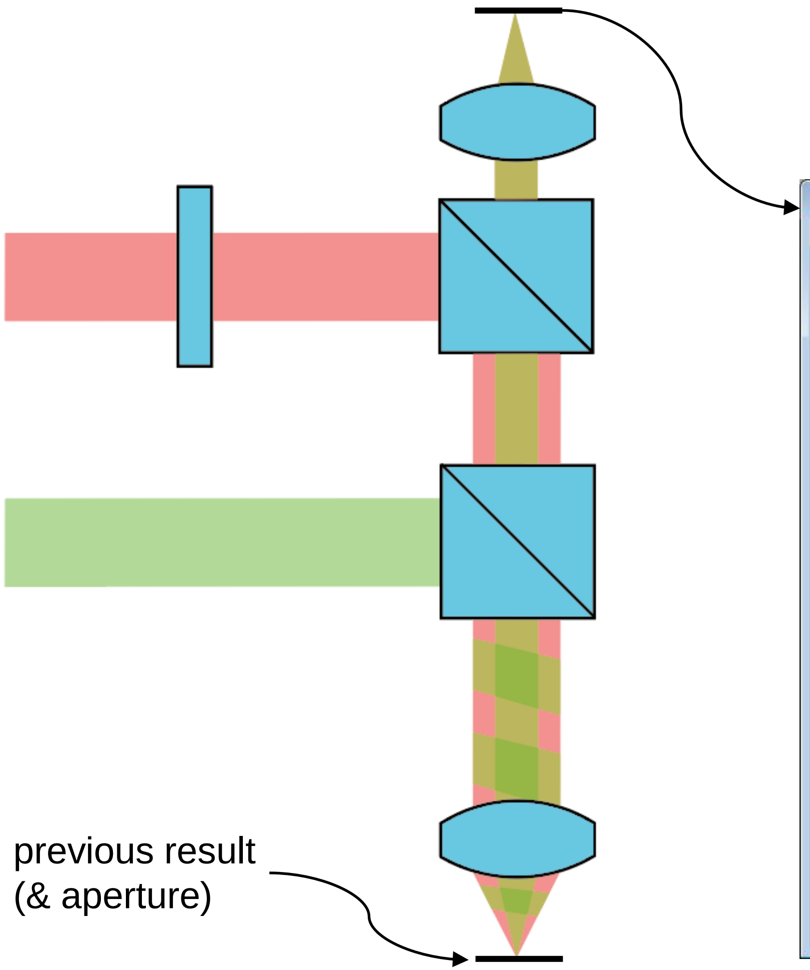


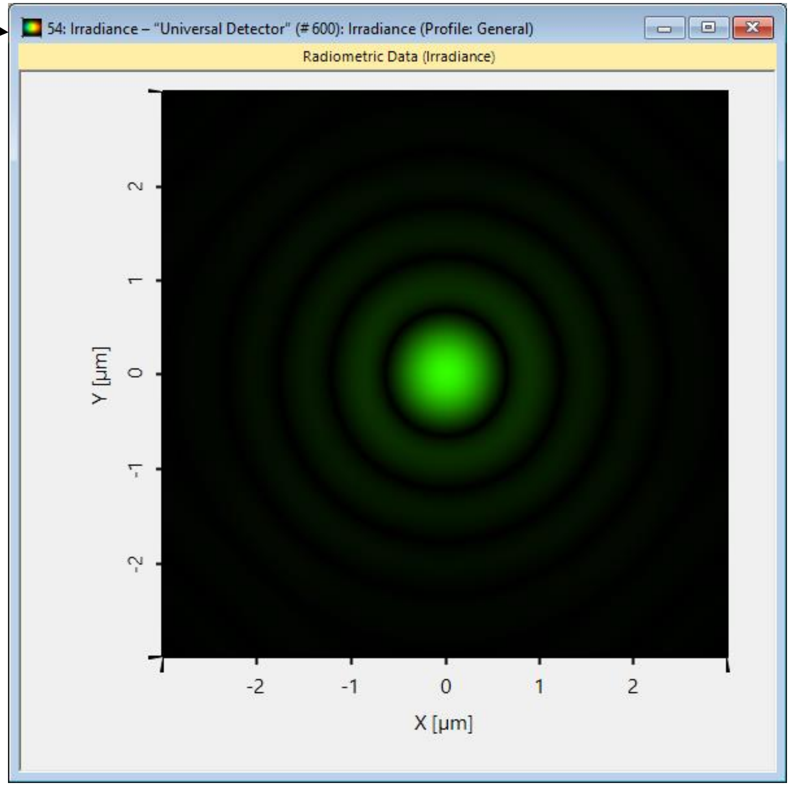


Note: As this simplified example does not include the actual fluorescence effect, we have normalized the two laser beams for visualization purposes.

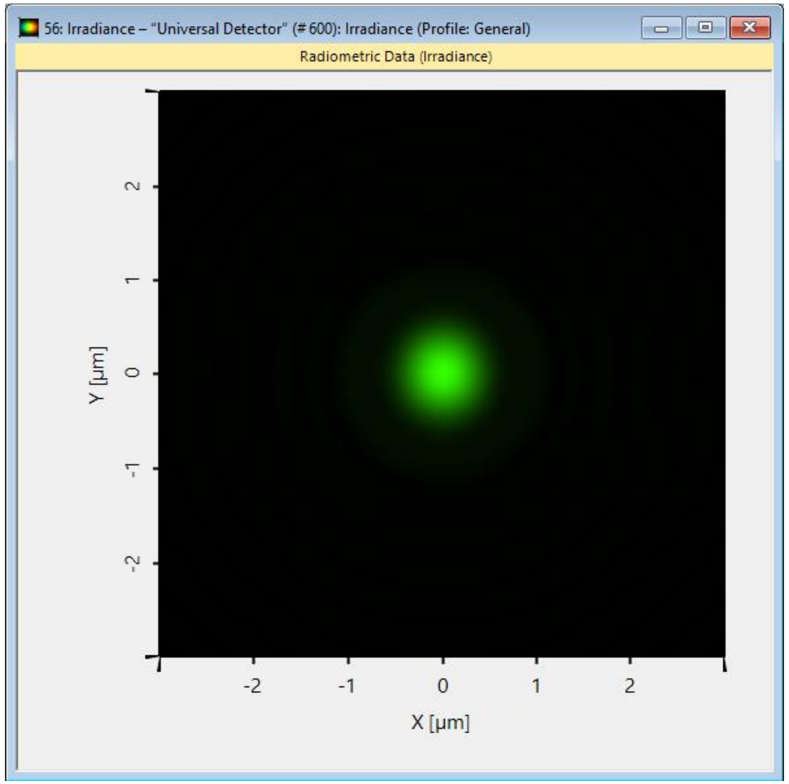
Stimulated Emission Depletion Effect

To approximate the effect of the saturated depletion, we applied an aperture effect on the result for the excitation laser at the focus position. The parameters of the aperture are roughly based on the focal profile of the depletion laser (600 nm diameter, 25% edge). Propagating back through the system to the detector plane reveals that the spot becomes significantly smaller due to this process.



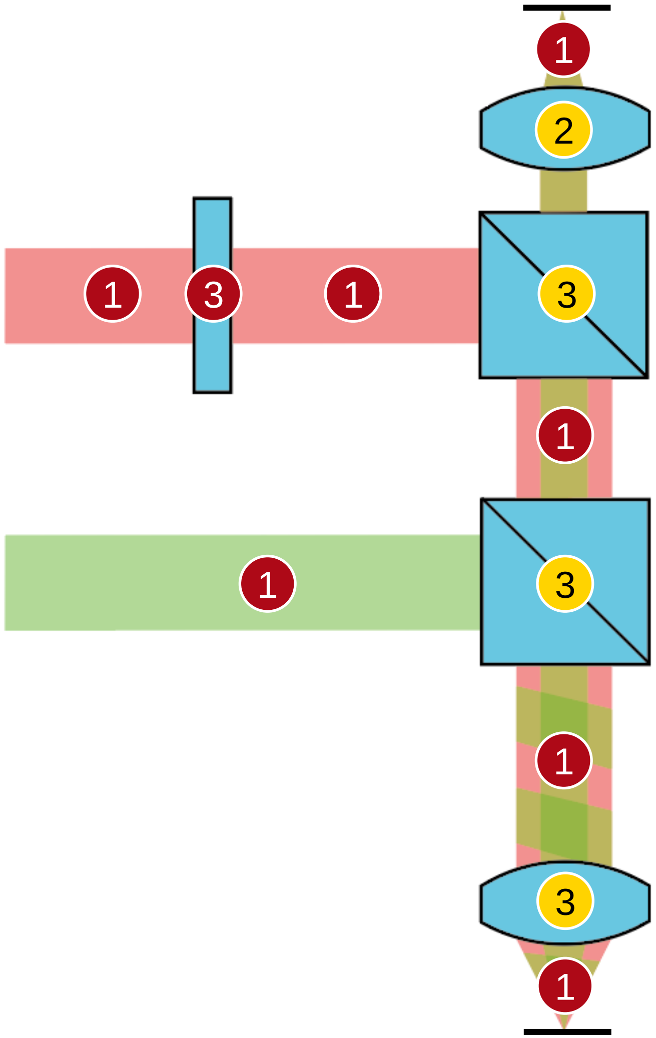


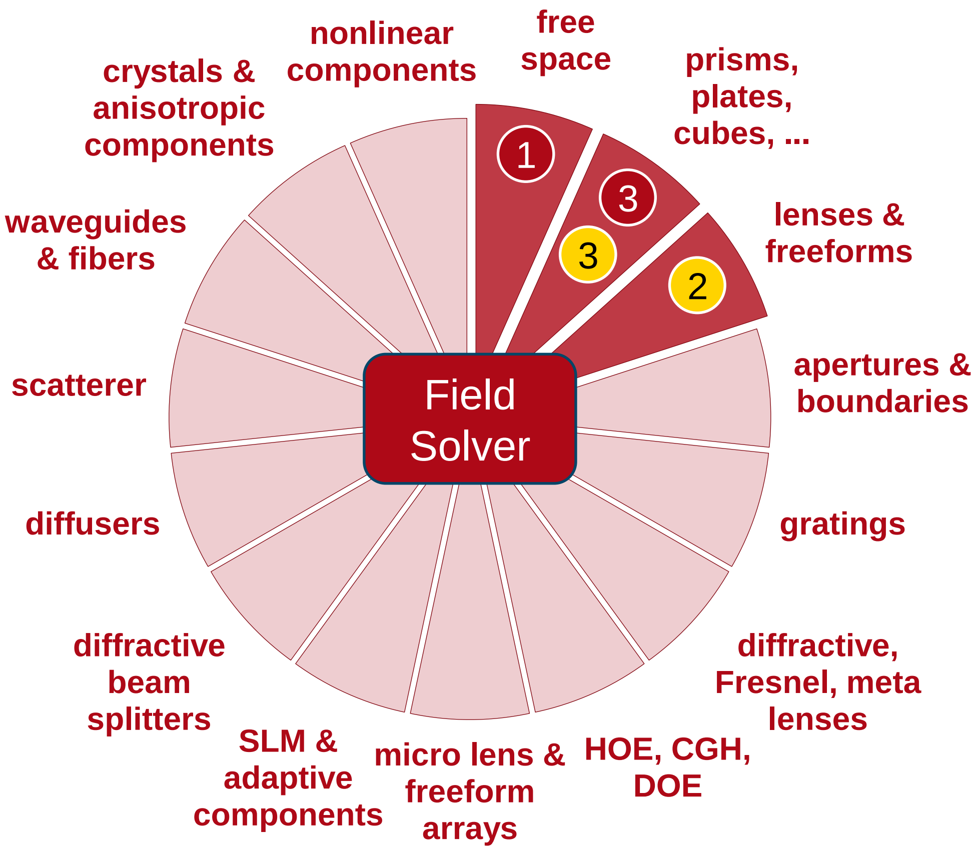
without aperture



with aperture

VirtualLab Fusion Technologies





idealized component

Document Information

title	Principle of Stimulated Emission Depletion (STED) Microscopy
document code	MIC.0024
document version	1.0
software edition	VirtualLab Fusion Basic
software version	2023.1 (Build 1.556)
category	Application Use Case
further reading	• Simulation of Multiple Light Source in VLF • Focusing of Gaussian-Laguerre Wave for STED Microscopy

Marketing Picture