

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



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

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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 (600nm diameter, 25% edge). Propagating back through the system to the detector plane reveals that the spot becomes significantly smaller due to this process.



VirtualLab Fusion Technologies



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Marketing Picture

