

# SHATTERING the DIFFRACTION LIMIT of LIGHT

## I. STRATEGIES

### IA. Volumetric-shaping of the excitation light

Most recent superresolution methods rely on the volumetric shaping of the excitation light (through a near-field aperture tip; periodic light gradients generated by interference, as in 4PI.)

### IB. Exploiting non-linear light-matter interactions

More specifically, using a nonlinear relationship between the excitation and the fluorescence emission

#### IB.1 Non-resonant Processes: Two- and multiphoton excitations

But they suffer from two main drawbacks.

- a) Fluorophores that emit in the visible require multiphoton excitation with doubled or tripled wavelength, which results in the doubling or tripling of the extent of the excitation spot (hence spoiling the resolution gained by the non linear process.)
- b) Multi-photon excitations are higher-order non-resonant processes. Consequently, their absorption cross sections are many orders of magnitude smaller than that of the linear one-photon process, which forces the use of intense short laser pulses for efficient excitation. The latter are phototoxic to the cell because they accelerate radical production, thereby limiting the available observation time before cell damage.

#### IB.2 Resonant processes:

Exploit spectroscopic properties of fluorophores to produce nonlinearities of large cross sections.

Previously fluorescence has been treated as a linear process. But Hell's group has been looking for ways to exploit the spectroscopic properties of fluorophores to produce non-linearities. This effort has led to the Stimulated Emission Depletion (STED).

**From reference:** S. Weiss, "Shattering the diffraction limit of light," PNAS 97, 8747 (2000)

## II. STIMULATED EMISSION DEPLETION (STED): Exploiting non-linearities in resonant processes

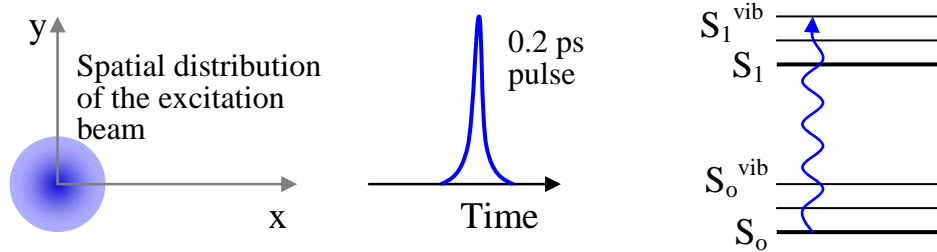
In 1994 Jan Wichmann and Hell published a theoretical paper on STED, outlining a concept to eliminate the resolution-limiting effect of diffraction without eliminating diffraction itself.

**Reference:** Stefan W. Hell and Jan Wichmann, "Breaking the diffraction resolution limit by stimulated emission: stimulated-emission-depletion fluorescence microscopy," *OPTICS LETTERS* **19**, 780 (1994).

STED exploit the selective quenching :

**a) Excitation process**

A **short pulse** 200 fs and  $\lambda_{exc} \sim 560 \text{ nm}$  excites the fluorophores from  $S_0$  into high vibrational states  $S_1^{vib}$ .



**Fig.1** Spatial (left) and temporal (center) distribution of the excitation beam. The diagram on the right side displays the energy levels of a typical fluorophore.  $S_0$  and  $S_1$  are the ground and first excited singlet states, respectively.  $S_0^{vib}$ , and  $S_1^{vib}$  are higher vibrational levels of these states. The excitation of the dye takes place from the relaxed state  $S_0$  to the states  $S_1^{vib}$ .

The intensity distribution of the excitation beam in the focal plane of the lens is determined by diffraction effects.

$$I_{exc}(r) \sim |J_1(\alpha r) / \alpha r|^2 \quad (\text{Joules per unit area per unit time.})$$

where  $r = (x^2 + y^2)^{1/2}$ , and  $J_1$  is the first order Bessel function.

The spatial extent of  $I_{exc}(r)$  determines the resolution of the microscope.

The efficiency of absorption is characterized by the absorption cross section coefficient  $\sigma_{01}$ .

$$\sigma_{01} \sim \text{in the } 10^{-16} \text{ to } 10^{-17} \text{ cm}^2 \text{ range}$$

If  $n_o$  is the population of the state  $S_0$ , the temporal population change due to stimulated absorption is given by,

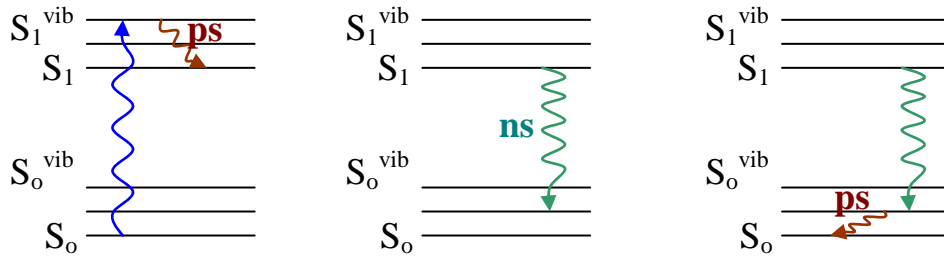
$$\frac{dn_o}{dt} = n_o \sigma_{01} I_{exc} / \hbar \omega$$

**b) Relaxation processes**

**Vibrational relaxations**  $S_1^{vib} \rightarrow S_1$  occurs in  $\sim 1$  to  $5 \text{ ps}$ .

**Fluorescence** by radiative transitions occurs due to transitions  $S_1 \rightarrow S_0^{vib}$ ; with an average fluorescence lifetime  $\tau_{fluor} \sim 2 \text{ n ps}$ .

This is three orders of magnitude slower than the vibrational transitions.



The transition from  $S_1$  to  $S_0^{\text{vib}}$  can also be induced by stimulated emission, which is

of particular interest here. The transitions  $S_{\text{vib}}$

$1 \rightarrow S_1$  and

$S_{\text{vib}}$

$0 \rightarrow S_0$  are vibrational relaxations. In the discussion of stimulated emission we can ignore the triplet state. Detailed reviews of dye properties are given by Lakowicz [12] and Schäfer [13].

Figure 1 also displays

$$I_{\text{exc}} = 1300 \text{ MW/cm}^2.$$

$$I_{\text{exc}}(r) / \hbar\omega \quad \text{photons per unit area per second,}$$

*quantifies the probability that an excitation photon arrives at  $r$ .*

Stimulated emission is the basis of laser action and one of the most widely applied physical phenomena. First reports of stimulated emission in organic fluorophores go back to Sorokin and Lankard [5] and to Schäfer and coworkers [6], who pioneered the development of the dye laser. The operational requirements in a laser are somewhat different than those for depletion of fluorescence. In a laser, the role of stimulated emission is to strengthen the beam by collecting stimulated photons, whereas in microscopy one is primarily interested in the depletion of the excited state by stimulated emission, irrespective of the population of the excited state.

When analyzing a sample containing organic fluorophores, STED exploits

- a) the depletion of a molecular fluorescent state through stimulated emission, and
- b) the fact that the product of two point-spread-functions (PSF) is narrower than a single PSF.

The first aspect is that it was experimentally demonstrated in 1999.

**References:**

Thomas A. Klar and Stefan W. Hell, "Subdiffraction resolution in far-field fluorescence microscopy," *OPTICS LETTERS*. **24**, 954, 1999.

Thomas A. Klar, Stefan Jakobs, Marcus Dyba, Alexander Egner, and Stefan W. Hell, "Fluorescence microscopy with diffraction resolution barrier broken by stimulated emission," *PNAS* **97**, 8206 (2000).