



KIT **STED: Confinement by "Inverse Saturation" or "Saturated Depletion"**

A Fluorescent (A) $\xrightarrow{k_{AB} = \sigma I}$ Non-fluorescent (B) $\xrightarrow{k_{BA}}$ Fluorescent (A)

B Intensity profiles $I(x)$ for spot sizes $l_s = 100 l_w$, $50 l_w$, $10 l_w$, and $500 l_w$. Scanning direction x .

C Energy level diagram showing Absorbing state (A), Fluorescent state (B), and Triplet or dark state (C). Transitions: Excitation, Fluorescence, Stimulated emission, Intersystem crossing.

Legend:
 ● STED
 ● GSD / photochromic switching
 ● Excitation saturation

Nanooptics 25/5 Hell et al., in Pawley (ed.), Biological Confocal Microscopy (2006)

KIT **STED Microscopy**

Spot volume: 12 attoliter (Confocal, 490 nm, 244 nm) vs. Spot volume: 0.67 attoliter (STED, 104 nm, 97 nm)

Fluorescence [a.u.] vs. I_{STED} [GW/cm²]. The graph shows a sharp drop in fluorescence as STED intensity increases, labeled "Saturated depletion".

References:
 S.W. Hell, J. Wichmann (1994), *Opt. Lett.* **19**, 780.
 T.A. Klar, S. Jakobs, M. Dyba, A. Egner, S.W. Hell (2000), *PNAS* **97**, 8206.
 S.W. Hell, M. Dyba, S. Jakobs (2004), *Curr. Opin. Neurobiol.* **14**, 599.

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KIT **Phase masks for STED beam**

donut focus
 a) 3D intensity plot
 b) Intensity profile
 c) Intensity profile
 d) phase mask
 e) Intensity profile vs. depletion peak intensity
 f) 3D intensity plot
 g) Intensity profile
 h) Intensity profile
 i) phase mask
 j) 3D intensity plot
 k) 3D intensity plot

anticipated voxel scaling

Joachim Fischer, Dissertation

Nanooptics 25/7

KIT **The Depletion Idea**

excitation intensity² depletion intensity relevant excitation

voxel width 230 nm

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