THEORY OF SUPER RESOLUTION – DETERMINISTIC AND STOCHASTIC METHODS UNIFICATION

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Introduction

Super resolution microscopy images are commonly acquired while no unique theory explains their resolution gain. Two methods, deterministic and stochastic, explain their gain compared to the diffraction limit, respectively with factors $\left(\sqrt{1+{}^{I}/_{I_{sat}}}\right)^{-1}$ and $\left(\sqrt{N}\right)^{-1}$ where I is the laser intensity, I_{sat} is the saturation power and N is number of measured photons [1-2]. Using simple ideal system equilibrium equations, we show the equivalence between these two factors. These equations also explain the apparent dark state at very high photon flux and show that the fluorescent probe concentration is the limiting factor for super resolution.

Dark state is the apparent result of depletion occurrence exceeding natural emission via fluorescence process

Simple equilibrium equations allow obtaining relative state population in function of laser photon flux (Figure 1). One process brings molecules to the excited state while two processes (depletion and natural fluorescence emission) bring the molecule back to the ground state. We show that the depletion (no emission of fluorescence) occurs more frequently at high power leaving fewer molecules to fluoresce but synchronously giving rise to blinking when emitting their fluorescence at high photon flux.

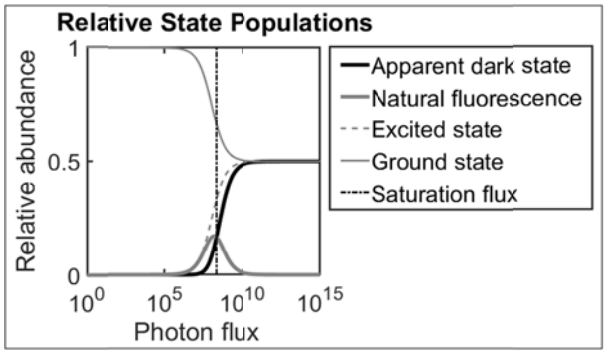

Figure 1. Relative state populations at different photon flux.

Concentration is the limitation for resolution fluorescence techniques

No matter the technique used (deterministic or stochastic) the resolution depends on fluorescence and is therefore limited by the specimen concentration. The closer the fluorescent molecules are, the smaller the resolution can be.

Conclusion

Better understanding of super resolution mechanisms might allow better controls over the fluorescence process and finding out new methods. We wish to explore how the apparent dark state is distributed over the whole process of photon emission, depletion and stimulated emission and prove that molecules' blinking is due to spontaneous stimulated emission.

References

[1] Hell, S. W. Microscopy and its focal switch. Nature Methods (2009).
[2] Thompson, R. E. *et al*. *Precise Nanometer Localization Analysis for Individual Fluorescent Probes*. Biophysical Journal. (2002).