

STIMULATED FLUORESCENCE EMISSION OCCURS AT SATURATION POWER

Dominic Fillion, Michael Housset
Microscopy Core Facility
Institut de Recherches Cliniques de Montréal
110, Avenue des Pins Ouest, Montréal (Québec) CANADA
E-mail: dominic.fillion@ircm.qc.ca

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Introduction

In order to support our theory for super resolution unification of deterministic and stochastic methods (manuscript in preparation), we subjected our specimen to an increasing ramp of photon flux. Measurement reveals an unexpected and unreported phenomenon occurring at saturation power. The result shows a resonant effect of fluorescence emission (more than 5 times greater than the maximum value) which we associate to a stimulated effect amplified by the proper rate of photons being brought to the fluorescent molecules.

Results

Paraformaldehyde-fixed HEK 293T cells overexpressing cytoplasmic green fluorescent protein (GFP) were labelled using Rabbit anti-GFP antibody and secondary goat anti-Rabbit secondary coupled to AF-647 and put in PBS-Cysteamine buffer solution [1]. Specimen was observed under a Zeiss Elyra system for time-lapse of 100 ms image acquisition at 1s frame rate with logarithmic increase of excitation laser power. Figure 1 shows the peak relative fluorescent at saturation excitation power along with the theoretical curve fit.

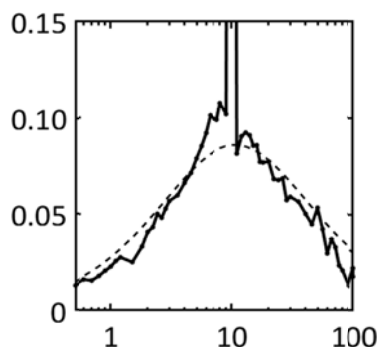


Figure 1. Relative fluorescence (maximum value = 1) in function of relative excitation power and predicted curve fit.

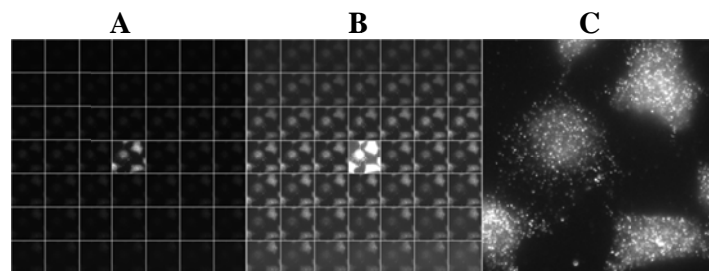


Figure 2. Raw image data (increasing power from left to right and up to bottom) are shown in tile (A), same tile images as A but amplified 5 times (B) and peak raw image acquisition (C) – center image from tile A.

Conclusion

The unexpected result shows that power is a frequency of photon number that we supply to our specimen at which the natural decay of fluorescence has resonance. This power ramp could be a way to precisely measure the time decay and also separate dyes. This result gives rise to many other unanswered questions which will be part of a forthcoming publication.

References

[1] Heilemann, Mike. *Subdiffraction-Resolution Fluorescence Imaging with Conventional Fluorescent Probes*.