Optimization of Advanced Live-Cell Imaging Through Red/Near-Infrared Dye Labeling and Fluorescence Lifetime-Based Strategies

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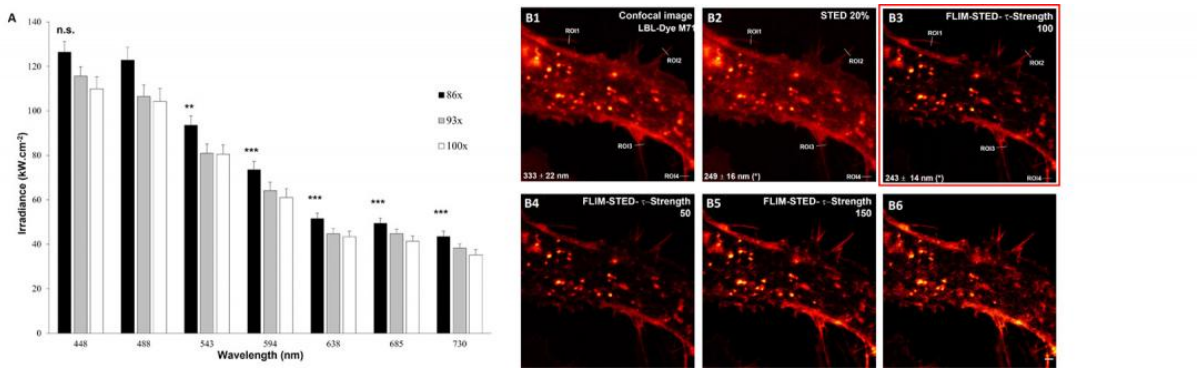
Abstract

Fluorescence microscopy is essential for a detailed understanding of cellular processes; however, live-cell preservation during imaging is a matter of debate. In this study, we proposed a guide to optimize advanced light microscopy approaches by reducing light exposure through fluorescence lifetime (t) exploitation of red/near-infrared dyes. Firstly, we characterized key instrumental elements which revealed that red/near-infrared laser lines at 86x (Numerical Aperture (NA) = 1.2, water immersion) objective allowed high transmission of fluorescence signals, low irradiance and super-resolution. As a combination of two technologies, i.e., vacuum tubes (e.g., photomultiplier) and semiconductor microelectronics (e.g., avalanche photodiode), type S, X and R of hybrid detectors (HyD-S, HyD-X and HyD-R) were particularly adapted for red/near-infrared photon counting and t separation. Secondly, we tested and compared lifetime-based imaging including coarse t separation for confocal microscopy, fitting and phasor plot analysis for fluorescence lifetime microscopy (FLIM), and lifetimes weighting for enhanced stimulated emission depletion (STED) nanoscopy, in light of red/near-infrared multiplexing. Mainly, we showed that the choice of appropriate imaging approach may depend on fluorochrome number, together with their spectral/lifetime characteristics and STED compatibility. Photon-counting mode and sensitivity of HyDs together with phasor plot analysis of fluorescence lifetimes enabled the flexible and fast imaging of multi-labelled living H28 cells. Therefore, a combination of red/near-infrared dyes labelling with lifetime-based strategies offers new perspectives for live-cell imaging by enhancing sample preservation through acquisition time and light exposure reduction



(A) Irradiance of WLL lines through 86×, 93× and 100× objectives. Thanks to LAS-X software, laser power at 448, 488, 543, 594, 638, 685 and 730 nm was set at 47 µW and measured through an optical power meter integrated in Argo-POWER slide. By considering wavelength (nm) and numerical aperture (unitless), irradiance values (kW.cm−2) were calculated and directly read on Daybook 3 software from Argolight. Each value represents the mean (± SEM) of three independent experiments. Significant irradiance differences between WLL lines (ns.; **p < 0.01; ***p < 0.001 vs 488 nm) were determined for 86× using an ANOVA test followed by a Tukey–Kramer multiple comparison test

(B) Input of FLIM in STED nanoscopy of living H28 cells labeled with red/near-infrared dyes. Confocal (B1) and STED (20% 775-nm depletion laser, B2) imaging of LBL-Dye M717-labeled H28 cells (ex 690 nm, 3%, HyD-X). (B3–B6) Impact of t Strength factors (50–200) on STED imaging. Red box indicates best parameters configuration. Significant lateral resolution differences between imaging approaches (*p < 0.05 vs confocal approach) were determined using an ANOVA test followed by a Tukey–Kramer multiple comparison test