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Enhanced Total Internal Reflection Fluorescence Microscopy using resonant dielectric multilayers

Total Internal Reflection Fluorescence (TIRF) Microscopy is based on the generation of evanescent waves on the surface of a glass coverslips enabling imaging with a depth of focus of about 100-200 nm. This makes TIRF microscopy a key instrument to observe cell – substrates contact region and to provide a detailing understanding of membrane particles dynamics. These 2 last decades, TIRF Microscopy has seen its importance increasing thanks to its coupling with single molecule localization microscopies (SMLM). However, the use of high NA objective amplifies the angular divergence of excitation beam which in turn provokes an uneven illumination (i.e. combination of both propagative and evanescent fields) when working at the critical angle. This results in a poor contrast of TIRF images. Excitation at higher incidence angles could solve this problem but the signal intensity is exponentially decreasing with increasing angles. Therefore, one has to compromise between contrast and signal intensity.

To overcome this limitation, we propose an optical method consisting of coating the glass coverslip with resonant dielectric multilayers. The latter, thoroughly optimized and designed, generates resonant modes that allows the local enhancement of the excitation field without increasing the noise level. Using a commercial TIRF microscopy, we carried out numerical and experiment studies to demonstrate the capability of these resonant dielectric multilayers to enhance fluorescent signal. We adjusted the optimization parameters in order to fabricate an adaptable coating with respect to the instrumental configuration. We finally exposed the enhanced fluorescence imaging of labeled viral particle and the assembly of HIV-1 Gag at the plasma membrane of living cells.

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Keywords: resonant dielectrics multilayers; fluorescent imaging; total internal reflection; evanescent wave

Session

Host-pathogen interactions

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