Enhanced axial position sensitivity for fluorescent molecules using nanostructured silver films

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We present a novel imaging technique with super-resolution axial sensitivity, based on changes in fluorescence lifetime above a nanostructured silver film (NSF). We have developed thin films of Ag nanostructures that can be easily manufactured over large continuous areas. By imaging a calibration sample through these films we have been able to empirically quantify changes in fluorescence lifetime above the NSF. We find these modifications are dominated by radiative rather than non-radiative processes, and fit well with a theoretical model: within 50 nm of the NSF we observe a two-fold reduction in radiative lifetime, with a commensurate increase in fluorescence intensity. From this it is possible to determine changes in the axial position of a fluorophore with a sensitivity of ~3nm. We demonstrate that the changes in lifetime can be used to monitor the position of a membrane receptor during endocytosis in carcinoma cells expressing eGFP.

Figures: (Left) Fluorescence lifetime (black) and normalized intensity (gray) as a function of axial distance above a NSF. (Right) Schematic showing CXCR4 mediated endocytosis of CXCL12. The NSF produces a spatial variation in the EGFP lifetime within the cell.