Dependence of Fluorescence Intensity on Tip-sample Separation in the Near-field Scanning Optical Microscope (NSOM)

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NSOM, a high-resolution technique that allows the simultaneous mapping of the topographical and optical properties of a sample surface, offers a spatial resolution beyond that of the classical diffraction limit. This improved resolution is obtained by utilizing a sub-wavelength light source maintained in the near-field of the sample surface. However, despite the high spatial resolution offered by this technique, the distance dependence of the sample fluorescence has not been examined in detail. The intensity of the fluorescence signal of fluorescent polystyrene microspheres, (40 nm red-orange spheres), embedded in layers of a transparent non-fluorescent polymer, polyvinyl alcohol (PVA), has been investigated as a function of the NSOM tip-sample separation. (Figure 1). Layers of PVA, each of a defined thickness, deposited onto the layer containing the spheres were used to vary the tip-sample separation in the range 10 – 460 nm. The illumination configuration of the NSOM was used to generate fluorescence (red-orange) following visible excitation at 568 nm. Analysis of the fluorescence images obtained for the thin films in the aforementioned range indicates that the fluorescence signal decays quasi-exponentially; in fact, there is a decrease of a factor of two when the tip is approximately 147 nm from the sample. The results obtained in this study are useful for the calibration of the NSOM and hence may lead to a better interpretation of the NSOM signals obtained for thick biological systems, such as cells.

Figure 1: Fluorescence signal of 40 nm red-orange spheres embedded in a thin film as a function of the NSOM tip-sample separation