

COMPLEX LATERAL ORGANIZATION IN LIVE CELL MEMBRANES REVEALED BY FLUORESCENCE CORRELATION SPECTROSCOPY IN SINGLE NANOMETRIC APERTURES

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We describe the development of a new methodology to probe the plasma membrane organization of living cells at the nanometric scale. We use single subwavelength apertures milled in a metallic film with radii between 75 and 250 nm to drastically limit the observed membrane area below the optical far-field diffraction barrier [1-3]. The new approach performs fluorescence correlation spectroscopy with increasing aperture sizes and extracts information on the diffusion process from the whole set of data. In particular, transient diffusion regimes are clearly observed when the probed area comes close to the size of the confining structures [4].

We investigate the diffusion dynamics of fluorescent lipid analogs and GFP-tagged proteins within living cell membranes. Transient diffusion regimes are observed when the probed area is close to the size of the confining structures in the plasma membrane, revealing nanometric membrane heterogeneities constraining the diffusion. Using numerical simulations, we identify the mechanism controlling the diffusion in the plasma membrane and give an estimate of the characteristic size of the nanometric heterogeneities. This technique allows a quantitative study of membrane domains such as lipid rafts. Compared to single particle tracking, our method combines the advantages of high spatio-temporal resolution and direct statistical analysis.

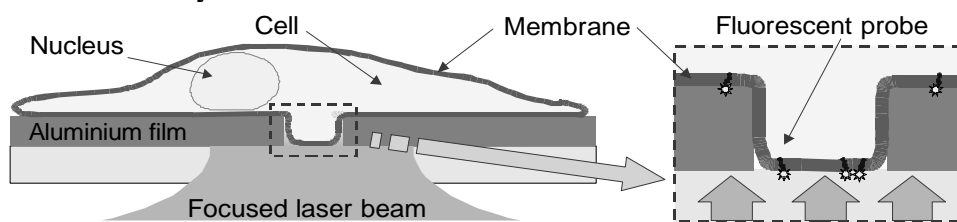


Figure 1 : Nanohole in an Al film to probe diffusion in a COS-7 cell membrane

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