Confocal Fluorescence Correlation Spectroscopy through a scattering layer

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Optical microscopy in presence of light scattering raises conceptual and practical issues, such as those encountered with biological tissues. In addition to imaging, optical measurements of molecular properties are also challenging. We have carried out Fluorescence Correlation Spectroscopy (FCS) experiments with free diffusing fluorophores, performed through two kinds of scattering layers:
i) in the first case, we considered a sparse scattering layer made of dielectric beads (from 3 to 15µm in diameter) that produce a partially developed speckle (Fig.1).

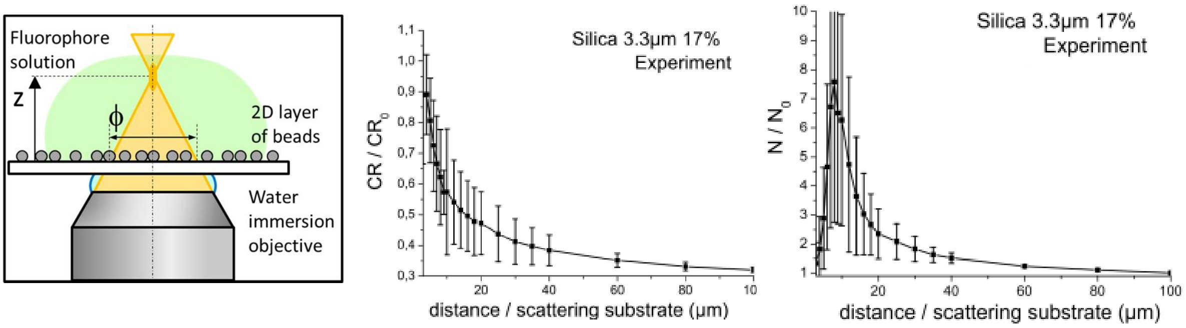

Figure 1 Left, principle of the FCS experiment in a fluorescent solution, through a scattering layer of dielectric beads; Middle, decay of fluorescence signal when going deeper in the solution; Right, maximum biasing effect on the number of molecules at a depth of about 2 bead diameters. Example for 3.3 µm silica beads.

We find that the fluorescence signal (Count Rate, CR) steadily decreases as the focus is moved away from the scattering layer. When focusing close to the surface only few beads scramble the wave front, inducing a strong increase of the mean number of molecules, N, within the confocal observation volume. The maximum bias is found at a characteristic distance of about two bead diameters. By contrast, the estimated number of molecules (and diffusion time) recovers its normal value when focusing deeper in the solution. Theoretical considerations demonstrate how these effects, due to diffraction and refraction by the scattering layer, depend upon the size and refractive index of the beads.
ii) in the second case, we used ground glasses producing a fully developed speckle, so that the pinhole cannot be used to filter out the non-ballistic photons. In this case, similarly to what is done to image behind a scattering medium [1], it is nevertheless possible to make profit of the spatial properties of the speckle, the autocorrelation of which is essentially the standard Point Spread Function. We will present preliminary results obtained by using molecules diffusing in 2D at the surface of an aqueous solution.

[1] O. Katz, P. Heidmann, M. Fink and S. Gigan. " Non-invasive single-shot imaging through scattering layers and around corners via speckle correlations," *Nature Photon.* **8**, 784-790 (2014)