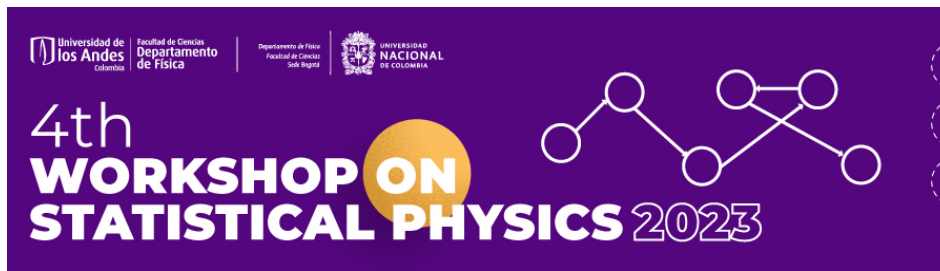


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Characterization of Fluorescence Correlation Spectroscopy (FCS) for Two-dimensional Diffusion Coefficient Measurements

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Thanks to Einstein's relation, it is known that two-dimensional diffusion coefficients give the amount of area that a particle under Brownian motion can cover in a determined time. In biological sciences, the diffusion coefficient is a relevant parameter to understand the motion of proteins and molecules providing quantitative insights into the mechanical properties of their microenvironment and their interaction with other molecules.

Among the available methods to measure these coefficients, *fluorescence correlation spectroscopy (FCS)* uses the fluorescence signal over time from the illumination volume in a confocal microscope that results from the random motion of fluorescent molecules. The analysis of time correlations in the fluorescence signal from FCS allow to quantitatively evaluate the concentration, interaction between molecules and the diffusion coefficient.

In this work, we characterize the FCS method in a model membrane system, allowing us to measure diffusion coefficients in a precise way. We use giant unilamellar vesicles (GUVs), a popular model for the study of the bilayer lipid membrane, composed of the phospholipid DOPC and the lipophilic fluorophore DiI. Using FCS we characterize the diffusion coefficient of DiI at 37 C and 45 C and corroborate the effect of temperature in molecule dynamics. To the best of our knowledge, this is the first implementation of FCS in a Colombian laboratory without use of any specialized software or external tools.

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