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Supporting Material

Exploring the Dynamics of Cell Processes through Simulations of Fluorescence Microscopy Experiments

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SUPPORTING MATERIAL

TABLE SI

Scenario	**Parameter**	**Simulation**	**ACF/CCF analysis**
Non-competitive binding	D_{red} ($\mu m^2/s$)	50.00	50.21 ± 0.16
	D_{green} ($\mu m^2/s$)	50.00	50.00 ± 0.19
	$k_{off,red}$ (1/s)	10.00	9.98 ± 0.27
	$k_{off,green}$ (1/s)	10.00	10.37 ± 0.26
Complex formation and binding	$D_{complex}$ ($\mu m^2/s$)	39.00	38.03 ± 1.63
	$k_{off,complex}$ (1/s)	10.00	11.78 ± 0.75

Errors in ACF or CCF analyses represent the standard error recovered obtained from 25 independent runs.

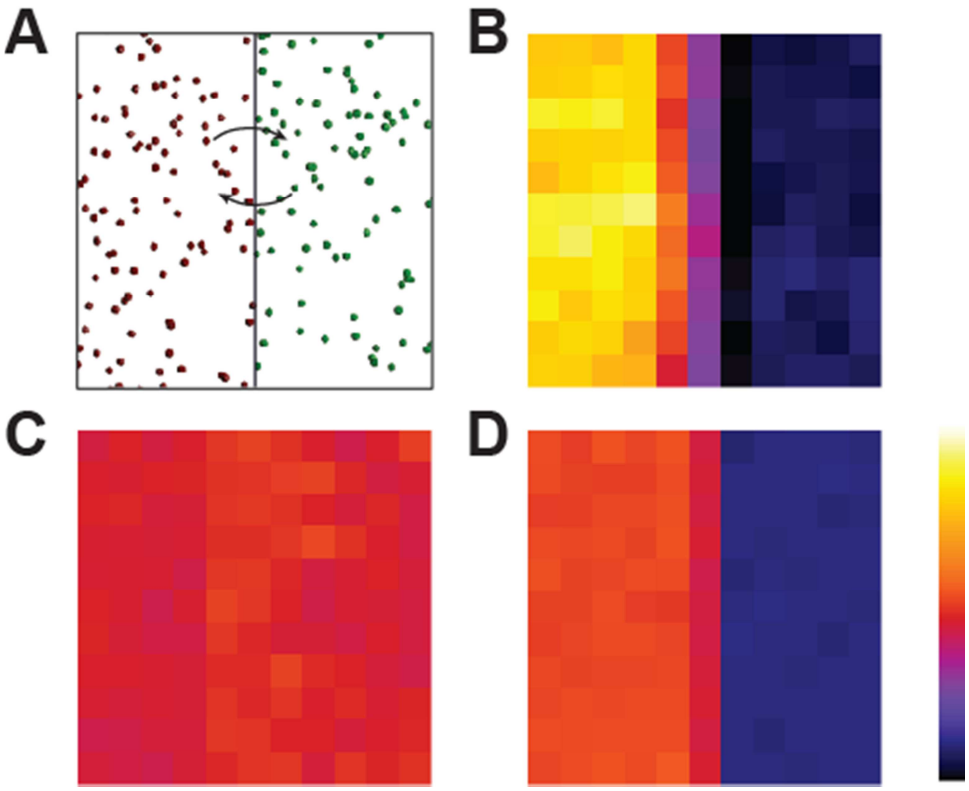

FIGURE S1 Multiplexing point-FCS measurements. The simulation space was considered as a cube divided into two compartments; a 2D scheme of this space is showed in (A). 385 molecules were released in each compartment (D=40 $\mu m^2/s$, ε = 1 cpm left side, D=10 $\mu m^2/s$, ε = 0.2 cpm right side) and the simulation was run with t_s = 10 µs during 5 s. The kinetic constant of the molecular conversion at the membrane was set to 10^9 s^{-1}. FERNET was set to collect the intensity traces in an array of 11x11 PSF. Equation 3 was fitted to the ACF calculated at each position to obtain pseudo-color maps of the diffusion coefficients (B) and number of molecules in the confocal volume (C). PCH analysis was performed at each position obtaining a pseudo-color map of brightness (D). Color-code bar represents D values from 8 to 60 $\mu m^2/s$; N values from 1 to 5 and ε values from 0 to 2 cpm (black to white), respectively.

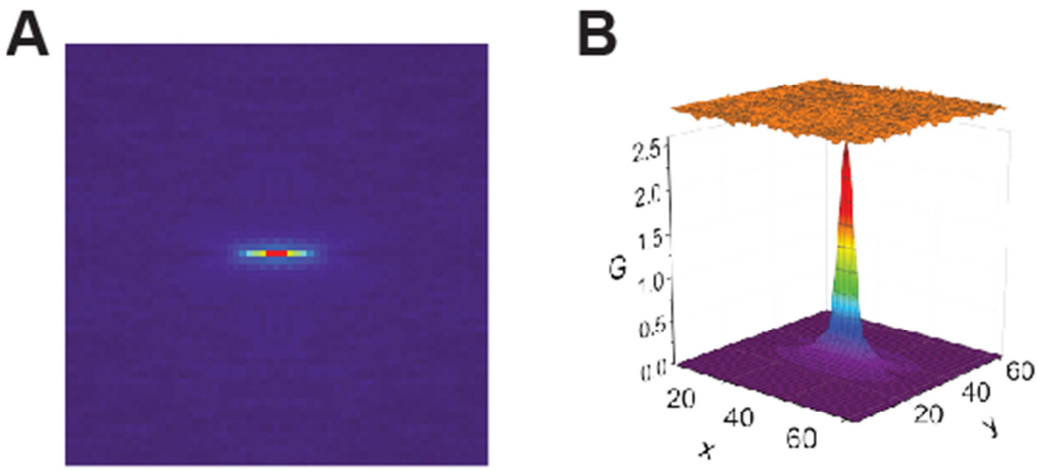

FIGURE S2 Raster image correlation spectroscopy. A similar simulation system to that described in Fig. 1 was considered and FERNET was set to a raster-scan mode. The RICS matrix was calculated from the simulated images (A) and was fitted as described in the text obtaining the best-fit surface (B) and the parameters presented in Table I. Top panel: residuals obtained from the fit.

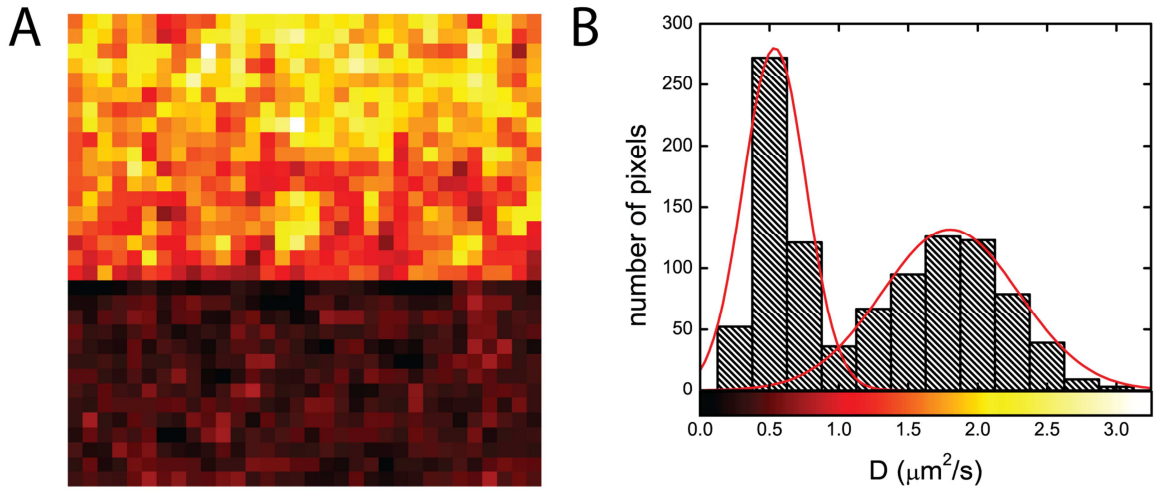

FIGURE S3 Simulations of SPIM-FCS (single plane illumination microscopy in combination with FCS). The simulation scenario was a cube divided into two compartments containing 24 particles/μm^3 each (D=2 $\mu m^2/s$, ε = 1 cpm top side, D=0.5 $\mu m^2/s$, ε = 1 cpm bottom side). The kinetic constant of the molecular conversion at the membrane was set to 10^9 s^{-1}.The illumination profile was considered uniform along the x-y plane and Gaussian (waist = 0.5 μm) in the perpendicular axis (1). MCell was run for 2 10^6 iterations and FERNET was set to simulate intensity traces in SPIM-FCS mode with a frame acquisition time of 1ms. The fluorescence emission was spatially binned to a pixel size of 0.24 μm, shot noise and Gaussian readout noise (σ = 5) was added to each pixel. The obtained image stack was processed with the imFCS plugin for ImageJ (2) to obtain the diffusion coefficient map (A, color code in panel B) and its corresponding histogram (B). This data was fitted with a bimodal Gaussian equation (red lines) recovering the following diffusion coefficients: 0.53±0.32 $\mu m^2/s$ and 1.80±0.68 $\mu m^2/s$ (mean±sd).

Supplementary File 1: User manual of FERNET toolkit.

Supplementary File 2: Parameters used for the simulations presented in Fig. 3

MOVIE S1: Simulating confocal imaging. MCell-Blender was programmed to simulate a complex geometrical model in which green fluorescent molecules are covalently bound to a filamentous structure (e.g. a microtubule), and red fluorescent molecules diffuse freely on the simulated space and can and attach to the filamentous structure and diffuse over it (e.g. microtubule associated proteins). FERNET was set to perform the raster-scan image acquisition in dual channel mode to simulate images acquired for this scenario in a confocal microscope.

Supplementary references

1. Wohland, T., X. Shi, J. Sankaran, and E. H. Stelzer. 2010. Single plane illumination fluorescence correlation spectroscopy (SPIM-FCS) probes inhomogeneous three-dimensional environments. Opt Express 18:10627-10641.
2. Sankaran, J., X. Shi, L. Y. Ho, E. H. Stelzer, and T. Wohland. 2010. ImFCS: a software for imaging FCS data analysis and visualization. Opt Express 18:25468-25481.