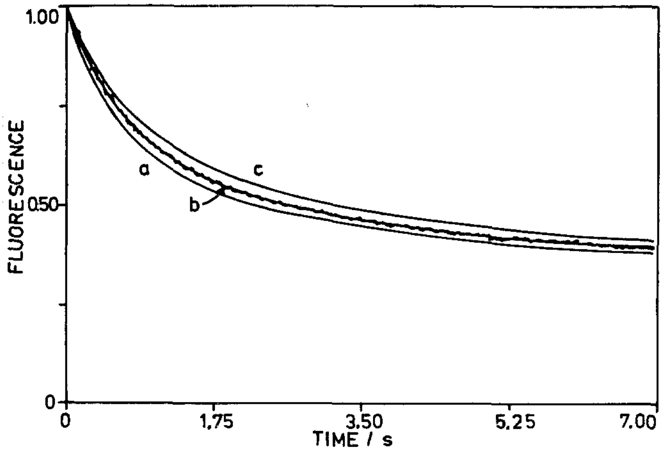
A New Method for the Study of Translational Diffusion in Single Cells

Reiner Peters (1,2), Klaus Schulten (3), and Axel Brünger (3)

(1) Zentrum der Biologischen Chemie, Universität Frankfurt, 6 Frankfurt 70, Theodor-Stern-Kai 7 (2) Max-Planck-Institut für Biophysik, 6 Frankfurt 71, Heinrich-Hoffmann-Straße 7 (3) Max-Planck-Institut für Biophysikalische Chemie, 34 Göttingen, Am Faßberg, GFR

Diffusion processes in single living cells have become accessible to analysis by introduction of the Fluorescence Microphotolysis (FM) methodology (1). Involving fluorescence microscopy and "photobleaching" the new method described here - Continuous Fluorescence Microphotolysis (CFM) - is similar to FM in some respect. Application of a new principle, however, shifts the emphasis from instrumentation to theory and data evaluation and improves the signal-to-noise ratio and the detection limit by orders of magnitude. CFM data on systems containing e.g. 3000 fluorophores can easily be obtained.

In CFM a small area e.g. of a fluorescently labelled membrane is irradiated by a laser beam. The beam power is adjusted such that a substantial irreversible decomposition of the fluorophores is induced. The decay of the fluorescence signal originating from the reaction area essentially depends on the reaction rate and on the rate by which new chromophores enter the reaction area from the surroundings. Model calculations have been performed for the case of a 2-dimensional system in which the diffusing particles are uniformly distributed at the beginning. Starting at zero time particles are eliminated in an area of the system by an irreversible first-order process. For this hitherto unsolved problem the time-dependent solution has been obtained. By fitting theoretical curves to experimental data (see fig.) values for the diffusion coefficient (and the reaction rate constant) have been obtained with high accuracy.



CFM-experiment on diffusion of di-octadecyl-indocarbo-cynine in lecithin vesicles. Radius of reaction area = 2µm.
Curve b: Experimental data with best fitting theoretical curve. Diffusion coefficient D = 1.350 $\mu m^2/s$; reaction rate constant K = 0.749/s.
Curves a and c: D = 1.100 and 1.600 $\mu m^2/s$, respectively; K = 0.749/s.

(1)Peters R.,Peters J.,Tews K.H.,Bähr W.(1974)Biochim.Biophys.Acta 367,282