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Fourier Transform Fluorescence Recovery after Photobleaching (FT-FRAP) with Patterned Illumination

A new Fluorescence Recovery After Photobleaching (FRAP) method uses Fourier transformation to increase accuracy and reduce noise, enabling high-throughput analysis of diffusion properties in complex and less homogeneous samples for research and pharmaceutical products.

Researchers at Purdue University have developed an improvement on the traditional methods of Fluorescence Recovery After Photobleaching (FRAP) using the Fourier transformation (FT-FRAP) to more accurately measure diffusion of molecules across complex matrices. In traditional FRAP, one spot is exposed to a high intensity light pulse causing that localized area to lose its fluorescence (bleaching). The recovery of fluorescence in that spot informs us about diffusion properties of the molecules. This method is most efficient only in homogenous samples and limited by high noise in measurements. FT-FRAP leverages an elegant mathematical model to quantify the recovery of the bleaching over a unique pattern of illumination. This method increases the power of the photobleach resulting in reduced noise and facilitating measurement of less homogeneous samples. This technology has been validated using fluorescent probes in both aqueous media and a glycerol/water solution. FT-FRAP is compatible with multiphoton excitation and can be retrofit into existing FRAP systems for automated high-throughput FRAP analysis. Traditional FRAP is being used to study the properties of biopolymers, quantification of protein aggregation and live-cell imaging. This innovation improves the ability to quantify diffusion gradients both on the research bench and for pharmaceutical products.

Advantages:

-Mathematical simplicity

-High signal to noise ratio

-Compatible with multi-photon excitation

Technology ID

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Category

Pharmaceuticals/Drug Discovery & Development
Biotechnology & Life Sciences/Analytical & Diagnostic Instrumentation

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Potential Applications:

-Study of the diffusion gradient at cell membranes

-Quantification of protein aggregation

-Live-cell imaging

Related Publication:

Anomalous Diffusion Characterization by Fourier Transform-FRAP with Patterned Illumination

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