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Comparison between iMSD and 2D-pCF analysis for molecular motion studies on *in vivo* cells: The case of the epidermal growth factor receptor

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ABSTRACT

Image correlation analysis has evolved to become a valuable method of analysis of the diffusional motion of molecules in every points of a live cell. Here we compare the iMSD and the 2D-pCF approaches that provide complementary information. The iMSD method provides the law of diffusion and it requires spatial averaging over a small region of the cell. The 2D-pCF does not require spatial averaging and it gives information about obstacles for diffusion at pixel resolution. We show the analysis of the same set of data by the two methods to emphasize that both methods could be needed to have a comprehensive understanding of the molecular diffusional flow in a live cell.

1. Introduction

In this article we describe two methods to analyze the same set of data. The iMSD (image Mean Square Displacement) method is used to obtain the diffusion law in small regions of interest (ROI) of the cell [1]. The term diffusion law indicates here that the iMSD plot can be fitted with a parabolic equation (flow), a straight line (pure diffusion), a function that bends at a given value of the iMSD (confined diffusion) or a combination of diffusion at a small spatial scale and slow apparent diffusion at a large spatial scale. These terms are discussed in [1]. The iMSD analysis provides information similar to the MSD obtained using single particle tracking, but is based on spatio temporal image correlation functions (STICS) and therefore do not require to isolate and track single particle. Since the correlation function is calculated in a given ROI, the diffusion law obtained refers to the ROI. The spatial resolution depends on the size of the ROI. In Fig. 1 we discuss the required size of the ROI as a function of the local diffusion coefficient. In Fig. 2 we show the concept of diffusion law in the contest of the iMSD method. The second method discussed in this article is named 2D-pCF (two dimensional pair correlation function) has the resolution of a pixel and provides the average path followed by molecules in the proximity of obstacle [2]. In the application of the 2D-pCF in article the 2D-pCF is not used to obtain values of the diffusion coefficient or the law of diffusion but it is used to visualize the path followed by molecules in the proximity of obstacles. The two approaches are complementary since they provide different information.

1.1. Theory/calculation

1.1.1. The purpose of the iMSD analysis

The purpose of the iMSD (image Mean Square Displacement) analysis is to determine the diffusion law in heterogeneous media. Commonly, the MSD is obtained in the single particle tracking from the analysis of a particle trajectory. Instead, for the iMSD, the MSD is obtained by a correlation function calculation without resolving the specific trajectory of a particle. The correlation function provides an estimation of the variance of a Gaussian describing the broadening of the spatial probability of finding a molecule in a given volume if it was at the center of the volume at time zero [1]. The Gaussian shape of the correlation function is a direct result of the Fick's second law for diffusion. In general, as molecules diffuse, they can be found at a distance from the origin that depends on the square of time, if the motion is pure Brownian diffusion. This is called the mean square displacement (MSD) which is a graph of the square of the displacement of a molecule as a function of time [3]. The slope of this graph is proportional to the diffusion

Abbreviations: 2D-pCF, two dimensional pair-Correlation Function; iMSD, image Mean Square Displacement; EGFR, epidermal growth factor receptor.

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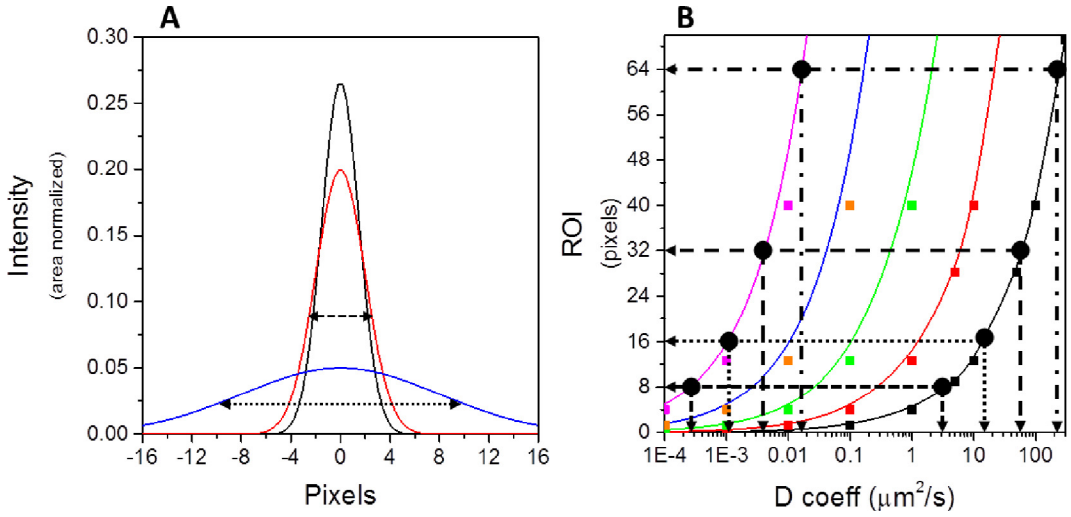

Fig. 1. A) Simulation of a Gaussian distribution for with a width of 3 pixels (black curve). The blue curve represents the spreading as a function of time of the original Gaussian function (black curve) by diffusion of a molecule with large diffusion coefficient. The dotted double arrow line represents FWHM for a fast diffusion (16 pixels wide). The red curve represents the spreading of the original Gaussian function (black curve) by diffusion of a molecule with slow diffusion coefficient. The spreading for the slow diffusion is almost unperceivable but the amplitude significantly decreases in the case of slow diffusion. B) Plot for the ROI (in pixels) in a camera-based image (pixel size 100 nm) needed to observe a change of a factor of 2 in the FWHM as a function of diffusion coefficient. From top to bottom the dashed/dotted lines indicate the measurable range in the diffusion coefficient (from 400 $\mu m^2/s$ to 3×10^{-4} $\mu m^2/s$) for a ROI (from 64 to 8 pixels) used in the iMSD analysis. From right to left (black to purple curves) the delay is increasing from 0.01 s black single frame, 0.1 s red, 1 s green 10 s blue, 100 s purple (delay after 10,000 frames). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

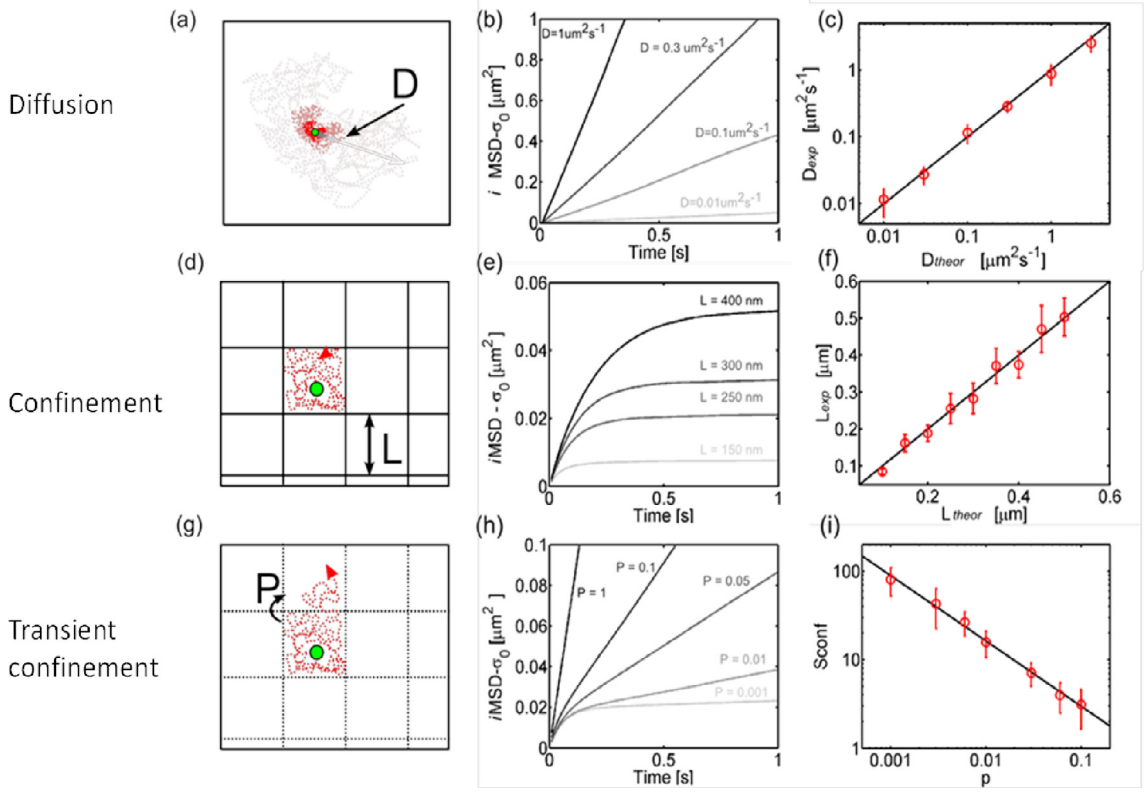

Fig. 2. iMSD analysis on simulated 2D diffusion. (a) Simulated condition: 2D isotropic diffusion, with diffusivity D. (b) iMSD is linear, with a higher slope for increasing D values. (c) Accordance between the theoretical D value and that recovered from the analysis. (d) Simulated condition: 2D isotropic diffusion in a meshwork of impenetrable barriers (probability P = 0 to overcome the barrier). (e) iMSD plot starts linear and then reaches a plateau that identifies the confinement area and the corresponding linear size L. (f) Accordance between the theoretical L value and that recovered from the analysis. (g) Simulated condition: 2D isotropic diffusion in a meshwork of penetrable barriers. Particles have probability P > 0 to overcome the barrier, thus generating a hop diffusion component. (h) iMSD plot starts linear (with a slope dependent on D_{micro}) and then deviates toward a lower slope which depends on P. (i) Calculated S_{conf} as a function of the imposed P. Part of this figure was previously published in [1].

coefficient. In the single particle tracking method, the MSD parameter is obtained in real space by tracking the position of particles one a time [3]. However, in heterogeneous media such as the cell interior, the distance from the center could be limited by the nature of the medium, barriers to diffusion, cavities and molecular interactions with fixed or mobile structures. Information about