Mapping Diffusion in a Living Cell Using the Phasor Approach

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Supplementary Information:

FIGURE S1. Relation between diffusion coefficient, correlation time and characteristic pixel dwell time. The correlation time and pixel dwell time was calculated for a PSF with 0.3 μm waist. The pixel dwell times are calculated based on 128 repeat measurements at each pixel. The faster pixel dwell times are accessible using the point scan mode and the slower pixel dwell times are accessible using the line scan mode.

FIGURE S2. Comparability between the diffusion measurements using RICS and phasor FCS approaches for GFP in DPBS. Panel A describes the fit and difference from the RICS analysis. The top part of this panel shows the difference between the autocorrelation and the fit, and appears random. The bottom part of the figure shows the autocorrelation function plotted in three dimensions. The magnitude of the autocorrelation function determines the height of the peak. In both top and bottom figures, horizontal correlation shift, ξ, is represented by the horizontal axis and the vertical axis represents the vertical correlation shift, ψ. The original correlation function and the fits are of same size, 64x64 pixel. Panel B shows the histogram of diffusion coefficients calculated from phasor FCS measurements. The maxima of this distribution, when fitted with the $\omega 0$ value recovered from RICS is at 91 $\mu m^2/s$, showing strong similarity to the value used for the RICS calibration, 90 $\mu m^2/s$.

FIGURE S3. The correlation between the RICS (A) and phasor FCS measurements (B) of the hIR-GFP labeled CHOK1 cells. The RICS measurement in two different areas yield a value of 0.18 $\mu m^2/s$ (green square) and 0.98 $\mu m^2/s$ (red square) which is similar to the values obtained by the stsFCS method and presented in the diffusion histogram (B, ii).

FIGURE S4. The inhomogeneity in the diffusion measurements of the hIR-GFP cells. Panel (A), (B) and (C) show the intensity maps, diffusion maps and the diffusion phasor plots, respectively. The panel (C) shows that the position and distribution of the points in the phasor histograms can be very different for the hIR-GFP in different cells.

Movie S1. 3D intensity map of the cell where the intensity was used as the wireframe. The height in Z axis represents the extent of intensity. The image was then color mapped according to the chosen cursors in (Fig. 3 C). The color of the mask at the peaks is mostly green, representing slow diffusion in the focal adhesion sites.

Figure S1

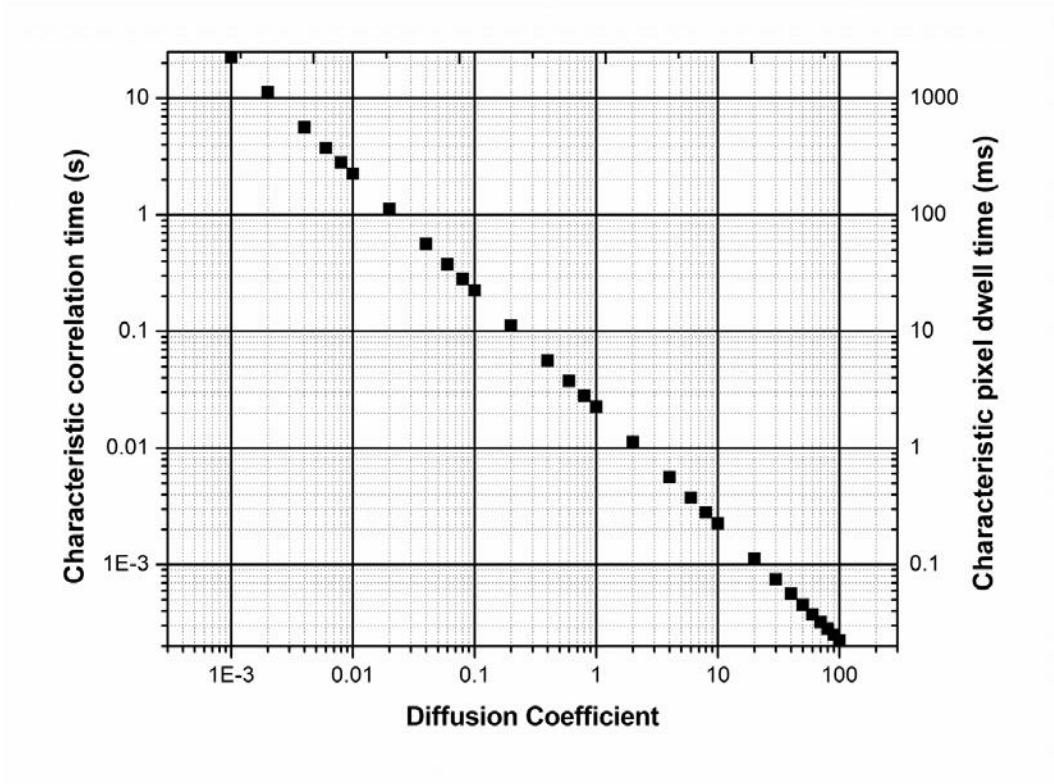
Characteristic correlation time (s)
10
1
0.1
0.01
1E-3
1000
100
10
1
0.1
Characteristic pixel dwell time (ms)
1E-3
0.01
0.1
1
10
100
Diffusion Coefficient

Figure S2

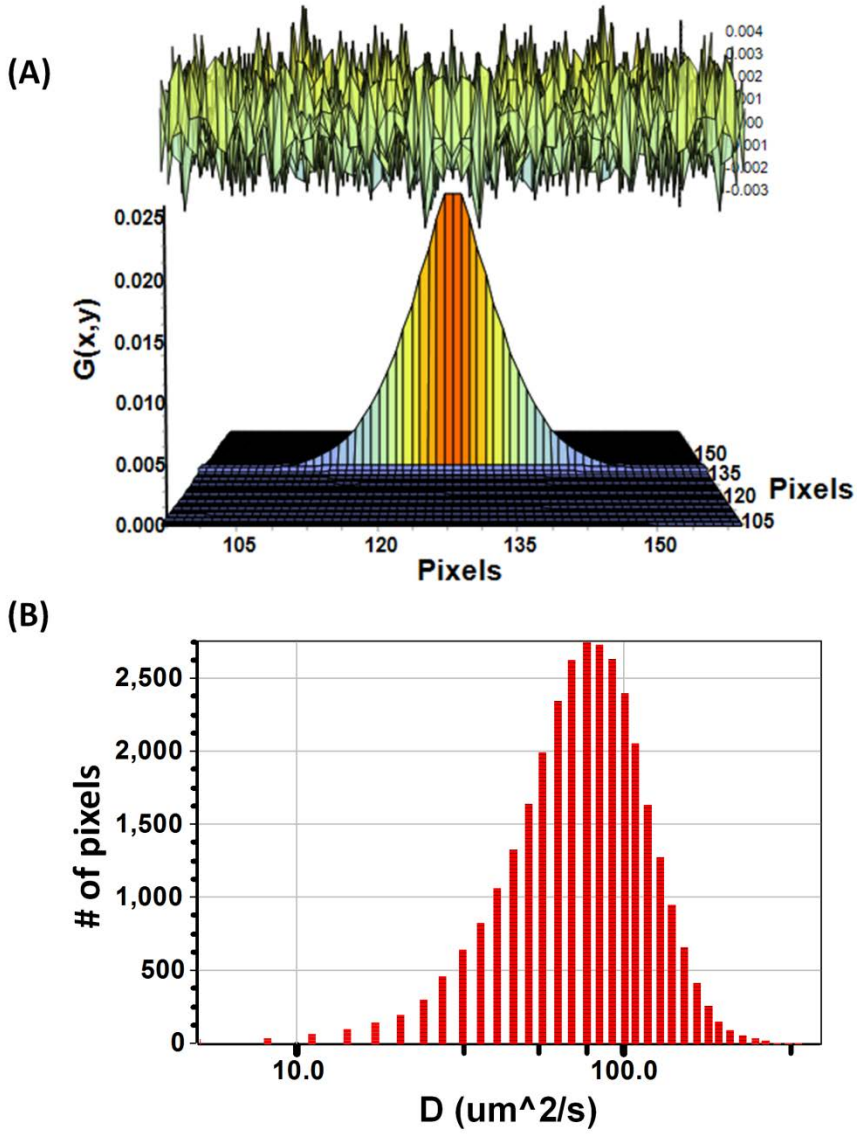
(A)
0.004
0.003
002
001
00
001
002
-0.003
0.025
0.020
0.015
0.010
0.005
0.000
G(x,y)
105
120
135
150
Pixels
150
135
120
105
Pixels
(B)
2,500
2,000
1,500
1,000
500
0
# of pixels
10.0
100.0
D (um^2/s)

Figure S3

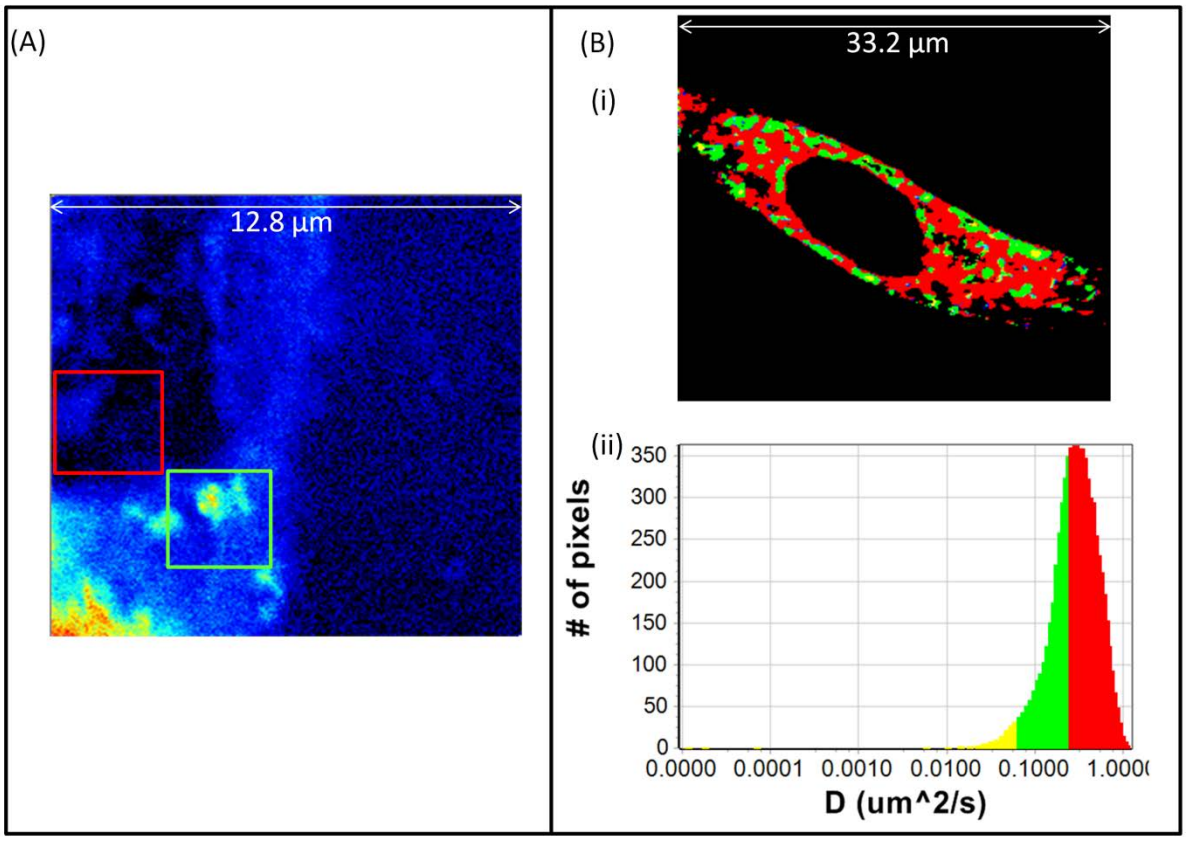
(A)
12.8 µm
(B)
(i)
33.2 µm
(ii)
# of pixels
350
300
250
200
150
100
50
0
0.0000
0.0001
0.0010
0.0100
0.1000
1.0000
D (um^2/s)

Figure S4