Biophysical Journal, Volume *119*

Supplemental Information

Anomalous Diffusion Characterization by Fourier Transform-FRAP with Patterned Illumination

Andreas C. Geiger, Casey J. Smith, Nita Takanti, Dustin M. Harmon, Mark S. Carlsen, and Garth J. Simpson

SUPPLEMENTAL INFORMATION
Anomalous Diffusion Characterization by Fourier Transform FRAP with Patterned Illumination

Andreas C. Geiger,[1] Casey J. Smith,[1] Nita Takanti,[1] Dustin M. Harmon,[1] Mark S. Carlsen,[2] and Garth J. Simpson[1]

[1]Department of Chemistry, Purdue University, 560 Oval Drive, West Lafayette, Indiana 47907, USA
[2]Jonathan Amy Facility for Chemical Instrumentation, Purdue University, 560 Oval Drive, West Lafayette, Indiana 47907, USA

Signal to noise ratio (SNR) considerations in point vs. FT photobleach

Direct comparisons between point-bleach and FT-FRAP measurements can be made by considering the SNR of the measurements during recovery. As noted in Section 2.5 of the manuscript, major increases in signal are potentially accessible in FT-FRAP by distributing greater photobleach excitation power over the entire field of view. For the purposes of direct comparison, we will assume a constant integrated bleach power for both comb-illumination FT-FRAP and point-bleach FRAP. For point-bleach analysis in the spatial domain, the illumination point-spread function (assumed for simplicity to be Gaussian) is convolved with a 2D time-varying Gaussian function, the standard deviation of which is dependent on the diffusion coefficient and time through **Equation (8)**. As the photobleach profile expands, the peak signal S measured at the center of the photobleach profile is reduced in time scaling as $S(t) = S_0 \cdot \sigma_b^2 / \left(\sigma_b^2 + 2Dt\right)$, generated by normalizing **Equation (8)** for an initial peak amplitude of S_0. An identical scaling arises for the SNR measured in a given pixel when fixing the integrated area of the photobleach peak and considering the increase in number of pixels over which the signal is distributed. For comparison, the integrated cross-section of the peak in the FT domain is independent of time, with a corresponding fixed noise power. The signal (and correspondingly the SNR) in a given harmonic decays exponentially with time with a time-constant given by $\tau = \left[\left(2\pi n\bar{\nu}^0\right)^2 D\right]^{-1}$. For a spatial period of 16 pixels between lines consistent with the experimental implementation of FT-FRAP and an initial photobleach width with a standard deviation of ~2 pixels, calculations are shown in **Figure S1** for the anticipated time-

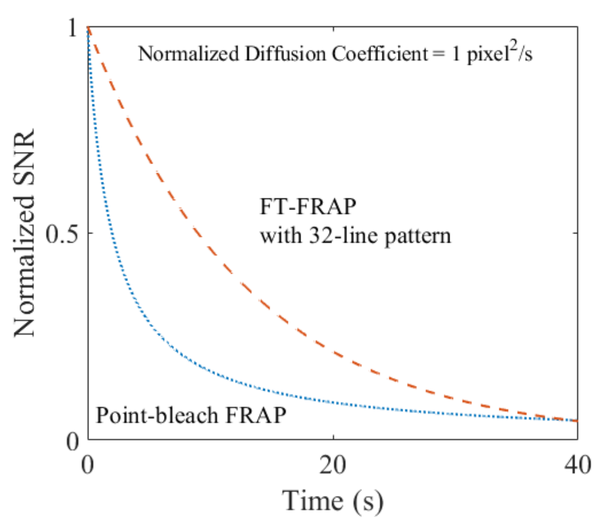

Figure S1. Decay of FRAP signal over time for point-bleach FRAP and FT-FRAP with 32-line patterned illumination. Signal rapidly degrades for point-bleach relative to comb-illumination even when normalized for total bleach power. In principle, the signal of FT-FRAP increases as the distance between photobleached lines increases.

dependence of the SNR for point vs. patterned illumination. As can be seen from the figure, even when normalized for total bleach power, the SNR rapidly degrades for point-bleach relative to comb-illumination. In principle, the integrated SNR of comb illumination can be further increased by increasing the distance between lines, thereby slowing the loss in the spatial harmonic. In practice, implementation may introduce additional 1/f noise contributions not explicitly considered herein.

Supplemental Video 1

The video shows an FT-FRAP experiment on FITC-polydextran (2 MDa) in 22 mg/mL hyaluronic acid. The left side of the video shows the microscope images during the experiment. The right side of the video shows the log of the 2D spatial Fourier transform of the microscope images on the left. During the period of 0:00−0:06, the baseline fluorescence is recorded. During the period of 0:07−0:09, the sample is photobleached with patterned illumination. During the period of 0:10−1:15, the fluorescence recovery is recorded. Note the puncta that appear in the log of the 2D-FT from the patterned photobleach at 0:10 and fade thereafter.