Supporting Information

Spatiotemporal Evolution of Fixed and Mobile Dopant Populations in Silica Thin-Film Gradients as Revealed by Single Molecule Tracking

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Example wide-field fluorescence videos and histograms depicting the measured diffusion coefficients for 1 d and 3 d dried gradient films are provided. A discussion of the methods used to generate simulated videos is given.
**Video S1.** Video (350 frames) depicting single molecule motion near the top (2 mm, TMOS end) of the gradient. Fluorescence in this video was excited using unpolarized light. The region shown is ~ 44 µm X 44 µm.

**Video S2.** Video (350 frames) depicting single molecule motion near the bottom (12 mm, MTMOS end) of the gradient. Fluorescence in this video was excited using unpolarized light. The region shown is ~ 44 µm X 44 µm.
Figure S1. Left) Histograms showing D values obtained from several hundred trajectories recorded at each point along the one-day dried silica film gradient. Right) Histograms showing frame-to-frame D values from the three-day dried silica film gradient. The solid curves depict Gaussian fits of the most rapidly moving population of mobile molecules. The y-scales in all but the 0 mm plots have been expanded to better show the mobile populations, truncating the immobile populations.
Results and Discussion

Gradient Structure and Heterogeneity. Simulated widefield videos were employed to model the behavior of molecules undergoing free two-dimensional diffusion in a perfectly homogeneous matrix. Simulations were performed using software written in-house in the National Instruments Labview programming environment. Simulated regions were identical in size to the regions imaged in experimental videos (i.e., 44 µm X 44 µm). A concentration of 40 molecules was maintained throughout each simulation. Individual molecules were initially positioned at random within the simulated region. A minority (40%) of the molecules were assigned to fixed locations, while the remainder moved about the region by free (unconfined) Brownian motion. The frame-to-frame step size (and direction) in the X and Y directions for each mobile molecule were selected at random from Gaussian distributions having a variance of 2Dt, where t is the frame time and D the diffusion coefficient of the mobile species. The simulations were run using a range of diffusion coefficients selected to mimic the positional variations in D along the gradients. D values of 0.018, 0.024, 0.025, 0.035, 0.051, 0.056 and 0.038 µm$^2$/s (top-bottom, respectively) were employed. All mobile molecules in individual simulations were assigned identical D values to model a homogeneous system. Each simulated molecule was also assigned a lifetime (i.e., time to photobleaching), generated at random from an exponential distribution having a mean lifetime of 15 s. The latter value closely approximates the mean trajectory length (14 ± 5 s) in the experimental data. To maintain a constant concentration of molecules, bleaching of one molecule triggered positioning of a new molecule at a random position within the simulated region. Background signal and Gaussian noise (simulating Poisson-distributed shot noise) were added to all simulated videos to obtain signal-to-noise and signal-to-background ratios that closely mimicked those observed in the
experimental data. The simulated videos were analyzed using procedures identical to those employed in analysis of the experimental data.