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Massively-parallel fluorescence correlation spectroscopy using a spinning disk confocal microscope DANIEL R. SISAN, RICH AREVALO, CAT GRAVES, RYAN MCALLISTER, JEFFREY S. URBACH, Dept. of Physics, Georgetown University — We describe an extension of fluorescence correlation spectroscopy (FCS) using a spinning disk confocal microscope. This approach can spatially map diffusion coefficients or flow velocities at up to $\sim 10^5$ independent locations simultaneously. Complex media—e.g., a tumor, cell nucleus, or extracellular matrix—are spatially-heterogeneous, making this spatially-resolved technique an ideal tool to understand hindered diffusion. There have been a number of recent extensions to FCS based on laser scanning microscopy. Spinning disk confocal microscopy, however, can be much faster at high resolution—potentially up to 1000 Hz at full resolution for the fastest available cameras—and without temporal delays between pixels. We show how to correct for a pixel size effect not encountered with standard or scanning FCS, and we introduce a method to correct for photobleaching. Finally, we apply this technique to microspheres diffusing in Type I collagen, which show non-trivial spatially varying diffusion caused by hydrodynamic and steric interactions with the collagen matrix.

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