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Single-molecule recycling in a nanochannel BO WANG, BRIAN K CANFIELD, LLOYD M DAVIS, University of Tennessee Space Institute and UT Knoxville — Use of a confocal fluorescence microscope for prolonged observation of a single fluorescently labelled molecule in solution can be accomplished by confining the solution to a nanochannel and alternating the flow so that the molecule is "recycled" in that it returns to pass again through the focused laser spot. To accomplish recycling experimentally, we have developed a custom microscope and LabVIEW real-time control system, which processes the stream of single-photon detection events to determine the transit time of a molecule and alternates the voltage that drives electrokinetic motion along the nanochannel after a set delay. We use fluidic devices that are themselves recycled from prior experiments and we describe methods for preparing the nanochannels. In initial investigations with fluorescently labeled proteins, sticking of molecules to the walls of the nanochannel limits experiments and we discuss studies on procedures for mitigating sticking. We give details on ongoing experiments using To-Pro-3 labeled dsDNA molecules in a pH 8.0 buffer. We also present Monte Carlo simulations to determine favorable experimental conditions for determining single-molecule diffusivities from the fluctuations in the intervals between successive detections. This provides a potentially accurate measure of molecular binding interactions for applications in pharmaceutical drug discovery research.

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