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Capabilities for measuring the diffusivity of a single molecule by recycling it in a nanochannel BO WANG, LLOYD DAVIS, University of Tennessee Space Institute — Analysis of the fractions of fluorescently labeled molecules with different diffusivities within a microliter drop of solution is often used for high-throughput screening of molecular binding interactions in pharmaceutical drug discovery research. Assays frequently employ fluorescence correlation spectroscopy, an ensemble technique that is able to resolve fast diffusing small ligands from those bound to much larger biomolecules with considerably slower diffusion. Single-molecule measurements have the potential to resolve species with different diffusivities and to count the numbers of molecules of each species. Single-molecule recycling in a nanochannel, which entails detection of bursts of fluorescence photons from the repeated passage of a molecule through a focused laser beam as the flow along a nanochannel is periodically alternated, can be used to determine the diffusivity of a single molecule from the fluctuations in the intervals between successive detections. We discuss Monte Carlo studies to determine favorable experimental conditions for determining single-molecule diffusivities, together with a weighted-sliding-sum photon burst detection algorithm for flow-control and maximum-likelihood based analysis of recycle times. We also discuss incorporation of the algorithms into our experimental apparatus for single-molecule recycling, which uses a LabView real-time system for photon count analysis and flow control.

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