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Separation and Characterization of DNA Molecules and Intermolecular Interactions in Pressure-Driven Micro Flow SARAH FRIEDRICH, Biomedical Engineering Department, Johns Hopkins University, TZA-HUEI WANG, Mechanical Engineering and Biomedical Engineering Departments, Johns Hopkins University — Pressure-driven flow in micron-sized diameter capillaries can be used to separate DNA molecules by size in a technique called Free Solution Hydrodynamic Separation. By coupling this technique with Cylindrical Illumination Confocal Spectroscopy, we have developed a highly sensitive and quantitative platform capable of separating DNA molecules by length over a large dynamic range (25 bp to 48 kbp) in a single run using only picoliters or femtograms of a DNA sample. The optical detection volume completely spans the capillary cross section, enabling highly efficient single molecule detection for enhanced sensitivity and quantification accuracy via single molecule counting. Because each DNA molecule generates its own fluorescent burst, these burst profiles can be further analyzed to individually characterize each DNA molecule's shape as it passes through the detection region. We exploit these burst profiles to visualize fluctuations in conformation under shear flow in microcapillaries, and utilizing combined mobility shift analysis, explore the complex relationship between molecular properties including length and conformation, hydrodynamic mobility, solution conditions including ion species and concentrations, and separation conditions including flow rate and capillary diameter.

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