Single Molecule Correlation Spectroscopy in Continuous Flow Mixers with Zero-Mode Waveguides

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Abstract:

This work illustrates how the use of a floor of zero-mode waveguides (ZMW) sustains sensitivity to diffusion measurements for fluorescence correlation spectroscopy (FCS) in high velocity flow channels, as occur in continuous flow microfluidic mixer (CFMM) designs. The basic idea is very simple: the floor of a CFMM is carpeted with an array of ZMWs which sample the local concentration of molecules at a particular region of the flow pattern but are shielded from the advection of the flow by the walls of the ZMW. Single molecules within a ZMW have a characteristic residence time given by their diffusion coefficient and the effective volume of the ZMW. Although above the entry of the ZMW the fluid is advecting, within the ZMW there is no advection and hence we expect the mean residence times in the ZMW waveguide, and hence the determination of the diffusion coefficient of the molecule, to be independent of the speed of the external flow.

Summary of Research:

This result has important consequences. CFMM designs allow studies of biological reaction and mixing kinetics with low reagent consumption and microsecond time resolution. The flow velocity profile assigns reaction times to different distances from inlets. Hydrodynamic focusing achieves sub-microsecond time resolution and mixing times less than 10 μs, enabling protein folding kinetic measurements. We show that combining zero-mode waveguides with fluorescence correlation spectroscopy in a continuous flow mixer avoids the compression of the FCS signal due to fluid transport at channel velocities up to ~ 17 mm/s. Thus zero-mode waveguides make FCS suitable for direct kinetics measurements in rapid continuous flow.

This work was done in collaboration with Rob Ilic and Harold Craighead of Cornell University.

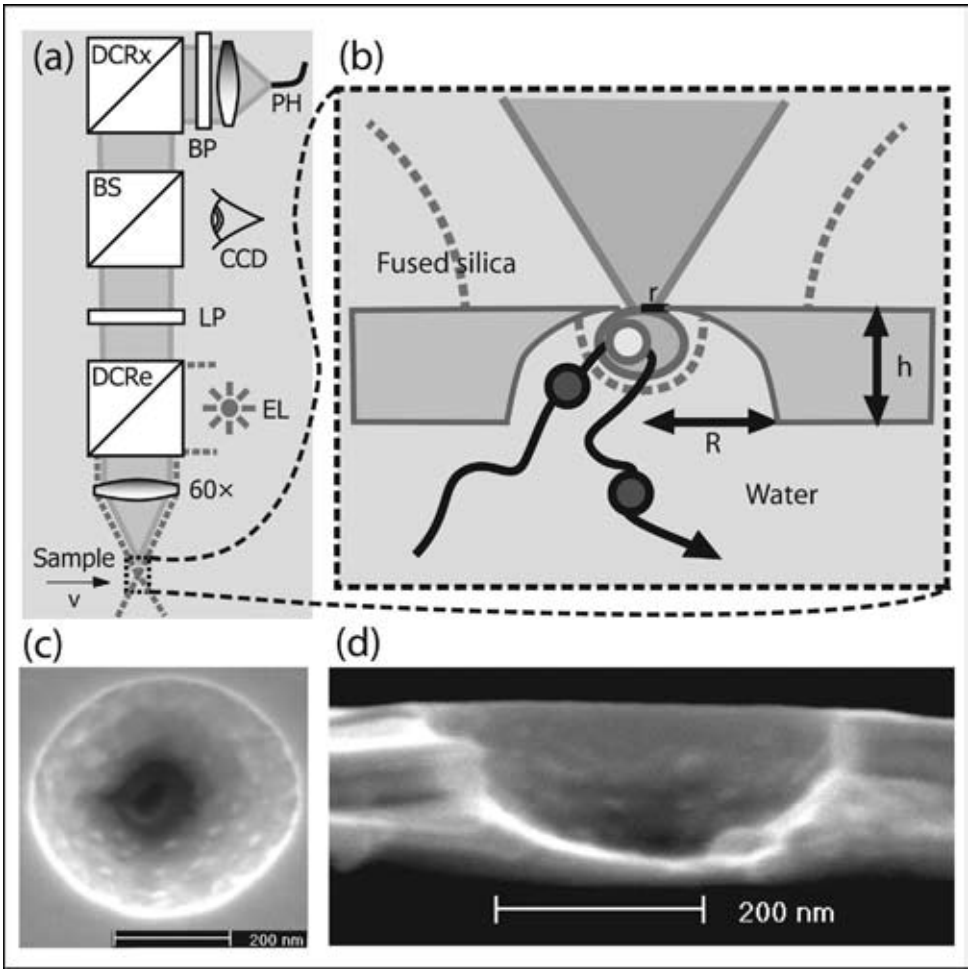

Figure 1: (a) and (b) A zero-mode waveguide method confines intensity fluctuations measurement to diffusers proximate to the metal substrate. (c) and (d) Repeated SEMs including those shown indicate typical scales h = 163.3 ± 8.8 nm, R = 209.3 ± 4.4 nm, and r = 26.1 ± 1.3 nm.