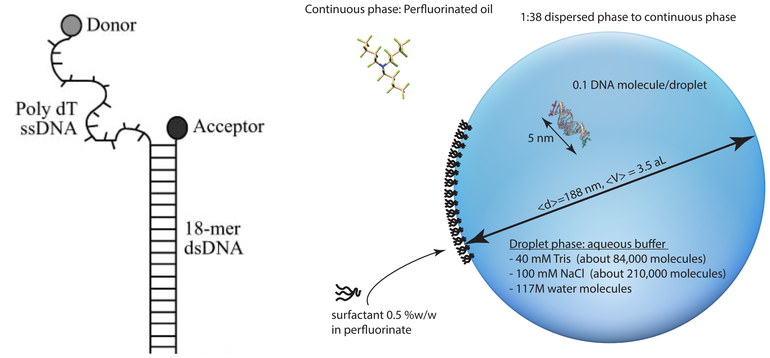
A MICROFLUIDIC PLATFORM TO STUDY UNTETHERED SINGLE-MOLECULE FLUORESCENCE TRAJECTORIES

Kieran P. Ramos, Lori S. Goldner
Department of Physics, University of Massachusetts Amherst

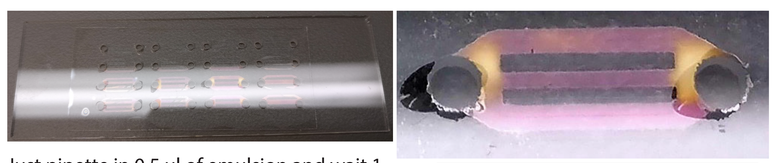
Abstract

We report on progress in the development of a simple microfluidic device that enables simultaneous measurement of multiple fluorescence trajectories from single-molecules confined within attoliter-volume water droplets. The device is designed for use with a total internal reflection fluorescence microscope with excitation through the objective. Similar to the observation of biomolecules tethered to a coverslip, we show that we can observe many molecular trajectories simultaneously using this device. In contrast to observations of surface-tethered molecules, molecules confined within droplets are free to diffuse in the droplets and are less likely to be perturbed by a heterogeneous glass surface. In this regard our method is analogous to confinement of molecules within surface-tethered liposomes. Unlike liposomes, attoliter-volume droplets can be mixed, potentially facilitating single-molecule sensitive reaction dynamics. Our current device is a first step, permitting parallel observations of equilibrium kinetics in static droplets. To date, we have observed and will report on fluorescence resonance energy transfer (FRET) trajectories from DNA oligomers confined to droplets in this device.

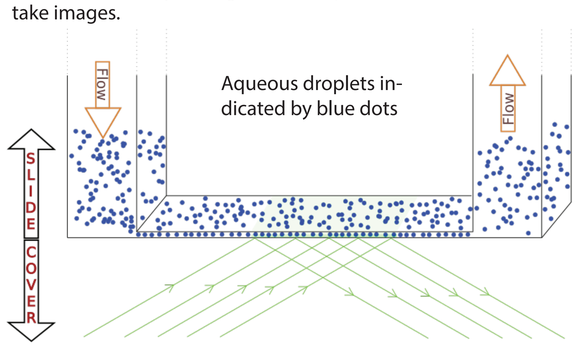
Sample



Passive Glass Microfluidic Device



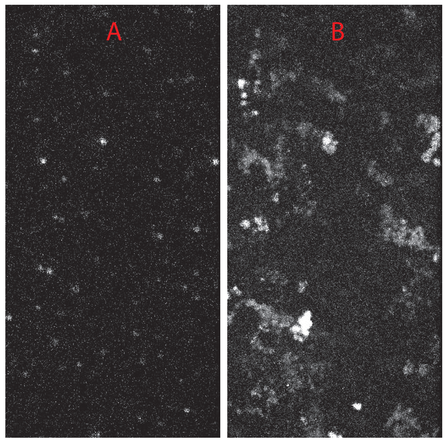
Just pipette in 0.5 ul of emulsion and wait 1 to 10 minutes (depending on viscosity) then take images.



Designed to be used in through objective total internal reflection fluorescence microscopy for reduced background and ease of use.

- Device channel height is nominally 200 to 300 nm
- Droplets in device can diffuse only in 2D
- Sample (contained in droplets) is always in evanescent wave

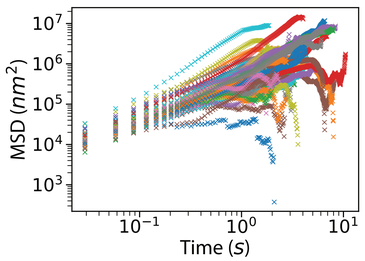
Measurement of Droplet Size *in situ*

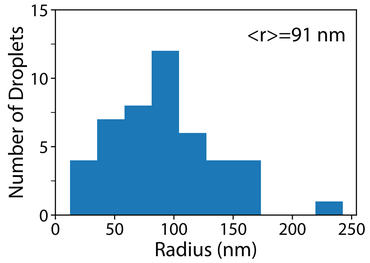


Images of droplets diffusing in the microfluidic device
- A: first frame of image sequence
- B: z-projection keeping maximum intensities
- 34.4 frames per second
- Droplet radii are calculated using the Stokes-Einstein relationship where the diffusion coefficient is determined by the Mean Squared Displacement (MSD)
- Diffusion coefficient, D, of each droplet is calculated from the slope of a linear fit to the first 10 data points of of the MSD trace

$$\mathrm{MSD} = \langle |r_i(t+\tau) - r_i(\tau)|^2 \rangle$$

$$\mathrm{MSD} = 4Dt, \qquad D = \frac{k_B T}{6\pi\eta r}$$





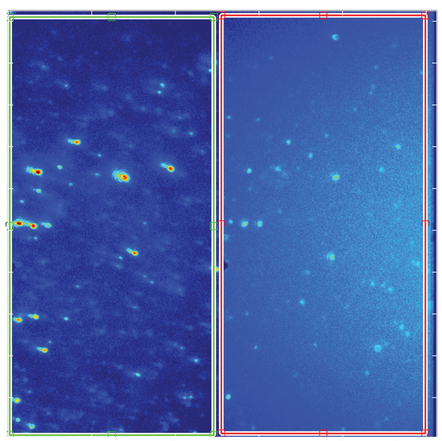
Fluorescence Resonance Energy Transfer (FRET)

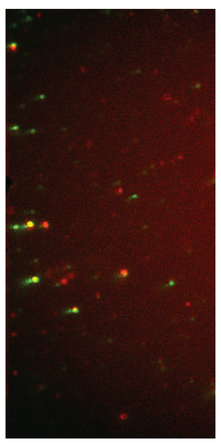
FRET Efficiency (Probability of Transfer)

$$E = \frac{1}{1 + (R/R_0)^6}$$

$$R_0^6 \propto \kappa^2 J \eta^{-4} Q_0^D$$

- Förster radius: R_0, usually around 5 nm
- Orientation: κ^2, usually taken to be 2/3
- Overlap: $J \propto \int q_{D,\lambda} \epsilon_{A,\lambda} \lambda^4 d\lambda$
- Refractive index: η, about 1.34
- Donor quantum yield: Q_0^D



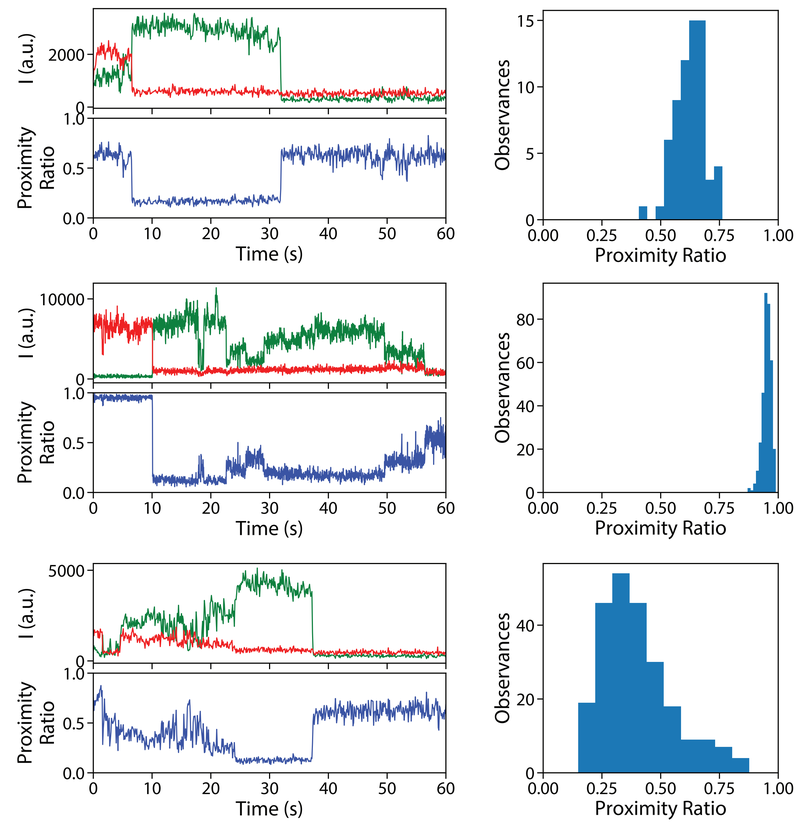


Preliminary Single-Molecule Trajectories

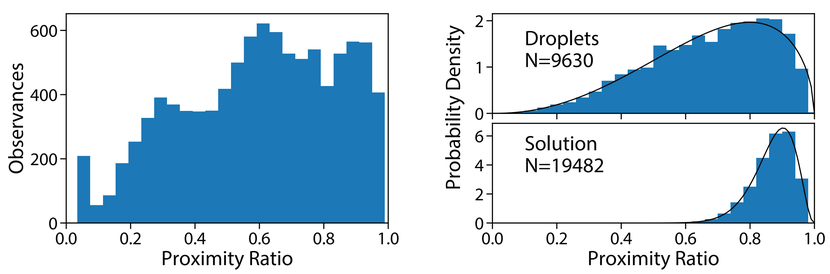
In this preliminary analysis, we used only droplets that were stationary within this device. The data are similar to what was observed for molecules confined in freely-diffusing droplets, but different from what is observed for the same molecule in solution. These data are very new and the origin of this difference is as yet unknown.

$$E = \frac{\langle P \rangle}{\gamma(1 - \langle P \rangle) + \langle P \rangle}$$

$$P = \frac{I_A}{I_D + I_A}$$



Left: proximity ratio of all 82 recorded single-molecule trajectories
Right: proximity ratio of same sample in droplets and in solution



Acknowledgements

This work was funding by NSF DBI-1152386.
Thanks to NEAGEP for their continuing support.
Thanks to Nina Zehfroosh for droplet tracking.

DBI-1152386