Nanoscale "Curtain Rods" for the Study of Protein-DNA Interactions

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In this work, we combine nanoscale engineering with single-molecule biology to probe the biochemical interactions between individual proteins and DNA. We observe in real time how proteins repair double-strand breaks and mismatches in DNA with unprecedented detail. For example, the Rad51 protein diffuses along DNA's helical axis due to thermal fluctuations in the surrounding solvent until it locates its target—a double strand break which it then repairs.[1] One key challenge in these experiments is collecting enough statistically relevant data in order to analyze reactions which are designed to be probed individually. Nanopatterning solves this problem.

"DNA curtains" are formed by flowing the DNA in a lipid bilayer across nanoscale barriers. The flow chamber is constructed from a fused silica slide patterned with sub-100 nm-wide, chromium barriers (Fig. 1) oriented perpendicular to the flow. These barriers mechanically tether DNA strands linked to a floating lipid bilayer. When buffer flow is applied, the DNA strands are elongated by the flow, and fluorescently-tagged proteins can be observed as they move along the DNA strands via diffusion. The barrier width is ~ 30 – 100 nm, with a measured error of 9 nm, meaning the resolution of this technique corresponds to ~26 base pairs of DNA.

The barriers are patterned on fused silica substrates by electron beam lithography and liftoff. A ~4 μL flowcell is assembled on the slide using a borosilicate glass cover slip (Fisher Scientific) and ~ 25 μm-thick double-sided tape (3M).[2] To illuminate the fluorescently-tagged proteins, Total Internal Reflectance Microscopy (TIRFM), shown schematically in Fig. 2, utilizes the evanescent field that is generated beyond a reflective surface present at the interface between two transparent materials with different refractive indices (e.g., a silica slide and the aqueous buffer solution). Figure 2 shows fluorescent images of DNA tethered to the barriers with the flow off (a) and on (b).

Our approach, which integrates state-of-the-art nanopatterning techniques into single-molecule biology experiments, allows us to study hundreds to thousands of protein-DNA interactions in a single TIRF field of view. This is an order-of-magnitude improvement over previously-reported manual observation techniques, which use highly irregular scratches to tether the DNA.[1,3] Using nanoscale features to tether DNA strands for observation revolutionizes the method by which protein-DNA interactions are observed.

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[2] T.K. Prasad, C.C. Yeykal, and E.C. Greene, J. Mol. Bio. **363**,3 (2006)
[3] A. Graneli, C.C. Yeykal, T.K. Prasad, and E.C. Greene, Langmuir. **22**,1 (2006)

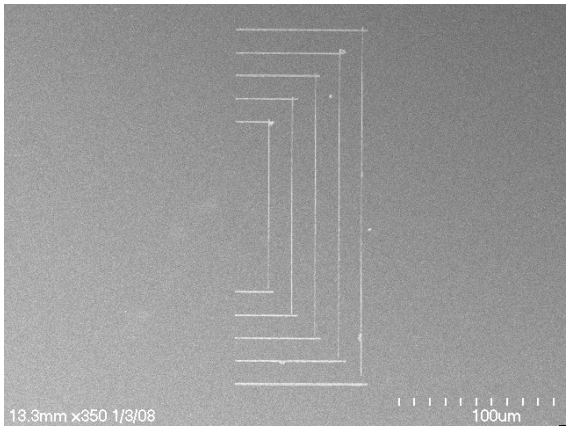


Figure 1. Pattern of Cr barriers for interrupting the lipid bilayer. Top and bottom brackets help corral the DNA so that it is evenly spaced and easily observable.

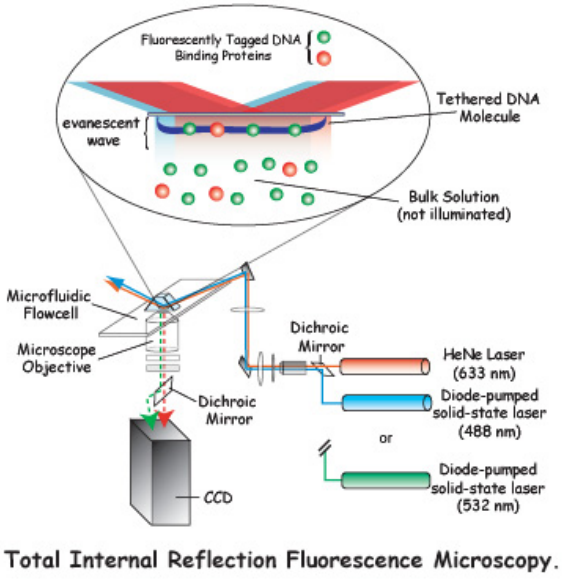
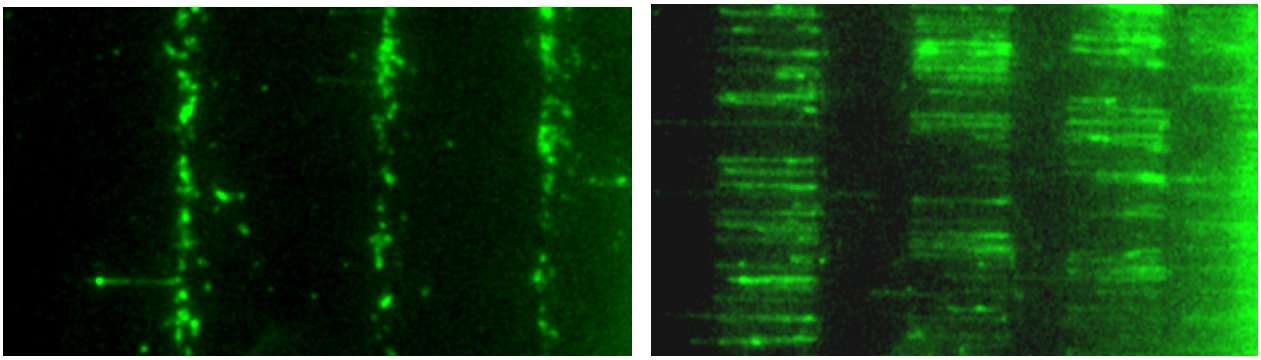


Figure 2. Schematic diagram of TIRFM setup. The barriers tether the DNA in the evanescent field.



(a) (b)

Figure 3. TIRF image of DNA tethered to barriers. (a) Flow off; DNA diffuses out of evanescent field in the absence of buffer flow. (b) Buffer flow on from left to right; DNA elongates and is visible with fluorescent dye. This figure shows three usable arrays of DNA curtains.