

Measurement of potential dependent DNA orientation on indium tin oxide surfaces by fluorescent-self interference microscopy

Philipp S. Spuhler¹, Laura Sola², Margo R. Monroe¹, Marcella Chiari², M. Selim Ünlü¹

¹Department of Biomedical Engineering, Boston University, Boston, MA 02215

²Istituto di Chimica del Riconoscimento Molecolare, CNR, Milano, Italy

Tel: (617) 353 5887, Fax: (617) 353 9917, e-mail: pspuhler@bu.edu

ABSTRACT

We present a platform for the measurement of DNA orientation on an Indium Tin Oxide surface. We apply an interferometric measurement technique called spectral self-interference fluorescent microscopy (SSFM) that allows precise distance measurements of fluorophores from the sensor surface. SSFM can be used to infer the orientation and conformation of bound biomolecules, such as double stranded DNA. High electric fields are induced at the sensor surface through application of an electric potential between the sensor and a counter-electrode in solution and resulting orientation changes of the immobilized DNA are observed. The platform is scalable to allow micro-arrayed, high-throughput measurement of bimolecular interactions.

Keywords: high throughput, DNA orientation and conformation, indium tin oxide surface chemistries, spectral self-interference fluorescence microscopy (SSFM), optical biosensor

1. INTRODUCTION

Understanding conformational dynamics and orientation of biomolecules is critical in determining their function. For double-stranded DNA, conformational changes result due to interactions with proteins such as histones, transcription factors and DNA-modifying enzymes. Regulation of gene expression, for example, in both prokaryotes and eukaryotes involves formation of specialized nucleoprotein structures, or snups (1). Within these complexes, distant segments of DNA are brought into close proximity to each other and, frequently, protein-induced DNA bends or kinks are formed. Understanding the DNA conformational changes and forces responsible for bending DNA to form specific complexes are thus of considerable biological significance.

Integration Host Factor (IHF) is an abundant protein that functions in several processes involving higher-order protein-DNA complexes, e.g., in replication, transcription, regulation, and a variety of site specific recombination systems. The steady-state IHF-DNA interaction is well characterized and, based on X-Ray crystallography, NMR, and AFM, it is well understood that IHF binds to DNA with high sequence specificity and bends the DNA 160° (2,3). We already demonstrated a platform to measure the kinetics of IHF-induced bending of surface bound DNA probes. In this platform, electric fields are used to orient immobilized DNA strands in a standing orientation to observe protein induced conformational changes through fluorophore-surface distance measurements (4).

To better quantify the orientation and conformation of the surface immobilized DNA we measure the fluorophore surface distance with self-interference fluorescent microscopy (SSFM). SSFM allows sub-nm measurements of the axial fluorophore-surface distance and we previously used SSFM to quantify DNA orientation tethered to an oxide surface (5). The SSFM platform consists of a non-conducting oxide layer on a silicon substrate. However, to electrically induce orientation changes of immobilized DNA the DNA must be bound to an electrically conducting surface (6). We incorporate an electrically conductive and optically transparent top layer of Indium Tin Oxide (ITO) on the SSFM surface.

2. Experiment

Figure 1 depicts the sensing principle. Fluorescent spectra from the tagged DNA yields the precise average height of the fluorophore with sub-nm precision (5). A 180 nm thick layer of ITO is deposited on the SSFM surface to provide an electrically conducting layer. The refractive index of ITO is somewhat higher than that of the silicon dioxide layer below it, however, the optical functionality of the ITO functionalized platform remains similar to the silicon dioxide on silicon SSFM platform.

The orientation of immobilized dsDNA is controlled through a voltage potential applied to the ITO surface with respect to electrically insulated ITO counter electrode. This results in the formation of an ionic double layer at the electrode surfaces and screens the electric field so that it is confined to a few nanometers from the electrode surface. For low potentials the voltage potential, as a function of distance from the electrode, reduces to the *Debye-Hückel* equation, $\psi \sim \psi_0 e^{-x/l_d}$, where the Debye length appears as the characteristic decay length of the potential (7). A large electric field results a few Debye lengths from the gold surface and exerts an electrostatic force on immobilized dsDNA that overcomes the Brownian motion, resulting in uniform alignment of the dsDNA in the electric field (8). As the contour length of the tethered dsDNA is significantly less than the

persistence length ($l_c \sim 27$ nm, $l_p \sim 50$ nm), the conformation is that of a rigid rod and orientation of the dsDNA probes, relative to the ITO surface, can be inferred (4).

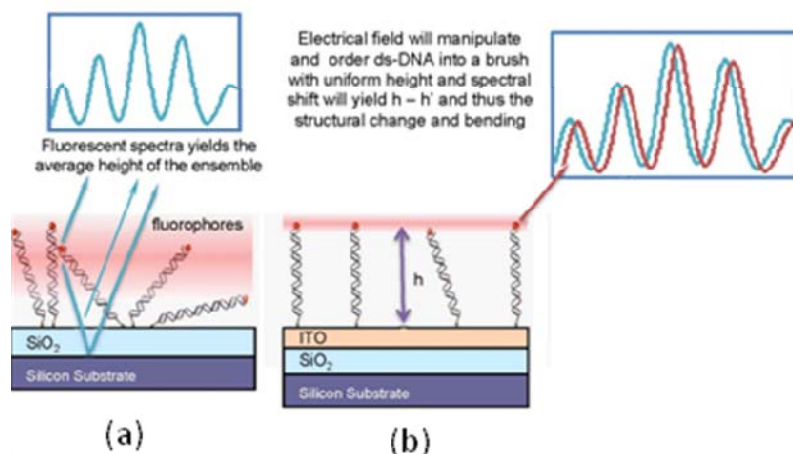


Figure 1. (a) SSFM maps the spectral oscillations emitted by a fluorophore located above a reflecting surface into a precise position determination of ensemble of fluorophores with sub-nm accuracy. (5) (b) Adding a transparent metal film on the top surface allows for utilizing electrical field to orient the ds-DNA as a uniform brush

3. Materials and Methods

3.1 Chip Fabrication, Functionalization & DNA Immobilization

A 180 nm thick film of Indium Tin Oxide is deposited (deposition rate of 2 \AA/s) using an RF magnetron sputtering system. Electrodes and counter electrodes were patterned using standard photolithography and etched in 50 % HCL. Au contacts were deposited (200 nm Au on 5 nm Ti) and patterned by liftoff. The chips were functionalized with a Copoly(DMA-NAS) polymer similar to what was reported previously (9), but which we customized to produce a thickness of less than 5 nm from the ITO electrode surface. $30 \text{ }\mu\text{M}$ 5'-amine-modified single-stranded 21 base-pair are hybridized with $30 \text{ }\mu\text{M}$ 5'-Atto647N 21 base-pair long DNA in sodium phosphate buffer (150 mM, 0.01% Triton, pH 8.5) at $80 \text{ }^\circ\text{C}$ for 1 hour. dsDNA are then microspotted (spot diameter $\sim 100 \text{ }\mu\text{m}$) on a polymer coated surfaces using a contact microarray spotter and incubated overnight in a humid chamber. The samples are then washed for 10 minutes in 1x PBS, 0.1 % tween, 10 minutes in PBS, rinsed with 10 mM NaCl.

3.2 SSFM Measurements

A detailed principle of operation and detailed setup of SSFM were reported previously (10). The system consists of a laser excitation source, a microscope and a spectrometer. The laser (HeNe, $\lambda=633$ nm) is coupled into the microscope objective (NA=0.12) and focused onto the sample. The fluorescent signal is then coupled to a spectrometer with 1800 grooves/mm grating for spectral acquisition and the signal is recorded using a thermoelectrically cooled CCD.

4. Results and Discussion

Height changes versus height at open potential were measured for 10 separate DNA spots in 10 mM NaCl solution (Figure 2). For positive applied potentials (+0.6 V vs. ITO counter electrode) the DNA is attracted to the surface resulting in a reduced fluorophore – surface height while a negative potential (-0.6 V) repels DNA from the surface, resulting in an increase in the fluorophore – surface height. The contour length for the 21mer dsDNA is 6.8 nm and the 1.3 nm change in fluorophore height for positive to negative applied potentials is lower than expected. This may be due to steric crowding between adjacent DNA strands (8). It may be possible to realize an increase in potential dependent switching by reducing the surface probe density.

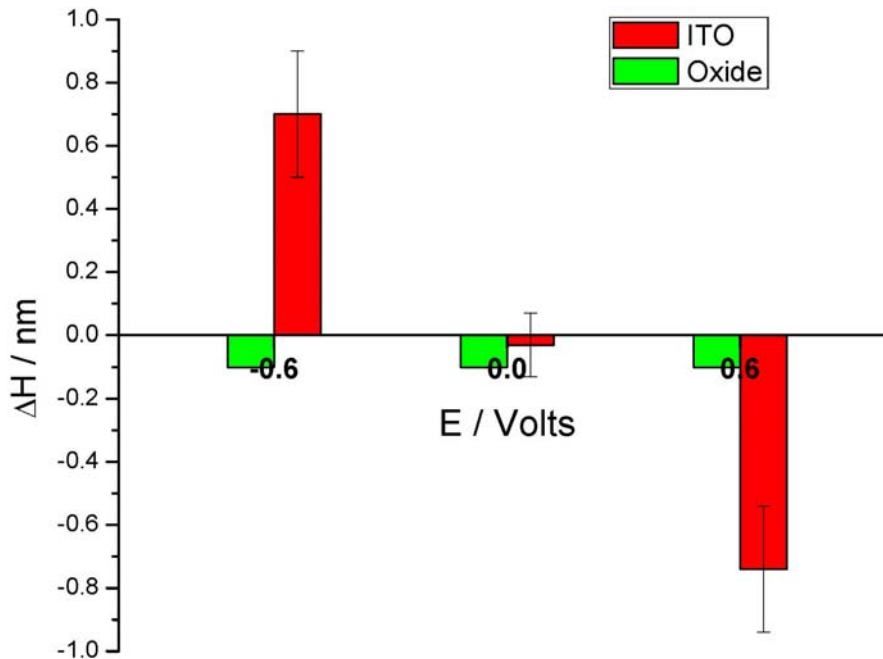


Figure 2. Average height change for an applied potential at 10 spots on a Indium Tin Oxide surface and for a reference spot on silicon dioxide. For positive applied potentials the DNA is pulled towards the surface and for negative applied potentials the DNA is repelled from the surface.

5. CONCLUSIONS

We demonstrate combined potential dependent orientation of dsDNA and sub-nm height measurement of fluorophores through SSFM on an ITO surface. The platform may be applied towards precise quantified measurements of DNA-protein complexes. The platform is scalable to allow high-throughput measurements; this will facilitate systematic studies of the effect of DNA sequence on protein induced conformation changes in DNA.

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