Spectral Self-Interference on Fluorescently Labeled DNA Monolayers

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Abstract: We have measured the height of a fluorescent label incorporated into immobilized DNA with sub nm resolution providing previously inaccessible insights into the structure of DNA monolayers on surfaces.

One of the characteristics of a DNA array is the availability of the single-stranded probes for hybridization with the target. The conformation of DNA molecules in an array may significantly affect the efficiency of hybridization. Immobilized molecules located farther away from the solid support are closer to the solution state and are more accessible for contact with dissolved analytes. The surface, especially a hydrophobic one, acts as a shield for probes positioned close to it because of the associated steric factors, and lack of diffusion of the bound molecules [1]. Unfortunately, little is known about the conformation of the DNA molecules immobilized on surfaces. This information can prove to be useful not only in the development and fabrication of DNA microarrays, but also in designing new applications.

Spectral Self-interference Fluorescence Microscopy (SSFM) [2] may be a powerful tool in studying the conformation of DNA molecules immobilized on a surface. In SSFM analysis of the spectral oscillations due to the self-interference from the direct and reflected emission of a fluorophore several wavelengths about a reflecting surface yields the vertical position of that fluorophore to within a few nanometers. If a fluorescent label is attached to the other, free end of the DNA, the height of the label can be determined by the fluorescence interference spectra modified by the reflection in a mirror buried in the substrate [3]. The height of the label determined by the emission spectra gives the average position of the free end of the DNA molecule immobilized on the surface.

We have placed a fluorescent label at one end of surface-bound single-stranded and double-stranded DNA molecules 21 and 50 nucleotides in length. Figure 1 illustrates the basic structure of DNA films attached to a silicon chip with an approximately 5μm spacer layer of silicon oxide.

The results obtained with SSFM are summarized in Figure 2. It indicates that the label attached to the free end of a surface-bound 21-mer oligo is located very close to the surface – at about 1nm. We also proved (data not shown) that the DNA-surface interaction under the conditions of the experiments is minimal, and that the molecules are unlikely to be lying flat on the surface. It is most likely that the oligos assume the conformation of a random coil.

The position of the label at the end of a 50-mer oligo is about 5.5 nm which is too high for a random coil, probably due to interactions between the adjacent DNA molecules which are larger than 21-mers.

Radiolabeling experiments showed that the average distance between adjacent

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DNA species immobilized on the surface is about 10 nm for both 21-mers and 50-mers. The efficiency of hybridization of the second strand is on the order of 50% for 21-mers and 90% for 50-mers.

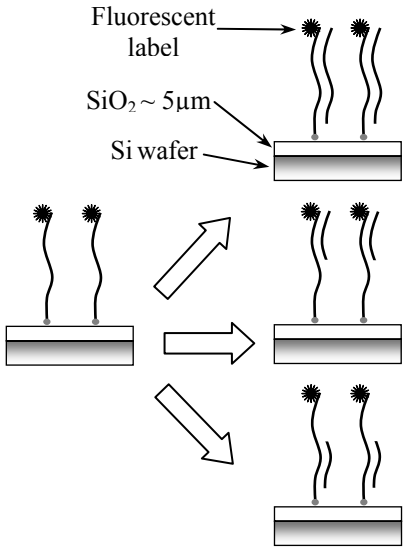

Figure 1. Schematic representation of the SSFM-ready chips with surface-bound fluorescently labeled ss- and ds-DNA.

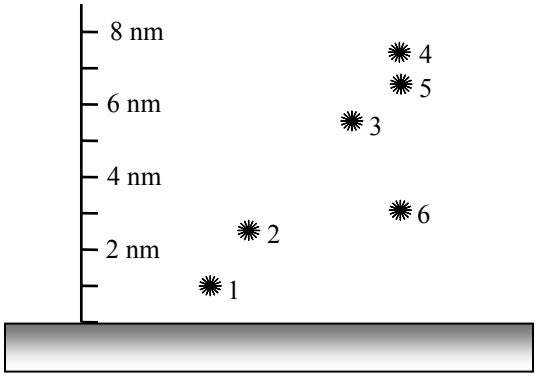

Figure 2. Position of the fluorescent label in the surface-bound DNA monolayer.1) ss 21-mer carrying a fluorescent label at the free end; 2) same after hybridization with a complementary 21-mer; 3) ss 50-mer; 4) ss 50-mer hybridized to an unlabeled complementary 50-mer; 5) ss 50-mer hybridized to an unlabeled 21-mer complementary to the distal end; 6) same, but with a 21-mer complementary to the proximal end;

Upon hybridization of the second strand, the DNA molecules extend as they are becoming double-stranded and assume the conformation of a molecular brush as seen in Figure 2.

A different result is observed when a short piece of DNA is hybridized to the proximal end of a long 50-mer chain. In this case, the end of the oligonucleotide dips down from 5.5 nm to 3 nm – an effect not observed when another complementary oligo of the same length is hybridized to the distal end of the 50-mer. The likely explanation is that the fluorescein molecule at the end of the DNA chain adheres to the double-stranded part, bringing the end of the oligo down.

SSFM carries obvious advantages over the other methods used for studying DNA conformation such as AFM, SPR, or ellipsometry. This method is non-contact, there are no limitations to applying it to chips submerged in a buffer, and it specifically determines the axial position of the labeled nucleotide only with sub nm accuracy. We will present a variety of experimental results on the conformation of single and double-stranded DNA using SSFM technique.

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