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# Probing DNA on Surfaces Using Optical Interference Techniques

*Mehmet Dogan, Julia R. Dupuis, Lev Moiseev, Anna K. Swan, Bennett B. Goldberg, M. Selim Ünlü, Charles R. Cantor*

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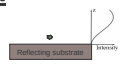
## Abstract

We have developed and patented [1] a technique, Spectral Self-interference Fluorescence Microscopy (SSFM), to determine the location of fluorescent molecules above a reflecting surface with nanometer precision. The method utilizes the spectral fringes produced by interference of direct and reflected emission from fluorescent molecules. The modified spectrum provides a unique signature of the axial position of the fluorophores. We have used the SSFM technique to determine the position of fluorescent markers attached to sub-cellular structures such as lipid bilayer membranes and DNA strands revealing conformational information. Using this technique we have estimated the height of single strand DNA, tilt angle of double strand DNA and amount of hybridization. With the patented SSFM technology, DNA hybridization and DNA-protein interactions can be studied in more detail in many areas such as molecular biological research as well as medical diagnostics, particularly cancer, genetic and infectious diseases. It is promising to be a valuable tool and have an impact in the growing DNA chip industry that utilize microarrays of DNA immobilized on surfaces.

## Current State of the Art

**Interference Techniques to Determine Position :**

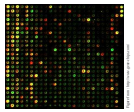
- Fluorescene Light Interference Contrast (FLIC) [2]
- Ellipsometer: non fluorescent technique




FLIC

**DNA Microarray technology [3] :**
**Parallel gene expression and processing**

- Gene analysis and discovery
- Disease diagnostics
- Drug discovery



DNA Microarray

## Challenges and Significance

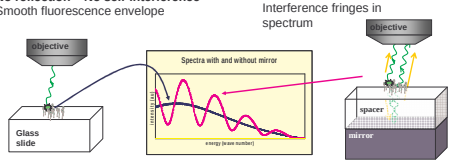
- Current DNA microarray technologies lack determining DNA conformation
- SSFM is capable of determining position of emitters that label DNA , thus reveals valuable information regarding conformation
- A Hyperspectral Fourier Transform Spectrometer (HS-FTS ) is being designed and built for parallel acqusition of spectra to perform SSFM on microarrays.

## Technical Approach: Spectral Self-interference Fluorescence Microscopy (SSFM)

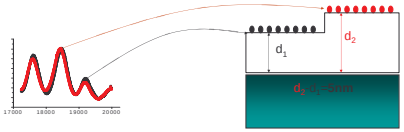
**MOTIVATION: Determining position of fluorescently labeled molecules with nanometer precision**

**No reflection – No self-interference**
Smooth fluorescence envelope

**Reflection – Self-interference**
Interference fringes in spectrum




**Self-interference Reveals Axial Position Information**

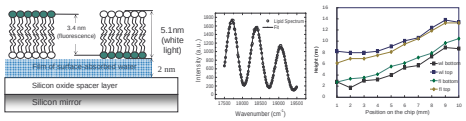


**Best fit to MODEL**
**fitting parameter: d, axial position**

Spectral Fringes → Axial Position

## Probing Lipid Bilayers with SSFM

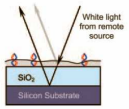
### (Artificial cell membranes)

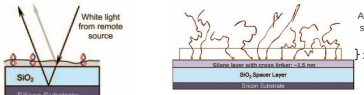



Probing fluorophore position in top or bottom leaflet of an artificial cell membrane

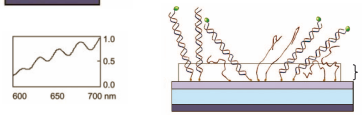
## Accomplishments up through Current Year: Probing DNA on Surfaces

### Estimating Amount of DNA Hybridization using White Light Spectral Interference




Average height of 50mer single strand from white light reflectivity :
} 2 nm optical thickness



Average height after hybridization :
} 3 nm optical thickness

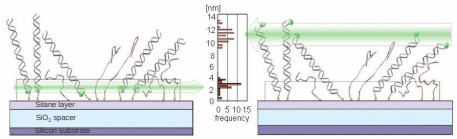



**White Light Interference Spectrum**

2nm optical thickness → 3nm optical thickness

50% hybridization
( Verified by radiolabeling )

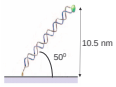
### DNA Conformation Studies




50 Base Pair Double Strand DNA
(Proximal end labeled)

50 Base Pair Double Strand DNA
(Distal end labeled)

| | |
|---|---|
| 50 bp double strand DNA | : 17 nm |
| Measured average length | : 10.5 nm |




$50^0$ double instead of average tilt angle of $30^0$ :

Steric hindrance

DNA as rigid rods on hinges

**In Progress : Probing DNA-Protein Interactions**

## Opportunities for Technology Transfer

### Microarry Industry Overview [4]

- \$ 450M, growing 10% each year
- \$ 1 Billion revenue projected for 2010

**Some of DNA microarray companies :**
Affymetrix Inc., Molecular Dynamics Inc., Nanogen, Protogene Labs, NimbleGen Inc.

**Industry members using microarrays:**

- Molecular Biological Research
- Healthcare
- Pharmacology for Drug Discovery

### Our proposed contribution to microarray technology:

Microarray detection with determining hybridization efficiency and position information of the fluorescent markers that leads to understanding of the DNA conformation

## Opportunities for Technology Transfer

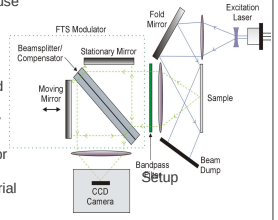
### Hyperspectral Fourier Transform Spectroscopy: SSFM on Microarrays

- We have demonstrated the proof of concept for SSFM
- We are now developing a hyperspectral Fourier transform spectrometer (HS-FTS) to demonstrate the SSFM principle with imaging on microarray chips

The instrument includes a small FTS modulator and a CCD camera to record the spatially resolved interferograms.

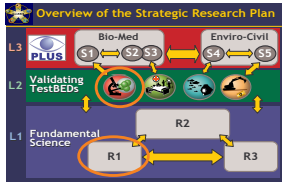
**Benefits of the system:**

- Good radiometric efficiency because of spectral multiplexing,
- Background rejection due to synchronous detection,
- Flexibility in spectral range by changing the spectral prefilter and sampling parameters,
- Flexibility in spectral resolution by changing the mirror stroke length.
- The HS-FTS is also being used for white light interferometry measurements of biological material bound to surfaces.




Setup

## Contribution to CenSSIS Research Thrusts




This work falls under CenSSIS Research Thrust R1(multispectral imaging ). The development of the HS-FTS nanoscope relates the project to the CenSSIS BioBED platform.

## Publications Acknowledging NSF Support

A.K. Swan, L.A. Moiseev, C.R. Cantor, B. Davis, S.B. Ippolito, W.C. Karl, B.B. Goldberg, and M. S. Ünlü, **"Towards nm resolution in fluorescence microscopy using spectral self interference,"** *IEEE Journal of Selected Topics in Quantum Electronics*, Vol. 9, No. 2, March/April 2003, pp. 294-300.

L.A. Moiseev, C.R. Cantor, I. Aksun, M. Dogan, B.B. Goldberg, A.K. Swan and M. S. Ünlü, "**Spectral self-interference fluorescence microscopy"** *Journal of Applied Physics*, Vol. 96, No. 9,1 November 2004, pp. 5311-5315

Lev Moiseev, Anna K. Swan, M. Selim Ünlü, Bennett B. Goldberg, C. Cantor, **"DNA Conformation on Surfaces Measured by Fluorescence Self-Interference**", PNAS , vol.103,no. 8, February 21, 2006

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(1) M. S. Unlu, A. K. Swan, B.B. Goldberg, S. Ippolito, L. Moiseev, S. Lipoff, and Y. Tong, "Spectral imaging for vertical sectioning," US Utility Patent filed 18 June 2003 (10/451,096)

(2)A. Lambacher, P. Fromherz, **"Fluorescence Interference-Contrast Microscopy on Oxidized Silicon using a Monomolecular Dye Layer"**,Appl.Phys.,A 63, 1996, pp. 207-216

(3) M. Schena, D. Shalon, R. W. Davis, and P.O. Brown **,"Quantitative Monitoring of Gene Expression Patterns with a Complementary DNA Microarray"** , Science 270 , 20 October 1995 , pp. 467-470

(4) "DNA Microarrays : A Market Update", June 2006, published by Biocompare

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