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:
• Fluorescence Light Interference Contrast (FLIC)(2)
• Ellipsometer: non-fluorescent technique

DNA Microarray technology (3):
J. Parallel gene expression and processing
Gene analysis and discovery
Disease diagnostics
Drug discovery

Challenges and Significance
• Current DNA microarray technologies lack determining DNA conformation
• SSFM is capable of determining position of emitters that label DNA, thus revealing valuable information regarding conformation

Our proposed contribution to microarray technology:
Microarray Industry Overview
• >450 M, growing 10% each year
• $1 Billion revenue projected for 2010

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

Opportunities for Technology Transfer

Spectroscopic Self-Interference Fluorescence Microscopy (SSFM)

MOTIVATION: Determining position of fluorescently labeled molecules with nanometer precision

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

No reflection – No self-interference
Smooth fluorescence envelope
Reflection – Self-interference
Fringes in spectrum

Self-interference reveals axial position information

In progress: Probing DNA-Protein Interactions

Probing Lipid Bilayers with SSFM
(Artificial cell membranes)

Probing DNA on Surfaces

Estimating Amount of DNA Hybridization using White Light Spectral Interference

In Progress: Probing DNA-Protein Interactions

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

Accomplishments up through Current Year:
Probing DNA on Surfaces

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 spatially resolved interferograms.

Benefits of the system:
• Good radiometric efficiency because of single detector multiplexing
• Background rejection due to synchronous detection
• Flexibility in spectral range by changing the spectral pretilter 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.

Contribution to CenSSIS Research Thrusts

Publications Acknowledging NSF Support


References


Contact Information

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