

# Proteomics Course

## LECTURE-36

### Label-free techniques: SPRi, Ellipsometry, Interference



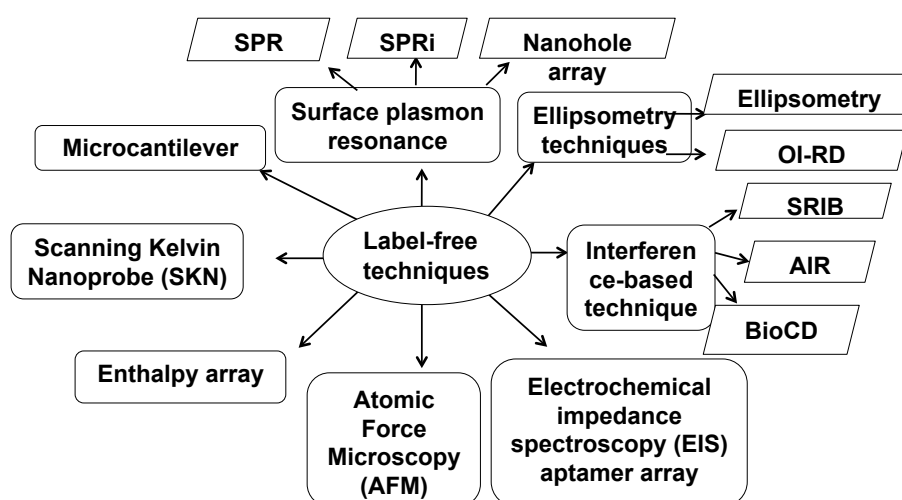
Dr. Sanjeeva Srivastava  
IIT Bombay



## Previous lecture

- Detection techniques
  - Label-based vs. label-free
- Label-free techniques
  - Surface plasmon resonance

## An overview of label-free techniques



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## Lecture outline

- Other promising label-free techniques
- SPR and related techniques
  - SPR imaging
  - Nanohole arrays
- Ellipsometry based techniques
  - Ellipsometry
  - Oblique incidence reflectivity difference (OI-RD)
- Interference-based techniques
  - Backscattering Interferometry Rectangular Channels (BIRC)
  - Spectral Reflectance Imaging Biosensor (SRIB)

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**(I) SPR and related  
techniques**

**Surface Plasmon Resonance  
Imaging (SPRi)**

## SPR Imaging

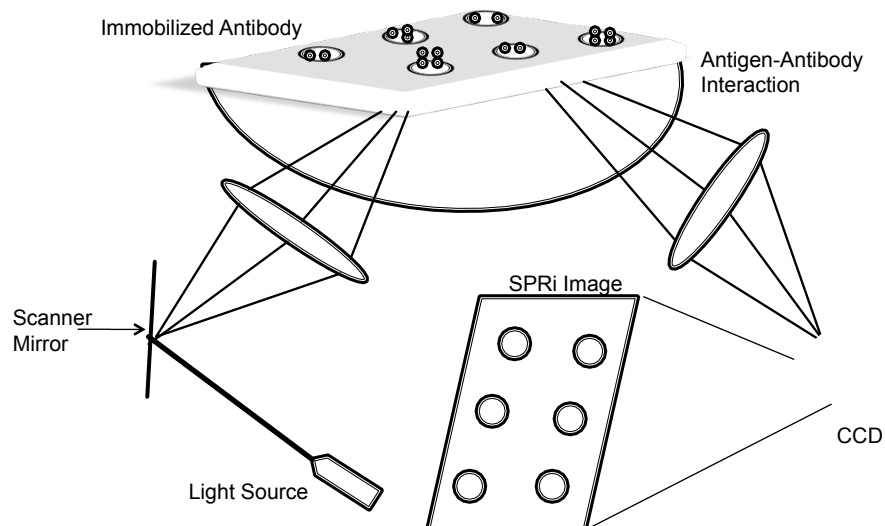
- Collimated, monochromatic beam of light illuminates sample assembly at a single incident angle near SPR angle, and light reflected from the surface is detected with charge coupled device to produce the SPR image
- SPRi fixes on a single-incidence angle to monitor reflection intensity for the whole array surface as a function of time

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## SPR imaging



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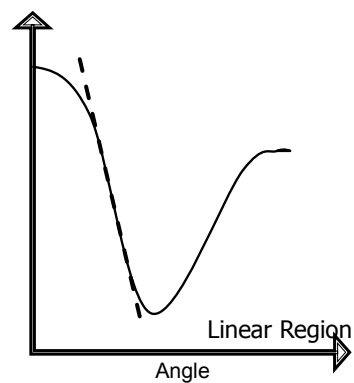
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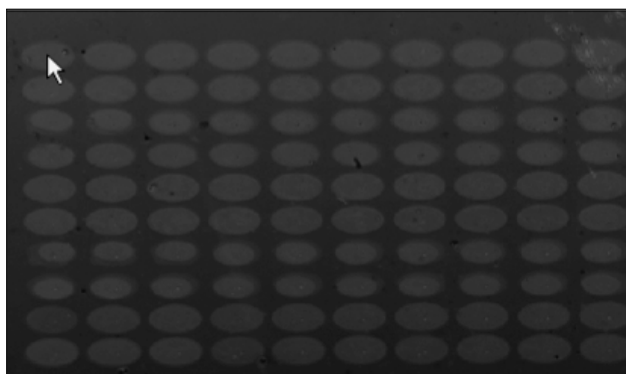
## SPR imaging

At fixed incident angle, spatial variations in refractive index due to presence of proteins adsorbates shift local resonant angle, which in turn changes reflected light intensity.

This linear region is directly proportional to optical angle.



## Image contrast



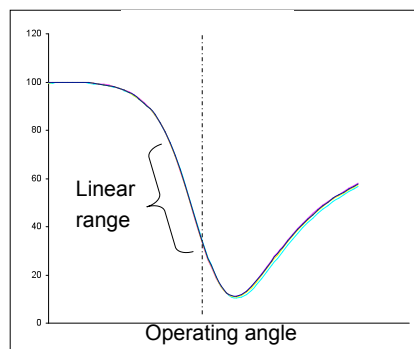
## SPRi experimental work-flow

### SPRi: experimental work-flow

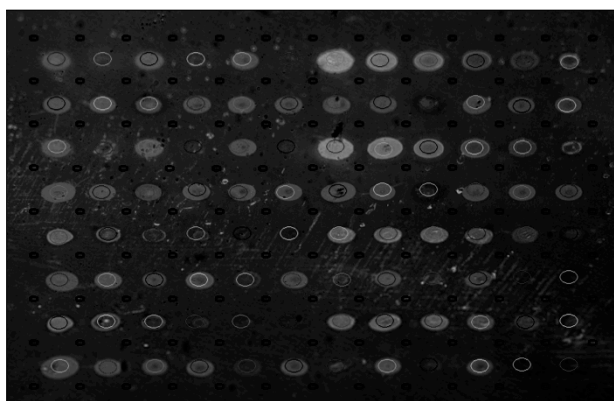
- Prepare and mount slide
- Load and prime samples
- Assign ROI's
- Determine operating angle
- Initiate data acquisition
- Record movie and generate data file
- Inject samples
- Save & export data

## Selecting an operating angle

- Measure the SPR curve
- Determine “linear range”
- Select an operating angle
- Bottom of linear range
- Typically ~ 30% reflectivity
- Select optics at this angle for your experiment



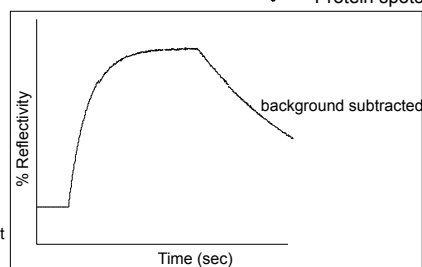
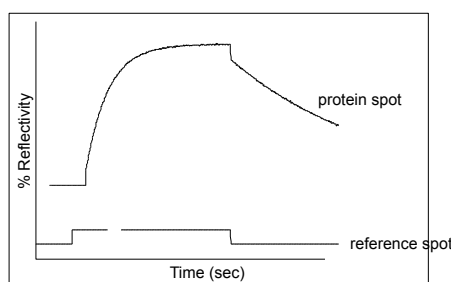
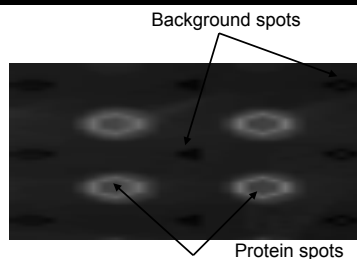
## Regions of Interest (ROI's)



ROI's define the regions of interest to measure

## Background Subtraction

- Background spots to remove bulk refractive index effects
- Buffer concentration
- Temperature change

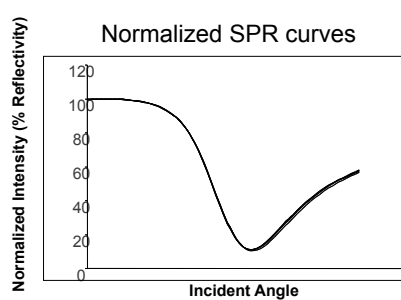
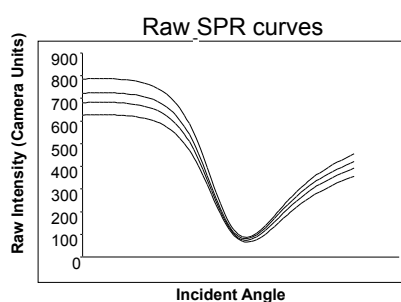


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## Normalized intensity (% reflectivity)



- At an angle very far away from SPR angle (optics position  $\sim 0$ ), 100% of the incident light is reflected
- 100% reflectivity used to normalize reflected light intensity so that all features have same sensitivity

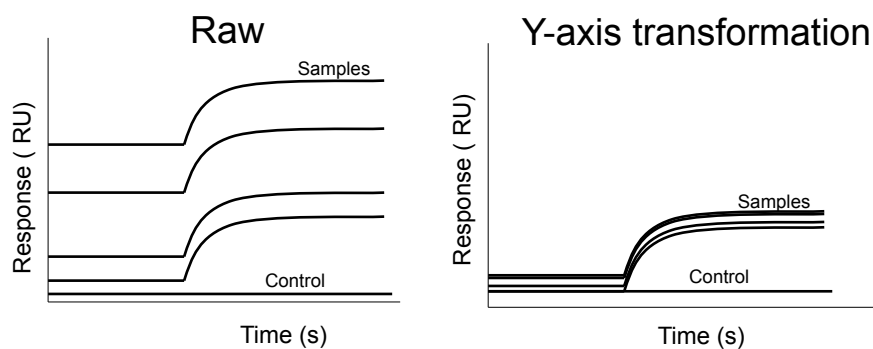
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## Data processing



Y-axis transformation to fit data (cropped and aligned)

## Kinetics and affinity determination

- Kinetics: rates of reaction
- Affinity: strength of binding
- $KD = K_d / K_a$ 
  - Dissociation constant (KD), on rate ( $K_a$ ), off rate ( $K_d$ )
- Kinetics as the rates of complex formation
- Fitting data according model

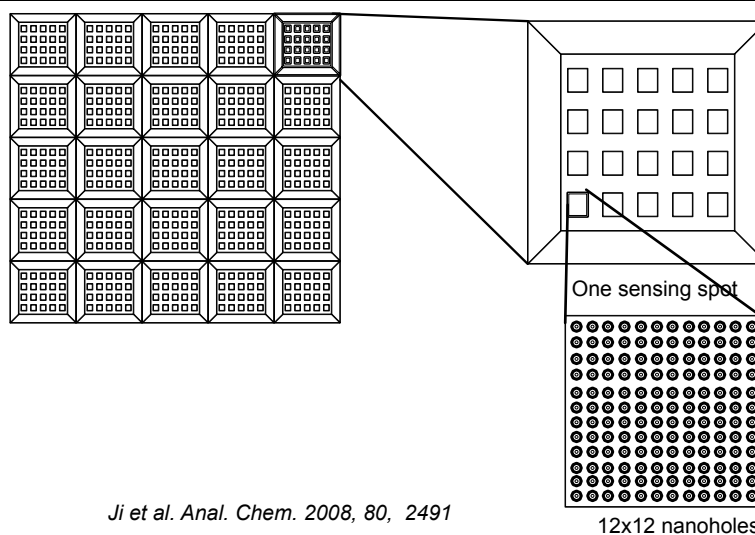
# **SPR Imaging Applications**

# **Nanohole arrays**

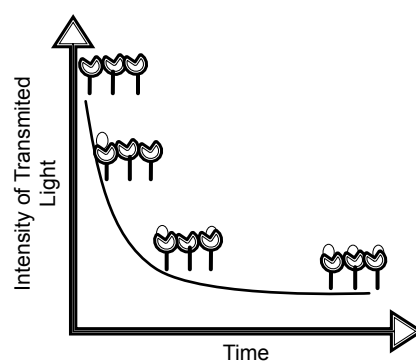
## Nanohole arrays

- Surface plasmons excited on both sides of metal surface resonantly coupled through the sub-wavelength holes
- It enhances the light transmission for a specific wavelength and makes nanohole arrays a potential surface based biosensor

## Nanohole arrays



## Nanohole arrays



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## Nanohole arrays: merits and demerits

- Merits
  - Real-time measurements
  - Multiplex analysis
  - Simple optical alignment
  - Miniaturization is possible
- Demerits
  - Insensitive to conformational changes
  - Limited to gold or silver surfaces

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# **Nanohole arrays Applications**

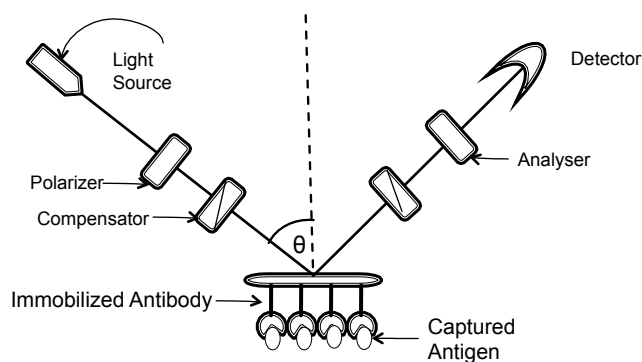
## **(II) Ellipsometry-based techniques**

# Ellipsometry

## Ellipsometry

- It is based on polarization state of reflected light
- Altered due to changes in dielectric property or refractive index of sample surface
- Imaging ellipsometry combines ellipsometer, microscopy and CCD camera
  - measures total protein content on solid surface

## Ellipsometry



*Jin et al. Conf. Proc. IEEE Eng. Med. Biol. Soc. 2004, 3, 1975-1978.*

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## Ellipsometry: merits and demerits

- Merits
  - Simple instrumentation
  - Not restricted to gold or silver
  - Cheaper than SPR based biosensors
  - HT capability
  - Simultaneous monitoring of entire microarray
- Demerits
  - Less sensitive

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# **Ellipsometry Applications**

# **Oblique incidence reflectivity difference (OI-RD)**

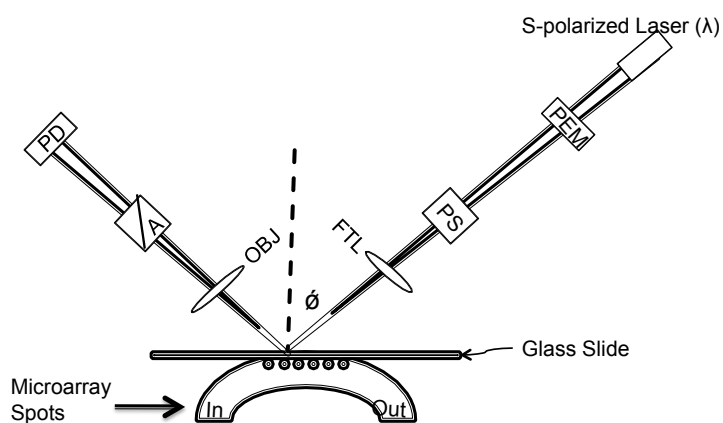
*Zhu et al. Appl. Opt. 2007, 46, 11890; Fei et al. Rev. Sci. Instrum. 2008, 79, 013708*



## Oblique incidence reflectivity difference (OI-RD)

- A form of ellipsometry in which, harmonics of modulated photocurrents are measured under suitable nulling conditions
- Changes in thickness or dielectric response due to a reaction, such as protein binding, provides a detectable signal

## OI-RD



## OI-RD: merits and demerits

- Merits
  - Higher sensitivity than imaging ellipsometry
  - Rapid detection
  - Real time measurement
  - HT affinity detection
- Demerits
  - Insensitive to conformational changes

# OI-RD Applications

## **(III) Interference-based techniques**

*Markov et al. 2004, J. Am. Chem. 126, 16659; Lin et al. 1997, Science, 278, 840*

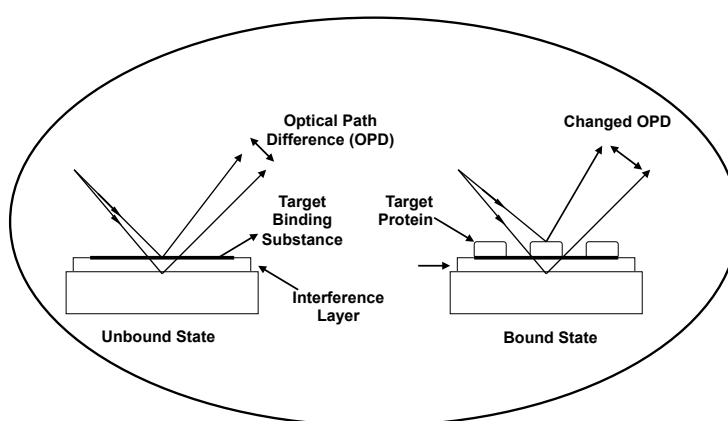
### **Interferometry**

- Basic principle – transformation of phase differences of wave fronts into observable intensity fluctuations known as interference fringes

## Interferometry

- Promising interferometric techniques include -
  - Spectral reflectance imaging biosensor (SRIB)
  - Dual-channel biosensor
  - SPR interferometry
  - On chip interferometric backscatter detection
  - Biological compact disc (BioCD)

## Interferometry

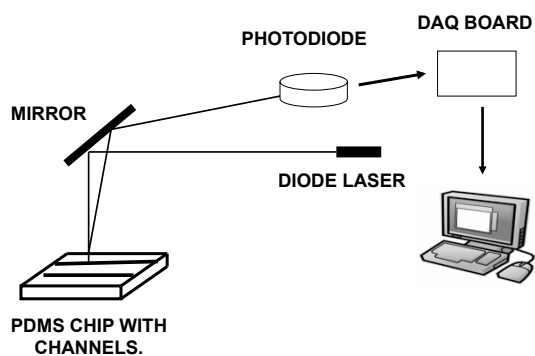


## **Backscattering Interferometry in Rectangular Channels (BIRC)**

### **Backscattering Interferometry in Rectangular Channels (BIRC)**

- High sensitivity interferometry performed within rectangular channels of micrometer size
  - formed in inexpensive [poly (dimethylsiloxane)] PDMS

## Backscattering Interferometry in Rectangular Channels



# BIRC Applications

## **BIRC: merits and demerits**

- Merits
  - Real-time measurements
  - Applicable for reversible and irreversible protein-protein interactions
  - Cost-effective
- Demerits
  - PDMS Chip
  - Surface preparations are multistep
  - Complex process which is time-consuming

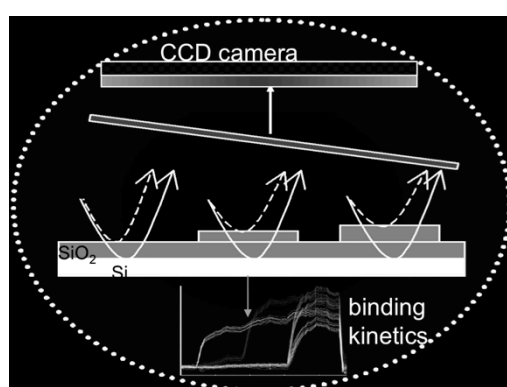
## **Spectral Reflectance Imaging Biosensor (SRIB)**

*Ozkumur et al. 2008, Proc. Natl. Acad. Sci. USA 2008, 105, 7988*

## Spectral Reflectance Imaging Biosensor

- SRIB, a label-free approach based on interference
- Changes in optical index as a result of capture of biological material on surface are detected using optical wave interference
- Directly monitors molecular binding interactions

## Spectral Reflectance Imaging Biosensor

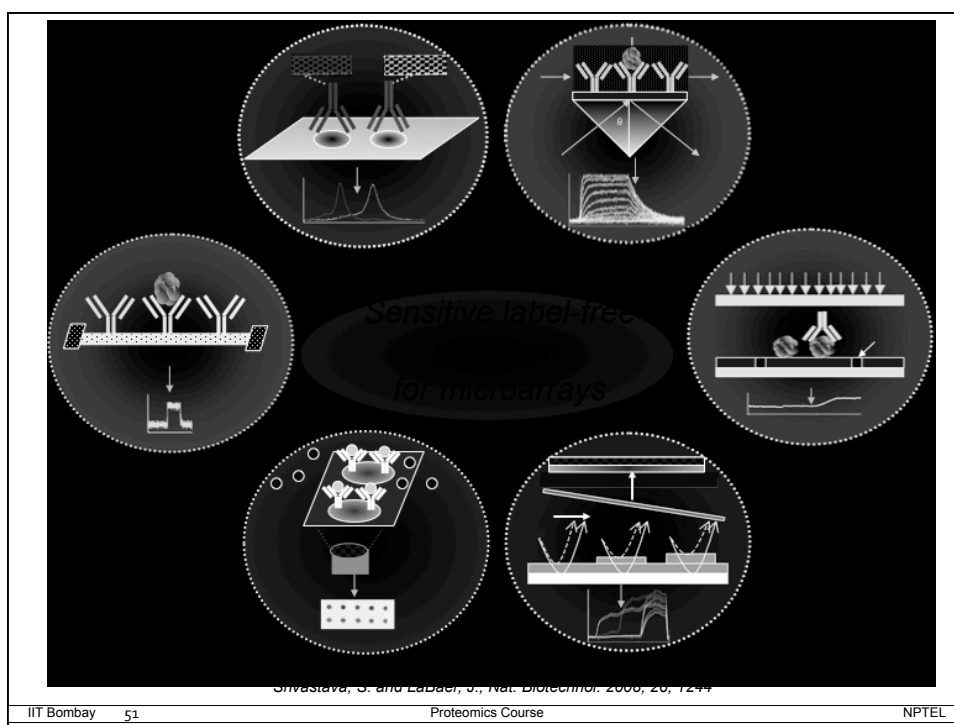




# SRIB Applications

## SRIB: merits and demerits

- Merits
  - Cost effective
  - Fast determination of binding kinetics
  - Easily implemented
  - HT capability
- Demerits
  - Suitable for only smooth layered substrates
  - Non-specific binding



## Summary

- Various promising label-free techniques
- SPR and related techniques
  - SPR imaging, Nanohole arrays
- Ellipsometry based techniques
  - Ellipsometry, OI-RD
- Interference-based techniques
  - BIRC, SRIB

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