

Proteomics Course

LECTURE-35 Label-free techniques: SPR and SPRi



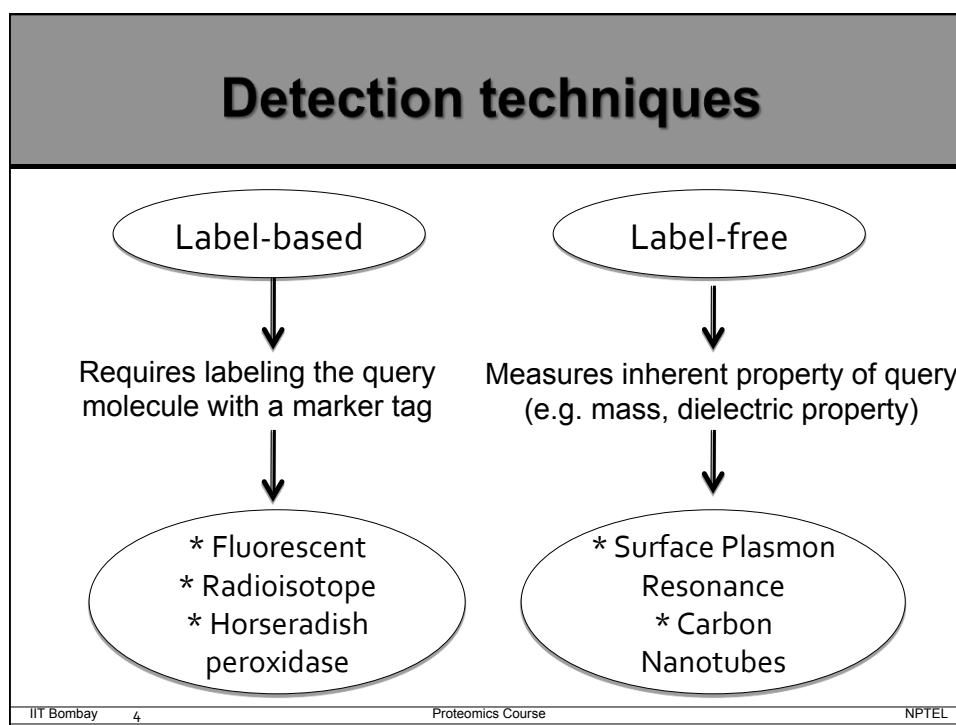
Dr. Sanjeeva Srivastava
IIT Bombay



Lecture outline

- Detection techniques
- Label-based vs. label-free
- Promising label-free techniques
 - SPR
 - SPRi

Detection techniques



Label-based vs. Label-free: strengths

Label-based



- + Widely used
- + Commonly available reagents & instruments

Label-free



- + Avoids tag-related issues
- + Allows real-time measurements

+ Strength

Label-based vs. Label-free: weakness

Label-based



- Tags interfere with function
- Tags are tedious to add
- Endpoint measurements

Label-free



- Typically less sensitive
- More costly

- Weakness

Label-free measurements

Label-free measurements

- Measure inherent property of query itself
 - Mass
 - Dielectric property etc.
- Avoids modifying interactors
 - No effect from conjugated fluorescent labels or radioactive material

Label-free measurements (2)

- Provide real-time reaction kinetics to determine dynamic parameters of biomolecular interactions
 - Kinetics, Affinities
- Protein functional behavior by developing models and wiring diagrams
- Different label-free techniques in various stages of development

Applications of real-time label-free detection

Protein interactions

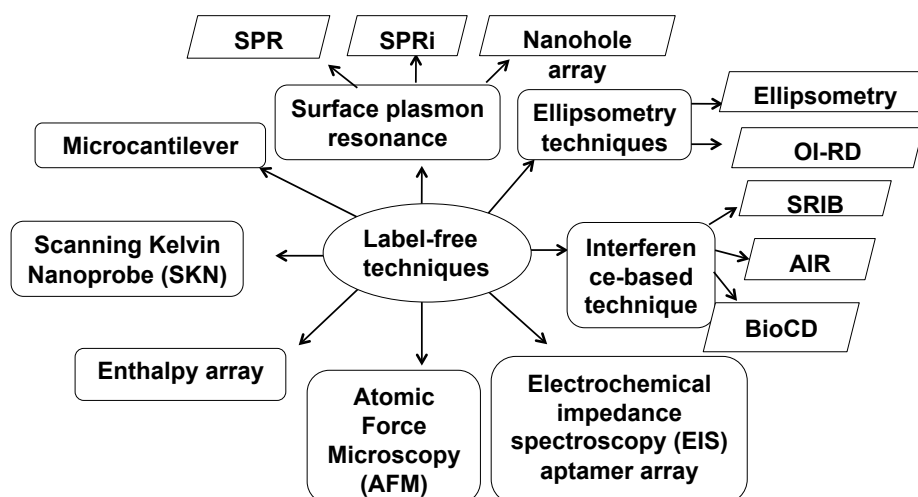
- Obtain kinetic profile for all interactors of a target
- Determine effects of mutation on kinetics of interactions
- Evaluate antibody performance (specificity, affinity, etc.)
- Study interactions with physiologically relevant KD

Applications of real-time label-free detection

Small molecule interactions

- Avoid need for direct labeling of small molecules or need for labeled competitive inhibitors
- Test binding selectivity against large number of targets
- Small molecule design to improve k_{on}/k_{off} or selectivity

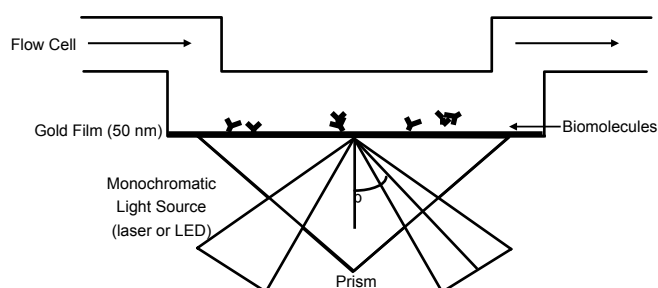
An overview of label-free techniques



Surface Plasmon Resonance (SPR)

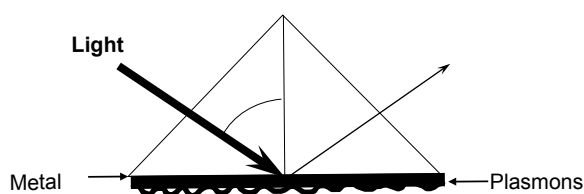
Surface Plasmon Resonance (SPR)

- Measures change in refractive index of medium directly in contact with sensor surface (e.g. gold)
- Medium in contact with surface is commonly an aqueous sample containing analyte “protein”



SPR (contd.)

- Plasmons - special electromagnetic waves that can be excited at certain metal interfaces, mostly gold & silver
 - generated on boundary of metal & external medium (e.g. air)
 - very sensitive to any change of this boundary (e.g. adsorption of biomolecules to the metal)

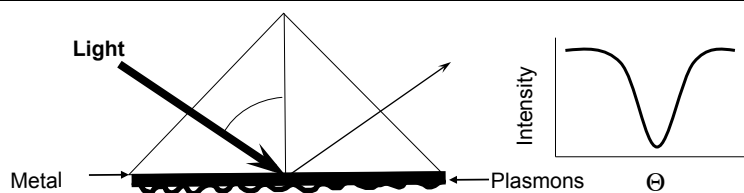


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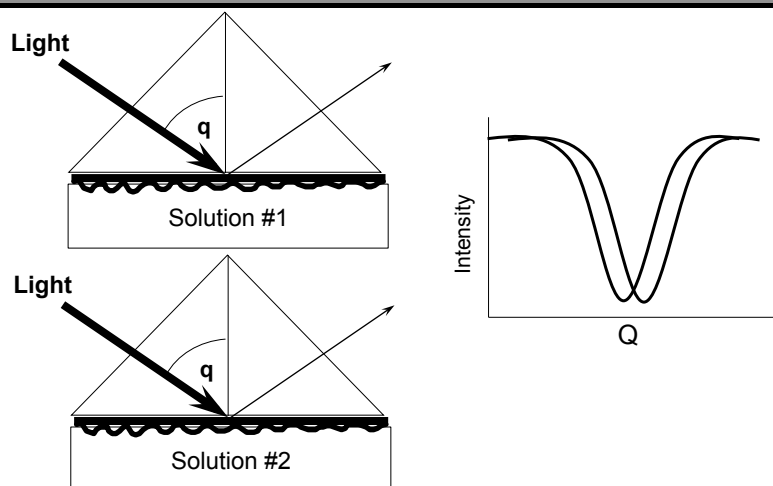
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SPR (contd.)



- A light beam impinge at interface between metal and media at a defined angle, “**resonance angle**”
 - *resonance angle* depends on refractive index in immediate vicinity of gold surface
- As material binds to the surface, the refractive index increases and the SPR curve shifts to higher angles

SPR angle



SPR angle depends on refractive index near the surface

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Real-time label-free detection by SPR

- Excitation of a planar surface with light excites surface plasmons and changes reflectivity
- Real-time label-free detection of binding events detected by measuring change in SPR reflectivity
- Changes in refractive index are monitored continuously
- Platform for real-time label-free detection

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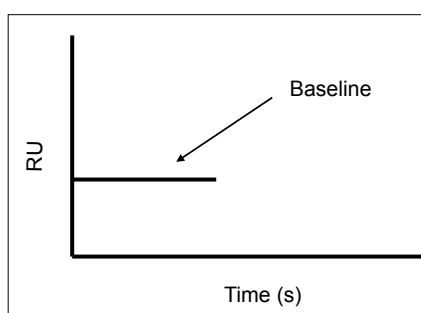
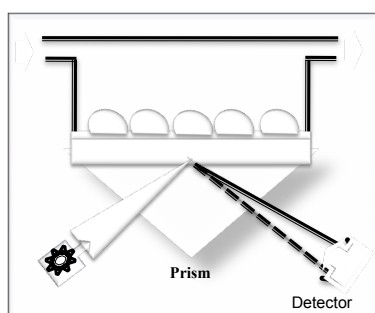
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SPR Sensorgram

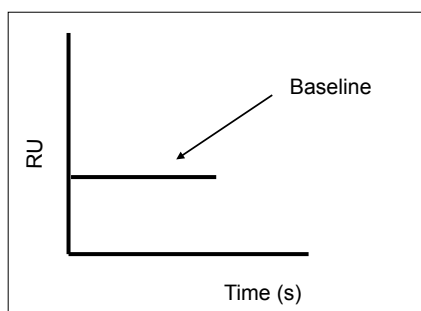
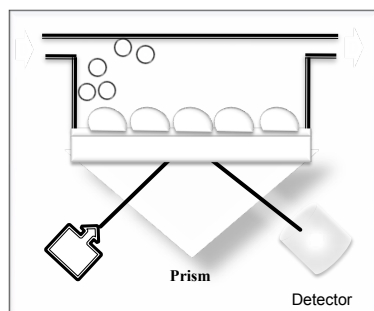
SPR Sensorgram (1)

Sensorgram - changes in the SPR signal versus time

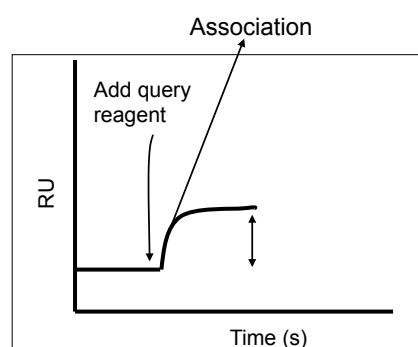
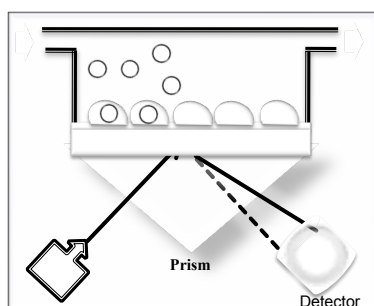


SPR Sensorgram (2)

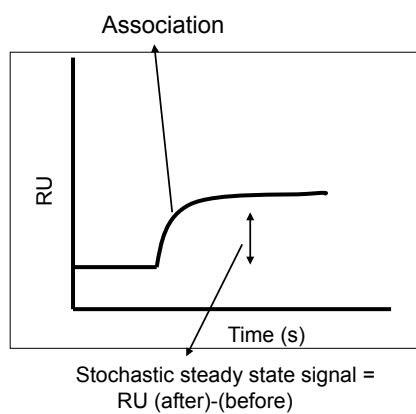
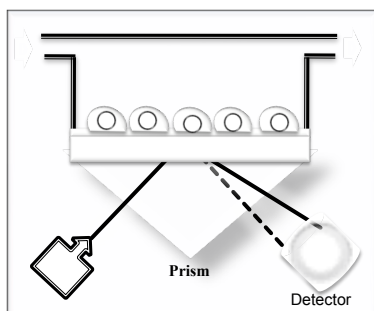
Sensorgram - changes in the SPR signal versus time



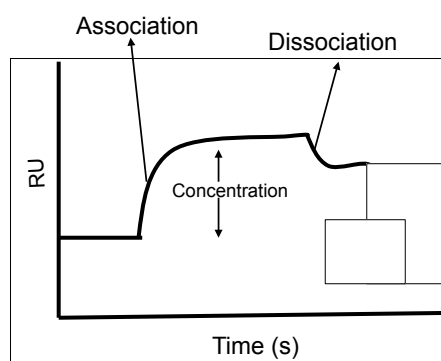
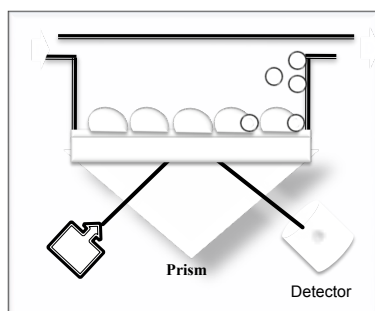
SPR Sensorgram (3)



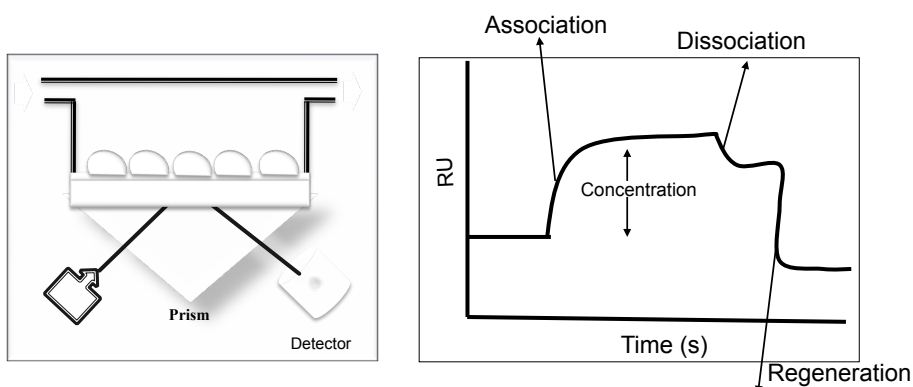
SPR Sensorgram (4)



SPR Sensorgram (5)



SPR Sensorgram (6)

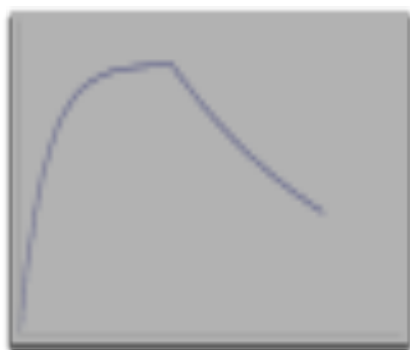


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SPR Sensorgram: what does it tell you?



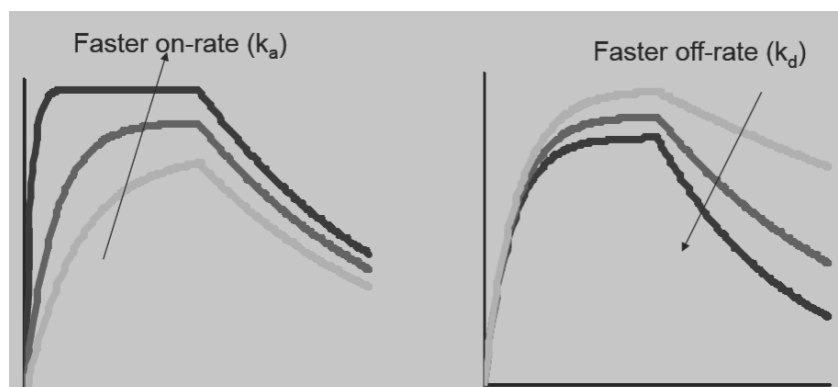
- Shape
- Kinetics (k_a , k_d)
- Concentration
- Amplitude
- Surface capacity
- Molecular weight

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SPR Sensorgram: what does it tell you?

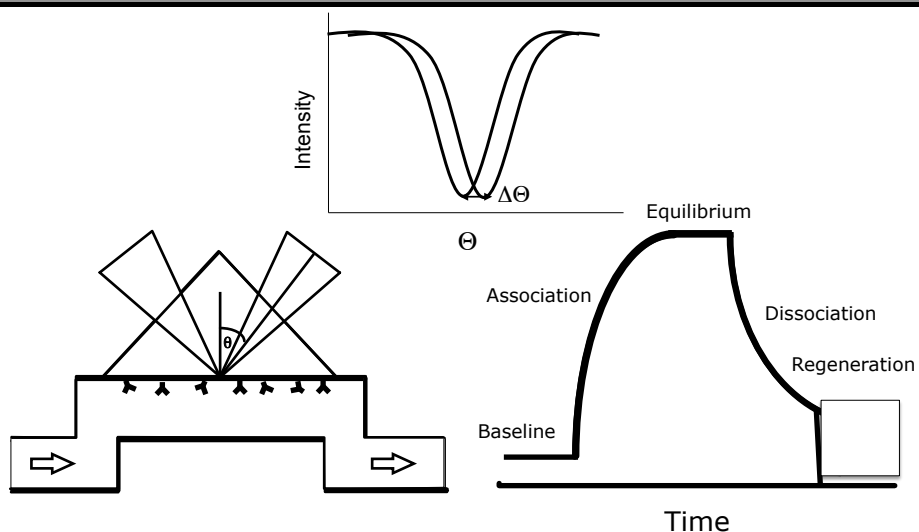


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SPR Assay



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SPR advantages

- Label-free
 - No need for tedious or expensive labeling protocols
 - Avoid potential labeling artifacts
- Direct
 - Measures binding of the actual analyte
- Measure binding kinetics and affinity (k_a , k_d , KD)
- Real-time
 - Allows user to watch the experiment as it happens
 - Not an “end point assay”

SPR limitations

- SPR detection relies on mass changes
- SPR detection decreases exponentially with distance from surface
- Estimated detection limit ~200 nm
- Limited to choice of metal which results in SPR

SPR: **SPR limitations (2)**

- Sample must be homogeneous
- Sample preparation and probe attachment to metal surface can be difficult
- Non-specific interactions also results into SPR signal
 - Need to ensure specific signal
 - Avoid bulk effects
- Refractive index is temperature dependent

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

Guideline for SPR experiment and data analysis

J. Mol. Recognit. 1999;12:279–284; *J. Mol. Rec.* 2002; 15:352-376

Guidelines

Experimental Preparation

Homogeneous reagents

- no aggregation
- no precipitation

Filter buffers

Degas buffers

Instrument cleaning

Guidelines (2)

Surface Capacity



Controls

- Empty surface
- Single component
- Baseline check

Guidelines (3)

Experimental Parameters



Injection at a fast flow rate (50-100 $\mu\text{L}/\text{min}$)

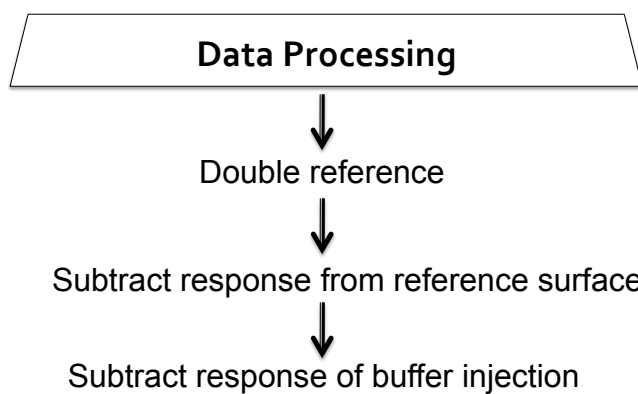


Analyze blanks of running buffer periodically

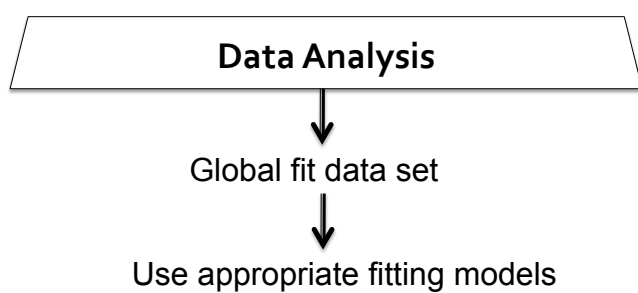


Replicates: slides and/or samples

Guidelines (4)

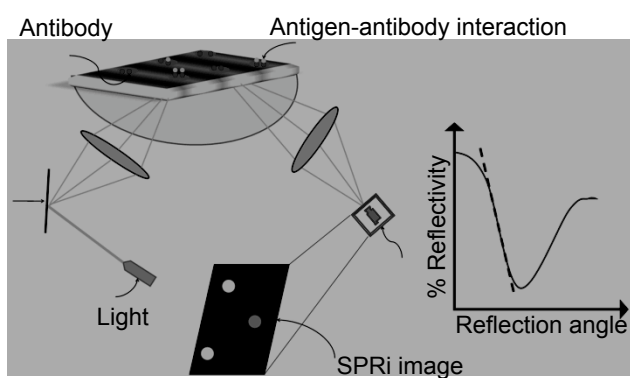


Guidelines (5)



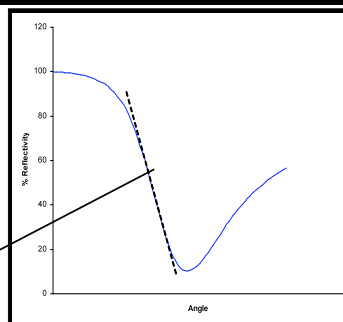
Surface Plasmon Resonance Imaging (SPRi)

SPR imaging



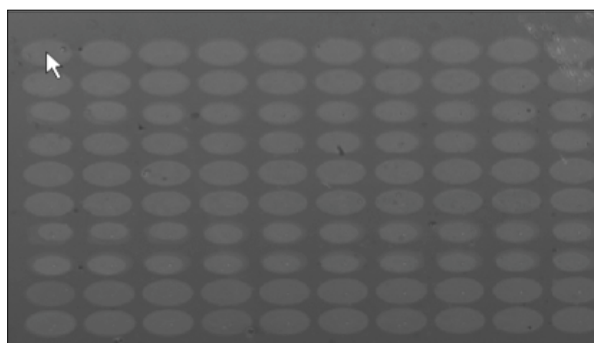
SPR imaging

- 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



- The linear region of the SPR curve quantitatively correlate changes in reflected light intensity with the amount of material on the surface
- This linear region is directly proportional to optical angle

Image contrast



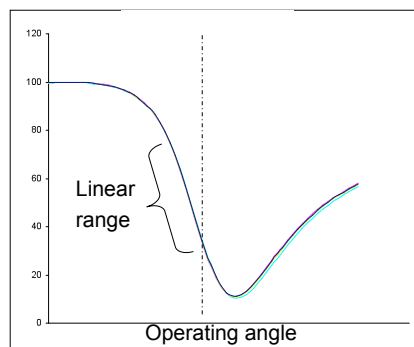
SPRi experimental work-flow

SPRi: experimental work-flow

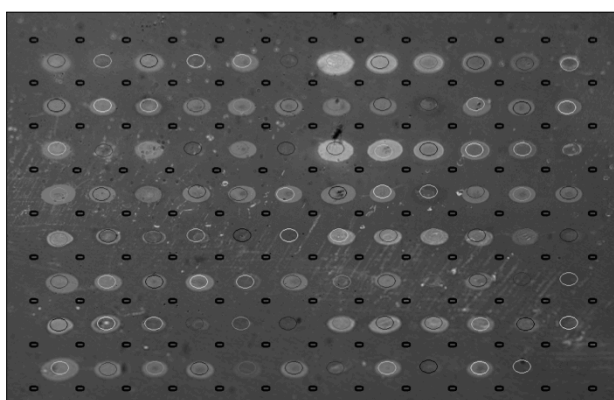
- Prepare & mount slide
- Load & prime samples
- Assign ROI's
- Determine operating angle
- Initiate data acquisition
- Record movie and generate data file
- Inject samples
- Save & export data

Selecting an operating angle

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



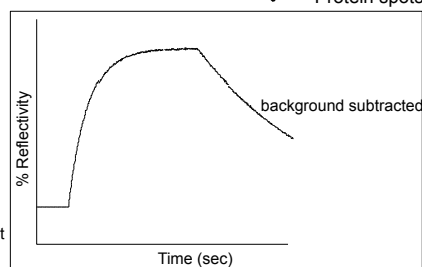
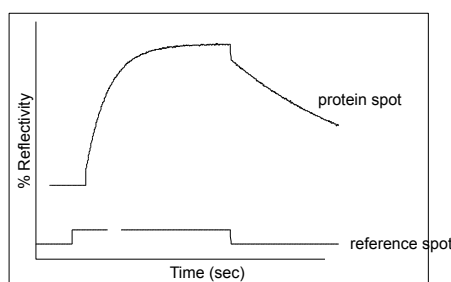
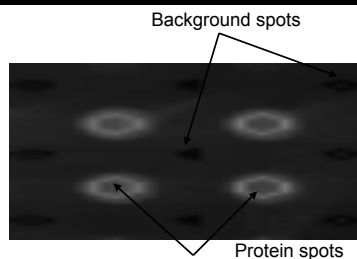
Regions of Interest (ROI's)



ROI's define the regions of interest to measure

Background Subtraction

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

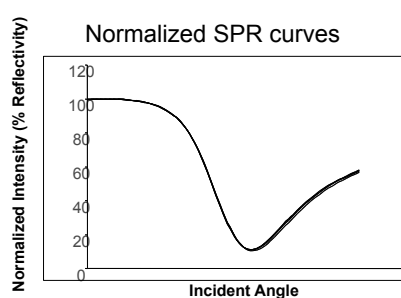
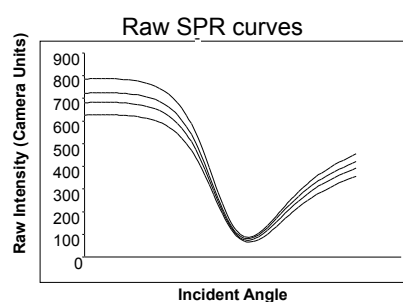


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Normalized intensity (% reflectivity)



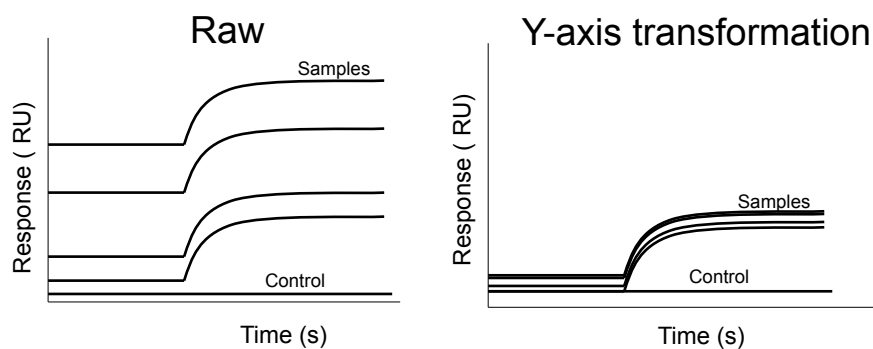
- At an angle very far away from SPR angle (optics position ~ 0), 100% of the incident light is reflected
- 100% reflectivity used to normalize reflected light intensity so that all features have same sensitivity

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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

Summary

- Label-based vs. label-free
- Promising label-free techniques
 - SPR

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