LECTURE-30

Surface Plasmon Resonance Imaging

Handout

PREAMBLE

SPRi-based label-free sensors, which depend on measurement of an inherent property (refractive index) of query molecules, are gaining popularity in HT proteomics due to their multiplexing capabilities. The basic working principle of SPRi is quite similar to that of SPR, which measures the changes in the refractive index. In SPR imaging (SPRi) a spatially resolved imaging device is introduced to SPR set-up for continuously monitoring the changes occuring on the surface to generate real-time kinetic data. SPRi-based biosensors are capable for instantaneous label-free analysis of several biomolecular interactions in a fast and HT manner (Reddy et al., 2012). In this lecture an introduction of SPRi, basic principle behind its operation, different applications, and advantages and disadvantages of SPRi in comparison to SPR will be discussed.

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OUTLINE OF LECTURE

I. Basic working principle of Surface Plasmon Resonance Imaging (SPRi)

II. Different applications of SPRi

(a) Study of biomolecular interactions

(b) Detection of cancer biomarkers

(c) Detection of biomarkers for other human diseases

(d) Screening of inhibitors of tumor targets

III. Advantages and disadvantages

IV. Conclusions

I. BASIC WORKING PRINCIPLE OF SURFACE PLASMON RESONANCE IMAGING (SPRi)

In SPRi the alterations in the refractive index of the medium directly in contact with sensor surface is measured (Fig 1).

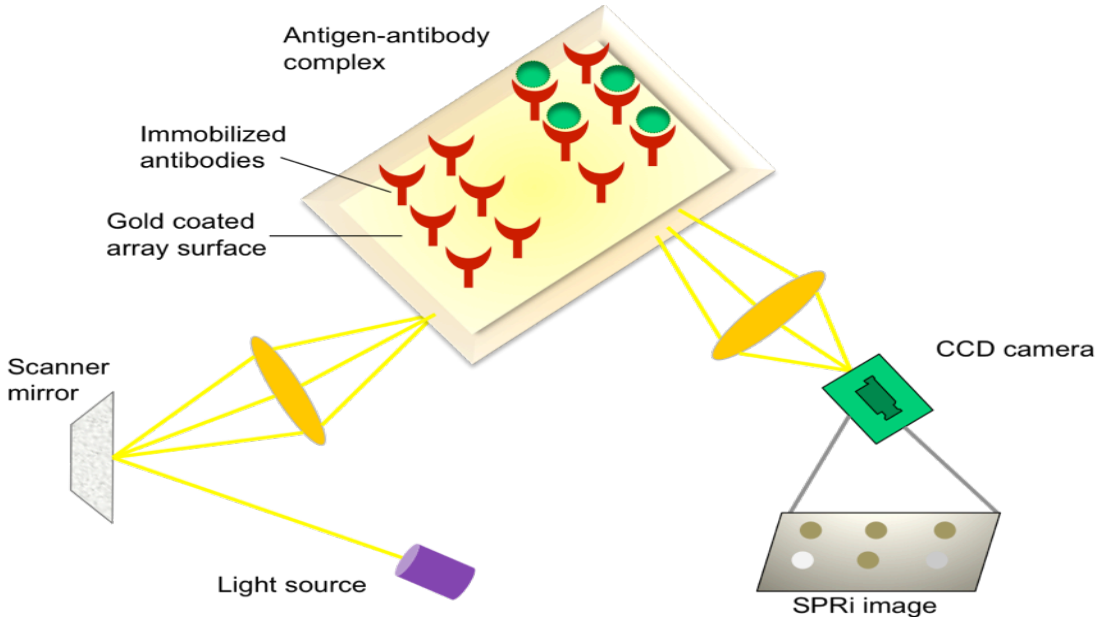

Fig 1. SPRi relies on measurement of changes in refractive index of the medium directly in contact with sensor surface.

In SPRi complete biochip surface is illuminated using a broad beam, monochromatic, polarized light and a CCD camera is used for simultaneous capturing of reflected light from each spot. In SPRi the intensity of the incident light as well as wavelength are kept constant, and the reflected light is determined at an optimum reflectance angle coming

from the metal interface. The complete array can be captured by coupled charge device (CCD) camera for HT studies. SPRi experimental flow includes preparation and mounting of the slide, loading prime samples, assignment of region of interests (ROIs), determination of operating angle and data acquisition (Fig 2).

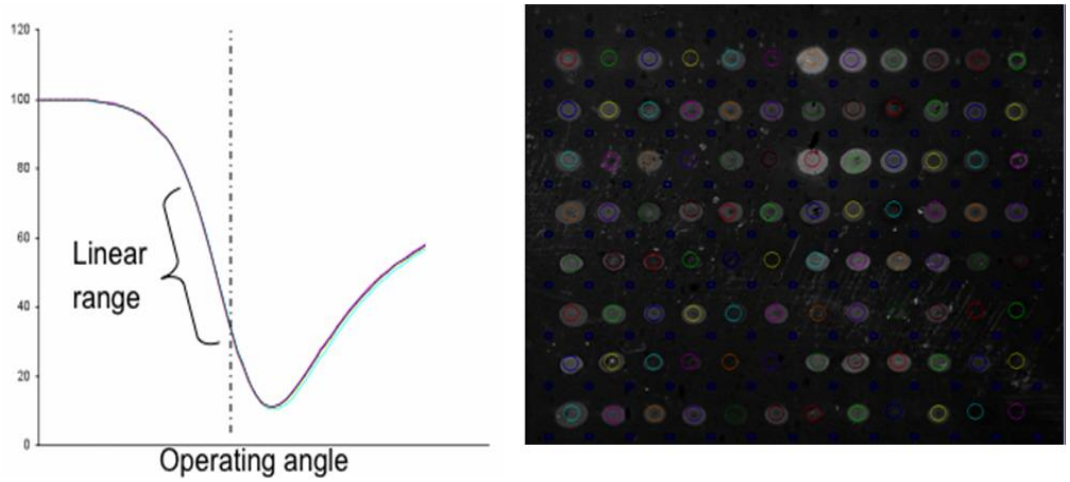

Fig 2. Selection of an operating angle and assignment of region of interests (ROIs) in SPRi experiment.

In SPRi experiments data generation is done in a real-time manner and generated data files are saved in proper format (as movies) and exported for further analysis.

Illustration: Surface Plasmon Resonance Imaging

A gold-coated glass array surface is used for immobilization of antibodies complimentary to the target protein of interest. A broad beam, monochromatic, polarized light originating from a suitable light source is used to illuminate the entire biochip surface with the help of mirrors placed at suitable angles that will reflect the light onto the surface. Reflected light from each spot on the array surface is captured by means of

a CCD camera and used to generate the SPRi image. Binding of target antigen to the antibody is detected in real-time due to the changes in intensity of reflected light from every spot on the array surface. Multiple biomolecular interactions can be studied simultaneously in a HT manner and changes occurring on the array surface can provide kinetic data about the interactions.

II. DIFFERENT APPLICATIONS OF SPRi

SPRi-based biosensors are attractive choices for detection of low-abundance analytes and biomolecular interactions in HT manner (Ray et al., 2010; Reddy et al., 2012). Over the last decade quite a few studies have used this label-free sensing platform for direct detection of potential maker proteins in human serum and other biological samples (Table 1). In a recent study, Ladd et al. has applied SPRi in combination with antibody array for detection of cancer biomarkers in buffer solution and diluted human serum (10%) samples (Ladd et al., 2009). The authors have employed SPR imaging sensor with polarization contrast and detected activated leukocyte cell adhesion molecule/CD 166 (ALCAM) and transgelin-2 (TAGLN2) biomarkers, down to ng/mL concentration (LOD 6 ng/mL and 3 ng/mL for ALCAM and TAGLN2, respectively) devoid of any significant cross-reactivity. Such studies testify the potential of SPRi-based biosensors for detection of biomarkers from complex biological samples.

However, the sensitivity obtained with actual biological samples are found to be less compared to that obtained with buffer solutions. To increase the sensitivity of SPRi-based biosensors different nanostructured materials, particularly quantum dots and gold nanoparticles are introduced for signal amplification. Application of nanoparticles in SPRi can effectively increase the detection limit and target antigen can be detected at pM level, which is not possible to achieve with conventional SPRi settings.

Table 1. Various applications of SPRi-based biosensors (selected examples)

Application	Studies (Reference)
(a) Study of biomolecular interactions	1. Protein-protein interactions (Natarajan et al., 2008) 2. Interactions of GST-fusion proteins (Yuk et al., 2006) 3. Structural changes with denaturation (Huang et al., 2006) 4. Adsorption and desorption of multiple proteins, protein–polymer interactions (Hook et al., 2009) 5. Antigen–antibody reactions (Xinglong et al., 2005)
(b) Detection of disease biomarkers and drug discovery	1. Cancer; β_2m and cysC (Lee et al., 2006); activated leukocyte cell adhesion molecule/CD 166 (ALCAM) and transgelin-2 (Ladd et al., 2009); PSA (Malic et al., 2011) 2. Auto-antibodies in sera of autoimmune patients (Lokate et al., 2007) 3. Drug discovery applications (Dong et al., 2008) 4. Screening of cancer inhibitors (Basappa et al., 2011)

III. ADVANTAGES AND DISADVANTAGES OF SPRi

Advantages:

The major advantage of SPRi over standard SPR is its multiplexing capabilities (ability to observe hundreds of reactions simultaneously). SPRi can be applied to perform interaction studies in an HT manner, which is not possible by conventional SPR. Additionally; other advantages of SPR-based biosensors are also applicable for SPRi.

- Label-free detection eliminates the requirement of any secondary reactants and long labeling process.
- Real-time measurements of biomolecular interaction and binding kinetics.
- Highly potential for multiplex analysis; so very well suited for high-throughput analysis.
- Both quantitative and qualitative measurements are possible.

Disadvantages:

Due to its HT capabilities, SPRi is promising for clinical research, but there are many limitations as well; such as

- Requires sophisticated instrumentation.
- Restricted to choice of metal (gold/silver surfaces).
- Changes in temperature affects refractive index and thereby efficiency of the measurement.
- Non-specific interactions affect the signal.
- Heterogeneous sample surface affects sensitivity.
- Not very effective in handling complex biological samples

IV. CONCLUSIONS

SPRi-based biosensors have shown their potential to measure kinetic reactions of biomolecular interactions in HT manner. These techniques have been found to be very efficient for real-time measurement of disease-related proteins in buffer *in vitro*. Although, some of the recent studies have testified the applicability of SPRi-based biosensors for direct detection of marker proteins in different biological fluids, including serum/plasma, saliva and urine. Issues regarding sensitivity and specificity remain to be explored further when complex biological samples are concerned. Due to the requirement of sophisticated instrumentation and restriction to gold/silver surfaces, the detection cost of SPRi-based sensors is very high, and not affordable for routine clinical diagnostics. If these basic limitations are circumvented successfully; SPRi could be one of the very attractive choice for HT proteomic research.

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