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Multispectral Imaging of a Biochip Based on Surface Plasmon Resonance

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1. Introduction

The Surface Plasmon Resonance (SPR) is a free label and real time biosensor. It is widely used to detect and characterize biomolecular interaction such as DNA/DNA [1], Protein/Protein [2] interactions. The SPR biosensors are capable of detecting small changes in the refractive index or thickness of thin layers induced by variation of concentration or interaction between biomolecules in the sensible surface. The SPR biosensor also called biochip is recently used to study multiple interactions in the surface. We can record the evolution of the signal in each interaction site on the surface using numerical imaging. In this present communication, we will present the Surface Plasmon Resonance Imaging (SPRI) as a powerful tool to detect biomolecular interaction between a biomolecule functionalized on a metallic film as a probe- and a biomolecule injected in the aqueous solution as a target.

The Kretschmann configuration [1] (Fig.1) is the most known method to excite SPR. It's based on the Attenuation of Total Reflection (ATR) of a TM polarized light on a metallic (gold or silver) thin film evaporated on a prism. The plasmon is excited by changing incidence angle at a fixed wavelength, or using multispectral light for a fixed incidence angle.

A great deal of information about nano-functionalization and macro-structure [3] of the sensible surface is acquired to compare and quantify with accuracy the response of the biochip. Precision and reliability of the measurements on the biochip are provided by a multidimensional approach (spatial, temporal, angle of incidence,

wave-length, and polarisability).

The goal of this work is to study the multispectral dimension to improve the response of the biosensors by the variation of refractive index for some wavelength. This variation is induced by integration of chromophore attached to target molecule. The chromophores such as Texas Red and Cy5.5 absorb, respectively, at 595 nm and 675 nm and this absorption induces changes in the refractive index in the plasmon spectrum.

Because, the chromophore exists only in the interaction site, we will have local variation of reflection to allow us to increase the image contrast that clearly separates and quantifies the useful signal.

2. Results

The simulation of spectral refractive index variation of the layer containing the chromophore cy5.5 which absorbs at 675 nm is presented in Fig.1.

To understand and work in the optimum multispectral conditions, and to excite plasmon and have pertinent information about the biological process, we first made some simulation couplings: the reflectivity to wavelengths and incidence angle (Fig.3); and the reflectivity to wavelength and refractive index (Fig.4).

The experiment is conducted first, on the target without label and second on the same target attached to Texas Red, and finally on the same target attached to Cy5.5. The results are presented in Fig.5.

The imaging treatment of the various chromophores' images will be presented and discussed later in a detailed paper.

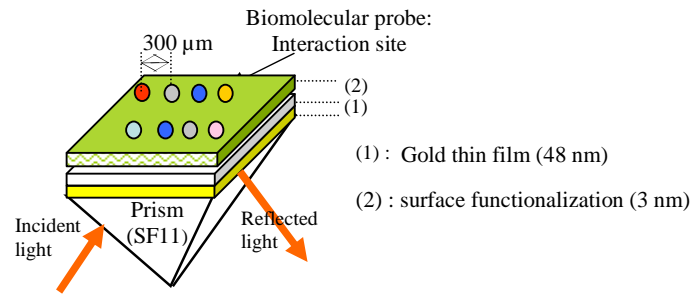


FIG.1: Kretschmann configuration of SPR transducer.

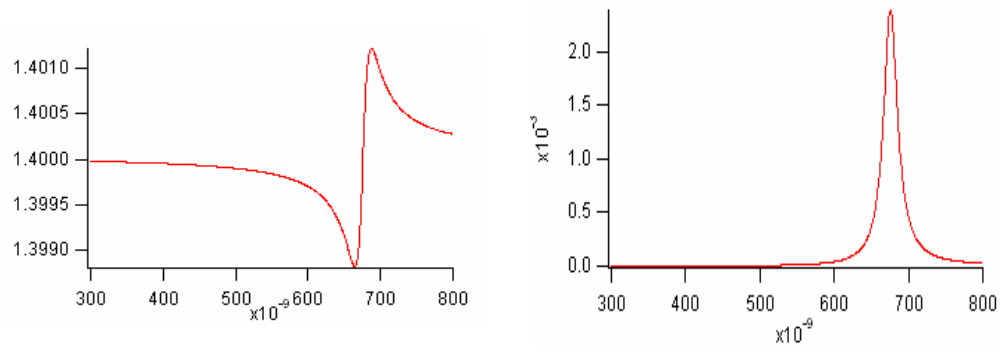


FIG.2: Simulation of refractive index variation of the chromophore Cy5.5.
(A): real part, (B): imaginary part using the Lorentz equation [4].

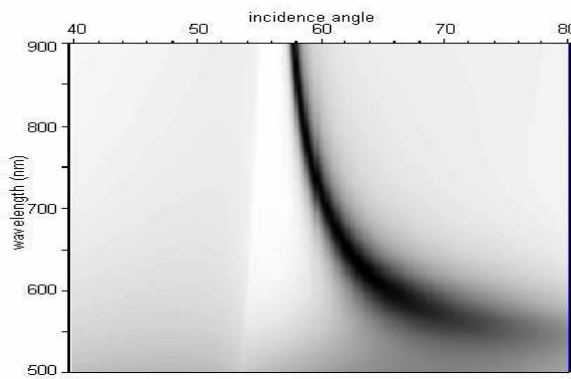


FIG.3: Variation of reflectivity of TM polarized light with wavelength and incidence angle.

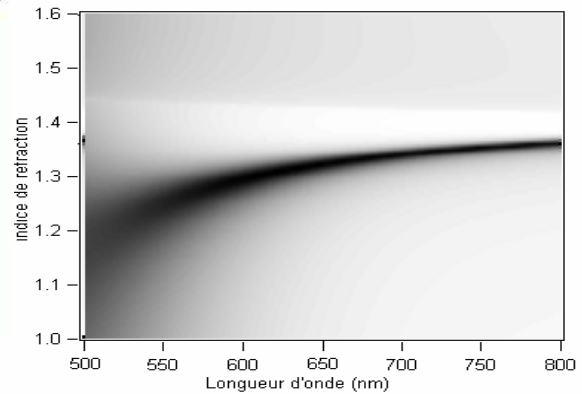


FIG.4: Variation of reflectivity of TM polarized light with wavelength refractive index.

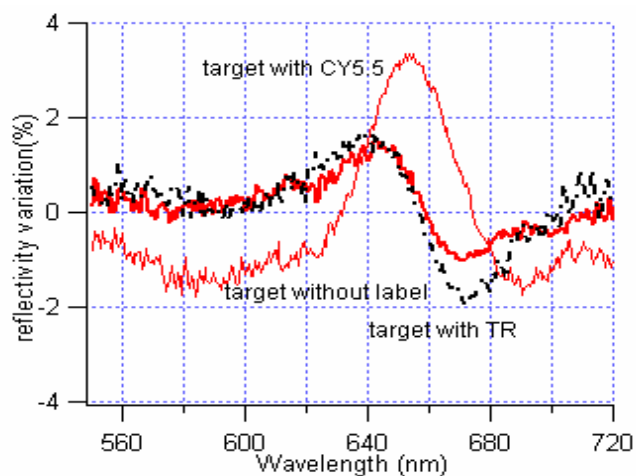


FIG.5: Variation of multispectral reflectivity on a biochip after for each chromophore (TR and CY5.5).

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