Supplemental Material to the article:

Myoglobin Immunoassay Utilizing Directional Surface Plasmon-Coupled Emission

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ABSTRACT

We described an immunoassay for the cardiac marker myoglobin on a thin silver mirror surface using surface plasmon-coupled emission (SPCE). SPCE occurs for fluorophores in close proximity (within about 200 nm) of a thin metal film (in our case, silver) and results into a highly directional radiation through a glass substrate at a well-defined angle from the normal axis. We used the effect of SPCE to develop a myoglobin

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immunoassay on the silver mirror surface deposited on a glass substrate. Binding of the labeled anti-myoglobin antibodies led to the enhanced fluorescence emission at a specific angle of 72^{o}. The directional and enhanced directional fluorescence emission enables detection of myoglobin over a wide range of concentrations from sub-normal to the elevated level of this cardiac marker. Utilizing SPCE allowed us also to demonstrate significant background suppression (from serum or whole blood) in myoglobin immunoassay. We expect SPCE to become a powerful technique for performing immunoassays for many biomarkers in surface-bound assays.

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THEORETICAL BASIS

The physical conditions for emission coupling to surface plasmon polaritons are complementary to better known surface plasmon resonance (SPR) and appearance of attenuated reflectivities. In our simple case we have a glass prism coated with a thin layer of metal film, sample layer and emerging medium (air) as shown in Figure 1B. The metallic thin-film is characterized by a complex refractive index, N_m, which include the refractive index n_m and the extinction coefficient k, ($N_m = n_m - ik$, where $i = \sqrt{-1}$). The reflectance of the multilayer system for monochromatic, linearly polarized light can be calculated from the following relationship involving the optical admittance [49-52]:

$$R = \left(\frac{y_Q - Y}{y_Q + Y}\right)\left(\frac{y_Q - Y}{y_Q + Y}\right)^* = \left(\frac{y_Q - Y}{y_Q + Y}\right)^2 \qquad \text{(S-1)}$$

where y_Q is the admittance of the incident medium (glass), * denotes complex conjugate and Y is given by:

$$Y = \frac{C}{B} \qquad \text{(S-2)}$$

where B and C are normalized electric and magnetic field, respectively, given by:

$$\begin{bmatrix} B \\ C \end{bmatrix} = \begin{bmatrix} \cos\delta_m & i(\sin\delta_m)/y_m \\ iy_m \sin\delta_m & \cos\delta_m \end{bmatrix} \begin{bmatrix} \cos\delta_s & i(\sin\delta_s)/y_s \\ iy_s \sin\delta_s & \cos\delta_s \end{bmatrix} \begin{bmatrix} 1 \\ y_0 \end{bmatrix} \qquad \text{(S-3)}$$

where

$$\delta_m = \frac{2\pi N_m d_m \cos\theta_m}{\lambda} = \frac{2\pi (n_m - ik_m) d_m \cos\theta_m}{\lambda}$$
$$\delta_s = \frac{2\pi N_s d_s \cos\theta_s}{\lambda} = \frac{2\pi n_s d_s \cos\theta_s}{\lambda} \qquad \text{(S-4)}$$

corresponds to the phase thickness of metallic layer (m) and sample layer (s) at the appropriate angle of incidence (θ) given by Snell's law for each interface and the wavelength (λ). The respective substrate admittances for p and s polarizations are given by:

$$\begin{aligned} y_Q &= n_Q / \cos\theta_Q \quad for\ p-polarisation \\ y_m &= (n_m - ik_m) / \cos\theta_m \\ y_s &= n_s / \cos\theta_s \\ y_0 &= n_0 / \cos\theta_0 \end{aligned} \qquad \text{(S-5)}$$

$$\begin{aligned} y_Q &= n_Q \cos\theta_Q \quad for\ s-polarisation \\ y_m &= (n_m - ik_m) \cos\theta_m \\ y_s &= n_s \cos\theta_s \\ y_0 &= n_0 \cos\theta_0 \end{aligned} \qquad \text{(S-6)}$$

The indexes Q, m, s and 0 relate to quartz, metal film layer, sample layer and emergent medium (air), respectively.

Relationships (1-6) describe the reflectance for a plane wave incident on layers of thin films as a function of the incident angle. The s-polarized modes (6) appear when the dielectric (sample) layer thickness is higher than 100 nm [49, 52, 53]. In our assay we will observed only p-polarized mode because the maximal thickness of the sample was only about 20 nm.

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