Ultra-sensitive immunoassay using VCSEL detection system

C.F.R. Mateus, M.C.Y. Huang, C.J. Chang-Hasnain, J.E. Foley, R. Beatty, P. Li and B.T. Cunningham

Highly sensitive protein characterisation on a new label-free biosensor system is reported. The system consists of a VCSEL, a plastic guided mode resonant filter and two *pin* detectors. It is suitable for measuring both static and dynamic interaction among proteins and can detect an antigen concentration as low as 1 pg/ml (6.7 femtoMolar).

Introduction: Protein-protein interaction is one of the most important subjects in molecular biology [1]. Bioassays have been developed to detect and quantify the biomolecular interactions with high sensitivity. There are two general categories of techniques: labelling with compounds and direct molecule identification. Label-free biosensor technologies are among the latter, which are desirable because of their non-intrusive nature and greatly simplified assay preparation.

Recently, we proposed a compact label-free biosensor system utilising a vertical cavity surface emitting laser (VCSEL) as the new detection system [2]. The biosensor (molecular binding surface) used here is a guided mode resonant (GMR) filter, which is made from a plastic sheet bonded to a bottomless 96-wells microplate [3]. The readout system consisted of one singlemode VCSEL and two *pin* detectors, which recognises and quantifies the binding event of the biomolecules. The biosensor configuration is shown in Fig. 1. The GMR has a narrow reflection wavelength (λ_{peak}), which varies greatly with the optical thickness (i.e. multiple of refractive index and physical thickness) of the biomolecules attached on top of its surface. The VCSEL and the two detectors are used to track this wavelength shift as materials are being deposited onto the GMR surface. In our previous publication [2], we showed that the VCSEL readout system could detect a minute variation of refractive index by immersing the GMR top surface with fluids having precise refractive index. In this Letter, we report the use of this system in immunoassay with a record high sensitivity to antigen concentration.

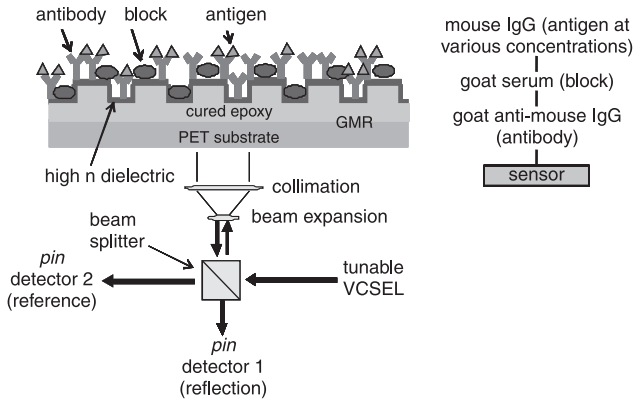

Fig. 1 *GMR biosensor with VCSEL based measurement system*

Tunable VCSEL and two *pin* detectors work as readout system for plastic GMR filter that is binding surface. Peak reflectivity from GMR is detected by correlating maximum normalised detector current with laser bias current

To map out the sharp reflectance peak of the GMR, the wavelength of the VCSEL is tuned by varying its bias current, which provides a fast, narrow range (2–3 nm) of wavelength. As the VCSEL wavelength is varied, the ratio of the two detector signals is used to monitor the reflection peak. Here, detector 1 is positioned to measure the reflection from the GMR substrate, while detector 2 provides the incident power normalisation. Temperature tuning is also used to augment the tuning range, extending it to 8–9 nm. Typically, the wavelength dependence of temperature and current tuning for a VCSEL is 0.08 nm/°C and 0.4 nm/mA, respectively. The dynamic range can be largely extended (~32 nm) by using a MEMS tunable VCSEL [4].

Mouse IgG capture immunoassay: In this Letter, we study the application of the VCSEL based label-free biosensor measurement system for immunoassay. The characterisation of protein interactions consists of the detection of the binding between an antibody (goat anti-mouse immunoglobulin (IgG)) and an antigen (mouse IgG), as shown in Fig. 1. Two different experiments were performed, to study the performance of the biosensor system. The dynamic experiment enables us to quantify the antibody-antigen binding as a function of time. Meanwhile, the static experiment enables us to quantify the resonant wavelength shift as a function of mouse IgG concentration.

The experimental protocol we used followed a standard mouse IgG capture immunoassay. First, we deposited the antibody protein by applying 100 µl/well of goat anti-mouse IgG (1.8 µg/ml), which was diluted in phosphate-buffered saline (PBS) solution, and incubated it for 1.5 h at room temperature. The incubation period allowed sufficient time for the antibody protein to bind to the GMR sensor surface. Then the excess solution was removed and the wells were washed with PBS-Tween. Next, we applied normal goat serum (200 µl/well of 3% goat serum in PBS) to block the vacant spots on the sensor surface not covered by the antibody and incubated the block solution for 2 h at room temperature. This blocking step is required to minimise false-positive errors from the measurements, since the subsequent antigen protein might bind to the sensor surface as well as to the antibody protein. Following the removal and wash procedures, we measured the spectral response of the GMR sensor, where this peak resonant wavelength became the reference of comparison for the subsequent antigen measurements. Lastly, we deposited the antigen protein by applying 100 µl/well of mouse IgG at various concentrations and incubated it at room temperature. Control wells were prepared simultaneously with identical steps except for the initial antibody protein deposition. Measurements were taken during and after the incubation period for the dynamic and static experiments, respectively.

In the dynamic measurement, the kinetics of the antibody-antigen binding for three different mouse IgG concentrations, each repeated for three wells, was continuously monitored during the incubation period with the time resolution of 5 s. Fig. 2 shows the normalised surface binding curves as a function of time, obtained by integrating the difference of the measured GMR spectral response for the starting time and that of a given time. It should be noted that the saturation value is antigen concentration dependent, and the normalisation performed here enables the comparison of surface binding time for different antigen concentrations. If required, this technique allows for ultrafast, dynamic monitoring in the nanosecond range, as the VCSEL can be modulated in GHz. As expected from results of typical kinetic binding experiments, most of the protein binding occurred rapidly at the beginning of the incubation period, followed by a gradual saturation. The 80% surface binding time for the goat anti-mouse IgG and mouse IgG proteins is about 300 s as indicated in Fig. 2, with a slight dependence on the mouse IgG concentration.

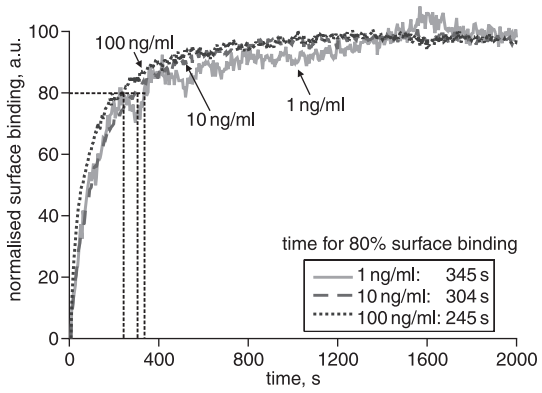

Fig. 2 *Dynamic measurement: surface binding against time for different antigen concentrations*

Most of protein binding occurred rapidly at beginning of reaction, followed by gradual saturation. The 80% surface binding time is ~300 s, with a slight dependence on mouse IgG concentration

In the static measurement, the resonance wavelength shift that resulted from the antibody-antigen binding was monitored for eight test wells with identical solutions and one control well (without the antibody protein) in the microplate. After the removal of the block solution, we started by applying mouse IgG solution with the smallest antigen concentration of 1 pg/ml, incubated it for 1.5 h at room

temperature, and measured the spectral response of the GMR biosensor. The wavelength shift of the control well was subtracted from those of the eight test wells. Then we removed the excess solution and added the next mouse IgG solution with the concentration of 10 pg/ml. This incubation-measurement-removal sequence was repeated for different mouse IgG solutions with their concentrations increased by 10X each time, up to 10 μg/ml. Fig. 3 shows the average of the resonant wavelength shifts for the eight wells, as a function of the mouse IgG concentration. The saturation observed was caused by the depletion of the available antibody-antigen binding sites, and it is expected that the saturation concentration can be increased by applying higher concentration of the antibody proteins initially. The high sensitivity of the biosensor system is shown from its ability to detect the smallest concentration of 1 pg/ml, which is equivalent to 6.7 fM (femtomole/litre) as molecules used have molecular weight of 150 kDalton. This result is more than one order of magnitude better than recent reports of sensors using surface plasmon resonance [5–7].

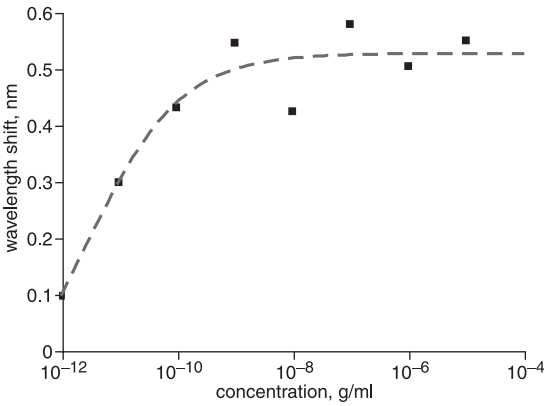

Fig. 3 *Static measurement: average resonant wavelength shift against antigen concentration*

The high sensitivity of VCSEL-based measurement system is shown through ability to detect smallest concentration of 1 pg/ml or 6.7 fM.

Conclusion: We have presented an immunoassay technique utilising a new ultra-sensitive, compact, fast label-free optical biosensor system that can be potentially low-cost. Specific biomolecules can be studied using this technique and their sensitivity determined based on the above results. We have demonstrated the suitability of this biosensor system for both static and dynamic measurements of molecular interactions and its capability of detecting protein concentration as low as 1 pg/ml (6.7 fM), which is about one order of magnitude better than other techniques reported in the literature.

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