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Biochip Group
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LAB-ON-A-CHIP BIOMOLECULAR INTERACTION MEASUREMENT SYSTEM WITH INTEGRATED MICROFLUIDICS AND SURFACE PLASMON RESONANCE IMAGING

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Abstract

We present a novel lab-on-a-chip approach for measuring biomolecular interactions of the well known model system (IgG - antiIgG interaction) using surface plasmon resonance (SPR) imaging. Surface plasmon resonance is a well established label free detection technique to measure real-time biomolecular interactions.

Introduction

The goal of this work is to develop a multi-analyte immunoassay based on SPR imaging. Microfluidics and fluidic transport are coupled to the SPR imaging platform. We demonstrate this concept with results measured from IgG and anti-IgG interactions.

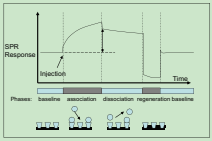


Figure 1: Typical sensorgram showing various steps involved in biomolecular interaction experiment

Baseline phase: Initially, running buffer is in contact with the sensor surface to establish the baseline.
Association phase: Sample containing the target component is injected, the capturing element on the sensor surface binds to the target component resulting in complex formation.
Dissociation phase: Upon injection of running buffer, target component as well as non specifically bound molecules dissociate from the surface.
Regeneration phase: Upon injection of low pH buffers, the remaining bound target components are removed.

Device design and working principle

Multi-ligands are spotted [1] and the biomolecular interaction is simultaneously monitored. We combine microfluidics with electrokinetic fluid transport [2] thus an automated lab on a chip approach for a biomolecular interaction measurements. See figure 2.
The bottom layer of the device contains the microarray (patterned gold islands) with entities for specific biomolecular interactions which will be detected in real time with imaging surface plasmon resonance (iSPR). (IBIS Technologies, Hengelo, Netherlands).

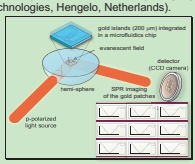


Figure 3. Principle of imaging SPR.

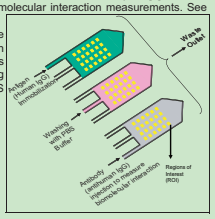


Figure 2. Three different steps involved in this biomolecular interaction experiment are shown in 3 different colours.

The gold islands of the microarray are exposed to polarized light at a certain controlled incident angle. The refractive index change is proportional to the amount of macromolecules (proteins) adsorbed at the surface. The biomolecular interaction between the immobilized antigen and the antibody in the serum results in a measurable surface plasmon resonance condition.

Conclusions

- The biomolecular interaction was successfully measured using the integrated microfabricated device on surface plasmon resonance imaging system.
- Future developments include immobilizing proteins on the iSPR surface with microfluidic based spotting as well as with commercially available spotters in order to acheive multi-analyte detection.
- It is our interest to transform this traditional kinetics appraoch to our new chip together with that of electrokinetics.

Microfabrication

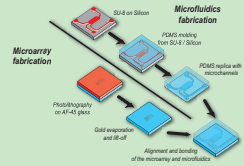
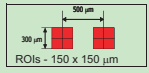


Figure 4. The chips were fabricated in the MESA+ cleanroom facilities and in the Biochip Group lab. The channel structures and the reservoir holes were patterned in PDMS while the SPR gold was patterned on the bottom plate, which is made of a glass with the same refractive index as the iSPR hemisphere (n= 1.52).

Chip dimensions:
Chip size - 15 x 15 mm
flow chamber - 5 x 4 mm
6 x 4 microarray - 3 x 2 mm

Gold island dimensions:



Results and discussion

The chip was placed in a fixture, containing four reservoirs with integrated Pt electrodes connected to a high voltage source. The applied voltages were controlled with a LabView program. The first inlet reservoir was filled with 1 mg/ml Human IgG, second inlet reservoir with PBS buffer (pH - 7.2) and the third inlet reservoir with 10 µg/ml anti-human IgG. The fourth reservoir was used as the waste container. The real time biomolecular interaction was measured using iSPR.

Figure 5. SPR Image of the gold microarray on the chip with Regions of Interest (ROI) for the measurement.

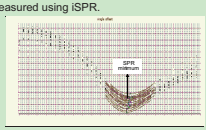


Figure 6. SPR dip obtained after the immobilization.

The "minimum" calculated from the SPR curve represents the quality of the surface. The shape of the SPR curve (Dip) shows the homogeneity of the protein immobilization on the gold surface.

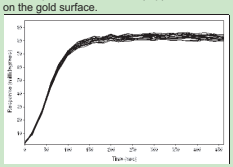


Figure 7. Human IgG - antihuman IgG interaction monitored by iSPR system on patterned gold islands with our new chip.

Amount of protein immobilized:
From calibration - 120 millidegrees response = 1 ng of protein immobilized on the square millimeter area.
The amount of protein immobilized in each ROI is 15 pg.

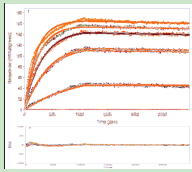


Figure 8. kinetics evaluation of Human IgG - antihuman IgG (various concentrations) interaction with conventional iSPR system

The kinetics are measured [3] for the model system using a normal SPR sensor chip. The results are described here. It is our interest to transform the kinetics approach to our new chip together with that of electrokinetics.

Human IgG was immobilized on the gold surface and 5 different antihuman IgG concentrations were injected to the chip.The interaction sensorgram was measured and the data was fitted to a simple 1:1 interaction model. The residual plot (lower) demonstrates the quality of data fitting. The results are: Association rate, k_a - 6.547xe^4 M^{-1}s^{-1}; Dissociation rate, k_d - 1.825e^{-5} s^{-1}; Affinity Constant, K_D - 278 pM.

References
[1] R.B.M. Schasfoort Proteomics-on-a-chip: the challenge to couple lab-on-a-chip unit operations. Expert Rev. Proteomics **1**(1), 123–132 (2004).
[2] G.A.J. Besselink et. al., "Electroosmotic guiding of sample flows in a laminar flow chamber", Electrophoresis, **25** 2004
[3] D.G. Myszka et al., "Survey of the year 2000 commercial optical biosensor literature", J. Mol. Recognit., 14 (2001) 273 - 284

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Research Interest: Lab on a Chip, Biomolecular Interactions, Kinetics Evaluation