IN-SITU SUB-MICROMETER SURFACE FLOW VELOCITY MEASUREMENT

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ABSTRACT

We have measured the flow velocity at a sub-micrometer distance from a flow channel wall by tracing the refractive index boundary of two consecutively flowed liquids. Using the surface plasmon resonance method to measure the surface proximity refractive index, we were able to measure the velocity without using additional tracer materials. This velocimetry can be performed while simultaneously measuring the reaction rate of surface immobilized biologically functioning molecules. Combining these two measurements enabled us to improve the fidelity of adsorption measurements of real samples undertaken using a capillary pumping biosensor by determining the flow velocity for each sample injection.

KEYWORDS: Flow velocity, surface plasmon resonance, capillary pump

INTRODUCTION

The flow velocity at a sub-micrometer distance from an immobilized antibody affects the observed antigen adsorption rate, because the parameters of mass transport, diffusion, and adsorption kinetics are all involved in the reaction (Fig. 1). However, the flow velocity at such a surface proximity depends on the surface roughness and the interaction between the sample liquid and the flow channel wall material even in a laminar flow condition [1]. Moreover, when microfluidic devices are used for sensor applications, the density and viscosity vary sample by sample and so the flow velocity must be determined or controlled. We developed disposable immunosensor devices based on capillary pumping for performing real-world sample measurements [2], but the flow velocity stability of this device was insufficient because we could only control the capillary force (pressure), and the viscosity range of real samples is very wide. Therefore, we needed to measure the surface flow velocity and the biological reaction simultaneously to obtain a reproducible sensor response.

On the other hand, the flow velocities in microfluidic devices have been determined mainly by trajectory analysis using a tracer particle or a marker (fluorescent or dye) molecule and the excitation mechanism of these markers [3,4]. Other methods have been reported including an electrochemical approach [5], and a

Figure 1: Surface flow velocity measurement and biological adsorption reaction. The full height internal flow velocity distribution of a micro fluidic sensor device is shown in the upper illustration. A magnified view near the channel wall is shown in lower illustration. When two liquids flowed from "up stream" to "down stream" the refractive index boundary (n1 and n2) of two consecutively flowed liquids (running buffer and sample) can be detected by SPR. The graph shows the calculated dependence of this refractive index boundary sensitivity on the wall-to-boundary distance. The heterogeneous reaction of immobilized antibody and antigen was influenced by the flow velocity less than 1000 nm from the channel wall. This local velocity can be measured by SPR.

technique using the laser Doppler effect [6]. These methods provide the precise flow velocity distribution of the internal volume of a flow channel. However, the flow velocity at less than a few micrometers from the flow channel wall was not readily obtained. This was because the wall position must be determined by these methods themselves, and/or intrinsically these methods require special instruments and materials for performing velocity measurements.

Based on the above requirement and limitation, we present a method for determining the flow velocity at nanometer distances from a flow channel wall. We used a surface plasmon resonance (SPR) method to selectively detect the flow conditions [7], and to determine antigen adsorption to the immobilized antibody on the flow channel wall. This flow velocity measurement method requires no markers and yields the in situ flow velocity at the location of the surface adsorption or other surface reactions are occurring. In this paper, we discuss the principle of this method and an antigen detection application that can be employed at the same time as a flow velocity measurement using a real sample.
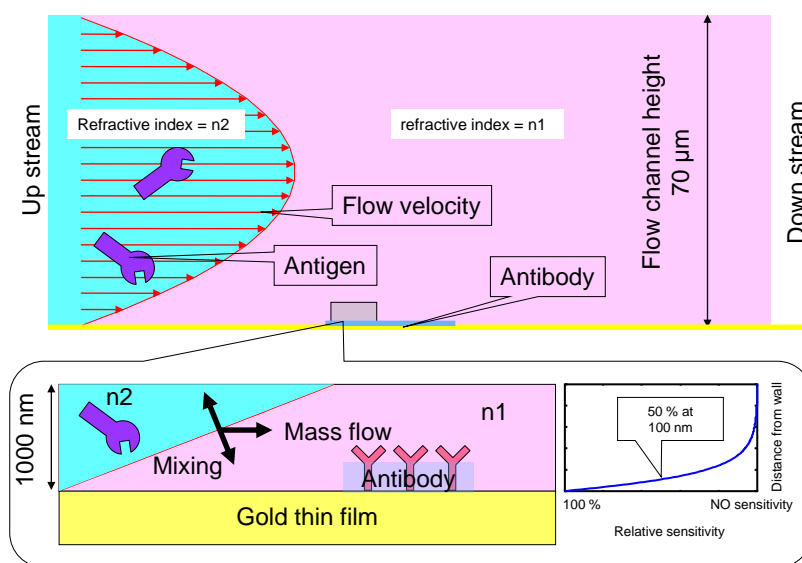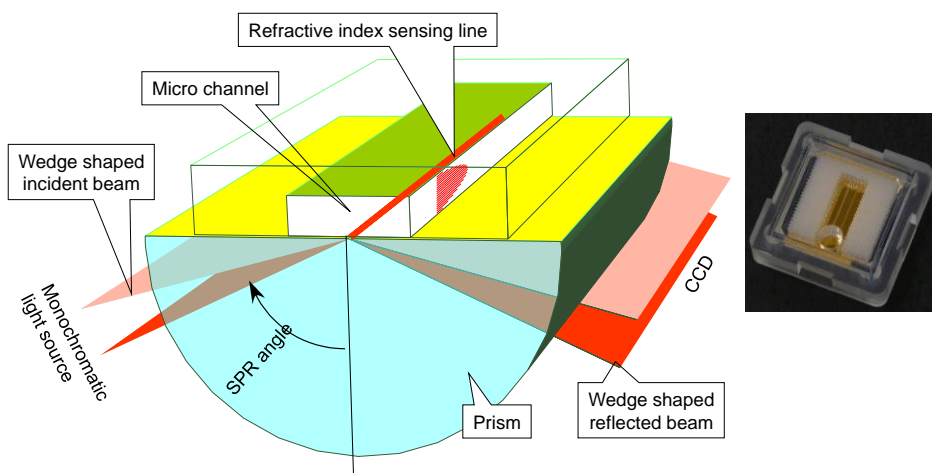
Figure 2: SPR optical system for time-space refractive index (RI) measurement. The RI of the bottom center of flow channel is measured. The picture shows a capillary force based micro fluidic device for a one-time use SPR chip.

EXPERIMENTAL

We used an SPR instrument SMART SPR (NTT Advanced Technology, Tokyo Japan). The optical setup of this instrument is shown in Fig. 2.

A wedge shaped line beam with a width of about 5 mm illuminates the thin gold film of the flow cell through the prism with an incident angle range of total internal reflection. The reflected light was detected with a 2D-CCD. One axis of the 2D-CCD corresponds to the stream-wise position and the other axis corresponds to the incident angle. The SPR angle that showed the minimum reflectance for each stream-wise position was calculated from a single image obtained with the CCD with a fixed time interval of 1 s. Therefore we could obtain the refractive index distribution along the stream-wise direction with a time resolution of 1 s. This resulted in an image as shown in Fig. 3. The flow cell we used is also shown in Fig. 2. This cell consisted of a base plate whose refractive index was the same as that of the prism of the SPR instrument, thin gold film formed on the flow channel side of the base plate, an adhesive tape with the flow channel pattern (thick-
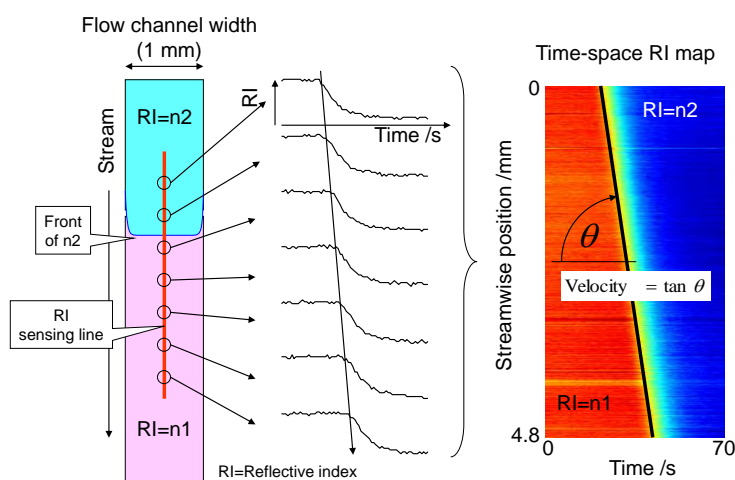

Figure 3: Surface velocity was calculated from the slope in the time-space refractive index (RI) map (right), which was obtained with the system in Fig. 2. The time-space RI map was constructed from the RI of stream-wise positions (left) and its time change.

ness of 70 μm), and a capillary pump lid. The gold film served as a refractive index sensing surface. We injected a blocking buffer solution and then a milk sample containing antigen. Because the refractive indices of the blocking buffer and the milk sample were different, the boundary between the two liquids advanced in the flow channel while they mixed with each other. The time taken to pass through the boundary at each stream-wise position was estimated from the time of the peak in the time differential of the SPR angle. The connected line of the peak time (Fig. 3) represents the advancing refractive index boundary, and so the tangent of this line represents the flow velocity.

RESULTS AND DISCUSSION

The refractive index sensitivity of SPR is limited to the reach of an evanescent wave. For example, when two liquids with different refractive indices form an interface above the sensing surface (wall), the detection sensitivity of the refractive index interface depends on the distance between the interface and the wall as shown in the graph in Fig. 1. The sensitivity drops 50 % at 100 nm from the wall. The cross section flow velocity profile of a micro flow channel is considered to be laminar. Under this condition, the first injected liquid (blocking buffer) was replaced by the second liquid (sample containing antigen) by diffusion, because the characteristic diffusion time of a sample that travels 100 nm is much less than 1 s (instrumental sampling interval). In general, the adsorption curve obtained with an SPR measurement in a thin layer flow channel is asso-
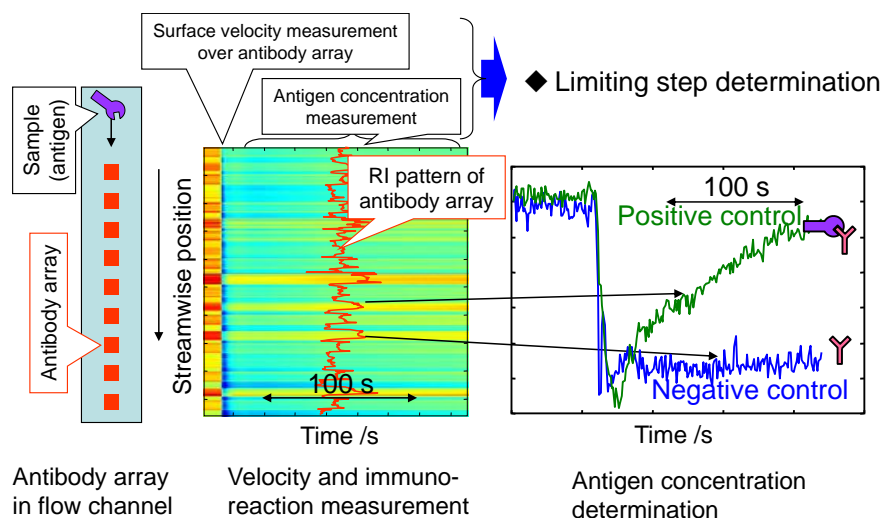
Figure 4 Simultaneous measurement of flow velocity and antigen-antibody reaction. Flow velocity was determined from RI front of injected sample (antigen) solution. The antigen-antibody reaction was measured from RI increase by antigen-antibody complex formation.

ciated with mass transport and diffusion [8, 9], and the reaction rate is determined by mass transport at the low velocity limit, and by adsorption at the high velocity limit. Figure 4 shows a typical example of a flow velocity measurement obtained with this method when the sample liquid was milk. Because the measured flow velocity in this condition was 1e-3 m/s, we could confirm that the reaction was adsorption limiting [9]. After the sample liquid had flowed through, the adsorption reaction rate was determined as shown in the graph in Fig. 4. We could perform these two measurements within 300 s. We were able to conduct this flow velocity measurement for each sample injection, and we could determine the rate limiting step even when the sample viscosity was changed over a wide range.

CONCLUSION

The nanoscale flow velocity of a capillary based pumping micro fluidic device was measured using an SPR system. This velocimetry can be integrated in a common SPR measurement and we successfully measured both adsorption and velocity in a single run. This result will provide a high fidelity measurement for an unstable flow rate but with low cost devices.

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