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Magnetophoretic position detection for multiplexed immunoassay using colored microspheres in a microchannel

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ABSTRACT

This paper demonstrates a new magnetophoretic position detection method for multiplexed immunoassay using colored microspheres as an encoding tool in a microchannel. Colored microspheres conjugated with respective capture molecules are incubated with a mixture of target analytes, followed by reaction with the probe molecules which had been conjugated with superparamagnetic nanoparticles (SMNPs). Under the magnetic field gradient, the resulting microspheres are deflected from their focused streamlines in a microchannel, and respective colored microspheres are detected using color charge-coupled device (CCD) in a specific detection region of the microchannel. The color and position of respective colored microspheres are automatically decoded and analyzed by MATLAB program, and the position was correlated with the concentration of corresponding target analytes. As a proof-of-concept, we attempted to assay simultaneously three types of biotinylated immunoglobuline Gs (IgGs), such as goat, rabbit and mouse IgGs, using colored microspheres (red, yellow and blue, respectively). As the capture molecules, corresponding anti-IgGs were employed and target analytes were probed using streptavidin-modified superparamagnetic nanoparticles. As a result, three analytes were simultaneously assayed using colored microspheres with high accuracy, and detection limits of goat IgG, rabbit IgG and mouse IgG were estimated to be 10.9, 30.6 and 12.1 fM, respectively. In addition, with adjustment of the flow rate and detection zone, the dynamic range could be controlled by more than one order of magnitude.

1. Introduction

The demand for multiplexed assay of important target analytes is high, and has recently attracted much attention (Plowman et al., 1999; Eriksson et al., 2000; Ferguson et al., 2000; McBride et al., 2003). Multiplexed assay provides quantitative information on the target analytes of great physiological significance in high throughput way, accelerating disease diagnosis and biomedical studies as well as screening of interesting biomolecules. As an approach to multiplexed assay, a microarray format has been widely used to distinguish different biomolecules in parallel (Christodoulides et al., 2002, 2005; Levit-Binnun et al., 2003; Li and Reichert, 2003; Peluso et al., 2003). However, conventional microarray has a major vulnerable point in that it is hard to provide an optimized reaction condition for individual contents to be analyzed. In addition, this approach requires long assay times and detection equipments for analysis of individual biomolecule (Nicewarner-Peña et al., 2001). To overcome these disadvantages, multiplexed microsphere-based suspension arrays have been recently reported using fluorescent molecules, quantum dots, photonic crystals and radio frequency as encoding tools (Moran et al., 1995; Fenniri et al., 2000; Medintz et al., 2005; Kuang et al., 2005). Due to the high flexibility in target selection, fast binding kinetics, good reproducibility and well-controlled binding conditions, microsphere-based suspension arrays have been of great interest in a multiplexed assay (Trau and Battersby, 2001; Nolan and Sklar, 2002).

Until now, most of the multiplexed microsphere-based assays have relied on fluorescent dyes for encoding of target analyte (Egner et al., 1997; Fulton et al., 1997; Battersby et al., 2000; Kim et al., 2008). Microspheres with fluorescent dyes are sensitive, but they have some limitations for application to multiplexed assay system. Fluorescence dyes tend to be quenched or bleached (Cunin et al., 2002; Stoermer et al., 2005). In addition, they have characteristics such as narrow excitation spectra and broad emission profiles (Kuang et al., 2005). The broad emission bands may make simultaneous evaluation of multiple probes difficult because of

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spectral cross-talk (Hermanson, 1996; Zhou et al., 2001). Recently, quantum dots were employed and embedded into polymer microspheres in order to take advantage of optical properties of quantum dots such as size-tunable light emission, resistance against photobleaching and simultaneous excitation of multiple fluorescence colors. Despite the advantages, however, quantum dots are difficult to be integrated into the microspheres reproducibly, and the surface modification chemistry is still under investigation. In addition, complex equipments such as confocal microscope and flow cytometry should be used for decoding the microspheres (Han et al., 2001; Zhou et al., 2001). As an alternative, new encoding methods, including graphical encoding (Evans et al., 2003; Zhi et al., 2003; Sha et al., 2006; Pregibon et al., 2007), physical encoding (Vaino and Janda, 2000; Dendukuri et al., 2006; Chung et al., 2007) and colored microspheres or nanoparticles (Schultz et al., 2000; Schuetz and Caruso, 2002; Lim et al., 2006; Matsubara et al., 2007; Gao et al., 2008), have been devised.

In this paper, we report a new magnetophoretic position detection method for multiplexed immunoassay using colored microspheres as an encoding tool in a microchannel. Our previous result demonstrated the utility of a magnetophoretic assay system based on the deflection velocity of microspheres associated with superparamagnetic nanoparticles (SMNPs) (Kim and Park, 2005; Hahn et al., 2007). As a proof-of-concept, we attempt to assay simultaneously three types of biotinylated immunoglobuline Gs (IgGs), such as goat, rabbit and mouse IgGs, using colored microspheres (red, yellow and blue, respectively). As the capture molecules, corresponding anti-IgGs are employed and target analytes are probed using streptavidin-modified SMNPs. Colored microspheres conjugated with respective anti-IgGs are incubated with a mixture of target analytes, followed by reaction with the SMNPs-modified probe molecules. The resulting microspheres are subjected to detection and analysis using color charge-coupled device (CCD) in a specific detection region of the microchannel under magnetophoretic assay condition. The position detection and decoding of colored microspheres are carried out by MATLAB program automatically and simultaneously. Consequently, the positions of respective colored microspheres are correlated with the concentration of corresponding target analytes. Details are reported herein.

2. Materials and methods

2.1. Materials

Rabbit anti-goat IgG, goat anti-rabbit IgG, and rabbit anti-mouse IgG were purchased from Sigma–Aldrich (St. Louis, MO). Normal goat IgG-biotin, normal rabbit IgG-biotin and normal mouse IgG-biotin were obtained from Santa Cruz Biotechnology, Inc (Santa Cruz, CA). SMNPs solution was obtained from Miltenyi Biotec (Bergisch Gladbach, Germany). Streptavidin was conjugated with SMNPs which consist of iron oxide and whose size was about 50 nm in diameter, including polymer coating and proteins on their surface. Tween 20 was purchased from Sigma–Aldrich. Three types of carboxylated microspheres, whose colors were red, yellow and blue, were bought from Polysciences, Inc (Warrington, PA). Their sizes were all 6 μm in diameter. Actually, the microspheres had coefficient of variation (CV) less than 10% for particle size.

2.2. Design and fabrication of a magnetophoretic chip

The magnetophoretic chip with a microchannel was fabricated by a conventional poly(dimethylsiloxane) (PDMS) (Sylgard 184;

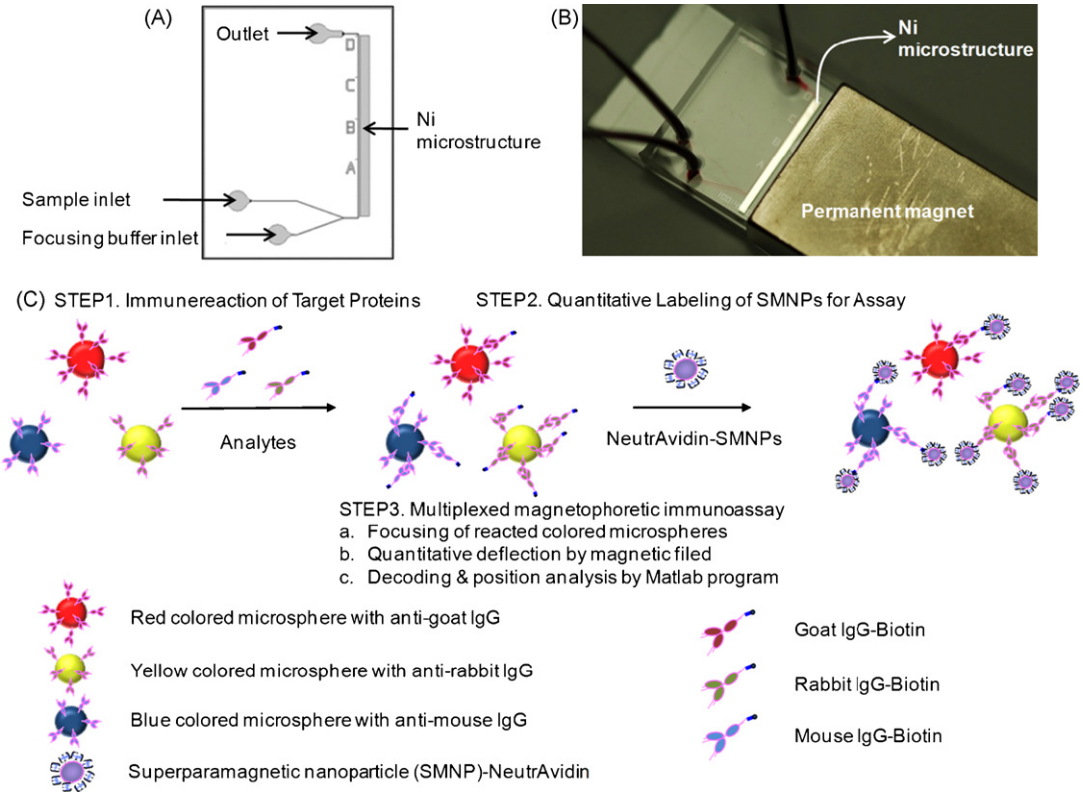

Fig. 1. Schematic of magnetophoretic multiplexed immunoassays using colored microspheres. (A) Design of a magnetophoretic chip which comprises four detection zones, A–D. The first detection zone A was used to obtain a calibration curve by magnetophoretic assay. (B) A photograph of the fabricated magnetophoretic chip. (C) Procedure of magnetophoretic immunoassay using colored microspheres. No washing steps were required in this assay. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Dow Corning, Midland, MI) molding process, and a Ni microstructure was electroplated on a Pyrex glass wafer. Detailed process was followed according to the previously reported method (Hahn et al., 2007). The negative photoresist SU-8 2010 (Microlithography Chemical Co., MA) was used for a PDMS mold. The height and width of the microchannel to be analyzed were 8.5 and 100 μm, respectively. The magnetophoretic chip has four detection zones whose pitch was about 4 mm between neighboring detection zones. The standard curve was made out through the first detection zone A in Fig. 1(A). The microfluidic device and Ni microstructure, whose height and width were 40 μm and 1 mm, were bonded as the previously reported process (Kang and Park, 2007). We introduced a Ni microstructure as the ferromagnetic material into the microfluidic device based on the fact that the ferromagnetic material concentrates the magnetic flux density under the external magnetic field (Hahn et al., 2007). The distance between the microchannel and Ni microstructure was adjusted to 100 μm. The design and photograph of the fabricated magnetophoretic chip are shown in Fig. 1(A) and (B).

2.3. Preparation of colored microspheres for immunoassay

To conjugate the anti-IgGs to the colored microspheres, a general conjugation chemistry involving 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) plus sulfo-N-hydroxysuccinimide (sulfo-NHS) was employed. First, 500 μL of red, yellow and blue colored microspheres (1.05×10^8 microspheres/each solution) were transferred to the respective microcentrifuge tube, separately. Colored microspheres were washed by centrifugation with deionized water at $10{,}000 \times g$ for 5 min three times and finally resuspended in 159 μL of 100 mM sodium phosphate buffer (pH 6.2) followed by sonication for 20 s. Then colored microspheres were activated by adding 65 μL of 50 mg/mL sulfo-NHS and 19.5 μL of EDC to a solution containing respective colored microspheres, and incubated for 30 min at room temperature. The activation was terminated by adding 3.6 μL of 14.3 M 2-mercaptoethanol to the reaction mixture to quench free EDC for 10 min. The resulting colored microspheres were collected by centrifugation at $10{,}000 \times g$ for 3 min at 4 °C, and the supernatant was carefully discarded. Washed with 10 mM of sodium phosphate buffer (pH 7.2) three times, the microspheres were sonicated for 20 s after resuspension in 100 μL of 10 mM sodium phosphate buffer (pH 7.2). The activated microspheres were incubated with 4 mg/mL of three different IgGs (red: rabbit anti-goat IgG; yellow: goat anti-rabbit IgG; blue: rabbit anti-mouse IgG) overnight in an automated mixer (Intelli Mixer, Korea) operating in shaking mode at 99 frequency, 20 rpm at room temperature. The conjugated colored microspheres were finally washed five times with phosphate buffered saline (PBS) buffer (pH 7.2) and resuspended in 1.2 mL of the same buffer. The colored microspheres were kept at 4 °C until further use. After conjugation, the reacted colored microspheres were prepared for immunoassay. First of all, the conjugated colored microspheres were counted by a hemacytometer (Marienfeld, Germany), and they were diluted into reaction buffer solution (1× PBS, 0.02% Tween 20, pH 7.4). The concentration of resulting respective solutions for immunoassay was prepared as follows: 1.28×10^3 red microspheres/mL for goat IgG-biotin, 1.2×10^3 yellow microspheres/mL for rabbit IgG-biotin and 1.33×10^3 blue microspheres/mL for mouse IgG-biotin.

2.4. Decoding and position analysis of colored microspheres

A program for microsphere analysis was written in MATLAB (The MathWorks, Inc., MA) and composed of four steps. First, the program selects captured images which contain microspheres, recognizes colored microspheres from background of the selected images, measures the positions of deflected colored microspheres, and finally determines the color of them. As a beginning step, captured images were converted into gray scale consisting of bright regions, dark regions, and background, where the darkest regions were the circumferences of microspheres. The positions of microspheres were measured by pinpointing the darkest pixels of images. It is very important to detect the center of mass of microsphere for exact color determination. Therefore, 17 pixels in long and wide small regions around the darkest pixels were selected. Noise could be ignored because these small regions were filled with microspheres. In these regions, pixels whose brightness was brighter than predefined value were selected. And then the center of mass of selected pixels was calculated.

To determine the color of microspheres, nine pixels around the center of mass of microspheres were used. The colors of nine pixels were decomposed into three RGB (red, green, blue) components. Using three RGB components as three axes, three-dimensional RGB space was constructed in which each RGB component of nine pixels was represented as each point. To find the boundary condition which determines the color of microspheres, the RGB space was divided into three separate spaces by two planes. Each space represented each color of microspheres as red, yellow and blue. Number of pixels of microspheres which were located in each space (red, yellow and blue) was counted and the color of microspheres was determined by whether the most pixels of microspheres were located in a certain space. By trial and error, the separation of RGB space was modified to maximize accuracy. These processes were performed iteratively with every image. Finally, the positions of each microsphere were determined with the distance from the left wall of a microchannel to the center of the mass of microspheres.

2.5. Instrumentation

The microfluidic device was set on an inverted microscope (Nikon TS100; Japan) with a 30 W halogen lamp. A color CCD (DS-2Mv; Nikon, Japan) was used to capture the movement of colored microspheres and a control program (NIS-Elements BR 2.30, SP4) of color CCD was used to obtain the images of colored microspheres in a way of time-lapse. The exposure time of color CCD was 3 ms, and time interval of the program for time-lapse images was 20 ms. For the hydrodynamic focusing of colored microspheres, 100 μL syringe (Hamilton Company, NV) was used to withdraw the fluid from outlet of the microfluidic device by a dual syringe pump (Pump 11 Pico Plus; Harvard Apparatus, Inc., MA). Magnetic field was applied by NdFeB 35 permanent magnet (Magtopia, Korea) with a dimension of 50 mm × 25 mm × 10 mm and Br = 12,000 G. The permanent magnet was placed 2 mm apart from the Ni microstructure.

2.6. Hydrodynamic focusing of colored microspheres in a microchannel

The focusing of colored microspheres is very important in this magnetophoretic position detection system. The reacted colored microspheres should be focused to the left wall of the microchannel from the instant of entry into the main microchannel 100 μm apart from Ni microstructure. For effective focusing without other equipments or structures in a microfluidic device for focusing, a method of withdrawing the fluid from the outlet was adapted. First, the microchannel was filled with reaction buffer solution from the outlet by syringe. After that, the reacted colored microspheres were injected through the upper inlet, and the buffer solution for focusing was injected through the lower inlet (Fig. 1(A)). Finally, syringe pump withdrew the entire solution to the outlet. In this case, two inlets were exposed in the atmosphere so that the pressure drop was only present between atmosphere and syringe. Due to the

constant pressure drop, there was almost no fluctuation in fluid so that the reacted colored microspheres were focused effectively. The result of focusing was 3.46 ± 1.89 μm from the left wall of the microchannel at a flow rate of 3 μL/h. When the flow rate was faster, the results of focusing were below this value.

2.7. Procedure of magnetophoretic immunoassay

The immunoassay used in this study had all-in-one reaction type without washing steps as shown in Fig. 1(C). The numbers of colored microspheres for magnetophoretic immunoassay were adjusted to 38,520 red microspheres for goat IgG-biotin, 35,850 yellow microspheres for rabbit IgG-biotin and 39,840 blue microspheres for mouse IgG-biotin in 70 μL of reaction buffer (1× PBS, 0.02% Tween 20, pH 7.4). After the two-fold serial dilutions of target analytes (goat IgG-biotin, rabbit IgG-biotin and mouse IgG-biotin) in reaction buffer, 10 μL of respective analyte sample solution was added to each reaction mixture containing respective anti-IgG-conjugated colored microspheres in 70 μL of the reaction buffer. The resulting solutions were incubated for 10 min at room temperature. After incubation, 5 μL of the solution containing the SMNPs conjugated with streptavidin was added to each mixture solution. Here, the volume and concentration of the solution of SMNPs were fixed. The mixture solutions were mixed and incubated for another 10 min at room temperature, followed by injection of these sample solutions into the microchannel. The focusing procedure of reacted colored microspheres was carried out as described above. To test the background level, 10 μL of a reaction buffer without analytes was added to a reaction mixture and incubated for 10 min. A flow rate of 3 μL/h was used for the standard curve, and the faster flow rates were applied to prove the adjustment of dynamic range only by the change of flow rate. Additionally, various detection zones were applied to control the dynamic range besides flow rates.

2.8. Multiplexed magnetophoretic immunoassay

To evaluate the reproducibility and multiplexing capability, that is, accuracy of decoding, multiplexed magnetophoretic immunoassays were carried out using different concentrations of three analytes and three types of colored microspheres, simultaneously. Known concentration of respective analyte was tested, and the results of positions for corresponding colored microsphere were compared with the values of standard curve.

3. Results and discussion

3.1. Decoding of colored microspheres

On the basis of the MATLAB program, we determined the color of detected microspheres and measured the positions of deflected microspheres in a microchannel. Using three RGB (red, green, blue) components as three axes, three-dimensional RGB space was constructed in which each RGB component of nine pixels was represented as each point in the space (Fig. 2). The RGB space was formed by reference database, in which they were established by capturing reference-colored microspheres which move at a flow rate of 3 or 5 μL/h in a microchannel. The RGB space was divided into three separate spaces by two planes. Two planes are as follows: (1) $240R - 174.9G - 76.1B + 1 = 0$ and (2) $5.9R - 209G + 171.4B - 1 = 0$. In these formulas, R, G, and B represent three color components in the

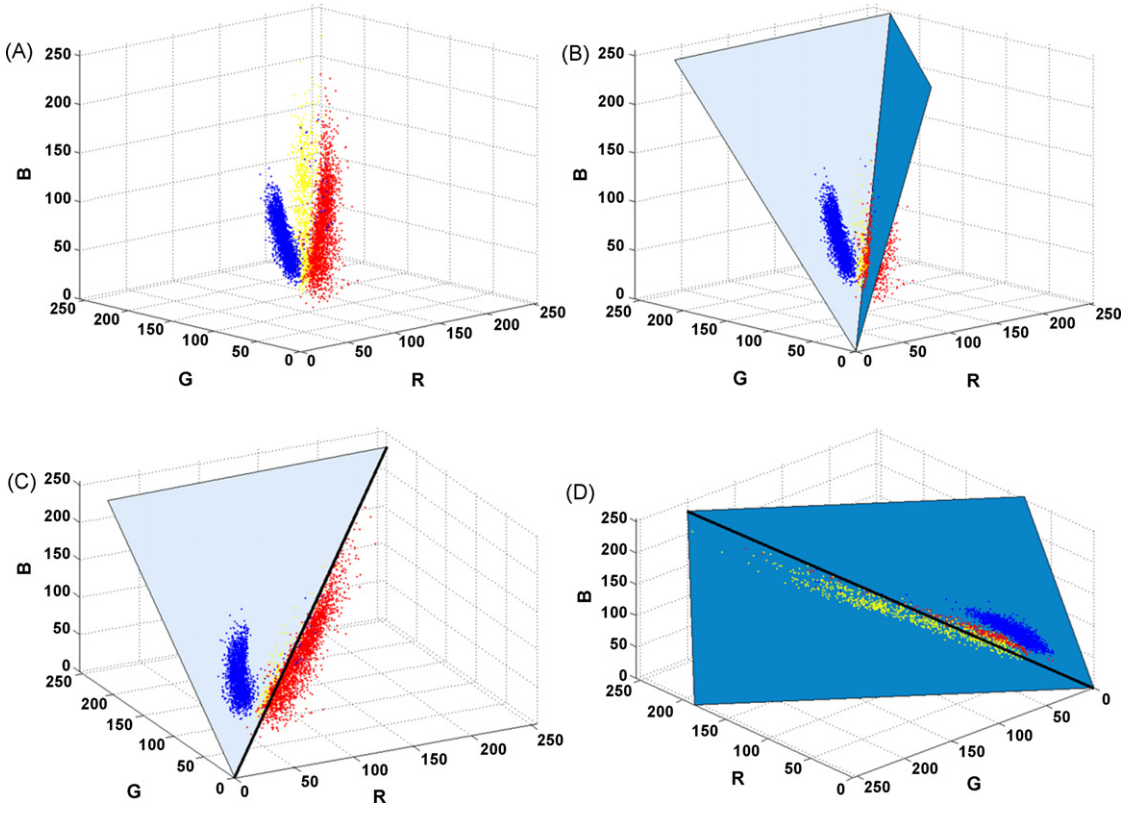

Fig. 2. The RGB (red, green, blue) space for decoding of colored microspheres. All figures were constructed from the MATLAB program. (A) Three colored colonies, including red, yellow and blue components, were formed by reference-colored microspheres. (B) Three colored colonies in the RGB space were divided by two planes: (1) $240R - 174.9G - 76.1B + 1 = 0$ and (2) $5.9R - 209G + 171.4B - 1 = 0$. Right plane (dark blue) and left plane (pale blue) represent plane (1) and (2), respectively. (C) Classification of red and yellow/blue components by plane (1) which is presented as the black line in the graph. (D) Classification of yellow and blue components by plane (2) which is shown by the black line. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

RGB space. By plane (1), the RGB space is divided into two spaces – red space and yellow/blue space. By plane (2), yellow/blue space is divided into two spaces – yellow space and blue space. In this study, nine pixels around the center of mass of microspheres were measured and the location of each pixel in a certain space was evaluated. As a result, the color of the microsphere was determined by the number of pixels evaluated. If most pixels among nine pixels of a colored microsphere are located below plane (1), the color of this microsphere is determined to be red. If most pixels measured are located above plane (1) and below plane (2), the color of this microsphere turns out to be yellow. Finally, if most pixels measured are located above plane (1) and (2), then the color of this microsphere becomes blue. If the number of pixels measured is 3:3:3 in a way of below plane (1):above plane (1) and below plane (2):above plane (1) and (2), the microsphere is excluded from the data. With these criteria, the colored microspheres whose colors were known were confirmed. Total of 1287 colored microspheres, including 728 red, 181 yellow and 378 blue microspheres, were evaluated. Among 728 red microspheres, 674 red ones, namely, 92.6% were correct. In cases of yellow and blue microspheres, only 6 yellow ones and 4 blue ones were estimated incorrectly. Consequently, this program showed 96.7% and 98.9% of accuracy for yellow ones and blue ones, respectively.

3.2. Magnetophoretic position detection

With the fabricated magnetophoretic chip, magnetophoretic immunoassays of respective target analyte were conducted. Three capture molecules, anti-goat IgG, anti-rabbit IgG and anti-mouse IgG were conjugated onto red, yellow and blue microspheres, respectively. The resulting respective colored microspheres were reacted with respective analytes, goat IgG-biotin, rabbit IgG-biotin and mouse IgG-biotin, followed by incubation with a solution of streptavidin-modified SMNPs. The flow rate used was 3 μL/h, and the reacted colored microspheres were analyzed in the first detection zone A as shown in Fig. 1(A). For the background test, three cases of control experiments without target analytes were carried out. The position result of control was 3.46 ± 1.89 μm from the left wall of the microchannel.

As shown in Fig. 3, the positions of respective colored microspheres were measured over ranges of concentration of goat IgG-biotin and mouse IgG-biotin from 6.7 to 666.0 fM and rabbit IgG-biotin from 26.6 to 799.2 fM. In case of goat IgG-biotin, the lowest concentration that was measured over the background was 6.7 fM and the corresponding position of red colored microsphere was 6.92 ± 2.22 μm. The lowest concentrations of rabbit IgG-biotin and mouse IgG-biotin were 26.6 fM and 6.7 fM which corresponded to 8.40 ± 2.46 μm and 6.58 ± 2.35 μm, respectively. As turned out in the previous work (Hahn et al., 2007), the positions of reacted colored microspheres were measured higher accordingly as the concentration of analytes was higher. The detection limits of goat IgG-biotin, rabbit IgG-biotin and mouse IgG-biotin were 10.9, 30.6 and 12.1 fM, respectively.

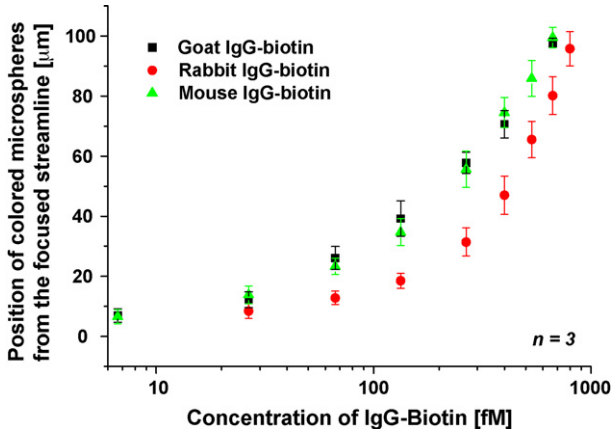

Fig. 3. Calibration curves for three types of IgGs by magnetophoretic immunoassay. The positions of colored microspheres from focused streamline were measured and correlated with the concentration of corresponding target analyte. The calibration curves were obtained at the first detection zone A and a flow rate of 3 μL/h. Assays were repeated three times.

3.3. Multiplexed magnetophoretic immunoassays

For evaluation of the reproducibility and multiplexing capability of this system, three immunoassays based on the multiplexed magnetophoresis were carried out as shown in Table 1. Known concentrations of three types of IgG-biotins were assayed so that the corresponding positions were measured. With these results of positions, the concentrations of analytes to be analyzed were again estimated from respective standard curves, reversibly. Finally, known concentrations were compared with the values estimated from the standard curves. Here reactions of each analyte were carried out individually. After reactions, all solutions were mixed and injected into the magnetophoretic chip followed by decoding of colored microspheres and quantitative analysis. In each assay, 5 min of analysis was sufficient to analyze all images obtained by microfluidic experiment with MATLAB program. The total analysis time was less than 30 min, including immunoreactions, decoding of colored microspheres, and position analysis. As a result, the concentrations estimated from three assays corresponded well to the known concentrations in an acceptable error range. In assay 1, the number of colored microspheres analyzed was 47 and all microspheres were decoded to the right color. In assays 2 and 3, 30 and 73 of colored microspheres were detected and only 2 and 5 of colored microspheres were decoded to the wrong color, respectively. Accordingly, the accuracy of decoding of colored microspheres was turned out to be 100% in assay 1 and 93% in assays 2 and 3, respectively. These results show that this magnetophoretic position detection system for multiplexed immunoassay works well.

3.4. Control of dynamic range by flow rate and detection zone

The developed system provides a possibility of controlling the dynamic range, depending on the concentration range of analytes by changing the flow rate or detection zone. The effect of flow rate is presented in Fig. 4. In this experiment, goat IgG-biotin was used to prove this feasibility. The positions of reacted red microspheres were measured through the first detection zone A of Fig. 1(A) and flow rates were changed from 3 to 7 μL/h. As shown in Fig. 4, the dynamic range was shifted to higher concentration of range as the flow rate increased. At a flow rate of 3 μL/h, the dynamic range of goat IgG-biotin was from 6.7 to 666.0 fM. Meanwhile, the dynamic range at a flow rate of 7 μL/h turned out to be from 133.2 fM to 1.9 pM. As the flow rate becomes higher, the microsphere is exposed to the magnetic field gradient for a shorter time. Consequently, the dynamic range at the faster flow rate was more extended to a broad range of analyte concentration, while calibration sensitivity, defined as the ratio of channel width to maximum detectable concentration, was decreased. In addition, the microsphere at the faster flow rate was detected at a lower position, even for the same concentration of analyte.

We also tested the effect of detection zone on the dynamic range for the mouse IgG-biotin at a flow rate of 3 μL/h (Fig. 5). The blue microspheres reacted with mouse IgG-biotins were analyzed at the four detection zones (A, B, C, and D). As expected, the difference in

Table 1
Reproducibility of magnetophoretic multiplexed immunoassays.

	Goat IgG-biotin	Rabbit IgG-biotin	Mouse IgG-biotin
Assay 1			
Concentration (fM)	26.6	799.2	266.4
Multiplexed immunoassay (μm)	17.00 ± 3.00	96.45 ± 5.09	59.44 ± 5.48
Fitting into the standard curve (fM)	37.8 ± 11.2	795.6 ± 41.0	285.7 ± 40.6
Assay 2			
Concentration (fM)	666.0	399.6	6.7
Multiplexed immunoassay (μm)	99.60 ± 0.91	45.50 ± 4.42	6.00
Fitting into the standard curve (fM)	682.6 ± 11.8	381.2 ± 37.2	4.5
Assay 3			
Concentration (fM)	266.4	26.6	666
Multiplexed immunoassay (μm)	56.80 ± 5.97	9.10 ± 3.00	98.70 ± 3.46
Fitting into the standard curve (fM)	258.3 ± 44.0	28.10 ± 9.80	654.5 ± 41.4

Accuracy of decoding: assay 1, 100%; assay 2, 93%; assay 3, 93%. No. of microspheres analyzed: assay 1, 47; assay 2, 30; assay 3, 73.

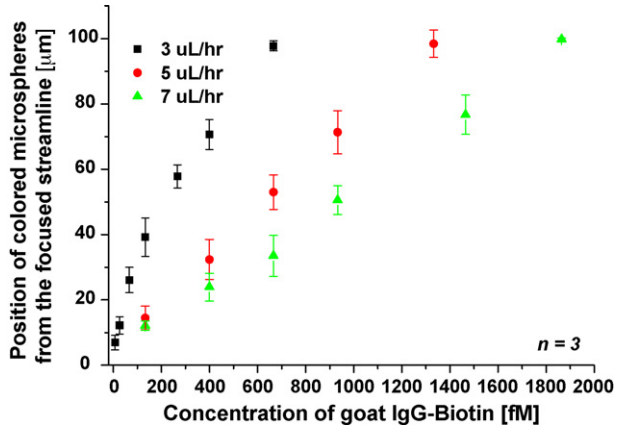

Fig. 4. Effect of the flow rate on the dynamic range. Goat IgG-biotin was used, and red microspheres that had been reacted with goat IgG-biotin were detected at the first detection zone A. Assays were repeated three times.

the detection zones was marginal at the low concentration range of analyte. But, higher concentration of analyte gave rise to the steeper slope in the graph. It seems that the reacted microspheres are more deflected as the microspheres are exposed to the magnetic field gradient for a longer time; in other words, as the detection zone is changed from A to D. In the first detection zone A, the position

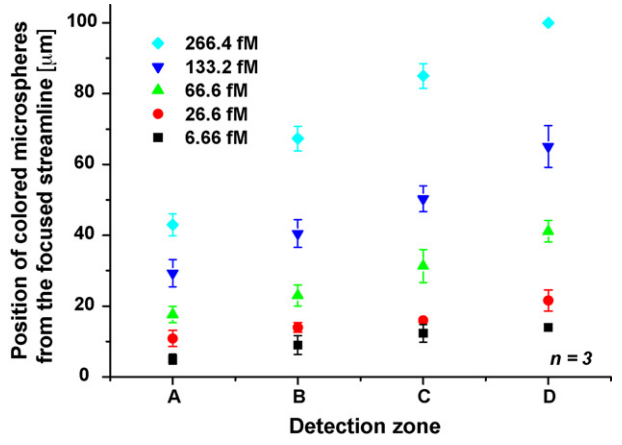

Fig. 5. Effect of the detection zone on the dynamic range. Blue microspheres that had been conjugated with mouse IgG-biotin were measured at various detection zones at a fixed flow rate of 3 μL/h. Four different detection zones were used, and the distance between the detection zones was about 4 mm. Assays were repeated three times.

detection shows higher dynamic range (up to 655.8 fM) than that of other detection zones. Meanwhile, although the position detection shows low dynamic range (up to 266.4 fM) in the last detection zone D, calibration sensitivity (0.33 μm/fM at the detection zone D) can be achieved higher than that of other detection zones (for example, 0.14 μm/fM at the detection zone A). From these results, we can predict the detectable concentration range and sensitivity of a target analyte, simply adjusting the detection zone in a microchannel. This is an advantage that can be expected without complicated adjustments such as change in magnetic field gradient or position of external permanent magnet.

4. Conclusion

We have demonstrated the magnetophoretic position detection method for multiplexed immunoassay. The positions of colored microspheres associated with SMNPs were well correlated with the concentration of target analytes. Colored microspheres were correctly decoded by the MATLAB program with high accuracy. In addition, analysis time was dramatically reduced due to automatic and simultaneous decoding of colored microspheres in a quantitative manner. The developed system has a potential to adjust the dynamic range by changing the flow rate or detection zone, showing the possibilities to detect analyte within sub-femtomolar range of concentration.

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