

Development of Microchip Electrophoresis-Integrated Nanoimprinted Photonic Crystal

Tatsuro Endo*, Kenzo Yamamoto, Kenji Sueyoshi and Hideaki Hisamoto

Department of Applied Chemistry, Graduate School of Engineering, Osaka Prefecture University
1-1 Gakuen-cho, Naka-ku, Sakai-shi, Osaka 599-8531, Japan

(Received February 25, 2015; accepted March 17, 2015)

Key words: photonic crystal (PhC), nanoimprint lithography (NIL), microchip electrophoresis, refractive index determination

In this study, microchip electrophoresis (MCE) was integrated with a two-dimensional photonic crystal (PhC) fabricated using nanoimprint lithography (NIL) for the detection of low-molecular-mass substances such as carbohydrates by determination of refractive index changes in solutions. The MCE-integrated PhC was fabricated using poly(dimethylsiloxane) (PDMS)-based micro-flow channels and NIL-based PhC. Different concentrations of sucrose solutions [0.5–20% (w/v), refractive index: 1.3337–1.3619] were introduced into a PDMS-based micro-flow channel by electro-osmotic flow (EOF), and the MCE-integrated PhC successfully detected fractional refractive index changes (0.0002) from the change in the optical characteristics of NIL-based PhC. Based on this detection principle, low-molecular-mass substances can be detected cost-effectively and easily without using a labeling procedure.

1. Introduction

In living organisms, low-molecular-mass substances such as carbohydrates play an important role in maintaining cellular functions.⁽¹⁾ In addition, carbohydrates have been studied for medical applications such as drug delivery.⁽²⁾ To realize carbohydrates for medical applications, the analysis of their function, chemical structure, and concentration is required. However, the analysis of carbohydrates presents several difficulties in that it requires labeling with reagents, such as a fluorescent dyes, by forming covalent bonds. In contrast, the analysis of carbohydrates without covalent labeling requires high-cost analytical instruments such as high-performance liquid chromatography (HPLC)⁽³⁾ and mass spectrometry (MS).⁽⁴⁾ These analytical

*Corresponding author: e-mail: endo@chem.osakafu-u.ac.jp

instruments require huge sample volumes, sophisticated techniques, and long analysis time. To overcome these disadvantages, micro-total analysis system (μ TAS)-based analytical devices fabricated using micromachining techniques have been actively produced.⁽⁵⁾ μ TAS-based analytical devices could overcome several disadvantages of conventional analytical instruments, such as huge sample volumes and sophisticated analysis techniques. However, previously reported μ TAS-based analytical devices still require high-cost instruments for analysis of unlabeled carbohydrates, such as capacitively coupled contactless conductivity detection (C^4D)-based systems⁽⁶⁾ and surface plasmon resonance (SPR).⁽⁷⁾ Considering these situations, we integrated microchip electrophoresis (MCE) and photonic crystals (PhC) to develop cost-effective, rapid, and simpler devices for the label-free analysis of carbohydrates.

MCE enables the introduction of small volumes of sample solutions with high reproducibility by using electrokinetic behavior.⁽⁸⁾ In addition, MCE-based devices can be adopted to establish a system of multiple analysis by enabling control of device design [flow channel dimensions (width and height) and construction]. On the basis of these features, MCEs have been widely applied in the analysis of low-molecular-mass substances.⁽⁹⁾

In addition, we focused on specific optical characteristics of nanostructures. Nanostructures, such as nanoparticles, exhibit specific optical characteristics [light absorption,⁽¹⁰⁾ fluorescence,⁽¹¹⁾ and diffraction⁽¹²⁾] that depend on the size and physical property of base materials. Furthermore, specific optical characteristics of nanostructures may be greatly modified by refractive index changes in the surrounding. Using this feature, novel chemical sensors and biosensors have been developed.^(13–15) On the basis of specific optical characteristics of nanostructures, we have developed novel chemical/biosensors using nanoimprint lithography (NIL)-based two-dimensional photonic crystals (2D-PhC).⁽¹⁶⁾ A 2D-PhC is a nanostructure-based optical device constructed of a periodic nanoarray of dielectric material such as polymers. A 2D-PhC can diffract and reflect a specific wavelength of light depending on the size, periodicity, and refractive index of its base material. Furthermore, the wavelength and strength of the reflection are shifted by the refractive index change of the surrounding environment that may be caused by antigen-antibody reaction⁽¹⁷⁾ or DNA hybridization.⁽¹⁸⁾

In addition, NIL is a novel, cost-effective fabrication techniques for producing nanostructures on polymers using a mold with high reproducibility.⁽¹⁹⁾ Basically, for the fabrication of 2D-PhC or nanostructure-based optical devices, high-cost fabrication instruments, such as electron beam lithography (EBL), focused ion beam (FIB), and reactive ion etching (RIE), have been widely used. In contrast, using NIL, 2D-PhC or nanostructure-based optical devices can be fabricated without using high-cost fabrication instruments. In view of these advantages of NIL, we refer to NIL-based fabrication techniques as “printable photonics”.⁽¹⁶⁾

In this study, a MCE-integrated 2D-PhC was developed for the analysis of carbohydrates. The MCE integrated 2D-PhC was used to detect different concentrations of sucrose and glucose.

2. Materials and Methods

2.1 Materials

For the fabrication of the MCE flow channel, SU-8 3050, SU-8 developer (Nippon Kayaku Co., Ltd., Tokyo, Japan), and poly (dimethylpolysiloxane) (PDMS) (SILPOT® 184) (Dow Corning Toray, Co., Ltd., Tokyo, Japan) were used. For the detection of carbohydrates as a test case, glucose and sucrose were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Ultrapure water (18.2 M Ω -cm) from Sartorius Stedim Biotech (Aubagne, Cedex, France) was used in all sample preparations.

2.2 Apparatus

To fabricate the triangular configured nanohole array 2D-PhC, a printable photonics technique based on NIL was carried out using a nanoimprint apparatus (X-300, SCIVAX Corp., Kanagawa, Japan). For the surface hydrophilization and bonding of the PDMS-based flow channel to the 2D-PhC, an O₂ plasma cleaner (CUTE-MP/R) [FEMTO Science (Gyeonggi-Do, Korea)] and UV/O₃ cleaner {UV253E [Filgen Inc. (Aichi, Japan)]} were used. For evaluating the optical characteristics of the MCE-integrated 2D-PhC, a UV-VIS spectrophotometer [SPM-002, wavelength range: 190–1100 nm (Photon control, Burnaby, BC, USA)], and optical power meter [8230E (ADC Corp., Tokyo, Japan)] were used. A tungsten halogen light source (HL-2000, wavelength range: 360–2000 nm) and an optical fiber probe bundle (R-200-7 UV/VIS, fiber core diameter: 200 μ m, wavelength range: 250–800 nm) were purchased from Ocean Optics (Dunedin, FL, USA).

2.3 Fabrication of 2D-PhC using printable photonics technology

To develop the MCE-integrated PhC, 2D-PhC (hole diameter, height, and distance: 230 nm) from cyclo-olefin polymer (COP) (thickness: 100 μ m), a printable photonics technique based on NIL was carried out using a nanoimprint apparatus (X-300, SCIVAX Corp., Kanagawa, Japan). From our previous study, this configuration of the 2D-PhC could detect the antigen-antibody and/or DNA hybridization highly sensitively.^(16–18,21) Hence, the same configuration of 2D-PhC was used in this study.

2.4 Fabrication of MCE-integrated PhC

The construction of the MCE-integrated PhC is shown in Fig. 1, along with photograph of the device. By integrating the MCE onto the 2D-PhC, the sample solution from the inlet can be introduced into the flow channel as a small volume plug by electro-osmotic flow (EOF), and the differences in refractive index between sample solution and loading buffer can be detected using the 2D-PhC as a reflection peak and an intensity change. Hence, the carbohydrate concentration can be measured without covalent labeling.

The fabrication method for the MCE-integrated PhC is shown in Fig. 2. The PDMS-based MCE flow channel was fabricated by the conventional rapid prototyping technique. The cross-channel MCE device consisted of three 10-mm-long channels and a 40-mm-long separation channel (500 μ m width and 30 μ m depth). The surfaces of the PDMS-

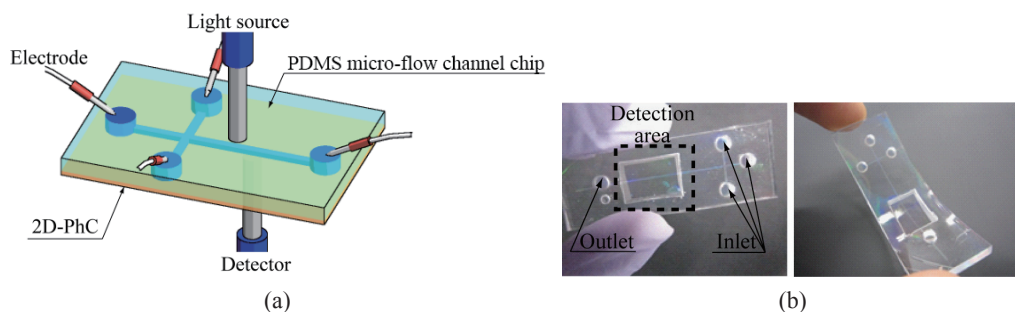


Fig. 1. (Color online) (a) Schematic illustration of the construction of an MCE-integrated PhC. (b) Photographs of an MCE-integrated PhC.

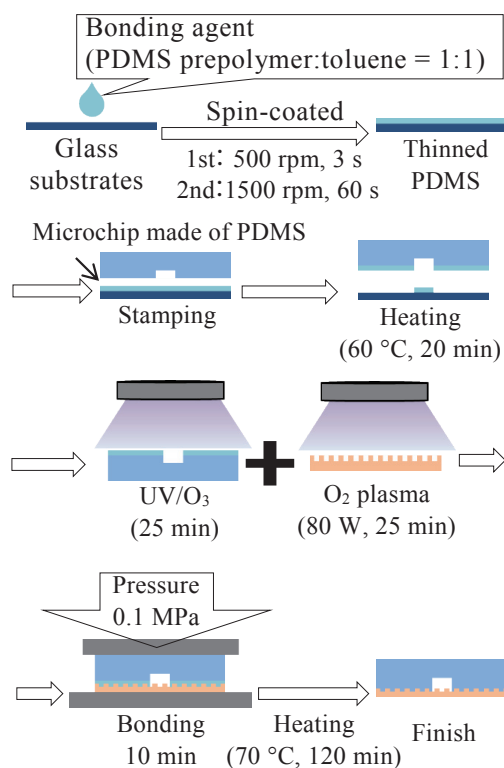


Fig. 2. (Color online) Fabrication method for an MCE-integrated PhC.

based MCE flow channel and the 2D-PhC were activated using O₂ plasma and UV/O₃ cleaner and bonded together via a bonding agent (PDMS prepolymer with catalyst: toluene = 1 : 1).⁽²⁰⁾ The MCE-integrated PhC was used to detect different concentrations of sucrose.

2.5 Optimization of optical setup for label-free detection of sucrose using MCE-integrated PhC

For the label-free detection of refractive index changes by EOF using an MCE-integrated PhC, the construction of an optical setup was investigated. From our previous study, we know that the optical setup affects the optical characteristics of the 2D-PhC based on how it is constructed.⁽²¹⁾ A schematic illustration of the optical setup is shown in Fig. 3. In this study, three optical setups {transmission [Fig. 3(a)], reflection [Fig. 3(b)], optical axis shift [Fig. 3(c)]} were constructed. In the case of optical axis shift, the fiber probes between the light source and the detector were separated by a (distance: 500 μm). Using these optical setups, the optical characterization of 2D-PhC and the optimization of optical setup were achieved.

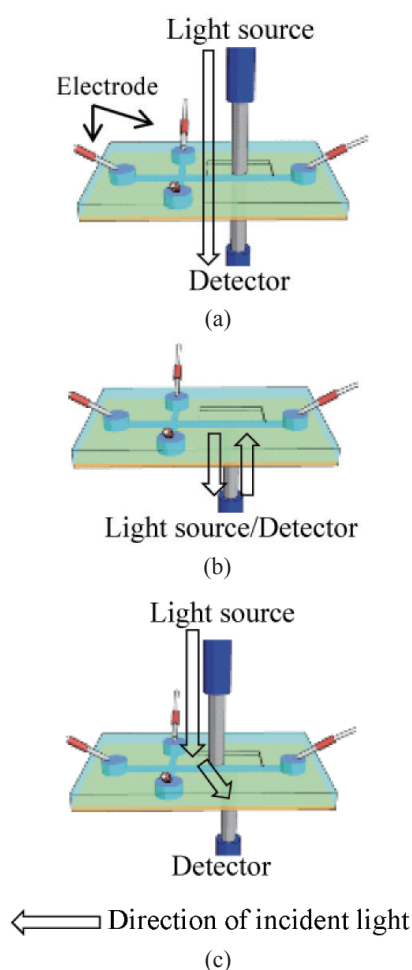


Fig. 3. (Color online) Experimental setup for detection of sucrose: (a) transmission, (b) reflection, and (c) optical axis shift.

2.6 Label-free detection of sucrose using MCE-integrated PhC

To evaluate the performance of the MCE-integrated PhC for the determination of refractive index changes between the loading buffer [10 mM HEPES buffer (pH: 8.2)] and different concentrations of glucose [5% (w/v)] or sucrose [0.5–20% (w/v)], solutions were introduced into the flow channel by EOF [0.5 kV (DC)].⁽²²⁾ The changes in optical characteristics between the sucrose plug and the loading buffer passing through the flow channel were monitored using an optical power meter set at 550 nm. In addition, for the evaluation of performance, the refractive indices of the sucrose solutions and loading buffer were also measured using a refractometer {PAL-RI [Atago Co., Ltd. (Tokyo, Japan)]}. Under these experimental conditions and procedure, the refractive index dependences of the optical characteristics were investigated using MCE-integrated PhC.

3. Results and Discussion

3.1 Optimization of optical setup for label-free detection of sucrose using MCE-integrated PhC

The optical characteristics using different optical setup are shown in Fig. 4. In the case of transmission [Fig. 4(a)] and reflection [Fig. 4(b)], the spectrum of the light source could be observed. To monitor the reflection peak wavelength from the 2D-PhC, the distance between the fiber probe tip and the 2D-PhC surface will be significantly affected. In this case, the depth of the flow channel (50 μm) and the thickness of PDMS (> 3 mm) determined the distance between the fibers and the 2D-PhC. Hence, the specific reflection peak could not be observed. In addition, the 2D-PhC was affected by the incident angle of light. The reflection peak wavelength of the 2D-PhC is based on Bragg's law. Hence, the specific wavelength associated with the 2D-PhC could not be observed with light irradiated in the perpendicular direction.

On the other hand, in the case of optical axis shift [Fig. 4(c)], the specific peak wavelength attributed to the 2D-PhC could be observed. In this study, the numerical

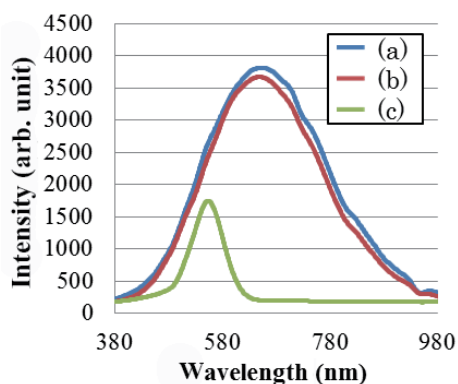


Fig. 4. (Color online) Optical characteristics of PhC using different optical setups: (a) transmission, (b) reflection, and (c) optical axis shift.

aperture of the optical fiber ($NA = 0.2$) was known. Hence, light was irradiated at an incident angle of approximately 11° . As a result, the specific wavelength from the 2D-PhC could be observed.

Furthermore, we found that the bulk layer (nonpatterned region) of the 2D-PhC acts as a waveguide. The irradiated light diffracts and is guided in the bulk layer. Hence, in the case of optical axis shift, the specific wavelength from the 2D-PhC could be observed. To confirm the function of the bulk layer of 2D-PhC as an optical waveguide, finite difference time domain (FDTD) {EEM-FDM [EEM Inc. (Saitama, Japan)]} analysis was performed (Fig. 5). In the FDTD analysis, incident light was diffracted and guided in the bulk layer of 2D-PhC. In addition, to monitor the guided wave, the distance between the light source and the detector was changed (500–1300 μm) [Fig. 6(a)], and the optical characterization was again carried out. By increasing the distance, the background light from the light source was reduced over 600 μm [Fig. 6(b)]. However, at larger incremental distances, the specific peak intensity decreased because of guided light intensity decay in the bulk layer.

3.2 Label-free detection of carbohydrates using MCE-integrated PhC

To verify the principle of detection, 5% (w/v) glucose solution ($n = 1.3395$) was introduced into the flow channel at different distances of fiber probes. The change in optical characteristics caused by introducing the solution using EOF for different optical fiber distances is shown in Fig. 7. A refractive index change between the loading buffer (HEPES buffer) and the glucose solution could be observed. In addition, by changing the distance between the fiber probes, the dependence of the intensity change on the distance could be observed. The distances of the fiber probes were 600–800 μm for the label-free detection of refractive index changes of sucrose solutions.

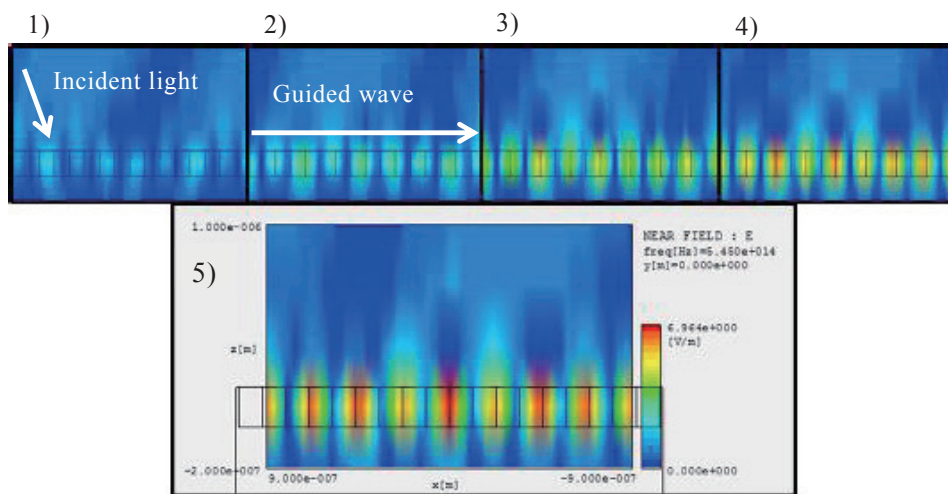


Fig. 5. (Color online) Results of FDTD simulation.

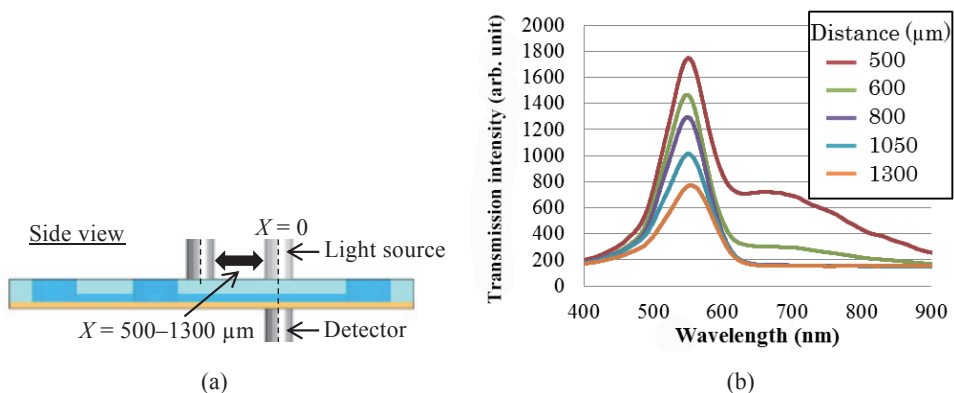


Fig. 6. (Color online) (a) Cross-sectional image of controlling fiber probe distances. (b) Optical characteristics at different fiber probe distances.

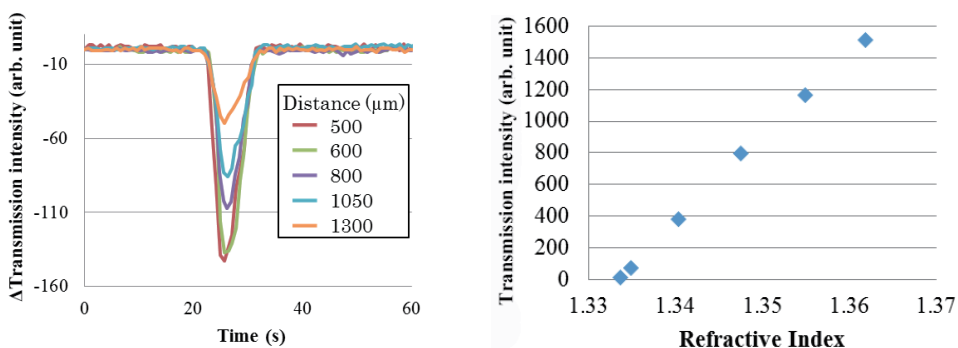


Fig. 7 (left). (Color online) Real-time changes in optical characteristics for different fiber probe distances for 5% (w/v) glucose solution using EOF.

Fig. 8 (right). (Color online) Sensitivity of detection of refractive indices of sucrose solutions.

Using the optimized optical setup, a label-free detection of sample solutions of a loading buffer ($n = 1.33335$) and sucrose [concentration: 0.5–20% (w/v) ($n = 1.33337$ – 1.3619)] was carried out as a test case. To monitor the refractive index change using the MCE-integrated PhC, changes in the time course optical characteristics at 550 nm were observed.

The sensitivity of the detection of refractive index is shown in Fig. 8. By introducing different concentrations of sucrose, MCE-integrated PhC could detect a refractive index change with a high linearity ($R^2 = 0.9992$). In addition, note that the MCE-integrated PhC could detect a fractional refractive index change of approximately 0.0002. For the detection of such a small change in refractive index, high-cost and highly sensitive optical setups are required. However, the MCE-integrated PhC can detect the refractive index change using a cost-effective and simple optical setup. From these results, an MCE-integrated PhC has great potential for the analysis of carbohydrates without involving sophisticated sample preparation and high-cost instruments.

4. Conclusions

In this study, an MCE-integrated PhC was developed using a rapid prototyping technique and printable photonics technology. In addition, the MCE-integrated PhC enabled the detection of changes in fractional refractive index without using a labeling procedure. Our results indicate that the MCE-integrated PhC has great potential for the analysis of low-molecular-mass substances, and may be particularly attractive for medical applications that require cost-effective analysis.

Acknowledgements

This work was supported by a Grant-in-Aid for Young Scientists (B) [KAKENHI WAKATE (B) 24750073] from the Japanese Ministry of Education, Culture, Science, Sports and Technology (MEXT), Asahi Glass Foundation, Murata Science Foundation, and the Ministry of Health, Labor and Welfare Grants-in-aid for Scientific Research.

References

- 1 K. Olden, R. M. Pratt and K. M. Yamada: *Proc. Natl. Acad. Sci. USA* **76** (1979) 3343.
- 2 B. Ernst and J. L. Manani: *Nat. Rev. Drug Discov.* **8** (2009) 661.
- 3 T. Ikegami, K. Horie, N. Saad, K. Hosoya, O. Fiehn, and N. Tanaka: *Anal. Bioanal. Chem.* **391** (2008) 2533.
- 4 K. E. Mutenda and R. Matthiesen: *Methods Mol. Biol.* **367** (2007) 289.
- 5 N. A. Lacher, K. E. Garrison, R. S. Martin and S. M. Lunte: *Electrophoresis*. **22** (2001) 2526.
- 6 W. K. T. Coltro, R. S. Lima, T. P. Segato, E. Carrilho, D. P. de Jesus, C. L. do Lago and J. A. F. Silva: *Anal. Methods* **4** (2012) 25.
- 7 H. Nakajima, Y. Harada, Y. Asano, T. Nakagama, K. Uchiyama, T. Imao, N. Soh and A. Hemmi: *Talanta* **70** (2006) 419.
- 8 V. Dolnik, S. Liu and S. Jovanovich: *Electrophoresis* **21** (2000) 41.
- 9 P. Schulze, M. Ludwig, F. Kohler and D. Belder: *Anal. Chem.* **77** (2005) 1325.
- 10 K. A. Willets and R. P. Van Duyne: *Ann. Rev. Phys. Chem.* **58** (2007) 267.
- 11 K. Brunner, U. Bockelmann, G. Abstreiter, M. Walther, G. Böhm, G. Tränkle and G. Weimann: *Phys. Rev. Lett.* **69** (1992) 3216.
- 12 S. V. Teeffelen, C. N. Likos and H. Löwen: *Phys. Rev. Lett.* **100** (2008) 108302.
- 13 T. Endo, K. Kerman, N. Nagatani, Y. Taamura and E. Tamiya: *Anal. Chem.* **77** (2005) 6976.
- 14 K. Kerman, T. Endo, M. Tsukamoto, M. Chikae, Y. Takamura and E. Tamiya: *Talanta* **71** (2007) 1494.
- 15 N. Li, X. R. Cheng, A. Brahmendra, A. Prashar, T. Endo, C. Guyard, M. Terebiznik and K. Kerman: *Biosens. Bioelectron.* **41** (2013) 354.
- 16 T. Endo, M. Sato, H. Kajita, N. Okuda, S. Tanaka and H. Hisamoto: *Lab Chip* **12** (2012) 1995.
- 17 T. Endo, S. Ozawa, N. Okuda, Y. Yanagida, S. Tanaka and T. Hatsuzawa: *Sens. Actuator. B: Chem.* **148** (2010) 269.
- 18 T. Endo, C. Ueda, H. Kajita, N. Okuda, S. Tanaka and H. Hisamoto: *Microchim. Acta* **180** (2013) 929.
- 19 L. J. Guo: *Adv. Mater.* **19** (2007) 495.
- 20 H. Wu, B. Huang and R. N. Zare: *Lab Chip* **5** (2005) 1393.
- 21 S. Aki, T. Endo, K. Sueyoshi and H. Hisamoto: *Anal. Chem.* **86** (2014) 11986.
- 22 X. Zhang, Q. Li, Z. Chen, H. Li, K. Xu, L. Zhang and B. Tang: *Lab Chip* **11** (2011) 1144.