< Reviews>

J. Flow Injection Anal., Vol. 30, No. 1(2013) 15–20

Optical Sensing Systems Suitable for Flow Analysis on Microchips

Rong Liu, Ryoichi Ishimatsu, Koji Nakano and Toshihiko Imato*

Department of Applied Chemistry, Graduate School of Engineering, Kyushu University, 744 Motooka, Nishi-ku, Fukuoka 819-0395, Japan

Abstract

The interest in downsized optical sensing systems suitable for microflow analysis on microchips has increased considerably due to the fact that analysis on the microchip has many advantages such as analytical time including reaction time is short, less consumption of reactants and separation time is also short. The analytical system built on the microchip is portable and suitable for on-site analysis for environmental analysis and bed-side diagnosis for clinical analysis. These systems are miniature microanalysis labs fabricated on a single substrate, and have numerous applications in chemistry and life science. This review focuses on the recent advances in the optical sensing systems used for microfluidic devices including fluorescence detection, absorbance detection and chemiluminescence (CL) detection, especially on the system based on the organic light-emitting diodes (OLED) and optical photodiodes (OPD). In this review, we demonstrate the developments and applications of the downsized optical sensing systems and point out exciting new approaches, and provide future tendency on this field.

Keywords Optical detection system, Miniaturization, Organic emitting diode (OLED), Organic photodiode (OPD)

1. Introduction

The microfluidic associated with Lab-on-a-Chip (LOC) or micro-total analysis systems (μ-TAS) has rapidly increased in complexity in resent years and applied in many aspects [1-4]. Although, during the past years, advances using optical sensing systems in this field have been developed very rapidly, much more research is required due to the demands of manipulating smaller and smaller amounts of sample volume such as nanoliter to combine with a thrust towards faster separations such as HPLC and so on. While with respect to detector systems for the microanalysis such as LOC and μ-TAS, and electrochemical detectors have been successfully miniaturized in order to be suitable for microfluidics devices. Optical detector systems which includes thermal lens and a laser have also applied to the microfluidics devices, because such optical systems can focus the light at very small area in the microchip. Since in these systems a somewhat large and expensive laser system with a microscope has been utilized, a much lower cost and much smaller optical system would be preferable to the micro-devices. Therefore with respect to microchip, integrating all the necessary elements to perform specific measurements on single chip is very important, so the light sources and detectors are the key components of any microfluidic LOC systems. These microdevices, by achieving reduction in instrument size are portable, promising for on-site and bed-side analyses, such as environmental monitoring of pollutants [5, 6], it is provide distinct advantages over conventional bulk systems. These include better control of reaction, multiple signal processing, shorter analysis time, higher analysis throughput, reduced consumption of reagents, and portability for field. Many researchers have summarized numerous methods in this field [7-16]. This review focuses on recent achievements in optical detection techniques used for microfluidic devices and the future prospects.

Fluorescence is one of the most important detection methods applied in analytical micro-systems commonly due to it high specificity and sensitivity. According to most of the published literatures, optical instrumentation for fluorescence detection is based on configuration and components from large laboratory instruments; for instance, laser-induced fluorescence (LIF) is most popular and easily adapted to the dimensions of microchips, in the previously work, so that nearly all electrophoresis-based devices utilize the LIF methods because the good sensitivity with low detection limits, providing the possibility of making detections in very small sample volumes and fast response is essential to resolve the peaks [17-19], Although the laser beam makes it easy to focus on very small detection volumes and obtain very high irradiation, resulting in one of the lowest limits of detection of any detection system, but the optical instrumentation surrounding lab-on-a-chip is bulky, expensive and dedicated for operation only inside laboratories. Afterwards, some of the researchers use lamp-based excitation systems to replace the laser, for example, high-output light-emitting diodes (LED) or organic light-emitting diodes (OLED) used as the light source and optical photodiode (OPD) as the detector have attracted a lot of attention due to its small size and low cost of production into microfluidic devices.

Absorbance measurement is another method to separations on microfluidic devices, an inherent shortcoming of these devices is the high limit of detection in absorption, because typically the channel depth employed as the optical path length, in most cases is smaller than the channel width and limited by the wafer or substrate thickness. To overcome this problem, several researchers have examined methods for increasing the pathlength on a microchip, such as fabricated Y- or Z-shaped absorbance detection cells [20, 21] to provide a long path parallel to the flow direction. For instance, Greg E. Collins and co-workers [22] have investigated a long pathlength, three-dimensional U-type flow cell and evaluated for improved absorbance detection on a glass microdevice within the plane of the glass device (126 μm long), the results indicated that detection limit for rhodamine B was determined to be 0.95 μM, which is nearly identical to the theoretical limit calculated by Beer's Law. However, to date most researches have been primarily limited to use optical system for fluorescence detection, and few study about detecting absorbance based sensing systems for microfluidic devices using optical component has been reported, In recent years there has been growing interest in this technique and further developments are aiming at improving the sensitivity in absorbance detection.

*Corresponding author. Tel.: +81 92 802 2889.
E-mail: imato@cstf.kyushu-u.ac.jp (T. Imato).

Chemiluminescence (CL) has proven to be a very sensitive and selective detection method for the research of separations because the light is generated by a chemical reaction, no excitation light source is required and a filter system to reduce the background is not necessary. In CL assays, emitted light is usually converted to an electrical signal with a photomultiplier tube (PMT) or a charge coupled detector (CCD) [23-30]. The large size and high voltage requirements of PMT and CCD detectors severely limit their use in compact portable instruments. With the development of LOC systems, in recent years, some researchers used optical photodiode (OPD) to take the place of PMT and CCD [24], to provide a promising route towards disposable, compact and portable devices for environmental-analytical applications.

This review summarizes the features and performances of optical detection methods mentioned above during the recent years, concludes with an assessment of future directions of optical sensing systems for integrated microfluideic devices, especially describes the detail about use OLED and OPD in microfluidic devices.

2. Laser-Induced Fluorescence (LIF) Technique

Laser-Induced Fluorescence (LIF) technique as a macro-scale optical detection, especially applied in spectrometric detection[31-41]. There have been published several reviews concerning the application of LIF to microanalysis [7]. Table 1 has showed the typical LIF method used in microfluidic technology during recent years.

Table 1 Analytical performance of microfluidic systems using LIF technology

Optical system	*Analyte*	*LOD**	Ref.
Laser, PMT	5-carboxy fluorescein	18 μM	[19]
Laser, CCD	Cd(II)	19.0 μg/L	[28]
LIFM, CCD	Immunoassay	0.46 ng/mL	[30]
Laser, CCD	DNA	0.1 ng/mL	[35]
Nd: YAG Laser	5-(and-6)-carboxy	–	[36]
Laser, CCD	β-galactosidase	–	[37]
Optical Fibers	Dye labeled antibody	0.5 nM	[38]
Laser	DBO, DAF-FM	4.3, 1.1 nM	[39]
Fiber Laser	Fluorescein	–	[40]
Laser, CCD	AF488-ANG	5 nM	[41]

*LOD: limit of lower detection

3. LED-based detection systems

An optical system based on LED as light sources, assembled on a microfluidic device, is one of the promising microanalytical systems due to the fact that it possesses many characteristics of small-size, low-cost, long lifetime, and low-power consumption. Indeed such optical systems are widely used in commercial instrumentation and household appliances [42-47]. In this review, some of typical examples of application of the optical systems on the microfluidics devices are listed in Table 2.

Table 2 Analytical performance of microfluidic systems using LED as the light source

Optical system	*Analyte*	*LOD**	Ref.
LED (365 nm), PMT	Ammonium	3.6×10^{-4} μg/mL	[43]
LED (470 nm), PMT	FITC, SF	0.25, 0.1μM	[44]
LED (501 nm), PMT	Myoglobin	1.5 ng/mL	[45]
LED (280 nm), PMT	DNA	4.85 μg/mL	[46]
LED (525 nm), PMT	Rhodamine B	0.95μM	[47]
LED (505 nm), OPD	Resorufin,IgA	5.0μM, 16 ng/mL	[57]

*LOD: limit of lower detection

Furthermore, the LED-based microfluidic system can provide satisfactory determinations, which proves that they are attractive for fast and efficient analysis. However, the beam intensity of LED is still less compared with laser. Therefore, future studies should pay more attention to enhance the excitation light intensity of LEDs and lower the baseline noise level.

4. OLED-based detection systems

Table 3 Analytical performance of microfluidic systems using OLED as the light source

Optical system	*Analyte*	*LOD**	Ref.
OLED, CCD	R-phycoerythrin	0.6 μg/mL	[48]
OLED (Alq_3, 530 nm), Photodiode	TAMRA	10 μM	[49]
OLED (PPV, 540 nm), CCD	HSA /AB 580	10 mg/L	[50]
OLED (530 nm), Photodiode	Fluorescence Dye	–	[51]
OLED (Alq_3, 510 nm), PMT	Rhodamine B	1 μM	[52]
OLED (Alq_3, 520 nm), PMT	Rhodamine 6G, Alexa 532	3 μM	[53]
OLED, PMT	R-phycoerythrin	38 ng/mL	[55]
OLED (512 nm), CCD	IgA	16.5 ng/mL	[56]

*LOD: limit of lower detection

In μ-TAS and LOC system fields, functional integration of optical components within monolithic substrates has only recently seen a spur of research and development efforts, as covered by some excellent literatures [48-54]. Organic light emitting diodes (OLED) have been employed as external light sources for fluorescence detection. Compared with inorganic LED, OLED has a flat surface, which makes it easy to integrate with microfluidic devices and is flexible to fabricate into many kinds of size and shape by photolithography techniques. Table 3 demonstrates analytical performance of microfluidic systems using OLED as the light source in the recent years. In this case,

the CCD and the PMT have become the most widely used to be the detectors, which gives excellent sensitivity, a wide dynamic range and a high detection frequency.

Yao [48] and co-workers used a green organic OLED and thin film interference filter as integrated excitation source is presented and applied to fluorescence detection of proteins. The achieved fluorescence signal of 300μM Rhodamine 6G is about 13 times as high as that without the excitation filter and 3.5 times for a perpendicular detection structure. Fig. 1 shows the optical set-up of OLED induced fluorescence detection system.

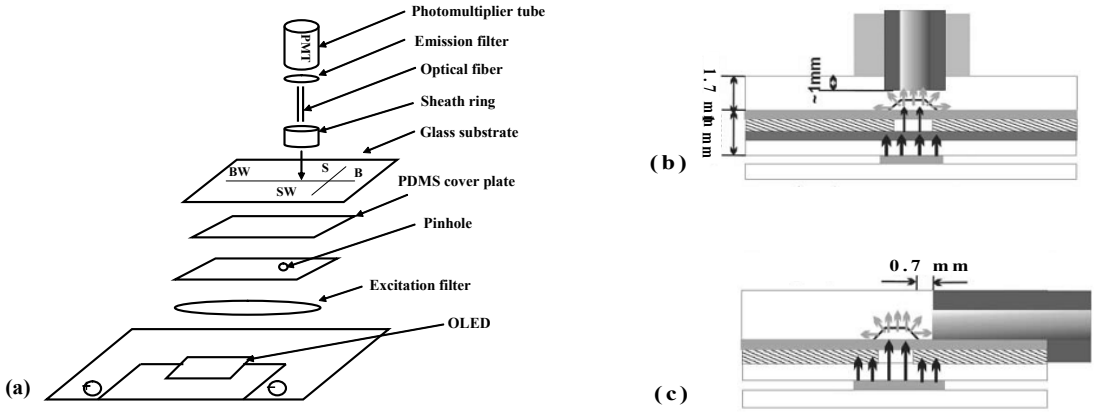

Fig. 1 Optical set-up of OLED induced fluorescence detection system: (a) detailed arrangement of each component, (b) side view of the structure with a coaxial optical fiber, and (c) side view of the structure with a perpendicular optical fiber [48].

This figure shows a layer-by-layer compact system consisting of a glass/PDMS microchip, pinhole and excitation filter, meanwhile OLED is designed and equipped with a coaxial optical fiber; for fluorescence detection a 300 μm thick excitation filter is employed for eliminating nearing 80% of the unwanted light emitted by OLED which has overlapped with the fluorescence spectrum of the dyes. The distance between OLED illuminant and microchannels is limited to ～1 mm for sensitive detection. This system has been used by Yao's team for fluorescence detection of Alexa 532, Rhodamine 6G and BSA conjugates in 4% liner polyacrymide (LPA) buffer.

Hofmann *et. al.,* [50] has reported the use of a thin-film OLED, which has a peak emission wavelength of 540 nm as an excitation source for microscale fluorescence detection. When the assay is performed in 800-μm deep and 800-μm wide microchannels on a PDMS microchip at flow rates of 20 μL/min, HAS concentrations down to 10 mg / g can be detected with a linear range from 10 to 100 mg/L. This sensitivity is sufficient for the determination of microalbuminuria (MAU). Ren [55] and his team also used OLED as the light source, chose the PMT instead of a CCD and compared them. Unlike conventional systems, no lenses, fibers or any mechanical components are required either. They reported that this novel simplified system has a broader linear range, higher sensitivity and higher efficiency in data collection. Fig. 2 showed the schematic diagram of the imaging mechanisms of CCD system and current work. The limit of detection (LOD) of R-phycoerythrin (PE) was 38 ng /mL.

At the same time, our group Nakajima [56] and co-workers have successfully combined OLED and CCD together for use in a flow-based enzyme-linked immunosorbent assay on a PDMS microfluidic device for the rapid determination of immunoglobulin A (IgA). Fig. 3 shows the fluorescence detection system and the calibration curve for IgA. The detection limit for IgA was 16.5 ng / mL. Compared with the conventional 96-well microtiter plate assay, the analysis time and the amounts of reagent and sample solutions could all be reduced.

To sum up, all of the research proved that OLED is promising light sources for microfluidic fluorescence detection systems, which have a very small size and are quite easy to integrate. However, the research of absorbance detection by using OLED has not been reported, and the OLED intensity and stability should be enhanced in the future with enormous efforts being directed in this area. In this case, we plan to explore these avenues further.

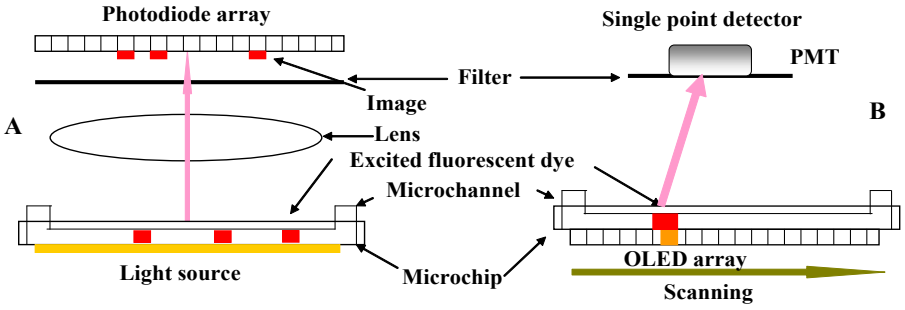

Fig. 2. A schematic diagram of the imaging mechanisms of CCD system and current work. The rectangles in yellow represent the lit OLED [55].

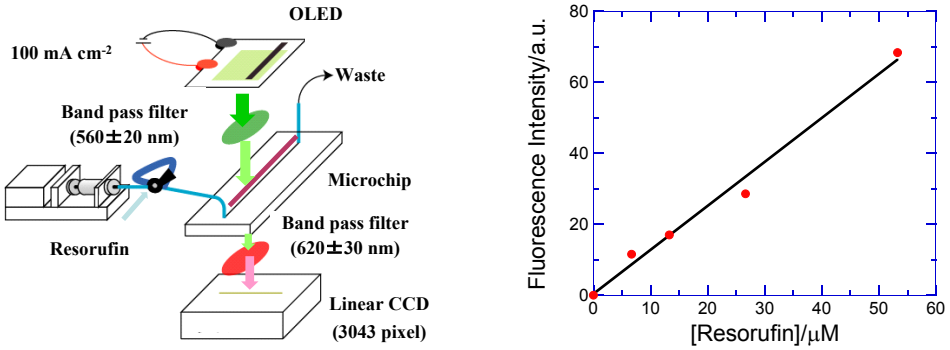

Fig. 3 Fluorescence detection system and calibration curve for IgA [56].

5. OPD-based detection systems

Table 4 Analytical performance of microfluidic systems using OPD as the optical detector

Optical system	*Analyte*	*LOD**	Ref.
OPD (P3HT / PCBM)	α-tocopherol, β-Carotene	9.4 μM, 1.87 μM	[24]
OPD (CuPc / C_{60})	PO-CL	~1 mM	[25]
LED (505 nm), OPD (CuPc / C_{60})	Resorufin, IgA	5.0μM, 16 ng/mL	[57]

*LOD: limit of lower detection

As we know, a variety of analytical applications require the sensitive detection of light originating, for example from CL or fluorescent systems. In CL assays, emitted light is usually converted to an electrical signal with PMT, the large size and high voltage requirements of PMT detectors severely limit their use in compact portable instruments. To overcome this problem, some researchers choose OPD instead of PMT, and the combination of OPD with microfluidic chips is a new research filed today and has many advantages. Hofmann [25] and co-workers were the first team to report the use of OPD as integrated optical detectors for microscale chemiluminescence. It was used to monitor a peroxyoxalate based chemiluminescence reaction (PO-OL) within a PDMS microfluidic device. The results showed that reproducibility was excellent with typical R.S.D. below 1.5% and the detection limit of ~1 mM and linearity over at least three decades. However, up to date, there are still fewer literature reported about use OPD as the detector. Table 4 shows the articles studied with OPD used in microfluidic systems in recent years, which can be searched on the internet.

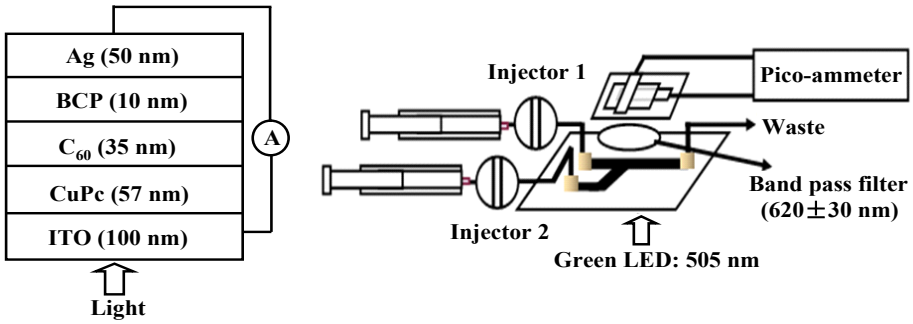

Fig. 4. Structure of the OPD and the schematic flow diagram of the fluorescence detection system [57].

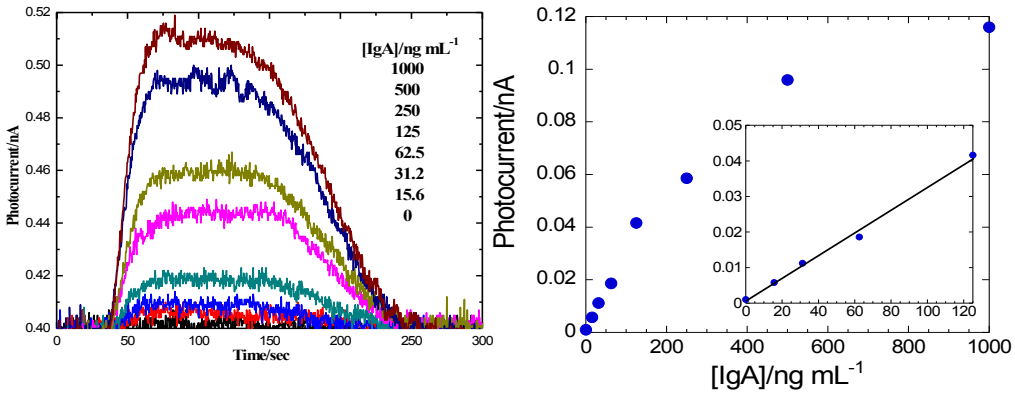

Fig. 5. Immunoasssay of IgA using the OPD as an optical detector for fluorescence. (a) Fluorescence response of OPD as photocurrent, when the Amplex Red solution was introduced into the microchip, where the sandwich immunoreaction was carried out. (b) Calibration curve for IgA by plotting the photocurrent of OPD, which was the average for the time period from 70 s to 120 s in the plateau region of the response signal, against the concentration of IgA [57].

Our laboratory have also done some research in this field, Miyake [57] and co-workers have combined green LED and OPD together to detected chemiluminescence generated from the reaction of luminol with horseradish peroxidase in the presence of H_2O_2, and the fluorescence from resorufin and IgA. The detection limits for resorufin and IgA were 5.0 μM and 16 ng/mL, respectively.

Fig. 4 showed the structure of the OPD and a schematic flow diagram of the fluorescence detection system. They fabricated this OPD from a hetero-junction which is comprised of two layers of C_{60} and a CuPc complex, which is usually used as a solar cell; the sensitivity of the OPD was sufficient for detecting CL with a power 0.1 μW/cm^2. Fig. 5 showed the immunoasssay of IgA using the OPD as an optical detector for fluorescence. Compared with above our laboratory's study, which used CCD as the detector, the use of OPD is more effective.

6. Coupling OLED and OPD detection systems

At last, some researches on combined OLED and OPD together optical microfluidic systems have been presented in table 2. To date, research on the development of optical systems for microfluidics using OLED and OPD is just at the initial stage, and the future progress would be expected as a result of the interdisciplinary efforts of analytical chemists and electro and mechanical engineers. OLED as light source can excite many different dyes, the combination of OLED and OPD has many advantages; these devices are compact, inexpensive, biodegradable, and an ideal candidate for disposable LOC applications. In recent researches, no more than 5 literatures can be found about it. Table 5 shows 2 articles which studied on OLED and OPD in detail in this field.

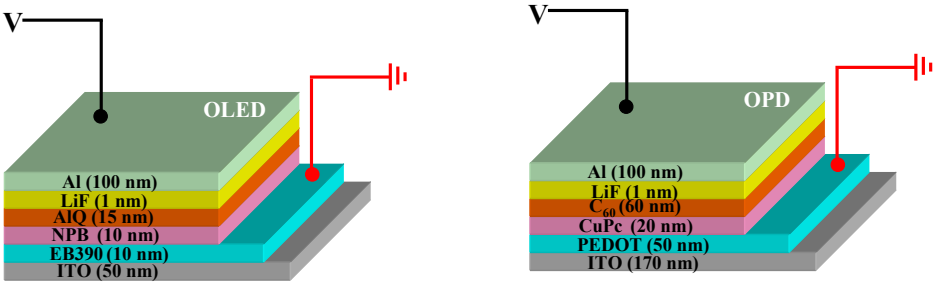

Fig. 6. Schematic illustration of the OPD (left) and OLED (right) structure [59].

Table 5 Analytical performance of microfluidic systems coupling OLED and OPD detection systems

Optical system	*Analyte*	*LOD**	Ref.
OLED (520 nm), OPD (CuPc / C_{60})	Rhodamine 6G, Fluorescein	100 nM, 10μM	[58]
OLED (520 nm), OPD (CuPc / C_{60})	Rhodamine 6G, Fluorescein	100 nM, 10μM	[59]

*LOD: limit of lower detection

For instance, Banerjee [58] and co-worker describe a novel, inexpensive approach to filtering out excitation light from the emission signal suitable for development into an integrated, high-sensitivity, low-cost, truly compact LOC quantitative fluorescence analysis device. This device uses OLED as the light source, and OPD as the detector and demonstrated detection limits of 10 nM. This represents a significant improvement over the results previously reported with the detectors. Another study is from Pais [59] and co-workers who use NPB/Alq_3 green OLED as the light source, CuPc/C60 thin-film OPD as the detector for on-chip fluorescence analysis; for the results, a limit of detection of 100 nM was demonstrated for Rhodamine 6G, and 10 μM for fluorescein. This suggests that an integrated microfluidic device, with an OLED excitation source, OPD detector and integrated polarizers, can be fabricated to realize a compact and economical lab-on-a-chip for point-of-care fluorescence assays. Fig. 6 showed the OLED and OPD structures in this research.

7. Conclusion

In summary, considering the number of publications during the recent years, nearly all electrophoresis-based devices still utilize LIF because it is an important tool for the detection of several analyses with its superior sensitivity. But, the large size and high voltage requirements of laser and detectors severely limit their use in compact portable instruments. In this case, μ-TAS or a LOC system, as a new research field has appeared. To date most researches has been primarily limited to fluorescence detection; however, UV/vis absorbance measurement only plays a minor role in microchip applications. Meanwhile, for the absorbance measurement, by noting Beer's law, the very short optical pathlengths of microfluidic devices will lead to the lower sensitivity, so the enhancement of pathlengths should be studied further. Moreover, the optical detection system based on the combination of OLED and OPD together has not been reported yet, so it can propose a new research topic in the future.

References

[1] C. Merfort, K. Seibel, K. Watty, M. Böhm, *Micro. Eng.*, **87**, 712 (2010).

[2] Y. Xu, X. G. Hu, J. Liang, J. X. Xun, W. W. Gu, T. M. Zhao, Z. Y. Wen, *Anal. Biochem.*, **394**, 1947 (2009).

[3] M. Ramuz, D. Leuenberger, L. Bürgi, *J. Polym. Sci., Part B: Polym. Phys.*, **49**, 80 (2011).

[4] T. Mayr, T. Abel, E. Kraker, S. Köstler, A. Haase, C. Konrad, M. Tscherner, B. Lamprecht, *Procedia Eng.*, **5**, 1005 (2010).

[5] E. Verpoorte, N. F. de Rooij, *Proc. IEEE*, **91**, 930 (2003).

[6] G. Medoro, *IEEE Sens. J.*, **3**, 317 (2003).

[7] B. Kuswandi, Nuriman, J. Huskens, W. Verboom, *Anal. Chim. Acta*, **601**, 141 (2007).

[8] S.T. Tu, X. Yu, W. Luan, H. Löwe, *Chem. Eng. J.*, **163**, 165 (2010).

[9] D. Xiao, L. Yan, H. Y. Yuan, S. L. Zhao, X. P. Yang, M. F.

Choi, *Electrophoresis*, **30**, 189 (2009).
[10] G. T. Roman, R. T. Kennedy, *J. Chromatogr. A*, **1168**, 170 (2007).
[11] S. Götz, U. Karst, *Anal. Biochem*, **387**, 183 (2007).
[12] K.R. Rogers, *Anal. Chim. Acta*, **568**, 222 (2006).
[13] M. Miró, E. H. Hansen, *Anal. Chim. Acta*, **750**, 3 (2012).
[14] R. Walczak, *Procedia Eng.*, **25**, 160 (2011).
[15] L. Malic, A. G. Kirk, *Sens. Actuators, A*, **135**, 515 (2007).
[16] W. R. Melchert, B. F. Reis, F. R. P. Rocha, *Anal. Chim. Acta*, **714**, 8 (2012).
[17] Z. H. Liang, N. Chiem, G. Ocvirk, T. Tang, K. Fluri, D. J. Harrison, *Anal. Chem.*, **68**, 1040 (1996).
[18] H. S. Moosavi, Y. T. Jiang, L. Lester, G. McKinnon, D. J. Harrison, *Electrophoresis*, **21**, 1291 (2000).
[19] K. B. Mogensen, N. J. Petersen, J. Hübner, J. P. Kutter, *Electrophoresis*, **22**, 3930 (2001).
[20] P. D. Ohlsson, O. Ordeig, K. B. Mogensen, J. P. Kutter, *Electrophoresis*, **30**, 4172 (2009).
[21] J. Riordon, M. Mirzaei, M. Godin, *Lab Chip*, **12**, 3016 (2012).
[22] G. E. Collins, Q. Lu, N. Pereira, P. Wu, *Talanta*, **72**, 301 (2007).
[23] W. Chen, W. Jie, Z. Chen, X. Jie, J. H. Xian, *Chinese J. Anal. Chem.*, **40**, 3 (2012).
[24] X. H. Wang, M. Amatatongchai, D. Nacapricha, O. Hofmann, J. C. de Mello, D. D.C. Bradley, A. J. de Mello, *Sens. Actuators, B*, **140**, 643 (2009).
[25] O. Hofmann, P. Miller, P. Sullivan, T. S. Jones, J. C. deMello, D. D. C. Bradley, A. J. deMello, *Sens. Actuators, B*, **106**, 878 (2005).
[26] E. Y. Pisarevskaya, E. V. Ovsyannikova, L. P. Kazanskii, N. M. Alpatova, *Russ. J. Electrochem.*, **46**, 1110 (2010).
[27] Z. J. Hu and A. J. Gesquiere, *J. Am. Chem. Soc.*, **133**, 20850 (2011).
[28] A. Kitano, A. Iiduka, T. Yamamoto, Y. Ukita, E. Tamiya, Y. Takamura, *Anal. Chem.*, **83**, 9424 (2011).
[29] M. D. Furtaw, D. Lin, L. Wu, J. P. Anderson, *Plasmonics*, **4**, 273 (2009).
[30] J. W. Park, V. Sunkara, T. H. Kim, H. Hwang, Y. K. Cho, *Anal. Chem.*, **84**, 2133 (2012).
[31] A. Bramati, J. P. Hermier, V. Jost, E. Giacobino, *Eur. Phys. J.*, **19**, 421 (2002).
[32] H. S. Moosavi, Y. T. Jiang, L. Lester, G. McKinnon, D. J. Harrison, *Electrophoresis*, **21**, 1291 (2000).
[33] Z. H. Liang, N. Chiem, G. Ocvirk, T. Tang, K. Fluri, D. J. Harrison, *Anal. Chem*., **68**, 1040 (1996).
[34] A. Kussrow, C. S. Enders, D. J. Bornhop, *Anal. Chem*., **84**, 779 (2012).
[35] R. Walczak, *Bio. Chip J.*, **5**, 271 (2011).
[36] S. Kuhn, K. F. Jensen, *Ind. Eng. Chem. Res.*, **51**, 8999 (2012).
[37] L. F. Cai, Y. Zhu, G. S. Du, Q. Fang, *Anal. Chem*., **84**, 446 (2012).
[38] F. Long, C. M. Gu, A. Z. Gu, H. C. Shi, *Anal. Chem*., **84**, 3646 (2012).
[39] Z. Z. Chen, Q. L. Li, Q. Q. Sun, H. Chen, X. Wang, N. Li, M. Yin, Y. X. Xie, H. M. Li, B. Tang, *Anal. Chem*., **84**, 4687 (2012).
[40] Y. Kazoe, K. Mawatari, Y. Sugii, and T. Kitamori, *Anal. Chem*., **83**, 8152 (2011).
[41] J. Choi, D. K. Kang, H. Park, A. J. deMello, S. Chang, *Anal. Chem*., **84**, 3849 (2012).
[42] M. Ramuz, D. Leuenberger, L. Bürgi, *J. Polym. Sci., Part B: Polym. Phys.,* **49**, 80 (2011).
[43] S. H. Xue, K. Uchiyama, H. F. Li, *J. Environ. Sci.*, **24**, 564 (2012).
[44] F. Yang, X. C. Li, W. Zhang, J. B. Pan, Z. G. Chen, *Talanta*, **84**, 1099 (2011).
[45] G. Ryu, J. S. Huang, O. Hofmann, C. A. Walshe, J. Y. Y. Sze, G. D. McClean, A. Mosley, S. J. Rattle, J. C. deMello, A. J. deMello, D. D. C. Bradley, *Lab Chip*, **11**, 1664 (2011).
[46] J. Z. Pan, B. Yao, Q. Fang, *Anal. Chem*., **82**, 3394 (2010).
[47] G. E. Collins, Q. Lu, N. Pereira, P. Wu, *Talanta*, **72**, 301 (2007).
[48] B. Yao, H. H. Yang, Q. L. Liang, G. A. Luo, L. D. Wang, K. N. Ren, Y. D. Gao, Y. M. Wang, Y. Qiu, *Anal. Chem*., **78**, 5845 (2006).
[49] Y. H. Kim, K. S. Shin, J. Y. Kang, E. G. Yang, K. K. Paek, D. S. Seo, and B. K. Ju, *J. Microelectromech. Syst.*, **15**, 1152 (2006).
[50] O. Hofmann, X. H. Wang, J. C. deMello, D. D.C. Bradley, A. J. deMello, *Lab Chip*, **5**, 863 (2005).
[51] C. Merfort, K. Seibel, K. Watty, M. Böhm, *Micro. Eng.*, **87**, 712 (2010).
[52] S. Kim, W. Choi, W. Rim, Y. Chun, H. Shim, H. Kwon, J. Kim, I. Kee, S. Kim, S. Y. Lee, J. Park, *IEEE Trans. Electron Dev.*, **58**, 3609 (2011).
[53] B. Yao, G. A. Luo, L. D. Wang, Y. D. Gao, G. T. Lei, K. N. Ren, L. X. Chen, Y. M. Wang, Y. Hu, Y. Qiu, *Lab Chip*, **5**, 1041 (2005).
[54] N. Misawa, S. Takeuchi, *IEE T. Electr. Electr. Eng.*, **6**, 97 (2011).
[55] K. N. Ren, Q. L. Liang, B. Yao, G. A. Luo, L. D. Wang, Y. D. Gao, Y. M. Wang, Y. Qiu, *Lab Chip*, **7**, 1574 (2007).
[56] H. Nakajima, Y. Okuma, K. Morioka, M. Miyake, A. Hemmi, T. Tobita, M. Yahiro, D. Yokoyama, C. Adachi, N. Soh, K. Nakano, S. H. Xue, H. L. Zeng, K. Uchiyama, T. Imato, *J. Sep. Sci.*, **34**, 2906 (2011).
[57] M. Miyake, H. Nakajima, A. Hemmi, M. Yahiro, C. Adachi, N. Soh, R. Ishimatsu, K. Nakano, K. Uchiyama, T. Imato, *Talanta*, **96**, 132 (2012).
[58] A. Banerjee, A. Pais, I. Papautsky, and D. Klotzkin, *IEEE Sens. J.,* **8** 621 (2008).
[59] A. Pais, A. Banerjee, D. Klotzkin, I. Papautsky, *Lab Chip*, **8**, 794 (2008).

(Received May 24, 2013)
(Accepted July 4, 2013)