Microelectronic Engineering 140 (2015) 1–5

Contents lists available at ScienceDirect

Microelectronic Engineering

journal homepage: www.elsevier.com/locate/mee

# Ultrafast laser microfabrication of a trapping device for colorectal cancer cells

Kwang-Ryul Kim [a], Hyun-Jin Kim [a], Hyun-Il Choi [b], Keong-Sub Shin [b], Sung-Hak Cho [c], Byoung-Deog Choi [a,*]

[a] Department of Electronic and Electrical Engineering, Sungkyunkwan University, Suwon 440-746, Republic of Korea
[b] Research Institute for Cell and Gene Therapy, SAM Medical Center, 9-Samdeok-ro, Manan-gu, Anyang-si, Gyeonggi-do 430-733, Republic of Korea
[c] Korea Institute of Machinery and Materials, 104, Sinseongno, Yuseong-Gu, Daejeon 305-343, Republic of Korea

ARTICLE INFO

*Article history:*
Received 12 November 2014
Received in revised form 2 April 2015
Accepted 23 April 2015
Available online 5 May 2015

*Keywords:*
Cell trapping device
Ultrafast laser
Colorectal cancer cells

ABSTRACT

Although celltrapping devices have been microfabricated and widely used for manipulation of bio cells, devices that trap multiple cells simultaneously are difficult to design because fabrication is complicated and time consuming. We designed and manufactured a microfluidic device using a polycarbonate plate with a flatness less than 200 μm and a gelatin-coated polyethylene terephthalate (PET) membrane. The device was used to capture colorectal cancer cells from one of the most common types of human malignant tumors. Microfluidic channels for the device were micromachined in minutes using a Computerized Numerically Controlled (CNC) engraving machine. We microfabricated multiple microholes on the PET membrane, which had a thickness of 13 μm, using an ultrafast, 1025 nm diode-pumped solid state femtosecond laser. The 100 microholes were drilled by spirally moving spot size of 4 μm laser beam. It is very important to obtain smooth and clean surface to avoid cell damages when they are trapped on the device. The relationship between the diameter changes of the microholes and variations in laser output power as well as laser fluence were investigated through parametric analysis. The average diameter of the holes increased exponentially with laser power. The gelatin-coated PET membrane was attached to the polycarbonate device and a syringe with a tube controlled negative pressure inside the channels of the cell-trapping device. Maintaining negative pressure inside the channels under the microholes on the PET membrane, colorectal cancer cells were dropped using the cell dropping pipette and successfully captured for manipulation under same environmental condition.

© 2015 Elsevier B.V. All rights reserved.

## 1. Introduction

Cell manipulation in microfluidic platforms can be applied to biomedical areas such as genomic mapping [1], oncology [2] and stem cell research [3]. Conventional cell studies are usually conducted with large population of cells, but this approach can lead to misunderstandings about cellular events. Therefore, studies on single-cell scale are necessary to obtain meaningful data [4].

A variety of methods are available for individual cell trapping [5] and can be classified as cell immobilization or contactless cell trapping. Hydrodynamic [6,7] or chemically induced cell trapping is a type of immobilization. Contactless cell trapping includes use of lasers, acoustics, dielectrophoresis [8–12] and magnetic trapping [13,14]. These devices have limited cell-capturing ability, depending on the cell type [13,15]. We focused on cell-capturing devices that use microfluidic channels. Studies on microfluidics published in recent years have described the underlying mechanisms of devices and their performances [16–18] Recently, femtosecond lasers, a microfabrication tool, have been used in cell-trapping devices [19,20]. Microholes can be fabricated in polyethylene terephthalate (PET) membranes without thermal damage because the pulse length of ultrafast lasers is less than 400 femtoseconds [21,22]. Since the cells are so delicate, rough surface of the holes on the trapping device may cause cell damages. Although some researchers fabricated cell trapping device using femto-second lasers, they failed to achieve very clean and smooth surface on the capturing holes [23].

Colorectal cancer (CRC) is one of the most common human malignant tumors with an age-adjusted incidence of 46.3 per 100,000 [24]. In the United States, CRC is the 4$^{th}$ common tumor after lung, prostate and breast cancers and 2$^{nd}$ common cause of cancer death after lung cancer [25]. Despite efforts of clinicians to find effective therapies against colorectal adenocarcinoma,

* Corresponding author.
*E-mail address:* bdchoi@skku.edu (B.-D. Choi).

http://dx.doi.org/10.1016/j.mee.2015.04.118
0167-9317/© 2015 Elsevier B.V. All rights reserved.

which is the leading cause of cancer-related mortality, its incidence and associated death rates continue to rise rapidly. According to molecular biology and pathology, CRC is developed from normal epithelial cells but histopathological and molecular properties are different from them. The risk factors for CRC are age, polyps, sedentary lifestyle, diet, obesity, family history of CRC, and Inflammatory Bowel Disease (IBD). Although surgical resection is only curative treatment, early detection and screening is of crucial importance. As the case stands, likelihood of cure is greater when disease is detected at early stage. The general methods to detect the colorectal tumor are Fecal Occult Blood Test (FOBT), flexible sigmoidoscopy and standard colonoscopy. However, these screening methods have disadvantages respectively. The FOBT and flexible sigmoidoscopy can not detect some polyps and cancers, and abnormal growths in the upper part of the colon will be missed. In addition, the standard (or optical) colonoscopy test is highly sensitive but it still may not detect all small polyps, nonpolypoid lesions, or cancers. Because the CRC is the leading cause of death and early stages can be detectable, the screening is very important for prevention of CRC. The CRC screening method using the cell trapping device is a novel approach for colorectal cancer detection and one of promising and non-invasive tool to detect Circulating Tumor Cells (CTCs) from small blood of patient. We designed a new cell trapping device to enhance the accuracy of diagnosis for early stage of colorectal cancer.

In this study, we made spot size of 4 μm beam using femtosecond laser and drilled spirally from the center to side very carefully on precise motorized stage. As a result, we fabricated very smooth and clean surface on the multiple holes for singular cell-capturing device which is not reported in former studies to manipulate CRC cells without damages. We fabricated aligned holes on gelatin-coated PET membrane filters using ultrafast femtosecond laser. Microfluidic channels were engraved on the polycarbonate to create negative pressure between the microfluidic device and the gelatin-coated PET membrane. CRC cells were captured by the smooth and clean membrane holes. We applied femtosecond lasers for a cell-capturing device and manufactured a reliable microfluidic device.

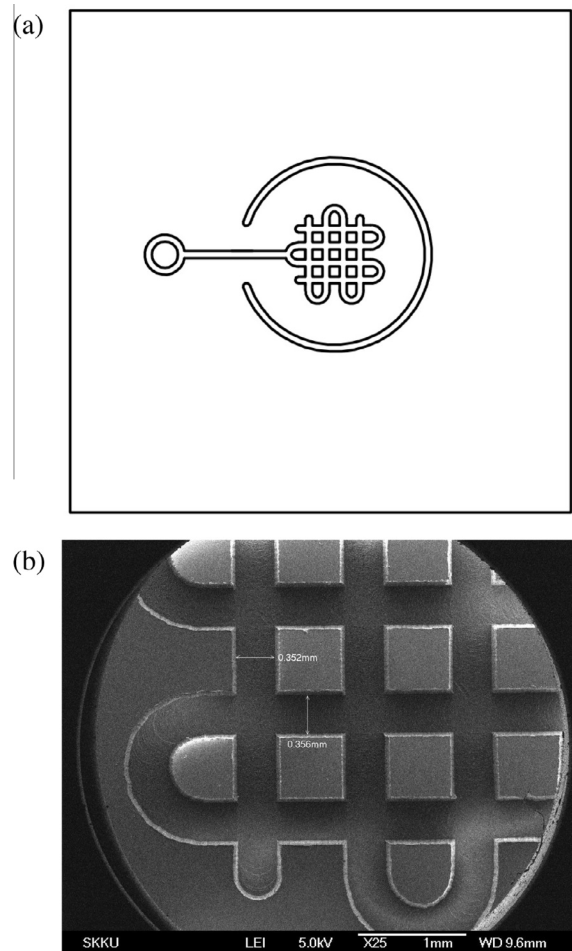



**Fig. 1.** (a) AutoCAD design for microfluidic device (b) SEM image of CNC engraved channel on the device.

## 2. Experiments

A device was designed and microfabricated to trap colorectal cancer cells. The device consisted of two components: a microfluidic device and a membrane filter. A polycarbonate plate was used to micromachine the microfluidic device, which was the device base. An AutoCAD design for the device is shown in Fig. 1(a). The microfluidic channels were prepared to maintain negative pressure for cell capture and a round hole on the left side was connected to the channels to create pressure. A CNC engraving machine was used to micromachine the polycarbonate microfluidic device. The movement speed of the machining tool was 300 mm/min with 15,000 rpm. The engraving depth was 500 μm and channel width was 350 μm. A scanning electron microscope (SEM) image for the CNC engraved channels is presented in Fig. 1(b). The drilled holes on the membrane filter will be aligned at the center of the cross area on the device for cell capturing.

A 1025 nm wavelength diode-pumped solid-state femtosecond laser was used to fabricate microholes on the membrane filter that was attached to the microfluidic device. The material for the filter was a SPI-Pore™ gelatin-coated PET membrane with a thickness of 13 μm. The gelatin coating protected cells held on the filter surface by negative pressure. Moreover, very smooth and clean surface are important to avoid cell damages from the negative pressures. The schematics of the femtosecond laser and the microfabrication system are shown in Fig. 2. Focusing lens, mirrors and attenuator are used for precise focusing and laser beam delivery. A JenLas D2.fs femtosecond laser (JENOPTIK) was prepared and equipped on the precise motorized stage. The laser had an average output power of 4 W and wavelength of 1025 nm. Pulse width was 380 femtoseconds and maximum pulse energy was 38 μJ at a pulse repletion rate of 100 kHz. Laser and beam specifications are shown in Table 1. The TEM00 beam mode was used for the fabrication. The laser beam was delivered through an attenuator and mirrors and focused by focusing lenses. The energy of the laser was controlled by the motorized attenuator between mirrors 3 and 4. Laser spot size of 4 μm and precise motorized stage were used to make

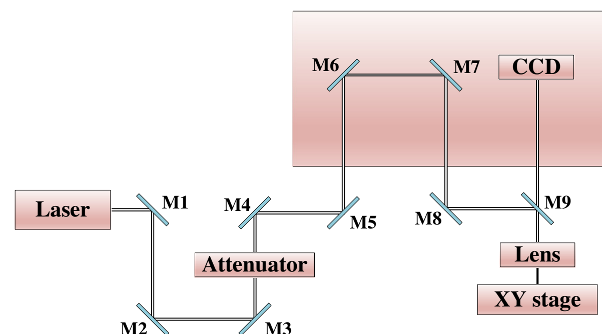



**Fig. 2.** Schematics of femtosecond laser microfabrication system.

**Table 1**
Femtosecond laser and beam specifications.

| Items | Specifications |
|---|---|
| Wavelength (nm) | 1025 |
| Pulse width (fs) | ~380 (up to 100 kHz) |
| Average output power (W) | 4 |
| Maximum pulse energy (μJ) | 38 at 100 kHz |
| Pulse repetition rate (kHz) | 30–200 |
| Beam mode | TEM00 |

microholes with diameters of 6–20 μm and to find out the relationship between the laser output power and average diameter of microholes. The laser beam spot was fixed and the motorized stage was moved spirally from the center to side to make smooth and clean surface.

The cross-sectional view of the device is presented in Fig. 3. The 13 μm thickness gelatin-coated PET membrane was attached and sealed on the microfluidic device using adhesive polyimide film. Microholes were laser microfabricated through the gelatin-coated PET membrane. Engraved channel depth was 500 μm and microfluidic device thickness was 1200 μm. Negative pressures inside the microfluidic device will capture the floating cells on the membrane. The experimental setup for cell trapping is shown in Fig. 4. After the membrane filter on the fluidic device was prepared, a suction hole on the left side was connected to syringe using the plastic tubes to generate negative pressure. The human colon cancer cell line HT-29 was provided by the American Type Tissue Culture Collection (ATCC, Manassas, VA, USA). The base medium for this cell line was ATCC-formulated McCoy's 5a Medium Modified (Catalog No. 30-2007). The cell line was cultured in McCoy's 5a Medium supplemented with fetal bovine serum at 10% at 37 °C in a humidified atmosphere with 5% $CO_2$, according to the culture method's recommendations. CRC cells were added to the celltrapping device by a celldropping pipette while the syringe created negative pressure in the channels. Cells floating over the microholes were captured on the holes by the negative pressure.

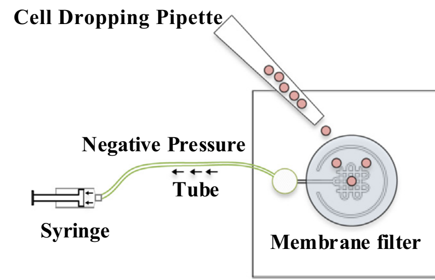
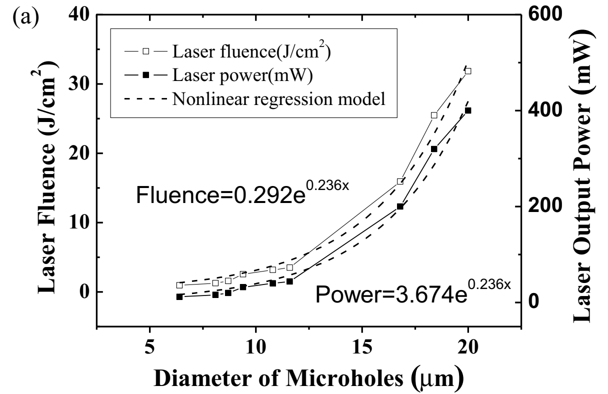



**Fig. 4.** Experimental set-up for the cell trapping.



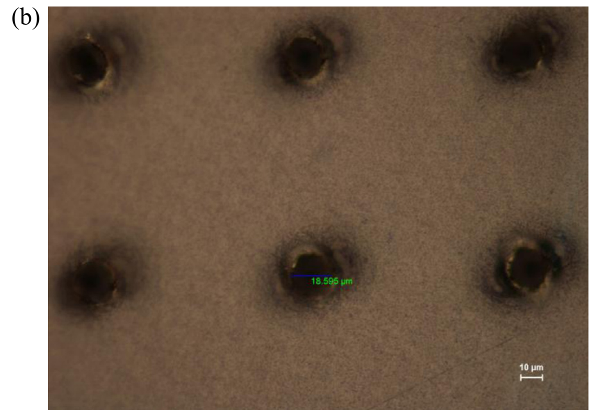




**Fig. 5.** (a) Diameter changes of the micro-holes in PET membrane according to the variation of laser output power and laser fluence (b) CCD image of the experimental results.

## 3. Results and discussion

An ultrafast laser was used to minimize surface damage of the microfabricated holes on the PET membrane. The relationship between the laser output power for pulses and the diameter of the microholes is presented in Fig. 5(a). Because the laser power is proportional to the laser fluence, the exponential relationship between the laser fluence and diameter of the microholes shows same trend with the relationship between laser power and diameter, although the coefficient values are different. The label for the laser fluence is printed on the left side of the axis in Fig. 5(a). Laser output power increased exponentially from 12 mW to 400 mW while the diameter of the microholes varied from 6 μm to 20 μm. Since the beam spot size was 4 μm and pulse repetition rate of the laser was 100 kHz, the laser energy fluence varied from 0.95 J/cm$^2$ to 31.83 J/cm$^2$ while the laser power changed from 12 mW to 400 mW. The exponent value of the exponential relationship is both 0.236 times diameters of the microholes and the coefficients for laser power and fluence are 3.674 and 0.292, respectively when we curve-fitted using the nonlinear regression method. Because the membrane was very thin as well as very soft, penetration depth was not concerned here. The hole was

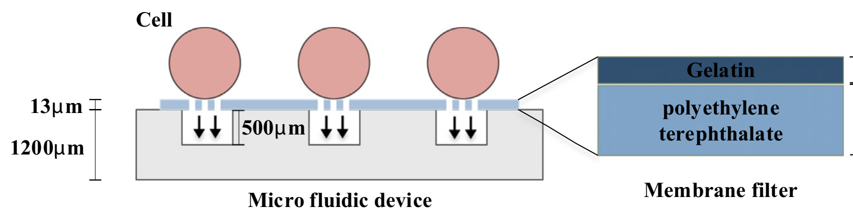



**Fig. 3.** Structures of the membrane filter and singular cell trapping device.

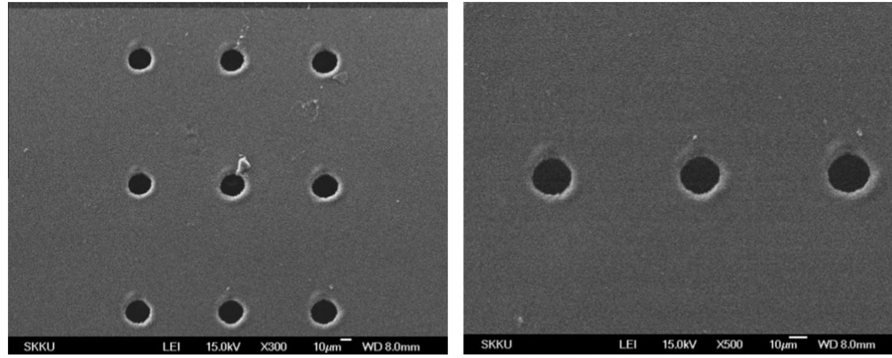



**Fig. 6.** SEM images of the laser microfabricated membranes.

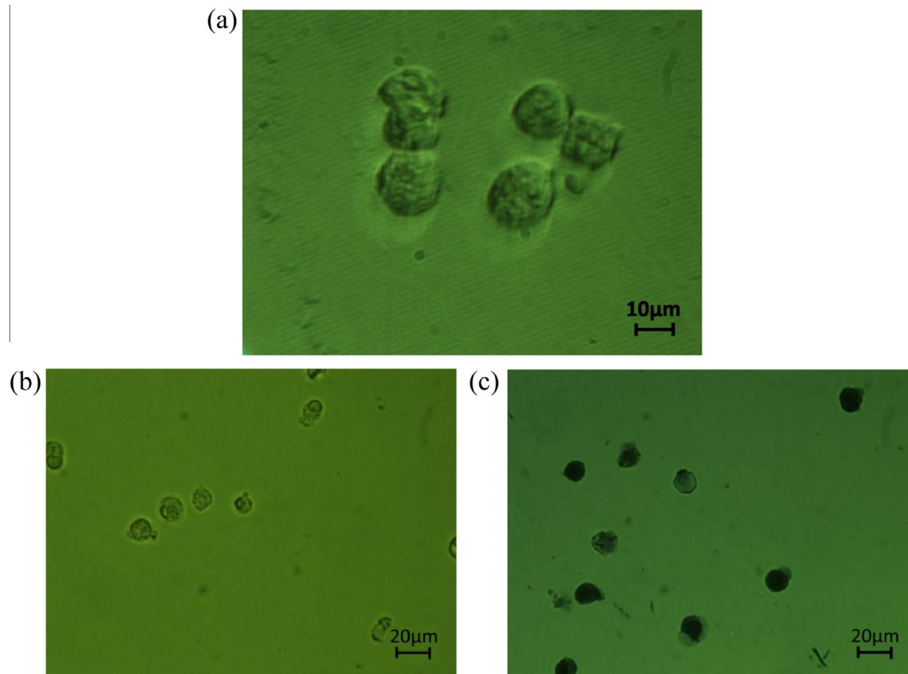



**Fig. 7.** Photomicrographs for colorectal cell line, HT29 (a) colorectal cancer cells, HT29 (×100 scale) (b) colorectal cancer cells (×50 scale) (c) dyed colorectal cancer cell using trypan blue. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

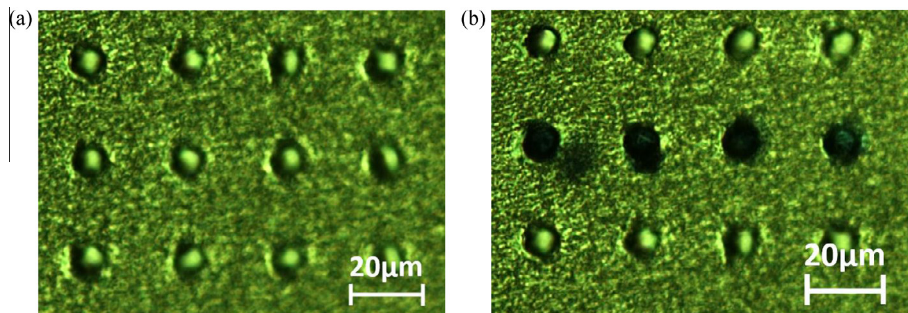



**Fig. 8.** (a) CCD image of capturing device and (b) CCD image of captured cells on the horizontal center line.

penetrated at even very low laser fluence of 0.95 J/cm$^2$. The laser microfabrication results are presented in Fig. 5(b). The hole-diameter was 19 μm. Since the pulse width was very short, thermal damage was very minimal. However, we can still observe some recast of slag and sharp edges near surface of the drilled hole. Because these problems were concerns for the safe trapping of the CRC cells, we changed the materials processing method from pulsed to spirally drilled. Maintain the beam spot size of 4 μm and output power of 32 mW which was laser fluence of 2.54 J/cm$^2$, we rotated the precise motorized stage five times from hole center to edge side after one pulse shot at the center of the hole. The SEM image of 3 × 3 matrix microholes which was spirally

drilled results was presented in Fig. 6. The result shows very low thermal distortion and precise machining of the PET membrane surface as well as smooth internal surfaces of the holes. The surface condition of the drilling holes is very important to capture the cells without physical damages. A 10 × 10 matrix was used to fabricate 100 holes on each PET membrane. The microhole size was determined as 10 μm to capture the CRC cells which show average diameter of 15 μm. Fig. 7(a) shows a microscopic image of CRC cells magnified by a factor of 100. The average diameter of the CRC cells which was used for our experiment was 15 μm. A microscopic image of CRC cells magnified by a factor of 50 is also presented in Fig. 7(b). Fig. 7(c) shows trypan blue-stained CRC cells captured by negatively pressured microholes on the membrane filter. Negative pressure was maintained by a syringe connected to suction hole on the membrane filter. We microfabricated diameters of 10 μm holes to capture the CRC cells on the device. A CCD image of the capturing device and captured cells on a horizontal center line are shown in Fig. 8(a) and (b). Maintaining negative pressure inside the channels between the polycarbonate plate and the PET membrane, stained CRC cells were dropped by a cell dropping pipette and three cancer cells were captured on the horizontal centerline. These CRC cells can be manipulated simultaneously which is very important consideration in cell manipulation experiments, because all cells can be treated under the same environment. Our results collectively indicated that targeting CRC cells with a trapping device was an effective and novel approach for diagnosis or treatment of CRC. Moreover, a cell-trapping device is a useful strategy for prescreening for CRC in human clinical specimens. However, further in-depth investigations are needed to establish the mechanism between metabolic profiles of cancer cells and the structure of CRC cells. We have also been able to apply this device in veterinary medicine for an animal cancer model as well as a human cancer one. Sufficient comprehension about the principles of operating of a device and the microenvironments of cancers are a rationale for using devices to study combinations of cancers to improve preclinical outcomes. Our initial findings show the potential applications of cell-trapping devices as a new category of cancer diagnostics.

## 4. Conclusion

We designed and manufactured a microfluidic device for capturing singular CRC cells using polycarbonate plates and gelatin-coated PET membranes. Microfluidic channels for the device were micromachined using a CNC engraving machine. Moreover, we microfabricated very clean and smooth surfaced multiple microholes on the PET membranes which were not reported yet using an ultrafast, 1025 nm diode-pumped solid-state femtosecond laser. The average diameter of the holes was exponentially increased with increase of the laser output power. A gelatin-coated PET membrane was attached to a polycarbonate device and a syringe with a tube controlled negative pressure inside the channels of the celltrapping device. While negative pressure was maintained inside the channels between the polycarbonate plate and the PET membrane, the CRC cells were dropped by a dropping pipette and captured on the microholes. We made the cell trapping device for diagnosis of cancer and this trial was first time in colorectal cancer detection. The diameters of microholes of the cell trapping device were 6–20 μm. Because we can easily control the size of the microholes, our cell trapping device can capture not only the solid tumor cells but also acute myeloid leukemia (AML) cells. Moreover, the cell-trapping device can be cultured the CRC cells for manipulation under same environmental conditions. The combination of a cell-trapping device and human cancer cells are very important for diagnosis and discovery of tumor biomarkers.

## References


[1] K. Jo, Y.L. Chen, J.J. de Pablo, D.C. Schwartz, Lab Chip 9 (2009) 2348–2355.
[2] M. Danova, M. Torchio, G. Mazzini, Exp. Rev. Mol. Diagn. 11 (2011) 473–485.
[3] D.C. Colter, I. Sekiya, D.J. Prockop, Proc. Natl. Acad. Sci. 98 (2001) 7841–7845.
[4] M.E. Lidstrom, D.R. Meldrum, Nat. Rev. Microbiol. 1 (2003) 158–164.
[5] A. Karimi, S. Yazdi, A.M. Ardekani, Biomicrofluidics 7 (2013) 021501.
[6] G. Vona, A. Sabile, M. Louha, V. Sitruk, S. Romana, K. Schütze, P. Paterlini-Bréchot, Am. J. Pathol. 156 (2000) 57–63.
[7] T.A. Crowley, V. Pizziconi, Lab Chip 5 (2005) 922–929.
[8] J. Nilsson, M. Evander, B. Hammarström, T. Laurell, Anal. Chim. Acta 649 (2009) 141–157.
[9] R. Pethig, Biomicrofluidics 4 (2010) 022811.
[10] S. Patel, D. Showers, P. Vedantam, T.R. Tzeng, S. Qian, X. Xuan, Biomicrofluidics 6 (2012) 034102.
[11] Z. Gagnon, J. Mazur, H.C. Chang, Biomicrofluidics 3 (2009) 044108.
[12] M. Muratore, V. Srsen, M. Waterfall, A. Downes, R. Pethig, Biomicrofluidics 6 (2012) 034113.
[13] R.M. Johann, Anal. Bioanal. Chem. 385 (2006) 408–412.
[14] S. Miltenyi, W. Müller, W. Weichel, A. Radbruch, Cytometry 11 (1990) 231–238.
[15] J.G. Yu, C.W. Lee, H.W. Choi, J. KWJS 28 (2010) 42–48.
[16] A.A.S. Bhagat, H. Bow, H.W. Hou, S.J. Tan, J. Han, C.T. Lim, Med. Biol. Eng. Comput. 48 (2010) 999–1014.
[17] J. Friend, L.Y. Yeo, Rev. Mod. Phys. 83 (2011) 647.
[18] A. Lenshof, T. Laurell, Chem. Soc. Rev. 39 (2010) 1203–1217.
[19] N. Bellini, K.C. Vishnubhatla, F. Bragheri, L. Ferrara, P. Minzioni, R. Ramponi, R. Osellame, Opt. Express 18 (2010) 4679–4688.
[20] F. Bragheri, L. Ferrara, N. Bellini, K.C. Vishnubhatla, P. Minzioni, R. Ramponi, I. Cristiani, J. Biophotonics 3 (2010) 234–243.
[21] K.R. Kim, Korean J. Mater. Res. 18 (2008) 247–252.
[22] R.R. Gattass, E. Mazur, Nat. Photonics 2 (2008) 219–225.
[23] Z. Fei, X. Hu, H. Choi, S. Wang, D. Farson, L. Lee, Anal. Chem. 82 (2010) 353–358.
[24] M.M. Center, A. Jemal, E. Ward, Cancer Epidemiol. Biomark. Prev. 18 (2009) 1688–1694.
[25] M.E. Forgue-Lafitte, A.M. Coudray, B. Bréant, J. Mešter, Cancer Res. 49 (1989) 6566–6571.