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## Thermoplastic microfluidic devices and their applications in protein and DNA analysis

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### Abstract

Microfluidics is a platform technology that has been used for genomics, proteomics, chemical synthesis, environment monitoring, cellular studies, and other applications. The fabrication materials of microfluidic devices have traditionally included silicon and glass, but plastics have gained increasing attention in the past few years. We focus this review on thermoplastic microfluidic devices and their applications in protein and DNA analysis. We outline the device design and fabrication methods, followed by discussion on the strategies of surface treatment. We then concentrate on several significant advancements in applying thermoplastic microfluidic devices to protein separation, immunoassays, and DNA analysis. Comparison among numerous efforts, as well as the discussion on the challenges and innovation associated with detection, is presented.

### 1. Introduction

Microfluidics is a platform technology that has been used for genomics, proteomics, chemical synthesis, environment monitoring, cellular studies, and other biomedical applications.<sup>1–3</sup> Benefits of conducting such a study in a microfluidic device may include low sample and reagent consumption, large surface-to-volume ratio, high-throughput, portability, and potential for point-of-care uses, making the device an alternative to a traditional bench-top apparatus.<sup>1–3</sup> One of the promises of the field is that multiple functions such as sample preparation may be integrated in the device, achieving the goal of “lab on a chip” (LOC). Although there have been significant advances in the field through a large number of research groups and efforts in the past two decades, the most of earlier promises including proliferation of commercial LOC systems “have not yet happened”.<sup>4</sup>

One of the reasons of the slow commercialization could be the device material used. Most pioneering works were carried out in silicon and glass devices<sup>5–8</sup> while many efforts and exciting development have been achieved in devices made from poly-(dimethylsiloxane) (PDMS).<sup>9–12</sup> Although each of these materials has been used in commercial products, they are generally considered as not ideal for the manufacturable process. For instance, PDMS is a great material for prototyping, especially in an academic setting, but it is “not readily or economically formed in high-throughput production.”<sup>13</sup>

Thermoplastics, including polycarbonate, poly(methyl methacrylate) (PMMA), cyclic olefin copolymer (COC), polystyrene, and poly(ethylene terephthalate), have been emerging as commercially viable fabrication materials, since they are amenable for industrial manufacturing and yet possess excellent optical properties such as low intrinsic fluorescence and broad visible transmittance. The process of manufacturing low-cost, high-volume plastic parts with micro-scale features is well-developed in other fields, exemplified by the ubiquitous compact disc (CD) that consists of microgrooves embossed on the back side of a thermoplastic.<sup>14</sup> Additionally, their biocompatibility (evidenced from plastic labwares), wide range of mechanical stiffness, and convenient prototyping methods outside clean-room environment allow rapid implementation of numerous research interests and thereby accelerate the advancement of microfluidics.

As a result, we chose to review the recent development of thermoplastic microfluidic devices, excluding those made from other polymers/plastics. We focused on protein separation for proteomics studies, immunoassays, and DNA analysis. For readers who are new to field, we presented the device design and fabrication methods so that they will understand the *pros* and *cons* of different methods and the efforts required before getting hold of a device for the endeavors they might pursue. For other readers, we discussed in length on the strategies of surface treatment and reviewed innovative detection approaches enabled by thermoplastics, both of which are important to their applications and to commercialization of microfluidics.

## 2. Device fabrication

Numerous merchant devices associated with various disciplines including biology, chemistry, medicine, and therapy, are made from thermoplastics. Commercially available thermoplastics, specifically PMMA,<sup>15–26</sup> COC,<sup>27–39</sup> and polycarbonate,<sup>40–44</sup> have also been utilized as the fabrication materials of a number of microfluidic devices over the past few years due to their optical properties, manufacturability, and other attributes mentioned above. The overall fabrication process and methods<sup>14,45–47</sup> are delineated below and illustrated in Fig. 1.

To start the fabrication process, a master or molding die must be generated first (Fig. 1a). One way is to use conventional photolithography and chemical etching to make a pattern in a glass, silicon, or other substrate, followed by electroplating to form a molding master.<sup>14,48</sup> This process can be classified into the LiGA (Lithographie-Galvanoformung-Abformung, German for lithography-electroplating-molding) process, though LiGA was originally associated with using X-rays produced by a synchrotron to create high-aspect ratio structures (UV-LiGA now also exists).<sup>49</sup> An alternative method of generating a molding master is to directly fabricate microstructures in a metal, including computer-numerically-controlled (CNC) precision milling, laser ablation, or other approaches.<sup>50</sup> After obtaining the molding master, a large number of high fidelity replicates can be made from the master in a relatively rapid manner as shown in Fig. 1b. Injection molding, compression molding, embossing are popular among the molding methods. The master can be repeatedly used with faithful replication.

For prototyping and low-volume device fabrication, direct patterning onto a thermoplastic substrate may be used without generating an expensive molding master and undertaking the time-consuming process. The direct fabrication methods include CNC milling, laser ablation and direct lithography,<sup>23,51–54</sup> all of which are suitable for producing high-aspect-ratio channels on a wide range of polymer surfaces regardless of their hardness. However, it should be noted that the direct fabrication techniques have the drawbacks of rough channel surfaces, curvature-shaped corners, and possible changes in the surface chemistry of the

polymer.<sup>39,54</sup> In addition, methods like lamination-based prototyping, wire-imprinting, and *in situ* photo-initiated polymerization have also been reported in the literature.<sup>36,55,56</sup>

To complete a device, a substrate with patterned microstructures must be sealed with a film or sheet to form channels or other closed architectures as shown in Fig. 1c. Before bonding, inlets and outlets can be created by drilling holes at the ends of channels. The bonding methods include thermal diffusion bonding,<sup>26,30</sup> plasma or ozone-activated bonding,<sup>36</sup> lamination,<sup>14,48,56</sup> solvent-assisted sealing,<sup>43,51,57-60</sup> and use of an adhesive layer.<sup>56,61</sup> The bonding process may often be viewed to be trivial, but it is a critical step to have a fully assembled, functional device. Using different bonding methods will affect the device quality and performance as discussed in the literature.<sup>62</sup> We compared these methods for the compatibility of a device with chemical and biological reagents to be used, the sustainable pressure for pumping, possible distortion of microstructures due to a high pressure/temperature or an additional adhesive layer, possibility to compensate the surface topology by filling micro-voids or smoothing surfaces, surface property changes due to exposure to solvent or others, and applicability of the bonding method to bonding two completely different types of thermoplastic sheets, as summarized in Table 1.

### 3. Surface treatment

Thermoplastics are generally hydrophobic. As a result, surface adsorptions exist when a sample contains proteins, peptides, cells, or other reagents with hydrophobic moieties. The interactions between these reagents and channel surfaces not only broaden the peaks when electrophoretic separation takes place, but also change the surface property and thus electroosmotic mobility; all of them degrade the separation resolution, efficiency and reproducibility. In addition, surface adsorption may cause appreciable sample loss. Therefore, a variety of surface treatment methods have been explored by a number of groups to diminish the effects of surface adsorption.

As in capillary electrophoresis, there are two major categories for surface treatment of thermoplastic channels: dynamic coating and permanent chemical modification. Dynamic coatings include the use of ionic or nonionic small molecules, as well as linear polymers, in the separation buffer. They have been used to prevent protein adsorption and suppress electroosmotic flow in a plastic microfluidic device. We have employed hydroxyethylcellulose to dynamically coat COC channels and found out that the electroosmotic mobility decreased by 72%.<sup>63</sup> Isoelectric focusing of proteins was carried out in the device. Shadpour and Soper employed the dynamic coating of methyl hydroxyethylcellulose for protein separation in a PMMA device.<sup>26</sup> The electroosmotic mobility was determined to be reduced from  $(1.56 \pm 0.05) \times 10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$  to  $(1.20 \pm 0.07) \times 10^{-5} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ . The peak capacity and theoretical plate height for a 10 mm separation distance were 59 and 0.87  $\mu\text{m}$ , respectively. Although the dynamic coating addresses the surface adsorption and is convenient to carry out, it requires the sample and buffer solution containing the reagents used for dynamic coating so that coating can be continuously replenished. For those applications coupled with a mass spectrometer in the downstream of the analysis, the use of dynamic coating becomes problematic since it could complicate the peak identification in the mass spectrum.

An alternative surface treatment method is permanent chemical modification by irreversibly attaching a moiety to polymer surfaces. For instance, Soper's research group reported formation of a layer of linear polyacrylamide on the channel surfaces of a PMMA device *via* an amide bond and they achieved high-resolution electrophoretic separation of single-stranded DNA with a single base pair resolution.<sup>64</sup> Polyacrylamide can also be coated on the surfaces of PMMA, COC and polycarbonate *via* photografting.<sup>32,65</sup> Other materials such as

poly(ethylene glycol) (PEG) have also been grafted onto the channel surfaces of a PMMA device for electrophoresis of proteins and peptides.<sup>17</sup> Separation efficiencies up to  $5.2 \times 10^4$  plates were obtained for a 3.5 cm long separation channel. The channel surfaces were often activated using ultraviolet/ozone or oxygen plasma before grafting.<sup>17,32,65</sup> COC surfaces may also be coated with silanes and silica.<sup>66,67</sup>

Permanent surface treatment also possesses shortcomings. One is that the coatings are often not sustainable in a harsh chemical environment and some covalent bonds (*e.g.* Si–O–C) are prone to hydrolyze in an aqueous environment. The other drawback is that the modification procedures are typically time-consuming and require skilled personnel. The fundamental approach to address the concerns is to create plastic materials that require no surface treatment. For instance, Lee's research group used monomers containing PEG moiety to form a polymer backbone that has desired properties without the requirement of surface treatment.<sup>55,68</sup> Three types of acrylate monomers were mixed at an appropriate ratio to form a copolymer substrate and the device was successfully demonstrated for separation of proteins and peptides. High theoretical plate numbers ( $4.7 \times 10^4$  plates per 3.5 cm) and the symmetrical shape of protein peaks indicate negligible interactions between the channel surface and proteins.<sup>55,68</sup> Along the same line, the authors also synthesized a monomer to create a PMMA derivative, namely poly(glycidyl methacrylate-*co*-methyl methacrylate).<sup>69,70</sup> A device made from this polymer was found to be superior to traditional PMMA in terms of surface treatment owing to the presence of epoxy functional groups in the backbone. Electrophoretic separation of multiple proteins and peptides was carried out in the devices with high separation efficiency and reproducibility.

Although the discussion above has been focused on the surface treatment for electrophoretic separation, these methods and/or their variations are generally applicable to other applications (since the goal is the same, for preventing molecules from being absorbed on thermoplastic surfaces). For instance, Zhao *et al.* oxidized the surfaces of COC using ozone to obtain carboxylic acid groups, followed by covalent binding for DNA microarray hybridization.<sup>71</sup> Similarly, Liu *et al.* modified the channel surface in a PMMA device using poly(ethyleneimine) to obtain amine groups, followed by glutaraldehyde treatment for antibody binding.<sup>72</sup>

In the light of the work discussed above, in particular the efforts by Lee's group,<sup>55,68–70</sup> the design and synthesis of new or modified polymer substrates specifically for microfluidic applications could be a very fruitful direction. They likely lead to materials with desirable physical and chemical properties for a wide range of applications targeted by the microfluidics community.

## 4. Protein analysis

### Two-dimensional protein separation

Protein separation and identification are important in both medical diagnostics and laboratory research. Among the techniques used for proteomics studies, two-dimensional gel electrophoresis (2DGE) remains "one of the most potent methods".<sup>73</sup> Conventional 2DGE consists of isoelectric focusing (IEF) as the first dimension and polyacrylamide gel electrophoresis (PAGE) as the second dimension. In analogy of DNA sequencing that has shifted from slab gel electrophoresis to capillary array electrophoresis,<sup>74,75</sup> 2DGE has been implemented in parallel microchannels in a device by a number of research groups.<sup>76,77</sup> The potential advantages of microfluidics-enabled 2DGE include faster electrophoresis and higher separation resolution as a result of higher electric fields applied to a microdevice, enhanced reproducibility due to elimination of gel-warping and other features associated with microfluidics.

Due to the aforementioned advantages of thermoplastics, efforts have been made in implementing 2DGE in thermoplastic devices.<sup>26,53,77–83</sup> Table 2 lists these efforts in the chronological order of the publications. An early example by Griebel *et al.* was a credit-card-sized PMMA device, which was comprised of an IEF slot in the top layer and 300 parallel PAGE channels in the bottom layer (Fig. 2a).<sup>79</sup> IEF was carried out using an immobilized pH gradient strip as in conventional 2DGE. The transfer of proteins from IEF to PAGE was carried out by a sequence of voltage controls on three electrodes (in the IEF slot and two buffer reservoirs); only a fraction of proteins were transferred due to the difference between macroscale IEF and microscale PAGE. The protein separation in parallel PAGE channels (Fig. 2b) is similar to the earlier work of DNA sequencing in 96 capillaries.<sup>84</sup> Although authors characterized the protein separation in each dimension as well as sample transfers between two dimensions, a 2D map of multiple proteins was not reported presumably due to the difficulty in the protein transfer between different scales.

Both IEF and PAGE were carried out in the microscale in several efforts later on. The key challenge to implement 2DGE in a microdevice is the interface design, which must (1) allow the introduction of two separation media into the respective dimension without cross-contamination and (2) fully transfer proteins from the first to the second dimension with negligible disturbance on protein bands. To address the challenge, different methods have been explored, including the use of viscous separation medium,<sup>78</sup> pseudo-valve arrays,<sup>81</sup> gel plugs in appropriate locations,<sup>53,83</sup> and narrower interface channels.<sup>85</sup> All of these interface designs demonstrated various degrees of success in the introduction of the separation media and the transfer of protein samples from the first to the second dimension.<sup>76</sup>

Rather than integrating one channel (first dimension) with an array of channels (second dimension), work has also been carried out by coupling two orthogonal channels, in which analytes eluted from one channel (as the first dimension) are consecutively transferred into the other channel (as the second dimension). The consecutive, sequential transfers of analytes between two dimensions require the waiting time, but the published results exemplified by the work of Soper and his colleagues are very impressive.<sup>82</sup> Fig. 3a shows their PMMA device consisting of a channel for PAGE and an orthogonal channel for micellar electrokinetic chromatography (MEKC). The effluents from the first dimension (PAGE) were transferred into the second dimension periodically, at a sampling frequency of 1 Hz, followed by MEKC separation. A 2D map of fetal calf serum proteins was developed within 30 min (Fig. 3b), yielding an average peak capacity of 2600. The peak capacity was three times larger than that obtained from the same sample using conventional 2DGE.

While the performance of some microfluidics-enabled devices is reaching to (or slightly better than) the level of conventional slab gels, it should be noted that one of the key advantages is its rapid analysis. The analysis time in the most work listed in Table 2 is 30 minutes or less. In contrast, conventional 2DGE is often completed in 2 days.

## Immunoassays

Immunoassays measure the concentration of analytes in a sample using specific immunological reaction of antibodies to their target antigens. They are extensively used in biomedical research, clinical diagnostics, food/water safety testing, and biological warfare defense. Among several modes of immunoassays, one common format is enzyme-linked immunosorbent assay (ELISA).

Efforts to implement ELISA in microfluidic devices have been made by several research groups.<sup>72,86–95</sup> Among them, thermoplastic devices have also been employed.<sup>72,92–95</sup> For instance, Liu *et al.* developed a PMMA device to determine  $\alpha$ -fetoprotein, a hepatocellular carcinoma biomarker from human serum.<sup>72</sup> The channel surface was modified with amine

groups, followed by covalent immobilization of  $\alpha$ -fetoprotein antibody. After antigen capture, a horseradish peroxidase-conjugated secondary antibody was used, followed by detection. Woolley's research group integrated an immunoaffinity column with electrophoretic separation in a PMMA device for detection of the same biomarker.<sup>95</sup> The purification step through the immunoaffinity column and the separation step *via* electrophoresis enable multiple biomarkers to be used for potential prognosis and diagnosis.

Detection of  $\alpha$ -fetoprotein using ELISA has also been implemented in a compact disc (CD)-shaped microdevice. Honda *et al.* exploited commercially available Gyrolab CD devices as an immunoassay platform for detection of several biomarkers in 200 nL of samples.<sup>96</sup> The 12 cm-diameter CD devices were made from polycarbonate and they were manufactured using injection molding.<sup>97</sup> As shown in Fig. 4a, there are 112 parallel analysis units in one CD. Each unit consists of various functional elements based on capillary action, centrifugal force, and hydrophobic barriers as illustrated in Fig. 4b. These elements enabled reagent/sample introduction, precise metering, and flow controls. The precision of 112 parallel volume definition operations was determined to be 0.75% CV (coefficient of variation) at 200 nL.

Compact disc is a unique microfluidic platform for running immunoassays. The rotation speed of a CD dictates the magnitude of centrifugal force, thus it can be adjusted to control the passage of flows in microstructures with different channel resistance and/or hydrophobic properties as exemplified in Fig. 4b. Moreover, it is possible to use the data-reading optics and electronics already incorporated in a conventional CD player. Yu's research group reported using a standard optical drive of an ordinary computer, without any modification of hardware or software, to demonstrate three different types of biochemical recognition reactions in a CD device.<sup>98</sup> Lee *et al.* described a PMMA-based CD device to perform ELISA for hepatitis detection in whole blood samples.<sup>93</sup> In addition to those mentioned above, a number of research groups as well as commercial entities have explored this platform for a variety of applications.<sup>99–102</sup>

## 5. DNA analysis

Polymerase chain reaction (PCR) is an important tool for genomics studies, forensic analysis, and medical diagnostics. Significant efforts have been made in carrying out PCR in a microfluidic device as reviewed by Landers and colleagues.<sup>103</sup> Some of these works were implemented in thermoplastic devices. For instance, Sundberg *et al.* fabricated a three-layer thermoplastic CD that consists of 1000 compartments for PCR.<sup>104</sup> Sample introduction to an individual compartment was enabled by centrifugal forces. A 300 bp plasmid DNA was amplified in each compartment of 33 nL and analyzed in 50 min for whole disc. Klapperich and coworkers described an integrated COC device and the associated instrument for the detection of bacteria (*Bacillus subtilis* was used).<sup>52</sup> The system conducted bacterial lysis, nucleic acid isolation, PCR, and fluorescent detection.

Bau's research group reported a polycarbonate device for real-time PCR.<sup>44</sup> The device allowed pre-storage of paraffin-encapsulated reagents in the PCR chamber and they were reconstituted when thermocycling was initiated after aqueous solutions were introduced. Detection was achieved by using waveguides to couple the device with a fluorescence reader. The authors also integrated PCR with other components in a thermoplastic cassette.<sup>105</sup> As shown in Fig. 5a, the cassette contained flexible pouches for reagent storage and valves for flow controls. The process operations included sample introduction, cell lysis, solid-phase extraction of nucleic acids, elution of the nucleic acids into a PCR chamber, and thermal cycling. Afterward, amplicons (PCR products) were transported onto an integrated

lateral flow strip, where detection took place. The functionality of the device was demonstrated by detecting the presence of HIV virus in saliva samples.<sup>105</sup>

A similar effort with a different design and detection scheme was reported by Liu *et al.*<sup>106</sup> As shown in Fig. 5b, a thermoplastic device consists of microfluidic mixers, valves, pumps, channels, chambers, heaters, and DNA microarrays. Cavitation micro-streaming was implemented to enhance mixing while thermally actuated microvalves and electrochemical pumps were integrated to regulate flows. Pathogenic bacteria detection from whole blood samples was demonstrated.<sup>106</sup>

As illustrated in Fig. 5b, DNA microarray is one of the means to analyze amplicons. However, conventional DNA microarrays are fabricated on glass slides and they are “not easily adaptable to integration into microfluidic systems”.<sup>71</sup> Therefore Zhao *et al.* studied polymeric materials and compared PMMA and COC with glass slides for DNA microarray hybridization.<sup>71</sup> They extracted RNA from four human respiratory viruses (IAV: influenza A virus, EV: enterovirus, RSV: respiratory syncytial virus, and MPV: metapneumovirus), followed by reverse transcriptase PCR. These samples went through DNA hybridization on both glass and plastic slides printed with DNA probes. Two thermoplastics, PMMA and COC (Zeonor 1060R) were modified to have carboxylic acid groups on the surface before use. As shown in Fig. 6, both materials had comparable performance with a glass slide. Yu’s research group also investigated DNA hybridization on polycarbonate substrates, which were activated using UV ozone treatment.<sup>107</sup> Comparable selectivity and efficiency of hybridization were observed relative to other materials.

It should be noted that all of these efforts have aims to be used in a clinic environment. As a result, potential cross-contamination of sequential samples is of concern if a device is reused. A disposable, low-cost device would be ideal for such applications. High-volume manufacturing capability in plastic parts with micro-scale features (*e.g.* CD) is one of the driving forces to explore thermoplastic microfluidics for DNA-based diagnostics.

## 6. Detection

Detection is often a challenge when a tiny amount of a sample is analyzed in a microfluidic device. Moreover, the device often possesses a significantly shortened path length when optical detection is used. According to Beer–Lambert law,  $A = abc$ , where  $A$  is the absorbance,  $a$  is the absorptivity,  $b$  is the optical path length, and  $c$  is the analyte concentration. The optical path length from mm-scale in a conventional cuvette to  $\mu\text{m}$ -scale in a microchannel dramatically reduces the detection sensitive, which is the reason that laser-induced fluorescence detection is often the default detection method for microfluidics. As a result, a variety of innovative detection methods have been explored as reviewed by Myers and Lee.<sup>108</sup>

Thermoplastics could offer additional advantage in addressing this challenge. Their facile fabrication procedures enable the device to possess optical-quality surfaces, integrate optic elements, and have specialized channel/well shapes. For instance, Ducree and colleagues fabricated a pair of triangle ridges (*i.e.*, V-grooves from the bottom surface of the substrate in Fig. 7a) in a CD device.<sup>109</sup> When a laser beam was directly at an incident angle exceeding the critical angle of the construction material (COC), total internal reflection occurred. As illustrated in Fig. 7a, light was guided to take place along the planar length rather than the vertical height of a channel. The increased optical path length in this integrated detection cell resulted in an improvement factor of 10 when they used the device for detection of glucose in blood. Similarly, Soper’s research group reported embedding a COC waveguide into a multi-channel PMMA device with an orthogonal configuration.<sup>110</sup> Light propagated through the COC waveguide and simultaneously illuminated 11 PMMA channels.

MacCraith's research group recently described a COC device consisting of an array of wells.<sup>111</sup> As shown in Fig. 7b, the wells were made in a shape of paraboloid, which substantially enhanced the fluorescence collection efficiency due to the phenomenon of supercritical angle fluorescence (SAF, which refers to a fact that substantial amount of fluorescence is emitted into angles above a critical angle). The SAF phenomenon also confined the fluorescence detection volume strictly to the close proximity of the paraboloid surface, thereby discriminating against fluorescence background from the analyte solution. The increased signal-to-noise ratio resulted in 20-fold improvement when they incorporated it with surface plasmon-coupled emission detection.

## 7. Conclusion

Microfluidics has been significantly advanced since the pioneering work in earlier 1990s.<sup>5-8</sup> The recent shift of the bulk of the research activities from silicon/glass to manufacturable thermoplastics could help the commercialization process of microfluidics. As discussed by Becker, a transition "from prototypes to volume manufacturing" must be a part of the development phase.<sup>112</sup> Many of the work discussed in this review will assist this transition and help bridge the gap between the research activities and commercialization efforts. The advantageous attributes of thermoplastics, strategies in surface treatment, demonstration of thermoplastic microfluidic devices for a range of applications, and innovative techniques to improve the detection sensitive are a part of the overall endeavors that will help eventually reach the holy grail of LOC.

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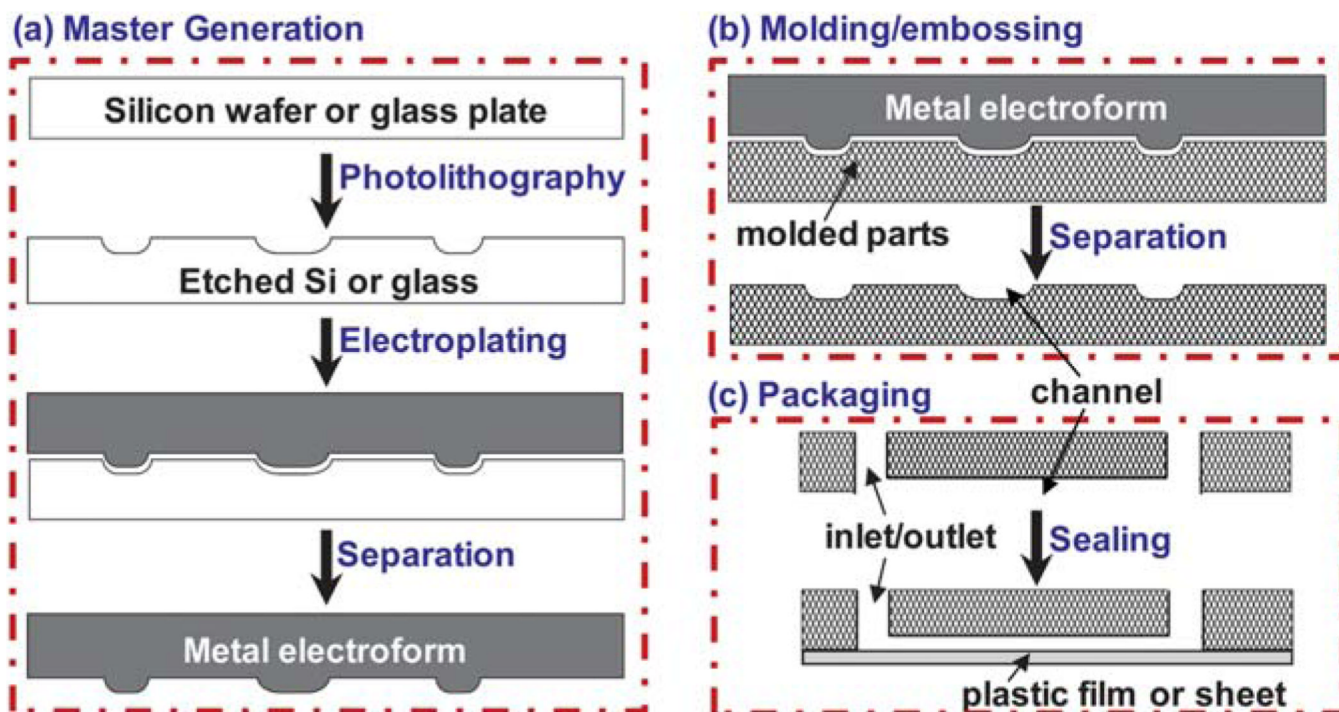
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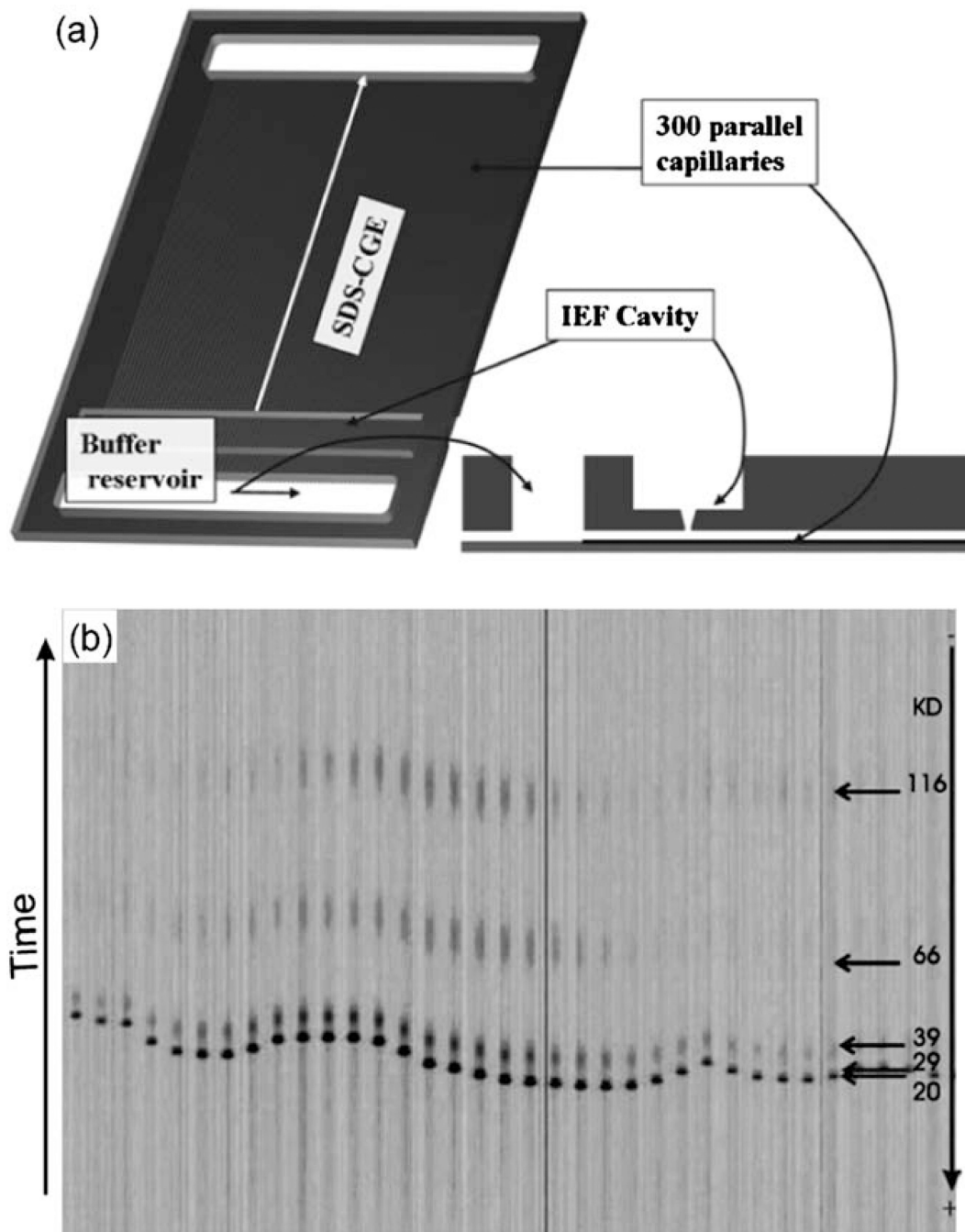
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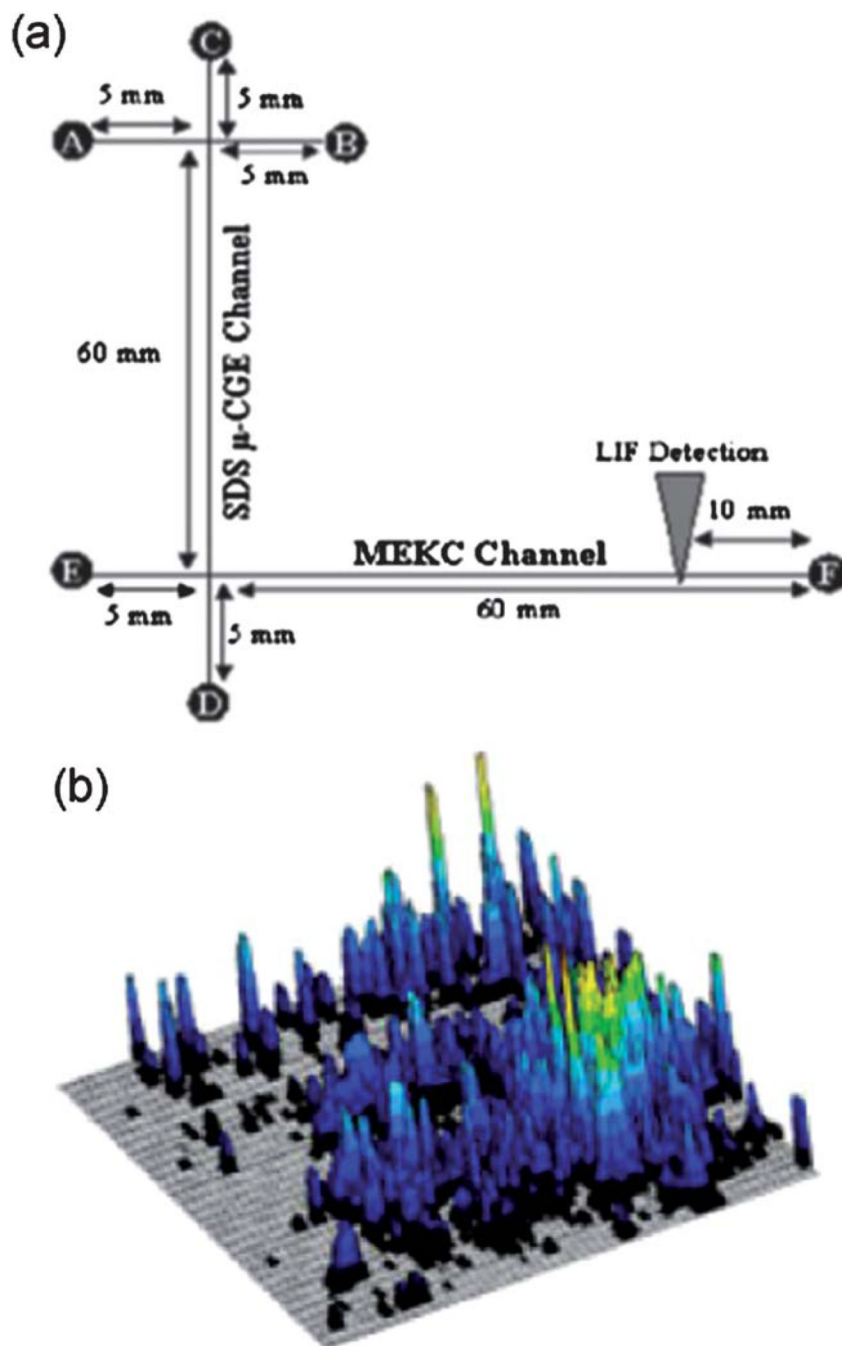


**Fig. 1.**

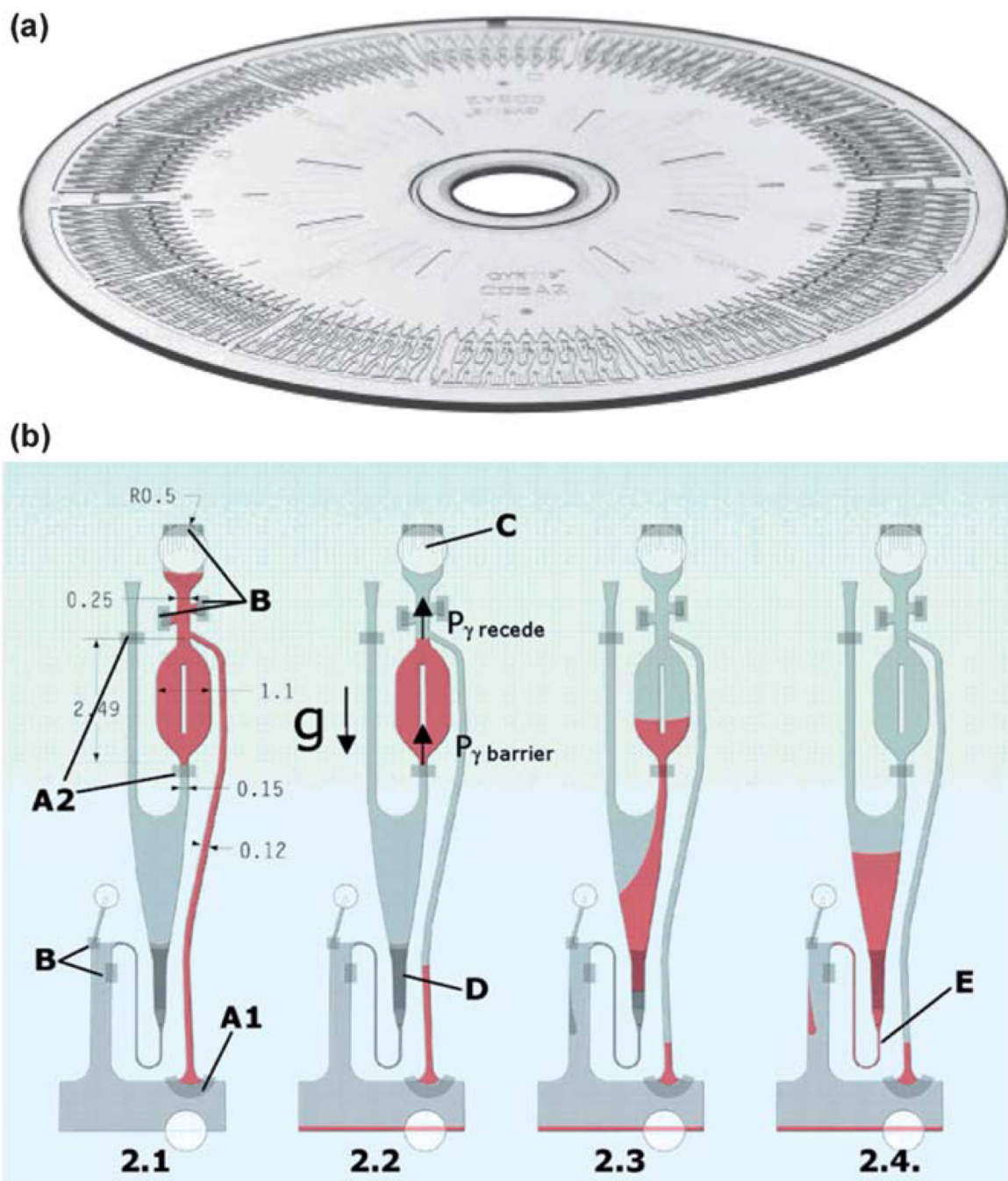
(a) The process of generating a master or molding die. A pattern of microfeatures (*e.g.* channels or chambers) is created in a silicon or glass substrate using photolithography. A metal mold (called electroform) is produced by electroplating on the patterned substrate. Note that the topology of the mold is exactly the opposite of the patterned silicon or glass substrate; a channel in the substrate becomes a ridge in the mold. (b) Plastic parts are fabricated using molding or embossing. The channels are in the direction out of the paper. Note that the topology of the plastic part is a negative image of the mold. Therefore, the plastic part has the exactly same shape as the patterned silicon or glass substrate. Repetitive molding would produce a large number of plastic parts without going through the photolithography steps for each individual device. (c) Packaging steps include drilling the inlet/outlet at the ends of each channel, followed by sealing the part with a plastic film or sheet. Shown in (c) is the molded part in (b) that is flipped vertically and then rotated 90° so that the channel is in the direction along the paper to show the inlet/outlet.



**Fig. 2.**  
 (a) Schematic representation of the PMMA device for 2DGE. The credit-card sized device (85 mm × 55 mm × 2 mm) contains a cavity for IEF and two buffer reservoirs that are connected by 300 parallel channels (50 μm × 50 μm × 64 mm) for PAGE. The IEF cavity and the PAGE channels are connected to each other through a 50 μm wide opening. (b) Electropherograms of six proteins in parallel PAGE channels after 1 cm separation. Adapted from ref. 79.



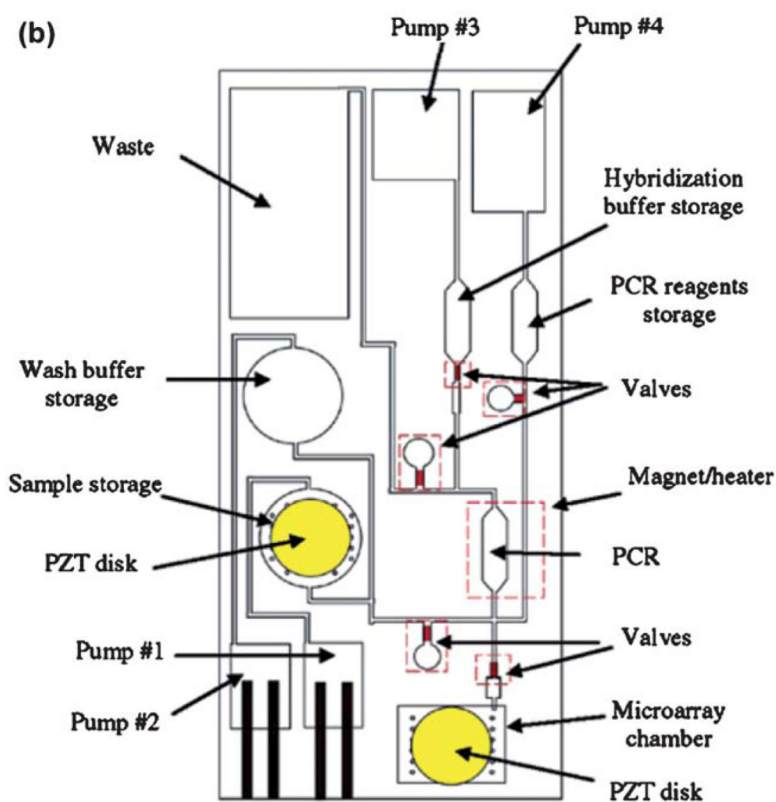
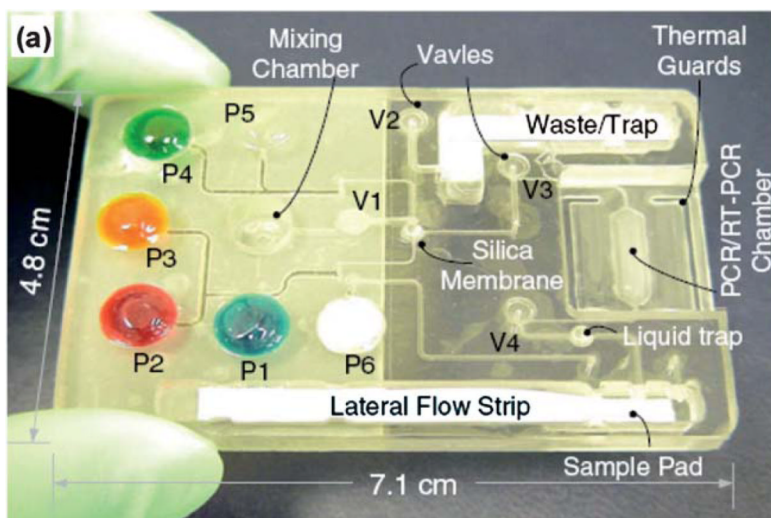
**Fig. 3.** (a) Layout of a device to carry out PAGE and MEKC. The channels were 50 mm deep and 20 mm wide. (b) 2D map of proteins from fetal calf serum. The sample was placed into reservoir A and electro-kinetically injected into the separation channel at  $200 \text{ V cm}^{-1}$ . PAGE and MEKC were performed at  $300$  and  $400 \text{ V cm}^{-1}$ , respectively. A 10 s separation time was utilized in the first dimension prior to performing the serial 10 s MEKC cycles. A total of 159 MEKC cycles were carried out. Adapted from ref. 82 with permission from John Wiley & Sons, Inc.



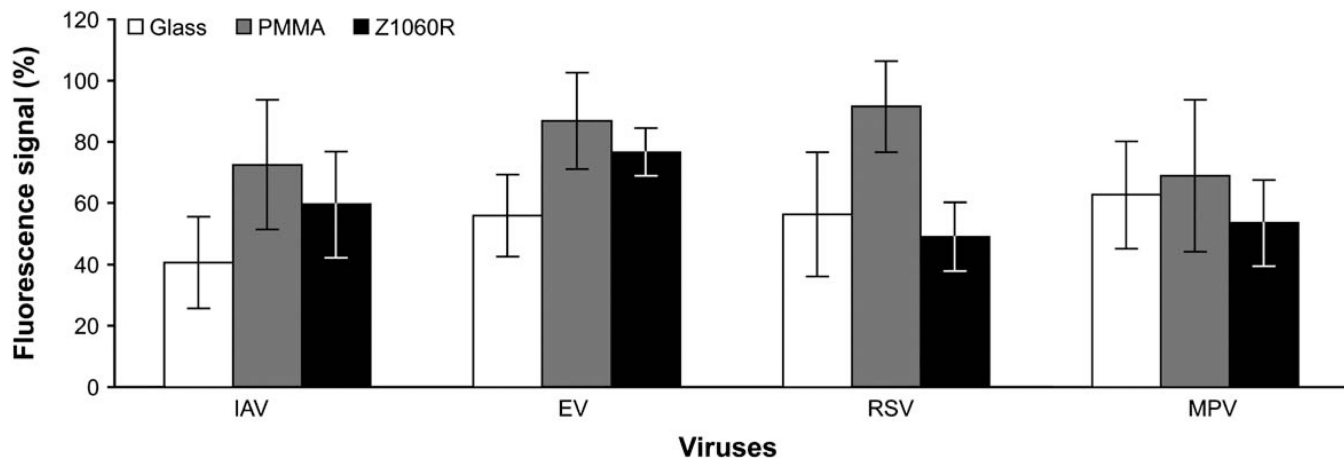
**Fig. 4.**

(a) Photograph of a Gyrolab CD device with 112 analysis units. (b) Exploded view of an analysis unit with hydrophobic barriers (A1 and A2), hydrophobic patches (B), capillary posts (C), and packed column (D). Part 2.1 shows liquid drawn in by capillary action. In part 2.2, the overflow channel was activated at 1000 rpm, removing excess liquid. Part 2.3 shows the volume (200 nL) defined within the chamber when a short pulse of a higher speed forced the liquid through the barrier. In part 2.4, a defined volume moved through the packed column at a constant flow rate by a spin speed program. Adapted from ref. 96 and 97.

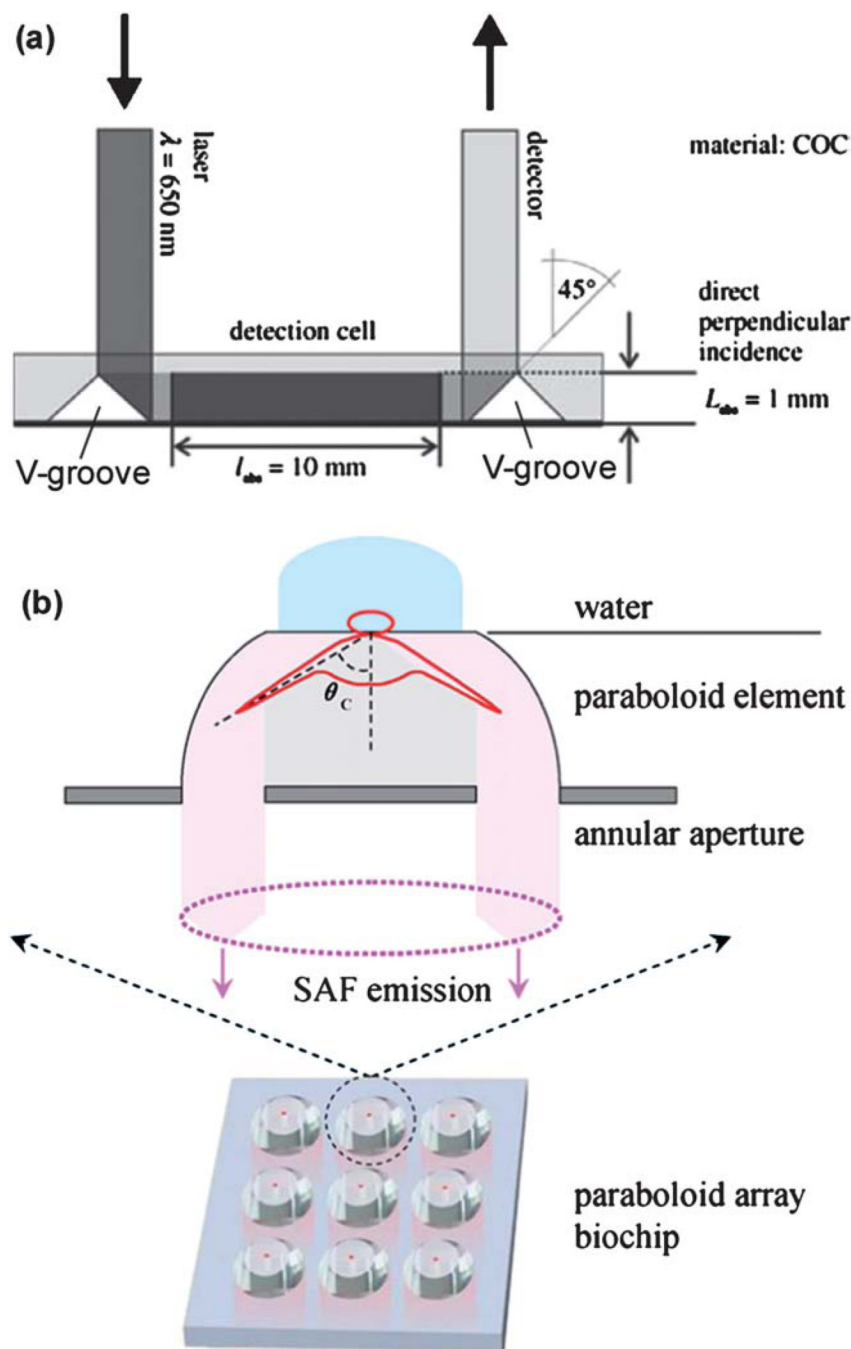




**Fig. 5.** (a) Photograph of a polycarbonate cassette, in which pouches were filled with dyes for better visualization and they were designed to store various reagents and buffers. Courtesy of Dr. Haim Bau (ref. 105). (b) Schematic of a polycarbonate device containing pumps, valves, PCR chambers and microarrays for detection. Adapted from ref. 106.



**Fig. 6.** Comparison among glass, PMMA, and COC (Zeonor 1060R) for flow-through microarray hybridizations. Each fluorescence signal represents an average from 36 spots on 3 slides and it is expressed as a percentage of the signal obtained from the corresponding control. The fluorescence signals are from Cy3-labeled amplicons hybridized to DNA microarray. Adapted from ref. 71 with permission from American Society for Microbiology.



**Fig. 7.** (a) Detection cell integrated in a CD device with an increased optical path length. The incident optical beam was guided by the V-groove on the left, through the detection cell, and reflected by the V-groove on the right before being detected. V-grooves were created from the bottom surface of the device. Courtesy of Dr. Jens Ducreé (ref. 109). (b) Layout of a  $3 \times 3$  COC array with paraboloid wells. Shown above the array is an exploded view of a well with supercritical angle fluorescence (SAF) emission. Courtesy of Dr. Colette McDonagh (ref. 111).

**Table 1**

Comparison of bonding methods for thermoplastic microfluidic devices

	Thermal bonding	Plasma or O <sub>3</sub> activated	Lamination	Solvent bonding	Adhesive bonding
Chemical compatibility	High	High	High	Low	Low
Pressure sustainability	Medium	Low	Medium-low	High	High-medium
Feature distortion	Yes	No	Depends	Depends	Yes
Topology compensation	Yes	No	No	Yes	Yes
Surface property change	No	Yes	No	Yes	Yes
Applicable to hybrid devices	No	Yes	No	Yes	Yes

**Table 2**List of efforts in two-dimensional protein separation in plastic microfluidic devices<sup>a</sup>

Device materials	Separation mechanisms	Separation time	Peak capacity	References
Polycarbonate	IEF-PAGE	10 min	1700	78
PMMA	IEF-PAGE	90 min	n/r	79
PMMA	PAGE-MEKC	12 min	1000	26
PMMA	IEF-PAGE	n/r	n/r	80
COC	IEF-PAGE	10 min	n/r	81
PMMA	IEF-PAGE	10 min	n/r	53
PMMA	PAGE-MEKC	30 min	2600	82
COC	IEF-RPLC	25 min	n/r	83

<sup>a</sup>Note: the efforts are listed in the chronological order. RPLC and n/r stand for “reverse phase liquid chromatography” and “not reported” while other abbreviations can be found in the text.