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Microfluidic Platforms for Gradient Generation and its Applications

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Abstract

Due to criticality of gradients in both chemical and biological fields, generating stable and controllable gradient concentration in microfluidics has significance such as analysis of cell migration, cancer metastasis, drug screening, chemotaxis and chemical synthesis. Integrated microfluidic chips are particularly amenable to gradient generation. Microfluidic chips functioning as concentration gradient have made great progress based on various principles. Diverse advanced microfluidic platforms have been developed as convection mixing-based gradient generators, laminar flow diffusion-based gradient generators, static diffusion-based gradient generator and geometric metering mixing-based gradient generator. In this review, we discuss recent advances and wide application of microfluidic gradient generators.

Keywords: Microfluidics; Concentration; Gradient generation cell biology

Introduction

Microfluidics is the science and technology of systems that process or manipulate small amounts of fluids, using channels with dimensions of tens to hundreds of micrometers. By manipulating the fluids and samples in the micro-channels, the individual operating units are interconnected to achieve specific experimental functions in the fields of biology, medicine, chemistry and the like. Microfluidics can achieve a variety of flexible operation of the unit and high throughput integration, such as cell culture, labeling, sorting and cracking, etc., and therefore it is also known as "lab on a chip".

Recently, microfluidics has been widely employed in many fields, due to following advantages. (i) Microfluidic channels are in the scale of several micrometers matching the scale of a cell, (ii) environment of a microfluidic multidimensional channel network is relatively independent. (iii) Mass transfer and heat transfer are fast in the micro-scale- microfluidic channels. (iv) Microfluidics can satisfy requirements of high-throughput analysis. (v) The multiple operational units of microfluidic platform can be combined flexibly, and have power to process a large number of tests in parallel. Microfluidic devices have been utilized in chemical and biological areas for generating gradient concentration. Traditional methods to generate concentration gradient in solution utilize pipet tip or reservoir in a gel were labour-some and limited [1]. Moreover, spatial resolution of concentration gradient generated by traditional methods is in the scale of several millimeters and difficult to control the gradient. In a sense, this gradient is not suitable for many analytical assays. Therefore, it is urgent to generate controllable and micro-scale concentration gradients [2].

Concentration gradient in life body determines various cell behaviors, such as inflammation [3], wound healing [4], cell growth, differentiation [5-7] and cancer metastasis [8]. One of the goals of *in vitro* cell models is to recapitulate tissue organization and cell signaling occurring *in vivo* in order to establish a research platform that is more physiologically relevant or higher throughput [9-11]. Concentration gradients in this context as cell secreted signals that are prevalent, and they diffuse into extracellular environment until they are removed by flows from vessels or degraded by enzyme. Many cellular processes have evolved to identify direction information encoded in gradients. For example, biomolecular concentration gradients have been involved in tumor-cell invasion in metastatic cancer [12,13]. Migration and differentiation of tumor cells is mainly determined by repellant or attractant factors. Chemotaxis of tumor cells has become a crucial issue in screening of cancer drugs, and its research has been promoted by the gradient generator for a period of time [14-16].

Using microfluidics to generate concentration gradient has many advantages in biological and chemical analysis. Among them utilizing microfluidic concentration gradient generator to generate gradients of compositions in solutions is the most popular. Gradient of compositions in solution has great usage in cell biology including cell growth and differentiation [17-28], axon guidance [29-32], neutrophil chemo-taxis [33-35], cell migration [36-39], cancer chemo-taxis [40], bacteria growth and chemo-taxis [41-44], cytotoxicity [45-54], optimization of reaction conditions [55] and bio-fabrication of chitosan membranes [56]. Microfluidic gradient generators have been developed such as convection mixing-based gradient generators [57-100], laminar flow diffusion-based gradient generators [101-119], static diffusion-based gradient generator [120-139] and geometric metering mixing-based gradient generator [140-145]. In addition to generate gradients of compositions in solutions [68,71,74,75,77-79,83,84,87,92,94,95,98,115,116,119,125,127-129,132-134,136,143-147], microfluidic gradient generators can produce gradients of physical-chemistry on surface [59,66,70,88,89,97,117,130,148-151], gradients of shear stress [152], and gradients of refractive index in solutions [153].

Most reviews on microfluidic gradient platforms are focused on biological application of drug screening, and limited emphasis on various gradient systems applied in optical systems. Here we described recent advances in the design and application of microfluidic gradient generators not only in the biological studies, but also about the optical systems.

Methods Applied in Microfluidic Gradient Generator

Microfluidic gradient generators can be classified into four

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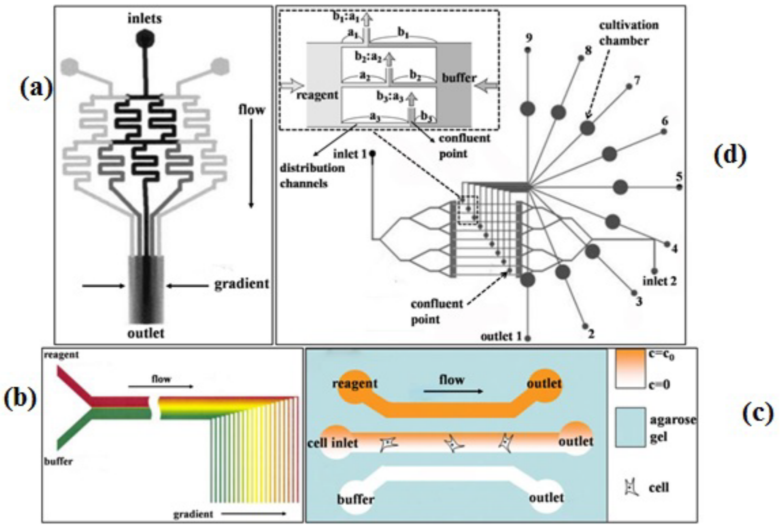

Figure 1: (a) Convection mixing-based gradient generator; two different solutions were introduced from top inlets and allowed to flow through splitting and mixing network. (b) Laminar flow diffusion-based gradient generators; two branches of different concentrations merge into one channel in which the gradient is generated transversally to the direction of the flow. (c) Static diffusion-based gradient generator; Solution of a fixed chemical concentration flows in the source channel while blank buffer flows in the sink channel. The chemical diffuses through the agarose gel membrane, and forms a linear gradient in the center channel. (d) Geometric metering mixing-based gradient generator; when the lengths of the distribution channels for liquids reagent and buffer were a and b, respectively, mixing ratio is b: a.

categories according to their gradient generating principles: convection mixing-based gradient generator, laminar flow diffusion-based gradient generators, static diffusion-based gradient generator and geometric metering mixing-based gradient generator. The convection mixing-based gradient generators consisted of a network of micro-channels with multiple branching points. Gradient generation in this network of micro-channels produced via convection mixing, where the fluid streams encountered repetition of splitting, mixing, and recombination. Microfluidic gradient generators can generate gradient distributions of biochemical molecules by controlling advective and diffusive transport processes in the microscale. The laminar flow diffusion-based gradient generator works by combining two streams of liquid at a junction into a main channel, and allowed to diffuse when they flow downstream neck to neck. After some fixed distances, a gradient has been produced that is perpendicular to the flow field. The static diffusion-based microfluidic gradient generator established gradient without convective flows in the channel by diffusing a reagent through a section with high fluidic resistance. The gradient generating mechanism of the geometric metering mixing-based gradient generator is simply that the geometry of micro-channels with the same flow rate determines the total amount of flow into the wells. And then, different ratios of buffer and reagent mixed to generate a gradient profile. The gradient generator is turned out to be a good platform for research in biology that reduced interference due to shear stresses.

Convection mixing-based microfluidic gradient generator

The most common convection mixing-based gradient generator is the Christmas tree or Tree-like Gradient Generator design firstly reported by Jeon et al. in 2000 as show in Figure 1a. Jeon and co-workers established a hydrofluoric acid [HF] gradient to etch a glass slide. They characterized gradient generation by analyzing depth proportional to HF concentration gradient [1]. The gradient generator was composed of a network of vertical and horizontal micro-channels with many junction points. In this network of micro-channels gradient generation through convection mixing, which the fluid streams encountered repetition of splitting, mixing, and recombination. The reagent fluid and the buffer fluid from two side inlets mixing at the junction point. After completely mixing, the recombination stream spilt into both sides of the horizontal channel. Through several repetitions of splitting, mixing, and recombination, different concentrations of the reagent of interest will be generated in different fluid streams. This design of the gradient generator is dominated by successive dilutions and diffusional mixing of parallel laminar flows. This gradient generator has been widely applied because it is controllable to generate different shapes of gradients and maintain it for a while. At the same time, the concentration generating part of this gradient generator is independent of the reaction analysis part and is easy to integrate with other chip modules to improve the analysis flux.

Laminar flow diffusion-based microfluidic gradient generator

The laminar flow diffusion-based gradient generator is another widely used gradient generation platforms since it has a simple channel structure. In this gradient generator, two [or more] fluids of different compositions conflate side by side in a channel due to the effect of laminar (Figure 1b). The structure of this gradient generator is typically Y channel (or multiple Y inlet channel), and each branch of the Y flows fluids of different compositions. Reagents contained in each laminar fluid mixed gradually driven by diffusion force and create a gradient that perpendicular to the flow field [154,155]. There are three advantages of this gradient generator, First, it has a simple channel structure and easy to fabrication [101]. Second, gradient concentration can be generated in a microscale that down to cellular level [156]. Third, gradient concentration can be maintained and controllable flexibly [157,158]. In spite of those advantages laminar flow-based gradients have some limitations. First, the constant flow caused shear stress was an interference factor for cell biology study. Second, the formation of gradient profile was critically affected by flow rate. Finally, they are untoward to large-scale integrate due to their typical structure [159]. Therefore, it is difficult to maintain a stable gradient and needs high precise flow rate control equipment [159]. For these reasons, application of laminar flow diffusion-based microfluidic gradient generator has been limited. Laminar flow diffusion-based gradient generator is capable of generating a concentration gradient stabilized in spatial and temporal. Also, the shape of the concentration gradient can be controlled by changing the geometry of the fluid channel or adjusting the fluid flow rate. However, laminar flow diffusion-based gradient generator also has some drawbacks, i.e., the flow of fluid will produce a fluid shear force that is detrimental to the cell. In addition, cell-secreting intercellular signaling factors are also taken away by flowing fluids.

Static diffusion-based microfluidic gradient generator

The static diffusion-based microfluidic gradient generator (Figure 1c) established gradient without convective flows in the channel by diffusing a reagent through a section with high fluidic resistance, such as multiple narrow micro-channels [160], micro-porous membranes [161], or gel walls [162]. This gradient generator can meet the need for (i) reducing interference of shear stress in cell biological tests. (ii) Easy to operate. (iii) Suitable for high-throughput assays [163]. The static diffusion-based gradient generator can achieve a wider range of geometrical gradient profiles, and shape of gradients and development of nonlinear could be controlled [127]. However, these gradients typically took long time to establish and provided less control of dynamic variation of gradient profile and diffusion distance. Nevertheless, static diffusion-based gradient generator has been generally accepted in cell biology assays due to improved usability. It becomes a trend that emerging from commercially available gradient platforms such as the Ibidi [164] or Bell-Brook [165], which are becoming "gold standards" for cell migration studies. The static diffusion-based microfluidic gradient generator effectively reduces the unnecessary flow of fluid present in the micro-channels. However, in the static diffusion-based microfluidic gradient generator, since the molecular diffusion is the determinant of the concentration gradient generation, the resulting concentration gradient shape is not easy to manipulate compared to the concentration gradient based on the laminar diffusion concentration generation of the chip.

Geometric metering mixing-based microfluidic gradient generator

The geometric metering mixing-based microfluidic gradient generator has two processes for generating gradients. First, introduced liquid flows were divided into several downstream flows geometrically through different distribution subchannels. Second, each divided flow met with the divided flow of buffer fluid and mixed at a joint point. The mixing ratio of the two flows depended on the geometry of the precisely designed distribution channels, without interference of flow rate. For example in Figure 1d, both flows divided into equal number of the distribution channels, and they are connected at the joint points. Basic principle for generating concentrations gradient was that two flow liquids are introduced from both sides continuously [142]. It is well known that a microchannel network is similar to analogy of a resistive circuit, in which the applied pressure P, the flow rate Q, and the channel hydrodynamic resistance R were respectively analogous to voltage V, electric current I, and resistance R, in Ohm's law [166-168]. When the flow rates, viscosity of two flows, widths and depths of the distribution channels are all uniform, length of every distribution channel will be proportional to its hydrodynamic resistance. Namely, ratio of lengths of the distribution channels is in inverse proportion to mixing ratio of two flows at a joint point. Thus, mixing ratio of the two flows will not be affected by diffusion coefficients of the molecules, diffusion length [channel width], and introduced flow rate. The greatest feature of geometric metering mixing-based microfluidic gradient generator is the ability to generate a concentration gradient quickly, which stabilized in spatial and temporal. However, there is fluid flow in the microchannels, and the resulting fluid shear force has an adverse effect on the cells.

Applications of Microfluidic Gradient Generator

Microfluidic gradient generator as an emerging technology platform can precisely control formation and direction of chemical concentration gradient. Utilizing these characteristics, concentration gradient of various substances could be generated and applied to different scientific fields. Here we summarized the gradient generator into following categories: gradients of the compositions in solution, gradients of physical-chemistry on the surface, gradients of shear stress in solutions, and gradients of refractive index in solution. In the following sections, generating of such gradient and its application will be discussed.

Generating gradients of compositions in solution

The gradient of compositions in solution has its own significance in

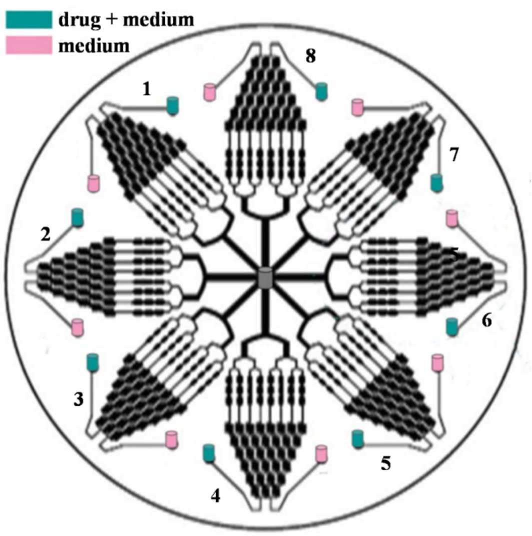

Figure 2: Schematic of the integrated microfluidic device for cell-based high content screening; the device consists of eight uniform structure units and each unit is connected by a common reservoir in the center of the device.

biochemical and chemical areas, especially in cell biology, including cell growth and differentiation [17-28], axon guidance [29-32], neutrophil chemo-taxis [33-35], cell migration [36-39], cancer chemotaxis [40], bacteria growth and chemotaxis [41-44], cytotoxicity [45-54], optimization of reaction conditions [55], and bio-fabrication of chitosan membranes [56].

Chung et al. fabricated a gradient generator platform to optimize proliferation and differentiation of neural stem cells (NSCs) *in vitro*. In the platform, cells are exposed to continuous flow of growth factor concentration gradient, thus, minimizing autocrine and paracrine signaling. Directional responses were reported by Bhattacharjee et al. of main mammalian neurons to the diffusion gradient of metrin *in vitro*. They concluded from their assays that most neurons extending axons during gradient application grow toward netrin source. Their data show that netrin acts as a growth factor for this same population of neurons sustained exposure of Human NSCs (hNSCs) to a gradient of a growth factor (GF) mixture containing epidermal growth factor (EGF), fibroblast growth factor 2 (FGF2) and platelet-derived growth factor (PDGF) for more than one week. NSCs stayed healthy throughout incubation period and more importantly, proliferated and differentiated in a graded and proportional fashion that varied directly with GF concentration [17,29]. Jeho et al. developed a device that can generate temporarily and spatially controlled gradients of chemokines and used this to study migration of human neutrophils in simple and complex interleudin-8 (IL-8) gradients (Figure 2) [33]. Barkefors et al. utilized a microfluidic chemotaxis chamber to study response of hill-shape gradient of fibroblast growth factor 2 (FGF2) and vascular endothelial growth factor A (VEGFA) to migration of endothelial cells. Analysis of cell migration at different gradient regions showed chemotaxis decreased when cells reached the high end of the gradient. Their findings indicate that gradient of chemokine growth factor may direct transition from endothelial cells to non-migratory phenotypes when endothelial cells approach source of growth factors [37]. Diao et al. developed a concentration gradient generator that produces a linear gradient without fluid shear force on the cell, ensuring that cell migration is caused by cell chemotaxis rather than by variations in fluid flow. With this device, they found that wild type *Escherichia coli* strain RP437 migrated toward attractant (e.g., L-asparate) and away from repellent (e.g., glycerol), while there was no change in bacterial distribution of RP437derivatives without motility capacity or chemotaxis. Their research demonstrated that *E. coli* absence of autoinducer-2-mediated quorum sensing response to chemoattractant L-aspartate was in some sense indistinguishable from the wild-type. This indicates that chemotaxis is isolated from this cell-cell communication model. Saadi et al. investigated migration of human metastatic breast cancer cells in different conditions using a microfluidic chemotaxis chamber that can generate multiple growth factor gradients simultaneously. They quantified and compared migration of breast cancer cells at 0–50 ng/ml and 0.1–6 ng/ml of epidermal growth factor (EGF) gradients. The results showed that the cells responded favorably to a gradient of 0–50 ng/ml. However, a shallow gradient of EGF could induce chemotaxis, and EGF can direct migration over a large gradient range, confirming potency of EGF as a chemo-attractant [40,41]. Ye et al. described an integrated microfluidic platform containing multiple concentration gradient generators for high-throughput studies of anti-cancer drug-induced apoptosis. This platform can extract maximum information from tumor cells in response to different concentrations of several drugs, with less time and minimal sample, which is of significance for cancer and basic biomedical research [54]. Damean et al. demonstrated that a microfluidic technology could compartmentalize and measure different chemical reactions in pL volumes simultaneously. This technique can be used to analyze a set of chemical reactions restricted in identical volumes (5 to 60 pL) of strings of water-in-oil droplets that contain different reactant concentrations. This technique provides a useful method for continuous and simultaneous analysis of multiple chemical reactions [55]. Luo et al. demonstrated an *in situ* pH gradient generation in microfluidics for freestanding and semi-permeable chitosan membranes bio-fabrication. In the microchannel, a pH gradient was formed at the converging interface between slightly acidic chitosan solution and mild basic buffer solution, and pH-stimuli-responsive polysaccharide chitosan was recruited to form a freestanding hydrophilic membrane. Thickness of fabricated chitosan membranes is 30-60mm, and uniform along the flow interface in the microchannels [56].

Generating a gradient surface for physical and chemical application

Growth and profilication of cells are not only affected by chemical agents in the external environment, but also by physicochemical properties of adherent substrates. Dertinger et al. described a general technique for generating a gradient of substrate-binding proteins with complex shapes. The gradient froming pure BSA to pure laminin were generated by the solutions within the microchannel and these proteins were adsorbed on the poly-L-lysine homogeneous layer. Rat hippocampal neurons were cultured on this substrate with a protein gradient. Optical imaging of these neurons revealed that the axon specification is directed toward increased surface density of laminin [169]. An integrated microfluidic gradient technology was developed by Zaari et al. which produce a microgradient-compliance substrate with photo-polymerization. They used the microfluidic chip to generate a concentration gradient solution of the hydrogel precursor and performed photopolymerization in a certain concentration of crosslinking agents. The cells were cultured on this microgradient-compliance substrate and they found that the spreading area of the cells increased rapidly in the region above the elastic threshold value [170]. Kreppenhofer et al. injected two polymers into the microfluidic chip for concentration gradient to produce different compositions of two polymerization mixtures, then polymerized into polymer monolithic with a gradient of a surface pore size. The chip was reversibly bonded by coating a curing agent. The polymerization mixtures solution in the chip is polymerized and then the chip is opened to obtain a 450 μm thick porous film with a pore size distribution in a gradient [171]. In brief, combination of a microfluidic technology and photopolymerization is a powerful tool to produce gradient-compliance substrates to study implication of cell response to the substrate mechanics (Figure 3).

Generating gradients of shear stress in solutions

Cells are sensitive to different microenvironmental factors, including mechanical forces and chemical gradients. Applications of microfluidic concentration gradient chips were focused on cell biology. Thus, *in vitro* physiological models of cells should take into account how cells sense and respond to microenvironmental factors. These problems can be solved by using a microfluidic system, which controls physical properties of the fluid at the micro-nano scale. Park et al. introduced a simple and general method to generate chemical concentration gradients and shear gradients in a single chip. In this system, we formed a chemical concentration gradient by diffusion, and a shear-force gradient in the interstitial level passively through a circular channel (Figure 4). They evaluated the system by incubating mouse L929 cells simultaneously under shear gradient and nutrient

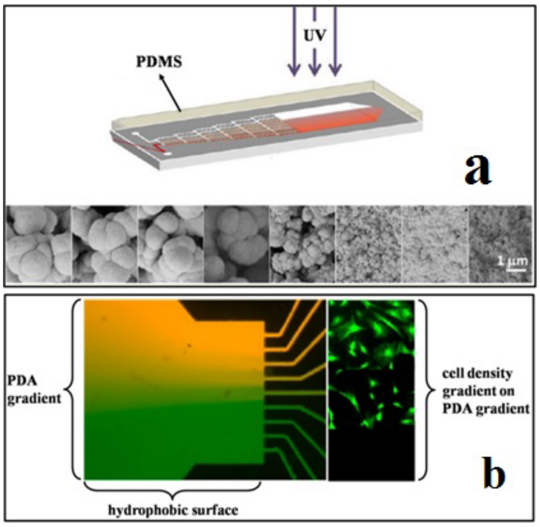

Figure 3: (a) Fabrication of polymer monolithic surfaces with a gradient of pore and polymer globule sizes from ~0.1 to ~0.5 μm defined by compositions of two polymerization mixtures injected into a microfluidic chip. (b) A microfluidic device was used to generate a covalently conjugated gradient of polydopamine (PDA), which changed wetabilty and surface energy of the substrate. The gradient was subsequently used to enable spatial deposition of adhesive proteins on the surface. When seeded with human adipose mesenchymal stem cells, the PDA-graded surface induced a gradient of cell adhesion and spreading.

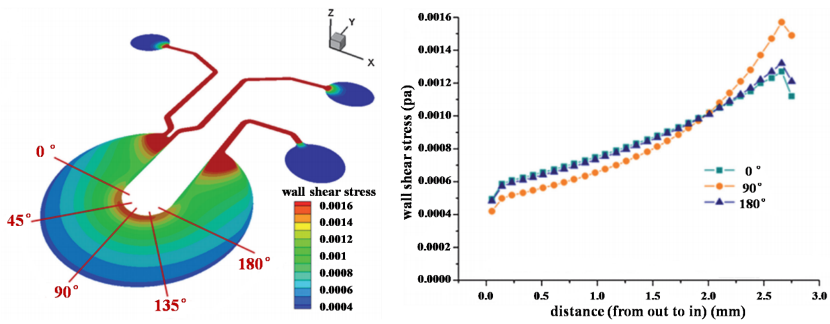

Figure 4: Wall shear stress distribution, with corresponding profiles (inset-right) measured at 0°, 90°, and 180°. The x-axis indicates distance from the outer rim. The same column numbers indicated in the x-axes of these graphs were used in subsequent experiments.

concentration gradient. It was found that shear stress had a major effect on cell arrangement, migration and migration rates. At the same time, the concentration gradient of nutrients affects proliferation [152].

Generating gradients of refractive index in solution

Recently, Zhao et al. first used a concentration gradient microfluidic chip to control mixing of ethylene glycol and deionized water to obtain a glycol solution with refractive index as expected (Figure 5). This solution could act as an optofluidic lens and has low spherical and low field curvature aberrations. They discussed the optimal refractive index profile that can suppress spherical and field curvature aberrations in optofluidic lenses, which would greatly improve fine of the focal spot and reduce focal length variation of the light source at different off-axis positions. This optical flow control lens with low spherical curvature and low field curvature distortion would find their applications in on-chip sample illumination, multiplexed detection and light manipulation [153].

Conclusion

Concentration gradient generator can serve for multiple experiments since it increases resolution of dose-response studies and reduces analysis time and other efforts. The microfluidic gradient generator would continue to evolve to address its existing problems or new challenges encountered in the application. Selection of a particular gradient generator platform requires considering experimental needs, such as maintaining low shear forces while keep the medium continuous perfusion, maintaining *in vivo* like conditions, retaining signal molecules and removing waste, etc. The microfluidic gradient generator could provide superior gradient control and gradient pattern when compared to the conventional gradient generation method. In addition, although the novel gradient design is important, it would give way to new applications of the gradient generator. These applications include integration with multiplexed drug screening, organs-on-a-chip, and some in other fields. The gradient generator platform can provide a microenvironment to investigate the miniaturized animal model and its response to chemical signals. Combined efforts would continue to benefit the field next few years to expand availability of these platforms and demonstrate their capabilities in a complex platform. In the future, Concentrating on reducing manufacturing and operational complexity would increase popularity rate of gradient generator platforms.

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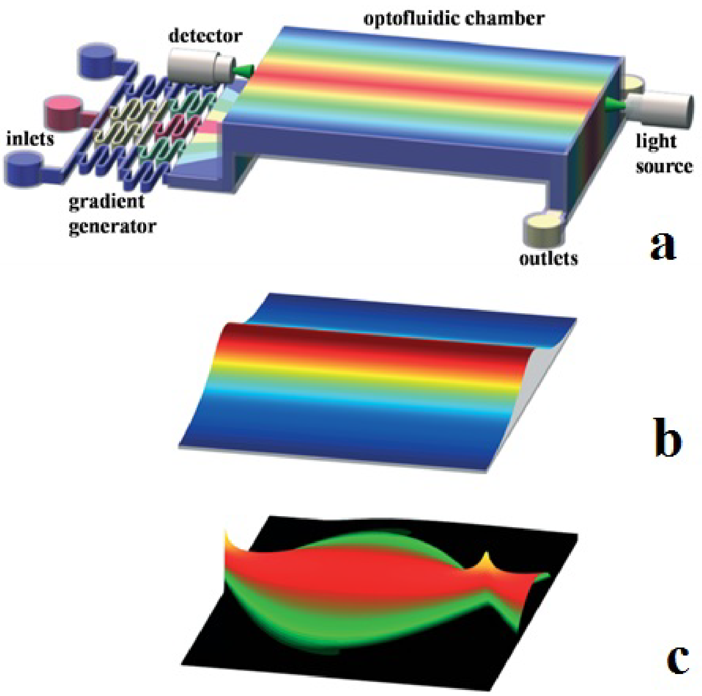

Figure 5: (a) Schematic illustration of the optofluidic chip. (b) Index profile and (c) the light propagation image in the optofluidic chamber.

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