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Mini Review

Microfluidic Systems for High Throughput Screening

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Abstract

High-throughput screening (HTS) is a method of scientific experimentation widely used in drug discovery and relevant to the fields of biology and chemistry. Microfluidic systems manipulate or process tiny volumes of fluids in channel with dimensions of tens to hundreds of micrometers and are well suited for HTS due to their small size, which allows massively parallel experimentation. The unique feature of fluids within microfluidic networks can give an insight on new way to resolve the current challenges of HTS and to revolutionize all stages in drug discovery. Herein, recent progress in both microarraying strategies based on microfluidics and novel microfluidic devices with high throughput rates will be discussed.

Keywords: Microfluidics, High throughput screening (HTS), Drug discovery, Surface patterning, Soft lithography

Introduction

Microfluidic systems manipulate or process tiny (10^{-9} to 10^{-18} liters) volumes of fluids in channel with dimensions of tens to hundreds of micrometers. These systems offer numerous advantages for chemical and biological analysis compared to standard laboratory instrumentation, which include low consumption of samples and reagents, low cost for mass production, short times for analysis, flexibility and dimensional precision, and separations and detections with high resolution and sensitivity[1-25]. They can probe physical phenomena and mechanisms that are not observable on the macroscopic scale as well. In addition, one key feature is the integration of different functions like sampling, sample pre-treatment, sample transport, biochemical reactions, analyte separation, product isolation and detection in a microchannel network. Therefore, microfluidic systems enable serial processing and analysis, and furthermore, can accomplish massive parallelization through efficient miniaturization and multiplexing. Further details of microfluidic physics are reviewed[1-4].

Recent advances in genomics, proteomics, cellomics, metabolomics and combinatorial chemistry have provided an opportunity for pharmaceutical industry to accelerate the pace of drug discovery using high throughput techniques[26-62]. The sequencing of the human genome has generated a number of new molecular targets with unknown function (new targets) as well as those with known function (established targets). Rapid progress in protein biochemistry and combinatorial chemistry has enabled the arrangement of chemical building blocks into all possible combinations, generating a library of millions of compounds. Cellomics and metabolomics provide more comprehensive pictures of cells under complex physiological parameters. The increase in experimental complexity demands the organization, interpretation and utilization of experimental data in a methodical and rational manner and so high throughput screening (HTS) technologies have emerged to meet this demand. High throughput screening is a method for scientific experimentation widely used in drug discovery and relevant to the fields of biology and chemistry. Modern HTS is composed of robotics, liquid handling devices, sensitive detectors, and software for data processing and control in order to perform millions of biochemical, genetic, proteomic or pharmacological tests on samples in parallel. It facilitates the understanding of human biological pathways that require large experimental data to study genes, proteins and metabolites. The primary ways to save time and minimize usage of chemical compounds are the miniaturization of existing technologies like high-density wells on assay plates and nanoliter dispensing systems. However, current robotic systems have been faced with several issues like high cost of the technology, poor reliability of data, standardization of data types, rapid and accurate dispensing of very small liquid volumes and quick evaporation of the dispensed liquid. Therefore, microfluidic systems are capable of resolving many of these issues and offer the possibility of technological breakthroughs in HTS. Herein, novel microfluidic technologies for developing HTS are reviewed.

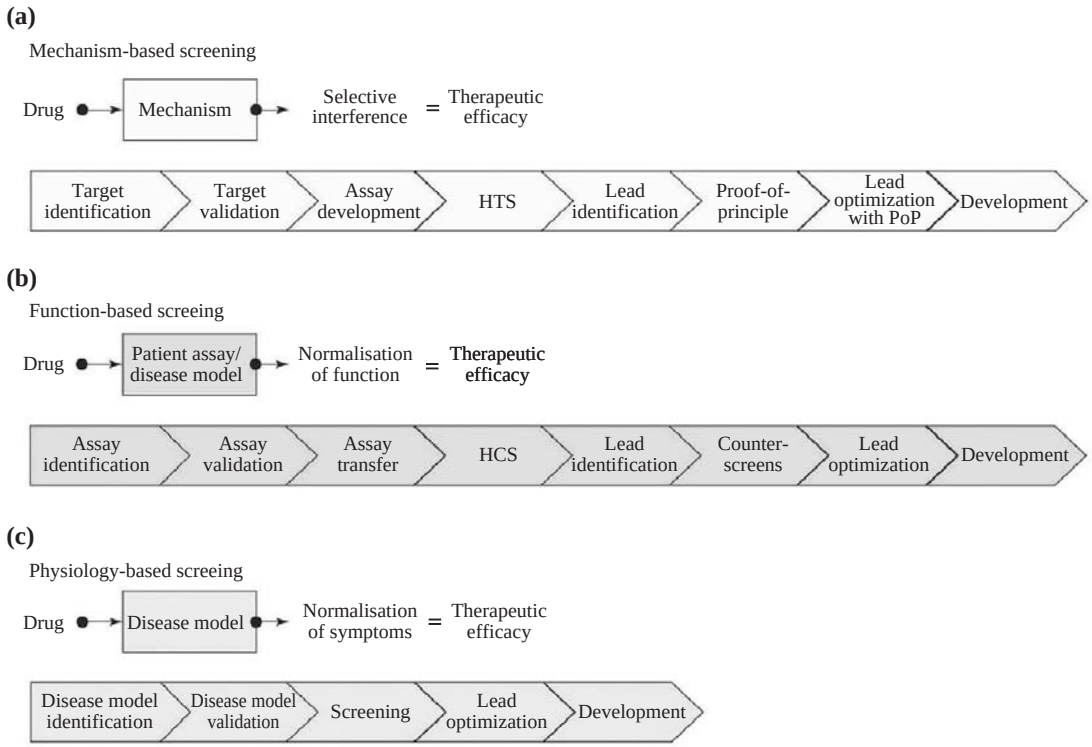


Figure 1. Drug discovery approaches: (a) mechanism-based drug discovery seeking to produce a therapeutic effect by targeting a specific mechanism, (b) function-based drug discovery seeking to induce a therapeutic effect by normalizing a disease-specific functional abnormality and (c) physiology-based drug discovery seeking to induce a therapeutic effect by reducing disease-specific symptoms or physiological changes[57].

Drug Discovery and Current HTS Systems

Drug discovery entails a series of serial and/or parallel processes including candidate identification, synthesis, characterization, screening and assays for therapeutic efficacy[13,34,45,46,49,56,57,59-61]. These processes are shown in Figure 1. First, both natural products and chemical libraries are screened to determine which compounds bind to a particular target protein or inhibit a particular enzymatic reaction. The successful compounds (called hits) that exceed a certain threshold value in a given assay are progressed into leads, which are confirmed by more complicated assessments of chemical integrity, accessibility, functional behavior, structure-activity-relationships (SAR) as well as bio-physicochemical and absorption, distribution, metabolism and excretion (ADME) properties. The lead series that demonstrate activity and selectivity in the secondary screens are tested in progressively more complex systems from cell to whole animals before reaching clinical trials. Thus, robust and fast methods are needed to vet an extremely large number of candidates and to minimize the attrition of chemical entities in the costly clinical phases of drug discovery. These demands have encouraged the development of HTS technologies including small molecule library design and assembly, robotics, assay development and data handling since 1980s. In present, libraries of millions of compounds are routinely screened with single compounds of high purity in 384- and 1536-well formats. Figure 2 shows the configuration of the integrated Ultra-High Throughput Screening System (UHTSS^{TM} platform, Aurora Biosciences, San Diego, CA, USA). One primary purpose of this system is the automated screening of over 100,000 compounds and 2400 re-tests per day in a miniaturized assay format.

Alternatively, microchip technologies offer other platforms for HTS, such as microarrays and microfluidic devices. Microarrays allow the simultaneous analysis of thousands of parameters within a single experiment and so have become crucial tools in drug discovery and life sciences research[30-32,35,39,55,58]. They are composed of immobilized biomolecules spatially addressed on planar surfaces, microchannels or microwells, or an array of beads immobilized with

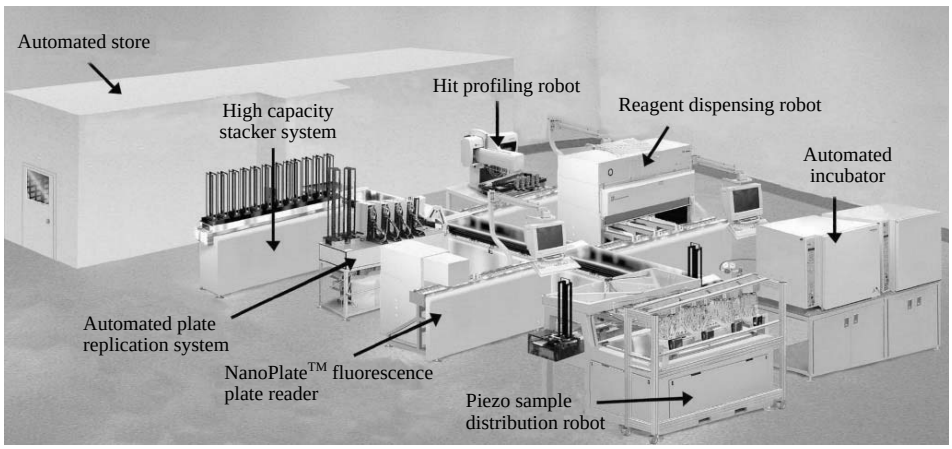
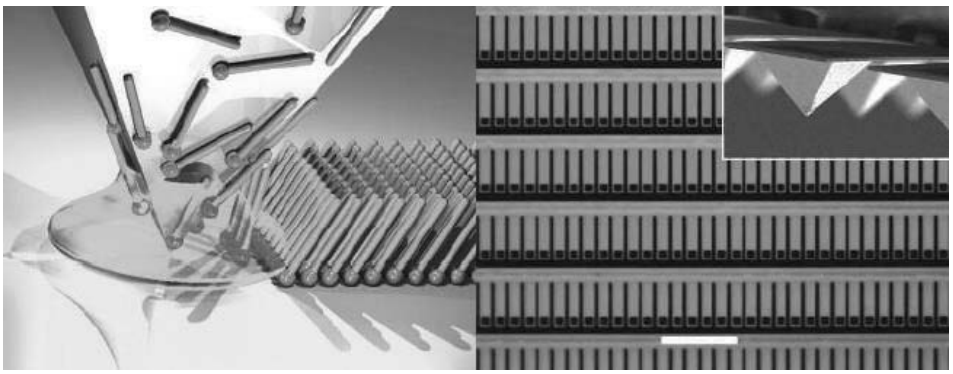


Figure 2. A schematic visualization of the UHTSS™ configuration (Aurora Biosciences, San Diego, CA, USA): a working compound solution store, an automated plate replication system, microfluidic dispensing systems, high-density 3456-well NanoWell™ assay plates, automated incubators and a high performance fluorescence plate-reader[27].

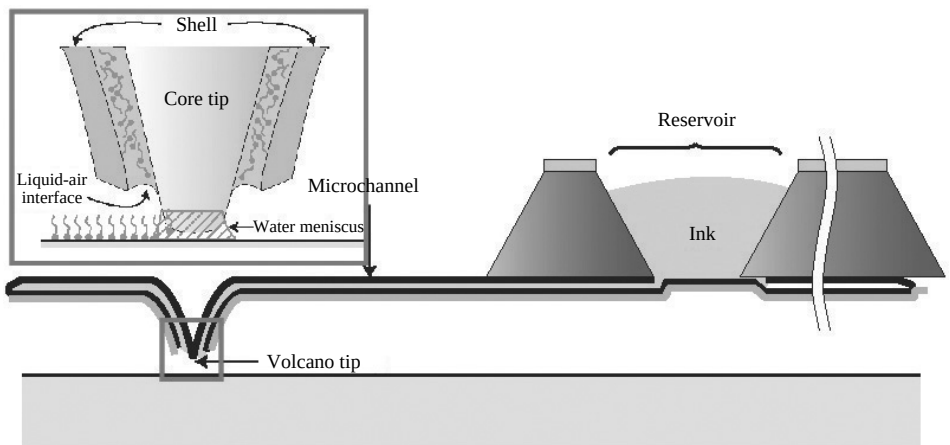
different biomolecules. Biomolecules commonly immobilized on microarrays include oligonucleotides, polymerase chain reaction (PCR) products, proteins, lipids, peptides and carbohydrates. Currently, in situ synthesized microarrays can be purchased or they can be custom fabricated in the laboratory. During the past few years, chemical compound microarrays with different surface chemistries and activation strategies have emerged in drug screening and discovery. By contrast, microfluidic devices can perform screening assays in a continuous flow that allows serial processing and analysis on a single chip. They require a series of generic components for introducing reagents and samples, moving the fluids within a microchannel network, combining and mixing them, and various other functional units such as valves, pumps, detectors, sorters and purifiers. To date, this field has focused on demonstrating concepts for these components and integrating them into a single chip and more details on microfluidic devices and their progresses can be found in several beautiful reviews[6-19]. Custom-made planar microfluidic chips and entire measuring systems have been launched commercially as well. For instance, Tecan's LabCD-ADMET™ system is a miniaturized turnkey system for the full automation of ADMET assays. GyroLab™ workstation sharing many of the benefits of the LabCD is a flexible benchtop instrument platform that automates almost every assay step from sample application to detection on the Gyros CDs. SpinX Technologies' LabBrick aims to automate all steps involved in optimizing and running assays, using 500 nl final volumes in a closed system. The testing of analyte on living cells is also an important part of high throughput drug discovery. Microfluidic devices enable manipulation of cells, maintenance of cellular environments close to physiological conditions typically found in biological systems, simultaneous characterization of cells under comparable conditions, and subsequent analysis. A fundamental paradigm shift in drug discovery, coined high-content screening by Cellomics, has motivated the development of cell-based high throughput assays to analyze individual cells and subcellular processes under complex physiological parameters simultaneously[13,15,20,34,46,55,57,59]. In addition, because of the long diffusion times and Taylor dispersion limitations associated with single phase flow, multiphase microfluidics has emerged to enhance and extend the performance of single phase microfluidic systems[17-19,23-25]. Therefore, because of several advantages of microfluidic technologies described above, the pharmaceutical industry is much interested in this technology and so investing substantially as a way to expand screening capacity at several stages in drug discovery. The unique feature of fluids within microfluidic networks can give an insight on new way to resolve the current challenges of HTS and to revolutionize all stages in drug discovery. Herein, recent progress in both microarraying stragtegies based on microfluidics and novel microfluidic devices with high throughput rates will be discussed.

Novel Surface Patterning

Inkjet and other non-contact droplet formation technologies are able to dispense nanoliter-sized drops onto surfaces, but they offer no control once the liquid has left the confinement of the ejection nozzle and, consequently, suffer from the problematic drying and spreading of the ink on the surface. Patterns can also be formed using pins or pen-like devices loaded with the appropriate solute and solvent to draw individual spots or lines. However, the fric-



(a)



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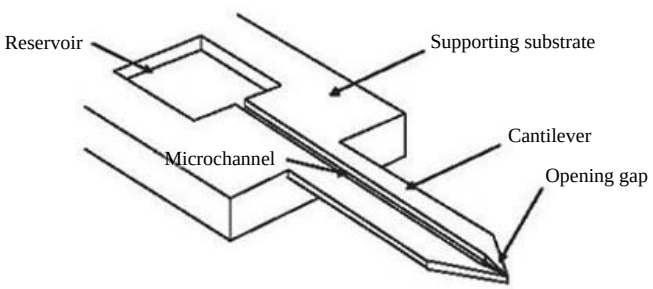
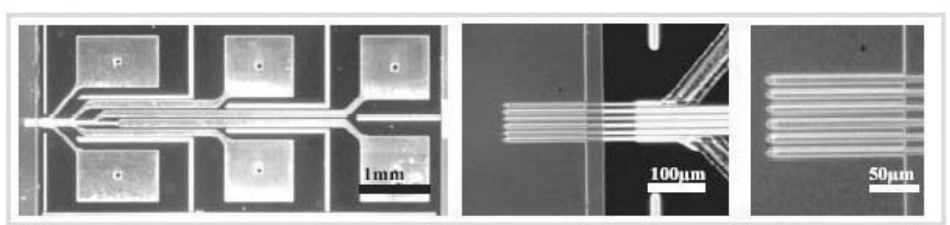
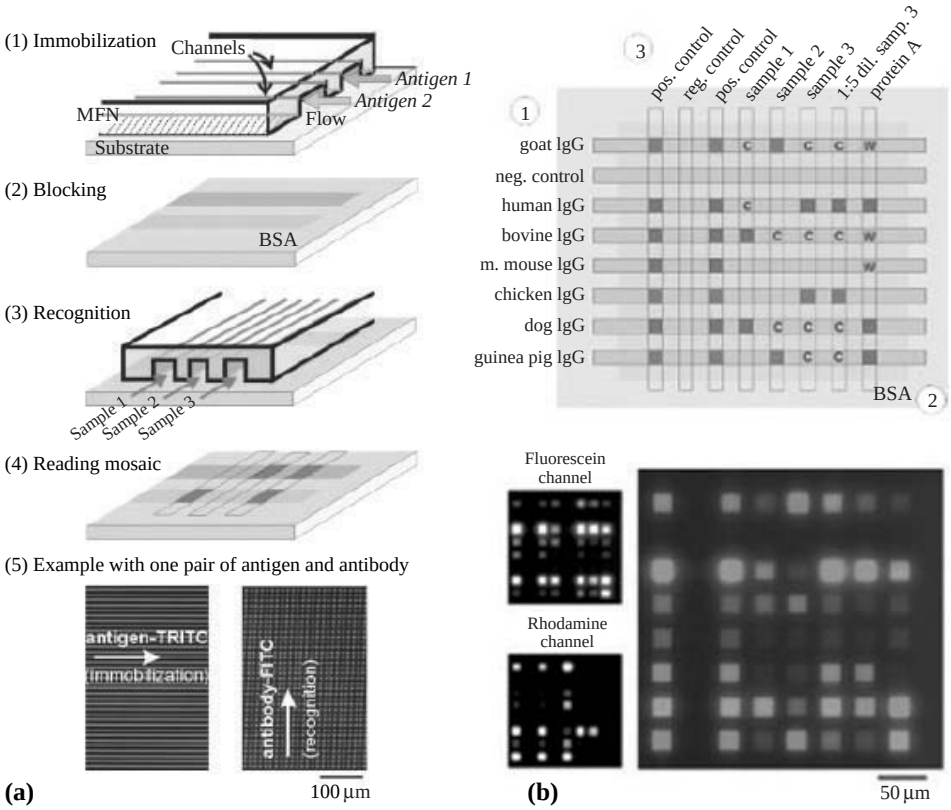




(c) Optical images of 6 cantilever SPT

Figure 3. Novel surface patterning: (a) Dip-pen nanolithography (DPN) (Left. Schematic of DPN, Right. Optical micrograph of a small portion of 55,000 cantilvers)[64], (b) Nanofoundation probe (NFP) (writing mechanism of the NFP device)[64,66] and (c) multiple cantilever array with open microfluidic channels (courtesy of E. Henderson).

tion and resulting mechanical wear between the pen and the substrate during drawing have limited the resolution of writing techniques to a few hundreds of micrometers. The extension of the pen concept to the micro- and nano-scale cantilevers is the direct-write dip-pen nanolithography (DPN) invented by C. Mirkin and co-workers (Figure 3a)[63-65]. In the DPN process, molecular inks coated on an AFM tip are transferred to a substrate while the tip is held or laterally moved along a surface as programmed. Features sizes



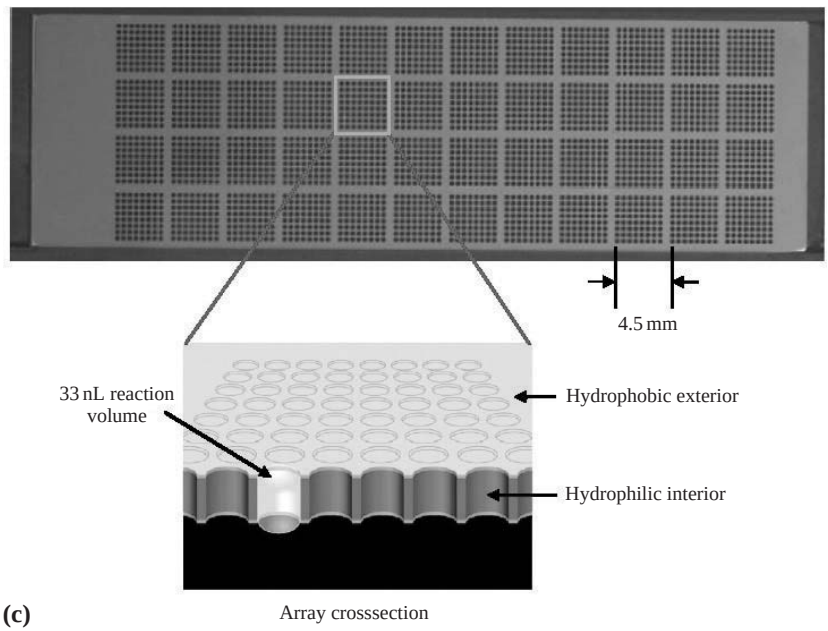


Figure 4. (a) A new concept of surface patterning based on a microfluidic channel[69], (b) combinatorial micromosaic immunoassay used to interrogate the binding between surface-immobilized receptors with fluorescently labeled detection antibodies[69] and (c) high-density array of through-holes with hydrophobic exterior and hydrophilic interior (courtesy of T.S. Kanigan).

less than 15 nm can be routinely patterned by molecular self-assembly of the ink on the substrate. In order to increase the throughput of DPN, two different approaches have been done: one with individually actuated tips including piezoelectric, thermoelectric and electrostatic mechanisms and the other with massive numbers of passive tips[64].

In the viewpoint of dispensing technologies, Espi-

nosa and co-workers[66-68] have demonstrated a novel microfluidic AFM probe called the Nanofountain Probe (NFP) with a sub-100 nm patterning capability. It consists of a hollow tip, integrated microchannels and on-chip reservoir (Figure 3b). When an ink solution is fed into the reservoir, it is driven by capillary action through the microchannel to the tip to form a liquid-air interface around the tip core. Molecules are transferred by diffusion from the interface to a substrate and a water meniscus is formed by capillary condensation. Although they demonstrated a multi-ink linear array of nanofountain probes containing 12 cantilevers probes[68], massively parallel probe arrays and integration of multiple reservoirs over large areas with multiple inks still are key challenges for high throughput screening applications. Meanwhile, Henderson and co-workers (Surface Patterning Tools (SPTs^{TM}), Bioforce Nanoscience Inc., Ames, IA, USA) have commercialized multiple microcantilevers with open microfluidic channels constantly delivering a supply of liquid to be transferred onto the surface for multiplexed biomolecular arrays. As illustrated in Figure 3c, fluids from the reservoir, down the channel, to the end of the cantilever where the channel narrows to a tiny gap. Upon contact with the surface, a small volume of liquid held in the gap by surface tension is directly transferred to the surface in an event typically requiring less than 1 ms. Capillary fluid flows down, the channel instantly replenishes the volume at the gap, and then the device is ready to write the next feature. They launched the Nano eNabler^{TM} system, which prints proteins and other biological and non-biological materials onto silicon and other surfaces with spot sizes from 20 μm and 1 μm.

Delamarche and co-workers[69] proposed a new format for surface patterning and biological assays using capillary forces. Fluids fill the microstructure of microfluidic networks (μFNs) due to the capillary pressure generated by the small dimensions of the channels and the hydrophilicity of their walls. One example for immunoassays using a μFN is shown in Figure 4a. A μFN patterns a series of antigens as narrow stripes onto a planar substrate. After a blocking step with bovine serum albumin (BSA), the antigens in each line may be recognized by specific analytes from a sample solution also guided over the substrate with a second μFN. This procedure is called a micromosaic immunoassay (μMIA). It places a series of ligands and analytes along micrometer-wide intersecting lines and thus provides a mosaic of signals from cross-reacted zones. Figure 4b shows the feasibility of μMIAs for high throughput screening. The usage of n and m microchannels yields $n \times m$ binding sites but only requires $n+m$ pipetting steps. They claimed that the μMIA would necessitate ~10 times less time, ~100 times less volume of samples and reagents, and ~1,000 times less area per site than an assay done using a conventional 1536-well plate. Recently, μMIAs and similar approaches have been developed to screen for biological analytes in combinatorial fashion with various detection schemes. Meanwhile, a conventional high-density microwell plate can be replaced with a nanoliter through-hole array (OpenArray^{TM}, BioTrove Inc., Woburn, MA, USA) shown in Figure 4c. A series of vapor and liquid deposition steps covalently attaches a polyethylene glycol (PEG) hydrophilic layer amine-coupled to the interior surface of each through-hole, and a hydrophobic fluoroalkyl layer to the exterior surface of the platen. Capillary pressure draws fluids into microchannels and surface tension holds the liquids in place, isolated from neighboring channels. The differential hydrophilic-hydrophobic coating facilitates precise loading and isolated retention of fluid in each channel. This platform can reduce capital cost of a thermocycler and a plate reader, technical labor and expense, reagent consumption and time compared to those of microwell-based HTS systems.

Novel Microfluidic Devices

One key feature of microfluidic systems is the integration of different functional units for sampling, sample pre-treatment, sample transport, biochemical reactions, analyte separation, product isolation and analysis in a continuous flow manner. The technology has centered on proof-of-concept studies for these components and it is still maturing. Two particular contributions have accelerated the development of an integrated microfluidic system. One is soft lithography in poly (dimethysiloxane) (PDMS), which is inexpensive, flexible, optically transparent down to 230 nm and compatible with biological studies, as a method for fabricating prototype devices[70-74]. This allows quicker, less expensive fabrication of prototype devices that test new ideas as compared to devices made from silicon and glass. The other is a simple method for fabricating pneumatically actuated valves, mixers and pumps by multilayer soft lithography (MSL)[21,75-78]. Monolithic elastomer actuators restrict fluid flow in a channel by pressurization of an adjacent channel under pressure. Figure 5 shows schematic operation mechanisms of monolithic elastomer valves and pumps. When pressure is introduced into the control channels, the elastic membranes expand into the fluidic channel. A regular valve hav-

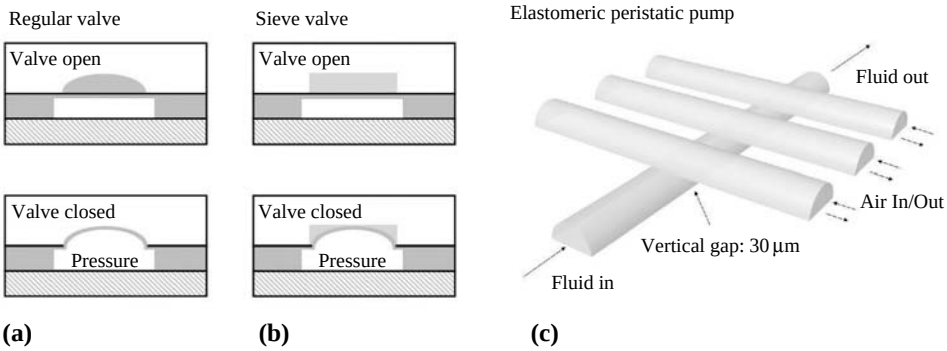


Figure 5. Schematic diagram of the operational mechanisms of (a) a regular valve having a round-profile fluidic channel, (b) a sieve valve having a square-profiled fluidic channel and (c) an elastomeric peristaltic pump from three valves arranged on a single channel.

ing a round-profiled fluidic channel makes the fluidic channel be completely sealed while a sieve valve having a square-profiled fluidic channel does be partially closed. A peristaltic pump with three elastomer valves works by applying air pressure sequentially in each air channel. These elastomer actuators have enabled the design and realization of large-scale integrated microfluidic systems for high throughput processing of smaller volumes with higher degrees of parallelization.

Quake and co-workers[76] have made enormous strides in multiplexing technology with MSL and developed elastomeric PDMS devices with thousands of valves and hundreds of individually addressable chambers (called microfluidic large-scale integration (mLSI)). They demonstrated a microfluidic system for highly parallel high-throughput screening which integrated 2056 valves with 256 compartments containing bacterial cells expressing an enzyme of interest that could be combined on a pairwise basis with 256 other compartments containing a fluorogenic substrate used to assay for a desired activity (Figure 6a). In following work, they developed mechanical valve-based systems for automation of serial and parallel bioprocesses including cell isolation, cell lysis, DNA purification and DNA recovery without any pre- or post-sample treatment on a single microfluidic chip (Figure 6b)[77]. They also implemented a microfluidic bioreactor having two alternating states: (a) continuous circulation and (b) cleaning and dilution. It allowed long-term monitoring of small populations of bacteria with single-cell resolution and unnatural behavior programmed by a synthetic 'population control' circuit over hundreds of hours (Figure 6c)[78]. Recently, the high-throughput microfluidic device containing 2400 unit cells controlled by 7233 valves have been developed to detect low-affinity transient binding events on the basis of the mechanically induced trapping of molecular interactions (Figure 6d)[79]. It enabled to map the binding energy landscapes of four eukaryotic transcript factors to predict *in vivo* function and to test basic assumptions about transcription factor binding.

Meanwhile, much effort has been made to develop miniaturized fluorescence-activated cell-sorting devices (μFACS) with high throughput rates by optical forces, electric fields, hydrodynamic valves and smart materials[80-90]. Wolff *et al.* designed and developed a novel pressure driven μACS device with advanced functional components including a chamber for holding and culturing the sorted cells and monolithically integrated waveguides for cell analysis (Figure 7a)[83]. They achieved a 100-fold enrichment of fluorescent beads at a throughput of 12,000 cells per second while sorting a mixture of fluorescent latex beads and chicken red blood cells. Wang *et al.* demonstrated a high throughput cell sorter with all-optical control switch for living cells (Figure 7b)[86]. The optical forces, which depend on the optical power and relative optical properties of the particle and its surrounding medium, were used to deviate the cell from the flow stream to a sufficient degree to that it was directed toward the target output channel. They showed that sorting runs of cell populations ranging from as few as 1,000 cells up to 280,000 cells can be completed in less than an hour. Renaud and co-workers have developed a microfabricated impedance flow cytometer that incorporates deflective dielectrophoresis barriers and controlled pressure driven liquids flows and characterizes cells by the ratio of the impedance magnitude at a high frequency to that at a low frequency (Figure 7c)[87]. More detailed information on novel flow cytometer based on microfluidic technologies can be found in Reference 5.

Laminar flow within microchannels can be exploited to deliver compounds to a local area of one cell, potentially allowing the evaluation of the stochastic nature of cellular responses, the effect of chemical gradients or drug interactions within a single cell[15,22,91-97]. Recently L.P. Lee and co-workers[91,92] have developed a microfluidic cell-culture array with an integrated concentration gradient generator for long-term cell-

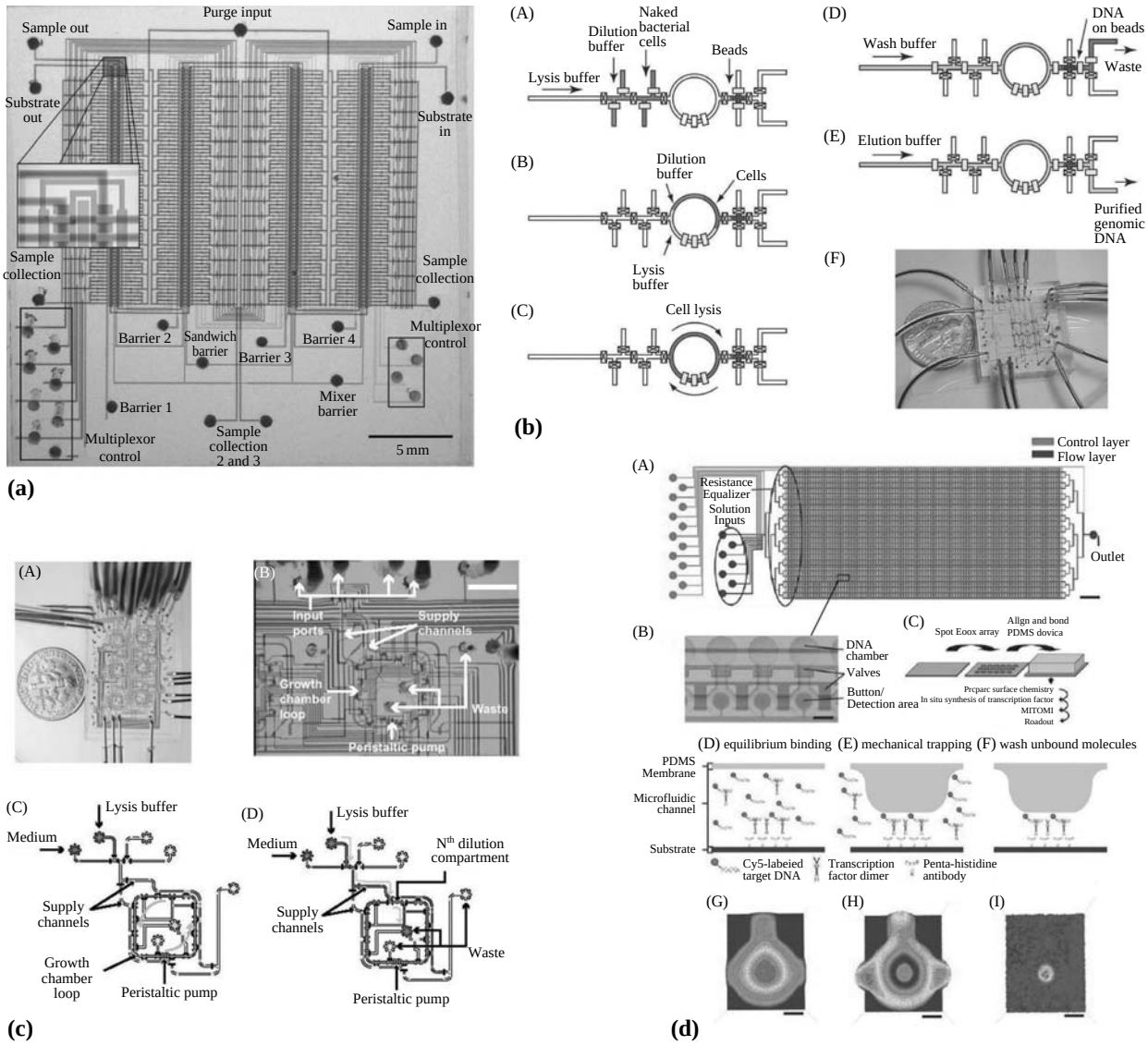


Figure 6. Microfluidic large-scale integration: (a) optical micrograph of a chip for parallelized high-throughput screening of fluorescence-based single-cell assays[76], (b) an integrated processor for DNA purification from cell culture to DNA recovery scheme[77], (c) microchemostats for long-term culture and monitoring of extremely small populations of bacteria with single-cell resolution[78] and (d) a high-throughput microfluidic platform to characterize DNA binding energy landscapes for transcription factors[79].

ular monitoring (Figure 8a). The gradient generation permits many growth conditions to be analyzed in a combinatorial fashion and so is well suited for high throughput experimentation, where a large number of conditions need to be screened in parallel with minimal consumption of analytes and reagents. They also demonstrated high-density regular arrays of single cells isolated in a purely hydrodynamic fashion within a microfluidic device (Figure 8b)[95]. Cells were held by obstacles incorporated into the channel while the fluid passed above and around both sides of the cells in the microchannel. The trapped cell on each obstacle can be used for biological studies on a single-cell level. Recently, high-content screening has been considered as a core method in the early stage of drug discovery for secondary compound screening. N. Ye *et al.* demonstrated an integrated microfluidic device for investigating cellular responses in human liver carcionoa (HepG2) cells under complex physiological conditions (Figure 8c)[97]. The device is composed of multiple drug gradient generators and parallel microscale cell chambers with the functions of liquid dilu-

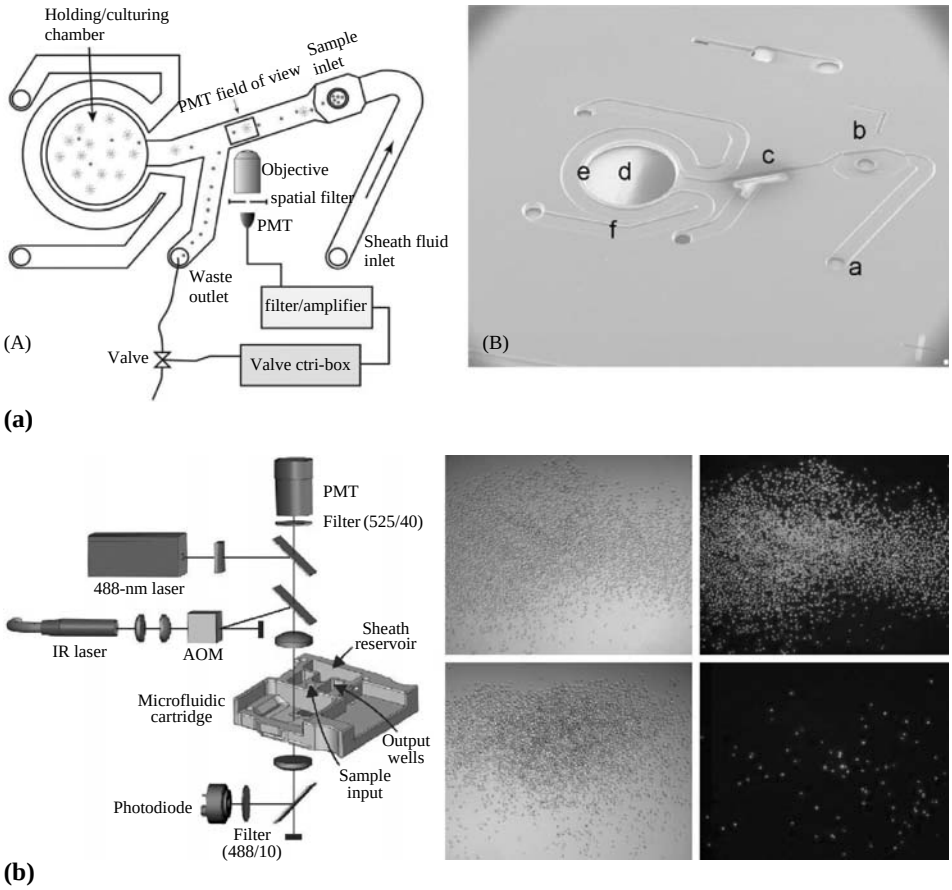


Figure 7. High-throughput cell sorter: (a) a micro cell sorter chip with an integrated holding/culturing chamber[83], (b) an optically switched microfluidic fluorescence-activated cell sorter[86] and (c) an integrated impedance flow cytometer[87].

tion and diffusion, cell culture, cell stimulation and cell labeling and so it enables to rapidly extract rich information related to cell response across several drug candidates and to reduce sample consumption and screening time.

The manipulation of multiphase flows is another application for high throughput rate microfluidic systems[13,17,19,23-25,98-102]. These systems enable the generation and manipulation of monodisperse bubbles and droplets of liquid phase in a continuous

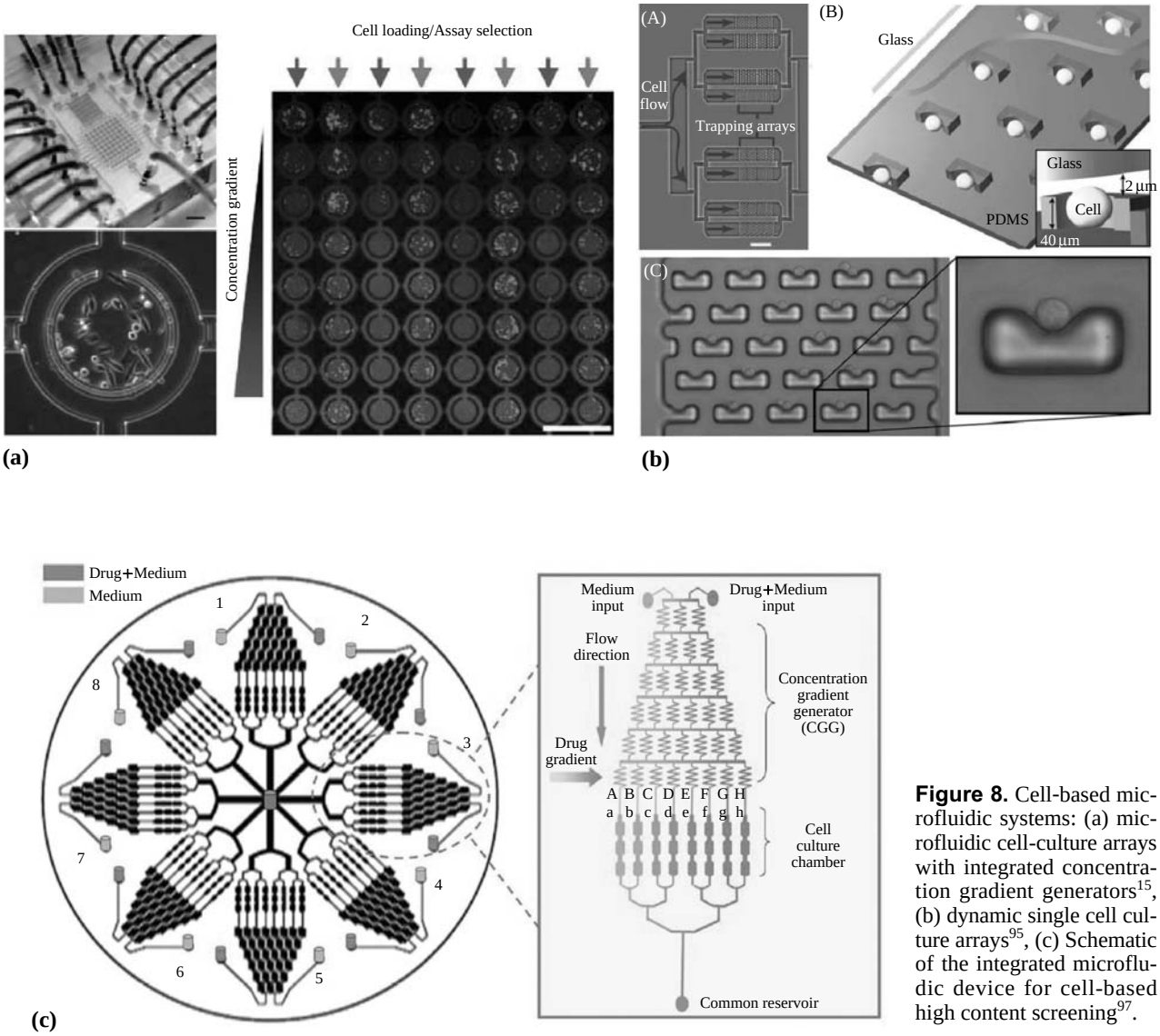


Figure 8. Cell-based microfluidic systems: (a) microfluidic cell-culture arrays with integrated concentration gradient generators[15], (b) dynamic single cell culture arrays[95], (c) Schematic of the integrated microfluidic device for cell-based high content screening[97].

stream, which can be artificial cells to understand the kinetics and biology behind life's fundamental reactions. These techniques have been applied to many different types of biochemical reactions that performed in parallel as well as in series because of enhancing reaction yields for mixing and mass transfer limited reactions and reducing the residence time distribution (RTD). For instance, single-cell enzymatic assays have been achieved by encapsulating single cells inside droplets (Figure 9a)[98]. A single droplet of controlled femtoliter volume was generated by specially designed T channels. Before the formation of the droplet, a single cell was selected from the aqueous solution and moved by optical trapping to the interface between the aqueous phase and the carrier fluid. These techniques make it possible to investigate each cell under controlled, physiologically relevant environments. Droplet-based microfluidics is also a useful tool for molecular evolution that is particularly interesting for the optimization of drug candidates with high throughput manner. Aqueous droplets from cell-sized compartments keep together the genes, the RNAs and proteins that they encode, and the products of their activity. *In vitro* expression of green fluorescent protein (GFP) to directly analyze the contents of individual droplets has been achieved on a microfluidic system (Figure 9b)[13]. W.-H Tan and S. Takeuchi achieved dynamic microarray using a trap-and-release mechanism due to the combination of fluidic resistance and optically generated microbubble (Figure 9c)[99]. The mechanism is as follows: When the trap is empty, the main stream flows along the

straight channel. Once the trap is occupied by a droplet, the main stream is redirected to the loop channel. To release the droplet, IR laser is focused on the aluminum pattern and so microbubble is generated because of localized heating. Meanwhile, Huebner *et al*. demonstrated protein expression by encapsulating single *E. coli* cells in droplets to express a yellow fluorescent protein (YFP) and they controlled cell occupancy in each droplet by the two flow rates of Luria-Bertani broth (LB medium) medium with cells

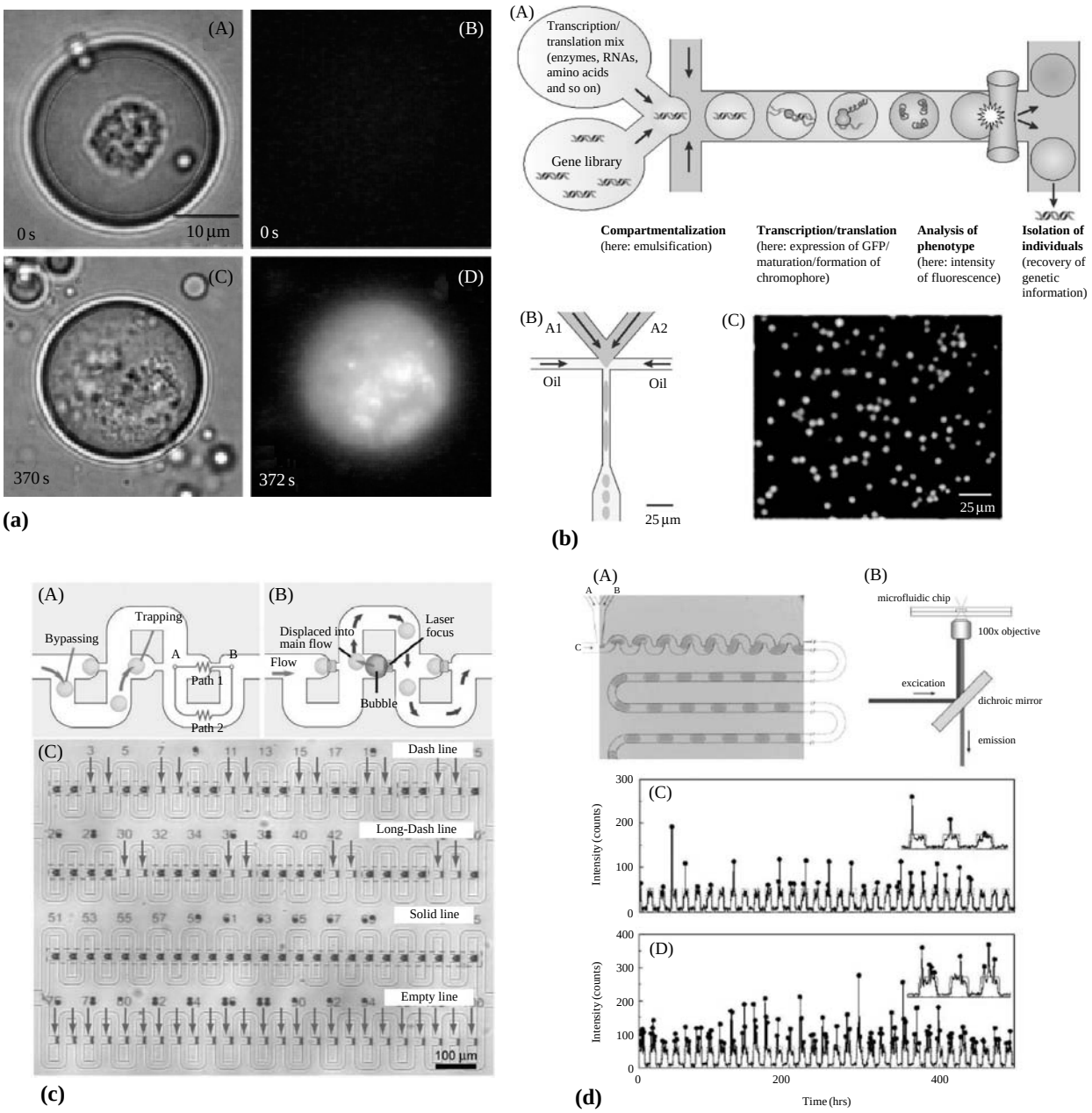


Figure 9. Droplet-based microfluidic systems: (a) Droplets formed with microfluidic channels (an enzymatic assay for a single mast cell within a droplet. A, C. bright-field images; B, D. fluorescence images)[98], (b) *in vitro* evolution of proteins in a microfluidic channels (A. the concept, B a schematic diagram of the microchip, C. fluorescence microphotograph of the droplets after GFP experession)[13], (c) a dynamic microarray based on a trap-and-release integrated microfluidic system (A. a microfluidic trap, B. release mechanism using microbubble, C. demonstration of the use of trap-and-release device in chemical microarrays applications)[99] and (d) quantitative detection of protein expression in single cells (A. optical images of the PDMS device, B. Schematic of the laser induced fluorescence optical setup, C, D. optical readout of 0.5s traces recorded under low and high suspension cell loading conditions[100].

and LB medium only at a Y-channel configuration (Figure 9d)[100]. M. Srisa-Art *et al*. demonstrated a high-throughput biological assay based on fluorescence resonance energy transfer (FRET) by attaching a FRET donor (Alexa Fluor 488) to streptavidin and labeling FRET donor (Alexa Fluor 647) on one DNA strand and biotin on the complementary strand at rates in excess of 1 kHz[101]. Laval *et al*. demonstrated droplet-based microfluidic devices for solubility screening of chemical compounds that allow the direct and quantitative reading of two-dimensional diagrams, temperature vs. composition[102]. The model experiments of adipic acid droplets were taken over less than 1 hr with showing better temperature control and faster screening than traditional methods.

Conclusions

The field of microfluidic systems for high throughput screening continues to mature. Before microfluidic devices replace existing assays and systems, several challenges still remain: standardization, interfacing between micro- and macro-worlds, ease of handling and robustness of systems, massive parallelization and cost. With these problems surmounted, future microfluidic systems will be capable of performing extensive processing and analysis for biological and drug discovery applications, on components ranging from DNA fragments to entire cells. The processing and analysis functions will be performed on a single chip with high throughput rates in a continuous flow manner and in near-physiological environments as close to in vivo conditions as possible. Furthermore, microfluidic systems combined with computational tools, nanobiotechnologies and information technologies will make technological breakthroughs in the near future. For instance, personalized medicine can be realized to treat an individual patient with the exact drug with optimal efficacy and safety.

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