Micromachines **2015**, *6*, 1249-1271; doi:10.3390/mi6091249

OPEN ACCESS

micromachines

ISSN 2072-666X

www.mdpi.com/journal/micromachines

Review

Recent Advances in Applications of Droplet Microfluidics

Wei-Lung Chou [1,†], Pee-Yew Lee [2,†], Cing-Long Yang [1], Wen-Ying Huang [3] and Yung-Sheng Lin [4,*]

[1] Institute of Occupational Safety and Hazardous Prevention, Hungkuang University, Taichung 43302, Taiwan; E-Mails: wlchou@sunrise.hk.edu.tw (W.-L.C.); sst790913@gmail.com (C.-L.Y.)

[2] Institute of Materials Engineering, National Taiwan Ocean University, Keelung 20224, Taiwan; E-Mail: pylee@mail.ntou.edu.tw

[3] Department of Applied Cosmetology, Hungkuang University, Taichung 43302, Taiwan; E-Mail: beca690420@sunrise.hk.edu.tw

[4] Department of Chemical Engineering, National United University, Miaoli 36003, Taiwan

[†] These authors contributed equally to this work.

* Author to whom correspondence should be addressed; E-Mail: linys@nuu.edu.tw; Tel.: +886-37-382-199; Fax: +886-37-382-189.

Academic Editors: Andrew deMello and Xavier Casadevall i Solvas

Received: 31 July 2015 / Accepted: 26 August 2015 / Published: 2 September 2015

Abstract: Droplet-based microfluidics is a colloidal and interfacial system that has rapidly progressed in the past decade because of the advantages of low fabrication costs, small sample volumes, reduced analysis durations, high-throughput analysis with exceptional sensitivity, enhanced operational flexibility, and facile automation. This technology has emerged as a new tool for many recently used applications in molecular detection, imaging, drug delivery, diagnostics, cell biology and other fields. Herein, we review recent applications of droplet microfluidics proposed since 2013.

Keywords: droplet; microfluidics; applications; detection; imaging; drug delivery; diagnostics; cell

---

1. Introduction

Microfluidics appeared in the early 1980s as a promising interdisciplinary technology and has since received considerable attention. Microfluidics involves volumes of fluid in the range of microliters to

picoliters, and demonstrates many advantages such as rapid mass delivery and heat transfer, and reduced reagent use and waste generation [1,2]. The reagent use can be reduced to nanoliters or less, and the reaction time can be reduced to mere seconds. Miniaturizing biological assays or processes on a chip has emerged as a hopeful technology [3–7].

Droplet microfluidics entails both continuous-flow emulsion-based droplet microfluidics and electrowetting-based droplet (also called discrete droplet or digital droplet) microfluidics as shown in Figure 1. Droplet formation of the continuous-flow microfluidics is the result of an emulsion created using two immiscible fluids, including liduid/liquid and gas/liquid systems. Various techniques, such as channel geometry (T-junction or flow-focusing) and dielectrophoresis, were well applied for good control of droplet generation. As for the electrowetting-based droplet, an electric field can change the interfacial tension between the liquid and the surface. Activation of the electrodes leads liquid wetting, and switching off the electrodes reverses. The change in the interfacial tension is capable of producing liquid finger and then breaking off from the reservoir to form a droplet.

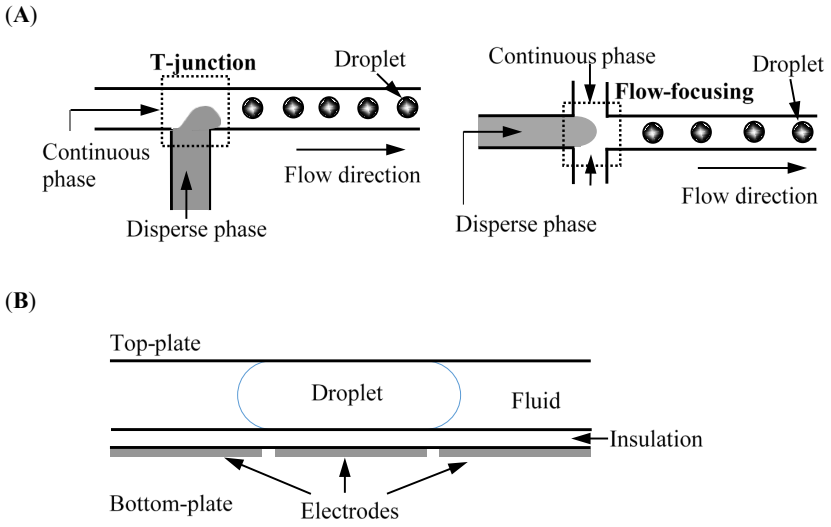

Figure 1. The two major types of droplet microfluidics. (**A**) Continuous-flow emulsion-based droplet microfluidics from T-junction and flow-focusing; (**B**) Electrowetting-based droplet microfluidics.

The microchannels in continuous-flow microfluidic devices have at least one dimension smaller than 1 mm. Droplets acting as individual compartments in the fluid are comparable in size with the apparatus itself. In contrast to that in macroscale channels, the capillary force in microchannels dominates the inertial effects, and capillary action dramatically alters system behavior. For obtaining fine control over the size and shape of droplets, several active and passive methods involving various techniques have been proposed [8,9]. As shown in Figure 2, droplet microfluidics is a rapidly growing field, and the number of publications (according to ISI Web of Knowledge) has increased substantially since 2010. Droplet microfluidics not only has most conventional microfluidic characteristics but also provides numerous superior advantages such as ultrahigh-throughput generation and manipulation of microreactors, implementation of ultrasmall reactors, expulsion of Taylor diffusion and sample dilution, minimization of sample absorption on channel walls, and enhanced mixing and mass transfer inside droplets [2].

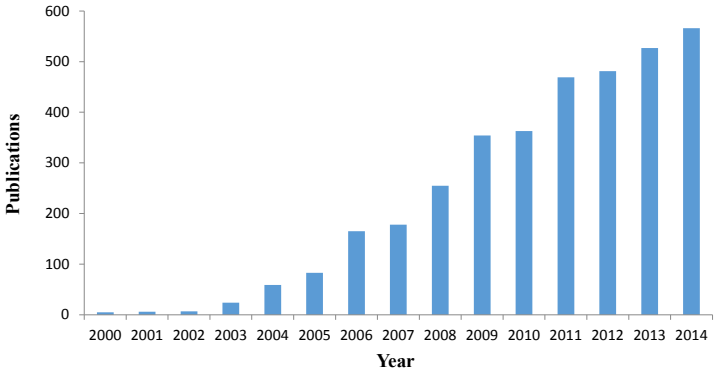

Figure 2. Number of publications on droplet microfluidics in the past 15 years.

Droplet microfluidics exhibits great promise for various applications in a broad spectrum of fields. Droplet-based platforms have been employed in chemical reactions, molecule synthesis, imaging, drug delivery, drug discovery, diagnostics, food, cell biology, and other applications. For example, they can be applied in polymerase chain reaction (PCR)-based analyses, enzyme kinetics and protein crystallization studies, cell cultures, functional component encapsulation, and small molecule and polymeric particle synthesis [10–15]. The fundamental features of these droplet-based microfluidic platforms with high-level integration include fine control of small sample volumes, reduced amounts of reagents and samples, reduced analysis times, improved sensitivity, lowered detection limits, increased high-throughput screening, enhanced operational flexibility [9].

Many researchers have conducted fundamental studies on droplet microfluidics [16]. Sarvothaman *et al.* developed a strategy that involves using fluoroalkyl polyethylene glycol copolymers to reduce protein adhesion, which causes droplet movements to fail [17]. Seiffert *et al.* proposed faster droplet production by delayed surfactant-addition to push droplet-based microfluidics to an industrially relevant scale [18]. Pirbodaghi *et al.* developed an accurate approach that entails using bright-field microscopy with white light illumination and a standard high-speed camera for studying the fluid dynamics of rapid processes within microfluidic devices [19]. Musterd *et al.* calculated the volume of elongated droplets in microchannels from a top-view image in interpreting experiments on reaction kinetics and transport phenomena [20]. Zantow *et al.* applied the Hough transform for image analysis to automatically determine microfluidic droplet sizes [21]. Luo *et al.* proposed a design method to reduce the number of control pins and facilitate the general purpose of digital microfluidic biochips [22]. Janiesch *et al.* found key factors for stable retention of fluorophores and labeled biomolecules such as antibodies, streptavidin, and tubulin proteins in droplet-based microfluidics [23]. Pan *et al.* studied optimization algorithms for designing digital microfluidic biochips [24]. Lai *et al.* developed an intelligent digital microfluidic processor for biomedical detection [25]. Huang *et al.* proposed a reactant and waste minimization algorithm for multitarget sample preparation on digital microfluidic biochips [26]. Chen *et al.* developed a reliability-oriented placement algorithm for reconfigurable digital microfluidic biochips by using a 3D deferred decision making technique to optimize bioassay completion times [27]. Maftei *et al.* proposed a module-based synthesis of digital microfluidic biochips with droplet-aware operation execution [28]. Luo *et al.* investigated error recovery by using a cyberphysical resynthesis technique to recompute electrode-actuation sequences on digital microfluidic biochips [29]. In addition, Isgor *et al.* developed

a scalable, portable, robust and high-sensitivity capacitive microdroplet content detection system using low-cost coplanar electrodes and off-the-shelf capacitive sensors for biochemical assay monitoring [30].

Considering the substantial size miniaturization and small component contents in droplet microfluidics, facilities and techniques that are more sensitive and powerful are required for detection and characterization of droplets in microfluidics. Lu *et al.* developed a microfluidic chip coupled with surface enhanced Raman scattering spectroscopy "lab-on-a-chip" system to rapidly detect and differentiate pathogens [31]. Kawano *et al.* developed a Darkfield Internal Reflection Illumination system for observing a microfluidic device containing microbubbles, fluorescent particles, or fluorescently labeled cells to overcome the limitation on conventional methods [32]. Kim *et al.* used optofluidic droplet interrogation device for ultrahigh-throughput detection of fluorescent drops at a rate of 254,000 drops/s [33]. Muluneh *et al.* demonstrated a handheld-sized device to monitor four independent channels with scaled-up to more than sixteen simultaneously [34].

Thus, increasingly more versatile applications of droplet microfluidics have been proposed, indicating marked matureness in various fields. In this article, we review the applications of droplet microfluidics, which are categorized as follows: molecular detection, imaging, drug delivery, diagnostics, cell biology, and other applications.

2. Molecular Detection

Droplet microfluidics revolutionizes molecular detection to replace cumbersome chemical laboratory experiments by using miniaturized and integrated systems [24] demonstrating the advantages of precise liquid handling for chemical assays, minimized reagent consumption, and maximized outputs for high-throughput configurations [35]. Droplet microfluidic systems have been applied in analytical detection by using various techniques for qualitative content analysis in droplets. Such analytical detection techniques entail image-based analysis, laser-based molecular spectroscopy, electrochemistry, capillary electrophoresis, mass spectrometry, nuclear magnetic resonance spectroscopy, absorption, and chemiluminescence detection [2].

Single molecule detection has been emphasized from examining the physical properties of biological macromolecules to extracting genetic information from DNA. Many protein biomarkers utilized for monitoring disease progression or healthy states are generally in complex samples at low concentrations [36]. However, the traditional macroscale detection of single molecule is often limited to measuring equilibrium states and is subject to background noises [37]. Therefore, isolating and analyzing single molecules at low concentrations in a complex mixture of biological samples are nearly impossible to implement [38]. Droplet microfluidics facilitates manipulating single molecules at the microliter scale and smaller, and has unique microscale fluidic characteristics for conducting single-molecule experiments with high sensitivities and throughput [35].

Oedit *et al.* thoroughly reviewed techniques used for bioanalytical applications over the past 3 years and discussed the involved merits and limitations, such as the growing popularity of throughput, small volume, disposability, and automation in bioanalysis [39]. In addition, Zeng *et al.* reviewed the recent progress of microfluidic design and applications in quantitative and systems biomolecular analysis including biomolecular interaction profiling, genomics and transcriptomics, proteomics, and clinical

diagnostics [40]. Other applications of droplet microfluidics in molecular detection are summarized in Table 1.

Table 1. Recent applications of droplet microfluidics in molecular detection.

Topic	Target	Remark	Ref.
A droplet-based fluorescence polarization immunoassay platform for rapid and quantitative analysis of biomarkers	Bovine angiogenin	Accurately determined the angiogenin concentration in cow's milk, and required a total sample volume of less than 1 nL.	[41]
A novel droplet dosing strategy-based versatile microscale biosensor for detection of DNA, protein, and ion	dsDNA, streptavidin, and Hg^{2+}	The contact-induced droplets dosing based on adsorption and desorption was developed to overcome the channel-fouling problem.	[42]
Specific detection of avidin-biotin binding using liquid crystal droplets	Bovine serum albumin, lysozyme, hemoglobin, and chymotrypsinogen	The $5CB_{PAA\text{-}biotin}$ droplets toward avidin were found to have high sensitivity, specificity, and stability.	[43]
Glucose sensor using liquid-crystal droplets made by microfluidics	Glucose	The biosensor detected samples under crossed polarizers at concentrations of 0.03 mM and 3-min response times.	[44]
Enzyme incorporated microfluidic device for *in situ* glucose detection in water-in-air microdroplets	Glucose	The fluorescence intensity linearly increased with glucose concentration up to 3 mM, and its detection limit was 6.64 μM.	[45]
Integrating bipolar electrochemistry and electrochemiluminescence imaging with microdroplets for chemical analysis	Quinones	Closed bipolar cell sensor could avoid the interference and cross-contamination between analyte solutions and electrochemiluminescence-reporting reagents.	[46]
Peptide nucleic acid molecular beacons for the detection of PCR amplicons in droplet-based microfluidic devices	*Olea europaea* L. and Roundup Ready soybean genes	Efficiently discriminated oligonucleotide sequences carrying single-base mutations at 100 nM.	[47]
A highly parallel microfluidic droplet method enabling single-molecule counting for digital enzyme detection	β-Galatosidase	An integrated microfluidic chip offered the feasibility of detecting single-enzyme molecules based on a digital counting method.	[48]
Digital microfluidic-enabled single-molecule detection by printing and sealing single magnetic beads in femtoliter droplets	β-Galactosidase	The fluorescent detection had a linear dynamic range of four orders of magnitude ranging from 10 aM to 90 fM.	[49]
Protein–protein interaction analysis in single microfluidic droplets using FRET and fluorescence lifetime detection	Bovine serum albumin, avidin, and streptavidin	Could be used for quantitative detection of molecules in direct and competitive assay formats within nM detection limits.	[50]

3. Imaging

The use of imaging analysis is increasing worldwide, thereby ensuring optimal research and clinical diagnosis. In imaging techniques, enhancing the clarity and quality of an image by using contrast agents is critical. Droplet microfluidics has been used in imaging applications for synthesizing monodisperse, size-controlled, and high-quality microparticles, such as microbubbles and volatile liquid droplets for echogenic particles [51–53].

Microbubbles are spherical particles with gas encapsulated in a shell, and have excellent biocompatibility at clinically relevant ultrasound frequencies for image contrast enhancement [54]. Traditional methods for generating microbubbles rely on bulk mechanical agitation, leading to a polydisperse size distribution. The polydisperse populations of microbubble sizes hinder the ability to cross the circulation and acoustic response. Microfluidics can achieve a fine degree of monodispersity and increase the overall yield of microbubbles for various applications [55].

With a high sensitivity and low detection limit, fluorescence is another frequently used tool for imaging. A new fluorescent molecule can perform different intracellular interactions with positive

and specific responses for visualizing the fluorescence process under analysis with a considerably low fluorophore concentration [56]. High-quality fluorescent CdTe:Zn^{2+} quantum dots of various emission spectra and other fluorescence probes can be synthesized in monodisperse polymeric microspheres by using an on-demand one-step process with droplet microfluidics [57]. Observing the particle size and concentration of fluorescent nanoparticles can clarify the nature of such particles in complex media. Many studies have used fluorescence to sort droplets or probe droplet-based microfluidic systems in high throughput [9].

For the biodistribution of microparticles *in vivo*, determining which organ or tissue traps the microparticles depends on the size, whereas determining the uptake efficiency depends on the uniformity [58]. Wang *et al.* reported using monodisperse radiolabeled microparticles from microfluidics for imaging of different organs and tissues [58]. The favorable properties of these microparticles demonstrated excellent performance in imaging according to their homogeneous and efficient retention. Other applications of droplet microfluidics in imaging are summarized in Table 2.

Table 2. Recent applications of droplet microfluidics in imaging.

Topic	Target	Remark	Ref.
Atom-economical *in situ* synthesis of $BaSO_4$ as imaging contrast agents within poly(*N*-isopropyl acrylamide) microgels using one-step droplet microfluidics	Microgel	Fourteen-nanometer crystallites of $BaSO_4$ as an X-ray imaging contrast agent were *in situ* synthesized with interlinking reactions.	[59]
Cloud-enabled microscopy and droplet microfluidic platform for specific detection of water	*Escherichia coli*	Magnetic beads conjugated with fluorescently labeled antibodies could selectively capture and isolate specific bacteria.	[60]
Live cell imaging compatible immobilization of *Chlamydomonas reinhardtii* in microfluidic platform for biodiesel research	*Chlamydomonas reinhardtii*	Provided real-time monitoring and analysis of lipid accumulation using single cell imaging for rapid optimization of microalgae culture conditions.	[61]

4. Drug Delivery

Droplet microfluidics establishes new frontiers and provides promising and powerful platforms for precise production of novel functional materials as drug delivery vehicles and drug molecules [8,16,58,62,63]. In the medical field, drugs can be supported to these fine particles, forming a type of drug delivery system for releasing drugs. The drug delivery vehicles have flexible delivery of existing drugs with improved performance [58]. The interaction force between drugs and carriers can control the drug release rate [64]. Protein and peptide therapeutics are typical examples of drug carrier systems in droplet microfluidics, because of their poor bioavailability in the gastrointestinal tract before reaching the bloodstream [65]. The main functions of drug carrier systems in pharmaceutics include (i) the immobilization process, (ii) protection against degradation, (iii) improved drug stability, and (iv) controlled drug delivery behavior [16].

Droplet microfluidics demonstrates great potential for production of complex drug systems of uniform size, monodisperse size distribution, and desired properties. Microfabricated drug delivery systems include emulsions, microparticles, microcapsules, and microgels [66]. Several recent applications of droplet microfluidics in drug delivery are summarized in Table 3.

5. Diagnostics

The performance of droplet microfluidics is suitable for miniaturized diagnosis platforms [58,63,66]. Droplet microfluidics can perform clinical laboratory tests by a part of reagents and in a short period. It has been shown in different fields to improve the diagnostic process for analyzed components, especially in proteomics and nucleic acid-based diagnosis [67]. For example, PCR devices of droplet microfluidics demonstrate many crucial advantages including portability, low reagent consumption, rapid heating/cooling, and a short assay time. Droplets for enzymatic assays can confine molecules and reactions to a small volume (picoliters to nanoliters), thereby reducing the number of mixing and washing steps [68].

Based on enzymatic reactions and cell cultures, Rosenfeld *et al.* investigated the performance metrics of droplet microfluidic systems. According to mature detection of nucleic acids in droplet microfluidics, a similar analysis can be applied to many other assay systems [68]. In addition, Kaler *et al.* reviewed electroactuation-based droplet microfluidics and its clinical diagnostic assays in nucleic acid amplification and real-time detection, immunoassays, and protein analysis [63]. Several recent applications of droplet microfluidics involving diagnostic chips are summarized in Table 4.

6. Cell Biology

Droplet microfluidics and new related techniques offer new possibilities for life science research. The basic principle of droplet microfluidic systems relies on unique liquid-handling capabilities and highly monodisperse aqueous droplets in an inert carrier oil flow. Therefore, each droplet is the functional equivalent of an independent microculture for cells. Encapsulated cells can remain viable for extended periods in droplets for additional cell-based assays and biochemical assays [69]. Rakszewska *et al.* well review recent developments in droplet microfluidics as a versatile tool for single-cell studies [70].

Droplet microfluidics in cell biology can be applied variously. Schlicht *et al.* developed a scalable and automated formation of arrays by using droplet-interface-bilayer techniques to imitate cell membrane processes [71]. Recently, Cao *et al.* thoroughly reviewed the toxicological screenings and applied sensing principles of organisms and cells inside microdroplets [72]. Despite the great progress of cell study in digital microfluidics, the mentioned technologies may impose potential biases. Au *et al.* recommended that digital microfluidic experiments involving cells be optimal for ensuring driving frequencies lower than 10 kHz and electrode sizes smaller than 5 mm to prevent DNA damage and changes in gene expression [73]. Table 5 lists recent applications of droplet microfluidics in cell biology.

Table 3. Recent applications of droplet microfluidics in drug delivery.

Topic	System	Remark	Ref.
Microfluidic-assisted engineering of polymeric microcapsules with high encapsulation efficiency for protein drug delivery	Polycaprolactone microcapsules encapsulating bovine serum albumin	The high encapsulation efficiency of proteins in the microcapsules reached 84%, and 30% of their content was released within 168 h.	[65]
Microfluidic assembly of multistage porous silicon-lipid vesicles for controlled drug release	Thermally hydrocarbonized porous silicon microparticle-lipid vesicle	The drug encapsulation efficiency was 19%, and the whole payload was released after only 6 h at pH 7.4.	[74]
Generation of uniform polymer-eccentric and core-centered hollow microcapsules for ultrasound-regulated drug release	Polydimethylsiloxane microcapsules encapsulating Rhodamine 6G and domperidone maleate	The system demonstrated the properties of a floating drug delivery system absorbed in the upper segments of the gastrointestinal tract for a long gastric residence time.	[75]
Synthesis of uniform core-shell gelatin-alginate microparticles as intestine-released oral delivery drug carrier	Core-shell gelatin-alginate	The fabricated microparticles could remain intact in gastric juice for more than 3 h, indicating effective protection in an acidic environment.	[76]
Controllable microfluidic fabrication of Janus and microcapsule particles for drug delivery applications	Poly(lactic-co-glycolic acid /poly(ε-caprolactone) microcapsule	The microparticles exhibited distinct degradation behavior, implying programmable drug delivery in different manners.	[77]
Core-shell structure microcapsules with dual-pH-responsive drug release function	Ampicillin loaded in the chitosan shell and diclofenac loaded in the alginate core	Demonstrated higher drug release efficiency than respective core or shell particles for dual-drug carriers.	[78]
Microfluidic-assisted generation of innovative polysaccharide hydrogel microparticles	Pectin-pectin (homo Janus) and pectin-alginate (hetero Janus) encapsulating bovine serum albumin	Facilitated studying the relationships between combined enzymatic hydrolysis and active release for anisotropic microparticles.	[79]
Microfluidic synthesis of monodisperse PEGDA microbeads for sustained release of 5-fluorouracil	Poly(ethylene glycol) diacrylate microbeads encapsulating 5-fluorouracil	The drug (0.1 to 0.5% *w*/*w*) demonstrated relatively fast elution in the first 12 h and continued to release over the next 156 h to effectively inhibit Huh-7 tumor cells *in vitro*.	[80]
Chitosan/agarose hydrogels: cooperative properties and microfluidic preparation	Chitosan and agarose composite hydrogels containing 5-fluorouracil	The hydrogels released 5-fluorouracil from chitosan/agarose macrogels with dual-pH and temperature properties.	[81]
Microfluidic fabrication of monodisperse biocompatible phospholipid vesicles for encapsulation and delivery of hydrophilic drugs or active compounds	Phospholipid vesicles encapsulating doxorubicin hydrochloride	The encapsulation efficiency was approximately 94%, and showed superior sustained release.	[82]

Table 4. Recent applications of droplet microfluidics in diagnostic chips.

Topic	Target	Remark	Ref.
Detecting and tracking nosocomial methicillin-resistant *Staphylococcus aureus* by using a microfluidic SERS biosensor	*Staphylococcus aureus*	A reliable detection and epidemiological surveillance of bacterial infections in a bacterial mixture at levels from 5% to 100% was developed.	[31]
Ultrarapid generation of femtoliter microfluidic droplets for single-molecule-counting immunoassays	Prostate-specific antigen	The femtodroplet system enabled a single enzyme molecule for prostate cancer to be detected within 10 min and reduced the concentration to 46 fM.	[83]
Microfluidic droplet-based liquid-liquid extraction and on-chip IR spectroscopy detection of cocaine in human saliva	Cocaine	Showed a 2−3-fold higher extraction efficiency compared with state-of-the-art H-filters.	[84]
A centrifugal microfluidic platform for point-of-care diagnostic applications	Plasma and blood cells	Achieved pumping and valving of fluids and generation of monodisperse droplets on lab-on-a-disk system.	[85]
An integrated CMOS quantitative-polymerase-chain-reaction lab-on-chip for point-of-care diagnostics	*Staphylococcus aureus*	The complementary metal-oxide-semiconductor-integrated circuit had a reliable and sensitive detection of *Staphylococcus aureus* from 1 to 104 copies per 1.2-nL droplet.	[86]
Picoliter droplet microfluidic immunosorbent platform for point-of-care diagnostics	Human anti-tetanus immunoglobulin G	Reduced the reagent volume by four orders of magnitude and the detection time from hours to minutes.	[87]
Analysis of single-nucleotide polymorphism in human angiogenin using droplet-based microfluidics	Human angiogenin	The detection of single-nucleotide polymorphism in the droplet could be performed using TaqMan probes on DNA samples amplified offline by using a conventional thermocycler rather an expensive real-time PCR system.	[88]
Magnetic bead droplet immunoassay of oligomer amyloid β for the diagnosis of Alzheimer disease using micropillars to enhance the stability of the oil-water interface	Oligomer amyloid β	The platform markedly reduced the assay time to 45 min and the amount of antibody usage to 10–30 ng per assay.	[89]
Droplet microfluidic chip based nucleic acid amplification and real-time detection of influenza viruses	Influenza A and C	The detection threshold of the chip-based qRT-PCR for detecting and quantifying viral nucleic acids was approximately five copies per PCR reaction.	[90]
Multiplex, quantitative, reverse-transcription PCR detection of influenza viruses using droplet microfluidic technology	Influenza A and B	The qRT-PCR process was found to be less than 10 RNA copies accomplished within 40 min.	[91]
Rapid detection of tuberculosis using droplet-based microfluidics	BlaC enzyme	For a 30-μm droplet size, the fluorescent intensity change could be detected after less than 1 h of incubation.	[92]

Table 4. *Cont.*

Topic	Target	Remark	Ref.
Development of a microfluidic-based optical sensing device for label-free detection of circulating tumor cells through their lactic acid metabolism	Circulating tumor cells	Could detect the targeted cancer cells without interference by the cell species.	[93]
Assembly-line manipulation of droplets in microfluidic platform for fluorescence encoding and simultaneous multiplexed DNA detection	Human immunodeficiency virus, variola virus	The result indicated that targets could be simultaneously detected using a time-saving process and without a complex dye-labelling process.	[94]
Digital microfluidic platform for the detection of rubella infection and immunity: a proof of concept	Rubella virus	For both rubella viruses IgG and IgM, the performance panel samples demonstrated 100% diagnostic sensitivity and specificity.	[95]
Rapid and reproducible analysis of thiocyanate in real human serum and saliva by using a droplet SERS-microfluidic chip	Human serum and saliva	The reaction required less than 15 s in the designed channel, which is at least 40-fold shorter than that for solid metallic substrates.	[96]
A novel microbead-based microfluidic device for rapid bacterial identification and antibiotic susceptibility testing	*Escherichia coli* O157	The immunocapture efficiency was 85%–92%, higher than 44%–86% of offline immunomagnetic separation.	[97]
Rapid detection of bacteriophages in starter culture using water-in-oil-in-water emulsion microdroplets	*Escherichia coli* BL21 and T7 phages	The lytic phage infection in a bacterial culture could be measured using a simple and inexpensive imaging approach in contrast to flow cytometry and PCR methods.	[98]
Rapid enumeration of phage in monodisperse emulsions	T4-LacZ and nonlytic M13	This quantification was robust and insensitive to environmental fluctuations in contrast to bulk assays.	[99]
Highly sensitive and homogeneous detection of membrane protein on a single living cell by using aptameric and nicking enzyme-assisted signal amplification based on microfluidic droplets	Protein tyrosine kinase-7	Used for constructing a high-throughput platform for detecting a single cell by using aptameric and enzyme-assisted amplification for membrane proteins.	[100]
A biocompatible open-surface droplet manipulation platform for detection of multi-nucleotide polymorphism	Multi-nucleotide polymorphism	The entire procedure required only 5 min and the total sample volume consumed in each operation was only 10 μL.	[101]
Topography-assisted electromagnetic platform for blood-to-PCR in a droplet	KRAS oncogene	Integrated automatic nucleic acid extraction (only 15 min) with real-time amplification detection of genetic targets.	[102]
Single-molecule quantitation and sequencing of rare translocations by using microfluidic-nested digital PCR	Lymphoblasts	Demonstrated quantitative measurement and single-molecule sequencing at extremely low levels ($<10^{-6}$) in healthy subjects.	[103]

Table 5. Recent applications of droplet microfluidics in cell biology.

Topic	Target	Remark	Ref.
Single-cell analysis and sorting by using droplet-based microfluidics	Mouse hybridoma cells	This protocol displayed the use of two-phase droplet-based microfluidics for high-throughput single-cell analysis and sorting.	[69]
Versatile microfluidic droplet array for bioanalysis	HL-60 cells	The novel regional hydrophilic chip demonstrated high-throughput screening in toxic tests of CdSe on cells, and a rapid biosensing approach for carcinoma embryonic antigen was developed.	[104]
Mixed hydrogel bead-based tumor spheroid formation and anticancer drug testing	Human cervical carcinoma (HeLa) cells	Multicellular tumor spheroids were formed in the microfluidic droplets, and the viability of cells encapsulated in the mixed hydrogel beads was higher than 90%.	[105]
Cell-based drug combination screening with a microfluidic droplet array system	A549 nonsmall lung cancer cells	The sequential operation droplet array technique provided flexible approach for performing cell-based screening, and the reagent consumptions were decreased by two to three orders of magnitude compared with traditional multiwell plates.	[106]
Digital microfluidics for time-resolved cytotoxicity studies on single nonadherent yeast cells	*Saccharomyces cerevisiae* strain BY4741	Could isolate single nonadherent cells and monitor their dynamic responses at a defined position over time for implementation of high-throughput cytotoxicity assays.	[107]
Droplet-based microfluidic platform for high-throughput, multiparameter screening of photosensitizer activity	*Escherichia coli*	Could detect both live and dead cells online to score cell viability and enable simultaneous measurement of many experiments including those on dark toxicity, photosensitizer concentration, light dose, and oxygenation levels.	[108]
Changing growth behavior of heavy-metal tolerant bacteria: media optimization using droplet-based microfluidics	*Bacillus sporothermodurans* and *Streptomyces tendae*	The nitrogen source between the light scattering and the fluorescence signal may be used for the production of fluorescent secondary metabolites.	[109]
Generation of monodisperse cell-sized microdroplets using a centrifuge-based axisymmetric coflowing microfluidic device	Yeast cells	After the encapsulation process, 87% of the yeast cells were alive in the monodisperse microdroplets.	[110]
Real-time image processing for label-free enrichment of *Actinobacteria* cultivated in picoliter droplets	*Actinobacteria*	Implemented high-throughput cultivation of soil-derived *Actinobacteria* and developed trigger imaging for picoliter droplet sorting.	[111]
Digital microfluidic processing of mammalian embryos for vitrification	Mammalian embryos	The benefits of this digital microfluidic device over conventional manual operation include cryoprotectant concentration gradient generation, automated operation, and feasibility of loading and retrieval of cells.	[112]

Table 5. *Cont.*

Topic	Target	Remark	Ref.
Single-cell forensic short tandem repeat typing within microfluidic droplets	Human lymphoid cells	Individual cells were efficiently encapsulated in nanoliter agarose droplets, serving as the reactors for PCR assays.	[113]
Microfluidic encapsulation of cells in alginate particles via an improved internal gelation approach	Antibody-secreting hybridoma cells (9E10 cell) and mouse breast cancer cells (M6C cell)	Two mammalian cell types were encapsulated with a viability of higher than 84% and grew well inside the microparticles.	[114]
A droplet-based heterogeneous immunoassay for screening single cells secreting antigen-specific antibodies	Alginate microbeads encapsulating antibody-secreting cells	Screened anti-TNF-alpha antibody-secreting cells from a mixture of cells in alginate microbeads as cell culture chambers.	[115]
Ultrahigh-throughput detection of single-cell β-galactosidase activity in droplets using microoptical lens array	*Escherichia coli*	This analytical throughput by a parallelized fluorescent detection compatible with droplet reinjection was larger than those obtained using flow cytometry.	[116]
New glycosidase substrates for droplet-based microfluidic screening	Cellobiohydrolase activity on model bacterial strains (*Escherichia coli* and *Bacillus subtilis*)	The fluorogenic substrates could be utilized to assay glycosidase activities in a broad pH range (4–11) and with incubation times of more than 24 h in droplet-based microfluidic systems.	[117]

7. Other Applications

In addition to the described applications in molecular detection, imaging, drug delivery, diagnostics, and cell biology, droplet microfluidics can be applied in many other fields. For example, droplet microfluidics can be used for monitoring the kinetics of reactive encapsulations occurring at the droplet interface to provide guidelines for generating microcapsules with soft interfaces [118]. Other applications are addressed as follows.

7.1. Particle Shaping

Advanced progress in microfluidics and other techniques have inspired the design of new microcarriers [12]. Shim *et al*. thoroughly reviewed the elaborate design strategies for microcarriers categorized by particle-type carriers, capsule-type carriers, and foldable carriers [119]. Microfluidics is a novel tool for particle shaping and is an improvement over the conventional mechanical shaping method. For example, two types of microgel-capsule structures, bulk microcapsules and core-shell structures, could be easily tailored by droplet-based microfluidic templating followed by subsequent droplet gelation [120]. Furthermore, droplet microfluidics has been applied in manufacturing poly(lactide-co-glycolide)/TiO_2 hybrid microparticles [121], silica microparticles [122], and polysaccharide hydrogel microparticles [79] of various shapes such as spherical, ellipsoidal, disk-like, and rod-like.

7.2. Food

The most common application of droplet microfluidics in foods entails the preparation of emulsions for providing accurate control over the droplet size and shape of internal structures [123]. Emulsion droplets can have a triggered release of flavor or other functional components, and they can also be used as solid particles for structural elements after the phase transition of the emulsion droplets [123]. Moreover, food microgel particles (typically biopolymer hydrogels) can be used for encapsulation of phytonutrients and prebiotics, satiety control, texture control, and targeting delivery in the gastrointestinal tract [124].

Therefore, droplets microfluidics has been proved to be a promising platform for numerous applications such as above-mentioned fields. Although the great progress in recent droplet microfluidics, there are still some concerns and challenges to overcome. For example, the current challenge is the numbering-up to produce large quantities of droplets, especially for more complex core-shell structures. Many used conditions in literatures are model samples and ideal conditions to demonstrate their feasibilities, and therefore there are considerable challenges to be a true device in the real world, especially for a robust and automated instrumentation. In addition, the ability to analyze droplet content qualitatively and quantitatively in the small-volume droplets is still a bottleneck that needs to be solved.

In the future directions, an integrated system is necessary to broaden their applications. For example, combining electrochemical methods, Raman spectroscopy, and mass spectrometry can contribute to a powerful analytical detection technique. The integration of actuators into microfluidic devices is effective for complex flow control and portability. The 3D mold elements can be applied for 3D structures in droplet trapping and sorting, fission and fusion, fluid mixing, and other manipulation. Besides, the

development of advanced sample pretreatment techniques will expand the range of possible applications in chemical and biological analysis to solve real-world problems.

8. Conclusions

Droplet microfluidics provides the benefits of miniaturization, automation, low reagent consumption, high sensitivity, and high-throughput for various applications. In this paper, we review the recent applications of droplet microfluidics since 2013, such as molecular detection, imaging, drug delivery, diagnostics, cell biology, and other applications involving particle shaping and food products. Many recent developments in various fields are tabulated for comparison. With many exciting possibility and opportunities, droplet microfluidics provides novel solutions to today's challenges in biology and medicine for advanced diagnostics and therapeutics. It also gives a promising platform for the next generation of ultrahigh-throughput screening and microsystems for applications. It can be expected that the growth of droplet-based microfluidics will contribute to future revolutions in the field of lab on a chip.

Acknowledgments

The authors gratefully acknowledge the support for this study by the Ministry of Science and Technology, Taiwan, under grant numbers NSC101-2628-E-241-013-MY3 and NSC102-2632-B-241-001-MY3.

Author Contributions

Yung-Sheng Lin designed the main parts and led the development of the paper. Wei-Lung Chou, Pee-Yew Lee, Cing-Long Yang, and Wen-Ying Huang performed the discussion. All authors reviewed the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

References

1. Puigmartí-Luis, J. Microfluidic platforms: A mainstream technology for the preparation of crystals. *Chem. Soc. Rev.* **2014**, *43*, 2253–2271. [CrossRef] [PubMed]
2. Zhu, Y.; Fang, Q. Analytical detection techniques for droplet microfluidics—A review. *Anal. Chim. Acta* **2013**, *787*, 24–35. [CrossRef] [PubMed]
3. Samiei, E.; Hoorfar, M. Systematic analysis of geometrical based unequal droplet splitting in digital microfluidics. *J. Micromech. Microeng.* **2015**, *25*, 055008. [CrossRef]
4. Oliveira, K.A.; Silva, P.B.M.; de Souza, F.R.; Martins, F.T.; Coltro, W.K.T. Kinetic study of glucose oxidase on microfluidic toner-based analytical devices for clinical diagnostics with image-based detection. *Anal. Methods* **2014**, *6*, 4995–5000. [CrossRef]
5. Jones, C.N.; Hoang, A.N.; Dimisko, L.; Hamza, B.; Martel, J.; Irimia, D. Microfluidic platform for measuring neutrophil chemotaxis from unprocessed whole blood. *J. Vis. Exp.* **2014**. [CrossRef] [PubMed]

6. Bernacka-Wojcik, I.; Lopes, P.; Vaz, A.C.; Veigas, B.; Wojcik, P.J.; Simões, P.; Barata, D.; Fortunato, E.; Baptista, P.V.; Águas, H.; *et al*. Bio-microfluidic platform for gold nanoprobe based DNA detection-application to mycobacterium tuberculosis. *Biosens. Bioelectron.* **2013**, *48*, 87–93. [CrossRef] [PubMed]
7. Peng, Z.; Young, B.; Baird, A.E.; Soper, S.A. Single-pair fluorescence resonance energy transfer analysis of mRNA transcripts for highly sensitive gene expression profiling in near real time. *Anal. Chem.* **2013**, *85*, 7851–7858. [CrossRef] [PubMed]
8. Vladisavljevic, G.T.; Khalid, N.; Neves, M.A.; Kuroiwa, T.; Nakajima, M.; Uemura, K.; Ichikawa, S.; Kobayashi, I. Industrial lab-on-a-chip: Design, applications and scale-up for drug discovery and delivery. *Adv. Drug Deliv. Rev.* **2013**, *65*, 1626–1663. [CrossRef] [PubMed]
9. Dressler, O.J.; Maceiczyk, R.M.; Chang, S.I.; DeMello, A.J. Droplet-based microfluidics: Enabling impact on drug discovery. *J. Biomol. Screen.* **2014**, *19*, 483–496. [CrossRef] [PubMed]
10. Sun, X.; Tang, K.; Smith, R.D.; Kelly, R.T. Controlled dispensing and mixing of pico- to nanoliter volumes using on-demand droplet-based microfluidics. *Microfluid. Nanofluid.* **2013**, *15*, 117–126. [CrossRef] [PubMed]
11. Lin, Y.S.; Yang, C.H.; Wu, C.T.; Grumezescu, A.M.; Wang, C.Y.; Hsieh, W.C.; Chen, S.Y.; Huang, K.S. A microfluidic chip using phenol formaldehyde resin for uniform-sized polycaprolactone and chitosan microparticle generation. *Molecules* **2013**, *18*, 6521–6531. [CrossRef] [PubMed]
12. Lin, Y.S.; Yang, C.H.; Hsu, Y.Y.; Hsieh, C.L. Microfluidic synthesis of tail-shaped alginate microparticles using slow sedimentation. *Electrophoresis* **2013**, *34*, 425–431. [CrossRef]
13. Huang, K.S.; Lin, Y.S.; Chang, W.R.; Wang, Y.L.; Yang, C.H. A facile fabrication of alginate microbubbles using a gas foaming reaction. *Molecules* **2013**, *18*, 9594–9602. [CrossRef]
14. Yang, C.H.; Wang, W.T.; Grumezescu, A.M.; Huang, K.S.; Lin, Y.S. One-step synthesis of platinum nanoparticles loaded in alginate bubbles. *Nanoscale Res. Lett.* **2014**, *9*, 277. [CrossRef] [PubMed]
15. Yang, C.H.; Huang, K.S.; Grumezescu, A.M.; Wang, C.Y.; Tzeng, S.C.; Chen, S.Y.; Lin, Y.H.; Lin, Y.S. Synthesis of uniform poly(d,l-lactide) and poly(d,l-lactide-co-glycolide) microspheres using a microfluidic chip for comparison. *Electrophoresis* **2014**, *35*, 316–322. [CrossRef]
16. Gañán-Calvo, A.M.; Montanero, J.M.; Martín-Banderas, L.; Flores-Mosquera, M. Building functional materials for health care and pharmacy from microfluidic principles and flow focusing. *Adv. Drug Deliv. Rev.* **2013**, *65*, 1447–1469. [CrossRef] [PubMed]
17. Sarvothaman, M.K.; Kim, K.S.; Seale, B.; Brodersen, P.M.; Walker, G.C.; Wheeler, A.R. Dynamic fluoroalkyl polyethylene glycol co-polymers: A new strategy for reducing protein adhesion in lab-on-a-chip devices. *Adv. Funct. Mater.* **2015**, *25*, 506–515. [CrossRef]
18. Seiffert, S.; Friess, F.; Lendlein, A.; Wischke, C. Faster droplet production by delayed surfactant-addition in two-phase microfluidics to form thermo-sensitive microgels. *J. Colloid Interface Sci.* **2015**, *452*, 38–42. [CrossRef] [PubMed]
19. Pirbodaghi, T.; Vigolo, D.; Akbari, S.; DeMello, A. Investigating the fluid dynamics of rapid processes within microfluidic devices using bright-field microscopy. *Lab Chip* **2015**, *15*, 2140–2144. [CrossRef] [PubMed]

20. Musterd, M.; Van Steijn, V.; Kleijn, C.R.; Kreutzer, M.T. Calculating the volume of elongated bubbles and droplets in microchannels from a top view image. *RSC Adv.* **2015**, *5*, 16042–16049. [CrossRef]
21. Zantow, M.; Dendere, R.; Douglas, T.S. Image-based analysis of droplets in microfluidics. *Eng. Med. Biol. Soc.* **2013**, 1776–1779.
22. Luo, Y.; Chakrabarty, K. Design of pin-constrained general-purpose digital microfluidic biochips. *Comput. Aided Des. Integr. Circuits Syst.* **2013**, *32*, 1307–1320. [CrossRef]
23. Jan-Willi, J.; Marian, W.; Gerri, K.; Jonathon, H.; Thomas, S.; Ilia, P.; Spatz, J.P. Key factors for stable retention of fluorophores and labeled biomolecules in droplet-based microfluidics. *Anal. Chem.* **2015**, *87*, 2063–2067.
24. Pan, I.; Mukherjee, R.; Rahaman, H.; Samanta, T.; Dasgupta, P. Optimization algorithms for the design of digital microfluidic biochips: A survey. *Comput. Electr. Eng.* **2013**, *39*, 112–121. [CrossRef]
25. Lai, K.T.; Yang, Y.T.; Lee, C.Y. An intelligent digital microfluidic processor for biomedical detection. *J. Sign Process Syst.* **2015**, *78*, 85–93. [CrossRef]
26. Huang, K.D.; Liu, C.H.; Lin, H.S. Reactant and waste minimization in multitarget sample preparation on digital microfluidic biochips. *IEEE Trans. Comput. Aided Des. Integr. Circuits Syst.* **2013**, *32*, 1484–1494. [CrossRef]
27. Chen, Y.H.; Hsu, C.L.; Tsai, L.C.; Huang, T.W.; Ho, T.Y. A reliability-oriented placement algorithm for reconfigurable digital microfluidic biochips using 3-D deferred decision making technique. *IEEE Trans. Comput. Aided Des. Integr. Circuits Syst.* **2013**, *32*, 1151–1162. [CrossRef]
28. Maftei, E.; Pop, P.; Madsen, J. Module-based synthesis of digital microfluidic biochips with droplet-aware operation execution. *ACM J. Emerg. Technol. Comput. Syst.* **2013**, *9*, 21. [CrossRef]
29. Luo, Y.; Chakrabarty, K.; Ho, T.Y. Error recovery in cyberphysical digital microfluidic biochips. *IEEE Trans. Comput. Aided Des. Integr. Circuits Syst.* **2013**, *32*, 59–72. [CrossRef]
30. Isgor, P.K.; Marcali, M.; Keser, M.; Elbuken, C. Microfluidic droplet content detection using integrated capacitive sensors. *Sens. Actuator B Chem.* **2015**, *210*, 669–675. [CrossRef]
31. Lu, X.; Samuelson, D.R.; Xu, Y.; Zhang, H.; Wang, S.; Rasco, B.A.; Xu, J.; Konkel, M.E. Detecting and tracking nosocomial methicillin-resistant staphylococcus aureus using a microfluidic SERS biosensor. *Anal. Chem.* **2013**, *85*, 2320–2327. [CrossRef] [PubMed]
32. Kawano, Y.; Otsuka, C.; Sanzo, J.; Higgins, C.; Nirei, T.; Schilling, T.; Ishikawa, T. Expanding imaging capabilities for microfluidics: Applicability of darkfield internal reflection illumination (DIRI) to observations in microfluidics. *PLoS ONE* **2015**, *10*. [CrossRef] [PubMed]
33. Kim, M.; Pan, M.; Gai, Y.; Pang, S.; Han, C.; Yang, C.; Tang, S.K. Optofluidic ultrahigh-throughput detection of fluorescent drops. *Lab Chip* **2015**, *15*, 1417–1423. [CrossRef] [PubMed]
34. Muluneh, M.; Kim, B.; Buchsbaum, G.; Issadore, D. Miniaturized, multiplexed readout of droplet-based microfluidic assays using time-domain modulation. *Lab Chip* **2014**, *14*, 4638–4646. [CrossRef] [PubMed]

35. Zhao, Y.; Chen, D.; Yue, H.; French, J.B.; Rufo, J.; Benkovic, S.J.; Huang, T.J. Lab-on-a-chip technologies for single-molecule studies. *Lab Chip* **2013**, *13*, 2183–2198. [CrossRef] [PubMed]
36. Cretich, M.; Daaboul, G.G.; Sola, L.; Unlu, M.S.; Chiari, M. Digital detection of biomarkers assisted by nanoparticles: Application to diagnostics. *Trends Biotechnol.* **2015**, *33*, 343–351. [CrossRef] [PubMed]
37. Streets, A.M.; Huang, Y. Microfluidics for biological measurements with single-molecule resolution. *Curr. Opin. Biotechnol.* **2014**, *25*, 69–77. [CrossRef] [PubMed]
38. Kang, D.K.; Ali, M.M.; Zhang, K.; Pone, E.J.; Zhao, W. Droplet microfluidics for single-molecule and single-cell analysis in cancer research, diagnosis and therapy. *TrAC Trends Anal. Chem.* **2014**, *58*, 145–153. [CrossRef]
39. Oedit, A.; Vulto, P.; Ramautar, R.; Lindenburg, P.W.; Hankemeier, T. Lab-on-a-chip hyphenation with mass spectrometry: Strategies for bioanalytical applications. *Curr. Opin. Biotechnol.* **2015**, *31*, 79–85. [CrossRef] [PubMed]
40. Zeng, Y.; Wang, T. Quantitative microfluidic biomolecular analysis for systems biology and medicine. *Anal. Bioanal. Chem.* **2013**, *405*, 5743–5758. [CrossRef] [PubMed]
41. Choi, J.W.; Kim, G.J.; Lee, S.; Kim, J.; deMello, A.J.; Chang, S.I. A droplet-based fluorescence polarization immunoassay (DFPIS) platform for rapid and quantitative analysis of biomarkers. *Biosens. Bioelectron.* **2015**, *67*, 497–502. [CrossRef] [PubMed]
42. Chen, J.; Liu, Y.; Ye, T.; Xiang, X.; Ji, X.; He, Z. A novel droplet dosing strategy-based versatile microscale biosensor for detection of DNA, protein and ion. *Sens. Actuator B Chem.* **2015**, *215*, 206–214. [CrossRef]
43. Khan, M.; Park, S.Y. Specific detection of avidin-biotin binding using liquid crystal droplets. *Colloid Surf. B Biointerfaces* **2015**, *127*, 241–246. [CrossRef] [PubMed]
44. Kim, J.; Khan, M.; Park, S.Y. Glucose sensor using liquid-crystal droplets made by microfluidics. *ACS Appl. Mater. Interfaces* **2013**, *5*, 13135–13139. [CrossRef] [PubMed]
45. Piao, Y.; Han, D.J.; Azad, M.R.; Park, M.; Seo, T.S. Enzyme incorporated microfluidic device for *in situ* glucose detection in water-in-air microdroplets. *Biosens. Bioelectron.* **2015**, *65*, 220–225. [CrossRef] [PubMed]
46. Wu, S.Z.; Zhou, Z.Y.; Xu, L.R.; Su, B.; Fang, Q. Integrating bipolar electrochemistry and electrochemiluminescence imaging with microdroplets for chemical analysis. *Biosens. Bioelectron.* **2014**, *53*, 148–153. [CrossRef] [PubMed]
47. Zanoli, L.M.; Licciardello, M.; D'Agata, R.; Lantano, C.; Calabretta, A.; Corradini, R.; Marchelli, R.; Spoto, G. Peptide nucleic acid molecular beacons for the detection of pcr amplicons in droplet-based microfluidic devices. *Anal. Bioanal. Chem.* **2013**, *405*, 615–624. [CrossRef] [PubMed]
48. Guan, Z.; Zou, Y.; Zhang, M.J.; Lv, J.; Shen, H.; Yang, P.; Zhang, H.; Zhu, Z.; Yang, C.J. A highly parallel microfluidic droplet method enabling single-molecule counting for digital enzyme detection. *Biomicrofluidics* **2014**, *8*, 014110. [CrossRef] [PubMed]
49. Witters, D.; Knez, K.; Ceyssens, F.; Puers, R.; Lammertyn, J. Digital microfluidics-enabled single-molecule detection by printing and sealing single magnetic beads in femtoliter droplets. *Lab Chip* **2013**, *13*, 2047–2054. [CrossRef] [PubMed]

50. Benz, C.; Retzbach, H.; Nagl, S.; Belder, D. Protein-protein interaction analysis in single microfluidic droplets using fret and fluorescence lifetime detection. *Lab Chip* **2013**, *13*, 2808–2814. [CrossRef] [PubMed]
51. Rademeyer, P.; Carugo, D.; Lee, J.Y.; Stride, E. Microfluidic system for high throughput characterisation of echogenic particles. *Lab Chip* **2015**, *15*, 417–428. [CrossRef] [PubMed]
52. Duncanson, W.J.; Arriaga, L.R.; Ung, W.L.; Kopechek, J.A.; Porter, T.M.; Weitz, D.A. Microfluidic fabrication of perfluorohexane-shelled double emulsions for controlled loading and acoustic-triggered release of hydrophilic agents. *Langmuir* **2014**, *30*, 13765–13770. [CrossRef] [PubMed]
53. Sheeran, P.S.; Rojas, J.D.; Puett, C.; Hjelmquist, J.; Arena, C.B.; Dayton, P.A. Contrast-enhanced ultrasound imaging and *in vivo* circulatory kinetics with low-boiling-point nanoscale phase-change perfluorocarbon agents. *Ultrasound Med. Biol.* **2015**, *41*, 814–831. [CrossRef] [PubMed]
54. Duncanson, W.J.; Kodger, T.E.; Babaee, S.; Gonzalez, G.; Weitz, D.A.; Bertoldi, K. Microfluidic fabrication and micromechanics of permeable and impermeable elastomeric microbubbles. *Langmuir* **2015**, *31*, 3489–3493. [CrossRef] [PubMed]
55. Dhanaliwala, A.H.; Chen, J.L.; Wang, S.; Hossack, J.A. Liquid flooded flow-focusing microfluidic device for *in situ* generation of monodisperse microbubbles. *Microfluid. Nanofluid.* **2013**, *14*, 457–467. [CrossRef] [PubMed]
56. Neto, B.A.; Carvalho, P.H.; Correa, J.R. Benzothiadiazole derivatives as fluorescence imaging probes: Beyond classical scaffolds. *Acc. Chem. Res.* **2015**, *48*, 1560–1569. [CrossRef] [PubMed]
57. Yu, X.; Cheng, G.; Zhou, M.D.; Zheng, S.Y. On-demand one-step synthesis of monodisperse functional polymeric microspheres with droplet microfluidics. *Langmuir* **2015**, *31*, 3982–3992. [CrossRef] [PubMed]
58. Wang, W.; Zhang, M.J.; Chu, L.Y. Functional polymeric microparticles engineered from controllable microfluidic emulsions. *Acc. Chem. Res.* **2014**, *47*, 373–384. [CrossRef] [PubMed]
59. Wang, Q.; Zhang, D.; Yang, X.; Xu, H.; Shen, A.Q.; Yang, Y. Atom-economical *in situ* synthesis of $BaSO_4$ as imaging contrast agents within poly(n-isopropylacrylamide) microgels using one-step droplet microfluidics. *Green Chem.* **2013**, *15*, 2222–2229. [CrossRef]
60. Golberg, A.; Linshiz, G.; Kravets, I.; Stawski, N.; Hillson, N.J.; Yarmush, M.L.; Marks, R.S.; Konry, T. Cloud-enabled microscopy and droplet microfluidic platform for specific detection of escherichia coli in water. *PLoS ONE* **2014**, *9*. [CrossRef] [PubMed]
61. Park, J.W.; Na, S.C.; Nguyen, T.Q.; Paik, S.M.; Kang, M.; Hong, D.; Choi, I.S.; Lee, J.H.; Jeon, N.L. Live cell imaging compatible immobilization of chlamydomonas reinhardtii in microfluidic platform for biodiesel research. *Biotechnol. Bioeng.* **2015**, *112*, 494–501. [CrossRef] [PubMed]
62. Seiffert, S. Small but smart: Sensitive microgel capsules. *Angew. Chem. Int. Edit.* **2013**, *52*, 11468. [CrossRef] [PubMed]
63. Kaler, K.; Prakash, R. Droplet microfluidics for chip-based diagnostics. *Sensors* **2014**, *14*, 23283–23306. [CrossRef] [PubMed]

64. Iwanaga, S.; Saito, N.; Sanae, H.; Nakamura, M. Facile fabrication of uniform size-controlled microparticles and potentiality for tandem drug delivery system of micro/nanoparticles. *Colloid Surf. B Biointerfaces* **2013**, *109*, 301–306. [CrossRef] [PubMed]
65. Pessi, J.; Santos, H.A.; Miroshnyk, I.; Yliruusi, J.; Weitz, D.A.; Mirza, S. Microfluidics-assisted engineering of polymeric microcapsules with high encapsulation efficiency for protein drug delivery. *Int. J. Pharm.* **2014**, *472*, 82–87. [CrossRef] [PubMed]
66. Zhao, C.X. Multiphase flow microfluidics for the production of single or multiple emulsions for drug delivery. *Adv. Drug Deliv. Rev.* **2013**, *65*, 1420–1446. [CrossRef] [PubMed]
67. Tasoglu, S.; Gurkan, U.A.; Wang, S.Q.; Demirci, U. Manipulating biological agents and cells in micro-scale volumes for applications in medicine. *Chem. Soc. Rev.* **2013**, *42*, 5788–5808. [CrossRef] [PubMed]
68. Rosenfeld, L.; Lin, T.; Derda, R.; Tang, S.K.Y. Review and analysis of performance metrics of droplet microfluidics systems. *Microfluid. Nanofluid.* **2014**, *16*, 921–939. [CrossRef]
69. Mazutis, L.; Gilbert, J.; Ung, W.L.; Weitz, D.A.; Griffiths, A.D.; Heyman, J.A. Single-cell analysis and sorting using droplet-based microfluidics. *Nat. Protoc.* **2013**, *8*, 870–891. [CrossRef] [PubMed]
70. Rakszewska, A.; Tel, J.; Chokkalingam, V.; Huck, W.T.S. One drop at a time: Toward droplet microfluidics as a versatile tool for single-cell analysis. *NPG Asia Mater.* **2014**, *6*, e133. [CrossRef]
71. Schlicht, B.; Zagnoni, M. Droplet-interface-bilayer assays in microfluidic passive networks. *Sci. Rep.* **2015**, *5*, 9951. [CrossRef] [PubMed]
72. Cao, J.; Köhler, J.M. Droplet-based microfluidics for microtoxicological studies. *Eng. Life Sci.* **2015**, *15*, 306–317. [CrossRef]
73. Au, S.H.; Fobel, R.; Desai, S.P.; Voldman, J.; Wheeler, A.R. Cellular bias on the microscale: Probing the effects of digital microfluidic actuation on mammalian cell health, fitness and phenotype. *Integr. Biol.* **2013**, *5*, 1014–1025. [CrossRef] [PubMed]
74. Herranz-Blanco, B.; Arriaga, L.R.; Mäkilä, E.; Correia, A.; Shrestha, N.; Mirza, S.; Weitz, D.A.; Salonen, J.; Hirvonen, J.; Santos, H.A. Microfluidic assembly of multistage porous silicon-lipid vesicles for controlled drug release. *Lab Chip* **2014**, *14*, 1083–1086. [CrossRef] [PubMed]
75. Huang, J.; Li, W.; Li, Y.; Luo, C.; Zeng, Y.; Xu, Y.; Zhou, J. Generation of uniform polymer eccentric and core-centered hollow microcapsules for ultrasound-regulated drug release. *J. Mater. Chem. B* **2014**, *2*, 6848–6854. [CrossRef]
76. Huang, K.S.; Yang, C.H.; Kung, C.P.; Grumezescu, A.M.; Ker, M.D.; Lin, Y.S.; Wang, C.Y. Synthesis of uniform core-shell gelatin-alginate microparticles as intestine-released oral delivery drug carrier. *Electrophoresis* **2014**, *35*, 336. [CrossRef] [PubMed]
77. Li, W.; Dong, H.; Tang, G.; Ma, T.; Cao, X. Controllable microfluidic fabrication of janus and microcapsule particles for drug delivery applications. *RSC Adv.* **2015**, *5*, 23181–23188. [CrossRef]
78. Yang, C.H.; Wang, C.Y.; Grumezescu, A.M.; Wang, A.H.J.; Hsiao, C.J.; Chen, Z.Y.; Huang, K.S. Core-shell structure microcapsules with dual pH-responsive drug release function. *Electrophoresis* **2014**, *35*, 2680. [CrossRef] [PubMed]

79. Marquis, M.; Davy, J.; Cathala, B.; Fang, A.; Renard, D. Microfluidics assisted generation of innovative polysaccharide hydrogel microparticles. *Carbohydr. Polym.* **2015**, *116*, 189–199. [CrossRef] [PubMed]
80. Xue, P.; Wu, Y.; Menon, N.V.; Kang, Y. Microfluidic synthesis of monodisperse pegda microbeads for sustained release of 5-fluorouracil. *Microfluid. Nanofluid.* **2014**, *18*, 333–342. [CrossRef]
81. Zamora-Mora, V.; Velasco, D.; Hernández, R.; Mijangos, C.; Kumacheva, E. Chitosan/agarose hydrogels: Cooperative properties and microfluidic preparation. *Carbohydr. Polym.* **2014**, *111*, 348–355. [CrossRef] [PubMed]
82. Kong, F.; Zhang, X.; Hai, M. Microfluidics fabrication of monodisperse biocompatible phospholipid vesicles for encapsulation and delivery of hydrophilic drug or active compound. *Langmuir* **2014**, *30*, 3905–3912. [CrossRef] [PubMed]
83. Shim, J.U.; Ranasinghe, R.T.; Smith, C.A.; Ibrahim, S.M.; Hollfelder, F.; Huck, W.T.S.; Klenerman, D.; Abell, C. Ultrarapid generation of femtoliter microfluidic droplets for single-molecule-counting immunoassays. *ACS Nano* **2013**, *7*, 5955–5964. [CrossRef] [PubMed]
84. Wagli, P.; Chang, Y.C.; Hans, K.; Homsy, A.; Hvozdara, L.; Herzig, H.P.; Sigrist, M.; De Rooij, N.F. Microfluidic droplet-based liquid-liquid extraction and on-chip IR spectroscopy detection of cocaine in human saliva. *Anal. Chem.* **2013**, *85*, 7558–7565. [CrossRef] [PubMed]
85. Hugo, S.; Land, K.; Madou, M.; Kido, H. A centrifugal microfluidic platform for point-of-care diagnostic applications. *S. Afr. J. Sci.* **2014**, *110*, 1–7. [CrossRef]
86. Norian, H.; Field, R.M.; Kymissis, I.; Shepard, K.L. An integrated cmos quantitative-polymerase-chain-reaction lab-on-chip for point-of-care diagnostics. *Lab Chip* **2014**, *14*, 4076–4084.
87. Golberg, A.; Yarmush, M.L.; Konry, T. Picoliter droplet microfluidic immunosorbent platform for point-of-care diagnostics of tetanus. *Microchim. Acta* **2013**, *180*, 855–860. [CrossRef]
88. Bardiya, N.; Choi, J.-W.; Chang, S.-I. Analysis of single nucleotide polymorphism in human angiogenin using droplet-based microfluidics. *BioChip J.* **2014**, *8*, 15–21. [CrossRef]
89. Kim, J.A.; Kim, M.; Kang, S.M.; Lim, K.T.; Kim, T.S.; Kang, J.Y. Magnetic bead droplet immunoassay of oligomer amyloid beta for the diagnosis of alzheimer's disease using micro-pillars to enhance the stability of the oil-water interface. *Biosens. Bioelectron.* **2015**, *67*, 724–732. [CrossRef] [PubMed]
90. Prakash, R.; Pabbaraju, K.; Wong, S.; Wong, A.; Tellier, R.; Kaler, K. Droplet microfluidic chip based nucleic acid amplification and real-time detection of influenza viruses. *J. Electrochem. Soc.* **2014**, *161*, B3083–B3093. [CrossRef]
91. Prakash, R.; Pabbaraju, K.; Wong, S.; Wong, A.; Tellier, R.; Kaler, K. Multiplex, quantitative, reverse transcription pcr detection of influenza viruses using droplet microfluidic technology. *Micromachines* **2015**, *6*, 63–79. [CrossRef]
92. Rosenfeld, L.; Cheng, Y.F.; Rao, J.H.; Tang, S.K.Y. Rapid detection of tuberculosis using droplet-based microfluidics. *Proc. SPIE 8976 Microfluid. BioMEMS Med. Microsyst. XII* **2014**. [CrossRef]
93. Chiu, T.K.; Lei, K.F.; Hsieh, C.H.; Hsiao, H.B.; Wang, H.M.; Wu, M.H. Development of a microfluidic-based optical sensing device for label-free detection of circulating tumor cells (CTCs) through their lactic acid metabolism. *Sensors* **2015**, *15*, 6789–6806. [CrossRef] [PubMed]

94. Chen, J.; Zhou, G.; Liu, Y.; Ye, T.; Xiang, X.; Ji, X.; He, Z. Assembly-line manipulation of droplets in microfluidic platform for fluorescence encoding and simultaneous multiplexed DNA detection. *Talanta* **2015**, *134*, 271–277. [CrossRef] [PubMed]
95. Ng, A.H.; Lee, M.; Choi, K.; Fischer, A.T.; Robinson, J.M.; Wheeler, A.R. Digital microfluidic platform for the detection of rubella infection and immunity: A proof of concept. *Clin. Chem.* **2015**, *61*, 420–429. [CrossRef] [PubMed]
96. Wu, L.; Wang, Z.; Zong, S.; Cui, Y. Rapid and reproducible analysis of thiocyanate in real human serum and saliva using a droplet sers-microfluidic chip. *Biosens. Bioelectron.* **2014**, *62*, 13–18. [CrossRef] [PubMed]
97. He, J.; Mu, X.; Guo, Z.; Hao, H.; Zhang, C.; Zhao, Z.; Wang, Q. A novel microbead-based microfluidic device for rapid bacterial identification and antibiotic susceptibility testing. *Eur. J. Clin. Microbiol. Infect. Dis.* **2014**, *33*, 2223–2230. [CrossRef] [PubMed]
98. Wang, M.S.; Nitin, N. Rapid detection of bacteriophages in starter culture using water-in-oil-in-water emulsion microdroplets. *Appl. Microbiol. Biotechnol.* **2014**, *98*, 8347–8355. [CrossRef] [PubMed]
99. Tjhung, K.F.; Burnham, S.; Anany, H.; Griffiths, M.W.; Derda, R. Rapid enumeration of phage in monodisperse emulsions. *Anal. Chem.* **2014**, *86*, 5642–5648. [CrossRef] [PubMed]
100. Li, L.; Wang, Q.; Feng, J.; Tong, L.; Tang, B. Highly sensitive and homogeneous detection of membrane protein on a single living cell by aptamer and nicking enzyme assisted signal amplification based on microfluidic droplets. *Anal. Chem.* **2014**, *86*, 5101–5107. [CrossRef] [PubMed]
101. Huang, C.J.; Fang, W.F.; Ke, M.S.; Chou, H.Y.; Yang, J.T. A biocompatible open-surface droplet manipulation platform for detection of multi-nucleotide polymorphism. *Lab Chip* **2014**, *14*, 2057–2062. [CrossRef] [PubMed]
102. Chiou, C.H.; Shin, D.J.; Zhang, Y.; Wang, T.H. Topography-assisted electromagnetic platform for blood-to-pcr in a droplet. *Biosens. Bioelectron.* **2013**, *50*, 91–99. [CrossRef] [PubMed]
103. Shuga, J.; Zeng, Y.; Novak, R.; Lan, Q.; Tang, X.; Rothman, N.; Vermeulen, R.; Li, L.; Hubbard, A.; Zhang, L.; *et al.* Single molecule quantitation and sequencing of rare translocations using microfluidic nested digital pcr. *Nucleic Acids. Res.* **2013**. [CrossRef] [PubMed]
104. Hu, S.W.; Xu, B.Y.; Ye, W.K.; Xia, X.H.; Chen, H.Y.; Xu, J.J. Versatile microfluidic droplets array for bioanalysis. *ACS Appl. Mater. Interfaces* **2015**, *7*, 935–940. [CrossRef] [PubMed]
105. Wang, Y.; Wang, J. Mixed hydrogel bead-based tumor spheroid formation and anticancer drug testing. *Analyst* **2014**, *139*, 2449–2458. [CrossRef] [PubMed]
106. Du, G.S.; Pan, J.Z.; Zhao, S.P.; Zhu, Y.; Den Toonder, J.M.; Fang, Q. Cell-based drug combination screening with a microfluidic droplet array system. *Anal. Chem.* **2013**, *85*, 6740–6747. [CrossRef] [PubMed]
107. Kumar, P.T.; Vriens, K.; Cornaglia, M.; Gijs, M.; Kokalj, T.; Thevissen, K.; Geeraerd, A.; Cammue, B.P.; Puers, R.; Lammertyn, J. Digital microfluidics for time-resolved cytotoxicity studies on single non-adherent yeast cells. *Lab Chip* **2015**, *15*, 1852–1860. [CrossRef] [PubMed]

108. Cho, S.; Kang, D.K.; Sim, S.; Geier, F.; Kim, J.Y.; Niu, X.; Edel, J.B.; Chang, S.I.; Wootton, R.C.; Elvira, K.S.; *et al*. Droplet-based microfluidic platform for high-throughput, multi-parameter screening of photosensitizer activity. *Anal. Chem.* **2013**, *85*, 8866–8872. [CrossRef] [PubMed]
109. Wetzel, K.; Cao, J.; Kothe, E.; Köhler, J.M. Changing growth behavior of heavy-metal tolerant bacteria: Media optimization using droplet-based microfluidics. *Eng. Life Sci.* **2015**, *15*, 327–332. [CrossRef]
110. Yamashita, H.; Morita, M.; Sugiura, H.; Fujiwara, K.; Onoe, H.; Takinoue, M. Generation of monodisperse cell-sized microdroplets using a centrifuge-based axisymmetric co-flowing microfluidic device. *J. Biosci. Bioeng.* **2015**, *119*, 492–495. [CrossRef] [PubMed]
111. Zang, E.; Brandes, S.; Tovar, M.; Martin, K.; Mech, F.; Horbert, P.; Henkel, T.; Figge, M.T.; Roth, M. Real-time image processing for label-free enrichment of actinobacteria cultivated in picoliterliter droplets. *Lab Chip* **2013**, *13*, 3707–3713. [CrossRef] [PubMed]
112. Pyne, D.G.; Liu, J.; Abdelgawad, M.; Sun, Y. Digital microfluidic processing of mammalian embryos for vitrification. *PLoS ONE* **2014**, *9*. [CrossRef] [PubMed]
113. Geng, T.; Novak, R.; Mathies, R.A. Single-cell forensic short tandem repeat typing within microfluidic droplets. *Anal. Chem.* **2014**, *86*, 703–712. [CrossRef] [PubMed]
114. Akbari, S.; Pirbodaghi, T. Microfluidic encapsulation of cells in alginate particles via an improved internal gelation approach. *Microfluid. Nanofluid.* **2014**, *16*, 773–777. [CrossRef]
115. Akbari, S.; Pirbodaghi, T. A droplet-based heterogeneous immunoassay for screening single cells secreting antigen-specific antibodies. *Lab Chip* **2014**, *14*, 3275–3280. [CrossRef] [PubMed]
116. Lim, J.; Vrignon, J.; Gruner, P.; Karamitros, C.S.; Konrad, M.; Baret, J.C. Ultra-high throughput detection of single cell β-galactosidase activity in droplets using micro-optical lens array. *Appl. Phys. Lett.* **2013**, *103*, 203704. [CrossRef]
117. Najah, M.; Mayot, E.; Mahendra-Wijaya, I.P.; Griffiths, A.D.; Ladame, S.; Drevelle, A. New glycosidase substrates for droplet-based microfluidic screening. *Anal. Chem.* **2013**, *85*, 9807–9814. [CrossRef] [PubMed]
118. Polenz, I.; Brosseau, Q.; Baret, J.C. Monitoring reactive microencapsulation dynamics using microfluidics. *Soft Matter* **2015**, *11*, 2916–2923. [CrossRef] [PubMed]
119. Shim, T.S.; Kim, S.-H.; Yang, S.-M. Elaborate design strategies toward novel microcarriers for controlled encapsulation and release. *Part. Part. Syst. Charact.* **2013**, *30*, 9–45. [CrossRef]
120. Seiffert, S. Microgel capsules tailored by droplet-based microfluidics. *Chem. Phys. Chem.* **2013**, *14*, 295–304. [CrossRef] [PubMed]
121. Tang, G.N.; Li, W.X.; Cao, X.D.; Dong, H. *In situ* microfluidic fabrication of multi-shape inorganic/organic hybrid particles with controllable surface texture and porous internal structure. *RSC Adv.* **2015**, *5*, 12872–12878. [CrossRef]
122. Yan, H.; Kim, C. Formation of monodisperse silica microparticles with various shapes and surface morphologies using double emulsion templates. *Colloid Surf. A Physicochem. Eng. Asp.* **2014**, *443*, 88–95. [CrossRef]
123. Maan, A.A.; Nazir, A.; Khan, M.K.I.; Boom, R.; Schroën, K. Microfluidic emulsification in food processing. *J. Food Eng.* **2015**, *147*, 1–7. [CrossRef]

124. Shewan, H.M.; Stokes, J.R. Review of techniques to manufacture micro-hydrogel particles for the food industry and their applications. *J. Food Eng.* **2013**, *119*, 781–792. [CrossRef]