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CRITICAL REVIEW

Microfluidics for food, agriculture and biosystems industries

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Microfluidics, a rapidly emerging enabling technology has the potential to revolutionize food, agriculture and biosystems industries. Examples of potential applications of microfluidics in food industry include nano-particle encapsulation of fish oil, monitoring pathogens and toxins in food and water supplies, micro-nano-filtration for improving food quality, detection of antibiotics in dairy food products, and generation of novel food structures. In addition, microfluidics enables applications in agriculture and animal sciences such as nutrients monitoring and plant cells sorting for improving crop quality and production, effective delivery of biopesticides, simplified *in vitro* fertilization for animal breeding, animal health monitoring, vaccination and therapeutics. Lastly, microfluidics provides new approaches for bioenergy research. This paper synthesizes information of selected microfluidics-based applications for food, agriculture and biosystems industries.

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Introduction

With a rapidly growing global population, there is significant demand for food, agriculture and biosystems research to deliver low-cost, low-environmental-impact and safe food, drink, and biomaterials. To this end, researchers have been focusing on developing new technologies to turn raw materials into food and biomaterials, and to improve food quality, quantity and safety. To address this complex set of engineering and scientific challenges in the agri-food industry, innovation is needed for new processes, products and tools. Microfluidic systems (a.k.a. micro

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Suresh Neethirajan was born in India in 1980. He received his PhD in Biosystems Engineering from the University of Manitoba, in Canada in 2009. Suresh was a JSPS postdoctoral fellow at the National Food Research Institute, Tsukuba, Japan before joining the Oak Ridge National Laboratories, USA as a Research Associate in 2010. His research interests include bioinstrumentation, bio-imaging, nanoscale science and engineering for biological and agricultural systems. Suresh is currently focusing on analyzing bacterial chemotaxis using microfluidic systems; and on understanding the adhesion kinetics of bacteria using nanoscale imaging techniques.

Isao Kobayashi

Isao Kobayashi was born in Gunma, Japan, in 1975. He received his PhD in agricultural and forest engineering from the University of Tsukuba, Japan, in 2003. He worked on micro-channel emulsification as a JSPS postdoctoral research fellow at the University of Tsukuba from 2003 to 2005. In 2005 he joined the National Food Research Institute, Japan as a researcher. Currently he is a senior researcher at National Food Research Institute, NARO, Japan. His current research interests include micro/nanofluidics, emulsification, food nanotechnology, and in vitro gastrointestinal digestion.

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total analysis systems (μTAS) or lab-on-a-chip (LOC)) are considered one of the top emerging technologies that will change the world market by having a profound impact on the economy and how we live and work.[1] In particular, they offer promising potential for applications in the food, agriculture and biosystems industries.

Microfluidics is commonly defined as the science and technology that process minute volumes of fluids using channels with dimensions of a few to hundreds of micrometres.[2] The field of microfluidic technology is interdisciplinary and covers a wide spectrum of disciplines including physics, chemistry, engineering and biotechnology. The principles of electrokinetics, electrohydrodynamics, and thermo-capillarity with small dimensional parameters in space and time for microfluidic systems help solve important scientific problems that are difficult using conventional technologies. The unique characteristics of microfluidic devices such as laminar flow, large surface-to-volume ratios, and surface tension and capillary effects at the micrometre scale enable more efficient methods for processing and analyzing complex samples. Moreover, compared to conventional fluidic systems, microfluidic devices require lower fabrication cost, power budget and chemical consumption, but improved analytical performance and biocompatibility. The overall market for microfluidics-based products is experiencing an annual growth rate of 15.5%[3] and forecasted to exceed US$ 3 billion in market revenues in 2014.[4] Based on our incomplete data, there are about 269 companies in 31 countries, 35 contract research organizations, and 118 university research groups worldwide, that are actively involved in developing methodologies, processes, tools and devices for microfluidic systems.[5]

Mitsutoshi Nakajima

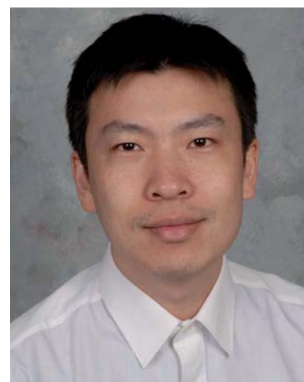
Mitsutoshi Nakajima was born in Kumamoto, Japan, in 1954. He received his PhD in Chemical Engineering from the University of Tokyo, Japan, in 1982. He worked on food engineering projects in Kyushu University (1980–1985), National Food Research Institute (1985–2007), and University of Tsukuba (2007–present). Currently he is the Director, Alliance for Research on North Africa (ARENA), and Professor, Division of Appropriate Technology and Sciences for Sustainable Development, Graduate School of Life and Environmental Sciences at the University of Tsukuba, Japan. His current research interests include food engineering, food nanotechnology, and micro/nano-process systems.

Saravanan Nandagopal

Saravanan Nandagopal was born in India in 1975. He received his Master's degree in Biochemical Engineering from the Institute of Technology at the Banaras Hindu University, in India in 2000. He has been a PhD student at the University of Manitoba under the supervision of Dr Francis Lin since May 2009. His current research interest is in investigating the guiding mechanisms of leukocyte migration and trafficking in lymphoid tissues and to derive microfluidics-based applications in the areas of biomedical and biosystems engineering.

Dan Wu

Dan Wu was born in China in 1976. She obtained her PhD in Physics from Huazhong Normal University, in China, in 2006. She then worked in the Huazhong University of Science and Technology as a lecturer in the Department of Physics from 2006 to 2009. She has been a postdoctoral fellow at the University of Manitoba under the supervision of Dr Francis Lin since October 2009. Her current research interest is in combining quantitative modeling and experimental approaches to explore the cellular mechanisms of cell migration in complex microenvironments.



Francis Lin

Francis Lin was born in China in 1975. He received his PhD in Physics from the University of California, Irvine, in USA in 2004. He then received his postdoctoral training at Stanford University School of Medicine from 2005 to 2008. He joined the University of Manitoba, Canada as an Assistant Professor in the Department of Physics and Astronomy in December 2008. His research interest is in applying biophysical and bioengineering approaches together with biological and immunological methods in understanding immune cell trafficking in complex tissue environments, and in developing microfluidic systems for biological and biomedical applications.

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Although applications of microfluidics in the food, agriculture and biosystems sector are relatively recent, it grows rapidly as evidenced by the number of relevant publications and patents over the last decade (Fig. 1).

In this paper, we review the existing applications and on-going research of microfluidic systems that are relevant to food, agriculture, and biosystems industries in the following five specific areas: (1) food safety, (2) food processing, (3) animal science, (4) plant production, and (5) biofuel production. In addition, the limitations of the current relevant microfluidics technologies and the future perspectives in light of the emerging needs of the agri-food market are discussed. Furthermore, to synthesize the current information of microfluidic technologies for their commercial applications in the agri-food and biosystems market, we list the companies involved in producing and commercializing microfluidic systems and devices for applications relevant to agriculture, food and bioprocessing industries in Table 1. The brand and the company names mentioned in the manuscript are for the purpose of information only and not intended as an act of promotion or endorsement.

I. Microfluidics for food safety

The most common food-borne pathogens are *Campylobacter jejuni*, *Escherichia coli* (*E. coli*) O157:H7, Shigella, *Listeria* and *Salmonella*. The worldwide economic impact of food-borne toxins producing illnesses and outbreaks are substantial and significant. *E. coli* O157:H7 and *Salmonella* pathogens alone have caused approximately 1.47 million food borne illnesses and 453 deaths in the United States in 2008, with an estimated $3.12 billion in associated medical costs, productivity losses, and costs of premature deaths.[6] Traditional methods for the detection of food borne pathogens rely on culturing of the bacteria onto agar plates which is time consuming. Microfluidic devices allow cheap, efficient, real-time temporal and spatial detection of the presence of residues, trace chemicals, antibiotics, pathogens and toxins in the food and water supply monitoring. With the lab-on-chip approach, it is possible to detect and quantify the infection within few minutes from food. Therefore, the quality monitoring can be done comprehensively from the farm to the fork encompassing all aspects of food production including transportation and food processing to retail and food service. With the microfluidic detection systems, it is possible to achieve zero tolerance standard of food pathogen detection.[7]

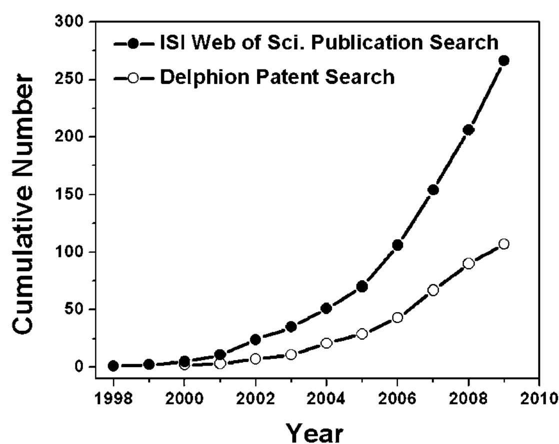

Fig. 1 Cumulative number of publications and patents of microfluidics-based technologies for food, agriculture and biosystems industries by year. Data is from publication search in ISI Web of Science and patent search in Delphion based on relevant key words.

The detection and estimation of pathogen concentration in the food and water samples are generally achieved by quantification of whole pathogen cells, metabolites release or pathogen specific protein/nucleic acid sequences. A microfluidic flow cell with embedded gold interdigitated array of microelectrodes (IDAM) integrated with magnetic nanoparticle-antibody conjugates has been developed to detect pathogenic bacteria in beef samples[8] (Fig. 2). This is a novel label-free impedance biosensor for the direct impedance measurement of bacterial cells without using redox probe or antibodies on the surface of electrodes. This microfluidic biosensor was able to detect as low as 1.2×10^3 cells of *E. coli* O157:H7 in beef samples in just 35 min.

In addition to pathogen and antigen detection, microfluidic devices can be used for pathogen sorting by isolating pathogens from suspended particle concentration mixture using dielectrophoresis. By converging fluid flow through alternating current electro-osmotic flow in a microfluidic device,[9] the target pathogens can be directed towards the stagnation points, while the suspended particles can be swept towards the outlet along the fluidic flow. It has been shown that bacterial cells inside a microfluidic channel can be captured efficiently through tailoring the orientation of the 3D electrodes and by creating a dielectrophoretic force field cage.[10] This device is capable of sorting and collecting three different types of pathogens at a rate of ~300 particles per second through 3D electrodes.[10]

Similarly, microfluidic systems are used for antigen detection. A prototype of integrated nanoporous silicon sensor array on a microfluidic platform has been developed for sensitive, rapid and simultaneous detection of multiple antigens for point-of-care applications in food industry.[11] Intercellular antigens of pathogenic bacteria (*e.g. Listeria monocytogenes*, *E. coli*) released by electric lyses of cells captured in the microfluidic device, bind to their antibodies immobilized on the inner surface of the silicon nanopores and can be detected by the change in the reflective interferometric spectra.

Microfluidic systems are also used for toxin detection. Deliberate or accidental contamination of food or drink with *Botulinum* neurotoxin (BoNT) is a form of bioterrorism and a concern for US homeland security. The current method of detection is through mouse bioassay which is sensitive, but slow, expensive, low throughput, and requires sacrificing animals. The Centers for Disease Control and Prevention, Atlanta and the National Center for Food Protection and Defense, St. Paul along with researchers from the University of Wisconsin-Madison have developed a microfluidic platform with high sensitivity, on-site portability and multiplexing capabilities for reliable *Botulinum* Neurotoxin detection in solution.[12,13] The device consists of input and detection ports interconnected by a microchannel (Fig. 3). The toxin sample is applied into the input port to catalyze the cleavage reaction of the fluorescent labeled peptide. The cleaved fluorescent labeled fragment diffuses into the detection port designed to facilitate evaporation of the solution and effectively pre-concentrate analyte before fluorescence detection. This evaporation led to 3-fold signal amplification over 35 min.

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Table 1 Companies producing and commercializing microfluidic devices and systems for applications in agri-food industries

Company Name and Location	Technology/Application	Website Address
Affymetrix Inc., Santa Clara, CA, USA	Biochips for sequencing the genomes of cattle that relate to commercially valuable traits such as disease resistance and leanness of meat	http://www.affymetrix.com
Agilent Technologies Inc., Santa Clara, CA, USA	Microfluidic platform (Bioanalyzer 2100) for sizing, quantification and quality control of DNA, RNA, proteins and cells. Example: quantifying the relative amount of fractions proteins in soybean cultivars	http://www.agilent.com
Akonni Biosystems Inc., Frederick, MD, USA	Gel-drop microarray platform for diagnosis of diseases and extracting nucleic acids from animals	http://www.akonni.com
Arryx, Inc., Chicago, IL, USA	BioRyx 200 is used to collect specified types of cells from a mixed suspension, manipulate cells for enhanced viewing, with applications in animal breeding	http://www.arryx.com/
Blue4Green, Enschede, The Netherlands	Microfluidic based hand-held tool for analysis at the point of animal care	http://blue4green.com/
Caliper Life Sciences Inc., Hopkinton, MA, USA	LabChip GX platform for high throughput screening and predictive assessments of biological and food product quality	http://www.caliperls.com
Dupont, Wilmington, DE, USA	Qualicon food safety sensor for testing food-borne bacteria using capillary electrophoresis	http://www2.dupont.com
Epigem Ltd, Redcar, UK	Fluence microfluidic chips for biochemical monitoring of food, soil and water	http://www.epigem.co.uk
EP. Tec Co., Ltd, Hitachi, Japan	Microchannel emulsification technology for producing monodisperse micro-dispersions including emulsions, microparticles, and microcapsules	http://eptec.jp/index.html (only in Japanese)
Fluidgm Corporation, San Francisco, USA	Microfluidic-based EP1 system for validating single-nucleotide polymorphism for testing cattle health	http://www.fluidgm.com
Fluigent, Paris, France	Tools for flow control in micro-channels; producing emulsion/droplets and food rheology	http://www.fluigent.com
Integram Plus Inc., UK	Microfluidic Pesticide Biosensor	http://www.integramplus.com
Lc Sciences, Houston, TX, USA	μParaflo microfluidics technology and microRNA discovery, detection and profiling for animals and plants	http://www.lcsciences.com
LioniX BV, Enschede, The Netherlands	Integrated optics and microfluidics based products for genomics, proteomics, cellomics for plants and animals	http://www.lionixbv.nl
Little Things Factory, Ilmenau, Germany	Micromixers and micro-reactors for applications in emulsions and biorefining	http://www.ltf-gmbh.de/de (only in German)
Microfluidics International Corporation, Newton, MA, USA	Microfluidizer high shear fluid processor, food processing applications	http://www.microfluidicscorp.com
Microfluidic Systems, Fremont, CA, USA	M-Band product offers biodefense, toxin or airborne pathogen detection and identification	http://www.microfluidicsystems.com/
Mikroglas Inc, Mainz, Germany	Microreactors for heat exchange, and other chemical applications	http://www.mikroglas.com
Micronit, Enschede, The Netherlands	Glass based lab-on-a-chip products for monitoring nutrients, and to sort plant cells to increase crop quality and production	http://www.micronit.com
miniFAB Pty Ltd, Victoria, Australia	Uses nano-bio-films to a microfluidic chip and incorporating it into a complete system for diagnostics. Examples include a device for detecting eye diseases by analyzing nanolitre tear samples of animals	http://www.minifab.com.au
Nanoterra, Inc., Cambridge, MA, USA	Portable analytical systems for food safety monitoring, pathogen detection in water, and for creating monodisperse droplets, foams, and colloids in food industries	http://www.nanoterra.com
NSG Precision Cells, Inc., Farmingdale, NY, USA	Quartz based microfluidic chips for use with micro-pumps, and other micro-machines with applications in chromatography and electrophoresis analysis	http://www.microfluidicchip.com
Qiagen, Hildon, Germany	Sample and assay technologies for food, animal pathogen testing	http://www.qiagen.com
Superior NanoBiosystems Inc., Washington, USA	Handheld device employing microfluidic amplification techniques for detecting bacteria in Oyster industry	http://www.superiornanobiosystems.com

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Table 1 (*Contd.*)

Company Name and Location	Technology/Application	Website Address
Syrris Ltd., Royston, UK	Microfluidic flow reactor manufacturer with applications in formulations, nanoparticle synthesis, and flow mixing	http://www.syrris.com
Takasago Electric Inc., Nagoya, Japan	Miniature chemically inert valves and pumps, plastic microfluidic chips for applications in food safety	http://www.takasago-elec.com
VitaeLLC, Madison, WI, USA	Microfluidic devices for culture, study, and manipulation of cells and embryos in assisted reproduction of livestock and cattle	http://www.vitaellc.com
XY Inc., Fort Collins, CO, USA	XY sex-selection technology (control of all sperm sorting) in non-human mammals, including cattle, horses, pigs using flow cytometry	http://www.xyinc.com/

The first generation of the device used a fluorescent substrate tethered to silica beads with relatively low sensitivity.[12] In the second generation of the device, the detection sensitivity was improved by tethering the substrate to a self-assembled monolayer on a gold surface and this device was able to detect as little as 3 pg mL^{-1} of the toxin in buffer.[13]

Miniaturized microfluidic versions of macroscopic assays such as sandwich type immunoassays[14] and Förster resonance energy transfer (FRET) fluorescence-based endopeptidase assays[15,16] provide clear advantages over conventional technologies which include the ability to operate in semiautomatic mode and a reduction of reagent consumption, facilitating field deployment. A suspended cantilever with built-in microfluidic channel has been demonstrated in a novel approach for weighing single nanoparticles, single bacterial cells and sub-monolayers of adsorbed proteins in water with sub-femtogram resolution.[17,18] This nanomechanical microfluidic resonator enables the measurement of mass with 100 ng of sensitivity and with quality factor of 15 000. The measurement was done in vacuum while the solution was flowing through the microchannels with the applications of the device focusing on direct detection of pathogens (both for food safety and animal health diagnosis) and mass density measurement of colloidal particles.

L-Glutamate is an important amino acid to be analyzed for food safety in consideration of the Chinese restaurant syndrome, Parkinson and Alzheimer diseases. On-chip-bead-based microfluidic systems provide over 91% selectivity in determining L-glutamate based on enzymatic recycling of substrate from food samples.[19] Plant-food-based antioxidants can be efficiently and rapidly determined using a microfluidic system based on a peroxyoxalate chemiluminescence assay.[20]

While the current single-function-based microfluidic systems successfully demonstrated their use in various food safety related applications, integration of key functions of food safety assurance such as sample pretreatment, assay operations and detection for biochemical analysis on a single microfluidic chip remains a major technical challenge. Interfaces for bridging microfluidic systems and the electronic readout instrument as well as embedding more flexible on-chip sample manipulations are required for practical applications.

II. Microfluidics for food processing

In food and bioprocessing industries, microfluidics has the potential to generate new products and processes by influencing food microstructure and thereby the rheology and functional properties of the final product. The laminar flow phenomena in microfluidic systems facilitates pressure-driven and electrokinetic flow of fluids in microchannels and thus provides a powerful platform for DNA sequencing, polymerase chain reaction (PCR) and immunoassays.[21] The solvent extraction in a microfluidic chip is expected to have higher efficiency due to shorter diffusion distance and relatively large interface area between water and organic streams inside the microfluidic channels. Microfluidic chips have been demonstrated as an efficient tool in the solvent extraction of bioactive compounds from plant based products such as strychnine.[22]

In the food and dairy industry, liquids and solids are mixed and blended for several reasons including dispersing gums and stabilizers in ice-cream mix or dairy products; and for dissolving salt and sugar in water to make brines. The characteristics such as fluid viscosity, fluid density, laminar/turbulence nature of fluid flow play a key role in an effective mixing. Microfluidics can address the challenge of mixing liquid with liquid and liquid with solids effectively[23,24] and can be integrated with food processing equipment for the production of highly concentrated nanoemulsions, nanosuspensions, nanoencapsulations and nanodispersions.

Microfluidic devices are also useful for preparing microporous calcium alginate gels. As an example, a microfluidic T-junction device was employed in the preparation of microporous calcium alginate gels by incorporating monodisperse air bubbles of 177 μm in diameter which would increase the volume to energy content ratio of the product and improve sample homogeneity.[25] The results of this project provide a method for manufacturing low-energy food products.

Oil-in-water and water-in-oil food emulsions such as mayonnaise and margarine respectively can be industrially produced by introducing energy through physical means in a mixer equipment, leading to shearing strains which will break up to form one phase into the other.[26] Microfluidic devices have the potential to dispense chemicals in a controlled manner to tailor the properties of foams and emulsions (*i.e.* microchannel emulsification (MCE)).[24,27,28] T-junction[29,30,31] and flow-focusing[32,33,34,35] are commonly used microfluidic channel configurations for droplet formation. In the T-junction configuration, a dispersed phase is introduced into a branch channel perpendicular to a main channel, and the continuous phase is also introduced into the main channel. Droplets are periodically formed just downstream

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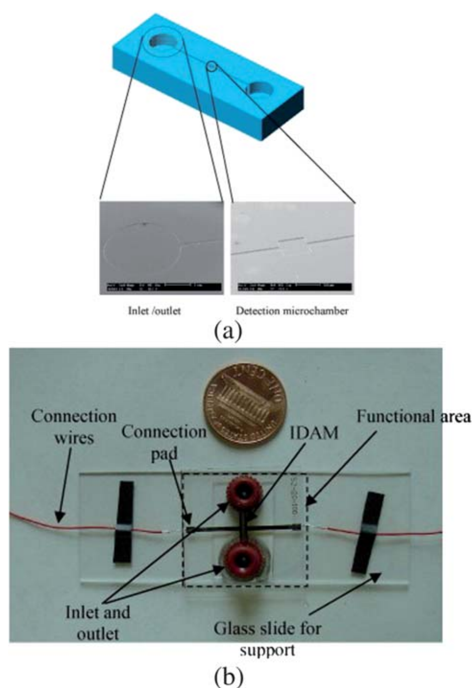

Fig. 2 Microfluidic device for bacteria detection in beef samples. (a) A microchannel with a detection micro chamber, and inlet and outlet channels. **(b)** An assembled microfluidic flowcell with embedded interdigitated array microelectrode and connected wires. Reprinted from ref. 8 with permission from Elsevier.

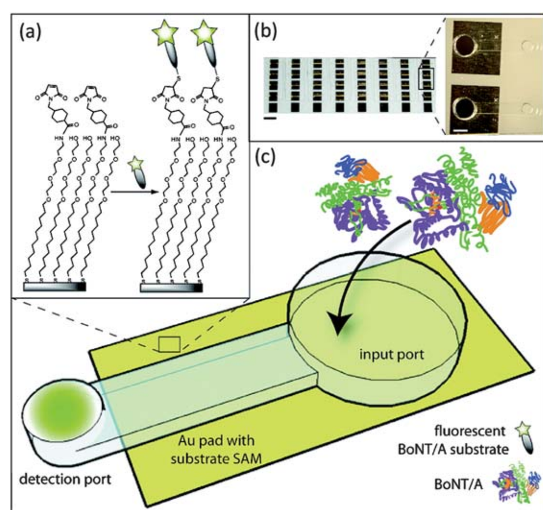

Fig. 3 Microfluidic sensor for toxin detection. (a) Self assembled monolayer (SAM) formation on Au yields mixed monolayers of amine- and hydroxyl-terminated alkanethiols presenting the BoNT enzymatic substrate. **(b)** PDMS microchannels on 40 arrayed Au pads (10.5 mm^2) with inset image representing two neighbouring channels. **(c)** BoNT is added at input port and incubated on SAMs, during which time it can cleave the immobilized substrate, releasing fluorescent fragments into solution. Flu-labeled fragments are concentrated at detection port *via* evaporation. Reprinted from ref. 13 with permission from the American Chemical Society.

the T-junction, which is driven by the pressure drop across the emerging dispersed phase at a low flow rate of the continuous phase or the shear stress due to a high flow rate of the continuous phase.[36] Y and cross-junction configurations are also efficient in terms of the droplet formation process.[37,38] In the flow-focusing configuration of the Y and cross-junction configuration, a dispersed phase is introduced into the center channel, and the continuous phase is introduced into two channels sandwiching the center channel. The streams of the dispersed and continuous phases are forced through a narrow region, causing the extension of the dispersed phase due to high shear stress by the rapidly flowing continuous phase.[32,33] The emerging dispersed phase was pinched off in or downstream the narrow region, leading to periodical droplet generation. Although the preceding microfluidic devices can vary the size of the resultant uniform droplets on the same chip,[30,35] this feature implies that the droplet size is sensitive to the flow rate of each phase.

Most of the microfluidic devices usually consist of one droplet formation unit, resulting in very low droplet productivity. A few microfluidic devices consisting of integrated droplet formation units have been recently developed for the mass production of uniform droplets.[38,39,40,41] However, the flow of the two phases must be precisely and equally controlled at all droplet formation units, which is expected to be not straightforward (especially for long-term operation). Two T junctions in a series can be used in a microfluidic arrangement for producing a water–oil–water (W/O/W) double emulsion.[42] The aqueous droplets to be enclosed are formed periodically upstream at the first junction where the internal surface of the channel is hydrophobic. At the downstream junction where the surface is hydrophilic, organic droplets enclosing the aqueous droplets are formed. By changing the flow rates and the wetting properties of the micro-channels (Fig. 4), various types of emulsions with different droplet sizes can be produced.

Kumacheva's group at the University of Toronto has designed consecutive flow-focusing configurations[43] in the microfluidic systems for preparing double emulsions. Droplets of an inner phase are formed at the upstream flow-focusing region, and then droplets of the middle phase enclosing smaller droplet(s) are formed at the downstream flow-focusing region. The Weitz group has used microcapillary devices for preparing double and triple emulsions.[44,45] The microcapillary devices, which consist of cylindrical injection and collection tubes nested within a square tube, enable the one-step formation of droplets enclosing a smaller immiscible droplet. In addition, the microcapillary devices do not require any surface modification of the tubes to prepare either W/O/W or the inverse, O/W/O emulsions. Although food researches using microfluidic droplet formation devices have not yet been conducted enough, we expect that new-class of complex food micro-dispersions, precisely controlled in size, shape, and structure, would be created using microfluidic techniques. The Nakajima group has studied MCE using microfluidic devices with unique channel configurations.[46,47]

Channel configurations for MCE can be classified into parallel microgrooves (grooved microchannel (MC) array, Fig. 5) and micro-through holes (straight-through MC array, Fig. 6). Here, the MCE device is mounted in a module filled with a continuous phase prior to emulsification, as the device is not chemically bonded on the glass plate. A dispersed phase is injected through

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a grooved or straight-through MC array into a deep flow channel filled with the continuous phase. Droplet formation for MCE is, in principle, different from that for the other microfluidic droplet formation devices. In MCE, droplets are periodically formed by the spontaneous transformation of the oil–water interface that passed through channels, even in the absence of the forced flow of the continuous phase.[48] The size of the uniform droplets obtained by MCE is not influenced by the flow rate below its critical value,[49,50] but driven by the pressure distribution of the dispersed phase that pass through the channels.[51] The current MCE devices can produce uniform droplets with a size of 1 to 200 μm.[52,53]

Scaling up of MCE devices has been attempted using integrated MC arrays for the mass production of uniform droplets.[54,55] This demonstrates the feasibility of parallelization of MCE modules, since droplet formation for MCE is robust and insensitive to the flow rate of each phase. Hence MCE is assumed to be a useful technique for the practical and long-term production of uniform droplets. To date, various kinds of food-grade materials (refined vegetable oils, a medium-chain triglyceride oils, essential oils, hydrophilic emulsifiers, proteins) have been examined for producing O/W, W/O, and W/O/W emulsions by MCE.[56] Monodisperse W/O/W emulsions can be produced as a two-step emulsification processes with homogenization as the first step followed by MCE.[57,58,59] Food grade monodisperse micro-dispersions such as solid lipid microparticles,[60] gelatin-gel microbeads,[61] core/shell microcapsules,[62] O/W emulsions coated by thin layers of charged molecules,[63] can be efficiently produced only through MCE technology. MCE serves as a promising potential in successfully producing uniform oil droplets containing functional lipids, such as β-carotene[64] and γ-oryzanol.[65] It is imperative in scaling up the MCE devices to realize mass production of monodisperse food-grade micro-dispersions.

Use of micro-nano-bubbles is an emerging area and has potential utility in the food industries by providing interfaces in the food ingredients to achieve their functionalities; in reducing the calorie intake[66] and as a disinfection and sterilization agent in food preservation applications.[67,68] Microfluidic technology will be offering solutions in near future in the formation as well as characterization of micro-nano-bubbles.

A continuous and a uniform 4 μm thickness organic encapsulated micro-bubbles of 110 μm diameter was produced[69] in a water flow multiphase microfluidic system. The generation rate of organic micro-bubble using this system was 40 numbers per second and the organic bubble is expected to apply as capsules of reactive gas handling in microfluidic system. It is also possible to produce micro-bubbles with defined geometry and bubble frequency using a simple co-flowing micro-channel.[70] Because of low power consumption, microfluidic bioreactors with the aid of fluidic oscillation appears to be one of the best techniques in the large scale generation of micro-nano bubbles.[71]

While microfluidic devices have the advantage of fluid control, infusion and mixing of liquids can be a challenge due to the low mixing efficiency in the small microfluidic channels and possible blocking of the channels by viscous samples. However, mixing in the microfluidic channels can be enhanced by acoustically inducing bubbles;[72–74] by introducing oppositely charged surface

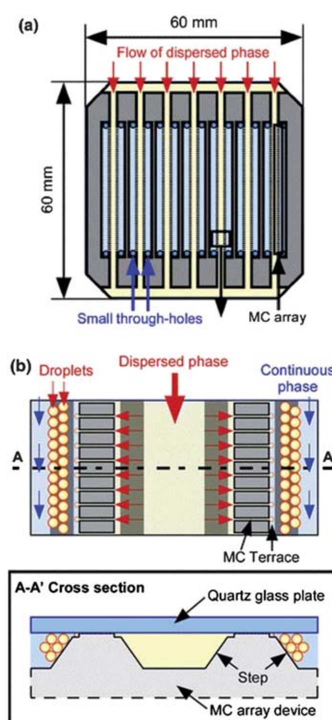

Fig. 5 Chip geometries for mass producing uniform fine droplets. Schematic top view of an MCE chip consisting of 14 MC arrays. (a) Solid circles denote the inlet through-holes for the continuous-phase liquid and the outlet through-holes for the emulsion product. **(b)** Schematic top and cross-sectional views of droplet generation *via* MC arrays on the chip. Reprinted from ref. 55 with permission from Springer.

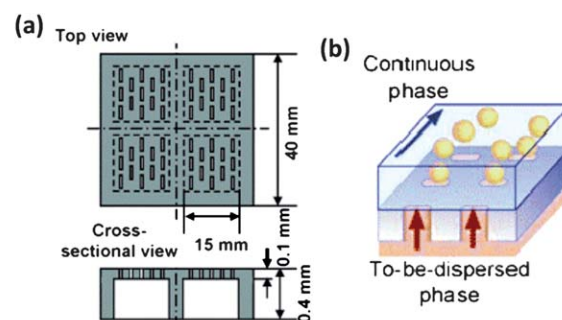

Fig. 6 Droplet generation using an MCE device. (a) Schematic of a silicon straight-through MC plate. **(b)** Schematic of the apparatus. Reprinted from ref. 54 with permission from the American Chemical Society.

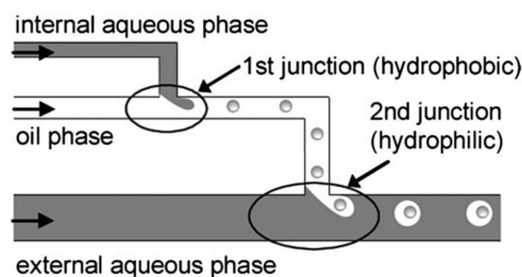

Fig. 4 Formation of double emulsions (W/O/W) using T-shaped microchannels. Reprinted from ref. 42 with permission from the American Chemical Society.

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heterogeneities to microchannel walls;[75] or by bas-relief structures on the channel floors.[76] For large volume production, it would be more feasible to use the microfluidic devices as integrated components with the macroscale modules instead of applying them as stand-alone systems.

III. Microfluidics for animal science

The microfluidic technology presents itself as a novel tool for solving problems in animal science and veterinary industry by bringing the benefits of miniaturization, integration and automation. Integration of microfluidics, MEMS (micro-electromechanical systems) and biological systems, a new class of systems called BIOMEMS, can help deliver drugs to specific sites in the animals.[76] BIOMEMS incorporates sealed channels, wells, fluidic ports and electrodes for delivery and analysis of cells, DNA and biomolecules. Smart disease treatment delivery system will contain sealed packages of the molecular coded drugs to be delivered to specific parts of the animal system.[77,78] This will help the farmers to reduce the costs of veterinary medicine and manage the health of livestock effectively by minimal usage of drugs.

Bovine mastitis is the inflammation of the mammary gland in cows and is a major concern for the dairy industry as it lowers milk yield, reduces milk quality and increases production costs. Microfluidic technology has already been applied for the detection of mastitis in the animal production systems.[79,80,81,82,83,84] Microfluidic-based biochip incorporating DNA amplification of genes has been developed for seven known mastitis-causing pathogens.[81] A novel microfluidic slide assembly using wedge design was developed[84] for detecting and quantifying leukocytes in milk for the purpose of disease detection and cell counting. The milk sample is mixed with a meta-chromatic substance to stain the leukocytes. The somatic cells are distributed evenly in the chip by capillary action and the stained cells are identified using fluorescence microscopy. This device has the advantage of having different reaction chambers, allowing the milk to be mixed with the dye.

A microfluidic device (Fig. 7) that integrates solid-phase extraction and nucleic acid sequence based amplification (NASBA) was developed[80] for the identification of low numbers of *E. coli*. By integrating and incorporating microfluidics to biochips, it is possible to determine several targets on one platform, which can improve assay efficiency, specificity and sensitivity for better mastitis detection and treatment.

Researchers have demonstrated the detection of melamine (adulterant) and Listeria monocytogenes (pathogenic bacteria) in milk using a disposable microfluidic device and an on-chip flow cytometer respectively.[85,86] Blue4Green, a spin-off company from the University of Twente, The Netherlands, is marketing a lab-on-a-chip system for testing animal blood or urine in the pasture to provide reliable veterinary diagnostics. This microfluidic system has the capability to measure the concentration of a number of minerals in blood or urine with capillary electrophoresis.

A microfluidic health monitoring device in a lollipop (Lollylab system) using saliva as sample for disease monitoring, pregnancy testing, hormone monitoring, detection of virus and strep throat infection in livestock animals, and monitoring of medications has been developed.[87] The chip is embedded with a candy shell that includes saliva stimulants. The chip accepts saliva and/or delivers fluids from ports that become exposed as the candy shell is dissolved in the mouth of animal. A drug reservoir with an electronically controlled microejector is also included in this device for timed drug delivery.

Microfluidic technology is also effectively used in animal science for simplifying *in vitro* fertilization procedures for livestock breeding. Beebe's group at Wisconsin has developed a microfluidic system[88,89] that physically sorts the sperm and eggs by controlling the flow of gases or liquids through a series of channels and valves. It is possible for breeders to use this technology to rapidly sequence the genomes of cattle, poultry, pig and sheep by considering the traits such as disease resistance and leanness of meat. The effect of delivery of pharmaceuticals, and feed supplements to the livestock can be precisely monitored and/or delivered through microfluidic technology.

Several challenges need to be overcome to further realize microfluidic systems for animal science applications. One such challenge is to improve the portability of the microfluidic systems to make them easier to use without the requirement of complicated and expensive control and readout instrument. As another challenge, the reliability and sensitivity of the microfluidic systems for various biological sample detection applications, and the throughput and efficiency of microfluidic sorting systems need to be further improved.

IV. Microfluidics for plant production

For meeting the goal of plant system biologists in successful modeling of living organisms, there is a need for accurate and comprehensive measurements in all aspects of biological processes. Currently, it is not possible to model flux through metabolic pathway without knowing the rate constants of enzymes, and the subcellular distribution of the enzymes and metabolites. The unique ability of microfluidic devices to produce concentration gradients coupled with advances in fluorescent substrates offers simplified solutions for studying enzyme reactions on a wide variety of plant responses.[90,91] The major

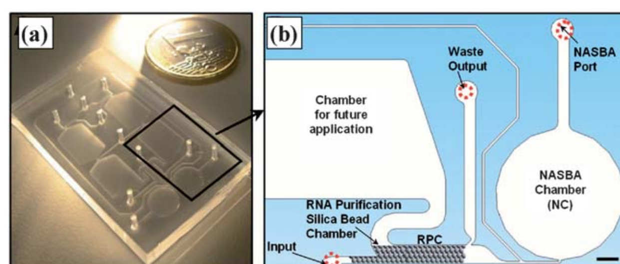

Fig. 7 Integrated microfluidic RNA purification chamber and real-time NASBA device. (a) Photograph of the device. The microfluidic architecture is mirrored to allow for 2 separate reactions with the same reagents, but different samples, to incorporate controls. **(b)** Single device architecture showing the distinct functional microfluidic modules: RNA purification chamber and real-time NASBA chamber. The remaining channels and chambers have been included for future integration of on-chip analysis. All channels and chambers are 80 μm high. Scale bar is 1 mm. Reprinted from ref. 80 with permission from the Royal Society of Chemistry.

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hurdle in structural biology studies involving ultra small volume screening of protein crystallization conditions was solved by a scalable microfluidic scheme called barrier interface metering (BIM).[90] The developed chip with BIM scheme had picolitre accuracy, negligible sample waste and complete insensitivity to the fluid properties. The device was able to implement 144 simultaneous (Fig. 8) metering and mixing reactions while requiring only two hydraulic control lines.

Bacteria *Xylella fastidiosa*, known for causing diseases in grapes, citrus, coffee, almond and alfalfa plants lives inside the xylem vessels of the plant host. Currently there are no effective methods to prevent or control the diseases caused by *Xylella fastidiosa*. To investigate and characterize the bacterial plant pathogen's molecular and biochemical aspects of infection processes and strategies, researchers have mimicked the plant xylem vessel using microfluidic chambers.[92,93] Using polydimethyl-siloxane (PDMS) microfluidic chambers, it has been shown that the migration of *X. fastidiosa* cells is directionally controlled against rapidly flowing currents of growth medium which helped to understand the colonization behaviour and migration of cells inside plant vascular systems.[93] A pilus is a hair like appendage found on the surface of bacteria. Pili connect a bacterium to another species or build a bridge between the interior of the cells for functions such as transfer of plasmids to provide antibiotic resistance. In understanding the role of pili in contributing to the adherence of *X. fasitdiosa*, traditional methods such as parallel-plate flow chambers, atomic force microscopy and laser tweezers had difficulty in obtaining data measurements due to large size of the chamber, and time consumption. A microfluidic device was developed to assess the drag forces necessary for detaching bacterial cells from a glass substratum.[92] The shear forces generated by flow through the microfluidic device was used to assess the degree to which two distinct pilus types, influence adhesion of bacteria to the glass substratum.

The advantages of the microfluidic devices over macroscale flow chambers include provision of a larger dynamic range of shear forces, a platform that is readily integrated with microscopy and an efficient system that can be assembled to mimic

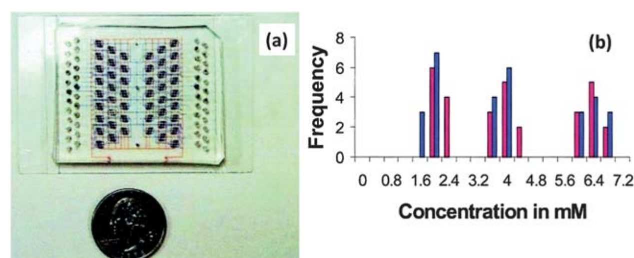

Fig. 8 Microfluidic device for producing robust and scalable fluid metering. **(a)** Prototype protein crystallization chip with 144 parallel reaction chambers (scale bars, 1 mm). **(b)** Histogram showing the insensitivity of BIM to fluid viscosity. BIM was used to combine 7 mM bromophenol blue sodium salt with water (η = 1 cP, 1 P = 0.1 Pa·s). Water measurements are shown in blue, and sucrose is shown in red. The variations in the concentration measurements (~10%) are comparable to those taken on solutions of known concentrations. Reprinted from ref. 90 with permission from the Proceedings of the National Academy of Sciences.

nanoscale and microscale features of plants. Glucosinolates are important natural products that occur in cruciferous plants, and have anti-cancer properties due to the enzyme modulation behavior. Microchip capillary electrophoresis can be effectively used to qualitatively determine glucosinolates from *Arabidopsis thaliana* seeds.[94] The method, which utilizes microchip with fluorescence detection, circumvents the multistep procedures of conventional techniques. The microchip is fabricated in poly(methyl methacrylate) and comprises of interconnected network of fluid reservoirs and microchannels. This study has demonstrated that microfluidics is an effective tool for metabolomics and targeted metabolic profiling applications.

Kumacheva's group at University of Toronto has developed a microfluidic approach to generate capsules of biohydrogels at room temperature with precise control of particle size distribution and internal structure.[95,96] Microcapsules with hydrogel shells that are formed by biopolymers can be used for the encapsulation and controlled release of pesticides or fertilizers. Thus, microfluidics becomes an enabling analytical technology for crop-based agriculture. Microfluidic devices have been developed for cultivating tobacco protoplasts and thus the microfluidic technology plays a vital role in the field of plant cell engineering and cell analysis.[97]

Agilent Technologies, Santa Clara is commercially selling a microfluidics based platform called Agilent 2100 Bioanalyzer for sizing, quantification and quality control of DNA, RNA, proteins and cells on a single platform. Food and grain industries have widely adopted this microfluidic based technology for defect identification such as sulfur deficiency and bug damage in wheat grain.[98] A device in the format of CD with microfluidics has been created to rapidly identify pathogens in the crops.[99] Plant diseases from fungal, bacterial and viral organisms can be rapidly identified using probe array and fast DNA sample hybridization in the microchannels of a microfluidic microarray assembly.[100]

Other applications of microfluidics for plant production systems include plant growth system monitoring, herbicide detection from photosynthetic membranes of higher plants,[101] study of xylem-inhabiting bacteria in plants,[102] folic acid content determination in food samples,[103] analysis of amino acids in Japanese green tea,[104] and electrochemical antioxidant sensing in apples, pears and wines.[105,106]

The general limitations of current microfluidics-based strategies for biological sample detection in low portability, special knowledge and skill requirements for system operation, and variations among different platforms also apply to the plant production related microfluidic systems. For example, while microfluidic systems can generate a broad range of chemical concentrations, most systems still require a large amount of chemicals and specialized instrument for fluid delivery and other manipulations. Key innovations are required to address these issues to further realize the potential of microfluidic systems in plant science and plant production industry.

V. Microfluidics for biofuel production

Biodiesel production is a hot research topic and its production from vegetable oils and alcohols is still a daunting task. Manufacturing of petroleum based products and fuels,

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herbicides, pesticides, and refining of ores involves multiphase reactors in any chemical and agricultural industry. Microfluidic-micro reactors offer enormous potential in solving key issues in this area. The smaller linear dimensions of microfluidic micro-reactors leading to increased specie gradients such as momentum flux, temperature and concentration results in rapid heat and mass transport, and short diffusion lengths.[107,108] The product yield from these microreactors will be higher because of better process control, high surface to volume ratio and faster system response.

A number of studies[109,110] examining transesterification of vegetable oils or animal fats with methanol to produce biodiesels in microreactors has been reported in the wake of escalating worldwide demand for energy. The rate of mass transfer is an influencing parameter in the transesterification reaction and can be efficiently optimized inside the microfluidic integrated microreactors. In comparison with a batch stirred reactor, microreactors offer greater conversion and selectivity within shorter reaction time.[108]

It is possible to produce very fast biodiesel production at high-throughput using microfluidic incorporated microreactors. Using multi laminated micro mixers (with channel dimensions (width × height) of 50 μm × 150 μm and 40 μm × 300 μm), biodiesel was produced through transesterification of cottonseed oil and methanol at a flow rate of 10 mL min^{-1} and a residence time of 17 s.[109] A 400 μm inner diameter fluidic microtube in a microreactor was used in producing biodiesel from trans-esterification of sunflower oil with a residence time of 100 s.[108] Microreactor fluid channels with sub-millimetre range (100 μm thickness) characteristic dimensions of the internal structure were employed by Oregon state university researchers[110] in producing biodiesels from soybean oil with conversion efficiency of 86% in less than 10 min. The research group claims that using microreactors, biodiesel could be produced between 10 to 100 times faster than traditional methods by eliminating the need for chemical catalysts upon coating the microchannels with a non-toxic metallic catalyst.

Microfluidics based techniques offer unique solutions in bio-energy research as it allows faster enzyme purification and analysis, providing an automated engineering method and in efficiently using smaller amounts of cell mass to produce proteins. Sandia National Laboratories have developed a technique[111] for high-throughput purification of minute amounts of native and recombinant proteins using a microfluidic extraction system (Fig. 9). This technique allows thousands of enzymes and their variants to be purified and screened rapidly which can significantly aid researchers as they search for the most optimal enzyme that meets biomass processing needs in breaking down cellulose into sugars. Enzymatic degradation of *p*-chlorophenol in a two-phase flow on a microfluidic device[112] demonstrates that the biochemical reactions can be efficiently performed by microfluidic technology.

The problems of heat and mass transfers due to exothermic reactions and catalyst attrition in the conventional reactors can be easily overcome by microchannel reactors. Further progress in developing the microfluidic technology for large scale production of biofuels will be determined by the optimization of the current microchannel reactors and the ability to scale up using multiple parallel microreactors.

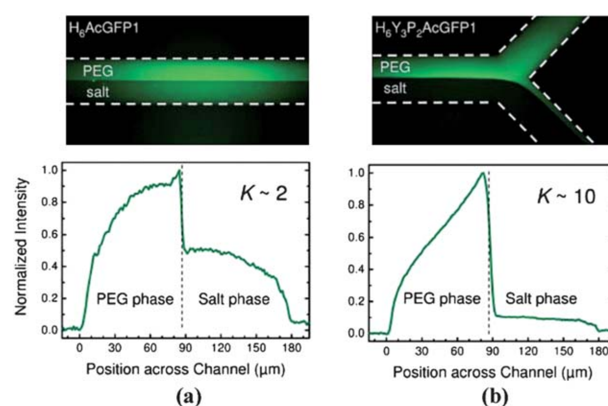

Fig. 9 Fluorescence micrographs illustrating partitioning of AcGFP1 (green fluorescent protein) with and without genetic modification with the Y_3P_2 partitioning tag. The micrographs were taken near the end of the channel, at ~320 mm from the inlet. **(a), (b)** The plots below the micrographs are normalized line profiles of fluorescence intensity, which can be used to calculate an apparent partition coefficient (*K*) for the AcGFP1. Reprinted from ref. 111 with permission from the Royal Society of Chemistry.

Future perspectives

As discussed in this paper, microfluidic technologies have clear advantages over conventional methods in the food, agricultural and biosystems related applications such as lower chemical and power consumption, higher throughput of synthesis, screening and processing of biological species, faster reaction and sample times, lower production costs, and would allow long-time continuous operation. These features are expected to enable increased profits and economic growth. The lessons learned from the successful microfluidic based ink-jet printers would be valuable in cheaper mass manufacturing of microfluidic devices for food, agriculture and biosystems industries. New micro-manufacturing approaches and multiplexing technologies pave the way for robust, inexpensive and high-throughput devices. Of course, these advantages are not globally applicable but are specific to applications (Table 2).

To realize the potential in the agri-food systems, the current microfluidic technologies need to overcome some common or specific limitations (Table 2). As repeatedly mentioned in the paper, a major challenge for the current microfluidic systems is in improving its portability. Further technical challenges include heat and mass transfer functions inside the microfluidic channels and their effect on the overall functionality. Another limiting factor is the requirement of specialized facility (*e.g.* cleanroom facility) for microfluidic device fabrication and characterizations. Consequently, high manufacturing costs of devices remains a hurdle for the microfluidic technology and its fusion in the food and biosystems industries. More flexible and easy fabrication methods without the clean room requirement are increasingly developed. In addition, there is an apparent resistance in adopting microfluidic technology from the manufacturers on the account of re-tooling the production facilities. However, this issue can potentially be overcome by the scalable, cheaper and robust fluidic systems that are being developed. The added advantage of using microfluidic component in the food

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Table 2 Advantages and limitations of microfluidic systems for agri-food and biosystems industries

Application area	Examples	Advantages	Limitations	References
Food safety	Pathogen and antigen detection	High sensitivity High speed High throughput	Lack of interfaces for bridging microfluidic systems and electronic read-out instruments	8,11
	Pathogen sorting	Label-free miniaturization		9,10
	Toxin detection	High sensitivity High speed High throughput	Lack of function integration	12,13
	Immunoassays	High sensitivity High throughput Low reagent consumption Ease of operation		14–20
Food processing	Food mixing	High efficiency	Lack of control of infusion and mixing of liquids	23,24
	Calcium alginate gels preparation	High throughput Improved homogeneity		25
	Microchannel emulsification	Operation Control	Not meeting the large scale production requirement	24,27,28
	Micro-nano-bubbles	Control of bubble generation		69,70,71
Animal science	Pathogen detection in animals	Miniaturization	Lack of portability	76–84
	In vitro fertilization	Precise monitoring Sorting function	Lack of operation efficiency	88,89
Plant production	Chemical reaction metering	High accuracy	Lack of portability	90,91
	Mimicking plant micro-environments	Provision of a larger dynamic range of shear forces Easy integration with microscopy Mimicking nano and microscale features of plants		92,93
	Microcapsules	Precise control of particle size distribution and internal structure	Reagent consumption	95,96
	Pathogen detection	Rapid identification		99,100
Biofuel production	Biodiesel production	High product yield	Lack of reactor optimization	107–110
	Enzyme purification and analysis	High throughput	Technical challenges in the large scale production requirement	111,112

processing equipment is that the processing unit can be easily and cheaply replaced, leading to increased fault tolerance capability of the equipment. Finally, knowledge gap in addressing and framing the standards of interfaces, operator training, and materials and channel geometries through synergistic collaboration between academic and industrial researchers is critically needed, and such effort is currently underway. The success of microfluidic technology adaptation in the food, agriculture and biosystems industry relies on the ease of use, the perception of the consumers, and the industrial acceptance.

Conclusions

Microfluidics is an important enabling technology that uniquely integrates various research areas for a broad range of scientific and commercial applications. The microfluidic technology offers novel approaches for solving crucial problems in the agri-food industry. The market is expected to expand significantly as the microfluidic technology has demonstrated sufficient and key benefits for the food, agriculture and biosystems industries. The penetration of microfluidics to the agri-food and biosystems market will require the technology validation from a manufacturability point of view, and addressing the knowledge gap in framing the standards. There is a need to improve the existing microfluidic technologies that are too complicated or expensive to integrate into a functional system. The new tools and devices have to perform at greater accuracy levels and higher levels of throughput than standard macro-scale automated equipment in order to progress beyond the laboratory into everyday world to solve problems of the agriculture, food and bioprocessing industries. Nonetheless, the room for growth, opportunities and innovation for microfluidic applications in the agri-food and biosystems industries is enormous.

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