Chemometrics and Intelligent Laboratory Systems 108 (2011) 64–75

Contents lists available at ScienceDirect

Chemometrics and Intelligent Laboratory Systems

journal homepage: www.elsevier.com/locate/chemolab

Lab-on-a-chip technologies for massive parallel data generation in the life sciences: A review

S.J. Trietsch, T. Hankemeier, H.J. van der Linden *

Division Analytical Biosciences, Leiden/Amsterdam Center for Drug Research, Einsteinweg 55, 2333CC, Leiden, The Netherlands
Netherlands Metabolomics Centre, Einsteinweg 55, 2333CC, Leiden, The Netherlands

ARTICLE INFO

Article history:
Received 27 October 2010
Received in revised form 4 March 2011
Accepted 9 March 2011
Available online 16 March 2011

Keywords:
Micro total analysis systems
Microfluidics
Chemometrics
Tutorial
Massively parallel

ABSTRACT

Lab-on-a-chip (LOC) technology is an important and rapidly developing research field focused on improving experimentation and analysis in the life sciences through miniaturization of full analytical systems into (monolithic) chip substrates. Since its emergence in the 1970s [1], the field has matured, gaining tremendous momentum in the last two decades. Miniaturization and integration of analytical processes on a chip can offer enormous advantages over existing technologies and can create a range of novel opportunities in the life sciences. The developments in the field have led to significant increases in analysis throughput, more than billion-fold sample volume reductions and increased separation efficiency. Despite its potential for the life sciences, the existence and the implications of LOCs are not widely known outside its community.
The aim of this review is to introduce scientists from different disciplines to LOC technology. We will discuss the most important LOCs, their physical operating principles and the unique benefits that can be gained through miniaturization. We will conclude this review with a discussion of the potential of LOCs for massive parallel data generation and their potential implications for the life sciences.

1. Introduction

This review will cover lab-on-a-chip (LOC) technology and the potential of this rapidly emerging field (Fig. 1) for the life sciences. As the name 'lab-on-a-chip' implies, this technology has the ambitious goal of fabricating entire laboratory analysis workflows in small devices, using technologies similar to microelectronic chip fabrication. To achieve this goal, these (micro-) fluidic 'chips' contain a fully-integrated network of micrometer-scale fluidic components, such as channels, reactors, pumps, chromatographic columns, and detectors. The field of LOC is highly multi-disciplinary, requiring expertise from many fields, including (bio-) chemistry, physics, electronics, (micro-) engineering and (micro-) fabrication. The high level of miniaturization results in faster analyses, higher separation efficiencies, reduced sample, reagent and solvent consumption, as well as allowing unique experiments such as single-cell genomics.

Due to their inherent small size (e.g. mm^2), many individual LOCs may be integrated into one larger device, still only several cm^2 in size, capable of performing many LOC-analyses in a rapid, parallel fashion. An analogy can be drawn with the use of integrated circuits in electronics versus the use of discrete electronic components. As the number of components and connections in an electrical circuit increases, the chance that the failure of a single connection or component causes malfunction of the entire circuit increases dramatically. This poses a practical limit to the maximum number of discrete components that can be combined in a single functional electronic device, known as the 'Tyranny of Numbers'. Integrated circuit fabrication solved this issue by downscaling the single discrete components, allowing monolithic integration of all components, removing error-prone mechanical connections between discrete components. The same principle applies to LOCs where the monolithic fabrication process eradicates conventional, leakage-prone couplings between discrete fluidic components. Furthermore, monolithic integration can significantly reduce dead volumes and dispersion associated with conventionally constructed analysis systems. As a result, almost arbitrarily complex analysis systems can be fabricated, capable of performing massive numbers of experiments in parallel, which would otherwise not be feasible. Another aspect of monolithic integration, analogous to integrated circuit fabrication, is the strong reduction of cost as large-scale mass fabrication is used.

An example of a monolithic, highly-parallel LOC, which shows the resemblance between electronic circuits and 'large-scale integrated' microfluidics is shown in Fig. 2. This device can perform 256 enzyme expression cell assays that could not be performed with conventional components. The 30×30 mm LOC contains thousands of channels and valves, connected in a highly intricate fluidic network, used to mix reagents and culture cells in 256 separate 750 pL chambers. Fabricating the analysis setup with conventional components (i.e. capillaries, pumps and valves) would literally cost tens of thousands of dollars while LOCs based on this technique are available for less than $100 [3].

* Corresponding author at: Gorlaeus Laboratories, P.O. Box 9502, Einsteinweg 55, 2300 RA, Leiden, The Netherlands. Tel.: +31 71 527 4509; fax: +31 71 527 4277.
E-mail address: lindenhjvander@lacdr.leidenuniv.nl (H.J. van der Linden).

0169-7439/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.chemolab.2011.03.005

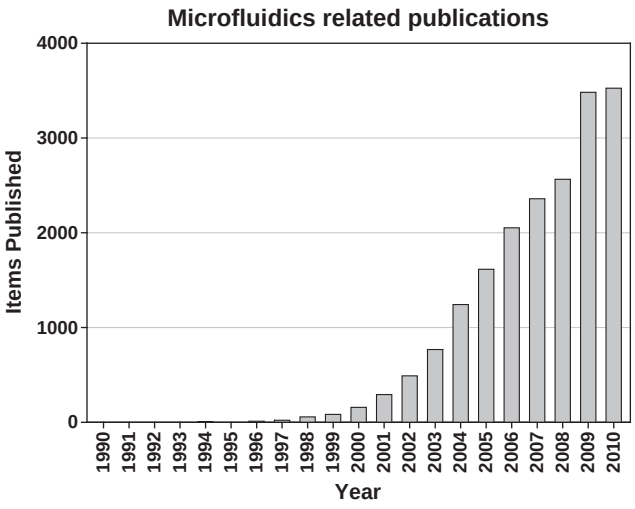

Fig. 1. Rapid growth of the number of microfluidics related publications [37].

2. Principles of downscaling

The advantages of labs-on-chips are based on the fact that miniaturization of analytical unit operations often improves the performance of analytical chemical systems as the dimensions of system components and physico-chemical principles scale positively with decreasing size (i.e. the principle of 'positive downscaling'). In addition, different physico-chemical phenomena are dominant at a small scale (i.e. laminar flow, diffusion and electro-osmotic flow), which are employed to improve performance or obtain unique methods of operation [6].

An obvious positive benefit of downscaling is found in the reduction of liquid volumes (i.e. samples, solvents and reagents) when performing analyses in an LOC system. Assuming x to be the characteristic length scale of a system component in an LOC system (e.g. a channel, reservoir or detection cell), the volume of the component scales as x^3. For a ten-fold reduction in the length scale x, a thousand-fold (10^3) reduction in volume is obtained. As the dimensions of LOC components are commonly about 100 times smaller than the components in conventional analysis systems, a million-fold volumetric reduction is obtained (i.e. a reduction from milliliters to nanoliters or even less). This allows analysis of extremely small volumes (e.g. single-cell analysis, forensic trace analysis, etc.), drastically reduces expensive reagent consumption and safely allows analysis with dangerous reagents like radio-active tracers used in assays.

The surface-to-volume-ratio (SVR) is another important scaling-dependent parameter. The SVR describes the ratio between liquid volumes in LOC components and the wall surface the liquid is contacting. When surface interactions between analytes in solution and the surfaces are important (e.g. in immuno-affinity assays, chromatographic interactions and electro-chemical detection), the SVR gives an indication of the efficiency of these interactions. The SVR commonly increases by a factor of more than ten thousand during downscaling and solute/wall interactions become dominant. This works advantageously in on-chip chromatography, but can lead to undesired results as aspecific adsorption of molecules may lead to analyte loss, carry-over and irreproducible chromatographic results. Aspecific adsorption in LOC systems can effectively be overcome by applying anti-adsorption coatings on the channel walls.

The flowing of fluids we commonly see in everyday life is characterized by turbulence (i.e. a flow pattern which constantly varies chaotically in time) as shown in Fig. 3 (left). In contrast, on the micrometer scale fluids flow in a stable, non-turbulent manner as fluid viscosity and not the fluid's inertia is dominant. An example of this non-turbulent, laminar flow is shown in Fig. 3 (right). In physics, the dimensionless Reynolds number [7] is used to predict flow behavior. Reynolds numbers above 2000 are turbulent in nature, Reynolds numbers below 1000 are laminar in nature while values between 1000 and 2000 describe flows alternating between laminarity and turbulence. The Reynolds number becomes very small in LOC systems and is often below one (i.e. the flow is completely laminar). A positive effect of laminarity is the high predictability of the fluid flows in LOCs which is used among others to create stable and accurate gradients in chemical concentrations in cell culture LOCs [8]. On the other hand, microscale mixing is very difficult as turbulent mixing does not exist and diffusion or special mixers like stagger-herringbone mixers [9] and chaotic advection mixers [10] need to be employed.

In contrast to on the macroscale, diffusion plays a dominant role on the microscale as diffusion lengths become very small. This is described by the Péclet number which relates diffusive mass transport

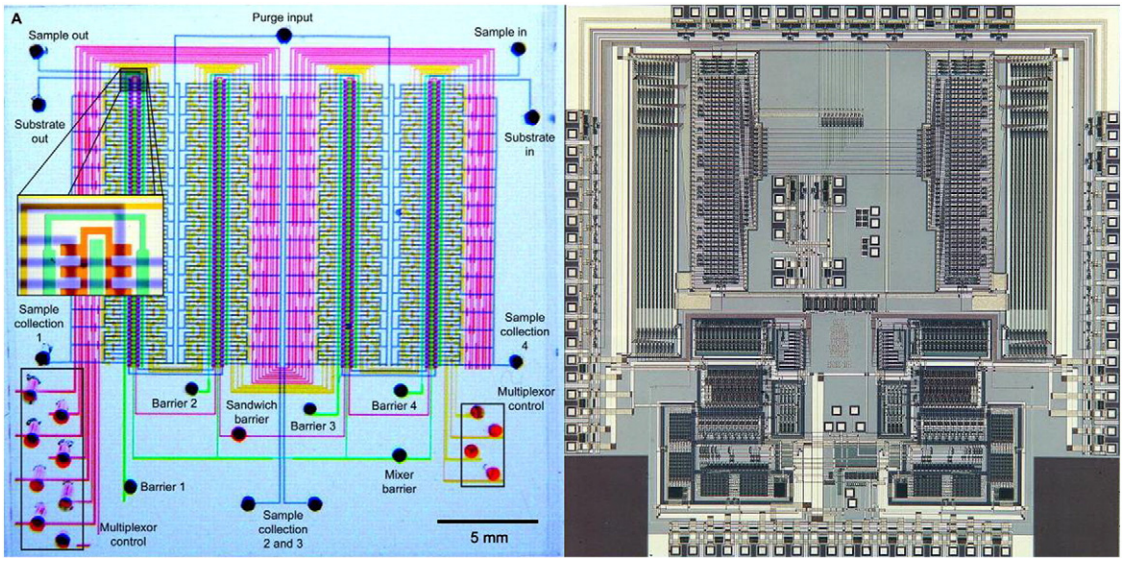

Fig. 2. A fluidic (left) and electronic (right) integrated circuit [4]. The fluidic integrated circuit shows a comparator chip, containing 2056 valves to mix reagents and assay for enzyme expression in cells in 256 separate 750 pL chambers. Adapted with permission from [5].

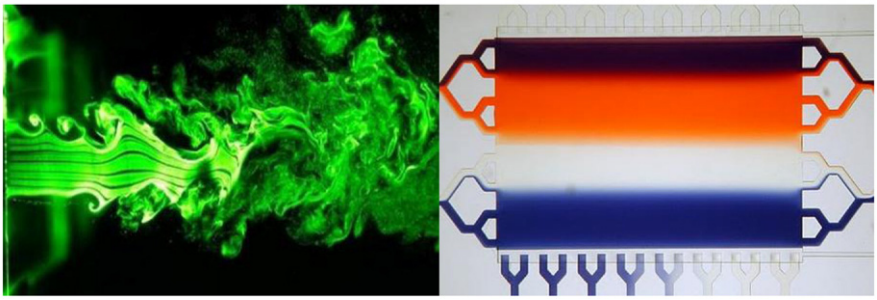

Fig. 3. Turbulence and laminar flow examples. (Right image courtesy of Greg Cooksey and Albert Folch, University of Washington, Bioengineering Dept) [11,12].

to advective mass transport. In LOCs the Péclet number is very small and diffusion becomes an important mode of mass transport. At this scale diffusion is used to effectively mix different liquids. Also diffusion is used to separate small molecules from larger objects like beads or cells in so-called H-filters [13,14] as shown schematically in Fig. 4.

The small Péclet numbers are also important in on-chip chromatography as the efficiency of diffusive mass transport from the analyte-carrying, liquid phase to the chromatographic solid phase greatly increases. This leads to better separations as higher flow rates can be used which limit peak broadening due to lateral diffusion during the separation.

3. A differentiation of lab-on-a-chip technologies

Lab-on-a-chip platforms may be categorized in a number of ways. A first categorization could be based on the separation mechanism that is employed or the detection principle that is used. However such a categorization puts different LOCs together which intuitively do not fit together. The authors have chosen a less obvious categorization, which in the end is more intuitive, namely one based on the fluid control mechanism employed. For each category, we will discuss illustrative examples which explain the working principles, the physics employed, the analytical workflows and the potential of these platforms for rapid, massive-parallel operation.

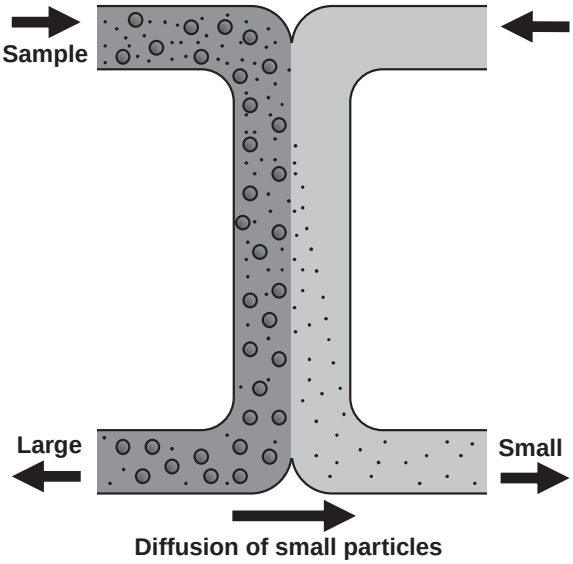

Fig. 4. Operating principle of microfluidic H-filters. A sample (e.g. blood) containing both large species (e.g. red blood cells or large proteins) and small species (e.g. salts or metabolites) is flushed, together with a laminar co-flow, through the H-filter. As the large species diffuse very slowly compared to the rapidly-diffusing small species, different-sized sample components can be separated in the H-filter.

It is not our aim to provide an exhaustive overview of all available LOC platforms. Instead we will focus on the most important platforms and for further reading the interested reader is referred to other reviews covering these topics [14–23]. It should finally be noted that the LOC field progresses rapidly and a number of these platforms may become outdated in just a few years as new platforms and technologies emerge.

3.1. Pressure-driven platforms

As the name suggests, pressure-driven LOCs control fluid movement with differential pressures commonly applied by mechanical pumps or gas pressure. An enormous variety of micropumps for LOCs has been developed over the decades, which are discussed in several reviews [24–26].

Pressure-driven LOC platforms are illustrated with the technology pioneered by Quake et al. in 1999 at the California Institute of Technology [27]. These platforms are made in the widely used, elastomeric silicone rubber poly-dimethyl siloxane (PDMS) by replication molding [28–31]. The LOCs consist of two thick layers of PDMS interspaced with a very thin, flexible PDMS membrane as depicted in Fig. 5. Microchannels in the bottom layer are used to supply gas pressure to on-chip microvalves which use the deflection of the thin membrane by the gas pressure to control liquid flow through channels in the top layer. By connecting the three microvalves in series, a peristaltic pump can be formed. In this way it is possible to create hundreds to thousands of microvalves and pumps on an LOC which measures a few square centimeters as is shown in Fig. 2 (left) [5].

These platforms hold the current record in the number of active components which can be integrated into one device. The device shown in Fig. 2 (left) has more than 2000 valves and more than 200 peristaltic micropumps, monolithically integrated to perform 256 single-cell analysis experiments in parallel. The challenge of individually controlling the valves and pumps is solved by connecting all the 256 experiments in parallel. In this way all experiments are run in synchronous fashion with only 18 external pressure valves. These PDMS-based LOCs are commercially available from the company Fluidigm™ [32].

Another example of pressure-driven LOCs is the Agilent HPLC-Chip, which is a miniature LC system that integrates all necessary

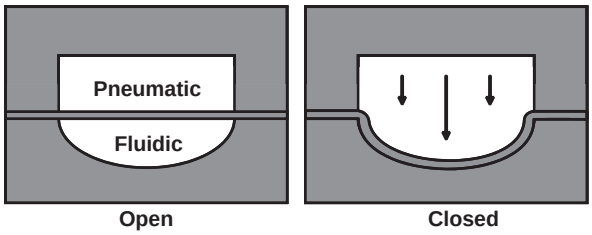

Fig. 5. Schematic of an elastomer microvalve. Pneumatic control channels are pressurized to deflect a membrane, closing of the fluidic channel.

chromatographic columns (both trapping columns and separation columns) and an electrospray interface for mass spectrometry into one small device (see Fig. 6).

Due to the positive effects of miniaturization, the LC-chip is capable of performing separations rapidly and efficiently, while at the same time allowing mass spectrometric analysis with the on-chip electrospray ion source. By efficient monolithic integration of the total system, dead volumes and peak dispersion have largely been removed. As a result the LOC is capable of very reproducibly generating good chromatographic separations.

3.2. Electrokinetically-driven platforms

Electrokinetic flow is an important fluid control technique in LOC devices. Electrokinetic flow is more commonly known from capillary electrophoresis (CE) where the electro-osmotic flow (EOF) is used to move fluid through capillaries. Briefly, as depicted in Fig. 7, positive ions in the solution accumulate near the negatively charged wall surface. An electric field is applied between the ends of the capillary to attract the layer of ions near the wall towards the negative electrode. As viscosity is dominant on these scales (i.e. <250 μm), the movement of the ion layer couples into the bulk liquid, resulting in a bulk flow (i.e. the EOF). Electrokinetically-driven LOC platforms often consist of photolithographically structured glass or silicon chips on which EOF is used for a variety of operations including pumping liquids around the device, injecting minute amounts of liquid accurately, etc [34–36]. By varying the electric field, the fluid flow rate can be set over a wide range and by measuring the current, the flow rate can be determined.

In addition to bulk fluid control, electrokinetics can be used for very efficient separations. The field affects the analyte ions in the solution, separating them based on their charge to size ratio. The smallest and most highly-charged ions migrate fastest towards their opposite pole. An important feature of EOF is the flat flow profile resulting from the wall-driven flow as depicted in Fig. 7. The flat flow profile causes significantly less peak broadening than the parabolic flow profile seen in pressure-driven flows, resulting in very efficient separations (routinely an order of magnitude better than liquid chromatography techniques) [2]. Electrokinetic separations can further be used for more complex separation or concentration techniques like isotachophoresis [38], isoelectric focusing [39], etc.

Fig. 6. Agilent LC–MS Chip™ [33].

Accurate sample injection on electrokinetically-driven LOCs is not trivial and an elegant injection method using EOF to fill precise injection volume geometries (i.e. a cross- or double T-injectors) is employed. Electrokinetic sample injection with a double-T injector is shown in Fig. 8. Briefly, by applying a voltage between the sample reservoir and the waste reservoir, the sample liquid is electrokinetically pumped across the injector (t = 0). The double-T geometry is used to accurately define the injection volume, obviating the need for valving. After loading, the sample volume is injected and separated into its constituents by applying a high voltage over the separation channel (t = 1). The double-T injector principle can be miniaturized another three orders (i.e. nanometer scale) as was recently done in our group [40]. In a nanofluidic electrokinetic separation device we performed a 400 femtoliter analysis of fluorescently-labeled amino acids spiked into a complex biomatrix. For a consideration of size: 400 femtoliter is less than the internal volume of a single 10 μm diameter cell.

Chip-based electrophoresis forms the basis of the commercially available Bioanalyzer 2100™ LOC platform by Agilent™ depicted in Fig. 9. A range of chips is available for different applications including RNA [41], DNA [42], and protein analysis [43] or performing on-chip cell staining and flow cytometry [44]. These well-characterized chips offer an off-the-shelf system for quickly performing various analyses. Predefined methods and ready-to-use reagent kits, combined with automated use of internal standards, readout and data analysis ensure very reproducible results, allowing researchers in the life sciences to tap into the potential of LOCs without needing any LOC expertise.

While exploiting many of the advantages microfluidics has to offer, the Bioanalyzer 2100™ platform does not perform massive parallel analyses. The Matthies group at Berkeley University showed several systems using radial [45] or parallel channel arrays [46] fabricated in glass. A high-throughput DNA sequencing system was developed consisting of 384 radial analysis channels on a 200 mm diameter glass disc, as depicted in Fig. 10. After sample preparation, this device is capable of genotyping 384 samples in parallel in only 7 min [47].

3.3. Centrifugally-driven platforms

Centrifugally-driven lab-on-a-chip platforms are an insightful example of the ingenious methods for fluid control which may be used in LOCs. Instead of mechanical or electrokinetic pumps, centrifugal forces are used which move liquids inside circular disks containing all necessary microfluidic components for chemical analysis. Such discs are commonly manufactured from plastics using injection molding or hot embossing. In excess of one hundred parallel analyses can be performed rapidly in an LOC the size of a compact disc (see Fig. 11).

The disc is processed in a robot-operated workstation which performs all pipetting operations and handles the whole disc-based analysis workflow. The elegance of the system lies in the fact that all parallel analyses are controlled with only one global parameter: the rotational speed of the disc. By rotating the disc at different speeds, the centrifugal force and thus all liquid movements in the disc can be adjusted.

Fig. 12 graphically depicts the operating principle of the system. The samples are pipetted into sample reservoirs positioned close to the center of the disc. By rotating the disc, the liquid is forced radially outward into metering reservoirs which accurately meter the analysis volume. Excess sample is transported to waste through overflow channels. Valves are created with so-called “hydrophobic stops” which consist of small channel sections that are locally covered with a hydrophobic coating which blocks the passage of the aqueous sample

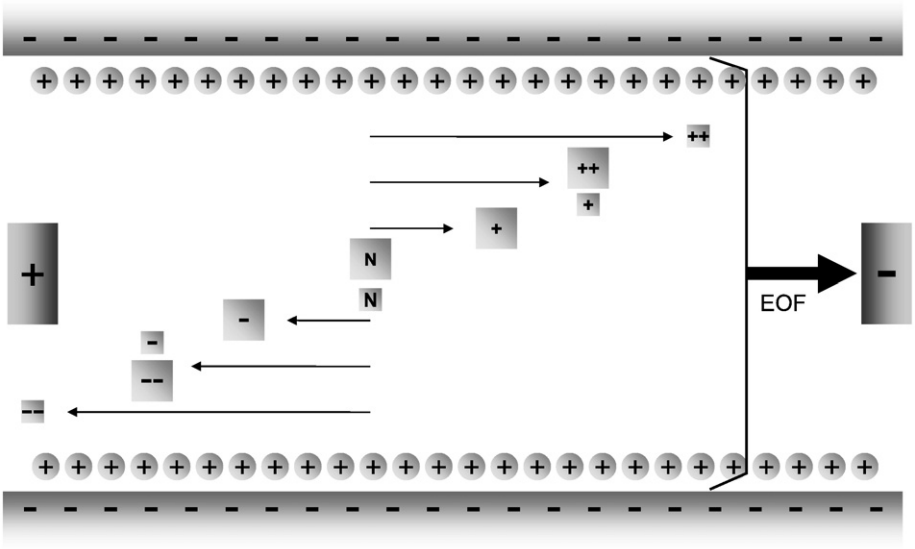

Fig. 7. Principle behind electro-osmotic flow, its flat flow profile and the mechanism of electrokinetic separation of ions based on their different size to charge ratios.

liquid. By increasing the rotation speed over a critical value, equivalent to the valve's "bursting pressure", the aqueous liquid is forced to cross the hydrophobic stop into the subsequent section of the analysis disc. On the system depicted in Fig. 11, 112 immunoassays can be performed in parallel, in less than one hour.

The disc-based analysis platforms have been commercially available for a number of years now from a number of manufacturers (i.e. Gyros and Tecan) and are in use by, for instance, pharmaceutical screening divisions at large pharmaceutical companies.

3.4. Digital Microfluidics

Digital microfluidics, also called droplet-based microfluidics [50–52] or segmented-flow microfluidics is another example of an ingenious exploit of working at the small scale. Two immiscible fluids are fed through a droplet-generating geometry to form a stream of highly monodisperse, separate droplets acting as individual micro-reactors as shown in Fig. 13. The droplets can be produced at high frequencies (i.e. kHz), with a very accurate volume going down to the femtoliter range.

The droplets can be manipulated to perform operations such as splitting [53], merging [50], mixing [54], counting, sorting [55], and droplet selection [56] These actions can be performed passively (i.e. using the chip geometry) or actively (e.g. electrically [57]). Because the droplets are produced so rapidly, this system is capable of performing massive amounts of discrete experiments (each contained in their individual droplet) in a small LOC. For example, by creating droplets from a liquid feed containing a chemical gradient, and subsequently merging these droplets with another droplet stream containing proteins, a huge range of protein crystallization conditions can be screened very rapidly. This screening technique is potentially a very significant step forward in the often tedious and time-consuming

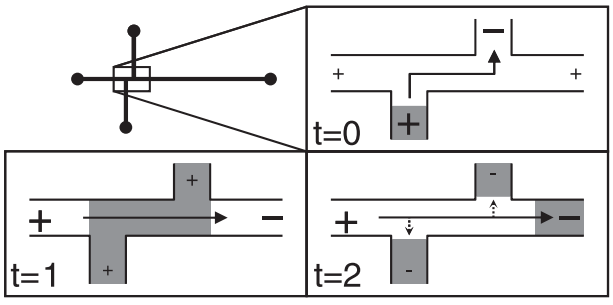

Fig. 8. Operating principle of electrokinetic injection using a double-T injector.

Fig. 9. The Agilent™ LabChip™ cartridge with a view of the electrokinetic glass chip that is inside. The sample is loaded on the cartridge which is inserted into the BioAnalyzer™ for automated analysis [33].

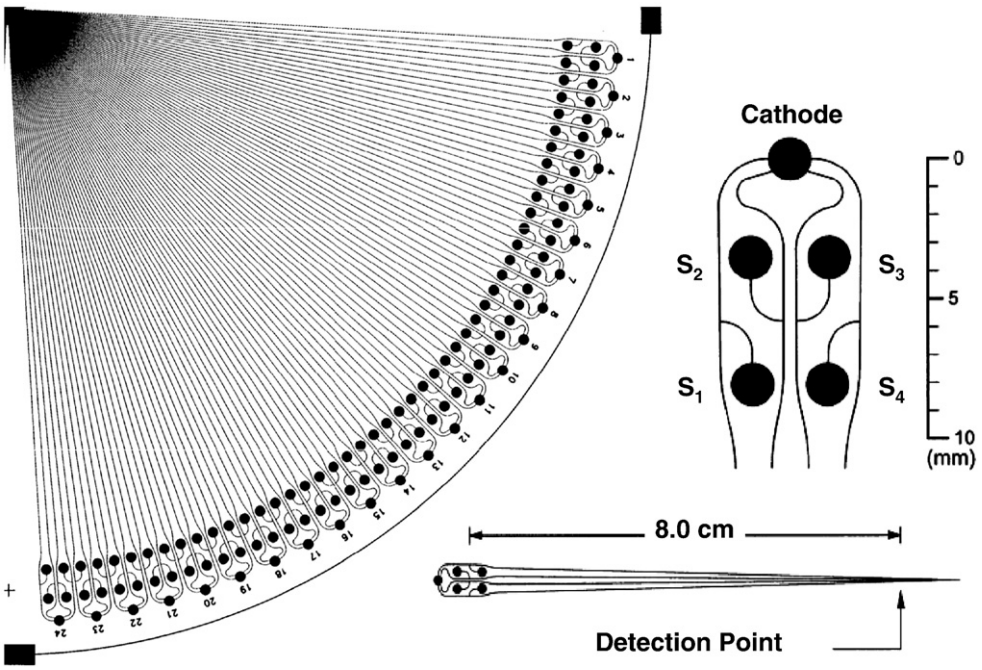

Fig. 10. Parallel genotyping device, with four sample reservoirs for each analysis channel and access for electrical connections. The transparency of the glass LOC allows detection to be performed using a rotary, confocal fluorescence scanner.
Adapted from [47].

selection of optimal protein crystallization conditions. In a similar fashion, optimal conditions can be screened for the chemical synthesis of compounds and compound libraries.

Although not operating in a parallel fashion, digital microfluidics can be used in a similar way to massive-parallel LOCs, to perform huge numbers of experiments. If droplets are created at 3 kHz and the entire analysis takes 100 s, 300,000 samples are processed at any one time on a single chip.

3.5. SlipChip™

Recently, the group of Professor Ismagilov at the University of Chicago has pioneered an ingenious method to perform microfluidics without the need for an elaborate setup. The system is based on two plates containing, fluids, wells and channels (often etched in glass). The two plates are positioned on top of each other and by slipping them, fluid-containing wells in the top plate are moved to new positions over reaction wells in the bottom plate, mixing the contained volumes as shown in Fig. 14. The liquid movement can thus be controlled manually instead of with expensive pumps. This principle can be applied to perform multi-step reactions on many samples in a simple manner. Although a recent development, several promising applications have been shown including nanoliter immunoassays [58], (digital) PCR [59,60] and water quality monitoring [61].

4. Detection in lab-on-a-chip systems

Downscaling in lab-on-a-chip platforms imposes unique challenges on detection of analyte species. For instance, UV/Vis absorbance detection, which is widely used in conventional analyses, suffers from downscaling. Due to the very small optical pathlength (often around 10–100 μm), the detector's signal-to-noise ratio (SNR) will be lower and can thus be insufficient for analyses that could be performed using UV/Vis on a conventional scale. General requirements for LOC detectors include: high sensitivity/SNR, capability of detection in μL–fL volumes, and fast response time (especially in the case of massive-parallel LOCs). Often on-chip integration of the detector is preferred, which can further complicate detection. Finally,

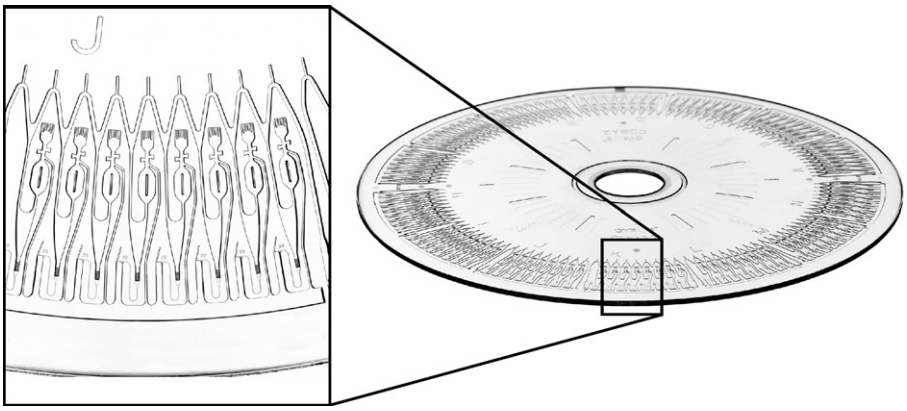

Fig. 11. A section of the Gyros™ BioaffyCD™ [48].

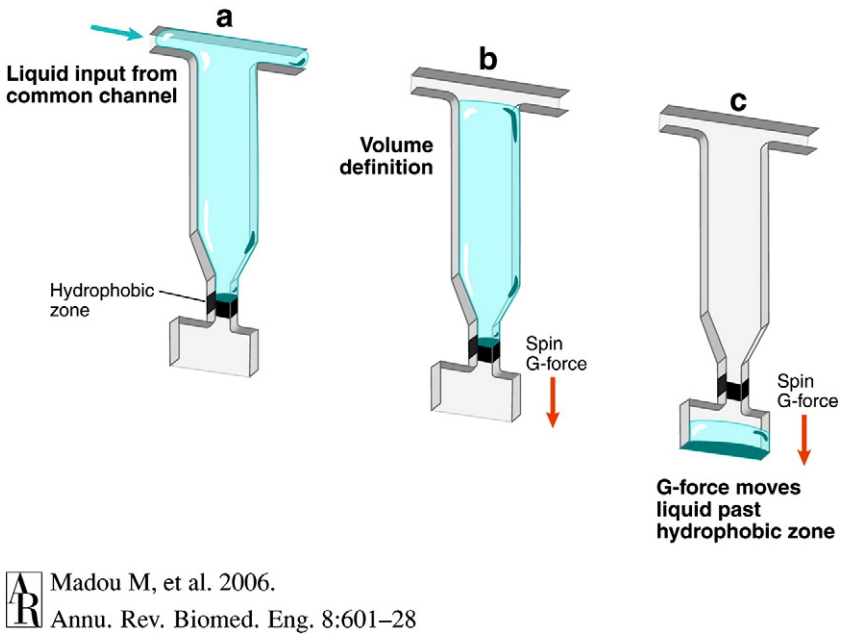

Fig. 12. A fluid dispenser in centrifugal microfluidics. The fluid fills up a reservoir with defined volume and is unable to pass the bottom valve until faster spinning of the disc provides the extra force necessary to overcome the hydrophobic barrier (Reproduced with permission from [49]).

techniques are needed which deliver information-rich spectra which can be used for compound identification. As a result, many well-known detection techniques are insufficient for LOC applications and therefore often exotic and innovative detection principles are used.

In this second part of the review we will discuss the most important LOC detection techniques as well as their effect on the data generation and processing.

4.1. Laser-induced fluorescence detection

As integration of detectors or sensors inside LOCs is often very challenging, often optical microscopy techniques are used. These techniques have a number of advantages including contactless detection, suitability for detection in extremely small volumes (down to fL) and very high sensitivity. The most common optical detection technique is laser-induced fluorescence (LIF) detection. Powerful laser light is used for excitation and high-quality optical filters used in combination with extremely-sensitive detectors (e.g. single-photon sensitivity photomultipliers) resulting in very low detection limits down to single molecules. In fact, LIF is such a sensitive technique that it compensates for the large decrease in detection volume caused by downscaling. The effectiveness of LIF is illustrated by the fact that most of the examples of LOCs presented above use LIF for detection.

While the most effective detection method, LIF does impose some limitations. It is limited to detection of autofluorescent or fluorescently-labeled compounds (which requires sample derivatization) and does not allow broad identification of different analytes as most biologically-relevant molecules do not fluoresce. The large dimensions of the detection setup and its inherent high cost limit the application of LIF to use in research laboratories and bench-top instrumentation. In these environments however LIF is by far the most widely used detection technique.

4.2. Surface-enhanced Raman spectroscopy

Raman spectroscopy is a vibrational spectroscopy technique that is based on the fact that a small portion of light is inelastically scattered from molecules (i.e. the wavelength is changed due to energy transfer to the molecule and scattered photons are emitted at longer wavelengths), resulting in a compound-specific Raman spectrum. Raman spectroscopy is an information-rich technique, but lacks

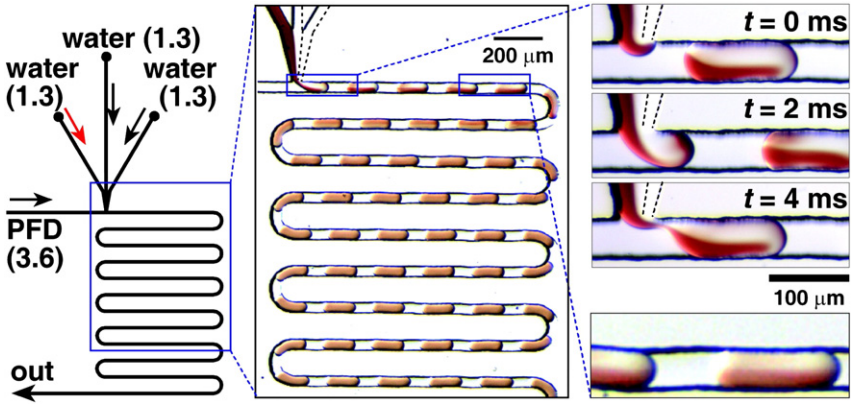

Fig. 13. A digital-microfluidics chip mixing two fluids in separate droplets at approx 250 Hz [53].

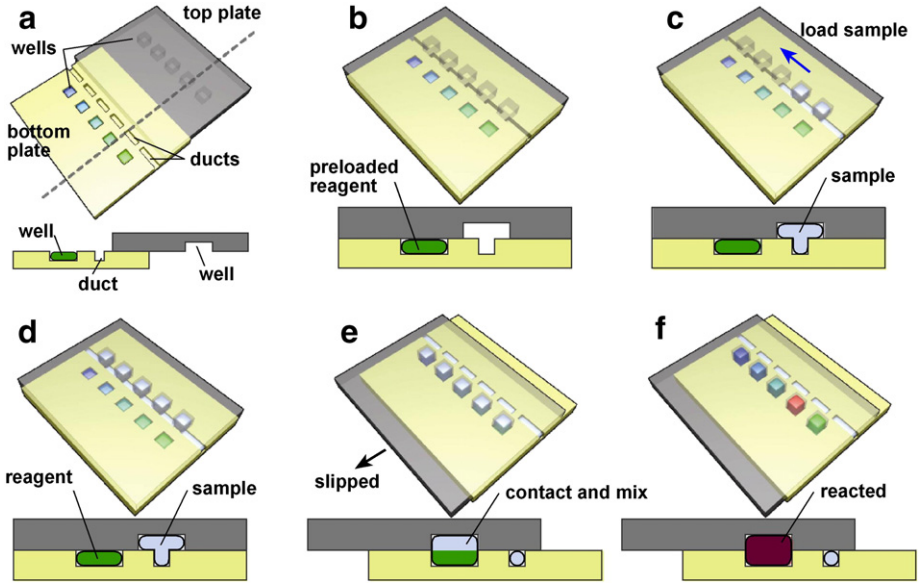

Fig. 14. Working principle of the SlipChip™ with (a) plates with pre-loaded wells and ducts (b) aligned chip ready for use (c and d) sample loading through the ducts of the bottom plate and wells of the top plate. (e) Slipping the top plate to move the sample from the duct to the separate wells. (f) Reaction of the mixed contents Reprinted with permission from [62].

sensitivity. The signal strength can however be greatly amplified using conductive (i.e. most often metallic like Au and Ag) nanostructures, resulting in a sensitive, information-rich detection technique called surface-enhanced Raman spectroscopy (SERS).

In SERS, metallic nanostructures manufactured on a surface are resonantly excited with an infrared laser, resulting in a highly-amplified electromagnetic field in the gap between the nanostructures. Molecules present in the gap are strongly excited by the electromagnetic field and the Raman signal strength is greatly improved. As this enhancement is confined within nanometers from the nanostructures, SERS measures very locally and is insensitive to downscaling. This makes SERS a very interesting information-rich detection technique for LOCs. The sensitivity of SERS is very much dependent on the quality of the nanostructures, but optimized SERS surfaces show in excess of one million-fold increases in sensitivity, compared to conventional Raman spectroscopy, resulting in detection limits down to single-molecules [63,64].

The compound-specific spectra acquired with SERS are comparable to infrared spectra, allowing for compound identification in addition to quantification. Although exotic, SERS is an important technique for LOC detection as its detection limit does not scale with size and SERS is one of the few detection techniques which allow compound identification. Currently, some challenges with this technique remain such as sub-optimal sensor response due to irreversible analyte binding the SERS nanostructures and the need for reproducible fabrication of SERS sensor surfaces with high sensitivities. It is foreseen that many of the challenges with SERS will be addressed in the near future as SERS is currently a very active area of research.

4.3. Surface plasmon resonance detection

Like SERS, surface plasmon resonance (SPR) detection is a near-field optical detection method. The technique uses the surface plasmon effect which occurs when polarized laser light at a specific angle of incidence (the surface plasmon angle) is reflected off a thin noble metal layer (e.g. gold) resulting in an absorbance at the SPR angle. Affinity molecules like antibodies or receptors are bound to the noble metal layer which is brought in contact with the sample solution. As ligand molecules bind to the receptors or antibodies, the refractive index near the metal layer is changed resulting in a change of the SPR angle. The change in SPR angle is a measure for the amount of ligand bound [65,66]. SPR lends itself well for LOC detection as the noble metal layers can be fabricated efficiently in LOCs and SPR is an optical detection technique which like SERS is insensitive to downscaling.

4.4. Mass spectrometry

Mass spectrometry (MS) has become one of the most important techniques in conventional chemical analysis. Although also used for analyte quantification, the true power of MS lies in its enormous compound identification capabilities based on the mass-to-charge ratio of analyte ions. These ions are commonly generated with the atmospheric pressure ionization technique electrospray ionization (ESI). In conventional ESI the analyte containing fluid is passed through a sharp capillary needle. A high voltage is applied to the needle tip causing fine spray of charged droplets to be emitted. As the solvent evaporates, the resultant ions are fed into the mass spectrometer.

Electrospray lends itself very well for interfacing LOCs with mass spectrometry. Commonly, the spray needle is monolithically integrated into the LOC as is the case with the Agilent LC–MS Chip shown in Fig. 15. As opposed to many techniques that suffer from downscaling, the ionization process is dramatically improved by downscaling electrospray to LOC dimension, resulting in more reproducible data, a better SNR, and a more linear detection response [67,68].

It is expected that the use of MS as a detection method for LOCs will become more widespread, as it is such a powerful identification technique and has excellent sensitivity for flow rates commonly encountered in LOCs.

4.5. Electrical detection

A number of different electrical detection methods are used in LOCs including conductivity detection, electrical impedance spectroscopy (EIS) and electrochemical detection (ECD). All these methods have the advantage that they use relatively simple electronic

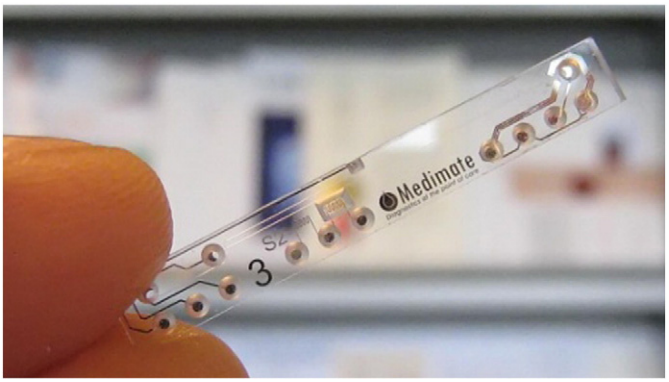

Fig. 15. Example of a commercially-available, glass LOC device for clinical applications. This commercial LOC rapidly determines plasma lithium concentration for bipolar patients by performing all necessary laboratory steps starting from whole blood.

detection setups, and that the electrical signals are easily controlled and measured with digital acquisition systems and computers.

Conductivity detection measures changes in the electrical conductivity due to changes in the composition of the liquid flowing through the detection setup. Commonly detection is done with high frequency (up to hundreds of kHz) AC signals applied between microelectrodes. Using conductivity detection the concentration of ions can be determined as is done in the LOC for plasma lithium concentration determination shown in Fig. 15.

Another electrical detection method, widely used for on-chip cytometry, is electrical impedance spectroscopy. Individual cells are exposed to a high-frequency electric field between two electrodes in a flow-through cell. As different cells influence the electric field according to their individual size and dielectric properties, the resultant changes in the electric signal can be used to identify different types of cells [69].

Electrochemical detection measures current generated by the electrochemical conversion of chemical species on the surface of integrated electrodes. Depending on the molecular properties of the compounds, different potentials are needed to induce the electrochemical reactions. The required potential is compound specific and may be used for compound identification. Although not very information rich, and not suitable for all molecules, ECD has a very high sensitivity that is independent of downscaling. The sensitivity of ECD was clearly demonstrated by the detection of the exocytosis of single histamine-containing vesicles from single mast cells [70].

4.6. Surface-acoustic wave detection

Surface-acoustic wave (SAW) detection uses the changes in surface propagation of acoustic waves as they propagate along the surface of on-chip detection regions [71]. Commonly, the surface of the detection region is modified with an affinity layer containing antibodies or receptors which bind to their respective substrates. Their added mass causes a change in the propagation of the acoustic surface waves. The change of the acoustic signal is a measure for substrate binding and can be used to selectively determine the concentration of a specific molecule in a sample mixture.

4.7. Cantilever detection

Cantilever detection uses micrometer-thick cantilever structures inside LOCs. One side of the cantilever is coated with a layer of affinity molecules like antibodies or receptors capable of specifically recognizing a molecular species in an analyte mixture. As the molecule of interest binds to the affinity layer on the cantilever a surface stress is developed which results in bending of the very thin cantilever structure. The amount of bending is a measure of the concentration of the analyte of interest and is measured optically by a laser beam or by integrated electrical sensors which respond to mechanical stress (piezoresistors) [72].

5. Massive-parallel (bio) analytical chemistry in LOC devices

In the preceding portion of the review we have covered a wide range of important subjects for LOC systems. We have introduced the basic physical phenomena and operation and detection principles of some of the major LOC platforms. It is again noted that it is not our aim to give an exhaustive review of all available techniques, but to give insights into the current possibilities and promise of the rapidly evolving field of microfluidics and to allow the reader to judge its impact on their own field. The final part of this review will address the question of how these LOC techniques are likely to impact the life sciences in the immediate and near future. These techniques are likely to affect the life sciences in three ways, namely by enhancing research on small samples or small biological systems, by greatly increasing throughput at reduced cost, and by enabling a much higher degree of hyphenation, to allow much more comprehensive measurements. We will discuss this in some detail, but future development will show which techniques will make it to widely used LOC techniques and can truly alter the way life sciences are performed.

5.1. Small samples

The most obvious result of miniaturizing analytical systems is their improved ability to work with minute sample volumes and very small biological systems. For example, the field of single-cell analysis is very complicated when using conventional techniques. The inherent size difference between the tools used to perform experiments and the studied cells can make seemingly simple operations tedious. This size discrepancy can also lead to a high degree of dilution, making detection difficult. Using conventional pipetting techniques, the minimum volumes that can be used accurately are normally in the μL range. When one cell is lysed in 1 μL of buffer, its contents are diluted in excess of 8 orders of magnitude, making even the most abundant analytes difficult to detect. Using LOCs, individual cells can be cultured in such small volumes that their contents or even the compounds they secrete will be kept in measurable concentrations. Additionally, it is possible to allow for communication between neighboring cells by having adjacent culture chambers in contact with each other, more closely resembling their natural environment.

The ability to work with such small biological systems will lead to unique insights into how single cells function and perform signaling in their native tissues (which may include cancerous tissues, brain tissues, organ tissues, etc.). In this and other ways it is expected that LOCs will deliver important contributions to genomics, transcriptomics, proteomics and metabolomics. With the advent of systems biology, LOC systems will probably have a strong impact in this important area of research as the functioning of organisms, organs, tissues and single cells can be studied in high resolution from the bottom up instead of the top down approaches used now.

5.2. High throughput

A second important impact these techniques will have is in the throughput of experiments and analyses. As LOC devices lead to less labor intensive, faster analyses, while consuming less chemicals and laboratory space, they can be scaled out in a massive-parallel fashion to increase the throughput by several orders of magnitude. The increase in throughput has a number of effects. First of all, experiments can be performed at lower cost. This economic aspect is further accentuated by the fact that LOC platforms use less sample, reagent and eluents. This will, for instance, also allow the performance

of large numbers of experiments which require very expensive chemicals. Experiments with dangerous chemicals like radioactive materials pose less of a risk allowing these experiments to be routinely performed.

Furthermore, a high degree of automation can be incorporated in LOCs. Similar to the 'Tyranny of Numbers', there is a practical limit to the number of pipetting steps a reasonable experiment can contain (i.e. the 'Tyranny of Pipetting'). This limit can be stretched by automated pipetting, but even then experimental errors and reagent consumption increase with the number of pipetting steps. Monolithically-integrated LOCs can alleviate this problem with intricate, multiplexed networks of channels that allow for the filling of hundreds of channels or reaction chambers with a sample from one common well. One pipetting step can thus replace hundreds of individual pipetting steps. Not only can one reagent be introduced in such a manner, but the network of channels can also be designed to generate gradients of mixtures or serial dilutions allowing for the rapid screening of a huge range of experimental conditions with only a few manual steps. Afterwards it is also possible to simultaneously perform many parallel operations like metering, pumping and mixing using only one controller. These aspects might be some of the most appealing features of LOC platforms as they reduce menial labor and increase productivity.

Performing large numbers of experiments in parallel would also ensure low experimental variability and thus higher data quality, addressing one of the great hurdles of large scale —omics studies.

An important corollary of the increase in throughput is that processing and evaluation of the data generated is most likely to become a bottle neck, as is already occurring with high resolution MS–MS data or DNA sequencing. When LOCs are used with a data-intensive detection technique like high-resolution mass spectrometry, the data bottleneck will become especially prominent.

Of course, if the data bottleneck challenges are alleviated as computing power is becoming exponentially cheaper and dedicated, high-quality analysis software is developed, the full benefits of LOC systems for the life sciences can be reaped. For instance chemical compound libraries may be screened at much higher rates at a much reduced cost, which is very important for lead compound screening in the pharmaceutical drug development process. Other important areas include (personal) genetic screening, the –omics sciences, and the screening of large patient cohorts.

5.3. Multiple hyphenation

As sample consumption, dead volumes, dispersion and connections can be greatly reduced by using integrated LOCs, it becomes possible to integrate multiple separation and detection techniques into one device. The value of hyphenating several techniques can already be seen in the field of mass spectrometry, where systems consisting of several chromatographic separations, a UV detector and multiple mass spectrometers are widely applied. The use of multiple separation and detection methods on one sample can significantly increase the coverage of an analytical system. Analytes that co-elute in one separation dimension will be separated on the second dimension and multiple detection methods will ensure that most compounds will be detected by at least one of the techniques.

Although not yet realized, this hyphenation could be put to extremes in LOC systems. A single sample could be split up and processed with different separation techniques, or multiple separation techniques can be performed sequentially in a single device. Afterwards a sample can be passed through several detectors finishing with a destructive technique like MS. It is thus not difficult to imagine extremely hyphenated techniques like LC–CE–SERS–LIF–EIS–MS (sic!). Gathering all this data in one experiment is preferable, not only because it is less labor intensive and consumes less sample, but also because it avoids inter-experimental variation.

This concept can be extrapolated even further to include a high degree of quality control in LOCs. Because of the method of manufacturing LOCs, it is not more difficult to integrate multiple electrodes in an LOC than it is to integrate one. One could thus integrate simple sensors to monitor for example, the temperature, pH, ion strength and other experimental conditions. Recording and potentially regulating all these experimental variables would allow for a large degree of control of the experimental conditions and thus significantly higher data quality.

It is envisioned that a new data format would be needed, to include all the experimental conditions and results, to allow for better comparison of results between experiments and especially between different laboratories. Such a standard, comprehensive data format would greatly increase the possibilities for meta-analyses of results of different studies, yielding large scale information. Especially in the —omics sciences, such analyses could be extremely useful to give insight into networks and pathways.

6. Conclusions

In this review we have introduced the basic concepts in microfluidics including the associated physics, the different microfluidic platforms, and the different detection techniques. Furthermore, we have discussed the concept of massive-parallel microfluidics, the potential for multiple hyphenations, and the opportunity for chemometrics. We hope that by reading this review, readers have gained a basic working knowledge on microfluidic systems, which may allow them to judge the impact on their own respective fields and potentially select the most suitable microfluidic technology for their endeavors.

Due to the wide range of physical phenomena available for fluid propulsion on the small scale, very different microfluidic platforms are available (e.g. electrokinetic, pressure driven and centrifugal platforms). Thus, specific platforms have different properties more suitable for certain applications. For example, digital microfluidics is very well suited for rapidly testing high numbers of different experimental conditions, but intrinsically does not allow for compound separation as is inherently possible in electrokinetic systems.

On the detection side, an even more impressive array of choices is available as the detectors may use the optical, chemical, mechanical, or electromagnetic domain. In addition it is possible to incorporate many detectors into one microfluidic device to achieve a multi-hyphenated device. As opposed to on the macroscale, monolithic integration can largely remove dead volumes and leakage-prone connections, allowing in-line coupling of multiple detectors without significant loss of performance. LOCs with multi-hyphenated detection could be very useful as they allow much more comprehensive screening of chemical samples. Especially the —omics sciences, which often process very complex samples, could benefit from such comprehensive screening methods to increase analyte coverage.

In addition to the inherent short run times seen in microfluidics, a significant benefit of microfluidic systems is their potential for performing many analyses of small volumes in parallel. Such massive parallel operation is very interesting for the life sciences as both sample throughput and reproducibility are drastically increased due to the parallel operation.

The massive amount of data generated by such techniques, does not only pose a challenge for chemometrics, but the increases in data quality and data coverage also offers huge opportunities for benefiting the life sciences with very reliable, comprehensive information.

Abbreviations

CE	Capillary electrophoresis
ECD	Electrochemical detection
EIS	Electrical impedance spectroscopy
EOF	Electro-osmotic flow

ESI	Electrospray ionization
LIF	Laser-induced fluorescence
LOC	Lab-on-a-chip
MS	Mass spectrometry
PDMS	Poly-dimethyl siloxane
SAW	Surface acoustic wave
SERS	Surface enhanced Raman spectroscopy
SNR	Signal-to-noise ratio
SPR	Surface plasmon resonance
SVR	Surface-to-volume ratio

Acknowledgments

This project was (co)financed by the Netherlands Metabolomics Centre (NMC) which is part of the Netherlands Genomics Initiative/ Netherlands Organization for Scientific Research.

References

[1] S.C. Terry, J.H. Jerman, J.B. Angell, A gas chromatographic air analyzer fabricated on a silicon wafer, IEEE Trans. Electron Devices 26 (1979) 1880–1886.
[2] ISI Web of Knowledge, Thomson Reuters, search results for "topic=(microfluidic*) or topic=(microtas) or topic=("micro total analysis system*") or topic=("lab on a chip") or topic=("lab on chip") or topic=(lab-on-a-chip) or topic=(lab-on-chip)" accessed on 21/02/2011.
[3] S.R. Quake, http://www.stanford.edu/group/foundry/pricing%20information.html,accessed on 10/10/2010.
[4] http://www.aztex.biz/general-computer/integrated-circuit-work/," "accessed on 10/11/2010.
[5] T. Thorsen, S.J. Maerkl, S.R. Quake, Microfluidic large-scale integration, Science 298 (2002) 580–584.
[6] T.M. Squires, S.R. Quake, Microfluidics: fluid physics at the nanoliter scale, Rev. Mod. Phys. 77 (2005) 977–1026.
[7] E.M. Purcell, Life at low Reynolds number, Am. J. Phys. 45 (1977).
[8] T.M. Keenan, A. Folch, Biomolecular gradients in cell culture systems, Lab Chip 8 (2008) 34–57.
[9] A.D. Stroock, S.K.W. Dertinger, A. Ajdari, I. Mezic, H.A. Stone, G.M. Whitesides, Chaotic mixer for microchannels, Science 295 (2002) 647–651.
[10] D.J. Beebe, G.A. Mensing, G.M. Walker, Physics and applications of microfluidics in biology, Annu. Rev. Biomed. Eng. 4 (2002) 261–286.
[11] S.R.H.L. Kim, S.J. Lee, Experimental study on the flow characteristics of sinusoidal nozzle jet, J. Korean Vis. 7 (2010) 28–34.
[12] A. Folch, LabART, http://faculty.washington.edu/afolch/FolchLabART.html, accessed on 12/9/2010.
[13] P. Yager, T. Edwards, E. Fu, K. Helton, K. Nelson, M.R. Tam, B.H. Weigl, Microfluidic diagnostic technologies for global public health, Nature 442 (2006) 412–418.
[14] T.H. Schulte, R.L. Bardell, B.H. Weigl, Microfluidic technologies in clinical diagnostics, Clin. Chim. Acta 321 (2002) 1–10.
[15] J.R. Kraly, R.E. Holcomb, Q. Guan, C.S. Henry, Review: microfluidic applications in metabolomics and metabolic profiling, Anal. Chim. Acta 653 (2009) 23–35.
[16] D. Mark, S. Haeberle, G. Roth, F.v. Stetten, R. Zengerle, Microfluidic lab-on-a-chip platforms: requirements, characteristics and applications, Chem. Soc. Rev. 39 (2010) 1153–1182.
[17] K.B. Mogensen, H. Klank, J.P. Kutter, Recent developments in detection for microfluidic systems, Electrophoresis 25 (2004) 3498–3512.
[18] J. Voldman, M.L. Gray, M.A. Schmidt, Microfabrication in biology and medicine, Annu. Rev. Biomed. Eng. 1 (1999) 401–425.
[19] S. Haeberle, R. Zengerle, Microfluidic platforms for lab-on-a-chip applications, Lab Chip 7 (2007) 1094–1110.
[20] N. Lion, T.C. Rohner, L. Dayon, I.L. Arnaud, E. Damoc, N. Youhnovski, Z.Y. Wu, C. Roussel, J. Josserand, H. Jensen, J.S. Rossier, M. Przybylski, H.H. Girault, Microfluidic systems in proteomics, Electrophoresis 24 (2003) 3533–3562.
[21] P.S. Dittrich, A. Manz, Lab-on-a-chip: microfluidics in drug discovery, Nat. Rev. Drug Discov. 5 (2006) 210–218.
[22] H. Becker, L.E. Locascio, Polymer microfluidic devices, Talanta 56 (2002) 267–287.
[23] G.M. Whitesides, The origins and the future of microfluidics, Nature 442 (2006) 368–373.
[24] N. Nguyen, H. XIAOYANG, C. TOH KOK, Mems-micropumps: a review: pump analysis and design, J. Fluids Eng. 124 (2002) 384–392.
[25] D.J. Laser, J.G. Santiago, A review of micropumps, J. Micromech. Microeng. 14 (2004) R35.
[26] W.O. Kwang, H.A. Chong, A review of microvalves, J. Micromech. Microeng. 16 (2006) R13.
[27] M.A. Unger, H.-P. Chou, T. Thorsen, A. Scherer, S.R. Quake, Monolithic microfabricated valves and pumps by multilayer soft lithography, Science 288 (2000) 113–116.
[28] D.C. Duffy, J.C. McDonald, O.J.A. Schueller, G.M. Whitesides, Rapid prototyping of microfluidic systems in poly(dimethylsiloxane), Anal. Chem. 70 (1998) 4974–4984.
[29] S.K. Sia, G.M. Whitesides, Microfluidic devices fabricated in poly(dimethylsiloxane) for biological studies, Electrophoresis 24 (2003) 3563–3576.
[30] J.C. McDonald, G.M. Whitesides, Poly(dimethylsiloxane) as a material for fabricating microfluidic devices, Acc. Chem. Res. 35 (2002) 491–499.
[31] J.C. McDonald, D.C. Duffy, J.R. Anderson, D.T. Chiu, H. Wu, O.J.A. Schueller, G.M. Whitesides, Fabrication of microfluidic systems in poly(dimethylsiloxane), Electrophoresis 21 (2000) 27–40.
[32] www.fluidigm.com,accessed on 08/10/2010.
[33] www.agilent.com,accessed on 10/09/2010.
[34] A. Manz, D.J. Harrison, E.M.J. Verpoorte, J.C. Fettinger, A. Paulus, H. Lüdi, H.M. Widmer, Planar chips technology for miniaturization and integration of separation techniques into monitoring systems: capillary electrophoresis on a chip, J. Chromatogr. 593 (1992) 253–258.
[35] D.J. Harrison, A. Manz, Z. Fan, H. Luedi, H.M. Widmer, Capillary electrophoresis and sample injection systems integrated on a planar glass chip, Anal. Chem. 64 (1992) 1926–1932.
[36] C.S. Effenhauser, A. Manz, H.M. Widmer, Manipulation of sample fractions on a capillary electrophoresis chip, Anal. Chem. 67 (1995) 2284–2287.
[37] D.C. Harris, Quantitative chemical analysis, 5th EditionW.H. Freeman and Company, New York, 1998.
[38] B. Jung, R. Bharadwaj, J.G. Santiago, On-chip million fold sample stacking using transient isotachophoresis, Anal. Chem. 78 (2006) 2319–2327.
[39] A.E. Herr, J.I. Molho, K.A. Drouvalakis, J.C. Mikkelsen, P.J. Utz, J.G. Santiago, T.W. Kenny, On-chip coupling of isoelectric focusing and free solution electrophoresis for multidimensional separations, Anal. Chem. 75 (2003) 1180–1187.
[40] K.G.H Janssen, J. Li, H.T. Hoang, R.J.B.H.N. v.d. Berg, H.S. Overkleeft, J.C.T. Eijkel, N.R. Tas, H.J. v.d. Linden, T. Hankemeier, Nanofluidic Isotachophoresis for attomole separations of amino acids in sub-picoliter volumes (in preparation).
[41] K. Dheda, Validation of housekeeping genes for normalizing RNA expression in real-time PCR, Biotechniques 37 (2004) 112.
[42] N.J. Panaro, P.K. Yuen, T. Sakazume, P. Fortina, L.J. Kricka, P. Wilding, Evaluation of DNA fragment sizing and quantification by the Agilent 2100 bioanalyzer, Clin. Chem. 46 (2000) 1851–1853.
[43] L. Bousse, S. Mouradian, A. Minalla, H. Yee, K. Williams, R. Dubrow, Protein sizing on a microchip, Anal. Chem. 73 (2001) 1207–1212.
[44] P. Tobias, L. Gerd, D.H.C. Samuel, N.W. Benjamin, D. Robert, B. Carsten, Detection of cellular parameters using a microfluidic chip-based system, J. Assoc. Lab. Automation 7 (2002) 85–89.
[45] B.M. Paegel, C.A. Emrich, G.J. Wedemayer, J.R. Scherer, R.A. Mathies, High throughput DNA sequencing with a microfabricated 96-lane capillary array electrophoresis bioprocessor, PNAS 99 (2002) 574–579.
[46] J.H. Aborn, S.A. El-Difrawy, M. Novotny, E.A. Gismondi, R. Lam, P. Matsudaira, B.K. Mckenna, T. O'Neil, P. Streechon, D.J. Ehrlich, A 768-lane microfabricated system for high-throughput DNA sequencing, Lab Chip 5 (2005) 669–674.
[47] C.A. Emrich, H. Tian, I.L. Medintz, R.A. Mathies, Microfabricated 384-lane capillary array electrophoresis bioanalyzer for ultrahigh-throughput genetic analysis, Anal. Chem. 74 (2002) 5076–5083.
[48] http://www.gyros.com, accessed on 11/10/10.
[49] M. Madou, J. Zoval, G. Jia, H. Kido, J. Kim, N. Kim, Lab on a CD, Annu. Rev. Biomed. Eng. 8 (2006) 601–628.
[50] S.-Y. Teh, R. Lin, L.-H. Hung, A.P. Lee, Droplet microfluidics, Lab Chip 8 (2008) 198–220.
[51] A. Huebner, S. Sharma, M. Srisa-Art, F. Hollfelder, J.B. Edel, A.J. deMello, microdroplets: a sea of applications? Lab Chip 8 (2008) 1244–1254.
[52] H. Song, D.L. Chen, R.F. Ismagilov, Reactions in droplets in microfluidic channels, Angew. Chem. Int. Ed. 45 (2006) 7336–7356.
[53] H. Song, J.D. Tice, R.F. Ismagilov, A microfluidic system for controlling reaction networks in time, Angew. Chem. 115 (2003) 792–796.
[54] A. Günther, M. Jhunjhunwala, M. Thalmann, M.A. Schmidt, K.F. Jensen, Micromixing of miscible liquids in segmented gas — liquid flow, Langmuir 21 (2005) 1547–1555.
[55] W. Shi, J. Qin, N. Ye, B. Lin, Droplet-based microfluidic system for individual caenorhabditis elegans assay, Lab Chip 8 (2008) 1432–1435.
[56] X. Niu, M. Zhang, S. Peng, W. Wen, P. Sheng, Real-time detection, control, and sorting of microfluidic droplets, Biomicrofluidics 1 (2007) 044101–044112.
[57] J.-C. Baret, O.J. Miller, V. Taly, M. Ryckelynck, A. El-Harrak, L. Frenz, C. Rick, M.L. Samuels, J.B. Hutchison, J.J. Agresti, D.R. Link, D.A. Weitz, A.D. Griffiths, Fluorescence-activated droplet sorting (FADS): efficient microfluidic cell sorting based on enzymatic activity, Lab Chip 9 (2009) 1850–1858.
[58] W. Liu, D. Chen, W. Du, K.P. Nichols, R.F. Ismagilov, Slipchip for immunoassays in nanoliter volumes, Anal. Chem. 82 (2010) 3276–3282.
[59] F. Shen, W. Du, E.K. Davydova, M.A. Karymov, J. Pandey, R.F. Ismagilov, Nanoliter multiplex PCR arrays on a slipchip, Anal. Chem. 82 (2010) 4606–4612.
[60] F. Shen, W. Du, J.E. Kreutz, A. Fok, R.F. Ismagilov, Digital PCR on a SlipChip, Lab Chip 10 (2010) 2666–2672.
[61] J.C. Gertsch, S.D. Noblitt, D.M. Cropek, C.S. Henry, Rapid analysis of perchlorate in drinking water at parts per billion levels using microchip electrophoresis, Anal. Chem. 82 (2010) 3426–3429.
[62] W. Du, L. Li, K.P. Nichols, R.F. Ismagilov, Slipchip, Lab Chip 9 (2009) 2286–2292.
[63] K. Kneipp, Y. Wang, H. Kneipp, L.T. Perelman, I. Itzkan, R. Dasari, M.S. Feld, Single molecule detection using surface-enhanced Raman scattering (SERS), Phys. Rev. Lett. 78 (1997) 1667–1670.
[64] S.M. Nie, S.R. Emery, Probing single molecules and single nanoparticles by surface-enhanced Raman scattering, Science 275 (1997) 1102–1106.
[65] J. Homola, S.S. Yee, G. Gauglitz, Surface plasmon resonance sensors: review, Sens. Act. B Chem. 54 (1999) 3–15.

[66] J. Homola, Present and future of surface plasmon resonance biosensors, Anal. Bioanal. Chem. 377 (2003) 528–539.
[67] A. Schmidt, M. Karas, T. Dülcks, Effect of different solution flow rates on analyte ion signals in nano-ESI MS, or: When does ESI turn into nano-ESI? J. Am. Soc. Mass Spectrom. 14 (2003) 492–500.
[68] E.T. Gangl, M. Annan, N. Spooner, P. Vouros, Reduction of signal suppression effects in ESI-MS using a nanosplitting device, Anal. Chem. 73 (2001) 5635–5644.
[69] T. Sun, S. Gawad, C. Bernabini, N.G. Green, H. Morgan, Broadband single cell impedance spectroscopy using maximum length sequences: theoretical analysis and practical considerations, Meas. Sci. Technol. 18 (2007) 2859.
[70] A. Albillos, G. Dernick, H. Horstmann, W. Almers, G.A. de Toledo, M. Lindau, The exocytotic event in chromaffin cells revealed by patch amperometry, Nature 389 (1997) 509–512.
[71] K. Länge, B. Rapp, M. Rapp, Surface acoustic wave biosensors: a review, Anal. Bioanal. Chem. 391 (2008) 1509–1519.
[72] A. Gupta, D. Akin, R. Bashir, Single virus particle mass detection using microresonators with nanoscale thickness, Appl. Phys. Lett. 84 (2004) 1976–1978.