University of Stavanger

‘Lab on a Chip’ Systems for Environmental Analysis

Rhituparna Paul Payel

Faculty of Science and Technology

MSc Thesis – August 2014

University of
Stavanger

Faculty of Science and Technology

MASTER'S THESIS

Study program/ Specialization: Masters in Environmental Technology/ Offshore Environmental Engineering	Spring semester,2014 √ Open / Restricted access
Writer: Rhituparna Paul Payel	.... (Writer's signature)
Faculty supervisor: Kåre B. Jørgensen External supervisor(s):	
Thesis title: 'Lab on a Chip' Systems for Environmental Analysis	
Credits (ECTS): 30	
Key words: Microfluidic LOC Lab On a Chip Environmental Analysis Microfluidic Systems	Pages: 52 + enclosure: NA Stavanger, 14/08/2014 Date/year

Acknowledgement

I would like to express my special appreciation and thanks to my Supervisor Dr. Kåre B. Jørgensen, you have been a tremendous mentor for me. I would like to thank you for encouraging my work throughout the whole period and for the guidance and support to give this thesis work a complete frame. Also thank you for making me a part of this thesis.

Last but not the least I would like to thank my family and friends, especially my husband for encouraging and supporting me throughout the process and for always being there for me.

Stavanger, August 2014 Rhituparna Paul Payel

Abstract

'Lab on a chip' refers to devices and methods for controlling and manipulating fluid flows at micro levels. These microfluidic devices used for manipulating and controlling fluids are widespread now, and are used in many scientific and industrial contexts. 'Lab on a chip' fabrication requires different geometries than the laboratory processes. It also depends on the interplays of multiple physical effects such as pressure gradients, electrokinetics, capillarity force etc. This thesis provides an overview of different techniques of flows in microdevices with focus on electrokinetics, mixing and dispersion, different microfluidic components, injection techniques, different sensing processes. It also focused here about the existing 'lab on chip' applications in industry and its future prospects. Lab on a chip system is growing very fast, replacing laboratory procedures and man work. It not only synthesizes chemicals efficiently but also carries out biological and chemical analyses. It has developed its applications in modern chemistry, clinical chemistry, fabrication, engineering and materials science, biology, physics, electronics, aiding communication and collaboration across disciplines. Most biological applications on lab on a chip are already commercialized in global market whereas for environmental chemistry, the field is still developing. Some established applications of lab on a chip in environmental chemistry and life science are discussed here.

Table of Contents

Title Page II
Acknowledgement III
Abstract IV
Abbreviations VI
1. Introduction 1
1.1 Motivations for lab on chip 1
1.2 'Lab on a chip' at present 2
2. Techniques and Components 3
2.1 Chemical Sensing Techniques 3
2.1.1 Spectrometry 3
2.1.2 Luminescence 6
2.1.3 Fluorescence 7
2.1.4 Capillary electrophoresis 8
2.1.5 Electrochemical 9
2.1.6 Laser 9
2.1.7 Mass Spectrometry 10
2.2 Components 11
2.2.1 Injectors 11
2.2.2 Transporters 12
2.2.3 Preparators 13
2.2.4 Mixers 15
2.2.5 Reactors 17
2.2.6 Separators 18
2.2.7 Controllers 19
2.2.8 Power Supply 20
3. Existing Applications of LOC 21
3.1 Application in Environmental Chemistry 21
3.2 Applications in Life Science 26
4. Adoption to new technologies and general issues to adoption 36
5. Conclusion 38
6. Expected Future Developments 38
References 39

Abbreviations

B.L	Bioluminescence
BM	Bone Marrow
CB	Cord Blood
CCD	Charged Coupled Device
CE	Capillary Electrophoresis
C.L	Chemiluminenscence
CMOS	Complementary Metal-Oxide Semiconductor
CV	Cyclic Voltammetry
DARPA	Defense Advanced Research Projects Agency
DEP	Di-Electrophoresis
DNA	Deoxyribonucleic acid
EMV	Electromagnetic valve
ESI-MS	Electro Spray Ionization Mass Spectrometry
FBB	Microfluidic BreadBoard
GPC	Gas-Phase Chromatography
HLA	Human Leukocyte Antigen
HPLC	High-Pressure Liquid Chromatography
LIBS	Laser Induced Breakdown Spectrometry
LASS	Laser Spark Spectrometry
LOC	Lab on a Chip
LOD	Limit of Detection
ME	Microchip Electrophoresis
MEMS	Micro Electromechanical Systems
MGA	Microfluidic Genetic Analysis
PAN	1-(2-pyridylazo)-2-naphtholor
PB	Peripheral Blood
PDMS	Poly Dimethylsiloxane
PMMA	Poly Methyl Methacrylate
PCR	Polymerase Chain Reaction
PET	Positron Emission Tomography
PZT	Piezoelectric Transducer
RBC	Red Blood Cell
RNA	Ribonucleic acid
SPE	Solid-phase extraction
WBC	White Blood Cell

1. Introduction

Lab on a chip is the science and technology of systems that process or manipulate microscopic amounts (10^{-9} to 10^{-18} liters) of fluids. It uses channels with dimensions of tens to hundreds of micrometers. The applications of micro fluidic technologies have been in study. They offer a number of different useful capabilities like the ability to use very little amount of samples and reagents; carry out separations and detections with high sensitivity and resolution; lower cost; minimum time for analysis; and the smaller analytical devices.

1.1 Motivation for lab on chip

The field of microfluidics is based on the development of four different sectors: molecular analysis, biodefence, molecular biology and microelectronics. The origins of microfluidics lie in microanalytical methods like gas-phase chromatography (GPC), high-pressure liquid chromatography (HPLC) and capillary electrophoresis (CE). These methods combined with the capability of the laser optical detection made it possible to gain high sensitivity and high resolution using very small amounts of sample. With the ongoing successes on these microanalytical methods, it is obvious now to widen the field with new, more compact and more versatile formats for them, and their applications in chemistry and biochemistry and biology.
The second motivation for the development of microfluidic systems rose after the end of the cold war. To counter the threats from chemical and biological weapons from terrorists, the Defense Advanced Research Projects Agency (DARPA) of the US Department of Defense supported a series of different programs in the 1990s aiming at the development of field-deployable micro fluidic systems to serve as detectors for chemical and biological threats. These programs worked as the main stimulator for the rapid growth of micro fluidic technology.
The third motivation came from the molecular biology. The explosion of genomics in the 1980s was followed by the initiation of other areas of microanalysis related to molecular biology like DNA sequencing. These required analytical methods with higher sensitivity and resolution and much greater throughput than had been contemplated in biology earlier. Micro fluidics approached to overcome these issues.
The fourth motivation was microelectronics. The main optimism of micro fluidics was photolithography and the associated technologies. These implementations had been so successful in silicon microelectronics and in micro electromechanical systems (MEMS). It would be directly applicable to micro fluidics. Some of the earliest work in fluidic microsystems used silicon and glass, but now-a-days, these materials have largely been replaced by plastics. For analyses of biological and chemical samples in water, glass and silicon fabricated devices are usually inappropriate. Silicon is expensive and difficult for visibility. For detection, ultraviolet light cannot be used because of this invisibility along with conventional optical methods. It is

easier to construct the components required for micro analytical systems in elastomers like PDMS (Poly Dimethylsiloxane)than in rigid materials[1].

1.2 'Lab on a chip' at present

At present, lab on a chip, together with new methods of fabrication has been able to utilize certain fundamental differences between the physical properties of fluids moving in large channels and those travelling though micrometer-scale channels [2-4]. There are now different enough methods of fabrication of 'lab on a chip' and a sufficient range of components which makes it possible to begin to apply lab on a chip systems to the resolution of problems. The most developed applications are screen conditioning like pH, ionic strength, compositions and concentrations. Cell biology and chemical synthesis; these two fields are the most common fields for lab on a chip applications. Cell biology entered a new phase with lab on a chip development. Jensen et al.[5] described different new tools useful for cell biologists. In chemical synthesis, lab on a chip technology fits naturally. But this adaption seems to be bit slow because of the flexibility of conventional apparatus and PDMS dissolves in many organic solvents [6]. The use of glass or silicon in the place of PDMS can resolve this issue but the fabrication of lab on chip with any of these materials are more difficult and challenging than that with PDMS.

2. Techniques and Components

2.1 Chemical Sensing Techniques

This section describes about different detection techniques used for 'Lab on a chip' analysis. These include spectrometry, luminescence, fluorescence, chromatography, electrochemical, mass spectrometry, laser and electrophoresis.

2.1.1 Spectrometry

This is mainly the analysis of electromagnetic radiation. Every chemical element absorbs photon at a certain wavelength. The measurement of this absorption is called absorption spectrometry.

I. <u>Absorption, Transmission and Concentration</u>

There is a relation between concentration of molecule in solution and photon absorption. This relationship is described by the Beer-Lambert-Bouger Law. According to that, Equation 2.1:

$$-\frac{dP}{Pz} = \sigma NA.\,dz \qquad \text{Equation 2.1}$$

Where, dP= Power absorbed by infinitesimal slab of thickness dz for monochromatic light

Pz= Power entering the slab

NA= Avogadro's number

Ơ = Effective absorption cross section of module

The amount of light absorbed is proportional to the amount of molecules in the medium which the light passes through. So, in the medium, the concentration of a molecule is directly proportional to the amount of light absorbed. The one drawback for this law is that it is only applicable when the molecules are evenly distributed and behaves differently from another absorbing species. The graphical representation in Figure 2.1 shows deviation caused by polychromatic light in the Beer-Lambert-Bouger Law.

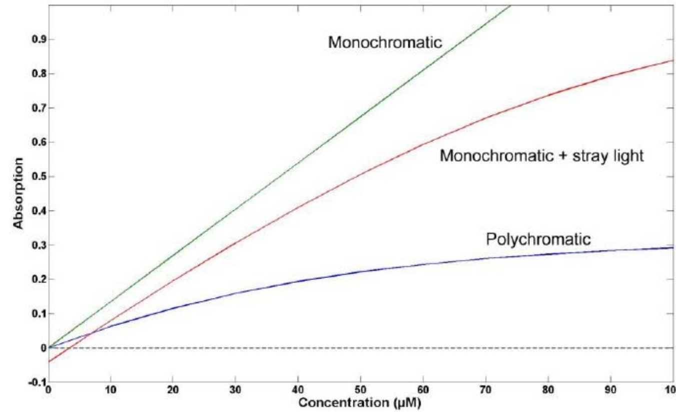


Figure 2.1: Deviation from the Beer-Lambert-Bouger law[7].

In commercial cases, the linear relationship between concentration and absorption remains valid until the absorption is greater than 3(excitation source filters used). Table 2-1 shows typical absorption performance data.

Table 2-1: Existing absorption spectrometry in lab on a chip analysis

Name	Detector material	Wavelwngth(nm)	Min. Absorption(A)
Shimadzu UV-3600	Pbs, InGaAs, PMT	185-3300	0.00003 [8]
Jasco V-670	PMT, Pbs	190-3200	0.002 [9]
DU 800	Si	190-1100	0.005 [10]
Hitachi U4100	PMT, Pbs	175-2600	0.002 [11]

II. Direct Spectrophotometry

The concentration of molecules, especially in sea water, like nitrate can be determined by calculating zero, first and second derivatives of absorption measurements. These absorption measurements are taken form UV-Vis spectrophotometry. Zero derivative gives measurement of absorption at a set wavelength whereas first derivative gives rate of change of absorption with wavelength and second derivative is the rate of change of first derivative. Figure 2.2a is a graph of absorption peak for nitrate at 210nm using the zero derivative. Both the first and second derivative are limited to nitrite detection in the solution of pH>5. This is because at more acidic environment nitrite becomes nitrous oxide and reduces the absorbance. This does not happen in sea water as sea water is alkaline. For the first derivative detection of nitrate, it is affected by the salinity of water[12] and other salts [13]. To minimize effect of other species on first derivative measurement, it is calculated using multiple wave lengths. Figure 2.2b shows nitrate having different absorption peaks at 203nm and 302nm which distorts the absorption level as spectra overlaps.

On the other hand, second derivative direct spectrometry has the efficiency of measuring nitrate containing other species as well without using any reagent. It also allows detection of nitrate and nitrite simultaneously [14, 15] as it is capable of separating those overlapped spectrums. The main disadvantage of direct spectrometry is that the derivation of spectra can amplify noise and highly dependent on the instrument condition.

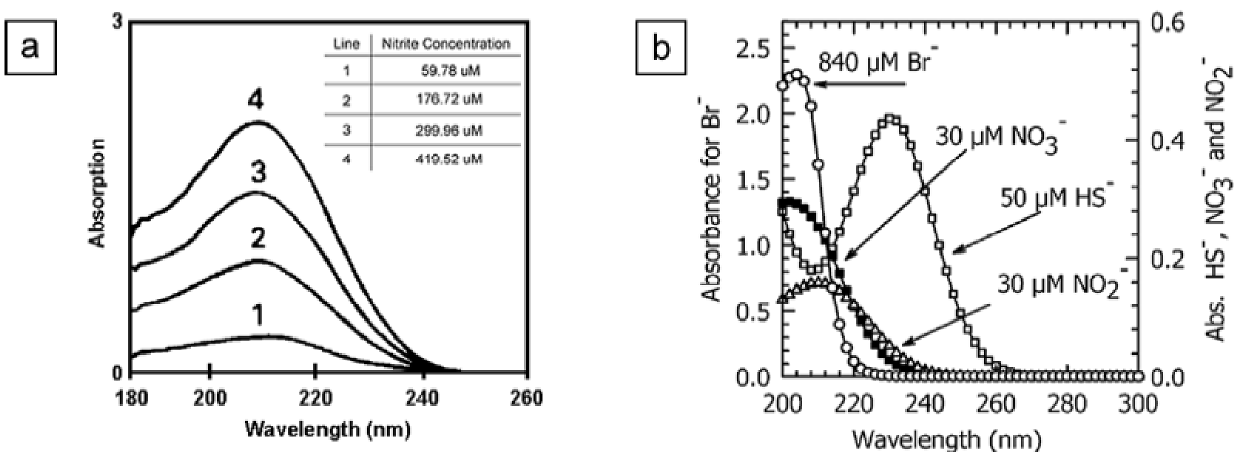
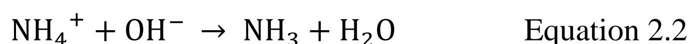


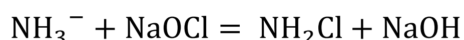
Figure 2.2: a. 210nm absorption peak for nitrate [15] b. comparison of nitrite and nitrate spectra [16].

III. Indirect(Colorimetric) Spectrophotometry

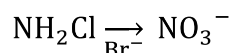
The mostly used colorimetric spectrophotometry is for Nitrite detection with Griess reaction [17]. Figure 2.3 shows the Griess reaction in which under acidic conditions nitrite reacts with the amino group of sulfanilic acid in the form of diazonium cation which is coupled to a-napthylamine [7]. Colorimetric spectrophotometry is also known as indirect spectrophotometry. Nitrate and ammonia is also detectable by indirect spectrophotometry using greiss reaction. Before detection, both of them have to be reduced to nitrite. Equation 2.2 illustrates that NaOCl transforms ${NH_3}^-$ to monochloramine (at pH>7.5) and in presence of bromide, this mono chloramine is reduced to nitrite. For nitrate, the reduction of nitrate to nitrite is done by cadmium. Equation 2.3 shows the reaction of nitrate with cadmium column.



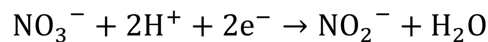
$${NH_4}^+ + OH^- \rightarrow NH_3 + H_2O \qquad \text{Equation 2.2}$$



$${NH_3}^- + NaOCl = NH_2Cl + NaOH$$



$$NH_2Cl \underset{Br^-}{\longrightarrow} {NO_3}^-$$



$$NO_3^- + 2H^+ + 2e^- \rightarrow NO_2^- + H_2O$$ Equation 2.3

$$Cd \rightarrow Cd^{2+} 2e^-$$

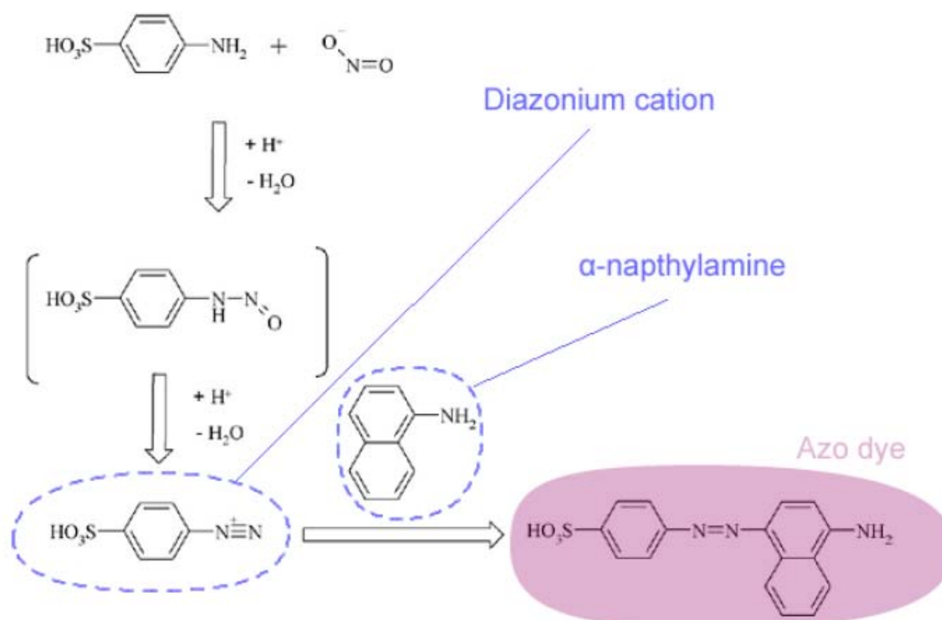


Figure 2.3: The Griess Reaction which shows under acidic condition, nitrite reacts with the amino group of sulfanilic acid to form Azo dye [18].

2.1.2 Luminescence

There are two most commonly used luminescence methods: Bioluminescence(B.L) and Chemiluminescence(C.L). B.L uses natural luminescence created in a living organism when chemical energy is converted to light energy [19, 20]. C.L uses luminescence from chemical or biochemical reaction. Figure 2.4 shows an overview of the mechanism leading to C.L. Here the reaction produces sufficient energy to excite an electron. This electron then decays to a lower energy state and emits a photon. C.L method is used for the detection of nitrite and nitrate in sea water[21]; but it can also be used to detect other elements or compounds like hydrogen peroxide, ammonium, chlorine, oxygen, copper, phosphate and sulphites. Very few chemical reactions can produce C.L. Because of limited selectivity of reagents, large sample volume, low measurement frequency, C.L method is not a commonly used method for in situ sensors.

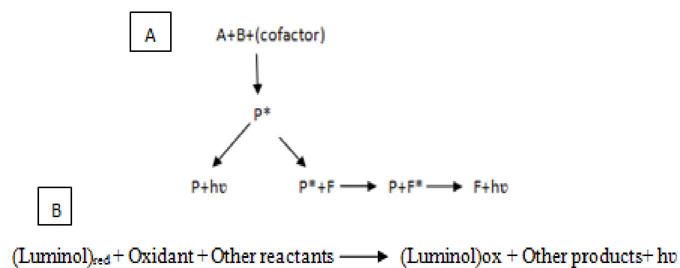


Figure 2.4: A. Mechanism of chemiluminescent reaction where A=substrate, B= Oxidant, P=Product, P*=Excited product, F= Flurophore, F*= Excited flurophor, hυ= radiation emission. B. General format of Luminol C.L reaction[19].

2.1.3 Fluorescence

Optical detection is considered as the most common detection method in lab-on-a-chip devices. Its integration simplicity has made this technique most popular. Additionally, light distribution on the chip enables integration of multiple individual detectors on a single chip [22]. In case of fluorescence detection, cells (labeled with fluorescent dye) emit light once they are excited with a light source (laser) [23, 24]. One main drawback for this is dye emission light usually gets overwhelmed by the excitation light in fluorescence detection. A band pass filter is used to overcome this [25, 26]. Klotzkin and Papautsky [27] proposed alternate solution with integration of a polarizer to isolate excitation light from the detector. Figure 2.5, Figure 2.6 shows techniques for fluorescence and polarizer analysis Banerjee described cross polarization strategy which is used with organic photodiode detectors in a fluorescence-based lab-on-a-chip [28] which is currently in use. Absorption spectroscopy [29, 30] is also widely used in optical detection due to its label-free environment. The intensity of a beam of light is measured before and after interaction with the analyte according to this technique. Absorption detection shows a lower sensitivity resulting in reduced limit of detection (LOD).

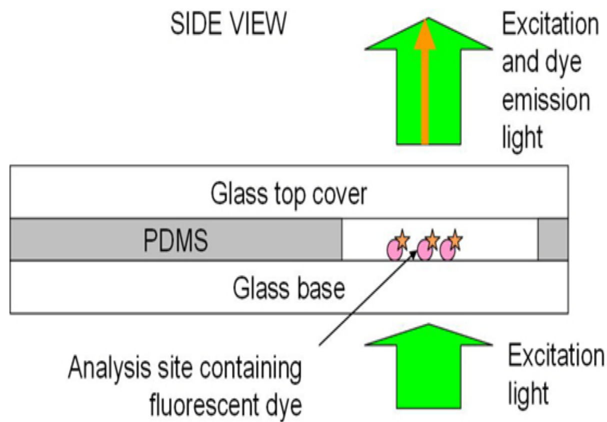


Figure 2.5: Schematic of a lab-on-a-chip (LOC) for fluorescence analysis[27].

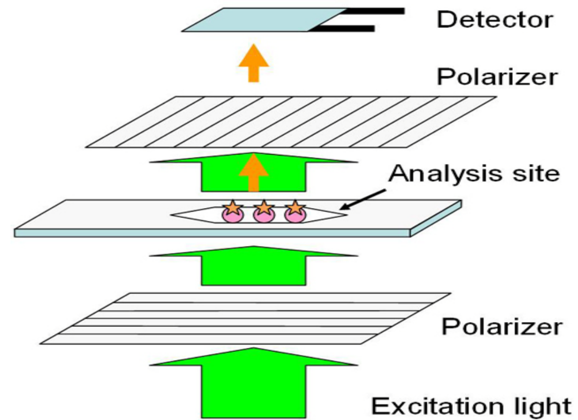


Figure 2.6: Schematic of the polarization filtering analysis system[27].

2.1.4 Capillary electrophoresis

Capillary electrophoresis (C.E) is the technique of separating ionic species by their charge and frictional forces. It was first used by Landers in 1995[31]. Figure 2.7 shows schematic of capillary electrophoresis. A voltage is applied across the electrodes in capillary, solute ions will move towards an electrode of opposite charge. Concentration of nitrate and relevant chemicals in water can be detected using CE method [32]. Success with CE is highly dependent on the buffer solution used and it is not easy to analyze anions in seawater [33]. Table 2-2 illustrates different modes of CE operation.

Table 2-2: Different Mode dependent CE operation[31]

Mode	Operating Principle	Analysis
Zone electrophoresis	Charge to mass ratio	Small ions, proteins, small molecules, DNA
Micellar electrokinetic	Charges species	Peptides, DNA, small molecules
Iso tachophoresis	Mobility	Peptide, protein and small molecules
Chromatography	Ratio and partitioning into micelles according to hydrophobicity	
Gel electrophoresis	Sleving based on charge to mass ratio	Peptides, proteins, DNA
Iso electric focusing	Iso electric point	Proteins and peptides

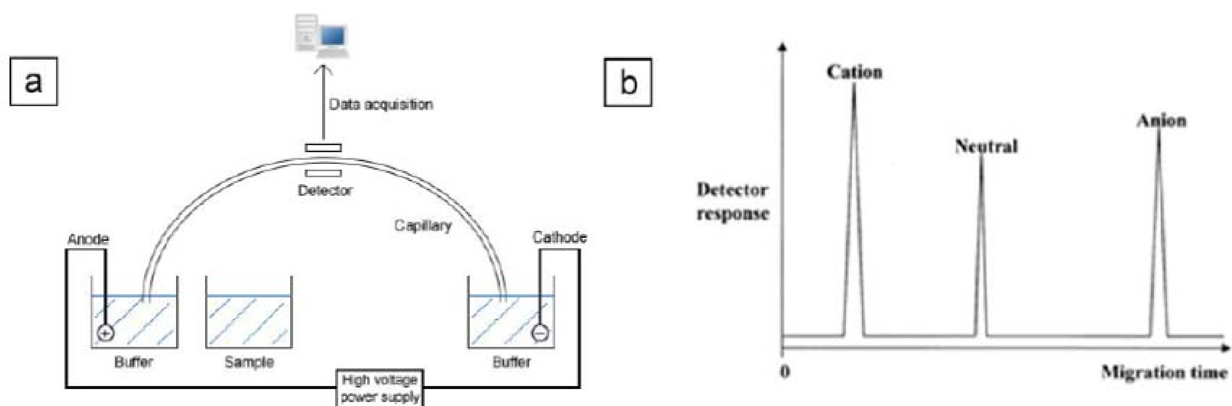


Figure 2.7: a) Overall setup of capillary electrophoresis; b) Electropherogram showing detector response over time with a potential applied across the electrodes[34].

2.1.5 Electrochemical

Electrochemical detection is comprised of three electrodes. The working electrode is dipped into the solution which contains analyte. To facilitate the electron transfer to and from the analyte, a voltage is applied. A reference electrode is also used as a reference to measure and control working electrodes. The auxiliary electrode gives the current needed to balance the current measured at the working electrode. This electrode is also named as counter electrode. To provide a conductive environment, additional electrolyte is added in the system. The ease of fabrication and integration of electrochemical cells with micro fluidic devices is the main advantage of this technique and making it suitable for biosensor systems that are based upon biochemical reactions for recognition [35].

Wei et al. described that working electrode potential is ramped linearly versus time in cyclic volumetric analysis[36]. When the potential reaches the set potential, the working electrode potential ramp is inverted. Again the potential of the working electrode is varied, and the resulting current from faradic processes occurring at the electrode is monitored as a function of time in chronoamperometry [35, 37]. According to Dong, a series of regular voltage pulses can be superimposed on the potential linear sweep or stair steps in differential pulse in voltametry technique [38]. During deposition, working electrode is electroplated with the analyte of interest where the species is oxidized from the electrode during stripping step[39].

2.1.6 Laser

Laser induction techniques are of two types: a) Laser induced breakdown spectrometry (L.I.B.S) and b) Laser spark spectrometry (L.A.S.S). These techniques began to be used since 1980 for single wavelength multi-element analysis. LIBS is now used for total spectral analysis in gas or

water enabling removal of performing thousands of measurements. Figure 2.8 shows the schematic of LIBS technique. This is the analysis of light emission which is produced when a pulsed laser beam put on a sample in order to create high temperature micro plasma[40]. Photosynthesis detectors collect the spectrum of light emission and record the intensity at a specific wavelength. LIBS is unsuitable for in situ deployment because of its size, power requirement and low sensitivity whereas in situ deployment is low cost and miniaturized sensors are used.

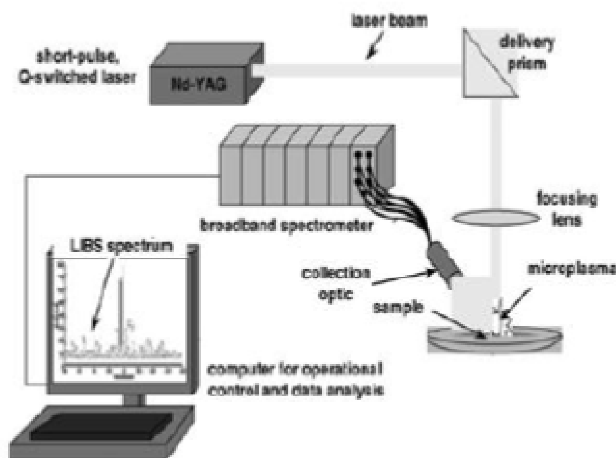


Figure 2.8: Schematic of LIBS[40].

2.1.7 Mass Spectrometry

Mass spectrometry takes in concern the ratio of charge to mass of particles. By this ratio, chemical composition of a compound is identified. This technique can provide molecular weight and structural information; so it is used in proteomics analysis. For electro spray ionization mass spectrometry (ESI-MS) detection of concentrated and resolved cytochrome c peptides, two Spellman high-voltage power supplies are given to deliver electric potentials of 18 KV to the glass vial and 3 KV to the micro dialysis junction to maintain an electric field of 300 V/cm over a 50 cm separation distance as well as induce the electro spray of peptides [41]. Yang et al first reported that polymer-based microchip have direct integration with an electro spray emitter and an internal gold electrode [42].

2.2 Components

The functionality of a lab on a chip system depends on: injector, transporter, preparator, mixer, reactor, separator, detector, controller, and power supply[39]. Figure 2.9 illustrates the component view of a lab on a chip system.

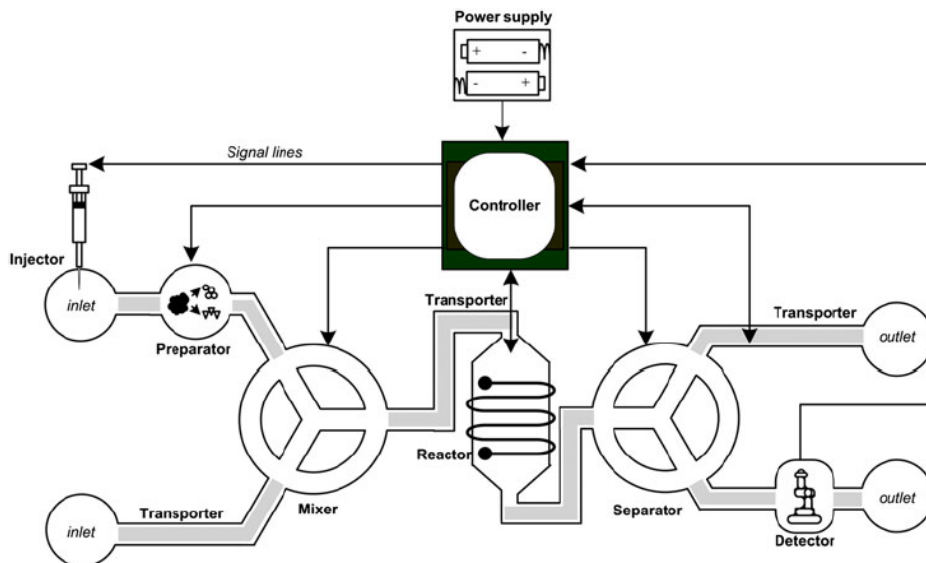


Figure 2.9: Lab on a chip component view[39].

2.2.1 Injectors

Injectors are used to ensure precise micro/nano liter volume delivery of sample or reagent into a lab on-a-chip process for analytical analysis. Injector principal depend on two different technologies:

I. <u>Between the external world and the lab-on-a-chip.</u>
II. <u>Among the internal components of the lab-on-a-chip.</u>

Table 2-3 shows different existing injection methods for lab on a chip system. These injectors have been developed and used in on-board of lab-on-a-chip devices. Syringe pumps and robotic pipettes are the most commonly used injectors. Precise sample volume delivery among different components is a most required for reaction and separation tasks in chemical and biological analysis on chip systems.

Table 2-3: Developed Injectors in existing lab on a chip system

Injection method	Approach	Remarks
External Injection	Robotic pipette	3μL delivery volume [43]
	Electronic pipette	100 nL–1μL range from a pipette loaded with 10μL [44]
	Syringe pipe	75 μL delivery volume [45]
	Metering chamber based on capillary burst valve	μL range to μL range delivery volume [46]
Among internal components	Volume measurement	Repeatability of standard deviation [47] 0.30% for the 50 μL dispenser 0.45% for the 100 μL dispenser 0.30% for the 150 μL dispenser
	Electronic hydrodynamic focusing	1 pL delivery volume [48]

2.2.2 Transporters

Fluidic transporters are used to control fluids flow sequence, flow duration, flow direction, and flow rate for fluid manipulation.

Table 2-4 gives examples of existing transporters in lab on a chip system. It can be categorized in the following two groups according to their actuation and driving force:

I. Active transporter

Active transporters require energy to actuate or give a driving force. Different kinds of driving forces are there like pneumatic, thermo-pneumatic, piezoelectric, hydrodynamic and electro osmosis, electrochemical and hydrostatic. Higher driving voltage is required for Pneumatic, thermo-pneumatic, and piezoelectric driven micro pumps and micro valves and these results in high power consumption. It is more complicated with the inclusion of a large voltage source which is usually bulky and inappropriate for point-of-care devices. On the other hand electrochemical pumping systems are preferable because they eliminate the design complexity and require a lower fabrication cost compared to their pressure-driven counterparts.

Table 2-4: Developed active and passive transporters in existing lab on a chip system

Driving Force	Parts	Maximum Flow rate
Pneumatic	Peristaltic pump and switch valves	10 mm/s to 14 mm/s depending on material[48]
	Serpentine-shaped micro-pump and 4 micro-valves	39.8 µL/min[49]
	Rotary pump with electromagnetic valves	170 µL /min at 30 psi [50]
	Spider-web peristaltic micro pump	8.4 µL /min at 150 kPa [51]
Thermo-pneumatic	Pump chamber and micro-valves	730 nL/min at 500 mW[52]
Piezoelectric	Peristaltic pump	1,500 µL /min [53]
Hydrodynamic and electro-osmosis	T-shaped micro channels with 4 reservoirs	0.2 mm/s[54]
Electrochemical	Micro-pump	0.8 ml/min[55]
	Syringe micro-pump and 2 check valves	0.74 mm/min[56]
Hydrostatic pressure (gravity driven)	Silica capillary	100 µL /min[57]

II. Passive transporter

Passive transporters neither require energy to actuate nor give a driving force. In the field of reactive cellular and tissues studies, diffusion mediated transporters are preferred because of their simplicity as they do not require any external force or power supplies. Various flow rates are achieved by manipulating Channel dimensions and geometries [58, 59]. Another passive transportation technique is gravity-driven pumping system which is able to maintain its flow rate regardless of the fluid in the reservoir [57]. Hydrostatic pressure is the key to generate gravity driven micro flow injection. As the gravity pump cannot generate steady flow, to ensure reproducibility of the micro flow injection analytical signals for two-phase segmented flow, reasonable liquid level difference and regular sample and solvent aspiration are needed. Absorption and capillary force are also common as passive transporter techniques [60].

2.2.3 Preparators

Sample pre-treatment is a mandatory step in molecular analysis especially in different kinds of amplification like DNA amplification. Also, residual material from the samples can inhibit the process reducing the efficiency of PCR (Polymerase Chain Reaction) amplification. These are also applicable to environmental and food samples collected for contaminants examination and pathogen detection. Integration of sample preparation on micro fluidic devices is challenging due to existence of the raw sample in different formats. Also the treatment and preparation of the sample is dependent on the type of analysis to be performed. Existing on-chip sample preparation protocols are filtration, pre-concentration, cell lysis and derivatization.

I. Filtration

Filtration is the most common technique to isolate the analyte of interest from the samples. Micro filters are the typical instrument for this. To strain wanted cells from the sample, filters structures can be designed. Filter can work in the form of membrane filters with holes diameter of 4–12 µm and thickness of 1–3 µm. Figure 2.10 shows different shapes of existing micro filters. These filters are used to filtrate airborne particles from airflows [61].

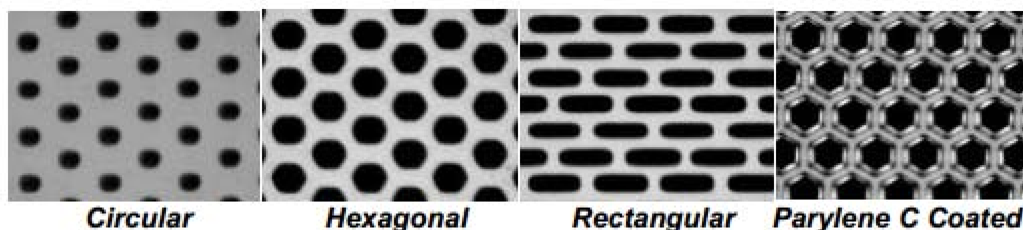


Figure 2.10: Micro machined membrane filters with hole sizes of 4~12 mm and thickness of 1~3mm. The opening ratio ranges from 4% to 45% [61].

Another filtration method is by using micro fabricated silicon structures[62]. Filtration by hydrophoretic strategy exploits the differences in size and deformability of cells. The practical implementation is in red blood cells filtration [63]. The most robust and effective system for filtration of larger particles(2 µm) in liquid is acoustic focusing as it requires low surface area [64]. Acoustic standing waves are generated via an integrated piezoelectric transducer (PZT) in the micro channel. Different driving voltage and particle size results in different focusing efficiency. By adjusting the driving conditions of the transducer, particles above a selected size can be removed.

The most common problems faced in filtration systems are due to clogging and surface adsorption.

II. Pre-concentration

Sample enrichment plays an important role in improving the detector sensitivity. Here comes the point of sample pre-concentration. This can be done by applying a voltage difference between sample reservoir and pre concentrator reservoirs based on the principle of field-amplified sample stacking [65]. Solid-phase extraction (SPE) creates a mobile phase and a stationary phase for mixture separation in medium. The desired or unwanted particle is retained at the stationary phase while the migrated particles through the stationary phase is either collected or discarded. Desired particles trapped at the stationary phase are collected by rinsing stationary chamber using appropriate solution. One frequently used SPE application is for isolation of nucleic acid which is a extraction from one liquid phase to another [66].

III. Cell lysis

For intracellular and single cell studies, lysing of cell ruptures the membrane cell to access their contents. Several cell lysis methods are used like mechanical, chemical, physic-chemical, enzymatic, optical, acoustic, and electrical. This selection depends on the downstream analysis to be performed [67].

IV. Derivatization

Derivatization is a sample preparation method which is frequently used in chemical analysis to convert a chemical compound into a product of similar chemical structure. This improves detector sensitivity and selectivity in laser-induced fluorescent detection in capillary electrophoresis. A comprehensive report describing different derivatization techniques has been published by Waterval et al [68].

2.2.4 Mixers

The main function of mixer is to mix multiple streams of fluids in micro channels of a lab on a chip. Mixing of multiple liquids in mixers is performed based on laminar diffusion under normal conditions. Table 2-5 shows different existing mixers on lab on a chip system.

Table 2-5: Mixers in existing lab on chip systems with different operating principles

Operating principle	Remarks
Magnetic	Maximum Mixing time is 0.5 s [69]
Ultrasonic	Turbulence occurs instantaneously after the ultrasonic vibration causes the mixing to spread nearly throughout the entire chamber after 2 s [70, 71]
Pneumatic	Mixing index is 96.3% [72]
Electrical	Mixing is achieved at <10 ms over a length of 200 µm[73]
Chaotic mixing	Complete mixing is achieved within <1 ms after the two liquids make contact [74]

Mixers can be classified into two groups according to the mixing force (A1, A2):

A1. Active Mixer

External forces are required to improve the mixing of input fluids. It is more expensive to develop integrate active mixers on a lab-on-a-chip though active mixers offer more rapid mixing of liquids in a short time interval. Several kinds of active forces are available like magnetic actuated [75] and PZT (piezoelectric transducer) generated ultrasonic vibration [71]. Figure 2.11 interprets the working process of active and passive mixer.

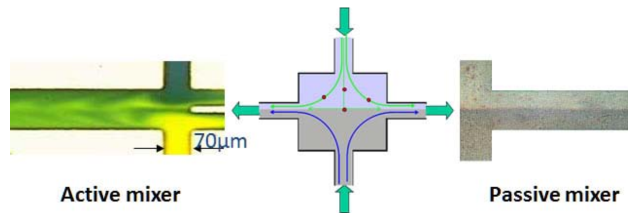


Figure 2.11: Active and passive mixer process[76]

A2. <u>Passive Mixer</u>

The mixing force is the flow characteristics to mix multiple fluids without applying any force. Passive mixers are more robust and stable. But longer period of time is required for ideal mixing. Though it is hard to obtain turbulent flow in the micro channel without external force, mixing can be still enhanced by secondary flow, swirling flow, and vortices [77-80].

According to the technique mixers use to mix fluids, it can be divided into three main categories (B1, B2, B3):

B1. <u>Serpentine</u>

These mixers are also known as Chicane mixers. This type of mixer is mostly used due to the easiness in fabrication and design. These are simple rectangular channels. The key working principle is diffusion along the boundary layer of the fluid. In one serpentine mixer, only a long single boundary layer is needed to complete mixing resulting in high back pressure Figure 2.12A shows typical serpentine mixer.

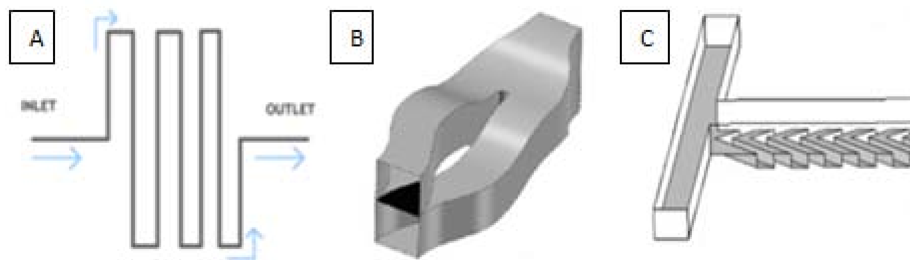


Figure 2.12: A. Serpentine Mixer B. Split and Recombine Mixer C. Herringbone Mixer

B2. <u>Split and Recombine</u>

Split and Recombine is the improved version of a serpentine mixer. The main principle here is to increase the number of diffusion boundary layers which results in mixing over a higher length. Both horizontal and vertical lamination can be used [81, 82]. It must be taken care of that

smearing is not increased due to offset fluid flows. Figure 2.12 B shows the illustration of Split and Recombine micromixer.

B3. Herringbone

This type of mixer was first introduced by Stroock et al [83, 84]. The channel grooves are designed for folding and stretching (helical flow) within the fluid and to increase the boundary level contact area [85-87]. This technique is not suitable for high resolution sensors as this produces smearing. The main advantage of this is that it gives lower pressure drop. Figure 2.12 C shows a typical illustration of the herringbone mixer.

Table 2-6 shows comparison of these three mixers at micro liter flow rate which is useful to determine suitable micro mixer for the system.

Table 2-6: Comparison of the performance of existing micro mixers

Type of mixer	Flow Rate(µL/min)	Mixing Length(mm)	Mixing level(%)
Serpentine	405.5	75	>90 [88]
	40	2.8	81 [89]
Split and Recombine-Horizontal	1.2	2.4	>90 [90]
Split and Recombine-Vertical	0.9	2.4	>90 [90]
Split and Recombine	3500	960	>90 [81]
Herringbone	20.4	9	>90 [83]
	1.1	0.44	80.5 [87]

2.2.5 Reactors

Reactor acts as controller for chemical or biological reaction. The reactor is usually equipped with heaters, sensors, or actuators to control and monitor the reaction process [39]. Reactors reduce cost and risk significantly as only minimal volume of sample and reagent is required for the reaction process. Table 2-7 shows existing reactors in lab on a chip system. Reactors are divided in three groups depending on types of function:

I. Gas Phase Reactor
II. Liquid Phase Reactor
III. Packed Bed Reactor

Table 2-7: Reactors in existing lab on a chip systems

Type of Reactor	Work done	Remarks
Gas-phase	Hydrogen production	Production of 88.19%. hydrogen from methanol [91]
Liquid-phase	Amide formation	After 1 h, 1.7 g of crude product (90% pure) were obtained. Reaction yield is 81% [92]
Packed-bed	Amie N-alkylation process	N-alkylation in ethanol: time taken is 150 s. Reaction yield of N ethylbenzylamine is 85%. Reaction yield of N-ethylaniline is 34% [93]

2.2.6 Separators

Separators are used to isolate sample and reagents after mixing or reaction processes. This can be used to perform selection of a pre-determined cell for analysis, or isolation of a large number of single cells where a pre-determined cell cannot be identified and determined. Micro-separation technique plays a significant role in chemical and biological analysis. Also, capillary electrophoresis (CE) is the mostly used trend in segregation of desired analyte. There are other techniques such as optical tweezers, magnetic, di-electrophoresis (DEP), iso tachophoresis, chromatography; capillary electrophoresis and laminar flow based filter are also emerging for lab on a chip analysis. Table 2-8 shows existing separators in lab on chip systems.

Table 2-8: Separators in Existing Lab on a Chip Systems

Method	Separation Target	Remarks
Optical tweezers	Homogenous mixtures	Count accuracy for homogenous mixture is 98-100% [94]
Magnetic Separation	Magnetic micro beads	Highest trapping ratio of 84.3% at a flow rate of 20 µL /min with Spiral micro-coil with magnetic pillar.[95]
Di-electrophoresis (DEP)	Human lung cancer cells, Beads mixture	Separation accuracy for viable cells is 84% and non-viable cells is 81% [49] For purity gained beads of 15 µm diameter is 96.8 ± 0.6%; and for 6 µm diameter is 99.5 ± 0.5% [96]
Iso tachophoresis	Tryptophan enantiomers	suitable for analysis of sample containing high concentration enantiomers [97]
Laminar flow	Erythrocytes and small fluorescent particles	About 80% of erythrocytes are collected [98]
Capillary electrophoresis	Organic peroxides	Separation is achieved within 120 s [99]

2.2.7 Controllers

Controllers manage all on-chip activities like control, data acquisition, and signal processing. They are also responsible for sending control signals to on-chip components such as actuators, heaters, and so on. They collect data from on chip sensors. A typical controller consists of microcontroller, communication interface, programmable memory, control circuitry, signal preconditioning circuitry and power interface circuitry. Table 2-9 shows different controller applications. Based on integration, controllers are of two types:

I. <u>On-Chip</u>

For lab on a chip technology, it is necessary to integrate all components on chip, not off chip. Lab on a chip for human immunodeficiency virus assay with an on-chip controller was proposed by Guo[100]. The proposed design consists of three modules: micro fluidic chip, sensor, actuators, executor, and a CCD imaging device. Advancement in micro fluidic and CMOS fabrication technology will make the incorporation of controllers in lab-on-a-chip analysis achievable.

II. <u>Off- Chip</u>

Pumps and valves integrated in lab-on-a-chip systems require control circuitry to perform their operations. Wang and Lee [51] developed control circuits and electromagnetic valves to control the motion of pumps. Tseng et al. [72] utilized a microcontroller to control the driving force of electromagnetic valves to generate pumping and mixing effects. A control system is also used in sample concentration and detection based on both insulator-based dielectrophoresis and electrical impedance measurement on lab-on-a-chips. This is shown in the work of Sabounchi et al. [101]. All types of low level and real time micro fluidic interfaces and data acquisition are done by microprocessors.

Table 2-9: Controllers in existing 'lab on a chip' systems

Integration of Controller	Remarks
On-chip	Actuators executor and Sensors [100]
Off-chip	Programmable high voltage power supply, HVS448 (Lab-Smith, Livermore, CA, USA); Laptop; Sequence software program (LabSmith); Modular power supply connections; Instrument communication; Impedance data acquisition and processing; A/D and D/A converters; 8-bit Rabbit 2000 microprocessor [101] ARM microprocessor [102] Electromagnetic valves, SMC Inc., S070M-5BG-32, Japan; Control circuit [51] Waveform generator (model 33220A; Agilent Technologies, Santa Clara, CA, USA); RF power amplifier (model 325LA; EIN); Lab view program [64]

2.2.8 Power Supply

Power supply is a mandatory thing for lab on a chip analysis. Without a power supply, on-chip components including transducers, actuators, and electronic circuits cannot be energized. Batteries are used as power source in portable lab-on-a-chip devices. On the other hand, lab-on-a-chip systems using electrophoresis or dielectrophoresis, require a very high voltage power supply. It can manipulate the voltage values also; for this high voltage programmable power supplies are needed. Tsai et al. [103] used a programmable high voltage power supply in order to generate the electrokinetic driving forces. This driving force is required for sample injection and separation steps in the CE process. A reusable integrated high voltage power supply for electro kinetic transportation of fluids is presented by Erickson et al. [104]. The self-containable and adjustable power supply was powered by an onboard cell battery.

3. Existing Applications of LOC

Lab on a chip technology is exhilarating the interest of scientists in every aspect. This technology is used not only to synthesize chemicals efficiently and economically but also to carry out biological and clinical analyses. It also performs combinatorial chemistry and carries out full-scale analyses from sample introduction to chemical separation and detection. All of these happen on a single, miniaturized device i.e the chip. The lab on a chip technology is growing very fast in both industrial and academic sectors. It has its applications in modern chemistry, clinical chemistry, fabrication, engineering and materials science, biology, physics, electronics, aiding communication and collaboration across disciplines. Lab on a chip also covers key areas such as: Combinatorial chemistry, Biotechnology, Electronics, Analytical chemistry, Environmental monitoring, Synthetic chemistry, Medical diagnostics and screening, Clinical chemistry, Genomics, proteomics, cellomics (DNA probes, PCR), Pharmaceuticals, Materials science, Engineering, Fluidics, Reactor technology, Fabrication, Robotics, Waste minimization. Biological applications on lab on a chip are already commercialized in global market whereas for environmental chemistry, the field is still developing. Some established applications of lab on a chip in environmental chemistry and life science are discussed here.

3.1 Application in Environmental Chemistry

3.1.1 Microfluidic pH analysis

Currently available laboratory methods are unstable for applications on in situ platforms. Microfluidic pH systems give the opportunity of pH analysis using simple design with low consumption of power and reagents. Such a pH microsensor with high precision and accuracy is being developed at National Oceanographic Center, Southampton, UK. The main indicator is sulfonephthalein (sulfonic acids derived from phthaleins) indicator where the pK value should be comparable to the expected pH of the indicator sea water solution as $pK-1 \leq pH \leq pK$ [105]. The microfluidic flow cell comprising the absorption cell as well as a static mixer is made of PMMA(poly methyl methacrylate). Four micro inert valves(Harvard Apparatus Nanomite, Kent, UK) are directly mounted on the chip and the syringe pumps controls fluid propulsion. The 10mm absorption cell has a volume of 5µL and this is connected to a light source and detector with two optical fibers. Figure 3.1 shows the schematic of microfluidic pH analysis. The light source is a tri color LED. This system was continuously on operation for over a month without any interruption except for maintenance [106]. Processing and analysis time takes only 6 minutes.

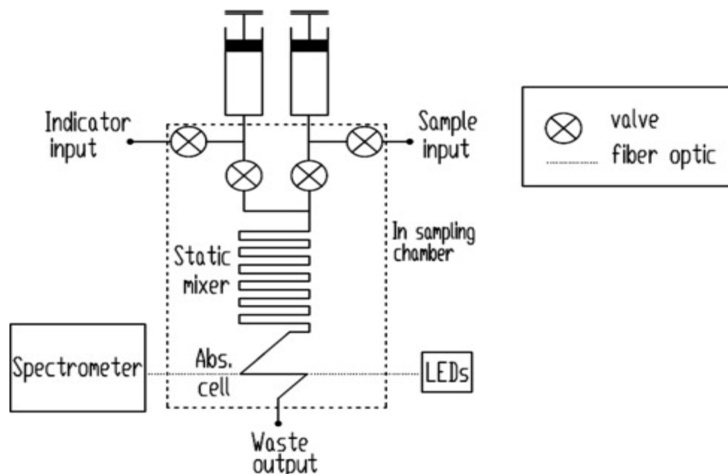


Figure 3.1: Microfluidic pH system analysis; sea water sample and indicator are pumped through two individual syringe pumps and mixed in the flow cell using a static mixer before reaching the absorption cell [106].

3.1.2 Sea water Nitrate and Nitrite Analysis

The most widely used method for nitrite detection is the Griess reaction. Nitrate and ammonia can also be detected using the same method but with some additional steps. For detection, both ammonia and nitrate shall be reduced to nitrite. The chemical reaction is shown in Figure 3.2. Under acidic conditions, nitrite reacts with sulfanilic acid and forms diazonium cation. This cation is then coupled to a α-napthylamine and forms colored chromophores named as azo dye. The absorption of azo dye is directly proportional to nitrite concentration.

The chip for nitrate and nitrite analysis contains three absorption cells: one 25 mm reference cell, one 25 mm measurement cell for concentration< 30 µM, and one 2.5-mm measurement cell for concentration>30 µM. The chip incorporates a fluidic manifold to permit selection of one of four standards: nitrate or nitrite, the sample, and a blank (artificial seawater or Milli-Q). 15 micro inert solenoid valves (LFNA1250325H, Lee Products Ltd., UK) mounted directly to the chip controls the fluid flow in the chip. Three titanium syringes: syringe 1 for sample/standard, syringe 2 for the buffer solution, and syringe 3 for the Griess reagent are also mounted on the chip. Dark PMMA reduces the amount of LED background light reaching the photodiode. When the cell is 2.5cm, additional background light rejection is gained by maximizing light passing through the cell and by spacing the LED and photodiode 14.5 mm apart from each other and using a 10.15 mm long 'light tube' to transmit light between the LED and the cell. For nitrite analysis, fluid is passed through the reference cell and mixed with Griess reagent. This mixture is then passed through the 0.25 mm long serpentine mixing channel. Absorption is determined in the two sequential measurement cells; separated by a milled groove to prevent cross-talk.

Figure 3.3 gives illustration of the LOC device for nitrate analysis. For nitrate analysis, before passing through the reference cell and mixing with the Griess reagent, fluid is combined with the

imidazole buffer(1,3-Diaza-2,4-cyclopentadiene and Glyoxaline) and passed through a 0.46 mm serpentine mixer and then again through an offchip cadmium tube (SEAL analytical, UK). This off chip cadmium tube can be fabricated as part of the chip in future [107]. The cadmium tube can be conditioned periodically to recover reduction efficiency by passing 5 mM copper sulfate solution for 2 min, 6% HCl for 4 min, and again in the copper sulfate solution for 6 min. these solutions are pumped through syringe 2 (in place of the buffer solution) and are selected using dedicated valves [108].

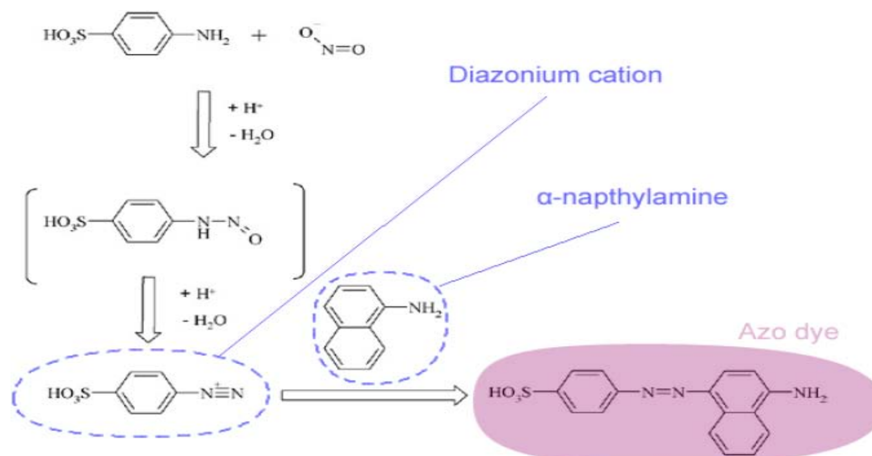


Figure 3.2: The Griess reaction under acidic condition.

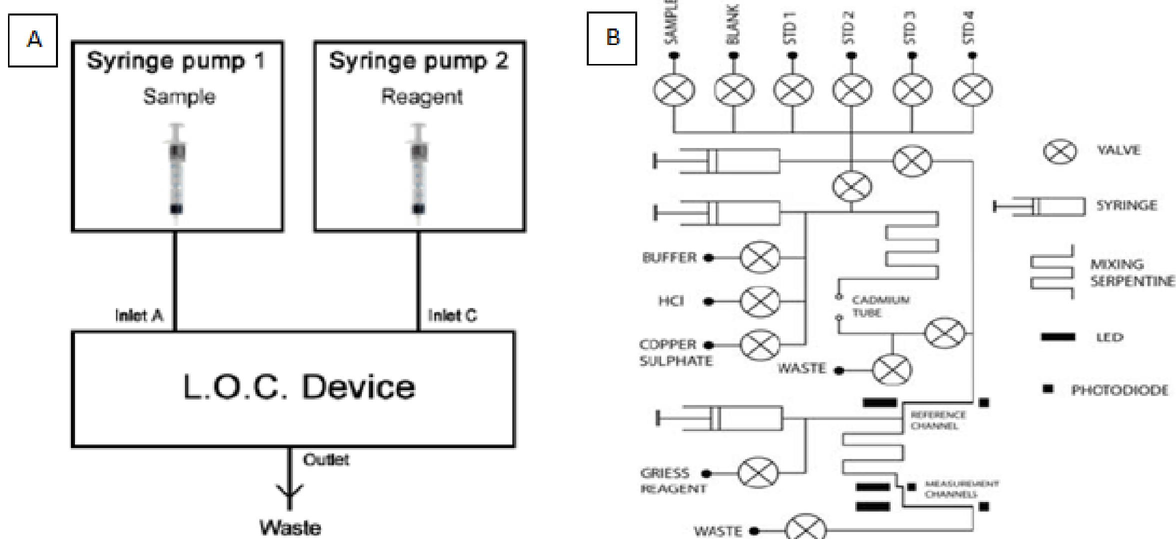


Figure 3.3: A. LOC Device experimental setup for nitrate analysis [7]; B. Fluidic path diagram indicating the three syringes, fifteen valves, and three absorption cells. Inputs for standard solutions are indicated as STD. Hydrochloric acid (6%) is denoted as HCl[108].

3.1.3 Detection of other chemicals in sea water

Apart from nitrite and nitrate, there are also other chemicals in the water column which are important for environmental analysis. Using the same technology as nitrate and nitrite detection, manganese, phosphate and silicate can also be detected in sea water. The basis technology for these detections is spectrometric techniques. In hydrothermal activity, manganese is most sensitive indicator, phosphate is an essential macronutrient for living organisms and silicate is also a macronutrient for fresh water environment [7].

Basic detection principles

I. <u>Manganse Detection</u>

Manganese detection is done by using the solubilisation method of Watanabe[109]. According to Watanabe, 1-(2-pyridylazo)-2-naphtholor (PAN) is solubilized by the surfactant triton X-100 and used as an effective colorimetric reagent. The sample is mixed up with a solution of PAN, Triton X100, Sodium Hydroxide, Boric acid and Desferrioxamine B [110]. The mixing ration will be 5.3:1. A Lab on a chip is necessary to do the setup for the detection method as illustrated in Figure 3.4. Desferrioxamine B is an iron specific chelating agent and it is used to suppress interference from iron. The effectiveness of this chelating agent depends on the grade used and its age. H_3BO_3 is dissolved in NaOH to make borate buffer. For manganese detection, PAN is mixed with Triton X-100 and MQ (mili Q) water until PAN is dissolved. At this stage, borate buffer was added with the mixer. After that, Desferrioxamine B solution is added when PAN is cooled as the sideramine(iron binding reagent) degrades very quickly when it is warm. Reacting with the sample, this PAN mixer forms PAN-Mn complex. The sample is first passed through the first mixer and then through the absorption cell 1. Reagent PAN-Mn complex is then added to the solution. The manganese is detected at absorption cell 2 as the absorption of solution is proportional to the concentration of manganese at 560nm.

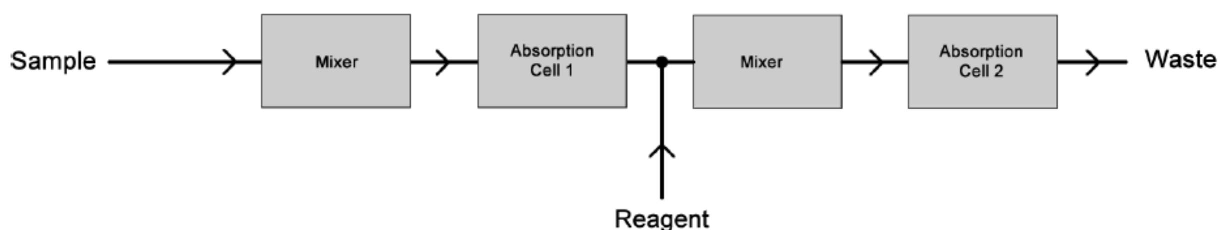


Figure 3.4: LOC device setup for detection of manganese: The reagent is a solution of 1-(2-pyridylazo)-2-naphtholor (PAN), Triton X100, Sodium Hydroxide, Boric acid and Desferrioxamine B[7].

II. Phosphate Detection

When phosphate in a sea water sample comes in contact with ammonium molybdate solution (pH=1.0) in presence of potassium antimony tartrate, antimonymolybdophosporous acid is formed. This acid works as reagent 1. Reagent 2, ascorbic acid is added to reagent 1 and reduces reagent 1 phosphomolybdenum blue complex [111]. Sea water sample is first passed through absorption cell 1 and then the mixer of the reagent 1 and 2 is added to it. At 710nm, this absorption of the solution is proportional to concentration of phosphate and detected in absorption cell 2. This whole process setup on the lab on chip is shown in Figure 3.5. The chemical reaction is as follows:

$$\text{Phosphate} + \text{Ammonium Molybdate} + \text{Potassum Anntimony tartrate}$$

$$\xrightarrow{\text{acidic solution}(H_2SO_4)} \text{Antimonylmolybdophosphorus acid}$$

$$\text{Antimonylmolybdophosphorus acid} + \text{Ascorbic acid} \rightarrow \text{PhosphoMolybdenum blue complex}$$

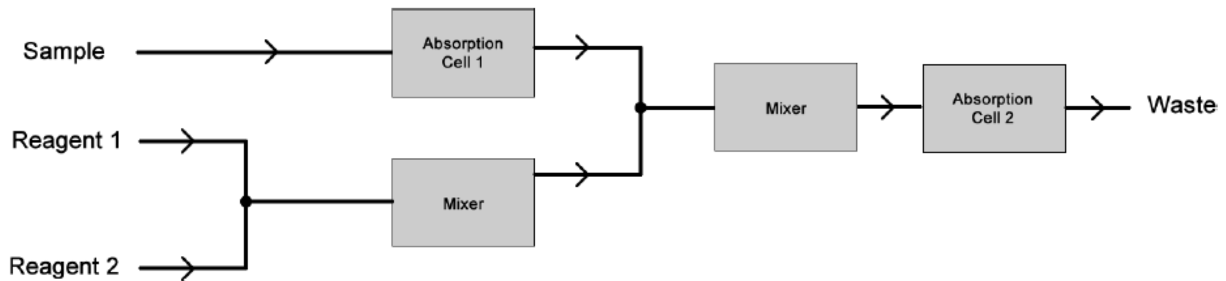


Figure 3.5: LOC device setup for detection of phosphate: reagent 1 is the solution of ammonium molybdate and potassium antimony tartrate; reagent 2 is ascorbic acid [7].

III. Silicate Detection

Silicate can be detected from a yellow silicomolybdate complex. This complex is formed when silicate reacts with ammonium molybdate. The yellow complex is then reduced to silicomolybdenum blue by ascorbic acid[112]. This whole process setup for 'lab on chip' is shown in Figure 3.6 . The reaction for the process can be as follows:

$$Sodium\ Silicate + Ammonium\ Molybdate + Oxalic\ acid \rightarrow Silicomolybdatae\ acid$$

$$Silicomolybdate\ acid + Ascorbic\ acid \rightarrow Silicomolybdenum\ blue\ complex$$

$$[MoO_4]^{2-} \rightarrow [Mo_7O_{24}]^{6-} \rightarrow [Mo_8O_{26}]^{4-} \rightarrow Larger\ complex$$

The sample is first passed through absorption cell 1 and mixed up with reagent 1, ammonium molybdate, in the mixer. Reagent 2, oxalic acid, is then mixed in after the first mixer and forms silicomolybdate acid. Reagent 3, ascorbic acid, mixes in with the silicomolybdate acid and reduce it to the silicomolybdenum blue complex which is detected in the absorption cell 2 at 600 or 800 nm. This absorption of the solution is proportional to concentration of silicate.

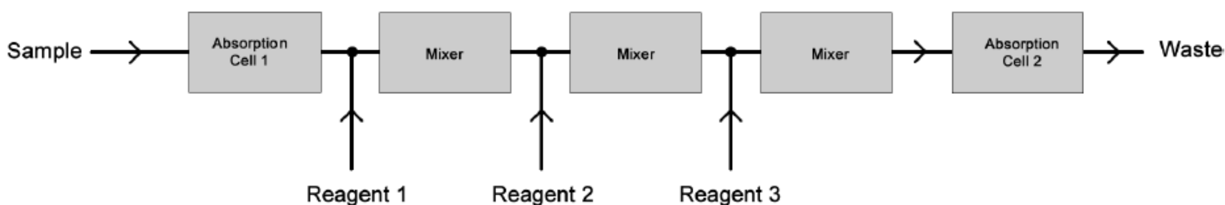


Figure 3.6: LOC device setup for detection of ailicate: reagent 1 is solution of ammonium molybdate; reagent 2 is Oxalic acid and reagent 3 is ascorbic acid[7].

3.2 Applications in Life Science

3.2.1 Hand-Held Microanalytical Instrument for Chip-Based Electrophoretic Separations of Proteins

Identification of proteins and other bio molecules can be done based on lab on a chip technology. The design, fabrication, and demonstration of hand-held microchip-based analytical instruments are already in process. The components on this lab in chip technology include a 2-cm-square fused-silica microfluidic chip, two independent separation modules incorporating interchangeable fluid cartridges, and a miniature laser-induced fluorescence detection module. Figure 3.7 illustrates the setup for analysis. A custom O-ring sealed manifold plate is used to connect the chip access ports to the fluid cartridges and a syringe injection port, and provides sample introduction and world-to-chip interface. A High-voltage power supply is programmed to provide bidirectional currents up to 100 µA at 5000 V. This enables real-time current and voltage monitoring and also facilitates troubleshooting and methods development. Picomolar (10^{-11} M) detection sensitivity of fluorescent dyes and nanomolar sensitivity (10^{-9} M) for fluorescamine-labeled proteins is allowed by laser-induced fluorescence detection[113].

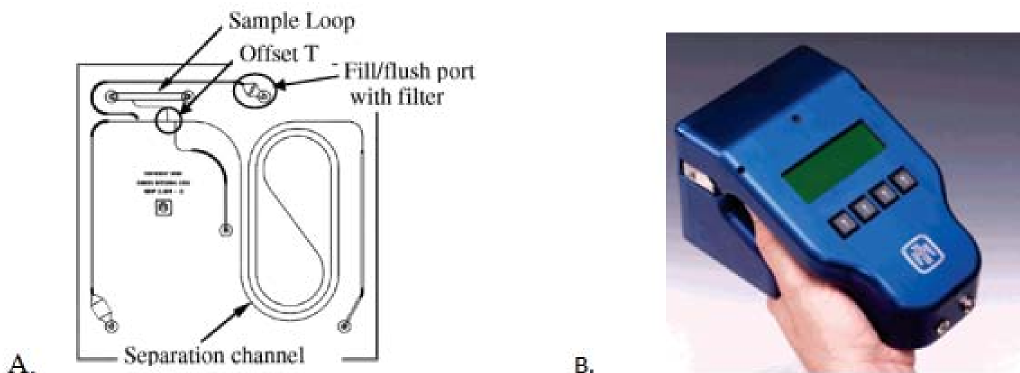


Figure 3.7: A: 2cm X 2cm microseparation chip including on chip sample introduction, inlet port filters, low dispersion turns, separation column, fluorescing silica substrate. B: The µChemLab instrument. The system integrates fluidics, lasers, optics, microseparation chips, electronic controls, data algorithms, HV power supplies, and a user interface into a hand-portable instrument[113].

3.2.2 A modular microfluidic architecture for integrated biochemical analysis

The architecture for bio-chemical analysis is conceptualized on two levels: 1) a single chip level, 2) 4 multiple chip module system level. At single chip level, the components are separated into two fundamental categories i.e passive fluid components including chemical reactors and channels and active electrochemical control structures consisting of sensors and actuators. The setup for the analysis involves a multifunctional chip where active and passive fluid components are fabricated. This multifunction chip is known as a FBB (Microfluidic BreadBoard). Figure 3.8 shows a schematic of the FBB. The FBB constitutes the foundation of second chip on which all the passive components are mounted to complete a LOC. An array of pneumatic valves is mounted on the FBB to create flow of the fluids in single fluid paths as well as multiple paths. The valves that control single paths are used for sample loading and reactor isolation whereas the multiple valve controls are used to select between different channels. All the valves are arranged symmetrically around each side of the FBB following simplified channel routing. One most common need for LOCs is a microreactor which mixes small volumes of two or more different solutions. A valve-assisted mixing system is built on the FBB. Figure 3.9 contains frames from a video that shows a pH-sensitive, colorimetric acid-base reaction used to demonstrate the operation of the mixing system. The basic solution was first saturated with a pH indicator Cresol red and then titrated with an acidic solution. This indicator fluid is yellow colored.

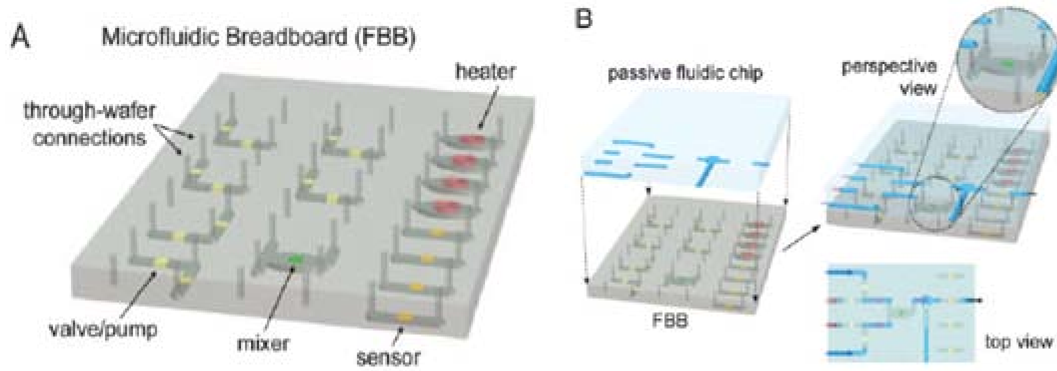


Figure 3.8: A. Microfluidic breadboard including electromechanical components and through water fluidic connections. B. A complete LOC created by bonding a passive fluidic chip with an FBB [114].

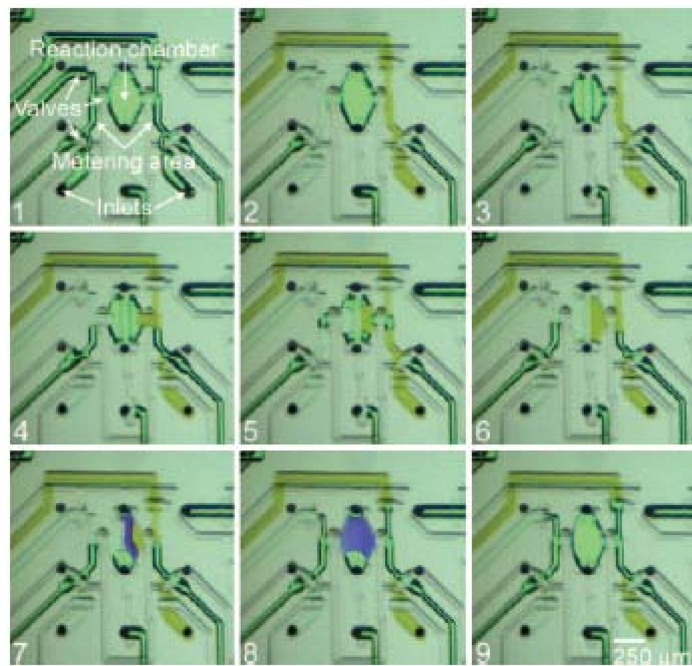


Figure 3.9: System for mixing picoliter-scale volumes of fluid; video frames illustrate the process of precise volumes of the fluid loading into either side of a microreactor and mixed by diffusion[114].

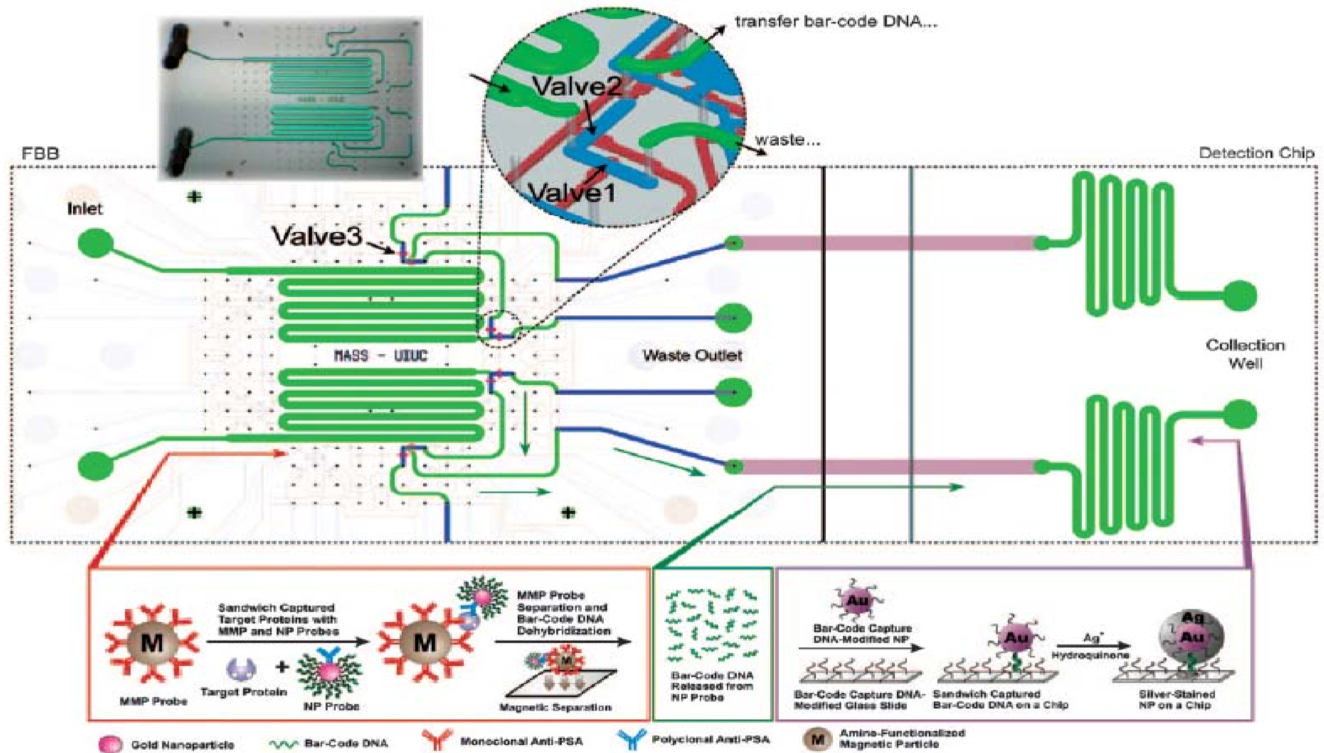


Figure 3.10: The System design for the detection of fPSA with the BBC protocol. The PSA is separated in the first stage of the device by flowing solutions from the inlet to the waste outlet with valve 1 open and valve 2 closed. Bar-code DNA provides indirect amplification which is released from the sandwich and transferred from the FBB to the detection chip. Valve 1 and valve 3 remains closed and valve 2 allows the bar-code transfer. The bar codes attach to the surface of the DNA modified the detection chip and are sandwiched with gold NPs to provide a direct optical readout of the result. The gold NPs becomes silver strained. [114].

Figure 3.10 illustrates the procedure for detection of fPSA with BBC protocol. The basic solution was flowed into separate sides of the valve controlled metering channels where specific volume measurements are taken. The measured volumes were then introduced into either side of the micro reactor. These volumes were separated by a valve oriented along the length of the chamber. This process was repeated until the desired volumes are achieved in the reactor (maximum 400 pL in each half). By Closing the chamber isolation valves and opening the central valve, the fluids are allowed to combine and mix by diffusion, producing a color change from yellow to purple and this change in color provide direct optical readout of the result. After desired mixing was achieved, the mixture was transported out of the chamber through FBB channels connected to a passive chip [114].

3.2.3 A fully integrated microfluidic genetic analysis system with sample-in–answer-out capability

A microfluidic genetic analysis system is developed with an integrated microfluidic device capable of accepting whole blood as a crude biological sample with the endpoint generation of a genetic profile. The microfluidic genetic analysis (MGA) system has a microchannel architecture with three functional domains, two for sample preparation as solid phase extraction (SPE) and PCR and one for microchip electrophoresis analysis (ME) (Figure 3.11). Five elastomeric normally closed valves are used which have direct flow from a single syringe pump and can localize the existing chemicals (Figure 3.11b). Valve V1 isolates the reagents used for DNA extraction in the SPE domain from the PCR chamber (because these are known as PCR inhibitors). The PCR domain is separated from the ME domain by V3 and V4 valves. These valves must be passivated to avoid protein fouling and deactivation of the Taq polymerase (thermostable DNA polymerase). Valves V3 and V4 isolates the two domains and/or pump amplicon (piece of DNA /RNA and artificially or naturally created from amplification/replication of DNA/RNA) from the PCR chamber, whereas valves V2 and V5 work as injector for the DNA standard from the marker reservoir. The sample is passed through the analysis channel for injection, and then the components are separated and detected by laser-induced fluorescence. V4 works as outlet valve and V3 is diaphragm valve. The elastomeric membrane valving process isolates each distinct functional region of the device. Using the resistive flow principle, the valve directs purified DNA and PCR reagents from the extraction domain into a 550-nl chamber for rapid target sequence PCR amplification. The injection process is pressure based and done repeatedly. These injections of nanoliter aliquots of amplicon along with the DNA sizing standard allow electrophoretic separation and detection providing DNA fragment size information. The presence of *Bacillus anthracis*(anthrax) in 750 nL of whole blood from living asymptomatic infected mice and of *Bordetella pertussis* in 1 μL of nasal aspirate from a patient suspected of having whooping cough were confirmed by the test done through this LOC device. It confirmed that it is because of the genetic profile [115].

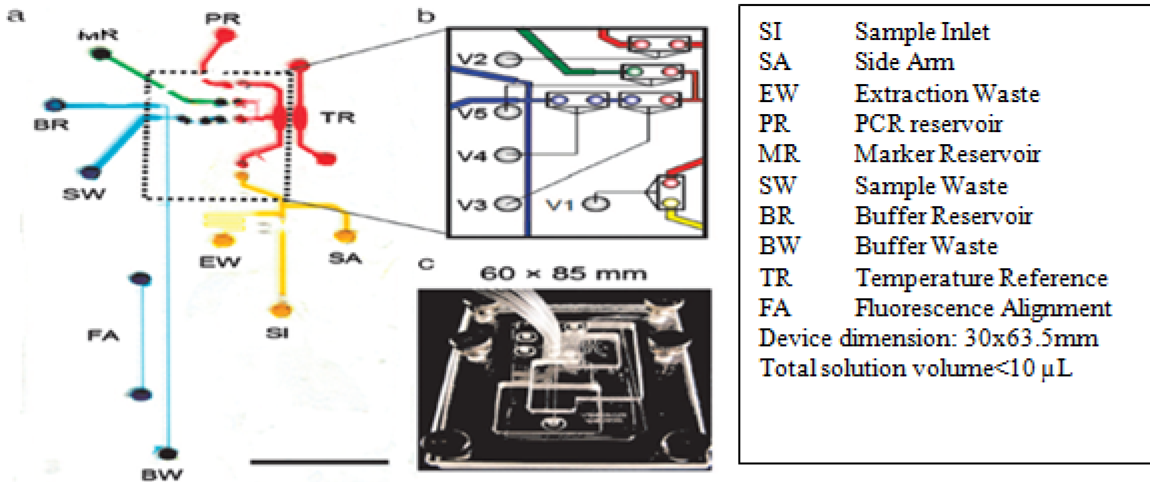


Figure 3.11: (a) Dyes are placed in the channels for visualization (Scale bar, 10 mm.). Domains for DNA extraction (yellow), PCR amplification (red), injection (green), and separation (blue) are connected through a network of channels and vias. The flow control region is indicated by a dashed box. (b) Schematic of flow control region. Valves are shown as open rectangles. (c) The Device assembled into the manifold [115].

3.2.4 Microfluidic systems for extracting nucleic acids for DNA and RNA analysis

Microfluidic components are integrated in a chip to perform isolation of deoxyribonucleic acid (DNA) from white blood cells and ribonucleic acid (RNA) from viruses in the blood. For DNA extraction, white blood cells (WBC) are captured and red blood cell (RBC) concentration is depleted. Hemoglobin of RBC works as a contaminant to inhibit downstream process of DNA amplification by PCR. For Viral RNA extraction, a submicron filter removes both RBC and WBC with a minimal reduction in the concentration of the viruses in plasma. In terms of requirement of basic microfluidic components, both DNA and RNA extractions uses similar equipments like mixers for mixing reagents, filters for capturing or separating the blood cells, and a binder for capturing and purifying the DNA/RNA molecules.

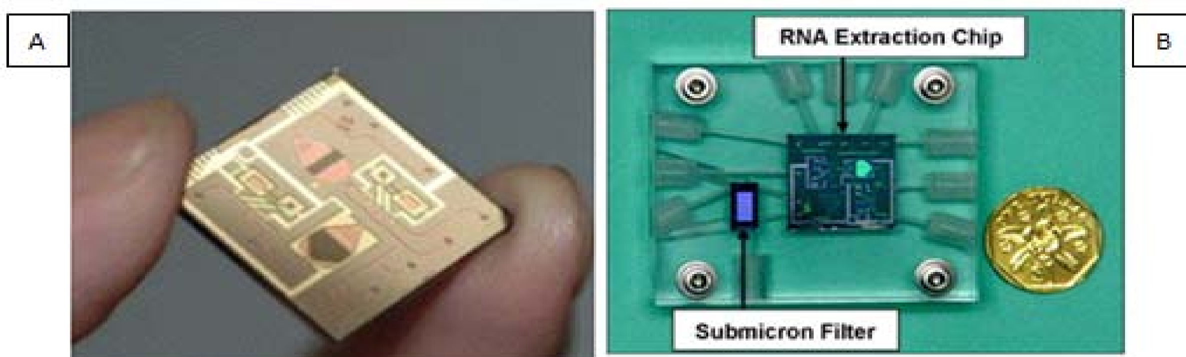


Figure 3.12: A) DNA extraction Chip; B)RNA extraction chip [116].

The microfluidic chip designed and fabricated for DNA extraction consists of an integrated mixer, two paraffin valves, a filter with 3-µm gap and a binder. W.C Hui et al[116] developed the microfluidc chip for analysis. Figure 3.12 shows chip setup for extraction of RNA and DNA. The Experimental setup included a test block to connect the microfluidic chips and the external microfluidic delivery system. The delivery system consists of several syringes containing the sample and the various reagents. A microlinear motion actuator is used to actuate the syringe to deliver the sample (µL) or reagent (µL). This actuator is controlled by a calibrated computer system. A microscope with a CCD video camera is used to monitor and capture both experimental parameters and video images. Figure 3.13 shows the basic experimental setup for DNA and viral RNA extractions. Besides, there are differences in the sizes of WBC and viruses; the concentration of the virus is typically much lower than WBC. This causes a much higher volume of blood for filtering to extract viral RNA, especially for detection of the viruses at an early stage of infection. With proper modifications of the protocols, it has been demonstrated that both the DNA and the viral RNA can be extracted successfully in the microfluidic device. The quality of the extracted samples was later verified by PCR and gel-electrophoresis [116]. Figure 3.14 shows a commercially available microfluidic system for DNA and RNA extraction.

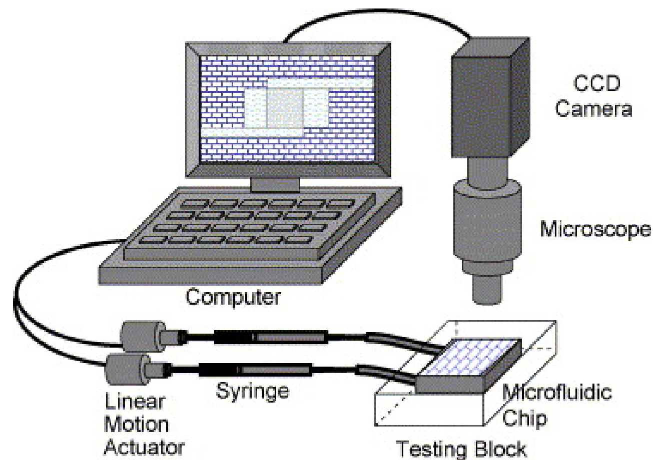


Figure 3.13: Basic experimental setup for DNA and viral RNA extractions[116].

Figure 3.14: The portable microfluidic system for: A. DNA extraction, B. RNA extraction[116] .

3.2.5 Integrated microfluidic system for electrochemical sensing of urinary proteins

The main function of the kidneys in a body is to filter blood to avoid metabolic wastes excreted out of the body in the form of urine. If the kidneys are functioning normally, they can retain or re absorb filtered protein molecules and get them back to the blood. Hence, for healthy human beings, proteins should not be detected in the urine. But, if the kidneys are damaged, the filtering mechanism becomes less effective making the proteins detectable in urine. Using an electrochemical method, it is easy to detect protein in the urine sample. By cyclic voltammetry (CV) method, the amount of the urine proteins can be accurately measured. A microfluidic chip integrated two-way spiral-shape micropump is used to facilitate the automatic transportation of the samples,. The spiral-shape micropump can drive the fluid flow inwards or outwards by using single electromagnetic valve (EMV). The spiral design makes it possible to drive samples through four microchannels giving provision of four parallel detection of protein with fewer samples. The electrochemical sensing module consists of four sets of urinary protein sensors. Each urinary protein sensor has three sensing electrodes: a working electrode, a counter electrode and a reference electrode. Figure 3.15 shows the illustration of electrodes. The conductive area of the working electrode is reduced when proteins are absorbed onto the working electrode, and thus the electric resistance increases. The change in electric current between the working and the reference electrodes is used for measurement of urinary proteins. Figure 3.16 shows the experimental setup for urinal protein analysis. The operational principle for electrochemical sensing of protein is the difference between the isoelectric point (ρl) value of protein and pH of urine. For the protein lysozyme, ρl=10.7. Thus it carries positive charge in urine (pH=5.5). So, the application of negative potential on the working electrode is it induces lysozyme to be absorbed onto the negatively charged surface. Similar thing happens with another protein albumin having ρl=4.7. The working pressure for both of the proteins is 20 psi and EMV frequency is 10 Hz. Pressure is an important factor for this analysis as it controls the flow of the liquid throughout the chip[117].

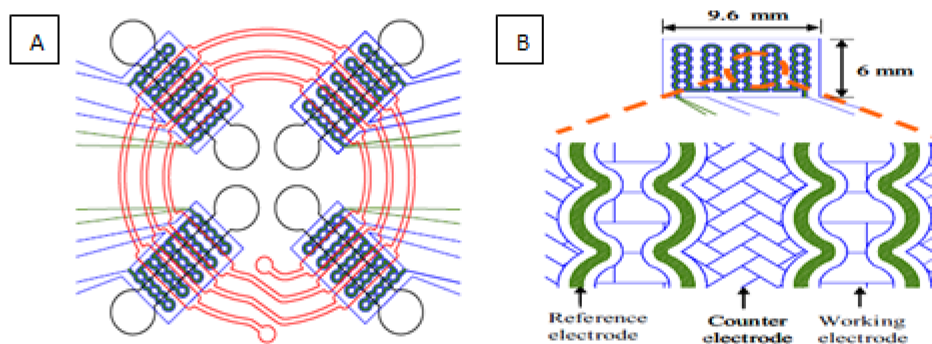


Figure 3.15: A. Four microchannels with a spiral pneumatic micropump B.An Electrochemical Sensing module[117].

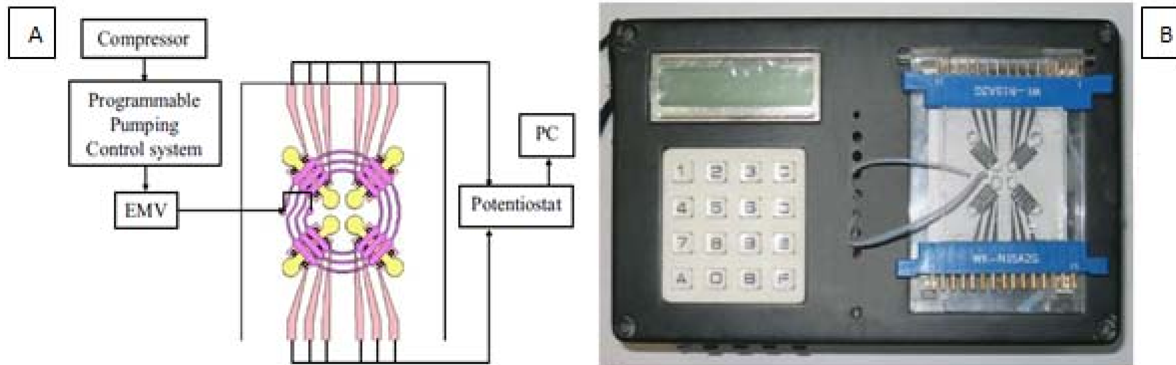


Figure 3.16: A.Experimental setup for urinal protein analysis B. Hand-held controller composed of a control circuit, an electromagnetic valve, an air compressor and a microfluidic chip [117].

3.2.6 An integrated microfluidic system for isolation, counting, and sorting of hematopoietic stem cells

Huei-Wen Wu et al[118] designed and developed an integrated microfluidic system which is capable of isolation, counting, and sorting of hematopoietic stem cells (HSCs). HSCs are very useful for cell therapies and are commonly recognized to express antigen CD34. This can be derived from PB(peripheral blood), BM(bone marrow) and CB(cord blood). HSCs from CB are widely used for transplantation and it can decrease human leukocyte antigen(HLA) mismatch from related family donor. All these increase the need to isolate HSCs from CB. HSCs are detectable in an automatic format by utilizing a magnetic-bead-based immunoassay. There are three functional modules: cell isolation, cell counting and cell sorting modules. All are integrated on a single chip by using microfluidic technology. Figure 3.17 illustrates the experimental setup of the microfluidc system for HSCs. The cell isolation module has four membrane type micromixer to bind the target stem cells, the magnetic beads with two pneumatic micropumps transport the sample, and an S-shaped channel isolate HSCs using a permanent magnet underneath. The counting and sorting of HSCs are carried out by the cell counting and sorting modules. Experimental results showed a separation efficiency of 88% for HSCs from cord blood within 40 min from a sample volume of 100 μl. Therefore, the development of this integrated microfluidic system is becoming promising for various applications such as stem cell research and cell therapy [118].

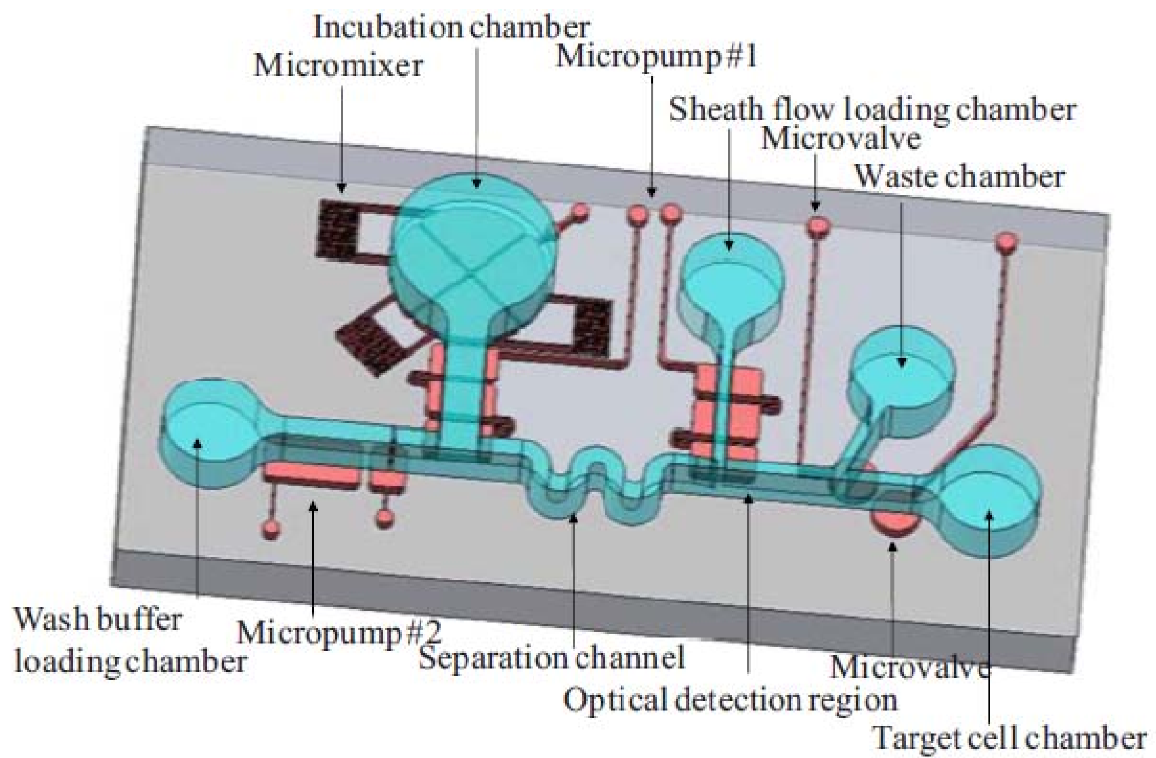


Figure 3.17: Experimental setup for isolation, counting, and sorting of hematopoietic stem cells[118].

4. Adoption to new technologies and general issues to adoption

There are many early-stage applications of microsystems containing fluids such as exploration of fluidic optics and cells, fabrication of microrobotic systems using hydraulic systems based on microfluidics, development of new types of organic synthesis in small-channel systems, fluidic versions of MEMS, development of technologies based on large arrays of detectors, high-throughput screening, and biomimetic systems with microfluidic components. The extension from microfluidic systems to nanofluidics compares the dimensions of the channels and the thickness of the layer of structured fluid at the walls of the device. These also enable exploration of properties of near-surface water, and ion and electrolyte transport at this interface. PDMS is biocompatible for which it may be possible to embed microfluidic devices in vivo for certain types of biomedically relevant analysis. Single-cell and single-molecule analysis require work with small volumes of sample, which allow the testing of fundamental assumptions of molecular chemistry, cell biology and biology. The development of new 'lab on a chip' tools for genomics, proteomics and metabolomics is proceeding very rapidly in research laboratories, and will provide a stimulus for large-scale production. There are, in principle, high-value applications for microfluidic systems. Developing these applications requires innovations in both microfluidics and in biomedicine or other fields. The development of different new types of bioassays for monitoring patient response to therapy is one such applications like development of assays for home testing, or for use in doctors' offices at early stages of disease (early detection of 'biomarkers), is a matter of second now [1]. Most recent efforts of 'lab on a chip' concerns devices along with the application in chemistry, biology and medicine. The developments of miniaturized systems are also considered for integrating the possibilities by microfabrication and microfluidics. The adoption of LOC in general research can be grouped in two categories: i) analytical studies in chemical, clinical, bio-clinical, bio analytical studies. And ii) true applications addressed in clinical, environmental, food, toxicological, agriculture and industrial fields [119].

To apply microfluidic systems in practice, there are new methods of fabrication, and a sufficient range of micro components. These have opened the door for application rather than simple demonstration of principles. The most highly developed 'lab on a chip' applications are their use to screen conditions such as pH, ionic strength and composition, co solvents and concentrations for protein crystallization [120-122]. Some of this technology is now commercially available. Other applications such as separations coupled to mass spectroscopy[123], high-throughput screening in drug development [124, 125], bioanalyses [126], examination and manipulation of samples consisting of a single cell [127, 128] or a single molecule [129, 130] and synthesis of 18F-labelled organic compounds for positron emission tomography (PET) [131] are demonstrated in laboratory. The development of practical 'lab on a chip' systems especially for bioanalysis is rapid [132-135]. This is due to the limited technology in two parts of analysis: sample preparation and detection. Samples used in analysis are of two types: environmental sampling (such as soil) and clinical samples (such as blood or faeces) and these are often diluted

or complicated. Samples must be converted to a form that is compatible with the intended analysis before they can be analyzed by microfluidic devices. The requirement to complete these tasks are sample dependent and not necessarily ’micro’ in scale. After a sample has been prepared and introduced into the analytical device and then processed, it must be detected. This detection is done on chip. The microfluidic chip is a small part of a system in which sample introduction and detection are much more complicated than the chip’s operation, but it detracts from the potential advantages of microfluidic devices. Other standard issues as pumping, valving and on-chip reagent storage also require better solutions than available ones so far.

5. Conclusion

Lab-on-a-chip systems facilitate miniaturization and integration of complex functions. 'Lab on a chip' systems automate repetitive laboratory tasks reducing the man work and time. Standardization in lab-on a chip system will improve the design process, reduce development cost and time, and simplify technology transfer among different areas. With the expansion of micro/nanofabrication technologies and micro/nanoelectromechnical systems development, it is expected that incorporation of all components on board of the chip will be accomplished and consequently benefit the environmental laboratory processes and the point-of-care diagnosis. Furthermore, the incorporation of nanotechnology in lab-on-a-chip systems will go one step ahead in realizing real-time sensing as well as label free and non-invasive therapeutic devices.

6. Expected Future Developments

Microfluidics has shown its growth with the rapid development of new methods of fabrication, and of the components. These components have been developed to replace the old laboratory equipments such as the micro channels that serve as pipes, and other structures that form valves [136, 137], mixers and pumps. All these are essential elements of micro chemical 'factories' on a chip. However, its impact on science has not yet been revolutionary. Revolutions in technology is still going on; regaining both a broad range of different types of components and subsystems, and their integration into complete, functional systems. The field of micro fluidics is in early adolescence, and still developments are going on in both of these essential requirements. In addition, it also opens doors to the integration of components into systems that can be used by the non-experts. As a field, it is a combination of unlimited promise, inflammation and incomplete assurance. This is a very exciting phase for the lab on chip systems, but still a lot of research is needed to find out exactly what it will be when the 'lab on a chip' systems grows up.

There are many interesting questions related to the fluid mechanics that come up with microdevices and many more that remain to be investigated. One main vision of microfluidics is the "chemical plant'' on a chip. In a more general viewpoint, it encompasses science and applications in chemistry, cellular biology, material science etc. Microfluidic technology offers a synthetic system for controlling transport and chemistry at different length scales below a few millimeters. These systems allows new challenges in fluid dynamics, transport, and engineering design for generations of researchers [2].

References

1. Whitesides, G.M., *The origins and the future of microfluidics.* Nature, 2006. **442**(7101): p. 368-373.
2. Stone, H.A., A.D. Stroock, and A. Ajdari, *Engineering flows in small devices: Microfluidics toward a lab-on-a-chip*. 2004. p. 381-411.
3. Beebe, D., G. Mensing, and G. Walker, *Physics and applications of microfluidics in biology.* Annual review of biomedical engineering, 2002. **4**(1): p. 261-286.
4. Squires, T.M. and S.R. Quake, *Microfluidics: Fluid physics at the nanoliter scale.* Reviews of Modern Physics, 2005. **77**(3): p. 977-1026.
5. Jensen, K.F., *Silicon-Based Microchemical Systems: Characteristics and Applications.* MRS Bulletin, 2006. **31**(02): p. 101-107.
6. Lee, J.N., C. Park, and G.M. Whitesides, *Solvent Compatibility of Poly(dimethylsiloxane)-Based Microfluidic Devices.* Analytical Chemistry, 2003. **75**(23): p. 6544-6554.
7. Taberham, A., *A Deap Sea Lab on a Chip Chemical Sensor*, in *Engineering, Science and Mathematics*. 2010, University of Southampton. p. 271.
8. *Shimadzu Scientific Instruments*. UV-Vis-NIR Spectrophotometer 2009; Available from: http://www.ssi.shimadzu.com/products/product.cfm?product=uv3600.
9. *JASKO UK limited*

V-670 UV-Visible NIR Spectrophotomer 2009; Available from: http://www.jasco.co.uk/UV_Visible_NIR_spectrophotometer.php.

10. *Beckman Coulter Limited*. DU 800 Spectrophotometer 2009; Available from: https://www.beckmancoulter.com/wsrportal/bibliography?docname=B2007-8367+DU800+Brochure.pdf.
11. *Hitachi High Technologies*. U-4100 UV-Vis-NIR Spectrophotometer 2009; Available from: http://www.hitachi-htc.ca/library/u4100-uvvisnir-spectrophotometer-continuously-variable-absolute-reflectance/transmittance.
12. Johnson, K.S. and L.J. Coletti, *In situ ultraviolet spectrophotometry for high resolution and long-term monitoring of nitrate, bromide and bisulfide in the ocean.* Deep Sea Research Part I: Oceanographic Research Papers, 2002. **49**(7): p. 1291-1305.
13. Wetters, J.H. and K.L. Uglum, *Direct spectrophotometric simultaneous determination of nitrite and nitrate in the ultraviolet.* Analytical Chemistry, 1970. **42**(3): p. 335-340.
14. Nagashima, K., X.-X. Qian, and S. Suzuki, *Second-derivative spectrophotometric determination of nitrite and nitrate at 10-8M concentrations.* Analyst, 1986. **111**(7): p. 771-775.
15. Suzuki, N. and R. Kuroda, *Direct simultaneous determination of nitrate and nitrite by ultraviolet second-derivative spectrophotometry.* Analyst, 1987. **112**(7): p. 1077-1079.
16. Finch, M.S., et al., *A low power ultra violet spectrophotometer for measurement of nitrate in seawater: introduction, calibration and initial sea trials.* Analytica Chimica Acta, 1998. **377**(2–3): p. 167-177.
17. Griess, *Coloring matters.* Journal of American Chemical Society, 1879(4): p. 126-126.

18. Tsikas, D., *Analysis of nitrite and nitrate in biological fluids by assays based on the Griess reaction: Appraisal of the Griess reaction in the l-arginine/nitric oxide area of research.* Journal of Chromatography B, 2007. **851**(1–2): p. 51-70.
19. Morais, I.P.A., I.V. Tóth, and A.O.S.S. Rangel, *An overview on flow methods for the chemiluminescence determination of phosphorus.* Talanta, 2005. **66**(2): p. 341-347.
20. Yaqoob, M., A. Nabi, and P.J. Worsfold, *Determination of nanomolar concentrations of phosphate in freshwaters using flow injection with luminol chemiluminescence detection.* Analytica Chimica Acta, 2004. **510**(2): p. 213-218.
21. Garside, C., *A chemiluminescent technique for the determination of nanomolar concentrations of nitrate and nitrite in seawater.* Marine Chemistry, 1982. **11**(2): p. 159-167.
22. Krawczyk, S., *Discussion on optical integration in Lab-on-a-Chip microsystems for medical diagnostics.* physica status solidi (c), 2003. **0**(3): p. 998-1012.
23. Ikuta, K., et al. *Finger-top total protein analysis system based on new biochemical IC chip*. in *Micro Electro Mechanical Systems, 2008. MEMS 2008. IEEE 21st International Conference on*. 2008.
24. Pipper, J., et al., *Clockwork PCR Including Sample Preparation.* Angewandte Chemie International Edition, 2008. **47**(21): p. 3900-3904.
25. Malic, L. and A.G. Kirk, *Integrated miniaturized optical detection platform for fluorescence and absorption spectroscopy.* Sensors and Actuators A: Physical, 2007. **135**(2): p. 515-524.
26. Yin, D., et al., *Planar optofluidic chip for single particle detection, manipulation, and analysis.* Lab on a Chip, 2007. **7**(9): p. 1171-1175.
27. Papautsky, D.K.a.I., *High-sensitivity integrated fluorescence analysis for microfluidic lab-on-a-chip.* SPIE Newsroom, 2007(2007).
28. Banerjee, A., et al., *A Polarization Isolation Method for High-Sensitivity, Low-Cost On-Chip Fluorescence Detection for Microfluidic Lab-on-a-Chip.* Sensors Journal, IEEE, 2008. **8**(5): p. 621-627.
29. Chandrasekaran, A. and M. Packirisamy, *Absorption detection of enzymatic reaction using optical microfluidics based intermittent flow microreactor system.* Nanobiotechnology, IEE Proceedings -, 2006. **153**(6): p. 137-143.
30. Zhu, L., C.S. Lee, and D.L. DeVoe, *Integrated microfluidic UV absorbance detector with attomol-level sensitivity for BSA.* Lab on a Chip, 2006. **6**(1): p. 115-120.
31. Landers, J.P., *Clinical capillary electrophoresis.* Clin Chem, 1995. **41**(4): p. 495-509.
32. Miyado, T., et al., *Simultaneous determination of nitrate and nitrite in biological fluids by capillary electrophoresis and preliminary study on their determination by microchip capillary electrophoresis.* J Chromatogr A, 2004. **1051**(1-2): p. 185-91.
33. Tsikas, D., *Analysis of nitrite and nitrate in biological fluids by assays based on the Griess reaction: appraisal of the Griess reaction in the L-arginine/nitric oxide area of research.* J Chromatogr B Analyt Technol Biomed Life Sci, 2007. **851**(1-2): p. 51-70.
34. Browning, S., *Potential applications for bio-sensing technology for environmental testing/monitoring.* Environmental Agency, 2008.
35. Choi, J.-W., et al., *An integrated microfluidic biochemical detection system for protein analysis with magnetic bead-based sampling capabilities.* Lab on a Chip, 2002. **2**(1): p. 27-30.

36. Wei, F., et al., *Electrochemical detection of low-copy number salivary RNA based on specific signal amplification with a hairpin probe.* Nucleic Acids Research, 2008. **36**(11): p. e65.
37. Popovtzer, R., et al., *Electrochemical detection of biological reactions using a novel nano-bio-chip array.* Sensors and Actuators B: Chemical, 2006. **119**(2): p. 664-672.
38. Dong, H., et al., *Sensitive electrochemical enzyme immunoassay microdevice based on architecture of dual ring electrodes with a sensing cavity chamber.* Biosensors and Bioelectronics, 2006. **22**(5): p. 621-626.
39. Lim, Y.C., A.Z. Kouzani, and W. Duan, *Lab-on-a-chip: a component view.* Microsystem Technologies, 2010. **16**(12): p. 1995-2015.
40. Harmon, R.S., et al., *Laser-induced breakdown spectroscopy – An emerging chemical sensor technology for real-time field-portable, geochemical, mineralogical, and environmental applications.* Applied Geochemistry, 2006. **21**(5): p. 730-747.
41. Gao, J., et al., *Integrated Microfluidic System Enabling Protein Digestion, Peptide Separation, and Protein Identification.* Analytical Chemistry, 2001. **73**(11): p. 2648-2655.
42. Yang, Y., et al., *A polymeric microchip with integrated tips and in situ polymerized monolith for electrospray mass spectrometry.* Lab on a Chip, 2005. **5**(8): p. 869-876.
43. Emrich, C.A., et al., *Microfabricated 384-lane capillary array electrophoresis bioanalyzer for ultrahigh-throughput genetic analysis.* Anal Chem, 2002. **74**(19): p. 5076-83.
44. Daniel, D. and I.G. Gutz, *Electronic micropipettor: a versatile fluid propulsion and injection device for micro-flow analysis.* Anal Chim Acta, 2006. **571**(2): p. 218-27.
45. Perch-Nielsen, I.R., et al., *Autonomous and 3D real-time multi-beam manipulation in a microfluidic environment.* Optics Express, 2006. **14**(25): p. 12199-12205.
46. Steigert, J., et al., *Integrated sample preparation, reaction, and detection on a high-frequency centrifugal microfluidic platform.* JALA - Journal of the Association for Laboratory Automation, 2005. **10**(5): p. 331-341.
47. Ahn, C.H., et al., *Disposable smart lab on a chip for point-of-care clinical diagnostics.* Proceedings of the IEEE, 2004. **92**(1): p. 154-173.
48. Fu, A.Y., et al., *An Integrated Microfabricated Cell Sorter.* Analytical Chemistry, 2002. **74**(11): p. 2451-2457.
49. Tai, C.-H., et al., *Automatic microfluidic platform for cell separation and nucleus collection.* Biomedical Microdevices, 2007. **9**(4): p. 533-543.
50. Lien, K.-Y., et al., *Purification and enrichment of virus samples utilizing magnetic beads on a microfluidic system.* Lab on a Chip, 2007. **7**(7): p. 868-875.
51. Wang, C.-H. and G.-B. Lee, *Automatic bio-sampling chips integrated with micro-pumps and micro-valves for disease detection.* Biosensors and Bioelectronics, 2005. **21**(3): p. 419-425.
52. Yoo, J.-C., et al., *A high performance microfluidic system integrated with the micropump and microvalve on the same substrate.* Microelectronic Engineering, 2006. **83**(4–9): p. 1684-1687.
53. Nguyen, N.-T. and X. Huang, *Miniature valveless pumps based on printed circuit board technique.* Sensors and Actuators A: Physical, 2001. **88**(2): p. 104-111.
54. Gao, J., X.-F. Yin, and Z.-L. Fang, *Integration of single cell injection, cell lysis, separation and detection of intracellular constituents on a microfluidic chip.* Lab on a Chip, 2004. **4**(1): p. 47-52.

55. Liu, R.H., et al., *Self-Contained, Fully Integrated Biochip for Sample Preparation, Polymerase Chain Reaction Amplification, and DNA Microarray Detection.* Analytical Chemistry, 2004. **76**(7): p. 1824-1831.
56. Suzuki, H. and R. Yoneyama, *Integrated microfluidic system with electrochemically actuated on-chip pumps and valves.* Sensors and Actuators B: Chemical, 2003. **96**(1–2): p. 38-45.
57. Cai, Z., et al., *A gravity driven micro flow injection wetting film extraction system on a polycarbonate chip.* Talanta, 2006. **68**(3): p. 895-901.
58. Cho, B.S., et al., *Passively Driven Integrated Microfluidic System for Separation of Motile Sperm.* Analytical Chemistry, 2003. **75**(7): p. 1671-1675.
59. Melin, J., et al., *A liquid-triggered liquid microvalve for on-chip flow control.* Sensors and Actuators B: Chemical, 2004. **100**(3): p. 463-468.
60. Schulte, T., R. Bardell, and B.H. Weigl, *Sample Acquisition and Control On-Chip Microfluidic Sample Preparation.* JALA - Journal of the Association for Laboratory Automation, 2000. **5**(4): p. XXI-XXII.
61. Chen, Y.-F., et al., *Microfluidic System for Biological Agent Detection*, in *Science and Art Symposium 2000*, A. Gyr, P. Koumoutsakos, and U. Burr, Editors. 2000, Springer Netherlands. p. 159-168.
62. Cady, N.C., et al., *Real-time PCR detection of Listeria monocytogenes using an integrated microfluidics platform.* Sensors and Actuators B: Chemical, 2005. **107**(1): p. 332-341.
63. Choi, S., et al., *Continuous blood cell separation by hydrophoretic filtration.* Lab on a Chip, 2007. **7**(11): p. 1532-1538.
64. Jung, B., et al., *Acoustic Particle Filter with Adjustable Effective Pore Size for Automated Sample Preparation.* Analytical Chemistry, 2008. **80**(22): p. 8447-8452.
65. Foote, R.S., et al., *Preconcentration of Proteins on Microfluidic Devices Using Porous Silica Membranes.* Analytical Chemistry, 2004. **77**(1): p. 57-63.
66. Bhattacharyya, A. and C.M. Klapperich, *Thermoplastic Microfluidic Device for On-Chip Purification of Nucleic Acids for Disposable Diagnostics.* Analytical Chemistry, 2005. **78**(3): p. 788-792.
67. Brown, R.B. and J. Audet, *Current techniques for single-cell lysis.* Journal of The Royal Society Interface, 2008. **5**(Suppl 2): p. S131-S138.
68. Waterval, J.C.M., et al., *Derivatization trends in capillary electrophoresis.* ELECTROPHORESIS, 2000. **21**(18): p. 4029-4045.
69. Grumann, M., et al., *Batch-mode mixing on centrifugal microfluidic platforms.* Lab on a Chip, 2005. **5**(5): p. 560-565.
70. Yang, Z., et al., *Active micromixer for microfluidic systems using lead-zirconate-titanate(PZT)-generated ultrasonic vibration.* ELECTROPHORESIS, 2000. **21**(1): p. 116-119.
71. Yang, Z., et al., *Ultrasonic micromixer for microfluidic systems.* Sensors and Actuators A: Physical, 2001. **93**(3): p. 266-272.
72. Tseng, H.-Y., et al., *Membrane-activated microfluidic rotary devices for pumping and mixing.* Biomedical Microdevices, 2007. **9**(4): p. 545-554.
73. Bottausci, F., et al., *An ultrashort mixing length micromixer: The shear superposition micromixer.* Lab on a Chip, 2007. **7**(3): p. 396-398.

74. Wong, S.H., M.C.L. Ward, and C.W. Wharton, *Micro T-mixer as a rapid mixing micromixer.* Sensors and Actuators B: Chemical, 2004. **100**(3): p. 359-379.
75. Suzuki, H., H. Chih-Ming, and N. Kasagi, *A chaotic mixer for magnetic bead-based micro cell sorter.* Microelectromechanical Systems, Journal of, 2004. **13**(5): p. 779-790.
76. Research, N.; Available from: http://fzhang.utsi.edu/Research.htm.
77. Sudarsan, A.P. and V.M. Ugaz, *Fluid mixing in planar spiral microchannels.* Lab on a Chip, 2006. **6**(1): p. 74-82.
78. Xia, H.M., et al., *Chaotic micromixers using two-layer crossing channels to exhibit fast mixing at low Reynolds numbers.* Lab on a Chip, 2005. **5**(7): p. 748-755.
79. Chen, H. and J.-C. Meiners, *Topologic mixing on a microfluidic chip.* Applied Physics Letters, 2004. **84**(12): p. 2193-2195.
80. Garstecki, P., et al., *Mixing with bubbles: a practical technology for use with portable microfluidic devices.* Lab on a Chip, 2006. **6**(2): p. 207-212.
81. Schonfeld, F., V. Hessel, and C. Hofmann, *An optimised split-and-recombine micro-mixer with uniform chaotic mixing.* Lab Chip, 2004. **4**(1): p. 65-9.
82. Kim, D.S., et al., *A serpentine laminating micromixer combining splitting/recombination and advection.* Lab Chip, 2005. **5**(7): p. 739-47.
83. Stroock, A.D., et al., *Chaotic mixer for microchannels.* Science, 2002. **295**(5555): p. 647-51.
84. Stroock, A.D., et al., *Patterning Flows Using Grooved Surfaces.* Analytical Chemistry, 2002. **74**(20): p. 5306-5312.
85. Fu, X., et al., *Research on staggered oriented ridges static micromixers.* Sensors and Actuators B: Chemical, 2006. **114**(2): p. 618-624.
86. Johnson, T.J. and L.E. Locascio, *Characterization and optimization of slanted well designs for microfluidic mixing under electroosmotic flow.* Lab Chip, 2002. **2**(3): p. 135-40.
87. Johnson, T.J., D. Ross, and L.E. Locascio, *Rapid Microfluidic Mixing.* Analytical Chemistry, 2001. **74**(1): p. 45-51.
88. H. Götze, S.G., L. Pagel, *Design and Testing of an static laminar diffusion mixer with convective cross mixture based on PCB technology.* Micromechanics Europe 2005, 2005(2005): p. 334-337.
89. Mengeaud, V., J. Josserand, and H.H. Girault, *Mixing Processes in a Zigzag Microchannel: Finite Element Simulations and Optical Study.* Analytical Chemistry, 2002. **74**(16): p. 4279-4286.
90. Koch, M., et al., *Two simple micromixers based on silicon.* Journal of Micromechanics and Microengineering, 1998. **8**(2): p. 123.
91. Pattekar, A.V. and M.V. Kothare, *A microreactor for hydrogen production in micro fuel cell applications.* Microelectromechanical Systems, Journal of, 2004. **13**(1): p. 7-18.
92. Kikutani, Y., et al., *Pile-up glass microreactor.* Lab on a Chip, 2002. **2**(4): p. 193-196.
93. Matsushita, Y., et al., *Photocatalytic reactions in microreactors.* Chemical Engineering Journal, 2008. **135, Supplement 1**(0): p. S303-S308.
94. Seo, S., et al., *Multi-color LUCAS: Lensfree On-chip Cytometry Using Tunable Monochromatic Illumination and Digital Noise Reduction.* Cellular and Molecular Bioengineering, 2008. **1**(2-3): p. 146-156.
95. Ramadan, Q., et al., *An integrated microfluidic platform for magnetic microbeads separation and confinement.* Biosensors and Bioelectronics, 2006. **21**(9): p. 1693-1702.

96. Choi, S. and J.-K. Park, *Microfluidic system for dielectrophoretic separation based on a trapezoidal electrode array.* Lab on a Chip, 2005. **5**(10): p. 1161-1167.
97. Ölvecká, E., et al., *Isotachophoresis separations of enantiomers on a planar chip with coupled separation channels.* ELECTROPHORESIS, 2001. **22**(15): p. 3347-3353.
98. Takagi, J., et al., *Continuous particle separation in a microchannel having asymmetrically arranged multiple branches.* Lab on a Chip, 2005. **5**(7): p. 778-784.
99. Wang, J., et al., *Capillary electrophoresis-electrochemistry microfluidic system for the determination of organic peroxides.* J Chromatogr A, 2002. **952**(1-2): p. 249-54.
100. Guo, W.P., X.M. Ma, and Y. Zeng, *Clinical laboratories on a chip for human immunodeficiency virus assay.* Conf Proc IEEE Eng Med Biol Soc, 2005. **2**: p. 1274-7.
101. Sabounchi, P., et al., *Sample concentration and impedance detection on a microfluidic polymer chip.* Biomedical Microdevices, 2008. **10**(5): p. 661-670.
102. Kornaros, G., et al. *Architecture of a Consumer Lab-on-Chip for Pharmacogenomics*. in *Consumer Electronics, 2008. ICCE 2008. Digest of Technical Papers. International Conference on*. 2008.
103. Tsai, C.-H., et al., *Experimental and numerical investigation into leakage effect in injectors of microfluidic devices.* ELECTROPHORESIS, 2006. **27**(24): p. 4991-4998.
104. Erickson, D., D. Sinton, and D. Li, *A miniaturized high-voltage integrated power supply for portable microfluidic applications.* Lab on a Chip, 2004. **4**(2): p. 87-90.
105. Byrne, R.H., et al., *Seawater pH measurements: an at-sea comparison of spectrophotometric and potentiometric methods.* Deep Sea Research Part A. Oceanographic Research Papers, 1988. **35**(8): p. 1405-1410.
106. Rérolle, V.M.C., et al., *Seawater-pH measurements for ocean-acidification observations.* TrAC Trends in Analytical Chemistry, 2012. **40**(0): p. 146-157.
107. Jannasch, H.W., K.S. Johnson, and C.M. Sakamoto, *Submersible, Osmotically Pumped Analyzer for Continuous Determination of Nitrate in situ.* Analytical Chemistry, 1994. **66**(20): p. 3352-3361.
108. Beaton, A.D., et al., *Lab-on-Chip Measurement of Nitrate and Nitrite for In Situ Analysis of Natural Waters.* Environmental Science & Technology, 2012. **46**(17): p. 9548-9556.
109. Watanabe, H., *Spectrophotometric determination of cobalt with 1-(2-pyridylazo)-2-naphthol and surfactants.* Talanta, 1974. **21**(4): p. 295-302.
110. Chin, C.S., K.S. Johnson, and K.H. Coale, *Spectrophotometric determination of dissolved manganese in natural waters with 1-(2-pyridylazo)-2-naphthol: application to analysis in situ in hydrothermal plumes.* Marine Chemistry, 1992. **37**(1–2): p. 65-82.
111. Zhang, J.Z., C.J. Fischer, and P.B. Ortner, *Optimization of performance and minimization of silicate interference in continuous flow phosphate analysis.* Talanta, 1999. **49**(2): p. 293-304.
112. Mullin, J.B. and J.P. Riley, *The colorimetric determination of silicate with special reference to sea and natural waters.* Analytica Chimica Acta, 1955. **12**(0): p. 162-176.
113. Renzi, R.F., et al., *Hand-Held Microanalytical Instrument for Chip-Based Electrophoretic Separations of Proteins.* Analytical Chemistry, 2004. **77**(2): p. 435-441.
114. Shaikh, K.A., et al., *A modular microfluidic architecture for integrated biochemical analysis.* Proceedings of the National Academy of Sciences of the United States of America, 2005. **102**(28): p. 9745-9750.

115. Easley, C.J., et al., *A fully integrated microfluidic genetic analysis system with sample-in–answer-out capability.* Proceedings of the National Academy of Sciences, 2006. **103**(51): p. 19272-19277.
116. Hui, W.C., et al., *Microfluidic systems for extracting nucleic acids for DNA and RNA analysis.* Sensors and Actuators A: Physical, 2007. **133**(2): p. 335-339.
117. Liu, C.-Y., et al., *Integrated microfluidic system for electrochemical sensing of urinary proteins.* Biomedical Microdevices, 2009. **11**(1): p. 201-211.
118. Wu, H.-W., et al., *An integrated microfluidic system for isolation, counting, and sorting of hematopoietic stem cells.* Biomicrofluidics, 2010. **4**(2): p. -.
119. Ríos, Á., M. Zougagh, and M. Avila, *Miniaturization through lab-on-a-chip: Utopia or reality for routine laboratories? A review.* Analytica Chimica Acta, 2012. **740**(0): p. 1-11.
120. Hansen, C.L., et al., *A robust and scalable microfluidic metering method that allows protein crystal growth by free interface diffusion.* Proc Natl Acad Sci U S A, 2002. **99**(26): p. 16531-6.
121. Shim, J.U., et al., *Using Microfluidics to Decouple Nucleation and Growth of Protein Crystals.* Cryst Growth Des, 2007. **7**(11): p. 2192-2194.
122. Zheng, B., et al., *A droplet-based, composite PDMS/glass capillary microfluidic system for evaluating protein crystallization conditions by microbatch and vapor-diffusion methods with on-chip X-ray diffraction.* Angew Chem Int Ed Engl, 2004. **43**(19): p. 2508-11.
123. Ramsey, R.S. and J.M. Ramsey, *Generating Electrospray from Microchip Devices Using Electroosmotic Pumping.* Analytical Chemistry, 1997. **69**(6): p. 1174-1178.
124. Dittrich, P.S. and A. Manz, *Lab-on-a-chip: microfluidics in drug discovery.* Nat Rev Drug Discov, 2006. **5**(3): p. 210-8.
125. Pihl, J., M. Karlsson, and D.T. Chiu, *Microfluidic technologies in drug discovery.* Drug Discovery Today, 2005. **10**(20): p. 1377-1383.
126. Sia, S.K. and G.M. Whitesides, *Microfluidic devices fabricated in poly(dimethylsiloxane) for biological studies.* Electrophoresis, 2003. **24**(21): p. 3563-76.
127. Werdich, A.A., et al., *A microfluidic device to confine a single cardiac myocyte in a sub-nanoliter volume on planar microelectrodes for extracellular potential recordings.* Lab Chip, 2004. **4**(4): p. 357-62.
128. Wheeler, A.R., et al., *Microfluidic Device for Single-Cell Analysis.* Analytical Chemistry, 2003. **75**(14): p. 3581-3586.
129. Dittrich, P. and A. Manz, *Single-molecule fluorescence detection in microfluidic channels—the Holy Grail in µTAS?* Analytical and Bioanalytical Chemistry, 2005. **382**(8): p. 1771-1782.
130. Stavis, S.M., et al., *Single molecule studies of quantum dot conjugates in a submicrometer fluidic channel.* Lab on a Chip, 2005. **5**(3): p. 337-343.
131. Lee, C.-C., et al., *Multistep Synthesis of a Radiolabeled Imaging Probe Using Integrated Microfluidics.* Science, 2005. **310**(5755): p. 1793-1796.
132. Auroux, P.A., et al., *Miniaturised nucleic acid analysis.* Lab on a Chip, 2004. **4**(6): p. 534-546.
133. Breslauer, D.N., P.J. Lee, and L.P. Lee, *Microfluidics-based systems biology.* Mol Biosyst, 2006. **2**(2): p. 97-112.
134. Huh, D., et al., *Microfluidics for flow cytometric analysis of cells and particles.* Physiol Meas, 2005. **26**(3): p. R73-98.
135. Suh, R., S. Takayama, and G.D. Smith, *Microfluidic Applications for Andrology.* Journal of Andrology, 2005. **26**(6): p. 664-670.

136. Hong, J.W. and S.R. Quake, *Integrated nanoliter systems.* Nat Biotech, 2003. **21**(10): p. 1179-1183.
137. Weibel, D.B., et al., *Torque-Actuated Valves for Microfluidics.* Analytical Chemistry, 2005. **77**(15): p. 4726-4733.