9 Integrated Multilayer Microfluidic Platforms with Silicon Architectures for Next-Generation Health Diagnostic Systems

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1 Definition of the Topic

Majority of research on lab-on-chip devices was on single layer devices. Stacking a combination of microfluidic layers to silicon architecture gives substantial advantage to integrate precise sensors, actuators, and control systems. Advantages of multilayer

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stack are: (i) multiple functions can be incorporated into single chip and (ii) simultaneous analysis of both macroscopic and microscopic properties, for example, characterizing blood as a bulk fluid and at the individual component level at the same time. Such integrated systems enable the applications that lead to development of comprehensive diagnostics system. Challenges for developing such devices are integrating multiple layers – a combination of biocompatible microfluidics and silicon architectures; individual automated systems that incorporate sensors, actuators, and control systems; development of rapid data analysis and management; and development of diagnostic metrics to manipulate the actuators based on the responses (feedback control). This chapter reviews existing literature and techniques to address the above challenges through the prospect of a state-of-the-art silicon integrated lab-on-chip device with advanced automation coupled with novel data analysis tools to address critical applications in healthcare.

2 Overview

Microfluidics is a crucial part for developing next-generation in vitro diagnostic systems. Integrating these systems with silicon architecture and using emerging machine learning and data analysis techniques is rapidly changing the medical diagnostics systems. In this chapter, we discuss very large-scale integrated microfluidic systems (VLSIMS) that are changing the scenario of point of care diagnostics – real-time personalized blood analysis and organs-on-chip (OOC) systems. We review novel fabrication and integration techniques available to develop these systems. Such integrated systems need rapid data analysis techniques to infer diagnostics; for example, understanding the overall information of the blood as a bulk fluid and also studying the individual components generate massive amount of data. These systems need to swiftly analyze this data and synthesize a comprehensive diagnostic information. Recent trends in machine learning and edge computing are also reviewed in this chapter.

3 Introduction

Lab-on-chip technology is essentially a miniaturized tool handling micro- or nanoliters of fluid to accomplish numerous applications. Microfluidic lab-on-chip technology has gained popularity in fields such as diagnostics, drug delivery, cell biology, computation, and reaction engineering. The immense popularity in detection or sensing, imaging, drug delivery, diagnostics, and cell biology is owed to its many advantages over conventional methods. It instills many advantages such as rapid analysis, high throughput, portability, low space, and sample requirement in various processes [1–3]. In cell biology, spatial and temporal control has been applied to the fundamental study of cell sorting and handling [3, 4]. This field has brought up a revolution in the discovery of nanoparticles due to ease in control of size and shape distribution within microchannels [1]. In addition, low reagent

consumptions and easy fabrication make these devices cost-effective and easy to implement [5]. The following paragraphs bring together the end use of microfluidics in point-of-care diagnostics field and the role of highly integrated microfluidic platforms in optimizing healthcare.

Ultra low cost microfluidic diagnostic devices fitting "ASSURED" criteria (i.e., affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free, deliver to the users who need them), set by the World Health Organization [6], could be pivotal in wellness and preventive care for the extreme point-of-care regime through self-monitoring by an untrained person. Beyond these, we propose here an advanced microfluidic lab-on-chip diagnostics companion for a trained medic at secondary point-of-care like a health center for effective and efficient treatment to increase success rate, speed up recovery, and reduce cost of treatment by preventing repeat visits.

In this data-driven era, advanced point-of-care (aPOC) diagnostics systems for personalized healthcare require rapid analysis of all biological parameters, measurable through minimally invasive techniques, to generate the datasets necessary to feed the cloud-based massive multiparameter analysis. Large-scale integrated and multiplexed microfluidic devices will address this gap [7]. Microfluidic large-scale integration includes thousands of micromechanical valves (actuators), sensors, and control systems on the same chip. Automation at this level is of designing many tiny robots that efficiently handle nanoliters of fluids and also precisely perturb these micro environments to enable simultaneous measurement of multiple parameters. Advanced semiconductor manufacturing technologies will allow for large-scale integration of such robots to a silicon architecture at the chip level for future mass produced microfluidic systems that enable challenging studies like whole blood analysis, organ-on-chip, cancer-on-chip, etc. Figure 9.1 shows the basic building blocks of such advanced systems and highlights the level of integration involved through an example case study of a hands-free rapid multifactor whole blood analysis system that can assist a physician to personalize healthcare for the patient.

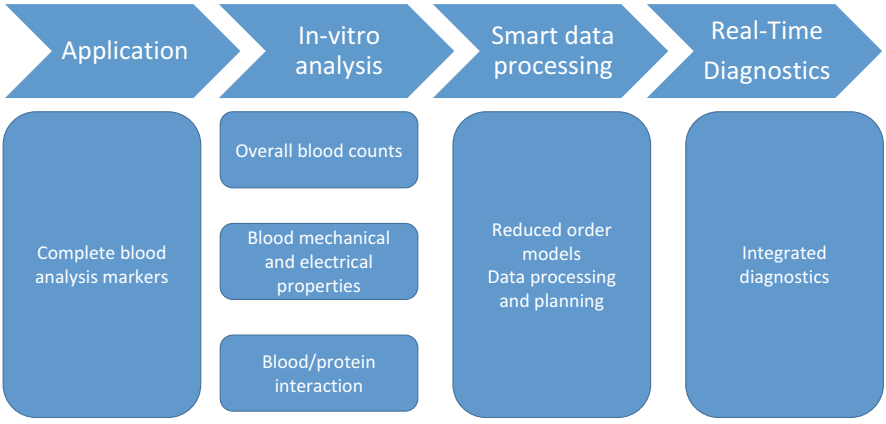

Fig. 9.1 Basic components for a complete blood analysis system

The suggested whole blood analysis application could include, in the first stage, a highly integrated microfluidic system that can simultaneously gather single cell level electrical, mechanical properties, imaging, scatter and fluorescence data for accurate cell classification and enumeration for RBCs, WBCs, platelets, rare cell statistics, etc. while simultaneously analyzing blood plasma for protein markers, free RNA and bulk properties like viscoelasticity, etc. Additional stages of analysis based on initial findings could include preemptive screening for platelet/WBC activation times [8], blood coagulation speeds, drug allergies, as well as other blood-based drug screening to feed a cloud-assisted real-time multifactor data analysis to autogenerate a powerful diagnostics report available during the current patient visit, thus enabling a personalized treatment plan.

This chapter introduces all the critical components like sensors, actuators, control systems, and data analysis tools necessary for such advanced integrated microfluidic systems. The chapter also discusses the current trends and advances in the fields of microfluidics/semiconductor fabrication and big data analysis that can be leveraged to enable large-scale integration and widespread adoption of systems like the whole blood-based diagnostic companion and organ/body-on-chips studies.

4 Experimental Methodology

Spatiotemporal (space and velocity) control of discrete nanoliter volume fluids in microchannels has promising benefits to cell biology, drug delivery, micro-reactors, and nanotechnology. Such applications of lab-on-chip technologies require easy and reliable manipulation of nanoliter volume of fluids. Sensors and actuators form an important part of an actively controlled microfluidic device. Over the recent years, several techniques are developed to simplify the fabrication and prototyping of these devices with novel kinds of sensing and actuation techniques. Innovations in the use of active control in these devices, from open loop to feedback, have widened the applications of lab-on-chip technology. Herein, we review and highlight the basic building blocks that are necessary to achieve complete automated lab-on-chip systems.

All the applications in microfluidics require the following basic components as seen in Fig. 9.2. This section summarizes the existing literature on understanding and fabricating these building blocks.

In microfluidics, controllers are used to regulate either droplet generation or droplet speed or target destination of individual cells or everything simultaneously. Controllers are microprocessing devices that can perform computation based on a predefined algorithm. Every control system has three main components: sensors, controller, and actuators. We identify the control variables (CV) and manipulated variables (MV) based on control applications. Sensors are instruments that can measure and report the CVs and MVs to the controller. The controller works either based on a predefined model that helps determine changes to MV to control the CV or an optimizer that predicts the model in real time to achieve control [9]. The

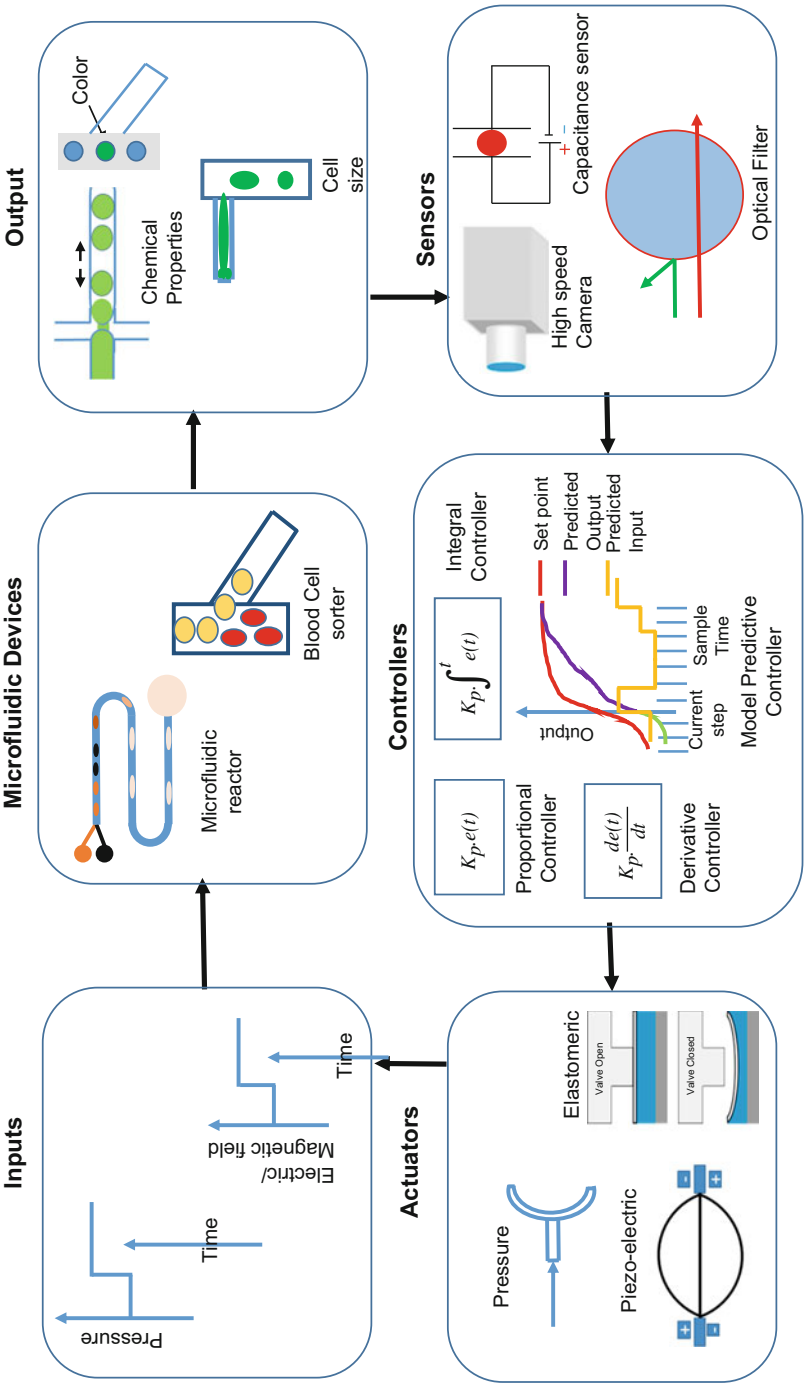

Fig. 9.2 Control system in microfluidics

controller takes inputs from the sensors and dictates the actuators to modify MVs. An actuator is the final element in control system that implements the controller's decision.

We define inputs and outputs with respect to the controller. A controller can have various inputs to communicate with a system to know if the process output is within predefined limit. Similarly, whenever a controller takes a decision to change the MV to adjust the system it communicates to the actuators through various signals, we call these signals as outputs of a controller. Typical examples of controller inputs are pressure, electric, or magnetic signals. Outputs from a controller are signals to the actuators. Typical examples of controller output are magnetic, electrical, or pressure based on the actuator. Most of the time it is electrical signal that is transduced by the actuator. Sensors are devices that communicate the real-time process output/state as an input to the controller. Sensors are of various types such as optical, electrical, and magnetic depending on the control variable. Actuator is the final piece in the controller puzzle. Actuators are mostly valves for flow control or some heat source for temperature control. Droplets can be controlled by flow control devices such as valves. Piezo-electric, pneumatic, and elastomeric valves solve most of the actuation goals in a microfluidic device.

Controllers integrated with sensors and actuators maintain the process under conditions favorable to the user. There are many choices of controllers available such as proportional, integral, derivative, and model predictive controller based on the way the controllers respond to error. We choose controllers based on the frequency of control action, limits on offset, and other constraints.

In this section, we look at the basic actuators, sensors, and controllers relevant to microfluidic systems.

4.1 Sensors and Actuators

A sensor is a physical device that measures system's parameters or variables dynamically. It can either be on-line for real-time data collection or offline for collecting data every few days. A sensor is typically selected based on its performance and reliability. A sensor will usually detect the location and/or composition of the cell/discrete fluid in the channel. In microfluidics applications, many on and off channel sensors are utilized to serve their purpose [10]. Most of the current research is directed toward optical techniques as they are easy to implement and operate in lab-scale microfluidic experiments.

Actuators, on the other hand, bring a change in a variable such that the pressure differs across a microchannel. Here, the actuator's response time after receiving a signal from a sensor plays a vital role in the overall speed and reliability of the system. These are necessary components of a control system. When designed according to system's need, they bring about the change necessary to achieve its objective. The usual approach in the recent works has been to manipulate flow either by varying hydrodynamic resistances or by a valve operated through electrical, mechanical, magnetic, or optical means. The force by which actuators are operated

in various microfluidic applications can be classified into pressure, mechanical, electrical, mechanical, and optical. Sensing in the field of spatial and temporal control of droplets is mostly carried out by optical means.

4.1.1 Electrical

Electric potential difference is widely used for actuation and detection techniques in digital microfluidics due to ease of its fabrication and operation. Actuations by electrical means are usually carried out by applying voltage from an external source. This voltage is supplied (for actuation) or captured (for detection) through various means such as integrated electrodes or wires inserted on the microchannels. The following passages summarize similar techniques in various applications of droplet microfluidics.

Flow cytometry applications such as cell sorting usually require specific reactions or cell properties leading to high preparation and reaction time. Microfluidic techniques overcome these limitations but have low throughput due to miniaturization. Hence, actuators with a high degree of control and ease in fabrication are employed.

Ahn et al. made high-speed microfluidic sorting devices separating water droplets flowing in oil streams. The first device consisted of the inverted Y-shaped channel with one leg shorter than the other and a pair of indium tin oxide electrodes (ITO) electrodes placed at the junction. The drops flow down the waste channel in the absence of electric field due to the low hydrodynamic resistance offered by its shorter length. As soon as the electrodes are activated or energized, the drops move to the collection channel with an average frequency of 1.6 kHz. This frequency was considerably improved to 4 kHz by using bidirectional manipulation. In this setup, the channel lengths were same, and an additional ITO electrode was placed to the left side of the junction. The water droplets moved to whichever side the electrodes were energized [11]. This methodology, however, was not tested on living cells. Additionally, automation using a simple on and off controller could improve the throughput considerably.

A feedback controlled highly sensitive detection and sorting system for living fluorescent cells (*E. coli* expressing R-phycoerythrin) was developed based on similar principles. The voltage supply activated electrodes placed at the separation junction as soon as the sensor detected the colored cells. The polarity thus generated separated the cells suspended in aqueous phase almost instantaneously [12]. A high throughput in cell sorting invariably increases voltage and power requirements. This limitation too was eliminated when Chen et al. achieved a high-speed automated cell sorting utilizing power as low as 0.1 mW, a voltage less than 10 V, and a throughput of 1000 particles per second. This automated sorting system was made of a fluorescence detection, real-time signal processing, and field programmable gate array (FPGA). The microfluidic input channel divides into a waste and two collection channels on the either side. As soon as a particle is detected by the optical system, a piezoelectric actuator (response time 0.1–1 ms) changes the flow dynamics by expanding and contracting and thus pushing the particles to the collection channels. *E. coli* sorting on the same device occurred at a speed of 330 cells/s with high sorting efficiency of 70% [13, 14]. All these devices can be grouped as linear systems where

the applied voltage is clearly the manipulated variable. This external voltage essentially changes the flow direction of suspended cells or droplets by either using their inherent polarity or hydrodynamics of carrier fluid. The on-off controllers employed in such systems seem to suffice, but more precise and reliable control can be achieved by using error-based controllers such as PID. This would, of course, require a thorough study on actuator's influence on flow hydrodynamics on a continuous time scale. Additionally, one would need to handle the nonlinearity hence imposed on the system.

Manifesting electrical actuators to study reaction kinetics in microchannels have been proved useful. Hans et al. used a metal electrode to measure the enzymatic kinetics of hydrogen peroxide (H_2O_2). The electrodes were strategically placed in the channel so that the microbubble creates a signal as soon as it touches both the electrodes. A calibration curve of H_2O_2 concentration vs electric signal strength was used to derive the concentration variation during the actual reaction. The data obtained were used to successfully derive Michaelis-Menten kinetics of H_2O_2. The major highlights of this work were low sample consumption (less than 50 μL), no fluorescence tagging was required, and ease in fabricating microfluidic channel with integrated electrochemical sensor [13, 14]. The inherent electrochemical property of hydrogen peroxide simplified the study. Similar studies on neutral molecules such as protein and organic compounds would require additional techniques such as inducing conductivity or polarity into the molecules. Metal electrodes are commonly used in electrochemical detection techniques but suffer from fouling and narrow potential range. Suea-Ngam et al. used carbon paste electrodes (CPEs), first of its kind, integrated on their microfluidic chip to detect dopamine (DA) and ascorbic acid (AA) in intravenous drugs. The authors determined the optimum voltage, droplet size, and total flow rate for the detection of these components in a micro bubble using their standard solutions. The detection limit in this technique was 20 μM requiring a small working area of 0.25 mm^2. The percentage errors in detection of DA in upamine, domine-250, and dopamax were 0.9, 1.69, and 1.46, respectively. The same for the detection of AA in ascorbic acid was 2.86 [15, 16]. The microfluidic platform resulted in a very high detection limit of the components with small errors when compared to existing batch processes. The low concentration of DA and AA (0.1 M each) in aqueous phase retained its polarity, and hence electric signals could be generated when these microbubbles came in contact with CPEs. As long as the samples are dilute, the electric detection techniques are fast and reliable. The detection technique alone is good enough to achieve the objectives of aforementioned work. These techniques can, however, be coupled with simple controllers to attain the additional purpose. For example, in reaction kinetic study a controller can extend the reaction time when hydrogen peroxide concentration is too low to be detected.

Fast and reliable electric actuation can also be achieved by electrowetting techniques. This kind of actuation uses polarizable and conductive microdroplets as an electrode to complete a circuit with a counter electrode powered by an external voltage source. In one such study, the authors first designed microchips with various configurations of electrodes and insulators. Finally, they demonstrated the

dependence of applied voltage to droplet's velocity. The average velocity of more than 10 cm/s could be achieved for a voltage range of 15–100 V. A threshold voltage has to be exceeded for droplets to start moving indicating a delay or lag in actuation time [15–17]. There are two important deductions in this work. First, the external voltage depends on the average linear velocity of the micro-droplets for different proportional scaling of electrode pitch, gap spacing, and droplet volume. The plots indicated nonlinear trends. Second, the velocity and displacement against time are also nonlinear. The inherent nonlinearity can further be complicated by implementing controllers in such devices. A suitable linear approximation can simplify the control problem where applied voltage can be used as a manipulated variable. As for the control variables, either of velocity and displacement can be chosen based on one's need.

Electrowetting can not only control droplets position but also its size at the point of generation. Link et al. [11] fabricated a microfluidic device to precisely control the droplet size at the point of its generation. The usual infusion methods use the interfacial tension between water and oil to form droplets, but the size of the drops cannot be controlled precisely. This work demonstrated that electrowetting techniques can help overcome this limitation. The external voltage applied to the electrodes fabricated within the device creates an electric field. This field capacitively charges oil and water interface and the water droplet is thus formed. This work clearly deduces the dependence of droplet size on applied voltage. At a constant water infusion rate of 20 nL/s, decreasing profiles of droplet sizes vs voltage applied were obtained for three different infusion rates of oil (80, 110, and 140 nL/s). Further, oppositely charged water droplets were fused together under similar device configurations [16, 17].

Controlling droplet size is not enough unless a uniform droplet size or volume can be obtained throughout the process. Bransky et al. created such uniform droplets (less than 3% deviation from average size) using a controllable piezoelectric actuator integrated on their microfluidic device. A cross and a T-junction with water and oil reservoirs (placed at a fixed height) were used separately to create droplets. The piezoelectric actuator was inserted into the PDMS device such that it rested a little away from the membrane. The external voltage amplitude of the actuator ranged from 30 to 120 V changing the droplet volume linearly from 260 to 950 pL [18, 19]. The linearity in applied voltage and droplet size ($R^2 = 0.993$) calls for a feedback control system for precise on-demand droplet generation of desired size or volume. The droplet size can be a controlled variable, while the voltage can be manipulated to move the actuator and hence the membrane at the oil and aqueous phase junction.

4.1.2 Optical

Optical means are popular in microfluidic manipulation and are mostly used as sensors. Since this field is still at experimentation level, an optical microscope attached to a CCD camera is an affordable and reliable sensor. Researchers integrate various techniques to improve the detection limit as summarized in the following paragraphs.

A high-end optical detection method using a confocal microscope, mirrors, and optical fibers was set up with the microfluidic channel to separate *E. coli* expressing R-phycoerythrin. The setup consisted of Ar ion laser, two dichroic mirror filters, optical fibers, band pass filters, avalanche photodiodes, and a pulse divider. The laser coupled into the microscope using dichroic filter excited the fluorescent cells. The light collected by objective was split into green and red spectral parts and directed into respective entrance slits by optical fibers. Additional band pass filters in these fibers ensured enhanced detection and suppression of background light. The signals were detected by avalanche photodiodes and split by a pulse divider for one part to reach the analyzer while the other reached the hardware processor which automatically controlled the sorting [12]. The use of filters at strategic positions ensured a high signal to noise ratio and hence a reliable detection system. However, the instrumentation is expensive and not portable from a commercial point of view. A laser-induced fluorescence detection was used by Cao et al. to sort encapsulated particles using a solenoid valve. A set of neutral density filters adjusted the intensity of laser beams focused on the channel. A dichroic beam splitter reflected them into the objective. Spectral filters improved the quality of fluorescent emission collected by the same objective. The lights collected were then converted into electrical signals using a PMT equipped with a low noise current preamplifier. A differential comparator processed the signal and fed it to a microcontroller [18, 19]. This technique uses similar working principles with more precise results but is limited to laboratory experiments.

Most optical sensing systems suffer from propagation loss within the channel. A high-intensity beam is a solution, but it is a challenge to determine an optimal intensity to excite living cells without incurred damage. Cho et al. coated the walls of their microfluidic cell sorter with amorphous Teflon. This not only created a waveguide during laser excitation of the mammalian cells, it ensured an optimum intensity to brighten the cell without any mutilation. The fluorescence thus emitted is collected by a microscope objective. The signals are then filtered consecutively by spatial and optical filters before entering a photomultiplier tube (PMT). The fluorescence signals when modulated by this spatial filter register different waveforms on PMT corresponding to different locations of the cells through the channel and thus tracking their path. The output from PMT is fed to a real-time control processor embedded with field programmable gate array (FPGA) which automates the mammalian cell sorting [20]. Microfluidic-based mammalian cell sorting imposes challenges such as high throughput, purity, and recovery of unstressed cells. HeLa, a kind of mammalian cells which express a fused histogen-green fluorescent protein, was sorted rapidly using optical switch control overcoming the above difficulties. The sorting device consists of a three-way microfluidic network (input, waste, and collection channels), a near-infrared laser for an optical switch, a visible wavelength laser for detection, and fluorescence measurement. The infrared laser deflects the detected cells to the collection channels using laser beams. The laser-on or response time of this device ranges from 2 to 4 ms with varying cell density in the input channel. The throughput ranges from 23 to 106 cells per second with purity as high as 98.5%. The total sorting time varied from 9 to 44 min [21]. Living cells are

delicate and can easily be damaged during analysis or experiments. The actuation by optical means other than being fast and reliable can be applied without distorting living cells.

Optical detection techniques are highly advantageous for monitoring microscale reactions for a simple reason that light can penetrate these devices easily. Lignos et al. manufactured lead sulfide (PbS) and lead selenide (PbSe) nanocrystals (NCs) in a droplet microfluidic system integrated with real-time detection to map the chemical reactions. The droplets exiting the channel after the reaction were optically excited using light emitting diodes (LED). The fluorescence spectra from individual droplets were then measured using a fiber-based spectrometer. The authors summarize the variations in the droplet spectra as the initial conditions such as substrate ratio, temperature, and residence time changes [22]. The inferences from these spectral emissions can be analyzed to make suitable control algorithms. The control techniques can drastically improve upon the reaction kinetics and hence the required product composition. A paper by Krishnadasan et al. formulated a control algorithm for the synthesis of nano-cadmium selenide (CdSe) QCs using microfluidic channels. The reacting particles in the channel were ignited using laser and the fluorescent spectra thus emitted were monitored using a CCD spectrometer. The data from variations in the emission spectra with a change in temperature and substrate injection rates were utilized to form a control algorithm which can drive the reaction to the desired product composition and size [23]. Maceiczyk and Demello suggested an on-demand synthesis of QCs (CdSe and CdSeTe) using similar optical detection system. They used the data from emission spectra of the reacting particles to formulate a model-based algorithm using Universal Kriging [24]. All these novel algorithms are yet to be visualized through experiments. Nevertheless, these spectral data can be used to predict reaction conditions in a microchannel without the need of setting the sensor up every time a QC is manufactured.

The online detection in nanomaterial manufacturing simplifies the kinetic studies for microscale reactions occurring in microchannels. The mode of detection can be photothermal, fluorescence, or absorbance spectroscopy depending on the sensitivity requirement for a given reaction. All of these methods give a comprehensive idea on the shape, size, and chemical composition of the formed nanoparticle [25, 26].

4.1.3 Pressure

Pressure actuation in microfluidic devices is brought about by using integrated or external valves. The opening and closing of these valves are operated through a vacuum or inert gas inlets. The following paragraphs bring together works that have used pressure actuation in manipulating droplets in the microchannel.

Sorting and trapping of EGFP fractions from pNB estrange (two different expressions of *E. coli* bacteria) were accomplished using pressure controlled mechanical valves. These valves were integrated on a T-shaped microfluidic channel using multilayer soft lithography techniques. An algorithm controlled the opening and closing of a set of five valves (three peristaltic and two switches) for trapping and reverse sorting. The valves responded in 5 microseconds. The recovery rate of EGFP fraction varied from 16% to 50% consuming 2 to 3 h [26]. The relation between the

cell velocity (control variable) and pump frequency (manipulated variable) is linear. Here, the valves are operated by an on and off the controller. An advanced controller such as PID can smoothly open and close the valves to control the cell's velocity with more precision, hence improving the sorting time and rate. Integration of such complex control system into the microfluidic channel is an area if explored can unlock new paradigms.

The sorting of living cells with no damage was achieved using pressure actuation. The system consists of a fluorescence detection method, a sorting microfluidic channel and an actuation channel. The dynamics of the flow in actuation channel is controlled by a solenoid valve. As soon as the desired cell is detected, the solenoid valves open, pushing the fluid in the actuation channel toward the collection channel. The cells move toward the waste channel by default due to its shorter length. This system has a sorting frequency as high as 50 Hz with 1 ms response time of the valve. The living cells could be sorted with a throughput of 30 cells per seconds with collection percentage of 93.7% [19]. Despite following an off-chip actuation system, the response time was very high. The clear reason behind that is having a separate detection and actuation channel in the device. That way, the cells and the particles are studied and manipulated individually. This will increase the overall sorting time, but the purity in sorting is very high as demonstrated. The feedback control used here is an on and off the system. A more complex control system can give better output in terms of manipulating cell's location. This, of course, would require a thorough study on the effects of applied pressure on flow hydrodynamics in the microchannel.

Steering of droplets in microchannels using an integrated valve in the single layered device can make microfluidic applications simpler. Abate and Weitz characterized such device relating the actuation pressure to the average flow velocity of droplets. Apparently, the linear relationship between the two indicates a simplified control problem. The actuator here was a membrane valve which is essentially a T-shaped channel placed perpendicular to one of the two branches to the main inlet. This membrane expands on the application of external pressure and blocks the branch which in turn changes its hydrodynamic resistances. This makes the droplets flow into the other branch [27]. The actuator response time, although not studied, would be low enough to build a feedback control for this problem. It can be a single input single output system with actuation pressure as manipulating variable and velocity of exiting droplets as control variables. The quantification of membrane contraction or expansion with varying actuation pressure would be an essential requirement.

The usual technique to generate droplet is to use an external device to pump oil and aqueous phase through channels. The surface tension between the two phases forms droplets at the junction. A 2009 work by Zeng et al. uses an integrated valve on T-shaped microchannel to generate droplets on demand. The aqueous and the oil phase flow under negative pressure applied at the end of the channel. The valve, placed at aqueous phase inlet, when closed, the water flow cuts off and a droplet is formed immediately at the T-junction. The response time of the microvalve (50 to 500 ms) is varied to decide on the optimum droplet size and volume (1.3 to 13.3 nL). The authors successfully show the linear dependence of microvalve open/response

time to the droplet size. Further, they create and fuse multiple droplets by extending the number of channels and valves [28]. The objective of this device here is to generate droplets on-demand by solely changing the opening of the microvalve. The relation between the valve opening time and the droplet size is linear, which means the system can be approximated to a first-order linear system. The actuation time here is represented in terms of the time it is taken by a valve to open completely which is at least 50 ms. It is then varied to understand the relationship between droplet size measured as its volume. It can be considered to be a step input system with an output represented by droplet size and the manipulated variable being the actuation time or valve opening itself.

4.1.4 Magnetic

Magnetic actuation in spatiotemporal control of droplets is an area less explored when compared to other techniques. The related works, although few, are promising from automation and control perspective and are summarized below.

Di-electrophoresis (electric manipulation of biological cells) is known to denature the cells to some extent. Lee et al. hence used electronically controlled magnetic field to spatially control the movement of bovine capillary endothelial cells (BCE). Their device consists of the distribution of micro-coils over a microfluidic channel connected to an integrated circuit (IC). This IC supplies current and controls the magnetic fields by activating the micro-coils (one at a time) to move the microfluidic bubbles to the desired location. At a single magnetic peak intensity of 15G, BCE containing magnetic beads could be trapped at the force of 50pN. It moved to a different location with an average speed of 6 μm/s by subsequent switching of alternate magnetic coils [29]. The writer demonstrated an excellent example of an integrated device which can single-handedly manipulate particle location in a micro-channel. Although the experiment was carried only for discrete movement of droplets, it can be extended for a continuous flow with real-time integrated control. It will require a detailed study on how the magnetic force (manipulated variable) affects the droplet position (controlled variable).

Manipulating cells in channels with precision and control is a sought after field for the wide range of applications it covers. Magnetic manipulation of fluids for splitting, exchange, trapping, and demulsification was achieved by Zhang et al. by using hydrophobic ferrofluid as a continuous phase in their microchannels. Magnets of size ranging from 1 to 3 mm were used to create magnetic fields. The change in effective magnetic field gradient broke the droplets in the main channel into three different ones which moved along the three branches. The ferrofluid not only acted as a hydrophobic continuous phase but also directed the droplet motion as its hydrodynamic changed with external magnetic field. Further, they demonstrated trap and release of water droplets in another device containing microwells. These wells stored the droplets when the ferrofluid repelled from the external magnetic field and released them when the external magnetic field repelled the fluid in wells [30]. This magnetic droplet merging, splitting, and trapping system can be made efficient and precise by implementing control techniques. The fluid motion actuating magnetic field can serve as a manipulative variable, while the interactive magnetic

force between the fluid and external field can be the control variable. This force plays a major role in droplet motion, thus a mathematical relation between the two would be essential to design control techniques.

4.1.5 A Comparative Study on Actuators

Optical detection methods are widely used in various application of microfluidics. The reason behind the same is a demand for high-speed detection systems of droplets of nano- and microscale sizes. The optical detection systems although following the same working principles can still be categorized based on the methodologies and excitation sources. It can either be on-chip meaning the sensor is integrated into the device or off-chip where a sensor is connected to the device. The detection technique in both on- and off-chip sensors can be classified into fluorescence, absorbance, laser-induced fluorescence (LIF), and chemiluminescence. Each of these techniques can be coupled with devices such as optical fibers, CCD cameras, or photomultiplier tubes (PMT) in an off-chip approach. On the other hand, waveguides, CMOS imager, or photodetectors are integrated on the device in an on-chip approach. Despite all the advances, these techniques have detection limits on practical time scale [10, 11]. Both on- and off-chip approaches are utilized in the spatiotemporal control of droplets. Synthesis of nanomaterials such as quantum dots is best monitored by optical means for the need of quick detection of the high-speed reactions. Algorithms to control nanomaterial manufacture in microchannels have been suggested based on the radiation emitted by the reacting particles as summarized under Sect. 4.1.1 of this chapter. However, active control using actuators is not yet studied in the literature. Actuators can be used in microchannels to precisely control reaction rates and microreactor (droplets) size to achieve desired nanomaterial structures with high accuracy.

The electric means are the most popular actuation techniques in droplet microfluidic owing to the high processing speed. Although in an application such as cell sorting that involves living bacterial or mammalian cell, pressure and magnetic actuations are a better choice over electrical to prevent cell deterioration. Electric actuation and optical detection are also favored in studying rapid kinetics over other techniques because the molecule under study exhibits polarizing property or is soluble in water. The same technique would not help kinetic study nonpolarizable or water insoluble compounds. More techniques are yet to be explored in this area.

Pressure and magnetic actuation systems are less explored when compared to electric and optical systems. Both on- and off-chip systems require external instrumentation which adds to the cost and reduces portability. Besides, the processing speed in such actuations is relatively low.

4.2 Fabrication

Since the standardization of microfluidic device fabrication [31], soft lithography has branched into many techniques to suit various applications. For instance, integrating micro sensors and actuators into devices has added new protocols to the classical

methods of fabrications. The devices designed vary in their fabrication methods. In the field of automation and control, the fabrication methods are broadly classified into the following.

4.2.1 Single Layer Soft Lithography

Soft lithography is widely used in fabricating microfluidic channels because of the ease in prototyping and handling. A transparency of the desired channel design is patterned on a spin-coated (with photoresist) silicon wafer using UV rays. This wafer (now called master) is covered with polydimethylsiloxane (PDMS) and cured in an oven at desired temperature. The mold hence formed is irreversibly bonded to a glass substrate. The microchannels are now ready for experiments. Single layered devices can be further categorized into those with or without integrated features. These additional features on the devices can be either in situ, meaning integrated into the channels, or ex situ, meaning connected to channels externally.

4.2.2 Ex Situ Features

In ex situ features, the channels are designed to have a simple additional channel or a reservoir to bring about actuation and detection from an external source. The dimensions of these features, although not optimized, are chosen to give the best actuation and detection speed.

The work by Dittrich and Schwille (2003) demonstrated how polarity switching can sort fluorescent cells in a T-shaped microchannel. They achieved a high sorting rate by inserting external electrodes at the junction of waste and collection channels. The additional feature on the channel was reservoirs. These reservoirs were punched before bonding to the glass slide and were used for inserting the external electrode wires. An electric controller connected to these wires changed the polarity as and when desired [12]. Another high-speed cell sorting using external solenoid valves was achieved by Cao et al. In addition to the inlets for water and oil, the device had an interrogation (23 μm wide) and actuation channels (80 μm wide and 2 mm long). The fluid flow in the actuation channel was controlled by the external valve, while the interrogation channel was used to observe the bubbles to be sorted [19]. Lignos et al. synthesized and observed semiconductor nanocrystals by adding an additional channel to their device. A three-channel inlet (for lead, sulfur, and carrier oil) was connected to an outlet. The outlet channel was continued to a heating zone where the reaction occurred, seen by a rapid color change in droplets. A channel free from oil (career fluid) followed the heating zone where the drops were excited by a light emitting diode (LED). The fluorescence spectra from individual droplets are measured through a fiber-based spectrometer to study the reaction kinetics.

4.2.3 In Situ (Integrated) Features

To achieve an objective, for instance, to control sorting of droplets, the additional feature in the microchannel can be integrated on the device itself in the form of valves or membranes. The actuation and detection speed gets improved. The device gets more portable eliminating the need for external devices.

The work by Zhang et al. achieved manipulation and collection of droplets using magnetic blocks of portable sizes (1 to 3 mm long). These blocks manipulated the carrier fluid containing magnetic nanoparticles and the flow hydrodynamics. The aqueous droplets under the influence of the flow could be relocated, split, trapped, and demulsified within the channel [30]. Zeng et al. used a T-shaped channel integrated with a microvalve to control droplet generation, size, composition, and their fusion. This pneumatic microvalve was constructed at the opening of the oil phase inlet. The flow of the oil and aqueous phase was controlled by regulating the pressure in evacuated bottles attached at the end reservoirs of the channels [28]. A flexible elastomeric membrane integrated on the device in a single layer adds to the many advantages of single layer soft lithography. The device consists of a main channel and a T-shaped control channel placed 13 μm apart. The fluid entering the control channel inlet compresses the main channel to change its hydrodynamic resistance and thus the velocity of the fluid in it changes [26, 27].

4.2.4 Double-Layer Soft Lithography

The devices in this technique are formed by bonding two layers of PDMS molds irreversibly first with each other and then on a glass substrate. Each layer has its own function to perform. Double-layered microfluidic channels almost always have integrated features that lead to either actuation or detection functionalities in them. Such features in spatiotemporal control of droplets are summarized below.

Fu et al. devised a control layer to integrate valves and pumps into their device. The channels meant for sorting cells were constructed on a different layer called fluidic layer. Control layer consisted of multiple lines ending with valves. The closing and opening of the valves were actuated by the flow of pressurized nitrogen and vacuum, respectively. The whole cell sorting process was automated by digitally controlling the valves through a fast Zener-diode circuit [26]. The pneumatic control setup was fabricated as previously described [32]. Guo et al. used the similar double-layered device to create a droplet on demand assay. The control layer is a set of single-layered microfluidic channels that ends with a valve at the crossing points of channels in liquid layer. The valves are actuated by compressed air with pressure around 400 kPa. This pressure is provided by electronic solenoid valves under the control of data acquisition module [33].

4.2.5 A Comparative Study on Fabrication Techniques

Integrated (in situ) features for actuation or detection are always at advantage over ex situ categories in both single- and double-layered microchannels due to their ease of handling and operation. The only advantage of a single-layered in situ feature over double layer is the less time consumed in fabrication. But as the system gets more complicated to handle more than two control functions, another layer to control the actuation device is desirable over single-layered in situ devices. As more researchers have progressed toward in situ features, there are many areas still unexplored. For example, the dimensions of these additional features are not optimized for their function. A fully automated detection-actuation-controller microfluidic system to control the position and velocity of droplets is yet to be invented.

Researchers are increasingly using droplet-based microfluidics-based systems for diagnosis (biomedical devices), reaction analysis, discovery of drugs, droplet-based display, and identification of cells. A lot of these applications require sorting or screening of droplets/cells, synchronization of droplets, and sometimes segregating and storing droplets. Though many passive techniques exist to control droplets, some applications need active control strategies using a sensor, a controller, and an actuator. In addition to the challenges involved in fabricating a droplet-based device, designing an active control system to improve the applications of droplet systems is a field on its own. Spatiotemporal dynamics of droplets in microfluidic networks can be quite complex to model and control as droplets have shown to exhibit interesting nonlinear dynamics in networks [34]. In the following sections, we describe and discuss the various controllers and their applications in digital microfluidics.

4.3 Multilayer Microfluidic Devices(MMD) and Integration with Silicon Layer

Multilayer microfluidic devices have been explored and demonstrated for applications that need more functionality than one layer can deliver, including organ on a chip [35, 36], devices needing more sophisticated structures like valves, pumps, etc. Techniques like anodic bonding, 3D printing and 3D printing with pattern transfer (printed transfer molding) [37, 38], adhesive bonding [39, 40], etc. have been employed to achieve multilayer devices of reasonable complexity. Often times these multilayer PDMS/glass/PMMA microfluidics have also been coupled with silicon layer to leverage the precision electronics (sensors, LEDS, etc.) semiconductor manufacturing can deliver [41, 42]. Silicon layers can include optical detectors, electrical/electrochemical sensors, LED and laser light sources, amplifiers, ADCs, noise filters, and signal processing elements [43, 44].

4.4 Controllers

A controller achieves an objective, usually maintaining a set point, with the help of sensors and actuators; process model present in the controller or a real-time model predictor enables a controller to adjust certain variables in order to achieve zero error situation. There are controllers that operate without any sensors or actuators such as open-loop controllers; they continue to operate autonomously without observing the state of the process. While there are controllers that compute the actions, on a limited frequency, based on the current state of the process or the predicted effect of disturbances, such as feedback and feedforward controllers.

In a microfluidic device that transports confined droplets, a feedback of the implemented controller action benefits the controller's ability to predict and correct future errors with precision. Feedback controllers such as proportional-integral-derivative (PID) and model predictive control (MPC) are powerful controllers that can take on complex tasks such as spatiotemporal control of droplets. Figure 9.2a, b,

and c illustrate the instances where an open-loop, on off, and MPC controllers can be used in microfluidic devices that involve droplet control. A controller operating based on changes in set-point is said to solve a servo problem, while a controller maintaining a set-point despite the process disturbances is said to solve a regulatory problem.

The first step in designing a controller is identifying the process inputs and outputs and modeling their dynamic behavior. In a feedback controller, controller input is the dynamic process output/state that is monitored to gauge the quality of the process, for example, droplet position, color, and volume. The controller inputs are usually referred to as control variables (CV). The input is compared with the set-point to compute the error. Controller output is the change in process input necessary to attain zero error in the process. The controller calculates it based on the dynamic process model and the error computed from the process; typical examples of controller output in a microfluidic device are change in inlet flow rate of oil and change in voltage to electrodes. We refer to controller inputs as control variables (CV) and outputs as manipulated variables (MV).

A mathematical model of process dynamics forms the basis of relation between controller input and output. Process models are developed based on the choice of control variables in the system. Hagen-Poiseuille law relating pressure drop and flow rate in a pipe is an example of a process model that can be used to control pressure drop in a channel by manipulating the flow rate.

Models used in droplet microfluidics involve variables (inlet flow rate, inlet pressure, inlet sequence) and parameters (length of channel, viscosity of fluid, density of fluid) that determine outputs like droplet traffic, droplet sizes, droplet positions, droplet velocity, etc.

4.4.1 Classification of Controllers

Controllers are either open loop or closed loop based on their nature of interaction with the system. An open-loop controller that is autonomous in nature does not keep track of the consequences of its control action, while a feedback controller continuously monitors the CVs for feedback on its control actions. A feed forward controller on the other hand predicts the effect of disturbances on a process before they are realized, to make control actions.

Open-Loop Controllers

Controllers that operate without feedback from a process are open-loop controllers. The decisions by an open-loop controller are completely independent of the current state of the process. These controllers are capable of solving simple and repetitive tasks. The processes involving uncertainty and complex dynamics cannot be controlled with these kinds of autonomous controllers.

In droplet microfluidics, droplet sorting based on charge does not require feedback. Link et al. [16, 17] have demonstrated the use of the electric field as an open-loop controller to sort positively charged droplets from the other droplets. This control method offers a lot of flexibility and speed as it involves switching electric field without moving parts and a very simple control logic. The large forces that can

be applied to a droplet make this technique robust for sorting of charged droplets. Neutral droplets could also be charged and controlled using this method [16]. Figure 9.3 shows sorting of positively charged droplets to one branch of a microfluidic network using the electric field.

Feedback Controllers

A controller that operates based on feedback from the process is a feedback controller. These controllers can monitor the process changes in the presence of disturbances and adjust the process inputs to maintain the CV at a set point. Feedback controller needs sensors that can continuously send data on the CV or on a state of the system that can help determine CV.

Feedback controllers are classified into proportional, integral, derivative, and model predictive controllers based on the nature of their control action. All of these controllers take action based on the process model and the control variable involved in the decision. Automation in chemical process plants has led to the development of these controllers, and through control researchers, they have found their way into digital microfluidics. We describe the nature and applications of these controllers under the coming sections.

Proportional Integral and Derivative Control (PID)

Proportional controllers increase or decrease the process input proportionally with the error in process output. Proportional controllers are easy to implement; however, they carry an unavoidable offset. For processes that require precise control, proportional integral controllers remove this offset by adding an integral time constant to the model. Most often PI controllers are enough to achieve control over processes,

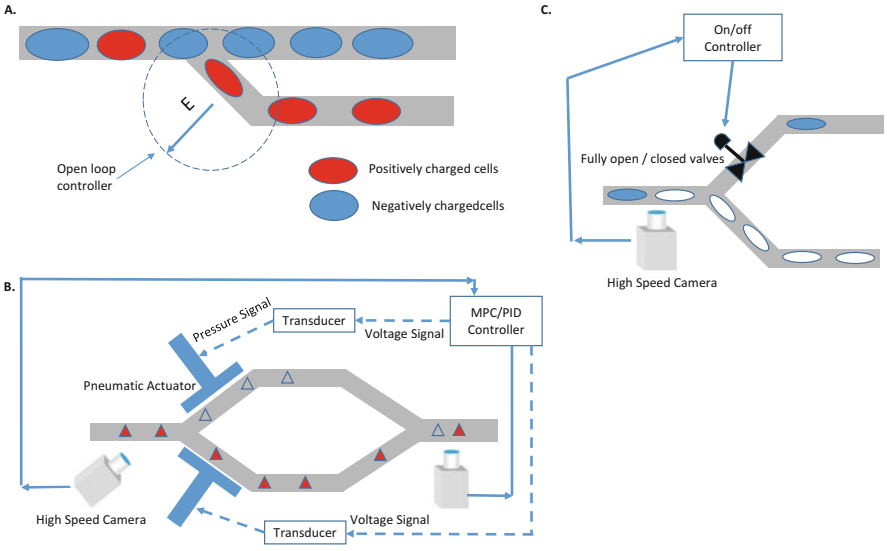

Fig. 9.3 A basic controller classification

while some processes have strict limits on oscillations in output where a derivative action is added to predict the error in advance and stabilize the process.

Feedback controllers have found some interesting applications in microfluidics. They are employed for precise droplet generation, fusion, and splitting. The dynamics of droplet transport in microfluidic networks presents a challenge in the selection and design of the controller.

All electronic droplet generation without moving parts uses feedback control to produce droplets of various sizes synchronously without a syringe. All electronic droplets generation performed using electrowetting on dielectric (EWOD) was demonstrated by Jian et al. in the year 2008 [45].

The droplet volume was continuously measured through capacitance, a technique established by Fair et al. The feedback of droplet volume is used to control the voltage across two sites. The error between the target capacitance and the real-time capacitance is minimized using a proportional-integral-derivative (PID) controller to achieve the generation of a droplet of the desired volume in the creation site. Figure 9.4a is a representation of the feedback process that was used in their work to generate small daughter droplets from a mother droplet in a reservoir. In this work, the hardware was designed to update the voltage outputs and measure droplet volume as fast as 1 ms in order to achieve actuation and sensing criteria for less than 1% volume control precision for a 225 nl droplet. A proportional controller could solve the problem very well but the oscillations in droplet volume, once the droplet is generated, is better controlled through the derivative action and the error accumulation due to capillary forces are dealt with the integral action of the PID controller. An error of less than 4% in the final droplet volume has been reported in the literature for this technique [46].

PI controllers are also used to control droplet lengths to produce droplets of various volumes in a T-junction. Flow rate ratio of continuous and dispersed phase is linearly related to the length of the droplet formed in the T- junction at a low capillary number ($C_a \leq 0.1$). PI controller implemented with this model that predicts the droplet size from the flow ratio achieved precisely controlled droplet generation; however, the control speed was limited by the speed of image processing [47], a classic example of sensors limiting the efficiency of the control system.

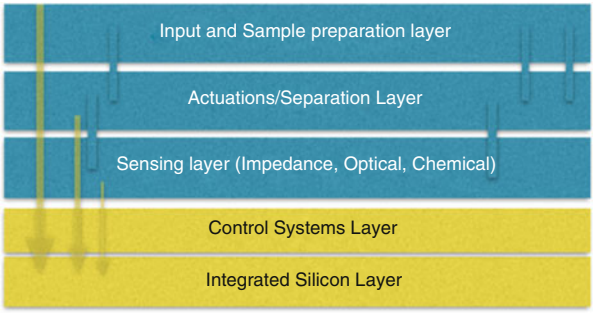

Fig. 9.4 Integrated microfluidic platform layers

Another example of PID controllers in droplet microfluidics is the spatiotemporal control of droplets with feedback. Improved stability and precision control are some of the benefits offered through these controllers. Yoko Abe et al. have demonstrated the use of feedback-based spatial control of droplets in a device filled with electro-conjugate fluid (ECF). ECF generates a powerful flow (ECF flow) when a high voltage is applied across a pair of electrodes in the device. The position of the droplet is sensed through image processing technique, and the controller decides the next voltage signal to drive the droplet closer to the target position. Closer the droplet to the target position, lesser the voltage applied to ECF. The block diagram in Fig. 9.4b captures the essence of control algorithms used in the device. The position is measured as a function of a pixel in the "x" coordinate (x_c) and compared along with the target coordinate (x_t). The error (ϵ) decides the voltage to be applied between the electrode pairs to move the droplets [48]. Image processing speed/optical detection limits the speed of the applied control technique. On-chip integration makes control easier with just the sensor as an external device.

Model Predictive Control (MPC)

Mathematical models describing the dynamic behavior of processes are nonlinear in nature. Yet, for the design of PID controllers, they are linearized around their target value introducing errors in control action. Though theses errors are tolerable in most of the processes, some operations like spatiotemporal control of droplets require the use of nonlinear models to achieve precise control.

Model predictive controller also works on nonlinear models, optimizing the input values needed to achieve minimum error taking into account all practical constraints. Most of the practical constraints such as sampling time and actuator limitations are accounted as constraints in an optimization algorithm to find the best actuation/control moves that can minimize the process error. MPC can handle multiple PIDs, processes with MIMO type models, and multiple disturbances. It is the complexity of the optimization problem that determines the efficiency of MPC.

Controllers based on MPC use the process model along with constraints to calculate an optimized set of actuations that will minimize the error in process output when implemented. Synchronization of droplets is an example of a nonlinear problem in digital microfluidics. Droplet synchronization calls for feedback and continuous actuation of the valves or other actuators. Maddala et al. [47] have simulated the use of MPC-based control algorithm for sort-synchronization of droplets in a microfluidic loop. Droplet positions are sensed through a high-speed camera, and optimal valve actuations are determined that will synchronize droplets at the outlet of the channel. Each time when an input is received, a new set of optimal actuations are determined and the first of them is implemented.

There are not enough applications of MPC in digital microfluidics. This type of controller in digital microfluidics can tackle the highly nonlinear and complex models observed in this field and can be seen as an opportunity for control researchers.

Fuzzy Logic Control

Fuzzy controllers are unique in the way they process the inputs received from the process. Unlike the other feedback controllers they do not have a process model that they use to determine the best manipulated variable. Instead they work very similar to the human operators in an industry. Fuzzy logic needs a well-defined table of outputs and inputs and some classification into different sets based on process need. They have fuzzification, processing, and defuzzification stages before they decide on the precise action to be implemented. In fuzzification, the input values are converted to linguistic terms such as high, low, cool, hot, etc., based on predefined sets in those names. Then they are processed to identify the corresponding fuzzy output such as increase the speed or decrease the flow rate, etc., to be applied to the process. In the end the output is defuzzified to a crisp output value that is applied to the process. They are very popular in the field of robotics where the encountered models are highly nonlinear. Fuzzy control logic works based on experience and heuristics. An unknown process cannot be handled with a fuzzy logic. Fuzzy controllers are referred to as intelligent controllers because of the way they process the inputs.

Fuzzy control has been used to control a pneumatic pump to manipulate positions of droplets in a biochip. Yung Chiang Chung et al. [49] have demonstrated the use of fuzzy logic-based controller in improving the extraction efficiency of DNA. The sample and buffer were moved at a necessary rate to achieve improved collision and better reaction efficiency through fuzzy logic-based controller. Jie Gao's [49, 50, 51] work on multi-droplet manipulation, moving, merging, and chasing using electrowetting and fuzzy logic-based feedback controller is another example of how fuzzy logic could be exploited to improve the applications of lab-on-chip technologies in the future.

4.4.2 Optimization

Optimization is the process of minimizing or maximizing an objective function within the defined constraints and few variables to manipulate. The variables that are allowed to change are decision variables whose choices will determine the best value of the objective function. In synchronizing droplets, exit time difference is the objective function, actuator speed the constraint, and the actuator positions as the decision variables. Optimization is also used in designing complex microfluidic networks to achieve a predefined objective such as combinatorial chemistry [52–54].

Complex nonlinear models like spatiotemporal dynamics of droplets need fast and reliable optimization techniques and call for the use of powerful controllers like MPC. Each droplet makes discrete decisions in a continuous flow system. Synchronizing droplets to form patterns then becomes a mixed integer nonlinear programming (MINLP) problem. Optimization of MINLP systems within a small period (time) available before a droplet makes the decision forms a key challenge in the application of MPC in real time. Maddala et al. [47] have shown that an optimization technique coupled with MPC could theoretically alter the spatiotemporal dynamics of droplets in a microfluidic device.

5 Key Research Findings

5.1 Whole Blood Analysis

Blood is a complex biological fluid comprising cellular components suspended in protein-rich plasma. These components including WBCs, RBCs, platelets, cell-free nucleic acids, exosomes, proteins, lactase, glucose, antibodies, other biomarkers [55, 56], and rare cells like circulating tumor cells (CTCs) [57], circulating mesenchymal cells (CMCs), circulating endothelial cells (CECs), and putative circulating stem cells (CSCs) perform distinct functions in vivo, and their enumeration and enrichment have been of enormous significance to clinical diagnosis and biological research as they are known to reflect the physiological state of the person, disease progression, and development of resistance to therapy [58]. Due to this and its 2 h in vitro half-life as a fluid, innumerable microfluidic studies have been published on blood with focus on study of one or more of these components as biomarkers for a particular use case. Microfluidic platforms have been used on blood samples for real-time cell enumeration and differentiation using optical sensors (imaging, scatter, FACS-style [59], spectroscopic, SERS [60], SPR) [10, 61] and electromagnetic sensors (resistive, capacitive, magnetic, electrochemical, piezoelectric, micromechanical, faradic) [62–70]. This classification information is also used to initiate cell separation/enrichment within the microfluidic devices using a variety of approaches including flow shaping [71, 72], pressure driven [73, 74], dielectric [71, 75], magnetic [76, 77], optical trapping [78–83], etc. Beyond these real-time cell level analysis technologies, many microfluidic technologies have been developed to perform blood-based analysis that need short incubation/processing times needed for applications like signal amplification and detection methods, including PCR, immunoassays [69, 84], biosensors, and cell/platelet activation/drug response [85–89] studies which provide further insights.

Here we envision a multilayer microfluidic device coupled with a silicon sensing layer with integrated real-time computation for simultaneous tracking of the largest feasible set of biomarkers/sensors with the hypothesis that multiparameter parallel data collection could lead to novel insights that are otherwise masked. The hypothesis draws strength from advanced semiconductor manufacturing technology that could deliver a single system-on-a-chip with integrated components including arrays of image sensors, solid state LED/photodetectors, precise electromagnetic components for measurement, amplification, noise-filtering, ADC, and signal processing, as well as direct integration into onboard CPU/GPU/FPGA/VPU for lossless real-time analysis coupled with the versatility of microfluidic platforms that can prepare and deliver the sample of interest to the sensors at the sensing layer.

In the use case of blood analysis, this would allow for simultaneous measurement of glucose, cell counts, and cell/platelet differentiation based on optical imaging [59], differentiation based on autofluorescence and fluorescence [69, 90–92], differentiation based of light scatter, light absorbance, electrical impedance [69], capacitance, blood plasma and cell lysate analysis for proteins [88], antibody and cell-free nucleic acids characterization through electrochemical analysis, surface plasmon resonance (SPR) [93, 94], surface-enhanced Raman scattering (SERS), on-chip spectroscopy [95, 96],

magnetic susceptibility, dielectrophoretic susceptibility, faradic catalytic amplification, and biosensors. Most of the external components not integrated into the microfluidic devices used in these analyses have been already implemented in silicon-based semiconductor devices for other commercial applications [61, 63, 85, 97–101]. With all the individual blocks already available and commercially implemented, it would merely be an engineering exercise of systems integration to package them for near-simultaneous measurement of multiple parameters on the same blood sample. We argue that the decision making often tends to be more accurate if we can increase the number of sensors/variables supplied to a system.

The multilayered device proposed would have a fluidic input layer, an actuation/separation layer, an optical sensing layer, an electrical sensing layer, and coupling layer on top of silicon layer [42, 92] with integrated sensors, electronics, optoelectronics, and compute elements. The layers are connected where necessary by: "Via's" that transport liquids/air from one layer to the other to move the analyte as well as the air necessary for valving [38]; electrical connectors for actuation/pumping and carrying back any additional sensors located outside the silicon layer; and optical waveguides [102] for tunneling light from the LED/laser sources on the silicon layer to the optical sensing layer as well as piping the light from the sample to the optical sensor arrays, photodetectors in the silicon layer. There could also be optical fibers linking layers where integrated waveguides are not adequate/feasible.

The fluidic input layer is the interface for all reagents/analytes [103] necessary for the device to function. It may also include modules for sample preparation [104], amplification, tagging/incubating, partitioning sample components, and waste collection/neutralization. The actuation/separation layer includes the actuators for the valves in the rest of the device as well as additional bulk separation modules to partition blood based on passive or field-based enrichment modules including filters, sieves, micropillars, electric/magnetic fields, etc. [105–108]. The optical sensing layer can include optics and modules to enable imaging flow cytometry and associated cell sorting [82, 109–115]/flow shaping [59, 116, 117], fluorescence activated cell sorting (FACS) [59], surface plasmon resonance (SPR) [93, 94], surface-enhanced Raman scattering (SERS) [118], on-chip spectroscopy [95, 96] that are coupled with the optical sensors in the silicon layer through waveguides and optical fibers. The electrical sensing layer contains modules setup for electromagnetic sensing (resistive, capacitive, magnetic, electrochemical, piezoelectric, micromechanical, faradic) [62–69]. This layer is designed to be closest to the silicon layer for the shortest possible interconnects before reaching the integrated electronics that perform signal amplification, noise filtering, and analog-to-digital conversion completed with minimum latency, losses, and noise associated with traditional setups that rely on off-chip signal processing. The compute layer includes the integrated microprocessors, graphics processors/vision processing units, network interface, and signal processing units that perform data analysis for flow control and classification analysis to generate a comprehensive diagnostic companion analysis for the medic. The compute layer provides real-time decisions to adjust sample preparation, process optimization, enrichment strategy, drug screening plan, and waste neutralization based on results at each

level of analysis. This could provide a customized report for each patient while also doing a multicomponent analysis to identify patterns leading to higher accuracy in diagnosis and medication.

5.2 Organs on Chip

Organs on chip (OOC) is a device that mimics organ level cell behavior in an in vitro environment [119, 120]. These devices are fabricated using microfluidic techniques and tissue cells are cultured in them thus mimicking the micro environments of in vivo conditions in vitro. The process is to grow tissue cells from different parts of the human body, for example, heart, liver, lung, muscle [121], on a PDMS chip to replicate human tissue barriers. The advantage of such systems is the ability to create precise micro environments of human tissue interactions. These micro environments can be exploited to understand the impact of specific chemicals/drugs. Such systems are crucial for personalized medicine and drug discovery. The next paragraphs give a high-level summary of various organs-on-chip platforms [121].

Blood vessel on chip is designed by coating the PDMS-based microfluidic channels with fibronectin [122] and flushed with endothelial growth media. The endothelial cells are pipetted into these channels and are incubated. The surface thus gets coated with endothelial cells. To make a cylindrical channel for blood flow, a capillary tube is inserted into the microfluidic channel, and after the endothelization, the capillary tube is removed leaving a circular channel to study the blood and endothelial interaction. Understanding blood vessel physiology has broad applications in predicting blood vessel bio-mechanics, fluid shear and stretch [123], wound healing [122], vascular injury [124], drug discovery, and hemostasis/thrombosis [122, 125].

Lung on chip [126] was build with soft lithography techniques that created an in vitro alveolar capillary interface of the human lung. This device was used to study the alveolar response to bacteria and nanoparticles. Fluorescent microscopy was used to quantify alveolar-capillary interface. Heart on chip [127, 128] muscular thin films are designed with flexible elastomeric polymers and anisotropic cardiac cells and tissues. The contraction and expansion of heart muscles is quantified by these deformations of the elastomeric polymer designed using microfluidics.

Designing these organs on chip with multilayer integration is challenging but gives unprecedented advantage in looking at all barriers wholistically. The current challenges for the organs on chip systems are: (i) interfacing multiple organs on single chip; (ii) measuring simultaneously optical, electrical, and chemical information; and (iii) measuring both the local and the global parameters simultaneously. Current chips are mostly limited to measuring optical properties. Measuring other mechanical properties of these systems such as electrical and mechanical properties gives better understanding of these systems. In situ continuous monitoring of the OOC is critical to use these chips to full potential.

These devices should not only have continuously monitoring sensors but also have feedback based actuators to precisely perturb the micro environments. The literature in developing such integrated OOC devices is very limited. Yu Shrike Zhang et al. [129] developed a modular OOC device that integrated sensors for monitoring micro environmental properties such as pH, O_2, and temperature; soluble biomarkers were monitored using electrochemical immuno-biosensors. Similar OOC optical properties are measured using fluorescent microscopes. The automation and integration of the sensors to the OOC gave uninterrupted continuous monitoring of a combined liver and heart on chip platforms.

5.3 Microfluidic Applications in Bionanomaterials and Nanomedicine

5.3.1 Bio-nanomaterials

Nanomaterials (size range of 1–100 nm) that are either entirely composed of biomolecules or partially built into them qualify as bionanomaterials. Bionanomaterials are being used for targeted drug therapy in cancer treatment research, for diagnostics, and in cosmetics [130]. Worapol et al. delivered siRNA to cancer cells in mice using biologically modified silica nanoparticles [130–132], and Inmaculada et al. developed magneto-plasmonic nanoparticles with potential applications in magnetic resonance imaging [133]. The above examples highlight the ongoing research in the development and application of bionanomaterials in clinical medicine. One of the biggest challenges in bionanomaterials research is the consistency in production from batch to batch. Microfluidic systems offer the advantage of tuning the size and quality for nanoparticle production without losing high rate of production. Due to the inherent small channels, microfluidic devices offer improved control over reaction conditions. Moreover, microfluidic systems have also enabled real-time characterization of nanoparticles during production [130]. These kinds of integrated systems will greatly reduce the time and money needed for clinical translation of nanoparticles. Jong-min et al. used 3D flow focusing in parallel microfluidic systems to synthesize poly(lactide-co-glycolide)-b-polyethyleneglycol (PLGA-PEG) nanoparticle that are currently in phase I clinical trials for prostate cancer treatment [134]. They reported a maximum production rate of 84 mg h^{-1} with eight parallel systems and tested their nanoparticles in mice for fluorescence. Increasing sophistication in the manufacture of bionanomaterials will enhance their success in biomedical applications. Highly integrated microfluidic devices with advanced process control and real-time optimization systems proposed in this chapter are poised perfectly to resolve challenges in production and characterization of bionanomaterials.

5.3.2 Nanomedicine

Nanomedicine primarily involves the use of nanoparticles to assist disease treatment. Major progress in nanomedicine has come in the form of targeted drug delivery through nanoparticles as carriers [130–132]. Highly unstable drugs and

biomolecules can also be delivered to target organs safely through nanocarriers. The progress in clinical translation of nanomedicine has not been keeping up with the pace of laboratory research due to problems associated with production, characterization, and in vivo testing of nanoparticles. Microfluidic technology is becoming in common in the development of lipid-based nanocarriers that are highly valued for their versatility in encapsulating both hydrophilic and hydrophobic loads. Alex Leung et al. [135] used microfluidic mixing to synthesize lipid nanoparticles with siRNA and identified a nanostructured core formed as a result of rapid microfluidic mixing. Organs-on-chip platforms enable ex vivo testing of nanoparticles for their organ selectivity [127, 128] and allow integration to mimic pharmacokinetics of human body. Cancer on chip models are also being developed to test cancer drugs and allow personalized medicine possibilities in future. Balabhaskar et al. developed a microfluidic platform to test the efficacy of two different nanopolymer-based drug delivery systems [136]. Advanced microfluidic systems with multiparameter analysis like the ones proposed here when developed to analyze cancer cells from patients could potentially enable personalized drug testing system.

5.4 Large-Scale Data Integration for Healthcare Diagnostics

With the advent of the Internet of Things (driven by low cost easily deployable sensing and connectivity) and associated advances in biosensors integration owing to an increasing number of use cases for disease diagnosis and drug screening, healthcare providers have access to mountains of data at their disposal to analyze and optimize healthcare at the individual level. Advanced multilayered microfluidic devices as proposed in Sect. 5.2 with multiplexed sensing also generate huge amounts of data that often need to be processed near-real-time to be able to complete all the many sequential analysis within the few hours of in vitro stability of biological fluids outside the body. For example, imaging flow cytometry techniques rely on ultrafast image processing (>100 fps) to extract cell properties like size, shape, color, and response to forces for cell classification in real time to feedback decisions necessary for downstream flow shaping or single-cell manipulation of the cells of interest for isolation and enrichment. Similar data processing speeds are needed for other sensing strategies including on-chip FACS, electrical impedance spectroscopy, absorbance/scatter, etc. to allow for high-speed classification needed for decision-making on the strategy downstream of each of these sensing locations in the process flow. However, these big data image/signal processing workloads have historically been very compute intensive leading to longer than optimal processing times (often hours) often ending up as offline analysis for post-mortem analysis. Adding to this challenge and as proposed in the multiplexed sensing strategy in Sect. 5.2, due to the inherent heterogeneity within human beings, genetic or otherwise, in their physiology and how they respond to stressants like disease, drugs, and other stimuli, as well as heterogeneity at the cell level due to age, disease, mutation etc., individual cell properties like size, shape, density, appearance, specific fluorescent markers, or susceptibility to magnetic, electrical, or light fields, and drug response in

isolation are not always adequate to classify with confidence and there is a need for multiparameter cross-correlation analysis. Recently, developments in the field of big data analysis, often driven by e-commerce firms for user data analysis, have started trickling into the field of healthcare to address a similar requirement: find trends/commonalities in extremely large, often incomplete, unstructured, and seemingly unrelated data piles with a large number of variables. Novel data processing approaches, enabled by huge advances in computational hardware capabilities, have pushed these data analyses to close to real time using machine learning and deep learning architectures where either the cell classification or cell identification or both is handled by pretrained models that can dole out classification at real time [115, 137–142]. This is not uncommon to the face detection algorithms widely deployed on smartphones or Movidius VPU chip–powered Intel compute stick/Google clip [143–145]. Recently this has also been demonstrated on an ultraportable raspberry Pi platform paving the path for compact, low power on-chip integration of the detection and the classification systems. In these systems, the training of the models is often performed in the cloud with crowd sourced data, and the on-chip compute systems are fed the trained models to perform inference and classify real time. Trained models are available for a variety of biological analysis including machine learning models for disease detection in liquid biopsies [146], cell counting [147], cancer screening [148, 149], cell feature extraction [115, 117], and flow shaping [115], while there is an increasing amount of literature/training/toolsets to retrain existing popular models for new systems with small datasets and achieve reasonable accuracies in classification [138, 140, 142, 150–152]

6 Conclusions and Future Perspectives

The exercise here was to conceptualize a one-stop advanced microfluidic analysis system and push to see how much we can do with one sample – with a goal of leveraging advances in computation and create an able virtual assistant to a doctor at point of care for customized, guess-free medical care that could optimize care and reduce cost and treatment time while increasing survival rate as well as patient comfort. We envision an advanced POC microfluidic device though systems integration and packaging via VLSI technology coupled to advances in sensor technology and computational technology to power a multiparameter co-analysis that could deliver valuable insights to dramatically improve decision making at the doctors office. In here, technologies developed in microfluidics systems are adapted to integrate with an extremely capable sensing and computation silicon layer (SOC). This can also cater to other applications like environmental sensing, food quality monitoring, and biosecurity applications. Inspiration is drawn from the IOT boom, advances in computational tools, both hardware (capable low power edge compute, extremely powerful cloud) and software (machine learning and DNN). A clear analogy would be quality-of-life improvement delivered by modern day smartphones that offer unreal artificial intelligence at the edge in the form of technologies that were recently science fiction and suggestions/interactions that are close to human like. Current day smartphones rely on up to 20 sensors to identify

context information of the user and provide very tailor-made and accurate suggestions and interactions/responses, often freaky. These include camera-based face recognition/image processing, speech recognition, fingerprint sensor, GPS, ultrasonic range finder, battery sensor, temperature sensor, humidity sensor, multi-axis accelerometer, light sensor, proximity sensor, etc. In this, the Google Assistant relies on a combination of sensors to recognize user spoken request and respond appropriately with human-like interpretation and relevance. While the speech recognition engine itself is a marvel with offline inference for some set of actions with an onboard neural network inference chip, Google relies on cloud-based neural networks to generate a highly relevant response by relying on a multitude of other variables that provide context to the conversation that allows it to make a meaningful and highly accurate interpretation of a much wide range of phrases with varying noise/accent. This context could be data from the many other sensors like the users' location, local weather, time of day, and recent searches. We also see a similar tactic applied improvement of face recognition where camera-based image detection is being assisted by additional sensors that provide depth information as well as deeper colors to allow for a better 3D representation as well as low light performance. This additional data coupled with a dedicated RAM and VPU for offline neural network inference is able to deliver real-time face/object/text recognition and better autofocus. Sometimes they leverage Phase-Detection AutoFocus (PDAF) coupled with some AI neural network wizardry to extract depth. DNNs these days can look at 200000 labels for classification with adequately trained models (Microsoft's Watson or other cloud sources training models). This is the age of "Internet of Things" where we are able to collect huge amounts of data needed to train neural network models to find patterns not imagined by humans. A smart diagnostics companion in Figs. 9.5

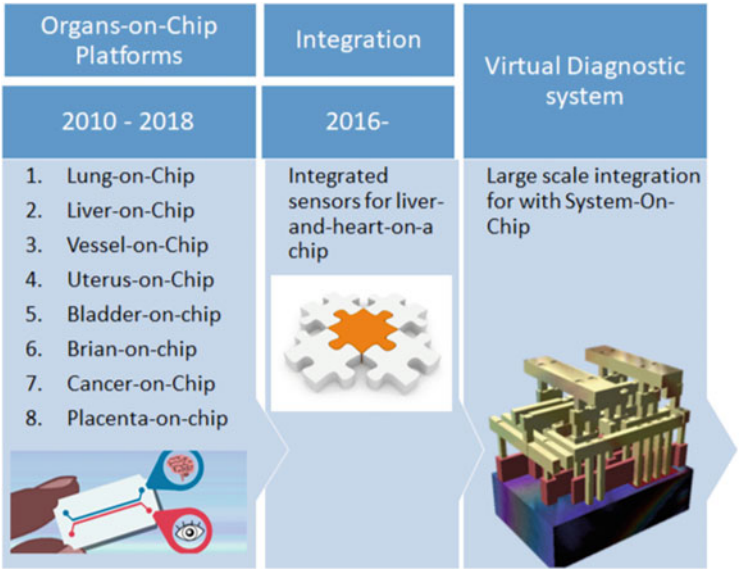

Fig. 9.5 Evolution and future of organs-on-chip platforms

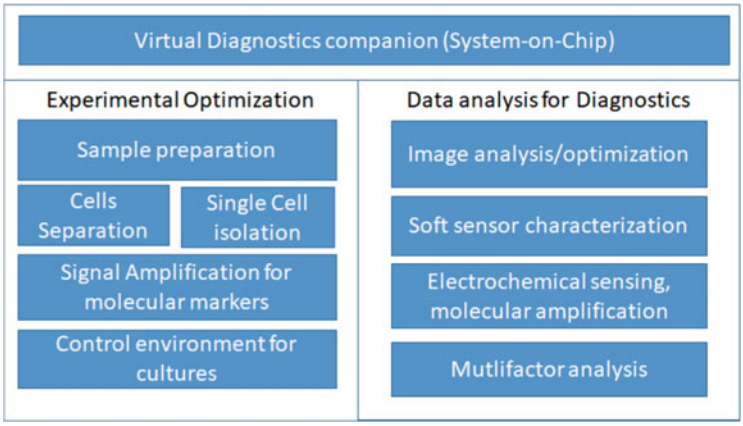

Fig. 9.6 Virtual diagnostics companion through and SOC integrated into a microfluidic VLSI platform

and 9.6 above provides a superior disease diagnosis by gathering all the evidence available to improve the context of a symptom, also preemptively screens/simulates all the alternate treatment paths before identifying the best possible path to speed up final diagnosis, optimizes reagent usage/cost while also preemptively screens appropriate drugs such as anticoagulants, antibiotics, and chemotherapy.

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