UC Riverside

UC Riverside Previously Published Works

Title

Recent developments in microfluidic large scale integration.

Permalink

<https://escholarship.org/uc/item/3jh0x2hh>

Authors

Araci, Ismail Emre
Brisk, Philip

Publication Date

2014-02-01

DOI

10.1016/j.copbio.2013.08.014

Peer reviewed

Recent developments in microfluidic large scale integration

Ismail Emre Araci¹ and Philip Brisk²

In 2002, Thorsen *et al.* integrated thousands of micromechanical valves on a single microfluidic chip and demonstrated that the control of the fluidic networks can be simplified through multiplexors [1]. This enabled realization of highly parallel and automated fluidic processes with substantial sample economy advantage. Moreover, the fabrication of these devices by multilayer soft lithography was easy and reliable hence contributed to the power of the technology; microfluidic large scale integration (mLSI). Since then, mLSI has found use in wide variety of applications in biology and chemistry. In the meantime, efforts to improve the technology have been ongoing. These efforts mostly focus on; novel materials, components, micromechanical valve actuation methods, and chip architectures for mLSI. In this review, these technological advances are discussed and, recent examples of the mLSI applications are summarized.

Addresses

¹ Department of Bioengineering, Stanford University, Stanford and Howard Hughes Medical Institute, CA 94305, USA

² Department of Computer Science and Engineering, Bourns College of Engineering, University of California, Riverside, CA 92521, USA

Corresponding author: Araci, Ismail Emre (earaci@stanford.edu)

Current Opinion in Biotechnology 2013, 25:60–68

This review comes from a themed issue on **Analytical biotechnology**

Edited by **Frank L Jaksch** and **Savaş Tay**

S0958-1669/\$ – see front matter, © 2013 Elsevier Ltd. All rights reserved.

<http://dx.doi.org/10.1016/j.copbio.2013.08.014>

Recent advancements in microfluidic technologies have created new opportunities in the fields of biology and chemistry through miniaturization and automation of common processes, including mixing, metering, trapping, reaction, filtering, and separation, among others. Microfluidic large scale integration (mLSI) enables the fabrication of microfluidic chips containing hundreds or more of these functions using well-established lithography techniques, similar in principle to electronic LSI in the semiconductor industry [1]. Small feature dimensions, abundant spatial parallelism, and control over fluid flow provide mLSI technology with advantages such as high throughput, precise metering, automation and low reagent consumption. The implications of mLSI technology can especially be seen in recent advances in single cell, genomic, and protein analysis.

The on-chip valve is the key component of mLSI, much like the transistor in semiconductor LSI. Typically, microfluidic valves are fabricated by multilayer soft lithography (MSL) using PDMS and are actuated by external pneumatic controllers. A valve requires an elastomeric membrane that is deflected to control the fluidic resistance; when the valve is open, the fluidic resistance has a minimum value, determined by channel dimensions and when the valve is closed, the fluidic resistance is increased to infinity because flow rate is reduced to zero. In other cases, fluidic resistance can be set between these two extremes by controlling the pressure values or channel shape. The application of this flow control principle has led to the development of many higher-level components including peristaltic pumps, rotary mixers, multiplexers, and sieve valves. PDMS-based mLSI is widely used and applicable, however the technology has not found a ‘killer application’ outside of a laboratory setting. Poor chemical resistance, a fundamental limitation of PDMS as a substrate material, causes swelling and defects when common solvents such as acetone or toluene are used as working fluids [2]. Another challenge is to realize facile valve actuation: although mLSI chips are small, pneumatic actuation requires complex external controls and a cumbersome chip-to-world interface, which has been compared, quite unflatteringly, to Medusa’s head [3]. Therefore, there is a growing effort to make mLSI technology more compatible with chemically resistant elastomers and to reduce the external components required for actuation.

In the first mLSI review published in 2007 [4], Melin *et al.* discussed economies of scale, parallelization strategies, multiplexing and multistep biochemical processing and highlighted several components as building blocks of mLSI technology. In a later review in 2010 [5], Mark *et al.* summarized applications of PDMS-based mLSI chips and discussed the strengths and limitations of mLSI as a substrate technology. Here, new components and recent advances in architecture and design methods will be reviewed to present readers with a broader perspective regarding the capabilities and usage cases for the most advanced mLSI devices to date. Table 1 summarizes the requirements, components and applications of mLSI as covered in this review.

Component-level developments section reviews new materials for realizing fundamental mLSI components, different actuation methods, and the most recent spate of mLSI components. In *Architectural developments* section, higher density and miniaturized components requiring reduced external control elements are reviewed; when

Table 1

Summary of the mLSI requirements, components and applications discussed in this review

Microfluidic large scale integration (mLSI)		
Requirements	Components	Applications
- Reliable material - User friendly actuation - Higher density - Less external components - Flexibility and programming - Automated design	Fundamental: - Push up, down, sieve valve - Normally closed valve - Structured valve - Cell trap High level: - Multiplexer, latch, pump, meter, mixer - Gradient generator and selector - Separation column - Agar filled chamber - Multilaminate mixer	- Single cell culturing and imaging - Single cell genomic and protein analysis - Cell microenvironmental interaction - Protein synthesis and characterization - Chemical and biological screening - Solid phase liquid chromatography

integrated together in single-chip architectures, these technologies increase throughput, dynamic range, and sensitivity for many applications. *Automated mLSI chip design and layout* section reviews recent attempts to automate the design and layout of mLSI chips. Current practice is to draw each layer of a device manually using commercial software such as AutoCAD. In recent years, there have been several notable academic efforts to automate the design and layout process. The final section reviews recently reported biological and chemical applications of mLSI technology.

Component-level developments

Any candidate material to replace PDMS for mLSI technology must possess transparency and elasticity as fundamental properties. One early candidate was photocurable perfluoropolyethers (PFPEs) which have been used to make on-chip valves [2]. PFPEs have low surface energy, Young's modulus and toxicity, and their high chemical resistance and gas permeability makes them attractive for mLSI; however, sensitivity to oxygen makes their processing difficult. More recently, a commercially available silicone with a PFPE backbone (SIFEL) was processed by MSL to make standard size (100 μm) valves [6]. These devices showed high solvent resistance while retaining the other favorable properties of PDMS, making them a promising candidate for future mLSI technologies. Viton [7], a commercial fluoroelastomer, can also be used as a membrane layer when bonded onto PMMA and cyclic olefin copolymer (COC) layers with plasma treatment and chemical assistance; the actuation pressure and bonding strength satisfied the requirements of mLSI.

Tuning the properties of PDMS by coating or doping can be a better strategy compared to replacing it with an alternative material especially when the goal is to reduce small molecule absorption; Parylene c coating [8] and silica nanoparticle doping by sol-gel treatment [9] have emerged as promising techniques. For example, the diffusivity of Rhodamine B can be reduced by a factor

of 19 in Parylene c caulked PDMS when compared to native PDMS [8].

The cost of pneumatic external controllers is a significant impediment to the widespread adoption of mLSI technology. Potential replacement technologies include electrostatic [10], magnetic [11], torque generated [12], Braille pin [13], thermal [14,15] and shape memory alloy (SMA) [3] actuation.

The minimum requirements to render an actuation technology suitable for mLSI are scalability and a fast (<1 s) response time. Thermal methods are scalable but have slow response times. For example, phase change microvalves using ohmic resistors as heat sources have response times of minutes [14]. Replacing the phase change element with a thermo responsive polymer (poly(N-isopropylacrylamide)) and reducing the thermal mass of the responsive element (100 μm valve scale) decreased the response time to seconds [15]. Favorably, the pressure resistance of microvalves in COC was very high (1350 psi), and could be optimized by modifying the crosslinker and tetrahydrofuran composition; unfortunately, the quartz halogen lamp used for heating has a large area (diameter = 1.3 cm), which severely limits the scalability.

Gu *et al.* showed that 320 vertically moving Braille pins can be integrated with PDMS to realize pumps and mixers; although short response time (120 ms) makes it promising, large valve diameter (900 μm) limits the scalability [13].

Another alternative valve technology uses an electrostatically operated PDMS membrane with metallic flexures, which can be driven by CMOS integrated circuits at a voltage of 15–20 V and frequency of 5 MHz [10]. The size of these valves was around 200 μm , permitting large scale integration. SMA actuation, too, simultaneously offers fast response time and small valve dimensions and also can be assembled directly on printed circuit boards [3].

Figure 1

(a) Picture of the 30-plex chip for long term gradient generation (top) and the description of flow-switching which provides the stable gradient under no-flow condition (bottom), reproduced by permission [24]. (b) Schematic of the gradient selector (left), multilaminar mixer (top, right) and the cross section of the multilaminar mixer (bottom, right), reproduced by permission [23]. (c) Picture of a 12-bit fluidic shift register, reproduced by permission [16].

One limitation of SMA actuation is its low pressure resistance (5–15 psi); moreover, both electrostatic and SMA actuation are incompatible with MSL and require complex fabrication techniques.

Alongside the search for better materials and simpler actuation technologies, new mLSI components have been introduced using standard materials and techniques. Improved designs for *fundamental components* include normally closed valves (NCVs) [16^{••},17], structured valves [18], cell traps [19], and capacitors and diodes (Figure 1a) [20,21]; *higher-level components* that have been introduced include a chamber array [22], a high performance separation column [23^{••}], a gradient selector (Figure 1b) [23^{••}], a long term gradient generator [24^{*}], a 2D spatial gradient controller [25], an agar filled chamber [26] and a mutilaminate mixer [27].

NCVs made of hybrid glass/PDMS materials [28] have been used for automated and quantitative single cell gene

expression analysis [29,30], but are millimeters in size, rendering them non-scalable. NCVs introduced by Devaraju *et al.* [16^{••}] achieve scalability, but require a flash curable polymer; this technology was used for building static gain valves and consequently for realizing microfluidic logic circuits. Monolithic PDMS NCVs can also be fabricated through selective deactivation of oxidized PDMS, and have been demonstrated for large scale integration [17]. Structured valves incorporate topographical features on the roof of a standard valve, which augment standard valves with additional functionalities, such as surface patterning or trapping [18].

High-level components comprise multiple valves or chambers to realize a specific function. For example, agar filled chambers for cell culturing [26] and high-performance separation columns for chromatography [23^{••}] require viscous liquids to fill in small microchambers. Such operations are realized using a series of valves that fill PDMS chambers with appropriate materials, and are

then closed permanently for sealing. Likewise, valves placed in various configurations can generate temporal [24[•]] or spatial control [25] over concentration gradients, while multilaminar mixers provide fast and efficient mixing using underpass channels (Figure 1b) [27].

As fluid volumes decrease, evaporation becomes a challenge, requiring hydration elements [31]. When chip complexity increases, on-chip nozzles [32[•]] can assist with product recovery. Such mLSI components play an important supporting role in larger and more comprehensive architectures.

Architectural developments

The term architecture is interchangeably used to define the chip cross-section or circuit design. Architectural improvement strategies focus on increasing *chip density*, decreasing the *reliance on external controllers* and designing *programmable chips* to reduce process time and complexity.

Chip density can be increased either through component miniaturization or the introduction of additional layers. The Quake research group at Stanford University has recently demonstrated integration of 1 million control elements onto a single chip is possible through microfluidic very large scale integration (mVLSI) [33[•]]. mVLSI has the potential to realize high dynamic range and reconfigurable chips for digital-PCR, digital-MDA or digital-ELISA applications.

It is possible to integrate more functionality into a fixed-area device by increasing the number of layers [23^{••},34^{••},35]. Inter-layer connections can be realized through lithographical methods [34^{••},35] or laser ablation [27]. Lithographical methods are simpler than laser ablation, but require fine-tuning of fabrication conditions when the device geometry changes.

Reducing the *dependency on external pneumatic controllers* can reduce the complexity of the chip-to-world interface for mLSI; this was the subject of two recent review papers [36,37]. Microfluidic logic circuits can eliminate the requirement of one external controller for each independently controlled valve set by utilizing a fluidic shift register [16^{••},35,38]. Researchers at Fluidigm recently demonstrated microfluidic NOT, NAND and NOR gates, flip-flops, oscillators, a self-driven pump and a 12-bit fluidic shift register (Figure 1c) [16^{••}].

A pressure-based serial digital to analog converter (DAC) [20] can eliminate the need for multiple pressure sources by generating arbitrary pressure values. Similarly, a frequency dependent flow control circuit can control several fluidic pathways with a single AC input. Leslie *et al.* demonstrated such a device which can select between

two fluids depending on the frequency of a time modulated pressure source (0.2 Hz or 7 Hz) [21].

For commercialization and mass production of mLSI, it is attractive to use a single software-*programmable chip* to execute any desired protocol. One such device was first developed by Jensen *et al.* [29] and recently improved to handle continuous processes with a greater number of inputs [39]. However, the larger hybrid PDMS/glass valve technology differentiates itself from mLSI and is more suitable for microliter scale fluid volume processing (the chamber volume is 120 nL); moreover, this technology requires one external valve per chamber, and it seems unlikely that any reduction is possible. Fidalgo *et al.* demonstrated an mLSI fluidic processor which has a 300-pL chamber volume, and uses 15 external valves to control 64 chambers [34^{••}]. The device demonstrated electrofluidic display, surface immunoassay, microformulation and cell culturing capabilities. Another recent programmable chip combines droplets and flow-based microfluidics using 95 individually addressable nL-volume storage chambers with pL-volume droplets; water immiscible oil is used as a carrier fluid, and the device supports 8 different reagents through a specialized metering module [32[•]]. The innovative introduction of bypass channels at the entrance of each chamber prevented undesirable droplet wetting, which eliminated cross-contamination. This device was successfully used for single cell sorting, culturing, genotyping and whole genome amplification (WGA) experiments. Specifically, environmental samples are used to explore genomic relationships within natural microbial communities.

Automated mLSI chip design and layout

Starting in the early 1980s, the semiconductor industry pioneered computer-aided design of semiconductor technology, otherwise known as electronic design automation (EDA) [40]. There have been recent academic efforts to develop EDA software for mLSI chips. These research efforts are still early-stage, and have not yet produced working software tools. Nonetheless, the potential for increased mLSI designer productivity is readily evident, and user-friendly mLSI EDA software will hopefully be released within the next 1–2 years.

The first relevant issue is how to specify the architecture of a mLSI chip; *hardware design languages (HDLs)* such as VHDL and Verilog have been semiconductor industry standards for decades; no such standard exists for mLSI chips. One recent academic proposal is the *Microfluidic Hardware Design Language (MHDL)* [41], which borrows much of its syntax from VHDL. An MHDL specification of an mLSI chip includes a list of components (e.g. valves, mixers, etc.) followed by a list of fluidic interconnections between components, which will become microchannels once the device is physically laid out. An example can be seen in Figure 2.

Figure 2

An AutoCAD layout of a simple mLSI chip (left), and an MHDL description of the same chip (right).

The second issue is how to physically lay out the mLSI chip, starting either from an MHDL (or equivalent) specification of the architecture, or alternatively, from a higher-level specification of a protocol for which the chip will be designed. Several academic research groups have investigated different aspects of these issues.

The first academic effort was Micado [42^{*}], an AutoCAD plug-in that generates the control layer of an mLSI device. Given a physical layout of the flow layer and a description of the fluid transfers to perform, Micado automatically generates the control layer by first, placing valves; second, determining independently activated subsets of valves that can share a control line; and third, generating the control layer routing network, which connects each control input to the valves that it controls. Although it performs useful optimizations, Micado falls short of being a complete end-to-end solution for the design and layout of mLSI chips.

A second effort is an ongoing research project carried out at the Technical University of Denmark [43–46], with

supporting efforts from researchers in Taiwan and Japan [47–49], which provides an algorithmic skeleton of an end-to-end design flow for mLSI chips. Starting from a high-level description of a biological protocol, algorithms are presented to: first, derive a graph-based representation of an mLSI chip that can execute the protocol [45,47–49]; second, automatically synthesize the protocol onto a graph-based representation of an mLSI chip [43,44]; third, physically place components in the flow layer, and route control channels between them [45]; and fourth, automatically generate the control layer [46]. Although complete in principle, this work has not yet been used to design a real-world mLSI chip that has been fabricated.

These research efforts have demonstrated the feasibility of the development of EDA tools for mLSI technology. The next step will be to produce a working tool that can increase mLSI designer productivity. In the long-term, the objective of such an effort would be to empower mLSI designer to create fully integrated chips whose complexity is several orders of magnitude beyond what

Figure 3

Performance comparison between the conventional 96-well approach and the two generations of click-chemistry screening platforms, reproduced by permission [62].

is feasible using the manual design methodologies that are prevalent today.

Biological and chemical applications

This chapter summarizes recent applications of mLSI components and technologies that have enabled scientists to develop a deeper understanding of a variety of chemical and biological phenomena. mLSI is particularly well suited for single cell analysis and manipulation; several excellent review articles have already summarized recent studies [50–52], which generally focus on single cell imaging [24[•],26,31,32[•],53–55] or single cell genomic/proteomic analysis [19,30,56[•],57[•],58,59].

An mLSI chip developed by Taylor *et al.* can perform 256 simultaneous time-lapse imaging experiments in 2048 single yeast cell traps for 32 different conditions [55]; however, in order to track cells over longer time scales, large chamber volumes and immobilization of cells under perfusion are required. In order to solve the immobilization problem, Lecault *et al.* used inverted chip geometry to image mammalian cells in 1600, 4 nL-volume chambers for over 60 hours [31]. In a more recent study, Frank *et al.* demonstrated generation and maintenance of thirty parallel gradients (ten independent chemical formulations in three repetitions) for 14-hours under no-flow conditions; a high-throughput, diffusion based gradient is

realized by temporal control provided by the on-chip valves. The chip is used to observe NFκB nuclear localization events in single mammalian cells for varying signaling molecule concentrations. The device enabled measurement of cell secretion effects at low concentrations [24[•]]. In another example, Falconnet *et al.* tracked yeast cells for multiple generations using a chip with polymerized agarose gel in 128 chambers for yeast cell immobilization, which allows tracking of 8 different genotypes under 16 different conditions [26].

For single cell genomic analysis, sorting, trapping, and lysing of the cell are typical mLSI chip functions. In a 2010 study, Fan *et al.* identified and sorted metaphase cells, released the chromosome by protein digestion of cytoplasm, and then randomly separated them in 48 chambers and amplified homologous copies of each chromosome from a single cell, following which the product is retrieved for genotyping [56[•]]. Whole genome haplotyping of single human metaphase cell as described in this study has applications in personal genomics.

Single cell protein secretion is another application of mLSI technology. Ma *et al.* realized multiplexed (>10 proteins) and quantitative measurement of secreted proteins from single cells [57[•]]. Immunosandwich assays of spatially encoded antibody barcodes in 1040,

3 nL-volume chambers are used for concentration measurements and multiplexing. A full barcode was representing a complete panel of protein assays and duplicate barcodes enabled statistics at the single cell level. The mLSI chip is used to assay the polyfunctionality of tumor antigen-specific T cells in the setting of an adoptive cell transfer therapy clinical trial for melanoma.

mLSI technology can also enable the study of microenvironmental interactions of cell populations. Liu *et al.* used a chip with 4 chambers connected by optional communication channels for monitoring cell-cell interactions. This experiment demonstrated the passive role but collaborative response of stromal cells while responding to signals from carcinoma cells [60].

Another field of application of mLSI is protein synthesis and characterization. In addition to high throughput, when married to DNA-to-protein arrays, mLSI has enabled multiplexed protein expression and utilization of advanced detection technologies, as summarized in the recent review by Maerkl on protein biochemistry [61].

Two recent examples of chemical applications are solid phase liquid chromatography [23^{••}] and *in situ* screening of click-chemistry [62]. Huft *et al.* integrated high performance separation columns with on chip sample loading, gradient generation, detection and sample recovery. The chip allows packing of resin slurry in 10 min under 30 psi pressure. This technology is promising for sequencing library preparation, cloning and sample fractionation for diagnostics. Wang *et al.* performed 1024 *in situ* click chemistry reactions in a proof of concept study. This second generation platform provides 20–50-fold sample economy when compared to 96-well plates. Figure 3 summarizes the advantages of mLSI for click-chemistry screening.

In conclusion, the high throughput and sample economy advantages of mLSI technology are highly attractive for single cell imaging and genomics, protein biochemistry and chemistry studies. Innovative architectural developments with the existing material, actuation combination and by using manual design strategies produced rapid results and enabled several noteworthy applications. In the meantime, researchers continue with advancements in material and actuation aspects for widespread and easier use of mLSI. Although promising, the implications of these advancements in real-world applications are yet to come.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Thorsen T, Maerkl SJ, Quake SR: **Microfluidic large-scale integration.** *Science* 2002, **298**:580-584.
2. Rolland JP *et al.*: **Solvent-resistant photocurable “liquid teflon” for microfluidic device fabrication.** *J Am Chem Soc* 2004, **126**:2322-2323.
3. Vyawahare S *et al.*: **Electronic control of elastomeric microfluidic circuits with shape memory actuators.** *Lab Chip* 2008, **8**:1530-1535.
4. Melin J, Quake SR: **Microfluidic large-scale integration: the evolution of design rules for biological automation.** *Annu Rev Biophys Biomol Struct* 2007, **36**:213-231.
5. Mark D *et al.*: **Microfluidic lab-on-a-chip platforms: requirements, characteristics and applications.** *Chem Soc Rev* 2010, **39**:1153-1182.
6. Devaraju NSGK, Unger MA: **Multilayer soft lithography of perfluoropolyether based elastomer for microfluidic device fabrication.** *Lab Chip* 2011, **11**:1962-1967.
- The chemically resistant elastomeric material SIFEL, was processed by simple MSL and demonstrated for standard micromechanical valve operation.
7. Ogilvie IRG *et al.*: **Chemically resistant microfluidic valves from Viton (R) membranes bonded to COC and PMMA.** *Lab Chip* 2011, **11**:2455-2459.
8. Lei Y *et al.*: **Studies on Parylene C-caulked PDMS (pcPDMS) for low permeability required microfluidics applications.** *Lab Chip* 2011, **11**:1385-1388.
9. Gomez-Sjoberg R *et al.*: **Biocompatibility and reduced drug absorption of sol-gel-treated poly(dimethyl siloxane) for microfluidic cell culture applications.** *Anal Chem* 2010, **82**:8954-8960.
10. Chang M-P, Maharbiz MM: **Electrostatically-driven elastomer components for user-reconfigurable high density microfluidics.** *Lab Chip* 2009, **9**:1274-1281.
11. Ghosh S *et al.*: **Oscillating magnetic field-actuated microvalves for micro- and nanofluidics.** *J Phys D: Appl Phys* 2009, **42**.
12. Hulme SE, Shevkoplyas SS, Whitesides GM: **Incorporation of prefabricated screw, pneumatic, and solenoid valves into microfluidic devices.** *Lab Chip* 2009, **9**:79-86.
13. Gu W *et al.*: **Computerized microfluidic cell culture using elastomeric channels and Braille displays.** *Proc Natl Acad Sci U S A* 2004, **101**:15861-15866.
14. Neumann C *et al.*: **Design and characterization of a platform for thermal actuation of up to 588 microfluidic valves.** *Microfluid Nanofluidics* 2013, **14**:177-186.
15. Chen G, Svec F, Knapp DR: **Light-actuated high pressure-resisting microvalve for on-chip flow control based on thermo-responsive nanostructured polymer.** *Lab Chip* 2008, **8**:1198-1204.
16. Devaraju NSGK, Unger MA: **Pressure driven digital logic in PDMS based microfluidic devices fabricated by multilayer soft lithography.** *Lab Chip* 2012, **12**:4809-4815.
- The authors demonstrated fluidic logic circuits with cascade-ability, feedback, programmability, bi-stability, and autonomous control capability. The described devices will potentially yield to smaller devices, higher clock rates and reduced external components.
17. Mosadegh B *et al.*: **High-density fabrication of normally closed microfluidic valves by patterned deactivation of oxidized polydimethylsiloxane.** *Lab Chip* 2011, **11**:738-742.
18. Vanapalli SA *et al.*: **Microfluidic valves with integrated structured elastomeric membranes for reversible fluidic entrapment and in situ channel functionalization.** *Lab Chip* 2009, **9**:1461-1467.
19. White AK *et al.*: **High-throughput microfluidic single-cell RT-qPCR.** *Proc Natl Acad Sci U S A* 2011, **108**:13999-14004.
20. Yu F, Horowitz MA, Quake SR: **Microfluidic serial digital to analog pressure converter for arbitrary pressure generation and contamination-free flow control.** *Lab Chip* 2013, **13**:1911-1918.
21. Leslie DC *et al.*: **Frequency-specific flow control in microfluidic circuits with passive elastomeric features.** *Nat Phys* 2009, **5**:231-235.

22. Jung S-Y, Liu Y, Collier CP: **Fast mixing and reaction initiation control of single-enzyme kinetics in confined volumes.** *Langmuir* 2008, **24**:4439-4442.
23. Huft J, Haynes CA, Hansen CL: **Microfluidic integration of parallel solid-phase liquid chromatography.** *Anal Chem* 2013, **85**:2999-3005.
The high efficiency chromatography chip integrates several novel mLSI components for execution of sample loading, mixing, gradient generation and selection, separation, detection and sample recovery.
24. Frank T, Tay S: **Flow-switching allows independently programmable, extremely stable, high-throughput diffusion-based gradients.** *Lab Chip* 2013, **13**:1273-1281.
Temporal control over concentration gradients allowed researchers to generate perfusion free, refreshable chambers to track mammalian cells over 14 hours at various stimulant concentration ranges.
25. Cooksey GA, Sip CG, Folch A: **A multi-purpose microfluidic perfusion system with combinatorial choice of inputs, mixtures, gradient patterns, and flow rates.** *Lab Chip* 2009, **9**:417-426.
26. Falconnet D et al.: **High-throughput tracking of single yeast cells in a microfluidic imaging matrix.** *Lab Chip* 2011, **11**:466-473.
27. Huft J et al.: **Three-dimensional large-scale microfluidic integration by laser ablation of interlayer connections.** *Lab Chip* 2010, **10**:2358-2365.
28. Grover WH et al.: **Monolithic membrane valves and diaphragm pumps for practical large-scale integration into glass microfluidic devices.** *Sens Actuat B: Chem* 2003, **89**:315-323.
29. Jensen EC, Bhat BP, Mathies RA: **A digital microfluidic platform for the automation of quantitative biomolecular assays.** *Lab Chip* 2010, **10**:685-691.
30. Toriello NM et al.: **Integrated microfluidic bioprocessor for single-cell gene expression analysis.** *Proc Natl Acad Sci U S A* 2008, **105**:20173-20178.
31. Lecault V et al.: **High-throughput analysis of single hematopoietic stem cell proliferation in microfluidic cell culture arrays.** *Nat Methods* 2011, **8**:581-593.
32. Leung K et al.: **A programmable droplet-based microfluidic device applied to multiparameter analysis of single microbes and microbial communities.** *Proc Natl Acad Sci U S A* 2012, **109**:7665-7670.
The programmable device combines the advantages of droplet and flow based microfluidics.
33. Araci IE, Quake SR: **Microfluidic very large scale integration (mVLSI) with integrated micromechanical valves.** *Lab Chip* 2012, **12**:2803-2806.
The authors demonstrated that micromechanical valves that are fabricated by MSL can be miniaturized down to 6 μm size, a reduction of more than an order of magnitude compared to standard valves. This can lead to an mVLSI chip density of about 1 million control elements per cm^2 .
34. Fidalgo LM, Maerkl SJ: **A software-programmable microfluidic device for automated biology.** *Lab Chip* 2011, **11**:1612-1619.
The authors have demonstrated a 64-node programmable mLSI chip analogous to field-programmable gate array (FPGA) in electronics. The device required only 15 external controller valves. Several low-level and high-level functionalities are demonstrated.
35. Weaver JA et al.: **Static control logic for microfluidic devices using pressure-gain valves.** *Nat Phys* 2010, **6**:218-223.
36. Mosadegh B et al.: **Next-generation integrated microfluidic circuits.** *Lab Chip* 2011, **11**:2813-2818.
37. Kim S-J et al.: **Microfluidic automation using elastomeric valves and droplets: reducing reliance on external controllers.** *Small* 2012, **8**:2925-2934.
38. Rhee M, Burns MA: **Microfluidic pneumatic logic circuits and digital pneumatic microprocessors for integrated microfluidic systems.** *Lab Chip* 2009, **9**:3131-3143.
39. Jensen EC et al.: **Digitally programmable microfluidic automaton for multiscale combinatorial mixing and sample processing.** *Lab Chip* 2013, **13**:288-296.
40. Conway L, Mead C: *Introduction to VLSI Systems.* Addison-Wesley; 1980.
41. McDaniel J et al.: **Design and verification tools for continuous fluid flow-based microfluidic devices.** In *Proceedings of the Asia and South Pacific Design Automation Conference*. 2013:219-224.
42. Amin N, Thies W, Amarasinghe SP: **Computer-aided design of microfluidic chips based on multilayer soft lithography.** In *Proceedings of the International Conference on Computer Design*. 2009:2-9.
The authors developed a freely available and open source AutoCAD plug-in called Micado, which assists with the automated design and control of mLSI chips. Micado automatically generates routes between punches and control valves while adhering to design rules specified by the foundry. Micado, and supporting documentation, can be downloaded from the following URL: <http://groups.csail.mit.edu/cag/micado/>.
43. Mihass WH, Pop P, Madsen J: **System-level modeling and synthesis of flow-based microfluidic biochips.** In *Proceedings of the International Conference on Compilers, Architecture, and Synthesis for Embedded Systems*. 2011:225-234.
44. Minhass WH, Pop P, Madsen J: **Synthesis for biochemical applications on flow-based microfluidic biochips using constraint programming.** In *Proceedings of the IEEE Symposium on Design, Test, Integration, and Packaging of MEMS/MOEMS*. 2012:37-41.
45. Minhass WH et al.: **Architecture synthesis of flow-based microfluidic large scale integration biochips.** In *Proceedings of the International Conference on Compilers, Architecture, and Synthesis for Embedded Systems*. 2012:181-190.
46. Minhass WH et al.: **Control synthesis for the flow-based microfluidic large-scale integration biochips.** In *Proceedings of the Asia and South Pacific Design Automation Conference*. 2013:205-212.
47. Dinh TA et al.: **A clique-based approach to find binding and scheduling result in flow-based microfluidic biochips.** In *Proceedings of the Asia and South Pacific Design Automation Conference*. 2013:199-204.
48. Tseng K-H et al.: **A network-flow based valve-switching aware binding algorithm for flow-based microfluidic biochips.** In *Proceedings of the Asia and South Pacific Design Automation Conference*. 2013:213-218.
49. Tseng K-H et al.: **A top-down synthesis methodology for flow-based microfluidic biochips considering valve-switching minimization.** In *Proceedings of the International Symposium on Physical Design*. 2013:123-129.
50. Lecault V et al.: **Microfluidic single cell analysis: from promise to practice.** *Curr Opin Chem Biol* 2012, **16**:381-390.
51. Yin H, Marshall D: **Microfluidics for single cell analysis.** *Curr Opin Biotechnol* 2012, **23**:110-119.
52. Mu X et al.: **Microfluidics for manipulating cells.** *Small* 2013, **9**:9-21.
53. Tay S et al.: **Single-cell NF-kappa B dynamics reveal digital activation and analogue information processing.** *Nature* 2010, **466**:267-271.
54. Gomez-Sjoeberg R et al.: **Versatile, fully automated, microfluidic cell culture system.** *Anal Chem* 2007, **79**:8557-8563.
55. Taylor RJ et al.: **Dynamic analysis of MAPK signaling using a high-throughput microfluidic single-cell imaging platform.** *Proc Natl Acad Sci U S A* 2009, **106**:3758-3763.
56. Fan HC et al.: **Whole-genome molecular haplotyping of single cells.** *Nat Biotechnol* 2011, **29**:51+.
The authors have described an mLSI chip capable of separating and amplifying homologous copies of each chromosome from a single cell. The presented approach has applications in personal genomics, single-cell genomics and statistical genetics.
57. Ma C et al.: **A clinical microchip for evaluation of single immune cells reveals high functional heterogeneity in phenotypically similar T cells.** *Nat Med* 2011, **17**:738-743.

The single cell barcoding chip is used for quantitative and multiplexed measurements of protein secretion from single T cells in high throughput. The platform is proved to be a useful tool for immune system monitoring in a clinical setting.

58. Marcy Y *et al.*: **Dissecting biological “dark matter” with single-cell genetic analysis of rare and uncultivated TM7 microbes from the human mouth.** *Proc Natl Acad Sci U S A* 2007, **104**:11889-11894.
59. Zhong J *et al.*: **A microfluidic processor for gene expression profiling of single human embryonic stem cells.** *Lab Chip* 2008, **8**:68-74.
60. Liu W *et al.*: **An integrated microfluidic system for studying cell-microenvironmental interactions versatily and dynamically.** *Lab Chip* 2010, **10**:1717-1724.
61. Maerkl SJ: **Next generation microfluidic platforms for high-throughput protein biochemistry.** *Curr Opin Biotechnol* 2011, **22**:59-65.
62. Wang Y *et al.*: **An integrated microfluidic device for large-scale in situ click chemistry screening.** *Lab Chip* 2009, **9**:2281-2285.