Microfluidic sensing: state of the art fabrication and detection techniques

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Abstract. Here we introduce the existing fabrication techniques, detection methods, and related techniques for microfluidic sensing, with an emphasis on the detection techniques. A general survey and comparison of the fabrication techniques were given, including prototyping (hot embossing, inject molding, and soft lithography) and direct fabrication (laser micromachining, photolithography, lithography, and x-ray lithography) techniques. This is followed by an in-depth look at detection techniques: optical, electrochemical, mass spectrometry, as well as nuclear magnetic resonance spectroscopy-based sensing approaches and related techniques. In the end, we highlight several of the most important issues for future work on microfluidic sensing. This article aims at providing a tutorial review with both introductory materials and inspiring information on microfluidic fabrication and sensing for nonspecialists. © 2011 Society of Photo-Optical Instrumentation Engineers (SPIE). [DOI: 10.1117/1.3607430]

Keywords: microfluidics; fabrication; sensing; optical; electrochemical; mass spectrometry; nuclear magnetic resonance.

Paper 11101VRR received Mar. 5, 2011; revised manuscript received May 30, 2011; accepted for publication Jun. 14, 2011; published online Aug. 4, 2011.

1 Introduction

With microfluidic/nanofluidic technology it is possible to operate on microscale or nanoscale liquids for controlling or sensing purposes. With the microfabrication techniques initially rooted from the microelectromechanical systems (MEMS) technology, this field will gradually develop into a discipline itself.\textsuperscript{[1–4]} The fundamental techniques for microfluidic or optofluidic device fabrication originate from the semiconductor industry and are based on silicon or glass materials. With the development of polymer-based fabrication techniques, polymer-based microfluidic devices become more prevalent with the advantages of being economic and easy to fabricate, and having material versatility and good system compatibility.

The early designs of the microfluidic controlling systems were mostly monolithic miniaturized components such as microvalves, micropumps, and micromixers.\textsuperscript{[5–8]} With further development, whole microfluidic systems were realized, which were made up of multiple elements to achieve certain functions. These parts comprise either of the monolithic microfluidic components of the microfluidic components with the external components/devices. Designs of those microfluidic devices, such as geometries and scales, have been modified and improved according to the applications for better performance.\textsuperscript{[9–11]} Further development requires more components to be coupled with the microfluidic system for increased system functionality. Optical components seem ideal, with the benefits of noncontact, fast response, compactness, high sensitivity, multiplex operation possibility, and so on. Self-contained microfluidic devices have generated impact in the point-of-care and global health.\textsuperscript{[12–14]} However, in order to achieve real self-contained miniature devices, it is ultimately required to have highly integrated systems. This means lowering or even eradicating the dependence on macro, bulky, external devices to perform control, detection, or analysis functions, by the development and integration of the necessary functional microcomponents. Examples recently demonstrated are the implementation of lensless microscopes of sub-pixel resolving ability on microfluidic platforms for on-chip imaging or diagnosis.\textsuperscript{[15–17]}

The term optofluidics was coined in 2003 at the California Institute of Technology in Pasadena to describe systems that combine optics and microfluidics/nanofluidics (or to say, optical microfluidics).\textsuperscript{[18–20]} The interaction between light and fluid provides possibilities of both versatile microsystems and a wide spectrum of applications.\textsuperscript{[21–23]} In the past decade, there has been a growing interest and development of optical microfluidics (optofluidics) and an explosion of publications in the field. Actually, we can classify all microfluidic devices to either controllers or sensors. Here, we would like to emphasis on the microfluidic sensors. After the review of fabrication techniques in Sec. 2 we will have a brief introduction of the existing microfluidic detection methods and techniques in Secs. 3 and 4. This is followed by a review of optofluidic sensing techniques in Sec. 5.

2 Fabrication Methods

In the early days, the fabrication of microfluidic devices mainly relied on techniques transferred from the conventional two-dimensional integrated circuit (IC) and silicon-based two- or three-dimensional MEMS processes. This includes photolithography, thin film metallization, and chemical etching. Later, glass based, glass-silicon, glass-polymer mixed microfluidic fabrication techniques, and devices started to garner more
interest. The glass materials were preferred partly for the biocompatibility toward the biomedical related application and the ideal surface characteristics where high temperature or strong solvents should appear (e.g., on-chip capillary electrophoresis-based operations). However, lack of optical transparency at interested wavelengths (for silicon), micromachining difficulties, and comparably high expenses for both silicon and glass materials have hampered their wider applications in microfluidics. Tremendous effort has been made to find alternative materials that are more cost-effective and easier for micromachining. With the development of related fabrication techniques in recent years, the polymer/plastic-based microfluidic systems has garnered more interest than its conventional competitors. In spite of comparatively weak bonding and structure deformation during device packaging processes, polymer materials still seem attractive due to the facts that: they are more economic compared with silicon and glasses, easier to be fabricated in/on, avoidance of high-temperature annealing and stringent cleaning, more system integration friendly (e.g., interconnections), and there exists a wider range of materials to be chosen for characteristics that are required for each specific application, such as good optical transparency, biocompatibility, and chemical or mechanical properties. Another important reason for the interest from both academia and industry on polymer microfluidic devices is the possibility of disposable microfluidic chips toward biomedical and clinical applications. These devices usually require low cost of fabrication, high volume production, good reproducibility, and versatility in design for a wide spectrum of specific applications.

Current methods for fabrication of microfluidic devices include prototyping techniques (includes hot embossing injection molding and soft lithography), and direct fabrication techniques such as laser photoablation or laser micromachining, photolithography/optical lithography, and x-ray lithography (Figs. 1 and 2). Table 1 presents the advantages and disadvantages of those techniques. Note that “photolithography” in this table refers to conventional photolithography that is mostly employed in IC industry for micrometer scale patterning. In fact, during the past decade photolithography techniques have progressed to achieve smaller feature patterning scale patterning. In fact, during the past decade photolithography that is mostly employed in IC industry for micrometer scale patterning. In fact, during the past decade photolithography can be gradually improved with the high quality masks of modern photolithography. Sub-100-nm fabrication resolution can also be achieved by composite layers of stamps. Other techniques were also used to obtain the soft lithography masters with nanometer scale features below 5 nm: such as to replicate those features from single-walled carbon nanotubes or from crystal fracture as soft lithography masters. For photolithography made masters for the soft lithography process, the recently reported resolution limit has been pushed to around 20 nm by Li et al.

3 Detection Methods

Integrated microfluidic devices involve the large-scale integration of various microfluidic components, such as microvalves, microchannels, micropumps, microfluidic mixers, and other elements to handle and control fluids at the microscale. They are frequently used for biological, chemical, and biomedical analysis. There has already been a considerable amount of insightful review articles on related topics. Various detection methods exist in the field of chemical, biological diagnosis, or analysis on microfluidic platforms.

The detection methods in microfluidics can be classified into three major types: optical methods, electrochemical methods, and mass spectrometry methods. Among these methods, optical and electrochemical methods are the most frequently utilized due to their selectivity and sensitivity. Other than the above major methods, approaches such as nuclear magnetic resonance (NMR) spectroscopy, magneto-resistive and acoustical methods are also coupled to microfluidics for sensing application.

As demonstrated in Fig. 3 typical optical detection methods comprise the direct detection by monitoring the light properties including fluorescence, absorbance, and luminescence-based methods, and the light property modulation detections such as surface plasmon
### Table 1 Microfluidic fabrication techniques.

<table>
<thead>
<tr>
<th>Methods</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hot embossing</td>
<td>Cost-effective, precise, and rapid replication of microstructures, mass production</td>
<td>Restricted to thermoplastics, difficult to fabricate complex 3D structures</td>
<td>56</td>
</tr>
<tr>
<td>Injection molding</td>
<td>Easy to fabricate complex geometry, fine features, and 3D geometries, low cycle time, mass production, highly automated</td>
<td>Restricted to thermoplastics, high cost mold, difficult to form large undercut geometries</td>
<td>58</td>
</tr>
<tr>
<td>Soft lithography</td>
<td>Cost-effective, able to fabricate 3D geometries, high resolution (down to a few nm)</td>
<td>Pattern deformation, vulnerable to defect</td>
<td>59, 63–66</td>
</tr>
<tr>
<td>Laser photoablation</td>
<td>Rapid, large format production</td>
<td>Multiple treatment sessions, limited materials</td>
<td>60</td>
</tr>
<tr>
<td>Conventional photolithography/optical lithography</td>
<td>High wafer throughputs, ideal for microscale features</td>
<td>Usually requires a flat surface to start with, chemical post-treatment needed</td>
<td>61</td>
</tr>
<tr>
<td>X-ray lithography</td>
<td>High resolution to fabricate nano-patterns, absorption without spurious scattering, able to produce straight smooth walls</td>
<td>Difficulties in master fabrication process, time consuming, high cost</td>
<td>62</td>
</tr>
</tbody>
</table>

resonance (SPR) detection. These methods usually involve techniques such as evanescent waves, SPR, interferometry, Raman spectrometry, fiber optics, and optical waveguides. Photonic crystals, optical cavity structures, and several optical techniques have also been reported to be integrated with the microfluidic system for sensing purposes, using one- and two-dimensional surface PCs (guided mode resonance filters) or three-dimensional photonic crystals or optical cavities or whispering gallery mode resonators and optical tweezers for cell related monitoring or fluidic rheological measurements. Electrochemical measurements are based on electrical property modulations of the analyte species that undergo redox reactions, and are usually employed for the detection of the electroactive species. They can be assigned to three categories: amperometries, potentiometries and conductometry measurements. The principles of these methods are demonstrated in Fig. 5. Amperometric detection

![Typical optical methods](https://www.spiedigitallibrary.org/journals/Journal-of-Biomedical-Optics)
is based on the fact that an applied voltammetric potential between a reference and a working electrode would cause the oxidation or reduction of the electroactive species in the vicinity, and an electrical current will be induced. Sensitive detection of the analyte(s) can be realized by the cyclic voltammogram(s) and current versus time curves of the electrode. In potentiometric detection, analyte detection is realized by monitoring the potential of an ion-selective electrode (usually a membrane) against a reference electrode. When selective ions pass through the membrane and a local equilibrium is established at the sensing interface, the resulting charge separation causes a potential between the working electrode and the reference electrode in relation to the species type and concentration. The principle of conductometric detection is that the conductivity of a zone is affected by the charged species in the zone. Different types of species would have their specific conductivity responses, which would also vary with different concentrations. It is the most commonly employed method in electrochemical measurements since it, in principle, can deal with all charged species of interest. The detection involves measuring the conductivity at a series of frequencies, both in conventional contacted conductivity detection and capacitively coupled contactless conductivity detection (C4D) methods such as potential gradient detection. Electrochemical detections are often used together with (capillary) electrophoresis operations (such as capillary electrophoresis separation) or in electrophoresis systems. Considering this, in this review the “electrochemical methods” actually include electrophoresis related work.

Mass spectrometry (MS) is able to perform highly selective detection by monitoring the trajectory of ions in electric and/or magnetic fields, which elucidate the mass and charge of the ions. Its most important application is in proteomic studies for protein separation and further identification from the fragmentation pattern of proteins. Identification after separation can be carried out in two ways. One is direct detection by combing through the database using the individual types of obtained protein(s). The more sophisticated way is carrying out tandem MS to get protein fragments/ions for sequence tagging. To date, several MS configurations have already been developed to be integrated with microfluidic devices for this application, for instance, electrospray ionization (ESI)-MS and matrix-assisted laser desorption ionization (MALDI)-MS. In 2006, another paradigm is ion trap mass spectrometry integrated with microfluidics for protein identification by Hardouin et al. Other systems were also reported, such as a chip-liquid chromatography (LC)-MS system for label-free profiling of human serum and a LC-ESI-MS system for...
multiple proteins detection from breast cancer cellular extract. The resolving ability of MS has recently been pushed down to the detection of a single molecular by Roukes’s group in Caltech using a nanoelectromechanical system-based MS (NEMS-MS) (Fig. 6). It can be expected that in the future when NEMS-MS meets microfluidics, a much more effective microfluidic-MS platform could be realized to analyze biological or chemical species (e.g., proteins or nanoparticles) at a single molecular level with the ability to operate in multiplexing and parallel modes.

Besides protein separation and identification, MS has also been applied to the quantitative detection in protein expression in various states (especially in disease states). However, the miniaturization and high sensitivity requirements have currently been one of the most significant technological hurdles. Yet, microfluidics seems to hold great promises for the related technological breakthrough: The micrometer scale geometries and smaller platform of microfluidic devices meet the enhanced sensitivity and system miniaturization need; the multi-channel geometries in microfluidics enable high throughput processing and multiplexing ability. Considering these, to couple with microfluidics could be the ultimate lab-on-a-chip solution for MS toward quantitative proteome applications. To our knowledge, there has not been any further report on MS-based microfluidic sensing and yet a lot of interesting research remains to be carried out.

Other than the three major categories, other methods such as the NMR spectroscopy have also been explored to be applied to microfluidic detection. It is a well-developed detection method in chemistry and life sciences, which employs the magnetic properties of nucleus or the chemical shift Zeeman effect and/or the Knight shift effect for detection purpose. NMR spectroscopy is able to detect biological and chemical analyte species such as proteins and nucleic acids. However, its application in microscale systems has been restricted by the low sensitivity of conventional NMR detection technique. Recently, this problem has been solved by hyperpolarization methods (e.g., to introduce the highly polarized para-hydrogen agent for signal enhancement). High resolution NMR for microfluidic systems was realized in 2007 by Pine’s group in UC Berkley on the study of multi-phase flows and catalyst deactivation (Fig. 7). Besides direct detection, the Pines’ group also pioneered the remote monitor work of NMR-based microfluidic detections. In 2007, they reported the remote monitoring of spin coherence transfer in chemical transformation and a double-phase encoded remote detector of the fluid diffusion through membranes. This technique is readily applicable mostly in hydrogenation reaction-related detection and imaging, and might be extended for more applications in microfluidics. However, the limited reaction time scale/polarization lifetimes remains a key technological bottle-neck for NMR-based microfluidic analytical detection. Besides, similar to MS spectroscopy, another key hurdle
is system miniaturization, in other words, to reduce the comparably large detector for better interface into microfluidics. In fact, not many related publications were reported afterward on NMR-based microfluidic detection. In spite of the technological challenges, NMR is still a competitive technique for microfluidic applications compared to optical or electrochemical methods: It does not have the optical accessibility issue for the region of interest (ROI) and the data acquisition time is much shorter, which makes time-resolved studies much easier; it has better detection persuasiveness than electrochemical methods that are limited to electroactive species. Hence, the solution to the weak NMR signal issue in microsystems, either by further signal enhancement or better detection implementations, will trigger a wider spectrum of lab-on-a-chip applications of NMR.

4 Sensing Techniques

In Sec. 3 we discussed general detection methods in microfluidics. Here, we would like to emphasize the microfluidics/optofluidics sensing techniques. A general review is presented in Table 2. From Table 2 we can see that the different methods have corresponding advantages and limitations. Some of them can be coupled with each other either to achieve certain phenomena for detection or to enhance sensor ability. For instance, evanescent waves are utilized with interferometers, optical fibers, fluorescence induction, and optical waveguides, in order to further enhance the detection sensitivity (e.g., in the form of a fiber-optic evanescent-wave approach or a fiber-optic localized plasmon resonance sensor). They are also widely used to induce surface plasmon resonance, which is essential in surface-enhanced Raman scattering. Cavities structures are used in localized plasmon resonance detections to improve sensing performance. Other detection methods were also actively studied for microfluidics, such as integrated optics components that can accommodate large arrays of compact optical channels and devices. One example is the laser induced fluorescence (LIF) detection, which is extensively used in microchip separations.

5 Outlook

The past decade has witnessed the progresses in microfluidics: more microfluidic system prototypes, increased device complexity, and more fabrication and sensing techniques have been developed or improved. However, microfluidics sensors are still in a formative stage and hold tremendous opportunities to be applied to a wider spectrum of fields and applications. We could expect the next drive engine to microfluidics sensors development would be based on:

(1) Complete integration for compactness and self-contained microsystems

While most of the current microfluidic systems are still based on microfluidic devices that are coupled with external macroscopic detectors or preliminary external detection platforms, it is of great significance to push the microfluidic systems down to a complete microscale level. The development of specific detection components for microfluidics and complete integration techniques would enable real compact and self-contained microfluidic systems.

(2) New and existing sensing principles and related technological advances

It is essential to explore for new sensing principles, or possible sensing components to be integrated with microfluidics while improving the sophistication of the microfluidic sensing systems. Ideal candidates should be both localized and sensitive (with minimized negative system perturbation), easy to fabricate and integrate, easy to assemble or even without post-fabrication assembly. More materials could also be considered, for instance, functionalized nanoscale particles, such as specialty nanocomposites (e.g., nanomaterials, nanocrystals, nanoparticles, nanocolloids, liquid crystals, quantum dots) and bio-/chemical-functionalized nanoscale materials or particles, could be incorporated with a microfluidic environment for flexible reconfiguration and system tuning for various sensing purposes.

Continuous research investments on optical, electrical, chemical, as well as mechanical, thermal, magnetic, or biological phenomena would open up new possibilities as well. In addition, technological advances in related fields such as electrical/optical/chemical instrumentation, microfabrication techniques, new material development, and processing techniques, would all contribute to the development of microfluidic sensors. Once the major barriers of existing sensing methods are cleared, such as the short life time and low resolution issue in NMR microfluidic sensors, the progress would be rapidly sped up.

(3) Theoretical work and software development

The study of integrated microfluidic sensors should be extended to more sophisticated simulation methods and elaborate algorithmic models for a deeper understanding at a theoretical level. For instance, a more complex model should be developed for the theoretical understanding of the shear stress acting on the particle in microflows, which includes the effect of the particle size and other parameters such as concentration and temperature. As in a real microfluidic suspension.
**Table 2** Optofluidic sensing techniques.

<table>
<thead>
<tr>
<th>Methods</th>
<th>Measurements/Applications</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Sensitivity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluorescence</td>
<td>Temperature, cell function, flow velocity, polymer dynamics</td>
<td>High selectivity, sensitivity</td>
<td>High cost, extensive calibration and mathematical corrections, lack of appropriate fluorophores</td>
<td>Down to single DNA level</td>
<td>74, 81, 83, 157 and 158</td>
</tr>
<tr>
<td>Mach-Zehnder interferometers</td>
<td>Refractive index, fluorescein concentration</td>
<td>High resolution, label free</td>
<td>Compromise between interaction lengths and sensitivity, multiplexing difficulties</td>
<td>Down to a few nano-molar concentration</td>
<td>74, 124, 126 and 159</td>
</tr>
<tr>
<td>Dual polarization interferometry</td>
<td>Refractive index, conformational change, layer thickness, average density</td>
<td>Label free, simultaneous multiple parameters detection</td>
<td>High cost, alignment difficulties, unsuitable for detection of large numbers of mutations</td>
<td>Down to sub-pico-molar concentration</td>
<td>127, 128 and 160</td>
</tr>
<tr>
<td>Surface enhanced plasmon resonance</td>
<td>Refractive index, molecular and chemical reactions, polymer dynamics</td>
<td>Superior sensitivity and selectivity, label free</td>
<td>Low throughput, system alignment difficulties, small penetration depth, large sensing area required, small molecular weight analytes sensing difficulties, specific and nonspecific interactions differentiate difficulties, surface sensitive</td>
<td>Down to nano-molar level, single molecules (e.g., DNA, RNA, proteins)</td>
<td>76, 80, 122 and 123</td>
</tr>
<tr>
<td>Localized (surface) plasmon resonance</td>
<td>Refractive index, binding events</td>
<td>Label free, highly localized, real-time monitoring, simple and cost-efficient detection equipments</td>
<td>Instability of the metal nanostructures’ morphology and optical properties</td>
<td>Down to pico-molar concentration, single DNA level</td>
<td>153, 156 and 161</td>
</tr>
<tr>
<td>Surface enhanced Raman spectroscopy</td>
<td>Molecular detection, investigation of the structure and function of large biomolecules, analysis of chemical processes</td>
<td>Superior sensitivity and selectivity, label free</td>
<td>Small penetration depth, surface quality dependence, denaturation problem for biomolecules, overlapping peaks</td>
<td>Down to single molecule level, sub-pico molar concentration</td>
<td>77, 129, 131 and 154 and 162</td>
</tr>
<tr>
<td>Fiber optics</td>
<td>Rotation, acceleration, electric and magnetic field measurement, temperature, pressure, acoustics, vibration, linear and angular position, strain, humidity, viscosity</td>
<td>Superior sensitivity, simultaneous multiple parameters detection, easy for integration, refractive index, chemical measurements, label free, environmental ruggedness, multiplexing ability, dynamic range and resolution</td>
<td>High cost, end-user unfamiliarity</td>
<td>Down to tens of nanometers, pico-molar concentration</td>
<td>78, 153, 163 and 164</td>
</tr>
<tr>
<td>Planar waveguides</td>
<td>Refractive index detection, study and detection of particles</td>
<td>High sensitivity, label free, compact configuration</td>
<td>Small penetration depth, limited for processes with diffusing particles</td>
<td>Single viruses and liposomes resolution</td>
<td>72 and 132</td>
</tr>
<tr>
<td>photonic crystals</td>
<td>Particle interactions (e.g., protein-protein, antibody-antigen and small molecule-protein interactions), cell-based assays, refractive index, concentration, absorption and conformation of biomolecules on a surface</td>
<td>Potentially high sensitivity, localized confinement, flexibility in sensor structures and related properties</td>
<td>Fabrication difficulties for higher order photonic crystals, related high cost, difficulties in high-throughput screening</td>
<td>Down to single molecule level (e.g., protein), nano-molar concentration</td>
<td>134, 136</td>
</tr>
<tr>
<td>Micro-ring and microsphere resonators, including those using whisper gallery modes (WGM)</td>
<td>Detection of virus, protein, DNA, or bacteria, detection of concentration, refractive index, binding events of molecules</td>
<td>Multiplexing feasibility for micro-ring resonators, high Q (microsphere resonators: Q &gt; 10^9, micro-ring using WGMs: Q &gt; 10^6)</td>
<td>Mass produce difficulties, multiplexing difficulties for microsphere resonators, lack robustness</td>
<td>Down to single molecule level</td>
<td>146, 147 and 163</td>
</tr>
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</table>
environment, considerable deviations have been observed in the experimental measurement results from the simulation with a pure fluidic system. Modeling of the fluidic dynamic characteristics, such as velocity, heat transfer, and shear stress inside microfluidic devices will also be beneficial.

Microfluidic work from the very beginning has been mainly among physical or more specifically fluid mechanical and lab-on-a-chip communities. However, the most important end-users are usually biological or chemical specialists, to whom the theoretical understanding, numerical simulation skills, and lab-on-a-chip know-how could be a huge hurdle. The accessibility of more user-friendly simulation software to nonspecialists could alleviate this problem and allow biologists and chemists to actually get actively involved in the early stage development of the sensors to better suit the actual demands.

Besides, the simulation software provides a more economical and quicker research and development loop by the pre-fabrication numerical tests for various designs or fabrication procedure optimization. In this way, the cost and time frame will be sharply reduced with refined theoretical simulations and modeling work carried out beforehand.

(4) Standard integration interfaces
Another important issue is that most of the current microfluidic sensing platforms are “one-design-one-application” types. It is not easy to modify or upgrade the systems or switch for multi-function operations. Usually the change of applications or the instruction of an additional task would simply mean redesigning a system. Thus, a standard microfluidic interface, that is friendly and flexible for various functional components to be easily integrated to, holds great promises for enhancing the adaptivity of microfluidic sensing systems. This will open up the possibility of dynamic microfluidic systems that support versatile modes of interfacing and operations.

References


