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Topical Review

Roadmap for optofluidics

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Abstract

Optofluidics, nominally the research area where optics and fluidics merge, is a relatively new research field and it is only in the last decade that there has been a large increase in the number of optofluidic applications, as well as in the number of research groups, devoted to the topic. Nowadays optofluidics applications include, without being limited to, lab-on-a-chip devices, fluid-based and controlled lenses, optical sensors for fluids and for suspended particles, biosensors, imaging tools, etc. The long list of potential optofluidics applications, which have been recently demonstrated, suggests that optofluidic technologies will become more and more common in everyday life in the future, causing a significant impact on many aspects of our society. A characteristic of this research field, deriving from both its interdisciplinary origin and applications, is that in order to develop suitable solutions a combination of a deep knowledge in different fields, ranging from materials science to photonics, from microfluidics to molecular biology and biophysics, is often required. As a direct consequence, also being able to understand the long-term evolution of optofluidics research is not easy. In this article, we report several expert contributions on different topics so as to provide guidance for young scientists. At the same time, we hope that this document will also prove useful for funding institutions and stakeholders to better understand the perspectives and opportunities offered by this research field.

Keywords: optofluidics, photonics, microfluidics, nanofluidics

(Some figures may appear in colour only in the online journal)

Contents

1. Introduction

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Optofluidics: an emerging and promising topic. The field of optofluidics is a relatively new one in the scientific panorama. Although the idea of using fluids to control light, e.g. by spinning-mercury mirrors, dates back to the 18th century, no liquid-mirror telescope was practically realized before the end of the 20th century. In particular, it is only over the last 15 years that the term 'optofluidics' has attracted significant attention, and that many groups have started devoting their research efforts to this field.

The 'young age' of this research field is demonstrated by the fact that the first works indexed on the Web of Science database and containing the word 'optofluidics' in the topic appears in 2005 [1, 2]. Since that moment, the attention devoted to the realization of systems exploiting the simultaneous control of fluidic conditions and optical beams has roared and other important seminal papers have appeared [3, 4]. In the following 12 years (2005–2016), the number of papers that can be found using the same criteria increased from 0 to 580. Currently, about 70 new papers with the word 'optofluidics' in the title are published every year, and the number of citations given to optofluidics papers is continuously growing, almost linearly, and increased from 20 in 2006 to about 1600 in 2016. As a result an increasing number of short schools for PhD students are currently being scheduled by universities, and topical conferences expressly dedicated to the field of optofluidics are being organized by important scientific associations (e.g. the EOS Conference on Optofluidics in Europe, the International Multidisciplinary Conference on Optofluidics in Asia, etc), so as to create meeting points for researchers with different backgrounds and interests [5–7].

Nowadays, optofluidic devices and applications are generally based on the integration of optical technologies (for sensing, actuation or imaging) within microfluidic (or nanofluidic) systems, allowing the use of small sample volumes in a highly controlled environment.

It is worth underlining that the field of optofluidics is doubly interdisciplinary, not only because of the competences required for the design and realization of the devices, but also because of the countless possibilities offered by these devices in the fields of chemical sensing, physical characterization and biomedical research. A consequence of the vastness of the optofluidics field is that writing a fully exhaustive Roadmap paper is an almost impossible task, and hence some techniques/applications, always inherent to the world of optofluidics, such as droplet-based optomechanics, optofluidic biolasers and optoelectronic tweezers, are not included in the current article, but they are covered in other papers [8–10].

Intended audience and aim of the Roadmap. The intended audience of this document are from three different categories of possible readers: young researchers, scientists from other disciplines, and optofluidics experts.

Young researchers starting their activity in optofluidics could significantly benefit from this article, as it represents a reference text, showing the possibilities and challenges of this specific research field. Additionally, the Roadmap also comes with a substantial bibliography making it easier to identify and access many helpful papers.

The Roadmap could also be useful for researchers who have already developed good expertise in a contiguous field, such as chemical sensing, microfluidic devices, high sensitivity molecule sensors, single-cell analysis, material science and high-precision diagnostics. For all of these areas, optofluidics can surely open new possibilities and scenarios and this Roadmap can help to unveil them.

Finally, for experienced researchers in the optofluidics field, the contributions included in this text by well-known experts give a reference point for the current state-of-the-art. The Roadmap gives them recent updates on scientific activities and an analysis of what other experienced researchers see as future perspectives.

The aim of this document is to show not only the current technologies and applications, but also promising approaches that still require research and technological development. It is, in any case, perfectly clear to the authors of this paper that what is now perceived as a challenge may, in such a rapidly evolving field, be achieved in one year, or even less, or may no longer be considered as a relevant target. Nevertheless, it is of great importance to define which research directions are now considered as the emerging and promising, so that it will be possible in the future to re-assess the validity of this analysis.

Another fundamental point that this Roadmap wants to stress, thanks to the different contributions, is the strong connections linking applications, materials, and methods. For this reason, contributions on all these aspects are included in the document, thus creating an article very different from more topic-specific reviews that limit their scope to detailed technology, such as imaging [11], sensing [12], actuations [13], or applications such as point-of-care diagnostics [14]. It is worth underlining that, even if many topics are discussed in each section of this Roadmap, the order of the different contributions has been selected to allow easy sequential reading.

Current status and challenges. This section briefly provides young researchers, and all those needing some introduction to the basics of optofluidics, with a short list of the main concepts useful for fully exploiting the Roadmap content, and a list of suggested introductory reading.

One of the basic ideas in optofluidics is that of taking advantage (at least in the majority of cases) of the laminar-flow regime that is obtained as a consequence of the typical dimensions and flow speed used in microfluidic systems. The strong laminarity of these systems is testified by their Reynolds number, defined by the equation below, where ρ is the fluid density ($\approx 10^3$ kg m^{-3} in case of room-temperature water), v is the fluid speed (generally in the range

10^{-5}–10^{-1} m s^{-1}), D is the microchannel diameter (of the order of 10^{-4} m) and μ is the dynamic viscosity (which for water is about 10^{-3} Pa s):

$$\boldsymbol{Re} = \frac{\rho \nu D}{\boldsymbol{\mu}}.$$

If we compare the maximum *Re* value achievable with the above reported numbers, 10, with the reference value of 3000, generally considered as the boundary between laminar flow ($Re < 2000$) and turbulent flow ($Re > 4000$), it is immediately understood that unless turbulence is artificially created a laminar flow is produced in these systems [15, 16]. Flow laminarity, which is a crucial point when well separated streams are needed, can become an issue when fluid mixing is required. In this case specific structures can be used to break the system laminarity [17, 18].

Another fundamental element in the optofluidics field is the good size-matching between biological objects (bacteria, cells, or organelles), microfluidic channels, and the diameter of optical beams in the visible or near-infrared spectral range. All these quantities are generally in the range between 0.1 μm and 500 μm and, while the size of biological objects is generally given data, it is often possible to tailor the microchannel width and optical beam diameter in order to obtain the desired values and fluidic/optical effects [19].

Additionally, when it is important to apply the samples forces matching those generally involved in biological processes, the radiation pressure exerted by optical beams can be used to trap, push or deform cells [20–26]. On the other hand, when much larger forces are required they can be produced by exploiting optically induced cavitation bubbles [27, 28], which have already been successfully demonstrated as an efficient actuation mechanism for different applications, ranging from cell sorting to droplet generation and fusion [29–31].

Finally, in optofluidics, thanks to the different responses exhibited by samples under analysis to optical or acoustic forces, it is also possible to use different actuation mechanisms as 'sensing mechanisms', enabling interesting selection and sorting strategies [32–36].

Some trends and challenges. Without going into the specific solutions that can be envisaged, there are three main challenges that future optofluidic devices should try to solve.

The first one deals with sample insertion and collection from a microfluidic system. Although the use of extremely reduced sample volumes brings several advantages, it is nevertheless evident that properly managing volumes in the μl range (or smaller) obviously requires precision and attention. Even more challenging is the collection of output samples in all those situations where sub-populations or cells (or even single cells) need to be extracted, preserving their viability and without the risk of losing them in microchip dead volumes.

A second challenge is that of achieving high performance and sensitivity while using low-cost, or disposable, systems. Such an achievement could allow the diffusion of optofluidic solutions in many environments where laboratory equipment is not always available.

The third challenge that many microfluidic structures are now trying to solve is that of creating easy-to-use devices. State-of-the-art microfluidic devices are generally tested on laboratory tables, requiring a lot of additional instruments (laser sources, thermal controllers, objectives, micropumps, cameras, etc) and must be operated by experienced researchers. The integration of all these functionalities in a simple microsystem would largely help the diffusion of these devices, making them user friendly and more suitable for point-of-care analysis. In particular, considerable attention has been paid in the last five years to the development of compact and efficient imaging systems, which will also be discussed in the following.

Acknowledgments. I want to thank Professor Ilaria Cristiani for her precious suggestions and Dr Jarlath McKenna for the great help in organizing and coordinating the article.

2. 3D optofluidic devices using femtosecond laser micromachining

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Relevance of the topic. Femtosecond laser micromachining (FLM) of transparent materials [37, 38] is a recent technique that has found wide application in optofluidic device fabrication. FLM is based on a non-linear absorption process, selectively triggered in the focal volume by ultrashort and tightly focused laser pulses (figure 1). Tailoring of the irradiation parameters can produce very different results in glass. In particular, one can achieve a gentle and localized change of the refractive index or self-assembled nanostructuring of the material. The first modification is extremely relevant as it allows drawing optical waveguides by suitably moving the glass substrate with respect to the laser focus. The second modification is also important for optofluidic applications since the nanostructured region has an enhanced selectivity to wet chemical etching (e.g. aqueous solutions of hydrofluoric acid). This means that an irradiated volume in this second regime will be preferentially etched away with respect to the unirradiated material, thus creating cavities and microfluidic networks inside the glass.

The first relevant advantage of this microfabrication technology for optofluidics is the capability to produce, with the same tool, both optical waveguides and microfluidic channels in glass. This puts the technology in a very favorable condition to combine these two elements in the same substrate and produce compact and complex optofluidic devices. The second advantage of this technology is its unique capability to produce 3D structures, taking advantage of the fact that the induced modification is highly localized to the focal volume and can thus be arbitrarily placed anywhere in the glass volume. A third advantage is the possibility of combining very different fabrication techniques with a single tool, from material property tuning (e.g. in waveguide writing), to selective material removal (e.g. for microchannel fabrication) and additive manufacturing by two-photon polymerization (e.g. for the creation of plastic microstructures embedded in the microfluidic channels [39, 40]). A final important advantage of FLM is its rapid prototyping capability; as for any maskless and direct-writing technique, a software design can be transferred onto a working prototype, with the possibility of simply and rapidly adjusting the design or to produce highly customized products.

Further advances in this technology will provide an unprecedented level of integration of functionalities in a single microsystem, with very short time-to-market of new products.

Current status and challenges. FLM has been widely exploited in the fabrication of optofluidic devices with the main aim of integrating optical detection and microfluidic handling of samples [38]. The use of optical waveguides to deliver a probing light and to collect output signals enables higher sensitivity, reproducible and alignment-free measurements, and multipoint sensing. Taking advantage of the 3D capabilities of the technology, it has been possible either to add specific components to already existing devices or to fabricate a complete microsystem using FLM. An example of the former case consists in adding optical waveguides to commercial microfluidic labs-on-a-chip to perform on-chip fluorescence or label-free detection of relevant biomolecules [38]. A different example of how FLM can be exploited to add specific components to already existing devices is the concept of lab-in-fiber [41]. In this case, the starting device is an optical fiber, where the cladding is typically underused. In this approach, FLM is exploited to add microchannels, cavities or additional optical waveguides to produce highly functionalized optical fibers that become self-consistent and sophisticated optofluidic sensors (figure 2(a)). The possibility of using FLM to fabricate the whole device further widens the design possibilities and fully unleashes the 3D capabilities of the technology. 3D microfluidic layouts have been exploited to achieve full-hydrodynamic focusing and, combined with laser-written optical waveguides, has enabled the demonstration of a 1 mm^3 cell counter, capable of counting up to 5000 cells s^{-1} [42]. Cell manipulation is another relevant application of FLM-produced devices (figure 2(b)). In fact, optical forces, exerted by the light coming from optical waveguides, can be exploited to trap and deform cells flowing in a microchannel, thus characterizing their mechanical response [43]. This analysis can discriminate between different cell populations (e.g. between healthy and cancer cells) without any markers (see section 11 for more details). Depending on the assay result, each cell can then be sorted into different output channels, again exploiting optical forces produced by a dedicated waveguide. The combination of 3D microfluidics and optical waveguides has been also exploited to produce a 3D-shaped nanoacquarium to study the behavior of

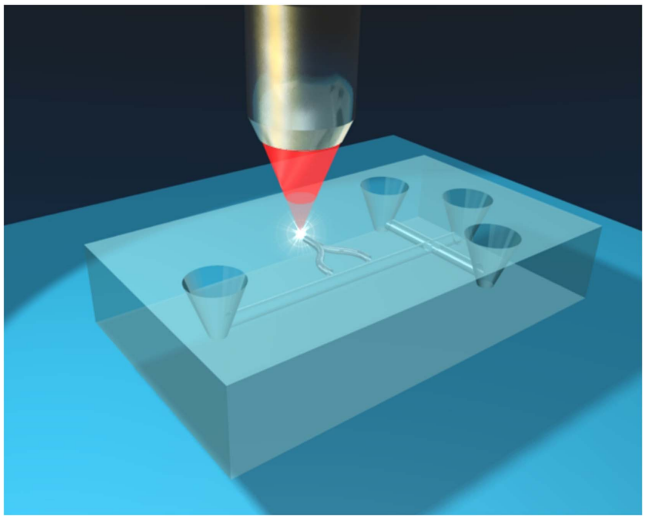

Figure 1. FLM can create optofluidic devices, encompassing optical waveguides and microfluidic channels, directly buried inside a glass sample.

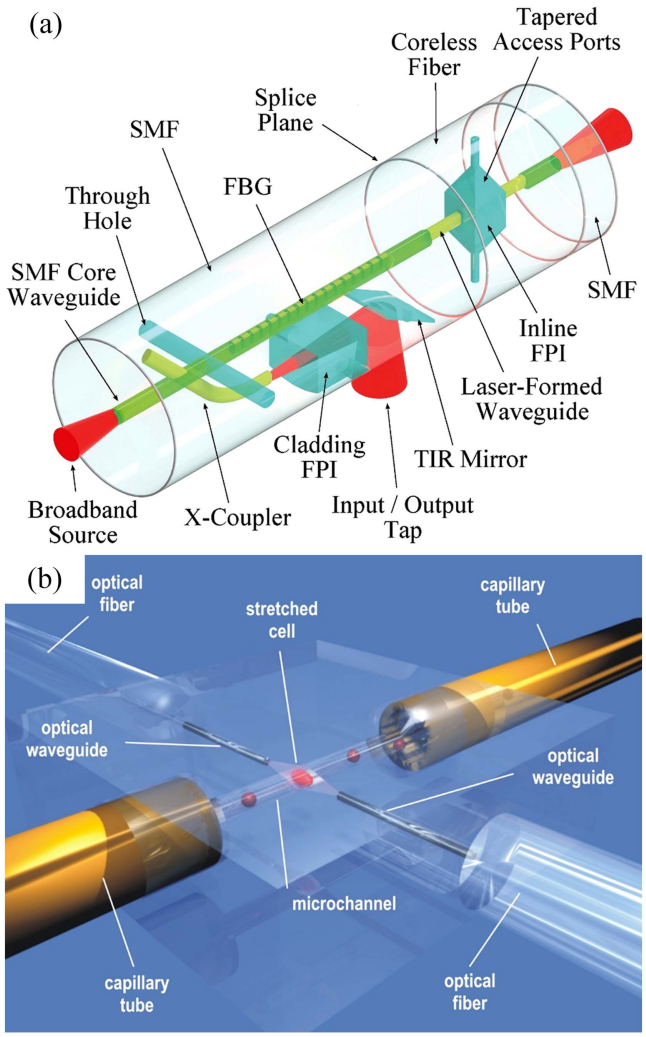

Figure 2. Two examples of optofluidic devices fabricated by FLM. (a) Lab-in-fiber: several optical and fluidic components can be integrated into the cladding of a fiber to perform optofluidic sensing; SMF: single-mode fiber, FBG: fiber Bragg grating, FPI: Fabry–Perot interferometer, TIR: total internal reflection. Reproduced from [41] with permission of The Royal Society of Chemistry. (b) Optical cell stretcher: optical forces exerted by light delivered by two optical waveguides allow characterizing the mechanical properties of flowing cells [43]. Reproduced from [43]. CC BY 3.0.

cyanobacteria in controlled conditions [44]. In particular, optical waveguides embedded in the chip were used to accurately monitor the CO_2 concentration in the nanoacquarium. Another 3D optofluidic device that allows sophisticated fluorescence imaging of biological samples has been the recent demonstration of selective plane illumination microscopy (SPIM) on-chip [45]. An optofluidic cylindrical lens was combined with a suitably shaped microchannel, allowing optical sectioning and 3D reconstruction of cellular spheroids in suspension. The biological sample is driven through the light sheet, created by the cylindrical lens, with a uniform microfluidic flow.

As already mentioned, additional functionalities can be integrated into the optofluidic device by two-photon polymerization (2PP). In particular, the 3D capability of FLM enables the 2PP-fabrication of plastic structures directly inside sealed microfluidic channels [39], with an approach that has been dubbed 'ship in a bottle' [40]. As an example, arrays of diffractive and refractive microlenses have been produced inside FLM-fabricated microfluidic channels to achieve white-light detection and counting of cells flowing in the microchannel [40].

A few technological challenges are still open to fully exploit this technology. The first one is FLM throughput; in fact, being a serial writing technique, it requires the time to irradiate each device and does not benefit from economy of scale. A second challenge is the possibility of processing different materials other than fused silica and Foturan glass. The third challenge is further miniaturization to the nanoscale.

Advances in science and technology to meet challenges. The challenges previously detailed have triggered a significant effort in the community and, although still open, there have already been encouraging results towards their solution. Regarding the scaling of fabrication capability, fine optimization of the processing window has already allowed writing velocities of the order of cm s^{-1} for several processes. In addition, the use of spatial light modulators has introduced the possibility of multifoci writing, thus enabling the production of several structures in parallel [46]. These approaches have still to be exploited fully to understand the ultimate limits, but FLM will also likely be competitive with standard microfabrication technologies for medium-sized production batches and not just for a few prototypes. Fused silica or Foturan glasses are excellent substrates for many optofluidic applications and in particular for biophotonic ones. However, enlarging the portfolio of materials amenable to FLM will be beneficial for targeting new optofluidic applications. A better understanding of the nanostructuring process, the basis of the microchannel formation, and its replication in other materials is an ongoing activity that still requires some effort. Finally, further miniaturization to the nanoscale could be the next big evolution of the technology. The 2PP technique is already able to produce nanoscale structures and recently nanofluidic channels have been demonstrated by FLM [44]. This fabrication scale is still largely unexplored; however FLM has the unique capability of easily connecting nanoscale structures to microscale ones, thus potentially improving the usability of new devices at this very small scale.

Concluding remarks and perspectives. FLM is a very powerful technique for the fabrication of optofluidic devices, allowing unique 3D layouts and an amazing level of integration of different functionalities. Its versatility opens up the way to many different applications, both for research and industry. In particular, in those fields where standardization is not too rigid, the design freedom allowed by FLM can produce revolutionary microsystems that will set new standards in the field.

3. Lithium niobate as an optofluidic platform

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Relevance of the topic. Droplet-microfluidics technology has paved the way to new approaches in chemical and biological analysis, exploiting parallel processing and high-throughput response [47]. Screening, rapid droplet sorting and biochemical microreactors have been demonstrated as well as bio-analytical assays, enzyme kinetics, cell analysis, encapsulation and sorting. Thanks to the exploitation of reduced volumes down to nanolitres and femtolitres, as well as reaction times shortened to seconds or less, chemical synthesis and complex particle fabrication up to drug delivery and diagnostic testing and biosensing have also been reported [48]. Although novel microfabrication techniques are continuously being developed to improve final performance, lower the device costs and differentiate functionalities with an appropriate fluidic interfacing scheme, fully integrated optomicrofluidics devices are far from being widely commercially available. The perspective of combining optical tool versatility with the potential of microfluidics has gained increasing interest. As already foreseen by Psaltis and co-workers in their pioneering work in [49], novel enabling technologies leading to true lab-on-a-chip systems have started being under debate where chemical, physical and biophysical sensors [50] could be integrated on multipurpose portable devices. To this aim, several challenges should be faced both from a scientific and a technological point of view. An optomicrofluidic portable device should in fact guarantee a trustworthy response, i.e. a reproducible and repeatable output that, minimizing false responses and artefacts, is easy-to-read/store and fast to calibrate. Finally, to be really competitive with fully optional bulky laboratory apparatus, it should be compact, low cost, easy to use and handle, and stable under different environmental conditions. At first sight, all these features seem difficult to achieve. The demand of reducing costs has therefore prevailed, leading to the spread of disposable devices and placing as a second priority the possibility of checking, monitoring and exploiting the device response in a longer timescale. However, in the last decade, draining the expertise in photonics, integrated optics and microelectronics MEMS processing into microfluidics has meant advances in overcoming and bypassing these limitations by the suitable combination of materials, preparation techniques and smart approaches, respectively [51].

Current status and challenges. In lab-on-a-chip systems, the integration of many stages is a key point in portable compact devices. Consequently, flexible and easily-molded materials (mainly polymers such as polydimethylsiloxane (PDMS), poly(methyl methacrylate)) and biocompatible substrates such as glass and silica have usually been used leading to commercially available disposable microfluidic systems. Optofluidics platforms have therefore been achieved by coupling both laser beams (including, in best cases, fiber coupling) and detection stages (often carried out by optical microscopy) to microfluidics. To this aim, prototypes able to detect, count, sort and analyze the droplets have therefore been investigated such as compact microflow cytometers (typical throughput of the order of several hundreds of cells per second and a typical sample purity of about 90%), velocity detectors [52], biosensors based on fluorescence measurements [53], just to cite a few. In these cases, polymers were frequently bonded to rigid materials: silicon, to provide a patterned substrate to anchor/align fibers to the microfluidic circuitries; silica or glasses to provide a solid base; lithium niobate (LN) to add surface acoustic wave (SAW) stages working as micropumps or mixers. Quite surprisingly, only in the last decade have significant advances been achieved by delivering monolithic, perfectly aligned, robust and portable optofluidic prototypes. Both microfluidic circuits and optical waveguides have been synergistically fabricated on the same substrate (fused silica) by FLM. Optically controlled photonic prototypes for manipulating micron-scale dielectric objects by light-driven phenomena have therefore been presented: optical tweezers and stretchers have been implemented with suitable focused laser beams, either externally or fiber-coupled, at a microfluidics level [54]. Similar results can also be achieved in LN crystal substrates but are currently underestimated (figure 3). Thanks to their excellent optical and electro-optical properties, high-quality optical waveguides, multiplexers, switches and optical

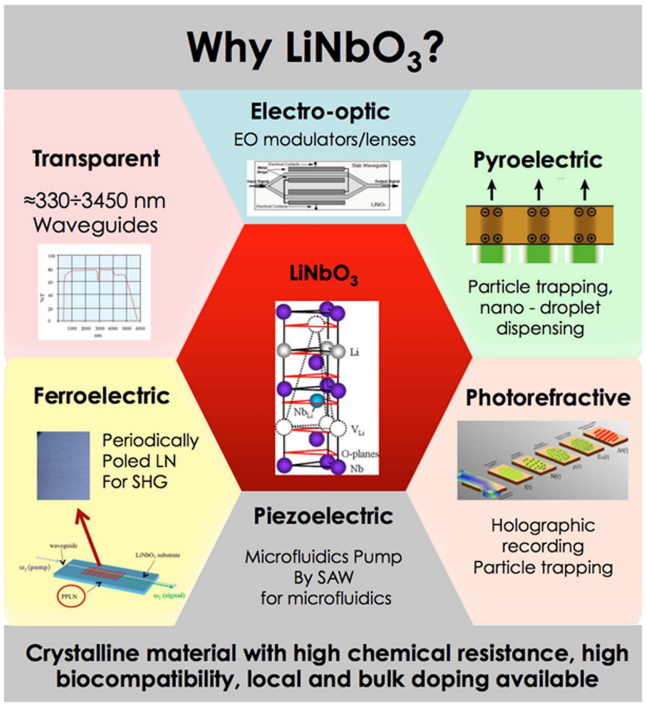

Figure 3. The main properties of lithium niobate ($LiNbO_3$).

modulators are commercially available, obtained mainly by Ti-in-diffusion technology. Their ferroelectric properties have been exploited to produce light sources by way of frequency conversion in periodically poled LN (PPLN) structures. Other applications have been: biocompatibility, photorefractivity for holographic recording, and induced local space-charge-fields to exploit dielectrophoretic and electrophoretic forces (either generated by inhomogeneous illumination [55] or heating [56]) as well as micromachining by high optical quality dicing [57]. This material therefore presents all the characteristics needed to host several different functionalities on the same substrate in a monolithic integrated optofluidic platform. This high level of integration represents a great challenge, but is required in portable lab-on-a-chip systems that can operate in a wide spectrum range, with tailored final response and real-time reconfigurability. Limiting factors mainly stem from either the tools for finely controlling the fluid flux and the optimization of the light coupling and detection within the microfluidic interface. Up to now expensive and bulky systems such as syringe or pressure based pumps have been used, whilst optical functionalities are often granted by using high-power or optical-bench working lasers. When optical fibers are integrated, instead, optical losses can represent a limiting factor for enhanced sensibilities. Hybrid configurations of polymers/durable materials have therefore been proposed to partially overcome this issue. Less investigated is the alternative solution of monolithic integration of several functionalities on the same substrate. Both these aspects somehow have hindered the full exploitation of optomicrofluidic benefits and potential so far.

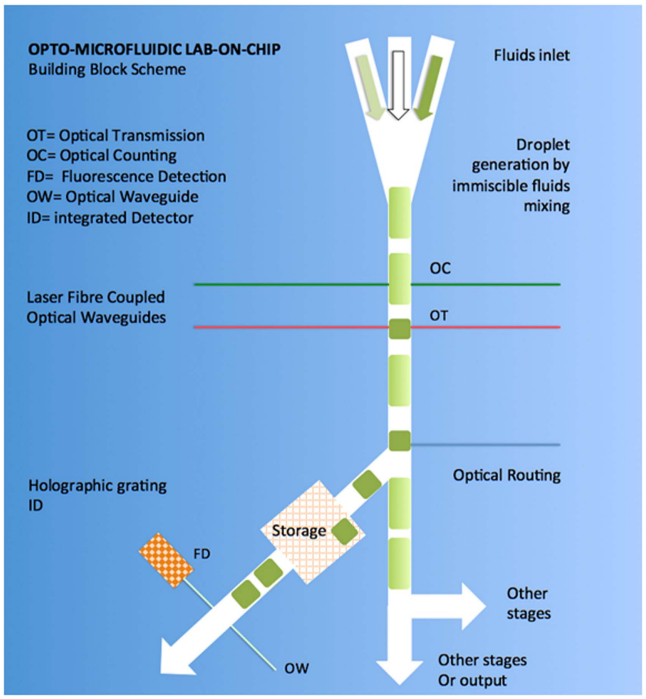

Figure 4. $LiNbO_3$-based optofluidic platform.

Advances in science and technology to meet challenges. In order to get a truly portable device some issues must be solved from a technological point of view. In integrated optomicrofluidics platforms, embedded or buried optical waveguides are needed. Up to now, only the FLM technique allowed one to reach such a result, but in fused silica and for a defined range of possible functionalities limited to this material's properties. Although some attempts have been made using $LiNbO_3$, FLM is still far from being truly exploited. As an alternative, LN could also be micro-machined and bonded to silica and to polymers, allowing hybrid configurations. Less sought, it seems, is $LiNbO_3$–$LiNbO_3$ bonding to get 3D configurations, which is still almost unexplored. Despite these technological challenges, the real advances in science are straightforward: droplet counting, sorting depending on the size or content, droplet routing and storage, all integrated on the same substrate with micropump fluid driving, can be foreseen. This is possible by implementing droplet optical transmission measurements and passage monitoring, as well as refractive index and/or fluorescence emission detection (eventually spectrally resolved by recording holographic gratings). Finally, waveguides could both confine light and allow for droplet routing and sorting, while storage could be achieved (figure 4). This challenge can be faced only by exploiting light-induced phenomena in the material, something possible in $LiNbO_3$ by way of its photorefractive and photovoltaic properties, even enhanced by suitable local doping. Consequently, tailored and reconfigurable setups could be designed, even in real-time, by way of feedback systems triggered by the optical waveguide response.

Concluding remarks and perspectives. Light-reconfigurable devices with multifunctional stages have not been proposed yet on the same substrate, nor have systems that activate a function in real time depending on the other stage feedback. Together with the miniaturization advantages, a fully integrated optofluidic platform promises to provide higher sensitivity and dynamic range, a finer control of the light power focused on the biological target, and a dynamical reconfigurability tailored depending on the final purpose. Integrated optics can provide a perspective and a systematic analysis of the role of different conditions of illumination and the relative real-time monitoring even of cumulative light-induced effects still unknown or not investigated. $LiNbO_3$ promises to be a valid alternative, as it has been demonstrated to host: efficient integrated optics stages; micropumps by SAW; second harmonic light generation by PPLN; particle manipulation stages and droplet routing by way of photovoltaic properties; engraved microfluidic circuitries using the Femto-laser technique; and high-quality dicing. Consequently, they represents an ideal gym floor for integrating all these functionalities together and achieving a portable fullly optional lab-on-a-chip device, tailored and reconfigurable by way of optical induced phenomena.

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4. Silk fibroin films for biophotonic and microfluidic applications

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Relevance of the topic. Microfluidics is a versatile technological platform for precise manipulation of fluids of extremely small volumes and has a broad range of biochemical and medical applications. PDMS, as one of the most commonly used microfluidic materials, has revolutionized the field by enabling out-of-clean-room and inexpensive fabrication and providing robust material properties to support a variety of applications from large-scale chemical synthesis to high-throughput bimolecular assays. However, its non-biofriendly fabrication process and its limitations in bulk functionalization have limited the utility of PDMS devices in the fields of tissue engineering and regenerative medicine. More recently, biologically relevant materials have been utilized to construct microfluidic devices, such as gelatin, collagen, alginate and silk. These natural biomaterials allow easy bulk and surface functionalization and exhibit fully compatible bio-interfaces. Among those materials, silk fibroin has unique physicochemical properties, including highly tunable mechanical stiffness and *in vivo* degradability as well as long-term stability in various medium environments. These attributes are essentially important for advanced applications of microfluidics in tissue-related research and *in vivo* implantation.

Current status and challenges. The first microfluidic device completely made of silk fibroin material was introduced in 2007 [58]. The microchannels were fabricated by aqueous casting of silk fibroin solution on a PDMS mold followed by water-stable treatment. The micromolded silk film was then laminated onto a flat silk film so closed microchannels were formed. Human hepatocytes were successfully cultured in the microchannels and perfused with cell culture medium for a prolonged period of time to demonstrate the biocompatibility of the devices. A new enzymatically crosslinked silk hydrogel material was later introduced and has also been utilized for microfluidics fabrication (figure 5). This material provides better tunability on mechanical properties to match different human tissues, bulk functionalization and cell encapsulation for artificial tissue/organ constructions [59]. A gelatin sacrificial molding method was used to create microchannels in silk hydrogel and a layer-by-layer assembly approach allowed the fabrication of multilayered 3D microfluidic devices. Pneumatic fluidic controls previously used in PDMS counterparts have also been implemented thanks to the elastic nature of the silk hydrogel, which allows automatic control of silk microfluidics forhigh-throughput large-scale devices. Human endothelial cells and fibroblast cells have been successfully cultured along the channel surface and in the bulk of the device, respectively, which demonstrated the utility of the silk hydrogel devices in potential tissue engineering applications.

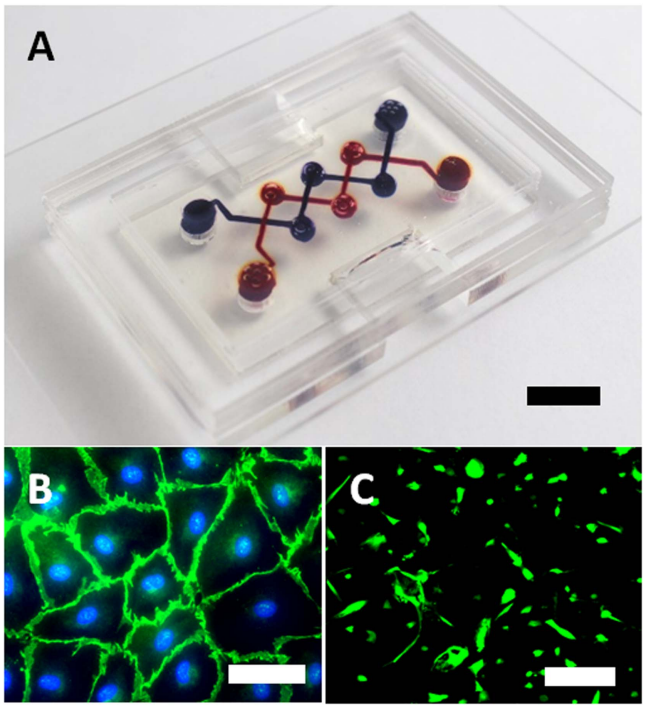

Figure 5. (A) A silk hydrogel microfluidic device with 3D microchannels. (B) Human endothelial cells cultured along the microchannel surface. (C) Human fibroblast cells cultured in the bulk of a silk hydrogel device. Scale bar shows 1 cm in (A), and 20 μm in (B) and (C). Reprinted from [59], Copyright 2016, with permission from Elsevier.

Silk microfluidics has made tremendous progress in the last decade, but challenges still remain to be addressed to bring this platform from research laboratories to hospitals and the clinical market. One of the key challenges is that current 3D silk microfluidic fabrication requires time-consuming layer-by-layer assembly and significant human intervention, which largely limits the throughput. Ideally, complex and multilayer silk microfluidics will be fabricated with automatic systems using either bottom-up (e.g. 3D printing) or top-down (e.g. 3D laser micromachining) approaches. Moreover, fabricating ultrafine features (from micrometer to nanometer) within the silk hydrogel is very challenging, which, if addressed, could allow the construction of high-density microvascular networks and highly integrated microfluidic devices.

Advances in science and technology to meet challenges. 3D printing is a promising technology that allows facile fabrication of complex 3D structures with minimal human intervention. We have recently developed a 3D bioprinter that is able to print room-temperature curable silk hydrogels into various 2D and 3D formats [60]. Ultrafast laser pulses of relatively low energy have also been utilized to directly carve high-resolution channels of micrometer dimensions within bulk silk hydrogels, which provides a promising solution to automatic fabrication of high-density silk microfluidics [61]. In addition to enzyme-induced crosslinking, light and electron beams have also been utilized to cure and pattern silk

hydrogel material under aqueous and biofriendly conditions, which could be potentially used to fabricate features of micrometer to nanometer resolution [62, 63].

Concluding remarks and perspectives. The next evolution of the field of microfluidics will be based on the integration of new classes of active materials as the bulk constituent of the devices. That is, the availability of materials that are biologically active and that can be integrated into living tissue, together with the possibility of incorporating dopant molecules, which can surround the volumes of fluid flowing through microchannels, with active environments that can define diffusive profiles into or out of the channnels, or can provide externally addressable functions. Future applications envisioned are biologically integrated fluidic devices such as smart tissue engineering platforms, active bulk doped microfluidics that embed optical and/or electronic functions, and dynamically reconfigurable, chemically active devices, extending the utility of the platform.

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5. Integration of microfluidics with silicon photonic sensors

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Relevance of the topic. With our rapidly advancing understanding of molecular biology, the interest in analyzing the molecular composition of various biological samples has increased dramatically in recent years. Medical diagnostics are, for example, increasingly based on the quantitative measurement of the concentration of certain biomolecules, referred to as biomarkers, in patient samples such as blood and urine. Currently, this type of analysis is predominately performed in a laboratory setting, due to stringent environmental control and the high skill level of operators required for reliable results. However, there is a growing need for reliable mobile bioanalysis, e.g. to tackle epidemics and biohazard monitoring on site.

The great success of current bio-analytical techniques is largely built on the exploitation of highly selective binding reactions of certain biomolecule pairs, e.g. certain proteins and nucleic acids, to perform biomolecule detection with high selectivity. In this way, we take advantage of the work already done by evolution on the fine tuning of the control systems of life. Alas, with this benefit comes a strict condition: we need to handle liquid samples, since biological binding reactions invariably take place in aqueous solutions.

Microfluidics refers to the technology of fluid manipulation on the microscale. From its inception almost 30 years ago [64], this field has benefited greatly from the microfabrication techniques of the microelectronics industry, but has recently developed its own toolset based on polymer microstructuring, often by casting or molding. The rapid development of microfluidics has been driven by the desire to automate and parallelize liquid sample handling for applications such as medical diagnostics, biological research, and drug development.

The result of a bioanalysis must be quantified, and optical detection is a particularly popular quantification approach, due to its large dynamic range, high spatial and temporal resolution, and strong resistance to electromagnetic interference. Silicon photonics is the study and application of integrated optical systems that use silicon as an optical medium, usually by confining light in optical waveguides etched into the surface of silicon-on-insulator (SOI) wafers. By fixing one part of the biological pairs mentioned above onto the surface of such a waveguide, the selective binding of the other part can be optically detected. The fixed molecule is referred to as a recognition element, the detected molecule as the analyte, and the technique as a whole as optical biosensing. Since the transparency window of silicon lies in the near- and mid-infrared, detection schemes based on refractive index changes upon biomolecule binding are most commonly applied. Fluorescence-based detection is uncommon, since very few fluorophores operate in the near- and mid-infrared.

The main benefit of silicon photonics is that by leveraging the investments made by the silicon-based microelectronics industry, silicon photonics can be efficiently manufactured on large diameter wafers using highly automated processes. Furthermore, silicon-based optical circuits can be made very compact on the SOI platform, due to the high refractive index contrast, which enables small waveguide bending radii. This permits efficient use of the wafer area, thereby keeping device costs down. For bioanalysis, this means that whole optical detector arrays can be integrated into a single microfluidic channel. Another benefit of silicon photonics is that electronics for readout and control can be integrated into the same substrate, yielding an unprecedented integration density. The main limitation of silicon photonics is that there are currently no monolithically integrated lasers available, but recent advances in germanium lasers on silicon [65] hold great promise for low-cost silicon photonic systems in the near future.

By combining the tools of silicon photonics and microfluidics, the basic toolset is in place for implementing robust mobile bioanalysis. A successful merger, however, requires overcoming a number of challenges outlined below.

Current status and challenges. To target mobile bioanalysis, the chosen integration technology should preserve the key benefits of silicon photonics mentioned above. This implies that both recognition element attachment and microfluidics integration should be done at wafer level, and without adding a significant wafer footprint. So far, such an approach has not been demonstrated.

The second fundamental challenge is the volume mismatch. For many important medical conditions, the concentration of the indicative biomarker analytes is very low in the sample liquid. Thus, for reliable detection, a certain minimum sample volume must be analyzed. For common cases, this results in sample volumes of 0.5 to 1 ml. Furthermore, most biosensing protocols call for washing with at least five times the sample volume of clean buffer liquid. For comparison, a silicon photonic chip of $10 \times 10\,\mathrm{mm}^2$ area covered with a uniform 0.1 mm thick liquid film holds only 10 μl of liquid. Any microfluidic channel network structured on top of such a chip will hold much less. Hence, a mobile silicon photonics biosensing instrument must be able to handle and store much more liquid than can be fitted on the silicon chip surface.

The third fundamental challenge is the sensitivity of the biological recognition elements to temperature and solvents. This is particularly true for the more interesting protein-based class of recognition elements that generally neither tolerate heating significantly above body temperature nor non-physiological solvents, which severely limits fabrication options. Nucleic acids, on the other hand, are more tolerant, but then there already exist many more alternative analysis methods for nucleic acids.

Figure 6 provides a schematic overview of the integration approaches that have been reported so far, as well as a few possibilities that have not been tried yet. The liquid handling methods can be classified into two major groups, based on

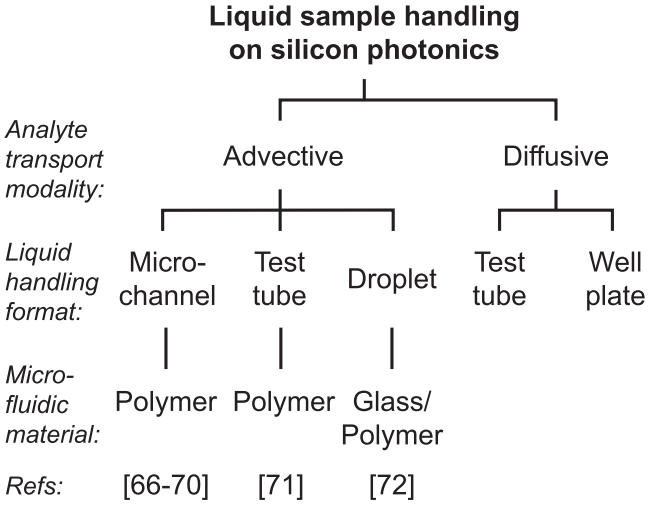

Figure 6. A schematic overview of the microfluidic integration approaches that have been reported, as well as a few new possibilities.

analyte transport modality, as advective or diffusive. The advective class is dominated by microfluidic channel based devices, usually employing pressure-driven flow in channels molded into polymers such as PDMS [66, 67] and thiol-enes [68], and transfer-bonded onto the silicon photonics. So far, only chip level processing, has been demonstrated. The critical aspect of this approach is how to seal the channels after recognition element attachment, without affecting the biomolecules. PDMS is difficult to dry-bond to silicon after biomolecule attachment, since the required oxygen plasma activation would destroy the biomolecules. Thiol-enes are more promising in this regard [69]. Another approach is to etch channels into a spin-coated perfluoropolymer and then seal them with clamped gaskets [70]. The drawback of this approach is that the poor alignment accuracy of the clamped gasket severely limits the integration density. Furthermore, a leak-tight seal will be difficult to achieve in a low-cost consumer operated device. Pressure has also been used to flow samples through orifices in silicon photonic chips glued to the bottom of open-ended plastic test tubes [71]. Advective droplet transport (digital microfluidics), which employs electrowetting, has also been reported [72], but the sample volumes that can be handled in this way are very small.

Due to their simplicity, diffusive transport devices are potentially very interesting for mobile biosensing and high-throughput screening applications. However, they will struggle with the low analyte concentrations of most medical diagnostic applications.

Advances in science and technology to meet challenges. To be able to structure microfluidic circuits directly on top of silicon photonic wafers, some advances in polymer science are needed. The ideal polymer should be patternable using standard photolithography, and allow for low-temperature dry bonding both to silicon and to other polymers. Using such a polymer, the alignment-critical first microfluidic channel layer could be made directly on the silicon photonics wafer, using spin coating, lithography, and development. Next, the biological recognition elements could be deposited in a serial process using a spotting robot, or preferably using a wafer-scale printing scheme with a patterned stamp. Then, a second, lower resolution lid layer could be transferred and dry-bonded to form closed channels with open vias aligned to the channel structure below. The lid layer could be injection molded into a thermoplastic, and thus also contain the large volume reservoirs necessary for milliliter-scale sample handling. Another alternative would be to add a third layer containing the large liquid reservoirs.

Currently, there exist polymers that have the potential to allow such a processing scheme. In particular, the class of photocurable thiolene-epoxy polymers [73] seems promising, due to their capability for high-resolution photopatterning and versatile low-temperature dry bonding.

Another potentially rewarding development of silicon photonics based biosensing would be to use IR fluorescence in combination with silicon photonics. This would enable the combination of the high signal to the background, possible with fluorescence-based sensing with the low-cost, high integration density optics possible with silicon photonics. There have already been recent developments in IR fluorophores, but combining them with silicon photonics is uncharted territory.

Concluding remarks and perspectives. Silicon photonics have already been commercially employed to biosensing in a laboratory setting. However, the big potential of the technology is to enable quantitative and reliable mobile bioanalysis, for example in a smartphone attachment. To get there, advances are necessary in wafer-scale polymer processing, as well as in wafer-scale biomolecule attachment. Furthermore, a monolithically integrated light source is also needed to realize this vision. Finally, even though optical sensing, and biosensing in particular, has been the driving force behind much of the recent work, the control and manipulation of light by fluids is a potentially rewarding application of the microfluidic integration technology discussed here.

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6. Holographic on-chip microscopy and tomography using lensless computational imaging

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Relevance of the topic. The recent advances in micro- and nanotechnologies have greatly enhanced our capability to build cost-effective and powerful chip-size microsystems that can handle, e.g. cells and biofluids. These lab-on-a-chip systems have wide applications in bioengineering and medicine. The integration of optical imaging and microscopy techniques with microfluidics can enable high-throughput detection and sensing of biological and/or chemical signals. To complement such microfluidics and lab-on-a-chip systems, optical microscopy tools need to undergo a transformation in terms of their size, cost and throughput, and computational on-chip imaging provides unique opportunities for this broad goal.

Current status and challenges. The optical interrogation of current microfluidic samples is by and large conducted by conventional light microscopy tools that use lenses for image formation, and have significantly smaller fields of view (FOV) than the size scale of a typical microfluidic chip and shallower depths of field (DOF) than the height of typical microfluidic channels. Moreover, the FOV as well as DOF of conventional microscopy scale down when switched to higher-magnification objective lenses (when higher resolution is needed), further reducing the available imaging area and volume within the sample. In addition, the bulkiness and high cost of these conventional microscopes present another obstacle to merging optical imaging with microfluidics for building lab-on-a-chip systems, especially for use at low-resource and field settings. This mismatch, caused by the limitations of traditional optical imaging modalities, prompts advances in microscopy.

Meanwhile, tomographic imaging techniques that enable sectional imaging have been of great interest to reveal 3D structures of various organisms. Sectional imaging modalities such as optical projection tomography, optical diffraction tomography, confocal microscopy and light-sheet microscopy, among others, can achieve high-resolution 3D optical imaging. However, these modalities are also limited by the aforementioned restrictions including low throughput, high cost and bulkiness.

Therefore, it is of great importance to develop high-resolution microscopic and tomographic imaging techniques that have small footprints and large sample imaging areas/volumes.

Lensless on-chip microscopy based on digital in-line holographic imaging offers a wide FOV and a large DOF within a low cost and compact device platform [74–83]. These features make it a promising solution for optofluidic microscopy applications [74, 79, 81]. In a holographic optofluidic microscopy (HOM) setup, the sample flows in a microfluidic channel, which is driven either by electrokinetic force or pressure. A partially coherent light source with a large illumination aperture (e.g. ~50–100 μm, filtered by a large pinhole or butt-coupled to an optical fiber section) is placed 50–100 mm (z_1 distance) above the sample microfluidic chip, while the microfluidic chip is placed directly on top of a CMOS image sensor. As a result, the sample of interest inside the microchannel is typically located at ~1 mm (z_2 distance) away from the imager chip. The illumination light that can be from a low-cost light-emitting diode (LED) acquires spatial coherence after free-space propagation over a distance of z_1, and the hologram recorded by the image sensor is formed by the interference of light scattered by the sample within the fluid with the unscattered light that is directly transmitted. Compared to laser sources, the use of a partially coherent light source (e.g. a simple LED) reduces speckle and multiple-interference noise, resulting in cleaner images without loss of resolution owing to on-chip imaging geometry. Moreover, the cost of the setup is reduced by using low-cost LEDs as light sources.

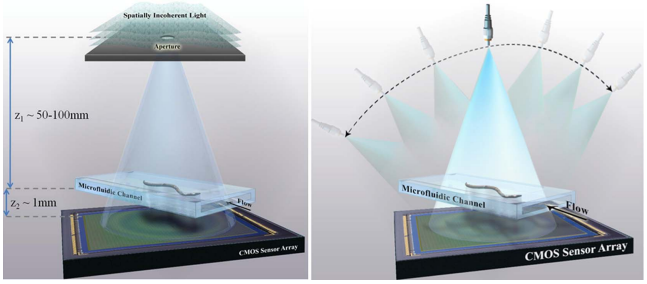

Figure 7. Left: schematic setup of holographic optofluidic microscopy (HOM). Reproduced from [74]. CC BY 3.0. Right: schematic setup of holographic optofluidic tomography (HOT). Reprinted from [83], with the permission of AIP Publishing.

An important advantage of using the holographic imaging principle is the capability to do single-shot depth-resolved amplitude and phase imaging of a large sample volume. The holographic patterns can be digitally refocused to different z distances, with particles/samples residing at different depths in focus.

When only one in-line hologram is used for reconstruction, the major limiting factor for the resolution is the pixel size, which is a disadvantage of using unit fringe magnification—a characteristic signature of an on-chip holographic microscope. However, the flow of the sample in a microfluidic channel (figure 7) can be leveraged to circumvent this limit imposed by the pixel size by capturing multiple holograms that are acquired at different time instances [74, 79, 81]. Due to the flow, these holograms generally have non-integer (sub-pixel) shifts with respect to each other. A high-resolution hologram that is compatible with all the sub-pixel-shifted low-resolution holograms can be calculated by using a pixel super-resolution (PSR) algorithm.

Using lens-free holographic on-chip imaging together with computed tomography principles, portable tomographic microscopes can also be built to achieve 3D sectional imaging capability [76, 79–81, 83]. In this method, the sample is illuminated from multiple angular positions ranging

between $\pm 50°$, which provides different perspectives of the samples. These images are holographically reconstructed, and a computed tomogram is generated by applying a filtered back-projection algorithm. Although the multiangle illumination increases the system complexity, it can still be implemented in a cost-effective and portable device by using multiple LEDs that are butt-coupled to optical fiber sections at different angular positions. PSR can be integrated onto the same platform by electromagnetic actuation of the optical fibers using coils and magnets [76]. This induced motion in the optical fibers results in sub-pixel shifts of the acquired holograms, and can be used to synthesize higher-resolution holographic images. With this approach, tomographic imaging of approximately 15 mm^3 has been achieved, with a spatial resolution of $<1\ \mu m \times <1\ \mu m \times <3\ \mu m$ in the x, y and z directions. Tomographic imaging of an entire *C. elegans* has been demonstrated using the same technique [80].

A unique advantage of the holographic optofluidic tomography (HOT) method (figures 7 and 8) is its extended DOF that is inherent to the holographic imaging principle. Unlike high-NA objective lenses that have a very shallow DOF, a tomogram of an object can be computed at any position within the imaged 3D volume of the microchannel. However, notice that the HOT technique assumes that the sample is a weak scattering object, and that the majority of the detected photons should have experienced, at most, a single scattering event.

A variant of the HOM and HOT methods was used for the dynamic 3D tracking of sperm cells over large volumes [77, 82]. In this system, two LED sources of different wavelengths (red and blue) simultaneously illuminate the microfluidic chamber from different perspectives (vertical and 45° tilted, respectively). This dual-wavelength illumination design enables digital separation of the two holograms of the same sperm cell, and the dual-angle design improves the cell localization accuracy in the z direction via triangulation. High-frame-rate lensless image sequences were taken to resolve the rapid motion of the sperm within a very large volume of $\sim 10\ \mu l$. Using this platform, rare trajectories of human sperm such as helical patterns were detected and quantified. A similar platform could be used to detect and track a wide variety of other microswimmers that exhibit 3D locomotion in fluids.

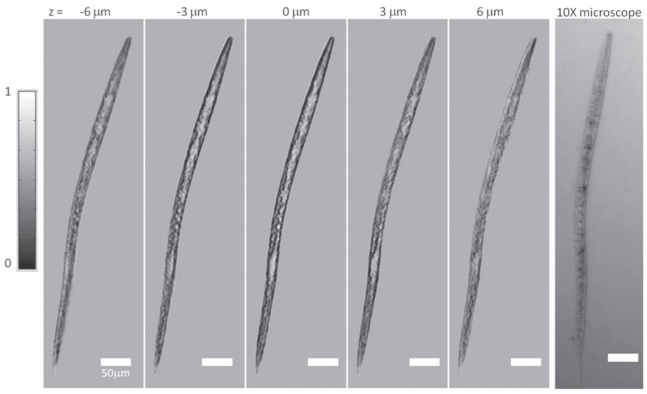

Figure 8. 3D imaging of *C. elegans* using HOT. Reprinted from [83], with the permission of AIP Publishing.

Concluding remarks and perspectives. Lensless holographic microscopy and tomography are promising techniques to complement microfluidic and lab-on-a-chip platforms as they present important advantages of, e.g. high throughput, low cost, simplicity and small footprints, making them ideally suited to be used in low-resource and field settings. Manipulation of liquid samples and rapid screening of large volumes of fluids are some of the applications that can benefit from these computational imaging techniques.

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7. Live cell imaging with optofluidics

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Relevance of the topic. Cell biologists and microscopists will often image individual live cells to establish the properties of a population of interest. However, these studies can be limited by the number of cells that can be practically examined due to the need to reposition, refocus and image each cell. As an alternative, flow cytometry can enable large throughput studies but typically requires a degree of instrument complexity. Perhaps a greater limitation is that flow cytometry makes it difficult to follow the fate of individual cells. As an alternative approach, optofluidics offers a unique opportunity to examine live cells and easily manipulate them in a high-throughput scheme.

Many microfluidic platforms have been developed for examining individual live cells. The earliest implementations often included an optical scheme for interrogating a cell, usually in the form of a fluorescent reporter [84]. As the technology matured, optics were also adapted for implementing cell sorting [85]. The development of optics integrated into the microfluidic platform gave birth to the field of optofluidics. The first key advances in this field were to enable high-resolution imaging within the microfluidic platform. These low-cost implementations offered fit for purpose alternatives to using a traditional microscope [86]. This approach combines microfluidics for sample manipulation with low-cost sensors for imaging, and employ reconstruction algorithms to obtain high-resolution images. Further development of these approaches for cell imaging in a compact, low-cost platform has enabled applications in new settings such as resource limited locations and point-of-care medical diagnosis. Below, recent advances in live cell imaging using optofluidics are discussed alongside a brief look at challenges in this field and suggestions on how future efforts may approach them.

Current status and challenges. The true advantage of optofluidics for live cell imaging is the ability to apply a range of well controlled, calibrated forces both to manipulate and characterize cells. These forces can be applied mechanically, electrically or optically to isolate and interrogate individual cells for study. Cells under examination in these devices are then typically profiled using an optical modality ranging from bright-field imaging to Raman spectroscopy to interferometry.

A recent example of this approach can be found in Lee and Liu [87] where individual cells are trapped at designated sites in a micropipette array using flow pressure. A calibrated syringe allows the pressure to be varied across the array and thus implement simultaneous micropipette aspiration assays on each trapped cell. This is a common technique in cell biology for assessing cell biomechanical properties by noting the degree to which a cell is drawn into a narrow capillary due to a given force. This technique is effective but can be quite laborious due to the need to manually trap and image each cell. In comparison, the optofluidic platform allows highly parallel studies using bright-field microscopy to assess cell deformation. In its initial demonstration, it was applied to distinguish breast cancer cells based on mechanical properties. One particular challenge of this approach is to keep the sample in focus during experiments. Here this is solved by integrating the platform with the microscope but other approaches are discussed below.

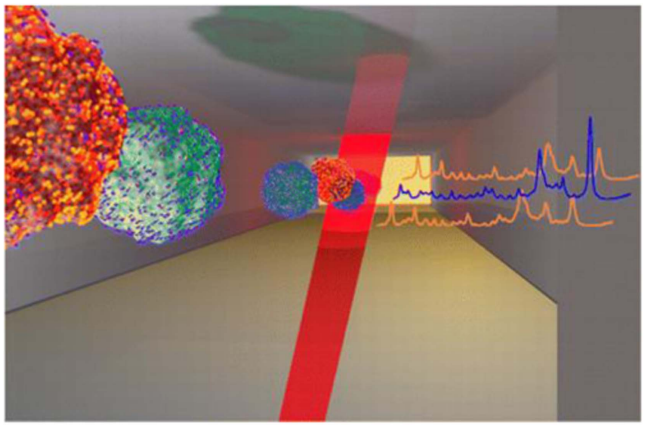

Figure 9. Surface-enhanced Raman spectroscopy tags allow rapid discrimination of cells in flow. Reprinted with permission from [89]. Copyright 2015 American Chemical Society.

Raman spectroscopy is a powerful technique for distinguishing cells by their biochemical properties. However, in general the Raman cross section is low, resulting in poor efficiency and the need for long integration times. Analysis of the Raman signal from cells inside an optofluidic platform must usually include a mechanism for trapping the cells using mechanical or electrophoresis approaches. Using laser tweezers to trap cells for Raman measurements has also been shown to be effective [88]. As an alternative approach, surface-enhanced Raman spectroscopy (SERS) has been used to discriminate cells in a microfluidic platform [89]. SERS increases the strength of a Raman signal by orders of magnitude, enabling more rapid detection. In this approach a SERS biotag is created, which is taken up by cells that are then introduced to the microfluid. The flowing cells can then be rapidly profiled using the biotag for sorting (figure 9).

Several efforts have sought to increase the rate of cell imaging as they flow through a device. While the use of today's high pixel count sensors can allow for highly parallel imaging approaches, these typically are limited by the low numerical aperture optics required to have a wide field of view. Light-sheet imaging approaches have shown promise for wide field and rapid throughput but are limited to detecting fluorescent tags. Novel imaging approaches, such as optical time stretch imaging, allows for more rapid imaging of cells [90], achieving rates comparable to flow cytometers. In this approach, the imaging information is spectrally encoded using a dispersed broadband pulse. Extensions to this approach have shown the ability to obtain phase information using an interferometry scheme but can have difficulty with maintaining cells in a focal plane. In addition, the complex

scheme may not be compatible with typical applications of microfluidics as point-of care devices.

Advances in science and technology to meet challenges. The continuing challenge in imaging of live cells in optofluidics is to achieve high throughput without sacrificing information or greatly increasing instrument complexity. Optofluidic devices become less efficient when there is a need to first trap each individual cell before analyzing it. Instead, developing methods in optofluidics seek to provide richer information while preserving throughput.

Holographic imaging offers a compelling way to obtain more information about cells in that it provides both phase and amplitude information on the optical field that has interacted with a cell. Unlike Raman spectroscopy or fluorescence, interferometric signals are quite robust, yet the method does present some challenges. For example, cell samples in a microfluid may present some turbidity, due to the presence of other cells or fouling biomolecules. To implement holographic imaging in this situation, a clean background image is needed for subtraction from later images. A recent study pointed the way to conquering turbidity through careful analysis of the holographic signal components [91]. The approach, termed multilook, allowed for successful imaging of cells in the face of a turbid sample in the microfluidic channel.

Another compelling aspect of holography is its ability to image the three-dimensional distribution of the refractive index, an indicator of the cell's mass distribution. However, this approach for refractive index cell tomography can require many camera exposures, typically from different angles, during which the cell must remain stationary. A recent advance has pointed the way to 3D holographic imaging in flow [92]. In this approach, a cell flows slowly past a high-speed camera, which captures the angular distribution of scattered light rather than a regular image. These data are then inverse transformed to deduce the 3D distribution. This advance was significant since it first demonstrated 3D tomographic imaging of cells in flow. However, it has some limitations, including requiring many exposures and significant computation time, indicating that further development is needed for high-throughput application. Further, this platform avoided the refocusing problem by confining cells to a channel just barely larger than their size, which could become easily clogged if debris were present.

As an alternative, our research group has shown that refocusing of holographic cell images can avoid the need for restrictive channel dimensions. In this approach holographic cell images are acquired and then digitally refocused by leveraging the fact that the complex optical field is obtained [93]. In this initial demonstration, it was shown that

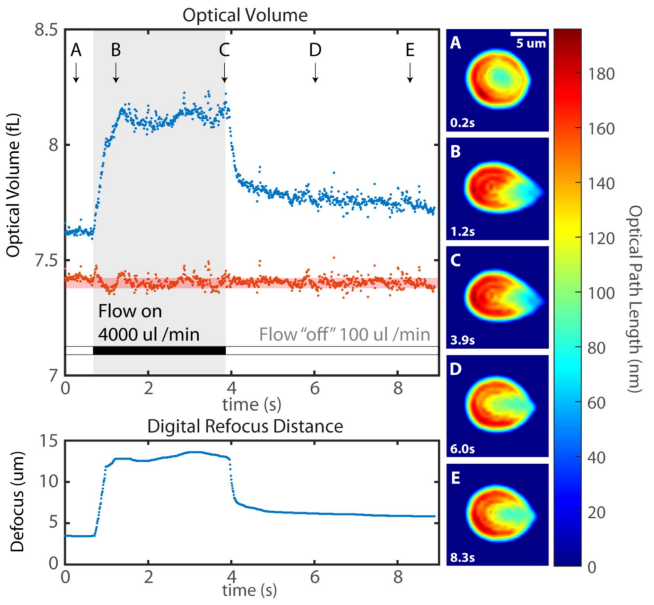

Figure 10. Digital refocusing of a single red blood cell image subjected to high flow rates. Top left: optical volume of red blood cells (RBC) with flow on and off (rates indicated on plot). Blue and red are before and after digital refocusing, respectively. Bottom left: defocus distance. Reprinted from [93]. CC BY 3.0.

quantitative comparison of red blood cells was much more accurate after refocusing (figure 10). We further employed this refocusing algorithm in two studies of red blood cells infected with *P. falciparum*, a parasite responsible for malaria. The first study used our quantitative phase spectroscopy technique [94] to analyze the hemoglobin content of individual red blood cells, revealing its consumption by the parasite [95]. A second study used machine learning to discriminate the presence of infection by using a set of cell descriptors, producing high enough sensitivity and specificity to suggest clinical utility [96]. Both of these studies employed the refocusing approach, which will be essential in the development of holographic techniques for studying cells using optofluidics.

Concluding remarks and perspectives. In summary, optofluidics offers unique capabilities for high-throughput cell studies. Many research efforts are underway to mine the richness of the optical spectrum to characterize cells while maintaining sufficient throughput to implement viable assays. New techniques such as holographic imaging can provide new capabilities but successful application can pose new challenges as well.

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8. Optofluidic microlenses: from tunable focal length to aberration control

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Relevance of the topic. Generating optical images using refractive lenses is still the most important practical use of optical systems. In principle only one single lens is needed to form an optical image and two to generate a zoom system. In practice, however, optical systems require a lot more than two lenses in order to guarantee a decent—ideally, diffraction limited—imaging quality. Combinations of several lenses are typically used to compensate imaging faults that arise from the various aberrations of each individual lens. If zoom or focus settings are to be changed, many of these lenses have to be displaced with respect to each other in a well-defined manner. This typically requires fairly precise and bulky mechanical displacement stages that are often not compatible with e.g. the spatial constraints in high tech devices such as mobile phones or endoscopes.

Optofluidic lenses provide a way out of this dilemma. Instead of translating lenses they adapt their imaging properties by adapting the shape of the lenses. This can be achieved by a variety of external control parameters including pressure variations, mechanical stresses, thermal expansion, and electrowetting [99]. While these approaches provide a fair degree of tunability of the focal length, most of them retain a spherical shape of the lens, as dictated by the mechanical equilibrium of free liquid surfaces in the absence of external forces. As a consequence, optical systems built of simple optofluidics lenses often display mediocre imaging quality due to uncompensated imaging faults such as spherical and other optical aberrations. High-quality adaptive imaging systems require new strategies for aberration correction in liquid microlenses while preserving the compactness of the design, i.e. without introducing mechanical stages to displace the lenses.

Current status and challenges. Standard optofluidic lens designs come in two basic variants, as illustrated in figures 11(a) and (b). In the first type, the edge of the lens, i.e. the three-phase contact line between the solid, the lens fluid (LF) and the ambient fluid (AF) is pinned along the edge of a physical aperture, figure 11(a). In this case, the curvature κ of the lens is determined either by the pressure difference $\Delta p_{\mathrm{L}} = 2\gamma\kappa$ between the two fluids or by the fixed volume of the lens fluid in combination with geometric constraints. The interfacial tension γ between the two fluids guarantees the spherical cap shape of the interface. (The densities of the lens and ambient fluid are typically matched in order to avoid gravity-induced distortions.) Pressure and/or volume can be changed by a variety of different actuation schemes, including piezoelectric transducers, thermal expansion, and electrowetting; see [99] for a recent review. In some approaches, the interface between the two fluids is covered by an elastomeric membrane, e.g. of PDMS with a thickness of a few tens of micrometers. For not too large deformations, the resulting shape of the interface in these 'liquid-filled' lenses is also approximately spherical cap-shaped with the elastic tension of the membrane taking over the role of γ.

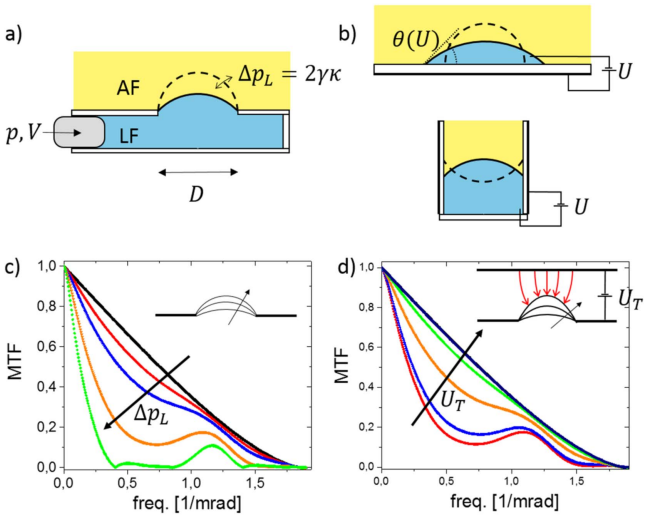

Figure 11. (a) Pressure-controlled optofluidics lens with pinned contact line. (b) EW-controlled optofluidics lens as a sessile drop (top) and in a cylindrical tube (bottom). (c) MTF for a spherical lens with fixed diameter D for increasing Δp_{L} and curvature (see inset). (d) MTF for fixed pressure and increasing top voltage U_{T} that induced increasing asphericity. Inset: electric field-induced surface deformation. Adapted from [98]. © 2016 Optical Society of America. CC BY 3.0.

In the second variant of optofluidics lenses, the three-phase contact line is free to move along the solid surface, but the contact angle θ is changed, figure 11(b). Variations in θ, in combination with the fixed radius and/or volume of the lens fluid, determine the curvature of the lens. Reversible, fast, and robust tuning of the contact angle over a wide range is best achieved using electrowetting (EW; see [101] for a review). EW requires a conductive lens fluid and a dielectric ambient fluid. It allows the variance of $\theta(U)$ by more than 90° within milliseconds with excellent reproducibility over millions of actuation cycles.

However, both approaches lead to spherical lenses for cylindrically symmetric geometries. As a consequence, such lenses always suffer from optical (in particular, spherical) aberrations that compromise the resulting image quality, as evidenced in plots of the modulus of the optical transfer function (MTF) for increasing curvature, figure 11(c), and in the resulting images, figure 12(a). Such deteriorations are particularly dramatic for lenses with high numerical aperture or small f-number, as they are frequently needed in miniaturized devices.

In the case of liquid-filled lenses, combinations of two differently curved surfaces in biconvex lenses were demonstrated to reduce both spherical [102, 103] and chromatic

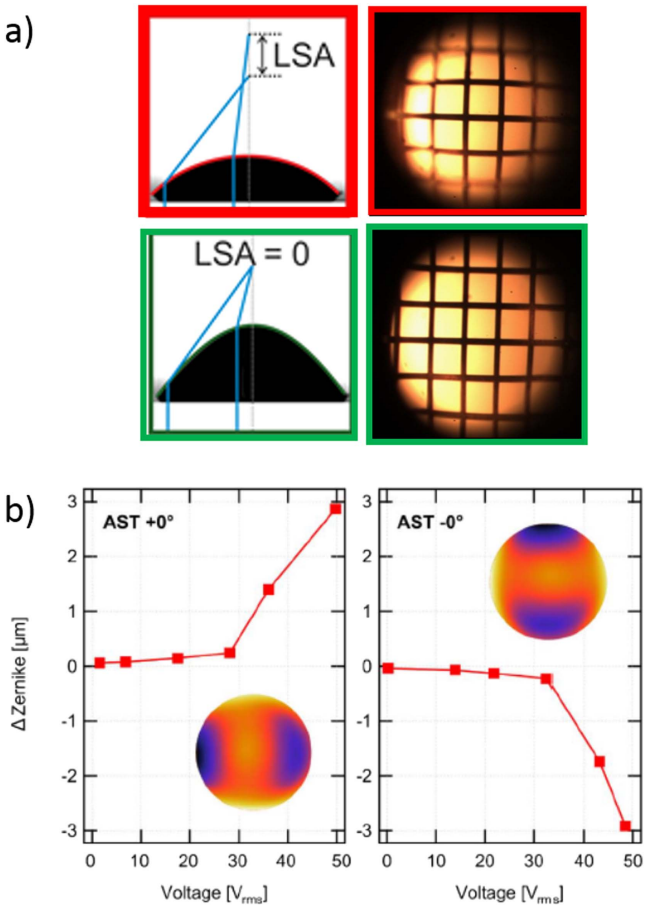

Figure 12. (a) Top: side view image (left) and image of a test grid (right) recorded with a spherical liquid lens with finite longitudinal spherical aberration (LSA); bottom: same as in (a) for an aspherical lens for negligible LSA deformed by optimized Maxwell stress. Adapted by permission from Macmillan Publishers Ltd: Scientific Reports [97], Copyright 2014. (b) Zernike coefficient for astigmatism in the vertical and horizontal directions obtained by EW with segmented electrodes in the azimuthal direction. Reproduced with permission from [100]. © 2016 Optical Society of America.

aberration. The different curvatures are achieved by using two membranes with different stiffness, resulting in different curvatures for the same applied pressure, or more recently with elastomeric membranes with a carefully designed radial thickness profile [104]. While preserving the elegance of a single actuator, the applied pressure, this fixed design of the membranes, and their non-linear elastic and hysteretic response, reduce to some extent the flexibility of this approach.

Advances in science and technology to meet challenges. To overcome these limitations, alternative actuation schemes that enable not only a variation of the overall average curvature of the lens, but also controlled deviations from the spherical shape, are needed. A few recent studies suggest addressing this problem using EW or a related electrical actuation in combination with suitably patterned electrodes. One of the first applications of this type was not intended for imaging optics but for (wide angle) beam steering. Smith *et al* [105] used square or rectangular cuvettes with EW-functionalized sidewalls that could be addressed individually. In this manner, they managed to generate flat liquid–liquid interfaces with a widely tunable tilt angle resulting in a $\pm 15^\circ$ beam steering capability.

Kopp and Zappe [100] recently presented a design in which they segmented the electrode along the inner side of a cylinder. In this case, the liquid–liquid interface assumes a general complex shape that is compatible with the local contact angles on the electrode and the requirement of a constant mean curvature in mechanical equilibrium. Using eight individually addressable electrode segments they managed to generate lenses with tunable focal length (7–12 mm) and tunable astigmatism in the 0, $\pm 45^\circ$, and 90° directions with amplitudes of up to 3 μm (Zernike coefficient) while keeping the amplitude of all other aberrations below 100 nm (figure 12(b)).

Mishra *et al* [97] presented a different approach, in which they used another electrode placed in the ambient dielectric above the liquid–liquid interface, see inset to figure 11(d). Application of a high voltage to that electrode generates a position-dependent electric field that pulls on the liquid–liquid interface with a Maxwell stress $\mathbf{\Pi}(\boldsymbol{r}) = \epsilon_0 \epsilon \boldsymbol{E}^2(\boldsymbol{r})/2$. The equilibrium shape of the liquid is set by balance between this position-dependent electrical stress and the resulting position-dependent local Laplace pressure and curvature of the interface. For an unstructured electrode on the top, the local curvature of the interface decreases monotonically from the optical axis towards the edge of the lens, similar to a perfect aspherical lens. Indeed, it turns out that the longitudinal spherical aberration can be completely eliminated, resulting in diffraction-limited MTF curves (figure 11(d)) as well as optical images (figure 12(b)).

Extensions of the method using segmented top electrodes, a homogeneous one, allows breaking the cylindrical symmetry and the generated lenses with tunable astigmatism [106], coma, and various other aberrations.

Concluding remarks and perspectives. EW and the related Maxwell stress-induced tuning of liquid–liquid interfaces provide very fast and flexible tools to generate tunable lens profiles with variable local curvature. The design of optofluidic devices that allows for correcting tilt, coma, astigmatism, and spherical aberration simultaneously with all-electrical actuation seems within reach, possibly best by combining the currently existing approaches discussed above. The extent to which different aberrations can be addressed completely independently needs to be explored. Improved geometric designs aided by numerical simulation and improved materials (e.g. low interfacial tension fluids) will help to improve the flexibility. Ideally, it is conceivable to achieve

almost arbitrary polarization-independent wavefront shaping on top of a lensing effect by using arrays of large numbers of individually addressable electrodes.

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9. Integration of reconfigurable photonics and microfluidics

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Relevance of the topic. Ever since the emergence of optofluidics in the early 2000s, reconfigurability has been one of the field's defining characteristics, afforded by the potential to replace and modify non-solid media to (rapidly) change the optical properties of a photonic device [107, 108]. At the time, reconfigurable devices were inspired by possible applications in optical communications. Thus, on-chip laser sources that could be tuned via swapping the active (liquid) gain medium or mechanically deforming the cavity were a natural early demonstration of the power of this approach [109]. Subsequently, reconfigurability was demonstrated in a number of other photonic elements, including switches [110], lenses [108], microring resonators [108], and spectral filters [108].

Current status and challenges. Reconfigurability remains one of the distinctive characteristics and a major thrust area for optofluidics. However, over the years, the main interest in terms of real-world applications has shifted towards chemical and biological analysis, in particular disease diagnostics. Here, some of the opportunities lie in the integration of photonic functions with microfluidic sample handling and the development of compact, powerful point-of-care devices. For example, recently single virus particles have been imaged on a smartphone-based platform [111]. At the same time, hybrid integration of silicon-based optofluidic chips with glass or PDMS-based microfluidic chips has emerged as a powerful approach to creating a single, optofluidic system that is optimized for both sample preparation and analysis [112, 113]. Using a combination of microvalve-controlled microfluidics and liquid-core waveguide-based fluorescence sensing, as shown in figure 13(a)(i), amplification-free detection of single Ebola nucleic acids was demonstrated with high specificity over the entire clinically relevant viral load range [114]. Moreover, multimode interference (MMI) waveguides were integrated on the same silicon chip platform to add multiplexing capabilities by creating color-dependent spot patterns in a liquid channel, as illustrated in figure 13(a)(ii) [115]. This approach was successfully used to distinguish three influenza subtypes on the single virus particle level [115].

These recent demonstrations are indicative of the power of waveguide-based optofluidic devices. It is, therefore, a natural question whether the well-established fluidic reconfigurability [107–110] can further enhance emerging optofluidic platforms for advanced biochemical analysis.

Advances in science and technology to meet challenges. Devices made from flexible materials such as PDMS offer the highest potential for dynamic reconfiguration, as their optical properties can be modified in at least three ways: mechanical pressure, altering the fluid in a channel, and by physical movement. These options are visualized in the left of figure 13(b). Recently, the first PDMS-based optofluidic platform that features all these options for dynamic reconfiguration was realized using a combination of fluid-filled microchannels (liquid-core waveguides) and solid-core PDMS waveguides in which the core and cladding are defined by PDMS layers with different precursor mixing ratios and, thus, different refractive indices (figure 13(b)) [116]. When properly fabricated, this allows for guiding light through micromillimeter-scale solid-core waveguides and interacting with liquid channels, much like established silicon-based devices [108, 114, 115]. A photograph of a completed device that features both a microvalve-based sample preparation region and an optical analysis region on the same chip is shown in the right of figure 13(b).

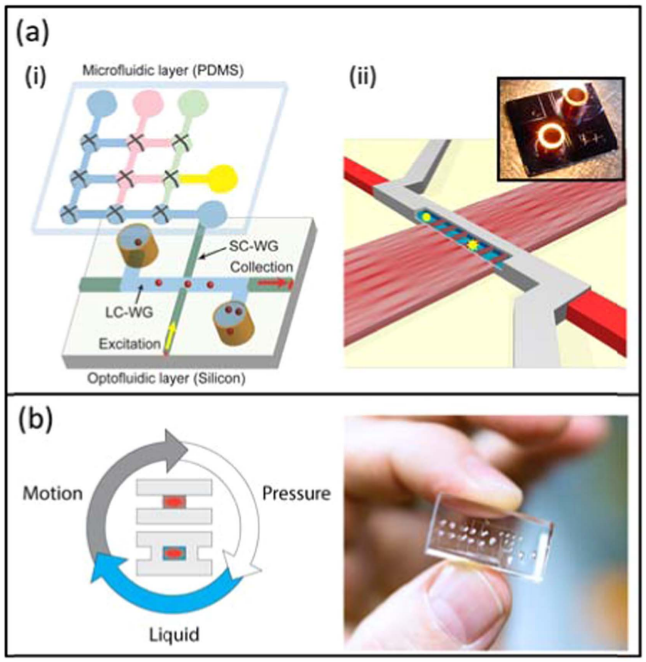

Figure 13. (a) Liquid-core waveguide optofluidic platform. (i) Schematic view of the hybrid integration of microfluidic and optofluidic chips [108], (ii) MMI waveguide creating multiple excitation spots in a liquid-core channel [109]; inset: photo of a silicon chip. (i) Reprinted by permission from Macmillan Publishers Ltd: Nature Photonics [108], Copyright 2011. (ii) Reproduced from [109]. CC BY 3.0. (b) Left: multiple ways for the dynamic reconfiguration of PDMS waveguides [110]; right: image of a PDMS waveguide chip. Reproduced from [110]. CC BY 3.0.

Figure 14 illustrates different aspects of this powerful new platform, highlighting the different reconfigurability options [116]. Figure 14(a) summarizes the implementation of an MMI waveguide whose properties can be controlled using the core fluid or mechanical pressure. Figure 14(a)(i) shows a schematic top-down view where light from a single-mode solid-core PDMS waveguide section (dark gray) enters a wide channel that can be filled with different liquids and acts as the MMI waveguide. When filled with ethylene glycol, an index-guiding MMI is formed. Fluorescent dye in the solution reveals the multimode interference pattern (figure 14(b)(ii),

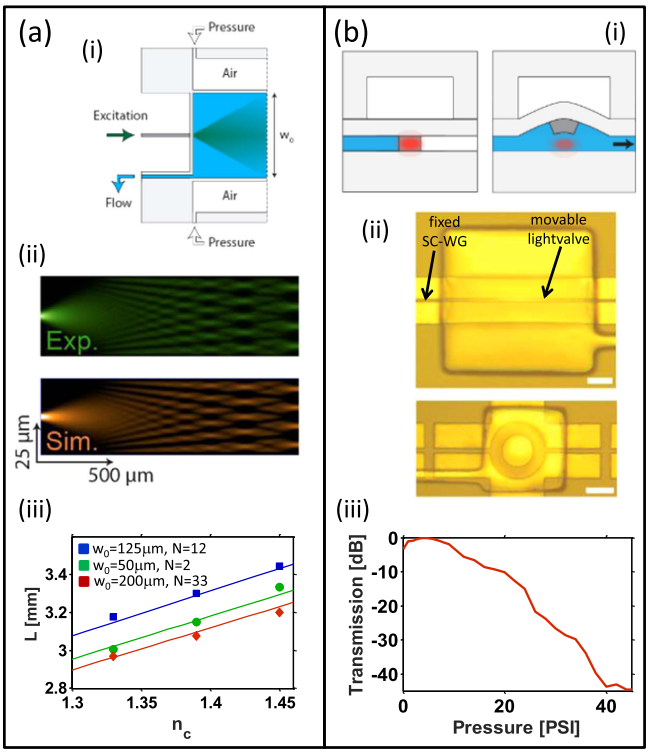

Figure 14. (a) Tunable MMI waveguide. (i) Schematic top-down view; (ii) image of the light pattern in the MMI section (top) in excellent agreement with simulations (bottom); (iii) tuning of spot pattern with core fluid index for different MMI widths [110]. (b) Actuatable light valves. (i) Schematic view of the cross section of the lifting-gate valve with central high-index layer for waveguiding; (ii) top-down images of the straight waveguide on-off switch (top) and ring-shaped light valve for particle trapping and analysis (bottom); (iii) pressure-dependent light transmission across the straight light valve switch from (ii) with high on–off ratio. Reproduced from [110]. CC BY 3.0.

top) in near-perfect match with the simulated behavior of this structure (figure 14(b)(ii), bottom). This spot pattern can be dynamically altered, either by changing the core liquid or by applying pressure in the air cavities surrounding the MMI, thus changing its width. Figure 14(a)(iii) shows how the location of different spot numbers along the MMI waveguide can be tuned with the index of the liquid in the MMI section.

A novel type of reconfiguration was demonstrated in this platform by re-envisioning the design and function of fluidic lifting-gate microvalves previously used for carrying out the distribution and preparation of biological sample materials [114]. Figure 14(b)(i) shows a schematic cross section of such a valve where a thin PDMS membrane sits on a substrate, thus blocking fluid flow (left). However, if the segment that seals the channel (dark gray) is made of high-index PDMS, the valve also acts as an optical waveguide, symbolized by the red confined mode in the figure. The structure now acts as a 'light valve' because lifting the central membrane up by applying negative pressure in the pneumatic top layer results in the light passing through fluid that now fills the space (figure 14(b)(i), right). This creates a new type of optical waveguide that can be individually controlled and dropped into fluidic channels at will. Figure 14(b)(ii) shows two examples of the vast number of possibilities that this principle creates. The top image shows a photograph of a straight light-valve segment that connects two fixed, solid-core waveguide sections. When resting on the substrate, light is passed with negligible loss, but upon pneumatic actuation (lifting up or pushing down), the optical path across the light valve becomes extremely lossy. Figure 14(b)(iii) shows how the transmission can be controlled by the (push-down) actuation pressure, and a tunable light switch with on-off ratios of up to 45 dB is created. This switch can be operated without degradation over 100 000 times.

At the bottom of figure 14(b)(ii), another version of the light valve is shown. Here, the movable segment is shaped as a ring with short waveguide stubs that interface with fixed solid-core waveguide sections on either end. This annulus was used as an actuatable trap for particles that flow through the fluidic channel when the ring is lifted. In this way, trapping and waveguide-based optical analysis of a controlled number of fluorescent beads and *E. coli* bacteria was demonstrated [116]. This shows that the light valve concept has tremendous flexibility both in geometrical design and functionality vis-a-vis transport and manipulation of both fluids and light.

Concluding remarks and perspectives. Dynamic reconfiguration can significantly expand the functionality and performance of waveguide-based optofluidic devices. This was illustrated with a PDMS-based platform that combines liquid-core and solid-core waveguides as well as fluidic sample handling in a single device. Light-guiding microvalves (light valves) that can be physically moved to alter fluid and light propagation further add to the optofluidic toolbox. It can be expected that additional novel functionalities will be demonstrated on this platform as well as using other optofluidic waveguide types such as slot waveguides, photonic-crystal waveguides, or laser-written waveguides.

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10. Optofluidic waveguides and resonators

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CNR-IREA

Relevance of the topic. Optofluidic waveguides offer the possibility of guiding light through a fluid that could be a liquid, gas or plasma. This peculiar property could be exploited in all applications in which strong light–matter interaction is required. In the last few years, promising demonstrations of the potentiality of optofluidic waveguides have been reported in sensing [117], non-linear photonics, atomic spectroscopy and quantum interference [118].

The main issue related to optofluidic waveguides is the difficulty in fulfilling the total internal reflection (TIR) condition when low refractive index fluids are used as core material [119]. TIR optofluidic waveguides based on nanoporous materials like aerogels or super hydrophobic surfaces have been proposed and successfully applied [120, 121]. Alternatively, optical confinement in a waveguide can also be based upon interference phenomena along the transverse direction [119]. Hollow core photonic-crystal (HCPC) fibers have been demonstrated both in 1D and 2D geometries. However, HCPC fibers require complex fabrication procedures and cannot be easily integrated with other components. Silicon-based integrated architectures have been proposed to develop waveguides that can be considered for planar optofluidics, like slot waveguides (SWs), Bragg waveguides (BWs) or antiresonant reflection optical waveguides (ARROWs) (see figure 15) [119]. The advantage of these approaches relies on the possibility of using them as building blocks of more complex integrated devices. In particular, the opportunity to make optofluidic resonators has attracted a lot of attention. These devices enable a strong increase in light–matter interaction, which is no longer limited by physical length but is related to the number of revolutions of light in the resonator, characterized by the quality factor Q.

Current status and challenges. Integrated optofluidic waveguides such as SWs, BWs and ARROWs offer very attractive potential for low loss, single-mode operation, which is highly desirable for building photonic circuits and systems. Moreover, the planar nature of these architectures lends itself to further integration of additional functionalities, such as microfluidics, in a similar manner to integrated electronics, leading to self-contained microsystems. SWs confine a substantial fraction of the optical power into nanometer voids thanks to the TIR effect. Such tight confinement in the subwavelength interaction area makes these waveguides very powerful for non-linear photonics and sensing applications [119]. Although SWs are extremely promising in detecting ultra-low concentrations, the fluidic transport scales unfavorably in nanofluidic channels, which means the integration of fluidic systems for sample manipulation still remains a challenging task. The implementation of integrated waveguides with a hollow

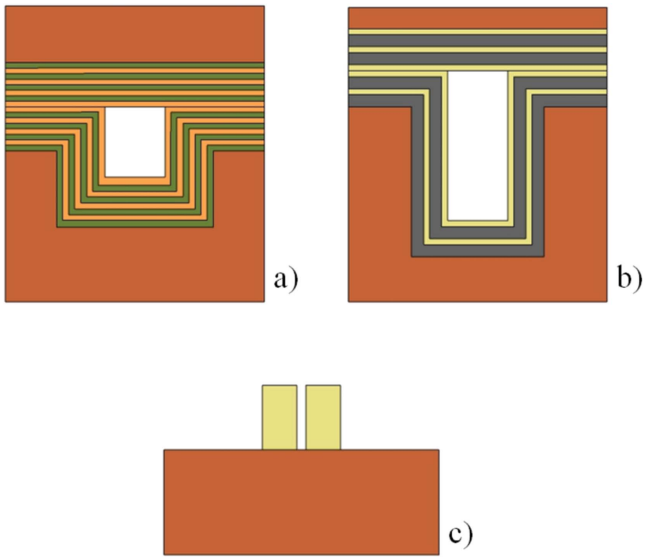

Figure 15. Schematic layout of (a) Bragg, (b) ARROW and (c) slot waveguides.

core is also possible by exploiting interference effects. BWs and ARROWs have been fabricated by creating multilayered dielectric claddings that act as high-performance reflectors. This confinement mechanism causes propagation losses due to not-ideal boundary reflectors, and thus requires an accurate design strategy. Air guiding BWs with core sizes of about $60 \times 2\ \mu m^2$ and propagation losses of few dB cm^{-1} have been demonstrated and bent section investigated. However, the implementation of BWs into a complex circuit layout for sensing purposes has not been fully demonstrated. Single-mode, linearly polarized, liquid-core ARROWs with a mode area of $4.5\ \mu m^2$ and low attenuations of about 1 dB cm^{-1} have been demonstrated by an optimized design of the waveguide structure. Nonetheless, further improvements are attainable for both BWs and ARROWs that could open up new application scenarios, especially in the field of sensing. An important concern in the waveguide performances is to reduce the core size in order to further shrink the mode field volume. A small mode volume can significantly enhance the light–matter interaction and increase the sensitivity by reducing the background optical noise. However, small core size poses tremendous design and fabrication challenges since they typically lead to a rapid increase in the propagation and bend losses. Until now, integrated optofluidic waveguides have been mostly designed to operate in the visible/near-IR spectral range with a liquid core ($n > 1.33$). Nonetheless, they can be designed to operate with a gaseous core ($n \approx 1$) and in a larger spectral window from the visible to the far-IR, providing that the cladding materials and the waveguide geometry are properly selected. In particular, the mid-IR (2.5–25 μm) is very interesting as it supports strong molecular absorption fingerprints, thus offering an interesting spectral window for gas/vapor spectroscopy as the hollow core can be used as a microchamber, enabling a strong sensitivity enhancement. Promising results with slot waveguides operating in the mid-IR for gas and liquid sensing

have been proposed [122]. In the case of ARROWs, when the core is filled with gases, the design optimization as far as fabrication is concerned turns into a non-trivial task, which is still being investigated.

A promising strategy for further improving the light–matter interaction is to increase the duration of the interaction by placing the target inside an optical cavity with a high - quality factor Q. Thus far, various types of optical resonators have been demonstrated in optofluidic format. Microdroplet resonators offer a unique integration between optics and microfluidics exhibiting a very high Q-factor [123, 124]. Although, recently, stable spheroidal shape water droplet resonators around a cylindrical stem have been fabricated, they suffer from several practical limitations related to the complexity associated with manipulating and controlling droplet structures and the difficulty of coupling light. More recently, hollow core optofluidic resonators in the form of a silica capillary have been demonstrated with high performance; however, they lack the possibility of easy planar integration [125, 126]. Integrated optofluidic resonators in the form of a planar ring have been obtained using SWs [127] and ARROWs [128] (figure 16), but also in the form of Fabry–Perot [8]. Each type of implementation has its own suitable application. Optofluidic resonators based on slot and hollow core waveguides, despite having moderate Q-factors (up to 10^4), open up interesting perspectives for planar integration. Nowadays, an increase in the Q-factor still remains a challenging task due to the leaky nature of the interference-based optofluidic waveguides and the problems associated with fabrication roughness in the nanometer-void of the SWs. The availability of high Q-factor resonators could represent a paradigm shift from passive to active sensors based on lasing action. Recent results have demonstrated that optofluidic lasers could result in a promising pathway towards ultrasensitive detection [8].

Advances in science and technology to meet challenges. The need to reduce the waveguides losses requires a two-fold approach. First, the development and optimization of new materials and refined fabrication processes. The proper selection of cladding materials and deposition processes has proven to effectively reduce waveguides losses. Second, the development of novel waveguide configurations able to overcome the limitations of existing designs. ALD has been demonstrated to be a powerful tool for reducing loss in ARROWs [129], and also for realizing novel slot waveguides [130]. New waveguide layouts are needed to extend the working range of optofluidic waveguides from the vis-IR to mid-IR region that can offer new integrated solutions to overcome the limited material choice of the integrated technologies in the mid-IR. As an example, suspended silicon slot waveguides have been proposed [122].

Concerning integrated optofluidic resonators, there are two main objectives to address in order to improve their performance and effectiveness. The first is to improve the quality factor of the resonator by optimizing the different optical elements (waveguide, bend regions, coupler), as also discussed above. The second is to provide a fine manipulation of the sample fluid by integrating suitable microfluidic systems. From this point of view, polymer materials offer several advantages for microfluidics, such as reduced cost and simplicity of fabrication. For this reason, hybrid silicon/polymer approaches appear as an optimal design strategy [113, 128], still requiring, however, considerable fabrication efforts to fully merge silicon photonics and microfluidics.

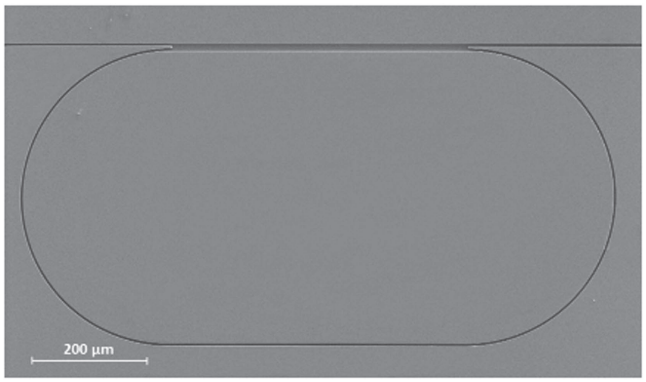

Figure 16. Scanning electron microscope image of an optofluidic ring resonator based on ARROWs.

Concluding remarks and perspectives. In perspective, optofluidic waveguides have the potential to play the same role as solid-core waveguides for conventional integrated photonic circuits. Promising performance has been demonstrated through the implementation of innovative waveguide layouts and high-yield fabrication processes. Ongoing progress is also focused on extending the range of applicability to the mid-IR range in order to address new applications, including gas sensing. The tasks of improving their performance and integration on a chip are now the main challenges that could be addressed in the near future by further development of state-of-the-art modeling and manufacturing. By taking advantage of recent innovative polymeric manufacturing techniques, hybrid silicon/polymer approaches will pave the way to the integration of additional functionalities on a chip. Moreover, polymer technologies could open up the prospect of high performance and low-cost all-plastic optofluidic microsystems, also suited to disposable applications.

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11. High-content physical phenotyping of biological objects with microfluidic dual-beam laser traps

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Relevance of the topic. Dual-beam laser traps (DBLTs) were the first type of optical trap, realized by Arthur Ashkin [20], and predate optical tweezers by two decades. Micromillimeter-sized dielectric objects, such as biological cells, are trapped by the combined scattering and gradient forces of two non-focused, counter-propagating Gaussian laser beams (see figure 17). The main advantages of DBLTs are that: (1) they can be simply realized by aligning two optical waveguides, such as single-mode optical fibers, (2) they can be combined with arbitrary imaging optics, and (3) the lack of focusing permits the use of high laser powers without cell damage ('opticution').

The latter aspect enables the controlled and non-destructive deformation of cells. When used for this purpose, DBLTs are called optical stretchers [25]. The cells' mechanical properties measured in this way have proven to be an important label-free marker of cell function, related to cancer progression, differentiation, cell cycle, infection, etc [134, 136]. The simplicity of the trap allows straightforward integration into microfluidic lab-on-a-chip environments [135] (see also section 2). Microfluidic optical stretchers (μOSs), thus, constitute a convenient technique for measuring cell mechanics at rates up to 10 cells min^{-1}, which compares favorably with other standard ways of measuring cell mechanics, such as atomic force microscopy (AFM) and micropipette aspiration, where 10 cells hour^{-1} are more typical.

However, with the recent advent of really high-throughput (1000 cells sec^{-1}) microfluidic techniques for the characterization of mechanical phenotypes that approach the analysis rates of conventional, fluorescence-based flow cytometers [138], the high-throughput aspect of the μOSs has lost some of its former appeal. Instead, the current focus is shifting to the high-content investigation of cells, and also of other biological objects, which are temporarily, but arbitrarily immobilized for a long time - until several different physical parameters important for cell function have been determined in detail. For example, full mechanical characterization, in particular steady-state viscosity, which is particularly important for relating to cell migratory processes [134], takes at least several seconds per cell, and inherently cannot be obtained in high-throughput, where the measurement time per cell is in the millisecond range.

Other physical characteristics of interest are the refractive index (RI) of cells, which can be used to track cell differentiation and other functional changes [131], or their local mass density, which could be important for phase separations and phase transitions in cells. The choice of laser wavelength and total power also determines water absorption, and with that the temperature in the volume of the trapped object [133]. By intentional use of this laser heating, additional thermodynamic properties of the trapped object can be investigated [132]. DBLTs can also be combined with other photonic techniques such as Raman and Brillouin spectroscopy and microscopy for additional chemical and mechanical information. Ideally, all these properties should be resolved in 3D inside of cells with diffraction-limited resolution so that either fast point-scanning, light-sheet or tomographic approaches become relevant. Finally, the investigation does not need to remain limited to entire cells. Other, often rather delicate, biological objects can also be gently handled, manipulated and investigated by optical forces in combination with photonic techniques. In this context, vesicles, isolated and artificial cell nuclei, mitotic spindles, or protein droplets come to mind.

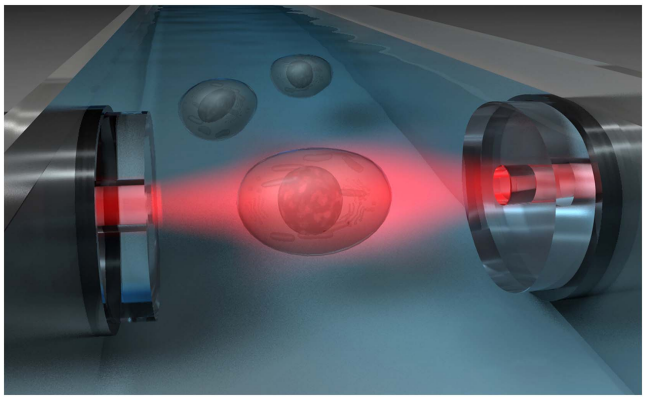

Figure 17. Principle of microfluidic dual-beam laser traps. Cells are serially trapped between counter-propagating laser beams in a microfluidic channel.

Current status and challenges. In order to realize the potential of DBLTs to deliver high-content physical information about cells, as outlined above, and considering the state-of-the-art in the field, there are a number of challenges to be met.

The optical trapping itself [25], the extraction of detailed viscoelastic properties [134], and the microfluidic integration [135] have been well elaborated. The temperature increase in the trap was until recently coupled directly to the amount of power used, since DBLTs were conveniently realized with ytterbium-doped fiber lasers operating at 1064 nm. Here, $\Delta T = 13\ \text{K W}^{-1}$ [133]. Due to the recent advent of alternative high-power fiber-based lasers, such as frequency-doubled erbium lasers, 780 nm sources are also now available, which reduce the temperature increase eight-fold and permit the decoupling of optically induced stress and temperature [132].

The combination of DBLTs with many different conventional imaging techniques, such as phase contrast microscopy, is straightforward. For fluorescence imaging, the limited usability of high-NA objectives—due to the significant distance between objective and trapped object, caused

by the diameter of the optical fiber and mounting on thick microscope slides—restricts the spatial resolution and light gathering ability of weaker fluorescence signals. While Ca^{2+} dyes and nuclear dyes can be readily imaged [132], sparser fluorescent signals from GFP proteins or fainter signals from fluorescently labeled surface markers pose considerable challenges at present.

For quantitative phase imaging, digital holographic microscopes are often used. Their integration with DBTLs, set up on a commercial microscope stand, is less straightforward, especially when a separate reference beam needs to be implemented. From such quantitative phase information, assuming spherical symmetry, which is often given for cells in suspension, a 2D integrated RI distribution and an overall average RI can be calculated [139]. In order to obtain the complete 3D distribution of RI, and mass density, tomographic reconstruction has to be used. For this, phase images need to be obtained from multiple angles. Here, the versatility of DBLTs offers at least two attractive solutions: (1) either cells can be trapped by a conventional DBLT with two counter-propagating Gaussian laser beams with cylindrical rotational symmetry and then rotated by fluid flow in the microfluidic channel, or (2) at least one of the Gaussian laser beams is replaced by a laser beam with higher-order modes that break the rotational symmetry, which leads to a preferential rotational alignment of the cell in the trap. The rotation of the laser modes then leads to the rotation of the trapped cell around the optical axis of the laser trap—and through the focal plane of the imaging microscope. When used for this latter purpose, DBLTs are also referred to as optical cell rotators [137] (see figure 18). An important challenge in this context is the translational and rotational stability of the trap, currently limiting the spatial resolution of any imaging.

While Raman microscopy has been researched and described extensively, Brillouin microscopy of biological cells has only recently been introduced. It promises to yield additional mechanical information via interrogation of the local phononic spectrum inside cells. The extraction of actual mechanical properties requires knowledge of the local density and RI, which can be provided by the simultaneous RI tomography as described above. A significant challenge is the small frequency shift away from Rayleigh scattering and the very low intensity of the Brillouin signal, which leads to long integration times, amplifying the problem of translational and rotational stability of the object in the trap.

Advances in science and technology to meet challenges. The challenge of high-quality fluorescence imaging in DBLTs can be addressed by optimized integration into specific lab-on-a-chip setups with specific consideration of the optical quality along the imaging axis and the minimization of the distance between objective and trapped object [135] (see also section 2). The use of more sensitive and faster fluorescence cameras, and an improved translational stability of the trap (see also below) will improve detection of weak signals and spatial resolution. The problem with incorporating quantitative phase microscopy can be solved by in-line interferometry, such as quadrilateral shearing interferometers. These are now commercially available, and can be mounted onto microscope stands just like other cameras.

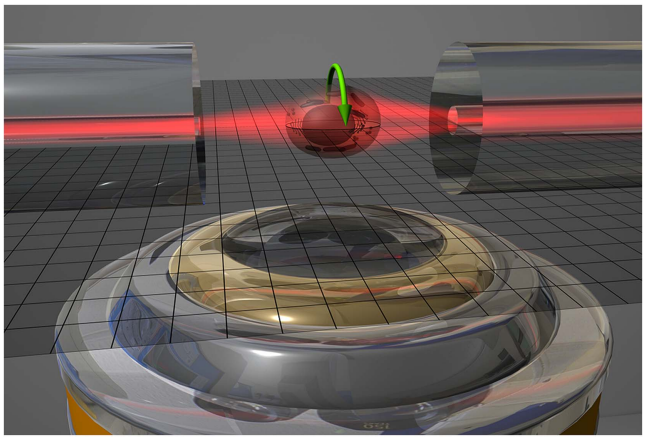

Figure 18. Cell rotation in an optical cell rotator can be combined with any arbitrary microscopic or spectroscopic technique for full physical characterization.

The challenge of sufficient translational and rotational stability of DBLTs can be approached by fast feedback loops, involving real-time analysis of the position and 3D RI distribution of the trapped object, and appropriate shaping of the light fields and their intensity using adaptive optics through the trapping fibers. The creation of complex light fields through multimode fibers using transfer-matrix operators has recently been demonstrated. This could also be used for illuminating cells with a light-sheet through the trapping fibers for improved fluorescence or even Brillouin microscopy. The problem of long integration times in Brillouin microscopy can potentially be ameliorated by stimulated Brillouin microscopy, and the use of line- or even light-sheet imaging.

Concluding remarks and perspectives. In summary, microfluidic DBLTs are ideal 'optical work benches' where cells can be temporarily held stationary, interrogated by a number of different photonic techniques for their complete physical phenotyping and then conveniently exchanged and sorted by microfluidic integration. Even though their high-throughput mechanical phenotyping appeal has faded with the recent advent of techniques that are 100 000 times faster, their true potential lies in high-content characterization, which is still to be realized. The best times of microfluidic DBLTs are yet to come.

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12. Integration of fiber-based tweezers and microfluidic systems

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Relevance of the topic. Over the last few decades, microfluidic technologies have demonstrated their potential for the realization of lab-on-a-chip systems. Lab-on-a-chip devices present integration and miniaturization of various probes and devices, along with parallelization of functions and analyses, which allow cost-effectiveness (e.g. thanks to low consumption of samples and chemicals) and automated high throughput, that are particularly beneficial to biomedical applications. In this context, considerable interest is attracted by implementing optical tweezers (OTs) in lab-on-a-chip devices, to support functions such as precise positioning of samples within the microchannel, single-cell manipulation and sorting. OTs are based on optomechanical forces, originating in exchange of momentum between photons and matter, that are exerted by a strongly focused optical beam on micro- or nanoparticles [141]. They have been used in many applications in biology and physics, for a wide variety of experiments ranging from trapping and manipulation of live bacteria and cells, to cooling, trapping and manipulation of single atoms [141]. Important results have also been achieved by combining OTs with other optical techniques, such as fluorescence and Raman spectroscopy. Early OT setups were based on tight focusing of a laser beam by means of high numerical aperture (NA) microscope objectives (usually NA $>$ 1) [141]. In order to overcome issues that arise in this scheme when trapping particles within thick samples or in turbid media, an effort to implement OTs based on optical fibers has been in place since the early 1990s. However, the realization of a fiber-based OTs (FOTs) forming three-dimensional optical traps has proven to be a challenging task, mainly because of the low NA of optical fibers. Indeed, three-dimensional optical trapping was initially demonstrated only with configurations made of at least two separated and opposing fibers. The early-proposed schemes based on a single fiber were essentially capable of only two-dimensional trapping, therefore were unable to stably trap particles in the middle of a fluid, or had impractically small trapping distances, making it difficult to trap a particle without physical contact. Only with more recent advances in technologies for microstructuring of the fiber tip has it become possible to design and fabricate OTs based on single optical fibers. FOTs have recently been demonstrated that present ready compatibility with microfluidic channels, and are best suited towards realization of fully integrated microfluidic chips. Future developments of FOTs for lab-on-a-chip devices could integrate, in addition to optical trapping, more advanced functions such as imaging and/or enhanced spectroscopies with plasmonic probes.

Current status and challenges. At the state-of-the-art, three-dimensional optical traps are mainly implemented inside microfluidic devices by using three different approaches: (1) with high-NA microscope objective-based setups; (2) with two counter-propagating beams, delivered by two optical waveguides (which could be optical fibers) positioned on opposite sides of the microfluidic channel; (3) with single optical fibers. Naturally only the two latter methods are capable of being integrated into microfluidic chips, and so compatible with the development of portable lab-on-a-chip devices. OTs based on two opposing waveguides are also called 'optical stretchers' and are mainly used to apply forces that deform the trapped particle, to probe its mechanical properties [142]. This particular type of integrated OT is considered in section 11 of this Roadmap.

Integrated OTs based on a single optical fiber, able to generate a three-dimensional optical trap far enough from the fiber tip to avoid contact with the sample, are currently possible only by the use of speciality fibers (bundles, multicore or annular core fibers) along with advanced methods to modify the fiber tip to overcome its intrinsic low NA [143]. Remarkably, the continuous progress made in the capability to structure the fiber tip has enabled ever more effective geometries for optical trapping. Indeed, several different fiber-tip modification methods have been proposed so far. Initially, tapering of a standard single-mode optical fiber to a very small tip size with the heating and pulling method, resulted in FOTs with a very small trapping distance [144]. More recently, high-precision fabrication techniques were used to implement an approach proposed in [145], where light beams having an annular arrangement (e.g. generated by an annular core fiber or carried by the different fibers of a bundle) are deflected by TIR towards a common crossing point, yielding the equivalent effect of a high-NA microscope objective, as needed for OTs (figures 19(a) and (b)). The TIR deflecting structures have been fabricated on the fiber tip by focused-ion beam milling [145] or, more recently, by two-photon lithography (TPL) (figure 19(c)) [140]. The latter fabrication method is significantly easier, more flexible and more cost-effective than the former. The use of a fiber bundle instead of an annular fiber allows specialization of different subsets of fibers for optical trapping and for simultaneous probing of the trapped sample (e.g. with fluorescence). A similar scheme, based on fiber annular core distribution and TIR-based deflection, has also been realized by the grinding and polishing technique [146].

As shown in [140], single-fiber-based OTs can be integrated into a microfluidic device (figure 19(d)), in a vertical configuration that allows the insertion of multiple FOTs at different locations within the same lab-on-a-chip device without interfering with the microfluidic channel network. Trapping within microfluidic channels has also been recently demonstrated with a tapered fiber creating a two-dimensional trap, under specific flow rate conditions [147], and with a tapered fiber for three-dimensional trapping but with the particle in contact with the tip [148].

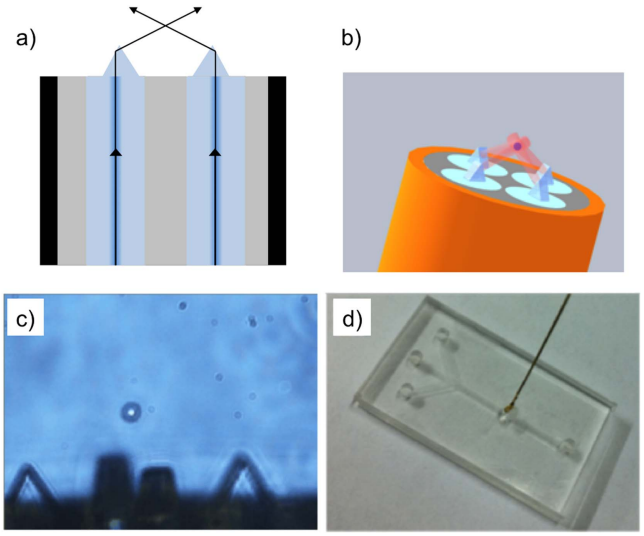

Figure 19. (a) Cross section of the FOT probe presented in [140]: beams propagating in two symmetrically positioned fibers are reflected at the interface between microprisms and the outer medium; (b) isometric view of the FOTs; (c) red blood cell trapped by the FOTs in hypotonic solution; (d) picture of a microfluidic chip where the FOTs have been inserted. Adapted by permission from Macmillan Publishers Ltd: Scientific Reports [140], Copyright 2013.

A few challenges still remain in achieving the goal of applying the full functionality of well-developed 'bulk' OTs in lab-on-a-chip devices. The first challenge is to allow all optical manipulation (translation in three dimensions, rotation) of trapped samples without moving the FOT probe. The second challenge is the creation of multiple optical traps with a single FOT within a microfluidic channel. Further challenges will be the addition of imaging capabilities to the FOT probe, e.g. by integrating multimode fibers [149], and of enhanced probing/sensing capabilities, e.g. with electrodes or plasmonic based structures.

Advances in science and technology to meet challenges. The addition of more advanced functionalities to single-fiber FOTs, for integrated lab-on-a-chip devices, will be possible with progress in the design and fabrication of complex photonic structures on the tip of optical fibers. In this regard, a key role in the TPL fabrication method is envisioned, as an extremely powerful tool for rapid and flexible prototyping of highly optical quality elements, with dimensions that range from a few to hundreds of micromillimeters. In particular, the development of a new design that further reduces the size of single-fiber FOTs will improve compatibility with small microfluidic channels and will allow for easier integration of other fibers, e.g. a multimode fiber for imaging, and/or other structures, e.g. for enhanced spectroscopy, within the FOT probe, to obtain an ultra-compact multimodal tool for lab-on-a-chip devices. Another important advance will be the combination with other technologies that can enable new functions, such as the use of spatial light modulators for fiber imaging [149] or the creation of dynamical multiple traps, which will also allow manipulation of the trapped particle.

Concluding remarks and perspectives. Integration of OTs into microfluidic channels will enable new functions in lab-on-a-chip devices. Optical fibers offer an excellent platform for miniaturization of OTs, so that they can be embedded in microfluidic circuits. Indeed, single-fiber FOTs have been recently reported, based on speciality fibers and TIR structures, and inserted in a microfluidic channel. Future developments will concern further miniaturization of FOTs, the addition of manipulation and multitrap capability, and the integration of new functionalities, such as imaging and enhanced spectroscopies, to the FOT probe.

Acknowledgments. The author would like to thank the following current and former colleagues for their valuable contributions in the joint work on the subject: Francesca Bragheri, Patrizio Candeloro, Gheorghe Cojoc, Ilaria Cristiani, Enzo Di Fabrizio, Lorenzo Ferrara, Paolo Minzioni, Gerardo Perozziello and Vijayakumar P Rajamanickam.

13. Acoustic prefocusing in optofluidic systems

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Relevance of the topic. The ability of acoustic fields to position small particles was discussed by Ernst Florens Friedrich Chladni (1756–1827), and is familiar to many scholars through the demonstration experiment named 'The Chladni plate'. When carried over to dimensions of typical microfluidic systems, the resonance phenomenon, nicely illustrated by the Chladni plate experiment, implies typical frequencies in the MHz range. Many research papers have in recent years demonstrated the ability of the effect, termed acoustophoresis, e.g. to sort cells according to their acoustic properties; readers unfamiliar with the topic may consult the tutorial series of papers in [150]. The second part of the topic, optofluidic systems, is the term currently used for microfluidic systems with embedded optical elements, be it a microlaser, or a passive or active optical element; the relevance of optofluidic systems at large is demonstrated by the collection of papers in this Roadmap. The benefits of the combination of the effects of acoustic and optical manipulation have been illustrated by active particle sorting [151], improved trapping efficiency in an optical stretcher setup [152] and access to complementary mechanical characteristics for the same single cancer cell [34]. Acoustic prefocusing in an optical stretcher setup is illustrated in figure 20. The results of references [34, 151, 152] suggest that further advances in the field may find both technological and biomedical applications.

Current status and challenges. The combination of acoustofluidics and optical manipulation has, to the best of the author's knowledge, only been applied in very few laboratories [34, 151, 152], with promising recent advances involving high-resolution imaging [153]. As discussed below, one may very well imagine inclusion of other characterization tools based on optics.

On the acoustics side, an interesting development is the recent introduction of the concept of iso-acoustic properties, whereby understanding and control in acoustic focusing and sorting has been tremendously widened [154]. A current challenge for the technology to be more accessible for applications is the choice of material for the construction of the microfluidic systems. Successful demonstrations of combined acousto- and optofluidics [34, 151, 152] are based on glass—a hard and highly resistant material with excellent optical properties. However, much current research explores the ability to construct micro- and optofluidic systems in polymer materials since micro- and even nanofluidic systems may be produced by large-scale production techniques such as injection molding or roll-to-roll imprinting, rather than the expensive and time-consuming clean-room processes required for the production of a single microfluidic system in glass [155]. In addition, the polymer material can be chosen with properties that match well both optical and biomedical requests. Unfortunately, acoustofluidics using plastic materials remain challenging due to the relatively low sound speed and acoustic impedance in most transparent plastics. This implies that acoustic energy may be transferred to, and lost in, the device itself, and that knowledge of the conditions for acoustic resonance, leading to large acoustic forces, requires detailed 3D modeling of the pressure fields in the entire device [156]. Such detailed models are in contrast to simple 1D models in which one assumes a large degree of reflection of the sound wave at the interface between the liquid in the microfluidic channel and the hard walls of the glass or silicon microfluidic device.

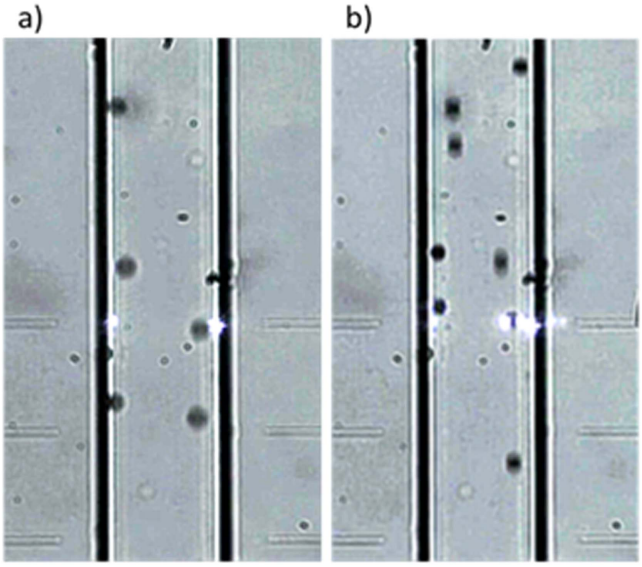

Figure 20. Microfluidic all-glass device with a liquid channel of a rectangular cross section; the channel is 100 μm wide. Details may be found in [152]. Shown are images of 5 μm diameter polystyrene beads in water, visualized by standard bright-field microscopy. Laser-written waveguides in the glass chip were in the focal plane; bright spots indicate scattered laser light coupled into two opposing waveguides. (a) Acoustic vertical focusing turned off. (b) Acoustic vertical focusing turned on. Scattering by trapping laser light on the beads illustrates nicely the acoustic prefocusing of the beads to the plane of the waveguides. [152] © Springer-Verlag Berlin Heidelberg 2015. With permission of Springer.

As discussed in section 3, lithium niobate offers an attractive alternative choice of material. In [153], a transparent transducer made out of $LiNbO_3$ with indium tin oxide electrodes was applied in a layered device. The experimental setup was demonstrated to be able to provide precise determination of both optical and acoustic forces on trapped silica microspheres.

Delivery or collection of light for optical manipulation or characterization by optical means is another point to decide on. One option is to have the light delivered by embedded optical fibers [157], or waveguides directly written into the microfluidic device [34, 152], suitable for optical traps based on two counter-propagating laser beams, or for collection of emitted light, e.g. for Raman spectroscopy of the sample. Another option, relevant for single-beam optical traps, is to rely on light delivery and light collection through an external microscope objective [151, 153]. When this latter option is chosen, one should be aware that the acoustic force field may be altered due to mechanical coupling between the immersion

oil and the microscope objective, and thus the entire microscope [153].

Advances in science and technology to meet challenges. As alluded to above, on the materials side, the combination of acoustophoresis and optofluidics would clearly benefit from the development of transparent polymer materials with larger acoustic contrast between device material and liquid—in addition to good optical quality and high chemical resistance. As this may not be realistic, the second best option is to develop and apply 3D modeling tools [156] that enable prediction of sound wave propagation in the entire experimental device, and thereby may suggest a design of the microfluidic device optimized for the simultaneous action of acoustic and optical forces.

The choice of a polymer material for the microfluidic device couples well with a desire to develop single-use devices. Waveguides produced by post-processing steps like DUV exposure, and/or other means to embed optical elements in a polymer chip produced by mass-production techniques, would clearly enhance the employability of optofluidic devices, with or without acoustophoresis. A challenge to resolve is, however, the typical loss of optical power of the order of 0.5–0.7 dB mm^{-1} in waveguides written by DUV or direct laser writing, in addition to the coupling losses into a square or rectangular waveguide. Alternatively, light coupling through standard optical fibers suffers much less from loss of optical power, and still offers a competitive solution, although manual assembly is required. With either choice of solution for light propagation in the microfluidic chip, the dream device would be a single-use polymer device that is mounted in a system or a holder that includes efficient and well-aligned coupling to light sources as well as an efficient coupling to a sound transducer; a simple sketch is provided in figure 21. In this respect, piezoceramic crystals may still be a wise choice for the acoustic transducer, although capacitive micromachined ultrasound transducers, which may even be controlled by light, may be an interesting alternative [158].

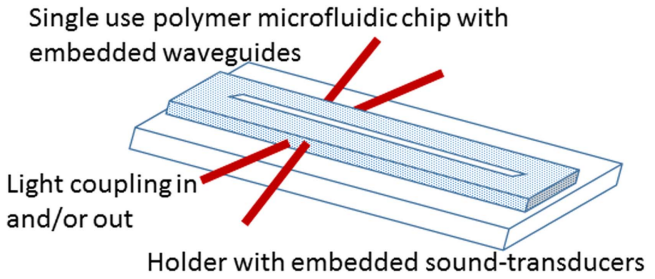

Figure 21. Simple sketch of a future device for simultaneous acoustic prefocusing, optical manipulation and spectroscopic characterization.

Concluding remarks and perspectives. As discussed, manipulation by both sound and light fields in microfluidic systems adds versatility to the optofluidics toolbox. We concentrated on merging tools of manipulation, applying both optical and sound waves, yet the perspectives include other uses of light, e.g. for spectroscopic characterization. One may foresee a combination of optical manipulation and/or spectroscopy on cells with particular iso-acoustic properties, with the perspective of learning many more details, including molecular, about the properties of such individual cells.

Acknowledgments. The author acknowledges discussions over the years with G Nava, T Yang, P Minzioni, I Cristiani, as well as with A Kristensen, and with H Bruus and his students. Financial support from the Carlsberg Foundation and COST MP1205 is highly appreciated.

14. Optofluidics for single-cell protein analysis

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Relevance of the topic. Proteins (i.e., macromolecules composed of chains of amino acid residues) perform key functions within organisms, including regulation of metabolic reactions, DNA replication, stimuli responsiveness, and molecule transportation [159]. As key components of biological organisms, the quantities and activities of proteins have been regarded as the most important indicators of biological activities, which are closely related to phenomena such as cellular differentiation, neuron transmission and disease progression [160].

Within the last few decades, a large number of characterization approaches (e.g. gel electrophoresis, immunoassay, chromatography and mass spectrometry) have been developed to estimate the quantities and activities of proteins [161]. These results have contributed to the rapid development of biological and medical sciences, which have significantly promoted the detection capabilities in clinical diagnosis [162]. However, these approaches are only capable of quantifying protein expressions from a large number of cells, which neglects the fact that most of the organisms are composed of cells with variations in their functionalities and thus protein expressions at the population level may mask key underlying mechanisms due to cellular heterogeneity.

Currently, fluorescent flow cytometry is the golden standard in the field of single-cell protein analysis, where single cells mixed with fluorescence-labeled antibodies are flushed into a fluorescent measuring domain composed of laser excitation and photomultiplier tube based fluorescent detection [163]. Based on this methodology, variations in single-cell protein expressions are closely correlated with tumor biology, microbiology, cellular differentiation and drug development (see figure 22). However, fluorescent flow cytometry can only provide an absolute quantitation of surface proteins of single cells, while it cannot quantify intracellular proteins due to the lack of calibration approaches, and thus it cannot translate raw fluorescent signals into copy numbers of intracellular proteins [164].

Optofluidics is the science and technology of the processing and optical detection of small amounts of fluids (10^{-9} to 10^{-18} liters) in channels with dimensions of tens of micrometers [2]. Since the dimensions of optofluidics are comparable to biological cells, optofluidics has been used for the manipulation of single cells with their protein expression levels quantified optically [165], and can be classified into four major types: (1) optofluidic fluorescent flow cytometry; (2) droplet-based optofluidic flow cytometry; (3) large-array microwells (microengraving), and (4) large-array microchambers (barcode microchips). In this section, we examine the advantages and limitations of each technique and discuss future research opportunities by focusing on two key parameters: absolute quantification and throughput. Note that other microfluidic approaches of single-cell protein analysis without key optical components are not included in this section.

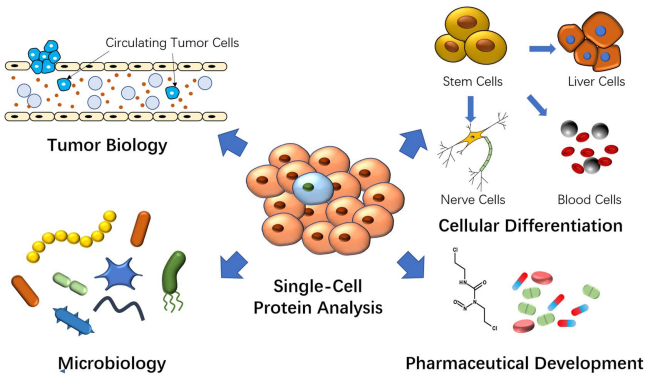

Figure 22. Variations in single-cell protein expressions are closely correlated with tumor biology, microbiology, cellular differentiation and pharmaceutical development.

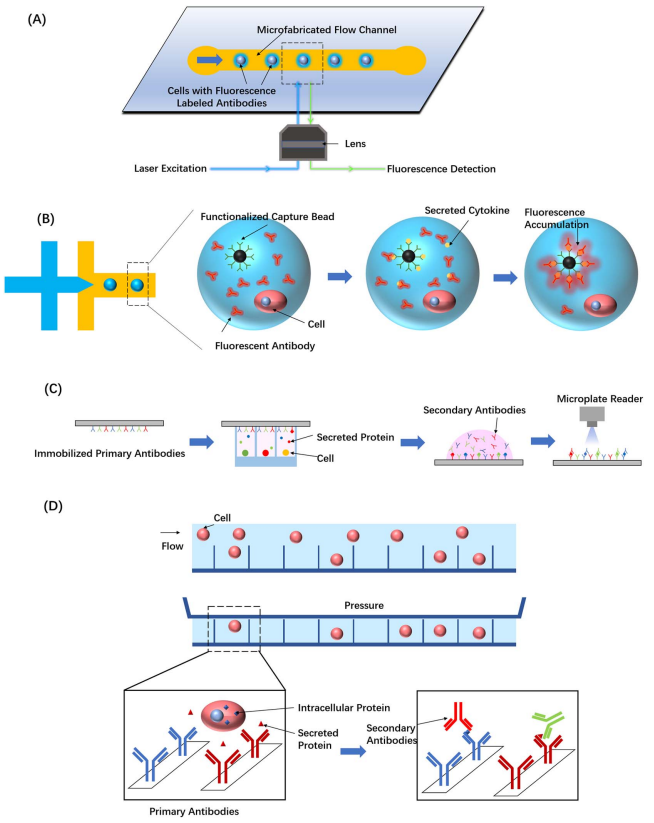

Figure 23. Key developments of optofluidic systems enabling high-throughput single-cell protein characterization, which includes (a) optofluidic fluorescent flow cytometry; (b) droplet-based optofluidic flow cytometry; (c) large-array microwells (microengraving); and (d) large-array microchambers (barcode microchips).

Current status and challenges. In optofluidics, fluorescent microflow cytometry was the first approach to quantifying single-cell protein expressions where single cells are mixed with fluorescence-labeled antibodies and flushed into microfabricated flow channels with fluorescent intensities measured [166] (see figure 23(a)). As a miniaturized version of conventional

fluorescent flow cytometry, microflow cytometry features low sample requirement of cells and reagents. However, this approach cannot quantify the copy number of intracellular proteins, due to the lack of effective calibration approaches.

In the second approach, droplet-based optofluidic flow cytometry was proposed where single cells, functionalized capture beads and fluorescence-labeled secondary antibodies are encapsulated in droplets; microbeads bind cytokines secreted by single cells, which further bind fluorescence-labeled secondary antibodies, leading to fluorescent signals [167] (see figure 23(b)).

Compared to conventional fluorescent flow cytometry, this approach can effectively assay secreted proteins at the single-cell line. However, due to the use of cytokine-capture beads, there is an uneven distribution of fluorescence within individual droplets (intensity peaks around individual beads), which poses obstacles in calibration and thus the secreted proteins cannot be effectively quantified.

In the third approach, large-array microwells ('micro-engraving') were proposed to isolate individual cells with copy numbers of secreted proteins quantified [168]. As shown in figure 23(c), single cells suspended in media are deposited into a large array of microwells, which are then sealed by a glass slide coated with a specific capture reagent. After certain periods of incubation, the glass slide is removed and the cells are further interrogated with laser-based fluorescence scanners. Although microengraving enables absolute quantification of proteins secreted by individual cells, compared to flow cytometry, it suffers from complicated processes and limited throughput.

In the fourth approach, large-array microchambers (single-cell barcoding microchips) were proposed to assay both cytosolic and membrane proteins of single cells [169]. As shown in figure 23(d), the single-cell barcoding microchips consist of thousands of individually addressed microchambers for single-cell trapping, followed by cell lysis, capture of targeted proteins by pre-printed antibodies on the surface of the chambers, which are further measured using immunosandwich assays. This approach is the most powerful optofluidic system in the field of single-cell protein analysis since it enables the absolute quantification of both surface and cytosolic proteins of single cells. However, since this approach relies on the confinement of single cells within chambers, it cannot be easily scaled up to further increase the throughput.

Advances in science and technology to meet challenges. Absolute quantification is a key requirement for single-cell protein assays since, without the capability of absolute quantification, the protein levels measured by different approaches cannot be effectively compared. From this perspective, new calibration approaches have to be developed in fluorescent microflow cytometry, enabling the translation of raw fluorescent signals to protein copy numbers.

Throughput is also a key factor in single-cell protein quantification. Although optofluidic large-array devices are featured with parallel analysis of single cells, which are capable of processing hundreds of single cells within one experiment, they still cannot be used to process cell samples with heterogeneity (e.g. tumor samples with at least one million cells) due to their limited throughputs. Thus, tremendous effort needs to be devoted to this field with the purpose of further scaling up these assays. As to microflow cytometry, further technical improvements should focus on the extension of this serial approach to function in parallel.

Concluding remarks and perspectives. In this study, we summarize key developments of optofluidic platforms enabling the quantification of single-cell proteins. Although significant improvements have been made within the last decade, an ideal tool of single-cell protein quantification featured with absolute quantification and high throughput is still not available.

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15. Optofluidics for DNA analysis

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University of Surrey

Relevance of the topic. Capillary electrophoresis (CE) is a powerful method for biomolecule separation and analysis. The sorting and sizing of DNA molecules within the human genome project [170] has largely beem enabled by CE separation and analysis. The human genome project has also led to the genetic mapping of various human illnesses [171]. The traditional techniques, e.g. using a bulk glass capillary filled with agarose gel [172], provide high separation resolution, but typically require rather long analysis times and bulky instrumentation.

These issues are addressed by the development of microchip CE. Integration of a lab-on-a-chip, thereby ensuring compactness and introducing the potential for mass fabrication, paves the way for device portability and in-field or point-of-care applications. Furthermore, DNA sequencing by microchip CE allows for cheap, high-speed analysis of low reagent volumes. In recent years, on-chip integration of DNA sequencing [173] and genetic diagnostics [174] have become feasible. One of its potential applications is the identification of genomic deletions or insertions associated with genetic illnesses.

Current status and challenges. Laser-induced fluorescence exploiting fluorescent dye labels is the most popular microchip CE monitoring technique. Conventional instruments use confocal setups to focus the excitation light and to collect the resulting fluorescence. Such schemes can provide high sensitivity down to a detection limit of ~200 fM [175], corresponding to merely eight molecules in the excitation volume, making plausible the vision of going down to interrogation at the single-molecule level. However, these schemes require accurate mechanical alignment of the optics to the microfluidic channels and are sensitive to mechanical vibrations and drifts. The need to use microchip CE in combination with a massive benchtop instrument, such as an optical microscope, frustrates many of the microchip CE advantages, in particular it strongly limits device portability and prevents in-field or point-of-care applications. In the future optical components, including light generation, transportation, interaction with the reagents, collection and detection, need to be integrated, partly directly, on the optofluidic chip.

Sizing accuracy poses an inherent challenge to CE, and particularly microchip CE, and achieving sufficient and reliable sizing accuracy for the envisaged DNA analysis is probably the biggest hurdle. Application of CE-based DNA sequencing in a lab-on-a-chip to identify genomic deletions or insertions associated with genetic illnesses, such as breast cancer or anemia, critically depends on the detection of single-base-pair insertions or deletions from DNA fragments in the diagnostically relevant range of 150–1000 base pairs. The sizing accuracy is related to DNA plug formation and depends on many factors, including the range of DNA fragment sizes to be separated, the choice of, and the changes in, the sieving gel matrix inside the microfluidic channel and its inner-wall coating, temperature and actuation voltage, as well as the choice of microfluidic channel cross section, dye label and, finally, the calibration procedure. A number of empirical studies have been performed to shed light on this complex situation, but results are sometimes not reproducible and existing models are typically inaccurate and unable to predict device performance.

Advances in science and technology to meet challenges. With the general advancement in microfabrication, significant technical progress has also been made in merging microfluidics and micro-optics in an optofluidic chip. For example, post-processing of commercial microfluidic chips by femtosecond laser writing of optical waveguides [37, 176] that can direct laser light to the interaction point and/or collect fluorescence from the excited dye labels and deliver it to the detection unit is a suitable method for optofluidic integration. This technique has the additional capability of writing three-dimensional optical structures into the microfluidic chip [177]. The light source and the detection unit are preferably placed in a small portable instrument that also includes the steering unit for the actuation voltages and to which the optofluidic chip can be precisely attached.

The technique of multiplex ligation-dependent probe amplification [178] allows for the simultaneous extraction and individual end-labeling of DNA fragments from independent human genomic segments. In order to exploit this technique, parallel optical processing of the prepared sample is required. Demonstrated parallel optical processing methods are modulation-frequency-encoded dual-wavelength laser excitation, fluorescence detection with a single ultrasensitive, albeit color-blind photomultiplier, and Fourier analysis decoding [179, 180]; see figure 24. To make the laser excitation of dye labels selective, each label must absorb light in a different wavelength range. Typical dye labels exhibit rather large absorption bandwidths, thereby strongly limiting the number of exclusive labels that can be employed simultaneously. Consequently, the extension of dual-wavelength analysis to a larger number of wavelengths faces the challenge of development of novel, narrow-excitation-band fluorescent labels. Alternatively, fluorescent labels with spectrally overlapping absorption bands can be excited with a single laser, thereby reducing the necessary infrastructure on the excitation side, and their individual fluorescence lines can be either detected by a color-sensitive detector array or spectrally dispersed and then detected by a detector array. However, any complication on the detection side that wastes fluorescence intensity or provides less sensitivity than a photomultiplier impairs the detection limit.

The dependence of sizing accuracy on the important system parameters must be investigated more systematically and its theoretical background needs to be improved. For example, recently it has been identified that the choice of fluorescent dye label can significantly influence the sizing

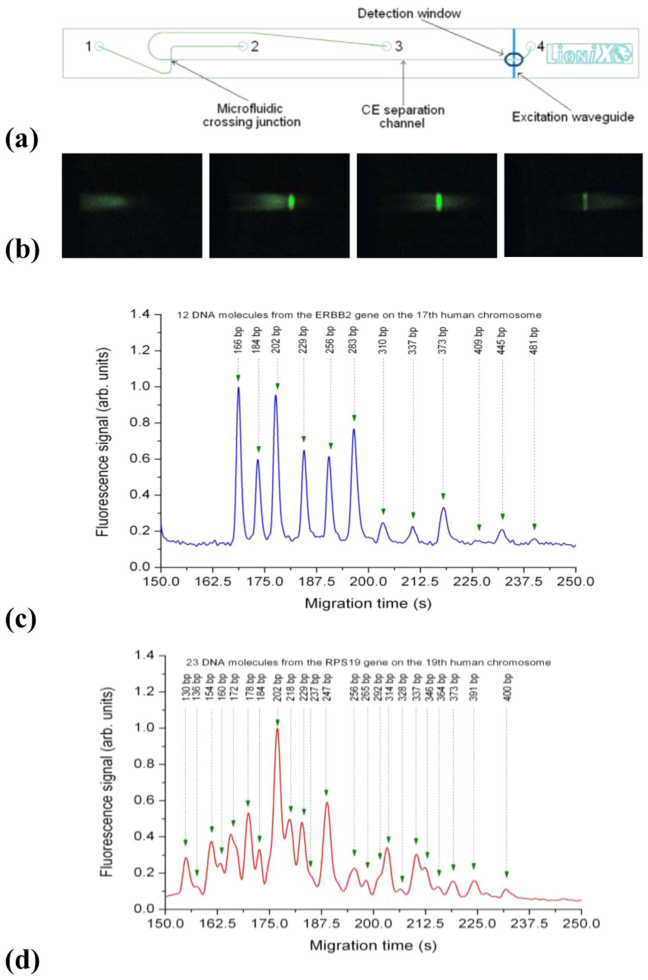

Figure 24. Multicolor fluorescence DNA analysis in an optofluidic chip. (a) Schematic diagram of the optofluidic chip. (b) Transient fluorescence from a molecule plug passing by the excitation waveguide (with background illumination outside the detection window). Reproduced from [181]. CC BY 3.0. Individual signals separated by Fourier analysis of (c) 12 DNA molecules from a breast cancer gene and (d) 23 DNA molecules from an anemia gene. Reproduced from [179] with permission from The Royal Society of Chemistry.

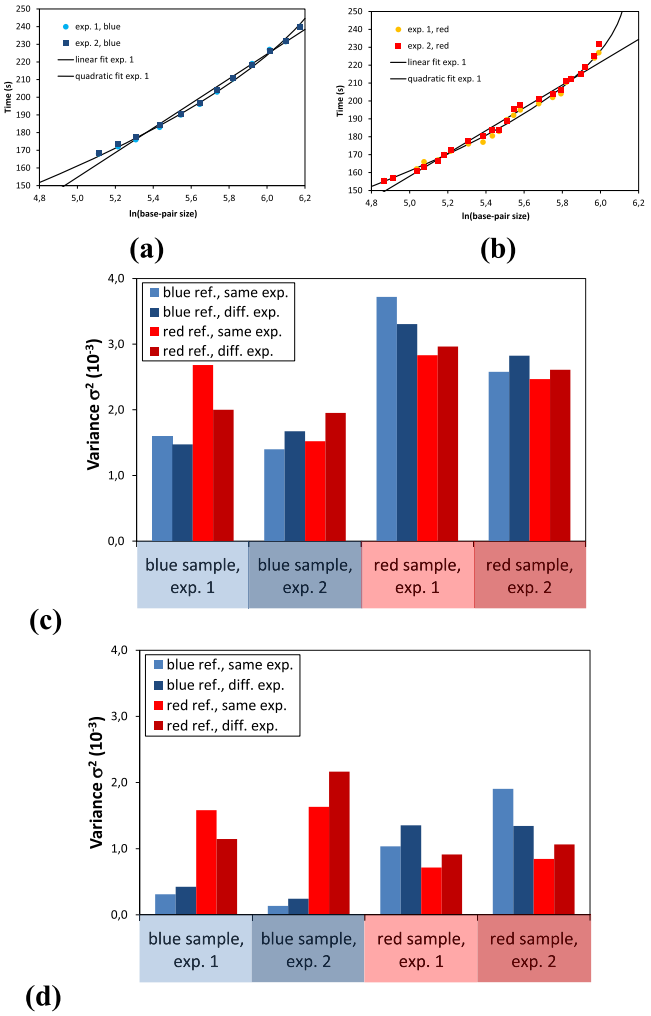

Figure 25. Migration time (linear scale) versus base-pair size (logarithmic scale) of (a) 12 blue-labeled and (b) 23 red-labeled DNA molecules simultaneously migrated and separated in experiment 1 (circles) and experiment 2 (squares). Linear and quadratic fits (solid lines) shown only for experiment 1. Variance σ^2 measured in experiment 1 or 2 from (c) the linear fit and (d) the quadratic fit, obtained from the measured reference data from the same or the different experiment. Reproduced from [182]. CC BY 3.0.

accuracy and that a proper choice can largely improve the accuracy [182]; see figure 25, but the reason for this effect is unknown and systematic investigations are lacking. In the same work it has been demonstrated that the calibration strategy influences the sizing accuracy [182]. On the one hand, it may not matter much whether the optofluidic device is calibrated by flow of a known reference sample in the same or in a separate experiment, and whether the DNA fragments of the reference sample are almost identical or slightly different from those of the unknown sample to be investigated. On the other hand, applying the same fluorescent dye label to the two samples appears to be crucial.

Most importantly, the dependence of migration time on base-pair size needs to be understood theoretically. The results displayed in figure 25 prove that a quadratic fit reproduces the measured data significantly better than a linear fit, indicating that the assumption of a simple logarithmic dependence of migration time on base-pair size underlying the production of DNA ladders with a logarithmic increase in base-pair size is not justified by the experiment [182]. Nevertheless, the sizing accuracy crucially depends on the applied law to fit the reference data. At present, in the absense of a proper theoretical understanding of DNA plug formation and flow mechanism the choice of fit function is merely a best guess and may even change with the underlying system parameters.

Choice of a suitable dye label, combined with reference calibration and sample investigation by fluorescent detection in consecutive experiments, and application of a best-guess fit function, has resulted in capillary electrophoretic separation of fluorescent-labeled DNA molecules in the 150−1000 base-pair range with single-base-pair resolution [182] (see figure 25), thereby paving the way towards the detection of

single-base-pair insertion or deletion in a lab-on-a-chip with low reagent volumes in a few-minute experiment.

Concluding remarks and perspectives. Through the efforts of many scientists in this research area, in-field and point-of-care applications have become feasible, but a considerable amount of work remains to be performed to better understand the dependence of sizing accuracy on the various system parameters by establishing a better theoretical framework, and to improve the measurement repeatability and reliability.

Acknowledgments. The author gratefully acknowledges collaboration with his co-workers C Dongre and H J W M Hoekstra, the Politecnico di Milano, LioniX B V and Zebra Bioscience B V.

16. Nanoscale optofluidics

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Relevance of the topic. Over the past few decades, optofluidic devices have been extensively exploited for novel functions and promising applications in chemical and biological analysis, imaging and energy by combining the merits of both fluids and optics in the microscale platforms [183, 184]. Recently, researchers have shown great interest in scaling down the channel size and studying both the fundamental and applied science on the nanoscale. When the channel dimension is comparable with the size of molecules, the fluid transport and molecular behaviors are different [185]. Up to now, the most promising applications and phenomena related to nanofluidic devices have been based on electrokinetic effects such as water desalination, fluidic diodes and energy harvesting. By contrast, the big potential of nanofluidics has not been exploited in the realm of optics. The functions of nanochannels in optofluidic devices are mainly in two aspects as shown in figure 26. First, the nanochannels provide a solution for local tunability over photonic circuits. Their sensitive response to the change in the refractive index can also be used for biosensing. Second, the nanochannels offer effective confinement of biomolecules to facilitate biological analysis, especially for DNA related genomic applications. Despite the great expectation of expanding optofluidics on the nanoscale, it leaves ample room not only for basic discovery but also for technical improvement in current directions.

Current status and challenges. Here, we discuss the state-of-the-art and highlight the technical barriers impeding the development of nanoscale optofluidics.

Although enormous progress has been made in the development of nanofabrication over recent years, fabricating well-defined nanochannels with low cost and high throughput is still a challenge. Typical nanofabrication is completed in a well-equipped clean room using a lithography process, such as electron-beam lithography or focused-ion beam lithography. It is usually expensive and laborious with a long processing time. Inspired by soft lithography with PDMS molding, nano-imprint lithography has been employed for large-scale fabrication of nanochannels. Although the replication method improves the throughput, it has limitations in fabricating the nanomolds. Researchers are looking for alternative materials and techniques for fabricating nanodevices in order to make them commercially viable.

Dynamic reconfiguration of photonic functions is one of the representative topics of optofluidics. It tunes the optical properties of the device by controlling fluid flows with various refractive indices. The nanochannels can infiltrate the liquid into well-defined defects of a photonic crystal [186], or form a tunable grating by filling liquid into the nanochannel array [187]. Introducing the nanofluidic modulation into the photonic circuits provides a powerful approach for realizing compact and reconfigurable optical functionalities with precise localized control. The major challenge for the tuning of photonic functions is nanofluidic handling. The volumetric flow rate of the liquids transported through the nanochannel is extremely low. Conventional transport methods, such as capillary filling, pressure drop or electroosmosis, are not effective.

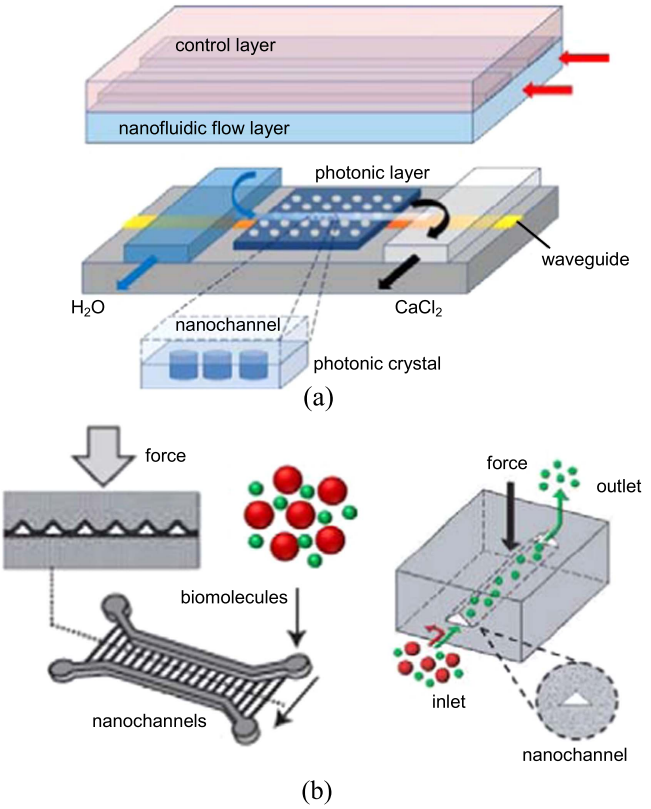

Figure 26. Illustration of nanochannel-based optofluidic devices. (a) Nanochannel bonded onto a planar photonic-crystal for optical tuning. Reproduced with permission from [186]. © 2006 Optical Society of America. (b) Nanochannels for biomolecule confinement. Adapted by permission from Macmillan Publishers Ltd: Nature Materials [189], Copyright 2007.

Another hot research topic on nanoscale optofluidics is the analysis and detection of a single molecule. Nanochannels are used to confine the sample volume to match the dimensions of the detection volume, increase its concentration for optical detection, or do precise manipulation of individual molecules, which enhance the sensitivity and resolution of the detection. Researchers have achieved length measurement, sizing and optical mapping of DNA by stretching or separating the molecules in nanochannels [188]. In most cases, the detection depends on fluorescent labeling, which is costly and time-consuming. Alternatively, scattering signals can also be used for detecting molecules in the nanochannels, which, however, has stringent requirements on the optical detection system. Improving the detection limit remains a bottleneck.

Although new phenomena keep being discovered, it is difficult to precisely probe the transport properties on the nanoscale and get good control over the nanoflow. The

existing techniques are mostly based on an electrical measurement. Optical detection may offer alternative strategies, but has to overcome several limitations in nanochannels, such as the influence of Brownian motion and wall effects.

Advances in science and technology to meet challenges. A revolution in nanofabrication is expected, just as the sprint in microfluidics following the development of PDMS-based soft lithography techniques. Recently, mechanical deformation of elastomeric materials (e.g. wrinkling, cracking and microchannel collapsing) was taken advantage of in fabricating nanochannels. These techniques are cost-effective and can adjust the channel size by controlling the stress applied on the materials [189]. Meanwhile, wide attention has been drawn towards bottom-up methods, in which the nanostructures are directly assembled by atoms and molecules. Novel materials, such as carbon nanotubes and graphene, have shown their unique properties in nanofluidic transport. One could exploit the newly emerging 3D printing technology to fabricate nanochannels in optofluidic systems.

Optical actuation is a burgeoning method for fluid control, which includes optical driven flow through the photothermal effect of nanoparticles, direct optical manipulation of particles/structures in the fluid, laser-induced cavitation, etc. Optically driven nanofluidic transport is insensitive to surface or solution conditions. Even though all the dynamic processes slow down due to the dominating surface forces, it is still possible to generate a high-speed jet by using a pulsed laser to drive flow, as shown in figure 27 [190].

In addition to developing a new optical detection concept and optimizing the optical system, sorting and pre-concentration of the targeted samples before detection is a feasible approach to improving the detection limit. Conventional approaches cannot precisely separate the samples. A more robust solution is offered by continuous flow separation of structures such as an entropic trap [191] and nanoscale deterministic lateral displacement pillar arrays [192], which have successfully demonstrated the sorting of DNA and exosomes. They can enhance the specificity of biomolecules, and therefore increase the sensitivity and resolution of detection. Advanced techniques are expected to increase the throughput of the separation and offer sophisticated manipulation of individual molecules.

A better understanding of mass transport at the nanoscale is required to reach the full potential of nanodevices. The next generation of cameras is expected to have higher frame rate and resolution, which will make it possible to improve flow characterization in the nanochannels. Combining other imaging techniques such as plasmonics and nanophotonics holds great promise to further improve detection. Moreover, optically induced perturbation on the nanoscale can be an alternative strategy to gain new insight into flow dynamics.

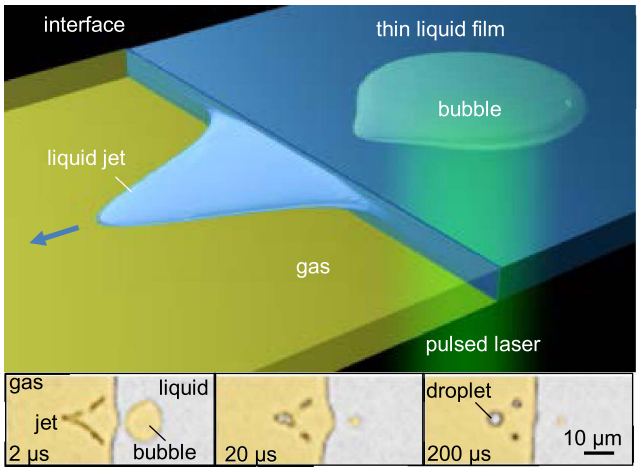

Figure 27. Jetting caused by the laser at a gas/liquid interface in a nanochannel. Adapted from [190] with permission from The Royal Society of Chemistry.

Nanophotonics-based optical manipulation may lead to an advantageous nanofluidic transport technique without the confinement of nanochannels [193].

The integration of nanofluidics and optical components in a fully automated system is a key point for transferring laboratory-based technologies into the real market. Advanced techniques are expected to integrate fluidic modulators, light sources and optical detection systems into the same miniaturized chip, as well as facilitate large-scale production with parallel nanochannels.

Concluding remarks and perspectives. Nanoscale optofluidics is an exciting frontier field, which carries the hope that new opportunities will emerge with reducing scales. At the point of application, genomic study is still a hot subject. Its potential in the detection and analysis of exosomes and viruses opens new windows into diagnostics and environment monitoring. The advanced techniques for optical detection and manipulation should be taken advantage of to go deeper on the fundamental research of nanofluidics such as flow characterization and precise control of flow on the nanoscale. Nanoscale optofluidics has the potential to bring breakthrough discoveries, new devices and functions by studying molecule level interactions.

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17. Optofluidic immunoassays

Chia-Chann Shiue and Shih-Kang Fan

National Taiwan University

Relevance of the topic. Integrating and combining optics and microfluidics, optofluidics [2] has been investigated to achieve precise and adaptive photonic components that generate, shape, route, switch and discriminate light for integrated optical systems. With spatial and temporal control of a fluid on the microscale, the optical properties can be altered and monitored; this capability has been developed further for imaging, light routing, biosensors, energy and other purposes. For applications in biological and chemical analysis, various mechanisms involving photonic detection have been studied in integrated optofluidic systems [194], including metallic nanohole array plasmonics, photonic crystals, photonic-crystal fibers, ring resonators, Mach–Zehnder interferometers, and Fabry–Perot cavities to sense the RI, which have been employed as label-free immunosensors. Beyond improving the performance of photonic sensing, the precise and automated manipulations of fluids and suspended particles with microfluidic techniques [195] facilitate assay protocols and enhance biomedical analyses. Among diverse analyses, an optofluidic immunoassay [196] is an essential and common practice in biomedical study and *in vitro* diagnosis (IVD) to analyze a target analyte, for example proteins and small molecules, by exploiting the sensitivity, specificity and affinity of antibody–antigen interactions.

Current status and challenges. Immunoassays are generally classified as homogeneous or heterogeneous. In homogeneous immunoassays, antigens and antibodies are dispersed and interact in solutions, whereas in heterogeneous immunoassays the target antigens or antibodies suspended in the solution are detected with specific antigens or antibodies immobilized on a solid support surface. Figure 28 shows various heterogeneous immunoassays that occur on a solid surface with appropriately immobilized and labeled antigen or antibody molecules that have been developed. In a competitive configuration, the sensing signal is inversely proportional to the amount of target antibody present in the sample solution. In the non-competitive manner of both sandwich and indirect configurations, a detectable signal from the labeled antibody or secondary antibody is proportional to the amount of target antigen. With optical labels, the signal can be detected with colorimetry, fluorescence, photoluminescence, chemiluminescence and electrochemiluminescence methods simply with a photodiode, photomultiplier tube (PMT), charge-coupled device (CCD) or CMOS image sensor. Without labeled molecules, various optical immunoassays were demonstrated, such as surface plasmon resonance, interferometers, ring resonators, and photonic crystals [197]. Other non-optical means were achieved using various techniques, for example electrochemical, piezoelectric (quartz-crystal microbalance and microcantilever), calorimetric methods and with field-effect transistors).

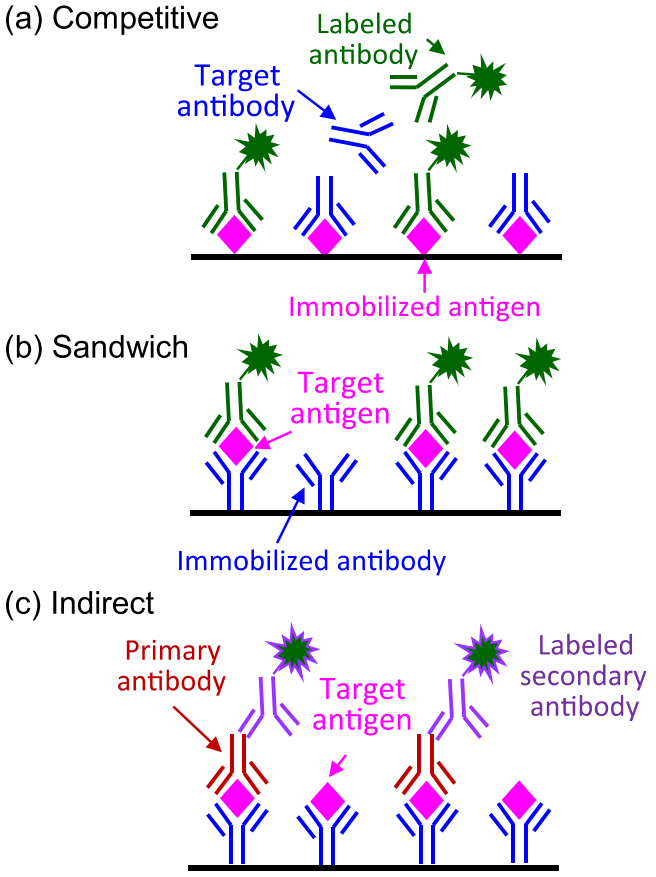

Figure 28. Heterogeneous immunoassay configurations.

Concurrently with advances in antigens/antibodies, labeled molecules of immunoassays and optical methods of detection, microfluidic device driven fluids, liquids or gas, typically along microchannels fabricated on silicon, glass or PDMS, to offer a miniaturized, automatic, rapid and cheap analysis with decreased consumption of reagents. Hatch *et al* investigated a homogeneous immunoassay conducted along T-shaped microchannels by flowing two fluid streams containing antibodies and labeled and target antigens [198]; the diffusion and interaction between the two streams under a laminar-flow regime with a small Reynolds number was monitored using a CCD to analyze the concentration of the target antigen. Although the detection was performed within 1 min, the laminar flow maintained by the continuous flow kept consuming the samples and reagents during the measurement. Depending on the tubing between the pump and chip, the dead volume might waste more sample and reagents.

Heterogeneous immunoassays with antigens or antibodies absorbed physically or immobilized covalently on a microchannel surface have also been studied. To simplify the fabrication of a microchannel, and modification and immobilization of a surface, conjugated microbeads, in particular magnetic particles, have been widely adopted for the development of optofluidic heterogeneous immunoassays that have been applied successfully to clinical samples; to

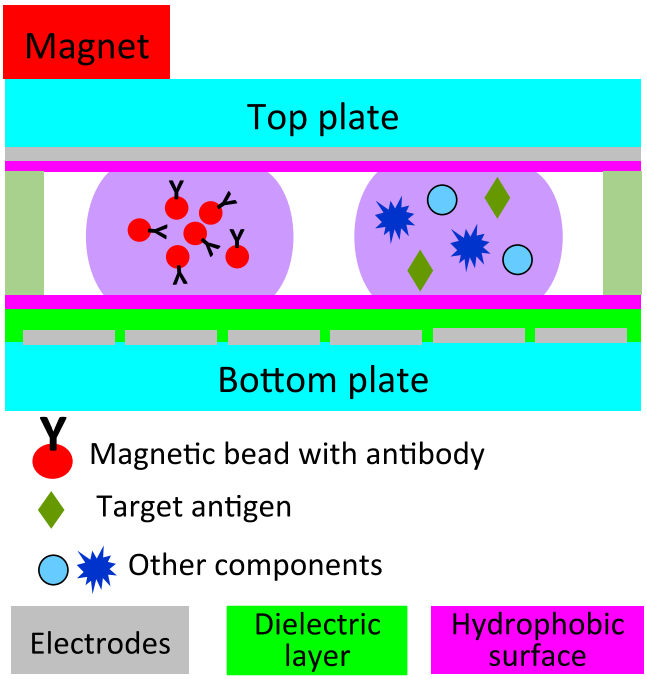

Figure 29. Parallel plate device configuration of a DMF platform performing a magnetic bead-based heterogeneous optofluidic immunoassay with EW driven droplets and precisely metered volumes.

complete the immunoassay, repeated microfluidic procedures, including mixing, incubation, and washing of samples and reagents with precisely metered volumes are necessary but challenging for microfluidic devices with fixed microchannels that are not reconfigurable during operation.

Advances in science and technology to meet challenges. An alternative means to perform optofluidic immunoassays is based on digital microfluidics (DMF) that has demonstrated promising DNA analysis, drug discovery and real-time biomolecular detection with precise, reprogrammable and individual control of multiple droplets. Among techniques of droplet actuation, EW is the most efficient, and has been widely adopted in DMF devices composed of parallel plates free from issues of complicated microchannel fabrication and dead volume. Figure 26 shows how droplets are generated, transported, mixed and split between two plates of glass, silicon or printed circuit board (PCB) on applying electric signals to the electrodes that are covered with a dielectric or hydrophobic layer. For instance, Sista *et al* reported a heterogeneous immunoassay to detect insulin and cytokine interleukin-6 (IL-6) on a DMF platform within 7 min [199]. A droplet of sample containing insulin or IL-6 was first mixed with the reagent droplet containing magnetic beads coated with a primary antibody, a secondary antibody labeled with alkaline phosphate (ALP) and blocking proteins. The antibody–magnetic bead–antigen sandwich complex was then collected and fixed on transporting the droplet near a permanent magnet placed either above or beneath the two plates for the following washing steps. The supernatant liquid was removed with droplet splitting; one part of the split droplet containing unbound components was discarded. The magnetic beads in the remaining droplet were resuspended on mixing with an additional fresh washing buffer droplet. The washing was repeated until all unbound components were removed. The chemiluminescence signal was eventually measured with a PMT on mixing the droplet containing the antibody-magnetic bead-antigen complex with a droplet of CL substrate, Lumigen APS-5. Such a DMF platform capable of automated fluid actuations to conduct multiple assay protocols has been applied to multiplex newborn screening.

With the sandwich heterogeneous immunoassay configuration using magnetic beads, the fluorescence signal from the fluorophore labeled with secondary antibody was enhanced with appropriately collected and gathered magnetic beads (diameter 6 μm) in a shallow fluidic space (height 10 μm) between plates [200]. The volume of the sample was 2.5 nl; the limit of detection was 15 pg ml^{-1} with coefficient of variability 3%. Moreover, without the top plate a sessile droplet typically performing as a liquid lens was applied to focus the fluorescence on the detector and to enhance the detection sensitivity [201]. The detection was performed in a compact and shielded light-proof box ($7 \times 7 \times 7$ cm^3) with all components, achieving a sensitive, cost-effective and portable optofluidic bioassay system.

Concluding remarks and perspectives. To date, various microchannel-based and droplet-based digital microfluidic techniques have been demonstrated for homogeneous and heterogeneous optofluidic immunoassays. Label-free photonic sensors generally require advanced fabrication techniques and are less suitable for the application of disposable IVD medical devices. DMF with modified magnetic beads to perform bioassays with individually driven droplets simplifies the fabrication and the antigen or antibody immobilization to realize optofluidic immunoassays for clinical diagnostics, enabling the performance of robust fluidic procedures with reliable droplet and magnetic-bead manipulation without volume variation, bead loss or biofouling. The integration of detectors in the DMF platform [202] to eliminate optical alignment is essential to the development of disposable cartridges for IVD.

18. Optofluidics and point-of-need diagnostics for precision medicine and global health

David Erickson

Cornell University

Relevance of the topic

Point-of-care and point-of-need diagnostics. Point-of-care diagnostics formally refers to the ability to identify the nature of an illness or health concern at the location where care is provided. From the engineer's or technologist's point of view, this typically concerns the development of devices or systems that can provide actionable information to a healthcare provider within a timeframe compatible with the likely patient–provider interaction. A variation on this, which we will focus on here, is point-of-need diagnostics. This differs from point-of-care in an important way, in that the best place for administering the diagnostic may not be where patient care is provided. There are numerous examples of this, including: mobile breast cancer screening programs, precision medicine where time tracking of health markers requires both sample collection and analysis to be done in a personalized unit, and infectious diseases monitoring where cases can originate far away from a proper healthcare site and preliminary screening can help ensure referral is done properly. The technologies that enable these diagnostics can exploit chemical, biological or physical measurements of health, be single-point measurement or involve monitoring over time, and address acute or chronic health issues.

Optics and optofluidics for point-of-care/need diagnostics. Since the early days of what we now call microfluidics, roughly the mid-1990s, point-of-care/need diagnostics were seen as one of the key applications. The concept was, and still is, simple; in the same way that modern integrated electronics enabled mobile, portable computers, microfluidics was going to put the power of a traditional analytical lab into a physician's office through the lab-on-a-chip.

When optofluidics first emerged as a formal term roughly ten years later, in the mid-2000s, diagnostics were still viewed as one of the major drivers. Indeed the seminal paper in the field by Psaltis *et al* [2] lists an anticipated market demand for the development of 'portable devices for environmental monitoring, medical diagnostics and chemical-weapon detection' as one of the key applications optofluidics was trying to realize.

The need for the 'fluidics' part of optofluidics in diagnostics is fairly obvious in that if one is taking, processing, and analyzing a biological sample (e.g. blood, sweat, saliva, biopsy, etc) there is likely to be at least some form of liquid-based sample processing involved. Additionally, optical methods, like florescence, have been used in lab-on-a-chip devices for many years prior to the advent of the term optofluidics. As such one could view the goal of the field in this area over the last dozen years as examining ways in which one might be able to: (1) use light and fluidics synergistically or (2) take advantage of advanced photonic/optical methods in ways that lead to better cost, reliability, deployment, accuracy or outcome advantages than would otherwise be possible.

Current status and challenges. There are numerous excellent examples of optofluidic diagnostic technology including recent work on diagnostics for concussion recovery [203], rapid single virus detection [204], and minimally invasive malaria diagnostics [205]. Limitations on the length of this article prevent providing a comprehensive review, so rather I will provide two case studies in involving precision medicine and global health. In the final subsection, I will point to some needed technological advancements that could have significant impact on the field.

Case study I: optofluidics for precision medicine. The US National Institutes of Health defines precision medicine as 'an emerging approach for disease treatment and prevention that takes into account individual variability in environment, lifestyle and genes for each person'. There are a number of ways in which optofluidics can play a number of roles in precision medicine with the most relevant to this article being to enable the point-of-need diagnostics that can enable the better, easier, and more accurate tracking of biomarkers at the individual level. Being able to fuse biomarker data with changes in behavior or environment is key to understanding how individuals react.

Given the current worldwide near-ubiquity of mobile technology, it is reasonable to pair these types of diagnostics with mobile technologies, such as mobile phones and tablets [206]. As one example, our group and the Mehta group in Nutritional Sciences at Cornell have been working on coupling optofluidics-based micronutrient level tracking diagnostics with mobile phones. Our 'NutriPhone', see figure 30(A), uses a specially developed paper microfluidics card coupled with an optofluidic reader system to enable individuals to easily track how changes in diet and lifestyle can affect circulating levels of different micronutrients. Monitoring nutritional outcomes is a particularly good candidate for personalized medicine because responses to changes in diet and lifestyle can be very individualized and often difficult to quantify the magnitude of changes *a priori*. To date, we have developed tests for vitamin D (25-OH-D_3) [207], iron (ferritin), vitamin B_{12} [208], and others.

Case study II: optofluidics for global health. Formally 'global health' refers to the health of everyone on the planet, independent of country of origin. When used in the context of technology development, however, it often refers to systems for use in so-called 'limited resource settings' or places where access to reliable infrastructure ideally required to operate the technology may not be available. In 2012, our group published a paper broadly describing the optofluidic opportunities within global health [209], including point-of-care diagnostics.

Numerous agencies and foundations have published criteria for what is required for a successful point-of-need diagnostic in limited resource settings. These requirements

Figure 30. (A) NutriPhone, personalized nutrition tracking, and (b) KS-Detect, SolarThermal PCR.

vary slightly depending on the source but most include requirements for devices to be: operable in locations with limited or no medical infrastructure, simple to operate and interpret, and able to provide actionable information with fidelity similar to what one could expect with the state-of-the-art test. Coupled with these technical requirements, there is an equally important need that the technology be coupled with a sustainable business/deployment model. This means that the system and any required consumables need to be able to be manufactured, serviced, distributed, and operated in a way that is compatible with the existing healthcare system. Since these requirements (including, but not limited to, price-point) vary from location to location, it is key to involve local expertise as early on in the process as possible.

An example of how we have used optofluidics to address some of these concerns is our KS-Detect system [210, 211] (see figure 30(B)). The technology focuses solar or other light through a shadow mask onto an optically absorbing microfluidic chip. The system is designed such that the projection of this light onto the chip creates a radial thermal pattern in steps whose temperatures match those required to conduct a PCR reaction. The channels on the microfluidic chip are designed so as to move the sample through the static temperature zones with the proper residence time. This technique, coupled with the use of a mobile phone to read and interpret the results, allows the device to be operated under conditions where power may not be available or reliable. This enables one to have more flexibility in performing the diagnosis at the true point-of-need rather than just the point-of-care. As can be seen in the above-mentioned papers, we have demonstrated the technology for the diagnosis of Kaposi's sarcoma.

Advances in science and technology to meet challenges. There are numerous opportunities for optofluidic technology in point-of-need diagnostics. The key technological and scientific advancements that will likely be required include: further and more seamless integration of optofluidic systems with mobile technology, reduction or elimination of the need for peripheral equipment needed to operate technology (e.g. tunable lasers), reduced material and fabrication costs for consumables to hit consumer-level or internationally acceptable price points, and an increased emphasis on the skill set of the end user resulting in lower complexity, easier to use devices and systems.

Concluding remarks and perspectives. A transformative change would be the ability to use optofluidic or optical/spectroscopic methods to reduce the invasiveness of diagnostics. This has proven difficult in the past, due to challenges with accuracy and high per-unit cost of the resulting instrumentation and thus technologies that can address these two challenges would be highly desirable.

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The author declares the following competing financial interests(s): DE has equity interest in VitaMe Technologies Inc. which is commercializing micronutrient diagnostic technology similar to that described herein.

19. Optofluidics in energy

David Sinton

University of Toronto

Relevance of the topic. With broad economic and environmental impacts, the energy sector is at the forefront of the minds of policy makers, technology developers and the general public. The dominant demand for global energy usage is in fluids (oil and gas) and the ultimate source of global energy is light (solar radiation). The tight integration of light and fluids via optofluidics thus presents various opportunities for current and future energy technologies. The small scale of traditional optofluidics technologies, however, is in stark contrast with the scale required for impact in the energy sector. Thus, as with microfluidics for energy applications, the challenge here is to develop optofluidic technologies that can leverage the integration of light and fluids fundamental to the area, while enabling scaling of the technology. The two established routes to scale are: leveraging already-scaled materials such as fiber optics (energy or energy conversion is the product), and employing optofluidics to inform processes at larger scales (information is the product).

An early vision for the field of optofluidics for energy was mapped in our perspective paper five years ago [212]. My focus here is the recent developments in these areas, newly emerging energy applications and the roadmap ahead to broader application of optofluidics in energy. Specifically, I first outline recent progress and a roadmap for optofluidics in photosynthetic and photocatalytic energy conversion—examples of tight integration of fluids and light—followed by emerging and hybrid approaches that leverage elements of the optofluidics toolbox for application in energy. In this short overview, I unfortunately cannot do justice to other exciting developments in this arena such as liquid lensing for solar energy collection, photothermochemical conversion, and solar steam generation, to name a few.

Current status and challenges. Photosynthesis is nature's method of converting light energy into chemical energy, and is well suited to optofluidics [212, 213]. Most of the challenges in this approach stem from the fundamental low efficiency of the photosynthetic process, effectively the overhead associated with biological machinery in series with losses of light energy at both the reactor scale and the organism scale. Although these challenges can be overcome to varying degrees with engineering, implementation increases cost in an already-strained sector [214]. Fuel production represents the largest volume potential market for photobioreactor products, but it is also provides the lowest margin. The low price of conventional hydrocarbons made biofuels a challenging market, and the economics became bleaker with the advent of unconventional oil and gas made possible by the combination of horizontal drilling and hydraulic fracturing in shale and tight oil reservoirs. As a result, biofuel producers and technology developers are focusing on niche high-value chemical outputs, such as neutraceuticals, in which optofluidic photobioreactors can better compete. Optofluidics has also enabled very controlled multiplexed experiments to determine optimal conditions for photosynthetic energy conversion in operations of all scales [215, 216].

Converting light energy into chemical energy using photocatalysts is an alternative to the biological approach. Integrating photocatalysts and fluid in optofluidic systems presents several attractive features, most notably enhanced transport of reactants to photocatalysts and products away, as well as leveraging the excellent control associated with optofluidics systems to ensure conditions throughout [217]. Analogous to the photosynthetic energy conversion (above), however, there are challenges of both cost and efficiency for optofluidics photocatalysis. Specifically, there is a mismatch between the goals of light collection (favoring large areas) and conversion (favoring dense reactors). This mismatch can be technologically addressed by separately collecting and concentrating solar energy and feeding light to, and distributing within, a dense reactor system using waveguides. There are associated challenges of minimizing losses and costs in this waveguiding approach, but there are exciting developments in this area.

Separating light collection and reactor functions by using conventional solar photovoltaics to collect and convert solar energy into electrical energy is another approach [218]. The result is not the tight integration of light and fluids germane to optofluidics, but the advantages are worth considering. Most notably, this approach leverages the massive scaling and cost reductions already achieved in solar photovoltaics, as well as the ease with which electrical output can be controlled and distributed. The fluids-half of the challenge is then in designing effective electrochemical reactors for which there is a long history from fuel cells and electrolyzers. Electrocatalytic conversion of CO_2 into (or *back* into) useful chemical feedstocks using solar-generated renewable electrons is an attractive approach that is gaining traction, particularly with advancements in both nanostructure electrocatalysts [219] and gas diffusion electrode cell designs [220].

Advances in science and technology to meet challenges. In the area of optofluidics photobioreactors, a promising approach is unifying light and fluid delivery within single materials, such as light-guiding gas-permeable membranes illustrated in figure 31 [221]. This approach removes the necessity for separate light/fluid infrastructure and associated costs. The next logical step in this progression of simplification might be to integrate immobilized photosynthetic organisms within such materials. Challenges of this approach are many, particularly as the one material must serve many separate functions, but there is an opportunity for cost reductions through the integration. A less exotic and more accessible approach would be to improve the optical function of existing medium- or large-scale facilities by, for instance, providing excitation light that is off-absorption-peak. Providing light that matches the absorption peak of the photosynthetic organism is a

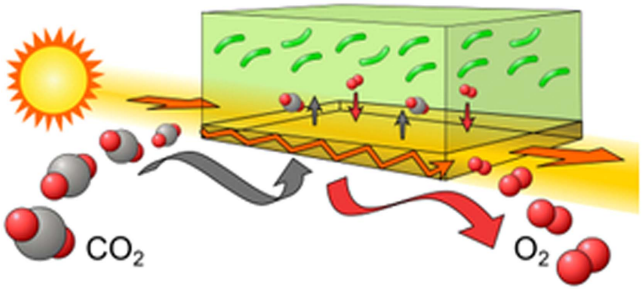

Figure 31. Breathable waveguides for combined light and fluid delivery to photosynthetic micro-organisms. Reprinted from [221], Copyright 2016, with permission from Elsevier.

common practice, which is grounded in the idea of feeding the organism the light it is tuned to absorb best. The result, however, is rapid absorption of light by the culture, and very poor light distribution, resulting in poor light use efficiencies overall. The penetration of various wavelengths into identical cultures is shown in figure 32. Illuminating cultures with wavelengths that are off-peak-absorbance, can allow much deeper penetration of the exciting light while maintaining photosynthetic productivity and boosting overall performance [222].

Photocatalytic reactors have made impressive advances over the last few years—both in the context of wastewater treatment and light-to-chemical energy conversion. The photocatalysis community has the challenge of engineering catalysts that efficiently manage both light utilization and chemical conversion while minimizing electron–hole recombination, bandgap, activity loss, toxicity and material cost. Despite these fundamental challenges there is much progress and reason for optimism in this area. Analogous to photosynthesis, there are also roles for optofluidics systems in informing both the synthesis of photocatalysts and how they might be used in larger systems, such as reactors with disperse photocatalytic nanoparticles [222].

Hybrid methods that separate solar-PV and electrocatalytic conversion have challenges to address particularly in the electrochemical reactions and reactors. Electrocatalytically upgrading CO_2 is energy intensive and advances are needed in catalysts and cells to increase Faradaic efficiency for intended products, increase current densities, and reduce the cost of catalysts. A challenge shared with photocatalysis is the production of long-chain hydrocarbons from CO_2 (something photosynthesis accomplishes). Although these long-chain liquid hydrocarbons are the preferred energy currency worldwide, producing them from CO_2 is a veritable, multistep photo/electrochemical challenge. Particularly with the increase in activity in this area of late, there is great potential for rapid advances.

The role of optofluidic systems to study and inform on photosynthetic and photocatatlyic processes is noted above. We see additional, broader opportunities for this approach here. Specifically, these same methods are very well suited to quantify the environmental impacts of anthropogenic CO_2 emissions on organisms and microcosms, in combination with light variables and/or a variety of local stressors. One can imagine independently controlled optofluidic reactors as multiplexed microgreenhouses, or micro-ecosystems, that can provide the massive amount of data necessitated by fundamentally complex, multivariable coupled interactions of the natural environment.

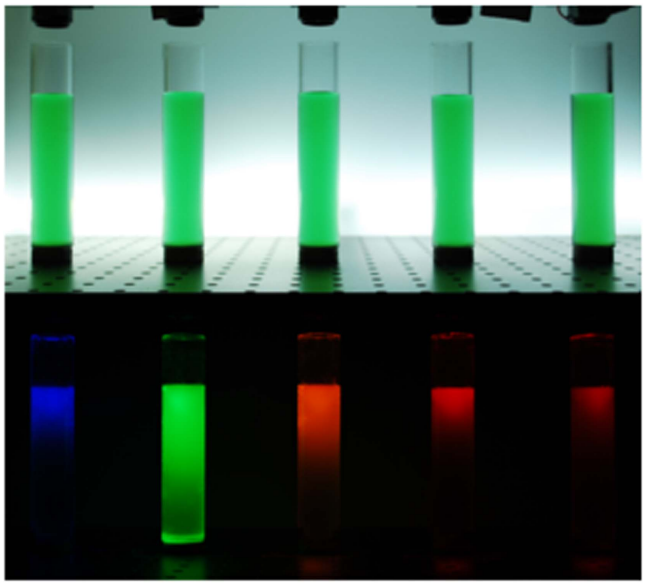

Figure 32. Demonstration of wavelength-dependant light penetration in dense cultures. The productivity of green light was ~4× that of red due to better light distribution of the weaker absorbed wavelength. [222] John Wiley & Sons. © 2017 Wiley Periodicals, Inc.

Concluding remarks and perspectives. A major barrier to wider application of renewable energy is the lack of storage. The optofluidic approaches outlined here aim to overcome this barrier by converting—directly or in hybrid—our most plentiful energy source, solar energy, into our most demanded energy form, liquid hydrocarbons. Converting these concepts and demonstrations into useful, scalable approaches will take a sustained effort from the science and engineering communities, as well as funding from policy makers and venture capital. This short overview covered some recent developments and opportunities in photosynthetic, photocatalytic and hybrid solar-PV-electrocatalysis approaches. All three of these approaches have shared challenges of cost and present opportunities for both fundamental science and engineering advancement, the most promising of which will keep cost minimization as the guiding and motivating principle.

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