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REVIEW ARTICLE

Lab on a chip technologies for algae detection: a review

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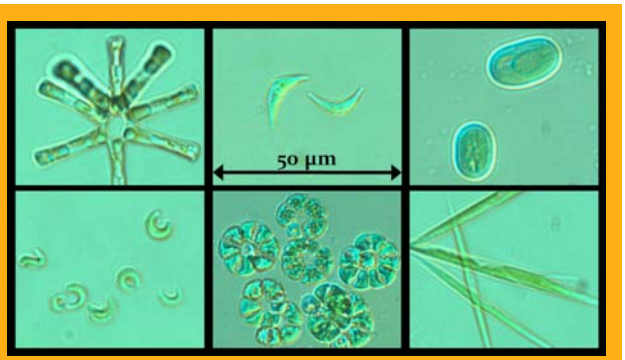
Over the last few decades, lab on a chip technologies have emerged as powerful tools for high-accuracy diagnosis with minute quantities of liquid and as tools for exploring cell properties in general. In this paper, we present a review of the current status of this technology in the context of algae detection and monitoring. We start with an overview of the detection methods currently used for algae monitoring, followed by a review of lab on a chip devices for algae detection and classification, and then discuss a case study based on our own research activities. We conclude with a discussion on future challenges and motivations for algae-oriented lab on a chip technologies.



Primary producers of oxygen with a wide range of shapes and sizes, algae are interesting and important organisms for detection and classification with lab-on-a-chip technologies.

1. Introduction

The research and development of lab on a chip devices – characterized by the miniaturization onto the microscale of laboratory equipment or functions – has grown rapidly over the past few decades. This growth was driven by a desire to mimic and indeed take advantage of the scalability of mass parallel microfabrication technology, with the goals of making small-scale, low-cost, portable devices [1].

Such lab on a chip devices typically contain some form of fluid transport, commonly in channels with cross-sectional lengths on the scale of tens or hundreds of micrometers, and with built-in functionalities including biological, optical, mechanical, electrical, or chemical-based sensing and actuation. Interested readers are referred to some of the many related reviews on, for example, optofluidics [2], on-chip cell handling [3], on-chip flow cytometry [4], and system integration and requirements [5, 6].

Here, we present an overview of the intersection of the lab on a chip field with the identification and classification of algae.

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1.1 Algae

Algae is a broad term, encompassing organisms from the micro- to the macro-scale. It is not itself a formal taxonomical term but rather a general classification of organisms which, though not necessarily related, share some morphological and ecological traits. In this paper, we are using the term "algae" to refer to submillimeter phytoplankton for simplicity. The term phytoplankton specifies organisms which are drifting in water, and which use light or energy from chemical reactions to convert inorganics into organic compounds, through photosynthesis or chemosynthesis. Algae are distinct from plants; they lack specialized tissue forming roots, leaves, stems, and vascular tissue, and their reproductive systems and life cycles are typically simpler.

Algae are found in a wide variety of water environments: in fresh or salt water, and over a broad range of values for pH, temperature, turbidity, oxygen levels, and carbon dioxide levels. Since algae get energy through photosynthesis, they are heavily affected by light conditions. Algae can be roughly grouped by their color – brown, red, golden, green, purple – which is related to the pigments they possess. Different colours have prominence in different geographic regions or environments, as certain species are more well-adapted to certain levels or wavelengths of light.

All algae contain chlorophyll *a*, which enables the conversion of carbon dioxide, water, and light into oxygen and carbohydrates. Algae also contain accessory pigments which work in conjunction with chlorophyll *a* to widen the range of wavelengths which can be used by the algae, as well as protective pigments which protect from photodamage [7]. These pigments – which can include carotenoids, xanthophylls, and the fluorescent phycobilins – have different wavelengths of peak light absorption, ranging from roughly 400 nm through 700 nm (see Table 2). The different phyta of algae possess different typical combinations of these pigments, lending them the colours which are often the basis for the informal names of the phyta (for example, "green algae" for the chlorophyta, "brown algae" for diatoms, and "red algae" for the rhodophyta) [8].

Algae can exist alone or as aggregates, with or without flagella for motion control. Algae surfaces feature a simple cell membrane, made of a lipid bilayer 7–8 nm thick. Additional structures and materials may also be present [8].

The density of algae is only slightly higher than that of fresh or sea water, generally between 1.03 and 1.10 g/cc. Algae need to control their depth to access light and to move to regions of undepleted nutrients. Some algae can control motion with flagella, while some others regulate their position by controlling their buoyancy with gas-filled structures inside the cell or by producing liquids with lower density than water [8].

It is evident that algae are a widely diverse set of organisms, and thus pose interesting challenges in their identification as well as interesting opportunities for their use.

1.2 Why algae detection?

Algae are primary producers of oxygen, and so are important organisms for understanding and monitoring the environment. While thousands of species of algae exist under many different combinations of local environmental conditions, local areas are typically dominated by a handful of species. The total biomass of algae and the relative concentrations of the dominant species vary in response to changing conditions, including the concentration of nutrients, the temperature, light conditions, and intentional or unintentional interventions by humans or other life forms. The dynamics of the species populations in a given body of water is thus a useful biomarker for changes occurring in the water and surrounding area [9]. In fact, the sensitivity of algae to their surrounding have led to them being touted as candidate organisms for biosensors [10].

Harmful algae blooms can occur when the local environment changes, particularly when the limiting nutrient (usually phosphorus or nitrogen) rapidly becomes available in higher supply. Such blooms can be particularly harmful if they consist of toxin-producing algae, such as the cyanobacteria (or blue-green algae). These cyanotoxins have been known to cause mass animal mortalities, and can have neurotoxic or hepatoxic effects in humans [11–14]. Even a bloom of non-toxin-producing algae can have serious consequences, as the accumulated biomass of the bloom can cause local oxygen depletion or habitat destruction by blocking light to submerged vegetation [15].

The formation of algae blooms are complex events, involving the interplay of many variables; better methods of monitoring the dynamics of the species and the local environment would help to detect, understand, and respond to such events [15–17]; as described by Anderson et al.,

"All too frequently, public perception of whether nutrient over-enrichment has reached undesirable levels has been based on acute, obvious or easily measured symptoms, such as high biomass algal blooms, massive fish kills, and oxygen deficits. Because of this focus, a broad array of indirect, chronic, often-subtle but serious impacts of nutrient pollution on aquatic ecosystems remain underemphasized and, in some cases, poorly understood." (from [15])

Further interest in algae monitoring comes from the need to inform and enforce government regulations, and from areas where algae are used for other

purposes. For example, a European Parliament directive requires the monitoring of recreational water quality, which can include the use of algae and cyanobacteria as markers thereof [18]. Concerns over the introduction of foreign algae species by the dumping of ship ballast water has also prompted the need for fast-response, portable algae species monitoring [19]. Algae have also been proposed and studied as a fuel source; farming algae for this purpose may also require an accurate understanding and monitoring of the species distribution.

1.3 Overview of this review

In this paper, we present an overview of the intersection of the lab on a chip (LOC) field with the identification and classification of phytoplankton. We first present an overview of the existing methodologies used for phytoplankton detection, focusing primarily on species identification at the single-cell level. Next, we present a review of the cases in which LOC technologies have been used to study phytoplankton; we concentrate primarily on species identification, but also briefly touch upon other applications. Most of these chip-based methods for identification use approaches or technologies which have also been applied to LOC problems other than algae detection. We illustrate this review with an example of a LOC device for algae detection, which relies on a novel laser processing technique. Finally, we conclude by discussing the challenges and perspectives for specialized LOC for algae.

2. Traditional algae identification methods

To illustrate which characteristics of algae have successfully been used for identification, and to find some inspiration for designing LOC technologies for algae detection, we provide an overview of existing macroscopic methods for algae classification. A summary of these techniques is given in Table 1.

Table 1 Overview of some traditional methods of algae classification.

Type	Method	Examples/ details
optical imaging	light microscopy, image recognition,	[20] [21–24]
spectroscopic (optical)	Raman spectroscopy, Fourier transform spectroscopy	[25–28]
fluorescence	flow cytometry	[29–37]
field volume measurements	satellite imaging, light scattering	[41, 42] [43]

2.1 Laboratory-based identification processes

The most common traditional and current method of single-cell algae identification is through light microscopy. For field monitoring, this involves collecting a sample, bringing it to the lab, fixing the cells, storing the sample, and then later imaging the sample to manually count and identify the species present. While this is a reliable method, it is too time-consuming to use for rapid monitoring [8].

Algae identification has also been attempted by use of image recognition methods. The majority of the work has been on stationary specimens on a microscope [21–24]. More recently, this has been attempted for moving algae in a stream of water [23]. The approach faces several challenges – large amounts of data are collected, and must be compared quickly against large libraries of shapes in computationally-heavy processes. For continuous monitoring of a moving algae sample, a fast and high-resolution camera with sufficient lighting is needed to obtain good images.

Pigments in algae have been used to distinguish between species or between different lines of the same species, as well as for characterization and study of algae. This can be done in-situ, looking at the pigments inside an intact cell (for instance, with Raman spectroscopy), or in bulk, after lysing a sample of cells, typically with a chromatography and mass spectrometry approach. Measuring the non-fluorescent pigments (such as the carotenoids) for algae classification offers more data than purely auto-fluorescent methods such as flow cytometry, but at the cost of a significantly slower and more complicated system. Raman spectroscopy, for example, has only been attempted a few times as a classification method, and on fairly small datasets [25, 26]. More common applications of Raman spectroscopy to algae are for compositional analysis or characterization of a fixed, concentrated sample of algae (for example, [27, 28]). An alternative spectroscopic method, Fourier Transform Infrared (FTIR) spectroscopy, was used to distinguish two very similar diatoms which are very difficult to differentiate by microscopy [38]; FTIR has also been used for classifying cyanobacteria [39].

In the past few decades, much work has been done on automating the task of algae classification. The most common method explored for the automated identification of individual algae is flow cytometry, which uses the autofluorescence of algae pigments to identify species. In flow cytometry, particles

are hydrodynamically focused into a narrow stream, passed in front of one or more interrogating light sources – typically a laser at a wavelength which will excite fluorescence in the algae – and the scattered and excited fluoresced light are measured. These light signatures are collected form a large number of samples and used to form clusters representing the different groups present within the sample.

The most common flow cytometric approach for algae is to excite fluorescence at a single wavelength and measure the fluorescence emission at multiple wavelengths (for example, [29–32]), although excitation at multiple wavelengths with the emission measured only at one has also been demonstrated for species classification [33–37].

2.2 *Field-deployable identification technologies*

The flow cytometry approach has been used for the few commercially available submersible algae monitoring devices. The submersible FlowCAM system acquires fluorescence measurements and photographs of particles in the size range 10–600 μm at a throughput rate of up to 3 mL/min. [40]. Designed to be deployed from – and tethered to – a fixed object, it requires 67 W power for normal operation and weighs 48 kg, with a volume of approximately 0.25 m^3. The fairly similar Cytobuoy system similarly provides a cluster analysis based on *in-situ* measurements of scattering and fluorescence of particles in water, with an option for triggering selective image capture as well [33, 34]. Compared with the typical performance of lab on a chip technology, these systems offer significantly higher throughput rate, although LOC devices may offer alternative benefits in terms of portability and cost.

Lastly, the identification of algae blooms and quantification of average algae biomass *in situ* is possible with satellite imaging and by measuring the absorption or scattering of light underwater. Both techniques can provide information about a large volume quickly, but at the cost of specificity, with satellite data typically providing an average measurement of the chlorophyll loading of water, or a breakdown by taxa but not at the species level [41, 42]. Scattering or absorption measurements can offer a measurement of the total amount of chlorophyll, but are heavily dependent on the type of algae present without offering usable species-wise specificity [43].

3. Algae on lab on a chip devices

The use and study of algae in microdevices is spread over several fields and applications, and is not restricted exclusively to detection. In Figure 1, we present a general overview of the uses of algae in microdevices, classifying the non-detection applications into two categories: the growth and manipulation of algae on-chip, and the on-chip use of algae to achieve some other purpose. In some cases, of course, these functionalities may be combined. The first category includes on-chip culturing [44, 45], imaging with an on-chip microscope [46], the manipulation of algae by hydrodynamic tweezers [47], and the lysis of algal cells [48], sometimes combined with a subsequent analysis of cell contents [49]. Work by Zhang et al. [50], for example, focused on detecting algal toxins with a microfluidic chip. The authors point to the current systems of cyanotoxin analysis – primarily through liquid chromatography – as offering a key target for miniaturization and portability improvements.

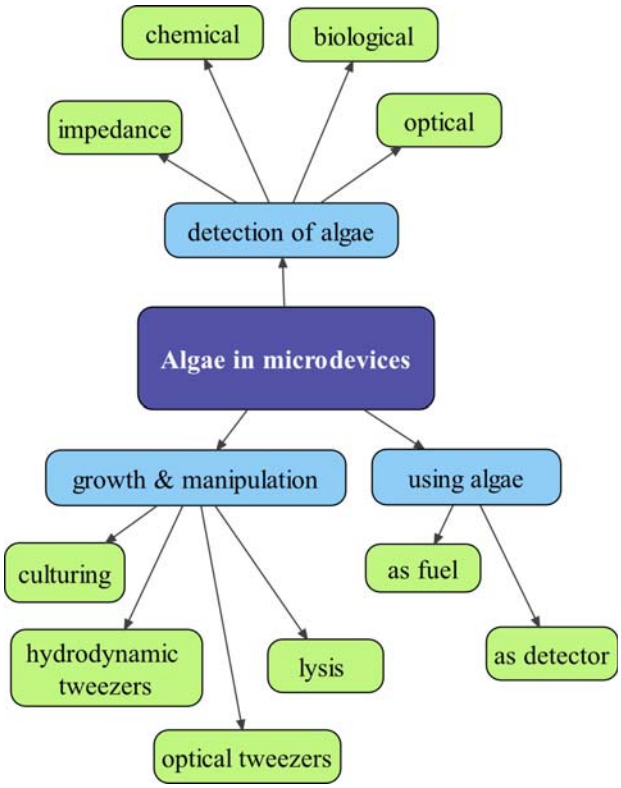


Figure 1 (online color at: www.biophotonics-journal.org) Overview of the use of algae in lab on a chip or MEMS devices. We classify the work in the literature into detection problems (the focus of this review), the growth and manipulation of algal cells, and the use of algae for other purposes.

The second category includes examples such as algae serving as biosensors or fuel. Algae have been used as on-chip biosensors through measuring, for example, optical [51], conductometric [52, 53], and amperometric [54] markers of enzyme inhibition in the algae.

There are many other potential applications of algae as biosensors which have been – or may be – implemented in a lab on a chip device. One overview of some such applications is presented in [10]. Algae have attracted attention as potential fuel sources and this interest has also translated down into the world of microdevices. In this capacity, algae have been used as a power source, for example, in a micro photosynthetic electrochemical cell [55].

For the purpose of this review – on the detection and classification of algae with microdevices – we explore the two most successful approaches demonstrated to date: impedance-based detection and optical detection.

3.1 Impedance-based detection

Impedance spectroscopy is a non-invasive electrical technique for measuring the size and dielectric properties of objects passing over electrodes which have an AC potential. Impedance spectroscopy has been implemented on lab on a chip devices, and applied to differentiate population in a microchannel. Common examples lie in sizing microbeads, or in differentiating between polymer microbeads and red blood cells [56–58]. Benazzi et al. [59] performed impedance spectroscopy on a mixed sample of three phytoplankton species, using a microdevice performing both impedance spectroscopy and fluorescence measurements.

Their device featured microelectrodes on a Pyrex substrate, which was underneath a channel made out of PDMS fabricated using a standard soft lithography fabrication process. The device was tested with three algae: *Isochrysis galbana*, *Synechococcus sp.*, and *Rhodosorus m.*, details of which are in Table 4 in the appendix. The microchannel was 11 μm high and 20 μm wide; since the algae sample stream was only focused in the width direction, the distance between the electrodes and the test item had to be controlled by limiting the channel height. Even so, the smallest of the algae were not detectable with the impedance spectroscopy measurements.

Measurements were performed at two frequencies, a lower one to correspond to cell size, and a higher frequency to measure membrane capacitance. The amplitude of the impedance signals compared against one another and against simultaneous fluorescence measurements (see below) were used to demonstrate the successful clustering of these characteristics by species. The volumetric flow rate is not specified, but from the time of flight values provided it is estimated to be on the order of 7 nL/s total. One variable which may present challenges to the impedance spectroscopy approach is the changing impedance characteristics of a "dirty" sample – one collected in the field – where the electrical properties of the medium and of any surrounding particles would be unknown.

3.2 Optical detection

Optical detection is a promising approach for algae identification, as algae have a variety of shapes and structures and contain coloured pigments necessary for photosynthesis. A common approach to optical identification of algae, especially in macro- and micro-scale flow cytometry approaches, is a cluster analysis in which a number of optical parameters of each cell are measured simultaneously. This is often implemented with the ratio of chlorophyll (CHL) to the algae size (as measured by side-scattering) and/or to some accessory pigments, typically phycoerythrin (PE) or phycocyanin (PC) (see Table 2).

The same group as performed the impedance spectroscopy (seen earlier) also implemented fluorescence-based microflow cytometry on chip. Their first device used free-space, non-integrated optics [59] and showed clear classification of a sample of three mixed algae species, with the results well-aligned to those obtained from a commercial flow cytometer. In a second device, the photosensitive polymer SU-8 was used to define channel walls and grooves for seven optical fibres [60] arranged around the channel. The fibres were used to illuminate the sample and to collect fluoresced and scattered light. This chip was able to simultaneously perform fluorescence and impedance cytometry, and was used to classify particles in a mixed sample containing one species of algae and two different types of microspheres with different sizes and fluorescent dyes.

An improved device was presented by Hashemi et al. [63, 64], featuring a similar optical setup but with flow focusing in two dimensions instead of only one. The flow was focused in the width direction by two inlets on either side of the inlet containing the sample. The height-wise flow focusing was achieved by grooves formed on the top and bottom walls of the channel, which directed some of the sheath fluid from the sides around the top and bottom of the algae-laden flow.

Table 2 Spectral ranges (approximate half-maximum values) of absorption and emission for some common autofluorescent algae pigments. Data compiled from [7, 60–62]

Pigment	Absorption maximum	Emission maximum
Chlorophyll-*a*	400–440 nm	660–690 nm
Chlorophyll-*b*	520–570 nm	640–670 nm
Phycocyanin	500–560 nm	630–670 nm
Phycoerythrin	470–510 nm	550–590 nm

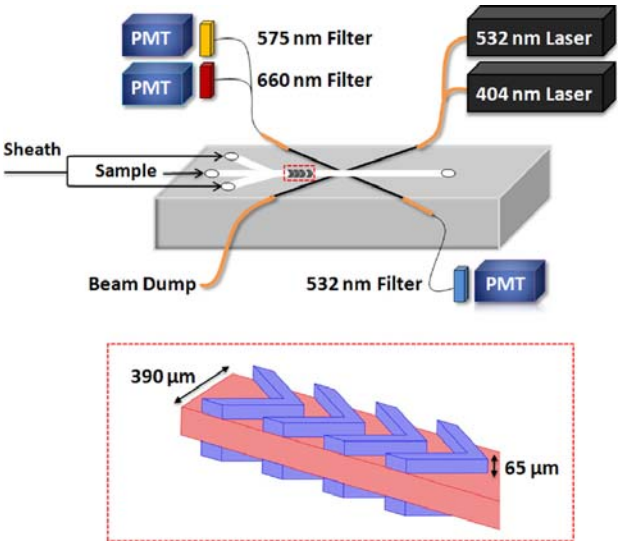


Figure 2 (online color at: www.biophotonics-journal.org) The microflow cytometer for algae identification presented by Hashemi et al. Two wavelengths of light illuminate the sample, while two wavelengths of fluoresced light and the scattered light are measured with photomultiplier tubes. The flow is focused first in-plane with the sheath flow inlets (top picture), and then in the out-of-plane direction by wrapping the sheath flow around the sample flow with grooves in the top and bottom of the channel (bottom picture). (Reprinted with permission from [63]. Copyright Biomicrofluidics, 2011, American Institute of Physics.)

The microflow cytometer featured optical fibers embedded in a PDMS chip, with external optical filters and photomultiplier tubes collecting the scattered and fluoresced light. In their first work, fluorescence was excited at 488 nm and three algae species were tested separately from one another in the microflow cytometer and in a commercial flow cytometer for comparison [64].

Three algae were tested unmixed (*Synechococcus sp.*, *Nitzschia dissipata*, and *Thalasstostra pseudonana*; see Table 4). When overlaid, the scatter plots comparing the various optical parameters show clustering by species, most distinctly in the plot comparing PE to CHL. In comparison to the commercial flow cytometer, similar trends were observed in the PE-CHL comparison for both devices, but the data comparing the CHL to side scatter and PE to side scatter are less consistent between the microflow cytometer and the commercial device. Nevertheless, the system was able to detect the small *Synechococcus* which was not detectable by the impedance spectroscopy based methods described earlier. The device used only one laser to excite the fluorescence, at a wavelength that did not strongly excite chlorophyll-*a*.

Later work (as in the schematic in Figure 2) used two excitation lasers, at 404 nm and 532 nm, with the scatter measured at 532 nm [63]. Four species were used; analysis of the CHL, PE, and side scatter signals showed significantly lower variation in results when the sample flow was more tightly focused within the sheath flow. The lowest flow rate used– providing the best results – was 167 nL/min. The mean values of the three parameters measured showed differentiation between species, although a significant overlap between species due to wide variability could make classification based on these three parameters alone a challenge.

4. Case study: an optofluidics device for algae identification based on algae shapes

Recent progress made in laser-assisted structuring of glass material have opened up new opportunities for lab on a chip devices in general [65–68], for flow cytometers in particular [69], and, consequently, for algae detection – as we will show in this section. Here we present an overview of our own work in this field, as an illustrative example of how this laser-assisted fabrication can enable new approaches to algae classification in lab on a chip devices.

The processing method consists of using a femtosecond laser to modify a piece of glass material throughout its volume so that exposed regions exhibit higher etching selectivity [70] and also, in certain glasses, a higher refractive index [71].

Femtosecond lasers pulses are characterized by ultra-high peak power (gigawatts/mm^2 or even terawatts/mm^2 when focused) leading to an unconventional type of laser-matter interaction. Non-linear phenomena play a significant role, as the modification induced in the material occurs only at the laser focal point.

This characteristic is of technological importance for the fabrication of lab on a chip devices: the material can be modified beneath its surface and anywhere throughout its volume. To form three-dimensional structures combining optical and fluidic functions, a two-step procedure is applied (Figure 3): laser exposure, and chemical etching. More details about the process can be found in other work [72, 73]. As demonstrated in [72], trenches (open-top channels) and waveguides can be written simultaneously by scanning the beam over the specimen. Under certain exposure conditions, the volume locally exposed to the femtosecond laser (the so-called Laser-Affected Zone or LAZ) experiences a slight increase of refractive index (Δn is typically in the order of 10^{-3}) but also an enhanced etching rate (up to 60 times faster than the pristine material).

In the second fabrication step, the part is etched in a low-concentration hydrofluoric acid (HF) bath. Concentrations between 2.5% and 5% are typically used. Etching time depends on pattern sizes and var-

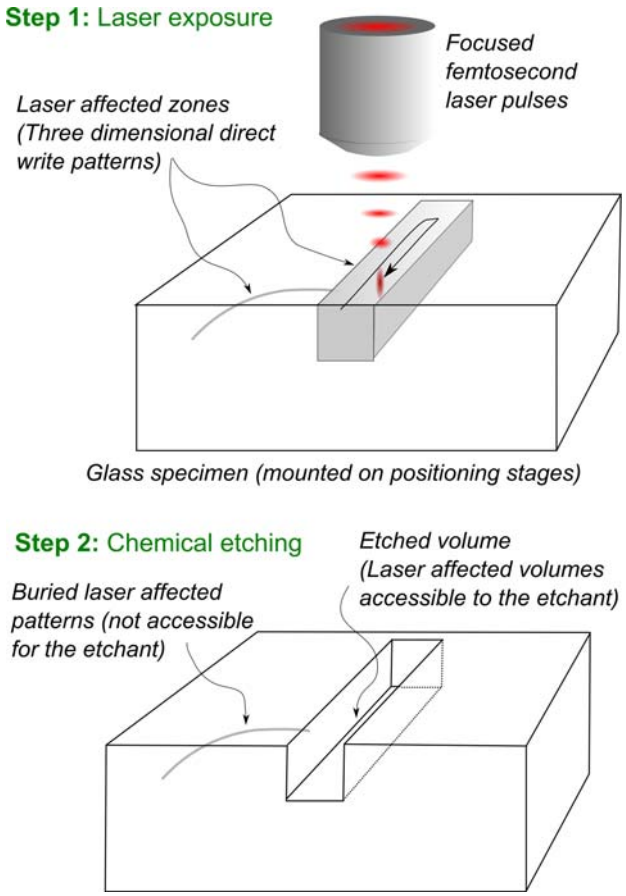


Figure 3 (online color at: www.biophotonics-journal.org) Optofluidics microfabrication using femtosecond lasers exposure followed by chemical etching. Step 1 – The material is exposed to femtosecond laser irradiation. The process is non-linear and exposure can take place anywhere in the material volume. Laser exposed material exhibits a higher etching rate than the pristine material as well as a higher refractive index. Step 2 – The substrate is etched. Laser written patterns that are accessible to the etchant are etched away. Buried patterns are preserved and can be used as optical elements such as waveguides.

ies from one hour to several hours for the deepest structures. Note that the laser polarization has a strong effect on the etching efficiency as reported by Hnatovski et al. [74]. To fabricate an optofluidic device, the etching process has to be carefully control so that the waveguide patterns are preserved [72] and not exposed to the etchant.

This direct-write, maskless and three dimensional manufacturing process is highly suitable for fused silica, a material of particular importance for biochips thanks to its very low to absent fluorescence background, its biocompatibility, and its chemical inertness. We have applied this micromanufacturing technology to algae detection and identification [75–77]. The optofluidic sensing principle is shown in Figure 4.

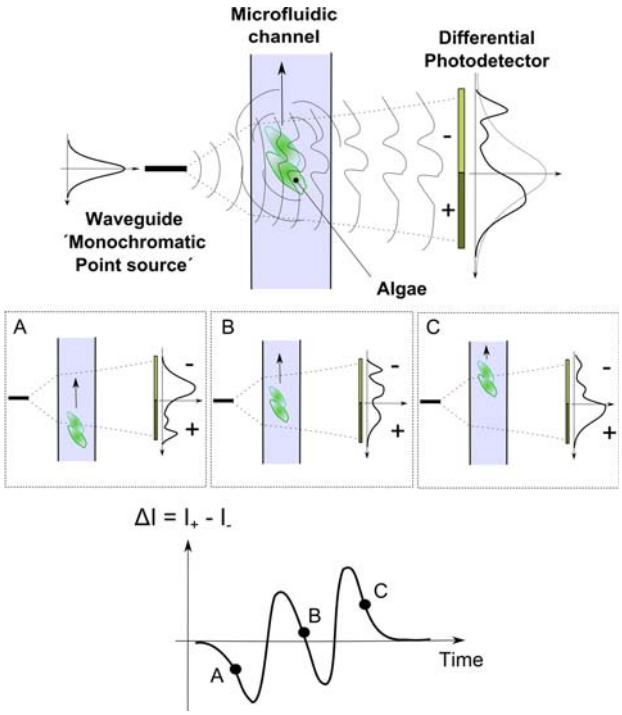
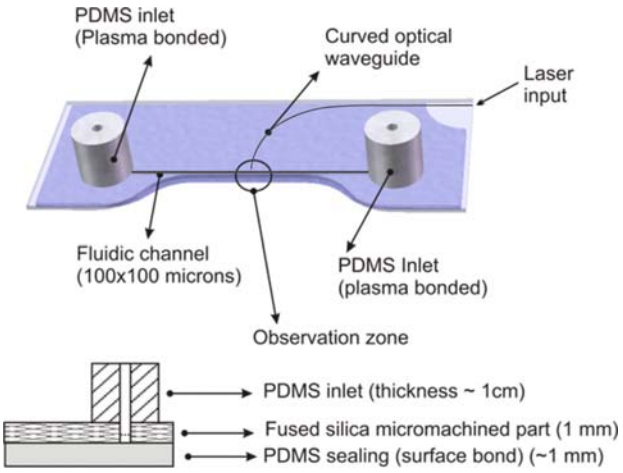


Figure 4 (online color at: www.biophotonics-journal.org) Sensing principle. A point source (consisting of a single mode waveguide guiding waves originating from a coherent, monochromatic laser source) emits a light beam that crosses a fluidic channel. The waveguide is located at a distance so that the beam illuminates a complete section of the channel. The numerical aperture of the waveguide is low enough so that the light beam crossing the channel is quasi-collimated. As an algae (or another object) crosses the light beam, it distorts the beam and modifies its intensity distribution. After crossing the channel, the light beam is projected on a photodetector which measures the difference of light intensity seen by two sectors of the photodetector. In time, the differential measurement produces a characteristic wavelet representative of the object flowing through the light beam.

It relies on the fluctuations in optical intensity induced by an algae crossing a monochromatic coherent light beam while travelling along a microfluidic channel. The time-dependant intensity fluctuations are measured on optical photodetector divided into sectors. The actual device [75] (shown in Figure 5) comprises a curved waveguide with a 8×8 μm cross section and a straight fluidic channel. To prevent uncoupled light from reaching the detector, the waveguide has a 90 degree curve. The 18 mm radius of curvature is dictated by the delta n (6.8×10^{-3}) of the waveguide. The waveguide ends perpendicular to the microchannel, which has a 100×100 μm cross section (see Figure 5). A laser emitting at 1550 nm is used to probe the fluidic channel via the waveguide. At this wavelength the waveguide is single mode. Losses are typically less than 0.1 dB/cm at 1550 nm.



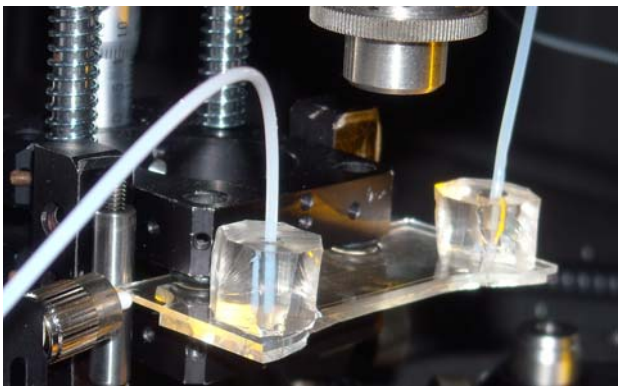


Figure 5 (online color at: www.biophotonics-journal.org) Top: Computer-Assisted-Drawing of the Sensor actual embodiment / Middle: device cross-section / Bottom picture: actual device. The fused silica micromachined substrates contains an open microfluidic channel and an optical waveguide buried in the material. The optical waveguide is bent to prevent improperly guided lights to propagate towards the fluidic channel. PDMS inlets are directly bonded to the glass substrate and used as fluidics interconnects. A PDMS layer is used to seal off the channel and can be removed to clean-up the device.

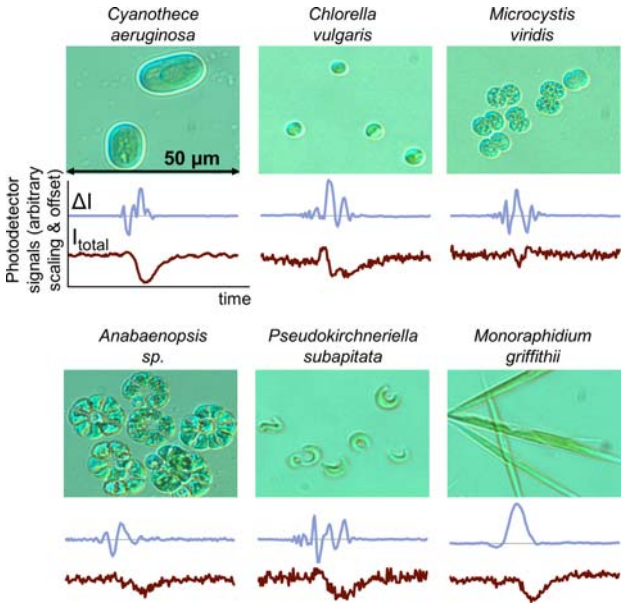


Figure 6 (online color at: www.biophotonics-journal.org) Example of differential intensity (blue top curve) and total intensity photodiode (red bottom curve) signals obtained from six species of algae, with corresponding micrographs, forming the basis of a library for comparison of data obtained by the optofluidic chip. Data collection was collected at 2 kHz for 100 ms; 70 ms of data are shown here, with the scaling on the *y*-axis in arbitrary units.

The waveguide is fabricated in the bulk of the substrate (buried at a depth of 50 µm from the surface) using femtosecond laser pulses that locally increase the refractive index of fused silica as described above. In the same laser writing step as that which defines the waveguide, a volume region defining the microchannel is modified. This region is later preferentially etched away in an HF bath to leave behind a surface with a trench removed (see Figure 3). A thick film for covering the trench is made from PDMS. PDMS fluidic interconnnects are permanently bonded to the substrate using an oxygen plasma process.

We have tested this sensing method on a collection of algae species out of which we built a database of waveforms recorded from the photodetector as various algae pass through the channel. In particular, we demonstrated that these waveforms contain sufficient information and are specific enough to given species to be used as discriminant information to uniquely identify them among other species or detritus [76, 77].

Single-species algal cultures were obtained from the Norwegian Water Research Institute, and diluted until there was typically only one algae cell in the detection region at a time. Figure 6 shows some examples of waveforms acquired for six different algae species. Features of the photodiode signals are extracted and used in a neural network pattern recognition algorithm [78]; the classification performance of this detection method is provided in Table 3. The robustness of the sensing method (i.e. false positives/false negatives versus accurate detection) is measured using images captured simultaneously as algae pass in front of the sensor. Despite the system's apparent simplicity, the information contained in the wavelets is sufficient to be able to correctly classify algae species in a mix of five species with an average accuracy above 75% (Table 3). Further performance improvements may be obtained by changes in the device design: for example, implementing flow focusing would help prevent the simultaneous incidences of multiple algae, and tailoring the channel size to a

Table 3 Results (confusion matrix) of neural network classification of five mixed species of algae in the optofluidic glass microdevice; correctly classified samples are in boldface on the diagonal. The species are S1: *Cyanothece aeruginosa*, S2: *Chlorella vulgaris*, S3: *Microcystis viridis*, S4: *Anabaenopsis sp.*, S5: *Monoraphidium griffithii*

		Predicted class				
		S1	*S2*	*S3*	*S4*	*S5*
Actual class	*S1*	**102**	0	0	4	2
	S2	0	**54**	8	0	10
	S3	0	34	**83**	10	5
	S4	3	0	2	**88**	2
	S5	1	18	6	7	**70**

particular target algae size range may further improve the signal to noise ratio.

In summary, lab on a chip approaches to algae identification have been demonstrated using photolithography-fabricated devices for fluorescence-based flow cytometry and impedance spectroscopy, and with a femtosecond laser fabricated device for laser illumination of algae with a two-pixel photodetector. The flow cytometric approach takes advantage of the autofluorescence of the pigments in algae, while the last method, presented in this case study, relies on the shape and structural differences in algae.

5. Discussion and challenges

Considering the importance of measuring the impact of human activities on our environment, there is a growing need for rapid, field-deployable and automated methods for monitoring algae species. Lab on a chip technologies offer substantial advantages for algae monitoring compared to classical large scale methods, among which are:

- The potential for a dedicated mass-producible instrument that can be field-deployable and/or used as sensors in water surveillance networks.
- The possibility for developing highly integrated and compact, miniaturizable instruments.
- The opportunity to analyse single algae cells.
- A higher level of automation in the detection process, which reduces the risk of measurement errors.

For biologists, LOC devices also provide a useful tool for investigating individual species and exploring their behaviour. As an illustration, K. Sugioka et al. demonstrated how a LOC device can be used for understanding the motility of the microorganism *Euglena Gracilis* [66]. In turn, algae studies are also of potential interest for new technological development. Diatoms in particular have fascinating highly organized nanostructures of potential interest for novel photonics band gap material [10, 79, 80].

Although early demonstrations (including ours) have exhibited the potential of LOC technologies for algae detection, there are challenges ahead among which are the use of these methods for high-throughput monitoring of watersheds, and the increased reliability and decreased cost of such devices. Among the most promising LOC technologies demonstrated for this purpose is the use of femtosecond laser processing for fabricating optofluidic components. This approach benefits from a versatile and integrated nature (multiple functionalities such as optics, mechanics and fluidics can fabricated in a monolithic material in a single process step [78]) combined with the outstanding physical properties of the substrate used (fused silica has nearly no self-fluorescence, high optical transparency over a broad spectrum and is chemically inert). The use of miniaturized instruments to detect and identify algae is still at its infancy but has a strong potential to be massively adopted in the near future.

Appendix

Table 4 Algae species used in lab on a chip classification experiments. Note: ESD stands for equivalent spherical diameter.

Species	Phyta	Size	Detection method	Reference
Isochrysis galbana	haptophyta	diameter 3–4.5 μm	impedance, fluorescence	[60]
Synechococcus sp.	cyanophyta	diameter 0.5–2.0 μm		
Rhodosorus m.	rhodophyta	diameter 4.5–7.0 μm		
Synechococcus sp.	cyanophyta	diameter: 0.8–1.5 μm	fluorescent microflow cytometry	[64]
Nitzschia dissipata	bacillariophyta	length: 12–85 μm width: 3–6 μm		
Thalasstostra pseudonana		length: 8–32 μm width: 4–14 μm		

Table 4 (continued)

Species	Phyta	Size	Detection method	Reference
Synechococcus sp.	cyanophyta	diameter: 0.8–1.5 μm	fluorescent microflow cytometry	[63]
Karenia brevis	dinophyta	diameter: 20–40 μm		
Alexandrium	dinophyta	length: 20–48 μm width: 18–32 μm		
Pseudo-nitzchia	bacillariophyta	length: 40–85 μm width: 2–4 μm		
Cyanothece aeruginosa	cyanophyta	length: 12–21 μm width: 9–14 μm	laser illumination for shape measurement	[77]
Chlorella vulgaris	chlorophyta	diameter: 4–8 μm		
Microcystis viridis	cyanophyta	length: 6–10 μm width: 4–7 μm		
Anabaenopsis sp.	cyanophyta	ESD: 7–12 μm		
Monoraphidium griffithii	chlorophyta	length: 100–200 μm width: 5–11 μm		

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