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Microfluidic technology for plankton research
Mathias Girault¹, Thomas Beneyton¹, Yolanda del Amo² and Jean-Christophe Baret¹

Abstract
Plankton produces numerous chemical compounds used in cosmetics and functional foods. They also play a key role in the carbon budget on the Earth. In a context of global change, it becomes important to understand the physiological response of these microorganisms to changing environmental conditions. Their adaptations and the response to specific environmental conditions are often restricted to a few active cells or individuals in large populations. Using analytical capabilities at the subnanoliter scale, microfluidic technology has also demonstrated a high potential in biological assays. Here, we review recent advances in microfluidic technologies to overcome the current challenges in high content analysis both at population and the single cell level.

Addresses
¹ Centre de Recherche Paul Pascal, Unité Mixte de Recherche 5031, Université de Bordeaux, Centre National de la Recherche Scientifique, 33600 Pessac, France
² Université de Bordeaux - OASU, UMR CNRS 5805 EPOC (Environnements et Paléoenvironnements Océaniques et Continentaux), Station Marine d’Arcachon, 33120 Arcachon, France

Corresponding author:
Baret, Jean-Christophe (jean-christophe.baret@u-bordeaux.fr)

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Introduction
Algae are located at the base of the trophic web. They support bivalve and fish recruitment rates as well as many other marine organism growth and hence significantly contribute to the local economy in numerous countries [1]. They are also used to produce various high-values chemical compounds such as biopharmaceuticals or cosmetics and therefore have a huge economical impact [2]. Among the algae, the phytoplankton are microscopic organisms which have benefited humankind since the earliest days of life on the planet, by contributing to the change of the atmosphere of the planet in the past. They are still responsible of more than 40% of the inorganic carbon fixation on the Earth, and play their significant role in climate control [3]. Those microorganisms are an important compartment ruling the fluxes of both oxygen and carbon at the world wide scale. However, evidences of substantial environmental changes, as illustrated by the effect of climate warming on surface ocean and on water column stratification, can lead to drastic changes of the plankton diversity and community structure at the surface of the Ocean [4]. The stratification of the Ocean and subsequent faster inorganic nutrient depletion in the surface layers due to phytoplankton uptake are expected to favor small picoplankton growth and microbial dominated food webs at the expense of larger microphytoplankton species and carbon export towards deep layers [5]. Therefore, modifications of biological carbon pump are expected at the global scale leading to important changes of the carbon export in the Ocean. However, some plankton species develop alternative strategies to survive to changing environments. For example, the activation of a set of enzymes to access the organic compounds as well as changes in the motility patterns can be efficient strategies to cope with nutrient limitation or predation.

Within a single population, these strategies are not activated in each cell suggesting a wide diversity in the physiological response of the cell living under the same environments. In addition, from an evolution viewpoint, adaptation to changes in environmental conditions arises from variants having genotypic and phenotypic properties different than the mean of the population. Therefore, a single cell approach unraveling statistically extraordinary behaviour is required to understand how plankton populations adapt to environmental changes. The diversity of these physiological adaptive responses of cells is not easily detected with classical sampling strategies which hide single event in the response of the population. Microfluidic technology has become a key technology to precisely control, manipulate and monitor small volume at the picoliter and nanoliter scales in a microfluidic chip device. The technology enables the miniaturization and parallelization of biochemical assays to achieve single cell level and high-throughput with low cost and time footprint. This miniaturization associated allows microorganism studies in the field in confined environments such as ships or space stations [6].

In this review, we present the recent developments in microfluidics dedicated to plankton research. We focus on how microfluidic platforms address the main challenges of
the field, such as analysis at a low density of organisms in environmental samples, difficulties to cultivate planktons, pre-concentrate, detect and sort them, and on the how analytical microfluidic platforms dedicated to the interactions between plankton and their environment are implemented (swimming speed of plankton, toxicity of phytoplankton, plankton-bacteria interactions and modes of nutrition of plankton).

**Analytical microfluidic platform**

**Culture of plankton in a microfluidic system**

Culturing bacteria or eukaryotic plankton in confined microfluidic chambers or water-in-oil (w/o) droplets offers several advantages over the traditional culturing methods. In particular, small droplets acting as nanoliter batch cultures act as trap for highly motile cells. Fragile cells can also be studied in a microfluidic device by encapsulating living cells in alginate-based hydrogels [7]. On-chip cultures conducted in a small height channel (<50 μm) reduces the culture chamber to a quasi two dimensional space and favours the observation of living plankton using classical microscope. In a continuous flow experiment, microfluidic technology enables the precise and dynamic control of the cellular environment. Dynamic modifications of nutrient conditions are created by a simple pulse of either nutrient-rich or nutrient exhausted medium. For example, Luke *et al.* measured in real-time the growth rate, cell size and chlorophyll *a* content of cyanobacteria in response to ammonium pulses in the microfluidic device [8]. They revealed that cyanobacteria growing in on-chip showed a dynamic response to the nutrient additions similar to those observed for natural conditions. They concluded that the on-a-chip culture method is suitable for probing physiological changes in a dynamically changing environment. Culturing plankton in confined environment, such as w/o droplets, also reveals the community interactions between two species. Especially, overyielding mechanism is shown when different species are encapsulated within the same droplet [9]. However, confined culture environments lead to a rapid consumption of nutrients [10]. As a consequence, cells cultivated within microfluidic devices could deviate from a natural to an unrealistic culture condition if no precautions are taken. The time scale for which the on chip growth rate on-chip differs from those measured with large culture condition is highly variable in the literature. This time spread from several hours up to 1 month (Figure 1) [11*,12]. This high variability is mainly explained by the physiology of the cells, the concentration of nutrients and the size of the incubation chamber (i.e. culture in a continuous flow or discrete w/o droplets). During the time period where microfluidic incubation mimics large flask conditions, the on-chip cell culturing is suitable to prospect the optimum of cell growth depending on the nutrient concentrations [13,14]. External factors such as the carbon dioxide concentration or light exposure can directly be tested in a microfluidic device [15,16**]. For example, the effect of light intensity on the growth of phytoplankton is measured using a microfluidic device composed of two independent and superimposed microfluidic networks: one layer for cell incubation and a second top layer where dark dyes flow with different concentrations to impact light intensity (Figure 1d). The light exposure can also be directly controlled by a liquid crystal display where each pixel of the screen matches an incubation zone containing plankton cells [17]. These original approaches assess several potential limiting factors and are highly suitable to test numerous combinations of environmental conditions in a single assay. Although on chip culture of plankton is suitable for testing effect of environmental parameters on the cell, discrimination of cells within the culture chamber is needed in order to focus on cells of interest. Such discrimination of cells in a microfluidic chamber is performed using either detection or recognition step.

**Detection and recognition of plankton**

If detection of focused cells within a culture presents obvious interest, the study of in situ coastal waters for seawater quality control or natural assemblage assay is also an important issue. For instance, detection of harmful algal bloom and identification of invasive species are crucial to correctly manage an ecological crisis and assess the risk of pollution. Over the past decades, the intensification of transport of goods by sea has increased the release of ship ballasts containing phytoplankton cells in coastal environments. Those cells, whether belonging to foreign or toxic species act as an inoculum released into the environment and can therefore become invasive and represent a threat for local species and economy. Ships are traditionally not equipped to analyse seawater and the crew members not formed to identify species trapped in the ballast. Because of the size of a microfluidic device, on-chip flow cytometers are proposed as a method to detect cells of interest in seawater such as ballast. This system should be transportable, affordable and easy to operate. A first approach is to consider that each particle in the ballast is potentially a cell. To achieve the detection of particles flowing in the channel, the simple difference in light intensity when a particle passes in the front of a detector is used for a fast cell count [18]. However, the presence/absence of objects is not suitable for a qualitative discrimination between cells and inorganic particles. In this context, Wang *et al.*, developed a compact and low cost microfluidic device capable to simultaneously detect three signals: the chlorophyll fluorescence, the side scatter and the resistance pulse sensing of microalgae cells [19]. The chlorophyll *a* signal discriminates inorganic particles or debris from living autotrophic cells. The resistance pulse sensing (change of electrical resistance between two electrodes) is used to determine the size of the microalgae cell flowing in the detection area and the side scatter is used for the cell roughness. Combination of
Droplet trapping using hydrostatic pressure. (a) Schematic of the experimental setup of the hydrostatic pressure head for trapping the droplets. (b) time-stamped images showing the formation of single algae culture plug (i–iv) and droplet arrays (v–x), and (c) the microchip after trapping 30 droplets. Reproduced, with permission from 11. The high-throughput microfluidic microalgal photobioreactor array. (d) The platform was composed of four layers – a light blocking layer, a microfluidic light–dark cycle control layer, a microfluidic light intensity control layer, and a microalgae culture layer. (e) Enlarged view of a single culture compartment having five single-colony trapping sites. (f) A single-colony trapping site composed of four micropillars. Reproduced, with permission from 16.

These three signals has been used to discriminate up to three photosynthetic living cells. Benazzi et al., also developed a high speed microfluidic platform to discriminate three species with a diameter >2 μm [20]. This microfluidic cytometer combines the measurement of impedance (as a proxy of the particle size) and different wavelength fluorescence signals (pigment content of the particles) to achieve cell discrimination at a flow rate of 3 cm s⁻¹. Pigment content is also used to identify five different species in the study of Schaap et al. [21]. The analysis, performed at the speed of 3–5 mm s⁻¹, is based on the generation of distinctive wavelets of fluorescence when the pigments of the cell are excited by a series of lasers.

Although most of systems discriminate well the cells in small height channels, the wide range of plankton size limits the detection efficiency. Plankton flowing at different depths in the microfluidic channel sometimes leads to a difficult setting of the optimum focus plane. Typically, only cells with a size lower than 2–3 μm are poorly detected in the channel and are often below the detection limit of a microfluidic flow cytometers. To optimize the detection of variable cell sizes, Maw et al., proposed to fit the size of the microfluidic channel in the detection area to the target size [22]. This system recognizes cells from small bacteria (Escherichia coli or Enterococcus faecalis, 1–1.5 μm) to microalgae (Platymonas subcordiformis, 20 μm). Another method consists in focusing the flow of particles in the channel in order to reduce their dispersion [23]. Upstream of the detection area, a series of chevron-like obstacles is added on the top and bottom of the channel. These obstacles progressively focus the flow of particles towards the center of the channel. This three-dimensional hydrodynamic focusing technique is capable to decrease the detection limit down to 1 μm (Synechococcus) but is also suitable for studying larger cells such as Karenia brevis (~50 μm) in a single chip.

Another approach to access the toxicity or pathogen risks consists in the distinction of living and dead cells. Detection of pigment presence as a proxy of autotrophic cell viability is insufficient in coastal region where the cellular debris overestimated the count of living cells. To discriminate living cells to dead ones, Song et al., developed a label-free method based on a capacitance change [24]. This robust method relies on the difference in the capacitance change between a living and a dead cell (living cells have a higher capacitance change than the sample suspension). In addition to cell viability, a precise detection of the presence of specific cells can also be needed for monitoring harmful invasive species in ballast waters and coastal environments. Microfluidic technology associated with qualitative polymerase chain reaction analysis is a powerful combination of techniques to accurately estimate the presence of target species or pathogen [25]. To detect-specific species showing environmental risks in coastal waters, Mahon et al., developed a chip composed of electrodes and carbon nanotubes functionalised with oligonucleotides [26]. The method consists in the injection of the sample containing amplified DNA in the chip device. The amplified DNA of the sample binds the DNA reverse complement. The binds lead to a modification of the impedance measured by the electrodes. These impedance modifications indicate that target species are present in a sample. To test this method, Mahon et al., successfully detect three invasive species using a
single device. Recently, a microfluidic PCR system has also been used to identify bacterial assemblages fixed on the plankton [27]. Both detection and recognition system makes possible the study of cell of interest in the incubation chamber. However, a pre-concentration of cells of interest in the chip are often needed for samples with low cell abundance.

**Pre-concentration of cells in a chip**

Excepted during large phytoplankton blooms or in specific culture conditions, cell concentration in a sample is commonly lower than one cell per nL (i.e. < 10^7 cells L^-1). The low cell abundances found in natural conditions lead to a low number of events of interest during an on-chip assay. For this reason, numerous methods are developed in order to pre-concentrate the sample directly on-chip. Sample filtrations through different small PDMS meshes are described in the literature. Filtration can be achieved by adding a series of small pillars disposed in a checked pattern in a large channel. By playing with the disposition of each pillar and the flow velocity, Singh et al., progressively rerouted larger cells to a collection channel located in the centre of the chip [28]. This smart method allows the separation of cells as a function of their sizes in a continuous flow device. Concentration of cells in a sample is also achieved by using a simple filter system. For example, Zhu et al. proposed a method based on the gap between a deep channel and a shallow one [29]. Microbial cells larger in size than the weir gap (1–4 μm) are hence concentrated in the deep channel whereas the liquid and smaller particles are flushed into the shallow channel. A series of small pores in the channel is also proposed as a solution to increase the concentration of cells in a sample. Shape of the filter can be tuned according to the morphology of the cells of interest. For example, Hønsvall et al. designed a series of trilobite shape-like filters to limit their clogging by chain-like microplankton species (such as *Chaetoceros* spp.) [30].

The filtration method developed by Hønsvall et al., allows an efficient plankton separation based on their sizes. However, the presence of large extracellular matrix produced by some plankton species (e.g. *Thalassiosira weiss-flogii*) can clog the filters in long duration experiments. To limit the progressive clogging of microfluidic devices with time, several studies avoid the use of obstacles in the channels. In absence of a filter type module, the concentration of plankton is performed using either AC electrophoresis or passive hydrodynamic forces (i.e. the inertial focussing method) [31]. The inertial focussing method mainly consists in a microfluidic device with a long channel. A long channel is needed to reach the equilibrium between the shear gradient lift force which pushes particles away from channel centreline and the surface-effect lift force which moves particles away from the channel. The stable equilibrium position within the cross section of the microchannel depends on the particle size. As a consequence, the long channel is disposed either in straight, serpentine or spiral to maximise the particle separation and minimize the place occupied on the chip. For example, Wang and Dandy used a single long serpentine channel (4 mm) to get a flow of concentrated cyanobacteria culture in a collection channel [32]. This approach suitable for harvesting a single strain of cells can be optimized to sort and accumulate particles depending on their sizes. By using inertial focussing method and Dean force, Millet et al., created a series of three spiral channels with different diameters in order to progressively cut-off the 100, 50 and 30 μm size-fractions in a sample [33]. To achieve the discrimination of particles, they connected, in a cascading way, the discard outlet of a large spiral channel to an inlet of a smaller spiral channel (Figure 2a). This process continuously re-injected the sample at the inlet of a spiral channel and improved the separation of particles as a function of their sizes. Although the inertial focussing method is popular in microfluidic applications, large plankton capable to swim can move in the channel cross section. As a consequence, the equilibrium between the shear gradient and the surface-effect lift forces is not reached. In this context, Kumano et al. managed to hydrodynamically trap highly motile cells [34]. The trap consists in a main channel splitting into two channels (a large channel similar in size as the main channel and a funnel type channel). The first cell flowing in the main channel is trapped in the funnel type channel and blocked the flow in it. Consequently, the subsequent cells are re-routed into the large channel until the next funnel type channel. By using a series of large and funnel type channels, the cells are progressively concentrated in the chip. Another strategy to increase the concentration of highly motile cells in a sample is to use traps which mimic the lobster pots or fish weirs [35]. Bouchillon et al., created numerous heart-shaped chambers where the motile plankton can easily enter inside the chamber but hardly escape (Figure 2b). This trapping method may be optimized by measuring the interactions between swimming cells and surfaces (i.e. the scattering angle after a contact with the wall of the channel) [36]. To summarize, pre-concentration of plankton in a microfluidic chip is performed as a function of the cell size or swimming speed and behaviour. Although pre-concentration methods are dedicated to increase the events of interest in a sample, they can not be accurate enough to isolate a specific cell in a population. To achieve a higher degree of discrimination of cell, a sorting system is required.

**Sorting plankton in a microfluidic device**

Sorting cells or droplets of interest in a microfluidic device is an effervescent research field where numerous methods have been developed and are continuously improved through the years and applications. These sorting methods are discriminated into three main approaches: the passive, the active and the hybrid.
Passive sorting systems

Among passives methods, the inertial technology described above can be optimized to sort morphologies of interest using both Dean and passive hydrodynamic forces. Schaap et al., created a microfluidic system capable to discriminate three plankton species with different morphologies (a high-aspect-ratio cylindrical Monoraphidium griffithii, a small spherical Chlorella vulgaris and a prolate spheroidal Cyanothece aerauginosa) [37]. This high throughout method can sort plankton with 77% separation efficiency (Figure 3a-d). Because of the high polydispersity in the cell dimensions, this value is lower than sorting efficiency of monodisperse microspheres tested under the same conditions. However, this method is suitable as a pre-sorting system and only need few devices (a pump and a chip designed according to the morphology of the cells of interest). Later, Syed et al., proposed an inertial sorting method to purify the Tetraselmis suecica culture contaminated by the diatom Phaeodactylum tricornutum [38]. They used a species-dedicated spiral-channel to separate these two species with a very high sorting efficiency (up to 95%) without affecting the cell viability. By using a long strait channel where sample and buffer flows in a laminar way, Godino et al., reported an impressive separation of eukaryotic plankton from bacteria (up to 99%) [39]. This smart principle used the difference of equilibrium between larger plankton cells (located in the centre of the channel) and smaller bacteria (close to wall of the channel). As a consequence, the large cells progressively left the flows of the contaminated culture sample and move in the direction of the centre of the channel where an uncontaminated buffer flows. This passive method is only suitable for large size difference between species such as microalgae and small bacteria. Another reported passive sorting method used the swimming behaviour of the plankton itself to separate the more active ones from the others. The study of Kim et al. used the combination of swimming speed and phototaxis to quickly isolate strains with improved photosynthetic efficiencies [40]. In a context of directed evolution, this method is able to quickly sort the cells with higher efficient photosynthetic activity in a mixture of 10,000 mutants. Within a population of cells with similar morphologies, this method enables the observation of intra-species variability of a particular response to stress conditions (i.e. the light intensity) and the sorting of the fittest cells capable to increase their lipid content. According to the passive sorting technology listed in this review, the main advantages of these approaches are that there are easy to use and cost-effective. However, the sorting parameters that is the morphology and the swimming speed of the cells are rather limited. The resolution of the sorting system limits the screening of cells with similar morphologies and is not suitable for working at the single cell level. Moreover, passive sorting systems are not triggered, which is leading to some contamination during the flow setting. In this context, an active sorting method capable to manipulate cells or droplets at a single object level is required.

Active sorting systems

Despite the numerous types of active sorting systems reported in the literature, the most extensive used system is the one based on dielectrophoresis force to deflect droplets or cells of interest into a collection channel. When the sorting condition is fulfilled, a pulse
Figure 3

(a) Schematic view of the experimental setup of passive sorting system. (b) Micrographs of the three different species of algae. (c) Examples of images captured at the end of the spiral channel (d) Normalized distributions of the algae across the channel cross section. Reproduced, with permission from 37. (e) Schematic view of a hybrid sorting system; Droplets are generated and focused to the side wall of the microchannel by oil phase flow from inlet 3. Then, single cell encapsulated droplet are separated by magnetic field. The scale bar is 200 μm. Reproduced, with permission from 45.

of high DC or AC voltage (typically more than 500 V) is applied on two electrodes located at each side of a Y-junction pulls of droplets into a collection stream. The main difference between the described systems is the sorting criteria. Popular systems in algal research use fluorescent label or take advantage of the auto-fluorescent pigment content of cells for screening cells of interest. In principle, fluorescence-activated droplet systems mimic the fluorescence-activated cell sorting (FACS) technology with a lower sorting speed. However, in contrast to continuous flow systems including flow cytometry technology, plankton encapsulated in water-in-oil droplets offer the advantage of maintaining the separation in small discrete microenvironments after the screening process. As the water-in-oil droplets of interest are separated from each other in the microfluidic channel, accurate throughput sorting method is performed. Best et al. used this robust method in order to sort all the droplets containing cells from the empty ones at a speed of 300 Hz and also sorted droplets containing cells depending on their chlorophyll a content at a speed up to 160 Hz [41**]. The sorting technology used a photomultiplier tube (PMT) to measure the fluorescence and a pulse of ~700 V to sort droplets of interest without any notable effect on the cell motility or viability (Figure 4). The absence of destructive effect of high voltage pulse on cells is important to control since water-in-oil droplet was reported as an efficient media for cell electroporation [42]. Interestingly, several physiological parameters such as cell viability as well as lipid content can be directly probed using the dielectrophoresis forces [43]. The advantages of these physiological parameters are that both the sorting criteria and object deflection in the channel are performed at the same time. For example, by adjusting the frequency of the AC voltage pulses Deng et al., sorted cells depending on their lipid content [43]. Finally, recent progress on high speed image processing system enables the sorting cell or droplets of interest depending on the morphology and the number of the encapsulated objects. The sorting speed of the image sorting systems (10 Hz) is slower than the fluorescence based active sorting system [12]. However, a wide range of sorting criteria can be used to separate the droplets (morphology, fluorescence, number of encapsulated objects). This method is suitable for numerous applications including cell identification,
growth rate assays as well as biological assay. Because of the high content image processing and microfluidic technology.

Hybrid sorting systems

An hybrid sorting method can be defined as a system capable to sort numerous events at a specific time. This system can not fully work at a single cell/droplet level even if the sorting technology can be optimized to reach this level. Hybrid systems include some parameters such as density, compressibility, or lipid content as sorting criteria. Acoustophoretic force is for example used to manipulate water-in-oil droplets depending on size, density and compressibility of objects flowing in the microfluidic channel. A typical example of droplet manipulation is the sorting of droplets containing numerous plankton inside [44]. The difference of cell concentration per droplet leads to a difference in the averaged density and/or compressibility for each droplet. The acoustophoretic force applied to each droplet deflects them to the pressure node when they pass through an acoustic standing wave field. This label-free sorting method enables the collection of droplets containing eukaryotic plankton cells or bacteria with a high growing rate (i.e. droplets containing high number of cells). A similar approach named magnetophoretic system allows the on-chip sorting of droplets containing cells from empty ones in the chip. This method uses a combination of magnet nanoparticles and a permanent magnet. In principle, the magnetophoretic system consists in the addition of magnet nanoparticles to the sample. When a cell is encapsulated in a water-in-oil droplet, its volume is not occupied by magnetic nanoparticles. The difference of number of nanoparticles between the cells containing and the empty droplets creates different magnetic forces when the droplets flow close to a permanent magnet (Figure 3e). This sorting system can sort droplets containing a single diatom from those empty droplets with >94% purity at a flow rate of 300 µL/h [45]. The difference in lipid content can also be used as sorting criteria since, AC voltage deflects cell depending on lipid concentrations [46]. By using the dielectrophoresis force and continuous flow device, Hadady et al., sort the high lipid content cells to the population with an efficiency of 75% [47]. Hybrid systems are suitable for applications mostly dedicated to cell density analysis or directed evolution experiments. However, biological assays and investigation of intra-species variability of responses to stress factors often need a higher degree of object manipulation. For these reasons, a more controllable screening method such as active sorting system is sometimes required to achieve a higher degree of purification.

Microfluidic platform for the measurement of cell-environment interactions

The second part of the review focuses on the interactions between plankton and their environments. The studied
interactions found in the literature revolve to the swimming speed of plankton, the diagnostic of the water quality, the microbial loops and the modes of nutrition of plankton as function of nutrient concentration and light.

The swimming speed of motile plankton species
Nychthemeral migration of plankton in the seawater column, light attenuation, nutrient concentrations and hydrodynamics are important factors leading to the heterogeneous distribution found within heterotrophic and autotrophic organisms in the ocean. Such patchiness distribution at smaller scale remains scarcely investigated in literature. In particular, the behaviour of eukaryotic plankton and bacteria in changing or turbulent environment is still not well understood. Phytoplankton dynamics in flow can be affected by multiple processes related to cell morphology and motility. Among the forces controlling the distribution at the lower scale, shear stress and small vortices are suggested to play key roles in this spatial heterogeneity of cell distribution. By trapping cells in a low volume, the microfluidic technologies help the observation of motile plankton and provide a suitable tool to perform a test bench of the effect of shear stress on living cells. Study of the pennate diatoms forming colonies (Bacillaria paradoxa) conducted in microfluidic tends to confirm the light-dark cycle of a high gliding speed during the light period and a non-motile and aligned forms during the dark period [48]. Interestingly, the period of vibration of the diatom colonies does not depend on the water flow speed. However, when the flow speed is high (1.1 mm s\(^{-1}\)), the amplitude of the vibration is higher than in still waters suggesting that diatoms move faster in flowing water. The motility speed and direction in a flow-controlled environment indicated that diatom cells automatically adjust their gliding directions to the direction of the flow. In less than 10 min, the direction of gliding is also rapidly adjusted to the direction of the flow offering the smallest resistance. This alignment of cells has also been observed for freshwater phytoplankton [49]. The alignment of cell is reported to depend on the species and link to the value of the shear rate (Figure 5) [50]. Typically, values of shear >1 s\(^{-1}\) are reported to have an effect on the distribution of flagellate species in a channel. The variability in the distribution of species remained not well understood purely, because the cell hydrodynamic profile (i.e. morphology, location of the flagella) cannot entirely explain their accumulations either in the low shear or high shear region. The modification of the flagellate beat pattern depending on the flow speed and cell preference probably plays a key role in the cell orientations. By using a microfluidic device and high speed camera, Chengala et al., found that Dunaliella primolecta can migrate across the stream without rotation. This-specific swim leads to a collective migration and a 2-D thin dispersion layer [51].

The motility of plankton is also investigated depending on the presence of obstacles. Wang et al., proposed to study the role of confinement and bending channel in a microfluidic device [52]. They showed that the speed of cells depends on the size of the microfluidic channel. In particular, they showed that the channel cross-sectional area to protozoan cross-sectional area had a statistically significant effect on the swimming speed (i.e. a small channel cross section leads to a low swimming speed of the cell). The swimming speed of Euplotes vanus measured on chip (70 \(\mu\text{m} \text{s}^{-1}\)) is significantly lower than previously reported in literature (430 \(\mu\text{m} \text{s}^{-1}\)). This is unexpected and addresses the question related to the appropriate channel size for measuring the cell motility [52]. The effects of obstacles on cells have also been used to characterize the bioluminescence of some plankton. Latz et al., investigated the latency between the impact of dinoflagellates on obstacles and the emission of a bioluminescence signal [53]. The minimum bioluminescence response latency is of interest as it represents the most rapid activity of signalling pathways. The results revealed that cell can trigger a bioluminescence flash in only 15 ms after a choc with an obstacle. The minimal latency between two flashes is 4 ms suggesting that reactivation involves a subsystem of the entire mechanosensory signalling pathway. The reasons of the emission of bioluminescence signal are still not fully understood but are probably due to the high shear stress closed to the obstacle which mimics a predation [53]. This relation between the predator and the prey is particularly complex at the scale of the cell. By using a microscale particle analysis in a microfluidic device, Gemmell et al., showed without the so called ‘wall effect’, that zooplankton can dissipate the vortices signature in 4 ms (new hatched N1) to >1s for the adult one [54]. These time values are in the same order of bioluminescence latency and seem to confirm that bioluminescence is a suitable escape technique for dinoflagellates. According to the rapid response of plankton to a stress such as predation, the swimming speed is also used to probe environmental conditions or pollutant effect on the cells.

Measurement of water quality using plankton
Motility as a survival strategy is an important mechanism of the cell and a factor of its resilience in an environment. As a consequence, both the swimming pattern and swimming speed are both a function of the physiology state of plankton as well as an indicator of environmental conditions. In this context, motility of the marine phytoplankton is used as a sensor for assessing pollutant toxicity. By trapping two marine phytoplankton species in a series of small incubation chambers, Zheng et al., tracked both the swimming pattern and speed of cells under eight different pollutant concentrations [55]. The different concentrations of pollutants are simultaneously generated by the diffusion of a solution containing a high
Figure 142

(a–d) Phase contrast microscopy of the four species of phytoplankton. (e) Probability density function of measured swimming speeds for each species. (f) Timeseries of the mean square displacement for each species. The solid line represents a linear fit to the diffusive regime, from which the effective translational diffusivity, \( D \), was obtained. (g) Schematic of the serpentine microfluidic device (left, plan view; right, isometric view, not to scale) showing the imaging plane (light blue) and the flow profile (red) at the channel mid-depth. (h) Flow velocity profiles measured at the mid-depth plane using cells as tracers (blue dots), fitted with a parabolic profile (red line). Error bars correspond to the standard error of the mean [50].

Concentration of pollutant into a sample with a low concentration of pollutant. The successive divisions of the channel allow to test of eight different concentrations flowing in different incubation chambers. The curvilinear, average path and straight line velocities are used to quickly characterize the swimming pattern and speed of plankton under stress. The half maximal effective concentration -which defined the response halfway between the baseline and maximum- is estimated in only two hours. Later, Feng \textit{et al.}, improved the system by parallelizing the operation and increasing the number of species tested at the same time [56]. However, an important dataset of plankton behaviour recorded under different controlled culture conditions (temperature, cell density and light conditions) is required. Once the calibration done, the results indicated that the microfluidic system is suitable for a rapid diagnostic of the water quality when the identification of the pollutant is not needed.

Reactive oxygen species (e.g. \( \text{H}_2\text{O}_2 \)) are important molecules in cell life and control numerous physiology processes within cells. Commonly observed in balance with antioxidant molecules when the cell is healthy, the increase of reactive oxygen species expresses a stress and can lead to the cell death. The generation of reactive
oxygen species is linked to the presence of abiotic factors (e.g. temperature, UV irradiation) as well as toxicants (e.g. metal and inorganic nanoparticles). The measurement of reactive oxygen species is commonly performed using fluorescent dyes. The fluorescent dyes can be combined with dielectrophoresis in order to stack cells in a 2-D film [57]. By taking advantage of microfluidic technology, Koman et al., developed a non-invasive method to continuously measure on-chip the concentration of extracellular H2O2 [58]. The results demonstrated a positive relationship between the kinetics of H2O2 generation and Cd2+ concentration. However, the balance between reactive oxygen species and antioxidant is re-established in 1 hour and suggested an efficient response of algae to a Cd2+ stress. The cumulative effect of a double consecutive exposition of the pollutant Cd2+ on the algae leads to a higher and faster generation rate of H2O2. These results obtained by the continuous monitoring of H2O2 generation highlighted the advantage of on chip devices by discriminating the different phases of cellular response to the pollutant such as the detection of inflammatory response.

Finally, fluorescence of microalgae is also used as an indicator of the toxicity of several chemical compounds such as herbicide or pesticide [59]. Lefevre et al., developed a portable fluorescent biosensor for detecting the herbicide Diuron with a very high sensitivity (7.5 nM) compared to the portable amperometric biosensor systems and transportable commercial fluorescence equipment [60]. However, the biosensor is dedicated to the detection of only one herbicide.

**Measurement of phycotoxins**

Due in part to the eutrophication of the coastal areas, harmful algal blooming becomes a common phenomenon at a worldwide scale. The bloom of planktonic species capable to produce toxins constitutes a threat for aquatic ecosystems, human health and activities. The measurement of toxin concentrations in the seawater is particularly important for preserving the health of ecosystem and managing the ecological risk for human health. Miniaturization and cost-affordable systems offered by the microfluidic technology are promising. However, the development of functional microfluidic systems including a full characterization of known toxins is still in development. The numerous series of complex reactions required for every toxin assay is complicated to integrate in a single microfluidic system.

However, several studies proposed some advanced microfluidic systems suitable for toxin measurement. For example, Wu et al., simply created a microfluidic chip capable to trap single cells and control their lyses by ultrasonication [61]. Although the system is purposed for this content, no biological assay is described in this study. In the same year, Zhang et al., developed a more advanced system for measuring three major cyanotoxins [62**]. They used conventional enzyme linked immunosorbent assay (ELISA) to detect and measure microcystin, saxitoxin and cylindrospermopsin (Figure 6). In addition to the good limit of detection (0.02 ng/ml), the main advantage of the microfluidics is the short time to analyse a sample (15 min for saxitoxin, and 25 min for microcystins and cylindrospermopsin analysis) compared to classical ELISA method (60 min for saxitoxin analysis, 115 min for microcystin analysis and 75 min for cylindrospermopsin analysis). This robust system consumes 3 orders less reagents and is suitable for on-field measurements. Recent progress in microfluidic fabrication enables the detection of toxin directly in food using an hybrid PDMS/paper chip device and aptamer functionalized with graphene oxide [63]. The detection of plankton toxin such as okadaic acid or brevetoxin can be performed in only 5minutes with a similar limit of detection as ELISA detection kits.

**Bacteria-plankton interactions**

Among the bacteria-plankton interactions, the swimming response of cell to chemical compounds (i.e. chemotaxis) is commonly observed in natural population. For example motile bacteria orientate their swimming direction along the gradient of organic material. The chemotactic response to a concentration gradient is well known to modify the dynamic of both phytoplankton and bacteria populations in culture. For example, the motile response of bacteria to some-specific amino acids, carbohydrates and prey was shown in cultures [64]. These chemotactic responses to some local conditions suggest the presence of a phycosphere where the heterotrophic bacteria interacts with algae products by being attracted and/or growing in this region. The concept of phycosphere where phytoplankton attract motile bacteria is demonstrated one year later by Bell and Mitchell, [65]. This concept of phycosphere suggests that a strong heterogeneous spatial distribution of microalgae and bacteria exists at very small scales. The manipulation of small volumes and single cell analysis make the microfluidics a suitable tool for monitoring interaction between cells at pondhe micron-scale. By using simple microfluidic devices and cell tracking system, Seymour et al., measured the chemotactic response to the nutrient pulses [66]. The study evidenced a rapid aggregation of bacteria (down to <10 s for Pseudoalteromonas haloplanktis) in the center of the channel where nutrient concentration is higher [67]. The aggregation of bacteria in the center of the channel persisted during 15~20 min suggesting a consumption of nutrients in this area. Interestingly, the extracellular cell products including the exudates released by toxic plankton also act as a strong chemotactant for bacteria (Figure 7) [68**]. The velocity response of bacteria to the extracellular products is highly variable and species dependant. This variability in not only linked to the swimming speed of bacteria but also depend on their chemoreceptor
sensitivity. For example, Miño et al., reported that only fast swimmer cells of the bacteria consumer, *Salpingoea rosetta*, are able to use the pH gradient to orient their swimming direction [69]. As the *S. rosetta* can also swim a lower speed, fast swimmers probably characterize cells in an active research phase. According to the fact that the high concentration of bacteria can induce local acidic pH, the attraction of *S. rosetta* to the region with low pH values suggested that this species used pH as an indicator of the presence of preys.

It should be noted that small pH changes may favour distinct groups of the bacteria at the community level and potentially create a shift in the microbial compositions [70]. Indeed, the alkaline conditions in marine environments are commonly considered as stress conditions for bacteria which need special physiological strategies to cope with it [71]. The acidic pH generated by bacteria also locally regulates the form of carbon present in the seawater. As phytoplankton preferential storage of inorganic carbon is the form of HCO$_3^-$, a research strategy to find the regions of high concentration of HCO$_3^-$ is expected for these cells. The region of neutral or alkaline pH containing a low concentration of bacteria is probably more suitable in terms of algae fitness especially since bacteria are commonly found to compete the plankton for nutrient acquisition. By using microfluidic channels filled with ion permeable agarose membrane, Choi et al., explored the chemotaxis of microplankton as a function of HCO$_3^-$ concentration [72]. The results obtained confirm that algae actively migrated to an optimum zone where HCO$_3^-$ is abundant (26 mM of HCO$_3^-$). This active research activity is dependant on circadian rhythm with a peak of activity in the dark...
period and a minimum during the light period. This peak of research activity for HCO$_3^-$ in the dark is explained by the need of *Chlamydomonas reinhardtii* to compensate the loss of CO$_2$ during its respiration phase. In addition to that reason, the maximum of chemotaxis for HCO$_3^-$ is linked to the maximum of chemotaxis for ammonium. As a consequence, the cell during the dark phase actively prospect the high carbon and nutrient sources and waits for the day light to initiate the photosynthesis and ammonium uptake/assimilation. In some extends, these results obtained in microfluidics may chemically explained the vertical migration observed in the seawater column.

Finally, both bacteria and plankton can be attracted by inorganic and organic compounds. The attractiveness of a chemical compound is function of the species and can vary at the single cell level. The swimming response of cell to chemical compounds is one method to find the optimum of growth for cell. However, marine cells need a set of sensitive chemoreceptors and an active propulsion method to reach the suitable area. These mechanisms of detection and motility are not ubiquitous among all groups and species. In this context, the development of methods probing the nutritional mode and growth optimum of low motility are needed.

**Modes of nutrition and preference for plankton growth**

Researches of modes of nutrition phytoplankton and growth optimum conditions are of particular interest for both fundamental knowledge on plankton behaviour and the biotechnology applications. The modes of nutrition of plankton are mainly autotrophy, mixotrophy and heterotrophy. The switch between different modes of nutrition within one single species is often linked to the nutrient or light availability in the seawater. For example, low concentrations of orthophosphate lead to an activation of a set of enzymes by some planktonic species. Among the enzymes activated during orthophosphate stress, the alkaline phosphatase is reported to be a good indicator of the physiological state and hence the mode of nutrition of the cell. To identify the mode of nutrition and evaluate the phosphate stress, alkaline phosphatase assays at the single cell level was developed in microfluidic device (Figure 8) [73**]. The results indicated that cell cultures have a similar timing in the activation of alkaline phosphatase (e.g. the switch in nutrition mode between the autotrophy and osmotrophy is activated within a coupled day at the community level). However, at a single cell level, a high variability in the kinetics of alkaline phosphatase activity has been measured under the same environmental conditions and suggested that each cell had different responses to a stress in orthophosphate nutrient.

This variability in response is also particularly important for research in biofuel production. Especially, since nutrition mode and nutritive stress leads to variable lipid content of microalgae cell. Nutrient concentration (i.e. the nitrogen starvation) is commonly reported to play a key role in the accumulation of lipid in the cell [74]. The high storage of lipid in algae can be promoted by a single stress in nitrate. However, combination of two different stresses (such as nitrate starvation-high temperature, nitrate starvation-high salinity as well as high temperature-high salinity) is also reported to stimulate the lipid production [75]. Similarly to the alkaline phosphatase activity results, the response of the plankton to a stress is highly variable in a population of a same species [76]. These high variations in lipid production led to the development of specific detection system suitable for the identification of the cells with the highest lipid content. Although the characterization of the lipid
content of algae can be measured using a Raman microspectroscopy, measurement of the fluorescence-based maximum quantum yield (Fv/Fm), a proxy of nitrate starvation, in culture of diatoms have also been developed [77, 78]. Later, Guo et al., developed a microfluidic device capable to simultaneously detect the fluorescence and to image cells at a frequency of 10 kHz [79]. They demonstrate the combination of the fluorescence of boron-dipyromethene (BODIPY) labelled cell with an optical parameter (opacity of cells) to efficiently detect the lipid content at the single cell level. The detection of optimum of lipid production under different nutrient condition was recently integrated into complex microfluidic analysis platforms (Figure 9) [80**]. With one of the most advanced microfluidic platforms, the culture of cells under different nutrient conditions, the cellular staining of lipids and the measurement of the lipid content at a single cell level was recently achieved [80**]. This recent development of microfluidic tools linked to the biotechnological applications opens the door to the higher degree of cell quantification and will probably be useful to unravel the complexity of the plankton ecology.

Conclusions and perspectives

Within the actual context of a global change, understanding the plankton behaviour and interactions of each cell with its changing environment is a challenge for the future in Oceanography. The intra-specific response of cells growing under the same conditions and the detection of active cells within the population or a community are hidden in measurements at the community level. In
this context, the rise of the high-content cell analysis will inexorably lead to the development of suitable single-cell technology in plankton research. Microfluidics is a key technology since the manipulation of small volumes at the subnanoliter scale combines both single cell cultivation and high content biological assays. The advanced microfluidic systems described in this review reveal the versatility of microfluidic platforms for plankton research. The confinement of a single cell trapped in small channels or in w/o droplets enables a fine control of cells and test various environmental scenarios in real-time. Stability of the w/o emulsion over time and reduced analytical volumes are the main advantages of microfluidic. Paradoxically, this small analytical volume is also the current weakness of microfluidic systems. The cell concentration of some plankton (such as diatom as well as dinoflagellate) can be very low in natural samples but several progresses in the pre-concentration of samples or sorting systems presented in this review already allow high throughput purification of cells. Integrating a series of modules such as cell concentration, biological assay, incubation, sorting and analysis is the next challenge for the technology. When controlled, such an integrated approach will enable the automation of complex biological workflows and decrease the potential errors done by the experimenter. Integration is therefore one of the next challenges to be tackled in the near future.

Conflict of interest statement
Nothing declared.

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