

Microfluidic technology for plankton research

Mathias Girault, Thomas Beneyton, Yolanda del Amo, Jean-Christophe Baret

▶ To cite this version:

Mathias Girault, Thomas Beneyton, Yolanda del Amo, Jean-Christophe Baret. Microfluidic technology for plankton research. Current Opinion in Biotechnology, 2019, 55, pp.134-150. 10.1016/j.copbio.2018.09.010. hal-02118999

HAL Id: hal-02118999 https://hal.science/hal-02118999

Submitted on 3 May 2019

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers. L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



ScienceDirect



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)

Current Opinion in Biotechnology 2019, 55:134-150

This review comes from a themed issue on Analytical biotechnology

Edited by Saulius Klimasauskas and Linas Mazutis

For a complete overview see the Issue and the Editorial

Available online 13th October 2018

https://doi.org/10.1016/j.copbio.2018.09.010

0958-1669/© 2018 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creative-commons.org/licenses/by-nc-nd/4.0/).

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 adaptative 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 laver 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 μ m [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 $2-3 \,\mu m$ 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 $1 \,\mu m$ (*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. $< 10^9$ 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- $4\,\mu\text{m}$) 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 electrokinetics 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 surfaceeffect 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 cvanobacteria 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. Miller *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.





(a) 3 spiral rectangular channels (500, 300 and 200 μ m high) are cascaded to fractionate a mix of particles with a wide range of diameter (100, 50 and 30 μ). Reproduced, with permission from 33. (b) Tiled micrograph images of a portion of a "tertiary series" sampler used for lab testing. Concentrations of *Cyclidium* sp. become greatly-enriched towards the 5th gallery in the series, in some cases, completely filling the terminal gallery with protists (inset). Reproduced, with permission from 35.

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 aeruginosa*) [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 tricor*nutum* [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 Yjunction 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-actived 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 waterin-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 \sim 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 dieletrophoresis 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,





Sorting *C. reinhardtii* low-chlorophyll cell-containing droplets from empty droplets. (a) Images taken of droplets before sorting; (b) 91% droplets collected in the "positive" channel contain cells whereas only 6% of negative droplets are occupied, false "negatives". Scale bar = $100 \,\mu$ m. (c) Images recorded during sorting show that cell-containing droplets are deflected into the "positive" channel (top panel), whereas empty droplets flow into the "negative" channel (bottom panel) 41.

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 $300 \,\mu$ L/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 intraspecies 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

Nycthemeral 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 eukarvotic 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 $>1 \text{ s}^{-1}$ 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 vannus measured on chip $(70 \,\mu m \, s^{-1})$ is significantly lower than previously reported in literature $(430 \,\mu m \, s^{-1})$. 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 >1 s for the adult one [54]. These time values are in the same order of bioluminescent 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





(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 *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. H_2O_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 $(0.02 \text{ ng ml}^{-1})$, 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 5 minutes 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 preys 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 tondhe 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 <10s 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 chemoattractant 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





(a) Schematic diagram of the competitive immunoassay in the immune-reaction columns. (b) Illustration of the chip operations to complete the immunoassay. The center circle area of the chip is graphical shown: each process of the reagent loading was controlled by two valves (Valve 1 and Valve 2). Status of the valves is clarified by different colours: red for action; gray for inaction. Reproduced, with permission from 62.

sensitivity. For example, Miño *et al.*, reported that only fast swimmer cells of the bacteria consumer, *Salpingoeca 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 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 HCO₃- 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 dependent on circadian rhythm with a peak of activity in the dark





(a) Swimming trajectories of *Silicibacter* sp. cells across the microfluidic channel following injection of f/2 growth medium as a chemoattractant (control). Each white path is the trajectory of a single bacterium. (b) Swimming trajectories of *Silicibacter* sp. cells within a band of *Synechococcus elongatus* extracellular products, demonstrating strong accumulation in the band of chemoattractant. Reproduced, with permission from 68.

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 prospects 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 of 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





Photomicrographs of labeled cells. Green labels show enzyme-labeled fluorescent compounds located at the alkaline phosphatase sites. Red labels are chlorophyll *a* pigments. (a) Typical fluorescence and bright-field images of four droplets containing single living labeled cells. (b) Difference in labeling between three dead cells with numerous labels at the surface (upper image) and a living cell with a single label located at the bottom of the flagellar apparatus (bottom image). (c) Example of the kinetics of a single living cell labeled in droplet. Reproduced, with permission from 73.

content of algae can be measured using a Raman microspectroscopy, measurement of the fluorescence-based maximum quantum yield (F_v/F_m), 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-dipyrromethene (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



Illustration of the droplet microfluidics based microalgae screening platform for analyzing microalgal growth and oil production. The platform is composed of three functional parts (*i*) the droplet generation/culturing region for culture and growth monitoring, (*ii*) the on chip staining region for tagging Nile red fluorescent dye to oil bodies of microalgae, and (*iii*) the rinsing/analysis region for oil quantification. Reproduced, with permission from 80.

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 scenarii 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 controled, 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.

Acknowledgements

We acknowledge financial support by the ERC (FP7/2007-2013/ERC Grant Agreement 306385 SofI), by the Region Aquitaine, and by the French '



Government Investments for the Future Programme, University of Bordeaux Initiative of Excellence (IDEX Bordeaux) [Reference Agence Nationale de la Recherche (ANR)-10- IDEX-03-02]. JCB acknowledges the support of the Institut Universitaire de France. We acknowledge support by the University of Bordeaux for a post-doc fellowship.

References and recommended reading

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

- of special interest
- •• of outstanding interest
- Stock CA, John JG, Rykaczewski RR, Asch RG, Cheung WW, Dunne JP, Friedland KD, Lam VWY, Sarmiento JL, Watson RA: Reconciling fisheries catch and ocean productivity. Proc Natl Acad Sci U S A, PNAS 2017, 114:E1441-E1449.
- Bilal M, Rasheed T, Ahmed I: Iqbal HMN: high-value compounds 2. from microalgae with industrial exploitability-a review. Front Biosci 2017, 9:319-342.
- Geider RJ, Delucia EH, Falkowski PG, Finzi AC, Grime JP, Grace J, Kana TM, La Roche J, Long SP, Osborne BA, Platt T, Prentice IC, Raven JA, Schlesinger WH, Smetacek V, Stuart V, Sathyendranath S, Thomas RB, Vogelmann TC, Williams P, Woodward FI: **Primary productivity of planet earth: biological determinants and physical constraints in terrestrial and aquatic habitats**. *Glob Change Biol* 2001, **7**:849-882. 3.
- Fu W, Randerson JT, Moore JK: Climate change impacts on net primary production (NPP) and export production (EP) regulated by increasing stratification and phytoplankton community structure in the CMIP5 models. Biogeosciences 2016, 13:5151-5170.
- Flombaum P, Gallegos JL, Gordillo RA, Rincón J, Zabala LL, 5 Jiao N, Karl DM, Li KWW, Lomas MW, Veneziano D, Vera CS, Vrugi JA. Martiny AC: Present and future global distributions of the marine Cyanobacteria Prochlorococcus and Synechococcus. Proc Natl Acad Sci U S A PNAS 2013, 110:9824-9829
- Fleming ED, Bebout BM, Tan MX, Selch F, Ricco AJ: Biological 6. system development for GraviSat: A new platform for studying photosynthesis and microalgae in space. Life Sci Space Res 2014, 3:63-75.
- Håti AG, Bassett DC, Ribe JM, Sikorski P, Weitz DA, Stokke BT: 7. Versatile, cell and chip friendly method to gel alginate in microfluidic devices. Lab Chip 2016, 16:3718-3727.
- Luke CS, Selimkhanov J, Baumgart L, Cohen SE, Golden SS, Cookson NA, Hasty J: A microfluidic platform for long-term 8. monitoring of algae in dynamic environment. ACS Synth Biol 2016, 5:8-14.
- Carruthers DN, Byun CK, Cardinale BJ, Lin XN: Demonstration of 9. transgressive overyielding of algal mixed cultures in microdroplets. Integr Biol 2017, 9:687-694.
- Boedicker JQ, Vincent ME, Ismagilov RF: Microfluidic 10. confinement of single cells of bacteria in small volumes initiates high-density behavior of quorum sensing and growth and reveals its variability. Angew Chem Int Ed 2009, 48:5908-5911

 Dewan A, Kim J, McLean RH, Vanapalli SA, Karim MN: Growth

 kinetics of microalgae in microfluidic static droplet arrays. Biotechnol Bioeng 2012, 109:2987-2996.

 They developed a microfluidic chip design suitable to store water in oil droplet containing cell. Aqueous droplets can be stored in PDMS microfluidic chip up to 1 month. Microfluidic chip can be used to study growth kinetics of single or multiple algae cells at a single droplet level. They reported that morphological information of cell can be used as proxy of the lipid content of cell.

12. Girault M, Hattori A, Kim H, Arakawa H, Matsuura K, Odaka M, Terazono H, Yasuda K: An on-chip imaging droplet-sorting system: a real-time shape recognition method to screen target cells in droplets with single cell resolution. Sci Rep 2017, 7:40072 http://dx.doi.org/10.1038/srep40072.

- 13. Bae S, Kim CW, Choi JS, Yang JW, Seo TS: An integrated microfluidic device for the high-throughput screening of microalgal cell culture conditions that induce high growth rate and lipid content. Anal Bioanal Chem 2013, 405:9365-9374.
- 14. Paik SM, Sim SJ, Jeon NL: Microfluidic perfusion bioreactor for optimization of microalgal lipid productivity. Bioresour Technol 2017 233 433-437
- 15. Xu Z, Wang Y, Chen Y, Spalding MH, Dong L: Microfluidic chip for automated screening of carbon dioxide conditions for microalgal cell growth. Biomicrofluidics 2017, 11:064104.
- 16. Kim HS, Weiss TL, Thapa HR, Devarenne TP, Han A: A
- microfluidic photobioreactor array demonstrating high-... throughput screening for microalgal oil production. Lab Chip 2014, 14:1415-1425.

They developed a multiple layers microfluidic device to continuously measure the growth of the plankton depending on 64 light intensity conditions. By using a single chip, the microfluidic platform can identify light conditions that showed maximum oil production that differed from conditions typically used in conventional cultures. This screening is achieved at 250 times higher throughput and 850 times less reagent consumption.

- 17. Graham PJ, Riordon J, Sinton D: Microalgae on display: a microfluidic pixel-based irradiance assay for photosynthetic growth. Lab Chip 2015, 15:3116-3124.
- 18. Machado LM, Mattiello I, Lopes RM, Carreño MNP: Microfluidic biochip for phytoplankton cell counting. IEEE Microelectron Technol Dev (SBMicro) 2013 http://dx.doi.org/10.1109/ SBMicro.2013.6676146.
- 19. Wang J, Zhao J, Wang Y, Wang W, Gao Y, Xu R, Zhao W: A new microfluidic device for classification of microalgae cells based on simultaneous analysis of chlorophyll fluorescence, side light scattering, resistance pulse sensing. *Micromachines* 2016, **7**:198 http://dx.doi.org/10.3390/mi7110198.
- 20. Benazzi G, Holmes D, Sun T, Mowlem MC, Morgan H: Discrimination and analysis of phytoplankton using a microfluidic cytometer. IET Nanobiotechnol 2007, 1:94-101.
- 21. Schaap A, Bellouard Y, Rohrlack T: Optofluidic lab-on-a-chip for rapid algae population screening. Biomed Opt Express 2011, 2:658
- 22. Maw MM, Pan X, Peng Z, Wang Y, Zhao L, Dai B, Wang J: A changeable lab-on-a-chip detector for marine non indigenous microorganisms in ship's ballast water. Micromachines 2018, 9 http://dx.doi.org/10.3390/mi9010020.
- 23. Hashemi N, Erickson JS, Golden JP, Ligler FS: Optofluidic characterization of marine algae using a microflow cytometer. Biomicrofluidics 2011, 5:032009.
- 24. Song Y, Li M, Yang J, Wang J, Pan X, Sun Y, Li D: Capacitive detection of living microalgae in a microfluidic chip. Sens Actuators B Chem 2014, 194:164-172.
- 25. Byappanahalli MN, Nevers MB, Whitman RL, Ishii S: Application of a microfluidic quantitative polymerase chain reaction technique to monitor bacterial pathogens in beach water and complex environmental matrices. Environ Sci Technol Lett 2015, 2:347-351
- 26. Mahon AR, Barnes MA, Senapati S, Feder JL, Darling JA, Chang HC, Lodge DM: Molecular detection of invasive species in heterogeneous mixtures using a microfluidic carbon nanotube platform. PLoS One 2011, 6:e17280 http://dx.doi.org/ 10.1371/journal.pone.0017280.
- Chun CL, Peller JR, Shively D, Byappanahalli MN, Whitman RL, Staley C, Zhang Q, Ishii S, Sadowsky MJ: Virulence and biodegradation potential of dynamic microbial communities associated with decaying Cladophora in Great Lakes. Sci Total Environ 2017:872-880 http://dx.doi.org/10.1016/j. scitotenv.2016.09.107.
- Singh V, Nguyen Q, Goettert J, Yemane D, Bargiel J, Lane C, 28. Stephenson F: Fabrication and characterization of HAR microfluidic device to concentrate microalgae Nanotechnology 2012: Electronics, Devices, Fabrication, MEMS, Fluidics and Computational - 2012 NSTI Nanotechnology Conference and Expo 2012:157-160.

- 29. Zhu L, Zhang Q, Feng H, Ang S, Chau FS, Liu WT: Filter-based microfluidic device as a platform for immunofluorescent assay of microbial cells. *Lab Chip* 2004, 4:337-341.
- Hønsvall BK, Altin D, Robertson LJ: Continuous harvesting of microalgae by new microfluidic technology for particle separation. *Bioresour Technol* 2016, 200:360-365.
- **31.** Yuan Q, Wu J, Greenbaum E, Evans BR: A resettable in-line particle concentrator using AC electrokinetics for distributed monitoring of microalgae in source waters. *Sens Actuator B Chem* 2017, **244**:265-274.
- Wang L, Dandy DS: A microfluidic concentrator for cyanobacteria harvesting. Algal Res 2017, 26:486-491.
- 33. Miller B, Jimenez M, Bridle H: Cascading and parallelising
 curvilinear inertial focusing systems for high volume, wide size
- distribution, separation and concentration of particles. Sci Rep 2016, 6:36386 http://dx.doi.org/10.1038/srep36386.

This study is a typical example of an effective passive sorting system. The system used a series of inertial focussing devices to discriminate cell depending on their morphologies.

- Kumano I, Hosoda K, Suzuki H, Hirataa K, Yomo T: Hydrodynamic trapping of Tetrahymena thermophila for the long-term monitoring of cell behaviors. Lab Chip 2012, 12:3451-3457.
- Bouchillon GM, Chau JF, McManus GB, Shor LM: Microfluidic
 passive samplers for *in situ* collection of live aquatic protists. Anal Methods 2014, 6:8350-8357.

This original pre-concentration method used fishery sciences to automatically concentrate aquatic protist cells in a trap. The method takes advantage of the motility of plankton to progressively trap motile cells. In lab testing, a marine ciliate Cyclidium sp. was concentrated from 600 cells per mL in a laboratory microcosm to above 2×10^8 cells per mL within individual microfluidic observation galleries.

- Kantsler V, Dunkel J, Polin M, Goldstein RE: Ciliary contact interactions dominate surface scattering of swimming eukaryotes. Proc Natl Acad Sci U S A 2013, 110:1187-1192.
- Schaap A, Dumon J, den Toonder J: Sorting algal cells by morphology in spiral microchannels using inertial microfluidics. *Microfluid Nanofluid* 2016, 20:125 http://dx.doi. org/10.1007/s10404-016-1787-1.
- Syed MS, Rafeie M, Vandamme D, Asadnia M, Henderson R, Taylor RA, Warkiani ME: Selective separation of microalgae cells using inertial microfluidics. *Bioresour Technol* 2018, 252:91-99.
- Godino N, Jorde F, Lawlor D, Jaeger M, Dusch C: Purification of microalgae from bacterial contamination using a disposable inertia-based microfluidic device. J Micromech Microeng 2015, 25:084002.
- Kim JYH, Kwak HS, Sung YJ, Choi HI, Hong ME, Lim HS, Lee JH, Lee SY, Sim SJ: Microfluidic high-throughput selection of microalgal strains with superior photosynthetic productivity using competitive phototaxis. *Sci Rep* 2016, 6:21155.
- 41. Best RJ, Lyczakowski JJ, Abalde-Cela S, Yu Z, Abell C, Smith AG:
- Label-free analysis and sorting of microalgae and cyanobacteria in microdroplets by intrinsic chlorophyll fluorescence for the identification of fast growing strains. Anal Chem 2016, 88:10445-11045.

By using a technology similar with the FACS, they presented a method for the screening and sorting of cyanobacteria and microalgae in microdroplets. The endogenous chlorophyll fluorescence in photosynthetic organisms is directly related to physiological properties within the cell. This direct and label-free detection method does not require cell staining or fluorescence labeling of cells, avoiding tedious, time-consuming and expensive sample preparation steps. The microfluidic system is able to efficiently deflect droplets containing low-chlorophyll cells in the collection channel (91%).

- Qu B, Eu YJ, Jeong WJ, Kim DP: Droplet electroporation in microfluidics for efficient cell transformation with or without cell wall removal. Lab Chip 2012, 12:4483-4488.
- Deng YL, Kuo MY, Juang YJ: Development of flow through dielectrophoresis microfluidic chips for biofuel production: Sorting and detection of microalgae with different lipid contents. *Biomicrofluidics* 2014, 8:064120.

- 44. Wang H, Kim HS, Guzman AR, Kim S, Devarenne T, Han A: Acoustophoretic droplet sorting system for high-throughput algal mutant library screening. 18th International Conference on Miniaturized Systems for Chemistry and Life Sciences October 26– 30 2014.
- Jo Y, Shen F, Hahn YK, Park JH, Park JK: Magnetophoretic sorting of single cell-containing microdroplets. *Micromachines* 2016, 7 http://dx.doi.org/10.3390/mi7040056.
- Deng YL, Chang JS, Juang YJ: Separation of microalgae with different lipid contents by dielectrophoresis. *Bioresour Technol* 2013, 135:137-141.
- Hadady H, Redelman D, Hiibel SR, Geiger EJ: Continuous-flow sorting of microalgae cells based on lipid content by high frequency dielectrophoresis. *AIMS Biophys* 2016, 3:398-414.
- Cai J, Chen M, Wang Y, Pan J, Li A, Zhang D: Culture and motion analysis of diatom, *Bacillaria paradoxa*, on a microfluidic platform. *Curr Microbiol* 2013, 67:652-658.
- Chen X, Zeng L, Wu Y, Gao Y, Zhao Y: Swimming characteristics of gyrotactic microorganisms in low-Reynolds-number flow: *Chlamydomonas reinhardtii*. Energy Ecol Environ 2017, 2:289-295.
- Barry MT, Rusconi R, Guasto JS, Stocker R: Shear-induced orientational dynamics and spatial heterogeneity in suspensions of motile phytoplankton. *J R Soc Interface* 2015, 12 http://dx.doi.org/10.1098/rsif.2015.0791.
- Chengala A, Hondzo M, Sheng J: Microalga propels along vorticity direction in a shear flow. Phys Rev E 2013, 87:052704.
- Wang W, Shor LM, LeBoeuf EJ, Wikswo JP, Taghon GL, Kosson DS: Protozoan migration in bent microfluidic channels. *Appl Environ Microbiol* 2008, 74:1945-1949.
- Latz MI, Bovard M, Van Delinder V, Segre E, Rohr J, Groisman A: Bioluminescent response of individual dinoflagellate cells to hydrodynamic stress measured with millisecond resolution in a microfluidic device. J Exp Biol 2008, 211:2865-2875.
- 54. Gemmell BJ, Jiang H, Buskey EJ: A new approach to microscale particle image velocimetry (μPIV) for quantifying flows around free-swimming zooplankton. J Plankton Res 2014, 36:1396-1401.
- Zheng GX, Li YJ, Qi LL, Liu XM, Wang XM, Yu SP, Wang YH: Marine phytoplankton motility sensor integrated into a microfluidic chip for high-throughput pollutant toxicity assessment. Mar Pollut Bull 2014, 15:147-154.
- Feng CY, Wei JF, Li YJ, Yang YS, Wang YH, Lu L, Zheng GX: An on-chip pollutant toxicity determination based on marine microalgal swimming inhibition. *Analyst* 2016, 7:1761-1771.
- Siebman C, Velev OD, Slaveykova VI: Two-dimensional algal collection and assembly by combining AC-dielectrophoresis with fluorescence detection for contaminant-induced oxidative stress sensing. *Biosensors* 2015, 5:319-336.
- Koman VB, von Moos NR, Santschi C, Slaveykova VI, Martin OJF: New insights into ROS dynamics: a multi-layered microfluidic chip for ecotoxicological studies on aquatic microorganisms. Nanotoxicology 2016, 10:1041-1050.
- Tahirbegi IB, Ehgartner J, Sulzer P, Zieger S, Kasjanow A, Paradiso M, Strobl M, Bouwes D, Mayr T: Fast pesticide detection inside microfluidic device with integrated optical pH, oxygen sensors and algal fluorescence. *Biosens Bioelectron* 2017, 88:188-195.
- Lefevre F, Chalifour A, Yu L, Chodavarapu V, Juneau P, Izquierdo R: Algal fluorescence sensor integrated into a microfluidic chip for water pollutant detection. *Lab Chip* 2012, 12:787-793.
- Wu C, Lillehoj PB, Sabet L, Wang P, Ho CM: Ultrasonication on a microfluidic chip to lyse single and multiple Pseudo-nitzschia for marine biotoxin analysis. *Biotechnol J* 2011, 6:150-155.
- 62. Zhang J, Liu S, Yang P, Sui G: Rapid detection of algal toxins by

• microfluidic immunoassay. Lab Chip 2011, 11:3516-3522. This is a typical microfluidic example showing the integration a complex biological analysis in a single microfluidic chip. The developed system is the first microfluidic method capable to quickly detect phycotoxines with a high sensitivity.

- 63. Weng X, Neethirajan S: Paper-based microfluidic aptasensor for food safety. J Food Saf 2018, 38:e12412.
- 64. Chet I, Fogel S, Mitchell R: Chemical detection of microbial prey by bacterial predators. J Bacteriol 1971, 106:863-867.
- Bell W, Mitchell R: Chemotactic and growth responses of marine bacteria to algal extracellular products. *Biol Bull* 1972, 143:265-277.
- 66. Seymour J, Ahmed T, Marcos, Stocker R: A microfluidic chemotaxis assay to study microbial behavior in diffusing nutrient patches. *Limnol Oceanogr Methods* 2008, 6:477-488.
- 67. Stocker R, Seymour JR, Samadani A, Hunt DE, Polz MF: Rapid chemotactic response enables marine bacteria to exploit ephemeral microscale nutrient patches. *Proc Natl Acad Sci U S A* 2008, 105:4209-4214.
- 68. Seymour J, Ahmed T, Durham W, Stocker R: Chemotactic
 response of marine bacteria to the extracellular products of

 response of marine bacteria to the extracellular products of Synechococcus and Prochlorococcus. Aquat Microb Ecol 2010, 59:161-168.

The microfluidic technology used in this study is simple but the results obtained are important in terms of ecology. The simple microfluidic system makes possible to test multiple ecological scenarii.

- 69. Miño GL, Koehl MAR, King N, Stocker R: Finding patches in a heterogeneous aquatic environment: pH-taxis by the dispersal stage of choanoflagellates. *Limnol Oceanogr Lett* 2017, **2**:37-46.
- Liu JW, Weinbauer MG, Maier C, Dai M, Gattuso JP: Effect of ocean acidification on microbial diversity and on microbedriven biogeochemistry and ecosystem functioning. Aquat Microb Ecol 2010, 61:291-305.
- 71. Padan E, Bibi E, Ito M, Krulwich TA: Alkaline pH homeostasis in bacteria: new insights. *Biochim Biophys Acta* 2005, 1717:67-88.
- 72. Choi HI, Kim JYH, Kwak HS, Sung YJ, Sim SJ: Quantitative analysis of the chemotaxis of a green alga, *Chlamydomonas reinhardtii*, to bicarbonate using diffusion-based microfluidic device. *Biomicrofluidics* 2016, **10**:014121.
- 73. Girault M, Beneyton T, Pekin D, Buisson L, Bichon S,
- Charbonnier C, Del Amo Y, Baret JC: High-content screening of plankton alkaline phosphatase activity in microfluidics. Anal

Chem 2018, **90**:4174-4181 http://dx.doi.org/10.1021/acs. analchem.8b00234.

They developed a microfluidic method to encapsulate phytoplankton and perform biological assay at a single cell level. The developed system focused on the alkaline phosphatase activity, an indicator of nutrient stress in numerous phytoplankton. The system can follow in real-time the kinetic of the alkaline phosphatase activity in every water in oil droplets containing plankton.

- Holcomb RE, Mason LJ, Reardon KF, Cropek DM, Henry CS: Culturing and investigation of stress-induced lipid accumulation in microalgae using a microfluidic device. Anal Bioanal Chem 2011, 400:245-253.
- 75. Kwak HS, Kim JYH, Woo HM, Jin E, Min BK, Sim SJ: Synergistic effect of multiple stress conditions for improving microalgal lipid production. *Algal Res* 2016, **19**:215-224.
- Lee DH, Bae CY, Han JI, Park JK: In situ analysis of heterogeneity in the lipid content of single green microalgae in alginate hydrogel microcapsules. Anal Chem 2013, 85:8749-8756.
- Erickson RA, Jimenez R: Microfluidic cytometer for highthroughput measurement of photosynthetic characteristics and lipid accumulation in individual algal cells. *Lab Chip* 2013, 13:2893-2901 http://dx.doi.org/10.1039/C3LC41429A.
- Samek O, Jonas A, Pilat Z, Zemánek P, Nedbal L, Tříska J, Kotas P, Trtílek M: A microfluidic chemotaxis assay to study microbial behavior in diffusing nutrient patches in vivo. Sensors 2010, 10:8635-8651.
- Guo B, Lei C, Ito T, Jiang Y, Ozeki Y, Goda K: A microfluidic chemotaxis assay to study microbial behavior in diffusing nutrient patches with fluorescence assisted optofluidic timestretch microscopy. *PLoS One* 2016, 11:e0166214 http://dx.doi. org/10.1371/journal.pone.0166214.

80. Kim HS, Guzman AR, Thapa HR, Devarenne TP, Han A: A droplet microfluidics platform for rapid microalgal growth and oil

production analysis. *Biotechnol Bioeng* 2016, **113**:1691-1701. This work is an excellent example of the integration of microfluidic modules to achieve both microalgae growth and oil production analyses. The single chip design involves the generation of droplets containing cells, culture of cells, synchronization of droplets containing cells with Nile red stained droplet, fusion of these two droplets to initiate the Nile red staining process, rinse the droplets, and an observation chamber to control the lipid content at a single cell level. This high-throughput droplet microfluidics-based microalgae screening platform is the first study where all steps occurring seamlessly on-chip.