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Review article

Advanced imaging for microalgal biotechnology

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ABSTRACT

The efficient operation of large-scale microalgae cultivation facilities requires continuous awareness of the culture condition. Although many conventional methods can be implemented in the laboratory, this goal can only be accomplished with non-invasive techniques such as spectral imaging based on the measurement of light backscattering of the culture surface. Several imaging methods are available, but we argue that developments in spectral approach will be among the essential breakthroughs for future advanced industrial-scale cultivation of microalgae.

The spectral methods initially developed for long-range (satellite and airborne) remote sensing of large water bodies are now increasingly employed for close-range monitoring of phytoplankton in natural ecosystems and large-scale microalgal cultures in open ponds and in closed photobioreactors. Similarly to high-throughput phenotyping which is now central to the progress of plant sciences, accelerated breeding, and precision farming, spectral imaging is gaining attention in microalgal biotechnology. Its power stems from the automated, rapid, non-invasive collection of large datasets, and the current advances in Machine Learning (ML). Their benefits include affordability, high information payload, and simplicity.

This review briefly presents imaging methods currently used in microalgal research, then focuses on spectral imaging. The background and biophysical foundation of remote sensing of communities and artificial monocultures is presented. Then, we elaborate on the methods for extracting relevant information from spectral images for monitoring of biomass accumulation, culture health, and target metabolites. Special attention was given to novel, trendy applications of ML to processing images and spectral data for the inference of actionable insights into the culture condition.

1. Introduction

In recent years, billions of dollars have been invested in biotechnologies involving prokaryotic cyanobacteria and eukaryotic algae (while cyanobacteria are not "true" microalgae, the term microalgae is used throughout for simplicity) [1,2]. The reason is that the microalgae-based products (e.g. high-value molecules, bioplastics, biofuels) and services (e.g. wastewater treatment) have supposedly low carbon footprints and a plethora of other benefits [3–7]. Consequently, commercial cultivation has intensified, and massive research is ongoing to develop microalgae-based environmental biotechnologies for bioproduct production, wastewater treatment, nutrient recovery, biofuel generation, and CO_2 mitigation [2,8]. To date, however, only a few microalgae species are currently domesticated and commercially cultivated, and the implementation of microalgae-based wastewater treatment is not common [2,8]. This is essentially due to numerous biological and engineering challenges that microalgae present for cultivation, resulting in high capital and/or operational costs [1,5,9,10]. Up-scaled cultivation of microalgae indeed requires large land areas and is sensitive to environmental and operational conditions [1,11,12]. To reach the highest productivity in terms of the biomass or related bio-products, light supply, nutrient availability, pH, and temperature are key operational parameters to optimize during cultivation [2,11]. While light supply and temperature are difficult to economically control at scale [13], other parameters can be controlled via online monitoring. The most common example is pH control via online pH monitoring and dynamic CO_2

Abbreviations: ANN, Artificial Neural Network; CNN, Convolutional Neural Network; Car, carotenoids; Chl, chlorophylls; HSRI, Hyperspectral reflectance images; ML, Machine Learning; RGB, Red Green Blue.

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injection [11,14].

Significant research is currently ongoing to develop advanced sensors and software to monitor microalgal cultures [15]. It is also expected that modelling effort, machine learning (ML), and the Internet of things (IoT) will enable automatic operation control to optimize microalgal productivity based on environmental conditions, culture conditions, and selected outputs [3,16]. New technologies have tremendous potential for monitoring biomass content and physiological status of the culture. As growth is highly dependent on the conditions experienced by the cells, it is critical that stresses, generally characterized by a change in morphology and/or pigments, are identified and mitigated as soon as possible in order to maintain productivity and prevent the loss of the culture [2,12,17]. While several instrumental approaches can be routinely used to monitor the status of a culture [18], automated, rapid, and non-invasive methods would be preferred. Imaging techniques and specifically spectral imaging techniques would offer such flexibility and efficiency [19,20].

This mini-review provides a concise outline of imaging-based approaches to monitoring of microalgal populations in artificial cultivation systems. Advantages and drawbacks of these approaches will be discussed briefly. The review then focuses on the foundations of spectral imaging and the potential use of this technology in microalgae farming. The issue of the knowledge-based selection of spectral features and processing techniques appropriate for different goals of large-scale culture monitoring is then considered. Finally, cases will be reviewed based on published reports describing the use of spectral imaging for solving real-life problems such as contaminant species detection, culture conditions assessment etc. We conclude that spectral imaging in combination with advanced approaches to the acquisition and processing of spectral images is a promising direction for monitoring industrial facilities for the cultivation of microalgae.

Noteworthily, phytoplanktonic organisms (including eukaryotic algae and prokaryotic cyanobacteria) are driving key biochemical cycles in aquatic environments but unfortunately can also lead to environmental damages as a result of intensifying anthropogenic eutrophication [21]. The development of tools such as remote sensing to detect phytoplankton in water has been ongoing for decades [22–24]. While further development is still needed for the technology to be applied globally [22], remote sensing has diverse applications either to characterize phytoplankton communities for biochemical modelling or to quantify their presence for water quality monitoring or harmful algae bloom surveillance (the latter being a critical step for bloom prevention/mitigation [25,26]). While the authors are aware of the use and technological development in imaging techniques for (global) phytoplankton ecological studies, this review focuses on the use of the spectral imaging for biotechnology.

2. Common methods available to monitor the status of microalgae cultures

The efficient operation of microalgal cultivation facilities requires continuous awareness of the culture condition regardless of the scale of the process. Particularly important parameters include biomass concentration, physiological condition (photosynthetic activity), biochemical composition (mostly pigment and lipid content) of the cells, and the presence of contaminating species [11,18,27,28]. This can be easily accomplished in cases of small-scale cultivation systems where the culture samples of limited volume would be representative of the whole culture. At a certain point during upscaling, especially in the case of (open) ponds, the single-point sampling approach ceases to yield comprehensive information about the whole facility so one needs to balance the sampling coverage and completeness of the information vs. the cost and labor of sampling and analysis.

Arguably, this problem can be solved with indirect measurements, e.g. on-line non-invasive sensing of the relevant parameters in situ [15]. The development of these solutions aims to achieve the acceptable (i) information payload, (ii) time resolution i.e., measurement frequency, and (iii) cost of the monitoring. Currently, the most widespread approach is monitoring of optical properties of the cultures, mainly the spectral intensity of the signal light back-scattered (reflected) by the culture [18]. This signal is loaded with valuable information about the microalgal cell population, although extraction and interpretation of this information might pose a challenge.

In laboratory conditions, the spectra of microalgal cell suspensions are commonly recorded by measuring the spectral transmittance of the samples, $T(\lambda)$, which is then represented as optical density or absorbance, Abs (λ). It can also be corrected for scattering to extract the spectral contribution of the pigments to the total attenuation of light by the cell suspension [29]. Notably, absorbance-based culture monitoring suffers from its limited dynamical range since the conventional spectrophotometers cannot reliably measure high optical density values typical of industrial cultivation systems. Further complications arise from cell self-shading [29,30]. However, the development of systems with inline dilution, ultra-high resolution, and broad bandwidth may solve those problems.

Other measurement methods can be routinely used to monitor a culture and its status (see summary in Table 1). Various abiotic (temperature, high light, pH, lack of nutrients) and biotic stresses (competition, viruses, bacteria, zooplankton, insects) can affect microalgae [28,31–33], and even when needed to trigger the production of certain molecules (e.g. lipids), these stresses led to a loss of productivity and a change in the biochemical composition of the cells [34]. Generally, stresses are characterized by a change in morphology, pigments, and the biosynthesis of stress-related products (e.g. exopolysaccharides, secondary carotenoids, reserve lipids [35]). As mentioned above, the dynamic nature of microalgae growth during cultivation means that rapid methods would be the preferred choice to detect an issue as early as possible during large-scale cultivation. Some of the methods that are currently available can be relatively simple and cheap, but require expertise, whereas others are costly and require time investments before data can be generated (Table 1). Therefore, there is a trade-off between efficiency and time.

Table 2 presents current imaging technologies that can be used for qualitative and quantitative detection of microalgae. While the main advantages of microscopy are the high optical resolution suitable for detailed observation of cell morphology, cellular structure, pigmentation, and possibility to use specific methods (e.g. fluorescence) for detection, this technique can be labor intensive, subjective, have limited spatial coverage, require significant training (i.e. to become an expert taxonomist), and is time constrained [39]. By contrast, advanced imaging technologies have high spectral resolution (i.e. fine distinction between similar species at a micro scale), but allow for a faster training process and can be streamlined with a high frequency of sampling in time and space [19,49,50,54].

Advanced imaging for microalgae monitoring enables precise measurement of parameters like chlorophyll content or the capture of spectral signatures linked to biochemical compounds (see example in Box 1). Consequently, these techniques could facilitate high-throughput screening for desirable traits, accelerate strain selection, and provide real-time insights into microalgae cultivation processes. If streamlined, spectral imaging could help to reduce costs related to microalgae cultivation [55] by improving the information payload (e.g. measuring growth as well as the physiological state of a culture) and availability (providing updates daily or hourly), ultimately helping in preventing the loss of cultures. In our view, it would be a suitable method for comprehensive monitoring i.e., to holistically study the desired traits and the environment of large (open-pond) cultivation facilities.

Table 1
Summary of the main methodologies used to monitor microalgal biomass and its physiological status.

Methodology	Description	Example
Dry weight and optical density	Biomass is the most relevant variable measured during cultivation because the biomass generally holds the value for commercial applications. Standard measurement methods such as dry weight and optical density are commonly used to measure the concentration of the biomass [18,36,37]. While the loss attributable to endogenous respiration needs to be considered at high biomass concentrations, an abrupt loss of biomass would indicate that a stress is occurring. However, a delayed recording of a statistically significant biomass loss during stress could mean that it is too late for preventive or even for corrective measures.	[36,37]
Cell counting	Cell counting using a haemocytometer is simple and easy, but this method can be prone to significant error and discrepancies between operators. However, combined with cell staining, this method also allows cell viability estimation [35]. Cell counting can be automated with the use of a flow cytometer [36,38], but this requires prior calibration with standardized particles. Cell concentration and morphology can also be measured with flow cytometry and the use of a specific dye can allow the measurement of cell viability, which provides significant physiological information [39].	[38]
Cell observations	Cell observations under an optical microscope can allow the identification of organisms present in the culture, morphological changes and/or the presence of intracellular structures [40,41]. However, this task can be time consuming and prone to error without training [39]. Advanced techniques such as transmission electron microscopy can also be useful for investigating morphological changes [38], but they are time-consuming, require expensive equipment and expertise.	[40,41]
Genetic marker	Genetic markers such as 16S or 18S RNA would provide an accurate species identification, but the samples need to be extracted, purified, sequenced, and identified [39,42,43]. Consequently, time could be an issue. MALDI TOF could fasten the identification process [44], however, the availability of extensive libraries is limited.	[39,44]
Biomarker	Biomarker molecules such as ATP, or DNA (and DNA degradation products) can provide an indication of cell lysis if their concentration is elevated in the cultures [35]. Several assays are available, but all of them require specific equipment and can be time consuming. Pigment composition of phytoplankton and fatty acids composition have been used to identify different phytoplankton at the class level [45]. However, chlorophyll *a* is the most common biomarker and the measure of absorbance generally targets chlorophyll *a*. As chlorophyll *a* is altered or even degraded by various factors, changes in the chlorophyll content and its fluorescence by leveraging the pulse amplitude modulation (PAM) technique can indicate the occurrence of a stress [33].	[33,45]
Imaging techniques	While imaging techniques can simply involve taking an image for further observation, automated techniques are being used and developed. For example, the FlowCAM system measures particle count, size, and morphology with Flow Imaging Microscopy [46]. Similarly, zooplankton that are unwanted during microalgal cultivation have been widely studied as water quality indicators. ZooScan, a laboratory-based scanner which analyses zooplankton preserved samples and ZooCAM, an in-flow imaging system designed for live zooplankton imaging, have been shown to successfully classify and quantify zooplankton (both instruments were shown to be complementary [47]).	[46,47]
Advanced imaging techniques	Advanced techniques such as spectral imaging are currently being developed in biotechnology due to their tremendous potential [20,48–53]. In particular, spectral imaging techniques and hyperspectral imaging involve spatially resolved measurements of the spectral intensity of light backscattered by the microalgal cultures. These measurements can be done remotely, so they are especially suitable for monitoring of large cultivation facilities. Essentially, this approach resembles the one used in remote sensing of vegetation or phytoplankton. It identifies spectral features sensitive to the content of a pigment or another trait in question, enhances the accuracy of microalgae classification based on selected relevant features, minimizes redundant information, and is insensitive to other traits of the studied culture. The reflectance-based approach retains sensitivity in a broad range of pigment concentrations; hence it can be used for monitoring high culture densities. Importantly, these approaches can be implemented with the use of UAV-borne reflectance imaging spectrometers. Both conventional and advanced imaging can be automated via modern ML-based algorithms.	This review

3. Advanced imaging techniques

3.1. Multi- and hyperspectral imaging

Depending on the spectral resolution determined by the number of available spectral channels, their widths, and the overlap between them, imaging techniques are categorized into “conventional” (RGB), multi-spectral (3–20 discrete spectral channels) and hyperspectral (ranging from several dozen to hundreds of continuous spectral channels), each with a different information payload (Box 1). Spectral image analysis leaped forward when the rapid progress in cheap computing power, image sensor hardware, and mathematical methods sparked the boom of machine learning (ML) [56]. Admittedly, both conventional and emerging approaches have their strengths and limitations.

In contrast to RGB pictures limited to the visible part of the electromagnetic spectrum, multi-spectral and hyperspectral imaging record a broader spectrum and allow the detection of specific features invisible to the naked eye (Box 1). These advanced imaging techniques are therefore powerful tools becoming more and more used for various applications including geology, agriculture, and biotechnologies, to name a few [23,54,57,58]. In the case of microalgal biotechnology, a useful parallel can be drawn with plant sciences where tremendous progress was made by the advent of high-throughput phenotyping—a comprehensive assessment of diverse traits constituting phenotype, in large populations of plants [59,60]. For example, hyperspectral imagers and machine learning techniques such as image processing with Support Vector Machines (SVMs) and Convolutional Neural Networks (CNNs) have been successfully developed to characterize phenotypic traits in plants such as diseases [61–63]. The concept of phenotyping is gaining attention of researchers in the field of microalgae biotechnology [48–52], especially for bioprospecting of strains, advanced laboratory evolution (ALE), and cultivation parameter optimization [64].

Table 2
Comparison of potential performance of different imaging methodologies regarding monitoring of microalgae communities and industrial cultures.

Approach	Manual (microscopy)	Semi-automated (flow cytometry)	Optical sensing (imaging)	
Parameter			Proximal (close range)[a]	Remote
Distance to the sensor	–	–	0–100 m	100–6000 m (airborne)–800 km (satellite)
Spatial resolution	–	–	0.1 mm–50 cm	0.5 m–1 km
Time resolution	~1 d	Arbitrary	Arbitrary	~15 d
Specific performance of surveillance	Several samples representative of limited culture volume	Several samples representative of limited culture volume	Spot based – up to 0.1 km^2 per day	0.1–10 mil. km^2 per day

[a] Including handheld sensors placed next to the measured object and sensors mounted on airborne devices such as UAVs.

3.2. Integration of deep learning and artificial intelligence for image analysis

Early approaches to digital image processing were heavily grounded in brightness and colour manipulation, as well as the creation of handcrafted features [96]. These techniques are extremely laborious and eventually were outpaced by machine learning (ML) algorithms, which find pertinent features in the data itself. ML algorithms are particularly well-suited for solving classification and detection problems such as identifying cells on a microscopic image, but they could also be used for regression problems such as deriving biochemical parameters from spectra.

Machine learning techniques fall into two broad categories: "classical" ML, comprised of algorithms such as Logistic Regression, Decision Trees, Random Forests (RF), eXtreme Gradient Boost (XGBoost), and Support Vector Machines (SVM), among others, and deep learning (DL), which utilizes artificial neural networks (ANNs) with a wide range of architectures (further details about some of these methods and their caveats can be found in the Online Supplementary S1). Classical ML algorithms have better explainability than their deep learning counterparts and tend to perform better with low volumes of training data, while ANN-based methods excel at finding complex, multi-scale patterns and are indispensable in image analysis. Regardless of the approach, input data in ML is viewed as a set of features comprising a multi-dimensional space, and the goal of ML algorithms is to find meaningful relationships between those features. Non-DL techniques are normally used with semantic features such as pH, turbidity and total nitrogen (see e.g. [97,98]), whereas ANNs are flexible and especially valuable in tasks such as object detection or image segmentation essential e.g. for taxonomic assignment [99].

A specificity of spectral imaging is the highly pronounced curse of dimensionality, or Hughes phenomenon [100]: Statistical learning becomes increasingly inefficient in high-dimensional spaces with collinear features, which is the case for hyperspectral images. Consequently, application of deep learning to spectral images requires feature selection either as a pre-processing step or as a core part of ANN architecture. Thus, selection of appropriate spectral bands for quantification of the productivity and/or taxonomic composition either of industrial cultures (or in natural phytoplankton communities) is of paramount importance to monitoring via spectral imaging (Box 1). The concept of optical monitoring is based on the existence of a tight relationship between the optical properties of the cell suspension and the changes in cell density, their biochemical and taxonomical composition during balanced growth and stress acclimation. This is the case e.g. for secondary carotenogenesis coordinated with the accumulation of lipids induced by nutrient deprivation and other stresses [32,101,102].

Finding the relationship between spectral data and biophysical and chemical characteristics is accomplished either by the traditional semi-analytical approach when the search for the spectral bands is guided by the spectroscopy of the phytoplankton cells and/or their constituents [103] or, in frame of emerging approach, by ML algorithms which can be completely "unaware" of the spectroscopic properties of the object. The latter is less relevant for the analysis of spectral images of bulk microalgal cultures which normally lack morphological features (their information payload is concentrated in the spectral dimension, [98]). On the other hand, image analysis with ML is very promising for automated identification of the target microalgae and contaminant species with optical microscopy including its microfluidic variant.

For identification of microalgae on microscopic images, segmentation tasks are the most pertinent; consequently, neural network architectures such as U-Net [104], Mask R-CNN [105], and, more recently, ViT [106] and their derivatives are effective. These architectures are based on intermediate representation of image and its regions as a compressed feature vector, followed by a "decompression" of said vector into a label map. So far, these techniques have not been commonly seen in microalgal research (some examples include [107,108]), but there is little doubt they will garner wide adoption in years to come.

Optimization of input features using deep learning algorithms remains a hot area of research. In particular, it combines supervised and unsupervised approaches to identify the most informative spectral ranges and perform band selection using Deep Belief Networks (DBNs) [109]. Alternatively, [110] used high-level CNN features to achieve explainability in band selection, while [111,112] leveraged the attention mechanism in CNNs for the same purpose. While extensive, this subfield of research presently yields more questions than answers. Still, without dimensionality reduction deep learning methods fail to generalize well [113].

Recent studies have also shown the potential for hyperspectral imaging in vivo: thus, [114] demonstrated deriving highly accurate and granular benthic maps from underwater hyperspectral imagery. In this study, the classification model performed well with F_1 (i.e. the harmonic mean of precision and recall) scores of 80 % ± 5 % with either 11 or 43 target labels, referring to either taxonomic units or substrate classes. Remarkably, the performance difference between ANN-based approach and RF classifier operating on preprocessed data was found to be minor, and ANN performance was substantially dependent on the size of the training set (~7–10 % improvement as the training set grew from ~1 million to ~6 million unique pixels).

Despite their power, ML-based image analyses have limitations. The most important is that these approaches seldom take into account actual optical properties of the culture (spectral absorption and backscattering) and function essentially as a "black box". Multiple authors have outlined the dire need in creating large, open annotated datasets for training and validation of ML algorithms [115–117]. This issue is further exacerbated in the case of spectral imaging, as there may be substantial variations in the spectrometer hardware, including vastly different numbers of spectral channels and resolution, both spectral and spatial, as well as other study parameters [118]. Neural network models do not provide information on the relationship between the spectral features distribution and system variables. As such, one cannot predict the target parameters (e.g., cell concentration) for different experimental conditions, and would have to repeat the entire set of procedures, from experiment to neural network learning, to predict the target parameter(s) if the experimental conditions are changed. This means that considerable efforts are still needed to develop broad methods to be used for commercial cultivation. At present, combining handcrafted

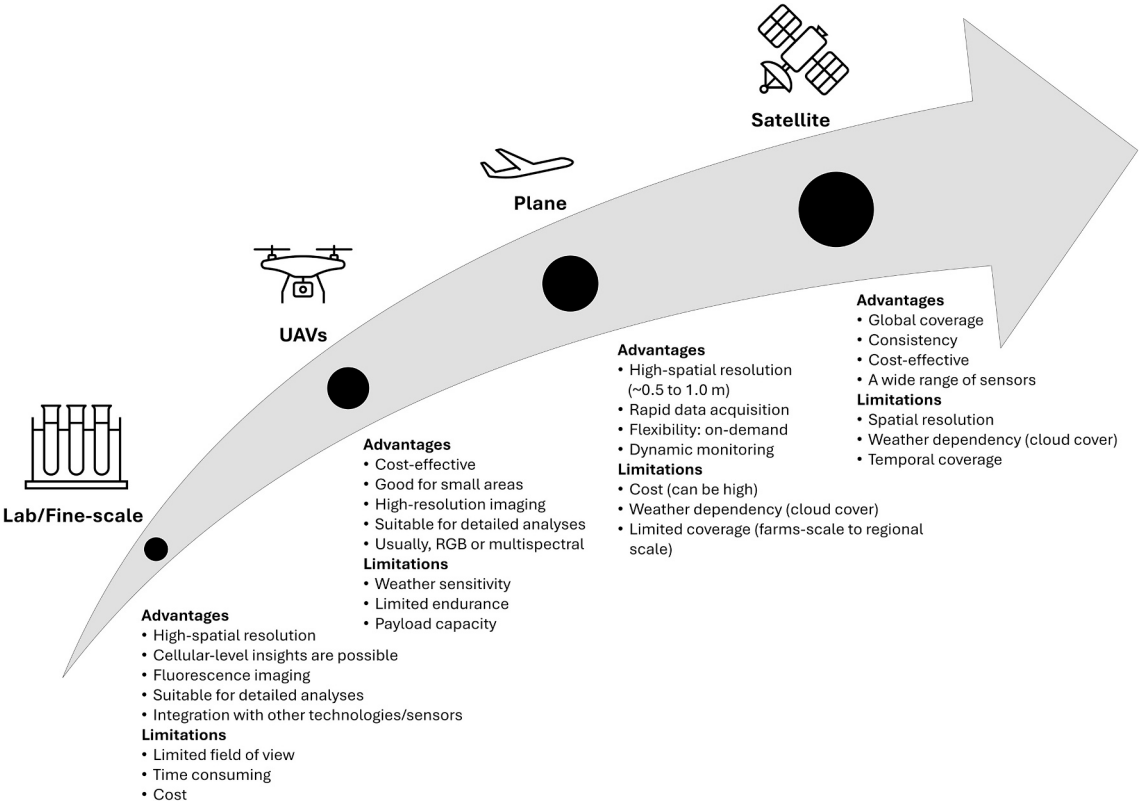
Box 1
Fundamentals of imaging data acquisition and processing

The mid-20th-century introduction of satellite technology marked a significant milestone, enabling large-scale environmental monitoring [57,65]. As technology progressed, airborne remote sensing emerged as a valuable complement to satellite observations [66–68]. Aircraft equipped with sensors offered higher spatial resolution and greater flexibility in data collection, especially beneficial for specialized applications like agriculture, forestry, and disaster response, which require detailed and timely information [69,70]. In recent years, proximal sensors have gained prominence, offering extremely high spatial resolution near the target area/system [71,72]. Currently, we are witnessing a bidirectional flow in platforms, with advancements in technology revitalizing the appeal of high-resolution satellite remote sensing [73–76]. Given the array of available options, it becomes crucial to categorize the essential steps associated with imaging acquisition and analysis as follows:

B1. Scale

The first step in utilizing imaging data is to define the acquisition scale distinctly. Whether through satellites or proximal sensing, the choice depends on the application and the specific research question being addressed, each offering distinct advantages, as shown in Fig 1.1.

Fig 1.1. The figure illustrates four different scales at which microalgae imaging can occur. The appropriate scale is selected based on the application and research question.

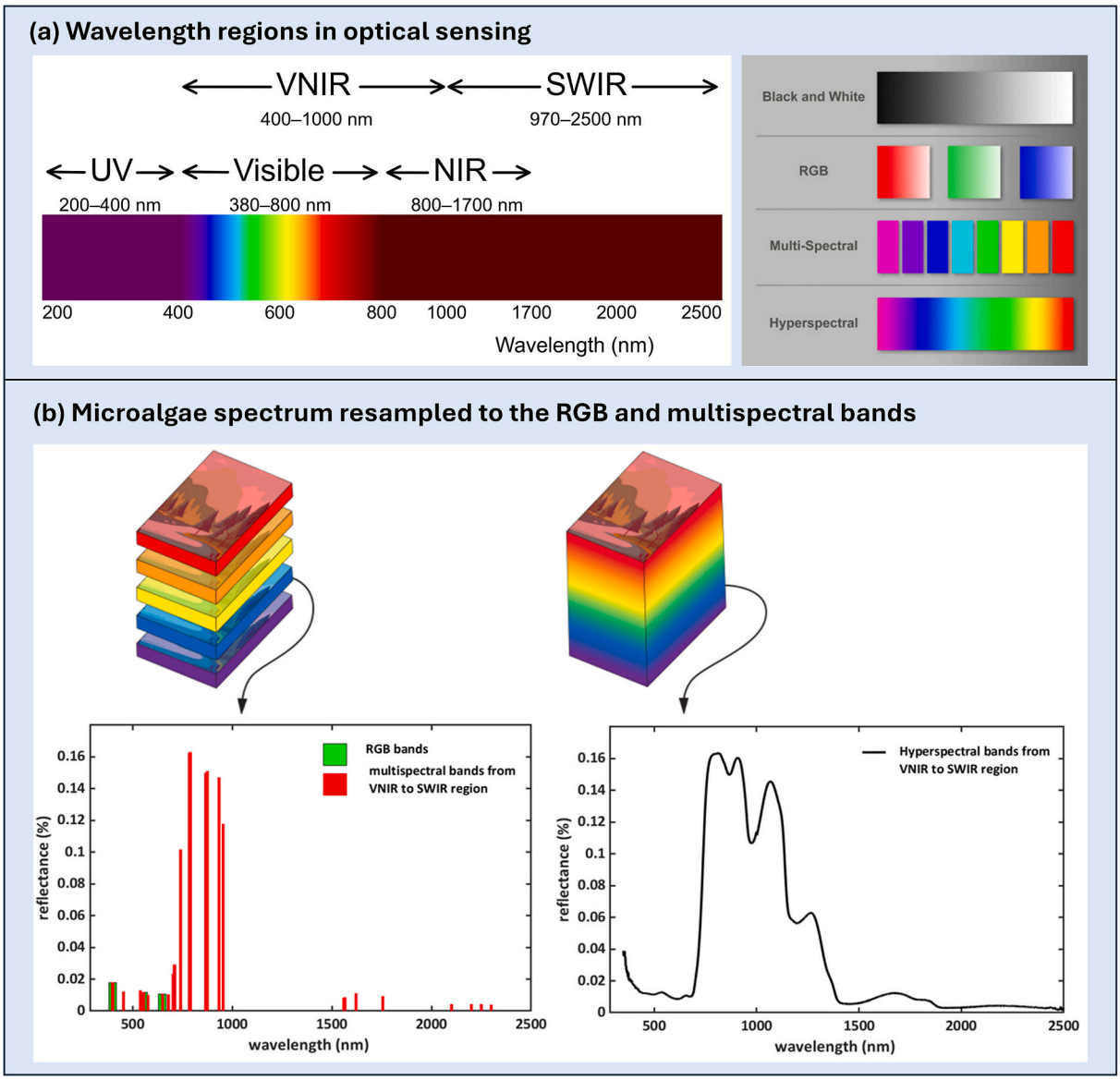


B2. Sensing technology

At different scales, various sensing technologies are available. Conventional colour sensor cameras are restricted to the 400–700 nm visible range, with three colour sensors (blue, green, and red) used to estimate the true colour of each pixel from an image [77,78]. Advanced multispectral cameras/sensors allow measuring the reflected light in the visible to near-infrared spectral range in discrete bands (Fig 1.2), revealing more distinct characteristics of a particular system. Increasing the number of channels being measured allow to determine the spectral profile of the system. A spectral imaging system produces a two-dimensional spatial array of vectors that represents the spectrum at each pixel location. The resulting three-dimensional dataset contains two spatial dimensions and one spectral dimension known as the data cube or hypercube. The third dimension stores the full spectral range, usually within 370–2500 nm, which comprises deep blue, blue, green, red, near infrared, and short wave infrared spectral regions [79,80]. It should also be noted that the operational spectral range is dependent on the semiconductor material used in the sensor, and imagers with wide spectral ranges leverage several detectors, increasing both capital and operational costs. For the visible and near-infrared range up to about 1100 nm, silicon-based sensors are used near-universally [81], while infrared detectors are more varied, common choices include germanium (Ge), indium gallium arsenide ($In_{1-x}Ga_xAs$), and mercury cadmium telluride (MCT, $Hg_xCd_{1-x}Te$), among others. These infrared sensors typically cover ranges of 900–1700 nm and beyond [82].

Spectral imaging has been instrumental for the development of advanced vegetation indices, growth indices, and environmental indices [54]. With hyperspectral data, one can generate spectral libraries of different plants and their surroundings to identify advanced traits of similar plants and, arguably, other photosynthetic organisms, at different locations, enabling generalizability in phenotyping [83,84]. However, both acquisition and processing of spectral images may prove to be challenging. While different sensor platforms — drone, airborne, satellite — have issues specific to their viewing geometry, the respective data processing pipelines are largely universal [85]. Below we give a brief overview of the required steps.

Fig 1.2. a) shows the range of the electromagnetic spectrum covered in (passive) optical remote sensing. b) depicts the difference between the contiguous spectrum of hyperspectral versus discrete bands in multispectral and RGB sensors.



B3. Pre-processing of data

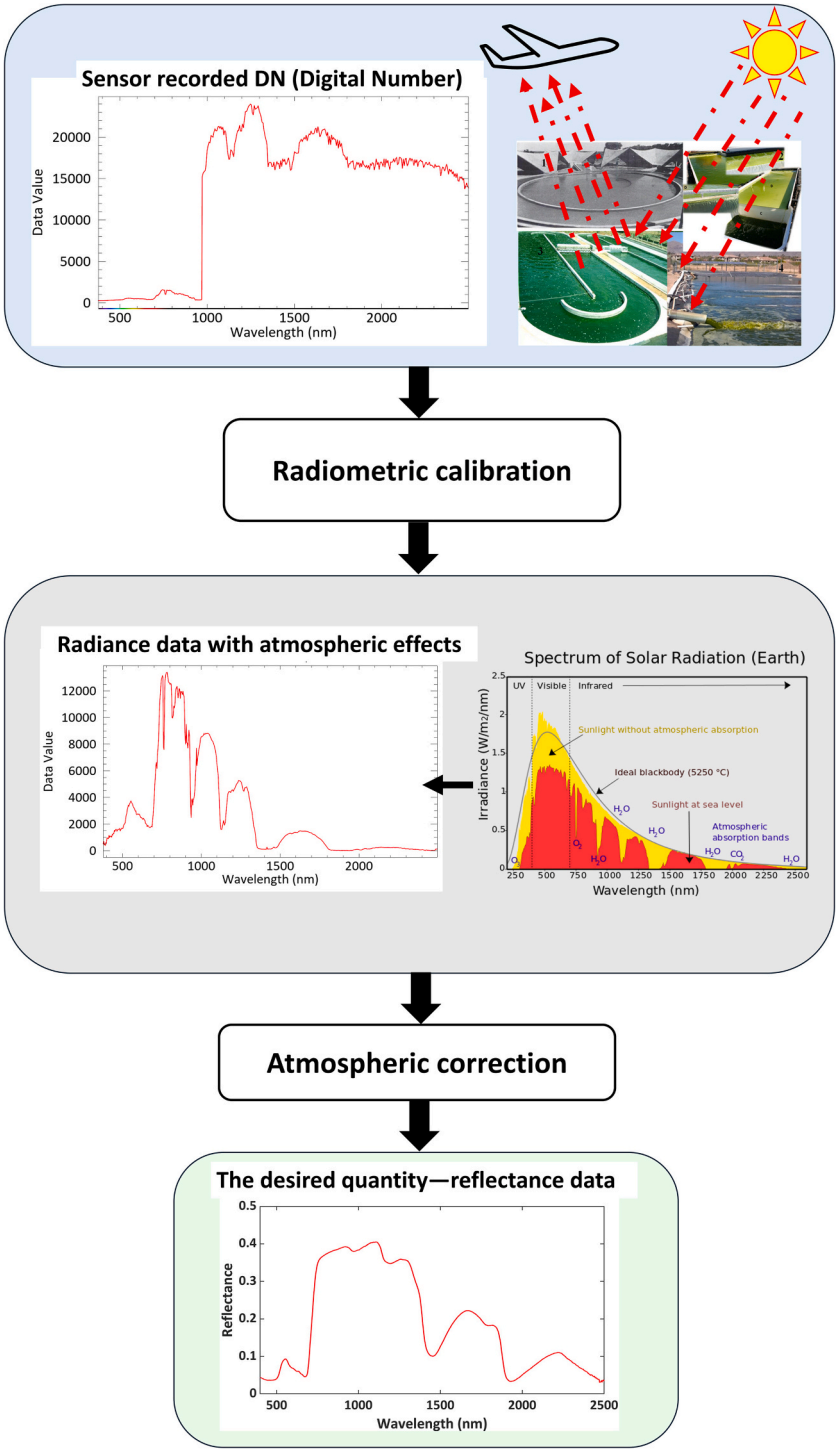
RGB, multispectral, or hyperspectral images are versatile and useful tools for obtaining both qualitative and quantitative information. However, data acquired from remote sensing platforms are not readily available for analysis, as it is not a reflectance product — i.e. it is expressed in terms of sensor units, not physical quantities. Factors such as sensor noise (including bias and offset), atmospheric conditions, viewing and illumination geometry, the contribution from background reflected radiation, and platform movements (roll, yaw, and pitch) [86,87], can all distort the measured radiation intensity. Thus, before utilizing remote and satellite sensing data, it is important to perform data pre-processing to remove these artifacts.

Depending on the application and goals, numerous pre-processing steps are available. We have outlined some of the standard pre-processing steps (Table 1.1) and discussed each type of correction below:

Radiometric calibration: Sensor-recorded data is referred to as Digital Number (DN), which is an electronic signal dependent on the radiometric resolution of the sensor. Each sensor requires calibration for its gain and offset for converting raw sensor measurements into radiance products, enabling accurate and reliable interpretation of the data (Fig. 1.3). Radiometric calibration is often performed using radiometric calibration parameters provided with the sensor. Reflectance standard measurements could be needed in the field (Table 1.1 and [86,88,89]).

Atmospheric correction: For passive remote sensing, where solar energy is the source of light when solar radiation propagates through the atmosphere, radiation is scattered and absorbed by the Earth's atmosphere. This effect is generally depicted in the radiance plot, where the absorption effect is apparent (Fig 1.3). Because atmospheric conditions vary significantly in space and time, it is critical to remove the atmospheric effect to prevent significant deviation in reflectance output. For instance, when conducting time-series analysis or monitoring changes over time, atmospheric correction ensures consistency in the reflectance values, allowing for more accurate analysis. Noteworthily, under laboratory conditions or with the use of sensors mounted on UAVs, atmospheric correction is generally not needed provided a reflectance standard is imaged simultaneously and/or a controlled light source is used. This adds to the value of close range sensing, since atmospheric correction is much harder to perform accurately than sensor-specific radiometric/geometric corrections [86,89].

Fig 1.3 Radiometric calibration and atmospheric correction components are shown for in-field conditions.



Geometric correction: Geometric correction aims to correct for the artifacts created by platform movements (roll, yaw, and pitch) that cause objects to "stretch".

After performing the standard calibration and correction processes the next tasks are to perform image enhancement and noise reduction. This is subject to the purpose of the enhancement, the sensor signal-to-noise ratio, and imaging conditions. Thus, this processing step is not always needed. There are several tools and coding platforms available to perform these tasks [90,91].

Table 1.1. Summary of the pre-processing steps needed for each type of sensors in proximal and remote sensing (● = required; ○ = optional; empty cell = not required). Image enhancement and noise reduction are optional steps for all type of sensors.

		Pre-processing step		
Acquisition	Sensor type	Radiometric calibration	Geometric correction	Atmospheric correction
Proximal (close range)	RGB	●		
	Multispectral	●		
	Hyperspectral	●		
Remote (long range)	RGB	●	●	○
	Multispectral	●	●	●
	Hyperspectral	●	●	●

B4. Data analyses

Feature extraction and machine learning (see also the Online Supplementary S1) are generally employed to create spectral libraries and reveal various characteristics of a system. This is further discussed below in relation to microalgae biotechnology.

B5. Case study — Non-destructive assessment of microalgae samples through proximal sensing

Hyperspectral imaging measures the reflectance of light from sample surfaces. With high spectral resolution, from visible to shortwave infrared (SWIR), the reflectance properties of photosynthetic organisms can reveal much about chemistry of their biomass [19,56,84]. Once calibration models are developed, hyperspectral analysis allows for non-destructive measurement of pigments, nutrient content, water status, and chemical composition.

The potential of hyperspectral for monitoring biomass concentration or growth phases has been demonstrated during several studies (Table 3). As another example, we used a case scenario of P-depleted vs P-replete microalgae. As microalgae can store P, their potential use for P removal from waste is currently of significant interest. This has been reviewed by others and was therefore used as an example [40,41,92]. The green alga *Chlamydomonas reinhardtii* grown for 5 days on low P medium were subsequently supplied with different P concentration (0, 20 and 40 mg-P · L^{-1}) and the spectral signatures of each culture was measured 20 h after P supplementation.

Data acquisition

Spectral measurements of *C. reinhardtii* cultures supplemented different concentration of phosphorus (0, 20 and 40 mg-P L^{-1}) were collected using an hyperspectral probe—ASD FieldSpec Pro (Analytical Spectra Devices, Inc., Boulder, CO, USA)—mounted on a stand, ensuring that the microalgae samples were positioned at a distance of 2 cm from the ASD probe. The ASD measures the Vis-NIR range (350–2500 nm), with a full-width half maximum of 3 nm in the Vis and 10 nm in the NIR, and an output of 1 nm spectral resolution.

Data pre-processing

In this case scenario, proximal sensing was used, the spectral data therefore do not require extensive pre-processing in comparison to hyperspectral from remote sensing as mentioned above. However, the ASD spectra were affected in reflectance values at the splice of the three sensors at 1000 and 1800 nm of the spectroradiometer. Consequently, an ASD splice correction was implemented using the ViewSpec Pro software which is based on the method described by [93].

Results and further processing

The reflectance of each culture is depicted in Fig 1.4 below, covering the VNIR region (<1000 nm) and SWIR region (>1000 nm), with a spectral resolution of 1 nm. While the three cultures visually appeared similar and all had an optical density of ~0.4 at 683 nm, they could be differentiated in their spectral signatures. Specifically, the higher the P supplied, the higher the reflectance. Because the differences in spectra were not related to biomass concentration, the clear difference between the spectra suggests a change in the biochemical composition of the microalgae when transitioning from P-depleted to P-replete conditions. Microalgae are known to have different biochemistry and to store significant amount of P as polyP during such transition [41,94,95]. Consequently, the spectral difference observed could be related to a change in biochemical composition of the cells including the accumulation of polyP. Critically, our study is preliminary, more samples (i.e. 5–10 samples per concentration) and analyses would be required. Further analysis including feature extraction and machine learning could then be employed to develop model to predict the P status of the cells and to reveal various characteristics related to P supply in microalgae.

Fig. 1.4. Spectral measurements of *C. reinhardtii* cultures supplied different concentrations of P (0, 20 and 40 mg-P · L^{-1}) in the spectral range of 350–1400 nm. Data represent the mean ± standard deviation from 10 ASD measurements.

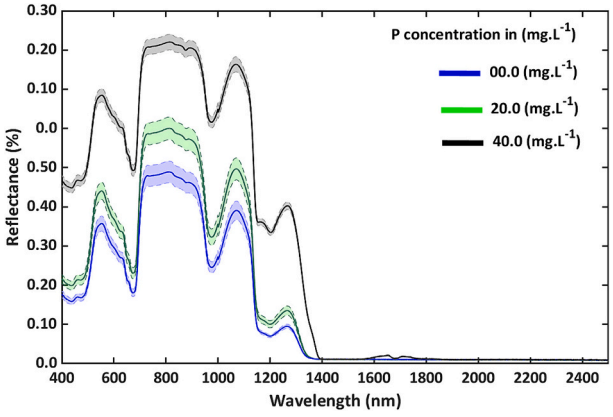


Table 3
Table summary of the studies that developed and used conventional and spectral imaging to measure growth and physiological state as well to detect contaminants in suspended microalgal cultures.

Cultivation system	Organism	Sensed parameters	Imaging equipment	Processing algorithm	Ref.
RGB/monochrome					
500 mL flask	A consortium (*Desmodesmus* sp., *Scenedesmus* sp., *Dictyosphaerium* sp., and *Klebsormidium* sp.)	Biomass concentration (g-DCW·L^{-1})	Smartphone dual camera	RGB to Luminance conversion and calibration vs. DW	[128]
Concentric tube airlift photobioreactor (5.45 L)	*Synechococcus* sp. PCC6301	Biomass concentration (OD_{600})	RGB (24-bit true colour, 320 × 240 pixels)	Light Distribution Image analysis vs. feedforward neural network with a backpropagation learning	[131]
Stirred tank	*Chlorella sorokiniana*	Light distribution in the vessel	Digital camera (COOLPIX S3100, Nikon)	Decomposition (brightness extraction)	[132]
Flasks (3 L)	*Chlorella vulgaris* KCTC AG10032, *Botryococcus braunii* UTEX 572, and *Ettlia* sp. YC001	Biomass concentration (g-DCW·L^{-1})	CCD camera (Sony NEX-7 with 100 mm F2.8 lens; Sony Corporation, Japan)	RGB colour/grayscale analysis	[36]
Panel photobioreactor (720 mL)	*Rhodobacter capsulatus* DSM 1710	Biomass concentration (OD_{660} and g-DCW·L^{-1}) and distribution in photobioreactor	Microsoft Webcam SCB-0340 N	RGB colour/grayscale analysis	[133]
Erlenmeyer flask (1 L)	*Isochrysis galbana* (clone T-ISO)	Cell number	Canon SD750 with 35–105 mm Canon Zoom lens	RGB/HIS analysis	[134]
Draught tube (1.5 L)	*Chlorella* sp. ATCC 14854	Lumostatic growth regime control	Canon 450D	RGB colour/grayscale analysis	[135]
Three types of PBR (cylindrical, 5 L; panel, 80 L; bag, 400 L)	*Chlorella vulgaris*	Biomass concentration (g-DCW·L^{-1})	Smartphone camera (Sony IMX214)	RGB colour/grayscale analysis (mean, median or mode)	[55]
ns[a]	*Planktothrix agardhii* CCNP 1305	Morphological change	YenCam HD camera (Yenway Microscopes)	Three different network architectures (AlexNet, 3ConvLayer and 2ConvLayer), four different optimizers (Adam, Adagrad, RMSProp and SDG) and five different image segmentations methods tested (Canny Edge Detection, Morphological Filter, HP filter, GrabCut and Watershed)	[31]
Spectral					
Bottles (2 L)	*Chlorella* sp. UTEX 2168, *Anabaena variabilis* ATCC 29413-U	Biomass concentration (g-DCW·L^{-1}), invasive species, culture health	RGB webcam (Logitech, Pro 9000)	RGB as tri-band multispectral simulation	[48]
Flasks (0.25 L)	*Microcystis* sp. CCAC 3504 B, *Synechococcus* sp. CCAC 2944 B, *Cryptomonas ovata* CCAC 0064, *Peridinium cinctum* CCAC 0102 B, *Desmodesmus maximus* CCAC 3524 B	Culture growth	SpecimIQ frame-based imaging hyperspectrometer	Ratio and difference spectral indices for NIR and RE: A/B, A/(A + B) or (A − B)/(A + B)	[51]
Flasks (0.25 L)	CCAC 3504 B *Microcystis* sp., CCAC 2944 B *Synechococcus* sp., CCAC 0064 *Cryptomonas ovata*, CCAC 0102 B *Peridinium cinctum*, and CCAC 3524 B *Desmodesmus maximus*	Contaminant presence, biomass concentration (g-DCW·L^{-1})	SpecimIQ frame-based imaging hyperspectrometer	Per-pixel (per-spectrum) processing of hyperspectral reflectance images with CNN	[50,53]
Flask (0.2 L)	*Nostoc* sp., *Scenedesmus almeriensis*, *Spirulina platensis* and *Chorella vulgaris*	Biomass composition	Minolta CM-3500d colorimeter	Normalized relative light absorption (for each absorbance band per sample) input to ANN	[136]
Beaker (0.25 L)	*Chlorella sorokiniana* (CCAP No. 211/8 K)	Biomass concentration (g-DCW·L^{-1})	Particle Track 400	Chord length distribution data processed with support vector regression and random forest regression modelling	[137]
ns	*Chlamydomonas reinhardtii*	Biomass concentration (cell·mL^{-1})	Miniature spectrometer USB6500pro	Fluorescence spectrum (1 nm between 660 and 760 nm) input to ANN	[138]
Harvested cells 1 mL in Eppendorf tube	*Chlorella vulgaris* (CV 211-11b)	Cell viability	Sysmex CyFlow Space flow cytometer, mounted with blue laser (488 nm), FSC, SSC detectors and three fluorescence channels (FL1: 536/40, FL2: 590/50 and FL3: 675/30 nm).	Clustering processing to segregate the three cell populations (active cells, non-viable cells and debris)	[38]
ns	*Chlorella vulgaris* and *Scenedesmus almeriensis*	Biomass classification	FlowCAM that combines flow cytometry, microscopy, and fluorescence detection techniques.	FlowCAM outputs images fed to ANN	[139]
ns	*Phaeocystis*, *Chlamydomonas* and *Chaetoceros*	Biomass classification, identification, and growth	A transmission hyperspectral microscopic imager system is mounted on a traditional microscope (RX50, SOPTOP, China)	ns	[52]

(*continued on next page*)

Table 3 (*continued*)

Cultivation system	Organism	Sensed parameters	Imaging equipment	Processing algorithm	Ref.
6-Well plates	*Chlamydomonas reinhardtii*	TiO_2 nanoparticle effect on culture performance	SeedReporter (PhenoVation B.V., Wageningen, The Netherlands) with a CCD-chip	Vegetation indices NDVI, ARI, CI	[129]
2.5 L to 11 m^3 PBRs	*Synechocystis* sp. and mixed cultures	Biomass concentration, growth phase and chlorosis	Sensor mounted on a Pika L camera system from Resonon	Spectronon software from Resonon with in-built library with image processing tools such as support vector machine and hyperspectral vegetation indices	[140]

[a] ns—not specified.

features such as spectral indices with the power of deep learning is the most promising approach for achieving robustness and overcoming this generalization bottleneck. In a similar vein, hybrid approaches incorporating physical considerations (e.g. noise equivalence [119] maybe used in the future to achieve better predictability and generalization of deep learning methods [120].

4. Advanced imaging in microalgal biotechnology research

The use of advanced (i.e. spectral) imaging techniques to detect or identify phytoplankton is not new and has been widely used for ecological studies [23]. For instance, satellite remote sensing has been widely applied to determine phytoplankton community structures via several missions [24,25,58,121,122]. While earlier methods focus on identifying dominant group, newer approaches try to identify groups with similar ecological roles (referred as phytoplankton functional types). These newer approaches use algorithms that retrieve phytoplankton size classes, size composition, or particle size distribution from the satellite data [24]. Please refer to [23], for an excellent review and guide on the different type of algorithm used in the field. Noteworthy, the taxa-specific algorithms that have been developed are currently not applicable globally and they can lack uniqueness [22]. Differentiating taxa can indeed be difficult when dominant species share similar pigment features [58]. As recommended by [22], the continuity of global missions with the development of algorithm will improve the versatility and accuracy of the data. Critically, the same need would apply to the field of microalgal biotechnology.

A similar approach to the one described above could be applied to cultivation at scale but utilizing sensors mounted on UAVs flying at low altitude. As summarized by Havlik, Lindner, Scheper and Reardon [123], the expansion of the proximal sensing of microalgae cultures was warranted by remarkable progress in the miniaturization and affordability of optical sensors becoming increasingly versatile, connectable, and adaptable. Although, proximal sensing refers to the collection of data from sensors that are positioned close to the objects, the definition of "close" is somewhat arbitrary and varies between authors. For example, while Mulder et al. [124] refers to the use of handheld devices for "proximal sensing", Sanaeifar et al. [125] includes UAVs flying at low altitudes (<10 m from the object) in that category. Key differences, however, lie in operational parameters - spatial resolution, revisit time, and dependence on weather conditions (Table 2). In contrast to remote sensing platforms, proximal sensors can be deployed at arbitrary times and allow for capturing fine details, but that comes at a cost to the data acquisition rate. However, being close to the objects, atmospheric interferences and other platform-related perturbations are less of an issue. That is, proximal data acquisition and processing is simpler than airborne remote platforms (e.g. planes, satellites) because fewer correction steps are needed (Box 1). However, microalgal suspensions are optically complex systems represented by particles (cells) consisting of diverse components (superficial structures, organelles, and cell subcompartments) with different refraction indices. Overlapping spectra and strong absorption of light by the pigments also add complexity. In remote sensing of vegetation, this complexity was overcome by development of efficient algorithms for retrieval of pigment content and composition from reflectance spectra, creating vegetation indices [126,127]. As described in Section 3.2, these algorithms are at the foundation of the quantitative interpretation of hyperspectral images.

While the amount of spectral information which would be necessary and sufficient for the retrieval of valuable information about microalgae culture is vigorously debated until now [58], evidence accumulated so far suggests that routine tasks such as pigment (chlorophyll) retrieval can be accomplished efficiently with multispectral data [48,128]. More complicated tasks such as distinguishing phycobilins of cyanobacteria and peridinin of dinoflagellates with strong overlap of their spectra are better solved with extensive hyperspectral datasets.

Nevertheless, as can be seen in Table 3, conventional and advanced imaging methods have been developed for monitoring of laboratory cultures from biomass quantification to the evaluation of the physiological status of the cells. For example, multispectral imaging was used to detect physiological changes in phytoplankton incubated with TiO_2 nanoparticles [129]. In the study of Adejimi et al. [49], hyperspectral transmittance spectroscopy was used to estimate cellular concentration (detection limit of 10^4 cells·mL^{-1}) and to differentiate between species. However, the team showed the need to test different algorithm for better performance with the SVM-classification algorithm that provided both quantitatively and qualitatively better predictions than the PLS-discriminant and single-wavelength regression algorithm. The development of ML techniques and algorithms is of paramount importance for extracting valuable information from advanced imaging techniques (as described in Section 3). While imaging methods are now increasingly employed in surveillance of large-scale industrial cultivation systems [130], more datasets are needed for validation and to create spectral databases that can be broadly used (similarly to ecological studies or plant sciences).

5. Index-based approach for processing microalgal culture spectral images

Hyperspectral vegetation indices (VIs) are widely used in agriculture and plant phenotyping to estimate for e.g. crop performance or plant traits [51,53,54]. The VIs are calculated as ratios or combinations of functions involving vegetative reflectance at different wavebands selected from different regions of the electromagnetic spectrum (e.g. the visible 400–700 nm; near infrared 700–1000 nm and shortwave infrared 1000–2500 nm). More than 500 hyperspectral VIs have been developed for plants [54], consequently, much can be learnt from this field.

The relationship of the amount of pigment-containing phytoplankton biomass vs. radiation absorption and hence with the amount of light backscattered in the direction of the detector is rapidly saturated in the spectral regions located near the maxima of pigment absorption spectra [141]. Therefore, there are non-linear changes of reflectance (backscattering) with the pigment content [142,143]. On the contrary, backscattering in the bands aside from the main maxima of pigment absorption spectrum are linearly related with pigment and/or biomass content of the culture. Therefore, wavelengths on the slope(s) e.g. on the long-wave Chl absorption maximum (so-called "red edge" region) are suggested for monitoring of dense cultures and vice versa (for monitoring of diluted cultures) [142,144,145]. Salmi, Eskelinen, Leppänen

and Pölönen [51] described a simple imaging setup and information extraction based on vegetation indices that could be used to monitor algal cultures. Consequently, spectral bands close to the red edge region i.e. the region of rapid change in the reflectance of vegetation in the near-infrared range of the electromagnetic spectrum, could potentially be used for microalgae as in higher plant. While efficient for biomass quantification or identification, the use of index in the red edge region may fail at identifying subtle patterns of the spectrum under specific conditions (e.g. stresses conditions triggering a change in pigments).

Different index may have to be developed for microalgae cultivation for the production of metabolites because of significant changes in pigments content. Stresses such as deprivation of nutrients, exposure to high light intensity as well as increased salinity are frequently used in biotechnology for the induction of the accumulation of carbon-rich reserves (starch and lipids) and/or valuable secondary carotenoids [3,32–34,146]. These stress responses trigger characteristic changes in the optical properties of microalgal cultures [102], mostly comprised by a decline of the absorption in the red region of the spectrum due to a decline in Chl content and an increase of the absorption in the blue-green region of the spectrum related to the accumulation of secondary carotenoids [146]. Therefore, an increase in the ratio of absorbance in the blue region to that in the red region can be used for monitoring of the buildup of valuable metabolites in the cultures of microalgae such as *Lobosphaera incisa* [147,148], *Nannochloropsis* sp. [32], *Dunaliella salina* [149], or *H. pluvialis* [101]. This approach would also allow to non-invasively quantify neutral reserve lipids whose biosynthesis is tightly linked to the biosynthesis of secondary carotenoids in stressed microalgae [150].

Timely detection of overstressing and subsequent damages to the cultures, which are likely to occur during outdoor cultivation, is important for taking efficient corrective actions. As in higher plants, excessive stress manifests itself by a synchronous bleaching of photosynthetic pigments (chlorophylls and primary carotenoids) [32,151]. In contrast to accumulation of Car in the course of stress response, the damage becomes apparent on the spectra as a synchronous decline of the absorbance in the red and blue regions.

Arguably, the knowledge outlined above could be used for extraction of quantitative information from the spectral images of microalgae cultures. To date, only a handful of methods have been developed for microalgae [51]. This is possibly related to the lack of comprehensive insights into the crucial features in terms of wavelength and their correlations with various biophysical and biophysiological attributes. This knowledge is critically needed to understand which spectral bands are significant for distinct microalgal characteristics.

6. Challenges

One of the main challenges related to spectral imaging techniques is the limited accuracy of target parameters estimation. Thus, in satellite-based remote imaging of phytoplankton the acceptable accuracy is ±35 %. This is clearly insufficient for biotechnology purposes where precision of ±5–10 % is required. In principle, this level of accuracy is achievable with the currently available "close-range" spectral indexes initially developed for processing of "point-based" spectral references [18]. Nevertheless, each proposed imaging method must be validated in terms of accuracy in a specific cultivation setup where it is intended to be used. This validation should span the whole range of culture growing stages from inoculation to harvest and the widest possible range of weather (ambient illumination) conditions to avoid the interference from the variation of incident solar radiation.

Another challenge is that ratio-based spectral indexes using fixed narrow-band reflectance are prone to errors due to optical complexity of the water containing diverse microalgal cells, especially when the cell density becomes relatively high and potentially experiencing stresses. Currently, detailed knowledge is almost lacking on the relation between microalgae spectral features and various biophysical and biophysiological attributes in mass cultivation facilities. Considering that publicly available datasets are limited (many datasets are kept private due to the substantial value and/or corporate restrictions of the collected data), the need for further spectral data and the creation of libraries has been raised by several authors [51,53,140].

Finally, one of the key challenge unspecific to the field of research is that different data processing method/algorithms of spectral data provides significant errors on parameters estimations [152]. Therefore, more sophisticated ML techniques are likely required to get most from spectral data. This is especially true when the changes in culture conditions and/or biomass composition manifest themselves as slight changes in spectral curve shape, frequently on the background of various interferences, making it hard to capture with a direct e.g. VI-based approach.

Despite all power behind conventional and ML-based imaging algorithms, they are "hardcoded" for specific species/cultivation background (as can be seen in Table 3, models have been developed for a handful of species and mostly on *Chlorella*). Changes in any of the parameters pushing the measured optical properties of the culture beyond the range encompassed by the initial training of the model will likely invalidate the results obtained from it. On one hand, this entails the hurdle of re-calibration and repeated training of the spectral image processing algorithms after significant changes in the cultivation conditions and/or composition of the industrially grown microalgae. On the other hand, the failure of a model might be employed as a useful alarm e.g. of contamination or grazer attack.

Of separate concern is generalization of the ML-based approach. It is well known that huge amounts of data are required to train ML models. Nevertheless, hyperspectral imaging providing large spectral datasets (hundred thousand and millions of spectra per image) has the potential to cope with this problem. One downside of this approach is the lack of standardization: Images acquired by different hyperspectral imagers are not directly comparable, which hinders data collection. Robust features such as VIs or biochemistry-informed spectral band selection are essential for building spectral databases for use in microalgal biotechnology. Still, significant work is needed to develop robust, scalable, and generalizable approaches for automated processing of spectral images with ML algorithms.

7. Conclusions and outlook

There is a growing consensus that scaling-up of the cultivation facilities in microalgal biotechnology will require a comprehensive monitoring approach which will give exhaustive information about the culture condition with sufficient resolution in time and space. It is clear now that this goal is not achievable with conventional wet lab methods, although they will undoubtedly remain as an important reference and benchmark e.g. in assessment of biochemical composition of the biomass and to generate input data for ML algorithms. Processing of remotely sensed spectral images for extraction of quantitative information about the cultivated microalgae has emerged as a promising alternative from the field of "classical" remote sensing of Earth. It became even more attractive after advent of efficient but affordable sensors and powerful ML-based algorithms for automated spectral data processing eliminating the need of an expert for routine acquisition and interpretation of the results (although it is required at the stage of the method development). Numerous studies confirmed the potential of spectral imaging application to lab- and pilot-scale cultures. The recent boom of UAVs makes possible the close-range monitoring of growing facilities, making UAVs the preferred choice for biotechnological applications.

At the same time, we need a deeper understanding of the microalgal culture optical properties and their changes during growth, stress-induced biosynthesis of the target metabolites as well as during the damage due to overstressing, contamination, and grazer feeding. It is needed particularly for validation of new models for spectral data processing and to provide a "sanity check" for advanced ML-based

approaches likely to be developed in the nearest future. Of special importance is the ability for detecting of the shifts in the taxonomical structure of the culture evident of contamination, grazing, stresses etc. Solving this problem will likely require more efficient ML approaches for spectral data treatment, but promising results have been already achieved.

There is a need for further research and development to improve the usability, robustness, and precision of the spectral imaging for monitoring of large-scale microalgal cultures. Thus, an effort is needed to ensure that the rapid technical progress in the hardware and data formats would not undermine the compatibility of new datasets with previously acquired ones so that all the data will remain accessible for training of the algorithms and retrospective analysis. The practitioners would welcome the development of dedicated systems for automated proximal sensing of large cultivation facilities with UAVs. Ideally, these systems should be capable of seamless integration into the current IT platforms used for the operation and control of the microalgae cultivation facilities.

Finally, extensive testing and implementation of the advanced imaging techniques for automated monitoring of large-scale microalgal cultivation facilities is required for further improvement of this methodology. It will also fuel the interest of the researchers to achieve further progress in this area and lift the current limitation related with basic understanding of the microalgal culture optics and efficient extraction of spatially resolved information from its images. Anyway, it is highly likely that the advanced imaging will constitute a core technology in the future of large-scale microalgae culture monitoring.

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M. Plouviez: Writing – review & editing, Writing – original draft, Conceptualization. **N. Bhatia:** Writing – review & editing, Writing – original draft. **B. Shurygin:** Writing – review & editing, Writing – original draft. **A. Solovchenko:** Writing – review & editing, Writing – original draft, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data used as an example in Box 1 is available on demand.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.algal.2024.103649.

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