

Multispectral imaging flow cytometry for microalgae biotechnology process monitoring

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ABSTRACT

The demand for tools to detect morphological and compositional changes of single cells is growing in the course of efficient development and optimization of biotechnological processes. Until now, the chemical composition of cells has been assessed by analyzing a pooled cell sample, which represents the average composition of the cell collection gathered. Individuals from a population can be analyzed using traditional flow cytometry. However, it is unable to resolve important aspects such as morphological characteristics and the distribution of chemical components within cells.

A combination of imaging flow cytometry and multispectral imaging bridges this gap. In the reported parameter research on the bio production of Astaxanthin (Ax) by the microalgae *Haematococcus pluvialis*, the potential of this Multispectral Imaging Flow Cytometry (MIFC) technique was evaluated and proven (HP). Only three spectral channels (446 nm, 532 nm, and 646 nm) were utilized to quantify the amount of substance and the molecular distribution of the key components chlorophyll (Chl) and Ax in multispectral imaging in transmission mode. The phase-contrast information provided by cellular structures and morphology could be readily distinguished from both. In general, the MIFC method's results are consistent with traditional measures, but they go into greater depth about morphological and compositional changes within the farmed cell population during cultivation and in response to applied stimuli.

Key Words: Multispectral imaging flow cytometry, Algae, Chemometrics, Biotechnology

INTRODUCTION

The microalga *Haematococcus Pluvialis* (HP) is a rich natural source of Astaxanthin (Ax), a secondary keto-carotenoid with a range of health-stimulating qualities. Different physical and chemical stress stimuli induced Ax biosynthesis, which was followed by morphological and physiological alterations and may be linked back to molecular regulatory systems. An intracellular Ax buildup of up to 5.9% per dry weight of the microalga can be produced depending on culture settings. Nitrate shortage in conjunction with excessive light stress is the most typical Ax induction criteria addressed in the literature. According to Niizawa, staggered administration of stress stimuli increases Ax accumulation rates by around 25%. To evaluate the spectral and morphological qualities of individual cells as well as the entire algal population, the state of the art for creating and improving such biotechnological culture systems combines two independent analytical methodologies. Chemical extraction methods and spectral point measurements, for example, are often used to determine Ax concentrations. Because the cells must be lysed first, their methods are frequently time-consuming and complicated. Furthermore, chemical analysis necessitates a large number of cells, which are lost throughout the process. As a result, the cells can no longer be used in any way. As a result, working with a small volume is crucial.

A cumulative signal from the entire sample is produced via laboratory photometric measurement of the individual dye components. The matching dye content is then associated with this value. An expert also performs morphological analysis and interpretation to categorize and assess the cells. This is a time-consuming technique that needs the skills and participation of a professional. Despite this, the microscopic assessment error rate is around 10%. The simultaneous recording and evaluation of the cells' spectral and morphological features are required for precise population analysis. Shifting from the existing subjective cell rating to objective criteria improves the analysis' comparability and repeatability. A more exact evaluation of the physiological status of the population at the individual cell level benefits both research and industry. These mistakes can be reduced and the accuracy of the analysis is greatly enhanced by combining high-throughput techniques like Imaging Flow Cytometry (IFC) with advanced analytical methods like deep learning or neural networks. For many years, IFC has been routinely employed to study huge cell populations. Immunology, cancer, hematology, microbiology, and biotechnology are the key application sectors for this approach. IFC was used by Hildebrand et al. to study wild algal populations in terms of morphology, cellular characteristics, and gene expression. IFC has the benefit of being able to detect abnormalities or cell

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alterations within a cell population fast and accurately. Existing IFC systems can take advantage of both the samples' morphological and fluorescence-based features. MIFC (Multispectral Imaging Flow Cytometry) is a technique for spatially resolving the spectral and morphological features of cells.

The potential and applicability of a MIFC technique as an analytical tool for cultivation process monitoring and strain optimization in biotechnology are investigated in this paper. The bio production of Ax by the microalgae HP was studied under various nutritional medium compositions and stressors. The MIFC method was used to determine the quantities and molecular distribution of the key components chlorophyll and Ax, as well as morphological information. The findings are compared to those acquired by traditional chemical analysis.

CONCLUSION

Parameter research on the bioproduction of Ax by the microalgae HP revealed the MIFC system's exceptional potential for biotechnology applications. The MIFC system allows cell populations to be quantified at the single-cell level in the growth medium without the need for labeling or disturbance. Chemical analysis has previously been used to assess the material composition of cells, however, this method is difficult and time-consuming and does not clarify important morphological aspects or molecular component distribution inside the cells.

The kind and order of the stress elements have also been demonstrated to have a direct impact on the rate of Ax product creation in the studies. Furthermore, it has been demonstrated that the measured CEV corresponds with the MIFC measured values. Thus, in terms of population analysis of the included individual cells, the provided MIFC system may be used for process monitoring in microalgae production. For this study, the sample volume can be lowered to 50 l. Machine vision and deep learning methods might be employed in the future to extract more information from the MIFC data. The MIFC system may also be utilized for morphological and spectral evaluation of technical microparticles, pollen, or microplastics due to the modularity of the optical system and the flexibility of the microfluidic. The presented application is interested in the spatial distribution of core components Chl and Ax within the cell. The phase contrast, as well as the cellular architecture and morphology of the cells, could be clearly and unequivocally differentiated. For cells with a particularly large quantity of Ax, however, there is no discernible differentiation between Ax and phase-contrast. The phase-contrast quantity can be allocated to the Ax based on the findings of the chemical extraction, even if it is not observable by MIFC measurement. MIFC analyses about 1900 cells in each sample on average. Quantitative distribution maps of the components in the cell, as well as image-based features and moments, are some of the measured values available with the accompanying data analysis tools. The frequency distribution of a measured value over the cell population is provided by MIFC.