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Smart Imaging Analysis Techniques for Bioprocesses in Liquid Environment

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DEDICATED TO

MY LOVELY MOTHER,

MY FATHER,

MY BROTHER,

AND MY SWEET SISTER

RAHAF

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Abstract

Nowadays, many developments allow the replacement of fish oil ingredients with the products yield from the biotechnological process of marine microalgae, which is used for the *ω-3* production to large amounts. *ω-3* fatty acids are used in protection against heart and cancer diseases.

For the improvement of biotechnological processes, an enhanced in-line imaging technology is strongly needed in order to track the morphological changes of the microalgae cells, which are important for the process yield, while the cells must be kept in a certain age.

As the microalgae cells live in submerged environment, the size parameter can differentiate between the biological cells, grain stones, and air bubbles. Indeed, particle size recognition is an important task in different applications those applied on microscopic imaging.

This thesis presents different methods for enhancing the automation of particles recognition, counting, and classifying using a recently developed SOPAT-System (Smart On-line Particle Analysis Technology), a photo-optical image acquisition device for in-situ microscopic imaging.

We have proposed three segmentation methods, namely, *template matching, edge detection and multi-thresholding,* and *iterative contrast enhancement and image adaptation,* to accurately identify the microalgae cells of the image. These methods are applied on different datasets as synthetic and real microscopic images of microalgae (low and high contrast).

The template matching method is applied on all datasets and approved that the segmentation results don't have accurate values. The second method, edge detection and multi-thresholding based segmentation, includes *image de-noising* using *Haar* wavelet transform, *image binarization* using edge detection and multi-thresholding methods, *image enhancement* using morphological operations, and *watershed transform*, alternatively, *circular Hough transform* for touched cells separation. This method proves that the microalgae particles could be recognized correctly with accuracy reaches up to 99 %. But, it is limited to high contrast images.

The third one, iterative contrast enhancement and image adaptation based segmentation is applied on all datasets. It includes *image de-noising*, *image normalization* by Contrast Limited Histogram Equalization method, *image enhancement* by morphological operations, a region of interest extraction using an integral filter, and active contour method, or, fuzzy c-mean, alternatively, Otsu's thresholding, circular Hough transform, or, watershed transform as segmentation step. The segmentation result of this method reaches up to 99 %.

Furthermore, for differentiating between the biological cells and others, the combination of *cell's size* and *cell's texture analysis* are measured for accurate classification based on segmentation process. The experimental results prove that the microalgae particles can be classified correctly with the accuracy reaches up to 100% compared to reference values.

List of Publications

1- Mayar A M A, Mohammed A-M S, Doaa A-K H, and Mohamed I R. Image Analysis for Particle Size Recognition of Bioprocesses in Liquid Environment. Asian Journal of Applied Science. 2016; Vol. 9, No. 4. pp: 170-177.

2- Mayar A M A, Mohammed A-M S, Doaa A-K H, and Mohamed I R. Image Segmentation and Particles Classification using Texture Analysis Method. Research on Biomedical Engineering. 2016; Vol. 32, No. 3. pp: 243-252.

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List of Abbreviations

1. AC- Active Contour
2. ACF - Auto-Correlation Function
3. ANN – Artificial Neural Network
4. BPNN - Back Propagation Neural Network
5. C. Cohnii – Crypthecodinium Cohnii
6. CCF - Cross-Correlation Function
7. CHT – Circular Hough Transform
8. CLAHE – Contrast Limited Adaptive Histogram Equalization
9. DHA – Docosahexaenoic Acid
10. DS - Dempster-Shafer
11. EM - Expectation-Maximization
12. EPA – Eicosapentaenoic Acid
13. FCM – Fuzzy C-Mean
14. FFNN - Feed Forward Neural Network
15. ISM – In-Situ Microscope
16. ISWT - Inverse Stationary Wavelet Transform
17. LOG – Laplacian of Gaussian
18. MAD - Mean Absolute Difference
19. MLFF - Multi Layer Feed Forward Neural Network
20. MLP - Multi Layer Perceptron
21. MRI - Magnetic Resonance Imaging
22. MSE - Mean Square Error
23. OCR - Optical Character Recognition
24. PCNN - Pulse Coupled Neural Network
25. PDE – Partial Differential Equation

26. PUFA – Polyunsaturated Fatty Acid
27. ROI – Region of Interest
28. SAD - Sum of Absolute Difference
29. SHD - Sum of Hamming Distance
30. SOM - Self Organizing Map
31. SOPAT – Smart On-line Particle Analysis Technology
32. SSD - Sum of Squared Difference
33. TEM - Transmission Electron Microscopy
34. TM – Template Matching

Chapter 1

Introduction

This chapter introduces the problem that is addressed in this thesis; how to develop a vision-based technique(s) for the improvement of a biotechnological process. The chapter includes the objective of the study, the conception of a framework, and concise description of thesis organization.

1.1 Motivation

Seafood (oily fish) plays important role in human's health. This importance is due to its unique composition of polyunsaturated fatty acids (*PUFAs*). Long-chain polyunsaturated fatty acids (*eicosapentaenoic* acid, *EPA*, ω-3, C20:5 and *docosahexaenoic* acid, *DHA*, ω-3, C22:6) are two important fatty acids in early and old age metabolism in humans [1]. They are used in prevention and protection against risky diseases i.e. heart, cancer, diabetes and depression diseases [2] [3] [4] and as nutritional supplements for people and marine organisms in liquid culture [5].

Actually, the growing sector of aquaculture drives an increasing demand for fish oil. Hence, the costs for fish oil are steadily increasing, currently reaching a maximum of over 2000 USD/t. Due to that, there are many efforts are made to replace the fish oil by the sources of organisms.

Large-scale microalgae production is studied for decades, given the wide variety of practical and potential metabolic products, such as food supplements, lipids, enzymes, biomass, polymers, toxins, pigments, tertiary wastewater treatment, and green energy products that can be obtained. These products are achieved by cultivating the microalgae on various mineral media, organic substrates, and synthetic waters [5].

Thus, many developments allow the replacement of fish oil ingredients with the products yield from the biotechnological process of marine heterotrophic microalgae, which is used for the ω-3 (*PUFAs*) production to large amounts up to (50 % of their dry weight) containing (30 - 70) % of DHA [1].

The microalgae *Crypthecodinium cohnii*, shown in Figure 1.1, is a small marine heterotrophic, identified as a good producer of DHA [6] [7]. The *C. cohnii* particles are grown in Liquid culture under ideal environments, in the dark at 27℃ in the normal glass vessel tank bioreactor [8]. Through its life cycle, two shapes of *C. cohnii* are observed; motile swimming cells and non-motile (cysts). Both forms can be differentiated by the size [6]. Out of one cyst, 1, 2, 4 or 8 swimming daughter or small cells can initiate inside the encapsulated cyst, as shown in Figure 1.1 [6] [9].