Image Segmentation of Microalgae Cells Using Fuzzy Logic for Edge Detection

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***Abstract*- One of the relatively unexplored field in microalgae technology is the use of digital image processing. Images of microalgae cells can be digitized to be able to perform various procedures to characterize and approximate their population counts. In digital image processing, images can be segmented to specify regions of interest that can be used for cell counting. However, microalgae cell structures differ from one specie to another. They vary in shapes, color, and even sizes. This results to declines in robustness of commonly used algorithm in image segmentation. A problem in choosing the suitable procedure to do so arises. In this paper, a method of segmenting images using fuzzy image analysis is proposed. The aim is to apply fuzzy logic in order to create a simple method of segmentation that can be useable for a wide variety of microalgae specimens based on the image gradient and color. Fuzzification is employed in order to overcome inhomogeneity in the images. The hardware is composed of a microscope, a webcam for image acquisition, and a computer for the image analysis. Although the target species of the study are microalgae cells that are used in the production of biofuel, the system will be implemented to multiple sample images and microalgae varieties.**

I. INTRODUCTION

Microalgae is one of the emerging technologies when it comes to renewable energy. As of March 2016, the world population is around seven billion, and is estimated to rise to around nine billion by 2050[1]. Thus, the demand for commodities such as fuel will greatly increase. In order to accommodate this demand without converting all available natural resources, sustainable production methods for energy is necessary. This arises to the need of integration of these processes in order to maximize production efficiency, and one of which is the problem with approximating the population density of microalgae cells.

There are already automated methods in counting microalgae cells population, which includes resistive-type counters and laser-based zone counting. In using resistive-type counters, the population is density is approximated by the varying electrical resistances of the sample. However, presence of bacteria and other microbial cells can affect the overall electrical resistances and may result to erroneous results. Laser-based zone counting on the other hand is very expensive and is mostly use for ultrasensitive analysis. [2] A relatively inexpensive automated method can be achieved by digital image processing, and it comes with a variety of remunerations. One of which is its akin to manual counting, which is considered as an accurate method and is widely used in the field of microscopy. The presence of bacteria in cultures will also not affect the results as it would in turbidity and resistive-type counter methods.[3] However, in order to perform cell counting, an effective image segmentation procedure must be established.

Organisms and culture conditions

The sample images will be acquired form a microalgae culture from a controlled system. Most microalgae cells that have high oil content have similarity in shapes, however they still differ in structure.

One of the species of microalgae that yields biofuel is Chlorella. It is a single-cell green algae that is spherical in shape, and has a diameter of approximately 2um. Chlorella is often preferred in biofuel production because of its abundance and relative ease in reproduction. Through photosynthesis, it can rapidly multiply requiring only sunlight, water, carbon dioxide, and a small amount of mineral. [4]

Dunaliella is a specie of the algae family Dunaliellaceae. [5] Dunaliella are unicellular oval shaped green algae, which are common in marine waters. It has a diameter of 9−11 μm. Similar to chlorella, these organisms are relatively simple to culture and do not clump or form chains. It is believed that the algae is sustained by the condensation of water vapor on hanging spider-webs. [6]. In this paper, images of chlorella and dunaliella, were used as specimens for testing the algorithm.

Fuzzy Logic System

Fuzzy logic is an approach to computing based on "degrees of truth" instead of the usual "true or false" of Boolean logic. With fuzzy logic, propositions can be represented with degrees of truthfulness. Fuzzy logic includes 0 and 1 as extreme cases of truth but also includes the various states of truth in between. Because of this, fuzzy logic is often regarded as similar to human logic. [7]

II. Proposed Methodology

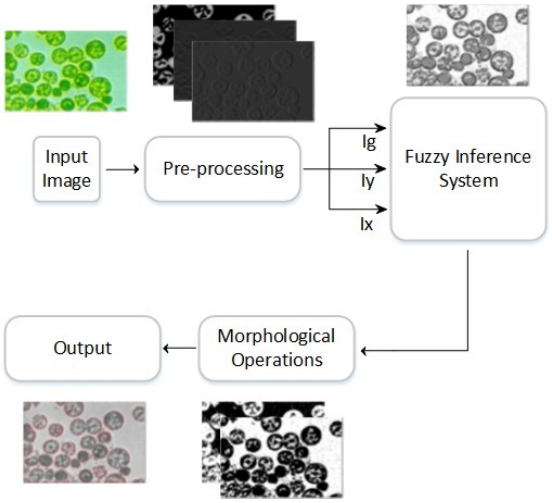

Figure 1. Proposed Methodology

Pre-processing

Image pre-processing can be done to improve the quality of the input image. Contrast enhancement may also be essential because the background most likely has a similar color to the target cells. Contrast enhancement will also help reduce the effect of noise from foreign particles and cells. However, pre-processing is mainly used in this paper to extract the necessary features that will be used as input parameters for the fuzzy inference system, which is the color and the gradient features.

Since microalgae cells used in biofuel production have innate green pigment, the color feature of the image can be used as a parameter to determine whether a certain pixel is a region of interest or not.[needed citation] In order to do so, the color image was decomposed into its red, green, and blue components. Further mathematical operations were done to extract the gray level intensities of the pixels that has a green color. The use of color as an input parameter is also helpful in discounting artifacts in the image that may be detected if gradient alone was used to segment the image.

Part of the pre-processing stage is the conversion of the color image into various gray levels. This is necessary in order to extract the gradient at the x-axis and y-axis of the image, which will also be used as inputs in the fuzzy inference system.

Fuzzy Inference System

The three inputs for the fuzzy inference system are the following: the gradient in the x-axis, the gradient in the y-axis, and the gray level of the green pixels of the image. Basically, the gradient inputs will be used to detect the edges of the image. However, using the gradients alone will also detect the edges of artifacts in the image. To compensate. The color feature was also used as a variable for the fuzzy inference system. The output of the fuzzy logic system is a binary image of the cell edges and cell regions.

Morphology

Morphological operations were done to further enhance the segmented image. In this stage, the output images from the fuzzy inference system is processed. Regions of interest that are too small are removed by morphologically opening the image. Since the fuzzy stage is practically used as edge and color detectors, certain regions of interest are not solid. Any part of the image that are not considered as background are then filled to complete the segmented image.

III. Simulation Results

Simulation is carried out using MATLAB R2015a.The steps of the proposed algorithm yields the following results.

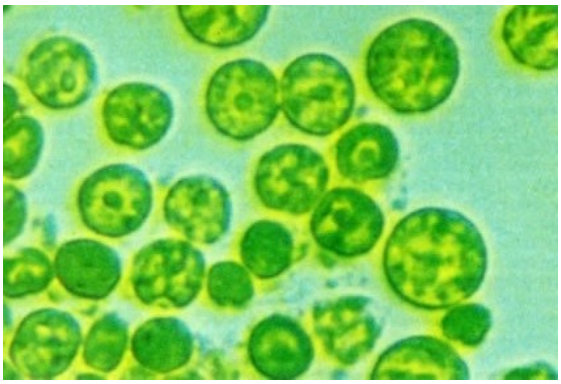

Figure 2. Light microscopy of Chlorella sp.

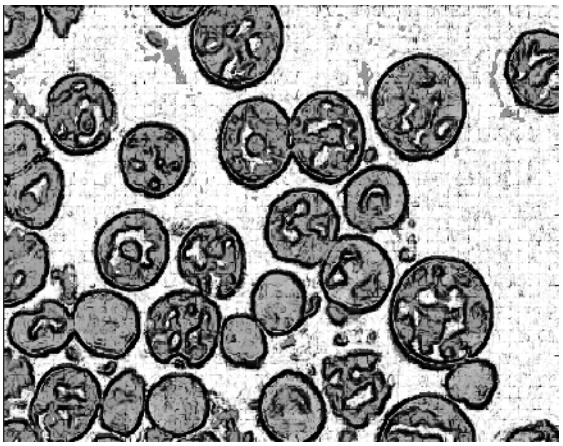

Figure 3. Fuzzy Inference System Output

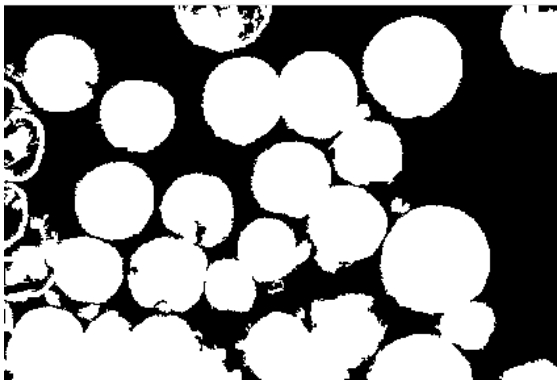

Figure 4. Morphological Operation

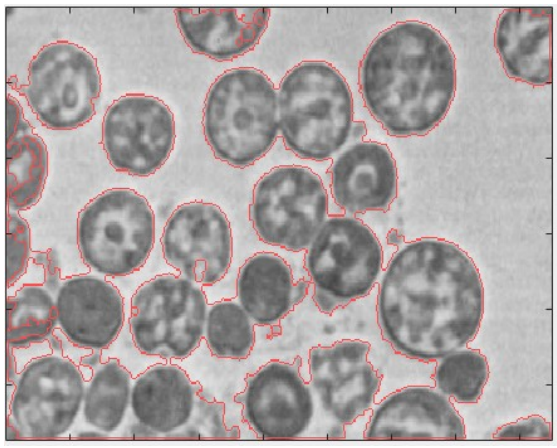

Figure 5. Segmented Image

IV. CONCLUSION

Although not yet perfect, a fuzzy logic approach proved to be viable in determining and segmenting microalgae cells. The use of the color feature were able to discount artifacts in the image that may have been detected if only the gradients were used. However, the algorithm is subject to improvement. The advantage of the method is that it uses fuzzy logic in image processing, which means that the fuzzy rules, along with the input and output membership functions can be fine-tuned to further improve the segmentation process, making the process flexible. Additional rules can also be implemented depending on the need to achieve good output in different types of microalgae species. This can be considered for future study, along with further tweaking of the morphology of the image after the segmentation process.

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