JVALUE BASED BIOMASS AND GROWTH RATE ESTIMATION OF DUCKWEED

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Thesis submitted in partial fulfillment of the requirements for the degree Master of Science in Electronic and Automation Engineering

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DECLARATION

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Name of the supervisor : Dr. Upeka Premaratne

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ABSTRACT

Duckweeds are known as Lemnaceae, comes under the family of small aquatic plants which grows forming a mat covering the surface of the water. Worldwide duckweeds are used as an effective wastewater treatment through conventional methods. These natural green plants remove the excess amount of nutrient or pollutants from the water body and maintain sustainable environmental conditions. *Spirodela polyrhiza*, *Lemna minor* and *Azolla pinnata* are some of the most popular duckweeds used in phytoremediation. Depending on the growing environment, these plants has ability to reproduce rapidly.

Rapid growth of duckweeds leads to dysfunction of water bodies and caused other problems. Because of that it is important to monitor the growth rate to control the growth and to avoid an excess duckweed. Traditional method of monitoring the growth rate by manually is laborious and time consuming. Automation of growth rate monitoring is important mostly for duckweed cultivation, modeling of waste water stabilization ponds and among researches.

Vision based image processing, used here to automate the growth rate monitoring of duckweeds. For that images of three plants were collected by capturing images from a camera once a two days. In this research two methods were used to estimate the green layer of the three plants *Spirodela polyrhiza*, *Lemna minor* and *Azolla pinnata*.

Here the biomass estimation of small fronded aquatic plants is performed by identifying the regions with texture using J- value which is homogeneity measure used in JSEG algorithm. To compare the accuracy alternative Green layer extraction (GLE) method was used.

The colour appearance of the surface of the three plants depends on light level, material properties, quality of the images and the view point. For each plant, it was done the green layer detection under two methods with three illuminance levels. Results were verified with the ground truth.

According to the results, it was calculated and compared the accuracy percentages and error percentages of two methods in different three illuminance levels. The mean accuracy under normal illumination for the proposed JVT method is *Spirodela polyrhiza is 85%,* for *Lemna minor* 82.93% and 83.71 % for *Azolla pinnata*. Furthermore, JVT method is robust enough to deal with different illuminance levels.

Finally, introduced JVT method effectively uses homogeneity measure known as the J- value to discriminate between the texture of the fronds of the plants from uniform water surface.

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LIST OF ABBREVIATIONS

JSEG	- J value based Segmentation
DB	- Data Base
JVT	- J Value Thresholding
HSV	- Hue Saturation Value
HIS	- Hue Saturation Intensity
LED	- Light Emitting Diode
GLE	- Green Layer Extraction
GT	- Ground Truth
IoU	- Intersection over Union

CHAPTER 1

INTRODUCTION

1.1 Project Overview

Duckweed small and fast growing aquatic plants belonging to *Lemnaceae* which is float on or just beneath the surface of water or slow-moving bodies of water and wetlands. These green plants mostly used as Phytoremediation which is directly use to remove, transfer, stabilize, and/or destroy contaminants in the soil surface water and groundwater. These duckweeds are commonly used in worldwide in the treatment of domestic and industrial waste waters. Due to their ability of Nitrogen and Phosphorus assimilation potential and their performance in the elimination of carbon pollution, duckweeds are popular as natural Phytoremediation [1].

Other than the removal of water contamination [2], world wild duckweeds are used as source of renewable energy. Because of forming a mat, it helps to mosquito prevention [3], preventing sunlight from the water surface helps to prevent algae growth [2]. Mostly in fish cultivation, duckweeds are used as natural animal feed as the protein supplement [2][4] [5][6]. Also used as organic fertilizer [1][4][7] and useful for bioremediation [8][9].

This plant has the ability to reproduce rapidly [5], depending on its growing environment temperature, light intensity, nutrients and pH. Monitoring the growth rate is important who is doing duckweed cultivation, because of rapidly growing plants, it covers the surface of water in high percentages. Then it helps to dysfunction of water bodies. To avoid an excess of biomass and detecting the death plants also important in duckweed cultivation. In Sri Lanka for the fish cultivation, mostly Thilapiya, it is used duckweed as their free feed which has high Nitrogen and high protein.

1.2 Motivation

Monitoring and observing features and growth of the plant is simple and reliable by using the manual method, but it is time consuming and there may be errors of the measurements.

When increasing the growth rapidly and covers water surfaces in high percentage, it also might induce dysfunction of that water bodies [10]. When harvesting the duckweeds from the cultivation ponds inappropriately, it cased to wrong purification and development of microalgae [10]. To control the growth of duckweeds by avoiding the excess duckweeds and to detect the plant diseases [11] monitoring is important. Image processing is the most suitable and accurate method to analysis of growth and automated plant identification [12][13][14][15].

It is important in Agriculture sector and among researches, to monitor the growth rate and develop a mathematical mode under various conditions like light intensity, temperature and concentration of nitrogen and phosphorus. The results of this kind of research will help to modeling of wastewater stabilization ponds [10].

The simplest and traditional method to monitor the growth rate is getting growth measurements at different times manually, it is more time consuming and laborious work. The use of computer vision for automation in biology has been significant recent interest. The main area of research has been in automated biomass estimation [16] [17] [18] of which a comprehensive review of its application in aquaculture can be found in [19].

1.3 Aim and Objectives

Aim

Develop a vision based algorithm for monitoring and growth rate estimation for *Spirodela polyrhiza*, *Lemna minor* and *Azolla pinnata*.

Objectives

- Robustly estimate the wet biomass of small fronded floating aquatic plants using the homogeneity measure of JSEG algorithm.
- Verify the robustness of the proposed method for different illuminance levels.

1.4 Contribution

Three duckweeds considered in this research are *Spirodela polyrhiza*, *Lemna minor* and *Azolla pinnata*. These three aquatic plants grow by forming a mat on water surfaces. When analyzing the green layer of the plant, it is difficult to determine fronds by manually. These plants do not have separated leaves, grows as blobs. Because of that it is difficult to determine green layer by counting leaves. There is no exact algorithm and methodology to determine the green layer of these kind of plants in vision techniques.

Implementing a robotic application for growth monitoring of these kind of unstructured environments like duckweed ponds is challenging task, mainly due to the variable illuminance levels and shadow effects due to sunlight incident angles [5].

Colour extracting method depends on light intensity and quality of the images. In this research, it is introduced a method independent of these parameters. When analyzing open and unstructured environments such as crop detection, images of plants in nature sense, it is required powerful computer analysis of image processing and segmentation algorithm [20]. Here, it is introduced JSEG algorithm for unsupervised segmentation of colour images of duckweeds. This algorithm estimates the biomass of the small fronded aquatic plants by identifying the regions with texture using the J- values. This introduced method robust enough to deal with the different illuminance levels.

To compare the accuracy of the algorithm, it was used another alternative method to extract the green layer of the plants based on image processing techniques such as HSV colour space, distance transformation and thresholding algorithms. MATLAB and python with OpenCV libraries were used in developing the image processing algorithms.

Accuracy level of the JSEG method was compared with the Green layer extraction method (GLE) and tested the accuracy with ground truth. It was done the growth rate analysis in different illuminance levels.

CHAPTER 02

LITERATURE SURVEY

2.1 Duckweed and Phytoremediation

Duckweeds are smallest aquatic flowering plants which grows on water surfaces by covering with the uniform green dense mat [21]. These plants do not have separate leaves and stems like other vascular plants. Simplest and smallest leaves of the duckweeds are called “fronds”. These are one of the fast growing plant on the earth which doubles their number of fronds within two to four days [21]. The growth of the duckweeds depends on factors like temperature, light intensity, salinity, pH and nutrients [10] [21].

Duckweeds like *Spirodela polyrhiza*, *Lemna minor* and *Azolla pinnata* are used as phytoremediation. These green plants are useful as conventional method to remove, transfer or stabilize contaminants in surface water, soil and groundwater [1]. Light intensity plays major role on growth of duckweeds [1].

2.2 Related Works

The most important step of the treatment process of the wastewater ponds is the growth of the duckweed. Very few number of research efforts were carried out to monitor the growth of duckweeds in different light intensities. For *Lemna minor* experiments were done in growth chambers which is made of transparent cubic tanks. In addition to fresh weight and dry weight measurements methods, it has done the growth rate monitoring by digital image processing [10]. Digital images taken by a camera processed by using two softwares. ACD-See software used to crop the image for pre- processing to get the area covered by the fronds. Green colour of the plant determined by manually. Automatic count was done according to the corresponding colour intensities red- green-blue. Green layer was between 100-255 and dead duckweed between 200-255. Image Pro-Plus used to get the statistical characteristics of the duckweed.

According to the results, *Lemna minor* growth comes under two phases. In first phase considered light intensities between 250 $\mu mol.m^{-2} s^{-1}$ and 300 $\mu mol.m^{-2} s^{-1}$ were optimal for duckweed growth. Progressive inhibition of the growth was observed between 250 $\mu mol.m^{-2} s^{-1}$ - 400 $\mu mol.m^{-2} s^{-1}$ intensities.

There are comparatively limited number of automatic biomonitoring methods to determine the growth of the duckweeds. Commonly it was used manual and semi-automated systems for that. Image analysis technologies tend to develop semi and fully automated systems for biomonitoring. Nikon Coolpix 995 digital camera was used to capture the images by maintaining the equal distance to the plant from the camera. Images were analyzed using Aphelion 3.02 and obtained the area of the green layer in pixels.

The algorithm starts to process the image by applying a median filter to eliminate the noise. The RGB components of the image used to convert the images in to monochromic images. Blue component was taken as it provides the highest contrast for the green plants. Then Black Top Hat (BTH) filter was used to better separation of the green layer. BTH filter enhances the dark objects of interest in a bright background. By applying a threshold filtered image was converted in to binary image. It is removed the small objects which is acting as noise. Green layer of the plant was outlined in black to estimate the surface area of the fronds covered by the line [22].

LemnaGrid is a computer software package founded in 1998. This package equipped with rich library of image processing functions. These functions have ability to drag and drop from the library panel to the grid designer window, to create image processing and image analysis algorithms by connecting them in logical manner [23].

As an application example digital images of *Lemna minor* samples can be loaded to the data base reader of the software.

Due to homogeneous white background, detecting green layer is easy by defining the white as the background. All others considered as foreground and get the binary image. The image masked is represent the green layer. By using the masked images, it determined surface area, morphologic parameters like center of mass, compactness and dimensions such as width and height. The result of the analysis is stored in a database with the DB Writer and can be retrieved from there for further analysis [23].

In order to count the number of fronds, first it converts the colour image into an intensity image with the HSI to gray converter. Edges between the fronds were determined using the canny edge detector. Here the frond detection is difficult task when growing the plants, because it is difficult to detect clear edges between fronds [23].

Duckweed Parameters Derived from the Image Processing

Estimating the green layer of the duckweeds, effectively can able to do with image processing tools more accurately. It is reproducible than visual inspection by human operators. Image processing permits capturing more accurate and quantitative parameters beyond the frond count than frequently monitoring qualitative data from visual scoring [23]. In addition to frond count, vision techniques enable to extract the parameters like sizes, shapes, and colors of the fronds. To estimate the growth more accurately image processing tends to determine the area of the fronds. The dynamics of the overall area of the visible fronds similarly link to growing or decreasing populations.

Image processing tools have ability to analysis the information on surface colour, it helps to distinguish the health condition of the plant whether the fronds are living or dead. When combining the characteristics derived from the image processing and the biological data, provides complete information on growth of duckweeds [23]. Accordingly, digital image processing is the most suitable and accurate method for estimate the biomass on duckweeds [10].

2.3 Object Detection

There are various types of object detection methods using machine vision and image processing. Segmentation means partitions an image into distinct regions containing each pixel with similar attributes. It means image segmentation is dividing the image in to set of regions that is easier to analyze.

Feature extraction and texture analysis methods are used for further processing of segmented images. In the area of computer vision, object detection technologies used to figure out the objects inside a photograph or video stream [24].

Object detection can be categorized in to several methodologies such as appearance based methods like edge matching, gray scale matching, gradient matching; feature based methods like edge, corner, blob and ridge detection [24].

Boundary or edge detection basically finds the edges or boundary of images and separates the object from the image [24]. Generally detecting the discontinuity of the gray scale images is done by edge base segmentation. Then it produces a binary image with edges while considering its background as the output of the image [25]. Among edge detection technologies most important techniques are Robert, Sobel, Prewitt, Canny operators in computer vision [25].

Canny edge detector is one of the best operator use to detect edges in the images. This is very effective operator to edge detection with good noise immunity and detects true edge points with minimum error. General criteria for edge detection includes,

1. Detection of edge with low error rate, which means that the detection should accurately done by maximizing the detecting real edge points while minimizing the non-edge points.
2. The edge point detected from the operator should accurately localize on the center of the edge.
3. A given edge in the image should only be marked once, and where possible, image noise should not create false edges.

The algorithm runs in five steps. 1) Smoothing; images taken from the camera contain some amount of noise. When finding the edges, noise can mislead the result. By applying a Gaussian filter, image can be soothed. The function Gaussian filter multiplies each pixel in the image by the kernel generated. It returns the smoothed image in a two dimensional array.

2) Finding gradients; by feeding the smoothed image through the Sobel operator with the derivative of the Gaussian in both the vertical and horizontal directions. 3) Non-maximum suppression; this is to convert the blurred edges in the image of the gradient magnitudes to sharp edges. This performed by considering only all local maxima in the gradient image and deleting everything rest. Finally, only local maxims should be marked as edges. 4) Double thresholding; Potential edges are determined by thresholding. Instead of using a single static threshold value for the entire image, the Canny algorithm introduced hysteresis thresholding. There are two threshold levels, high and low. 5) Edge tracking by hysteresis; final edges are determined by suppressing all edges that are not connected to a very strong edge [26].

Thresholding is an easy and convenient way to perform segmentation on the basis of the characteristics of the image, like different intensities or colors in the foreground and background of an image. The simplest technique is replacing each pixel in an image with a black pixel if the image intensity is less than some fixed constant T, or a white pixel if the image intensity is greater than that constant. Thresholding creates binary images from grey level ones by turning all pixels below some threshold to zero and all pixels above that threshold to one. This can be used in object detection.

Other most popular segmentation technique is region based segmentation. Similarity-based approaches group similar pixels into different homogenous regions. Examples are region growing, region splitting, region merging [27].

CHAPTER 03

METHODOLOGY

There are less number of researches regarding the growth rate monitoring of duckweeds. To analysis of the growth, finding the existing data bases of image series of duckweeds was difficult task. The initial step of this research was image acquisition. In this study it was considered three types of duckweeds *Spirodela polyrhiza*, *Lemna minor* and *Azolla pinnata* are used for experiments.

Overview of the project in figure 3.1 as follows,

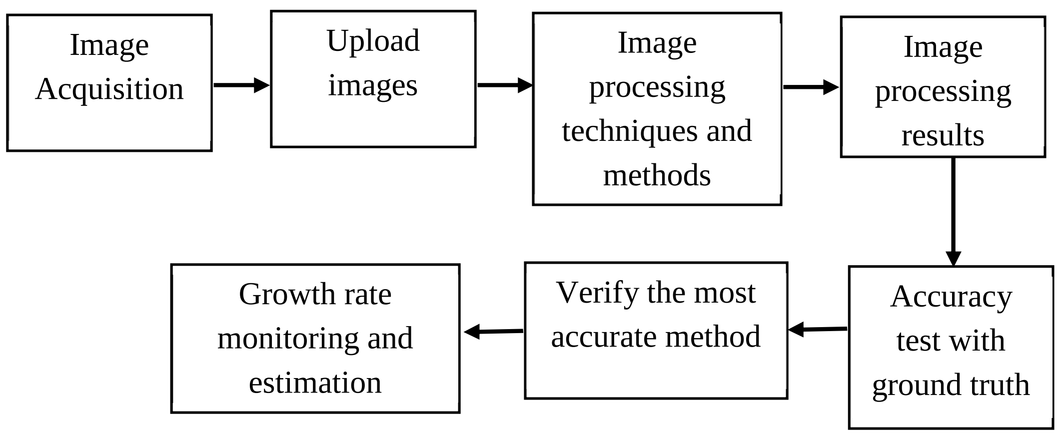


Figure 3.1- Overview of the project

Most important step of the system overview is image acquisition. Because, it should maintain same camera resolution, position of the camera and the illuminance level throughout the period of image capturing. Then image processing techniques used to analyses the images. Accordingly verified the most accurate method that can be used to estimate the growth rate of the relevant duckweed species. With various illuminance levels, there are no exact algorithms to determine the green aquatic plants which is known as duckweed.

In the project it is considered three duckweeds, *Spirodela polyrhiza*, *Lemna minor* and *Azolla pinnata* do not have separated fronds. Since duckweeds grow by spreading over the surface of their medium, surface area of the plant is a useful means of estimating growth as well. Because of the nature of the plant it is difficult to count number of leaves. Therefore, area of the plant is considered to determine the growth rate.

Colour detection gives errors on area determination with different illuminance levels. When the illuminance level high actual green layer tends to determine as another colour. Because of that some of the particular areas may not be taken as the green layer. It wasn't a successful method to determine the green layer of the plant.

This project based on the algorithms developed by using image processing techniques. Novel method of detecting the homogeneity of the image with given color texture pattern called JSEG algorithm is used in this project to extract green layer (surface area) of the plant. To check the accuracy of the algorithm, it is used another method for same procedure by using morphology transformation, thresholding algorithms and distance transformations. In the developing phase, it is used MATLAB and Python with OpenCV libraries to develop the algorithms. Then the most accurate method was identified by comparing with the ground truth. Main steps of the system development as follows,

3.1 Image Acquisition

The experiments were carried out under indoor environmental conditions and growth rate was analyzed for specimens growing in municipal water with no addition of fertilizer or chemical. Same camera resolution was used to capture images under controlled illuminance levels. Illuminance levels were controlled by using two LED panels under 16 and 18 VDC.

The images of *Spirodela polyrhiza* were captured by using HD720p, 3MP, Logitech HD webcam during nearly two months once a two days with the resolution of 1920x920. Manually captured images save on a data base respectively. Logitech HD webcam powered by USB port enables user to capture images when required. To maintain the same quality of the images, camera is mounted on a stand which can maintain the same distance from the camera to plant and maintained the same water level of the container of the plant. Experimental setup was as follows in figure 3.2,

Figure 3.2 - Experimental setup

When capturing the images, images were taken for normal illumination and the controlled illumination with 16 and 18 V respectively at 1000 lux and 3800 lux .

Images for *Lemna minor* and *Azolla pinnata* were captured using Arducam-F Shield V2 Camera Module Shield which is a universal camera control board with OV2640 camera board controlled by Arduino UNO. It is a plug and play camera control interface ignoring the complexity of the camera consists library with ready to use software source code. This supports 0.3MP- 5MP camera modules with different formats like RAW, RGB, YUV, JPEG and supports slandered Arduino boards. ArduCam shields consists with built in SD card socket which can store the images and videos captured by the camera module. Images can be captured with several resolutions, for the experiment it was used resolution as 1600x1200.

Figure 3.3 - OV2640 2MP camera with ArduCam shield

OV2640 is a 2MP camera board used with ArduCam shield in figure 3.3. This can be effectively use by setting the parameters on Arduino code.

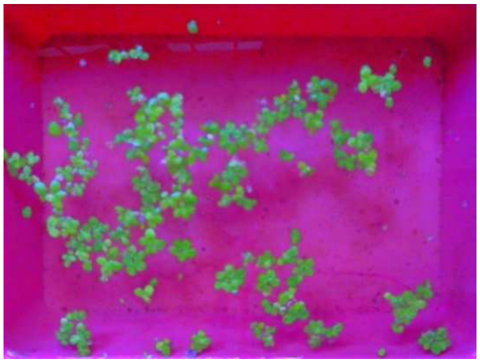
By using above two cameras required image data sets were collected.

3.1.1 Input Images

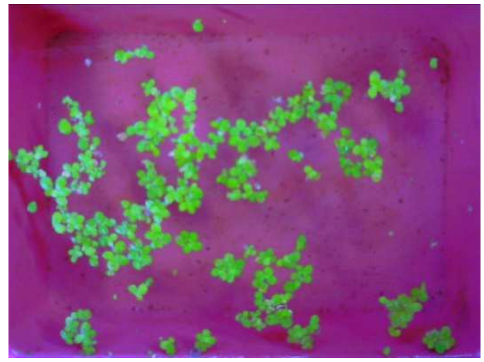
Images were taken under three controlled illuminance levels. Three duckweeds have their own characteristics of the plant.

3.1.1.1 *Spirodela polyrhiza*

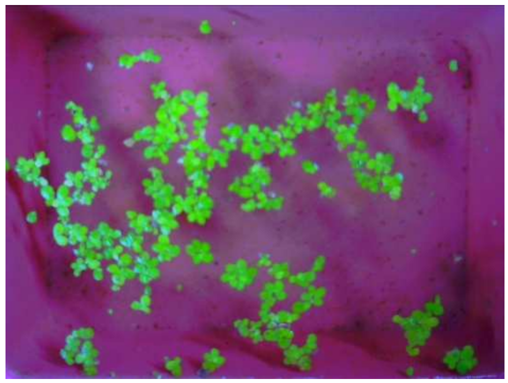
Worldwide this green duckweed can found in many kind of water habitat which grows by forming a mat on the water surface. This plant having smooth, round and flat disc fronds which are one half to one centimeter wide. Total number of 75 images were captured including 25 per each illuminance level, corresponding to a total no of 50 days growth. Sample input images for different illuminance levels for *Spirodela polyrhiza* are in figure 3.4.



(a) Normal illumination



(b) Controlled illumination at 1000 lux

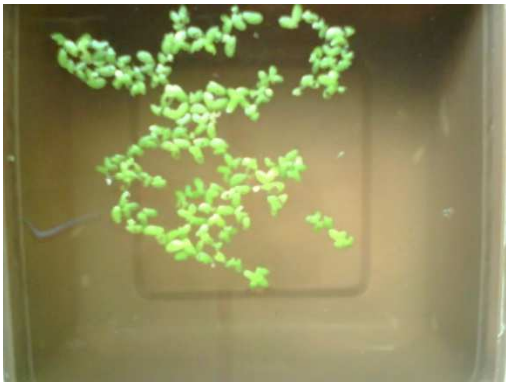


(c) Controlled illumination at 3800 lux

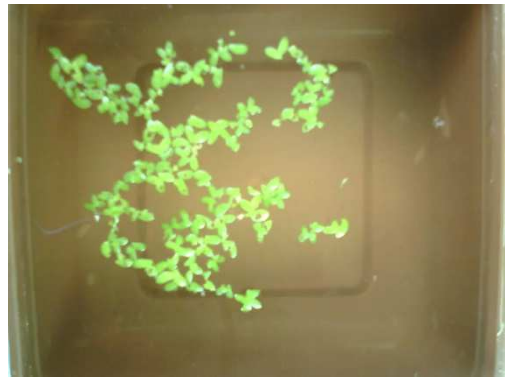
Figure 3.4- Input image samples for *Spirodela polyrhiza* in different illuminance levels

3.1.1.2 *Lemna minor*

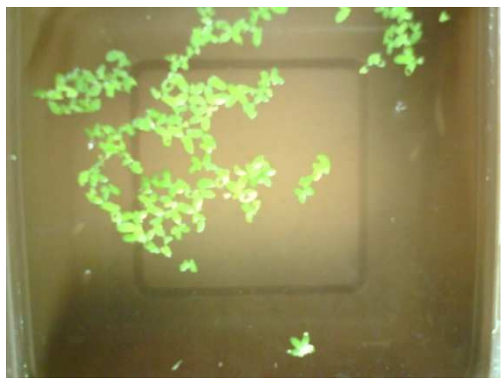
This also a kind of floating aquatic plant which grows in fresh water habitats. Each plant having 1-4 oval shaped leaves. Fronds of the plants are 1–8 mm long and 0.6–5 mm broad, light green, with small air spaces to assist flotation. Here the total no of 45 images were captured including 15 per each illuminance level over a 30-day period. Sample input images for different illuminance levels for *Lemna minor* are in figure 3.5.



(a) Normal illumination



(b) Controlled illumination at 1000 lux

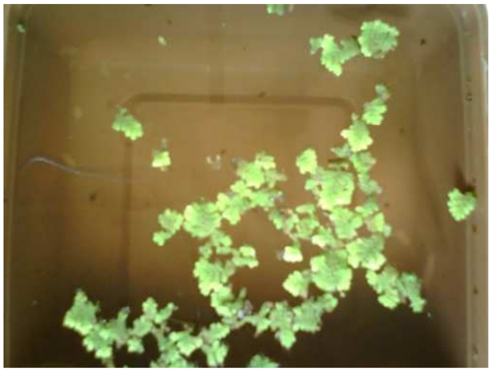


(c) Controlled illumination at 3800 lux

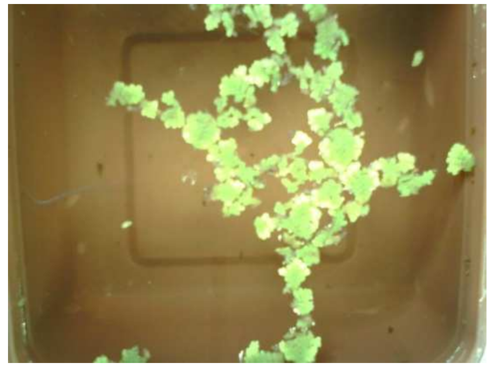
Figure 3.5 - Input image samples for *Lemna minor* in different illuminance levels

3.1.1.3 *Azolla pinnata*

Azolla pinnata is one of the species of aquatic green plant which grows forming a mat on surface of the water. This comes under small fern with triangular fronds up to 2.5cm in length. These fronds are made up with round or overlapping leaves each in 1-2 mm long. Fronds are mix of green, blue-green, or dark red colors with a velvety appearance. The total no of 30 images were captured including 10 per each illuminance level over a 20-day period. Sample input images for different illuminance levels for *Azolla pinnata* are in figure 3.6.



(a) Normal illumination



(b) Controlled illumination at 1000 lux

(c) Controlled illumination at 3800 lux

Figure 3.6 - Input image samples for *Azolla pinnata* in different illuminance levels

3.2 Biomass Estimation

Duckweed growing ponds mostly open and unstructured environments, plants identification in that kind of environments, it is required powerful computer analysis image processing method with segmentation algorithm. Here the biomass estimation of aquatic plants was performed by identifying the regions with texture using the J-value. The J- value is a homogeneity measure used in JSEG algorithm for image segmentation. To compare the accuracy of the J- Value Thresholding (JVT) method another alternative method called Green Layer Extraction (GLE) method used as in [18] to determine the biomass of the plants.

3.3 Ground Truth

It was used two methods JVT and GLE method to determine the frond area of the images captured for three plants, *Spirodela polyrhiza*, *Lemna minor* and *Azolla pinnata* in different illuminance levels.

Most important part of the research is accuracy test of above two methods. Ground truth scenario is the most popular and crucial in accuracy testing. Hence, to obtain the ground truth, manually masked the images by using Adobe Photoshop with Magic wand and colour tools. Manually marked fronds of the plants coloured in black colour, while background remaining white. As the masked image is a binary image, it was calculated the black colour pixels by using MATLAB code. Manually segmented frond area is taken as the ground truth for accuracy testing.

Then the ground truth is used to get the true extracted biomass from above two methods considering Intersection over union (IoU). IoU is calculated for all output images of the above two methods considering overlapping area of the ground truth and the output images.

3.4 Biomass Estimation Methods

3.4.1 J value Thresholding Method

JSEG algorithm is complex segmentation method which the computational method evaluates and predict environmental characteristics such as shadows, brightness, colour elements, complex object composition and inhomogeneous region colors for texture [20]. Here the biomass estimation of small fronded aquatic plants is performed by identifying regions with texture using the J- value, which is homogeneity measure of JSEG algorithm [28].

Variation of the colour pattern within the image referred to as image texture. In an image there may be regions with high level of texture, these regions taken as typically non-homogenous. Considering the floating aquatic plants, surface of the water is highly uniform, untextured while the fronds of the plants are highly textured. Thus a texture based thresholding method efficiently discriminate fronds and the water surface. Previous studies have shown the effectiveness of the J-value in localization [29]. Accordingly, JSEG is an unsupervised quantization algorithm for colour images [30].

This algorithm segments images without manual parameter adjustment for each image and simplifies texture and color properly [31].

The J-value calculation starts by assigning every RGB value a unique class depending on its colour. Due to the large number of unique combinations for a typical 24 bit RGB image, the image has to first dequantized to eight levels to get the manageable number of classes. It means JSEG algorithm segments color images with uniform regions which having set of pixels to the same colour to generate clusters in the class.

Then, the image pixel colors are replaced by their corresponding color class label and the newly established image of labels is called a class-map. The class-map also a special kind of texture composition. It means JSEG algorithm simplifies color and texture of images effectively.

J value quantifies the homogeneity of a region by comparing the distance between different classes over the distance between the members within each class.

In order to calculate J-value, Z is defined as the set of all N data points (pixels) in the class map.

A pixel is taken as a position vector in the x and y directions as follows,

$$z = (x, y), \quad \text{where } z \in Z$$

The mean of the all Z elements m is,

$$m = \frac{1}{N}\sum_{z \in Z} z$$

C is the number of classes obtained in the quantization. Then Z is classified into C classes.

Z_i are the elements of Z belongs to class *i* , where ,

i = 1, 2, ………., C

Let, m_i be the mean of the N_i data points in Z_i ,

$$m_i = \frac{1}{N_i} \sum_{z \epsilon Z_i} z$$

S_T is the sum of quantized image points within the average in all Z elements,

$$S_T = \sum_{z \epsilon Z} \|z - m\|^2$$

S_W is the total variance of points belongs to the same class,

$$S_W = \sum_{i=1}^{C} \sum_{z \epsilon Z_i} \|Z - m\|^2$$

The J value is given by,

$$J = \frac{S_T - S_W}{S_W}$$

The J- value quantifies the homogeneity of a region by comparing the distance between different classes over the distance between the members within each class. If the image is uniform the J- value will be close to zero while the textured non-homogeneous area closes to one. Typically, a textured region is highly heterogeneous. Hence, the J- value is a good metric for its detection.

The matrix containing the J values of nxn regions of an image is known as the JImage. To development of the algorithm, it was used image processing based MATLAB functions. For an example, to verify the steps of the algorithm, it was considered the image of *Spirodela polyrhiza* in normal illumination as follows,

3.4.1.1 Class Image

According to the colour quantization algorithm figure 3.7 a) Original image is quantized and quantized colours are assigned the labels. Set of image pixels quantized to a same colour is known as colour class. Then the actual image pixel colours replaced with their corresponding colour class labels. Resultant image called class map or class image. Figure 3.7 b) shows the class image of figure 3.7 a) Since a 24 bit colour image requires 16777216 classes, quantization is done in 8 levels [32]. Here it can clearly identify that the textured green area has replaced with a specific colour class label.

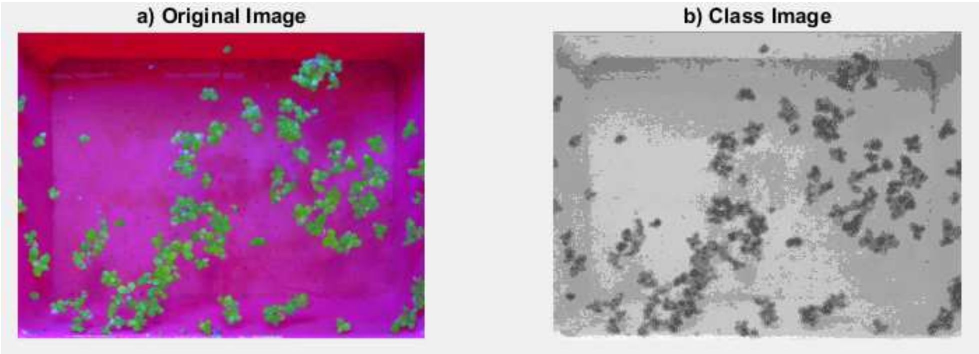


Figure 3.7 - (a) Original image and (b) the class map

3.4.1.2 J- image

J-image in figure 3.8 is a gray scale image whose pixel values are the J values calculated over local windows centered on the pixels [27]. J- image contains the texture related to the green layer.

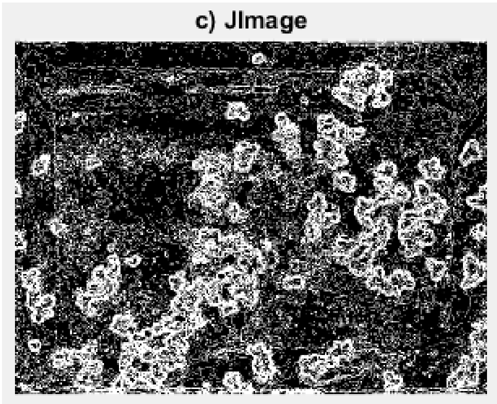


Figure 3. 8- JImage

The size of the local window determines the size of image regions that can be detected. Windows of small size are useful in localizing the intensity/color edges, while large windows are useful for detecting texture boundaries [33]. Here, the window size 4x 4 window is used. The JSEG algorithm continues by computing, for each pixel, a J value and produce the J-image.

3.4.1.3 Binary JImage

Next step is to extract the green layer from the J-image. In order to threshold the J-image to get the binary image of extracted green layer, manually selected the threshold value as 0.6 using the data curser on MATLAB. After thresholding the binary image of J-image obtained is in figure 3.9.

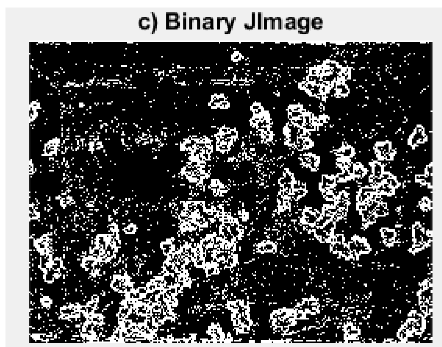


Figure 3.9 - Binary J-image

3.4.1.4 Biomass Extracted Image

To get the true biomass estimated image, it was obtained the overlapped image with ground truth and the output binary JImage. Accordingly obtained true biomass extracted image as follows in Figure 3.10.

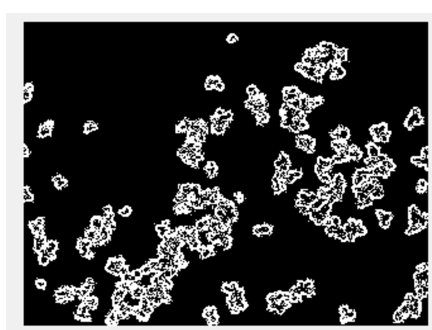


Figure 3.10 - Filtered J-Image

3.4.1.5 Green Layer Percentage Calculation

Using the filtered J-image, white pixels were taken as green layer while black pixels as back ground. Green layer percentage was calculated correspond to the number of white and black pixels.

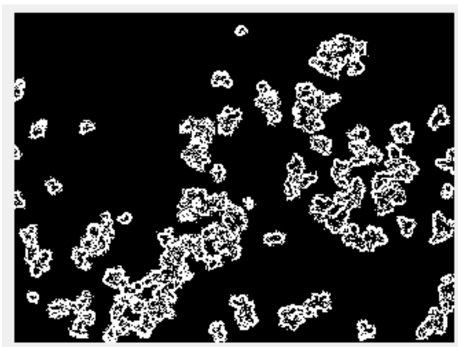
Sample calculation for *Spirodela polyrhiza* in normal illumination as follows,

No. of white pixels (X) of Green layer	=	11439
No. of total pixels (Y) of the image	=	76800
Percentage of green layer	=	$\frac{X}{Y} \times 100$
	=	14.89%

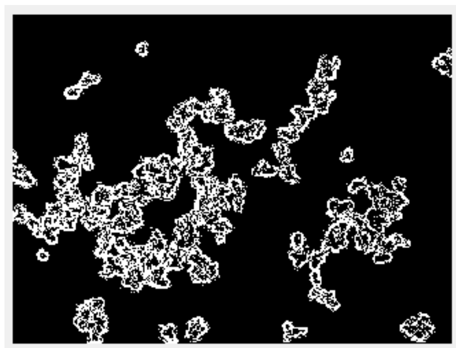
In this way, green layer is calculated for three plants under three illuminance levels.

3.4.1.6 Filtered J-images for same Image in Different Illuminance Levels

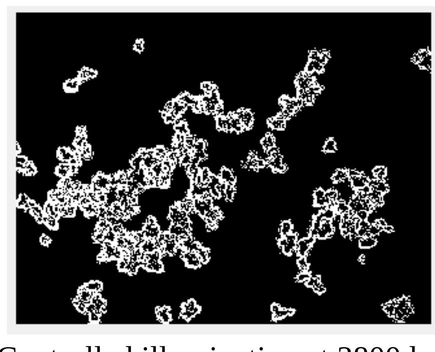
Under three illuminance levels filtered J-images for same image as follows in figure 3.11.



(a) Normal illumination



(b) Controlled illumination at 1000 lux



(c) Controlled illumination at 3800 lux

Figure 3.11 - Filtered J-images for different three illuminance levels

3.4.1.7 Resultant Images for Other Two Plants

Same J- value thresholding used to segment the other two types of plants.

Lemna minor

It is used the JVT method with 4x4 window size to get the texture boundary. 0.2 is used as the threshold value. Resultant images for normal illumination is in figure 3.12.

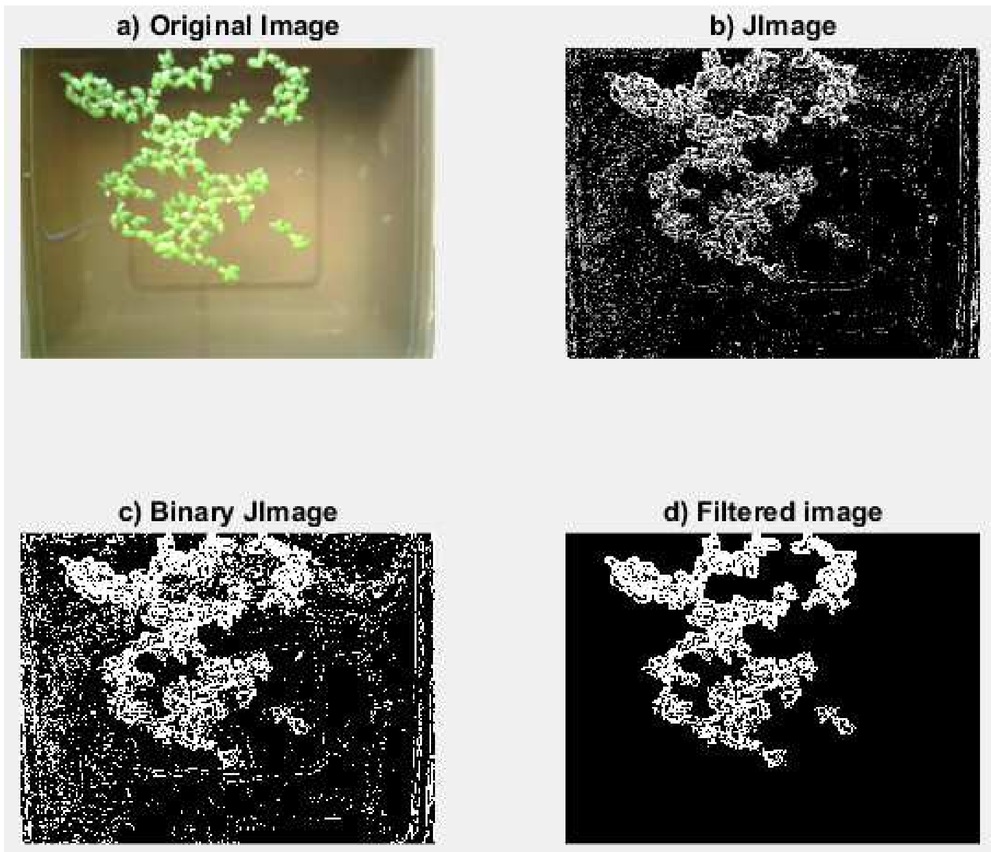
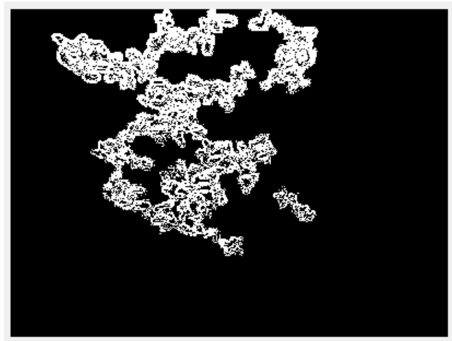
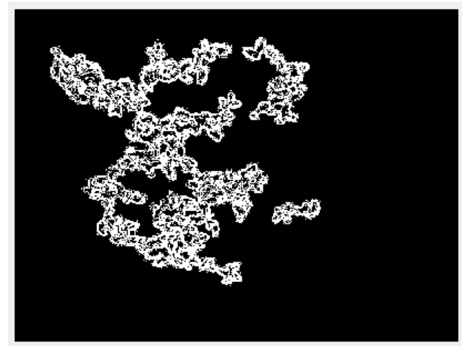


Figure 3.12 - Resultant images for *Lemna minor* in JVT method

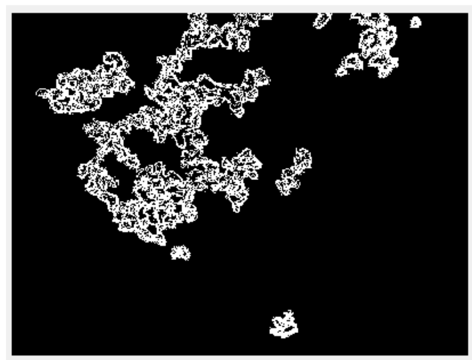
Similarly, it was taken the green layer percentage for three illuminance levels. Filtered green layer extracted images for same image in different three illuminance levels are in figure 3.13 as follows,



(a) Normal illumination



(b) Controlled illumination at 1000 lux



(c) Controlled illumination at 3800 lux

Figure 3.13 - Filtered J-Images for different illuminance levels

Azolla pinnata

It was used 8x8 window size to get texture boundary and 0.6 as the threshold value. Resultant images for normal illumination as follows in figure 3.14.

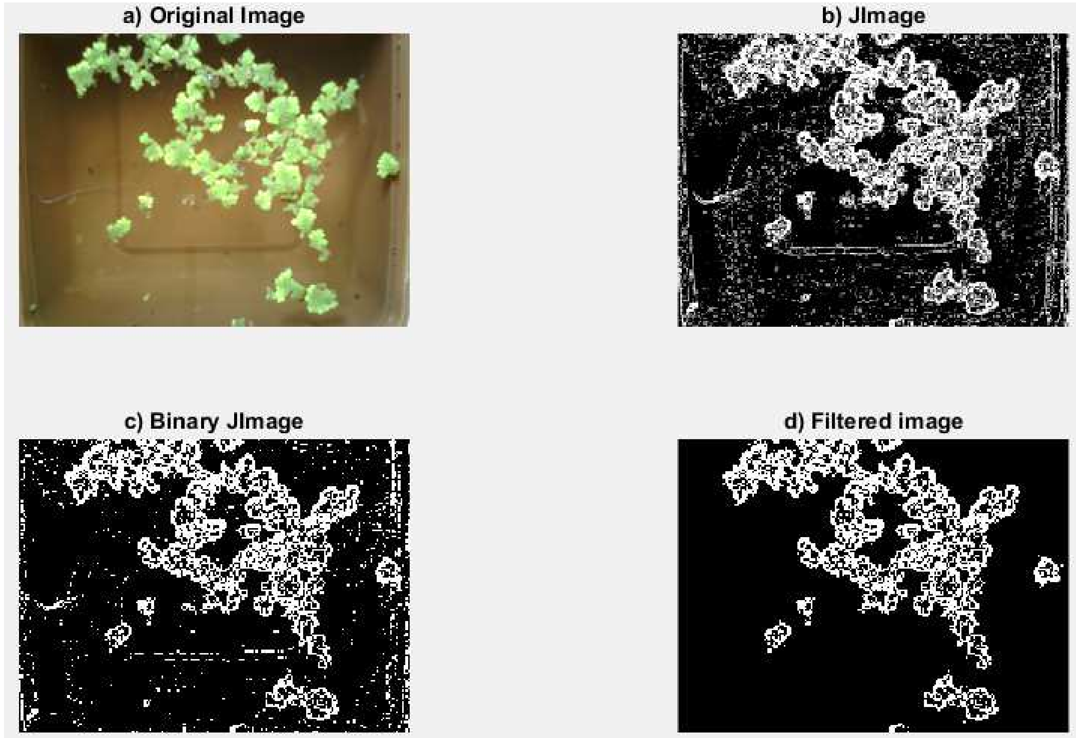
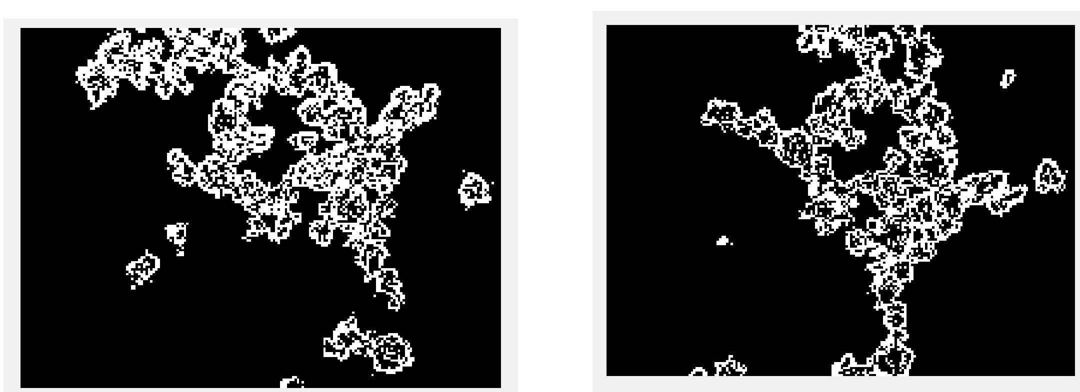
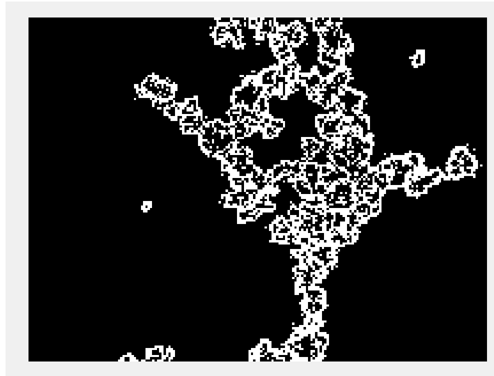


Figure 3.14 - Resultant images for *Azolla pinnata* in JVT method

Filtered green layer extracted images for same image in different three illuminance levels as follows in figure 3.15.



(a) Normal illumination (b) Controlled illumination at 1000 lux



(c) Controlled illumination at 3800 lux

Figure 3.15 - Filtered J-Images for different illuminance levels

3.4.2 Green Layer Extraction Method

In order to compare the accuracy of the JVT method with another alternative method, it was used colour detection method to extract the green layer. Main steps of the of the GLE method shown in figure 3.16. Implemented the method with flask based web application

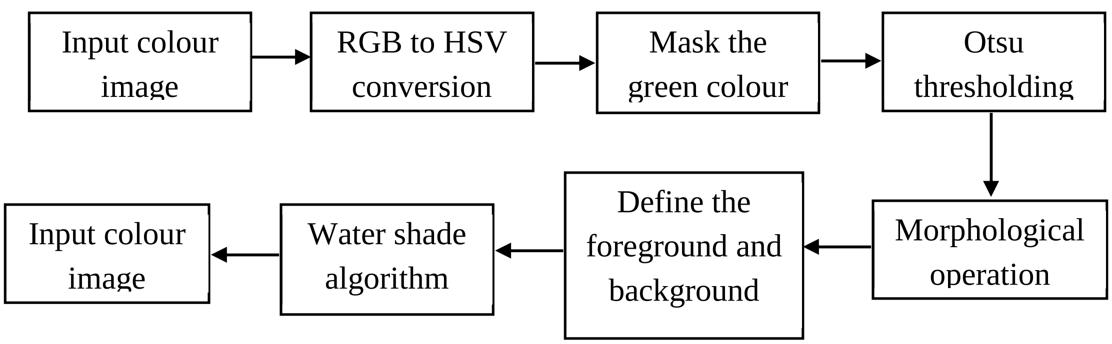
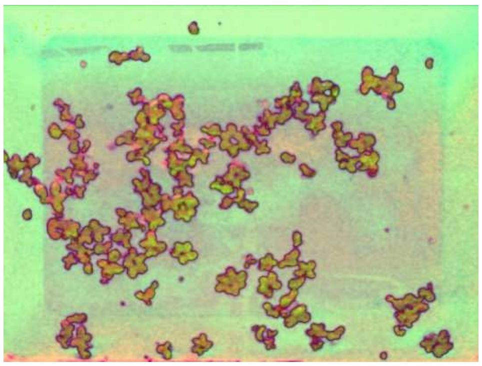


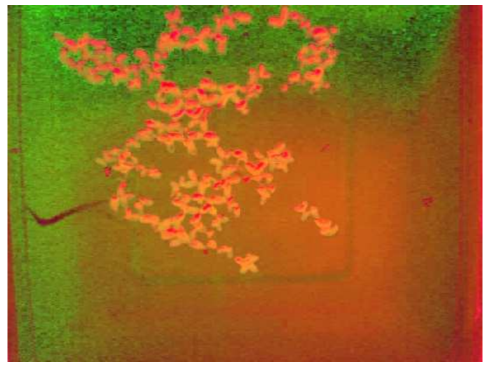
Figure 3.16 - Steps of the GLE method

3.4.2.1 Input Image and Conversion to HSV Colour Space

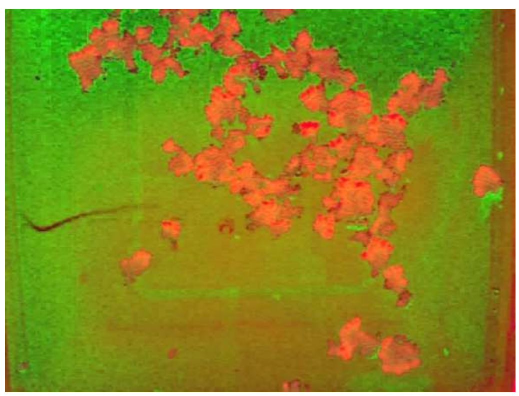
The images captured from the camera mentioned in above Figure 3.4, 3.5 and 3.6 were used as true colour or RGB images. RGB colour space describes colours in terms of amount of red, green and blue. HSV color space describes colors in terms of the Hue, Saturation, and Value. While RGB space defines colour in terms of a combination of primary colours, HSV describes colors similarly to how the human eye tends to perceive color. Importance of HSV space is the separation of chromatic (Hue and Saturation) and achromatic (Value) information [34]. The HSV colour space widely used in image processing applications since it clearly separates light and chromatic information [35]. HSV transformed image for three plants indicated in figure 3.17



(a) *Spirodela polyrhiza*



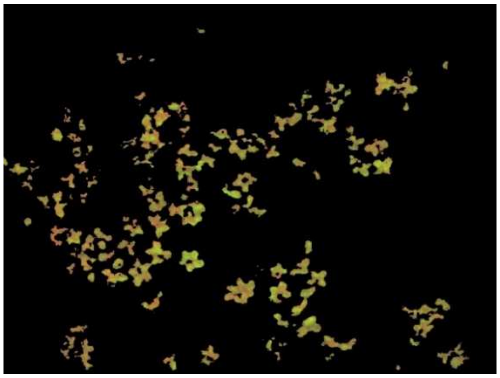
(b) *Lemna minor*



(c) *Azolla pinnata*

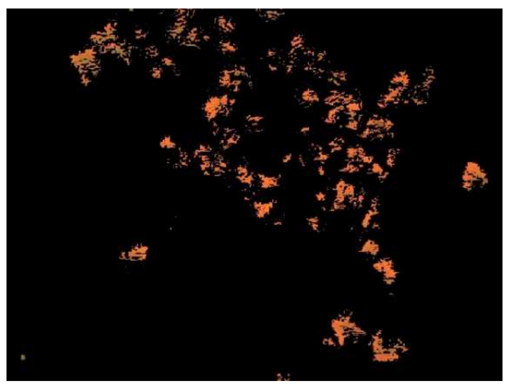
Figure 3.17 - HSV transformed images

In HSV space hue, saturation and value ranges are normalized in to range of [0-255]. HSV value for green colour is obtained by using python command as [60,255,255]. Accordingly it was taken lower and upper bounds respectively as [60-sensitivity,100,50] and [60+sensitivity,255,255], because of fronds of the plants includes quite dark colour green also.Here the sensitivity was taken as 15. To get the HSV threshold for green colour, it was used two lower and upper bounds considering the sensitivity by changing only the hue values. Then thresholded the HSV image to get only the green layer of the plants. Thresholded images of the three plants for normal illumination is in figure 3.18.



(a) Spirodela Polyrhiza

(b) *Lemna minor*



(c) *Azolla pinnata*

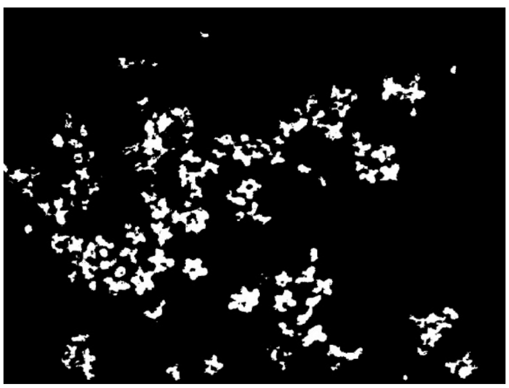
Figure 3.18 – Thresholded image

3.4.2.2 Otsu's Thresholding

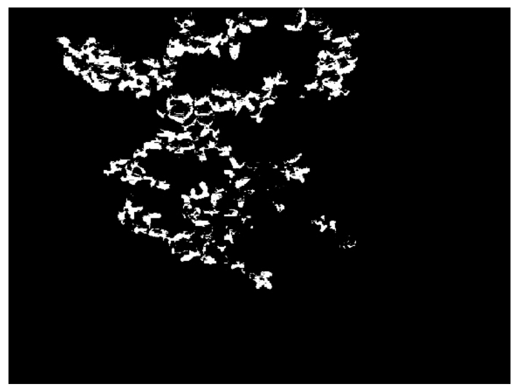
It was used Otsu's thresholding as the global threshold technique for gray scale images to get the binary image [36]. Binarization means separate the gray scale image pixel values in to two groups. Gray scale image carries only luminance or intensity information which the pixel values between 0 to 255.

In order to obtain the binary image, above figure 3.18 HSV thresholded image converted into RGB. Then converted in to gray scale image.

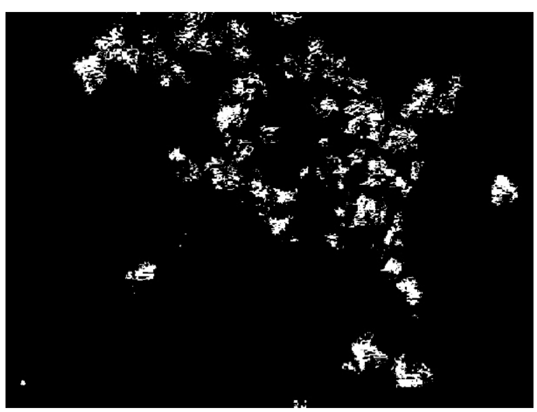
Otsu's binarization creates a histogram known as bi-model histogram which has two peaks for foreground and background pixels. As the next step, it calculates the optimum threshold by separating the two classes by minimizing the intra- class variance and maximizing the inter-class variance. Then it automatically calculates a threshold value between the two peak values. Thresholded binary image in figure 3.19.



(a) *Spirodela polyrhiza*



(b) *Lemna minor*



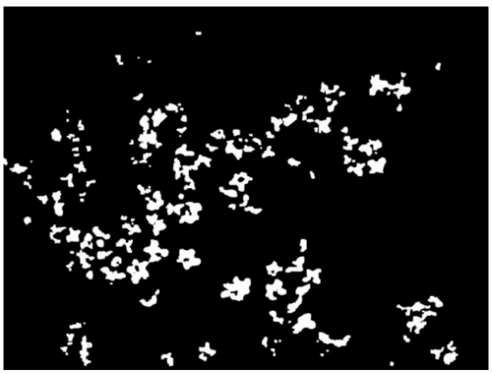
(c) *Azolla pinnata*

Figure 3.19 - Otsu's Thresholded binary image

3.4.2.3 Morphology Transformation

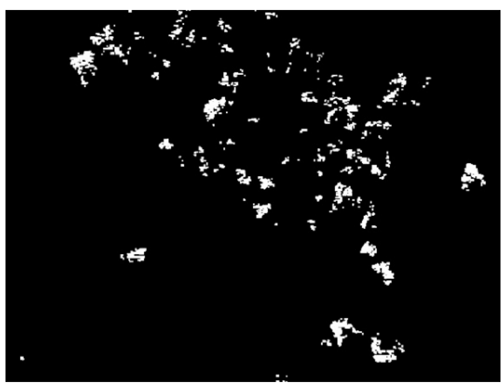
Morphological transformation is simple operation which based on the characteristics of image shape in binary images. This needs two inputs original binary image and structuring element or kernel which decides the nature of operation. The basic morphological operators are erosion, dilation, opening and closing.

To remove the white noise in the figure 3.19, it is used morphological opening. Morphological transformed image is in figure 3.20.



(a) *Spirodela polyrhiza*

(b) *Lemna minor*

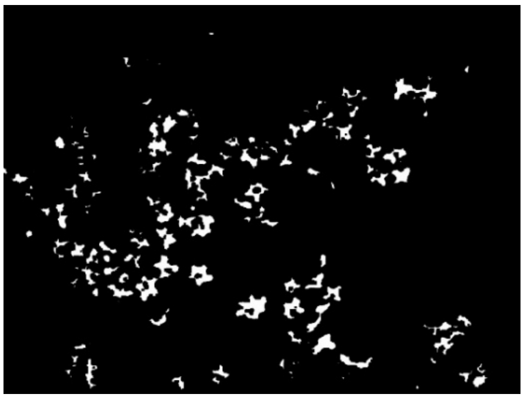


(c) *Azolla pinnata*

Figure 3.20 - Morphological transformed image

The region near to center of leaves are sure foreground and region much away from the object are background. Boundary region of the extracted green layer is the not sure area. Accordingly, to get the sure area for the fronds, it was used erosion and dilation.

Erosion removes the boundary pixels to extract the sure area of morphological transformed image. Then it was sure the remaining region belongs to foreground. Sure foreground image is in figure 3.21.



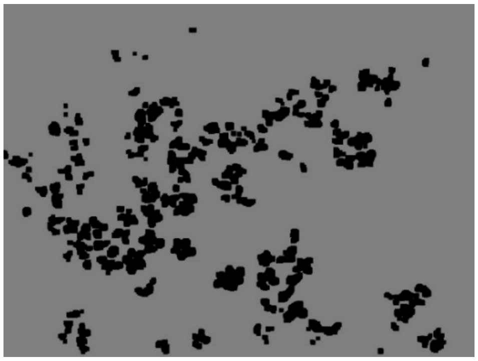
(a) *Spirodela polyrhiza*

(b) *Lemna minor*

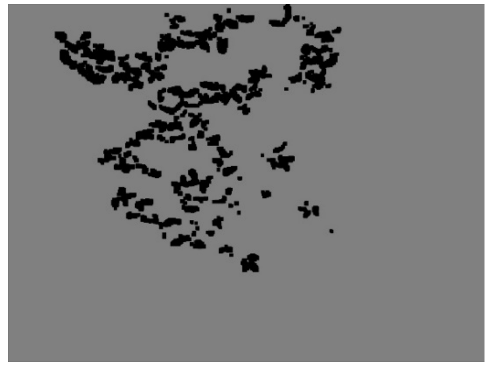
(c) *Azolla pinnata*

Figure 3.21 - Sure foreground

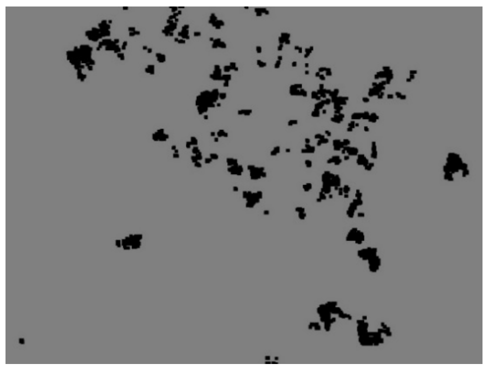
To find the sure background area, dilate the morphological transformed image. Dilation increases object boundary to background. This make sure to define sure background. Sure background image is in figure 3.22.



(a) *Spirodela polyrhiza*



(b) *Lemna minor*



(c) *Azolla pinnata*

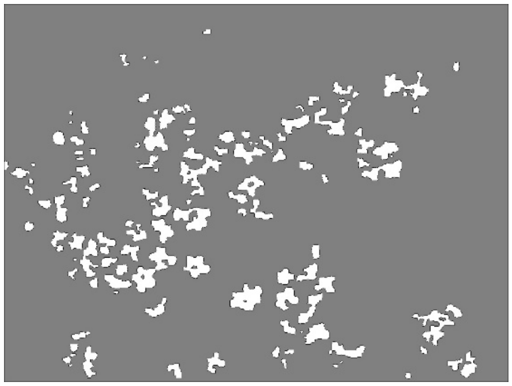
Figure 3.22 - Sure background

3.4.2.4 Marker Image for Watershed Algorithm

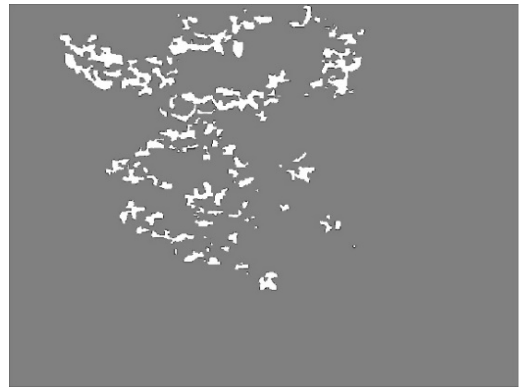
Marked the foreground, background and not sure area in marker image. Finally applied the Watershed algorithm to convert result back to unit8 image. Not sure area is determined by watershed algorithm.

The watersheds concept is one of the classic tools in the field of topography [37]. Any gray scale image act as a topographic surface where high intensity denotes peaks and hills while low intensity denotes valleys [37]. Like water fills the valleys, every isolated local minima filled with different coloured labels. Since the water rises from the bottom of the valley as it fills, depending on the peaks of the gradients surrounded by, the water from different valleys tend to overspill by merging the different colors of labeled water.

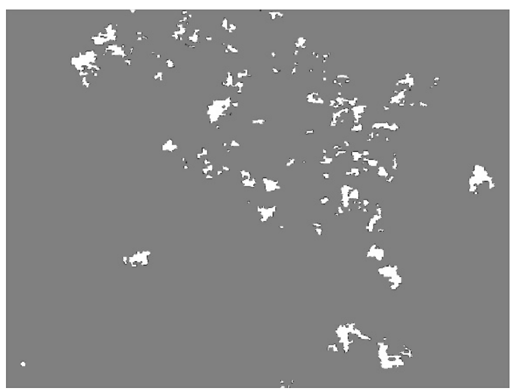
Therefore, in order to avoid the merging, the barriers are built in the locations whilst the water fills until all the peaks are in the range of protected barrier. Applying the watershed algorithm, in figure 3.23 shows the green layer extracted image.



(a) *Spirodela polyrhiza*



(b) *Lemna minor*

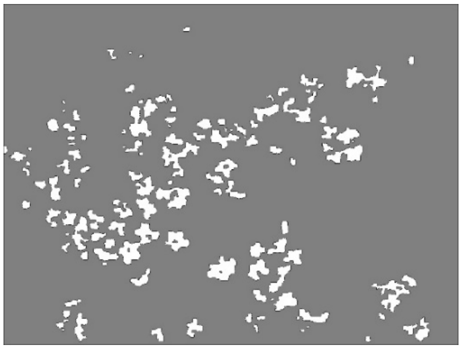


(c) *Azolla pinnata*

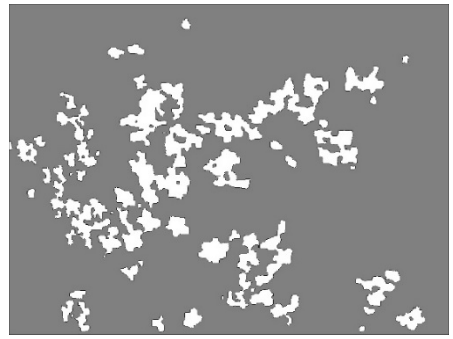
Figure 3.23 - Green layer extracted image after the watershed algorithm

While the white pixels are taken as green layer (fronds of the plant) and gray colour pixels are taken as back ground. Accordingly, it was calculated the percentage of the green layer. By following the same procedure, it was done the calculations for three plants in different three illuminance levels.

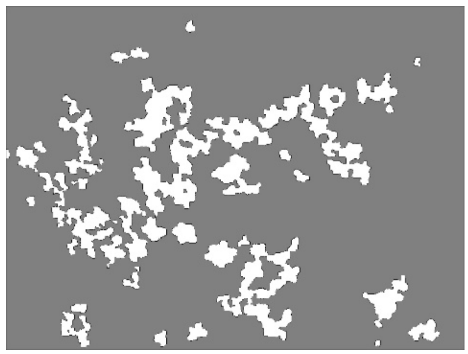
Green layer extracted images for three different illuminance levels for *Spirodela polyrhiza* are in figure 3.24.



(a) Normal illumination



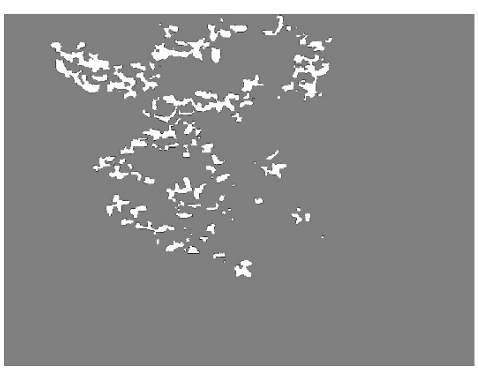
(b) Controlled illumination at 1000 lux



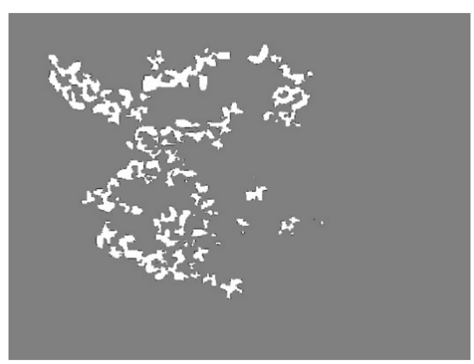
(c) Controlled illumination at 3800 lux

Figure 3.24 -Green layer extracted images for different illuminance levels for *Spirodela polyrhiza*.

Green layer extracted images at three different illuminance levels for *Lemna minor* as follows in figure 3.25.



(a) Normal illumination

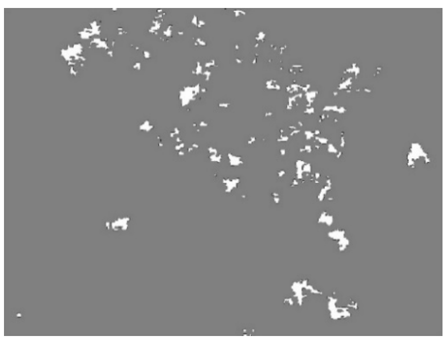


(b) Controlled illumination at 1000 lux

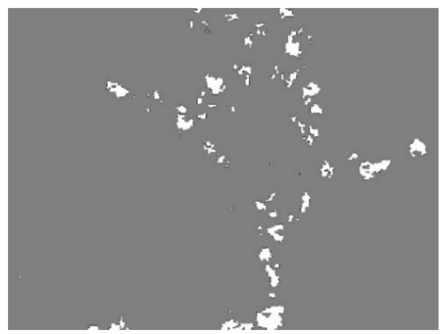
(c) Controlled illumination at 3800 lux

Figure 3.25 - Green layer extracted images for different illuminance levels for *Lemna minor*.

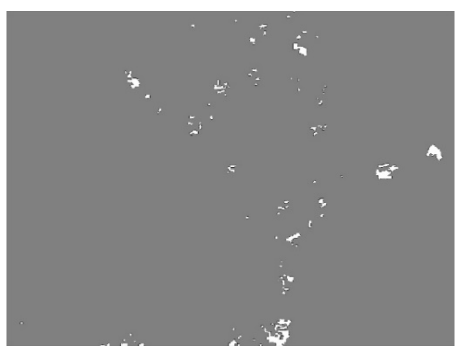
Green layer extracted images for three different illuminance levels for *Azolla pinnata* as follows in figure 3.26.



(a) Normal illumination



(b) Controlled illumination at 1000 lux



(c) Controlled illumination at 3800 lux

Figure 3.26 - Green layer extracted images for different illuminance levels for *Azolla pinnata*.

3.5 Growth Rate Estimation

Throughout the project, tried to monitor the growth rate in different illuminance levels for different three plants. Main objective was to check the accuracy of JVT method to estimate the biomass of different aquatic plants. According to the data gathered and processed, growth rate estimation discussed in CHAPTER 4.

CHAPTER 04

RESULTS

This chapter summarized the results obtained through out the project for both JVT and GLE methods. Accuracy testing was done with the ground truth and the Intersection over Union (IoU) and estimated the growth rate.

4.1 Ground Truth Proof

It was done the ground truth proof for three plants *Spirodela polyrhiza*, *Lemna minor* and *Azolla pinnata*. To estimate the biomass (green layer), it was considered the overlapped area with the ground truth and the all output images of the two methods.

Throughout the analysis main objective was to identify the performance of the algorithms under different illuminance levels. Fronds of three aquatic plants are different in shape, size, colour and frond surface quality. It was checked how these parameters effect for both GLE and JVT methods. It was considered three image data sets under different illuminance levels, to find out the performance of the algorithms under various conditions as follows,

4.1.1 Image Data Set 1

Image data set 1 for *Spirodela polyrhiza*, captured in different three illuminance levels which is controlled by using two LED panels. Considered illuminance levels were normal illumination, controlled illumination at 1000 lux and 3800 lux. 3MP Logitech web camera used to capture the images with resolution of 1280x960.

Then the two algorithms were used to estimate the biomass (green layer) of the plant under three illuminance levels. With the help of manually segmented images (ground truth) & IoU accuracy was calculated at three illuminance levels and it is indicated in table 4.1.a, b and c. To compare the both methods, minimum, maximum and mean values were obtained to check the accuracy and error percentages are indicated in table 4.2 and 4.3.

4.1.2 Image Data Set 2

Image data set 2 is for *Lemna minor*. Images were captured under three illuminance levels using arducam shield with 2MP camera module with resolution of 1600x1200. This used to analyze, how the accuracy percentage varies according to the quality of images with different camera. Accuracy was tested with ground truth & IoU is in table 4.4.a,b and c. Minimum, maximum and mean values for accuracy and error percentages are indicated in table 4.5 and 4.6.

4.1.3 Image Data Set 3

Azolla pinnata is the third image data set used for accuracy testing. Similarly, it was used arducam shield with 2MP camera module to capture the images with resolution of 1600x1200. Accuracy tested with ground truth and IoU is in table 4.7.a,b and c. Minimum, maximum and mean values obtained to check the accuracy and error percentages are indicated in table 4.8 and 4.9.

4.2 Accuracy Calculation for Image Data Set 1

This is for *Spirodela polyrhiza* under three illuminance conditions.

Table 4.1.a - Accuracy calculated in normal illumination.

	Ground Truth		Normal Illumination							
			JVT				GLE			
	Total Pix = 1228800		Total Pix = 76800				Total Pix = 1228800			
Day	GL in pixels	GL %	GL in pixels	GL %	ACC %	Error %	GL in pixels	GL %	ACC %	Error %
1	216346	17.61	11269	14.67	83.34	16.66	96965	7.89	44.82	55.18
3	234170	19.06	11803	15.37	80.65	19.35	107273	8.73	45.81	54.19
5	233932	19.04	10840	14.11	74.14	25.86	106452	8.66	45.51	54.49
7	230184	18.73	11051	14.39	76.82	23.18	105495	8.59	45.83	54.17
9	220129	17.91	11536	15.02	83.85	16.15	103902	8.46	47.20	52.80
11	225881	18.38	11545	15.03	81.78	18.22	103446	8.42	45.80	54.20
13	230200	18.73	12063	15.71	83.84	16.16	95387	7.76	41.44	58.56
15	253987	20.67	11911	15.51	75.03	24.97	104989	8.54	41.34	58.66
17	261105	21.25	16119	20.99	98.77	1.23	107089	8.71	41.01	58.99
19	263571	21.45	15475	20.15	93.94	6.06	112504	9.16	42.68	57.32
21	269205	21.91	15855	20.64	94.23	5.77	127925	10.41	47.52	52.48
23	265612	21.62	16146	21.02	97.26	2.74	134384	10.94	50.59	49.41
25	282445	22.99	17273	22.49	97.85	2.15	137589	11.20	48.71	51.29
27	288560	23.48	13869	18.06	76.90	23.10	104520	8.51	36.22	63.78
29	298963	24.33	17038	22.18	91.18	8.82	146866	11.95	49.13	50.87
31	329173	26.79	17881	23.28	86.91	13.09	151327	12.32	45.97	54.03
33	339988	27.67	17615	22.94	82.90	17.10	161301	13.13	47.44	52.56
35	356405	29.00	15633	20.36	70.18	29.82	131839	10.73	36.99	63.01
37	367679	29.92	20209	26.31	87.94	12.06	185588	15.10	50.48	49.52
39	393906	32.06	21317	27.76	86.59	13.41	193746	15.77	49.19	50.81
41	414383	33.72	21320	27.76	82.32	17.68	194669	15.84	46.98	53.02
43	431594	35.12	24893	32.41	92.28	7.72	208550	16.97	48.32	51.68
45	461500	37.56	23989	31.24	83.17	16.83	222050	18.07	48.11	51.89
47	463345	37.71	24742	32.22	85.44	14.56	228885	18.63	49.40	50.60
49	540542	43.99	26249	34.18	77.70	22.30	255407	20.79	47.25	52.75

Table 4.1.b - Accuracy calculated for Controlled illumination at 1000 lux

Day	Ground Truth		Controlled illumination at 1000 lux							
			JVT				GLE			
	Total Pix = 1228800		Total Pix = 76800				Total Pix = 1228800			
	GL in pixels	GL %	GL in pixels	GL %	ACC %	Error %	GL in pixels	GL %	ACC %	Error %
1	216346	17.61	10260	13.36	75.88	24.12	147492	12.00	68.17	31.83
3	234170	19.06	11005	14.33	75.19	24.81	165657	13.48	70.74	29.26
5	233932	19.04	9915	12.91	67.81	32.19	157608	12.83	67.37	32.63
7	230184	18.73	10926	14.23	75.95	24.05	163903	13.34	71.21	28.79
9	220129	17.91	9768	12.72	71.00	29.00	150559	12.25	68.40	31.60
11	225881	18.38	12677	16.51	89.80	10.20	171778	13.98	76.05	23.95
13	230200	18.73	10995	14.32	76.42	23.58	148692	12.10	64.59	35.41
15	253987	20.67	10336	13.46	65.11	34.89	157004	12.78	61.82	38.18
17	261105	21.25	13728	17.88	84.12	15.88	133697	10.88	51.20	48.80
19	263571	21.45	12714	16.55	77.18	22.82	131664	10.71	49.95	50.05
21	269205	21.91	13175	17.15	78.30	21.70	150350	12.24	55.85	44.15
23	265612	21.62	12905	16.80	77.74	22.26	157794	12.84	59.41	40.59
25	282445	22.99	14698	19.14	83.26	16.74	168201	13.69	59.55	40.45
27	288560	23.48	13553	17.65	75.15	24.85	166096	13.52	57.56	42.44
29	298963	24.33	13842	18.02	74.08	25.92	179039	14.57	59.89	40.11
31	329173	26.79	14591	19.00	70.92	29.08	203672	16.57	61.87	38.13
33	339988	27.67	14660	19.09	68.99	31.01	201911	16.43	59.39	40.61
35	356405	29.00	15755	20.51	70.73	29.27	214331	17.44	60.14	39.86
37	367679	29.92	17500	22.79	76.15	23.85	221914	18.06	60.36	39.64
39	393906	32.06	16750	21.81	68.04	31.96	233871	19.03	59.37	40.63
41	414383	33.72	17555	22.86	67.78	32.22	248057	20.19	59.86	40.14
43	431594	35.12	22208	28.92	82.33	17.67	275658	22.43	63.87	36.13
45	461500	37.56	22390	29.15	77.63	22.37	309794	25.21	67.13	32.87
47	463345	37.71	21191	27.59	73.18	26.82	307084	24.99	66.28	33.72
49	540542	43.99	23386	30.45	69.22	30.78	333150	27.11	61.63	38.37

Table 4.1.c - Accuracy calculated for controlled illumination at 3800 lux

Day	Ground Truth		Controlled illumination at 3800 lux							
			JVT				GLE			
	Total Pix = 1228800		Total Pix = 76800				Total Pix = 1228800			
	GL in pixels	GL %	GL in pixels	GL %	ACC %	Error %	GL in pixels	GL %	ACC %	Error %
1	216346	17.61	10370	13.50	76.69	23.31	171012	13.92	79.05	20.95
3	234170	19.06	11170	14.54	76.32	23.68	181137	14.74	77.35	22.65
5	233932	19.04	11225	14.62	76.77	23.23	180926	14.72	77.34	22.66
7	230184	18.73	11447	14.90	79.57	20.43	179384	14.60	77.93	22.07
9	220129	17.91	11399	14.84	82.85	17.15	178854	14.56	81.25	18.75
11	225881	18.38	11671	15.20	82.67	17.33	182621	14.86	80.85	19.15
13	230200	18.73	11923	15.52	82.87	17.13	182156	14.82	79.13	20.87
15	253987	20.67	12227	15.92	77.02	22.98	188635	15.35	74.27	25.73
17	261105	21.25	13752	17.91	84.27	15.73	166043	13.51	63.59	36.41
19	263571	21.45	13418	17.47	81.45	18.55	175446	14.28	66.56	33.44
21	269205	21.91	14281	18.60	84.88	15.12	193318	15.73	71.81	28.19
23	265612	21.62	13541	17.63	81.57	18.43	192441	15.66	72.45	27.55
25	282445	22.99	14937	19.45	84.62	15.38	205094	16.69	72.61	27.39
27	288560	23.48	15237	19.84	84.49	15.51	217031	17.66	75.21	24.79
29	298963	24.33	15333	19.96	82.06	17.94	227268	18.50	76.02	23.98
31	329173	26.79	16403	21.36	79.73	20.27	241576	19.66	73.39	26.61
33	339988	27.67	16455	21.43	77.44	22.56	252879	20.58	74.38	25.62
35	356405	29.00	17485	22.77	78.49	21.51	265364	21.60	74.46	25.54
37	367679	29.92	17768	23.14	77.32	22.68	273224	22.24	74.31	25.69
39	393906	32.06	18674	24.32	75.85	24.15	286506	23.32	72.73	27.27
41	414383	33.72	19411	25.27	74.95	25.05	301311	24.52	72.71	27.29
43	431594	35.12	23137	30.13	85.77	14.23	315636	25.69	73.13	26.87
45	461500	37.56	23171	30.17	80.33	19.67	345504	28.12	74.87	25.13
47	463345	37.71	23491	30.59	81.12	18.88	349529	28.44	75.44	24.56
49	540542	43.99	23972	31.21	70.96	29.04	376283	30.62	69.61	30.39

According to the image data set gathered, biomass was extracted for both JVT and GLE methods in different three illuminance levels. Figure 4.1 depicted the percentages of green layer with respect to ground truth. In JVT method, there is not much variation in accuracy, compared to ground truth in three illuminance levels while GLE method having considerable variation.

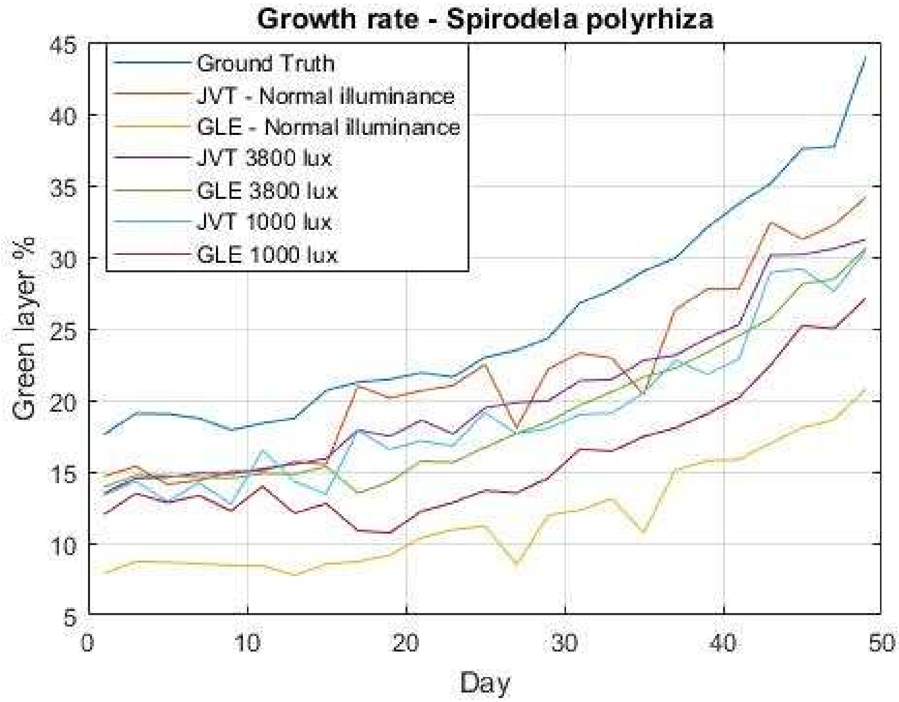


Figure 4.1 - Green layer percentage of both JVT and GLE methods

In figure 4.2 it shows, the green layer depicted in normal illumination for *Spirodela polyrhiza* in both JVT and GLE methods. When capturing the images day to day, illuminance level of each day not constant. Although it varies time to time, it was not much effect on green layer extraction in JVT method. With compared to ground truth GLE method has much variation in detecting the green layer.

When it falls different illuminance levels on the surface of the fronds of *Spirodela polyrhiza*, the green colour of the fronds appear in different clours. Because of that GLE method not recognize the green colour with appearance of colour variation.

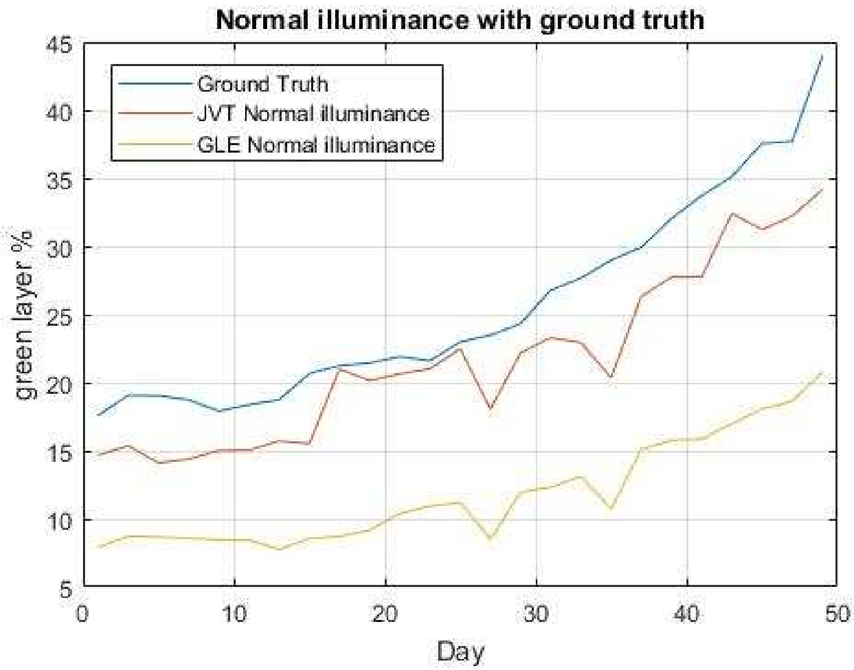


Figure 4.2 - Green layer variation for normal illumination level.

Figure 4.3 clearly show in JVT method with different illuminance levels, it is not much varied the green layer extraction with compared to ground truth.

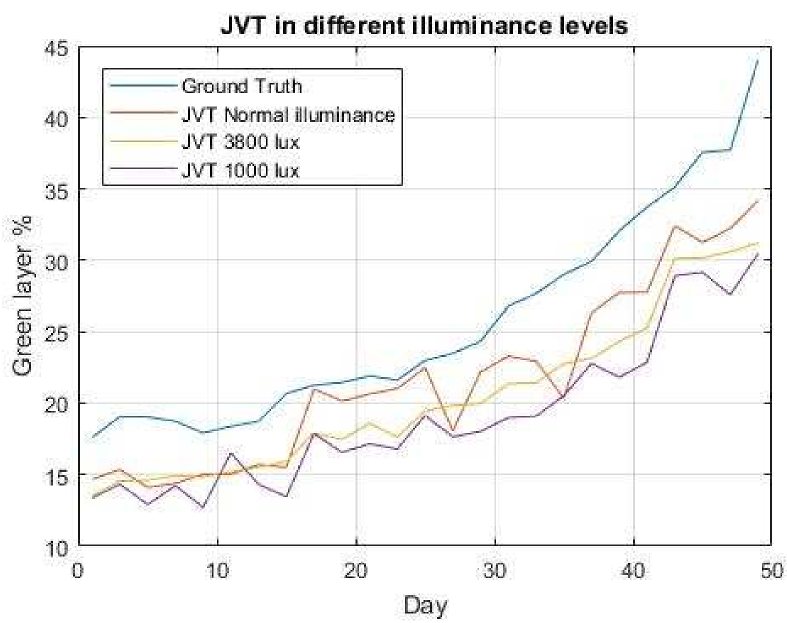


Figure 4.3 - Green layer extraction in different illuminance levels in JVT method

In accordance to the GLE method, in figure 4.4, there was considerable variation in green layer percentage than the JVT method. When in higher illuminance levels figure 4.4 shows that the percentage reaches to the ground truth value. Accordingly, GLE method depends on the illuminance level while JVTmethod not depend on environment physical characteristics, such as color elements, complex objects composition, shadows, brightness and inhomogeneous region colors for texture [20].

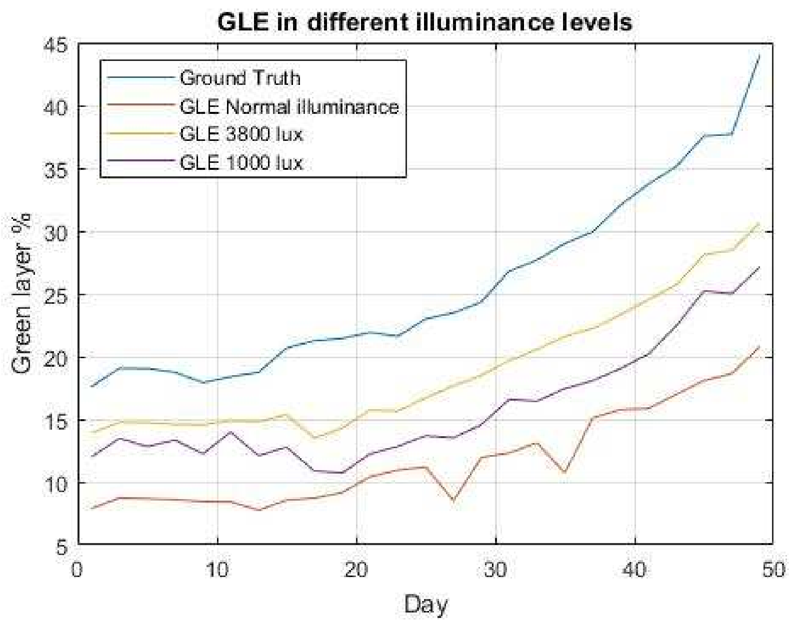


Figure 4.4 - Green layer extraction in different illuminance levels in GLE method

4.2.1 Accuracy Detection for *Spirodela polyrhiza.*

The detection accuracy was determined by obtaining the ground truth proportion of green pixels (obtained with IoU) in the image and comparing it to the proportional results obtained by the JVT and GLE. In accordance to the table 4.1.a,b and c accuracy comparison of the JVT and GLE methods, the results summarized in minimum, maximum and mean values indicated in table 4.2.

Table 4.2 - Accuracy comparison for *Spirodela polyrhiza*

Accuracy %	**Normal Illumination**		**3800 lux**		**1000 lux**	
	JVT	**GLE**	**JVT**	**GLE**	**JVT**	**GLE**
Min	70.18	36.22	70.96	63.59	65.11	49.95
Max	98.77	50.59	85.77	81.25	89.80	76.05
Mean	85.00	45.75	80.00	74.42	74.88	62.47

Table 4.3 - Error % comparison for *Spirodela polyrhiza*

Error %	**Normal illumination**		**3800 lux**		**1000 lux**	
	JVT	**GLE**	**JVT**	**GLE**	**JVT**	**GLE**
Min	16.66	55.18	23.31	20.95	24.12	31.83
Max	22.30	52.75	29.04	30.39	30.78	38.37
Mean	15.00	54.25	20.00	25.58	25.12	37.53

According to the summery on table 4.2, JVT method has the higher accuracy percentage than the GLE method in all illuminance levels. Considering the JVT method mean accuracy percentages of normal illumination, controlled illumination at 1000 lu**x** and 3800 lu**x** are respectively 85%, 74.88% and 80%. GLE method is based on the colour, while JVT method based on the texture composition. It is proofed here the texture is not depending on the external environmental characteristics like luminance level. Accordingly, GLE method having low accuracy than the JVT method.

Table 4.3 indicates the error percentages for *Spirodela polyrhiza* for three illuminance levels. When it considers the error percentages, mean error percentages of normal illumination, controlled illumination at 1000 lu**x** and 3800 lu**x** are respectively 15%, 25.12% and 20%. But in GLE method it is between 25 -55%. Considering the two ranges, JVT method having low error rate below 26%.

4.3 Results for Image Data Set 2

This is for *Lemna minor* under three illuminance conditions. This is also a green aquatic plant which has different appearance than the *Spirodela polyrhiza* in colour, shape and size. This plant was used to check the accuracy of two algorithms for different plants while using different camera to capture the images. 2MP arduino based camera module used here which can take images in different quality than above used Logithch webcam. Accuracy calculation on biomass estimation is in table 4.4.a,b and c.

Table 4.4.a - Accuracy calculated for Normal illumination

Day	**Ground Truth**		**Normal illumination**							
			JVT				**GLE**			
	Total Pix = 1920000		Total Pix =120000				Total Pix = 1920000			
	GL pixels	GL %	GL pixels	GL %	ACC %	Error %	GL pixels	GL %	ACC %	Error %
1	175782	9.16	10935	9.11	99.53	0.47	104973	5.47	59.72	40.28
3	283741	14.78	14922	12.44	84.14	15.86	104906	5.46	36.97	63.03
5	306201	15.95	14579	12.15	76.18	23.82	106922	5.57	34.92	65.08
7	333058	17.35	17388	14.49	83.53	16.47	104197	5.43	31.28	68.72
9	396122	20.63	16996	14.16	68.65	31.35	62137	3.24	15.69	84.31
11	448085	23.34	20823	17.35	74.35	25.65	138631	7.22	30.94	69.06
13	481333	25.07	23624	19.69	78.53	21.47	98582	5.13	20.48	79.52
15	553204	28.81	27175	22.65	78.60	21.40	85191	4.44	15.40	84.60
17	585462	30.49	30676	25.56	83.83	16.17	99478	5.18	16.99	83.01
19	697764	36.34	35075	29.23	80.43	19.57	80239	4.18	11.50	88.50
21	840161	43.76	43975	36.65	83.75	16.25	53707	2.80	6.39	93.61
23	843940	43.96	46978	39.15	89.06	10.94	48338	2.52	5.73	94.27
25	916434	47.73	50929	42.44	88.92	11.08	25522	1.33	2.78	97.22
27	1001020	52.14	52711	43.93	84.25	15.75	53268	2.77	5.32	94.68
29	982596	51.18	55362	46.14	90.15	9.85	21104	1.10	2.15	97.85

Table 4.4.b - Accuracy calculated for controlled illumination at 1000 lux

Day	**Ground Truth**		**Controlled illumination at 1000 lux**							
			JVT				**GLE**			
	Total Pix = 1920000		Total Pix = 120000				Total Pix = 1920000			
	GL pixels	GL %	GL pixels	GL %	ACC %	Error %	GL pixels	GL %	ACC %	Error %
1	175782	9.16	6961	5.80	63.36	36.64	57675	3.00	32.81	67.19
3	283741	14.78	13124	10.94	74.01	25.99	126805	6.60	44.69	55.31
5	306201	15.95	14583	12.15	76.20	23.80	134306	7.00	43.86	56.14
7	333058	17.35	17471	14.56	83.93	16.07	136869	7.13	41.09	58.91
9	396122	20.63	19224	16.02	77.65	22.35	90811	4.73	22.93	77.07
11	448085	23.34	21036	17.53	75.11	24.89	90757	4.73	20.25	79.75
13	481333	25.07	24478	20.40	81.37	18.63	104823	5.46	21.78	78.22
15	553204	28.81	28756	23.96	83.17	16.83	105283	5.48	19.03	80.97
17	585462	30.49	32827	27.36	89.71	10.29	84510	4.40	14.43	85.57
19	697764	36.34	38320	31.93	87.87	12.13	104162	5.43	14.93	85.07
21	840161	43.76	47233	39.36	89.95	10.05	103003	5.36	12.26	87.74
23	843940	43.96	51074	42.56	96.83	3.17	42180	2.20	5.00	95.00
25	916434	47.73	53027	44.19	92.58	7.42	150486	7.84	16.42	83.58
27	1001020	52.14	55552	46.29	88.79	11.21	50610	2.64	5.06	94.94
29	982596	51.18	58154	48.46	94.69	5.31	152487	7.94	15.52	84.48

Table 4.4.c - Accuracy calculated for controlled illumination at 3800 lux

Day	**Ground Truth**		**Controlled illumination at 3800 lux**							
			JVT				**GLE**			
	Total Pix = 1920000		Total Pix = 120000				Total Pix = 1920000			
	GL pixels	GL %	GL pixels	GL %	ACC %	Error %	GL pixels	GL %	ACC %	Error %
1	175782	9.16	10523	8.77	95.78	4.22	93692	4.88	53.30	46.70
3	283741	14.78	12083	10.07	68.14	31.86	103132	5.37	36.35	63.65
5	306201	15.95	14379	11.98	75.13	24.87	100993	5.26	32.98	67.02
7	333058	17.35	17703	14.75	85.04	14.96	118818	6.19	35.67	64.33
9	396122	20.63	19527	16.27	78.87	21.13	77255	4.02	19.50	80.50
11	448085	23.34	19997	16.66	71.40	28.60	79273	4.13	17.69	82.31
13	481333	25.07	24264	20.22	80.66	19.34	109160	5.69	22.68	77.32
15	553204	28.81	29736	24.78	86.00	14.00	133357	6.95	24.11	75.89
17	585462	30.49	33833	28.19	92.46	7.54	123927	6.45	21.17	78.83
19	697764	36.34	39215	32.68	89.92	10.08	134860	7.02	19.33	80.67
21	840161	43.76	47738	39.78	90.91	9.09	150823	7.86	17.95	82.05
23	843940	43.96	52422	43.69	99.39	0.61	177555	9.25	21.04	78.96
25	916434	47.73	55025	45.85	96.07	3.93	255127	13.29	27.84	72.16
27	1001020	52.14	57739	48.12	92.29	7.71	204171	10.63	20.40	79.60
29	982596	51.18	60199	50.17	98.02	1.98	336229	17.51	34.22	65.78

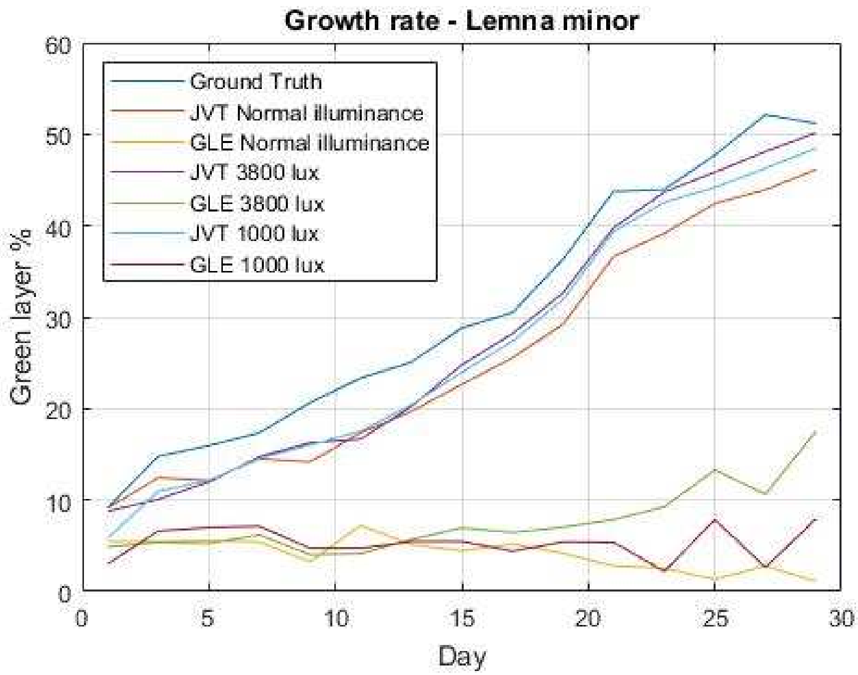


Figure 4.5 - Green layer extraction in three illuminance levels for both methods for *Lemna minor*

Figure 4.5 depicted the green layer extraction in three different illuminance levels for both JVT and GLE methods for *Lemnar minor*. Here it clearly shows in figure 4.5, how the accuracy deviates in both methods with respect to the ground truth. Because of the variation of colour due to various illuminance levels GLE method gives low accuracy on detecting green layer compared to the ground truth. It can clearly identify JVT method has much accuracy compared to the ground truth.

Lemna minor has light green colour fronds. When it falls light on it, surface of the fronds tends to glow. Because of that GLE method difficult to identify the green colour by giving low green layer percentage with respect to ground truth. As well as quality of the image also effect on the accuracy. Although it was used different camera to capture the images, it was not effected on JVT method. It gives higher accuracy percentage on green layer extraction even under low image quality.

4.3.1 Accuracy Detection for *Lemna minor*

According to the image data gathered and accuracy calculated Table 4.5 summarized the accuracy in minimum, maximum and mean values. For JVT method, mean accuracy percentages of normal illuminanion, controlled illumination at 1000 lux and 3800 lux are respectively 85.93%,83.68% and 86.67% while GLE method having low accuracy between 19 – 27%.

Table 4.5 - Accuracy comparison for *Lemna minor*

Accuracy %	**Normal illuminance**		**3800 lux**		**1000 lux**	
	JVT	**GLE**	**JVT**	**GLE**	**JVT**	**GLE**
Min	68.65	2.15	68.14	17.69	63.36	5.00
Max	99.53	59.72	99.39	53.30	96.83	44.69
Mean	82.93	19.75	86.67	26.95	83.68	22.00

Table 4.6 - Error % comparison for *Lemna minor*

Error %	**Normal illuminance**		**3800 lux**		**1000 lux**	
	JVT	**GLE**	**JVT**	**GLE**	**JVT**	**GLE**
Min	0.47	97.85	0.61	46.70	3.17	55.31
Max	31.35	40.28	31.86	82.31	36.64	95.00
Mean	17.07	80.25	13.33	73.05	16.32	78.00

According to table 4.6, the mean error percentages for JVT method are below 18% in all illuminance levels while GLE having up to 80%. Compared to GLE method, JVT method is more accurate in estimating the biomass.

4.4 Results of Image Data Set 3

This is for *Azolla pinnata* under three illuminance conditions. This green plant is not like *Spirodela polyrhiza* and *Lemna minor*, triangle in shape and frond colour varies to green to brown colour. When is grows older, fronds turned in to brown colour.

Image data set gathered and accuracy calculation is in table 4.7.a,b and c while depicted in figure 4.6.

Table 4.7.a - Accuracy calculated for Normal illumination

Day	GT		Normal illumination							
			JVT				GLE			
	Total Pix = 1920000		Total Pix = 30000				Total Pix = 1920000			
	GL in pixels	GL %	GL in pixels	GL %	ACC %	Error %	GL in pixels	GL %	ACC %	Error %
001	386547	20.13	4686	15.62	77.59	22.41	82040	4.27	21.22	78.78
002	417753	21.76	4752	15.84	72.80	27.20	24522	1.28	5.87	94.13
003	450258	23.45	5417	18.06	77.00	23.00	33469	1.74	7.43	92.57
004	474569	24.72	6114	20.38	82.45	17.55	36411	1.90	7.67	92.33
005	528471	27.52	6899	23.00	83.55	16.45	85390	4.45	16.16	83.84
006	566521	29.51	7381	24.60	83.38	16.62	118283	6.16	20.88	79.12
007	656032	34.17	8940	29.80	87.22	12.78	179453	9.35	27.35	72.65
008	771184	40.17	10547	35.16	87.53	12.47	143636	7.48	18.63	81.37
009	787992	41.04	11369	37.90	92.34	7.66	181336	9.44	23.01	76.99
010	887053	46.20	12922	43.07	93.23	6.77	223997	11.67	25.25	74.75

Table 4.7.b - Accuracy calculated for Controlled illumination at 1000 lux

Day	GT		Controlled illumination at 1000 lux							
			JVT				GLE			
	Total Pix = 1920000		Total Pix = 120000				Total Pix = 1920000			
	GL in pixels	GL %	GL in pixels	GL %	ACC %	Error %	GL in pixels	GL %	ACC %	Error %
001	386547	20.13	3732	12.44	61.79	38.21	72163	3.76	18.67	81.33
002	417753	21.76	4423	14.74	67.76	32.24	94902	4.94	22.72	77.28
003	450258	23.45	4949	16.50	70.35	29.65	83577	4.35	18.56	81.44
004	474569	24.72	5450	18.17	73.50	26.50	106444	5.54	22.43	77.57
005	528471	27.52	6730	22.43	81.50	18.50	156770	8.17	29.66	70.34
006	566521	29.51	7782	25.94	87.91	12.09	174390	9.08	30.78	69.22
007	656032	34.17	8796	29.32	85.81	14.19	267342	13.92	40.75	59.25
008	771184	40.17	9929	33.10	82.40	17.60	248357	12.94	32.20	67.80
009	787992	41.04	10733	35.78	87.17	12.83	299518	15.60	38.01	61.99
010	887053	46.20	12159	40.53	87.73	12.27	307442	16.01	34.66	65.34

Table 4.7.c - Accuracy calculated for Controlled illumination at 3800 lux

Day	GT		Controlled illumination at 3800 lux							
			JVT				GLE			
	Total Pix = 1920000		Total Pix = 120000				Total Pix = 1920000			
	GL in pixels	GL %	GL in pixels	GL %	ACC %	Error %	GL in pixels	GL %	ACC %	Error %
001	386547	20.13	3859	12.86	63.89	36.11	25763	1.34	6.66	93.34
002	417753	21.76	4648	15.49	71.21	28.79	45547	2.37	10.90	89.10
003	450258	23.45	5313	17.71	75.52	24.48	41192	2.15	9.15	90.85
004	474569	24.72	5793	19.31	78.12	21.88	63622	3.31	13.41	86.59
005	528471	27.52	7226	24.09	87.51	12.49	117285	6.11	22.19	77.81
006	566521	29.51	8575	28.58	96.87	3.13	150604	7.84	26.58	73.42
007	656032	34.17	9253	30.84	90.27	9.73	138699	7.22	21.14	78.86
008	771184	40.17	10428	34.76	86.54	13.46	177616	9.25	23.03	76.97
009	787992	41.04	11354	37.85	92.22	7.78	282277	14.70	35.82	64.18
010	887053	46.20	12764	42.55	92.09	7.91	331112	17.25	37.33	62.67

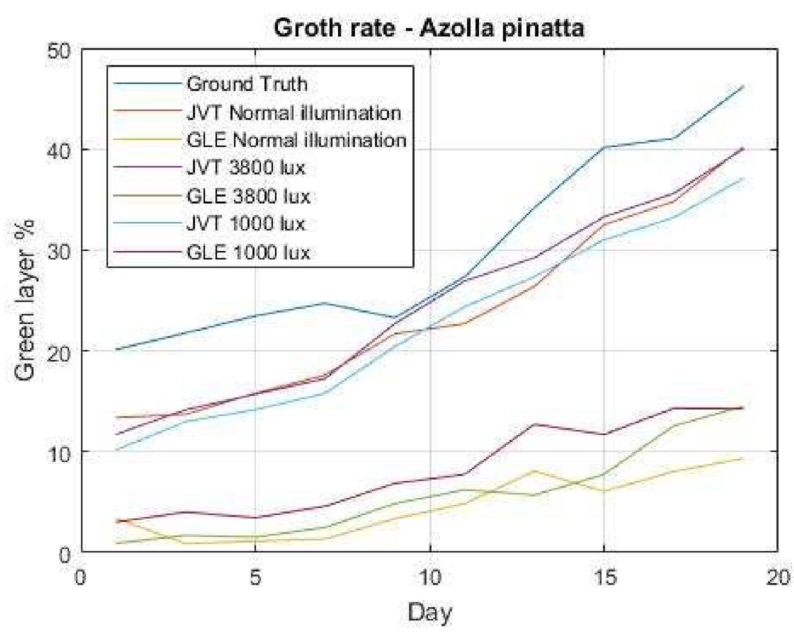


Figure 4.6 - Green layer extraction in three illuminance levels for both methods for *Azolla pinnata*.

4.4.1 Accuracy Detection for *Azolla pinnata*

In table 4.8, it is summarized accuracy calculated in minimum, maximum and mean values.

Table 4.8 - Accuracy comparison for *Azolla pinnata*

Accuracy %	**Normal illumination**		**3800 lux**		**1000 lux**	
	JVT	**GLE**	**JVT**	**GLE**	**JVT**	**GLE**
Min	72.80	5.87	63.89	6.66	61.79	18.56
Max	93.23	27.35	96.87	37.33	87.91	40.75
Mean	83.71	17.35	83.42	20.62	78.59	28.85

Table 4.9 - Error % comparison for *Azolla pinnata*

Error %	**Normal illumination**		**3800 lux**		**1000 lux**	
	JVT	**GLE**	**JVT**	**GLE**	**JVT**	**GLE**
Min	6.90	76.36	1.46	68.60	10.80	62.82
Max	37.07	95.98	41.82	95.46	49.53	85.27
Mean	16.29	82.65	16.58	79.38	21.41	71.15

Here, in both methods there is a reduction of mean accuracy value compared to other two plants. Specially in GLE method because of the colour variation, it gives low mean accuracy percentage. Velvety surface area of the *Azolla pinnata* also a reason for that. But in JVT method, there is a higher accuracy compared to GLE method. Because of in JVT method based on the texture based segmentation, it is not effects the surface appearance for biomass estimation.

With compared to *Spirodela polyrhiza* and *Lemna minor*, *Azolla pinnata* has higher error rate as indicated in table: 4.9.

4.5 Accuracy of the Introduced Methods

Comparison and interpretation of the results and advantages and disadvantages of the system are the important final outcomes of the research. Ground truth proof accuracy test was used to identify the reliability methodologies under controlled, specific environment and various conditions with different camera resolutions.

According to the accuracy comparison of three plants, as indicated in table 4.3, 4.5 and 4.8 JVT method has considerable accuracy than the GLE method. One of the unsuccessfulness of the GLE method is, it is mainly focused on extracting the green colour of the plants. Most of the time, it is not recognized the fronds which near to yellow, blue and brown colour. The main drawback of the GLE method is dependence on the illuminance level. When it falls the light in different levels, it tends to appear the frond surface colour in different way due to glow, shadow, brightness and other environmental characteristics.

GLE method based on the HSV transformation, obtaining the mask, morphological transformation and water shade algorithm. To get the better performance of this method, it is required more sophisticated pre and post processing.

JVT method segment the colour image with homogeneous regions to generate clusters in the colour class. And segments images of natural scenes, without manual parameter adjustment for each image and simplifies texture and color. This segmentation does not depend on color elements, complex objects composition, shadows, brightness and inhomogeneous region colors for texture. Here the water surface is highly homogeneous and fronds of the plant is inhomogeneous. Thus texture based thresholding efficiently differentiate the fronds of the plant and the water surface.

According to the accuracy calculations in table 4.2, 4.5 and 4.8, it clearly shows the JVT method does not depend on illuminance level and the quality of the images. It is based on the texture.

4.6 Correlation Results

The robustness to illumination is analyzed using the Pearson Correlation Coefficient (PCC) for different illumination levels for the given method and the ground truth (GT). The PCC results are given below for the JVT and GLE respectively in Table 4.10 and 4.11.

According to the correlation analysis, it was investigated the relationship between ground truth and the two methods used under three different illuminance levels.

4.6.1 PCC for JVT Based Estimation

Table 4.10 - PCC for JVT based estimation for different illumination levels

Species	Method	PCC Results		
		GT	JVT (Normal)	JVT (1000 lux)
Spirodela polyrhiza	JVT (Normal)	0.954	-	-
	JVT (1000 lux)	0.964	0.966	-
	JVT (3800 lux)	0.981	0.969	0.983
Lemnar minor	JVT (Normal)	0.993	-	-
	JVT (1000 lux)	0.997	0.997	-
	JVT (3800 lux)	0.993	0.998	0.998
Azolla pinnata	JVT (Normal)	0.998	-	-
	JVT (1000 lux)	0.992	0.991	-
	JVT (3800 lux)	0.987	0.985	0.999

4.6.2 PCC for GLE Based Estimation

Table 4.11 - PCC for GLE based estimation for different illumination levels

Species	Method	PCC Results		
		GT	GLE (Normal)	GLE (1000 lux)
Spirodela polyrhiza	GLE (Normal)	0.971	-	-
	GLE (1000 lux)	0.961	0.952	-
	GLE (3800 lux)	0.961	0.952	0.942
Lemnar minor	GLE (Normal)	-0.816	-	-
	GLE (1000 lux)	-0.031	-0.116	-
	GLE (3800 lux)	0.820	-0.831	0.309
Azolla pinnata	GLE (Normal)	0.908	-	-
	GLE (1000 lux)	0.965	0.945	-
	GLE (3800 lux)	0.956	0.894	0.927

The results indicate that JSEG method is more robust than GLE to variations in illumination since it shows consistently high results for the ground truth as well as among different illumination levels. All of the PCC values for the JVTare in excess of 95%. On the other hand, the GLE method exhibits low and negative correlations in some cases.

Accordingly, the results indicate that JVT method is more robust than the GLE to variations in illumination since it shows consistently high results for the ground truth as well as among different illumination levels.

CHAPTER 5

CONCLUSION

Throughout the project growth rates were monitored for *Spirodela polyrhiza*, *Lemna minor* and *Azolla pinnata*. At each stage obtained results were compared with a reference (Ground truth) and considering the Intersection over Union (IoU) to verify the project is driving in the correct path. According to the results, obtained the growth rates of three plants by JVT and GLE methods, under three different illuminance levels normal illumination, controlled illumination at 1000 lux and 3800 lux.

Research introduced a new method of automated estimation of the wet biomass of above three plants. The method uses a homogeneity measure known as the J- value to discriminate between the texture of the fronds of the plants from the uniform water surface.

The proposed method is highly accurate compared to the alternative GLE method. For the JVT method, the mean accuracy under normal illumination for *Spirodela polyrhiza is 85%*, for *Lemna minor* 82.93% and 83.71 % for *Azolla pinnata* while GLE method having low accuracy percentages. Because of JVT method is independent with illuminance level, texture, shadows and brightness.

The PCC results show that, it is highly robust to variations in lighting with the resulting value ranging from 95% - 99%. It can be concluded that the vision based image processing techniques successfully used for the estimation of growth rate analysis of *Spirodela polyrhiza*, *Lemna minor* and *Azolla pinnata* under different illuminance levels.

In terms of future works, the main focus has to be on adapting this method for use outside laboratory conditions such as flowing water and growth of filamentous algae. It can also be tried on floating aquatic plants with large fronds such as *Salvinia molesta* and *Pistia stratiotes*. Calibration of the models using measured the moisture content of the fronds is another important line of investigation.

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